

Highly Photostable and pH-Sensitive Nanosensors

Zhenzhen Lin ¹, Fang Hu ², Gang He ^{3,*}, Youjun Yang ², Yujun Liao ⁴, Xiao Luo ^{5,*} and Xu-dong Wang ^{1,*}

¹ Department of Chemistry, Human Phenome Institute, Fudan University, Shanghai 200438, China

² Shanghai Frontiers Science Center of Optogenetic Techniques for Cell Metabolism, State Key Laboratory of Bioreactor Engineering, Shanghai Key Laboratory of Chemical Biology, School of Pharmacy, East China University of Science and Technology, Shanghai 200237, China

³ State Key Laboratory for Strength and Vibration of Mechanical Structures, Frontier Institute of Science and Technology, Xi'an Jiaotong University, Shaanxi, Xi'an 710054, China

⁴ Department of Neurosurgery, Huashan Hospital of Fudan University, 12 Middle Wulumuqi Road, Shanghai 200040, China

⁵ Shanghai Engineering Research Center of Molecular Therapeutics and New Drug Development, School of Chemistry and Molecular Engineering, East China Normal University, Dongchuan Road 500, Shanghai 200241, China

* Correspondence: ganghe@mail.xjtu.edu.cn (G.H.); xluo@ecnu.edu.cn (X.L.); wangxudong@fudan.edu.cn (X.W.)

1. Immobilizing pHI Inside mSiNPs

1.1. Method A

CTAB (150 mg), ddH₂O (3.0 mL) and 25 μ L ddH₂O containing 25% *v/v* triethanolamine was added into a 25 mL flask. The reaction was under vigorous stirring and heated up to 60 °C. 30 min later, cyclohexane (0.8 mL) was added. After stabilization for 5 min, TEOS (200 μ L) and pHI-silane (272 μ L) was added dropwise into the mixture and kept under stirring for 2 h. Next, TEOS (40 μ L) was added and kept under stirring for 6 h. The preparation of pHI-silane is same with that in experimental section.

1.2. Method B

CTAB (75 mg), sodium salicylate (16.8 mg), ddH₂O (5 mL) and triethanolamine (12 μ L) was added into a 25 mL flask. The mixture was sonicated for 30 min and then immersed in an oil bath at 80 °C for 1 h. Then, TEOS (250 μ L) and pHI-silane (272 μ L) was added dropwise into the mixture and kept under stirring for 2 h. Next, TEOS (50 μ L) was added and kept under stirring for 8 h. The preparation of pHI-silane is same with that in experimental Section.

Finally, both in method A and B, the mixture was centrifuged at 17,000 g for 10 min and washed with 95% ethanol for five times. The nanoparticles pHI@SiO₂ were redispersed in 95% ethanol at a concentration of 10 mg·mL⁻¹.

2. In Vitro Fluorescence Measurement

The nanoparticles pHI@mSiO₂ were dispersed in 100 mM Britton-Robinson buffer at a concentration of 1.0 mg·mL⁻¹. The excitation wavelength was at 545 nm. The slit widths of excitation and the emission on the spectrometer were set at 10 nm.

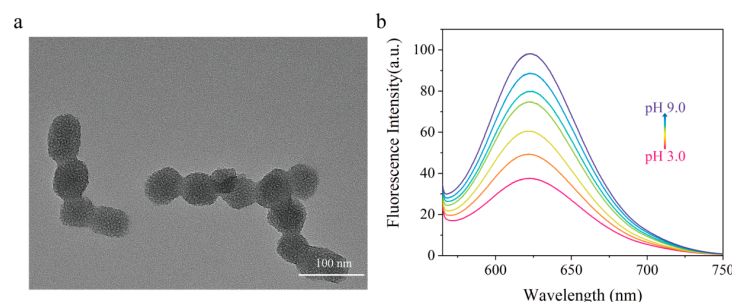


Figure S1. The transmission electron microscopic image and fluorescence spectra of pHI@SiO₂ (prepared by method C—the method used in the manuscript).

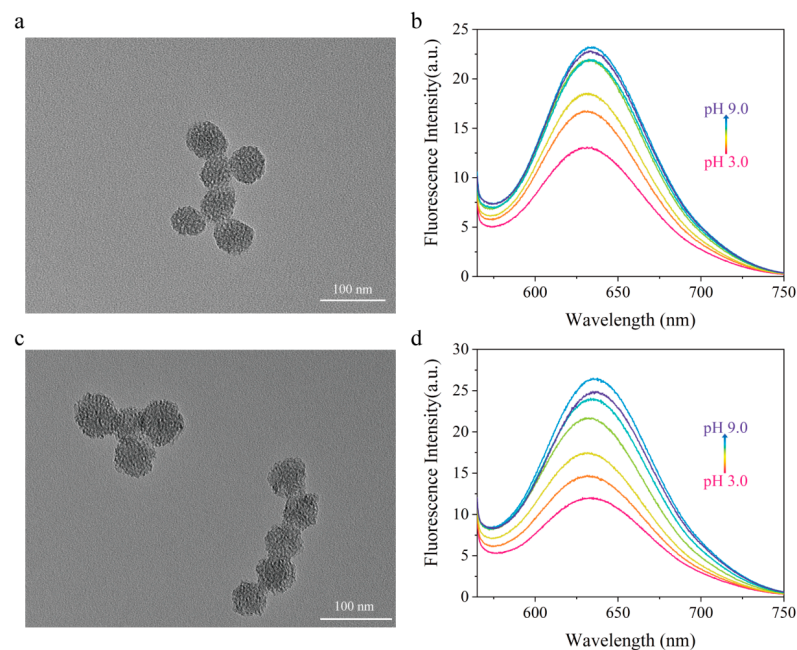


Figure S2. The transmission electron microscopic image and fluorescence spectra of pHI@SiO₂ (a, b prepared by method A; c, d prepared by method B).

Table S1. Optimization of molar ratios of pHI and pHD immobilized in the nanosensors.

Entry	pHI@mSiO ₂	pHD
1	0.15 μ mol	0.20 μ mol
2	0.15 μ mol	0.15 μ mol
3	0.15 μ mol	0.11 μ mol

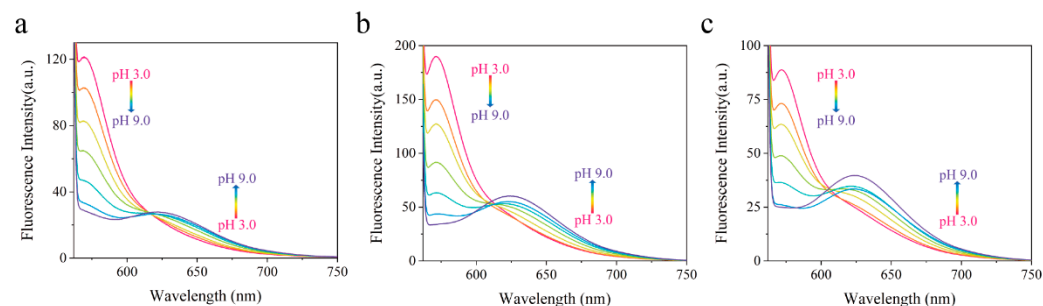


Figure S3. The pH responses of pHI@mSiO₂-pHD synthesized with different molar ratio of pHI and pHD. a) entry 1; b) entry 2; c) entry 3.

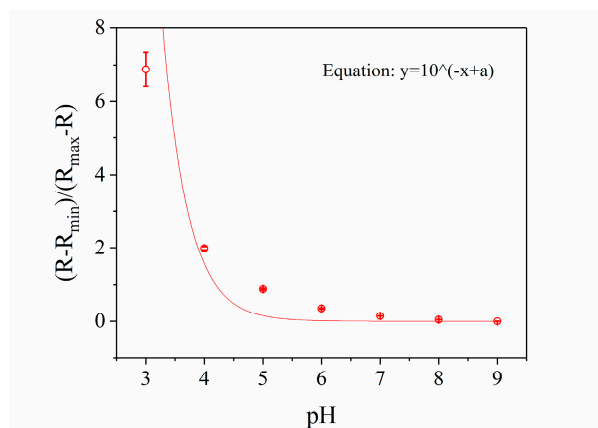


Figure S4. pH-dependent fluorescence intensity ratio $\frac{I - I_{\min}}{I_{\max} - I}$ of the nanosensors, the curve was fitted according to equation 2, which cannot describe the response of the nanosensor.

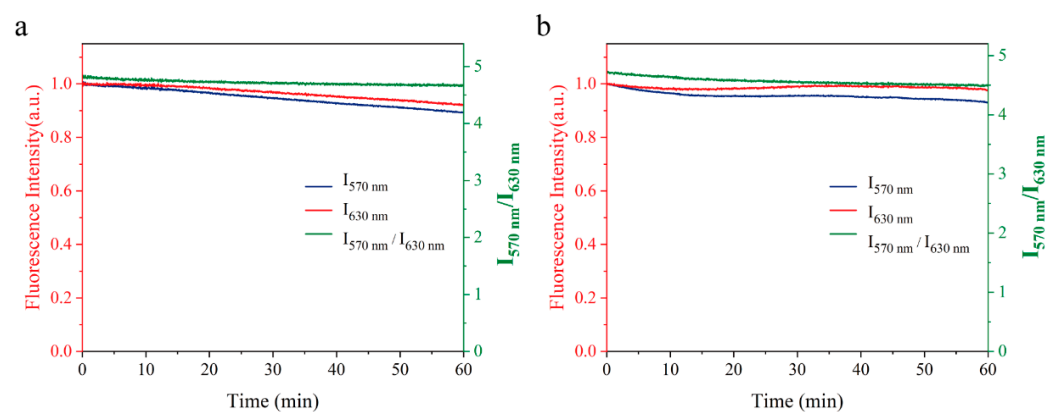


Figure S5. The photostability of the nanosensors in the solution with various ionic strengths. **a)** in BR buffer (50 mM, pH = 3.00). **b)** in BR buffer (200 mM, pH = 3.07).

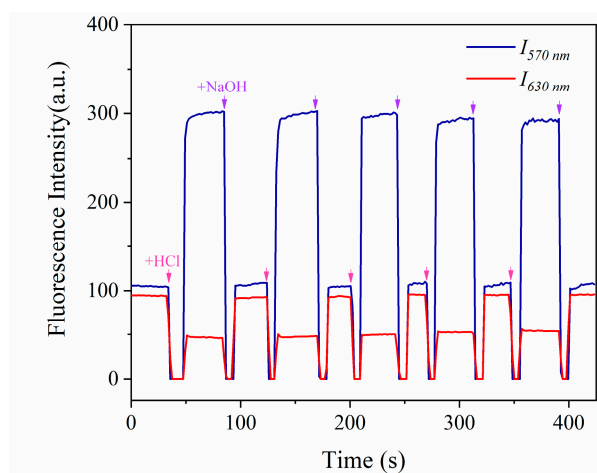


Figure S6. The reversibility of the nanosensors. The solution of nanosensors in continuous sensing of 1 M HCl/NaOH for 5 cycles.

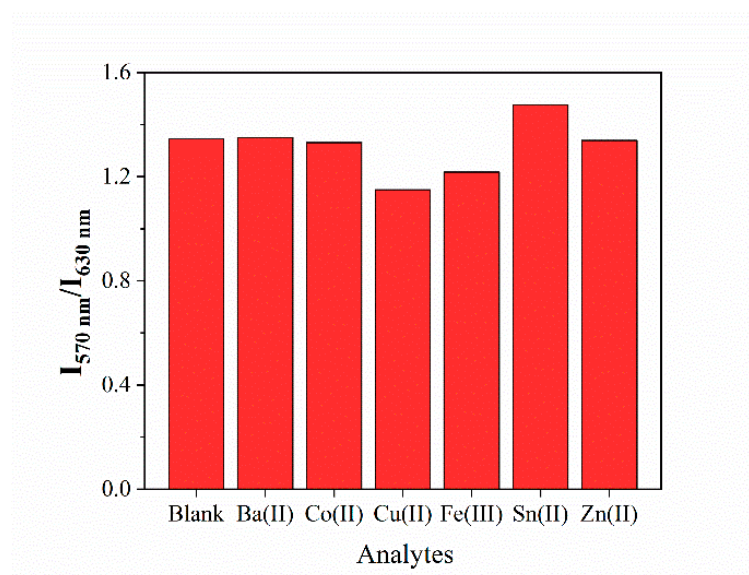


Figure S7. Fluorescence intensity ratio ($I_{570\text{ nm}} / I_{630\text{ nm}}$) of nanosensors in the presence of various heavy metal ions at a concentration of 50 mg·L⁻¹.

^1H NMR (600 MHz, CD_3OD) for pHI.

