Physikalisches Kolloquium des Fachbereich Physik
Arnimallee 14, D-14195 Berlin
Freitag, 28. Mai 2021, 15 h s.t., Online-Veranstaltung

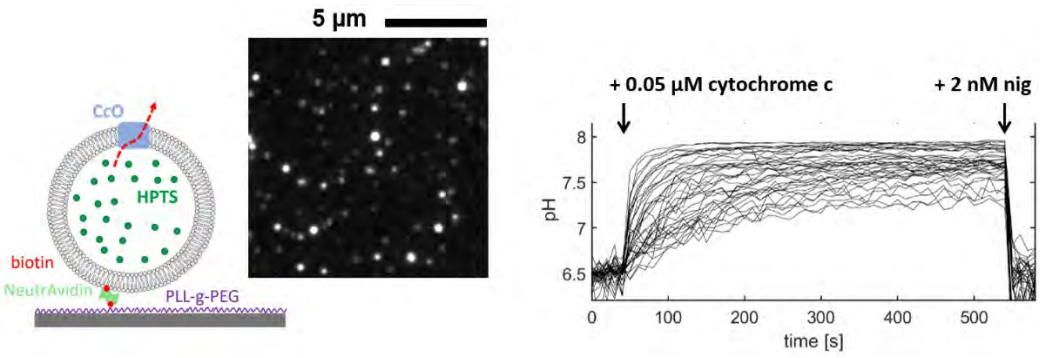
Probing Catalytic Activity and Interactions of Single Proteins Using Optical Microscopy

Stephan Block, Emmy-Noether-JRG "Bionanointerfaces" (stephan.block@fu-berlin.de), Institute of Chemistry and Biochemistry, Freie Universität Berlin, Germany

In order to arrive at a molecular understanding of the interplay between protein structure and function, it is necessary to quantify both properties with high accuracy. While this is, in principle, solved for protein structure, the quantification of protein function is in many cases a major challenge, as it often requires to probe the minute action of single proteins, *i.e.*, to operate with single-molecule resolution. In addition, many proteins show rather complex behaviors, which generate heterogeneities across the probed proteins and thus requires high throughput measurements to accurately sample the entire protein ensemble. Both requirements, *i.e.*, single-molecule resolution and high data throughput, are often mutually exclusive in single-molecule methods, therefore calling for new approaches.

In this talk, I will demonstrate that optical microscopy can be used to massively parallelize the quantification of protein function, while maintaining single-molecule resolution. A first example addresses the *catalytic activity of heme-copper oxidases* [1], such as the cytochrome *c* oxidase (C*c*O), which are transmembrane proteins that take up electrons and protons and thereby generate an electrochemical gradient across the hosting membrane that drives ATP synthesis. I will show that reconstituting single C*c*Os into 100 nm liposomes filled the pH-sensitive dye pyranine enables to quantify proton uptake of a single C*c*O and for 100s of single C*c*Os in parallel (see figure). Such measurements reveal that C*c*O exhibits populations with different protonation dynamics and enable to study the impact of lipid composition and pH gradient on the proton uptake dynamics.

I will demonstrate, in addition, that weak and/or complex interactions can also be probed by optical microscopy [2-4]. By employing the interaction of interest to link nanoparticles to an interface, it is possible to quantify this interaction by tracking single nanoparticle motion at interfaces. The concept enables to probe the equilibrium *binding dynamics of viruses* interacting with lipid bilayers (serving here as artificial cell membranes), providing information on the recruitment of receptors by viruses and how binding dynamics is altered by addition of antivirals. Supplemented with microfluidics, the concept also allows to probe the *mechanical stability of single macromolecules* with yet unprecedented accuracy (sub-nm spatial and sub-pN force resolution) and high data throughput, which is demonstrated by quantifying unfolding and refolding equilibria of proteins involved in the mechanobiology of cells.



References: [1] Hugentobler KG, Block S et al. *International Journal of Molecular Sciences* **21**, 6981 (2020); [2] Wallert M, Block S et al. *Small* **16**, 2004635 (2020); [3] Müller M, Block S et al. *Nano Letters* **19**, 1875-1882 (2019); [4] Block S et al. *Nature Communications* **7**, 12956 (2016).