Supporting Information

Regulation of α-Chymotrypsin Activity on the Surface of Substrate-Functionalized Gold Nanoparticles

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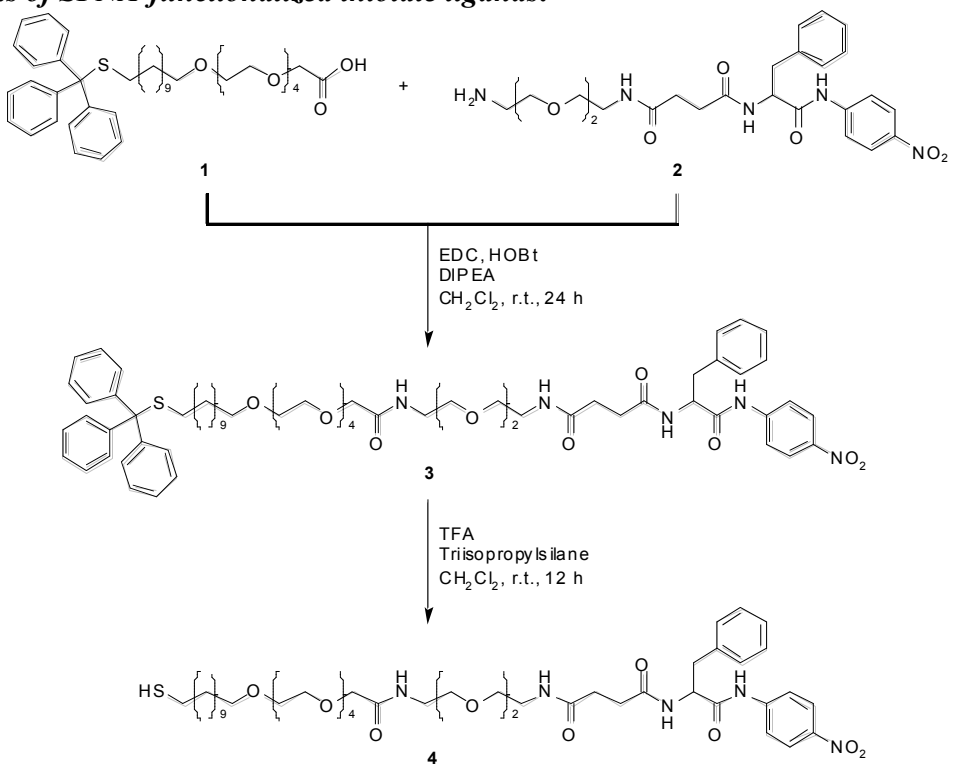
I. Synthesis of SPNA-functionalized thiolate ligands:



Scheme S1. Synthetic scheme for thiolate SPNA-functionalized ligand.

Trityl protected acid **1**[1] and SPNA derivative **2**[2] were prepared according to previous reports.

Synthesis of Ligand 3: Trityl protected acid **1** (681 mg, 1 mmol) was dissolved in dry CH_2Cl_2 (30 mL), which was cooled with ice bath. Diisopropylethylamine (DIPEA, 130 mg, 1 mmol), *N*-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC, 191 mg, 1 mmol) and 1-hydroxybenzotriazole (HOBt, 135 mg, 1 mmol) were added and the mixture was stirred for 10 min. Subsequently, SPNA derivative **2** (516 mg, 1 mmol) was added and the reaction proceeded at room temperature for 24 h. The mixture was poured into water (300 mL) and extracted with CH_2Cl_2 (50 mL × 4). The organic layers were combined and washed successively with citric acid solution (10%, 2×), water (1×), sodium bicarbonate (2×), water (1×) and brine (2×) and dried over anhydrous sodium sulfate. After filtration and removal of the solvent, the residue was charged on SiO_2 column eluting with CH_2Cl_2/CH_3OH (95:5). The desired band was collected and evaporated to dryness. Yield 700 mg (59%).

^{1}H NMR ($CDCl_3$, TMS): δ 9.58 (s, 1H, –NH–), 8.16 (d, ^3J = 9.1 Hz, 2H, Ar-NO_2), 7.96 (d, ^3J = 9.1 Hz, 2H, Ar-NO_2), 7.40 (m, 6H, Ar), 7.35~7.15 (m, 15H, Ar + –NH–), 6.75 (t, ^3J = 5.3 Hz,

1H, –NH–), 6.40 (d, ^{3}J = 8.3 Hz, 1H, –NH–), 5.93 (q, 1H, –CH<), 3.97 (s, 2H, –OCH$_2$–), 3.70~3.55 (m, 22H, –CH$_2$–), 3.50 (m, 4H, –CH$_2$–), 3.42 (m, 4H, –CH$_2$–), 3.27 (d, 2H, Ar-CH$_2$–), 2.90~2.40 (m, 2H, –CH$_2$–), 2.36 (m, 2H, –CH$_2$–), 2.13 (t, ^{3}J = 7.3 Hz, 2H, –SCH$_2$–), 1.55 (m, 2H, –CH$_2$–), 1.38 (m, 2H, –CH$_2$–), 1.20 (m, 14H, –CH$_2$–).
MS (ESI): *m*/*z* 1200.7 $[M+Na]^+$ (calcd for $C_{65}H_{87}N_5O_{13}S$ 1177.6).

Synthesis of Ligand 4: Trityl protected lignd **3** was dissolved in CH_2Cl_2 (30 mL) and trifluoroacetic acid (TFA, 2 mL) and triisopropylsilane (1 mL) were added successively. The reaction mixture was stirred at room temperature for 12 h. The solvent and excess TFA were removed under reduced pressure. Purification was achieved on flash chromatography with EtOAc/CH_3OH (90:10) as eluent. Yield 350 mg (63%).
^{1}H NMR ($CDCl_3$, TMS): δ 9.84 (s, 1H, –NH–), 8.16 (d, ^{3}J = 9.3 Hz, 2H, Ar-NO$_2$), 7.95 (d, 3J = 9.3 Hz, 2H, Ar-NO$_2$), 7.49 (s, 1H, –NH–), 7.28 (m, 3H, Ar), 7.22 (m, 2H, Ar), 7.00 (s, 1H, –NH–), 6.54 (d, ^{3}J = 7.8 Hz, 1H, –NH–), 4.95 (q, 1H, –CH<), 3.98 (s, 2H, –OCH$_2$–), 3.70~3.30 (m, 30H, –CH$_2$–), 3.24 (m, 2H, –CH$_2$–), 2.70 (m, 1H, –CH$_2$–), 2.52 (q, 2H, –SCH$_2$–), 2.43 (m, 3H, –OCH$_2$–), 1.58 (m, 4H, –CH$_2$–), 1.26 (m, 14H, –CH$_2$–).
MS (ESI): *m*/*z* 958.9 $[M+Na]^+$ (calcd for $C_{46}H_{73}N_5O_{13}S$ 935.5).

Fabrication of SPNA-Functionalized Gold Nanoparticle: 1-Pentanethiol protected gold nanoparticles (*d* = 2 nm, 40 mg) were dissolved in CH_2Cl_2 (10 mL) and ligand **4** (150 mg) in CH_2Cl_2 (5 mL) was added subsequently. The mixture was stirred at room temperature for 2~3 days. The precipitates formed were collected by centrifugation and washed thoroughly with CH_2Cl_2. ^{1}H NMR spectrum shows that the incorporation of the ligands onto the nanoparticle surface causes the signal broadening (Figure S1). No free ligand was detected in the system. The dark solid was dried under high vacuum to remove the solvent. The nanoparticle is highly soluble in DMSO, partially soluble in dichloromethane or methanol, and insoluble in hexane or water. In this context, it is dissolved in a DMSO/EtOH (1:2) to make a stock solution (40 μM). Based on the UV absorption of SPNA moiety at 320 nm (cf. Figure S2), it is estimated that there are *ca*. 90 SPNA-functionalized thiolate ligands on each nanoparticle.

II. Experimental Details:
Zeta-potential. The stock solution of SPNA-functionalized nanoparticle (40 μM, in DMSO/EtOH) was diluted with Tri/HCl buffer solution (100 mM) to make a 1 μM solution. In another experiment, **NP_SPNA** (1 μM) was incubated with ChT (10 μM) for 24 h to create the corresponding negatively charged derivative. The zeta-potentials were measured on a MALVERN Zetasizer Nano ZS instrument. Three rounds of assays have been performed and the average values were reported.

Activity Assays. All of the experiments were performed in Tris/HCl buffer (5 mM, pH 7.4). To detect the time-dependence of ChT activity in the presence of **NP_SPNA**, ChT (3.2 μM), **NP_SPNA** (0.8 μM) and corresponding substrate (**S1**-**S4**, 2 mM) were mixed together and the product formation was immediately followed every 15 s for 60 min at 405 nm with a microplate reader (EL808IU, Bio-Tek Instruments, Winooski, VT). The absorbance was converted to a concentration scale by a molar absorption coefficient of 9800 M^{-1} cm^{-1} for 4-nitroaniline (pNA).[3] In the regulation study, the concentrations of **NP_SPNA** and substrate **S1** were kept as 3.2 μM

and 2 mM, respectively, while the concentration of ChT varied from 3.2 to 19.2 μM. N_α-Bezoyl-DL-arginine 4-nitroanilide hydrochloride (BANA, 1.0 mM) and *N*-succinyl-Ala-Ala-Ala-*p*-nitroanilide (SAAANA, 0.5 mM) were used as the chromogenic substrates of trypsin and elastase, respectively. **NP_TCOOH** was prepared according to the reported procedure.[2]

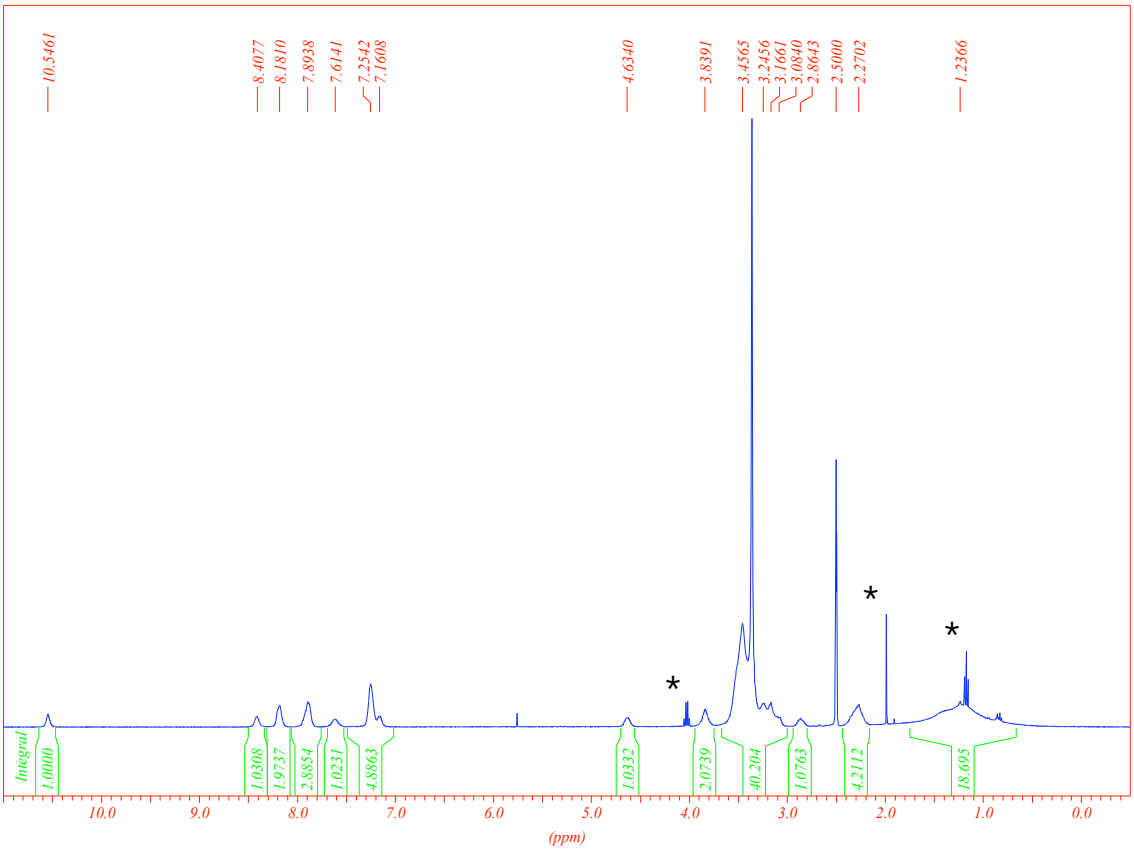


Figure S1. ^{1}H NMR spectrum of **NP_SPNA** in DMSO-*d6*. The asterisks mark the proton signals of concomitant ethyl acetate.

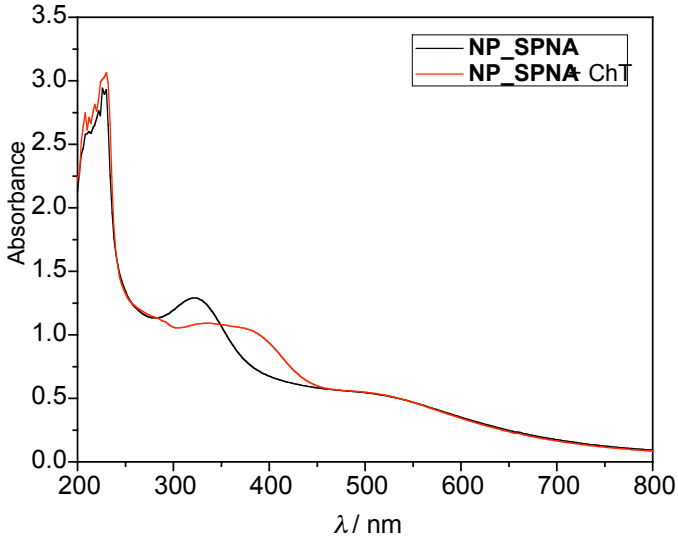


Figure S2. UV/Vis absorption spectra of **NP_SPNA** (0.5 μM) and the mixture of **NP_SPNA** and ChT after incubation for 20 h.

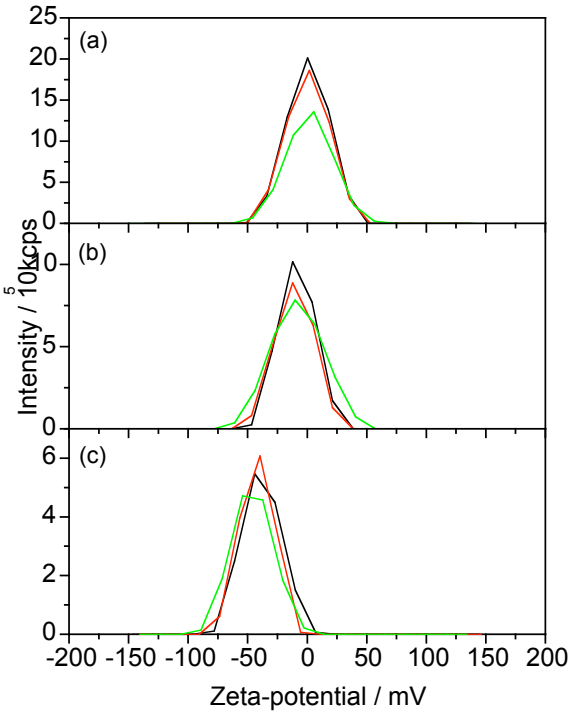


Figure S3. Zeta-potential distribution of **NP_SPNA** (a), ChT (b), and **NP_SPNA** after incubation with ChT (c) in Tri/HCl buffer solution (pH 7.4, 100 mM) at 25 °C.

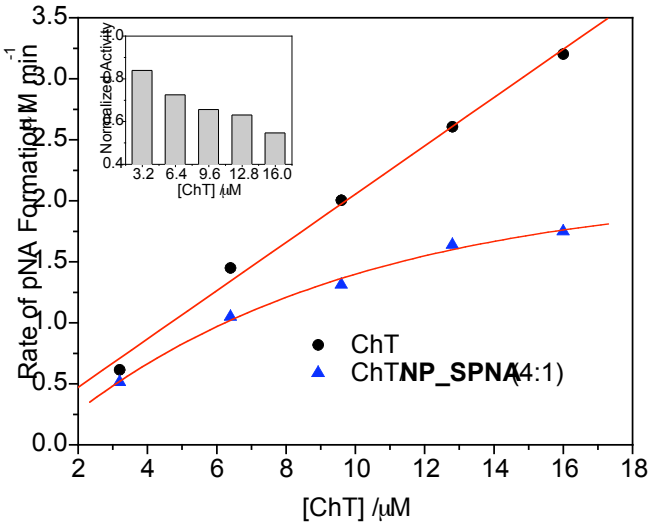


Figure S4. Generation rate of pNA from substrate **S1** in the presence of various concentrations of ChT and ChT/**NP_SPNA** (ratio 4:1, incubated for 30 min). (Inset) The activity of ChT/**NP_SPNA** was normalized to that of free ChT at the same concentration.

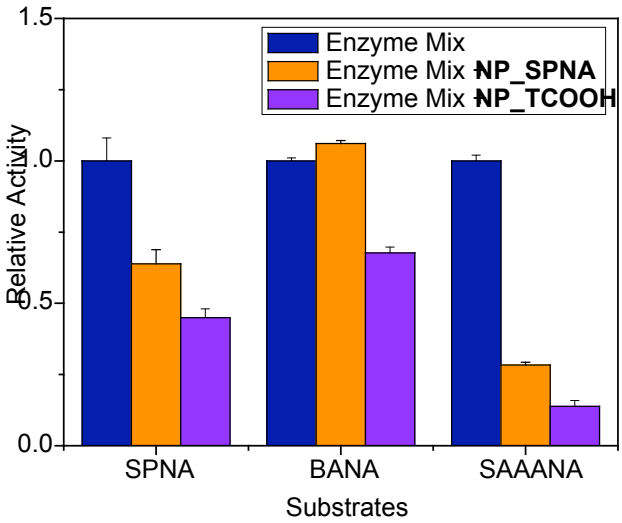


Figure S5. The relative activity of a mixture of ChT (3.2 μM), trypsin (1.0 μM) and elastase (250 nM) in the presence of **NP_SPNA** (2.0 μM) or **NP_TCOOH** (2.0 μM), The incubation period is 2 h. The substrates SPNA (2.0 mM), BANA (1.0 mM) and SAAANA (0.5 mM) were used to monitor the activity of ChT, trypsin, and elastase, respectively.

[1] (a) B. T. Houseman and M. Mrksich, *J. Org. Chem.*, 1998, **63**, 7552–7555; (b) C.-C. You, M. De, G. Han and V. M. Rotello, *J. Am. Chem. Soc.*, 2005, **127**, 12873–12881.
[2] R. Hong, T. Emrick and V. M. Rotello, *J. Am. Chem. Soc.*, 2004, **126**, 13572–13573.
[3] N. Sträter, L. Sun, E. R. Kantrowitz and W. N. Lipscomb, *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 11151–11155.