Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information
For
Amphiphilic multi-charged cyclodextrins and vitamine K co-assembly as synergistic coagulant

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1 Materials and Methods

Materials

All the reagents and solvents were commercially available and used as received unless otherwise specified purification. 8-Hydroxypyrene-1, 3, 6-trisulfonic acid, trisodium salt (HPTS), 1-buthylimidazole and 1-octylimidazole were purchased from Sigma-Aldrich. Heparin, hyaluronic acid and alginic acid were purchased from Macklin.

UV/Vis spectroscopy

UV-Vis spectra were recorded in a quartz cuvette (light path 10 mm) on a Shimadzu UV-2450 UV-Vis spectrophotometer equipped with a dual cuvette peltier accessory and a temperature controller (TCC-240A).

Fluorescence spectroscopy

Steady-state fluorescence measurements were recorded in a conventional quartz cuvette (light path 10 mm) on a Cary Eclipse equipped with a Cary single-cuvette peltier accessory.

TEM, measurements

TEM images were recorded on a Philips Tecnai G2 20S-TWIN microscope operating at an accelerating voltage of 200 keV. The sample for TEM measurements was prepared by dropping the solution onto a copper grid. The grid was then air-dried.

DLS measurements

The samples were examined on a laser light scattering spectrometer (BI-200SM) equipped with a digital correlator (TurboCorr) at 636 nm at a scattering angle of 90u. The hydrodynamic radius (R_h) was determined by dynamic light scattering

experiments.

Zeta potential measurements.

Zeta potential values were determined on a Brookheaven ZetaPALS (Brookheaven Instrument, USA) at 25 °C. The instrument utilizes phase analysis light scattering to provide an average over multiple particles. Doubly distilled water was used as the background electrolyte for zeta potential measurements.

The NMR spectra and mass spectra measurement

^{1}H and ^{13}C NMR data were recorded on a Bruker AV400 spectrometer. Mass spectra were performed on a Varian 7.0T FTICR-MS (ESI-FTMS).

aPTT activity measurements

All of the aPTT activity measurements and samples incubation was carried out at 37 °C. 500 μL test sample was taken from 2.5 mL of citrated plasma (plasma: sodium citrate 9:1) as control. UFH and LWMH were added to the remaining 2.0 mL of citrated blood, incubated for 2 min and fractioned into 400 μL samples. Then the appropriate amount of AMCD solution was added to the remaining samples. The samples were incubated for 5 min and centrifuged at 2500 rpm for 10 min. The plasma (100 μL) was added to 100 μL of aPTT reagent solution, incubated for 4 min, and then 100 μL of 0.02 M $CaCl_2$ solution was added. Once a clot formed the timer was stopped and the clotting time was recorded.

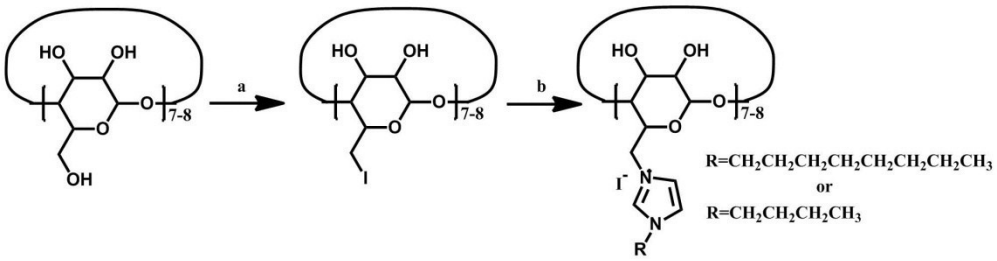
The preparation of AMCD-VK co-assembly and the VK release

The AMCD-VK co-assembly solutions were prepared by a film hydration method. In brief, AMCD and VK were dissolved in 2 mL chloroform. Chloroform was

evaporated using a rotary evaporator to create a thin lipid film. This film was re-suspended in 2 mL water after 0.5 h of stirring at 55 ºC and sonicated for another 0.5 h at the same temperature. The solution was then dialyzed for 3 hours to remove the unloaded VK.

The VK released experiment was carried out by adding heparin to the solution of AMCD-VK co-assembly and stirring for 5 mins. Then the solution was dialyzed for 3 hours to remove the released heparin.

2 Syntheses of AMCDs



Scheme S1. Synthetic routes of **AMCDs.** a) triarylphosphines, iodine and DMF; b) 1-buthylimidazole or 1-octylimidazole.

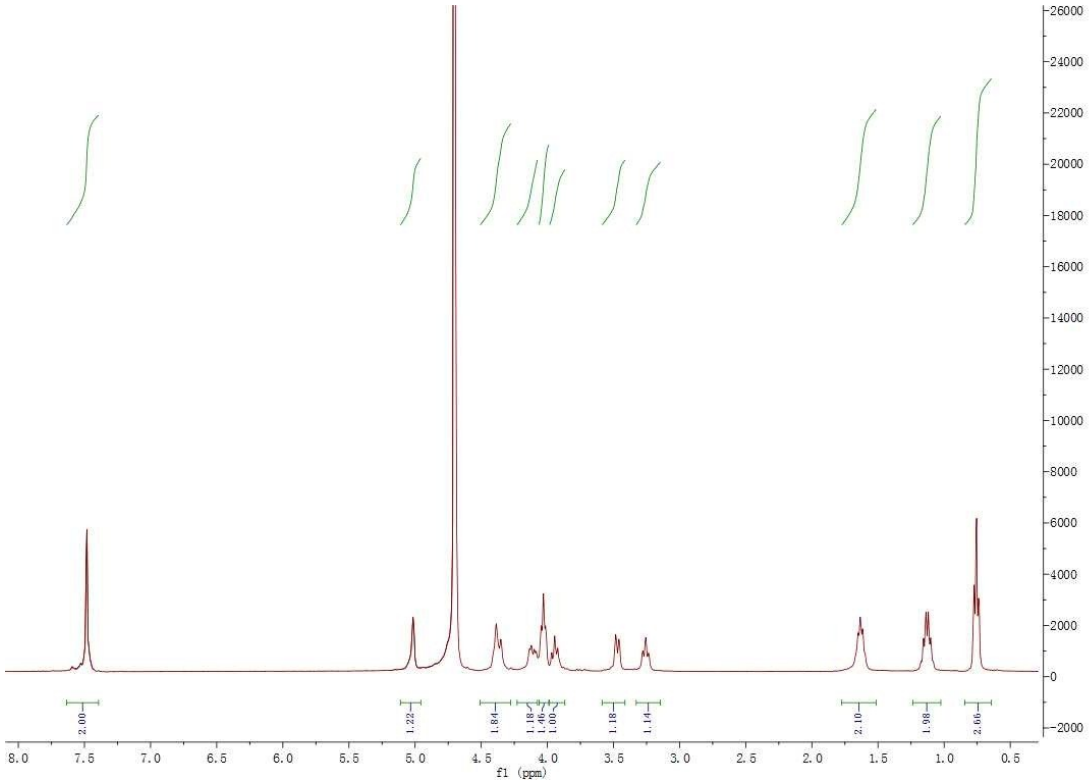
The AMCD was synthesized according to the following general procedure. Octakis (6-deoxy-6-iodo)-γ-cyclodextrin (200 mg) was dissolved in 1-octylimidazole (20.0 mL). The reaction mixture was stirred at 80 °C under argon atmosphere for 2 days. After that the resultant solution was poured into acetone (100 mL) and the precipitate formed was collected by filtration. The filter was recrystallized from water to give a light yellow solid.

Synthesis AMCD-γ4C

^{1}H NMR (400 MHz, D2O) δ: 7.53 (s, 2H), 5.02 (s, 1H), 4.51 – 4.28 (m, 2H), 4.11 (dd, J = 14.2, 5.5 Hz, 1H), 4.03 (t, J = 6.5 Hz, 2H), 3.94 (t, J = 9.1 Hz, 1H), 3.47 (d, J = 9.9 Hz, 1H), 3.26 (t, J = 8.9 Hz, 1H), 1.77 – 1.51 (m, 2H), 1.24 – 1.03 (m, 2H), 0.76 (t, J = 6.6 Hz, 3H).

^{13}C NMR (100 MHz, D_2O) δ: 136.75, 123.22, 123.07, 101.92, 82.17, 71.90, 71.60, 69.06, 49.83, 49.52, 31.26, 18.77, 12.58.

ESI-FTMS m/z: [M - I] +, found 3042.52.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1.98
2.06
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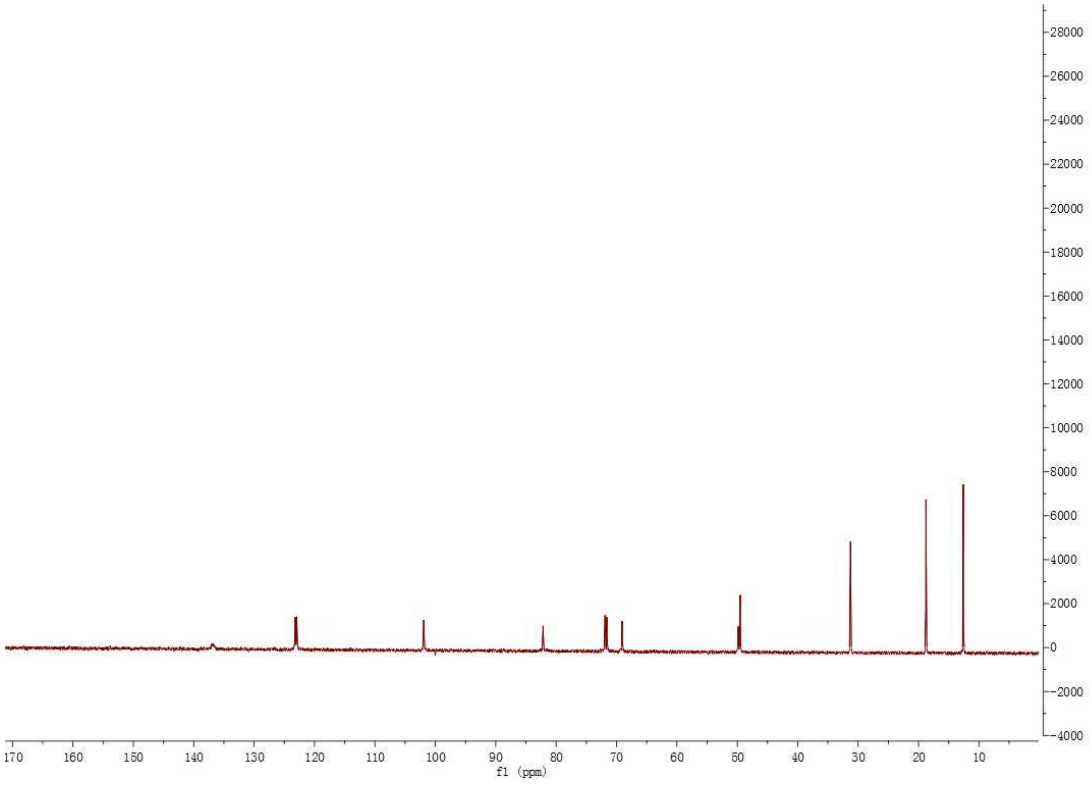
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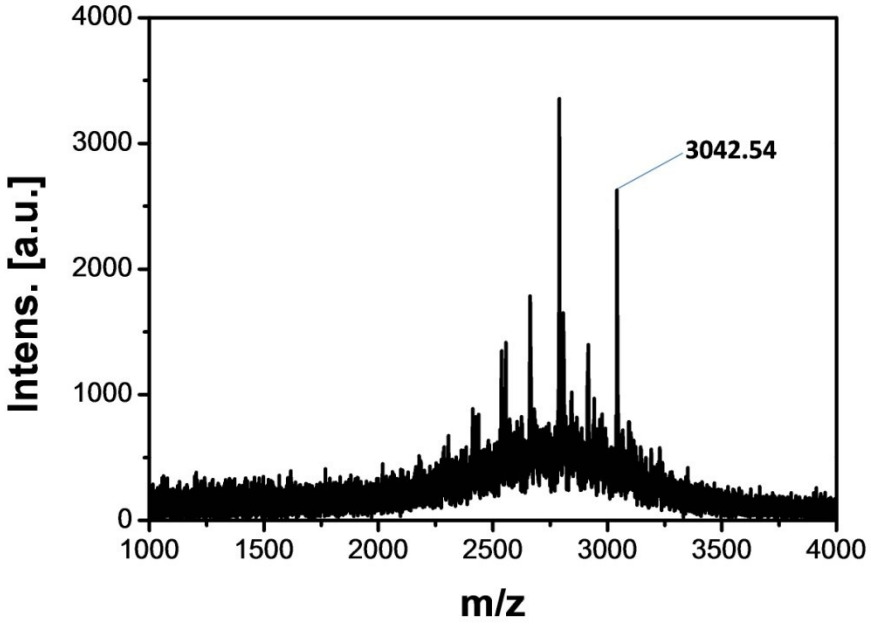
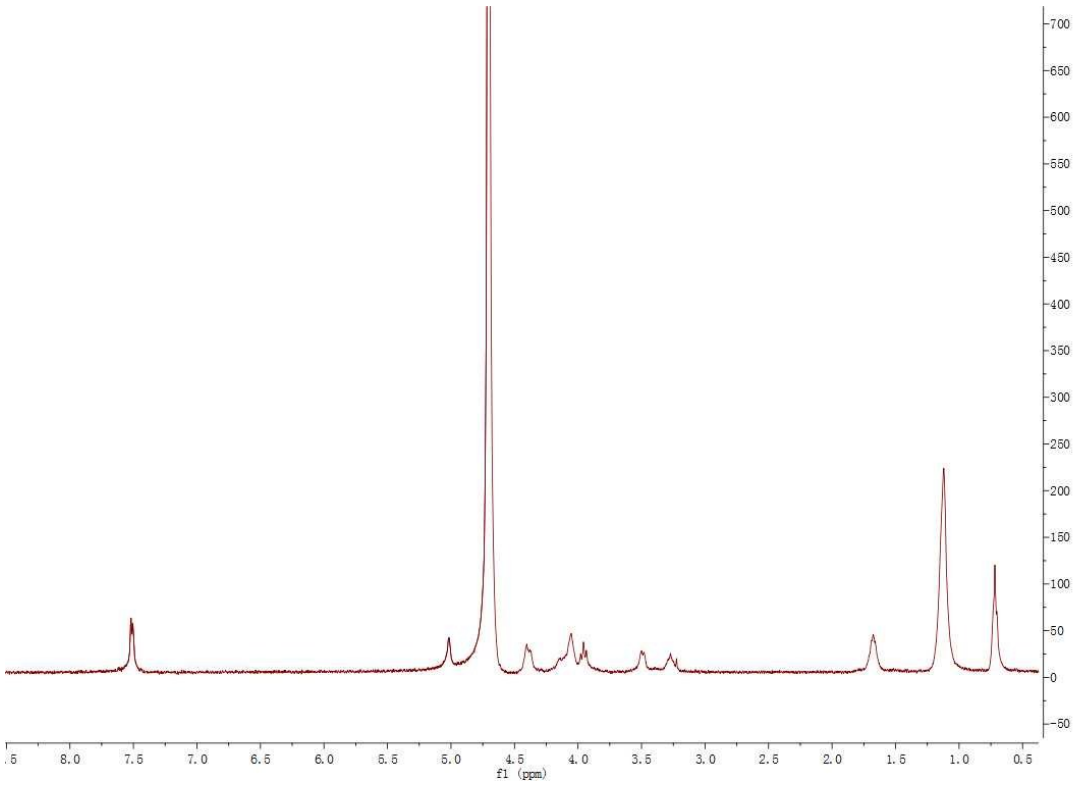


Figure S1. (a) ^{1}H NMR spectrum of **AMCD-γ4C** in D_2O, 400 MHz, 25 °C, and (b)^{13}C NMR spectrum of **AMCD-γ4C** in D_2O, 100 MHz, 25 °C, and (c) ESI-FTMS of **AMCD-γ4C**.

Synthesis AMCD-γ8C

^{1}H NMR (400 MHz, D_2O) δ: 7.63 (s, 2H), 5.07 (s, 1H), 4.37 (m, 2H), 3.84 (m, 4H), 3.55 (d, 1H), 3.38 (t, 1H), 1.67 (m, 2H), 1.12 (m, 10H), 0.70 (t, 3H).

^{13}C NMR (100 MHz, D_2O) δ: 137.69, 123.89, 122.56, 101.80, 82.42, 71.82, 69.29, 49.81, 49.11, 30.85, 29.96, 28.53, 22.47, 19.97, 13.74.



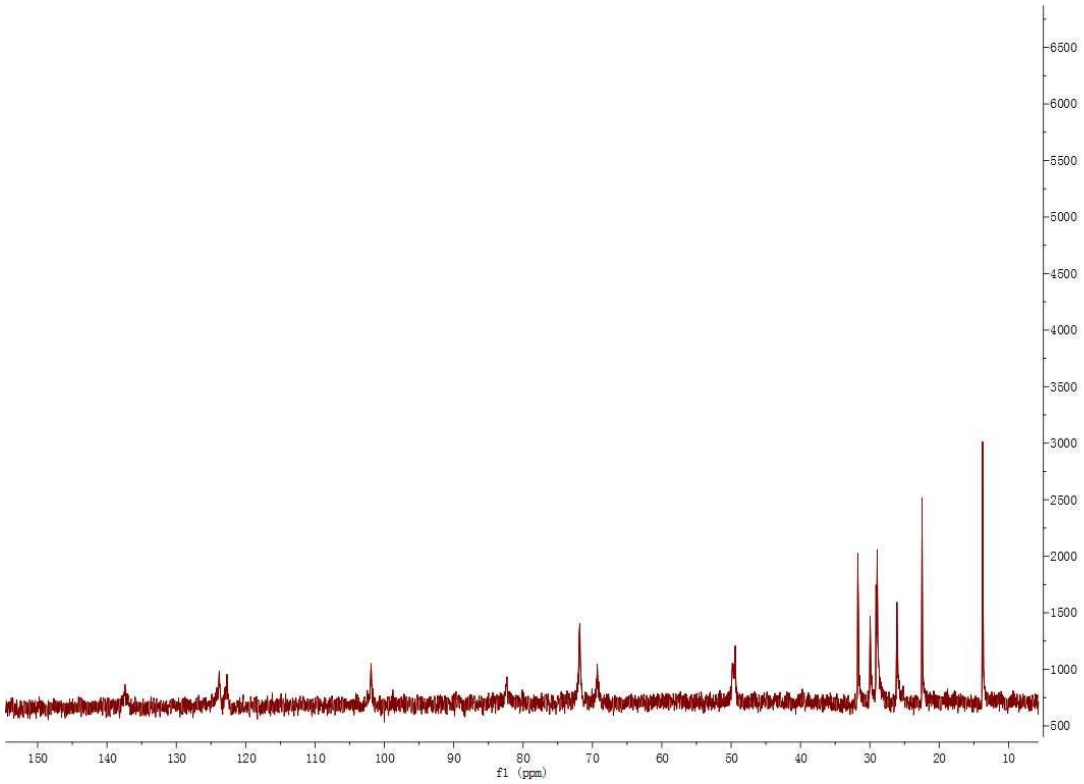


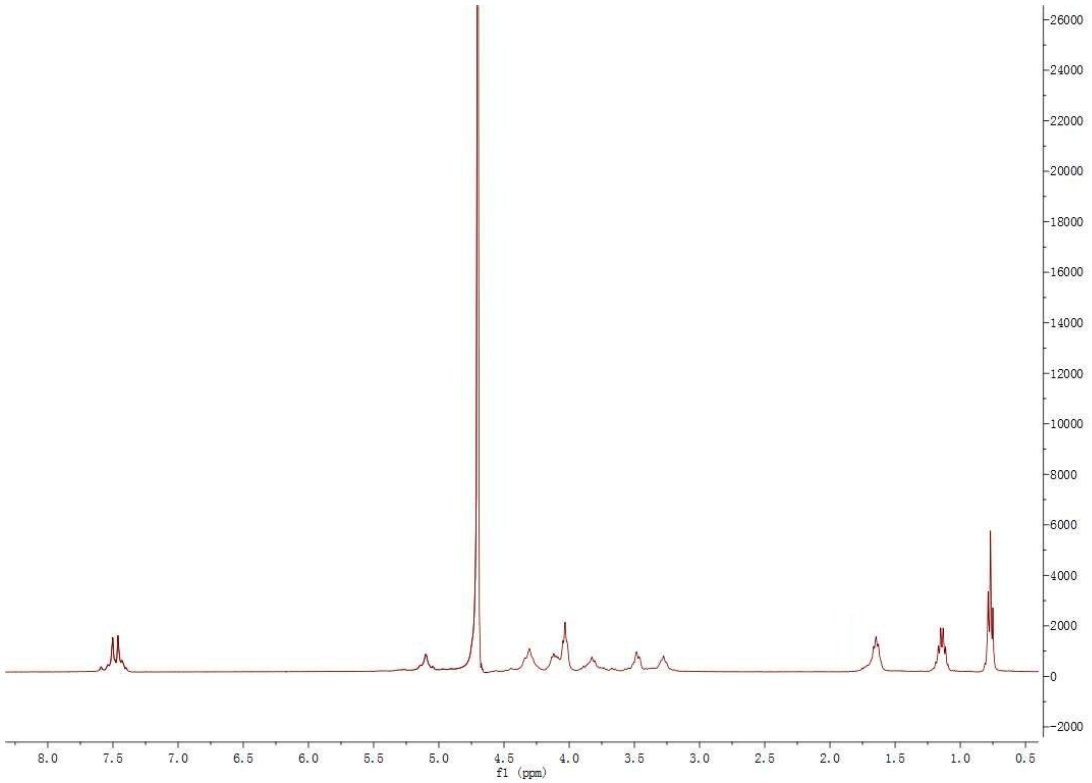
Figure S2. (a) ^{1}H NMR spectrum of **AMCD-γ8C** in D_2O, 400 MHz, 25 °C, and (b)^{13}C NMR spectrum of **AMCD-γ8C** in D_2O, 100 MHz, 25 °C, and (c) ESI-FTMS of **AMCD-γ8C**.

Synthesis AMCD-β4C

^{1}H NMR (400 MHz, D_2O) δ: 7.55 (s, 1H), 7.45 (s, 1H), 5.11 (s, 1H), 4.31 (m, 2H), 4.12 (dd, 1H), 4.05 (t, J = 6.8 Hz, 2H), 3.83 (t, 1H), 3.48 (d, J = 8.0 Hz, 1H), 3.28 (t, 1H), 1.80 – 1.55 (m, 2H), 1.15 (m, J = 14.9, 7.5 Hz, 2H), 0.77 (t, J = 7.4 Hz, 3H).

^{13}C NMR (100 MHz, D_2O) δ: 136.75, 123.25, 122.85, 101.02, 81.36, 71.59, 68.58, 49.73, 49.46, 31.21, 18.73, 12.55.

ESI-FTMS m/z: [M - I] +, found 2646.48.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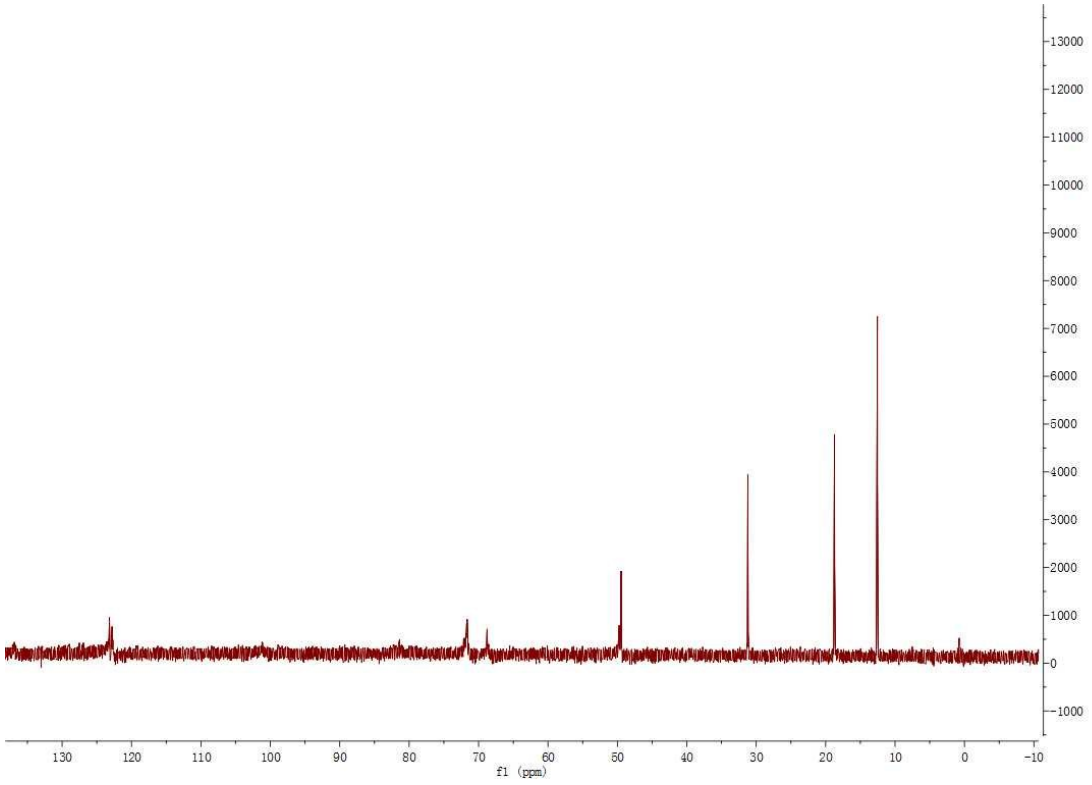
f1 (ppm)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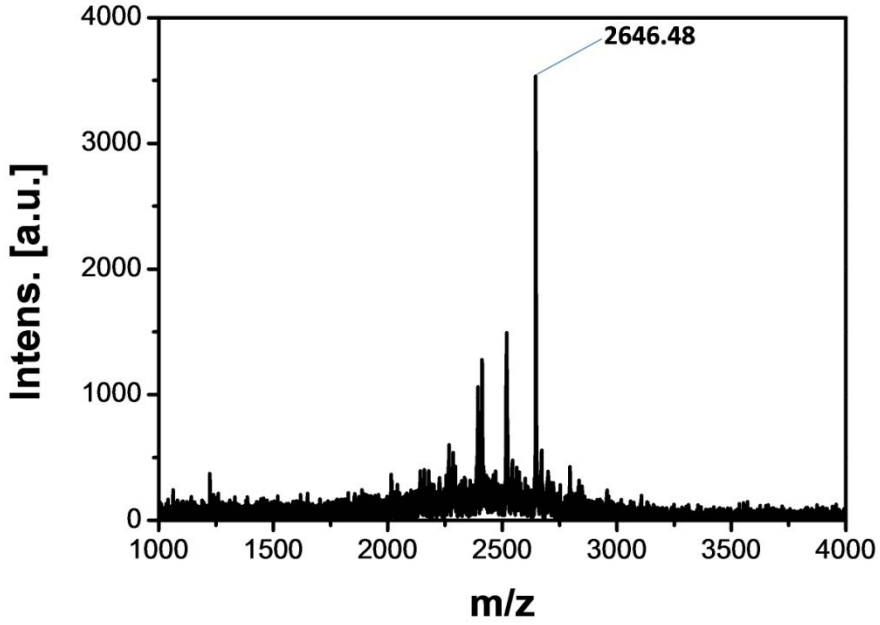

Figure S3. (a) ^{1}H NMR spectrum of **AMCD-β4C** in D_2O, 400 MHz, 25 °C, and (b)^{13}C NMR spectrum of **AMCD-β4C** in D_2O, 100 MHz, 25 °C, and (c) ESI-FTMS of **AMCD-β4C**.

Synthesis AMCD-β8C

^{1}H NMR (400 MHz, D_2O) δ: 7.51 (s, *J* = 5.8 Hz, 2H), 5.02 (s, 1H), 4.40 (m, 2H), 4.20 – 4.00 (m, 3H), 4.00 – 3.89 (t, 1H), 3.50 (d, 1H), 3.25 (t, 1H), 1.68 (m, 2H), 1.12 (m, 10H), 0.72 (t, 3H).

^{13}C NMR (100 MHz, D_2O) δ: 137.69, 123.59, 122.53, 101.40, 82.48, 71.98, 71.23, 68.74, 50.13, 49.48, 31.56, 29.48, 28.85, 25.73, 22.28, 13.38.

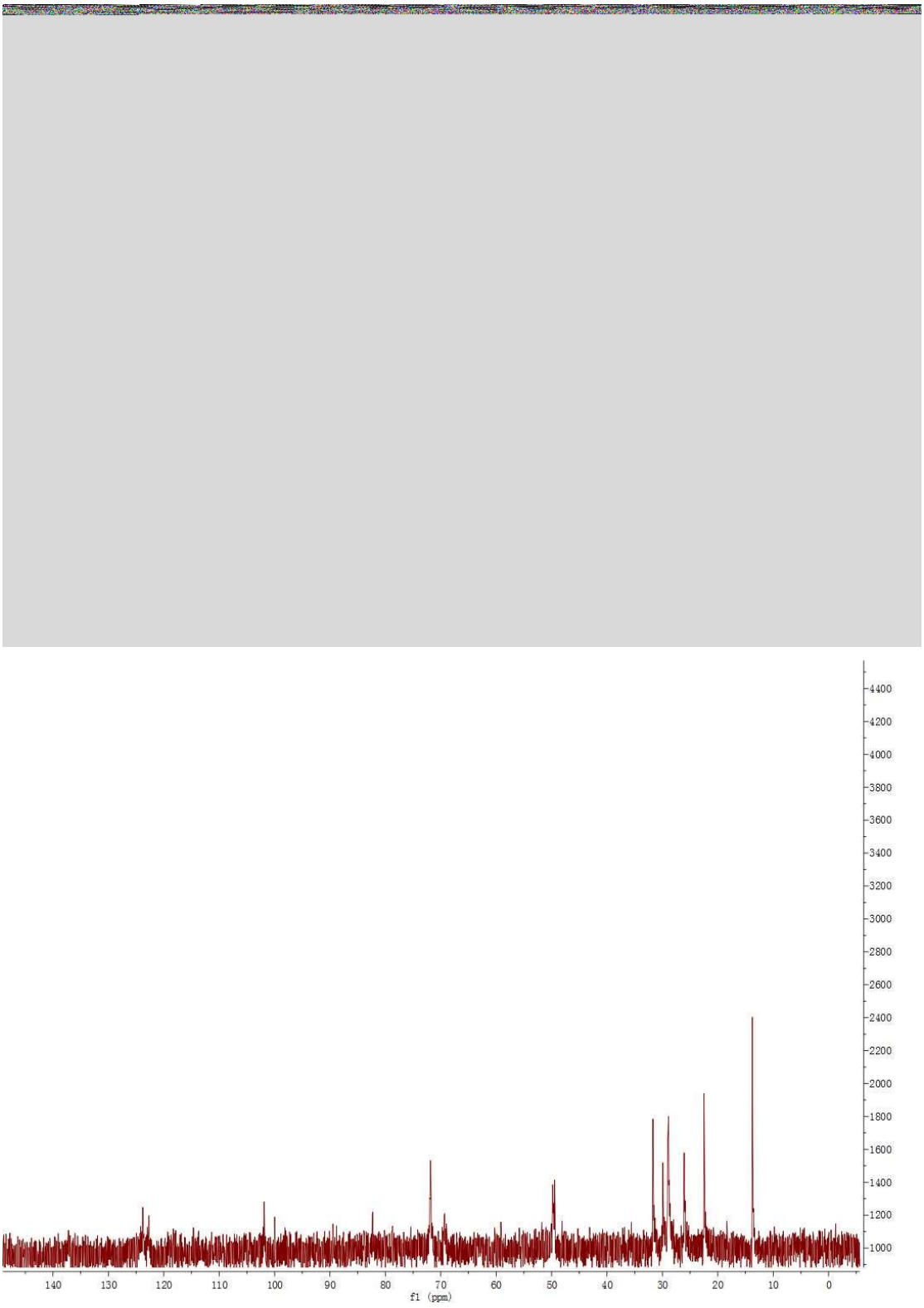

Figure S4. (a) ^{1}H NMR spectrum of **AMCD-β8C** in D_2O, 400 MHz, 25 °C, and (b)^{13}C NMR spectrum of **AMCD-β8C** in D_2O, 100 MHz, 25 °C, and (c) ESI-FTMS of **AMCD-β8C**.

3 Characterization of the binding affinity and selective between AMCD and Heparin

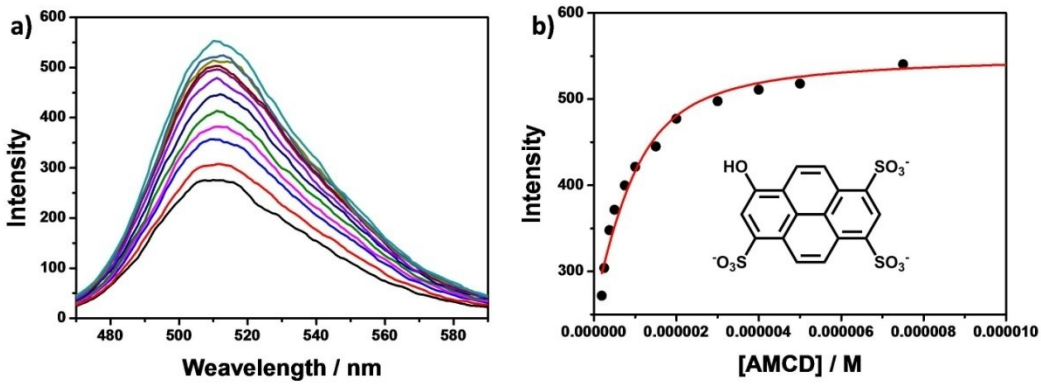

Figure S5. a) Direct fluorescence titration of HPTS (1.0 μM) with AMCD (up to 10 μM), λ_{ex} = 450 nm in 10mM Tris•HCl buffer PH=7.4. b) The associated titration curve at λ_{em} = 515 nm and the fit according to a 1:1 binding stoichiometry.

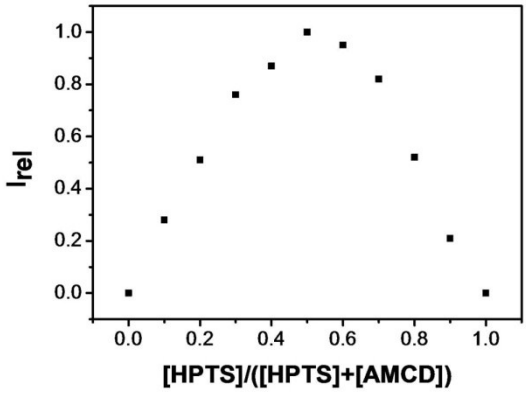

Figure S6. Job's plot for **AMCD** with **HPTS** (λex = 450 nm, λem = 515 nm, [**AMCD**] + [**HPTS**] = 4.0 μM) in 10mM Tris•HCl buffer PH=7.4.

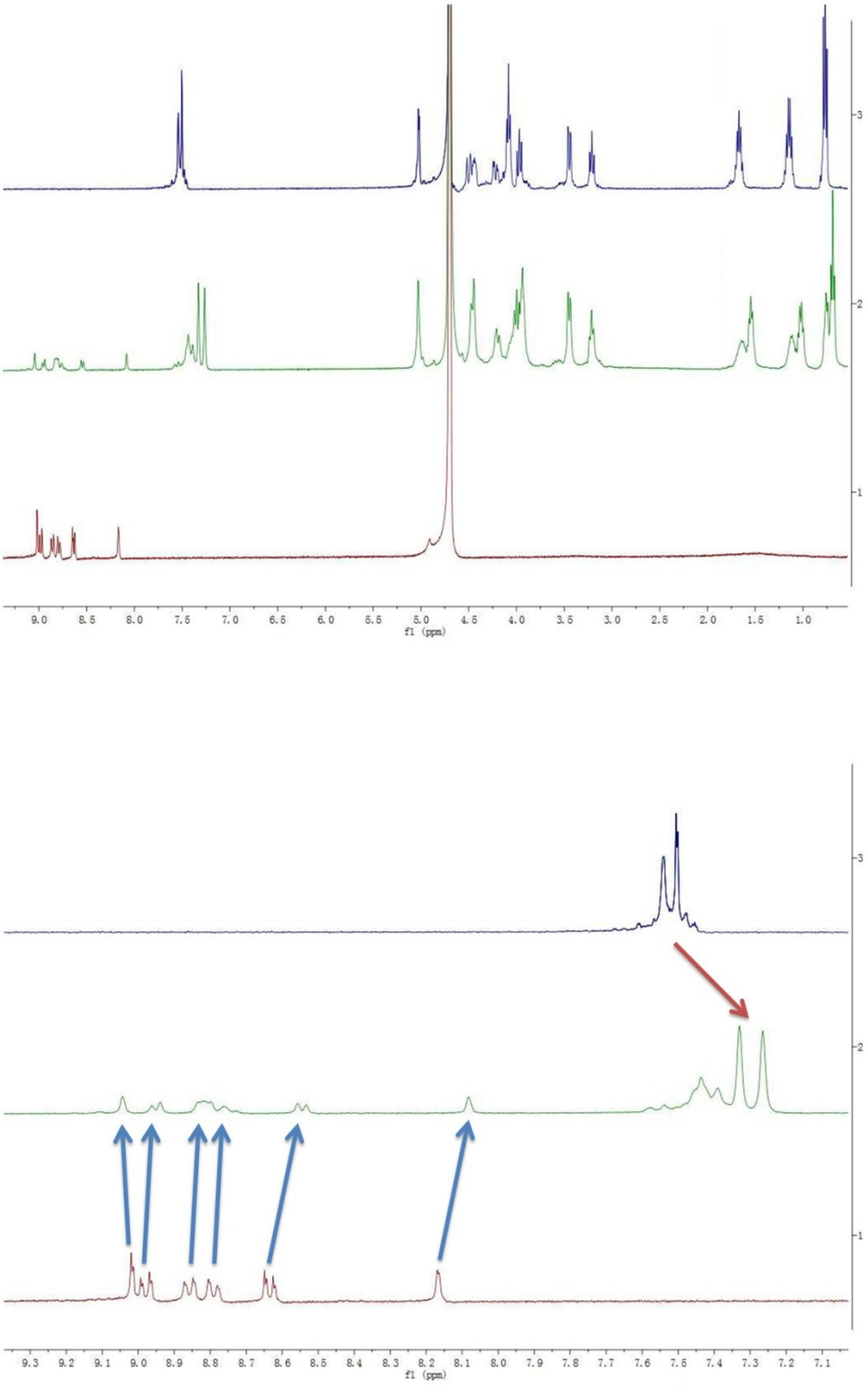

Figure S7. (a) ^{1}H NMR spectrum of **AMCD-γ8C-HPTS** in D_2O, 400 MHz, 25 °C; (b) Partial enlargement of (a).

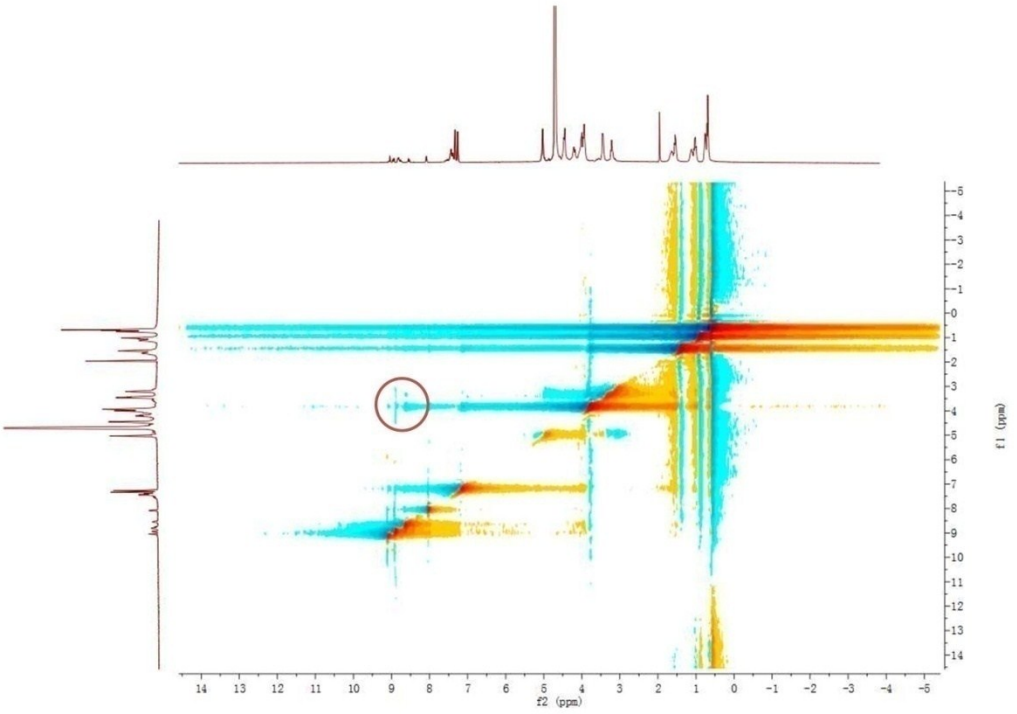

Figure S8. 2D Roesy of **AMCD-HPTS** in D_2O, 400 MHz, 25 °C.

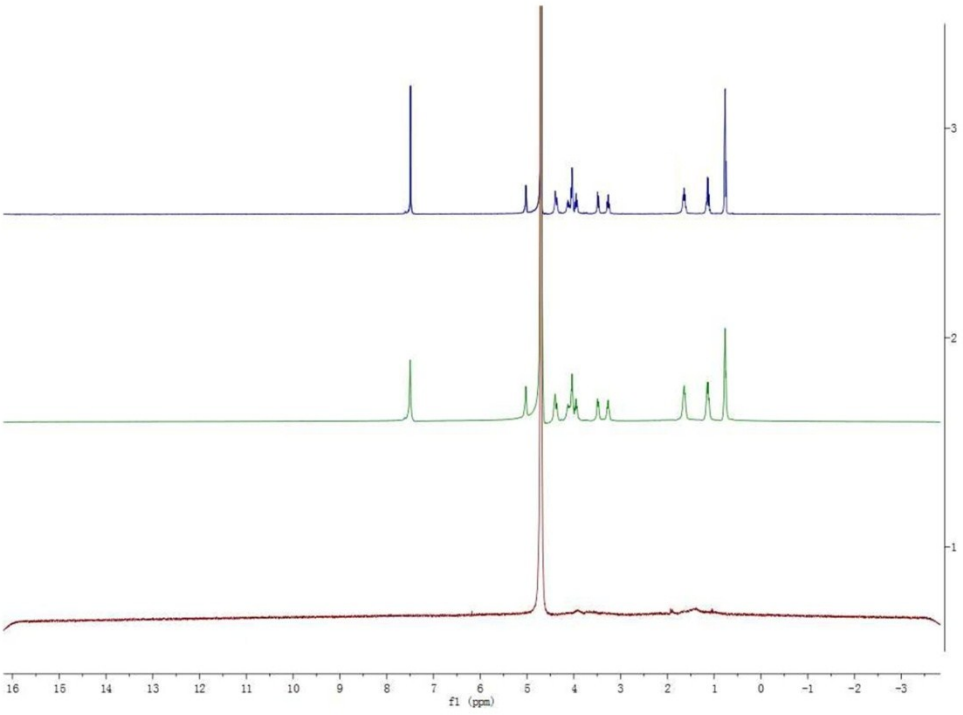

Figure S9. ^1H NMR spectrum of **AMCD-heparin** in D_2O, 400 MHz, 25 °C.

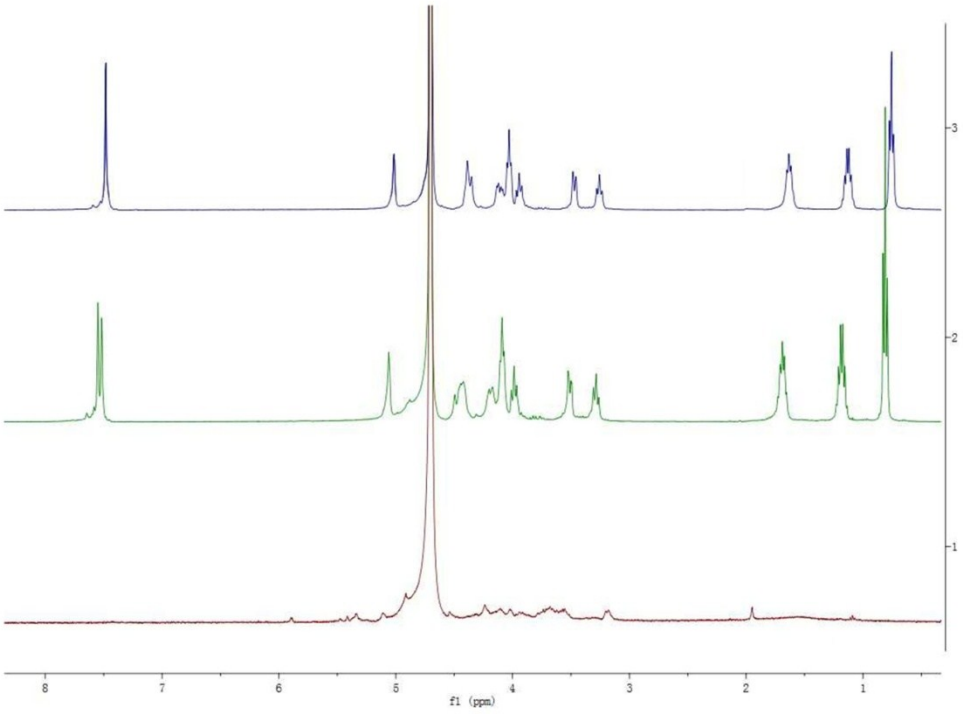

Figure S10. ^{1}H NMR spectrum of **AMCD-LMWH** in D_2O, 400 MHz, 25 °C.

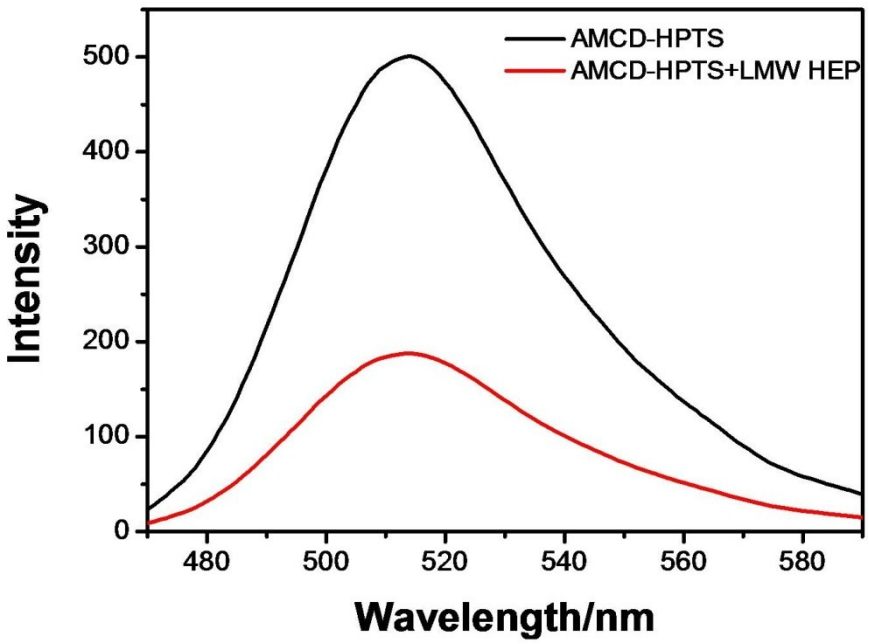

Figure S11. Fluorescence spectra of **AMCD-HPTS** with the addition of **LMWH** in 10mM Tris•HCl buffer PH=7.4.

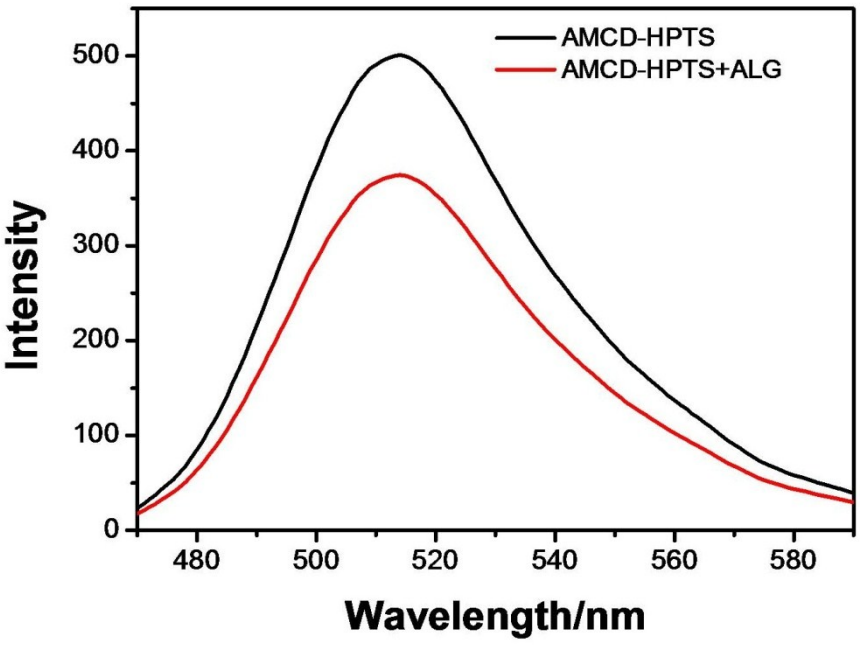

Figure S12. Fluorescence spectra of **AMCD-HPTS** with the addition of **ALG** in 10mM Tris•HCl buffer PH=7.4.

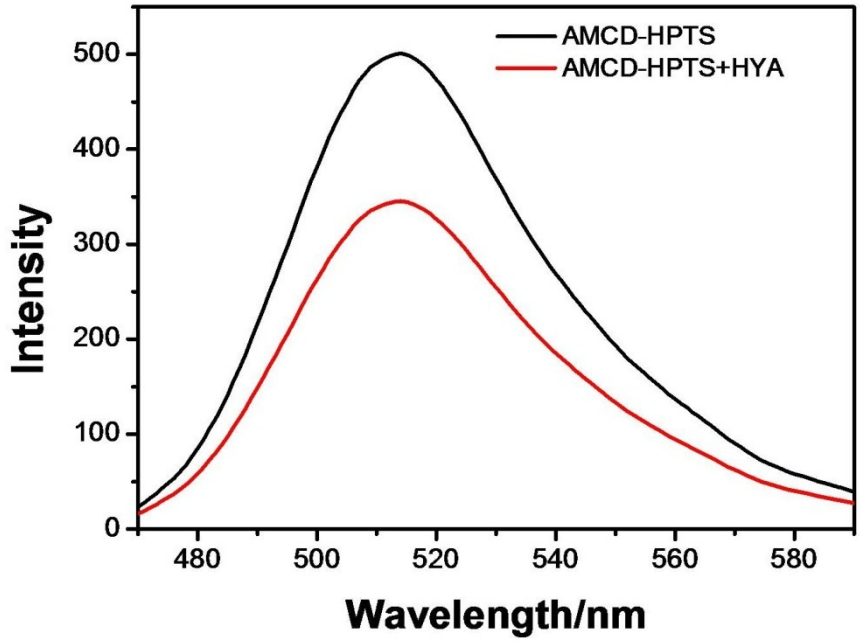

Figure S13. Fluorescence spectra of **AMCD-HPTS** with the addition of **HYA** in 10mM Tris•HCl buffer PH=7.4.

4 Characterization of coagulant effects

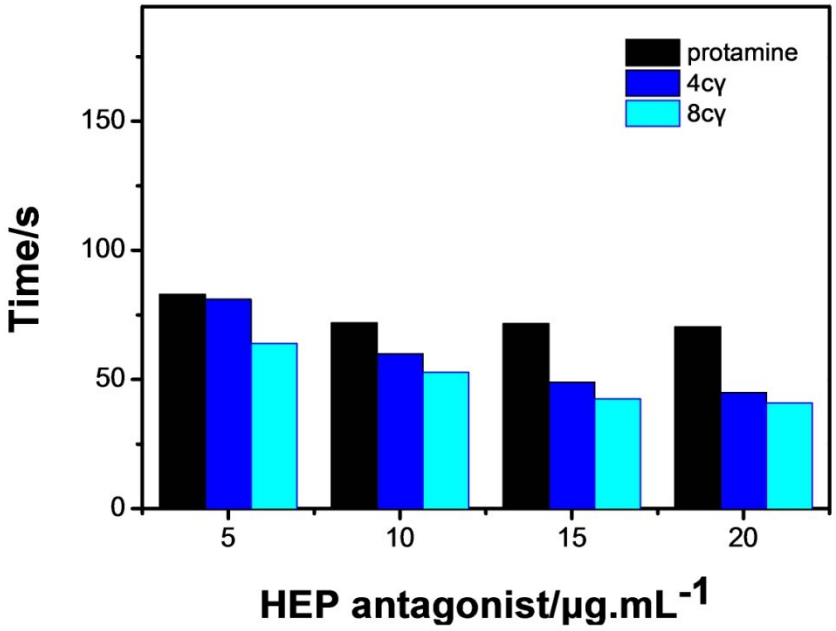

Figure S14. aPTT clotting assay of **LMW heparinized** plasma**.**

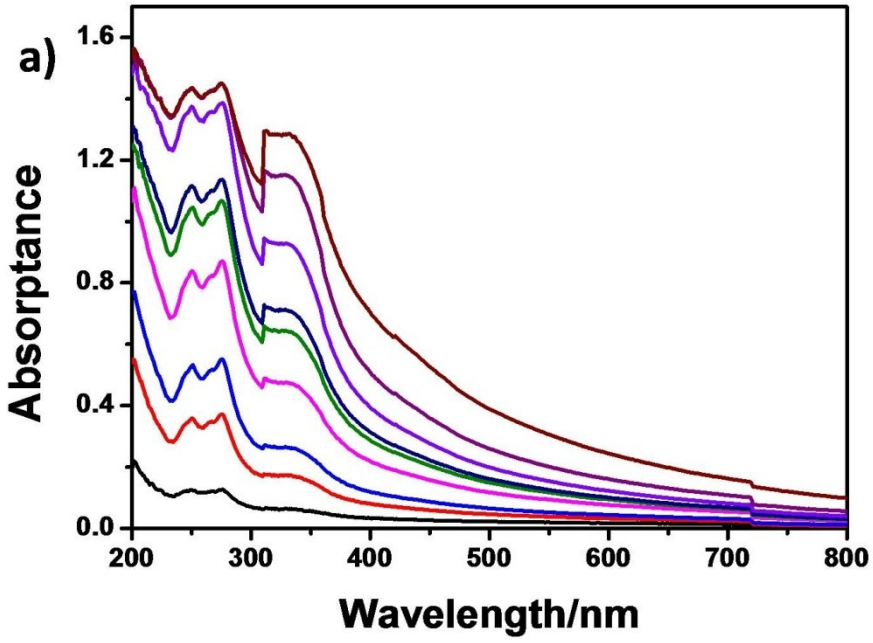

Figure S15. The UV-vis absorption spectra of variable concentration of **vitamin K**

(up to 25 μM).

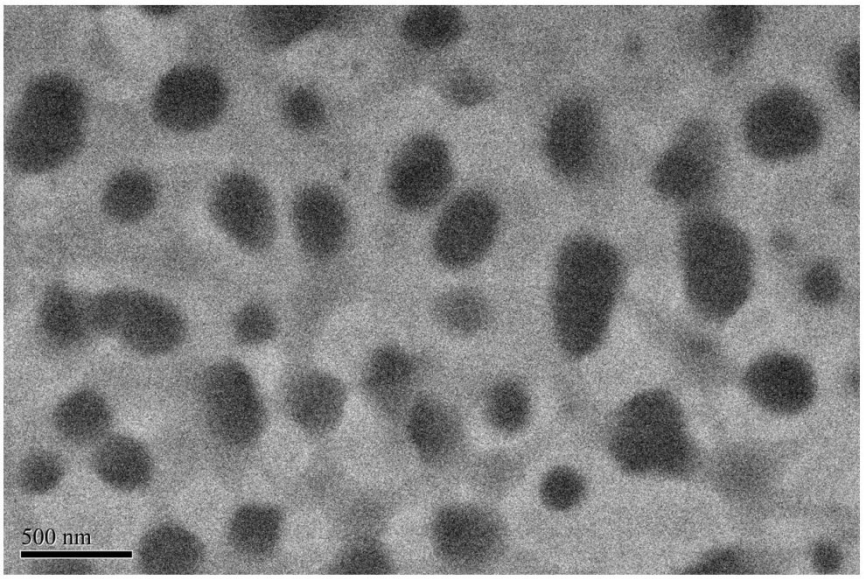

Figure S16. TEM images of **AMCD-VK** co-assembly.

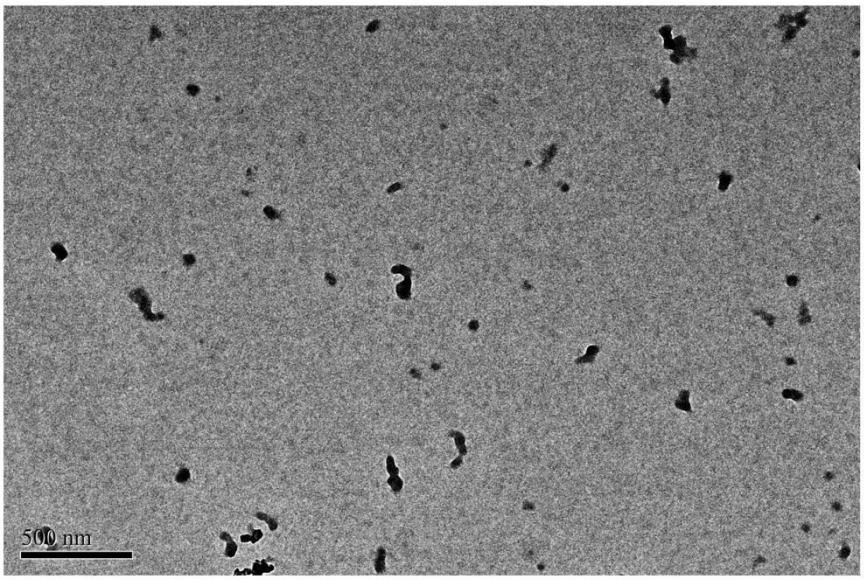

Figure S17. TEM images of **Heparin.**