Advanced Physical and Computational Techniques to Investigate Protein Dynamics

723. WE-Heraeus-Seminar

26 – 28 April 2021
Online via MeetAnyway

WILHELM UND ELSE
HERAEUS-STIFTUNG

Introduction

The Wilhelm und Else Heraeus-Stiftung is a private foundation that supports research and education in science with an emphasis on physics. It is recognized as Germany's most important private institution funding physics. Some of the activities of the foundation are carried out in close cooperation with the German Physical Society (Deutsche Physikalische Gesellschaft). For detailed information see https://www.we-heraeus-stiftung.de

Aims and scope of the 723. WE-Heraeus-Seminar:

The remarkable advances in physical and computational techniques over the past decade have revolutionized our understanding of the conformational dynamics of macromolecules. As such, our focus is to tightly integrate the various theoretical and experimental approaches currently employed in the field, combining their inherent capabilities and overcoming their specific limitations in order to arrive at a more unified concept of protein dynamics and function.

With this seminar we aim at providing a platform for scientists from diverse experimental and theoretical fields to discuss the most recent scientific discoveries and novel technologies under development, as well as the most outstanding challenges in the field. The seminar will include more than twenty invited speakers covering the following areas:

- X-ray crystallography and free electron laser for probing dynamics
- Magnetic resonance techniques
- Fluorescence and electron microscopy
- Free energy landscapes and protein dynamics
- Multi-scale dynamics and Markov models
- Combined interdisciplinary approaches to overcome the spatiotemporal gap in protein dynamics.

The main goals are to improve interaction and mutual understanding between the different areas of computational simulations and experimentation and to provide an inspiring environment for sparking innovation and establishing new collaborations.

The best four posters will be awarded prizes and will be selected for an oral presentation

Introduction

Scientific Organizers:

Prof. Christian Freund	Freie Universität Berlin, Germany E-mail: chfreund@zedat.fu-berlin.de
Prof. Frank Noé	Freie Universität Berlin, Germany E-mail: frank.noe@fu-berlin.de

Seminar Coordinator:

Dr. Esam Abualrous	Freie Universität Berlin, Germany E-mail: e.abualrous@fu-berlin.de

Administrative Organization:

Dr. Stefan Jorda Elisabeth Nowotka	Wilhelm und Else Heraeus-Stiftung Postfach 15 53 63405 Hanau, Germany Phone +49 6181 92325-12 Fax +49 6181 92325-15 E-mail nowotka@we-heraeus-stiftung.de Internet: www.we-heraeus-stiftung.de

Program

Monday, 26 April 2021

08:30 – 09:00	**Technical check**	
09:00 – 09:30	Christian Freund Frank Noé	**Welcome**
	Stefan Jorda	**About the Wilhelm and Else-Heraeus Foundation**
Session I:	*NMR methods for probing dynamics*	
09:30 – 10:15	Christian Freund	**Dynamic aspects of antigen presentation by MHC class II molecules**
10:15 – 11:00	Michael Sattler	**Combining NMR and integrative structural biology to study dynamics and allostery in protein complexes**
11:00 – 11:45	Birthe B. Kragelund	**Dynamic complexes of intrinsically disordered proteins by NMR spectroscopy**
11:45 – 12:30	*LUNCH BREAK*	
12:30-13:30	*Cultural Event: Virtual Tour in Museum Barberini*	
13:30-14:00	*COFFEE BREAK*	
14:00 – 16:00	**Poster session I**	
16:00 – 16:30	*COFFEE BREAK*	

Program

Monday, 26 April 2021

16:30 – 17:15	Nikolaos Sgourakis	**Dynamics of MHC-I molecules in the antigen processing and presentation pathway**
17:15 – 18:00	Lewis E Kay	**The important role of dynamics in the function and misfunction of molecular machines**
18:00 – 18:45	Julie D. Forman-Kay	**NMR insights into phase separation of a CAPRIN1 intrinsically disordered protein region**
18:45 – 19:30	Roundtable discussion I: **Perspectives of NMR**	

Program

Tuesday, 27 April 2021

Session II: *Molecular dynamics simulation and free energy landscapes*

09:00 – 09:45	Frank Noé	**Exploring protein dynamics with Markov modeling and deep learning**
09:45 – 10:30	Kresten Lindorff-Larsen	**Biophysical experiments and biomolecular simulations: A perfect match?**
10:30 – 11:00	*COFFEE BREAK*	
11:00 – 11:45	De Fabritiis	**Neural networks potentials for molecular simulations**
11:45 – 12:30	Cecilia Clementi	**Designing molecular models by machine learning and experimental data**
12:30 – 13:30	*LUNCH BREAK*	
13:30 – 14:30	*Cultural event: Virtual Musical Performance*	
14:30 – 16:30	**Poster session II**	
16:00 – 16:30	*COFFEE BREAK*	

Program

Tuesday, 27 April 2021

16:30 – 17:15	Carlo Camilloni	**Modelling biomolecules structure and dynamics by combining small-angle X-ray scattering and computer simulations**
17:15 – 18:00	Bert de Groot	**The molecular dynamics of potassium channel gating**
18:00 – 18:45	Rommie Amaro	**A glycan gate controls the opening of the SARS-CoV-2 spike protein**
18:45 – 19:30	Roundtable discussion II**:** **Future aspects of theoretical approaches**	

Program

Wednesday, 28 April 2021

Session III: *Structure and dynamics of macromolecular systems*

09:00 – 09:45	Oliver Daumke	**The mechanism of dynamin in membrane constriction - From static snapshots to a dynamic model**
09:45– 10:30	Christian Spahn	**tba**
10:30 – 11:00	*COFFEE BREAK*	
11:00 – 11:45	Ben Schuler	**Probing the dynamics and interaction mechanisms of disordered proteins with single-molecule spectroscopy**
11:45 – 12:30	Enrica Bordignon	**Probing conformational heterogeneity of ABC transporters in vitro and in cells via EPR**
12:30 –13:30	*LUNCH BREAK*	
13:30 – 14:15	Markus Wahl	**RNA polymerase dynamics during transcription termination and anti-termination**
14:15 – 15:00	Alke Meents	**Fixed target serial crystallography for studying protein dynamics**
15:00 –15:45	**4 best poster presentations**	
15:45 –16:15	*COFFEE BREAK*	

Program

Wednesday, 28 April 2021

16:15 – 17:00	Brian Baker	**Dynamic allostery in class I major histocompatibility complex proteins**
17:00 – 17:45	Stefan Raunser	**Bringing life into frozen proteins to elucidate molecular mechanisms**
17:45 – 18:30	Roundtable discussion III: **Towards cellular structural biology**	
18:30	Christian Freund Frank Noé	**Final remarks**

End of the Symposium

Posters Session Monday

1	Esam Abualrous	**Natural polymorphism modulates the susceptibility of MHC class II proteins to HLA-DM**
2	Reid Alderson	**Structural basis for the dysregulation of HSP27 in Charcot-Marie-Tooth disease**
3	Robert Arbon	**Assessing the generalizability of adaptive sampling policies**
4	Ernest Awoonor-Williams	**Modelling the binding free energy of peptidomimetic inhibitors to SARS-CoV-2 M^{pro}**
5	Cory Ayres	**Force pull quasiequilibrium: investigating the impact of force on tcr/peptide-mhc stability and immunological recognition**
6	Hossein Batebi	**Formation of a β2AR*-Gs^{GDP} intermediate complex**
7	Miriam Bertazzon	**Investigation of the functional role of CD2BP2**
8	Johann Biedermann	**Investigation of cation permeation through AMPA receptors by molecular dynamics simulations**
9	Yasemin Bozkurt Varolgünes	**Allostery in proteins as point-to-point telecommunication in a network: frequency decomposed signal-to-noise-ratio and channel capacity analysis**
10	Matteo Castelli	**Exploring the effects of post-translational modifications through computational approaches**
11	Liwei Chang	**Inferring protein folding pathways from accelerated molecular dynamics**

Posters Session Monday

12	Yaoyi Chen	**Machine learning implicit solvent method for molecular dynamics**
13	Moumita Dasgupta	**Biophysical characterization of interactions of T cell receptor with viral antigens derived from lymphocytic choriomeningitis virus**
14	Andrea Di Luca	**How global motions are involved in the deactivation of complex I**
15	Kathrin Funck	**Structural and functional explorations of the MICOS complex**
16	Toni Giorgino	**Nanobody interaction unveils structure, dynamics and proteotoxicity of the Finnish-type amyloidogenic gelsolin variant**
17	Giulia Glorani	**An unusual aspartic acid cluster in the reovirus attachment fiber σ1 mediates stability at low pH**
18	Sebastian Günther	**Temperature jump X-ray crystallography to analyze protein dynamics in macromolecular crystals**
19	Shozeb Haider	**Allosteric communication in Class A β-lactamases**
20	Nandan Haloi	**Role of the internal loops in gating of outer membrane porins**
21	Tim Hempel	**Molecular mechanism of inhibiting the SARS-CoV-2 cell entry facilitator TMPRSS2 with camostat and nafamostat**
22	Miriam Jäger	**Predicting ion channel conductance from dissipation-corrected TMD and LE simulations**

23	Eoin Kearney	**Molecular dynamics simulations of uranyl adsorption on montmorillonite clay with desferrioxamine-B**
24	Grant Keller	**Determinants of antigenicity in tumor neoepitopes for the development of personalized/multiple peptide vaccines**
25	Christopher Kolloff	**NOE spin diffusion in large biomolecules**

Poster Session Tuesday

26	Huan Lan	**Exchange catalysis by tapasin exploits conserved and allele-specific features of MHC-I molecules**
27	Eric Lang	**How can molecular modelling support de novo protein design, and vice versa?**
28	Stefano Leoni	**Advanced molecular dynamics simulations for drug discovery**
29	Stephanie Linker	**Polar/apolar interfaces modulate the conformational and kinetic behavior of cyclic peptides and catalyze their passive membrane permeability**
30	Mohamed Marzouk Sobeh	**Investigating the dissociation process and Binding energy of the DBD-p53/DNA complex by PaCS-MD and MSM**
31	Samuel Musson	**Deep learning protein conformational space with convolutions and latent interpolations**
32	Daniel Nagel	**MSMPathfinder: Finding Pathways of Markov State Models**
33	Rainer Nikolay	**Snapshots of native pre-50S ribosomes reveal a biogenesis factor network and evolutionary specialization**
34	Jeffrey Noel	**Summary of the structural biology of dynamin**
35	Oscar Palomino Hernandez	**On the impact of Tyr-39 for the structural features of α-synuclein and for the interaction with small molecules**
36	Guillermo Pérez Hernández	**mdciao: Analysis of molecular dynamics simulations using residue neighborhoods**

Poster Session Tuesday

37	Matthias Post	**Memory kernel estimation from constrained MD simulations**
38	Victor Principe	**Improving MM/PBSA binding affinity calculations using machine learning**
39	Lluís Raich	**Discovery of a hidden transient state in all bromodomain families**
40	Tatiana Rosales	**Understanding the implication of peptide register shifts in TCR cross-reactivity**
41	Joseph Rudzinski	**Automated identification of collective variables and metastable states from molecular dynamics data**
42	Florian Seufert	**Investigation of different protonation states on ADGRL1 flap dynamics**
43	Jiale Shi	**Novel elastic response in twist-bend nematic models**
44	Kamolrat Somboon	**Computational simulations reveal substrate translocation pathway through hydrophobic transporters**
45	Marija Sorokina	**Structural models of ACE2 with RBD of SARS-CoV-2 Spike protein**
46	Rene Staritzbichler	**SmoothT unbiased construction and visualization of transition pathways, linking monte carlo and molecular dynamics simulations**
47	Matthias Stein	**Accurate receptor-ligand binding free energies from fast QM conformational chemical space sampling**

Poster Session Tuesday

48	Hao Tian	**Deciphering the allosteric process of the phaeodactylum tricornutum aureochrome 1a LOV domain**
49	Felix Wiggers	**Diffusion of the disordered E-cadherin tail on β-catenin**
50	Romina Wild	**Counter transport of potassium ions in the human serotonin transporter**
51	Jayasubba Reddy Yarava	**Probing the site-specific backbone dynamics of YadA autotransporter in microcrystals and native membranes using solid-state NMR spectroscopy**

Abstracts of Talks

(in alphabetical order)

A glycan gate controls the opening of the SARS-CoV-2 spike protein

Rommie E. Amaro[1]

[1]*University of California, San Diego, La Jolla, CA 92093-0340*

I will discuss the use of rare event sampling methods in molecular simulations on more extreme scales, to study the spike components in SARS-CoV-2. After introducing the system, I will describe our weighted ensemble (WE) simulations of the fully glycosylated spike ectodomain. The WE simulations allowed us to characterize more than 300 continuous, kinetically unbiased RBD opening pathways. Together with biolayer interferometry experiments, we reveal a gating role for the N-glycan at position N343, a finding that was only possible by careful examination of the functional transition between the open and closed states of the spike. I will also touch on current limitations for determining kinetics of the spike protein, and other similarly complex systems.

References

[1] Casalino, L., Gaieb, Z., Goldsmith, J.A., Hjorth, C.K., Dommer, A.C., Harbison, A.M., Fogarty, C.A., Barros, E.P., Taylor, B.C., McLellan, J.S., Fadda, E., Amaro, R.E. Beyond Shielding: The Roles of Glycans in the SARS-CoV-2 Spike Protein, *ACS Cent. Sci.*; doi: 10.1021/acscentsci.0c01056 (2020)

[2] Sztain, T., Ahn, S.-H., Bogetti, A.T., Casalino, L.C., Goldsmith, J.A., McCool, R.S., Kearns, F.L., McCammon, J.A., McLellan, J.S., Chong, L.T., Amaro, R.E., A Glycan Gate Controls Opening of the SARS-CoV-S Spike Protein, bioRxiv 2021.02.15.431212; doi: https://doi.org/10.1101/2021.02.15.431212 (2021)

Dynamic allostery in class I major histocompatibility complex proteins

Cory M. Ayres and Brian M. Baker

Harper Cancer Research Institute
Department of Chemistry and Biochemistry
University of Notre Dame, Notre Dame, IN USA

Major histocompatibility complex (MHC) proteins bind and present peptides and other small molecules to T cells of the immune system. Peptide/MHC complexes are recognized by a variety of immune receptors, including T cell receptors, coreceptors, and large families of diverse natural killer receptors. Mounting evidence suggests that peptide cargo can tune the molecular motions of class I MHC proteins, impacting how various immune receptors bind. Here we explore this “dynamic allostery” in detail using molecular dynamics simulations coupled with a range of experimental observations.

Probing conformational heterogeneity of ABC transporters in vitro and in cells via EPR

E. Bordignon

[1]Ruhr University Bochum, Faculty of Chemistry and Biochemistry, Bochum, Germany

Site-directed spin labeling EPR enables detection of conformational changes in proteins with almost no restriction in the environmental conditions. Key information for structural analysis is provided by changes in the dynamics and water accessibility of spin-labeled sites and by interspin distances between selected pairs of labels, the latter being detected by pulse dipolar techniques (mostly via DEER or PELDOR). Here we will focus on the use of EPR to sample the conformational plasticity of ABC transporters in different environments. Besides the conventional method of spin labeling the transporters in vitro, that enables the identification of conformational equilibria in the presence of nucleotides[1,2,3], we will show the potential of a novel approach exploiting the specificity of spin-labeled nanobodies as conformational reporters of wild type ABC transporters in native cellular membranes and in cells.
In a proof of principle study[4], we used two distinct gadolinium-labeled nanobodies against the heterodimeric ABC exporter TM287/2884. Thanks to a “cocktail” of state- and non-state-specific nanobodies binding to different sites of the protein, we were able to obtain a fingerprint distance of the outward-facing state of the transporter and follow the conformational cycle of the unlabeled wild type protein in isolated cellular membranes. The nanobody-assisted DEER strategy is then applied to the homodimeric ABC exporter MsbA[5] to gather information on its structural plasticity in detergent micelles, liposomes, nanodiscs, E. coli native membranes and E. coli cells. Our results show a strong dependency of the transporter’s conformational equilibria on the environment, proving the need of structural studies in native membranes.

References

[1] M.H. Timachi et al. eLife 6, e20236 (2017)
[2] H. Göddeke et al. JACS **140** (13), 4543-4551(2018)
[3] C.A.J. Hutter et al. Nat. Commun. **10** (1), 1-13 (2019)
[4] L. Galazzo et al. PNAS **117** (5) 2441-244 (2020)
[5] L. Galazzo et al., in preparation

Modelling biomolecules structure and dynamics by combining small-angle X-ray scattering and computer simulations

C. Paissoni[1] A. Jussupow[2], and C. Camilloni[1]

[1] *University of Milano, Milano, Italy*
[2] *Technical University Munich, München, Germany*

Biomolecules in solution can be characterized by a different extent of conformational dynamics, depending on the specific system and experimental conditions. While the dynamics of single-domain proteins under native conditions is generally limited to fluctuations around a well-defined structure, fully disordered proteins can only be described as statistical ensembles of conformations. Between these cases, multidomain proteins connected by linker regions can populate multiple states generally characterized by a different size. Small-angle X-ray scattering (SAXS) experiments provide low-resolution but valuable information about the dynamics of biomolecular systems, which could be ideally integrated into molecular dynamics (MD) simulations to accurately determine conformational ensembles of flexible proteins. The applicability of this strategy is hampered by the high computational cost required to calculate scattering intensities from three-dimensional structures. I will present the Metainference strategy to integrate experiments and simulations, a hybrid approach to efficiently calculate SAXS and show you different approaches to describe the dynamics of biomolecules and what we can learn about their function [1-4].

References

[1] Jussupow, A. et al. Sci Adv 6, eabc3786 (2020).
[2] Paissoni, C., et al. J Chem Theory Comput 16, 2825–2834 (2020).
[3] Paissoni, C., et al. J Appl Crystallogr 52, 394–402 (2019).
[4] Kooshapur, H. et al. Nat Commun 9, 2479 (2018).

Designing molecular models by machine learning and experimental data

Prof. Cecilia Clementi
Department of Physics
Freie Universität Berlin
Germany

The last years have seen an immense increase in high-throughput and high-resolution technologies for experimental observation as well as high-performance techniques to simulate molecular systems at a microscopic level, resulting in vast and ever-increasing amounts of high-dimensional data. However, experiments provide only a partial view of macromolecular processes and are limited in their temporal and spatial resolution. On the other hand, atomistic simulations are still not able to sample the conformation space of large complexes, thus leaving significant gaps in our ability to study molecular processes at a biologically relevant scale. We present our efforts to bridge these gaps, by exploiting the available data and using state-of-the-art machine-learning methods to design optimal coarse models for complex macromolecular systems. We show that it is possible to define simplified molecular models to reproduce the essential information contained both in microscopic simulation and experimental measurements.

The mechanism of dynamin in membrane constriction - From static snapshots to a dynamic model

O. Ganichkin[1], R. Vancraenenbroeck[2,3], G. Rosenblum[3], H. Hofmann[3], AS Mikhailov[4,5], Jeffrey K. Noel[1,4], Oliver Daumke[1,6]

[1]Crystallography, Max Delbrück Center for Molecular Medicine, Berlin, Germany
[2]Department of Structural and Molecular Biology, University College London, London, United Kingdom
[3]Department of Structural Biology, Weizmann Institute of Science, Rehovot, Israel
[4]Department of Physical Chemistry, Fritz Haber Institute of the Max Planck Society, Berlin, Germany
[5]Computational Molecular Biophysics, WPI Nano Life Science Institute, Kanazawa, University, Kanazawa, Japan
[6]Institute for Chemistry and Biochemistry, Free University of Berlin, Berlin, Germany

Dynamin is a mechano-chemical GTPase that assembles at the neck of clathrin-coated vesicles and catalyzes membrane scission in a GTPase-dependent reaction. Here, I will review previous structural data on dynamin from our and other groups. I will then describe our efforts toward translating the structural snapshots into a dynamic model of membrane constriction. We incorporated our experimental rate measurements and single molecule FRET-based force measurements into a model that resolves individual powerstrokes within a protein filament wound around a deformable membrane tube.

Neural networks potentials for molecular simulations

G. De Fabritiis

[1]University Pompeu Fabra, Barcelona, Spain
[2] ICREA, Barcelona, Spain

Molecular dynamics simulations provide a mechanistic description of molecules by relying on empirical potentials. The quality and transferability of such potentials can be improved leveraging data-driven models derived with machine learning approaches. Here, I present a framework for molecular simulations with mixed classical and machine learning potentials. I will show some initial applications to learning an ab-initio potential, performing an end-to-end training and finally learning and simulating a coarse-grained model for protein folding.

References

[1] Doerr, S., Majewski, M., Pérez, A., Krämer, A., Clementi, C., Noé, F., Giorgino, T., & Fabritiis, G.D. (2020). TorchMD: A deep learning framework for molecular simulations. ArXiv, abs/2012.12106.

The molecular dynamics of potassium channel gating

Bert de Groot[1]

[1]Max Planck Institute for Biophysical Chemistry, Göttingen, Germany

Ion channels facilitate the passive, selective permeation of ions such as sodium, potassium and chloride across biological membranes and as such are essential for cellular electrical signalling. Molecular dynamics simulations are used to study ion permeation across potassium channels at the atomic level. Together with crystallographic analyses and electrophysiological experiments these provide insight into the mechanisms of permeation of potassium, as well as the complex and subtle conformational changes involved in the gating of these channels.

NMR insights into phase separation of a CAPRIN1 intrinsically disordered protein region

T.H. Kim[1,2], B. Tsang[1,2], B.J. Payliss[2], M.L. Nosella[1,2], L. Wong[2], L.E. Kay[2,1], J.D. Forman-Kay[1,2]

[1] *The Hospital for Sick Children, Toronto, Ontario, Canada*
[2] *University of Toronto, Toronto, Ontario, Canada*

The critical role of protein and nucleic acid phase separation in regulating biology is increasingly recognized, as is the contribution of intrinsically disordered protein regions (IDR) to this phase separation. We have utilized NMR to investigate IDR phase separation, focusing on the C-terminal low-complexity region of CAPRIN1 as well as the low-complexity region of FMRP. NMR data provide insights into dynamic interactions within condensed protein states that illuminate how phase separation regulates biology.

References

[1] T.H. Kim, B. Tsang, R.M. Vernon, L.E. Kay and J.D. Forman-Kay, Science **365**, 825 (2019).
[2] L.E. Wong, T.H. Kim, D.R. Muhandiram, J.D. Forman-Kay and L.E. Kay, JACS **142**, 2471 (2020).
[3] T.H Kim, B.J. Payliss, M.L. Nosella, I.T.W. Lee, Y. Toyama, J.D. Forman-Kay and L.E. Kay, Submitted (2021).

Dynamic aspects of antigen presentation by MHC class II molecules

Christian Freund

Freie Universität Berlin, German

Internalized protein antigens derived from pathogens are degraded in the late endosome and loaded onto host-specific Major histocompatibility (MHC) class II proteins, a process referred to as antigen processing and presentation. Stable MHC-peptide complexes then serve as recognition units for T cell surveillance, potentially invoking the hosts immune response. The mechanism underlying the competitive binding of peptides to MHC molecules in the late endosome involves the exchange catalyst HLA-DM that provokes the formation of high-affinity complexes. In this talk, the importance of protein dynamics for peptide editing will be highlighted and reveal conformational plasticity as a hallmark of catalyzed peptide exchange.

The important role of dynamics in the function and misfunction of molecular machines

Lewis E. Kay
Departments of Molecular Genetics, Biochemistry and Chemistry
University of Toronto
Toronto, Ontario, Canada

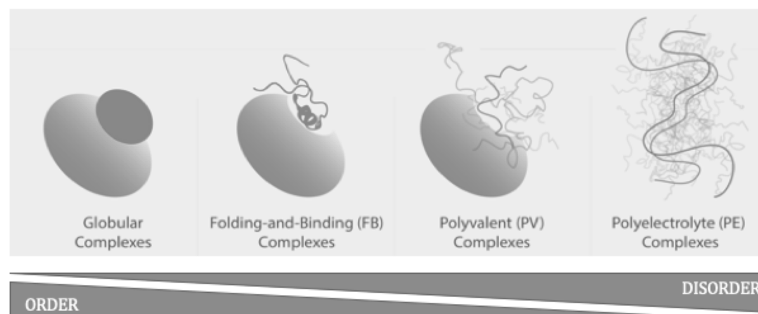
Protein molecules play critical roles in cellular function and they catalyze many of the biochemical reactions that are necessary for life. The three-dimensional shapes of these molecules are crucial for guiding proper function and they can change with time due to interactions with other molecules, various stresses on the cell or simply the result of random fluctuations. Although very detailed static pictures of protein molecules have been produced using traditional biophysical tools, macromolecular function and misfunction is, in many cases, intimately coupled to flexibility and knowledge of molecular motions therefore becomes critical. For the past 3 decades my laboratory has developed biophysical techniques, focusing on solution based Nuclear Magnetic Resonance spectroscopy for the study of biomolecular dynamics. A brief description of some of the methods we have derived will be given along with examples to illustrate the critical importance of dynamics to protein function and misfunction.

Dynamic complexes of intrinsically disordered proteins by NMR spectroscopy

B.B. Kragelund

Structural Biology and NMR Laboratory, Linderstrøm-Lang Centre for Protein Science, Department of Biology, University of Copenhagen, Denmark
E-mail: bbk@bio.ku.dk

Intrinsically disordered proteins (IDPs) (or –regions (IDRs)) are functional while existing in broad ensembles of near iso-energetic conformations. Despite their lack of tertiary structure, IDPs are involved in communication with other molecules forming associations ranging from binary, discrete complexes to large multicomponent assemblies. Due to their dynamic nature, IDPs expand the types of association possible, further enabling functional regulations by very different mechanisms. The fast dynamics characteristic of IDPs may persist in their complexes and the degrees of disorder within the complex can therefore vary greatly. We have been exploring the functional role of disorder in a diverse set of proteins ranging from small soluble IDPs to large IDRs in membrane proteins, combining NMR spectroscopy with other biophysical methods as SAXS, neutron diffraction, computation and cell biology [1–78]. I will address functional disorder and highlight how NMR spectroscopy has been an essential method for these investigations as well as a critical contributor in the understanding of disorder dependent biology.



References

[1] Paraskevopoulos, K. et al., Mol. Cell, **53**(3), 453-6 (2014)
[2] Haxholm, G. et al., Biochem. J **468**, 495–506. (2015)
[3] Bugge, K, et al., Nat. Comm. **7**, 11578. (2016)
[4] Hendus-Altenburger, R., et al., BMC Biol. **14**, 31 (2016)
[5] Borgia, A., Borgia, M. B., Bugge, K, et al., Nature, **555**, 61–66 (2018)
[6] Chhabra, Y, et al., Oncogene, **37** (4), 489-501(2018)
[7] Hendus-Altenburger, R, et al., Nature Comm **10** (1), 3489 (2019)
[8] Schenstrøm, SM, et al., Cell Rep, **25** (4), 862-870 (2019)

Biophysical experiments and biomolecular simulations: A perfect match?

K. Lindorff-Larsen[1]

[1] *Linderstrøm-Lang Centre for Protein Science, Univ. of Copenhagen, Denmark*

Biological macromolecules are dynamic entities and I will discuss methods and applications for how simulations and experiments can be used synergistically to study protein and RNA dynamics [1,2]. Functional protein motions are often described as an exchange between a ground state structure and minor states. The structural and biophysical properties of these transiently and sparsely populated states are, however, difficult to study, and an atomic-level description of those states is challenging. Using proteins with extensive NMR data available as test systems, we have shown how enhanced sampling simulations can be used to capture accurately complex conformational changes in proteins, and I will briefly discuss such examples [3,4]. Despite recent progress, one may still find that a simulation does not quantitatively match experiments. Then, experiments and simulations may be combined in a very direct fashion to provide a description of the molecular motions that combines the details of atomic simulations with the accuracy afforded by experiments [1,2]. The resulting conformational ensembles may provide novel insight into biomolecular systems that are not obtainable by simulations of experiments alone. I will discuss how this may be achieved [5], and give examples of the application of such approaches using both NMR and small-angle scattering experiments to describe both proteins [6,7] and RNA [8,9], and possible future approaches to include timescales of motion [10]

References

[1] Bottaro, Sandro, and Kresten Lindorff-Larsen. "Biophysical experiments and biomolecular simulations: A perfect match?." *Science* 361 (2018): 355-360.

[2] Orioli, Simone, et al. "How to learn from inconsistencies: Integrating molecular simulations with experimental data." *Prog Mol Biol and Transl Sci* Vol. 170, 2020. 123-176.

[3] Wang, Yong, Elena Papaleo, and Kresten Lindorff-Larsen. "Mapping transiently formed and sparsely populated conformations on a complex energy landscape." *Elife* 5 (2016): e17505.

[4] Henriques, João, and Kresten Lindorff-Larsen. "Protein dynamics enables phosphorylation of buried residues in Cdk2/Cyclin A-bound p27*." Biophys J.* (2020): 2010-2018

[5] Bottaro, Sandro, Tone Bengtsen, and Kresten Lindorff-Larsen. "Integrating molecular simulation and experimental data: A Bayesian/maximum entropy reweighting approach." *Structural Bioinformatics*. Humana, 2020. 219-240.

[6] Larsen, Andreas Haahr, et al. "Combining molecular dynamics simulations with small-angle X-ray and neutron scattering data to study multi-domain proteins in solution." *PLoS Comput Biol* 16 (2020): e1007870.

[7] Bengtsen, Tone, et al. " Structure and dynamics of a nanodisc by integrating NMR, SAXS and SANS experiments with molecular dynamics simulations." *eLIFE* (2020): e56518.

[8] Bottaro, Sandro, et al. "Conformational ensembles of RNA oligonucleotides from integrating NMR and molecular simulations." *Sci Adv* 4.5 (2018): eaar8521.

[9] Bottaro, Sandro, et al. "Integrating NMR and simulations reveals motions in the UUCG tetraloop." *Nucleic Acids Res* 48 (2020): 5839-5848.

[10] Kümmerer, Felix, et al. "Fitting side-chain NMR relaxation data using molecular simulations." *bioRxiv* (2020).

Fixed target serial crystallography for studying protein dynamics.

S. Guenther, P. Reinke and A. Meents

DESY, Center for Free-Electron Laser Science,
Notkestrasse 85, 22607 Hamburg, Germany

Macromolecular X-ray crystallography allows determining the structure of proteins at high resolution in 3D. Whereas conventional X-ray crystallography is based on rotation images from one or a few relatively large crystals, in serial crystallography (SX) still images from a few hundred to several thousands of microcrystals are recorded and subsequently merged into one dataset. Thus in SX much shorter exposure times down to a few femtoseconds can be applied, which allows following protein dynamics and enzyme reactions on the same time scales. Moreover, in contrast to conventional crystallography SX experiments are typically performed at room temperature enabling observation of protein motions typically hidden in experiments at cryo-conditions.

For SX highly reliable sample delivery requiring only small amounts of sample has remained one of the bottlenecks. Fixed target sample delivery is one of the most powerful methods. Here microcrystals are immobilized on a solid support and then systematically scanned through the X-ray beam [1]. We have developed the Roadrunner goniometer, which allows collecting a complete serial X-ray data set at 1 kHz sample exchange rate in less than 2 minutes [2]. In combination with very low background scattering levels achievable with our approach this method is ideally suited to study protein dynamics and enzyme reactions in a highly efficient and reliable way.

Using the Roadrunner goniometer we have performed several time resolved diffraction experiments at both synchrotrons utilizing a polychromatic ‘pink’ X-ray beam and at XFELs [1,3]. Different laser-pumping schemes have been applied to trigger protein motion and reactions. These include optical laser pumping experiments of the photoactive yellow protein PYP and temperature jump experiments induced with ns-duration IR pulses on Ribonuclease A. Here I will provide an overview of the method followed by a few recent application examples.

References

[1] Roedig,P., et al., “High-speed fixed-target serial virus crystallography” Nature Methods 14, 805+, (2017).

[2] Tolstikova A. et al,. “1 kHz fixed-target serial crystallography using a multilayer monochromator and an integrating pixel detector“ IUCRJ 6(5), 927-937 (2019).

[3] Meents, A. et al., “Pink-beam serial crystallography”. Nature Comm. 8, (2017).

"Bringing life into frozen proteins to elucidate molecular mechanisms"

Stefan Raunser

Department of Structural Biochemistry – Max Planck Institute of Molecular Physiology, Otto-Hahn-Str. 11, 44227 Dortmund

raunser@mpi-dortmund.mpg.de

Facilitated by recent technological breakthroughs, the Nobel prize-winning technique electron cryomicroscopy (cryo-EM) has become a versatile and extremely powerful tool to solve routinely near-atomic resolution three-dimensional protein structures. In my presentation, I will explain how we combined single particle cryo-EM with molecular dynamics simulations, NMR, EPR, single molecule FRET, TIRF microscopy and biochemistry to elucidate the molecular mechanism of action of Tc toxins from the insect-pathogenic bacterium *Photorhabdus luminescens*. Furthermore, I will present our latest developments in structural cell biology using focused-ion-laser beam milling and electron cryo tomography.

Combining NMR and integrative structural biology to study dynamics and allostery in protein complexes

Michael Sattler[1,2]

[1] *Institute of Structural Biology, Helmholtz Center Munich, Neuherberg, Germany*
[2] *Bavarian NMR Center, Department of Chemistry, Technical University of Munich, Garching, Germany*

Most eukaryotic proteins are comprised of multiple structural domains connected by linkers of variable length and rigidity. We combine solution NMR spectroscopy and small angle scattering (SAXS, SANS) with crystallography and cryo-EM in integrative structural biology approaches to study the conformational dynamics of multidomain proteins and the roles of the connecting linkers. Studies with multidomain RNA binding proteins (RBPs) and the multidomain chaperone Hsp90, will be discussed.
The molecular functions of multi-domain proteins often rely on dynamic structural ensembles and can be controlled by population shifts between inactive and inactive conformations. This is not visible in static structures. The domains in these proteins are often connected or flanked by intrinsically disordered regions, where posttranslational modifications can further modulate the molecular interactions to regulate the biological activity. Integrative structural biology combining solution techniques, especially NMR spectroscopy, can help to unravel the molecular recognition, dynamics and regulation of protein complexes.

References

[1] Kang H-S *et al. An autoinhibitory intramolecular interaction proof-reads RNA recognition by the essential splicing factor U2AF2*, PNAS **117**, 7140–7149 (2020).
[2] Jagtap, PKA *et al. Selective Inhibitors of FKBP51 Employ Conformational Selection of Dynamic Invisible States*, Angew. Chemie **58**, 9429–9433 (2019).
[3] Kooshapur, H. *et al.* Sattler M *Structural basis for terminal loop recognition and stimulation of pri-miRNA-18a processing by hnRNP A1*, Nature Commun **9**, 2479 (2018).
[4] Mackereth CD et al. Sattler M *Multi-domain conformational selection underlies pre-mRNA splicing regulation by U2AF*, Nature **475**, 408–411 (2011).

Probing the Dynamics and Interaction Mechanisms of Disordered Proteins with Single-Molecule Spectroscopy

Benjamin Schuler[1]

[1]University of Zurich, Zurich, Switzerland

The functions of proteins have traditionally been linked to their well-defined three-dimensional, folded structures. It is becoming increasingly clear, however, that many proteins perform essential functions without being folded. The lack of structure in these intrinsically disordered proteins (IDPs) and the resulting pronounced long-range dynamics and conformational heterogeneity have limited our understanding of their physical properties and functional mechanisms. Single-molecule FRET combined with nanosecond FCS and molecular simulations provides new opportunities for investigating these questions, for defining the properties of IDPs in terms of polymer-physical concepts, and for identifying their interaction mechanisms. I will illustrate the surprising interaction mechanisms that these proteins exhibit and how they may affect molecular communication and regulation in biology.

Dynamics of MHC-I Molecules in the Antigen Processing and Presentation Pathway

[1,2]Andrew C. McShan, [3]Christine A. Devlin, [1,2]Hau V. Truong, [3]Erik Procko and [1,2]Nikolaos G. Sgourakis

[1]Center for Computational and Genomic Medicine, Department of Pathology and Laboratory Medicine, The Children's Hospital of Philadelphia
[2]Department of Biochemistry and Biophysics, Perelman School of Medicine, University of Pennsylvania, 3401 Civic Center Blvd, Philadelphia, PA, 19104, USA
[3]Department of Biochemistry and Cancer Center at Illinois, University of Illinois, Urbana, IL 61801, USA.

The class-I proteins of the major histocompatibility complex (MHC-I) capture and display antigenic peptides derived from the processing of intracellular targets to cytotoxic T-cells and natural killer cells for immune surveillance. Human MHC proteins, referred to as human leukocyte antigens (HLA), are highly polymorphic with over 10,000 distinct allotypes identified to date. Despite structural similarities, allelic sequence diversity has been shown to influence interactions with dedicated molecular chaperones, Tapasin and TAPBPR, along the MHC-I antigen processing and quality control pathway. Mounting biophysical and functional data highlight the role of protein dynamics in fine-tuning MHC-I/chaperone interactions for proper folding and peptide selector function. This seminar will present insights from NMR experiments designed to probe dynamic motions spanning a range of timescales and degrees of freedom, combined with deep mutational scanning for probing functionally important residues on both MHC-I and chaperone structures. Taken together, our results outline a paradigm where the intrinsic conformational plasticity of key MHC-I surfaces determine chaperone recognition, and peptide repertoire selection.

References

[1] A.C. McShan, C.A. Devlin et al., *Nature Communications, accepted*
[2] A.C. McShan, C.A. Devlin et al., *Proc Natl Acad Sci USA*, **116 (51)**, 25602-25613 (2019)
[3] A.C. McShan et al., *Nature Chemical Biology,***14**, *811–820* (2018)

A cold look at the formation and activation of the bacterial large ribosomal subunit

R. Nikolay[1], T. Hilal[1,2], S. Schmidt[3], B. Qin[1], S. Lauer[1], T. Mielke[4], E. Deuerling[3] and C.M.T. Spahn[1]

[1] Institut für Medizinische Physik und Biophysik, Charité, Berlin, Germany
[2] Research Centre for Electron Microscopy, FUB, Berlin, Germany
[3] Molekulare Mikrobiologie, Universität Konstanz, Konstanz, Germany
[4] Max Planck Institute for Molecular Genetics, Berlin, Germany

Ribosome biogenesis is a fundamental multi-step cellular process in all domains of life. It involves the production, processing, folding, and modification of rRNAs and r-proteins. During assembly, the rRNAs and r-proteins undergo a series of highly ordered interactions and binding events ensuring cooperativity and leading to the formation of active ribosomal subunits [1]. *In vivo* numerous biogenesis factors tune and regulate this process. The core of the ribosome and certain features of ribosome assembly are evolutionary conserved, but there are significant differences among the domains of life as well.
Here we employ multiparticle cryo-EM to study the assembly process of the bacterial large ribosomal 50S subunit [2, 3]. The *E. coli* 50S subunit consists of 23S rRNA, 5S rRNA and 33 r-proteins. We exploit the unique possibility of the bacterial system to study assembly not only *in vivo* but also by an *in vitro* total reconstitution assay not available for eukaryotic systems [4]. Thus, we can compare structures of ribosomal precursor particles obtained *ex vivo* from the complex context of a living cell, and from a minimal *in vitro* system with controllable external parameters. Our *in vitro* structures monitor the blueprint of assembly that is encoded in the ribosomal components itself and identify the folding of the rRNA-based peptidyl transferase center as the final critical step along the 50S assembly pathway. Our *ex vivo* structures provide mechanistic insight into how the GTPase ObgE, in concert with biogenesis factors YjgA, RluD, RsfS, facilitates the maturation of the 50S functional core. Our structural study of ribosome assembly from complementary perspectives provides insights into the molecular mechanisms involved and reveals both conserved and divergent evolutionary features of ribosome biogenesis between prokaryotes and eukaryotes.

References

[1] Z. Shajani et al., Annu. Rev. Biochem. **80**, 501–526 (2011)
[2] R. Nikolay, Mol. Cell **70**, 881–893 (2018)
[3] R. Nikolay, Mol. Cell **81**, 1200-1215 (2021)
[4] K.H. Nierhaus, Biochimie **737**, 39-55 (1991)

RNA polymerase dynamics during transcription termination and anti-termination

M. C. Wahl[1,2]

[1]Freie Universität Berlin, Structural Biochemistry, Berlin, Germany
[2]Helmholtz-Zentrum Berlin, Macromolecular Crystallography, Berlin, Germany

The interplay of transcription termination and continued transcription elongation (anti-termination) is a pervasive gene regulatory principle in bacteria. We employ structural biochemical techniques, in particular cryogenic electron microscopy and single particle analysis, to elucidate the structural basis underlying transcription termination, anti-termination and RNA polymerase recycling processes in bacteria [1-5]. I will report on our recent findings with a particular focus on the structural remodeling of RNA polymerase and associated transcription factors in these functional contexts.

References

[1] N. Said, T. Hilal, N.D. Sunday, A. Khatri, J. Bürger, T. Mielke, G.A. Belogurov, B. Loll, R. Sen, I. Artsimovitch, M.C. Wahl, Science **371**, eabd1673 (2021)

[2] H.H. Pei, T. Hilal, Z. Chen, Y.H. Huang, Y. Gao, N. Said, B. Loll, J. Rappsilber, G.A. Belogurov, I. Artsimovitch, M.C. Wahl, Nat Commun **11**, 6418 (2020)

[3] Y.H. Huang, T. Hilal, B. Loll, J. Bürger, T. Mielke, C. Böttcher, N. Said, M.C. Wahl, Mol Cell **79**, 1024-1036.e5 (2020)

[4] F. Krupp, N. Said, Y.H. Huang, B. Loll, J. Bürger, T. Mielke, C.M.T. Spahn, M.C. Wahl, Mol Cell **74**, 143-157 (2019)

[5] N. Said, F. Krupp, E. Anedchenko, K.F. Santos, O. Dybkov, Y.H. Huang, T.C. Lee, B. Loll, E. Behrmann, J. Bürger, T. Mielke, J. Loerke, H. Urlaub, C.M.T. Spahn, G. Weber, M.C. Wahl, Nat Microbiol **2**, 17062 (2017)

Abstracts of Posters

(in alphabetical order)

Natural polymorphism modulates the susceptibility of MHC class II proteins to HLA-DM

Esam T. Abualrous[1,2] , Sebastian Stolzenberg[2] , Jana Sticht[1] , Frank Noe[2] , and Christian Freund[1]

Protein Biochemstry, Inistitute of Biochemstry, Freie Universität Berlin, Berlin, Artificial Intelligence for the Sciences, Inistitute of Mathematics and Informatics, Freie Universität Berlin, Berlin

Antigen-specific recognition of peptide-major histocompatibility class II complexes (pMHCIIs) by CD4+ T cells represents a key feature of adaptive immunity. Professional antigen-presenting cells express a subset of different alleles of heterodimeric MHCII proteins on their surface. Initially, the class II-associated invariant chain peptide (CLIP) occupies the MHCII-binding groove, and can later be replaced in the endosomal compartments for higher affinity antigens. The peptide exchange process is catalyzed by the nonclassical MHCII molecule HLA-DM (DM) [1, 2]. The major histocompatibility (MHC) locus is the most polymorphic of the human genome, with hundreds to thousands of variants (alleles) expressed within the human population. Here we investigate how polymorphism affects the mechanism of peptide binding/exchange in the context of the antigen presentation process.

References:

1. Wieczorek, M., et al., Front Immunol., 2017. 8:292
2. Wieczorek, M., et al., Nat. Commu., 2016. 7:13224

Structural basis for the dysregulation of HSP27

in Charcot-Marie-Tooth disease

T. Reid Alderson[1,2,§], Elias Adriaenssens[3‡], Bob Asselbergh[4], Vincent Timmerman[3], Andrew J. Baldwin[1], Justin L. P. Benesch[1]

[1]Department of Chemistry, Physical and Theoretical Chemistry Laboratory, University of Oxford, South Parks Road, Oxford, OX1 3QZ, UK

[2]Laboratory of Chemical Physics, National Institutes of Health, Bethesda, MD, 20892, USA

[3]Peripheral Neuropathy Research Group, Department of Biomedical Sciences, Institute Born Bunge, University of Antwerp, 2610 Wilrijk, Antwerpen, Belgium

[4]Histology and Cellular Imaging, Neuromics Support Facility, VIB-UAntwerp Center for Molecular Neurology, VIB and University of Antwerp, Antwerpen, Belgium

§ Present address: Department of Biochemistry, University of Toronto, 1 King's College Circle, Toronto, ON M5S 1A8, Canada

‡ Present address: Max Perutz Labs, University of Vienna, Dr. Bohr-Gasse 9, 1030 Vienna, Austria

HSP27 is a human molecular chaperone with important roles in homeostasis. Dysregulation of the activity or expression of HSP27 can lead to cancers, neurodegenerative diseases, and neuropathies. For instance, over 30 heritable mutations in HSP27 cause Charcot-Marie-Tooth (CMT) disease, the most common inherited disorder of the peripheral nervous system. However, the structural basis for the dysregulation of HSP27 in disease has been hindered by the formation of large, dynamic oligomers that are recalcitrant to traditional biophysical approaches. Here, we show that the dissociated HSP27 monomers that are present at physiological concentrations become highly active chaperones[1]. With NMR spectroscopy, we determined the structural basis for this hyperactivation and found that the monomeric chaperone undergoes an ordered-to-partially-disordered transition[1,2]. We further observed that a severe CMT disease-causing mutation abrogates chaperone activity and drastically increases the size of HSP27 oligomers formed *in vivo*, including in motor neurons differentiated from patient-derived stem cells[3]. Using NMR spectroscopy, we determined that the mutation weakens an intra-HSP27 interaction involving the structured α-crystallin domain and a short linear motif known as the IxI/V motif near the C-terminus. We observed that other, HSP27-interacting proteins with an IxI/V motif bind with higher affinity to the CMT disease-causing variant of HSP27. Our results provide a mechanistic basis for a disease-causing mutation in HSP27 and suggest that the IxI/V motif plays an important, regulatory role in modulating protein-protein interactions.

References:

1. Alderson TR, …, Bax A, Benesch JLP*, Baldwin AJ*. *Nat. Commun.* **10**, 1068 (2019).
2. Alderson TR, Ying J, Bax A, Benesch JLP*, Baldwin AJ*. *J. Mol. Biol.* **432**, 3033-3049 (2020).
3. Alderson TR[§], Adriaenssens E[§], …, Timmerman V*, Baldwin AJ*, Benesch JLP*. *EMBO J*, e103811 (2021).

Assessing the generalizability of adaptive sampling policies

R. Arbon, R. Wiewiora and J. Chodera

MSKCC, New York, USA

Simulating proteins is expensive, environmentally damaging and time consuming. For unbiased exploration of conformational space using distributed computer systems (such as Folding at Home [1]) adaptive sampling [5] (AS) has been shown to speed up both exploration [2] and targeted discovery e.g., of protein folding pathways [3]. AS proceeds in a series of sampling epochs, where the starting configurations of the current epoch depend on information learned from previous epochs (restart policy). Comparisons of a small number of restart policies have been tested on benchmark systems [2,3]. However, our ability to choose appropriate policies for arbitrary systems is so far limited because of the lack of information on the policies generalizability. This work answers the question: “Given an arbitrary (metastable) Markov process, what is the most appropriate AS policy?”. To do this, we use a recently proposed algorithm for creating random Markov matrices with given eigenvalue spectra [4]. This algorithm is at the heart of our Python package ‘adaptive’ which evaluates AS policies from a statistical perspective. ‘adaptive’ currently measures the performance of AS policies in terms of state discovery i.e., graph cover times. The posterior distribution of cover times is estimated by averaging over Markov matrices with the same eigenvalue spectrum but different stationary distributions, and then optionally over matrices with different metastable spectra. Future work will optimize AS hyperparameters using Bayesian optimisation, incorporate new cost functions suitable for high performance computing, and use graph neural networks and reinforcement learning to design new policies based on similar work on graph exploration [6].

References

[1] M. Shirts et. al., Science, **290**, 1903 (2000)
[2] E. Hruska, et. al., J. Chem. Phys, **149**, 244119 (2018)
[3] M. Zimmerman et. al., J. Chem. Theory Comput. **11**, 5747 (2015)
[4] L. Ciampolini et. al., HAL Archives-Ouvert, hal-01016804 (2014)
[5] N. Singhal et. al., J. Chem. Phys. **123**, 204909 (2005)
[6] H. Dai et. al., arXiv:1910.12980 (2019)

Modelling the Binding Free Energy of Peptidomimetic Inhibitors to SARS-CoV-2 M^{pro}

Ernest Awoonor-Williams [1]

[1]*Memorial University of Newfoundland, Department of Chemistry, St. John's, NL, Canada.*

As of April 2021, COVID-19 has infected over 300 million people and caused over 2.8 million deaths worldwide. Currently, there are very limited therapeutics for the treatment of the disease. The main protease (M^{pro}) of the virus responsible for COVID-19 — SARS-CoV-2, is an attractive target for the treatment of the disease. This is because inhibiting the M^{pro} blocks a key piece of the self-replication machinery of the virus. In an effort to contribute towards the global fight against COVID-19, I will present the results of binding free energy calculations that were performed on two recently crystallized structures of the M^{pro} target (i.e., N3, *Nature* **2020**, *582*, 289–293; and alpha-ketoamide 13b, *Science (80-.).* **2020**, *368*, 409–412.). The calculations employ an integrated approach consisting of alchemical absolute binding free energy calculations and QM/MM calculations. Our results show that this integrative approach can predict both the non-covalent and covalent binding energies of the M^{pro} inhibitors well.[1] We anticipate that this information could prove useful in the rational design and evaluation of potent SARS-CoV-2 M^{pro} inhibitors for targeted antiviral therapy.

References

[1] Awoonor-Williams, E. and Abd Al-Aziz A. Abu-Saleh, *Phys. Chem. Chem. Phys.,* **2021**, 23, 6746–6757.

Force Pull Quasiequilibrium: Investigating the Impact of Force on TCR/peptide-MHC Stability and Immunological Recognition

Cory M. Ayres[1], Brian D. Evavold[2], Steven A. Corcelli[1], Brian M. Baker[1]

1. Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana
2. Division of Microbiology and Immunology, Department of Pathology, University of Utah, Salt Lake City, UT

The driving force of adaptive cellular immunity involves the recognition of peptide antigens bound and presented by major histocompatibility complex (MHC) proteins by T-cells through the use of their T-cell receptors (TCRs). Significant effort has been dedicated to understand the mechanisms involved in the recognition and discrimination of antigenic peptides by various TCRs, including but not limited to investigation of crystal structures, TCR/peptide-MHC binding affinity as well as extensive molecular dynamics simulations. While much insight has been gleaned from these studies, they have been limited to detailing molecular recognition in solution and neglect to address the potential impact that cellular machinery may have within the binding interface of these two molecules. In particular, both scanning of the antigen presenting cell by the T-cell, as well as the retrograde flow derived from cytoskeletal reorganization, impart a pulling force at the binding interface of the TCR and peptide-MHC. It has been experimentally demonstrated that various TCRs display a differential response to this pulling force, with some TCRs exhibiting a longer bond lifetime under application of said force - a phenomenon known as a catch bond. To investigate the mechanisms of these catch bonds, we have performed molecular dynamics simulations of two different T-cell receptors bound to the same peptide-MHC ligand. Experimentally, while differing by only one amino acid, these two TCRs display a significantly varied response to force, notably by exhibiting a peak bond life time at two distinct forces. Accordingly, simulations were performed while applying an unbinding force of different magnitudes and directions. In agreement with experiment, our simulations recapitulate the differential bond lifetimes of the two TCR/peptide-MHC complexes while under force. Further, our simulations detail a potential mechanism of how these catch bonds arise. Through calculation of linear interaction energies between the T-cell receptor, peptide-MHC and interfacial solvent, in simulations in which catch bond behavior is observed we are able to identify a partially unbound quasiequilibrium state which is more energetically favorable than the original fully ligated conformation.

Formation of a β2AR*-Gs^{GDP} intermediate complex

Hossein Batebi,[1] Guillermo Pérez Haernández,[5] Johanna K. S. Tiemann,[4] Xiangyu Liu,[3] Jesper Mosolff Mathiesen,[4] Brian Kobilka,[2,3] Peter W. Hildebrand,[1,5,*]

[1] *Universität Leipzig, Medizinische Fakultät, Institut für Medizinische Physik und Biophysik, Leipzig (Germany)*
[2] Stanford University School of Medicine, Department of Molecular and Cellular Physiology, Stanford, (USA)
[3] Tsinghua University, Beijing Advanced Innovation Center for Structural Biology, Beijing (China)
[4] University of Copenhagen, Department of Drug Design and Pharmacology, Copenhagen (Denmark)
[5] Charité - Universitätsmedizin Berlin, Institut für Medizinische Physik und Biophysik, Berlin (Germany)
* *Corresponding author. PWH peter.hildebrand@medizin.uni-leipzig.de.*

X-ray crystallography and cryo-electron microscopy structures of receptor G protein complexes show hallmarks of receptor and G protein activation. In all these complexes, the C-terminal α5 helix of Gα binds deeply into the binding pocket of the activated receptor (R*). In contrast, recent hydrogen-deuterium exchange mass spectrometry and X-ray footprinting mass spectrometry data of the β2-adrenoceptor Gs system show that α5 remains dynamic for a long period of time suggesting an intermediate GDP-bound β2AR*-Gs^{GDP} complex as the first specific receptor G protein interaction[1]. The existence of an intermediate R*-G^{GDP} complex was initially demonstrated by kinetic and single-molecule fluorescence resonance energy transfer analysis[2,3]. The recent X-ray structure of the β2AR construct with a fused Gαs carboxyl-terminal 14 amino acid peptide (PDB-id: 6E67) shows a binding mode of α5 that significantly differs from the X-ray structure of nucleotide-free β2AR*-Gs (PDB-id: 3SN6)[4]. In order to investigate whether that mode of interaction represents the β2AR*-Gs^{GDP} complex, we analyzed the spontaneous formation of the β2AR*-Gs^{GDP} complex in long all-atomistic classical molecular dynamics simulations. Results are discussed with regards to the role of structural intermediates for coupling specificity and for receptor catalyzed G protein nucleotide exchange.

References

[1] Du, Y. et al. *Cell* 2019, ***177* (5)**, 1232-1242.e11.
[2] Scheerer, P. *Proc. Natl. Acad. Sci. U. S. A.* 2009, ***106* (26)**, 10660–10665.
[3] Gregorio, G. *Nature* 2017, ***547* (7661)**, 68–73.
[4] Liu, X. *Cell* 2019, ***177* (5)**, 1243-1251.e12.

Investigation of the functional role of CD2BP2

Miriam Bertazzon[1], Almudena Hurtado Pico[1], Carlos Plaza[2], Stephanie Kliche[3], Benno Kuropka[1], Marco Preußner[4], Fan Liu[5], Ingo Schmitz[2], Florian Heyd[4], and Christian Freund[1]

[1]*Freie Universität Berlin, Department of Biochemistry, Protein-Biochemistry Berlin*
[2]*Helmholtz Centre for Infection Research, Braunschweig*
[3]*Institut für Molekulare und Klinische Immunologie, Magdeburg*
[4]*Freie Universität Berlin, Department of Biochemistry, RNA-Biochemistry, Berlin*
[5]*Leibniz-Forschungsinstitut für Molekulare Pharmakologie, Berlin*

Eukaryotic cells have evolved several mechanisms for RNA processing, with pre-mRNA splicing being one of the most complex. Splicing removes non-coding regions from the pre-mRNA, leaving the coding regions to be translated into protein. Modulation of this splicing mechanism gives rise to alternative events that increase protein diversity with a strong impact on cellular function.
Pre-mRNA splicing is catalyzed by a complex molecular machinery known as the spliceosome, which undergoes several conformational and compositional rearrangements comprised of stepwise cross-intron assemblies and disassemblies during a continuous splicing cycle. Although the last several years have seen the characterization of the mechanism of this stepwise process (1), there remain many unresolved questions about the dynamic interactions implicated in these rearrangements, as well as their role in the modulation of splicing.
In particular, we investigate the role of the splicesomal protein CD2BP2 which is part of the U5 snRNP (2). Moreover, this protein was already demonstrated to be essential for cell differentiation in many organisms (3-6).
Therefore, with the aim to understand the role of CD2BP2 in differentiation and gene regulation, genetically modified mice lacking CD2BP2 in T-cells expressing the CD4 receptor were generated. Those mice show T-cells subtypes unbalance in thymus, spleen and lymph nodes. Furthermore, RNA was extracted from CD4+ T-cells and RNA-Seq analysis was performed. The data clearly indicates that CD2BP2 is involved in gene regulation, in fact several Differentially Expressed Genes (DEGs) and Differentially Spliced Variants (DSVs) were detected.

References

1. Wan R, Bai R, Shi Y. Curr Opin Struct Biol.; 59:124-33, (2019).
2. Laggerbauer B, Liu S, *et al.* RNA.; 11(5):598-608, (2005).
3. Wang C, Gupta P, *et al.* Nucleic Acids Res.; 45(3):1488-500 (2017).
4. Wang C, Wilson-Berry L, *et al.* Dev Dyn.; 241(3):505-21, (2012).
5. Geiger JA, Carvalho L, *et al.* PLoS One.; 6(11):e28349, (2011).
6. Albert GI, Schell C, *et al.* J Mol Cell Biol.; 7(5):402-14, (2015).

Investigation of Cation Permeation through AMPA Receptors by Molecular Dynamics Simulations

J. Biedermann[1,2,3], S. Braunbeck[1,2], H. Sun[3] and A. Plested[1,2]

[1] Molecular Neuroscience and Biophysics, Leibniz FMP Berlin, Berlin, Germany
[2] Cellular Biophysics, Humboldt University Berlin, Berlin, Germany
[3] Structural Chemistry and Computational Biophysics, Leibniz FMP Berlin, Berlin, Germany

AMPA Receptors (AMPAR) belong to the family of ionotropic glutamate receptors and are responsible for fast excitatory signal transduction at vertebrate synapses. They are crucial for all higher brain functions and defects in AMPARs can lead to severe neurodevelopmental disorders. We performed extensive molecular dynamics simulations (over 20 microseconds) of the transmembrane domain of a putative open AMPAR structure[1]. These simulations revealed that the structure is indeed open and molecular dynamics (MD)[2] are able to simulate ionic current through the ion channel structure derived from cryogenic electron microscopy. Permeation proceeded at physiological rates during the simulations, but depended on the force field used. We cross validated permeation rates of the monovalent cations potassium, sodium and caesium with single channel recordings. MD simulations of permeation of those ion types showed that the narrowest part with two glutamines in each subunit play the most important role in cation permeation. Rather than a single permissive structure, the selectivity filter of the ion channel is flexible, and allows different ions to permeate using distinct mechanisms but at similar rates.[3]

References

[1] E. Twomey, M. Yelshanskaya, R. Grassucci,J. Frank, A Sobolevsky, nature **549(7670)**, 60.65 (2017)

[2] C. Kutzner, H. Grubmüller, B. de Groot, U. Zachariae, Biophysical Journal **101 (4)**, 809-817 (2009)

[3] J. Biedermann, S. Braunbeck, H. Sun, A. Plested, PNAS, **118(8**), 2021

Allostery in Proteins as Point-to-Point Telecommunication in a Network: Frequency Decomposed Signal-to-Noise-Ratio and Channel Capacity Analysis

Yasemin Bozkurt Varolgunes[1,2], **Joseph F. Rudzinski[1]**, **Alper Demir[2]**

[1]*Max Planck Institute for Polymer Research, Mainz, Germany*
[2]*Koc University, Istanbul, Turkey*

Allostery in proteins is a phenomenon in which the binding of a ligand induces alterations in the activity of remote functional sites. This can be conceptually viewed as point-to-point telecommunication in a networked communication medium, where a signal (ligand) arriving at the input (binding site) propagates through the network (interconnected and interacting atoms) to reach the output (remote functional site). The reliable transmission of the signal to distal points occurs despite all the disturbances (noise) affecting the protein. Based on this point of view, we propose a computational frequency-domain framework to characterize the displacements and the fluctuations in a region within the protein, originating from the ligand excitation at the binding site and noise, respectively. We characterize the displacements in the presence of the ligand, and the fluctuations in its absence. In the former case, the effect of the ligand is modeled as an external dynamic oscillatory force excitation, whereas in the latter, the sole source of fluctuations is the noise arising from the interactions with the surrounding medium that is further shaped by the internal protein network dynamics. We introduce the excitation frequency as a key factor in a Signal-to-Noise ratio (SNR) based analysis, where SNR is defined as the ratio of the displacements stemming from only the ligand to the fluctuations due to noise alone. We then employ an information-theoretic (communication) channel capacity analysis that extends the SNR based characterization by providing a route for discovering new allosteric regions. We demonstrate the potential utility of the proposed methods for the representative PDZ3 protein.

Exploring the effects of post-translational modifications through computational approaches.

G. Colombo[1,2] and M. Castelli[1]

[1]*University of Pavia - Department of Chemistry, Via Taramelli 12, 27100 Pavia (Italy)*
[2] *Istituto di Scienze e Tecnologie Chimiche "Giulio Natta" - SCITEC, CNR*
Via Mario Bianco 9, 20131 Milano (Italy)

Hsp90 plays a pivotal role in the folding pathway of client proteins implicated in diverse diseases, including cancer. This function requires the assembly of multiprotein complexes involving cochaperones and clients. Hsp Organizing Protein (Hop) is an essential cochaperone that facilitates substrate transfer between the Hsp70 and Hsp90 molecular chaperones. Hop functions are regulated through phosphorylation at unique sites[1]. Single point mutations are able to negatively affect the interaction with Hsp70, leaving Hsp90 affinity uncompromised. We investigated six different Y354 point mutations in order to elucidate atomic detailed mechanism at the base of the weaker affinity between phosphorylated-Hop (pHop) and Hsp70. We ran more than 60 μs of combined all-atom molecular dynamics simulation with the aim to extensively explore Hop dynamic behaviour. In this context, we analysed the essential dynamics of Hop through Principal Component Analysis (PCA) and its internal dynamics through Distance Fluctuation (DF) analysis. A consensus between these analyses and Clustering methodologies allows us to distinguish two different conformational ensembles among different mutations, namely an open and a closed conformation. Phosphorylated-Hop is the only variant that populates both the open and the closed state. The closed conformation is incompatible with the open functional form observed in multiproteic complexes [2]. To provide information on the kinetics of the outlined structural ensemble we are implementing stochastic methods to develop a Markov state model useful to the determination of a kinetic ensemble of WT Hop and pHop.

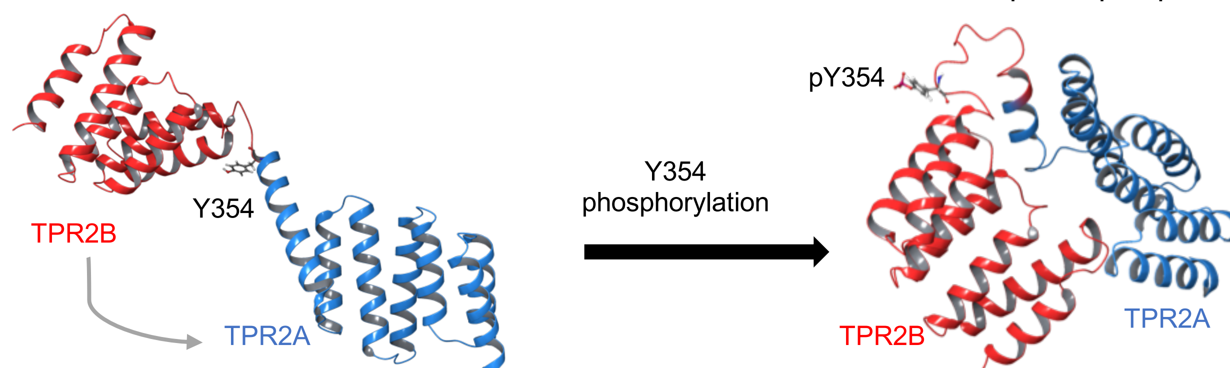


Figure 1: Schematic representation of transition from open to closed conformation.

References

[1] Buchner et al; EMBO reports, Vol 16, 2, (2015)
[2] D.A. Agard *et al*; bioRxiv (2020); (doi: https://doi.org/10.1101/2020.11.05.370247).

Inferring protein folding pathways from accelerated molecular dynamics

Liwei Chang[1] **and Alberto Perez**[1]

[1]*Department of Chemistry, University of Florida, Gainesville, FL 32611*

There are two puzzles encoded in a protein sequence: what is the 3D structure encoded by the sequence and the second what are the pathways that allow the protein to fold so fast. The first problem of protein structure has seen a recent breakthrough from machine learning. The second problem, the protein folding problem, remains a challenge both experimentally and computationally. All-atom molecular dynamics simulation has the potential to solve protein native structure and find folding pathways simultaneously, however, it is still very expensive even with specialized hardware. In this work, we explore new ways of predicting pathways using advanced sampling strategies. We build on the successes of our MELD (Modeling Employing Limited Data) pipeline in structure prediction to infer folding pathways within hours to days using standard GPUs. The strategy incorporates native structure information as restraints to quickly draw inference on intermediates along protein folding pathways. We have tested our method on protein G & L and their mutants, the result correctly captures mutation effect on folding behavior for both pair of proteins and agrees well with latest experiment.

Machine Learning Implicit Solvent Method for Molecular Dynamics

Yaoyi Chen[1], Andreas Krämer[1], Nicholas E. Charron[2, 3, 4], Brooke E. Husic[1], Cecilia Clementi[2, 3, 4, 5], and Frank Noé[1, 4, 5]

[1] *Department of Mathematics and Computer Science, Freie Universität, Berlin, Germany*
[2] *Department of Physics, Rice University, Houston, Texas 77005, USA*
[3] *Center for Theoretical Biological Physics, Rice University, Houston, Texas 77005, USA*
[4] *Department of Physics, Freie Universität, Berlin, Germany*
[5] *Department of Chemistry, Rice University, Houston, Texas 77005, USA*

Accurate modeling of the solvent environment for biological molecules is crucial for computational biology and drug design. In order to achieve longer time scales for larger system sizes in molecular dynamics (MD) simulations, it is common practice to incorporate implicit solvent models to reduce computational cost. However, current models are often insufficiently accurate compared to their explicit solvent counterparts. In the present work, we develop a more accurate implicit solvent method for MD simulations based on the theory of multiscale coarse-graining. Inspired by the machine-learned coarse-grained force field CGSchNet [1], we use a graph neural network (ISSNet) to model the implicit solvent potential of mean force. ISSNet can learn from explicit solvent simulation data and be readily applied to MD simulations. We compare the solute conformational distributions under different solvation treatments for two peptide systems. The results indicate that ISSNet models can outperform widely-used generalized Born and surface area models [2] in reproducing the thermodynamics of small protein systems with respect to explicit solvent. The success of this novel method demonstrates the potential benefit of applying machine learning methods in accurate modeling of solvent effects for in silico research and applications in biomedical disciplines.

References

[1] B. E. Husic, N. E. Charron, D. Lemm, J. Wang, A. Pérez, M. Majewski, A. Krämer, Y. Chen, S. Olsson, G. de Fabritiis, F. Noé, and C. Clementi, J. Chem. Phys. **153**, 194101 (2020)

[2] A. Onufriev, D. Bashford, and D. A. Case, Proteins **55**, 383 (2004)

Biophysical Characterization of Interactions of T Cell Receptor with Viral Antigens derived from Lymphocytic Choriomeningitis Virus

Moumita Dasgupta, **Brian M. Baker**

Department of Chemistry and Biochemistry, Harper Cancer Research Institute, University of Notre Dame, Notre Dame, IN 46617

How T-cell receptors (TCRs) bind peptide/major histocompatibility complexes (pMHC) and their associated structural dynamics is of crucial importance for understanding cellular immunity. For example, recognition of viral antigens present on infected cells by cytotoxic T lymphocytes is necessary for clearance of the viral infections. However, to evade anti-viral immune responses, viral escape variants with single amino acid mutations in key antigens occurs. The current study investigates the binding affinities and structural changes associated with the interactions of the P14 TCR with different peptide epitopes derived from the lymphocytic choriomeningitis virus (LCMV) presented by the class I MHC protein H-2D^{b}. As, *in vivo*, TCR-pMHC interactions are also under tensile forces which can increase the lifetime of the protein-protein complex, we also explore the formation of "catch bonds" between the TCR and pMHC, and how these are modulated by viral escape variants. Altogether, our study aims to identify the intersection between binding affinity, structural dynamics, and catch bonds in order to help develop a comprehensive model incorporating parameters which influence T cell recognition.

HOW GLOBAL MOTIONS ARE INVOLVED IN THE DEACTIVATION OF COMPLEX I

Andrea Di Luca[1] **and Ville R.I. Kaila**[2]

[1]Department of Biology, University of Fribourg,
Chemin du Museé 10, 1700 Fribourg, Switzerland
[2] Department of Biochemistry and Biophysics, University of Stockholm,
Svante Arrhenius väg 16C, 10691 Stockholm, Sweden

Complex I (NADH:ubiquinone oxidoreductase) is a redox-driven proton pump that serves as a primary electron entry point in aerobic respiratory chains. In absence of substrate, complex I undergoes in some species an active-to-deactive (A/D) transition, entering a deactive state (D) that shows a low turnover rate [1]. Recent Cryo-EM structures of the complex in both A and D states shed light on some of the conformational changes involved in this transition [2,3]. By employing Elastic Network Models (ENM), we highlight how these structural changes are related to low-frequency collective motions of the enzyme [4,5]. We identify high-strain regions of the enzyme during the transition and relate these to the elements that may transduce redox energy into the proton pumping machinery.

References

[1] Blaza JN, Vinothkumar KR, Hirst J. Structure of the Deactive State of Mammalian Respiratory Complex I. Structure **26**:312–319 (2018)

[2] Zhu J, Vinothkumar KR, Hirst J. Structure of mammalian respiratory complex I. Nature **536**:354–358 (2016)

[3] Agip AA, Blaza JN, Bridges HR, Viscomi C, Rawson S, Muench SP, Hirst J. Mitochondria in Two Biochemically Defined States. Nat Struct Mol Biol **25**:548–556 (2018)

[4] Di Luca A, Kaila VRI. Global collective motions in the mammalian and bacterial respiratory complex I. BBA-Bioenerg **1859**:326–332 (2018)

[5] Di Luca A, Kaila VRI. Molecular strain in the active/deactive-transition modulates domain coupling in the respiratory complex I. BBA-Bioenerg. *In press.*

Structural and functional explorations of the MICOS complex

Kathrin Funck[1,2], Manuel Hessenberger[3], Tobias Bock-Bierbaum[1], Oliver Daumke[1,2]

[1]Max-Delbrück-Center for Molecular Medicine, Robert-Rössle-Straße 10, 13125 Berlin, Germany
[2] Institute of Biochemistry, Freie Universität Berlin, Takustraße 6, 14195 Berlin, Germany
[3] Karl Landsteiner Privatuniversität für Gesundheitswissenschaften, Dr.-Karl-Dorrek-Straße 30, 3500 Krems an der Donau, Austria

Mitochondria are highly dynamic organelles, which are involved in many vital biological processes including ATP production and programmed cell death. Defects in mitochondrial dynamics are linked to neurodegenerative diseases such as Parkinson's disease. The mitochondrial contact site and cristae organizing system (MICOS) complex is a large protein complex located in the inner mitochondrial membrane close to crista junctions. MICOS has a crucial function in mitochondrial membrane architecture and ensures that the cristae membrane remains attached to the inner boundary membrane. This hetero-oligomeric protein complex consists of at least six components in yeast, which can be divided into two subcomplexes: Mic10/Mic26/Mic27/Mic12 and Mic60/Mic19. However, the exact composition of MICOS as well as the interaction interfaces have not been clarified yet.
The Daumke group analyzed the interaction between Mic60 and Mic19 and found that the *Chaetomium thermophilum* (thermophilic fungus) Mic60-Mic19 subcomplex can deform liposomes into thin tubules in vitro. Furthermore, a lipid binding site crucial for mitochondrial ultrastructure was identified in the soluble intermembrane space-exposed part of Mic60. The aim of my PhD thesis is to continue the investigation of the architecture of the MICOS complex and to determine the mechanism by which MICOS contributes to the formation of crista junctions. Here, I will present my latest results of the interaction between Mic60 and Mic19 and discuss how Mic60 might function in the formation of crista junctions. Characterization of the MICOS complex will contribute to a molecular understanding of neurodegenerative diseases, in which MICOS components are over-expressed or mutated.

References

[1] M. van der Laan et al., Curr Opin Cell Biol **41**, 33-42 (2016)
[2] N. Pfanner et al., J Cell Biol **204**, 1083-1086 (2014)
[3] M. Hessenberger et al., Nat Commun **8**, 15258 (2017)

Nanobody interaction unveils structure, dynamics and proteotoxicity of the Finnish-type amyloidogenic gelsolin variant

Toni Giorgino [a,b], Davide Mattioni [a,c,1], Amal Hassan [b,1], Mario Milani [a,b], Eloise Mastrangelo [a,b], Alberto Barbiroli [d], Adriaan Verhelle [e], Jan Gettemans [f], Maria Monica Barzago [c], Luisa Diomede [c], Matteo de Rosa [a,b]

[a] Istituto di Biofisica, Consiglio Nazionale delle Ricerche, Milano, Italy
[b] Dipartimento di Bioscienze, Università degli Studi di Milano, Milano, Italy
[c] Department of Molecular Biochemistry and Pharmacology, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, 20156 Milan, Italy
[d] Dipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente, Università degli Studi di Milano, Milano, Italy
[e] Department of Molecular Medicine, Department of Molecular and Cellular Neuroscience, Dorris Neuroscience Center, The Scripps Research Institute, La Jolla, CA 92037, USA
[f] Nanobody Lab, Department of Biochemistry, Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium
[1] These authors contributed equally to this work.

AGel amyloidosis, formerly known as familial amyloidosis of the Finnish-type, is caused by pathological aggregation of proteolytic fragments of plasma gelsolin. So far, four mutations in the gelsolin gene have been reported as responsible for the disease. Although D187N is the first identified variant and the best characterized, its structure has been hitherto elusive. Exploiting a recently-developed nanobody targeting gelsolin, we were able to stabilize the G2 domain of the D187N protein and obtained, for the first time, its high-resolution crystal structure. In the nanobody-stabilized conformation, the main effect of the D187N substitution is the impairment of the calcium binding capability, leading to a destabilization of the C-terminal tail of G2. However, molecular dynamics simulations show that in the absence of the nanobody, D187N-mutated G2 further misfolds, ultimately exposing its hydrophobic core and the furin cleavage site. The nanobody's protective effect is based on the enhancement of the thermodynamic stability of different G2 mutants (D187N, G167R and N184K). In particular, the nanobody reduces the flexibility of dynamic stretches, and most notably decreases the conformational entropy of the C-terminal tail, otherwise stabilized by the presence of the Ca2+ ion. A Caenorhabditis elegans-based assay was also applied to quantify the proteotoxic potential of the mutants and determine whether nanobody stabilization translates into a biologically relevant effect. Successful protection from G2 toxicity in vivo points to the use of C. elegans as a tool for investigating the mechanisms underlying AGel amyloidosis and rapidly screen new therapeutics.

An unusual aspartic acid cluster in the reovirus attachment fiber σ1 mediates stability at low pH

Glorani G., **Ruwolt M., Holton N., Neu U.**

1 Freie Universität Berlin, Institute for Chemistry and Biochemistry, Biochemistry of Viruses, Takustr. 6, D-14195 Berlin, Germany

Reovirus encounters different environments as it migrates through the gastrointestinal tract before infecting susceptible cells in the gut epithelium [1]. Following engagement of mammalian glycans and JAM-A by the attachment protein σ1, reovirus is internalized into the endosomes [2-3]. σ1 is a trimeric filamentous protein anchored to the viral capsid at its N-terminus, followed by an α-helical coiled-coil region named Tail, a tightly entwined Body, and a globular Head [4]. During infection, σ1 undergoes conformational changes. An attractive candidate for a molecular switch is the highly conserved aspartic acid cluster forming the trimer interface at the bottom of the σ1 Head domain. According to structural data and modelling, at neutral pH, D345 in the aspartic acid cluster must be unusually protonated to accomplish σ1 trimer stabilization. However, there is no direct evidence on how changes in environmental pH affect σ1 conformation, stability, and trimeric organization [5-7].
We have recombinantly expressed and purified different T3D σ1 proteins spanning the Head and Body-Head domains, both wild-type sequences and carrying the mild D345N mutation, and investigated their thermostability and oligomerization state over a broad range of pH, including the very low pH of the stomach. We then determined their melting temperatures via differential scanning fluorimetry, identifying the pH of optimum stability of all the constructs to be 5, a value close to the pH of the endosome and jejunum. By performing analytical size exclusion chromatography we found that the T3D σ1 Head detrimerizes at high pH, confirming protonation of the aspartic acid cluster. The effect of the D345N mutation at the trimer interface was found to particularly influence the stability of σ1 trimer at neutral and low pH, upon incubation at room temperature.
Altogether, our findings suggest that the aspartic acid at the trimer interface of the σ1 Head domain is required for its trimeric stability, especially in a low pH environment.

References

[1] Di Cani, Gut, 2018
[2] Prota, Proc Natl Acad Sci, 2003
[3] Chappell, J. Virol, 1997
[4] Reiter, PLoS Pathogen, 2011
[5] Chapell, EMBO J, 2002
[6] Campbell, J. Virol, 2005
[7] Cavalli, Biophys J, 2004

Temperature jump X-ray crystallography to analyze protein dynamics in macromolecular crystals

S. Günther[1], A. Tolstikova[1], O. Yevanov[1], H. Chapman[1], R. Henning[2], M. Levantino[3], and A. Meents[1]

[1]Center for Free-Electron Laser Science, Hamburg, Germany
[2]Center for Advanced Radiation Sources, The University of Chicago, Chicago, USA
3 European Synchrotron Radiation Facility, Grenoble, France.
E-mail: sebastian.guenther@desy.de

Protein dynamics are the basis of protein function, whether it is binding to ligands or facilitating enzymatic reactions. Motions on the femtosecond to millisecond scale determine how proteins behave ranging from bond vibrations to large scale domain movements. X-ray crystallography offers the highest spatial resolution of macromolecules. However, these structures represent only a temporally averaged snapshot of the most populated conformations. Various other methods are available that probe protein dynamics experimentally. But they lack high spatial resolution. Time-resolved X-ray crystallography has begun to discern transient conformations of macromolecular structures. Here, the system is removed from is equilibrium by laser light or addition of ligands. While the temporal resolution of ligand binding is generally hampered by the diffusion of ligands into the crystal, mostly to the millisecond range, activation by laser light can resolve femtosecond processes.
Infrared laser-induced temperature jump combined with X-ray solution scattering demonstrated to be able to unveil hidden conformational dynamics in an enzyme [1]. In contrast to solution scattering, X-ray crystallography provides a much higher spatial resolution down to the atomic level. Here we combine temperature jump of protein crystals with pink beam serial crystallography to remove the well characterized enzyme Ribonuclease A from its conformational equilibrium. We take snapshots of the structure from micro- to milliseconds to follow the changes in the protein crystals. The first analysis shows no major shift in the observed conformation of the protein. Globally we detect an increase in the average atomic displacement ("temperature") factor reaching a maximum within a few microseconds and a cool-down after a few milliseconds. A closer look at individual residues reveals an unequal rise and fall of temperature factors within the protein. If these time-resolved changes indeed reflect the dynamic behavior of RNase A, has yet to be established. This method has the potential to shed light on the protein dynamics of virtually any crystallizable protein at high spatial and temporal resolution.

References

[1] Thompson, M.C., Barad, B.A., Wolff, A.M. et al. Temperature-jump solution X-ray scattering reveals distinct motions in a dynamic enzyme. Nat. Chem. **11**, 1058–1066 (2019). https://doi.org/10.1038/s41557-019-0329-3F

Allosteric communication in Class A β-lactamases

I. Galdadas[1], S. Qu[1], A.S.F. Oliveira[2], E. Olehnovics[1], A.R. Mack[3], M.F. Mojica[3], P.K. Agarwal[4], C.L. Tooke[2], F.L. Gervasio[5], J. Spencer[2], R.A. Bonomo[3], A.J. Mulholland[2] and S. Haider[1]

[1]*University College London, London, UK*
[2] *University of Bristol, Bristol, UK*
[3]*Case Western Reserve University, Cleveland, USA*
[4]*Oklahoma State University, Stilwater, USA*
[5]*University of Geneva, Switzerland*

The rise in antimicrobial resistance is a growing global public health crisis. New strategies to overcome resistance are urgently needed, which will ultimately be facilitated by a deeper understanding of the mechanisms that regulate β-lactamases. Understanding allostery and tools to identify it, would offer promising alternative strategies to inhibitor development. Through a combination of equilibrium and nonequilibrium molecular dynamics simulations, we identify allosteric effects and communication pathways in two prototypical class A β-lactamases, TEM-1 and KPC-2, which are important determinants of antibiotic resistance. This study uses a combination of equilibrium and nonequilibrium simulations to reveal allosteric communication networks and how allosteric ligands affect enzyme activity. The nonequilibrium simulations reveal pathways of communication operating over distances of 30 Å or more, where the propagation of the signal occurs through cooperative coupling of loop dynamics. The results predict positions of clinically observed variation relating to the spectrum of action of these enzymes. This suggests that clinically important variation may affect, or be driven by, differences in allosteric behavior, providing a mechanism by which amino acid substitutions may affect the relationship between spectrum of activity, catalytic turnover and potential allosteric behavior in this clinically important enzyme family.

Role of the internal loops in gating of outer membrane porins

Nandan Haloi,[†,‡] Archit Kumar Vasan,[†,‡] Po-Chao Wen,[†,‡,¶] Paul Hergenrother,[§] Diwakar Shukla,[‖,†,‡] and Emad Tajkhorshid,[¶,†,‡]

[†]NIH Center for Macromolecular Modeling and Bioinformatics, Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign, Urbana, IL61801, USA
[‡]Center for Biophysics and Quantitative Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

[¶]Department of Biochemsistry, University of Illinois at Urbana-Champaign, Urbana, IL61801, USA

[§]Department of Chemistry, University of Illinois at Urbana-Champaign, Urbana, IL 61801,USA

[‖]Department of Chemical & Biomolecular Engineering, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

Multi-drug resistant Gram-negative bacteria are becoming an increasing public health concern and the development of new drugs that can readily traverse the lipopolysaccharide-coated outer membrane (OM) of these pathogens is very challenging. Antibiotics that are able to traverse this OM typically do so through OM porins; thus, understanding the permeation properties of these OM porins would be instrumental to the development of new antibiotics. A key feature of several OM porins is their ability to undergo conformational changes between functionally distinct open and closed states to regulate transport. It has been suggested for many of these porins, such as OmpF in *E. coli*, that these conformational changes are mediated by dynamics of internal loop, L3; however, support for this hypothesis remains limited. Using extensive molecular dynamics simulations and Markov state models, we show that a large-scale motion of L3 indeed gives rise to distinct open and closed states of the pore. We found that gating transitions are mediated by two acidic residues E117 and D121in L3. Movement of E117 to a cluster of basic residues (B-face) mediates transition from the open state and the movement of D121 to the B-face mediates complete closure of the pore. The key residues involved in the gating transitions are highly conserved across diffusion porins from various gram-negative bacteria suggesting the generality of our results. Additionally, mutations of several key B-face residues to neutral or acidic residues have been shown to increase substrate permeability in previous experiments. Our results suggest that these mutations reduce the E117 and D121 attraction towards the B-face and hence decrease the probability of pore closure transitions, leading to an increased likelihood of the open state. This hypothesis was further tested by introducing mutations in the B-face residues of OmpF. Overall, we propose a model indicating that permeability of OM porins depends on dynamic equilibrium between open and closed conformations. This model provides novel mechanistic insight on the mechanisms by which OM porins might confer antibiotic resistance to gram-negative bacteria.

Molecular mechanism of inhibiting the SARS-CoV-2 cell entry facilitator TMPRSS2 with camostat and nafamostat

Tim Hempel[1,2], Lluís Raich[1,†], Simon Olsson[1,3,†], Nurit P. Azouz[4,5], Andrea M. Klingler[4], Markus Hoffmann[6,7], Stefan Pöhlmann[6,7], Marc E. Rothenberg[4], and Frank Noé[1,2,8,*]

[1]*Freie Universität Berlin, Department of Mathematics and Computer Science, Berlin, Germany*

[2]*Freie Universität Berlin, Department of Physics, Berlin, Germany*

[3] *Chalmers University of Technology, Department of Computer Science and Engineering, Göteborg, Sweden*

[4]*Division of Allergy and Immunology, Cincinnati Children's Hospital Medical Center, Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH, USA*

[5]*Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH, USA*

[6]*Infection Biology Unit, German Primate Center – Leibniz Institute for Primate Research, Göttingen, Germany*

[7]*Faculty of Biology and Psychology, University Göttingen, Göttingen, Germany*

[8]*Rice University, Department of Chemistry, Houston, TX, USA*

The entry of the coronavirus SARS-CoV-2 into human lung cells can be inhibited by the approved drugs camostat and nafamostat. Here we elucidate the molecular mechanism of these drugs by combining experiments and simulations. *In vitro* assays confirm that both drugs inhibit the human protein TMPRSS2, a SARS-Cov-2 spike protein activator. As no experimental structure is available, we provide a model of the TMPRSS2 equilibrium structure and its fluctuations by relaxing an initial homology structure with extensive 330 microseconds of all-atom molecular dynamics (MD) and Markov modeling. Through Markov modeling, we describe the binding process of both drugs and a metabolic product of camostat (GBPA) to TMPRSS2, reaching a Michaelis complex (MC) state, which precedes the formation of a long-lived covalent inhibitory state. We find that nafamostat has a higher MC population than camostat and GBPA, suggesting that nafamostat is more readily available to form the stable covalent enzyme–substrate intermediate, effectively explaining its high potency. This model is backed by our *in vitro* experiments and consistent with previous virus cell entry assays. Our TMPRSS2–drug structures are made public to guide the design of more potent and specific inhibitors.

[Published as *Hempel et al, Chem. Sci. 2021*, https://doi.org/10.1039/D0SC05064D.]

Predicting ion channel conductance from dissipation-corrected TMD and LE simulations

M. Jäger[1] and S. Wolf[1]

[1] *Biomolecular Dynamics, Institute of Physics, Albert Ludwigs University, 79104 Freiburg, Germany*

E-mail: miriam.jaeger@physik.uni-freiburg.de

Ion channels control information transfer and vital functions in the human body. To gain insight into their molecular mechanisms of ion transfer and to predict conductance characteristics, we here applied dissipation-corrected targeted MD [1] to potassium ions moving through a Gramicidin channel as test system. Performing a non equilibrium PCA on backbone dihedral angles we find coupled protein-ion dynamics occurring during ion transfer. Using free energies and friction profiles along the channel obtained from the targeted MD simulations as input for Langevin equation simulations [2] with an external electric field, we explicitly predict I-V curves as macroscopic observables. These curves exhibit good agreement with their experimental counterparts when taking into account polarization effects within the channel.

References

[1] Wolf, S., Stock, G., *J. Chem. Theory Comput.* **14**, 6175–6182 (2018)

[2] Wolf, S., et al., *Nat Commun* **11,** 2918 (2020)

Molecular Dynamics Simulations of Uranyl Adsorption on Montmorillonite Clay with Desferrioxamine-B

V. Erastova[1] and E. Kearney[2]

[1]University of Edinburgh, Edinburgh United Kingdom
[2]University of Edinburgh, Edinburgh United Kingdom

Uranyl (UO_2) is a key component of nuclear waste and its disposal is of principal concern due to its toxicity, radioactivity and long residence time. Clay minerals are an important site of research for uranyl remediation, as they are ubiquitous in the environment and have useful adsorptive properties, such as swelling as seen in montmorillonite clay. Desferrioxamine-B is an organic siderophore secreted by plants to increase iron uptake and is used to treat iron overdose in humans. It has been shown to complex uranyl via its hydroxamic acid groups.[1] This project seeks to examine the interlayer behavior of desferrioxamine-B using molecular dynamics, as this is hard to assess experimentally.[2] The overall aim of this study is to improve uranyl adsorption at high pH values.

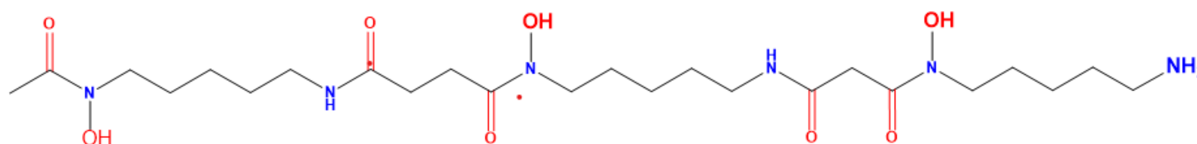


Figure 1 – Desferrioxamine-B. Charge variant groups in bold.

References

[1] L. Mullen, C. Gong, K. Czerwinski, J. Radioanal. Nucl. Chem., **273(3)**, 683-688 (2007)

[2] J. Suter, R. Anderson, C. H. Greenwall, P. Coveney, Jour. Mater. Chem., **19(17)**, 2482-2493 (2009)

Determinants of Antigenicity in Tumor Neoepitopes for the Development of Personalized/Multiple Peptide Vaccines

G. Keller[1], H. Ebrahimi-Nik[2], P. Srivastava[2], B. Baker[1].

1. Dept. of Chem & Biochem, Harper Cancer Research Institute, University of Notre Dame
2. Dept. of Immunology, University of Connecticut School of Medicine

Class I MHC proteins bind and present short peptides to T cells of the immune system. Vaccines incorporating peptides incorporating mutations present in the cancer genome, or “neoantigens,” represent a low-cost, broadly applicable type of cancer immunotherapy which can sensitize the immune system to tumors, break tolerance, and confer persistent immunologic memory with fine specificity. However, predicting which candidate neoantigens will likely be immunogenic is imprecise, which limits applicability in treating spontaneous tumors. We previously demonstrated prediction of peptide-MHC immunogenicity from physicochemical characteristics of the peptide in its structural context are more accurate than predictions from common sequence-based tools. Our estimation of these characteristics, however, is limited by available three-dimensional structures and the accuracy of computational structural predictions.

In this study, we identified a group of mutant peptides presented by class I MHC in a murine cancer model and rationalized their immunogenicity with molecular modeling, as predicted peptide binding affinity alone could not explain reactivity. After solving the crystal structure of one pair of neoantigens, we developed a method to improve peptide-MHC structural modeling using multiple model generation and post-selection based on energetic features of the peptide-MHC. When evaluated on 99 crystal structures of peptides bound to the prevalent human class I MHC, HLA-A*02:01, our method exhibited significantly higher accuracy than conventional approaches to model selection. These results are of significant interest for improving our ability to qualitatively determine the “differences from self” an MHC-presented peptides, but are also of translational interest in producing realistic structural and energetic signatures for predicting immunogenicity.

References

[1] H. Ebrahimi-Nik et al. 2019. Mass spectrometry–driven exploration reveals nuances of neoepitope-driven tumor rejection. JCI Insight.

NOE spin diffusion in large biomolecules

Christopher Kolloff[1], **Adam Mazur**[1], **Jan K. Marzinek**[2], **Peter J. Bond**[2], **and Sebastian Hiller**[1]

[1]Biozentrum, University of Basel, Basel, Switzerland
*[2] Bioinformatics Institute (BII), Agency for Science, Technology and Research (A*STAR), Singapore*

NMR spectroscopy provides a unique perspective on the structure, dynamics, and function of proteins. With the advent of biochemical methyl labelling techniques, it is possible to study fundamental biological processes of large proteins using NOE spectroscopy. The establishment of a comprehensive methyl–methyl network, however, requires measuring spectra at long mixing times, where the spin diffusion effect prevails. Although the spin density in methyl-labelled proteins is already significantly reduced, second-order magnetisation transfers render a quantitative interpretation of NOESY cross-peaks difficult. We explore the key factors that need to be accounted for in the calculation of experimentally observed NOESY cross-peaks using a combination of different structural templates, full-relaxation matrix analysis, MD-derived ^{1}H–^{1}H S^2 order parameters, and Markov state models. On the one hand, a structural representation is needed that reflects the time-averaged signal observed in NOESY spectra. On the other, it is important to discriminate between fast (sub-τ_c) and slow (supra-τ_c) conformational exchange to account for motional effects. Once established, this approach can be applied to a plethora of different studies, such as structure-based assignment methods or the investigation of conformational change.

Exchange catalysis by tapasin exploits conserved and allele-specific features of MHC-I molecules

Huan Lan[1], Esam T. Abualrous[1,5], Jana Sticht[1,2], Laura Maria Arroyo Fernandez[1], Tamina Werk[1], Christoph Weise[1,2], Martin Ballaschk[3], Peter Schmieder[3], Bernhard Loll[4] & Christian Freund[1]*

[1]*Laboratory of Protein Biochemistry, Institute for Chemistry & Biochemistry, Freie Universität Berlin, Thielallee 63, 14195 Berlin, Germany.*

[2]*Core Facility BioSupraMol, Institute for Chemistry & Biochemistry, Freie Universität Berlin, Takustr. 3, 14195 Berlin, Germany.*

[3]*Leibniz-Forschungsinstitut für Molekulare Pharmakologie, Robert-Roessle-Str. 10, 13125 Berlin, Germany.*

[4]*Laboratory of Structural Biology, Institute for Chemistry & Biochemistry, Freie Universität Berlin, Takustr. 6, 14195 Berlin, Germany.*

[5]*Artificial Intelligence for the Sciences, Department of Mathematics and Computer Science, Freie Universität Berlin, Arnimallee 14, 14195 Berlin, Germany.*

Abstract

The repertoire of peptides presented by major histocompatibility complex class I (MHC-I) molecules on the cell surface is tailored by the ER-resident peptide loading complex (PLC) that contains the exchange catalyst tapasin. Tapasin promotes the replacement of suboptimal antigens by high-affinity peptides and thereby fosters the formation of thermodynamically stable complexes fit to serve as T cell antigens once transported to the surface. Here, we analyzed the changes in MHC-I dynamics in the presence of tapasin by biophysical methods and propose the catalyst to exploit two essential features of MHC-I: First, tapasin recognizes a conserved allosteric site underneath the α_{2-1}-helix of MHC-I, “loosening” the F-pocket region that binds to the C-terminus of the peptide. Second, tapasin’s scoop loop$_{11-20}$ targets the F-pocket by its critical L18 residue, enabling competitive peptide binding. Residue K16, emerging later during evolution, plays an accessory role for MHC-I allotypes bearing an acidic F-pocket.

How can molecular modelling support de novo protein design, and vice versa?

Eric J. M. Lang[1], Derek N. Woolfson[1,2], and Adrian J. Mulholland[1]

[1] *School of Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS, UK*
[2] *School of Biochemistry, University of Bristol, University Walk, Bristol, BS8 1TD, UK*

By allowing to study engineered biomolecules at an atomic level of detail over a wide range of time scales, molecular modelling provides a unique opportunity, not only to support the design of *de novo* proteins, but also to help interpreting experimental results. Moreover, because they are highly tunable and can be reduced to minimal, stripped-down functioning units, *de novo* proteins can also foster the improvement of molecular modelling methods by testing the limit of their accuracy and applicability.

Here, we present recent examples of how molecular modelling can support de novo protein design and vice versa. First, we describe how molecular dynamics (MD) simulations and time-dependent quantum mechanical calculations helped to shed light on the molecular mechanisms that govern the binding of a fluorescent dye to a set of de novo α-helical barrels (αHBs) and to explain a puzzling induced circular dichroism band observed experimentally. We then detail how constant pH molecular dynamics has been used to rationalize the stability of αHBs designed with ionizable residues pointing toward their lumen. Finally, we show how a family of small de novo peptides presenting a highly controllable degree of helicity, can be used to probe the accuracy of protein force field and implicit solvent model combinations, providing important practical recommendations regarding the use of these modelling methods.

Advanced molecular dynamics simulations for drug discovery

S. Leoni[1], **D. Wragg**[2] **and A. Casini**[2]

[1]*Cardiff University, School of Chemistry, CF10 3AT, Cardiff, UK*

[2] *Department of Chemistry, Technical University of Munich, 85747 Garching bei München, Germany*

Drug discovery can nowadays rely on computational approaches, which can focus on specific drug binding sites and substrate-molecule interactions modes. While the molecular docking paradigm can provide some guidance in identifying and developing novel drugs, a complete mapping of the free energy of drug-substrate interaction is an emerging paradigm towards rational drug design. The free energy landscape paradigm allows for an explicit assessment of factors affecting interaction strength, including entropic contributions, which are essential to understand binding mode competition, as well as conformational degrees of freedom and the role of solvation. To exemplify this paradigm, we have focused on DNA secondary structure G-quadruplexes [1], which can control oncogene expression, and are therefore important targets in cancer research. The specific binding of gold carbene complexes to G4 was investigated by metadynamics, to assess drug-substrate bonding specificity and variety of binding poses. Metadynamics explores chemical processes along selected reaction coordinates, so-called collective variables (CV), efficiently achieving comprehensive scans of the totality of binding modes over the whole DNA G4 system. This makes for an overall unbiased mechanistic exploration of the system of interest, one that not only allows for a detailed investigation of binding mechanisms, but also includes the relative free energy ranking of all possible binding modes. Computationally, the strategy can be focused on identifying suitable CVs, which can drive the exploration of local conformational changes and global binding modes. This provides a simulation platform, which can be used to screen novel drugs, by testing their competition directly on the substrate, to identify the best candidate. Furthermore, the approach can be transferred to other systems [2-4], including membrane proteins, for a complete reconnaissance of mechanisms underlying small molecule drug transport and their action.

References

[1] D. Wragg, A. de Almeida, R. Bonsignore, F. E. Kühn, S. Leoni, A. Casini, Angew. Chem. Int. Ed. **57**, 14524 (2018).

[2] D. Wragg, A. de Almeida, A. Casini, S. Leoni, Chemistry **25**, 8713 (2019).

[3] D. Wragg, A. Casini, S. Leoni, Advances in Bionanomaterials II, 64 (2019).

[4] D. Wragg, S. Leoni, A. Casini, RSC Chemical Biology **1**, 390 (2020).

Polar/apolar interfaces modulate the conformational and kinetic behavior of cyclic peptides and catalyze their passive membrane permeability

S. M. Linker [1], C. Schellhaas[1], B. Ries[1] and S. Riniker[1]

[1] Laboratory of Physical Chemistry, ETH Zürich, Zürich, Switzerland
E-mail: linker@phys.chem.ethz.ch

Cyclic peptides are attractive alternatives to linear peptides as therapeutics to target proteins with large and flat binding sites because they promise better passive cell membrane permeability and metabolic stability [1,2]. However, cyclization is often not sufficient for peptidic drugs to have oral bioavailability and designing peptides with good membrane permeability and solubility remains a challenge.

We use molecular dynamics simulations and Markov state models (MSM) of cyclic peptides in order to rationalize the origin of their membrane permeability. Previous work has shown that an important determinant for this permeability is a peptide's conformational behavior in the water phase [3]. In this study, we focus on the conformational behavior of cyclic peptides at the polar/apolar interface of water and chloroform that mimics the cell membrane. Our findings support the hypothesis that only peptides in the so-called 'closed' conformation, where polar groups are shielded from the environment, are able to permeate through the apolar region of a membrane.

In addition to internal coordinates such as backbone torsions, we included external coordinates in our MSM that describe the positioning of the peptide in respect to the interface. The inclusion of these external coordinates was crucial to detect two states with distinct kinetic properties in the MSM. Those two states share the same peptide conformation but differ in the positioning of the peptide in respect to the interface. The polar/apolar interface catalyzes the interconversion from an open to the closed conformation, a key event for permeation. Based on our simulations, we show how specific amino acids and different local environments influence this closing event. These findings highlight the role of interfaces in protein and peptide dynamics and may be helpful in the design of future generations of membrane permeable cyclic peptides.

References

[1] C. Adessi, C. Soto, Current medicinal chemistry **9**, 963-978, (2002)
[2] A. T. Bockus, et al. Journal of medicinal chemistry **58**, 4581-4589 (2015)
[3] J. Witek, et al. Journal of chemical information and modeling **59**, 294-308 (2018)

Investigating the dissociation process and Binding energy of the DBD-p53/DNA complex by PaCS-MD and MSM

Mohamed Marzouk[1,2] and Akio Kitao[1]

[1] Tokyo Institute of Technology, Tokyo, Japan

[2] Ain Shams University, Cairo, Egypt

The binding of biological molecules to other molecules plays a crucial role in understanding their function, in mechanisms of diseases, and in the development of drug design. The binding of the p53 DNA binding domain (DBD-p53) to DNA is essential for the defensive role of p53 as a “guardian of the genome” against cancer. Recently, our group used the enhanced sampling technique, parallel cascade selection molecular dynamics (PaCS-MD)[1] in combination with a Markov state model (MSM) to generate dissociation pathways of the small complex MDM2/p53 transactivation domain (TAD-p53), and then calculated binding free energy and kinetic rates in good agreement with experimental values[2]. However, applying such a method and observing the dissociation pathways for larger systems is still challenging. Therefore, in the current study, we used the same technique to study the dissociation pathways of DBD-p53, which binds to a specific sequence of DNA, forming a DBD-p53/DNA complex. We used 10 parallel MD simulations in PaCS-MD to generate 75 dissociation pathways of the complex, which allowed us to investigate the key residues that have impact during the dissociation process. Then, using vector coordinates as reaction coordinates, we built 3D MSM, to generate free energy landscape of the dissociation of the complex. Our results showed free-energy-favorable directions during the dissociation. The combination of PaCS-MD/MSM was shown to be useful in the investigation of dissociation pathways of a large protein/DNA complex and can assist in the calculation of binding energy as well.

References

1. Harada, R. & Kitao, A. *J. Chem. Phys.* **139**, (2013).
2. Tran, D. P. & Kitao, A. *J. Phys. Chem. B* **123**, 2469–2478 (2019).

Deep learning protein conformational space with convolutions and latent interpolations

S. C. Musson[1], V. K. Ramaswamy[1], C. G. Willcocks[2], M. T. Degiacomi[1]

[1]Department of Physics, Durham University, Durham DH1 3LE
[2] Department of Computer Science, Durham University, Durham, DH1 3LE

Determining the different conformational states of a protein and the transition paths between them is key to fully understanding the relationship between biomolecular structure and function. This can be accomplished by sampling protein conformational space with molecular simulation methodologies. Despite advances in computing hardware and sampling techniques, simulations always yield a discretized representation of this space, with transition states undersampled proportionally to their associated energy barrier. We present a convolutional neural network that learns a continuous conformational space representation from example structures, and loss functions that ensure intermediates between examples are physically plausible. We show that this network, trained with simulations of distinct protein states, can correctly predict a biologically relevant transition path, without any example on the path provided. We also show we can transfer features learned from one protein to others, which results in superior performances, and requires a surprisingly small number of training examples.

References

[1] V. K. Ramaswamy, S. C. Musson, C. G. Willcocks, M. T. Degiacomi. Phys. Rev. X **11**, 011052 (2021)

MSMPathfinder: Finding Pathways of Markov State Models

D. Nagel[1], **A. Weber**[1], **and G. Stock**[1]

[1]*Institute of Physics, Albert-Ludwigs-University Freiburg*

In numerous fields of research, the population dynamics of states is described in terms of a master equation or a Markov state model (MSM). Given Markovian dynamics, we can define a transition matrix T_{ij} for a certain lagtime τ_{lag} which determines completely the time evolution of the system. Often we are interested in the pathways of the MSM, that lead from an initial to a final state. E.g., in protein folding these paths account for the mechanism the molecular reaction evolves. These paths naturally arise in a Markov chain Monte Carlo simulation, where we draw random numbers which determine the next step depending on the transition matrix T_{ij}. The catch is the slow convergence.

As a remedy, Vanden Eijnden and co-workers have proposed transition path theory. However, this method is designed to only give the most important pathways correctly. In systems of biological interest, e.g. protein folding, allostery, etc., many pathways may arise, and may also be important to understand the underlying mechanism. To cope with these problems, we suggest a new method—MSMPathfinder—which directly considers the path probabilities. In contrast to Markov chain Monte Carlo, it samples the path space more efficiently and gives a well-defined error. We demonstrate the performance and discuss the insights revealed by adopting the folding of villin headpiece.

References

[1] D. Nagel, A. Weber, and G. Stock, J. Chem. Theory Comput., 16, 7874 (2020)

Snapshots of native pre-50S ribosomes reveal a biogenesis factor network and evolutionary specialization

Rainer Nikolay[1], Tarek Hilal[2], Sabine Schmidt[3], Bo Qin[1], David Schwefel[1], Jörg Bürger[4], Thorsten Mielke[4], Justus Lörke[1], Elke Deuerling[3] and Christian Spahn[1]

[1]Institut für Medizinische Physik und Biophysik, Charité – Universitätsmedizin Berlin, Berlin, Germany

[2]Freie Universität Berlin, Research centre for electron microscopy, Fabeckstr. 36a, 14195 Berlin, Germany

[3]Molekulare Mikrobiologie, Universität Konstanz, Germany

[4]Microscopy and Cryo-Electron Microscopy Service Group, MPI for Molecular Genetics, Berlin, Germany

The biogenesis of small and large ribosomal subunits is a multistep process that involves trans-acting factors, referred to as ribosome biogenesis factors. While in eukaryotic systems numerous of these factors have been identified and analyzed genetically, biochemically and structurally in the past two decades, the function and molecular mechanism of biogenesis factors in prokaryotic systems remains mostly unexplored. Here, we used an E. coli strain harboring the biogenesis factor ObgE fused with a StrepII affinity-tag to isolate authentic pre-50S particles, which were subjected to cryo-EM analysis. Using multiparticle refinement, we obtain a variety of states allowing us to visualize their molecular evolution.

In addition to ObgE, we identify the factors YjgA, RluD and RsfS, which altogether form a network of interacting biogenesis factors that assists in the transitioning of a pre-50S particle to maturity.

Even though the final processes in the maturation of bacterial pre-50S and eukaryotic pre-60S subunits are apparently very similar, the involved factors differ substantially, which further opens new avenues for the development of molecular therapies targeting ribosome assembly.

Quantification and demonstration of the constriction-by-ratchet mechanism in the dynamin molecular motor

Oleg Ganichkin[a], Renee Vancraenenbroeck[b], Gabriel Rosenblum[c], Hagen Hofmann[c], Alexander S. Mikhailov[d], Oliver Daumke[a,e], and Jeffrey K. Noel[a]

[a]*Max Delbrück Center for Molecular Medicine, Berlin, Germany*
[b]*University College, London, United Kingdom*
[c]*Weizmann Institute, Rehovot, Israel*
[d]*WPI Nano Life Science Institute, Kanazawa, Japan*
[e]*Free University of Berlin, Berlin, Germany*

Dynamin oligomerizes into helical filaments on tubular membrane templates and, through constriction, cleaves them in a GTPase-driven way. Structural observations of GTP-dependent cross-bridges between neighboring filament turns have led to the suggestion that dynamin operates as a molecular ratchet motor. However, the proof of such mechanism remains absent. Particularly, it is not known whether a powerful enough stroke is produced and how the motor modules would cooperate in the constriction process. Here [1], we characterized the dynamin motor modules by single molecule (sm) FRET and found strong nucleotide-dependent conformational changes. Integrating smFRET with molecular dynamics simulations allowed us to determine the forces generated in a power stroke. Subsequently, the quantitative force data and the measured kinetics of the GTPase cycle were incorporated into a model (characterized in the absence of GTP here [2]) including both a dynamin filament, with explicit motor cross-bridges, and a realistic deformable membrane template. In our simulations, collective constriction of the membrane by dynamin motor modules, based on the ratchet mechanism, is directly reproduced and analyzed. Functional parallels between the dynamin system and actomyosin in the muscle are seen. Through concerted action of the motors, tight membrane constriction to the hemi-fission radius can be reached. Our experimental and computational study provides an example of how collective motor action in megadalton molecular assemblies can be approached and explicitly resolved.

References

[1] Noel JK, et al. bioRxiv doi: https://doi.org/10.1101/2020.09.10.289546

[2] Noel JK, Noe F, Daumke O, and Mikhailov AS (2019) Polymer-like model to study the dynamics of dynamin filaments on deformable membrane tubes. Biophys. J. 117, 1870-91.

On the impact of Tyr-39 for the structural features of α-synuclein and for the interaction with small molecules

Oscar Palomino-Hernandez[#,1,3,7,8], **Fiamma A. Buratti**[#,2], **Pamela S. Sacco**[2], **Giulia Rossetti**[1,4,5], **Paolo Carloni**[*,1,3,6], **Claudio O. Fernández**[*,2,9]

[1] *Institute for Neuroscience and Medicine (INM-9) and Institute for Advanced Simulations (IAS-5), Forschungszentrum Jülich, 52425 Jülich, Germany.*
[2] *Max Planck Laboratory for Structural Biology, Chemistry and Molecular Biophysics of Rosario (MPLbioR, UNR-MPIbpC) and Instituto de Investigaciones para el Descubrimiento de Fármacos de Rosario (IIDEFAR, UNR-CONICET), Universidad Nacional de Rosario, Rosario, Argentina.*
[3] *Faculty of Mathematics, Computer Science and Natural Sciences, RWTH Aachen, 52425 Aachen, Germany.*
[4] *Department of Oncology, Hematology, Oncology, Hemostaseology, and Stem Cell Transplantation University Hospital Aachen, RWTH Aachen University, Pauwelsstraße 30, 52074 Aachen, Germany.*
[5] *Jülich Supercomputing Center (JSC), Forschungszentrum Jülich, 52425 Jülich, Germany*
[6] *Institute for Neuroscience and Medicine (INM-11) Forschungszentrum Jülich, 52425 Jülich, Germany.*
[7] *Computation-based Science and Technology Research Center, The Cyprus Institute, Nicosia 2121, Cyprus.*
[8] *Institute of Life Science, The Hebrew University of Jerusalem, Jerusalem 91904, Israel.*
[9] *Department of NMR-based Structural Biology, Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, D-37077 Göttingen, Germany.*
[#] *These authors contributed equally to the manuscript.*

Recent studies suggest that the N-terminal region of α-synuclein (αS) plays a critical role for both the normal function and pathological dysfunction involved in Parkinson's disease.

In this work, we perform expanded ensemble simulations for a comparative analysis between structural features of human αS and its Y39A, Y39F and Y39L variants. We show that removing aromatic functionality at position 39 of monomeric αS lead to protein variants populating more compact conformations, conserving its disordered nature and secondary structure propensities.

Contrasting with the subtle changes induced by mutations on the protein structure, removing aromaticity at position 39 impacts strongly on the interaction of αS with the potent amyloid inhibitor phthalocyanine tetrasulfonate (PcTS). Parallel-tempering well-

tempered metadynamics simulations shed light on the nature of the binding on position 39, and its selectivity with other aromatic moieties in fragments of monomeric αS.

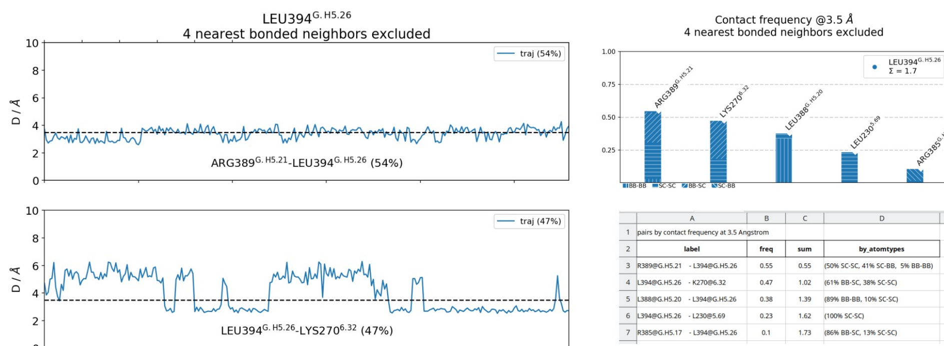
Our findings further support the role of Tyr-39 in forming essential inter and intramolecular contacts that might have important repercussions for the function and dysfunction of αS, and the nature of ligand binding in position 39 for αS.

mdciao: Analysis of Molecular Dynamics Simulations

Using Residue Neighborhoods

G. Pérez-Hernández[1] **and P. W. Hildebrand**[1,2]

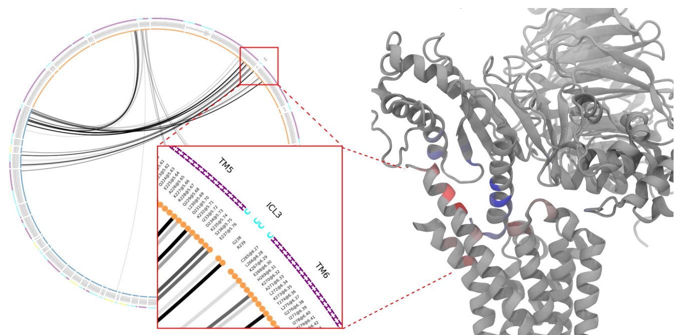
[1]Institute for Medical Physics and Biophysics, Charité Universitätsmedizin Berlin, Berlin, Germany
[2]Institut für Medizinische Physik und Biophysik, Medizinische Fakultät, Universität Leipzig, Leipzig, Germany



	A	B	C	D
1	pairs by contact frequency at 3.5 Angstrom			
2	label	freq	sum	by_atomtypes
3	R389@G.H5.21 - L394@G.H5.26	0.55	0.55	(50% SC-SC, 41% SC-BB, 5% BB-BB)
4	L394@G.H5.26 - K270@6.32	0.47	1.02	(61% BB-SC, 38% SC-SC)
5	L388@G.H5.20 - L394@G.H5.26	0.38	1.39	(89% BB-BB, 10% SC-SC)
6	L394@G.H5.26 - L230@5.69	0.23	1.62	(100% SC-SC)
7	R385@G.H5.17 - L394@G.H5.26	0.1	1.73	(86% BB-SC, 13% SC-SC)

We present mdciao, an open-source command line tool and Python Application-Programmers- Interface (API) for easy, one-shot analysis of molecular simulation data. It uses a distance-cutoff to compute frequent neighbors, tracking the underlying interactions between residues. The added value to this simple computation comes from wrapping it in a user-friendly command-line-interface (CLI) which simultaneously simplifies most decisions for non-expert users while keeping customizability for expert ones. It produces paper-ready figures and tables that can be automatically annotated with consensus nomenclature like the Ballesteros-Weinstein or Common-G-protein-Nomenclature. The readout of the so-called neighborhoods and contact frequencies are presented in a number of familiar graph types like contact matrices, flareplots, histograms and beta-colored 3D molecular structures.

mdciao also ships with an API for Python users to have programmatic access to many core- and/or helper- functions beyond those of the pre-packaged command-line-tools.



mdciao is fully documented, unit-tested and developed under continuous integration. The package is published under the GNU Lesser General Public License v3.0 or later, and is readily available for download under https://github.com/gph82/mdciao . A manuscript is in preparation.

Memory Kernel Estimation from Constrained MD Simulations

M. Post[1], **S. Wolf**[1] **and G. Stock**[1]

[1] *Institute of Physics, Albert-Ludwigs-University Freiburg*

Usually, the modeling of biophysical systems necessitate very long molecular dynamics simulations to sample all important configurations and transitions, with biologically relevant timescales ranging from few femtoseconds to multiple seconds. The complexity is then reduced by some form of dimensionality reduction, resulting in a description of the system by a set of a few collective coordinates. Then, the essential dynamics can be described by the (generalized) Langevin equation.

If the aforementioned sampling problem is circumvented by an enhanced sampling method, can this Langevin dynamics still be recovered? We study the special case of constraining one reaction coordinate to enforce slow transitions. First, the Free energy can be recovered by use of Jarzynskis equality; not only along this reaction coordinate, but also in general for a set of collective variables. The position dependent memory kernel or friction can be recovered through the constraint force auto-correlation. We study the influence of the constraint and the pulling rate on the friction estimate.

Improving MM/PBSA binding affinity calculations using machine learning

V. Principe[1] and A.S.J.S. Mey[1]

[1] *University of Edinburgh, David Brewster Road, Edinburgh, EH9 3FJ, UK*

Protein function can be regulated through the design of small molecule inhibitors that bind to the protein non-covalently. To understand if a small molecule is an effective inhibitor for a protein target, measuring the free energy of binding is essential. This can be done either experimentally or computationally. Computational determination of free energies of binding can help decide which compounds to test experimentally for their protein-ligand binding efficacy. However, computational methods, even state-of-the-art alchemical free energy methods, lack accuracy and are very computationally costly. The accuracy of computational methods can be improved by using quantum mechanical calculations, but this can be a lengthy process to which point there is no advantage over experimentally measuring binding free energies. Machine learning (ML) has been shown to predict molecular properties to quantum mechanical accuracy at a fraction of the computational cost [1]. This has the possibility to hugely increase fidelity of binding affinity calculations, without expending practicality. In the present study, we aim to use ML to improve fidelity of free energy calculations using MM/PBSA. The data used for this study forms part of a larger protein-ligand benchmarking data set partially introduced by Wang et al. [2]. We use MD simulations of the protein tyrosine kinase 2 with 16 ligands as a starting point for the reference MM/PBSA calculations. MD simulations are run using AMBER force fields through OpenMM, with MM/PBSA post-processing for free energy of binding analysis. The machine-learnt neural network potential ANI-2x, which has been shown to predict molecular energies of organic molecules to QM accuracy, will be used to provide an improved description of the ligands in the MM/PBSA calculations. Improvements over the traditional AMBER gaff2 forcefield are expected.

References

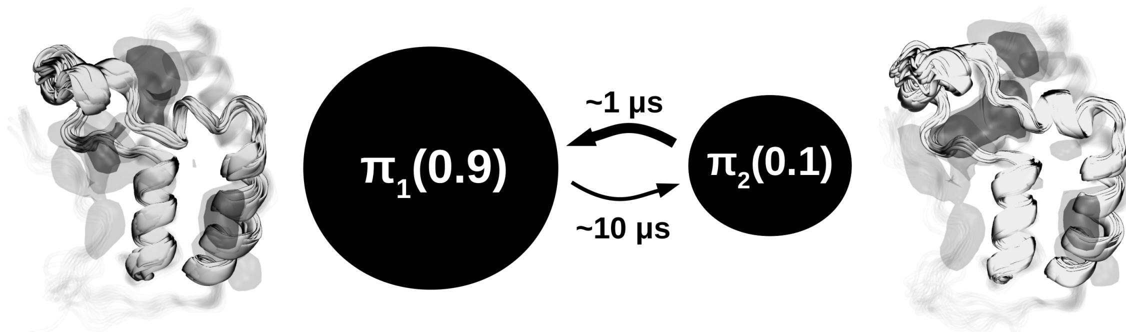
[1] Smith *et al.,* Chem. Sci., **8**, 3192-3203 (2017)
[2] Wang *et al.*, J. Am. Chem. Soc., **137**, 2695-2703 (2015)

Discovery of a Hidden Transient State in all Bromodomain Families

Lluís Raich,[1] Katharina Meier,[2] Judith Günther,[3] Clara D. Christ,[3] Frank Noé[1,4,*] and Simon Olsson[5,*]

[1]Department of Mathematics and Computer Science, Freie Universität Berlin, Berlin, Germany; [2]Computational Molecular Design, Pharmaceuticals, R&D, Bayer AG, 42096 Wuppertal, Germany; [3]Computational Molecular Design, Pharmaceuticals, R&D, Bayer AG, 13342 Berlin, Germany; [4]Department of Chemistry, Rice University, Houston, TX 77005; and [5]Department of Computer Science and Engineering, Chalmers University of Technology, Gothenburg, Sweden.

Bromodomains (BDs) are small protein modules that interact with acetylated marks in histones [1]. These post-translational modifications are pivotal to regulate gene expression, making BDs promising targets to treat several diseases [2]. While the general structure of BDs is well known, their dynamical features and their interplay with other macromolecules are poorly understood, hampering the rational design of potent and selective inhibitors. In this communication I will show how we combined extensive molecular dynamics simulations, Markov state modeling, and available structural data to reveal a transiently formed state that is conserved across all BD families [3]. It involves the breaking of two backbone hydrogen bonds that anchor a flexible loop with a rigid α-helix, opening a cryptic pocket that partially occludes the one associated to histone binding. We also analyzed more than 1.900 experimental structures and unveiled just two adopting the hidden state, explaining why it has been previously unnoticed and providing direct structural evidence for its existence. Our results suggest that this state is an allosteric regulatory switch for BDs, potentially related to a recently unveiled BD-DNA binding mode.



References

[1] P. Filippakopoulos *et al.* Cell **149**, 214 (2012)
[2] E. A. Morrison *et al.* Nat. Commun. **8**, 16080 (2017)
[3] L. Raich *et al.* Proc. Natl. Acad. Sci. U.S.A. **118**, e2017427118 (2021)

Understanding the Implication of Peptide Register Shifts in TCR Cross-Reactivity

Tatiana Rosales[1] and **Brian M Baker[1]**

[1]Dept. of Chemistry & Biochemistry and Harper Cancer Research Institute, University of Notre Dame.

T cells of the immune system use specialized receptors (T cell receptors, or TCRs) to recognize self and non-self peptides bound and presented by major histocompatibility complex (MHC) proteins. Studies of TCR-peptide/MHC interactions have provided considerable insights into the determinants of TCR recognition, including TCR preferences for peptides, and the 'rules' of TCR recognition and subsequent immune responses. However, the ability for some peptides to adopt multiple conformations within MHC binding grooves complicates these rules. One example is the potential for peptides to alter their conformation upon binding. Although small peptide conformational changes have been seen in previous studies, we recently observed a dramatic peptide reorganization upon TCR binding, yielding a TCR-peptide/MHC interface wholly different from that anticipated by the structure of the unbound peptide/MHC complex. Upon recognition by the TCR DMF5, the MMWDRGLGMM decamer peptide presented by the class I MHC protein HLA-A2, undergoes a dramatic "register shift," moving from a decameric to a nonameric conformation, with large changes in the peptide center and at the C-terminal end. Here, through stability measurements, binding studies, and MD simulations, we examine the role that peptide composition plays in this behavior and whether features that promote peptide register shifting might be present in other systems. The fundamental insights provided by these studies will expand our understanding of the nature T cell specificity, cross-reactivity, and fundamental aspects of immune recognition.

Automated identification of collective variables and metastable states from molecular dynamics data

Yasemin Bozkurt Varolgüneş[1], Atreyee Banerjee[1], Tristan Bereau[1,2], Joseph F. Rudzinski[1]

[1]*Max Planck Institute for Polymer Research, 55128 Mainz, Germany*
[2]*Van 't Hoff Institute for Molecular Sciences and Informatics Institute, University of Amsterdam, Amsterdam 1098 XH, The Netherlands*

Extracting insight from the enormous quantity of data generated from molecular simulations requires the identification of a small number of collective variables whose corresponding low-dimensional free-energy landscape retains the essential features of the underlying system. Data-driven techniques provide a systematic route to constructing this landscape, without the need for extensive a priori intuition into the relevant driving forces. In particular, autoencoders are powerful tools for dimensionality reduction, as they naturally force an information bottleneck and, thereby, a low-dimensional embedding of the essential features. While variational autoencoders ensure continuity of the embedding by assuming a unimodal Gaussian prior, this is at odds with the multi-basin free-energy landscapes that typically arise from the identification of meaningful collective variables. In this work, we incorporate this physical intuition into the prior by employing a Gaussian mixture variational autoencoder (GMVAE), which encourages the separation of metastable states within the embedding. The GMVAE performs dimensionality reduction and clustering within a single unified framework, and is capable of identifying the inherent dimensionality of the input data, in terms of the number of Gaussians required to categorize the data. We illustrate the approach on two challenging examples: a disordered peptide ensemble and polymeric polymorphism.

Investigation of different protonation States on ADGRL1 Flap Dynamics

Florian Seufert[1], **Hossein Batebi[1]**, **Peter W. Hildebrand[1]**

[1] *Institute for Medicinal Physics and Biophysics, University of Leipzig, Germany*

Adhesion G-Protein Coupled Receptors (aGPCR) are a family of 33 receptors exhibiting large and variable N-terminal extracellular regions. The defining feature of aGPCRs is the conserved GPCR-autoproteolysis inducing domain (GAIN domain), mediating autoproteolytic cleavage at the GPCR proteolytic site (GPS).[1,2] A tethered agonist (TA) between the GPS and the seven-transmembrane region activates aGPCR, however the mechanism of TA exposure to the seven-transmembrane region remains elusive.[3–6]

In a recent study, GAIN domains were investigated using molecular dynamics (MD) simulations of GAIN domain crystal structures and homology models combined with *in vitro* labeling experiments. It was shown that the GPS region is constitutively solvent-accessible, mediated by two surrounding flexible protein regions termed flaps.[7] Our studies proposed two glutamate residues being part of solvent-exposure mediating flaps in ADGRL1, exhibiting high flexibility in MD simulations. With the change in position, changes in residue pKa are expected in closed and open flap states. However, such changes cannot be easily simulated by classical MD based on non-polarizable force fields and unbreakable bonds. Therefore, protonation states of residues must be determined before running the simulation. We show that by changing the initial protonation state pattern of two glutamate residues in ADGRL1, GAIN domain dynamics are affected and state-dependent differences in pKa of the acidic side chains can be observed, indicating that protonation state determination of flexible residues needs to be treated in detail to promote reliability of dynamics observed in MD simulations.

References

[1] Prömel, S. *et al.* The GPS Motif Is a Molecular Switch for Bimodal Activities of Adhesion Class G Protein-Coupled Receptors. *Cell Rep.* **2**, 321–331 (2012).

[2] Araç, D. *et al.* A novel evolutionarily conserved domain of cell-adhesion GPCRs mediates autoproteolysis. *EMBO J.* **31**, 1364–1378 (2012).

[3] Araç, D., Sträter, N. & Seiradake, E. Understanding the Structural Basis of Adhesion GPCR Functions. in *Handbook of Experimental Pharmacology* vol. 234 67–82 (Springer New York LLC, 2016).

[4] Zhu, B. *et al.* GAIN domain–mediated cleavage is required for activation of G protein–coupled receptor 56 (GPR56) by its natural ligands and a small-molecule agonist. *J. Biol. Chem.* **294**, 19246–19254 (2019).

[5] Wilde, C. *et al.* The constitutive activity of the adhesion GPCR GPR114/ADGRG5 is mediated by its tethered agonist. *FASEB J.* **30**, 666–673 (2016).

[6] Demberg, L. M. *et al.* Activation of adhesion G protein-coupled receptors: Agonist specificity of Stachel sequence-derived peptides. *J. Biol. Chem.* **292**, 4383–4394 (2017).

[7] Beliu, G. *et al.* Tethered agonist exposure in intact adhesion/class B2 GPCRs through intrinsic structural flexibility of the GAIN domain. *Mol. Cell 81*(**5**), 905-921.e5., (2021).

Novel elastic response in twist-bend nematic models

Jiale Shi[1] , Hythem Sidky[1] and Jonathan K. Whitmer[1]

[1]*Department of Chemical and Biomolecular Engineering, University of Notre Dame, Notre Dame, IN 46556, USA*

Bent-shaped liquid crystals have attracted significant attention recently due to their novel mesostructure and the intriguing behavior of their elastic constants, which are strongly anisotropic and have an unusual temperature dependence. Though theories explain the onset of the twist-bend nematic phase (N_{TB}) through spontaneous symmetry breaking concomitant with transition to a negative bend (K_3) elastic constant, this has not been observed as yet in experiments. There, the small bend elastic constant has a strongly non-monotonic temperature dependence, which first increases after crossing the isotropic (I)–nematic (N) transition, then dips near the nematic (N)-twist-bend (N_{TB}) transition before it increases again as the transition is crossed. The molecular mechanisms responsible for this exotic behavior are unclear. Here, we utilize density of states algorithms in Monte Carlo simulation applied to a variant of the Lebwohl–Lasher model which includes bent-shaped-like interactions to analyze the mechanism behind elastic response in this novel mesostructure and understand the temperature dependence of its Frank–Oseen elastic constants.

References

[1] J. Shi, H. Sidky, J. K. Whitmer. Soft Matter **15**, 8219 (2019)

Computational Simulations Reveal Substrate Translocation Pathway through Hydrophobic Transporters

Kamolrat Somboon[1], Prof. Bert van den Berg[2], and Prof. Syma Khalid[1]

[1]University of Southampton, Southampton, UK
[2] Newcastle University, Newcastle, UK

BTEX (benzene, toluene, ethylbenzene and xylene) is a group of monoaromatic hydrocarbons which naturally occurs in crude oil. Despite of the negative effects on human health, BTEX are continually produced worldwide by petrochemical industry. Due to the limitations and burden economic costs of abiotic methods to clean-up BTEX, bacterial biodegradation has been focused as an alternative way to eliminate the environmental contaminants. To improve the efficiency of BTEX entry to bioremediating bacteria, the mechanism of BTEX transport through the outer membrane of Gram-negative bacteria, which is rich in lipopolysaccharides (LPS), need to be clarified.
The FadL family of proteins are known to enable the passage of a range of hydrocarbons across the outer membrane[1,2]. Here, we focus our attention on TodX from Pseudomonas putida which has been shown to facilitate passage of toluene and benzene. Based on the X-ray structure, there are two plausible pathways for substrate transport through TodX; either through the long axis, called the "classical mechanism" or through the lateral pore in the barrel, called the "lateral diffusion mechanism". In order to identify which mechanism is more likely, atomistic level molecular dynamics simulations were performed. Free energy calculations and steered MD simulations reveal substantial barriers to the movement of monoaromatic compounds through the classical route. Our results provide predict that TodX can allow the substrate to move through the lateral opening in the barrel wall, into lipid bilayer where the substrate can spontaneously diffuse to the periplasmic space. These are supported by the simulation results performed on TodX mutants and CymD protein. Furthermore, we identify the likeliest route taken through the lateral gate. This work is part of the Somboon *et al*, Uptake of Monoaromatic Hydrocarbons During Biodegradation by FadL channel-mediated Lateral Diffusion, *Nature Communications*, DOI: 10.1038/s41467-020-20126-y.

References

[1] B. W. Lepore, M. Indic, H. Pham, E. M. Hearn, D. R. Patel and B. van den Berg, Proc. Natl. Acad. Sci. U. S. A., **108,** 10121-6 (2011)
[2] B. Van Den Berg, Curr. Opin. Struct. Biol., **15**, 401–407 (2005)

Structural models of ACE2 with RBD of SARS-CoV-2 Spike protein

M. Sorokina[1,2,3], **JMC Teixeira**[4], **S. Barrera-Vilarmau**[5], **R. Paschke**[3,6], **I. Papasotiriou**[2], **JPGLM Rodrigues**[7] **and PL Kastritis**[1,6,8]

[1] Institute of Biochemistry and Biotechnology, Martin Luther University Halle-Wittenberg, Halle/Saale, Germany
[2] RGCC International GmbH, Zug, Switzerland
[3] BioSolutions GmbH, Halle/Saale, Germany
[4] Program in Molecular Medicine, Hospital for Sick Children, Ontario, Canada
[5] Institute of Advanced Chemistry of Catalonia, Barcelona, Spain
[6] Biozentrum, Martin Luther University Halle-Wittenberg, Halle/Saale, Germany
[7] Department of Structural Biology, Stanford University, Stanford, USA
[8] Interdisciplinary Research Center HALOmem, Charles Tanford Protein Center, Martin Luther University Halle-Wittenberg, Halle/Saale, Germany

The current outbreak of SARS-CoV-2 poses a threat to global health and economy. Development of therapies for treatment of coronavirus infections for prevention of possible future epidemics is of paramount importance. For that, the interaction biophysics between surface spike glycoprotein (S protein) and Angiotensin-converting enzyme 2 (ACE2), an epithelial receptor that both SARS-CoV and SARS-CoV-2 use to enter the host cells, of different species has to be understood.

To this end, first we collected information regarding naturally occurring variants of the human ACE2 (hACE2) and built 242 structural models of these variants bound to the receptor binding domain (RBD) of the SARS-CoV-2 S protein and refined their interfaces with HADDOCK.[1] Our dataset includes 140 variants of hACE2 representing missense mutations found in genome-wide studies, 39 mutants with reported effects on the recognition of the RBD, and 63 predictions after computational alanine scanning mutagenesis of hACE2-RBD interface residues. Sequence variations of analyzed hACE2 variants were compared with variants from other known datasets identifying important hACE2 mutations that must be monitored and understood further to accelerate the design of therapeutics against SARS-CoV-2, as well as to contribute to prevention of possible future coronaviruses outbreaks.

Next, 3D models of ACE2 from 28 different species, which are likely to encounter humans in a variety of residential, industrial, and commercial settings, bound to RBD of SARS-CoV-2 were created.[2] Interfaces of the models were refined with short molecular dynamics simulations, and the resulting structures were scored using the HADDOCK score. This analysis provides a hypothesis why some species are not susceptible to the infection with SARS-CoV-2. The results can be used when building predictors for susceptibility to coronavirus infections.

References

[1] M. Sorokina *et al.*, Sci Data **7,** 309 (2020)
[2] João P. G. L. M. Rodrigues *et al.*, PLoS Comput Biol **16**, 12 (2020)

SmoothT

Unbiased Construction and Visualization of Transition Pathways, linking Monte Carlo and Molecular Dynamics Simulations

René Staritzbichler and **Peter Hildebrand**

Institute of Medical Physics and Biophysics, Leipzig, Germany

Simulating Molecular Dynamics is computationally expensive and thus limited to rather isolated extracts of the global picture. Monte-Carlo based methods, on the other hand, are strong in revealing a global perspective and can sample even complex transition energy-landscapes. Here, we introduce a software tool, SmoothT, which provides a way to connect these methods and to exploit the strengths of both approaches in terms of a multi-scale strategy. SmoothT uses as input an ensemble of conformations calculated by *low-resolution* Monte-Carlo methods, such as docking or folding. From this ensemble, SmoothT constructs a pathway with the minimum (pseudo) energy barrier. The nodes of the resulting pathways can be used as starting conformations of subsequent *high-resolution* Molecular Dynamics simulations. The method is unbiased in the sense that there is no initial assumption on how the pathway is expected to look like.

Accurate receptor-ligand binding free energies from fast QM conformational chemical space sampling

E. Boz[1] and M. Stein[1]

[1]*Molecular Simulations and Design Group, Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany.*

Small molecule receptor-binding is dominated by weak, non-covalent interactions such as van-der-Waals, hydrogen bonding or electrostatics. Calculating these non-covalent ligand-receptor interactions is a challenge to computational means in terms of accuracy and efficacy since the ligand may bind in a number of thermally accessible conformations. The conformation/rotamer ensemble sampling tool (CREST) uses an iterative scheme to efficiently sample the conformational space and calculates energies using the semi-empirical 'Geometry, Frequency, Noncovalent, eXtended Tight Binding' (GFN2-xTB) method. This combined approach is applied to blind predictions of the modes and free energies of binding for a set of 10 drug molecule ligands to the cucurbit[n]urils CB[8] receptor from the recent 'Statistical Assessment of the Modeling of Proteins and Ligands' (SAMPL) challenge including morphine, hydromorphone, cocaine, fentanyl, ketamine and others. For each system, the conformational space was sufficiently sampled for the free ligand and the ligand-receptor complexes using the quantum chemical Hamiltonian. A multitude of structures makes up the final conformer-rotamer ensemble, for which then free energies of binding are calculated. For those large and complex molecules, the results are in good agreement with experimental values with a mean error of 3 kcal/mol. The GFN2-xTB energies of binding are validated by advanced density functional theory calculations and found to be in good agreement. The efficacy of the automated QM sampling workflow allows the extension towards other complex molecular interaction scenarios.

Deciphering the Allosteric Process of the Phaeodactylum tricornutum Aureochrome 1a LOV Domain

Hao Tian[1] and Peng Tao[1]

[1]Department of Chemistry, Center for Research Computing, Center for Drug Discovery, Design, and Delivery (CD4), Southern Methodist University, Dallas, Texas, United States of America

The conformational-driven allosteric protein diatom *Phaeodactylum tricornutum* aureochrome 1a (PtAu1a) differs from other light-oxygen-voltage (LOV) proteins for its uncommon structural topology. The mechanism of signaling transduction in PtAu1a LOV domain (AuLOV) including flanking helices remains unclear because of this dissimilarity, which hinders the study of PtAu1a as an optogenetic tool. To clarify this mechanism, we employed a combination of tree-based machine learning models, Markov state models, machine learning based community analysis, and transition path theory to quantitatively analyze the allosteric process. Our results are in good agreement with the reported experimental findings and reveal a previously overlooked Cα helix and protein linkers as important in promoting the protein conformational changes. This integrated approach can be considered as a general workflow and applied on other allosteric proteins to provide detailed information about their allosteric mechanisms.

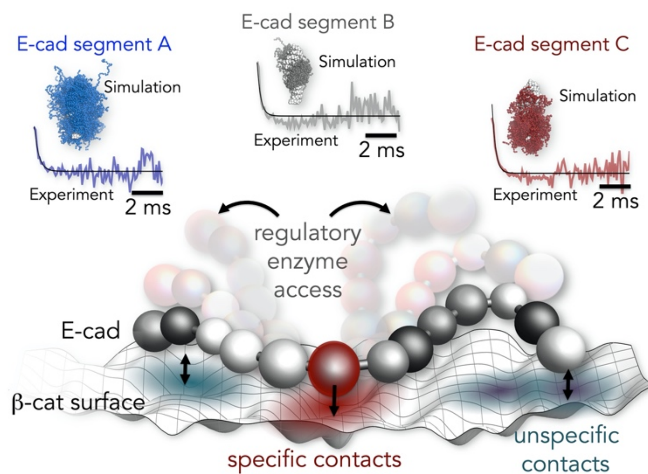
Diffusion of the disordered E-cadherin tail on β-catenin

Felix Wiggers[1], Samuel Wohl[2], Artem Dubovetskyi[1], Gabriel Rosenblum[1],

Wenwei Zheng[3] and Hagen Hofmann[1]

[1]Department of Structural Biology, Weizmann Institute of Science, Herzl St. 234, 76100 Rehovot, Israel
[2]Department of Physics, Arizona State University,
Tempe, AZ 85287, USA, College of Integrative Sciences and Arts, Arizona State University, Mesa, AZ 85212, USA

Specific protein interactions typically require well-shaped binding interfaces. Here, we report a cunning exception. The disordered tail of the cell-adhesion protein E-cadherin dynamically samples a large surface area of the proto-oncogene β-catenin. Single-molecule experiments and molecular simulations resolve these motions with high resolution in space and time. Contacts break and form within hundreds of microseconds without dissociation of the complex. A few persistent interactions provide specificity whereas unspecific contacts boost affinity. The energy landscape of this complex is rugged with many small barriers (3 – 4 kBT) and reconciles specificity, high affinity, and extreme disorder. Given the roles of β-catenin in cell-adhesion, signalling, and cancer, this Velcro-like design has the potential to tune the stability of the complex without requiring dissociation.



Diffusion of an IDP on its folded ligand. Single-molecule FRET experiments and molecular simulations identify extreme disorder in the complex between E-cadherin (E-cad) and β-catenin (β-cat). The complex allows access to regulatory enzymes without requiring dissociation

References

[1] **Diffusion of the disordered E-cadherin tail on β-catenin.** Wiggers F., Wohl S., Dubovetsky A., Rosenblum G., Zheng W. and Hofmann H. (2021) bioRxiv 2021.02.03.429507

Counter transport of potassium ions in the human serotonin transporter

Romina Wild[1,2,3], Andrea Di Luca[2,3], Ina Bisha[2], Ville R. I. Kaila[2,3]

[1]Molecular and Statistical Biophysics Group at Scuola Internazionale Superiore di Studi Avanzati, Via Bonomea, 265, 34136 Trieste TS, Italy.
[2]Center for Integrated Protein Science Munich at the Department of Chemistry, Technical University of Munich, Lichtenbergstr. 4, D-85748 Garching, Germany.
[3]Department of Biochemistry and Biophysics, Stockholm University, 10691 Stockholm, Sweden.

The human serotonin transporter (hSERT) is a solute-carrier (SLC) protein that recycles serotonin back into the presynaptic neuron after chemical stimuli. Impairment of serotonin reuptake results in several patho-physiological conditions, and hSERT has thus become a central target of many antidepressant drugs. However, despite recently resolved structures of inhibitor-bound hSERT , its sodiumchloride-dependent transport and K^+/H^+ -dependent counter transport mechanism still remains unclear. On the basis of single topology free energy perturbation calculations, we propose putative potassium binding sites that might stimulate relaxation of hSERT back to the outward-open state.

Probing the site-specific backbone dynamics of YadA autotransporter in microcrystals and native membranes using solid-state NMR spectroscopy

Jayasubba Reddy Yarava[1], Marcella Orwick-Rydmark[2], Barth-von Rossum[1], Dirk Linke[2], and Hartmut Oschkinat*[1]

[1]Leibniz-Forschungsinstitut für Molekulare Pharmakologie, 13125 Berlin, Germany; Freie Universität Berlin, 14195 Berlin, Germany,

[2] Department of Biosciences, University of Oslo, Oslo, Norway

Membrane proteins perform many important biological functions such as molecular transport, protein trafficking and signaling pathways. Determining the dynamic picture of membrane protein motions in native lipids (outer-membrane) is of utmost important to understand their actual biological activity. YadA (Yersinia Adhesin A) is a member of the family of trimeric autotransporter adhesins, which are important pathogenicity factors that mediate adhesion to host cells and tissues in such diverse diseases as diarrhea, urinary tract infections, or airway infections.[1,2] The structure of YadA was determined in both microcrystals and native membranes using solid-state NMR spectroscopy. The present study aims to determine the similarities and differences in YadA protein motions in two environments such as micro-crystals and outer-membranes.

Here, we use ^{15}N spin-lattice relaxation rates (R_1) and ^{15}N, ^{13}C' spin-lattice relaxation rates in the rotating frame ($R_{1\rho}$) to probe the site-specific backbone dynamics as well as peptide-plane motions of YadA. The experimental data were fit into two theoretical relaxation models such as simple model-free (SMF) and extended model-free (EMF) formalisms. The SMF model mainly reflects the information related to collective motions of the protein whereas EMF provides both slow (nano-second) and fast motions (pic-second) of the protein. The differences and similarities in the amplitude of motions as well as the time-scale of motions (slow and fast) of both environments will be discussed. The measurements on both the samples were performs on a 900 MHz spectrometer with a sample spin rate of 60 kHz (MAS).

References

(1) S.A. Shahid, B. Baridaux, W.T. Franks, L. Krabben, M. Habeck, B. van Rossum, D. Linke, Nature Methods, **9**, 1212 (2012)

(2) S.A. Shahid, S. Markovic, D. Linke, B. van Rossum, Nature Scientific Reports, 2, 803 (2012)