Emerging Theoretical Approaches to Compliment Single-Particle Cryo-Electron Microscopy

Trieste, Italy | October 21–25, 2024

Biophysical Society

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October 2024

Dear Colleagues,

We would like to welcome you to the Biophysical Society Thematic Meeting entitled, *Emerging Theoretical Approaches to Complement Single-Particle Cryo-Electron Microscopy*. This thematic meeting seeks to explore the interface between computational biophysics and cryo-EM, highlighting the breadth of work that spans these two fields, and encouraging new synergies. Our goal for this meeting is to maximize the potential of computations and experiments in the field of single-particle cryo-EM.

Overall, this conference will feature 32 posters, 35 lectures, and bring together over 80 scientists from a wide range of backgrounds and expertise. We hope that this meeting will not only provide a place to share your recent findings, but also to help promote new collaborations, helpful discussions, and future connections.

We invite you all to actively take part in the discussions following each talk, the poster sessions, and the informal exchanges that will be possible during the coffee breaks and meals. We also hope that you will also enjoy the beautiful city of Trieste!

We would like to thank our generous sponsors: CECAM-IT-SISSA, Structura Biotechnology, Agouron Institute, QRB Discovery Journal, and Mark III Systems in conjunction with NVIDIA for their support of this meeting.

The Organizing Committee
Rommie Amaro
Gabriel Lander
Alessandra Magistrato
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Biophysical Society Code of Conduct, Anti-Harassment Policy

The Biophysical Society (BPS) is committed to providing an environment that encourages the free expression and exchange of scientific ideas. As a global, professional Society, the BPS is committed to the philosophy of equal opportunity and respectful treatment for all, regardless of national or ethnic origin, religion or religious belief, gender, gender identity or expression, race, color, age, marital status, sexual orientation, disabilities, veteran status, or any other reason not related to scientific merit.

All BPS meetings and BPS-sponsored activities promote an environment that is free of inappropriate behavior and harassment by or toward all attendees and participants of Society events, including speakers, organizers, students, guests, media, exhibitors, staff, vendors, and other suppliers. BPS expects anyone associated with an official BPS-sponsored event to respect the rules and policies of the Society, the venue, the hotels, and the city.

Definition of Harassment
The term "harassment" includes but is not limited to epithets, unwelcome slurs, jokes, or verbal, graphic or physical conduct relating to an individual's race, color, religious creed, sex, national origin, ancestry, citizenship status, age, gender or sexual orientation that denigrate or show hostility or aversion toward an individual or group.

Sexual harassment refers to unwelcome sexual advances, requests for sexual favors, and other verbal or physical conduct of a sexual nature. Behavior and language that are welcome/acceptable to one person may be unwelcome/offensive to another. Consequently, individuals must use discretion to ensure that their words and actions communicate respect for others. This is especially important for those in positions of authority since individuals with lower rank or status may be reluctant to express their objections or discomfort regarding unwelcome behavior. It does not refer to occasional compliments of a socially acceptable nature. It refers to behavior that is not welcome, is personally offensive, debilitates morale, and therefore, interferes with work effectiveness. The following are examples of behavior that, when unwelcome, may constitute sexual harassment: sexual flirtations, advances, or propositions; verbal comments or physical actions of a sexual nature; sexually degrading words used to describe an individual; a display of sexually suggestive objects or pictures; sexually explicit jokes; unnecessary touching.

Attendees or participants who are asked to stop engaging in harassing behavior are expected to comply immediately. Anyone who feels harassed is encouraged to immediately inform the alleged harasser that the behavior is unwelcome. In many instances, the person is unaware that their conduct is offensive and when so advised can easily and willingly correct the conduct so that it does not reoccur. Anyone who feels harassed is NOT REQUIRED to address the person believed guilty of inappropriate treatment. If the informal discussion with the alleged harasser is unsuccessful in remedying the problem or if the complainant does not feel comfortable with such an approach, they can report the behavior as detailed below.

Reported or suspected occurrences of harassment will be promptly and thoroughly investigated. Following an investigation, BPS will immediately take any necessary and appropriate action. BPS will not permit or condone any acts of retaliation against anyone who files harassment complaints or cooperates in the investigation of same.

Reporting a Violation
Violations of this Conduct Policy should be reported immediately. If you feel physically unsafe or believe a crime has been committed, you should report it to the police immediately.

To report a violation to BPS:

- You may do so in person at the Annual Meeting at the BPS Business Office in the convention center.

- You may do so in person to BPS senior staff at Thematic Meetings, BPS Conferences, or other BPS events.

- At any time (during or after an event), you can make a report through http://biophysics.ethicspoint.com or via a dedicated hotline (phone numbers listed on the website) which will collect and relay information in a secure and sensitive manner.

Reported or suspected occurrences of harassment will be promptly and thoroughly investigated per the procedure detailed below. Following an investigation, BPS will immediately take any necessary and appropriate action. BPS will not permit or condone any acts of retaliation against anyone who files harassment complaints or cooperates in the investigation of same.

Investigative Procedure

All reports of harassment or sexual harassment will be treated seriously. However, absolute confidentiality cannot be promised nor can it be assured. BPS will conduct an investigation of any complaint of harassment or sexual harassment, which may require limited disclosure of pertinent information to certain parties, including the alleged harasser.

Once a complaint of harassment or sexual harassment is received, BPS will begin a prompt and thorough investigation. Please note, if a complaint is filed anonymously, BPS may be severely limited in our ability to follow-up on the allegation.

- An impartial investigative committee, consisting of the current President, President-Elect, and Executive Officer will be established. If any of these individuals were to be named in an allegation, they would be excluded from the committee.

- The committee will interview the complainant and review the written complaint. If no written complaint exists, one will be requested.

- The committee will speak to the alleged offender and present the complaint.

- The alleged offender will be given the opportunity to address the complaint, with sufficient time to respond to the evidence and bring his/her own evidence.

- If the facts are in dispute, the investigative team may need to interview anyone named as witnesses.

- The investigative committee may seek BPS Counsel's advice.

- Once the investigation is complete, the committee will report their findings and make recommendations to the Society Officers.

- If the severity of the allegation is high, is a possible repeat offense, or is determined to be beyond BPS's capacity to assess claims and views on either side, BPS may refer the case to the alleged offender's home institution (Office of Research Integrity of similar), employer, licensing board, or law enforcement for their investigation and decision.

Disciplinary Actions

Individuals engaging in behavior prohibited by this policy as well as those making allegations of harassment in bad faith will be subject to disciplinary action. Such actions range from a written warning to ejection from the meeting or activity in question without refund of registration fees, being banned from participating in future Society meetings or Society-sponsored activities, being expelled from membership in the Society, and reporting the behavior to their employer or calling the authorities. In the event that the individual is dissatisfied with the results of the investigation, they may appeal to the President of the Society. Any questions regarding this policy should be directed to the BPS Executive Officer or other Society Officer.

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GENERAL INFORMATION

Registration/Information Location and Hours

The meeting will take place at the International School for Advanced Studies (SISSA), located at Via Bonomea 265 in Trieste, Italy. The BPS Registration Desk, to pick up your badge and meeting materials, will be located in the Aula Magna Sala Attesa during the following times:

Monday, October 21	16:30 – 18:30
Tuesday, October 22	8:00 – 18:00
Wednesday, October 23	8:00 – 18:00
Thursday, October 24	8:00 – 15:00
Friday, October 25	8:00 – 12:00

Instructions for Presentations

(1) Presentation Facilities:

A data projector will be available in the Aula Magna Lecture Hall. Speakers are required to bring their own laptops and adaptors. It is recommended to have a backup of the presentation on a USB drive in case of any unforeseen circumstances. Speakers are advised to preview their final presentations before the start of each session.

(2) Poster Session:

1) All poster sessions will be held in the Aula Magna Sala Attesa.

2) A display board measuring 95 cm by 135 cm (portrait orientation) will be provided for each poster. Poster boards are numbered according to the same numbering scheme as listed in the E-book.

3) Posters will slide into the poster boards, and no mounting materials are required. However, we recommend bringing some (push pins, tape, etc.) in case they are needed.

4) There will be formal poster presentations on Monday and Tuesday from 16:20 – 18:20. Please refer to the daily schedule for your formal presentation date and time. Two hours (120 minutes) have been allotted for poster presentations each day. Presenting authors with odd-numbered poster boards should present during the first 60 minutes, and those with even-numbered poster boards should present during the last 60 minutes.

5) During the assigned poster presentation sessions, presenters are requested to remain in front of their poster boards to meet with attendees.

6) All posters left uncollected at the end of the meeting will be discarded.

Note Pads/Pens

Society pens will be provided, however please bring your own note pad.

Meals, Coffee Breaks, and Socials

The opening mixer, coffee breaks and luncheons will be served in the Aula Magna Foyer. The Wednesday evening social dinner will be held at 8:00 PM (20:00) in the restaurant of the Savoia Excelsior Palace Hotel, located at Riva del Mandracchio 4.

Smoking

Please be advised that the International School for Advanced Studies (SISSA) is a non-smoking facility.

Name Badges

Name badges will be given to you when you check-in at the Registration Desk in the Aula Magna Sala Attesa. Badges are required to enter all scientific sessions, poster sessions, and social functions. Please wear your badge throughout the conference.

Internet

Wi-Fi will be provided at the venue. Information will be available at the registration desk.

On-Site Contact Information

If you have any further requirements during the meeting, please contact the meeting staff at the registration desk from October 21-25 during registration hours.

In case of emergency, you may contact the following:

Dorothy Chaconas
Phone: 301-785-0802
Email: dchaconas@biophysics.org

Erica Bellavia
Phone: 571-435-7669
Email: ebellavia@biophysics.org

Emerging Theoretical Approaches to Compliment Single-Particle Cryo-EM

Trieste, Italy
October 21-25, 2024

All scientific sessions will be held in the Aula Magna Lecture Hall unless otherwise noted.

PROGRAM

Monday, October 21, 2024

16:30 – 18:30	**Registration/Information**	**Aula Magna Sala Attesa**
16:30 – 18:30	**Opening Mixer**	**Aula Magna Foyer**

Tuesday, October 22, 2024

8:00 – 18:00	**Registration/Information**	**Aula Magna Sala Attesa**
8:30 – 8:40	**Welcome and Opening Remarks** Giulia Palermo, University of California, Riverside, USA	
Session I	**Structure and Dynamics** Giulia Palermo, University of California, Riverside, USA, Chair	
8:40 – 9:15	Manidipa Banerjee, Indian Institute of Technology, Delhi, India ***Structural Dynamics of a Non-Enveloped Virus During Disassembly***	
9:15 – 9:50	Yuji Sugita, RIKEN Center for Biosystems Dynamic Research, Japan ***Integrative Modeling of Protein Structure and Dynamics Using MD Simulations and Experimental Data***	
9:50 – 10:25	Hong Zhou, University of California, Los Angeles, USA ***AI-Based Methods to Overcome the Preferred Orientation and Missing-Wedge Problems in Cryo-EM and Cryo-ET***	
10:25 – 10:55	**Coffee Break**	**Aula Magna Foyer**
Session II	**Methodological Advances in Cryo-EM** Alessandra Magistrato, International School for Advanced Studies, Italy, Chair	
10:55 – 11:30	Paulina Dominiak, University of Warsaw, Poland ***Exploiting the Full Potential of Cryogenic Electron Microscopy Maps***	
11:30 – 12:05	Jose-Maria Carazo, Spanish National Center for Biotechnology, Spain ***Zernike3d and Hetsiren for Conformational and Compositional Heterogeneity Analysis of Cryo-EM Images***	
12:05 – 14:00	**Lunch**	**Aula Magna Foyer**
Session III	**Cryo-EM Applications** Joanna Trylska, University of Warsaw, Poland, Chair	
14:00 – 14:35	Abhishek Singharoy, Arizona State University, USA ***Ice-Cream: Integrated Cyberinfrastructure for Ensemble Cryo-EM Applications & Modeling***	

14:35 – 15:10	Audrone Lapinaite, Arizona State University, USA ***Understanding the Molecular Mechanism of Genome Editors to Enhance their Precision and Targeting Scope***	
15:10 – 15:30	Ivaylo Ivanov, Georgia State University, USA * ***Molecular Architecture and Functional Dynamics of the Pre-Incision Complex in the Nucleotide Excision DNA Repair Pathway***	
15:30 – 15:50	Matthew Holcomb, Scripps Research Institute, USA * ***CRYOXKIT: Incorporation of Experimental Structural Density into Autodock for Improved Pose Prediction***	
15:50 – 16:10	Milosz Wieczór, Gdańsk University of Technology, Poland * ***Inferring the Conformational Landscape of Multistate Protein Assemblies Using Coarse-Grained Sampling of Transition Pathways***	
16:20 – 18:20	**Poster Session I**	**Aula Magna Sala Attesa**
18:20	**Dinner on Own**	

Wednesday, October 23, 2024

8:00 – 18:00	**Registration/Information**	**Aula Magna Sala Attesa**
Session IV	**Structure and Dynamics** Rommie Amaro, University of California, San Diego, USA, Chair	
8:30 – 9:05	Janusz Bujnicki, International Institute of Molecular and Cell Biology, Poland ***Computational Modeling of RNA 3D Structures and Interactions – With and Without the Use of Experimental Data***	
9:05 – 9:40	Pilar Cossio, Flatiron Institute, USA ***Probability Distributions from Individual Cryo-EM Images and Molecular Dynamics***	
9:40 – 10:15	Massimilliano Bonomi, Pasteur Institute, France ***Protein Structural Ensembles from 3D and 2D Cryo-EM Data***	
10:15 – 10:45	**Coffee Break**	**Aula Magna Foyer**
Session V	**Cryo-EM Applications** Gabriel Lander, Scripps Research Institute, USA, Chair	
10:45 – 11:20	Elizabeth Kellogg, St. Jude Children's Research Hospital, USA ***New Frontiers in Understanding and Improving RNA-Guided DNA Integration Using Cryo-EM and Machine Learning***	
11:20 – 11:55	Sebastian Glatt, Jagiellonian University Krakow, Poland ***Translational Control of Eukaryotic Gene Expression***	
11:55 – 12:15	Mingxu Hu, Tsinghua University, China * ***CRYOPROS: Addressing Preferred Orientation in Single-Particle Cryo-EM Through AI-Generated Auxiliary Particles***	
12:15 – 14:10	**Lunch**	**Aula Magna Foyer**

Session VI	**Methodological Advances in Cryo-EM** Giulia Palermo, University of California, Riverside, USA, Chair	
14:10 – 14:45	Paul Emsley, MRC Laboratory of Molecular Biology, United Kingdom ***Model-Building, Refinement and Validation With COOT***	
14:45 – 15:20	Sergio Cruz León, Max Planck Institute of Biophysics, Germany ***Enabling Visual Proteomics by High-Confidence 3D Template Matching***	
15:20 – 15:40	Elsa Posani, International School for Advanced Studies, Italy * ***All Atom Molecular Dynamics Simulations Enable Ensemble Refinement of Flexible and Mismodelled Cryo-EM Derived RNA Structures***	
15:40 – 16:00	Colin Kinz-Thompson, Rutgers University-Newark, USA * ***Measuring the Biomolecular Information Content Present in Structural Data***	
16:00 – 16:20	Lorenzo Casalino, University of California, San Diego, USA * ***Harnessing Molecular Simulations to Design Stabilized Sars-Cov-2 S2 Antigens***	
16:20 – 18:20	**Poster Session II**	**Aula Magna Sala Attesa**
20:00 – 22:00	**Banquet Dinner**	**Savoy Hotel Restaurant**

Thursday, October 24, 2024

8:00 – 15:00	**Registration/Information**	**Aula Magna Sala Attesa**
Session VII	**Structure and Dynamics** Joanna Trylska, University of Warsaw, Poland, Chair	
8:30 – 9:05	Natalie Strynadka, University of British Columbia, Canada ***Structure-Guided Drug Discovery Targeting Antibiotic Resistance Mechanisms in Staphlococcus Aureus***	
9:05 – 9:40	Florence Tama, RIKEN Center for Computational Science, Japan ***Exploring Continuous Conformational Variability Via Cryo-EM Single-Particle Imaging and MD Simulations***	
9:40 – 10:15	David Taylor, University of Texas at Austin, USA ***Reengineering CRISPR-Cas Effector Complexes***	
10:15 – 10:45	**Coffee Break**	**Aula Magna Foyer**
Session VIII	**Cryo-EM Applications** Gabriel Lander, Scripps Research Institute, USA, Chair	
10:45 – 11:20	Wojciech Galej, European Molecular Biology Laboratory, France ***Structural Studies of the Pre-mRNA Splicing Machinery***	
11:20 – 11:40	Vivek Sharma, University of Helsinki, Finland * ***Molecular Insights into Mitochondrial Energy Production by Integrating Cryo-Electron Microscopy and Biochemistry with Computer Simulations***	
11:40 – 12:00	Erik Thiede, Flatiron Institute, USA * ***Towards Quantitative Recovery of Probability Densities from Cryo-EM***	

12:00 – 14:00	**Lunch**	**Aula Magna Foyer**
Session IX	**Methodological Advances in Cryo-EM** Joanna Trylska, University of Warsaw, Poland, Chair	
14:00 – 14:35	James Fraser, University of California, San Francisco, USA ***Uncovering Protein Ensembles: Automated Multiconformer Model Building for Cryo-EM Proteins, Nucleic Acids, Solvent, and Ligands***	
14:35 – 15:10	Mateusz Sikora, Jagiellonian University, Poland ***Integrative Modeling of Glycoproteins, Lessons from the Pandemic***	
15:10	**Free Time/Dinner on Own**	

Friday, October 25, 2024

8:00 – 12:00	**Registration/Information**	**Aula Magna Sala Attesa**
Session X	**Structure and Dynamics** Lorenzo Casalino, University of California, San Diego, USA, Chair	
8:30 – 9:05	Michele Vendruscolo, University of Cambridge, United Kingdom ***Determination of Protein Structural Ensembles Using Cryo-Electron Microscopy***	
9:05 – 9:40	Erik Lindahl, Stockholm University, Sweden ***Modeling Conformational Transition of Proteins by Combining Cryo-EM, Alphafold and Molecular Simulations***	
9:40 – 10:15	Isabelle Rouiller, University of Melbourne, Australia ***Characterizing the Conformational Landscape of the Hexameric VCP Complex from 2D Cryo-EM Images Using Molecular Dynamics Simulation***	
10:15 – 10:45	**Coffee Break**	**Aula Magna Foyer**
Session XI	**Cryo-EM Applications** Alessandra Magistrato, International School for Advanced Studies, Italy, Chair	
10:45 – 11:05	Jakub Rzeszótko, Max Planck Institute of Molecular and Cell Biology and Genetics, Germany * ***Advancing Structural Insights into Small Membrane Transporters Through SP-Cryo-EM: Unraveling the Regulatory Mechanisms of SLC26 Family***	
11:05 – 11:25	Nathan Bernhardt, National Institutes of Health, USA * ***What Determines the Dwell-Time of a Bound Lipid? Interpreting Lipid Densities in Structural Data Using Molecular Dynamics Simulations and Specialized Tools for Their Analysis***	
11:25 – 11:45	Tatiana Shugaeva, KTH Royal Institute of Technology, Sweden * ***Accurate Protein Fitting into Cryo-EM Maps Using Multiple Conformers Generated by Alphafold2***	
11:45 – 12:00	**Closing Remarks and *Biophysical Journal* Poster Awards** Giulia Palermo, University of California, Riverside, USA	

**Short talks selected from among submitted abstracts*

SPEAKER ABSTRACTS

STRUCTURAL DYNAMICS OF A NON-ENVELOPED VIRUS DURING DISASSEMBLY

Milan Kumar Lokshman[1]; Gourav Shrivastav[2]; Kirti Suhag[1]; Kimi Azad[1]; Manish Agarwal[3]; **Manidipa Banerjee**[1];
[1]IIT Delhi, Kusuma School of Biological Sciences, New Delhi, India
[2]IIT Delhi, Chemical Engineering, New Delhi, India
[3]IIT Delhi, CSC, New Delhi, India

Objective: The objective of the study is to establish the stepwise conformational alterations in the capsid of a model non-enveloped virus, Flock House Virus (FHV), during disassembly. Non-enveloped icosahedral viruses have highly stable and symmetric capsids; however, dynamic structural changes are needed for release of genome during cellular entry. A molecular level understanding of the disassembly pathways can be utilized to devise methods for globally effective chemical inactivation strategies. Methods: To identify disassembly intermediates in vitro, Differential Scanning Calorimetry (DSC) and PaSTRy assay was utilized at different conditions mimicking cellular entry. Altered particles were subjected to cryoelectron microscopy and single particle analysis. Mixed populations of intermediates were sorted and subjected to icosahedral or asymmetric reconstruction. In parallel, whole capsid simulations were carried out to identify dynamic regions and hydrophobic barriers to genome release. Results: Mature and immature forms of FHV were subjected to incremental heating in DSC, which resulted in the identification of two disassembly intermediates of mature FHV. The immature particle did not undergo disassembly-related conformational changes. PaSTRy assay at low pH conditions resulted in the identification of several intermediate states, including empty capsids. Single particle reconstructions of disassembling particles indicated conformational alterations including puffing of particles triggered by movement of subunits, and major alterations at symmetry axes, particularly the 5-fold and the 2-fold axes of symmetry. Asymmetric reconstructions indicated directional genome release from the 2-fold axes of symmetry, suggesting structural differences in sequentially identical capsid proteins occupying different positions in the capsid. All atom simulations of the whole capsid supported the cryoEM studies by indicating the existence of favored pathways within the capsid for externalization of flexible components (4). Conclusion: Our studies indicate that cryoelectron microscopy and whole capsid, all-atom simulation studies can be combined to create a molecular roadmap for identifying the stages of virus disassembly.

INTEGRATIVE MODELING OF PROTEIN STRUCTURE AND DYNAMICS USING MD SIMULATIONS AND EXPERIMENTAL DATA

Yuji Sugita[1,2,3]; Mao Oide[1,4]; Teppei Ikeya[5]; Yutaka Ito[5];
[1]RIKEN, Cluster for Pioneering Research, Wako, Japan
[2]RIKEN, Center for Computational Science, Kobe, Japan
[3]RIKEN, Center for Biosystems Dynamics Research, Kobe, Japan
[4]Osaka University, Institute for Protein Research, Suita, Japan
[5]Tokyo Metoropolitan University, Department of Chemistry, Hachioji, Japan

Multi-domain proteins often have flexible conformations in the solution or the cell. Although X-ray crystal structures of these proteins are available, inconsistency between the crystal structures and other experimental measurements has often been observed. We aim to solve such a problem by developing an integrative modeling approach based on MD simulations and several experimental data. In our integrative modeling approach, we rely on MELD (Modeling Employing Limited Data), a Bayesian method, to selectively use only reliable experimental data. We apply this method to the solution structure ensembles of Growth factor receptor bound protein 2 (GRB2), which is one of the critical proteins to recognize the tyrosine phosphorylation of the epidermal growth factor receptor (EGFR). Our integrative modeling method successfully resolved the inconsistency between X-ray crystal structures, solution nuclear magnetic resonance (NMR), and small-angle X-ray scattering (SAXS) experiments. It also provides representative structures with their statistical weights.

AI-BASED METHODS TO OVERCOME THE PREFERRED ORIENTATION AND MISSING-WEDGE PROBLEMS IN CRYOEM AND CRYOET

Hong Zhou[1,2]; Yun-Tao Liu[1,2]; Hongcheng Fan[1,2]; Jason J Hu[1,2];
[1]University of California, Los Angeles, Microbiology, Immunology and Molecular Genetics, Los Angeles, CA, USA
[2]University of California, Los Angeles, California NanoSystems Institute, Los Angeles, CA, USA

While advances in single-particle cryoEM have enabled the structural determination of macromolecular complexes at atomic resolution, particle orientation bias (the so-called "preferred" orientation problem) remains a complication for most specimens. Existing solutions have relied on biochemical and physical strategies applied to the specimen and are often complex and challenging. Previously, we have developed a neuron network-based software package, called IsoNet, to generate isotropic tomograms by correcting for the artefacts due to the missing-wedge problem in cryogenic electron tomography (cryoET). IsoNet is now completely re-designed for single-particle analysis (spIsoNet), by employing an end-to-end self-supervised deep-learning strategy to address the preferred orientation problem. Using preferred-orientation views to recover molecular information in under-sampled views, spIsoNet improves both angular isotropy and particle alignment accuracy during 3D reconstruction. We demonstrate spIsoNet's capability of generating near-isotropic reconstructions from representative biological systems with limited views, including ribosomes, β-galactosidases, and a previously intractable hemagglutinin trimer dataset. spIsoNet can also be generalized to improve map isotropy and particle alignment of preferentially oriented molecules in subtomogram averaging. Therefore, without additional specimen-preparation procedures, spIsoNet provides a general computational solution to the preferred orientation problem.

EXPLOITING THE FULL POTENTIAL OF CRYOGENIC ELECTRON MICROSCOPY MAPS

Paulina M Dominiak[1];
[1]University of Warsaw, Biological and Chemical Research Centre, Department of Chemistry, Warszawa, Poland

Cryogenic electron microscopy (cryo-EM) experiments yield three-dimensional images of electrostatic potential of molecules. Currently, these images are only used to obtain information about the position of atoms in space. However, there is much more information in them. The electrostatic potential carries information about the delicate balance between the positive potential generated by atomic nuclei and the negative potential generated by electron density. Thanks to this, it is very sensitive to changes in the protonation of functional groups, the state of metal oxidation, and the redistribution of electron density due to the formation of chemical bonds and intermolecular interactions. All these phenomena can be studied by analyzing images from an electron microscope. However, to make this possible, appropriate electrostatic potential models are needed, which will allow for reliable verification of the hypotheses through direct comparison with experimental data. During the lecture, I will present electron density models, and therefore electrostatic potential models, that have been developed over the years in the field of quantum crystallography, focusing on X-ray diffraction experiments [1]. These models are currently being adapted for electron diffraction [2] and microscopy [3], which will enable the full potential of these experiments to be explored.[1] Kulik M, Dominiak PM (2022) Comp. Struct. Biotech. J., 20, 6237-6243 [DOI: 10.1016/j.csbj.2022.10.018][2] Kulik M, Chodkiewicz ML, Dominiak PM (2022) Acta Cryst. D 78, 1010–1020 [DOI: 10.1107/S2059798322005836][3] Bick T, Dominiak PM, Wendler P (2024) BBA Advances, 5, 100113 [DOI: 10.1016/j.bbadva.2024.100113]The National Science Center, Poland, provided the funding for the research presented in this work under the grants 2020/39/I/ST4/02904 and 2017/27/B/ST4/02721.

ZERNIKE3D AND HETSIREN FOR CONFORMATIONAL AND COMPOSITIONAL HETEROGENEITY ANALYSIS OF CRYO-EM IMAGES

Jose Maria Carazo[1]; David Herreros[1]; James Krieger[1]; Marcos Gragera[1]; Carlos Oscar S Sorzano[1];
[1]CNB-CSIC, Department of Structural Biology, Madrid, Spain

We present two different approaches to analyze Electron Microscopy (cryo-EM) images with the goal to detect conformational and/or compositional structural changes at the specimen level in a continuous manner. One makes use of the notion of flexibility fields and expansion into a certain bases (Zernikes3D), precisely modulating biologically relevant motions by integrating bonded and non-bonded sterochemical constraints in the motion fields. In contrast, the other method directly works at the image/volume level (HetSIREN). In both cases the optimization is carried on following an Encoder/Decoder approach. Zernikes3D is well suited for conformational heterogeneity, while HetSIREN addresses both conformational and compositional changes. It is to be noted that for Zernikes3D we can perform an inversion (reconstruction) process that explicitly takes into account the deformation field, while for HetSIREN this task is implicitly performed by the neural network. We will present results of Zernikes3D and HetSIREN in several systems, focusing on the study of the conformational changes happening on Epidermal Growth Factor Her2 upon binding of the therapeutic antibody Trastuzumab as well as on the complex GR-HsP90-FKBP51 in the context of a collaboration with Prof. David Agard laboratory. Additionally, we introduce a novel method for comparing conformational spaces with diverse characteristics within a unified consensus space. This approach not only simplifies the identification of potential discrepancies between spaces generated by different algorithms, but also enables the refinement of conformational states based on reliability metrics derived directly from the consensus space. Through this uniquely flexible consensus framework, we aim to facilitate researchers with tools to better interpret and trust the results produced by the latest conformational heterogeneity algorithms emerging in the field.

ICE-CREAM: INTEGRATED CYBERINFRASTRUCTURE FOR ENSEMBLE CRYO-EM APPLICATIONS & MODELING

Abhishek Singharoy;
[1]Arizona State University, Biodesign Institute, Tempe, AZ, USA

Integrative modeling is an area of rapid methodological developments, wherein, atom-resolved structures of biological systems are determined by merging data from multiple experimental sources with physics and informatics-based approaches. These elegant fitting, learning and inferencing methodologies have been successful in resolving a range of structures, starting with soluble and membrane proteins up to sub-cellular complex architectures. The integrative models routinely make it to top positions at the CASP, EMDB and PDB competitions, serving a diverse cross-section of the Biophysics community. Yet, the dearth of cyberinfrastructure renders these modeling tools unresponsive to parallelization and scale necessary to model longer timescales and larger multi-domain structures. We are designing and implementing the Integrated Cyberinfrastructure for Ensemble CRyo-Em Applications & Modeling (ICE-CREAM) to support a wide range of temporal duration representing the uneven data resolutions that underpin the multi-model picture of cryo-EM density. This implementation will break free of the traditional high-performance computing execution model that assumes singular jobs and static execution of tasks and data, to one that is fundamentally designed for data-integration and assimilation across different scales, quality and sparsity.

UNDERSTANDING THE MOLECULAR MECHANISM OF GENOME EDITORS TO ENHANCE THEIR PRECISION AND TARGETING SCOPE

Audrone Lapinaite[1];
[1]Arizonia State University, Tempe, Arizona, USA

No Abstract

MOLECULAR ARCHITECTURE AND FUNCTIONAL DYNAMICS OF THE PRE-INCISION COMPLEX IN THE NUCLEOTIDE EXCISION DNA REPAIR PATHWAY

Ivaylo Ivanov[1]; Jina Yu[1]; Chunli Yan[1]; Tanmoy Paul[1]; Susan E Tsutakawa[2]; Chi-Lin Tsai[3]; Samir Hamdan[4]; John A Tainer[3];
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Nucleotide excision repair (NER) is a genome maintenance pathway critical for human health. NER repairs a vast array of structurally unrelated DNA lesions caused by ultraviolet radiation, reactive oxygen species, environmental carcinogens, and chemotherapeutic agents such as cis-platinum. Despite numerous biochemical and genetic studies, knowledge of the inner workings of the complex NER protein machinery remains fragmentary. By synthesizing cryo-EM and cross-linking mass spectrometry (XL-MS) data with computational modeling, MD simulations and AlphaFold2 predictions, we elucidate the structure and dynamics of a critically important state of the NER machinery – the pre-incision complex (PInC). Our analyses yield key mechanistic insights into PInC's assembly and regulation, the structural basis of XPF and XPG nuclease coordination, and the licensing of the NER dual incision. Using graph-theoretical algorithms we also build dynamic network models of the PInC, which powerfully elucidate the etiology of devastating human genetic syndromes. Notably, we find that xeroderma pigmentosum (XP) and Cockayne syndrome (CS) disease mutations cluster at key interfaces of PInC's dynamic communities, impacting NER protein stability, functional dynamics, DNA binding, nuclease licensing, and community integrity.

CRYOXKIT: INCORPORATION OF EXPERIMENTAL STRUCTURAL DENSITY INTO AUTODOCK FOR IMPROVED POSE PREDICTION

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Recent advances in structural biology have led to the publication of a wealth of high-resolution x-ray crystallography and cryoEM structures, including those containing complexes with small molecules of interest for drug design. While it is common to incorporate information from the atomic coordinates of these complexes into docking (e.g. pharmacophore models or scaffold hopping), there are limited methods to directly leverage the underlying density information. This is desirable because it does not rely on the determination of relevant coordinates, which may require expert intervention, but instead interprets all density as indicative of regions to which a ligand may be bound. To do so, we have developed CryoXKit, a tool to convert experimental densities from either cryoEM or x-ray crystallography into a biasing potential on heavy atoms during docking. Using this structural density guidance implemented with AutoDock-GPU, we found significant improvements in redocking and cross-docking, important pose prediction tasks, compared with the unmodified AutoDock4 force field. Failures in cross-docking tasks are additionally reflective of changes in positioning of pharmacophores in the site, suggesting it is a fundamental limitation of transferring information between complexes. We additionally found, against a set of targets selected from the LIT-PCBA dataset, that rescoring of these improved poses leads to better discriminatory power in a virtual screening setting for selected targets.

INFERRING THE CONFORMATIONAL LANDSCAPE OF MULTISTATE PROTEIN ASSEMBLIES USING COARSE-GRAINED SAMPLING OF TRANSITION PATHWAYS

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An increasing number of molecular systems solved with cryo-electron microscopy is characterized by the presence of multiple conformational states, either discrete or with a continuum of flexible geometries. While advanced clustering algorithms allow for discerning and refining individual conformations, the information about the pathways connecting these discrete states is usually unavailable due to the low statistical weights of the conformational intermediates, and the well-established effects of finite-rate cooling. To address this, we repurpose a physics-based coarse-grained discrete molecular dynamics (dMD) engine, augmented with a Maxwell-demon acceptance criterion and a metadynamics-like enhanced sampling algorithm, to rapidly explore viable conformational transitions between experimentally resolved endpoints. The pool of candidate intermediates can then be scored and subsampled based on multiple criteria, from Bayesian estimation of consistency with the raw cryo-EM images to knowledge-based potentials, and input into fully atomistic string-based pathway optimization methods.

CHARACTERISING THE CONFORMATIONAL LANDSCAPE OF THE HEXAMERIC VCP COMPLEX FROM 2D CRYO-EM IMAGES USING MOLECULAR DYNAMICS SIMULATION

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The different conformations adopted by protein complexes in solution are captured in cryo-EM data. However, retrieving this information remains challenging. Classical image analysis methods rely on averaging, which is inadequate for obtaining detailed information on the flexible regions of proteins. The calculated EM maps have poorly defined or invisible densities for regions of complexes that undergo continuous conformational changes. To analyse the conformational dynamics of proteins from cryo-EM data, we have developed a method that combines Normal Mode Analysis and Molecular Dynamics simulation to deform/fit 3D structures to match the 2D cryo-EM images data. Using this method, called Molecular Dynamics for Single Particle Analysis of Continuous Conformational hEterogeneity or MDSPACE [1], we analyzed the conformational variability in the hexameric AAA + ATPase p97/VCP, a protein complex with a six-fold rotational symmetric core surrounded by six flexible N-domains [2]. This approach enabled us to detect and characterize the swaying moving of a ~30KDa domain, the N-domain of VCP, by up to 60° around a central position [3]. It also enabled us to analyze the cooperativity of the six N-domains within the VCP hexamer. Moreover, cluster analysis of the 3D models fitted to the 2D images revealed the presence of an unexpected conformation adopted by very few particles in the data set (approximately 2%). This study demonstrates the application of MDSPACE in analysing the continuous conformational changes in partially symmetrical protein complexes, systems notoriously difficult to analyse due to the alignment errors caused by their partial symmetry. References: 1. Vuillemot, R., et al., J Mol Biol, 2023: p. 167951; 2. Valimehr, S., et al., Biomolecules, 2023. 13(5); 3. Valimehr, S., et al., Int J Mol Sci, 2024. 25(6).

PROBABILITY DISTRIBUTIONS FROM INDIVIDUAL CRYO-EM IMAGES AND MOLECULAR DYNAMICS

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No Abstract

PROBABILITY DISTRIBUTIONS FROM INDIVIDUAL CRYO-EM IMAGES AND MOLECULAR DYNAMICS

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No Abstract

NEW FRONTIERS IN UNDERSTANDING AND IMPROVING RNA-GUIDED DNA INTEGRATION USING CRY-EM AND MACHINE LEARNING

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No Abstract

TRANSLATIONAL CONTROL OF EUKARYOTIC GENE EXPRESSION

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My Max Planck Research Group studies different translation control mechanisms, which regulate the production of specific sets of proteins by chemical modifications of tRNA molecules. Every protein in the cell is produced by the ribosome, which uses transfer RNA (tRNA) molecules to translate the sequence information coded in mRNAs into correctly assembled poly-peptide chains. The lab is focusing on understanding the molecular mechanisms that lead to the specific base modifications in anticodons of tRNAs. These modifications have a strong influence on the efficiency and accuracy of the codon-anticodon pairing and therefore regulate the translational rates and folding dynamics of protein synthesis. Recent findings have shown that alterations of these modification pathways play important roles in the onset of certain neurodegenerative diseases and cancer. We mainly use X-ray crystallography (MX) and cryogenic electron microscopy (cryo-EM) to obtain snapshots of the involved macromolecular machines and analyse their reaction intermediates at atomic resolution. Subsequently, we employ different complementary in vitro and in vivo approaches to validate and challenge our structural observations. Furthermore, we have started working on other (t)RNA modification pathways and elucidate the structure of folded RNA molecules directly by cryo-EM. Furthermore, we aim to understand how these post-transcriptional modifications affect ribosomal decoding and translation elongation by directly imaging translating ribosomes at atomic resolution. Last but not least, we develop novel structural, biochemical and biophysical approaches to study structured RNA domains. In summary, our work contributes to the fundamental understanding of eukaryotic gene expression and its complex regulatory mechanisms.

CRYOPROS: ADDRESSING PREFERRED ORIENTATION IN SINGLE-PARTICLE CRYO-EM THROUGH AI-GENERATED AUXILIARY PARTICLES

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Preferred orientation presents a persistent challenge in cryo-EM single particle analysis. Despite extensive experimental study, computational analysis of preferred orientation artifacts from real-world datasets remains an important yet underexplored perspective. In this study, after analyzing datasets exhibiting preferred orientation, we identified misalignment as the primary challenge. This led us to develop cryoPROS, a computational framework designed to address this issue by co-refining synthesized and experimental data. Utilizing a self-supervised deep generative model, cryoPROS synthesizes auxiliary particles to effectively eliminate misalignment errors in experimental particles through a co-refinement process, achieving near-atomic resolution with the untilted HA-trimer dataset. With extensions, cryoPROS resolved high-resolution structures of two membrane proteins, P001-Y and Na_X, affected by preferred orientation and micelle effects, and uncovered a new state of hormone-sensitive lipase dimer within a dataset afflicted by both preferred orientation and heterogeneity. Extensive experiments validated the robustness of cryoPROS and its negligible risk of model bias.

MODEL-BUILDING, REFINEMENT AND VALIDATION WITH COOT

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The interactive model-building tool, Coot, has recently has been reworked in two major ways: (i) The interface has been rebuilt practically from scratch using a new toolkit (ii) The program has been split to provide a re-usable library. The consequences of (i) are that Coot has a modern dynamic/interactive attractive GUI. Considerably more thought has been given to the layout of tools and their usability in this version than had been the case in previous versions. This version of Coot is also able to exploit multi-core processors, for validation tools such as atom overlaps, rotamer and Ramachandran plot probabilities so that they are now interactive (i.e. dynamically respond to modification of the model). The upgrade to the graphics means that informative textures are now available, density maps with large radius can be rotated with ease and Coot now uses frame-buffer techniques such as screen-space ambient occlusion and shadows.The consequences of (ii) allows (1) compilation using WebAssembly to provide the computational "back-end" of "Coot on the Web" a.k.a "Moorhen" (moorhen.org) (2) a "headless" (i.e. no-GUI) interface to Coot tools as a python module. This module can be plugged into Blender to provide structural biology tools and representations as if they were built-in."Fo-Fc-style" difference maps have been a useful tool when modifying molecular models in the light of x-ray data. This has become more useful still with the recent addition of maps that dynamically update in the light of the current model. Cryo-EM data-sets and models are considerably larger (typically) and so similar interactivity is not possible. However, we will show that, using GEMMI-based tools, something useful can be achieved. Coot is Free Software (recently it has become part of Debian GNU/Linux) and is available free of charge.https://www2.mrc-lmb.cam.ac.uk/personal/pemsley/coot/

ENABLING VISUAL PROTEOMICS BY HIGH-CONFIDENCE 3D TEMPLATE MATCHING

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By creating detailed 3D maps of the interior of cells, visual proteomics aims to understand the spatial interactions between macromolecular complexes in their native environment. Cryo-electron tomography (CryoET) offers a powerful approach for imaging the interior of cells with fully preserved context. However, the complex and noisy nature of CryoET data hinders feature identification. In my talk, I will present our recent advances in high confidence 3D template matching (hcTM) for CryoET. hcTM enables automated, objective, and comprehensive detection of a wide range of cellular structures within crowded cells. The high-confidence nature of our approach opens doors to unexpected biological discoveries and the computational modelling of realistic subcellular systems. By enabling the reliable detection of individual complexes in different conformational states, we can establish robust connections between their functional state, spatial localization, cellular context, and even cell type and species. Our work sets the stage for analyzing individual molecular events inside living cells with defined statistical confidence.

ALL ATOM MOLECULAR DYNAMICS SIMULATIONS ENABLE ENSEMBLE REFINEMENT OF FLEXIBLE AND MISMODELLED CRYO-EM DERIVED RNA STRUCTURES

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RNA molecules span a great variety of biological functions, from genetic information storage to catalysis. This can be achieved thanks to the highly heterogeneous conformational ensembles that these molecules can adopt [1]. With the advent of the single particle cryogenic electron microscopy (cryo-EM) technique, it is possible to access large and highly flexible RNA macromolecules at near atomic-level resolution, thus allowing to fully assess their structural and functional features. Still, given that standard refinement tools assume that all the collected images are associated to a single structure, the most mobile regions are very challenging to solve. We tackle these problems by integration of molecular dynamics simulations and experimental density maps, using as a test case a group IIB intron ribozyme [2]. We show that a refinement done assuming a single structure leads to group II intron structural models with nucleobase base pairs that are either non-properly paired or in disagreement with the experimental potential density map. Interestingly, incorrect base pairing is also present in the deposited structure [2]. We solve this problem by using metainference-based [3] ensemble refinement, along with ad hoc restraints to enforce the correct base pairing. The use of an ensemble refinement approach enables the natural RNA dynamics to be reconstructed, preserving the proper interactions.[1] J.A. Doudna, T.R. Cech, Nature, 418: 222-228 (2002).[2] D.B. Haak et al., Cell, 178: 612-623 (2019).[3] M. Bonomi et al., Science Advances, 2: 3 (2016).

HARNESSING MOLECULAR SIMULATIONS TO DESIGN STABILIZED SARS-COV-2 S2 ANTIGENS

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The effectiveness of COVID-19 vaccines, rooted in the full-length prefusion-stabilized SARS-CoV-2 spike protein, is challenged by the continual emergence of variants of concern accumulating sequence modifications in the immunodominant S1 subunit. A possible solution to this limitation lies in the spike's S2 subunit, known for its evolutionary conservation across sarbecoviruses and ability to elicit a robust immune response. Yet, the inherent instability of S2 remains a significant hurdle to its application in vaccine design. To tackle this challenge, we used weighted ensemble molecular dynamics simulations to examine the conformational plasticity of the S2 trimer and inform the design of tryptophan cavity-filling mutations aimed at stabilizing the trimer in a closed prefusion conformation. Alchemical non-equilibrium free energy calculations of three engineered S2 variants in the closed state revealed a stabilizing energetic contribution imparted by the tryptophan substitutions. Experimental assays, including cellular expression and differential scanning fluorimetry, validated the computational predictions by demonstrating increased expression yields and enhanced thermostability for the engineered S2 variants. Furthermore, these stabilizing mutations facilitated the determination of a high-resolution cryo-EM structure of the S2 trimer in its closed prefusion conformation, revealing an extended network of new, stabilizing hydrophobic interactions also observed in the simulations. Finally, we characterized the immunogenicity of the engineered S2 antigen, demonstrating its ability to elicit neutralizing responses against sarbecoviruses. Leveraging a simulation-driven approach and supported by experimental and cryo-EM structural data, our findings pinpoint specific cavity-filling substitutions that enhance the stability of the SARS-CoV-2 S2 trimer, making it a promising antigen that could potentially be incorporated into viable vaccine platforms.

STRUCTURE-GUIDED DRUG DISCOVERY TARGETING ANTIBIOTIC RESISTANCE MECHANISMS IN STAPHLOCOCCUS AUREUS

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No Abstract

EXPLORING CONTINUOUS CONFORMATIONAL VARIABILITY VIA CRYO-EM SINGLE-PARTICLE IMAGING AND MD SIMULATIONS

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No Abstract

REENGINEERING CRISPR-CAS EFFECTOR COMPLEXES

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No Abstract

STRUCTURAL STUDIES OF THE PRE-MRNA SPLICING MACHINERY

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No Abstract

MOLECULAR INSIGHTS INTO MITOCHONDRIAL ENERGY PRODUCTION BY INTEGRATING CRYO ELECTRON MICROSCOPY AND BIOCHEMISTRY WITH COMPUTER SIMULATIONS

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In several organisms [1], enzyme-catalyzed translocation of ions across the membrane drives the generation of ATP. The membrane-bound respiratory complexes of the mitochondrial electron transport chain catalyze redox-driven proton pumping, but the molecular mechanisms have remained unknown. The respiratory complexes coalesce together to form higher order assemblies called the mitochondrial supercomplexes [1], but the functional basis of these also remain debated. Here, results from recent multiscale computer simulations on isolated mitochondrial complexes and supercomplexes will be presented, which helps in delineating the molecular basis of energy production in mitochondria and bacteria. Coarse-grained and atomistic molecular dynamics (MD) simulations on high-resolution cryo-EM structures of mitochondrial supercomplexes [2] provide insights on the coupled dynamics of proteins and membrane and their interactions. Respiratory complex I and Mrp-type antiporter structures are studied with long time-scale atomistic MD simulations and hybrid QM/MM free energy calculations [2-4] to decipher the energetics of quinone redox chemistry and protonation dynamics. We discuss an approach of combining pKa prediction with cryo-EM density map analysis that can help in improved atomic modeling of the density data [5] and deeper understanding of molecular mechanisms of bioenergetic enzymes [6]. [1] M. Wikström, C. Pecorilla, V. Sharma, The Enzymes, 54 (2023) 15-36.[2] Y.-Ch. Shin, P. Latorre-Muro, A. Djurabekova, O. Zdorevskyi, Ch. F. Bennett, N. Burger, K. Song, Ch. Xu, V. Sharma, M. Liao, P. Puigserver. bioRxiv (2024)[3] Y. Lee, O. Haapanen, A. Altmeyer, et al., Nature Communications, 13 (2022) 6091.[4] O. Zdorevskyi, A. Djurabekova, J. Lasham, V. Sharma, Chemical Science 14 (2023) 6309-6318.[5] J. Lasham, A. Djurabekova, V. Zickermann, J. Vonck, V. Sharma, The Journal of Physical Chemistry B 128 (2024) 2304-2316.[6] A. Djurabekova, J. Lasham, O. Zdorevskyi, V. Zickermann, V. Sharma, Biochemical Journal, 481 (2024) 499–514.

TOWARDS QUANTITATIVE RECOVERY OF PROBABILITY DENSITIES FROM CRYO-EM

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Many biological molecules are structurally heterogeneous. In principle, cryo-electron microscopy (cryoEM) gives us the tools to extract this heterogeneity since the snap-freezing process traps biomolecules in conformations close to the ones they adopt in solution. This means that, modulo the effects of freezing on the conformational ensemble, we should be able to quantitatively recover conformational probabilities from cryo-EM. Unfortunately, cryo-EM's low signal-to-noise ratio, as well as the complexity of proteins' conformational landscapes, makes this a challenging task. Prior work that attempts to quantitatively recover the probabilities of protein conformations often finds itself hamstrung by the computational expense of comparing conformational hypotheses with images and of generating conformational hypotheses through simulation. Here, we discuss our progress towards addressing these problems. We first discuss our work in using fast algorithms to accelerate comparisons of cryo-EM images with conformations. By filtering out comparisons that don't effect the final result, we can improve the scaling from linear to logarithmic in the size of the hypothesis-space, leading to substantial improvements in speed. Next, we discuss our initial work in using of generative machine learning to capture the conformational ensemble. Using generative machine learning, we can generate physically-meaningful conformations efficiently and without requiring long molecular simulations.

UNCOVERING PROTEIN ENSEMBLES: AUTOMATED MULTICONFORMER MODEL BUILDING FOR CRYO-EM PROTEINS, NUCLEIC ACIDS, SOLVENTS, AND LIGANDS

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No Abstract

INTEGRATIVE MODELING OF GLYCOPROTEINS, LESSONS FROM THE PANDEMIC

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Glycans, complex sugars covalently attached to proteins, affect protein stability and function, participate in 'self' recognition, and modulate protein-protein interactions. The glycosylation machinery is frequently hijacked by pathogens, which hide their proteins behind a "glycan shield", making them inaccessible to the immune system and complicating pharmacological interventions. Unlike many biomolecules, glycans do not typically form secondary structures and remain highly mobile, posing a challenge for traditional structural biology techniques. In our research, we combined molecular dynamics simulations with cryo-electron tomography and atomic force microscopy to understand how glycans affect viral fusion proteins, particularly the SARS-CoV-2 spike protein. We discovered a surprising flexibility of the spike protein [1,2] and predicted new antibody binding sites accessible through the dynamic glycan shield [3], which can aid in designing novel vaccines. Additionally, we developed a simplified, open-source method GlycoSHIELD for rapidly predicting glycan shielding with minimal computing power. This method has been applied to refine existing cryo-EM maps of glycoproteins [4]. [1] B Turoňová, M Sikora, C Schürmann, WJH Hagen, S Welsch et al., In situ structural analysis of SARS-CoV-2 spike reveals flexibility mediated by three hinges. Science, 370(6513) 2020 [2] R Zhu, D Canena, M Sikora, M Klausberger, H Seferovic, et al., Force-tuned avidity of spike variant-ACE2 interactions viewed on the single-molecule level; Nat Comm, 13(7926) 2022 [3] M Sikora, S von Bülow, FEC Blanc, M Gecht, R Covino, G Hummer. Computational epitope map of SARS-CoV-2 spike protein. PLoS computational biology, 17(4) 2021 [4] Y-X Tsai, N-E Chang, K Reuter, H-T Chang, T-J Yang et al., Rapid simulation of glycoprotein structures by grafting and steric exclusion of glycan conformer libraries. Cell ;187(5):1296-1311.e26 2024

DETERMINATION OF PROTEIN STRUCTURAL ENSEMBLES USING CRYO-ELECTRON MICROSCOPY

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Achieving a comprehensive understanding of the behaviour of proteins is greatly facilitated by the knowledge of their structures, thermodynamics and dynamics. All this information can be provided in an effective manner in terms of structural ensembles. A structural ensemble can be obtained by determining the structures, populations and interconversion rates for all the main states that a protein can occupy. To reach this goal, integrative methods that combine experimental and computational approaches provide powerful tools. In this context, cryo-electron microscopy has become over recent years an invaluable resource to bridge the gap from order to disorder in structural biology. I will provide a perspective of the current challenges and opportunities in determining protein structural ensembles, and describe an integrative approach to combine cryo-electron microscopy data with molecular dynamics simulations using Bayesian inference and metadynamics.

MODELING CONFORMATIONAL TRANSITION OF PROTEINS BY COMBINING CRYO-EM, ALPHAFOLD AND MOLECULAR SIMULATIONS

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Single-particle cryo-electron microscopy has potential to provide a much richer view of molecular structure than traditional methods, in particular since the method is often capable of reconstructing multiple states from the same sample. However, it is still common that we cannot identify some states, and that minority classes corresponding to intermediates drown in noise. Even for cases where we are able to model molecular flexibility in terms of continuum descriptions of density, it is considerably more challenging to achieve true time resolution e.g. of kinetics. I will discuss our approaches to combine cryo-EM with simulation-based Markov State Models to resolve complex conformational transitions of ligand-gated ion channels in particular, new methods to efficiently refine molecular structure into cryo-EM density in simulations without requiring constraints on secondary structure, and how we are using generative methods such as AlphaFold to improve the efficiency of these approaches and predict how e.g. ligand binding or pH changes selectively stabilize specific states.

COMPUTATIONAL MODELING OF RNA 3D STRUCTURES AND INTERACTIONS - WITH AND WITHOUT THE USE OF EXPERIMENTAL DATA

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Ribonucleic acid (RNA) molecules are master regulators of cells. They play key roles in many molecular processes: transmitting genetic information, sensing cellular signals, relaying responses, and even catalyzing chemical reactions. The function of RNA, especially its ability to interact with other molecules, is encoded in its sequence. To understand how these molecules carry out their biological tasks, we need detailed knowledge of RNA structure, dynamics, and thermodynamics. The latter largely determines how RNA folds and interacts within the cellular environment. Experimentally determining these properties is challenging. Several computational methods have been developed to model the folding of RNA 3D structures and their interactions, mainly with proteins. However, these computational methods are nearing their limits, especially when the biological implications demand calculations of dynamics beyond a few hundred nanoseconds. For researchers facing such challenges, a more effective approach is to use coarse-grained modeling. This reduces both the data points and computational effort to a feasible level, while retaining as much essential information as possible. I will present strategies for computational modeling of RNA 3D structures and their interactions with other molecules. These strategies use a suite of methods from my laboratory, based on the SimRNA program. Our methods employ coarse-grained representations of molecules, utilize the Monte Carlo method for sampling conformational space, and use statistical potentials to approximate energy. They also help identify conformations that match biologically relevant structures. Specifically, I will discuss computational methods to determine RNA structure using low-resolution experimental data, such as chemical probing and electron microscopy. References 1. Ponce-Salvatierra, A. et al. Biosci. Rep. 39, BSR20180430 (2019)2. Boniecki, M. J. et al. Nucleic Acids Res. 44, e63 (2016)3. Rocha de Moura, T., et al. Nucleic Acids Res. 52(6): 3419–3432 (2024)

ADVANCING STRUCTURAL INSIGHTS INTO SMALL MEMBRANE TRANSPORTERS THROUGH SP-CRYOEM: UNRAVELING THE REGULATORY MECHANISMS OF SLC26 FAMILY

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The structural characterization of small membrane proteins via SP-CryoEM presents unique challenges. Additionally, the dynamic conformational landscape of membrane transporters adds to the complexity. Our research group tackles these obstacles through an approach combining extensive sample preparation and biochemical characterization, and the application of conformation-specific nanobody binders. This methodology, coupled with SP-cryoEM and advanced in vitro transport assays, enables us to study the structure-function relationships within the Solute Carrier Family 26 (SLC26). The SLC26 family is crucial for maintaining ion homeostasis and pH regulation, critical for numerous physiological processes, particularly during development. These secondary transporters facilitate the translocation of various anions across cellular membranes. Its C-terminal cytoplasmic STAS domain plays important role for their functionality, including trafficking, activity regulation, and protein interactions. My research is dedicated to investigating the regulatory mechanisms of the STAS domain and its evolutionary trajectory. This investigation is crucial for understanding the broader functional dynamics of the SLC26 family and holds potential for therapeutic advancements targeting SLC26-related disorders such as chondrodysplasia and deafness. Furthermore, this project underscores the significance of exploring prokaryotic SLC26 homologs. Our discovery of a novel dimer interface in the cyanobacterial bicarbonate transporter BicA provides fresh insights into the regulatory role of the STAS domain. By leveraging cutting-edge SP-CryoEM and innovative biochemical strategies, our work advances the frontier of membrane protein research. It offers promising avenues for therapeutic intervention and deepens our understanding of protein structure-function relationship.

WHAT DETERMINES THE DWELL-TIME OF A BOUND LIPID? INTERPRETING LIPID DENSITIES IN STRUCTURAL DATA USING MOLECULAR DYNAMICS SIMULATIONS AND SPECIALIZED TOOLS FOR THEIR ANALYSIS

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Structural studies of membrane proteins often report detection of phospholipid molecules, but in many cases of interest, it is unclear how to discriminate between bona-fide regulatory sites and non-specific densities, as the lipid headgroups that should be conferring specificity are often unresolved. This lack of detail implies that the stabilization of these lipids on the protein surface owes to other factors. In this study, we show that the dynamics of phospholipids at the protein-membrane interface is primarily dictated by the protein topography and its influence on the lipid chains. That is, long-lasting complexes form at sites where the features of this topography protect individual lipids from being displaced by their neighbors. This conclusion stems from a novel framework for identifying important interactions stabilizing bound lipids based on molecular dynamics simulations and a suite of specialized analysis tools. We apply this methodology to both the Kv2.1 and KcsA potassium channels, whose mechanisms are known to be modulated by specific lipid types. In both cases, structural data reveals lipid densities, clearly resolved for the chains but not for the headgroups. Our simulations closely replicate the experimental data, and reveal it largely reflects the topographical features of the protein surface within the hydrophobic span of the membrane, rather than the potential for interactions between polar protein sidechains and lipid headgroups. We conclude with a discussion of the broader implications of these findings and the origins of specificity in lipid regulatory mechanisms.

ACCURATE PROTEIN FITTING INTO CRYO-EM MAPS USING MULTIPLE CONFORMERS GENERATED BY ALPHAFOLD2

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Recent advances in cryo-electron microscopy have enabled near-atomic resolution structures of challenging protein targets. Refining an initial atomistic model into a target density map is one of the key reconstruction steps, where molecular dynamics simulations can be used to achieve a good balance between physical plausibility and map fitting scores. However, proteins with multiple functional states often remain a challenge for simulation-based refinement methods, in particular when map resolution is not high enough to build de-novo models. Here, we introduce a new refinement approach in which 1) numerous initial models (instead of a single one) are generated by stochastic subsampling the multiple sequence alignments (MSA) space in AlphaFold2, 2) resulting models are subjected to structure-based clustering, and 3) multiple density-guided molecular dynamics simulations are performed from the centroid structures. Our method shows better fitting accuracy compared to single starting point scenarios. This improvement is demonstrated for the epsilon subunit of ATP-synthase, CGRPR, LAT1, and ASCT2. Each of these proteins undergoes significant conformational changes. The results suggest that simulation-based refinement combined with AlphaFold2-MSA-subsampling might both make it possible to model transitions between states and build models of states with smaller populations in cryo-EM data. This could be especially beneficial for drug design applications because it might help in the identification of compounds that selectively stabilize specific states.

POSTER ABSTRACTS

Tuesday, October 22
POSTER SESSION I
16:20 – 18:20

Posters are available for viewing only during their scheduled date of presentation. Below are the formal presentation times. Presenting authors with odd-numbered poster boards should present from 16:20 – 17:20 and those with even-numbered poster boards should present from 17:20 – 18:20. The presenters listed below are required to remain in front of their poster boards to meet with attendees.

Odd-Numbered Boards 16:20 – 17:20 | Even-Numbered Boards 17:20 – 18:20

Aupic, Jana	**1-POS**	**Board 1**
Catapano, Lucrezia	**2-POS**	**Board 2**
Fellmeth, Thomas	**5-POS**	**Board 5**
Haloi, Nandan	**6-POS**	**Board 6**
Jardón-Valadez, Eduardo	**9-POS**	**Board 9**
Jin, Hong	**10-POS**	**Board 10**
Lapenta, Fabio	**13-POS**	**Board 13**
Lee, Sun Joon	**14-POS**	**Board 14**
Mitusinska, Karolina	**17-POS**	**Board 17**
Moller, Elissa	**18-POS**	**Board 18**
Parise, Angela	**21-POS**	**Board 21**
Pokorná, Pavlína	**22-POS**	**Board 22**
Santiago-McRae, Ezry	**25-POS**	**Board 25**
Silva, Tomás F.D.	**26-POS**	**Board 26**
Van Heerden, Dewald	**29-POS**	**Board 29**
Volkan-Kacso, Sandor	**30-POS**	**Board 30**
Yeo, Joel	**33-POS**	**Board 33**

Posters should be set up on the morning of Tuesday, October 22 and removed by 18:30 on Wednesday, October 23. All uncollected posters will be discarded.

1-POS Board 1

MONOVALENT METAL IONS IN PRE-MRNA SPLICING

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Splicing, i.e. excision of non-coding introns and ligation of coding exon regions, is an integral step of messenger RNA (mRNA) maturation. The splicing reaction is catalyzed by the spliceosome, an intricate and highly dynamic ribonucleoprotein complex that relies on a two-Mg^{2+}-ion motif to perform phosphodiester bond breakage and formation. Cryogenic electron microscopy (cryo-EM) studies of the spliceosome complex furnished unprecedented insights into the functional mechanism of splicing, identifying conformational and compositional changes that govern the process. Conversely, the complete classification of all metal cofactors is still an ongoing challenge, despite their relevance for spliceosome folding and catalysis. All-atom molecular dynamics (MD) simulations, particularly in the hybrid quantum-classical (QM/MM) regime, can importantly complement cryo-EM data by distinguishing the precise location and identity of metal ions and disentangling their dynamic and functional behavior along the splicing cycle. Here, we employ QM/MM MD to characterize the role of second shell K^+ ions in splicing. We show that the K^+ ion positioned in the direct vicinity of the two-Mg^{2+}-ion motif promotes the first splicing reaction by rigidifying the active site and stabilizing the substrate in the pre- and post-catalytic state via formation of key hydrogen bonds. Furthermore, an additional dynamic K^+ ion, present during the second splicing step, is paramount for 3' splice site selection, governing the correct positioning of the intron-exon boundary at the active site. Taken together, our findings suggest monovalent ions play an instrumental and multifaceted role in RNA processing.

2-POS Board 2

MOORHEN AND BLENDCOOTAN APPLICATION OF THE COOT LIBRARIES FOR THE WEB AND IN BLENDER

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The interactive molecular modelling program, Coot, has recently undergone a redesign so that the libraries are available via a Python API and JavaScript/Web Assembly. These libraries provide the modelling, refinement, validation and analysis functions of Coot in the web-based application Moorhen (moorhen.org). Moorhen is a client-side-only app. This means it is 100% private. The validation tools provide scores for rotamers, Ramachandran and density fit analysis. Tools particularly useful for cryo-EM include molecular placement with jiggle-fit and real-space refinement model-morphing using local distance restraints. Molecular graphics include ribbon diagrams, molecular surfaces and chemistry-aware bond representation (aromaticity and delocalisation). The lighting model includes screen-space ambient occlusion and shadows. Additionally, the Python API offers a framework to integrate Coot functions into Blender, enabling macromolecular model and map representations within the 3D graphics program. All of the meshes/molecular representations that one can create in Moorhen can be generated in BlendCoot. By exploiting the ray tracing renderer of Blender, it is possible to generate pleasing/realistic images and animations.

5-POS Board 5

REPRODUCTION OF TAU FIBRIL FORMATION USING COARSE-GRAINED MOLECULAR DYNAMICS SIMULATIONS AND VALIDATION VIA CRYOGENIC ELECTRON MICROSCOPY

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Tau is a well-known intrinsically disordered protein (IDP), found hyperphosphorylated in Alzheimer's disease (AD) in human brains. Under pathological conditions, tau undergoes hyperphosphorylation, leading to tau fibrillation. [1] The most abundant forms of tau fibrils are the paired helical filaments (PHFs) and the straight filaments (SFs), which are structural polymorphes. Further tauopathies like Pick's disease or Parkinson's disease, show different disease-specific conformations of tau fibrils. [2] Molecular dynamics simulations can provide insights on the time-evolution of tau fibril formation on molecular level. The predicted fibril structures will then be validated using single-particle cryogenic electron microscopy (cryo-EM). All simulation will be performed on coarse-grained (CG) level of resolution because it allows to extend the lengths and time scale of the system by orders of magnitudes compared to all-atom simulations. [3] For tau fibril structure determination, cryo-EM is the method of choice, because it is a well-established high-resolution structural biology method that provides insights on near-atomistic level. The importance of the choice of different CG force fields in combination with the water model was investigated. SIRAH was found to give a fair description of the conformational ensemble of monomeric tau and can accurately reproduce NMR data, such as the gyration radius. The collapsing nature of the CG force field is shown to be overcome by strengthening the water-protein interactions. The polarizable Martini force field for both, protein and water leads to less collapsed conformations. Analysis of the interaction surface of the PHF of AD indicates, that salt bridges between 331Lys and 338Glu play an important role for their stability. Furthermore, the effect of buffer conditions, especially the choice of the ions on the stability of both, the PHF and SF will be discussed. Understanding the mechanism of tau fibrillation is the key to understand the neurodegenerative process of AD and related tauopathies. The computational resources in IT4Innovations were granted by the Ministry of Education, Youth and Sports of the Czech Republic through the e-INFRA CZ (ID:90254). This project was supported by the Brno Ph.D. Talent Scholarship – funded by the Brno City Municipality, Czech Republic and by the European Union's Horizon Europe 2020 program under the grant agreement No. 101087124 – ADDIT-CE. [1] E. M. Mandelkow and E. Mandelkow, Biochemistry and cell biology of tau protein in neurofibrillary degeneration. Cold Spring Harb Perspect Biol., 4:ea006247, 2012.[2] B. Falcon et al., Tau filaments from multiple cases of sporadic and inherited alzheimer's disease adopt a common fold. Acta Neuropathol, 136(5):699–708, 2018.[3] P. Latham and B. Zhang, Unifying coarse-grained force fields for folded and disordered proteins. Current Opinion in Structural Biology, 72:63–70, 2022.

6-POS Board 6

MODELING OF NICOTINE WITHIN THE DIFFUSE ELECTRON DENSITY: TO DESIGN EFFICIENT PERIPLASMIC BINDING PROTEIN-BASED BIOSENSORS

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Intensity-based fluorescent nicotine-sensing fluorescent sensors are developed to test the hypothesis that nicotine enters the cytoplasm and organelles at the concentrations relevant to the brain of smokers and vapers. This entry allows nicotine to act as a pharmacological chaperone for nascent nicotinic acetylcholine receptors in the endoplasmic reticulum. This process leads to the upregulation thought to play a key role in nicotine dependence. To develop sensitive sensors, it is crucial to obtain a structural understanding of nicotine-bound sensor complexes, which are often hampered by the diffused electron density of the ligand, possibly due to the inherent dynamics. Here, we developed a molecular dynamics (MD) simulation-based workflow to assign a nicotine-bound pose in the X-ray map of nicotine-bound iNicSnFR3a. The protocol involves 1) exhaustive exploration of drug conformations, 2) clustering of the energy minimized conformations, 3) launching of parallel MD simulations from each cluster, 4) re-clustering the MD data, and 5) analysis of cluster populations and cross-correlation calculations with the X-ray map. The newly identified nicotine-bound complex was further subjected to multi-microsecond scale MD simulation that showed how a helix, linking the ligand binding site to the fluorophore, appears tilted in the newly designed sensor relative to the older one, likely altering allosteric network(s). Our computational findings were further verified by thermal stability characterization using differential scanning fluorimetry experiments. Overall, we showed how interactive computational and experimental approaches can be used to improve the sensitivity of periplasmic binding protein-based nicotine biosensors for measurements in biofluids to understand nicotine dependencies.

9-POS Board 9

STRUCTURAL INSIGHTS AND COMPUTATIONAL DESIGN OF LIGAND-MEDIATED FUNCTION RECOVERY IN D408Y AND I423T MISSENSE MUTANTS OF THE HUMAN FOLLICLE-STIMULATING HORMONE RECEPTOR

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Follicle-stimulating hormone receptor (FSHR) plays an essential role in reproductive function. Mutations in the structure of this GPCR may result in impaired receptor expression and function due to misfolding and intracellular retention of the defective receptor, leading to reproductive disorders. The present study took advantage of the recent cryo-electron microscopy (Cryo-EM) structure of the FSHR, which was further refined applying molecular dynamics (MD) simulations to elucidate the structural dynamics and signal transduction mechanisms of the wild-type receptor. Using these structures, we then applied computational biophysics approaches to test whether a novel allosteric agonist may restore expression and function of mutant FSHR variants. Our refined MD simulations revealed key conformational changes and interactions within the transmembrane (TM) regions of FSHR, highlighting potential allosteric binding sites critical for receptor activation. The small molecule (CAN1405; CanWell Pharma Inc., Woburn, MA, USA) showed promising binding affinity and efficacy for rescuing expression and function of FSHRs with clinically relevant missense mutations. Trafficking defective mutations in the ectodomain of the FSHR failed to rescue expression of the misfolded receptor in response to the allosteric agonist, whereas those mutations in the TM domain (mainly at TM2 and the extracellular loop 2) favored trafficking of the misfolded FSHR to the cell surface plasma membrane in response to the allosteric agonist. Biochemical studies showed that the rescued FSHRs responded to the orthosteric agonist in terms of cAMP production and phosphorylation of MAPK/ERK1/2. These findings provide a comprehensive structural and dynamic frame work for understanding FSHR signal transduction and offer a promising therapeutic strategy for treating mutation-induced receptor dysfunction. This study underscores the synergistic potential of Cryo-EM and computational biophysics in drug discovery and precision medicine, offering novel insights and new therapeutic avenues for reproductive health disorders. [Study supported by grant IN208323 from the PAPIIT-UNAM, Mexico (to A.U.-A.)].

10-POS **Board 10**

MOLECULAR FUNCTIONS OF DRG GTPASES IN PROMOTING PROTEIN SYNTHESIS IN TRANSLATION

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Maintaining proper protein homeostasis is essential for cell physiology. The ribosome and GTPases, which are two of the most ancient and critical cellular molecules, are central players in protein synthesis and its regulation. Here we report the discovery of a new general translation factor that targets stalled ribosomes and promotes protein synthesis in an evolutionarily conserved manner. We show that the essential bacterial Obg GTPases are distant homologs of eukaryotic and archaeal Drg proteins and serve critical roles in promoting efficient protein translation in stalled ribosomes. Through in vivo characterization, including cross-species complementation of cells where ribosomes are induced to stall by addition of either the antibiotic anisomycin or exogenous mRNA harboring a long poly(A) sequence, we demonstrate that a conserved function of Drg proteins is to alleviate ribosomal stalling during translation. Our data show that bacterial Obg rescues stalled ribosomes in both Saccharomyces cerevisiae and human cells lacking endogenous Drgs, as does supplementation of the respective endogenous Drg proteins from yeast and human cells. Furthermore, the presence of ObgE and GTP stimulates peptidyl transfer, the key catalytic function of the ribosome, suggesting a possible molecular mechanism of this GTPase to enhance translation in stalled ribosomes. Combined with the interactions revealed by the cryoEM structure, our results show that the Drg protein is a new general translation factor that directly affords cells from three domains of life a new form of translation regulation.

13-POS **Board 13**

CRYO-EM STRUCTURE OF THE 8 MDA HUMAN VAULT PARTICLE AT 3.2 ÅNGSTROM

Fabio Lapenta[1,2]; Jana Aupic[3]; Simone Marrancone[2]; Sonia Covaceuszach[6]; Gangupam Bhavani[2]; Alexandre Durand[4]; Nils Marechal[4]; Dihia Moussaoui[7]; Claudia d'Ercole[2]; De March Matteo[2]; Ario de Marco[2]; Davide Cotugno[5]; Gianni Frascotti[5]; Paolo Tortora[5]; Alessandra Magistrato[3]; Alberto Cassetta[6];
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Vaults are large ribonucleoprotein (RNP) particles with a size of 70 nm × 40 nm × 40 nm. Initially reported in 1986, vaults have a distinct ovoidal architecture made of 2 symmetric 39-meric shells composed of the major vault protein (MVP). Despite its large size and the high levels of conservation of the vault components, the precise role of this complex in the cell is not yet fully understood. Here, we report the first cryoelectron microscopy (cryoEM) reconstruction of the human vault complex at 3.2 Å obtained with a standard workflow in cryoSPARC and provide a comparison to the previously published structures of its murine counterpart. Additionally, we investigated the interaction and binding site to the human onco-suppressor protein PTEN with Grating-Coupled Interferometry (GCI) and Small Angle X-ray scattering (SAXS), which showed a strong affinity (kd of 1 μM) in presence of 10 mM of Ca(II), this supports the role of the vault as molecular scaffold and transport of the onco-suppressor protein.

14-POS **Board 14**

KIR CHANNEL DYNAMICS REVEALED BY MOLECULAR DYNAMICS SIMULATIONS

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Inwardly rectifying potassium (Kir) channels are important regulators of membrane potential in both excitable and non-excitable cells. Various diseases arise from aberrant Kir channel activities, which are controlled by a complex interplay of regulatory lipids, small molecules, and auxiliary proteins. Despite recent advancements in cryo-electron microscopy (EM) that have greatly enhanced our understanding of Kir channel structures, their conformational dynamics remain elusive. This study employs atomistic molecular dynamics (MD) simulations in order to 1) unveil intrinsic conformational dynamics of Kir2.1 channels and 2) elucidate how these dynamics are modulated by the primary agonist, phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$). The simulations show that $PI(4,5)P_2$ potentiates a clockwise twisting motion in the cytoplasmic domain of each subunit, leading to opening of the channel at a gate formed by the four TM2 helices at the bottom of each transmembrane domain, consistent with a recent EM-based structure of $PI(4,5)P_2$-bound open Kir6.2 channel. $PI(4,5)P_2$ also suppresses intrinsic 2-fold anticorrelated motions between the two pairs of diagonally opposed subunits while enhancing positive correlation among all four subunits, thus facilitating channel opening and explaining the basis of cooperative gating by $PI(4,5)P_2$. In conclusion, the atomistic MD simulations reveal the intrinsic conformational dynamics of Kir channels and demonstrate how the primary agonist modulates these dynamics, collectively facilitating channel opening.

17-POS Board 17

REFINING AND ASSIGNING METAL IONS OF RNA STRUCTURES

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The presence of metal ions in the biomolecular structure is crucial to its function. For complex RNA-based structures, magnesium ions are of utmost importance due to their role in catalysis, folding and stability. They are also the most often found ion in such structures. However, around 40% of the ribosomal structures deposited in the Protein Data Bank database do not contain a single Mg2+ site. Moreover, the number of Mg2+ ions in RNA-based structures is mostly insufficient to neutralize the negative charge of the nucleic acids of the system. Identification of Mg2+ ion sites is challenging due to the fact that magnesium has the same number of electrons as water and sodium, and neither of which can be distinguished from Mg2+ by difference electron density maps alone. Therefore, in our study, we employed a machine learning (ML) approach to first identify incorrectly assigned ions and to predict the correct placement of metal ions in a set of ribozyme structures. All ion sites were examined using a ML approach in order to determine if the ion type was assigned correctly. Classical molecular dynamics (MD) simulations were used to observe the behavior of the metal ions combined with the same ML approach to determine the ion type during the course of the simulation. Finally, the metal ion positions were superimposed with ML predictions of metal ions placement in order to corroborate the observations from MD simulations. Surprisingly, we were able to identify metal ions which most likely are incorrectly assigned. Poorly coordinated metal ions were more likely to dissociate from their binding site, while those which were assigned correctly and coordinated by more ligands were more likely to stay in their site during the simulation.

18-POS **Board 18**

POLYMER-EXTRACTED STRUCTURE OF THE MECHANOSENSITIVE CHANNEL MSCS REVEALS LIPID-MEDIATED MECHANISM OF INACTIVATION

Elissa Moller[1,2,3]; Madolyn Britt[2,3]; Fei Zhou[1]; Hyojik Yang [4]; Andriy Anishkin[3]; Robert Ernst[4]; Sergei Sukharev[3,5]; Doreen Matthies[1];
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Membrane protein structure determination is technically challenging and further complicated by the removal or displacement of lipids, which can result in non-native conformations or a strong preference for certain states at the exclusion of others. This is especially applicable to mechanosensitive channels (MSC's) that evolved to gate in response to subtle changes in membrane tension in the lipid bilayer. E. coli MscS, a model system for MSC gating, is an inner membrane protein that opens when external osmolarity changes cause water influx and stretches the membrane. The efflux of osmolytes through these channels reduces the osmotic gradient and prevents cell lysis, enabling bacteria to colonize osmotically challenging host environments and survive transmission through fresh water. As a tension sensor, MscS is very sensitive and highly adaptive. It readily opens under super-threshold tension and closes upon tension reduction, but under lower tensions, it slowly inactivates and can only recover after tension release. Existing cryo-EM structures do not explain the entire functional gating cycle of open, closed, and inactivated states. A central question in the field has been the assignment of the frequently observed non-conductive conformation to either a closed or inactivated state. In this study we solved a 3 Å cryo-EM structure of MscS in native nanodiscs obtained via extraction with the novel Glyco-DIBMA polymer, eliminating the detergent solubilization and lipid removal step common to all prior structures. We observe densities of endogenous phospholipids between the transmembrane helices, stabilized by electrostatics interactions. Through mutations we examine the functional effects of their destabilization, illustrating a novel lipid-mediated inactivation mechanism based on an uncoupling of the peripheral tension-sensing helices from the gate. The use of this polymer increased the predictive power of our cryo-EM structure, allowing us to associate the solved conformation with the inactivated state of the multi-state MSC MscS.

21-POS Board 21

ELUCIDATING THE INTRICATE REGULATORY MECHANISMS OF RHO GTPASES CANCER ISOFORMS THROUGH COMPUTER SIMULATIONS

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Rac1 (Ras-related C3 botulinum toxin substrate 1), a member of the Rho-GTPase family, regulates cell adhesion, morphology, and movement, and is over-expressed in various types of cancers. An elegant and intricate system of protein-protein interaction networks regulates the function of Rho-GTPases. GTPase-activating proteins (GAPs) and guanine exchange factors (GEFs) repeatedly promote the exchange between GTP and GDP, thereby allowing the switch between the active and inactive forms of the Rho GTPase.The cryo-EM structures of DOCK proteins and their small-GTPase complexes have provided insights into the GEF activity mechanism. However, the regulatory mechanism of DOCKs by partner proteins and the signal transduction events responsible for Rho-GTPase regulation in larger macromolecular assemblies remain elusive. Due to their key regulatory role, Rho GTPases are the object of cancer-associated mutations, and following the recent approval of Sotorasib, they are emerging as amenable targets for the discovery of small molecule covalent inhibitors.In this contribution, the regulatory mechanisms of Rac1 are revealed by all-atom simulations. Several model systems are compared, starting with the homodimeric cryo-EM structure of ELMO1-DOCK5-Rac1 (1). The monomer and the DOCK5-Rac1 complex bound to GDP and in the apo form are also investigated. Additionally, a comparison between the different wild-type systems, the most frequent Rac1P29 mutations, and the Rac1b splice variant is done. Different types of simulations reveal: (i) the molecular details of the main functional motifs of Rac1 and Rac1 pathological isoforms; (ii) the structural and dynamical features of ELMO1-DOCK5-Rac1 complexes; (iii) the GTP binding affinities and the residence time of the GDP product within the catalytic pocket of wild-type and Rac1 cancer isoforms [2]; and (iv) ELMO1-DOCK5-Rac1 membrane interactions.(1)Kukimoto-Niino, Mutsuko, et al. "Cryo-EM structure of the human ELMO1-DOCK5-Rac1 complex." Science advances 7.30 (2021): eabg3147.(2)Parise, Angela, and Alessandra Magistrato. "Assessing the mechanism of fast-cycling cancer-associated mutations of Rac1 small Rho GTPase." Protein Science 33.4 (2024): e4939.

22-POS Board 22

AUGMENTING CRYO-EM DATA ON DYNAMIC SYSTEMS WITH ALL-ATOM SIMULATIONS: EXPLORING THE MECHANISTIC CONTRIBUTION OF PRP2 HELICASE TO SPLICING CYCLE PROGRESSION

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Premature messenger (pre-mRNA) transcripts undergo splicing before becoming mature funcational RNAs. The splicing process is promoted by spliceosome, a large and dynamic macromolecular RNA-protein assembly. Splicing occurs though multiple consecutive steps which are accompanied by significant compositional and conformational changes of the spliceosome. Responsible for driving this spliceosomal remodeling are specific helicase proteins. Here, we focus on transition from the initially assembled state into catalytic competent spliceosome; a process driven by the PRP2 helicase. We employed all-atom molecular dynamics simulations to augment a Cryo-EM study (Schmitzova et al., 2023) by refining all-atom structure of the splicesomal core in the Baqr state and reconstructing its structural dynamics. We simulated two variants of the system on a micro-second time-scale: spliceosome with PRP2 helicase mechanically stalled in its presumably final state, and a modeled system with PRP2 free to process. The simulations predict dynamic interactions between the helicase and spliceosomal proteins responsible for pre-mRNA selection, which were not resolved in the Cryo-EM structure. Analysis of the movement correlation in the simulated systems suggests that the helicase facilitates large-scale structural rearrangements not only by translocating along the pre-mRNA strand, as revealed by the Cryo-EM structure, but also by actively releasing some of the per-mRNA-bound splicing factors. For instance,we observe strong coupling between SF3B1 ring opening dynamics and the movements of the helicase's catalytic domain. Further analyzes and simulations are ongoing to gain more robust sampling and understand coupling between the PRP2 helicase action and the catalytic site. Taken together, our simulations extend the static Cryo-EM structure by predicting all-atom-resolution structural ensembles and reconstructing functional dynamics of this highly plastic system.

25-POS **Board 25**

IDENTIFICATION OF EM-RESOLVED LIPID FRAGMENTS USING STREAMLINED ALCHEMICAL FREE ENERGY PERTURBATION (SAFEP)

Ezry Santiago-McRae[1]; Thomas T Joseph[2]; Wayland Cheng[3]; Jérôme Hénin[4]; Grace Brannigan[1,5];
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Over the past ten years, the number of cryo-EM structures with partially resolved lipids has increased dramatically. Lipid binding sites may be important both for native protein function and as potentially drugable sites. Although cryo-EM data can indicate the presence of ordered lipids, those lipids are rarely identifiable from the density alone. We use our Streamlined Alchemical Free Energy Perturbation protocol (SAFEP) for computationally estimating free energies of phospholipid binding (ΔGbind) to identify lipids and compare affinities across sites and protein conformations. There are several challenges posed by the binding of phospholipids to membrane proteins: 1) lipid flexibility makes traditional restraint schemes less effective, 2) slow relaxation of lipids slows convergence of the free energy estimates, and 3) lipid-water phase separation necessitates careful interpretation of any lipid ΔGbind. Addressing the first two issues required simple, but highly designed restraints combined with thorough sampling. The phase separation of the system was addressed theoretically by framing the results in terms of binding probabilities - leaving any "standard" binding free energy as an intermediate result. Convergence of all calculations was monitored by several metrics as any one metric was been found to be fallible. We use lipid binding by Erwinia Ligand-Gated Ion Channel (ELIC) as a model system. ELIC is a GABA-activated, prokaryotic member of the pentameric ligand-gated ion channel (pLGIC) protein family. Like other pLGICs, ELIC is known to be modulated by its lipid environment through unknown mechanism(s). By a combination of relative and absolute binding free energy calculations we were able to identify a partially resolved lipid as POPG, determine that POPC is a non-binder to the site, POPE can compete for the site at higher mole fractions, and POPG binds with greater affinity to the open conformation of ELIC.

26-POS **Board 26**

CONSTANT PH METADYNAMICS SIMULATIONS IN THE STUDY OF RNA OLIGOMERS

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RNA molecules have a wide range of biological functions due to their highly flexible structures. Their flexibility stems from complex H-bonding networks defined by both canonical and non-canonical base pairs. With some non-canonical base pair interactions requiring (de)protonation events to either stabilize or perturb H-bond networks. Constant pH molecular dynamics (CpHMD) methods provide a reliable tool to describe the conformational space of dynamic structures and to obtain robust calculations of pH-dependent properties (i.e. pK_a). However, pH-sensitive methods have rarely been explored in the field of nucleic acids, despite growing biological evidence concerning pH regulation of certain motifs' H-bond networks. In this work, we present an extension of the stochastic CpHMD method to RNA from the standard XOL3 AMBER force field and demonstrate the accuracy of our method to reproduce pK_a's of RNA oligomers. Poly-U trimers and pentamers with a single central titrable site were characterized for method validation. To tackle their high degrees of freedom, we have integrated a well-tempered (wt) metadynamics approach into the CpHMD methodology (CpH-MetaD). The CpH-MetaD technique significantly expanded the sampled conformational space, allowing for more robust and accurate estimates of the oligomers' pK_a shifts with respect to the increased phosphate content: 0.5 (A3mer to A5mer); 0.4 (C3mer to C5mer); 0.6 (U3mer to PolyU); 0.5 (G3mer to G5mer). The predicted pK_a values – A[3,5]mer: 3.6 (0.1)/4.1 (0.2); C[3,5]mer: 4.6 (0.3) / 5.0 (0.2) ; G[3,5]mer – 10.1 (0.3) / 10.6 (0.2); U[3,5]mer: 9.9 (0.1)/10.5 - and relative shifts are in good agreement with experimental data. Nucleobase stacking and electrostatic interactions with phosphate groups clarify the intramolecular phenomena which dictate the experimentally observed pK_a shifts. This work highlights the robustness and accuracy of CpHMD/CpH-MetaD applied to RNA oligomers, and the proton binding affinity sensitivity to phosphate group content in the RNA backbone.

27-POS Board 27

PARAMETERIZING THE COVALENT INTERMEDIATES OF THE NITRILASE SUPERFAMILY

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Proteins of the nitrilases superfamily (which include amidases) have a structurally conserved active site grouping comprising two glutamates, a lysine, and a cysteine. All postulated mechanisms involve covalent modification of the cysteine. Such covalently modified amino acids cannot be modeled by the best available modeling tools used in the field. To address this shortcoming, we developed a procedure that enables the optimization of these amino acids using the Interactive Structure Optimization by Local Direct Exploration (ISOLDE) package incorporated in UCSF ChimeraX. In addition to coordinates that conventional docking programs rely on, ISOLDE employs electron density to minimize protein structures. The AmberTools23 suite of programs was used to generate force field parameters and semi-empirical (AM1-BCC) charges for the covalently modified cysteine residues of the thioester and thioimidate intermediates, enabling their minimization in ISOLDE.We previously characterized the amidase from Nesterenkonia sp. (NitN) that forms a dimer and displays activity against a range of amide substrates that are hydrolyzed to form ammonia and the corresponding carboxylic acid. To investigate the energy barrier involved in the deprotonation of the catalytically active cysteine by the conserved glutamate (i.e., Cys-S-H + Glu-COO(-) --> Cys-S(-) + Glu-COOH), truncated models of the active site were extracted from the molecular mechanics-minimized protein structures and further investigated using density functional theory.The ωB97X-D/def2-TZVP energy profiles indicate that Cys-S-H + Glu-COO(-) is approximately 5 kJ/mol lower in energy than Cys-S(-) + Glu-COOH). In addition, there is an ~20 kJ/mol energy barrier that must be overcome for the conventional base-catalyzed nucleophilic attack of the cysteine on an amide substrate to hold true. Furthermore, we have generated the natural bond orbitals (NBOs) of various docked amide and nitrile substrates and their thioester and thioimidate intermediates. Preliminary results indicate the correct stereoelectronic orientation for the progression of the enzymatic reactions.

30-POS Board 30

RELATION BETWEEN STRUCTURE AND RATE CONSTANTS IN SINGLE-MOLECULE ROTATION OF F1-ATPASE

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In this work we describe a method for extracting, from F1-ATPase controlled rotation experiments, the angle-dependent rate constants for nucleotide binding and release without assuming a particular functional form. Then an analysis of angle-dependent conformational changes using recent Cryo-EM structures is provided. To establish the relation between the rate constants and structure, we employ an angle-dependent theory of nucleotide binding. Using the theory it is shown how the concerted and angel-dependent binding of ATP and release of ADP post-hydrolysis occur in concert both in uni-site occupancy and multi-site conditions. The theory, applied to single-molecule rotation data, also reveals a short-lived state after ATP binding and before the ADP release in a different site. It is concluded that the concerted kinetics is due to a strong structural correlation in the binding cleft opening un one subunit with the closing of the cleft in another subunit in the $\alpha_3\beta_3$ ring.

33-POS Board 33

GHOSTBUSTER - A DIFFRACTION TOMOGRAPHY ALGORITHM FOR CRYO-EM PARTICLE REFINEMENT

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Continual advances in methods development for single particle imaging in cryogenic electron microscopy (cryoEM) have paved the way towards ever-increasing resolution for three-dimensional (3D) particle reconstruction. For thicker particles, one has to further correct for the effects of multiple scattering within the particle. This is commonly associated with the correction of the Ewald sphere curvature, and existing algorithms have recently been successful in pushing the resolution limit beyond what was possible. However, the physics of cryo-EM image formation is better understood as in-line holography. This implies that the phases of the complex-valued field arriving at the detector are lost upon the measurement of particle images. Therefore, the Fourier coefficients inferred from these phase-less images, which are subsequently used in Ewald sphere curvature correction, cannot fully remove the effects of multiple scattering. This results in ghost-like artifacts in the reconstructed particle. Here, we develop an end-to-end diffraction tomography algorithm, named Ghostbuster, as an alternative approach to minimize the appearance of these ghost artifacts. Ghostbuster refines the 3D particle through batch stochastic gradient descent by minimizing the error between the estimated images from a multislice-based forward model and actual cryo-EM measurements. By accounting for the effects of multiple scattering, Ghostbuster demonstrates improved reconstructed particle resolution beyond traditional Ewald sphere curvature correction algorithms in existing state-of-the-art software for both simulated and experimental datasets.

Wednesday, October 23
POSTER SESSION II
16:20 – 18:20

Posters are available for viewing only during their scheduled date of presentation. Below are the formal presentation times. Presenting authors with odd-numbered poster boards should present from 16:20 – 17:20 and those with even-numbered poster boards should present from 17:20 – 18:20. The presenters listed below are required to remain in front of their poster boards to meet with attendees.

Odd-Numbered Boards 16:20 – 17:20 | Even-Numbered Boards 17:20 – 18:20

Di Marco, Salvatore	**3-POS**	**Board 3**
Dingeldein, Lars	**4-POS**	**Board 4**
Hoff, Samuel	**7-POS**	**Board 7**
Janoš, Pavel	**8-POS**	**Board 8**
Kubo, Shintaroh	**11-POS**	**Board 11**
Languin-Cattoën, Olivier	**12-POS**	**Board 12**
Marotta, Federico	**15-POS**	**Board 15**
Miceli, Marcello	**16-POS**	**Board 16**
Novi Inverardi, Giovanni	**19-POS**	**Board 19**
Oide, Mao	**20-POS**	**Board 20**
Rozza, Riccardo	**23-POS**	**Board 23**
Sahoo, Abhilash	**24-POS**	**Board 24**
Svetina, Saša	**27-POS**	**Board 27**
Tang, Wai Shing	**28-POS**	**Board 28**
Yadav, Priya	**32-POS**	**Board 32**

Posters should be set up on the morning of Tuesday, October 22 and removed by 18:30 on Wednesday, October 23. All uncollected posters will be discarded.

3-POS Board 3

RNA-MEMBRANE INTERACTIONS: ELUCIDATING SEQUENCE SPECIFICITY VIA ENHANCED SAMPLING TECHNIQUES

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Mounting evidence indicates that RNA molecules are also present on the extracellular surface of living cells. Moreover, RNA-membrane interactions are emerging as a key player in natural and synthetic biological systems, such as virions and lipid-based RNA delivery systems, respectively. While preliminary studies suggest these interactions are sequence-specific and mediated by factors such as divalent cations, RNA secondary structure and membrane phase, the molecular terms which drive RNA-membrane interactions remain poorly understood. Here, we use enhanced sampling molecular dynamics to elucidate the molecular principles governing RNA-membrane interactions, considering the membrane both in their fluid and gel-like phases. In particular, we focused on characterizing the interactions between model membranes and a different set of model systems ranging from simple nucleosides to G-quadruplexes structures. G-rich RNA strands have been demonstrated (Czerniak, Tomasz, and James P. Saenz. "Lipid membranes modulate the activity of RNA through sequence-dependent interactions." Proceedings of the National Academy of Sciences 119.4 (2022)) to have a strong affinity for the gel membrane. For these model systems, we estimated affinities and binding modes, validating our results on the basis of currently available experimental data. Thus, our work will highlight the molecular principles governing RNA-membrane association, broaden our understanding of RNA biological roles and could help the design and delivery of mRNA-based vaccines.

4-POS Board 4

AMORTIZED IDENTIFICATION OF BIOMOLECULAR CONFORMATIONS IN CRYO-EM USING SIMULATION-BASED INFERENCE

Lars Dingeldein[1,2]; David Silva-Sánchez[4,6]; Luke Evans[4]; Nikolaus Grigorieff[8,9]; Edoardo D'Imprima[7]; Roberto Covino[1,3]; Pilar Cossio[4,5];
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[8]University of Massachusetts Chan Medical School, RNA Therapeutics Institute, Worcester, MA, USA
[9]Howard Hughes Medical Institute, Chevy Chase, MD, USA

Comparing experimental measurements to theoretical models is a fundamental component of scientific investigations. Often, this means comparing observations from simulators that capture the important physics to experimental observations. The aim is to gain a mechanistic understanding by identifying model parameters that can reproduce the experimental data. However, inferring these parameters is often challenging and time-consuming. To tackle these issues, new machine learning-based approaches known as Simulation-Based Inference have been developed, although they have not yet been applied to Cryo-Electron Microscopy (Cryo-EM). Here, we introduce a new method (cryoSBI) using Simulation-based inference to approximate the Bayesian posterior distribution. We utilize Neural Posterior Estimation, a technique that directly approximates the Bayesian posterior using a simulator (e.g., a Forward Model) and a neural density estimator. The key advantage is that the cryoSBI training only happens once with simulated data. Afterwards, inference for each particle takes just one forward pass through the neural network. This provides a significant advantage as the posterior is amortized: the particle pose and imaging parameters do not need to be estimated, resulting in a considerable computational speedup compared to explicit likelihood methods. Through extensive experiments with synthetic and real cryo-EM data, cryoSBI extracts molecular conformations from observations while providing a meaningful measure of confidence in the inferred parameter.The primary advantage of cryoSBI lies in its direct estimation of the posterior distribution, circumventing the computationally intensive pose estimation typically associated with cryo-EM. This leads to a substantial reduction in computational overhead compared to traditional explicit likelihood methods. CryoSBI is especially beneficial in situations that involve large conformational changes and large amounts of experimental data, as is often the case in cryo-EM.

7-POS Board 7

EXTRACTING PROTEIN ENSEMBLES FROM CRYO-EM 2D DATA AND RECONSTRUCTED 3D MAPS

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Cryo-EM has proven to be an instrumental technique for determining the structures of biomolecules. As the resolution obtainable through cryo-EM increases, it has become possible to generate high-quality single-structure atomic models of proteins from the reconstructed 3D cryo-EM maps to represent proteins and other biomolecules. However, as proteins occupy a variety of functionally important conformation states, they cannot be accurately described by single model representations. Critical information pertaining to protein conformational heterogeneity is present in both reconstructed 3D maps and 2D particle image sets but is often not fully utilized during model generation or is not properly extracted from the particle images and 3D maps. To accurately describe proteins as entities which occupy an ensemble of structural states, new approaches must be developed to accurately extract information on biomolecule conformational heterogeneity present in sets of cryo-EM particle images and reconstructed 3D maps. Here we demonstrate the utility of two Bayesian inference based integrative approaches, BioEM and EMMIVox, to determine conformational ensembles of proteins from both 2D particle images and reconstructed 3D maps. We utilize BioEM with coarse-grain molecular dynamics to gain insights into the dynamic nature of a multidomain bacterial toxin and a myosin protein from 2D particle images. Additionally, we apply the newly developed tool EMMIVox to explore functionally important conformational heterogeneity describing the continuous dynamics of glutamine synthetase using high-resolution (2 Å) 3D reconstructed maps from cryo-EM. These approaches allow us to obtain novel insights into the structure-dynamic-function relationships of these important systems. Further development and application of pipelines which extract and utilize the entirety of the data generated from cryo-EM will be critical to accurately describe structurally complex and dynamic biomolecules. EMMIVox is now available in the Integrative Structural and Dynamical Biology module of the open-source, freely-available PLUMED library (www.plumed.org).

8-POS Board 8

CLOSING OF THE SF3B SPLICING COMPLEX: INSIGHT FROM SIMULATIONS WITH MACHINE-LEARNED COLLECTIVE VARIABLES

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RNA splicing is a crucial stage in gene expression wherein introns are removed from pre-RNA and exons are joined to form mature RNA, either protein-encoding mRNA or functional non-coding RNA. The spliceosome, a complicated ribonuclear machine, plays a crucial role in precise pre-RNA splicing [1] and if deregulated can lead various diseases. [2] Spliceosome is composed of five small nuclear RNAs (snRNA) and tens of associated protein factors, which all undergo dynamical conformational changes. One of the important conformational transitions is the formation of the B^{act} spliceosome associated with closing of the SF3b complex upon recognition of the branch point sequence, during which the pre-RNA is engulfed and the bulged branch point adenosine is positioned in a specific pocket [3]. Cryo-EM studies have made tremendous strides in shedding light into the splicing process [3]. However, they mostly provide static snapshots, while computational techniques can uncover the dynamical transformation between different stages in the splicing cycle and thus help fill-in the gaps between experimental results. The conformational change that the SF3b complex undergoes is essential for understanding key aspects of splicing, including the mechanism of small-molecule splicing modulators [5]. However simulating it is challenging due to the size and complexity of the system. To overcome this challenge, we apply the deepLDA machine learning approach [6] to obtain a collective variable that can describe such a complex conformational transformation. Set of distances and angles between distinct part of the SF3b complex [7] are used as the input parameters that get compressed by deepLDA into a single collective variable able to discriminate the open and closed states of SF3b complex. Using this collective variable with enhanced sampling methods we are able to simulate the opening/closing of the SF3b complex and obtain key mechanistic insight into spliceosome, including the effect of splicing modulators. [1] Wilkinson, M. E., Charenton, C., & Nagai, K. (2020). RNA splicing by the spliceosome. Annual review of biochemistry, 89, 359-388. [2] Borišek, J., Casalino, L., Saltalamacchia, A., Mays, S. G., Malcovati, L., & Magistrato, A. (2020). Atomic-level mechanism of pre-mRNA splicing in health and disease. Accounts of Chemical Research, 54(1), 144-154. [3] Sun, Chengfu. "The SF3b complex: splicing and beyond." Cellular and Molecular Life Sciences 77.18 (2020): 3583-3595 [4] Tholen, J., & Galej, W. P. (2022). Structural studies of the spliceosome: bridging the gaps. Current Opinion in Structural Biology, 77, 102461. [5] Rozza, R., Janoš, P., Spinello, A., & Magistrato, A. (2022). Role of computational and structural biology in the development of small-molecule modulators of the spliceosome. Expert Opinion on Drug Discovery, 17(10), 1095-1109. [6] Bonati, L., Rizzi, V., & Parrinello, M. (2020). Data-driven collective variables for enhanced sampling. The journal of physical chemistry letters, 11(8), 2998-3004. [7] Melo, Marcelo CR, et al. "Generalized correlation-based dynamical network analysis: a new high-performance approach for identifying allosteric communications in molecular dynamics trajectories." The Journal of Chemical Physics 153.13 (2020): 134104.

11-POS Board 11

CRYO-EM AND THEORETICAL STUDIES HIGHLIGHT THE CRITICAL ROLE OF OUTER JUNCTIONS IN NATIVE DOUBLE MICROTUBULES

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Cilia, important cellular structures, rely on a microtubule-based framework known as the axoneme, composed of intricate networks of microtubule-inner proteins (MIPs). This study uses cryo-electron microscopy to investigate the MIPs found in Tetrahymena thermophila's microtubule structures, identifying 42 distinct MIPs. Crucial insights are gained into the diverse roles of MIPs, including assembly and stability of the doublet outer junction. Knocking out CFAP77, a key component of the outer junction, reduces Tetrahymena swimming speed and beat frequency, underscoring its significance in cilia function.

12-POS **Board 12**

OVERCOMING SLOW MG^{2+} DYNAMICS IN RNA SIMULATIONS

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Magnesium ions have a crucial role in shaping RNA structural and catalytic properties [1]. Unfortunately, identification of Mg^{2+} binding sites using X-ray crystal diffraction and Cryo-EM is notably challenging, and has been shown to be biased [2]. Additionally, RNA folding and ion binding are coupled, highly dynamical processes that are not easily captured by standard refinement procedures, which generally aim at inferring a static molecular model.Molecular Dynamics (MD) simulations are an ideal complementary tool for studying the interplay between ions and nucleic acids, but they greatly suffer from the slow binding kinetics and overall low diffusivity of divalent cations. In his work, we develop a methodological approach to accelerate Mg^{2+} dynamics while maintaining the system's exact thermodynamics. We optimize a set of Mg^{2+} Lennard-Jones parameters to accelerate Mg-water and Mg-RNA binding kinetics while maximizing overlap with the original ensemble, allowing the direct use of Hamiltonian Replica-Exchange methods as already available in most MD softwares. Additionally, we implement a simulated-tempering variant that bypasses the need for a ladder of replicas, and replaces it with an appropriate re-weighting of the simulated trajectory. This allows to scale up the method to a large number of ions without requiring a large number of replicas. Moreover, this procedure can be seamlessly combined to other strategies relying on multiple replicas, such as Metainference [3], in order to incorporate available experimental information and investigate the intricate relationship between Mg^{2+} ions and RNA structural dynamics.[1] Bowman et al., Curr. Opin. Struct. Biol., 22:262-272 (2012)[2] Auffinger et al., RNA, 27:243-252 (2021)[3] Bonomi et al., Sci. Adv., 2(1): e1501177 (2016)

15-POS Board 15

A KINETIC MODEL FOR TRANSLATION ELONGATION FROM THE STEADY-STATE DISTRIBUTION OF RIBOSOME INTERMEDIATES

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Protein synthesis is one of the fundamental processes of molecular biology, with the ribosome playing a crucial role. Recently, it became possible to explore the intermediate states of translation elongation directly within living cells using cryogenic electron tomography (cryo-ET). Specifically, this gave us a picture of the occupancy of each state in the model organism Mycoplasma pneumoniae. Nevertheless, cryo-ET is still unable to provide a dynamic, time-resolved view of biological processes. In this work, we develop a kinetic model of translation elongation that integrates data from biochemical experiments in E. coli and transfers this information to M. pneumoniae under a "minimal evolution" assumption. This approach allows us to estimate the transition rates between intermediates in a way that can account for the observed steady-state distribution. Our findings indicate that the translation elongation cycle in M. pneumoniae is considerably slower and more error-prone than in E. coli. Furthermore, the mycoplasma ribosome has a marked tendency to remain in the non-rotated pre-translocation state, compared to the E. coli ribosome. Finally, we investigate whether the model can explain the effect of ribosome-targeting antibiotics such as chloramphenicol. Our methodology can potentially be extended to other biological processes where both cryo-ET and biochemical datasets are available, even if they are derived from different organisms. This allows for the estimation of the process dynamics in vivo, complementing the static cryo-ET snapshots.

16-POS Board 16

UNDERSTANDING THE MOLECULAR CONSEQUENCES OF THE R1611W MUTATION IN ALSIN'S VPS9 DOMAIN: EXPERIMENTAL AND COMPUTATIONAL PERSPECTIVES

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The expression of mutated forms of the Alsin protein has been linked to Infantile Onset Ascending Hereditary Spastic Paraplegia (IAHSP), a rare neurodegenerative disease. One of the mutations identified as pathological is located in Alsin's Vacuolar Protein Sorting 9 (Vps9). The Alsin's Vps9 domain works as an exchange factor (GEF) for Rab5, and evidence suggests its involvement in the process of Alsin-mediated endosome formation. More in detail, it was observed that the missense mutation that produces the expression of a tryptophan instead of an asparagine (R1611W), in the Vps9 domain alters the oligomeric state of Alsin and its GEF functions affecting vesicular trafficking. An understanding of the molecular structure of the Vps9 domain in the Wild Type (WT) and the mutated case may help to understand the mechanisms involved in the onset of the disease. In this work, a complementary in-vitro/in-silico approach was implemented to shed light on the VPS9 domain secondary structure and understand the effects of the R1611W mutation on the isolated VPS9 domain of Alsin. Expression and purification of WT and Mutant VPS9 constructs were performed in the E. coli expression system. The circular dichroism and the size exclusion chromatography showed altered folding and a different oligomerisation behaviour because of the single-point mutation. Structure prediction and multiscale molecular dynamics, both all-atom and coarse-grained, were employed to elucidate the structure-to-function altered behaviour observed in the experiments. The mutation appears to induce conformational changes that alter the protein's structure and its ability to oligomerise. This study lays the groundwork for understanding how R1611W alters the function of the VPS9 domain and for developing an experimental protocol to obtain stable constructs for experimental structure determination experiments such as cryo-electron microscopy.

19-POS Board 19

ATOM PROBE TOMOGRAPHY AS NEW TOOL TO DETERMINE THE STRUCTURE OF PROTEINS: A COMPUTATIONAL AND EXPERIMENTAL STUDY

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Atom Probe Tomography (APT) has been recently proposed as an alternative procedure to determine three-dimensional structures of proteins. APT consists of a spatially–resolved mass-spectrometry technique, whereby a biological specimen is first embedded by an amorphous silica matrix, and subsequently evaporated under irradiation of short laser pulses, upon application of a strong electric field to direct ejected ions towards a detecting surface. Building on APT, a funded EIC-Pathfinder consortium, named MIMOSA, aims to provide a new approach to 4D microscopy, enabling high spatial resolution and chemical sensitivity by employing terahertz (THz) source lasers. Both the embedding procedure and the subsequent laser irradiation step present several challenges that can be addressed and inspected with the available computational techniques. We have proposed a Molecular Dynamics protocol aimed at depicting the early stages of the embedding process of proteins in a solution of water and polymers of orthosilicic acid, taken as precursor of the silica matrix. APT requires that the silica primer remains bio compatible, keeping the native structural features of the system at hand while condensing into an amorphous, glassy-like coating. If on one hand we observed a negligible influence of orthosilicic acid polymers on the dynamics of stable folded domains, on the other they seem to reduce the fluctuations of intrinsically disordered domains and loops. We have also recently developed a computational protocol based on Time–Dependent Density Functional Theory (TDDFT), aimed at describing the orthosilicic acid under electric field and THz laser irradiation. In fact, the combined effects of electric fields and laser pulses on the matrix complex are still not properly deciphered. We explored different values of electric field and laser frequencies, reconstructing a phase diagram characterizing the ion–field evaporation process of the silanol groups of orthosilicic acid, matching confirmed experimental data.

20-POS **Board 20**

MD-BASED CONFORMATIONAL INFERENCE OF SINGLE-PARTICLE CRYOEM IMAGES USING STOCHASTIC 3D RECONSTRUCTION

Mao Oide[1,2]; Chigusa Kobayashi[3]; Yuxia Zhang[4,5]; Satoshi Watanabe[5,6]; Kenji Inaba[5,6]; Yuji Sugita[2,3,7];
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In single-particle cryo-electron microscopy (cryoEM), we can observe a vast number of single-particle biomolecular images, each embedded in vitreous ice and consequently trapped in a conformation within a structural ensemble. These particle images enable the analysis of discrete structural heterogeneity, continuous motions, and the conformational landscape of biomolecules. Several studies have explored integrative methods using molecular dynamics (MD) simulations to support the analysis of complicated and anharmonic dynamics, such as state transitions. Typically, this type of analysis infers the conformation of each particle image by comparing it with MD structures. However, even integrative approaches struggle to handle particle images with low signal-to-noise ratios. To address this issue, we are investigating the applicability of 3D reconstruction as a denoising process prior to conformational inference. In this presentation, we will introduce our approach that incorporates stochastic 3D reconstruction into MD-based conformational inference. We will also present the application results of this method on both test and experimental cryoEM data, and discuss further challenges for its implementation.

23-POS **Board 23**

MOLECULAR MECHANISM OF ZINC-CATALYZED COVALENT SPLICING MODULATION

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The pre-messenger RNA (pre-mRNA) splicing process is catalyzed by the spliceosome, an intricate biochemical machinery comprising hundreds of proteins and five small nuclear RNAs (snRNAs). Pre-mRNA splicing consists of joining the coding regions (e.g., removing non-coding ones (introns) to form protein-coding and long-coding RNAs. Faithful recognition of key signaling sequences is key for splicing fidelity and the onset of diseases [1]. The heptameric SF3b complex, a component of the spliceosome, plays a crucial role in the early stages of splicing by recognizing the branch point sequence. SF3b is also the target of known splicing modulators [2]. Of particular interest is the Spliceostatin A (SSA) that has been recently demonstrated in cryo-EM studies to covalently bind to cysteine of a zinc finger of PHF5A (a core SF3b complex component) and strongly interferes with intron-exon recognition in cancer cells. In this study we shed light on the reaction mechanism of SSA covalent binding to PHF5A by means of classical and quantum molecular dynamics simulations. We identify the peculiar features that lead to SSA covalent binding to PHF5A as well as the chemical-physical characteristics exploitable for efficiently targeting the SF3b complex with SSA-like drugs. [1] Rozza, R., Janoš, P., Spinello, A., & Magistrato, A. (2022). Role of computational and structural biology in the development of small-molecule modulators of the spliceosome. Expert Opinion on Drug Discovery, 17(10), 1095-1109. [2] Cretu, C., Gee, P., Liu, X., Agrawal, A., Nguyen, T. V., Ghosh, A. K., ... & Pena, V. (2021). Structural basis of intron selection by U2 snRNP in the presence of covalent inhibitors. Nature Communications, 12(1), 4491.

24-POS Board 24

PREDICTING PROBLEMS CAUSED BY THE AIR-WATER INTERFACE IN CRYO-ELECTRON MICROSCOPY: INSIGHTS FROM MOLECULAR DYNAMICS SIMULATIONS

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Cryo-electron microscopy (cryo-EM) has revolutionized structural biology and biophysics by enabling high-resolution analysis of diverse biomolecular structures. However, a persistent challenge in single particle cryo-EM is the phenomenon of preferred particle orientation, which limits the accuracy of three-dimensional structural reconstructions and hinders interpretation. This issue is often exacerbated by the interaction of proteins with the air-water interface (AWI) during specimen preparation. In this study, we employ coarse-grained molecular dynamics simulations to investigate this complex interplay between proteins, detergents, and the AWI. Specifically, we focus on the preferential partitioning and orientation behavior of proteins to the AWI and the influence of detergents with specific biophysical properties on this process. By mimicking the conditions encountered during cryo-EM sample preparation, we shed light on the mechanisms governing preferred orientations and partitioning at the AWI. This work advances our fundamental biophysical understanding of protein interactions at the AWI, and also offers practical implications for improving cryo-EM sample preparation protocols.

27-POS **Board 27**

A NONSPECIFIC CATION MEMBRANE CHANNEL PIEZO1 EMBEDDED IN A LIPID VESICLE

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Piezo1 is a mechanosensitive protein known to respond to mechanical forces acting on cellular membranes. Several cryo-EM studies of solubilized Piezo1 revealed that it is a homotrimer whose three subunits construct its propeller-like shape by forming three spatially separated blades and a common central part containing the channel hole. A promising approach to study the mechanism of Piezo1 action are more recent cryo-EM studies of Piezo1 molecule embedded in a phospholipid vesicle [1-4]. They show that Piezo1 affects the shape of the vesicle membrane and also that the curvature of the surrounding membrane affects its own shape. The interpretation of these experiments requires the application of the theory of vesicle shape [3]. Here we describe the basics of this theory and report on the improvement of previous analyses [3-4] that makes it possible to identify from the measured vesicle shapes distinct Piezo1 conformations. [1] Y-C. Lin et al., Nature, 573, 230 (2019). [2] X. Yang et al., Nature, 604, 377 (2022). [3] C. A. Haselwandter et al., Proc. Nat. Acad. Sci, 119, e2208027119 (2022). [4] C. A. Haselwandter et al., Proc. Nat. Acad. Sci, 119, e2208034119 (2022).

28-POS Board 28

REFINEMENT A PYTHON PACKAGE FOR CRYO-EM IMAGE-TEMPLATE LIKELIHOOD ENSEMBLE

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Extracting conformational heterogeneity from cryo-electron microscopy (cryo-EM) data is challenging, especially for heterogeneous and flexible samples where 3D classification fails. To tackle this challenge, we previously proposed a Bayesian ensemble refinement approach for estimating the conformational ensemble probability density using structures MD simulations and a set of cryo-EM particle images. Here, we present our recent efforts in applying the ensemble refinement framework on experimental datasets by introducing a computationally efficient algorithm for evaluating the image-to-structure likelihood for large image sets and ensembles. We packaged the algorithm in a user-friendly Python workflow. This work presents a valuable tool for quickly extracting conformational ensemble probabilities from experimental cryo-EM images.

32-POS **Board 32**

DECIPHERING PURINE METABOLIC REGULATION IN E. COLI ADENINE PHOSPHORIBOSYLTRANSFERASE (APRT): A MULTI-METHOD APPROACH

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Nucleoside analogues have been invaluable in treating cancer, viral, and fungal diseases. However, the challenges of multidrug resistance, dormancy, and latency in bacterial treatments have prompted their screening as antibacterial targets in nucleotide metabolism. The salvage pathway of nucleotide synthesis provides an energy-efficient supply of nucleotides, maintaining cellular pools, adapting to nutrient-starved conditions, and ensuring antibiotic tolerance. Adenine phosphoribosyltransferase (APRT), salvage pathway enzyme, catalyzes a reversible reaction converting adenine into adenosine monophosphate (AMP). In proteobacteria, a large class of human pathogenic bacteria, the mechanistic regulation of APRT remains underexplored. This study aims to decipher the regulation of purine metabolism by APRT in this class and design potential inhibitors.We determined atomic-resolution structures of the apo and substrate-bound (adenine, AMP) forms of E. coli APRT using X-ray crystallography, revealing unique mechanistic regulations of substrate binding within the active site. Our crystallographic and isothermal calorimetry data concluded a new mode of adenine binding which is PRPP-independent binding of adenine in the active site, contrary to the other APRTs which are well known PRPP-dependent binding of adenine. The catalytic residues which we identified in enzyme assay are 14 Å away from the nucleobase ring in determined monosubstrate bound forms. Therefore, we are employing a scaffold-based cryo-EM approach to capture a transition state where both adenine and PRPP are bound. The cryo-EM data, analyzed with MD simulations and existing crystallographic, enzymatic, and thermodynamic data, will uncover metastable and transition states in proteobacterial APRT activity. Targeting these states with inhibitors offers a promising strategy for antimicrobial interventions.