both of our sQDs were able to enter the synaptic cleft, allowing us to observe and measure constrained diffusion at the synapses in live neurons. This demonstrates the suitability of our sQDs as labels for glutamate receptors to study their diffusion dynamics. Furthermore, since both colors of our sQDs are the same size, we can use them together to compare different kinds of receptors, such as AMPA and NMDA receptors. In addition, we are presenting the optical properties of these size-equalized QDs (SE-QDs) under two-photon excitation. Since QDs have exceptionally large multi-photon absorption cross sections, we are able to achieve wide-field two-photon fluorescence imaging for a much faster imaging rate than that of confocal scanning microscopy. In two-photon wide field images, we achieved 2.3 nm, 2.5 nm, and 3.8 nm localization accuracy of 605 nm, 620 nm, and 725 nm QD, respectively, with 800 nm excitation. Furthermore, we found that the photostability of SE-QDs increased 10-fold with oxyrase (10%, with 20 mM sodium lactate). This allows low background, high penetration imaging of biological systems.

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Synaptic Protein Dynamics Measured by Fluorescence Correlation Spectroscopy

Francesco Colaci, Francesca D'autilia, Alberto Diaspro, Paolo Bianchini, Luca Lanzanò, Andrea Barberis.
Italian Institute of Technology - iit, Genova, Italy.

The activity dependent adjustment of synaptic strength (synaptic plasticity) involves the reorganization of post-synaptic proteins. The fast diffusion of synaptic proteins has been shown to play an important role in such molecular rearrangements, but the dynamic events that lead to the formation of molecular complexes are still poorly understood. Taking advantages of Single Particle Tracking (SPT) and FRAP approaches, our group has recently demonstrated that during inhibitory long term potentiation stimuli (iLTP) Gephyrin and GABAA receptors are accumulated at post-synaptic inhibitory sites. In addition we have shown that the synaptic clustering of the scaffold protein gephyrin do not precede the synaptic inclusion of GABA receptors. However, the coordinated dynamics of gephyrin and GABAA receptors outside and inside the synapse remain to be elucidated. To investigate the diffusion dynamics of the main synaptic proteins we exploited here Fluorescence Correlation Spectroscopy (FCS), an established technique to study the mobility of molecules and to measure the protein diffusion parameters with high statistics in unperturbed live cell, in both the plasma membrane and the cytoplasmic space. We calibrated an FCS method (FCS) to study the coordinated multilevel organization of receptors and adhesion molecules that laterally diffuse on the plasma membrane and the scaffold proteins that diffuse in the cytoplasmic space. Our data suggest that, the scaffold protein freely diffuse in the extrasynaptic space and is suddenly immobilized at both synaptic site and extrasynaptic clusters. This approach may clarify the coordinated diffusion of membrane synaptic proteins and cytoplasmic scaffold proteins thus clarifying the mechanisms of synaptic clustering in basal conditions and during synaptic plasticity.

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A New Method to Detect Intra- and Extracellular Conformational Changes in Ion Channels with High Temporal Resolution

Matthias Wulf, Stephan A. Pless.
Drug Design and Pharmacology, University of Copenhagen, Copenhagen, Denmark.

Ligand-gated ion channels (LGICs) and voltage-gated ion channels (VGICs) are membrane proteins that represent important drug targets. While electrophysiological techniques allow the distinction between open, closed and desensitized/inactivated states, it is not capable to directly detect conformational changes that might precede, accompany or follow the gating or ligand binding events of an ion channel. The simultaneous detection of current and emission from a fluorescent label in voltage-clamp fluorometry (VCF) has proven a powerful technique to overcome this limitation by allowing to monitor conformational rearrangements at the channel gate and in a protein domain of interest. However, ligand application in VCF is slow and limited to extracellular domains. Here, we developed a novel, ultrafast patch-clamp fluorometry (ufPCF) setup for use with both LGICs and VGICs. A combination of a digital mirror device, an emCCD camera and a piezo-driven perfusion tool enable us to achieve a time resolution of around 1 ms for ligand application and fluorescence detection. Importantly, ufPCF can be used in both outside-out and inside-out patches, irrespective of the position of fluorescent dye within the protein. For example, ufPCF allows easy access to the intracellular side of extracellularly labeled channels in inside-out patches, enabling the modulation of intracellular conditions while monitoring rearrangements in extracellular domains (and *vice versa*). Further, our ufPCF can be used with fluorescent unnatural amino acids, which can be introduced anywhere in the protein. Overall, we find this method to be highly versatile and to generally result in larger fluorescence changes compared to VCF. We anticipate ufPCF to significantly aid our understanding of the pharmacology and molecular function of both LGICs and VGICs and we intend to extend its use to studies using transporters and pumps.

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Designing Neuronal Optical Voltage-Sensing Probes using Artificial Proteins

Martin J. Iwanicki, Geetha Goparaju, Christopher C. Moser, Sergei A. Vinogradov, Bohdana M. Discher.
Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA, USA.

Communication between neurons in neuronal networks is associated with changes in neuronal membrane potentials. The ability to image these changes on sub-ms timescales is vital for better understanding of inter-neuronal connections. Inorganic probes have been developed for this purpose and proved to be fast enough to detect neuronal action potentials with sufficient time resolution. However, these probes non-selectively partition into membranes other than plasma membranes and they cannot be targeted to specific cells. Protein-based optical voltage-sensing probes can address this problem, but current genetically encoded voltage indicators (GEVIs) lack behind the inorganic probes in speed and amplitude. Here, we present the design and characterization of new sensors that are based on artificial proteins (maquettes). These artificial membrane maquettes contain four membrane-spanning α-helices that are linked into a single protein chain and form electron transfer chain across a lipid bilayer. To facilitate *in vitro* characterization, they have been expressed in high yields in inclusion bodies of *E. coli*, purified and refolded in charged and uncharged detergent micelles, as well as in lipid vesicles. Circular dichroism studies revealed 70% α-helicity in SDS and no melting in high temperatures and common denaturants, indicating their strong structural stability. The maquettes assemble in different membrane environments and bind 3 hemes upon assembly in vesicles (two hemes strongly with 150 nM affinity and third one more weakly with $k_d \sim 1$ μM). The redox midpoint potentials of the three hemes at pH=8.0 are −38, −150, and −203 mV, suggesting that they will be in mixed oxidation states when incorporated in resting plasma membranes. We are currently conjugating these maquettes with fluorescent proteins and nanoparticles to enable fast electron and energy transfers that will lead to strong fluorescent signals deltaF/F as a function of transmembrane voltage.

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Photoacoustics as a New Modality for Recording Membrane Potential Changes

Haichong K. Zhang[1], Ping Yan[2], Jeeun Kang[1], Diane Abou[3], Hanh N.D. Le[4], Abhinav K. Jha[3], Daniel Thorek[3], Jin Kang[4], Arman Rahmim[3], Dean F. Wang[3], Emad M. Boctor[3], Leslie M. Loew[2].
[1]Computer Science, The Johns Hopkins University, Baltimore, MD, USA, [2]R.D. Berlin Center for Cell Analysis and Modeling, University of Connecticut School of Medicine, Storrs, CT, USA, [3]Radiology, The Johns Hopkins Medical Institutes, Baltimore, MD, USA, [4]Electrical and Computer Engineering, The Johns Hopkins University, Baltimore, MD, USA.

Voltage sensitive dyes (VSD) are designed to monitor the membrane potential by detecting fluorescence change in response to neuronal or muscle electrical activity. However, fluorescence imaging is limited by depth of penetration and high scattering losses which leads to low sensitivity in vivo systems for external detection. In contrast, photoacoustic (PA) imaging, an emerging modality, is capable of deep tissue, noninvasive imaging by combining near infrared light excitation and ultrasound detection. In this work, we develop the theoretical concept whereby the fluorescence quenching of the voltage-dependent dye fluorescence leads to a reciprocal enhancement of PA intensity. Based on this concept, we synthesized a novel near infrared photoacoustic VSD (PA-VSD) whose PA intensity change is sensitive to membrane potential. In the polarized state, this cyanine-based probe enhances PA intensity while decreasing fluorescence output in a lipid vesicle membrane model. With a 1-9 μM VSD concentration, we measured a PA signal increase in the range of 14% to 23%, and observed a corresponding signal reduction in fluorescence emission. We used our theoretical model to predict the expected PA intensity increase of the dye, given certain fluorescence and absorption properties. The predicted values showed a good agreement with the experimental values. These results not only demonstrate the voltage sensing capability of the dye, but also indicate the necessity of considering both fluorescence and absorption spectral sensitivities in order to optimize the characteristics of improved photoacoustic probes. Together, our results demonstrate photoacoustic sensing as a potential new modality for sub-second recording and external imaging of electrophysiological and potentially neurochemical events as in vivo brain cortex.