Two-color Single-Molecule Switching Nanoscopy in Live Cells

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The performance of single-molecule switching nanoscopy (SMSN) relies to a large extent on the photophysical properties of fluorophores. Fluorescent proteins (FPs) are inherently suitable for live-cell imaging, and mEos3.2, one of the best photoswitchable FPs, has already been used successfully in live-cell nanoscopy with sub-second time resolution [1]. Compared with FPs, chemical dyes can emit far more photons and therefore achieve higher localization precisions. But most dyes are not live-cell compatible, either because they are not membrane-permeable or because they depend on external additives to produce the required on-off switching behavior. Recently, a spontaneously blinking dye, HMSiR, has been reported [2], which provides a good opportunity to perform two-color super-resolution imaging in live cells.

In this presentation, we describe the optimization of HMSiR live-cell SMSN imaging. We tested HMSiR photoswitching under different imaging conditions in in vitro single-molecule experiments and found that a laser intensity of ~10 kW/cm² at a frame rate of 400 Hz yields the best results. We further present live-cell SMSN movies using HMSiR with Halo and SNAP-tags of clathrin-coated pits and mitochondria (Figure 1). Finally, we demonstrate that HMSiR and mEos3.2 can be combined to carry out two-color super-resolution imaging in live cells.

Figure 1: The performance of HMSiR in live-cell super-resolution imaging. (A) Super-resolution image of clathrin-coated pits labeled with HMSiR by SNAP-tag. (B) Super-resolution image of mitochondria labeled with HMSiR by Halo-tag.