Supporting Information

Facile control of the self-assembled structures of polylysines having pendent mannose groups *via* pH and surfactant

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1. Materials

Acetonitrile was dried over CaH_2 and distilled under reduced pressure. Potassium thiocyanate (99%, Alfa Aesar), tetrabutylammonium iodide (98%, Alfa Aesar) and Concanavalin A (*Con A*, 99%, Sigma) were used as received. 2,3,4,6-*O*-acetyl-*α*-D-mannosyl bromide was synthesized according to a literature procedure.[1] Polylysine was prepared via the ring-opening polymerization (ROP) of ε-benzyloxycarbonyl-L-lysine *N*-carboxyanhydride (NCA)[2] initiated by *n*-butylamine, followed by the acidolysis of benzyl carbamate groups.[3]

2. Characterization

^{1}H NMR spectra were recorded in $CDCl_3$, D_2O and DMSO-d_6 on a Bruker ARX-400 spectrometer or a Varian Gemini 300 spectrometer, and tetramethylsilane (TMS) was used as the internal reference for chemical shifts. FTIR spectra were recorded on a Nicolet Magna-IR 750 spectrometer. Transmission electron microscopy (TEM) observations were conducted on a JEOL JEM-100CXII electron microscope at an acceleration voltage of 100 kV. Samples were deposited onto the surface of 200 mesh Formvar-carbon film-coated copper grids. Excess water was wicked away, and the grids were dried at room temperature. The image contrast was enhanced by negative staining with 2.0 wt% uranyl acetate in aqueous solution. Circular dichroism (CD) spectra were recorded at 25 °C with a Jasco J-810 (Tokyo, Japan) spectrophotometer. UV-Vis spectra were performed on a Shimadzu 2101 UV–vis

spectrometer. The AFM images were taken on DiMultimode V SPM (Veeco Instrument Inc.) equipped 100μm scanner with FESP AFM probe (Veeco Instrument Inc., nominal spring constant k = 2.8 $N \times m^{-1}$) at tapping mode.

3. Synthetic procedures

3.1 Synthesis of 2,3,4,6-O-acetyl-α-D-mannopyranosyl isothiocyanate[4]

A mixture of potassium thiocyanate (3.56 g, 36.6 mmol), tetrabutylammonium iodide (6.76 g, 18.3 mmol), and molecular sieve (4 Å, 11 g) in anhydrous acetonitrile (150 mL) was stirred at room temperature for 3 h. Then the 2,3,4,6-tetra-*O*-acetyl-*α*-D-mannopyranosyl bromide (7.50 g, 18.3 mmol) in acetonitrile was added dropwise to the solution and the mixture was refluxed until the reaction was complete as detected by TLC. Then the mixture was filtered, and the filtrate was concentrated by rotary evaporation. The residue was purified by flash chromatography (silica gel, ethyl acetate/petroleum ether=2:3, v/v) to obtain 2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl isothiocyanate (4.51 g, 63%) as a yellow solid..

^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ = 2.02-2.19 (4s, 12H, CH_3); 4.09 (m, 1H); 4.12-4.18 (dd, 1H); 4.27-4.33 (dd, 1H); 5.26-5.34 (m, 3H); 5.57 (s, 1H).

3.2 Modification of polylysine with 2,3,4,6-O-acetyl-α-D-mannopyranosyl isothiocyanate and the deprotection process, general procedure[5]

Polylysine (100 mg, 0.768 mmol -NH_2, 1 equiv), mannosyl isothiocyanate (90 mg, 0.230 mmol, 0.3 equiv) were dissolved in anhydrous DMF (7 mL). The solution was stirred at 50°C for 24 h. The resulting mixture was poured into diethyl ether and filtered. The final product was dried under high vacuum.

A suspension of P(AcM/Lys-*co*-Lys) in MeOH/H_2O(1/2, 15 mL) and 1 M sodium methoxide (1 mL) was stirred at room temperature for 1 h until all the solid had dissolved. The solution was neutralized with Amberlite IR-120 cation exchange resin and filtered. Methanol was evaporated under reduced pressure, and the resulting aqueous solution was purified by dialysis against H_2O (MW cutoff 1000 Da) for three days after which the product could be recovered by freeze-drying. The yield was

quantitative.

4. Preparation of Vesicles and Rod-like Micelles

The following protocol for vesicle preparation was adopted. Copolymer (2.0 mg) was dissolved into dilute aqueous HCl (pH 2; 20 mL; the initial copolymer concentration was 0.1 mg/mL) and stirred for 2 h. Then the pH was slowly adjusted over a 40 minute period from pH 2 to pH 11 using 0.10 M aqueous NaOH. The final copolymer concentration after pH adjustment was about 0.023 mg/mL.

In order to prepare rod-like micelles, 1 mM SDS solution was added dropwise into 1 mL of an aqueous copolymer solution (c=0.2 mg/mL, pH=4) in steps of 100 μL at constant stirring yielding solutions with SDS/lysine ratios of 0.75. After that, the solution was continuously stirred for 24 h at 20°C.

5. Dye Loading and Elution Experiment

A loading method involved mixing 1 mL of a 0.1 mg/mL copolymer **2** solution (pH=2) with 0.1 mL of a 5 mM calcein solution (pH=9.16). Following the vesicle preparation protocol described above, the pH was gradually raised to pH 11 by adding 0.1 M aqueous NaOH, and the solution was allowed to stand for 48 h prior to dialysis. The calcein-vesicle mixture was dialyzed against 200 mL of 0.1 M carbonate buffer at pH 10.83 for 16 h to remove excess calcein from the solution. The final calcein concentration in the vesicle solution after dialysis was 0.24 mM as determined by visible absorption spectrophotometry using a calibration curve constructed from aqueous calcein solutions of known concentration (see Fig. S7). A control solution without any copolymer **2** was prepared by simply adding 0.1 mL of 5 mM calcein solution (pH=9.16) to 2.0 mL of pH 10.83 carbonate buffer. This sample was not dialyzed prior to elution.

The elution experiments were carried out immediately after the calcein-vesicle samples were dialyzed. 1.0 mL of dye-loaded vesicles solution or the control solution was added to the dialysis tube and dialyzed against 10 mL of carbonate buffer at pH 10.83 and 20°C. The elution bottle was kept agitated at a constant stirring speed. After

suitable time intervals of typically 30-60 minutes, 1.0 mL of solution was periodically removed to determine the calcein concentration at 497 nm by UV-Vis spectrometer using the calibration curve shown in Fig. S12. This extract was replaced by the addition of 1.0 mL of fresh carbonate buffer and the new calcein concentration was recalculated.

6. Lectin Recognition Study

The concentrations of vesicle solutions previously used for TEM were set at 0.04 mg/mL. *Con A* was dissolved in buffer solution (0.05 M Tris-HCl, pH 7.10, 1 mM $CaCl_2$, 1 mM $MnCl_2$) with different concentrations. Equal volumes of the two solutions were mixed. The lectin recognition properties of these aggregates were initially monitored by following the changes of transmittance at 350 nm with time.

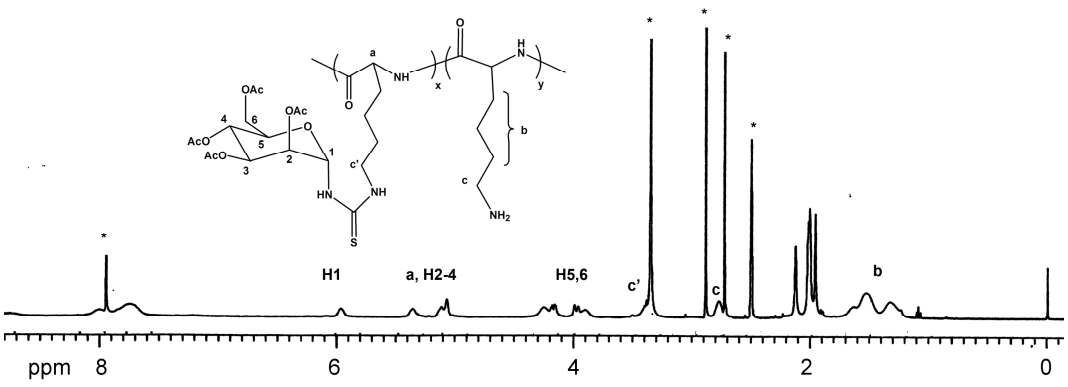

Fig. S1 ^{1}H NMR spectrum of P(AcM/Lys-*co*-Lys) in d_6-DMSO (*: DMSO, H_2O, DMF).

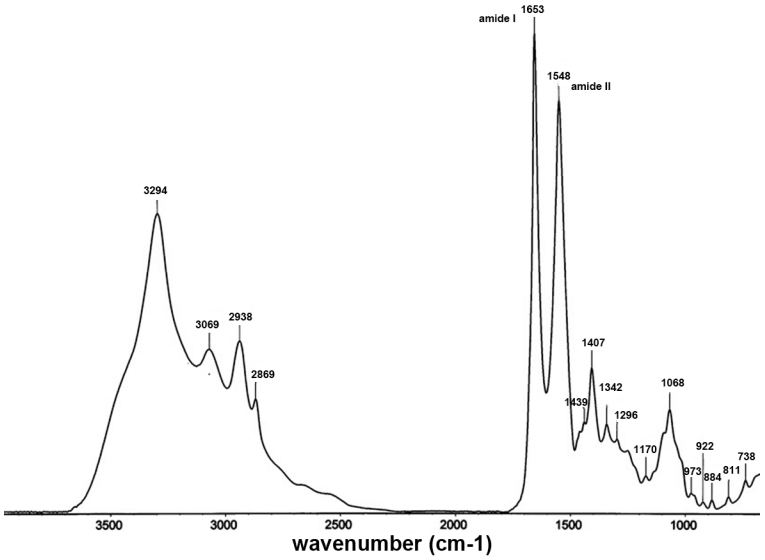

Fig. S2 FTIR spectrum of copolymer **1**

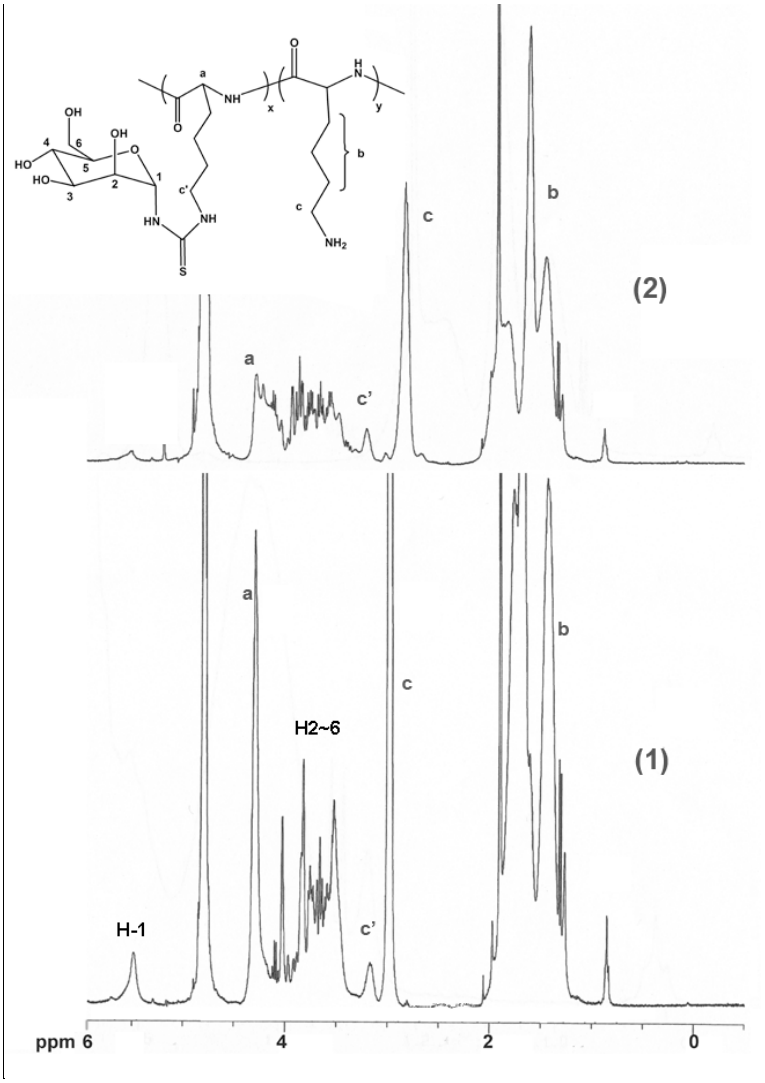

Fig. S3 ^{1}H NMR spectra of copolymer **1** in (1) D_2O, pH=7; (2) in D_2O +NaOD, pH=10.

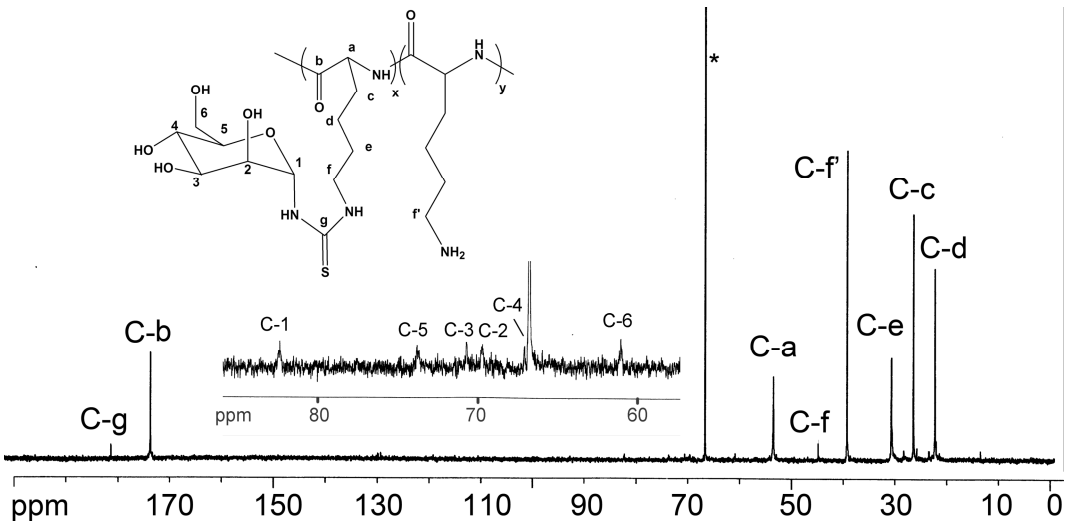

Fig. S4 ^{13}C NMR spectrum of copolymer **1** in D_2O (*: dioxane was added as a standard substance).

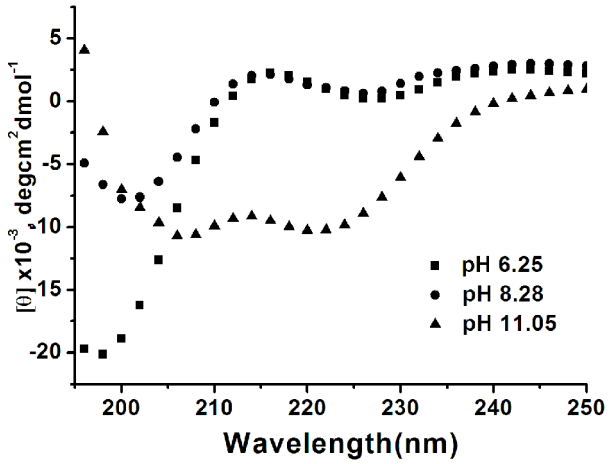

Fig. S5 Circular dichroism spectra of copolymer **1** at different pH. The copolymer concentration is 0.2 mg/mL.

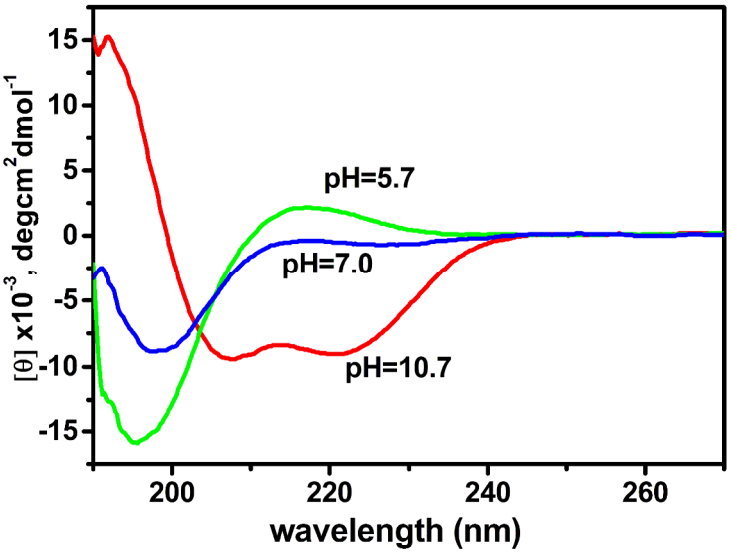

Fig. S6 Circular dichroism spectra of polylysine (DP=32). The polymer concentration is 0.1 mg/mL.

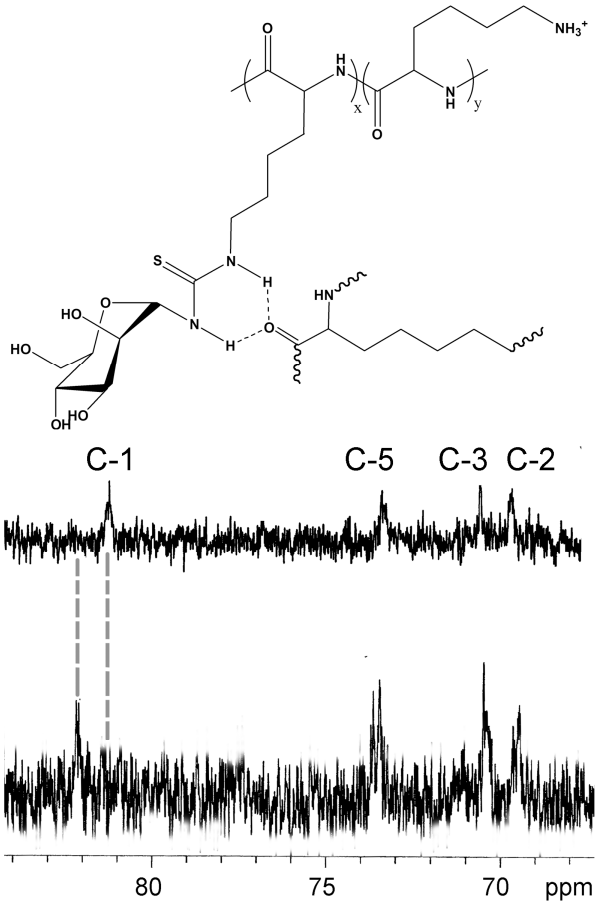

Fig. S7 Stacked selected regions of the ^{13}C NMR spectra of the copolymer **1** in the absence (upper) and presence (1 M, lower) of urea in D_2O at 298 K.

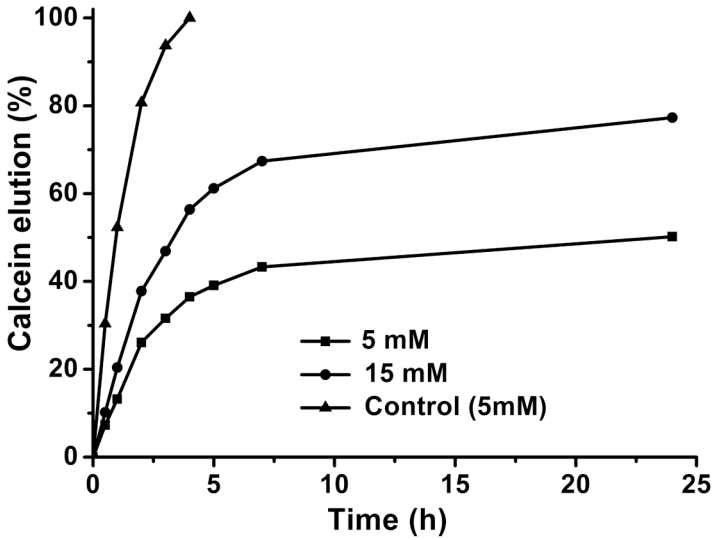

Fig. S8 Calcein elution profile from copolymer **2** vesicle solution (pH 10.14 carbonate buffer at 20°C).

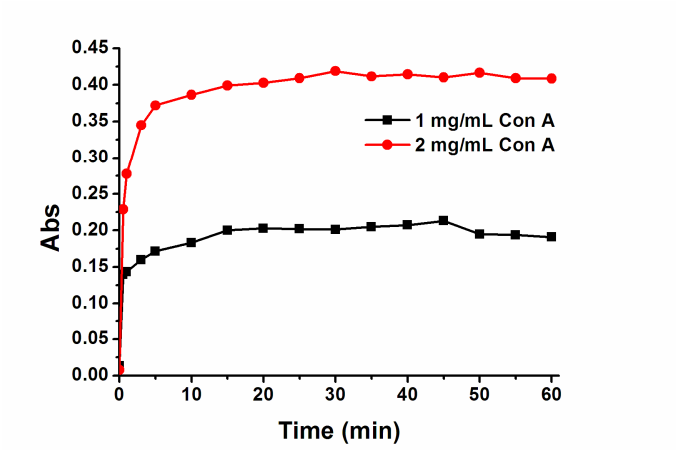

Fig. S9 Absorption of the mixed aqueous solution of *Con A* and copolymer **1** vesicular aggregates at 350 nm as a function of time at room temperature, pH = 10. The initial concentration of copolymer **1** is 0.04 mg/mL.

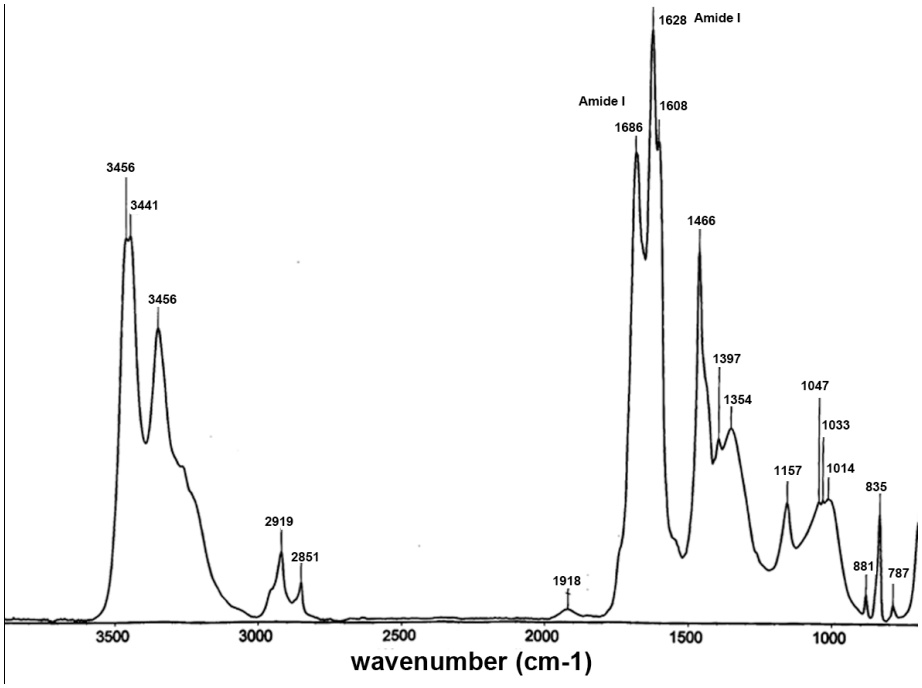

Fig. S10 FTIR spectrum of lyophilized precipitates of copolymer **1**

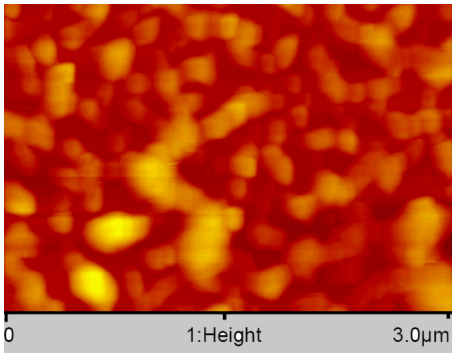

Fig. S11 AFM image for aggregates prepared from 0.2 mg/mL copolymer **2** aggregate in pH=4 aqueous solution by adding 1 mM SDS with SDS/lysine ratio=3/4.

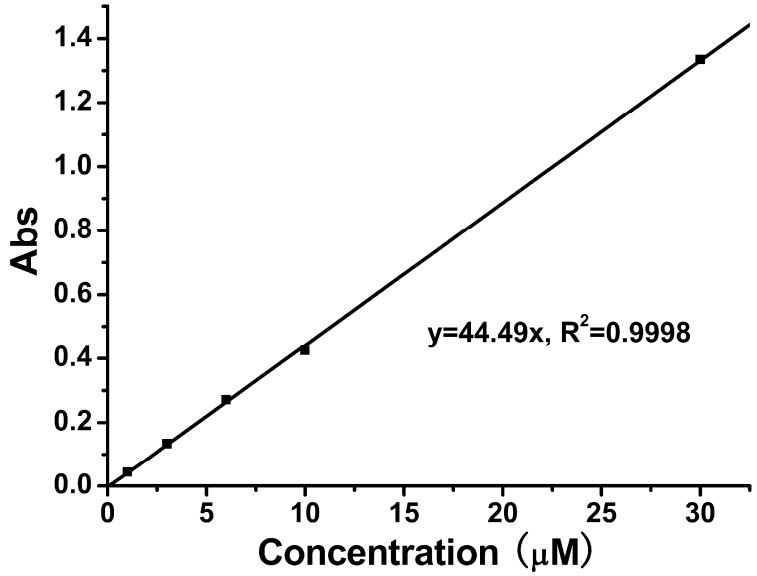

Fig. S12 Calibration curves of calcein determined in aqueous carbonate solution at pH 10.83 as measured at 497 nm by UV-Vis spectrometer.

References

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