Literature Report

Reporter: Zhang Yue

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Genetically Encoded Supramolecular Targeting of Fluorescent Membrane Tension Probes within Live Cells: Precisely Localized Controlled Release by External Chemical Stimulation

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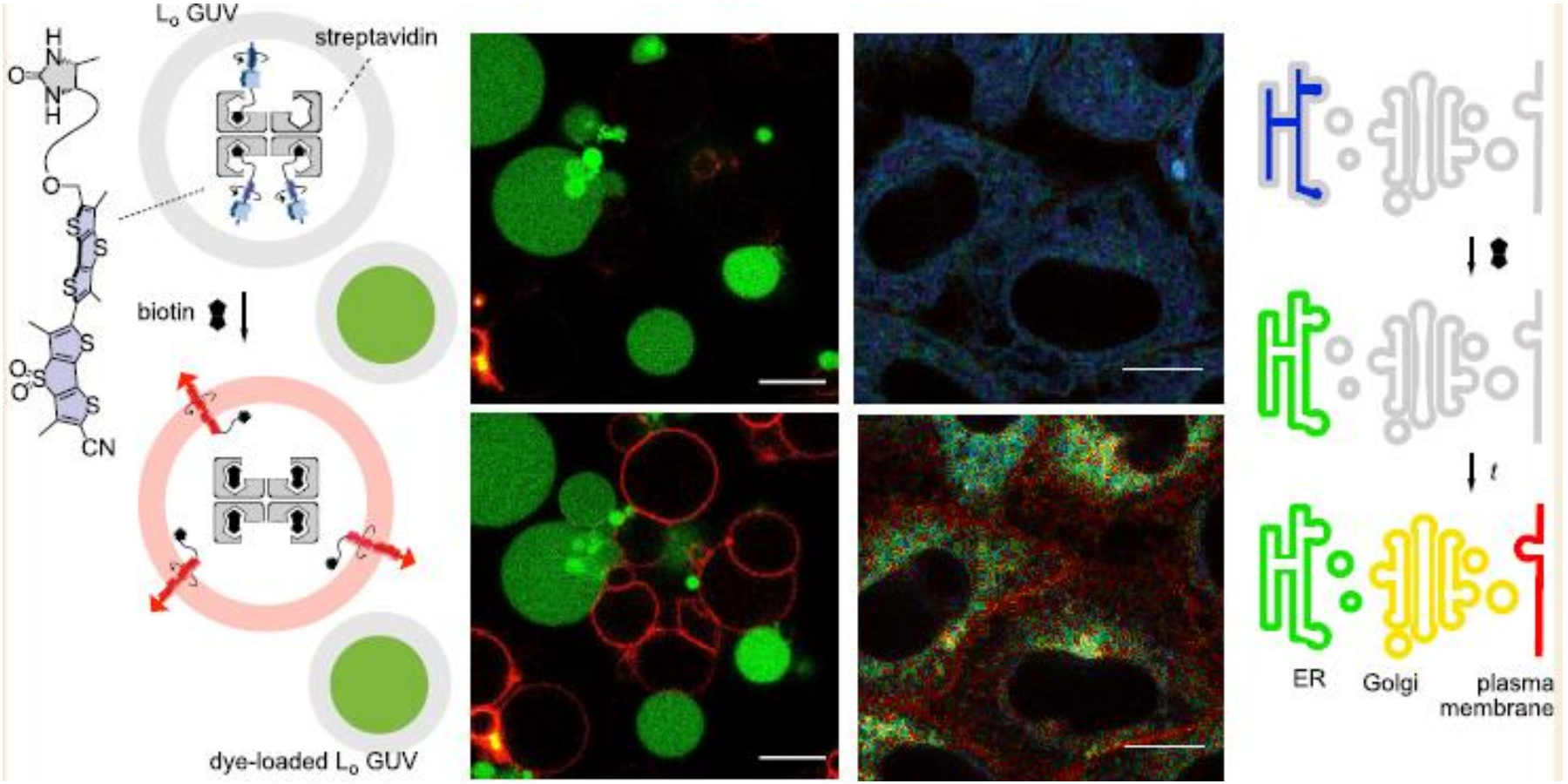


Introduction

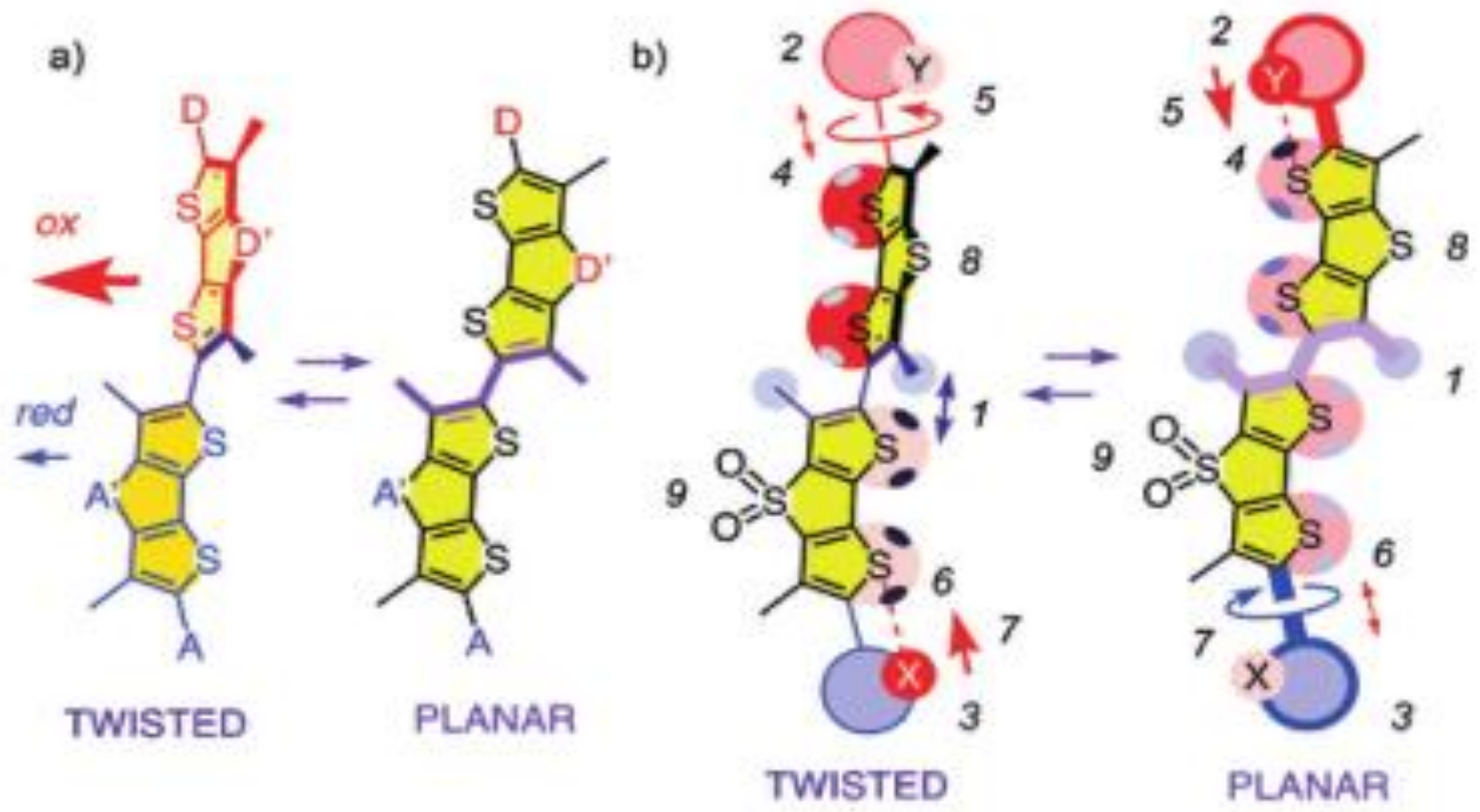
Stefan Matile

Stefan Matile is a Full Professor in the Department of Organic Chemistry at the University of Geneva and a founding member of the National Centre of Competence in Research (NCCR) Chemical Biology and the NCCR Molecular Systems Engineering.

RESEARCH INTERESTS

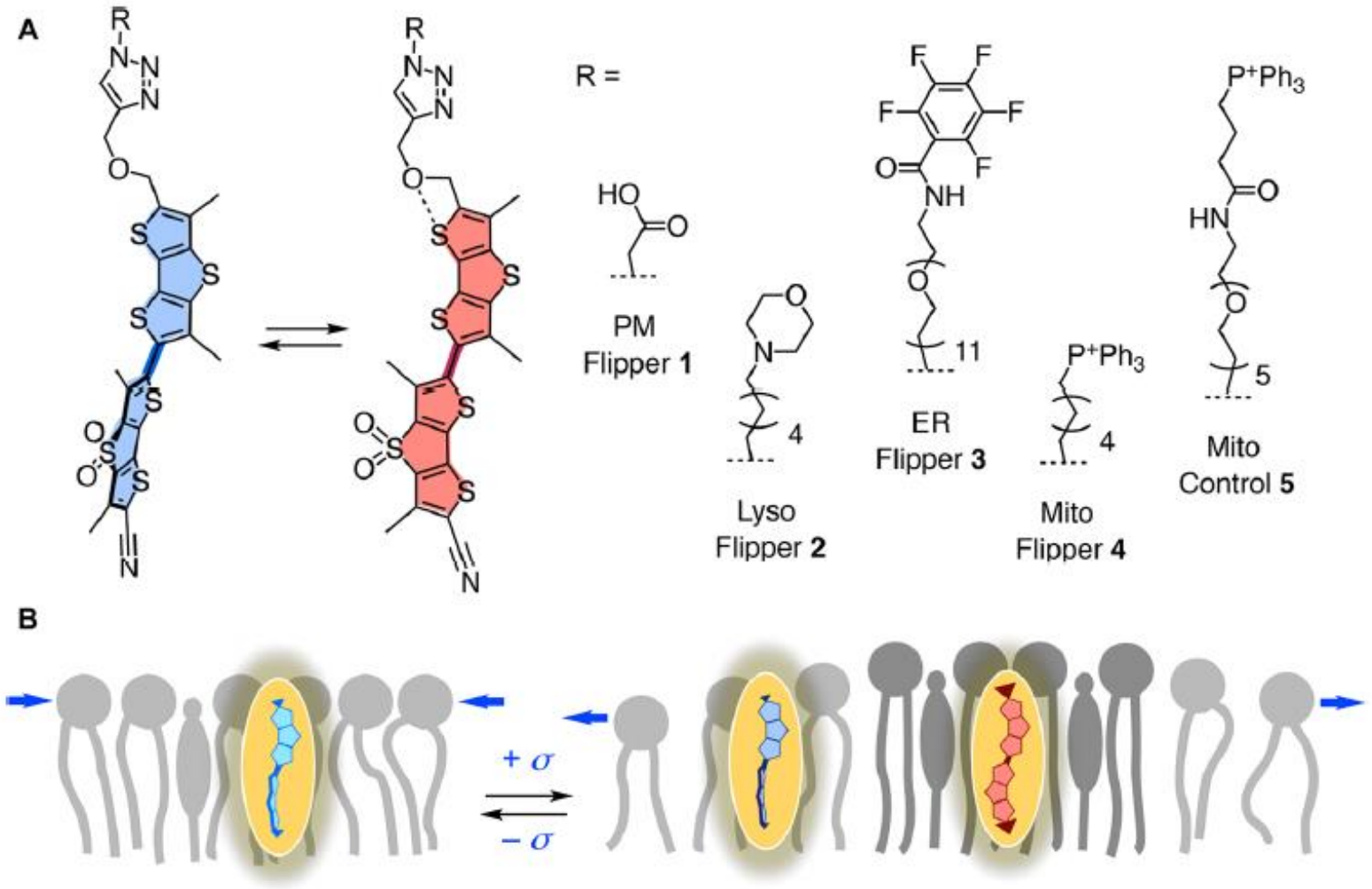
At the interface of synthetic organic, biological and supramolecular materials chemistry. Emphasis is on functional supramolecular chemistry.

Introduction



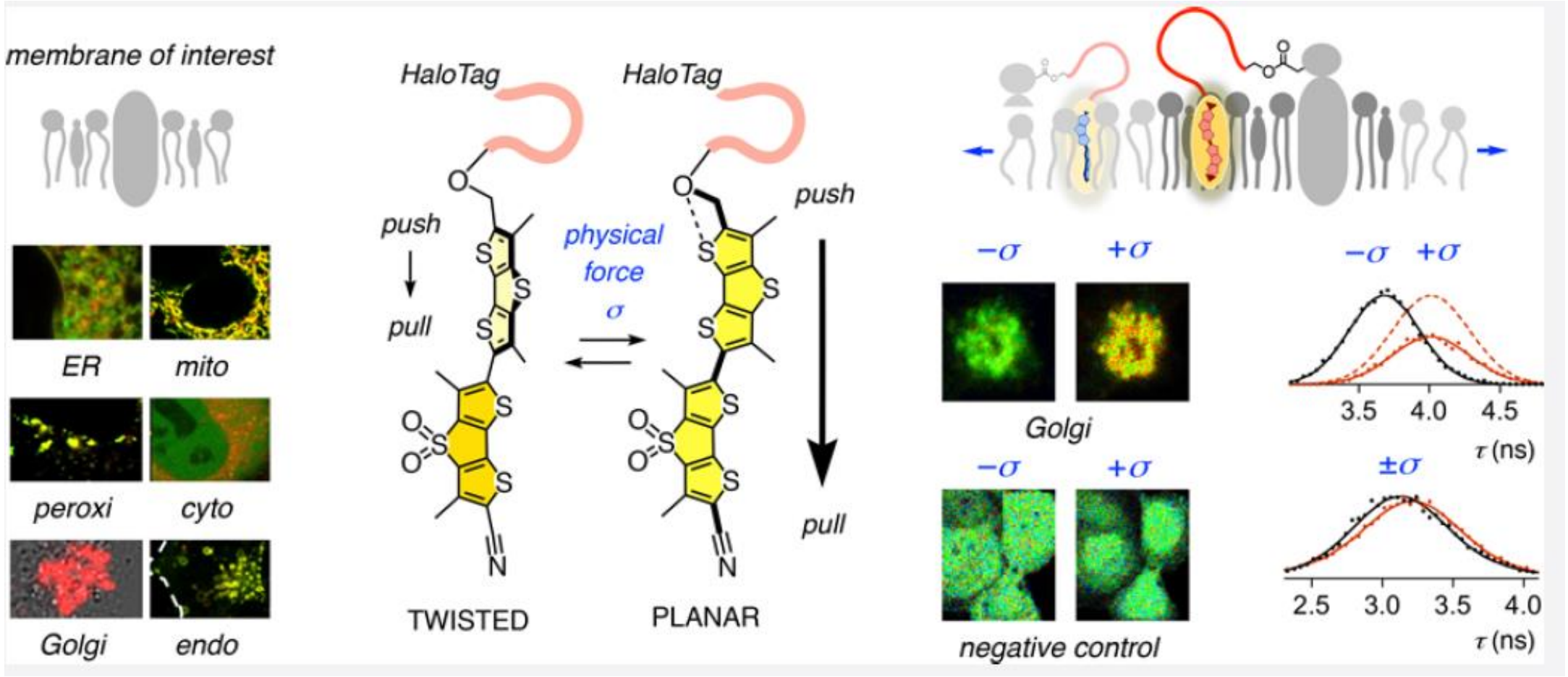
Angew.2019.44.15752-15756

Flipper-TR



J. Am. Chem. Soc. 2019, 141, 3380−3384

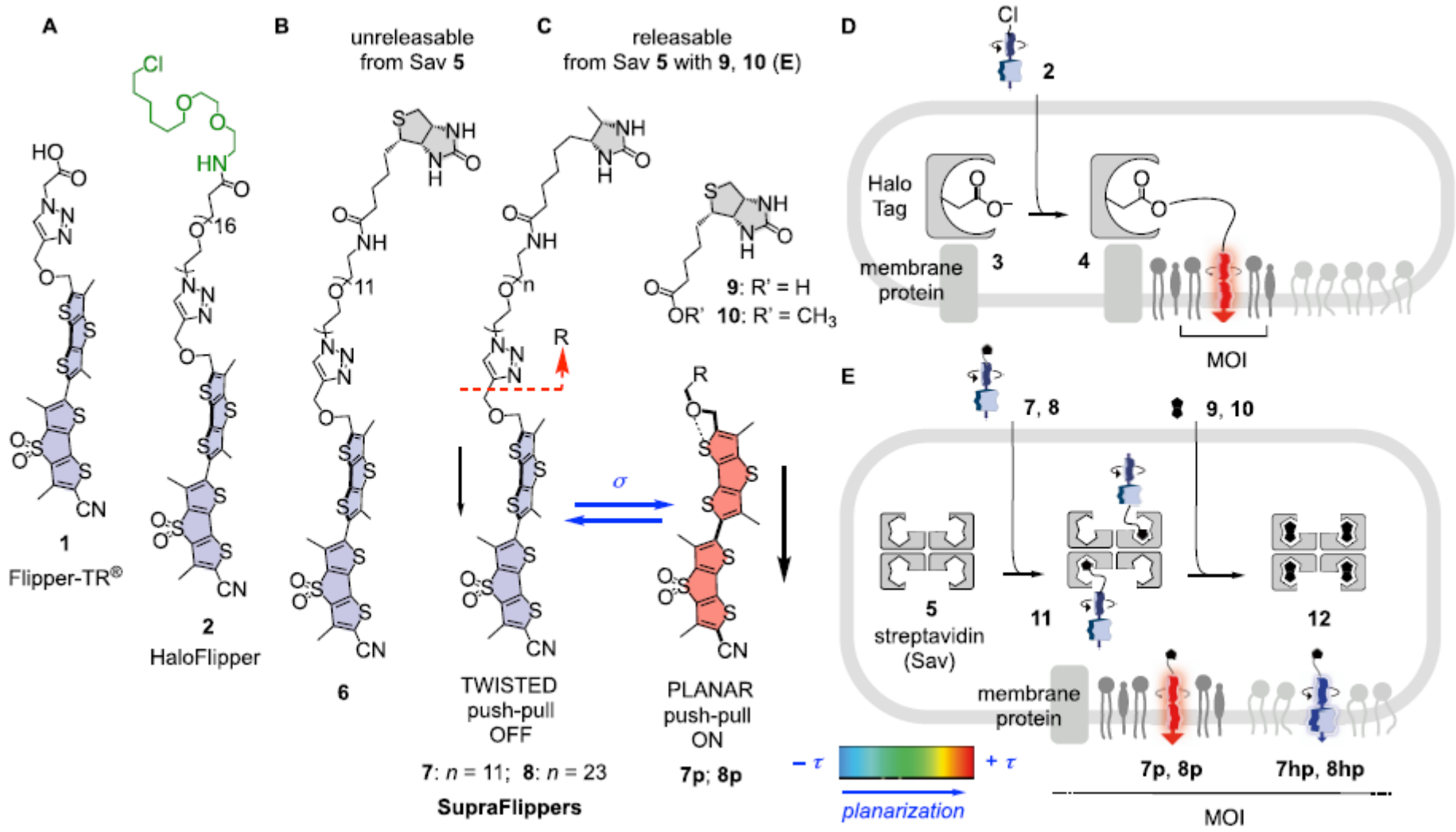
HaloFlippers



ACS Cent. Sci. 2020, 6, 1376−1385

SupraFlippers

SAV:链霉亲和素是与亲和素有相似生物学特性的一种蛋白质



Release in Artificial Model Systems

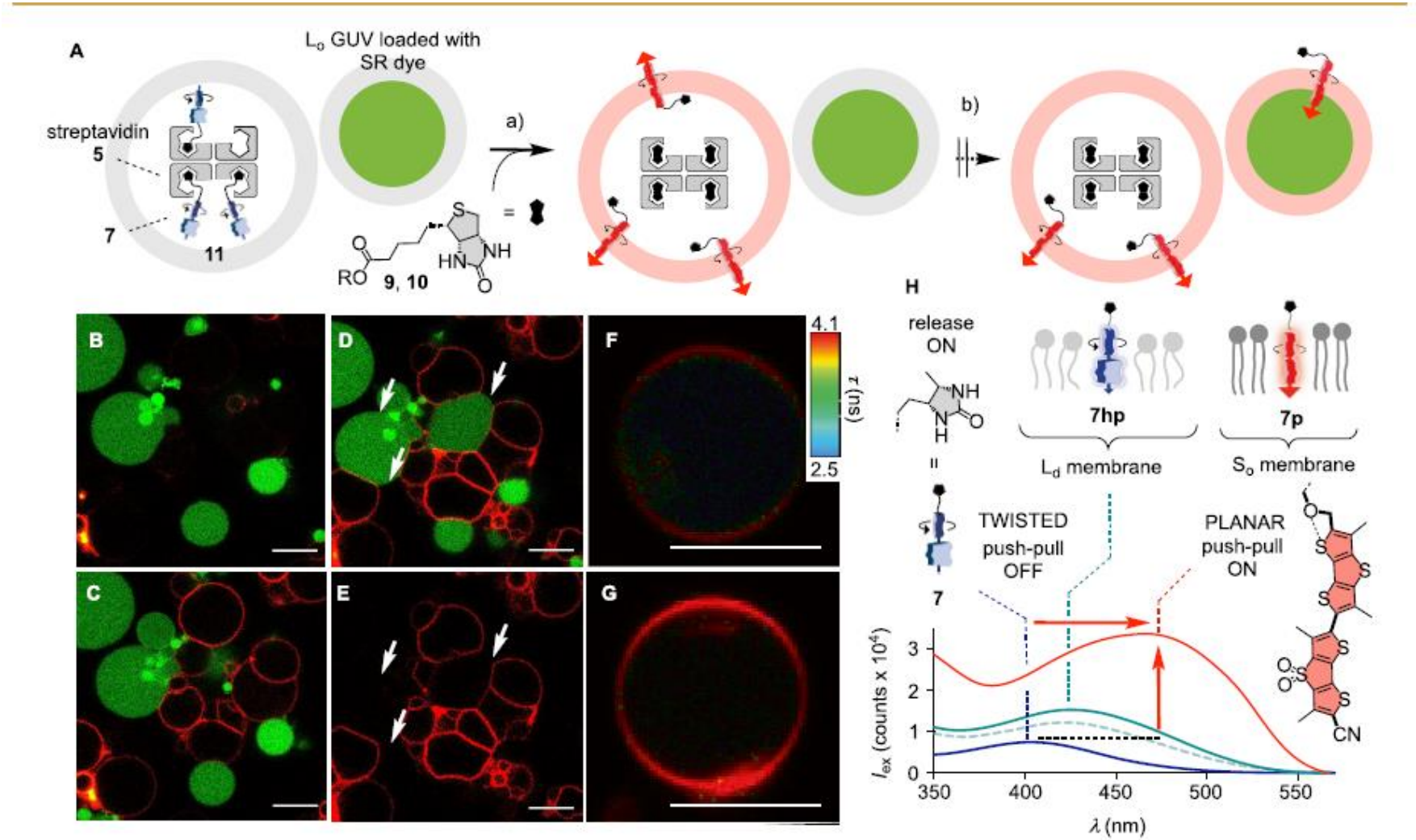


Figure 2. (A) Schematic representation of model studies using GUVs. After the addition of biotin **9** or **10** to GUVs loaded with **11** or SR (a), SupraFlipper 7 dissociates from Sav and partitions in the membrane of GUVs loaded originally with **11**, without diffusing to SR-loaded GUVs over time (b). (B–E) Merged CLSM images of GUVs loaded with SR (1 μM, green channel) and **11** (15 μM, 3 eq of 7, red channel) before (B), 5 min (C), and 1 h (D) after addition of **10** (100 μM). (E) Same image as part D, only in the red channel. Arrows point to the membrane of SR-loaded GUVs, which did not show fluorescence in the red channel, even after time. (F–G) FLIM images of one GUV loaded with **11** (15 μM, 3 equiv of 7) before (F) and (G) 6 min after addition of **10** (100 μM). Scale bars: 10 μm. (H) Excitation spectra of probes 7 (solid) and 8 (dashed, both 100 nM) in DPPC LUVs (75 μM) at 25 °C (red, S_o) or 55 °C (mint, L_d) and in buffer at 25 °C (blue).

Targeting and Controlled Release in Living Systems

KDEL序列是内质网驻留信号序列，凡是含有这个序列的蛋白质都会被滞留在内质网中。

SBP:Sav-binding peptide

质粒p2与14一起编码13的表达

GFP channel

Flipper channel

Bright field image

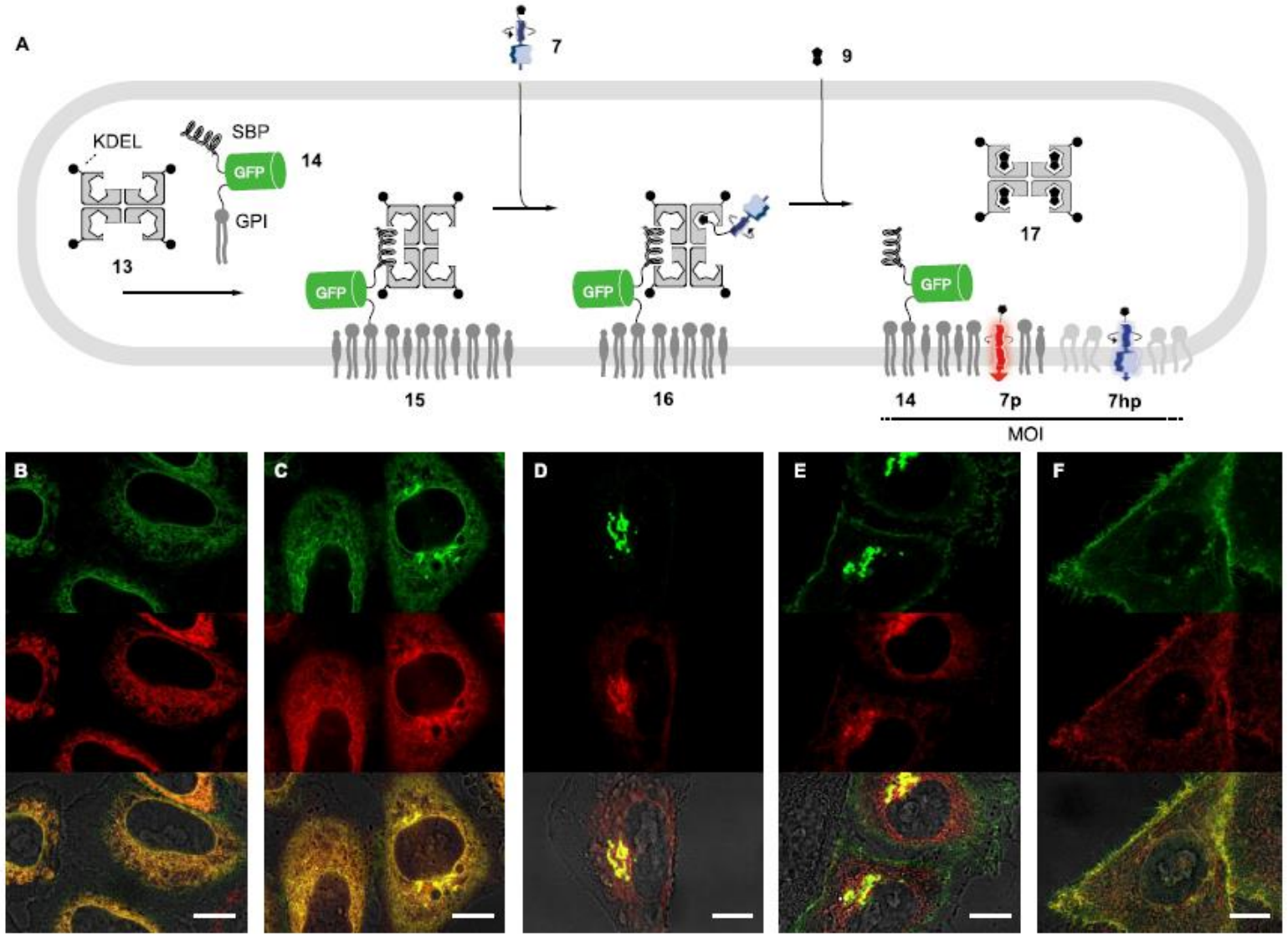


Figure 3. (A) Release of SupraFlipper 7 in the ER with spatiotemporal control: SupraFlipper 7 binds to the empty sites of complex 15, composed of Sav 13 equipped with KDEL ER retention sequences and SBP-GFP-GPI 14 in the ER, and is released together with 14 upon addition of biotin 9. (B–F) CLSM images of HeLa cells transfected with p2 after incubation with 7 (100 nM, 1 h) in the GFP channel (green, top) and flipper channel (red, middle) and merged with the bright field image (bottom) (B) 10, (C) 50, (D) 65, (E) 90, and (F) 100 min after addition of biotin (9, 40 μM). Brightness and contrast are not equivalent in all images; scale bars: 10 μm.

Merging HaloTag Technology and SupraFlippers

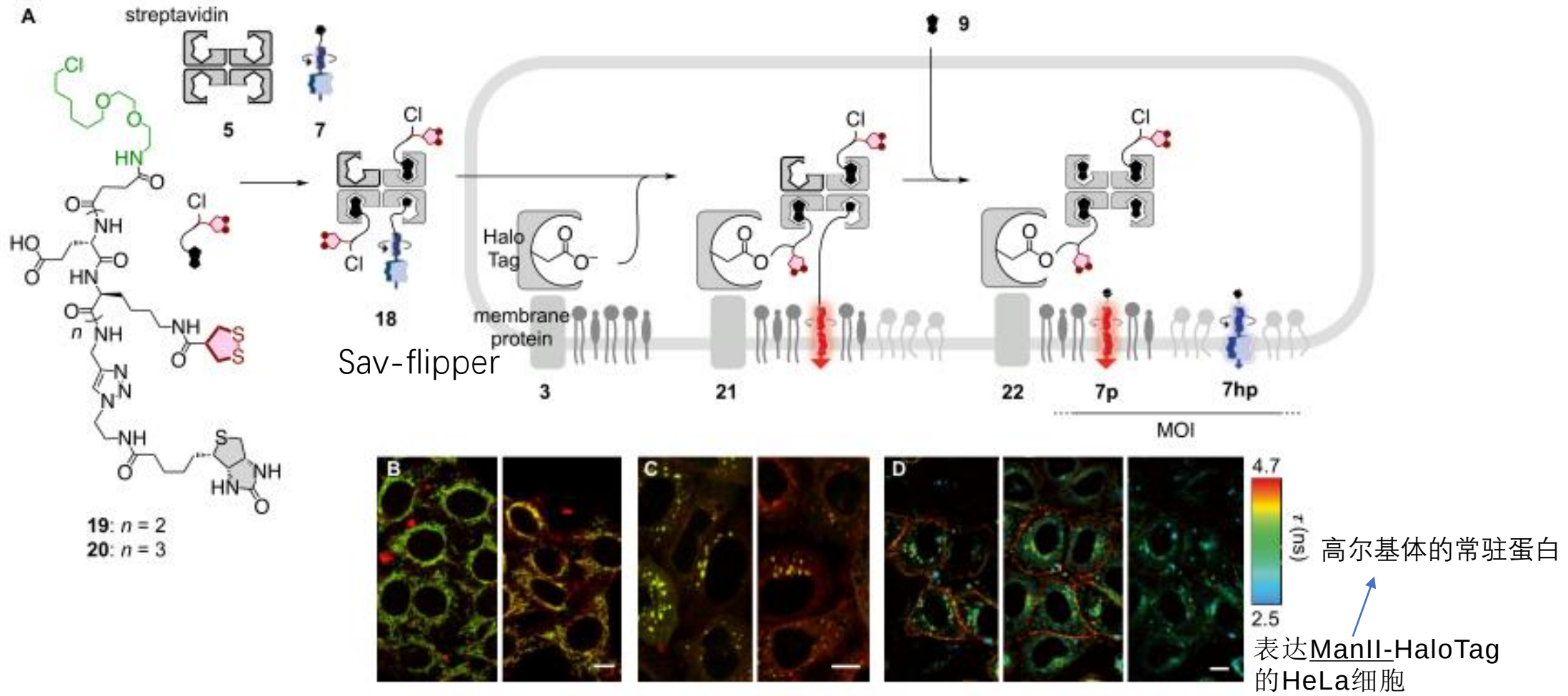


Figure 5. (A) Strategy to combine SupraFlippers and HaloTag technology. Mixing of chloroalkylated transporters **19** or **20** with wild-type Sav **5** and SupraFlipper **7** yields complex **18** (showing the stoichiometry of mixing), which can cross the plasma membrane and find HaloTag **3** to form complex **21**. After the addition of biotin **9**, SupraFlipper **7** is released, with complex **21** left behind. (B) Merged CLSM images (flipper red; GFP green) of HGM cells (HaloTag and GFP on mitochondria) incubated with complex **15** (5 μM, 2 h) before (left) and 1 h after the addition of biotin (40 μM, right). (C) Same as part B, using HeLa cells expressing GTS-HaloTag-meGFP (**p3**, Golgi, partially fragmented due to transfection of **p3**). (D) FLIM images of HeLa cells expressing ManII-HaloTag (**p4**, Golgi partially fragmented due to transfection of **p4**) and incubated with complex **18** (2 μM, 2 h) before (left), 60 min after addition of biotin (40 μM, middle) and after the application of hyperosmotic stress (0.5 M of sucrose, right). Scale bar: 10 μm.

CONCLUSIONS

In artificial model systems, the best SupraFlippers efficient partitioning into the closest membranes upon the addition of biotin, without diffusing into other membranes.

In cells expressing KDEL-tagged Sav in the ER lumen,colocalization studies with a protein reporter demonstrate the specificity of the probe.

In FLIM, SupraFlipper remains insensitive to membrane order and changes in membrane tension before the addition of biotin. Upon release, the probe becomes operational and reports on the nature of the MOI.