Supplementary Information for

Quantitative Study on the Antifreeze Protein Mimetic Ice Growth Inhibition Properties of Poly(ampholytes) Derived from Vinyl-Based Polymers

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SEC analysis of poly(aminoethyl methacrylate)

Below are representative traces for the SEC of the PAEMAs. Molecular weights below that expected from NMR/conversion were observed, as discussed in the main manuscript. This was ascribed to a combination of polymer 'sticking' to the column and internal amidation by backbiting of the amine onto the ester, which is a well-known side reaction for PAEMA upon storage in aqueous solution.

Figure S1 shows the SEC trace obtained for $PAEMA_{11k}$ showing two peaks at molecular weights significantly below that expected from conversion and end-group analysis. Similarly, Figure S2 shows SEC for $PAEMA_{35k}$ showing a similar bimodal distribution at very small molecular weights. Further evidence that these molecular weights are incorect is that the polymers could be dialysed (in acidic media to prevent amidation) against 10kDa MWCO dialysis tubing, without significant mass loss.

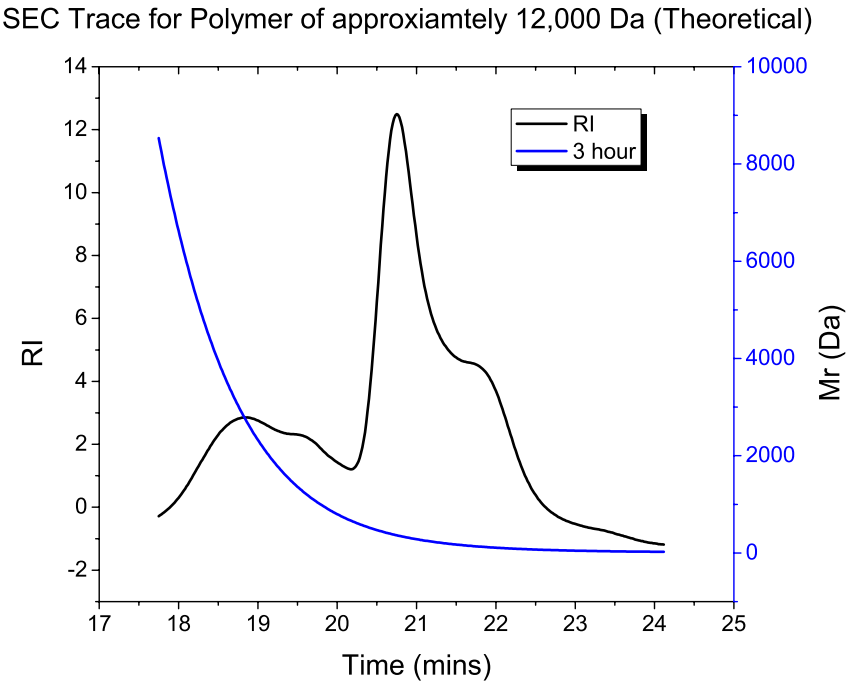

Figure S1. SEC trace of PAEMA M_N = 11 kg.mol^{-1} (by NMR).

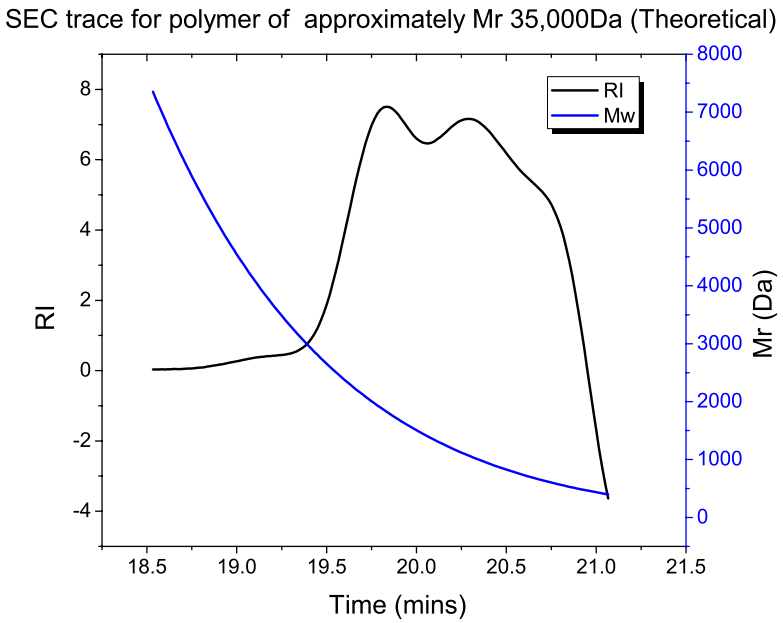

Figure S2. SEC trace of PAEMA M_N = 35 kg.mol^{-1} (by NMR).

It was also attempted to functionalize the amine side chains, to obtain a non-reactive polymer. This was done by addition of Boc-anhydride, and the obtained SEC (in THF) trace is shown below. A rather disperse, but mono-modal peak is obtained at ~ 20 kg.mol^{-1}, which is in line with the NMR measurements, suggesting the polymer does have the anticipated chain length. The dispersity can be accounted for by the incomplete functionalization of the amine groups, leaving some cationic groups which still have strong column interactions.

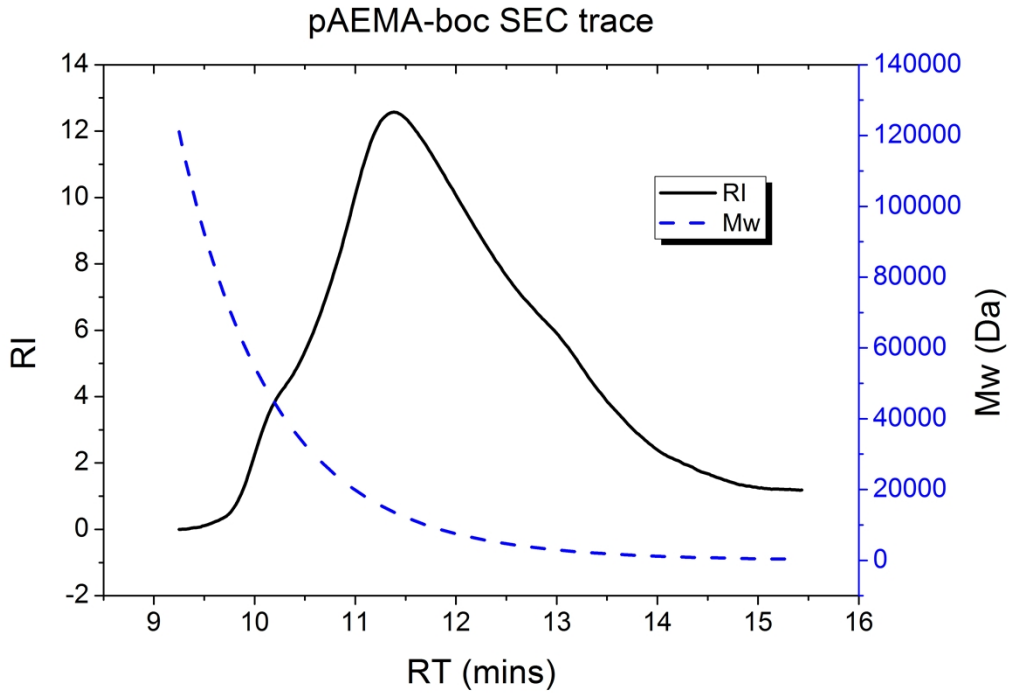

Figure S3. SEC (THF) of Boc-PAEMA M_N = 33 kg.mol^{-1} (by NMR).

Synthesis of Carbohydrate-Centered Poly(ampholytes)

The reaction scheme is shown in Figure S4, below, and full synthetic details are also included.

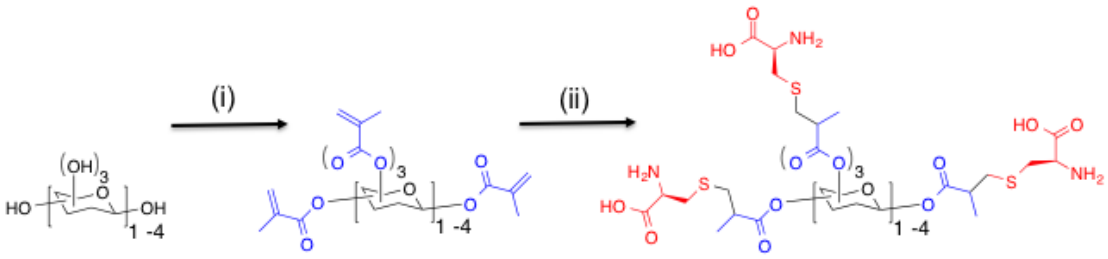

Figure S4. (i) Methacrylic anhydride/pyridine; (ii) Boc-Cys, then TFA/DCM.

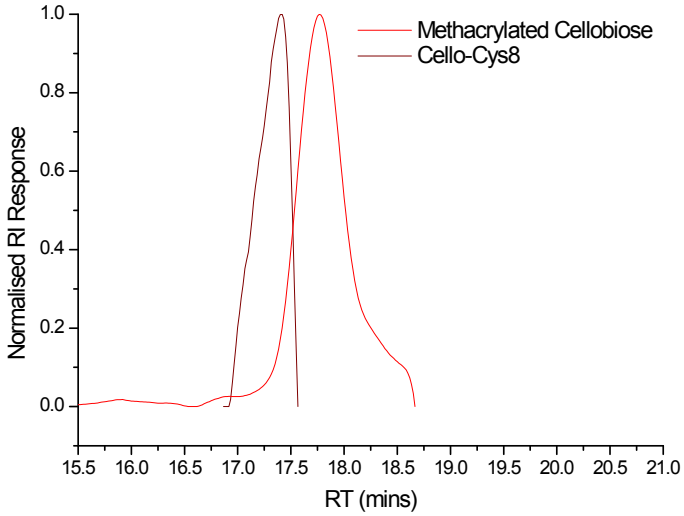

Figure S5. SEC (DMF) showing decrease in rentention time upon addition of Boc-Cysteine

Synthetic Procedures.

All reactions were undertaken in a similar manner. Representative examples using glucose core is described.

Glucose pentamethacrylate

Glucose (5.0500g, 27.75mmol, 1eq), methacrylic anhydride (21.5252g, 0.14mol, 5.1eq) and pyridine (125ml) were stirred at room temperature overnight before being heated to 60°C for 3 hours. Upon cooling, dichloromethane (50ml) was added and the product washed with 0.1M HCl (40x50ml), water (2x50ml), brine (50ml) and saturated $NaHCO_3$ (2x50ml) before being dried with $MgSO_4$. The solvent was then removed under vacuum at 40°C to yield a clear sticky product which was purified from THF and water (2.9101g, 20% yield). ^{1}H NMR ($CDCl_3$, 400MHz) δ (ppm): 6.45 (d, *J*=3.8Hz, 0.5H, β anomer), 6.27-6.03 (m, 5H, C=H_1H_2), 5.87 (d, J=8.0Hz, 0.5H, α anomer), 5.76-5.44 (m, 6H, 5 C=H_1H_2, 1 C$H_{glucose}$), 5.39-5.09 (m, 2H, C$H_{glucose}$), 4.44-4.15 (m, 3H, C$H_{glucose}$, C$H_{2\ glucose}$), 2.01-1.84 (m, 15H, CH_3); ^{13}C NMR ($CDCl_3$, 600MHz, PENDANT) δ (ppm) 166.9-164.9 (*C*=O), 135.7-134.8 (R_2C=CH_2), 128.2-126.4 (R_2C=CH_2), 92.3-92.1 (β anomer), 89.8-89.7 (α anomer), 76.1-68.2 (C$H_{glucose}$), 62.8-62.0 (C$H_{2\ glucose}$), 18.2-18.0 (CH_3); IR υ (cm^{-1}) 1721 (s, C=O ester), 1637 (m, C=C), 1453 (m, CH_2/CH3 bend), 1317 (m, CH_3 bend), 1292 (m, alkyl C-H), 1140 (s, C-O stretch); ESMS (positive mode) m/z 475.1 $[M(4mer)+Na]^+$ $C_{22}H_{28}O_{10}$, m/z 543.2 $[M(5mer)+Na]^+$ $C_{26}H_{32}O_{11}$; GPC (DMF) M_n 432, M_w 442, M_w/M_n 1.02.

Penta-Cysteine Glucose methacrylate.

Methacrylated glucose (0.0521g, 96μmol, 1eq), Boc-cysteine (0.1143g, 250μmol, 2.6eq), tributyl phosphine (drop, catalytic) and benzylamine (drop, catalytic) were dissolved in DCM (3ml) and stirred at 45°C for 72 hours. DCM (10ml) was then added and the product washed with water (2x20ml) and brine (20ml) before the

solvent was removed under vacuum. The product (Gluco-Cys5) was then purified in THF and water. IR υ (cm^{-1}) 2960 (m, O-H), 2927 (m, C-H stretch), 1720 (s, C=O ester), 1637 (m, C=C), 1454 (m, CH_2/CH3 bend), 1318 (m, CH_3 bend), 1160 (s, C-O stretch), 751 (m, C-S); GPC (DMF) M_n 946, M_w 1190, M_w/M_n 1.26. The product was then dissolved in DCM, and TFA (4 drops) added to remove the boc protecting group. The product was then dialysed and the water removed by freeze drying to yield a sticky solid (0.0072g, 7 %yield)

Cellobiose core M_n = 2126, M_w = 2153, M_w/M_n 1.01. **Stachyose** core GPC (DMF) M_n = 2467, M_w = 2575, M_w/M_n 1.04.

Ice Recrystallization Assay

Some example micrographs are shown below.The COOH degree of functionalization is indicated, with 50 % clearly have smaller grains compared to the others. Micrographs are for **P7, P11, P16** respectively at 15 $mg.mL^{-1}$.

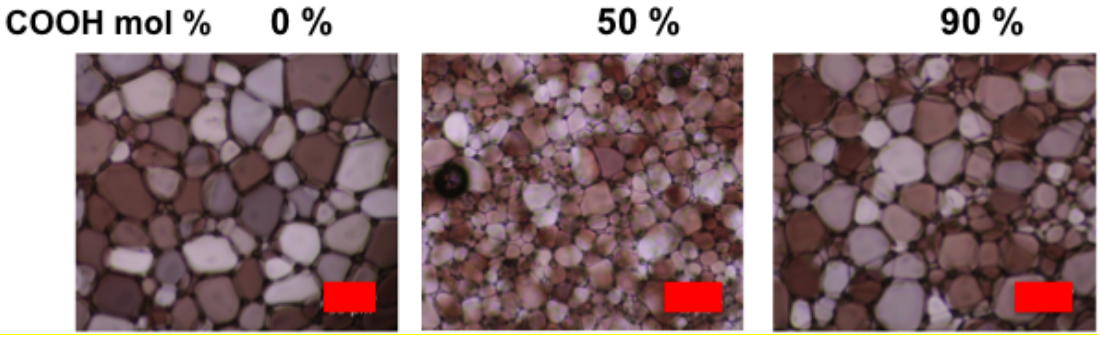

Figure S5. Example ice recrystalisation assay micrographs for (left to right) P7, P11, P16 at 15 mg.mL-1, after 30 minutes annealing at -6 °C.