Electronic Supplementary Information (ESI) for

Detection of mixed organophosphorus pesticides in real samples using quantum dots/bi-enzyme assembly multilayers

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Part S1 Preparation and characterization of the proposed biosensor

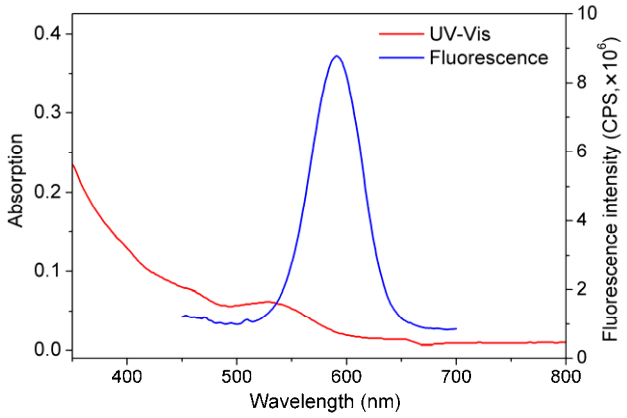

Fig. S1 UV-vis spectra (red line) and fluorescence spectra (blue line) of $(PAH/QDs)_8(PAH/PSS)_3(PAH/ChO_x)_6(PAH/AChE)_3$ multilayer.

Part S2 Biosensing mechanism of QDs/ChOx/AChE multilayer

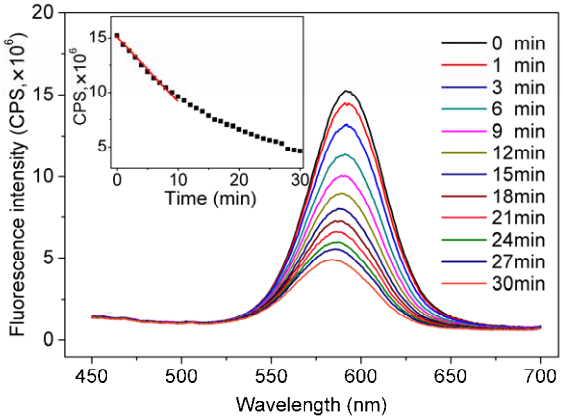

Fig. S2 Time-dependent fluorescence changes upon the interaction of 8 bilayers of PAH/CdTe QDs in the absence and presence of 0.02 mM H_2O_2 at room temperature.

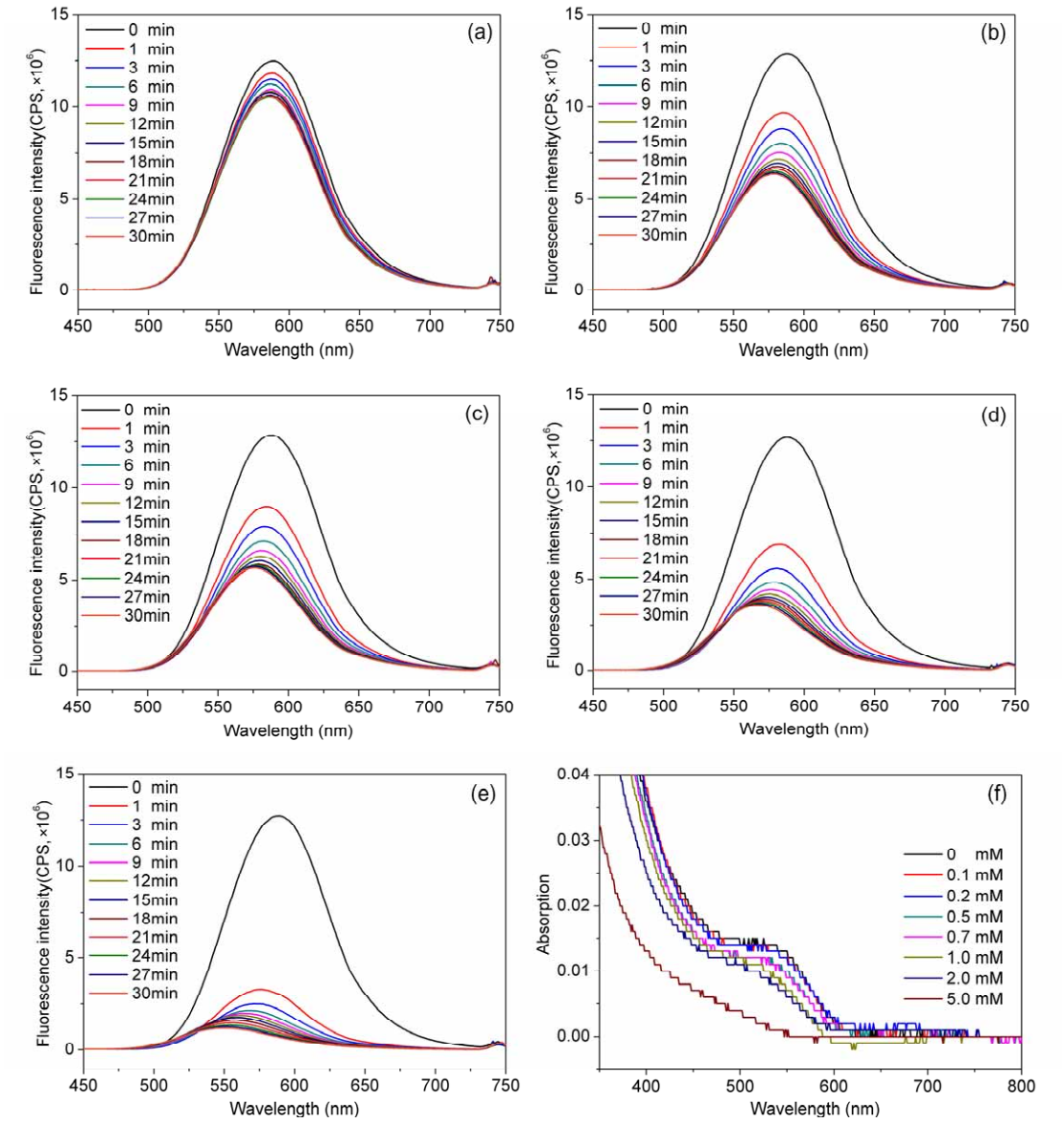

Fig. S3 Time-dependent fluorescence changes upon the interaction of CdTe QD solution in the absence and presence of (a) 0.1 mM, (b) 0.5 mM, (c) 1.0 mM, (d) 2.0 mM, and (e) 5.0 mM H_2O_2. (f) UV-vis spectra of CdTe QD solution after interacting with different concentration of H_2O_2 at room temperature. All measurements were performed in a 20 mM phosphate buffer solution, pH = 8.0. The samples were excited at 380 nm, and the exciting slit and the emission slit were both 5 nm, respectively.

Part S3 Optimal AChE and ChOx immobilizing conditions

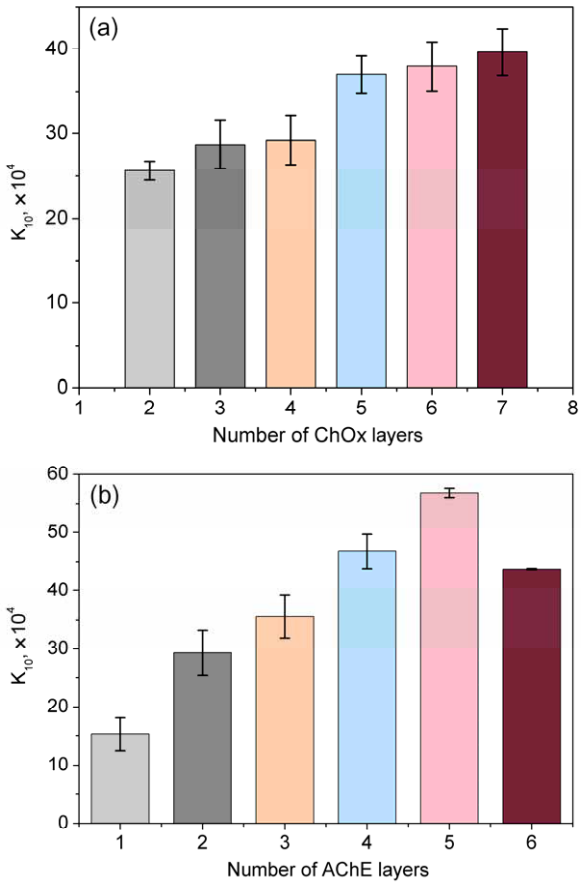

Fig. S4 (a) Absolute quenching rate of the fluorescence intensity at 592 nm within 10 min as a function of number of ChOx layers. All measurements were performed in a 20 mM phosphate buffer solution, pH = 8.0, and 4 mM choline. (b) Absolute quenching rate of the fluorescence intensity at 592 nm within 10 min as a function of number of AChE layers on the top of $(PAH/QDs)_8(PAH/PSS)_3(PAH/ChOx)_6$. All measurements were performed in a 20 mM phosphate buffer solution, pH = 8.0, and 2 mM acetylcholine.

Part S4 Stability of the proposed biosensor

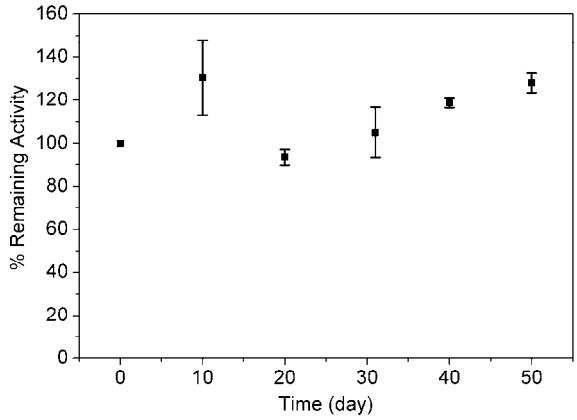

Fig. S5 Stability of the biosensor.

Immobilization of AChE into polyelectrolyte multilayer can effectively stabilize enzymes against unfolding forces. This effect can be observed by testing the long term stability of the sensors. A stock of $(PAH/CdTe)_8(PAH/PSS)_3(PAH/ChOx)_3(PAH/AChE)_3$ multilayers were prepared and stored at -20 ºC. At certain time intervals, the samples were taken out and the remaining activity of enzyme was analyzed under the optimal conditions. Remain enzyme activity (%) is determined from the below equation:

$$\text{Remain enzyme activity (\%)} = K_{10\ \text{stored}}/K_{10\ \text{freshly}} \times 100\% \quad \text{(S1)}$$

Where, $K_{10\ \text{stored}}$ and $K_{10\ \text{freshly}}$ are the absolute quenching rate of the biosensor stored for defined time and the freshly prepared biosensor, respectively. As shown in Fig. S5, all the immobilized enzymes into polyelectrolyte multilayers remained their bioactivity and the response of the biosensor decreased by only 10% compared with the initial response after 1 month of storage. An even slight increase of the AChE activity at the initiate storage might be attributed to the changes of the enzyme's three-dimensional structure inside multilayers, which were already observed in other biosensor systems. So it is safely concluded that polyelectrolyte multilayer provides an efficient micro-environment for enzyme stabilization, which will benefit the applications of many types of biosensors.

Part S5 Comparing the performance of the proposed biosensor with other AChE-based sensors

Table S1 Comparison of the proposed biosensor and other AChE-based sensors for LOD.

Sensor(enzyme)[a]	Signal substrate	Transduction technology	Application (analyte/detection limit)	Ref.
ChOx (A. sp.) and AChE (E. eel) immobilized on electrode	H_2O_2	electrochemical	aldicarb: 60 pM	2002[1]
AChE (hs) immobilized in sol-gel	bromcresol purple	absorption spectra/fiber-optic	dichlorvos: 23.53 nM	2002[2]
Free AChE (E. eel)	2-butyl-6-(4-methyl-piperazin-1-yl)-benzo[de]isoquinoline-1,3-dione)	fluorescence	dichlorvos: 113.13 nM, paraoxon: 43.60 nM	2004[3]
AChE (E. eel) modified thick film strip electrode	thiocholine	electrochemical	paraoxon: 0.5 nM	2005[4]
AChE (D. m) immobilized in liposome	pyranine	fluorescence	dichlorvos: 0.20 nM (I25) paraoxon: 0.67 nM (I25)	2007[5]
AChE (E. eel) immobilized on electrode	thiocholine	electrochemical	paraoxon: 0.1 nM	2010[6]
AChE (E. eel) immobilized on electrode	thiocholine	electrochemical	dichlorvos: 11.31 pM	2010[7]
Mutants AChE (N. b) on gold disposable electrochemical printed (DEP) chips	thiocholine	electrochemical	paraoxon: 36 nM (I20)	2010[8]
AChE (E. eel) and ChOx (A. sp.) multilayers on glass	H_2O_2	fluorescence	Paraoxon:2.75 pM Dichlorvos: 2.09 pM Parathion: 4.82 pM	This work

[a] AChE (E. eel) is AChE from Electrophorus electricus. AChE (hs) is AChE from horse serum. AChE (D. m) is AChE from Drosophila melanogaster. Mutants AChE (N. b) is AChE from Nippostrongylus brasiliensis. ChOx (A. sp.) is ChOx from Alcaligenes sp..

Table S2 Comparison of this study and other AChE-based sensors for detecting real samples.

Sensor (enzyme)[a]	Signal molecular	Readout (technology)	Application (analyte)[b]	Recovery (%)	Ref.
AChE (E. eel) immobilized on electrode	thiocholine	electrochemical	carbaryl in peach (5 ppm), orange (7 ppm), carrot (0.5 ppm); propoxur in sweet pepper (3 ppm)	109 ± 4.0 116 ± 8.7 125 ± 2.3 122 ± 10.4	1999[9]
AChE (be) immobilized on polyurethane foam	DTNB	thermal lens spectrometry/ flow-injection analysis (FIA)	paraoxon in tap water (10 ppm), orange juice (10 ppm), apple juice(10 ppm)	76 ± 0.08 60 ± 0.1 51 ± 0.05	1999[10]
AChE (E. eel) immobilized on electrode	thiocholine	electrochemical	paraoxon in infant food (5 ppm)	104%	2002[11]
AChE (hs) immobilized in sol-gel	bromcresol purple	absorption spectra/ fiber-optic	dichlorvos in ground water (10 ppb); No data for fruits or vegetables	91.5 ± 3.8	2002[2]
AChE (D. m) immobilized in liposome	pyranine	fluorescence	dichlorvos in water (0.221 ppb); No data for fruits or vegetables	detection limit	2007[5]
AChE (bs) immobilized on filter paper	DTNB	colorchange/ naked eyed	diazinon oxon in apple juice (0.1 ppm) (extracted with hexane)	detection limit	2007[12]
AChE (E. eel) and ChOx (A. sp.) multilayers on glass	QDs	fluorescence	dichlorvos in apple (22 ppb, 2.2 ppb); paraoxon in apple (22.67 ppb, 2.27 ppb)	105.78 ± 2.08 106.52 ± 4.01 97.77 ± 6.67 103.01 ± 9.58	This work

[a] DTNB is abbreviation of 5,5%-dithio-bis(2-nitrobenzoic acid). AChE (E. eel) is AChE from Electrophorus electricus. AChE (be) is AChE from bovine erythrocytes. AChE (hs) is AChE from horse serum. AChE (D. m) is AChE from Drosophila melanogaster. AChE (bs) is AChE from bovine serum. ChOx (A. sp.) is ChOx from Alcaligenes sp..

[b] ppm: $mg \cdot L^{-1}$, ppb: $\mu g \cdot L^{-1}$, ppt: $ng \cdot L^{-1}$.

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