Electronic supplementary information

Biochromic silole derivatives: a single dye for differentiation, quantitation and imaging of live/dead cells

Sijie Chen,[†ab] Jianzhao Liu,[†ac] Shouxiang Zhang,[†d] Engui Zhao,[a] Yee Yung Yu,[a] Roozbeh Hushiarian,[d] Yuning Hong*[ad] and Ben Zhong Tang*[ade]

[a]*Department of Chemistry, Hong Kong Branch of Chinese National Engineering Research Center for Tissue Restoration and Reconstruction, Institute of Molecular Functional Materials, State Key Laboratory of Neuroscience, Division of Biomedical Engineering, and Division of Life Science., The Hong Kong University of Science and Technology, Kowloon, Hong Kong, China. Email: tangbenz@ust.hk*
[b]*Ming Wai Lau Centre for Reparative Medicine, Karolinska Institutet, Hong Kong*
[c]*MOE Key Laboratory of Macromolecular Synthesis and Functionalization, Department of Polymer Science and Engineering, Zhejiang University, Zheda Road 38, Hangzhou 310027, China*
[d]*Department of Chemistry and Physics, La Trobe Institute for Molecular Science, La Trobe University, Melbourne, Victoria 3086 Australia. Email: Y.Hong@latrobe.edu.au*
[e]*HKUST-Shenzhen Research Institute, Hi-tech Park, Nanshan, Shenzhen 518057*
[f]*China NSFC Center for Luminescence from Molecular Aggregates, SCUT-HKUST Joint Research Institute, State Key Laboratory of Luminescent Materials and Devices, South China University of Technology, Guangzhou 510640, China*
† *These authors contribute equally.*

pH Sensing

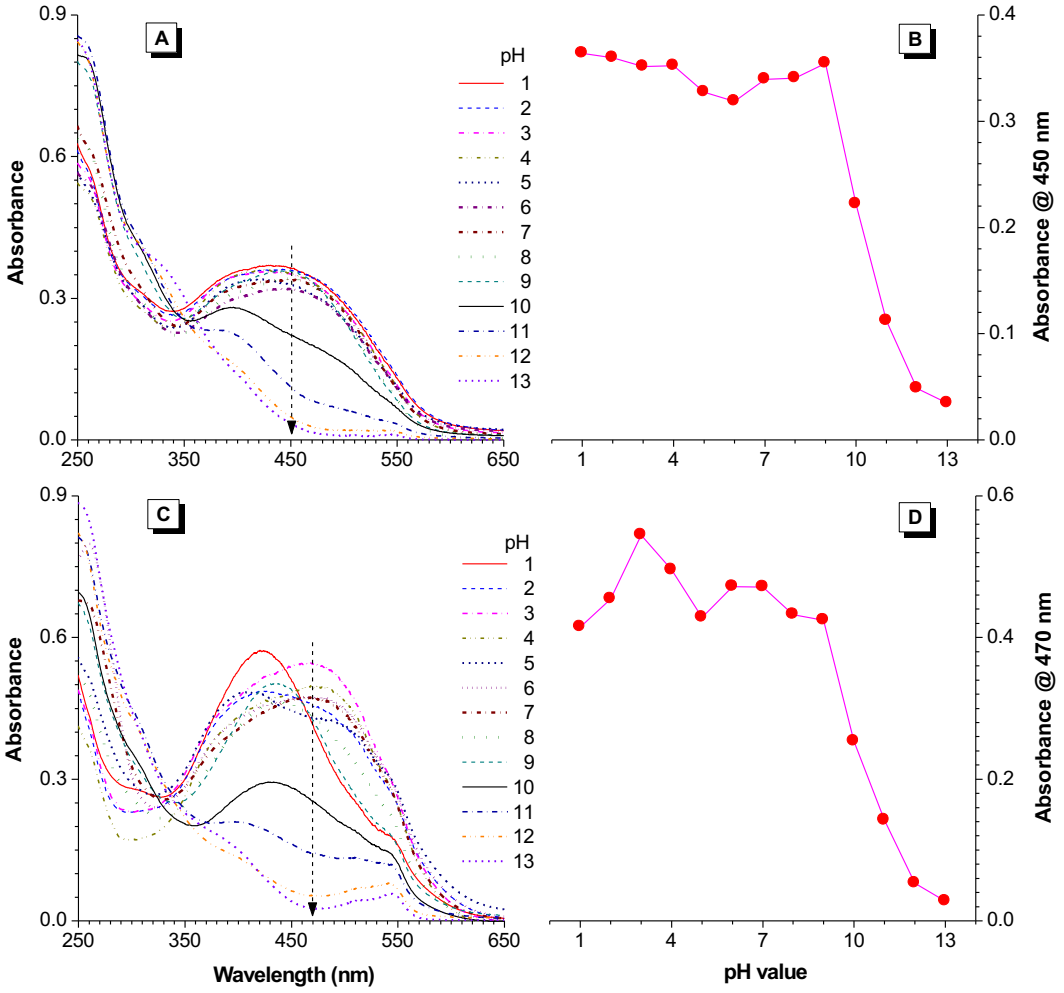

Fig. S1 Absorption spectra of (A) Silo-Cy and (B) Silo-2Cy in aqueous buffer solutions with different pH. (B, D) Absorption values at wavelengths of 450 nm for Silo-Cy and 470 nm for Silo-2Cy versus pH value. [dye]: 3×10^{-5} M.

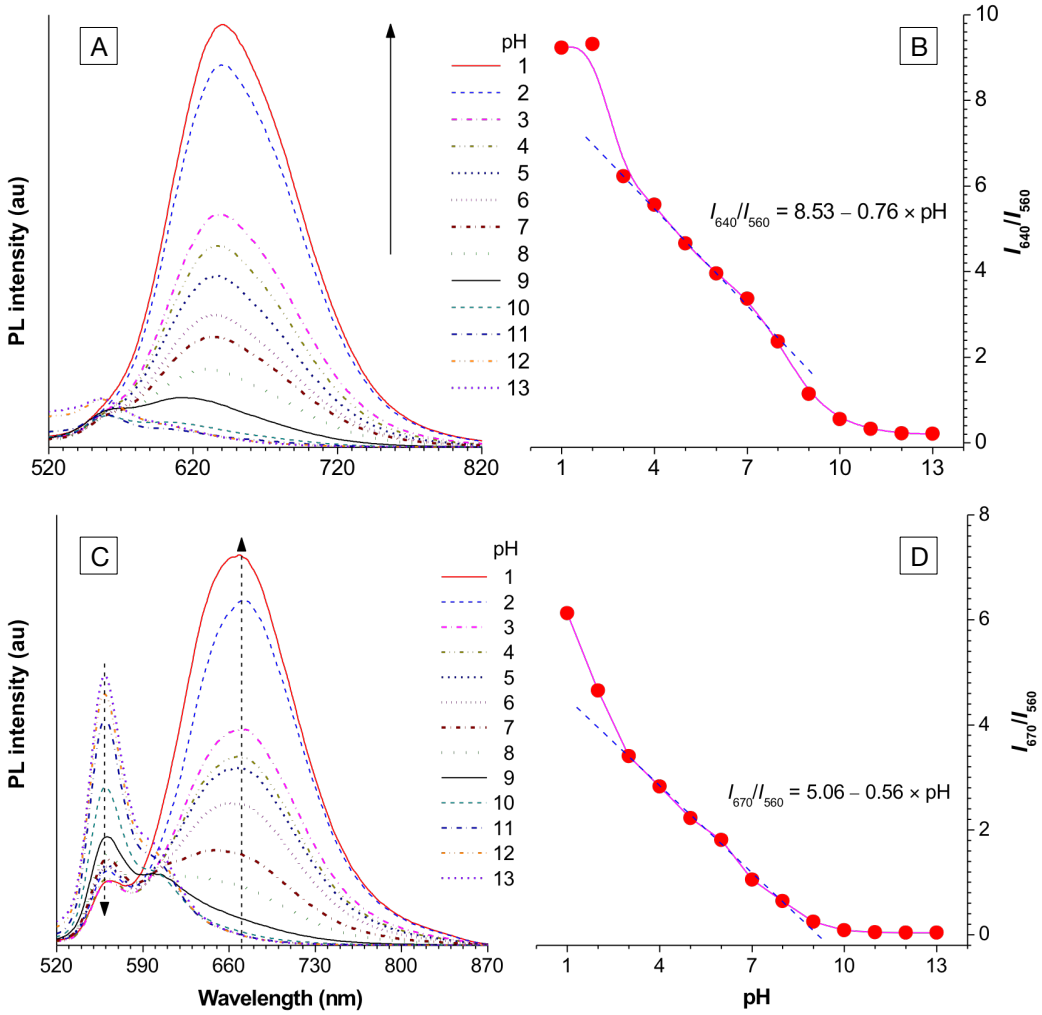

Fig. S2 (A, C) PL spectra of Silo-Cy and Silo-2Cy, respectively, in aqueous buffer solutions with different pH values excited at 470 nm. (B, D) Plots of I_{640}/I_{560} of Silo-Cy and I_{670}/I_{560} of Silo-2Cy versus pH value, respectively. I_{500}, I_{640}, and I_{670} denote PL intensities at wavelengths of 500, 640, and 670 nm, respectively. In panel B and D, linear regression curve was fitted in the pH range from 3 to 8. [dye]: 3×10^{-5} M.

Cell Viability

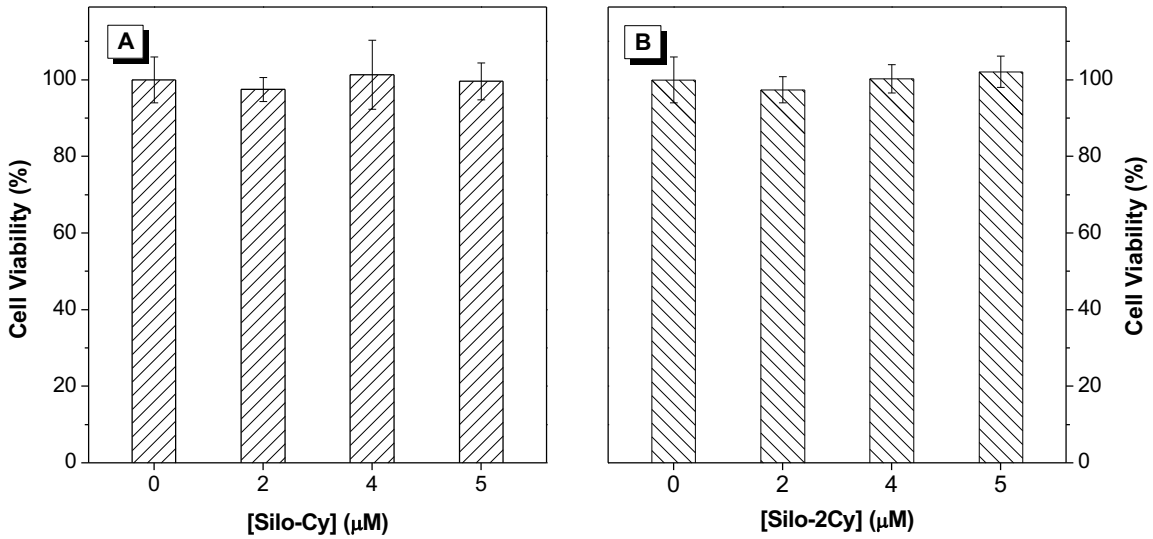

Fig. S3 Cytotoxicity of (A) Silo-Cy and (B) Silo-2Cy evaluated on HeLa cells by MTT assay.

Live Cell Imaging

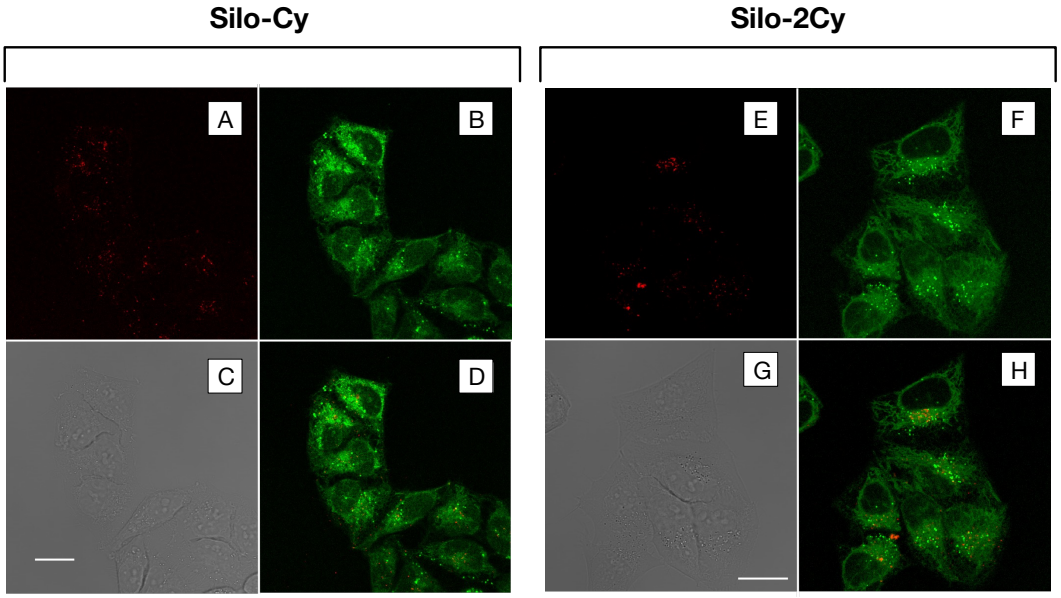

Fig. S4 Confocal images of HeLa cells incubated with Silo-Cy and Silo-2Cy for 2 h under excitation of (A, E) 488 and (B, F) 405 nm. (C, G) Bright-field image. (D, H) Image merged from those in panels A, B, C and E, F, G, respectively. Scale bar: 20 μm. [dye] = 4×10^{-6} M.

The role of lipid

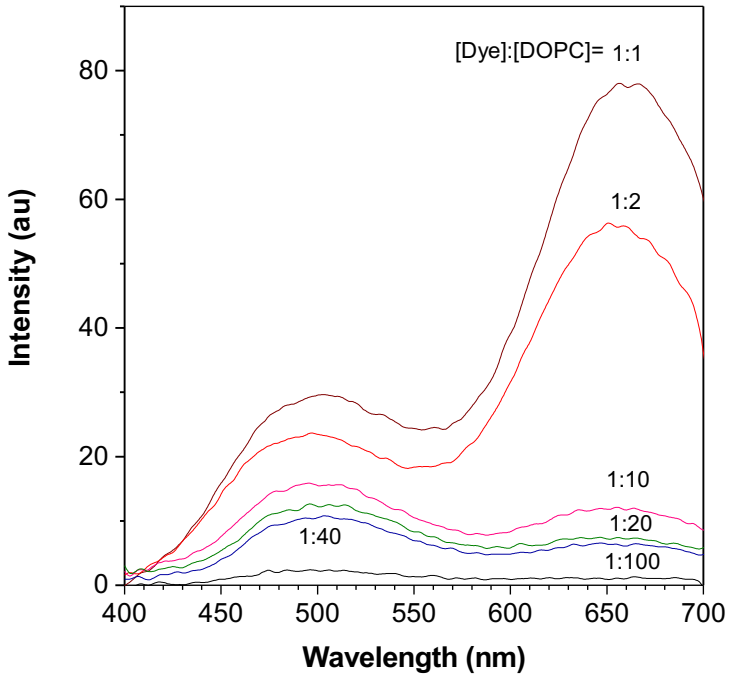

Fig. S5 PL spectra of different amount of Silo-2Cy in the presence of same amount of DOPC. [DOPC] = 0.1 mM. λ_{ex}: 370 nm.

Bleedthrough test of TO-PRO-3

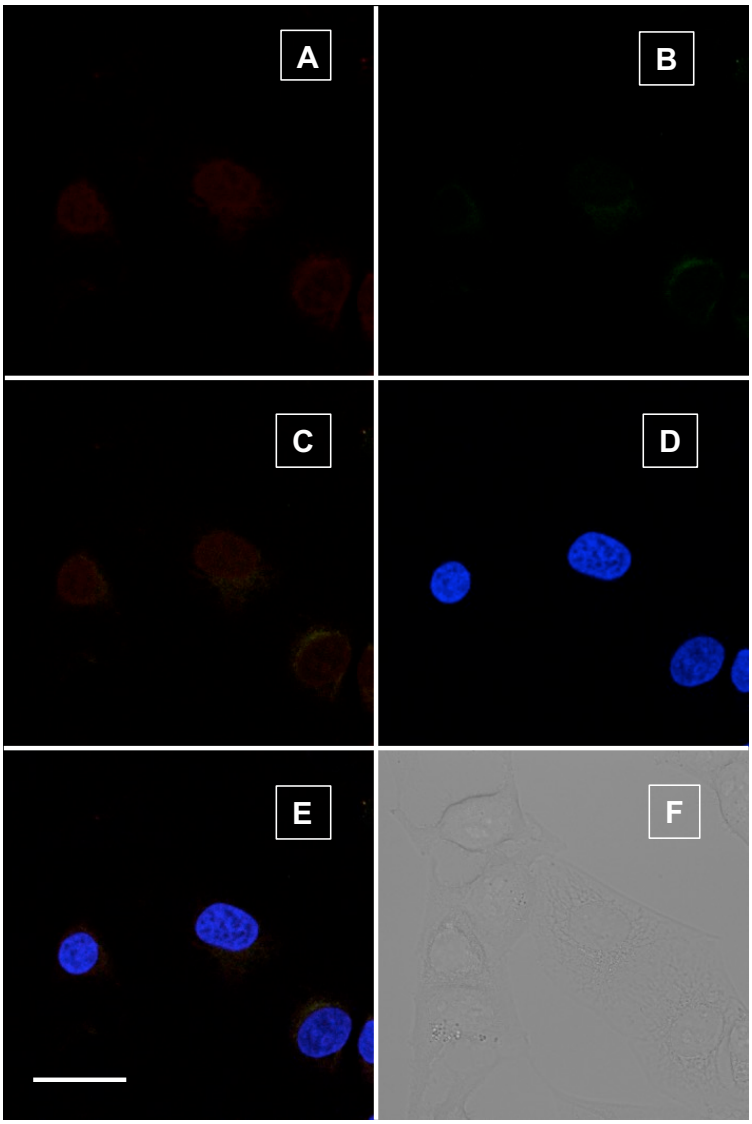

Fig. S6 TO-PRO-3 stained dead and live cells imaged with confocal microscope using the same parameters as Figure 4. (A) and (B) are the red channel and green channel of Silo-Cy, respectively. (C) Image merged from panels A and B. (D) TO-PRO-3 channel. (E) Image merged from panels A, B and D. (F) bright field image. Scale bar: 30 μm

Flow cytometric assay

Table S1 Fold of fluorescence intensity change of Silo-Cy or Silo-2Cy stained cells upon exposed to different stimuli in the corresponding channels.

Silo-Cy	Green channel			Red channel		
Blank	0.07	0.07	0.07	0.33	0.31	0.31
UV	3.18	2.94	2.81	3.15	2.99	2.94
Nocodazole	4.45	4.38	4.69	7.74	7.46	8.56
Heat	6.67	6.35	6.52	24.41	25.25	23.82
Ethanol	9.77	9.21	10.13	33.65	31.65	32.67

Silo-2Cy	Green channel			Red channel		
Blank	0.57	0.57	0.56	0.72	0.69	0.69
UV	2.15	2.09	2.10	2.64	2.64	2.69
Nocodazole	3.22	3.75	3.65	5.05	5.93	5.64
Heat	4.62	4.61	4.65	11.32	11.47	11.60
Ethanol	7.54	7.21	7.49	15.31	14.65	14.89

Each change fold is calculated by the ratio of median stress fluorescence to average of median negative control fluorescence.

Table S2 Raw data of fluorescence intensity of Silo-Cy or Silo-2Cy stained cells upon exposed to different stimuli in the corresponding channels in biological triplicate.

Silo-Cy	Green channel									
	Negative control fluorescence			Average	Stress fluorescence			Blank fluorescence		
UV	3983	4041	3886	3970.0	12611	11666	11171	N/A		
Nocodazole	4049	3878	4116	4014.3	17862	17572	18808			
Heat	5404	5544	5616	5521.3	36812	35082	36023			
Ethanol	6559	6645	6926	6710.0	65556	61831	67999	477	480	471

Silo-Cy	Red channel									
	Negative control fluorescence			Average	Stress fluorescence			Blank fluorescence		
UV	1722	1658	1702	1694.0	5336	5060	4976	N/A		
Nocodazole	1579	1489	1767	1611.7	12467	12018	13796			
Heat	2013	2064	2044	2040.3	49800	51521	48607			
Ethanol	2283	2373	2262	2306.0	77586	72987	75343	752	722	718

Silo-2Cy	Green channel									
	Negative control fluorescence			Average	Stress fluorescence			Blank fluorescence		
UV	582	576	591	583.0	1251	1220	1225	N/A		
Nocodazole	639	631	630	633.3	2040	2377	2310			
Heat	682	674	666	674.0	3113	3107	3132			
Ethanol	814	871	840	841.7	6349	6067	6308	477	480	471

Silo-2Cy	Red channel									
	Negative control fluorescence			Average	Stress fluorescence			Blank fluorescence		
UV	735	740	747	740.7	1953	1953	1994	N/A		
Nocodazole	828	817	798	814.3	4116	4831	4594			
Heat	864	864	857	861.7	9751	9884	9996			
Ethanol	1008	1063	1057	1042.7	15967	15277	15527	752	722	718

Blank fluorescence is measured only once together with ethanol treated cells simultaneously, and is not measured repeatedly for other stressors, shown as N/A.