

Conformational Ensembles from Experimental Data and Computer Simulations

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Berlin, Germany | August 25–29, 2017



Organizing Committee

Helen Berman, Rutgers University

Andrea Cavalli, Institute for Research in Biomedicine

Gerhard Hummer, Max Planck Institute for Biophysics

Kresten Lindorff-Larsen, University of Copenhagen

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Dear Colleagues,

We would like to welcome you to the Biophysical Society Thematic Meeting on *Conformational Ensembles from Experimental Data and Computer Simulations*. We have assembled an exciting program, which consists of a mix of computation, theory, and a broad range of methods in experimental structural biology with a focus on methods and applications for studying the structural dynamics of biomolecules by integrating experiments and simulations. This meeting aims to bring together scientists from across disciplines to advance integrative structural biology into the “dynamic age”.

The program features 25 invited speakers, 12 short talks selected from contributed posters, and 120 contributed posters. Over 160 participants from around the world will be in attendance to share and discuss their ideas. We hope that the meeting will not only provide a venue for exchanging recent exciting progress, but also promote fruitful discussions and foster future collaborations.

Harnack Haus lies in the center of the “German Oxford” of Berlin, which is characterized by its excellent scientific institutions. Established in 1929 as guest house and conference venue of the Kaiser Wilhelm Society in Berlin-Dahlem, it quickly distinguished itself as an international scientific club, hosting prominent scientists, artists, industrialists, and politicians. After World War II, it was used as an officers’ club by the U.S. Armed Forces, until its return to the Max Planck Society in 1994. Today, the Max Planck Society draws inspiration from the venue’s founding history, which we invite you to explore in the Harnack Haus’ exhibition installations, located in the foyer areas.

Thank you all for joining this meeting, and we look forward to enjoying this event with you!

Sincerely,

Helen Berman, Andrea Cavalli, Gerhard Hummer, Kresten Lindorff-Larsen
The Organizing Committee

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GENERAL INFORMATION

Registration/Information Location and Hours

Registration will be located at the Reception Desk in the Planck Lobby. Registration hours are as follows:

Friday, August 25	12:00 – 18:00
Saturday, August 26	8:30 – 18:00
Sunday, August 27	8:30 – 18:00
Monday, August 28	8:30 – 18:00
Tuesday, August 29	8:30 – 12:30

Instructions for Presentations

(1) Presentation Facilities:

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

(2) Poster Sessions:

- 1) All poster sessions will be held in Meitner Hall.
- 2) A display board measuring 144 cm (4 ft. 7 in.) high by 116.5 cm (3 ft. 8 in.) wide will be provided for each poster. Poster boards will follow the same numbering scheme as listed in the E-book.
- 3) Poster boards require pushpins or thumbtacks for mounting. Authors are expected to bring their own mounting materials.
- 4) There will be formal poster presentations on Saturday, Sunday, and Monday. Posters will be available for viewing during their scheduled presentation date only. During each day, odd-numbered posters will be displayed from 16:30 – 17:15, and even-numbered posters will be displayed from 17:15 – 18:00.
- 5) During the assigned poster presentation sessions, presenters are requested to remain in front of their poster boards to meet with attendees.
- 6) All presenters must remove their poster by 18:00 on the day of their scheduled presentation. Posters left uncollected at the end of the evening will be disposed.

Meals and Coffee Breaks

There will be a two-hour Welcome Reception on Friday, August 25 from 18:00 – 20:00. This reception will be held in the Einstein Lounge & Terrace.

Coffee breaks will be served in the Planck Lobby and Meitner Hall.

Lunches will be served in the Restaurant, which is located on the basement level of the building. Additional seating will be available in Laue Hall. Lunch is provided on August 26, 27, 28, and 29.

On Sunday, August 27, a barbeque banquet dinner will be provided at 18:00, upon the conclusion of the evening's poster session. Dinner will be served at the Restaurant Terrace, with seating also available in the Restaurant and Einstein Lounge. Should inclement weather occur, this event will take place in the Restaurant and Laue Hall.

Internet

Wi-Fi will be available throughout all areas of Harnack Haus. Attendees will receive a passcode at registration for logging in to the network.

Smoking

Please be advised that smoking is not permitted inside Harnack Haus or the meeting facilities. Smoking is permitted in designated outside areas.

Name Badges

Name badges are required to enter all scientific sessions, poster sessions, and social functions. Please wear your badge throughout the conference.

Contact Information

If you have any further requirements during the meeting, please contact the meeting staff at the registration desk from August 25-29 during registration hours.

In case of emergency, you may contact the following:

Erica Bellavia, BPS Meeting Coordinator
ebellavia@biophysics.org

Front Desk, Harnack Haus
+ 49 30 841 33 800

Conformational Ensembles from Experimental Data and Computer Simulations

Berlin, Germany
August 25-29, 2017

PROGRAM

Friday, August 25, 2017

12:00 – 18:00	Registration/Information	Planck Lobby
14:00 – 14:15	Opening Remarks Kresten Lindorff-Larsen, University of Copenhagen, Denmark	Goethe Hall
Session I	Disordered Protein Ensembles Gerhard Hummer, Max Planck Institute for Biophysics, Germany, Chair	
14:15 – 15:00	Adriaan Bax, NIH, NIDDKD, USA <i>An NMR View of Folded and Unfolded Proteins and Their Transient Intermediates</i>	
15:00 – 15:45	Tanja Mittag, St. Jude Children's Research Hospital, USA <i>Sequence Determinants of the Conformational Properties of an Intrinsically Disordered Protein Undergoing Multi-site Phosphorylation</i>	
15:45 – 16:15	Coffee Break	Meitner Hall & Planck Lobby
16:15 – 17:00	Teresa Head-Gordon, University of California, Berkeley, USA <i>New Methods for Generating and Evaluating Conformational Ensembles</i>	
17:00 – 17:30	Paul Robustelli, D.E. Shaw Research, USA* <i>Developing Force Fields for the Accurate Simulation of Both Ordered and Disordered Protein States</i>	
18:00 – 20:00	Welcome Reception	Einstein Lounge & Terrace

Saturday, August 26, 2017

8:30 – 18:00	Registration/Information	Planck Lobby
Session I (cont.)	Disordered Protein Ensembles William Eaton, NIH, USA, Chair	

9:00 – 9:45	Martin Blackledge, Institut de Biologie Structurale, France <i>Large-scale Protein Conformational Dynamics from NMR and Molecular Simulation. From Fundamental Biophysics to Biological Function</i>	
9:45 – 10:30	Birthe Kragelund, University of Copenhagen, Denmark <i>Dynamics and Disorder in Class I Cytokine Receptors</i>	
10:30 – 11:00	Coffee Break	Meitner Hall & Planck Lobby
Session II	Integrative and Hybrid Methods Helen Berman, Rutgers University, USA, Chair	
11:00 – 11:45	Andrej Sali, University of California, San Francisco, USA <i>Integrative Modeling of Multiple States of Macromolecules</i>	
11:45 – 12:30	Alexandre Bonvin, Utrecht University, The Netherlands <i>High-resolution, Integrative Modelling of Biomolecular Complexes from Fuzzy Data</i>	
12:30 – 13:00	Ji-Joon Song, Korea Advanced Institute of Science and Technology, South Korea* <i>Structural Insights into the Architecture of Human Importin4_Histone H3/H4_Asf1a Complex and Its Histone H3 Tail Binding</i>	
13:00 – 14:00	Lunch	Restaurant
Session III	Interpreting Experiments Through Molecular Simulation Andrea Cavalli, Institute for Research in Biomedicine, Switzerland, Chair	
14:00 – 14:45	Cecilia Clementi, Rice University, USA <i>Incorporating Experimental Data in Long Timescales Macromolecular Simulations</i>	
14:45 – 15:15	Michael Feig, Michigan State University, USA* <i>Dynamics of Proteins Under Crowded Conditions in Simulations and Experiments</i>	
15:15 – 16:00	Arianna Fornili, Queen Mary University of London, United Kingdom* <i>In Silico Identification of Rescue Sites by Double Force Scanning</i>	
16:00 – 16:30	Coffee Break	Meitner Hall & Planck Lobby
16:30 – 18:00	Poster Session 1	Meitner Hall

Sunday, August 27, 2017

8:30 – 18:00	Registration/Information	Planck Lobby
Session III (cont.)	Interpreting Experiments Through Molecular Simulation Andrea Cavalli, Institute for Research in Biomedicine, Switzerland, Chair	
9:00 – 9:30	Shang-Te Danny Hsu, Academia Sinica, Taiwan* <i>Structural Basis of Substrate Recognition and Chaperone Activity of Ribosome-associated Trigger Factor Regulated by Monomer-dimer Equilibrium</i>	

9:30 – 10:00	Jana Selent, Pompeu Fabra University, Spain* <i>Functional Dynamics of the Distal C-tail of Arrestin</i>	
10:00 – 10:30	Massimiliano Bonomi, University of Cambridge, United Kingdom* <i>Integrative Structural and Dynamical Biology with PLUMED-ISDB</i>	
10:30 – 11:00	Coffee Break	Meitner Hall & Planck Lobby
Session IV	Interpreting X-Ray Scattering Data Marie Skepö, Lund University, Sweden, Chair	
11:00 – 11:45	Jill Trehwella, University of Sydney, Australia <i>Modelling Conformational Ensembles from Small Angle Scattering</i>	
11:45 – 12:30	Jochen Hub, Georg-August University Göttingen, Germany <i>Bayesian Refinement of Protein Structures and Ensembles Against SAXS Data by Using Molecular Dynamics</i>	
12:30 – 13:00	Lee Makowski, Northeastern University, USA* <i>Comparison of the Global Dynamics of Proteins as Assessed by WAXS and MD</i>	
13:00 – 14:00	Lunch	Restaurant
Session V	Bayesian Methods for Generating Ensembles Martin Blackledge, Institut de Biologie Structurale, France, Chair	
14:00 – 14:45	Michael Habeck, Max Planck Institute for Biophysical Chemistry, Germany <i>Bayesian Modeling with Ensemble Data</i>	
14:45 – 15:30	Collin Stultz, Massachusetts Institute of Technology, USA <i>What Does It Mean for a Protein to be Disordered? Insights from Experiment and Molecular Simulations</i>	
15:30 – 16:00	Simon Olsson, Freie Universität Berlin, Germany* <i>Bridging the Gap Between Stationary and Dynamic Data Through Augmented Markov Models</i>	
16:00 – 16:30	Coffee Break	Meitner Hall & Planck Lobby
16:30 – 18:00	Poster Session 2	Meitner Hall
18:00 – 22:00	Dinner	Restaurant & Restaurant Terrace

Monday, August 28, 2017

8:30 – 18:00	Registration/Information	Planck Lobby
Session VI	Interpreting X-Ray Diffraction and EM Data Alexandre Bonvin, Utrecht University, The Netherlands, Chair	

9:00 – 9:45	Henry van den Bedem, Stanford University, USA <i>Resolving Catalytic Motions and Dynamics of Isocyanide Hydratase from X-Ray Crystallography</i>	
9:45 – 10:30	Michael Wall, Los Alamos National Laboratory, USA <i>Diffuse X-Ray Scattering to Model the Protein Conformational Ensemble</i>	
10:30 – 11:00	Coffee Break	Meitner Hall & Planck Lobby
11:00 – 11:45	James Fraser, University of California, San Francisco, USA <i>Birth of the Cool: Protein Allostery by Multi-temperature Multi-conformer X-Ray Crystallography</i>	
11:45 – 12:30	Pilar Cossio, Max Planck Institute of Biophysics, Germany <i>Hybrid Models and Bayesian Analysis of Individual EM Images: An Alternative for Challenging EM Data</i>	
12:30 – 13:00	Gydo Van Zundert, Schrodinger, USA* <i>Objectively and Automatically Building Multi-conformer Ligand Models in Electron Densities</i>	
13:00 – 14:00	Lunch	Restaurant
Session VII	Integrating Heterogeneous Data Andrej Sali, University of California, San Francisco, USA, Chair	
14:00 – 14:45	Tanja Kortemme, University of California, San Francisco, USA <i>Systematic Perturbation of a Fundamental Biological Switch</i>	
14:45 – 15:30	Thérèse Malliavin, Institut Pasteur, France <i>From High-resolution Protein Structures to Information About Functional Dynamics</i>	
15:30 – 16:00	Claus Seidel, Heinrich Heine University, Germany* <i>Quantitative Integrative FRET Studies Unravel the Dynamic Structural Ensemble of the Large GTPase hGBP1 Required for Oligomerization</i>	
16:00 – 16:30	Coffee Break	Meitner Hall & Planck Lobby
16:30 – 18:00	Poster Session 3	Meitner Hall

Tuesday, August 29, 2017

8:30 – 12:30	Registration/Information	Planck Lobby
Session VII (cont.)	Integrating Heterogeneous Data Kresten Lindorff-Larsen, University of Copenhagen, Denmark, Chair	
9:00 – 9:45	Justin MacCallum, Calgary, Canada <i>Inferring Protein Structure from Sparse and Unreliable Experimental Data</i>	

9:45 – 10:30	Enrico Ravera, University of Florence, Italy <i>Averaged Experimental Data: From Algebra to Biology</i>	
10:30 – 11:00	Coffee Break	Meitner Hall & Planck Lobby
11:00 – 11:45	Charles Schwieters, National Institutes of Health, USA <i>Computation of Structural Ensembles from NMR and Other Data</i>	
11:45 – 12:15	Naomi Latorraca, Stanford University, USA* <i>Mechanism of Substrate Translocation in an Alternating Access Transporter</i>	
12:15 – 12:30	Closing Remarks and BJ Poster Awards Presentation Gerhard Hummer, Max Planck Institute for Biophysics, Germany	Goethe Hall
12:30 – 13:30	Lunch	Restaurant

* *Contributed talks selected from among submitted abstracts*

SPEAKER ABSTRACTS

An NMR View of Folded and Unfolded Proteins and Their Transient Intermediates

Adriaan Bax

NIH, NIDDKD, Bethesda, MD, USA

No Abstract

Sequence Determinants of the Conformational Properties of an Intrinsically Disordered Protein Undergoing Multi-site Phosphorylation

Tanja Mittag

St. Jude Children's Research Hospital, Memphis, TN, USA

No Abstract

Sequence Determinants of the Conformational Properties of an Intrinsically Disordered Protein Undergoing Multi-site Phosphorylation

Teresa Head-Gordon

University of California, Berkeley, Berkeley, CA, USA

No Abstract

Developing Force Fields for the Accurate Simulation of Both Ordered and Disordered Protein States

Paul Robustelli¹, Stefano Piana¹, David E. Shaw^{1,2}.

²Columbia University, New York, NY, USA. ¹D. E. Shaw Research, New York, NY, USA,

Molecular dynamics (MD) simulation can serve as a valuable complementary tool to experiments in characterizing the structural and dynamic properties of ordered and disordered proteins. The utility of MD simulation depends, however, on the accuracy of the underlying physical models (“force fields”). We present here an extensive benchmark study to systematically assess the ability of commonly used MD force fields to reproduce NMR, SAXS, and FRET data for a number of ordered and disordered proteins. We found that, while the properties of folded proteins are generally well described in simulation, large discrepancies exist between simulation and experiment for disordered proteins, which is significant given that a large fraction of proteins are partially or completely disordered under physiological conditions. We subsequently developed a new water model, TIP4P-D, that better balances electrostatic and dispersion interactions, resulting in significantly improved accuracy in the description of disordered states, but slightly degraded results for ordered proteins. Guided by experimental measurements from folded proteins, fast-folding proteins, weakly structured peptides, and disordered proteins, we are further optimizing force fields to more accurately simulate proteins across the order-to-disorder spectrum.

**Large-scale Protein Conformational Dynamics from NMR and Molecular Simulation.
From Fundamental Biophysics to Biological Function**

Martin Blackledge

Institut de Biologie Structurale, Grenoble, France

No Abstract

Dynamics and Disorder in Class 1 Cytokine Receptors

Birthe Kragelund

University of Copenhagen, Copenhagen, Germany

No Abstract

Integrative Modeling of Multiple States of Macromolecules

Andrej Sali

University of California, San Francisco, San Francisco, CA, USA

No Abstract

High-resolution, Integrative Modelling of Biomolecular Complexes from Fuzzy Data

Alexandre Bonvin.

Utrecht University, Utrecht, Netherlands.

The prediction of the quaternary structure of biomolecular macromolecules is of paramount importance for fundamental understanding of cellular processes and drug design. In the era of integrative structural biology, one way of increasing the accuracy of modelling methods used to predict the structure of biomolecular complexes is to include as much experimental or predictive information as possible in the process.

We have developed for this purpose a versatile information-driven docking approach HADDOCK (<http://www.bonvinlab.org/software/haddock2.2>). HADDOCK can integrate information derived from biochemical, biophysical or bioinformatics methods to enhance sampling, scoring, or both. The information that can be integrated is quite diverse: interface restraints from NMR, mutagenesis experiments, or bioinformatics predictions; shape data from small-angle X-ray scattering and, recently, cryo-electron microscopy experiments.

In my talk, I will illustrate HADDOCK's capabilities with various examples. I will also introduce the concept of explorative modelling in which the interaction space defined by a limited number of restraints is systematically mapped, which allows, for example, to identify false positive restraints from MS cross-link experiments. We have developed for this purpose the DisVis web server available from: <http://milou.science.uu.nl/services/DISVIS>

Structural Insights into the Architecture of Human Importin4_Histone H3/H4_Asf1a Complex and Its Histone H3 Tail Binding

Jungmin Yoon, **Ji-Joon Song**.

Korea Advanced Institute of Science and Technology (KAIST), Daejeon, South Korea.

Importin4 is responsible for transporting histone H3 and H4 in complex with the histone chaperone Asf1a to the nucleus for de novo chromatin assembly. Importin4 recognizes the nuclear localization sequence (NLS) located at the N-terminal tails of histones. Here, we analyzed the structure and interactions of human Importin4, histones and the histone chaperone Asf1a by cross-linking mass spectrometry (XL-MS), X-ray crystallography, negative-stain electron microscopy (EM), small-angle X-ray scattering and integrative modeling. The XL-MS data showed that the C-terminal region of Importin4 interacts exclusively with the N-terminal tails of histone H3. We determined the crystal structure of the C-terminal part of Importin4 bound with histone H3 tail, thus revealing that the acidic path in Importin4 accommodates histone H3 NLS and that histone H3 Lys14 is the primary residue interacting with Importin4. Furthermore, we present the structure of the Importin4_Histone H3/H4_Asf1a complex computed through an integrative modeling approach. Overall, this work provides structural insights into how Importin4 recognizes histones and their chaperone complex.

Incorporating Experimental Data in Long Timescales Macromolecular Simulations

Cecilia Clementi

Rice University, Houston, TX, USA

No Abstract

Dynamics of Proteins Under Crowded Conditions in Simulations and Experiments

Michael Feig^{1,2}, Grzegorz Nawrocki¹, Po-hung Wang⁴, Isseki Yu⁴, Takanori Kigawa^{2,3}, Yuji Sugita^{2,4}.

¹Michigan State University, East Lansing, MI, USA, ²RIKEN, Kobe, Japan, ³RIKEN, Yokohama, Japan, ⁴RIKEN, Wako, Japan.

Crowding in cellular environments results in constant non-specific interactions between macromolecules impacting their stability and dynamics. Altered dynamics involves both retarded diffusional motions and altered conformational sampling as a result of crowding. An analysis of computer simulations of crowded protein solutions ranging from homogeneous solutions of small model proteins such as villin to large cytoplasmic models is presented and compared to experimental data, primarily from NMR spectroscopy. The integration of simulation and experiment offers new insights into the transient weak associations of proteins under crowded conditions that result in reduced translational and rotational diffusion rates and the possibility of expanding the native-state conformational ensemble under dilute conditions towards non-native states. The comparison between simulations and experiment also offers an opportunity to critically evaluate the ability of current force fields to accurately capture the interactions of proteins under realistic cellular conditions.

In Silico Identification of Rescue Sites by Double Force Scanning

Matteo Tiberti¹, Alessandro Pandini², Franca Fraternali^{3,4,5}, **Arianna Fornili**^{1,5}.

¹Queen Mary University of London, London, United Kingdom, ²Brunel University London, London, United Kingdom, ³King's College London, London, United Kingdom, ⁴The Francis Crick institute, London, United Kingdom, ⁵The Thomas Young Centre for Theory and Simulation of Materials, London, United Kingdom.

Deleterious amino acid changes in proteins can be compensated by second-site rescue mutations. These compensatory mechanisms can be mimicked by the binding of small molecules, so that the position of rescue mutations can be used to identify possible druggable regions on the protein surface for the reactivation of damaged mutants¹.

Here we present the Double Force Scanning (DFS) method², the first general computational approach to detect rescue sites that use compensatory mechanisms mediated by backbone dynamics. The method is based on an elastic network model and on the application of external forces to mimic the effect of mutations. All the possible residue pairs in the protein are scanned and a rescue effect is detected when the simultaneous application of forces at the two sites affects the protein structure less than a force at a single site. The second-site residues that make the protein structure most resilient to the effect of single mutations are then identified.

We tested DFS predictions against two datasets containing experimentally validated and putative evolutionary-related rescue sites, finding a remarkably good agreement between predictions and reference data. Indeed, half of the experimental rescue sites in the tumour suppressor protein p53 was correctly predicted by DFS, with 65% of remaining sites in contact with DFS predictions. Similar results were found for other proteins in the evolutionary dataset. Finally, we show how the prediction of rescue sites can be used to identify potential pockets for the binding of reactivating drugs.

1. Wassman C.D., et al. (2013) Nat Commun, 4, 1407-1409
2. Tiberti M., Pandini A., Fraternali F., Fornili A., submitted.

Structural Basis of Substrate Recognition and Chaperone Activity of Ribosome-associated Trigger Factor Regulated by Monomer-dimer Equilibrium

Chih-Ting Huang¹, Yun-Tzai Lee^{1,2}, Shih-Yun Chen¹, Yei-Chen Lai³, Meng-Ru Ho¹, Yun-Wei Chiang³, **Shang-Te Danny Hsu**^{1,2}.

¹Academia Sinica, Taipei, Taiwan, ²National Taiwan University, Taipei, Taiwan, ³National Tsing Hua University, Hsinchu, Taiwan.

Trigger factor (TF) is a highly conserved bacterial chaperone that binds as a monomer via the ribosome binding domain (RBD) to the exit tunnel of the ribosome to facilitate co-translational folding of nascent polypeptide chains. Free TF however, exists in a monomer-dimer equilibrium in solution with a dissociation constant comparable to its physiological concentration. Using fluorescence anisotropy and nuclear magnetic resonance (NMR) spectroscopy, we established quantitatively that TF preferentially recognizes peptide segments enriched with aromatic and positively charged amino acids to form fuzzy complexes through binding to four distinct sites in TF. Paramagnetic NMR analysis indicated that three of these substrate binding sites within TF are sequestered upon dimer formation mediated by RBD. Small angle X-ray scattering (SAXS) demonstrated that the dimeric assembly of TF in solution deviates significantly from the previously reported crystal structure. We therefore devised an integrated approach using structural restraints derived from paramagnetic NMR, pulsed electron paramagnetic resonance, chemical cross-linking and SAXS to determine the solution structure of TF dimer in an antiparallel configuration. Our structural and functional analyses suggested that the dynamic equilibrium of the oligomeric state of TF is important for maintaining the balance between substrate binding and chaperone activities on the one hand, and preventing excessive exposure of hydrophobic surface on the other hand. Furthermore, the RBD of TF plays a dual role in regulating the three-state equilibrium between self-association and ribosome binding.

Functional Dynamics of the Distal C-tail of Arrestin

Martha Sommer¹, Ciara C.M. Lally¹, Brian Bauer¹, **Jana Selent**².

²Pompeu Fabra University, Hospital del Mar Medical Research Institute, Barcelona, Spain. ¹Institute of Medical Physics and Biophysics (CC2), Charité Medical University, Berlin, Germany,

Arrestin proteins regulate the large and diverse family of G protein-coupled receptors (GPCRs). Arrestins have an elongated structure consisting of two clam shell-like domains and a long C-terminal tail (C-tail). In crystal structures of arrestin, the proximal C-tail is observed to interact extensively with the N-domain, thereby stabilizing the basal state. However, the highly flexible and negatively charged distal C-tail is not visible in the crystal structures. Displacement of the entire C-tail by the phosphorylated receptor C-terminus is believed to activate arrestin for receptor binding.

In this study, we have applied a combination of computational and biophysical methods in order to investigate the structural dynamics of the arrestin distal C-tail. Molecular dynamics simulations show the distal C-tail sampling a wide conformational space within the concave surface of the N-domain, and one favoured placement was identified by cluster analysis. Both the placement and flexibility of the distal C-tail were verified using site-directed fluorescence methods applied to arrestin-1. The interaction between the distal C-tail and the N-domain is primarily electrostatic, and salt or binding of inositol-6-phosphate disrupts this interaction. We have further identified a functional “hinge”, which divides the relatively stable proximal C-tail from the flexible distal C-tail. Importantly, we observe that pre-complex formation with the phosphorylated receptor displaces the arrestin C-tail up to the hinge, and full-C-tail displacement occurs only upon transition to the high-affinity complex. These results imply a step-by-step displacement of the arrestin C-tail during formation of the arrestin-receptor complex.

Integrative Structural and Dynamical Biology with PLUMED-ISDB

Massimiliano Bonomi¹, Carlo Camilloni², Michele Vendruscolo¹.

¹University of Cambridge, Cambridge, United Kingdom, ²Technische Universität München, Garching, Germany.

Accurate structural models of biological systems can be obtained by properly combining multiple sources of information, such as experimental data and a priori physico-chemical knowledge [1]. Here we present PLUMED-ISDB, an open-source, freely-available module of the popular PLUMED library [www.plumed.org; 2], which enables the simultaneous determination of structure and dynamics of conformationally heterogeneous systems by integrating experimental data with a priori information. This integration is achieved using metainference [3], a general Bayesian framework that accounts for both noise in the data and their ensemble averaged nature. PLUMED-ISDB implements different types of experimental data, such as several NMR observables, FRET, SAXS and cryo-electron microscopy data [4], and enables modelling structure and dynamics of individual proteins, protein complexes, membrane proteins, RNA, and DNA, using a variety of enhanced sampling methods and resolutions of the system.

[1] M. Bonomi, G. T. Heller, C. Camilloni, M. Vendruscolo, Principles of protein structural ensemble determination. *Curr. Opin. Struct. Biol.* 42, 106-116 (2017).

[2] G. A. Tribello, M. Bonomi, D. Branduardi, C. Camilloni, G. Bussi, PLUMED 2: New feathers for an old bird. *Comp. Phys. Comm.* 185, 604-613 (2014).

[3] M. Bonomi, C. Camilloni, A. Cavalli, M. Vendruscolo, Metainference: A Bayesian inference method for heterogeneous systems. *Sci. Adv.* 2, e1501177 (2016).

[4] S. Hanot et al., Multi-scale Bayesian modelling of cryo-electron microscopy density maps. *bioRxiv* doi: 10.1101/113951 (2017).

Modelling Conformational Ensembles from Small Angle Scattering

Jill Trehella², Wojciech Potrzebowski¹, Ingemar André¹.

¹Biochemistry & Structural Biology, Lund University, Lund, Sweden, ²The University of Sydney, Sydney, Australia.

We have applied a Bayesian approach to ensemble modelling against solution Small-Angle Scattering (SAS) and NMR chemical shift data for our two domain NMR solution structure of Δ mC2 (PDB:2KDU Michie et al. 2016, Structure 24, 2000) from cardiac Myosin Binding Protein C (cMyBP-C). Δ mC2 has two folded domains linked by 7 highly flexible amino acids that are the surprisingly also highly conserved and include severe disease-linked mutation sites. We postulate it to be a polymorphic binding domain that interacts with multiple proteins to regulate muscle action in the sarcomere.

The small-angle scattering (SAS) from proteins in solution samples the ensemble average of the randomly oriented structures, and ensemble modelling for proteins with flexible regions against SAS data is increasingly popular. However, the smooth SAS profile can typically be defined by as few as 10-15 points, and the ensemble model has many more degrees of freedom. Typically, a very large ensemble (10,000 or more) is generated within some constrained set, and a population weighted sub-set of structures is identified that predicts a profile that best-fits the data. Representative structures are selected based on clustering analysis to aid in visualizing the nature of the ensemble, but their accuracy and what minimal set is justified by the data are outstanding questions.

The alternate Bayesian approach assigns a posterior probability for the population weight of each structure in an ensemble. As a result, uncertainty in the parameters of the ensemble can be quantified so that inferences can be made using standard statistical methods. We will present the results of our Bayesian approach using SAS or NMR chemical shift data alone, and SAS plus chemical shift data, and the effects of the quality and size of structural library on the selected models and their populations.

Bayesian Refinement of Protein Structures and Ensembles Against SAXS Data by Using Molecular Dynamics

Jochen Hub.

Georg-August University Goettingen, Göttingen, Germany.

Small-angle X-ray scattering is an increasingly popular technique used to detect protein structures and ensembles in solution. However, the refinement of structures and ensembles against SAXS data is often ambiguous due to the low information content of SAXS data, unknown systematic errors, and unknown scattering contributions from the solvent. We offer a solution to such problems by combining Bayesian inference with molecular dynamics simulations and explicit-solvent SAXS calculations. The Bayesian formulation correctly weights the SAXS data versus prior physical knowledge, it quantifies the precision or ambiguity of fitted structures and ensembles, and it accounts for unknown systematic errors due to poor buffer matching. The method further provides a probabilistic criterion for identifying the number of states required to explain the SAXS data. The method is demonstrated by refining ensembles of a periplasmic binding protein and of the large chaperone heat shock protein 90 (Hsp90).

[1] Shevchuk and Hub, Bayesian refinement of protein structures and ensembles against SAXS data using molecular dynamics, submitted

[2] Chen and Hub, *Biophys. J.*, 108, 2573–2584 (2015), *Biophys. J.*, 107, 435-447 (2014), *J. Phys. Chem. Lett.*, 6, 5116–5121 (2015)

Comparison of the Global Dynamics of Proteins as Assessed by WAXS and MD

Hao Zhou², Hugo Guterres³, Carla Mattos³, **Lee Makowski**^{1,3}.

¹Northeastern University, Boston, MA, USA, ²Northeastern University, Boston, MA, USA,

³Northeastern University, Boston, MA, USA.

Wide-angle x-ray solution scattering (WAXS) is highly sensitive to changes in protein dynamics. Comparison of observed scattering with that predicted for a rigid protein provides information about the spatial extent of interatomic distance fluctuations. This information is quantitated as the standard deviation of interatomic distance as a function of interatomic distance. Referred to here as a sigma-r plot, this metric can be estimated from WAXS and from molecular dynamics (MD) trajectories. Comparison of sigma-r plots from WAXS and MD can assess the degree to which an MD simulation represents a structural ensemble. It also makes possible demonstration of the self-consistency of dynamic behavior as assessed by experimental and computational approaches. Where comparison reveals inconsistencies it may provide clues to their origin: They may be caused by errors in the model for the solution structure of the protein; inaccuracies of computed trajectories; or impact of experimental conditions on the structure and/or dynamics of the protein.

We demonstrate the power of this approach by analysis of WAXS data from several proteins including HIV protease and three isoforms of ras. We show that the observed structural fluctuations of HIV protease are of greater extent than exhibited in 100 nsec MD simulations, suggesting that the MD trajectories are of inadequate length to fully explore the solution ensemble. Joint computational and experimental studies of H-ras and K-ras demonstrate extraordinary consistency between calculated and observed estimates of protein dynamics, validating the accelerated MD studies of these molecules. By contrast, inconsistencies between calculated and observed estimates of dynamics in N-ras suggest that the crystal structure of N-ras may not be an adequate representation of its solution structure. These examples demonstrate the power of the sigma-r plot for assessment of global dynamics of proteins.

Bayesian Modeling with Ensemble Data

Michael Habeck

Max Planck Institute for Biophysical Chemistry, Göttingen, Germany

No Abstract

What Does It Mean for a Protein to be Disordered? Insights from Experiment and Molecular Simulations

Collin Stultz

Massachusetts Institute of Technology, Cambridge, MA, USA

No Abstract

Bridging the Gap Between Stationary and Dynamic Data Through Augmented Markov Models

Simon Olsson, Frank Noé.

Freie Universität Berlin, Berlin, Germany.

Structural biology is rapidly moving towards a paradigm characterized by data from a broad range of experimental and computational data. Each of these are potentially sensitive to structural changes across multiple time and length scales. However, a major open problem remains: devise inference methods which optimally combine all of these different sources of information into models amenable to human analysis. There has been a considerable number of contributions to achieve this, however, reconciling information which is dynamic in nature - that is, time-series, correlation functions etc - with stationary information, remains difficult. To this end, we introduce augmented Markov models (AMM). The approach marries concepts from probability theory and information theory to optimally balance multiple sources of data - and since these models are mathematically equivalent to Markov state models, a broad suite of techniques is already available to facilitate their analysis. Through a number of examples we show how the use of AMMs results in accurate models of thermodynamics and kinetics of a number of protein systems. We therefore consider AMMs to constitute an important first step towards developing truly mechanistic, data-driven models in integrative structural biology.

Resolving Catalytic Motions and Dynamics of Isocyanide Hydratase from X-ray Crystallography

Henry Van den Bedem.

Stanford University, Menlo Park, CA, USA.

Biomolecules rely on accessing transient, excited states to interact with their partners or perform their biochemical functions. Advances in experimental techniques such as X-ray crystallography and NMR spectroscopy have resulted in unprecedented access to structural snapshots of the conformational landscapes of proteins, RNA, and their binding partners. However, these snapshots often present themselves as spatiotemporally averaged data. Resolving averaged, sparse, and heterogeneous data into constituent, structural contributions remains a formidable challenge. We have developed computational procedures to resolve biomolecular ensembles, collective motions and allostery directly from X-ray crystallography, measured at ambient temperature, as well as NMR spectroscopy data. We present results for several proteins, their ligands, and RNA.

We applied our procedures to probe the catalytic motions of isocyanide hydratase (ICH), a 230-residue homodimeric enzyme that hydrates diverse isocyanides to yield N-formamide. Oxidation of the catalytic nucleophile by irradiation forms a sulfenic acid that resembles the proposed thioimidate intermediate of ICH catalysis. The altered electrostatic environment weakens a critical hydrogen bond, which results in large conformational rearrangements of the active site. To examine how formation of a catalytic intermediate alters the structure and fast dynamics in ICH, we designed a radiation-dose perturbation series for X-ray diffraction, from minimal radiation exposure at the LCLS, to maximum radiation-induced oxidation at a synchrotron at ambient temperature. These data sets reveal a striking shift of the conformational ensemble around the active site, including a 2Å displacement of an α -helix, as the catalytic intermediate forms. Analysis of X-ray crystallography-derived order parameters reveal widespread changes in dynamics throughout the protein.

Diffuse X-ray Scattering to Model the Protein Conformational Ensemble

Michael Wall

Los Alamos National Laboratory, Los Alamos, NM, USA

No Abstract

Birth of the Cool: Protein Allostery by Multi-temperature Multi-conformer X-ray Crystallography

James Fraser

University of California, San Francisco, San Francisco, CA, USA

No Abstract

Hybrid Models and Bayesian Analysis of Individual EM Images: An Alternative for Challenging EM Data

Pilar Cossio^{1,2}, Gerhard Hummer².

¹University of Antioquia, Medellin, Colombia, ²Max Planck Institute of Biophysics, Frankfurt, Germany.

Electron microscopy (EM) provides projections images of individual biomolecules. Unhampered by the need to obtain crystals, and without the system size limits faced in nuclear magnetic resonance studies, EM is a true single-molecule technique at near-native conditions. To harness this potential, we developed a method to extract structural information from individual images of dynamic molecular assemblies. The Bayesian inference of EM (BioEM) [1] method uses a likelihood-based probabilistic measure to quantify the degree of consistency between each EM image and given model ensembles. These structural models can be constructed using hybrid-modeling or obtained from molecular dynamics simulations. To analyze EM images of highly flexible molecules, we propose an ensemble refinement procedure, and validate it with weighted ensembles from simulations and synthetic images of the ESCRT I-II supercomplex. Both the size of the ensemble and its structural members are identified correctly.

The BioEM posterior calculation is performed with a highly parallelized, GPUaccelerated computer software [2] resulting in a nearly ideal scaling both on pure CPU and on CPU+GPU architectures. This enables Bayesian analysis of tens of thousands of images in a reasonable time, and offers an alternative to 3D reconstruction methods by its ability to extract accurate population distributions for highly flexible structures and their assemblies.

[1] Cossio, Hummer. (2013) *J. Struct. Biol.* 184: 427-37.

[2] Cossio, et al. (2017) *Compu. Phys. Commun.* 210, 163-171.

Objectively and Automatically Building Multi-conformer Ligand Models in Electron Densities

Gydo Van Zundert¹, Daniel Keedy², Pooja Suresh², Amelie Heliou³, Kenneth Borrelli¹, Tyler Day¹, James Fraser², Henry Van den Bedem¹.

¹Schrodinger, New York, NY, USA, ²UCSF, San Francisco, CA, USA, ³Inria, Palaiseau, France,

⁴SLAC National Accelerator Laboratory, Menlo Park, CA, USA.

Structure-based drug design is often challenged by an inadequate understanding of the conformational dynamics of ligands and their receptors. X-ray-crystallography is generally the method of choice for resolving the structure of macromolecular molecules and investigating the binding pose of ligands. While the electron density represents a time-averaged representation of the underlying conformational ensemble, in the majority of cases the data are interpreted to represent a single conformation at unit occupancy. Temperature factors inadequately account for atom position ambiguity and thermal motion from their averaged positions. Multiple, alternative ligand conformations are under-represented, even in high resolution datasets. Moreover, the impact of alternative conformations for ligands remains underexplored. The presence of different binding poses for ligands would have important consequences for rational drug design and a fundamental understanding of the underlying binding mechanisms.

Here, we show that evidence for alternative ligand poses is common in the PDB, including for pharmaceutically highly relevant targets. In addition, we introduce a fast, automated method for building multi-conformer ligand models in electron densities by hierarchically sampling and building the ligand's degrees of freedom. We rely on powerful, state-of-the-art solvers to identify a minimal set of conformations to collectively explain the density and for determining the individual occupancies. This new tool provides an objective view on the ligand's structural heterogeneity, while paving the way for a deep investigation of its impact on rational drug design.

Systematic Perturbation of a Fundamental Biological Switch

Tanja Kortemme.

UCSF, San Francisco, CA, USA.

Cellular protein-protein interactions can be highly interconnected. Because of this complexity, it is often difficult to extract quantitative information on how each interaction contributes to distinct or overlapping cellular functions, and, moreover, how changes to individual interactions result in altered function or disease. We are developing an experimental platform for studying perturbations to multi-functional network “hub” proteins by combining high-throughput in vivo genetic interaction screening technology (Epistatic MiniArray Profile (E-MAP)) with mass-spectrometry and biophysical assays. Our case study protein is the highly-conserved multi-functional Gsp1/Ran GTPase switch that controls key eukaryotic processes. The approach first engineers defined perturbations to Gsp1/Ran protein-protein interactions by amino acid point mutations (“edge perturbations”). The second step determines the functional effects of these perturbations at the cellular and organism level in the model *S. cerevisiae*. We find that E-MAPs have a resolution that enables us to identify quantitative functional differences in vivo between individual point mutations, even those between different amino acid substitutions of the same residue. Our analysis reveals several classes of observed phenotypes that could be explained by the underlying biophysical perturbations of the on/off balance of the fundamental GTPase switch and considerable allosteric effects in the system.

From High-resolution Protein Structures to Information About Functional Dynamics

Therese Malliavin.

CNRS/Institut Pasteur, Paris, France.

High-resolution protein structures give important information on their function. Nevertheless, the discrete picture of conformational space provided by these structures do not permit to infer a complete vision of the protein functional dynamics. Besides, the enhanced sampling approaches allow a more rapid exploration of the conformational space and thus a better predictive power on the aspects of functional dynamics. Here, we will describe the application of such an approach to several proteins playing a significant biological role.

In the context of antibiotics resistance, VanA catalyzes the formation of D-Ala-D-Lac instead of the vancomycin target D-Ala-D-Ala. This reaction requires the opening of the so-called "omega-loop". Enhanced sampling coupled to clustering and graphs building provide a coarse-grained pattern of this opening (Duclert-Savatier et al, 2016).

The toxin adenylyl cyclase AC from *Bordetella pertussis* is activated by calmodulin. An high-resolution crystallographic structure is available for activated AC, whereas the inactive state of AC has not been up to now, amenable to high-resolution structural studies. The development of enhanced sampling approaches (Cortes-Ciriano et al, 2015) coupled to an analysis of the biophysical measurements on inactive AC, permits to propose series of protein conformations in agreement with the experimental knowledge on AC.

The histidine kinase CpxA belongs to a two-component system, which serves in *Escherichia coli* to couple environmental stimuli to adaptive responses. The stimuli transmission is performed via conformational transitions of the HAMP and DHP domains, for which various models are available. A combination of molecular dynamics simulations (Martinez et al, 2016), enhanced sampling approaches and fitting to experimental data made possible to probe the relevance of these models.

Cortes-Ciriano, Bouvier, Nilges, Maragliano, Malliavin. JCTC 11, 2015.

Duclert-Savatier, Bouvier, Nilges, Malliavin. JCIM 56, 2016.

Martinez, Duclert-Savatier, Betton, Alzari, Nilges, Malliavin. Biopolymers 105, 2016.

Quantitative Integrative FRET Studies Unravel the Dynamic Structural Ensemble of the Large GTPase hGBP1 Required for Oligomerization

Claus A. Seidel¹, Thomas-Otavio Peulen¹, Christian Herrmann², Carola S. Hengstenberg², Andreas Stadler³, Johann P. Klare⁴.

¹Heinrich Heine University, Duesseldorf, Germany, ²Ruhr University Bochum, Bochum, Germany, ³Forschungszentrum Jülich, Jülich, Germany, ⁴University of Osnabrück, Osnabrück, Germany.

Fluorescence spectroscopy and imaging are important biophysical techniques to study dynamics and function of biomolecules in vitro and in live cells. However, often our view of molecular function is still formed, to a significant extent, by traditional structure determination showing many detailed static snapshots of biomolecular structures. Recent fluorescence experiments added a dynamic perspective by showing the heterogeneity and flexibility of molecular structures, visualizing transiently populated conformational states and identifying exchange pathways. We introduced multi-parameter fluorescence detection (MFD) [1] and multi-parameter fluorescence image spectroscopy (MFIS) [2] to register all eight characteristic fluorescence parameters in a single measurement for gaining maximum resolution of specific fluorescence information on the biomolecule. The application of fluctuation spectroscopy allows us to resolve system properties such as diffusional properties and kinetic networks. The use of more than one fluorophore per molecule opens additional opportunities arising from photon densities, coincidences and dipolar coupling by Förster Resonance Energy Transfer (FRET) to study the stoichiometry and structure of biomolecular systems [4]. We applied our techniques to resolve the conformational ensemble and map the structural dynamics of the large GTPase [5] human Guanylate binding protein 1 (hGBP1) [5] during oligomerization in vitro and in live cells [5].

[1] *Anal. Chem.* 78, 2039-2050 (2006).

[2] *Photochem. Photobiol. Sci.*, 8, 470-480 (2009).

[3] *Nat. Methods* 9, 1218-1225 (2012).

[4] *Curr. Opin. Struct. Biol.* 40, 163-185 (2016).

[5] *eLife* 5, e11479 (2016).

Inferring Protein Structure from Sparse and Unreliable Experimental Data

Justin MacCallum

University of Calgary, Calgary, AB, Canada

No Abstract

Averaged Experimental Data: From Algebra to Biology

Enrico Ravera¹, Luca Sgheri², Giacomo Parigi¹, Claudio Luchinat¹.

¹University of Florence, Sesto Fiorentino, Italy, ²National Research Council (CNR), Sesto Fiorentino, Italy.

Calculating the expected experimental observables (e.g.: NMR, EPR, SAXS,...) from a given conformation, or ensemble thereof, is a trivial task. On the contrary finding the relative population of different conformers that compose the natural ensemble from the averaged experimental observables is an ill-posed inverse problem that admits an infinite number of solutions. Several approaches have been provided over the years to address this problem: here we will examine the relations among different approaches[1] and we will present results on some biological entities as obtained by a largest-weight approach.[2]

[1] Ravera, Sgheri, Parigi, Luchinat, *Phys. Chem. Chem. Phys.*, 2016, 18(8), 5686-5701;
Bonomi, Heller, Camilloni, Venduscolo, *Curr. Opin. Struct. Biol.*, 2017, 42, 106-116.

[2] Cerofolini, Fields, Fragai, Geraldes, Luchinat, Parigi, Ravera, Svergun, Teixeira, *J. Biol. Chem.*, 288, 30659-30671; Andralojc, Ravera, Salmon, Parigi, Al-Hashimi, Luchinat, *Phys. Chem. Chem. Phys.*, 2016, 18(8), 5743-5752; Andralojc, Hiruma, Liu, Ravera, Nojiri, Parigi, Luchinat, Ubbink, *Proc. Natl. Acad. Sci. USA*, 2017, 114(10), E1840-E1847.

Computation of Structural Ensembles from NMR and Other Data

Charles Schwieters

NIH, Bethesda, MD, USA

No Abstract

Mechanism of Substrate Translocation in an Alternating Access Transporter

Naomi Latorraca^{1,2,3}, Nathan Fastman^{2,3}, Ron Dror^{1,2,3}, Liang Feng^{2,3}.

¹Stanford University, Stanford, CA, USA, ²Stanford University, Stanford, CA, USA, ³Stanford University, Stanford, CA, USA.

Transporters shuttle molecules across cell membranes by alternating among distinct conformational states. Fundamental questions remain about how transporters transition between states and how such structural rearrangements regulate substrate translocation. We captured the translocation process by crystallography and unguided molecular dynamics simulations, providing an atomic-level description of alternating access transport (Latorraca et al, Cell 2017). Simulations of a SWEET-family transporter initiated from an outward-open, glucose-bound structure reported here spontaneously adopt occluded and inward-open conformations. Strikingly, these conformations match crystal structures, including our inward-open structure. Mutagenesis experiments further validate simulation predictions. Our results reveal that state transitions are driven by favorable interactions formed upon closure of extracellular and intracellular “gates” and by an unfavorable transmembrane helix configuration when both gates are closed. This mechanism leads to tight allosteric coupling between gates, preventing them from opening simultaneously. Interestingly, the substrate appears to take a “free ride” across the membrane without causing major structural rearrangements in the transporter.

POSTER ABSTRACTS

POSTER SESSION I**Saturday, August 26, 2017****16:30 – 18:00****Meitner Hall**

Posters are available for viewing only during their scheduled date of presentation. Below are the formal presentation times. The presenters listed below are required to remain in front of their poster boards to meet with attendees during the designated times.

Odd-Numbered Boards 16:30 – 17:15 | Even-Numbered Boards 17:15 – 18:00

Annick, Dejaegere	1-POS	Board 1
Axen, Seth	2-POS	Board 2
Baker, Brian	3-POS	Board 3
Ball, K. Aurelia	4-POS	Board 4
Berntsson, Oskar	5-POS	Board 5
Budday, Dominik	6-POS	Board 6
Bunster, Marta	7-POS	Board 7
Cembran, Alessandro	8-POS	Board 8
Cesari, Andrea	9-POS	Board 9
Chang, Chun-Chun	10-POS	Board 10
Chevallier, Augustin	11-POS	Board 11
Chrisman, Ian	12-POS	Board 12
Christensen, Mikkel	13-POS	Board 13
Ciemny, Maciej	14-POS	Board 14
Ciemny, Maciej	15-POS	Board 15
Cragnell, Carolina	16-POS	Board 16
Daday, Csaba	17-POS	Board 17
Day, Tyler	18-POS	Board 18
Degiacomi, Matteo	19-POS	Board 19
Domene, Carmen	20-POS	Board 20
Dong, Hao	21-POS	Board 21
Dutta, Sutapa	22-POS	Board 22
Eilert, Tobias	23-POS	Board 23
Enderlein, Joerg	24-POS	Board 24
Erlenbach, Nicole	25-POS	Board 25
Fadda, Elisa	26-POS	Board 26
Feng, Chi-Jui	27-POS	Board 27
Flores-Canales, Jose	28-POS	Board 28
Fossépré, Mathieu	29-POS	Board 29
Frezza, Elisa	30-POS	Board 30

Fukuda, Ikuo	31-POS	Board 31
Gandhi, Neha	32-POS	Board 32
Girodat, Dylan	33-POS	Board 33
Globisch, Christopher	34-POS	Board 34
Götz, Alexander	35-POS	Board 35
Granata, Daniele	36-POS	Board 36
Ha-Duong, Tâp	37-POS	Board 37
Hashem, Shaima	38-POS	Board 38
Hays, Jennifer	39-POS	Board 39
Hoffman, Falk	40-POS	Board 40

Posters should be set up on the morning of Saturday, August 26 and removed by 18:00 on Saturday, August 26. All uncollected posters will be discarded.

1-POS Board 1

Integrative Modelling of Nuclear Receptor Proteins

Jérôme Eberhardt, Roland H. Stote, **Annick Dejaegere**.
IGBMC - Strasbourg University, Illkirch, France.

Nuclear hormone receptors (NRs) are ligand-dependent transcriptional regulators that have a central role in regulating development and homeostasis in metazoan. Their molecular regulation is linked to their ability to undergo allosteric structural changes upon signaling hormone binding, which leads to activation or repression of regulated genes. Besides ligand binding, nuclear receptors are regulated by different signals including post-translational modifications. However, for these latter signaling events, the underlying molecular mechanism of regulation is still poorly understood.

Although structural snapshots of essential structures along the regulation pathway of NRs have been obtained largely by crystallographic studies of their structured DNA binding (DBD) and ligand binding (LBD) domains, regulation is also linked to changes in the structural dynamics of the receptor. The characterization of these structural dynamical effects is crucial to our understanding of the allosteric mechanisms occurring in these receptors.

In recent years, mass spectrometry based hydrogen-deuterium (HDXMS) exchange has emerged as the method of choice to characterize NRs structural dynamics. However, even if empirical correlations have been established between HDXMS and functional effects of NRs ligands, the underlying conformational landscape has not been characterized. We use molecular simulations coupled with experimental data to characterize the conformational dynamics of nuclear receptors and their role in functional regulation. In particular, we identified transient conformations of the retinoic X receptor (RXR) using accelerated molecular dynamics simulations and showed that phosphorylation of the RXR ligand binding domain affects the underlying conformational landscape. To validate our conformations, we developed a protocol that permits us to calculate hydrogen-deuterium exchange data. This protocol is of general use to interpret HDMX data and to relate the observed exchange to transient conformations.

2-POS Board 2

Determining the Information Content of Second Harmonic Generation Spectroscopy for Modeling Conformational Changes of Macromolecules

Seth D. Axen¹, Bason Clancy², Joshua Salafsky², Andrej Sali¹.

¹University of California, San Francisco, San Francisco, CA, USA, ²Biodesy, Inc., South San Francisco, CA, USA.

When modeling conformational changes of proteins, often the structure of a single conformation or of individual domains is known or can be inferred from structures of homologous proteins. A number of structural techniques are available for modeling additional conformations in response to perturbations. However, many of these techniques suffer from limitations on the size of the system, are performed under non-physiological conditions, are difficult to perform, or are low-throughput. A promising new technique uses the nonlinear optic phenomenon Second Harmonic Generation (SHG) in a highly sensitive assay for conformational changes in proteins that informs relative orientations of structural components. In this work, a computational method is developed for modeling conformational changes of ideal rigid body systems using SHG data combined with distance restraints. By varying the relative density of simulated data and degrees of freedom of modeled systems, the relative information content of SHG data is estimated, and the experimental constraints necessary to increase this information content are determined, directing future SHG experiments.

3-POS Board 3

Peptide Tuning of MHC Protein Energy Landscapes: A Key Aspect of Antigen Presentation and Recognition in the Immune System

Cory Ayres, **Brian Baker**.

University of Notre Dame, Notre Dame, IN, USA.

Major histocompatibility complex (MHC) proteins bind and "present" peptides for recognition by T cells of the immune system. Through a combination of structural biology and solution biophysics, we previously demonstrated that different peptides "tune" the energy landscapes of MHC proteins. The consequences of this tuning on the motional properties of MHC proteins impacts recognition by T cell receptors of the immune system. To explore this phenomenon in more detail, here we describe the creation and analysis of a large library of molecular simulations of different peptides bound to the most common class I MHC protein, HLA-A2. Nearly 100 different peptide/HLA-A2 complexes were simulated for 1 microsecond, generating a rich dataset of MD data for the same protein bound to different peptides. We identified key residues that are important in tuning of HLA-A2 dynamics and show that the HLA-A2 energy landscape is modulated such that protein dynamics are altered across the protein. Altered dynamics are transmitted not only to sites that interact with T cell receptors, but sites that interact with other activating and inhibitory immune receptors and components of the peptide loading and editing machinery. We propose that modulation of the energy landscape of MHC proteins is a key aspect of antigen presentation and recognition in the immune system.

4-POS Board 4

Conformational Ensembles of the HIV Vif Complex

K. Aurelia Ball¹, Lieza Chan¹, Eliese Tierney¹, David Stanley³, Matthew Jacobson², John Gross².

¹Skidmore College, Saratoga Springs, NY, USA, ²University of California San Francisco, San Francisco, CA, USA, ³University Of California Berkeley, Berkeley, CA, USA.

Like many viruses, HIV hijacks the host cell's apparatus for normal protein ubiquitination and degradation, using it to eliminate antiviral proteins. Understanding how a virus recruits and targets the ubiquitination complex is critical for developing therapeutics to prevent it. One HIV protein responsible for this hijacking is Virion infectivity factor (Vif). Vif is intrinsically disordered but loses flexibility as it binds more host proteins, a process that may be crucial for function. We are investigating the complex formed with Vif and the host proteins Elongin B (EloB), Elongin C (EloC), and Core-Binding Factor subunit beta (CBF-beta) to determine what remaining conformational flexibility Vif retains as part of this large complex with folded proteins. Using molecular dynamics simulations, we have found that this Vif-host protein complex exhibits large-scale conformational changes and occupies alternate conformational states. These conformational dynamics are altered when additional proteins such as Cullin 5, part of the ubiquitination complex, or APOBEC3F, an antiviral protein targeted by Vif for ubiquitination, are bound to the complex. We observe a reduction in large scale motions of the complex and a reduction in the sampling of the conformational landscape when an additional protein is bound. Additionally, we find that the partially disordered C-terminus of EloB is important for the structural stability of the complex. These computational results are supported by methyl-labeled NMR spectroscopy. The alternate conformations sampled in our simulations are important for better understanding the function of the complex in ubiquitinating APOBEC, as well as how a disordered protein like Vif can affect the dynamics of a larger complex of folded proteins.

5-POS Board 5

Signal Transduction in *Drosophila* Cryptochrome Revealed by Experimentally Guided Molecular Dynamics Simulations

Oskar Berntsson¹, Ryan Rodriguez², Erik Schleicher², Sebastian Westenhoff¹.

¹University of Gothenburg, Göteborg, Sweden, ²University of Freiburg, Freiburg, Germany.

Cryptochromes are blue light photoreceptor proteins found in plants and animals. They are the main photoreceptor in the circadian clocks of insects and plants and are also required for magnetoreception in birds and insects. A flavin chromophore within the protein matrix is responsible for the blue light sensitivity. Upon illumination an electron is transferred a series of tryptophan residues to the chromophore, creating a long lived radical pair. Eventually the signal is relayed through the protein, causing detachment of the carboxy terminus. Using a combination of molecular dynamics simulations and time-resolved X-ray solution scattering we investigate the signal transduction pathway in *Drosophila melanogaster* cryptochrome (*DmCry*). Our unpublished data show that *DmCry* gets compressed within the first 300 ns following light exposure. This compression lasts about 1 ms before the protein extends and the carboxy terminal part of the protein detaches. Our analysis reveals how the protonation state and hydrogen bonding of a conserved histidine residue relays the signal from the chromophore to the carboxy terminus. This is one of the most comprehensive structural models for cryptochrome signal transduction to date and it provides insights into the effect of blue light on the circadian cycle.

6-POS Board 6

Hierarchical, Structural Basis for Motions Encoding HDXMS Data

Dominik Budday¹, Sigrid Leyendecker¹, Henry Van den Bedem².

¹University of Erlangen-Nuremberg, Erlangen, Germany, ²Stanford University, Menlo Park, CA, USA.

Hydrogen-Deuterium Exchange Mass Spectroscopy (HDXMS) can provide important experimental insights into functional dynamics based on neutron exchange between protein and solvent. Differences in Deuterium exchange by the protein between wild-type and mutants, or across different members of a protein family can relate structural dynamics with function. However, the long time-scales of HDXMS experiments often make data interpretation challenging. The availability of computational methods capable of resolving these spatio-temporal scales could result in broader adoption of HDXMS.

Here, we adapt Kino-Geometric Sampling (KGS) to provide a structural basis for motions with hierarchically increasing hydrogen bond violations. Our geometric approach encodes hydrogen bonds as holonomic constraints, imposing collective motions on the dihedral degrees of freedom to maintain cycle-closure. A singular value decomposition of the constraint Jacobian ranks independent, orthonormal motion modes by constraint violation, reminiscent of normal modes that describe functionally relevant motions at low eigenfrequencies. The method is based on the hypothesis that violations of the hydrogen bond network geometrically encoded in KGS is related to functional, dynamic exchange in the molecule. Our kinematic, time-independent analysis is very fast and applicable to proteins and RNA, making it suitable to study motions across spatio-temporal scales in a matter of seconds.

Predictions from KGS hierarchical motions on exchanging hydrogen bonds show qualitative agreement with HDXMS measurements in the α -subunit of protein Gs. We further compare our predictions with the Start2Fold database which provides a collection of sparse data on folding cooperativity and stability of proteins measured by HDXMS and related experimental methods. Initial results are promising and strengthen our hypothesis, displaying a fast, yet detailed computational tool to interpret and predict hydrogen-deuterium exchange in macromolecules.

7-POS Board 7

Structural Studies of a Phycobilisome

Marta Bunster, Maximiliano Figueroa, José Martínez-Oyanedel, Jorge Dagnino-Leone, Aleikar José Vásquez.
n/a, Concepción, Chile.

The structure of a Phycobilisome is strictly related to its function of light harvesting and energy transfer towards the photoreaction center. Phycobilisome is a macro complex of phycobiliproteins and linker proteins that in *Gracilaria chilensis*, an eukaryotic red algae, is associated to thylakoids.

Each phycobilisome is formed by a CORE of Allophycocyanin from which radiate RODS formed by Phycocyanin and Phycoerythrin. A common feature of all phycobiliproteins is that they are formed by alfa/beta heterodimers that oligomerize to trimers or hexamers originating ring structures that are piled up as in an antenna. Each phycobiliprotein contains chromophores bound to specific cysteine residues.

We have been studying this complex by using experimental approaches such as molecular biology and biochemistry techniques, biophysical approaches such as X-ray crystallography, and spectroscopy, as well as molecular simulations *in silico*.

Electron microscopy provide evidences for a three cylinders core of Allophycocyanin, 5 to 6 rods of Phycoerythrin and Phycocyanin in the PBS. The three dimensional structures of all the phycobiliproteins were determined by X ray diffraction, and their association to form rods and the core was approached by *in silico* and *in vitro* studies. Variation of subunits and the presence of linkers were also approached by transcriptomics, biochemical techniques and spectroscopy. All this information is presented in a model for the structure and function of the phycobilisome of *Gracilaria chilensis*.

8-POS Board 8

Conformational Landscape of Dystrophin's Actin Binding Domain 1 by Molecular Dynamics Simulations

Benjamin Horn¹, Michael Fealey², Anne Hinderliter¹, David Thomas², **Alessandro Cembran**¹.
¹University of Minnesota Duluth, Duluth, MN, USA, ²University of Minnesota, Minneapolis, MN, USA.

The primary role of dystrophin is to stabilize the membrane of muscle cells against the mechanical forces deriving from muscle contraction and relaxation; its absence or mutation lead to various forms of muscular dystrophy. Dystrophin is connected at the protein's N-terminus to the actin protein through the actin binding domain 1 (ABD1), formed by two calponin-homology (CH) domains connected by a linker. Double electron-electron resonance experiments indicate that the ABD1 domain of dystrophin switches from a compact to an extended conformation upon binding to actin. We hypothesize that hydrophobic interactions are the main driving force promoting the conformational transition toward the compact ensemble in the absence of actin. To test this hypothesis we performed molecular dynamics simulations of the ABD1 domain of dystrophin, of the ABD1 domain of the utrophin homolog, and of dystrophin's ABD1 mutants. Our results confirmed that disruption of the hydrophobic interactions leads to a destabilization of specific compact conformations, but also showed that the compact ensemble of the protein is resilient to hydrophobic-to-hydrophilic mutations. On the contrary, mutations that affect the overall charge of the two CH domains had a much more significant impact on the equilibrium, and led to a larger shift toward the extended state. Together, these data indicate that electrostatic interactions play an important role in the extended to compact conformational transition, and that the complementarity in hydrophobic interactions characterizes the specific compact conformations that are stabilized.

9-POS Board 9

Refining Molecular Dynamics Simulations of RNA Using Solution NMR Data

Andrea Cesari, Alejandro Gil Ley, Giovanni Bussi.
SISSA, Trieste, Italy.

RNA structure and dynamics play a fundamental role in non-coding RNAs and significantly affect functions such as gene expression inhibition, splicing, and catalysis. Molecular dynamics is a computational tool that can be in principle used to investigate RNA structure and dynamics at atomistic resolution. However, its capability to predict and explain experimental data is limited by the accuracy of the employed potential energy functions, also known as force fields. Recent works have shown that state-of-the-art force fields could predict unphysical conformations that are not in agreement with experiments. The emerging strategy to overcome these limitations is to complement molecular dynamics with experimental data included as restraints. Solution NMR data are particularly useful since they provide averages over the conformations explored on the experimental time scale and ultimately give access to RNA dynamics. We here propose a scheme based on the maximum entropy principle to combine bulk experiments with molecular dynamics simulations explicitly taking into account experimental errors[1]. This scheme allows to generate conformational ensembles based on a standard force field, that is used as a prior, and in agreement with experimental data. In addition, the method can be extended to adjust force fields in a chemically-consistent manner allowing transferable corrections to be obtained. The resulting RNA force field is then validated on a number of noncanonical structures.

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10-POS Board 10

Combining In Silico and In Vitro Investigations for the Study of Selective Targeting of the Hepatitis C Virus to Its Host

Chun-Chun Chang^{1,2}, Hao-Jen Hsu³, Je-Wen Liou^{1,4}.

¹Tzu Chi University, Hualien, Taiwan, ²Tzu Chi Medical Center, Hualien, Taiwan, ³Tzu Chi University, Hualien, Taiwan, ⁴Tzu Chi University, Hualien, Taiwan.

Hepatitis C virus (HCV) is one of the most important causes of human liver diseases. However, HCV infection is extremely species-specific, only human and chimpanzee can be infected. This makes the study of the HCV infection on other animal models almost impossible. Previous studies indicated that interactions between the HCV E2 glycoprotein and CD81 on the human cells are crucial for HCV infection. The main purpose of this study is to find out decisive factors for the species-specific interactions at molecular levels by employing the in silico techniques including structure model construction and molecular dynamic simulation. The binding of the HCV E2 protein on human and rat surface markers were also compared and analyzed. in vitro experiments including surface plasmon resonance measurements and cellular binding assays were followed for the validations of the in silico results. Based on the in silico studies, two binding regions on the HCV E2 loop domain were identified to be important for the interactions with CD81s, and the one of the two might be the determinant factor for the species-specificity. MM/PBSA binding free energy calculations indicated that the E2/CD81 binding process might follow a two-steps model, which involves the initial binding of human-specific region of E2 to the CD81, followed by E2 orientation change to allow the binding of the other E2 region. The amino acid sequence derived from the human-specific, stronger binding E2 region might be used as a template for the possible development of HCV inhibiting synthetic peptide drugs, and the techniques demonstrated here might be an excellent example for the study of species-specific virus/host interactions.

11-POS Board 11

Density of State Estimates for Conformational Ensembles Using an Improved Wang-Landau Algorithm

Augustin Chevallier, Frederic Cazals.
INRIA, Sophia Antipolis, France.

Generalized ensemble methods have proven effective to explore the energy landscape of complex systems. Among the available options, the Wang-Landau (WL) algorithm implements a random walk in energy space, from which the density of states is obtained.

However, even though several improvements have been proposed, notably on the choice of proper bin size and random walk [1], obtaining effective convergence using Wang-Landau is still challenging. Practically, differences in flatness and high dimensional effects (measure concentration) are major hurdles for convergence.

To address these problems, we introduce two novel strategies based upon non symmetric random walks and the exploitation by the random walk of local geometric features of the landscape.

In addition, there exists very few versatile implementations of the Wang-Landau algorithm. We provide such an implementation, in C++, decoupling all key ingredients of the algorithm (physical system, data structures, flat histogram rule, etc). The code, which is to be released in the Structural Bioinformatics Library [3], is used to obtain results on peptides and a model protein (BLN69), whose energy landscapes have been studied elsewhere [4,5]. Convergence speedups of several orders of magnitude are obtained.

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J. of Computational Chemistry, 37 (8), 2016.

12-POS Board 12

Examining the Conformational Ensemble in the Peroxisome Proliferation-activating Receptor Gamma (PPAR γ) Ligand Binding Domain

Ian M. Chrisman¹, Michelle D. Nemetchek¹, Travis S. Hughes^{1,2,3}.

¹University of Montana, Missoula, MT, USA, ²University of Montana, Missoula, MT, USA,

³University of Montana, Missoula, MT, USA.

Relatively little is known about the conformational ensemble in the ligand binding domains (LBD) of nuclear receptors, a type of transcription factor. They are often assumed to exist in two-state systems where ligand binding causes conformational change to direct transcriptional activation or repression. In this study we employ ¹⁹F NMR and chemical exchange saturation transfer (CEST) to examine the conformational states of the LBD of the nuclear receptor PPAR γ , in both an apo state as well as bound to a variety of drugs. Fluorine NMR is employed through the covalent attachment of a fluorine probe to a cysteine introduced in the AF2 region, a region of the protein important for binding other effector proteins (coregulators). In an apo state the AF2 region exists in a wide variety of conformations which are in intermediate exchange. These conformations are the components of two distinct NMR peaks which are in slower exchange. Molecular simulations suggest the possible structure of the faster exchanging conformations. With all ligands examined there exist two or more conformations of the LBD as determined by objective deconvolution of the NMR signal into Lorentzian populations. This is seen even in the presence of covalent ligands. We observe very slow exchange (<1s⁻¹) between conformations detected as well separated ¹⁹F NMR peaks and differences in the ¹⁹F probe solvent exposure in these distinct conformations. Such results indicate that the conformational ensemble of PPAR γ LBD supports multiple distinct conformations in a single protein state and a two-state protein conformation model may be unsuitable for studying of this and other nuclear receptors. Finally, there is a significant correlation between conformation chemical shift and coregulatory peptide binding, indicating that distinct NMR observed conformations are also functionally distinct.

13-POS Board 13

Modelling the Self-Assembly of Islet Amyloid Polypeptide

Mikkel Christensen^{1,2,2}, Katrine K. Skeby^{1,2}, Birgit Schiøtt^{1,2}.

¹Aarhus University, Aarhus, Denmark, ²Interdisciplinary Nanoscience Center, Aarhus, Denmark.

Islet amyloid polypeptide (IAPP) is an amyloid peptide that forms structures with a cytotoxic activity; thereby decreasing the number of insulin producing cells in patients with type II diabetes mellitus. While the nature of the cytotoxic structures is unknown; mature amyloid fibrils of IAPP have been isolated from living tissue and non-selective and membrane-perforating pores of IAPP has been observed in experiments. Many researchers seek to learn about these structures to find a way to slow down the development of the disease. With all-atom molecular dynamics simulations combined with the Highly Mobile Membrane Mimetic Model (HMMM) with enhanced sampling of membrane dynamics, we have investigated the membrane interaction and self-assembly of this IAPP peptides. The focus is on the initial self-assembly and how lipid membranes induce the transformation from monomers to oligomers. We have observed the transition from alpha-helical monomers to stable beta-sheet containing assemblies. With this we have identified regions of the peptide that are important for the inter-peptide recognition and for connecting the peptides in beta-sheets. These results support current experimental findings and can be used to explain experimental results in atomistic models, and inspire future experimental and computational studies of the peptide.

14-POS Board 14

CABS-dock Web Server for Protein-Peptide Docking with Large-scale Flexibility of a Protein Receptor

Maciej Ciemny^{2,1}, Karolina Dawid^{2,1}, Mateusz Kurcinski¹, Maciej Blaszczyk¹, Andrzej Kolinski¹, Sebastian Kmiecik¹.

¹University of Warsaw, Warsaw, Poland, ²University of Warsaw, Warsaw, Poland.

Protein-peptide interactions may involve large-scale conformational changes of a protein receptor, that are challenging to study either by experiment or computationally¹. Here, we present our method for flexible protein-peptide docking – the CABS-dock²⁻⁴. The method performs a blind global search for a binding site combined with an on-the-fly folding of a fully flexible peptide, while the receptor backbone fluctuates around its input conformation. In CABS-dock, the flexibility of chosen protein regions may be assigned as fully flexible by a user to allow for large-scale conformational changes. This method was applied to modeling of the MDM2/p53 complex, in which the receptor contains intrinsically disordered regions of significant length¹. The obtained results matched well the experimental data and provided new insights into the possible role of unstructured receptor regions. CABS-dock is available as a web server at: <http://biocomp.chem.uw.edu.pl/CABSdock>.

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15-POS Board 15

CABS-flex Web Server for Fast Simulations of Flexibility of Globular Proteins

Maciej Ciemny^{2,1}, Karolina Dawid^{2,1}, Mateusz Kurcinski¹, Maciej Blaszczyk¹, Andrzej Kolinski¹, Sebastian Kmiecik¹.

¹University of Warsaw, Warsaw, Poland, ²University of Warsaw, Warsaw, Poland.

Investigating of the conformational flexibility of protein structures is crucial for understanding their functions. Large timescales of protein molecular motions limit the applicability of standard molecular dynamics methods to this problem. Our method - CABS-flex^{1,2} – implements a highly efficient, coarse-grained approach for prediction of fluctuations of the protein structures. The resulting structural ensembles reflect the flexibility of investigated protein and provide a picture complementary to the results obtained from molecular dynamics simulations³ as well as NMR conformational ensembles². The CABS-flex methodology is also successfully used for efficient simulations of protein flexibility in predictions of protein-peptide complexes^{4,5} and protein aggregation properties⁶. The CABS-flex web server is freely available at: <http://biocomp.chem.uw.edu.pl/CABSflex/>.

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16-POS Board 16

Modelling of the Flexible Protein Histatin 5

Carolina Cragnell, Marie Skepö.
Lund University, Lund, Sweden.

The existence of functional disordered (unstructured) proteins has been recognized for many years. However, due to the classical structure-function paradigm, the functional role of intrinsically disordered proteins has only recently been recognized. Biochemical evidence has since shown that these proteins are functional, and that the lack of a folded structure is related to their function.

We would like to present results from a combined experimental and theoretical study, where the aim is to develop a model for flexible proteins and to relate the lack of structure of the proteins in solution with their function and structure when adsorbed to surfaces. For this purpose, we are combining atomistic and coarse-grained modelling, with simulation techniques such as molecular dynamics and Monte Carlo simulations. The simulations are compared with SAXS experiments of a model protein (Histatin 5).

We will show simulated scattering curves that are in good agreement SAXS. At high salt concentration, Histatin 5 behaves as a neutral polymer, and at low salt concentration, a repulsive peak is obtained at low q . In the latter regime, it is the net charge of the protein that is of importance for the inter molecular interaction and not the charge distribution. Preliminary results also indicates that the peptide is more stretched out in low pH solutions as well as in presence of divalent ions such as Zn^{2+} , Mg^{2+} , and Ca^{2+} . This indicate that electrostatic interactions indeed are important for Histatin 5 bulk structure.

17-POS Board 17

Coarse-grained Modelling of Conformational Changes in Focal Adhesion Kinase upon Oligomerization

Csaba Daday^{1,2}, Frauke Gräter^{1,2}.

¹Heidelberg Institute for Theoretical Studies, Heidelberg, Baden-Württemberg, Germany,

²Heidelberg University, Heidelberg, Baden-Württemberg, Germany.

Focal adhesion kinase (FAK) is a membrane-bound protein found in focal adhesion sites, the kinase domain of which is autoinhibited in equilibrium by the so-called FERM domain. It has been observed experimentally that FAK is recruited by PIP₂, a highly negative lipid, and this binding is accomplished through a basic patch of the protein (four cationic residues).

In previous work, we have shown in conjunction with FRET experiments that small conformational changes of FAK happen upon binding PIP₂[1], although significant mechanical force as well as the PIP₂ binding is also required to activate the kinase domain[2].

In recent experiments (unpublished data) it has also been shown that FAK can form regular assemblies on PIP₂-enriched membranes, with potential effects on its conformation and activity.

We perform large-scale coarse-grained (CG) simulations on 25 copies of FAK on a PIP₂-enriched membranes to observe and further understand FAK clustering. We find spontaneous FAK oligomerization with implications for FAK's scaffolding function at focal adhesions. Our data also point to a too strong protein-PIP₂ interaction in the MARTINI force field, for which we suggest a correction.

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18-POS Board 18

The Use of Conformational Ensembles in Virtual Screens

Tyler Day¹, Brandi Hudson², Levi Pierce³, Goran Krilov¹, Jeremy Wilbur³, Jonathan Weiss³, James Fraser².

¹Schrodinger, Inc, New York, NY, USA, ³Relay Therapeutics, Cambridge, MA, USA. ²University of California, San Francisco, San Francisco, CA, USA,

Structure-based virtual screening is a well-established protocol for identifying the initial chemical matter of a drug discovery program. However, due to the inherent hardness of structure-based scoring functions, the nature of the chemical matter identified by virtual screens is strongly influenced by the protein structure employed. Conventional crystallographic refinement produces just a single protein conformation consistent with a particular data set. In some cases, an additional set of alternate coordinates for a small number of residues can be modeled into high resolution data. This lack of conformational diversity can lead directly to a similar lack of diversity in the chemical matter found during a virtual screen. To overcome this limitation, we show how using multiple crystal structures within a single virtual screen can lead to a significant improvement in not only the number of hits identified, but also their chemical diversity. As an alternative approach, since multiple crystal structures are not always available, we have constructed conformational ensembles from individual crystallographic data sets. We find that this approach can lead to an increase in the number and diversity of hits, provided key ensemble members are identified and included in the screen. This ensemble approach opens up the possibility of identifying novel chemical matter that binds to lower occupancy, but still energetically accessible, conformational states of the receptor. Such states would never be evident in a single-structure representation of the experimental data, thereby missing the opportunity to identify this novel chemical matter.

19-POS Board 19

Mass Spectrometry Based Modelling of Macromolecular Assemblies

Matteo T. Degiacomi, Justin L. Benesch.
University of Oxford, Oxford, United Kingdom.

Small Heat-Shock Proteins (sHSP) are present in all kingdoms of life, and form assemblies spanning a continuum of structures from mono- to polydisperse, with variable architecture. These can bind unfolding proteins, preventing their potentially harmful denaturation. Since sHSP oligomers can bind several proteins simultaneously, ensembles of $\gg 100$ sHSP:target stoichiometries are often observed. This dynamic nature, seemingly critical to their cellular function, makes these proteins intractable by most conventional biochemical approaches. Mass Spectrometry (MS) is one of the few techniques able to separate these complexes and assess them individually. By exploiting MS structural data, we generate plausible polyhedral models for sHSPs, and describe a possible binding mode to their targets.

This work led to the development of novel computational tools of general applicability. These include an accurate method to predict cross-linkable amino acids, adopting an ensemble representation to account for both cross-linker and protein flexibility. Furthermore methods have been developed to calculate the collision cross-section of electron density maps and to arrange molecules according to arbitrary topologies. These are all integrated in BiobOx, a Python package allowing the analysis and manipulation of molecular structures and ensembles at an atomistic, super coarse-grain, or electron density level.

Overall, we show that protein ensemble representations coupled with MS data can be successfully exploited for the modelling of protein assemblies and their interactions with specific substrates.

20-POS Board 20

Insight into Oxygen Diffusion in Oxygenases

Carmen Domene,
Kings College London, London, United Kingdom.

The 2-oxoglutarate (2OG) dependent oxygenases are a superfamily of non-haem iron dependent oxygenases, most of which use the Krebs cycle intermediate, 2-oxoglutarate (2OG), as a cosubstrate. We are interested in understanding these enzymes for their ability to catalyse synthetically difficult or 'impossible' reactions (e.g. the stereoselective hydroxylation of unactivated carbon-hydrogen bonds), for their diverse physiological roles, and for their links to disease. These enzymes have important roles in oxygen sensing, collagen biosynthesis, fatty acid metabolism and in the epigenetic regulation of gene expression.

There are estimated to be 60-80 human 2OG oxygenases. Defining functions for all human '2OG oxygenases' at biochemical, cellular, and physiological level is crucial. Given the mechanistic and structural conservation amongst 2OG oxygenases, we are attempting to define the molecular features that are responsible for their reaction with O₂. As part of this, a combined computational and experimental approach has been followed. Crystal structures have been obtained, small-molecule inhibitors of 2OG oxygenases have been identified, and computational mechanistic studies using equilibrium and non-equilibrium computer simulations have been carried out. An overview of this project will be given in this presentation.

21-POS Board 21

Molecular-level Computer Simulations on the Self-assembled Peptides with Catalytic Activity

Ruiheng Song, Guixiang Zeng, Wenfei Li, **Hao Dong**, Wei Wang.
Nanjing University, Nanjing, China.

Molecular self-assembly is ubiquitous in nature. Exploiting the principles of self-assembly could help people to create new materials with novel structures and functions. Amyloid fibrils made up with self-assembled short peptides have well-defined structures, which are able to form functional motif. In this work, we employed multi-scale molecular simulations to study amyloid-forming short peptides, which were reported to have remarkable activity to mimic the carbonic anhydrase. The building block is a 7-peptide with alternate hydrophobic and hydrophilic residues: the hydrophobic side-chains are able to have stable packing interactions with each others, and the hydrophilic histidine side-chains is capable of forming active sites for the catalytic reactions. Firstly, we explored the aggregation of peptides into organized structure as a consequence of hydrogen bonding and hydrophobic packing interactions among the subunits. Molecular simulations suggested that the sequence of the peptide greatly affects the pattern of the assembled structure, and therefore influences its catalytic activity. Based on the self-organized structure, we did systematic conformational searching on the rotamer library of histidine side-chain which are exposed to the solvent molecules, and identified two new coordination forms which are different from the structure in the active site of carbonic anhydrase. We further explored the atomic mechanism of the catalytic reaction taking place at the interface, compared it with the reaction happening at the active site of carbonic anhydrase, and proposed how to improve the catalytic activity of the artificial material.

22-POS Board 22

Temporal Correlations Among Functionally Important Distant Residues of Ubiquitin

Sutapa Dutta, Mahua Ghosh, Jaydeb Chakrabarti.

S. N. Bose National Centre for Basic Sciences, Salt Lake City, Kolkata, West Bengal, India.

Long distant coupling between distant binding sites in bio-macromolecules is a fundamental challenge in uncovering their functions. We study the microscopic basis of such communication through time dependent dihedral cross-correlation functions among spatially separated yet functionally important residues of a small protein, named ubiquitin which participates in degradation of mis-folded protein in eukaryotes. We perform 1.05 micro-second long all atom Molecular Dynamics simulation. We observe that the dihedral angles of the residues possess non-trivial temporal cross-correlations with asymmetry with respect to exchange of the dihedrals, having peaks at low frequencies with time scales in nano-seconds and an algebraic tail with a universal exponent for large frequencies. We show the existence of path for temporally correlated degrees of freedom among the functional residues. We explain the qualitative features of the cross-correlations through a general mathematical model. The generality of our analysis suggests that temporal cross-correlation functions may provide convenient theoretical framework to understand bio-molecular functions on microscopic basis.

23-POS Board 23

Fast-NPS - An Analysis Tool to Obtain Structural Information from Single-Molecule FRET Measurements

Tobias Eilert, Jens Michaelis.

University of Ulm, Ulm, Baden-Württemberg, Germany.

Fast-Nano-Positioning-System (Fast-NPS) is an analysis software package that can be used to analyze *single-molecule FRET* (smFRET) data to obtain quantitative structural information of macromolecules in their natural environment. In the algorithm a Bayesian model gives rise to a multivariate probability distribution describing the uncertainty of the structure determination (Muschielok and Michaelis 2011).

Since Fast-NPS aims to be an easy-to-use general purpose analysis tool for a large variety of smFRET networks, we established a *Markov Chain Monte Carlo* based sampling engine, that approximates the target distribution and requires no parameter specification by the user at all. In previous works this method has already been used to study the position of the exiting RNA from the eukaryotic RNA polymerase II (Andrecka et al. 2008) and to investigate the influence of the transcription factor TFIIB on the position of the nascent RNA (Muschielok et al. 2008). Further, the position of the non-template and upstream DNA in yeast Polymerase II transcription elongation complexes (Andrecka et al. 2009) and the architecture of a minimal Polymerase II open promoter complex (Andrecka 2009) were analyzed. Moreover, the NPS was also applied to shed light on the archaeal initiation complex (Nagy et al. 2015).

Since the molecular surrounding of a dye molecule effects its spatial mobility and thus the smFRET efficiency, our current developments focus on the specific description of dye models only driven by data taken from experiments, i.e. time-resolved anisotropy and fluorescence lifetime measurements.

Further, a recent progress is the Bayesian analysis of the relative orientation of several macromolecules, i.e. rigid body docking guided by smFRET measurements. After a clustering step, the uncertainty in structure determination can be visualized by ensembles of rigid bodies in a static picture or in a video.

24-POS Board 24

**Photoinduced Electron-transfer Fluorescence Correlation Spectroscopy of the
Conformational Dynamics of Intrinsically Disordered Proteins**

Joerg Enderlein.

Georg August University, Goettingen, Lower Saxony, Germany.

Intrinsically disordered proteins (IDP) form a large and functionally important class of proteins that lack an ordered three-dimensional structure. IDPs play an important role in cell signaling, transcription, or chromatin remodeling. The discovery of IDPs has challenged the traditional paradigm of protein structure which states that protein function depends on a well-defined three-dimensional structure.

Due to their high conformational flexibility and the lack of ordered secondary structure, it is challenging to study the flexible structure, dynamics and energetics of these proteins with conventional methods. In our work, we employ photoinduced electron-transfer (PET) combined with fluorescence correlation spectroscopy (FCS) for studying the conformational dynamics of one specific class of IDPs: phenylalanine-glycine rich protein domains (FG repeats) which are dominant building blocks within the pore of nuclear pore complexes. We use the peculiarity of the fluorescent dye Atto655 that, when excited, it shows a quasi-instantaneous and high-efficient electron transfer to a tryptophan in direct contact with the dye. By placing one tryptophan at a specific position within a peptide chain that is labeled at its end with Atto655, one can use FCS for measuring the contact pair formation rate between the labeled peptide end and the tryptophan position. By moving the tryptophan position along the peptide chain, one thus maps the conformational dynamics of the full peptide. We observe point contact formation rates of a few hundred nanoseconds, and see a strong variation of the rate on the distance between dye and tryptophan, as well as on total length of the peptide chain. We demonstrate that PET-FCS is particularly useful for the measurement of fast intramolecular conformational dynamics of small biomolecules where conventional methods such as single-molecule Förster Resonance Energy Transfer fail due to steric reasons.

25-POS Board 25

Conformational Dynamics of Nucleic Acids by Orientation Selective PELDOR

Nicole Erlenbach¹, Lukas S. Stelzl², Gerhard Hummer^{2,3}, Thomas F. Prisner¹.

¹Goethe University, Frankfurt am Main, Germany, ²Max Planck Institute of Biophysics, Frankfurt am Main, Germany, ³Goethe University, Frankfurt am Main, Germany.

PELDOR (Pulsed Electron Electron Double Resonance)¹ experiments on nucleic acids with rigid spin-labels provide highly accurate distance and orientation information, which can be used to study their structure and dynamics.² PELDOR experiments on dsDNA, with the rigid spin label Ç, a cytidine analogue, have already revealed a twist-stretch motion.³

Molecular dynamics (MD) simulations can act as a 'computational microscope' to resolve the dynamics of individual atoms. However, for a long time the application of MD to nucleic acids was hindered by the lack of accurate force fields. Experimental PELDOR signals can be used as sensitive benchmark data for the evaluation of MD simulations on nucleic acid molecules. In contrast to older force fields, signals calculated from MD simulations with the new parmbsc1⁴ and OL15⁵ force fields closely match the experimental PELDOR traces, which confirms that these force fields are significant improvements in the computational description of DNA. This evaluation can be combined with answering the fundamental question as to the nature of the conformational dynamics of DNA. MD simulations show that dsDNA undergoes bending and twist-stretch motions in solution.

For dsRNA, the comparison of initial PELDOR experiments with MD simulations shows larger deviations between experiments and theory, suggesting that further optimization of the force fields is required for accurate description.

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26-POS Board 26

Molecular Recognition of Intrinsically Disordered Motifs and the Design of High(Er) Affinity Biomimetic Binders

Elisa Fadda.

Maynooth University, Maynooth, Ireland.

Conformational disorder is a distinctive and functional feature of many proteins involved in regulatory and signalling pathways, where intrinsically disordered regions (IDRs) are often involved in transient and reversible protein-protein interactions (PPI)[1,2]. This class of PPIs differ significantly from obligate PPIs in that they usually involve small surface areas, and varying degrees of folding-on-site upon binding. Both of these aspects contribute to modulate binding affinity[3]. Conformational plasticity is an architectural advantage for proteins involved in structuring reversible macromolecular assemblies and also to confer broad binding specificity towards a variety of different receptors[4]. Therefore understanding the structure, energetics, and dynamics of molecular recognition of specific disordered motifs at the atomistic level of detail can allow us to consider reversible PPIs as targets for the design of high affinity biomimetic binders. In this work I will describe how extensive sampling via molecular dynamics (MD) simulations can reveal important clues that help our understanding of the molecular recognition of specific disordered motifs, thus can inform the biomimetic design process. More specifically I will discuss the molecular basis for the binding promiscuity of the extreme C-terminus of the p53 tumour suppressor, and how we are implementing the biomimetic design strategy in the case of the Xeroderma pigmentosum complementation group A (XPA)[5, 6], a key scaffolding protein involved in the nucleotide excision repair (NER) pathway.

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27-POS Board 27

Assessment of Peptide Conformational Landscape and Dynamics by Amide I' Infrared Spectroscopy and MD Simulations

Chi-Jui Feng, Andrei Tokmakoff.
University of Chicago, Chicago, IL, USA.

Amide I' spectroscopy has growing capabilities for probing complex protein structures including intrinsically disordered regions. With amide I' spectroscopic maps, one can structurally interpret experimental spectra from MD trajectories, providing a route to disordered protein ensemble refinement. However, few studies have quantitatively evaluated the quality of these spectroscopic simulations and their ability to interpret protein conformational distributions and dynamics. Such studies will help understand how much structural and dynamical information can be obtained from these methods.

As a proof of principle study, we compared experimental and simulated IR and 2D IR spectra of Ala-Ala and Ala-Ala-Ala. In Ala-Ala, the correlation time of the amide I' frequency-frequency correlation function (FFCF) is related to the fluctuating hydrogen bonding environments to the amide group. 2D IR spectra of Ala-Ala-Ala provide information on the average angle between the two amide carbonyl groups, giving constraints on underlying conformational distributions. Comparing experimental spectra with spectra simulated from several force fields, we are able to characterize the conformational distribution of trialanine and also assess the predictions of these force fields. We show that its structure is dominated by ppII conformer, with minor population of β conformer.

These analytical tools are being developed to interpret more complicated protein spectra in combination with site-specific isotope labeling. We use human insulin as an illustration to understand the structural changes during the monomer-dimer transition, with the focus on the dissociation of the inter-chain β -sheet, and the hinging motion and disorder of the monomer B-chain.

28-POS Board 28

Molecular Dynamics Simulations of Kinesin Eg5 using a Structure Based Model

Jose C. Flores-Canales^{1,2}, Keehyoung Joo^{1,2}, Steven P. Gross^{3,1}, Jooyoung Lee^{1,2}.

¹Korea Institute for Advanced Study, Seoul, South Korea, ²Korea Institute for Advanced Study, Seoul, South Korea, ³University of California, Irvine, Irvine, CA, USA.

Eg5 belongs to the kinesins family of molecular motors, which move along microtubules by using the energy of ATP hydrolysis. Eg5 has an essential role in mitotic cell division, and as such, disruption of its function has a considerable interest for development of new drugs for oncogenic treatment. While usually a tetramer, here we studied a truncated Eg5 dimer, consisting of two catalytic domains, each of which is joined by a neck linker to one terminus of a coiled-coil domain. Based on multiple experiments, it is generally believed that kinesins move along microtubules using a hand over hand mechanism, involving the binding of ATP to the nucleotide-binding region, ATP hydrolysis, and subsequent release of ADP. Importantly, these changes are coupled to the docking state of the neck-linker structural motif. Docking of the latter transmits mechanical force between the two catalytic domains. However, this model does not provide a detailed dynamic description of the coupling between multiple states of the neck-linker and nucleotide-binding region. In this work, we present a structure based model and molecular dynamics (MD) simulations of Eg5.

Eg5's X-ray structures in ATP- and ADP-bound states have suggested that conformational changes in the nucleotide-binding site are coupled to both the generation of force and to motion along microtubules. To gain insight on the coupling of ligand binding and generation of force, we have carried out multiple independent MD runs of the transition from the ATP- to ADP-bound state. We constructed a dual-basin structure-based model using Lorentzian restrains. Analysis shows that there is a weak coupling of switch I and the neck-linker. Furthermore, principal component analysis shows that there are multiple pathways of transition from ATP- to ADP-bound state. An interpretation of this pathway diversity for Eg5 is provided.

29-POS Board 29

Minimalist Coarse-grained Models for Double-stranded DNA Fragments: A Comparative Survey

Mathieu Fossépré. Mathieu Surin.
UMONS, Mons, Belgium.

Computational molecular modelling approaches such as Molecular Dynamics (MD) play an increasingly important role for studying structure, dynamics, and function of biomolecular systems and to complement experimental results [1]. Despite the constant development of computer performances, the size of biomolecular systems and the phenomenological timescales required to consider most biological phenomena are still out of reach. By merging a set of atoms into one bead, coarse-grained (CG) models permit much faster simulations of large and complex biomolecular systems on the microsecond timescale. Consequently, there was a renewed interest in the use of coarse-grained (CG) models for biopolymers in the last decade, leading to a large variety of CG DNA models with various spatial resolutions, mapping schemes, and interaction potentials [2,3]. In this study, we compared a selection of generic CG models for DNA, as a prerequisite to simulate DNA/polymer complexation. We focused our analysis on minimalist DNA models, i.e., a class of CG models using a single or a few beads for each nucleotide. CG models were applied on DNA sequences of various lengths, ranging from 17 to 100 base pairs, on the microsecond timescale by using MD simulations techniques. The performance of CG models is evaluated in terms of the dynamical and mechanical properties of DNA fragments and on the range of applicability of these properties. Ultimately, this comparison between CG models will be helpful to further develop models in order to understand the complexation and aggregation mechanisms of DNA with conjugated polyelectrolytes [4].

[1] J.R. Perilla et al., *Curr. Opin. Struct. Biol.* 31 64 (2015)

[2] M.G. Saunders et al., *Annu. Rev. Biophys.* 42 73 (2013)

[3] P.D. Dans et al., *Curr. Opin. Struct. Biol.* 37 29 (2016)

[4] J. Rubio-Magnieto et al., *Soft Matter* 11 6460 (2015)

30-POS Board 30

Application of Internal Normal Mode Analysis to the Study of Protein Flexibility

Elisa Frezza, Richard Lavery.
CNRS/ Univ. Lyon I, LYON, France.

Normal mode analysis provides information on the equilibrium modes accessible to a system, within a harmonic approximation. It has been used for several decades in studying classical physical phenomena, as well as the flexibility of proteins. In the past decade it has also become a tool for exploring functional motions and it has been demonstrated that low frequency motions play an important role in biological processes. We have carried out normal mode analyses with two different strategies depending on the choice of the independent variables: either considering Cartesian (CCS) or internal (ICS) coordinates. The latter is advantageous since it leads to an important reduction in the number of variables (by freezing higher-frequency bond and angle deformations) and by extending the range over which the conformational energy hypersurface can be assumed to be harmonic. In the case of N bodies, inter-bond distances and angles have to be taken into account. Despite the advantages coming from the use of ICS, a transformation to CCS is often useful to gain insight into the overall structural changes occurring in the system. This transformation must be made so that the ICS dynamics reflect only internal motions of the molecules and no external (overall translational or rotational) motions are introduced. We have applied internal coordinate normal mode analysis (iNMA) to a systematic investigation of the changes in protein conformation upon binding either another protein or a small ligand, using different protein representations. We have also compared the flexibility deduced from normal modes with all-atom molecular dynamics simulations. iNMA is shown to be an effective and fast tool for predicting large conformational changes and for providing information on the key torsions involved in the global movements.

Levitt, M. et al. *J.Mol.Biol.* 1985, 181, 423.
Sunada, S. and Go, N. *J.Comput.Chem.* 1984, 16, 328

31-POS Board 31

Non-Ewald Method for Accurately and Efficiently Calculating Electrostatic Interactions in Molecular Simulations

Ikuo Fukuda¹, Narutoshi Kamiya², Kota Kasahara³, Han Wang⁴, Shun Sakuraba⁵, Haruki Nakamura¹.

¹Osaka University, Suita, Osaka, Japan, ²University of Hyogo, Kobe, Japan, ³Ritsumeikan University, Kusatsu, Japan, ⁴Institute of Applied Physics and Computational Mathematics, Beijing, China, ⁵The University of Tokyo, Tokyo, Japan.

A larger system and longer time steps are necessary to conduct a realistic molecular simulation, but they are hardly realized in current computational environments. The most time-consuming part of the simulation is the calculation of long-range interactions of particles. In particular, appropriate treatment of the electrostatic interaction is critical, since the simple truncation cannot be used due to the slow decay of the Coulombic function. Thus, there is strong demand to calculate the electrostatic interactions with high accuracy and low computational cost.

For this purpose, we have developed the Zero-multipole summation method (ZMM) [1]. In this method, the periodic boundary condition, which can potentially cause artifacts in particular for heterogeneous systems, is not necessary, and the Fourier-part evaluation, which is typically the bottleneck for high performance computation, is not needed, in contrast to conventional Ewald-based methods. Instead, a suitably defined simple pairwise function, which differs from the original Coulombic function, is used with a cutoff scheme. The underlying physical idea is simple that certain electrostatic neutrality is attained in a biological or condensed matter system [2]. This idea is realized by a mathematical foundation to generate a new pairwise function. The accuracy and efficiency of the ZMM has been validated in fundamental systems as well as heterogeneous biomolecular systems, including DNA and protein. In the presentation, we will provide the theory and numerical results on the ZMM, and discuss how the treatment of the electrostatic calculations seriously affects simulation results.

- [1] I. Fukuda, *J. Chem. Phys.* 139, 174107 (2013); I. Fukuda et al., *ibid.* 140, 194307 (2014); H. Wang et al., *ibid.* 144, 114503 (2016); K. Kasahara, et al., *Biophys. Physicobiol.*, 13, 209 (2016).
[2] I. Fukuda and H. Nakamura, *Biophys. Rev.* 4, 161 (2012).

32-POS Board 32

Structural Investigation on the Intrinsic Disordered Region of the HCV Protein NS5A and Its Role in Viral RNA Replication

Neha S. Gandhi¹, Marie Dujardin^{2,3}, Vanesa Madan⁴, François-Xavier Cantrelle², Robert Schneider², Helene Launay², Guy Lippens^{2,6}, Ralf Bartenschlager⁴, Xavier Hanouille².

¹Queensland University of Technology, Brisbane, Queensland, Australia, ²CNRS UMR 8576, Université Lille1, Villeneuve d'Ascq, Hauts de France, France, ³CNRS UMR 5086, Université Lyon 1, Lyon, Auvergne-Rhône-Alpes, France, ⁴University of Heidelberg, Heidelberg, Baden-Württemberg, Germany, ⁵INSERM U 1068, CNRS UMR 7258, Aix-Marseille University, Marseille, Provence-Alpes-Côte d'Azur, France, ⁶University of Toulouse, Toulouse, Occitanie, France.

The Non-structural 5A (NS5A) protein of hepatitis C virus (HCV) plays an important role in cellular and viral processes [1]. We have performed Gaussian accelerated molecular dynamics (MD) and nuclear magnetic resonance (NMR) to investigate the native conformational preferences of the wild type NS5A-D2 (residues 308 to 327) peptide and its mutants (P306A, P310A, P315A, P319A; P320A, A311G, D316E and DY316-317EN) from JFH1 strain. We used dihedral principal component analysis (dPCA) to map a conformational ensemble of fast interconverting conformations and further constructed dPCA-based free energy landscapes (FES) of these peptides. The analysis of the FES and chemical shifts showed that the motif Pro314–Trp316 does not randomly explore the conformational space in solution except for the A311G NS5A-D2 peptide. Our simulation captured intrinsic conformational characteristics of proline-rich NS5A-D2 that are in good agreement with the NMR data like presence of a prominent “turn” [2]. Indeed, our results indicate that the absence of a turn in the A311G NS5A-D2 mutant does not allow HCV RNA replication in a cell-based assay. Our current work highlights that very small structural motifs like a turn present in intrinsically disordered proteins are essential for performing a very specific function.

References 1). Ross-Thriepland and Harris, *Gen. Virol.*,727-738, 2015. 2). Dujardin, M et al., *J. Biol.Chem.*, 19104–19120, 2015.

33-POS Board 33

Mapping the Structural Mechanism of the Dopamine 2 Receptor Allosteric Signaling Pathway with Molecular Dynamics and Evolutionarily Coupled Residues

Dylan Girodat, Hans-Joachim Wieden.

Alberta RNA Research and Training Institute (ARRTI), University of Lethbridge, Lethbridge, AB, Canada.

Intramolecular allosteric signaling is required for the proper function of G-protein coupled receptors (GPCRs). Treatment for a number of neurodegenerative disorders such as Parkinson's disease and schizophrenia involve modulation of GPCR activity. As such, novel therapeutics for these disorders can be rationally designed to target the allosteric signaling pathway of GPCRs aiming to modulate their activity. However, the mechanism and structural basis of how allosteric signaling is mediated through GPCRs from the allosteric site to the distal functional site is unknown. Recently, Sung et al., reported that the identity of distal amino acids in the Dopamine 2 Receptor (D2R) are evolutionarily coupled which can only be explained as residues that are connected through the D2R allosteric signaling pathway¹. We used these coupled residues in conjunction with Molecular Dynamics (MD) simulations to describe the D2R allosteric signaling pathway.

Here we present a computational method that analyzes correlations between amino acid dynamics in MD simulations to describe allosteric signaling. Specifically we use parallel motions between proximal C α 's of amino acids in conjunction with network theory to construct 2D maps of allosteric pathways. Using this approach we have described a likely pathway by which the D2R processes allosteric signals and have gained insight into how residues in the D2R are evolutionarily coupled contributing to observed functional characteristics of the receptor. Our work provides a transferable framework to analyse the allosteric regulation of membrane-bound receptors, enabling the rational design of both receptors with desired behaviors as well as small molecules and peptides that can modulate their function.

¹Sung Y, et al. Intramolecular allosteric communication in dopamine D2 receptor revealed by evolutionary amino acid covariation. PNAS 2016; 113: 3539-3544

34-POS Board 34

Multiscale simulations of partially disordered systems: Representing environment-induced helix-coil transitions

Christoph Globisch¹, Cahit Dalgicdir², Mehmet Sayar³, Christine Peter¹.

¹University of Konstanz, Konstanz, Germany, ²University of Darmstadt, Darmstadt, Germany,

³Koç University, Istanbul, Turkey.

Coarse grained (CG) models are widely used to study peptide self-assembly and nanostructure formation. One of the recurrent challenges in CG modeling is the problem of limited transferability. A crucial question for peptides is whether a model reproduces the molecule's conformational response to a change in its molecular environment. Examples are conformational transitions between a rather disordered and an ordered state upon a change in pH value or due to the presence of a soft apolar/polar interface. To handle such transitions CG models mostly utilize auxiliary interactions to aid secondary structure formation. Such interactions take care of properties of the real system that are per se lost in the coarse graining process such as dihedral-angle correlations along the backbone or backbone hydrogen bonding. Since the CG models are designed to emphasize certain conformational propensities they may destroy the ability of the model to respond to stimuli and environment changes. This points out how important it is to investigate whether they impede transferability.

To analyze such processes in combined atomistic/CG manner a common characterization of the shallow conformational free energy landscapes is needed which is dominated by a huge number of metastable and often ill-defined minima. Dimensionality reduction methods such as multidimensional scaling-like embedding (sketch-map) can be applied to compare the phase space sampled at both resolutions (atomistic/CG), either to judge the success of elevated sampling techniques and possibly guide further simulations, or to monitor the response of the systems to external stimuli.

35-POS Board 35

Teaming up Molecular Dynamics Simulations with Mass-Spectrometry and ssNMR to Reveal the Dynamic Architecture of the Amyloid Precursor Protein's Transmembrane Domain

Alexander Götz¹, Hannes Heinel², Philipp Högel³, Alexander Vogel², Dieter Langosch³, Daniel Huster², Christina Scharnagl¹.

¹Technical University of Munich, Garching, Germany, ³Technical University of Munich, Freising, Germany. ²University of Leipzig, Leipzig, Germany,

Alzheimer's disease (AD) is characterized by accumulation of toxic β -amyloid ($A\beta$) in the brain and neuronal death. $A\beta$ peptides of different lengths are produced by stepwise proteolytic cleavage within the transmembrane domain (TMD) of the amyloid precursor protein (APP) by γ -secretase. $A\beta$ toxicity is related to fragment length, which correlates with cleavage at $\epsilon 48$ or $\epsilon 49$. Mutations located in the C-terminal domain of APP (TM-C) shift production towards the longer, aggregation-prone $A\beta 42$, associated with early-onset familial AD (FAD). No FAD mutations are known for the N-terminal domain (TM-N) as well as the central GGTV hinge region. A highly anisotropic TMD fluctuation pattern defines a hierarchically organized substrate dynamic. To further investigate the dynamic architecture of the APP TMD, a joint approach of molecular dynamics simulations, mass-spectrometry and solid-state nuclear magnetic resonance is used, comparing wild-type (WT) APP with designed G38L, G38P and the I45T FAD mutant. The TMD's intrinsic dynamics is studied in POPC and POPE/POPG bilayers, while the environment of substrate bound in γ -secretase's aqueous active site is mimicked by a TFE/H₂O mixture. No mutant enhances helix unwinding at the scissile bonds locally. Rather, G38 mutants affect fluctuations in TM-N, while increased fluctuations upstream the ϵ -sites in I45T are consistent with our results for other FADs. Different solvents induce mainly differences of the extent of TMD fluctuations. Lipid composition does not impact the TMD's internal dynamics, but enforces different overall rotational dynamics. Since TM-C dynamics is associated with disease's onset, while TM-N dynamics is not, we propose a model where processing of the substrate utilizes the hierarchy of its TMD flexibility: Binding-induced stiffening of TM-N promotes the functional importance of motions localized in the cleavage domain.

36-POS Board 36

From Sequence to Function: Coevolving Amino Acids Encode Structural and Dynamical Domains

Daniele Granata^{1,2}, Luca Ponzoni³, Kresten Lindorff-Larsen¹, Cristian Micheletti³, Vincenzo Carnevale².

¹University of Copenhagen, Kobenhavn, Denmark, ²Temple University, Philadelphia, PA, USA, ³SISSA, Trieste, Italy.

Amino acids interactions within protein families are so optimized that the sole analysis of evolutionary co-mutations can pinpoint pairs of contacting residues. It is also known that evolution conserves functional dynamics, i.e., the concerted motion or displacement of large protein regions or domains. Is it, therefore, possible to use a pure sequence-based analysis to identify these dynamical domains? We introduce a general co-evolutionary coupling analysis strategy and apply it to a curated sequence database of 800 protein families. As soon as few hundreds of sequence are available for the coupling inference, the sequence-based method partitions amino acids into clusters spatially separated but individually compact, when represented on the relative native structure. Remarkably, these "Evolutionary Domains" are also highly correlated with proteins dynamical domains, encoding their structural dynamics. Finally we discuss relevant applications to comparative analysis within ion channels family and to the identification of allosteric communication between domains in specific enzymes.

37-POS Board 37

Functional Role of the TCTP Intrinsically Disordered Region Investigated by MD Simulations and NMR Experiments

Florian Malard¹, Nadine Assrir¹, Ewen Lescop¹, Mouad Alami², Samir Messaoudi², **Tâp Ha-Duong²**.

¹CNRS, Gif-sur-Yvette, France, ²Université Paris-Sud, Châtenay-Malabry, France.

The translationally controlled tumor protein (TCTP) is involved in several biological processes. To exert its various functions, TCTP is brought to interact with many other biomolecules. Notably, TCTP residues S46 and S64 can be phosphorylated, impacting its binding with other proteins. Moreover, TCTP can sequester calcium to block the calcium-dependent apoptosis process. However, little information on the quaternary structures of TCTP complexes are available, impeding the full understanding of the mechanisms by which TCTP performs its functions.

The protein three-dimensional structure is composed of a core domain and an intrinsically disordered loop which contains a highly conserved TCTP signature. The objective of this study is to explore this disordered loop conformational ensemble, using molecular dynamics simulations in combination with NMR experiments, to gain a better insight into its functional role.

The TCTP conformational ensemble was studied under four conditions: without or with calcium, with residue S46 phosphorylated, and with both S46 and S64 phosphorylated. Simulations show that, in the absence of calcium, the non-phosphorylated and pS46 protein have overall similar conformational ensemble. The double phosphorylated pS46-pS64 protein is more extended, whereas calcium induce a more compact structure. However, in all conditions, the disordered loop has a similar extent, but is more or less bound to the core domain, hiding accessible surface area from TCTP partners. Simulations also permit to identify calcium binding sites on TCTP which were partially confirmed by NMR experiments.

In conclusion, the TCTP intrinsically disordered loop might play the role of a switch which could interfere with the binding of its multiple partners. Its conformational states depend on the presence of calcium and on the phosphorylation of residues S46 and S64.

38-POS Board 38

Modulation of Cardiac Myosin Dynamics by Omecamtiv Mecarbil

Shaima Hashem, Matteo Tiberti, Arianna Fornili.
Queen Mary University of London, London, United Kingdom.

Cardiac myosin II is an allosteric protein involved in the contraction of the heart muscle. Mutations in this protein are responsible for the emergence of several cardiac diseases like hypertrophic and dilated cardiomyopathies, which can lead to heart failure and sudden cardiac death. Therapies based on small-molecule effectors of myosin have recently started to be explored and they seem promising. In particular, the sarcomeric modulator Omecamtiv Mecarbil (OM) is currently being tested in clinical trials for the treatment of heart failure.

The binding site of OM on cardiac myosin has been recently unravelled by X-ray crystallography. The drug was shown to bind to a deep pocket close to key regions of the motor domain involved in the propagation of motion from the actin-binding cleft to the converter and lever arm regions. This suggests that a possible role for OM is to increase the coupling between these regions and hence their efficiency in propagating structural changes. Moreover, the drug was found to induce subtle conformational changes in distant regions close to the ATP binding site of myosin, indicating the presence of allosteric effects.

The goal of our project was to study the effect of OM on myosin dynamics in order to elucidate its mechanism of action using molecular modelling and Molecular Dynamics (MD) simulations. In particular, we examined the dynamical correlations between the different structural elements of the motor domain. A stronger coupling was observed between the converter-lever arm domain and the rest of the protein upon OM binding, with preferential pathways connecting the OM binding site and distant regions such as the upper 50K domain.

This research is supported by the British Heart Foundation and the UK High-End Computing Consortium for Biomolecular Simulation, HECBioSim.

39-POS Board 39

A Simulation-Guided Method to Select Optimal DEER Experiments to Refine Highly Flexible Conformational Ensembles

Jennifer M. Hays¹, Marissa Keiber², Linda Columbus², Peter M. Kasson^{3,1}.

¹University of Virginia, Charlottesville, VA, USA, ³University of Virginia, Charlottesville, VA, USA. ²University of Virginia, Charlottesville, VA, USA,

Determining the structural basis of flexible molecular recognition is experimentally challenging because many techniques that capture multiple conformational populations provide sparse rather than complete data on the conformational ensemble. Selecting a set of optimal experiments to best refine the conformational ensemble therefore remains an important challenge. The binding of Opa *Neisserial* virulence protein to its human host receptor (CEACAM) exemplifies these flexible recognition processes. Although Opa has long loops that have been shown by NMR to be loosely structured, these same loops still bind CEACAM with high affinity. In order to refine the Opa-CEACAM conformational ensemble, we have developed a model-free, information-theoretic approach for guiding double electron-electron resonance (DEER) experiments that 1) uses a mutual information distance metric to rank pairs of residues based on how well they refine a conformational ensemble and 2) identifies a set of highly informative pairs that perform well under this metric. Specifically, we utilize the data from initial ensemble simulations of Opa₆₀ to identify a set of maximally-informative and minimally-redundant (mRMR) pairs, measure the distance distributions of those pairs using DEER, incorporate the experimental distributions into restrained-ensemble MD simulations, and demonstrate that the set of high-scoring mRMR pairs better reduces the conformational search space than a set of experimentalist-selected pairs. This systematic approach provides a way to both efficiently refine flexible receptor-ligand complexes and help elucidate fundamental physical principles of receptor-ligand binding.

40-POS **Board 40**

Closing the Gap Between NMR Relaxation and Molecular Dynamics Simulations of Methyl Dynamics in Proteins

Falk Hoffmann¹, Mengjun Xue², Lars Schäfer¹, Frans Mulder².

¹Ruhr-University Bochum, Bochum, Germany, ²University of Aarhus, Aarhus, Denmark.

Molecular dynamics (MD) simulations and nuclear magnetic resonance (NMR) spin relaxation experiments have become increasingly powerful to study protein dynamics at atomic resolution due to steady improvements in physical models and computation power. Good agreement between generalized Lipari-Szabo (S^2_{NH}) order parameters derived from experiment and simulation is now observed for the backbone dynamics of a number of proteins. Unfortunately, the agreement for side chains, as e.g. probed by $S^2_{\text{CH}_3}$ for methyl-containing side chains, is much poorer. In this work we discuss several issues with methyl side chains that need to be addressed to close the gap between NMR and MD. Accounting for protein tumbling is the single most important factor to obtain a good agreement. In our hands, the application of improved water force fields with an appropriate way of including anisotropic overall protein tumbling improves the prediction of experimentally measured dynamic observables by MD simulations. We demonstrate these aspects for T4 lysozyme as an example. Our results guide the way for extracting the most accurate parameters that describe protein side chain dynamics and report on conformational entropy from the NMR relaxation data.

POSTER SESSION II

Sunday, August 27, 2017

16:30 – 18:00

Meitner Hall

Posters are available for viewing only during their scheduled date of presentation. Below are the formal presentation times. The presenters listed below are required to remain in front of their poster boards to meet with attendees during the designated times.

Odd-Numbered Boards 16:30 – 17:15 | Even-Numbered Boards 17:15 – 18:00

Hugel, Thorsten	41-POS	Board 1
Ivanovic, Milos	42-POS	Board 2
Jain, Alok	43-POS	Board 3
Jeschke, Gunnar	44-POS	Board 4
Johansson, Kristoffer	45-POS	Board 5
Johnson, Michael	46-POS	Board 6
Jordan, E. Joseph	47-POS	Board 7
Kekenes-Huskey, Peter	48-POS	Board 8
Kirmizialtin, Serdal	49-POS	Board 9
Klose, Daniel	50-POS	Board 10
Koefinger, Juergen	51-POS	Board 11
Koehler Leman, Julia	52-POS	Board 12
Kovács, Bertalan	53-POS	Board 13
Krah, Alexander	54-POS	Board 14
Kunze, Micha	55-POS	Board 15
Lau, Andy	56-POS	Board 16
Li, Ya-Tzu	57-POS	Board 17
Liu, Wei	58-POS	Board 18
Londergan, Casey	59-POS	Board 19
Mabrouk, Mahmoud	60-POS	Board 20
Marinelli, Fabrizio	61-POS	Board 21
McAnany, Charles	62-POS	Board 22
Melvin, Ryan	63-POS	Board 23
Mioduszewski, Lukasz	64-POS	Board 24
Miranda, Williams	65-POS	Board 25
Mochena, Mogus	66-POS	Board 26
Mori, Takaharu	67-POS	Board 27
Moritsugu, Kei	68-POS	Board 28
Nemetchek, Michelle	69-POS	Board 29
Nerenberg, Paul	70-POS	Board 30

Nilsson, Lennart	71-POS	Board 31
Oleniecki, Tymoteusz	72-POS	Board 32
Orioli, Simone	73-POS	Board 33
Pandini, Alessandro	74-POS	Board 34
Peach, Robert	75-POS	Board 35
Pearce, Nicholas	76-POS	Board 36
Peters, Günther	77-POS	Board 37
Petkeviciute, Daiva	78-POS	Board 38
Pinamonti, Giovanni	79-POS	Board 39
Pomès, Régis	80-POS	Board 40

Posters should be set up on the morning of Sunday, August 27 and removed by 18:00 on Sunday, August 27. All uncollected posters will be discarded.

41-POS Board 1

Dynamic Structure of the Protein Hsp90 Solved by a Hybrid Approach Based on Single Molecule FRET

Thorsten Hugel.

n/a, Freiburg, Germany.

Most molecular machines alternate dynamically between multiple conformations. Common techniques are not ideal to study such conformational dynamics on relevant time scales from micro-seconds to several seconds.

Here we present a hybrid approach based on single molecule FRET combined with X-ray crystal structure data and simulations. This approach enables us to simultaneously access structure and dynamics of a multi-domain protein in solution [1]. We applied this method to solve the dynamic structures of the heat shock protein Hsp90 dimer in solution. The previously unknown open state of yeast Hsp90 is represented by an ensemble of conformations with inter-domain fluctuations of up to 25 Å.

In addition, we show how multicolor single molecule FRET allows us to identify microscopic states in transient complexes. Conformational dynamics and nucleotide binding are simultaneously detected for Hsp90. Their correlation is quantified using 3D ensemble hidden Markov analysis, in and out of equilibrium [2,3].

[1] B. Hellenkamp, P. Wortmann, F. Kandzia, M. Zacharias, T. Hugel. Multidomain structure and correlated dynamics determined by self-consistent FRET networks. *Nat Meth*;14(2):174-180 (2017).

[2] S. Schmid, M. Götz and T. Hugel. Single-Molecule Analysis beyond Dwell Times: Demonstration and Assessment in and out of Equilibrium. *Biophys J.* 111:1375-1384 (2016)

[3] M. Götz, P. Wortmann, S. Schmid and T. Hugel. A multi-color single molecule FRET approach to study protein dynamics and interactions simultaneously. *Method Enzymol* 581, 487-516 (2016)

42-POS Board 2

Atomistic Structures of Detergent Micelles Refined Against X-ray Solution Scattering Data

Milos T. Ivanovic, Jochen S. Hub.
Georg August University, Goettingen, Germany.

Detergent micelles have been studied using a range of methods, including small-angle neutron scattering (SANS), small-angle X-ray scattering (SAXS), high frequency rheology, NMR self-diffusion, fluorescence techniques, as well as density, viscosity and dielectric constant measurements. Such methods provide data of limited information content and low spatial resolution. Therefore, simplified continuum models of certain ad-hoc symmetries, such as symmetrized ellipsoids, were fitted to such data, but the data alone was insufficient to infer atomic models. Molecular dynamics (MD) simulations were instead used to derive atomic models of detergent micelles. However, since simulations were not yet directly compared with structural experimental data, it remained unclear whether force field imperfections bias the structure and shape of the simulated micelle. Hence, methods that integrate experimental data into MD simulations are needed to derive reliable atomic models of micelles. Here, we derived atomistic models of two maltoside micelles, n-Dodecyl- β -D-Maltoside (DDM) and n-Decyl- β -D-Maltoside (DM) at temperatures between 10 and 70°C, by combining experimental SAXS data with all-atom MD simulations. We incorporated the SAXS data as a energetic restraint into MD simulations, allowing us to refine micellar structures against structural data. Because all SAXS calculations were based on explicit-solvent models, the calculations involve accurate physical models for the hydration layer and the excluded solvent, thereby avoiding any solvent-related fitting parameters and, in turn, enabling highly predictive structural modelling. The study highlights that the combination of experiments and simulations provides more detailed and reliable structures of soft matter systems, as compared to each of the methods alone.

43-POS Board 3

Morphology Control in Peptide Nanostructures: Multiscale Simulation Study of Penta Peptide Aggregation

Alok Jain¹, Narendra K. Mishra², Christoph Globisch¹, Sandeep Verma², Christine Peter¹.

¹University of Konstanz, Konstanz, Germany, ²Indian Institute of Technology Kanpur, Kanpur, India.

Development of new well ordered, functional biomaterials based on the underlying principal of self assembly has immense application in nanotechnology, nanomedicine and tissue engineering. Peptide based nano-materials are not only biocompatible but also their properties can be altered easily by slight changes in environmental conditions and/or side- chains of amino-acids. Herein, we report a multiscale simulation study of penta peptides that exhibit very different morphologies upon altering a single amino acid. Atomistic simulations identified governing factors that lead to specific peptide morphology such as peptide flexibility vs rigidity, role of dimerization and the partitioning of hydrophobic side chains. The study was extended with coarse grained simulations. That allowed general conclusions about the mechanistic origin of the different morphologies. Our systematic study with different backbone beads and supportive pseudo dihedral angles illustrate the importance of very careful and delicate selection of coarse grained parameters to reproduce the chemical and structural properties of the system.

44-POS Board 4

The RigiFlex Approach to Modelling Structure Ensembles with Restraints from Different Techniques

Gunnar Jeschke.

ETH Zurich, Zurich, Switzerland.

The objective of this study is the development of a modelling approach for proteins and protein-RNA complexes that consist of rigid domains with separately determined structure and flexible linkers. The ensemble shall not depend on the quality of a molecular force field, but shall rather include uncertainty from lack of experimental restraints as well as from disorder of flexible chain sections and disorder in the rigid-domain arrangement. Such an ensemble can inform on potential further experiments that can distinguish between individual conformations or rigid-domain configurations.

The rigid-body problem is solved first by defining three reference points per rigid domain. Distance distribution measurements between such reference points can overdetermine this problem and provide information on domain arrangement disorder via the distribution width. The solution space of reference polyhedra is efficiently sampled by distance matrix geometry. Since the rigid bodies are chiral, their position and orientation is uniquely defined by the three reference points. Any further restraints, such as clash avoidance, maximum length of the flexible linkers, auxiliary distance restraints, and small-angle x-ray or neutron scattering data can be used to prune the initial ensemble. The flexible linker ensembles can then be modelled individually by an approach that we introduced previously for peptide chains and that can take into account further restraints.

RigiFlex was applied to a preliminary experimental restraint set for the complex of the human polypyrimidine tract binding protein with a virus internal entry site RNA. We found that the RNA linkers, but not the peptide linkers restrain the structure and that small-angle neutron scattering data are valuable already at a stage where the number of distance distribution restraints is clearly insufficient.

45-POS Board 5

Conformational Variation in Computational Protein Design

Kristoffer E. Johansson, Kresten Lindorff-Larsen, Jakob R. Winther.
University of Copenhagen, Copenhagen, Denmark.

Conformational dynamics are essential for the function of most enzymes. To realize the full potential of computational protein design and engineering, the methods must be able to handle conformational dynamics. We have investigated the significance of minor conformational variations in a state-of-the-art computational design method. Surprisingly, the minor conformational variations have a direct influence on the ability to produce soluble and stably folded protein. Our results suggest that design methods are sensitive below the lengths scale of thermal ensembles and thus that these can and should be considered in computational sampling and optimization of amino acid sequences.

46-POS Board 6**MM-GBSA, Gaussian and Electronic Circular Dichroism (ECD) Determination of Absolute Configuration and Binding Efficacy of Benzimidazole-Based FabI Inhibitors**

Michael E. Johnson^{1,2}, Jinhong Ren¹, Tina L. Mistry¹, Pin-Chih Su¹, Shahila Mehboob².

¹University of Illinois at Chicago, Chicago, IL, USA, ²Novalex Therapeutics, Inc., Chicago, IL, USA.

We have reported benzimidazole-based compounds to be potent inhibitors of FabI for both *F. tularensis* and *S. aureus* (FtFabI, SaFabI), making them promising antimicrobial hits. Optically active enantiomers exhibit markedly differing affinities toward FtFabI. The IC₅₀ of benzimidazole 6-(-) is ~100x lower than the (+)-enantiomer, with similar results for enantiomers of 7. Determining the absolute configuration (AC) for these optical compounds and elucidating their binding modes is important for further design. Electronic Circular Dichroism (ECD) calculated by quantum methods has become important in AC determination of optical compounds. The AC of 6-(-)/(+) and 7-(-)/(+) were determined by comparing experimental spectra and theoretical DFT simulations of ECD at the B3LYP/6-311+G(2d, p) level using Gaussian09. Comparison of experimental and calculated ECD spectra indicates that the *S* configuration corresponds to the (-)-rotation for both compounds 6 and 7, while the *R* configuration corresponds to the (+)-rotation. Further, MD simulations and MM-GBSA binding free energy calculations for these two pairs of enantiomers with FtFabI show much tighter binding MM-GBSA free energies for 6-*S* and 7-*S* than for their enantiomers, 6-*R* and 7-*R*, consistent with experimental observations that the (-)-enantiomers were more active. The results are consistent with the ECD determination of the *S* configuration corresponding to (-) and the *R* configuration corresponding to (+). Finally, fifteen benzimidazoles, including these optically active compounds were subjected to systematic MD simulations and MM-GBSA predictions for SaFabI binding. The predicted absolute configuration is further confirmed by the resulting coefficient of R²=0.80 between experimental and MM-GBSA predicted binding free energies. Thus, our computational studies allow us to assign (+)-(*R*)- and (-)-(*S*)-compounds 6 and 7, and to further evaluate structural changes to improve efficacy.

6 = 1-(1-(3,4-dichlorophenyl)ethyl)-1,5,6,7-tetrahydroindeno[5,6-d]imidazole

7 = 1-(1-(3,4-dichlorophenyl)ethyl)-5,6,7,8-tetrahydro-1H-naphtho[2,3-d]imidazole

47-POS **Board 7**

Molecular and Metadynamics to Predict Activation in Experimentally Validated Kinase Domain Mutations

E. Joseph Jordan¹, Ravi Radhakrishnan^{1,2}.

¹The University of Pennsylvania, Philadelphia, PA, USA, ²The University of Pennsylvania, Philadelphia, PA, USA.

Kinase proteins are important in cell growth and division and are frequently mutated in cancer. Numerous studies have shown a good correlation between increased catalytic efficiency in mutant kinases and increased ability of mutant cell lines to grow in cell culture. Kinases have distinct active and inactive conformations and this makes them amenable to simulations to predict whether mutations bias the conformation to the active state, which should correlate with increased catalytic efficiency. In this study, we run molecular dynamics simulations of a series of experimentally validated mutants from BRAF, HER2, and ALK kinases and compare the dynamics to those of wild type simulations as assessed by both molecular and metadynamics. This allows us to make predictions of the effect of mutations on kinase activation with greater than 60% accuracy using simple metrics like changes in hydrogen bond occupancy during the course of a simulation. We believe that this method could eventually be used in guiding personalized medical treatments as both the number of observed mutations and the number of available drugs increases going forward.

48-POS Board 8

Competition and Cooperation of Electrostatic-Steering and Conformational Dynamics in the Binding of Calcineurin's Intrinsically-disordered Recognition Domain with Calmodulin

Bin Sun², Erik C. Cook², Trevor P. Creamer², **Peter M. Kekenos-Huskey²**.
¹n/a, Lexington, USA, ²University of Kentucky, Lexington, KY, USA.

Calcineurin (CaN) is a serine/threonine phosphatase that regulates a variety of physiological and pathophysiological processes in most mammalian tissue. It has been established that the calcineurin (CaN) regulatory domain is highly disordered when inhibiting CaN, yet it undergoes a disorder-to-order transition upon binding calmodulin (CaM) to activate the phosphatase. The prevalence of polar and charged amino acids in the RD domain implicate electrostatic interactions in mediating CaM binding, yet it unclear whether properties of the RD conformational ensemble, such as its effective volume and accessibility of its CaM binding motif help or hinder its ability to participate in protein-protein recognition events. In the present study, we investigated via computational modeling the extent to which electrostatics and structural disorder co-facilitate or hinder CaM/CaN association kinetics. We examined several peptides containing the CaM binding motif, for which lengths and amino acid charge distributions were varied, to isolate the contributions of electrostatics versus conformational diversity to predicted, diffusion-limited association rates via microsecond-scale molecular dynamics (MD) and Brownian dynamics (BD) simulations. Our results indicate that the RD amino acid composition and sequence length influence both the dynamic availability of conformations amenable to CaM binding, as well as long-range electrostatic interactions to steer association. These findings provide intriguing insight into the interplay between conformational diversity and electrostatically-driven protein-protein association involving CaN, which are likely to extend to wide-ranging processes regulated by intrinsically-disordered proteins.

49-POS Board 9

Dramatic Shape Changes Occur as Cytochrome c Folds

Serdal Kirmizialtin¹, Felicia Pitici², Alfredo Cardenas³, Ron Elber^{2,3}, Devarajan Thirumalai².
¹New York University Abu Dhabi, Abu Dhabi, United Arab Emirates, ²The University of Texas
Austin, Austin, TX, USA, ³The University Of Texas Austin, Austin, TX, USA.

The protein folding has been the focus of intense study. Cytochrome c (cyt c) has been a model protein for folding studies and extensive experimental work has been done on the folding of cyt c demanding computations to characterize the structural transitions and the corresponding times during the folding process. Here, we use simulation methods to create a conformational ensemble that describe the folding of cyt c. Atomically detailed folding trajectories generated by the Stochastic Difference Equation in Length fitted to time resolved Small Angle X-ray experiments. We were able to find a linear relationship between average trajectory length and real time of the folding process that allowed us to monitor the changes in the folding process from microseconds to seconds in unprecedented atomic detail that is otherwise impossible. We identify folding intermediates and characterize the stages of folding both in the form of global shape and size changes and in the form of secondary and tertiary fold formation kinetics.

50-POS Board 10

Rotamer Libraries for Spin and Fluorescence Labels Aid Structural Interpretation of Experimental Data

Daniel Klose¹, Svetlana Kucher², Heinz-Juergen Steinhoff², Gunnar Jeschke¹.

¹ETH Zurich, Zurich, Switzerland, ²University of Osnabrueck, Osnabrueck, Germany.

In structural biology, the conformation of biomolecular complexes can be investigated by techniques that require covalent modification of protein side chains or nucleotides with labels, such as spin or fluorescence labels. The interpretation of such experimental data generally benefits from taking the label's spatial distribution into account. We previously established rotamer libraries for nitroxide spin labels, such as MTSSL, as a rapid approach that yielded good agreement with experimental data with only 0.2 nm standard deviation.

Here, we extended this approach to significantly larger labels comprising up to eleven dihedral angles as found in fluorescence labels as well as in recently developed spin labels. We applied Monte Carlo sampling to efficiently generate large ensembles of label conformations, followed by clustering into a significantly smaller, yet representative, set of conformations, a rotamer library. These pre-computed libraries can be used to calculate the spatial distribution of a label within seconds on a desktop computer and thereby facilitate the comparison of experimental data to atomic structures, even for screening large ensembles or for refinement of structural models.

51-POS Board 11

Bayesian Ensemble Refinement by Replica Simulations and Reweighting

Juergen Koefinger, Gerhard Hummer.

Max Planck Institute of Biophysics, Frankfurt am Main, Germany.

Ensemble refinement attempts to overcome the respective limitations of experiment and simulations when it comes to the inference of structural ensembles of highly flexible and dynamic macromolecules. Most experimental measurements provide averages over ensembles of structures (NMR) and additionally often provide only low-resolution structural information (SAXS/SANS, FRET, DEER). In contrast, bioinformatics and simulations can provide high-resolution ensembles of structures but suffer from systematic and sampling uncertainties. In replica simulations, experimental measurements can be used to steer simulations to explore configurations space more efficiently. However, the number of replicas, and thus the maximum size of the ensemble, is strongly limited by the available computational resources. In contrast, reweighting allows us to adapt the statistical weights of large ensembles of structures efficiently but it will fail if the overlap with the true ensemble is insufficient. By combining these two approaches, we overcome their respective limitations. We show that replica simulations [Best and Vendruscolo, *J. Am. Chem. Soc.* 2004] and the Ensemble Refinement of SAXS method (EROS) [Rozycki et. al, *Structure* 2011] both aim to find the optimal solution of the same underlying Bayesian formulation of ensemble refinement. In the Bayesian Inference of ENsembles method (BioEn) [Hummer and Koefinger, *J. Chem. Phys.* 2015] we first perform replica simulations to explore configurations space. From these simulations, we generate a reference ensemble using a bin-free version of WHAM, which we then refine while striking a balance between our prior knowledge, summarized in the reference ensemble, and the new experimental data.

52-POS Board 12

Fast High-Resolution Refinement of Large Proteins and Membrane Protein Complexes in Rosetta

Julia Koehler Leman^{1,2}, Jeffrey J. Gray³, Richard Bonneau^{1,2}.

¹Simons Foundation, New York, NY, USA, ²New York University, New York, NY, USA,

³Johns Hopkins University, Baltimore, MD, USA.

Protein structure refinement is one of the most often used modeling applications, especially for high-resolution refinement of structures from homology modeling, de novo structure prediction, loop modeling; to create starting models for protein design, protein-protein docking or ligand docking; to refine structures into density maps from crystallography and cryoEM; to create all-atom representations from coarse-grained models; and to model protein conformational flexibility. Here, we present a new high-resolution refinement algorithm implemented into the Rosetta software suite. Since Rosetta was historically developed for small, soluble proteins, the gold-standard refinement algorithm in Rosetta, FastRelax, was neither routinely used, nor benchmarked on large to very large, asymmetric proteins. When applied to larger complexes, such as membrane proteins, FastRelax becomes prohibitively expensive. We show that our new RangeRelax algorithm does not only speed up refinement of complexes up to ~5000 residues by up to 60-fold, but it also results in fewer clashes and better stereochemistry. RangeRelax is available for soluble and for membrane proteins and can be easily integrated into larger modeling protocols.

53-POS Board 13

Generation and Assessment of Dynamic Structural Ensembles of Parvulin-type Peptidyl-Prolyl Isomerases by Externally Restrained Molecular Dynamics Simulation

Bertalan Kovács¹, András Czajlik¹, Perttu Permi², Zoltán Gáspári¹.

¹Pázmány Péter Catholic University, Budapest, Hungary, ²University of Jyväskylä, Jyväskylä, Finland.

Parvulins are a subtype of peptidyl-prolyl isomerases (PPIase) that catalyze the isomerization of the peptide bond preceding proline residues. They play a critical role in several biological processes like chromatin remodeling, transcription and nuclear receptor signaling, as well as in protein folding. While their three dimensional structure and the location of the active site is well-defined, the exact mechanism of their catalytic activity remains elusive. It is generally accepted that there is no breaking and reforming of the peptide bond, instead, isomerisation occurs through a twisted intermediate state. A recent analysis of dynamic structural ensembles of cyclophilin A proposed an electrostatic handle mechanism facilitated by the polarity of the carbonyl group of the peptide bond.

In the presented study, externally restrained dynamical structural ensembles were generated using experimentally determined NOEs and S2 parameters for three distinct parvulins: SaPrsA, TbPin1 and CsPinA. The resulting ensembles show good agreement with the experimental parameters, including chemical shifts, but also show significant differences in the dynamics of the three enzymes.

Analysis of the predominant motional modes in both the restrained and unrestrained ensembles as well as the role of the WW-domain in Pin1-type parvulins compared to the non-Pin1-type suggest a catalitically determining motion in the enzyme activity. It is concluded that modulation of the extent and dynamics of the identified motion accounts for the differences in the function of the distinct parvulins. The presented results were recently published in Czajlik et al. 2017, Sci Rep.

54-POS Board 14

Structural and Mechanistic Insights into the E Subunit from Bacterial ATP Synthases

Alexander Krahl, Changbong Hyeon.

Korea Institute for Advanced Study (KIAS), Seoul, South Korea.

The central energy conversion machinery in all living cells, the ATP synthase, uses an electrochemical ion gradient to synthesize ATP, the main energy source in all living cells, by a catalytic rotational motion. Vice versa it can pump protons, by hydrolyzing ATP, rotating in the opposite direction. The ϵ subunit from bacterial ATP synthases undergoes a large conformational change from the ATPase inhibitory up- to the non-inhibitory down state upon ATP binding. However, the ATP binding affinity of ϵ subunits from different organisms is dramatically different, from 4 μ M (thermophilic *Bacillus* PS3) to 20 mM (*Escherichia coli*), while others may not bind ATP at all (e.g. *Mycobacterium tuberculosis*). We use MD simulations to clarify reasons for the different ligand binding affinities of the ϵ subunit from different organisms. In this work, we obtain the ATP binding site structure of the ϵ subunit from *Escherichia coli*, deriving molecular reasons for the dramatically decreased binding affinity compared to the ϵ subunit from thermophilic *Bacillus* PS3. Furthermore, we observe that the protonation state of one carboxylate group is essential to allow ATP binding, inducing a conformational change and ATP binding of one of the key binding residues - thus the mechanistic modes of the ϵ subunit from distinct bacterial organisms are controlled differently.

55-POS Board 15

Coupling Nuclear Magnetic Resonance and Molecular Dynamics Simulations to Study Protein Dynamics

Micha Kunze, Kresten Lindorff-Larsen.
University of Copenhagen, Copenhagen, Denmark.

Symbiotic use of high-resolution ensemble measurements such as NMR and molecular dynamics (MD) simulations is crucial for an in-depth understanding and visualization of a proteins structural ensemble.

My presentation focuses on applications where we use experiment and simulation in tandem to understand the structure-dynamics-function relationship of different proteins. We use experimental data to restrain and correct MD simulations, e.g. chemical shift or NOE data [1,2]. In this way we can gain insight into the mechanism of allosteric enzyme regulation [1] or the structural ensemble of peptides [2].

Vice versa we use simulations to guide experiments, by e.g. suggesting mutations to alter enzymatic activity [3] or by suggesting specific experiments to probe conformational changes observed in simulations.

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56-POS Board 16

Conformational Dynamics of the Cop9 Signalosome-Cullin-2 RING E3 Ligase Supercomplex Probed by Hybrid Mass Spectrometry

Andy M. Lau¹, Sarah V. Faulk², Chloe Martens¹, Zainab Ahdash¹, Edward P. Morris², Argyris Politis¹.

²Institute of Cancer Research, London, United Kingdom. ¹King's College London, London, United Kingdom,

The Cullin-2 (CUL2) scaffold, along with RING-box binding protein 1 (RBX1) forms the core of a Cullin-2-RING Ligase (CRL2) complex. Further association with the VHL-EloB/C (VBC) adaptor complex, the CRL2VBC is responsible for cellular modulation of oxygen-dependent processes through regulation of the HIF-1 α transcription factor. The activity of CRL2^{VBC} is regulated by the conjugation of the ubiquitin-like protein, NEDD8 (N8), through a process known as neddylation. Deactivation is achieved through complexing with the hetero-octameric Cop9 Signalosome (CSN) assembly which removes N8 from the CUL2 scaffold. Despite the importance of CSN-mediated CRL deactivation in regulating critical cellular processes, the precise and ubiquitous deactivation mechanism of CSN remains elusive to traditional structural biology techniques. Using a hybrid mass spectrometry (MS)-based strategy employing native, ion-mobility, chemical cross-linking and hydrogen deuterium exchange (HDX)-MS, combined with molecular dynamics simulations and integrative modelling with cryo-EM mapping, we have documented the highly dynamic conformational responses of CSN upon stimulation by both neddylated and deneddylated CRL2^{VBC}. Native MS has confirmed the formation of the CSN-CRL2^{VBC} supercomplex, while a mutation in the CSN5 subunit has allowed the generation of the CSN-CRL2^{VBC}~N8 deneddylation intermediate. Integrative modelling of subunit connectivities via chemical cross-linking MS and electron density maps via cryo-EM have identified discrete architectures of CSN-CRL2^{VBC} and CSN-CRL2^{VBC}~N8. Using differential HDX-MS and molecular dynamics simulations, we have further characterised the multiple layers of conformational dynamics imposed by binding of CRL2^{VBC} and CRL2^{VBC}~N8. Our results show that neddylation of CRL2^{VBC} directly influences the mode of binding to CSN, while the deneddylation mechanism of CSN5 is indifferent to the neddylation status.

57-POS **Board 17**

Conformational Change of Dopamine D3 Receptor Complex Induces GDP Dissociation from Gai Protein for Signal Transmission

Ya-Tzu Li, Hao-Jen Hsu.

Tzu-Chi University, Hualien, Taiwan.

G protein-coupled receptors (GPCRs), one of the largest superfamily of membrane proteins, can recognize most external stimuli in different signal transduction pathways responsible for a vast majority of physiological responses. Dopamine is an important neurotransmitter in the central nervous system that plays a critical role in movement, cognition and emotion. Dysfunction of dopamine receptors which belong to GPCR family A may lead to severe nervous diseases. In general, when dopamine binds to the dopamine receptor, it induces a conformational change of the receptor to recruit Ga protein association. GDP is then dissociated from Ga protein. However, the atomic-level activation mechanism of dopamine receptor from ligand binding to G protein dissociation for signal transmission remains unclear. In this research, based on our previous study, dopamine-bound full-length dopamine D3 receptor (D3R) with homology-modeled N-terminus taken from 1.8 μ s MD simulations was docked to GDP-bound Gai for signal transmission during the activation. The multi-microsecond MD simulations showed that dopamine-Gai-GDP-bound D3R have large fluctuations in TMs 1, 3, 5 and 7 to enlarge the cytoplasmic binding site of D3R for Gai association. Helix α 5 of Gai flattened to embed into ICL3 when compared with the dopamine-Gai-bound D3R complex system. The approach of helix α 5 to β 6 of Gai and the flipping out of switch I and II of Gai may induce GDP dissociation from Gai. The internal water channel gradually formed during the D3R activation process, which is similar to other GPCRs. The findings of this study elucidate how D3R assumes its active conformation, and could prove valuable in drug design for the treatment of nervous system-related diseases.

58-POS Board 18

Matching Pursuit Genetic Algorithm for Structure Characterization of Large Intrinsically Disordered Proteins

Wei Liu, Daiwen Yang.

National University of Singapore, Singapore, Singapore.

Structure characterization of intrinsically disordered proteins (IDPs) remains a key obstacle in understanding their functional mechanism. Due to the highly dynamic feature of IDPs, structure ensembles instead of static unique structures are often derived from experimental data. Determination of a structure ensemble usually uses a combinatorial optimization strategy, which selects an optimal ensemble to fit the data from a structure pool without prior experimental information. The search space of the combinatorial optimization problem could be extremely huge, as it's an exponential function of the ensemble size and a power function of the pool size. In such a case, conventional algorithms become less efficient to find a good solution within appropriate computational time. Here we present a matching pursuit genetic algorithm (MPGA), which uses matching pursuit (MP) for search space reduction and genetic algorithm (GA) for optimization. A sub-pool is selected from the original pool based on diverse criteria, and a structure ensemble is selected from the sub-pool by GA. Like MP, the sub-pool is sequentially adjusted according to the differences between the experimental and back-calculated data, and then utilized in next round GA. This process is iterated until the outcome converges. We demonstrate that the MPGA method outperforms other state-of-art algorithms in structure ensemble selection from a large pool (>1.3 million) of p130CasSD, an IDP with 306 amino acids.

59-POS **Board 19**

Constructing Ensembles Using Site-Specific Vibrational Spectroscopy Probe Groups

Casey H. Londergan, Rosalind J. Xu, Kristen L. Kelly, Shannon R. Dalton, Alice R. Vienneau, Daniel M. Konstantinovsky.
Haverford College, Haverford, PA, USA.

Vibrational spectroscopy has an inherent advantage over other experimental techniques for ensemble determination due to its very fast intrinsic time scale (10s of fs to a few ps), which means that protein conformational changes are in slow exchange in vibrational spectra and the full conformational distribution is thus present in some form in the spectrum. A recently popularized approach uses functional groups with unique vibrational frequencies (i.e. the CN stretch of nitriles) as reporters of the environment around specific sites in proteins. The infrared or Raman lineshapes of these probe groups contain the local structural distribution, and the fast intrinsic time scale also means that there is a direct match between the decay times of the vibrational correlation functions and the time steps in all-atom molecular dynamics simulations. We have placed the SCN vibrational probe group into several proteins, including model peptides, alpha synuclein, calmodulin, and fuzzy viral complexes. The SCN group in particular is surprisingly non-perturbative in most cases and this lack of perturbation suggests that it could be placed in many systems, including directly along protein-protein and protein-membrane binding interfaces. Our extensive experimental data provides new and previously unreported information about the range of environments around specific sites in these proteins, especially about the dynamic structures of bound protein-protein and protein-membrane complexes. We have also performed initial molecular dynamics simulations intended to provide an interpretive guide to the data, and it appears that there is at least semi-quantitative agreement between simulated probe solvent exposure distributions and the CN frequencies and lineshapes of the SCN group. While there are current challenges associated with making a direct connection between vibrational probe data and simulations, this general two-pronged strategy is a promising new methodology for determining and representing protein ensembles.

60-POS Board 20

Elucidating Structure and Conformational Changes with Cross-Linking/Mass Spectrometry (CLMS)

Michael Schneider², **Mahmoud Mabrouk**¹, Kolja Stahl¹, Oliver Brock¹, Juri Rappsilber^{3,2}.
¹Technische Universität Berlin, Berlin, Germany, ²Technische Universität Berlin, Berlin, Germany, ³University of Edinburgh, Edinburgh, United Kingdom.

Protein structure and dynamics are studied typically outside the native context needed for correct function and possibly also folding. Cross-linking/mass spectrometry (CLMS) is a maturing structure analysis tool, poised to overcome this limitation. Under native conditions, cross-linkers can convert proximity into chemical bridges that can be observed experimentally. CLMS can reveal the structure of individual proteins, probe protein assemblies, and deliver proteome-wide information. Our labs recently added novel chemistry to drastically improve the density of data obtained.

Leveraging CLMS data computationally remains challenging since it contains noise and averaged data. We recently demonstrated that using high-density CLMS data, which increases the amount of cross-links by an order of magnitude, enables the de novo reconstruction of the human serum albumin domains. The key to this success is the complementary integration of experiment and computation: We were able to utilize CLMS data with a degree of noise that would normally be rejected using a noise intolerant conformational space search algorithm. Our method is novel in two key aspects: first, it models cross-link constraints with a modified Lorentz function allowing it to be robust to wrongly predicted cross-links. Second, it leverages cross-links to guide the search towards promising regions which are then searched using an unbiased all-atom energy function.

Combining high-density cross-linking with quantitative experimental setups hold a high potential to elucidate protein dynamics. We have recently made use of CLMS to reveal subtle conformational changes in the complement protein C3 and its activated cleavage product C3b. Yet, the development of computational methods for interpreting this data is still in its early stages. We see the upcoming conference as an ideal chance to disseminate our findings to a panel of experts and discuss the future of this novel type of experimental data.

61-POS Board 21

Molecular Breakdown of DEER Data from Self-learning Atomistic Simulations

Fabrizio Marinelli, Giacomo Fiorin, José Faraldo-Gómez.
n/a, Bethesda, USA.

Double Electron-Electron Resonance (DEER) has become a landmark technique to investigate bio-molecular structure and dynamics. DEER allows obtaining the distance distributions between spin-labels attached to a biomolecule and in contrast to X-ray crystallography and NMR spectroscopy, DEER is neither limited by the need of crystallization nor by the size of the biomolecule. This notwithstanding, it is often not straightforward to interpret DEER data as it reflects a plethora of molecular conformations and rotameric states of the spin-labels. Several strategies to disentangle this variability have been put forward recently, either based on approximate structural models or on atomistic simulations. Both kinds of approaches however rely on probability distributions that are inferred from the actual measured data and do not take into account the experimental noise. Building upon the maximum entropy principle, we present an adaptive simulation framework to minimally bias an atomistic simulation to sample a conformational ensemble that reproduces the DEER data within the experimental uncertainty. Our approach has been formulated either to target directly the DEER time signal within the experimental noise or to reproduce DEER distributions within the confidence intervals. We first test the performance of this approach for the spin-labeled T4 lysozyme. Then, we apply it to investigate the conformational dynamics of the apo VcSiaP binding protein, that undergoes an open to close conformational change upon substrate binding. The results indicate a wider opening of the VcSiaP apo state compared to both the X-ray structure and standard MD simulations, underlying that the proposed technique is a powerful tool to structurally characterize DEER experiments and to investigate the dynamics of biomolecules.

62-POS Board 22

A Simulation-based Approach to the Dynamical Basis of Hfq-RNA Interactions

Charles McAnany, Sooraj Achar, Kimberly Stanek, Cameron Mura.
University of Virginia, Charlottesville, VA, USA.

The bacterial Sm protein, known as Hfq, acts as a generic RNA chaperone that facilitates interactions between two RNA strands, typically a noncoding small RNA (sRNA) and a regulatory target (e.g., an mRNA). Many sRNAs play key roles in post-transcriptional regulation, including protein translational control and RNA decay pathways. While a decade of crystal structures have provided static snapshots of Hfq and Hfq-RNA complexes, and biochemical studies have supplied valuable information about Hfq function, the physicochemical behavior of RNA and Hfq, as they interact across their molecular surfaces, remains unexplored. Hfq self-assembles into hexameric rings, with two distinct RNA-binding regions. One side of the ring (the *distal* face) binds U-rich RNA, while the other (*proximal*) face binds A-rich RNAs. Our recent crystal structure of an *Aquifex aeolicus* Hfq reveals, in addition to the proximal and distal sites, a conserved *lateral* RNA site on the periphery of the ring. This lateral site binds U-rich RNA with lower affinity than the proximal site. To see how RNA interacts with Hfq, we are pursuing an extensive suite of μ sec-scale MD simulations. By using steered MD to drive two nucleotides of RNA toward the lateral site, our simulations start with a physically plausible, partially-bound state. We then simulate the unconstrained system to examine RNA interactions with the neighboring protein surface, guided by specific questions such as: Can RNA simultaneously bind both the lateral and distal (or lateral and proximal) sites? How stable and persistent (thermodynamically and kinetically) are RNA interactions with the lateral site? These simulations will illuminate, in molecular detail, the fundamental mechanism of Hfq-mediated RNA annealing.

63-POS Board 23

Lukasz Mioduszewski, Marek Cieplak.
Polish Academy of Sciences, Warsaw, Poland.

Gluten proteins do not seem to have one clearly defined tertiary structure, and can form covalently and non-covalently joined megadalton-sized complexes. They form a mechanochemical network, responsible for the viscoelastic properties of wheat dough. The properties can be characterized by the dynamic Young modulus $G^* = G' + G''$, which describes the response to small-amplitude oscillating deformation: G' for the in-phase (elastic) part and G'' for the out-of-phase (viscous) part. The main goal of this work is to present a model that can recreate this elastic response of gluten in computer simulations. The existing theories of gluten elasticity point out the crucial role of hydrogen and disulfide bonds between different gluten protein chains. The theories provide some predictions that can be incorporated into a simple coarse-grained model of gluten. In the model amino acids are represented as pseudoatoms, connected harmonically to form protein chains. Non-bonded interactions include Lenard-Jones potential, which mimics hydrogen bonding, and a dynamic potential for disulfide bonds. Initial chain conformations are generated randomly, and then evolve according to the simplified potential, forming large complexes. The results were obtained by periodically deforming the box containing gluten proteins and recording the response force. The amplitude of the response force seems to increase, indicating strain hardening, an effect observed in experiments. It is accompanied by changes in the protein network structure: the number of inter-chain hydrogen and disulfide bonds increases. The connection between those conformational changes and system response to deformation is discussed, as well as the ability of simple models to predict properties of large complexes of disordered proteins.

64-POS Board 24

A Modern Approach to Determining and Displaying Conformational Ensembles

Ryan L. Melvin^{1,2}, Ryan C. Godwin¹, Jiajie Xiao¹, William G. Thompson¹, Kenneth S. Berenhaut², Freddie R. Salsbury Jr¹.

²Wake Forest University, Winston Salem, NC, USA. ¹Wake Forest University, Winston Salem, NC, USA,

The ensemble nature of biopolymers makes arbitrary parameter choices when selecting micro and macro-states a significant source of bias and uncertainty. Most partitioning methods require users to either have some *a priori* knowledge about the system to be clustered or to tune parameters through trial and error. Here we present non-parametric uses of two modern clustering techniques suitable for first-pass investigation of data sets containing multiple structural ensembles. After determining partitions, displaying ensembles in static print media remains a challenge. Using a single representative conformation of a biopolymer rather than an ensemble of states mistakenly conveys a static nature rather than the actual dynamic personality. Here we suggest a standardized methodology for visually indicating the distribution width, standard deviation and uncertainty of ensembles of structural states with little loss of the visual simplicity of displaying a single representative conformation. This method includes a dynamic element in that it clearly distinguishes between isotropic and anisotropic motion of polymer subunits. We also apply this method to ligand binding, suggesting a way to indicate the expected error in many high throughput docking programs when visualizing the structural spread of the output. We also discuss how these methods apply to any macromolecular data set with an underlying distribution, including experimental data such as NMR structures.

65-POS **Board 25**

Modeling the Partition of Carvedilol in Lipid Bilayers Using All-Atom Molecular Dynamics Simulations

Williams E. Miranda, Van A. Ngo, Sergei Y. Noskov.
University of Calgary, Calgary, AB, Canada.

Heart disease is the primary global cause of deaths with 17.3 million fatalities each year. Despite the tremendous efforts for finding solutions for heart arrhythmias, current drugs have dangerous side-effects, increasing rather than decreasing the fatal probabilities. Carvedilol is a β -blocker that has shown to have encouraging antiarrhythmic effects. Recent experimental evidence suggest that carvedilol modulates the activity of the cardiac ryanodine receptor (RyR2), a membrane protein responsible for calcium homeostasis in cardiac cells. This suggests that the drug must traverse the cytoplasmic membrane to reach RyR2 which is located in the sarcoplasmic reticulum. Although there are experimental studies on carvedilol partitioning in model membranes, no atomistic insight into this process is currently available. In this work, we aim to study the partition of carvedilol into lipid bilayers using all-atom molecular dynamics simulation (MD). We performed a systematic quantum-based parameterization for carvedilol. Then, we used umbrella sampling, replica exchange and steered MD simulations to thoroughly sample conformational ensembles of carvedilol during the partition process, and to obtain converged free energy profiles. Our preliminary results from umbrella sampling simulations show small energetic barriers for the partitioning process of the drug in its neutral state. We seek to explore the energetic relations between rotations of carvedilol with respect to the distance from the lipid bilayer. We also aim to simulate the partition for charged carvedilol for comparison with the neutral one. These atomistic simulations will provide insights at the molecular level on how carvedilol interacts with the lipid membrane, as a first step to understand its action mechanism on RyR2.

66-POS Board 26

Large-scale DFT Calculation of Double-Stacked Beta Sheet Conformations

Mogus Mochena¹, Stacyann Nelson¹, Anant Paravastu².

¹Florida A&M University, Tallahassee, FL, USA, ²Georgia Institute of Technology, Atlanta, FL, USA.

Understanding biological self-assembly pathways and controlling them is a challenging problem, but is of great importance both from fundamental point view of deciphering complex biological processes on one hand and technological applications on the other. Here we have studied quantum mechanically how beta strands self-assemble into beta sheets to gain understanding of the self-assembly mechanism in simple designer peptide nanofiber RADA16-I formed from double -stacked beta sheets. According to Sawaya et al [1], there are eight distinct structures that result from association of beta strands as they form stacked beta sheets. RADA16-I was designed to form when the beta sheets associate in ant-parallel conformations. [2] However, recent detailed NMR studies show that the strands combine in parallel conformations with a registry shift of 2 residues. [3] Classical molecular dynamics calculations were performed to determine the stable structure among the different conformations. Unfortunately, the molecular dynamics simulations predict that most of the structural possibilities are stable, and only experiments could identify what structures are formed in solution. We have performed linearly scaled density functional theory (DFT) calculations on symmetric structures as suggested by Sawaya et al to determine stable conformations.

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67-POS Board 27

Efficient Parallel Computation for Flexible Fitting of Cryo-EM Density Map

Takaharu Mori^{1,2}, Osamu Miyashita³, Yuji Sugita^{1,2,3}.

¹RIKEN Theor. Mol. Sci. Lab., Wako-shi, Japan, ³RIKEN AICS, Kobe-shi, Japan. ²RIKEN iTHES, Wako-shi, Japan,

Single-particle cryo-electron microscopy (cryo-EM) is one of the powerful experimental techniques to determine structures of biomolecules at near atomic resolutions. The method does not require crystallization of the sample, and the 3D structure of the target biomolecule is reconstructed from a large number of 2D images. Molecular dynamics (MD) simulations have been often used to construct 3D structures by fitting the all-atom model to low-resolution cryo-EM density map. One of the major problems in such a flexible fitting simulation is that we need large computational costs if we tackle big molecules like ribosome and protein complexes. Recently, we have developed our in-house MD program package (GENESIS), which supports various types of replica exchange molecular dynamics methods (REMD) as well as conventional MD methods for large systems including hundreds million atoms [1]. In this study, we introduced a new REMD algorithm for cryo-EM fitting, where the force constants of the biasing potential are exchanged between a pair of replicas. The method can automatically adjust the strength of the biasing force in each replica to avoid overfitting issues [2]. We also proposed a new parallel computing algorithm for cryo-EM fitting with hybrid MPI/OpenMP schemes, where the simulation system is decomposed into several domains according to the number of atoms in the local spaces. We show performance of GENESIS in cryo-EM fitting simulations for large systems such as membrane proteins and ribosomes.

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[2] O. Miyashita, C. Kobayashi, T. Mori, Y. Sugita and F. Tama, J. Comput. Chem. DOI: 10.1002/jcc.24785

68-POS Board 28

Multiscale Enhanced Sampling for Glucokinase

Kei Moritsugu¹, Tohru Terada², Akinori Kidera¹.

¹Yokohama City University, Yokohama, Japan, ²The University of Tokyo, Tokyo, Japan.

Free energy landscapes derived from all-atom protein conformational ensembles have played an important role for elucidating protein functional dynamics with high structural and energetic resolution. Since the characteristic time scale of biologically relevant processes such as protein structural changes far exceeds the feasible computational time, the calculations of protein free energy landscapes require the acceleration of conformational samplings and mapping along the reaction coordinates or the pathways of such structural changes. Here, a multiscale molecular dynamics simulation method, “multiscale essential sampling (MSES)”, has been proposed which enables full conformational samplings of large proteins at atomic resolution including explicit solvent. In MSES, the sampling of a full-dimensional model is enhanced by coupling with accelerated dynamics of the associated coarse-grained model (CG), together with a multicopy scheme, Hamiltonian replica exchange, to remove the biasing potential in MSES. CG is then useful for determining the sampling region according to our purpose, and can be suitably defined by prior knowledge such as experimental data.

MSES has been further extended for maximizing the CG driving force and applied to large systems in solution such as intrinsically disordered protein, protein complex, and protein-ligand interaction. Here, a recent application has been presented to glucokinase, an enzyme that facilitates phosphorylation of glucose for the regulation of carbohydrate metabolism. Conformational ensembles of glucokinase with and without bound glucose were fully calculated by MSES and found to be extended ranging from closed to open and super-open structures, which is consistent with the previous SAXS experiments. The result clarified the structural basis of positive cooperativity for the activity of glucokinase in response to glucose concentration that originates from a high energy barrier between the closed and open structures relating to the helix-coil transition of an interfacial helix.

69-POS Board 29

Coregulators Select Conformational States from Drug Specific Peroxisome Proliferator-activated Receptor Gamma (PPAR γ) Conformational Ensembles

Michelle Nemetchek^{1,2,3}, Ian M. Chrisman^{1,2}, Travis S. Hughes^{1,2,3}.

²The University of Montana, Missoula, MT, USA, ¹The University of Montana, Missoula, MT, USA, ³The University of Montana, Missoula, MT, USA.

Nuclear receptors are transcription factors that, when bound to agonists, cause transcription of regulatory genes. Peroxisome Proliferator-activated receptor gamma (PPAR γ) is a nuclear receptor that is an important target of many FDA-approved type II diabetes drugs. When agonist bound, PPAR γ recruits the coactivator Mediator complex subunit 1 (MED1), which is part of the mediator complex and triggers transcription of PPAR γ -regulated genes. These genes control adipogenesis and lipid storage, and may enhance insulin sensitivity. When bound to inverse agonists, PPAR γ complexes with Nuclear Receptor Corepressor I (NCoR1) and is blocked from transcriptional activation. By using Time-Resolved Förster Resonance Energy Transfer (TR-FRET), PPAR γ binding drugs are shown to produce differing affinities for MED1 and NCoR1 peptides which contain the essential nuclear receptor binding motif. We covalently label PPAR γ with a fluorine containing probe on the AF2 surface (the surface where coregulators have been demonstrated to interact) and use Fluorine-19 (19F) Nuclear Magnetic Resonance to determine how coregulator binding affects this surface. The AF2 surface conformations are highly similar for both full agonist bound and full agonist and MED1 co-bound states, similarly inverse agonists induce conformations that are relatively unchanged by NCoR1 binding. Our data also suggest that coregulators select conformations from a preexisting ensemble which is highly drug specific. This work links structure of the LBD to the functional outcome of transcription.

70-POS Board 30

Conformational Effects of Threonine Phosphorylation in Proline-rich Disordered Motifs

Gabriel A. Lucero¹, **Paul S. Nerenberg**^{2,3}.

²California State University, Los Angeles, Los Angeles, CA, USA, ³California State University, Los Angeles, Los Angeles, CA, USA. ¹California State University, Los Angeles, Los Angeles, CA, USA,

The experimental literature regarding the conformational effects of threonine (Thr) phosphorylation in intrinsically disordered proteins/regions presents an apparent paradox. In some contexts, Thr phosphorylation appears to stabilize beta conformations, while in others – including a proline-rich region of the tau protein – it appears to stabilize PPII conformations. In this work we present MD simulations of several small peptide systems that suggest that the identity of the residue immediately C-terminal to the Thr phosphorylation site is the primary determinant of how the conformational ensembles of these peptides change upon phosphorylation. These data help resolve the aforementioned paradox by demonstrating that the existence of a proline residue in this position prevents the formation of a phosphate-to-backbone hydrogen bond that would otherwise stabilize beta conformations. This in turn has implications for the role of phosphorylation in proline-rich motifs, which are both often disordered and recognition sites for protein-protein interactions. Finally, our simulations provide specific, testable hypotheses that can be confirmed – or falsified – with appropriate NMR experiments.

71-POS Board 31

Effects of Change-of-Function Mutations on Helix Stability in the Intrinsically Disordered T1-Core Activation Domain of the Glucocorticoid Receptor

Lennart Nilsson, Evdokiya Salamanova, Joana Paulo, Alok Juneja, Anthony Wright.
Karolinska Institutet, Huddinge, Sweden.

Intrinsically disordered proteins hold important functions in the cell, such as regulation of transcription and translation, signalling, storage of small molecules and self-assembly of macromolecular units in active complexes. The lack of a compact 3D-structure or folding only upon binding to their targets is related to the specific rôle of the unstructured regions. The glucocorticoid receptor belongs to a family of ligand-inducible nuclear receptors, and two of its domains (tau1 and tau2) have shown conserved activity after they have been removed from the receptor entity[1]. The disordered core region of the tau1-domain consists of 58 amino acids. It carries most of the activity and has shown a helical propensity in hydrophobic solvents. We have investigated structural effects of change-of-function mutations[2] in the tau1-core transactivation domain in the glucocorticoid receptor. Based on our previous experience with the A β -peptide[3], where we have also identified small peptide like molecules that stabilize the helix conformation of A β , we have performed hundreds of 200+ ns molecular dynamics simulations to correlate the experimental activities of the wild type and mutant peptides to their helical propensity and their effect on activity.

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3. Ito, M., Johansson, J., Strömberg, R., and Nilsson, L. (2012) Effects of Ligands on Unfolding of the Amyloid β -Peptide Central Helix: Mechanistic Insights from Molecular Dynamics Simulations, *PLoS ONE* 7, e30510.

72-POS Board 32

Local Descriptors of RNA Structures

Tymoteusz Oleniecki^{2,1}, Agnieszka Mykowiecka³, Pawel Daniluk^{1,4}.

¹Mossakowski Medical Research Centre Polish Academy of Sciences, Warsaw, Poland,

²University of Warsaw, Warsaw, Poland, ³University of Warsaw, Warsaw, Poland, ⁴University of Warsaw, Warsaw, Poland.

Local Descriptors of Structure (LDS) have been proven useful in comparison and classification of protein structures [1]. In this approach a protein is divided into overlapping fragments representing neighbourhoods of each residue according to the chosen definition of inter-residue contact. This allows to treat structures as non-rigid objects leaving out residue order, but putting emphasis on architectural similarities [2]. Aim of this study was to generalize descriptor approach to other biopolymers i.e. nucleic acids.

We have prepared two sets of RNA structures: a non-redundant set of 96 pure RNA structures to develop and validate contact criteria on (CCV), and a set of nearly one thousand RNA chains, which differ either in sequence or in structure, used for proper study (NR-RNA). We have also designed a set of geometrical contact criteria of different accuracy and computational efficiency, and assessed their feasibility by measuring contact preservation and genericity of descriptors extracted from CCV. We have selected the simplest and most general criterion, which was physico-chemically accurate. We used that criterion to compute a set of LDSs based on structures in NR-RNA. Finally we assessed similarity of these LDSs and clustered them.

We have obtained a database of representative recurrent RNA structural patterns present in PDB/NDB which, like their protein counterparts, could be useful in classification and statistical analysis of RNA structures as well as prediction of tertiary structure.

These studies were supported by National Science Centre (DEC-2011/03/D/NZ2/02004), and by the BST-176600/BF (task 22) funds of Faculty of Physics, University of Warsaw. Computations were carried out using infrastructures financed by the POIG.02.03.00-00-003/09 (Biocentrum-Ochota) and POIG.02.01.00-14-122/09 (Physics at the basis of new technologies) projects.

73-POS Board 33

Dimensional Reduction of Markov State Models from Renormalization Group Theory

Simone Orioli^{1,2}, Pietro Faccioli^{1,2}.

¹University of Trento, Trento, Trento, Italy, ²Trento Institute for Fundamental Physics, Trento, Trento, Italy.

In this work we used Renormalization Group (RG) theory to define a rigorous algorithm to cluster the microstates of a Markov State Model into macrostates. The result is a lower-dimensional Master Equation which yields the optimal reduced Markovian description of the system's relaxation kinetics. To illustrate and validate our method we analyze a number of test systems of increasing complexity, ranging from synthetic toy models to atomistic molecular dynamics simulations. In all cases, the low-dimensional Markov State Model is found to reproduce the kinetics of the original model with very high accuracy, with a relative error of at most a few percent.

74-POS Board 34

Using Experimentally-derived Local States to Drive the Sampling of Global Conformations in Molecular Dynamics Simulations

Alessandro Pandini¹, Matteo Tiberti², Arianna Fornili².

¹Brunel University London, Uxbridge, United Kingdom, ²Queen Mary University of London, London, United Kingdom.

Introduction

Conformational changes associated with protein function often occur at timescales inaccessible to unbiased Molecular Dynamics (MD) simulations, consequently different approaches have been developed to accelerate their sampling. Here we investigate how knowledge of experimental backbone conformations preferentially adopted by protein fragments, as contained in pre-calculated libraries known as Structural Alphabets (SA)[1], can be used to explore the landscape of global protein conformations in MD simulations.

Methods

SAs were successfully used to analyze protein dynamics after simulation[2,3]. Here we define a novel SA-based Collective Variable (CV_{SA}) to bias the sampling of backbone conformations of protein fragments towards recurring local states[4] found in experimental structures.

Results

We find that: a) Enhancing the sampling of native local states allows recovery of global folded states, both in Metadynamics and in Steered MD, when the local states are encoded by strings of SA letters derived from the native structures. b) Global folded states are still recovered when the information on the native local states is reduced by using a low-resolution SA, where the original letters are clustered into macrostates. The macrostates provide the approximate shape of the fragments, while sampling with the atomistic force field allows the structure to adopt the native conformation of the specific amino acid sequence. c) SA strings derived from collections of experimental structural motifs can be used to sample alternative conformations of pre-selected regions. We recently extended our approach combining the CV_{SA} with contact prediction from residue coevolution methods.

References

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3. Pandini A., Fornili A., Fraternali F., Kleinjung J., *Bioinformatics* 29:2053 (2013).
4. Pandini A., Fornili A., *JCTC* 12:1368 (2016).

75-POS Board 35

Bridging the Gap Between Markov Stability Theory and Protein Dynamics Experiments at All Timescales

Robert Peach¹, David Klug¹, Sophia Yaliraki¹, Keith Willison¹, Mauricio Barahona², Liming Ying³.

¹Imperial College, London, United Kingdom, ²Imperial College, London, United Kingdom, ³Imperial College, London, United Kingdom.

Objective: The hierarchy of timescales over which protein dynamics occurs is difficult to probe both experimentally and computationally where motions cover approximately ten orders of magnitude in timescale. We have developed Markov Stability, an atomistic graph theoretical method that is able to explore protein dynamics across all of these temporal and spatial scales by finding communities of atoms that are biologically relevant. Markov Stability is able to provide information regarding the timescale of dynamics and has the ability to identify functionally and dynamically important residues. We compare the computational results with single-molecule FRET and Fluorescence correlation spectroscopy (FCS) to both test the computational predictions and to calibrate a relationship between measured physical parameters and Markov Stability.

Methods: The main methods used were Markov Stability (an atomistic graph theoretical community detection method), single-molecule FRET and FCS on a confocal microscope. The experimental system is Aquifex Adenylate Kinase, a well-studied and understood system and an ideal proving ground for experimental tests of the Markov Stability method.

Results: Computational mutagenesis identified a number of residues that altered protein dynamics and molecular stability. The key result was the straight line correlation between the computational score of a mutation and the shift in population equilibrium as measured by single-molecule FRET. Additionally we have found a correlation between Markov time and the dynamical rates of subdomain motion obtained using FCS. Furthermore, a correlation between melting temperatures and predicted scores were identified in the core domain.

Conclusions: We have experimentally validated the predictions from Markov Stability analysis of Adenylate Kinase by showing a linear relationship between measured and calculated parameters. In order to do this we successfully identified key functional residues through computational mutagenesis providing a tool that can be used for protein engineering.

76-POS **Board 36**

A Multi-crystal Parameterisation Method for Separating Atomic and Molecular Disorder in Crystallographic Experiments

Nicholas M. Pearce, Piet Gros.

University of Utrecht, Utrecht, Utrecht, Netherlands.

Diffraction experiments result in a temporal and spatial average over many molecules in a crystal. Atomic Displacement Parameters (ADPs) model harmonic deviations from the average coordinates arising through thermal motion or crystal imperfections. The ADP for a particular atom therefore comprises contributions from multiple sources, including: crystal-dependent disorder; collective, molecule-dependent, “rigid body” motions; and any individual motions of the atom. Large-scale crystal-dependent factors can be considered an artifact of the crystallographic experiment, but collective and individual motions of atoms within a crystal may reveal subtle and biologically relevant protein motions.

Translation-libration-screw (TLS) models are well-established as a method for describing collective motions of groups of atoms in a crystal. In general, however, the separation of the overall observed motion into the different contributions (crystal, rigid body, atomic) is ambiguous, since crystal-dependent factors cannot be uniquely separated from crystal-independent factors. Furthermore, overfitting is ever-present, and the complexity of a ADP model is dictated by the resolution of the crystallographic data.

To overcome the intrinsic obstacles of parameterising disorder in a single crystallographic dataset, we present a multi-dataset ADP-parameterisation approach for modelling atomic disorder: by characterising the ADPs across a series of datasets simultaneously, using a series of TLS models, we separate crystal-dependent and crystal-independent parameters. This results in a hierarchical model of motion, allowing e.g. atomic motions of a sidechain to be decoupled from the large-scale motions of the whole molecule. This approach is validated by both a reduction in the R-free/R-work gap across the set of datasets and a decrease in R-free: the multi-dataset parameterisation thus not only limits overfitting, but increases overall model quality.

77-POS **Board 37**

Unfolding Pathway of Human Serum Albumin Studied by Isothermal Chemical Denaturation and Molecular Dynamics Simulations

Rikke L. Knudsen, Alina Kulakova, Sowmya Indrakumar, Pernille Harris, Jens T. Bukrinski, **Günther H. Peters.**

Technical University of Denmark, 2800 Kgs. Lyngby, Denmark.

Protein aggregation is one of the grand problems of biophysics. It is highly complex, sensitive to initial conditions, operates on a wide range of timescales and its products range from dimeric proteins to macroscopic fibrils. Unwanted aggregation, for instance, has implications in biotechnology, where protein aggregation leads to reduced yield in bioprocessing, and human health, where aggregation-based diseases such as Alzheimer, Parkinson, and Huntington affect millions of people. Hence, an understanding of the molecular mechanisms underlying protein aggregation is of great relevance in diverse research fields such as medicine, pharmacy, food science and industrial biotechnology. The challenge is when proteins are exposed to conditions that are far from physiological conditions and where stability and solubility become critical. The aim of the current study is to gain better fundamental understanding of the relation between protein properties and their role in protein aggregation propensity, and to explore on a molecular level how solution conditions (e.g. pH) affect protein stability. We have chosen human serum albumin as a model system to study the pH-induced unfolding of the protein applying isothermal chemical denaturation (ICD) and classical molecular dynamics simulations. ICD measurements are performed at different pHs and concentrations of the denaturing agents urea and guanidine hydrochloride. The study was supplemented by classical molecular dynamics simulations performed at different pHs to provide a molecular understanding of the unfolding pathway and to identify specific regions in the proteins that act as hotspots driving protein instability and hence protein aggregation.

78-POS Board 38

Using the cgDNA Coarse-grain Model to Generate Sequence-dependent DNA Configuration Ensembles

Daiva Petkeviciute^{1,2}.

¹Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland, ²Kaunas University of Technology, Kaunas, Lithuania.

The cgDNA coarse-grain model and its associated cgDNAmc Monte Carlo code [1, 2, 3] can generate large equilibrium ensembles of configurations of naked B-DNA fragments in solution. The appropriate length of such a fragment can vary from 10 to 10⁴ base pairs, with any DNA sequence being specified. For sequence length of 300 base pairs 1 million configurations can be generated in approximately three minutes on a single processor [3]. The resulting ensembles are strongly dependent on the sequence, e.g. as manifested in both the average shape and flexibility. The model parameter sets are trained on libraries of Molecular Dynamics simulations, that are many orders of magnitude more computationally demanding than the cgDNAmc code. At the length scale of tens of bases model predictions can be compared with X-ray crystal structure and NMR data, and the fits are shown to be in rather good agreement [2]. Another target application of the model is estimating looping and cyclisation j factors [4]. Here we use the cgDNAmc to study various notions of DNA persistence length [3].

[1] A sequence-dependent rigid-base model of DNA. O. Gonzalez, D. Petkeviciūtė, J. H. Maddocks, *J. Chem. Phys.* 138 (5), 2013.

[2] cgDNA: a software package for the prediction of sequence-dependent coarse-grain free energies of B-form DNA. D. Petkeviciūtė, M. Pasi, O. Gonzalez and J. H. Maddocks, *Nucleic Acids Res.* 42 (20), 2014.

[3] Sequence-dependent persistence lengths of DNA. J. S. Mitchell, J. Glowacki, A. E. Grandchamp, R. S. Manning and J. H. Maddocks, *J. Chem. Theory Comput.*, 2016. DOI: 10.1021/acs.jctc.6b00904.

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79-POS **Board 39**

Kinetics of RNA Unzipping: Insights from Density-Peak Clustering Applied to Core-set Markov State Models

Giovanni Pinamonti^{2,1}, Fabian Paul², Frank Noe², Alessandro Laio¹, Giovanni Bussi¹.

¹International School for Advanced Studies, SISSA, Trieste, Italy, ²Freie Universitaet, Berlin, Germany.

We present a novel approach to the construction of a Markov state model that describes the dynamics of a biomolecular system, starting from atomistic MD simulations. We make use of the unsupervised density peak (UDP) clustering algorithm, introduced by Rodriguez and Laio [2014] and further developed by d'Errico et al. [2017]. We combine this algorithm with time-lagged independent component analysis (TICA) [Molgedey and Schuster, 1994] in order to define the microstates of the system and we next compute the transition probabilities between them using a “core-set approach” [Buchete and Hummer, 2008]. We test this approach by studying the process of the fraying of the terminal base pair in a RNA double helix, characterizing the different pathways involved and the sequence dependence of the process timescales.

80-POS Board 40

Conformational Ensembles of Phase-Separating Elastin-Like Peptides from NMR and Molecular Simulations

Quang Huynh^{1,2}, Sean Reichheld¹, Sarah Rauscher^{2,3}, Simon Sharpe^{1,2}, **Régis Pomès**^{1,2}.
¹Hospital for Sick Children, Toronto, ON, Canada, ²University of Toronto, Toronto, ON, Canada, ³Max Planck Institute, Goettingen, Germany.

Elastin endows skin, arteries, the lung, and the uterus with extensibility and elasticity. Elastin and elastin-like peptides are structurally disordered and self-aggregate via liquid phase separation. Despite extensive study, the structural basis for the self-assembly and the mechanical properties of elastin remains unclear. As an essential step towards elucidating the structural ensemble of elastin, we combine molecular dynamics simulations and NMR spectroscopy to study an elastin-like peptide modelled after the sequence of alternating hydrophobic and cross-linking domains of elastin. Computational and spectroscopic results are in excellent agreement. Although the peptide is highly disordered, it possesses a significant propensity for local secondary structure. The cross-linking domains are characterized by fluctuating α -helical structure, whereas the hydrophobic domains form sparse and transient hydrogen-bonded β -turns. As a result, the individual domains are collapsed but not compact, and they remain disordered and hydrated despite their predominantly hydrophobic character. These findings resolve long-standing controversies regarding the structure and function of elastin and afford insight into the physical and structural basis for the phase separation of disordered proteins.

POSTER SESSION III

Monday, August 28, 2017

16:30 – 18:00

Meitner Hall

Posters are available for viewing only during their scheduled date of presentation. Below are the formal presentation times. The presenters listed below are required to remain in front of their poster boards to meet with attendees during the designated times.

Odd-Numbered Boards 16:30 – 17:15 | Even-Numbered Boards 17:15 – 18:00

Potrzebowski, Wojciech	81-POS	Board 1
Primus Dass, Kingsley Theras	82-POS	Board 2
Qiao, Shenglan	83-POS	Board 3
Reichel, Katrin	84-POS	Board 4
Reißer, Sabine	85-POS	Board 5
Reymer, Anna	86-POS	Board 6
Rozycki, Bartosz	87-POS	Board 7
Schroeder, Indra	88-POS	Board 8
Schuetz, Denise	89-POS	Board 9
Shehu, Amarda	90-POS	Board 10
Shimizu, Hirofumi	91-POS	Board 11
Shinobu, Ai	92-POS	Board 12
Singh, Jasdeep	93-POS	Board 13
Sommer, Martha	94-POS	Board 14
Stolzenberg, Sebastian	95-POS	Board 15
Stornes, Morten	96-POS	Board 16
Tang, Chun	97-POS	Board 17
Thalhamer, Anja	98-POS	Board 18
Tiemann, Johanna	99-POS	Board 19
Topal, Busra	100-POS	Board 20
Uluca, Boran	101-POS	Board 21
Völker, Jens	102-POS	Board 22
Wang, Yong	103-POS	Board 23
Wang, Jun	104-POS	Board 24
Wang, Wei	105-POS	Board 25
Weiel, Marie	106-POS	Board 26
Wereszczynski, Jeff	107-POS	Board 27
Wieden, Hans-Joachim	108-POS	Board 28
Wiewiora, Rafal	109-POS	Board 29
Wlodarski, Tomasz	110-POS	Board 30

Won, Jonghun	111-POS	Board 31
Wrabl, James	112-POS	Board 32
Xu, Rosalind	113-POS	Board 33
Xu, Huafeng	114-POS	Board 34
Yenenler, Asli	115-POS	Board 35
Zeppelin, Talia	116-POS	Board 36
Zhang, Zhiyong	117-POS	Board 37
Zhao, Yani	118-POS	Board 38
Zhou, Hao	119-POS	Board 39
Buck, Matthias	120-POS	Board 40

Posters should be set up on the morning of Monday, August 28 and removed by 18:00 on Monday, August 28. All uncollected posters will be discarded.

81-POS Board 1

Exploring Protein Association Pathways with Time-resolved SAXS and SANS

Wojciech Potrzebowski^{1,2}, Ingemar Andre²,

²Lund University, Lund, Sweden. ¹European Spallation Source, ERIC, Copenhagen, Denmark,

Protein performs its biological functions by interacting with other proteins. Protein complexes, which are formed as a result of these interactions, consist of two or more components that associate along specific pathways - protein association pathways. The association pathway from monomer to oligomer is critical in a range of biological processes and thus it is of a vital importance to elucidate both atomic-resolution structures of intermediates along the pathway as well as the structure of the final state. Although considerable progress has been made in using experimental and computational techniques to determine start and final structural states, we have a limited understanding of what happens in between.

By enabling both time resolution and structural detail Time-Resolved Small Angle X-ray/Neutron Scattering (TR-SAXS/TR-SANS) is uniquely suited to interrogate complex self-assembly reactions and to provide a molecular understanding of self-assembly pathways. However, the analysis of such data is complicated because scattering arises from a mixture of many components, the information content in each spectrum is limited and there is no framework for simultaneous analysis of data from different data sources. The similar problem is faced when resolving conformational ensembles from small angle scattering data.

To overcome this problem we developed a method that combines a computational structural modeling (which delivers atomic-resolution structures) with experimental data (which provides information about the population of different states). The method applies Bayesian probabilistic model to analyze scattering data from mixtures of oligomeric species. The method allows for a modeling large structural ensembles, it can be used to assess uncertainty of all modeling parameters and enables minimization of over-fitting. We demonstrated that ensembles determined with this approach explain experimental data to a higher degree and are less prone to over-fitting than the current state-of-art methods used to analyze data.

82-POS Board 2

In Silico Study on the Activation Mechanism of Chemokine Receptor CXCR4 in Complex with Chemokine CXCL12 and G α_i Protein

Kingsley Theras Primus Dass, Hao-Jen Hsu.
Tzu Chi University, Hualien, Taiwan.

The chemokine receptor CXCR4 belonging to rhodopsin superfamily of GPCR is commonly expressed in immune cells and central nervous system. Engagement of CXCR4 in regulating the cell migration and developmental process make CXCR4 an important drug target. Higher expression of CXCR4 on the cancer cells recruits CXCL12 leading to the metastatic condition. So far, many studies have reported the interaction between CXCR4 and CXCL12 but failed to explain the detailed signal transduction of CXCL12 to CXCR4. In this study, we investigated the binding and activation mechanism of CXCR4 in complex with CXCL12 and G α_i by docking and molecular simulations. CXCL12 was docked to the full-length CXCR4 with homology modeled N-terminus. Two preferable poses, Pose-36 and Pose-597, were selected for further multi-microsecond MD stimulations. MM/PBSA binding free energy calculations revealed Pose597 has lower binding free energy than Pose-36. For the complex of Pose-597, the simulation showed that the TM1, TM6, TM5 and ECL 3 underwent large fluctuations and a kink formation in TM7 was observed. Moreover, to explore the complete activation mechanism of CXCR4, G α_i with GDP bound was docked to the CXCR4 complex after 1.5 us MD simulations. The MD stimulation of the ternary complex showed that the N-terminus of G α_i fluctuated to cause its helix α_5 approaching the cytoplasmic pocket of CXCR4, increasing the distance between the two domains of G α_i , which leads to the release of GDP. CXCR4 with the mutated L244^{6.40}N was developed to examine the activation without its ligand CXCL12 binding, which showed that the N-terminus of mutant CXCR4 flipped out for signal transmission. These results give a detailed understanding about the signal transmission through G α_i , which would be helpful in the development of anticancer drugs.

83-POS Board 3

Solution X-ray Scattering Data Can Reveal Three-dimensional Atomic-level Structural Diversity in Ensemble Measurements

Shenglan Qiao, Gundolf Schenk, Derek Mendez, Sebastian Doniach.
Stanford University, Stanford, CA, USA.

Proteins are flexible molecules whose function has evolved based on their ability to sample a variety of conformations. Methods for studying their structures and dynamics need to be capable of probing mixtures of conformations. X-ray scattering by ensembles of particles in solution at an x-ray free electron laser (xFEL) is well suited for this task, but extracting atomic-resolution three-dimensional structural information is challenging. Computing angular correlations, a natural extension of small and wide-angle scattering known as correlated x-ray scattering (CXS), may be used to infer occupancies in models of 3D structures. We present an approach that uses modeling to disentangle angular correlations in solution scattering data contributed by distinct conformations in structurally diverse ensembles; once separated, we can use each angular correlation for refining the structure of its respective component in the ensemble. To illustrate our approach, we describe results from both physical and simulation experiments. In a proof-of-principle experiment, we collected solution scattering data at an xFEL from gold nanoparticles. Analysis of angular correlations, combined with structural models supported by electron microscopy data, successfully isolates correlated signals from two structurally distinct populations; the CXS signals reveal 3D structural differences in the two types of co-existing nanoparticles. We present simulations showing that, based on one of the two atomic models for beta-2 adrenergic receptor (B2AR) conformations, the molar concentration and angular correlations of the second conformation of B2AR can be recovered from CXS data simulated from an ensemble that contains both conformations. By demonstrating its ability to provide atomic-level structural information of components in a mixture and their respective concentrations, we establish CXS as a methodology for studying protein structural dynamics in large ensembles.

84-POS Board 4

Inferential Determination of the Dimeric Structures of TMD0 of TAPL from DEER and PRE Data

Katrin Reichel^{1,2}, Benesh Joseph³, Christoph Bock⁴, Lukas S. Stelzl¹, Jürgen Köfinger¹, Lars V. Schäfer², Rupert Abele⁴, Thomas Prisner³, Gerhard Hummer¹.

¹Max Planck Institute of Biophysics, Frankfurt, Germany, ²Ruhr-University Bochum, Bochum, Germany, ³Goethe-University, Center for Biomolecular Magnetic Resonance, Frankfurt, Germany, ⁴Goethe-University, Institute of Biochemistry, Frankfurt, Germany.

One of the largest membrane protein families is the ATP-binding cassette (ABC) transporter family. Besides their core domain, which performs nucleotide binding and the actual translocation process, some of the ABC transporter have an additional N-terminal transmembrane domain, called TMD0. To elucidate its homodimeric configurations, TMD0 was expressed separately from the core domain and investigated by spectroscopic techniques. In our study, we combine spin-label distance measurements from NMR paramagnetic relaxation enhancement (PRE) and double electron-electron resonance (DEER) experiments with ensemble refinement. These experimental observables seem particularly valuable to detect even sparsely populated configurations. To avoid artifacts, we explicitly model spin-label conformations and the ensemble of structures is directly refined against the nuclear relaxation rates from the PRE measurements and the DEER signals. To perform ensemble refinement, we generate an ensemble of dimeric structures, and subsequently refine the ensemble by reweighting all structures using EROS[1], a maximum-entropy method with Bayesian interpretation [2]. By exploring various numerical approaches to solve the underlying high-dimensional optimization problem, we identify configurations of the TMD0 that are consistent with experiments and our prior expectations.

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85-POS Board 5

Combining Molecular Dynamics and NMR to Characterize Excited States of an RNA Hairpin

Sabine Reißer¹, Peter Podbevšek², Silvia Zucchelli¹, Stefano Gustincich¹, Giovanni Bussi¹.
¹Scuola Internazionale Superiore di Studi Avanzati (SISSA), Trieste, Italy, ²National Institute of Chemistry, Ljubljana, Slovenia.

An inverted SINEB2 element embedded in a non-coding RNA was identified as crucial for the enhancement of mRNA translation under cellular stress [1]. Deletion studies showed, that a terminal 29 nt hairpin is important for this function. To reveal its tertiary structure, 125 NOEs were recorded from NMR experiments and translated into distance restraints for structure refinement [2].

The refined structures were used as a starting point for molecular dynamics simulations. In a replica exchange simulation with 20 replicas, the Hamiltonian (i.e. electrostatic, van-der-Waals, and dihedral potentials) of the hairpin was gradually scaled down to enhance sampling, while adaptive restraints according to the maximum-entropy principle were applied to satisfy the NOE distances as an average over the unbiased replica [3]. The resulting ensemble had an improved agreement with the NOE restraints, compared with the initially refined set of structures. A clustering analysis based on the ϵ RMSD - a metric which measures structural similarity by analyzing base-pairing and stacking interactions [4], rather than absolute atomic coordinates like the RMSD - yielded several conformational states, some of which had shifted base pairs with respect to the initial set of structures. Averaging over the structures within one cluster, it became evident that some NOEs are satisfied in specific clusters, while not in others, leading to the conclusion that the NOE signal represents an average over different conformations exchanging rapidly on a timescale below what can be resolved by NMR. Using our approach, the population of these excited states could be quantified, and the single conformations can be studied towards their structure-function relationship.

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86-POS Board 6

Twisting DNA

Anna Reymer^{1,2}, Krystyna Zakrzewska², Richard Lavery².

¹University of Gothenburg, Gothenburg, Sweden, ²Université Lyon 1 / CNRS UMR 5086, IBCP, Lyon, France.

Ability of DNA to dynamically change its superhelical state is central to many biological functions, including regulation of gene expression, repair, and packaging in the cell. To address conformational mechanics of DNA during supercoiling transitions we designed a new structural constraint, implemented in PLUMED free energy library environment package, which can be used in complement with standard all-atom molecular dynamics software. The constraint controls the value of total twist between any two base-pairs in a DNA molecule, while it does not restrict any other DNA helical parameter. The constraint can be applied to DNA molecules of any length and curvature, alone or in complex with other molecules. This allows for the first time to study DNA in conditions resembling its in vivo state, where DNA's topology is substantially restricted. As a proof of concept, we applied the restraint to four different linear DNA molecules, changing their superhelical density from -0.15 to +0.15, which corresponds to under- or overwinding by 5 degrees per base pair step. DNA's response to the torsional stress is discontinuous – certain dinucleotide steps are more susceptible to modifying their twist through coupled changes in the phosphodiester backbone. This allows the remaining base pair steps to stay close to a canonical B-form conformation despite the overall torsional restraint. These findings constitute a new aspect of how DNA sequence can contribute to biological regulation mechanisms.

87-POS **Board 7**

Large, Dynamic, Multi-protein Complexes - Molecular Simulations and SAXS Experiments

Bartosz Rozycki.

n/a, Warsaw, Maryland, Poland.

Many biological functions are carried out by large and dynamic protein complexes, which are built of multiple domains that are tethered together by intrinsically disordered polypeptide segments. Examples range from cell signaling to protein sorting and trafficking. Despite their importance in molecular biology, there is currently no single method which can provide information on the overall structure of such protein systems: They are not directly accessible to X-ray crystallography due to the presence of the intrinsically disordered regions (although the folded domains can be crystallized individually). They are also not accessible to NMR techniques due to their large molecular weights. In addition, their inherent flexibility make them practically inaccessible to cryoEM. Notable examples, with great potential applications in biofuel production, are cellulosomes. They are complex multi-enzyme machineries which efficiently degrade plant cell-wall polysaccharides. While many of their individual domains have been characterized structurally by crystallography and NMR methods, the overall conformations of cellulosomal components have been studied by low-resolution methods, including small angle X-ray scattering (SAXS).

A number of SAXS experiments exploring the solution structures of the cellulosomal proteins have evidenced that the intrinsically disordered linkers provide conformational flexibility which gives rise to the spatial liberty of the individual globular domains. But the static X-ray scattering methods only indirectly give access to information about conformational flexibility. We combine molecular simulations with SAXS experiments to extract additional, dynamic properties of these proteins. Using this approach, we gain information not only about the distributions of shapes and dimensions of these proteins, but also about such quantities as the probabilities of inter-domain contacts and the end-to-end distance distributions for the flexible linkers. Our results thus provide detailed pictures of the conformational ensembles of the cellulosomal proteins.

88-POS Board 8

Correlating Ion Occupation and Voltage-Dependent Selectivity Filter Gating Obtained from Functional Data of a K⁺ Channel

Oliver Rauh¹, Ulf-Peter Hansen², Gerhard Thiel¹, **Indra Schroeder**¹.

¹Technical University of Darmstadt, Darmstadt, Germany, ²Christian-Albrechts-University of Kiel, Kiel, Germany.

The conformational transition between conducting and non-conducting states (“gating”) in the selectivity filter of potassium channels is influenced by the occupation of the ion binding sites inside the filter. This has been shown by numerous functional, structural and computational studies (e.g. 1–3). However, which of the structural findings applies to which electrophysiological observation is not always clear. Here, we show that classical kinetic modelling – when based on current structural knowledge - is able to provide a bridge between structural/computational data and electrophysiology.

The viral K⁺ channel K_{CVNTS} served a model system(4). It closely resembles the pore domain of more complex K⁺ channels in structure and function and shows a fast, strongly voltage-dependent gating process at negative membrane potentials. Channels were expressed *in vitro* and reconstituted into planar lipid bilayers. Because the voltage-dependent gating process is faster than the temporal resolution of bilayer experiments, extended beta distribution analysis(5) was employed to determine the open channel current and the rate constants of gating.

From current structural knowledge, a kinetic model for the ion flux was derived and fitted to the single-channel IV curves. Specific states within the conduction cycle could be correlated with the voltage-dependent rate of channel closing of K_{CVNTS}.

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89-POS Board 9

Conformational Flexibility of Multi-Domain Proteins Determined by Pulsed EPR

Denise Schuetz^{1,3}, Sina Kazemi^{2,3}, Kniss Andreas^{2,3}, Oliver Mirus⁴, Eva M. Brouwer⁴, Peter Günert^{2,3}, Thomas Sommer^{5,6}, Enrico Schleiff⁴, Volker Dötsch^{2,3}, Thomas F. Prisner^{1,3}.
²Goethe-University, Institute of Biophysical Chemistry, Frankfurt, Hessen, Germany, ³Goethe-University, Center for Biomolecular Magnetic Resonance, Frankfurt, Hessen, Germany, ⁴Goethe-University, Dept. of Molecular Cell Biology of Plants, Frankfurt, Hessen, Germany, ⁵Max-Delbrück Center for Molecular Medicine, Berlin, Berlin, Germany, ⁶Humboldt-Universität Berlin, Institute for Biology, Berlin, Berlin, Germany. ¹Goethe-University, Institute of Physical and Theoretical Chemistry, Frankfurt, Hessen, Germany,

Pulsed Electron-Electron Double Resonance (PELDOR / DEER)[1] spectroscopy in combination with site-directed nitroxide labeling [2] is frequently used to gain distance restraints in the range of 1.8 and 6 nm. [3] The distance and flexibility of the spin labeled protein domains are encoded in the PELDOR time trace. Thereby, the intrinsic flexibility of the spin label itself could be an obstacle for structural modelling, if the flexibility of the label is large compared to the flexibility of the protein domains. Here, we present the investigation of two multi-domain proteins by the 4-pulse DEER experiment [3]. First, the N-terminal polypeptide transport-associated (POTRA) domains of anaOmp85 [4], is a rigid three domain protein giving well-defined PELDOR restraints. These restraints are used for refining the x-ray structure [5], revealing a strong impact of the spin label flexibility on the accuracy of structural refinement. Second, K48-linked diubiquitin [6], is a highly flexible two-domain protein on which the spin label flexibility is of minor impact. The recently developed 7-pulse Carr-Purcell PELDOR sequence [7] is applied to extended polyubiquitin chains to study their high intrinsic flexibility. CP-PELDOR enables to extend the PELDOR time window, thereby providing increased accuracy of the observable distance distributions.

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90-POS Board 10

Guiding Stochastic Optimization Algorithms with Experimental Data to Model Protein Energy Landscapes and Structural Transitions

Amarda Shehu.

n/a, Fairfax, USA.

Some of the most complex human disorders are driven by DNA mutations that percolate to protein dysfunction. While it is known that mutations percolate to dysfunction by changing the energy landscape and in turn the structural dynamics of a protein, quantifying changes to the landscape and dynamics of a protein in response to a mutation remains elusive. Even reconstructing the energy landscape of the healthy, wildtype form of a protein is currently out of reach. While the challenges to wet- and dry-laboratory techniques are different in nature, they all relate to the fact that the dynamics of interest, corresponding to structural transitions on the energy landscape, spans disparate spatio-temporal scales.

Recent work in our laboratory is exploiting the wealth of accumulated structural data on a protein's variants to address some of the outstanding challenges to in-silico models of equilibrium dynamics. Stochastic optimization algorithms are developed in our laboratory to build detailed, yet resource-aware maps of protein energy landscapes. These algorithms exploit experimental data to make informed algorithmic decisions such as variable selection and variation operators. The algorithms first construct unstructured, sample-based maps of a protein's energy landscape, and then enrich such maps with connectivity information to obtain the connected landscape. The latter can provide information on any structural transitions of interest, as well as yield summary statistics on dynamics. Studies on several proteins show this approach is promising and can reconstruct landscapes that currently remain beyond the reach of molecular dynamics and monte carlo-based approaches. Results on specific proteins of importance to human disorders make the case that the computed, connected landscapes advance our understanding of the role of dynamics on how mutations percolate to dysfunction and even provide directions of relevance for novel therapeutics.

91-POS Board 11

Single-Molecule Recordings of Gating Motions of KcsA Potassium Channels at Submillisecond Time Resolution

Hirofumi Shimizu.

University of Fukui, Yoshida-gun, Fukui, Japan.

Until now, structural analyses of ion-channel proteins have shown static pictures at atomic resolutions. However, these pictures had no time stamps, and their structural stabilities were obscure. In this work, we adopted the diffracted X-ray tracking method for KcsA potassium channels to show time-stamped dynamic pictures of the conformational changes in the form of a movie. Here, the channel was fixed on a glass plate at the extracellular side in an upside-down orientation, while a gold nanocrystal was attached to the cytoplasmic side as a probe. The synchrotron white X-ray beam was directed perpendicular to the sample plate as the observation light, while a diffraction spot from the attached nanocrystal was tracked through the two-dimensional X-ray detector. In this geometry, the motions of the spots were translated into those of the channels; the circumferential and radial motions indicated the twisting and bending motions of the channels, respectively. Although we previously reported the global twisting motions upon gating of the KcsA channels at video rate, the time resolution was insufficient to reveal the entire picture of the gating motions. To resolve this problem, we recently introduced an X-ray focusing mirror and a high-speed X-ray detector system to the SPring8; these components enabled us to record the motions with wide spatial range at a submillisecond time resolution. By using this refined measurement system, the gating motions were recorded continuously in real time, providing information on the stabilities of the structures in transition states upon gating. The high spatial and temporal resolutions enabled the evaluation of single molecular fluctuations and conformational changes. In this session, we will present recent our data, which is expected to provide a contribution to the integrative structural biology of potassium channels.

92-POS **Board 12**

Refining Binding Free Energies of Docked Complexes by Sampling Configurations During Molecular Dynamics Simulations

Ai Shinobu, Kazuhiro Takemura, Akio Kitao.

Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo, Japan.

Molecular docking is a widely used computational tool for predicting protein-protein complex structures. Evaluating relative energies of generated complex models is a crucial step in the process of selecting near-native structures. A method previously developed in our group, evaluates the binding free energy of a complex (ΔG_C) as the sum of its conformational energy and solvation free energy, the latter is calculated using the energy representation method (the ERMOD method) which requires short molecular dynamics (MD) simulations of the initial and final states. Using this method, ΔG_C values were calculated for a series of docked complex models, producing lower values for models that resemble the crystal structure. This shows that including an explicit solvation term is important when evaluating energies of complexes. In the current study, we aim to refine relative ΔG_C values for a set of protein-protein complex models by subjecting them to all-atom MD simulations in explicit water. Multiple configurations produced by the simulations are carefully selected and used as new configurations for which ΔG_C values are recalculated. Our results show that solvation free energies decrease for the configurations collected during MD, the conformational energies increase, to a lesser degree, resulting in an overall decrease in ΔG_C . This suggests that proper hydration of the system achieved by MD simulations is a crucial step for generating native-like complex structures. Moreover, the calculated ΔG_C values are lower for MD configurations which are closer to the crystal structure, making this procedure useful for selecting the native-like configurations.

93-POS **Board 13**

Mechanistic Insights into Modulation of Amyloid Pathways by DNA Intercalators

Jasdeep Singh, Bishwajit Kundu.

Indian Institute of Technology Delhi, New Delhi, New Delhi, India.

Amyloid structures share a sequence independent core consisting of a cross- β spine stabilized through inter-molecular H-bonding networks, with their initial assembly derived by hydrophobic and aromatic interactions. Co-operative balance of similar interactions also confer stability and integrity to DNA duplexes present in living systems. Structural perturbations of both assemblies, by planar molecules or intercalators rely on their ability to interfere with this balance. Although several reports on small molecule based amyloid intervention exist, perturbation of amyloids by planar, DNA intercalating moieties have not been studied yet. The present work investigates detailed mechanism(s) of these hetero-molecular interaction that may modulate amyloid assembly by disturbing the aforesaid interacting forces. Herein, we employed four different DNA intercalators to understand if their non-native hetero-molecular associations could modulate amyloid forming pathways. Through microsecond scale simulations, we show that each molecule individually is capable of interfering with native aggregation landscape of a steric zipper from diabetes associated amyloid precursor protein (hIAPP). Further, the simulation estimates were experimentally tested and validated with other disease associated amyloid systems including gelsolin, prion and lysozyme. Experimental verification using spectroscopic studies and electron microscopy showed that intercalators indeed stabilize monomeric and prefibrillar assemblies, reducing their ability to transform into structured supra-molecular amyloids. Our results conclusively establish dominant role of aromatic associations in diverting course of amyloid assembly process at the expense of stabilizing H-bond networks. Overall, our study provides comprehensive theoretical and experimental insights that would pave ways for designing newer anti-amyloid therapeutics.

94-POS Board 14

C-edge Loops of Arrestin Function as a Membrane Anchor

Ciara Lally¹, Brian Bauer¹, Jana Selent², **Martha Sommer**¹.

¹Charité Medical University, Berlin, Germany, ²Pompeu Fabra University, Hospital del Mar Medical Research Institute, Barcelona, Spain.

Over 800 different G-protein-coupled receptors (GPCRs) are present in the human body and regulate a wide variety of sensory and physiological responses. Signalling of these membrane proteins is attenuated by a two-step mechanism entailing receptor phosphorylation by a kinase followed by receptor binding by the protein arrestin. During formation of the arrestin–receptor complex, arrestin interacts with the phosphorylated receptor C terminus in a pre-complex, which activates arrestin for tight receptor binding. Although the first crystal structure of a GPCR–arrestin complex was recently published [1], the structure of the pre-complex and how it transitions to a high-affinity complex is poorly understood. Here we present molecular dynamics simulations and site-directed fluorescence experiments on arrestin-1 interactions with the GPCR rhodopsin, showing that loops within the C-edge of arrestin function as a membrane anchor. Activation of arrestin by receptor-attached phosphates is necessary for C-edge engagement of the membrane, and we show that these interactions are distinct in the pre-complex and high-affinity complex in regard to their conformation and orientation. Our results expand current knowledge of C-edge structure and further illuminate the conformational transitions that occur in arrestin along the pathway to tight receptor binding [2].

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95-POS Board 15

MHC Class II Complexes Sample Intermediate States Along the Peptide Exchange Pathway

Sebastian Stolzenberg², Marek Wieczorek¹, Jana Sticht¹, Sebastian Günther^{1,3}, Christoph Wehmeyer², Zeina El Habre¹, Miguel Álvaro-Benito¹, Frank Noé², Christian Freund¹.

²Computational Molecular Biology Group, Institute for Mathematics, Freie Universität Berlin, Berlin, Berlin, Germany, ¹Protein Biochemistry, Institute for Chemistry and Biochemistry, Freie Universität Berlin, Berlin, Berlin, Germany, ³Institute of Human Virology, University of Maryland School of Medicine, Baltimore, MD, USA.

The presentation of peptide-MHCII complexes (pMHCII) for surveillance by T cells is a well-known immunological concept in vertebrates, yet the conformational dynamics of antigen exchange remain elusive. By combining NMR-detected H/D exchange with Markov modelling analysis of an aggregate of 275 microseconds molecular dynamics simulations, we reveal that a stable pMHCII spontaneously samples intermediate conformations relevant for peptide exchange. More specifically, we observe two major peptide exchange pathways: the kinetic stability of a pMHCII's ground state defines its propensity for intrinsic peptide exchange, while the population of a rare, intermediate conformation correlates with the propensity of the HLA-DM-catalysed pathway. Helix-destabilizing mutants designed based on our model shift the exchange behaviour towards the HLA-DM-catalysed pathway and further allow us to conceptualize how allelic variation can shape an individual's MHC restricted immune response.

96-POS **Board 16**

Coarse Grained Monte Carlo Simulations of Polyelectrolyte-nanoparticle Complexation

Morten Stornes, Binamra Shrestha, Rita S. Dias.

NTNU - Norwegian University of Science and Technology, Trondheim, Norway.

The formation of complexes between polyelectrolytes (PEs), and other charged macroions have received much attention in recent years. Studying these systems are important to understand e.g. DNA condensation and protein-membrane interactions, and PE-nanoparticle complexes have potential applications in gene and drug delivery systems.

Of special interest are weak (annealed) PEs which, contrary to strong (quenched) polyelectrolytes, have a pH dependent ionization. In addition, annealed PEs exhibit charge mobility, where protons are mobile along the chain, giving rise to non-uniform charge profiles and charge patches. This charge mobility has previously been shown to increase the binding between oppositely charged species, such as DNA or protein adsorbing on membranes.

We have studied the complexation of one and two charged nanoparticles with multiple PE chains of opposite charge, to better understand the influence of chain concentration and length on the PE ionization with increasing pH, the effect chain ionization has on bridging between nanoparticles and the effect of charge mobility on the PE-nanoparticle adsorption. This has been done using Monte Carlo simulations. Rather than using more detailed all-atomic models or molecular dynamics simulations, the simpler coarse grained Monte Carlo approach allows us to focus on the importance of the electrostatic interaction between constituents without large computational costs.

It is found that the PE ionization decreases with increasing concentration relative to the nanoparticle, and the combination of chain length and concentration can significantly influence the ionization. Contrary to previous studies, we also find that quenched PEs adsorb better than annealed PEs on the nanoparticle at similar degrees of ionization. It is suggested that this is due to the difference in PE stiffness compared to other studies, and the formation of PE loops.

97-POS **Board 17**

Preferred Conformational States of Retroviral Capsid Protein Visualized

Chun Tang, Wen-Xue Jiang, Xu Dong, Zhou Gong, Zhu Liu.
Chinese Academy of Sciences, Wuhan, Hubei, China.

Discovered back in 1901, the Rous sarcoma virus (RSV) is a archetypal retrovirus. Several thousand copies of the RSV capsid (CA) protein interact with each other to form a nanoparticle with a variety of morphologies. A RSV CA comprises two domains, namely the N-terminal domain (NTD) and the C-terminal domain CTD, which are connected by a short linker. The previous cryoEM study has indicated that in the final CA assembly, specific interactions exist between the NTD and the CTD and between the CTD and the CTD from adjacent CA molecules. On the other hand, the previous NMR study has shown that the NTD and the CTD tumble independently in solution. To assess whether there is already any preference for the orientation between the two CA domains and to understand how CA molecules assemble to form an enclosed capsid, we carried out the study on the structure and dynamics of RSV CA protein, with conjoined use of single-molecule fluorescence resonance energy transfer (smFRET), small angle X-ray scattering, and NMR lanthanide pseudo-contact shift (PCS), which I will present at this meeting. We found that an RSV CA protein can exist in two alternative conformational states with the domains arranged differently. We call them the “up” and the “down” conformational states. Significantly, the “down” conformation can be dissipated at increasing concentration of the CA protein, while the “up” conformation closely resembles the CA structure found in RSV capsid assembly by cryoEM. As such, the preferred quaternary arrangements of the CA protein likely dictate the final outcome of the assembly product.

98-POS Board 18

Partial Folding of Intrinsically Disordered Plant LEA Proteins is Required for Membrane Binding and Stabilization

Anja Thalhammer¹, Anne Bremer², Carlos Navarro-Retamal³, Gary Bryant⁴, Wendy González³, Dirk K. Hincha².

¹University of Potsdam, Potsdam, Germany, ²Max-Planck Institute of Molecular Plant Physiology, Potsdam, Germany, ³Universidad de Talca, Talca, Chile, ⁴RMIT University, Melbourne, Australia.

Late embryogenesis abundant (LEA) proteins accumulate in seeds and vegetative plant tissues, especially after exposure to abiotic stresses and in desiccation tolerant bacteria and invertebrates. Their expression is directly linked to cellular dehydration as arising during freezing or desiccation. Most LEA proteins are intrinsically disordered under fully hydrated conditions and fold during drying. We focus on two cold-induced *Arabidopsis thaliana* LEA proteins, COR15A and COR15B. Functionally redundant, COR15A and COR15B stabilize membranes during freezing *in vitro* and *in vivo* while they do not stabilize selected enzymes during freezing *in vivo*. Both proteins are disordered in solution, but fold into amphipathic α -helices in the dry state, as shown by circular dichroism (CD) and fourier-transform infrared (FTIR) spectroscopy and *in silico* analysis. The unfolding process of both COR15 proteins after transfer to water was modeled by Molecular Dynamics simulations, using homology and threading modelling approaches and showed quantitative agreement with experimental data. In water, unfolding was driven by a break of intramolecular and concomitant formation of protein-water H-bonds. We used glycerol as a low-molecular weight crowding agent to model reduced cellular water availability. Experimentally, we found a concentration dependent gain of α -helical structure in solutions containing glycerol. Unfolding of COR15A and COR15B as assessed by Molecular Dynamics simulations was reduced in glycerol-containing systems, indicating that structural stabilization can be explained by preferential exclusion of glycerol from the protein backbone. FTIR spectroscopy, X-ray diffraction and Molecular Dynamics simulations further revealed that COR15A associates with artificial membranes exclusively in an at least partially folded state. Overall, our findings indicate an initial dehydration-induced folding step is necessary to render the COR15 proteins competent for membrane interaction. A second folding step takes place during membrane association.

99-POS Board 19

MDSrv: Viewing and Sharing Molecular Dynamics Simulations on the Web

Alexander S. Rose^{2,3}, **Johanna K. Tiemann**^{1,3}, Peter W. Hildebrand^{1,3}.

¹Institute of Medical Physics and Biophysics, Leipzig, Germany, ²San Diego Supercomputer Center, San Diego, CA, USA, ³Institute of Medical Physics and Biophysics, Berlin, Germany.

Molecular dynamics (MD) simulations are these days an established and widely used tool to investigate the time resolved motions of biological macromolecules. Sharing of the resulting coordinate trajectories for visualization is complicated by their size, the requirement of having special software set-up and the knowledge of the software usage. However, as research groups and collaborations become increasingly interdisciplinary, it is desirable to make sharing of MD trajectories easier to facilitate discussions and further analyses. In contribution, we present MDSrv, a tool to serve trajectories of molecular coordinates and visualize them in a web browser by employing the NGL Viewer [1]. It supports trajectories formats from different MD software including *xtc/trr* (GROMACS), *nc/netcdf* (AMBER) or *dcd* (NAMD) files. The trajectories can be served within a local network to co-workers or over the Internet to colleagues all over the world. For interactive viewing of the MD simulations only a web browser is needed and no installation of any MD-specific software is required. The trajectories and the NGL Viewer web application are served by a Python module readily installable from PyPI that can be run locally or deployed to a web-server. Together, the client and the server component make data from MD simulations accessible to a wide audience of researchers and students. MDSrv promotes concepts for publishing MD data along with publications of their analysis similar to classical structural biology data published in the PDB.

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100-POS Board 20

PolyQ Tracts as Efficient C-capping Elements for Coiled-coils

Busra Topal¹, Albert Escobedo¹, Jesús García¹, Oscar Reina¹, Camille Stephan-Otto Attolini¹, Xavier Salvatella^{1,2}.

¹Institute for Research in Biomedicine (IRB Barcelona), Barcelona, Spain, ²Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.

Poly-glutamine (polyQ) tract expansions have been linked to a variety of neurodegenerative diseases. The conservation of such sequences points to a relevant role, which is suggested to involve their organization into secondary structure elements. For the particular case of the androgen receptor (AR) we recently reported that the Leu-rich segment N-terminal to the polyQ tract acts as a helical N-capping sequence that propagates helicity into the tract itself [1]. Based on that, we have acquired in vitro CD and NMR as well as in silico MD data on a battery of peptides showing that the helicity of the sequence positively correlates with the number of glutamines in the tract up to the values found in the average human population (16-25 residues, depending on ethnicity), and that helix stabilization depends on glutamine sidechain-mediated hydrogen bonds. This supports a C-capping role for the polyQ tract, as a minimum number of glutamine residues is required to stabilize the helicity while further growth of the tract is detrimental because of increased aggregation rates. Proteome analysis shows that regions predicted to fold into coiled-coils are highly enriched in adjacent sequences N-terminal to polyQ tracts, thus providing the grounds for a general role of such tracts as C-caps for these helical elements.

[1] Eftekharzadeh et al. Sequence Context Influences the Structure and Aggregation Behavior of a PolyQ Tract. *Biophys J.* 2016 Jun 7;110(11):2361-6.

101-POS Board 21

Conformational Ensembles of α -synuclein in the Different Conformational States Studied by DNP-Enhanced NMR at Low Temperature

Boran Uluca^{1,2}, Hamed Shaykhalishahi^{1,2}, Dusan Petrovic¹, Thibault Viennet^{1,2}, Franziska Weirich^{1,2}, Aysenur Guenelalan¹, Birgit Strodel^{1,2}, Manuel Eitzkorn^{1,2}, Wolfgang Hoyer^{1,2}, Henrike Heise^{1,2}.

¹Forschungszentrum, Jülich, Germany, ²Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany.

Dynamic Nuclear Polarization (DNP) overcomes the inherently low sensitivity of magnetic resonance methods by transferring high spin polarization of unpaired electrons to surrounding nuclei. Low-temperature Nuclear Magnetic Resonance (NMR) spectra usually suffer from severe line broadenings due to freezing out different conformations [1]. While this is usually accounted for as an unwanted side-effect of DNP-NMR, these inhomogeneously broadened lines also contain valuable information about conformational ensembles of (disordered) proteins.

To study conformational ensembles of intrinsically disordered proteins, both experimental and computational methods have evolved. For the experiments reported here, we have chosen α -synuclein as a model protein and the large-scale conformational flexibility is investigated both DNP-Enhanced NMR and by a molecular dynamics (MD) simulations. We have studied the conformational ensemble of α -synuclein in frozen solution under different conditions: in the fully disordered form, in the fibrillated form with flexible ends, and in contact with lipid bilayers in the form of nanodiscs (in different ratio protein/nanodisc). We could probe the conformational ensembles of all valine residues in α -synuclein by selectively labeling the sample with [2-¹³C]-glucose [2].

102-POS Board 22

The Role of Dynamic Conformational Ensembles in Base Excision Repair and Triplet Repeat DNA Expansion

Jens Völker¹, Vera Gindikin¹, G. Eric Plum², Kenneth J. Breslauer¹.

¹Rutgers, The State University of New Jersey, Piscataway, NJ, USA, ²IBET, Inc, Columbus, OH, USA.

Trinucleotide repeat DNA sequences of type (CNG)_n can undergo uncontrolled expansion resulting in debilitating neurological diseases such as Huntington's and Myotonic dystrophy type I. DNA expansion appears inevitable when the repeat length exceeds a threshold value, $n \sim 35$. It has been postulated that uncontrolled expansion occurs as a consequence of the propensity of large repeat DNA sequences to adopt alternative single strand structures, such as bulge loops, in competition with normal duplex DNA, thereby leading to aberrant metabolic processes during DNA replication, recombination, and repair. Using a combination of calorimetric and spectroscopic techniques in conjunction with modeling, we present evidence that a bulge loop structure forming within the confines of larger repeat domains can occupy multiple, nearly isoenergetic loop positions, called rollamers, thereby creating an ensemble of metastable loop isomers in dynamic exchange. Such dynamic ensembles of bulge loops provide an intriguing thermodynamic basis for the unexplained threshold phenomenon associated with expansion of DNA repeat domains. We further demonstrate how a common mutagenic, oxidative DNA lesion (8oxodG) and its base excision repair (BER) intermediate (abasic site), both implicated in repeat DNA expansion in mouse models of Huntington's disease, each alter the rollamer ensemble. Our prior studies on static, lesion-containing repeat bulge loops revealed the preferred lesion containing repeat loop isomers to be poor substrates for APE1, a key BER enzyme. Based on these collective observations, we postulate that the impact of DNA damage on ensemble distribution and dynamics of DNA bulge loops is a significant factor in aberrant repair outcomes leading to DNA expansion, and ultimately disease states. Our results emphasize the importance of considering dynamic conformational ensembles in DNA repair outcomes, thereby providing a biophysical basis for an observed biological outcome.

103-POS Board 23

Linking Kinetics and Thermodynamics of Biomolecular Conformational Transformations and Ligand Binding

Yong Wang, Joao M. Martins, Kresten Lindorff-Larsen.
University of Copenhagen, Copenhagen, Denmark.

The accurate calculation of thermodynamics and kinetics in biomolecular conformational transformations and ligand binding is a problem of critical importance but tremendous challenges in computational biology and computer-aided drug design. Instead of pursuing a one-shot solution, for example by long equilibrium molecular dynamics simulations, one usually adopts a divide-and-conquer strategy by which the binding free energy (ΔG) is calculated by enhanced sampling methods or alchemical methods, while the binding rate (k_{on}) is obtained from binding events with high ligand concentrations. From known ΔG and k_{on} , the unbinding rate k_{off} can be estimated analytically. In this work, we seek the possibility to address the thermodynamics-kinetics problem through a novel route by which the kinetics (both k_{on} and k_{off}) is calculated first and subsequently is used to estimate ΔG . By taking a simple two-state and a four-state model system as examples, we show that such 'kinetic' ΔG can reach promising consistence with thermodynamic ΔG obtained from free energy profiles with a mean absolute error of 0.6 kcal/mol. The feasibility is further supported by the application on the binding of a cavity mutant of T4 lysozyme with benzene in which $\Delta G_{binding}$ values from kinetics, free energy perturbation method and experiments are all in good agreement. The approach both sheds light on the accuracy of methods for calculating kinetics and further provides a generally useful test for the internal consistency of kinetics and thermodynamics. We also expect it to be useful for estimating thermodynamic properties in cases where equilibrium sampling or alchemical methods are difficult to apply, for example in the case of conformational exchange.

104-POS Board 24

The Connections Between Coherent Fluctuations and Native Structures of Proteins

Wang Jun^{1,2,3}, Qian-Yuan Tang¹, Wei Wang^{1,2,3}.

¹Nanjing University, Nanjing, Jiangsu, China, ²National Lab of Solid State Microstructure, Nanjing, Jiangsu, China, ³Collaborative Innovation Center of Advanced Microstructures, Nanjing, Jiangsu, China.

Recently, it is observed that the structural fluctuations of globular proteins exhibit long-range correlations which resemble the behavior around critical point. This kind of dynamic behaviors are universal for all globular proteins. What are the physical sources of such kind of behaviors? Are there sequential or structural signatures for this kind of dynamic behaviors? The connections between structure (sequence) and the fluctuation dynamics may help to understand the characteristics and evolution of natural proteins. In present work, the coherent fluctuations and the features of landscapes are connected based on a physical consideration. To exemplify this kind of connection, the fluctuations of proteins are analyzed with elastic network models. It is found that the proteins also exhibit highly correlated fluctuations starting from only the native conformations determined through X-ray diffraction. The scale-free behavior is also true for the B-factor in X-ray experiment. This clearly demonstrates the connection between coherent fluctuation and the features of vibrational spectrums of proteins. Some structural analysis demonstrate that the coherent fluctuations may comes from some special features of structure and interactions. These results are consistent with previous simulations and bioinformatics studies, and may help to understand the design principle of natural proteins.

105-POS Board 25

Critical Behaviors of Structural Fluctuations in the Native States of Proteins

Wang Wei^{1,2,3}, Qian-Yuan Tang¹, Yang-Yang Zhang¹, Jun Wang^{1,2,3}, Dante Chialvo⁴.

¹Nanjing University, Nanjing, China, ²National Lab of Solid State Microstructure, Nanjing, China, ³Collaborative Innovation Center of Advanced Microstructures, Nanjing, China,

⁴Universidad Nacional de San Martin, Buenos Aires, Argentina.

The three-dimensional folded structures of proteins, known as native states, make proteins capable of performing related biological functions. To achieve such performance, the structure of the native state of a protein must be susceptible enough to sense the signal and switch to another structure, but also be stable enough to warrant functional specificity and structural robustness. This means a coexistence of high susceptibility and stability for the protein around its native state, which is apparently competing since high susceptibility implies large fluctuations and thus small stability in general, and vice versa. Does the balance of such competition result in a certain kind of critical behavior in proteins? Based on protein structural ensembles determined by NMR, we study the position fluctuations of residues by calculating distance-dependent correlations and conducting finite-size scaling analysis. The fluctuations exhibit high susceptibility and long-range correlations up to the protein sizes. The scaling relations between the correlations or susceptibility and protein sizes resemble those in other physical and biological systems near their critical points. These results indicate that, at the native states, motions of each residue are felt by every other one in the protein. We also find that proteins with larger susceptibility are more frequently observed in nature. Overall, our results suggest that the protein's native state is critical.

106-POS Board 26

Integration of SAXS Data into Biomolecular Simulations

Marie Weiel, Ines Reinartz, Alexander Schug.
Karlsruhe Institute of Technology, Karlsruhe, Germany.

The cardinal aim of structural analyses in molecular biology and biophysics is to reveal an interrelation between macromolecular structure and conformational changes on the one hand and function of biological macromolecules on the other hand. Biological small angle X-ray scattering (SAXS) is an experimental technique for structural characterization of macromolecules and complementary to common high-resolution methods such as X-ray crystallography and NMR spectroscopy. To date, SAXS data are often interpreted by ambiguous reconstruction of low-resolution three-dimensional models from one-dimensional scattering intensities or assembly of rigid high-resolution elements. However, with large structural rearrangements being involved, these methods do not yield satisfying results. We include the limited information from SAXS into molecular dynamics (MD) simulations using native structure-based models (SBM). A particular initial structure, e.g. from X-ray or NMR methods, is defined as the global energetic minimum in a minimally frustrated single-basin energy landscape dominated by native interactions. The resulting description in terms of a smooth energy funnel can provide rich information about complex processes and is computationally efficient. In order to incorporate information from SAXS, a bias term is added to the SBM potential so that conformations reproducing the experimental target data are energetically favoured. In this vein, SAXS data may be reasonably interpreted whilst simultaneously retaining chemical knowledge and sampling power of molecular force fields. Running SAXS-guided MD simulations of a protein in some known initial configuration, one can obtain a well-grounded atomistic structure of the protein in another conformation corresponding to the experimental data by dynamically fitting the starting model to the SAXS intensity. Giving fast and reliable structure predictions for transiently populated conformations and related conformational changes, we hope to make a significant contribution to unraveling the relation between macromolecular structure and function.

107-POS Board 27

Combining Small Angle X-ray Scattering Experiments with Accelerated Molecular Dynamics Simulations to Determine the Conformational Ensemble of Tri-ubiquitin Chains

Jeff Wereszczynski.

Illinois Institute of Technology, Chicago, IL, USA.

Small angle X-ray scattering (SAXS) has become an increasingly popular structural technique for characterizing the ensemble of solution states of flexible biomolecules. However, data resulting from SAXS is typically noisy and low-dimensional and may therefore be difficult to interpret without additional structural knowledge. In principle, this information can be provided by molecular dynamics (MD) simulation, but conventional MD trajectories rarely sample sufficient phase space to probe the range of structures that contribute to the observed experimental data. Accelerated MD (aMD) can overcome these sampling inadequacies by introducing a bias to the underlying energy landscape that lowers the height of energy barriers and encourages conformational transitions, albeit at the cost of distorting the Boltzmann distribution of states. Here, we present a method for combining the results of aMD simulations with experimental SAXS data to accurately model the relative populations of representative solution states. Scattering states are first identified from aMD trajectories, and their populations are then re-weighted against empirical data through a Bayesian Monte Carlo approach. Special care is taken to avoid ensemble over-fitting by iteratively considering increasing subsets of scattering states along with the associated Akaike Information Criterion, and by reducing experimental data to the Shannon sampling limit. We apply this technique to several ubiquitin trimers and find that aMD trajectories typically outperform conventional MD simulations in both goodness-of-fit and model convergence speed. Furthermore, we observe that different ubiquitin linkages yield distinct ensembles, which points to their unique roles in biological signaling. These methods are being implemented in the “SASSIE” webserver, which aims to provide an easy-to-use modeling interface for interpreting data from scattering experiments.

108-POS Board 28

Should I Stay Or Should I Go – Integrating Molecular Dynamics Simulations and Biochemical Data Provides Insight into the Structural Basis of Biomolecular Decision Making

Hans-Joachim Wieden^{1,2}, Dylan Girodat^{1,2}.

¹University of Lethbridge, Lethbridge, AB, Canada, ²Alberta RNA Research and Training Institute, Lethbridge, AB, Canada.

Gene expression strongly relies on the rapid and accurate responses of a large number of protein and ribonucleoprotein (RNP) complexes to a variety of inputs. Two examples for this are the RNA processing machinery and ribosome-dependent protein synthesis. Their critical role for survival of bacterial cells makes these processes promising targets for antibiotics. On this background, understanding the structural dynamics of the involved proteins and RNP complexes is pivotal for the development of novel antibiotic strategies that utilize the modulation of their structural dynamics. Here we report data from our recent work on RNaseE (1) and Elongation Factor Tu (2,3) using an approach that combines Molecular Dynamics (MD) simulations with experimental methods that provide mechanistic information derived from detailed kinetic studies using rapid-kinetics (stopped-flow), high-throughput biochemical assays, and next generation sequencing. Our findings explain how in RNaseE conformational dynamics contributes to the selection of the cleavage site two nucleotides downstream of a uracil (U₊₂), as well as to triggering its catalytic activity. Furthermore, we demonstrate how structural dynamics is utilized by P-loop GTPases such as Elongation Factor Tu to facilitate nucleotide selection and to fine-tune nucleotide binding properties. In line with the role that structural and conformational dynamics plays for the function of translational GTPases, we also have investigated, by developing computational methods that combine MD simulations with rapid kinetics data, how the conformational ensemble distribution targeted by different types of ligands can provide opportunities to modulate the functional characteristics of the respective biomolecular complex.

(1) Chao, Y., et al. (2016) *Mol. Cell* 65(1): 39-51.

(2) De Laurentiis, E.I., (2016) *J. Biol. Chem.* 291: 23136-48.

(3) Mercier, E., et al. (2015) *Sci. Rep.* 5: 7677.

109-POS Board 29

Conformational Dynamics of Histone Lysine Methyltransferases by Millisecond-timescale Molecular Dynamics on Folding@home

Rafal P. Wiewiora^{1,2}, Shi Chen^{3,2}, Minkui Luo³, John D. Chodera¹.

¹Computational and Systems Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA, ²Tri-Institutional PhD Program in Chemical Biology, Weill Cornell Medicine, New York, NY, USA, ³Chemical Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA.

Epigenetic regulation is essential for eukaryotic organisms in processes spanning from embryo development to longevity. Histone lysine methyltransferases (HKMTs) are amongst the key players that control these processes. HKMT dysregulation via mutation or altered expression has been implicated in many cancers' initiation, maintenance, aggressiveness and metastasis. Furthermore, roles of HKMTs in aging and drug addiction have been shown in animal models. Development of selective inhibitors for many members of this protein family remains an unmet need. Conformational dynamics have been observed or proposed at both cofactor- and substrate-binding sites of most HKMTs; this structural plasticity has a crucial impact on the shapes and druggabilities of pockets in HKMTs and on inhibitor design.

Here we present multiple-millisecond aggregate timescale Molecular Dynamics simulations, collected on Folding@home, for the SETD8 methyltransferase. Hypotheses for the dynamics within the catalytic cycle of SETD8, based on the available and two new crystal structures, were tested. In addition to apo simulations started from all crystal structures, hypothetical 'chimeric' homology models (assembled from domains of the protein from multiple crystal structures) were constructed and propagated in simulations; moreover a whole-catalytic-cycle set of simulations, comprising all possible combinations of the co-factor SAM, by-product SAH and histone H4 peptide, were conducted. Here we present Markov State Models of the conformational landscapes of multiple catalytic cycle states of SETD8, based on ~6 ms aggregate simulation time. Furthermore, planned verification of the computational results via biochemical experiments is presented.

110-POS Board 30

Computational Studies of Co-translational Protein Folding and Misfolding on the Ribosome

Tomasz Wlodarski^{1,2}, Lisa D. Cabrita², Christopher A. Waudby², Anaïs M. Cassaignau², Annika Deckert³, Xiaolin Wang⁴, Abid Javed², Michele Vendruscolo¹, John Christodoulou².

¹University of Cambridge, Cambridge, United Kingdom, ²University College London, London, United Kingdom, ³Max Delbrück Center for Molecular Medicine, Berlin, Germany, ⁴Max Planck Institute for Biophysical Chemistry, Göttingen, Germany.

How a protein acquires its biologically active structure is a key biological question, since folding is closely linked to a wide range of cellular processes, and where aberrant folding can result in aggregation, a process that have been strongly implicated in several human disorders, including Alzheimer's and Parkinson's diseases. Currently, there is very little understanding of the differences between folding that occurs on the ribosome relative to that of isolated proteins, as there are very few high-resolution structural and dynamical studies detailing the process of co-translational folding.

In order to change this picture I have been using bioinformatics, structure-based models and all-atom molecular dynamics simulation, where I combine enhanced sampling methods (metadynamics) with structural restraints from various NMR and CryoEM experiments to study two model ribosomal nascent chain (RNC) systems: (a) the immunoglobulin-like domain and (b) alpha-synuclein. First system provides understanding of the folded-unfolded transition in a single globular domain, whereas second is offering unique insights into the capacity for an unfolded nascent chain (the first state visited by all nascent chains) to sample conformational space, as well as understand the role of the ribosomal surface as a protective strategy to guard against misfolding. These models offer the unique capacity to for the first time comprehensively characterise both structure and dynamics during co-translational protein folding in an unprecedented level of atomistic detail.

111-POS Board 31

Improved GPCR Loop Structure Prediction by *Ab Initio* and Template-Based Sampling with Triaxial Loop Closure

Jonghun Won, Gyu Rie Lee, Chaok Seok.
Seoul National University, Seoul, South Korea.

Extracellular loops (ECLs), especially the second extracellular loop (ECL2), of G-protein-coupled receptors (GPCRs) are often involved in GPCR functions. However, structure prediction of ECL2 is more difficult than that of globular protein loops because of its long length. Accordingly, several researchers have reported *ab initio* methods specialized for ECLs. In this research, a new ECL2 structure prediction method that uses both *ab initio* and template-based sampling is developed. Noting that ECL2 structures of related GPCRs are similar, adequate structural templates are first detected among GPCRs with experimentally resolved structures. The template loop structures are then attached to the framework structure and the loops are closed by triaxial loop closure, an analytical loop closure method. *Ab initio* loop sampling is also conducted using fragment assembly and triaxial loop closure. The proper geometry of the conserved disulfide bridge between the third transmembrane helix and ECL2 is maintained during sampling by applying triaxial loop closure. Final model structures are ranked and selected by the GALAXY energy. Performance of this method was tested on ECLs of 28 GPCR subtypes with available experimental structures. The method showed outstanding accuracy compared to other available *ab initio* methods such as CABS. This method can provide an accurate loop structure or an ensemble of structures that may be used for further experimental or computational studies related to GPCR function or molecular design targeting GPCR.

112-POS Board 32

Experimental Characterization of "Metamorphic" Proteins Predicted from an Ensemble-Based Thermodynamic Description

James Wrabl^{1,2}, Jordan Hoffmann^{1,3}, Mark Sowers^{2,4}, Vincent Hilser^{1,2}.

²Johns Hopkins University, Baltimore, MD, USA, ¹Johns Hopkins University, Baltimore, MD, USA, ³Harvard University, Boston, MA, USA, ⁴University of Texas Medical Branch, Galveston, TX, USA.

The emerging biological phenomenon of "metamorphic" proteins, single amino acid sequences that adopt two physiologically distinct structures and functions, challenges current prediction methods largely reliant on sequence similarity. To address this problem, we develop an innovative metric for sequence-structure compatibility, using energetic information derived from an experimentally validated ensemble-based description of protein thermodynamics. The simulated ensemble's unique information, *i.e.* the locations of high and low stability, enthalpy, and entropy regions within a protein, is reduced to an eight-symbol code that permits efficient scoring of any structure against any amino acid sequence. Ensemble-based information from both native and denatured states is incorporated, with separate calibration of Gaussian probability models for background scores in each state. High-identity sequences, previously demonstrated *in vitro* to adopt either *Streptococcus* protein G_A or G_B folds, were correctly recapitulated, demonstrating that this ensemble-based compatibility metric indeed reflected the energetic determinants of fold. To further test this model, ten arbitrarily chosen uncharacterized members of the high-identity sequence space were expressed and purified; nine were found to be consistent with their predicted folds as assessed by circular dichroism spectroscopy. Several additional designed proteins, each containing a single Glycine mutation, appear to enable a fold switch between the G_A and G_B conformational ensembles. Complete biophysical characterizations and structure determinations are underway to confirm these conclusions. Since this ensemble-based scoring framework is applicable to any desired fold, it may be practically useful for the future targeted design, or large-scale proteomic detection, of novel metamorphic proteins.

113-POS Board 33

**Cyanylated Cysteine as an Infrared Reporter of Calmodulin-Ligand Interactions:
Experimental Measurements, Molecular Dynamics Simulations and Multi-level
Calculations of IR Lineshape**

Rosalind J. Xu, Casey H. Londergan.
Haverford College, Haverford, PA, USA.

The structure and dynamic conformational ensembles of calmodulin (CaM), with and without Ca^{2+} and a 26-residue synthetic peptide (M13) comprising the binding domain of skeletal muscle myosin light chain kinase, were studied by inserting an artificial SCN probe group at different sites along both the CaM and M13 chains. The IR CN stretching absorption frequency of the probe group displays solvatochromic shifts due to different polarity and hydrogen bonding environments. Comparisons between CN stretching frequencies of each mutant in the different binding states of the CaM/M13 complex showed varying changes in degree of probe solvent exposure upon binding. To gain a more quantitative understanding of IR frequencies and lineshapes at each mutated site, all-atom molecular dynamics (MD) simulations including the artificial probe group were performed using a modified AMBER99SB forcefield, and solvent-accessible surface areas of SCN probe at each mutated site were calculated. Two methods were attempted for IR lineshape simulation: a QM/MM method adapted from Layfield and Hammes-Schiffer and a solvatochromic effective fragment potential (SolEFP) method adapted from Blasiak and Cho. Despite challenges in a mature and direct theory-experiment connection, we have nevertheless determined some new fine-grained details about the structural ensembles of CaM under different conditions and we also have constructed a dual experimental/simulation methodology that could be applied to various protein systems.

114-POS Board 34

Protein Evolution Under a Computational Microscope

Huafeng Xu¹, David E. Shaw¹,

¹D. E. Shaw Research, New York, NY, USA, ²Columbia University, New York, NY, USA.

Protein evolution is often driven by changes in the amino acid sequence that alter the affinity and specificity of the protein for its binding partners. Mutations that do not appear to affect the specific interactions between the protein and its binding partner can have a surprisingly large effect on the binding affinity. We have used long-timescale molecular dynamics (MD) simulations to study how such mutations affect the binding affinities in two examples of protein evolution: 1) the affinity maturation of a broadly neutralizing antibody lineage against influenza hemagglutinin, in which two divergent maturation pathways have led to mature antibodies that potentially neutralize a broad range of H1 influenza viruses, and 2) the adaptation of influenza hemagglutinin, in which hemagglutinins of avian strains have accrued mutations that favor binding to human receptors over avian receptors (a requirement for human transmission). In the affinity maturation study, our simulations contributed to the finding that the affinity increase in the mature antibodies was primarily attributable to the stabilization of the CDR H3 loop in the antigen-binding conformation. In the hemagglutinin adaptation study, our simulations suggested that the human receptor adopts a dynamic ensemble of diverse conformations in binding to hemagglutinin, including a novel conformation not yet seen in crystallography. We identified mutations in an avian hemagglutinin that we predicted would favor the formation of new specific interactions with the human receptor in the novel binding conformation, and then experimentally verified increased affinity of the mutant hemagglutinin for the human receptor. Results from these studies suggest that conformations generated by MD simulations, including some which have not been previously identified by experimental structural determination, may help unravel the structural mechanism underlying the evolution of proteins, and serve as starting points for engineering biomolecular complexes with enhanced affinity and specificity.

115-POS Board 35

Modeling Structural Properties and Thermostability of the Active Conformation of Lipase from *Geobacillus Thermocatenulatus*, BTL2, in Organic Solvent

Asli Yenenler^{1,4}, Alessandro Venturini², Cahit Burduroglu³, Osman Ugur Sezerman⁴.

¹Sabancı University, İstanbul, Turkey, ²ISOF-CNR, Bologna, Italy, ³Acıbadem University, İstanbul, Turkey, ⁴Acıbadem University, İstanbul, Turkey.

Lipase enzymes have been widely used in different fields of biotechnology for many years. Even if they are generally used in aqueous environment for hydrolysis reactions, their usage in organic solvent promises high-level development at industrial level allowing a broad range of different esterification and transesterification reactions. Yet, the presence of organic solvent brings structural and enzymatic limitations to lipases that have to be overcome for their effective usage. In this work, we have investigated the effects of toluene solvent on the structure of BTL2 from *Bacillus thermocatenulatus* through MD simulations over 30 ns. By comparing the behavior of BTL2 in aqueous and in hydrophobic media, we have detected the parts of the protein more affected by the presence of toluene. In general, the organic solvent increases the rigidity of the enzyme. A significant example of our analysis is the unusual packing of catalytic Ser114. Further, we assess the change in secondary structure and destabilization tendency of BTL2 in the hydrophobic environment with STRIDE analysis and FoldX calculations. These results suggest that the presence of toluene molecules leads to some structural changes that affect the packing of BTL2 and eventually limiting its enzymatic ability. To overcome this problem, we have added a layer of water (5%) around BTL2 to provide the required structural flexibility. Moreover, point mutations like Gly116Pro, Gly116Pro_Gly319Pro (double mutation) and, Glu271Ala, and Asn317Ala to increase structural stability and to provide the required flexibility of the protein, have been carried out. We think that this study will allow further progress in understanding the behavior of these enzymes in organic solvent and then allow their industrial exploitation.

116-POS Board 36

A Direct Interaction of Cholesterol with Monoamine Transporters Prevents Their Out-to-Inward Transition

Talia Zeppelin¹, Lucy K. Ladefoged³, Steffen Sinning², Xavier Periole¹, Birgit Schioett^{1,3}.
¹Aarhus University, Aarhus C, Denmark, ²Translational Neuropsychiatry Unit, Aarhus C, Denmark, ³Aarhus University, Aarhus C, Denmark.

Monoamine transporters (MATs) carry out neurotransmitter reuptake from the synaptic cleft, which is a key step targeted in treatment of neurological disorders. Cholesterol, a major component of the synaptic plasma membrane, has been shown to exhibit a modulatory effect on MATs, and recent crystal structures of the dopamine transporter (DAT) in the presence of two conserved cholesterol molecules substantiate the hypothesis of a direct protein-cholesterol interaction. We performed extensive all-atom molecular dynamics (MD) simulations of DAT with and without cholesterol bound. In the absence of cholesterol, DAT undergoes structural changes reflecting early events of transport: transition to an inward-facing conformation. In contrast, in the presence of cholesterol these conformational changes are inhibited presumably by a stabilizing interaction of cholesterol at the intracellular side of TM5. We further provide evidence, from using coarse-grained MD simulations, that the cholesterol sites observed in the DAT crystal structures are conserved in all human MATs, suggesting that this effect might extend to the entire family.

117-POS Board 37

Determining Structural Ensembles of Flexible Biomolecules Using Small-angle X-ray Scattering and Computer Simulations

Junhui Peng, Yonghui Zhang, Bin Wen, Peng Cheng, **Zhiyong Zhang**.
University of Science and Technology of China, Hefei, Anhui, China.

Large biomolecules, such as proteins, RNA, DNA, and their complexes, are generally flexible with large scale conformational changes in solution, which are closely related to their biological functions. Small-angle X-ray scattering (SAXS) has made substantial progress over the past decades and has become more and more popular among structural biologists. SAXS is particularly useful in characterizing the flexibility of a large biomolecule because the scattering profile contains the information of multiple conformations of the biomolecule and their relative population in solution. In recent years, a notion of ‘integrative structural biology’ has been proposed, which aims to determine the biomolecular structure and characterize its flexibility by combining complementary high and low resolution experimental data using computer simulations. Our work focus on the development and application of multi-scale computer simulation methods integrating SAXS and other structural data to investigate conformational dynamics of large biomolecules. we have determined structural ensembles of several multi-domain proteins and protein complexes by integrating SAXS data into various simulation techniques, such as all-atom molecular dynamics simulations, enhanced sampling techniques, and coarse-grained modeling. We have also developed a couple of new computational tools, which aim to (1) easily integrate any low-resolution structural data including SAXS to construct atomic models of large biomolecules, and (2) efficiently perform SAXS-oriented ensemble refinement for flexible biomolecules. Multi-scale simulations integrating SAXS data have enabled us to characterize conformational changes of large biomolecules and their assemblies accurately, which contributes to the study of the relation between structural dynamics and biological function.

118-POS Board 38

Structural Changes in LTP1 Isoforms from Beer at Air-water Interfaces

Yani Zhao, Marek Cieplak.

Institute of Physics, Polish Academy of Sciences, Warsaw, Poland.

We use a coarse-grained model to study the conformational changes in two barley proteins, LTP1 and its ligand adduct isoform LTP1b, that result from their adsorption to the air-water interface. The model introduces the interface through hydrophobicity indices. This model is justified by all-atom simulations. The choice of the proteins is motivated by making attempts to understand formation and stability of foam in beer. We demonstrate that both proteins flatten out at the interface and can make a continuous stabilizing and denser film. We show that the degree of the flattening depends on the protein – the layers of LTP1b should be denser than those of LTP1 – and on the presence of glycation. It also depends on the number (≤ 4) of the disulfide bonds in the proteins. The geometry of the proteins is sensitive to the specificity of the disulfide bonds. We provide estimates of the volume of cavities of the proteins when away from the interface.

119-POS Board 39

Sigma-r Plots - A Fast and Intuitive Way to Visualize the Global Properties of Molecular Dynamics Trajectories

Hao Zhou¹, Shangyang Li¹, Lee Makowski².

¹Northeastern University, Boston, MA, USA, ²Northeastern University, Boston, MA, USA.

The increasing availability of computational MD simulation software and high performance computing platforms makes possible generation of nano- to micro- second trajectories which contain substantial information about the internal motions of proteins, but pose challenges of storage, sharing and analysis of the data. In order to take advantage of these huge data sets, condensing this information into a form that is intuitive becomes an essential task. Here we discuss the merits of the sigma-r plot, a plot of the averaged standard deviation of intermolecular distances of each atom pair in an MD trajectory as a function of intermolecular distance. This representation reduces the four dimensional data (3D space + simulation time) to a single, one dimensional plot that exhibits the average range of motion at different length scales within a macromolecule. A sigma-r plot can be generated for all atoms or for C-alpha atoms only; it can be used for the entire molecule or individual domains. Here we demonstrate that a sigma-r plot can distinguish differences in the global dynamic behavior of the four major SCOP fold classes. We also show that differences in domain structure and molecular weight produce recognizable features in sigma-r plots. Plots generated from trajectories with longer simulation time reflect more complete sampling of the structural ensemble. Sigma-r plots generated from all atom positions make possible facile comparison to experimental measures such as the x-ray solution scattering.

120-POS Board 40

From Single Structures to Ensembles: Application of the Galaxy Program Suite to BPTI, Ubiquitin and the RBD of Plexin-B1; Validation Against NMR Data

Gyu Rie Lee², Minkyung Baek², Sangwoo Park², Choak Seok², **Matthias Buck**^{1,2}.

¹Case Western Reserve University, Cleveland, OH, USA, ²Seoul National University, Seoul, South Korea.

It is now widely accepted that an accurate representation of a protein structure should incorporate a description of internal as well as protein loop dynamics. Lindorff-Larsen and colleagues suggested that protein structures can be refined by NMR derived order parameters, S^2 , by including this measure and/or RDCs as an additional restraint in the refinement protocol[1]. More recently it has become clear that the amplitude of local motions can be predicted with considerable accuracy by considering the surrounding atoms and the packing environment they may provide[2]. Nevertheless, the prediction of loop motions, if not distinct loop conformations has remained a challenge.

We apply here the Galaxy Suite of Programs (GalaxyLoop and GalaxyVoyage)[3] to several test-cases: BPTI and Ubiquitin, which have been extensively characterized by NMR spectroscopy well as by molecular dynamics simulations(e.g.[4,5]). For BPTI it was important to consider both disulphide bond chirality and internal water molecules, but good agreement with the predominant conformational states could be obtained. Ubiquitin motions emphasized small displacements, whereas the ubiquitin-like RhoGTPase Binding Domain has long loops inserted into an ubiquitin fold. Some of these loops undergo considerable motions on the ps-ns timescale as measured by NMR relaxation[6], which suggested the NMR derived structure needed substantial local refinement[7]. These examples suggest that the implementation of ensemble refinement into the Galaxy Suite of Programs should be successful.

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[7] Tong Y, et al., Structure 2008 16:246-58