Integrative dynamic structural biology with multi-parameter fluorescence spectroscopy and super-resolution FRET microscopy

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We combine super-resolution microscopy via stimulated emission depletion (STED) and Multi-parameter Fluorescence Image Spectroscopy (MFIS) [1,2] to reach molecular resolution with sub-nanometer precision for studying biomolecules and their complexes. While STED-MFIS captures the spatial and temporal information of the cellular context with a resolution down to 20 nm, the concurrent measurement of Förster resonance energy transfer (FRET) between an excited donor and acceptor provides a zoom with Ångström precision. Thus, super-resolution FRET microscopy exploits these synergies to reach molecular resolution. I will present the complete workflow from (I) super-resolution FRET microscopy over (II) FRET-specific data analysis to (III) integrative FRET-restrained structural models. MFIS uses multi- parameter fluorescence detection (MFD) [1] to capture the complete fluorescence information on the biomolecules by registering all eight characteristic fluorescence parameters in a single measurement. This allows us to study the formation of homo- and hetero-complexes by homo- and hetero-FRET in live cells simultaneously.
Using FRET restraints and computer simulations, we established an automated workflow to generate integrative structural models of biomolecular assemblies that can be deposited in the new protein data bank, PDB-dev [3, 4]. We studied Guanylate binding proteins (GBPs) that undergo a conformational transition for GTP-controlled oligomerization to exert their function as part of the innate immune system of mammalian cells - attacking intra-cellular parasites by disrupting their membranes. We identified GBP's intrinsic flexibility, a GTP-triggered association of the GTPase-domains and an assembly-dependent GTP-hydrolysis as functional design principles that control their reversible oligomerization in polar assemblies and the subsequent formation of condensates [5]. Further examples for dynamic structural biology via integrative FRET studies is the mapping of the dynamic exchange network in chromatin fibers by studying a 12-mer nucleosome array (approx. 2,5 MDa) [6] and the detection of a so far hidden functionally important conformational state in the enzyme T4 Lysozyme [7].

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