Electronic Supporting Information

Fluorescence turn-on detection of a protein using cytochrome *c* as a quencher

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Experimental Section

Materials

The FAM fluorophore labeled lysozyme aptamer (FAM-aptamer: 5′-FAM-TTC ATC AGG GCT AAA GAG TGC AGA GTT ACT TAG-3′) and the FAM fluorophore labeled PDGF aptamer (5′-FAM-AGG CTA CGG CAC GTA GAG CAT CAC CAT GAT CCT-3′) were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Cytochrome *c* (cyt *c*), horseradish peroxidase (HRP), bovine serum albumin (BSA) were purchased from Bio Basic Inc. (BBI, Markham, Canada). Lysozyme and thrombin were purchased from Beijing Dingguo Biotechnology Co., Ltd (Beijing, China). Hemoglobin and platelet-derived growth factor (PDGF) were purchased from Worthington Biochemical Corporation (Lakewood, USA). Protamine (from salmon) was purchased from Sangon Biotech Co., Ltd. (Shanghai, China). S1 nuclease was purchased from Fermentas Inc. (MBI, Canada). Other reagents were all of analytical grade and used without further purification. Water was doubly distilled and purified by a Milli-Q system (Millipore, Billerica, MA, USA).

Instrumentation

Fluorescence experiments were carried out on a Fluoromax-4 spectrofluorometer (Horiba Jobin Yvon Inc., USA). Sample solutions were excited at 490 nm and fluorescence emission spectra were recorded with slits for excitation and emission both of 2.5 nm. All emission spectra were collected at an ambient temperature of 22 ^{o}C.

Assay conditions

Lysozyme detection: 5 mM Tirs-HCl (pH 9.0), 20 nM FAM-aptamer, 80 nM cyt *c*, and different concentrations of lysozyme. Final sample volume: 2 mL. Assay conditions for the selectivity study: FAM-aptamer: 20 nM; cyt *c*: 80 nM; proteins: 100 nM each; buffer: 5 mM Tris-HCl (pH 9.0).

PDGF detection: 5 mM Tirs-HCl (pH 9.0), 20 nM of the FAM labeled PDGF aptamer, 200 nM cyt *c*, and different concentrations of PDGF. Final sample volume: 400 μL. Assay conditions for the selectivity study: FAM labeled PDGF aptamer: 20 nM; cyt *c*: 2 μM; proteins: 50 nM each; buffer: 5 mM Tris-HCl (pH 9.0). A higher concentration of cyt *c* was used to suppress the nonspecific binding of lysozyme and protamine.

S1 nuclease digestion: 2 mM HAc-Ac (pH 4.6), 100 nM Zn^{2+}, 400 nM FAM-aptamer, 20 U S1 nuclease. Final sample volume: 500 μL. Reaction temperature: 37 ºC. Reaction time: 1 h. S1 nuclease inactivation conditions: at 85 ºC for 10 min.

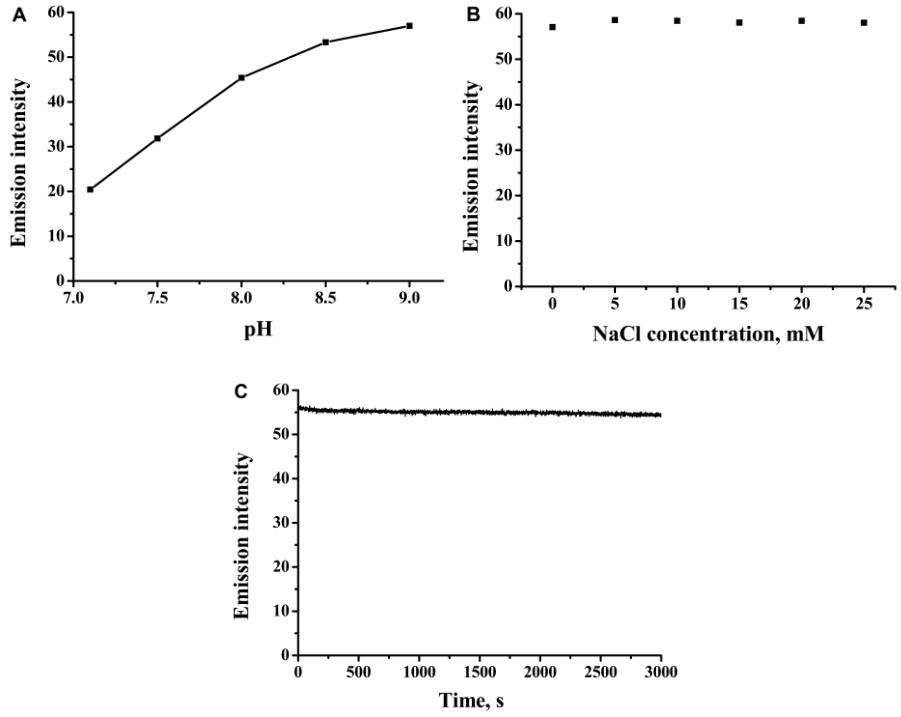

Figure S1. Emission intensity changes of the FAM-aptamer at 515 nm as a function of the assay solution pH value (**A**), NaCl concentration (**B**), or incubation time (**C**). Conditions: FAM-aptamer: 20 nM; (**B**) and (**C**): in 5 mM Tris-HCl buffer (pH 9.0).

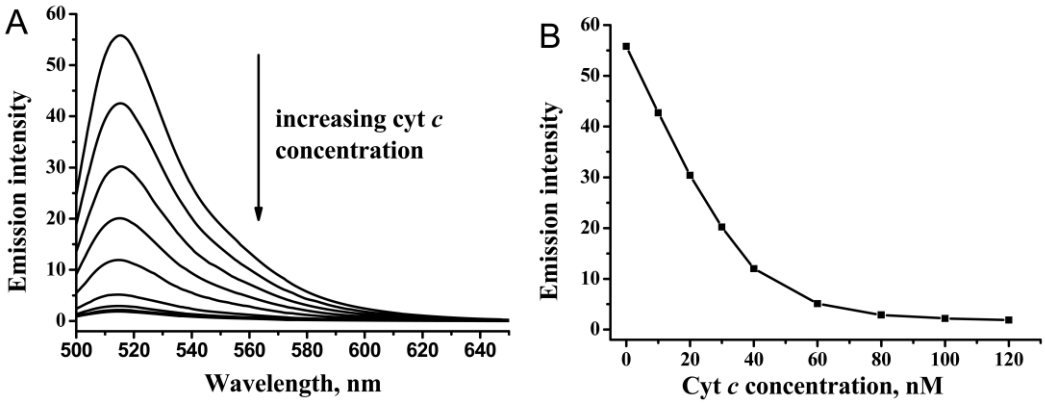

Figure S2. Changes in emission spectrum (**A**) and emission intensity at 515 nm (**B**) of the FAM-aptamer as a function of cyt *c* concentration. FAM-aptamer: 20 nM; buffer: 5 mM Tris-HCl (pH 9.0).

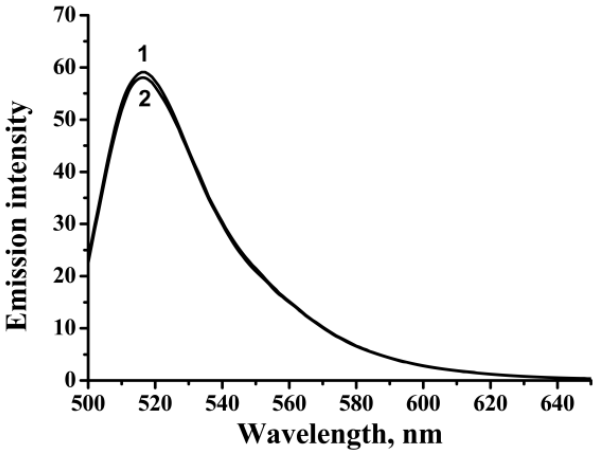

Figure S3. The emission spectra of the FAM-aptamer digested with S1 nuclease (curve **1**), and the digested FAM-aptamer mixed with cyt *c* (curve **2**). FAM-aptamer: 20 nM; cyt *c*: 80 nM; buffer: 5 mM Tris-HCl (pH 9.0).

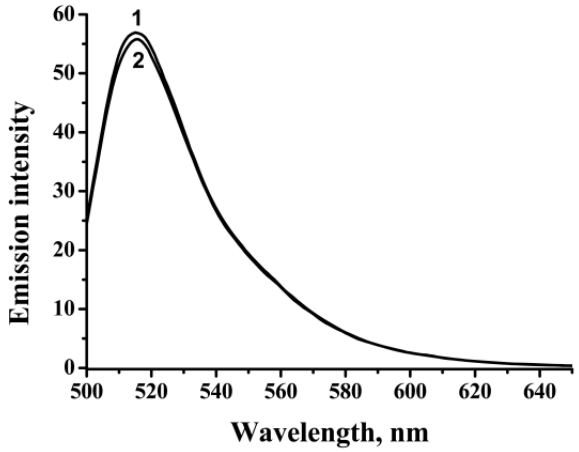

Figure S4. The emission spectra of the FAM-aptamer (curve **1**), and the FAM-aptamer mixed with hemoglobin (curve **2**). FAM-aptamer: 20 nM; hemoglobin: 100 nM; buffer: 5 mM Tris-HCl (pH 9.0).

Our results show that bovine serum albumin (BSA), horseradish peroxidase (HRP), and thrombin show little quenching of the FAM-aptamer fluorescence. Quenching of the FAM fluorescence requires binding between the nucleic acid aptamer and the

protein, and the electron transfer between the fluorophore (FAM) and the quencher (the heme cofactor of cyt *c*). BSA and thrombin do not contain a quencher cofactor, regardless of whether the protein binds to the aptamer, these proteins would not quench the FAM fluorophore. Hemoglobin and HRP are heme-containing proteins. However, they are negatively charged proteins (pI values: 6.8 and 7.2, respectively, Ref. 8b and *J. Biochem. Tech.* 2009, **1**, 92-95) under our experimental conditions. Our results suggest that their binding to the FAM-aptamer is weak, thus the quenching effect is also quite weak.

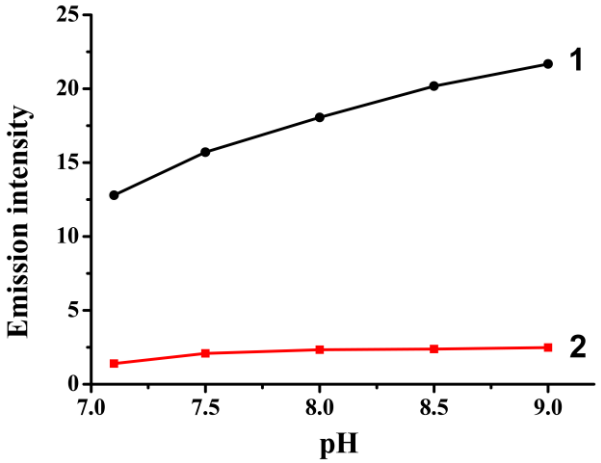

Figure S5. Changes in emission intensity of the assay mixtures of the FAM-aptamer and cyt *c* at 524 nm with (curve **1**) and without (curve **2**) the addition of lysozyme at different buffer pH values. FAM-aptamer: 20 nM; cyt *c*: 80 nM; lysozyme: 100 nM; buffer: 5 mM Tris-HCl.

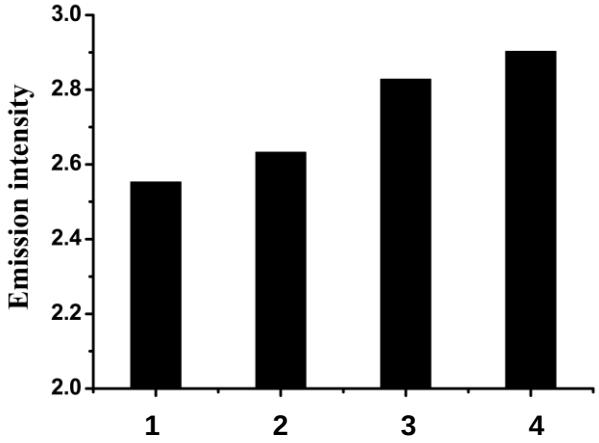

Figure S6. Changes in emission intensity at 524 nm upon the addition of increasing concentrations of lysozyme to the assay mixture of the FAM-aptamer and cyt *c*. Columns 1-4: 0, 0.2, 0.5, 1.0 nM lysozyme, respectively. FAM-aptamer: 20 nM; cyt *c*: 80 nM; buffer: 5 mM Tris-HCl (pH 9.0).

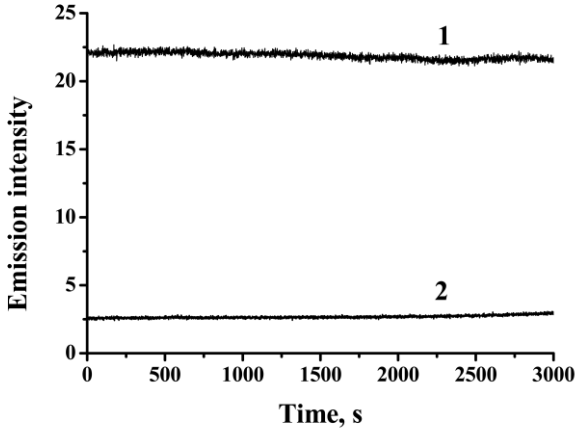

Figure S7. Real-time emission intensity changes at 524 nm of the mixtures of the FAM-aptamer and cyt *c* with (curve **1**) and without (curve **2**) the addition of lysozyme. FAM-aptamer: 20 nM; cyt *c*: 80 nM; lysozyme: 100 nM; buffer: 5 mM Tris-HCl (pH 9.0).

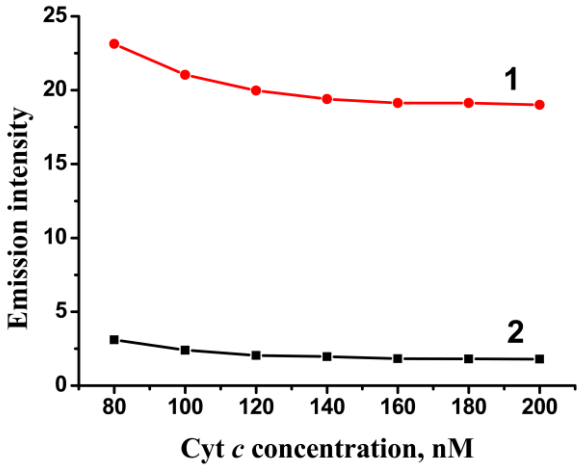

Figure S8. Changes in emission intensity of the FAM-aptamer at 524 nm at different cyt *c* concentrations. Curve **1**: with the addition of lysozyme; Curve **2**: without the addition of lysozyme. FAM-aptamer: 20 nM; lysozyme: 100 nM; buffer: 5 mM Tris-HCl (pH 9.0). The results show some decrease of the FAM fluorescence recovery upon the addition of increasing concentrations of cyt *c*. However, the influence is quite moderate.