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Monitoring Matrix Metalloproteases Based on the Selective Interaction between Ir(III) Solvent Complex with Histidine-rich Peptide

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Materials and apparatus

Magnetic beads (MBs) (monodispersed magnetite microspheres: NH_2-modified Fe_4O_3 nanosphere with the size of 200−300 nm and the concentration of 5 $mg.mL^{-1}$. 231.53 MW) and 1,10-phenanthroline were bought from Sigma-Aldrich. Tris(hydroxymethyl) minomethane (Tris), $MgCl_2$, KCl, HCl, NaCl and other agents with analytical grade were bought from Sinopharm Chemical Reagent Co., Ltd. and used without further treatment. Matrix metalloproteinase 9 (MMP-9) was obtained from Sino Biological Inc. Prostate specific antigen (PSA), fibronectin (FN), thrombin (TB) and insulin were purchased from Sigma-Aldrich for the selectivity experiment. All the polypeptides (Table S1) were synthesized by Shanghai Sangon Biotechnology Co., Ltd. and were dissolved in Tris buffer (50 mM Tris, 150 mM NaCl, pH = 7.5). A Hitachi F-4600 fluorescence spectrofluorimeter was used for luminescence measurements. The luminescent intensity of the detection system was recorded with $\lambda_{ex/em}$= 350/490 nm. UV absorption spectra were tested by a PERSEE TU-1901 spectrophotometer.

Preparation of MCF-7 cell lysate

Cancer cell lysates of MCF-7 was obtained through the procedures of revival, incubation and disruption, and the whole process took about three days. The density of cells was 1×10^7 cell per mL which was determined by cell counting chamber (Bio-

system Medical Technology, Shanghai, Co., Ltd). The disruption of cells was achieved by the ultrasonic crasher noise isolating chamber and sonifier JY92-II in TCNB buffer (50 mM Tris, 10 mM $CaCl_2$, 150 mM NaCl, 0.05% (w/v) Brij®35, pH = 7.5) (Bcientz Biotechnology Co., Ltd).

Preparation of His-rich polypeptides modified magnetic beads (MBs)

His-rich polypeptide modified MBs were prepared according to the reported method[1, 2], and the involved reaction was presented in Fig. S1. Firstly, 120 mg maleic anhydride and 5 μL NH_2- modified MBs (21.6 mM) were dissolved in 10 mL $CHCl_3$. The mixture was reacted for 24 hours at room temperature. Then, 15 mL acetic anhydride and 136 mg NaOAc were added in the obtained reaction solution and refluxed for 24 h. After magnetic separation and washing with Tris buffer, the maleimide-modifided MBs was obtained. Secondly, 250 μL Tris buffer (50 mM Tris, 150 mM NaCl, pH = 7.5) containing 1 mM polypeptide was quickly introduced in the above prepared maleimide-modifided MBs at 37 ºC for 2 h. Through the covalently reaction between the mercapto- group of polypeptide and the maleimide moieties on the surface of the MBs, His-rich polypeptides modified MBs were achieved. Lastly, the final product was washed three times with Tris buffer (50 mM Tris, 150 mM NaCl, pH=7.5) to remove the uncoupled peptide followed by magnetic separation. The prepared His-rich polypeptides modified MBs were dispersed in 500 μL Tris buffer (50 mM Tris, 150 mM NaCl, pH = 7.5) with the concentration of 0.216 mM (this concentration is based on the initial amount of MBs), and were stored in −20 ºC for after use.

Preparation of Ir(III) Solv-1

Ir[(2-phenylpyridine)$_2$(CH_3CN)$_2$]CF_3SO_3 was synthesized according to the literature method [3, 4]. In brief, [Ir_2(2-phenylpyridine)$_4Cl_2$] was mixed with 2.0 equivalents of silver triflate ($AgCF_3SO_3$) in 25 mL acetonitrile and stirred at room temperature under a nitrogen atmosphere for 15 h. The mixture was filtered and washed with two portions of ether (2×30 mL) to yield titled product.

Optimization experiments

For the optimization of the sequence of His-rich peptides, three kinds of His-rich peptides with different AA sequences (Table S1) were used in this experiments. In order to simplify the optimization, the luminescent responses of 3 μM of Ir(III) Solv-**1** towards three kinds of unmodified peptides (100 μM) were investigated without the addition of MMP-9 and the after magnetic separation.

In the optimization of pH, the concentration of Ir(III) Solv-**1** and the reaction time between MMP-9 with His-rich peptides, relative intensity ($F-F_0$) was used to express the signal enhancement under different detection condition. Where, F means the luminescent signal when the detection system contains MMP-9, while F_0 means the luminescent signal in the absence of MMP-9.

For the optimization of pH for the detection system, in the present of 1 nM of MMP-9, 25 μL polypeptide-modified MBs (0.216 mM) were added to the reaction system under different pH (5.5, 6.5, 7.5, 8.5, 9.5). After the magnetic separation, 3.0 μM of Ir(III) Solv-**1** was added to the supernatant and the luminescence responses of the detection system were tested.

For the optimization of the concentration of Ir(III) Solv-**1**, various concentrations of Ir(III) Solv-**1** (1.0, 2.0, 3.0, 4.0 and 5.0 μM) were added to the supernatant which was obtained from the reaction system of polypeptide-modified MBs and MMP-9 (1 nM) by magnetic separation. After mixing, the luminescent signals of the detection system were recorded.

For the optimization of the reaction time between MMP-9 with His-rich peptides, the reaction systems of the polypeptides-modified MBs (25 μL, 0.216 mM) and MMP-9 (1 nM) were reacted at different reaction time (0, 10, 20, 30, 60, and 90 min). After the magnetic separation, the Ir(III) Solv-**1** (3 μM) was added to the supernatant, and then the luminescent intensities of the detection system were tested.

Detection of MMP-9 in buffered solution

Firstly, MMP-9 (100 nM) was activated by 1 mM 4-Aminophenylmercuric acetate (APMA) in TCNB buffer (50 mM Tris, 10 mM $CaCl_2$, 150 mM NaCl, 0.05% (w/v) Brij®35, pH = 7.5) and incubated at 37 ºC for 24 hour. 25 μL of His-rich polypeptides modified MBs (0.216 mM) and various concentrations of MMP-9 were incubated in Tris buffer (50 mM Tris, 150 mM NaCl, pH = 7.5) at 37 °C for 1 h, in which MMP-9 could specifically cleave the His-rich polypeptides at its recognition site (between G and L). After magnetic separation, 3 μM of Ir(III) Solv-**1** was added in the supernatant fraction to carry out the subsequent emission testing. In this detection, the total volume was 500 μL, so all of the mentioned concentrations are based on 500 μL.

Detection of MMP-9 in MCF-7 cell lysate

25 μL of His-rich polypeptides modified MBs (0.216 mM) was added in Tris buffer (50 mM Tris, 150 mM NaCl, pH=7.5) containing certain volume of MCF-7 cell lysate (0, 1.25, 1.875, 3.125, 6.25 μL) which is positively expressed MMP-9. As the final volume of the mixture was 500 μL, it means that the cell lysate were diluted 400, 267, 160, 80 times in this detection, respectively. So, five different densities (0, 25, 37.5, 62.5, 125 $\times 10^3$ cells/mL) of MCF-7 were tested. Then, the following procedures were the same as the detection procedures in buffered solution.

Screening the inhibitor of MMP-9

To confirm that the detection platform can screen MMPs' inhibitor, the MMP-9 inhibitor (1,10-phenanthroline, Phen) was added into the detection system, and the luminescent responses of the detection system were investigated. The activated MMP-9 (1 nM) and various concentrations of Phen (0, 1, 2, 5, 7.5, 10 μM) were added to the reaction system at 37 °C for 1 h. Subsequently, the supernatant was served as the reaction solution for the next magnetic separation. The luminescence spectrum of the detection system was investigated upon the addition of Ir(III) Solv-**1** into the supernatant. The IC_{50} of Phen for MMP-9 (1 nM) was estimated through the changes of luminescence in the presence of different concentrations of Phen.

Selectivity and anti-interference studies

To test the selectivity and anti-interference of the detection platform, the luminescent responses of the detection system to four kinds of proteins (prostate specific antigen , fibronectin, insulin and thrombin) were investigated in parallel under the same conditions. All the proteins' concentration was 10 nM, and the concentration of MMP-9 was 1 nM. The detection procedures were the same as the detection performed in buffered solution.

Table S1 The peptides used in this detection

Peptide	Sequence
1	CPLGLPKGGHHH
2	CPLGLPHKGHGH
3	CPLGLPHHHKGG

Table S2 The various MMPs detection strategies developed in these years

Method	Detection limit	Real samples	Recyclability	Reference
Detection of plasma MMP-9 via a fast and simple electrochemical magneto-immunosensor.	13 pg/mL	Blood plasma	No	[5]
Disposable MMP-9 sensor based on the degradation of peptide crosslinked hydrogel films using electrochemical impedance spectroscopy	15 ng/mL	Not mentioned	No	[6]
Peptide microarray-based fluorescence assay for simultaneously detecting	60 pg/mL	Cell secreted	Not mention	[7]

matrix metalloproteinases	(MMP-9), 45 pg/mL (MMP-2)	MMPs	ed	
Simultaneous detection of multiple targets for investigating cellular migration and invasion with a multicolor fluorescent nanoprobe	Not mentioned	MCF-10A cells	Not mentioned	[8]
Micropatterned surfaces functionalized with electroactive peptides for detecting protease release from cells	60 pM	Serum containing RPMI-1640 media	No	[9]
Hydrogel-framed nanofiber matrix integrated with a microfluidic device for fluorescence detection of matrix metalloproteinases-9	10 pM	Cocktail solutions	Yes (for microfluidic system)	[10]
Au−Se-bond-based nanoprobe for imaging MMP-2 in tumor cells under a high-thiol environment	1.7 ng/mL	HepG2 cell and MCF-7 cell	Not mentioned	[11]
PtNPs as scaffolds to regulate interenzyme distance for construction of efficient enzyme cascade amplification for ultrasensitive electrochemical detection of MMP-2	0.03 pg/mL	Diluted healthy human serum.	No	[12]
Poly(m-phenylenediamine)-based fluorescent nanoprobe for ultrasensitive detection of matrix metalloproteinase 2	32 pM.	Human serum samples, colorectal cancer cell	No	[13]

Peptide microarray-masked metal enhanced fluorescence assay for multiple profiling of matrix metalloproteinases activities	12.2 fg/mL (MMP-2), 60 pg/mL (MMP-3), 0.22 pg/mL (MMP-7), 102 fg/mL (MMP-9), 0.68 ng/mL (MMP-14)	Clinical thyroid tissue	No	[14]
Upconversion fluorescence resonance energy transfer based biosensor for ultrasensitive detection of matrix metalloproteinase-2 in blood	Not mentioned	Human plasma and whole blood	No	[15]
An electrochemical peptide cleavage-based biosensor for matrix metalloproteinase-2 detection with exonuclease III-assisted cycling signal amplification	0.15 pg/mL	Diluted healthy human serum	No	[16]

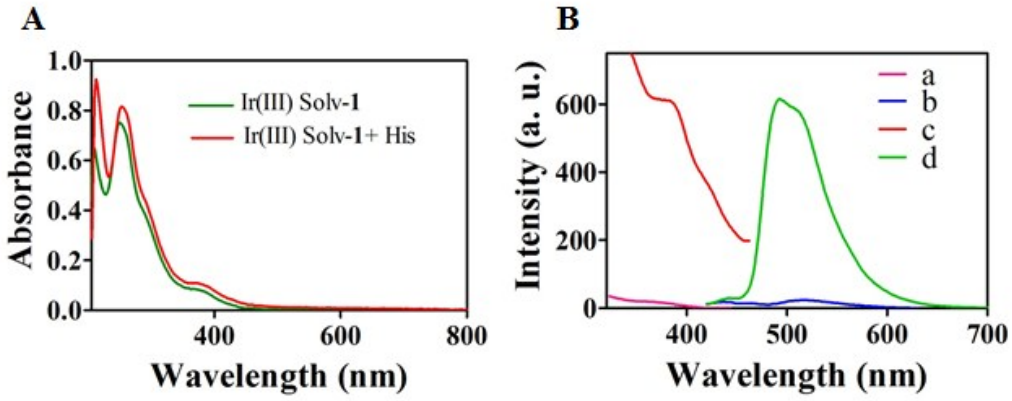

Fig. S1 (A) The absorbance spectra of Ir(III) Solv-**1** (15 μM) in the presence/absence of (50 μM) His in Tris-HCl buffer. (B) The excitation and emission spectra of Ir(III) Solv-**1** in the presence/absence of (50 μM) His in Tris-HCl buffer. a: excitation of Ir(III) Solv-**1**, b: emission of Ir(III) Solv-**1**, c: excitation of Ir(III) Solv-**1** + His, d: emission of Ir(III) Solv-**1** + His.

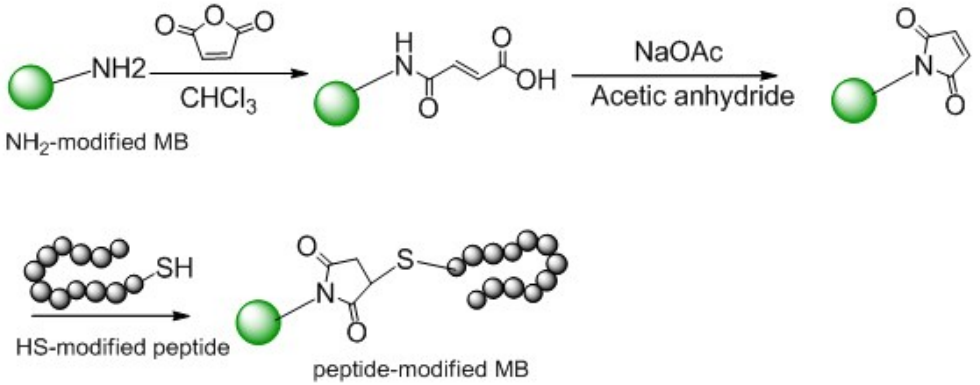

Fig. S2 The synthetic route for peptide modified MBs used in this work.

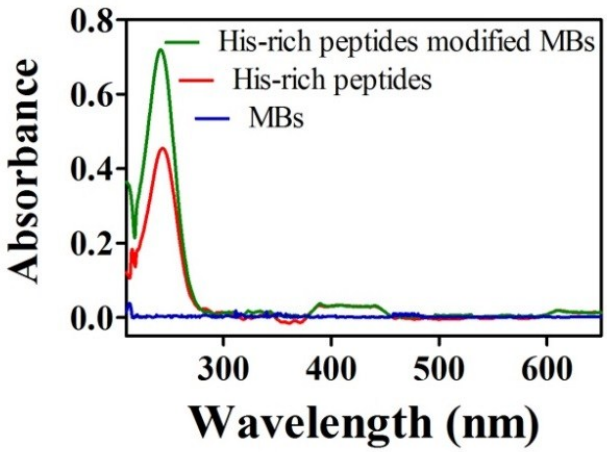

Fig. S3 UV absorption spectra of MBs, His-rich peptides and His-rich peptides modified MBs.(The concentrations of MBs, His-rich peptides, His-rich polypeptides modified MBs were 10.8 μM, 100 μM and 10.8 μM, respectively).

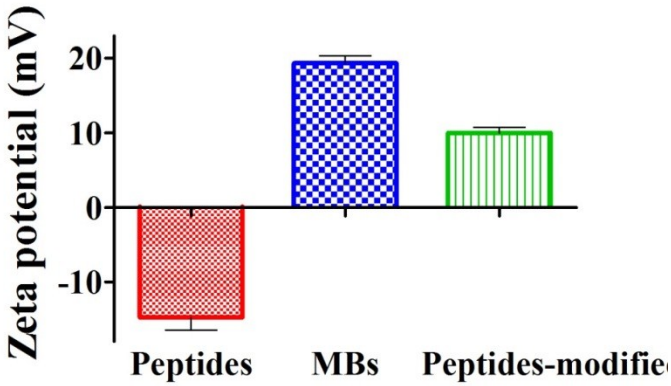

Fig. S4 Zeta-potentials of (a) His-rich peptides, (b) maleimide-modifided MBs and (c) His-rich peptides modified MBs, respectively.

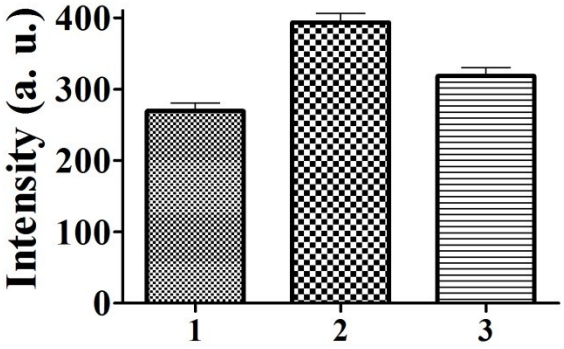

Fig. S5 Optimization of the sequences of His-rich peptides (The concentrations of Ir(III) Solv-**1** and peptides were 3.0 μM and 100 μM, respectively. The reaction time was 60 min. The pH was 7.5.). Among these three kinds of peptides, peptide **2** presented the highest luminescence intensity ascribed to the suitable location of His in it which could offer the best binding sites for Ir(III) Solv-**1**. In the next experiments, peptide **2** was employed.

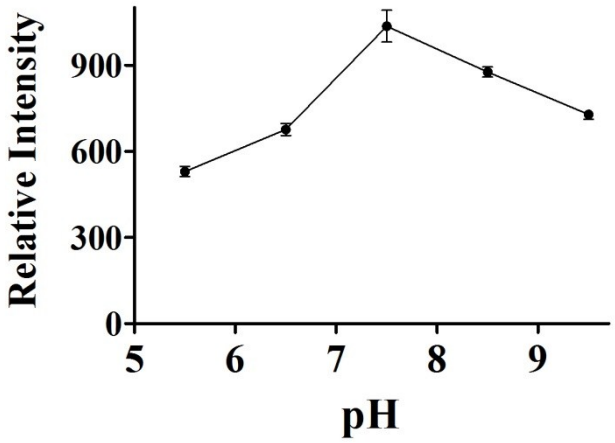

Fig. S6 Optimization of the pH (5.5, 6.5, 7.5, 8.5 and 9.5) for the detection system. (The concentrations of Ir(III) Solv-**1** and MMP-9 were 3.0 μM and 1 nM, respectively. The reaction time was 60 min). The optimal pH for this biosensor should be 7.5. A higher or lower pH may influence the combination between Ir(III) Solv-**1** with His, and hence influence the luminescence intensity of the detection.

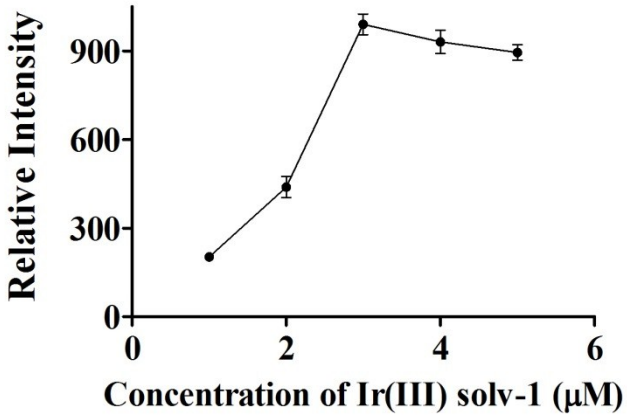

Fig. S7 Optimization of the concentration of Ir(III) Solv-**1**. (The concentration of MMP-9 was 1 nM, the reaction time was 60 min, and the pH was 7.5). 3 μM of Ir(III) Solv-**1** could present the highest luminescence signal. Too low level of Ir(III) Solv-**1** would not present enough of binding molecules for His-rich peptide binding, while too high level of Ir(III) Solv-**1** can cause the waste of the complex.

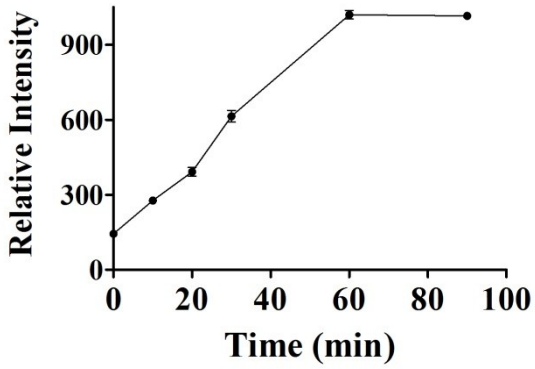

Fig. S8 Optimization of the reaction time between MMP-9 with His-rich peptides. (The concentrations of Ir(III) Solv-**1** and MMP-9 were 3.0 μM and 1 nM, respectively. The pH was 7.5). The reaction time should be 60 min since further increasing the cleavage time no luminescence change was observed.

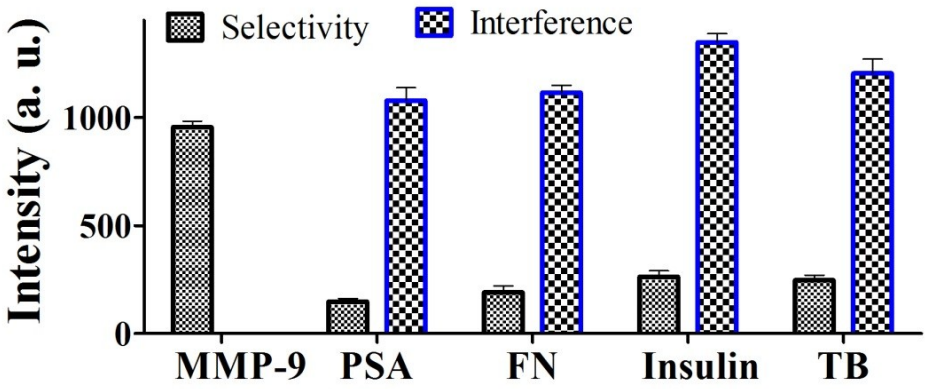

Fig. S9 The selectivity and anti-interference of the detection platform in the presence of PSA, FN, insulin and TB (The concentrations of Ir(III) Solv-**1**, MMP-9, and other protenis were 3.0 μM, 1 nM and 10 nM, respectively. The reaction time was 60 min. The pH was 7.5).

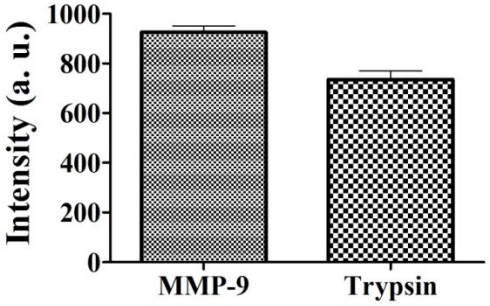

Fig. S10 The luminescence intensity of the detection system in the presence of MMP-9 and trypsin, respectively (The concentrations of Ir(III) Solv-**1**, MMP-9 and trypsin were 3.0 μM, 1 nM and 1 nM, respectively. The reaction time was 60 min. The pH was 7.5).

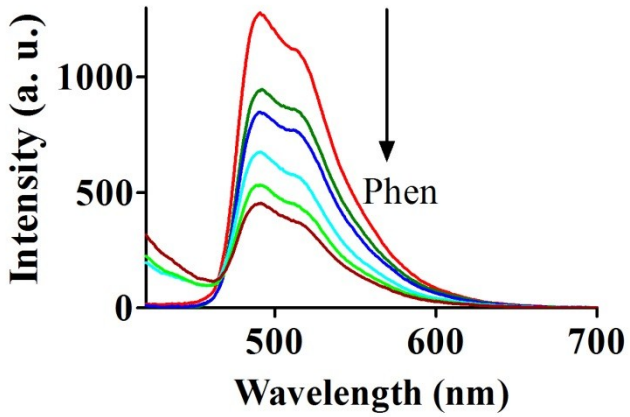

Fig. S11 The luminescence responses of the detection system in the presence increased concentrations (0, 1, 2, 5, 7.5, 10 μM) of Phen. (The concentrations of Ir(III) Solv-**1** and MMP-9 were 3.0 μM and 1 nM, respectively. The reaction time was 60 min. The pH was 7.5).

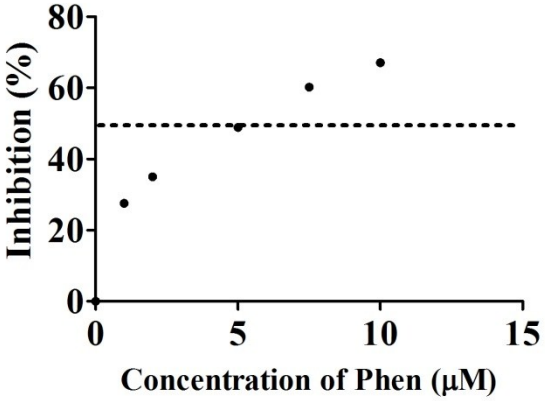

Fig. S12 Estimation the IC_{50} of Phen towards 1 nM of MMP-9

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