Wednesday
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10:30 s.t.

Dr. Antonio Politi

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Quantitative mapping of endogenously fluorescently tagged proteins using FCS-calibrated 4D live cell imaging

The ability to tag a protein at its endogenous locus with a fluorescent protein (FP) allows for quantitative understanding of protein dynamics at the physiological level. Genome editing technology, e.g. CRISPR/Cas9, has now made this powerful approach routinely applicable to widely used mammalian cells and many other model systems, opening the possibility to systematically and quantitatively map the cellular proteome in four dimensions. Confocal time-lapse microscopy in 3D (4D imaging) is a useful tool to investigate spatio- and temporal protein dynamics, however it lacks the required quantitative power to make absolute measurements. Fluorescence correlation spectroscopy (FCS) on the other hand provides quantitative measurements such as protein concentrations and diffusion coefficients, but lacks the ability for routine 4D spatial and temporal sampling. I will present an automated experimental and data analysis workflow to combine both methods and obtain quantitative imaging data in high throughput. We discuss the potential of the method using endogeneously GFP tagged nuclear pore proteins as example protein of interest. Finally, I will show how the method can be used to create a comprehensive quantitative 4D mapping of several proteins important for cell mitosis.

Hosts: Dirk Görlich, Melina Schuh, Péter Lénárt

Large Seminar Room, Administration Building
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