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Quantum Dot Sensors and Nanoactuators in Living Cells

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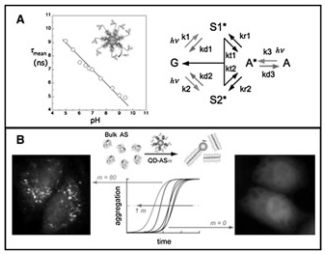
Quantum dots (QDs) are unique probes due to their special properties (brightness, photostability, narrowband emission and broadband absorption), and excellent bio(chemical)compatibility for imaging structures and functions of living cells. When functionalized with ligands, they enable the recognition of specific targets and the tracking of dynamic processes for extended periods of time, detecting biomolecules with a sensitivity extending to the single molecule level. Here we present nanosensors and nanoactuators based on QDs in which the multivalency of the particle plays an essential role in determining the functionality and sensing characteristics of the nanodevices. Two examples are discussed:
(i) luminescent pH nanosensing QDs calibrated and measured by FLIM (Fig. A). The underlying process is bidirectional (a novel feature) FRET between the QD and the conjugated sensor with pH-sensitive spectral properties[1].
(ii) nanoactuators consisting of streptavidin QD to which biotinylated alpha-synuclein (AS) has been conjugated (Fig. B). These constructs are very efficient intiators and sensors of AS aggregation *in vitro* and in live cells[2] .



[1] G. Menendez, in preparation.
[2] M. J. Roberti, M. Morgan, L. I. Pietrasanta, T. M. Jovin, E. A. Jares-Erijman, *J. Am. Chem. Soc*. (2009) 131: 8102-8107; in preparation.

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Nonlinear Structured-Illumination Microscopy Using Photo-Switchable Labels

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Periodically structured illumination light can extend the resolution of a light microscope beyond the classical limit by an amount equal to the spatial frequency, k1, of the illumination structure [1]. The wavelength and the laws of physics limit the set of frequencies that can be physically generated in a light intensity field in the same way as the set of frequencies that can be observed. Consequently, k_1 cannot be much greater than the classic resolution limit itself, and therefore the resolution extension cannot exceed a factor of two.
However, dramatically greater resolution extension is possible if the emission rate is made to be nonlinear with the incoming illumination intensity. This nonlinearity can cause the effective excitation to contain harmonics at multiples of k_1, with correspondingly multiplied resolution-enhancing ability [3].
Photo-switching of fluorophores is a nearly ideal source of such nonlinearity. This phenomenon has produced modest resolution improvements when applied on one dimension and analyzed with real-space methods [4]. We are using it to develop high-resolution microscopy of 2D samples based on our frequency-space-based approach to nonlinear structured-illumination microscopy. Here we describe recent progress toward multidimensional super-resolution of biological samples using this technique.
[1] M.G.L. Gustafsson, ''Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy,'' *J. Microsc.***198**, (2000).
[2] R. Heintzmann, T.M. Jovin, and C. Cremer, ''Saturated Patterned Excitation Microscopy - a Concept for Optical Resolution Improvement,'' *J. Opt. Soc. Am. A*. **19**, (2002).
[3] M.G.L. Gustafsson, ''Nonlinear structured-illumination microscopy: wide-field fluorescence imaging with theoretically unlimited resolution,'' *Proc. Nat. Acad. Sci. USA* **102**, (2005).
[4] M. A. Schwentker, H. Bock, M. Hofmann, S. Jakobs, J. Bewersdorf, C. Eggeling, and S.W. Hell, ''Wide-Field Subdiffraction RESOLFT Microscopy Using Fluorescent Protein Photoswitching'', Microscopy Res. & Techn. **70**, (2007).

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Reversibly Switchable Fluorescent Proteins

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Recently, reversibly switchable fluorescent proteins (RSFPs) have become a new branch of the green fluorescent protein (GFP) like family. RSFPs may be reversibly switched between a fluorescent and a non-fluorescent state by irradiation with light of distinct wavelengths; the key structural event of this switch is a cis / trans isomerization of the chromophore which is accompanied by changes in the protonation state of the chromophore. These proteins may be applied in sub-diffraction resolution microscopy as well as in novel protein tracking schemes or even as data storage elements.
Consequently, the creation of novel RSFPs with unique characteristics for the respective applications is of great importance. To this end, the combination of rational and random mutagenesis together with an automated screening system allowed us to create several new RSFPs. These include rsCherryRev, Padron and bsDronpa. rsCherryRev, the first red fluorescent monomeric RSFP, was successfully applied in a live cell sub-diffraction resolution microscopy experiment to visualize the movement of the endoplasmic reticulum. Padron, a green RSFP with reversed switching behavior, in combination with the RSFP rsFastLime allowed for multilabel single detection color microscopy, while bsDronpa, a Dronpa variant with a broad excitation spectrum, was used for two-color sub-diffraction resolution microscopy.

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Photoswitching Mechanism of Cyanine Dyes

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Photoswitchable fluorescent probes have been used in recent years to enable super-resolution fluorescence microscopy by single-molecule imaging. Among these probes are red cyanine dyes, which can be reversibly photoconverted between a fluorescent and a dark state for hundreds of cycles before permanently photobleaching, yielding several thousand detected photons per switching cycle. While these properties make them excellent probes for super-resolution imaging, the photochemical mechanism by which switching occurs has yet to be elucidated. In this study we determine the mechanism of photoswitching by characterizing the kinetics of dark state formation, and the spectral and structural properties of the dark state. The rate of switching to the dark state depends on the concentration of the primary thiol in solution and the solution pH, in a manner quantitatively consistent with the formation of an encounter complex between the cyanine dye and ionized thiol prior to their conjugation. Using mass spectrometry, we identify the photoconversion product as a thiol-cyanine adduct in which covalent attachment of the thiol to the polymethine bridge disrupts the original conjugated pi electron system of the dye. Such an understanding could prove useful for creating new photoswitchable probes with improved properties.

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Fluorescence Enchancement from Single Molecules Confined in SiO2 Nanochannels

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A large challenge in biophysics when studying single molecules using fluorescence microscopy is to obtain a signal that is clearly detectable above the background noise. Ways to improve or optimize the fluorescence signal is therefore of great interest. We here study DNA extended in 320 nm deep funnel-shaped SiO2 nanochannels with a width ranging from 40nm to 600nm. The DNA is stained with a fluorescent dye (YOYO-1) and we show that the total emission from the DNA varies significantly with the dimensions of the channels and has a peak intensity at half the wavelength of the emitted light. Measurements at varying salt concentrations, where the same confinement leads to different extension of the DNA, confirm that it is solely the geometry of the channel that governs the enhancement effect, ruling out alternative explanations, such as self-quenching. By using polarizers on the emission side we can investigate the light polarized parallel and perpendicular to the channel separately and we see that they show vastly different behavior with the peak in emission only detected in the light polarized parallel to the channels. We will discuss how our data may be explained by cavity-resonance effects when the lateral dimensions of the channels coincide with half the wavelength of the emitted light. Our results suggest that it is possible to fine-tune the size and shape of the nanochannels to maximize the number of photons collected from the molecule under study, for example when studying DNA interacting with single DNA-binding proteins where maximizing the photon budget is paramount.