imaging to determine affinity by visualizing single molecular bindings on nano-size dendrons. Using DNA hybridization as a demonstrator, an AFM sensor adorned with a cognate binding strand senses and localizes target DNAs at nanometer resolution. The equilibrium dissociation constant of capturing DNA duplexes was obtained, yielding 2.4×10^{-10} M. Our label-free single molecular biochemical analysis approach evidences the utility of recognition imaging and analysis in quantifying biomolecular interactions of just a few hundred molecules. Acknowledgements. This work was supported by the Korean National Research Foundation (NRF)-Austrian Science Fund (FWF) Joint Research Program I 3173, FWF project V584-BBL, and the Austrian National Foundation for Research, Technology and Development and Research Department of the State of Upper Austria.

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Photoacoustic Selective Plane Illumination Microscopy

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The photoacoustic effect (PA) is a physical phenomenon involving the generation of sound waves following light absorption in a material sample. The photon absorption and subsequent non-radiative relaxation of the chromophores induces a rapid isochoric heating. Thus, it increases the pressure within the sample inducing a thermoelastic expansion, and the emission of a pressure wave called a photoacoustic wave[1]. By utilizing low ultrasonic scattering, PA imaging enables high-resolution, deeply penetrating imaging in biological tissue. The base of the contrast in photoacoustic imaging is the different absorption coefficients of tissue components or suitable transgene labels in the sample. However, since transgene probes are few and they have a poor efficiency, there are few applications to living animals and processes at the cellular and subcellular levels. Reversibly switchable fluorescent proteins (rsFPs) have had a revolutionizing effect on life science imaging due to their contribution to optical nanoscopy as agents able to improve contrast-to-noise ratio and spatial resolution[2]. Since PA requires pulsed illumination and depends on signal generation via nonradiative energy decay channels, rsFPs optimized for fluorescence imaging may not be ideal for PA due to competitiveness between light emission and heating[3]. The aim of this project is the development of a novel approach in photoacoustic microscopy working both on the engineering of probes and setup. The probes belong to two different families of photochromic proteins: GAF3[4], and two novel mutants of GFPs obtained adding a fluorescence-decreasing mutation to wildQ/wildQT proteins[5]. We will apply novels rsFPs in a brand new photoacoustic system based on Selective Plane Illumination Microscope (paSPIM).

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Biophysical Characterization of Exosomes Based on their Unique Dielectric Properties

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Exosomes belong to the cell-secreted extracellular vesicles family with diameters of ~30-100 nm, released from many cell types into the extracellular space. From there, they are taken up by neighboring or distant cells and subsequently modulate functions of the recipient cells. Exosomes are composed of a lipid bilayer membrane containing various receptors and tetraspanin surface markers. They also encapsulate nucleic acids, proteins, and lipids in the lumen and their components reflect their cellular origin. Thus, when the secreting cells are in abnormal microenvironments, the compositional changes are reflected in the secreted exosomes, thereby sending the wrong message to the recipient cells. Exosomes are often characterized based on their size by Nanoparticle Tracking Analysis, Transmission Electron Microscopy, or Flow Cytometry techniques. Although size-based characterization has served as an initial step for exosome detection, it has not been able to distinguish exosomes by their cells of origin or functions and thus, exosomes are often get lysed or labeled for downstream genomic or proteomic analyses which adds extra cost and time to the procedure, not to mention the destruction of the vesicles' structure. Here, for the first time, a new class of electrical impedance measurement system has been developed to non-invasively characterize exosomes from different cell of origins. The characterization was based on the exosomes' unique dielectric properties as their biogenesis was subjected to systematic changes under different culture conditions. The principle of the impedance measurement was adapted from Maxwell's Mixing Theory to analyze the dielectric property of cells. Extracted exosomes from different origins were measured and the result showed distinguishable impedance at frequencies higher than 1MHz, which represented the difference between the membrane and cytosolic compositions. This technique can be further evolved as a diagnostic tool for characterizing the pathogenic exosomes.

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Improved Split Fluorescent Proteins for the Visualization of Endogenous Proteins and Synapses

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Self-complementing split fluorescent proteins (FPs) are split FPs whose fragments associate spontaneously in cells to form a functional FP, which have demonstrated wide applications including detection of protein expression, scaffolding of protein assemblies and visualization of cell-cell interactions. In particular, the small fragment of split GFP1-10/11 and other split FP1-10/11 constructs (including split mNeonGreen21-10/11, sfCherry21-10/11, and PAsfCherry21-10/11) can be easily integrated into any gene locus of interest, which enables the generation of endogenously-tagged cell line libraries for the systematic analysis of protein function and localization under almost-native condition. However, newly developed self-complementing split FPs have suffered from suboptimal fluorescence signal compared to its full-length counterpart. By investigating the complementation process, we have found two approaches to improve the overall brightness of split fluorescent proteins: one is promoting FP1-10 and FP11 complementation through SpyTag/SpyCatcher interaction and the other is screening with more stringent complementation efficiency in directed evolution. The latter approach has generated two split sfCherry3 variants with substantially enhanced overall brightness, facilitating endogenous protein labeling by gene editing. Based on sfCherry3, we have further developed a new red-colored trans-synaptic marker called Neuroligin-1 sfCherry3 Linker Across Synaptic Partners (NLG-1 CLASP) for multiplexed visualization of neuronal synapses in living animals. Similarly, we improved yellow-green colored split FP by directed evolution and rational design. The former strategy yielded mNG31-10/11 variants with enhanced brightness in E.coli and better complementation profile in mammalian cells. Finally, we rationally introduced mClover mutations into Split GFP1-10. The brightness of this chimera protein was recovered by directed evolution, generating a new split fluorescent protein, CloGFP1-10/11

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Spatially Compartmentalized Phase Regulation in the Ca^{2+}-cAMP-PKA-Oscillatory Circuit

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Signaling networks are spatiotemporally organized in order to sense diverse inputs, process information, and carry out specific cellular tasks. In pancreatic β cells, Ca^{2+}, cyclic adenosine monophosphate (cAMP), and Protein Kinase A (PKA) exist in an oscillatory circuit characterized by a high degree of feedback, and this circuit is instrumental in mediating and potentiating pulsatile insulin secretion. Here, we describe a novel mode of regulation within this circuit involving a spatial dependence of the relative phase between cAMP, PKA, and Ca^{2+}. We show that nanodomain clustering of Ca^{2+}-sensitive adenylyl cyclases drives oscillations of local cAMP levels within the membrane nanodomain to be precisely in-phase with Ca^{2+} oscillations, whereas Ca^{2+}-sensitive phosphodiesterases maintains out-of-phase oscillations within the general plasma membrane, providing a striking example and novel mechanism of cAMP compartmentation. Disruption of this precise in-phase relationship perturbs Ca^{2+} oscillations, suggesting that the relative phase within an oscillatory circuit can encode specific functional information. This example of a signaling nanodomain utilized for localized tuning of an oscillatory circuit has broad implications for the spatiotemporal coordination of many biological networks.