Deciphering complex energy landscape and kinetic network from single molecules to cells : a new challenge to make theories meet experiments

3-8 September 2017, Dijon

Organizers :

Patrick SENET, Université de Bourgogne
Tamiki KOMATSUZAKI, Hokkaido University

Schedule

September 3 (Sunday)
16:00-18:00 Registration
19:00 **Welcome apéritif**

September 4 (Monday)
Session Chair: Gia Maisuradze
8:40-8:45 Welcome (Patrick, Tamiki)
8:45- 9:30 Robert Jernigan
9:30-10:00 Break (30')
10:00-10:45 Takahisa Yamato
10:45-11:30 Patrick Senet
12:00-14:00 **Lunch**

Session Chair: Matt Comstock
14:00-14:45 Katsumasa Fujita
14:45-15:30 Douglas Shepherd
15:30-16:10 Break (40')

Session Chair: Dmitrii Makarov
16:10-16:55 Matt Comstock
16:55-17:40 Roland Stote

September 5 (Tuesday)
Session Chair: Tamiki Komatsuzaki
8:45- 9:30 Atsushi Mochizuki
9:30-10:00 Break (30')
10:00-10:45 M. Dauchez
10:45-11:30 Kenji Okamoto
12:00-14:00 **Lunch**

Session Chair: Takahisa Yamato
14:00-14:45 Mikko Karttunen
14:45-15:30 Paul Grassein
15:30-16:10 Break (40')
16:10-16:55 Jerelle Joseph
16:55-17:40 Fabio Sterpone
19:00 Wines & cheese tasting

September 6 (Wednesday)
Session Chair: Steve Presse
8:45- 9:30 Dmitrii Makarov
9:30-10:00 Break (30')
10:00-10:45 Sosuke Ito
10:45-11:30 David Allouche
12:00-14:00 **Lunch**

Session Chair: Sosuke Ito
14:00-14:45 Steve Presse
14:45-15:30 Gilad Haran
15:30-16:10 Break (40')

Session Chair: Benjamin Schuler
16:10-16:55 Ken Ritchie
16:55-17:40 Kingshuk Ghosh

September 7 (Thursday)
Session Chair: Gilad Haran
8:45- 9:30 Benjamin Schuler
9:30-10:00 Break (30')
10:00-10:45 Satoshi Takahashi
10:45-11:30 Tamiki Komatsuzaki
12:00-14:00 **Lunch**
14:30 car to Beaune (excursion)
19:00 Wine tasting and banquet in Beaune

September 8 (Friday)
Session Chair: Katsumasa Fujita
8:45- 9:30 Aymeric Leray
9:30-10:00 Break (30')
10:00-10:45 Adrien Nicolai
10:45-11:30 Gia Maisuradze
11:30-12:00 Open Questions & Discussions (Benjamin Schuler, Dmitrii Makarov, Ken Ritchie, Takahisa Yamato, Mikko Karttunen, Alain Dereux, etc)

Titles of the talks

MONDAY

Building Landscapes from Known Structures, Robert Jernigan (jernigan@iastate.edu)

Protein Dynamics: Energy Exchange Network in the Folded State and Mechanical Unfolding, Takahisa Yamato (yamato@nagoya-u.jp)

Protein dynamics and function: all-atom molecular dynamics meets experiments, Patrick Senet (psenet@u-bourgogne.fr)

Raman microscopy: a new tool for molecular imaging of cells and tissues, Katsumasa Fujita (fujita@ap.eng.osaka-u.ac.jp)

Inferring system dynamics from stop motion single-cell data, Douglas Shepherd (douglas.shepherd@ucdenver.edu)

High-resolution optical trapping and fluorescence investigations of protein folding complexity, Matt Comstock (comstock@pa.msu.edu)

Integrating molecular dynamics simulations and spectroscopy to study biomolecular dynamics, Roland Stote (rstote@igbmc.fr)

TUESDAY

Structural analysis of sensitivity of chemical reaction networks, Atsushi Mochizuki (mochi@riken.jp)

Numerical simulations of extracellular matrix peptides and proteins, M. Dauchez (manuel.dauchez@univ-reims.fr)

Single-molecule FRET measurement for EGFR-RAS-MAPK signal transduction pathway, Kenji Okamoto (okamotok@riken.jp)

Protein conformations by computer simulations, Mikko Karttunen (mkarttu@uwo.ca)

Response of Glutathione S-Transferases upon substrate binding: a dynamical approach of flexibility, Paul Grassein (paul.grassein@gmail.com)

Exploring Large-scale Conformational Changes in Proteins: The Potential Energy Landscape Approach, Jerelle Joseph (jaj52@cam.ac.uk)

Toward microscopic simulations of cell-like environments. A Lattice Boltzmann Molecular Dynamics strategy, Fabio Sterpone (sterpone@ibpc.fr)

WEDNESDAY

Transition path times reveal memory effects and anomalous diffusion in the dynamics of protein folding, Dmitrii Makarov (makarov@cm.utexas.edu)

Thermodynamics of information on biochemical signaling networks, Sosuke Ito (sosuke.ito@es.hokudai.ac.jp)

Euclidean Variable Neighborhood Search: A method for large computation protein design, David Allouche (david.allouche@toulouse.inra.fr)

Infinity makes the cut: Bayesian nonparametrics for protein biophysics, Steve Presse (spresse@asu.edu)

Understanding fast functional dynamics of proteins with single-molecule FRET and HMM, Gilad Haran (gilad.haran@weizmann.ac.il)

High-speed single molecule tracking of proteins in E. coli, Ken Ritchie (kpritchie@purdue.edu)

Using polymer physics to investigate the folded and unfolded proteome, Kingshuk Ghosh (Kingshuk.Ghosh@du.edu)

THURSDAY

Binding without folding: Extreme disorder and dynamics in a high-affinity protein complex, Benjamin Schuler (schuler@bioc.uzh.ch)

Dynamics of protein folding studied by single molecule fluorescence measurements at microsecond resolution, Satoshi Takahashi (st@tagen.tohoku.ac.jp)

Energy landscapes learned from single molecule FRET time series: Role of Photobleaching, Tamiki Komatsuzaki (tamiki@es.hokudai.ac.jp)

FRIDAY

Temporal and spectral analysis of enhanced Raman scattering data for accessing sub-molecular information, Aymeric Leray (aymeric.leray@u-bourgogne.fr)

Computational investigation of the translocation of single polypeptide through MoS2 nanopores from ionic conductance dynamics, Adrien Nicolaï (adrien.nicolai@u-bourgogne.fr)

Recent developments in elucidating mechanisms of protein misfolding diseases, Gia Maisuradze (gm56@cornell.edu)

Building Landscapes from Known Structures

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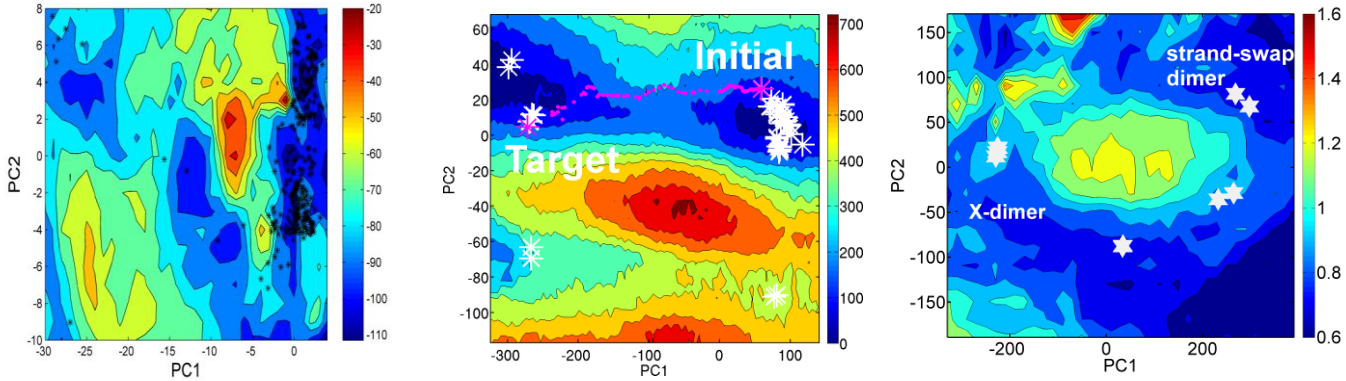

A B C

Three cases of landscapes derived from experimental sets of structures. *A) HIV-1 Protease where open forms are shown on the left and closed forms on the right as black stars, B) GroEL subunit with ATP bound forms on the right and ADP forms on the left as white stars, and C) E-cadherin structures as white stars.*

Sets of experimental protein structures usefully describe the conformations accessible for a given type of protein structure. These structures can include unbound, bound, open, closed, and mutant structures, in general. Overall it is important to have more than 50 related structures, but there are many proteins where there are already more than 100 structures [1]. The first few principal components (PCs) of these sets provide a set of coordinates capturing the most important motions. The energetics of these are usefully described with coarse-grained empirical potential functions that were derived from sets of protein structures, to include sets of 4-proximate amino acids [2]. These potential functions generally place the experimental structures within favorable positions on the PC space. Recently we have also derived coarse-grained empirical entropies from such sets of structures based on the types of amino acid contacts that are broken or formed [3]. These empirical landscape approaches have been useful for a variety of problems – assessing the effects of directed forces from exothermic reactions such as ATP hydrolysis [4], defining pathways for conformational transitions, and interpreting single molecule experiments [5].

References

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Protein Dynamics: Energy Exchange Network in the Folded State and Mechanical Unfolding

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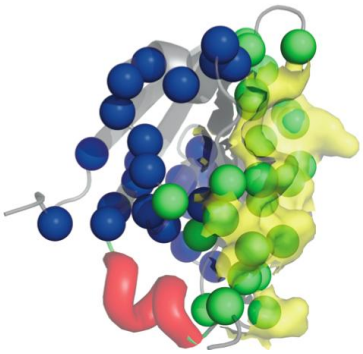

Figure 1. The predicted dynamic subdomain architecture of PDZ3: two distinct regions, represented by blue and green spheres, are connected by the α3 helix (red tube) whose removal significantly reduces the ligand binding affinity. The yellow surface represents the binding pocket.

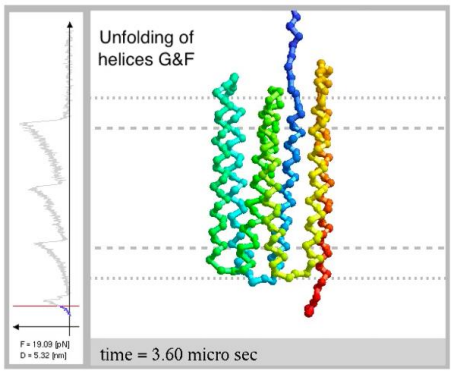

Figure 2. A snapshot of coarse grained simulation of bacteriorhodopsin. The simulated F–D curve is shown in the left window in parallel with the molecular movie (Supporting material, ref [1]).

Protein function is regulated not only by the structure but also by physical dynamics and thermal fluctuations. We used the CURP program to calculate the energy flow within the PDZ3 domain of the neuronal protein PSD-95, and the results were used to illustrate the energy exchange network of inter-residue interactions based on atomistic molecular dynamics simulations[1–3]. The removal of the α3 helix is known to decrease ligand affinity by 21-fold without changing the overall protein structure (Fig. 1). Interestingly, we demonstrated that the helix constitutes an essential part of the network graph.

To study the forced unfolding mechanism of bacteriorhodopsin, we performed coarse grained Brownian dynamics simulations (Fig. 2) [4]. As a result, the C-G simulation has successfully reproduced the key features, including peak positions, of the experimental F-D curves in the literature. Furthermore, we investigated the relationships between the energy barrier formation on the forced unfolding pathways and the force peaks of the F-D curves.

References

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Protein dynamics and function: all-atom molecular dynamics meets experiments

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Protein dynamics and protein folding are essential for a protein to function. Partial or total unfolding of a protein is related to the initiation of undesirable protein aggregation leading to misfolding diseases as for example the Alzheimer disease. Understanding protein unfolding is therefore essential.

In the first part of the talk, we present the simulations of the thermal unfolding of the fast downhill folder gpW between 280 K and 380 K using all-atom molecular dynamics (MD), and we predict the chemical shifts (CS), δ_i, of $^{13}C^{\alpha}$ atoms along the amino-acid sequence as a function of the temperature. The choice of the downhill folder gpW is motivated by the recent measure of CS of 180 atomic probes of gpW as function of the temperature. The CS exhibit jumps near the melting temperature, leading the authors to interpret the CS curves as local denaturation curves. Previous works shown that MD simulations using current force fields reproduce the qualitative behaviour of the CS of gpW and the weak cooperativity of its folding/unfolding transition but did not provide an interpretation of the physical meaning of the denaturation curves based on CS. Thanks to the present large-scale all-atom MD simulations of gpW, we answer the following fundamental questions: 1) Can we define a **local native state** from MD simulations based on internal coordinates and relate this local state to the measure of the CS and to the nativness of the protein? 2) How a local native state is related the global folding/unfolding of the protein? 3) Why some CS curves fail to be a reporter of the global unfolding state of a protein? 4) How are correlated local native states to each other?

In the second part of the talk, we present the interpretation of acoustical modes of proteins (at frequencies lower than 100 GHz) measured in recent single-molecule experiments and we discuss briefly the existence of intrinsically localized modes (soliton type) in proteins.

Raman microscopy:
a new tool for molecular imaging of cells and tissues

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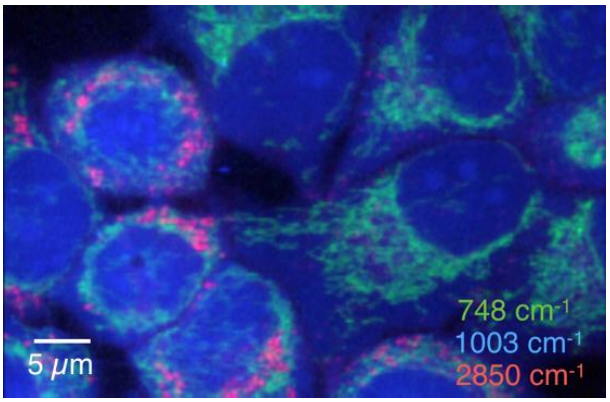

Raman image of living HeLa cells. The distributions of cytochrome c, phenylalanine, and lipids are visualized by using the Raman peaks at 748, 1003, and 2850 cm^{-1}, respectively.

Raman spectroscopy has been used for material analysis in various research fields. The capability of detection molecular vibrations enables to detect molecular species, conditions, and their environment in a sample to understand the function and characteristic of target materials. The recent development of techniques for highly sensitive Raman detection has expanded the application of Raman spectroscopy to the biomedical field. In particular, Raman microscopy can obtain spatial distributions of biological molecules base on their vibrations and provide various methodologies to analyze complicated biological phenomena. In our research, we have developed a Raman microscope that can rapidly image molecular distribution in a live cell [1,2], and studied the application to the analysis of molecular dynamics during cellular events, such as apoptosis, cell division and differentiation [2-5]. We measured Raman spectra during those cellular events and found that Raman spectra can represent the cell state during the activities, indicating that Raman microscopy can be used to investigate cell status without labeling. We also developed a technique to observe small molecules by using alkyne as a tag [6]. Due to its small size, alkyne can be used to tag small molecules without strong perturbation of molecular functions. The unique Raman band of alkyne allows us to image the alkyne-tagged molecules separately from endogenous molecules. We have demonstrated alkyne-tag Raman imaging of EdU localized in cell nuclei, quantitative measurement of intracellular ubiquinone, and imaging of the microdomains in an artificial lipid raft [7].

References

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Inferring system dynamics from stop motion single-cell data

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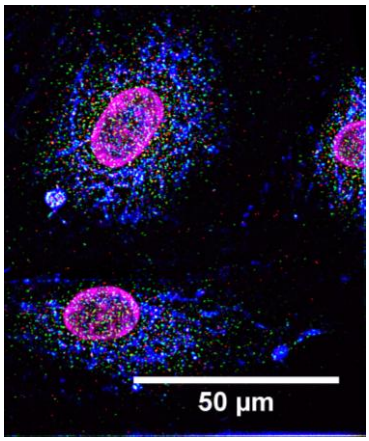

VEGF RNA (green), eNOS RNA (red), actin cytoskeleton (blue), and nuclei (purple) in fixed human umbilical cord derived endothelial progenitor cells.

Single-molecule, single-cell measurements have rapidly altered our understanding of the role of heterogeneity in gene and protein expression during development. However, multiple sources of experimental uncertainty complicate the use of these methods in determining the role of environmental stimuli in patterning cellular genotype and phenotype. Technical noise inherent in single-molecule RNA fluorescence in situ hybridization (smFISH, [1-3]) may lead to erroneous RNA counting. Under sampling of non-Gaussian spatiotemporal changes in cellular genotype and phenotype, due to limited-throughput imaging experiments, may lead to erroneous model and parameter identification [4]. Here, I present a high-throughput GPU based framework for robust image analysis of smFISH data. Utilizing this framework, we analyze RNA expression in human umbilical cord blood derived endothelial progenitor cells (EPC). We find that EPC derived from infants delivered at term have a different pro-angiogenic gene and protein expression profile from EPC derived from infants delivered prematurely [5]. Using these data, I will present a framework to predict the “next best” experiment to minimize uncertainty in signaling network and parameter identification using discrete single-molecule, single-cell data.

References

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High-resolution optical trapping and fluorescence investigations of protein folding complexity

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We present high resolution optical tweezers measurements of single protein molecules folding and unfolding under force. Recent developments in high spatial resolution optical tweezers have enabled measurements of e.g., motor protein motion at the angstrom scale. We can trade this high spatial resolution for increased time resolution, down to 10's of microseconds. This increased resolution has the potential to directly reveal complexity beyond two-state modeling for small, computationally tractable model proteins. We present results from investigations of two small (~60 aa) proteins: the B1 domain of protein G (PG) and the human Yes-associated protein (hYap). For PG, standard, out-of-equilibrium force ramp measurements show transitions between two apparently unique folded and unfolded states in agreement with polymer modeling and simple two-state modeling. However, force distributions suggest the presence of intermediate states or multiple folding pathways. Long duration fixed trap position measurements directly reveal equilibrium folding and unfolding reactions and confirm complexity in unfolding and the presence of intermediates. For hYap, we are able to observe sub-millisecond folding and unfolding at low forces in force ramp experiments. Finally, we present a novel method of measuring single protein folding under zero force that utilizes our combined fluorescence and optical tweezers capabilities.

Integrating molecular dynamics simulations and spectroscopy to study biomolecular dynamics

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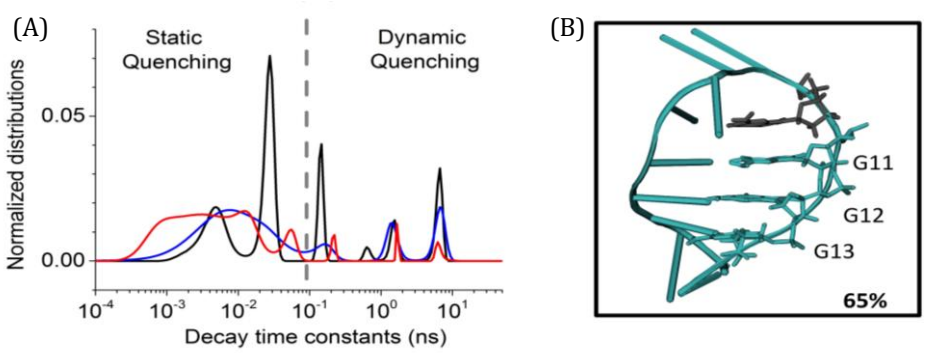

(A) Distribution of fluorescence decay times of three 2AP substituted DNA hairpins. The vertical dashed line represents the frontier between dynamic and static quenching. (B) Representative structure from MD simulations of 2AP substituted hairpin subject to static quenching.

Molecular dynamics (MD) simulations were integrated with results from optical spectroscopy to get more comprehensive information on the structural dynamics of biomolecules. In the first study (1), MD simulations and time-resolved fluorescence (TRF) spectroscopy were combined to quantitatively describe the conformational landscape of 2 amino-purine (2AP) labeled constructs of a short DNA hairpin targeted by retroviral nucleocapsid proteins of HIV-1 implicated in the viral reverse transcription. The populations obtained from MD were compared with those inferred from experimental lifetime amplitudes obtained by TRF. Cluster analysis further identified predominant conformations that were consistent with the fluorescence decay times and amplitudes, providing a structure-based rationalization for the unusually wide range of fluorescence lifetimes. In the second study, MD simulations and far-infrared (far-IR) spectroscopy were combined to study peptide binding by the second PDZ domain (PDZ1) of MAGI1, which has been identified as an important target for the Human Papilloma virus. Both the experimental and calculated far-IR spectra showed a red shift of the low-frequency peaks upon peptide binding. The calculations show that this is coincident with an increased number of hydrogen bonds formed as the peptide augments the protein β-sheet. We further investigated the contribution of surface-bound water molecules to the far-IRspectrum and we identified potential pathways for allosteric transmission of effects of binding.

References

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Structural analysis of sensitivity of chemical reaction networks

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In living cells, chemical reactions are connected by sharing their products and substrates, and form complex network systems, e.g. metabolic network. It is believed that biological functions arise from dynamics based on the networks, and that regulations of biological functions are realized by modulations of enzymes. One experimental approach to study such network systems is sensitivity analysis where amount or activity of the enzymes is perturbed and responses (concentrations of chemicals or fluxes in the system) are measured. However, due to the complexity of systems, it is unclear how a network structures influences behaviors of the system. In this study, we introduce a mathematical method, named *structural sensitivity analysis*, to determine responses of chemical reaction systems to the perturbation of enzyme amount/activity based only on network structure. From analyses we found that (1) qualitative responses at a steady state are determined from topological information of network only. We also found that (2) response patterns, e.g., distribution of nonzero responses of chemical concentrations in the network, exhibit two characteristic features, localization and hierarchy, depending on the structure of networks and position of perturbed reactions. Finally, we found (3) a general law which directly connects a network topology and response patterns, and governs the characteristic patterns of responses. These results imply that network topology is an origin of biological adaptation and robustness. This theorem, which we call the law of localization, is not only theoretically important, but also practically useful for examining real biological systems. We apply our method to several hypothetical and real life chemical reaction networks, including the metabolic network of the E. coli metabolic network.

References

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Numerical simulations of extracellular matrix peptides and proteins

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The extracellular matrix (ECM) is composed of very large extracellular molecules and macromolecules secreted by cells; it is a biochemical reservoir and it provides structural supports to the surrounding cells. The composition of ECM varies according to multicellular structures and cellular types. Macromolecules of the ECM are often very huge macromolecules constituted of numerous multi-globular domains and patterns, with fibrous structures and/or glycosylated molecules. These domains and patterns could adopt numerous structures and have a fabulous adaptability and most of the time a large flexibility to perform their functions. Most of the three-dimensional structures at the atomic and molecular levels are provided by experimental data from crystallographic and NMR experiments. Both of these methodologies lead to some structure/function relationships and to an understanding of their roles.
Nevertheless, the dynamical aspects are up to now missing from the structure/function/dynamic relationships. In this presentation, we will describe some of our results obtained using two types of numerical simulations: molecular dynamics simulations and molecular docking experiments. We will discuss the interactions and motions observed in proteins in the case of some metalloproteinases (MMPs) and their natural inhibitors TIMPs. MMP2 and MMP9 cleave the elastin molecule into small fragments: the elastin-derived peptides (EDP). We will focus on the specific structural behaviour of these EDP. Some of these peptides interact with the Elastin Receptor Complex (ERC) and particularly with the Elastin Binding Protein (EBP). We will present how molecular docking could explore these interactions and propose new sequences designed *in silico* that could theoretically bind to EBP. Last, the use of "serious games" as a future tool used for the modelling of large systems and interactions will be briefly presented. All these examples result from the works done in our group in conjunction with experimental and biological data obtained by colleagues who worked on different pathologies as for instance cancers, cardiovascular diseases or diabetes and obesity (ref 1-7 below). The simulated data try to take into account as much as possible these biological data in order to validate different models.

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Single-molecule FRET measurement for EGFR-RAS-MAPK signal transduction pathway

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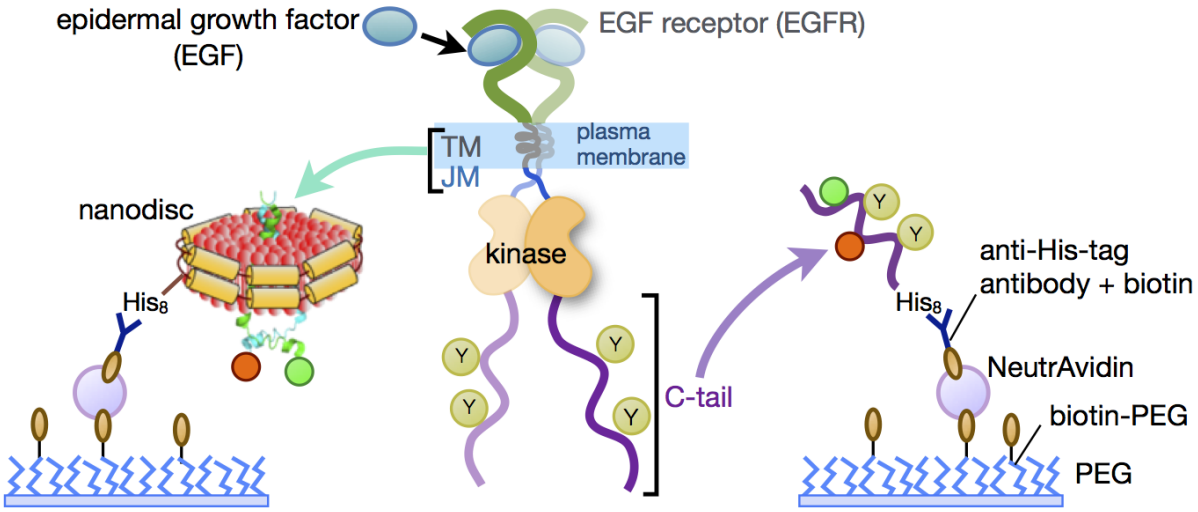

EGFR (center) is a transmembrane receptor protein. Fragment molecules are immobilized on polyethylene glycol (PEG)-coated glass surface via avidin-biotin coupling for *in vitro* smFRET measurement. TM-JM domain is buried in nanodisc (left). C-tail domain is intrinsically disordered (right). Green and red circles represent the donor and the acceptor dyes, respectively.

Most of proteins change their conformation when they work. Epidermal growth factor receptor (EGFR)-RAS-mitogen-activated protein kinase (MAPK) signaling pathway also involves such structural dynamics. EGF binding on extracellular domains of EGFR dimers allosterically activates intracellular kinase domains. Subsequent autophosphorylation of binding sites for downstream effector proteins on C-tail domain may induce conformational change to regulate binding kinetics. We conducted single-molecule Förster resonance energy transfer (smFRET) imaging measurements using fragment molecules from EGFR. Transmembrane (TM)-juxtamembrane (JM) domains were buried in nanodiscs to investigate charge interaction between the plasma membrane and the JM domain. C-tail domain, which is thought to be intrinsically disordered, was measured to study the effect of tyrosine phosphorylation on polypeptide structure. We also developed data analysis method based on the hidden Markov model (HMM) for FRET time series and applied it to spontaneous branch migration of Holliday junction (HJ) DNA.[1,2] In addition, we have tried the smFRET measurement of diffusing molecules in living cells to obtain the structural distribution of cytosolic Raf protein.

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Protein conformation by computer simulations

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The lack the secondary structure poses a significant challenge to computational modeling of intrinsically disordered proteins (IDPs). Mapping the free energy landscape with multiple shallow local minima is, however, vital for understanding their conformations and binding properties. A further challenge comes from the fact that the conformational ensemble is also highly dependent on conditions such as pH. Metadynamics is a family of techniques that can provide information about the conformational ensemble (albeit with its own limitations). One of the main issues in metadynamics simulations is the choice collective variables (CVs) that are required for efficient sampling. We have studied the choices and number of CVs together with several variants of metadynamics, namely well-tempered metadynamics (WT-META), and bias-exchange metadynamics (BE-META). We then study β-hairpin conformation of the Neh2 peptide and the binding of nrf2 and PTMA to Kelch. The latter is a key player in cellular response to oxidative stress and binding hub for multiple intrinsically disordered proteins. The extensive sampling enabled by metadynamics shows that the coupled folding–binding mechanism may be the dominant mode in binding to Kelch. We also discuss aspects related to force fields and coarse-grained simulations. In particular, we assess the free energy of peptides‘ entry into membranes.

Response of Glutathione S-Transferases upon substrate binding: a dynamical approach of flexibility

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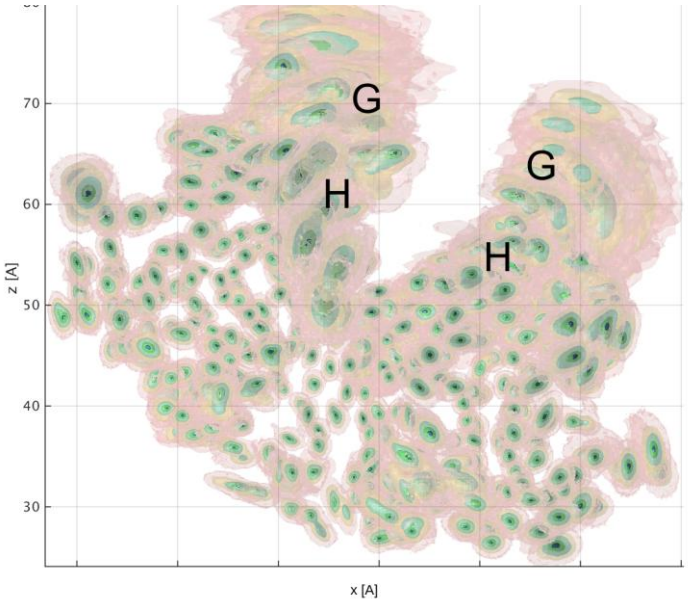

Equilibrium C_α density plot of hGSTA1-1 from a 2.7 µs all-atom simulation. Glutathion (G) and ligand (H) binding sites position are indicated.

In the world of enzymes, human Glutathione S-transferases (GSTs) have a quite uncommon selectivity. While most enzymes are chemoselective, this detoxifying protein catalyses the conjugation of glutathione with a wide variety of xenobiotic substrates. XRD structures of native GST dimers feature approximate positions for atoms of the binding site, indicating high flexibility of some parts of GSTs, which could explain their multi-target activity.

The aim of this work is to build a linear response theory of the GST structure to ligand interaction by extracting key information about the complex dynamics of the protein. All-atom molecular dynamics simulations of the protein in explicit solvent were performed without glutathione nor ligand (APO). The µs-long trajectories were analyzed using proven techniques such as normal mode analysis and principal component analysis. The modes are interpreted together and serve as a guide for a new method that, similarly to liquids, relies on particle density to compute the linear response and predict interactions.

Exploring Large-scale Conformational Changes in Proteins: The Potential Energy Landscape Approach

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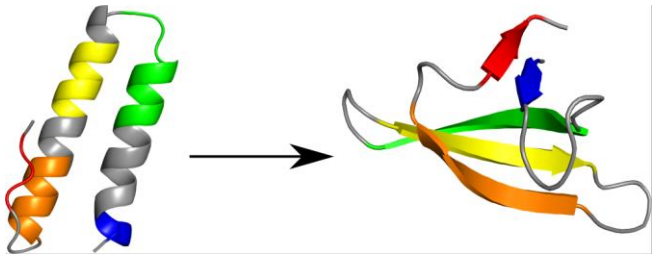

All-α to all-β structural transition in RfaH-CTD

Large-scale structural transitions in proteins often involve multiple degrees of freedom. Therefore, it is usually difficult to simulate these transitions at biologically relevant time scales using conventional computer simulation techniques. The potential energy landscape (PEL) framework represents an attractive alternative for characterising the structural features, thermodynamics and kinetics of such transitions. This approach utilises geometry optimisation, which is inherently time-independent, to construct kinetic transition networks for the process of interest. The C-terminal domain (CTD) of the bacterial transcription factor RfaH provides an interesting example, where RfaH-CTD undergoes a dramatic all-α to all-β transition. In the all-α state RfaH-CTD interacts with the N-terminal domain (NTD) and functions as a regulator of transcription by masking the RNA polymerase binding site. However, upon dissociation from the NTD, the CTD refolds into the all-β state and aids in the activation of translation. Accordingly, this system provides a useful model for testing protein simulation techniques and for elucidating protein structural transitions in general. In this talk, I will discuss how the PEL approach can be used to compute the free energy surface and analyse the mechanics of (re)folding for the RfaH-CTD structural transition at atomistic resolution.

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Toward microscopic simulations of the cellular environment

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Proteins work in an extremely heterogeneous and crowded environment. Indeed 5 to 40 % of the intracellular total volume is occupied by large biomolecules [1]. This crowding condition affects both the mobility and stability of a protein. Understanding the molecular details of protein's life in the cell is therefore considered a challenge for modern biophysics and molecular biology [2]. Unfortunately, the experimental manipulation of the intracellular milieu is very difficult, as well as the in silico characterization of systems with such a large size and spread of length- and time-scales [3].
We have recently developed a multi-scale multi-physics approach that enables us to investigate proteins in cell like environments via computer simulations [4]. This approach combines molecular and fluid dynamics and allows to include naturally hydrodynamic interactions in water free coarse-grained model for biomolecules at the quasi-atomistic resolution. In this talk we will describe the methodology and showcase the ensemble of applications currently on the way. This includes the study of amyloid peptide aggregation (the hallmark of neurodegenerative diseases like Alzheimer), the protein unfolding under shear flow, the mobility of proteins in crowded environment.

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Title: Transition path times reveal memory effects and anomalous diffusion in the dynamics of protein folding

Abstract

Although theoretical considerations and computer simulations suggest complexity of protein dynamics, experimental studies of protein folding can usually be interpreted using the simple picture of one-dimensional diffusion along a reaction coordinate. In this talk I will argue that this picture breaks down when applied to single-molecule measurements of transition path times, which became possible in recent years. I will further discuss alternative models that capture memory effects and provide a more accurate description of transition path ensembles (at least those observed in molecular simulations).

Thermodynamics of information on biochemical signaling networks

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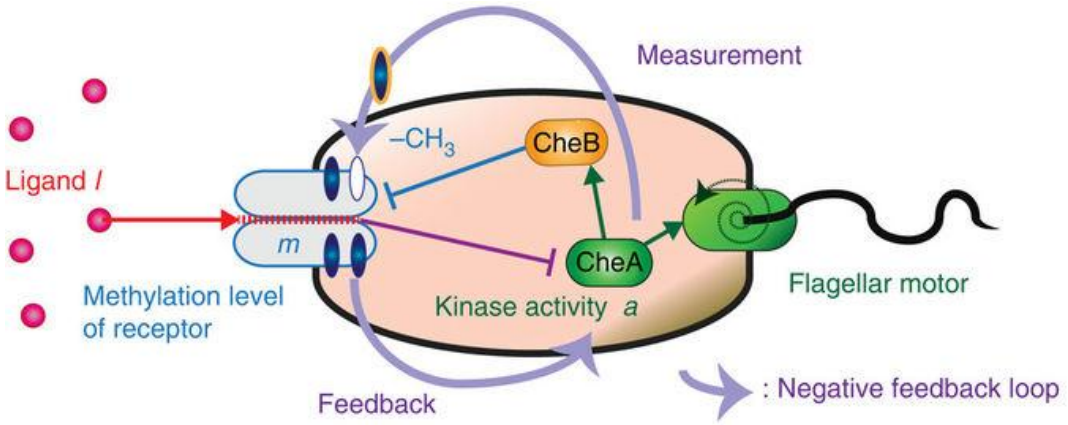

Figure 1:Schematic of adaptive signal transduction of E. coli bacterial chemotaxis.

Unified theory of information and thermodynamics has been intensively discussed for nonequilibrium stochastic dynamics from the viewpoint of a thought experiment known as Maxwell's demon.

In a series of our studies [1-5], we have obtained the generalized second law of thermodynamics with information transfer, that is applicable to a broad class of dynamics such as autonomous biochemical reaction [1]. To prove the generalized second law of thermodynamics with information transfer, we have used a probabilistic graphical model known as Bayesian network for a mathematical description of complex nonequilibrium stochastic dynamics.

Furthermore, we have also applied the generalized second law of thermodynamics with information transfer to biochemical signal transduction [2], e.g., sensory adaptation in E. coli bacterial chemotaxis (see also Figure 1).
From the view point of thermodynamics of information, we can quantitatively discuss a fundamental bound of information transfer in a biochemical sensor.

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Euclidean Variable Neighborhood Search: A method for large computation protein design

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Computational protein design (CPD) is an important tool for biotechnology still under development. Early applications led to proteins with novel ligand-binding functions, novel enzyme activity, and proteins that were completely "redesigned": around 2/3 of their sequence was mutated, yet their structure and stability were retained. In the last few years, CPD has allowed the creation of new protein folds, completely new enzymes, and the assembly or deassembly of multiprotein complexes. CPD methods are mainly characterized by (a) the energy function, (b) the description of the folded protein's conformational space, (c) the treatment of the unfolded state, and (d) the search method used to explore sequences and conformations.

Graphical model and cost function network have been recently introduced as new search methods for CPD[1,2] search allowing to find the global minimum energy conformation (with optimality proof). However, it can solved problems with less than 100[3] and 48[4] mutations respectively with Rosetta and Amber forcefield and Dunbrack[5] and Tuffery[6] rotamers library. In this work, we introduce a new search method for CPD dedicated to large instances. The method, based on Variable Neighborhood Search[7] (VNS), uses (partial) tree search in order exhibit the solutions with the lowest energy.

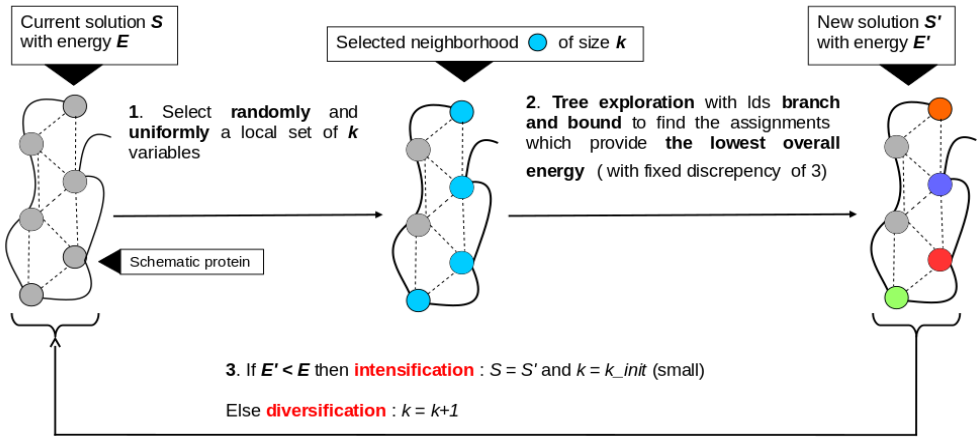

fig 1: schematic representation of variable neighborhood search algorithm

In order to improve its behavior, we implemented a new heuristic taking advantage of the euclidean information provided by the pdb structure for neighbor variable selections. This last one used in conjunction with logarithmic size incrementation of the neighborhood size improves significantly the VNS behaviors. The resulting algorithm, called euclidean VNS, is more robust. It also outperforms Replica-Exchange Method for global minimum energy search. Furthermore, the method can provide direct correlation between energy improvement and protein structure, allowing to identify energetic hot spots in the protein backbone.

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Infinity makes the cut:
Bayesian nonparametrics for protein biophysics

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As theorists, we draw trends and make predictions on protein function and interactions from models of protein dynamics. One route to modeling protein dynamics involves the bottom-up, molecular simulation, approach. In this approach, approximate classical potentials are used to simulate short time local protein motions in order draw insight on protein dynamics at longer time and larger length scales. Here we take a different route. Instead we present a top-bottom approach to building models of protein dynamics. The approach we present exploits a novel branch of Statistics – called Bayesian nonparametrics (BNPs) – first proposed in 1973 and now widely used in data science as the important conceptual advances of BNPs have become computational feasible in the last decade. BNPs are new to the physical sciences. They use flexible (nonparametric) model structures to efficiently learn models from complex data sets. Here we will show how BNPs can be adapted to address important questions in protein biophysics directly from the data which is often limited by factors such as finite photon budgets as well as other fluorophore artifacts in addition to data collection artifacts (e.g. aliasing, drift). More specifically, we will show that BNPs hold promise by allowing complex spectroscopic time traces (e.g. sm-FRET, photon arrivals) or images (e.g. single particle tracking) to be analyzed and turned into principled models of protein motion – from diffusion to conformational dynamics and beyond.

Understanding fast functional dynamics of proteins with single-molecule FRET and HMM

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The catalytic mechanisms of complex biological machines may involve a combination of chemical steps and conformational transitions. The latter are oftentimes hidden to traditional biochemical investigations, but can be exposed by single-molecule experiments. Single-molecule FRET (smFRET) is an ideal tool to probe the conformational dynamics accompanying functional dynamics of biological machines. We recently developed a novel photon-by-photon hidden Markov model (HMM) analysis that facilitates studying conformational dynamics even on the microsecond time scale [1].

We first applied our new method to the domain closure reaction of the enzyme adenylate kinase. Surprisingly, we found that the bound enzyme opens and closes its domains much faster than the unbound enzyme, and two orders of magnitudes faster than the turnover rate of the enzyme! This exciting finding, which radically deviates from previous observations on adenylate kinase, led us to suggest that multi-substrate enzymes use numerous cycles of conformational rearrangement as a means to optimize the mutual orientation of their substrates for reaction.

Our photon-by-photon HMM method was also applied to study the functional dynamics of the disaggregating machine ClpB. This machine is comprised of six identical subunits arranged as a barrel. A coiled-coil domain resides on the outside surface of each subunit, and this domain has been implicated as a control element of the machine, to which the co-chaperone DnaK binds. We used smFRET together with HMM to show that the coiled-coil domain resides in several conformational states, which are interchanging on a sub-millisecond time scale. We related some of these conformational states to well-known functional states of the machine.

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High-speed single molecule tracking of proteins in E. coli

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All living cells are encapsulated by an outer envelope, which contains a fluid phospholipid-based membrane, that structurally delineates the inside of the cell from its environment. Embedded within this membrane is the machinery (proteins) required for the cell to sense and interact with its environment. As such, one expects that there are mechanisms in place to control the location and mobility of the membrane proteins in the fluid membrane in order to perform complex and critical tasks. In this talk, I will present our group's recent single molecule mobility studies into the (dynamic) organization of the cellular membranes of *E. coli* bacteria performed at acquisition rates up to 1 kHz. Examples will include the structure of the polar region of the inner membrane as seen by the serine chemoreceptor Tsr and investigations into interactions of the iron transporter FepA in the *E. coli* outer membrane with the inner membrane protein TonB.

Modeling the folded and unfolded proteome using polymer physics

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We will use polymer physics principles to model both the folded and unfolded proteome (the collection of diverse proteins inside an organism). Specifically, we will focus on two topics. First, we will address a long-standing question in protein science: how do thermophilic proteins -- extracted from organisms that live at high temperature -- can withstand temperatures much higher than their mesophilic counterparts, obtained from organisms that live near room temperature. This extreme thermal tolerance in thermophilic (folded) proteins is particularly intriguing given they share high structural and sequence similarity with their mesophilic counterparts. Supported by coarse-grain theory and all-atom simulations, we will show charge patterning in the folded and unfolded ensemble is key to understand this puzzle. On the next topic, we will demonstrate the important role of charge patterning to model conformational heterogeneity in the Intrinsically Disordered proteins (IDP). Guided by analytical theory and all-atom simulations, we will show how subtle mutations or post-translational modifications, as few as two amino acids, can significantly alter IDP conformations giving us new insights to manipulate IDP function.

Binding without folding:
Extreme disorder and dynamics in a high-affinity protein complex

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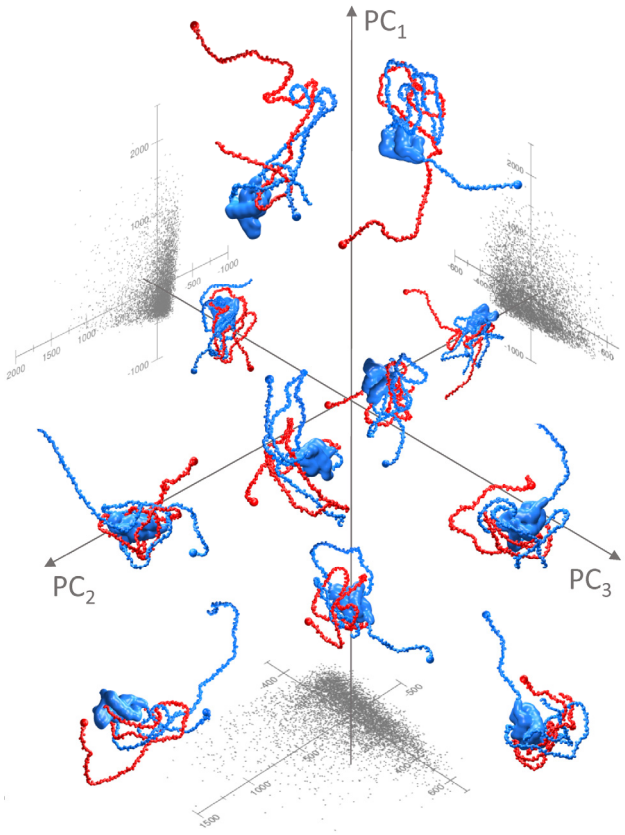

Examples of configurations of H1 (blue) and ProTα (red) in their high-affinity complex from molecular simulations based on the experimental data

Molecular communication in biology is mediated by protein interactions. According to the current paradigm, the specificity and affinity required for these interactions are encoded in the precise complementarity of binding interfaces. Even proteins that are disordered under physiological conditions or that contain large unstructured regions commonly interact with well-structured binding sites on other biomolecules. Here we demonstrate the existence of an unexpected interaction mechanism: The two intrinsically disordered human proteins histone H1 and its nuclear chaperone prothymosin α associate in a one-to-one complex with picomolar affinity, but they fully retain their structural disorder, long-range flexibility, and highly dynamic character. Based on the close integration of single-molecule experiments, NMR, and molecular simulations, we obtain a detailed picture of this complex and show that the interaction can be explained by the large opposite net charge of the two proteins without requiring defined binding sites or interactions between specific individual residues.

Dynamics of protein folding studied by single molecule fluorescence

measurements at microsecond resolution

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Single molecule fluorescence spectroscopy (SMFS) is a powerful technique for the investigation of protein folding dynamics. However, the time resolution of the conventional method of SMFS is limited to a few milliseconds. To improve the time resolution, we developed a line-confocal microscope, which enables us to obtain the time resolution of 20 microsecond and the observation time of a few milliseconds for the single molecule FRET efficiency time series [1].

We first investigated the B domain of protein A (BdpA) [2]. We doubly labeled BdpA with donor and acceptor fluorophores, and detected single molecule FRET time series. We obtained traces having high and low FRET efficiencies, which were assigned to the native and unfolded states, respectively. The distribution assigned to the unfolded state was broad and split into two components near the unfolding midpoint. The distribution assigned to the native state was narrow but showed a gradual shift as the changes in the denaturant concentration. These data, demonstrating a partial swelling of the native state and the presence of several substates in the unfolded state, indicated the complexity of the folding transition of a single-domain protein.

We next investigated the unfolding transition of ubiquitin [3]. We observed two distributions that were assigned to the native and unfolded states. At 0 M concentration of urea, the observed width was narrow and comparable to the theoretical width estimated from the average photon numbers. In contrast, the width for the denatured state at 4 and 8 M urea was obviously broader than the theoretical width. Accordingly, the data suggested that the denatured ubiquitin are heterogeneous and that the each component of the unfolded state possess the lifetime longer than several milliseconds.

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Energy landscapes learned from single molecule FRET time series: Role of Photobleaching

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Energy landscapes are one of the most pervasive concepts in understanding protein dynamics and functions. It is one of the most intriguing subjects to trace single molecules by being mapped onto the energy landscape along single molecule time series one observes in e.g., fluorescence resonance energy transfer (FRET) measurements. It should be noted that unavoidable experimental errors such as photon statistics, photobleaching and the finiteness the sampling of the time series impose a major impediment to developing a method to resolve this problem. In addition, the question of how energy landscape can be mapped from state network so as to be free from any projection and from multiple pathways is also one of the central subjects.

In my talk I briefly overview Local Equilibrium State analysis that extracts a set of local equilibrium states and free energy landscape with taking into account experimental errors and the finiteness in the sampling of the time series, and discuss the role of photobleaching in constructing the energy landscape.

Temporal and spectral analysis of enhanced Raman scattering data for accessing sub-molecular information

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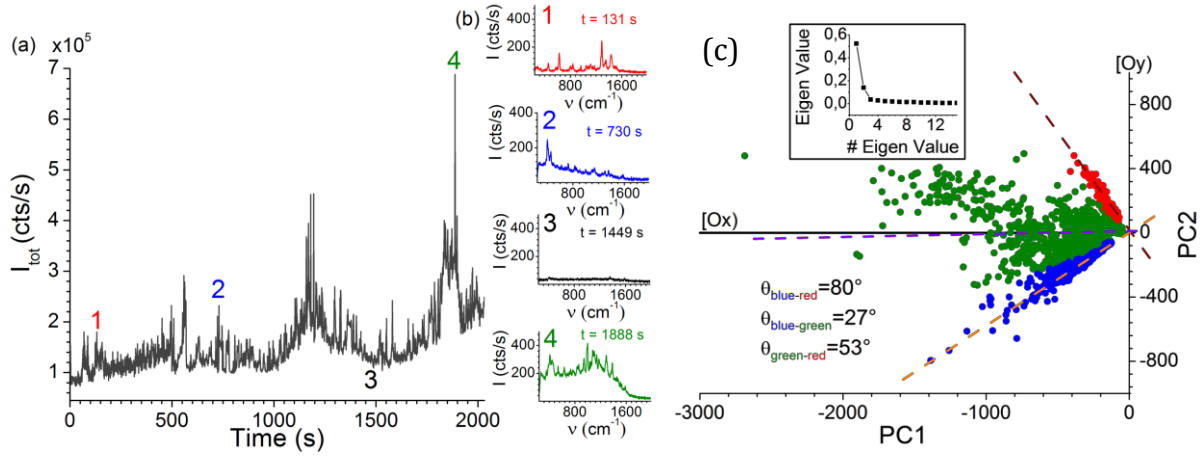

(a) Temporal fluctuations of SERS response of cysteine on raspberry-like gold nanoparticles. Some examples of SERS spectra are shown in (b). Principal component analysis enables to sort these spectra (c).

It is well known that localized surface plasmon at metallic surface is able to enhance spontaneous Raman scattering signals with several orders of magnitude. This effect called surface enhanced Raman scattering (SERS) is increasingly used in biosensors because it has the ability to record the spectroscopic imprint of individual molecules [1]. Due to its high sensitivity and specificity, SERS based sensor collects a large number of spectral and temporal data whose robust and reliable analysis is challenging.

In this talk, I will introduce different methods that we used for sorting accurately the numerous fluctuating spectra acquired with SERS based biosensors. By exploiting the temporal dynamics, we can also obtain quantitative information about the concentration and the number of emitters for instance. Our main objective is to reconstruct the ensemble average from the dynamics of successive individual data [2]. This approach has been applied on various analytes, from small molecules (0.3kDa) to large proteins (66kDa) and has been exploited for accessing sub-molecular information.

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Computational investigation of the translocation of single polypeptide through MoS_2 nanopores from ionic conductance dynamics

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Keywords: solid-state nanopores, protein sequencing, MD simulations

Solid-state nanopores have emerged as versatile devices for single molecule detection and sequencing [1]. As the sequence of biological molecule such as DNA or proteins is fundamental for a better understanding of the biological mechanisms, which govern cellular behavior and disease, the efforts to decode the sequence in humans is of strong interest. The idea behind nanopores sequencing is based on experimental ultrafast monitoring of the ionic current through nanometer-sized channels [2]. Because the channel conductance of the ionic flow through nanopores scales inversely with the membrane thickness, few-atoms thick materials are ideal candidates with an expected high signal-to-noise ratio [3]. Beyond graphene, transition metal dichalcogenides such as molybdenum disulfide (MoS_2) are potentially advantageous due to their rich optoelectronic and mechanical properties. This study reports the results of all-atom molecular dynamics simulations that investigate the feasibility of using MoS_2 nanopores for protein sequencing. First, we investigate the dynamics of KCl ions through MoS_2 nanopores using non-equilibrium molecular dynamics (NEMD) simulations. MoS_2 nanopores with different diameters, from 1.0 to 3.0 nm and nanoporous membranes with different thicknesses, from single-layer to trilayers MoS2 are studied. We provide open pore benchmark signals of such devices for further translocation simulations/experiments [4]. Second, we investigate the translocation of peptides made of Lysine residues associated with other types of residues such as hydrophobic amino acids. Due to the fact that Lysine residues are positively charged, the translocation process of peptides containing Lysine tags through MoS_2 nanopores in the presence of an electric field may be better controlled. Using NEMD, we study the diffusion of different peptides of biological interest in bulk 1M KCl, on the top of the MoS_2 surface and finally inside the nanopores during the translocation process. The aim is to estimate the performances of MoS_2 nanopores for protein sequencing applications.

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Recent developments in elucidating mechanisms of protein misfolding diseases

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Neurodegenerative disorders, such as Alzheimer's, Parkinson's, Huntington's, and Creutzfeldt-Jakob's disease, are caused by incorrect protein folding followed by the aggregation and accumulation of protein deposits in neuronal cells. Moreover, protein folding intermediates are associated with the formation of amyloid fibrils, which are responsible for neurodegenerative diseases. Therefore, elucidation of the important sites and mechanisms for amyloid fibril formation, the origins of formation of intermediates, and finding ways to prevent them are very important. All these problems will be addressed in this presentation by investigating (i) the folding trajectories of the 37-residue triple β-strand WW domain from the Formin binding protein 28 (FBP28) and its six mutants (L26D, L26E, L26W, E27Y, T29D, and T29Y);[1] (ii) the entire computationally-modeled process of Aβ fibril elongation;[2] (iii) the molecular dynamics of two forms, monomeric and tetrameric, of α-synuclein.[3] Experimental validation of theoretical findings in these studies will also be presented.[4]

References

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