# Telluride Science Research Center

# Protein Dynamics Workshop

# 2019

Doug Tobias, Shigehiko Hayashi and

Martin Blackledge

**Participants**

Adams, Ellen – Ruhr University, Bochum (DE)
Anfinrud, Philip – National Institutes of Health (USA)
Blackledge, Martin – Institut de Biologie Structurale (FR)
Brüschweiler, Rafael – Ohio State University (USA)
Fenimore, Paul – Los Alamos National Laboratory (USA)
Grubmuller, Helmut, Max Planck Institute Göttingen (DE)
Hayashi, Shigehiko – Kyoto University (JP)
Kitao, Akio – Tokyo Institute of Technology (JP)
McDermott, Ann – Columbia University (USA)
Mizutani, Yasuhisa – Osaka University (JP)
Mulder Frans – University of Aarhus (DK)
Palmer, Arthur – Columbia University (USA)
Stefano Piana-Agostinetti  D.E.Shaw (USA)
Priyakumar, U. Deva – Int Instt of Information Technology, Hyderabad (IN)
Sanabria, Hugo – Clemson University (USA)
Seidel, Claus – University Düsseldorff (DE)
Straub, John – Boston University (USA)
Thielges, Megan – Indiana University (USA)
Tobias, Doug – University of California, Irvine (USA)
van Thor, Jasper – Imperial College London (UK)

Monday 8th July

7.30-8.30 Breakfast at TSRC

8.30-9.00 Opening comments (Martin Blackledge, Doug Tobias, Shigehiko Hayashi, TSRC)

***Moderator Martin Blackledge***

9.00-9.50 **ATOMISTIC SIMULATION OF BIOMOLECULAR FUNCTION: RIBOSOMAL TRANSLATION AND LIGAND BINDING HETEROGENEITY**

Helmut Grübmuller, Max Planck Insitut für Biophysikalische Chemie

9.50-10.20 Break

10.20-11.10 **PROTEIN INTERACTIONS AND DYNAMICS BY NANOPARTICLE-ASSISTED NMR AND IMPROVED MD SIMULATIONS WITH NEW FORCE FIELD**

Rafael Brüschweiler, Ohio State University

11.10-12.00 **ATOMISTICALLY DECIPHERING FUNCTIONAL PROCESSES OF TRANSPORTER AND REDOX PROTEINS WITH MOLECULAR SIMULATIONS**

Shigehiko Hayashi, Kyoto University

Lunch break (not provided)

Free time

***Moderator Frans Mulder***

14.00-14.50 **PROTEIN DYNAMICS OF A LIGHT-DRIVEN SODIUM ION-PUMPING RHODOPSIN**

Yasuhisa Mizutani, Osaka University

14.50-15.40 **OPTICAL CONTROL OF PROTEIN STRUCTURAL DYNAMICS BY ULTRAFAST TR-SFX**

Jasper van Thor, Imperial College London

Tuesday 9th July

7.30-9.00 Breakfast at TSRC

Free time

***Moderator Shigehiko Hayashi***

13.30-14.20 **LARGE SCALE SIMULATIONS OF FOLDED AND DISORDERED PROTEINS**

Stefano Piana-Agostinetti, D.E. Shaw Research

14.20-15.10 **UREA ASSISTED UNFOLDING – REVISITING THE NATURE OF UREA-AROMATIC/ALIPHATIC INTERACTIONS AND THEIR ROLE IN OTHER BIOLOGICAL SYSTEMS**

U. Deva Priyakumar, International Institute of Information Technology, Hyderabad

15.10-15.40 Break

***Moderator Rafael Brüschweiler***

15.40-16.30 **PROBING THE GENESIS, STRUCTURE AND ASSEMBLY OF AMYLOID PROTEINS**

John Straub, Boston University

16.30-17.20 **INTEGRATIVE DYNAMIC STRUCTURAL BIOLOGY WITH FLUORESCENCE SPECTROSCOPY**

Claus Seidel, Heinrich-Heine-Universität Düsseldorf

18.30-19.15 Town talk

Wednesday 10th July

7.30-9.00 Breakfast at TSRC

***Moderator Doug Tobias***

9.00-9.50 **PROTEINS, PROTONS, THEIR SOLVATION, AND DYNAMICS**

Paul Fenimore, Los Alamos National Laboratory

9.50-10.40 **DYNAMICAL PROPERTIES OF WATER IN THE HYDRATION SHELL OF PROTEINS**

Ellen Adams, Rohr University, Bochum

10.40-11.10 Break

11.10-12.00 **DYNAMIC COMPLEXES OF PROTEIN REDOX PARTNERS ILLUMINATED VIA 2D IR SPECTROSCOPY**

Megan Thielges, Indiana University Bloomington

Lunch break (not provided)

***Moderator Martin Blackledge***

14.00-14.50 **PROTEIN DYNAMICS FROM NMR SPECTROSCOPY AND MD SIMULATIONS**

Art Palmer, Columbia University

14.50-15.40 **USING NMR RELAXATION TO MAKE NMR RELAXATION REDUNDANT**

Frans Mulder, Aarhus University

15.40-16.10 Break

***Moderator Art Palmer***

16.10-17.00 **PROTEIN DYNAMICS STUDIED BY SOLID STATE NMR: FUNCTIONAL GATING OF AN ION CHANNEL**

Ann McDermott, Columbia University

17.00-17.50 **TIME-RESOLVED SAXS/WAXS STUDIES OF BIOMOLECULES IN SOLUTION FOLLOWING A TEMPERATURE JUMP**

Philip Anfinrud, National Institutes of Health

18.00 TSRC Picnic

Thursday 11th July

7.30-9.00 Breakfast at TSRC

Free time

***Moderator John Straub***

15.00-15.50 **SIMULATING PROTEIN COMPLEX FORMATION AND DISSOCIATION**

Akio Kitao, Tokyo Institute of Technology

15.50-16.40 **ANOMALOUS SUB-DIFFUSION OF A LARGE PROTEIN IN THE PLASMA MEMBRANE**

Doug Tobias, University of California, Irvine

16.40-17.30 **COMPLEX DYNAMICS AND DYNAMIC COMPLEXES: NMR STUDIES OF THE ROLE OF LARGE SCALE MOTION IN PROTEIN FUNCTION**

Martin Blackledge, Institut de Biologie Structurale UGA-CEA-CNRS, Grenoble

17.30-18.00 Summing up, Selection next committee

Friday 12th July

Breakfast at TSRC

Free time

**Atomistic Simulation of Biomolecular Function: Ribosomal Translation and Ligand Binding Heterogeneity**

Lars V. Bock, Michal Kolar, Andrea C. Vaiana, Andreas Russek, Benjamin von Ardenne, Helmut Grubmüller

Max Planck Institute for Biophysical Chemistry, Theoretical and Computational Biophysics Department, Göttingen, Germany

Ribosomes are highly complex biological nanomachines which operate at many length and time scales. We combined single molecule, x-ray crystallographic, and cryo-EM data with atomistic simulations to elucidate how tRNA translocation, the action of antibiotics, and frameshifting work at the molecular level. We describe a new combined allosteric mechanism for erythromycin-induced translational stalling of the antibiotics sensor peptide ErmB, as well as a free energy model that can explain and predict frameshifting efficiencies. Using streptavidin/biotin as a model system with super-strong affinity, we show that the underlying free energy landscape which governs ligand binding and unbinding can be extracted from combined atomic force microscopy (AFM) and force probe simulation data, which covers loading rates of 11 orders of magnitude.

[1] Arenz S, Bock LV, ... , Grubmüller H, Vaiana AC, Wilson DN. Nature Comm. 7, 12026 (2016)
[2] Fischer N, ..., Stark H. Nature 540 (7631), 80-85 (2016)
[3] Bock LV, Blau C, Vaiana AC, Grubmüller H. Nucl. Acids Res. 43 (14), 6747–6760 (2015)
[4] Bock LV, ..., Grubmüller H. Nature Struct. Molec. Biol. 20 (12), 1390-1396 (2013)
[5] Bock LV, Kolar MH, Grubmüller H. Curr. Opin. Struct. Biol. 49, 27–35 (2018)
[6] Huter P et al. Mol. Cell 68 (3), 515–527 (2017)
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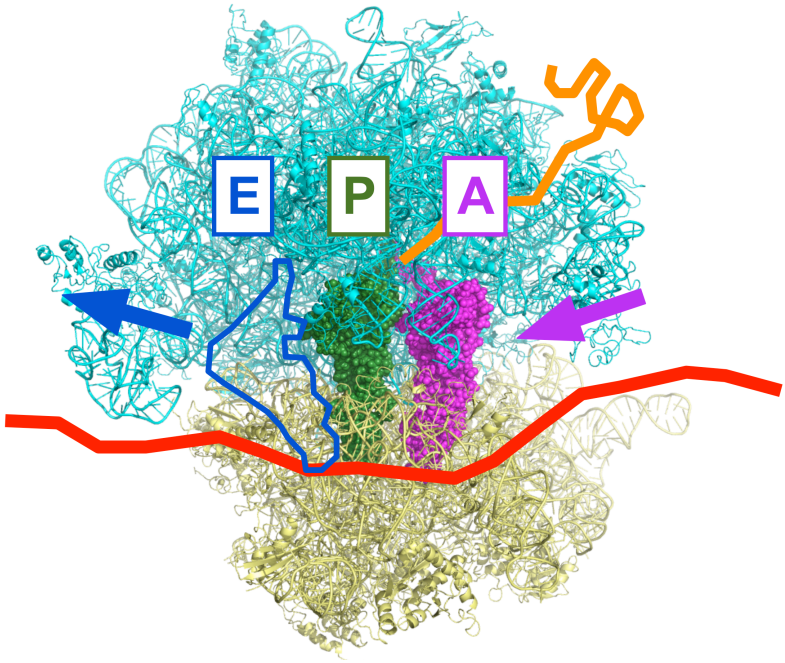

**Protein interactions and dynamics by nanoparticle-assisted NMR and improved MD simulations with new force field**

Rafael Brüschweiler

Department of Chemistry & Biochemistry and Biological Chemistry & Pharmacology, The Ohio State University, Columbus, Ohio 43210, U.S.A.

Interactions between biological and synthetic materials and proteins in a folded or unfolded state can be sensitively monitored by biomolecular NMR spectroscopy. I will discuss recent progress in the description of protein-nanoparticle interactions using nuclear spin relaxation data to understand site-specific interaction propensities. An analytical and fully transferable model is presented for the prediction of the interaction strengths between nanoparticles and intrinsically disordered proteins as a function of the polypeptide sequence. Conversely, the presence of nanoparticles can help monitor protein flexibility. Interpretation of these and other data by molecular dynamics (MD) simulations requires accurate molecular mechanics force fields that adequately present both folded and disordered protein regions. A new MD force field along with applications is presented that shows good promise to fill this gap.

**Atomistically Deciphering Functional Processes Of Transporter And Redox Proteins With Molecular Simulations**

Shigehiko Hayashi

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Functional processes of transporter and redox proteins are often fulfilled by dynamic and global molecular conformational changes of complex protein systems which correlate with local molecular events at ligand binding sites and reaction centers. Hence the multi-scale functional coupling of local chemical events with protein global molecular dynamics need to be revealed for understanding of molecular nature of protein functions. In this talk, I will present our recent studies on photo-activation processes of a channelrhodopsin (ChR) photo-sensitive ion transporter and redox processes of cytochrome c (cyt c) and photosystem II (PSII) by a hybrid QM/MM free energy geometry optimization technique, which allows one to optimize electronic wave function and molecular geometry of a reaction center at the ab initio quantum chemistry level of theory on a free energy surface constructed with statistically extensive conformational ensemble of the protein environment obtained by long-time MD simulations. I will also present an atomistic MD study of alternating access of mitochondria ADP/ATP transporter with a linear response path following (LRPF) method which is a biasing MD technique accelerating global protein conformational changes coupled to local ligand binding events.

I will first present an atomic structural model of a chimeric ChR in a precursor state of the channel opening. The photo-activated structure features extensive tilt of the chromophore accompanied by redistribution of water molecules in its binding pocket which is absent in previously known photo-activated structures of analogous proteins, and manifests a photo-activated ion conduction pathway which is markedly different from a previously proposed one. I will also present theoretical investigations of redox processes of cyt c and PSII which include transition metal complexes as reaction centers. Through ab initio QM/MM free energy geometry optimizations for many combinations of redox and protonation states of the reaction centers free from difficult force field determination for the electronically complex reaction centers, we successfully revealed significant structural differences of the redox centers with the different redox and protonation states. Finally, I will present an atomic structural model of alternating access of ADP/ATP transporter undergoing large conformational changes of the entire protein, which has successfully predicted an X-ray crystallographic structure recently reported.

**Protein Dynamics of a Light-Driven Sodium Ion-Pumping Rhodopsin**

Yasuhisa Mizutani

Osaka University

Time-resolved resonance Raman spectroscopy is a powerful tool to examine protein structure evolving from the earliest moments such as the picosecond regime through the evolution of complexity toward time scales that are highly relevant to biological function such as the microsecond regime or millisecond regime. In this talk, I present our work on structural dynamics of a light-driven sodium ion-pumping rhodopsin KR2 upon chromophore photoisomerization. The chromophore structures of the intermediates and their relevance to the ion transport mechanism are discussed.

**Optical control of protein structural dynamics by ultrafast TR-SFX**

Jasper J. van Thor

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The new ability to perform ultrafast time resolved X-ray crystallography in pump-probe mode [1] raises a number of fundamental questions regarding the assignment, control and analysis of light-induced differences that are measured [2][3][4][5][6]. I will discuss methods of analysis and experimental design that allow quantification and control of non-linear photochemical reactions under typical conditions of femtosecond time resolved TR-SFX[1][4]. This analysis has now also been extended to retrieve the explicit orientation-dependence of transient populations by crystal optics calculations [3]. The high peak power required for successful pump-probe TR-SFX experiments also provide an opportunity, to measure and control low frequency ground state and excited state vibrational coherence[2][3]. Future high repetition rate XFEL instruments such as LCLS-II may advance the technique to the practical application of the X-ray crystallographic equivalent of an impulse time-domain Raman measurement of vibrational coherence at high frequencies [3]. In addition, I will present a first experimental demonstration of optical control of protein structural dynamics using femtosecond TR-SFX at near-atomic resolution.

References

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[2] C. Hutchison and J.J. van Thor. (2017). Int. Rev. Phys. Chem. 36, 117-143
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[4] C. Hutchison et al.(2016) Chemical Physics Letters, Vol: 654, Pages: 63-71
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[6] C. Hutchison et al. (2017) Int. J. Mol. Sci. 18(9), 1918; doi:10.3390/ijms18091918

**Urea Assisted Unfolding – Revisiting The Nature Of Urea-Aromatic/Aliphatic Interactions And Their Role In Other Biological Systems**

U. Deva Priyakumar

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Urea is a chemical denaturant that assists protein and RNA unfolding and shifts the folding equilibrium towards their unfolded states. A number of studies that attempted to understand the molecular mechanism of this phenomenon have been reported during the last three decades. As shown by several groups, dispersion type interactions seem to be largely responsible for this phenomenon, but the way by which urea interacts with these groups is not known. We have investigated the different possible modes of interactions between urea and aromatic/aliphatic groups of amino acid residues using molecular dynamics simulations, free energy calculations and high-level quantum mechanical calculations. The first part of the presentation will deal with the urea-aromatic interactions briefly and discuss the commonalities between urea assisted protein and RNA unfolding. It has been experimentally shown that among all the functional groups in proteins, urea interacts strongly with the aromatic groups. We will discuss our attempts at providing structural, thermodynamic and kinetic basis of these novel interactions. Free energy calculations on the unfolding of Trp-cage miniprotein reveal the importance of urea-aromatic interactions in the unfolding process. The primary mode of interaction between the two is shown to be stacking interactions in addition to hydrogen bonded and NH-π interactions. This was further quantified by thermodynamic integration and quantum mechanical calculations. Lifetime calculations based on μs long MD simulations reveal these to be long lasting in the ps timescale.

In the second part of the presentation, we will discuss the effect of aqueous urea on the aliphatic side chains. One of the intriguing aspects of these interactions is the stabilization of solvent exposed states of alkyl chains via CH-π interactions! Energetic, structural and dynamic aspects of these interactions in the context of urea assisted unfolding will be discussed. Finally, the relevance of these interactions in other biological systems such as urea transport via transporters and damaged DNA with urea lesions along with prevalence of these modes of stabilization in experimental structures will be presented. We will attempt to demonstrate the necessity of the same interactions that are responsible for stabilization of solvent exposed aromatic/aliphatic groups in achieving urea transport and in maintaining the structural integrity of damaged DNA with urea moiety.

In summary, we will present the role of novel and unusual interaction between urea and aromatic/aliphatic groups in different facets in biology and the possible role of these interactions in increasing the binding affinity/specificity of drug-receptor interactions.

**Probing the genesis, structure and assembly of amyloid proteins**

John Straub

Boston University

I will describe ongoing work on a number of systems related to amyloid protein genesis, association, and aggregation.
(1) Serum Amyloid A (SAA) is a highly conserved protein, found in organisms ranging from sea cucumbers to humans, with both intrinsically disordered regions and structured domains. Elevated levels of SAA have been linked to inflammatory disease, including atherosclerosis, rheumatoid arthritis, and amyloid A amyloidosis. In response to inflammation, SAA is hypothesized to be redirected from its role in routine lipid transport to lipid removal and recycling. However, structural and mechanistic details of SAA in its normal and pathological functions are still unknown. I will describe our recent attempts using all-atom (AA) and coarse-grained (CG) models to understand how SAA structure supports its native function and transition to amyloid.
(2) Considerable progress has been made, using experiments and computations, to decipher the general principles governing the mechanism of formation of oligomers and fibrils of amyloid proteins implicated in diseases, including the amyloid-β protein (Aβ) associated with Alzheimer's disease (AD). However, the identification of the link between protein aggregation and the systems of disease at the molecular level has proved elusive. The biogenesis of Aβ starts with interaction of the Amyloid Precursor Protein (APP) with secretases in the presence of membrane. Subsequently, interactions with cholesterol determine the route to oligomer formation and the extent of cytotoxicity. I will discuss ongoing work to systematically investigate the biogenesis of Aβ, its propensity toward aggregation, and putative mechanisms of cytotoxicity in order to highlight critical areas for future research.

**Integrative dynamic structural biology with fluorescence spectroscopy**

Claus A.M. Seidel,[1] Mykola Dimura,[1,2] Hugo Sanabria[1], Katherina Hemmen,[1] Thomas-Otavio Peulen,[1] Dmitro Rodnin,[1] Holger Gohlke[2]

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Förster Resonance Energy Transfer (FRET) studies on the ensemble and single-molecule level probe biomolecular structure and dynamics and identify coexisting conformational states. Integrative structural biology solves biomolecular structures by combining data from different sources, which individually would be insufficient. I will especially discuss the striking similarities of the measurement and analysis techniques between fluorescence, EPR and NMR spectroscopy [1]. Here, we combine FRET experiments with computer simulations to overcome their sparsity. I will discuss recent methodological developments in integrative structural modelling by satisfying spatial restraints on networks of FRET pairs (hybrid-FRET) [2,3]. We introduce new procedures for: (i) an automated FRET experiment design tool determining most informative FRET pairs for structural modeling using prior structural knowledge (https://github.com/Fluorescence-Tools), (ii) a protocol for efficient FRET-assisted computational structural modelling at multiple scales (http://nmsim.de), (iii) choice of appropriate models for describing the dye behavior and consequences for designing appropriate labels, (iv) use of all eight characteristic fluorescence parameters as a vectorial local and dynamic structure information, and (v) a new quantitative quality estimate for judging the accuracy of determined structures. I will present applications simulations and real experiments of our techniques to proteins such as T4 Lysozyme (T4L) [4]. We used 33 variants with single-FRET pairs to resolve three T4L conformers via their characteristic distance sets. Screening the known T4L structures, revealed that T4L in solution mainly adopts the known open and closed states in exchange at 4 µs. A newly found minor state, undisclosed by at present more than 500 crystal and NMR structures of T4L and sampled at 230 µs, may be actively involved in the product release step in catalysis. Integrative dynamic FRET studies of larger systems will presented as well: (1) Mapping the intrinsic flexibility of Guanylate binding proteins (approx. 65 kDa) that enables conformational transitions to exert their function as part of the innate immune system of mammalian cells [5]; (2) dynamic architecture of chromatin fibers by studying a 12-mer nucleosome array (approx. 2,5 MDa) [6].

Importantly, we released together with the PDB the initial version of the fluorescence (FLR) dictionary extension (https://github.com/ihmwg/FLR-dictionary) on the Integrative/Hybrid Modeling (IHM) working group GitHub site. Now, fluorescence-restrained structural models can be deposited at PDB-Dev. Finally, I will discuss the striking similarities of the measurement and analysis techniques between fluorescence, EPR and NMR spectroscopy.

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**Proteins, Protons, their Solvation, and Dynamics**

Paul Fenimore

Los Alamos National Laboratory

Many protein functions follow the activation enthalpy of near-by bulk solvent dynamics ($\alpha$) and hydration dynamics ($\beta_n$). Bulk solvent dynamics are well-known to arise from Debye-like relaxations in liquids and are macroscopically evident as viscosity. In contrast, hydration dynamics at protein surfaces are known to predict a range of functionally-relevant protein motions, but their structural basis is murky. Using a customized analysis code, we have analyzed all high-resolution, high-occupancy X-ray and neutron structures in the PDB for their water and proton content. The large excess of solvated proton species coordinated to and near protein surfaces shows solvated protons from both neutron and X-ray structures, have a strong propensity to coordinate hydrophilic groups, have geometries reminiscent of Zündel and Eigen cations, with a tail of unusual coordination species, and show some striking homology between proteins. These observations of proton solvation at protein surfaces are consistent with the ($\beta_n$) process showing a large H/D kinetic isotope effect.
I hope to also discuss quasi-elastic neutron scattering, the role of anisotropy in determining the Q-dependence of quasi-elastic scattering, and a possible explanation for the origin of the energy dependence of both Mössbauer absorption and neutron quasi-elastic spectra. These processes are related to dynamical light scattering, and a comparison of the physics while not identical is highly instructive.

**Dynamical Properties of Water in the Hydration Shell of Proteins**

Ellen Adams

Rühr Univeristy, Bochum

In recent years the importance of the aqueous solvent in influencing protein structure, function, and dynamics has been recognized. Coupling of water molecules to the protein surface results in an interfacial region in which water molecules within this region have distinctly different properties than bulk water. However, the structure and dynamics within this interfacial region are still not easy to access experimentally. Terahertz (THz) spectroscopy has been shown to be a powerful tool to investigate solvent dynamics in bulk solutions. Radiation in the THz regime is directly sensitive to the low frequency collective intermolecular hydrogen-bonding vibrations of water (0.3-6 THz or 10-200 cm-1), and thus to any changes in the hydrogen-bonding network. Changes in these sub-picosecond collective motions, such as protein-water interactions, result in changes in the measured THz absorption. Individual hydrations shells of proteins have been shown to contribute largely to structure-function relationships and ultimately modulate the binding properties of proteins. Here the role of solvation dynamics in processes such as electron transport in protein complexes and enzymatic catalysis will be investigated.

**Dynamic Complexes of Protein Redox Partners Illuminated via 2D IR Spectroscopy**

Megan Thielges,

Indiana University

Transient protein complexes are crucial for sustaining dynamic cellular processes. Such complexes, for example, are formed by protein redox partners for mediating efficient electron transport. However, their heterogeneous and dynamic nature makes the complexes experimentally challenging to fully capture. To overcome this issue, we have combined linear and two-dimensional infrared spectroscopy, which provide high temporal and spatial resolution, and incorporation of vibrational reporter groups that provide frequency-resolved absorptions for selectively characterizing their local environments in proteins. My talk will share how we have applied this approach toward better understanding the complexes of redox partners cytochrome P450 and putadiredox and plastocyanin and cytochrome *f*.

**Protein dynamics from NMR spectroscopy and MD simulations.**

Arthur G. Palmer, III

*Biochemistry and Molecular Biophysics, Columbia University, 701 West 168th Street, New York, NY 10032, U.S.A.*

NMR spectroscopy is a powerful experimental approach for characterizing protein conformational dynamics on multiple time scales, while molecular dynamics (MD) simulation is the only method capable of describing full atomistic details of protein dynamics. Methods for characterizing dynamics on picosecond-nanosecond and on microsecond-millisecond time scales, emphasizing the information content provided by multiple static magnetic fields, are illustrated by applications to the enzyme ribonuclease H, the yeast transcription factor GCN4, and the dimerization domain of E and N cadherin. These data are interpreted by MD simulations, particularly aimed at deconvoluting contributions to side chain conformational fluctuations. Theoretical approaches generalize two-state descriptions of the $R_{1\rho}$, Carr-Purcell-Meiboom-Gill, and CEST experiments to N states with arbitrary kinetic topologies, facilitating applications to complex biological phenomena. Simple models, developed in the 2017 Telluride School, illustrate the connection between NMR relaxation models and statistical models of chemical kinetics.

Contact Information.

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**Using NMR relaxation to make NMR relaxation redundant**

Frans Mulder

University of Aarhus, Denmark

I will describe our recent efforts to narrow the gap between experimental (nuclear spin relaxation) and computational (MD simulation) descriptions of protein dynamics. The overarching goal of this effort is to obtain access to accurate estimates of protein configurational entropy from simulation.
To reach this goal we first aid in the improvement of the MD force fields to the level that they faithfully predict experimental NMR relaxation data. Although good agreement for the rather rigid main chain is already possible, the invariably more mobile side chains are a lot more challenging.
In order to compare nuclear spin relaxation and MD simulations with minimal assumptions, we compare the two at the level of the spectral density function. We have overcome several hurdles to make this comparison robust, including (i) removing experimental artefacts in methyl $^{2}H$ relaxation experiments; (ii) treating anisotropic overall protein tumbling in simulation and in relaxation data analysis; and (iii) re-estimation of the energy barriers for methyl rotation in computation.
Ultimately, it is hoped that improvements in MD computations will help us leave the practice of gleaning protein dynamics from very approximate descriptors, like Lipari-Szabo order parameters, behind us.

Narrowing the gap between experimental and computational determination of methyl group dynamics in proteins.
**Hoffmann** F, Xue M, Schäfer LV, Mulder FAA.
Phys Chem Chem Phys. 2018 Oct 3;20(38):24577-24590. doi: 10.1039/c8cp03915a.

Accurate Methyl Group Dynamics in Protein Simulations with AMBER Force Fields.
**Hoffmann** F, Mulder FAA, Schäfer LV.
J Phys Chem B. 2018 May 17;122(19):5038-5048. doi: 10.1021/acs.jpcb.8b02769.

# PROTEIN DYNAMICS STUDIED BY SOLID STATE NMR: FUNCTIONAL GATING OF AN ION CHANNEL

Ann McDermott

*[1]Biochemistry and Molecular Biophysics, Columbia University, 701 West 168th Street, New York, NY 10032, U.S.A.*

**Time-resolved SAXS/WAXS studies of biomolecules in solution following a temperature jump**

Philip Anfinrud, Hyun Sun Cho, Friedrich Schotte, Valentyn Stadnytskyi, Shuang Li, and Jinwei Zhang

National Institutes of Health, NIDDK
Bethesda, MD

We have developed the infrastructure required to record time-resolved x-ray scattering patterns from biomolecules in solution over a broad range of scattering angles ($0.02 < q < 5.2$ **.**) and temperatures ($-20 < T < 120$ °C) following a sudden (< 10 ns), large temperature jump (~20 °C).
We have used this infrastructure to investigate the dynamics of quaternary structure changes in carbonmonoxy hemoglobin, and found that its R state is represented by at least two quaternary conformations that interconvert on the microsecond time scale. To further demonstrate the capabilities of this infrastructure, we investigated a series of short RNA hairpins and discovered, unexpectedly, a propensity to dimerize at sub-millimolar concentrations. We characterized thermodynamic parameters and kinetic rate coe**ffi**cients corresponding to hairpin dimer dissociation/formation and hairpin duplex formation/melting.
The time-resolved structural details that can be extracted from these SAXS/WAXS studies goes well beyond that accessible with other biophysical techniques, and provides a wealth of information that can be used to assess the validity of potentials used in molecular dynamics simulations.

**Simulating protein complex formation and dissociation**

Akio Kitao

School of Life Science and Technology, Tokyo Institute of Technology

Formation and dissociation of protein complexes are essential processes in biological systems, however, observation of these processes at atomic resolution is challenging. Molecular simulations are useful methods to investigate molecular mechanisms of protein dissociation and association *in silico*. We recently developed efficient molecular simulation methods to investigate the mechanisms, which include a combination of parallel cascade selection molecular dynamics (PaCS-MD) and Markov state model, and concentrated ligand docking (ColDock). PaCS-MD comprises cycles of multiple unbiased MD simulations using a selection of MD snapshots as the initial structures for the next cycle. We will show that PaCS-MD can facilitate the observation of protein complex formation and dissociation. ColDock consists of multiple independent molecular dynamics simulations in which ligands are initially distributed randomly around a protein at relatively high concentration. This condition significantly increases the probability of the ligand exploring the protein surface, which induces spontaneous ligand binding to the correct binding sites within a short MD. The results of ColDock indicate that plausible protein-ligand complex structures can be easily generated by conducting the ColDock procedure using standard MD simulation software.

**Anomalous sub-diffusion of a large protein in the plasma membrane**

Doug Tobias

University of California, Irvine

The cellular environment presents a number of challenges to a diffusing macromolecule: it is labyrinthine, crowded, and sticky. Consequently, the amount of progress a diffusing macromolecule makes in a given amount of time, as quantified by its mean-squared displacement (MSD), is significantly less in the cellular than in a dilute bulk environment. For Brownian motion or normal diffusion, the ensemble-averaged MSD increases linearly with time. For anomalous diffusion the MSD is a power law function of time, while for anomalous sub-diffusion, the power-law exponent is less than one. In the cellular environment, anomalous sub-diffusion is, well, normal. In this talk I will present an analysis of experimental single-particle tracking data on the anomalous sub-diffusion of a large (~290 kD) protein, the mechanosensitive Piezo1 channel, in the plasma membrane of mouse embryonic fibroplast cells. The analysis includes a variety of statistical tests aimed at elucidating the underlying sub-diffusive mechanisms, and attributing them to specific cellular features. Our long term goal is to connect the membrane mobility of Piezo1 to its function.

**Complex Dynamics And Dynamic Complexes: NMR Studies Of The Role Of Large Scale Motion In Protein Function**

Nicola Salvi, Serafima Guseva, Wiktor Adamski, Aldo Camacho-Zarco, Damien Maurin, Malene Jensen, Sigrid Milles, & Martin Blackledge

*Protein Dynamics and Flexibility by NMR, Institut de Biologie Structurale UGA-CEA-CNRS, Grenoble, France*

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Proteins are inherently dynamic, exhibiting conformational freedom on many timescales,[1] implicating structural rearrangements that play a major role in molecular interaction, thermodynamic stability and biological function. Intrinsically disordered proteins (IDPs) represent extreme examples where flexibility defines molecular function. IDPs exhibit highly heterogeneous local and long-range structural and dynamic propensities, sampling a much flatter energy landscape than their folded counterparts, allowing inter-conversion between a quasi-continuum of accessible conformations.[2] We are combining multifield NMR relaxation measurements and ensemble MD approaches to develop a unified description of the dynamics of IDPs as a function of environmental conditions.[3-5]

In spite of the ubiquitous presence of IDPs throughout biology, the molecular mechanisms regulating their interactions with physiological partners remain poorly understood. We use NMR spectroscopy to map these complex molecular recognition trajectories at atomic resolution, from the highly dynamic free-state equilibrium to the bound state ensemble. Examples include the replication machinery of Measles virus, where the highly (>70%) disordered phosphoprotein initiates transcription and replication exploiting weak interactions with ordered and disordered domains of the nucleoprotein,[6,7] the nuclear pore, where weak interactions between the nuclear transporter and highly flexible chains containing multiple ultra-short recognition motifs, facilitate fast passage into the nucleus.[8] and large-scale domain dynamics in Influenza H5N1 polymerase are essential for import into the nucleus of the infected cell.[9]

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