Electronic Supplementary Information

Multifunctional small molecule for controlled assembly of oligmeric nanoparticles and crosslinked polymers

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Figure S1. Absorption spectra (500-700 nm due to the light scattering) of self-assembled condensation products of **1** at 1 mM in water with (Red) or without (black) 4 equiv. of TCEP at pH 7.4.



Figure S2. (a) SEM and (b) TEM images of the nanoparticles assembled by the condensation products of **2** at 1 mM treated with 2 equiv. of TCEP at pH 7.4.

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Figure S3. MALDI mass spectrum of the condensation mixture of 1 mM of **2** treated with 2 equiv. of TCEP at pH7.4.



Figure S4. MALDI mass spectrum of the peak at the retention time of 20.7 min on the HPLC trace of figure 3b.



Figure S5. MALDI mass spectra of the peaks at the retention times of (a) 22.0 min, (b) 23.7 min, (c) 25.7 min, (d) 26.9 min, and (e) The broad peak at 29.5 min on the HPLC trace in figure 3c.

Supplementary Table S1. HPLC conditions for the purification of Cys(SEt)-Lys(Cys(SEt))-CBT (1) and its oligomers after condensation.

Time (minute)	Flow (mL/min.)	H ₂ O %	CH ₃ OH %
0	3.0	80	20
3	3.0	80	20
30	3.0	20	80
32	30	20	80
33	3.0	80	20
35	3.0	80	20

Supplementary Table S2. HPLC condition for the purification of Lys-CBT (B).

Time (minute)	Flow (mL/min.)	H ₂ O %	CH ₃ OH %
0	3.0	30	70
3	3.0	30	70
35	3.0	0	100
37	3.0	0	100
38	3.0	30	70
40	3.0	30	70

Supplementary Methods

General methods. Synthesis and characterization of monomers are in the online Supplementary information. All the starting materials were obtained from Adamas or Sangon Biotech. Commercially available reagents were used without further purification, unless noted otherwise. All other chemicals were reagent grade or better. HPLC analyses were performed on an Agilent 1200 HPLC system equipped with a G1322A pump and an in-line diode array UV detector using an Angilent Zorbax 300SD-C₁₈ RP column with CH₃OH (0.1% of TFA) and water (0.1% of TFA) as the eluent. ¹H NMR spectra were obtained on a 300 MHz Bruker AV 300. MALDI-TOF/TOF mass spectra were obtained on a time-of-flight Ultrflex II mass spectrometer (Bruker Daltonics). Dynamic light scattering (DLS) measurement was taken on a Zeta Sizer Nano Series (Malvern Instruments). Scan electron micrographs (SEM) were obtained on JEOL-JSM-6700F electron microscope at an accelerating voltage of 5.0 kV. Transmission electron micrographs (TEM) were obtained on a JEOL 2010 electron microscope, operating at 100 kV. The cryo-dried samples were prepared as following: a copper grid coated with carbon was dipped into the suspension and placed into a vial, which was plunged into liquid nitrogen until no bubbles were apparent. Then, water was removed from the frozen specimen by a freeze-drier.

Syntheses and Characterizations

The preparations of compound **1** and **2** were described as below; 2-cyano-6-aminobenzothiazole (CBT) was synthesized following the literature method (White, E. H., Worther, H., Seliger, H. H., McElroy, W. D. Amino analogs of firefly luciferin and biological activity thereof. *J. Am. Chem. Soc.* **1966**, *88*, 2015-2019).

Preparation of Cys(SEt)-Lys(Cys(SEt))-CBT (1): Scheme S1. Synthetic route for compound 1.



Synthesis of Boc-Lys(Boc)-CBT (A): The isobutyl chloroformate (109 mg, 0.8 mmol) was added to a mixture of Boc-Lys(Boc)-OH \bullet DCHA (422mg, 0.8 mmol) and MMP (4-methylmorpholine,

151mg, 1.5 mmol) in THF (5.0 mL) at 0°C and the reaction mixture was stirred for 30 min. 2-cyano-6-aminobenzothiazole (140 mg, 0.8 mmol) was added to the reaction mixture and further stirred for 1 h at 0 °C then overnight at room temperature. The pure product of **A** (yield: 78 %) was obtained after normal flash chromatography (eluent: ethyl acetate: petroleum ether = 1 : 4). HPLC trace: single peak (Fig. S6a). UV (50% methanol) λ_{max} : 218, 324 nm (Fig. S6b). MS: calc. M⁺ = 503.22, obsvd. ESI-MS: m/z 502.16 [(M-H)⁻].



Figure S6. (a) HPLC trace, and (b) UV spectrum of compound A.

Synthesis of Lys-CBT (**B**): The Boc protecting group of **A** (302 mg, 0.6 mmol) was cleaved with 95% TFA in CH₂Cl₂ for 2 h at room temperature to produce compound **B** in good yield (92%) after HPLC purification. ¹HNMR of compound **B** (d-CDCl₃, 300MHz, Fig. S7): 8.03 (s, 1 H), 7.58 (d, J = 8.9 Hz, 1 H), 7.33 (d, J = 8.8 Hz, 1 H), 4.07 (t, J = 6.1 Hz, 1 H), 2.86 (t, J = 6.9 Hz, 2 H), 1.92 (t, J = 6.5 Hz, 2 H), 1.58 (d, J = 6.9 Hz, 2 H), 1.40 (dd, J₁ = 7.4 Hz, J₂=14.4 Hz, 2 H). MS: calculated for C₁₄H₁₈N₅OS [(M+H)⁺]: 304.1232; obsvd. HR-MALDI-TOF/MS: m/z 304.1234.



Figure S7. ¹HNMR spectrum of compound **B**.

Synthesis of Boc-Cys(SEt)-Lys(Boc-Cys(SEt))-CBT (C): Compound **B**, Boc-Cys(SEt)-OH•DCHA (333.16mg, 0.72 mmol), HBTU (273 .05mg, 0.72 mmol), and HOBt (97.29 mg, 0.72 mmol) in DMF (3 mL) were stirred overnight in presence of DIPEA (116.32 mg, 0.9 mmol). The pure product **C** (yield, 53%) was obtained after normal flash chromatography (eluent: ethyl acetate: petroleum ether= 1: 4) ¹HNMR of compound **C** (d-CD₃OD, 300MHz, Fig. S8): 8.69 (s, 1 H), 8.14(d, J = 8.3 Hz, 1 H), 7.74 (d, J = 8.3 Hz, 1 H), 4.53 (dd, J₁ = 2.7 Hz, J₂ = 7.5 Hz, 1 H), 4.43 (dd, J₁ = 3.8 Hz, J₂ = 8.2 Hz, 1 H), 4.30 (dd, J₁ = 3.4 Hz, J₂ = 8.9 Hz, 1 H), 3.03-3.23 (m, 4 H), 2.81-2.95 (m, 2 H), 2.68-2.78 (m, 4 H), 1.80-1.96 (m, 2 H), 1.54-1.61 (m, 2 H), 1.46-1.48 (d, J = 5.7 Hz, 20 H), 1.28-1.34 (m, 6 H). MS: calc. M⁺ = 829.25, obsvd. ESI-MS: m/z 829.78 [(M+H)⁺].



Figure S8. ¹HNMR spectrum of compound **C**.

Synthesis of Cys(SEt)-Lys(Cys(SEt))-CBT (1): Deprotection of **C** with 95% TFA in DCM for 2 h at room temperature yielded crude product of **1**. Pure compound **1** (yield: 89%) was obtained after HPLC purification. ¹HNMR of compound **1** (d-D₂O 300MHz, Fig. S9a): 8.21 (s, 1 H), 7.72 (d, J = 9.0 Hz, 1 H), 7.42 (d, J = 8.9 Hz, 1 H), 4.50 (t, J = 6.6 Hz, 1 H), 4.32 (t, J = 5.8 Hz, 3 H), 4.08 (t, J = 6.4 Hz, 3 H), 2.92-3.33 (m, 6 H), 2.37-2.51 (m, 4 H), 1.79-1.81 (m, 2 H), 1.51 (m, 2 H), 1.36 (m, 2 H), 0.94-1.03 (m, 6 H). HPLC trace: single peak (Fig. S9b). IR (KBr) ν_{max} 3425, 2927, 2232, 1672, 1578, 1529, 1478, 1401, 1202, 1135, 838, 723, 614, 426 cm⁻¹ (Fig. 4a). UV (50% methanol) λ_{max} : 218, 324 nm (Fig. S9c). MS: calculated for C₂₄H₃₆N₇O₃S₅ [(M+H)⁺]: 630.1483; obsvd. HR-MALDI-TOF/MS: m/z 630.1485.



Figure S9. (a) ¹HNMR spectrum, (b) HPLC trace, and (c) UV spectrum of compound **1**.

Preparation of Cys(SEt)-Lys(Biotin)-CBT (2)¹:

Scheme S2. Synthetic route for compound 2.

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Synthesis of **D**: The isobutyl chloroformate (109 mg, 0.8 mmol) was added to a mixture of Boc-Lys(Fmoc)-OH (375 mg, 0.8 mmol) and MMP (4-methylmorpholine, 151mg, 1.5 mmol) in THF (5.0 mL) at 0 °C under N₂ and the reaction mixture was stirred for 20 min. The solution of 2-cyano-6-aminobenzothiazole (140 mg, 0.8 mmol) was added to the reaction mixture and further stirred for 1 h at 0 °C then overnight at room temperature. Water (50 mL) was added and the reaction mixture was extracted with ethyl acetate (2 X 100 mL). The combined organic phase was dried by Na₂SO₄ and then evaporated. The pure product **D** (yield: 39 %) was obtained after normal flash chromatography (eluent: AcOEt : Hexane = 1 : 1).

Synthesis of compound **E**: The Boc protecting group of **D** was cleaved with 95% TFA in CH₂Cl₂ for 2 h. Precipitated from the cleavage solution using cold diethyl ether, the amino CBT compound **E** was obtained in good yield which was directly used for next step reaction. The mixture of **E** (300 mg, 0.6 mmol), Boc-Cys(SEt)-OH•DCHA (334 mg, 0.72 mmol) and HBTU (300 mg, 0.72 mmol) in DMF (3 mL) was stirred overnight in presence of DIPEA (100 mg, 0.9 mmol). After normal workup, the compound **F** was obtained. Treated with 50% DIPEA in DMF overnight, the desired amino compound **G** was obtained after HPLC purification. MS: calc. $M^+ = 566.7$, obsvd. ESI MS: m/z 567.0 [M⁺].

Synthesis of compound **2**: The mixture of amino compound **G** (67.4 mg, 0.129 mmol), Biotin-NHS (52.9 mg, 0.155 mmol), HBTU (98.2 mg, 0.259 mmol), and DIPEA (10 μ L) in DMF (2 mL) was stirred for 50 min at room temperature, and then purified by HPLC to yield compound **H**. Deprotection of **H** with 95% TFA in DCM in the presence of 1% triisopropylsilane at room temperature for 2 h yielded compound **2** after HPLC preparation. HPLC trace: single peak (Fig.

S10a). UV (50%methanol) λ_{max} : 218, 324 nm (Fig. S10b). MS: calculated for C₂₉H₄₁N₈O₄S₄ [(M+H)⁺]: 693.2134; obsvd. HR-MALDI-TOF/MS: m/z 693.2135.



Figure S10. (a) ¹HNMR spectrum, (b) HPLC trace, and (c) UV spectrum of compound 2.

Scheme S3. Preparation of the oligomers of 1.



Synthesis and characterization of oligners of 1: 10 μ L 0.4 M TCEP was dropped into 1 mL solution of 1 at 1 mM and stirred for 0.5 h. Then 10 μ L solution of 1.2 M Na₂CO₃ was added. The white emulsion was formed 5 mins later. MALDI-TOF mass analysis indicated that the majority of the oligomers are the dimers of 1 (e.g., 1-D-3). MS: calc.M⁺=984.21, obsvd. MALDI-TOF MS: m/z 985.296 [(M+H)⁺].

Scheme S4. Preparation of crosslinked polymer of 1-D-3.



Synthesis and characterization of crosslinked polymers of **1-D-3**: The oligomers of **1** was dissolved in methanol and applied on a HPLC column for separation. Main peaks on the HPLC trace were identified with MALDI-TOF mass analysis. The peak at the retention time of 25.7 min (**1-D-3**) was collected and evaporated at 50 $^{\circ}$ C to yield yellowish, polymeric powders.

Scheme S5. Degradation of polymers.



Reduction of crosslinked polymers of 1-D-3: The polymers of 1-D-3 was partially reduced by TCEP to yield 1-D-3, as showed in scheme S5 and proved in figure 3d&e.

Reference:

[1] Liang, G. L.; Ren, H. J.; and Rao, J. H. Nat. Chem. 2010, 2: 54-60.