Supplementary Information

Experimental Procedures

Materials and Apparatus

All t-butyloxycarbonyl (Boc)- and 9-fluorenylmethoxycarbonyl (Fmoc)-PNA monomers were purchased from PANAGENE Inc. (Daejeon Korea), Rink Amid MBHA resin was purchased from CSBio (Shanghai) Ltd (China); The 29-mer DNA oligonucleotide (TBA_{29}: 5'-AGT CCG TGG TAG GGC AGG TTG GGG TGA CT-3') was ordered from Jie Li Biology Inc. (Shanghai, China). The purchased DNA was dissolved in high purified water without further purification. The partially complementary PNA oligonucleotide (AAC CTG CCC T) was manually prepared by the method of solid-phase peptide synthesis and characterized according to our previously published literature.[1] All the PNA oligomers were purified by high-quality pre-packed column (Agilent Eclipse XDB-C18) and characterized by mass analysis. Their purities were greater than 99% according to the HPLC (Agilent). The mass spectra were obtained on an AB Sciex LC-Q-TOF 4600 spectrometer. The concentrations of DNA and PNA were determined by absorbance at 260nm (DeNovix DS-11 Spectrophotometer) with extinction coefficients calculated from DNA nearest neighbour values. Thrombin from human plasma, bovine serum albumin, IgG (Immunoglobulin G), 1,2-Ethanedithiol, the dye $DiSC_2(5)$ were purchased from Sigma-Aldrich (Mainland, China). HQ fetal bovine serum was purchased from TransGen Biotech Co., LTD (Beijing, Mainland, China). Acetic anhydride (Ac_2O), dichloromethane (DCM), N,N-diisopropylethylamine (DIEA), N,N-dimethylformamide (DMF), Kaiser test reagents, N-methyl-2-pyrrolidinone(NMP), trifluoroacetic acid (TFA), triisopropylsilane, Tris(hydroxymethyl) aminomethane, diethyl ether anhydrous, potassium chloride, methanol, acetonitrile, O-benzatriazole-N,N,N',N'-tetramethylammoniumhexafluorophosphate (HBTU), piperidine, pyridine, 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU) were purchased from Aladdin (Shanghai, Mainland, China). High purity water (18MΩ) was generated from a Millipore (Merck) Milli-Q water system. Stock solutions of $DiSC_2(5)$ dye was prepared in methanol. Unless otherwise stated, all the experiments were carried out in 10mM Tris-HCl buffer (pH 7.4).

Thrombin sensing procedure

First, 10 μL TBA_{29} (100 μM, in water) and 10 μL PNA (100 μM, in water) were added into 470 μL Tris-HCl buffer (10 mM, pH 7.4), and the resulting solution was heated to 95 °C and then allowed to cool to room temperature over a period of ca. 1 h. Then, 10 μL varying concentrations of $DiSC_2(5)$ was added into the above prepared PNA/TBA_{29} solution and incubated for 5 min at room temperature to get the PNA/TBA_{29}•$DiSC_2(5)$ complex. The resulting samples were tested with a UV-vis spectrometer.

For the detection of thrombin, 5 μL of different concentrations of thrombin or other proteins was added into 495 μL PNA/TBA_{29}•$DiSC_2(5)$ complex solution (containing 10 μL 100 μM PNA, 10 μL 100 μM TBA_{29}, 5 μL 3 mM $DiSC_2(5)$, and 470μL Tris-HCl buffer) and incubated for 5 min at room temperature. Then, the resulting samples were tested with a UV-vis spectrometer.

For the detection of thrombin in fetal bovine serum, the fetal bovine serum was first diluted 10 times with Tris-HCl buffer (10 mM, pH 7.4), and then the resulting Tris-HCl buffer was used to prepare the PNA/TBA_{29}•$DiSC_2(5)$ complex according to the procedures described above. Then, 5 μL of different concentrations of thrombin was added into 495 μL PNA/TBA_{29}•$DiSC_2(5)$ complex solution (containing 10 μL 100 μM PNA, 10 μL 100 μM TBA29, 5 μL 3 mM $DiSC_2(5)$, and 470μL serum buffer).

Absorbance signals were measured after 5 minutes incubation at room temperature.

Absorbance Measurements

Absorbance measurements were performed using a Cary 300 UV-Vis spectrophotometer (Agilent) with a 1 cm path length quartz cuvette. The absorption spectra of the solution were measured in the wavelength range from 700 to 500 nm.

Circular Dichroism Measurements

CD spectra were recorded on a Chirascan spectrophotometer (Applied Photophysics Ltd., Leatherhead, UK). The optical chamber of CD spectrometer was deoxygenated with dry purified nitrogen (99.99%) for 5 min before use and kept the nitrogen atmosphere during experiments. The CD spectra were obtained by taking the average of at least three scans with a bandwidth of 1.0 nm.

To characterize the structure of PNA/TBA_{29} hybrid, 200 μL of 5 μM PNA/TBA_{29} sample was prepared in 10 mM Tris buffer (pH 7.4), and the CD spectra were recorded from λ = 200 to 320 nm before and after the addition of 150 nM thrombin.

To measure the induced CD signal of $DiSC_2(5)$, 1 μL of varying concentrations thrombin was added into 200 μL PNA/TBA_{29}•$DiSC_2(5)$ complex solution (containing 5 μM PNA/TBA_{29} and 60 μM $DiSC_2(5)$) and incubated for 5 min at room temperature. Then, the CD spectra were recorded from λ = 500 to 700 nm.

Reference：

[1] C. Zhao, T. Hoppe, M. K. H. G. Setty, D. Murray, T. W. Chun, I. Hewlett, D. H. Appella, *Nat. Commun.* **2014**, *5*, 5079.