Literature Report

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Small-Molecule Fluorescent Probes for Live-Cell Super-Resolution Microscopy

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他目前的研究方向:

1. 开发半合成荧光传感蛋白来检测活细胞中的关键代谢产物。
2. 蛋白相互作用表征方法的发展与应用。
3. 控制活细胞中影响蛋白功能的小分子生成。
4. 应用于细胞生物学的新型光谱探针的合成。
5. 结核病候选药物的机理研究。

Introduction

Examples of small molecule fluorophores used in live-cell SRM:

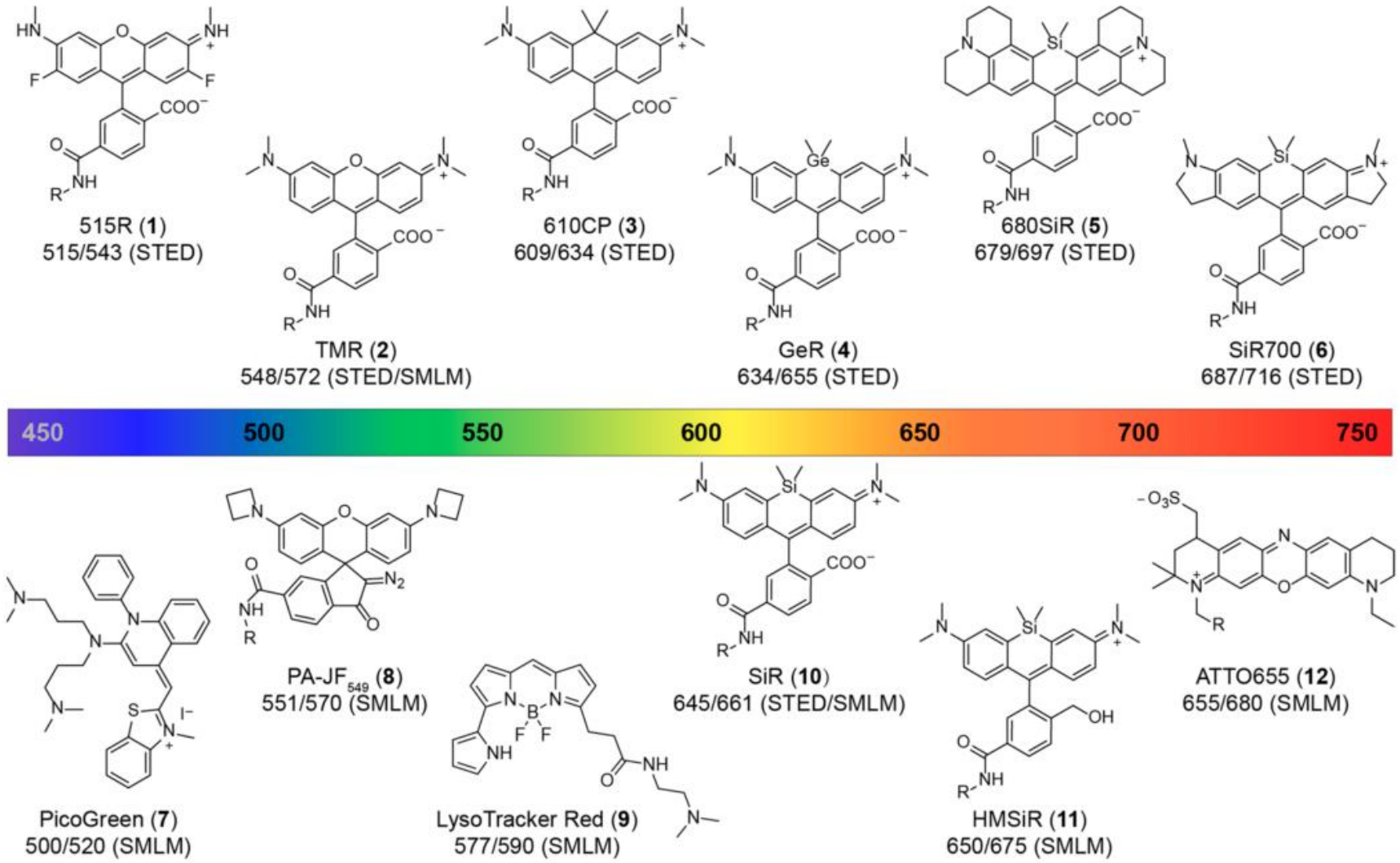


Figure 1. Examples of small-molecule fluorophores used in live-cell SRM. Absorption and emission maxima are given underneath the structures (abs/em). In parentheses, the application in which these fluorophores were used is listed. R depicts the position where the fluorophores are substituted for labeling purposes. Reference: 515R (**1**),[22] TMR (**2**),[23,24] 610 CP (**3**),[22,25] GeR (**4**),[26,27] 680 SiR (**5**),[25] SiR700 (**6**),[28] PicoGreen (**7**),[29] PA-JF$_{549}$ (**8**),[30] LysoTracker Red (**9**),[31] SiR (**10**),[32] HMSiR (**11**),[33] and ATTO655 (**12**).[34]

Introduction

Approaches suitable for live-cell labeling of biomolecules with synthetic fluorophores:

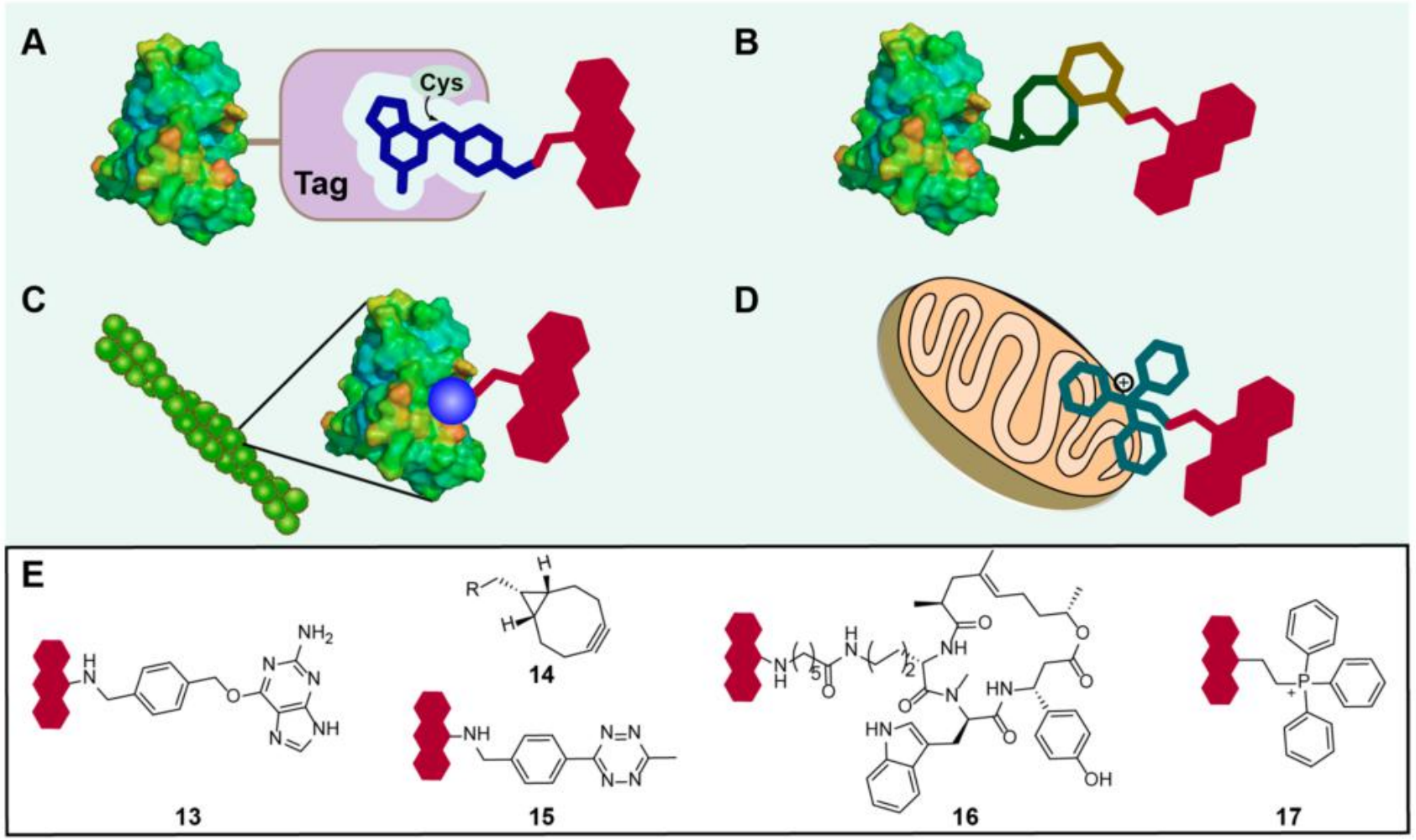


Figure 2. Overview of approaches suitable for live-cell labeling of biomolecules with synthetic fluorophores. (A) Fusion of proteins with self-labeling protein tags such as SNAP-tag, Clip-tag or HaloTag. (B) Exploiting click chemistry to site-selectively label proteins or lipids. (C) Labeling through ligands that specifically bind to biomolecules. (D) Labeling of organelles such as mitochondria. (E) Structures of ligands used in the labeling strategies (A–D). **13** SNAP-tag ligand benzylguanine. **14** Strained alkyne and tetrazine **15** used in click chemistry. **16** F-Actin-binding ligand jasplakinolide. **17** Triphenylphosphine used to stain the mitochondrial membrane. R in **14** depicts the ligand needed for metabolic incorporation or an unnatural amino acid used.

Introduction

Three main pathways of how small molecule fluorophores can enter cells:

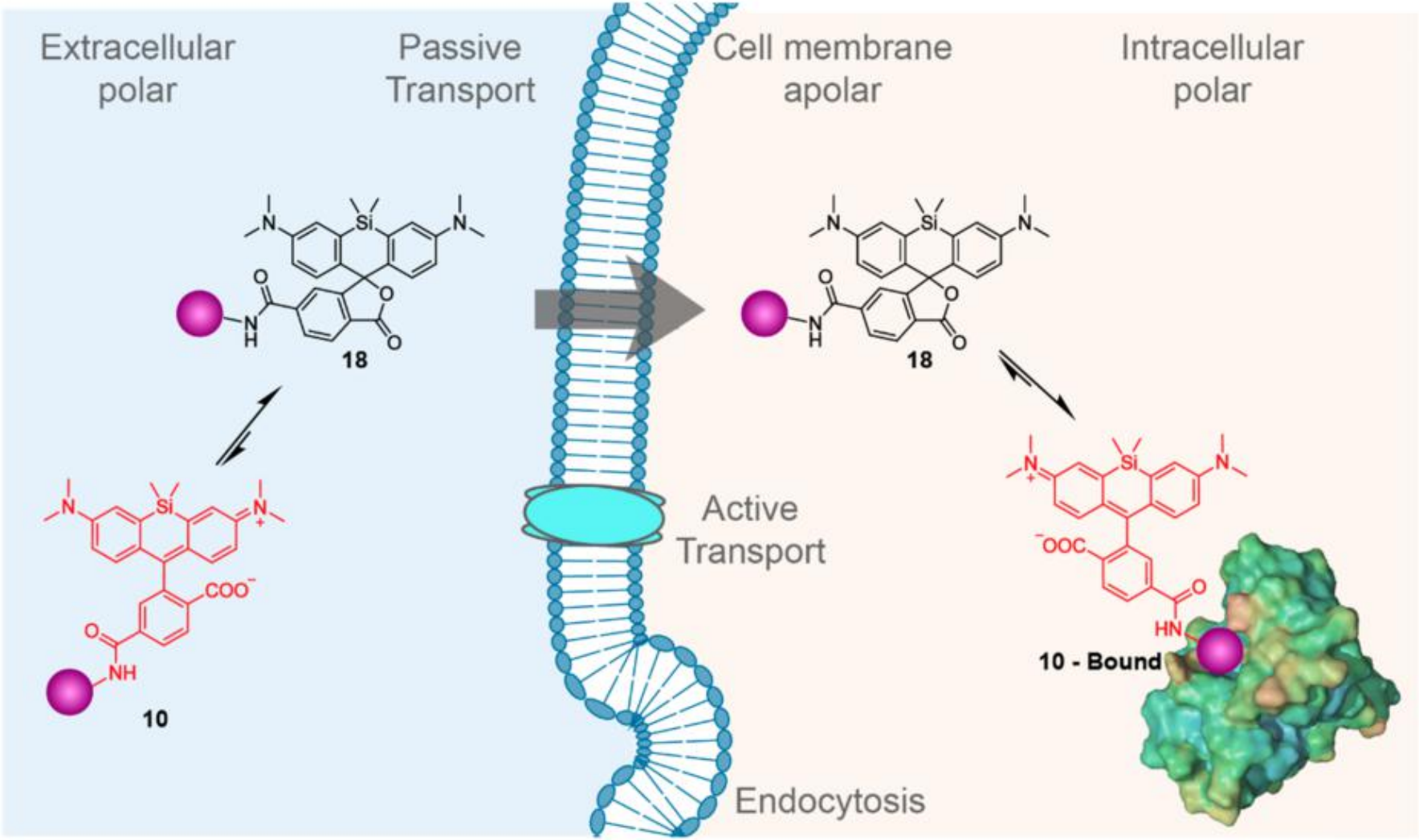
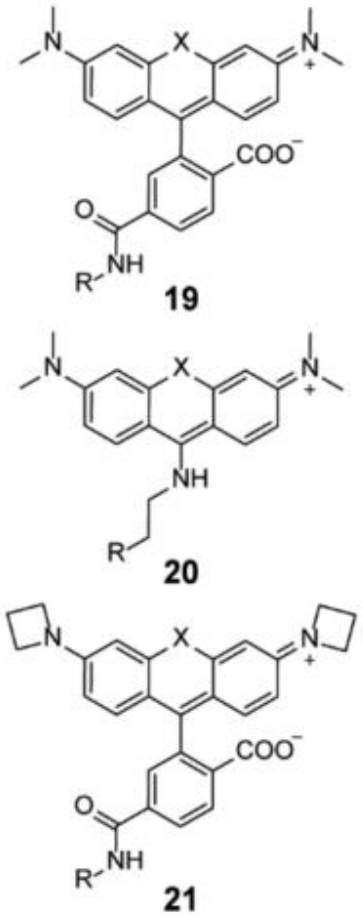


Figure 3. Uptake of fluorophores into cells. The three main pathways of how small-molecule fluorophores can enter cells are depicted. In addition, the spirolactone equilibrium of rhodamines is depicted, using a SiR-carboxyl-based probe as a representative example. Note that the uncharged spirolactone **18** enters the cell through passive transport and that binding of the probe to its target shifts the equilibrium to the fluorescent zwitterion **10**.

Introduction

Photophysical properties of selected small molecule fluorophores:

A



B

Probe	X	ε ($M^{-1}cm^{-1}$)	$\lambda_{abs}/\lambda_{em}$ (nm)	Φ	Ref.
19a	O	78,000[a]	548/572[a]	0.41[a]	24
19b	CMe_2	121,000[a]	606/626[a]	0.52[a]	24
19c	$GeMe_2$	97,000[b]	634/655[b]	0.43[b]	26
19d	$SiMe_2$	141,000[c]	643/662[a]	0.41[a]	24
20a	CMe_2	28,000[d]	459/599[d]	0.55[d]	52
20b	$GeMe_2$	24,000[d]	454/618[d]	0.17[d]	52
20c	$SiMe_2$	17,000[d]	458/623[d]	0.28[d]	52
20d	SO_2	13,000[e]	509/647[e]	0.15[e]	52
21a	O	101,000[a]	549/571[a]	0.88[a]	24
21b	CMe_2	99,000[a]	608/631[a]	0.67[a]	24
21c	$SiMe_2$	152,000[c]	646/664[a]	0.54[a]	24

Figure 4. (A) Chemical structures and (B) photophysical properties of selected small-molecule fluorophores. R groups show where the fluorophores are substituted for labeling purposes. [a]10 mM HEPES buffer, pH 7.3. [b]10 mM PBS, pH 7.4. [c]Ethanol containing 0.1% (v/v) trifluoroacetic acid. [d]10% methanol/PBS (10 mM, pH 7.4). [e]10% acetonitrile/H_2O.

Introduction

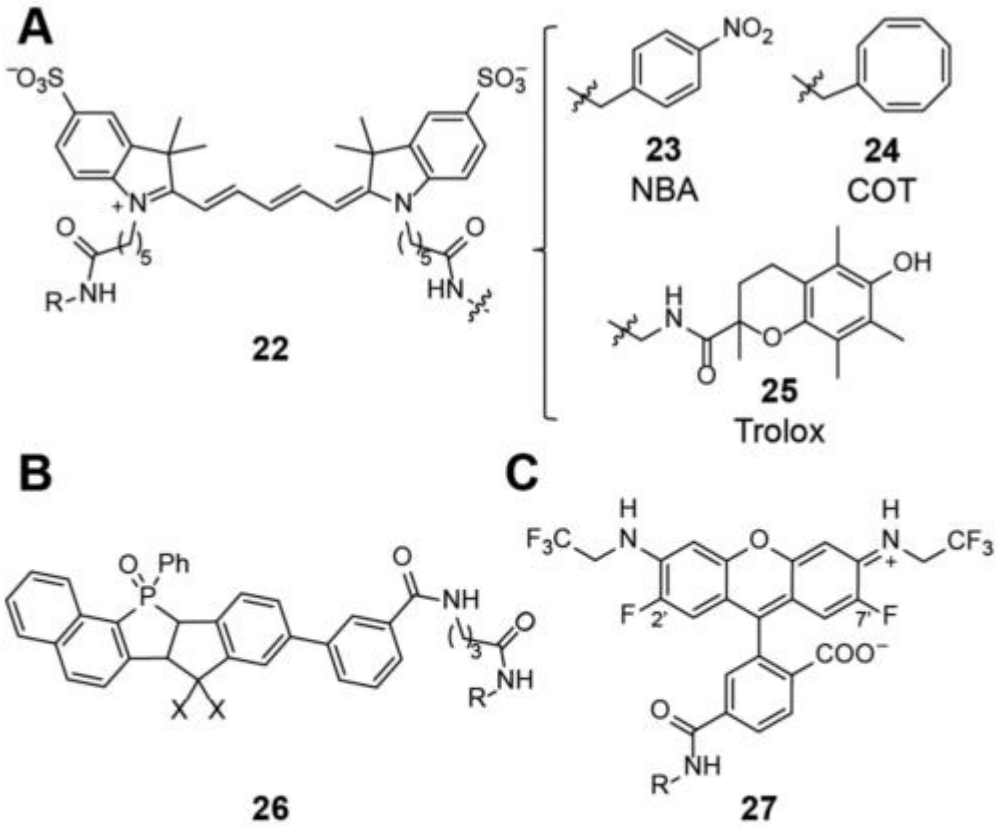


Figure 5. (A) Representative structure of TSQ-conjugated Cy5 derivative **22** along with the structures of TSQs. (B) Phosphine oxide-fused photostable fluorophore **26**. X in **26** represents chemical groups increasing hydrophilicity. (C) Fluorinated photostable rhodamine **27**. R groups show where the fluorophores are substituted for labeling purposes.

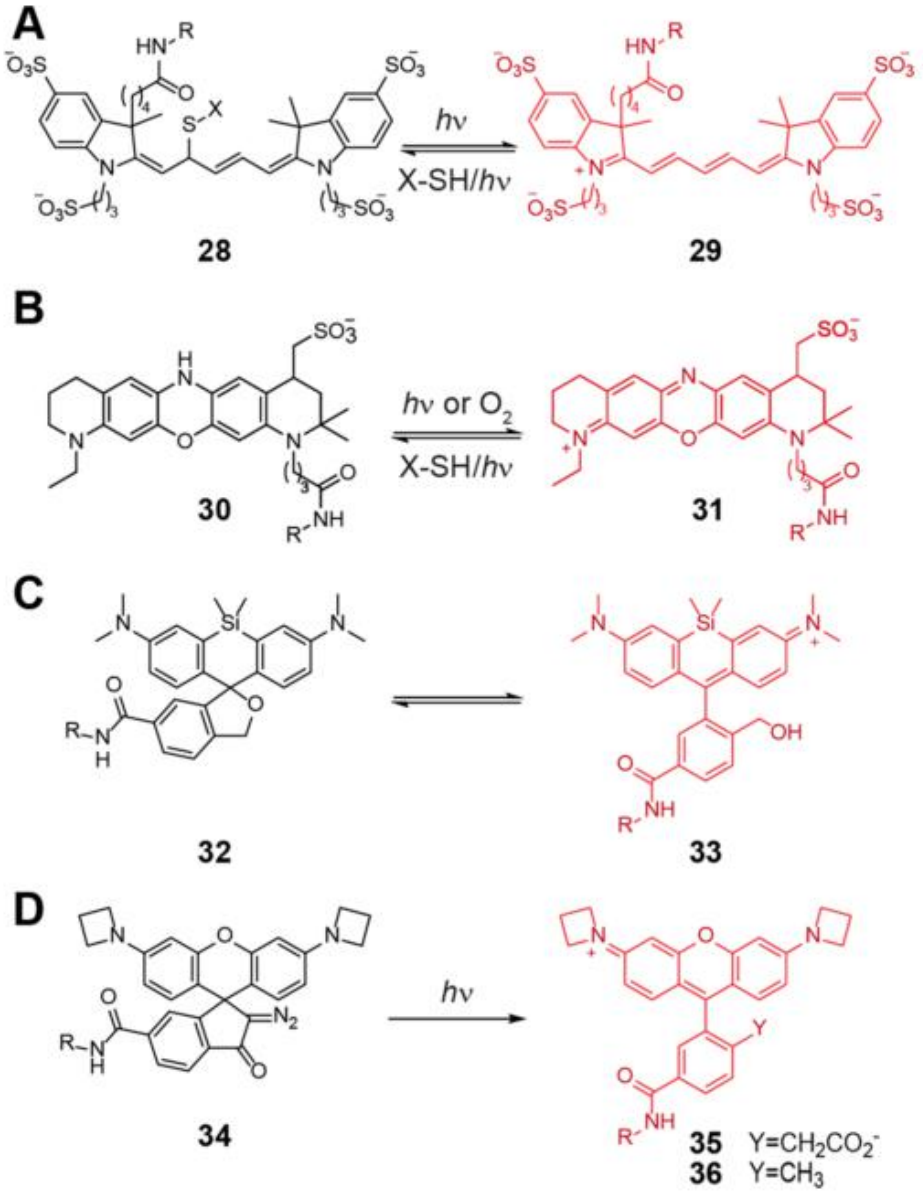


Figure 6. Fluorophores used for photoswitching or photoactivation: (A) Alexa647, (B) Atto655, (C) HMSiR, and (D) diazoketone rhodamine. X-SH represents thiol-containing compounds. R groups show where the fluorophores are substituted for labeling purposes.

Introduction

Applications of small molecule fluorescent probes in live-cell SRM:

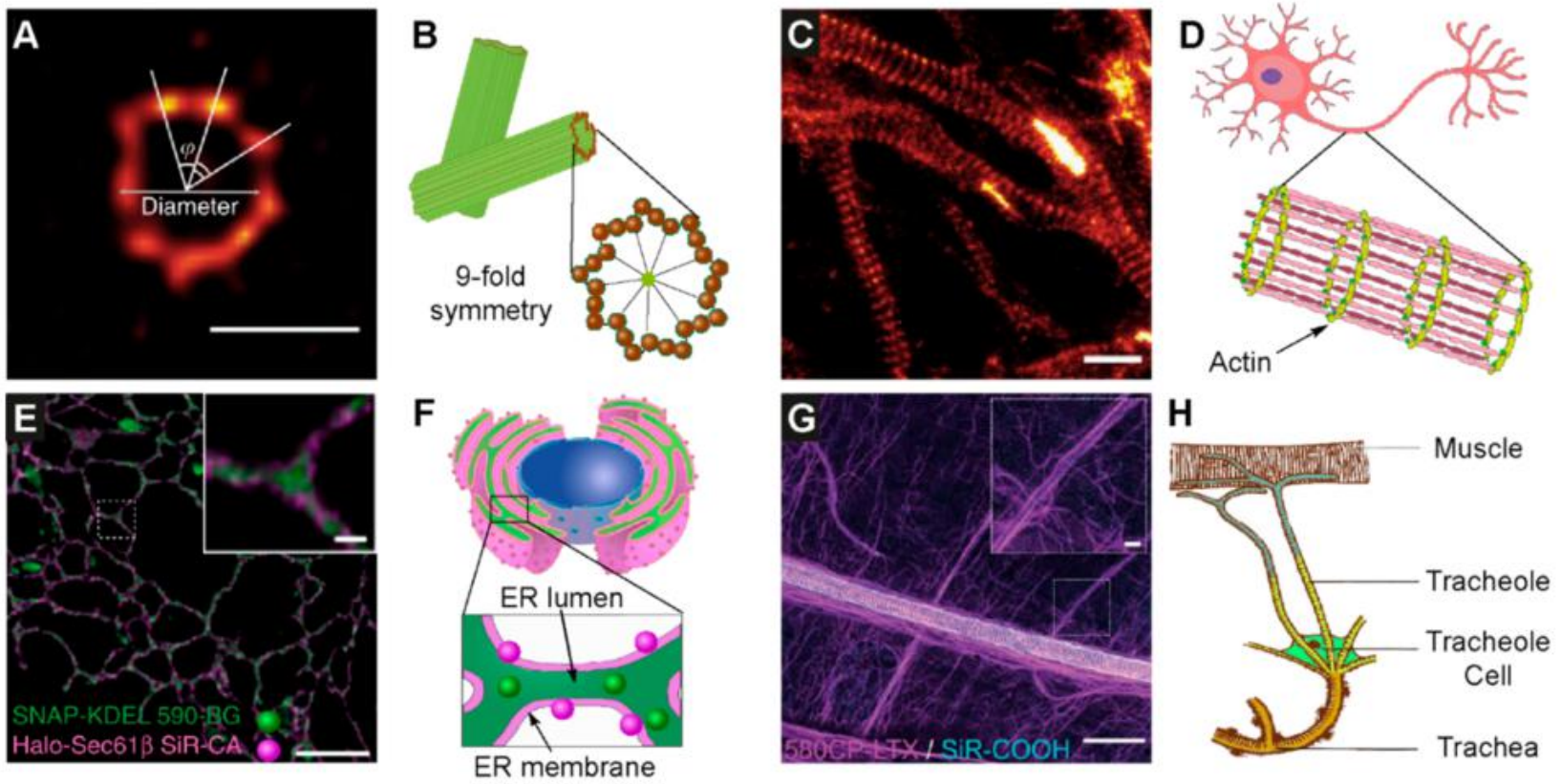


Figure 7. Live-cell STED imaging with small-molecule fluorescent probes. (A) STED image of centrosomal microtubules stained with SiR-tubulin. The modulation in brightness along the rim of the cylinder (with polar angle ϕ of 40°) is a consequence of the 9-fold symmetry of centrioles. Scale bar: 200 nm. (B) Cartoon of structure of a centriole with 9-fold symmetry structure. (C) Live-cell STED image of rat hippocampal neurons stained with SiR-actin, revealing the presence of actin rings. Scale bar: 1 μm. (D) Cartoon of the periodic, ring-like actin structure found in neuronal cells. (E) COS-7 cells expressing Halo-Sec61b (ER membrane) and SNAP-KDEL (ER lumen) fusion proteins labeled with ATTO590 and SiR respectively. Scale bar: 2 μm. (F) Scheme of ER structure showing ER lumen and membrane. (G) Two-color image of body wall muscle microtubule network stained with carbopyronine-tubulin and tracheoles stained with SiR-carboxyl. Inset shows zoom-in image of the region shown in panel as a white rectangle. Image shows microtubule network following tracheole. Scale bars: 5 mm in the large field of view and 1 mm in the zoom-in image. (H) Cartoon of tracheole structure. Panel A and C: Adapted by permission from ref 48. Springer Nature Copyright 2014. Panel E: Adapted from ref 71, published by Springer Nature under CC BY 4.0. Panel G: Adapted from ref 27, published by the Royal Society of Chemistry under CC BY-NC 3.0.

Introduction

Live-cell SMLM imaging with small molecule fluorescent probes:

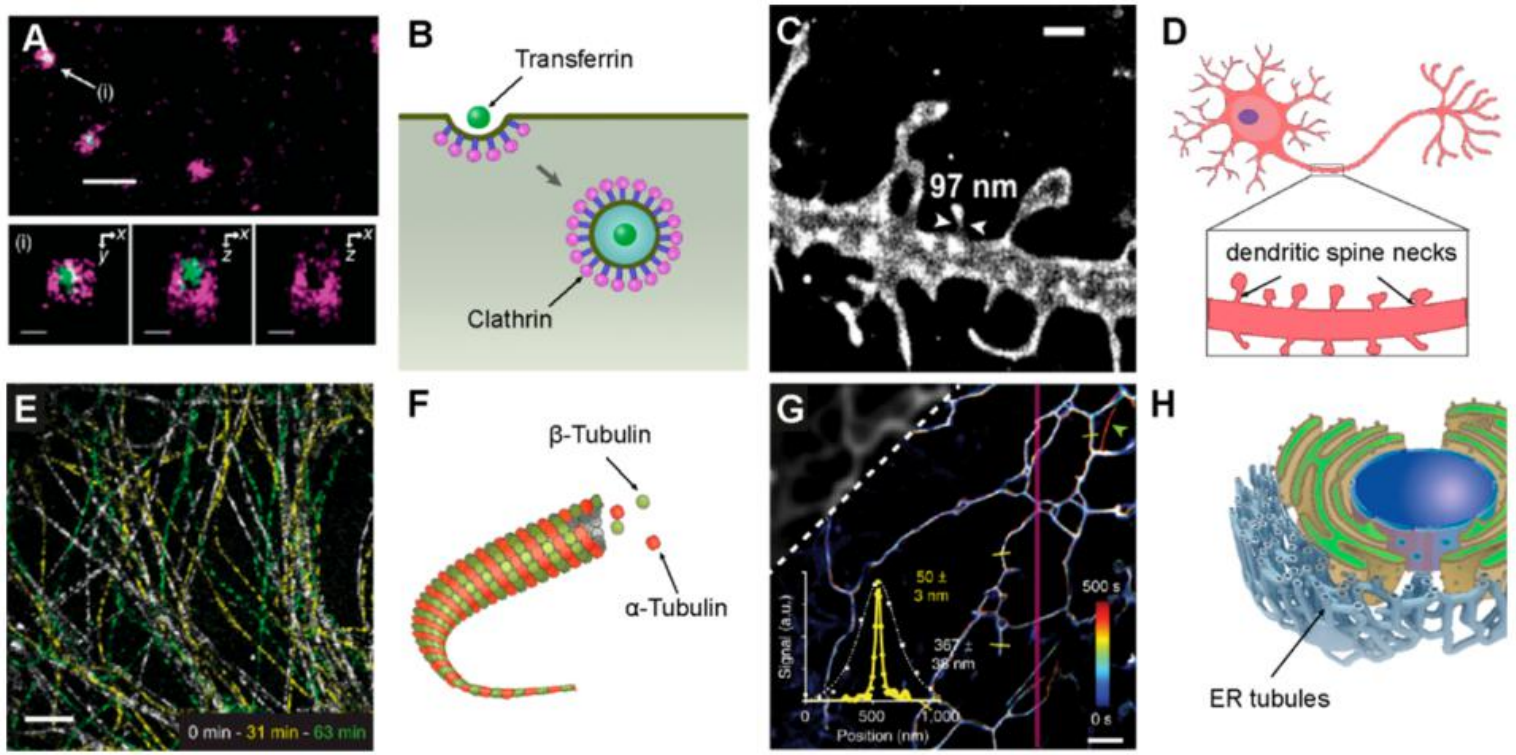


Figure 8. Live-cell SMLM imaging with small-molecule fluorescent probes. (A) Dual-color SMLM image of clathrin coated pits labeled with Alexa647 (magenta) and transferrin labeled with Alexa568 (green); (top image) *xy* projection, (lower left) *xy* cross section, (lower middle) *xz* cross section through the middle of the pit, (lower right) cross section through the middle of the pit clathrin channel only. Scale bars: 500 and 100 nm, respectively. (B) Cartoon of an invaginating clathrin coated pit together with its transferrin cargo. (C) SMLM image of the plasma membrane labeled with DiI in a hippocampal neuron. Scale bar: 1 μm. (D) Cartoon of hippocampal neuron showing dendritic spine necks. (E) β-Tubulin−HaloTag fusion proteins expressed in Vero cells were labeled with HMSiR−Halo. Sequential acquisition of super-resolution images of microtubules at 0 min (white), 31 min (yellow) and 63 min (green). Scale bar: 2 μm. (F) Cartoon of tubulin structure forming with α-tubulin and β-tubulin. (G) SMLM image of the ER in HeLa cells. Upper left corner shows the diffraction limited image. Color indicates the time between 0 and 500 s of the localization. Averaged line profiles from the four yellow lines are shown in an insert. The profiles show fwhm values of 50 ± 3 nm (super-resolution image, yellow) and 367 ± 38 nm (diffraction-limited image, white dashed). Scale bar: 1 μm. (H) Cartoon of ER with periphery tubules. Panel A: adapted by permission from ref 23, published by Springer Nature Copyright 2011. Panel C: adapted from ref 31, published by the National Academy of Sciences under CC BY-NC-ND. Panel E: adapted by permission from ref 33. Springer Nature Copyright 2014. Panel G: adapted by permission from ref 97. Springer Nature Copyright 2017.