Supporting information

Affinity Binding-Induced Hg^{2+} Releasing and Quantum Dot Doping for General, Label-Free, and Homogenous Fluorescence Protein Assay

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Table S1. The used nucleic acid base sequences in the experiment[a]

Name	Sequence (5′ to 3′)
Motif A for anti-Dig antibody	Dig-AAAAAAAAAAAAAAA**GCGTCTTGACGC**
Blocking strand B	**GCGTCTTGACGC**
Motif C for anti-Dig antibody	**GCGTCAAGACGC**AAAAAAAAAAAAAAA-Dig
Strand A with no Dig label	AAAAAAAAAAAAAAA**GCGTCTTGACGC**
Strand C with no Dig label	**GCGTCAAGACGC**AAAAAAAAAAAAAAA
Motif A for streptavidin	Biotin-AAAAAAAAAAAAAAA**GCGTCTTGACGC**
Motif C for streptavidin	**GCGTCAAGACGC**AAAAAAAAAAAAAAA-Biotin
Motif A for thrombin	*AGTCCGTGGTAGGGCAGGTTGGGGTGACT*AAAAAAA AAAAAAAA**GCGTCTTGACGC**
Motif C for thrombin	**GCGTCAAGACGC**AAAAAAAAAAAAAAA*GGTT GGTGTGGTTGG*

[a]The underlined letters in motif A and blocking strand B show two T-T mismatches. The bold letters in motif A and C indicates mutual base complementarities. The italic letters in motif A and C (for thrombin) represents the corresponding aptamer sequences.

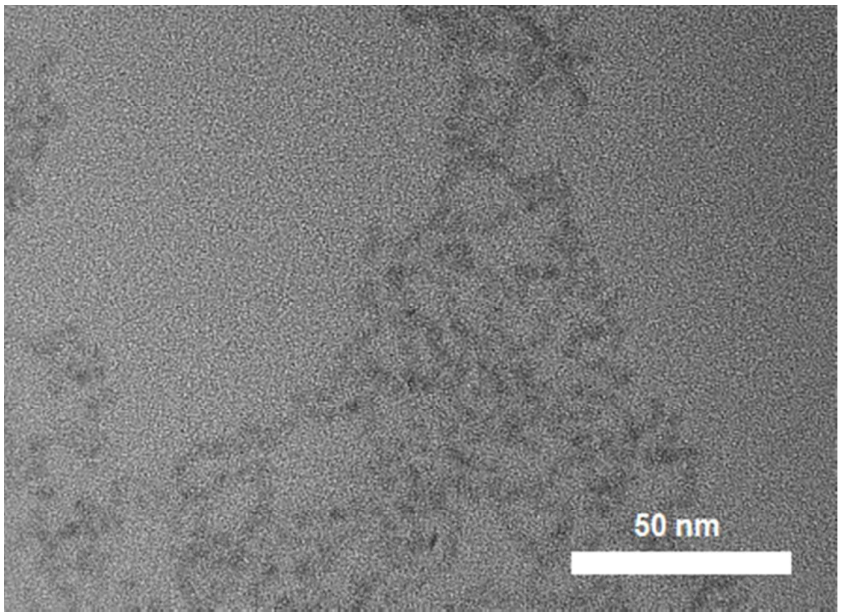

Figure S1. A large scale TEM images of synthesized ZnSe QD

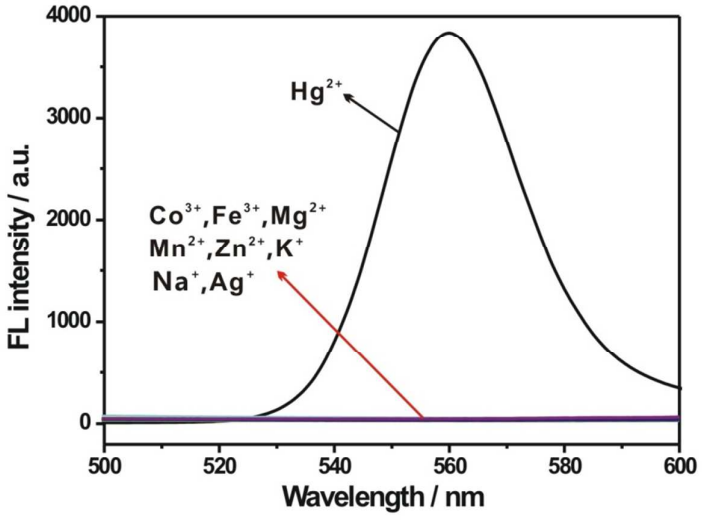

Figure S2. Fluorescence spectra of ZnSe QD mixed with different metal ions. The Hg^{2+} concentration is 20 nM and all other metal ions have a same concentration of 1 μM.

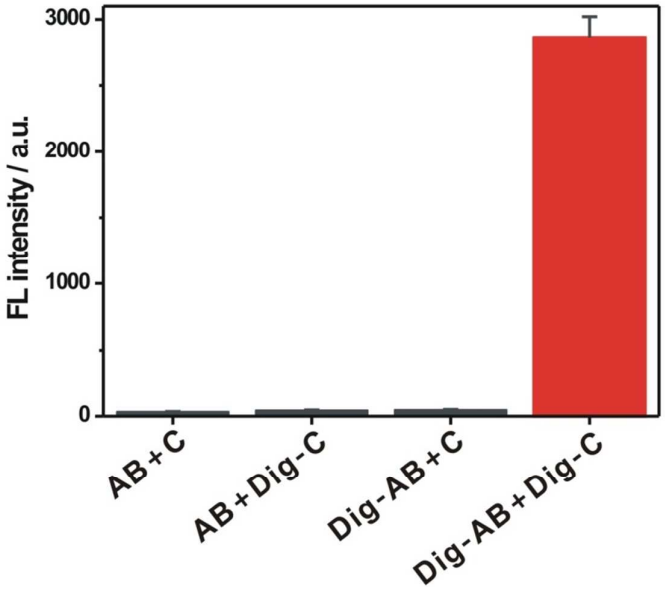

Figure S3. Fluorescence responses toward 10 nM anti-Dig antibody by using motif A and C that were conjugated with or no Dig ligands. Error bars show standard deviations of three repetitive measurements.

Table S2. The detection performance comparison toward anti-Dig antibody by current method with these reported methods

Method	Detection limit	Dynamic range	Strategy	Ref.
Electrochemistry	1 nM	1 ~100 nM	DNA-based electrochemical switch	1
Fluorescence	1 nM	1 ~ 20 nM	DNA-based strand displacement competition reaction	2
Electrochemistry	5 nM	0 ~ 20 nM	Redox-tagged oligonucleotide probes	3
Fluorescence	5.6 nM	10 ~ 125 nM	Steric hindrance inhibition of strand displacement	4
Electrochemistry	0.1 pM	0.1 pM ~ 0.1 nM	DNAzyme migration behavior and gold nanoparticle amplification	5
Fluorescence	1 nM	0 ~ 20 nM	Assembly of an RNA mimic of green fluorescent protein	6
Electrochemilum inescence	0.72 nM	1 ~ 100 nM	Steric hindrance inhibition of electron transfer	7
Fluorescence	0.33 nM	0 ~13.3 nM	Binding-induced cascade dissociation of kissing Complex	8
Nanopore sensor	0.5 nM	0 ~ 50 nM	Triplex molecular beacon	9
Electrochemistry	0.67 nM	1 ~ 25 nM	DNA-mediated strand displacement	10
Colorimetry	0.039 nM	0.5 nM ~ 1 μM	DNAzyme-based conformational switching	11
Fluorescence	0.034 nM	0 ~ 5 nM	Affinity binding-induced Hg^{2+} releasing and Quantum dot doping	This work

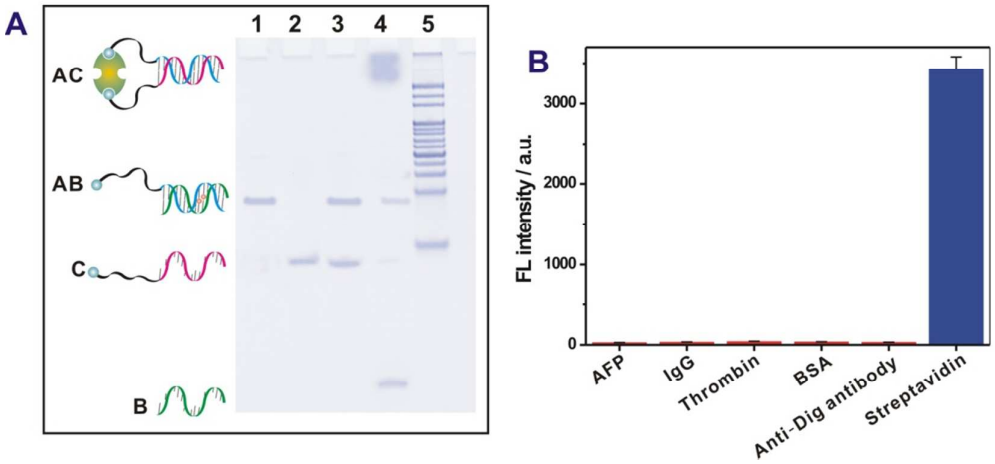

Figure S4. (A) Native PAGE characterization toward streptavidin binding-induced DNA strand displacement reaction: lane 1, 2 μM duplex AB; lane 2, 2 μM motif C; lane 3, 2 μM duplex AB and 2 μM motif C; lane 4, a mixture containing 2 μM duplex AB, 2 μM motif C, and 1 μM streptavidin; lane 5, low molecular DNA ladder. (B) Selectivity test of the fabricated protein biosensor for streptavidin against other different proteins including AFP, IgG, thrombin, BSA, and anti-Dig antibody. Each tested protein has a concentration of 10 nM

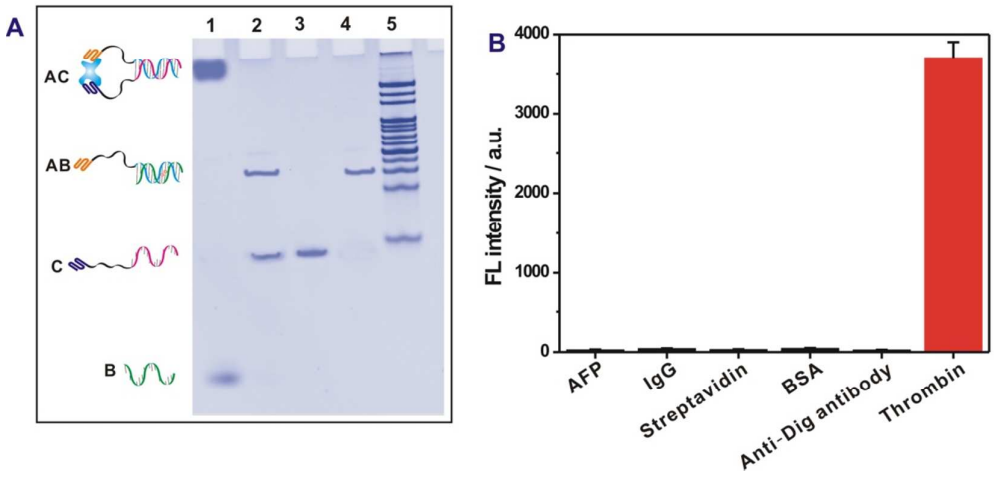

Figure S5. (A) Native PAGE characterization toward thrombin binding-induced DNA strand displacement reaction: lane 1, a mixture containing 2 μM duplex AB, 2 μM motif C, and 1 μM thrombin; lane 2, 2 μM duplex AB and 2 μM motif C; lane 3, 2 μM motif C; lane 4, 2 μM duplex AB; lane 5, low molecular DNA ladder. (B) Selectivity test of the fabricated protein biosensor for thrombin against other different proteins including AFP, IgG, streptavidin, BSA, and anti-Dig antibody. Each tested protein has a concentration of 10 nM

Table S3. The detection performance comparison toward thrombin by currently developed biosensor with some reported methods

Method	Detection limit	Strategy	Ref.
Fluorescence	0.2 nM	Nanomaterial-based amplification	12
Fluorescence	0.18 nM	Upconverting phosphors-carbon nanoparticles FRET	13
Fluorescence	2.5 nM	AuNPs-based fluorescence enhancement of dye	14
Fluorescence	1.1 nM	SYBR Green I dye	15
Fluorescence	0.3 nM	DNA–MoS_2 nanosheet	16
Fluorescence	0.15 nM	Bovine serum albumin-capped CdS quantum dots	17
Fluorescence	0.76 nM	$[Ru(bpy)_2(o\text{-}mopip)]^{2+}$ and graphene oxide	18
Fluorescence	1 nM	Aptamer-functionalized silver nanocluster DNA probes	19
Fluorescence	0.1 nM	Catalytic DNA hairpin assemblies	20
Fluorescence	0.0313 nM	Graphene fluorescence resonance energy transfer	21
Fluorescence	0.01 nM	nicking enzyme assisted signal amplification	22
Fluorescence	0.025 nM	Affinity binding-induced Hg^{2+} releasing and Quantum dot doping	This work

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