Electronic Supplementary Material (ESI) for Nanoscale.

Electronic Supplementary Information

Bottom-up on-surface synthesis based on click-functionalized peptide bundles

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Materials and methods

Materials. Amino acids and resin were purchased from Jil Biochemical Co., Ltd (Shanghai, China). All organic solvents were purchased from Dingsheng Limited Liability Company (Chengdu, China). 4-maleimide butyric acid was purchased from Block Chemical Technology Co., Ltd (Shanghai, China). Single-ended modified alkynyl PEG (PEG-alkynyl 10kDa), 4-pentynoic acid, 2-azidoacetic acid, and dopamine were bought from Jiankai Co., Ltd. (Shanghai, China). The mica sheets were purchased from a mirror instrument. CCK8 was purchased from Glpbio company (California America). Mouse epithelioid fibroblasts cell line (L929) was purchased from the Chinese Academy of Science Cell Bank (Shanghai, China). Triisopropylsilane (TIPS), ethylenedithiol (EDT), O-(1H-6-chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HCTU) and N,N-diisopropylethylamine (DIPEA), ethyl(hydroxyimino)cyanoacetate (Oxyma), N,N′-diisopropylcarbodiimide (DIC), tris (2-carboxyethyl) phosphine (TCEP), and piperidine were purchased from Adamas Reagent Co., Ltd.

Synthesis and characterization of DA-SH (Scheme S1 and Figure S1). First, 100 mg amino resin (0.06 mmol) was weighed and added to 5 mL DMF,and the solution was swelled for 30 min. Subsequently, The resin protection group was deprotected using 20% piperidine in DMF for 20 min (10mL, 3 times). Then, the DMF solution of Fomc-Cys (Trt)-OH (0.17 mmol), DIEA (0.425 mmol), and HCTU (0.17 mmol) was added to the resin for coupling, and the solution was shaken for 3 hour. After coupling, The Fmoc amine-protection group was deprotected using 20% piperidine in DMF for 20 min (10mL, 3 times). Then, the DMF solution of DIEA (0.75 mmol), HCTU (0.3 mmol), and 3,4-dihydroxyphenylacetic acid (0.3 mmol) DMF solution was added to the resin. Finally, the resin was washed with DMF (10 mL, 3 times) and dichloromethane (DCM, 10 mL, 2 times). The resin was cleaved for 3 h using a cleavage solution consisting of 92.5 % trifluoroacetic acid (TFA), 2.5 % triisopropylsilane (TIPS), 2.5 % ethylenedithiol (EDT) and 2.5 % deionized water. The cleavage solution was precipitated in ice ether to give the pale yellow solid product DA-SH. ^{1}H NMR (400 MHz, CD3OD, δ) of DA-SH : 6.91-6.64 (m, 5H), 4.50 (t, 1H), 3.85-3.76 (m, 1H), 3.54-3.46 (m, 1H), 2.91-2.76 (m, 2H) ppm (AV-400, Bruker, USA).

Synthesis of peptides 1, peptides 2, peptides 3, and peptides 4 (Scheme S2 and Scheme S3). The amino acid sequence of all the peptides was synthesized by solid phase peptide synthesis (SPPS) on a Liberty blue microwave peptide synthesizer (CEM) at a scale of 0.25 mmol from the C-terminus to the N-terminus using rink amino resin based on the Fmoc standard protocol. The coupling reaction of the resin with a mixture of amino acids (0.2 mmol), Oxyma (1 mmol), and DIC (0.5 mmol) was carried out by microwave heating at 90 ° C for 2 minutes. Then the resin was washed with DMF for 4 times between each steps. The Fmoc amine-protection group was deprotected using 20% piperidine in DMF for 0.5 min at ~90°C via microwave heating. After the synthesis on peptide synthesizer, the DMF solution of N-(3-carboxypropyl) maleimide (1 mmol), HCTU (1 mmol), and N, N-diisopropylethylamine (2.5 mmol) was added to the resin for the final coupling, and the solution was shaken for 1 hour. The same solution was added again to achieve dual coupling. Finally, the resin was washed with DMF (10mL, 3 times) and dichloromethane (DCM, 10mL, 2 times). Then, the resin was cleaved for 3 hours using cleavage solution consisting of 95 % trifluoroacetic acid (TFA), 2.5 % TIPS, and 2.5 % deionized water, then the cleavage solution was precipitated in ice ether to give the crude product of peptide 1.[1] Replacing N-(3-carboxypropyl) maleimide with 4-pentynoic acid and 2-azidoacetic acid at the final coupling step, the crude products of peptide 3 and peptide 4 were obtained. Peptide 2 was directly subjected to general SPPS on a microwave peptide synthesizer. The resulting resin was then directly cleaved for 3 hours using a pyrolysis solution consisting of 92.5 % TFA, 2.5 % TIPS, 2.5 % EDT, and 2.5 % deionized water, and the cleavage solution was precipitated in ice ether to give the crude product of peptide 2.

Synthesis and characterization of DA-alkynyl and DA-N_3 (Scheme S4 and Figure S4). DMF solution (1mL) containing DIPEA (0.25 mmol), HCTU (0.1 mmol), and 4-pentynoic (0.1 mmol) was added to the DMF solution of

dopamine (0.1 mmol, 1mL), then the mixed solution was shaken at room temperature for 3h. After the reaction, the solution was precipitated in ice ether to give the brownish-yellow product DA-alkynyl. ^{1}H NMR (400 MHz, CD_3OD, δ) of DA-alkynyl: 6.78-6.74 (m, 2H), 6.63-6.61 (m, 1H), 4.05 (q, 1H), 3.51 (q, 1H), 3.22-3.15 (m, 2H), 3.01-2.92 (m, 3H), 2.88-2.72 (m, 2H) ppm.
Replacing 4-pentynoic acid with 2-azidoacetic acid, DA-N_3 was obtained through the same synthesis steps. ^{1}H NMR (400 MHz, CD_3OD, δ) of DA-N_3: 6.77-6.75 (m, 2H), 6.64-6.61 (m, 1H), 4.05 (q, 1H), 3.51 (q, 1H), 3.22-3.15 (m, 1H), 3.10-2.88 (m, 3H) ppm.

Preparation of rigid rods on mica sheet using bundlemer 3 and bundlemer 4. We prepared two separate solutions, which were 1 mmol peptide 3 and peptide 4 in phosphate buffer (pH 6.25, 2 mL), respectively, for the formation of bundlemer 3 and bundlemer 4 (Figure S4). Next, a piece of clean mica sheet was put into DA-N_3 solution (0.1 mmol, 2 mL, pH 7), and the resulting solution was shaken for 6 hours. Then we took out the mica sheet and washed it with deionized water. The washed mica sheets were placed in aqueous solutions containing bundlemer 3 (2 mL). At the same time, $CuSO_4$ (0.002 mmol) and sodium ascorbate (0.01 mmol) were added as catalysts to the bundlemer solution and the resulting solution was shaken for 12 hours. Then the mica sheet was taken out and washed with deionized water, and placed in aqueous solution containing bundlemer 4 (2 mL). $CuSO_4$ (0.002 mmol) and sodium ascorbate (0.01 mmol) were added to the solution and the resulting solution was shaken for 12 hours. Then the mica sheet was taken out for characterization after drying.

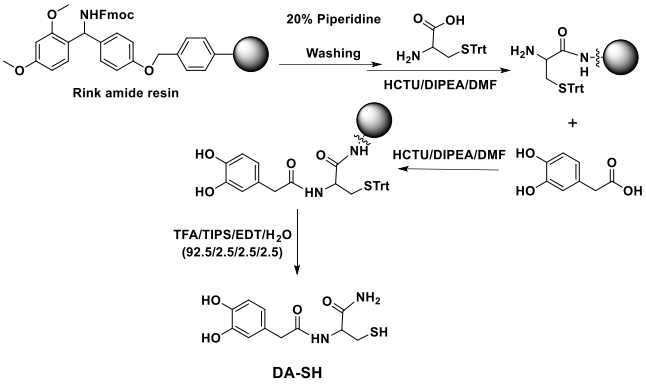
Preparation of bundlemer-PEG and rod-PEG. PEG-alkynyl (0.001 mmol) aqueous solution (2 mL) was prepared first. Then the mica sheets with one bundlemer or rigid rod composed of three bundlemers on the surface were also prepared. PEG-alkynyl solution was added to the bundlemer or rigid rod grafted mica sheets, and $CuSO_4$ (0.002 mmol) and sodium ascorbate (0.01 mmol) were added as catalysts. The resulting solutions were shaken for 12 hours. Then the mica sheets were taken out for characterization after drying. Or the mica sheets were placed in 1 mol hydrochloric acid solution (0.5 mL) for 1h, and the resulting solutions were then adjusted the pH to neutral for characterization.

AFM characterization. We use the Dimension ICON instrument from the Bruker company (Germany) to test with the ScanAsyst Air ultra-sharp tip. We use a clean mica sheet as the substrate for sample deposition, and the mica sheet is fixed on the slide with double-sided glue. The sample solution was prepared in advance, and 5 μL of the solution was added on the mica sheet for AFM characterization after air-drying.

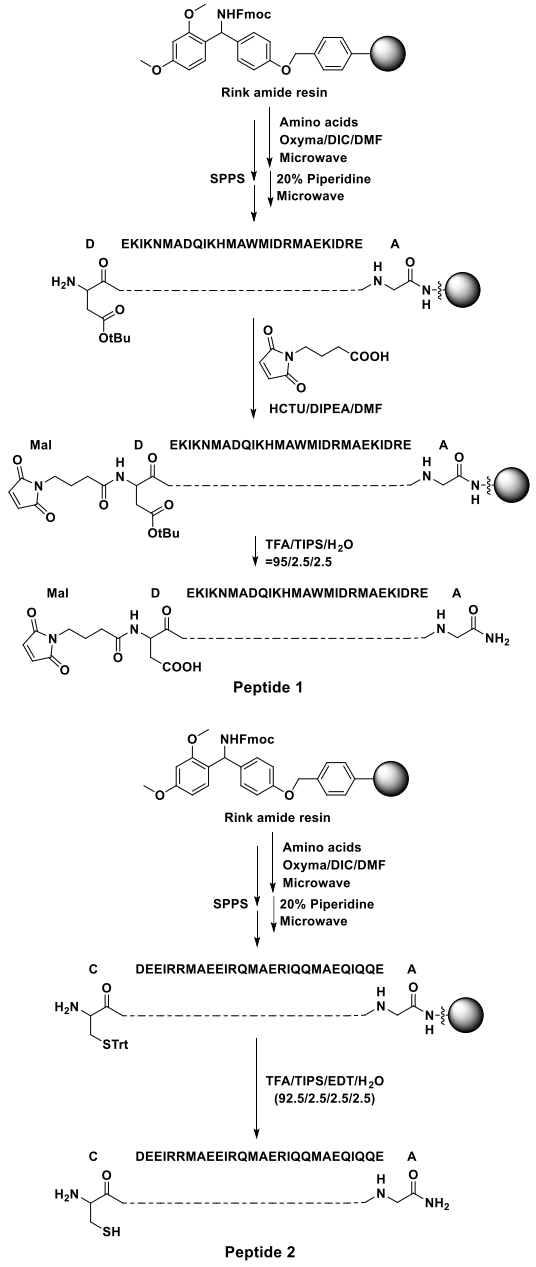
Zeta potential characterization of rod-PEG. Zeta potential was detected by DLS analysis using a Malvern Zetasizer (Nano ZS Malvern, UK). Rod-PEG was pre-prepared in an aqueous solution of a suitable concentration, and 1 ml of the solution was added to a cuvette for direct testing.

Biocompatibility of rod-PEG. L929 cells were uniformly inoculated to the 96-well plates and cultured for 24 hours. Subsequently, Dulbecco's modified eagle medium containing different concentrations of rod-PEG were used as the medium to incubate cells for 36 hours, then the cell viability was quantified using the commercial CCK8 cell proliferation assay kit by following the manufacturer’s procedure.

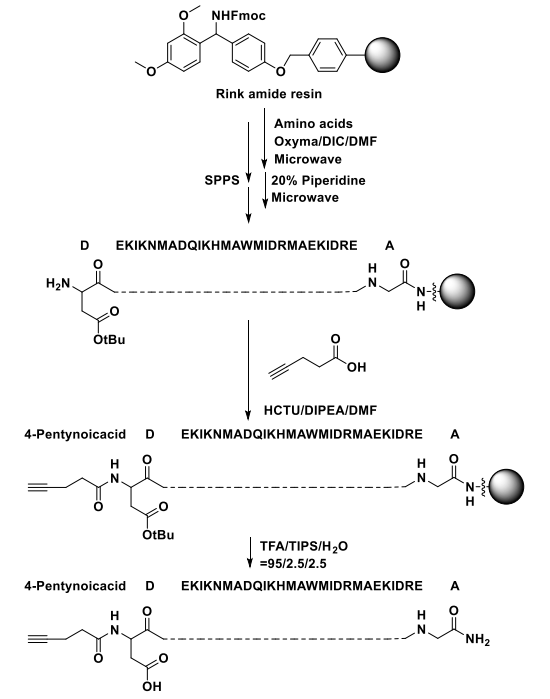
Sch. S1 Synthesis of DA-SH



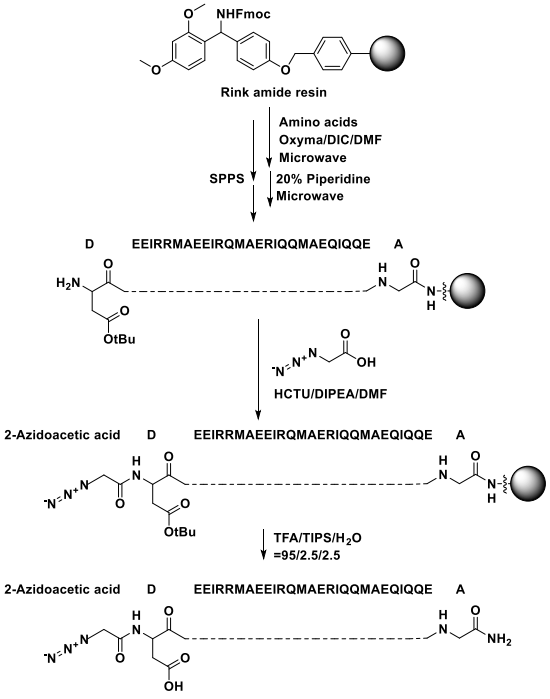
Sch. S2 Synthesis of peptides 1 and peptides 2



Sch. S3 Synthesis of peptides 3 and peptides 4

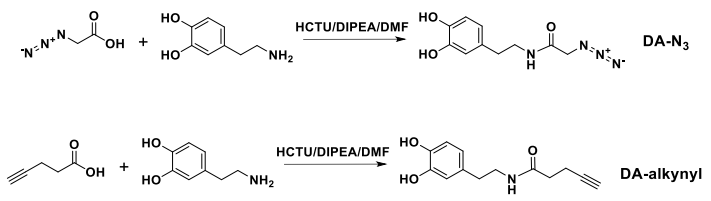


Peptide 3



Peptide 4

Sch. S4 (A) Synthetic route to make DA-alkynyl. (B) Synthetic route to make DA-N_3.



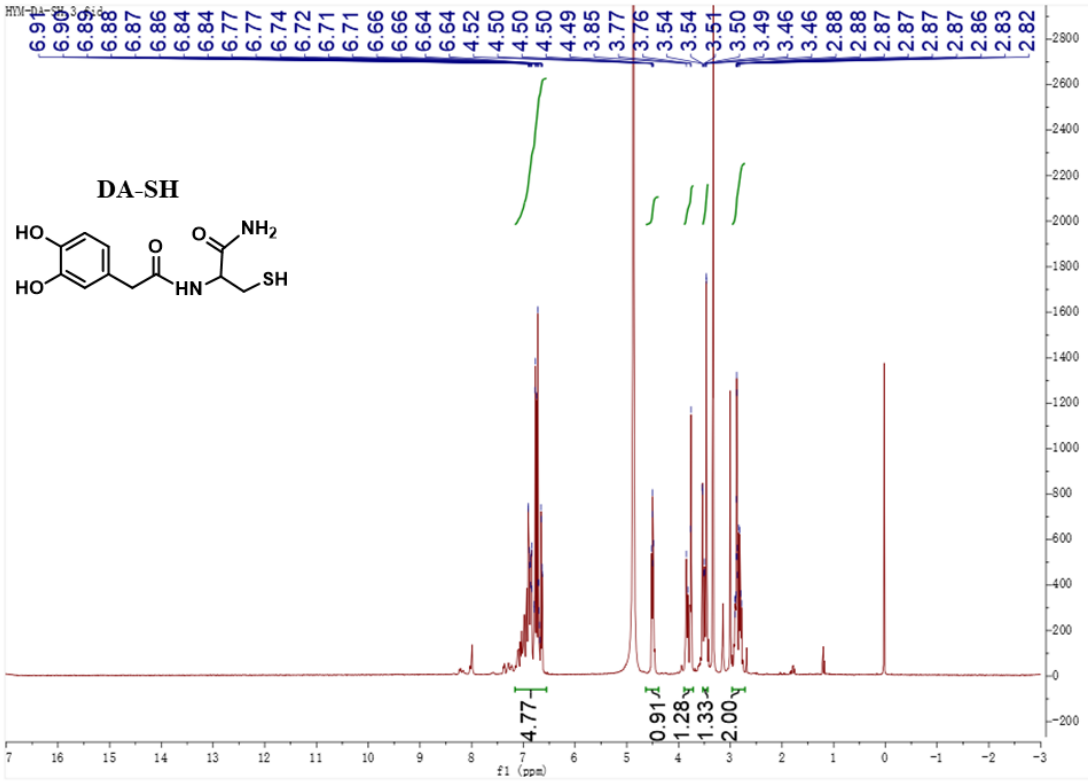


Fig. S1 ^{1}H NMR (400 MHz, CD_3OD) spectrum of DA-SH.

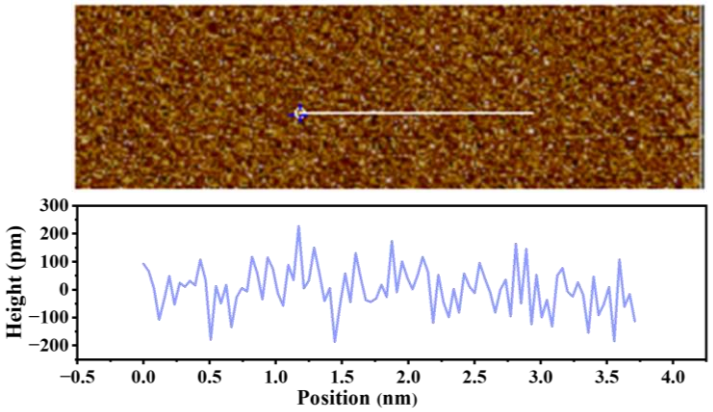


Fig. S2 The AFM image of untreated mica sheet surface.

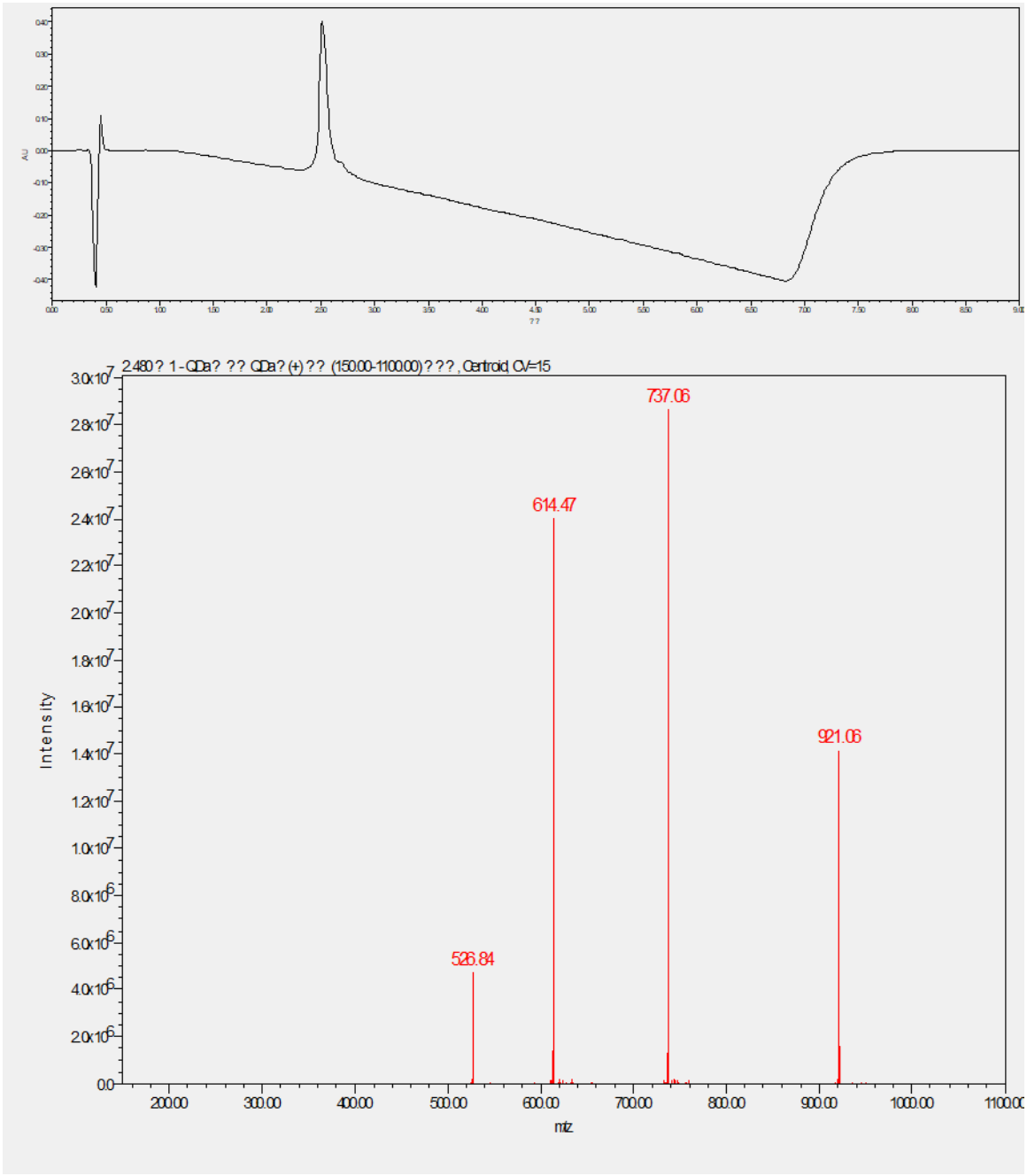


Fig. S3 UPLC diagram (top) and mass spectra (bottom) of peptide 1. Calculated mass is 3683 Da. (Mass spectral data were collected on a Waters K13 QSM 178A mass spectrometer)

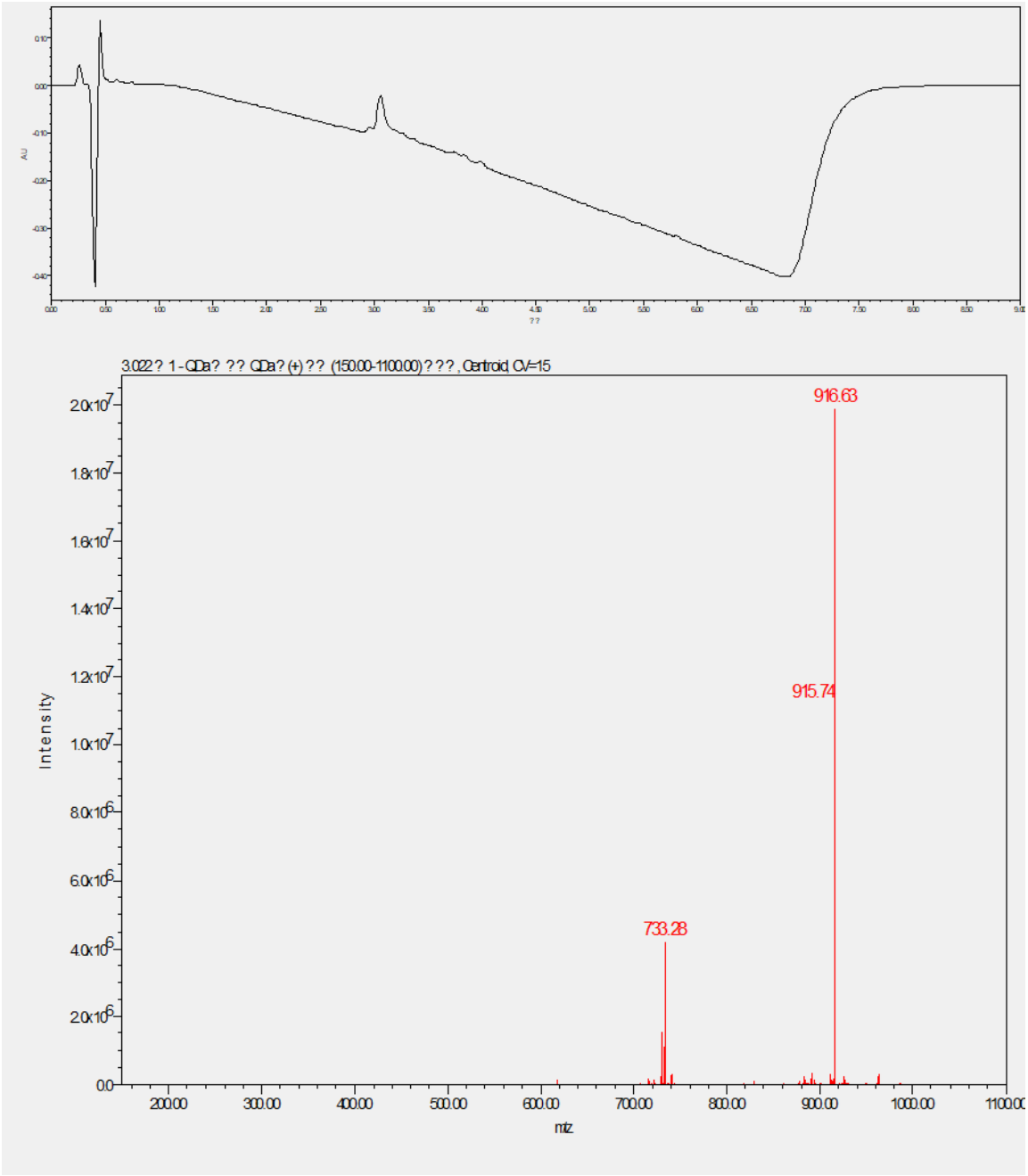


Fig. S4 UPLC diagram (top) and mass spectra (bottom) of peptide 2. Calculated mass is 3663 Da. (Mass spectral data were collected on a Waters K13 QSM 178A mass spectrometer)

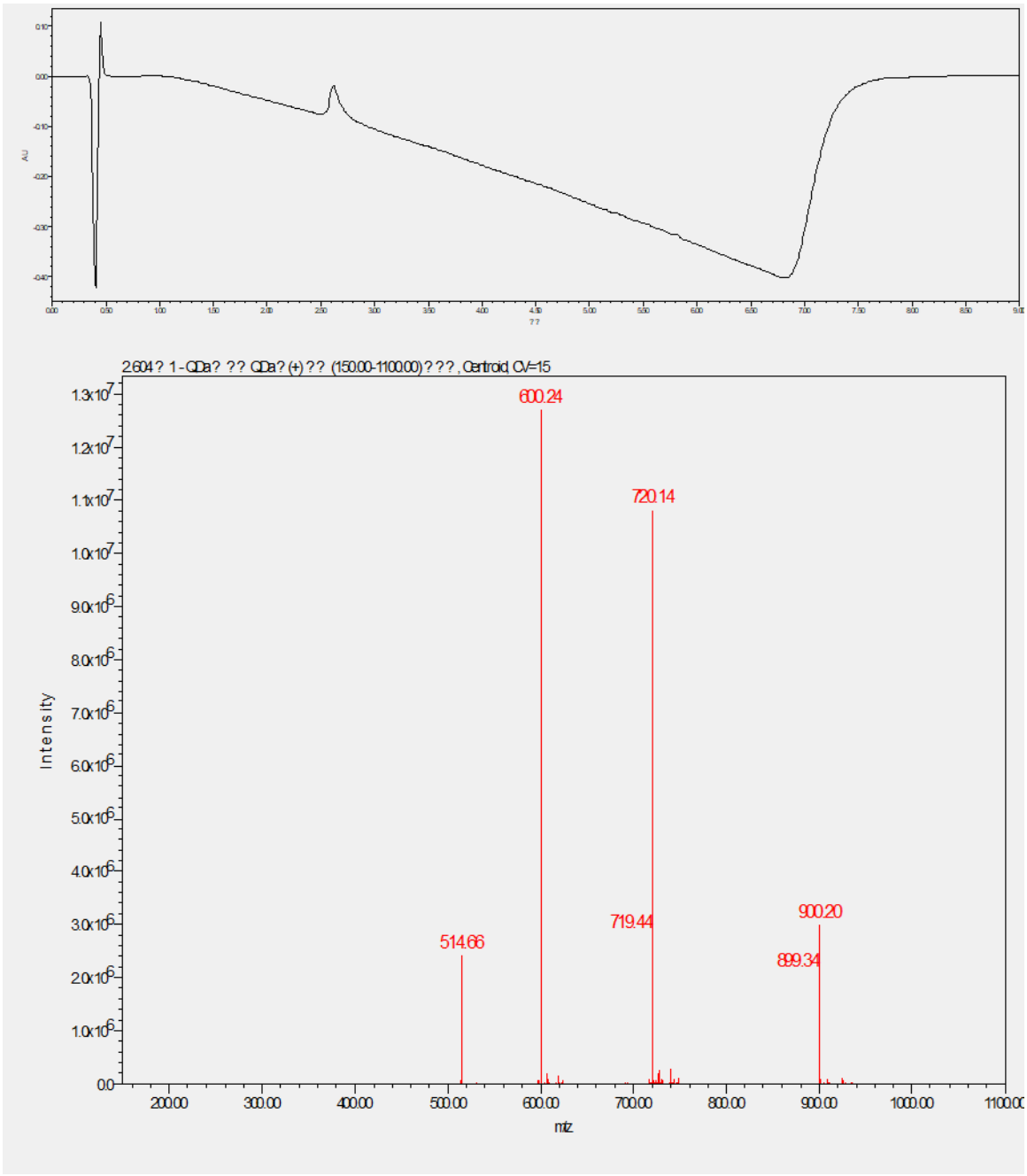


Fig. S5 UPLC diagram (top) and mass spectra (bottom) of peptide 3. Calculated mass is 3597 Da. (Mass spectral data were collected on a Waters K13 QSM 178A mass spectrometer)

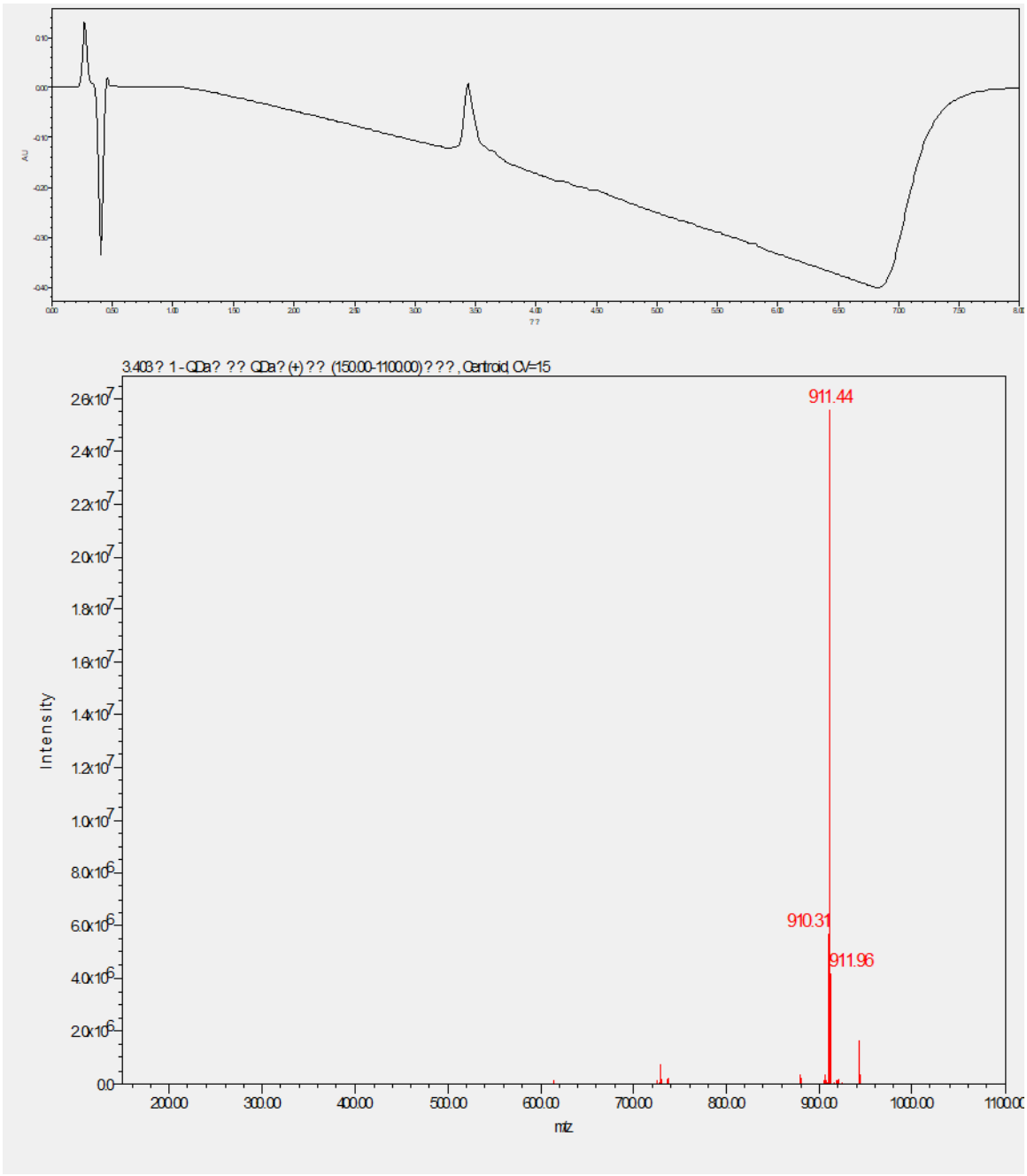


Fig. S6 UPLC diagram (top) and mass spectra (bottom) of peptide 4. Calculated mass is 3643 Da. (Mass spectral data were collected on a Waters K13 QSM 178A mass spectrometer)

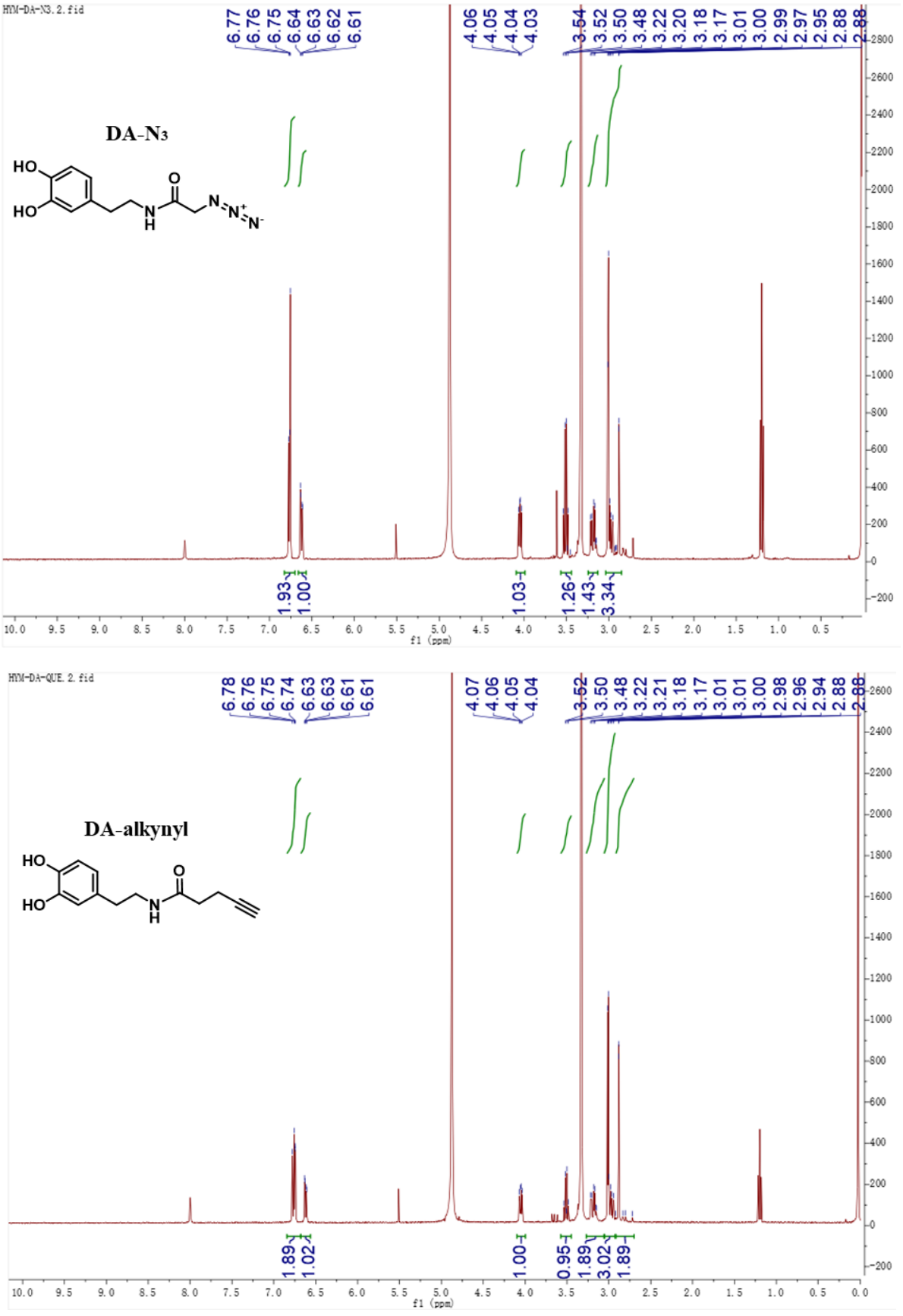


Fig. S7 ^{1}H NMR (400 MHz, CD_3OD) spectrum of DA-N_3 (top) and DA-alkynyl (bottom).

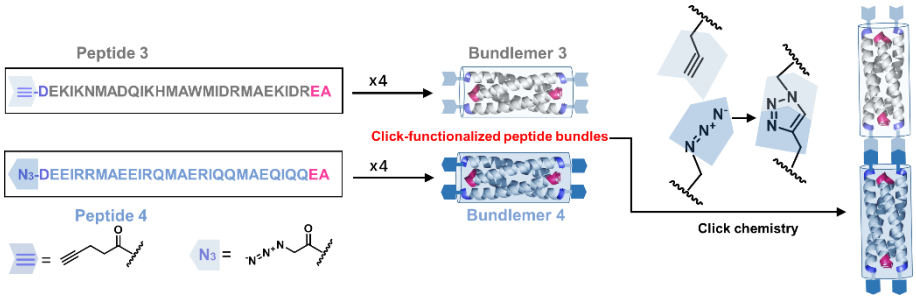


Fig. S8 The folding of peptide 3 and peptide 4 to form bundlemer 3 and bundlemer 4, respectively, and the CuAAC click chemistry between bundlemer 3 and bundlemer 4 to produce rigid rod.

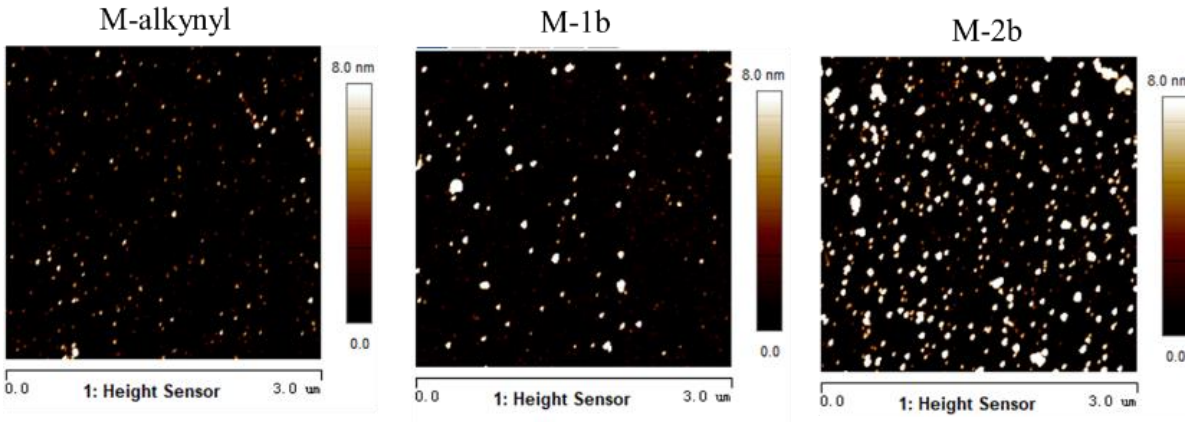


Fig. S9 The AFM images of rigid rods prepared by CuAAC click chemistry using bundlemer 3 and bundlemer 4 on the mica sheet. M-alkyne is the AFM image of the DA-alkynyl modified mica sheet, M-1b is the AFM image of bundlemer 4 on the mica sheet, and M-2b is the AFM image of the rigid rods composed of two bundlemers on the mica sheet.

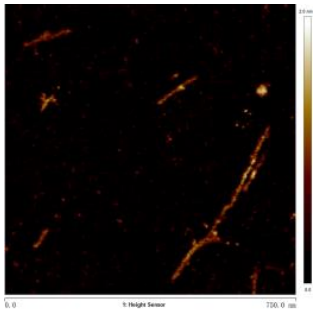


Fig. S10 AFM image of the nano-hyperstructure formed by self-assembly of the released bundle-PEG

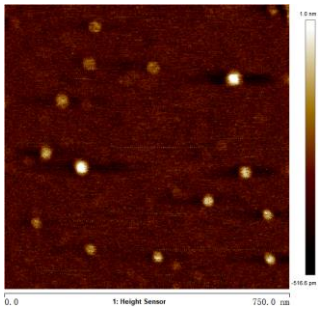


Fig. S11 AFM image of the nano-hyperstructure formed by self-assembly of the released rod-PEG.

References

1. D. Wu, N. Sinha, J. Lee, B. P. Sutherland, N. I. Halaszynski, Y. Tian, J. Caplan, H. V. Zhang, J. G. Saven, C. J. Kloxin and D. J. Pochan, Nature, 2019, 574, 658-662.