Telluride Science Research Center
2023Protein Dynamics Workshop
July 24-28, 2023
Arthur Palmer and Takahisa Yamato

Participants (19 lectures)

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Workshop Schedule
(All sessions are at the Intermediate School, 725 West Colorado Ave.)

Sunday, July 23, 5:00-6:30 pm
All-Telluride Science Meet and Greet
Location: **Oak** – **250 W San Juan Avenue (at the base of the gondola)**
This is a good chance to meet up with fellow participants prior to your meeting. A staff member will be on hand to welcome you and distribute badges.

Monday, July 24

7:30-8:30 Breakfast at TSRC (Intermediate School)

8:30-9:00 Opening comments

Session 1: John Straub, Moderator

9:00-9:50	Takahisa Yamato	Energy and heat flow in proteins
9:50-10:20	Break	
10:20-11:10	Rafael Brüschweiler	Quantitative prediction of ensemble dynamics, shapes and contact propensities of intrinsically disordered proteins
11:10-12:00	Nozomi Ando	Correlated atomic displacements in proteins

12:00-14:00 Lunch (not provided)

Session 2: Toshifumi Mori, Moderator

14:00-14:50	Akio Kitao	Rotational mechanisms of bacterial flagellum and roles of water
14:50-15:40	Douglas Tobias	Voltage-dependent structural dynamics of the Hv1 Proton Channel
15:40-16:10	Break	
16:10-17:00	John Straub	Understanding amyloid protein aggregation from the perspective of the monomer conformational ensemble

Tuesday, July 25

7:30-9:00 Breakfast at TSRC (Intermediate School)

9:00-14:00 Free time

Session 3: Ilme Schlichting, Moderator

14:00-14:50	Megan Thielges	Site-selective infrared spectroscopy to investigate protein conformations and dynamics
14:50-15:40	Philip Anfinrud	Probing biomolecule structure and dynamics as a function of temperature via time-resolved small- and wide-angle X-ray

		scattering
15:40-16:10	Break	
15:10-17:00	Tomotaka Oroguchi	Optimization of MD-derived conformational ensemble in information content space and its application to experimental X-ray solution scattering data

18:30-19:20 Telluride Science Town Talk

THE TRUE COLORS OF CANCER: Imaging New Biomarkers with Light, not Labels
Stephen Boppart, M.D, PH.D
Professor and Grainger Distinguished Chair in Engineering
University of Illinois Urbana-Champaign

Location: Telluride Conference Center in Mountain Village
Cash Bar, Doors Open at 18:00.

Wednesday, July 26

7:30-9:00 Breakfast at TSRC (Intermediate School)

Session 4: Megan Thielges, Moderator

9:00-9:50	Martin Weik	Photoswitchable fluorescent proteins: What we have learnt through time-resolved serial crystallography
9:50-10:40	Jasper van Thor	Coherence Structure from non-linear optical crystallography and Coherent control of ultrafast structural dynamics with femtosecond X-ray crystallography
10:40-11:10	Break	
11:10-12:00	Paul Fenimore	TBA

12:00-14:00 Lunch break (not provided)

Session 5: Jasper van Thor, Moderator

14:00-14:50	Ilme Schlichting	Mechanism and dynamics of fatty acid photodecarboxylase
14:50-15:40	Takayuki Nishizaka	Insights into the mechanism of ATP-driven rotary motors from direct torque measurement
15:40-16:10	Break	
16:10-17:00	Ryota Iino	Single-molecule analysis and engineering of molecular motor proteins

17:30-19:30 All Telluride Science Picnic

Free BBQ, Beer, Wine and Non-Alcoholic Beverages. Friends and Family are invited free of charge.

Location: Tent behind the Intermediate School.

Thursday, July 27

7:30-9:00 Breakfast at TSRC (Intermediate School)

9:00-14:00 Free time

Session 6: Takahisa Yamato, Moderator

14:00-14:50	Toshifumi Mori	Dissecting the heterogeneous dynamics of proteins during folding and catalysis
14:50-15:40	Francesca Massi	The CCCH-type zinc finger proteins: functional insights from an ancient family of post-transcriptional regulators
15:40-16:10	Break	
16:10-17:00	Shigehiko Hayashi	Atomistically deciphering functional activation processes of transporter proteins with molecular simulations
17:00-17:50	Arthur Palmer	Conformational dynamics in molecular recognition

17:50-18:20 Summing up, selection of next organizing committee

Friday, July 28

7:30-9:00 Breakfast at TSRC (Intermediate School)

Workshop Abstracts

Correlated Atomic Displacements in Proteins

Nozomi Ando
Chemistry & Chemical Biology
Cornell University

Correlated motions in proteins arising from the collective movements of residues have long been proposed to be fundamentally important to key properties of proteins, from catalysis and allostery to evolvability. Recent breakthroughs in structural biology have made it possible to capture proteins undergoing complex conformational changes, yet the subtle and intrinsic fluctuations within a single conformation remain one of the least understood facets of protein structure. For many decades, the analysis of diffuse X-ray scattering held the promise of animating crystal structures with such correlated motions. With recent advances in both X-ray detectors, this long-held promise can now be met. In this talk, I'll describe our recent work in interpreting this signal and discuss how it can be used to probe structural fluctuations that give rise to fundamental protein properties.

Probing Biomolecule Structure and Dynamics as a Function of Temperature via Time-resolved Small- and Wide-Angle X-ray Scattering

Philip Anfinrud, Hyun Sun Cho, Friedrich Schotte, and Eli Worth
National Institutes of Health, NIDDK, Laboratory of Chemical Physics

Life flourishes over a broad range of temperatures spanning from around -2 Celsius for arctic fish to 122 Celsius for hyperthermophillic *Methanopyrus kandleri*. To understand how biomolecules function over such a broad temperature range, it is crucial to develop methods capable of probing temperature-dependent structure and structure dynamics at extreme temperatures. To that end, we continue to refine x-ray scattering methods recently developed on the BioCARS beamline at the Advanced Photon Source to probe biomolecule structural dynamics in solution. For example, Small Angle X-ray Scattering (SAXS) data can unveil biomolecule size and shape as well as biomolecule-biomolecule interactions. Moreover, Wide Angle X-ray scattering (WAXS) data can be used to characterize sample temperature and secondary structure. We have acquired temperature-dependent and time-resolved x-ray scattering data from proteins of various sizes as well as from various RNA constructs including hairpins and dumbbells. The structural details extracted from these SAXS/WAXS studies extend beyond that accessible with other biophysical techniques, and provide a wealth of information that can help unveil mechanistic details of protein folding/unfolding and function.

Quantitative Prediction of Ensemble Dynamics, Shapes and Contact {ropensities of Intrinsically Disordered Proteins

Rafael Brüschweiler
Departments of Chemistry & Biochemistry and Biological Chemistry & Pharmacology
The Ohio State University

Intrinsically disordered proteins (IDPs) are highly dynamic systems that play an important role in cell signaling processes and their misfunction often causes human disease. Proper understanding of IDP function not only requires the realistic characterization of their three-dimensional conformational ensembles at atomic-level resolution but also of the time scales of interconversion between their conformational substates. Large sets of experimental data are often used in combination with molecular modeling to restrain or bias models to improve agreement with experiment. It is shown for the N-terminal transactivation domain of p53 (p53TAD) and Pup, which are two IDPs that fold upon binding to their targets, how the latest advancements in molecular dynamics (MD) simulations methodology produces native conformational ensembles by combining replica exchange with series of microsecond MD simulations. They closely reproduce experimental data at the global conformational ensemble level, in terms of the distribution properties of the radius of gyration tensor, and at the local level, in terms of NMR properties including ^{15}N spin relaxation, without the need for reweighting. The IDP ensembles were analyzed by graph theory to identify dominant inter-residue contact clusters and characteristic amino-acid contact propensities. These findings indicate that modern MD force fields with residue-specific backbone potentials can produce highly realistic IDP ensembles sampling a hierarchy of nano- and picosecond time scales providing new insights into their biological function.

Atomistically Deciphering Functional Activation Processes of Transporter Proteins with Molecular Simulations

Shigehiko Hayashi
Graduate School of Science
Kyoto University, Japan

Activation processes of protein functions are often fulfilled by dynamic and global molecular conformational changes of complex protein systems which correlate with local molecular events at chemically active sites. Hence the multi-scale functional coupling of local chemical events with protein global molecular dynamics needs to be revealed for understanding of molecular nature of protein functions.

In this talk, I will present our recent studies on activation processes of photo-sensitive ion transporters by a hybrid QM/MM free energy geometry optimization technique, which allows one to optimize electronic wave function and molecular geometry of the chemically active sites 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 [1].

We succeeded in theoretically predicting photo-activated structural changes of a photo-sensitive cation membrane channel, C1C2 channelrhodopsin, which remarkably differ from previously known conformational changes in other homologous proteins [2] and were verified by a recent time resolved serial femtosecond X-ray crystallography measurement [3]. We also simulated photo-activation processes of anion membrane transporters and revealed significant conformational changes of the channels. We first

examined protonation states of carboxy groups in the channel which strongly affect transport properties of ions. Through excitation energy calculations of various mutants, we determined the protonation states which are consistent with experimental evidence. We also succeeded in modeling a photo-activated channel opening structure by the hybrid QM/MM free energy optimization with a long-time MD simulation. Free energy profiles of the ion transport in the channel opening state were then obtained by umbrella sampling MD simulations, revealing atomistic dynamics and energetics of the ion transport process.

I will also present an atomistic molecular dynamics (MD) study of alternating access of mitochondria ADP/ATP transporter with a linear response path following method [4,5] which is a biasing MD technique accelerating global protein conformational changes coupled to local ligand binding events. The proposed structural model of the alternating access state has been shown to successfully predict at atomic resolution an X-ray crystallographic one reported later.

[1] Hayashi et al., *Annu. Rev. Phys. Chem.* **68**, 135 (2017). [2] Cheng et al., *Biophys. J.*, **115**, 1281 (2018). [3] Oda et al., *eLife*, **10**, e62389 (2021). [4] Tamura and Hayashi, *J. Chem. Theory and Comput.*, 11, 2900-2917 (2015). [5] Tamura and Hayashi, *PLOS ONE*, 12, e0181489 (2017)

Single-molecule Analysis and Engineering of Molecular Motor Proteins

Ryota Iino
Institute for Molecular Science
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Molecular motors, an important class of molecular machines, harness various energy sources to move unidirectionally [1]. The operational principles of molecular motors are distinct from those of man-made macroscopic motors, because they have nanoscale dimensions and generally work in a solution environment where viscosity is dominant. Under these low Reynolds number, overdamped conditions, they cannot rely on inertia to sustain motion. Furthermore, they are continually agitated by random Brownian motion, which provides both challenges and opportunities for the unidirectional motion.

To understand the operational principles of molecular motor proteins, we are developing single-molecule imaging techniques which enable visualization of their fast and dynamic motions with microsecond time resolution and angstrom localization precision [2, 3]. Furthermore, to understand their design principles and to outperform natural ones, we are engineering non-natural molecular motor proteins by using saturation mutagenesis [4], hybrid/chimera generation [5], and computational design [6]. In this seminar, I will overview our approaches by introducing our results on rotary and linear motors such as V-ATPase [7, 8], processive chitinase [9], kinesin [10], and dynein [11].

1. Iino R, et al., ***Chem Rev***, 120, 1-4 (2020)
2. Ando J, et al., ***Biophys J***, 115, 2413-2427 (2018)
3. Ando J, et al., ***ACS Photonics***, 6, 2870-2883 (2019)
4. Visootsat A, et al., ***ACS Omega***, 5, 26807–26816 (2020)
5. Baba M, et al., ***PNAS***, 113, 11214-11219 (2016)
6. Kosugi T, et al., ***Nat Chem***, in press
7. Iida T, et al., ***J Biol Chem***, 294, 17017-17030 (2019)
8. Otomo A, et al., ***Proc Natl Acad Sci USA*** 119, e2210204119 (2022)
9. Nakamura A, et al., ***Nat Commun***, 9, 3814 (2018)

10. Isojima H, et al., ***Nat Chem Biol***, 12, 290-297 (2016)
11. Ando J, et al., ***Sci Rep***, 10, Article number: 1080 (2020)

Rotational mechanisms of bacterial flagellum and roles of water

Akio Kitao
School of Life Science and Technology
Tokyo Institute of Technology, Japan

Many pathogenic bacteria, such as *Escherichia coli* and *Salmonella*, use bacterial flagella to propel. Bacterial flagellum is a large molecular machine, whose length can be 10 micrometers long, and it is an assembly of ~30,000 molecules of more than 30 different proteins. For cell motility, bacterial flagellum employs a transport apparatus to construct a giant molecular assembly, converts ionic gradient energy into rotational force, and transmits the rotational force via a flagellar rod and hook (universal joint) to rotate the flagellar filament (screw). The rotation is controlled by chemotaxis signals, and cell motility is regulated by changing the helical nature of the flagellar filament.

We investigated the mechanisms by which the flagellar rod transmits torque supported by the flagellar bushing, allowing rotation at low energy cost with large-scale molecular dynamics simulations. 40 nanosecond molecular dynamics simulations were performed from the initial states of 140 different rotation angles of rod-hook complex. The free-energy landscape of the rotation and the time required for the rotation were calculated by the Markov state model. As a result, it was found that the rod-hook complex can rotate at ~ 1900 Hz, which is consistent with the time obtained experimentally so far. We are currently analyzing the interactions between the rod-hook complex and the LP ring mediated by water molecules in between, which enables rotations at low energy cost while maintaining the structure of the large flagellar complex.

The CCCH-type Zinc Finger Proteins: Functional Insights from an Ancient Family of Post-transcriptional Regulators

Francesca Massi
Biochemistry and Molecular Biotechnology
UMass Chan Medical School

CCCH-type tandem zinc finger (TZF) domains are found in many proteins that are involved in transcriptional regulation and splicing. We have discovered that these TZF domains have evolved to have different propensities toward intrinsic disorder and recognize diverse RNA sequences. We investigate how the structure and dynamics of these TZF domains impact differential RNA recognition, localization, and turnover and allow for regulation of the protein activity.

Dissecting the Heterogeneous Dynamics of Proteins During Folding and Catalysis

Toshifumi Mori
Institute for Materials Chemistry and Engineering
Kyushu University, Japan

While the structures of proteins have been critical for understanding functions, the importance of dynamics has become evident over the last decades. Molecular dynamics simulation is a powerful tool in studying the structures and functions of biomolecules in molecular detail, but most computational studies have focused on the static (free energy) and kinetic perspectives while often omitting the dynamic aspect. Furthermore, how protein dynamics participate in enzyme catalysis have been highly debated [1,2].

To decipher the role of dynamics in protein function, we have been developing theoretical approaches and studying the dynamic aspect of proteins using molecular dynamics simulations. Here I discuss the dynamics and its role behind protein folding [3,4] and enzyme catalysis [5,6,7]. The analyses of protein folding trajectories shows that protein folding occurs via multiple pathways, and furthermore, the transitions occur in a highly heterogeneous manner. The studies of the enzymatic reaction catalyzed by the peptidyl-prolyl isomerase Pin1, show that protein conformational transition precedes the reaction event, i.e., isomerization [5]. The lag in protein and ligand dynamics in enzyme catalysis occurs due to the separation of the temporal scales. Thus, the distinctive protein conformation necessary for the reaction to proceed is only set up as a conformational excited state. Further analysis of the slow dynamics of Pin1 indicates that changes in the equilibrium distribution of the Pin1's conformational ensembles before and after the reaction results in a seeming coupling of protein dynamics and chemical reactions during catalysis [7,8].

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2. A. Warshel, A. R. P. Bora, *J. Chem. Phys.* **144**, 180901 (2016).
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4. T. Mori, S. Saito, *J. Phys. Chem. B* **120**, 11683 (2016)
5. T. Mori, S. Saito, *J. Phys. Chem. Lett.* **10**, 474–480 (2019).
6. T. Mori, S. Saito, *J. Chem. Theory Comput.* **16**, 3396–3407 (2020).
7. T. Mori, S. Saito, *J. Phys. Chem. B* **126**, 5185–5193 (2022)
8. W. Labeikovsky et al., *J. Mol. Biol.* **367**, 1370–1381 (2007).

Insights into the Mechanism of ATP-driven Rotary Motors from Direct Torque Measurement

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Department of Physics, Gakushuin University, Japan

Motor proteins are molecular machines that convert chemical energy into mechanical work. In addition to existing studies performed on the linear motors found in eukaryotic cells, researchers in biophysics have also focused on rotary motors such as bacterial flagellar motor (BFM) and F_1-ATPase. The research group including Nishizaka contributed to correlate all chemical states to specific mechanical events by visualizing single chemical reactions under the advanced optical microscope [1-3]. From the structural point of view, conformational changes of β-subunit [4,5] and behaviours of the γ-shaft [6-8] were also addressed through a series of microscopy techniques at the single molecular level. Recent

studies showed that there exists another ATP-driven protein motor in life: the rotary machinery that rotates archaeal flagella (archaella). None of the archaellar motility structure is homologous to any BMF proteins. Rotation speed, stepwise movement, and variable directionality of the motor of *Halobacterium salinarum* were described in previous studies [9]. We further presented the experimental work discerning the molecular mechanism underlying how the archaellar motor protein FlaI drives rotation by generation of motor torque [10]. In combination, those studies found that rotation slows as the viscous drag of markers increases, but torque remains constant at 160 pN·nm independent of rotation speed. Unexpectedly, the estimated work done in a single rotation is twice the expected energy that would come from hydrolysis of six ATP molecules in the FlaI hexamer. To reconcile the apparent contradiction, a new and general model for the mechanism of ATP-driven rotary motors [11] will be discussed.

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3. Adachi, K., K. Oiwa, M. Yoshida, T. Nishizaka, K. Kinosita, Jr., *Nat Commun*, 2012. **3**: p. 1022.
4. Masaike, T., F. Koyama-Horibe, K. Oiwa, M. Yoshida, T. Nishizaka, *Nat Struct Mol Biol*, 2008. **15**(12): p. 1326-33.
5. Sugawa, M., K. Okazaki, M. Kobayashi, T. Matsui, G. Hummer, T. Masaike, T. Nishizaka, *Proc Natl Acad Sci USA*, 2016. **113**(21): p. E2916-24.
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7. Naito, T.M., T. Masaike, D. Nakane, M. Sugawa, K.A. Okada, T. Nishizaka, *Sci Rep*, 2019. **9**(1): p. 7451.
8. Hasimoto, Y., M. Sugawa, Y. Nishiguchi, F. Aeba, A. Tagawa, K. Suga, N. Tanaka, H. Ueno, H. Yamashita, R. Yokota, T. Masaike, T. Nishizaka, *Biophys J*, 2023. **122**(3): p. 554-564.
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10. Iwata, S., Y. Kinosita, N. Uchida, D. Nakane, T. Nishizaka, *Commun Biol*, 2019. **2**: p. 199.
11. Nishizaka, T., T. Masaike, D. Nakane, *Biophys Rev*, 2019. **11**(4): p. 653-657.

Optimization of MD-derived Conformational Ensemble in Information Content Space and its Application to Experimental X-ray Solution Scattering Data

Tomotaka Oroguchi
Department of Physics, Keio University, Japan

The information on protein conformational ensemble is essential for understanding the mechanisms of protein function. MD simulation is a powerful technique to acquire such information. However it is often difficult for MD simulations to provide a conformational ensemble that can reproduce experimental data, due to the low reliability of force fields and low efficiency of conformational sampling. Therefore, optimization of the MD-derived conformational ensemble is required to achieve consistency with experimental data. In this study, we propose an optimization method using the information content space. In the method, the ensemble model as a probability density distribution is first projected on the

information content space. Then the model is iteratively moved toward the optimized model in this space according to a newly introduced Lagrangian, which was constructed based on the consistency between the model and experimental data. To evaluate the validity of the algorithm, simulations of the optimization process assuming X-ray solution scattering (SAXS) as an experiment were performed using test conformational ensembles generated via coarse-grained MD. These simulations demonstrated the possibility of obtaining true conformational ensembles using our method with SAXS data. We also applied the method to experimental SAXS data of glucosamine kinase, and revealed the changes in conformational ensemble on substrate binding.

Conformational dynamics in molecular recognition

Arthur Palmer
Biochemistry and Molecular Biophysics
Columbia University

Recognition of substrates and other ligands is an essential part of the mechanisms of biological function of proteins, in processes including catalysis, regulation, and signal transduction. Interactions between proteins and other molecules frequently require conformational changes in either protein or target or both, or more precisely a shift in the conformational ensemble between the apo and holo states of the system. NMR spectroscopy and molecular dynamics (MD) simulations are complementary experimental and computational methods, respectively, for describing conformational dynamics of proteins with atomistic and temporal resolution. The latest results in on-going studies of the ribonuclease H superfamily and the GCN4 transcription factor will be presented as model systems for elucidating linkages between conformational dynamics and molecular recognition by proteins.

Mechanism and Dynamics of Fatty Acid Photodecarboxylase

Ilme Schlichting
Max Planck Institute for Medical Research, Germany

Light is important for organisms from all domains of life, serving as an energy resource or carrier of information initiating intra- or intercellular signaling. Photosensitive proteins, endowed with a light-absorbing chromophore, enable this. Detailed insights, including the initial events on the ultrafast time scale, can be obtained by various forms of spectroscopy and computation. However, direct structural information necessary to understand the underlying molecular mechanisms has been inaccessible until recently. The unique properties of X-ray free electron lasers open the sub-ps time domain for time-resolved crystallography using small crystals that can be efficiently photolyzed, thus providing access to the long sought-after excited state and intermediate structures. Photodecarboxylation is a well-established reaction in chemistry; however, no photo-enzymatic equivalent was known until the discovery of Fatty Acid Photodecarboxylase (FAP), a flavin containing photoenzyme [1]. The enzymatic mechanism was investigated in detail by a large interdisciplinary consortium [2]: decarboxylation occurs directly upon reduction of the photo-excited flavin by the fatty acid substrate. Along with flavin reoxidation by the alkyl radical intermediate, a major fraction of the cleaved carbon dioxide unexpectedly transforms in 100 ns into another species, assigned to be bicarbonate based on IR-spectroscopy performed on cryogenic samples. Despite a great deal of insight into

the catalytic mechanism and the role of two strictly conserved residues for substrate stabilization and functional charge transfer [2], a number of questions remain. To address these, including the nature of the transiently generated CO_2-derivative we performed a follow-up time-resolved serial femtosecond crystallography experiment at SwissFEL spanning time-delays from ns to ms after photoexcitation. The results will be discussed.

[1] Sorigué et al An algal photoenzyme converts fatty acids to hydrocarbons, Science 357: 903-907 (2017)
[2] Sorigué et al Mechanism and dynamics of fatty acid photodecarboxylase, Science 372, **eabd5687** (2021)

Understanding Amyloid Protein Aggregation from the Perspective of the Monomer Conformational Ensemble

John Straub
Boston University

A crucial step in the aggregation of amyloidogenic sequences is the transition from a relatively disordered state to assembly-competent structures referred to as N* states. The assembly-competent N* states share key structural characteristics with the protein monomer in the fibril environment as observed in experiments. Using a coarse-grained model for intrinsically disordered proteins (IDPs), we demonstrate that the N* states exist as rare excited states on the monomer free energy landscape. By relating the populations of the N* states to fibril formation time scales, using an empirical relation derived from lattice simulations, we quantitatively correlate the probability of observing the N* states with the relative aggregation propensities. Using kinetic transition networks, we observe that the N* states are obligatory along the favored assembly routes. I will discuss recent applications of N* theory to explore the origins of polymorphism in fibrils composed of amyloid-β (Aβ) peptide and fibrils composed of fused-in-sarcoma (FUS) peptide.

Site-selective Infrared Spectroscopy to Investigate Protein Conformations and Dynamics

Megan Thielges
Department of Chemistry
Indiana University

To fully uncover the role of protein dynamics in function, experimental approaches must contend with both the complex spatial heterogeneity of proteins and the rapid interconversion of potentially important states. To address these challenges, our group combines the inherent temporal resolution of linear and two-dimensional infrared spectroscopy with the spatial resolution afforded by site-selective incorporation of vibrational reporter groups. This enables detection of individual vibrations to probe specific locations and capture of even rapid dynamics throughout proteins. I will share how we apply this approach to investigate the functional role of dynamics in protein molecular recognition and catalysis.

Voltage-dependent structural dynamics of the Hv1 Proton Channel

Andrew Geragotelis,[1] Mona L. Wood,[1] Hendrik Goeddeke,[1] Quinn Nguyen,[1] Eric K. Wong,[1]
J. Alfredo Freites,[1] Liang Hong,[2] Parker D. Webster,[2] Francesco Tombola,[2]
and Douglas J. Tobias[1]
Departments of Chemistry[1] and Physiology and Biophysics[2]
University of California, Irvine

The voltage-gated Hv1 proton channel is a ubiquitous membrane protein that plays roles in a variety of cellular processes, including proton extrusion, pH regulation, production of reactive oxygen species, proliferation of cancer cells, and increased brain damage during ischemic stroke. Like voltage-gated metal ion channels, Hv1 contains a voltage-sensing domain (VSD), but it lacks a separate ion-conducting pore domain. The functional unit of Hv1 is a dimer in which the monomers are connected by a coiled-coil domain (CCD). Proton conduction occurs within each of the two VSDs and monomeric constructs lacking the CCD also conduct protons. The Hv1 VSDs open and close in response to changes in the transmembrane (TM) electric potential. In order to elucidate the voltage-dependent structures and the transition between the closed and open states of Hv1, we carried out multi-microsecond molecular dynamics (MD) simulations of the human Hv1 (hHv1) monomer under physiologically relevant applied electric fields in an explicit membrane environment. The crystal structure of the mouse Hv1 chimera (mHv1cc), which shares a sequence identity of 65% with hHv1 in the TM regions, was used as a template for homology modelling of hHv1. In simulations of the unpolarized and hyperpolarized/resting states, the hHv1 monomer contains a complex salt-bridge network and extensive internal hydration. In response to a depolarizing membrane potential the voltage-sensing S4 helix undergoes a large outward displacement, leading to changes in the VSD internal salt-bridge network, resulting in a reshaping of the proton permeation pathway and a significant increase in the hydrogen-bond connectivity throughout the channel. The total gating charge displacement associated with this transition is consistent with experimental estimates. Docking calculations confirm the proposed mechanism for the inhibitory action of the first known Hv1 blocker, 2-guanidinobenzimidazole, derived from electrophysiology measurements and mutagenesis. Our depolarized structural model was also validated with the formation of a metal bridge between residues located in the VSD. Taken together, our results suggest that the structures sampled in our MD simulations are representative of the closed and open states of the Hv1 proton channel. Preliminary results from our on-going efforts to model the Hv1 dimer will also be presented.

Coherence Structure from Non-linear Optical Crystallography and Coherent Control of Ultrafast Structural Dynamics with Femtosecond X-ray Crystallography

Jasper van Thor
Imperial College, London

Non-linear spectroscopy measures the electronic and nuclear configuration but lacks molecular structure information. X-ray crystallography determines molecular structure and can also measure its dynamics, but lacks the electronic and nuclear configuration information. Therefore crystallography can learn from spectroscopy and spectroscopy can learn from crystallography.

We have demonstrated a coherent control experiment of vibrational coherence with ultrafast X-ray crystallographic observation [1]. The photoisomerisation reaction of a fluorescent protein chromophore includes ultrafast barrier crossings and the avoidance of

internal conversion processes. The relationship between experimentally observed ultrafast nuclear motions and the biological reaction coordinate is not known. High resolution pump-probe X-ray crystallography measurements reveal complex sub-Ångström ultrafast motional dynamics and hydrogen bonding rearrangements in the active site. However, we demonstrate that the measured motions are not part of the reaction coordinates but instead arise from impulsively driven coherent vibrational processes of the electronic ground state. We apply the analysis of impulsive stimulated Raman spectroscopy and coherent control methodology to the real-space observation of low frequency vibrational dynamics and displacements, that we have measured by ultrafast X-ray crystallography. Using the 'Tannor-Rice' coherent control method it is shown that ultrafast motions can be strongly amplified. This demonstrates that the conventional pump-probe measurements, without application of optical control, are dominated by electronic ground state displacements that are unrelated to the reactive photoisomerisation coordinate. We present the first application of coherent control using X-ray crystallography in combination with coherence theory that has application to the real space wavepacket observation. This allows the unambiguous assignment of the structural changes observed in ultrafast X-ray crystallography.

Coherence analysis of non-linear spectroscopy can add structural information in the x-ray crystallographic frame if measurements are made on oriented single crystals [1-5]. The third order response of crystals is generally different from measurements of isotropic solutions because each coherence path that contributes to the measured field scales to the ensemble average of the four-point correlation functions of the four field-dipole interactions involved in the respective Feynman paths. The application of a symmetry operator to the point correlation function allows the explicit calculation of the structural dependence of all contributing Feynman diagrams and the total signal field [5]. This level of structural analysis is possible for non-linear optical crystallography but is also has applications for time resolved serial femtosecond x-ray crystallography.

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Photoswitchable Fluorescent Proteins: What We Have Learnt Through Time-resolved Serial Crystallography

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Reversibly photoswitchable fluorescent proteins (RSFP) are essential tools in advanced fluorescence nanoscopy of live cells (such as PALM and RESOLFT). They can be repeatedly toggled back and forth between a fluorescent (*on*) and a non-fluorescent (*off*) state by irradiation with light at two different wavelengths.

We combined time-resolved serial femtosecond crystallography (TR-SFX), ultrafast absorption spectroscopy in solution and in microcrystals and simulation methods to study *off*-to-*on* photoswitching mechanisms in the RSFP rsEGFP2 on the nano- to picosecond time scale. *Off*-to-*on* photoswitching proceeds *via* excited-state isomerisation, as evidenced by capturing an intermediate with a fully twisted chromophore 1 ps after photon absorption [1], followed by ground-state chromophore deprotonation [2]. Through the TR-SFX studies we discovered that the *cis* chromophore of rsEGFP2 isomerizes to two distinct *off*-state conformations, *trans1* and *trans2*, located on either side of the V151 side chain that was shown to transiently retract on the picosecond time scale during *off*-to-*on* photoswitching [1]. Reducing or enlarging the side chain at this position (V151A and V151L variants) leads to single *off*-state conformations (*trans1* and *trans2*, respectively [3]). The V151A variant was shown to exhibit higher photoswitching contrast, both *in vivo* and *in vitro*, a property of prime interest for RESOLFT nanoscopy [3]. Our studies shed light on the structural dynamics involved in *off*-to-*on* photoswitching and suggests a means to rationally improve fluorescent proteins for nanoscopy applications. Currently, we are studying the influence of the pump-laser fluence on structural changes seen in rsEGFP2, a much-debated issue in the TR-SFX field.

The work presented involves a multi-national consortium composed by the authors of references [1 – 3].

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Energy and Heat Flow in Proteins

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Although thermal energy transport is among the essential biophysical properties of proteins, its relationship with protein structure, dynamics, and functions is still elusive [1]. Within thermally fluctuating protein molecules, tightly packed amino acid residues interact with each other through heat and energy exchanges [2]. The structures of folded proteins are highly inhomogeneous, giving rise to anisotropic and non-uniform flow of thermal energy during conformational fluctuations. To illustrate such features of proteins, we have developed a theoretical framework and computer software (https://curp.jp) for analyzing local thermal transport properties based on the autocorrelation function formalism. This method has been applied to a wide range of biophysical problems [3–7].

Regarding thermal energy transport mechanism of proteins, there still remains an ongoing debate as to what determines dominant pathways [8, 9]. Recently, we applied our computational method to a small protein domain, villin headpiece [9], and evaluated residue-wise thermal conductivities [10]. As a result, we observed their distinct residue-type dependence [11]. Furthermore, we calculated residue-residue thermal conductivities and evaluated the importance of the contact distance, the variance of contact distance, and H-bonding occurrence probability by using the random forest model, a machine learning algorithm [12].

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