Straightforward access to glycosylated, acid sensitive nanogels by host-guest interactions with sugar modified pillar[5]arenes

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CONTENT

1. Materials

Acrylic acid (AA), potassium persulfate (KPS) and concanavalin A (Con A) were purchased from Sigma-Aldrich. The monomer *N*-[(2,2-dimethyl-1,3-dioxolane)methyl]acrylamide (DMDOMA), the crosslinker (propane-2,2-diylbis(oxy))bis(ethane-2,1-diyl)diacrylate (CL), 6-acryloyloxyhexylpyridinium chloride (AHPC) and sugar pillar[5]arene were synthesized according to previous reports.[1-5]

2. Instrumentation

Proton NMR (^{1}H NMR) spectra were measured at room temperature in $CDCl_3$ or D_2O on a Bruker Avance 300 MHz. The chemical shifts are given in ppm. Dynamic light scattering (DLS) was performed on a Zetasizer Nano ZS (Malvern Instruments, Herrenberg, Germany). For this purpose, nanogel suspensions (1 mg mL^{-1}) were measured at 25 °C (λ = 633 nm) at an angle of 173° after an equilibration time of 120 s. The size distribution of the nanospheres was calculated applying the nonlinear least-squares fitting mode.

Scanning electron microscopy (SEM) imaging was conducted with a Zeiss LEO 1530 Gemini utilizing the lnlense detector. Therefore, one droplet of a 0.7 mg mL^{-1} nanogel suspension was placed on a silicon wafer and dried at room temperature.

3. Preparation of nanogels (NG)

The synthesis of NG is described as followed: 1.6 g (9 eq.) DMDOMA, 259 mg (1 eq.) AHPC and 51 mg CL (2 mol% *vs.* monomers) were dissolved in 160 mL phosphate buffer solution (50 mM, pH 7.4). The solution was degassed with argon for 40 min, and the flask was placed in a pre-heated oil bath at 70 °C. Subsequently, 14 mg KPS in 3 mL degassed H_2O was quickly added to initiate the polymerization. After 16 hours, the reaction was cooled to room temperature and dialyzed in a phosphate buffer solution (10 mM, pH 7.4) for 2 days (molar mass cut off 12000 to 14000 g mol^{-1}) to obtain a 10 mg mL^{-1} suspension.

For the preparation of pillar[5]arene loaded nanogels, GP-NG is exemplarily described: 0.1 mL (1 mg) NG solution and 1.5 mg (1 eq.) GP were mixed and diluted to 1 mL gaining GP modified nanogel (GP-NG) for the complexation, hydrolysis, and binding experiments

4. Complexation and hydrolysis investigation by NMR spectroscopy

Briefly, three suspensions or solutions (NG (5 mg mL^{-1}), NG (5 mg mL^{-1}) + 1 eq. GP, 1 eq. GP) were prepared in D_2O, respectively. Subsequently, all the samples were measured by ^{1}H

NMR spectroscopy at room temperature. The same method was also applied for the complex formation of NG and CP.

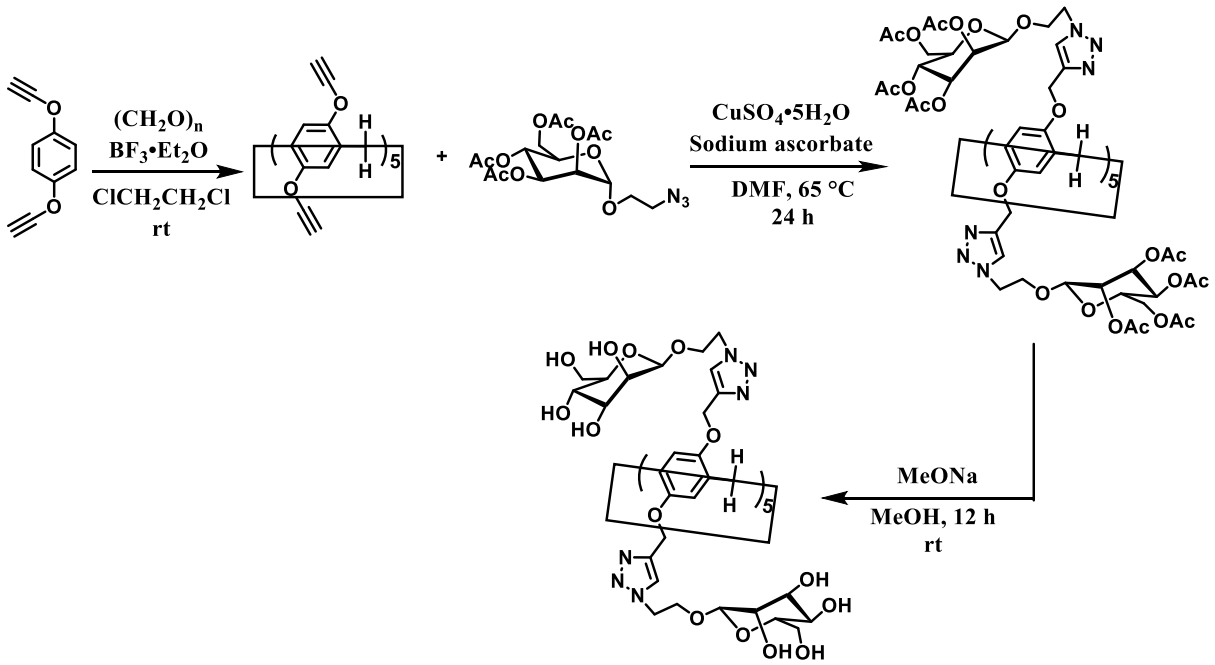
2 mg mL^{-1}nanogel solutions with 1 eq. of GP in D_2O (acetate buffer, 10 mM, pH 5.1) were prepared in NMR tubes and incubated at 37 °C. At each time point, samples were measured by ^1H NMR spectroscopy, and the integral of the signal of the acetate buffer was set as 100 (internal standard) to calculate the degree of hydrolysis using the increasing integral of the appearing acetone peak.

5. Investigation of the selective binding of sugar pillar[5]arenes modified nanogels to Con A

Con A (2.5 mg mL^{-1}) in a buffer solution (10 mM, pH 7.4, 0.1 mM $CaCl_2$ and $MnCl_2$) was added to the sugar pillar[5]arene modified nanogels and measured by DLS with a fixed attenuator and position. Additional control experiments were performed with Con A and pure NG or CP.

Further competition experiments between MP-NG, Con A and methyl-D-mannose were prepared as follows: 1 mg mL^{-1} (1 mL) NG was first mixed with 1 eq. (1.5 mg) MP providing MP-NG complexation, then, 30 μL of Con A (2.5 mg mL^{-1}) was added into the mixture. Afterwards, 10 μL methyl-D-mannose (100 mg mL^{-1}) or CP (38 mg mL^{-1}) was added under process control by DLS.

6. Schemes and figures



Scheme S1 Schematic representation of the route for sugar pillar[5]arene.

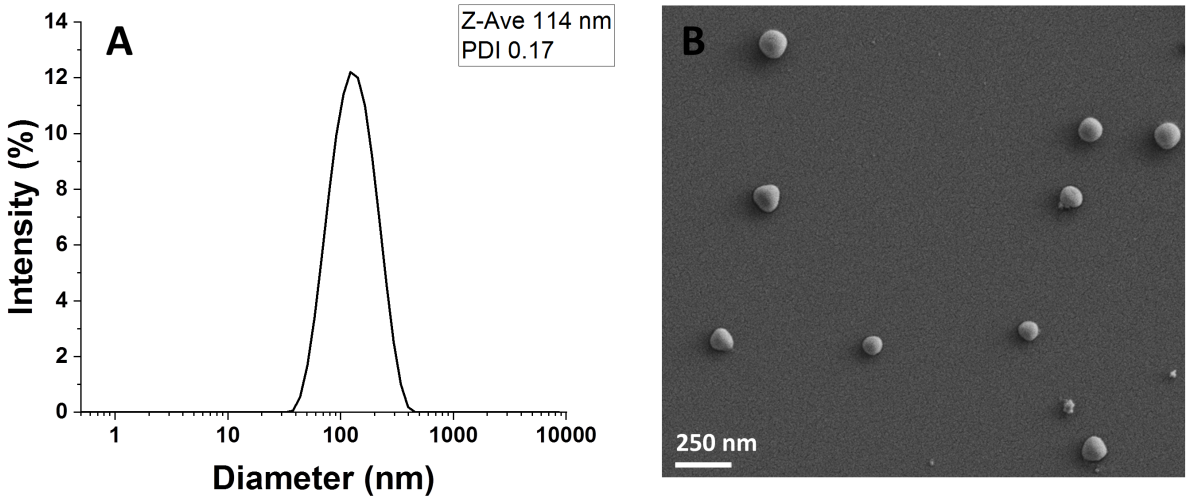


Figure S1 Characterization of pure nanogels (NG) by A) DLS and B) SEM.

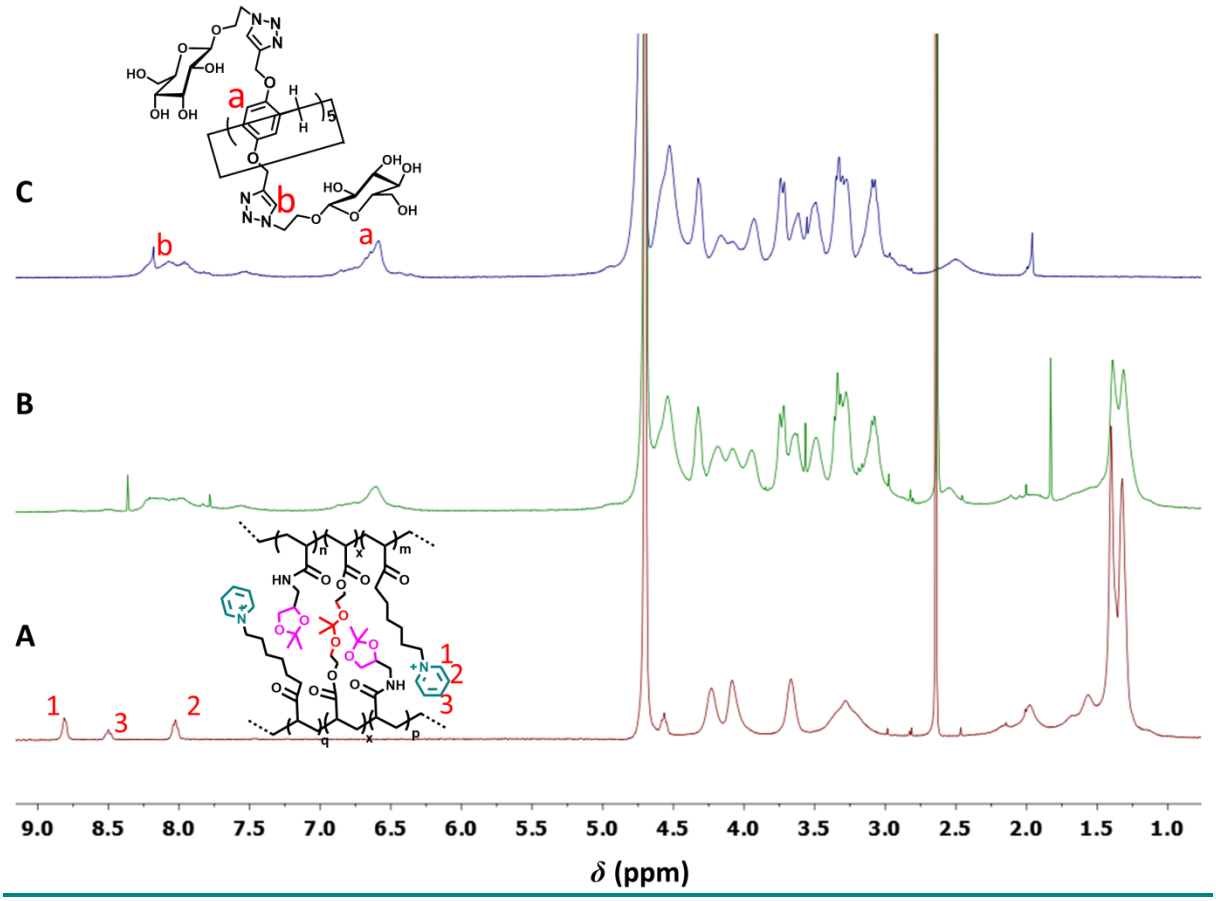


Figure S2 ^{1}H-NMR (D_2O, 300 MHz) spectrum of A) nanogel (5 mg mL^{-1}), B) NG (5 mg mL^{-1}) + 1 eq. GP and C) GP.

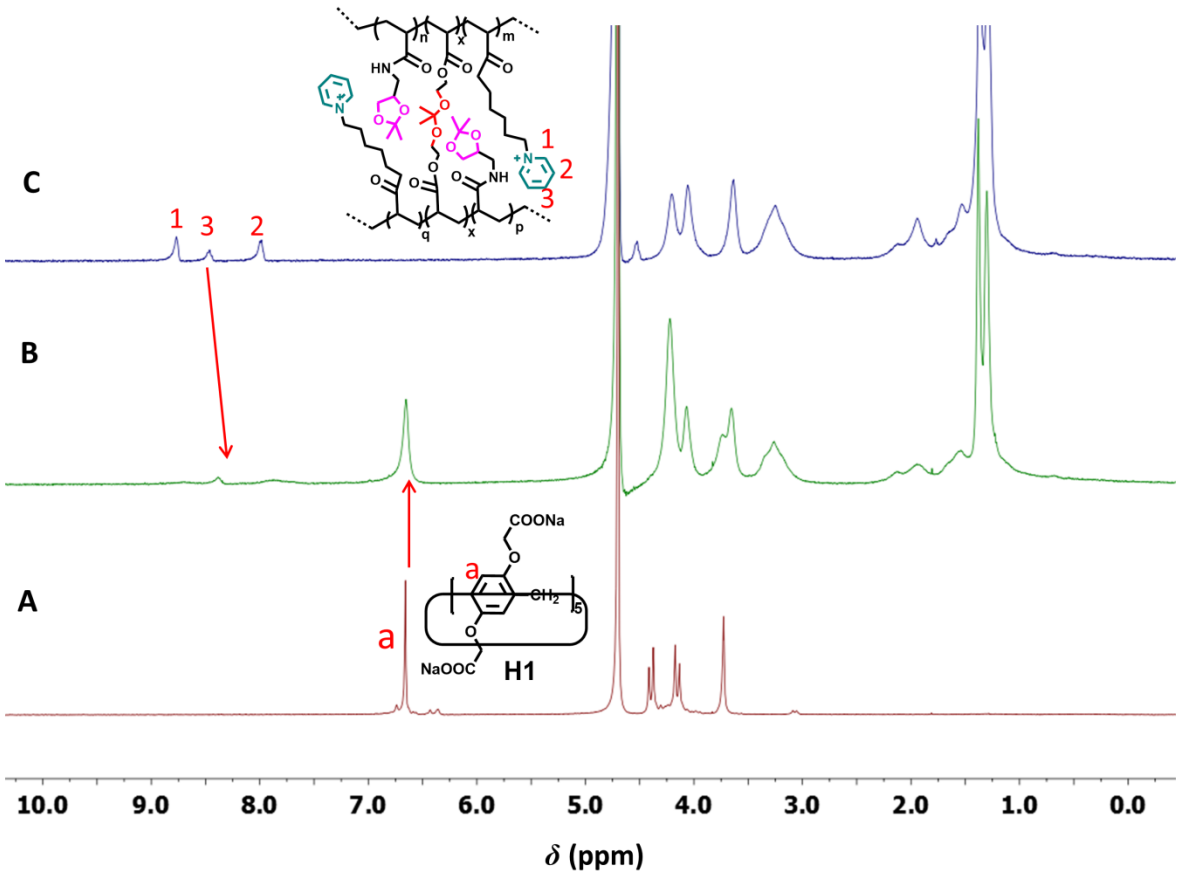


Figure S3 ^{1}H-NMR (D_2O, 300 MHz) spectrum of A) CP, B) nanogel (5 mg mL^{-1}) + 1 eq. CP and C) nanogel (5 mg mL^{-1}).

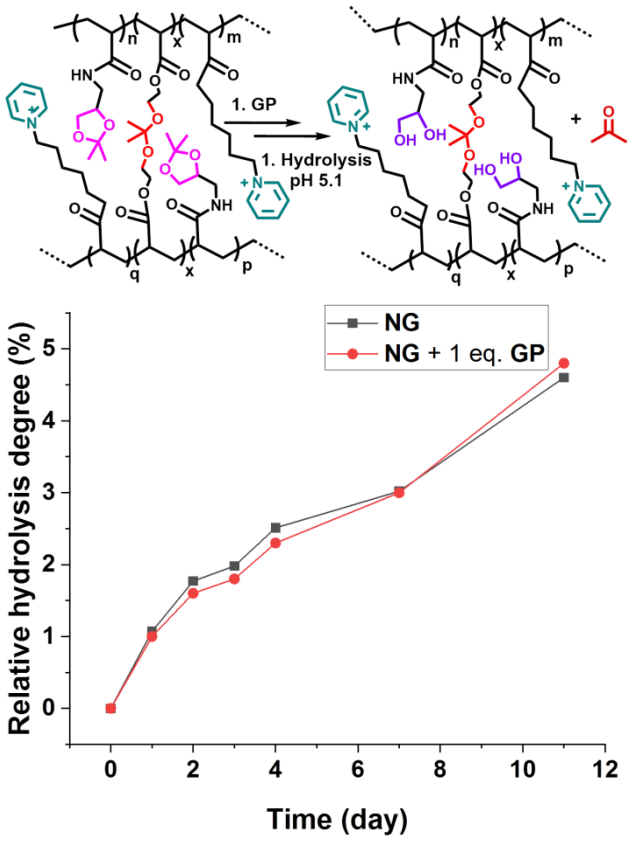


Figure S4 Time dependent hydrolysis of nanogels with 1 eq. GP in acetate buffer (pH 5.1, 10 mM) determined by ^{1}H NMR (D_2O, 300 MHz).

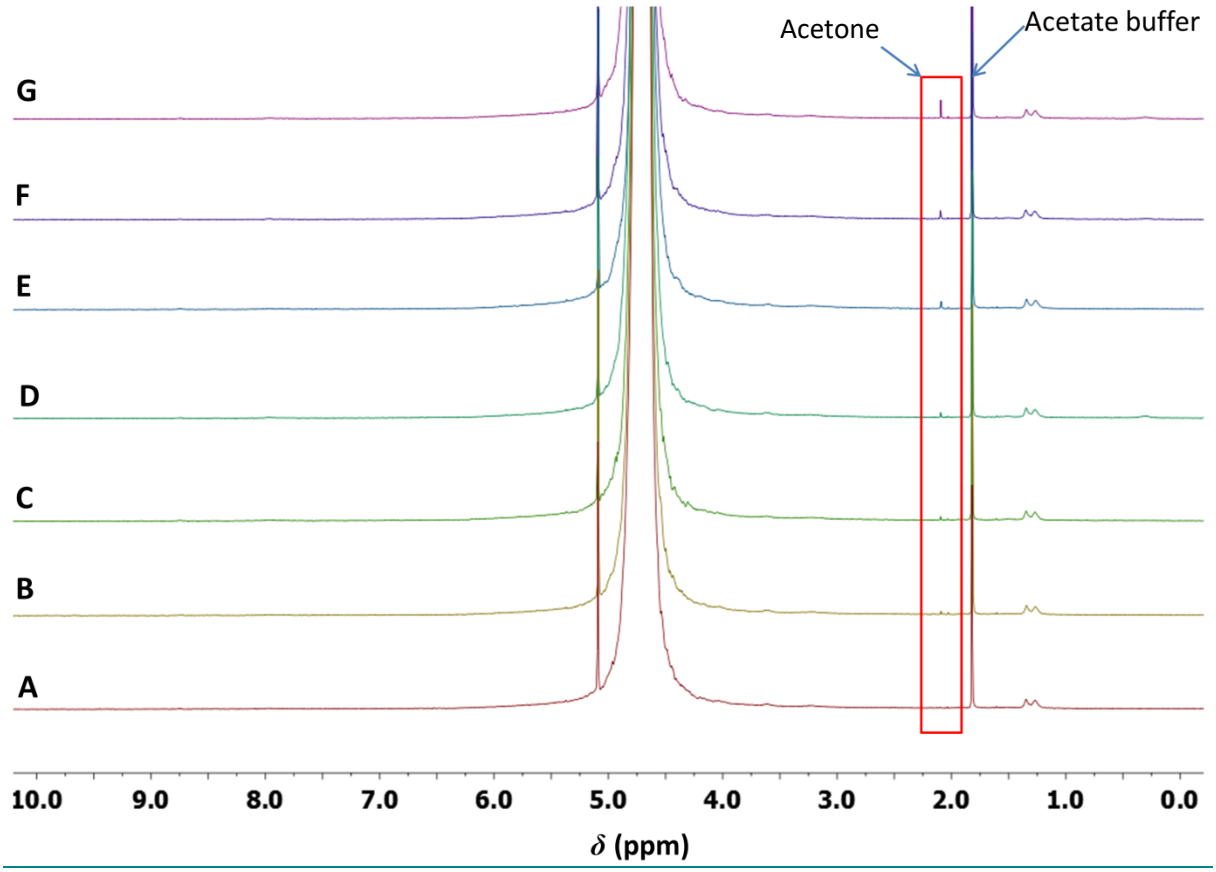


Figure S5 ^{1}H-NMR (D_2O, 300 MHz) spectra during the hydrolysis of nanogel (5 mg mL^{-1}) in pH = 5.1 after A) 0 h, B) 24 h, C) 48 h, D) 72 h, E) 96 h, F) 168 h and G) 264 h.

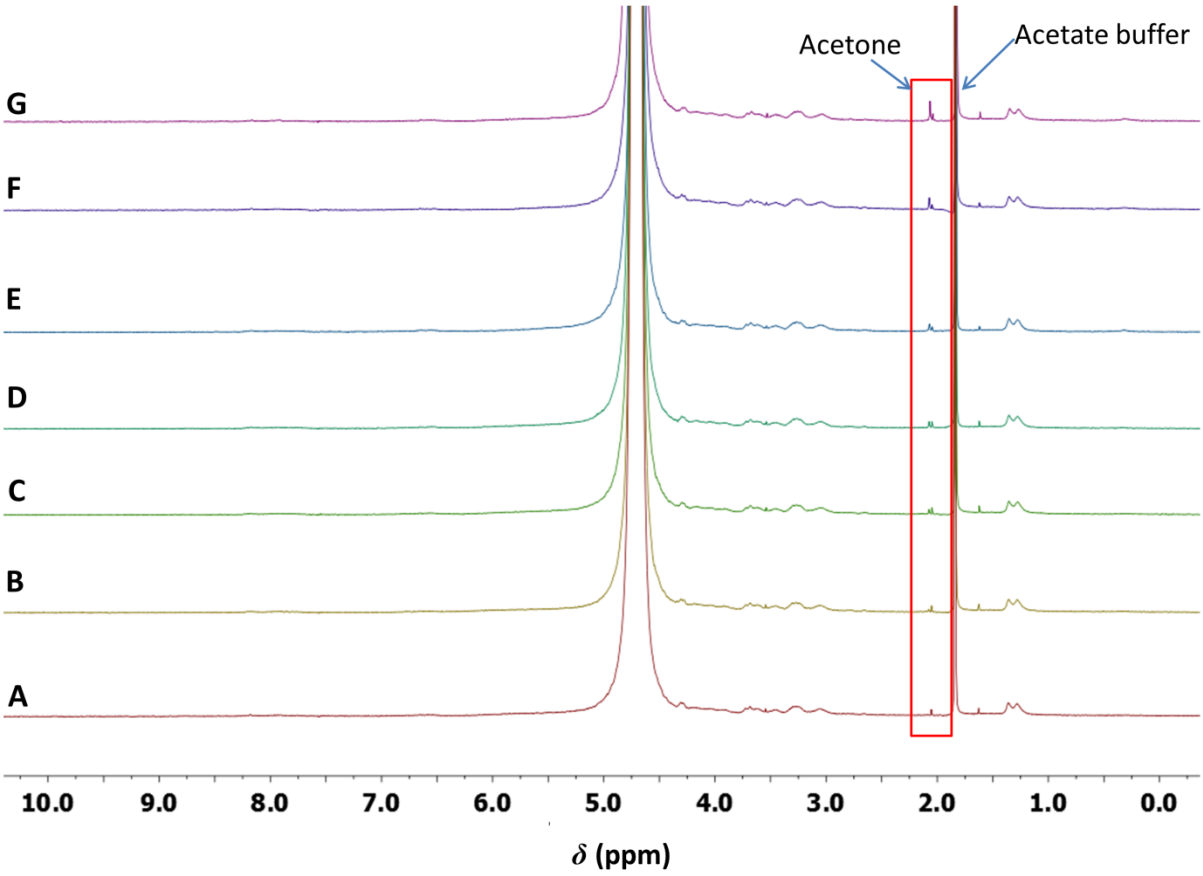


Figure S6 ^{1}H-NMR (D_2O, 300 MHz) spectra during the hydrolysis of nanogel (5 mg mL^{-1}) + 1 eq. **GP** in pH = 5.1 after A) 0 h, B) 24 h, C) 48 h, D) 72 h, E) 96 h, F) 168 h and G) 264 h.

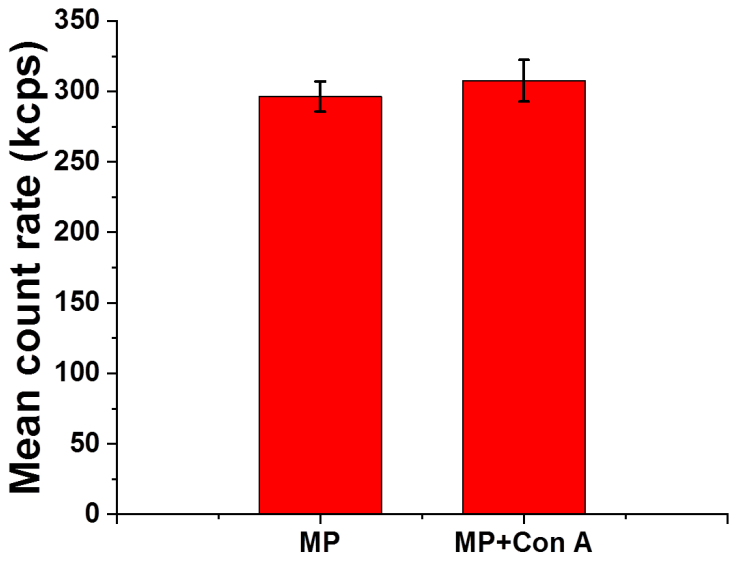


Figure S7 Selective binding studies of pure mannose-pillar[5]arene (MP) with or without Con A in buffer solution (10 mM, pH 7.4, 0.1 mM $CaCl_2$ and $MnCl_2$) by DLS at room temperature.

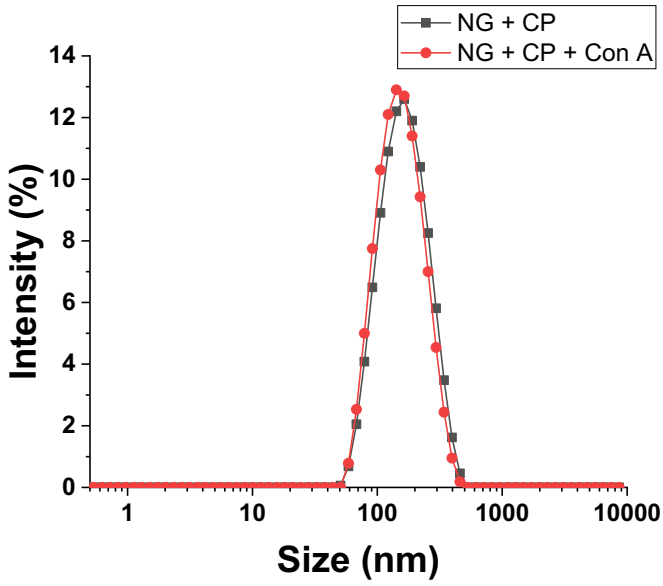


Figure S8 Selective binding studies of nanogel (NG) with or without CP and Con A in buffer solution (10 mM, pH 7.4, 0.1 mM $CaCl_2$ and $MnCl_2$) by DLS at room temperature.

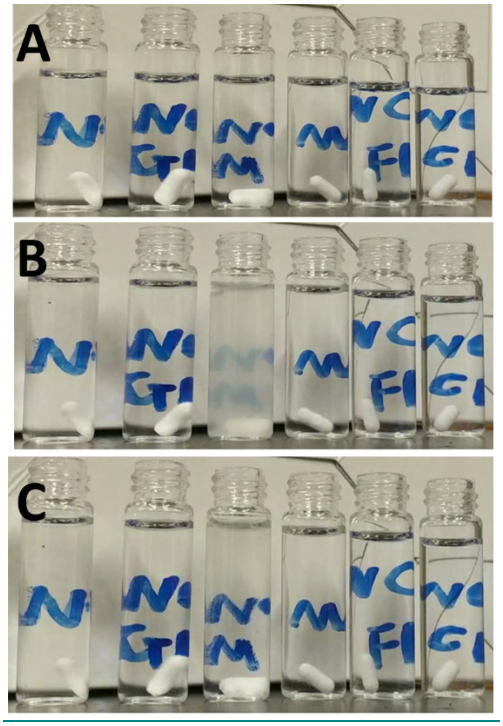


Figure S9 Solution of nanogels A) before addition of Con A, B) after addition of Con A, C) binding with Con A after addition of methyl-D-mannose in PBS at room temperature: nanogel, **NG-GP**, **NG-MP**, **MP**, **NG-FP** and **NG-CP** (from left to right).

7. References

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