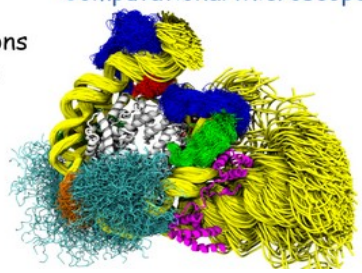
ISQBP
THE INTERNATIONAL SOCIETY OF QUANTUM BIOLOGY & PHARMACOLOGY

2026 President's Meeting
June 15-18, Cluj-Napoca, Romania
Auditorium Maximum of the Babeș-Bolyai University

Breaking barriers

Biomolecular Simulations
Artificial Intelligence
Quantum Computing
Drug Design

Computational Microscope



Breaking barriers with the computational nanoscope

Abstract Book

Organizers

The International Society of Quantum Biology and Pharmocology

Babes-Bolyai University

ISQBP President

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Abstract book

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Welcome

As the acting President of the ISQBP I would like to welcome you to the 2026 President's meeting which is hosted and co-organized by the Babeş-Bolyai University. The meeting will take place in Cluj-Napoca, a marvelous city located in the heart of Transylvania, right at the edge of the Carpathian Mountains. Cluj-Napoca may be less known in the world but it is the second largest city in Romania, hosting some of the most prestigious universities in the country. I have recently made Cluj my home after spending 24 years abroad and the two main reasons for choosing it were the high quality academic environment and the quality of life. Of course, there are many aspects that need to be improved, but at the moment I am very happy with my decision.

ISQBP is a very important community for me. It was at the ISQBP President's meeting in Como (Italy) in 2004 when I received my first award for a poster presentation as a graduate student. I enjoyed very much a very open community that welcomed me as a newcomer and supported me and my scientific work. Since 2004, I attended almost all ISQBP meetings and always enjoyed the informal and welcoming atmosphere. It has been a great honor to be elected Vicepresident in 2022 in an edgy moment of my career and for this election I am very grateful to the entire ISQBP community. A special thanks goes to Alex MacKerell for nomitating me. As a result of that election I became President in 2024. I am very excited to host this year's meeting in a location that soon will be hopefully known for scientific excellence in our field.

I would like to thank the leadership of the Babeş-Bolyai University for considering this meeting as a strategic meeting and giving us the access to this amazing venue. Also, many thanks to Radu-Silaghi Dumitrescu, the President of the Scientific Council of our University for all his support and to my colleagues from the Doctoral School of Integrative Biology for welcoming me and giving me the chance to lead the school. Finally, many thanks to all members of my research group for their commitment to our research and for all the help with the organization of this meeing.

And thank you all for making this meeting a meeting to remember ENJOY THE SCIENCE AND THE CITY !

Yours sincerely, Vlad Cojocaru

Abstract book

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Information

Website

The meeting website is at https://isqbp2026.com . Here you can find all relevant information for the conference, including the abstracts of the invited talks, and the biographies of the 2026 ISQBP award winners.

Registration desk

We are aiming to keep the registration desk occupied by one organizer throughout the meeting. However, if you don't find anyone at the desk, please feel welcome to call or write a What's Up message to 0772209522 (Vlad Cojocaru). In addition, there will be another phone number available at the desk. Please consider that during the talks, a message is better and please introduce yourself.

Please return your badges to us before departure.

Venue

The venue of the meeting is the historic Auditorium Maximum of the Babes-Bolyai University. The venue is used both for scientific and artistic events. Often it is hosting music concerts and the local philarmonic.

It is located in the building of the Academic College, next to the main building of the university in the heart of the Cluj's old city center. The street address of the Academic College is Mihail Kogalniceanu street 5, 400394 Cluj-Napoca. Check the website https://isqbp2026.com/venue/ for a Google Maps link.

Getting to and around

In general, Google Maps is reasonably accurate in finding the public transport connections within the city. Just add your destination (e.g. Auditorium Maximum for the conference venue) and let the Maps navigate you.

Public transportation is quite good and reliable within the city.

Very convenient are car sharing options such as Bolt and Uber (book directly through their apps). Local Taxis are also an option and can be ordered by phone if you don't want to deal with the apps and you know your departure and arrival street addresses. Here are some numbers: Nova Taxi - telefon: (+40)264 949, Diesel Taxi - telefon: (+40)264 946, Napoca Taxi - telefon: (+40)264 953, ProRapid Taxi - telefon: (+40)264 948, TerraFan Taxi - telefon: (+40)264 944, Nova Taxi - telefon: (+40)264 949

To get from the airport to the city center you can take the Bus no A1E which is a direct, express line departing from within the airport terminal. However, bus A1E only goes every hour. Alternatively, if you just walk out of the terminal, on the right side of the roundabout there is a regular bus stop. From there you can take bus number 5, 8, or M41 at regular, short intervals. They are slower than A1E but will all take you to the city center, to slighly different locations (e.g. M41 takes to the station "Opera", whereas 5 and 8 to the station "Constanta"). From where the buses stop you, you may have another 10-15 min walking or you may change the bus.

Alternatively, you can use Uber and Bold car sharing apps or local Taxis. Taxis located at the airport are in general reliable but if they ask you for a price bigger than 15 EUR (approx 75 RON please double check with other drivers)

The city center of Cluj-Napoca is walkable. The entire west-east area between the Cluj arena stadium, the Central park and the National theater and the Orthodox cathedral can be walked either on pedestrian streets or on sidewalks. You can also reach the botanical garden by walking (a short walk up the hill from the university) and the fortress park for great views over Cluj (longer walk up the hill).

Internet access

At the conference venue you have internet access through Eduroam or through the following credentials.

SSID: "FSE"; Password: "fseubbcluj"

Presentations

You can connect you laptop with an HDMI cable to the presentation screen. We will also have a laptop available in case you don't wish to connect with your own laptop. Please note that our laptop runs on Linux. Therefore, if you wish to avoid any formatting risks, please have a PDF prepared.

Invited talks are 20 min + 5 min discussion, while selected talks are 12 min + 3 min discussion. Please keep on time respecting the discussion time slots. Its great to have questions and answers and it helps keeping with the tight program and with the predictibility of the time slots. We hope it will not be necessary but the chairs will have the mission to stop you when your time is up.

Posters

Posters are in A0 format and should be hanged according to the numbers assigned in the abstract book. All posters will be available for the entire period of the meeting in the foyer. Therefore there should be sufficient time for extensive discussions at the posters

For organizational reasons, we recomment the presenters of odd poster numbers to be available for presentation on Jun 16 and those of even poster numbers on June 17.

There will be 3 poster prizes awarded during the gala dinner. The jury wiill visit only during the official poster sessions.

Coffee breaks

Coffee breaks and the drink & snack at the poster sessions are oferred by our in-house university catering service Piramida

Lunch

Lunch on June 16 is offerred by our in-house university catering service Piramida. Please do not walk away on this day as lunch is prepared for all participants and if many walk away there will be a lot of left-over which should be avoided.

Lunch on June 17 is not offerred at the venue because we wanted to allow you to have a nice walk through the city and have lunch at one of the many local restaurants on a nice terrace if weather is kind to us.

Gala dinner

The conference gala dinner on June 17 will take place at the CLUJANA FESTA which is situated along the Somes river and is specialized in organizing events. The address is Str. Tăbăcarilor 15 C, 400139 Cluj-Napoca

The dinner will last until max 2 am.

Clujana Festa can be easily reached by public transport using Bus No. 1 (from stop "Strada Memorandului Sud" to stop Piata 1 Mai), 52 (from stop "Strada Memorandului Sud" to stop "Rasaritului") or 50 (from stop "Piata Cipariu" to stop "Rasaritului". From the last bus stop, its a 10-15 min walk to the actual location. See Google maps route on our website.

You can also group and use Uber or Bolt.

For the return after the dinner to the city, the best option is car sharing (Uber Bold) unless you want to depend on the late bus scheduling (last bus departing at 22:45 from Piata 1 Mai).

You should count on a 30 min ride from the conference venue to the dinner venue and you should aim to be at 19:45 at the dinner.

If you want to have a good walk through the city (maybe along the river), its about 45-50 min walk from the conference venue to Clujana Festa.

ISQBP Award in Computational Biology

The winner of the 2026 ISQBP Award in Computational Biology is **Prof. Dr. Modesto Orozco** for his outstanding scientific contributions to decoding DNA structure and dynamics at multiple scales and in different genomic contexts.

Graduate (M.Sc.) in Chemistry from the Universitat Autònoma de Barcelona (1985). PhD in Biochemistry from the same university (1990). Pre-Doctoral research fellow of the Spanish MEC. Departament de Bioquímica - Universitat de Barcelona (1987-1989). Assistant Professor of Biochemistry at the same university (1989-1990). Professor of Biochemistry and Molecular Biology. Departament de Bioquímica. Universitat de Barcelona. 1991-2001. Invited Scientist. Department of Chemistry. Yale University 1991-1993. Full Professor of Biochemistry and Molecular Biology. Departament de Bioquímica. Universitat de Barcelona 2002-present. Director Molecular Modelling and Bioinformatics Unit (2002-) IRB Barcelona. Director of the Life Sciences Department. Barcelona Supercomputing Center (2005-2015). Integrative Research Node coordinator IRB Barcelona.

Counsellor and Advisor of different Scientific Societies and Granting Agencies. Associated Editor WIRES Computational Molecular Sciences. Advisory Editor of Theoretical Chemistry Accounts: Theory, Computation and Modelling (2005-2014). Member of the Editorial board of: Journal of Computational Chemistry, Journal of Chemical Theory and Computation, Phys.Chem.Chem.Phys., J.Mol.Recog., J. Mol.Graphics and Modeling, Nucleic Acids Res., and Chem.

Modesto Orozco's research activity is focused on the theoretical study of biological systems and is reflected in close to 500 papers published in international peer-reviewed journals of the highest impact His publications have collected more than 35000 citations with an h-index of 93, the highest for a computational chemist in Spain.

Among other awards, in 1997 he got the "Diaz de Santos" National Award for young scientist, the Distinción Investigadora de la Generalitat de Catalunya (Annual award of Science of the Catalan Science Ministry) in 2000, the FEBS Anniversary Prize of the

Gesellschaft für Biochemie und Molekularbiologie in 2001, the Fundación Marcelino Botín fellowship (2007), the Brucker award for research in biophysics (2010), as well as the ICREA Academy award for excellence in research and the Advanced Grant of the European Research Council.

Modesto Orozco is founder and president of Nostrum Biodiscovery.

ISQBP Gilda Loew Lectureship

The 2026 ISQBP Gilda Loew Lecture is awarded to **Dr. Zoe Cournia** for her outstanding contributions in various scientific topics which were at the core of Gilda Loew's interests.

Dr. Cournia is Director of Research at the Biomedical Research Foundation, Academy of Athens. She graduated from the Chemistry Department, University of Athens in 2001 and received her PhD at the University of Heidelberg in Germany in 2006. She then worked as a postdoctoral researcher at the Chemistry Department, Yale University, USA, on computer-aided drug design with Prof. Bill Jorgensen. She has been awarded with the American Association for Cancer Research Angiogenesis Fellowship (2008), the "Woman of Innovation 2009" Award from the Connecticut Technology Council, USA, the Marie Curie Fellowship from the European Union (2010), the "Outstanding Junior Faculty Award" from the American Chemical Society (2014) and the first "Ada Lovelace Award" from the "Partnership for Advanced Computing in Europe" (2016). She was a member of the Infrastructure Advisory Group (INFRAG) of the European High Performance Computing Joint Undertaking in 2018-2021 and in 2022 she was appointed as the Greek representative in the Innovative Health Initiative Joint Undertaking. She is an Executive Editor with the Journal of Chemical information and Modeling, American Chemical Society and the national representative of Greece in the Division of Computational and Theoretical Chemistry in the European Chemical Society.

Abstract book

2026 President's Meeting
June 15-18, Cluj-Napoca, Romania

Program

Monday • 15.06.2026

13:30 - 14:00 Opening ceremony
13:30 – 13:40 Adrian-Olimpiu Petruşel – Vicerector Babeș-Bolyai University
13:40 – 13:50 Radu Silaghi-Dumitrescu – President Scientific Council Babeș-Bolyai University
13:50 – 14:00 Vlad Cojocaru – President ISQBP

SESSION I:
NUCLEIC ACIDS ACROSS SCALES AND TIMES
(CHAIR: VLAD COJOCARU)

14:00 - 14:25 Jan Huertas | University of Cambridge, UK | "Multiscale simulations reveal molecular mechanisms of chromatin organisation"
14:25 - 14:50 Bojan Žagrović | Max Perutz Labs & University of Vienna, Austria | "RNA-protein interactions and the structure of the genetic code"
14:50 - 15:15 Anna Panchenko | Queen's University, Canada | "Key Challenges in Modeling Protein–DNA Interactions in Chromatin"
15:15 - 15:30 Roxana Geanina Vasarhelyi | Babeș-Bolyai University, Cluj-Napoca, Romania | "Conformational plasticity modulates RNA recognition by tandem RRM proteins"

15:30 – 16:00 COFFEE BREAK

16:00 - 16:25 Remo Rohs | University of Southern California, USA | "Integrating deep learning and physics-based methods to study molecular interactions"
16:25 - 16:50 Ioan Andricioaei | University of California, Irvine, USA | "Computing large-and-slow DNA motions from fast sampling of rare conformational transitions"
16:50 - 17:05 Maria Julia Maristany | University of Cambridge | "Nucleosome Mechanics Shape the Energy Landscape of DNA Unwrapping"

17:05 – 17:30 REFRESHMENTS/SNACKS

ISQBP AWARD IN COMPUTATIONAL BIOLOGY LECTURE
17:30 – 18:10 **Modesto Orozco** | IRB Barcelona, Spain | "A Journey Through DNA"

18:10 – open FREE TIME (POSTERS/DINNER/NETWORKING)

Tuesday • 16.06.2026

SESSION II: FORCE FIELDS FOR BIOMOLECULES
(CHAIR: ANA NICOLETA BONDAR)

09:00 - 09:25 **Alex MacKerell** | University of Maryland, USA | "Ongoing developments in the Classical Drude Oscillator Polarizable Force Field"
09:25 - 09:50 **Jiří Šponer** | Institute of Biophysics, Brno, Czech Republic | "Atomistic Molecular Dynamics Simulations of Nucleic Acids: Force Field Advances and Issues"
09:50 - 10:15 **Thomas Cheatham III** | University of Utah, USA | "Over 30 years of RNA simulation - lessons learned, force fields, modified nucleotides and salt."
10:15 - 10:30 **Leo Christanell** | Ludwig-Maximilians-Universität, München, Germany | "An Improved Force Field Parameterization of DNA-Metal Ion Interactions that Preserves the tRNA Anticodon Loop Structure"

10:30 – 11:00 COFFEE BREAK

11:00 - 11:25 **Petr Jurečka** | Palacký University Olomouc, Czech Republic | "New AMBER Force Fields for More Reliable DNA and Protein–DNA Simulations"
11:25 - 11:50 **Paulo Cesar Telles de Souza** | Laboratoire de Biologie et Modélisation de la Cellule, CNRS, Lyon, France | "From Mechanism to Design: Coarse-Grained and Machine-Learning Approaches to Lipid Nanoparticle Delivery"
11:50 - 12:15 **Adrian Roitberg** | University of Florida, USA | "10+ years of General Machine Learning Interaction Potentials. Ubi eramus? Ubi sumus? Quo imus?"
12:15 - 12:30 **Valentin Gradisteanu** | OneAngstrom, Grenoble, Frace | "Understanding pKa shifts through Local Environment Analysis"

12:30 - 14:00 LUNCH AND POSTERS

SESSION III: BIOMOLECULAR INTERACTIONS AND COMPLEXITY (CHAIR: NICOLAE VIOREL BUCHETE)

14:00 - 14:25 **Nathalie Reuter** | University of Bergen, Norway | "Allosteric control of lipid transfer proteins by membrane lipids"
14:25 - 14:50 **Ana Nicoleta Bondar** | University of Bucharest, Romania | "Dynamic hydrogen-bond networks for G Protein Coupled Receptor activation"
14:50 - 15:15 **Bert de Groot** | Max Planck Institute for Multidisciplinary Sciences, Goettingen, Germany | "Alchemical binding free energy calculations for ligand binding, protein allostery, post-translational modifications, and more"
15:15 - 15:30 **Victor Reys** | Utrecht University, The Netherlands | "Structural modelling and binding affinity prediction of the Human PDZ-PBM interactome"

15:30 - 16:00 COFFEE BREAK

16:00 - 16:25 **Martin Zacharias** | Technical University of Munich | "Protein interaction modulation studied by ligand design and molecular simulations"
16:25 - 16:50 **Marco De Vivo** | Istituto Italiano di Tecnologia, Genoa, Italy | "Decoding Biochemical Complexity with Simulations and AI-Enhanced Sampling"
16:50 - 17:05 **Tom Vlaar** | Ghent University, Belgium | "Kinetics of enantiomeric amino acid permeation through chiral phospholipid membranes via path sampling "
17:05 - 17:30 **Ioana Ilie** | University of Amsterdam | "AI-enhanced molecular design of peptides regulating Bax activation"
17:30 - 17:55 **Paolo Carloni** | Forschungszentrum Jülich, Germany | "Neurological Disease-Linked Mutations: A Multiscale Approach"

17:55 – 19:30 POSTER SESSION I (with snacks/refreshments)

19:35 - open FREE TIME (DINNER/NETWORKING)

Wednesday • 17.06.2026

SESSION IV: BIOMOLECULAR REACTIONS FROM QUANTUM TO CLASSIC AND BEYOND (CHAIR: DRAGOS HORVATH)

09:00 - 09:25 **Fahmi Himo** | Stockholm University, Sweden | "Quantum Chemical Modeling of Enzyme Enantioselectivity"
09:25 - 09:50 **Jean-Philip Piquemal** | Sorbonne Université, 75005 Paris, France | "A Quantum Foundation Model for Accurate Atomistic Simulations in Drug Design"
09:50 - 10:15 **Alexey Aleksandrov** | Institut polytechnique de Paris, France | "Molecular Simulation and Experimental Insights into the Catalytic Mechanism of Fatty Acid Photodecarboxylase"
10:15 - 10:30 **Nigel Richards** | Foundation for Applied Molecular Evolution, Alachua, Florida, USA | "Quantum Chemical Insights into Arginine Sidechain Modification"

10:30 - 11:00 COFFEE BREAK

11:00 - 11:25 **Ruibin Liang** | Texas Tech University, USA | "Understanding the quantum dynamics of photoactive proteins through multiscale simulations"
11:25 - 11:50 **Radu Silaghi Dumitrescu** | Babeș-Bolyai University, Cluj-Napoca, Romania | "Do you even own a proton? Awkward spectroscopic quirks with reactivity implications for hemoprotein ferryls"
11:50 - 12:15 **Jan Brezovsky** | Adam Mickiewicz University, Poznań, Poland | "Cracking the code of enzyme tunnels: understanding, predicting, and engineering hidden pathways"
12:15 - 12:30 **Dan T Major** | Bar-Ilan University, Israel | "An AI Framework for Reaction Mechanism Discovery in Enzymes- A Case Study of Carbocation Reaction Networks and Enzyme Docking"

12:30 - 14:00 FREE TIME (CITY EXPLORATION/LUNCH)

14:00 - 14:25 **Ulf Ryde** | Lund University, Sweden | "Estimating ligand-binding affinities with quantum-mechanical methods"
14:25 - 14:50 **Johan Aqvist** | Uppsala University, Sweden | "Computational Design of the Temperature Dependence of Enzyme Reactions
14:50 - 15:15 **Maciej Szaleniec** | Jerzy Haber Institute, Polish Academy of Sciences, Krakow, Poland | "Tungsten-containing enzymes – new tools in the biotech toolbox. Modelling and challenges"
15:15 - 15:45 COFFEE BREAK

SESSION V: FRONTIERS IN DRUG DESIGN
(CHAIR: RADU SILAGHI DUMITRESCU)

15:45 - 16:10 **William Jorgensen** | Yale University, USA | "What's New with Force Fields and Free Energy"
16:10 - 16:25 **Inés Sabine Rahali** | Université Paris Cité, France | "PockFlex: a web server for flexibility-aware binding site identification and prioritisation from structural ensembles"
16:25 - 16:50 **Dragos Horvath** | Universite de Strasbourg, France | "The Chemical Library Space: From Compound Chemography to Management of Portofolio of DNA-Encoded Libraries"
16:50 - 17:05 **Stefan Ivanov** | Faculty of Pharmacy, Medical University of Sofia, Bulgaria | "An Efficient Computational Chemistry Approach to Generating Negative Data for Virtual High-Throughput Screening Validation "

ISQBP GILDA LOEW LECTURE
17:05-17:45 **Zoe Cournia** | Biomedical Research Foundation, Academy of Athena, Greece | "Allostery in Drug Discovery: From MD to ML"

17:45 - 18:15 ISQBP AFFAIRS MEETING

17:45 - 18:15 POSTER SESSION II (with snacks/refreshments)

19:45 - 02:00 CONFERENCE GALA DINNER | LOCATION: CLUJANA FESTA
See page 6 for details

Thursday • 18.06.2026

SESSION VI: FRONTIERS IN BIOMOLECULAR SIMULATIONS
(CHAIR: IOANA ILIE)

09:00 - 09:25 **Carol Post** | Purdue University, West Lafayette, IN, USA | "Phosphorylation influence on protein conformational equilibrium with connections to functional outcomes"

09:25 - 09:50 **Joanna Trylska** | University of Warsaw, Poland | "Molecular dynamics in multi-dimensional space reveals how mutations reshape neomycin binding to the riboswitch"
09:50 - 10:15 **Charles L. Brooks III** | University of Michigan, USA | "Novel high-throughput methods for free energy calculations"
10:15 - 10:30 **Nicolae Viorel Buchete** | University College Dublin, Ireland | "Bridging Single-Protein Biophysics and Nanoparticle Protein Corona Signatures"
10:30 - 10:45 **Cosmin Alexandru Bugeac** | Institute of Biochemistry, Romanian Academy | "Structure-guided engineering of conformational constraints in the HCV E2 glycoprotein for epitope-focused vaccine design"

10:45 - 11:10 COFFEE BREAK

11:00 - 11:15 **Parham Rezaee** | Gent University, Belgium | "Molecular dynamics to estimate biological kinetics: a new path sampling method with replica exchange of Hamiltonians "
11:15 - 11:30 **Laurentiu Spiridon** | Institute of Biochemistry, Romanian Academy, Bucharest, Romania | "Advanced Sampling Methods for Protein–Ligand Interactions"
11:30 - 11:55 **Klaus Liedl** | University of Innsbruck, Austria | "From Canonical Structures to Conformational Ensembles: Physics-Based Modeling of Antibody Structure, Dynamics, and Developability in Solution"
11:55 - 12:20 **Elinor Haglund** | University of Hawaii, Manoa, USA | "Structural Plasticity in Chemokines Driven by Native and Non-Native Disulfide Bonds"
12:20 - 12:45 **Roland Stote** | Institute of Genetics and Molecular and Cellular Biology, Strasbourg, France | "Nuclear Receptors in Motion: Shape-Shifts and Dynamics"

12:45 - 13:15 CLOSING REMARKS / POSTER PRIZES

Abstracts – Oral Presentations

Multiscale simulations reveal molecular mechanisms of chromatin organisation

Jan Huertas
Yusuf Hamied Department of Chemistry, University of Cambridge, United Kingdom

In eukaryotic cells, DNA is packaged into chromatin, a highly compact and dynamic structure whose organisation regulates gene expression by modulating DNA accessibility. Chromatin architecture is a multiscale problem: Local nucleosome conformations and interaction networks give rise to higher-order fibre organisation and mesoscale domains with distinct material properties. Understanding how these emergent chromatin states arise from underlying molecular interactions remains a central challenge to understand genome regulation. In this talk, I will present two recent efforts that use multiscale simulations to dissect the physical principles underlying chromatin organisation. In the first study, we focus on understanding chromatin condensates, by integrating cryo-electron tomography (cryo-ET) with our coarse-grained chromatin models. These simulations resolve chromatin fibers inside droplets at single amino-acid and base-pair resolution and reveal how linker DNA length controls nucleosome geometry, interaction networks, and material properties through distinct histone tail–mediated contacts. In the second story, we dig deeper into how the structures of chromatin fibers are modulated by other proteins. I will describe how the pioneer transcription factor Oct4 can reshape chromatin fibres beyond the scale of a single nucleosome. Using coarse-grained molecular dynamics simulations of full-length Oct4 interacting with nucleosome fibres of varying nucleosomal repeat lengths, we show that Oct4 enhances DNA accessibility not by global decondensation, but by driving chromatin into compact, liquid-like states in which nucleosomes breathe, reorient, exchange neighbours, and transiently expose DNA. Together, these studies establish how chromatin architecture, dynamics, and accessibility emerge from multivalent molecular interactions, and illustrate how multiscale simulations can provide mechanistic insight into chromatin regulation at submolecular resolution.

RNA-protein interactions and the structure of the genetic code

Bojan Žagrović
Laboratory of Molecular Biophysics, Max Perutz Labs & University of Vienna, Austria

Recently, we have characterized a robust, statistically significant matching between the nucleotide-density profiles of mRNA coding sequences and the nucleotide-affinity profiles of the protein sequences they encode. These results support the long-standing proposal that the genetic code evolved from direct interaction preferences between amino acids and the appropriate nucleotides. Moreover, these findings suggest a possibility of direct, complementary, co-aligned interactions between mRNAs and their autogenous proteins even in present-day cells, especially if both are unstructured, with implications extending to different facets of nucleic-acid/protein biology. In this talk, I will discuss the computational and experimental tests as well as functional implications of this proposal. Finally, I will provide evidence that proteins may in general interact with RNAs that are compositionally related to their own autogenous mRNA, as a simple, yet powerful organizational principle behind the structure of RNA-protein interaction networks in the cell.

Key Challenges in Modeling Protein–DNA Interactions in Chromatin

Anna Panchenko

Queen's University, Kingston, Ontario, Canada

The key challenges in modeling of protein–DNA interactions in chromatin arise from its multiscale, dynamic, and heterogeneous nature, the coupling between DNA sequence-dependent mechanics and nucleosome organization, and the cooperativity among many DNA binding factors. Such a complex nature of protein–DNA interactions in chromatin makes them difficult to study using experimental approaches alone. Therefore, we develop integrative approaches that combine atomistic molecular dynamics simulations and molecular modeling with various experimental methods, such as Cryo-ET, NMR spectroscopy, MNase-seq, and binding assays. Our findings show how DNA modifications and protein missense mutations can impact protein-DNA binding and chromatin dynamics.

Integrating deep learning and physics-based methods to study molecular interactions

Remo Rohs

University of Southern California, USA

Research on molecular interactions increasingly relies on the ability to generate large amounts of data in biological experiments and on the exponential growth of computing power. The combination of data and computation forms the basis for the recent development of AI-based computational biology methods. My lab develops such tools based on molecular structure, with the goal of answering biological questions related to gene regulation, nucleic acid structure, protein-nucleic acid binding, and drug design. I will discuss DeepPBS, a method for predicting protein-DNA binding specificity from structural data, and DrugHIVE, an approach for designing drug-like molecules that are not available in current drug libraries. I will further discuss a hybrid pipeline that integrates AlphaFold predictions and molecular simulations with our DeepPBS method. This multiscale framework successfully captures conformational flexibility and the modulation of DNA structure through protein binding, providing new insights into transcription factor binding specificity and creating a roadmap for integrating deep learning and physics-based methods to study molecular mechanisms.

Computing large-and-slow DNA motions from fast sampling of rare conformational transitions

Ioan Andricioaei

University of California, Irvine, USA

The molecular machinery of biological cells works on one DNA at a time, and therefore these processes are to be fundamentally understood at the single-molecule level. In my talk, I will present computer simulation results obtained by my research team that address two difficulties in modeling such events. They stem from the long spatial scales on which the DNA strand relaxes and, separately, from the long relaxation time of the underlying kinetics.

Two frameworks, both based on path integrals, developed for these two problems will be showcased. One uses molecular-dynamics derived parameters to scale up the dynamics on the micrometer-microsecond scale via the Kirchhoff theory for elastic rods. The other allows for the enhanced calculation of long-time kinetics in complex systems and is based on a stochastic path integral formalism: assigning weights to trajectories of artificially biased dynamics allows for the calculation of time-correlation functions for the unbiased system of interest via reweighting.

Biography
Ioan Andricioaei, a native of Romania, studied physics and biophysics at the A. I. Cuza University of Iasi. He then pursued graduate studies at Boston University and completed his Ph.D. in 1999 under the supervision of John Straub, followed by postdoctoral work at Harvard University with Martin Karplus. Andricioaei was then on the faculty at the University of Michigan, before moving to the University of California, Irvine in 2008, where he is Professor of Chemistry. His research explores theoretical topics at the interface between molecular biophysics and physical chemistry.

A Journey Through DNA

Modesto Orozco

IRB Barcelona, Spain

DNA is the carrier of genetic information and perhaps the molecule that best defines life as we know it. Yet, for me, it is something more: a fascinating physical problem in which multiple time and length scales coexist. In DNA, biology emerges from a myriad of sub-ångström details that ultimately map onto a meter-long molecular fiber. Approaching DNA from the perspective of physics requires integrating multiple levels of resolution within a coherent framework. This is a formidable challenge: it demands navigating across different physical models while incorporating experimental measurements at the core of theoretical and computational approaches. In this talk, I will summarize our experience over several decades developing tools to simulate nucleic acids, with special emphasis on DNA. I will also share my enthusiasm for how the next generation of methods may allow us to decipher, with increasing clarity, the fundamental molecular principles underying life.

Ongoing developments in the Classical Drude Oscillator Polarizable Force Field

Alex MacKerell

Computer-Aided Drug Design Center, Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, Baltimore, MD, 21230, USA

The Drude polarizable force field (FF) uses a simple auxiliary particle, the Drude oscillator, linked via a harmonic spring to the nuclei of non-hydrogen atoms to model the electrons in the system. The induced polarization response is modelled by the Drude particles relaxing in the surrounding electric field for each configuration of the real atoms, thereby solving the Born-Oppenheimer approximation. While conceptually simple, this model allows for accurate treatment of electronic polarization in a computationally efficient manner, with a 2.6 to 4-fold increase in computational costs over additive force fields. Drude FF parameters have now been developed for proteins, nucleic acids, selected lipids and carbohydrates as well as a range of cofactors and drug-like molecules. Ongoing efforts involve improving the treatment of the protein and nucleic acids, including the equilibrium between folded and unfolded states and their interactions with their environments. In addition, efforts are focused on the development of the Drude General Force Field (DGenFF) for small molecules.

Atomistic Molecular Dynamics Simulations of Nucleic Acids: Force Field Advances and Issues

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Atomistic molecular dynamics (MD) simulations are an important tool for understanding the structural dynamics of nucleic acids and their complexes with proteins and ligands. I will provide a brief overview of our recent MD studies of selected systems, including interactions of RNA recognition motif protein domains with single-stranded RNA and the basic principles governing the folding landscapes of guanine quadruplex structures. I will then place particular emphasis on the limitations of atomistic MD, namely persistent force-field uncertainties and the often underestimated limitations of collective-variable–based

enhanced sampling protocols. Finally, I will comment on recent developments in the OL series of modifications of AMBER nucleic acid force fields, discuss how simulation outcomes depend on the choice of water model, and rationalize why it can sometimes be justified to use goal-specific force-field modifications that sacrifice broad transferability in favor of accurately describing a specific class of nucleic acid systems.

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Over 30 years of RNA simulation - lessons learned, force fields, modified nucleotides and salt

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Starting with the Cornell et al. “ff94” force field and the implementation and parallelization of the particle mesh Ewald method, the first papers from my PhD with Peter Kollman demonstrated stable simulations of DNA, RNA, and proteins, investigated the A-B transition in water, ethanolic mixtures, and with hexaaminecobalt (III), and characterized RNA and

RNA-DNA duplex structure. The results were encouraging, however we had not fully converged the simulations. As we got to bigger and faster computers (Cray T3D, Cray T3E, IBM SP-2, …) longer and longer simulations started to expose deficiencies in the AMBER nucleic acid force fields. From ~1998-recent time, the community has worked hard to overcome those deficiencies. Recent work highlights testing of Mg2+, development of force field parameters for natual and unnaturally modified nucleotides, and further force field testing on a variety of systems.

New AMBER Force Fields for More Reliable DNA and Protein–DNA Simulations

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The latest addition of the OL family of force fields, OL24, improves the description of the A/B-DNA equilibrium. Existing AMBER force field variants underpopulate the north (C3′-endo) sugar pucker, compromising simulations of protein–DNA systems requiring B-to-A transitions. The OL24 refinement of deoxyribose puckering brings the A/B equilibrium into better agreement with experimental data. The description of canonical B-DNA, DNA–RNA hybrids, and protein-induced conformational adaptation is also improved. Combined with the previous α/γ correction of OL21, OL24 provides a coherent framework for general DNA and protein–DNA simulations. We also assess the compatibility of OL force fields with Electronic Continuum Correction (ECC) charge scaling and the usefulness of Lorentz-Berthelot combining rules.

From Mechanism to Design: Coarse-Grained and Machine-Learning Approaches to Lipid Nanoparticle Delivery

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Lipid nanoparticles (LNPs) are central to the delivery of RNA-based therapeutics, yet their rational design remains challenging due to the complex coupling between composition, structure, and pH-dependent molecular interactions. Experimental approaches, while essential, are often costly and provide limited structural resolution, whereas atomistic simulations remain computationally demanding[1,2].

In this talk, I will present recent advances in coarse-grained (CG) molecular dynamics simulations that provide mechanistic insight into RNA encapsulation, pH-driven structural transitions, and endosomal escape in LNPs. Using validated Martini 3 models[3] together with refined lipid parameters and an expanded library of ionizable lipids, sterols, and PEGylated components4,5, these simulations reveal how different protonation states regulate lipid–RNA interactions, internal LNP organization, and membrane fusion pathways during endosomal trafficking.

Building on this mechanistic framework, I will further show how molecular descriptors extracted from CG simulations can be integrated with machine-learning approaches to predict delivery efficiency and accelerate formulation screening. Together, these multiscale and data-driven strategies enable a transition from molecular understanding to predictive design of next-generation lipid nanoparticle delivery systems.

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10+ years of General Machine Learning Interaction Potentials. Ubi eramus? Ubi sumus? Quo imus?

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When one wants to work in a field where it is important to sample molecular conformations according to a distribution (e.g. Boltzmann), the standard view has been that one can either sample properly (i.e keep many structures, for which you need cheap computational methods) OR have accurate weights (i.e. Good energy calculations, which are expensive), but never both.
This general idea goes back to Dirac, who in 1929 wrote that we (theoretical physicists) need to design methods that are computationally tractable, but as accurate as possible.
In the early 2000's, an idea came to life asking: "Can a machine learning method learn quantum chemistry?" More precisely, can it learn and predict energies and forces for molecular systems with the same accuracy as actual QM calculations? Surprisingly, the answer was YES, for a given molecular system. This enables the breaking of the problem described above. One could calculate energies very accurately, but at a very low cost, which enabled sampling!
In 2016, my group asked the follow up question: Can an ML method learn to predict energies and forces for ANY substance, given enough data? The answer was again, yes! We showed that one can create a Neural Network that can learn from a large dataset of diverse structures and predict E and F with high accuracy for compounds outside the training dataset.
I will present some of the history of the field (Ubi eramus?), show some of our more recent results (Ubi sumus?), and discuss some ideas as to where we might be going (Quo imus?").

Allosteric control of lipid transfer proteins by membrane lipids

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Lipid transfer proteins (LTPs) transfer lipids between intracellular membranes through non-vesicular mechanisms and regulate lipid homeostasis. Despite a wealth of structural data, little is known about the mechanisms by which LTPs desorb lipids from well-organized membranes. We investigated 3 LTPs from the family of StAR-related lipid transfer domains (STARD): STARD2 (phosphatidylcholine transfer domain), STARD4 (sterol transfer domain), and STARD11 (ceramide transfer domain in CERT multidomain protein). We used a combination of multiscale molecular simulations and free energy calculations to investigate the mechanisms for extraction (or release) of their cargo lipids from (or into) their donor (or acceptor) membranes. We used multicomponent lipid bilayers modeling the plasma membrane and relevant organelle membranes. The simulations revealed similarities in binding orientation of the three proteins to lipid bilayers but also striking differences in their sensitivity to lipid composition. We propose detailed models for the mechanism of lipid uptake/release in START domains and highlight mechanistic differences between structurally similar proteins [1-4]. Our models are supported by experimental data.

Dynamic hydrogen-bond networks for G Protein Coupled Receptor activation

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G Protein Coupled Receptors use hydrogen(H)-bond networks to couple ligand binding with protein conformational dynamics, activation, and the binding of cytoplasmic interaction partners. These long-distance H-bond networks are typically mediated by water molecules, tend to be dynamic, and can include titratable residues whose protonation changes during receptor function. To study the role of dynamic protein-water hydrogen-bond networks in GPCRs, we developed graph-based algorithms and tools. DNET, our most recent computational workflow and graph-based tool, enables efficient computations of protein-water H-bond networks, estimates of pKa values for titratable residues of the H-bond network, and potential of mean force for pairwise distances between residues that are nodes of the graph. We apply DNET to study the assembly and role of internal H-bond networks in a visual receptor of interest as optogenetic tool. From multiple simulations of wild-type and mutant receptors we find that about half of the H-bond network at the ligand-binding site consists of interactions with one conformational mode; the other half of the network consists of interactions that are more dynamic, with two or more conformational modes. H-bonds with more than one conformational mode could help facilitate the propagation of changes in receptor structure and dynamics during activation.

Alchemical binding free energy calculations for ligand binding, protein allostery, post-translational modifications, and more

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Alchemical free energy calculations have come of age. These calculations explore physical paths not experimentally accessible and provide unprecedented accuracy in the prediction of processes as diverse as protein thermostability and ligand binding free energies. Based on the PMX framework coupled to the GROMACS molecular dynamics engine, results of high-throughput relative as well as absolute ligand binding free energies are presented, in addition to applications of allostery in a GPCR and the effect of post-translational modifications in protein aggregation.

Protein interaction modulation studied by ligand design and molecular simulations

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Protein molecules and the interaction with other biomolecules are essential for many biological processes including signal transduction, immune reactions and many diseases. Recent machine learning and deep learning methods allow one to predict the structure of proteins in complex with other biomolecules and also with organic ligands. Complex formation of proteins often results in new pockets at the interface that can be targets for binding of complex stabilizing compounds. We have developed and evaluated molecular simulation approaches to identify such putative binding cavities. In combined with ligand and cyclic peptide design methods our efforts also focus on identifying possible new compounds binding at protein-protein complex interfaces. In addition, the realistic evaluation and scoring of putative binders is of critical importance. The application of rapid knowledge-based as well as molecular simulation based free energy calculations to score designed binders will also be discussed.

Decoding Biochemical Complexity with Simulations and AI-Enhanced Sampling

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In my contribution, I will illustrate our recent efforts to understand complex chemical processes in biochemistry through molecular simulations. I will present and discuss our work on dissecting the molecular mechanisms by which enzymes perform their complex functions. Examples will include the mechanism of DNA translocation by polymerase (Pol) enzymes, a critical step in Pol-mediated nucleic acid polymerization. The goal is to understand how complex chemical processes often involve critical conformational changes that are difficult to capture with equilibrium molecular dynamics or simple collective variables for enhanced-sampling simulations. I will explain how these problems were tackled through advanced simulations and AI-guided enhanced sampling, decoding molecular mechanisms that align well with the experimental evidence. Our efforts demonstrate how such approaches can address (bio)chemical questions of increasing complexity, where identifying the proper collective variable is inherently challenging.

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AI-enhanced molecular design of peptides regulating Bax activation

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Protein–protein interactions govern most cellular decisions but remain challenging to modulate because their interfaces are large, dynamic and often considered "undruggable."

Peptides offer a powerful way to rewire PPIs by mimicking or competing with natural interaction motifs, providing high affinity and specificity at otherwise inaccessible surfaces. Here, we introduce a computational framework for de novo design of peptide binders that integrates a generative protein design model with physics-based molecular simulations1,2. We focus on the pro- apoptotic Bcl-2 family member Bax, a key executor of apoptosis whose dysregulation is implicated in cancer and neurodegenerative diseases and target it with helical and cyclic peptides as potential therapeutic agents. Our approach couples a generative model, used to traverse the binder design landscape and propose candidate Bax-binding peptides, with atomistic molecular dynamics (MD) simulations that provide explicit physical evaluation of binding affinity and complex stability. Our results show that short MD simulations of designed binders enable a more informative in silico screening step than standard sequence- or structure-based heuristics, which become insufficient in the regime of highly capable generative models; this is validated by follow-up extensive MD and enhanced sampling (umbrella sampling) calculations of binding free energies. On this basis, we construct a surrogate model that maps peptide sequence features to physically motivated metrics, including MD- derived binding free energies, enabling fast prediction of binding affinity for new designs without additional simulations. Finally, we integrate the surrogate into an active learning loop, replacing explicit MD scoring with learned physical proxies, and perform iterative peptide design. As a proof of concept, we show that the resulting peptides bind Bax with high predicted affinity, populate the canonical hydrophobic groove (BH1 domain) and an alternative site near the BH3 domain (α2 helix), and restrict the flexibility of the α1–α2 loop associated with activation. Together, these results establish a digital, simulation-informed paradigm for peptide design against Bax and provide a generalizable route to embed physical realism into generative binder design.

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Neurological Disease-Linked Mutations: A Multiscale Approach

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I will present applications on protein complexes and RNAs, which may demonstrate the usefulness of different simulation techniques (from QM/MM and atomistic MD to systems biology). These methods may provide insight into the mechanisms by which mutations lead to aberrant neurological processes. I will conclude by discussing our recent efforts to combine molecular and neuronal simulations to predict changes in neural spikes caused by ion channel variants.

Quantum Chemical Modeling of Enzyme Enantioselectivity

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With density functional theory methods, it is today possible to design large models of enzyme active sites and investigate detailed reaction mechanisms and origins of various selectivities with high accuracy. Indeed, many mechanistic problems have been addressed and solved for a wide range of enzymes using the so-called quantum chemical cluster approach. We have in recent years employed this methodology to study enzymes of biocatalytic interest, with particular focus on enzymes utilized in asymmetric synthesis [1-3]. This talk will give a brief account of the cluster methodology and discuss recent examples of relevance for enantioselective biocatalysis.

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A Quantum Foundation Model for Accurate Atomistic Simulations in Drug Design.

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While artificial intelligence has revolutionized the prediction of static protein structures, characterizing their dynamics and interactions with drug candidates remains a computational bottleneck. Here, we introduce FeNNix-Bio1 [1], a foundation machine learning model designed to power accurate, reactive atomistic simulations of biological systems at an unprecedented speed and scalability. Trained exclusively on synthetic quantum chemistry data, FeNNix-Bio1 accurately captures complex condensed-phase phenomena such as ion solvation and subtle liquid water properties for which it outperforms state-of-the-art specialized force fields. In this presentation, I will illustrate its versatility across a full spectrum of drug design applications, including the calculation of hydration free energies (HFEs), the reversible folding of small proteins, the simulation of protein-ligand absolute binding free energies and chemical reactions. Notably, FeNNix-Bio1 sets a new standard for the precise prediction of HFEs for the more than 600 molecules of the Freesolv dataset, providing sub-kcal/mol accuracy. By enabling scalable, quantum-accurate molecular dynamics without the need for manual parametrization, FeNNix-Bio1 bridges the gap between static structure prediction and dynamic biological reality.

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Molecular Simulation and Experimental Insights into the Catalytic Mechanism of Fatty Acid Photodecarboxylase

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Fatty acid photodecarboxylase (FAP) is a recently discovered photoenzyme that converts fatty acids into hydrocarbons through a light-driven radical mechanism. In this talk, I will show how we combine molecular dynamics (MD) and quantum mechanics/molecular mechanics simulations with time-resolved spectroscopic observations to elucidate key mechanistic aspects of FAP function. MD simulations reveal a strong dependence of active-site organization on substrate chain length, with short-chain substrates inducing tighter pocket packing aided by the alkane product acting as a co-catalyst, thereby explaining the unexpectedly high activity toward medium-chain fatty acids. In contrast, substrate-free simulations show expansion of the flavin-binding pocket and increased water penetration, rationalizing ultrafast spectroscopic evidence for hydrated electron formation following photoexcitation of the flavin anion radical. Combined experimental and simulation results further reveal details of the FAP photocycle, including transformation of the CO_2 coproduct into bicarbonate at cryogenic temperatures, while this pathway is suppressed under physiological conditions, explaining the strong temperature dependence observed experimentally. Overall, by integrating simulations with spectroscopy, we demonstrate how protein dynamics and active-site electrostatics govern light-driven radical chemistry in FAP.

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Quantum Chemical Insights into Arginine Sidechain Modification

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A variety of important biological processes are underpinned by the enzyme-catalyzed modification of arginine side chains, including the regulation of gene expression, subversion of the immune system by bacterial pathogens, and a method of buffering ATP concentration in cells. This lecture will briefly outline recent insights, obtained by DFT analyses of quantum

chemical cluster models for arginine kinase1 and the three families of protein arginine methyltransferase (PRMT), into how arginine side chains are activated for reaction. Our findings, when taken together with recent work by Rovira and co-workers on arginine N-glycosylation,2 suggest that arginine-modifying enzymes employ similar strategies for activating the cationic guanidinium moiety despite being evolutionarily unrelated.

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Understanding the quantum dynamics of photoactive proteins through multiscale simulations

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A fundamental understanding of how photochemical reactions interact with proteins is crucial for advancements in the biological and biomedical sciences. However, it remains elusive how the protein environment modulates these reactions and how the reactions induce structural changes in the protein. Molecular simulation is indispensable to answering these questions. However, the multiscale nature of these processes poses significant challenges for traditional computational methods. In this talk, I will discuss our recent efforts to overcome these computational challenges, focusing on the multiscale simulation of light-induced reactions in protein environments such as photoinduced electron transfer in cryptochrome and photoisomerization of molecular photoswitches in various proteins. Our simulations elucidate how the ligand-protein interactions and chemical modifications on the ligand influence the pathway, kinetics, and quantum yields of

photochemical reactions. These novel insights are critical for improving the design of photoactive biomolecular systems.

Do you even own a proton? Awkward spectroscopic quirks with reactivity implications for hemoprotein ferryls

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The structure and reactivity of biological ferryl centers generally entails three coordinates: (1) the highly-covalent and electronically-robust S=1 double-bonded Fe(IV)-oxo unit, (2) its occasional magnetic coupling and ensuing illuminating particularities such as the two-state reactivity (TSR), and (3) its protonation in hemoproteins where the axial ligand is an anionic cysteine thiolate or tyrosine phenolate. Occasional x-ray diffraction and spectroscopy reports explore situations where exceptions or additions to these coordinates can be argued. Among these are some unusually long iron-oxygen distances in some ferryl crystal structures of histidine-ligated heme proteins, and some accompanying spectroscopy data that raises the intriguing question of whether two ferryl systems with differing Fe-O distances can have (near?)-identical properties when probed spectroscopically. We discuss here such experimental data and explore the topic using, DFT, TD-DFT, QM/MM and ab initio dynamics in conjunction with new experimental data where in some cases exceptions to the three rules/coordinates can be argued, including excited-state chemistry. Case studies in heme peroxidases, globins, and chlorite dismutase are presented (1–5)

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Cracking the code of enzyme tunnels: understanding, predicting, and engineering hidden pathways.

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Molecular transport through protein tunnels governs ligand access to buried active sites [1], a process critical for understanding binding kinetics and optimizing drug residence times [2,3]. Yet capturing the full complexity of tunnel networks requires extensive sampling that generates massive trajectory datasets [4, 5], analyses of which represent a methodological challenge. To overcome this, we developed TransportTools library [6,7] capable of systematic characterization of transient tunnels and explicit tracking of molecular migrations across thousands of parallel simulations. Building on this, we developed knowledge-based seeding strategies that incorporate prior structural information about tunnel geometries into adaptive sampling workflows [5]. This approach dramatically improves sampling consistency for complete (un)binding pathways, enabling reliable estimation of koff/kon ratios that match experimental measurements even for complex systems with multiple relevant, functional tunnels. Applying this framework at scale, we generated and analyzed 1.6 µs adaptive simulations of 40 diverse enzymes (five EC and four structural classes). This dataset of tunnel dynamics covers over 450 distinct transient tunnels, detailing almost 2.5 million water transport events via more than 90 million tunnel conformations. Finally, such approaches can reveal molecular principles behind the effect of mutations in ABCG transporters [8] and drive protein engineering of N-terminal hydrolases by re-designing molecular gates controlling access to their binding site [9, 10].

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Estimating ligand-binding affinities with quantum-mechanical methods

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One of the largest challenges of computational chemistry is to estimate the binding free energy of a small molecule to a biomacromolecule (e.g. a drug candidate to its receptor). Currently, the best results are typically obtained by free-energy perturbation (FEP) methods, with free energies estimated by exponential averaging, thermodynamic integration or Bennett acceptance ratio [1]. Such methods require extensive sampling and therefore they have been mainly used with molecular-mechanics (MM) methods. However, it is well-known that such methods have severe limitations. Therefore, there have been quite some interest to improve binding-affinity estimates using quantum-mechanics (QM) methods [2]. We have tried to employ QM methods to in FEP estimates of binding affinities for both proteins and host–guest models. Initial attempts failed, because the perturbations did not converge [3, 4]. We have compared various reference-potential methods with explicit QM/MM FEP calculations [5]. They former are based on FEP calculations at the MM level, combined with MM QM/MM FEP calculations. We have tried to avoid QM/MM simulations by employing single-step exponential averaging or non-Boltzmann Bennett acceptance ratio method. For convergence, about 700 000 QM energy calculations were needed [6]. However, more reliable and accurate results are obtained with explicit QM/MM simulations [5], although

they are very expensive. We have shown how they can be sped up by employing many short FEP calculations [7] or by use non-equilibrium simulations and Jarzynski's equality [8]. These calculations still require millions of QM calculations. An alternative is to instead use structures minimized by QM/MM. We have developed and tested such methods for host–guest systems and proteins [9-11]. They are appreciably cheaper, but the accuracy is also worse.

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Computational Design of the Temperature Dependence of Enzyme Reactions

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Cold-adapted enzymes from psychrophilic species are generally among most highly optimized enzymes found in nature. They thus invariably outperform their orthologous mesophilic counterparts, in terms of activity, below and around room temperature. It is therefore not so surprising that no successful attempts to enhance the activity of cold-adapted enzymes appear to have been reported yet. We will address this problem for two bacterial enzymes from arctic environments to illustrate our strategy. In the case of a small extracellular lipase, our results show that it is possible to construct variants, with just a few mutations, that both markedly increase the catalytic rates over the entire examined temperature range and also move the temperature optimum upwards. Of particular interest here is the fact that our most efficient cold-adapted enzyme variant only has mutations that are 15-20 Å away from the reaction center. This underscores the high degree of optimization of the native enzyme and thus suggests that mutation sites for further improvement may have to be sought at more distant amino acid positions.

Tungsten-containing enzymes – new tools in the biotech toolbox. Modelling and challenges

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The incorporation of tungsten pterin complexes into the active site of enzymes enormously expands the available repertoire of possible chemical transformations. The central metal facilitates two-electron redox processes due to stable IV and VI oxidation states and acts as a Lewis acid (with its open ligation position), which can directly activate a bound reagent. Meanwhile, the other ligands of the W ions participate in the reactions by (i) providing additional means to activate recalcitrant bonds (e.g. in homo or heterolytic C-H cleavage),

(ii) enabling hydroxylations without molecular oxygen (by O transfer to activated intermediates), (iii) introducing strong acid/base catalysis (accepting protons or enabling proton transfer electron transfer processes), (iv) tuning the W redox potentials by varying their positions, or (v) even participate directly in the redox processes by providing a reservoir for additional electrons. Moreover, many W-enzymes contain additional redox-active cofactors assembling into "nanowire" structures, which connect the W-cofactors with other active sites within the enzyme. As a result, many chemically highly challenging reactions are catalysed by W-enzymes, such as direct reduction of aromatic rings, reduction of CO2 to formate or of non-activated carboxylic acids to aldehydes, hydroxylation of alkylaromatic or heterocyclic compounds, hydration of acetylene to acetaldehyde, or oxidation of molecular hydrogen. In the presentation, we will show our recent results on parametrization of the AMBER force field for the tungsten cofactor, as well as highlight several challenges that make this process difficult. Furthermore, we will present our latest results on QM-modelling of the reaction pathway for aldehyde oxidoreductase from Aromatoleum aromaticum, that not only is able to oxidise aldehydes but also molecular hydrogen. We will highlight the problem with establishing the oxidation state and the coordination mode of the heavy metal by structural techniques, as well as the oxidation states of the metallopterin cofactor. We will also present our working hypothesis on the activation of molecular hydrogen by the W-cofactor.

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Evolution of Solution-Phase Free-Energy Calculations

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Free-energy calculations in solution have had a revolutionary effect on computational chemistry. Calculations for more than model systems only became possible in the 1980s through increased computer resources, the development of general Monte Carlo and molecular dynamics software, viable force fields for water, organic and biomolecular systems, and through the discovery of the potential precision of free-energy perturbation (FEP) calculations.[1] Applications rapidly expanded to include fundamental solution thermodynamics, solvent effects on equilibria, activation barriers for reactions in solution, host-guest binding, and, eventually, drug lead optimization. While continuing to make methodological advances, our group became increasingly focused on prospective studies in which FEP calculations are used to efficiently evolve weak screening hits to potent enzyme inhibitors with low-nM or pM activity.[2] The presentation will note some key early studies as well as recent technical advances and discoveries of extraordinarily potent enzyme inhibitors.[3]

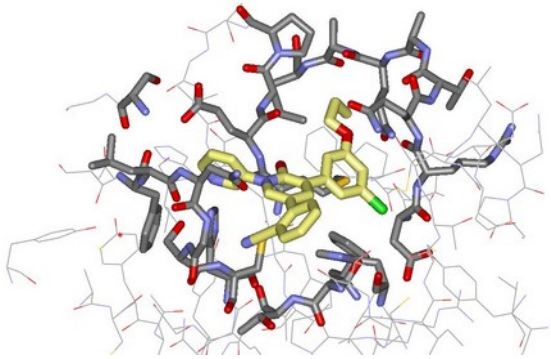

Figure 1. *Rendering from a 1.8-Å crystal structure for a complex with the main protease of SARS-CoV-2* (PDB ID 7L11). *Carbon atoms of the ligand are in yel*

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The Chemical Library Space: From Compound Chemography to Management of Portofolio of DNA-Encoded Librarie

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The development of DNA-Encoded Library (DEL) technology introduced new challenges for the analysis of chemical libraries. Comparing two such libraries by pairwise compound comparisons is way too time-consuming. A chemical library should be considered as a stand-alone chemoinformatic object – represented both as a collection of independent molecules, and yet an individual entity – in particular when they are inseparable mixtures, like DELs. Herein we introduce the concept of Chemical Library Space (CLS) in which resident items are individual chemical libraries. We define and compare four vectorial library representations obtained using Generative Topographic Mapping (GTM). These allow effective comparison of libraries, with the ability to tune and chemically interpret the similarity relationships. In particular, property-tuned CLS encodings enable to simultaneously compare libraries with respect to both property and chemotype distribution. We apply the various CLS encodings for the selection problem of DELs that optimally "match" a reference collection (here ChEMBL28), showing how the choice of the CLS descriptors may help to fine-tune the "matching" (overlap) criteria. Even so, library enumeration and mapping required to obtain their vectorial representations may become a bottleneck as soon as sizes of billions of coumpounds come into play. However, Artificial Intelligence (AI) can be used to bypass this lengthy enumeration process. A graph-convolutional Combinatorial Library Neural Network (CoLiNN) was developed to predict the vectorial representation of any combinatorial library from the reaction rules and building block sets directly, with excellent accuracy and in times shortened by several orders of magnitude. The CLS is therefore useful for the management of a portfolio of libraries, just like Chemical Space (CS) helps managing a portfolio of molecules. Given the steadily growing number of DEL designs, the CLS becomes "crowded", and requires analysis tools beyond pairwise library comparison. An option is the cartography of CLS on meta-(μ)GTMs

– "meta" to remind that these are maps of the CLS, itself based on responsibility vectors issued by regular CS GTMs. 2,5K DELs and ChEMBL (reference) were projected on the μGTM, producing landscapes of library-specific properties. These describe both inter-library similarity and intrinsic library characteristics in the same view, herewith facilitating the

selection of the best project-specific libraries. Hence, the proposed CLS is a new efficient way for polyvalent analysis of thousands of chemical libraries. Selection of an easily accessible compound collection for drug discovery, as a substitute for difficult to produce reference library, can be tuned for either primary or target-focused screening, also considering property distributions of compounds. Alternatively, selection of libraries covering novel regions of the chemical space with respect to a reference compound subspace may serve for library portfolio enrichment.

Allostery in Drug Discovery: From MD to ML

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Allosteric regulation is a fundamental biological mechanism that can control critical cellular processes via allosteric modulator binding to protein distal functional sites. These protein allosteric sites are increasingly targeted in drug discovery because they can enable selective and specific modulation with fewer side effects [1]. However, predicting protein allosteric mechanisms and binding sites remains challenging due to the limited available data and the inherent complexity of allostery [2]. In this talk, we will discuss using Molecular Dynamics simulations and Machine Learning to uncover allosteric mechanisms of membrane proteins [3,4] and to target protein-membrane interfaces for allosteric drug design. As molecular simulations require abundant computational resources to identify protein-protein and protein-membrane interfaces, we describe an ensemble machine learning methodology to predict protein-membrane interfaces of peripheral membrane proteins [5] and present a drug design pipeline for drugging protein-membrane interfaces using the DREAMM (Drugging pRotein mEmbrAne Machine learning Method) web-server https://dreamm.ni4os.eu.[6] To support the development of allosteric site prediction models, we assemble an updated database of over 3,000 allosteric sites in protein structures, integrating multiple sources of annotations across diverse protein families [7].

Using these data, we develop AlloPockets, a deep learning model that predicts allosteric sites using comprehensive protein descriptors derived from sequence, structure, and dynamics (0.66 MCC, 0.83 F1 score). AlloPockets outperforms existing machine learning allosteric site prediction tools in a benchmark of 10 other allosteric prediction tools. Feature importance analysis indicates that AlloPockets captures hydrophobicity and complex dynamical properties that enable robust identification of allosteric pockets across conformational states. Together, these findings highlight the potential utility of AlloPockets for prospective structure-based allosteric drug design. Data, code, and models for allosteric site prediction are available at https://github.com/zoecournia/AlloPockets.

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Phosphorylation influence on protein conformational equilibrium with connections to functional outcome

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The function of multidomain proteins such as those in cellular signaling is often regulated by post-translational phosphorylation. The localization of proteins within cells as well as the levels of enzymatic activity are both tightly controlled to output the proper functionality. One component of regulation is the control of inter-domain structure through phosphorylation. To best understand these fundamental processes, we scrutinized conformational behavior of μs-timescale molecular dynamics simulations in concert with

NMR relaxation to gain insight into the physical basis of responses to phosphorylation. Analyses elucidated regulation mechanisms of protein-protein interactions relevant to localization. This talk will explain an entropy-driven phospho-sensor so far unique to Syk tyrosine kinase. We discovered a network of interactions that includes a central triad of charged residues that switches salt-bridge partners in response to phosphorylation. We envision the design of biosensors derived from principles of the Syk network. In addition, how linker phosphorylation of FAK-FERM kinase alters linker dynamics to enable protein-protein interactions critical for coordinating cellular events underlying cell adhesion and migration will be presented. Given the disease relevance of both Syk and FAK kinases, knowledge on functional regulation by phosphorylation is projected to inform future drug development efforts.

Molecular dynamics in multi-dimensional space reveals how mutations reshape neomycin binding to the riboswitch

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Riboswitches are structured RNA elements that regulate gene expression upon ligand binding and represent promising targets for antimicrobial strategies. The N1 riboswitch, engineered to selectively recognize neomycin—an aminoglycoside antibiotic—serves as a model for RNA–small molecule recognition. Using all-atom molecular dynamics simulations in multi-dimensional space combined with enhanced sampling techniques, we characterized the complete association pathway of neomycin to the riboswitch and elucidated how single-point mutations alter this process. Our studies revealed that the riboswitch recognizes neomycin through a conformational selection mechanism, where mutations alter RNA flexibility and reshape the conformational ensemble available for binding. The subsequent induced fit arises from the interactions of neomycin with the RNA backbone, causing backbone rearrangement. Crucially, mutations shift the RNA– neomycin distance at which each step occurs. This approach uncovered that mutations do not merely weaken binding affinity but alter the association pathway, modifying intermediate states and transition barriers. These findings provide an atomistic-level understanding of how sequence variations modulate RNA–ligand recognition.

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Novel high-throughput methods for free energy calculations

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The rapid exploration of functionally relevant chemical spaces associated with the design and refinement of small molecules as potential therapeutic agents and in the design of protein sequences in functional biologics are key to the identification of new or improved treatments for disease. Multi-site λ-Dynamics (MSλD) is a rigorous statistical mechanically framework of computationally based free energy methods that enable such calculations. MSλD facilitates the exploration of combinatorically complex chemical spaces at a level of precision that mirrors conventional pairwise free energy approaches but utilizes only a fraction of the computer wall time. In this talk, we will illustrate the basic formalism and computational infrastructure of MSλD through examples to protein-ligand optimization problems, in which both protein sequence and ligand substituents are being explored simultaneously as well as new applications to protein sensor design involving optimization of protein-protein interface interactions. These case studies will illustrate the ability to explore vast chemical spaces with the rigorous free energy methods of MSλD. Beyond the current developments within the MSλD methodology, we will describe the ability to integrate modern machine learning interfaces that present high-level quantum mechanical within the ML framework into purely Python workflows using pyCHARMM.

Bridging Single-Protein Biophysics and Nanoparticle Protein Corona Signatures

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Rapid advances in computational hardware, molecular simulations, and AI-driven modelling are transforming our ability to probe protein behaviour across multiple length and time scales. In this talk, I will present recent work from our group that leverages these developments to connect fundamental single-protein properties with emergent mesoscale phenomena. Our results include the identification and validation of transition states in intrinsically disordered proteins using high-resolution molecular dynamics simulations—focusing on amyloidogenic peptides such as amyloid-beta and human islet amyloid polypeptide (hIAPP). We also introduce a set of robust mesoscopic biophysical descriptors derived from a diverse library of single-domain proteins, enabling systematic comparisons across structural classes. Building on these insights, we explore protein adsorption and protein-corona formation on nanoparticles and other nano-engineered materials under biologically relevant conditions. Together, these efforts contribute to a unifying framework that links the kinetic and structural features of individual protein domains to the collective behaviour of larger assemblies—from homo- and hetero-oligomers to complex nanoparticle-associated coronas. By integrating state-of-the-art simulation with emerging AI methodologies, we aim to accelerate predictive modelling of protein aggregation, interaction specificity, and nano–bio interface behaviour.

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Advanced Sampling Methods for Protein–Ligand Interactions

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Introduction: Efficient and reliable sampling of protein–ligand systems remains a central challenge in molecular simulation due to the complex, high-dimensional nature of biomolecular energy landscapes and the presence of high energy barriers between metastable states. We present Generalized Coordinate Hybrid Monte Carlo (GCHMC [1]), an unbiased sampling method that combines Hamiltonian Monte Carlo dynamics with Gibbs sampling to generate statistically rigorous samples from the canonical ensemble, implemented in Robosample software package [2]. The method is formulated in internal coordinates with rigid-body representations, enabling natural treatment of constrained molecular motion and facilitating large-scale conformational changes relevant to binding processes.

Materials and Results: GCHMC alternates deterministic Hamiltonian propagation in generalized coordinates with stochastic Gibbs updates of selected degrees of freedom, ensuring detailed balance while improving exploration of configurational space. This approach allows efficient sampling of both ligand flexibility and protein–ligand relative motion without the use of biasing potentials. The internal-coordinate formulation further reduces numerical stiffness associated with bonded interactions and supports stable integration of constrained systems. The program achieves high performance through the use of efficient algorithms derived from robot mechanics. In addition to accurate pose prediction and binding free energy estimation, the method enables computation of the full potential of mean force (PMF) along relevant binding coordinates, providing detailed characterization of the thermodynamic landscape associated with protein–ligand binding.

Conclusions: We evaluate the method on CASF diverse set of protein–ligand complexes [3] spanning different sizes, flexibilities, and interaction types. The results demonstrate robust sampling performance and consistent convergence of thermodynamic observables across systems, highlighting the potential of GCHMC as a practical and scalable framework for unbiased molecular simulation of biomolecular binding.

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From Canonical Structures to Conformational Ensembles: Physics-Based Modeling of Antibody Structure, Dynamics, and Developability in Solution

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Therapeutic antibodies constitute one of the fastest growing classes of biologics, yet their function is governed by conformational dynamics that are poorly captured by static crystal structures or canonical loop classifications. Over the past three decades, antibody modeling has evolved from knowledge-based approaches toward physics-based molecular simulations that explicitly describe motions in solution. Here, we present a computational framework that combines atomistic molecular dynamics, enhanced sampling, and kinetic state models to resolve conformational ensembles of antibody complementarity-determining regions (CDRs) on micro- to millisecond timescales. These ensembles replace single "canonical" structures and enable quantitative predictions of binding-competent states, paratope geometries, and mechanisms ranging from conformational selection to induced fit. Incorporating improved force fields, polarization effects, and rigorous solvation thermodynamics allows robust free-energy estimates and reliable structure–property relationships. We demonstrate how ensemble-based descriptions improve docking accuracy, rationalize humanization and framework effects, and quantify developability features such as electrostatics and hydrophobicity using grid inhomogeneous solvation theory. Across diverse systems, physics-based predictions of loop conformations and biophysical properties are reproducible and computationally tractable, with typical turnaround times of one to two weeks per antibody. Together, these advances establish

conformational ensembles in solution as a new paradigm for antibody modeling, bridging molecular physics with therapeutic design and enabling predictive, mechanistic insights for next-generation biologics.

Structural Plasticity in Chemokines Driven by Native and Non-Native Disulfide Bonds

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Disulfide bonds stabilize protein structure, guide folding, enable redox regulation, and create opportunities for functional and structural plasticity. While native disulfide bonds define the canonical functional fold of proteins, non-native disulfides can stabilize alternative conformational states that may modulate, alter, or even impair biological activity. The regulatory and pathological consequences of these alternative disulfide configurations remain poorly understood. In this work, we use the chemokine Interleukine-8 (IL-8) as our model system containing four cysteines forming two disulfide bonds. Chemokines are small, disulfide-rich signaling proteins that regulate immune cell trafficking through receptor activation and gradient formation. Conserved disulfide bonds stabilize the chemokine fold, yet alternative disulfide connectivities can arise under oxidative stress. A combination of molecular dynamics (MD) simulations with in vitro and in cell biological assays was used to understand the role of native versus non-native disulfides in IL-8. Our results show that native disulfide connectivity encodes structural precision beyond thermodynamic stability, fine-tuning core packing and conformational integrity to support proper chemokine function. By defining how alternative disulfide patterns reshape structure without globally destabilizing the fold, this work provides new mechanistic insight into disulfide-driven functional plasticity under physiological and oxidative stress conditions.

Nuclear Receptors in Motion: Shape-Shifts and Dynamics

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Nuclear receptors are ligand-regulated transcription factors that control key physiological processes, including metabolism, development, and cellular homeostasis. Although experimental structures have revealed detailed static snapshots of these proteins, the functionality of nuclear receptors relies on their ability to explore a wide range of conformations and dynamics. Classical molecular dynamics simulations, and particularly enhanced sampling methods, allow one to explore conformational space beyond known static structures, but often validation of the results is lacking. An important way to validate molecular dynamics simulation is by connecting with experimental results.
Following an introduction to the role of structural dynamics in nuclear receptor function, this talk will present ongoing efforts in our team that aim to integrate molecular dynamics simulations with novel experimental approaches, such as far-IR spectroscopy and hydrogen/deuterium exchange mass spectrometry. The synergy developed in such an approach can further enhance the understanding of conformation dependent protein dynamics, while at the same time, providing atomistic information for the interpretation of the experimental results.

Abstracts - Posters

01. PockFlex: a web server for flexibility-aware binding site identification and prioritisation from structural ensembles

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The identification of protein binding sites is a fundamental step in structure-based drug design. Conventional pocket detection methods perform well on individual protein structures, but proteins are inherently dynamic, with binding site shape, accessibility, and druggability changing across conformations, limiting the reliability of single-structure analyses.

PockFlex is a web server designed to analyse pockets across protein structural ensembles and support the reconstruction, characterisation, and prioritisation of recurrent binding site organisations. Applicable to ensembles derived from molecular dynamics simulations, multiple experimental structures, or protein structure predictions, PockFlex detects pockets independently in each conformation, retains those overlapping a user-defined region of interest, and groups them across the ensemble by residue-level similarity.
This residue-centred clustering framework identifies recurrent binding site clusters, quantifies residue recurrence and variability, and distinguishes persistent from transient binding site regions across the ensemble. Pocket-level druggability, predicted using the PockDrug workflow, is summarised at the cluster level to support binding site prioritisation under conformational variability while preserving access to individual pocket scores.

The web application provides interactive, residue-level insights into pocket organisation, variability, and druggability in structural ensembles. The web server is free and open to all users, without login requirement, at https://pockflex.rpbs.univ-paris-diderot.fr/.

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02. Chromatin Folding Dynamics from Feature-optimized Adaptive Sampling of MSMs

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Chromatin, composed of DNA wrapped around histone and non-histone proteins, exhibits dynamic and heterogeneous folding that regulates both gene expression and nuclear architecture. Quantitative analysis of these processes is often hampered by intrinsic self-association and the interplay of numerous in vivo factors. To address this, we investigate a minimal fibre comprising tetra-nucleosome arrays to delineate the thermodynamics and kinetics of chromatin folding. Using a chemically specific coarse-grained model implemented in OpenMM, coupled with adaptive sampling and Markov state models (MSMs), we map conformational ensembles and transitions between metastable states while systematically varying DNA linker length and sequences. Simulations with this new coarse-grained model, benchmarked against experimental measurements and established theoretical frameworks, reveal free-energy landscapes that depend strongly on linker length. MSMs resolve metastable states and free-energy landscapes projected onto physically interpretable collective variables, highlighting slow modes in inter-nucleosome rearrangements. We further employ deep-learning-based optimisation of low-dimensional reaction coordinates, yielding MSMs with enhanced kinetic fidelity. This integrated computational framework provides a systematic, scale-bridging interrogation of chromatin folding mechanisms that remain challenging to probe experimentally, and clarifies how DNA geometry modulates self-association and energy barriers during the early stages of chromatin organization.

03. Mechanistic Basis of pH-Dependent Binding in Engineered Anti-CD3 Antibodies

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pH-dependent antibodies can enhance tumor selectivity by exploiting acidic microenvironments. Here, we investigated the mechanisms underlying pH-dependent binding in engineered anti-CD3 antibodies derived from 40G5c using simulation approaches. Mutations in CDR-L3 (e.g., Q90LE, T89LD) do not directly contact the antigen but instead modulate paratope dynamics. At physiological pH, pH-sensitive variants sample broader conformational ensembles, reducing the population of binding-competent states. In contrast, acidic conditions induce rigidification of key antigen-binding loops (CDR-L1 and CDR-L3), favoring binding-compatible conformations. In addition, altered interdomain interactions lead to shifts in VH–VL orientation in specific variants. These findings support a conformational selection mechanism, where pH-dependent binding arises from ensemble reshaping rather than direct electrostatic effects, and provide a framework for rational design of conditionally active antibodies.

04. Integrated Multiscale Strategies for Targeting the Bacterial Ribosome: From Virtual Screening to Allosteric Modulation

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The rapid emergence of antibiotic resistance necessitates innovative strategies to discover new therapeutics targeting essential bacterial processes such as protein synthesis. The bacterial ribosome, a highly dynamic protein–RNA complex, offers multiple orthosteric and allosteric binding opportunities that remain underexplored. In this work, we present an integrated computational and experimental framework to identify both small-molecule and peptide-based inhibitors of the E. coli ribosome.

Our approach begins with structure-based virtual screening targeting the 30S decoding center using consensus docking (Glide and AutoDock Vina) and interaction fingerprinting to capture aminoglycoside-like binding patterns. This strategy is extended to the peptidyl transferase center (PTC) through a multi-stage pipeline incorporating pharmacophore filtering and MM-GBSA binding free energy calculations on truncated ribosome models, improving computational efficiency while preserving key interactions. Experimental validation identified Mitoxantrone (IC_{50} = 14.10 ± 0.38 µM) as a translation inhibitor with activity comparable to Clindamycin, supporting drug repurposing potential (1,2). To further expand the chemical space, we developed a multiscale peptide discovery workflow combining SiteMap-based pocket identification, consensus peptide docking (Glide and rDock), and all-atom and coarse-grained MD simulations. This enabled the identification of peptide candidates targeting multiple ribosomal regions, including the decoding center,

PTC, and intersubunit bridge B8. A cyclic peptide scaffold exhibited multi-site binding capability, suggesting a promising lead for antimicrobial design. Dynamic cross-correlation and residue interaction network analyses revealed long-range allosteric communication pathways within the ribosome (3). Most recently, network-based analysis using RinPy has been applied to the 70S ribosome to further characterize key hub residues and intersubunit signaling (4).

Overall, this study establishes a scalable framework integrating virtual screening, multiscale simulations, and experimental validation to accelerate the discovery of ribosome-targeting therapeutics, with broad applicability to complex RNA and protein–RNA systems.

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05. Decoding Oct4-nucleosome interactions from molecular dynamics simulations

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In eukaryotic cells, DNA is packaged into chromatin inside the nucleus, with the nucleosome serving as its fundamental structural unit. Oct4, a pioneer transcription factor, plays a crucial role in regulating DNA accessibility by engaging with nucleosomal DNA - the fundamental unit of chromatin, in which DNA is wrapped around a core of histone proteins. Understanding interaction of Oct4 with nucleosomes provides valuable insights into its role in gene regulation. We analyze the structural and dynamic aspects of Oct4-nucleosome interactions using classical molecular dynamics simulations, focusing on an Oct4-nucleosome complex lacking the unstructured regions of the histones (histone tails). We previously showed that Oct4 binds and opens this nucleosome in a histone tail dependent manner (Huertas et al., 2020, MacCarthy et al., 2022). Removing the histone tails allows us to separate the direct effect of Oct4 binding on nucleosome dynamics from that of histone tails. Thus, we analyzed classical molecular dynamics simulations of Oct4-bound tailless nucleosomes containing the ESRRB DNA sequence. In these systems, Oct4 binds through its POUS domain at SHL +5.5, and each simulation was extended to 5.2 µs, corresponding to 10.4 µs of sampling per ensemble. These ensembles show substantial heterogeneity, with some trajectories sampling partially open states and others reaching more expanded open conformations. Importantly, Oct4 binding promotes asymmetric nucleosome opening, occurring predominantly at the 3′ DNA end. This study contributes to the growing understanding of how pioneer transcription factors reshape chromatin architecture,

facilitating gene regulation. Our computational approach highlights the intricate dynamics of Oct4-mediated nucleosome remodeling, offering mechanistic insights into chromatin plasticity.

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06. Elucidating the Mechanisms of Sox2-Mediated Nucleosomal Conformational Transition

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Pioneer transcription factors (PFs) play a critical role in development, stem cell pluripotency, and cell-fate determination by recognizing DNA targets within nucleosome-occupied chromatin and initiating chromatin opening. However, the molecular basis by which structurally distinct PFs engage nucleosomal DNA and remodel local chromatin architecture remains poorly understood. Among them, Sox2 is a pioneer transcription factor that binds to chromatinized DNA and induces cell identity changes. It regulates gene expression by specifically targeting DNA sequences, triggering local chromatin opening and recruiting chromatin remodelers and co-activators. The available structure of Sox2-Nucleosome complex suggests that Sox2 induces a large opening of the nucleosomes (Dodonova et al., 2020); however, these structures lack complete linker DNA and intrinsically disordered histone tails, leaving the detailed atomistic mechanism of opening unresolved. Recently it was shown that Sox2-nucleosome conformational dynamics is strongly influenced by nucleosomal DNA flexibility, and histone-tail interactions (Orsetti et al., 2025, Moos et al., 2026). Here, we performed all-atom molecular dynamics simulations of complete free and

Sox2-bound nucleosomes with the synthetic DNA (177bp) and including the intrinsic disordered histone-tails. Our simulations reveal that Sox2-induced opening depends on the structural model and binding-site occupancy: while one model remains compact, simultaneous Sox2 binding at SHL ±2 in a second model promotes a more expanded and flexible nucleosome conformation, leading to a partially open state. We further find that persistent histone tail-DNA contacts, particularly involving the H3 and H2A and H2B tail, restrict linker DNA motion and may oppose nucleosome opening. However, community network analysis indicates that this partial opening is linked to an allosteric interaction network, which drives asymmetric dispersion of the DNA linkers. Together, these results provide an atomistic framework for understanding how Sox2 remodels nucleosomes and may guide future strategies to control cell identity transitions, with potential applications in cell-based regenerative therapies.

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07. Structural and Functional Effects of P-Glycoprotein Missense Mutations: Preliminary Insights

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P glycoprotein (P-gp) is a transmembrane efflux pump essential for cellular protection and a major contributor to multidrug resistance, limiting the efficacy of treatments such as

anticancer therapies. Although widely studied, the structural impact of missense mutations remains insufficiently understood. This study provides a preliminary evaluation of how such mutations may influence local physicochemical properties and substrate interactions.
Forty five missense variants reported in the literature were analyzed. Hydrophobicity and flexibility changes were evaluated using ProtScale, while local structural stability was assessed by comparing hydrogen bonding patterns between native and mutant residues in Chimera. To identify residues involved in substrate recognition, PLIP was applied to nine experimentally determined P gp structures. Based on these results, nine transmembrane

mutations were modeled on the 7A6C structure, docked with elacridar using SwissDock, and re-analyzed with PLIP to examine mutation specific effects on ligand binding. Hydrophobicity and flexibility profiles showed localized alterations across multiple variants and only 20 of the 45 mutated residues preserved their native hydrogen bonding patterns. PLIP analysis of experimental structures identified nine mutations directly involved in substrate binding, including M986V and F978V, which interact with Paclitaxel and Tariquidar, respectively. Docking and PLIP analysis of the mutant models showed that elacridar retains a conserved hydrophobic-aromatic interaction core, with variants such as L305P, A980P, Y853N and M986V producing only localized rearrangements.
These findings indicate that missense mutations can introduce localized changes in flexibility, hydrophobicity and structural stability and may alter substrate recognition by reshaping specific ligand interaction patterns. Together, the results highlight that while the overall binding architecture of P-gp is robust, certain transmembrane mutations can modulate interaction networks in ways that may influence transport efficiency and multidrug resistance behavior.

08. Investigating the conformational flexibility and binding mechanisms of tandem RRM proteins

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Proteins containing tandem RNA Recognition Motifs (RRMs) are essential for RNA regulation, yet the way these domains coordinate to achieve specificity remains a complex question. The Dead End protein (DND1), a key regulator of germline cell fate, utilizes two RRMs to bind AU-rich RNA in a non-canonical manner: only one RRM employs the standard

binding interface, while the second motif acts like a scaffold in a non-canonical way. To characterize the structural features of this interaction, atomistic molecular dynamics (MD) simulations were performed on the DND1-RNA complex and compared with other tandem RRM systems to identify general binding principles.

The simulations show that the DND1-RNA complex is inherently flexible, sampling different relative orientations between the two RRM domains and deviating from initial experimental structures. While RNA binding restricts inter-domain movement, the complex maintains significant plasticity. The simulations suggest that the cooperative action of both RRM domains is necessary to restrict RNA dynamics and maintain sequence specificity, which is largely stabilized by base-stacking interactions. By comparing these findings with other tandem RRM proteins, it appears that such flexibility may allow these proteins to adopt different binding mechanisms depending on the RNA sequence or the presence of partner proteins. This work suggests that dynamic, versatile RNA binding interfaces are a central feature of how tandem RRM proteins function in various biological contexts (1).

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09. Vaccine Development Using Computational Approaches for Multidrug-Resistant Bacteria

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Antimicrobial resistance among ESKAPE pathogens represents a growing global health threat and highlights the urgent need for effective vaccine strategies against multidrug-resistant bacteria. Identifying antigenic epitopes capable of inducing protective immune responses is a fundamental step in the development of rational and broadly protective vaccines. However, the discovery of reliable epitope candidates remains particularly challenging for rapidly evolving pathogens with high levels of antimicrobial resistance.

This work focuses on the identification and evaluation of potential antigenic epitopes in bacterial proteins using integrated computational approaches. Proteins with experimentally validated epitopes are employed as benchmark systems to assess predictive performance and to characterize structural features associated with immunogenic regions. Computational structural modeling and comparative analyses are applied to evaluate how accurately surface-exposed regions corresponding to known epitopes can be reproduced and to identify factors that influence the reliability of predicted antigenic sites. Additionally, variability across independently generated structural models is analyzed to assess prediction consistency and to identify regions of structural uncertainty that may affect epitope identification.
Antigen–antibody interactions are further analyzed to evaluate epitope accessibility, structural stability, and binding affinity. Ultimately, this work aims to identify promising epitope candidates and contribute to the rational development of broadly protective vaccines targeting multidrug-resistant bacterial pathogens.

10. How Ikaros-family proteins dimerize

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Two C-terminal zinc fingers (ZF5 and ZF6) mediate dimerization of Ikaros family transcription factors. Homologous ZFs define putative dimerization domains in a larger family of proteins, which include the Drosophila patterning factor Hunchback, and the human proteins Pegasus and Trps1. How these ZFs mediate dimerization is unknown. We used Alphafold modeling combined with molecular dynamics simulations and experimental validation to elucidate how the Ikaros C-terminal ZFs dimerize. This project used Alphafold to predict the structure of a protein that is resistant to experimental characterization and the resulting structural information needs to be validated experimentally, in a manner similar to validating an experimental structure. In the course of devising mutants for experimental testing, we compared several popular methods and came across discrepancies between them that deserve attention. After careful selection of mutants, and validation of the dimer architecture, our data revealed the structural basis of Ikaros dimerization and showed that Ikaros proteins from the two deuterostome branches have evolved distinct strategies to stabilize dimers.

11. Structure-guided engineering of conformational constraints in the HCV E2 glycoprotein for epitope-focused vaccine design

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Introduction: Envelope glycoproteins of RNA viruses are frequently characterized by extensive sequence variability, conformational heterogeneity, and dense glycosylation — properties that collectively facilitate immune evasion. When a single sequence region can adopt multiple structural states, generating a focused antibody response against a defined epitope becomes difficult. The E2glycoprotein of hepatitis C virus (HCV) illustrates this challenge, as its inherent structural plasticity shapes both receptor engagement and epitope accessibility. To address this, we applied structure-guided protein engineering to selectively stabilize the 412–423 antigenic region, with the aim of restricting its conformational landscape and altering its immunogenic behavior.
Methods: Structural models of HCV E2 in its native form were derived from multiple crystal structures representing different conformational states. A set of candidate mutations was systematically designed, generating a panel of structural variants for comparative analysis. Conformational dynamics were characterized through molecular dynamics simulations in implicit solvent using the OpenMM engine. Dynamic and structural metrics - including RMSD/RMSF profiles, pairwise inter-residue distance distributions, and solvent-accessible surface areas — were computed to compare stability and flexibility across variants. A subset of computationally prioritized candidates was subjected to experimental characterization.
Results: Molecular dynamics simulations show that targeted structural constraints reduce local conformational flexibility without disrupting the overall fold of the glycoprotein. Systematic comparison across variants allowed identification of candidates with notably more restricted dynamic behavior relative to the native protein. Preliminary experimental data indicate changes in antigen recognition patterns in vitro, alongside evidence of distinct antibody elicitation profiles in vivo.

Conclusions: This work demonstrates that computationally designed structural constraints can effectively narrow the conformational distribution of a flexible viral epitope. Integrating structure-based mutation design with molecular simulations and experimental readouts represents a viable strategy for rational epitope stabilization in the context of vaccine development against conformationally heterogeneous viral antigens.

12. In Silico Investigation of the Tissue Transglutaminase – Fibronectin Interaction as a Therapeutic Target in Ovarian Cancer

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Ovarian cancer represents a major global health concern, ranking as the eighth most common cancer and the eighth leading cause of cancer-related death among women worldwide. In this context, identifying molecular interactions that drive tumor progression is essential for the development of new therapeutic strategies. Tissue transglutaminase (TG2) has emerged as an important contributor to ovarian cancer biology, being involved in extracellular matrix remodeling, cell adhesion, migration, and epithelial–mesenchymal transition. One of the key interactions through which TG2 may promote these processes is its association with fibronectin (FN), a major extracellular matrix protein involved in tumor cell attachment and dissemination. Therefore, disrupting the TG2–FN interaction represents a promising therapeutic approach that could interfere with tumor–matrix communication and reduce the invasive potential of ovarian cancer cells.[1][2]
The current work investigates the TG2–FN interaction using an in silico molecular modeling strategy. Structural models of TG2, FN, and the TG2–FN complex were generated and analyzed to assess the putative interaction interface. Molecular docking is used to evaluate the binding mode of known TG2/FN inhibitors and to assess their potential capacity to interfere with the TG2–FN interaction.[2] These known inhibitors serve as positive controls for validating the structural models and the docking workflow. Following model validation, the same computational pipeline can be applied to newly proposed inhibitor derivatives in order to identify compounds with improved predicted binding affinity and favorable interaction profiles.

Overall, the current work combines protein modeling, protein–protein docking, ligand docking, and binding free energy estimation to explore the molecular basis of TG2–FN inhibition in ovarian cancer. By validating the computational model against known inhibitors, this work aims to establish a reliable pipeline for screening novel TG2-targeting compounds, which would expedite the screening process.

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13. Robosample: A Gibbs - HMC Framework for Scalable Exploration of Multimodal Biomolecular Distributions

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Efficient sampling of high-dimensional, highly multimodal probability distributions remains a central challenge in biomolecular simulation, particularly for complex free energy landscapes. We address this problem using Robosample [1], a high-speed robot-mechanics molecular simulation software combining atomistic and rigid-body dynamics within a Gibbs sampling framework coupled to Hamiltonian Monte Carlo [2]. Its formulation allows for efficient decomposition of molecular degrees of freedom and physically consistent exploration of conformational space using Gibbs block updates, improving convergence in challenging systems.
We present here some of Robosample capabilities using several examples. First, simulations on a curated set of proteins with NMR-resolved structures reveal structured patterns of correlation in internal coordinates, informing principled Gibbs block selection strategies. Second, we evaluate docking performance on the CASF-2016 benchmark, alongside targeted studies of ligand binding to the receptor for advanced glycation end products (RAGE), including the inhibitor FPS-ZM1. Finally, we show how Robosample achieves

substantial performance improvements through extensive engineering efforts, including profile-guided optimization, post-link optimization, memory-access restructuring, and OpenMM-based hardware acceleration. These results clearly demonstrate that Robosample is an efficient and scalable framework for sampling complex biomolecular systems.

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14. Efficient sampling of rare conformational shifts through the complementary usage of enhanced sampling methods

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High energy barriers make large conformational shifts unfeasible, even on specialized hardware. Sampling these shifts is mandatory when studying the effect of a given modification upon the equilibrium between known states. For instance, when describing the effect a mutation has on the relative probability of an open state versus a closed state.

Enhanced sampling methods have been previously described and used [1] to overcome such barriers. Before the advent of LLMs, the implementation and the analysis of the subsequent data required both a deep understanding of the underlying theory and a non-trivial time investment. Currently, these methods can be quickly implemented through popular Molecular Dynamics software in a straight-forward method, along with a data analysis workflow. In the present work, OpenMM [2] is used. To showcase system is the E2 protein of the HCV polyprotein, which has multiple crystallized states (RCSB IDs: 8RJJ/8U9Y). These differ by the orientation of three domains – the N-terminal hairpin, the following helical domain which becomes unstructured upon transition, and the CD81-binding loop. The 8RJJ conformation was subjected to 1 μs of MD which did not manage to reach the 8U9Y state. Consequently, multiple enhanced sampling methods were employed – simulated annealing, Temperature Replica Exchange, Steered Molecular Dynamics and Metadynamics. Out of the described methods, only the Metadynamics approach was able to sample both conformations as well as produce a physically plausible pathway.

Using the above methods, we can gain insight into the soft degrees of freedom involved in conformational change, more specifically, the combination of DoFs that define the transition pathway. Using software that allows oversampling of soft DoFs, such as Robosample [3], which leverages algorithms developed in the field of robotics and implements them together with Gibbs sampling, it is possible to selectively oversample a set of DoFs and achieve conformational transition without the need to bias the Free Energy Surface.

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15. In Silico Characterization of Cannabidiol Derivatives for GPR55 Modulation in CNS Disorders

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G protein-coupled receptor 55 (GPR55), expressed in the central nervous system, is involved in neurodevelopment, motor control, and neuroinflammation. These make it a promising therapeutic target for disorders such as Parkinson's disease, chronic pain, and psychiatric conditions. Often referred to as the "third cannabinoid receptor" due to its functional relationship with cannabinoid receptors CB1 and CB2, GPR55 has been proposed as a target for cannabidiol (CBD) and its derivatives.
Here, we used molecular docking, molecular dynamics (MD) simulations, and ADMET profiling to evaluate CBD and thirteen CBD derivatives in relationship with GPR55. Docking was performed using AutoDock Vina on the cryo-EM structure of GPR55 (PDB ID: 8ZX5), with the binding site defined by the co-resolved ligand. All compounds showed favorable binding affinities (−9.2 to −7.2 kcal/mol), interacting with a hydrophobic pocket formed by residues Tyr101, Phe102, Tyr106, Ile156, Phe169, Met172, Trp177, Pro184, Leu185, Leu270, and Met274.

Binding stability was further assessed through 100 ns MD simulations of the top-ranked ligands (3″-HOCBD, THC, and CBL) using NAMD 3.0.2 with CHARMM36/CGenFF in an explicit POPC membrane, solvated environment, and physiological ionic strength (150 mM NaCl). All complexes remained stable, exhibiting ligand-specific conformational rearrangements within the binding pocket.
ADMET predictions indicated favorable drug-like properties, including compliance with Lipinski's rule of five, suitability for oral administration, and predicted blood–brain barrier permeability.

Overall, these results suggest that CBD derivatives may act as promising modulators of GPR55, providing a computational framework for the development of novel therapeutics targeting neuroinflammatory and neurodegenerative disorders.

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16. Integrative Modeling and Molecular Dynamics of the MHC-I Peptide Loading Complex

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The Major Histocompatibility Complex class I (MHC-I) peptide loading complex (PLC) is a multi-protein assembly that selects and loads antigenic peptides for cell-surface presentation. Despite structural insights from cryo-electron microscopy, the dynamic mechanisms underlying peptide accommodation and complex stability remain poorly understood due to the system's size, flexibility, and membrane association.

In this study, an integrative computational model of the PLC was developed based on the HLA-B sequence (IMGT/HLA Acc No: HLA00132), combining homology modeling, constrained docking, and glycosylation to reconstruct a dimeric assembly. Two antigenic peptides, LEEYNSHQS and YMDGTMSQV, were selected to evaluate sequence-dependent effects on peptide loading. Molecular dynamics simulations were performed using OpenMM under NPT conditions (300 K, 1 bar), applying the ff19SB force field for proteins and GLYCAM for glycans, in both implicit and explicit solvent environments. Three systems were analyzed: the peptide-free PLC and two peptide-associated complexes, with peptides initially positioned near the binding groove to allow spontaneous accommodation.
Simulations of 100 ns indicate stable inter-domain organization of the PLC, accompanied by flexibility in peptide-binding regions. Analysis of conformational fluctuations and interaction patterns suggests the presence of electrostatic and hydrophobic regions contributing to structural stability and peptide recognition. Differences in peptide-dependent conformational behavior were observed, indicating that sequence-specific dynamics may influence binding efficiency and antigen presentation. Ongoing simulations incorporate an explicit membrane environment using NAMD, enabling the study of membrane–protein interactions.
Overall, this work establishes a computational framework for investigating PLC function and supports the role of dynamic adaptation in peptide loading.

17. Rational Design of Tanshinone Derivatives Targeting the Exosite of Cathepsin K

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Cathepsin K is a key protease involved in osteoclast-mediated collagen degradation and represents an important therapeutic target in disorders associated with excessive bone resorption [1]. Active site inhibitors have shown severe adverse effects, therefore development of ectosteric inhibitors has become a promising strategy to selectively

suppress the collagenolytic activity of the enzyme while preserving its other functions [2]. In this work, novel tanshinone derivatives were designed and experimentally evaluated as potential exosite inhibitors of cathepsin K. This study combined synthetic chemistry, biological evaluation and computational modeling to investigate structure–activity relationships within a series of nitrogen containing tanshinone analogues inspired by the tricyclic pharmacophore of tanshinone derivatives. The synthesized compounds were evaluated for their ability to inhibit the collagenolytic activity of the cathepsin K–chondroitin 4-sulfate complex. Molecular modeling techniques, involving molecular docking, molecular dynamics simulations as well as binding free energy calculations, were used to characterize ligand binding and rank promising targets. The obtained results indicate that polar substitution at the C-2 position and compact nitrogen containing motifs favour inhibitory activity and stable exosite binding. Overall, the experimental and computational data were consistent and support tanshinone-based scaffolds as promising starting points for the development of selective cathepsin K ectosteric inhibitors.

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18. Mapping the Dynamic Conformational Landscapes of PPARγ

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Peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand-regulated nuclear receptor crucial to metabolic regulation and inflammation, making it a key therapeutic target. Its activity is tightly linked to the conformational state of its ligand-binding domain (LBD), particularly the dynamic behavior of helix 12 (H12), which modulates coactivator recruitment. While static structures have provided snapshots of active or repressive conformations, the full spectrum of PPARγ dynamics remains incompletely characterized. In this study, we systematically investigated the conformational landscape of the PPARγ LBD using a combination of molecular dynamics (MD) and enhanced sampling strategies. Explicit-solvent MD simulations revealed limited structural flexibility, with conformations largely trapped in a narrow basin. In contrast, implicit-solvent simulations and excitation-based methods — including Molecular Dynamics with excited Normal Modes (MDeNM) and Variational Mode Sampling (V-MOD) — significantly broadened the sampled conformational space, capturing large-scale motions relevant to functional transitions.

Despite their diverse sampling characteristics, these methods consistently revealed that H12 undergoes continuous, rather than discrete, rearrangements. This dynamic continuum challenges the classical notion of well-defined functional states and motivates the development of contact-based metrics and tailored clustering strategies for future analyses.

Keywords
Nuclear receptors; PPARγ; molecular dynamics; conformational landscape; MDeNM; enhanced sampling; helix 12 dynamics

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19. Modeling α-Amino-β-CarboxyMuconate-ε-Semialdehyde Decarboxylase Substrate Binding

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α-Amino-β-CarboxyMuconate-ε-Semialdehyde Decarboxylase (ACMSD) is a key metalloenzyme involved in the modulation of the NAD+ biosynthesis via the tryptophan metabolism. This makes it a prime target for the treatment of diseases associated with NAD+ deficiency, such as neurodegenerative or kidney diseases. ACMSD is active as a homodimer, and its structure has been determined experimentally. Although the active site of this enzyme, which contains a zinc center, is well-known, the reaction mechanism remains unclear, particularly how the substrate binds to the enzyme. Mutation data provide insights into this binding mode but do not allow us to determine the orientation of the substrate within the enzyme's active site. First, various approaches were used to determine the substrate's conformation within the active site: a deep learning method such as AlphaFold 3, a physical docking method such as Autodock Vina, and density functional theory calculations using ORCA to model the active site and its substrate. We attribute key roles to arginines R51 and R239*, which interact with the two carboxylate functions of the ligand, respectively. Based on the active site model, the entire enzyme structure was reconstructed using deep learning structure prediction tools. Molecular dynamics simulations were then used to study the dynamics of four different configurations of the enzyme/substrate complex. The evolution of key interactions between the substrate and other residues are studied, as well as the zinc center coordination. The results offer insight into the most likely substrate binding modes. This provides a starting point for studying the reaction mechanism of ACMSD and guiding the design of new inhibitors of this enzyme.

20. Tracking Conformational Transitions in Malarial PfCRT Using Enhanced Sampling Simulations

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Malaria is a life-threatening, endemic vector-borne parasitic disease, responsible for an estimated 263 million cases and 597,000 deaths in 2023[1]. The widespread use of antimalarial drugs such as chloroquine (CQ) and piperaquine (PPQ) has driven the emergence of drug-resistant, highly virulent strains of Plamsodium falciparum, particularly in tropical and subtropical regions. Resistance to these therapies is largely attributed to specific point mutations in the P. falciparum chloroquine resistance transporter (PfCRT),

located on the membrane of the parasite's digestive vacuole. These mutations alter PfCRT function, enabling mutant variants to recognize and efflux multiple antimalarial drugs from the vacuole, thereby preventing them from reaching their intended molecular targets. PfCRT, like other transmembrane transporters, operates via an alternating access mechanism[3], undergoing global conformational changes between inward-facing (IF), outward-facing (OF), and occluded (OC) states. These transitions are modulated by protonation and/or ligand binding. However, the molecular determinants governing these conformational changes remain poorly understood, largely due to the lack of experimental structural information for the OF state.
To elucidate the conformational landscape underlying PfCRT dynamics, we employ an integrated workflow combining machine learning with both conventional and enhanced-sampling molecular dynamics simulations. Using a machine learning-informed collective variable, in conjunction with On-the-fly Enhanced Sampling (OPES) simulations, we first map the free energy landscape of apo-PfCRT, characterizing the previously unresolved OC and OF states. Comparative analysis of residue contacts between the IF and OF states reveals an extended interaction network spanning the transporter, many components of which have been implicated in modulating PfCRT sensitivity to chloroquine.

We further extend this approach to ligand-bound systems, employing OPES simulations of CQ- and PPQ-bound PfCRT to resolve key protein-ligand interactions in the OC and OF states. Together, these simulations provide an atomistic description of the conformational states sampled during the transport cycle and offer a mechanistic framework for the alternating access behavior of PfCRT.

21. Computing protein-ligand residence time by using the τ Random Acceleration Molecular Dynamics (τRAMD) methodology in conjunction with clustering and classification approaches

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Protein-ligand residence time (τ), defined as the inverse of the dissociation rate (τ=1/koff), is often a good predictor of drug efficacy in the non-equilibrium conditions of living organisms. τRAMD (τ Random Acceleration Molecular Dynamics) has been developed for efficiently computing relative residence times and ranking different ligands according to their ligand-target τ.1 The RAMD method uses an additional randomly oriented force applied to the ligand to accelerate its dissociation during molecular dynamics (MD) simulations. In this study, we evaluated the ability of the τRAMD methodology to rank ligands according to their residence time on two different sets of drug-target complexes and one set of peptide-antibody complexes. The Histamine H1 receptor (H1R), a G-Protein Coupled Receptor, in a complex with its antagonists and an E3 ubiquitin-protein ligase, Cbl-b (Casitas B-lineage lymphoma proto-oncogene b) in complex with its inhibitors were used as examples of drug-target systems. Additionally, we report on the application of τRAMD to complexes where the ligands are highly flexible repeating 20-amino acid peptide sequences from the Plasmodium falciparum Circumsporozoite Surface Protein (CSP) and the target proteins are mutants of a high affinity antibody. The τRAMD methodology was applied using a new automatic clustering pipeline, which separates and classifies all relevant ligand and protein conformations according to the correlation of the computed residence time with

experimental measurements. The results demonstrate the applicability of τRAMD to diverse protein-ligand complexes and the value of the clustering approach for identifying the conformations and mechanisms contributing to the protein-ligand dissociation kinetics.

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22. Kinetics of enantiomeric amino acid permeation through chiral phospholipid membranes via path sampling

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Cell membranes, composed of phospholipid bilayers, act as selective barriers that enable the compartmentalization essential to life. Understanding the permeation of small molecules across membranes is therefore crucial for a wide range of biological and pharmacological processes. In this work, the permeation rates of amino acid enantiomers are investigated, focusing on proline. Permeation across a 5:1 DOPC–POPC membrane is examined. Experimentally, biologically relevant L-amino acids have been shown to permeate membranes up to six times faster than their D-counterparts. However, the role of membrane chirality in this stereoselective permeation remains only partially understood. By elucidating the molecular determinants underlying this behavior, this study aims to provide mechanistic insight relevant to the design of chiral therapeutics and membrane-permeable prodrugs.
In molecular dynamics (MD) simulations, such permeation events are rare, making them computationally demanding to observe directly and to quantify kinetically. A substantial reduction in simulation cost, with an accurate determination of rate constants can be achieved using infinity-replica exchange transition interface sampling (∞-RETIS). ∞-RETIS generates new trajectories from an initial path through shooting moves that are accepted or rejected according to the Metropolis–Hastings criterion. By allowing effectively infinite exchanges between trajectory ensembles, the method significantly enhances sampling efficiency without introducing external bias potentials. Moreover, ∞-RETIS increases exchange efficiency without incurring steep factorial scaling, thereby offering improved

computational performance while maintaining accuracy. As a result, the generated reactive pathways preserve the true system dynamics, enabling both qualitative and quantitative analysis of rare events. Using the path sampling methodology, the permeation of proline could be investigated with atomic detail, and it was found that the proximity to the chiral centers of the phospholipids are a dominant factor in the different permeation behavior between L- and D-enantiomers.

23. An integrated protocol for relating Hydrogen-Deuterium exchange data to protein conformational ensembles

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Proteins do not maintain a single static 3D structure, but instead, exist in a dynamic equilibrium, constantly fluctuating between various conformations. While experimental structural determination – for example by X-ray crystallography – will capture one or a few stable structures, there is ample experimental and computational evidence on the existence of transient structures that likely play critical roles in protein function.

An important experimental method for characterizing protein structural dynamics is hydrogen-deuterium exchange, which was historically performed by NMR and now has been revived by mass-spectrometry methods that allow the study of much larger proteins.

For interpreting these data in terms of conformational ensembles, molecular dynamics (MD) simulations are extremely valuable, yet many significant conformational changes occur on timescales that are not routinely accessible, if at all, with conventional MD techniques.

In this study, we present a protocol based on well-tempered metadynamics (WTmetaD) to explore transient fluctuations of protein structure that are relevant for interpreting Hydrogen-Deuterium exchange data. The protocol was tested on the protein ubiquitin, which has been extensively characterized through both computational and experimental methods. We highlight important points of attention concerning the choice of collective

variables for efficient exploration of the conformational landscape. We also discuss important parameters relevant to the calculation of Hydrogen-Deuterium exchange rates from computational trajectories. Our data show that the WTmetaD trajectories successfully sample the functional states of ubiquitin identified in independent NMR studies and that the overall conformational distribution aligns well with HDX data. The protocol is general and versatile, offering a robust framework for studying protein dynamics across various systems.

24. Molecular dynamics to estimate biological kinetics: a new path sampling method with replica exchange of Hamiltonians

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Many molecular processes, such as membrane permeation and protein-ligand unbinding, proceed via spontaneous transitions between metastable states separated by free energy barriers. When these barriers are high, such transitions become rare events occurring on timescales far beyond those accessible to conventional molecular dynamics (MD) simulations.

Path sampling methods, including transition interface sampling (TIS), provide an effective framework to address this limitation by focusing on sampling the reactive trajectories rather than the full equilibrium that includes non-reactive trajectories. TIS employs Monte Carlo moves, such as the shooting move, to generate ensembles of trajectories that satisfy detailed balance in the Markov chain in trajectory space. This enables the estimation of the rate constant at a significantly reduced computational cost. However, in systems exhibiting multiple reaction channels, TIS may suffer from sampling inefficiencies, as transitions between distinct pathways are often hindered by barriers in the orthogonal degrees of freedom, limiting adequate exploration.

In this work, we introduce a novel methodology that uses replica exchange between two Hamiltonians, aimed at enhancing sampling efficiency in such complex systems. The approach alternates between two Hamiltonians: a high-level Hamiltonian as the "main" model that ensures accurate dynamical behavior, and a computationally inexpensive "helper" model that facilitates rapid exploration of phase space. By allowing exchanges of Hamiltonians between replicas, while ensuring detailed balance, the method promotes transitions between otherwise weakly connected regions of phase space, thereby improving the sampling of diverse reaction pathways.
Preliminary simulation results demonstrate that the introduced Hamiltonian replica exchange move significantly enhances the exploration of phase space, i.e. trajectories sampling across distinct regions more efficiently. Furthermore, comparative analysis indicates that simulations incorporating the Hamiltonian swap move achieve this with fewer force evaluations. Our new Hamiltonian replica exchange scheme for TIS is therefore a promising method for studying the kinetics of rare events.

25. On the determinants of electron transfer reorganization energy in a cytochrome P450: cytochrome b5 complex. A combined quantum mechanics and molecular dynamics simulation study

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The electron transfer steps in the catalytic cycle of cytochrome P450 (CYP) enzymes, ubiquitous proteins with key roles in processes such as drug metabolism and steroidogenesis, are often rate-limiting. To predict ET rates from atomistic molecular dynamics simulations using Marcus theory, values of the reaction free energy ΔG0 and the reorganization free energy λ are required from either experiments or computations. For the reduction of cytochrome P450 17A1 (CYP17A1) by the secondary redox protein cytochrome b5 (CYb5), a critical step in the regulation of steroidogenesis, experimental measurements of λ are not available. We here describe the computation of λ for this system from a combination of molecular mechanics/molecular dynamics simulations and quantum mechanics computations. Our results show that a quantum mechanical treatment of the redox-active cofactors is necessary, even though the surrounding protein and solvent, which are modeled classically, contribute most to the reorganization energy. The values of λ

computed for structural ensembles corresponding to two predicted binding modes of the proteins are 1.23 and 1.16 eV. We find that the λ values computed for the individual soluble globular domains of the two proteins sum to approximately the λ values computed for the membrane-bound CYP17A1-CYb5 complex, indicating that additivity can be invoked in a computationally efficient approach to estimating λ values for such protein–protein complexes.

26. Exploring the Conformational Dynamics of HIV-2 Protease through the HMM-SA structural alphabet

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HIV-1 and HIV-2 are two etiological agents of AIDS (acquired immunodeficiency syndrome). While HIV-1 is prevalent worldwide, HIV-2 remains largely confined to West Africa, infecting between 1 and 3 million people. Current antiretroviral therapies are primarily designed for

HIV-1, yet HIV-2 exhibits intrinsic resistance to several of these drugs, underscoring the need for more selective therapeutic strategies. HIV-2 protease (PR2), a key enzyme for viral maturation, represents an important drug target. To better understand the mechanisms underlying HIV-2 resistance to protease inhibitors, we first explored the structural diversity of PR2 by characterizing and comparing the 19 available structures in the Protein Data Bank (PDB). These studies highlighted regions with conserved conformations that are crucial for structure and function. In addition, this work enabled us to characterize regions capable of deforming upon ligand binding and led to a detailed mapping of the inhibitor binding site, identifying critical residues involved in ligand specificity. However, these studies only partially accounted for the intrinsic flexibility of PR2, which plays a crucial role in ligand binding. PR2 adopts different conformational states: the semi-open form, observed in the absence of a ligand, and the closed form, induced upon inhibitor binding. These conformations were initially identified based on crystallographic structures. In this work, we investigated the intrinsic conformational dynamics of apo PR2 in its semi-open state using three independent all-atom molecular dynamics simulations (0.5–1 μs each). Analyses were performed by encoding each generated conformation into structural letter sequences via the HMM-SA structural alphabet [1-3], followed by time-lagged independent component analysis (TICA). This multiscale approach captures local conformational variability while simultaneously identifying global conformational states, revealing key transitions and novel structural states that could be exploited for the design of more effective HIV-2 protease inhibitors. More broadly, it provides a general analysis pipeline applicable to other proteins of interest.

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27. Design of compounds targeting the Group IIC Intron during Self Assembly Process

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Group IIC introns represent the smallest and most streamlined subclass of group II introns, retaining the essential structural and catalytic elements required for self-splicing. While recent studies identified intronistat B as a small-molecule inhibitor that blocks both the first and second catalytic steps by targeting the conserved catalytic core, we are also investigating an alternative strategy to disrupt intron splicing by interfering with its hierarchical self-assembly. In particular, recent mechanistic studies have shown that docking of subsequent domains dynamically induces opening of the D1 "closed" conformation, highlighting this conformational transition as a potential target for allosteric inhibition of splicing. In this work, classical molecular dynamics simulations were performed to sample the conformational landscape of the D1 closed state. The resulting trajectories were analyzed using Pocketron to identify transient and druggable allosteric pockets. Several candidate pockets were detected, with the top-ranked site located at a conformational "gate" involved in D1 opening. This pocket was subsequently subjected to virtual screening and docking of small molecules evaluated with three different scoring functions. Selected candidate compounds have been prioritized and are currently under experimental evaluation.

28. A novel antiviral strategy targeting SARS-CoV-2 non-structural protein 13

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We present a novel antiviral strategy targeting SARS-CoV-2 non-structural protein 13 (NSP13), a multifunctional helicase essential for viral replication and suppression of host

innate immunity. NSP13 not only participates in the replication–transcription complex (RTC) but also inhibits type I interferon (IFN-I) signaling through interaction with the host kinase TBK1. Disrupting these protein–protein interactions represents a promising therapeutic approach. Using molecular dynamics simulations, structural interaction mapping, and convolutional neural network-based binding free energy predictions, we examined whether fragments derived from the viral cofactor NSP8 can competitively bind NSP13. Our results identify the N-terminal fragment of NSP8 (residues 1–87, NSP8-N) as a high-affinity binder that occupies key NSP13 residues involved in both RTC assembly and TBK1 interaction. Structural comparisons reveal significant overlap between NSP8-N and TBK1 binding interfaces on NSP13, supporting a competitive inhibition mechanism. Binding free energy estimates further indicate that NSP8-N interacts with NSP13 more strongly than both native NSP8–NSP13 complexes and the NSP13–TBK1 complex. Functional validation in poly(I:C)-stimulated A549 cells demonstrates that while NSP13 suppresses IFN-β expression, co-expression with NSP8 or NSP8-N restores interferon signaling to near-control levels. In contrast, the NSP8 C-terminal fragment does not produce this effect, consistent with its distinct, non-overlapping binding site. Collectively, these findings support a dual model in which NSP8-N sequesters NSP13, preventing both its incorporation into the RTC and its interaction with TBK1, thereby impairing viral replication and restoring host immune responses. The NSP8 N-terminal α-helical region emerges as a promising scaffold for the development of peptide-based or peptidomimetic antivirals targeting conserved NSP13 interaction surfaces.

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29. RxnNet-EnzyDock: An AI Framework for Reaction Mechanism Discovery in Enzymes-A Case Study of Carbocation Reaction Networks and Enzyme Docking

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Understanding complex chemical reaction cascades—both in solution and within enzyme active sites—remains a central challenge in chemistry. Scalable exploration of their thermodynamic and kinetic landscapes requires automated approaches, yet systematic investigation of multistep reaction networks involving highly reactive intermediates, such as carbocations, is still difficult. Here, we present an integrated artificial intelligence-assisted framework for reaction mechanism discovery that combines RxnNet, an automated reaction network generation platform [1], with EnzyDock, a mechanistic docking tool tailored for enzymatic systems [2-3]. RxnNet constructs mechanistically informed reaction networks using heuristic rules augmented with domain-specific chemical knowledge, including stereochemistry, regiochemistry, conformational preferences, and isotope labeling, and couples these networks with on-the-fly quantum chemical evaluations to identify intermediates and transition states. Integration with EnzyDock enables efficient exploration of enzyme-catalyzed reactions by incorporating active-site constraints and substrate binding modes. We demonstrate the capabilities of this approach on carbocation-driven reaction cascades in terpene synthase (TPS) enzymes, which generate structurally complex natural products. The method successfully recovers known multistep mechanisms and provides insight into how enzyme active-site architecture modulates carbocation reactivity, substrate preference, and initial cyclization events that determine the overall reaction trajectory. This combined EnzyDock–RxnNet approach enables scalable and mechanistically grounded prediction of complex reaction pathways at significantly reduced computational cost, providing a foundation for the rational design of chemical transformations and enzyme function.

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30. Parameterization of the tungsten cofactor – enabling molecular modeling of tungstoenzymes in AMBER force field

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Tungsten-containing enzymes enable low-redox-potential reactions, making them a promising direction for green catalysis. Aldehyde oxidoreductase from Aromatoleum aromaticum (AORAa) is an enzyme involved in cellular detoxification of bacterial cells NAD+-dependent oxidation of aldehydes to carboxylic acids. Meanwhile, the reverse process, i.e. reduction of carboxylic acids, is highly interesting for the pharmaceutical and perfumery industries. Propitiously, in addition to aldehyde oxidation, AORAa catalyzes also H2 oxidation, which can be coupled to the reduction of acids to valuable aldehydes or NAD+ to NADH. The latter activity enables clean coenzyme recycling. As the AOR H2-oxidation is a completely novel process, its mechanism still remains a mystery to be elucidated by combined experimental and theoretical methods. Meanwhile, multiscale modeling and MD simulation of AORAa is challenged due to the scarcity of appropriate force field parameters for the tungstopterin cofactor (W-co) and limited knowledge on the coordination of the W

atom in the enzyme's active sites. While it is known that central metal is most stable at IV and VI oxidation states, ligand composition at different stages of reaction is still the topic of debate. Additionally, the pterin ligands of W-co are believed to be redox non-innocent, attaining two distinctive redox states, which multiply potential variants for parametrization. In this work, we describe the parameterization of W-co for both the oxidized WVI and reduced WIV states. Moreover, we introduce a set of dihedral parameters aimed at a better description of the geometric features of tetrahydropterin or 10,10a-dihydropterin. The parameterization protocol included initial geometry optimization, frequency and vibrational analysis performed at the DFT level of theory, followed by testing of obtained parameters in MD simulation. The validation of parameters was conducted at two levels – as a statistical analysis of geometry from the stable MD trajectory (with respect to literature and EXAFS data), and by comparison of geometries from representative structures, which were cooled down to 0K, with results of QM/MM geometry optimization.

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31. Seeing it through - exhaustive exploration of transport tunnel networks in haloalkane dehalogenases.

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Although challenging to study, transport tunnels are major contributors to the efficiency and selectivity of enzymes with buried active sites. These tunnels exhibit distinct geometrical and chemical properties that govern the passage of substrates, products, and solvent. As dynamic entities, their properties fluctuate constantly. Haloalkane dehalogenases (HLDs) are a family of enzymes with deep active sites that established themselves as model systems for research of intramolecular transport. Among the studied HLDs, LinB and DhaA have been described most robustly with proposed primary and auxiliary tunnels. However, knowledge regarding transport pathways in HLDs is fragmented across multiple sources, often differing

in approaches, sampling scales, and molecules studied (substrates, products or solvents). In this study, we leverage increased computational capabilities to build a uniform foundation of knowledge regarding transport in HLDs. Using the recent method of tunnel seeding for efficient simulation of transport events [1], we applied adaptive sampling molecular dynamics to describe the transport of selected substrates (1,2-dibromoethane, water) and products (2-bromoethanol, and bromide ion). We amassed 100 μs of simulation data for each enzyme-ligand combination, representing a dataset of unprecedented scale for HLDs. We describe tunnel geometries and ligand transport events, focusing on comparisons between enzymes and transport differences between water, substrates, and products. Our results uncover previously unreported pathways in both enzymes and provide quantitative measures of their engagement in transport processes. Employing Markov state models, we identified metastable states of transport, providing a basis for describing ligand competition effects during this process. This work updates previous insights with modern sampling scales and new methods, enabling a more complete understanding of dynamical transport processes and tunnel networks, offering insights transferable to ligand binding in pharmacologically relevant targets.

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32. Structural dynamics of a cryptic TSP-1:CD47 interface enable structure-guided targeting in the tumor microenvironment

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Thrombospondin-1 (TSP-1) is a multifunctional extracellular matrix glycoprotein involved in various physiopathological processes and is considered a key therapeutic target within the tumor microenvironment. The interaction between TSP-1 C-terminal domain and its

membrane receptor CD47 is crucial to support tumor growth and immune evasion [1].

Previous computational studies based on in vacuo modeling [2] suggested that the CD47-binding site on TSP-1 could be buried, requiring an opening of TSP-1 to interact with a specific region of CD47. These results led to the development of the TAX2 peptide, an antagonist that disrupts the TSP-1:CD47 interaction, inhibits tumor progression in preclinical models, and is currently entering a first-in-human clinical trial [3]. A better understanding of the TSP-1 opening mechanism could guide the development of next-generation antagonists that block its interaction with CD47 receptor.

Using classical all-atom molecular dynamics (MD) simulations, we explored the intrinsic dynamics of each protein considered separately, in a realistic environment. While CD47 exhibited various orientations of its extracellular domain, TSP-1 C-terminal domain did not display any opening motion. These data confirmed that such opening requires larger collective motions, which classical MD may not capture.

To address this, a bias-exchange metadynamics protocol was applied to TSP-1 in water, combining the four lowest-frequency normal modes as collective variables to predict the opening of TSP-1 and the mutual orientation of the protein partners. This enhanced sampling method successfully revealed previously uncharacterized conformational states of TSP-1 and provided a structural basis for peptide design.

These TSP-1 open conformations were subsequently used for AI-based de novo peptide binder design using diffusion models. The resulting peptide candidates were further evaluated using coarse-grained MD simulations, MM-PBSA calculations, and structural alignment analyses. Several peptides targeting the TSP-1:CD47 interface have been identified and are currently undergoing experimental validation. Altogether, this integrative computational strategy provides a framework for structure-based design of next-generation antagonists targeting the TSP-1:CD47 complex.

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33. Modeling nucleosomes: from FASTA to fully atomistic structures

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The nucleosome-pioneer transcription factor (PTF) complex encompasses a wide range of features, from the histone core to flexible histone tails and interacting transcription factors. Among these, intrinsically disordered regions such as histone and transcription factor tails present a particular challenge, as they require specialized approaches to accurately capture their conformational variability. There are several methods that can generate comformational ensembles of the nucleosome and it's tails. These methods can be classified into homology modelling, physics based methods and AI tools. While each

approach offers distinct advantages, they often fall short in consistently generating complete, fully atomistic models that integrate both ordered and disordered regions within a unified framework. Here, we present an automated pipeline designed to generate nucleosome–PTF complexes by integrating multiple modeling strategies. By combining

homology modeling[1] for the core and physics based/AI tools[2,3] for sampling the conformational space of the tails, this pipeline enables the construction of fully atomistic models starting from the FASTA sequence.

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34. Systematically Improvable and Locality Accelerated Enzymatic Reactivity Modeling

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Quantum mechanics/molecular mechanics (QM/MM) is the de facto method for modelling chemistry in large molecular systems, particularly in enzyme catalysis. However, the requirement of a large QM region for converged kinetic and thermodynamic properties limits the scale of QM/MM simulations by their cost. Our development of the local embedded subsystem (LESS) approach [1] enables hybrid and double hybrid DFT accuracy at the cost of a few core hours, even with large QM sizes up to ca. 400 QM atoms. The applicability of LESS has been demonstrated on the challenging phosphate-catalytic enzyme Ras [2], as well as examples of homogeneous and heterogeneous catalysis, resulting in up to 90-fold speed-up, with a few tenths of a kcal/mol embedding error. The speed LESS provides ultimately allows reaction path optimisations and free energy simulations. We organise the takeaways from our extensive QM/MM studies into a Locality Accelerated and Systematically Improvable (LASI) scheme [3] designed to provide affordable computation of free energies. Locality is exploited at two levels in LASI: in LESS, it provides the acceleration that is crucial for sampling; and local natural orbital (LNO) based CCSD(T) ensures that relative energies are obtained within chemical accuracy (1 kcal/mol). With these tools, we advance the reliability of QM/MM calculations so that they can be compared to experimental kinetic and thermodynamic measurements.

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35. Assessing DNA Force Fields: A/B Conformational Equilibria, Sugar Puckering, and the Role of Water Models

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Accurate modeling of DNA conformational equilibria remains a key challenge in molecular simulations. The balance between A- and B-DNA forms, closely linked to sugar puckering, is essential for processes such as protein–DNA recognition and the stability of DNA/RNA hybrid duplexes. However, commonly used force fields often exhibit systematic biases in this equilibrium, which can affect conformational sampling and the behavior of biomolecular complexes. Here, we present a systematic assessment of selected current DNA force fields with respect to their ability to describe sugar puckering and the A/B conformational equilibrium in canonical DNA duplexes, DNA/RNA hybrids, and protein–DNA complexes. Our results show that a recently introduced parameter set (OL24) significantly improves the description of the A/B equilibrium by shifting it toward a more realistic balance and increasing the population of A-like conformations, while maintaining an accurate representation of canonical B-DNA. In addition, we evaluate the impact of different explicit water models on canonical DNA duplexes, focusing on selected conformational equilibria and structural features, including A/B balance and terminal fraying. Our results demonstrate that both force field parameterization and solvent representation play a critical role in determining these properties and should therefore be carefully considered for reliable modeling of DNA structure and dynamics.

36. Antibody Fv Interface Hydration as Driving Force of Germline Pairing

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Antibodies are increasingly used as biotherapeutics due to their high specificity, and they find their application in the treatment of cancers and autoimmune diseases. To be eligible drug candidates, antibodies must have appropriate physicochemical properties such as solubility, clearance and stability. These characteristics are highly dependent on the molecular architecture of the antibody. Human antibodies are Y-shaped immunoglobulins that consist of two identical heavy chains and two identical light chains. Modern sequencing

technologies have allowed to identify which heavy chain-light chain pairings occur in the human immune system. However, the physicochemical principles governing gene selection and heavy–light chain pairing probabilities remain poorly understood. In particular, the role of enthalpic and entropic contributions, as well as the role of interfacial hydration and solvation effects at the variable fragment interface, remain unclear. This project aims to investigate the physicochemical properties of different germlines and their influence on the pairings and their probabilities. Particular attention will be dedicated to investigating the effect of pairing different heavy and light chain germlines in the variable fragment, which contains the sequences directly responsible for antigen binding. To achieve this, I will employ a range of state-of-the-art computational techniques, including molecular dynamics simulations and free energy calculations, to better characterize the thermodynamics and kinetics of germline pairings. By improving our understanding of the molecular characteristics underlying heavy–light chain pairing, this work aims to contribute to the rational design and optimization of therapeutic antibodies.

37. A Systematic Dimer Configuration Dataset for Non-Bonded Force Field Parameterization

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Non-bonded interactions remain a persistent bottleneck in the accuracy of classical nucleic acid simulations. However, current parametrization efforts can be constrained by the limited availability of comprehensive quantum mechanical (QM) reference data specifically tailored to the intermolecular landscape of biomolecules, and nucleic acids in particular.

Our aim is to develop a special-purpose dataset to support the development of machine learning potentials for biomolecules. The dataset will focus on noncovalent interactions, providing high-quality data for efficient parameterization of interaction potentials. Our methodology samples the six-dimensional intermolecular configurational manifold using automated roto-translational sampling and adopts a modular and easily extensible architecture. Special attention is paid to quality of the dataset in terms of low redundancy and high completeness. The resulting configurations are characterized using DFT (ωB97M-V/def2-QZVPPD) to produce high-fidelity interaction energies and forces across the

sampled space. This ongoing work aims to provide a reproducible foundation for force field development based on QM reference data. Specifically, the dataset is intended to support both machine learning-based parametrization and direct force field refinement, including the optimization of Lennard-Jones and other non-bonded parameters governing intermolecular interactions.

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38. Computational Predictions of the Binding Activity of Endocrine Disrupting Compounds to the Estrogen Receptor Alpha

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Molecular docking, a well-established computational method to aid drug discovery, has found widespread use in endocrinological applications to study the interaction between environmental pollutants and ligand activated receptors. This interaction is the primary mode of action of endocrine disrupting compounds (EDCs) which pose an increasing risk to human and environmental health. By screening large chemical spaces and identifying substances of high concern for further testing, molecular docking can improve the effectiveness and sustainability of chemical risk assessment. Moreover, it is often used to provide predictions for environmentally relevant species with little validation, due to the lack of experimental data for non-model organisms. However, the scoring functions used in molecular docking only provide a simplified approximation of ligand binding to efficiently evaluate binding poses. And while more rigorous approaches, such as relative binding free energy (RBFE) calculations, have gained significant usage for drug development applications, they are rarely applied to toxicological studies.

Here, we provide an assessment of the applicability domain of molecular docking as a new approach methodology to facilitate next generation risk assessment in environmental toxicology and compare its performance to relative binding free energy calculations using an alchemical approach. We show that molecular docking is able to accurately predict binding poses to the estrogen receptor alpha (ERa) for different chemical classes, when receptor flexibility is considered. However, we also demonstrate a lack of correlation between the docking score and experimental measures of affinity and transactivation potency. As an alternative, RBFE calculations were tested on a congeneric series of bisphenols, showing a higher sensitivity towards small structural changes. Taken together, our results suggest that more rigorous approaches than those currently in use are required to facilitate environmental toxicology.

39. Active targeting of folate receptor-alpha by conjugates of folate and antifolates visualized by atomistic molecular dynamics

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Drug delivery systems based on active targeting are nowadays a practical tool for alleviating side effects of chemotherapeutics. The current study summarizes the outcome from atomistic molecular dynamics simulations describing the targeting behavior of a set of vector ligands encompassing two forms of folate and four antimetabolites. The models progress from salinated free ligands or vector-drug conjugates to these entities simulated in the presence of a membrane-anchored folate receptor-alpha at physiological conditions. Trajectories with lengths of up to 1 s are generated from up to 4 independent runs. The experimentally detected targeting specificity of the free ligands is captured and rationalized at the molecular level by different balance of H-bonding, van der Waals and electrostatic attraction, delineating a unique fingerprint of each vector. The effect of loading the chemotherapeutic doxorubicin as bioactive cargo on the binding efficiency of the vector molecules is traced and the importance of optimizing the carrier composition for efficient binding is highlighted. The observed trends provide clues for construction of prospective biocompatible folate-based conjugates for active targeting delivery of doxorubicin.

40. HSulf Endosulfatases (title assigned by organizers / not provided in the form)

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A sulfatase is an enzyme responsible for modulating the sulfated state of macromolecules, such as heparan sulfate proteoglycan (HSPG), by cleaving the ester-sulfate or sulfamate bond and thus releasing sulfate. It is involved in cellular metabolism and developmental cell signaling. The cysteine or serine in the active site is converted to formylglycine (FGL) to perform enzymatic activity. Among the 17 known human sulfatases, endosulfatases 1 (HSulf-1) and 2 (HSulf-2) were discovered in 2002 [1]. These two HSulf isoforms are composed of three domains: the catalytic domain (CAT), the hydrophilic domain (HD), and the C-terminal domain (Cter). One of the main differences is that HSulf-1 tends to suppress cancer development, whereas HSulf-2 is overexpressed in numerous cancers, particularly breast cancer, making it a promising therapeutic target. Structural exploration of HSulf-2, especially of the ligand-binding pocket, is essential for the design of inhibitors as potential anticancer drug candidates [2]. However, no experimentally resolved structure of HSulf-2 has yet been uploaded to the Protein Data Bank (PDB). One alternative is to use the state-

of-the-art AI-based structure predictors to predict the apo form of HSulf-2, and the holo form with polysaccharide ligands, to especially reveal the binding cleft of the HD. Molecular dynamics simulations are run to study the dynamic profile of the HD and the potential binding pockets. This study sheds light on the IDR-binding mechanism of the HD and contributes to the eventual design of inhibitors targeting it.

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41. An Improved Force Field Parameterization of DNA-Metal Ion Interactions that Preserves the tRNA Anticodon Loop Structure

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Electrostatic interactions of nucleic acids and metal ions critically affect the structure and functionality of DNA and RNA in solution. It has therefore been a constant effort of molecular dynamics (MD) simulations of aqueous nucleic acid systems to improve the parameterization of the non-bonded interactions between metal ions and the biomolecular surface, in particular for doubly charged ions, like Mg2+. However, non-polarizable, state of the art force fields overestimate contact ion pair (CIP) formation with the phosphate groups of nucleic acids and it is therefore an open question to derive adequate non-bonded force field parameters for the simulation of nucleic acids in solution. Using experimental and ab initio (GIAO-DF-LMP2) simulated 31P chemical shifts we have quantified the concentration dependent interaction between Mg2+ and the phosphate backbone of nucleic acids, indicating a preference for solvent separated ion pairs (SSIP) over contact ion pair formation [1]. Building on this new benchmark data set, we developed a force field parameterization approach that unifies preceding approaches by fitting directly to ab initio data. Missing polarization effects and mismatched surface-ion interactions can be accounted for within the 12-6-4 Lennard-Jones model with additional induced dipole term [2]. Alternatively, the screening of charges in condensed liquid phase is effectively accounted for in a mean field way via a rescaling of charges [3]. We show that the use of non-integer ion charges and a 12-6-4 Lennard-Jones potential allows to derive improved nucleic acid-ion parameter sets. Extensive benchmark MD simulations demonstrate that the new force field substantially improves the polyanionic properties of nucleic acids. First, we find qualitative agreement for the expulsion of bi-valent ions by mono-valent ions in ion counting experiments. Furthermore, the force field improves the description of the complex loop structures of tRNAPhe compared to the crystal structure, where Mg^{2+} plays a crucial role in stabilizing the formation of the anticodon loop.

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42. CGChrom, a comprehensive multiscale model for the representation of active chromatin

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We introduce CGChrom, a comprehensive multiscale model for the representation of active chromatin. CGChrom integrates a highly accurate coarse-grained (CG) description of DNA derived from the original CGenerate potential with a hybrid CG framework for DNA-binding proteins and a streamlined version of our low-resolution nucleosome model. Calibrated to accurately reproduce DNA–protein interactions as well as the general structural and mechanical properties of chromatin fibres with sequence specificity, this framework enables, for the first time, base-pair-resolution simulations of chromatin dynamics beyond conventional approaches. Unlike existing models that primarily consider histones, CGChrom explicitly incorporates DNA-binding proteins and Transcription Factors thereby capturing key features of active chromatin organization and dynamics.

43. An Efficient Computational Chemistry Approach to Generating Negative Data for Virtual High-Throughput Screening Validation

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In a hit discovery context, structure-based virtual high-throughput screening (VHTS) pipelines typically employ a funnel-shaped model where the search begins with a large number of small molecules which are subjected to increasingly sophisticated, computationally costly, and theoretically rigorous techniques on a progressively decreasing

pool of molecules. Scoring all ligands and ordering them by score produces a monotonic score vs. rank function; marking the positions of the actives produces a plot known as a recovery plot [1]. In an ordered list of scores, a hypothetical perfect estimator would place all active ligands at the top of the list. Real scoring tools are assessed by their recovery capabilities – the more actives they place near the beginning of the list, the greater their utility in a drug discovery setting. The first mistake practitioners often make during validation is that they evaluate performance as a whole, i.e. for the entire pipeline rather than for each step and element individually. In this way, they inadvertently hurt performance by unwittingly allowing suboptimal, unproductive or even counterproductive steps to remain undetected in their workflow.

The second serious flaw often lies in the composition of the active and decoy sets which practitioners use to validate their pipelines. For validation to be robust and reliable, decoys should match actives as closely as possible in as many aspects as possible. This has given rise to several generations of validation sets that address previously reported shortcomings of earlier collections. This is an iterative and expensive method of curating validation sets that leaves ample scope for discrepancies between actives and decoys to creep in. In silico isomerization offers an attractive alternative for generating decoys for drug-like compounds that naturally mitigates many of these discrepancies [2]. We show that isomerization can produce molecules that have hydrogen bond acceptor, donor, rotatable bonds counts, charge and surface area distributions that match more closely experimental actives than experimental decoys. While these are properties that receive a lot of attention in drug design, we also show that isomerization can produce decoys that are positioned closer to actives in property hyperspace than current experimental decoys which tend to be highly dissimilar from the actives. The latter is a significant shortcomin that has thus far remained unreported and unaddressed. Moreover, by randomizing ligands across published experimental structures, one can generate validation sets with a perfect match in properties between actives and decoys because the actives and decoys are the exact same set of molecules. This constitutes the ultimate test for ABFE pipelines which are believed to be approaching maturity. Further still, recovery analysis is an excellent test for method development in computational chemistry because it is completely orthogonal to many of the metrics used in that development. For instance, one might reasonably ask whether force field modifications, hydrogen mass repartitioning, solvation free energies [3], ligand strain or entropy calculations would improve recovery during VHTS [1,2,3]. Many more such questions are yet to be asked and answered.

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44. Computational protein design in Proteus

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Computational protein design (CPD) aims to create proteins with new properties. Applications include the design of new catalytic reactions, new peptide ligands, vaccines, and new materials. A key element of CPD is the energy or scoring function used to discriminate the sequences and conformations. We use an energy function combining molecular mechanics (MM) with generalized Born (GB) solvation along with approximations that make the model pairwise decomposable. Our CPD approach is implemented in the Proteus software. The use of a physics-based energy function ensures a certain transferability and explanatory power to the model. An ambitious problem, often used to evaluate CPD approaches, is the redesign of full protein sequences in which the sequence of all positions is optimized at the same time. We obtained good results previously, with protein cores similar to natives and Superfamily recognition of the sequences close to 100%. A possibility to further improve our results consists of reducing the errors due to the pairwise decomposition of the solvation terms. Our group has proposed a "fluctuating dielectric boundary" (FDB) approach allowing an exact decomposition of the GB term. It was previously applied only to the sidechains. The goal of the present work is to extend the GB FDB approach to the whole protein and apply it to the single-position redesign of protein sequences. A notable improvement in the quality of designed sequences is obtained. This allows our Proteus program to have one of the most realistic electrostatic models among CPD approaches.

45. Blocking the Gate: MD Simulations Reveal How a Tunnel Entrance Mutation Disrupts Transport in an ABCG Transporter

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ATP-binding cassette transporters of the G subfamily (ABCG) play essential roles in plant physiology by transporting diverse metabolites across cellular membranes, contributing to homeostasis and environmental responses. Understanding their structure-function relationships could inform strategies for improving crop stress tolerance. (1) The ABCG46 from Medicago truncatula mediates export of phenylpropanoids, specialized metabolites, essential for pathogen resistance. Previously, we revealed the first molecular basis of ABCG46 multispecificity and identified F562, located in the central tunnel region, as a residue critical for substrate transport. (2) In this follow-up study, we investigated how a mutation at the tunnel entrance leads to loss of transport function, motivated by experimental evidence of uncoupling of transport and ATPase activity in the closely related ABCG36 transporter from Arabidopsis thaliana carrying equivalent substitution. (3) Using molecular dynamics (MD) simulations, we examined the structural and energetic consequences of mutating a residue positioned at the site of initial substrate entry to the transmembrane region of ABCG46.

Simulations were performed using an AlphaFold3 model embedded in a membrane environment, allowing detailed analysis of substrate access pathways (tunnels). Comparative simulations of the wild-type and mutant transporter revealed mutation-induced changes in the conformations of transmembrane helices, tunnel geometry, and thermodynamically unfavorable substrate transport to the central cavity through the tunnel. These structural alterations provide a mechanistic explanation, supported by the experimentally confirmed loss of transport activity in ABCG46. They highlight the critical role of the tunnel entrance in substrate gating and have implications for interdomain communication in ABCG transporters.

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46. Mechanistic Insights into Antibiotic Hydrolysis and Inhibition of β-Lactamases via Multiscale Simulations

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β-lactam antibiotics are widely used to treat bacterial infections and have saved numerous human lives. However, the effectiveness of β-lactam antibiotics is rapidly declining due to the emergence of antibiotic resistance, mainly driven by β-lactamases (BLs), enzymes produced by bacteria to hydrolyse the β-lactam ring, leading to inactivation of the drug.

Therefore, the development of strategies to combat antibiotic resistance caused by BLs is urgently needed.
Class D β-lactamases, in particular OXA-48-like enzymes, have emerged as a clinical concern due to their carbapenemase activity. Experimental data show that OXA-48 variants carrying mutations in the β5-β6 loop (residues 213-218 in OXA-48) differ significantly in hydrolytic activity towards imipenem (a carbapenem antibiotic) and in the inhibition potency of diazabicyclooctane (DBO) inhibitors.

Multiscale QM/MM simulations were first applied to investigate the molecular origin of differential enzyme activities toward imipenem. Reaction simulations using efficient semi-empirical QM/MM umbrella sampling simulations indicate that the deacylation of imipenem is most efficient when the deacylating water acts as a hydrogen bond donor to imipenem. Dynamics simulations show that the mutations in the β5-β6 loop subtly alter the active site hydrogen bond network. In OXA-48 and -517, Thr213 in the β5-β6 loop helps stabilize the hydrogen bond network when the deacylating water acts as a donor, as revealed by QM/MM potential energy calculations. This residue is absent in OXA-163 and -405, which explains the higher enzyme activity of OXA-48 and -517: QM/MM activation energies correlate perfectly with experimental kinetic data when assuming the relevant hydrogen bond network.
Next, QM/MM simulations were applied to the same enzyme variants with two DBO inhibitors, avibactam and nacubactam. The second step of DBO acylation was identified as rate-limiting using high-level QM/MM potential energy calculations. Umbrella sampling of the rate-limiting step then revealed a trend consistent with experimental data: nacubactam exhibits a lower covalent inhibition potency (higher energy barrier) than avibactam, resulting from the anti-catalytic electric field generated by nacubactam. Notably, an electrostatic repulsion between the positively charged tail of nacubactam and Arg214 in OXA-48 β5-β6 loop explains the large differences in nacubactam and avibactam potency here. Additionally, when taking into account non-covalent binding energies (estimated from MM MD simulations), our computational data accurately reproduce the relative order of experimental IC50 values available for three variants, and thus enables prediction of inhibitor potency for OXA-517. Overall, our work demonstrates how multiscale simulation can identify the detailed molecular basis for changes in hydrolytic and inhibitory activities of β-lactamase variants, offering detailed insights into how subtle changes in the active site alter the dynamics and reaction efficiencies related to antibiotic resistance.

47. Benchmarking AI Structure Prediction Tools for TCR-pHLA Drug Design

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T-cell receptors (TCRs) are an emerging class of cancer therapeutics, offering broad target recognition and the ability to eliminate malignant cells directly. T-cell-based biologics leverage affinity-enhanced TCRs to engage low-abundance antigens and promote CD3-mediated recruitment of endogenous T-cells for highly specific cytotoxic responses. As the development of these therapies expands, computational approaches are increasingly employed to predict TCR–target interactions, reducing experimental demands and accelerating discovery cycles. A key opportunity lies in applying deep-learning-based structure prediction models to streamline early-stage development. These tools support large-scale, accurate evaluation of binding modes, affinity, and off-target specificity—without the need for protein expression or crystallographic data.
This study systematically benchmarks several open-source models, functionally analogous to AlphaFold, for predicting TCR–pHLA complex structures. We assess their ability to reproduce canonical CDR loop topologies and intermolecular contacts, and to recapitulate experimentally measured affinity trends using computational scoring functions. The findings can inform the development of a robust, accessible in silico pipeline for TCR drug design. However, deep-learning based structure prediction remains a challenge for the highly variable loops involved in binding, indicating the importance to specifically tailor machine-learning tools for immuno-oncology applications.

48. Title N.A.

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Conventional chemotherapy has been widely applied in combating cancer over the past decades. Unfortunately, this treatment suffers from many drawbacks, mainly multiple drug resistance, non-specific targeting and adverse side effects [1]. The need to improve therapeutic targeting on malignant cells and suppress unwanted side effects leads to the development of systems that possess the ability to deliver specific chemotherapeutics to tumor sites. The design of drug delivery systems (DDSs), implemented within an active targeting context, relies on binding the chemotherapeutic agent to a vector molecule that steers its delivery and release to specific types of cells by recognizing their cellular components [2].

Folate receptor-α (FRα) is a membrane-anchored protein with high affinity for folate, which mediates the endocytic internalization, critical for DNA synthesis and cell proliferation. The expression of FRα is typically limited in normal tissues but the receptor is rather overexpressed on various types of neoplastic cells, such as ovarian, lung and breast, making it a suitable target for folate-based conjugates for targeted drug delivery [3].
Within the framework of active targeting, a set of promising folate-based conjugates are investigated for their implementation as vector constructs for receptor-specific delivery of the chemotherapeutic doxorubicin. This study aims to obtain molecular-level information on the structure and targeting behavior of this set of folate conjugates in physiological conditions through multi-step atomistic molecular dynamics simulations. Comparative structural analysis of the different conjugates in saline enables the identification of prospective spacer-linker combinations, as well as the key intramolecular interactions governing the structural flexibility of the complexes. Prominent π-stacking turns out to be the leading driving force for the conformation of the conjugates, thereby limiting the accessibility to the folate fragment. Optimization through modification or reduction of the spacer and linker fragments shows that functionalization of the spacer relieves this problem. The most promising candidates are to be tested against FRα as targeting drug delivery systems.

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49. Multisite λ Dynamics in Academic Drug Design Projects

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Over the last four years we have used multisite λ dynamics (MSλD) for calculating protein-ligand relative binding free energies in our academic projects. The projects involved three different targets of pharmaceutical relevance with varying but interconnected goals.

First, we explored the use of MSλD in retrospective and prospective affinity predictions for Human Neutrophil Elastase (HNE) and Proteinase 3 (PR3) respectively, both drug targets for chronic lung inflammation. Four different force field combinations were evaluated for retrospective predictions from which the hybrid CHARMM36m-OPLS-AA force field (FF) predicted highest correlation. The prospective predictions for PR3 with this hybrid FF revealed the polypharmacological potential of the existing HNE inhibitors.

Next, we proposed a λ-dynamics based methodology for accurately ranking alternate ligand binding poses. We tested the validity and predictive power of our approach using two pharmaceutically relevant targets (HNE and Leishmania major N-myristoyltransferase) and eight compounds from experimentally characterized congeneric series. For each target, our approach correctly ranked the known X-ray poses to be more favorable than alternative flipped poses.

Currently, we are applying MSλD and relative binding free energy calculations in a de-novo drug design setting for targeting the FabF enzyme, a novel target for new antibiotics.

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50. Structural modelling and binding affinity prediction of the Human PDZ-PBM interactome

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PDZ domains are involved in major cellular functions, such as the localization of cellular elements and the regulation of pathways. They do this by interacting with partner proteins PDZ binding motifs (PBM), which are C-terminus linear motifs. With 266 PDZ domains and up-to 5000 potential PBMs that can be found in the human proteome, the corresponding interactome could involve more than 1 million putative interactions. Not only endogenous proteins are interacting with PDZ domain, but also pathogens are hijacking this system to better navigate through the cell, making the understanding of the underlaying interacting network of great interest. New experimental methods, specialized in the detection of low affinity detection of interactions, are now deciphering the specificities of such PDZ-PBM transient complexes by gathering binding affinities for more than 400 PBMs over the 266 human PDZ domains, leading to more that 65 thousand experimental binding affinities.

While experimental data requires specialized setup and tedious work, in silico predictions together with the training of machine-learning models can bridge the gap between available binding affinities and the complete description of the interactome involved with the human PDZome. In this work, we first performed a systematic structural modelling of the Human PDZ-PBM interactome, generating an unprecedented proteome scale database composed of millions of complexes. Later described as graphs, and learned through graph convolutional neural network, with the DeepRank2 framework, we built both binary classification and binding affinity regression models, able to accurately profile any C-terminal peptide on the Human PDZome. Finally, we predicted the binding affinities for the entire human PDZ interactome. While waiting for experimental validation, we already backed confident predictions using available literature information by aggregating protein-protein interaction databases, Gene Ontology terms and reseach articles mined by large-language models.

The accurate prediction of the interactions involving the human PDZ domains will not only complement our understanding of the complexity to maintain homeostasis in the human cell

but also allow the deciphering of pathways used by pathogens to infect the cell and exploit its function, and therefore envision potential new therapeutic solutions.

51. Title N.A.

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Molecular recognition is a phenomenon central to many chemical and biological processes, such as enzyme catalysis and drug binding. However, molecular recognition involves a delicate balance of non-covalent interactions, which is often not trivial to disentangle. To this end, computational approaches can dissect molecular interactions and help guide their rational design and optimization. Here, we present a statistics-based algorithm (HydroSEA) that extracts optimal interatomic interaction sets from protein-ligand complexes contained in the Protein Data Bank (PDB). This information is used to reconstruct the probabilities of observing each amino acid in specific positions with respect to the ligand in the target structure binding pocket. This is achieved through three steps: (i) the starting ligand is divided into small chemical moieties using a fragmentation algorithm; (ii) each moiety is searched within the PDB, and the resulting structural data are voxelized and averaged to characterize the ideal chemical environment for that given fragment; (iii) using this analysis, a scoring grid is constructed for the target structure, indicating what amino acid mutation can generate better scores, thus improving ligand binding at the considered pocket. We validate the algorithm using a curated dataset of hydrolase enzyme mutants for which experimental binding affinities are available in literature. This analysis demonstrates that HydroSEA can efficiently classify amino acid mutations effects, thus supporting its application to enzyme design.

52. Insights into Ionic Liquid-Enzyme Interactions for Enhanced Multienzyme Catalysis

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Understanding how solvent microenvironments regulate enzyme cascades remains a major challenge in biocatalysis. In this work, computational and experimental approaches were combined to investigate how stoichiometrically engineered hydrated ionic liquids (ILs) modulate the structure, dynamics and catalytic efficiency of the glucose oxidase–horseradish peroxidase (GOx-HRP) cascade.

Computational analysis revealed that tuning the cholinium-to-anion stoichiometry generates pH-switchable IL environments, which significantly alter enzyme surface electrostatics and interaction patterns. Molecular docking identified preferential binding of IL anions near catalytically relevant regions of HRP and GOx, while molecular dynamics simulations revealed stabilization of dynamic regions surrounding the HRP active site. These simulations further suggested that specific IL compositions maintain catalytically competent conformations by modulating local flexibility and enzyme–enzyme interface interactions. Kinetic modeling and free-energy analysis demonstrated that the optimized IL environment reduces the transition-state free energy barrier and enhances catalytic efficiency of the cascade. Consistent with computational predictions, experimental studies showed up to 25-fold enhancement in cascade catalytic efficiency and increased thermal stability in hydrated cholinium phosphonoacetate systems. Additionally, thermodynamic stability curves and circular dichroism experiments confirmed improved enzyme stability under thermal stress.

Together, this integrated computational-experimental study reveals how ionic liquid composition can be used to engineer enzyme microenvironments, providing a framework for rational design of solvent systems that enhance multienzyme catalysis and stability.

53. Tunnels as Drug Targets: A New Paradigm in Drug Discovery for Proteins with Buried Active Sites ?

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Many enzymes possess buried active sites that are accessible through tunnels connecting the catalytic pocket to the protein surface. While these tunnels play a critical role in substrate transport and product release, they remain underexplored as targets for drug discovery. Targeting tunnels rather than catalytic pockets offers an alternative strategy to modulate protein function, particularly for proteins whose active sites are highly conserved, prone to resistance-conferring mutations, or require strong metal-chelating inhibitors that often suffer from poor selectivity.

In this work, this concept is investigated using UDP-3-O-acyl-N-acetylglucosamine deacetylase (LpxC), an essential enzyme in lipid A biosynthesis in Gram-negative bacteria, as a model system. Traditional LpxC inhibitors rely on zinc-chelating groups such as hydroxamates, which suffer from poor selectivity and toxicity due to interactions with human metalloproteins. To explore alternative binding regions, protein tunnels in LpxC were mapped using Caver on molecular dynamics (MD) simulations of the zinc-bound enzyme, enabling identification of dynamically accessible transport pathways beyond those observed in static structures. This analysis revealed an alternative tunnel distinct from the canonical tunnel and from regions occupied by known inhibitors. To evaluate the potential of these tunnels for ligand binding, a library of FDA-approved drugs was screened using CaverDock on tunnel ensembles derived from MD snapshots. CaverDock simulations provided energy profiles of ligand migration along the tunnels. Top ranked candidates were subsequently rescored using consensus scoring and were subjected to molecular dynamics simulations to evaluate their stability within the tunnel.

This workflow resulted in the identification of ten compounds exhibiting consistent low-energy profiles across multiple tunnel conformations and stable occupancy within the tunnel region. Notably, none of the identified compounds contain classical zinc-chelating groups. These compounds will be experimentally evaluated against bacterial strains, followed by LpxC inhibition assays. Beyond the specific case of LpxC, this study represents one of the first systematic efforts to directly utilize protein tunnels as primary targets for virtual screening. The results highlight the potential of tunnel-centric strategies for identifying inhibitors against proteins with buried active sites, opening new opportunities for structure-based design.

54. Molecular Insights into Crystallophore-Assisted Protein Nucleation from All-Atom Simulations

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Crystallophores are lanthanide-based complexes that act as molecular glues and efficient nucleating agents, promoting protein crystallization, with terbium-based variants referred to as Xo4s. As the formation of well-diffracting protein crystals is a critical prerequisite for structure determination, understanding the molecular mechanisms underlying Xo4-assisted nucleation is of considerable interest. Previous studies have revealed extensive networks of supramolecular interactions between proteins and Xo4s, highlighting their versatile binding behavior at protein surfaces. Efforts to further enhance nucleation efficiency and crystal detection have also led to the development of a new variant, the Imaging-crystallophore (Im-Xo4), which combines nucleating capability with intrinsic imaging properties.

While these interaction networks highlight binding versatility, they do not fully explain why different Xo4 variants display varying crystallization efficiencies. In this study, we employ all-atom molecular dynamics (MD) simulations to characterize binding modes, residue-level interactions, and dynamical changes induced upon Xo4 association. Particular emphasis is placed on identifying protein surface regions preferentially involved in binding and assessing their impact on protein conformatAnalysis of the resulting conformational ensembles

indicates that Xo4 binding stabilizes persistent intermolecular contacts and reduces conformational flexibility, as evidenced by decreased residual fluctuations. This behavior suggests a narrowing of accessible conformational states, reflecting a reduction in configurational entropy.

Together, these results support a mechanistic picture in which Xo4s promote nucleation by coupling specific intermolecular interactions with entropy-driven confinement of protein conformational ensembles. This work provides molecular-level insight into crystallophore-assisted protein crystallization and highlights the utility of MD simulations in dissecting nucleation mechanisms.

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55. Holliday junctions (Title N.A.)

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Holliday junctions (HJs) are noncanonical four-way DNA structures that play central roles in homologous recombination, DNA repair, and the design of DNA-based nanomaterials. Their ability to adopt multiple conformational states makes them particularly attractive as programmable structural motifs, but also complicates a detailed understanding of their structural dynamics and sequence-dependent behavior. Elucidating the mechanisms governing HJ conformational transitions and their consequences for supramolecular assembly remains essential for both biological insight and the rational design of DNA nanostructures. In our work, we combine molecular dynamics (MD) simulations with insights from crystallographic studies of immobile Holliday junctions to investigate how junction structure, dynamics, and sequence composition influence higher-order DNA assemblies. These computational insights complement experimental studies of immobile HJ sequences used as central nodes in self-assembling DNA crystals. Crystallographic screening of all 36 immobile junction sequences demonstrates that, contrary to previous

assumptions, the majority are capable of forming crystalline lattices and the nucleotides adjacent to the junction significantly influence crystallization outcomes. A subset of sequences fails to crystallize entirely. MD simulations indicate that these "fatal" junctions consistently lack two discrete ion-binding sites that appear crucial for stabilizing crystal contacts. We have subsequently used MD simulations to characterize spontaneous conformational transitions between open and closed HJ states. These simulations reveal complex rearrangement pathways involving previously unrecognized “half-closed” intermediates that bridge the canonical conformations. Enhanced sampling methods were further employed to map the free-energy landscape governing these transitions. Because commonly used force fields tend to overstabilize the stacked junction state, we implemented a system-specific correction that enables the observation of spontaneous opening–closing transitions in unbiased simulations, allowing a more realistic description of junction dynamics. Together, these results highlight the intricate interplay between sequence, conformational dynamics, and ionic interactions in determining the structural behavior of Holliday junctions and their ability to form ordered assemblies. Understanding these relationships provides a framework for improving computational models of branched DNA and offers practical guidance for the rational design of DNA crystals and other junction-based nanostructures.

56. The Erlangen National High-Performance Computing Center

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The Erlangen National High-Performance Computing Center (NHR@FAU) at FAU Erlangen-Nürnberg was established in 2021 as a national center for HPC at German universities. Together with eight other institutions, it forms the NHR-Alliance. NHR@FAU operates large-scale HPC systems and provides HPC services, related user support, and HPC training to members of German universities.

A strong focus of NHR@FAU lies on atomistic simulations and it also provides tailored hardware solutions in this area. As a key component of the NHR program, it offers exceptional competence and conducts extensive research in the field of atomistic

simulations of molecular structures, with broad applications in chemistry, life sciences, materials science, and physics.

With bundled atomistic structure simulation expertise, NHR@FAU helps users to select and use atomistic simulation methods in an HPC environment and actively accompanies and coordinates the development of high-performance simulation codes. An interdisciplinary approach promises not only synergy effects, e.g., through the exchange and joint development of simulation and evaluation tools, but in particular a cross-fertilization of materials and life sciences, which often use the same or similar simulation techniques.

The HPC research activities at NHR@FAU focus on performance engineering and modelling, performance tools, and research software engineering. NHR@FAU investigates and further develops hardware-efficient building blocks, programming concepts, and numerical algorithms for scalable, efficient, and robust iterative sparse matrix applications and stencil-based solvers on large-scale HPC systems.

A further core project is the education and lifelong training of scientists and engineers. The close cooperation among theory, simulation, and experiment, which has a long tradition in Erlangen, ensures that the training is not aimed specifically at modelers, but also made available to experimental colleagues. This is of particular importance in the light of increasing digitalization in science. NHR@FAU makes an essential contribution to the key technologies of scientific computing and scientific software development through the sustained concentration of methodological competence in both the application and development of computer codes and their hardware-related optimization.

57. Allosteric regulation in the kinesin-5 motor domain investigated by molecular dynamics simulations across 4 catalytic states

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Human kinesin-5 is a dumbbell-shaped tetrameric protein regulating the formation of the bipolar spindle during cell mitosis. Each monomer features a motor domain, whose binding and unbinding to tubulin is regulated by sequential ATP hydrolysis. This domain is also a primary oncotarget for several highly specific antimitotic agents currently in clinical trials: most of these, including drug filanesib, target an allosteric pocket conformed by Loop-5 (L5) and helices α2 and α3. In this communication [1], we report an investigation on how filanesib can disrupt normal allosteric regulation in kinesin-5. After conducting microsecond-long Molecular Dynamics simulations of the ATP-bound, ADP-bound, apo, and ADP- and filanesib-bound catalytic states, we apply two approaches—distance fluctuation analysis [2] and the Shortest Path Map [3]—to identify and compare in detail their different allosteric fingerprints. Simulations confirm that filanesib affects domains responsible for microtubule binding, and stabilises the kinesin-5 – ADP interaction; remarkably, we could show that this is partly due to L5 being locked into a conformationally restrained state that is typical of the apo and ATP-bound forms (both highly affine to nucleotide binding). These findings were not immediately inferable from previous literature or available crystal structures alone, and we believe they could guide the development of smarter allosteric kinesin-5 inhibitors, as well as rationalising the insurgency of drug-resistant mutants.

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58. Multiscale cell correlation (Title N.A.)

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As non-covalent association processes are ubiquitous in biology, their quantification is crucial. One approach for calculating binding free energies consists of energy-entropy

methods. The energy of a system gives information concerning the strength of molecular interactions and can be easily computed, while entropy relates to dynamics within the system, but is often overlooked and more difficult to calculate in a computationally efficient and accurate manner. However, assessing this term is also important for understanding these processes as protein and solvent dynamics have been established to play a significant role in governing whether binding occurs. Multiscale cell correlation (MCC) yields the entropy of a system by discretizing configuration space into different length scales, as well as vibrational and topographical terms, thus giving rise to lower order terms which are easier to compute. This approach allows for detailed insights into contributions to entropy changes occurring upon binding, as well as for scalability and fast convergence. Another advantage of this method is that it treats all molecules in a system equivalently and hence, can be used for a complete analysis of the system, including both solutes and solvent. [1,2,3] The thermodynamics of the streptavidin-biotin system, widely studied due to its very high binding affinity, as well as those of two protein-protein complexes binding to small molecule antagonists have been studied. MCC has allowed a detailed breakdown of changes in entropy terms of the protein-protein complex, ligands and solvation water molecules. Furthermore, the different length scales have allowed for assessing local entropy changes and gaining insights into residues' individual contributions to binding.

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59. Understanding pKa shifts through Local Environment Analysis

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Protonation states modulate biological function. Weak acids exchange protons with the environment in an equilibrium described by the acid dissociation constant (pKa).

Unfortunately, pKa values are highly sensitive to environmental factors (such as pH and ionic strength) and neighboring molecules (such as residues, pigments, and lipids), which can cause significant shifts from the intrinsic pKa values measured in aqueous media. Moreover, changes in protonation state can trigger substantial conformational changes, further increasing the complexity of determining pKa values

Because pKa corresponds to a macroscopic ensemble of conformations, computational methods must be able to sample the conformational space accessible to the biological system across different pH conditions. One way to achieve this is to perform all-atom constant-pH molecular dynamics (CpHMD) simulations. Specifically, CpHMD explores the coupled motions of titratable groups in both deprotonated and protonated states at different pH values, ultimately yielding a titration curve from which a CpHMD pKa value can be obtained.

In this work, we aim to deepen our understanding of the relationship between pKa shifts and the local environment. By decomposing CpHMD titration curves into several ideal titration curves, we identify key environmental factors that drive pKa shifts. This is especially important, as it may advance data-driven pKa prediction methods and enable the development of new property-driven enhanced-sampling methods.

60. Nucleosome Mechanics Shape the Energy Landscape of DNA Unwrapping

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In eukaryotic cellular nuclei, DNA is tightly packaged into chromatin. Chromatin's basic unit, the nucleosome, is composed of DNA wound around a protein histone core. The spatiotemporal organisation of chromatin packaging is a key regulator of gene expression, but the physicochemical mechanisms that govern this packaging remain poorly understood.

In this study, we focus on force-induced nucleosome unwrapping: the process by which the DNA-histone complex is disassembled upon applied force. Using enhanced coarse-grained molecular dynamics simulations of over 40 distinct nucleosome systems, we quantitatively

characterise the components that contribute to the energy landscape of an unwrapping curve: electrostatic, thermal, and mechanical energies. Through a physicochemical lens, we provide insight into the role of histone tails, sequence variations, and post-translational modifications during the unwrapping process. Finally, we quantify the biochemical features that govern the asymmetric response of the unwrapping process under external forces, with remarkable agreement with experimental data.

Our results reveal that the mechanical properties of DNA alone, such as its flexibility, can modulate nucleosome unwrapping. Our data also highlight the key role of PTMs as regulators of force-induced unwrapping profiles. Our findings provide a detailed description of the molecular interactions that contribute to nucleosome stability and delineate a multi-scale computational framework to probe the effects of DNA mechanical properties on other mechanisms of chromatin organisation.

61. From Docking to Molecular Dynamics of Metabolite Binding in Antibody Recognition Sites

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Antibodies are viewed as cofactor-free recognition proteins, yet crystal structures have revealed that small molecules can occupy antibody binding pockets [1]. A notable example is the 'yellow antibody' IgG(GAR), whose fragment antigen-binding (Fab) region contains a tightly bound riboflavin molecule in the antigen-binding site, motivating the broader question of whether abundant serum metabolites can associate with antibodies and modulate antigen binding [2, 3]. Here, we present a computational workflow to investigate the binding of physiologically relevant small molecules (e.g. riboflavin, ascorbate, urate, glucose) to antibody structures and to assess where such binding is most likely to occur and how it influences antigen recognition. Our study comprises three stages:

1. Detailed interaction analysis of riboflavin in the original yellow antibody.

2. Blind docking and rescoring of selected metabolites to antibody fragments to generate binding-site maps.

3. Structural mining of antibody structures in the Protein Data Bank to identify recurrent small-molecule binding poses across diverse Fv/Fab frameworks.

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62. Ostia: A GPU-Accelerated Blind Docking Engine Built on the AMBER Force Field Framework

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Ostia is a rigid-body molecular docking engine that performs fully blind protein surface scanning using GPU acceleration. It reads standard AMBER topology and coordinate files (.prmtop/.inpcrd) directly, supporting any AMBER force field combination—e.g. ff14SB/ff19SB for proteins, GAFF/GAFF2 for ligands, and MCPB.py-derived parameters for metalloenzymes. This native AMBER compatibility ensures that docked poses can be carried forward into molecular dynamics (MD), ligand Gaussian accelerated MD (LiGaMD), or free energy perturbation calculations without re-parametrization.

The search places the ligand on a translation grid covering the entire protein surface and evaluates a tunable number of uniformly distributed rotations (Shoemake sampling on SO(3)) at each grid point, yielding hundreds of millions to billions of candidate poses per system, all scored on the GPU through custom CUDA kernels. The scoring function is fully configurable, offering standard 12–6 or softened 4–8 Lennard-Jones potentials, constant or distance-dependent dielectric models, directional 12–10 hydrogen bonds, and an optional Eisenberg–McLachlan desolvation term. Spatial binning preserves the top candidates from

every surface region, which then undergo rigid-body energy minimization and RMSD clustering.

So far, validation on 37 protein–ligand complexes spanning the Astex Diverse Set, cross-validated supplementary targets, multi-binding-site systems, and large proteins (3 013–16 472 atoms) shows that Ostia identifies the crystallographic binding pocket in 100% of cases (37/37) in fully blind mode, with both scoring variants (with and without desolvation) achieving this result. The median runtime is approximately 50 minutes per system on a single NVIDIA RTX 4060 consumer GPU. By unifying docking and MD under the AMBER framework, Ostia enables a direct docking-to-dynamics pipeline suited for binding site discovery, pose refinement, and downstream free energy estimation.

63. Rationales for the choice of metals for super-reduced biological metal centers: cobalt in cobalamin vs. nickel in F430

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Reported here are DFT calculations on porphyrin, corrin, and corphin complexes with formally metal (I) states of cobalt, rhodium, iron, manganese, or nickel, complemented by QM/MM calculations on enzyme models, and exploring the available spin states, with the goal to understand factors controlling the availably of super-reduced metal(I) states on such biologically-relevant systems. In the porphyrin systems, the preferred spin states offer no clear case of metal(I) electronic description – but rather metal(II) coupled with porphyrin anion radicals; this is in line with the fact that porphyrin is not used in biology for mechanisms/reactions entailing super-reduced metals. The corrin, somewhat less conjugated than the porphyrin, affords a clear-cut ground-state S=0 Co(I) complex, with essentially no delocalization of the extra electron onto the macrocycle. None of the neighbors of cobalt in the 3rd period afford clear super-reduced ground states with corrin – in line with cobalamin's choice of metal in biology, cobalt. The least conjugated system, the corphin (as seen in F430), now affords a metal(I) state even in the case of Ni. Moreover, in fact, only Ni affords a ground state with clear super-reduced character – in line with F430's choice of metal in vivo.

64. RAG2 Acidic Hinge: Conformational Sampling and Functional Implications

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RAG1 and RAG2 constitute the RAG recombinase tetramer, a molecular machinery essential to V(D)J recombination and the generation of antigen receptor diversity in T and B lymphocytes. Within this complex, RAG1 is responsible for DNA recognition and cleavage, while RAG2 serves as a regulatory cofactor.

Current evolutionary models place the RAG recombinase as a domesticated transposon. Related mobile elements have notable structural resemblance to RAG, yet lack several components, most notably the RAG2 acidic hinge, which has emerged as a key regulatory element safeguarding genomic integrity through suppression of transposition. [Martin et al. (2020) Mob DNA; Martin et al. (2023), Mol Biol Evol; Zhang et al. (2012), Nature]. Despite its functional importance, the intrinsic flexibility of this region has precluded its mapping in resolved structures and how this region inhibits transposition represents a critical unresolved question.

To investigate the potential functions of the acidic hinge and elucidate how it may inhibit transposition, a RAG tetramer model incorporating the acidic hinge was constructed and subjected to molecular dynamics simulations using OpenMM, with the aim of characterizing the conformational ensemble adopted by this intrinsically disordered region in the context of the tetramer.

Cluster analysis revealed preferential localization of the acidic hinge above or along the edges of the RAG1 groove — the site responsible for target DNA capture. This is consistent with the current mechanistic model of transposition inhibition: occupancy of the groove by the acidic hinge would sterically obstruct target DNA from descending into the active site and adopting the U-shaped conformation required for transposition. A further conformational cluster of interest positions the acidic hinge at the RAG1/RAG2 lateral interface. The RAG complex is not a rigid assembly — it undergoes conformational rearrangements throughout the catalytic cycle. Following DNA cleavage, the complex remains bound to the cleaved DNA ends, and target DNA capture requires the two subunits to flex relative to one another, widening access to the catalytic site. Insertion of the acidic

hinge at this interface would introduce an allosteric mechanism, restricting this opening motion and trapping the complex in a closed or semi-closed state in which transposition cannot proceed — even though the catalytic site itself remains structurally intact and functional.

Taken together, these findings support a model in which the acidic hinge acts as a dynamic regulatory element that inhibits transposition through multiple, potentially concurrent mechanisms. Several of the insights emerging from this analysis are currently under experimental validation, while others are being considered for future investigation.

65. Decoding antibody framework regions: sequence, structural fingerprints, and modeling foundations

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Antibody aggregation is a persistent liability in therapeutic development, driven in part by insufficient scaffold stability. Current AI/MD aggregation predictors overlook the contribution of framework regions (FR), while full-antibody modeling tools — anchored on hypervariable complementarity-determining regions (CDRs) and hinges — often introduce FR inaccuracies that can erode overall stability. Both problems call for a systematic, data-driven account of FR sequence and structural space. We developed a pipeline extracting information on >3,000 human and mouse immunoglobulin structures (cryo-EM, X-ray) from SAbDab, stratified by interaction state, chain, and domain (Fv, Fab, Fc, full Ig). AHo-aligned, deduplicated, outlier-filtered FR sequences were profiled (PSSM heatmaps) and clustered (30–90%, MMseqs2), visualized as dendrograms, distance heatmaps, and network graphs. Secondary structure assignment yields contact maps and hydrogen-bond fingerprints (binary and donor-acceptor coded) per entry and similarity clustering; bond-angle-torsion analyses and cross-peptide angle computation provide backbone geometry distribution, which was followed by similarity clustering. A unified structural registry consolidates all features and yields a maximally diverse representative set across sequence, secondary structure, and backbone geometry. Structure prediction benchmarking has been initiated with RaptorX-Single, ABodyBuilder-2, and Ibex, with ABodyBuilder-3 and AlphaFold-3 to follow.

This empirical characterization of FR sequence and structural space, together with a curated multi-feature representative set, provides a foundation for principled cluster-based alignment and a robust modeling workflow for full antibodies and antibody-antigen complexes.

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66. Impact of Molecular Size, Concentration, and Temperature on Lignin Derivatives' Skin Membrane Interactions

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Lignin, a widespread natural biopolymer, holds significant promise for the development of sustainable and safe healthcare products. In this study, we employed molecular dynamics simulations and free energy calculations to investigate how lignin derivatives interact with lipid membranes that mimic human skin. Focusing on a synthetic lignin derivative, our research reveals that molecular size, concentration, and temperature critically influence the insertion, interaction dynamics, and localization of lignin compounds within membrane models. These factors determine key behaviors such as rapid membrane penetration, hydrogen bonding, aggregation, and surface adherence. Our insights elucidate the molecular mechanisms of lignin derivative interactions with skin-like membranes, emphasizing the crucial roles of molecular size and concentration in optimizing these compounds for bio-based skincare and transdermal therapeutic applications.

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67. NEGF-DFT Direct Evaluation of Intraprotein Electron Transfer Paths within Myoglobin

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Electron transport within proteins is typically rationalised through Marcus-Theory frameworks that compare initial and final electronic states. We extend the Non-Equilibrium Green's Function / Density Functional Theory (NEGF-DFT) formalism — established in single-molecule electronics and previously applied to enzyme active sites — to whole-protein scale, mapping electron delivery from the antioxidant caffeate to the catalytic iron of myoglobin. Three docking-derived routes are decomposed into atom-resolved paths and ranked by step-wise conductance, revealing that proximity to the heme, not residue identity, dictates path efficiency.

68. Coarse-grained molecular dynamics simulation of liquid-liquid phase separation of intrinsically disordered proteins

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Liquid-Liquid Phase Separation (LLPS) is a spontaneous process in which molecules within a solution undergo phase separation, leading to the formation of distinct liquid phases and the emergence of droplets or condensates. These phenomena are particularly noteworthy in eukaryotic cells, where certain condensates, devoid of a lipid membrane and termed membraneless organelles, play diverse roles. Notable examples include nucleoli, Stress granules, etc[1,2].

Intrinsically Disordered Proteins (IDPs), with highly dynamic structures and the ability to facilitate multivalent interactions, are closely associated with phase transitions and condensate formation [3]. However, the intricate interplay of intramolecular and

intermolecular interactions can be modulated by environmental factors such as temperature, salts concentrations, pH, etc.

Thermoresponsive IDP-based polymers, exhibiting reversible changes in solubility based on temperature, provide a promising avenue to address certain disease challenges. The temperature-transferable Coarse-Grained model, specifically optimized in this study, proves effective in reproducing both Upper Critical Solution Temperature and Lower Critical Solution Temperature behaviors of IDP LLPS [4].

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69. Computational methods applied to understanding structure-function relationships in sterol homeostasis in the Niemann-Pick Type C protein

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Niemann–Pick type C (NPC) disease is a fatal autosomal recessive neurodegenerative disorder caused by mutations in the NPC1 or NPC2 proteins. Both proteins are essential for intracellular cholesterol trafficking, and mutation of either can lead to the accumulation of cholesterol and other lipids in late endosomes and lysosomes. Nonetheless, the molecular basis of sterol transport and its disruption by mutation in NPC1 or NPC2 is still not fully understood, and many disease-associated NPC variants remain uncharacterised. Addressing this gap is essential for understanding the structure-function relationship in NPC1 and NPC2 and ultimately, for designing therapeutic strategies.The 4.00 Å all-atom structure of the human NPC1-NPC2 complex (PDB ID: 6W5V) provides a structural framework for studying disease-associated variants in cholesterol trafficking. Using

computational modelling and molecular dynamics simulations, we analyse selected NPC2 variants to define their structural and mechanistic roles in cholesterol transfer. This work is now being extended to different lysosome-relevant bilayer compositions to determine how the membrane environment influences protein interactions and cholesterol egress. In parallel, machine learning approaches, like Gaussian process models, are being applied to NPC1 using experimental data and features derived from pre-trained protein large language models to identify uncharacterised pathogenic variants. Selected NPC1 variants will subsequently be investigated by simulation and experiment to determine how these mutations alter molecular interactions and perturb cholesterol transfer. Together, these complementary approaches provide an integrated mechanistic framework for understanding how mutations in NPC1 and NPC2 disrupt cholesterol egress and cause NPC disease.

70. Insights into the Binding of Carbon Monoxide to V-Nitrogenase: A QM/MM Investigation

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Nitrogenases are enzymes that reduce atmospheric nitrogen (N_2) into bioavailable ammonia (NH_3), thereby sustaining the global nitrogen cycle and supporting life on Earth. Their ability to activate and reduce the highly stable N≡N triple bond under ambient conditions distinguishes them from the industrial Haber–Bosch process, which requires high temperatures and pressures. Nitrogenases are classified into three major types based on the heterometal present in their active-site cofactor: molybdenum nitrogenase (Mo-nitrogenase), vanadium nitrogenase (V-nitrogenase), and iron-only nitrogenase (Fe-nitrogenase), containing the FeMo-cofactor (FeMoco), FeV-cofactor (FeVco), and FeFe-cofactor (FeFeco), respectively. Each class exhibits distinct catalytic properties and substrate reactivities.

Among these, Mo-nitrogenase is the most extensively studied and has provided the foundation for understanding biological nitrogen fixation. In recent years, however, increasing attention has focused on V-nitrogenase because of its unique ability to reduce carbon monoxide (CO) predominantly into ethylene. A particularly intriguing feature of V-

nitrogenase is its ability to bind CO in the resting state, defined as the enzyme's state prior to catalytic turnover. Importantly, the bound CO species remains catalytically competent and can undergo further reduction under turnover conditions, suggesting that this binding mode represents a catalytically relevant intermediate. This behaviour sharply contrasts with Mo-nitrogenase, where CO binds only under turnover conditions and primarily acts as an inhibitor rather than a substrate.

The recent availability of high-resolution crystal structures of V-nitrogenase provides an excellent platform for computational investigations into its catalytic mechanism. In this work, we employ hybrid quantum mechanics/molecular mechanics (QM/MM) calculations combined with the broken-symmetry (BS) approach to investigate the electronic structure of the FeVco active site. The aim of this study is to develop an atomistic understanding of the electronic and magnetic factors governing CO binding and reduction. Such insights could contribute to the future design of bio-inspired catalytic systems for sustainable fuel production and carbon conversion technologies.

71. Connecting Lipopolysaccharide Regulation and Lipid A Dephosphorylation: Structural Insights into the LapB–YejM Complex and LPS-YejM interaction

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Lipopolysaccharide (LPS) homeostasis is essential for Gram-negative bacterial viability and antibiotic resistance. While LapB is a recognized regulator of LPS biosynthesis and YejM has been implicated in Lipid A dephosphorylation, the molecular relationship between these proteins remains unclear.

To investigate a potential connection between LapB and YejM, we constructed a structural model of a LapB–YejM complex using available experimental structures, structural overlap analysis, protein–electrondensity docking, and completion of unresolved regions. The resulting assembly was analyzed for interface compatibility and substrate accessibility. To explore opportunities for chemical modulation of the YejM-LPS interaction, virtual screening

of Enamine in-stock collection compound-libraries was performed against selected sites of the YejM-LapB complex, selecting promising candidates for experimental testing.

Our experimental results and integrative structural model suggest a functional association between YejM phosphatase activity and LPS binding. The identified small molecules represent promising chemical probes for experimental inhibition of the LPS–YejM interaction and may contribute to future antibacterial drug discovery efforts.

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72. A Multiscale Simulation Workflow for Solvated Molecular Systems in ChemShell: Application to Orthosilicic Acid Condensation

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We present a guided workflow in the ChemShell package for multiscale simulation of molecular systems in homogeneous solution. The workflow packs solute and solvent molecules of desired molar ratios into a simulation box, performs classical MD equilibration using DL_FIELD and DL_POLY following a 3-step protocol, and extracts snapshot structures for subsequent QM/MM calculations. We validated the workflow by modelling orthosilicic acid in an acidic water–methanol mixed solvent at pH <1 and 333.15 K. The equilibrated system of 20205 atoms reproduces key structural observables in close agreement with experimental data. A QM/MM model of 8026 atoms is constructed and a local reactant minimum is located at the B3LYP-D3/6-31+G**/OPLS2005 level of theory, revealing two orthosilicic acid monomers at a distance favourable for nucleophilic condensation, bridged by an intricate hydrogen-bond network involving the solvent and hydronium. Well-

tempered QM/MM metadynamics at the GFN2-xTB/OPLS2005 level yields a dimerization free energy barrier of 15.6 kcal/mol (65.3 kJ/mol), in reasonable agreement with the experimental activation energies of 50–59 kJ/mol (12–14 kcal/mol) reported for silica condensation kinetics, and reveals a stepwise oligomerization mechanism. The results demonstrate that the workflow provides an effective route from system preparation through to QM/MM optimisation and free energy calculations for chemically complex solution environments.

73. Spectroscopic Evaluation of the Interaction of Myoglobin with Biomedically Relevant Compounds

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Reported here is an investigation of the redox behavior of myoglobin (Mb) in the presence of several bioactive compounds with biomedical relevance. These include the well-known Pt(II) drug, cisplatin, oxoplatin (a Pt(IV) compound representative for a newer-generation drugs based on platinum), capric acid, fenbufen, plumbagin and fisetin. These molecules were selected based on their structural diversity and documented biological effects, such as anti-inflammatory, chemotherapeutic, or antioxidant properties. The study aimed to evaluate how these compounds affect the structural and redox properties of Mb, particularly in terms of their capacity to bind to the protein and modulate its oxidation state. Spectroscopic methods (UV-Vis absorption, fluorescence, and NMR) and docking calculations were used to monitor binding and modifications of the redox state of the Mb in reactions such as autoxidation, nitrite-induced autoxidation, and peroxide-induced damage. These experiments are designed to parallel previous findings with similar experiments on hemoglobin with small molecules of biomedical/therapeutic relevance[1-4]. While some compounds induced minimal or no spectral changes - suggesting limited interaction or redox impact, others displayed distinct redox effects, with plumbagin promoting oxidation while fisetin exerted a stabilizing, protective influence on the oxygenated form of myoglobin. These findings suggest that plumbagin may act as pro-oxidant by promoting myoglobin oxidation, whereas fisetin interacts with Mb in a manner that stabilizes the

oxygenated form and mitigates oxidative conversion. The observed variability highlights the complex nature of small molecule-protein interactions and underlines the importance of assessing individual compound behavior in biochemical systems.

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74. To be added

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75. When Different Solutions Fit the Same Spectrum: Insights on Structural Ambiguity from Multi-Spectroscopic Inverse Problem

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We are living through a renaissance in molecular spectroscopy because advanced experimental techniques, DFT calculations, and powerful computational resources have become widely accessible. As a consequence, structure determination increasingly relies on matching experimental spectra with DFT-simulated spectra. Yet, for flexible molecules, multiple distinct conformational ensembles may reproduce the same experimental spectrum equally well, highlighting the finite structural resolution of spectroscopic techniques.

To investigate this problem, we studied the flexible molecule citronellol using an inverse-analysis framework in which DFT-derived conformer populations were optimized to reproduce experimental VCD, IR, ROA, and Raman spectra. Genetic Algorithms (GA), Particle Swarm Optimization (PSO), and Pattern Search optimisation techniques were employed to investigate the multiplicity of solutions compatible with the experimental data.

When each spectroscopic technique was considered individually, multiple distinct solutions reproduced the experimental data with comparable quality. Nevertheless, all optimization methods converged toward similar regions of the solution space. In contrast, simultaneous fitting of multiple spectroscopic observables led to substantially greater agreement between the independently optimized solutions.

These results demonstrate that spectral agreement alone does not necessarily imply a unique structural solution. More importantly, they suggest that combining complementary spectroscopic observables increases the effective structural resolution of the inverse problem, thereby reducing structural ambiguity and improving the reliability of spectroscopic structure determination.