ESAB 2024
EUROPEAN
SOUTH ATLANTIC
BIOPHYSICS
CONGRESS

Book of Abstracts

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Welcome letter

The Organizing Committee presents the first International Biophysics Congress **"European South Atlantic Biophysics Congress (ESAB 2024)"** that will take place in Donostia-San Sebastián, from June 5 to 7, 2024. This conference is the first event of an agreement between the **Spanish Biophysical Society (SBE)**, the **French Biophysical Society (SFB)** and the **Portuguese Biophysical Societies (SPBf)**, to organize regular conferences promoting biophysics, especially among young scientists, in southwestern Europe. Our goal is to promote this event biennially as a complement to the Congress organized by the **European Biophysical Societies' Association (EBSA)** by increasing connections within the biophysics community. In this context, the next Congresses will take place in Montpellier in 2026 promoted by SFB, and in Portugal in 2028 promoted by SPBf (location to be determined).

This first edition will highlight the most innovative contributions developed in the field of Biophysics, giving visibility to a wide range of lines of research, both experimental and theoretical, related to biophysics. Specifically, scientists from diverse backgrounds – chemists, biochemists, biologists, and physicists – work together to carry out cutting-edge fundamental and translational research in biophysics with the aim of improving the understanding of biological processes and developing new applications based on this understanding, including new tools in biomedicine.

ESAB 2024 research conference will be held at the heart of Donostia-San Sebastian city, facing the sea. Kursaal Congress Centre, an avant-grade building designed by Rafael Moneo, won the Mies van der Rohe Prize for the best building in Europe in 2001. This time, Kursaal Congress Center will have the honour of hosting an invaluable audience of biophysicists. Indeed, we are pleased to present an outstanding program in the field with 3 internationally renowned plenary speakers and 20 invited speakers, 29 oral and 27 flash communications, 6 platinum sponsors talks, more than 130 scientific posters and 11 exhibition booths. The Conference will also celebrate the three biophysical societies awards, as such the SBE prizes: the "Manuel Rico" - Bruker España, "E. Pérez Payá - Prospera Biotech- SBE 40" and "Hawk Biosystems - SBE 33" Prizes for senior investigators, those up to 40 years old and those up to 33 years old, respectively. The SFB award that nominates SFB young researcher Prize and the SPBf award that nominates the SPBf Young Biophysicist prize. The conference also organizes a Congress Satellite Workshop Event – Young Scientists Seminar (ESAB-YSS), which will be held at Donostia International Physics Center (DIPC), on June 4-5, 2024, focused on graduate students, postdoctoral researchers and other early-career scientists attending the ESAB Congress.

In addition, ESAB 2024, wants you to enjoy this unique location, exchange of ideas, social events, and the best atmosphere! ESAB 2024 Congress had the collaboration of local research centers active in Biophysics including the main local organizer, CIC biomaGUNE and local collaborators such as CIC bioGUNE, DIPC, and Biofisika Institute. ESAB 2024 Congress has been supported by the Basque Government, the Gipuzkoa Provincial Council, Donostia-San Sebastian Convention Bureau, Kursaal Donostia-San Sebastián, and Fomento Donostia-San Sebastián. We thank to all our sponsors and exhibitors, such as Lumics GmbH, Fidabio, Paralab S.L., Abberior Instruments GmbH, Impetux Optics S.L., Azbil Testar S.L.U., Cube biotech GmbH, Labclinics S.A., The Protein Society (TPS), Nanion Technologies GmbH, ARBRE Research Infrastructure, Scharlab S.L., LI-COR, and Termo Fisher Scientific. The three Societies will give their awards in the two awards sessions, and Scharlab S.L. and ARBRE will sponsor the best oral presentation and best poster prizes.

We expect that this Congress will establish itself as a reference meeting in Europe for a large and diverse community of researchers working in Biophysics, with special focus on young biophysicists. We are setting up an inspiring event that will provide plenty of opportunities for interaction and networking.

We look forward to seeing you in Donostia!
Ongi etorri Donostiara!

Aitziber L. Cortajarena
President of the Organizing and Scientific Committee (SBE)

José María Valpuesta
President of the Spanish Biophysical Society (SBE)

Coralie Bompard
President of the French Biophysical Society (SFB)

Armindo Salvador
President of the Portuguese Biophysical Society (SPBf)

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Coralie Bompard President of the French Biophysical Society (SFB)
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Hélène Deméné, Centre de Biologie Structurale.

MontpellierPortuguese Biophysical Society (SPBf)
Armindo Salvador, Center for Neuroscience and Cell Biology-University of Coimbra
Manuela Pereira, University of Lisbon

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Aitor Manteca, CIC biomaGUNE, San Sebastián

Sponsors & Organizers

Organizers

SBE Spanish Biophysical Society

SFB Société Française de Biophysique

SPBf Sociedade Portuguesa de Biofísica

EBSA Biophysics in Europe

Local Organizer

Main organizer

CIC bioma GUNE
MEMBER OF BASQUE RESEARCH & TECHNOLOGY ALLIANCE

Collaborators

CIC bioGUNE
MEMBER OF BASQUE RESEARCH & TECHNOLOGY ALLIANCE

DIPC Donostia International Physics Center

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Programme

Wednesday 5th June 2024

14:00 Registration

14:30 - 16:00 Plenary Session, Plenary Room

14:30 Opening Ceremony

15:00 Plenary Lecture, Chair: Jose Maria Valpuesta, Aitziber L. Cortajarena

Functional insights from the visualization of large protein complexes regulating human gene expression
Prof. Eva Nogales, University of California Berkeley, USA

16:00 - 16:30 Coffee break

16:30 - 18:30 Parallel Sessions

Session I: *Cell and tissue biophysics: Mechanobiology, Single cell biophysics*, Plenary Room,
Chairs: Nuno Santos, Coralie Bompard

16:30 Invited speaker
FRET as a tool to probe the nanoscale environment of membrane proteins
Dr. Fabio Fernandes, Universidade de Lisboa, Portugal

17:00 Invited speaker
Tissue flows and patterns during symmetry breaking of multicellular systems
Dr. Pierre-François Lenne, CNRS Research Director, Aix-Marseille University, France

17:30 Platinum Sponsor
Cell membrane and intracellular mechanics inside cells, tissues and living organisms using SENSOCELL optical tweezers and TimSOM Micro-rheology
Oriol Nos Aguilà, Impetux Optics S.L., Spain

17:45 Oral contribution
Unraveling native complexity: Single molecule characterization of perinatal titin by force spectroscopy of tissue-purified proteins
Inés Martínez-Martín, Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain

18:00 Oral contribution
Exploring the role of GalNAc glycosylations at the cellular and molecular level
S. Masó-Orriols, University of Vic, Barcelona, Spain

18:15 Flash Talk
Mechanobiology of the secretory pathway: Golgi export responds to external mechanical cues
Javier Vera Lillo, ICFO- -Institut de Ciencies Fotoniques, Spain

18:20 Flash Talk
Two-layer elastic models for single-yeast compressibility with flat microlevers
F. Argoul, Université de Bordeaux, Talence, France

18:25 Flash Talk
Nanomechanics of cell-derived matrices in Collagen VI-related Congenital Muscular Dystrophies
Marina I. Giannotti, IBEC, Barcelona, Spain

Session II: *Disordered proteins, Liquid-liquid Phase Separation and Aggregation*, Room III,
Chairs: Véronique Receveur, Nunilo Cremades

16:30 Invited speaker
Modulators of Tau aggregation
Dr. Yann Fichou, Institut Europeen de Chimie et Biologie (IECB-CNRS), University of Bordeaux, France

17:00 Invited speaker – joint partnership with the Biophysical Society of Argentina
Unlocking the Parkinson's Puzzle: Oligomerization, Membrane-Curvature Sensitivity, and Phase Behavior of Alpha-Synuclein
Dra. María Soledad Celej. President of the Biophysical Society of Argentina, Research Center in Biological Chemistry, CONICET-UNC, Córdoba, Argentina

17:30 Oral contribution
Disordered proteins interact with the chemical environment to tune their protective function during drying
Thomas Boothby, Department of Molecular Biology, University of Wyoming, Laramie, WY, USA

17:45 Oral contribution
Conformational dynamics and liquid-liquid phase separation of the endocytic protein Eps15
Andromachi Papagiannoula, Leibniz-Forschungsinstitut für Molekulare Pharmakologie im Forschungsverbund, Berlin, Germany

18:00 Platinum Sponsor
Breaking Cryo-TEM barriers at lower kV- Introducing Tundra with a Falcon-C detector
Dhirendra Singh, EMEA, Life Sciences

18:15 Flash Talk
Labile assembly of a tardigrade protein induces biostasis
Silvia Sánchez-Martínez. Department of Molecular Biology, University of Wyoming, Laramie, WY, USA

18:20 Flash Talk
Unraveling the role of crowding-driven biomolecular condensates in bacterial cell division
Silvia Zorrilla. Centro de Investigaciones Biológicas Margarita Salas (CIB, CSIC), Madrid, Spain

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18:00-18:30 Outreach event, Ruiz Balerdi Hall. Kutxa Fundazioa, 4th floor, TABAKALERA
Healthy weight? Science has all the answers
Prof. Félix M. Goñi

18:30-20:30 Welcome Cocktail

Thursday 6th June 2024

9:00 - 10:00 Plenary Session, Plenary Room

9:00 Plenary Lecture, Chair: Coralie Bompard
Engineering protein stability and solubility, from monomers to inclusion bodies
Prof. Elizabeth Meiering, University of Waterloo, President of the Protein Society, Canada

10:00 - 10:30 Coffee break

10:30 - 12:30 Parallel Sessions

Session III: *Protein structure, integrative structural biology, dynamics and function,* Room I
Chairs: Claudio Soares, Jose Maria Valpuesta

10:30 Invited speaker
Where to start : the dynamic adventure of identifying start codons in mRNAs
Dr. Israel Fernández, Biofisika Institute (CSIC, UPV/EHU), Spain

11:00 Invited speaker
Exploring Ataxin-3 Pathogenic Aggregation with Supramolecular Inhibitors: Clues for SCA3 therapies
Dr. Sandra Ribeiro Macedo, IS3, Porto, Portugal

11:30 Platinum Sponsor
FIDA technology – Combining structural and functional information,
Hasse Hedeby, Marketing Manager, FidaBio, Denmark.

11:45 Flash Talk
The Pseudomonas aeruginosa effector Tse5 forms membrane pores disrupting the membrane potential of intoxicated bacteria
David Albesa-Jové, Instituto Biofisika, Leioa, Spain.

11:50 Flash Talk
ESV1 and LESV, two new non-enzymatic proteins involved in new stages in the biosynthesis of starch granules
Coralie Bompard, Université de Lille, CNRS, Lille, France.

11:55 Flash Talk
High resolution structure of a T=219 giant virus,
Sara Otaegi-Ugartemendia, Centro Nacional de Biotecnología (CNB- CSIC), Spain.

12:00 Flash Talk
How can protonation influence conformational switching: the curious mechanism of diphtheria toxin translocation (T-) domain
Nuno F. B. Oliveira, Universidade de Lisboa, Portuga.

12:05 Flash Talk
Structural basis for functional complexes between heptameric GroEL chaperonin and tetrameric plastid Cpn20 cochaperonin
Alberto G. Berruezo, Instituto Biofisika, Spain.)

12:10 Flash Talk
A novel BioID strategy for in vivo detection of protein-protein interactions
Laura Sen-Martín, Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Spain.

12:15 Flash Talk
The genetic architecture of hydrophobic protein cores through deep sequence space
Albert Escobedo, Centre for Genomic Regulation (CRG), Spain.

12:20 Flash Talk
Bag1 has a key role in the Hsp70-assisted, proteasome-mediated degradation pathway
Jorge Cuéllar, Centro Nacional de Biotecnología (CNB-CSIC), Spain.

12:25 Flash Talk
Molecular mechanism of a bacterial Retron
Arturo Carabias, Novo Nordisk Foundation Centre for Protein Research, University of Copenhagen, Denmark.

Session IV: *Biophysics of Nucleic Acids,* Room II, Chairs: Eric Ennifar, Fernando Moreno Herrero

10:30 Invited speaker
Beyond the helix: probing the structure of supercoiled DNA using high- resolution atomic force microscopy
Dr. Alice Pyne, University of Sheffield, UK.

11:00 Invited speaker
Monitoring tRNA maturation and identifying modification circuits with NMR spectroscopy
Dr Pierre Barraud, Institut de Biologie Physico-Chimique, (IBPC), CNRS-Université Paris Cité, France.

11:30 Platinum Sponsor
Towards the real-time observation and manipulation of biological processes: from single molecule to whole cells
Dr. Vincenzo Mascoli, LUMICKS, The Netherlands

11:45 Oral contribution
Exploring Long Non-Coding RNA secondary structures through AFM imaging
Eva M. Martín Cuevas, Spanish National Center of Biotechnology (CNB-CSIC), Spain.

12:00 Oral contribution
Single molecule studies of the role of FBH1 in fork reversal
Javier Mendia-García, Centro Nacional de Biotecnología (CNB), CSIC, Spain.

12:15 Flash Talk
Monitoring of a DNA double helix formation: a multi-approach benchmark study
Carmelo di Primo, University of Bordeaux, CNRS, INSERM, France

12:20 Flash Talk
Characterization of the real-time kinetics of the activities of the human mitochondrial DNA helicase at the single-molecule level
Ismael Plaza, Instituto Madrileño de Estudios Avanzados en Nanociencia, Spain

12:25 Flash Talk
DNA curtains-based approach for fluorescence microscopy-correlated Optical Tweezers
Sara de Bragança, Spanish National Center for Biotechnology (CNB), CSIC, Spain

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12:30 - 13:40 Awards I Session (SBE Awards)

12:30 SBE Awards presentation

12:35 `Manuel Rico´-Bruker España Prize
Kinetic stabilization of translation-repression condensates by a neuron- specific microexon
Dr. Xavier Salvatella, ICREA Research Professor and Principal Investigator, Laboratory of Molecular Biophysics, Institute for Research in Biomedicine (IRB), Spain

13:05 'E.Pérez Payá-Prospera Biotech & BCN peptides'-*SBE 40 Prize Bridging the scales in molecular biophysics via multipronged theoretical approaches*
Salvatore Assenza, Soft Matter and Physics of Biopolymers, UAM & IFIMAC, Madrid, Spain

13:25 Hawk Biosystems-SBE 33 Prize
Imaging cell membrane biophysics
Pablo Carravilla, Scilifelab, Karolinska Institutet, Stockholm, Sweden

13:40 - 14:30 Lunch break

14:30 - 16:30 Poster Session

16:30 - 17:00 Coffee break

17:00 - 19:00 Parallel Sessions

Session V: *Applied biophysics: for industry, biotechnology, health and green transition,* Plenary Room, Chairs: Aitziber L. Cortajarena, Cécile Formosa

17:00 Invited speaker
Challenges to make the Atomic Force Microscope a biomedical device
Dr. Etienne Dague, Research Director of CNRS (LAAS-CNRS, ELIA equip), Université de Toulouse, France

17:30 Invited speaker
Photosynthetic proteins in action! Towards a real-time investigation of how light-harvesting is regulated in plant
Dr. Nicoletta Liguori, ICFO, Spain

18:00 Platinum Sponsor
New techniques in biophysics research
Dr. Andrea Zapater, Product Specialist BIO, Paralab-bio, Barcelona, Spain

18:15 Oral contribution
Deciphering enzyme kinetics at the confined space
Fernando López-Gallego, CIC biomaGUNE, Spain

18:30 Oral contribution
Biophysical and microscopic approaches to unravel the mechanism of action of peptide-based constructs for wound healing
Mariana Ferreira, LAQV-REQUIMTE/FCUP, Portugal

18:40 Flash Talk
Engineering Nanobodies for Biotechnological Applications
Gabriel Ortega, CIC bioGUNE, Spain

18:45 Flash Talk
Protein-based conductive inks: challenges in the design and production
Javier Porcayo-Loza, CIC biomaGUNE, Spain

18:50 Flash Talk
Detecting diseases-related oligonucleotides sequences: a multi-technique approach for label-free sensing
Silvia Maria Cristina Rotondi, University of Genova, Italy

18:55 Flash Talk
Engineering of an anti-CD20 antibody through site-selective chemical modification to improve its therapeutic potential
Sara Insausti, Instituto Biofisika (CSIC-UPV/EHU), Spain

Session VI: *Computational biophysics: Simulations, Artificial Intelligence, Machine learning, and Design,* Room II, Chairs: Armindo Salvador, David de Sancho

17:00 Invited speaker
Conformational space exploration and reactivity of minimal ribozymes for autocatalytic networks
Dr. Guillaume Stirnemann, École Normale Supérieure & CNRS, France

17:30 Invited speaker
Beyond the Active Site: Designing Efficient Enzymes Using Correlation and Evolutionary Information
Dr. Silvia Osuna, Universitat de Girona, Spai

18:00 Oral contribution
Computational/Experimental Synergy for the Study of Supramolecular Peptide Materials
Ivan Sasselli, Centro de Física de Materiales (CFM), CSIC-UPV/EHU, Spain

18:15 Oral contribution
Unravelling the Structure and Dynamics of Biomolecules via Computational Modeling
Florence Tama, RIKEN Center for Computational Science, Kobe, Japan

18:30 Oral contribution
Stochastic dilution and recovery of epigenetic marks
Ander Movilla Miangolarra, John Innes Centre, Department of Computational and Systems Biology, United Kingdom

18:45 Oral contribution
Integrative Computational Modeling of Biochemical Processes: The Role of Protonation States and Conformational Dynamics in Ligand-Binding and Enzymatic Catalysis,
Helena Girame, Institute of Computational Chemistry and Catalysis, University of Girona, Girona, Spain

19:00 Biophysical Society Assembly (closed event)

19:15 City visit and walk to Monte Urgull
Get together from Kursaal to "Polvorín Bar terrace" (flexibility to join at any time).
First departure at 19:15h and second departure at 20:00h from Kursaal (after SBE assembly)

Friday 7th June 2024

9:00 - 10:00 Plenary Session, Plenary Room

9:00 Plenary Lecture, Chair: Armindo Salvador
The emergence of ecological function
Prof. Álvaro Sánchez, Institute for Functional Biology & Genomics, CSIC-University of Salamanca, Spain

10:00 - 10:30 Coffee break

10:30 - 12:30 Parallel Sessions

Session VII: *Synthetic biophysics: self- organized and biomimetic systems,* Plenary Room
Chairs: Beatriz Ibarra-Molero, Edurne Rujas

10:30 Invited speaker
Active Vesicles: Nanocontainers for temporally regulated cargo delivery
Dr. Mohit Kumar, Assistant Professor, University of Barcelona, Senior Researcher, Institute for Bioengineering of Catalonia (IBEC), Spain

11:00 Invited speaker
Sperm-carrying micromotors for gynecological cancer and infertility interventions
Prof. Mariana Medina Sánchez, CIC nanoGUNE, Spain

11:30 Oral contribution
Nanostructural characterisation of glycosylated protein biomarkers interaction with lipid bilayer membranes: basis for biosensor development
Beatrice Barletti, Institut Laue-Langevin, Grenoble, France

11:45 Oral contribution
Surpassing substrate-enzyme competition by compartmentalization
Susana Carregal-Romero, CIC biomaGUNE, Spain

12:00 Oral contribution
A Designed Imaging Scaffold Breaks the Barrier to High-Resolution Structure Determination of Small Proteins by Cryo-EM
Roger Castells-Graells, UCLA-DOE Institute for Genomics and Proteomics; Los Angeles, US

12:15 Flash talks
Exploring membrane fusion mediated by amphiphilic gold nanoparticles
Beatrice Leonardini, Department of Physics, University of Genoa, Italy

12:20 Flash talks
Tuning the dimensionality of supramolecular functional materials based on the rational design of biomolecules
Laura Perez-Chirinos, CIC biomaGUNE, Spain

12:25 Flash talks
Designing model photosystem for tracking protein dynamics in chromophore´s light harvesting properties
Gonzalo Pérez Serrano, IMDEA Nanociencia, Madrid, Spain

Session VIII: *Membrane and membrane proteins (receptors, ion channels and transporters)*, Room III,
Chairs: Manuela Pereira, Marlene Martinho

10:30 Oral contribution
Dissecting metabotropic glutamate receptor activation by multicolor single molecule FRET
Emmanuel Margeat, Centre de Biologie Structurale (CBS), Univ. Montpellier, CNRS, INSERM, Montpellier, France

11:00 Oral contribution
Mechanism for ring biogenesis and lipid membrane repair: Vipp1
Adai Colom, Biofisika Institute (CSIC, UPV/EHU) Leioa

11:30 Oral contribution
Effects of LLPS on the cell membrane and actin remodelling
Claire Buchanan, Instituto Biofisika, Spain

11:45 Oral contribution
The ancient mechanism of membrane compartmentation of the glycosyltransferase enzyme MurG discovered by HS-AFM
Elodie Lafargue, DyNaMo U1325-Université Aix-Marseille, Marseille, France

12:00 Oral contribution
Towards the amino acid sensing mechanism of a lysosomal amino acid transporter
Jose Luis Vázquez-Ibar, Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), France

12:15 Oral contribution
Optical control of cellular membrane fluidity using photoswitchable lipids
Noemi Jiménez-Rojo, Department of Biochemistry & Molecular Biology University of the Basque Country (UPV/EHU)

12:30 - 13:30 Awards II Session (SFB, SPBf Awards, IUPAB), Plenary Room

12:30 SFB Award: SFB Young Researcher Prize
Understanding the influence of surface state on Pseudomonas aeruginosa surface twitching: experiment and modelling
Yeraldinne Carrasco-Salas, Laboratoire Interdisciplinaire de Physique, Grenoble, France

12:55 SPBf award: SPBf Young biophysicist 2024 prize
Membrane remodelling by active rotary ATP synthases
Marcin Makowski, Universidade de Lisboa, Portugal

13:20 IUPAB announcements

13:30 - 14:30 Lunch break

14:30 - 16:30 Poster Session II

16:30 - 17:00 Coffee break

17:00 - 19:00 Parallel Sessions

Session IX: *Advanced Bioimaging*, Plenary Room, Chairs: Lorena Redondo, Carlo Manzo

17:00 Invited speaker
Conformational dynamics of SARS-CoV-2 spike protein modulates the binding affinity to ACE2
Dr. Felix Rico, Associate Professor in Physics Department, Aix-Marseille University & INSERM, France

17:30 Invited speaker
3D Genome folding and topology in the eye of the STORM
Dr. Marie Victoire Neguembor, CRG, Barcelona, Spain

18:00 Oral contribution
Particle-tracking, super resolution microscopy and machine learning reveal the spatial-temporal regulation of exocytosis,
Sebastián Ortiz, Universitat Pompeu Fabra, Barcelona, Spain

18:15 Oral contribution
Tuning in to nanoscale membrane dynamics with near-field optical antennas
Thomas S. Van Zanten, INMA-Instituto de Nanociencia y Materiales de Aragón, Zaragoza, Spain

18:30 Oral contribution
Spatiotemporal Dynamics of Protein Export from the Endoplasmic Reticulum,
Roger Pons-Lanau, ICFO-Institut de Ciencies Fotoniques, The Barcelona Institute of Science and Technology, Barcelona, Spain

18:45 Flash talk
Protein-stabilized nanomaterials as MRI contrast agents: one size does not fit all, but versatility does,
Gabriela Guedes, CIC biomaGUNE, Spain

18:50 Flash talk
Visualizing Molecular Dynamics with High-Speed Tip-Scanning Atomic Force Microscopy
André Körnig, JPK BioAFM, Bruker Nano GmbH, Am Studio 2D, 12489 Berlin, Germany

18:55 Flash talk
Effects of microbial glycolipids on phospholipid membranes by Atomic Force Microscopy (AFM)
Yulia Fok, Aix-Marselle Universite, INSERM-CNRS, France

Session X: *Biological Physics*, Room III, Chairs: Antoine Loquet, Javier Buceta

17:00 Invited speaker
The microtubule/kinesin active nematic system: An intriguing biophysical material
Dr. Francesc Sagues, Full Professor at the Department of Materials Science and Physical Chemistry, University of Barcelona, Spain

17:30 Invited speaker
Botton-up construction of life-like synthetic cells
Dr. Jean-Christophe Baret, Institut de Biologie Physico-Chimique, Paris

18:00 Platinum Sponsor
Abberior STED and MINFLUX: Super-resolution improves in all directions
Frédéric Eghiaian, ABBERIOR INSTRUMENTS GmbH, Germany

18:15 Oral Contribution
Ionic Conductivity in Engineered Protein Thin Films
Lisa Almonte, Applied Physics Department & IUMA, University of Alicante, Spain

18:30 Oral Contribution
Droplet-based improvement of artificial metalloenzymes
Aitor Manteca, CIC biomaGUNE, Spain

18:45 Oral Contribution
Patterning and Mechanics in Bacterial Filaments
Javier Buceta, Theoretical and Computational Systems Biology Program Institute for Integrative Systems Biology (I2SysBio), CSIC-UV, Valencia, Spain

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19:00 Concluding Remarks and Prizes, Plenary Room
Best oral presentation award sponsored by Scharlab
Best poster awards sponsored by ARBRE Research Infrastructure

20:00 Conference Dinner: Muka Restaurant

Abstracts

Abstracts: Oral Contribution & Flash Talks

Unraveling native complexity: Single molecule characterization of perinatal titin by force spectroscopy of tissue-purified proteins.

Inés Martínez-Martín[1]*, Elías Herrero-Galán[1], Jorge Alegre-Cebollada[1]

[1] Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain
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Single-molecule force spectroscopy methods have emerged as a powerful tool for unraveling the mechanical properties of biological molecules and their modulation by biochemical cues. However, these techniques generally rely on the production of recombinant proteins that lack the native modifications of the molecule. In addition, very large proteins cannot be expressed recombinantly, and their study often resorts to the characterization of small fragments. A striking example is titin, a > 3MDa sarcomeric protein with important mechanical functions and whose properties can be modulated by posttranslational modifications [1]. Biochemical studies show that cysteine oxidation in titin increases after birth, but distinguishing between non-crosslinking and crosslinking modifications such as disulfide bonds is challenging for conventional bulk techniques. This distinction is crucial, as the chemical nature of the modification determines its effect in the mechanics of the protein. To overcome this limitation, we have implemented an approach in which titin is purified from a mouse model containing a HaloTag insertion that allows the covalent tethering of the molecule to a surface [2]. Using atomic force spectroscopy and magnetic tweezers we have specifically probed the presence of disulfide bonds in embryonic and postnatal titin. We have observed that the proportion of domains containing disulfide bonds increases in postnatal animals, which can contribute to the adaptation of myocardial stiffness to the requirements of the postnatal stage. Considering these results, we anticipate that the application of strategies probing native molecules extracted from tissues will facilitate the understanding of the physiological mechanisms governing the mechanical properties of proteins.

References

[1] E. Herrero-Galán, I. Martínez-Martín, C. Sánchez-González, N. Vicente, E. Bonzón-Kulichenko, E. Calvo, C. Suay-Corredera, M.R. Pricolo, Á. Fernández-Trasancos, D. Velázquez-Carreras, C.B. Careaga, M. Abdellatif, S. Sedej, P.P. Rainer, D. Giganti, R. Pérez-Jiménez, J. Vázquez, J. Alegre-Cebollada, Redox Biol 52 (2022) 102306.

[2] J.A. Rivas-Pardo, Y. Li, Z. Mártonfalvi, R. Tapia-Rojo, A. Unger, Á. Fernández-Trasancos, E. Herrero-Galán, D. Velázquez-Carreras, J.M. Fernández, W.A. Linke, J. Alegre-Cebollada, Nat Commun 11 (2020) 2060.

Exploring the role of α5β1 glycosylations at the cellular and molecular level

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Glycosylation is a common post-translational modification in membrane proteins that has a significant impact on cellular function. Despite a deep knowledge of the mechanisms of glycosylation, little is known about how it affects the spatiotemporal organization and interactions of proteins at the plasma membrane.

Recent studies have shown that mutations of glycosylation sites of the α5β1 integrin exhibit altered proliferation [1] and migration rate [2]. The α5β1 integrin functions as a fibronectin receptor, participating in cell-matrix adhesion and cell signaling [3].

To investigate the role of α5β1 integrin glycosylation at multiple scales, we used a combination of microscopy and biochemical methods on wild-type and mutated HeLa cells. We generated cell lines stably expressing the α5 integrin subunit with mutated glycosylation sites and performed deep-learning-assisted cell proliferation and migration assays to determine the effect of mutations on cellular functions.

Additionally, we inserted a HaloTag at the C-terminus of the α5 subunit, allowing us to covalently tag the protein with bright fluorescent dyes. We used single-molecule imaging and single-particle tracking to quantify the lateral motion of the integrin and revealed different diffusion features associated with changes in glycosylation.

Furthermore, co-immunoprecipitation of the α5 subunit and mass spectroscopy analysis identified proteins that differentially and selectively interact with wild-type and mutated forms of the subunit, revealing significant differences between four different mutation genotypes.

Overall, our experiments suggest a link between the effect of glycosylation at the molecular scale and alterations of function observed at the cellular level.

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Disordered proteins interact with the chemical environment to tune their protective function during drying

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The conformational ensemble and function of intrinsically disordered proteins (IDPs) are sensitive to their solution environment [1,2]. The inherent malleability of disordered proteins combined with the exposure of their residues accounts for this sensitivity [1,2]. One context in which IDPs play important roles that is concomitant with massive changes to the intracellular environment is during desiccation (extreme drying). The ability of organisms to survive desiccation has long been linked to the accumulation of high levels of cosolutes such as trehalose or sucrose as well as the enrichment of IDPs, such as late embryogenesis abundant (LEA) proteins [3,4] or cytoplasmic abundant heat soluble (CAHS) proteins [5,6]. Despite knowing that IDPs play important roles and are co-enriched alongside endogenous, species-specific cosolutes during desiccation, little is known mechanistically about how IDP-cosolute interactions influence desiccation tolerance. Here, we test the notion that the protective function of desiccation-related IDPs is enhanced through conformational changes induced by endogenous cosolutes. We find that desiccation-related IDPs derived from four different organisms spanning two LEA protein families and the CAHS protein family, synergize best with endogenous cosolutes during drying to promote desiccation protection. Yet the structural parameters of protective IDPs do not correlate with synergy for either CAHS or LEA proteins. We further demonstrate that for CAHS, but not LEA proteins, synergy is related to self-assembly and the formation of a gel. Our results demonstrate that functional synergy between IDPs and endogenous cosolutes is a convergent desiccation protection strategy seen among different IDP families and organisms, yet the mechanisms underlying this synergy differ between IDP families. This is important as IDPs are ubiquitous across biology and function in key developmental processes and disease states that are concomitant with large changes in intracellular chemistry. Understanding how disordered proteins interact and evolve with the solution environment will provide insights into these biological mechanisms and phenomena.

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Conformational dynamics and liquid-liquid phase separation of the endocytic protein Eps15

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Eps15, one of the early initiators of clathrin-mediated endocytosis, is responsible for locally concentrating downstream components on the membrane surface and it permits dynamic rearrangement of proteins within the budding vesicle. Oligomerization of Eps15 promotes the assembly of Eps15 into liquid-like protein droplets that catalyzes endocytosis. Its N-terminal EH domains are involved in intracellular trafficking and cell signaling. They bind with particularly low affinities and specificity to Asn-Pro-Phe (NPF) motifs, that are present in many endocytic proteins. We investigated the interaction between individual EH domains within Eps15, as well as the full EH-domain (comprising EH1, EH2 and EH3), with the intrinsically disordered region of endocytic protein Dab2, using NMR spectroscopy. We observe a high level of binding promiscuity and specific interaction modes of the three EH-domains, as well as of the full EH-domain with implications for multi-site binding events contributing to clathrin-mediated endocytosis.

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Exploring Long Non-Coding RNA secondary structures through AFM imaging

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Long noncoding RNA molecules (lncRNAs) are emerging as key players in several cellular processes and have been associated with multiple diseases in humans. However, the majority of lncRNAs remain structurally uncharacterized and the relation between the structure and function is still scarce. In this study, we used Atomic Force Microscopy (AFM) to capture insights into the secondary structure and conformational dynamics of RNA molecules, operating both within air and liquid environments. Using the 5′ proximal region of Sars-CoV-2 genome and other beta coronaviruses as a proof of concept, we demonstrate our capability of observing the secondary structure of RNA molecules [1]. Subsequently, we developed a novel approach for synthesizing lncRNA molecules appended with polyA tails at their 3′ and 5′ termini. This modification not only facilitates the precise localization of intact full-length molecules but also enables the discernment of reproducible molecular features, thus advancing our capacity for structural characterization. Additionally, we have established an image analysis framework to extract structural details from AFM images. We focus on the analysis of the lncRNA CONCR, which is involved in the sister-chromatid cohesion process [5]. Through the application of our methodological framework, based on previous work [2-4], we have unraveled the structural landscape of CONCR, delineating structured domains and estimating nucleotide ranges based on volumetric distributions. Notably, our acquisition of AFM movies in liquid environments provides dynamic insights into the structural dynamics and RNA flexibility. This work highlights the AFM advantages in deciphering the global conformation of RNAs, which in combination with other high-resolution techniques like cryo-EM and Chemical Probing can offer a deeper understanding of the functional roles of lncRNAs.

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Single molecule studies of the role of FBH1 in fork reversal

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The reactivation of stalled DNA replication forks is crucial for the maintenance of genomic integrity, involving complex pathways and hundreds of proteins [1]. One of these pathways is fork reversal, which involves the unwinding and reannealing of fork DNA strands to form a four-way junction, mitigating DNA damage. In humans, four helicases have been identified as the drivers of this process: SMARCAL1, ZRANB3, HLTF and FBH1 [1,2]. The three former ones are described as annealing helicases, given that they do not exhibit the canonical strand separating helicase activity [3-5]. In contrast, FBH1 stands out due to its processive unwinding activity in vitro. Thus, the specific mechanisms and implications of FBH1's activity in fork reversal remain less understood.

In this work, we have characterized the helicase and ssDNA translocation activities of FBH1 at different forces at the single molecule level using magnetic tweezers and hairpin DNA molecules. Moreover, we have conducted an assay with a stalled replication fork-like substrate to understand the role of FBH1 in fork reversal. We show that FBH1 can unwind the lagging strand of the model replication fork when a ssDNA gap is present on it. Moreover, once the lagging strand has been unwound, we are able to detect a decrease in the extension of the DNA molecule that could be related with the fork reversal activity of the protein. Thus, we propose a model in which the initial lagging strand unwinding by FBH1 may induce a structural change in the fork that allows FBH1 to catalyze the reversal of the fork.

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Deciphering enzyme kinetics at the confined space

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Understanding the kinetics of immobilized enzymes is crucial for optimizing enzyme functionality at solid material interfaces. This study investigates the spatiotemporal migration of His-tagged enzymes within porous agarose microbeads under various storage and reaction conditions. A novel assessment toolbox tracks intraparticle protein migration and kinetics across porous supports, revealing the effect of different migration patterns [1], spatial organization, and molecular crowding [2] on the enzyme functionality. For example, we studied the effects of protein migration and spatial distribution of self-sufficient heterogeneous biocatalysts on enzyme activity and stability. This immobilized biocatalyst does not require the exogeneous addition of cofactors (NAD(P)H, FAD, PLP…), where His-tagged cofactor-dependent enzymes and phosphorylated cofactors are co-immobilized on cationic polymer-coated agarose microbeads. Fluorescence recovery after photobleaching, time-lapse microscopy, and image analytics elucidate reversible interactions between adsorbed phosphorylated cofactors and carrier polycations, facilitating confined cofactor access to immobilized enzyme active sites. The relationship between apparent Michaelis-Menten kinetic parameters and enzyme density in confined spaces reveals a negative impact of molecular crowding on enzyme performance. Notably, intraparticle enzyme kinetics are influenced by enzyme spatial organization, highlighting the importance of multiscale characterization in understanding enzyme functionality within confined spaces. Overall, this study underscores the significance of kinetic analysis in optimizing enzyme immobilization strategies for enhanced performance in various biotechnology applications.

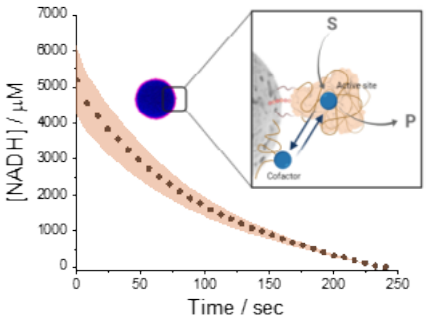

Figure 1. Single-particle analysis to determined the apparent kinetics of alcohol dehydrogenase (red) co-immobilized with NADH (blue) on porous agarose surfaces.

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Biophysical and microscopic approaches to unravel the mechanism of action of peptide based constructs for wound healing

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Chronic wounds are characterized by a prolongated inflammatory phase of the wound healing process, usually triggering collagen destruction, and systematically exhibiting bacterial infections (including multidrug-resistant bacteria). Antimicrobial peptides (AMPs) have been widely explored as alternatives to conventional antibiotics, due to their broad spectrum of action at very low concentrations and to the lower chances of antimicrobial resistance development [1]. Gomes et al. designed dual-action peptides (hybrid constructs) comprising an AMP (3.1, with amino acid sequence KKLLKWLLKLL) and a collagen-boosting peptide (PP4, with amino acid sequence KTTKS) covalently linked. These peptides, 3.1-PP4 and its isomer PP4-3.1, combine antimicrobial action and faster healing, being promising for the treatment of infected wounds [2, 3]. In vitro studies previously disclosed that 3.1-PP4 has improved activity against Gram(-) bacteria and PP4-3.1 exhibits a broad spectrum of action [2, 3]. In this work, we combine biophysical and microscopic techniques (spectrofluorimetric, dynamic light scattering, confocal laser scanning microscopy and atomic force microscopy experiments) to clarify the peptide-bacterial membrane interactions and unveil their mechanism of action (MOA) against Escherichia coli and Staphylococcus aureus, using mimetic model systems (large and giant unilamellar vesicles – LUVs and GUVs) and bacterial susceptible strains (E. coli ATCC 25922 and S. aureus ATCC 29213). The results revealed different effects resultant from the action of 3.1-PP4 and PP4-3.1 in bacterial membranes, suggesting a different MOA.

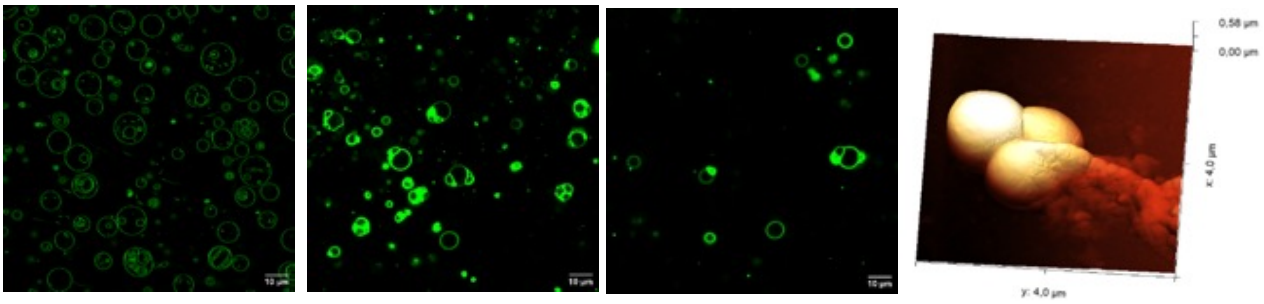

Figure 1 The effect of peptide PP4-3.1 in Gram(+) bacteria: (A-C) confocal microscopy images of GUVs of POPC:POPG (1:1)- 2% NBD-DPPE and (D) atomic force microscopy images of S. aureus ATCC 29213.

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Computational/Experimental Synergy for the Study of Supramolecular Peptide Materials

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Supramolecular peptide assemblies (SPAs) hold great promise in the realms of nanotechnology and biomedicine, particularly as scaffolds for tissue regeneration. Leveraging the innate capacity of short peptides to self-assemble into structured entities with adjustable properties (Figure 1a), SPAs have garnered significant interest. While the study of SPAs draws upon techniques adapted from their protein counterparts, SPAs present distinct challenges. Notably, they grapple with structural ambiguity at the molecular level due to a dearth of representative crystal structures and their inherently lower order. Consequently, the design of SPA materials with desired properties remains a formidable task, given the intricate interplay between peptide sequence, structure, and bioactivity. The fusion of experimental and computational methodologies offers a promising avenue to unravel this complexity and deepen our understanding of SPAs.[1] This symbiotic approach has unveiled molecular motion as a pivotal determinant of bioactivity, driving advancements in optimizing materials for spinal cord injury regeneration (Figure 1b).[2] However, ongoing challenges persist, particularly in elucidating the effects of sequence on structure and function, deciphering the interplay of monomers in systems composed of different monomers (co-assemblies), and identifying critical factors for functional behavior.[2] In computational studies, it is crucial to develop protocols that accurately model SPAs and capture parameters that reliably correlate with experimental findings.[3] Addressing these hurdles stands as a critical frontier in leveraging computational tools to unlock the full potential of SPAs.

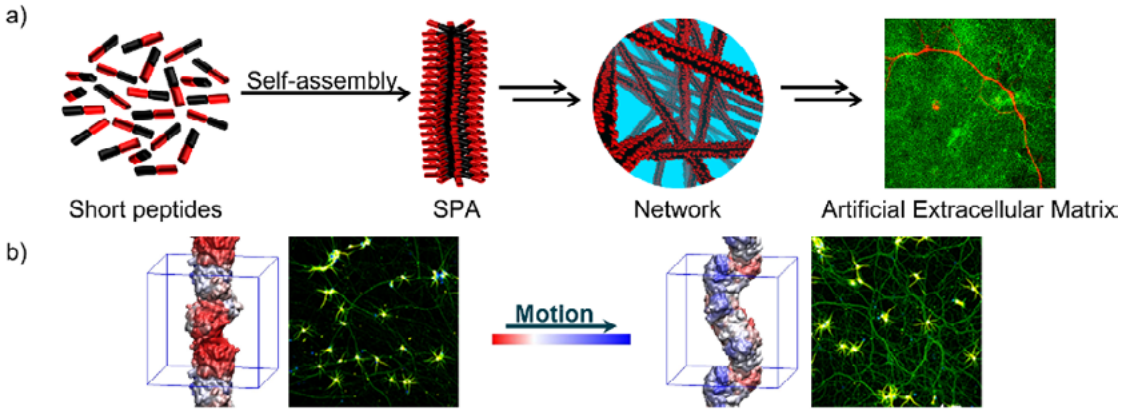

Figure 1 (a) Scheme of the self-assembly of short peptides into a SPA to then form a network that acts as artificial extracellular matrix. (b) Computational prediction and effect of molecular mobility in the bioactivity of SPAs.

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Unraveling the Structure and Dynamics of Biomolecules via Computational Modeling

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To fully understand biological functions, high-resolution biomolecular structures are required, which can be obtained through diverse experimental methods such as X-ray crystallography and, more recently, cryo-electron microscopy (cryo-EM). While these techniques provide valuable structural insights, elucidating the dynamic of these biomolecules is also essential, given that some molecules display inherent structural flexibility through spontaneous large-domain motions. A thorough comprehension of such dynamics involves characterizing various conformational states at the atomic level. Cryo-EM is particularly well-suited for studying dynamics, as each captured image theoretically represents a distinct conformational state, provided that corresponding atomic models can be constructed for each state. To bridge the gap between 2D images and atomic models, guided molecular dynamics simulations can be employed to explore the conformational space embedded within the 2D EM data [1]. These computational tools facilitate the analysis of large-scale cryo-EM experiments, where a significant volume of data is collected without specific experimental conditions that would constrain the molecule into a particular conformation, which would be necessary for achieving high-resolution structures. We will introduce these methods and discuss their applications to specific biological systems.

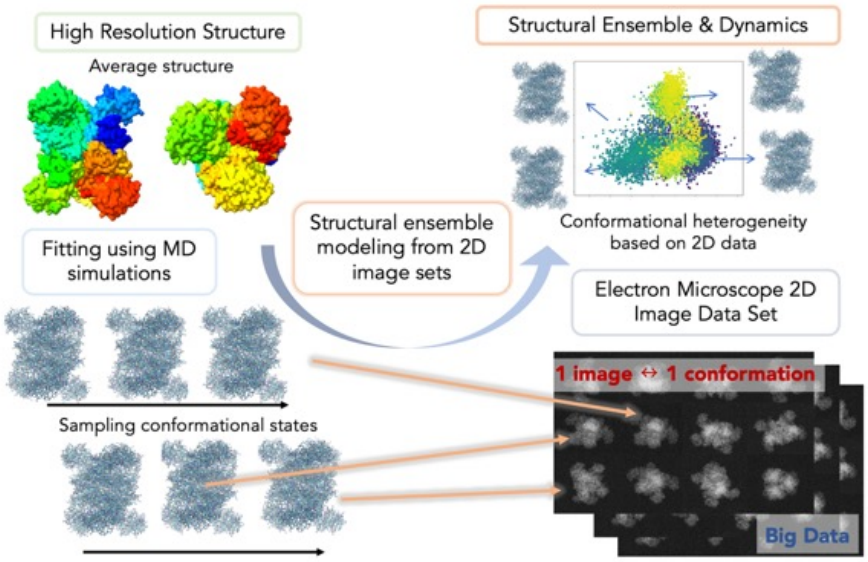

Figure 1: Elucidating conformational ensemble from large-scale cryo-EM experiments and MD simulations.

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Stochastic dilution and recovery of epigenetic marks

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Covalent modifications of histone tails play a major role in genetic regulation, activating or repressing genes. Upon mitosis, an identical copy of the DNA is created but histones are newly synthesized, which do not necessarily carry the same covalent modifications. Thus, daughter cells, in general, do not have the same histone modification profile at the beginning of the cell cycle as the mother cell did at the end of its cell cycle. However, gene expression profiles are typically very robust and inheritable, and the histone modification profiles tend to recover as the cell cycle proceeds. In this talk, I will explore mathematically, through a combination of stochastic simulations and dynamical systems, models that help us explain this recovery behaviour. In addition to a general model of histone mark inheritance, I will focus on a tractable pair of genes in yeast, by means of a more detailed model that can quantitatively recapitulate experimental data. These models, in combination with the experimental data, allow us to obtain important insights underlying the robustness of gene expression in cells. This approach makes quantitative predictions regarding how the fidelity of inheritance of the transcriptional state varies as a function of the size of the gene; how a longer gene, in general, has more histones that can enhance the inheritability, but also dilutes the effect of special genetic regions that promote the silencing of the locus. These predictions were then validated with previously published data and with experiments specially designed to test these predictions.

Integrative Computational Modeling of Biochemical Processes: The Role of Protonation States and Conformational Dynamics in Ligand-Binding and Enzymatic Catalysis

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Enzymes modulate their dynamics and function in response to environmental changes, including binding, catalysis, or pH alterations [1]. Understanding this response is crucial to fully comprehend underlying molecular mechanisms that govern biochemical processes, and then harness this information to improve the rational design of novel biocatalysts. However, the transient nature of these processes hinders the experimental characterization of functionally relevant states. Computational modeling has become a well-established approach to provide structural, dynamical, and mechanistic insights into biomolecular processes at atomic resolution [2]. Nonetheless, conventional molecular dynamics (MD) simulation methods, based on classical molecular mechanics, are rigid in adapting to environmental perturbations. For instance, the protonation states of titratable residues are fixed throughout the simulation, neglecting their associated equilibrium. These aspects can limit the reliability of MD simulations when studying certain biochemical processes.

In this work, we will discuss how protonation states, protein electrostatics, and conformational dynamics impact ligand binding and enzymatic catalysis. First, we will demonstrate how the assignment of protonation states determines the computational modeling of protein-ligand binding in the trypsin-benzamidine model system [3]. A constant-pH approach was employed to account for protonation state changes during the MD simulations, which supplied an improved description of benzamidine recognition and binding. Afterwards, we will combine MD simulations, data analysis techniques, and electrostatic modeling to rationalize the difference in chemoselectivity among a set of methyltransferases that are evolutionarily related. The study found that non-conserved active site residues control the local electrostatic environment, which alters the conformational ensemble and ultimately affects the catalytic function.

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Nanostructural characterisation of glycosylated protein biomarkers interaction with lipid bilayer membranes: basis for biosensor development

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The study and understanding of protein-lipid interactions is of great importance specially to optimise the sensitivity and reliability of biosensing techniques to identify protein biomarkers, in particular when present in their glycosylated forms which are currently challenging to recognise with present methods [1].
In the project we investigated at nanostructural level the interaction of glycosylated protein biomarkers with biologically relevant lipid bilayer membranes using neutron reflectometry, particularly to identify how different lipids and glycosylation can affect this interaction to build a potential biosensor that is aimed for early detection of scarce biomarkers in blood samples.
The glycosylated proteins of interest for the study are soluble vascular-endothelial cadherin (sVE) and alpha-fetoprotein, two clinical biomarkers found in the blood for the detection of vascular abnormalities and liver cancer respectively [2,3,4].
Neutron reflectometry (NR) results, together with QCM-D complementary data, showed significant changes in the lipid bilayer after the injection of glycosylated proteins, alpha-fetoprotein and sVE, while smaller changes were reported in presence of non-glycosylated protein, BSA. We highlighted that the kinetic has an important role for the interaction and we proved that the interaction behavior is influenced by the lipid composition of the system as well as the degree of glycosylation on the protein. In addition, we found interesting results comparing the interaction behavior of non-glycosylated BSA and glycated-BSA.
Thanks to the study and glycosylation chemistry, it was possible to set the basis for the development of a lipid-based protein-biosensor in collaboration with the industrial partner of the project.

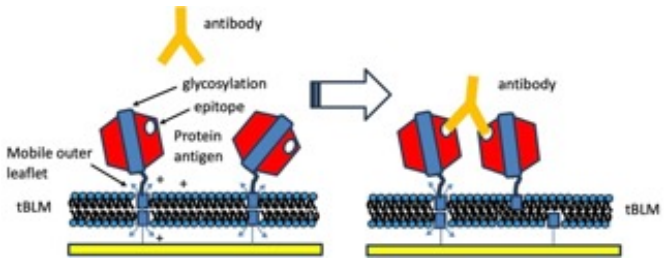

Fig. 1: Scheme of the lipid-based protein-biosensor

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Surpassing substrate-enzyme competition by compartmentalization

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Nature employs enzyme compartmentalization to create distinct chemical environments, facilitating concurrent enzyme reactions within cellular metabolic networks. However, emulating such nanostructured architectures poses challenges, particularly when competing enzymes operate on the same substrate [1-2]. In this study, we present a novel approach to fabricate soft hybrids, segregating two oxidoreductases with distinct kinetics for NADH. Encapsulation of the less competitive enzyme within polymeric capsules, capable of NADH recruitment, is followed by assembly onto porous agarose microbeads harboring the more competitive enzyme. This hybrid system enables simultaneous enzyme activity within a single reaction medium, a feat unattainable in non-compartmentalized setups. Our findings underscore substrate recruitment as a potent strategy for constructing dynamic enzymatic networks [3]. Additionally, operando single-particle analysis elucidates the impact of enzyme spatial arrangement on hybrid performance, emphasizing the significance of functional variability in compartmentalized systems. Integration of this approach into a model cell-free biosynthetic cascade enhances (S)-β-hydroxybutyrate production from vinyl acetate, yielding twice the titer achieved in non-compartmentalized systems. This versatile strategy holds promise for generating compartmentalized cell-free biosynthetic pathways and multienzyme cascades, addressing challenges posed by enzyme competition.

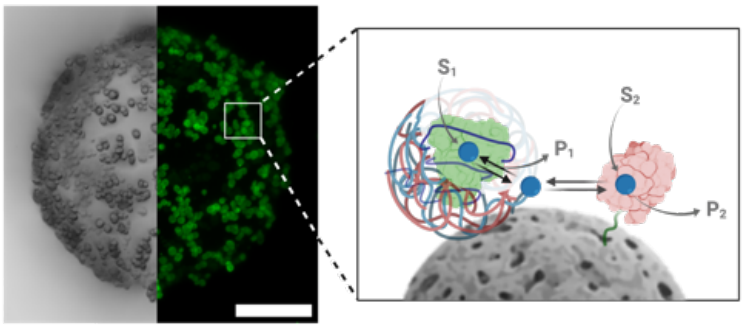

Figure 1. Immobilization of two competing enzymes and effect of compartmentalization.

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A Designed Imaging Scaffold Breaks the Barrier to High-Resolution Structure Determination of Small Proteins by Cryo-EM

Roger Castells-Graells[1]*, Kyle Meador[2], Mark A. Arbing[1], Michael R. Sawaya[1], Morgan Gee[2], Duilio Cascio[1], Emma Gleave[3], Judit É. Debreczeni[3], Jason Breed[3], Karoline Leopold[4], Ankoor Patel[4], Dushyant Jahagirdar[4], Bronwyn Lyons[4], Sriram Subramaniam[4], Chris Phillips[3], Todd O. Yeates[1,2]

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Recent technical advances have made cryo-electron microscopy (cryo-EM) an attractive method for atomic structure determination, but problems of low signal-to-noise prevent routine structure determination of proteins smaller than about 50 kDa. We have developed symmetric protein imaging scaffolds to display and solve the structure of small proteins. In earlier work (Liu Y, et al., 2019), we demonstrated the design of a novel protein cage scaffold with sufficient rigidity and modularity to reach an imaging resolution of 3.8 Å for a 26 kDa protein. In the present work, we use molecular engineering techniques to further rigidify a new cryo-EM imaging scaffold, enabling 3 Å or better resolution imaging to be achieved, even for very small proteins. We apply this system to the key cancer signaling protein KRAS (19 kDa in size), obtaining four structures of oncogenic mutational variants by cryo-EM. Importantly, a structure for the key G12C mutant bound to an inhibitor drug (AMG510) reveals significant conformational differences compared to prior data in the crystalline state. The findings highlight the promise of cryo-EM scaffolds for advancing the design of drug molecules against small therapeutic protein targets in cancer and other human diseases.

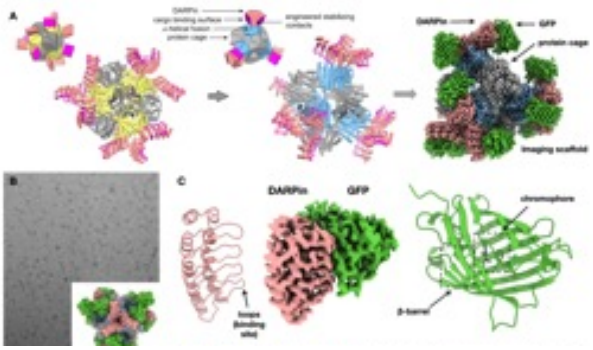

Figure 1. Rigidified modular cryo-EM imaging scaffolds. A. (left) A scheme for a previously described scaffold (Liu et al. 2019), based on a self-assembling protein cage, displayed protruding DARPin domains as modular binders via continuous alpha helical fusions. B. Cryo-EM micrograph of the rigidified imaging scaffold bound to GFP (model shown in inset) C. (middle) A view of the final density map covering the DARPin and its bound GFP protein. Ribbon models of the two components are shown on the sides.

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Dissecting metabotropic glutamate receptor activation by multicolor single molecule FRET

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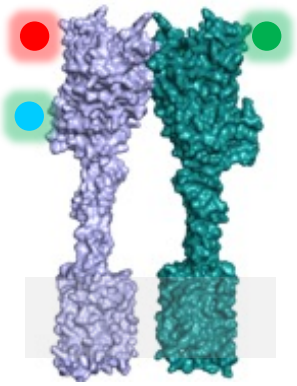
Metabotropic glutamate receptors (mGluR) regulate neuronal excitability and synaptic transmission by sensing L-glutamate – the major excitatory neurotransmitter in the central nervous system. Their crucial role for synaptic function makes them attractive targets for the treatment of numerous neurological and psychiatric diseases including for instance anxiety, depression, schizophrenia and addiction.

To explore mGluR activation, we monitored its conformational changes using single molecule two and three color FRET with submillisecond resolution. We established multiple, minimally invasive conformational FRET sensors through bioorthogonal double labeling of two reactive and different non-canonical amino acids after incorporation by genetic code expansion. These sensors report on the initial step of mGluR2 activation, including the reorientation of the upper and the lower lobes of the venus flytrap domain (VFT) in an intersubunit fashion and its closure in an intrasubunit fashion. By studying ligand induced conformational changes on single receptors, we show that the natural full agonist glutamate is sufficient to close the VFTs but a synergy with synthetic positive allosteric modulators or the G protein, is required for full activation [1,2].

Our study highlights the power of minimally invasive, ncAA-based, bioorthogonal labeling to dissect domain-specific conformational rearrangements of single GPCRs using smFRET.



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Towards the amino acid sensing mechanism of a lysosomal amino acid transporter

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The proline-glutamine loop repeat-containing protein 2, PQLC2, is a lysosomal membrane transport protein responsible of exporting cationic amino acids from the lysosomal lumen to the cytoplasm [1]. In addition, PQLC2 is a membrane receptor that recruits at the lysosomal surface and only under cellular cationic amino acid starvation, a three-protein complex (CSW) [2]: the GTPase-activating proteins C9ORF72 and SMCR8, and WDR4 the anchor between PQLC2 and the CSW complex. CSW's lysosome localization is associated with cellular processes like autophagy, lysosome biogenesis or down-regulation of Toll-like receptors [3]. The molecular basis of PQLC2/WDR41 recognition, key for the lysosomal recruitment of the CSW complex are not yet understood, despite several cryo-EM structures of the CSW complex are available. Pull-down assays using cellular extracts indicate that this interaction is mediated by a short peptide motif located in a loop that extends from a WDR41 α-propeller (loopWDR41) [4]. Here, we present direct evidences of this interaction using purified PQLC2 and a synthetic loop-WDR41 peptide. We first applied a consensus mutagenesis approach to engineer a recombinant version of rat PQLC2 with sufficient stability in detergent micelles after purification. Interestingly, PQLC2 seems to assemble as homotrimer like other members of the same PQ-loop family of transporters. We then exploited Electron Paramagnetic Resonance (EPR) spectroscopy to assess the direct interaction between the engineered PQLC2 and loop-WDR41 labelled with a single nitroxide spin-label at various positions. These experiments uncovered the specificity of some residues within loop-WDR41 required for PQLC2 binding as well as the role of PQLC2 substrates on PQLC2-loop-WDR41 recognition.

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Optical control of cellular membrane fluidity using photoswitchable lipids

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Lipids are heterogeneously distributed across the evolutionary tree with lipidomes evolving in parallel to organismal complexity. At the cellular level, lipids are differentially distributed across organelles and membranes are asymmetric and laterally heterogeneous. This lipid asymmetry at different scales indicates that these molecules may play very specific molecular functions in biology, many of which remain still unknown. Lipidomics approaches combined with quantitative cell and chemical biology tools can help us understand better membrane biology and uncover new lipid functions in cells. Here, we introduce an optical approach to manipulate membrane fluidity based on exogenous synthetic fatty acid with an azobenzene photoswitch, termed FAAzo4. This generates photoswitchable PC analogs (AzoPC), which are predominantly located in the endoplasmic reticulum (ER). Irradiation causes a rapid photoisomerization that increases membrane fluidity with high spatiotemporal precision. We use these 'PhotoCells' to study the impact of membrane mechanics on protein export from the ER and demonstrate that this two-step process has distinct membrane fluidity requirements. Our approach represents an unprecedented way of manipulating membrane fluidity in cellulo and opens novel avenues to probe roles of fluidity in a wide variety of biological processes.

Mechanism for ring biogenesis and lipid membrane repair: Vipp1

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ESCRT-III proteins drive membrane remodeling through dynamic filament assembly. We investigate the transition from planar to 3D structures, focusing on bacterial Vipp1. Using FAST-AFM (Fig. 1a,c) and CRYO-EM (Fig. 1b), we observe Vipp1 polymerizing into sheets and spirals from membrane defects. Real-time imaging reveals spiral convergence into central rings crucial for membrane budding (Fig. 1a and c). Our study emphasizes the role of membrane defects in Vipp1 polymerization and underscores the importance of ring structures in lipid membrane interaction and repair. Examining Vipp1's morphological shifts between polymers, we identify helical filament architecture and twisting as key factors for transitioning between planar and 3D forms (Fig. 1c). The structural parallels with ESCRT-III hint at broader implications for understanding geometric changes in ESCRT-III filaments during the shift between 2D and 3D configurations[1] .

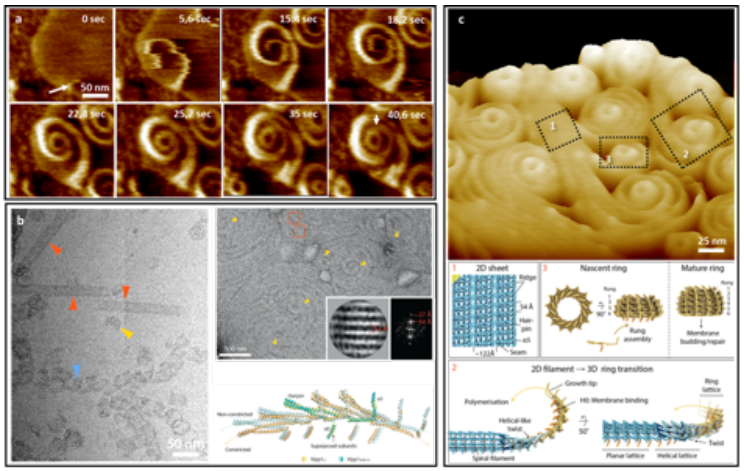

Figure 1 a)Fast-AFM image showing ring biogenesis from a spiral. b) Cryo-EM reveals Vipp1 helical filaments. NS-EM exhibits 2D planar spirals and sheets on a lipid. c)AFM image (top) and model of Vipp1 planar sheet, spiral and 3D ring biogènesis.

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Particle-tracking, super resolution microscopy and machine learning reveal the spatial-temporal regulation of exocytosis.

Sebastian Ortiz[1*], Marta Puig[1], Philipp Hoess[2], Sasha Meek[1], Markus Mund[2], Alma Vivas[3], Raffaele Coray[3], Daniel Castaño[3] , Jonas Ries[2,4], Carlo Manzo[5], Oriol Gallego[1]

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Fluorescence microscopy provides unique opportunities to capture molecular processes in vivo. Unfortunately, these methods are limited by intrinsic spatial and temporal resolution and by the sensitivity detection. These technical restraints challenge the imaging of dynamic biological processes which typically occur at nanometers and millisecond scales. Constitutive exocytosis (hereafter exocytosis) is a paradigmatic fundamental cellular process that, despite being long-studied, remains largely unknown at the mechanistic level. Exocytosis is a highly conserved vesicle-trafficking pathway that follows subsequent steps controlled by different proteins. Despite being identified decades ago, how these proteins are regulated in time and space, as well as their stoichiometry, remain mysterious. Here we present an approach that integrates particle tracking and Single Molecule Localization Microscopy (SMLM) to resolve the dynamics of the exocytic machinery. Tracking events of exocytosis at milliseconds rates allowed us to obtain a timeline of representative proteins controlling the main substeps of exocytosis. SMLM and machine learning, allowed us to classify in situ super-resolution images (within ~20 nm resolution) of single exocytotic events based on a geometrical model derived from cryo-correlative light-electron microscopy. Finally, using correlative-SMLM we time-resolve the higher-order structure formed by multiple exocyst copies. With the docking of the vesicle to the plasma membrane, 6 exocyst cluster in a small patch of around 26 nm of radius, which 4.5 s later switch to a ring-like structure of 40 nm of radius and ~8 exocysts on average. These results were further validated by yeast genetics and suggest a new molecular function for the AAA ATPase Sec18.

Tuning in to nanoscale membrane dynamics with near-field optical antennas

Thomas S van Zanten[1]*, Maria Sanz-Paz[2], Pamina M Winkler[2], Satyajit Mayor[3] and Maria F Garcia-Parajo[3]

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Probing single molecule events at the nanoscale in living cells requires nanometric observation volumes together with sub-millisecond temporal resolution. Because of their properties in confining and enhancing light at the nanoscale, plasmonic nanoantennas are ideally suited for investigating biological processes at high spatial resolution. I will present two possible configurations to obtain nanoscale diffusion dynamics of proteins at the membrane of living cells from near-field antennas.
The first [1], is a broadband aluminium-based nanoantennas carved at the apex of near-field probes. This design allowed multicolour excitation in order to simultaneously record fluorescence fluctuations of dual-colour labelled transmembrane receptors known to form nanoclusters. Fluorescence cross-correlation studies revealed transient interactions between individual receptors in regions of 50 nm. Moreover, the high signal-to-background ratio provided by the antenna illumination permitted the direct detection of fluorescent bursts arising from the passage of individual receptors underneath the antenna. Remarkably, by reducing the illumination volume below the characteristic receptor nanocluster sizes, the molecular diffusion within nanoclusters is resolved and distinguished from nanocluster diffusion.
In a second configuration [2], we have implemented a simple wide-field illumination scheme together with sCMOS readout to simultaneously interrogate nanoscale volumes from 225 antennas in a single measurement. Recording at 1 kHz allowed multiplexed dynamic measurements from 50 nanoantennas simultaneously with a temporal resolution dictated by the camera framerate and the photons emitted per molecule during a single passage. As proof-of-concept, we demonstrate the capability for high-throughput arrayed detection of single molecule dynamics at the nanoscale in the membrane of living cells.

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[2] Winkler & van Zanten et al., arXiv: 2201.12068

Effects of LLPS on the cell membrane and actin remodelling

Claire Buchanan[1]*, Kexin Zhu[2], Yubexi Correa[3], Marián Alonso[1], Manuchar Gvaramia[1], Luke Clifton[4], Anton Le Brun[5], Adai Colom[1], Yansong Miao[2], & Marité Cárdenas[1,3]

[1]Instituto Biofisika, Spain. [2]Nanyang Technological University, Singapore. [3]Malmö University, Sweden. [4]ISIS neutron and muon source, England. [5]Australia's Nuclear Science and Technology Organisation, Australia.

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Actin remodelling is a crucial cellular response to both external or internal stimuli, effecting cell motility, cytokinesis, and cell signalling. Recently, actin remodelling has been found to involve liquid-liquid phase separation (LLPS), with coacervation occurring at the surface of the membrane, likely acting to upregulate critical proteins and adjust their activity. Actin remodelling in plants can also be activated by bacterial invasion, such as protein XopR found in phytobacteria, which has a strong affinity to the actin binding protein actin nucleator type I formin, initiating acting remodelling. By adjusting the NaCl buffer concentration, XopR can be easily switched between a dilute (150mM NaCl) and LLPS forming (50mM NaCl) conditions, allowing the interaction of both conditions to be analysed. Preliminary data indicates that cell membrane stiffness is a crucial factor in coacervation at the cell membrane, although the mechanism is unclear. The relationship between membrane stiffness and coacervation will be investigated by using neutron reflectometry (NR), quartz crystal microscopy (QCMD), and atomic force microscopy (AFM) to create a data set of the effect of protein XopR on supported lipid bilayers (SLB). QCMD will allow the adhesion of XopR to the SLB to be observed, AFM will allow for effect of XopR on the SLB to be monitored in real-time, while NR with selective deuteration will allow the detailed analysis of structural change in the SLB due to XopR, as well as providing information on the nature and origins of the coacervation (i.e. is coacervation 2D or 3D, and is it seeded at the membrane?).

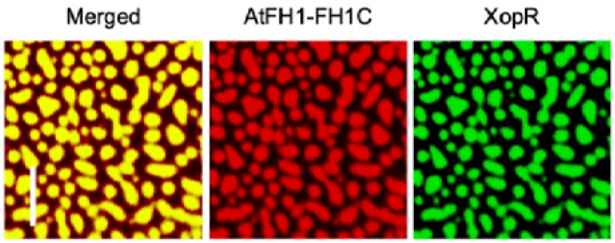

Figure 1. TIRF images on a POPC SLB sequentially exposed to 5 uM AtFH1-FH1C and 5 uM XopR. The formation of co-localized domains indicates LLPS at the membrane. Similar domains, albeit of different shape, where observed on the surface of plasma membranes in vivo.

The ancient mechanism of membrane compartmentation of the glycosyltransferase enzyme MurG discovered by HS-AFM

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Bacterial membranes have distinct components and are exposed to different environments, such as temperature variations, compared to eukaryotic cells. Microdomains of increased fluidity, known as Regions of Increased Fluidity (RIFs), have been observed in bacterial membranes and may act as platforms for the assembly of proteins. However, the exact mechanisms governing the organization of proteins in bacterial membranes are still not fully understood [1,2]. It is possible that protein assembly may not always rely on lipid segregation or pre-existing lipid domains, and other factors, such as electrostatic interactions between proteins, protein concentration, and lateral pressure, may be important.

This study used High-Speed Atomic Force Microscopy (HS-AFM) [3] to investigate the partitioning of MurG glycosyltransferases from Gram positive and Gram negative bacteria into lipid bilayers. Surprisingly, the MurG proteins were found to partition the membrane without requiring any preliminary lipid-induced partitioning. This study reveals important information about the membrane organisation of MurG proteins in phospholipid bilayers, providing insight into how these proteins help structure the bacterial membrane.

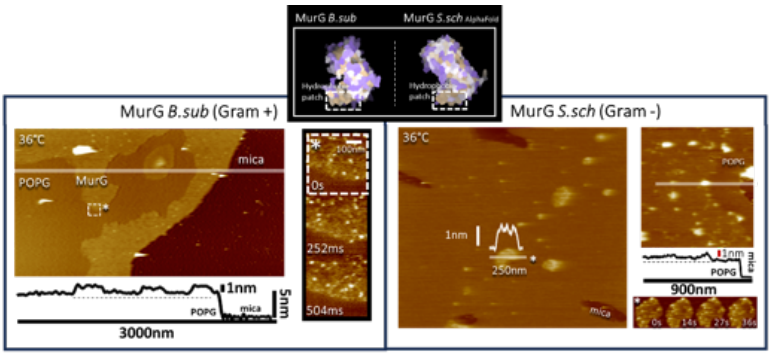

Figure 1: Comparison of the formation of partitioning in the POPG bilayer by two MurG proteins from Gram + and Gram - bacteria. Topohgraphic images of POPG supported bilayer after addition of MurG protein - left MurG Bacillus subtilis, right MurG Salmonella schwarzengrund - and showing the diffusion of the protein in these partitioning. Black part, representation of the membrane interaction region of MurG proteins by AlphaFold.

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Tuning in to nanoscale membrane dynamics with near-field optical antennas

Thomas S van Zanten[1]*, Maria Sanz-Paz[2], Pamina M Winkler[2], Satyajit Mayor[3] and Maria F Garcia-Parajo[3]

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Spatiotemporal Dynamics of Protein Export from the Endoplasmic Reticulum

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Intracellular trafficking is essential for the homeostasis of eukaryotic cells. The secretory pathway, which is entered by a third of all proteins, is responsible for processing and targeting proteins to their final destinations. A crucial step in the secretory pathway is the export of proteins from the endoplasmic reticulum (ER), which happens at specialized domains called ER exit sites (ERESs) [1]. Although the foundations for these processes were laid decades ago, there are still a number of unanswered questions regarding ER protein export. For example, it is not entirely clear how large proteins are exported from the ER, nor how ERESs are originally formed and maintained. Resolving these conundrums requires unraveling the dynamic organization of ER export in space and time. We are currently exploiting recent advances in biophysical and single-molecule fluorescence microscopy tools to provide insights into such processes. I will present an approach in which, by combining trafficking synchronization assays [2], intracellular single particle tracking photoactivated localization microscopy (iSPT-PALM) in living cells [3,4], and state-of-the-art machine learning-based analyses [5], can provide quantitative information about the spatiotemporal organization of secretory cargo proteins on their export from the ER. Altogether, our research will help characterize and provide a clearer understanding of a fundamental mechanism of intracellular communication.

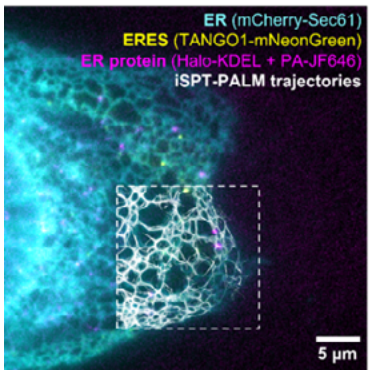

Figure 1. Representative image of an iSPT-PALM experiment on U2OS cells showing the ER (mCherry-Sec61, cyan), the ERES (TANGO1-mNeonGreen, yellow), and an ER protein at the single-molecule level (Halo-KDEL + PA-JF646, magenta, trajectories in white).

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Ionic Conductivity in Engineered Protein Thin Films

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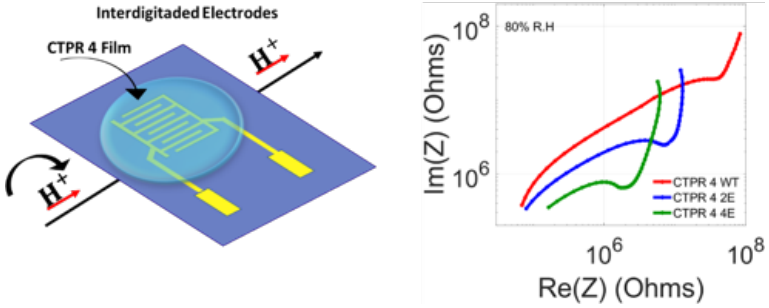
Protein-based conductive materials are promising for next-generation bioelectronics due to their versatility, biocompatibility, and biodegradability. Protein films composed of engineered consensus tetratricopeptide repeat (CTPR)[1] – proteins with a high content of negatively charged amino acids – have been demonstrated to be ionic conductors [2], with the dominant mechanism being protonic conduction. Proton transport within proteins is of interest for designing biocompatible energy storage devices or intelligent devices such as biosensors; raising the interest on improving protein film conductivities that can make them suitable for applications. In this work, we present an electrical characterization of thin films (≈ 100-200 nm thickness) prepared using engineered CTPR protein variants modified by different rates of glutamic acid substitution. From impedance spectroscopy measurements of CTPR films drop-casted on interdigitated electrodes, we find an increase of ionic conductivity with increasing the substitution rate, which suggests an increase in protonic carrier concentration. Additionally, we investigate the impact of NaCl doping on the ionic transport of the films, finding also an improved conduction performance of engineered variants. Finally, we explore the variations of the mechanical properties of the films with the amino acid substitution. Our results suggest that engineered proteins, such as modified CTPR scaffolds, can generate appropriate materials for bioelectronics applications.



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Droplet-based improvement of artificial metalloenzymes

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The advancement of enzyme alternatives is crucial for enhancing and facilitating novel processes in biotechnology and industry. Artificial metalloenzymes (ArMs) represent a fusion of protein scaffolds with metal elements, such as metal nanoclusters or metal-containing molecules, exhibiting specific catalytic properties that can be adapted. In this study, we engineered an ArM using the consensus tetratricopeptide repeat (CTPR) scaffold, introducing a distinct histidine residue to coordinate the hemin cofactor. Our findings demonstrate that this tailored system displays robust peroxidase-like catalytic activity when it is coordinated to a hemin molecule [1]. The expression of the scaffold and subsequent hemin coordination were achieved through recombinant expression in bulk and via in vitro transcription and translation systems within water-in-oil drops. The synthesis of this system in emulsio lays the foundation for enhancing its properties through droplet microfluidic screenings [2,3]. Using RosettaLigand software and entropy analyses, we have developed an extensive library containing 60,000 mutants. This library is founded on the binding residues within the pocket, enhancing its specificity and potential applications. Screening the generated library allows for the exploration of protein combinatorial space, leading to enhancements in the catalytic activity of the ArM.

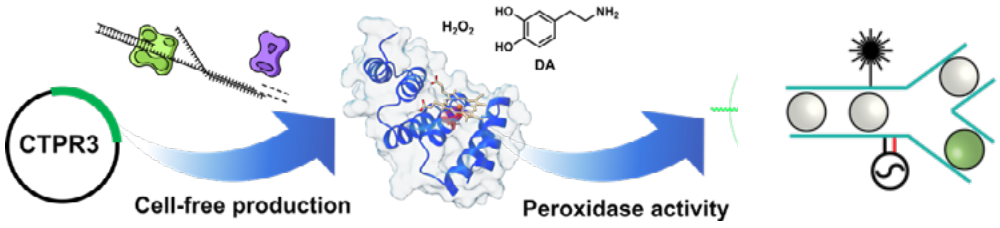

Figure 1. A hemin-based ArM with peroxidase activity. The fabrication of this ArM in emulsio paves the way for droplet-based screenings.

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Patterning and Mechanics during Bacterial Filamentation

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Bacteria employ diverse survival tactics in response to environmental stress, including challenges posed by antibiotic treatments. Among these strategies, filamentation stands out as a well-studied phenomenon where cells elongate instead of undergoing division. This adaptive behavior, crucial for bacterial survival, is also linked to their virulence. Our current investigation delves into the intricate interplay between gene expression patterns and mechanical forces during filamentation in E. coli. Specifically, we explore how the spatial and temporal distribution of MinD, a key protein involved in halting cell division, is affected by mechanical stress. Through quantitative fluorescent time-lapse microscopy observations, we report that MinD oscillations tend to avoid regions experiencing heightened mechanical stress. Furthermore, through manipulation of filament curvature in micropatterned agarose settings, we have identified the threshold at which mechanical stress triggers alterations in Min patterning. To elucidate the underlying mechanism driving this avoidance behavior, we conducted Fluorescence Recovery After Photobleaching (FRAP) experiments across regions with varying curvatures within the filaments. Our results indicate that MinD membrane diffusion is notably slower in areas exhibiting the highest curvature. To further validate our findings and explore the proposed mechanism from FRAP experiments, we have developed computational models to simulate bacterial filament behavior. Collectively, our study suggests that bacterial filaments possess a mechanosensing capability through the Min oscillatory system, which ultimately impacts the bacterial division process in response to diminishing stress signals.

Mechanobiology of the secretory pathway: Golgi export responds to external mechanical cues

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Cells interact with their environment through focal adhesions (FAs), molecular platforms bridging the extracellular matrix (ECM) and the cytoplasm. These structures serve as mechanosensors of ECM rigidity and as initiators of different signaling cascades, establishing feedback communications between the ECM and the cell interior to regulate mechanosensing and mechanoresponse. This back-and-forth signaling requires a regulated delivery of mechanosensing components to the plasma membrane (PM), suggesting that the Golgi apparatus is a mechanosensitive organelle. Indeed, recent studies have shown that mechanical cues modulate the levels of components involved in Golgi lipid homeostasis [1] and transport carrier formation [2]. Importantly, the molecular identity of the carriers trafficking cargoes to FAs overlaps with that of CARTS [3], a class of Golgi-derived vesicles that contain the transmembrane protein TGN46. These data let us hypothesize a role for CARTS in delivering mechanosensing components from the Golgi to FAs. Here, by combining a stretching device to mechanically challenge cells and advanced imaging techniques, we reveal that external stimuli modify the rate at which post-Golgi carriers are formed. Interestingly, we show that those vesicles are delivered at or close to FAs. Notably, mechanical cues emanating from physiological processes, such as cell spreading or migration, also affect the production of CARTS, as compared to cells seeded on passive substrates where cell spreading is abrogated. Finally, we have studied the mechanisms governing the communication between the PM and the Golgi. All in all, our results suggest a role of the secretory pathway in mechanotransduction.

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Two-layer elastic models for single-yeast compressibility with flat microlevers

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Among natural kingdoms, plants, fungi and unicellular microorganisms (yeasts, bacteria, algae ...) differ from multicellular animals by their intracellular structure and a rigid wall that reinforces the extracellular membrane and can sustain quite high turgor pressure (from 0.5 MPa in exponential growth phase to 1.5 MPa in the stationary phase for yeast) [3]. These cells regulate their volume, depending on the drop of pressure between the intracellular (□) and the extracellular media (□), the difference □P = □ − □ is defined as the turgor pressure. This wall is not a static structure, as it is expected to be dynamically remodeled according to growth stage, division cycle, environmental osmotic pressure and ageing. It is therefore of great interest to study the mechanics of these organisms, but they are more difficult to study than other mammalian cells, in particular because of their small size (radius of a few microns) and their lack of an adhesion machinery. Since the early nineties, the atomic force[4] microscope (AFM) emerged as a powerful tool because of its ability to probe biomaterials from nanometre scales (biomolecules) up to several tens of micron scales (subcellular organelles, cells, multicellular organisms) with forces in the range of tens to hundreds of nanoNewtons, under near physiological conditions [2]. Using flat cantilevers, we performed compression experiments on single yeast cells (S. cerevisiae) on poly-L-lysine-coated grooved glass plates, in the limit of small deformation using an atomic force microscope (AFM). Thanks to a careful decomposition of force-displacement curves, we extracted local scaling exponents that highlight the non-stationary characteristic of the yeast behavior upon compression [1]. Our multi-scale nonlinear analysis of the AFM force-displacement curves provides evidence for non-stationary scaling laws. We report here an original model which reproduce the experimental observations, based on a two-component elastic system, where each layer follows a different scaling law.
This work has a broad interest for modeling the mechanics of walled-cells more generally. These intrinsic nonlinearities of unicellular wall mechanics at small deformation depths (small compared to the cell diameter, but not its wall thickness) should be taken into account for mechanical modelling of larger deformation (plastic) regimes. This modelling brings an additional perspective to the structural characterisation previously performed with electron microscopy, and raises the question of the interplay of unicellular wall mechanics with the proliferation conditions (carbon source, growth stage, environment, confinement).

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Nanomechanics of cell-derived matrices in Collagen VI-related Congenital Muscular Dystrophies

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Collagen VI-related congenital muscular dystrophies (COL6-RDs) encompass a range of incurable neuromuscular disorders. These conditions stem from the deficiency or dysfunction of microfibrillar collagen VI (COL6) in the extracellular matrix (ECM) of connective tissues, due to autosomal pathogenic variants in any of the three major COL6 genes and manifest across a clinical spectrum. The utilization of cell-derived matrices (CDMs) technology in creating personalized pre-clinical models of COL6-RDs enables the direct observation of ECM fibrillar organization in patient-derived samples. This allows for the reconstruction of single protein fibrils within the CDMs and the comparison across various phenotypes [1], revealing altered arrangement of COL6, Fibronectin and Fibrillin-1 for COL6-RD patients compared to healthy individuals.

Here, we expanded our investigation to the nanomechanical properties of these models using atomic force microscopy-based force spectroscopy (AFM-FS), seeking to identify particular mechanical features for the COL6-RDs. We determine the Young's elastic modulus value (E) of the CDMs, which serves as an indicator of its stiffness. Our findings reveal a variation in the ECM elastic modulus value with patient phenotypes. We further investigate how such AFM-FS studies can evaluate the success of Nucleic Acid Therapeutics for COL6-RDs [2]. After genetic correction, a restoration of patient CDM mechanical properties to those of healthy CDMs was obtained. Thus, we introduce a novel functional readout specific to COL6-RDs, offering potential for improved diagnosis and prognosis. This emphasizes the potential of nanomechanical analysis as a prospective clinical tool, serving as a functional biomarker for tracking COL6-RD progression and therapeutic response.

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Labile assembly of a tardigrade protein induces biostasis

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Tardigrades are microscopic animals that survive desiccation by inducing biostasis. To survive drying tardigrades rely on intrinsically disordered CAHS proteins, which also function to prevent perturbations induced by drying in vitro and in heterologous systems [1, 2]. CAHS proteins have been shown to form gels both in vitro and in vivo, which has been speculated to be linked to their protective capacity [3,4]. However, the sequence features and mechanisms underlying gel formation and the necessity of gelation for protection have not been demonstrated. Here we report a mechanism of fibrillization and gelation for CAHS D similar to that of intermediate filament assembly. We show that in vitro, gelation restricts molecular motion, immobilizing and protecting labile material from the harmful effects of drying. In vivo, we observe that CAHS D forms fibrillar networks during osmotic stress. Fibrillar networking of CAHS D improves survival of osmotically shocked cells. We observe two emergent properties associated with fibrillization; i. prevention of cell volume change and ii. reduction of metabolic activity during osmotic shock. We find that there is no significant correlation between maintenance of cell volume and survival, while there is a significant correlation between reduced metabolism and survival. Importantly, CAHS D's fibrillar network formation is reversible and metabolic rates return to control levels after CAHS fibers are resolved. This work provides insights into how tardigrades induce reversible biostasis through the self-assembly of labile CAHS gels [5].

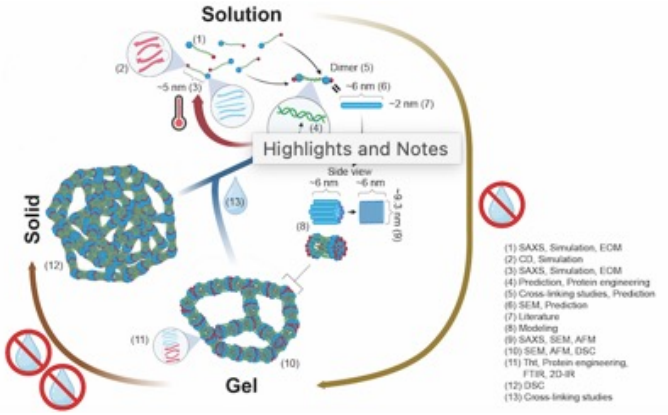

Figure 1: Model of in vitro CAHS D gel formation. Working model of CAHS D dimerization and gel formation. Numerical annotations note where evidence for model elements come from.

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Unraveling the role of crowding-driven biomolecular condensates in bacterial cell division

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Initially described in eukaryotic cells, biomolecular condensates arising from phase separation are becoming increasingly recognized as key players in the organization of intracellular space and the regulation of cell cycle events in bacteria [1]. These dynamic structures appear to be crucial for bacterial fitness and to withstand stresses such as antibiotic treatment. Thus, they represent interesting potential targets to fight antimicrobial resistance. We have identified homotypic and heterotypic biomolecular condensates in cell-like environments assembled by FtsZ, a GTPase that constitutes the scaffold of the cell division ring in most bacteria [2]. These membraneless compartments are strongly promoted by macromolecular crowding, mostly locate at the lipid interface inside microfluidics microdroplets, and interconvert with FtsZ polymers in response to GTP addition/depletion. Combining synthetic biology reconstitution and orthogonal biophysical approaches, we have dissected how selected positive and negative regulators of division ring formation modulate the switch between biomolecular condensates and polymers [3]. Our results show that phase separation may contribute to the mechanisms bacteria have developed for the precise positioning of the FtsZ ring at the cell center, essential for correct division into two equal daughter cells and, hence, for survival.

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The Pseudomonas aeruginosa effector Tse5 forms membrane pores disrupting the membrane potential of intoxicated bacteria

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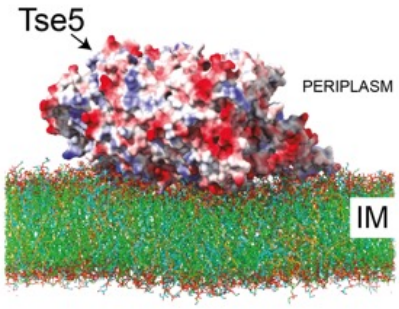
Bacterial competition is a significant driver of toxin polymorphism, which allows continual compensatory evolution between the toxin and the resistance developed to overcome it. Bacterial Rearrangement hot spot (Rhs) toxins represent a widespread example of toxin polymorphism. Rhs toxins are organised into three domains, each having a specific function. The N-terminal domain targets the toxin to a secretory pathway, while the toxicity is localised in the C-terminal domain. Recently, we combine structural, biophysical and in vivo techniques to investigate the molecular function of the Pseudomonas aeruginosa type VI secretion system (T6SS) exported effector Tse5. We will present recent results demonstrating that the C-terminal toxin (Tse5-CT) is a pore-forming toxin that can transport ions across the membrane, causing membrane depolarisation and bacterial death [1]. Remarkably, we will provide the first structural and functional insight into an Rhs protein that encapsulates and delivers a pore-forming toxin to the membrane of competing bacteria [2].



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ESV1 and LESV, two new non-enzymatic proteins involved in new stages in the biosynthesis of starch granules

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Starch is the major energy storage compound in plants. Whether it is transient or stored, it is accumulated in the form of insoluble, semi-crystalline granules. The structure of these granules is related to the structure of the main component: amylopectin. Amylopectin consists of linear polymers of glucose units linked by α1,4 bonds, forming double helices that combine to form the semi-crystalline lamellae of the granules, and α1,6 branching points that form the amorphous lamellae. This particular structure of amylopectin allows the starch granules to be structured. Two new proteins, LESV and ESV1, have been characterized and are involved in the phase transition of amylopectin (LESV) or in the maintenance of the granule structure (ESV1) [1]. These proteins share a tryptophan-rich domain folded into an antiparallel α-sheet that is particularly well suited to bind amylopectin double helices. In this talk we will present the structural study of these proteins alone and in interactions with starch polyglucans using integrative structural biology approaches [2]. We will show, as well, that LESV, in contrast to ESV1 can intervenes during amylopectin biosynthesis.

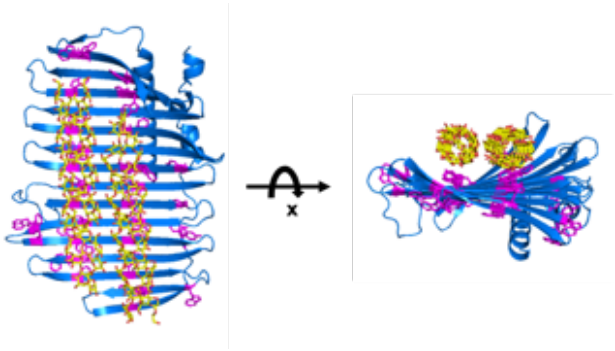

Figure 1: Molecular model of the complex between C-terminal domain of LESV and amylopectin double helices. Conserved Trytophan residues and Amylopectin double helices are represented by sticks and colored purple and by atom types respectively[2].

References

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High resolution structure of a T=219 giant virus

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The Double Jelly Roll (DJR) lineage is one of the four structural lineages of icosahedral viruses [1]. With 10-1500 kbp genomes and 60-500 nm capsid diameters, the DJR lineage contains the most complex icosahedral viruses discovered to date [2]. Prymnesium kappa virus RF02 (PkV-RF02) is a DJR virus with a 583 kbp linear dsDNA genome and 200 nm diameter capsid. PkV-RF02 infects a haptophyte alga, and is closely linked to other algal infecting viruses in the Mesomomiviridae family, that belongs to the same order as the amoeba infecting viruses (Imitervirales) [3,4]. Using mass spectrometry, we have identified 140 different proteins in purified PkV-RF02 virions.
Achieving high resolution in cryo-electron microscopy (cryo-EM) for giant virus particles is challenging [5], and our PkV-RF02 icosahedrally averaged map was limited to ~5 Å resolution. This was enough to show the T=219 overall capsid organization (Figure 1). This architecture was previously observed only for Phaeocystis pouchetii virus (PpV01) [6].
Using block-based reconstruction methods, we have overcome the resolution limitations and achieved ~3 Å in capsid blocks. We have identified the major capsid protein (MCP) and the penton protein, and we are currently tracing the polypeptide chains in the high-resolution maps. We have observed two conformations of the penton protein, indicating the presence of two different kinds of vertices. We also observed densities corresponding to minor capsid proteins (mCPs), which reinforce the capsid and connect it to the virus internal membrane (Figure 1).

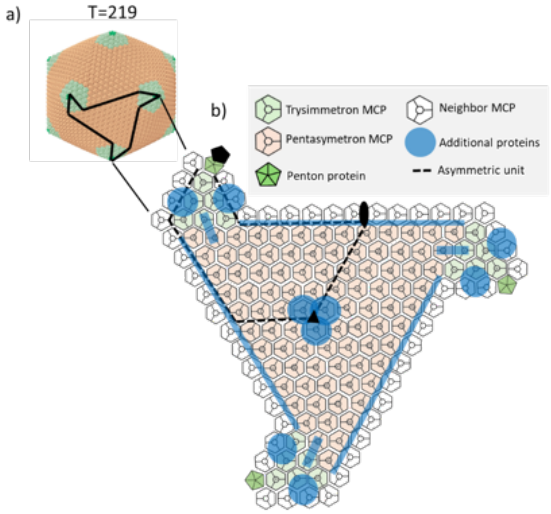

Figure 1. (a) PkV-RF02 capsid highlighting one facet of the icosahedron. (b) Schematic figure of a facet of the icosahedron and elements composing it. MCP, major capsid protein.

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How can protonation influence conformational switching: the curious mechanism of diphtheria toxin translocation (T-) domain

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Toxins and viruses excel at penetrating our membrane bilayers, often achieving this by pH-dependent conformational transitions [1,2]. One such case is found in the translocation domain (T-domain) of the diphtheria toxin, which uses to its benefit the acidification of the endosome as a translocation trigger for delivering its catalytic domain to the cell. Although the overall process is known, including the trigger role of His residues protonation [3], the molecular details of these translocation steps are not clear. Using constant-pH MD simulations, we intend to describe, at the molecular level, the effects of pH in key residues of the wt T-domain, the H223Q, H257Q, and E259Q single mutants, and the H223Q/H257Q double mutant. Combining pKa calculations and protonation data from our CphMD and NMR data from our collaborators it was possible to identify crucial pH-dependent features of the starting stages of the conformational transition of T-domain translocation. We confirmed the crucial role of the protonation in key histidines and how they impacted the activity of the mutated T-domains, while simultaneously revealed the presence of a strong latch-type mechanism between residues H223 and E259, which can modulate the trigger of the translocation domain.

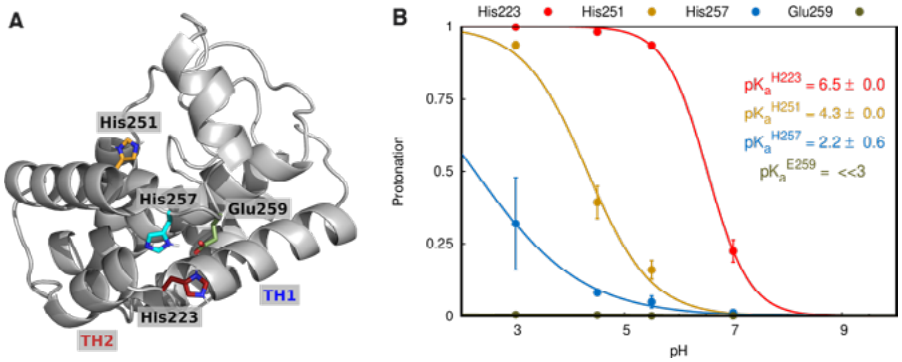

Figure1: Structural representation of the Wild type T-domain with the key Histidine residues highlighted (A) and pKa curves (B) of residues H223 (red), H251(yellow), and H257 (blue), and E259 (olive), obtained from CpHMD simulations.

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Structural basis for functional complexes between heptameric GroEL chaperonin and tetrameric plastid Cpn20 cochaperonin

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Members of the ATP-driven group I Cpn60 chaperonin family work together with a Cpn10 cochaperonin to assist the folding, translocation, and assembly of native proteins in the cytoplasm of prokaryotes, and in the endosymbiotic organelles of eukaryotes, such as mitochondria, chloroplasts and apicoplasts. GroEL-GroES from E. coli is the paradigm for group I chaperonin-cochaperonin systems. The apicoplasts of the parasites T. Gondii and P. Falciparum contain a single Cpn60-Cpn20 chaperonin system making them potentially vulnerable to chaperonin targeted anti-toxoplasmosis and anti-malaria drugs. Although the Cpn60 subunits in these apicoplasts form presumably heptameric rings as in GroEL, the cochaperonin Cpn20 consists of two tandem GroES-like Cpn10 domains. How would Cpn20 subunits assemble to work with a heptameric chaperonin ring is yet to be elucidated. Here we demonstrate that Cpn20 cochaperonins from T. Gondii (TgCpn20) and P. Falciparum (PfCpn20) can function together with GroEL in assisting the refolding of substrate proteins. The ADP:BeF3-bound cryo-EM structures of GroEL-TgCpn20 and GroEL-PfCpn20 exhibit a 14:(4)2 subunit stoichiometry, with only seven GroES-like Cpn10 domains contacting each of the seven GroEL subunits in each ring. This pseudo-C7 molecular symmetry solves the stoichiometry mismatch between GroEL and Cpn20 subunits and ensures the complexes can assist substrate protein folding.

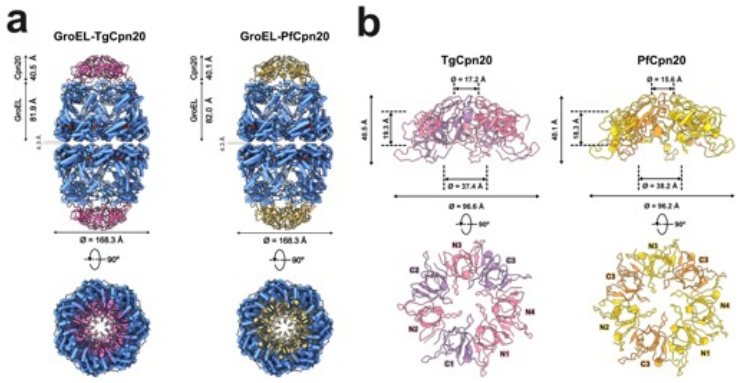

Figure 1 (a) Cartoon representation of the structures of GroEL-TgCpn20 (left) and GroEL-PfCpn20 (right). (b) Cartoon representation of the TgCpn20 (left) and PfCpn20 (right) tetrameric cochaperonin lids.

A novel BioID strategy for in vivo detection of protein-protein interactions

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Missense variants in the sarcomere protein cMyBP-C are a common cause of hypertrophic cardiomyopathy (HCM), a cardiac genetic disorder characterized by asymmetric hypertrophy and impaired diastolic function. While approximately 50% of these variants are associated with protein destabilization or mRNA splicing alterations [1], the underlying pathomechanism for the remaining 50% remains unknown. A plausible hypothesis to explain how these non-haploinsufficiency missense variants in cMyBP C lead to HCM could involve the presence of perturbed protein-protein interactions (PPIs) disrupting the normal mechanical integrity of the sarcomere. Here, we present a new strategy to study protein-protein interactions in the crowded and highly ordered environment of the cell based on the use of conventional proximity-dependent biotin identification (BioID) [2] in combination with the precise use of nanobodies (single variable domain on a heavy chain (VHH) antibodies) targeting the central C3 domain of cMyBP-C, a hotspot for pathogenic HCM missense variants. Steps to achieve this purpose involve: (1) production of the appropriate synthetic VHHs against the C3 domain using an optimized synthetic intrabody library [3], (2) screening of their intracellular expression and localization, (3) fusion of selected VHHs with the BioID-miniTurbo enzyme in a plasmid allowing expression in an adeno-associated virus (AAV), and (4) infection of cardiomyocytes with AAV to identify biotinylated candidate protein interactors by mass spectrometry. We expect our method to be widely applicable, since it does not depend on genetic manipulation of the system, is easily adaptable to be applied to different proteins regardless of their size or cellular localization, and can also be used in vivo.

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The genetic architecture of hydrophobic protein cores through deep sequence space

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Residues that make up the protein core are key at driving folding, defining the fold architecture and conferring thermodynamic stability. How flexible are the combinations of core residues that evolution has shaped in different protein folds? How much do they depend on the rest of the sequence? How complicated is the energetic landscape of the core, and what are the energetic couplings that constrain evolution? Using a deep mutational scanning (DMS) approach coupled to a protein stability readout [1, 2], we systematically measured the stability of thousands of combinatorial core variants in a range of small domains, to explore how many are functional and how diverse they are. We found that the stability landscape ruggedness varies with some proteins tolerating a significant number of multiple core mutations and some not. Using a neural network [3], we fitted thermodynamic models of the folding equilibrium accounting for single mutation effects (G) and pairwise energetic couplings (G) that suffice to capture nearly all variance in the data, providing evidence that the genetic architecture of protein cores is remarkably simple and nearly devoid of relevant high-order interactions. In FYN SH3, this holds true for function preservation, since pairwise interactions also suffice to explain changes in binding affinity for its ligand in high-order variants. For all cases we tested, we also found that even single mutations outside the core can improve the tolerance towards and rescue detrimental core mutations. Altogether, this advances our understanding of protein stability and has implications for bioengineering and clinical genomics.

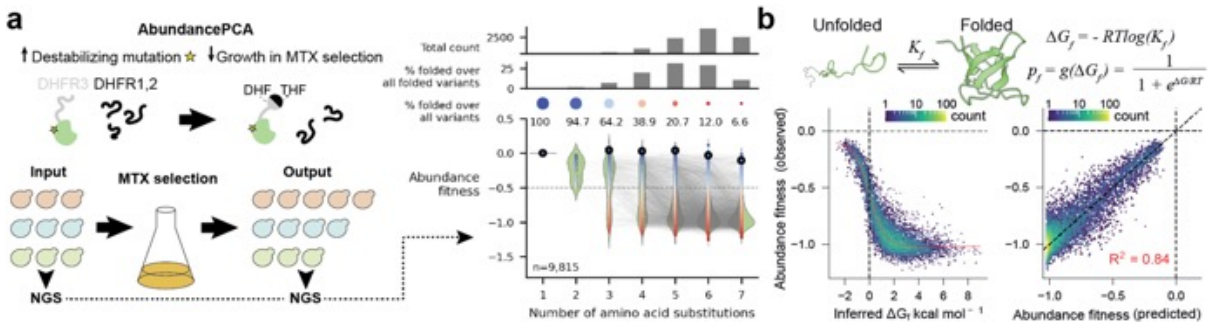

Figure 1 (a) AbundancePCA is a DMS readout we used to measure abundance fitness for thousands of high order core variants (right). (b) A simple second order two-state model of folding equilibrium yields high predictive performance in unseen variants.

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Bag1 has a key role in the Hsp70-assisted, proteasome-mediated degradation pathway

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Eukaryotic cells maintain cellular proteostasis through intricate protein quality control systems that orchestrate chaperone-mediated protein folding and protein degradation systems, preventing potential harm from accumulated misfolded proteins. The chaperone system attempts to refold the abnormal proteins and solubilize aggregated proteins; if unsuccessful, these aberrant proteins are removed through the protein degradation system.

In the ubiquitin-proteasome system (UPS), covalent conjugation of poly-ubiquitin chain on substrates guides specific protein degradation by 26S proteasome. The degradation process is initiated by recognition of ubiquitinated substrates by the 19S regulatory particle (RP), which is then followed by unfolding and translocation of substrates through a channel into the catalytic chamber 20S core particle (CP) that executes protein degradation Six AAA+ (ATPases associated with diverse cellular activities) ATPases subunits, Rpt1-6, unfold substrates by processive threading through a central channel driven by sequential ATP hydrolysis. Aside from the canonical subunits, proteasome function is finely tuned by transiently associated cofactors including UBL-UBA proteins which are involved in recruiting polyubiquitinated substrates to the 26S proteasome.

On the other hand, Bag1 has been shown to interact with Hsc70/Hsp70 to modulate the chaperone activities. Through an ATP-driven conformational cycle, HSP70 can recognize misfolded proteins, promote refolding, prevent protein aggregation, and resolubilize protein aggregates. Despite their many different roles, all members of the Hsp70 family contains two highly conserved structural domains: the substrate binding domain (SBD) and the nucleotide binding domain (NBD). The ADP/ATP switch is catalyzed by a group of cochaperones called nucleotide exchange factors (NEF), which bind to the NBD and favor ADP release from the active site and ATP re-uptake. Bag1 is one of such NEFs and contains both UBL and BAG domains and interacts with the 26S proteasome through the UBL domain to degrade unfolded proteins. However, how cochaperone Bag1 bridges between refolding system and protein degradation system, and how Bag1 enhances degradation of unfolded proteins remain unanswered.

In this work, using cryoelectron microscopy (cryoEM) and different biochemical and biophysical techniques, we have revealed that Bag1 plays a key role in Hsp70-mediated, proteasome-dependent protein degradation, not only by physically linking Hsp70 to the proteasome (through its subunit Rpn1), thus facilitating protein delivery to the latter, but also by inducing a series of conformational changes in the proteasome regulatory domain that facilitate the client protein degradation.

Molecular mechanism of a bacterial Retron

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Retrons are reverse transcriptase-carrying prokaryotic immune systems that protect bacteria against phages. Retrons are formed of three components: A reverse transcriptase (RT), a non-coding RNA (ncRNA), and one or several effector proteins with diverse enzymatic activities. Typically, the RT synthesizes a multi-copy single-stranded DNA (msDNA) from the ncRNA template, a feature that has been exploited for gene editing and synthetic biology applications. Upon phage infection, the effectors induce cell dormancy or death, preventing the phage from spreading throughout the population – a phenomenon known as abortive infection (Abi). To avoid cellular toxicity in the absence of infection, retron effectors are frequently kept in low-activity states, and RT and ncRNA/msDNA are believed to play a neutralizing role in this process. Despite the recent advances in the Retron field, the molecular events underlying immunity are poorly understood. Here, we reveal the molecular mechanism of a Retron [1]. First, we characterized the activity of the effector in vitro and during phage infection. CryoEM structures of the Retron complex illustrate that the msDNA stabilizes a filament in which the effector is kept in a low-activity state (Figure 1). Interestingly, msDNA's mutations induce the release of the effector from the complex and cause toxicity, underscoring the msDNA role in immunity. In addition, we identified and characterized a phage-encoded Retron inhibitor that suffices to offset the immune response. Collectively, our work outlines the structural basis of the Retron defense system and highlights the intricate interplay between bacterial defense systems and phages.

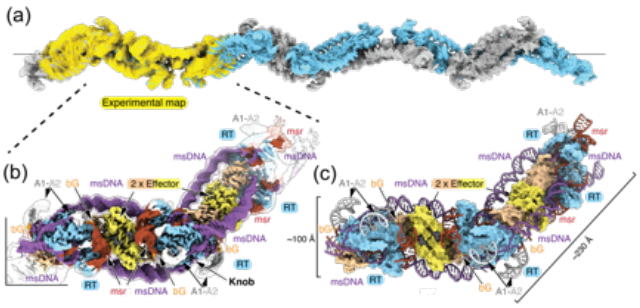

Figure 1. (a) Retron filament model with 10 segments. (b) CryoEM map of a two-segment retron filament. (c) Atomic model of a two-segment retron filament.

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Monitoring of a DNA double helix formation: a multi-approach benchmark study

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In both basic or applied research the characterization of binding parameters of complexes involving biological macromolecules remains a crucial step to understand function, to relate it to the structure, to evaluate whether a specific ligand is of interest for therapeutic purposes, or to validate a molecular probe for biosensing or for more fundamental studies. The determination of the dissociation equilibrium constant, KD, and if possible the kinetics and the thermodynamic parameters of the binding reaction, is often seen as the Holy Grail. The last decade has seen the emergence of new manufacturers offering biosensing instruments that suit the needs of researchers. There is now a wide range of instruments based on different physical principles capable of measuring binding affinities. The goal of the present benchmark study was to analyze how different technical setups performed for analyzing a very simple model, the formation of a 10-mer DNA double helix, the stability of which can be easily tuned by temperature, ionic strength or single point mutations. The results show that the measured dissociation constant, KD, depends to a significant extent on how the DNA formation helix is monitored.

This work was partly supported by the Euskampus Bordeaux LTC Sarea program (BIOMINT)

Characterization of the real-time kinetics of the activities of the human mitochondrial DNA helicase at the single-molecule level

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Human mitochondrial DNA is replicated by a minimal replisome that consists of DNA polymerase gamma (PolG), DNA helicase (TWINKLE) and mitochondrial single-stranded DNA binding protein (mtSSB). TWINKLE is a ring-shaped helicase that uses energy from NTPs to couple unidirectional translocation (5′ to 3′) along single-stranded DNA with unwinding of the duplex DNA. Here, we used optical tweezers to determine the real-time kinetics of TWINKLE DNA unwinding activity and its regulation by the stability of the DNA fork and mtSSB. Our results show that TWINKLE DNA unwinding kinetics are dominated by frequent transient inactive states or pauses. We found that these pauses are mainly caused by the interaction of the N- and C-terminal domains of the helicase with the displaced strand. Interestingly, mtSSB modulates TWINKLE DNA unwinding kinetics by decreasing specifically the pause occupancy. In addition, we used optical tweezers combined with confocal fluorescence microscopy (Figure 1) to show that, in the presence of ATP, TWINKLE loads on dsDNA and rapidly diffuses along this polymer until encountering the fork position.

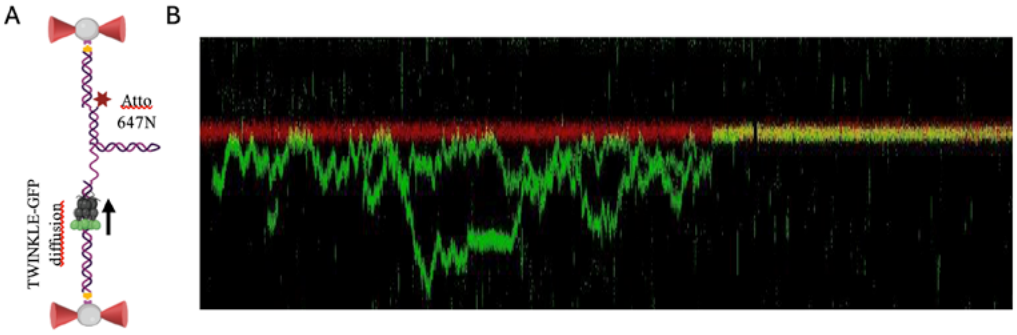

Figure 1. A) In C-trap® , a single DNA hairpin is tethered between two optical trapped beads at constant tension, the DNA hairpin is localized by annealing with labelled oligo. B) Kymograph showing the hairpin localization and diffusion of TWINKLE-GFP along dsDNA until encountering of the DNA fork.

DNA curtains-based approach for fluorescence microscopy-correlated Optical Tweezers

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The pertinent combination of optical tweezers and confocal scanning microscopy has revolutionized single-molecule research. It is now possible to manipulate and visualize the interaction of proteins and RNAs with individual DNA molecules in real time. However, when it comes to studying DNA double-strand breaks and their repair, the typical setup lacks accessible DNA ends, which creates a research gap [1].
To address this issue, we have developed a DNA curtains-based approach where a branched DNA structure is immobilized in between the two optically trapped beads. This branched construct can have up to 8 bifurcations from where 7 kbp-long dsDNA chains emerge. Using this new approach and employing labeled proteins/RNAs, the loading or binding of the proteins to the DNA ends as well as the unwinding and translocation events can be directly evaluated from confocal fluorescence imaging (1D or 2D). As proof of application, we have successfully observed the loading and threading of the Ku heterodimer onto the DNA branches, and we have obtained visual confirmation of the destructive action of the helicase/nuclease AddAB on dsDNA molecules with free ends.

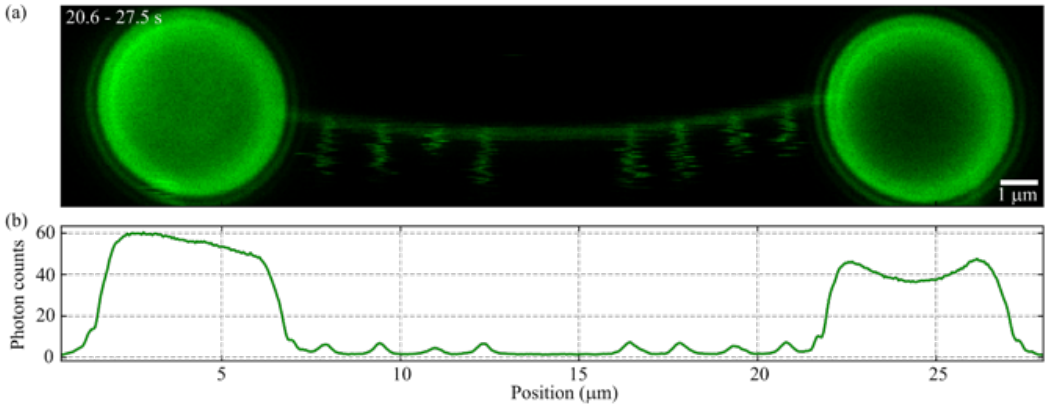

Figure 1. A) In C-trap® , a single DNA hairpin is tethered between two optical trapped beads at constant tension, the DNA hairpin is localized by annealing with labelled oligo. B) Kymograph showing the hairpin localization and diffusion of TWINKLE-GFP along dsDNA until encountering of the DNA fork.

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Engineering Nanobodies for Biotechnological Applications

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Tailoring proteins to suit specific applications represents an important challenge in biomolecular engineering, and will improve their use in many biotechnological applications. Among the large set of proteins of biotechnological relevance, nanobodies stand out due to their versatility and simplicity. Derived from camelid single-chain antibodies, these minimalistic antibodies offer antigen-binding capabilities very similar to canonical antibodies, but with a much smaller size (12 kDa). Nanobodies have thus found widespread application in, for example, immunotherapy, biosensing, imaging and drug delivery.

Research in our lab focuses on developing engineering strategies to improve the biophysical properties of nanobodies [1]. By combining phylogenetic and structural analysis, computational and artificial intelligence tools, and experimental characterization, we have enhanced the stability and solubility of nanobodies against drugs, hormones and biomarkers. This ability to optimize nanobodies rationally will facilitate their implementation in biotechnologies by, for example, improving their use as therapeutics, and facilitating their recombinant production.

We have also succeeded in engineering new functions into nanobodies. By tuning their stability, we have engineered nanobodies undergoing binding-induced conformational changes. These nanobody-based conformational receptors enable more direct signal transduction upon target binding, thus facilitating the development of reagent-less biosensors for continuous monitoring [2]. Leveraging this, we have developed a biosensor for the hormone chorionic gonadotropin, a biomarker used to monitor pregnancy.

This work shows our ability to engineer nanobodies, and potentially other proteins, to enhance their properties and functional versatility. Our research thus holds fundamental implications in understanding protein biophysics, as well as an applied biotechnological perspective.

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Protein-based conductive inks: challenges in the design and production

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Traditional electronics significantly contribute to global environmental pollution due to the slow degradability and high toxicity of their components [1]. Therefore, developing biocompatible, biodegradable, and more sustainable components is necessary for the advancement of green electronics. In this context, protein-based conductive materials have significant potential due to their intrinsic biocompatibility and biodegradability capabilities. Engineered Consensus tetratricopeptide repeat (CTPR) proteins are stable and robust proteins [2] with a remarkable degree of tunability. The versatility stems from the structure of the CTPR unit, consisting of a helix-turn-helix motif codified by 34 amino acids, with only eight conserved positions essential for the correct folding [3]. Here, we demonstrate how rational engineering in protein design, specifically through glutamic acids and tryptophan substitutions, enhanced ionic and electronic conductivity, respectively, without affecting the structural features of engineered CTPRs. We also developed a bioreactor-based gram-scale production of CTPR proteins by fermentation, which is critical to enable the formulation of protein-based conductive inks and hydrogels. In summary, this work aims to illustrate how an integrated approach effectively addresses the limitations encountered during the production of protein-based conductive inks, paving the way for the development of biocompatible and sustainable conductive ink materials.

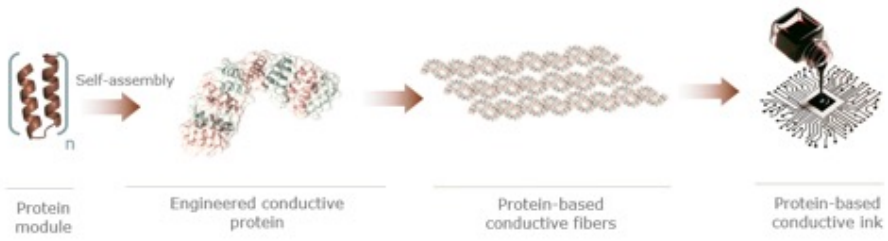

Figure 1. Self-assembly of CTPR-engineered proteins for their use in protein-based conductive inks.

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Detecting diseases-related oligonucleotides sequences: a multi-technique approach for label-free sensing

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We present a comprehensive study for the development of a label-free sensor platform for the detection of oligonucleotide sequences. This platform is based on the hybridization of thiol-tethered DNA strands self-assembled on flat gold substrates. Target sequence detection was demonstrated using the RdRp-Helicase sequence of SARS-CoV-2.
Through time-resolved measurements with quartz crystal microbalance with dissipation (QCM-D) and spectroscopic ellipsometry (SE), we monitored the molecular immobilization of probe DNA strands and the hybridization with the target sequence. By combining SE measurements with QCM-D data, we established a pathway for the quantification of the assay through the calibration of SE data.
Dynamic and static optical measurements also demonstrated the selectivity and recovery properties of the sensing platform. To improve the platform sensitivity, we exploited surface plasmon-enhanced ellipsometry (SPEE), which combines SE with surface plasmon resonance (SPR).
The interpretation of SE data requires a proper effective optical model. This model, supplemented by information on film thickness (scanning probe nanolithography) [1], and surface composition (X-ray photoemission spectroscopy, XPS), enabled the clear spectral identification of UV DNA resonances and the formation of the gold/thiolate interface [2]. Furthermore, analysis of UV absorptions and optical thickness, compared with QCM-D data, allowed us to assess the effect of hybridization-induced hypochromism of immobilized DNA absorbers. Although hypochromism has been commonly observed in various experiments involving molecular solutions, our study represents the first attempt, to our knowledge, to characterize this phenomenon for DNA monolayers immobilized on a surface [3].

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Engineering of an anti-CD20 antibody through site-selective chemical modification to improve its therapeutic potential

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Monoclonal antibodies constitute a rapidly expanding class of biologics used in the treatment of cancer, autoimmune, and infectious diseases. Improving their binding affinity is crucial for enhancing potency and minimizing required doses for efficacy. This challenge is particularly pronounced for antibodies targeting poorly accessible external epitopes on integral membrane proteins, such as the tumor-associated antigen CD20, the focal point of the monoclonal antibody Rituximab, approved for treating B cell malignancies. In this study, we employed a synthetic aromatic grafting method to amplify the therapeutic potential of Rituximab. Specifically, we introduced a polycyclic aromatic compound at an antibody position, juxtaposed to the membrane interface upon epitope binding. The selectively modified Rituximab demonstrated an order of magnitude higher potency than the original version in inducing tumor cell death through complement activation. Confocal microscopy revealed that this enhancement was correlated with an improved recognition of CD20 on the surface of B cells. Furthermore, the modified Rituximab maintained a favorable pharmacokinetic profile following intraperitoneal infusion in SCID mice, positioning it as a promising candidate for future treatment development.

Exploring membrane fusion mediated by amphiphilic gold nanoparticles

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Lipid membrane fusion plays a crucial role in fundamental cellular processes, such as cargo transport, reproduction, and communication. Within the biological milieu, the regulation of fusion events relies on highly specific factors, including a wide range of specialized proteins. Similarly, various synthetic systems can be developed to design controlled and selective membrane fusion mechanisms. We demonstrated that membrane-embedded amphiphilic gold nanoparticles (AuNPs) can be employed as synthetic fusogens of lipid vesicles exploiting the cooperative effects of AuNPs with a core diameter smaller than the lipid bilayer, cholesterol-containing vesicles, and divalent ions [1,2]. Currently, our focus is on unraveling the interplay between lipid vesicle curvature and AuNPs size in the fusion process. Combining fluorescence spectroscopy assays and computational techniques, we studied the fusogenic properties of AuNPs of distinct sizes (2.4 nm and 4.8 nm - core diameter) interacting with large and small unilamellar vesicles containing a biologically relevant cholesterol percentage. Our experimental and computational results revealed that small AuNPs promote vesicle fusion regardless of the membrane curvature [3]. Conversely, large AuNPs did not exhibit fusogenic properties with low curvature membranes but suggest a tendency to induce fusion events with more curved membranes. Overall, these results indicate that, by fine-tuning all these physicochemical parameters, AuNPs are a promising synthetic fusion machinery for the development of tailored drug delivery tools.

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Tuning the dimensionality of supramolecular functional materials based on the rational design of biomolecules

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Peptide-based supramolecular assemblies have been widely studied for their inherent ability to self-organization, making them useful for applications in nanotechnology.[1] Combining these structures with other molecules opens a new perspective for the design of complex functional materials.[2] Here, we aim to build complex architectures combining a versatile engineered protein, which has potential applications in bioelectronics and nanotechnology, with self-assembled fibers.[3] For this purpose, the protein is modified to incorporate one or two peptides in one or both termini, respectively. This modification will drive the co-assembly with the fibers, resulting in one-dimensional co-assemblies when the protein has one peptide, and an extended network on a second dimension when the protein has two peptides, acting as a fiber cross-linker. The material design was carried out by screening peptide sequences using molecular dynamics (MD) simulations to optimize their co-assembly with the engineered proteins. The resulting architectures were experimentally characterized to prove the change in dimensionality with the number of peptides linked to the protein. The combination of the experimental and computational techniques provided a better understanding of each component in the system, facilitating further rational functional modifications. Therefore, versatile hybrid materials have been created using biocompatible building blocks without introducing any artificial chemical groups or bonds. These materials can be customized to suit specific needs in the fields of bioelectronics and biomedicine.

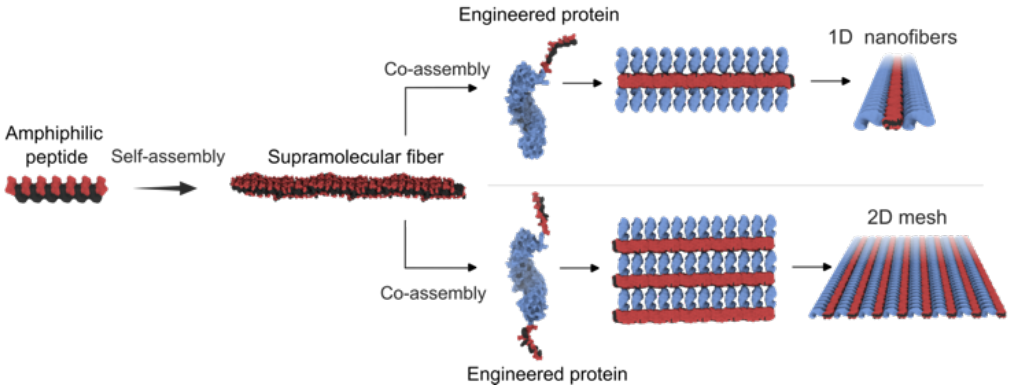

Figure 1. Rational design of hybrid materials. The co-assembly between the self-assembled fibers and the engineered protein with one peptide incorporated in the N-termini results in 1D hybrid nanofibers (top). The co-assembly between the self-assembled fibers and the engineered protein with two peptides incorporated in both termini results in a 2D hybrid mesh (down).

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Designing model photosystem for tracking protein dynamics in chromophore´s light harvesting properties

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Studies in natural photosystems suggest that a correlation between protein dynamics and chromophore´s excited state relaxation is key for efficient light harvesting. However, showing the impact of these protein dynamics in chromophore´s light-harvesting properties is challenging due to the huge complexity of the natural systems. Artificial photosystems have emerged as a solution to perform mechanistic studies by simplifying the effects that occur in natural light-harvesting processes. However, the chromophores used in most cases have linkers that attach them to the protein structure, giving flexibility and rotational freedom to the chromophore that obscures the role of protein dynamics in the photosystem.1 In this work, I proposed a new approach to generate the artificial photosystem based on the incorporation of the chromophore as an unnatural amino acid (UAA). We hypothesize that the direct incorporation of the chromophores into the protein backbone will allow us to track protein dynamics in real-time. To show that, we designed two photosystem models one in which the chromophore is incorporated directly into the backbone as a UAA and the other where the chromophore is attached to the backbone via a linker (Figure 1). These designs will allow us to compare whether there are significant differences in the light-harvesting properties of the chromophore depending on whether a linker is present or not. This new approach will allow the development of mechanistic studies from a completely different angle, revealing how protein dynamics affect the light-harvesting properties of chromophores.

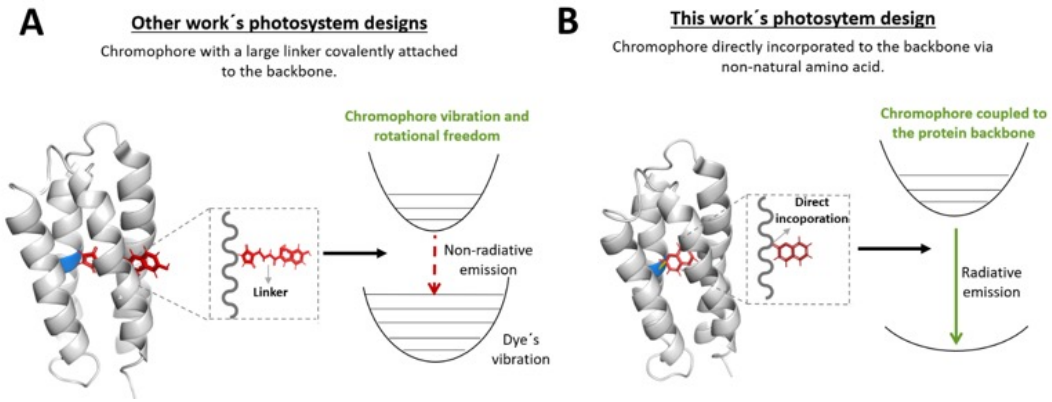

Figure 1: (a) Artificial photosystem model with an Alpha4 protein (grey) linked to coumarin-maleimide via click chemistry (red), implying weak coupling, less emission. (b) Alpha4 protein (grey) with coumarin bound as a non-natural amino acid (red), indicating strong coupling, more emission.

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Protein-stabilized nanomaterials as MRI contrast agents: one size does not fit all, but versatility does

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Magnetic Resonance Imaging (MRI) is a widely used diagnostic method, often requiring the use of contrast agents to enhance image contrast in diseased regions. However, existing contrast agents lack specificity and rely on deviations in anatomy or physiology to create contrast. As a result, there is a pressing demand for the development of more specific and targeted contrast agents.
Protein-stabilized nanomaterials have emerged as promising candidates in biomedicine due to their biocompatibility, stability, and ease of preparation.[1] Consensus tetratricopeptide repeat (CTPR) proteins offer a versatile platform for nanomaterial development, facilitating the introduction of metal coordination sites and targeting or therapeutic motifs without compromising structural integrity.[2] Herein, we designed two sets of CTPR proteins sharing a metal-coordination domain and further engineered to display either a tankyrase-binding peptide (TBP)[3] or a KEAP1-binding peptide (Figure 1).[4] These motifs are well-studied modulators of tankyrase and Nrf2-KEAP1 axis, two relevant targets in pathologies such cancer and neurodegenerative diseases. These proteins were used to stabilize iron oxide nanoparticles (IONPs), which demonstrated improved relaxivities while preserving the high binding affinities towards their targets, indicating that neither the synthesis process nor the presence of the nanomaterials interferes with binding capacity of the protein scaffold. Nrf2-IONPs also demonstrated ability to bind KEAP1 in cellular environment with a functional therapeutic activity.
These findings highlight the potential of protein-stabilized nanomaterials as multifunctional MRI contrast agents, offering prospects for the development of an effective theranostic agent.

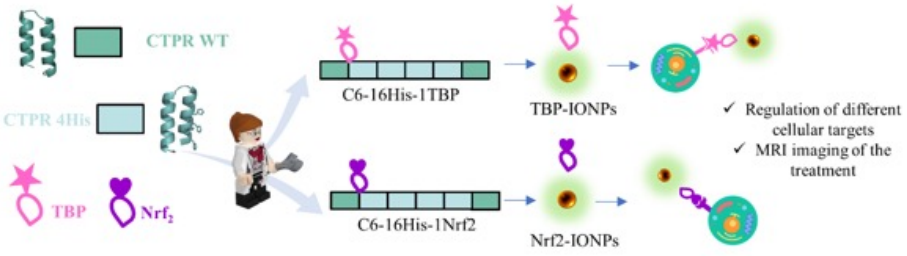

Figure 1. CTPR proteins are used as building blocks to engineer new proteins bearing both metal coordination sites and different binding peptides (TBP and Nrf2). These proteins allowed the synthesis of Prot-IONPs with different targeting and therapeutic capabilities simultaneously allowing for MRI imaging.

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Visualizing Molecular Dynamics with High-Speed Tip-Scanning Atomic Force Microscopy

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Biological systems exhibit high structural and functional dynamics on molecular scales. Understanding kinetic principles behind structural changes at that scale is of critical importance when studying samples ranging from single membrane proteins to complex macromolecular systems, to accurately develop novel therapeutic applications. We have used high-speed tip-scanning atomic force microscopy (AFM) with a kilohertz linerate to visualize molecular dynamics by enabling temporal resolution on the sub-100-milisecond scale. The use of a tip-scanning AFM, as compared to a sample-scanning system, enables high-resolution correlation experiments with advanced optical techniques. We will give two examples in which high-speed tip-scanning AFM was applied for studying of structural transitions and biomolecular dynamics in samples, containing triangular DNA origamis and photosensitive surfactants.

DNA origami structures serve as a functional template in multiple artificial and native molecular systems. We studied the development of order in 2D DNA triangular Rothemund lattices. By mobilizing the DNA origami adsorption on mica with varying buffer composition we looked at the temporal dependence between lattice order development and Na+ ion content in the studied sample with a temporal resolution of 1 frame/s.

We monitored the structural photosensitive transition of photosensitive surfactants under external light-induced deformation. By simultaneous high-speed AFM measurements and switching the external wavelength illumination from 365 nm to 546 nm and vice versa, we could monitor and induce a reversible structural transition within the studied sample in real-time.

Effects of microbial glycolipids on phospholipid membranes using Atomic Force Microscopy (AFM)

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Microbial glycolipids are biosurfactants comprising a hydrophilic saccharide moiety coupled to a hy drophobic fatty acid. Their antimicrobial properties are raising an increasing interest as they might be alternatives to antibiotics, offering biodegradability and low ecotoxicity, and their production is based on renewable-resource substrates. Antimicrobial activity involves changes and rupture of the cellular mem brane inducing lysis. However, their mechanism of action is still not well-known. It might rely on the interaction of the saccharide moiety with the bacteria! membrane and posterior penetration thanks to the lipophilic moiety [1]. The current project aims to study the effect of glycolipids on supported lipid bilay ers using atomic force microscopy (AFM), to better understand the underlying molecular mechanisms at the nanoscale.
We observed the dynamic evolution of model phospholipid membranes after injection of two glycolipids. We also characterized the effect of glycolipids on the (nano)mechanical properties of membranes (Figure 1). Using a similar approach, supported!amellar structures can be formed by mixing phospholipids and glycolipids to form vesicle in suspension. We are exploring a wide range of phospholipid:glycolipid ratios to better understand their interaction s and properties. These insights into the effect of microbial glycolipids on model membranes at the nanometre scale can contribute to a better understanding of their biological activity, as well as their potential use as nanocarriers.

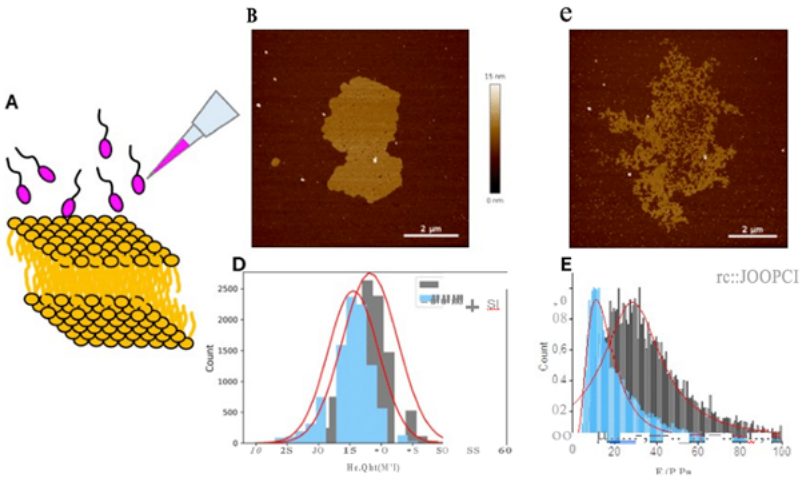

Figure I : A) Glycolipid injection on a phospholipid membrane. B) AFM topography image of a DOPC lipid membrane before and C) after adding sophorolipid (SL) solution . D) Histograms of the height of the membrane . and E) of the Young Modulus

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Abstracts: Awards

Kinetic stabilization of translation-repression condensates by a neuron-specific microexon

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The inclusion of microexons by alternative splicing is frequent in neuronal proteins. The roles of these sequences are in most cases unknown, but changes in their degree of inclusion are associated with neurodevelopmental diseases. We recently found that the decreased inclusion of a 24-nucleotide neuron-specific microexon in CPEB4, an RNA-binding protein that regulates translation through cytoplasmic changes in poly(A) tail length, is linked to idiopathic autism spectrum disorder (ASD) [1]. Why this microexon is required and how small changes in its degree of inclusion generate a dominant-negative effect on the expression of ASD-linked genes is not clear. Here we show that neuronal CPEB4 forms condensates that dissolve upon depolarization, a transition associated with a switch from translational repression to activation. Heterotypic intermolecular interactions between the microexon and a cluster of histidine residues kinetically stabilize the condensates by competing with homotypic interactions between clusters, that otherwise lead to the irreversible aggregation of CPEB4 [2]. We conclude that the microexon is required in neuronal CPEB4 to preserve the reversible regulation of CPEB4-mediated gene expression in response to neuronal stimulation.

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Bridging the scales in molecular biophysics via multipronged theoretical approaches

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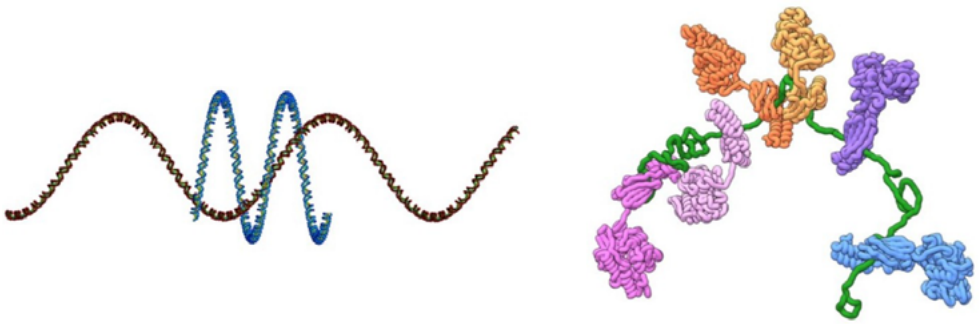
Biological macromolecules epitomize the concept of emergence of complexity: despite the fact that all their physical details are concealed in their (sub)atomistic description, novel behavior is unveiled as larger time and length-scales are considered. A mechanistic understanding of how the features at lower scales are propagated into large-scale phenomena can be obtained by combining complementary approaches. In this talk, I will speak about recent efforts in our group where this approach is employed in different contexts. First, I will address some of our results on the equilibrium conformational and mechanical properties of nucleic acids, e.g. the theoretical prediction on the different elastic response between double-stranded DNA and RNA [1], and our simulations on the exotic elastic behavior of phased A-tracts [2]. The second part of the talk will instead be devoted to the characterization of the mechanical action of the Hsp70 chaperone in its housekeeping tasks, enabled by ATP hydrolysis [3,4]. This latter example shows the potential of multipronged approaches to tackle biophysical problems with non-equilibrium nature.



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Imaging cell membrane biophysics

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Microscopy provides a straightforward method to image live cells, and when combined with environment-sensitive dyes it allows for quantification of membrane biophysical properties, such as fluidity or diffusion. Traditional implementations, however, have faced numerous limitations, including low spatial resolution, fast photobleaching, probe internalisation, and non-specific labelling. My research focuses on implementing developments in microscopy and chemical biology tools to reliably measure membrane biophysical properties in living cells with unprecedent resolution and specificity. These advancements have allowed us to gain mechanistic insights into the molecular processes underlying significant biomedical challenges, such as HIV-1 infection and rare neurodegenerative diseases.

Understanding the influence of surface state on Pseudomonas aeruginosa surface twitching: experiment and modelling

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Bacterial adhesion to a solid surface is the first step of surface colonization and subsequent biofilm formation. Our research aims to understand better the surface motility mechanism of Pseudomonas aeruginosa, an opportunistic pathogen that colonizes various substrates, including living tissues and inert materials. To this aimed, we first studied how twitching, a motility mechanism orchestrated by appendices called Type-IV pili, is affected by substrate stiffness (Figure 1 (a)), and the consequences on the organization of bacterial colonies. We subsequently rationalized our findings by developing a model of twitching on soft substrates which balances the pulling force induced by pilus retraction with the friction force between the bacterial body and the surface, and the surface elastic deformation [1]. To confirm this model of regulation of twitching by the surface physical properties, we have then designed a methodology for measuring adhesion forces that integrates microfluidics and optical microscopy. By combining surface colonization studies at low shear rates with orientation and detachment and orientation assays at higher shear rates (Figure 1 (b)), we can assess the relative adhesion strengths of the cell body and the pili and, through appropriate modelling, connect them to the bacteria twitching velocity. Our findings demonstrate that changing the chemical functionalization of the surface permits manipulating this equilibrium and test our theoretical model of bacterial movement.

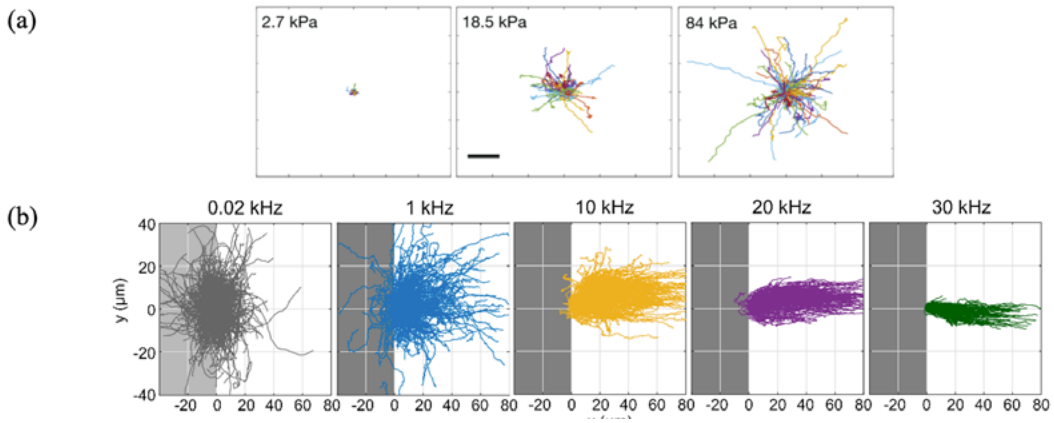

Figure 1 (a) Bacterial tracks on soft (2.7 kPa), intermediate (18.5 kPa) and stiff (84 kPa) hydrogel polyacrylamide. Scale bar: 10 µm [1]. (b) Bacterial tracks for several shear rates show a random movement of bacteria moving at a low shear rate and directional movement at a high shear rate on glass substrates.

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Membrane remodeling by active rotary ATP synthases

Marcin Makowski [1,2,*], David Valdivieso González [3,4], M. Pilar Lillo [5], Francisco J. Cao-García [6,7], Manuel N. Melo [2], Víctor G. Almendro-Vedia[3,4] , Iván López-Montero [3,4,8]

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The universal transmembrane nanomotor protein ATP synthase catalyzes the synthesis of ATP through the rotatory movement of its membrane-spanning subunit. Mitochondrial ATP synthases arrange as dimers at the highly curved rims of cristae. Conversely, monomeric bacterial ATP synthases locate at the flat surface of the plasma membrane and lack the dimerization subunits of their mitochondrial homologs. We here explore a putative link between the rotatory movement of ATP synthases and their preference for curved membranes. To this purpose, we have used bacterial ATP synthases reconstituted in giant unilamellar lipid vesicles (GUVs). Pulling experiments with GUVs show that active ATP synthases concentrate at the highly curved regions of the pulled nanotubes. On the other hand, passive ATP synthases showed no preference for the curved regions of the giant vesicle. To gain a molecular perspective of this process, we performed molecular dynamics simulations that verified the curvature-seeking behavior of rotating ATP synthases. Furthermore, our simulations suggest that the curvature sorting of ATP synthases is concurrent with reversible and frequent protein-protein contacts. The formation of these transient dimers relies on the hydrophobic mismatch produced at the vicinity of the rotating proteins. Overall, our results suggest a new functional role for the rotational movement of ATP synthases in their dynamic self-assembly in biological membranes.

Abstracts: Posters

Session I: Cell and tissue biophysics

Understanding the influence of surface state on Pseudomonas aeruginosa surface twitching: experiment and modelling

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Bacterial adhesion to a solid surface is the first step of surface colonization and subsequent biofilm formation. Our research aims to understand better the surface motility mechanism of Pseudomonas aeruginosa, an opportunistic pathogen that colonizes various substrates, including living tissues and inert materials. To this aimed, we first studied how twitching, a motility mechanism orchestrated by appendices called Type-IV pili, is affected by substrate stiffness (Figure 1 (a)), and the consequences on the organization of bacterial colonies. We subsequently rationalized our findings by developing a model of twitching on soft substrates which balances the pulling force induced by pilus retraction with the friction force between the bacterial body and the surface, and the surface elastic deformation [1]. To confirm this model of regulation of twitching by the surface physical properties, we have then designed a methodology for measuring adhesion forces that integrates microfluidics and optical microscopy. By combining surface colonization studies at low shear rates with orientation and detachment and orientation assays at higher shear rates (Figure 1 (b)), we can assess the relative adhesion strengths of the cell body and the pili and, through appropriate modelling, connect them to the bacteria twitching velocity. Our findings demonstrate that changing the chemical functionalization of the surface permits manipulating this equilibrium and test our theoretical model of bacterial movement.

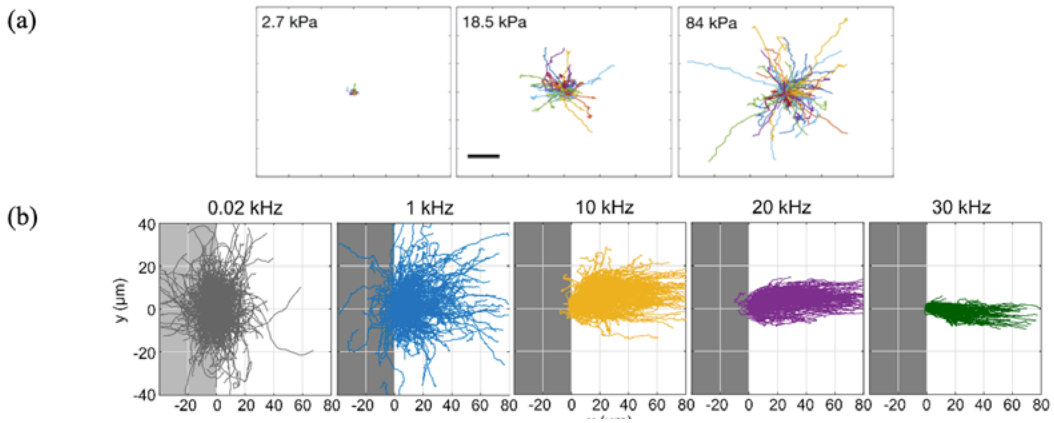

Figure 1 (a) Bacterial tracks on soft (2.7 kPa), intermediate (18.5 kPa) and stiff (84 kPa) hydrogel polyacrylamide. Scale bar: 10 µm [1]. (b) Bacterial tracks for several shear rates show a random movement of bacteria moving at a low shear rate and directional movement at a high shear rate on glass substrates.

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Extending the analogy between intracellular motion in mammalian cells and glassy dynamics

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The cell cytosol is a heterogeneous system responsible for intracellular environment. organization (e.g., organelle distribution). A similarity between the motion in the cell cytosol and that in glassy systems is now emerging for complex eukaryotic cells [1]. To investigate this analogy, we focused on the motion of mitochondria, the cell's energy-supplying organelles, in different cell types (HEK 293, HeLa, and neuronal-like HT-22 cells) [2]. To then broaden the generality of this analogy, we included observations on lysosome motion (in HEK 293 cells) [2]. Fluorescently labelled organelles were followed at 50 ms intervals in the three different cell lines in two dimensions using time-lapse fluorescence microscopy. Single organelle trajectories were determined using the ImageJ (Fiji) plugin TrackMate. The trajectories were later analyzed through various stochastic concepts (mean square displacement, step size distributions, etc) to characterize the motion. The results show that both organelles and in all cell types exhibit a non-Gaussian displacement distribution (figure 1a).

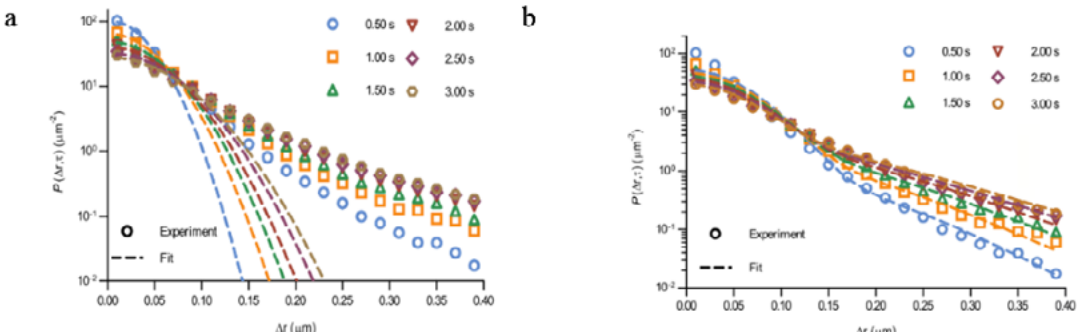

Figure 1: Experimental displacement distributions with a. Fit of a Gaussian distribution to the data b. Fits of a model describing glassy motion to the experimental data. Adapted from [2]

Furthermore, the organelle motion shares multiple features with glassy systems, such as heterogeneous motion, non-ergodic movement and decoupling of persistence and exchange time. To then look deeper into possible mechanisms, preliminary investigations on the influence of different factors, such as ATP levels, cytoskeleton integrity and intracellular crowding were conducted. In conclusion, our observations demonstrate the general validity of glassy motion as a model for motor-protein-driven motion in mammalian cells (figure 1b) and shed light on potential cellular mechanisms at the base of this dynamics.

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VEGF-A differentially influences fibroblast migration and receptor spatiotemporal organization as a function of cell density

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Vascular endothelial growth factor-A (VEGF-A) is a vital growth factor with significant implications for endothelial cells, including its role in angiogenesis induction and cell migration promotion. Throughout the wound-healing process, VEGF-A acts as a chemoattractant, drawing other cell lineages towards the site of injury [1].

In this study, we used optical microscopy to investigate the effect of VEGF-A on human dermal fibroblast migration with a multiscale approach. Experiments performed on cell monolayers and individual cells were complemented with single-molecule imaging of receptors involved in cell adhesion and migration (the integrin α5β1), and signaling (VEGFR-1).

Our results revealed that the VEGF-A treatment led to increased velocity in individual cell migration. In contrast, VEGF-A treatment slowed down the collective migration of fibroblasts. Furthermore, VEGF-A produced a nontrivial effect on the nanoscale dynamics and organization of cell surface receptors, with a dependence on cell density. Notably, we established a correlation between receptor dynamics and alterations in Hippo pathway signaling, a critical regulator of gene transcription linked to proliferation, migration, and contact inhibition of growth [2].

Our study underscores the intricate interplay between VEGF-A signaling, cell-cell interactions, adhesion, and migration, shedding light on the involvement of the Hippo pathway in non-endothelial cell migration regulation. These findings reinforce the importance of understanding the diverse roles of VEGF-A in different physiological and pathological settings.

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Redox-induced spatial and functional differentiation of the peroxiredoxin/thioredoxin system in the cytosol of human cells

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H_2O_2 regulates important physiological processes such as cell proliferation, inflammation, and apoptosis, but its regulatory mechanisms remain unclear. In the cytosol of human cells, its concentrations and dynamics are determined by the peroxiredoxin/thioredoxin system, which confines it to micro-domains. Here we used a reaction-diffusion model that for the first time considers the differential properties of human peroxiredoxins 1 and 2 (Prdx1/2) and the thioredoxin redox cycle to analyze the spatial distribution and dynamics of these components [1]. Results show that the Prdx1 sulfenates and disulfides are more localized than the corresponding Prdx2 forms. The thioredoxin disulfides are also localized. As the H_2O_2 supply rate (vsup) approaches and then surpasses the maximal rate of the thioredoxin/ thioredoxin reductase system (V), these concentration gradients attenuate, and then vanish. At low vsup the Prdx concentration determines the H_2O_2 concentrations and gradient length scale, but as vsup approaches V, the thioredoxin reductase activity gains influence. A differential mobility of peroxiredoxin disulfide dimers vs. reduced decamers enhances the redox polarity of the cytosol: as vsup approaches V, reduced decamers are preferentially retained far from H_2O_2 sources, attenuating the local H_2O_2 buildup. Substantial total protein concentration gradients of both Prdx emerge under these conditions, and the concentration of reduced Prdx1 far from the H_2O_2 sources even increases with vsup. Altogether, the properties of Prdx1/2 and thioredoxin are such that localized H_2O_2 supply induces a redox and functional polarization be- tween source-proximal regions (redox microdomains) that facilitate peroxiredoxin-mediated signaling and distal regions that maximize antioxidant protection.

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Mechanobiology of the secretory pathway: Golgi export responds to external mechanical cues

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Cells interact with their environment through focal adhesions (FAs), molecular platforms bridging the extracellular matrix (ECM) and the cytoplasm. These structures serve as mechanosensors of ECM rigidity and as initiators of different signaling cascades, establishing feedback communications between the ECM and the cell interior to regulate mechanosensing and mechanoresponse. This back-and-forth signaling requires a regulated delivery of mechanosensing components to the plasma membrane (PM), suggesting that the Golgi apparatus is a mechanosensitive organelle. Indeed, recent studies have shown that mechanical cues modulate the levels of components involved in Golgi lipid homeostasis [1] and transport carrier formation [2]. Importantly, the molecular identity of the carriers trafficking cargoes to FAs overlaps with that of CARTS [3], a class of Golgi-derived vesicles that contain the transmembrane protein TGN46. These data let us hypothesize a role for CARTS in delivering mechanosensing components from the Golgi to FAs. Here, by combining a stretching device to mechanically challenge cells and advanced imaging techniques, we reveal that external stimuli modify the rate at which post-Golgi carriers are formed. Interestingly, we show that those vesicles are delivered at or close to FAs. Notably, mechanical cues emanating from physiological processes, such as cell spreading or migration, also affect the production of CARTS, as compared to cells seeded on passive substrates where cell spreading is abrogated. Finally, we have studied the mechanisms governing the communication between the PM and the Golgi. All in all, our results suggest a role of the secretory pathway in mechanotransduction.

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Abstracts: Posters

Session II: Disordered proteins, Liquid-liquid Phase Separation and Aggregation

The Modulatory Effects of CHCHD10 protein in TDP-43's Liquid-Liquid Phase Separation Properties and Aggregation Dynamics

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Amyotrophic Lateral Sclerosis (ALS) presents a significant challenge in neurodegenerative disease research, with its complex etiology only partially understood. Among the proteins implicated in ALS, TDP-43 is well-studied for its role in the ~90% of sporadic cases, known for its propensity for liquid-liquid phase separation (LLPS)[1], aggregation and amyloid formation[2]. Recently, CHCHD10 has emerged as a novel protein involved in the disease[3], suggesting new pathways of pathogenesis. This study aims to explore the interaction between CHCHD10 and TDP-43, focusing on how CHCHD10 influences TDP-43's LLPS dynamics and aggregation behavior, which are critical in ALS development.

Utilizing a combination of Nuclear Magnetic Resonance, Cryo-Electron Microscopy, and in silico modeling, we delved into the structural and biophysical relationships between CHCHD10 and TDP-43. Our investigations aimed to uncover the effects of CHCHD10 mutations on the LLPS and aggregation properties of TDP-43. Preliminary results reveal that mutations in CHCHD10 significantly modulate TDP-43's phase behavior and aggregation, indicating a novel mechanism through which these proteins may drive ALS pathology.

The findings from our research shed light on the complex interplay between CHCHD10 and TDP-43, underscoring the importance of their interaction in ALS pathology. By identifying key mechanisms of protein aggregation and phase separation influenced by CHCHD10, we highlight novel therapeutic targets. This work not only advances our understanding of ALS but also opens new avenues for developing treatments, emphasizing the critical role of protein-protein interactions in the disease's progression.

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Disordered proteins interact with the chemical environment to tune their protective function during drying

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The conformational ensemble and function of intrinsically disordered proteins (IDPs) are sensitive to their solution environment [1,2]. The inherent malleability of disordered proteins combined with the exposure of their residues accounts for this sensitivity [1,2]. One context in which IDPs play important roles that is concomitant with massive changes to the intracellular environment is during desiccation (extreme drying). The ability of organisms to survive desiccation has long been linked to the accumulation of high levels of cosolutes such as trehalose or sucrose as well as the enrichment of IDPs, such as late embryogenesis abundant (LEA) proteins [3,4] or cytoplasmic abundant heat soluble (CAHS) proteins [5,6]. Despite knowing that IDPs play important roles and are co-enriched alongside endogenous, species-specific cosolutes during desiccation, little is known mechanistically about how IDP-cosolute interactions influence desiccation tolerance. Here, we test the notion that the protective function of desiccation-related IDPs is enhanced through conformational changes induced by endogenous cosolutes. We find that desiccation-related IDPs derived from four different organisms spanning two LEA protein families and the CAHS protein family, synergize best with endogenous cosolutes during drying to promote desiccation protection. Yet the structural parameters of protective IDPs do not correlate with synergy for either CAHS or LEA proteins. We further demonstrate that for CAHS, but not LEA proteins, synergy is related to self-assembly and the formation of a gel. Our results demonstrate that functional synergy between IDPs and endogenous cosolutes is a convergent desiccation protection strategy seen among different IDP families and organisms, yet the mechanisms underlying this synergy differ between IDP families. This is important as IDPs are ubiquitous across biology and function in key developmental processes and disease states that are concomitant with large changes in intracellular chemistry. Understanding how disordered proteins interact and evolve with the solution environment will provide insights into these biological mechanisms and phenomena.

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Study of the interaction between an intrinsically disordered region of the Scaffold Protein Jip-2 and Jnk-1 Kinase

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Scaffold proteins, containing primarily intrinsically disordered regions (IDRs), are able to link folded subunits within multidomain proteins, serving as flexible connectors. These, play key roles in assembling signaling, facilitating their localization, and coordinating feedback signals for pathway regulation[1]. JNK Interacting Protein 2 (JIP-2), a scaffold protein in the mitogen-activated protein kinase (MAPK) pathway, warrants exploration for its interaction with c-Jun N-terminal kinase (JNK), crucial in various cell signaling pathways and acknowledged for its interaction with the D-motif of the JIP-1 scaffold protein[2].

This research targets the intrinsically disorder domain of JIP-2, to confirm and understand the interaction with JNK-1, whose mechanistic and regulatory details are still unclear. By using NMR, we aim to study all the IDR of this scaffold protein through the divide-and-conquer approach, since it allows the NMR assignment of the complete region by overlapping the individual assignment of smaller constructs.

This study has focused on the region 100-240 of JIP-2, with the presence of a D-motif. 2D and 3D NMR experiments allowed its complete assignment. Then, the derived chemical shifts were used to determine the secondary structure propensity of this region. NMR titration experiments with JNK-1 allowed us to determine the specific residues of JIP-2 involved in the kinase interaction, where two different regions were identified.

Understanding this interaction is vital to enhance knowledge of key signaling pathways in neurodegenerative and cardiovascular diseases, cancer, and other conditions where these proteins are present or involved.

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Functional reconstitution of bacterial cell division machineries in microfluidics cytomimetic platforms reveals the interplay between biomolecular condensates and the lipid membrane

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Functional reconstitution of molecular machines in microdroplets allows to discern the effect of physiological factors such as macromolecular crowding, microenvironments and membranes in their operation. The encapsulation of different combinations of elements of the E. coli division machinery using microfluidics technology evidenced their structural and functional organization in droplets where the boundary mimics the lipid composition of the intracellular membrane. We could determine the possible role of the membrane in the assembly and localization of biomolecular condensates formed by FtsZ (GTPase key in cell division), SlmA (effector of the nucleoid occlusion system negatively regulating Z ring assembly) and the DNA sequences specifically recognized by the latter [1]. The location of the biomolecular condensates at the membrane seems to be modulated by their composition. Thus, for these heterotypic condensates it is possibly related with the membrane binding of SlmA we identified [2], being maintained in the presence of a positive regulator of the Z ring assembly counteracting the effect of SlmA [3] and basically lost in homotypic FtsZ condensates [4]. Additionally, we could characterize the differential distribution of biomolecular condensates and FtsZ polymers resulting from their nucleotide-dependent switch, both in homogeneous crowding [3] and in intracellular compartmentation models using binary phases [1]. Our results evidence the advantages of using this kind of technological approximations to identify new players within the biomolecular interaction networks and better characterize, in a quantifiable and reproducible manner, their effects.

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Conformational dynamics and liquid-liquid phase separation of the endocytic protein Eps15

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Eps15, one of the early initiators of clathrin-mediated endocytosis, is responsible for locally concentrating downstream components on the membrane surface and it permits dynamic rearrangement of proteins within the budding vesicle. Oligomerization of Eps15 promotes the assembly of Eps15 into liquid-like protein droplets that catalyzes endocytosis. Its N-terminal EH domains are involved in intracellular trafficking and cell signaling. They bind with particularly low affinities and specificity to Asn-Pro-Phe (NPF) motifs, that are present in many endocytic proteins. We investigated the interaction between individual EH domains within Eps15, as well as the full EH-domain (comprising EH1, EH2 and EH3), with the intrinsically disordered region of endocytic protein Dab2, using NMR spectroscopy. We observe a high level of binding promiscuity and specific interaction modes of the three EH-domains, as well as of the full EH-domain with implications for multi-site binding events contributing to clathrin-mediated endocytosis.

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Labile assembly of a tardigrade protein induces biostasis.

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Tardigrades are microscopic animals that survive desiccation by inducing biostasis. To survive drying tardigrades rely on intrinsically disordered CAHS proteins, which also function to prevent perturbations induced by drying in vitro and in heterologous systems [1, 2]. CAHS proteins have been shown to form gels both in vitro and in vivo, which has been speculated to be linked to their protective capacity [3,4]. However, the sequence features and mechanisms underlying gel formation and the necessity of gelation for protection have not been demonstrated. Here we report a mechanism of fibrillization and gelation for CAHS D similar to that of intermediate filament assembly. We show that in vitro, gelation restricts molecular motion, immobilizing and protecting labile material from the harmful effects of drying. In vivo, we observe that CAHS D forms fibrillar networks during osmotic stress. Fibrillar networking of CAHS D improves survival of osmotically shocked cells. We observe two emergent properties associated with fibrillization; i. prevention of cell volume change and ii. reduction of metabolic activity during osmotic shock. We find that there is no significant correlation between maintenance of cell volume and survival, while there is a significant correlation between reduced metabolism and survival. Importantly, CAHS D's fibrillar network formation is reversible and metabolic rates return to control levels after CAHS fibers are resolved. This work provides insights into how tardigrades induce reversible biostasis through the self-assembly of labile CAHS gels [5].

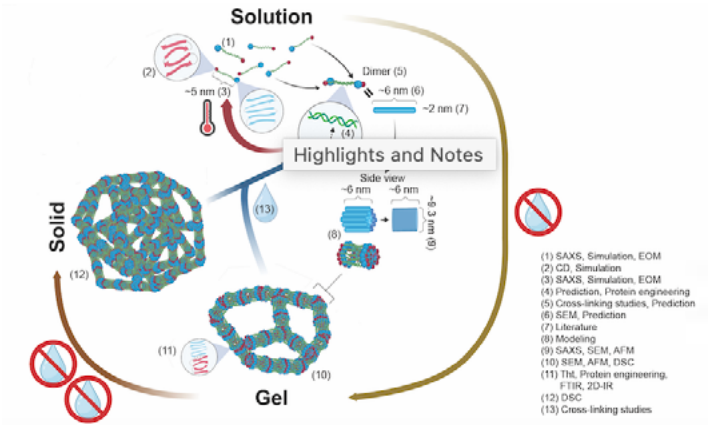

Figure 1: Model of in vitro CAHS D gel formation. Working model of CAHS D dimerization and gel formation. Numerical annotations note where evidence for model elements come from.

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Tau selectively aggregates on membranes and induces membrane damage

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Tau is an amyloid protein implicated in various diseases collectively known as tauopathies, including Alzheimer's disease and frontotemporal dementia. In pathological conditions, tau can disassemble from microtubules and accumulate in the cytosol of neuronal cells, leading to the formation of amyloid fibers. The precise mechanism underlying tau pathogenicity remains elusive.
Previous investigations have highlighted critical aspects: (i) tau's tendency to aggregate into fibers [1] or bind [2] when interacting with negatively charged lipids, (ii) its ability to form structured species upon contact with anionic membranes [3], and (iii) the potential disruption of the membrane upon tau binding [4].
In this study, we examine the disease-associated P301L mutation of the 2N4R isoform of Tau and its effects on phosphatidylcholine (PC) and phosphatidylserine (PS) lipid bilayers mimicking the inner neuronal membrane. To address this, we have combined polarized ATR-FTIR (Fourier-transform infrared in attenuated total reflection), plasmon waveguide resonance (PWR) and atomic force microscopy (AFM) real time imaging to characterize tau-membrane interactions.
Our findings reveal that the Tau protein can induce damage to both PC and PS lipid bilayers, albeit through seemingly distinct mechanisms. Tau exhibits a robust interaction with anionic lipid membranes, resulting in bilayer disruption followed by the accumulation of protein in various aggregates, from flat "carpet"-like patches to fibrillary structures reminiscent of amyloids. In contrast, Tau's interaction with zwitterionic bilayers is influenced by their fluidity. This study deepens our understanding of Tau's multifaceted interactions with lipids, shedding light on its role in tauopathies and the potential mechanisms underlying its membrane-related toxicity.

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Conformational Preferences in the C-terminal Tail of GroEL

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The chapereonin GroEL and its mitochondrial homolog mHsp60 are large barrel-like oligomeric proteins [1]. Chaperonins facilitate folding by isolating nascent chains in their hollow interior and undergoing conformational transitions driven by ATP hydrolysis [1]. Due to their vital importance, the structure of GroEL and its homologs have been extensively studied by X-ray crystallography and CryoEM, e.g. [2,3]. The protein assembly consists of one or two rings each of which contains seven subunits. Each subunit has three folded domains and a twenty-four residue C-terminal extension. Whereas this C-terminal tail has been reported to bind and stimulate the folding of the client protein [4], it appears to be invisible or poorly resolved, which suggests that it is disordered. The objective of this study is to characterize conformational preferences in the C-terminal tails of GroEL and mHsp60 using circular dichroism and nuclear magnetic resonance spectroscopy methods supported by MD simulations. The results suggest that these C-terminal extensions are not wholly disordered but adopt preferred conformations which may have relevance for the chaperonins' activities.

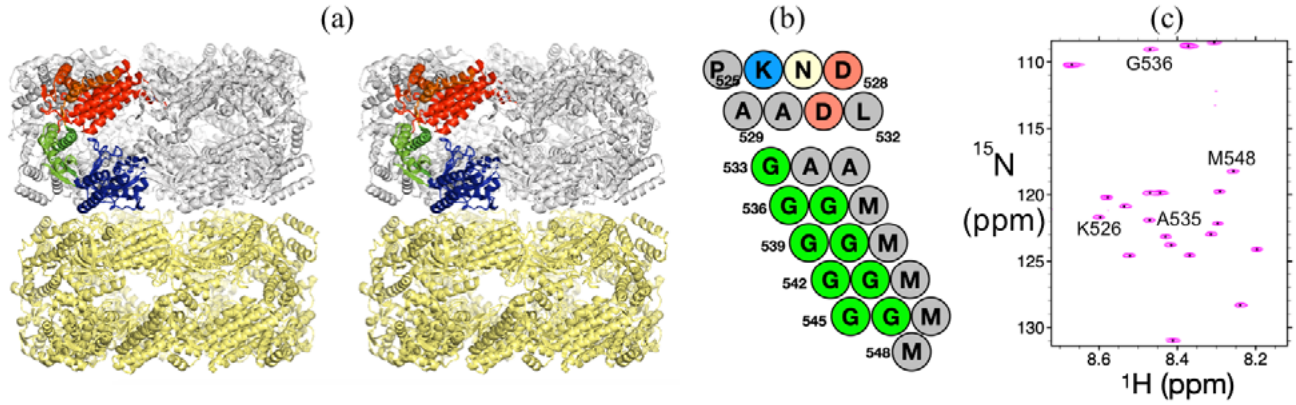

Figure 1 (a) Crosseyed stereo view of GroEL (PDB 2EU1 [2]), showing the seven subunits of the lower barrel in gold and six subunits of the upper barrel in silver. The apical, intermediate and equatorial domains of one subunit are colored red, green and blue, respectively. (b) Residues of the GroEL C-terminal tail, which are invisible to X-ray diffraction or CryoEM. Polar (yellow) cationic (blue), anionic (red), hydrophobic (gray) and glycine (green) residues are colored. (c) 2D ^{1}H-^{15}N HSQC NMR spectrum of the C-terminal tail of GroEL. Some assigned signals are labeled.

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Acknowledgement

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Unraveling the role of crowding-driven biomolecular condensates in bacterial cell division

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Initially described in eukaryotic cells, biomolecular condensates arising from phase separation are becoming increasingly recognized as key players in the organization of intracellular space and the regulation of cell cycle events in bacteria [1]. These dynamic structures appear to be crucial for bacterial fitness and to withstand stresses such as antibiotic treatment. Thus, they represent interesting potential targets to fight antimicrobial resistance. We have identified homotypic and heterotypic biomolecular condensates in cell-like environments assembled by FtsZ, a GTPase that constitutes the scaffold of the cell division ring in most bacteria [2]. These membraneless compartments are strongly promoted by macromolecular crowding, mostly locate at the lipid interface inside microfluidics microdroplets, and interconvert with FtsZ polymers in response to GTP addition/depletion. Combining synthetic biology reconstitution and orthogonal biophysical approaches, we have dissected how selected positive and negative regulators of division ring formation modulate the switch between biomolecular condensates and polymers [3]. Our results show that phase separation may contribute to the mechanisms bacteria have developed for the precise positioning of the FtsZ ring at the cell center, essential for correct division into two equal daughter cells and, hence, for survival.

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Abstracts: Posters

Session III: Protein structure, integrative structural biology, dinamics and function

Intramolecular crosslinking glycation in titin stiffens cardiomyocytes

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Protein glycation is a hallmark of diseases like diabetes and aging. A common feature of these conditions is cardiac tissue stiffening, partly by accumulation of extracellular crosslinking advanced glycation end products (AGEs). However, modification of extracellular proteins fails to explain why cardiomyocytes themselves become stiffer. Here, we demonstrate that methylglyoxal, a major inducer of protein glycation, induces cardiomyocyte stiffening. Based on the observation that titin is glycated in aged myocardium, we have examined the mechanistic link between titin glycation and cardiomyocyte stiffening using high-resolution, single-molecule protein nanomechanics profiling by Atomic Force Spectroscopy. Our single-molecule data show that methylglyoxal induces substantial formation of intramolecular crosslinks in titin domains, which become remarkably stiffer as a result of reduced contour length, and, unexpectedly, enhanced mechanical folding. We speculate that intramolecular crosslinks in intracellular proteins with mechanical roles can contribute to altered mechanical properties of tissues beyond the myocardium.

The impact of the membrane-mimetic micelles on the interaction between α-synuclein and Cu(II)

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α-Synuclein (αS) is a presynaptic protein whose aggregates are considered as a hallmark of Parkinson's disease (PD) [1]. Although its physiological function is still under debate, it is widely accepted that its functions are mediated by its interaction with membranes [2, 3]. The association of αS with phospholipid membranes occurs concomitant to its folding from its monomeric, unfolded state towards an antiparallel amphipathic α-helix [4]. Besides this, copper ions can also bind αS and modify its aggregation propensity. The effect of Cu(II) on the lipid-αS affinity and on the structure of the membrane-bound αS have not yet been studied. This knowledge is relevant to understand the molecular pathogenesis of PD.

Therefore, we have here applied different biophysical techniques to study the affinities between Cu(II) and the micelle-bound αS, as well as the effect of these cation on the structure of micelle-bound αS. Our results show that Cu(II) did not affect the α-helical structure of the micelle-bound αS. However, the micelle-bound αS displays different Cu(II) binding sites than unbound αS. In any case, sodium docecyl sulphate -micelles reduce the stability of the αS complexes with Cu(II). Finally, we have observed that the micelle-bound αS is still able to prevent the Cu(II)-catalysed oxidation of neuronal metabolites (e.g. ascorbic acid) and the formation of reactive oxygen species, thus this binding does not impair its biological function as part of the antioxidant machinery.

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Trigger Factor Chaperone Demonstrates Multifaceted Interactions with 70S Ribosomes

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High-Speed Atomic Force Microscopy (HS-AFM) has emerged as a pivotal instrument in contemporary biological research, providing real-time imaging capabilities with nano escale resolution for the dynamic observation of molecular processes. In this work, HS-AFM is employed to study the interaction between the ribosome 70S and the trigger factor (TF) under physiological conditions. The trigger factor, recognized as a chaperone protein integral to the folding of nascent chains during protein synthesis [1] has conventionally been elucidated in a canonical 1:1 stoichiometry with the ribosome [2]. However, our findings challenge this paradigm by revealing the concurrent binding of multiple trigger factors to the ribosome at different regions.
This surprising finding hints at a complex interaction between the ribosome and the trigger factor, encouraging further investigation to gain a deeper insight into the intricate molecular mechanisms that govern protein synthesis regulation. The potential varied functions of the trigger factor in translating ribosomes, extending beyond its established roles in nascent chain folding, may include contributing to the stability of the ribosome, adding a nuanced dimension to its cellular functions.
This unexpected observation implies a more intricate collaboration between the ribosome and the trigger factor than previously assumed, raising new inquiries into the mechanisms governing protein synthesis control in cells.

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The role of the copper ion in the long-distance charge transport between plastocyanin and photosystem I

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Charge exchange is the essential process that maintains cellular respiration and photosynthesis, moving electrons between redox cofactors through a series of electron transfer (ET) steps. Even without redox cofactors, proteins constrained between electrodes support electron transport (ETp) through the protein matrix, as the charges housed by the redox sites in ET are provided by the electrodes. Still, it is unknown whether protein ETp mechanisms apply to the interprotein medium present under physiological conditions. We simulate the interprotein ET-like situation facing at nanometer proximity electrode-bound protein partners. In this configuration, we measured current at distances as long as 12 nm through the aqueous solution for a redox cognate pair of the respiratory chain [1].
We study interprotein interaction [2] and charge exchange between plant photosystem I (PSI) and its soluble redox partner plastocyanin (Pc) and address the role of the Pc copper center (Figure 1). Using electrochemical scanning tunneling spectroscopy current-distance and blinking measurements, we quantify the spatial span of charge exchange between individual Pc/PSI pairs and ETp through transient Pc/PSI complexes [3]. Lacking the redox center, Pc_{apo} can exchange charge with PSI at longer distances than with the copper ion (Pc_{holo}). Conductance bursts associated with Pc_{apo}/PSI complex formation are higher than in Pc_{holo}/PSI. Thus, copper ions are not required for long-distance ETp but regulate its spatial span and conductance. Findings suggest that the redox center in Pc may not be necessary for charge exchange interprotein ET in aqueous solutions, challenging the canonical view of tight complex binding between redox protein partners.

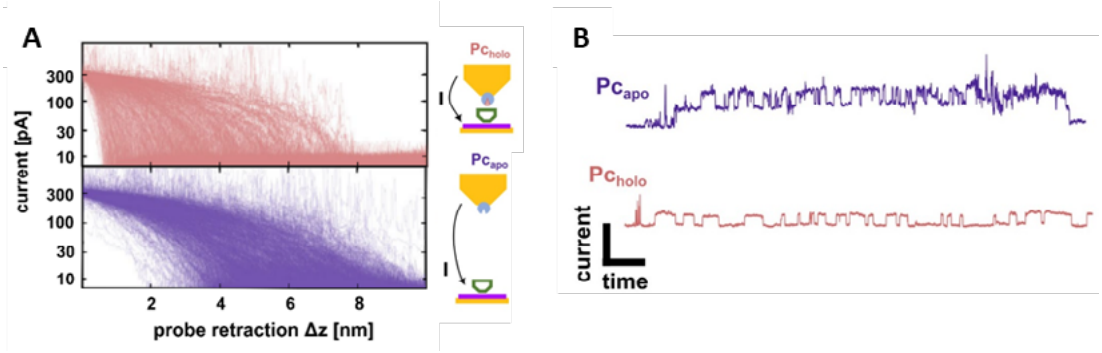

Figure 1. Electrochemical scanning tunneling spectroscopy for Pc_{apo}/PSI and Pc_{holo}/PSI: representative current-distance (A) and blinking (B) records.

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Dual binding of RSV NS1 to both TAD-binding faces of MED25 contributes to RSV virulence and interferon antagonism

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Respiratory syncytial virus (RSV), responsible for pneumonia in infants, elicits a weak innate immune response. This is partially mediated by the non-structural NS1 protein. We and others found that NS1 interacts with the MED25 subunit of the Mediator complex [1,2,3]. We showed that NS1 binds to the MED25-ACID domain, targeted by transcription activators. This suggests that NS1 could modulate host transcription by an unknown mechanism, since NS1 lacks a DNA binding domain. NS1 contains a C-terminal helix α3, involved in modulation of host gene expression [2]. By NMR we showed that α3 peptide binds to the "H2" transactivation domain (TAD)-binding face of MED25-ACID with micromolar affinity. However, the ITC-measured affinity of full-length NS1 was 15 nM. Structural predictions for a heterodimer by AlphaFold2 hinted at dual binding of NS1 to both TAD-binding faces "H1" and "H2" (Figure 1), corroborating binding observed for the NS1α3 deletion mutant to MED25-ACID. To investigate the individual roles of NS1 subdomains, we generated mutant recombinant rRSV, using NS1α3 or NS1 mutated in the core domain at the heterodimer interface. These NS1 mutants displayed significantly weaker interaction with MED25-ACID than WT NS1. Compared to wild-type rRSV, rRSV mutants were attenuated and induced increased production of several interferon-stimulated genes. In parallel, structural analysis of NS1 by NMR revealed significant conformational plasticity that likely drives interactions with partner proteins versus self-assembly. Taken together, our results suggest that a strong interaction with MED25-ACID, achieved by cooperative binding of α3 and the core domain, is correlated to antiviral response antagonism.

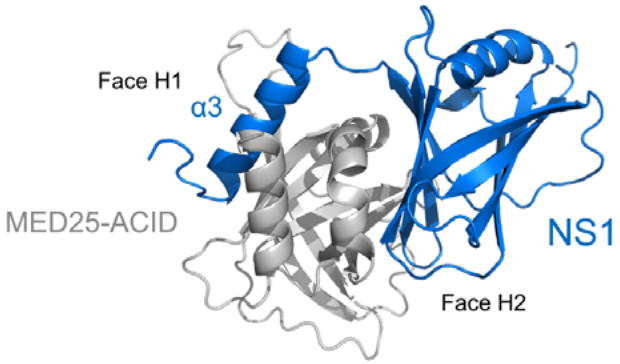

Figure 1 Heterodimer model generated by AlphaFold2 displays dual binding of NS1 to MED25-ACID.

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Molecular bases of the neurodevelopment syndrome CTNNB1

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CTNNB1 is a rare disease that manifests in childhood with developmental and speech delay as well as intellectual disability, amongst other symptoms. It is caused by de novo germinal mutations in the CTNNB1 gene, coding for b-catenin, an elongated protein built of helical repeats. b-catenin is critical in embryogenesis, particularly of the nervous system, by virtue of two main functions: On the one hand, it has a central role as a transcription factor in the Wnt/b-catenin signalling pathway, which regulates in cells the balance between proliferation and differentiation. On the other hand, b-catenin forms part of the adherens junctions between cells, which provide mechanical support in epithelial and neuronal tissues and are critical for synapses formation and plasticity.
Multiple CTNNB1-associated mutations, distributed along the entire length of the gene, have been described. Most prevalent ones generate truncated variants of b-catenin that may be unstable, unable to fold, or display altered functions i.e. probably resulting in a loss of function in cells. The precise consequences of the pathogenic mutations on the functionality of b-catenin are however unknown.
In order to explore the effects of the CTNNB1 mutations on b-catenin folding, stability and interaction with certain ligands, we are expressing recombinant variants corresponding to various patients, and characterizing their conformational stability through circular dichroism spectroscopy. The results will help to understand the genotype / phenotype correlation and the molecular bases of this disease, shedding light on a potential treatment.

Mechanistic insight into the sequential dsDNA cleavage by SpCas12f1

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Miniature CRISPR-Cas12f1 effector complexes have revolutionized genome engineering due to simplified cellular delivery, with only ~400-500 aa and one single active nuclease domain. Nevertheless, the intricate working mechanisms of these systems remain enigmatic. Our investigation focused on Cas12f1 from Syntrophomonas palmitatica, aiming to elucidate the fundamental mechanisms governing its target recognition, R-loop formation and DNA degradation. Single-molecule measurements employing magnetic tweezers allowed us to explore target recognition dynamics. Key findings include the identification of an optimal spacer length of 18 nucleotides, where R-loop stability peaked at approximately 90%, a key factor for efficient DNA cleavage. Spacer lengths deviating from this optimum exhibited significantly reduced stability, emphasizing spacer length's critical role in Cas12f1 function. In addition, analysis of nuclease-dead complexes revealed a multi-step unwinding process, often spanning minutes, characterized by intermediate states. Cleavage measurements unveiled a stepwise process involving unwinding, nicking, and eventual cleavage, accompanied by transient intermediate states and clamping.

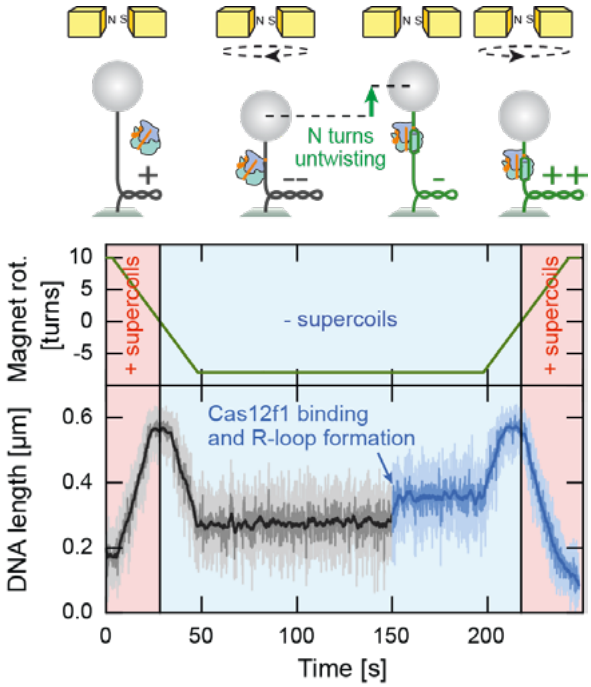

Figure 1. Magnetic tweezers experiment to study R-loop formation by SpCas12f1 under supercoiling. Representative trace of DNA length of a negatively supercoiled molecule, showing a jump as a result of R-loop formation by SpCas12f1.

Furthermore, denaturing PAGE experiments employing fluorescently labelled oligoduplexes provided critical insights into the impact of blocking the (non-) target strand on cleavage rates, uncovering the intricate sequential DNA cutting mechanism and its efficiency. Our research offers a comprehensive understanding of Cas12f1 effector complexes, bridging the gap between molecular structure, target recognition and DNA cleavage.

Structural basis for regulation of a CBASS- CRISPR-Cas defense island by a transmembrane anti-σ factor and its ECF σ partner

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CRISPR-Cas and CBASS are widespread bacterial antiviral systems. How their expression is coordinated with invading signal sensing remains intriguing. We present the first high-resolution structure of a full-length transmembrane anti-σ (DdvA), with a large (~900-residue) periplasmic TPR-CHAT domain, bound via its ~70-residue cytoplasmic domain to its ECF σ (DdvS). Importantly, DdvA-DdvS regulate expression of a locus containing two predicted CBASS and a type III-B CRISPR-Cas system that, we show, endows phage defense. The DdvA periplasmic domain adopts a separase/craspase-type TPR-CHAT fold in a striking arrow-shaped dimeric architecture poised for signal-induced caspase-like activity for potential autocleavage of DdvA that, when lacking its TPR-CHAT domain, undergoes RseP-mediated intramembrane proteolysis. The DdvA cytoplasmic domain, a CHCC-type zinc-bound three-helix bundle, interacts with both DdvS s_2 and s_4 domains undergoing an unprecedented s_4-induced helix extension that, with zinc-binding, is crucial for DdvS trapping and proper control of the CBASS-CRISPR-Cas defense island. Our study thus yields structural-mechanistic insights into membrane anti-σ-ECF σ-regulation of an antiviral response.

Studying photosystem conformation landscape by protein loop engineering

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Natural photosystems show remarkable efficiency in light-harvesting due to specific interactions between chromophores and protein structures. Protein conformational changes play a key role in modifying the chromophore's energy landscape to optimize it for efficient light harvesting 1. These conformational changes are favored by the presence of unstructured protein structures called loops that link different structural motifs, providing flexibility and dynamics to the photosystem. The impact of the loops on shaping the protein conformation and their mechanistic role remains unknown due to the enormous complexity of natural photosystems. In this work, we propose an innovative approach to studying the protein loops by developing easily manipulable biohybrid assemblies as model photosystems. The engineered biohybrids are formed by a de novo α-helix protein and a covalently attached pyrene in the protein pocket (Figure 1A). A close interaction between phenylalanine in the protein pocket and the pyrene triggers a strong bio-chrome coupling in the bio-hybrid, which closes its conformation due to the flexibility of its loop. In this bio-hybrid, we modified the flexibility and length of one loop by adding from 4 to 8 glycines or prolines. Through spectroscopic methods, we demonstrated that long and rigid loops strengthen the bio-chrome coupling between the pyrene and phenylalanine, closing the conformation. In contrast, a short and rigid loop weakens it, leading to an open conformation (Figure 1B). Our study highlights the active role of loop in the protein conformational landscape in photosystems. It shows the potential of our engineered bio-hybrid as a model photosystem to study unknown questions in photosystem mechanisms.

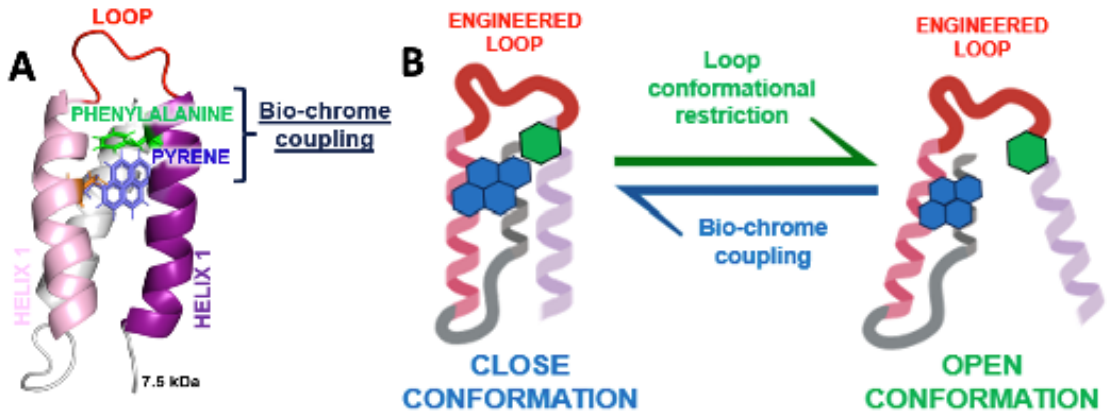

Figure 1: (a) Engineered biohybrid model with a closed conformation. (b) The conformational equilibrium of the model. The conformation depends on the strength of the biochromic coupling and the conformational restriction imposed by the loop.

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Human HELB promotes RAD51 nucleoprotein formation by removing RPA

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Human DNA helicase B, HELB, is a human helicase with diverse roles in DNA repair and replication. Its precise function is still a matter of discussion, with different studies pointing towards both positive and negative regulatory roles in homologous recombination and the recovery from replicative stress. Here, we have combined bulk and single-molecule approaches to characterise the biochemical activities of HELB protein, with a particular focus on its interactions with Replication Protein A (RPA)-single-stranded DNA (ssDNA) and RAD51-ssDNA filaments. HELB efficiently removes RPA from ssDNA [1] in contrast to RAD51, which impairs HELB translocation. The higher binding affinity of RPA for ssDNA compared with RAD51 does not favour the formation of the RAD51 nucleoprotein filaments. However, at low RPA concentrations, the removal of RPA by HELB promotes the loading of RAD51 and the formation of the nucleoprotein filament, observed by an increase of the extension of the ssDNA tether. Experiments with a related bacterial translocase, which also removes RPA from DNA, show that it too facilitates binding of RAD51. However, unlike HELB, it does not promote the formation of an ordered RAD51 nucleoprotein filament. Therefore, our results suggest that a specific interaction between HELB and RPA helps to promote the nucleation and assembly of the RAD51 filament.

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Characterisation of the effect of methylglyoxal-derived advanced glycation end products in titin nanomechanis

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Posttranslational modifications of the sarcomeric protein titin are key to explain the mechanical properties of the myocardium. Diabetes-derived cardiomyopathies, such as diabetic cardiomyopathy, are characterized by an increased passive stiffness of the myocardium, where advanced glycation end products (AGEs) have been shown to be a major cause of the mechanical alteration of the extracellular matrix proteins. These molecules arise from the high levels of glucose and glucose-by products, such as methylglyoxal. Nevertheless, mechanical perturbation of passive stiffness of the cardiomyocytes remains not fully understood. In this work, we focus on the alteration of titin nanomechanics by methylglyoxal-induced AGEs as a cause of the increased passive stiffness of the cardiomyocytes. For this purpose, we make use of single-molecule force spectroscopy techniques such as magnetic tweezers and atomic force microscopy to study the mechanical modulation of titin produced by methylglyoxal-derived modifications under physiological forces. Moreover, we employ these same techniques to explore the optimal conditions and the exact residues involved in these reactions. Additionally, we seek novel methods to revert AGEs formation in titin. Finally, we study the presence and the effect of AGEs in cells treated with methylglyoxal.

The Basque Resource for Electron Microscopy: Towards in situ structural biology

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Cryo-electron microscopy (cryo-EM) allows the visualization of the structure, to atomic resolution in some cases, of biological specimens –macromolecules, organelles, cells, and tissues– in a quasi-physiological environment. The Basque Resource for Electron Microscopy (BREM) is dedicated to unraveling the structural basis of biological processes and human disease pathogenesis through high-resolution cryo-EM. Serving both national and international researchers from academia and industry, BREM offers access to cutting-edge instrumentation and expert guidance.
The elucidation of macromolecular complexes and structures in situ in a cellular context is a long-term aspiration of structural biology. Correlative Light and Electron Microscopy (CLEM) has the potential to achieve this goal, which requires preparing vitrified lamellae of cell regions of interest whose thickness does not exceed 200 nanometers in a field of view of several microns. For the preparation of lamellae BREM has just installed a ThermoFisher Aquilos 2 cryo-FIB-SEM, which also includes an integrated epifluorescence microscope for correlative cryo-microscopy.
BREM also hosts a Krios G4 cryo Transmisssion Electron Microscope (cryo-TEM) paired with Gatan´s BioContinuum Imaging Filter and a K3 direct electron detector device, as well as a phase plate, a Falcon III direct electron detector device and Ceta-D CMOS camera for diffraction data collection. This cryo-TEM is specially designed for all types of data collection, from single protein particles to cryo-electron tomography of lamellae, at the highest possible resolution. In addition, BREM has highly qualified staff to operate the equipment and provide comprehensive user support for three-dimensional structure determination.

Conformational dynamics of influenza A virus ribonucleoprotein complexes during RNA synthesis revealed by HS-AFM

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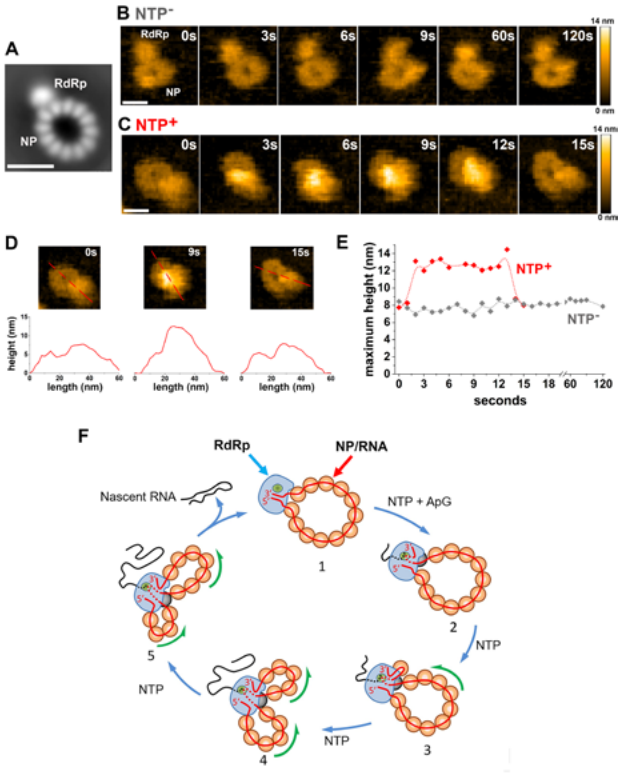
Viral ribonucleoproteins (vRNPs) are the cornerstones of viral proliferation as they form the macromolecular complexes that are responsible for the transcription and replication of most single-stranded RNA viruses. The influenza A virus polymerase catalyzes RNA synthesis within the context of vRNPs where the genomic viral RNA (vRNA) is packaged by the viral nucleoprotein (NP). We used high-speed atomic force microscopy and electron microscopy to study the conformational dynamics of individual IAV recombinant RNPs (rRNPs) during RNA synthesis. The rRNPs present an annular organization that allows for real-time tracking of conformational changes in the NP-vRNA template caused by the advancing polymerase. We demonstrate that the rRNPs undergo a well-defined conformational cycle during RNA synthesis, which can be interpreted in light of previous transcription models. We also provide the first estimates of average RNA synthesis rates in a vRNP and their dependence on nucleotide concentration and stability of the nascent RNA secondary structures. Furthermore, we provide evidence that vRNPs can perform consecutive cycles of RNA synthesis, accounting for their ability to recycle and generate multiple copies of RNA.



HS-AFM captures the conformational cycle of individual rRNPs during RNA synthesis. A) 2D average of EM images of individual rRNPs (scale bar = 20 nm). B) and C) Successive HS-AFM video frames (1 fps) of a single rRNP in the reaction buffer without and with NTPs, respectively (scale bar = 20 nm). D) Height profiles of frames 0, 9 and 15 s from C. E) Variation of the maximum height of individual rRNPs vs. time. F) Schematic diagram of the conformational cycle of rRNPs during RNA synthesis based on HSAFM and EM analysis.

Exploring the structural dynamics of SbcCD: a DNA repair nanomachine

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Maintenance of genome integrity relies on the efficient detection and repair of DNA double-strand breaks (DSBs). The Mre11-Rad50 complex serves as an initial responder to DSBs. The bacterial homologs of the Rad50-ATPase and Mre11-nuclease are known as SbcC and SbcD, respectively. Cryo-EM and atomic force microscopy studies captured various distinct configurations of SbcCD, both free and in complex with DNA [1-3]. However, the dynamics of SbcC coiled-coil arms and how they coordinate the active sites of SbcD dimers during DNA repair remain unclear.
To answer these questions, we directly visualize SbcCD structural dynamics using high-speed atomic force microscopy (HS-AFM) imaging at nanometer spatial and subsecond temporal resolutions. We majorly observed complexes with open coiled-coil arms while persistently connected at the hook region. The addition of ATP triggered the closure of the arms resulting in a ring-shaped configuration via SbcC-ATPase domains. Strikingly, we observed that coiled-coil regions have the ability to intertwine into a compact rod-shaped configuration and can undergo sudden relaxation events, indicating a possible pathway to release elastic energy for triggering large conformational changes. These findings may provide novel insights into the structural dynamics of SbcCD and will be important in understanding the SbcCD interaction with DNA.

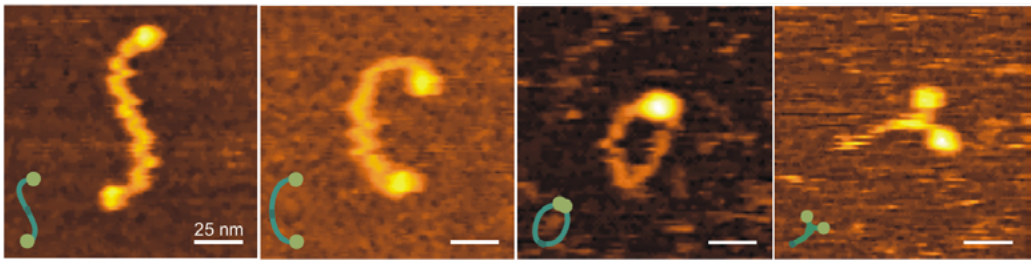

Figure 1. Real-time dynamics of SbcCD observed using HS-AFM imaging on mica surfaces in near physiological conditions.

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Droplet microfluidics as a screening and selection tool for epitope libraries

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Epitopes are small protein regions in antigens that can be recognized by antibodies by noncovalent bounds [1]. Predicting the recognition between antigen-antibody and finding new epitopes is extremely important. Therefore, it is crucial to develop rapid and efficient techniques to screen epitope libraries. One approach is to create a randomized epitope library and use droplet microfluidics to screen it against target antibodies to achieve high affinity binders [2]. Microfluidics is a high-throughput technology for screening and selection of mutant libraries. In microfluidic chips it is possible to generate, direct and manipulate water-in-oil droplets that act as reaction vessels [3]. In this project, we use modified versions of the fluorescence-activated droplet sorting built on an inverted microscope, that allows the selection of the drops for analysis [4]. For the fluorescence readout of the epitope-antibody recognition, the epitope library is fused to the green fluorescent protein. We are exploring two different microfluid approaches. One based on the selection of the drops and fluorescence anisotropy, where the polarization of the fluorophore (GFP-epitope) depends on its binding to a larger molecule (antibody) [5]. The second approach is based on the generation of microbeads conjugated with the target antibody, and non-immobilized GFP-epitope, both encapsulated in drops. An epitope-antibody binding event results in clustering of the fluorescence around the bead, causing a distinct fluorescence peak within the drop. Both microfluids approaches have potential for epitope discovery, showing great promise in the development of new antibody detection technologies and vaccines, and in the study of immune-related diseases.

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Sensing differences on the mechanical properties of encapsulins with a Quartz Crystal Microbalance

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The Quartz Crystal Microbalance with Dissipation monitoring (QCM-D) is a highly sensitive and versatile tool for studying adsorption and mechanical properties of soft materials on surfaces immersed in liquid [1]. Encapsulins are self-assembled icosahedral protein nanocompartments with important roles in numerous biological processes and biotechnological applications. Their mechanical properties are still poorly understood despite being essential to their biological function [3]. This study investigates the mechanical properties of two geometrically identical encapsulin homologs from different bacterial species that inhabit different ecosystems. Specifically, we examine homologs from the hyperthermophilic Thermotoga maritima and the pathogenic Mycobacterium tuberculosis. The QCM-D signal is dominated by the hydrodynamic stress at the vibrating resonator surface [2]. Experimental results are rationalized using the new Virtual-QCM (VQCM) software, which uses the immersed boundary method and elastic network models for flexible structures in liquids. This software allows the user to tune relevant experimental parameters to relate the QCM signal with the analyte properties, facilitating the difficult interpretation of this sensible technique. Our experimental results detect a significant change in the resonant frequency between both encapsulin species while the energy dissipation remains similar. These differences can be explained by a difference in the stiffness of the encapsulins.

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Mechanical properties and dynamics of nsP3 Helical scaffolds assembly

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Chikungunya virus (CHIKV) is a positive single stranded RNA virus that replicates its genome by a viral replication complex (RC) for a successful infection. The RC consists of four non-structural proteins: nsP1, nsP2, nsP3 and nsP4. In this work we studied nsP3 which contains three different domains: alphavirus unique domain (AUD), hypervariable domain (HVD) and macromolecular domain (MD). nsP3 forms helicoidal macrostructures, called helical scaffolds (HSs) that form part of the replication complex of Chikungunya virus and are responsible of assembling macromolecular traps in the cytoplasm. In our work, we used High-Speed Atomic Force Microscopy (HS-AFM) and Transmission Electron Microscopy (TEM) to investigate these macrostructures and its mechanical disassembly process. Our findings indicate that helical scaffolds are brittle structures and present low mechanical stability. Furthermore, when we analyzed nsP3 in its monomeric state, we observed that the first oligomerization step of the nsP3 HSs is by interaction of nsP3 monomers forming triskelios or arcs that form rings. After, nsP3 monomers form lattice aggregation. Finally, we observed that the different domains of the protein play different roles in assembly and stability of nsP3.

Deciphering the Structural Dynamics of the Transcription Factor-DNA Interaction: Insights from EMSA, SAXS, and Limited Proteolysis

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Our research investigates the structural and functional dynamics of a transcription factor in Saccharomyces cerevisiae, known for its role in repressing hypoxic genes under aerobic conditions and aiding in mitochondrial respiration and viability when protein import is impaired. This protein, characterized by an HMG-box domain and extensive disordered regions, is suggested to undergo structural organization upon binding DNA and protein partners. Through Electrophoretic Mobility Shift Assays (EMSA), we confirmed the protein's capacity for DNA binding, and subsequent Small-Angle X-ray Scattering (SAXS) analyses provided insights into conformational changes, revealing a transition to a more compact and globular structure upon DNA interaction. To further explore the protein's structural characteristics, we employed limited proteolysis in the presence and absence of DNA, aiming to isolate stable, globular fragments suitable for crystallization. This approach identified a significant ~15 kDa fragment protected by DNA binding, which was subsequently purified via size-exclusion chromatography. From the purified protein, we will conduct crystallization screenings to identify conditions under which the fragment can crystallize, aiming to facilitate a detailed structural analysis and gain insights into its function and interaction with DNA.

Single-molecule characterization of DNA binding and bridging by human CtIP

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The human DNA repair factor CtIP plays a critical role in initiating the resection of double-stranded DNA breaks for repair through homologous recombination, primarily by binding and bridging DNA molecules [1,2]. However, the structural mechanisms underlying these functions remain unclear. In this work, we focus on single-molecule approaches to characterize the mechanism of DNA binding and bridging by CtIP. Using a magnetic tweezer assay [3], we investigated wild-type and variant CtIP proteins to unveil the DNA binding domains and assess the impact of mutations linked to inherited human diseases. Single-molecule relax-stretch experiments demonstrated CtIP-mediated bridging of distant DNA segments. However, together with bulk binding assays we established that DNA binding alone is insufficient for bridging, requiring the tetramerization through the N-terminal domain. Variant CtIP proteins associated with Seckel and Jawad syndromes exhibit compromised DNA binding and bridging capabilities [4]. Using optical tweezers combined with confocal microscopy we determined that CtIP binds DNA non-specifically and diffuses over thousands of nucleotides [4]. Further experiments to assess CtIP diffusion at different forces and ionic strengths, and binding of fork structures in trans will be presented and discussed.

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Scientific opportunities in MX beamlines at ALBA

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ALBA, a 3-GeV synchrotron radiation facility, which serves global academic and industrial users, has two beamlines dedicated for macromolecular crystallography (MX):BL13-XALOC is a versatile MX beamline, offering photon fluxes up to $2.5{\cdot}10^{12}$ ph/s and an adjustable beam size between 50 × 7 µm2 and 300 × 100 µm2 in the 5.2 – 22 keV energy range [1]. Recent upgrades include an unattended data collection protocol for automatic sample measurements and a high viscosity extrusion injector. This injector has been successfully used to obtain high-quality SSX data [2] but also for time-resolved SSX approaches [3]. Additionally, the detector has been upgraded to a Dectris Pilatus 3 X, reducing acquisition time to ~10 ms, allowing for the study of processes.
BL06-XAIRA, a microfocus beamline (4.0 – 14 keV), set to launch user experiments by year-end, offers a highly stable micrometric X-ray beam (3 × 1 µm2 FWHM at 1 Å wavelength, adjustable down to 1 × 1 µ m2 and up to at least 10 × 10 µm2) [4]. It aims to support various microfocus MX experiments, featuring a vertical single-axis diffractometer for oscillation data collection. Additionally, it will have a dedicated stage for fixed-target SSX experiments. The enclosed end station, enables experiments in cryogenic conditions, room temperature or helium atmosphere (reducing background noise and flux loss). Thanks to a dual CC/ML monochromator, the beamline can deliver fluxes at sample position $>3.10^{13}$ph/s, allowing time-resolved SSX experiments in millisecond ranges, supported by a Dectris Eiger2 XE 9M detector with a 1 kHz maximum frame rate.

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Characterization of Glycine-Rich Polyproline II Helical Bundle Domains and its application in Machine Learning

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Machine learning methods have succeeded brilliantly in predicting the conformation of most well-folded proteins [1]. However, their success is patchy with glycine-rich polyproline II helical bundle domains. These folded domains can be composed of >50% glycine residues which are generally predicted to be disordered but in fact form robust, ordered structures with distinct characteristics [2], such as the formation of networks of chains in a 2D bilayer or honeycomb pattern perpendicular to the helix where every chain interacts with up to 6 neighbors. To better understand these protein domains, we have compared the several glycine-rich polyproline II helical bundle domains from several natural proteins with different functions and diverse evolutionary origins. We find that these domains: 1) are well-packed and have few cavities, 2) preferentially aligned their helices in an anti-parallel configuration which may favor macro-dipole interactions, 3) are tolerant of different connecting loops and flanking sequences and 4) are stabilized by 3D networks of N-H···O=C and Cα-H···O=C hydrogen bonds [3]. These factors set them apart from α-helices and β-sheets, which could help improve machine learning methods and guide the design of novel proteins incorporating glycine-rich PPII helical domains.

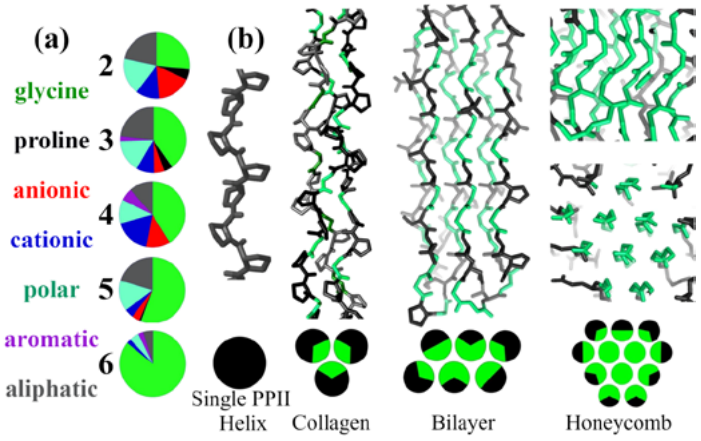

Figure 1 (a) aa composition of studied PPII helical domains by number of neighboring helices. (b) typical patterns formed by PPII helical domains.

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Mechanism of activation of the guanine nucleotide exchange factor C3G: non-canonical roles of Crk adaptor proteins

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C3G (RapGEF1) is a guanine nucleotide exchange factor (GEF) that activates the small GTPase Rap1. The GEF activity of resting C3G is self-repressed by an intramolecular interaction [1]. C3G is activated by stimuli that signal through tyrosine kinases, and activation requires Crk adaptor proteins (CrkL and CrkII). Here, we present a detailed characterization of C3G activation. Binding of Crk proteins to four Pro-rich motifs (P1 to P4) of C3G is essential for the recruitment of C3G to signaling sites and the release of autoinhibition [2]. Sites P1 and P2 are required for translocation to the plasma membrane, while activation requires binding of CrkL/CrkII to sites P3 and P4. C3G is phosphorylated by Src family kinases. In the absence of Crk proteins, phosphorylation of C3G by Src only caused marginal activation. Yet, phosphorylation of C3G resulted in a stronger activation by Crk proteins. In addition, activation of phospho-C3G required lower concentrations of CrkL/CrkII than for unphosphorylated C3G, coupling Crk-binding to C3G activation. Thus, the main role of the Tyr-phosphorylation is to prime C3G for activation by Crk proteins. Unexpectedly, effective activation of phospho-C3G requires a secondary low affinity interaction between at least one of the P3-P4-bound CrkL/CrkII molecules with a phospho-site in C3G via the SH2 domain. The detailed description of the mechanisms of C3G activation paves the way to identify alterations of the C3G-Rap1 pathway in diseases. Our results also highlight the importance of adaptor-independent roles and low-affinity interactions of adaptor proteins in signaling.

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C-Cbl interaction with C3G through the adaptor protein CrkL

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C-Cbl is a proto-oncogenic E3 ubiquitin ligase that targets proteins for degradation. C-Cbl attenuates the signaling of the small GTPase Rap1, by regulating the guanine nucleotide exchange factor C3G, and Crk adaptor proteins (CrkL and CrkII). C3G and c-Cbl interact via CrkL in B and T cells, platelets, and chronic myeloid leukemia cells. Crk proteins contain an SH2 domain that binds to phospho-Tyr sites (pY), such as pY700 and pY774 in c-Cbl [1], a first SH3 domain (SH3N) that binds to Pro-rich motifs in C3G [2,3] and other proteins, and a C-terminal SH3C domain that does not bind to Pro-rich sequences. Despite the functional relevance of the interactions between c-Cbl, CrkL, and C3G, the architecture of C3G-CrkL-c-Cbl complexes remains largely unknown. We have characterized the interactions within these complexes using pull-down assays and purified C3G, CrkL, and c-Cbl. The formation of ternary complexes required the phosphorylation of c-Cbl by kinases of the Src family. C3G has four Pro-rich motifs (P1 to P4) for CrkL binding. Only P1 and P2 sites in C3G were involved in the formation of complexes with CrkL and phospho-c-Cbl. We further analyzed the binding of CrkL to pY700 and pY774 of c-Cbl. We employed isothermal titration calorimetry (ITC) to measure the binding affinity of CrkL to a phosphorylated C-terminal fragment of c-Cbl. CrkL binds to pY700 and pY774 with sub-micromolar affinity. Our results suggest that C3G, CrkL, and c-Cbl form stable complexes that may serve as a link between Rap1 signaling and protein targeted degradation pathways.

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Insights into the complex multi-capsid protein assembly of hypersaline membrane-containing icosahedral phage SSIP-1

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Salisaeta icosahedral phage 1 (SSIP-1) is a halophilic bacteriophage that infects Salisaeta sp. SP9-1 bacteria. It was found in a water sample from ponds at Sedom (Israel) where water of Dead Sea is diluted with water of the Red Sea leading to a hypersaline water (50-100 g/L salt). Life in such extreme environments is challenging and the study halophilic viruses and their hosts can provide us with insights into the living conditions on the "early" Earth or other planets.
Aalto et al. [1] showed that SSIP-1 is a tailless membrane-containing bacteriophage, whose dsDNA genome is enclosed by a lipidic membrane underneath its capsid. SSIP-1 possesses a proteinaceous capsid composed of capsomers displaying a pseudo-hexameric morphology triangulation number T = 49. However, the resolution achieved at the time by cryo-EM (12.5 Å) limited the virion structure analysis.
We have reconstructed the whole virion (~900 Å in diameter) at 3.5 Å resolution by cryo-EM resolving the 3D structure of the four different capsid proteins composing the proteinaceous shell.
Here, we provide insights into the principle governing the assembly mechanism of such large and complex bacteriophage.

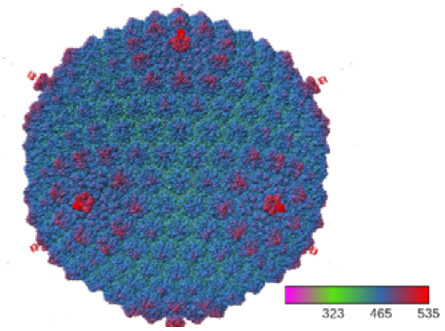

Figure 1. (Left) SSIP-1 full virion capsid density map coloured by radius. (Right) Central section of the virus where internal membrane bilayer and genome organization are clearly visible.

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Structural and functional insights into allosteric communication in the modulation of KirBac1.1 by pH

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Potassium (K^+) channels are specialized proteins which can specifically permeate K^+ ions across the membrane. The study of KirBac1.1, a prokaryotic inward rectifier model channel, which shares a similar sequence and structure with its eukaryotic counterparts (Kir), provides valuable insights into the structure and function of these proteins. Importantly, anionic phospholipids such as PIP_2, cholesterol and pH modulate their function though its complex CTD domain, although the molecular basis of these processes and their inter-dependence are not completely understood. In this study, different functional and structural assays using the intrinsic fluorescence of the protein, have been carried out focusing on the pH effect. Functionally, the channel is open at neutral but closed at acidic pH, being just slightly selective for K^+ vs Na^+. Structurally, we have observed that this pH variation produces conformational changes in different regions of the protein, both in the selectivity filter and the inner gate. In fact, the binding affinity of K^+ and Na^+ to the selectivity filter strongly change when pH is varied, showing the dynamics of this structure and the allosteric communication with the inner gate. Understanding the molecular basis of these processes will allow the discovery of new allosteric sites towards which to design more selective and effective drugs to combat diseases in which ion channels are involved.

The genetic architecture of hydrophobic protein cores through deep sequence space

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Residues that make up the protein core are key at driving folding, defining the fold architecture and conferring thermodynamic stability. How flexible are the combinations of core residues that evolution has shaped in different protein folds? How much do they depend on the rest of the sequence? How complicated is the energetic landscape of the core, and what are the energetic couplings that constrain evolution? Using a deep mutational scanning (DMS) approach coupled to a protein stability readout [1, 2], we systematically measured the stability of thousands of combinatorial core variants in a range of small domains, to explore how many are functional and how diverse they are. We found that the stability landscape ruggedness varies with some proteins tolerating a significant number of multiple core mutations and some not. Using a neural network [3], we fitted thermodynamic models of the folding equilibrium accounting for single mutation effects (DDG) and pairwise energetic couplings (DDDG) that suffice to capture nearly all variance in the data, providing evidence that the genetic architecture of protein cores is remarkably simple and nearly devoid of relevant high-order interactions. In FYN SH3, this holds true for function preservation, since pairwise interactions also suffice to explain changes in binding affinity for its ligand in high-order variants. For all cases we tested, we also found that even single mutations outside the core can improve the tolerance towards and rescue detrimental core mutations. Altogether, this advances our understanding of protein stability and has implications for bioengineering and clinical genomics.

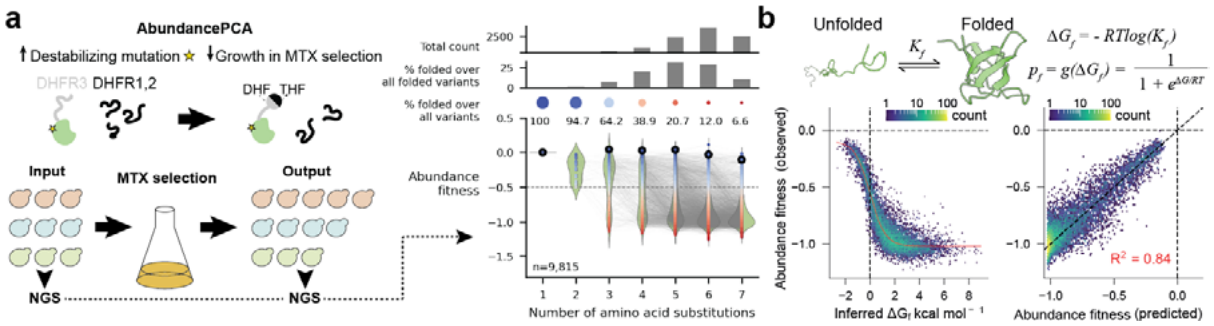

Figure 1 (a) AbundancePCA is a DMS readout we used to measure abundance fitness for thousands of high order core variants (right). (b) A simple second order two-state model of folding equilibrium yields high predictive performance in unseen variants.

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Aphafold model of the protein CP12 from diatom challenged by a combination of Site-Directed Spin Labeling Electron Paramagnetic Resonance, Small angle X-ray Scattering and Molecular Dynamics

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The chloroplastic protein CP12 is a conditionally disordered protein ubiquitous in photosynthetic organisms. In plants, green microalgae and cyanobacteria, it is known to regulate the Calvin cycle upon day-night cycle. It is disordered in reduced conditions (dark), and gains structure in oxidized conditions (light) [1]. We have recently identified a new CP12 from the model diatom Thalassiosira pseudonana that presents atypical structural features. Diatoms are the major component of phytoplankton and are responsible for 20% of the global CO_2 fixation on Earth. Because of their complex evolutionary history, their regulation of the Calvin cycle significantly differs from other photosynthetic organisms and remains enigmatic.
Unlike all the other CP12 studied so far, this diatom CP12 is dimeric, with a predicted central coiled coil and looks folded [2]. However, the 3D-model generated by AlphaFold does not account for our SAXS data.
We have therefore used Site-Directed Spin Labeling combined with Electron Paramagnetic Resonance (SDSL-EPR) spectroscopy to explore its structure and dynamics based on this model. With specific labeling of several mutants of CP12 we analized the local structural dynamics of various regions of the protein. Additionally, using double electron-electron resonance (DEER) experiments, we measured inter-label distance distributions and experimentally demonstrated the antiparallel orientation of the coiled coil. Finally, we refined the Alphafold model by using harmonic restrained all-atom molecular dynamics simulations to generate ensembles of conformations that meet the DEER and SAXS data. This unique combination of biophysical approaches allowed us to decipher the structural and dynamic behaviour of this atypical protein.

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Dissecting the Triatoma virus genome

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Keywords: viral particle αprotein assembly αprotein stability

Triatoma virus (TrV) is a member of the insect virus family Dicistroviridae and consists of a small, non-enveloped capsid that encloses its positive-sense ssRNA genome. TrV virus like particles (VLPs) have been previously obtained after genome release from native virions [1]. The capsid polyprotein has also been obtained in a baculovirus-based expression model [2] and in E.coli.
Each of the structural proteins of the capsid, VP1, VP2 and VP3 can be expressed in bacteria to high levels. Also the 3C protease and the RNA-dependent RNA polymerase from non-structural ORF1 have also been produced and purified. The stability and solubility of the individual proteins under different buffer conditions has been investigated. A variety of protein expressions constructs for used in multiple host systems are being generated. Ultimately, we aim to identify the best viral capsid functionalization loci and determine the size of insertions that do not compromise VLPs assembly and stability. Development of a novel VLP platform suitable for versatile functionalization will pave the way for multiple applications.

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Figure 1. Arrangements of individual subunits (VP1, VP2 and VP3) within capsid pentamers
Ribbon representation of major viral proteins empty capsids (pdb: 3NAP).

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New insights on the effect of methylglyoxal on the conformation, function and aggregation propensity of α-synuclein

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The connection between hyperglycemia and Parkinson's disease (PD) is increasingly recognized, possibly mediated by the glycation of neuronal proteins such as α-synuclein. This intrinsically disordered protein is the main component of the Lewy bodies characteristic of PD1. Despite being involved in many different processes, its main biological function seems to be related to synaptic vesicle trafficking2. Although there is evidence of glycation's involvement, its precise consequences remain unclear, hindering the understanding of the diabetes-PD link.

In this study, we investigate how methylglyoxal, a highly reactive cytoplasmic carbonyl compound3, influences α-synuclein conformation, aggregation, and synaptic vesicle transportation. Our results prove that methylglyoxal induces Lys-Lys crosslinking, forming the methylglyoxal-derived crosslinking Advanced Glycation End Product MOLD. Although α-synuclein's overall conformation remains unchanged, methylglyoxal drastically reduces its propensity to aggregate, inhibiting the formation of soluble oligomers and insoluble fibrils. Furthermore, methylglyoxal disrupts α-synuclein's ability to bind, cluster, and fuse synaptic vesicles, affecting dopamine release, and thus neurotransmission.

In conclusion, our findings shed light on the molecular mechanisms linking hyperglycemia to PD. Methylglyoxal-induced glycation alters α-synuclein behavior and impairs its function in synaptic vesicle dynamics. Understanding these processes could inform targeted therapies to mitigate PD pathology in diabetic individuals. Further exploration of metabolic-neurodegenerative interactions offers promising avenues for PD management in susceptible populations.

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Allosteric modulation of the CXCR4:CXCL12 axis by targeting receptor nanoclustering via the TMV-TMVI domain

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CXCR4 is a widely expressed chemokine receptor crucial for regulating leukocyte trafficking and arrest under various physiological and pathological conditions. Its functions extend to organogenesis, HIV-1 infection, and tumorigenesis. Despite the therapeutic promise of CXCR4 antagonists, only one, plerixafor (AMD3100), targeting the ligand-binding site, has been successfully translated to clinical use. Recent advancements in imaging and biophysical methodologies have deepened our comprehension of the membrane organization and dynamics of CXCR4.
Upon activation by CXCL12, CXCR4 undergoes changes at the membrane, reducing the presence of monomers/dimers while promoting the formation of large immobile nanoclusters critical for accurate cell orientation toward chemoattractant gradients. Activation of CXCR4 involves a specific structural motif characterized by residues on transmembrane helices V (TMV) and VI (TMVI) of the receptor. Leveraging this motif, we conducted in silico molecular modeling followed by in vitro screening of a compound library to identify negative allosteric modulators of CXCR4 that do not disrupt CXCL12 binding.
Through this approach, we discovered AGR1.137, a small compound that disrupts CXCL12-mediated receptor nanoclustering and dynamics. AGR1.137 also impedes cells' ability to sense CXCL12 gradients both in vitro and in vivo, while leaving ligand binding and receptor internalization unaffected.

Insights into the dual activation mechanisms of SNX17 for Retriever recruitment

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Endosomes function as cellular sorting stations, orchestrating the fate of membrane proteins, referred to as cargo, by directing them towards lysosomal degradation or facilitating their recycling to different destinations. The recycling pathway contributes to the regulation of protein distribution within the plasma membrane, which is crucial for maintaining cellular homeostasis. Indeed, disruptions in this process are linked to the pathogenesis of various human diseases, including Alzheimer's and Parkinson's diseases. Retriever, a recently identified multiprotein complex is implicated in the recycling of cargos from endosomes back to the plasma membrane. The cargo adapter protein SNX17 is crucial in this process due to its role in recruiting Retriever to endosomal membranes. We have reconstituted in vitro the recruitment of Retriever to membranes using recombinant proteins and liposomes. Through biophysical assays and site-directed mutagenesis based on AlphaFold2 modeling, we have demonstrated that the C-terminal tail of SNX17 forms an intramolecular autoinhibitory interaction with its cargo binding pocket, preventing its interaction with Retriever. This autoinhibited state is overcome either by SNX17 binding to selective cargo or its association with membranes containing phosphatidylinositol-3-phosphate. The released C-terminal tail of SNX17 engages with the VPS35L-VPS26C interface. The proposed model illustrates two complementary activation mechanisms that could promote the interaction between SNX17 and Retriever at endosomes for cargo recycling.

Abstracts: Posters

Session IV: Computational biophysics

Computational design of Second-Generation Huisguenases based on CTPR protein

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Designed Consensus TetratricoPeptide Repeat (CTPR) proteins [1] showed enzymatic behavior towards 1,3-dipolar reaction between an imine and a ϖ-deficient dipolarophile in THF solution to form unnatural nitroproline esters. Furthermore, the diastereocontrol of the reaction depended on the sequence of the enzyme, yielding the racemic mixture [2]. This work aims to reengineer the CTPR protein to make it enantiomeric selective in the catalysis of the unnatural proline amino acids. To do so, we present a knowledge-based-high-throughput computational protocol that test thousands of sequence variations near the catalytic center. Sequences are generated through Rosetta software by performing docking calculations that allow sequence design with the precursor of the enantiomer of interest (azomethine ylides, referred to as 3a and 3a'). Afterwards, the conformational space of each enantiomer are explored in the presence of each candidate sequence, through a series of docking and molecular dynamics simulations. Given that several mutations were applied to the sequences, the structures of the candidate sequences were validated with AlphaFold2.

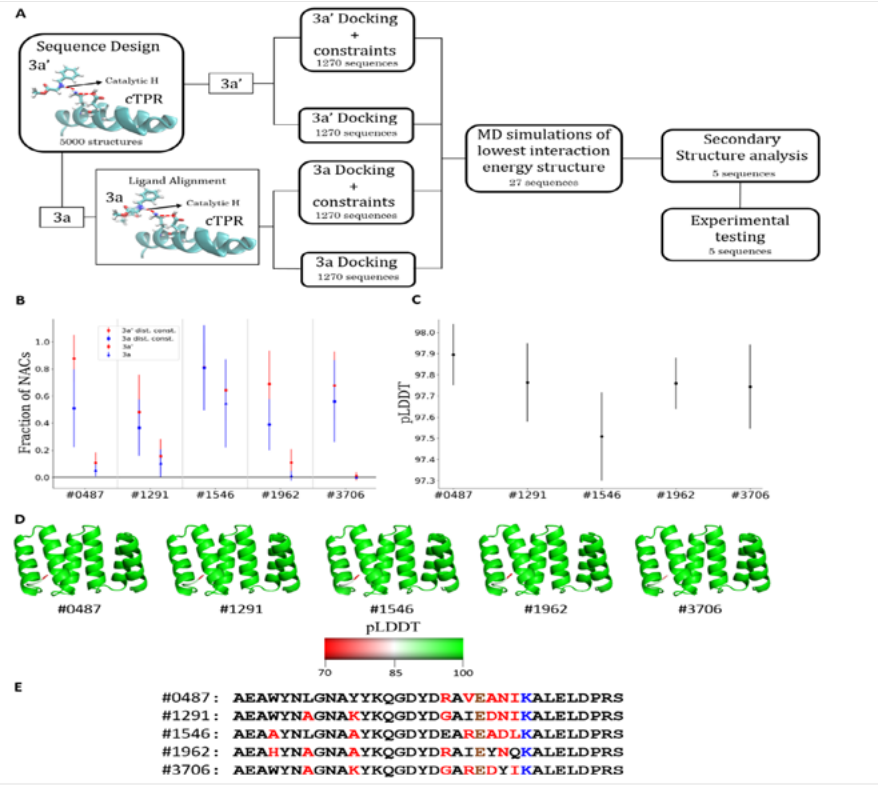

Figure 1: Computational protocol used for reengineering the CTPR proteins.

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Extending the stochastic titration CpHMD method to AMBER14SB for acid-sensing ion channels modeling

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Acid-sensing ion channels (ASICs) are voltage-insensitive, proton-gated cation channels in membranes (Figure 1), widely expressed across the central and peripheral nervous systems, that are involved in physiological processes ranging from nociception to brain ischemia [1]. ASICs are activated by extracellular acidosis and ligands can act as antagonists or agonists for the channel's affinity for protons [2]. To discover ASIC activity modulators, one must understand the pH effects on the protein channel that result in altered cation membrane permeability. Constant-pH Molecular Dynamics (CpHMD) methods are pivotal to describe pH and its effects on the conformational space of biological systems [3]. The stochastic titration CpHMD (st-CpHMD) method has shown excellent performance over the years [3,4]. Until recently, our implementation of this method only supported the GROMOS 54A7 [3] and the CHARMM36m force fields [4], but we have now extended this method to also support AMBER 14SB, a force field particularly suited for studying disordered proteins and membrane channels. Using this method, we have started modulating ASICs activity at different pH values. We will present the preliminary results and some of the caveats that were surpassed to render the st-CpHMD method fully AMBER 14SB compatible.

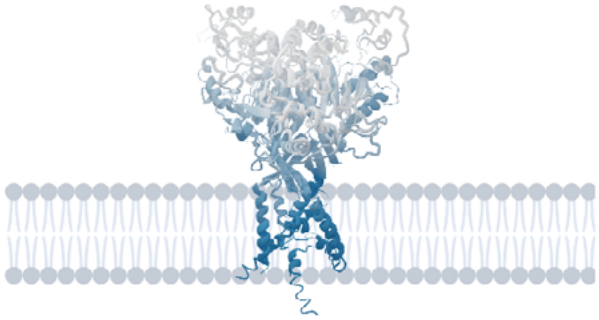

Figure 1. Cartoon representation of the ASIC protein.

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Adsorption-driven deformation and footprints of the RBD proteins in SARS-CoV-2 variants on biological and inanimate surface

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Computational Biophysics has provided a broad and deep understanding of molecular-scale phenomena, during the recent pandemics, the direct transmission of the SARS-CoV-2 has elucidated the mechanisms of infection for the different variants that contributed to the development of vaccines and therapies. However, respiratory viruses, carried through airborne microdroplets, frequently adhere to surfaces, including plastics and metals. Our understanding of the interactions between viruses and materials remains limited, particularly in scenarios involving polarizable surfaces. Here, we investigate the role of receptor-binding domain (RBD) mutations on the adsorption of SARS-CoV-2 to hydrophobic and hydrophilic surfaces employing molecular simulations. To contextualize our findings, we contrast the interactions on inanimate surfaces with those on native-biological interfaces, specifically the ACE2 receptor. Notably, we identify a twofold increase in structural deformations for the protein's receptor binding motif onto the inanimate surfaces, indicative of enhanced shock-absorbing mechanisms [1]. Furthermore, the distribution of amino acids (landing footprints) on the inanimate surface reveals a distinct regional asymmetry relative to the biological interface. Despite the H-bonds formed at the hydrophilic substrate, the simulations consistently show a higher number of contacts and interfacial area with the hydrophobic surface. In contrast, the adsorption of delta and omicron to hydrophilic surfaces was characterized by a distinctive hopping pattern. The novel shock-absorbing mechanisms identified in the virus adsorption on inanimate surfaces could lead to current experimental efforts in the design of virucidal surfaces and sensors.

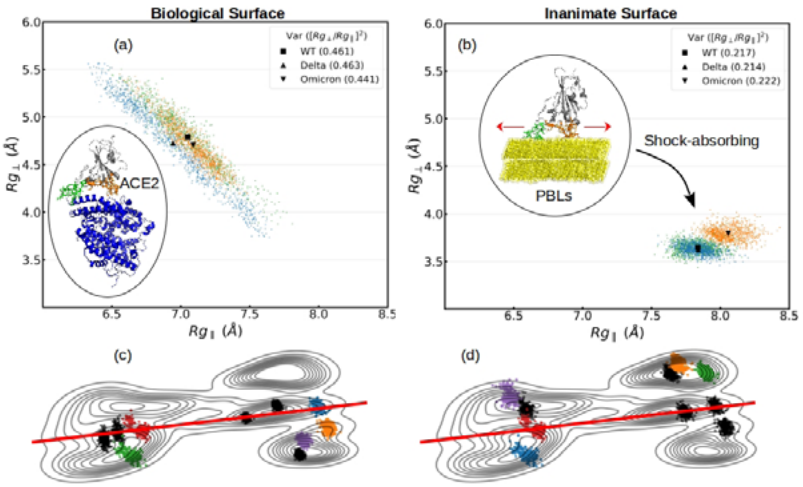

Figure 1: (a,b) Perpendicular versus parallel Radius gyration and (c,d) the landing footprints of the group 1 of the RBM (depicted in green in insets) onto the (a,c)ACE2 and PBL0 (b,d). ratio on the legend [2].

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Engineering artificial fluorescent proteins for biological light-emitting diodes

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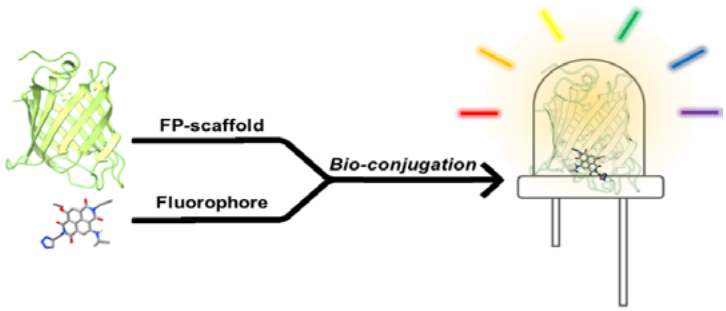
Artificial fluorescent proteins (AFPs) composed of red-emitting fluorophores stabilised within designed protein scaffolds have been developed to address the ever-growing need for efficient and environmentally sustainable lighting. Current white light-emitting diodes (WLEDs) use toxic and difficult-to-source materials, leading to high production costs and significant ecological impact. Protein-based light down-converters for integration into WLEDs provide an attractive eco-friendly alternative to the currently chemical-based components.

Taking inspiration from natural fluorescent proteins, like the UnaG-bilirubin complex, protein scaffolds with large internal cavities/pockets were screened for their capability to accommodate selected large polyaromatic dye molecules with desired photophysical properties, followed by rational scaffold redesign to increase the compatibility with the selected fluorophores. Reengineered through physics, statistics and artificial intelligence-based computational tools, α-barrel-based protein scaffolds were tailored for pocket complementarity with the fluorophore chemical frameworks. This optimization aimed to maximize host-guest interactions and shield the dye from the environment after covalent chemical bioconjugation. Specifically, the project explores the (de novo) design of proteins with central cavities with a focus on achieving reasonable binding affinity, thermodynamic stability and structural integrity. This approach aims to produce a variety of tailored AFPs with distinct spectral characteristics, contributing to the dev



Schematic diagram showing the bio-conjugation strategy for the generation of new AFPs in the development of Bio-LEDs

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Developing a Computational Protocol for Modelling Multidomain and Transmembrane Receptor Dimers: A TLR2-TLR1 Case Study

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Understanding the molecular mechanisms of innate immunity receptor activation and modulation is of paramount importance for designing effective treatments for infectious diseases, autoimmune disorders, and cancer. Particularly, mammalian Toll-like receptors (TLRs) are key players in innate immune response to injuries through the effective recognition of pathogen-associated molecular patterns [1]. TLRs can be identified by the distinctive horse-shoe-like fold of their extracellular domain (ECD), which is followed by a single transmembrane (TM) α-helix and concludes in an intracellular domain (ICD). For activation, TLR2 dimerizes with TLR1 or TLR6 upon recognition of bacterial tri- or di-acylated lipoproteins through the ECD dimer. Based on our previous work [2,3], we here report the development of a computational protocol to tackle the modelling of a multidomain TLR heterodimer in membrane environments. We have combined homology modelling, molecular docking, standard and enhanced-sampling all-atom molecular dynamics simulations, and thermodynamics calculations to explore the dynamic behaviour and conformational changes of TLR domains separately to then provide the most plausible and complete 3D structure of the active TLR2-TLR1 heterodimer in a membrane. In particular we have gathered evidence of differential behaviour of TM domains depending on the membrane composition and we have characterized for the first time the particular structures of juxtamembrane (JM) domains which are critical for signal transduction. We have also done an exhaustive exploration and classification of possible ICD heterodimers that culminated in a clear dimerization mode. All together, these results aid in the understanding of the activation mechanism of TLRs to promote the development of new TLR modulators [4].

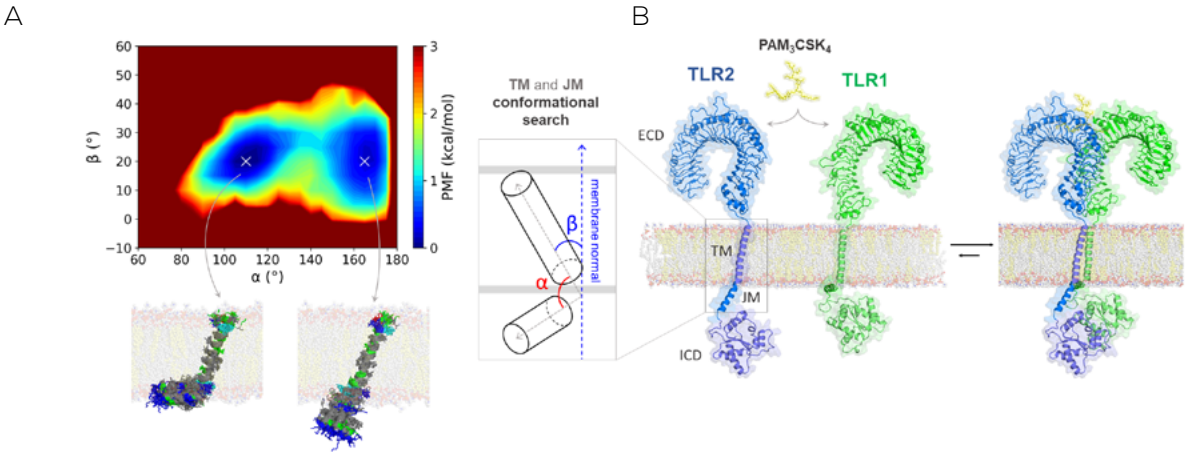

Figure 1 (a) Part of the computational protocol: Exploring the conformational space of TLR2 TM and JM domains through potential of mean force (PMF) calculations. (b) Full-length TLR2-TLR1 heterodimer in membrane model.

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Computational design and validation of ACE2-based inhibitors against SARS-CoV-2

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In the quest to contain the spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), targeting viral entry became a promising therapeutic approach [1,2]. Here, triple helical bundle antiviral proteins (ARMYs) were computationally designed and optimized to target Spike protein receptor-binding domain (RBD) and block it from binding angiotensin-converting enzyme 2 (ACE2). Therefore, the design strategy considered the molecular details of the RBD-ACE2 binding, by incorporating the two adjacent α-helices from ACE2, which accounts for most of the interactions with the RBD, into the backbone scaffolds. Aiming to stabilize the two interacting helices, a short loop and a third helix were inserted into the scaffold. For the interface design, the amino acid sequences were designed to optimize target binding, folding and stability. This resulted in five candidate proteins. Further structural validation was performed using structural prediction methods. Experimental assays were performed and demonstrated that four of the five designed proteins (ARMYs 1-4) effectively bound to the RBD with nanomolar affinity, comparable to the ACE2 affinity for Spike and successfully blocked SARS-CoV-2 infection. Thus, these protein designs showed great potential as viral therapeutics. Further molecular dynamics simulations of the designs were performed in the unbound and the bound state to the RBD to assess their stability and the key interactions between these and the RBD that may have affected the binding affinity, respectively. As expected, ARMY 5 demonstrated a distinct dynamic behavior from the others. The knowledge gained from this work led to a new workflow for anti-SARS-CoV-2 design and validation.

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One- and Two- Electron Reductions in MiniSOG and their Implication in Catalysis

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In the last years, unconventional bioorthogonal catalytic activation of anticancer metal complexes through flavin and flavoprotein photocatalysis has been explored. This reactivity relies on a two-electron redox reaction initiated by photoactivated flavin. Notably, in the context of flavoproteins, our recent findings indicate that site mutagenesis can effectively modulate and enhance catalytic activity in miniSOG.Latest results reveal that specific mutations at positions 50 and 103 alter the redox properties of the flavin, influencing the reactivity of miniSOG.Therefore, our main objective in this work is to characterise the flavin's coordination to the protein in the different mutated variants of miniSOG and analyze how it affects the reactivity of the flavin. By means of computational chemistry tools, we areable to adscribe the experimentally observed modulation of the reactivity to fundamental physicochemical characteristics of the system, particularly (i) the competition between single and double reduction of flavin and (ii) the likelihood of electron transfer from the protein to flavin [1-3].

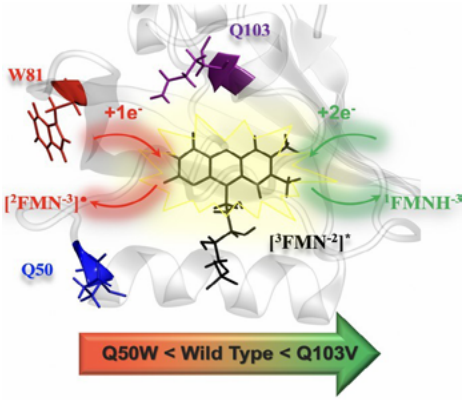

Figure 1. The efficiency of miniSOG as a catalyst for metallorganic complex activation.

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Abstracts: Posters

Session V: Applied biophysics: for industry, biotechnology, health and green transition

Biophysical Study of the Antibiotic Activity-Enhancing Characteristics of Antimicrobial Peptides.

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INTRODUCTION

Antimicrobial peptides (AMPs) are ancient evolutive agents of innate immune system. AMPs are characterized for a small size, amphipathic properties, and broad spectrum of action. The great value of these peptides is that can be designed to target prokaryotic cell membranes and hinder the development of bacterial resistance mechanisms.

MATERIALS AND METHODS

Peptide-lipid interactions were studied with rational designed AMPs (LAM-A, LAM-B, LAM-C, LAM-D, LAM-E) in model membranes by two assays: binding assay and surface plasmon resonance (SPR). Tryptophan fluorescence was read for determinate the presence of peptide linked with membrane. SPR was used to determined which peptide sequence had more affinity to model membranes. Antibiogram and bacteria viables were performed with the most interesting peptides (LAM-A and LAM-D) to determinate their antibiotic potential.

RESULTS

SPR analysis shows that, depending on hydrophilicity and charge, antimicrobial peptides have the highest affinity for model membranes. Furthermore, a specific amino acid has been identified as being related to the affinity difference. On the other hand, binding assays show that AMPs with a combination of hydrophobic and hydrophilic amino acids exhibit a similar presence in lipid vesicles; it seems that some peptides need to oligomerize to increase their affinity. Finally, the two best AMPs were tested on bacteria, demonstrating antibiotic activity.

CONCLUSIONS

1. Hydrophilicity seems to contribute to an increase in the peptide affinity to lipid membranes.
2. The charge of amino acids could favour the formation of oligopeptides.
3. Peptides with high affinity for model membranes exhibit antibiotic activity in bacteria.

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Structural characterisation of protein- nanoparticle complexess relevant for biomedicine

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Nanoparticles (NPs) are widely studied in biomedical applications; Today it is well established that when NPs get in contact with the bloodstream, proteins adhere to their surface, forming the so-called protein corona [1]. This interaction can alter the physicochemical properties of the NPs and affect their internalization, circulation time, final destination and toxicity [3]. In particular, fluorinated NPs are proposed as excellent candidates in the field of 19F MRI based diagnosis [2]. Thus, learning about their fate in the presence of protein corona is crucial for their future development into a clinical tool. We propose to study the changes suffered by the NPs in the presence of HSA (human serum albumin) by SAXS (small angle X-ray scattering) and FCS (fluorescence correlation spectroscopy) since we hypothesize that the binding of the proteins affect the nanoparticle structure.

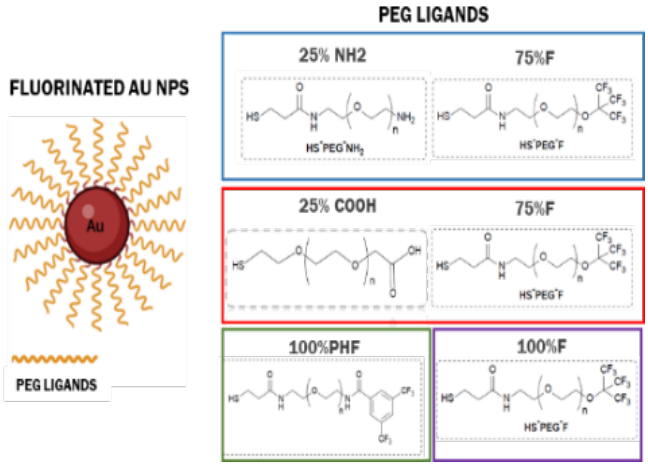

Figure 1. (a) SAXS results of fluorinated Au NPs (NP-F) incubated with increasing concentrations of HSA. (b) FCS results of HSA in combination with NP-F.

We synthesized fluorinated gold (Au)NPs with a core-shell structure, SAXS studies on NPs before and after incubation with increasing concentrations of HSA were performed at P12 Hamburg, DESY (Figure 1a). This set of data confirms the presence of a colloidal disperse system and a slight reduction or no increase in NP diameter upon incubation with HSA. To demonstrate the protein binding to NPs, FCS was performed to follow changes on HSA diffusion coefficient due to their adsorption into NPs surface (Figure 1b). Diffusion coefficient can be directly related with the radius of HSA using Stokes Einstein relation. The data suggests that binding of HSA to Au NPs is minimal but we observe a small compaction of the PEGylated shell on the NPs in the presence of HSA. This could be due to very fast HSA desorption kinetics in a way that the effect observed is a consequence of molecular collisions.

In conclusion, SAXS allows to study changes underwent by nanoparticles due to the presence of HSA, while FCS allow us to observe changes in the diffusion coefficient of fluorescent HSA due to its interaction with NPs. Both measurements are complementary and important to fully understand the effects of protein serum on NPs structure. The NPs used have small Au Cores of only 2 nm in radii, we plan to test a larger core radii to determine if the observed effect is due to the coatings used or the NP size instead.

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Engineering of an anti-CD20 antibody through site-selective chemical modification to improve its therapeutic potential

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Monoclonal antibodies constitute a rapidly expanding class of biologics used in the treatment of cancer, autoimmune, and infectious diseases. Improving their binding affinity is crucial for enhancing potency and minimizing required doses for efficacy. This challenge is particularly pronounced for antibodies targeting poorly accessible external epitopes on integral membrane proteins, such as the tumor-associated antigen CD20, the focal point of the monoclonal antibody Rituximab, approved for treating B cell malignancies. In this study, we employed a synthetic aromatic grafting method to amplify the therapeutic potential of Rituximab. Specifically, we introduced a polycyclic aromatic compound at an antibody position, juxtaposed to the membrane interface upon epitope binding. The selectively modified Rituximab demonstrated an order of magnitude higher potency than the original version in inducing tumor cell death through complement activation. Confocal microscopy revealed that this enhancement was correlated with an improved recognition of CD20 on the surface of B cells. Furthermore, the modified Rituximab maintained a favorable pharmacokinetic profile following intraperitoneal infusion in SCID mice, positioning it as a promising candidate for future treatment development.

Biosensors based on quantum dots

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Disruption of chemical reactions in living organisms can lead to various serious diseases, such as blood coagulation with subsequent thrombosis or disruption of cell metabolism leading to cancer. Due to the unique photophysical properties of quantum dots (QDs), such as: high quantum yield of photoluminescence (PL), long-lived PL lifetimes, high extinction coefficient, broadband PL in the visible spectral range, size quantization effects, ability to chemically modify functional groups on their surfaces, QD-based sensors exhibit high sensitivity in the detection of specific analytes in chemical and biochemical fields [1].

This work focuses on the generation of the sensors based on ternary AgInS2 (AIS) core and AIS/ZnS core/shell QDs for the detection disorders of chemical compounds in living organisms via non-radiative Förster resonance energy transfer mechanism from the energy donor to the energy acceptor (Figure 1) [2]. The absence of the toxic metals in the composition of the AIS QDs, make them more biocompatible and environmentally friendly compared to the commonly used Cd or Pb based QDs, and in the same time AIS QDs retain all the unique photophysical properties of traditional QDs. The stabilization of AIS QDs with L-glutathione tripeptide increases the ability to functionalize the QDs with different biologically active molecules (antibodies or DNA) and also allows to control the number of molecules on the surface of the QDs. Functionalization of the QDs with biologically active molecules will allow to detect and identify the presence of specific molecules or biomarkers in the sample. Due to the high sensitivity of QDs will able to detect low concentrations, which is particularly important in diagnostics where early detection may be a prerequisite for treatment. Thus, QDs-based biosensors could reveal new opportunities for more precise and sensitive diagnostics.

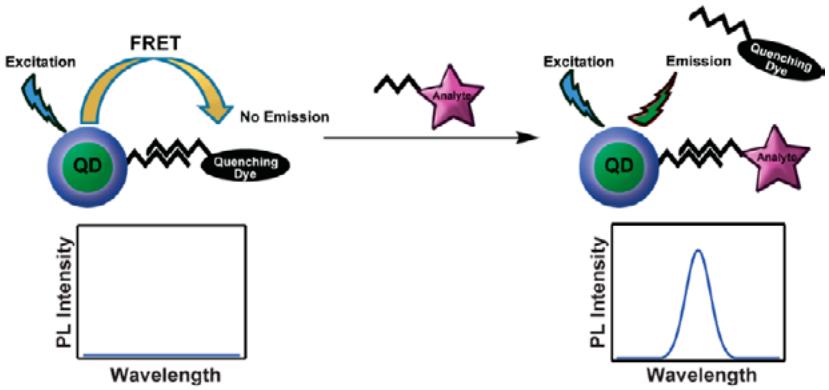

Figure 1 Competitive displacement of a quenching dye by the analyte removes the FRET interaction, resulting in QD emission recovery [3].

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Kinetic and biophysical characterisation of gold-based artificial enzymes

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Artificial metalloenzymes allow to enhance the properties exhibited by natural enzymes or introduce new functionalities altogether. Metalloenzymes are constructed from the incorporation of an abiotic metal co-factor into a protein scaffold. The use of CTPR (Consensus Tetratricopeptide Repeat) proteins as scaffolds has been previously reported due to their robustness, super-stability, high module engineerability, and the capacity to complex metal ions [1]. Gold catalysts allow a rapid functionalisation of substrates with alkyne functionalities, such as alkyne cyclo-isomerisation [2]. The goal of this study is to organise new-to-nature synthetic pathways capable of transforming simple substrates into complex products with high added value. To this aim, we produce artificial metalloenzymes based on CTPR proteins coordinating gold. The chosen transformation is a gold-mediated cyclo-isomerisation of pentinoic acid as a model alkyne substrate, coupled to an enzymatically driven lactone hydrolysis. Consistent gold ratios and gold oxidation states within the protein-gold hybrids have been achieved with reproducible catalytic activity. The artificial metalloenzymes will be assembled into solid materials and the immobilisation for potential uses to obtain the highest activity and selectivity will be optimised [3]. Therefore, this project combines protein engineering with immobilisation techniques towards the aim of producing catalytic biofilms.

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An emerging nanozyme class for à la carte enzymatic-like activities based on protein-metal nanocluster hybrids

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Some of the most challenging chemical reactions found in nature are catalyzed by enzymes that accommodate metal cofactors, which are coordinated by specific amino acids of the protein skeleton. The objective of this study is to create a library of catalytic biomaterials with tailored features which will compete versus natural enzymes in many chemical biology applications. To achieve this, we imitated the nature strategy in which metalloproteins incorporate metal cofactors into their protein structures. We selected repeat proteins, namely, consensus tetratricopeptide repeat (CTPR) protein modules, as scaffold to support the growth and stabilization of a range of metal nanoclusters synthesized in situ via a green synthetic approach.[1] A total of 24 protein-templated nanozymes were synthesized, and their peroxidase-like activity tested versus a wide range of the chromogenic substrates. These nanozymes were found to be more resistant to hydrogen peroxide and had greater thermostability compared to natural peroxidases such as horseradish peroxidase. The protein-templated nanozymes thus represent a highly advantageous alternative to natural enzymes. Finally, we fabricated films of the nanozymes, which could be easily fabricated and reused in numerous catalytic cycles without any significant reduction in their catalytic performance. Moreover, we explored additional enzymatic-activities such as catalase and oxidase, exhibiting the promising potential of these new nanomaterials to replace enzymes that cannot withstand harsh conditions neither perform multiple enzymatic activities simultaneously.[2] We foresee that the development of novel metal-biocatalysts with à la carte catalytic activities through protein engineering and molecular biology tools will have a significant impact of the field of protein-protected metal nanozymes for novel applications.

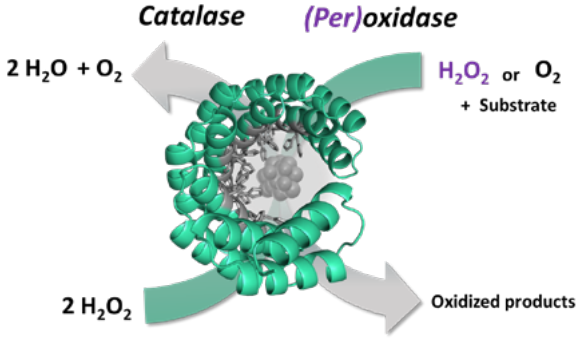

Figure 1. Illustration of engineered metalloproteins mimicking multiple enzymatic reactions.

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Development of a novel antibody/membrane hybrid-based platform for drug delivery.

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Antibody-drug conjugates (ADCs) have become a significant component of cancer therapies, but the emergence of resistance mechanisms poses challenges to their clinical efficacy. The strategy of transporting multiple drugs per antibody is crucial for delivering a potent therapeutic dose to eliminate tumors and reduce resistance, but it often leads to lower solubility and rapid clearance from the bloodstream. To overcome this, we developed an innovative platform, termed "backpacked-antibody" (B-Ab), consisting of an IgG scaffold engineered to incorporate a lipid nanodisc, which resembles a "backpack" for efficient adsorption of multiple drugs. The IgG and the nanodisc scaffolding proteins self-assemble into disc-shaped particles, which specifically recognize tumor cells and efficiently internalize upon receptor binding. Water-insoluble chemotherapeutic agents can be incorporated into the nanodisc lipid bilayer hydrocarbon core, while less hydrophobic drugs can be loaded on the surface through chemical conjugation with lipid head groups. Due to their higher loading capacity, these molecules demonstrate improved efficacy in blocking cell growth compared to conventional ADCs. Additionally, they were well tolerated and exhibit long circulation times in vivo. In contrast to ADCs, their decay over time is independent of the loaded payload. This technology holds the potential to yield biologics with broader therapeutic effects than conventional approaches.

Repeat RNA-binding proteins for splicing modulation

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Transcriptome editing has opened an array of possibilities for new therapeutic strategies, such as the modulation of mRNA splicing using antisense oligonucleotides (ASOs), a therapy already approved for spinal muscular atrophy (SMA) [1]. However, the use of ASOs presents some disadvantages, and is important to explore alternative strategies, such as engineered RNA-binding repeat proteins. RNA-binding repeat proteins, such as the pentatricopeptide (PPR) and the Pumilio and FBF (PUF) families, can selectively bind specific RNA sequences based on an amino acid code [2, 3]. Each repeat recognizes a single RNA base, enabling the design of modular proteins programmed to bind specific sequences with high affinity.
Here, we design a PPR and a PUF protein that specifically bind a sequence in SMN2 mRNA to induce exon 7 inclusion as a therapeutic strategy to correct splicing in SMA [4]. To obtain stable scaffolds as backbone to design RNA-binding proteins, we use a consensus PPR sequence [3], and design a de novo consensus PUF sequence based on natural modules. These newly engineered proteins efficiently modulate SMN2 splicing and are promising tools for transcriptome editing in other diseases.

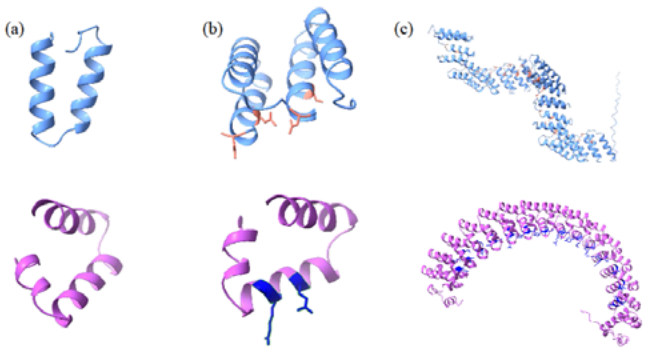

Figure 1 (a) Consensus PPR (blue) and PUF (pink) protein single repeat. (b) RNA binding residues in consensus PPR and PUF repeats. (c) SMN2 mRNA binding PPR and PUF full proteins.

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Arming monoclonal antibodies with cytotoxic peptides for cancer treatment

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Monoclonal antibodies stand out as the fastest-growing class of biologics for human cancer treatment. Nevertheless, limitations such as tissue accessibility, antigen heterogeneity, and the emergence of tumor evasion mechanisms constrain their clinical efficacy. To tackle these challenges, we have developed a new biotherapeutic for the selective and safe delivery of cytotoxic peptides at the tumor site, endowing these molecules with an additional killing mechanism. Specifically, we designed membranolytic peptides with anti-tumor activity and strategically placed them within an immunoglobulin G (IgG) scaffold. To achieve this, we integrated the lytic peptides in frame into flexible linkers, which connected the heavy and light chains of the antibody, and were flanked by matrix metalloproteinase (MMP) cleavage sites. Validating this innovative approach, we demonstrate that, in this format, the peptide remains as an inactive pro-peptide and that, in the presence of metalloproteinase, it is released into its membranolytic active form. This fusion of functionally distinct molecular components holds promising potential for advancing immunotherapy.

Rational design of all-protein-based conductive system

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The new era of wearable devices will be characterized by an increasing presence of components originating from or inspired by biological systems. Platforms for bioelectronics including printable circuits, information processing and energy storage devices require structurally robust bio-compatible materials able to transport charge carriers (either electrons or protons) efficiently.
In this study, focusing on the primary structure, we present the design of new conductive CTPR proteins. Engineered Consensus TetratricoPeptide Repeat proteins (CTPR) can be used for the development of conductive biomaterials owing to their high level of structural stability and inherent biocompatibility. CTPR proteins are composed of sequential 34-amino acid-long helix-loop-helix motifs, with only 8 highly conserved residues responsible for the structure, allowing high sequence variability for protein rational design.[1] Conductivity can be introduced into the CTPR proteins by the addition of charged residues, specifically Glu, to facilitate ion conductivity, aromatic residues, particularly Trp, to enable electron conductivity,[2] or bioconjugation with conductive functionalities like TEMPO using cysteine-maleimide chemistry[3,4]. The possibility to extend the CTPR sequence in tandem up to 20 repeats and the propensity for self-assembly allows the explore the protein length to optimize the transduction of the conductive properties from a molecular level to the macroscopic level, resulting in final well-ordered all-protein materials.

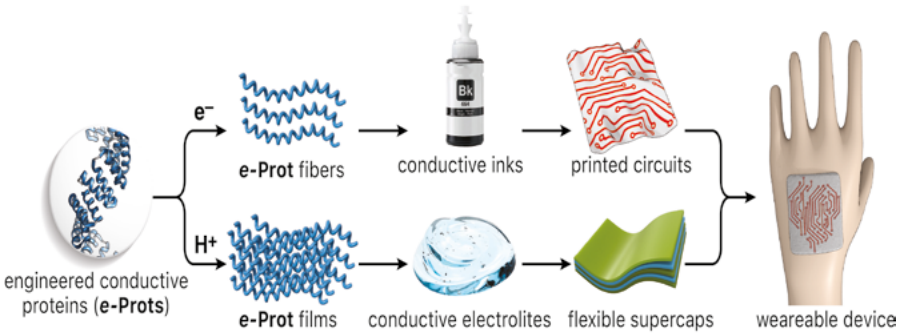

Figure 1. Illustration the e-Prot project roadmap.

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Tailor-made antiviral biopharmaceuticals: From computational design to experimental validation

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The recent COVID-19 pandemic has highlighted the devastating consequences of unpreparedness for such events, not only on public health but also on social and economic levels. As it is not possible to accurately predict which virus(es) will cause the next pandemic(s), it is of utmost importance to find new solutions that allow the effective targeting of a broad range of viruses and have easy and cost-effective discovery, development, production and validation process trajectories.
Within the scope of the BioPlaTTAR project (La Caixa Foundation HR22-00722), we have built an integrated platform for biopharmaceutical development, to quickly and efficiently respond to viral threats in a streamlined pipeline, ranging from rational design to in vivo validation. This pipeline starts from the computational design of a diverse set of protein leads that block targets on the viral surface; then takes these designs, produces them in a high-throughput expression platform, and selects top leads after several rounds of physical-chemical characterization and in vitro and in vivo validation of activity. This pipeline has been validated in three consecutive rounds of design and experimental evaluation leading to the development of several miniproteins that bind to the SARS-CoV-2 receptor binding domain with high affinity (in the low nm range) and were shown to neutralize this virus in high-throughput neutralization assays using ACE2-expressing cells. The combination of artificial intelligence and physics based modelling methods, as well as the close integration of computational and experimental data have been key factors for the consecutive improvement of this platform.

Recruitment of specific lipid interactions driven by bulky aromatics after epitope binding potentiates neutralizing HIV-1 MPER antibody

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Establishment of interactions with envelope lipids is a cardinal feature of broadly neutralizing antibodies (bnAbs) that recognize the membrane-proximal external region (MPER) of HIV. Here, we sought to elucidate the mechanism underlying the improvement of 10E8 bnAb neutralization potency, produced after grafting bulky aromatic compounds at the Fab framework region that accommodates the viral membrane. Binding evaluation using Fab-mVenus fluorescent fusions in quantitative microscopy of single vesicles, demonstrated that conjugation with synthetic aromatics could improve recognition of the membrane-inserted MPER helix, without boosting spontaneous partitioning into bona fide surrogates of the plasma membrane. Lipid-dependence analyses in membranes emulating the lipid composition of the thicker and more rigid viral envelope revealed distinctive roles for cholesterol (Chol) and phosphatidylethanolamine (POPE) in the mechanism of binding potentiation. Accordingly, all-atom simulations carried out on Fab 10E8 and its chemical conjugates, LC.S65C-Lin3 and LC.S65C-Fus4 in complex with MPER-Transmembrane Domain (TMD) helices, supported the preferential interaction with Chol and POPE of the compounds derivatized with Fabs. These small head-group lipids appear to accumulate at the compressed Fab-membrane interface and favor inserted conformations of the FRL3 and CDRL1. Furthermore, preferential interactions with Chol molecules congregated by the compounds appear to help positioning sphingomyelin with its polar head-group inserted into a previously described phospholipid-binding Fab pocket. These evidences support the recruitment of specific viral membrane lipid interactions by bulky aromatics and validate targeted chemical modification as a method to optimize MPER antibodies.

Tuning the Optical Properties of Au Nanoclusters by Designed Proteins

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Gold nanoclusters (AuNCs) exhibit interesting photoluminescent properties that can be endowed with biomolecular recognition and biocompatibility when stabilized with proteins. The interplay between the optical features of AuNCs and the function added by the protein makes them ideal candidates for generating hybrid protein-inorganic nanomaterials. Previous research on protein-stabilized AuNCs has focused on the use of natural proteins to grow AuNCs. However, the exploitation of engineered proteins enables fine-tuning of the photoluminescent assets of AuNCs[1]. In this study, we use rational protein engineering of modular protein scaffolds for capping of non-emissive, non-passivated naked AuNCs, resulting in a fast and easy method for the synthesis of customizable and emissive protein-AuNC nanomaterials[2]. We can tune the photoluminescent properties of the final hybrid module by selecting appropriate coordination residues grafted on the same protein scaffold. We investigated the effects of ligands and coordination bonds using time-resolved photoluminescence and X-ray absorbance spectroscopies, providing insight into the mechanisms behind the emerging properties of these hybrid materials. Furthermore, this versatile strategy opens new avenues for the on-demand synthesis of not only photoluminescent hybrids for a wide spectrum of optical applications, but also catalytic[3], magnetic[4] and electrochemical[5] metal-protein hybrids with applications in biosensing and medical imaging. Finally, we present the application of these protein-metal hybrids tailored for the detection of antibodies involved in infectious diseases by further engineering the modular protein scaffold.

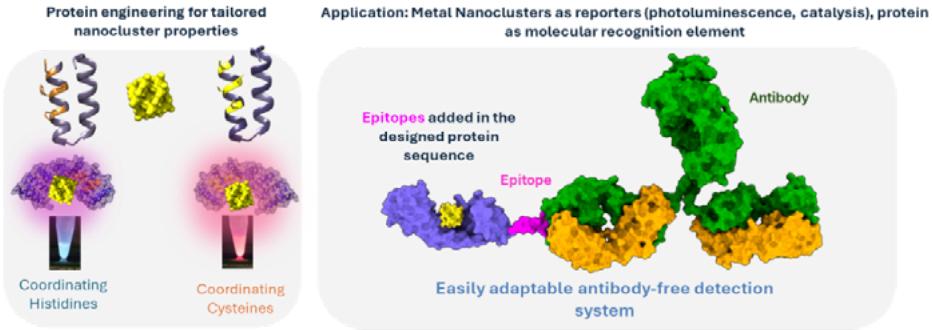

Figure 1: Left: Protein engineering for metal nanocluster formation. Right: application of metallic nanoclusters on engineered protein for antibody detection.

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β-catenin mutants causing CTNNB1 neurodevelopment syndrome.

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CTNNB1 is a rare disease that manifests in childhood with developmental and speech delay as well as intellectual disability, amongst other symptoms. It is caused by de novo germinal mutations in the CTNNB1 gene, coding for *β*-catenin, an elongated protein built of helical repeats. *β*-catenin is critical in embryogenesis, particularly of the nervous system, by virtue of two main functions: On the one hand, it has a central role as a transcription factor in the Wnt/*β*-catenin signalling pathway, which regulates in cells the balance between proliferation and differentiation. On the other hand, *β*-catenin forms part of the adherens junctions between cells, which provide mechanical support in epithelial and neuronal tissues and are critical for synapses formation and plasticity.
Multiple CTNNB1-associated mutations, distributed along the entire length of the gene, have been described. Most prevalent ones generate truncated variants of *β*-catenin that may be unstable, unable to fold, or display altered functions i.e. probably resulting in a loss of function in cells. The precise consequences of the pathogenic mutations on the functionality of *β*-catenin are however unknown.
In order to explore the effects of the CTNNB1 mutations on *β*-catenin folding, stability and interaction with certain ligands, we are expressing recombinant variants corresponding to various patients, and characterizing their conformational stability through circular dichroism spectroscopy. The results will help to understand the genotype / phenotype correlation and the molecular bases of this disease, shedding light on a potential treatment.

Gold-based liposomal nanoparticles for potential use in chemotherapy and photothermal therapy

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In recent years, researchers have become increasingly interested in the development of multifunctional nanoformulations, which combine nanomaterials with different functionalities, for their ability to better address the treatment of complex diseases. Among all these nanomaterials, gold nanoparticles (AuNPs) stand out for their characteristic surface plasmon resonance. As a result of this phenomenon, AuNPs absorb energy at a certain wavelength, which depends on their shape and size, and subsequently transform it into heat through various non-radiative processes, allowing local hyperthermia. This property, together with their excellent biocompatibility have made these nanoparticles excellent candidates for photothermal therapy (PTT). The incorporation of AuNPs into liposomes, considered one of the most successful nanocarriers for drug delivery in chemotherapy, could lead to nanostructures potentially capable of simultaneously providing PTT and chemotherapy [1]. In the present study, we have developed different nanoformulations that integrate both nanomaterials into a single entity and characterized in detail their properties, as well as their suitability to transport and release drugs after irradiation. Specifically, we have fabricated spherical gold nanoparticles and nanoprisms and attached them to thermosensitive liposomes that exhibit phase transitions near 41°C. Drug release experiments carried out with the fluorescent probe carboxyfluorescein as a hydrophilic drug model confirmed the capability of these nanohybrids to release drugs when triggered by NIR laser irradiation, as well as the possibility to modulate the release kinetics by controlling the exposure time and laser power. This work was funded by Project PID2022-138507OB-I00 and PID2020-118485-RB-I00, from Spanish MICIN

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Engineering of Antibodies for Targeted Drug Delivery

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Currently, monoclonal antibodies are key in cancer treatment: their recognition of tumor-associated antigens stimulates a specific immune response that is able to eliminate cancer cells or interferes with signaling pathways crucial for their survival and proliferation. Within the last decades, these antibodies have been engineered to carry cytotoxic drugs to achieve selective delivery, minimizing off-target toxicity and broadening the therapeutic window of chemotherapeutic agents. The antitumor efficacy is often correlated to the quantity of drugs delivered by the antibody. Hence, to enhance the amount of drug delivered to the site of action, in this study we employed a maleimide chemical reaction to conjugate a model antibody, commonly employed in cancer treatment, with a nanostructured lipid carrier (NLC). These nanoparticles are comprised of a solid matrix combined with a liquid lipid (oil) to create a non-crystalline matrix able to transport poorly soluble compounds. Specifically, we demonstrate that the resulting particles are monodisperse (with a PDI of 0.27), have a hydrodynamic radius of 160 nm, and exhibit high specificity for the target epitope. Additionally, these particles exhibited high stability over time. Our current objective is to load these nanoparticles with the antitumoral drug topoisomerase I inhibitor SN-38, and demonstrate the capacity of these particles to efficiently target tumor cells.

A pioneering approach for enhanced therapeutic antibody development

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Bispecific antibodies act as a molecular bridge between two different antigens, providing these molecules with novel functionalities. Encouraged by their potential clinical benefits, bispecific antibodies have entered a golden period of development. However, technical challenges associated with production yields, stability, and purity hinder the broader clinical application of these molecules. In this study, we developed a new strategy to efficiently assemble bispecific antibodies through engineering of the Fc domain. Through an iterative process of protein engineering, expression, and bispecific quantification, we have identified a condition that precludes the formation of byproducts, resulting in the formation of only properly assembled bispecific molecules. We employed several biophysical tools to demonstrate that the resulting molecules are functional, thermostable, and that the 3D organization of their Fc fragment resembles that of conventional antibodies. This strategy has the potential to advance the field of bispecifics towards biologics with more favorable manufacturing properties compared to current strategies.

Designed protein-nanocluster hybrids for specific detection of antibodies

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Nanomaterials have become increasingly relevant in sensing applications due to their remarkable features, such as fluorescence and catalytic properties. In this research project, we focused on the development of a new versatile platform based on protein-nanocluster (NC) hybrids with defined properties for biorecognition and their implementation in bioanalytical systems. For that purpose, consensus tetratricopeptide repeat proteins (CTPR) have been engineered to incorporate both antibody-binding region and nanomaterial-coordination modules within the same protein scaffold, to create a unique sensing probe that enables the recognition and detection of antibodies of interest. The repeat protein scaffolds of CTPR have been proven as versatile templates for the synthesis and stabilization of fluorescent gold [1,2], and catalytic bimetallic gold/platinum NCs [3]. Such modules have been combined with the Hepatitis C virus (HCV) and Z-domain epitopes which were rationally selected as model binder sequences. The resulting protein-nanomaterial hybrids have been successfully employed as a modular sensing tool with optical and catalytic properties that enable the specific and sensitive optical detection of HCV and IgG antibodies in the nanomolar range.

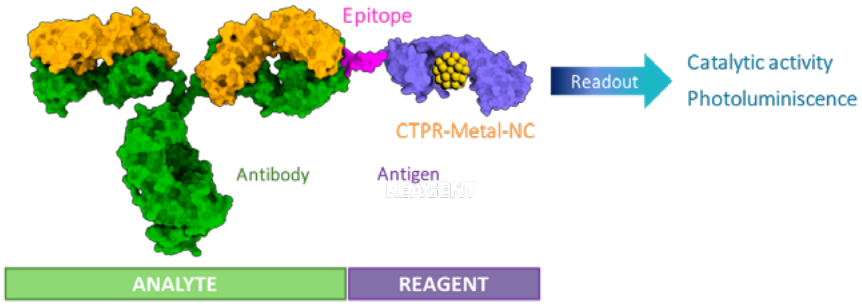

Figure 1. Schematic illustration of the CTPR-NC hybrids containing an epitope region for antibody recognition.

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An Artificial Intelligence Approach to Engineer Super-Stable Nanobodies

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Nanobodies are the smallest antibodies that retain full antigen-binding function. Derived from the heavy-chain-only antibodies from camelid and sharks, their smaller size, monomeric state and fold simplicity render them more suitable for a diverse range of biotechnological applications, such as drug delivery, immunotherapy, biosensors, and imaging [1].
Motivated the biotechnological potential of nanobodies, we aimed to improve their biophysical properties. The improvement of their thermostability and solubility will facilitate their implementation in biotechnological platforms, as well as their durability in the biological and cellular environment. Additionally, it will also facilitate the recombinant production of nanobodies, which is often challenging because of their low expression yield and tendency to aggregate [2].
We have developed and validated experimentally a rational engineering strategy to improve the stability, solubility and production yield of nanobodies. For this purpose, we have used phylogenetic and structural analysis, along with new deep learning–based protein engineering tools [3]. To make the approach generalizable to different nanobodies, we have focused our engineering efforts in the conserved region, thus maintaining binding affinity and specificity intact. We have then expressed and purified the resulting variants, and evaluated experimentally their thermostability, folding reversibility, solubility, expression yield and ligand binding. For all nanobodies tested, we have found remarkable improvements in thermostability and, in most cases, also in solubility and expression, while retaining the original binding affinity.
Overall, our approach offers a rational and generalizable strategy to engineer nanobodies with enhanced biophysical properties, which will facilitate their production and their use in biotechnologies.

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Overcoming the flavin – dehydrogenase inactivation in a chemoenzymatic catalyst through enzymatic immobilization

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In a previous work [1], we assembled a supported (organo)enzymatic system in which an alcohol dehydrogenase catalyzes enzymatic oxidations while a flavin derivative (FMN) performs as a NAD^+ cofactor regeneration system for the enzyme. This was possible because the co-immobilization and confinement of both elements on a solid material increased the electron transfer efficiency from the substrates to the cofactors, making possible the NAD^+ recycling demanded for alcohol oxidation in dark conditions, otherwise impossible using free cofactors and enzymes. Finally, the reusability of the system was tested, showing some limitation due to the enzyme's inactivation and lixiviation from the support after three cycles.
In this work, we investigate the interactions between FMN and the alcohol dehydrogenase to improve the system. Interestingly, we discovered that the flavin was responsible for the enzyme's inactivation and studied the molecular mechanism behind this inactivation. The FMN generates an oxidative microenvironment in the support that leads to the oxidation of the cysteine's residues in the protein's surface, a phenomenon that previously has been reported in different operative conditions [2]. To solve such incompatibility, we propose two different spatial organizations (figure 1) capable of enhancing the performance of the system and its operational reusability.

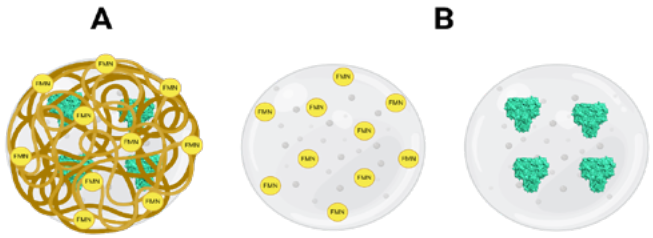

Figure 1 Spatial organizations studied: (A) The protein is separated by a polymer coating from the flavin, externally immobilized into the polymer, (B) the protein and the flavin are physically separated into two different supports.

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Engineering Protein Folding for Real-Time, Continuous Biosensing

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Continuous, real-time biosensors measure molecular analytes without specialized equipment or personnel, allowing fully personalized medicine [1]. Most biosensing technologies, however, require multistep protocols that prevent their use for continuous, real-time measurements. And the few that do, such as the glucose continuous monitor, are not generalizable to other analytes.
In response, we are developing a new sensing technology that is reagent-less, selective enough to work in biological fluids, and generalizable to many different targets. To achieve this, we use nanobodies, single domain antibodies capable of binding with high affinity and specificity many different analytes [2]. Inspired by the biophysics of conformational signaling, we are engineering nanobodies to couple ligand binding to a folding structural change. To design them, we combine artificial intelligence and computational prediction tools, as well as biophysical and structural characterization.

As proof of concept, we have developed a nanobody-based conformational receptor for chorionic gonadotropin hormone, a biomarker of pregnancy, which we have used to develop a fluorescent biosensor. The convenient, real-time measurement of pregnancy hormones will improve the monitoring of pregnancy, enabling early detection of miscarriage in high-risk pregnancies, or the monitoring of embryo implantation during fertility treatments.
Given the great versatility of nanobodies and conformational signaling, our approach is generalizable to many different target molecules. This will facilitate the development of biosensors capable of continuous, real-time measurements, which will greatly improve health monitoring, but it will also enable responsive biomaterials or smart imaging probes.

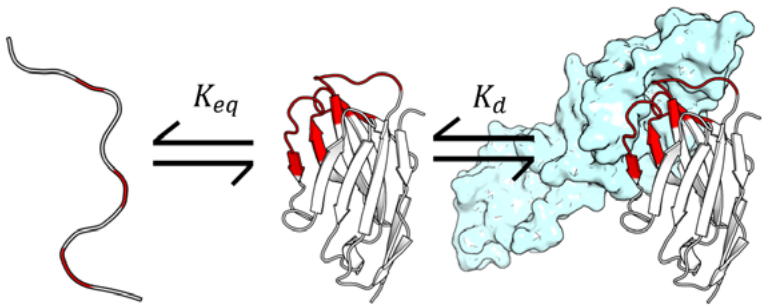

Figure 1. Binding-induced folding of a nanobody.

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Protein-Metal nanocluster hybrids for added value catalytic reactions

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Proteins are versatile biocatalysts that can carry out a wide range of reactions in nature. These biomolecules are building blocks consisting of amino acids or monomers. Molecular biology has made it possible to develop new tools such as protein engineering to modify their intrinsic properties. In this context, repeat proteins, and in particular CTPRs (Consensus Tetratricopeptide Repeat Proteins), have attracted our attention due to their modular nature, high mutability, robustness, and stability [1].
We have previously designed CTPRs to encode specific metal coordination sites, which enables their application in the synthesis of metal nanoclusters [2]. Metal-protein hybrids use proteins as templates to generate controlled nanostructures, endowing these proteins with the characteristic properties of the nanomaterials. It has been reported that these hybrid structures are capable of exhibiting, for example, catalase, peroxidase and oxidase activity [3,4]. Taking advantage of the versatile properties offered by these nanomaterials, the aim of this research is to use these artificial protein-nanocluster hybrids to mimic the catalytic activity of natural enzymes such as methane monooxygenases which oxidize methane to methanol, or alcohol dehydrogenases which oxidize alcohols to aldehydes or ketones, providing these hybrids with interesting properties for chemical reactions with added value for industrial applications.

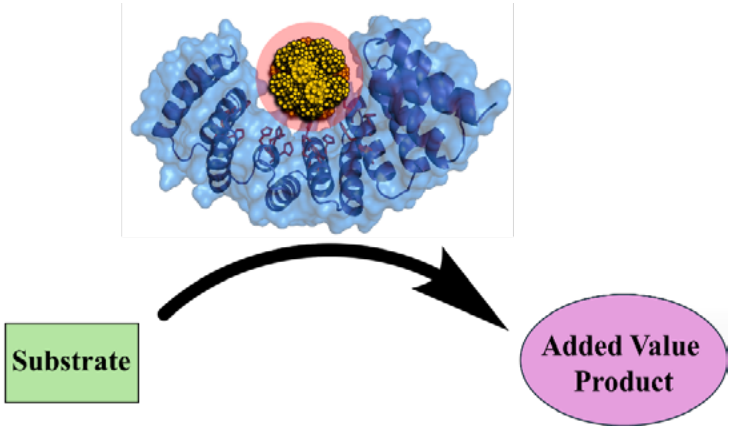

Figure 1 Schematic illustration of catalytic activity exhibited by CTPR stabilized nanoclusters.

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Engineering of new enzymes for the development of biocatalytic systems

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Protein engineering enables the rational design of proteins with particular functionalities. Biocatalysis and nanotechnology stand out as two research areas profoundly influenced by the progress made in protein engineering [1][2]. In prior research, an artificial Huisgen (3+2) cycloaddition-catalysing enzyme based on the Consensus Tetratricopeptide Repeat protein (CTPR) was engineered, able to catalyse 3+2 cycloadditions. Depending on protein designs, the cycloaddition reaction was achieved with varying degrees of diastereoselectivity [3]. The principal aim of this research is to develop a new generation CTPR-based biocatalysts with enhanced diastereoselectivity product control. These protein-based catalysts will be computationally engineered to position substrates in predetermined orientation to maximise the desired stereochemistry in the cycloaddition products. This is anticipated to enable the controlled synthesis of cycloadducts with unprecedented precision and selectivity, allowing the production of materials not accessible through conventional chemical synthesis, or by naturally occurring enzymes.

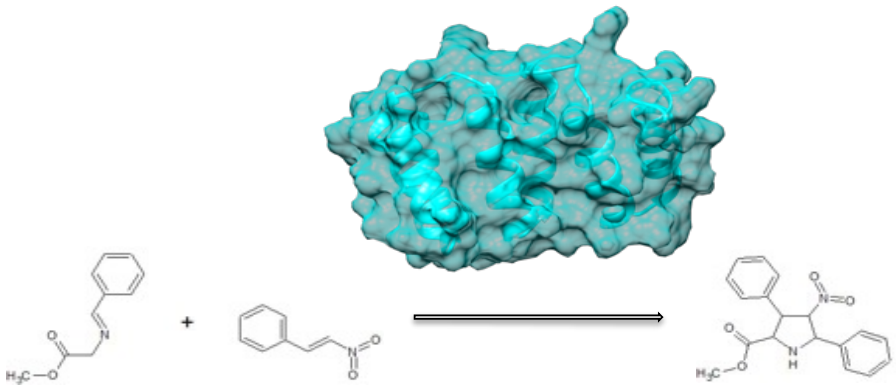

Figure 1. CTPR-based biocatalysis. Computational approach to rationally modify the CTPR sequence to acquire a specific diastereoselectivity and efficiency of the catalyzed Huisgen (3+2) cycloaddition.

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Engineering protein tools for combination therapies

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The use of combination therapies to overcome some of the main limitations for cancer treatment, such as the acquisition of resistances, has emerged as a promising alternative in the fight against this disease. Consensus tetratricopeptide repeat protein (CTPR) is an engineered protein with great stability and robustness that also allows plenty of freedom for the addition of functional mutations, making it an excellent scaffold for the combination of different therapeutic elements in one platform [1]"ISSN":"15204898","abstract":"ConspectusThe last decades have witnessed unprecedented scientific breakthroughs in all the fields of knowledge, from basic sciences to translational research, resulting in the drastic improvement of the lifespan and overall quality of life. However, despite these great advances, the treatment and diagnosis of some diseases remain a challenge. Inspired by nature, scientists have been exploring biomolecules and their derivatives as novel therapeutic/diagnostic agents. Among biomolecules, proteins raise much interest due to their high versatility, biocompatibility, and biodegradability.Protein binders (binders. Protein binding domains that recognize target proteins in tumours can be added to the CTPR for biological inhibition therapies [2]. Moreover, the development of hybrids of these proteins with metal nanoclusters (NCs) aims to achieve a synergistic effect, as the protein would offer colloidal stability and protection to the NCs, while these would offer photothermal properties and specific catalytic activities [3, 4]the goal is to fabricate robust and highly efficient peroxidase-like nanozymes that can ultimately be assembled into films for their easy reuse in catalytic cycles. Nanozymes are designed by mimicking the strategy adopted by metalloproteins to accommodate metal cofactors within their protein structure. The engineered consensus tetratricopeptide repeat (CTPR.

Photothermal therapy (PTT) consists on the induction of cell death through hyperthermia caused by the NCs when irradiated in the near infrared region (NIR). However, this can lead to an increase in the production of reactive oxygen species (ROS) by the cells, which may damage healthy tissue around the treated area.

Here we present the design of multifunctional CTPR-nanomaterial hybrids based on Au:Pt NCs with photothermal properties and specific catalytic activities, in particular catalase activity. We demonstrate their ability to induce cell death in tumor cell lines. Moreover, we evaluate the potential of the catalytic activity of the NCs to reduce ROS produced by the cells, demonstrating their beneficial antioxidant properties for effective and safe therapeutic applications.

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IL-PP4, a dual-action peptide for wound healing: biophysical characterization

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Antimicrobial resistance is a spiralling worldwide concern, as ineffective treatment of infections may lead to serious or even life-threatening conditions. Alternatives to conventional antibiotics have been explored to fight this problem, e.g. antimicrobial peptides [1]. Recently, Gomes et al. designed peptide-based constructs, aiming at their application for the treatment of infected wounds, providing antimicrobial action and faster healing. Peptide IL-PP4 combines an antibacterial ionic-liquid (IL) with the collagen-boosting peptide (PP4, with amino acid sequence KTTKS), exhibiting in vitro antibacterial, antifungal, and collagenesisinducing activity [2]. In this work, we aim at understanding the biophysical profile and the mechanism of action (MOA) of ILPP4 against Gram(+) and Gram(-) bacteria, using POPC:POPG (1:1) and total extract of Escherichia coli liposomes as bacterial mimetic model systems. Peptidebacterial membrane interaction was studied through the determination of the partition constant by spectrofluorimetry, and by dynamic light scattering and confocal laser scanning microscopy experiments, to understand possible aggregation effects in lipid vesicles [3]. Atomic Force Microscopy was further used to elucidate the MOA of the peptide against Staphylococcus aureus ATCC 29213 and E. coli ATCC 25922. Biophysical and microscopical results revealed that ILPP4 has a different MOA in Gram(+) and Gram(-) bacteria. In E. coli, IL-PP4 induces aggregation and disaggregation of lipid vesicles, establishing electrostatic interactions and adsorption on membrane surface, and subsequent penetration, possibly acting by the "carpet model". In S. aureus no aggregation effects were observed, and the results point out for a "toroidal-pore model".

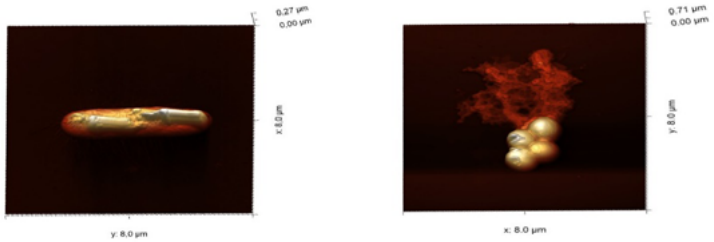

Figure 1 Representative AFM images of the antibacterial effect of peptide IL-PP4 on E. coli ATCC 25922 (left) and S. aureus ATCC 29213 (right).

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Abstracts: Posters

Session VI: Biophysics of Nucleic Acids

Uncovering the Mechanical Properties and Folding of Single-Stranded RNA using Optical Tweezers: A Study on Influenza A Viral ssRNA Genomes

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The mechanical characteristics of single-stranded RNA (ssRNA) play a pivotal role in shaping its structure, and therefore, its function. While the elastic behavior of other nucleic acids like ssDNA and dsDNA has been extensively explored, the determinants governing the folding of naturally occurring ssRNA molecules remain elusive. To bridge this knowledge gap, we utilized optical tweezers to mechanically manipulate individual ssRNA molecules. As a model of biologically active ssRNA we employed individual segments from Influenza A genome. We measured their average extensions under varying forces and ionic compositions. We have extracted information about the basic parameters that define the mechanical properties of ssRNA. Furthermore, we proposed a theoretical model that elucidates the interplay between base pairing, base stacking and electrostatic interactions in ssRNA folding. Our findings pave the way for a deeper understanding of the problem of RNA folding.

Characterization of SLA RNA promoter from Dengue virus and its interaction with the viral non-structural NS5 protein

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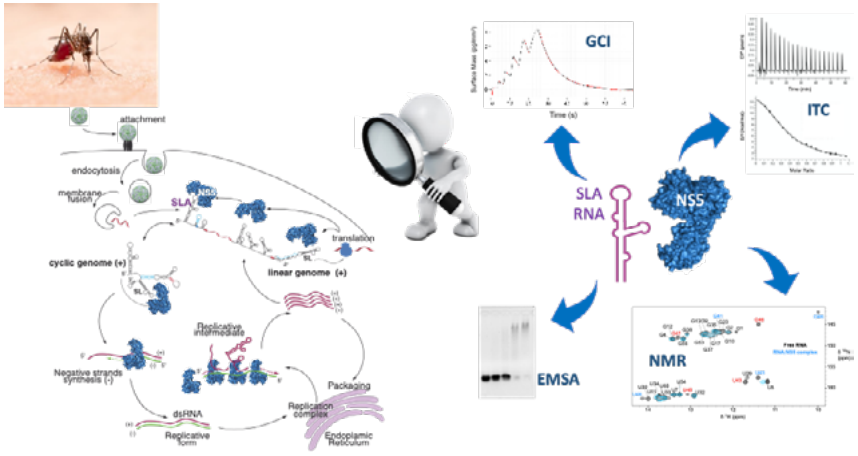
The Dengue virus (DENV) is the most significant arthropod-borne viral pathogen in humans with 400 million infections annually. DENV comprises four distinct serotypes (DENV-1 to -4) which complicates vaccine development [1]. Any of the four serotypes can cause clinical illness but with distinctive infection dynamics. Variations in sequences identified within the four genomes induce structural differences in crucial RNA motifs that were suggested to be correlated to the degree of pathogenicity among DENV-1 to -4 [2,3]. In particular, the RNA Stem-loop A (SLA) at the 5'-end of the genome, acts as a key regulator of the viral replication cycle by interacting with the viral NS5 polymerase to initiate the minus-strand viral RNA synthesis and later to methylate and cap the synthesized RNA [4-6]. The molecular details of this interaction remain not fully described. Here, we report the solution secondary structures of SLA from DENV-1 to -4. Our results highlight that the four SLA exhibit structural and dynamic differences. Secondly, to determine whether SLA RNA contains serotype-specific determinants for the recognition by the viral NS5 protein, we investigated interactions between SLA from DENV -1 to -4 and DENV2 NS5 using combined biophysical approaches. Our results show that NS5 from DENV2 is able to bind SLA from other serotypes, but that other viral or host factors may be necessary to stabilize the complex and promote the catalytically active state of the NS5. By contrast, we show that a serotype-specific binding is driven by specific interactions involving conformational changes within the SLA RNA.



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Development of novel vivo-like DNA nanotransducers for the analysis of conformational changes upon binding to target proteins

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Understanding the link between the conformational changes and the function of biomolecules is fundamental to address the challenging task of unravelling the mechanism of complex biological processes. In the past years there have been a noticeable progress on techniques to study the complex molecular conformations [1-2]; however, resolving the dynamics and the conformational changes occurring upon binding of such biomolecules are still a challenge. Biological molecular machines like DNA can modify their conformation to interact with different biomolecules, being able to act as a DNA nanotransducer (DNA-NT). In this work we aim to build a DNA-NT that presents two functions: (i) capacity to detect and bind specific proteins and (ii) ability to produce a measurable conformational change upon binding, allowing the analysis of molecular dynamics as they occur in-vivo biological processes.
The general control nonderepressible 4 (GCN4) and α-synuclein have been selected as target proteins to be used as a model of diagnostic biomarkers to validate the technology for the transcription factor and neurodegenerative disorders, respectively. Protein-binding oligonucleotides sequences and aptamers have been selected from the literature and they have been inserted in a ~3 kbp plasmid (pUC19) to build the DNA-NT. The ability of the protein-binding DNA construct to detect both target proteins has been confirmed by Electrophoretic Mobility Shift Assay (EMSA) and the conformational changes produced in the DNA-NT upon binding will be explored by using advanced spectroscopic techniques in combination with bioinformatics approaches to predict 3D structures of conformational states.

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How temperature impacts the activity of the mitochondrial replisome, a single-molecule approach

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Mitochondria are eukaryotic organelles that are essential for energy generation and key to diverse cellular processes including apoptosis, innate immunity, and metabolism. Mitochondria harbour their own DNA (mtDNA), which is replicated by a protein machinery (replisome) comprised by three core factors: the DNA polymerase holoenzyme (Polg), the Twinkle helicase, and the mitochondrial single-stranded DNA-binding protein (mtSSB). Malfunction of any of these three proteins results in defects in mtDNA replication and mitochondrial activity, which in turn are linked to devastating mitochondrial diseases and other prominent disorders such as Parkinson's and Alzheimer's disease, autism spectrum disorders, diabetes, and various cancer types [1].
mtDNA replication takes place inside the inner membrane of the mitochondria, a compartment that was thought to stay at the same temperature as the rest of the cell. However, recent evidence based on thermoresponsive fluorescent probes has shaken this paradigm, with some authors observing temperature differentials higher than 10°C between mitochondria and cytosol [2]. While this is still a contentious notion under discussion, we aimed to shed some light into the effects of increasing temperature on the real-time kinetics of the mitochondrial replisome operation.
Here, we used optical tweezers with a temperature controller [3] to study at the single-molecule level the real-time kinetics of DNA replication activity of the DNA polymerase Polg in isolation and in combination with other replisome proteins. Preliminary results already shown the strong effect of increasing temperatures on the activities of these proteins.

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Mg-Ion detection from DNA-Origami buffer solutions

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DNA-Origami nanostructures (DONs) are an emerging technology with huge potential in research areas such as medicine, single-molecule diagnostics and Lab-on-a-chip devices. The successful preparation of DONs depends on various aspects such as cations, buffers and pH [1]. It was found, that Mg^{2+} cations are the most important cations for DON stability, as these neutralize the negative charge of the densely packed phosphate backbone of the DNA structures [2]. Different usecases require different buffers, such as phosphate buffered saline (PBS) or Tris buffers. Therefore, it is of high importance to quantify and control the amount of Mg^{2+} cations in DON solutions. In this paper we describe a method to detect Mg^{2+} cations from Tris and PBS buffer solutions. This method is based on the thiazole yellow g reaction [3-5]. The detection and quantification of Mg^{2+} is suited for use with DONs as it works with small sample volumes and is not interfered by commonly used buffers. The method is designed to allow for screening and to be easily adopted in biological laboratories as it only requires a plate-reader. Mg^{2+} quantification was successful in Tris buffer at various pH values (6, 8.4 and pH 10) while for PBS buffer at pH 9 an estimation was possible for a range of 0.1 – 30 mM Mg^{2+}. This is due to the partial precipitation of Mg^{2+} after reaction with free hydroxy ions. It was found, that for PBS buffers the amount of free hydroxy ions is higher than for Tris buffers.

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Monitoring of a DNA double helix formation: a multi-approach benchmark study

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In both basic or applied research the characterization of binding parameters of complexes involving biological macromolecules remains a crucial step to understand function, to relate it to the structure, to evaluate whether a specific ligand is of interest for therapeutic purposes, or to validate a molecular probe for biosensing or for more fundamental studies. The determination of the dissociation equilibrium constant, K_D, and if possible the kinetics and the thermodynamic parameters of the binding reaction, is often seen as the Holy Grail. The last decade has seen the emergence of new manufacturers offering biosensing instruments that suit the needs of researchers. There is now a wide range of instruments based on different physical principles capable of measuring binding affinities. The goal of the present benchmark study was to analyze how different technical setups performed for analyzing a very simple model, the formation of a 10-mer DNA double helix, the stability of which can be easily tuned by temperature, ionic strength or single point mutations. The results show that the measured dissociation constant, K_D, depends to a significant extent on how the DNA formation helix is monitored.

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The mutation R107Q alters mtSSB ssDNA compaction ability and binding dynamics

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Mitochondrial single-stranded DNA-binding protein (mtSSB) is essential for mitochondrial DNA (mtDNA) replication. Recently, several mtSSB variants have been associated with autosomal dominant mitochondrial optic atrophy and retinal dystrophy. We have studied at the molecular level the functional consequences of one of the most severe mtSSB variants, R107Q. We first studied the oligomeric state of this variant and observed that the mtSSBR107Q mutant forms stable tetramers in vitro. On the other hand, we showed, using complementary single-molecule approaches, that mtSSBR107Q displays a lower intramolecular ssDNA compaction ability and a higher ssDNA dissociation rate than the WT protein. Real-time competition experiments for ssDNA-binding showed a marked advantage of mtSSBWT over mtSSBR107Q. Combined, these results show that the R107Q mutation significantly impaired the ssDNA-binding and compacting ability of mtSSB, likely by weakening mtSSB ssDNA wrapping efficiency. These features are in line with our molecular modeling of ssDNA on mtSSB showing that the R107Q mutation may destabilize local interactions and results in an electronegative spot that interrupts an ssDNA-interacting-electropositive patch, thus reducing the potential mtSSB-ssDNA interaction sites.

Mesoscale Modelling of Thrombus Formation using smoothed Dis- sipative Particle Dynamics

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Mathematical modeling and numerical analysis are fundamental allies in medical applications, such as understanding thrombus formation dynamics and exploring therapeutic interventions for clotting disorders. We propose a computational scheme for modeling blood coagulation using Advection- Diffusion-Reaction (ADR) equations.

Our computational scheme employs a particle-based representation, adapt- ing the smoothed Dissipative Particle Dynamics (sDPD) method [1] to cap- ture fluid momentum transport at mesoscales. Additionally, we account for diffusion and reaction phenomena among multiple coagulation species through compositional-field variables associated with each particle. Notably, our model faithfully reproduces concentration profiles observed in blood ex- periments reported previously [2, 3], validating its accuracy.

Our approach facilitates the exploration of diverse scenarios, spanning from homogeneous to heterogeneous conditions, both static and dynamic. Par- ticularly in dynamic scenarios, we explore the impact of the Peclet number on species concentration distribution across the domain. This analysis offers promising prospects for applications in in-vivo and in-vitro systems, as well as in multiscale models involving medical devices.

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Abstracts: Posters

Session VII: Synthetic biophysics: self-organized and biomimetic systems

Effect of precursor conformation on the topology of protein and polypeptide single chain nanoparticles.

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Single chain nanoparticles (SCNPs) are unimolecular polymer chains folded or collapsed via intra-molecular cross-linking under high dilution, leading to sparse conformations and a topological polydispersity similar to that of intrinsically disordered proteins (IDPs). Currently, there is great interest in expanding this technology to biodegradable and biocompatible polymers, including proteins. For this, we fabricated BSA-SCNPs via intramolecular cross-linking of denatured bovine serum albumin (BSA) using disuccinimide ester linkers that mainly react with lysine moieties in a polypeptide. SANS measurements demonstrated that the denatured protein progressively shrinks along with a lowering of the scaling exponent by cross-linking, thus allowing for size control of the BSA-SCNPs [1].
To extend SCNPs to polypeptides, it is important to understand the role of the chain conformation of the precursor on the resulting SCNP morphology. For this, we have systematically varied the solvent conditions (pH, salt and denaturant concentrations) of BSA and the homopolymer polylysine solutions as well as the cross-linker concentration and studied the resulting SCNPs by dynamic and static light scattering as well as small angle neutron scattering. Our results indicate that the precursor conformation has an effect on the SCNP morphology. In particular, we found that more extended precursor conformations are able to collapse more as the intramolecular cross-links are increased. In addition, a longer cross-linker is more effective in chain compaction due to its ability to form larger intramolecular loops.

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Sub-crystalline protein assemblies through crystal contact engineering

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Protein crystal lattice contact modulation allowed to synthesise crystalline two-dimensional planar and one-dimensional tubular assemblies, based on consensus tetratricopeptide repeat (CTPR) proteins. CTPR proteins fold into rigid right-handed superhelices [1]. The specific fold creates a regular repeating surface that can be used to impose molecular order on the nanoscale, allowing the construction of functional nanomaterials [2]. Supramolecular protein assemblies are highly desired for nanoscale applications as a means to confer order on the molecular level, with considerable effort spent on the development of self-assembling proteins [3]. The predominantly employed approach relies on artificial design of interacting protein surfaces to sustain the protein assemblies, grafted on protein scaffolds of suitable topology to support desired assemblies. However, during protein crystallisation proteins naturally establish contacts with their neighbouring protein units to maintain macroscopic three-dimension scaffolds, i.e. crystals. Targeted disruption of the contacts restricts crystal lattice growth in selected directions, resulting in lower-dimension assemblies with crystalline order. The large library of deposited crystal structures provides an abundance of targets for sub-crystalline assembly design. Thus, crystal contact engineering presents a promising rapid and attractive approach for the design of ordered protein assemblies.

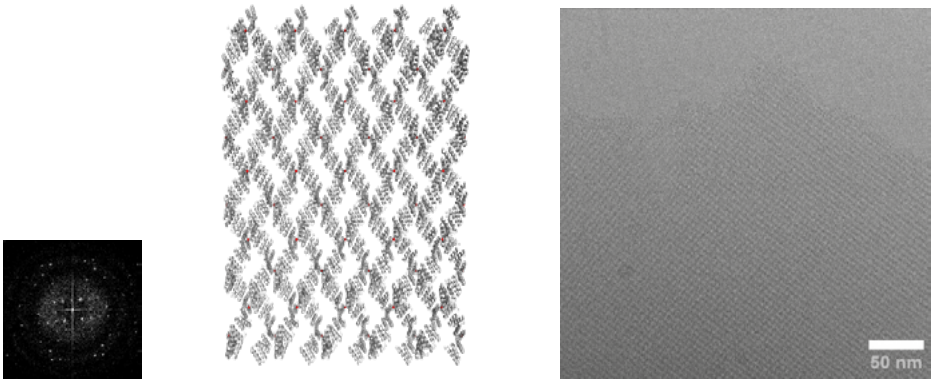

Figure 1. A model of a two-dimensional assembly (left) and cryo-electron micrograph of the assembly (right; inset contains the Fourier transform).

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Abstracts: Posters

Session VIII: Membrane and membrane proteins

Rheological properties of model pulmonary surfactant films under breathing-like conditions

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The respiratory dynamics depend on the activity of pulmonary surfactant, the surface active agent in charge of diminishing the surface tension at the air-liquid interface of the alveoli to make breathing dynamics possible. This complex is composed mainly by lipids (90 %) and a small proportion of specific proteins (10 %). The major lipid component of surfactant is the disaturated phospholipid DPPC. DPPC is responsible of sustaining the lowest surface tensions needed at the end of expiration to avoid alveolar collapse. Other phospholipids, such as unsaturated PC and PG species, as well as cholesterol, are needed to modulate rheological properties of surfactant along its secretion, surface-associated action and recycling throughout the respiratory cycle. Among surfactant proteins, hydrophobic SP-B and SP-C are crucial for surfactant biophysical activity, although the mechanism by which they improve this activity remains unclear [1]. In this work, the behaviour of model mixtures mimicking pulmonary surfactant composition has been studied in a radial trough, an especial equipment in which interfacial lipid and lipid/protein films can be formed and subsequently expanded and compressed at physiologically relevant breathing ranges in order to study their response to dilatational deformation [2]. The results indicate how the presence of hydrophobic proteins is necessary for a proper reduction of surface tension, even in the presence of supra-physiological amounts of DPPC, and how the combination of both proteins leads to a better response to the dilatational stimuli. Furthermore, we have also studied whether the presence of cholesterol affects to the behaviour of surfactant films.

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Nanomechanics of Glucosylceramide enriched membrane domains in lipidosis.

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Gaucher disease (GD) is a lipidosis caused by several mutations on the gene encoding acid α-glucosidase, leading to aberrant accumulation of glucosylceramide (GlcCer) in cells [1]. Despite the genetic, biochemical, and medical levels of GD are well characterized, how GlcCer accumulation in membranes alters cell biology and biomechanics processes is poorly understood.
Here we used atomic force microscopy (AFM) and AFM-based force spectroscopy (AFM-FS) to investigate the nanomechanical properties of model supported lipid bilayers (SLBs), composed of different combinations of dioleoyl-phosphocholine (DOPC), sphingomyelin (SM_E) and cholesterol (Chol), with and without GlcCer, to simulate the excess of GlcCer in GD. We determined the breakthrough force (F_b) of the SLBs, as an indicator of the lateral interactions between lipid molecules [2]. Our results show that in, GlcCer fully segregates from DOPC:GlcCer SLBs into well-defined, rigid domains, reflected by an extremely high F_b, which are even released from the membrane at high concentrations (Fig. 1, a). When SM_E is present, GlcCer integrates into SM_E domains, increasing the packing. The fluidifying effect of Chol is observed when comparing DOPC:SM_E:Chol in presence, or absence, of 10-20% GlcCer. The lipidic packing of these domains increased with GlcCer concentration, but never reaching individual GlcCer F_b levels (Fig. 1, b). In general, GlcCer leads to an increased packing and mechanical resistance of the membrane domains.

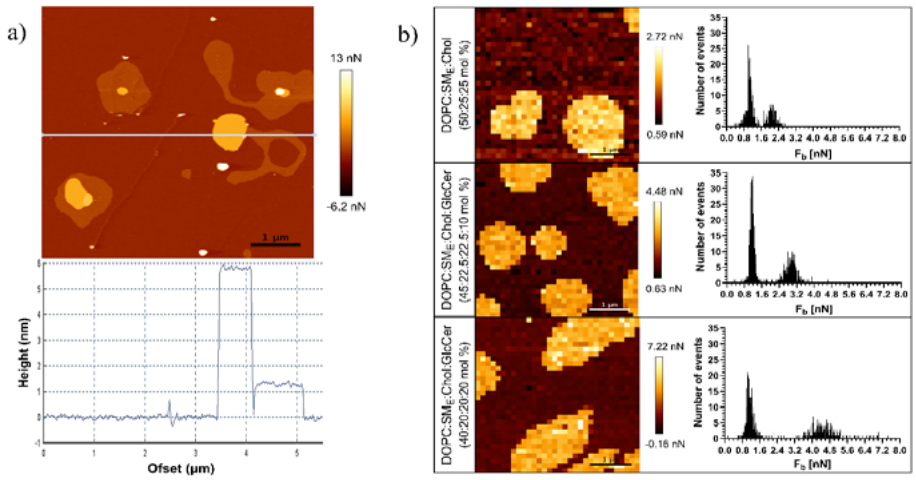

Figure 1 a) AFM topographical image, with its corresponding profile, of DOPC:GlcCer SLB (80:20 mol %). b) F_b map and distribution for DOPC:SM_E:Chol SLB with 0, 10 and 20 mol% GlcCer. Image and maps area: 4 x 4 mm.

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Assembly mechanisms of the plant protein remorin into membrane nanodomains

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Plant-specific REMORINs (REMs) are crucial proteins involved in plant defense against viral propagation by regulating cell-to-cell connectivity. They are tightly associated with the clustering of nanodomains at the plasma membrane, driven by specific protein-protein and protein-lipid interactions. REMs can be classified into 6 groups, containing a membrane-associating C-terminal anchor (REM-CA), neighboring a coiled-coil domain that is followed by an intrinsically disordered N-terminal region (IDR). We have recently contributed to understanding the precise underlying mechanisms of nanodomain clustering by REMs, involving interactions of REM-CA with specific phosphatidylinositol phosphates (PIPs) [1, 2]. Moreover, our data have revealed that StREM1.3's nanodomain clustering depends on REM's oligomerization behavior [3], and on the phosphorylation status in the IDR [4]. We now address the role of the structural divergence between the different REM groups and found that REMs rely on diverse sequence motif arrangements and REM-CA sequences. We investigate REM's structural and dynamic organization based on domain-specific analysis and their positioning in the context of the three-domain protein. Bioinformatics analysis suggests implications of motif distribution in the regulation of nanodomain clustering. Based on 3D structure determination of REM-CAs of different REM groups by NMR, we discovered the REM-CA structural diversity, further highlighting the role of sequence adaptation and structure modulation to control membrane association and nanodomain formation.

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Characterization of antipsychotic interactions with the lipid membrane

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Antipsychotics (APs) are used for treatment of several psychiatric disorders, such as schizophrenia. They exert their activity mainly by interacting with dopaminergic receptors (notably, D2R), where they modulate their activation and subsequent signalling cascades [1]. We hypothesize that the antipsychotics can access the receptor via the lipid membrane in complement to the direct interaction, since this has been postulated for another ligand with another dopaminergic receptor (dopamine - D3R) [2]. Therefore, we are currently exploring the partitioning of two antipsychotics: chlorpromazine and clozapine (D2R ligands) in lipid membranes and their impact on physical and mechanical properties of the membrane.
Lipid model membranes that mimic the synaptic cell membrane have been used as well as more simplistic models with the intent of exploring the effect of negative charge and cholesterol on the interaction. Different biophysical approaches have been used, such as: isothermal titration calorimetry, differential scanning calorimetry, molecular dynamics simulation and sulforhodamine leakage assay. Our results indicate that both antipsychotics highly partition to the membrane, being mostly located around the headgroup region where they tend to increase membrane ordering while decreasing ordering along the fatty acid chain region. Subtle differences in membrane remodeling between the two APs were observed.

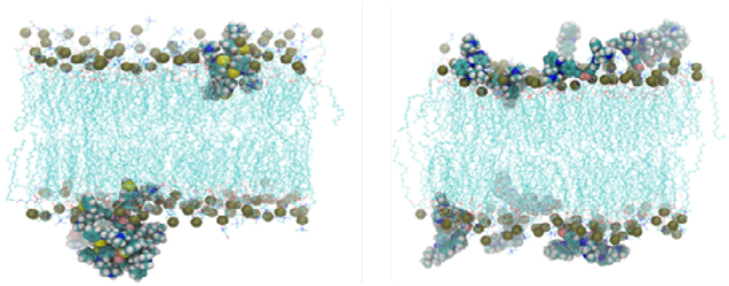

Figure 1: Chlorpromazine (left) and clozapine (right) interaction with a synaptic lipid membrane mimic – snapshot obtained by all-atom molecular dynamics simulation.

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Revealing the mechanism of the lipid storage disorder Niemann-Pick C by combining multi-omics and biophysical imaging

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Niemann-Pick Type C (NPC) is a rare neurodegenerative disorder caused by mutations in the NPC intracellular cholesterol transporters 1 and 2 that result in cholesterol and sphingolipid accumulation in the lysosome. The subsequent pathogenic cascade responsible for the broad range of NPC symptoms remains unknown. We hypothesise that such an unbalanced lipid distribution in the cell would alter the biophysical properties of cellular membranes, which may have a pathological implication. Using advanced imaging, we quantify the lipid order and tension of multiple organelles in fibroblasts obtained from NPC patients, healthy volunteers and mice. Our analyses confirm that indeed NPC mutations induce changes in the biophysical properties of cell membrane. To investigate the metabolic response mechanisms to such imbalance, we performed transcriptomic and lipidomic analyses and identified dysregulated pathways involved in lipid metabolism. In future experiments, we will assess whether by interfering with such pathways we can revert the NPC phenotype and find potential treatments for this fatal lipid storage disorder.

Optimizing the membrane protein system size for realistic MD simulations

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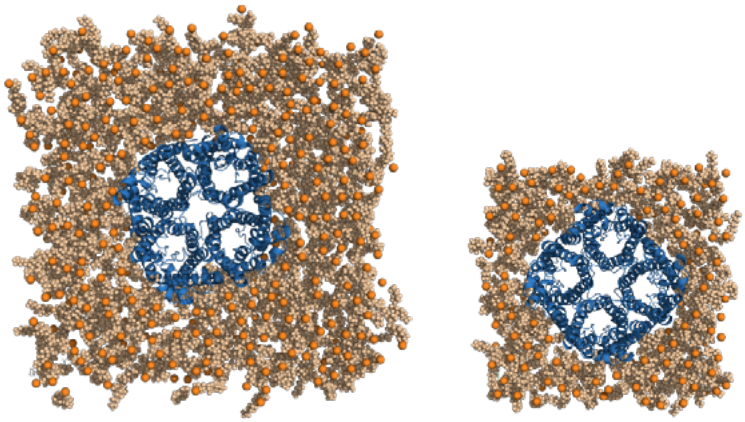
Aquaporins (AQPs) are responsible for permeating solutes across membranes. They can be divided into two subgroups: classical aquaporins, strictly selective for water, and aquaglyceroporins, those permeable to water and glycerol. The identification of AQP function modulators has been a difficult task due to the low target druggability and the unsuitability of the commonly used computational approaches. The crystallographic structures of AQPs often present inadequate conformations due to the crystal packing, which impairs the binding of the best modulator candidates. Despite attempts to use computational approaches to mitigate the mentioned problems [1], a fundamental issue remains: are we using the most adequate models, parameters, and force fields to simulate these membrane proteins?
In this work, we initially focused on studying the impact of different membrane sizes on AQPs' stability and function, followed by addressing the impact of different force fields. For this, we set up Molecular Dynamics (MD) simulations with the solved structure of the aquaglyceroporin hAQP7 (6QZI) [2] in a POPC lipid bilayer of different sizes (160, 200, 300, 400, and 500 lipids) as shown in Figure 1, with the Amber ff14SB and the CHARMM 36m forcefields. This protocol helped us understand the conformational behavior of the protein and identify the minimal lipid embedding environment required for a fully functional protein. These results will be useful for the identification of structural features that regulate the function of hAQP7, and later on of specific and efficient functional modulators that could be explored as therapeutic approaches for different diseases.



(a) (b)

Figure 1 (a) hAQP7 colored in blue embedded in a 500 POPC lipid bilayer, colored orange. (b) hAQP7 colored in blue embedded in a 200 POPC lipid bilayer, colored orange.

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Synergy between amphipathic helices and conical lipids in membrane shape transition.

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Proteins involved in generating membrane curvature typically contain an amphipathic helix on a membrane interacting site. This helix is believed to act as a 'wedge' that induces or assists in outward membrane bending when it is shallowly inserted into the lipid bilayer [1]. However, recent research has challenged this idea, suggesting that protein steric interactions (crowding) are be primarily responsible for membrane bending, while the role of the amphipathic helix has been reduced to anchoring in the membrane [2]. Here we examine the ability of H0 helix of the Epsin N-therminal homology domain (ENTH), along with designed peptides with a similar length and hydrophobic profile, to cause membrane shape transition. Our findings indicate that while the studied peptides exhibit a significant positive spontaneous curvature when embedded in the lipid monolayer, they have little effect on the flat shape of the membrane. Therefore, a high surface density of peptides and low lateral tension are necessary to induce spontaneous tubulation of membranes. However, including conical lipid PE in the membrane significantly facilitates the destabilization of its flat shape, reducing dramatically the critical peptide coverage and increasing lateral tension that can resist membrane shape transition. Furthermore, the radii of the forming tubes showed a strong dependence on the amount of PE, decreasing as it increased. We suggest a theoretical model that considers the curvature-composition coupling for both amphipathic peptides and conical lipids [3] to explain their synergy in membrane shape transition. This work was supported by Russian Science Foundation (grant # 22-15-00265).

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Biophysical evaluation of aquaporin-3 and aquaporin-5 modulation of cell stiffness, cell-cell adhesion and cell migration

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Aquaporins (AQPs) are transmembrane proteins that mediate the transport of water, glycerol, and small neutral solutes across cell membranes [1]. AQPs are overexpressed in cancer, being involved in cancer cell proliferation, migration, angiogenesis and metastasis [2]. Our previous study with AQP3-, AQP5-, and double-silenced human pancreatic cancer cells showed morphological alterations and lower cell-cell adhesion, with AQP5 influencing cell stiffness and membrane fluidity [3]. These findings suggest that these AQPs can impact tumor progression by modulating cell biomechanical properties. With this work, we developed a cell-based platform of HEK-293T (HEKT) cells overexpressing individually the isoforms most associated with cancer: AQP3 (water and glycerol channel) and AQP5 (water channel) to investigate how their overexpression can modulate biological processes and cell membrane features. After cell model validation, we assessed their impact on morphological properties through atomic force microscopy (AFM) imaging. AQP5-overexpressing cells exhibited a higher roughness and area, with no differences for AQP3-HEKT cells. Using AFM-based force spectroscopy, we evaluated the influence of these AQPs on cell stiffness and cell-cell adhesion. AQP3-HEKT cells showed higher cell stiffness and lower cell-cell adhesion. In contrast, AQP5-HEKT cells demonstrated higher elasticity and cell-cell adhesion. Afterwards, we investigated the effect of AQP3 and AQP5 overexpression on cell migration, proliferation and adhesion. Both AQPs promoted cell migration and impaired cell adhesion to matrix, with no differences for cell proliferation. Further studies are needed to understand the mechanisms underlying the roles of AQP3 and AQP5 on cell membrane properties and their impact on biological processes crucial for tumorigenesis.

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Characterization of potential viroporins encoded by African Swine Fever Virus

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African swine fever virus (ASFV) is the causative agent of a highly contagious disease affecting domestic and wild swine within vast regions of Europe and Asia. The genome of the virus encodes for several integral membrane proteins (IMPs) displaying features of structural viroporins. Here, we characterize experimentally three potential candidates, namely: (i) B117L, a single pass IMP with its N-terminus facing the ER lumen, i.e., reminiscent of class IA viroporins; (ii) B169L, containing a helical hairpin anchored to the ER membrane with both terminal ends facing the lumen of the organelle, and thus reminiscent of class IIA viroporins; and (iii) EP84R, also anchored to ER membrane through a helical hairpin, but adopting a topology similar to that of class IIB viroporins, i.e., with both ends facing the cytoplasm. For these three proteins we compare: (i) oligomer formation capacity upon cell expression; (ii) conformations adopted by the predicted transmembrane helices reconstituted in lipid bilayers that emulate the ER membrane; (iii) permeabilization of ER-like single vesicles (pore-forming activity); and (iv) ion conductance changes measured in ER-like planar bilayers (ion-channel activity). Collectively our data support viroporin-like assistant roles in ASFV replication for IMPs B117L and B169L, but not for EP84R.

T-cell monitoring during Immune Synapse formation by multi-protein micropatterning

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T-cells are one of the main characters of our immune system, responsible for detecting pathogens and orchestrating the immune response against them. During interactions with antigen-presenting cells, membrane lipids and proteins such as TCR or CD4, undergo reorganization, forming three concentric rings and giving rise to a specialized structure known as the Immune Synapse (IS).
Despite its significance, the mechanisms underlying this reorganization and the role of membrane mechanics in IS formation remains poorly understood.
Using specific multi-protein micropatterning system, we have functionalized surfaces (Fig.1a) mimicking IS, where cells can attach and interact with immobilized molecules such as T-cell receptor activating proteins. This approach, combined with semi-automatized image segmentation, allows us to track single cells in real-time and monitor changes in cell morphology, actin cytoskeleton, and membrane mechanics on surfaces functionalized with different molecules through fluorescent lifetime imaging (FLIM) and confocal microscopy (Fig.1b).

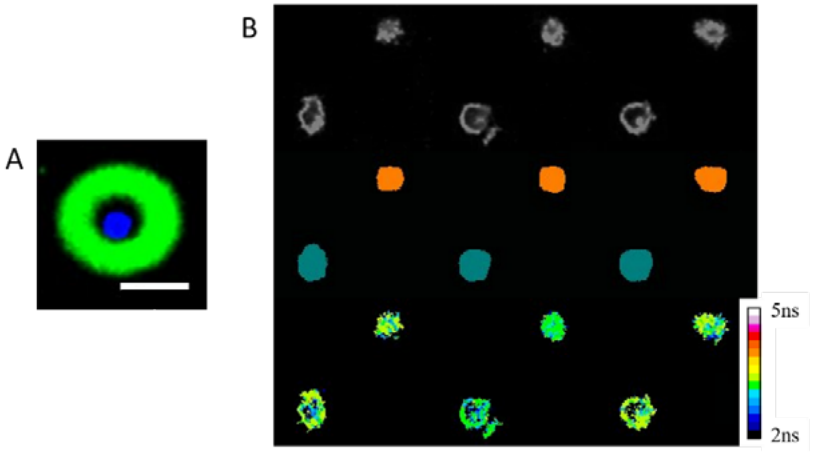

Figure 1: IS formation on concentric micropatterns. A) Micropattern of two concentric rings with fibronectin-488 (blue), in the central ring and fibronectin-647 (green) in the peripheral one. Scale bar: 10µm. B) Jurkat cells, stained with Flipper-TR membrane tension reporter, on functionalized surface (top panel), the corresponding segmented mask (middle panel), and FLIM image (bottom panel).

Rottlerin as an aquaporin-3 inhibitor for cancer therapy

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Aquaporins (AQPs) are specialized transmembrane protein channels that facilitate the diffusion of water, glycerol and other small solutes across cell membranes [1]. However, some aquaporin isoforms such as AQP1 and AQP3, are aberrantly expressed in different types of cancer tissues [2,3]. Thus, these proteins are considered potential drug targets prompting the discovery of AQP inhibitors for cancer therapy. Since most inhibitors reported so far have high toxicity and poor selectivity, there is an urgent need to discover efficient modulators for therapeutical use. This led us to develop an innovative structure-based in silico computational workflow to find AQP inhibitors, where a polyphenol compound was identified. Rottlerin was tested for its inhibitory effect on AQP1 (water channel) and AQP3 (water and glycerol channel) by measuring water and glycerol permeability in human red blood cells (RBCs), that endogenously express these AQPs, using stopped-flow spectroscopy. Rottlerin strongly inhibited glycerol permeability via AQP3 (IC_{50} = 6.7 ± 2.97 μM). To validate the results obtained in RBCs, yeast cells were transformed with a plasmid encoding individually human AQP1 (hAQP1) and AQP3 (hAQP3). Rottlerin showed no effect on yeast-hAQP1 activity but induced pronounced yeast-hAQP3 inhibition (73% ± 2.14), corroborating the results obtained in RBCs. Rottlerin seems to establish strong and consistent interactions with several residues at the extracellular surface region of AQP3, acting as a stereochemical cap that blocks glycerol from accessing the pores of the protein [4].
Our data shows that Rottlerin strongly inhibits AQP3 activity, revealing its potential as a new drug for cancer therapeutics.

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Dynamin-2 functional promiscuity in membrane remodeling is controlled by punctual mutations in its Pleckstrin-homology domain

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Dynamin2 GTPase (Dyn2) is a crucial player in clathrin-mediated endocytosis. Dyn2 is tetrameric in cytoplasm and self-assembles into functional units upon membrane binding. Most of the neurogenerative pathology-related mutations in Dyn2 are point mutations in its pleckstrin homology domain. Here, we have asked how such punctual mutations affect the overall functionality of Dyn2. We first reconstituted the Dyn2 self-assembly process using membrane nanotubes (NT) and vesicles and characterized it using single-molecule fluorescence and cryo-electron microscopy. On NTs, WT Dyn2 first forms small subhelical oligomers, which are already curvature active and display pronounced curvature sensing properties. Conical lipids and GTP promote their further self-assembly into helical machinery, mediating the NT scission.
However, in the presence of large unilamellar vesicles (LUVs), we observed an alternative self-assembly pathway where the subhelical oligomers form membrane tethering complexes mediating LUV-NT binding. Reconstitution of tethering in the LUV system revealed that conical lipid species, divalents, GTP, and SH3 binding partners of Dyn2 control lipid mixing. We further revealed that the disease-related point mutations in the PH domain alter the balance between fission and tethering/fusion activities of Dyn2, pointing to the physiological relevance of its membrane tethering activity in cells.

Self-assembly and functions of curved Annexing V lattices

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Annexin V (AnV) is a versatile cytoplasmic protein involved in various cellular membrane processes, from membrane wound healing to formation of protective anticoagulant shields around apoptotic cells. AnV binding to membranes is triggered by factors like calcium spikes or sudden increases in local membrane charge. It is known that AnV shows a preference for planar membranes, where trimers assemble into intrincated lattices on the membrane surface. However, its behavior on curved membranes and the functional significance of these curved lattices are not well understood. To study the formation of AnV lattices on positively curved membranes, we used synthetic lipid membrane nanotubes (NT) pulled from planar reservoirs and NT networks formed on custom micropillar arrays. Through quantitative fluorescence microscopy and conductance measurements, we discovered that AnV exhibits weak curvature preferences due to its complex membrane interface. Small pieces of protein, such as oligomers, monomers or trimers can spontaneously partition from planar to curved membrane, in contrast with earlier association of AnV shape with negative membrane curvature, leading to a progressive accumulation of the protein into cylindrical rigid-elastic scaffolds. These scaffolds not only shaped the NT geometry but also facilitated NT fission under force or shear stress. Our findings suggest that AnV could operate on positively curved membranes, with its curved lattices acting as both stabilizing scaffolds and mechanosensitive regulators of membrane fission, determining AnV role in cellular processes.

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Rheological properties of model pulmonary surfactant films under breathing-like conditions

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The respiratory dynamics depend on the activity of pulmonary surfactant, the surface active agent in charge of diminishing the surface tension at the air-liquid interface of the alveoli to make breathing dynamics possible. This complex is composed mainly by lipids (90 %) and a small proportion of specific proteins (10 %). The major lipid component of surfactant is the disaturated phospholipid DPPC. DPPC is responsible of sustaining the lowest surface tensions needed at the end of expiration to avoid alveolar collapse. Other phospholipids, such as unsaturated PC and PG species, as well as cholesterol, are needed to modulate rheological properties of surfactant along its secretion, surface-associated action and recycling throughout the respiratory cycle. Among surfactant proteins, hydrophobic SP-B and SP-C are crucial for surfactant biophysical activity, although the mechanism by which they improve this activity remains unclear [1]. In this work, the behaviour of model mixtures mimicking pulmonary surfactant composition has been studied in a radial trough, an especial equipment in which interfacial lipid and lipid/protein films can be formed and subsequently expanded and compressed at physiologically relevant breathing ranges in order to study their response to dilatational deformation [2]. The results indicate how the presence of hydrophobic proteins is necessary for a proper reduction of surface tension, even in the presence of supra-physiological amounts of DPPC, and how the combination of both proteins leads to a better response to the dilatational stimuli. Furthermore, we have also studied whether the presence of cholesterol affects to the behaviour of surfactant films.

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Controlling membrane electroporation via membrane nanotubes

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Membrane nanotubes (NTs) spontaneously emerge from planar membrane reservoirs in response to diverse mechanical and chemical stimuli. The network of NTs linked to the reservoir significantly influences its apparent mechanical characteristics, thereby becoming an integral component of the mechano-transduction systems associated with membrane reservoirs such as the plasma membrane. However, the structural integrity of the NT-reservoir system under an acute external stress has not been explored. In this study, we utilized electroporation as a tool to assess the stability of an NT pulled from a planar lipid bilayer against tensile-like lateral stress. A moderate electric field was applied to induce transient pores in the NT and reservoir membranes, allowing us to determine the probability of pore formation as a function of membrane curvature, tension, and lipid composition. Our findings revealed that the efficiency and outcomes of the poration process in this system depend on the curvature and elastic properties of the NT membrane, with clear implications for the role of NTs in regulating the membrane response to external stress.

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Effect of PEGylation on pulmonary surfactant-driven interfacial vehiculization of drug delivery systems

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Coating the surface of nanoparticles with polyethylene glycol (PEG), or "PEGylation", is a well-established approach to improve the efficiency of drug and gene delivery to target cells and tissues. Different PEGylated nanoparticles have been designed for pulmonary drug delivery since PEGylation protects nanocarriers from pulmonary immune system, enhances mucopenetration and facilitates sustained drug release. However, PEGylation may interfere with the interfacial vehiculization of nanoparticles along the air/liquid interface of the respiratory airways as it is promoted by pulmonary surfactant, a lipoprotein complex that facilitates the work of breathing by forming films that decrease the surface tension at the alveolar surface. Therefore, the aim of this work was to assess the effect of PEGylation on the interfacial vehiculization of liposomes composed of dipalmitoylphosphatidylcholine and lysophosphatidylcholine. To that end, we characterized the interfacial activity of PEGylated liposomes, its interaction with surfactant components and its vehiculization by surfactant using two surface balance-based set ups: an adsorption Wilhelmy balance and a double-balance setup connected through the interface. Finally, to evaluate whether the incorporation of PEGylated nanomaterials could compromise surfactant function, we investigated their impact into surfactant performance using a captive bubble surfactometer.

Exploring the Influence of Surfactant Protein C in Lung Homeostasis and Immune Defense: Insights from Vesicle Uptake Studies

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Alveoli are covered by pulmonary surfactant (PS), a lipoprotein mixture located at the air-liquid interface, which optimizes respiratory mechanics and prevents lung collapse by decreasing surface tension. In addition, PS contributes to the immune defense against harmful microorganisms and particles present in inhaled air. However, oxidative stress environment modifies PS constituents in the long term. Because of that, a balance between the secretion and processing of PS is maintained by type II epithelial cells and alveolar macrophages.
Surfactant protein C (SP-C) could play a role in this process due to its ability to induce strong membrane curvature that triggers the formation of small vesicles enriched in SP-C that would be internalized by alveolar cells. In this work, we present the study of the role of SP-C in the recycling of surfactant material as a key process in lung homeostasis, as well as in the regulation of the innate defense of the respiratory system. To do this, we have investigated the SP-C-mediated cellular uptake of synthetic unillamelar vesicles with different lipid compositions. In addition, the intracellular localization of lipid and SP-C once the vesicles are internalized have been examined using cell organelle markers. For this purpose, confocal microscopy and flow cytometry experiments have been carried out using different alveolar cell lines.

Universal principles of ESCRT-driven membrane remodeling deciphered from Asgard archaea

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Membrane remodeling is fundamental for intracellular communication and molecular uptake at the plasma membrane. Within eukaryotic cells, the ESCRT-III protein machinery orchestrates membrane remodeling at several subcellular locations, participating in scission and repair during the nuclear envelope reformation, multivesicular body formation, lysosomal membrane repair, and plasma membrane during cytokinetic abscission. Interestingly, ESCRT-III's evolutionary roots extend to the closest relative of eukaryotes being Asgard archaea.

In this study, by phylogenetic analysis we first categorize Argard ESCRT-III proteins into two main groups that are sequentially recruited for remodeling: first, ESCRT-IIIB as the early subunits, and second, ESCRT-IIIA as the late subunits. Progression of membrane remodeling by the sequential recruitment of the subunits is mediated by the ATPase ESCRT-IIIC upon ATP hydrolysis.

We further reconstitute a minimal in vitro system using purified proteins and model membranes to analyze the structural, biochemical and biophysical features involved in remodeling by the Asgard ESCRT-III machinery. Our results demonstrate how ESCRT-IIIB is initially recruited to the membrane and self-assembles independently on the initial membrane curvature. We further confirmed its polymeric flexibility by solving its structure at atomic resolution using cryo-EM. We next showed by electron and fluorescence microscopy how highly curved helical ESCRT-IIIA filaments are recruited to pre-assembled ESCRT-IIIB, then mediating constriction and fission of a membrane neck upon ATP hydrolysis by ESCRT-IIIC.

By exploring the functional principles of Asgard ESCRT-III proteins on model membranes, our results provide a comprehensive analysis of all the steps involved in membrane remodeling by ESCRT-III in a simplified but realistic manner, as Asgard are the closest organisms relative to eukaryotes. We ended by proposing a model that reconciles what is known and what was missing about ESCRT-III activity.

(1-Deoxy)ceramides in bilayers containing sphingomyelin and cholesterol

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The discovery of a novel sphingolipid subclass, the (1-deoxy)sphingolipids, which lack the 1-hydroxy group, has attracted considerable attention [1,2]. They differ in their properties from the canonical (or 1-hydroxy) sphingolipids and they are toxic when accumulated in cells, inducing neurodegeneration and other dysfunctions. (1-Deoxy)ceramides, (1-deoxy)dihydroceramides, and (1- deoxymethyl) dihydroceramides, the latter two containing a saturated sphingoid chain, have been studied in this work using differential scanning calorimetry, confocal fluorescence and atomic force microscopy, to evaluate their behavior in bilayers composed of mixtures of three or four lipids. When compared to canonical ceramides (Cer), all C16:0 (1-deoxy)Cer show a lower miscibility in mixtures of the kind C16:0 sphingomyelin/cholesterol/XCer, where XCer is any (1-deoxy)ceramide, giving rise to the coexistence of a liquid-ordered phase and a gel phase. The latter resembles, in terms of thermotropic behavior and nanomechanical resistance, the gel phase of the C16:0 sphingomyelin/cholesterol/C16:0 Cer mixture [3]. Differences are seen between the different C16:0 XCer under study in terms of nanomechanical resistance, bilayer thickness and bilayer topography. When examined in a more fluid environment (bilayers based on C24:1 SM), segregated gel phases are still present. Probably related to such lateral separation, XCer preserve the capacity for membrane permeation, but their effects are significantly lower than those of canonical ceramides. Moreover, C24:1 XCer show significantly lower membrane permeation capacity than their C16:0 counterparts. The above data may be relevant in the pathogenesis of certain sphingolipid-related diseases, including certain neuropathies, diabetes, and glycogen storage diseases.

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Membrane fusion properties of the ATG8 autophagy protein LC3C

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Autophagy activation is usually monitored by the de novo formation of a double-membrane structure called phagophore, which expands to engulf intracellular contents before closing to form the mature autophagosome (AP) [1]. (AuTophaGy related) ATG8 protein family members participate at various stages of AP formation. ATG8 are ubiquitin-like proteins that, in order to carry out their function, must be covalently anchored to phosphatidylethanolamine (PE), a lipid present in the AP membrane. The present study compares the capacity to induce lipid-vesicle tethering and fusion of two ATG8 family members, LC3B and LC3C, with model membranes [2]. LC3B is the most thoroughly studied ATG8 protein. It is generally considered as an autophagosomal marker and a canonical representative of the LC3 subfamily. LC3C is less studied, but recent data have reported its implication in various processes, crucial to cellular homeostasis. In our previous studies, the activity of lipidated LC3C to induce vesicle tethering and fusion has been reported [3]. The results in this poster show that LC3C induces higher levels of tethering and of intervesicular lipid mixing than LC3B. As the N-terminus of LC3C is different from that of the other family members, various mutants of the N-terminal region of both LC3B and LC3C were designed, and their activities compared. It was concluded that the N-terminal region of LC3C was responsible for the enhanced vesicle tethering, membrane perturbation and vesicle-vesicle fusion activities of LC3C as compared to LC3B. The results suggest a specialized function of LC3C in the AP expansion process. In summary, LC3C displayed higher vesicle aggregation and fusogenic activities than LC3B, and this difference appeared to be associated to the N-terminal region of LC3C.

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Increased binding of LC3/GABARAP autophagy proteins to cardiolipin-containing membranes in the presence of ceramide

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The role of ceramide (Cer) in autophagy is unclear, but CL and Cer have been proposed to coexist in mitochondria under certain conditions favoring this catabolic process [1]. Among autophagy-related proteins, LC3/GABARAP constitute a protein family with important roles in autophagosome formation, binding future cargo organelles and promoting autophagosome growth. The involvement of specific lipids in this process is poorly understood. A biophysical analysis of bilayers composed of eSM, DOPE, CL, and/or Cer has been performed to understand the relevance of CL + Cer coexistence. Bilayers were studied by differential scanning calorimetry, confocal fluorescence microscopy, and atomic force microscopy. Upon the addition of CL and Cer, one continuous phase and two segregated ones were formed. Assuming that phase separation at the nanoscale is ruled by the same principles acting at the micrometer scale, it is proposed that Cer-enriched rigid nanodomains, stabilized by eSM:Cer interactions formed within the DOPE- and CL-enriched fluid phase, result in structural defects at the rigid/fluid nanointerfaces, thus hypothetically facilitating LC3/GABARAP protein insertion [2]. When the interaction of LC3/GABARAP proteins with phospholipid monolayers and bilayers based on phosphatidylcholine or on sphingomyelin is studied, CL is found to be essential for the protein interaction with such bilayers, as measured through gradient centrifugation experiments, while ceramide markedly increases binding. Giant unilamellar vesicles examined under confocal fluorescence microscopy reveal that ceramide segregates laterally into very rigid domains, while GABARAP binds only the more fluid regions, suggesting that the enhancing role of ceramide is exerted by the minority of ceramide molecules dispersed in the fluid phase [3].

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Solvent-assisted deposition of supported lipid bilayers

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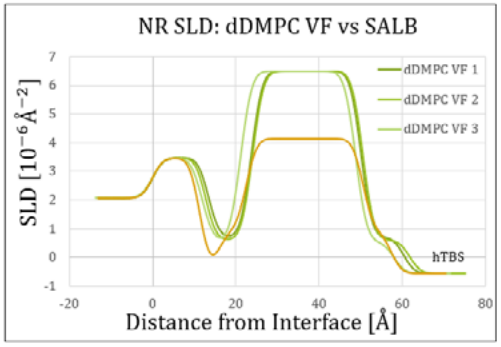
Supported Lipid Bilayer (SLB) are cell membrane mimics with widespread use in the biophysics field. However, common and simple methods for SLB preparation, such as vesicle fusion (VF), are limited in lipid compositions and solid supports. Ferhan et. al. proposed a solvent-assisted lipid bilayer (SALB) method capable to surpass these limitations. The authors identify key experimental parameters for successful SLB formation using QCM-D (quartz crystal microbalance with dissipation-monitoring) [1]. The procedure involves dissolving lipid samples in a water-miscible organic solvent, preferably isopropanol, flushing the solution on a solid support, and slowly exchanging the organic solvent with water [1]. Despite the many reports on SALB, it is unclear if some organic solvent remains trapped in the SLB after deposition and if the structure of the SLB remains comparable to that by VF. We used Neutron Reflectometry (NR) to determine the optimal POPC concentration and flowrate for SLB formation using the SALB method, which gave ≥95% coverage. We showed that 7.2% of Isopropanol remains in the tail layer, by comparing the SLD of deuterated (d) DMPC-SLBs prepared by VF and SALB. We plan to form SALBs with different lipid types (including PC with 40 mol% Cholesterol and atypical mixtures such as POPE:POPG, POPE:Cardiolipin), and probe them against the antimicrobial peptide Melittin, which is well studied using SLBs formed via VF [2]. It is important to demonstrate that the properties/ behavior of the SALB are not affected by the alcohol trapped in the tail region, especially when monitoring subsequent interactions with (bio)molecules/nanoparticles.



Sample	Layer	SLD [10^{-6}Å^{-2}]	Thickness [Å]	Buffer [%]
dDMPC VF	Heads	1.89*	9.2-11.2	48.4-54.7
	Tails	6.49°	26.4-27.7	0.1-0.2
dDMPC SALB	Heads	1.89*	6.8	39.4
	Tails	5.99	28.0	28.2

* Fixed value; ° linked over 3 dDMPC SLBs done by VF;
Only the dDMPC done by SALB contains a water layer.

Table 1 SLD [10^{-6}Å^{-2}], thickness [Å] and buffer content [%] of the head and tail layer of the 4 dDMPC SLBs done by vesicle fusion (VF) as well as of the SLB done by solvent-assistance (SALB).

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Effects of microbial glycolipids on phospholipid membranes using Atomic Force Microscopy (AFM)

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Microbial glycolipids are biosurfactants comprising a hydrophilic saccharide moiety coupled to a hy drophobic fatty acid. Their antimicrobial properties are raising an increasing interest as they might be alternatives to antibiotics, offering biodegradability and low ecotoxicity, and their production is based on renewable-resource substrates. Antimicrobial activity involves changes and rupture of the cellular mem brane inducing lysis. However, their mechanism of action is still not well-known. It might rely on the interaction of the saccharide moiety with the bacteria! membrane and posterior penetration thanks to the lipophilic moiety [1]. The current project aims to study the effect of glycolipids on supported lipid bilay ers using atomic force microscopy (AFM), to better understand the underlying molecular mechanisms at the nanoscale.
We observed the dynamic evolution of model phospholipid membranes after injection of two glycolipids. We also characterized the effect of glycolipids on the (nano)mechanical properties of membranes (Figure 1). Using a similar approach, supported!amellar structures can be formed by mixing phospholipids and glycolipids to form vesicle in suspension. We are exploring a wide range of phospholipid:glycolipid ratios to better understand their interaction s and properties. These insights into the effect of microbial glycolipids on model membranes at the nanometre scale can contribute to a better understanding of their biological activity, as well as their potential use as nanocarriers.

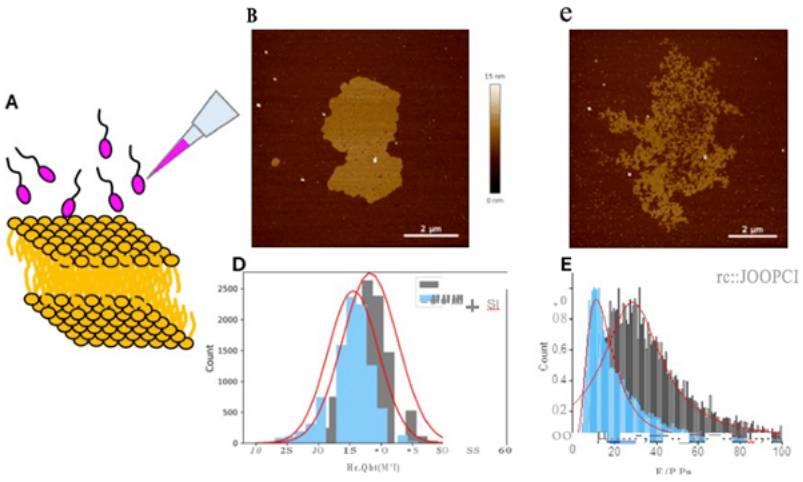

Figure I : A) Glycolipid injection on a phospholipid membrane. B) AFM topography image of a DOPC lipid membrane before and C) after adding sophorolipid (SL) solution . D) Histograms of the height of the membrane . and E) of the Young Modulus

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Molecule Tracking to unravel the role the bilayer plays in FtsZ active assembling

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FtsZ is a cytoskeletal protein crucial for bacterial cell division. It actively self-assembles into a filamentous structure (Z-ring) that forms at the division site using energy from GTP hydrolysis. In this study, we utilize single molecule tracking (SMT) to investigate the effects of FtsZ association to the membrane and subsequent filamentation on the lipid bilayer examining the diffusional behaviour of the lipids and the protein. To this aim, we used a FtsZ mutant (FtsZ-His) on supported lipid bilayers composed of POPC:DGS-NTA(Ni), where FtsZ-His can associate with the bilayer providing surface coverage. To determine the diffusion coefficient of either single fluorescent lipids or protein monomers we used fluorescently labeled lipid (DOPE) at a very low proportion (10-8 mol · mol-1), and fluorescently labelled FtsZ monomers at the same proportion.
Our results reveal that FtsZ association with the membrane even before filamentation has an effect on the bilayer itself, resulting in the slow down of lipid diffusion. Generalised Polarisation measurements showed that FtsZ did not increase lipid packing, and thus, it is not responsible for the slow down. Instead, we hypothesize the existence of local protein-lipid interactions that are revealed by single-molecule measurements. In addition, single-molecule tracking of FtsZ showed that protein monomers kept diffusing on the membrane upon filamentation, as two distinct diffusing populations were detected: some protein copies with diffusing with the same coefficient as before filamentation and some diffusing slower.

The conserved MPER-TMD region of SARS-CoV-2 S protein as a potential nucleic-acid vaccine

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4 years after the first cases of coronavirus disease were reported, its causative agent, SARS-CoV-2, remains in circulation, exhibiting noteworthy genetic and antigenic evolution within its spike protein (S), which is the main antigenic determinant of this virus and thus, the antigen of choice in vaccine design. Specifically, mutations are selected within the accessible S1 subunit, especially, in the immunodominant receptor binding domain. Consequently, although Omicron XBB.1.5 based vaccines now in use elicit cross-reactive responses against currently circulating SARS-CoV-2 variants, constant surveillance and update of the antigens included in the different vaccine platforms is needed. In an attempt to circumvent this problem, in this work, we analyze the immunogenicity of the membrane-proximal external region (MPER) of the S2 subunit, a highly conserved region across the Orthocoronavirinae subfamily in a SARS-CoV-2 infected human cohort and report that, although weak, a portion of the patients do elicit antibodies against this region. Moreover, we characterize its structure in a low-polarity environment and in lipid membranes and show its fusogenic potential. These results confirm its suitability as a potential vaccine candidate. Finally, considering this data and the potential impact of the lipid membrane on the antigenic structure of this region, we have designed and assessed the expression in the membrane of eukaryotic cells of a conserved S2-derived DNA sequence including the MPER-TMD region, which was delivered through solid lipid nanoparticles. The results obtained support the feasibility of designing a nucleic-acid vaccine targeting this region.

Impact of cannabidiol on pulmonary surfactant structure and function

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To increase the psychoactive effects of cannabis, Marijuana smokers tend to inhale more deeply and hold their breath longer than cigarette smokers. This smoking technique may potentiate the interaction of cannabinoids with pulmonary surfactant, a lipoprotein complex that covers the alveolar epithelium and lowers surface tension at the air/liquid interface to values close to 0 mN/m, avoiding atelectasis and facilitating respiratory mechanics. The aim of this work was to characterize the possible interaction of cannabidiol (CBD), a key ingredient of cannabis, with pulmonary surfactant. Results obtained with the captive bubble tensiometer show that CBD hampers the performance of native surfactant, inhibiting its surface activity. Adsorption experiments to preformed lipid monolayers show that CBD strongly interacts with dipalmitoylphosphatidylcholine (DPPC), the major lipid component of pulmonary surfactant. As a result, the lipid film became more fluid as determined by epifluorescence microscopy and compression isotherms. In summary, a preferential interaction of CBD with DPPC may be involved in the pathogenesis of respiratory disorders related to inhalation cannabis use.

The role of palmitoylation on the aggregation state of Pulmonary Surfactant protein C (SP-C): a time-resolved fluorescence study.

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Pulmonary surfactant (PS) is a lipid-protein complex that reduces surface tension at the air-liquid interface in the alveoli. Surfactant Protein C (SP-C) plays a crucial role in rearranging the lipids within PS during breathing. The N-terminal segment of SP-C, a lipopeptide consisting of 35 amino acids, contains two palmitoylated cysteines that impact the molecule's stability and structure. The C-terminal region includes a transmembrane α-helix with an ALLMG motif, supposedly analogous to a well-studied dimerization motif in glycophorin A. In this work, the oligomerization state of SP-C in membrane systems has been studied using fluorescence spectroscopy techniques. We have performed self-quenching and FRET assays to analyze dimerization of native palmitoylated SP-C and of a non-palmitoylated recombinant version of SP-C (rSP-C) using fluorescently labeled versions of either protein reconstituted in different lipid systems mimicking pulmonary surfactant environments. Our results [1] reveal that doubly palmitoylated native SP-C remains monomeric in membranes lacking compartmentalization. In contrast, non-palmitoylated recombinant SP-C exhibits dimerization, potentiated at high concentrations, especially in membranes with lipid phase separation. These findings suggest that palmitoylation could play a crucial role in stabilizing the monomeric α-helical conformation of SP-C. Depalmitoylation, high protein densities as a consequence of membrane compartmentalization, and other factors may all lead to the formation of protein dimers and higher-order oligomers, which could have functional implications under certain pathological conditions and contribute to membrane transformations associated with surfactant metabolism and alveolar homeostasis.

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Mechanism of action and lipid-mediated synergistic interactions of antimicrobial peptides: New regulatory options for membrane proteins?

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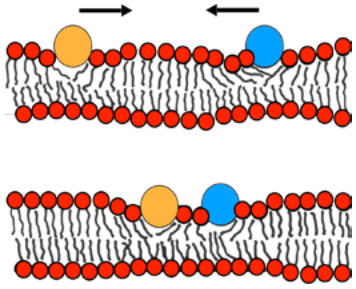
Biophysical and structural studies of peptide-lipid interactions, peptide topology and dynamics have changed our view how antimicrobial peptides insert and interact with membranes. Clearly, both the peptides and the lipids are highly dynamic, change and mutually adapt their conformation, membrane penetration and detailed morphology on a local and a global level. As a consequence, the peptides and lipids can form a wide variety of supramolecular assemblies in which the more hydrophobic sequences preferentially, but not exclusively, adopt transmembrane alignments and have the potential to form oligomeric structures similar to those suggested by the transmembrane helical bundle model. In contrast, charged amphipathic sequences tend to stay intercalated at the membrane interface, where they have been found to adopt mesophase structures in a lipid dependent manner. Although the membranes are soft and can adapt, at increasing peptide density they cause pronounced disruptions of the phospholipid fatty acyl packing. At increasing local or global concentrations the peptides result in transient membrane openings, rupture and ultimately lysis.
Interestingly mixtures of peptides such as magainin 2 and PGLa which are stored and secreted naturally as a cocktail exhibit considerably enhanced antimicrobial activities when investigated together in antimicrobial essays but also in pore forming experiments applied to biophysical model systems. Our investigations reveal that these peptides do not form stable complexes but act by specific lipid-mediated interactions and through the nanoscale properties of phospholipid bilayers. Notably, a quantitative idea about the strength of the lipid packing interactions can be obtained when comparing the peptide topologies in DMPC and POPC bilayers. The data are suggestive that similar new regulatory mechanisms exist also for larger membrane proteins.



The Figure illustrates the 'lipophobic effect'. In order to reduce the total amount of disorder of the lipid fatty acyl chains the in-plane oriented peptides pack more closely along the membrane surface. This can result in an attractive force between the peptides, similar to hydrophobic interactions in aqueous solution.

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Role of the transmembrane segment of the SARS-CoV-2 Spike protein in the viral-host membrane fusion process.

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Viral-host membrane fusion is a key early step for establishing infection in enveloped viruses such as SARS-CoV-2. Although membrane fusion is energetically favourable, free energy is required to overcome the kinetic barriers due to repulsive hydration forces that exist when two membranes approach each other [1]. Specifically in SARS-CoV-2 this free energy comes from the refolding of the metastable pre-fusion state of the trimeric Spike (S) protein to a stable post-fusion state where the transmembrane domain (TMD) and fusion peptide (FP) may interact, resulting in membrane fusion [2,3]. This transition between the pre- and post-fusion states is driven by a host furin-like protease and the host serine protease TMPRSS2 that allows the conformational changes needed for binding of the S protein trimer to the host ACE2 receptor and insertion of the fusion peptide (FP) in the host membrane respectively [4]. In this work we explore the role of the S protein TMD in membrane fusion process by investigating the effect of S protein trimerization to recognise ACE2. To this end, we have tested different TMD mutants (from alanine insertions to whole TMD replacements) of the full-length S protein using the Bimolecular Multicellular Complementation (BiMuC) assay (Figure 1) [5,6]. This assay allows qualitative (via fluorescence microscopy) and quantitative determination of the ability of S protein to fuse mammalian cell membranes and form syncytia. Furthermore, preliminary data about the crosstalk between the TMD and FP will be presented.

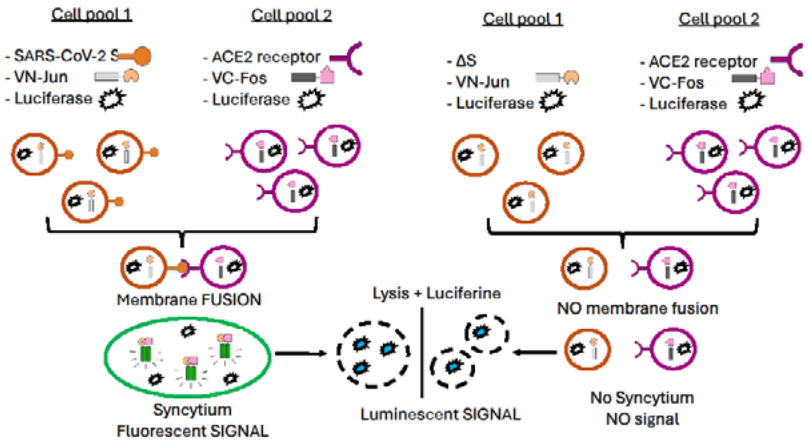

Figure 1 Schematic representation of the Bimolecular Multicellular Complementation (BiMuC) assay.

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Tuning the Assembly of Proteins and Peptides in Lipid Bilayers

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Interactions between peptides and lipids play a pivotal role in orchestrating intricate processes within cellular membranes, such as cell signaling and viral fusion. They are fundamentally important for maintaining membrane integrity and regulating membrane protein functions. Moreover, short peptides have sparked considerable interest in recent years due to their applications in nanomedical innovations propitiated by their ability to interact with biological systems [1]. Understanding these processes requires studying these interactions at a molecular level, which is empowered by computational tools, such as Molecular Dynamics (MD) simulations. While all-atom (AA) MD simulations can accurately model peptide conformation, they become computationally expensive when studying large systems and long timescales. Instead, coarse-grained (CG) models allow for the necessary conditions to model these systems. However, accurately reproducing the behavior of these peptides requires identifying their most stable conformations using AA-MD, which are then mapped into the CG model. We employ the MARTINI CG force field, developed to simulate the behavior of lipid membranes and proteins [2, 3] to comprehend the mechanisms underlying the interactions and structural assemblies of peptides on lipid membranes. Our aim is to use this understanding to engineer proteins and peptides that assemble into novel configurations, thereby designing membrane complexes to improve existing drug delivery systems.

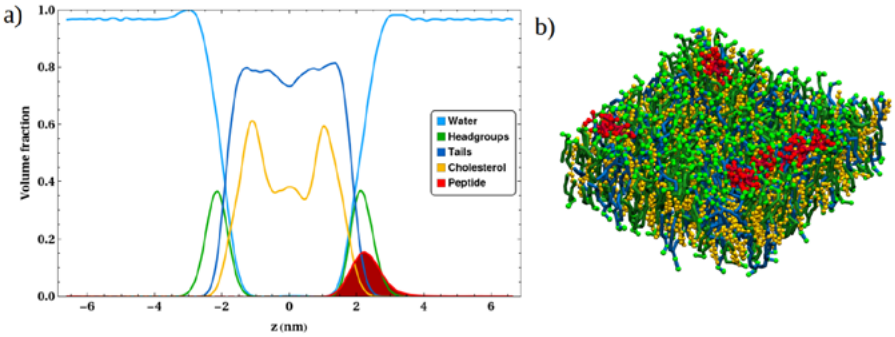

Figure 1. POPC:POPS:CHOL 3.2:5 lipid bilayer with five peptides bound to the interface.
a) Volume fraction profile normal to the bilayer distribution. b) Snapshot of the lipid membrane after 5μs of simulation. Solvent is omitted for clarity.

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Photosensitized manipulation of membrane tension triggers lipid phase separation and raft protein sorting

The lateral organization of the plasma membrane is fundamental to regulating a wide range of cellular processes. Compartmentalized regions or rafts are enriched with specific lipids that modulate the properties of the membranes and drive protein sorting. Novel methods and tools enabling the visualization, characterization and/or manipulation of lipid rafts are crucial to link the biophysical and mechanical properties of the membrane with cell functions. Flipper, a commercially available fluorescent membrane tension probe, has become a reference tool for quantitative membrane tension studies using Fluorescence Lifetime Imaging Microscopy (FLIM). However, concerns of Flipper regarding cell phototoxicity have also arisen. Here, we report that Flipper embedded into lipid membranes photosensitizes singlet oxygen ($^1O_2$) under blue light, producing lipid hydroperoxides that alter the properties and organization of lipid membranes. We first combine the reporting and photosensitizing abilities of Flipper to simultaneously induce and visualize changes in the membrane tension by FLIM imaging. We show that lipid hydroperoxidation increases membrane tension and triggers phase separation in model and biological membranes. Strikingly, the segregated domains retain the sorting ability of intact phase-separated membranes, directing raft and non-raft proteins into ordered and disordered regions, respectively, in contrast to radical-based photo-oxidation reactions that disrupt protein partitioning. Moreover, the localized production of $^1O_2$ at the illuminated area provides high spatiotemporal control of the photo-oxidation reactions, leaving the surrounding membranes unaltered. Our results uncover additional properties of Flipper that should be considered in light-based tension measurements and open new avenues for optical control and visualization of lipid raft formation and directed protein sorting.

Lipids as Drug Discovery Probes in TRPV channels

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Membrane proteins are integral to diverse biological processes, such as transport and cellular signaling, their activity often governed by lipid bilayer composition and properties. Lipids bind to specific sites on these proteins, regulating function, trafficking, and interactions. However, the intricate nature of these interactions, compounded by experimental challenges, impedes full elucidation of lipid-mediated regulation mechanisms. In our study, we utilized coarse-grained molecular dynamics simulations (CG-MD) to investigate lipid-binding sites and interactions across all six transient receptor vanilloid channels (TRPVs), employing a comprehensive asymmetric membrane composition of 10 lipids. CG-MD simulations on Cryo-EM TRPV apo structures not only validated known binding sites for cholesterol and phosphatidylinositol-(3,4)-biphosphate but also unveiled potential binding sites for these lipids and others. By correlating known TRPV drug binding sites with identified lipid binding sites, both established and putative, we could assign specific residues, offering fresh insights for rational drug design targeting TRPV channels. Our research underscores CG-MD simulations' effectiveness in uncovering potential regulatory regions on membrane proteins, providing specific insights into TRPV's lipid-protein interactions. This expansion of our understanding of native membrane protein regulation complexity lays a foundation for future drug development efforts targeting lipid-binding sites on TRPVs and other membrane proteins.

Computational Dissection of Peptide Translocation Using Computational Electrophysiology and Adaptive Steered Molecular Dynamics

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The cell membrane is a highly selective and dynamic barrier that encloses the contents of all living cells, while regulating the flux of species between intra- and extra cellular compartments. Composed predominantly of phospholipids, it gains its selectively permeable nature. Cell penetrating peptides (CPPs) are small, positively charged peptides capable of traversing the cell membrane without inducing cellular toxicity [1]. CPPs demonstrate great potential in the delivery of various cargo such as proteins, nucleus acids, or nano particles, providing CPPs with substantial potential across various fields. The penetration mechanisms described are passive diffusion, pore formation, translocation, and endocytosis [2].
To assess whether a peptide possesses CPP-like capabilities, in vivo experiments can be performed. Nonetheless, this method does not allow the description of the translocation mechanism, which can be achieved through molecular dynamics (MD) simulations. Regrettably, to the best or our knowledge, there are no existing tools to conduct such unbiased MD experiments. In this study, we introduce a novel approach involving a double membrane composition to perform Computational Electrophysiology (CompEL) [3] for identifying peptides with these abilities. Besides, we compare it with adaptive Steered Molecular Dynamics (aSMD) combined with conventional Molecular Dynamics (cMD), a method that we have previously proposed in a recent paper (manuscript currently under review).
To conduct such study, we have chosen four canonical CPPs: nona-Arginine (Arg9), Model Amphipathic Peptide (MAP), Transportan 10 (TP10), and Translocating Peptide 2 (TP2). In this research, we have been able to observe undirected peptide translocation, pore formation, lipid bilayer reorganization, and peptide insertion.

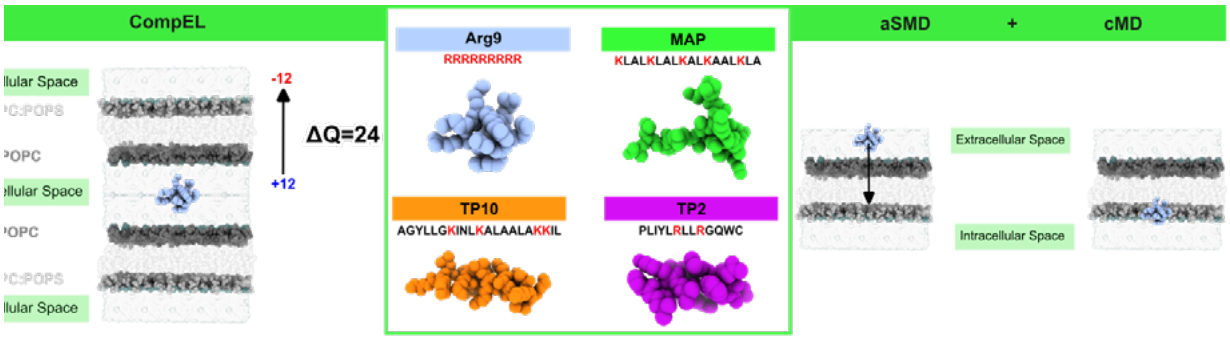

Figure 1 Illustrative representation of the two computational methods studied in this research: Computational Electrophysiology (CompEL) and adaptive Steered Molecular Dynamics (aSMD) in combination with conventional Molecular Dynamics (cMD).

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Fluorescence quenching reveals the distribution of two synergistic antimicrobial peptides on a lipid membrane surface

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Antibiotic resistance is regarded as one of the most urgent challenges in public health and necessitates comprehensive efforts to combat it. Antimicrobial peptides offer a viable and promising alternative to traditional antibiotics by targeting bacterial cells through diverse pathways that are less susceptible to resistance development.
Fluorescence quenching is used to determine the peptides' distribution on membrane surfaces. The focus of this work lies on two cationic amphipathic peptides from the magainin family: PGLa and magainin 2, which partition into the membrane interface and interact with biological membranes synergistically. Hereby, one peptide helps the other to perforate the lipid membrane. Synergism is also observed in antimicrobial assays. Understanding their interaction with each other provides new insights into how membrane-active antibiotic peptides work.
Peptide labeling at different positions along the PGLa sequence (1, 10, 16, 21) was performed by introducing the diaminopropionic acid (Dap) which intrinsically carries a fluorophore (nitrobenzoxadiazole, NBD) coupled to the amide of the side chain. This labeling scheme allows one to establish a model for the distribution of the peptide helices which are oriented along the membrane surface. Fluorophores quench each other in a radius of 1 nm. Using N-terminal labeled PGLa it has been demonstrated previously[1], that PGLa and magainin 2 assemble into defined supramolecular structures. The formation of such assemblies is confirmed here, even at high peptide dilution. Furthermore, we observe that the presence of magainin 2 alters PGLa's distribution on the surface. Interestingly, magainin 2 seems to prevent PGLa-Dap10-NBD clustering, while the N-terminus, as well as the position 16 more likely remain close to each other.

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The G protein-coupled receptor (GPCR) are membrane proteins which act as key players in cell to cell communication and control most of physiological cell responses. Vasopressin receptors are GPCR regulating water homeostasis. In particular, the V2 type is expressed in renal cells, where it governs water reabsorption. V2R has long resisted to crystallization trials and cryoEM characterization, because of intrinsic flexibility. We had previously used NMR to characterize its different structural compartments [1-3]. In this work, we analyse the conformational landscape of the V2R in distinct pharmacological conditions using liquid-state NMR spectroscopy by monitoring signals from ^{13}C-methyl-labelled lysines. In particular, we investigate the structure and dynamics changes upon binding to different ligands ranging from agonist to antagonists, non-selective or selective (biased) towards the G-protein signalization pathway. Our results outline common features as well as distinct particularities with other GPCRs. We also used specific NMR restraints, ranging from STD (Saturation Transfer Difference) to paramagnetic ones, to decipher the pose of the endogenous vasopressin ligand onto the low-resolution structures of V2 obtained by cryoEM [4], but also to determine the docking pose of a biased ligand in molecular dynamic simulations, revealing the mechanistic details of V2R biased activation [5]. We will discuss the generalization potential of our approach to other receptors and how V2R biased activation mechanism elicited in this study compares to that of the mu receptor to opioid which we recently studied [6-7].

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Exploring labetalol permeation across lipidic membranes: Insights from the pH variation assay

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The major barrier for permeation is the lipid bilayer, and most xenobiotics permeate this barrier by passive permeation. Understanding unassisted permeation behaviour of bioactive molecules across lipidic membranes is crucial for optimizing drug delivery and enhancing therapeutic efficacy. In the pH variation assay, the permeation of weak acids and bases is followed through changes in the pH of the aqueous medium inside liposomes encapsulating a pH sensitive fluorescent probe, being one of the few methodologies with wide applicability for drugs and biological ligands[1].
Labetalol is a beta-blocker used to treat hypertension that possesses two ionizing groups, an aliphatic amine and a phenolic group (pK_a=9.8 and 8.0, respectively, as calculated by MarvinSketch). At pH=7, the rate of equilibration of Labetalol across lipid membrane is 2-orders of magnitude slower than that of drugs containing an amine group only (Figure 1), in spite of the similar relative abundance of neutral form and comparable lipophilicity ($CLogD_{Labetalol}$=1.3 and $CLogD_{Propanolol}$=0.73). A comprehensive characterization of labetalol interactions with distinct lipid membranes was performed to identify the reasons for the distinctive behaviour. This included the partition coefficient for the lipid membranes, changes in ionization upon association with the membrane, and effect of pH and temperature on membrane-affinity and rate of permeation. The results were analysed with the detailed kinetic model recently developed by us[2], to rationalize the contribution of the distinct ionization species to the affinity for the membrane, to the rate of translocation through the membrane non-polar centre, and to the overall permeability coefficient.

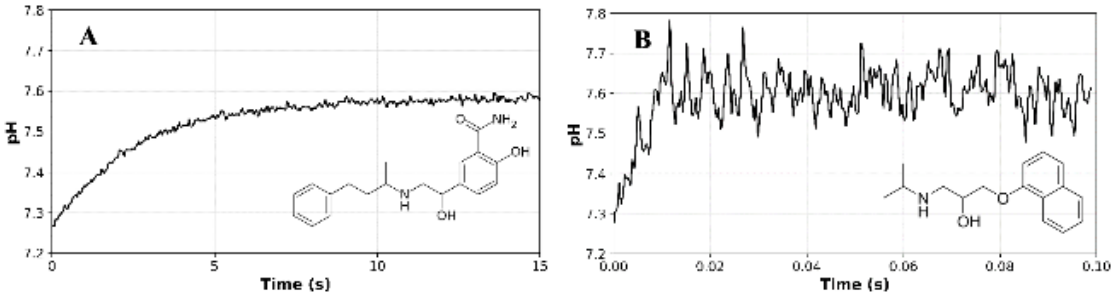

Figure 1. pH variation inside of the POPC liposomes upon permeation of A) Labetalol and B) Propanolol.

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Hoechst 33342 acid/base properties and interaction with POPC membranes

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The fluorescent probe H33342 possesses 5 ionizing groups and is commonly used to stain the DNA of living cells. To accomplish this, it needs to interact with and permeate through cell membranes, despite its high overall charge at physiological pH values. The aim of this work is to evaluate H33342 properties and better understand the mechanisms of interaction of this probe with lipidic membranes.
H33342 acid-base properties were assessed by absorbance and fluorescence spectroscopy.
The effect of pH in the association of H33342 with lipid bilayers were measured using a combined experimental and computational approach. The partition of H33342 to POPC lipid membranes was quantified using fluorescence spectroscopy and isothermal titration calorimetry measurements. Interaction of the most stable isomer with POPC bilayers was studied by both unrestrained and umbrella sampling molecular dynamics simulations (MD).
Both experimental and computational results indicate that the partition coefficient of H33342 displays a small variation over a wide pH range, not exceeding one order of magnitude. The enthalpy variation upon partition to the membrane suggests efficient hydrogen bonding between the probe and the lipid, namely, for the +2 form, which was confirmed by MD. The relatively high lipophilicity of charged species contrasts with the decrease in their general hydrophobicity as estimated from octanol/water partition. [1]

This work clearly shows that lipophilicity cannot be directly estimated from solute hydrophobicity ($LogP_{oct}$ and $LogD_{oct}$), highlighting the importance of considering acid-base properties and the association with lipid bilayers when predicting the affinity for biomembranes.

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Langmuir monolayers with real cell membranes and their interaction with doxorubicin anticancer drug

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Langmuir monolayers of lipid mixtures have typically been used to mimic half of cell membranes and study their interactions with other substances, such as anticancer drugs, under controlled conditions [1,2]. However, the composition of biological membranes is complex and diverse making it difficult to replicate. Thus, it would be convenient to start exploring the formation of Langmuir films with membranes extracted from real cells [3].
In order to maintain all the components of the membrane, we create stable Langmuir films with membranes extracted from human breast adenocarcinoma cells (line MCF-7) and healthy fibroblasts. The membranes were characterized via their surface pressure–mean molecular area isotherm and AFM images, and the effect of doxorubicin anticancer drug in both films was analyzed by introducing the drug into the subphase while recording the changes in surface pressure and morphology of the films.
Finally, Langmuir monolayers were created using lipid mixtures based on the lipidic composition of breast tumor cells found in literature [4,5], to validate the use of lipidic models. The results obtained on models with and without doxorubicin in the subphase were compared with those obtained with real membranes to determine their degree of reliability.

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Abstracts: Posters

Session IX: Advanced Bioimaging

Protein-stabilized iron nanoclusters as novel MRI contrast agents

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Gadolinium-based MRI contrast agents are commonly used in clinical research but present drawbacks such as high toxicity [1]. Protein-stabilized iron nanoclusters (FeNC) are an emerging alternative for manufacturing novel MRI contrast agents. For this purpose, consensus tetratricopeptide repeat (CTPR) proteins were employed, leveraging their modular nature to allow the introduction of metal coordination sites without substantial impact on the protein structure, subsequently stabilizing iron atoms into nanoclusters. These proteins were previously engineered to introduce cysteine-based metal binding sites [2]. The complex formed exhibit remarkable relaxivity properties allowing their examination as Magnetic Resonance Imaging (MRI) contrast agents. In this work, we focused in developing T_1-based positive contrast agents considering the accurate signal and brightness in clinical applications.

CTPR-FeNC can be further engineered to bind relevant molecular targets, such as the chaperone heat shock protein 90 (Hsp90), which has been described as a component of the TGFαsignaling cascade. This protein (Hsp90) is overexpressed in several pathologies such as fibrosis and certain cancers [3]. The CTPR employed in this work presents, apart from the metal binding site, a C390 module which inhibits the Hsp90. This provides antitumoral and antifibrotic effects, showcasing its potential therapeutic value in targeting cancer and fibrosis. In this study, we have evaluated the viability in cancerous and fibrotic cell lines and its functionality in 3D in vitro fibrotic models.

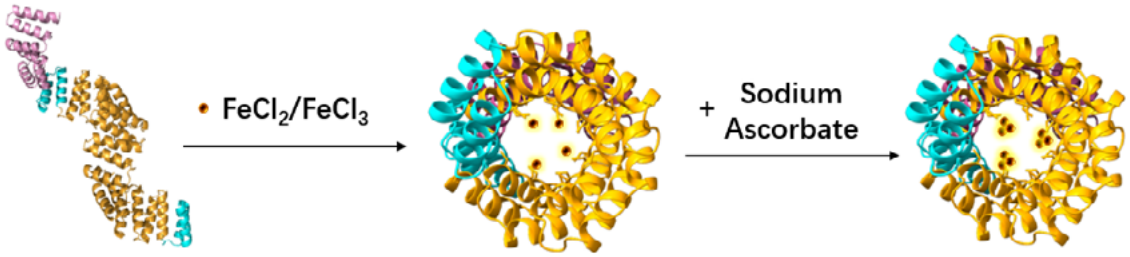

Figure 1 Protein scaffold (CTPR) with Cysteine domain (yellow) for metal binding and module for Hsp90 inhibition (pink).

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Multifunctional Protein-stabilized Nanomaterials for Theranostics Applications

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Protein-stabilized nanomaterials have emerged as highly promising tools for the development of personalized multifunctional biologics, offering significant potential in both biotechnological and biomedical applications. Particularly noteworthy are designed metal coordination proteins that seamlessly integrate safety and biocompatibility with the versatile functionalities of metallic nanoparticles [1]. Among these, Consensus Tetratricopeptide Repeat (CTPR) proteins stand out for their robustness and mutational permissiveness, thanks to their repetitive structure allowing the incorporation of metal coordination sites without causing a significant structural impact [2].

In this work, CTPR proteins were modified to include cysteine-based metal coordination sites for Gd and Fe incorporation. Subsequently, these proteins were used for the synthesis of protein-stabilized nanomaterials (Prot-NCs) with relaxometric properties, enabling their use as contrast agents for Magnetic Resonance Imaging (MRI). Furthermore, the versatility of these proteins allows the incorporation of a CTPR390 recognition module, exhibiting specific binding to the Hsp90 protein. Hsp90 is an essential molecular chaperone for cell survival, and its inhibition by CTPR390 is being explored as a potential antifibrotic and anticancer treatment. Thus, this strategy enables the integration of a therapeutic protein module with a nanomaterial-stabilizing module within a single molecule, yielding a multifunctional nanocomposite. In this context, following comprehensive characterization and in vitro evaluation, the functionality of Prot-NCs was assessed in complex in vitro 3D fibrotic models and an orthotopic mouse cancer model.
Overall, this work serves as a proof of concept, revealing the versatility and potential of Prot-NPs in biomedical applications through the development of MRI contrast agents with targeting ability.

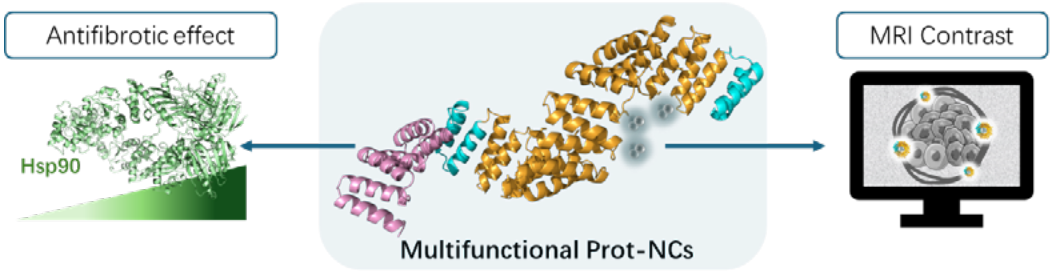

Figure 1 Schematic illustration of the multifunctional behavior of Prot-NCs. Main image showed the Protein scaffold (CTPR) with cysteine domain (yellow) for metal (grey) binding and the recognition module CTPR390 (pink) for Hsp90 inhibition.

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Multiphoton imaging of structural collagen for assessment of antifibrotic effect of CTPR390 nanodrug in engineered human heart tissue

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The use of engineered connective tissue (ECT) with human adult cardiac cells embedded in collagen matrix which mimic the 3D arquitecture of the heart, has been used in this study as a drug screening platform.[1] While preserving the cardiac functionality the ECT is utilized to explore the mechanical, biochemical, and structural features of the tissue when exposed to an experimental anti-fibrotic drug. The ECT undergoes fibrosis when exposed to the main pro-fibrotic protein, Transforming Growth Factor α(TGFα). [2]
CTPR390 consists of small engineered protein domains, which effectively binds and inhibits TPR protein-protein interactions with the TGFαreceptor complex, thereby impeding fibrosis. [3]

The study demonstrated a correlation between collagen orientation at the nanoscale with fibrosis within the ECT, allowing differentiation between well-organized collagen structures in TGFα-activated ECT and less organized or disoriented collagen in control ECT and TGFα-activated ECT treated with CTPR390.
Overall, these findings enhance our understanding of collagen's role in tissue biomechanics and provide valuable insights for therapeutic interventions targeting fibrotic conditions.

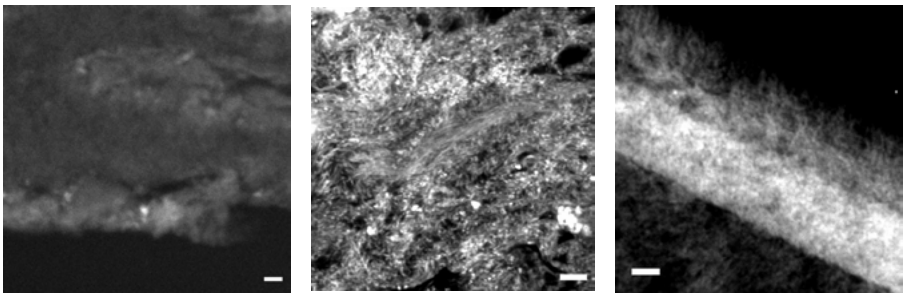

Figure 1. Multiphoton image of collagen (I) in Control EC, TGFα-activated ECT and TGFα-activated ECT post-CTPR390 treatment (TGFα-CTPR390). The scale bar is 50 micra. Image was acquired with LSM 880 NLO (Zeiss) coupled with MaiTaiDeepSee multiphoton laser using 40x (W, 1.4) objective.

In summary engineered tissues, play an increasingly vital role in minimizing the use of laboratory animals and advancing personalized medicine approaches. The inclusion of 3D architectures in culture systems enhances the physiological and pathological relevance of these models which can be monitored by multiphoton optical microscopy in a non-invasive way.

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Towards Volumetric Imaging of Mechanically Stress Propagation in developing C. elegans Embryos

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Mechanosensation is the ability of specialized cells, known as mechanoreceptors, to detect and transduce external mechanical stimuli into electrochemical signals. Through the process of mechanotransduction, these stimuli trigger a cascade of intracellular responses, including altering ion concentrations (i.e., Ca^{2+}) and activating signaling pathways [1, 2]. Thank to this process, common to all living organisms and fundamental for survival, we are able to experience the external world. Considering its crucial importance, any dysfunction in mechanotransduction can have profound consequences, potentially leading to neurological and developmental disorders. A detailed knowledge about the onset and timing of mechanosensation would facilitate our understanding of neurodevelopment and its dysfunctions.
However, studying mechanotransduction in living, developing organisms is still challenging due to the lack of techniques to simultaneous apply and measure precise mechanical insults, and observe their consequences on nervous system activity. Here, I aim to overcome these challenges by developing an advanced engineering system to apply, observe, and study the effects of mechanical stimulation on touch receptor neurons (TRN) in Caenorhabditis (C.) elegans embryos.To achieve precise and reproducible mechanical stimulation, we utilize a fiber-optics-based nanoindenter [3], mounted on an open-top light-sheet microscope (OT-LSFM) [4]. This customized OT-LSFM enables rapid, three-dimensional acquisitions, reduces photodamage during long-term imaging, and provides mechanical access to the sample.
The final goal of this investigation is to understand the timeframe during which TRNs, already present in the embryo from early stages, develop their mechanosensitive properties despite the lack of apparent exposure to external forces in the eggshell.

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Abstracts: Posters

Session X: Biological Physics

Electrical Characterization of Tryptophan-Substituted Peptides Assemblies

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Bioelectronic materials, bridge biological and electronic systems, offering transformative potential in diverse applications ranging from biotechnology to biomedical devices [1,2]. Proteins and peptides, with their inherent biocompatibility and self-assembly capabilities, have emerged as promising candidates for constructing bioelectronic interfaces. Peptide nanostructures offer a platform for precise engineering of electronic functionalities at the nanoscale. In this context, the integration of aromatic amino acids, such as tryptophan, can increase ϖ-stacking interactions within peptides, thereby influencing their electronic properties[3].
In this study, we explored the self-assembly capabilities of different tryptophan-substituted peptides. We performed an electrical characterization under vacuum and ambient conditions. Our findings suggest that the selective inclusion of tryptophan residues in the peptide sequences resulted in enhanced interactions, which in turn yield substantial changes to their electrical properties.

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The role of the tubular topology in the biomechanics and disassembly of Tobacco Mosaic Virus

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Tubular viruses have recently awakened interest as a template for nanostructures, especially the Tobacco Mosaic Virus (TMV) [1]. However, despite the multiple researchers in this field, little is known about the specifics of its mechanical disassembly [2]. Here we present how the capsid responds to single indentations [3] and sustained forces, exploring mechanical fatigue through a combination of experimental data and coarse-grain simulations. Our experiments show how TMV disassembles through the progressive detaching of proteins along the whole capsid, forming rifts that grow until nothing remains. Mechanical fatigues combined with nanoindentations reveal how the central hollow tube concentrates the mechanical stress on the upper half of the virus, enhancing its resilience and producing a buckling effect until the inner walls collide and the capsid completely collapses.

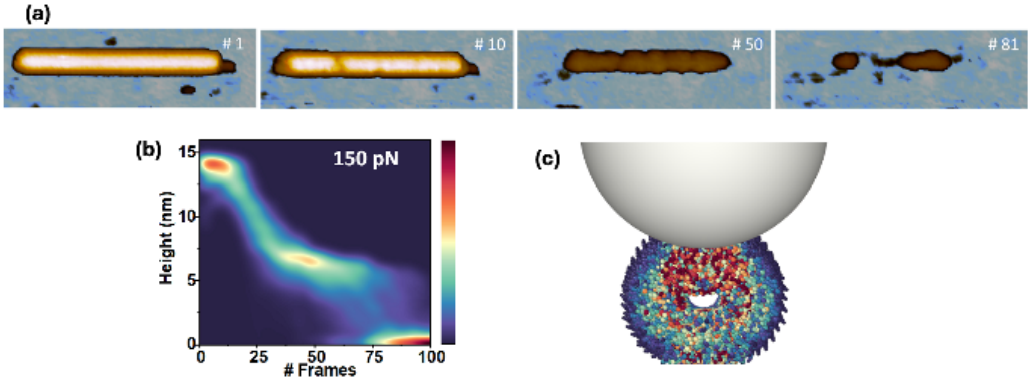

Figure 1. (a) Mechanical fatigue on TMV particle: #1 first frame, #10 beginning of disassembly, #50 disappearance of the upper half, and #81 final remains. (b) Density plot of the mechanical fatigue showing a stable step once the upper half is lost. (c) Coarse-grain simulation of the stress distribution of an AFM tip against the protein capsid (axial view).

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