Quantitative FRET studies and integrative modeling unravel the structure and dynamics of biomolecular systems in vitro and in live cells

Claus A.M. Seidel

Chair for Molecular Physical Chemistry, Heinrich Heine University, Universitätsstraße 1, 40225 Düsseldorf, Germany.
E-mail: cseidel@hhu.de

Fluorescence spectroscopy and imaging are important biophysical techniques to study dynamics and function of biomolecules in vitro and in live cells. However, often our view of molecular function is still formed, to a significant extent, by traditional structure determination showing many detailed static snapshots of biomolecular structures. Recent fluorescence experiments added a dynamic perspective by showing the heterogeneity and flexibility of molecular structures, visualizing transiently populated conformational states and identifying exchange pathways. This dynamic personality is an essential aspect for understanding their function. We introduced multi-parameter fluorescence detection (MFD) [1] and multi-parameter fluorescence image spectroscopy (MFIS) [2]. We register all eight characteristic fluorescence parameters in a single measurement to gain maximum resolution of specific fluorescence information on the biomolecule. The five intrinsic fluorescence properties of a fluorophore such as spectral properties in absorption and in fluorescence, brightness and quantum yield, lifetime and anisotropy report on its local environment. Three fluorescence parameters are connected to the system. The application of fluctuation spectroscopy allows us to resolve system properties such as diffusional properties and kinetic networks. The use of more than one fluorophore per molecule opens additional opportunities arising from photon densities, coincidences and dipolar coupling by Förster Resonance Energy Transfer (FRET) to study the stoichiometry and structure of biomolecular systems. We introduce a workflow for hybrid-FRET modeling that automates integrative structural modeling and experiment planning to put hybrid-FRET on rails [4]. We applied our techniques to the large GTPase human Guanylate binding protein 1 (hGBP1), to unravel the structural and dynamical intermediates during hGBP1 oligomerization by high-precision FRET in vitro and in live cells [5].

References:

[1] Widengren et al.; Anal. Chem. 78, 2039-2050 (2006).
[2] Weidtkamp-Peters et al.; Photochem. Photobiol. Sci., 8, 470-480 (2009).
[3] Kalinin et al.; Nat. Methods 9, 1218-1225 (2012).
[4 Dimura et al.; Curr. Opin. Struct. Biol. 40, 163–185 (2016).
[5] Kravets et. al.; eLife 5, e11479 (2016).