Development of functional fluorescent probes and simple instrumentation for single-molecule studies of proteins

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Ligand binding and conformational changes of biomacromolecules play a central role in the regulation of all cellular processes. It is important to understand how both are coupled and what their specific role is in biological function. In the first part of my talk, I will highlight how the characterization of conformational heterogeneity and real-time kinetics of conformational changes by single-molecule fluorescence spectroscopy[1] allows to discern the temporal order of ligand-protein interactions and conformational changes in protein model systems (induced fit vs. conformational selection)[2-4]. Secondly, I will introduce essential enabling technology (self-healing dyes[5,6]) that facilitate such investigations. I will introduce the underlying concepts of intramolecular photostabilization, discuss our most recent applications in super-resolution microscopy[7] and future plans related to non-covalent assembly of functional fluorescent dyes on biomolecules. Finally, I will show how such single-molecule investigations, which currently still require sophisticated optical laboratories, can become possible at the biochemistry bench. For this we introduce a compact and versatile 3D-printed microscopy platform that allows to assemble many different fluorescence imaging modalities (confocal and video detection) and with that facilitates single-molecule or single-particle detection and high-resolution fluorescence imaging outside of specialized optical labs.

References: