Literature Report

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EDGE ARTICLE

Enhancing biocompatibility of rhodamine fluorescent probes by a neighbouring group effect

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Chem. Sci-2020

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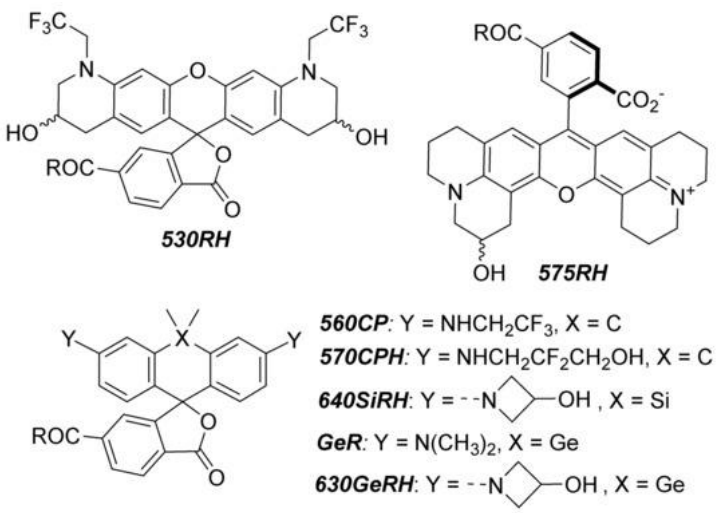
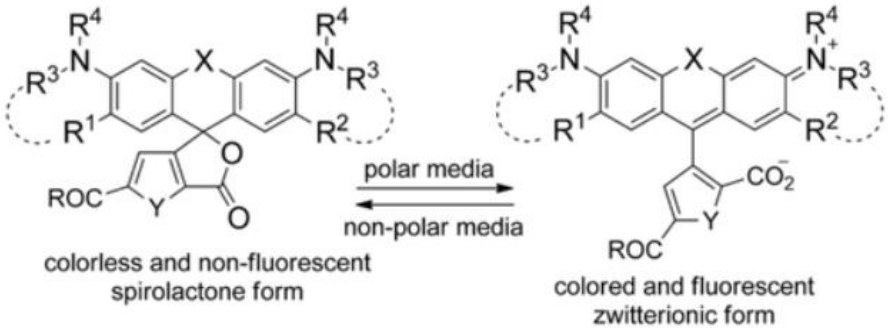
Chromatin Labeling and Imaging

- Creation of new biocompatible fluorescent chromatin probes.
- Application of the newly developed probes for imaging and establishment of image analysis pipelines.
- Elucidation of the chromatin structure and dynamics in living cells during cell cycle or external stimulation

罗丹明开关环研究背景



Chemistry – A European Journal-2017-12114



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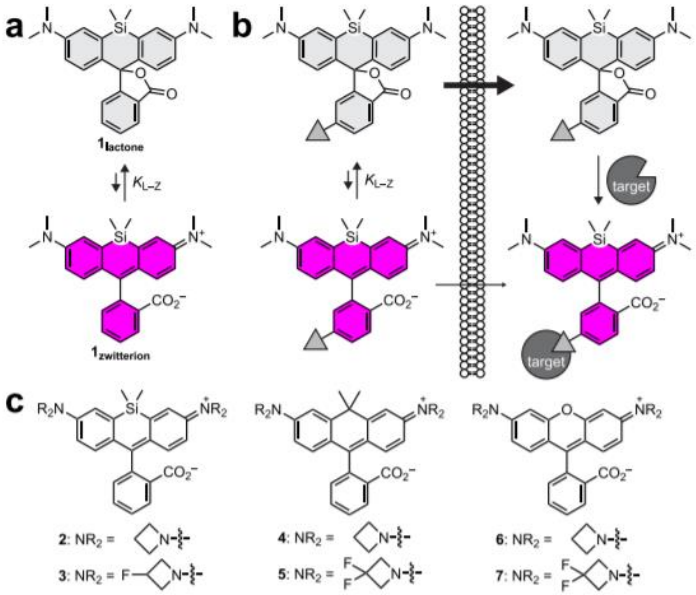
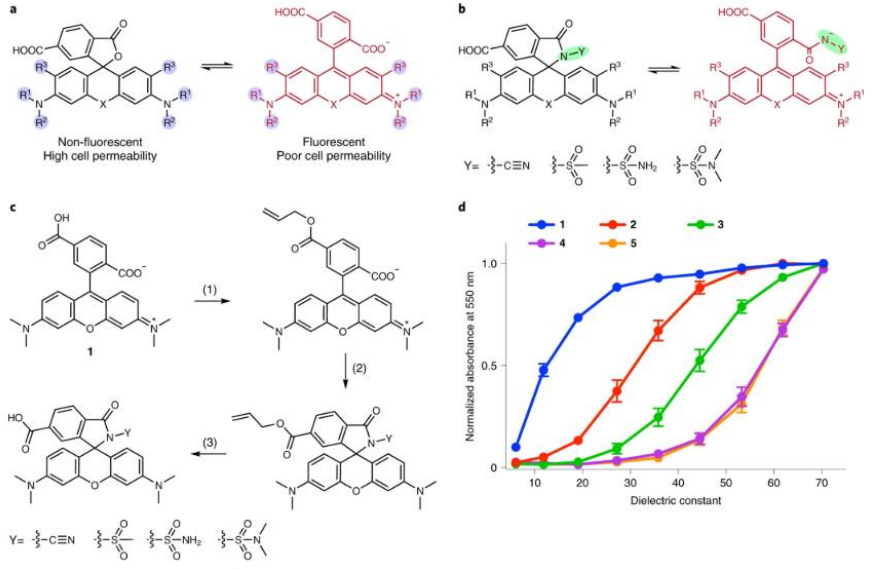
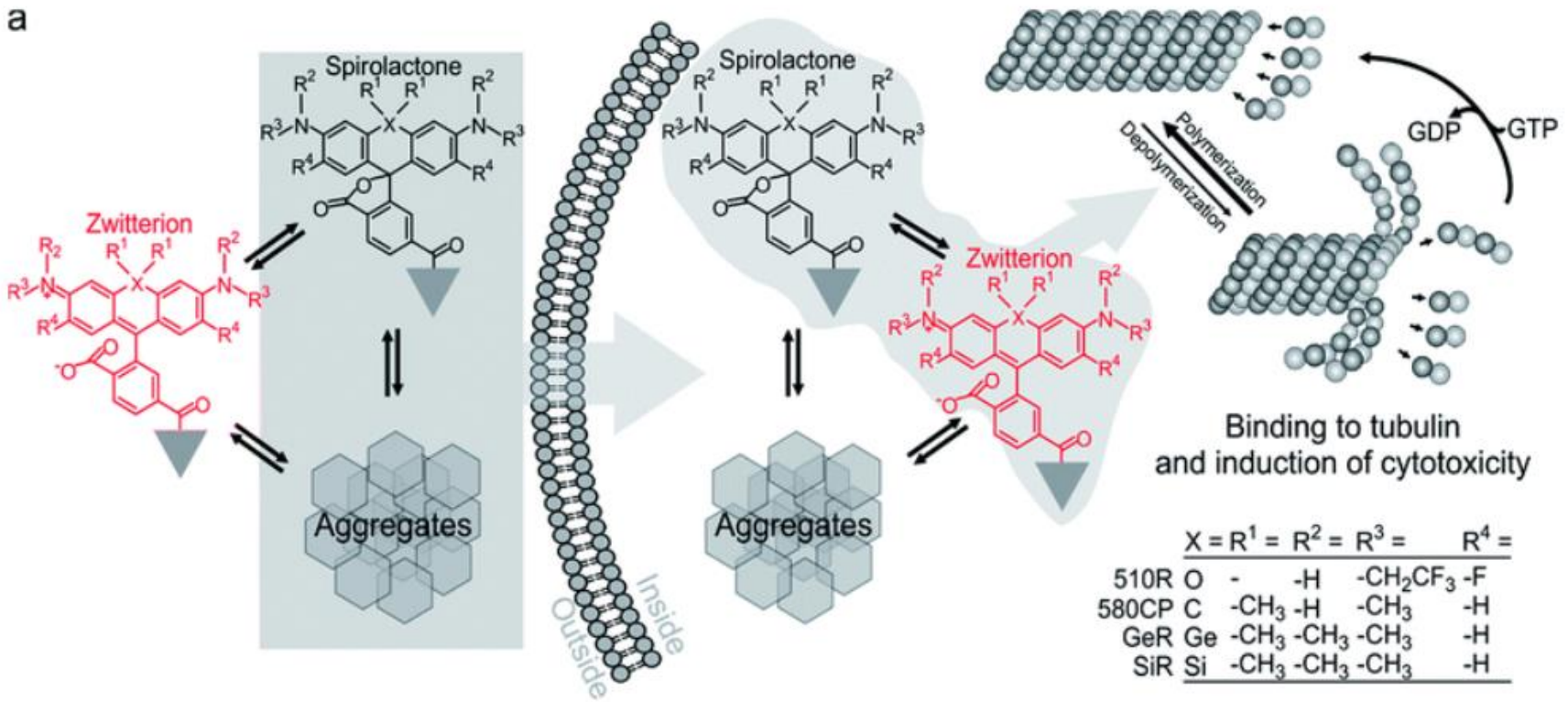


ACS CentSci-2019-1602



Nat. Chem-2020-165

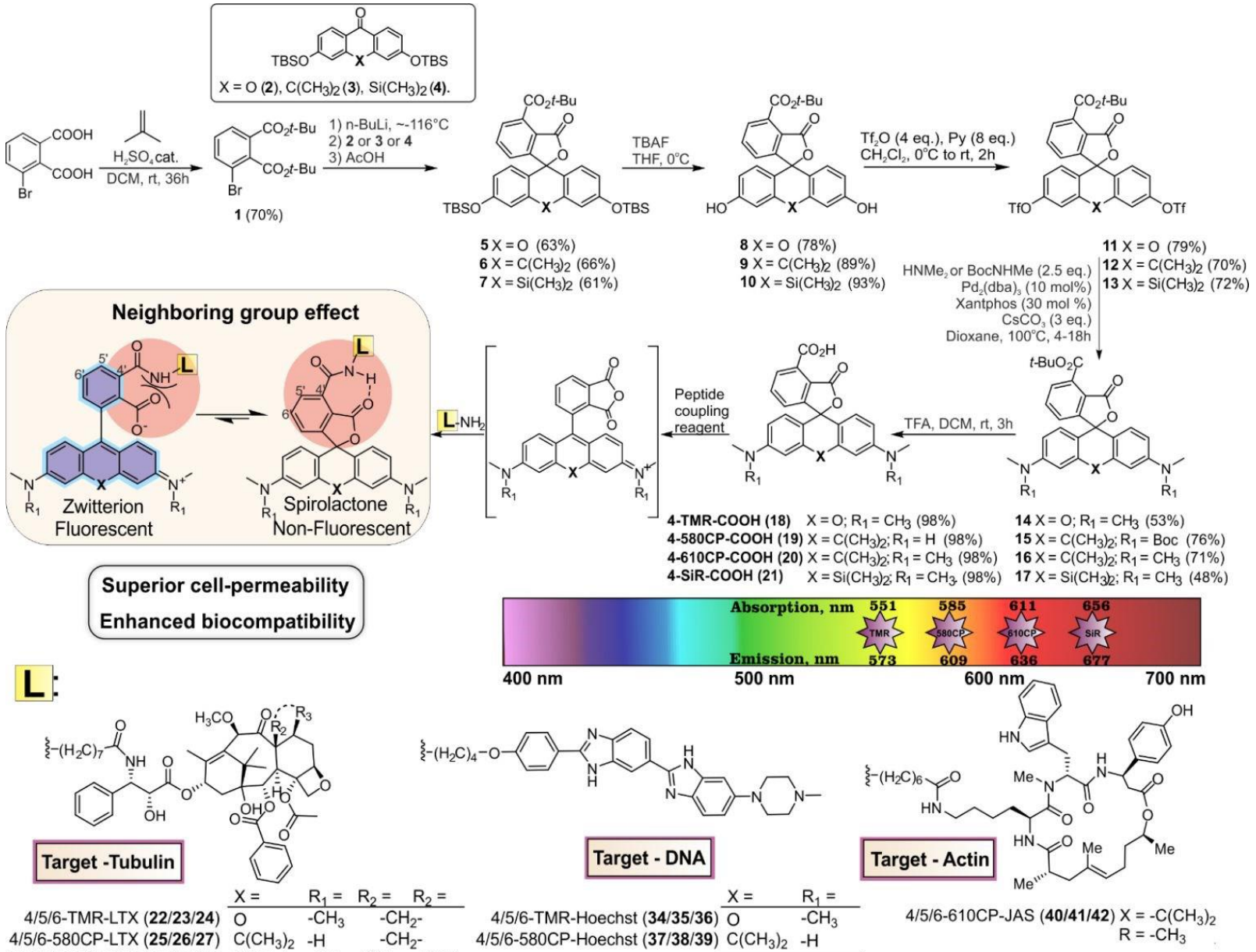
罗丹明开关环研究背景



	X =	R^1 =	R^2 =	R^3 =	R^4 =
510R	O	-	-H	$-CH_2CF_3$	-F
580CP	C	$-CH_3$	-H	$-CH_3$	-H
GeR	Ge	$-CH_3$	$-CH_3$	$-CH_3$	-H
SiR	Si	$-CH_3$	$-CH_3$	$-CH_3$	-H

Chem. Sci-2018-3324

分子设计


X = O (2), C(CH3)2 (3), Si(CH3)2 (4).
1 (70%)
1) n-BuLi, ~-116°C
2) 2 or 3 or 4
3) AcOH
H2SO4 cat.
DCM, rt, 36h
TBAF
THF, 0°C
Tf2O (4 eq.), Py (8 eq.)
CH2Cl2, 0°C to rt, 2h
5 X = O (63%)
6 X = C(CH3)2 (66%)
7 X = Si(CH3)2 (61%)
8 X = O (78%)
9 X = C(CH3)2 (89%)
10 X = Si(CH3)2 (93%)
11 X = O (79%)
12 X = C(CH3)2 (70%)
13 X = Si(CH3)2 (72%)
HNMe2 or BocNHMe (2.5 eq.)
Pd2(dba)3 (10 mol%)
Xantphos (30 mol %)
Cs2CO3 (3 eq.)
Dioxane, 100°C, 4-18h
Neighboring group effect
Zwitterion
Fluorescent
Spirolactone
Non-Fluorescent
L-NH2
Peptide coupling reagent
TFA, DCM, rt, 3h
4-TMR-COOH (18) X = O; R1 = CH3 (98%)
4-580CP-COOH (19) X = C(CH3)2; R1 = H (98%)
4-610CP-COOH (20) X = C(CH3)2; R1 = CH3 (98%)
4-SiR-COOH (21) X = Si(CH3)2; R1 = CH3 (98%)
14 X = O; R1 = CH3 (53%)
15 X = C(CH3)2; R1 = Boc (76%)
16 X = C(CH3)2; R1 = CH3 (71%)
17 X = Si(CH3)2; R1 = CH3 (48%)
Superior cell-permeability
Enhanced biocompatibility
Absorption, nm 551 585 611 656
Emission, nm 573 609 636 677
TMR
580CP
610CP
SiR
400 nm
500 nm
600 nm
700 nm
L:
Target -Tubulin
Target - DNA
Target - Actin
4/5/6-TMR-LTX (22/23/24) X = O R1 = -CH3 R2 = -CH2-
4/5/6-580CP-LTX (25/26/27) X = C(CH3)2 R1 = -H R2 = -CH2-
4/5/6-610CP-CTX (28/29/30) X = C(CH3)2 R1 = -CH3 R2 = -CH3 R3 = -OCH3
4/5/6-SiR-CTX (31/32/33) X = Si(CH3)2 R1 = -CH3 R2 = -CH3 R3 = -OCH3
4/5/6-TMR-Hoechst (34/35/36) X = O R1 = -CH3
4/5/6-580CP-Hoechst (37/38/39) X = C(CH3)2 R1 = -H
4/5/6-610CP-JAS (40/41/42) X = -C(CH3)2
R = -CH3

机制验证

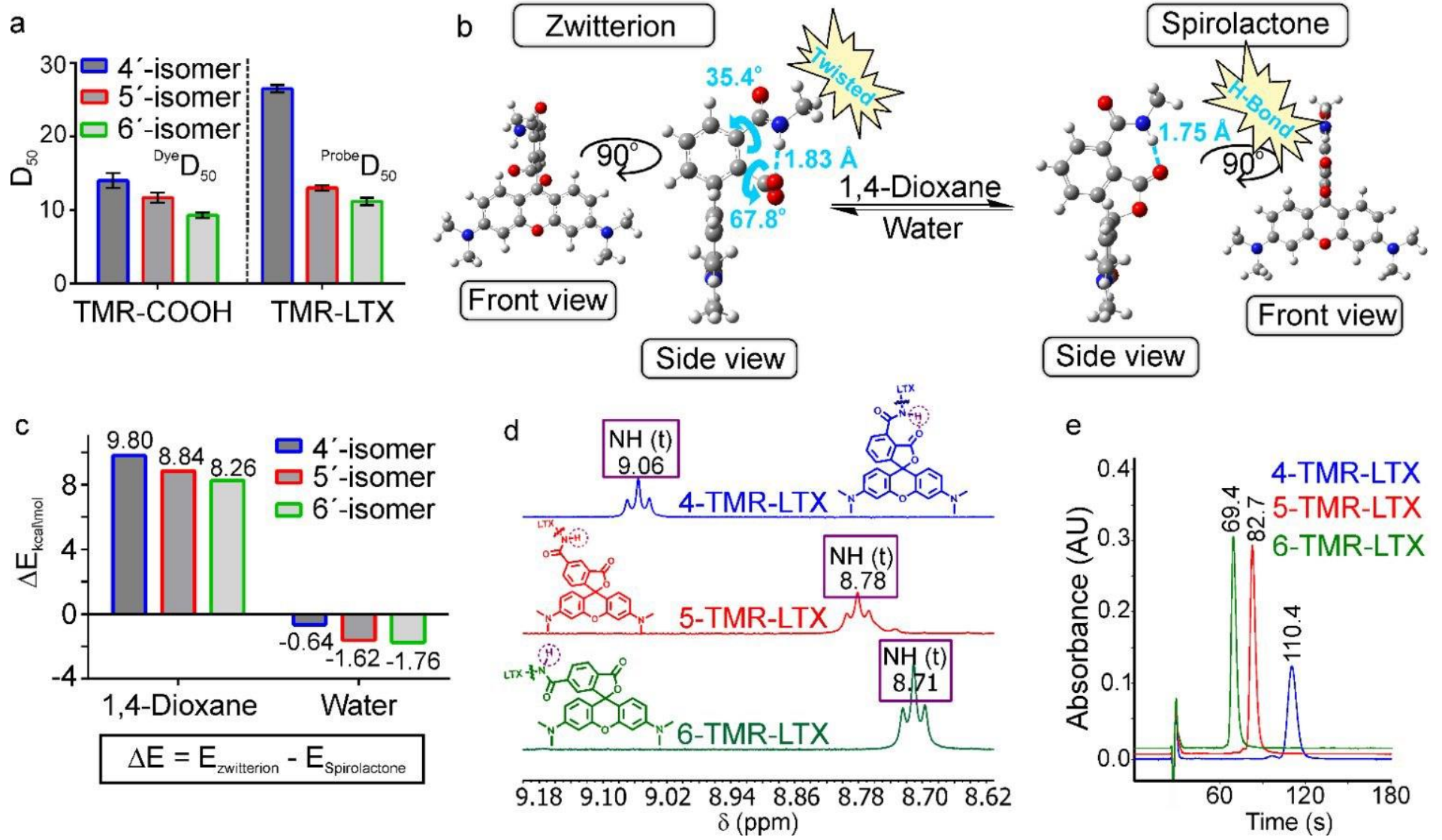
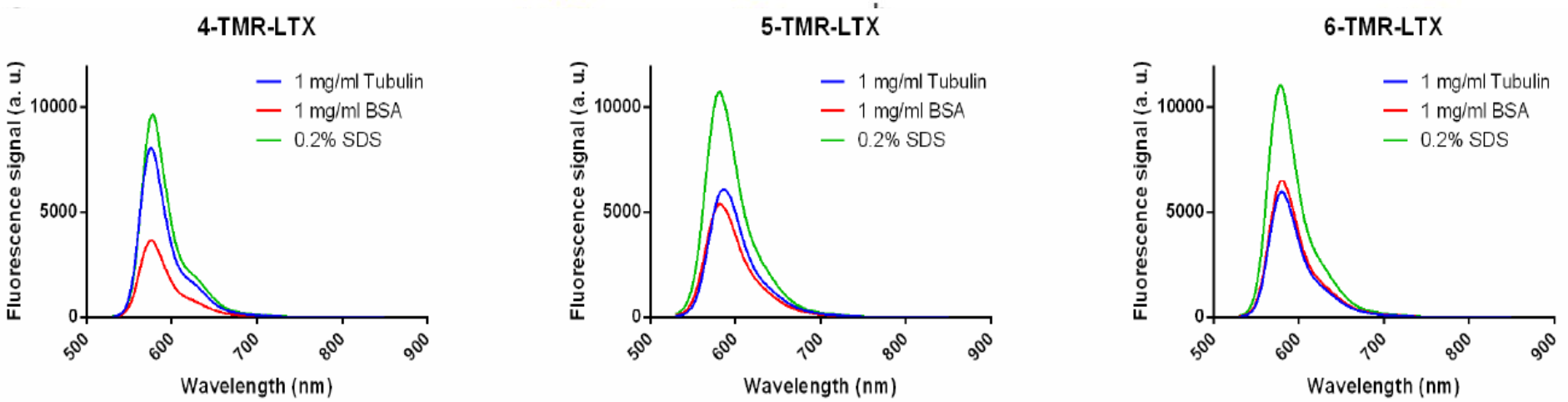
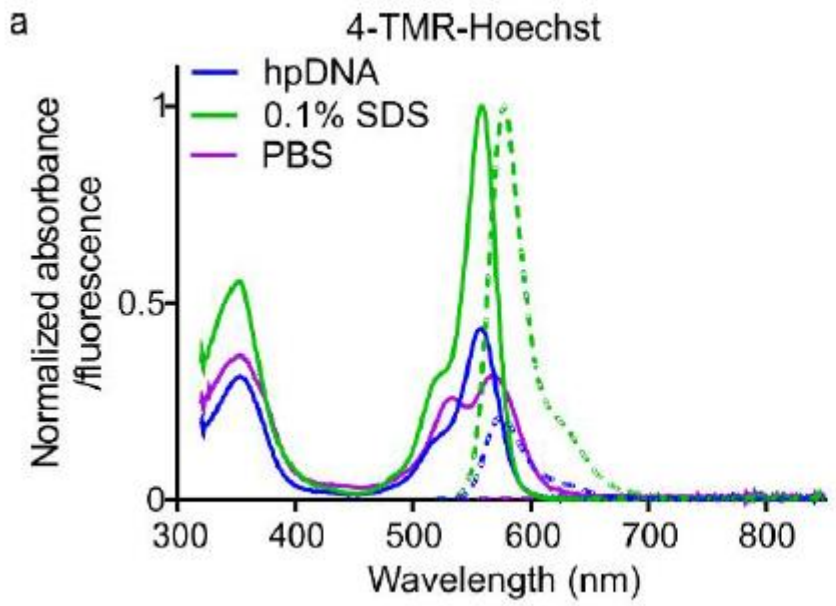
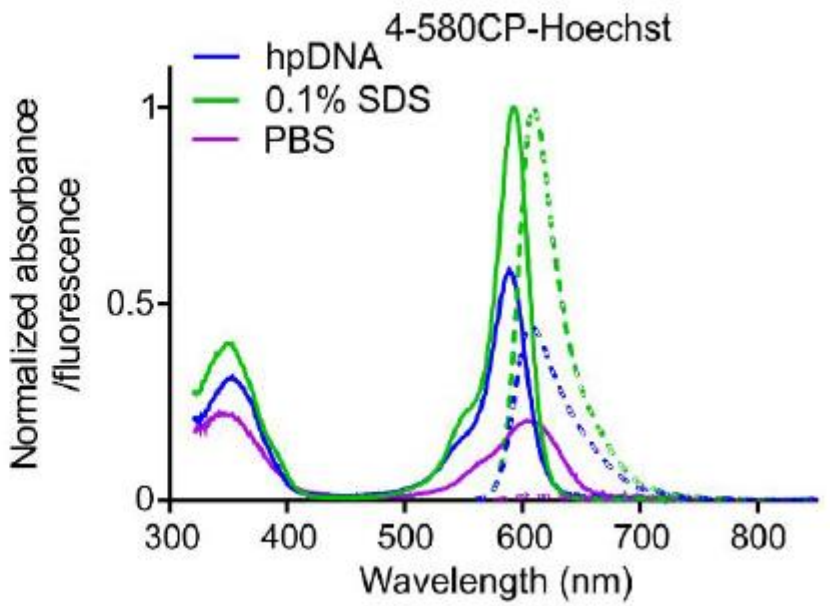


Fig. 2. Neighbouring group effect in the fluorescent probes. **a**, $^{Dye}D_{50}$ values of positional isomers of **TMR-COOH** and $^{probe}D_{50}$ of **TMR-LTX**. **b**, DFT optimized geometries of a model 4´-regioisomer fluorescent probe in spirolactone and zwitterion forms with truncated linker and targeting moiety. **c**, DFT calculated potential energy differences between spirolactone and zwitterion of model 4'/5'/6'-regiosomer probes in 1,4-dioxane and water environment. **d**, Chemical shifts of the amide proton of **TMR-LTX** regioisomeric probes. **e**, Comparison of retention times of **TMR-LTX** regioisomeric probes in HPLC analysis with SB-C18 column under isocratic elution conditions (75:25 MeOH : H_2O 25mM $HCOONH_4$ pH = 3.6).

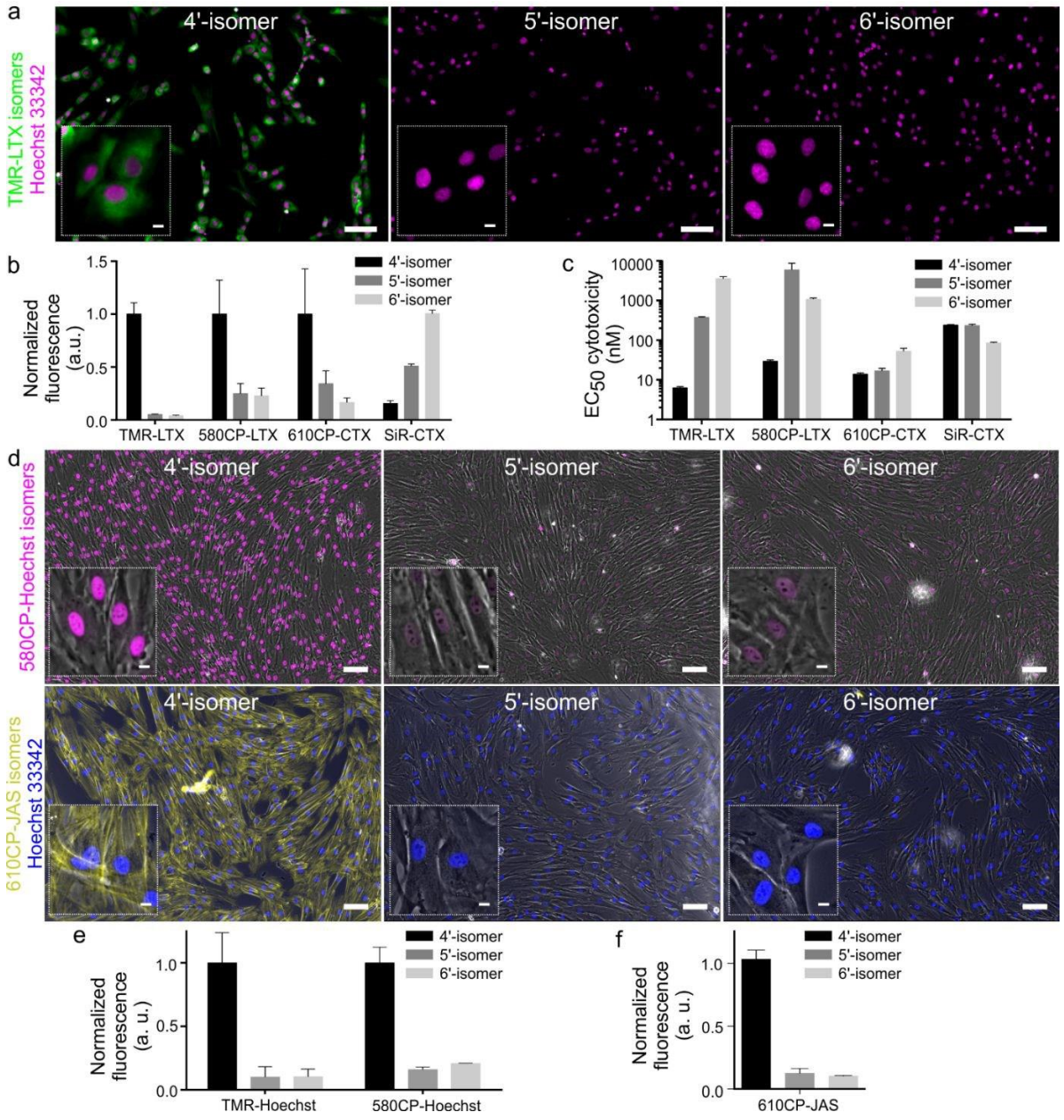
开关环性质测试


4-TMR-LTX
5-TMR-LTX
6-TMR-LTX
Fluorescence signal (a. u.)
10000
5000
0
500
600
700
800
900
Wavelength (nm)
1 mg/ml Tubulin
1 mg/ml BSA
0.2% SDS


a
4-TMR-Hoechst
hpDNA
0.1% SDS
PBS
Normalized absorbance /fluorescence
1
0.5
0
300
400
500
600
700
800
Wavelength (nm)


4-580CP-Hoechst
hpDNA
0.1% SDS
PBS
Normalized absorbance /fluorescence
1
0.5
0
300
400
500
600
700
800
Wavelength (nm)

亚细胞器免洗成像



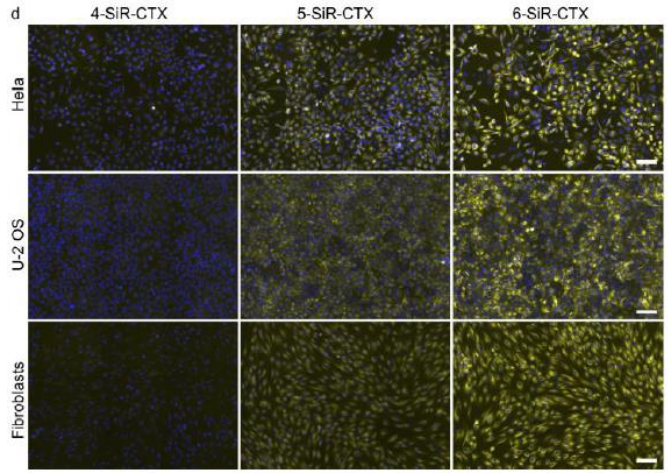


Fig. 3. Imaging performance of fluorescent probes based on rhodamine isomers. **a**, Wide-field fluorescence microscopy of living primary fibroblasts stained with 100 nM **TMR-LTX** isomers for 1h at 37°C. Cells were washed once with HBSS and imaged in DMEM growth media. Insets shows zoomed-in images. Scale bars: 100 µm (large field of view), 10 µm (inset). Hoechst staining is shown in cyan and all tubulin probes are in magenta. **b**, Quantification of fluorescence signal in the cytoplasm of living cells stained with tubulin probes. Data is presented as mean ± s.e.m., N = 3 independent experiments, each time n > 100 cells were quantified. **c**, Cytotoxicity of tubulin fluorescent probes presented as half maximal effective concentration (EC50) after 24h incubation at 37°C in growth media. Cytotoxicity was determined as fraction of cells containing less than a single set of genetic material (sub G1 DNA content). Data is presented as mean ± s.e.m., N = 3 independent experiments, each time n > 100 cells were quantified. **d**, Wide-field microscopy images of living primary fibroblasts. The cells were stained with 100 nM **4/5/6-580CP-Hoechst** (magenta) or 100 nM **4/5/6-610CP-JAS** (yellow) for 1h at 37°C, washed once with HBSS and imaged in DMEM media. Inserts show zoomed-in images. Scale bars: 100 µm (large field of view), 10 µm (insets). Overlay with phase contrast (grey) images are shown. **e**, Quantification of DNA probes fluorescence signal in the nuclei. Data presented as mean ± s.d., N = 3 independent experiments, each n > 100 cells. **f**, Quantification of **4/5/6-610CP-JAS** fluorescence signal in the cytoplasm of living cells. Data presented as mean ± s.d., N = 3 independent experiments, each n > 100 cells.

共聚焦成像及其动态

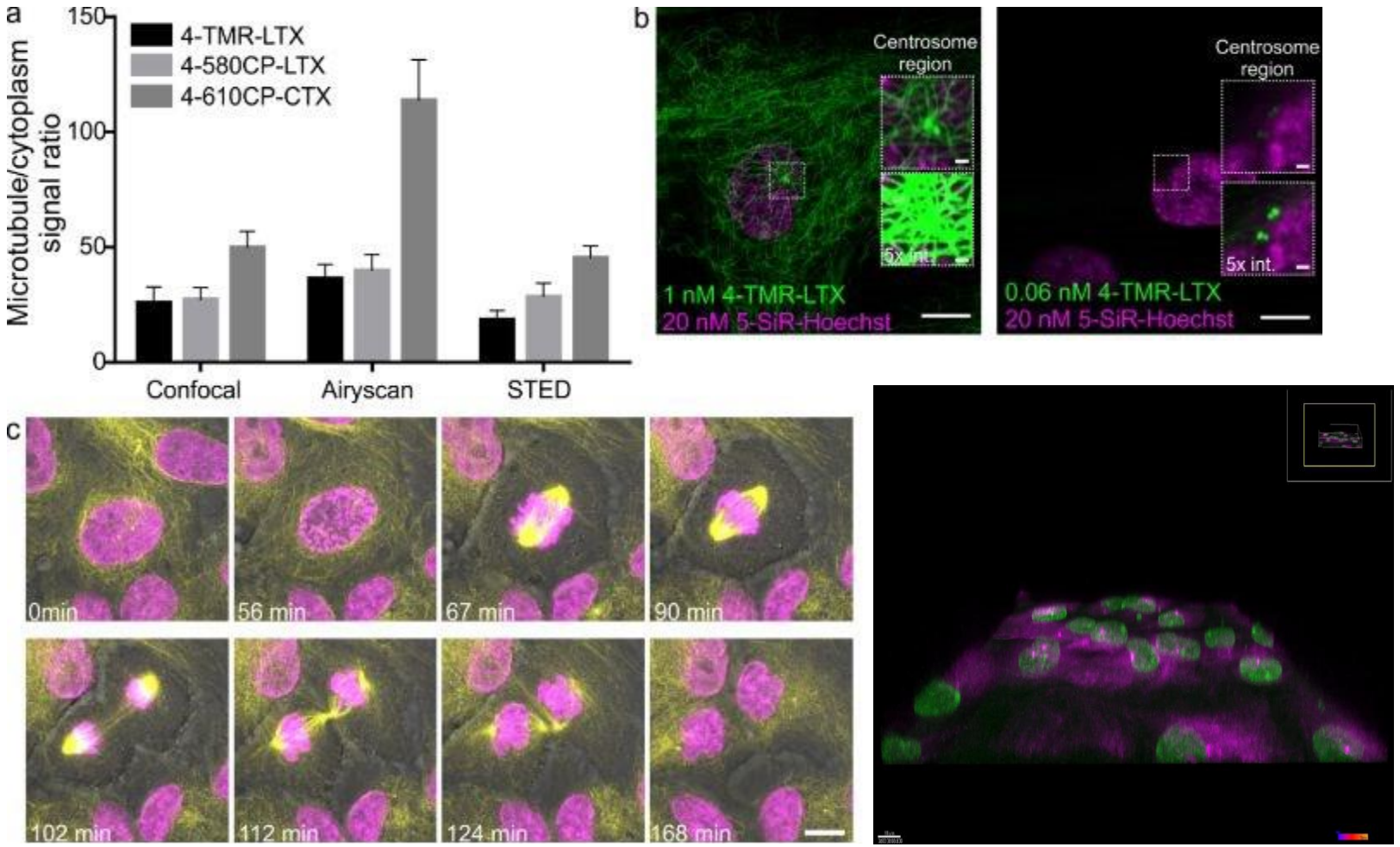


Fig. 4. Confocal and Airyscan imaging of living cells stained with rhodamine 4'-isomer probes. **a**, Single microtubule and cytoplasm fluorescence signal ratio in living human fibroblasts stained with 100 nM of indicated probe for 1h at 37°C and imaged without probe removal. Data is presented as mean ± s.d., N ≥ 3 independent fields of view, each time n ≥ 20 microtubules. **b**, Zeiss Airyscan images of human primary fibroblasts stained with **5-SiR-Hoechst** and **4-TMR-LTX** for 24 h at 37°C in growth media at indicated concentrations. Images acquired without probe removal. **c**, Cell cycle of human primary fibroblasts stained with 1 nM **4-TMR-LTX** (yellow) and 10 nM **5-610CP-Hoechst** (magenta). Scale bar: 10 µm. Overlay with phase contrast (grey) images are shown. **d**, Three-colour ZEISS Airyscan image of living HeLa cell at metaphase stained with 3 nM **4-TMR-LTX** (yellow), 20 nM **5-SiR-Hoechst** (magenta) and 1000 nM **6-510R-JAS** (cyan). Scale bar: 1 µm.

STED超分辨成像

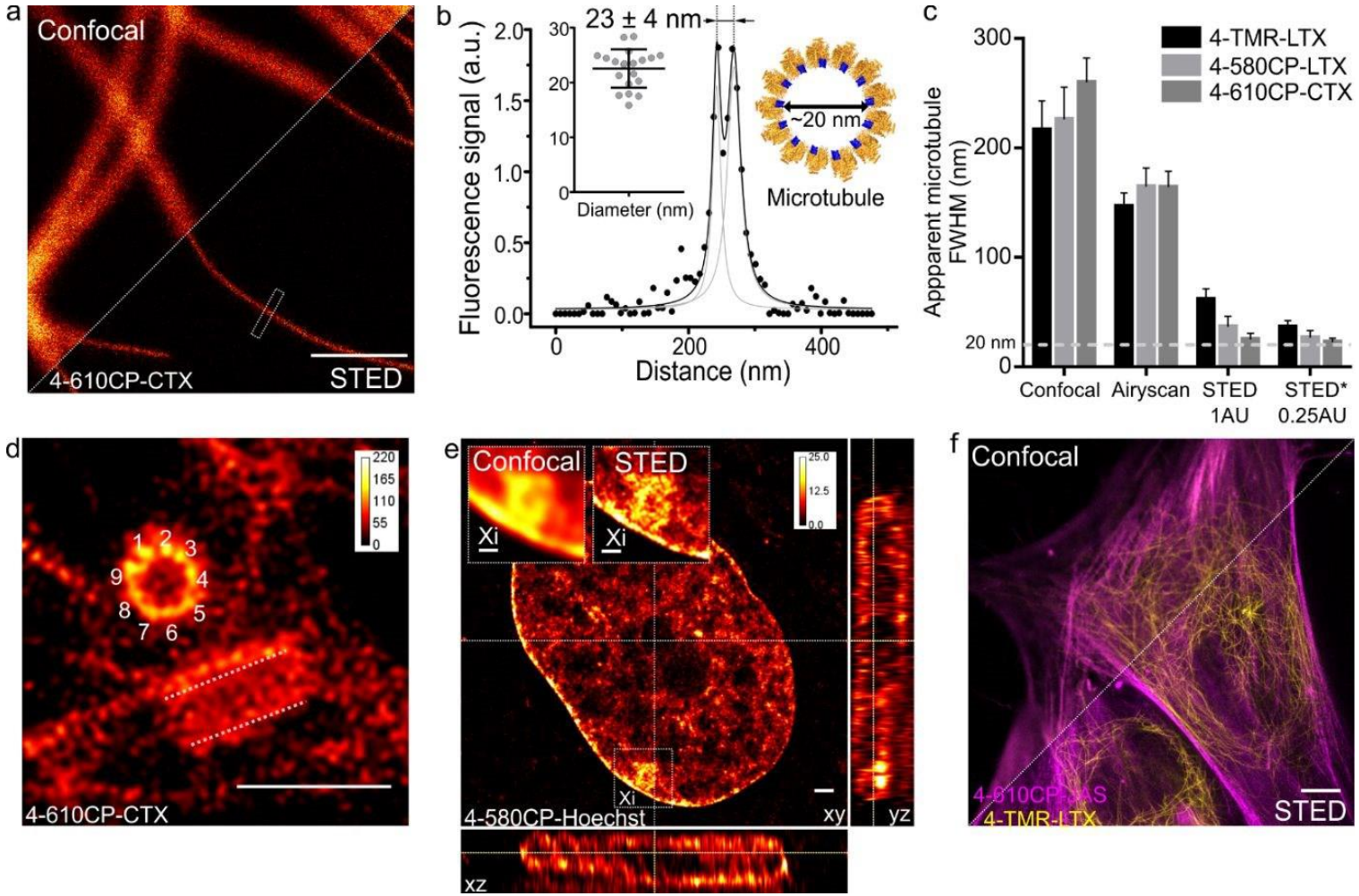


Fig. 5. STED nanoscopy imaging of living cells stained with rhodamine 4'-isomer probes. **a,** Confocal and STED images of microtubules in human fibroblasts taken with 0.25 AU pinhole. The cells were stained with 100 nM **4-610CP-CTX** for 1h at 37°C. Scale bar: 1 µm. **b,** Fluorescence intensity profile at the rectangle in panel **a**. Insets show measured (mean ± s.d., N = 20) and predicted diameter from cryo-electron microscopy model of tubulin (orange) bound to paclitaxel (blue). **c,** Apparent microtubule FWHM measured by different microscopy methods. Human fibroblasts stained with 100 nM probes for 1h at 37°C. * - diameter measured between two peaks of fitted intensity profile. Data presented as mean ± s.d., N ≥ 3 independent fields of view, each time n ≥ 10 microtubules. **d,** Nine-fold symmetry of centriole resolved in the deconvolved STED DyMIN[25] image of U-2 OS cell stained with 1 µM **4-610CP-CTX** for 1h at 37°C. White dashed lines mark a second centriole. Scale bar: 1 µm. **e**, Deconvolved STED image of human female fibroblast nucleus stained with 100 nM **4-580CP-Hoechst** showing the inactivated X chromosome (Xi). Insets - zoomed-in confocal and STED images of Xi region. Scale bars: 500 nm (insets), 1 µm (main image). **f,** Two-colour STED no-wash image of human fibroblasts stained with 100 nM **4-610CP-JAS** and 10 nM **4-TMR-LTX** for 1h at 37°C. Scale bar: 10 µm.