Exploiting dynamics and position of single molecule events allows imaging-based proteomic and structural analyses inside cells

Human cells rely on the concerted interplay among many thousands of different proteins to function and most of these act as part of dynamic multimolecular complexes. Protein abundance and interactions are generally analyzed by proteomic techniques that average over cell populations. Structural insights on the other hand can typically only be gained after isolation and crystallization or high concentration of individual proteins in vitro. These methods therefore have limitations to capture dynamic properties of protein abundance, interactions and their structure in situ, which are essential to understand how they function physiologically.

To obtain proteomic data in living cells, we established a new single molecule-based approach, high-throughput fluorescence correlation spectroscopy (HT-FCS), by automating acquisition and analysis of (cross-) correlation spectroscopy of fluorescently tagged proteins. HT-FCS enabled us to quantify protein abundance, mobility and interactions inside single living cells. We demonstrate the potential of the technology for live cell proteomics in two proof-of-concept applications. First, we studied the complex formation and chromatin binding of more than 50 nuclear proteins in human cells, by acquiring more than 60,000 single molecule fluctuation measurements. Second, investigation of the cell cycle dynamics of a key regulatory mitotic kinase complex, Aurora B and its regulator INCENP revealed how the cell cycle rise in kinase concentration first triggers formation of a pre-complex, before the fully activated kinase complex is formed in mitosis.

To obtain structural insights, we combined superresolution microscopy of fluorescent tags on proteins with single particle averaging. In the first proof of complex application to the nuclear pore, we could resolve the orientation of the largest subcomplex in the nuclear pore, the Y-shaped Nup107-160 complex with a precision below 1 nm and show that the method is in principle applicable to all large protein complexes using two color superresolution imaging. More recently we have been able to extend this method to 3D views of the nuclear pore.

We expect that the throughput and statistical robustness of single molecule data, that HT-FCS and particle averaged superresolution microscopy provides, will make them widely used technologies for systems biology, *in vivo* proteomics and in situ structural biology.