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Supporting information

Affinity Purification of Multifunctional Oligomeric Ligands Synthesized via Controlled Radical Polymerization

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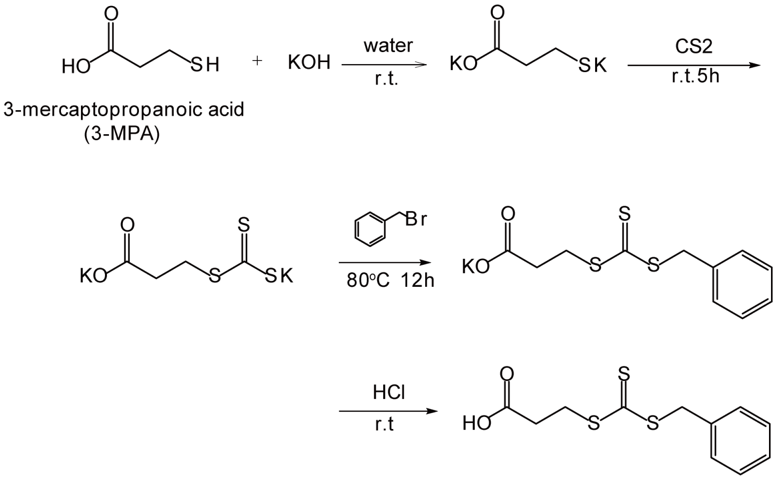
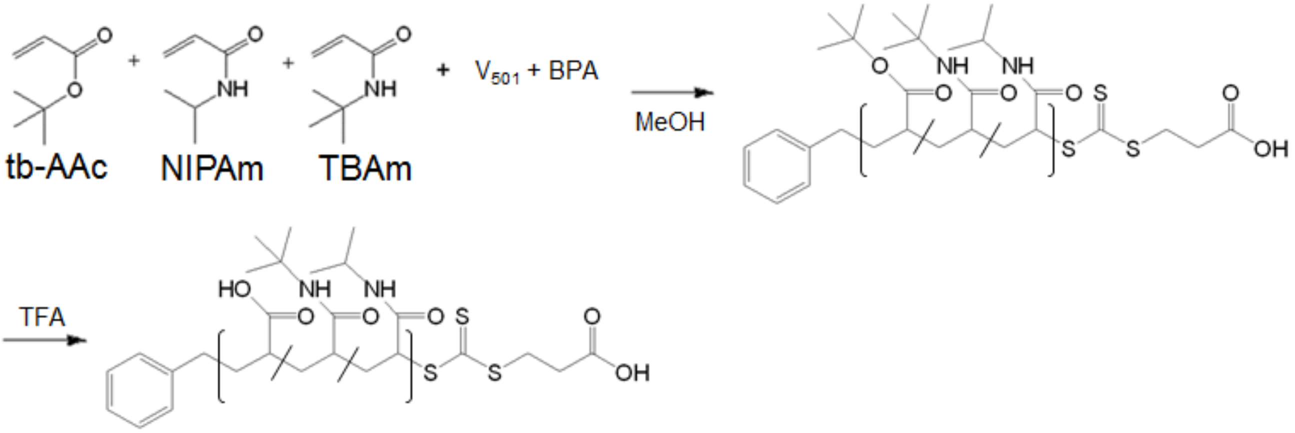
1. Preparation of the chain transfer agent (benzylsulfanylthiocarbonylsulfanyl propionic acid; BPA)



Scheme S1

Benzylsulfanylthiocarbonylsulfanyl propionic acid (BPA) was prepared as previously reported[1,2]. 3-Mercaptopropionic acid (10 mL, 115 mmol) was added to an aqueous solution of potassium hydroxide (12.9 g in 125 mL H_2O, 230 mmol). CS_2 (15.3 mL) was subsequently added dropwise and the solution was stirred for 5 h at room temperature. Benzyl bromide (13.65 mL, 55.4 mmol) was then added. The mixture was stirred at 80 ºC for 12 h, then at room temperature for an additional 2 h. Chloroform (150 mL) was then added followed by the slow addition of 150 mL HCl (1 M in H_2O). The aqueous layer was extracted with chloroform (3 x 100 mL), while the organic layer was washed with distilled water (3 x 100 mL). Finally, the organic layer was dried over $MgSO_4$. BPA was dissolved in a small volume of ethylene acetate and recrystallized in hexane. Yield: 70 %.

2. Preparation of polymer ligands



Scheme S2

Reversible addition-fragmentation chain-transfer (RAFT) was used to synthesize polymer ligands with a narrow molecular weight distribution. BPA and V-501 were used as the chain transfer agent and radical initiator, respectively. *N*-isopropyl acrylamide (NIPAm) was recrystallized from hexane prior to use. *Tert*-butyl acrylate (*tb*-AAc, 0.15 M), NIPAm (1.05 M), *N*-*tert*-butylacrylamide (TBAm, 0.3 M), BPA (0.05 M) and V_{501} (0.01 M) were dissolved in methanol. Solutions were degassed in three cycles of freeze-evacuate-thaw, sealed in an ampoule, and heated at 70 °C for 3 h in an oil bath. Products were precipitated twice with brine and once with distilled water at 50 °C then dried in vacuo at 50 °C. Conversion rates were calculated from ^{1}H NMR in MeOD prior to the purification process. Compositions and molecular weights were determined by ^{1}H NMR and GPC (10 mM LiBr, DMAc) after the purification process. The purified polymer ligands were deprotected with dried TFA for 3 h then dried in vacuo at 50 °C. The conversion rate was 96% and the yield before deprotection was 83%.

3. ^{1}H NMR of polymer ligands

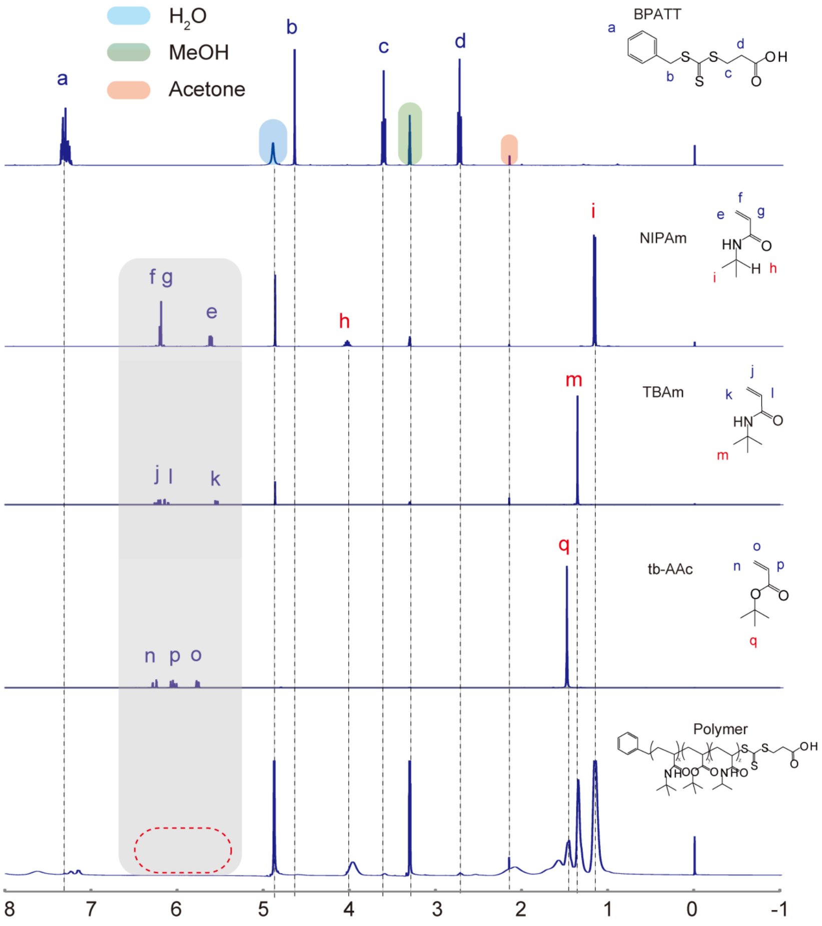


Figure S1. ^{1}H NMR of BPATT, monomers and ALs containing NIPAm 21-mer, TBAm 6-mer and *tb*-AAc 3-mer after purification (400 MHz, methanol-d_4).

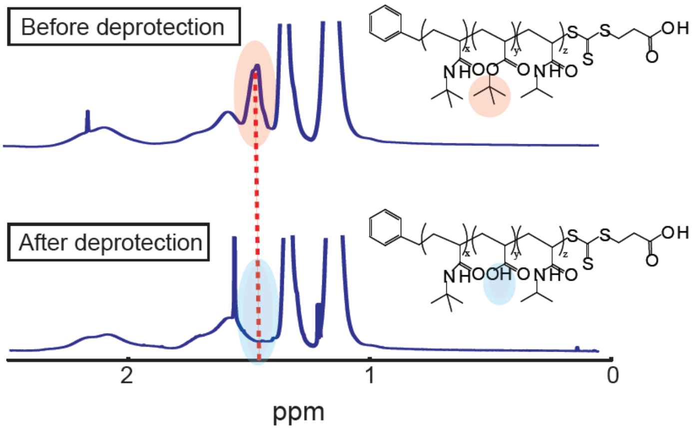
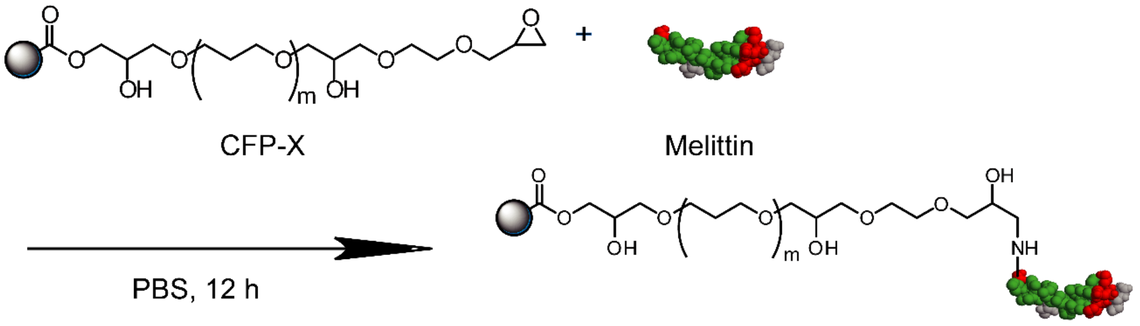


Figure S2. ^{1}H NMR of ALs containing NIPAm 21-mer, TBAm 6-mer and *tb*-AAc 3-mer before and after deprotection (400 MHz, methanol-d_4).

4. Peptide immobilized beads



Scheme S3

Melittin and magainin 1 were immobilized on the solid support (hydrophilic methacrylic polymer beads; CFP-X) as shown in scheme S3. CFP-X (10 g) was washed three times with 20 mL of phosphate buffer (PBS; 35 mM phosphate, 150 mM NaCl, pH 7.4). CFP-X in PBS (10 mL of a 50 vol% slurry) was mixed with 1.5 mL of peptide solution (PBS, 1.5 mM) then incubated for 12h at room temperature. The beads were then washed three times with PBS. Any remaining epoxides were quenched using 10 mM NaOH solution (incubated for 10 min at room temperature). Immobilized peptides were quantified using UV absorbance (280 nm) before and after incubation with CFP-X.

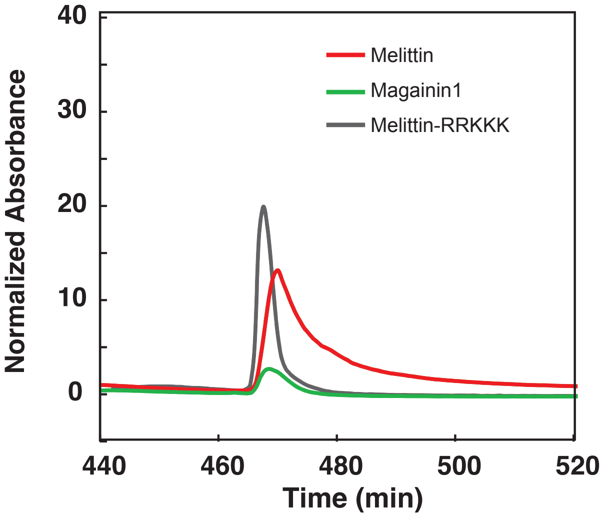


Figure S3. Chromatogram of cold elution process of the affinity purification process monitored by UV detector (280nm). Randomly copolymerized oligomers (2 mg/mL in PBS) were loaded onto melittin-immobilized beads (red), magainin 1-immobilized beads (green) and melittin RRKKKK-immobilized beads (gray).

References

1. M. H. Stenzel, T. P. Davis, *J. Polym. Sci. A.*, **40**, 2002, 4498.

2. H. Lee, Y. Hoshino, Y. Wada, Y. Arata, A. Maruyama, Y. Miura, *J. Am. Chem. Soc.*, 2015, **137**, 10878.