Supporting Information

Fluorescence Probe Techniques to Study the Interaction between Hydroxylated Polybrominated Diphenyl Ethers (OH-PBDEs) and Protein Disulfide Isomerase (PDI)

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Protein disulfide isomerase (PDI) from bovine liver was purchased from SIGMA (St. Louis, MO). All hydroxylated PBDEs, including 4-OH-2,2',3,4'-BDE(4-OH-BDE-42), 6-OH-2,2',4,4'-BDE(6-OH-BDE-47), 2-OH-2,4,4'-BDE(2-OH-BDE-28), 6'-OH-2,2',4,4',5-BDE (6'-OH-BDE-99), 3-OH-2,4,-BDE(3-OH-BDE-7), were purchased from AccuStandard (New Haven, CT, USA). Bisphenol A (BPA), 3, 3', 5-triiodo-thyronine (T3), fluorescein isothiocyanate (FITC) were purchased from Sangon Inc. (Shanghai, China). Other chemicals employed were analytical reagent grade. Double distilled water (Milli-Q, Millipore, resistance 18.2 M U) was used throughout the experiments. All the OH-PBDEs were dissolved in acetonitrile. The structures of some chemicals used were listed in Fig. S1.

The fluorescence conjugate F-T3 was synthesized according to the previously reported method.[s1,s2] Briefly, One volume 20mg/mL FITC was reacted with two volumes 20mg/mL T3 in a triethylamine / water /pyridine medium (0.1:1.5:9, v/v/v) for 1 h at room temperature. Crude products were then precipitated with 20 volumes 0.2M ammonium acetate buffer (pH 4.0), and then collected by centrifugation (10 min, 1000 g). The suspension was discarded and the precipitate was washed by suspending in 20 volumes distilled water followed by centrifugation as before. Then the product was redissolved in 8 volumes NH_4HCO_3 (0.05M), if necessary, a small amount of ammonia solution will help. Then aliquots (0.5mL) were applied to a Sephadex G-75 column (3×30cm) equilibrated with NH_4HCO_3 (0.05M, pH 8.5). The impurities were removed from

the column by passing NH_4HCO_3 (0.05M), and the desired labeled T3 product was then eluted from the column with distilled water and either freeze-dried to a yellow powder or stored frozen(-20℃)

The reaction was conducted in Tris-NaCl buffer (50mM Tris−HCl/100mM NaCl, pH7.4). The concentration of F-T3 was determined by measuring the absorbance at 490nm with $7.8\times10^4 M^{-1}cm^{-1}$, the molar extinction coefficient.

All fluorescence measurements were carried out using a FLS 920 Steady State and Time Resolved Fluorescence Spectrophotometer (Edinburgh Instruments, UK). Different volumes of F-T3 were reacted with different volumes of PDI, for finally required concentration. After incubation for 30 min at 4 ℃, the solution was transferred into a quartz micro-cuvette, and the fluorescence emission spectrum of each solution was recorded at room temperature. A control sample without PDI was also recorded. The excitation wavelength was 490 for F-T3, and the intensity at 516 nm was plotted.

The competitive binding assay was used to study the binding reaction between PDI and OH-PBDEs. For the assay, 200 nM F-T3 and 5 nM PDI were mixed in a total volume of 190 μL and reacted for 30 min at 4 ℃. Then, 10 microL of different concentrations of OH-PBDEs were added and incubated for 10min. The fluorescence intensity at 516nm before and after OH-PBDEs addition was measured. The content of acetonitrile in the final solution was kept below 1.2% to avoid solvent effect.

Circular dichroism spectroscopy is widely used in the determination of protein secondary structure and also a powerful tool to study protein-ligand binding interaction. Protein conformational changes are often caused by ligand binding and accompanied by changes in CD spectrum. As shown in Figure S4, with the addition of 200nM T3 into 200nM PDI, the sharp positive peak at 195nm obviously increased which is probably due to the absorbance of the small molecules in that area, while the negative peak at 210-220nm remained unchanged. The same result can be obtained by adding F-T3 in place of T3, suggesting that the protein is folded and the binding does

not affect the protein structure.

References

S1. D. S. Smith, FEBS Lett., 1977, 77, 25-27.

S2. X. M. Ren and L. H. Guo, Environ. Sci. Technol., 2012, 46, 4633-4640.

Fig.S1

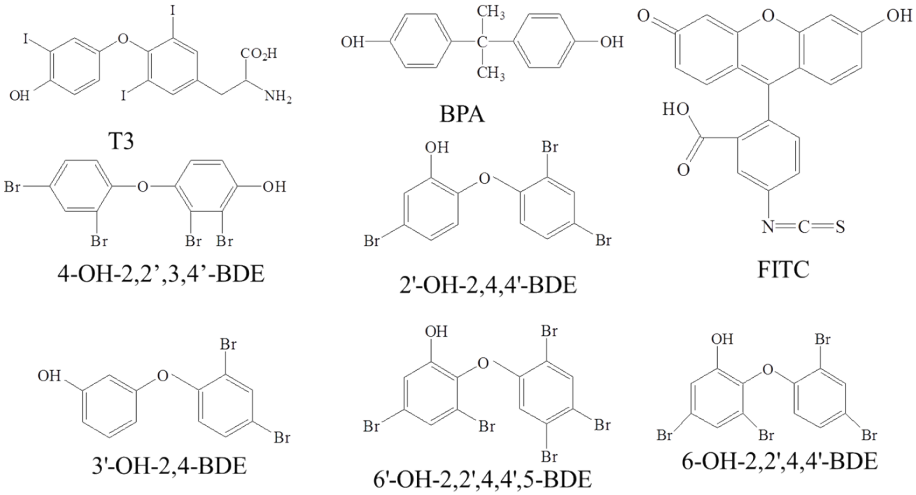

Fig. S1 Structures of the compounds used in the assay.

Fig.S2

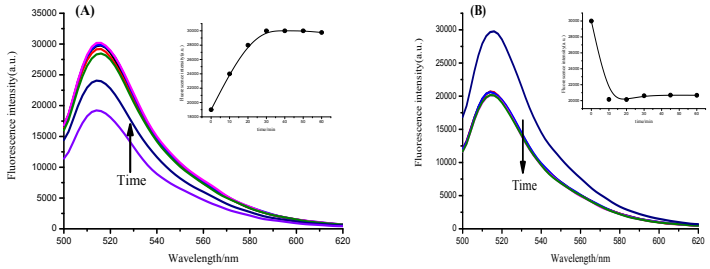

Fig. S2 The influence of different incubation time to direct binding assay between PDI and F-T3. (B) The influence of different incubation time to competitive binding assay of OH-PBDEs

Fig.S3

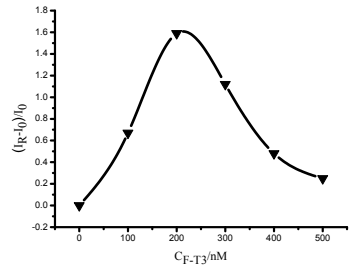

Fig. S3 Fluorescence intensity ratio at different F-T3 concentrations, using a fix PDI 5nM.

Fig.S4

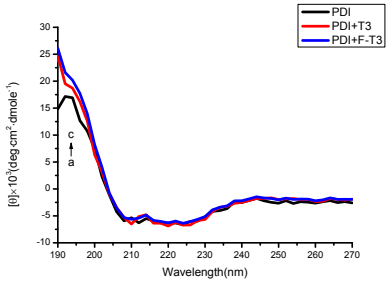

Fig.S4 Circular dichroism spectra of (a)PDI, (b)PDI+T3, (c)PDI+F-T3.

Fig.S5

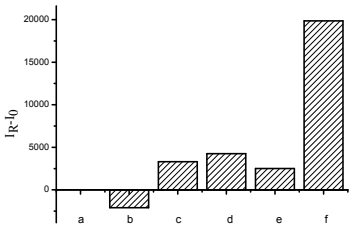

Fig. S5 Selectivity for F-T3combined with PDI and other enzymes. (a)F-T3; (b)F-T3+1.0×10^{-6} mol L-1 Invertase; (c)F-T3+1.0×10^{-6} mol L-1 Pyrophosphatase; (d)F-T3+5.0×10^{-7} mol L-1 Platelet-Derived Growth Factor(PDGF); (e)F-T3+5.0×10^{-7} mol L-1 Thrombin; (f)F-T3+5.0×10^{-9} mol L-1 PDI.

Fig.S6

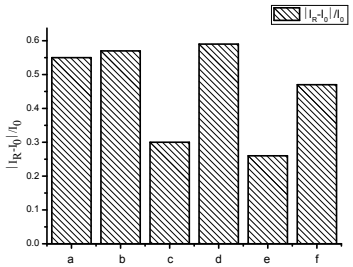

Fig. S6 The comparison of different targets (a)4-OH-BDE-42, (b)BPA (c)6-OH-BDE-47, (d)2-OH-BDE-28, (e)6'-OH-BDE-99 (f)3-OH-BDE-7.