Smart fluorescent probes for superresolution imaging

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It is generally accepted that living cells make extensive use of spatial compartmentalization and structuring to limit the interactions that can occur, giving rise to e.g. signaling specificity. In this presentation I will discuss our recent work on trying to visualize biosensor responses in live cells with a spatial resolution below the diffraction limit. Our efforts thus far have centered on the use of stochastic optical fluctuation imaging (SOFI) [1] combined with engineered sensor variants that possess appropriate single-molecule fluorescence dynamics. After introducing the fundamentals of the imaging and the fluorophores, I will discuss two recent results. In the first study, we developed an interaction sensor based on bimolecular fluorescence complementation (BiFC), by creating variants of a photochromic fluorescent protein. Using this label we could visualize protein interactions at a spatial resolution of about 100 nm. In the second study, we developed a variant of AKAR (PKA activity reporter) that encoded the activity of PKA into changes in fluorescence dynamics. Using SOFI, we could read out this kinase activity with about 100 nm spatial resolution and a temporal resolution of a few second. In this way we observed dynamic PKA activity microdomains or 'hot spots', whose existence was corroborated with STORM-based experiments using antibodies selective for PKA phosphorylation sites.

References

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[3] Mo et al, Genetically encoded biosensors for visualizing live-cell biochemical activity at super-resolution, Nature Methods 2017