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Supporting Information

Polypeptide induced perylene probe excimer formation and its application for the noncovalent ratiometric detection of matrix metalloproteinase activity

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Experimental

1.1 Measurements

^{1}H NMR spectra were obtained using a Bruker AVANCE 400 Fourier transform (400 MHz) NMR spectrometer. Absorption spectra were obtained using a Cary 50 Bio Spectrophotometer (Varian Inc., CA., USA). Fluorescence spectral changes were recorded using a fluoromax-4 spectrometer (Horiba Jobin Yvon Inc., USA). The fluorescence spectra were tested at room temperature of 25 ℃ with a cuvette optical length of 10 mm, if not otherwise specified. The excitation wavelength for fluorescence detection was 495 nm and the slit for both excitation and emission was 6 nm. The emission spectra were corrected against photomultiplier tube (PMT) response. For brevity, the emission spectra were normalized (I_{545} value =1).

The vacuum drying oven was purchased from Jinghong Experimental Equipment Co., Ltd. (Shanghai, China). Ultrasonic Cleaner was purchased from Ultrasonic Instruments Co., Ltd. (Kunshan, Shanghai). The electric thermostatic water tank was purchased from Yiheng Technology Co., Ltd. (Shanghai, China). The analytical balance and pH meter were purchased from Sartorius Scientific Instruments Co., Ltd. (Beijing, China). Sterilization pot was purchased from Boxun Industrial & Commerce Co., Ltd., Medical Equipment Factory (Shanghai, China). The filter was purchased from Pall Filter Co., Ltd. (Beijing, China). The centrifuge was purchased from Scilogex Co., Ltd. (USA). Pipette guns were purchased from Thermo Fisher Scientific Co., Ltd. (Shanghai, China). Ultrapure water preparation instrument was a Milli-Q

A10 filtration system (Millipore, Billerica, MA, USA). Storage and buffer solutions used for testing were filtered and sterilized (120 ℃, 20 min).

1.2 MMP Activation

The buffer used for the activation of all recombinant human MMPs in the present work was 50 mM Tris, 10 mM $CaCl_2$, 150 mM NaCl, 0.05% (w/v) Brij-35, pH 7.5 (TCNB buffer). 1 μg/mL of MMP-1 was activated by APMA at a final concentration of 1 mM in a water bath for 2 h and then frozen for storage. 1 μg/mL of MMP-2 was activated by APMA at a final concentration of 1 mM in a water bath at 37 ℃ for 1 h and then frozen for storage; 10 μg/mL of MMP-3 was activated by 5 μg/mL of α-chymotrypsin in a water bath at 37 ℃ water for 30 min. Next, the activation was terminated by adding PMSF at a final concentration of 2 mM, and then frozen for storage; 1 μg/mL of MMP-7 was activated by APMA at a final concentration of 1 mM in a water bath at 37 ℃ for 1 h, and then frozen for storage; 1 μg/mL of MMP-9 was activated by APMA at a final concentration of 1 mM in a water bath at 37 ℃ for 24 h, and then frozen for storage. 1 μg/mL of MMP-13 was activated by APMA at a final concentration of 1 mM in a water bath at 37 ℃ for 2 h and then frozen for storage.

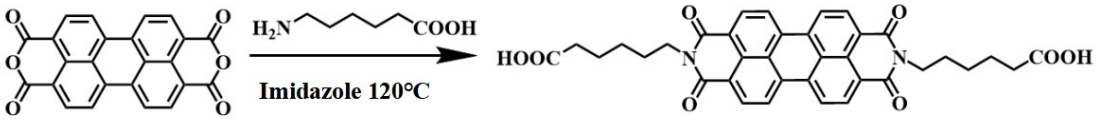

Figure S1. Schematic diagram of PC1 synthesis.

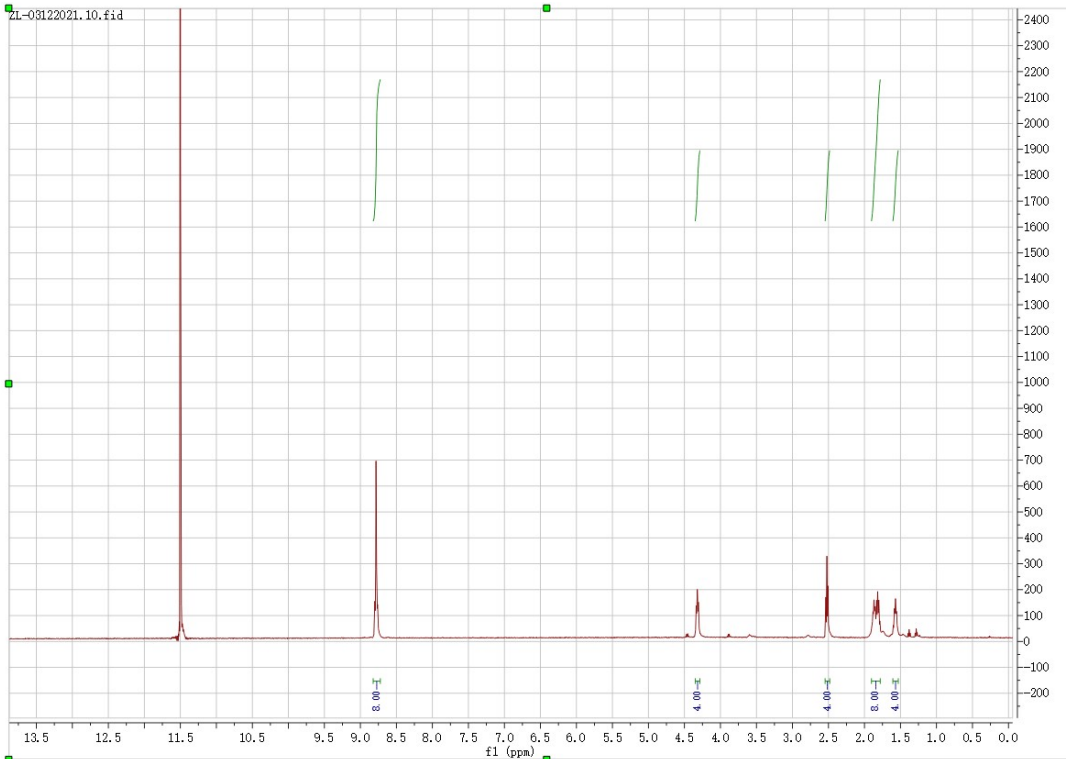

Figure S2. ^{1}H-NMR spectra of PC1.

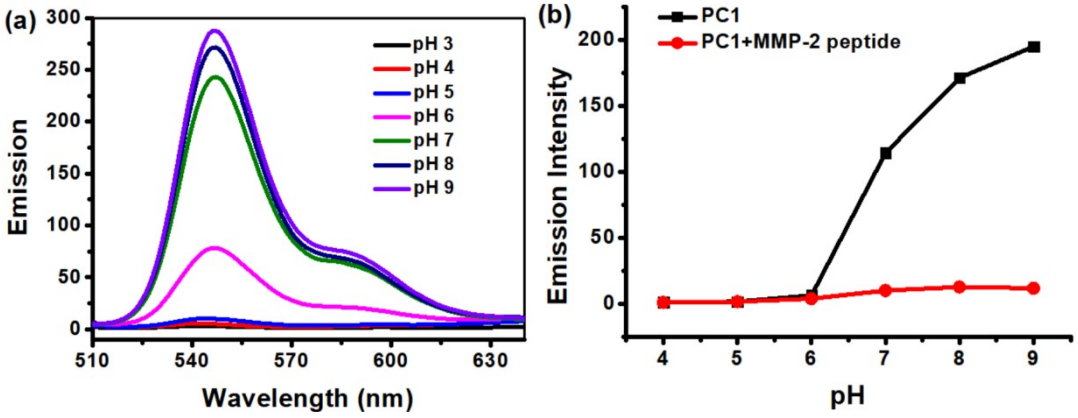

Figure S3. (a) Emission intensity changes of PC1 (10 μM) with assay solution pH. (b) Scatter plot of I_{545} value of 10 μM PC1 with assay solution pH in the presence and absence of 2.5 μM MMP-2 peptide substrate.

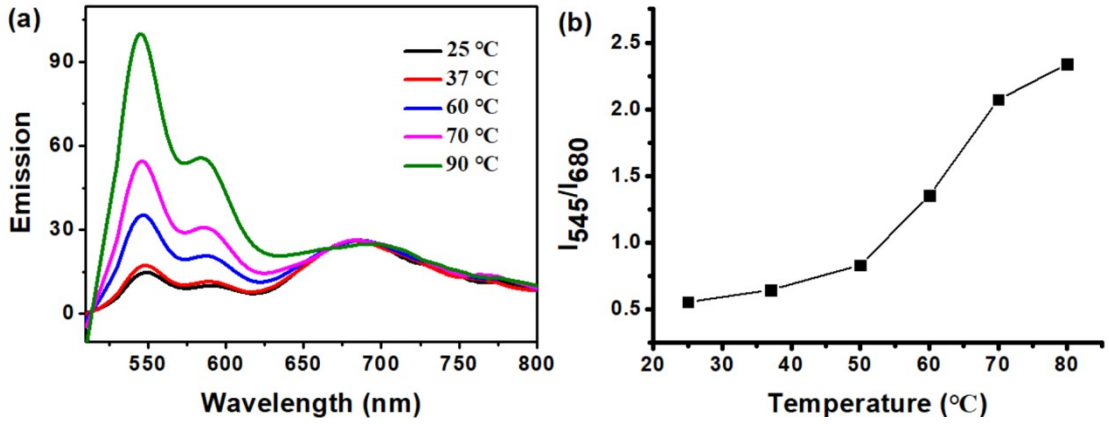

Figure S4. (a) Emission intensity changes of PC1 (10 μM) with assay solution temperature in the presence of 2.5 μM MMP-2 peptide substrate. (b) Scatter plot of I_{545}/I_{680} value of 10 μM PC1 with assay solution temperature in the presence of 2.5 μM MMP-2 peptide substrate.

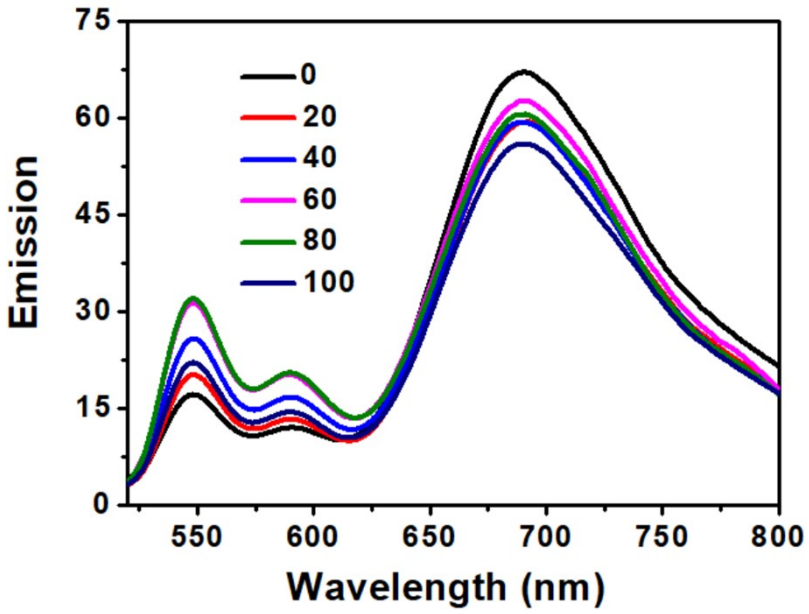

Figure S5. Emission intensity changes of PC1 (10 μM) with assay solution NaCl concentrations (mM) in the presence of 2.5 μM MMP-2 peptide substrate.

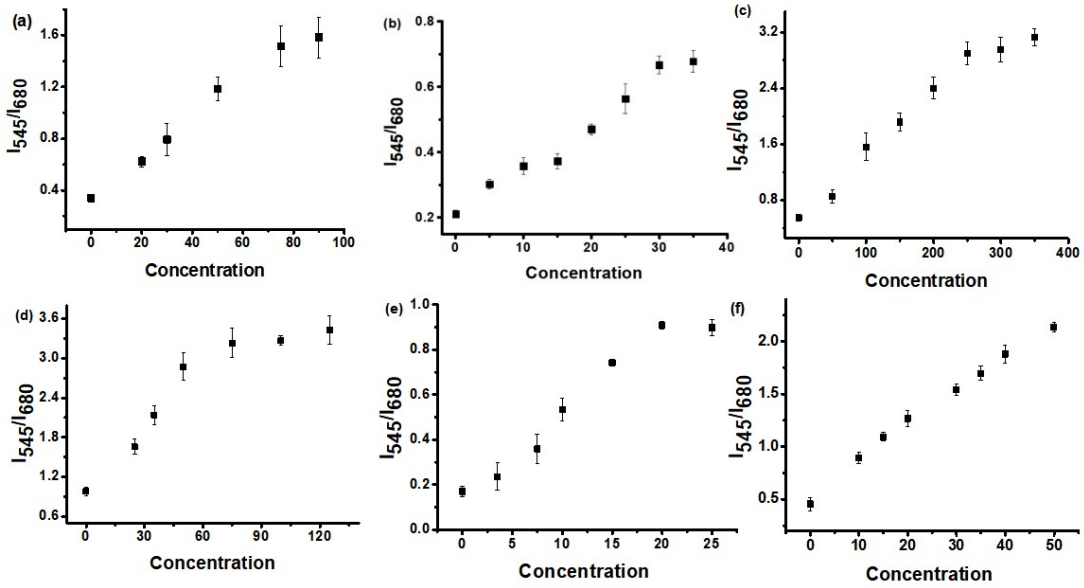

Figure S6. Scatter plot of the I_{545}/I_{680} value changes after adding different concentrations of MMP (in ng/mL). (a) MMP-1, (b) MMP-2, (c) MMP-3, (d) MMP-7, (e) MMP-9 and (f) MMP-13.

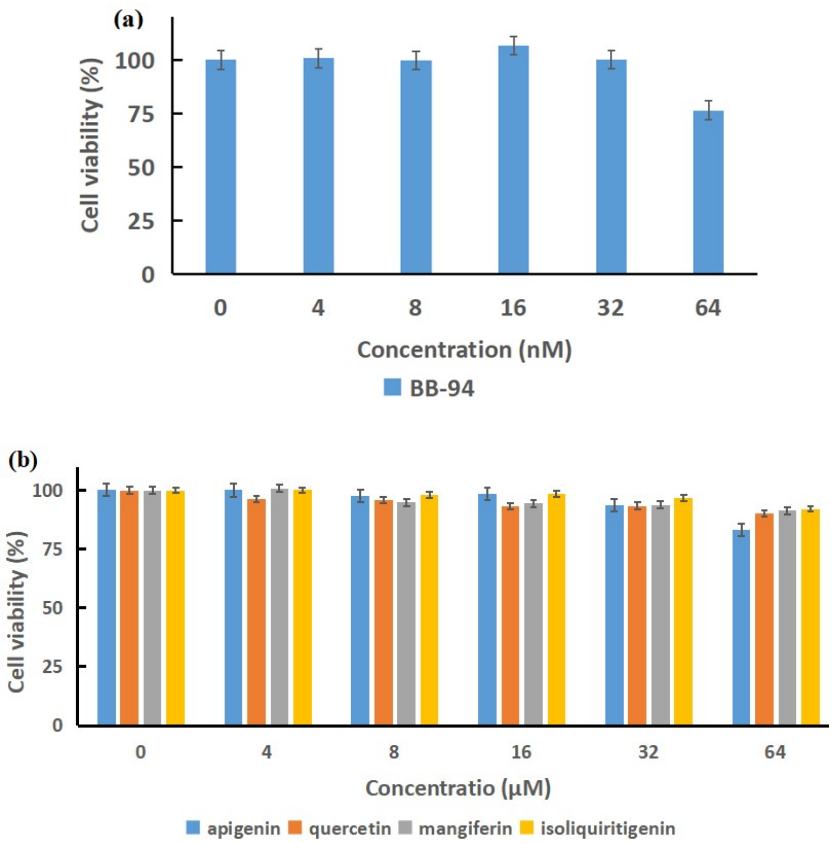

Figure S7. Toxicity of different concentrations of drugs on HT-1080 cells. (a) BB-94 (b) bule for apigenin, orange for quercetin, gray for mangiferin, yellow for isoliquiritigenin.

Table S1. Concentrations of peptide substrate and MMP in the MMP activity assay.

MMP	Peptide substrate concentration (μM)	MMP concentration (ng/mL)
MMP-1	1.5	0, 20, 30, 40, 50, 60, 80, 90, 100, 120
MMP-2	2.75	0, 5, 10, 15, 20, 25, 30, 35
MMP-3	2.0	0, 50, 100, 150, 200, 250, 300, 350
MMP-7	1.5	0, 25, 35, 50, 75, 100, 125
MMP-9	2.5	0, 3.5, 7.5, 10, 15, 20, 25
MMP-13	2.5	0, 10, 15, 20, 30, 35, 40, 50

Table S2. The limit of detection (LOD) and linear range of MMPs activity based on monomer/excimer ratio fluorometric method.

MMP	LOD, ng/mL	Linear range, ng/mL	Linear regression equation (and R^2 value)
MMP-1	4.8	20 – 100	I_{545}/I_{680} = 0.016 C + 0.33 (0.99)
MMP-2	2.2	5.0 – 30	I_{545}/I_{680} = 0.014 C + 0.21 (0.98)
MMP-3	16.0	50 – 250	I_{545}/I_{680} = 0.0093 C + 0.52 (0.99)
MMP-7	6.0	25 – 75	I_{545}/I_{680} = 0.032 C + 0.97 (0.97)
MMP-9	1.7	4.0 – 20	I_{545}/I_{680} = 0.038 C + 0.16 (0.99)
MMP-13	5.5	10 – 40	I_{545}/I_{680} = 0.034 C + 0.54 (0.99)

* ‘C’ stands for the concentration of MMP in ng/mL.

Table S3. Assay performance of reported biosensors for determination of MMP activities (NA: not available).

Target	Sensing platform	Transduction Type	Detection Limit	Detection range	Ref.
MMP-3	Gold array	SPR	NA	50 ng/mL -20 μg/mL	S1
MMP-7	AuNP-JR2EC	Colorimetry	0.1 μg/mL	0 - 2 μg/mL	S2
MMP-7	AuNP-Pep-QDs	Fluorescence	10 ng/mL	10 ng/mL - 5 μg/mL	S3
MMP-7	UCP-Pep-FITC	Fluorescence	13.9 ng/mL	0.01 -1 μg/mL	S4
MMP-7	rGO-peptide	Electrochemistry	10 ng/mL	10 ng/mL - 1 μg/mL	S5
MMP-7	Carboxy AuNPs	Colorimetry	0.28 μg/mL	0.084 - 1.46 μg/mL	S6
MMP-7	AuNPs/BCTOT-Eu^{III}/AMC	Fluorescence	1.1 ng/mL	0.04 - 0.4 μg/mL	S7
MMP-3	Perylene probe	Fluorescence	6 ng/mL	50 - 250 ng/mL	This Work
MMP-7			16 ng/mL	25 -75 ng/mL	

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