Electronic Supplementary Information

Fluorescence Resonance Energy Transfer between an Anionic Conjugated Polymer and a Dye-labeled Lysozyme Aptamer for Specific Lysozyme Detection

Experimental Section

FAM labeled lysozyme aptamer was synthesized by Sigma-Genosys. It has a sequence of 5'-FAM-ATC TAC GAA TTC ATC AGG GCT AAA GAG TGC AGA GTT ACT TAG. Lysozyme aptamer was reported to show high affinity to lysozyme with a dissociation constant of 31 nM.[1] Hen egg white lysozyme, BSA, human trypsin and human plasma thrombin were from Sigma-Aldrich.

The lysozyme aptamer (0.5 μM) was first heated at 80°C in 100μL incubation buffer (20mM Tris, 0.1M NaCl, 5mM $MgCl_2$, pH 7.5) for 4min, which was then allowed to slowly cool down to RT.[2, 3] For lysozyme assay, lysozyme of appropriate concentrations was added to the annealed aptamer solution and incubated at RT for 30 minutes. The solution was then transferred into 1 mL of detection buffer (5mM Tris, pH 8.0) with the addition of an appropriate amount of PFP-SO_3Na before FRET measurement. The FRET study was carried out upon excitation of PFP-SO_3Na at 370nm, and the emission spectra were collected between 400-650 nm. Control experiments with lysozyme aptamer and PFP-SO_3Na in the presence of nonspecific proteins including BSA, trypsin and thrombin were carried out under the same conditions. The artificial samples were prepared by adding a certain amount of lysozyme into the fetal bovin serum (FBS), saliva, and urine, respectively. In addition to the common contents such as water and electrolytes, FBS contains various proteins and enzymes as well as peptides, carbohydrates, lipids, growth factors and hormones; saliva contains various enzymes, mucus and antibacterial

compounds; urine contains metabolic wastes such as urea, and organic compounds. FBS was obtained from sigma. FBS was obtained from sigma. Saliva and urine were freshly taken from human. Before use, the media were centrifuged at 12000 rmp for 20 minutes to get the supernatant. For lysozyme detection in biological media, 10μL of the artificial sample was incubated with 0.5 μM preannealed lysozyme aptamer in 100μL of incubation buffer at RT for 30 minutes. The solution was then diluted with 1 mL of detection buffer before the addition of PFP-SO_3Na for FRET measurement. The fluorescence measurement was performed on Perkin-Elmer LS-55 fluorometer equipped with a xenon lamp excitation source and a Hamamatsu (Japan) 928 PMT. The FRET measurement is performed under excitation at 370nm, which is the maximum absorption of PFP-SO_3Na. The excitation beam intensity has been automatically corrected with a correction file that is provided by the instrument supplier. Photographs were taken with an Olympus C-5060 digital camera under excitation of 365nm by a handhold UV lamp.

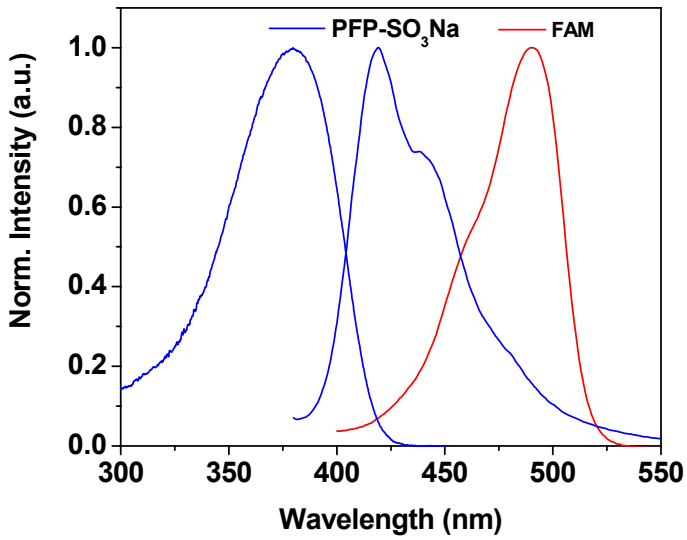

Figure S1. The normalized absorption and emission spectra of PFP-SO_3Na and the absorption spectrum of FAM in 5mM Tris buffer, pH = 7.5.

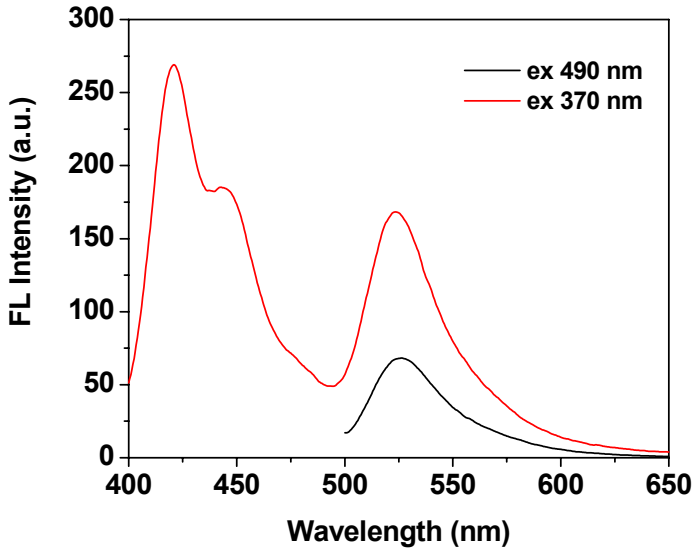

Figure S2. Fluorescence spectra of PFP-SO_3Na/lysozyme aptamer/lysozyme complexes in solution upon excitation at 370 nm (red). Direct excitation of the solution at 490 nm in the absence of PFP-SO_3Na is shown in black. [lysozyme aptamer] = 4.5×10^{-8} M, [lysozyme] = 2.4 μg/mL and [PFP-SO_3Na] = 4.6×10^{-7} M in 5 mM Tris buffer.

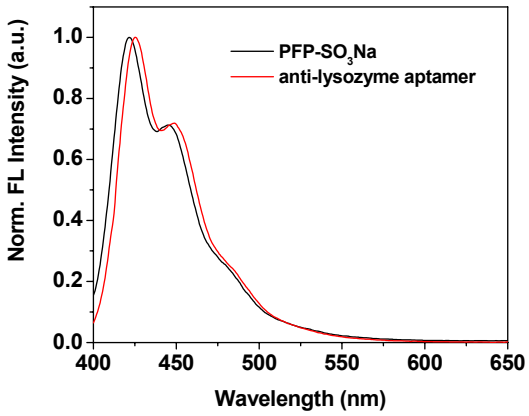

Figure S3. Normalized fluorescence spectra of PFP-SO_3Na, and PFP-SO_3Na with lysozyme aptamer. [lysozyme aptamer] = 4.5×10^{-8} M, [lysozyme] = 2.4 μg/mL and [PFP-SO_3Na] = 4.6×10^{-7} M in 5 mM Tris buffer.

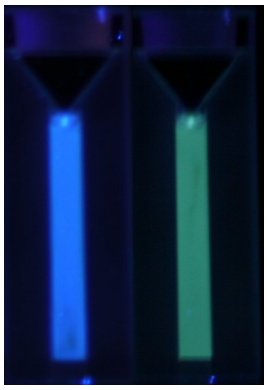

Figure S4. Photographs taken with a handhold UV lamp under 365nm excitation. Left: PFP-SO_3Na with lysozyme aptamer only; Right: PFP-SO_3Na with lysozyme aptamer in the presence of lysozyme. [lysozyme aptamer] = 5.0×10^{-7} M, [lysozyme] = 26.5 μg/mL, [PFP-SO_3Na] = 4.6×10^{-7} M in 5 mM Tris buffer.

Reference

1. J. C. Cox and A. D. Ellington, *Bioorg. Med. Chem.*, 2001, **9**, 2525-2531.
2. A. K. H. Cheng, B. Ge and H. Z. Yu, *Anal. Chem.*, 2007, **79**, 5158-5164.
3. M. C. Rodriguez, A. N. Kawde and J. Wang, *Chem. Commun.*, 2005, 4267-4269.