Top-Collaboration Support Project International Lecture
also as Nikon Imaging Center at Hokkaido Univ. International Lecture

DATE November 19th (Thu), 2015

PLACE Confference Room (1F), Research Institute for Electronic Sciences (RIES), Hokkaido University (N20, W10, Kita-ku, Sapporo, Japan)

PROGRAM

14：00 - 14：05 Tomomi Nemoto, Professor, Hokkaido University
"Introduction"

14：05 - 15：05 U. Valentin Nägerl, Professor, University of Bordeaux
"Imaging neurons and synapses at the nanoscale by STED microscopy"

15：05 - 15：15 (Coffee Break)

15：15 - 16：15 Peilin Chen, Research Fellow, Academia Sinica
"Nanoparticles for *in vitro* and *in vivo* Optical Imaging"

16：15 - 16：25 (Coffee Break)

16：25 - 17：25 Patrick Sanet, Professor, University of Bourgogne
"The free-energy landscape: an unifying concept to understand protein dynamics"

17：25 - 17：30 Tamiki Komatsuzaki, Professor, Hokkaido University
"Closing remark"

Imaging neurons and synapses at the nanoscale by STED microscopy

U. Valentin Nägerl

Professor of Neuroscience and Bioimaging, group leader

Interdisciplinary Institute for Neuroscience,

University of Bordeaux, France

Neurons and glia span an incredibly ornate and beautiful morphology, that allow them to connect with one another through functional junctions (synapses) and to hold thousands of conversations at the same time. Given the high speed of electrical signals (action potentials), the brain represents a parallel-processing supercomputer with unfathomable combinatorial power and bandwidth.

As many of the anatomical structures that mediate electrical signals in the brain (such as dendritic spines, axons and perisynaptic glial processes) are too small to be visualized by conventional light microscopy, it has been very difficult to study how any dynamic changes in these basic circuit elements might shape signal transmission and neural computation in the brain.

The advent of super-resolution microscopy has opened up huge experimental opportunities in this regard, making it possible to directly image and dissect structural and functional processes inside nanoscale neural compartments in living brain tissue.

Stimulated emission depletion (STED) microscopy was the first fluorescence microscopy technique to break the classic diffraction barrier of light microscopy. It is an ensemble imaging technique that uses a pair of lasers for controlling the excitation state of fluorescent molecules in a targeted manner over nanoscale distances, achieving nanometric spatial resolution deep inside scattering brain tissue.

I will review our progress in developing STED microscopy for nanoscale imaging deep inside three-dimensional brain tissue. I will then describe a couple of neurobiological applications concerning the activity-dependent plasticity of synaptic structures that regulate neuronal function in living brain slices, specifically: 1) dynamic changes in dendritic spines during synaptic plasticity and their impact on biochemical compartmentalization and electrical signaling; 2) activity-dependent remodeling of axon morphology and its impact on action potential propagation in unmyelinated hippocampal axons.

Nanoparticles for *in vitro* and *in vivo* Optical Imaging

Peilin Chen

Research Center for Applied Sciences, Academia Sinica, Taiwan

In this talk, some recent developments in the advanced optical imaging systems in our group will be discussed. We will first present the development of various nanoparticles for sensing the pH value in cellular environment. By surface functionalization schemes, it is possible to control the location of nanoparticles in cells allowing us to track the local pH value around the nanoparticles inside cancer cells. The acidification process of nanoparticles in the endocytosis process can be recorded. Such technique can be extended to detect the local chemical reaction inside living cells [1]. As for in vivo imaging, we have utilized the multi-photon microscopy to investigate the disease models with the help of nanoparticles. We will discuss the changing biodistribution of nanoparticles in a mouse model of inflammation. Since inflammation alters basic physiology, increases vessel permeability and modulates the immune system, we theorized that systemic inflammation would alter nanoparticle distribution within the body. We have found that inflammation affects nanoparticle distribution by multiple mechanisms, in a size dependent manner [2]. We will also discuss cardioprotective drugs to be actively delivered without relying on the EPR effect. To mimic the platelet interactions with the circulating monocytes during post-myocardial infarction (MI), platelet-like proteoliposomes (PLPs) were fabricated using purified human platelet membrane proteins and DOPC lipids. Intravital multiphoton imaging revealed PLPs had better targeting for the tissue injury site compared to the plain liposomes control. When being injected at 72 hours of reperfusion, which is the number of the circulating monocytes peaked at, there was significantly more PLPs at the infarcted heart than the controls. Moreover, cobalt protoporphyrin (CoPP) encapsulated in PLPs (PLPs-CoPP) were shown to improve the cardiac function in murine model of MI while reducing the adverse effect of the encapsulated drug [3].

References

[1] TJ Chou et al, Nanoscale 7 (2015) 4217.

[2] KH Chen *et al*, Nanoscale 7 (2015) 15863.

[3] BC Chen *et al* (Submitted).

The free-energy landscape: an unifying concept to understand protein dynamics

Patrick Senet

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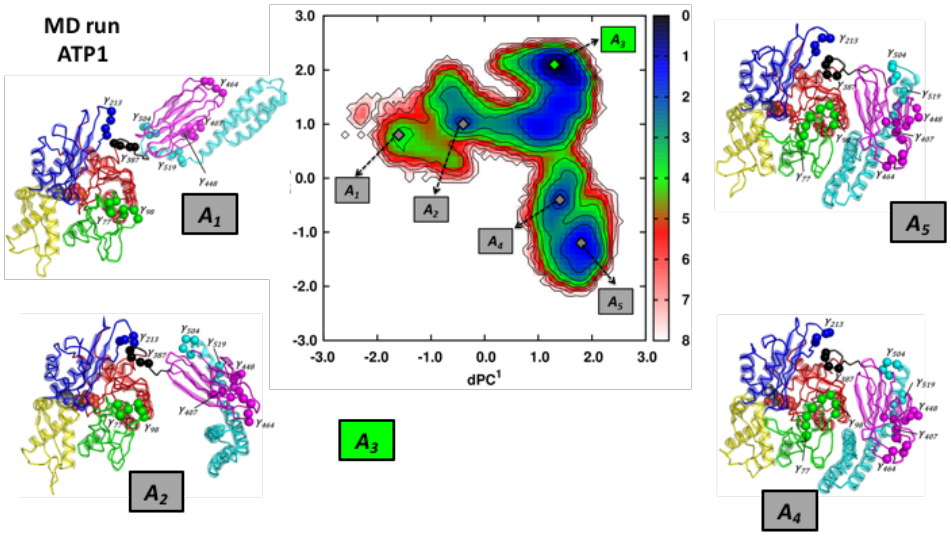

Effective Free-Energy-Landscape of Hsp70 [4]

Nowadays, numerical simulations of proteins at the atomic scale are performed on typical microseconds of duration. These simulations produce a very detailed description of the protein dynamics. For example, an all-atom molecular dynamics simulation of a protein of a duration of one microsecond generates typically an ensemble of 10^9 to 10^{11} atom coordinates if the protein structures are recorded every picosecond. How to extract from this big data set the information relevant to the biological function of the protein? How to detect the key amino-acids involved in the protein conformational dynamics? In this lecture, we introduce the concept of the protein free-energy landscape (FEL) and we show that it holds the key to understand the conformational dynamics of proteins. We illustrate the application of the FEL to understand the conformational changes induced by ATP binding to Hsp70 using all-atom molecular dynamics simulations of this protein nanomachine.

References

[1] Senet P, Maisuradze GG, Delarue P, Foulie C, Scheraga HA, Proc Natl Acad Sci USA 2008; 107: 19844-49

[2] Cote Y, Maisuradze GG, Delarue P, Scheraga HA, Senet P. New Insights into Protein (Un)Folding Dynamics. J Phys Chem Lett 2015; 6: 1082-86

[3] Cote Y, Senet P, Delarue P, Maisuradze GG, Scheraga HA. Nonexponential decay of internal rotational correlation functions of native proteins and self-similar structural fluctuations. Proc Natl Acad Sci USA 2010; 107: 19844-49

[4] Nicolaï A, Delarue P, Senet P. Decipher the Mechanisms of Protein Conformational Changes Induced by Nucleotide Binding through Free-Energy Landscape Analysis: ATP Binding to Hsp70. PloS Comput Biol 2013; 9: e1003379