Literature Report

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Article

Spontaneously Blinking Fluorophores Based on Nucleophilic Addition/Dissociation of Intracellular Glutathione for Live-Cell Super-resolution Imaging

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CAREER HISTORY:
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研究领域：化学生物学
主要研究兴趣之一是开发用于生物和医学研究的新型小分子光功能工具，包括检测活细胞和动物中各种事件的荧光探针。

Introduction

2014年诺贝尔化学奖给了三个物理学家：艾力克·贝齐格（Eric Betzig）、斯特凡·W·赫尔（Stefan W. Hell）和W·E·莫纳（W. E. Moerner），以表彰他们对于发展超分辨率荧光显微镜做出的卓越贡献。他们的突破性工作使光学显微技术进入了纳米尺度，从而使科学家们能够观察到活细胞中不同分子在纳米尺度上的运动。

图1 2014 年诺贝尔化学奖得主：（左）艾力克·贝齐格（Eric Betzig），（中）斯特凡·W·赫尔（Stefan W. Hell），（右）W·E·莫纳（W. E. Moerner）。

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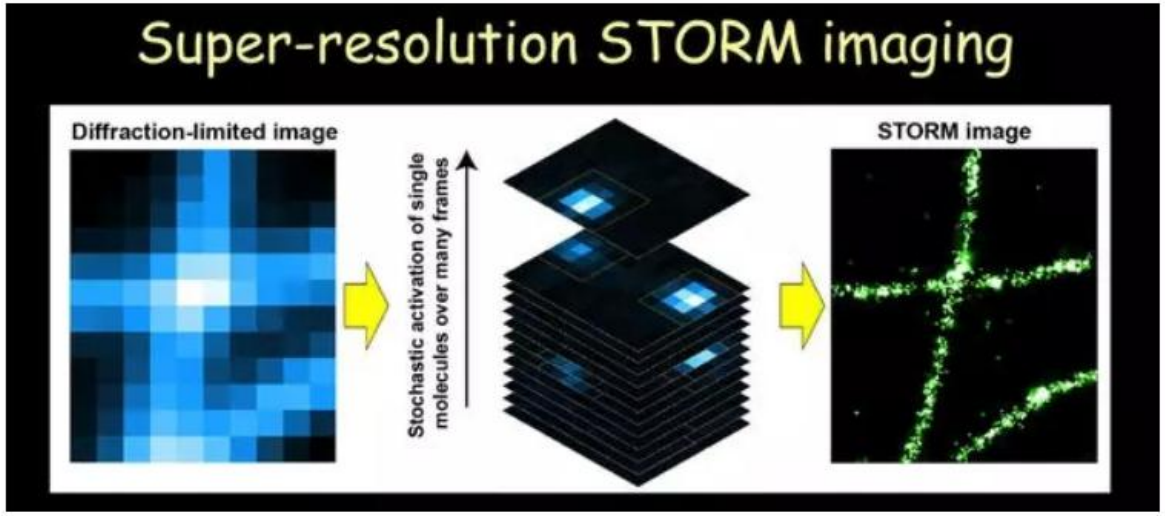
超分辨光学与普通的光学显微镜相比，它突破了衍射极限（即200 nm大小，也称之为阿贝极限，约为所使用可见光波长的一半），将人类用光学成像方法所表示的分辨率极限从几百纳米提升到了几纳米。相比于电子显微镜（分辨率约为50 nm）和扫描电子显微镜（分辨率约为0.01 nm），超分辨光学以其独特的非侵入性和高度特异性的多色标记，非常适合于活细胞的成像。

2006年，哈佛大学教授庄小威提出随机光学重建显微技术（Stochastic Optical Reconstruction Microscopy，STORM）。STORM在原理上属于基于单分子定位技术的超分辨显微成像。基于单分子定位的超分辨技术的核心是，如果图像上的点不是同时亮起来，也就是不会有两个靠的很近的点同时亮，就可以通过定位的方式实现超分辨。虽然一次定位只能得到少数几个分子，但是通过数千张图片对数十万个单分子定位，就可以获得一张高分辨率的图像。



STORM超分辨原理图

Introduction

该工作为了扩展自发闪烁荧光团的分子设计策略，开发了一种在活细胞中自发闪烁的新原理，该原理建立在他们之前的发现基础上，即由于细胞内谷胱甘肽(GSH)对蒽环的第9个碳原子的可逆基态亲核攻击，一些蒽环荧光团在有色荧光形式和无色非荧光形式之间转换(Figure 1a)。在这种热平衡条件下, GSH加合物缺乏背景荧光将有利于在SMLM中实现高的定位精度。为了利用这种分子间反应进行SMLM，必须确保只有一小部分荧光团被打开，然后以适当的动力学随机恢复到非荧光状态。因此，需要寻找合适的候选荧光团。

优化两个至关重要的参数：（1）GSH的解离常数值（$K_{d,GSH}$），这样一小部分的荧光团就会在生理GSH浓度范围内处于荧光状态，以避免信号重叠。（2）荧光形式的寿命（τ，直到荧光解离形式恢复为非荧光GSH加合物形式的持续时间），以匹配显微镜相机的曝光时间，以便可以检测到足够的光子以进行精确定位。

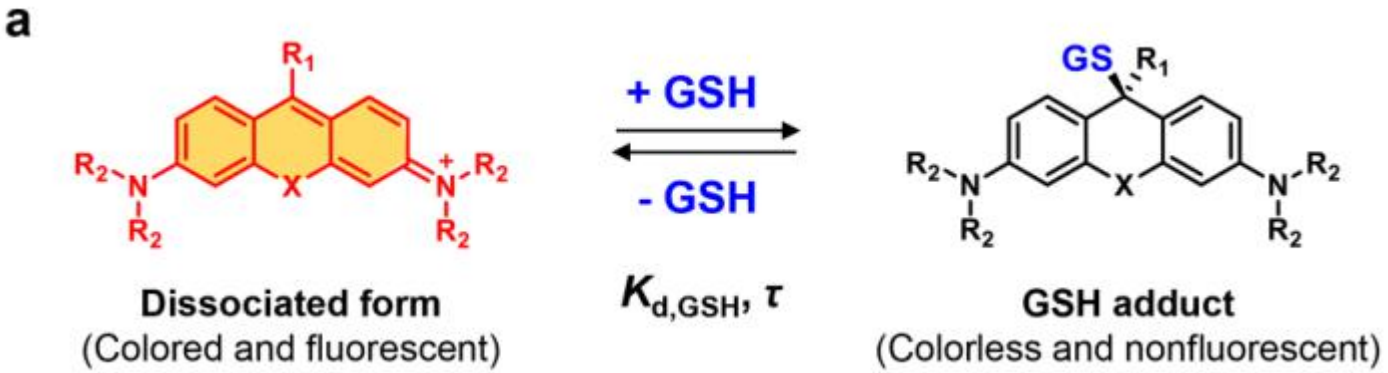


Figure 1a Fluorescence switching based on intermolecular nucleophilic addition and dissociation of GSH to and from xanthene derivatives as a novel mechanism of fluorescence blinking for SMLM. Xanthene derivatives can convert between the fluorescent dissociated form and the nonfluorescent GSH adduct form. $K_{d,GSH}$ is the dissociation constant toward GSH, and τ is the lifetime of the dissociated form.

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筛选得到了4个$K_{d,GSH}$值在1−100 μM范围内的候选者 (9Phe SiP600、SiP650、CP550、CP600)。在这些衍生物中，SiP650、CP550和CP600表现出足够高的量子产率 (Φ_f分别为0.39、0.70和0.49)。在剩下的三个候选中，他们选择了SiP650（近红外）和CP550（绿色）作为不同颜色的候选荧光团支架。

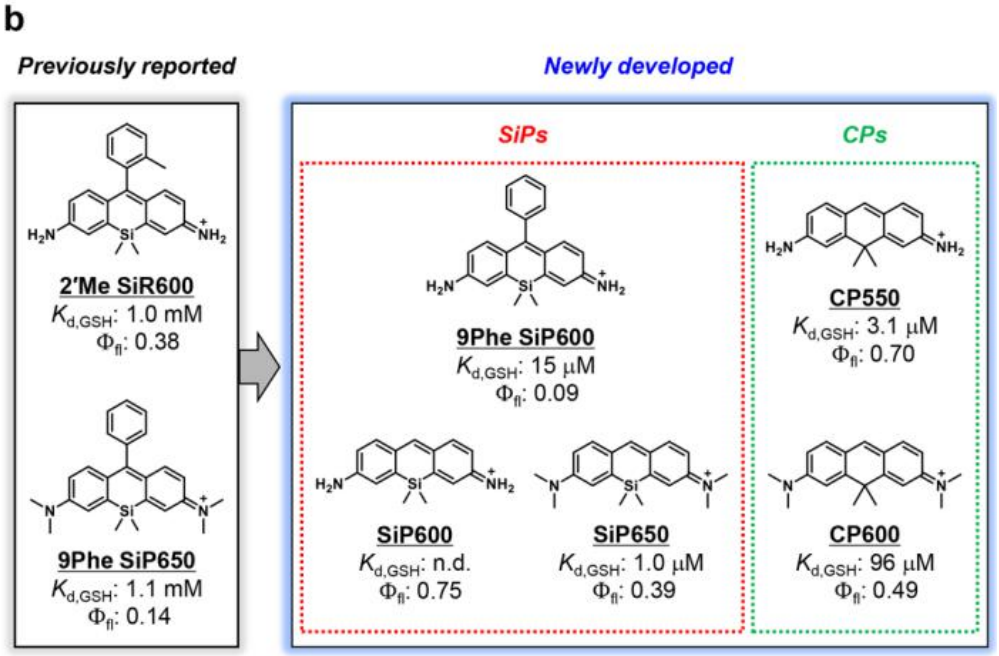


Figure 1b

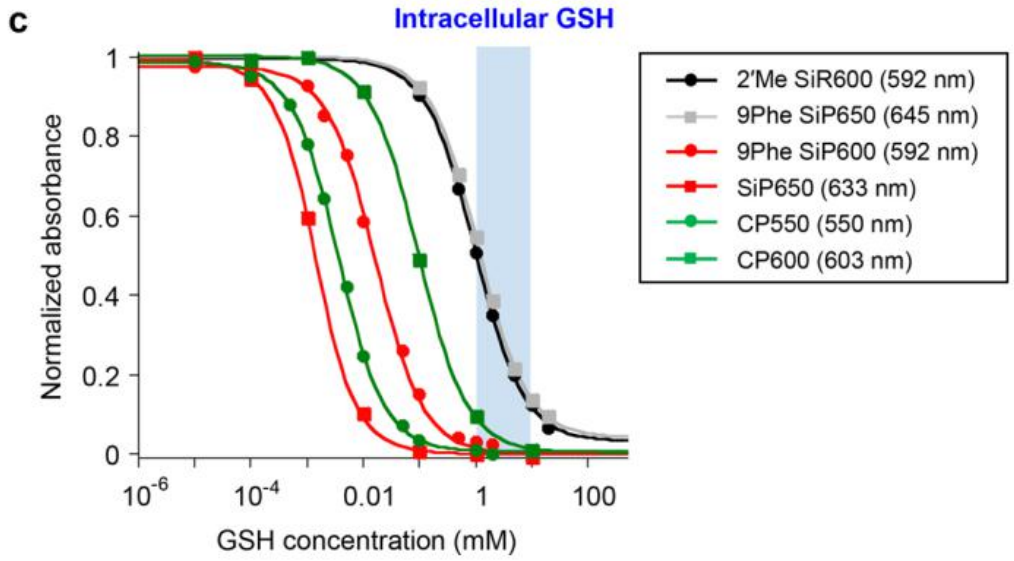
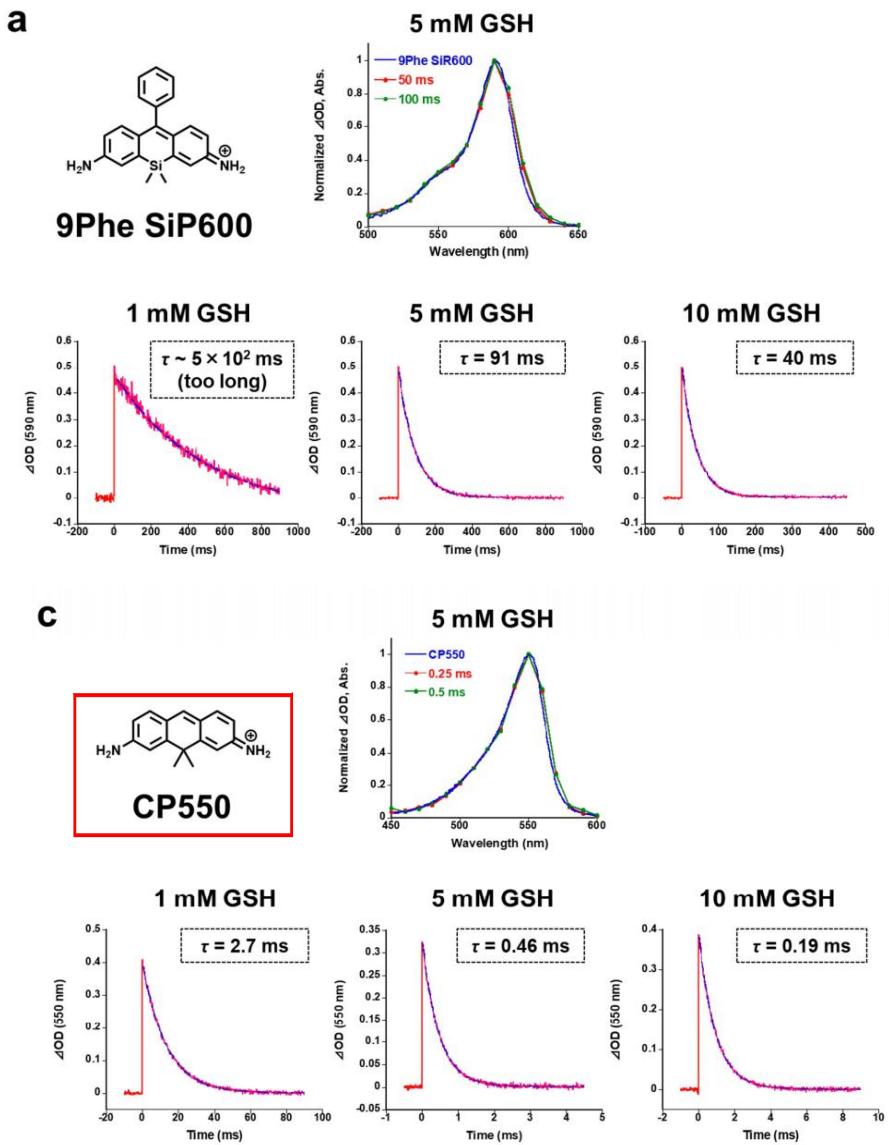
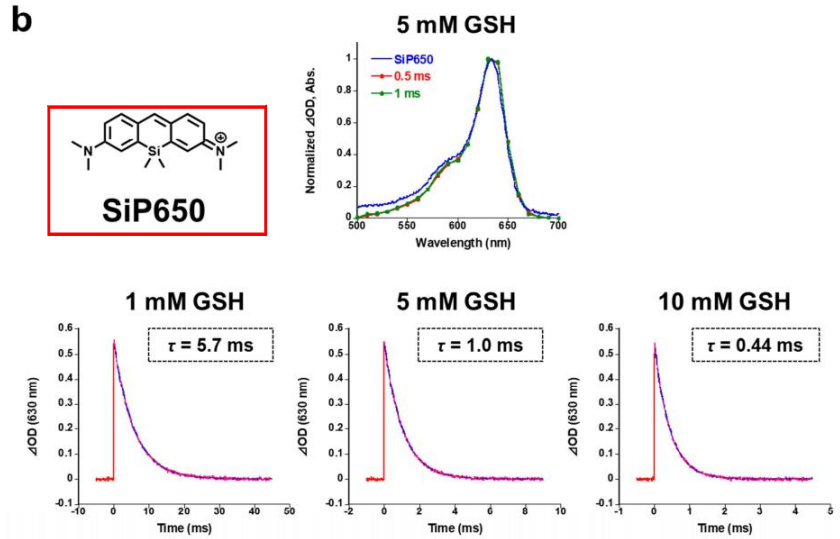


Figure 1c

Figure 1b Preparation of new xanthene derivatives with sufficiently low $K_{d,GSH}$ values. Chemical structures of candidate fluorophores based on silicon pyronine (SiP) and carbopyronine (CP) scaffolds are shown with the measured $K_{d,GSH}$ values and fluorescence quantum yields (Φ_f). Figure 1c Dose−response curves of the candidate fluorophores versus GSH.

Introduction





Absorption and fluorescence spectra and GSH titration curves of SiP650 and CP550 derivatives. Absorption (left) and fluorescence (middle) spectra and GSH titration curves (right) of small-molecular derivatives SiP650-BA (a) and CP550-BA (c) and of HaloTag-bound fluorophores SiP650-HaloTag (b) and CP550-HaloTag (d), obtained in the same manner as Figure S1.

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在SiP650和CP550中引入了HaloTag的配体单元，以制备SiP650-Halo和CP550-Halo，然后将它们与纯化的HaloTag蛋白偶联，提供SiP650-HaloTag和CP550-HaloTag作为荧光团−蛋白结合物(图Figure2a)。

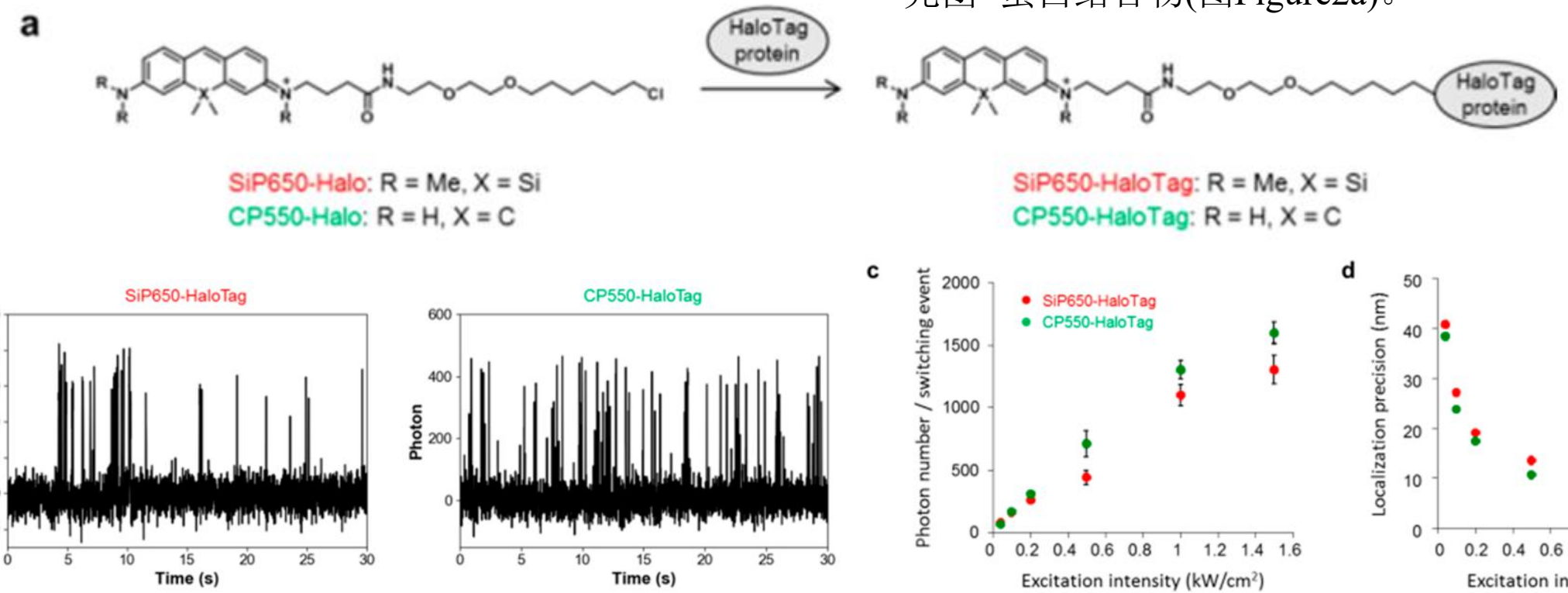

Figure 2. Evaluation of switching properties of the HaloTag protein−fluorophore conjugates by single-molecule fluorescence imaging. (a) Chemical structures of SiP650- and CP550-based HaloTag ligands, and labeling of purified HaloTag proteins to prepare fluorophore−protein conjugates (SiP650-HaloTag, CP550-HaloTag). (b) Single-molecule fluorescence time traces of SiP650-HaloTag (left) and CP550-HaloTag (right). (c, d) Excitation intensity dependence of photon number per switching event (c) and lateral localization precision (d) of SiP650-HaloTag (red) and CP550-HaloTag (green) (mean ± SE, $N = 416–12802$). Single-molecule imaging was performed in 10 mM sodium phosphate buffer (pH 7.4) containing 5 mM GSH. Excitation 647 nm (100 W/cm^2 for panel b) for SiP650 and 561 nm (100 W/cm^2 for panel b) for CP550. Exposure 8.8 ms/frame.

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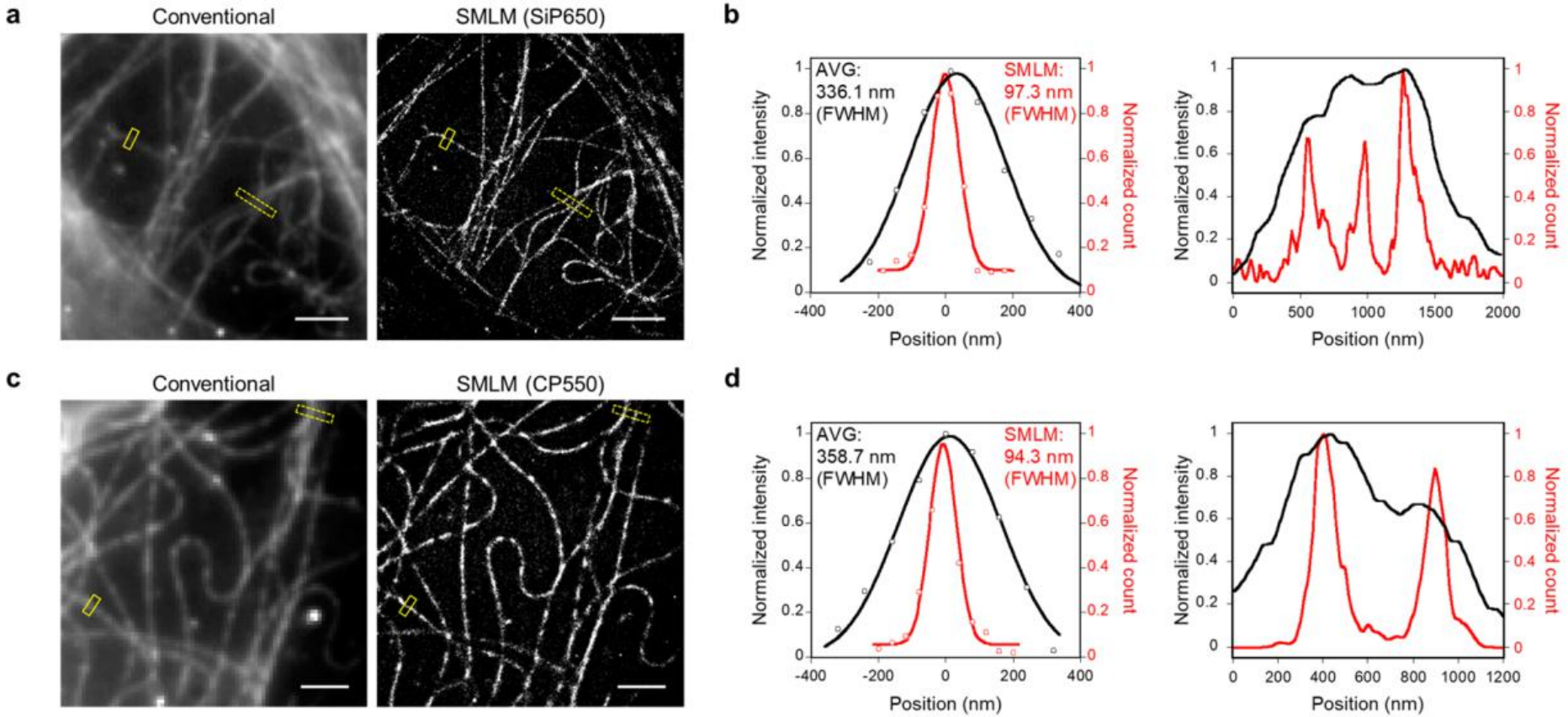
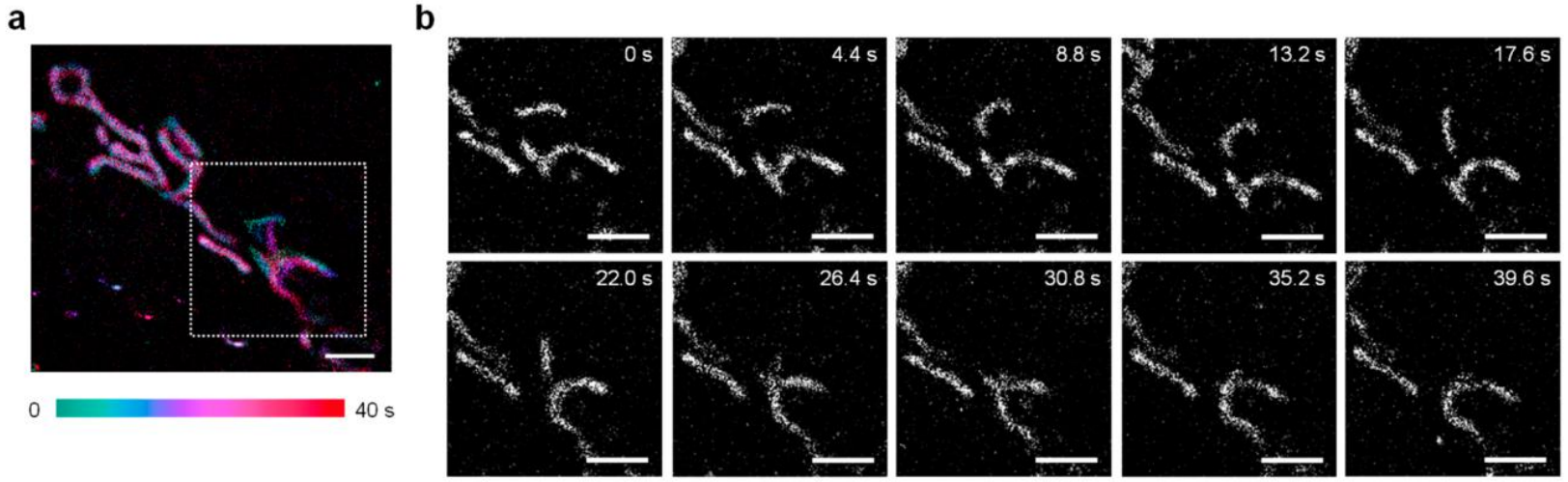
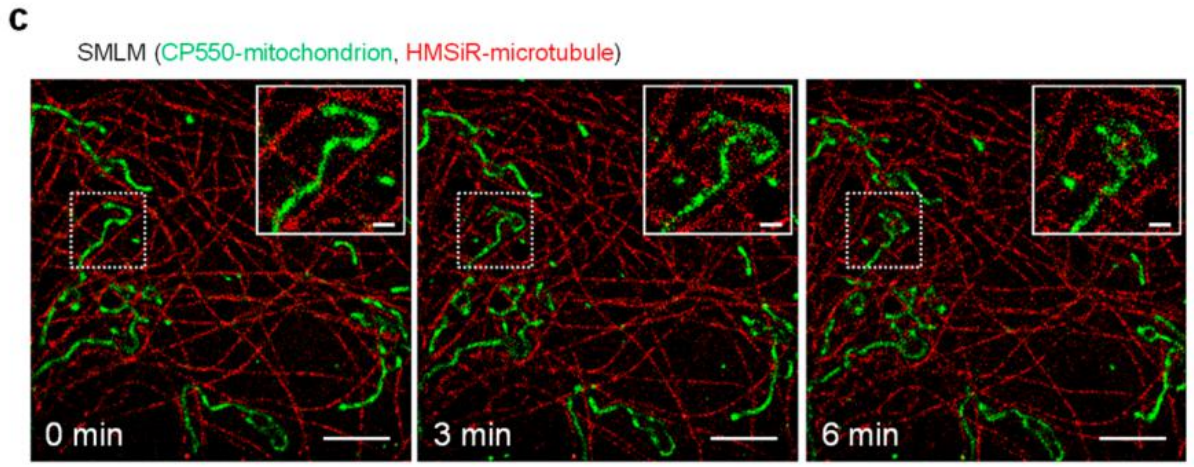


Figure 3. Live-cell SMLM with SiP650-Halo and CP550-Halo. β-Tubulin−Halo fusion proteins were transiently expressed in Vero cells and labeled with SiP650-Halo (a) or CP550-Halo (c) for 30 min. Imaging was performed in cell culture medium (DMEM) after washing. (a, c) Conventional images (averaged projection images, left) and SMLM images (right). Excitation 647 nm (200 W/cm^2) for panel a and 561 nm (200 W/cm^2) for panel c. Acquisition 8.8 ms/frame, 2000 frames (17.6 s) for panel a and 1000 frames (8.8 s) for panel c. (b, d) Transverse profiles of fluorescence intensity in the conventional images (black) and localizations in the SMLM images (red) corresponding to the regions outlined by the solid yellow lines (left) and by the dotted yellow lines (right). Panels b and d correspond to panels a and c, respectively; fwhm = 347.4 ± 25.5 nm (conventional) and 109.2 ± 8.4 nm (SMLM) (mean ± SE, $N = 6$) for panel b and 339.3 ± 11.1 nm (conventional) and 100.4 ± 5.1 nm (SMLM) (mean ± SE, $N = 6$) for panel d. Scale bars 3 μm (a) and 2 μm (c).

Introduction



CP550-BnClPy作用于线粒体的延时活细胞SMLM。可视化了线粒体的动态变化过程。



多色超分辨率成像：
用CP550-BnClPy标记了线粒体定位的SNAP-tag，用HMSiR-Halo标记了β-tubulin-Halo-tag，HMSiR-Halo是他们之前报道的基于分子内螺环开关的近红外发射自发闪烁的荧光团。