**Single-molecule biology and visualization of endocytosis**

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I will present our current work in live cells based on dynamic imaging that combines single molecule fluorescence microscopy with single-object tracking to understand the regulation of the clathrin endocytic machinery involved in communication of cells with its environment, in pathogen invasion and viral infection, in cell growth control and cancer, and in the biogenesis of organelles.

Coated pits assemble by growth of a clathrin lattice, linked by adaptors to the underlying membrane and finish by the GTP-driven dynamin-mediated scission of the tubular neck connecting the pit from the plasma membrane. The large-scale disassembly of the clathrin coat then follows, mediated by the ATP-driven molecular clamp Hsc70 and its cofactor auxilin. The formation of clathrin coated pits and coated vesicles typically lasts ~ 45 - 55 s, requires the organized recruitment of hundreds of different protein molecules and displays high stochasticity. How does this process start? To answer this question we investigated the first 5 seconds in the life of a clathrin-coated pit using live-cell TIRF imagingwith single-molecule EGFP sensitivity and millisecond temporal resolution. Unbiased object identification and trajectory tracking, together with a statistical modeling yielded the numbers and arrival times of individual proteins to endocytic clathrin coated pits. We found that clathrin coated pits initiate by two sequential events, each typically involving the coordinated arrival of one clathrin triskelion and two AP2 adaptors. PI-4,5-P2 is essential for initiation whereas the accessory proteins FCho1/2 are not.