

## Supporting Information

### Nanoparticles Assembled via pH-Responsive Reversible Segregation of Cyclodextrins in Polyrotaxanes

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#### Experimental Section

*Materials and Methods.* High-purity water (Milli-Q (MQ) water) with a resistivity greater than 18 MΩ-cm was obtained from an in-line Millipore RiOs/Origin water purification system. The pH of solutions was measured with a Mettler-Toledo MP220 pH meter. αCD (>99.5%) was obtained from Kanto Chemicals (Japan). Mica discs were obtained from

ProSciTech (Australia). Phosphate buffered saline (PBS) tablets, sodium acetate (99+%), *N,N*-dimethylformamide (DMF, 99+%), dimethylsulfoxide (DMSO, 99.98%), anhydrous pyridine (99%), citric acid (99+%), lysozyme from chicken egg (>90%), dialysis tubing (D0530-100FT) with a MWCO of 12 400, and succinic anhydride (99+%) were obtained from Sigma-Aldrich (Australia) and used as received. Bis(*o*-pyridyldisulfide2-(poly(ethylene glycol)) (OPSS-PEG-OPSS; Mw 20 kDa) was purchased from Creative PEGWorks (Winston Salem, NC, USA) and used as received. The capping thiol **3** was synthesised according to a previously reported procedure.<sup>[1,2]</sup> Nuclear magnetic resonance (NMR) spectra were recorded using a 400 MHz Varian INOVA system at 25 °C. Spectra were referenced to residual proton resonances of the deuterated solvent. Chemical shifts are reported as parts per million (ppm) downfield from external tetramethylsilane (TMS). Fourier transform infrared (FTIR) spectra were recorded on a Varian 7000 FTIR spectrometer with a Specac MKII Golden Gate single-reflectance diamond ATR attachment with KRS-5 optics and a heated top plate maintained at 25 °C. PRX solutions were dropped and dried onto the diamond ATR crystal, and spectra were collected in the absorbance mode from 4000 to 400 cm<sup>-1</sup> at a resolution of 2 cm<sup>-1</sup>. Dynamic Light Scattering (DLS) measurements were performed on a Nano ZS model ZEN3600 with a scattering angle of 173°. Atomic force microscopy (AFM) images were obtained with an MFP-3D Asylum Research instrument. Typical scans were conducted in AC mode with ultrasharp SiN gold-coated cantilevers (NT-MDT). Transmission electron microscopy (TEM) images were obtained with a FEI Tecnai TF20 transmission electron microscope operated at 200 kV.

*Synthesis of PRX 4.* OPSS-PEG-OPSS **1** (Mw = 20 kDa, 0.100 g, 0.013 mmol) was dissolved in H<sub>2</sub>O (0.2 mL) and added to a concentrated solution of  $\alpha$ CD **2** (0.288 g, 0.294 mmol) in H<sub>2</sub>O (1.5 mL). Within several minutes a white precipitate was formed, indicating the formation of PPRX assemblies. Over the course of the following hour a gel

formed. The suspension was sonicated for 30 min, shaken (1 rpm) for 16 h at 24 °C and freeze dried to give the PPRX as a white powder (0.388 g), which contained a fraction of non-threaded  $\alpha$ CD. Compound **3** (0.012 g, 0.029 mmol) and the PPRX (0.388 g) were placed in a tube (2 mL) and then DMF (0.36 mL) was added. The mixture was vortexed until a thick yellow slurry was formed (ca. 2 min). The suspension was sonicated for 1 h and left to sit for 12 h. Acetone (1.5 mL) was added on top of the slurry, and the resulting precipitate was washed with acetone ( $5 \times 1.5$  mL) and H<sub>2</sub>O ( $2 \times 1.5$  mL). The crude product was freeze dried, dissolved in DMSO (0.5 mL) and precipitated with H<sub>2</sub>O (2.0 mL); this cycle was repeated to ensure that all non-threaded  $\alpha$ CDs and PEG were removed. The precipitate was freeze dried to give PRX **4** (0.059 g) as a white powder. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): (s, 4H, Ph); 5.66 (br s, 6H, OH-2 of  $\alpha$ CD); 5.50 (br s, 6H, OH-3); 4.80 (br s, 6H, H-1 of  $\alpha$ CD); 4.43 (br s, 6H, OH-6 of  $\alpha$ CD); 3.74-3.27 (b, 78H, H-6a,b, H-3, H-5, H-4, H-3 of  $\alpha$ CD); 3.51 (s, 1818H, -OCH<sub>2</sub>CH<sub>2</sub>O- of PEG) ppm. The number of threaded  $\alpha$ CDs per PEG chain was determined to be 63 by integration of the CH-1 signal at 4.80 ppm and the PEG methylene protons at 3.51 ppm (Fig. S1).

*Synthesis of PRX 5.* PRX **5** was prepared accordingly to a previously described protocol.<sup>[1-3]</sup> PRX **4** (0.059 g) and succinic anhydride (0.055 g, 1.5 equivalent to CDs primary alcohols) were dissolved in anhydrous pyridine (0.5 mL). The solution was sonicated for 60 min and left to react for 16 h. The solution was precipitated into diethyl ether (5 mL); the precipitate was isolated via centrifugation (12 000 g, 1 min), and dissolved in 200 mM phosphoric acid solution (1 mL) at pH 7. Subsequently, the sample was dialyzed against water at pH 7 using a regenerated cellulose membrane (MWCO = 12.4 kDa), and then freeze dried to afford PRX **5** (0.050 g). The degree of substitution of CDs with carboxyethylester was determined by comparing the integration values between CH-1 of  $\alpha$ CD and ethyl peaks (2.65 ppm and 2.48 ppm) of carboxyethylester groups. <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O): 6.75 (s, Ph), 5.12~5.03 (b, C<sub>1</sub>H

of  $\alpha$ CD), 4.52~3.50 (b, C3H, C5H, C6H, C2H, and C4H of  $\alpha$ CD), 3.72 (s, -OCH<sub>2</sub>CH<sub>2</sub>O- of PEG), 2.65 (b, CH<sub>2</sub>COO-), 2.48 (b, CH<sub>2</sub>COO) ppm (Fig. S2).

*Synthesis of PRX 6 and 7.* PRX 6 and 7 were prepared accordingly to a previously described protocol.<sup>[1-3]</sup> The PPRX was formed with a saturated solution of  $\alpha$ CD containing 224 equivalent of  $\alpha$ CD for 1 PEG equivalent. The resulting PPRX was blocked with 3-mercaptopropion-(3,4,5-trimethoxybenzylamide) as described for the synthesis of PRX 4 to obtain PRX 6. The precursor to PRX 7, PRX 6, was modified with succinic anhydride following a protocol identical to the modification of PRX 5 (<sup>1</sup>H-NMR spectra: Fig. S3 and S4).

*DLS Analysis.* Sample solutions were prepared by dissolving PRX 5 in MQ water or buffered MQ water (0.1 to 1 mg mL<sup>-1</sup>) and centrifuging at 20 000 g for 3 min to remove any possible contaminant that could affect the measurement. PRX 5 self-assemblies were formed by adding acid from a 200 mM citric acid solution at pH 2 or a solution of 1 M HCl to the centrifuged solution.

*AFM Analysis.* Samples were prepared by dropping 3  $\mu$ L of a solution of PRX 5 (0.025 mg mL<sup>-1</sup>) in 10 mM HCl or in MQ water on a freshly cleaved mica disc followed by drying at 24 °C. The edge of the dried droplet was imaged.

*TEM Analysis.* Samples were prepared by dropping 1  $\mu$ L of a solution of PRX 5 (0.025 mg mL<sup>-1</sup>) in 10 mM HCl or in MQ water on formvar-coated 300-mesh copper grids (ProSciTech) followed by washing with 10 mM HCl solution or MQ water. After blotting, the sample was left to dry at 24 °C prior to analysis.

*pH Dependent Behaviour of PRX 5.* From a stock solution of PRX 5 in MQ water (5 mg mL<sup>-1</sup>) diluted solutions were prepared (0.1 mg mL<sup>-1</sup>) with a buffered solution of sodium acetate for measurements at pH 5, 4 and 3, citric acid at pH 2, and HCl solutions at pH 1 and 0. For  $\zeta$  potential measurements the samples were diluted in the same manner and

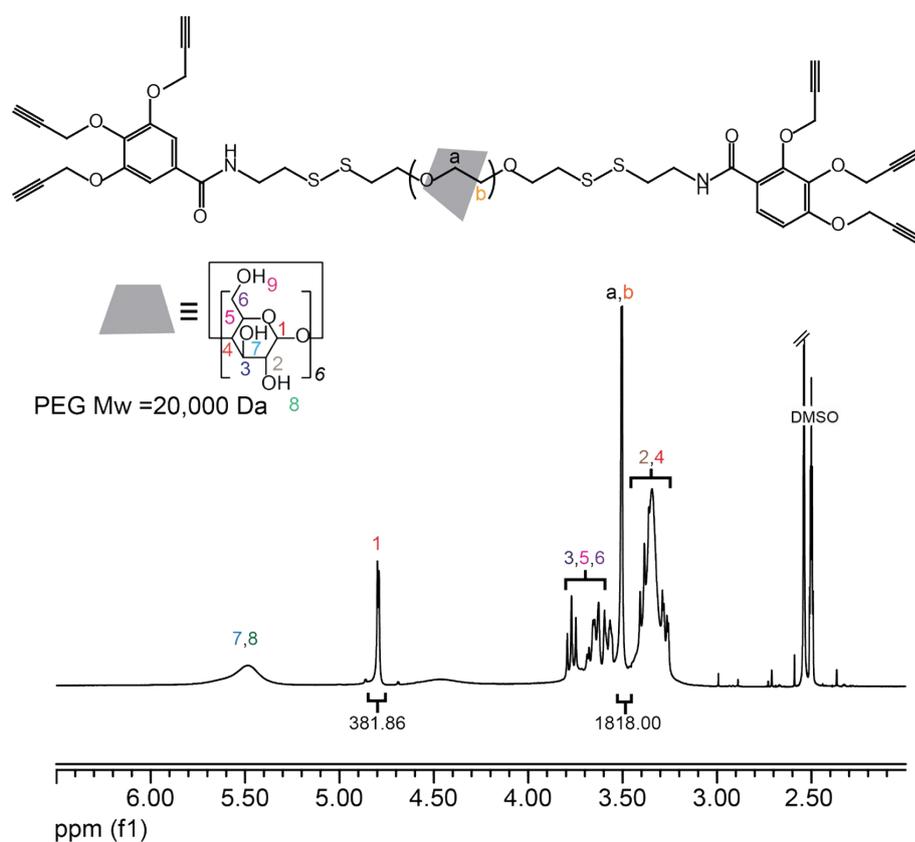
measured against 5 mM solutions of buffer except for pH 1 and 0 where the molarities of the acid were 0.1 M and 1 M, respectively.

*pH Cycling Study.* A solution of 1 mg mL<sup>-1</sup> of PRX 5 was prepared in water and centrifuged at 20 000 g for 1 min in order to remove any possible contaminant. The resulting solution was adjusted to pH 2 by the addition of citric acid (2 μL, 0.1 M). Adjustment of the pH of this mixture to 7.4 was performed by dialysis against PBS. Adjustment of the pH to 2 was performed by the addition of citric acid. After each pH adjustment, the size distribution of the suspension was measured by DLS.

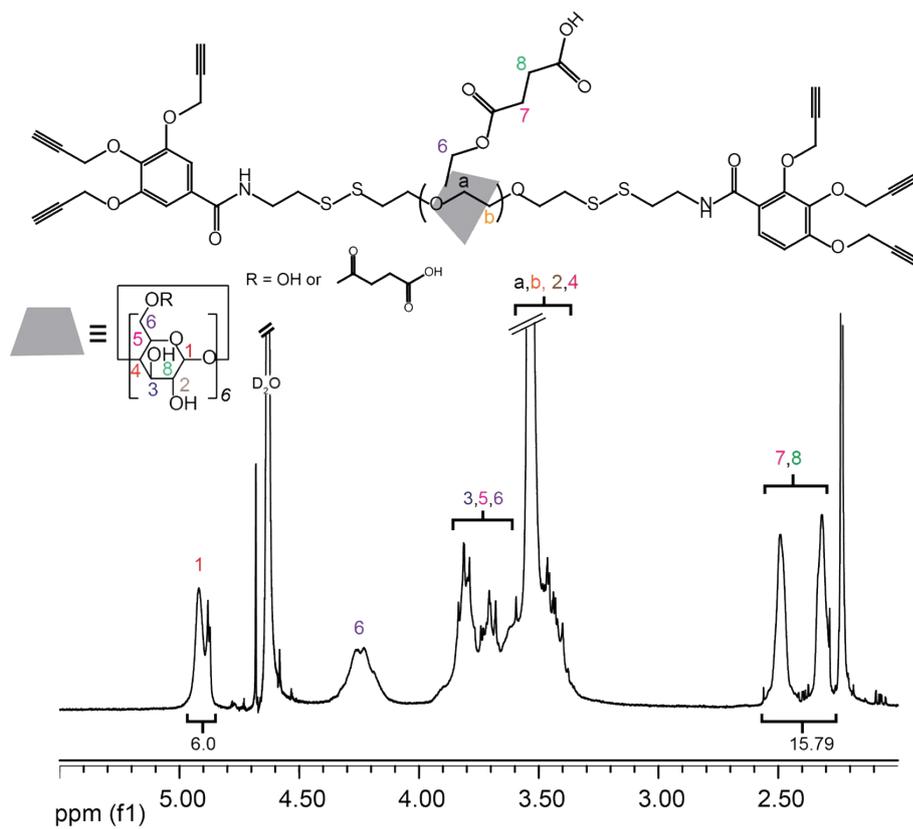
*Protein Association Experiments.* Solutions of lysozyme (1 mg mL<sup>-1</sup>) and PRX 5 (1 mg mL<sup>-1</sup>) were prepared in either 0.1 M of citric acid adjusted to pH 2 or in PBS adjusted to pH 7.4. The solutions were centrifuged for 2 min at 20 000 g to remove any possible contaminant. PRX 5 solutions at pH 2 were prepared from a solution of PRX 5 (aq.) centrifuged for 2 min at 20 000 g followed by acidification to pH 2 with HCl. The 1:1 v:v lysozyme PRX 5 mixtures were prepared by mixing equal quantities of both solutions at a given pH; this was followed by DLS measurements. The reversibility of the lysozyme-PRX 5 association/dissociation was assessed via the following procedure. Starting with the 1:1 mixture in PBS at pH 7.4, a 100 mM solution of citric acid at pH 2 was added until the pH was 2, followed by DLS measurements. The pH of the resulting mixture was adjusted to 8 by the addition of 100 mM solution of phosphoric acid. This was followed by DLS measurements. Subsequently, a 100 mM solution of citric acid was added to adjust the pH to 2 and the dispersion was measured by DLS.

*FTIR Measurements.* Solutions of SA-PRX 5 were prepared at 1 mg mL<sup>-1</sup> and pH adjusted using 2 M solutions of HCl or NaOH. After thoroughly washing the crystal with water, ethanol, acetone and water, 10 μL of the solution was deposited on the FTIR instrument crystal and left to dry for several hours before measurements.

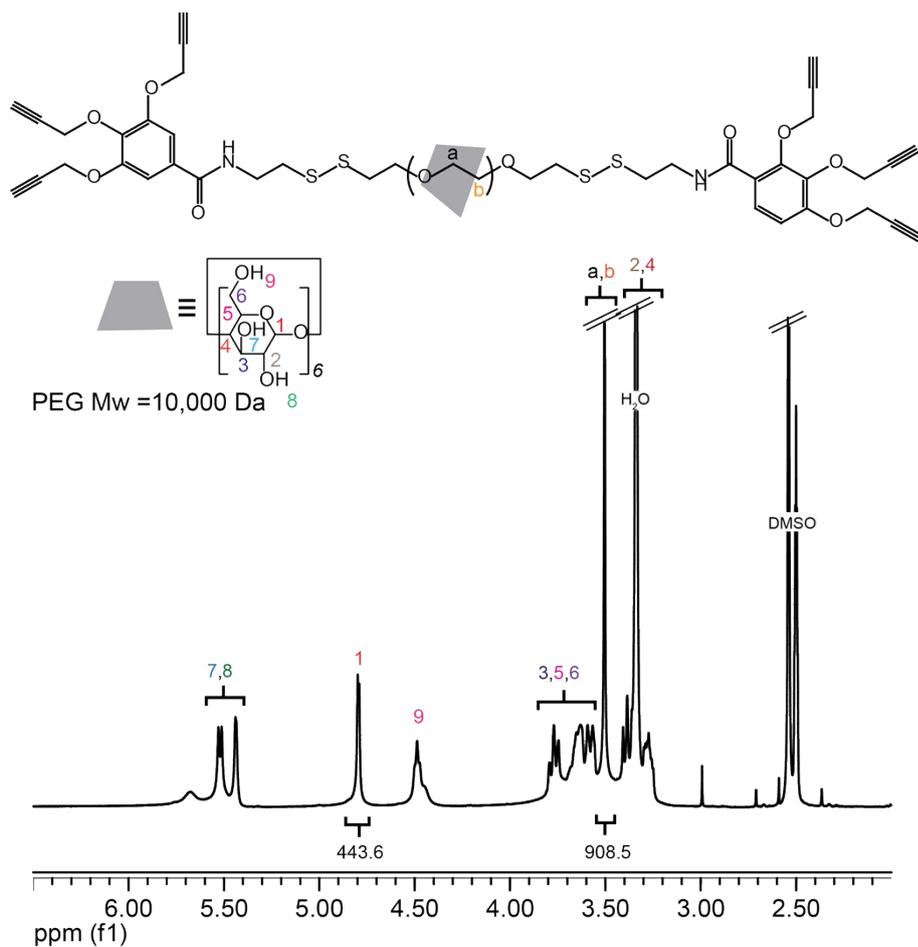
Mixture of SA-PRX 5 and Triton-X. Solutions of Triton-X were prepared at 0.9, 10 and 200 mg mL<sup>-1</sup>, and mixed with a PRX 5 solution at pH 2 in order to obtain mixtures at fixed concentrations. The final concentration of PRX 5 was 0.3 mg mL<sup>-1</sup>. The average sizes were obtained by DLS.



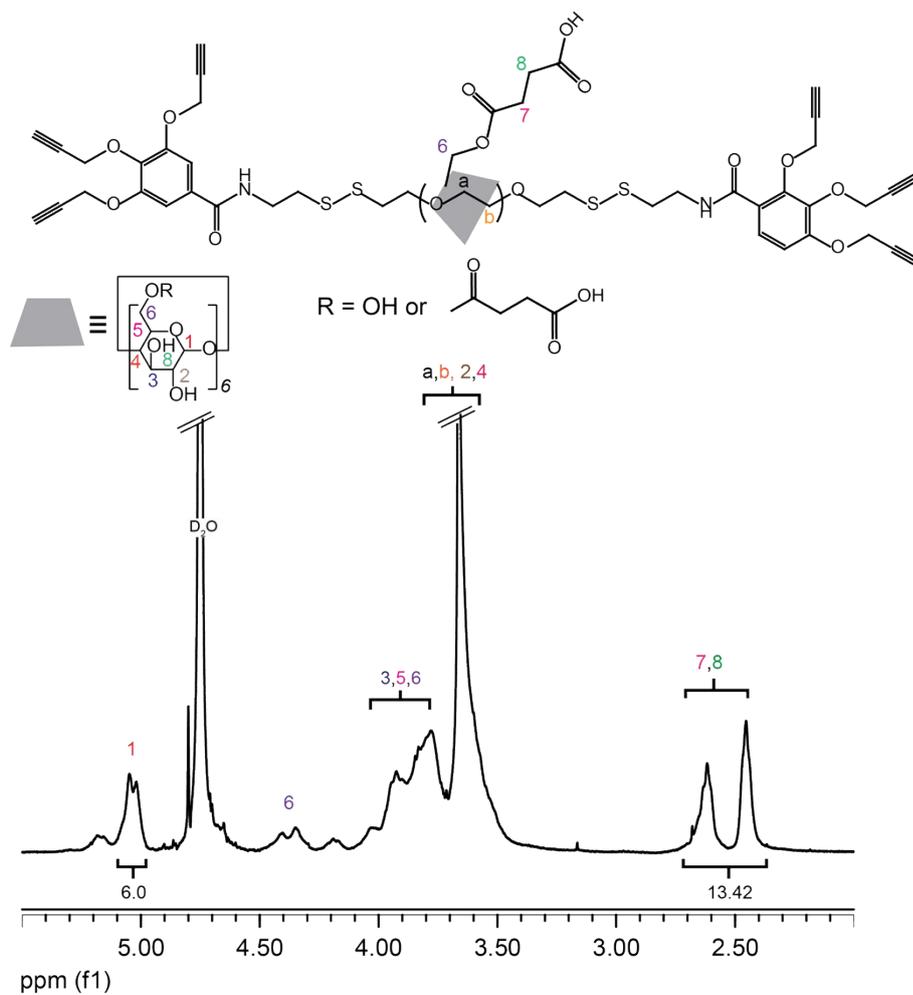
**Fig. S1** <sup>1</sup>H NMR spectrum (DMSO-*d*<sub>6</sub>, 400 MHz) of αCD/ PEG<sub>20kDa</sub> PRX, PRX 4.



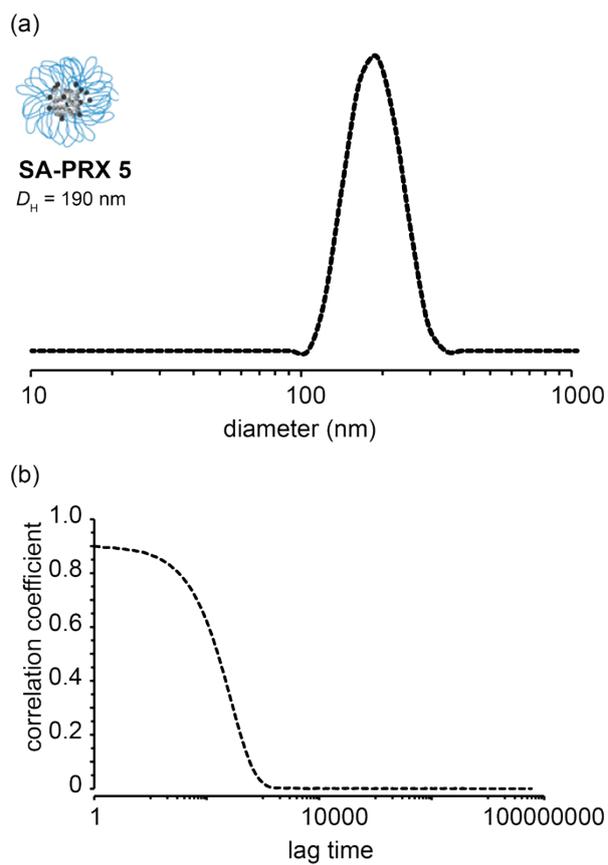
**Fig. S2**  $^1\text{H}$  NMR spectrum ( $\text{D}_2\text{O}$ , 400 MHz) of  $\alpha$ CD/ PEG<sub>20kDa</sub> PRX with carboxyethyl ester modification, PRX 5.



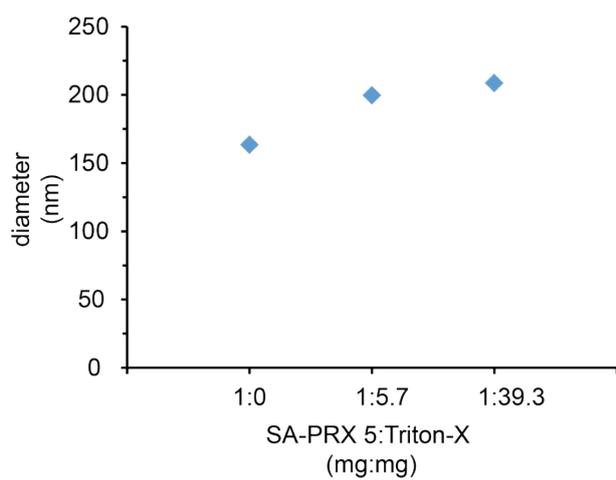
**Fig. S3** <sup>1</sup>H NMR spectrum (DMSO-*d*<sub>6</sub>, 400 MHz) of  $\alpha$ CD/ PEG<sub>10kDa</sub> (PRX 6), precursor to PRX 7.



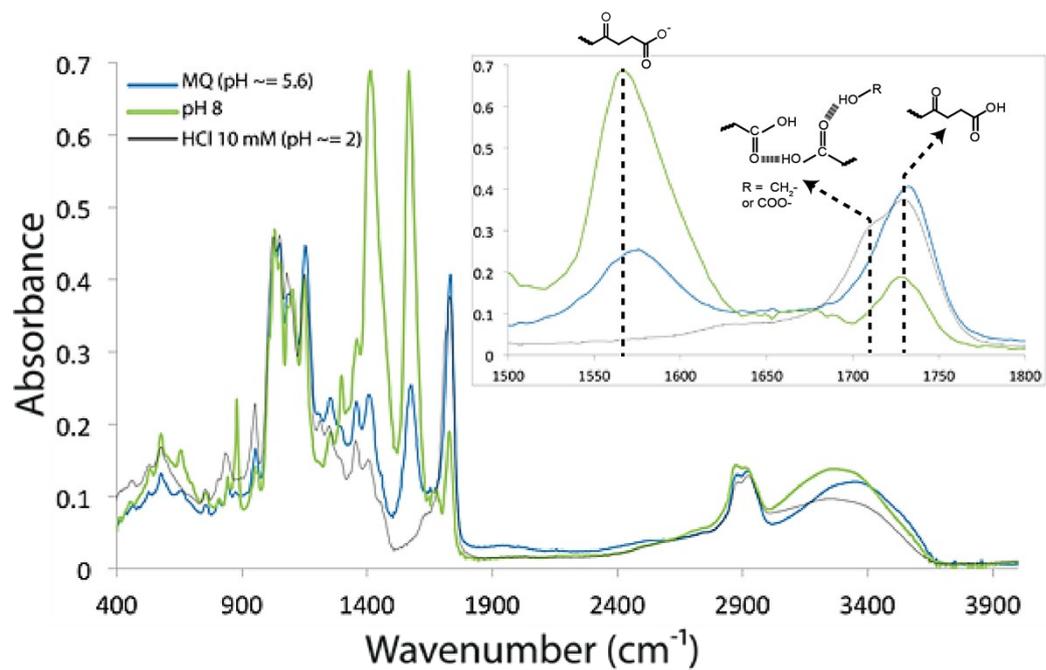
**Fig. S4**  $^1\text{H}$  NMR spectrum (D<sub>2</sub>O, 400 MHz) of  $\alpha$ CD/ PEG<sub>10kDa</sub> with carboxyethyl ester modification, PRX 7.



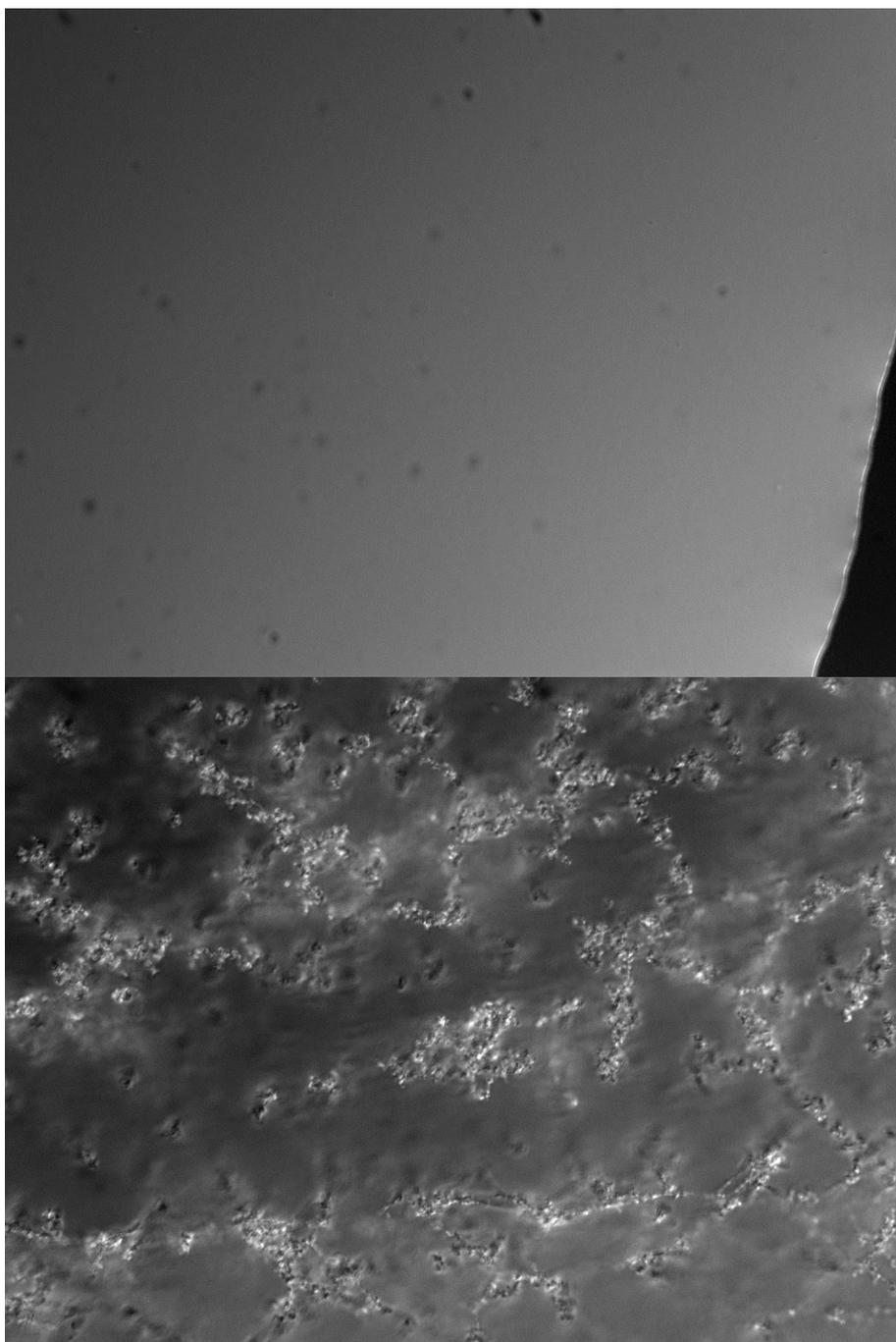
**Fig. S5** (a) DLS (intensity) analysis of SA-PRX 5 at pH 2 and (b) the corresponding correlogram.



**Fig. S6** DLS (intensity) size measurements at different ratios of SA-PRX 5 : Triton-X (mg:mg).



**Fig. S7** FTIR data for PRX **5** spotted from MQ water solution (pH 5.6, blue trace), pH 8 solution (green trace) and 10 mM HCl solution (pH 2, black trace). The inset shows a scheme suggesting the carbonyl chemical environment.



**Fig. S8** PRX 7 formed using PEG of  $M_w$  10 kDa with a high threading ratio (65%) and the same extent of carboxyethylester modification per CDs as PRX 5. Dissolved in MQ water (pH 5.6) (top) or in 100 mM HCl (pH 2) (bottom).

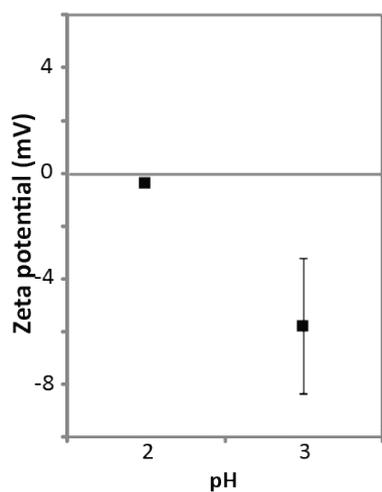


Fig. S9 Zeta potential measurements of SA-PRX 5 at pH 2 and 3, respectively.

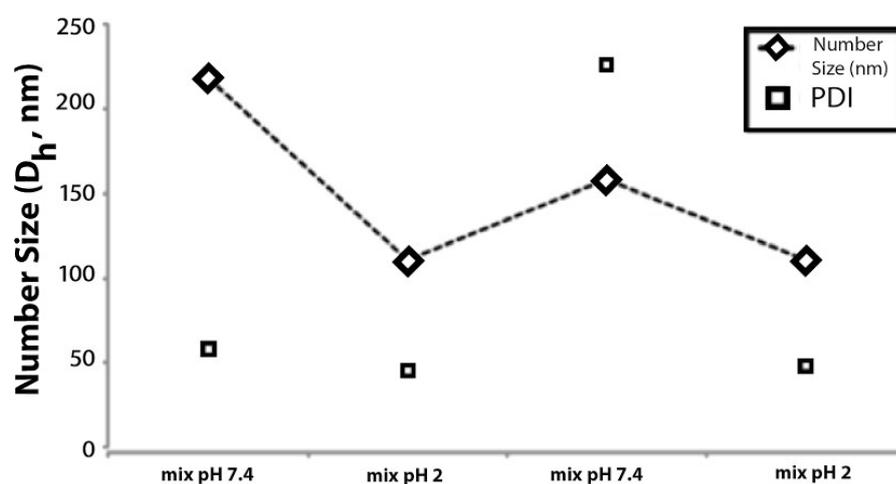


Fig. S10 DLS studies showing pH-reversible interaction between SA-PRX 5 and lysozyme.

## REFERENCES

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