This journal is © The Royal Society of Chemistry 2012

Supporting Information

Microstructured fluorescent biosensor based on energy migration for selectively sensing of metalloprotein

Lei Wang,*[a] Yang Liu,[a] Jia-xiang Yang,[b] Xu-tang Tao*[a] and Zhi Liu[a]

*[a] State Key Laboratory of Crystal Materials, Shandong University, Jinan, 250100, P. R. China, Fax: +86 531 88574135; Tel: +86 531 88364963; E-mail: *icmwl@sdu.edu.cn*; *txt@sdu.edu.cn*

[b] Department of Chemistry, Key Laboratory of Inorganic Materials, Anhui University, Hefei 230039, P.R. China

1. Experimental section

Materials: The saline solution of ferritin from equine spleen, the lyophilized powder of myoglobin from equine heart (≥90%), cytochrome c from equine heart (≥95%), lysozyme from chicken egg white (≥90 %,), thrombin from bovine plasma (40-300 NIH units/mg protein), α-chymotrysin from bovine pancreas (≥40 units/mg protein), pepsin from porcine gastric mucosa (3,200-4,500 units/mg protein) and albumin from bovine serum (≥98.0%) were purchased from Sigma-Aldrich and used as received without further purification. THF was HPLC grade and obtained from Merck. Nanopure water was prepared with a Milli-Q Plus water system. Phosphate buffered Saline (PBS, pH 7.4, 10 mM) was used throughout the experiments. DSFO[s1] and 3B2B[s2] were synthesized in our laboratory. Alumina (AAO) membranes with a pore size of 20 nm were bought from Whatman International Ltd.

Preparation of DSFO-doped 3B2B microparticles: Classical reprecipitation method was employed in our work. Typically, a stock solution of 3B2B (2×10^{-3} M) and DSFO (2×10^{-3} M) was prepared in THF, respectively. The solutions then filtered through a 0.22 μm micro filter for further use. A solution (200 μL) of 3B2B and DSFO with the DSFO/3B2B molar ratio (1/50) in THF were mixed homogeneously and then injected quickly into 4 mL PBS with vigorous stirring for 10 minutes, whereupon a yellowish colloid was obtained.

Characterization: Steady-state fluorescence measurements were performed on a Hatachi F-4500 spectrofluorometer using the excitation at 369 nm. For fluorescence studies, aqueous suspension of DSFO-doped 3B2B nano/microparticles (50 μM) was incubated with various concentrations of protein (0-50 μM) in 10 mM phosphate buffer (pH 7.4) and the measurements were taken after 30 minutes. The spectra were recorded using a 375 μL quartz cuvette. The UV-Vis absorption spectra were recorded on a Varian spectrophotometer (cary 50) using quartz cells. Transmission electron microscopy (TEM) characteristic was performed on the JEOL JEM-100CX at an accelerating voltage of 100 kV. Scanning electron microscope (SEM) measurements were performed on a Hitachi S-4800 field emission scanning electron microscope. To enhance the conductivity of the sample, a layer of platinum was sputtered at a current of 15 mA and a pressure of 8 mm Hg. Fluorescence microscopic (FM) images were obtained using an Nikon Eclipse 80i fluorescence microscope equipped with a Nikon Coolpix digital camera excited using UV light. The as-prepared colloidal dispersion was filtered by an AAO membrane and washed with DI water for several times and then the samples on the surface of the AAO membrane were redispersed in water. One drop of dispersion was transferred to the carbon-coated grid by pipetting for the TEM, SEM and FM observation.

2. Results

This journal is © The Royal Society of Chemistry 2012

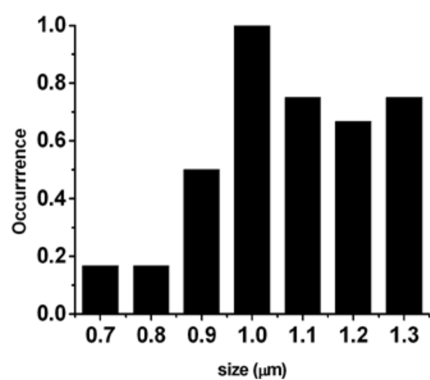

Fig. S1 Histogram plot of particle size obtained from the SEM and TEM images in Fig. 1.

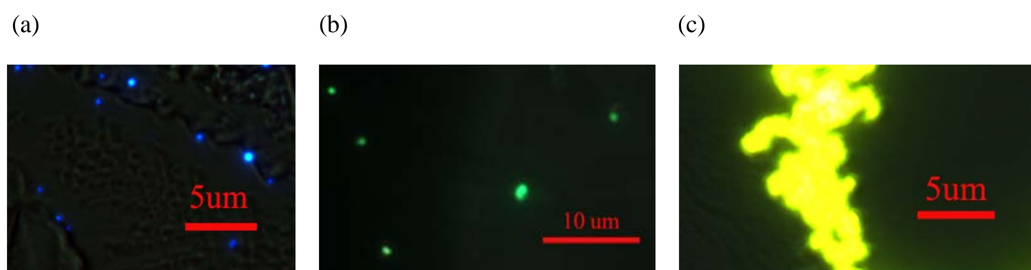

Fig. S2 Fluorescence microscopic images of particles of (a) 3B2B; (b) 2% DSFO-doped 3B2B; (c) DSFO excited with UV light (330-380 nm).

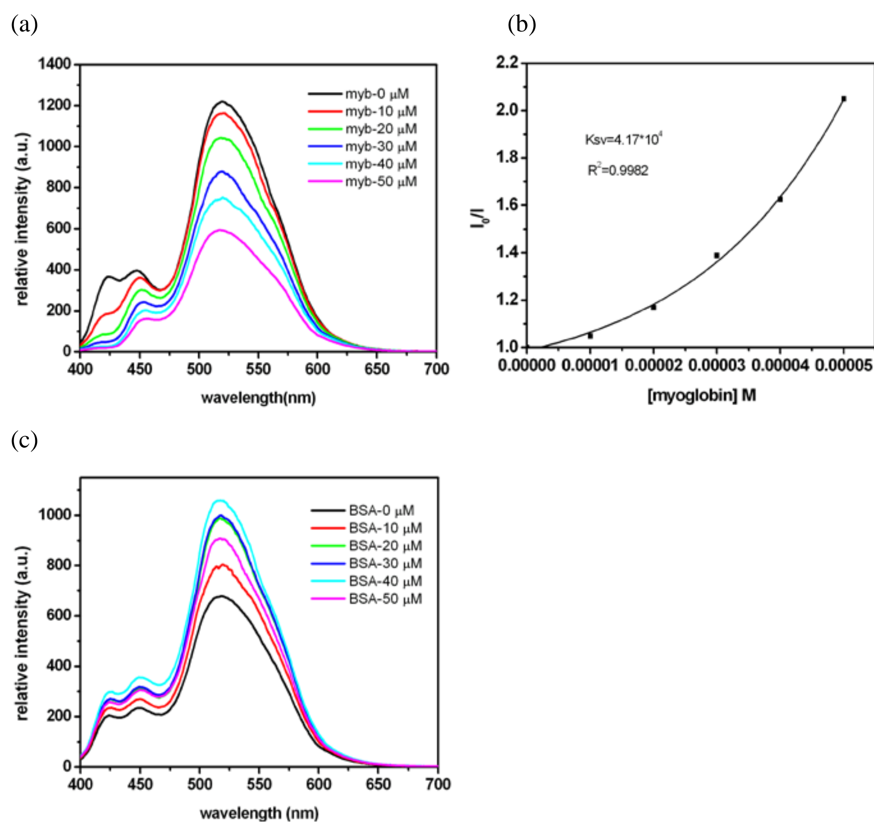

Fig. S3 (a) Fluorescence spectra of the **DMPs** with various concentrations of metalloprotein (myoglobin) in 10 mM PBS (pH 7.4). (b) Corresponding Stern-Volmer plot.[s3] (c) Fluorescence spectra of the **DMPs** with various concentrations of non-metalloprotein (BSA) in 10 mM PBS (pH 7.4).

This journal is © The Royal Society of Chemistry 2012

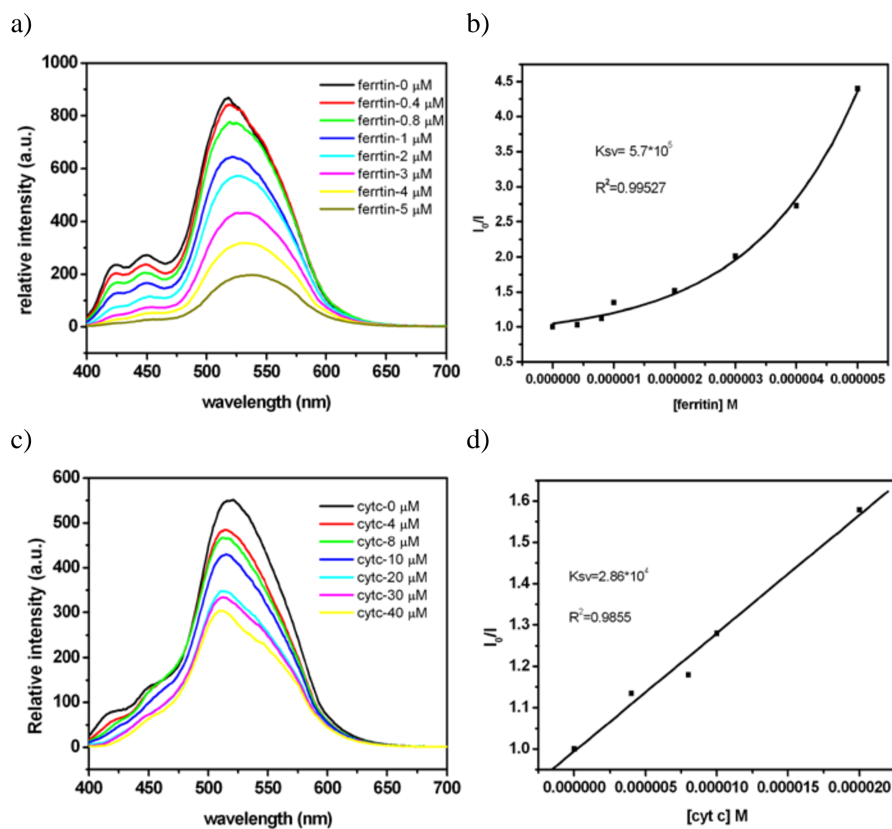

Fig. S4 (a) Fluorescence spectra of the **DMPs** with various concentrations of metalloprotein (ferritin) in 10 mM PBS (pH 7.4). (b) Stern-Volmer plot of the fluorescence spectra shown in a).[s3] (c) Fluorescence spectra of **DMPs** with various concentrations of metalloprotein (cytochrome *c*) in 10 mM PBS (pH 7.4). (d) Stern-Volmer plot of the fluorescence spectra shown in c)

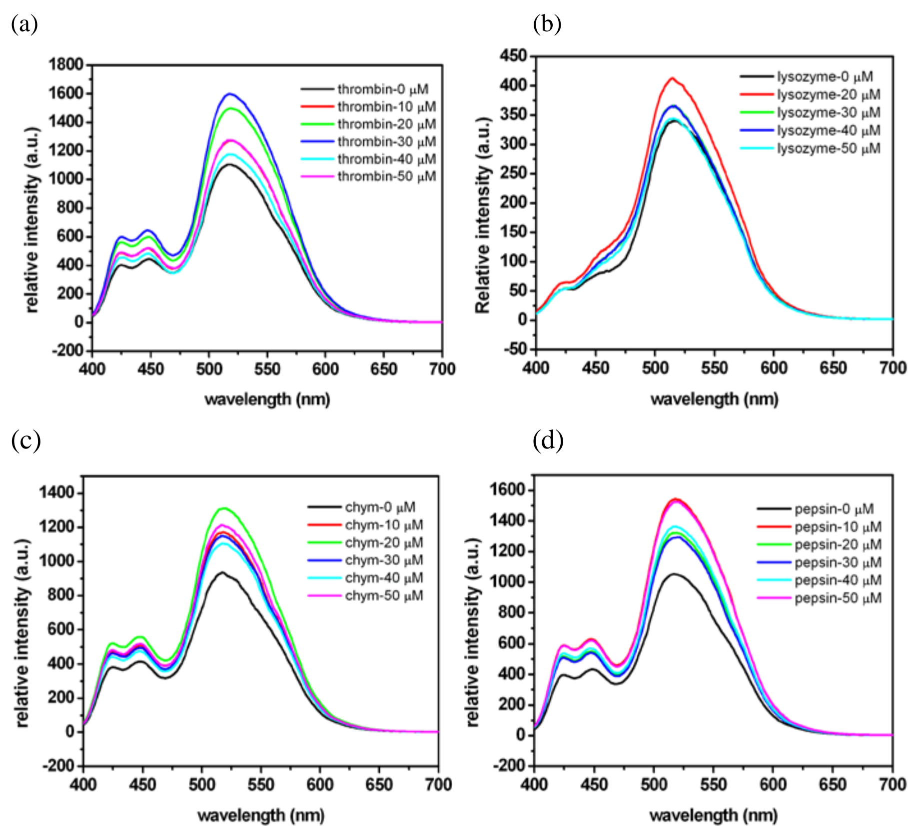

Fig. S5 Fluorescence spectra of the **DMPs** with various concentrations of non-metalloprotein (a) thrombin (b) lysozyme (c) α-chymotrysin (d) pepsin in 10 mM PBS (pH 7.4)

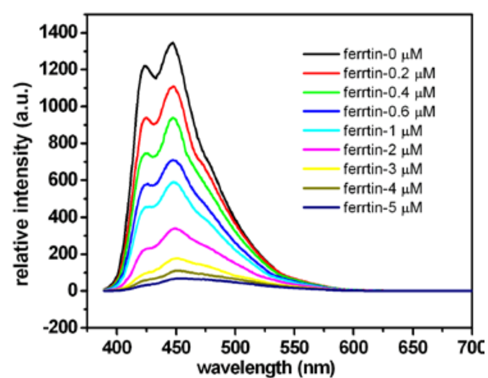

Fig. S6 (a) Fluorescence spectra of the aqueous suspension of pure 3B2B with various concentrations of metalloprotein (ferritin) in 10 mM PBS (pH 7.4).

Reference：

S1 Y. Liu, X. Tao, F. Wang, J. Shi, J. Sun, W. Yu, Y. Ren, D. Zou, M. Jiang, *J. Phys. Chem. C*, 2007, **111**, 6544.

This journal is © The Royal Society of Chemistry 2012

S2 Y. Liu, X. Tao, F. Wang, X. Dang, D. Zou,Y. Ren, M. Jiang *Organic Electronics*, 2009, **10**, 1082.

S3 In the presence of myoglobin and ferritin, the Stern-Volmer plot show superlinear behavior due to the sphere of action mechanism. A linear Stern-Volmer relationship will be observed if either static or dynamic quenching is dominant. Situations in which the two processes are competitive give rise to superlinear Stern-Volmer data. In the DMPs : myoglobin/ferritin (quencher) system, there are always quenchers within a energy or electron transfer distance from one of the DMPs, i.e. there are always quenchers within the sphere-of-action of the DMPs. In this regime, the quenching is expected to increase superlinearly with the quencher concentration.