Electronic Supplementary Material for CrystEngComm

Supporting Information for

Morphology Control of Lysozyme Crystal Shape by Different Block Copolymers**

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1. Materials

Monomers 2-(Dimethylamino)-ethyl methacrylate (DMAEMA, Aldrich), Poly(ethylene glycol) methyl ether methacrylate(PEGMA, Aldrich) and Poly(ethylene glycol) methacrylate(PEGMA-OH, Aldrich) were passed through a basic alumina column and then distilled prior to polymerization. Monomer n-butyl methacrylate(BMA) were distilled before polymerization. 1,1,4,7,10,10-hexamethyl triethylenetetramine (HMTETA, Aldrich, 98%), ethyl 2-bromoisobutyrate(EbiB, Aldrich, 98%) were used as received. All other reagents and solvents were used without further purification unless stated. Lysozyme purchased from Genview without any purification were made up as 40 mg/ml stock solution in 0.1 M Hepes buffer at pH 7 and kept at -20℃ when not immediately used. The polymers were also dissolved in 0.1 M Hepes buffer, concentrations of polymer solutions used are 5%, 10%, 15% and 20% at pH 7. The tacsimate contained 1.36 M malonate, 0.25 M citrate, 0.12 M succinate, 0.3 M DL-malate, 0.4 M acetate, 0.5 M formate, and 0.16 M DL-tartrate were dissolved in nonionic water.

Synthesis of block copolymers

Block copolymers were prepared by sequencial addition of different block monomers. Commonly, the first kind of monomers was polymerized in THF solution at room temperature for 6h under nitrogen, using CuBr/HMTETA as the catalyst and EBiB as the initiator. Then the second kind of monomers was added into the reaction mixture, reacted for another 6h, and then the third kind of monomers was added. After the reaction, the mixture was passed through a basic alumina column to remove Cu complexes using acetone as the eluent. The products were recovered by removing the eluent and then precipitated into a large excess of diethyl ether. Each precipitated polymer product was dialyzed against MWCO 3500 membrane in deionized water and lyophilized.

2. Protein crystallization

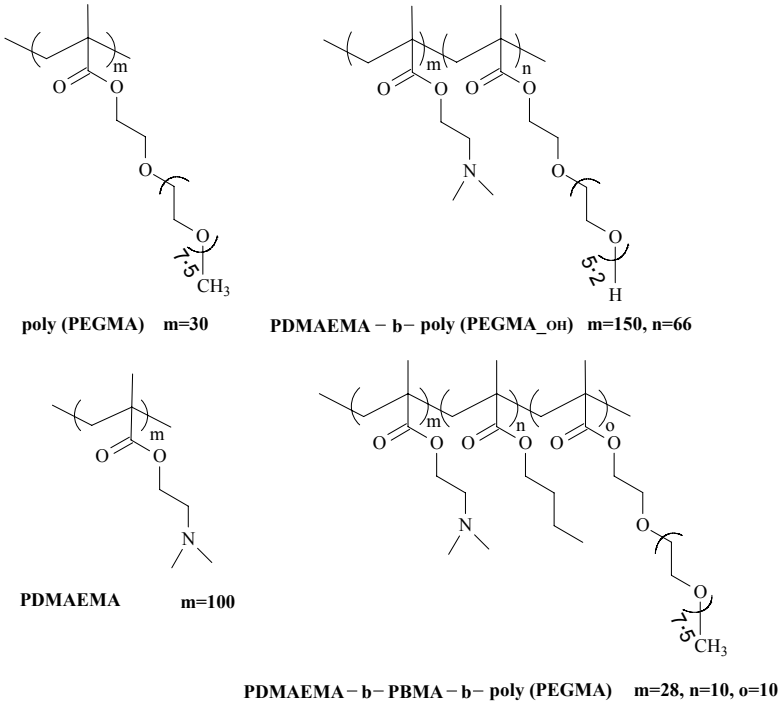
Crystallization trials were carried out by hanging drop vapor diffusion methods in 24 well plates and maintained at room temperature over five-day period. In trial one, According to Alexander Mcpherson, we used 50%(w/w) tacsimate to mix with polymer solutions in equal volumes, and that a volume of lysozyme stock solution that was equal to the total volume of the mixtures were added. Thus droplet contained 2μl of the mixtures, reservoirs were 1ml 30% PEG4000. In trail two, we only mixed the polymer solutions at any concentrations and the lysozyme stock solution with equal volume, thus the droplet contained 2μl of lysozyme and polymer mixtures, reservoirs were 1 ml 30% PEG4000 in 0.1 M Hepes buffer at pH 7. An NIKON ECLIPSE TE2000-U microscope with an attached digital camera was used to obtain most of the photographs after five days.

3. Charaterization

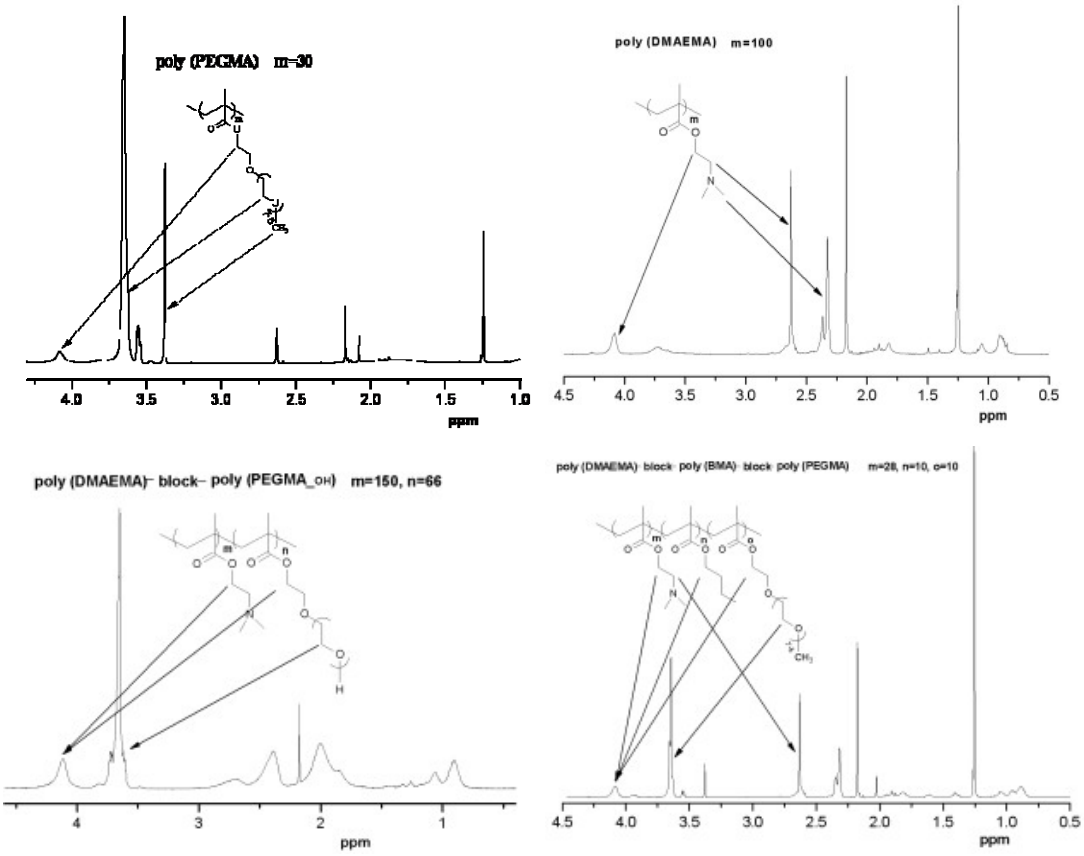
The composition of block copolymers were determined by 1H NMR spectroscopy recorded on a Varian Unity plus-400MHz instrument using D_2O or $CDCl_3$ as solvents. The molecular weight and polydispersity index for each block copolymer were measured with a Waters 1525 gel permeation chromatography(GPC) equipment consisted of four Polymer Standards Service columns (102 Å, 103 Å, 104 Å, 105 Å) in series coupled to a Waters 2414 differential refractometer. Each sample was dissolved in DMF, the mobile phase, immediately injected onto the column, and eluted at 1.0mL/min.

The size and morphology of polymers self-assembled in aqueous solution were analyzed by TEM images. Commonly polymers were dissolved in deion water at a concentration of 5mg/mL. Then a total of 10μL of the solution was dispensed onto 300-mesh carbon-coated copper grid and left air dry. The samples were viewed in a Philip TZOST transmission electron microscope.

Nanostar Small-Angle X-ray Scattering was used to analyze the polymer self-assemble in solution. SAXS samples were prepared by dissolving 10% Polymers into 0.1M HEPES buffer or 10% polymer solution mixed (1:1) with 50% tascimate. Data were collected on the spectrometer($0.00071 < q < 0.94736$ $Å^{-1}$ where $q = 4\pi \sin(2\theta/2)/\lambda$ and 2θ is the scattering angle). This wide q range probes structure on length scale from a few nanometers to a few hundred nanometers.



Scheme S1. Chemical structures of block copolymers poly(PEGMA), PDMAEMA, PDMAEMA-b-poly(PEGMA-OH) and PDMAEMA-b-PBMA-b-poly(PEGMA).



FigS1*. 1H NMR spectra of different block copolymers (1) poly(PEGMA) (2) PDMAEMA (3) PDMAEMA-b-poly(PEGMA-OH) (4) PDMAEMA-b-PBMA-b-poly(PEGMA)*

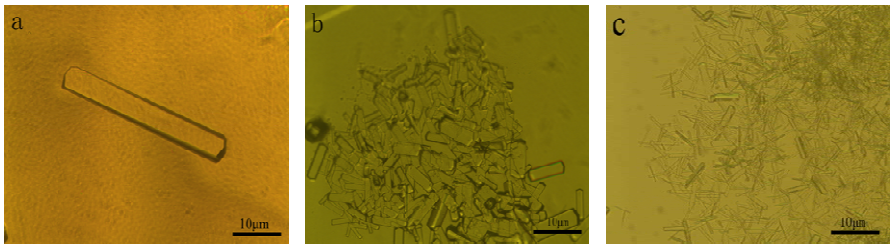


Figure S2. optical microscope images of lysozyme crystals obtained at different magnification from different precipitant: a), 30% PEG4000 and 50% tascimate; b), 10% PDMAEMA and 50% tacsimate ; c), 10% PDMAEMA-b-PBMA-b-Poly(PEGMA) and 50% tacsimate. All the crystals obtained are rod-shaped.