Electronic Supplementary Information

Virus-templated FRET platform for rational design of ratiometric fluorescent nanosensors

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Materials

β-Cyclodextrin (β-CD), succinic anhydride (SA), pyridine, 4-dimethylaminopyridine (DMAP), rhodamine B (RhB), 1-adamantanemethanol, 1-adamantanamine hydrochloride, *N*-hydroxysuccinimide (NHS) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC·HCl) were purchased from Aladdin Chemistry Co., Ltd., Shanghai, China. Fluorescein isothiocyanate (FITC) was purchased from J & K Scientific Co., Ltd., Beijing, China. Dubelcco's Modified Eagle's Medium (DMEM) was purchased from Thermo Fisher Scientific Inc. Other reagents were local commercial products and used as received.

Preparation of M13-β-CD/Ada-FITC/Ada-RhB

M13-β-CD. M13-β-CD was prepared from bacteriophage M13 and NHS-activated β-CD-COOH as our previous report.[1]

Ada-RhB. Ada-RhB was prepared from RhB and 1-adamantanemethanol as our previous report.[2]

Ada-FITC. FITC (20.0 mg, 0.05 mmol), 1-adamantanamine hydrochloride (9.3 mg, 0.05 mmol), triethylamine (28.0 μL, 0.2 mmol), DMSO (10 mL) and 100 mM pH 9.5 sodium carbonate buffer solution (10 mL) were mixed and stirred at room temperature for 24 h. The resulting Ada-FITC was separated by silica gel column chromatography (CH_3OH/CH_2Cl_2, 1/5) as an orange solid (16.9 mg, 62 %). ^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 8.26 (s, 1 H), 7.84 (d, J = 8.0 Hz, 1 H), 7.17 (d, J = 8.0 Hz, 1 H), 6.73 (d, J = 8.6 Hz, 2 H), 6.70 (d, J = 2.3 Hz, 2 H), 6.58 (dd, J_1 = 8.6 Hz, J_2 = 2.3 Hz, 2 H), 2.07 (s, 3 H), 1.74-1.64 (m, 6 H), 1.54 (s, 6 H).

M13-β-CD/Ada-FITC/Ada-RhB. M13-β-CD was first incubated with various molar ratios of Ada-FITC and Ada-RhB in 10 mM pH 7.8 K-phos buffers for 30 min at 4 °C, and purified by precipitation twice with PEG_{8K} and NaCl to give the M13-β-CD/Ada-FITC/Ada-RhB.

Table S1 The approximate number of Ada-FITC and Ada-RhB anchored on a M13-β-CD particle

$n_{Ada\text{-}FITC}$: $n_{Ada\text{-}RhB}$	approximate values of Ada-FITC per M13-β-CD particle	approximate values of Ada-RhB per M13-β-CD particle
6 : 0	1416	0
4 : 2	859	242
3 : 3	577	503
2 : 4	456	835
0 : 6	0	1410

Characterization of M13-β-CD/Ada-FITC/Ada-RhB

UV-Vis spectra of M13-β-CD/Ada-FITC/Ada-RhB K-phos buffer solutions (10 mM, pH 7.8) were acquired on a

TU-1901 spectrometer (Beijing Purkinje General Instrument Co., Ltd.). For transmission electron microscopy (TEM) analysis, a drop of M13-β-CD/Ada-FITC/Ada-RhB aqueous solution was placed on a carbon-coated grid for 30 seconds and most of the solution was then removed by filter paper, which was then stained with uranyl acetate and observed by a JEOL JEM-1011 microscope operating at an accelerating voltage of 100 kV. The fluorescence spectra were acquired on a Hitachi F-2500 FL spectrophotometer. The pH-dependent ratiometric response of the M13 nanosensor was determined based on the fluorescence emission ratio ($I_{515\ nm}/I_{580\ nm}$) of M13-β-CD/Ada-FITC/Ada-RhB K-phos buffer solution (10 mM) as a function of pH (3.4 ~ 9.4). Time-resolved fluorescence decay measurements were performed on a time-correlated single photon counting FLSP-920 system with excitation at 377 nm and detection at 515 nm.

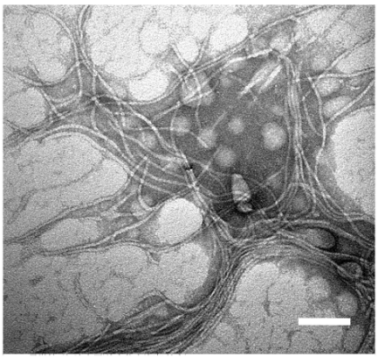

Fig. S1 TEM image of uranyl acetate-stained M13-β-CD/Ada-FITC/Ada-RhB ($n_{Ada\text{-}FITC}$: $n_{Ada\text{-}RhB}$ = 1 : 1). The scale bar represents 100 nm.

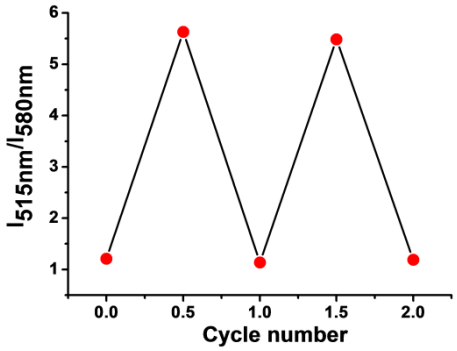

Fig. S2 The fluorescence emission ratios ($I_{515\ nm}/I_{580\ nm}$) of M13-β-CD/Ada-FITC/Ada-RhB ($n_{Ada\text{-}FITC}$: $n_{Ada\text{-}RhB}$ = 1 : 2) when pH was changed from 5.0 to 8.4 repeatedly.

Intracellular imaging

RAW 264.7 macrophages were seeded into a 24-well plate at a density of 1.5 ×10^4 cells mL^{-1} in 500 μL of DMEM supplemented with 10 % FBS, 100 units mL^{-1} of penicillin and 100 μg mL^{-1} of streptomycin. After 24 h of cell attachment, the cells were incubated with DMEM containing M13-β-CD/Ada-FITC/Ada-RhB for 12 h. Then the media were removed. The cells were washed three times with PBS buffer and observed by a LSM 700 confocal laser scanning microscope imaging system (Carl Zeiss). With excitation at 488 nm, the cells were imaged by collecting fluorescence channel of 510-520 nm (Ada-FITC) and 575-585 nm (Ada-RhB).

1. L. Chen, X. Zhao, Y. Lin, Z. Su and Q. Wang, *Polym. Chem.*, 2014, **5**, 6754-6760.
2. L. Chen, X. Zhao, Y. Lin, Y. Huang and Q. Wang, *Chem. Commun.*, 2013, **49**, 9678-9680.