ABSTRACTS

AIAS Symposium: Cutting Edge Technologies for neurobiology

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Chemical strategies for labeling and controlling neurotransmitter receptors

Protein labeling/imaging is now one of the powerful methods to analyze the protein functions in living systems. My group has been interested in development of chemistry-based strategies to selectively modify, visualize and regulate a target protein (a protein-of-interest (POI)) under live cell conditions. Here I would like to discuss two methods, both of which rely on the recognition-driven chemical reactions. The first method exploits a pair of a reactive short peptide tag and a corresponding synthetic probe, where the short peptide is fused to POI and the protein labeling is enhanced by proximity effect between the peptide tag and the synthetic probe. The second method is so-called ligand-directed chemistry (LDchem). In LDchem, a labeling reagent is designed which consists of a ligand for POI, a probe and a reactive (cleavable) linker. LDchem allows for the protein-selective and site-selective labeling in live cells. The target proteins are being now extended to various membrane-bound proteins such as folate receptor, GPCR and neurotransmitter receptors (Glutamate receptor (GluR) and GABA receptor (GABAR). My group also succeeded in the structure-based rational incorporation of a new allosteric site into GluRs for their activation by coordination chemistry. Such new chemical approaches may contribute to fundamental understanding of the roles of these receptors, in addition to diagnostic or pharmaceutical application.

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Dimerization techniques to resolve intracellular signaling paradox

Complexity in signaling networks in cells including neurons is often derived from co-opting particular sets of molecules for multiple operations. Understanding how cells achieve such sophisticated processing using a finite set of molecules within a confined space –what we call the "signaling paradox"- is critical to cell biology and bioengineering as well as the emerging field of synthetic biology. We have recently developed a series of chemical-molecular tools that allow for inducible, quick-onset and specific perturbation of various signaling molecules. The present technique has been employed to unravel several important, previously unresolved questions regarding the regulatory mechanisms of potassium ion channels, the membrane targeting mechanisms of small GTPases and positive feedback machinery in neutrophil migration. Using this novel technique in conjunction with fluorescence imaging and biochemical analysis, we are currently further dissecting intricate signaling networks in living cells. In particular, we investigate mechano-chemical coupling mechanisms underlying the initiation of neutrophil chemotaxis (known as symmetry breaking), as well as spatio-temporally compartmentalized signaling of Ras and membrane lipids such as phosphoinositides. In parallel, we also try to understand how cell morphology affects a biochemical pathway inside cells. Ultimately, we will generate completely orthogonal machinery in cells to achieve existing, as well as novel, cellular functions. Our synthetic, multidisciplinary approach will elucidate the signaling paradox in cells created by nature.

Sarah A. Lemprière, Centre for Clinical Brain Sciences, University of Edinburgh, UK

Using a novel knock-in model to map synaptic Arc across the mouse brain

Arc/Arg3.1 is an activity-regulated gene which is involved in multiple forms of synaptic plasticity. Arc expression has previously been used as a marker of recently-activated neurons, particularly for the investigation of neuronal circuits underlying memory formation(1). Further to this, the Arc protein is localised specifically to recently-activated synapses(2), opening up the possibility that it could be used as a marker of activity at the single synapse level. Arc also localises to the nucleus, and recent evidence suggests that the subcellular localisation of Arc protein affects its role in synaptic plasticity(3). In order to investigate the subcellular distribution of Arc in vivo we have developed a knock-in mouse line where endogenous Arc protein is tagged with the Venus fluorescent protein. This mouse model has enabled us to visualise the precise localisation and accumulation of Arc protein at individual synapses and nuclei. We have combined this mouse line with high-throughput and high-resolution microscopy, followed by automated image analysis methods, to map Arc accumulation at the single synapse level across whole sections of mouse brain. This method allows the identification of these synaptic accumulations along with the quantification of their density of distribution, their size, shape and fluorescence intensity. We found that increasing neuronal activity with seizure led to an increase in both the synaptic and nuclear accumulations of Arc, but these followed different time courses. Using this method, we also observed that an antidepressant dose of the NMDA receptor antagonist ketamine induced a long-lasting increase in synaptic Arc accumulations, whereas a higher non-antidepressant dose induced a decrease.

Tomonori Takeuchi, Department of Biomedicine, The Danish Research Institute of Translational Neuroscience (DANDRITE), and Aarhus Institute of Advanced Studies, Aarhus University, Denmark

Memory modulation by light

It is difficult to remember what you had for dinner a few weeks ago. Everyday memories, including our experiences and remembered facts, are automatically encoded in the hippocampus, and then decay very rapidly (Morris et al., EJN, 2006). In contrast, many people have vivid memories of the first dinner date with their partner, including details like the name of the restaurant and the food they had. What makes memories last? Memory retention can be boosted by a mechanism that involves novelty-associated dopamine release in the hippocampus (Wang et al., PNAS, 2010).

Recent our studies (Takeuchi et al., Nature, 2016) revealed that projections from neurons in the locus coeruleus to the hippocampus can drive the environmental novelty-associated enhancement of memory retention through non-canonical release of dopamine in the hippocampus. Our results are complemented with the subsequent finding of direct detection of dopamine co-release from hippocampal axons of the locus coeruleus (Kempadoo et al., PNAS, 2016).

These studies also raise a possibility that the impact of distinct novel experiences which by their very nature bare minimal relationship to past experiences ('distinct novelty') may differ from novel experiences that share some commonality with past experiences ('common novelty'). Projections from neurons in the ventral tegmental area to the hippocampus might mediate common novelty which modulates the memory retention with a narrow time window. We now propose that memory of events accompanied by novelty can be selectively retained through two distinct dopaminergic mechanisms, depending on the nature of the novel experience itself (Yamasaki and Takeuchi, Neural Plasticity, 2017; Duszkiewicz et al., Trends in Neuroscience, 2018).

Marco Tripodi, MRC-LMB Cambridge, UK

Life-long genetic and functional access to neural circuits using Self-inactivating Rabies virus
Neural networks are emerging as the fundamental computational unit of the brain and it is becoming progressively clearer that network dysfunction is at the core of several psychiatric and neurodegenerative disorders. Yet, our ability to target specific networks for functional or genetic manipulations remains limited. Monosynaptically restricted G-deleted Rabies virus facilitates the investigation of neural circuits' structure by the selective labelling of first-order presynaptic partners. However, despite the transformative role of ΔG-Rabies in defining the anatomical organization of neuronal circuits, its implementation in long-term functional studies, as well as for the genetic manipulation of neural networks, is largely prevented by its inherent cytotoxicity. To overcome this limitation, we engineered the Rabies virus genome so to eliminate its toxicity by modulating viral proteins stability. We reversibly targeted the viral proteins to the proteasome using a conditionally cleavable proteasome-targeting domain.
We identified one configuration that gave origin to a Self-inactivating ΔG-Rabies virus (SiR), which is completely silenced following the primary infection, thereby preventing cytotoxicity, while providing permanent genetic access to the mapped neural elements via a recombinase-mediated event triggered soon after infection. We show that SiR infected neurons retain unaltered physiological properties, functional connectivity and normal synaptic function several months following the primary infection and, likely, for the entire life of the animal. Furthermore, we used SiR to perform in vivo 2-photon calcium imaging of V1 neurons projecting to V2, showing that network-dependent computational properties of V1 neurons, such as their orientation tuning, remains unaltered long after SiR infection. The development of the SiR virus gives, for the first time, permanent genetic access to neural networks with no adverse effects on neural physiology, circuit function and circuit-dependent computations. This opens new horizons in the functional investigation of neural circuits and potentially represent the first approach to experimentally follows neural circuits remodelling in vivo. We will present the latest advancements in the development of this technology.

Keisuke Yonehara, DANDRITE, Nordic EMBL, Biomedicine, Aarhus University

Visual motion processing from retina to visual cortex in mice
Visual system is organized in a hierarchical structure, in which extracted sensory information by the retinal parallel circuits is transmitted to downstream neuronal stages via diverging and converging pathways. Computation of visual motion is one of the most fundamental functions of visual system and critical for animal´s survival. However, we are still far from understanding how visual motion is processed by neuronal circuits in different visual areas for mediating relevant visually guided behaviors. My lab combines multi-disciplinary experimental approaches such as molecular biology, transcriptome analysis, mouse genetics, two-photon imaging, electrophysiology and behavioral analysis in order to link genes, cell types, circuits and behavior by using the mouse motion vision as an experimental model. In this talk I will present recent findings from our lab aiming at understanding the function of visual motion circuits in the retina and visual cortex in the mouse.

Kevin Briggman, Research Center Caesar, Germany

Cellular resolution connectomics
The mapping of synaptic connectivity among neurons is required to constrain computational models of brain circuits. We have focused on the use of automated large-scale 3D electron microscopy to reconstruct complete microcircuits in the brain, particularly in the mammalian retina. I will describe the latest technical advances in this field as well as our recent results that explored the implications of species-specific wiring differences in the retina.