reannealing activity observed was extremely strong. In particular, Uvsw was able to reanneal complementary ssDNA strands against very high forces (around 20-30 pN) without a reduction in its rate of reannealing. These force measurements gave us some insight on the translocation mechanism of the motor and allowed us to estimate its kinetic step size. Finally, we also tested at the single-molecule level the ability of Uvsw helicase to resolve stalled forked-DNA structures by generating a chicken foot structure. We found that Uvsw helicase was capable of performing fast Holliday junction migration.

1SM-05 Functional Membrane Protein Dynamics

Morten Jensen (*D. E. Shaw Research*)

Long molecular dynamics (MD) simulations, which recently reached the millisecond timescale for the first time, may prove a powerful tool in advancing the understanding of protein function. Here I will discuss our MD studies of the conformational changes associated with ion channel voltage gating. Our results reveal an atomic-level structural mechanism applicable to the entire $\text{Na}^+/\text{K}^+/\text{Ca}^{2+}$ voltage-gated ion channel superfamily, reconciling much apparently conflicting experimental data.

1SM-06 シナプス内信号伝達、2光子励起蛍光寿命イメージング、神経生物学 “単一シナプスにおける信号伝達の可視化”

Signaling in synapses, Neurobiology, 2-photon Fluorescence Lifetime Imaging “Signal transduction in single dendritic spines”

Ryohei Yasuda^{1,2} (¹Duke University Medical Center, ²Howard Hughes Medical Institute)

Learning and memory is believed to be caused by long-term potentiation (LTP) - long-term strengthening of synaptic transmission in the brain. It has been known that intracellular signaling triggered by Ca^{2+} elevation in a synapse underlies LTP. However, how the transient Ca^{2+} elevation, which lasts only ~0.1 s, is translated into long-lasting (lasting more than an hour) synaptic change has remained elusive. To address this question, we have developed a new technique to visualize enzymatic activity in single synapses undergoing LTP in brain tissue. Using this technique, we succeeded in monitoring the activity of several signaling pathways important for LTP. Our results indicated that signaling activity is subjected to a complicated spatiotemporal regulation: some signals are compartmentalized in single synapses, while others spread to affect a short stretch (micrometers) of dendritic segments including multiple synapses. This rich spatiotemporal regulation plays an essential role in coordinating cellular events in different dendritic micro-compartments. Further, we found that Ca^{2+} elevation in a synapse is relayed in several different stages. First, Ca^{2+} signals are integrated by a signaling protein CaMKII, of which activity decays over ~10 s. Then, Cdc42 and RhoA are activated by CaMKII, and relay this transient signal into signals lasting more than half an hour. These long-term signals are likely to serve as biochemical memory storage in synapses.

1SM-07 構造変化と流れが生み出す機能

Functions generated by the structural change and the associated flow

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Flow around microorganism and molecular machine is different from the flow around insects, e.g. butterflies and dragonflies. At the scale of insects, the inertia of flow is dominant, which causes separation from the boundary layer. As a result, insects' flight is achieved by the dynamical interaction between separation vortices and wings, which is much different from the case of airplane in which the wing motion is steady. Such difference plays an important role in the function of insects' flight - large lift generation mechanism and high controllability -. On the other hand, the flow at the scale of microorganism is dominated by the viscous effect; the governing equation is mathematically simpler. However, the simpleness restricts the boundary motion to generate functions. For instance, to generate net propulsion for a microorganism, the boundary motion must be described by at least two degree of freedom. In this talk, I would like to talk about how creatures in nature generates (or can generate) functions by using the surrounding flow. The topics may be molecular machine propulsion, sol transport in amoeba, and insect flight, etc.

1SM-08 揺らぎの定理による生体モーターの駆動力測定

Fluctuation theorem applied to bio-motors

Kumiko Hayashi (*Sch. Eng., Appl. Phys., Tohoku Univ.*)

The fluctuation theorem (FT) was first advocated by Evans *et al.* in 1993, and is one of new relations on non-equilibrium fluctuation in the field of non-equilibrium statistical mechanics. It represents the property of entropy production in a small (stochastic and fluctuating) system. In the 2000s, FT has been applied to the wide range of physical and biological systems including RNA hairpin systems [1]. Finally last year, we succeeded in applying FT to the molecular motor $\text{F}_1\text{-ATPase}$ [2]. Making the most of a fluctuation property in non-equilibrium, the method to measure the driving force of a small motor by use of FT was established [2]. Recently, the method is applied further to another motor protein V_1 [3]. Because the measurement method using fluctuation is a non-destructive one, it seems suitable to be used in an observation *in vivo*. In the end, we would like to show the application of FT in a living cell. We are measuring a driving force acting on a mitochondria transported in the axons of PC12 cells.

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[3] K. Yokoyama, J. Kishikawa, K. Hayashi, E. Uner, H. Noji and H. Konno, in preparation.

1SQ-01 Folding and Dynamics of Globular Proteins Studied by time resolved FRET Methods

Elisha Haas (*Bar Ilan University*)

We introduced methods based on time-resolved FRET experiments in the ensemble and single molecule modes combined with protein engineering and site directed labeling methods. These approach yields intramolecular distance distributions, domain motions, diffusion coefficients of segments' ends and sub-populations. Fast changes of these characteristics during conformational transitions such ligand binding and the folding/unfolding transitions are determined by fluorescence correlation spectroscopy, single molecule FRET and the “double kinetics” methods. Using these methods we studied the folding of globular proteins and obtained data which support the “loop hypothesis”. We hypothesize that protein folding starts by formation of few non-local interactions which form loop closure soon after the hydrophobic collapse. We suggest that proteins fold more like “global first, local second” rather than “local first, global latter”. We further studied the ensemble properties of segments of the intrinsically disordered protein $\alpha\text{-Synuclein}$ and found fine differences in the extent of disorder of segments of this protein's backbone. Single molecule FRET methods are being used in studies of the conformational dynamics of the multidomain protein Adenylate kinase. Sub-millisecond domain motions were detected by the measurements. Ultrafast subdomain dynamics was monitored by FRET-FCS experiments. This assortment of FRET based methods enables us to detect the elementary steps of the mechanism of folding and action of the protein molecules.

1SQ-02 微小管上の分子モーターの振る舞い: 1分子、数分子、多分子モーターの運動特性

Behaviors of motor proteins on microtubule as single, several and many molecules

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Dynein and kinesin are microtubule-based motor proteins that work in trafficking of a large variety of cargos within the cell. While kinesin moves toward the plus end of microtubules, dynein moves toward the opposite end. They are both two-headed but structurally very different, and dynein is a huge and complex molecule whose machinery has not been well elucidated yet. We report here several kinds of experiments about the *in vitro* motile properties of these molecules moving as single, several or many molecules on microtubules. Firstly, both the single molecules of dynein-1 and kinesin-1 moved processively on the edge of zinc-sheet, demonstrating that only one protofilament is sufficient to support the processive walking of dynein-1 and kinesin-1. Secondly, when two molecules of non-processive Ncd (kinesin-14) were linked together, it moved processively along microtubules for more than 1 micrometer, indicating the cooperativity between two Ncd molecules. Thirdly, the velocity