2021 Literature report VI

Reporter: Wu Shaowei

Date: 2021-11-25

ACS chemical biology

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Letters

Blinking Fluorescent Probes for Tubulin Nanoscopy in Living and Fixed Cells

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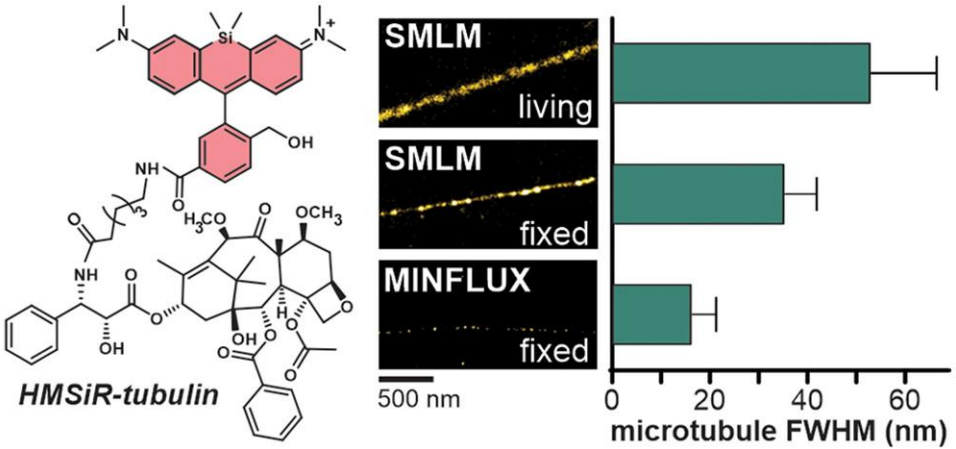

Author

Gražvydas Lukinavičius

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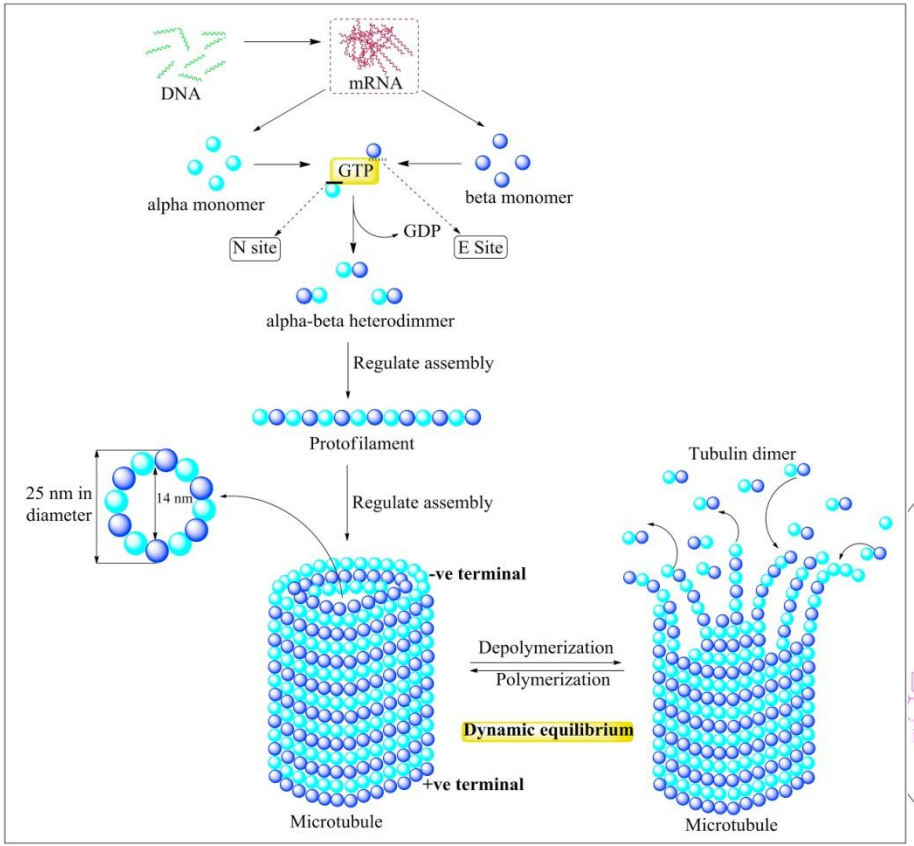
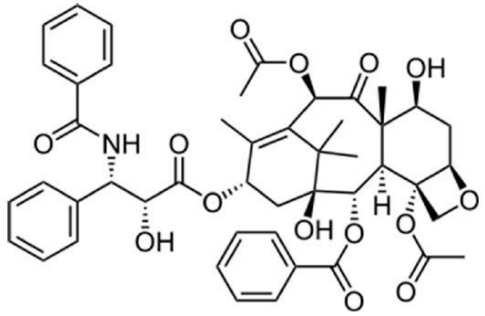
Chromatin Labeling and Imaging

- Create of the new biocompatible fluorescent probes targeting biomolecules.
- Explore new labeling methods for efficient labeling of biomolecules.
- Apply of the newly developed probes for imaging and establishment of image analysis pipelines.
- Image of dynamic processes in living cells during cell cycle or external stimulation

Introduction





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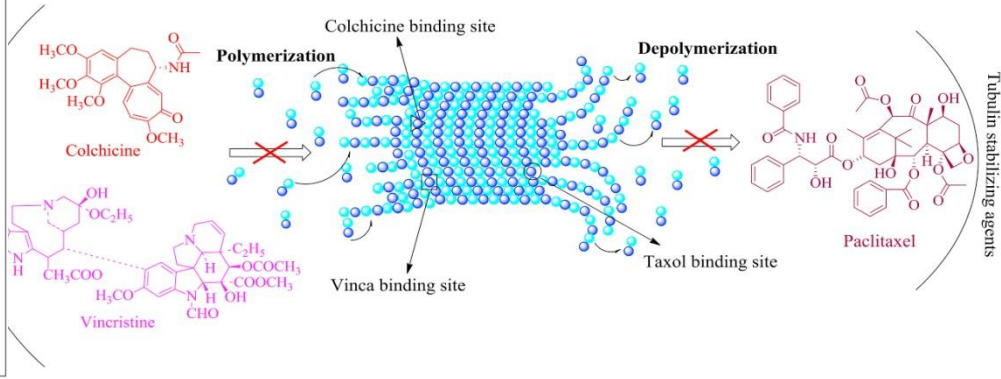
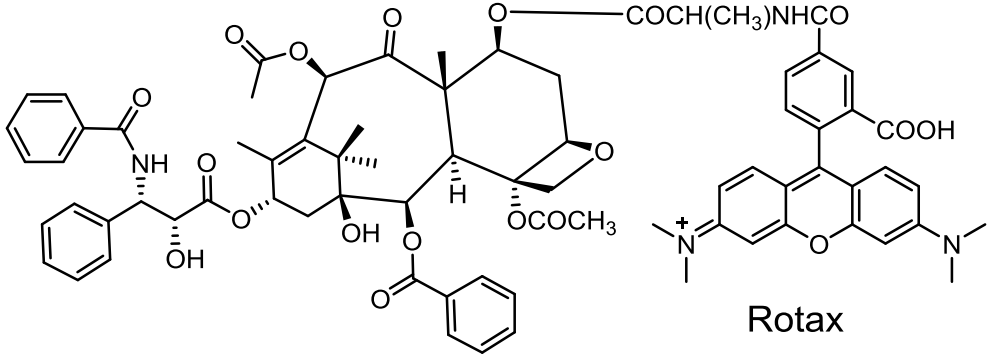
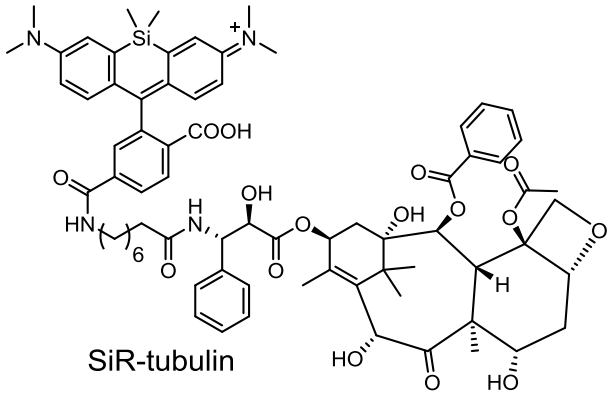


European Journal of Medicinal Chemistry **2019** 310

Introduction



Cell Motil. Cytoskeleton **1998** 73



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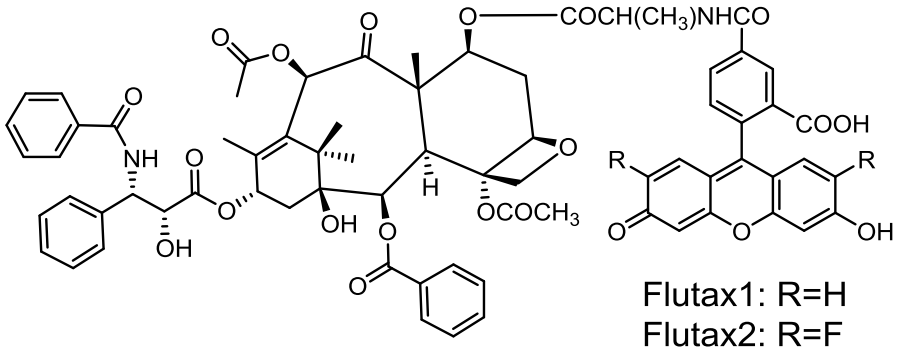
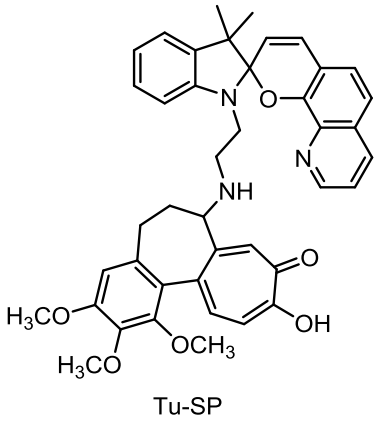
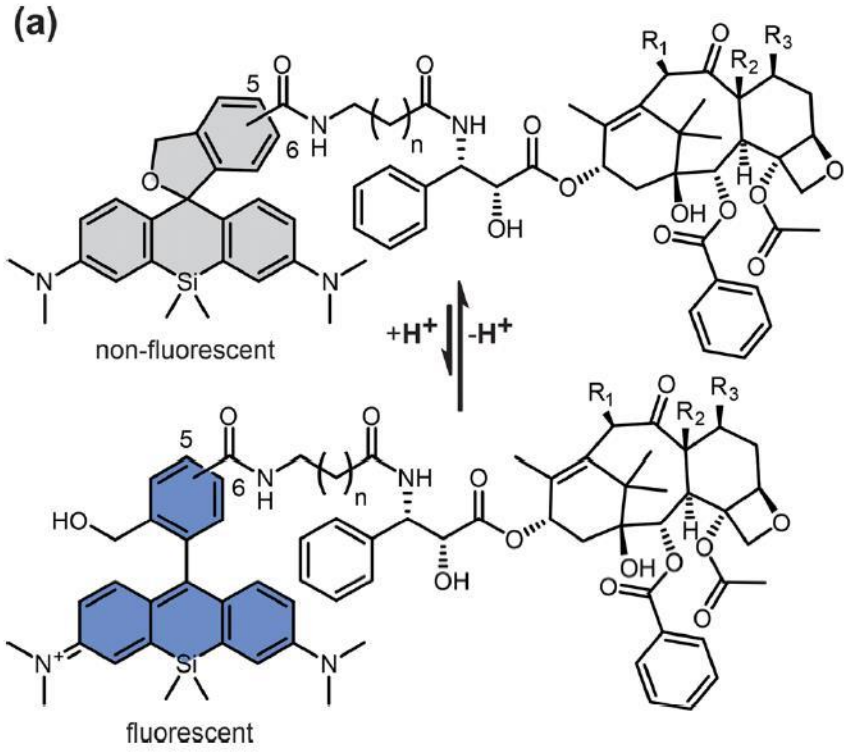


J. Biol. Chem. **2000** 26265



Anal. Chem. **2015** 5216

Strategy



(b)

probe	isomer	parent	R_1	R_2	R_3	n
1	6'	CTX	$-OCH_3$	$-CH_3$	$-OCH_3$	2
2	5'	CTX	$-OCH_3$	$-CH_3$	$-OCH_3$	4
3 HMSiR-tubulin	6'	CTX	$-OCH_3$	$-CH_3$	$-OCH_3$	4
4	6'	CTX	$-OCH_3$	$-CH_3$	$-OCH_3$	6
5	6'	DTX	-OH	$-CH_3$	-OH	6
6	6'	LTX	-OAc		$-CH_2-$	4

(c)

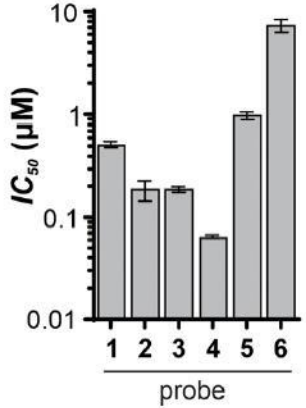


Figure 1. Tubulin probes synthesized in this study. (a) General structure of the probes, showing spirocyclization of the fluorophore that is responsible for spontaneous blinking. (b) Structure and naming convention of the probes. (c) Toxicity in HeLa cells after 24 h incubation with the probes (mean ± SD, N = 3).

Characterization

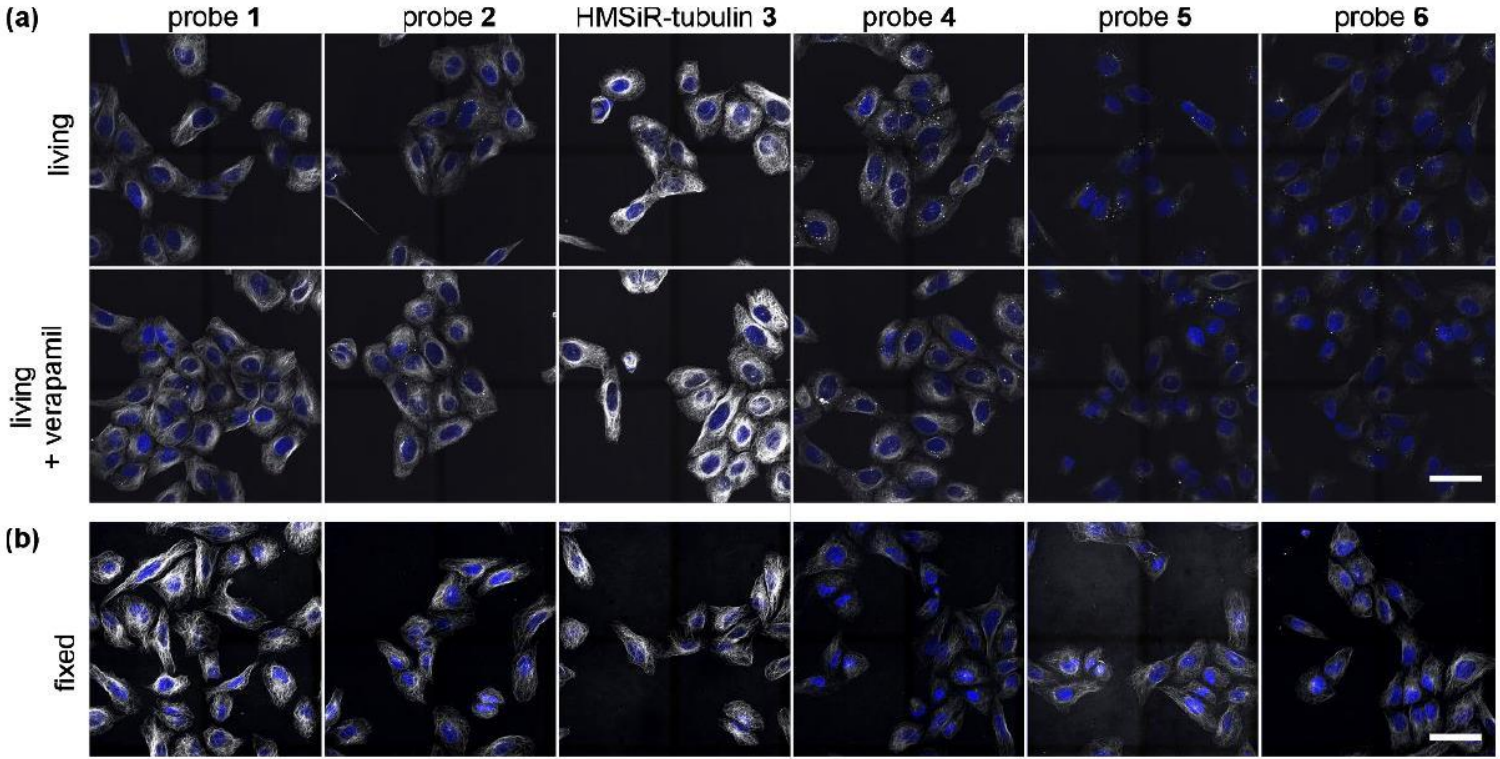
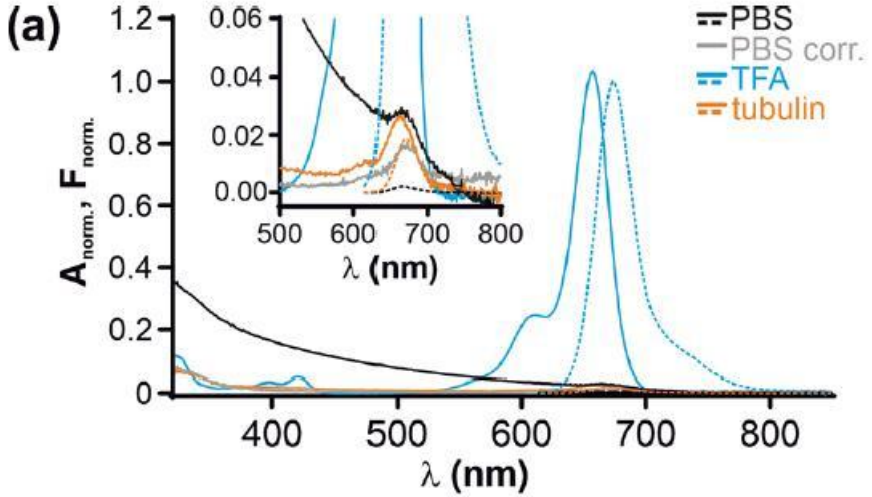


Figure S1. Staining U-2 OS cells with tubulin probes. (a) Living U-2 OS cells were incubated with 300 nM probe, +/-10 μM verapamil and 1 μg/ml Hoechst 33342 in DMEM medium with FBS for 1h at 37° C and imaged on confocal spinning disk microscope without washing. (b) The cells were fixed as described in Methods section and incubated with 100 nM probe and 0.1 μg/ml Hoechst 33342 in PEM buffer for 30 min. at room temperature. In all cases, maximal intensity projections of 39 planes (living cells) or 41 planes (fixed cells), acquired with a step size of 200 nm, are shown. Probe channel is greyscale, Hoechst 33342 is blue. Scale bar – 50 μm.

Characterization



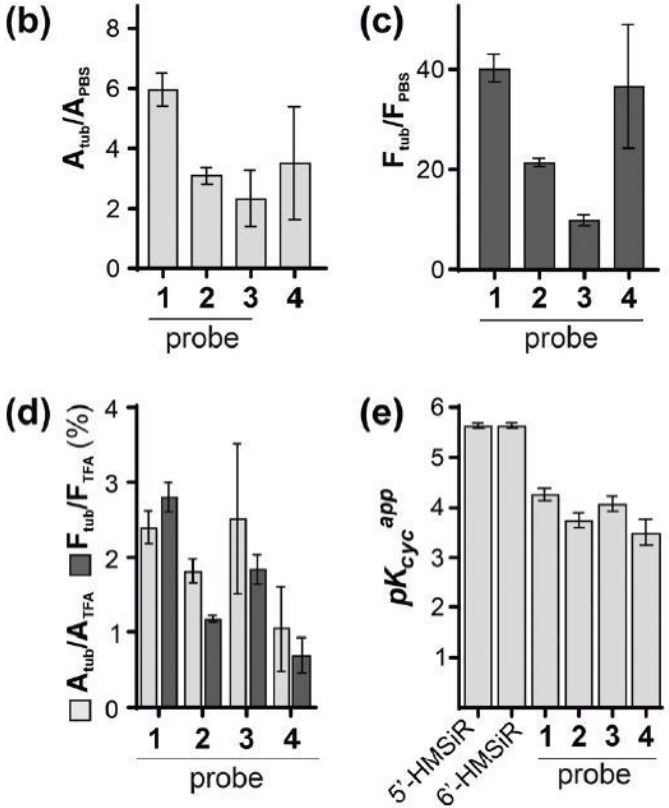


Figure 2. Properties of HMSiR CTX-based probes. (a) Normalized absorbance (solid line) and fluorescence (dashed line) spectra of 3. PBS corr., absorbance spectrum corrected for light scattering. The inset zooms in on weak signals in aqueous buffers. (b, c) Absorbance (659 nm) and fluorescence (673 nm) increase upon tubulin binding, as compared to PBS. (d) Percent of absorbing and fluorescent probe when bound to tubulin. Maximum values were determined in ethanol + 0.1% TFA; mean ± SD, N = 3 or 4. (e) Apparent cyclization constants (pH at which half of molecules are in the spiroether state) of 5′- and 6′- regioisomers of free dyes and HMSiR CTX probes.

Characterization

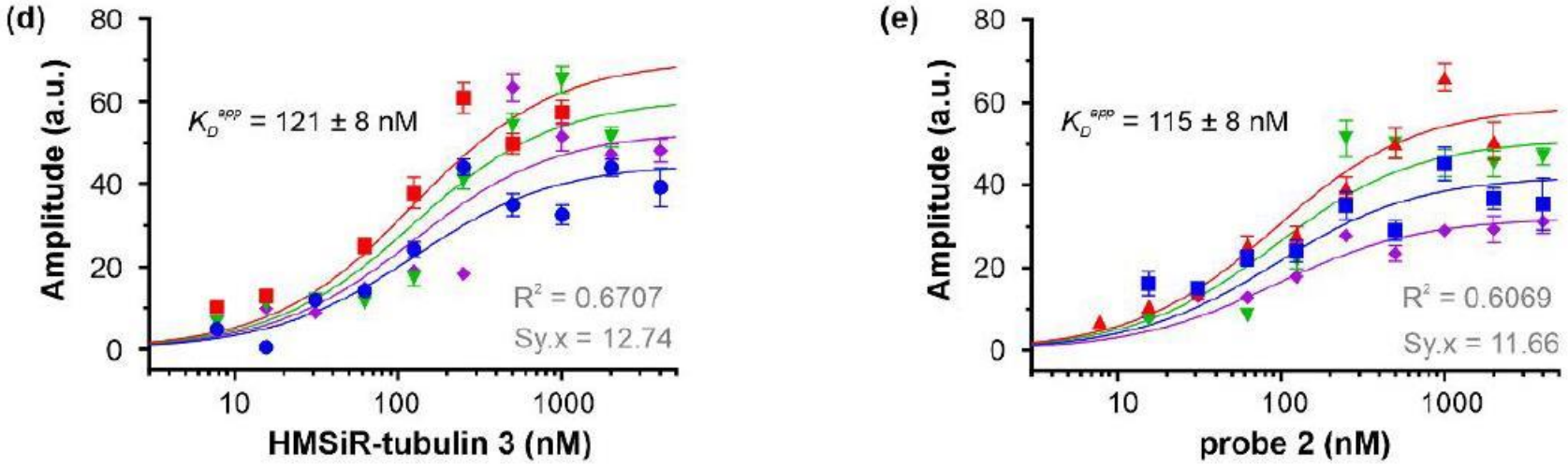
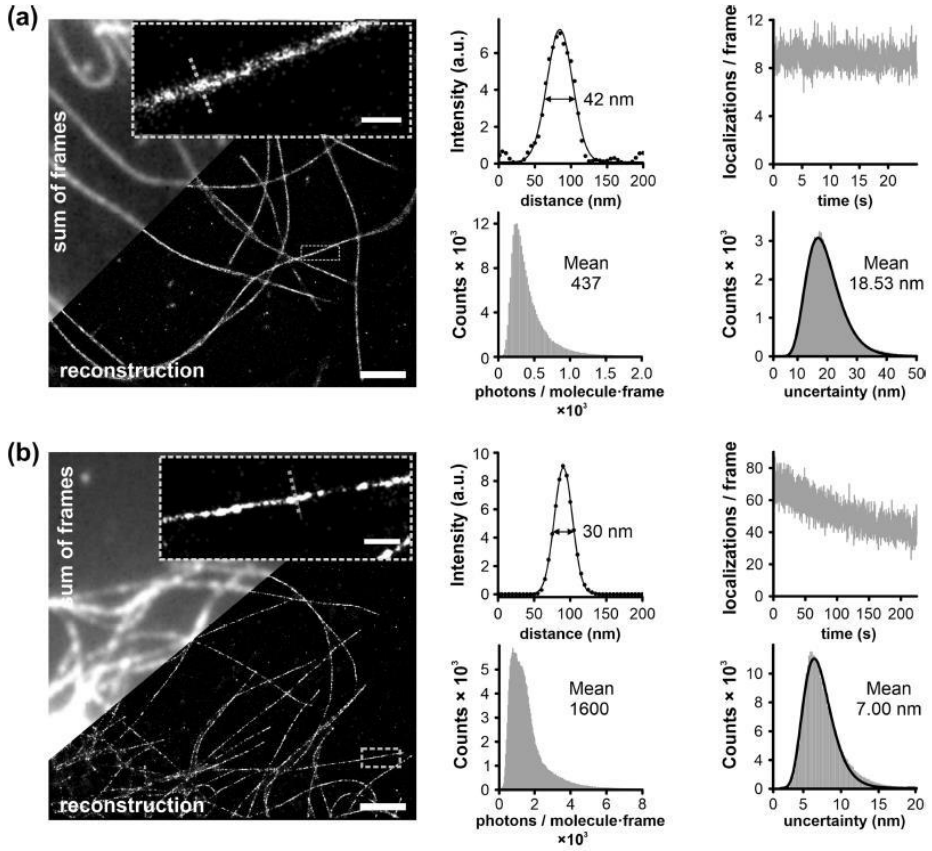


Figure S7. Determination of probe affinity to a single microtubule on fixed U-2 OS cells. (d, e) The data from 4 independent experiments were globally fitted to the equation for a single site binding with shared K_D^{app} *and unconstrained Fmax, that was fitted individually for each data set.* The data points are shown as mean ± SEM. The fitted K_D^{app} ± *SE of the fit.*

SMLM imaging



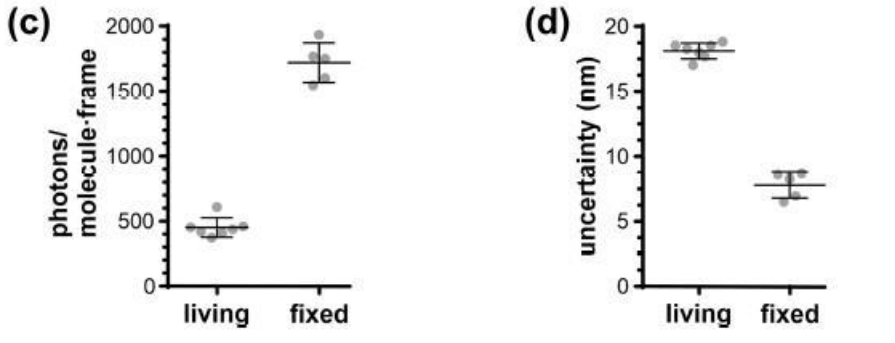
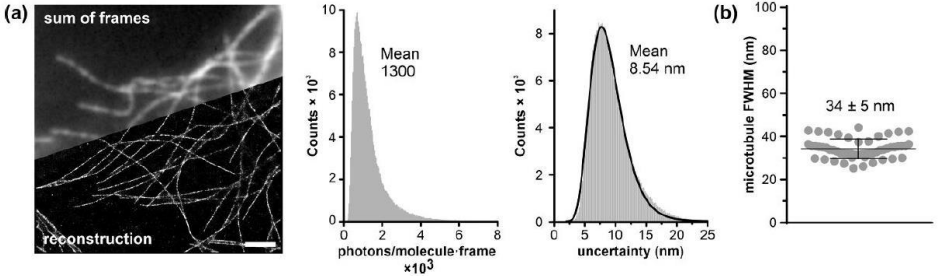


Figure 3.SMLM imaging of microtubules in living and fixed U-2 OS cells stained with HMSiR-tubulin (3): (a) Living cells, (b) Fixed cells. Zoom-in



fixed samples. The data is from 6 and 7 fields of view, from 2 independently prepared samples.

STED & MINFLUX imaging

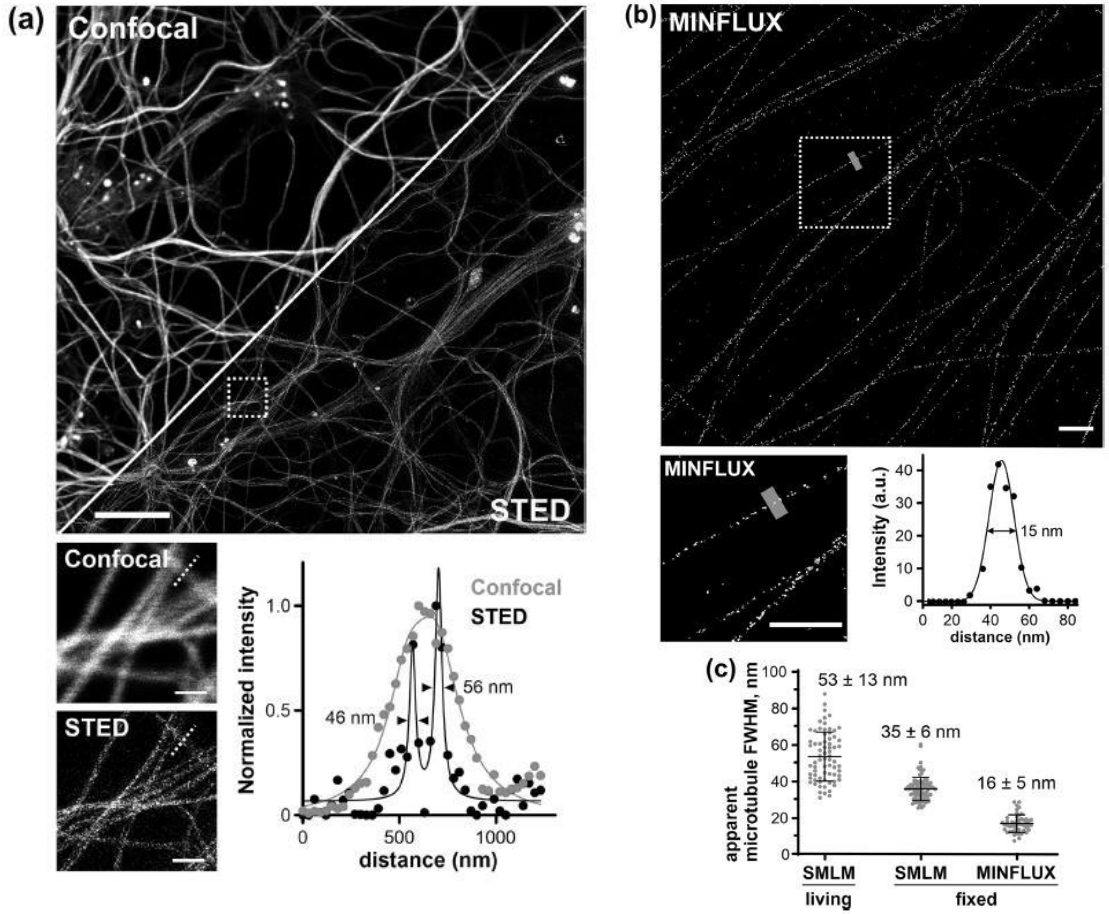


Figure 4. HMSiR-tubulin (3) performance in 2D-MINFLUX and 2D STED nanoscopy. (a) Confocal and 2D STED with 775 nm images of living mouse neurons stained with 300 nM HMSiR-tubulin (3). Scale bar 10 μm; inset 1 μm. Numbers represent fwhm. (b) MINFLUX image of microtubules in fixed U-2 OS cells stained with 20 nM HMSiRtubulin (3) acquired in 3 h and rendered with 4 nm pixel size. Scale bar 500 nm. Line profile of the region (200 nm in width) shown in the inset. (c) Microtubule fwhm measured by SMLM and MINFLUX nanoscopy. MINFLUX data are from 5 fields of view, 2 independently prepared samples.

Summary

- 首个细胞微管靶向的透膜的自闪烁荧光探针
- 通过对linker长度和定位基团的选择实现探针的透膜性和选择性
- 该探针可用于SMLM、STED和MINFLUX等多种超分辨成像方式