Electronic Supplementary Information

Synthesis and Effectiveness of Functional Peptide-based RAFT Agents

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1. Materials

4-Cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid (CDTPA) was prepared as previously reported.[1] *N,N*-dimethylaminoethyl methacrylate (DMAEMA; purchased from Aldrich), oligo(ethylene glycol) methyl ether methacrylate ($OEGMA_{475}$, M_n ~ 475 g mol^{-1}; purchased from Aladdin), and *n*-butyl methacrylate (BMA; purchased from J&K Chemical) monomers were purified by stirring in the presence of inhibitor-remover. 1,1'-Azobis(cyclohexanecarbonitrile) (VAZO-88; ACCN) initiator (Aldrich, 98 %) was used as received. Trifluoroacetic acid (TFA), *N,N*-diisopropylethylamine (DIEA), triisopropylsilane (TIPS), were used as received from J&K Chemical. Fmoc-amino acid derivatives (Fmoc-Arg(Pbf)-OH, Fmoc-Asp(tBu)-OH, Fmoc-Gly-OH, Fmoc-Ser(tBu)-OH), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), 2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU , 1-Hydroxy-7-azabenzotriazole (HOAt) and Rink amide AM resin (loading: 0.89 mmol/g) were used

as received from CS Bio Co. All solvents used were obtained from J&K Chemical.

2. Instrumentation

NMR spectra were recorded on a Bruker Avance III 400 MHz spectrometer in $CDCl_3$ or MeOD. Purification of products was performed on a preparative Shimadzu HPLC system (LC 20 AR，SIL, SPD) using a Dr. Maisch column (Reprosil-Pur Basic-C18, 250 x 10 mm, 5 um) with a flow rate of 4 mL/min and a UV-detection at 254 nm (eluents: A: 0.1% TFA in water and B: 0.1% TFA in acetonitrile). Mass spectrometry was performed on a Shimadzu LC-MS 2020 system with an electrospray ionization probe and a Waters Xevo G2 Qtof MALDI-TOF-MS. Analysis of the molecular weight of polymer samples were determined by a Shimadzu GPC system (using low dispersity PS as standards) equipped with a SIL-20A auto sampler, a 20 A refractive index detector, three Shodex KF-805L columns (8 × 300 mm, 10 μm, 5000 Å) and one Shodex KF-801 column (8 × 300 mm, 6 μm, 50 Å) using *N,N*-Dimethylacetamide (containing 2.1 g/L Lithium Chloride) as eluent at 80 °C with a flow rate of 1 mL min^{-1}. Peptide was synthesized by a peptide automatic synthesizer (136 XT, purchased from CS Bio Co.).

3. Synthetic Procedures

3.1 Synthesis of peptide functionalized RAFT agents

In general, oligopeptide was synthesized via fluorenylmethoxycarbonyl (Fmoc) standard solid-phase supported peptide synthesis method on Rink amide AM resin (0.45 mmol). The *N*-terminal Fmoc-protecting group was removed by using 20% piperidine in DMF. For the coupling of Fmoc-protected amino acids, 4 equivalents of amino acid, 3.8 equivalents of HBTU, and 8 equivalents of DIEA were used. After all the amino acids were linked to the resin, CDTPA was conjugated to the terminal of the oligopeptide using HATU/HOAt/DIEA for activation. Then the conjugates were cleaved by 2 h treatment with a cleavage cocktail of TFA/TIPS/water (95: 2.5: 2.5).

In particular, the crude RGD-based RAFT agent could be separated by three times of precipitation in diethyl ether. The purification of crude peptide was performed by

reverse-phase HPLC using a 25-100% buffer B gradient over 35 min. Analysis of product was performed on the same HPLC system with a Shimadzu column (5µm, 4.6 × 250 mm), employing a 10-90 % buffer B gradient at a flow rate of 1 mL/min.

The synthesis procedures for GFLG and cell-penetrating peptide (TP 10) based RAFT agents were similar to that of RGD based RAFT agent. The purification of GFLG based RAFT agent was performed by reverse-phase HPLC using a 30-90 % buffer B gradient over 40 min. TP 10 based RAFT agent was purified using the same column with a 20-100% buffer B gradient over 40 min.

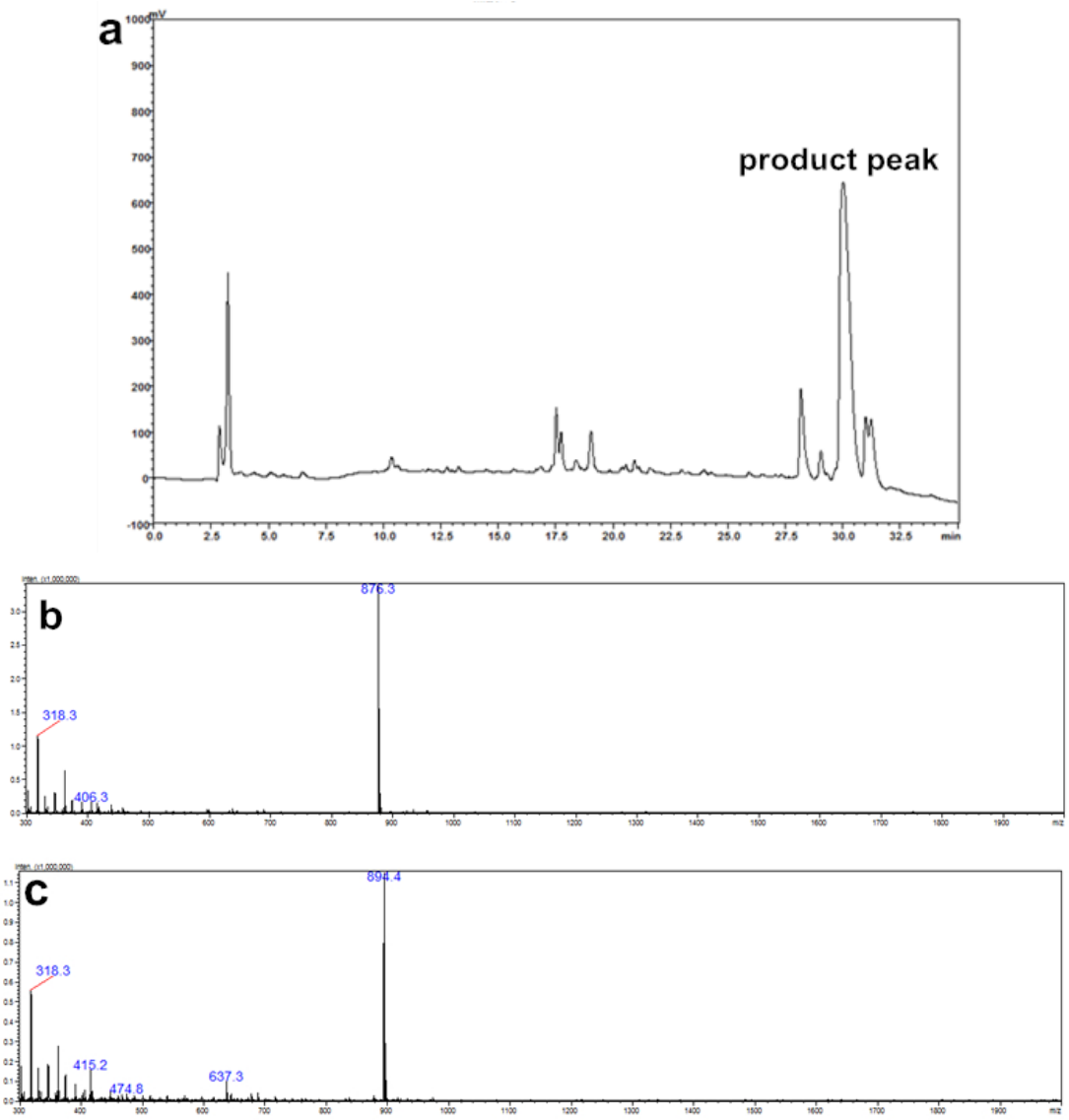

Fig. S1 (a) HPLC profile of crude RGD-based RAFT agent, (b) ESI-MS of purified RGD-based RAFT agent, (c) ESI-MS of RGD-based RAFT agent treated with pH 9 sodium carbonate buffer.

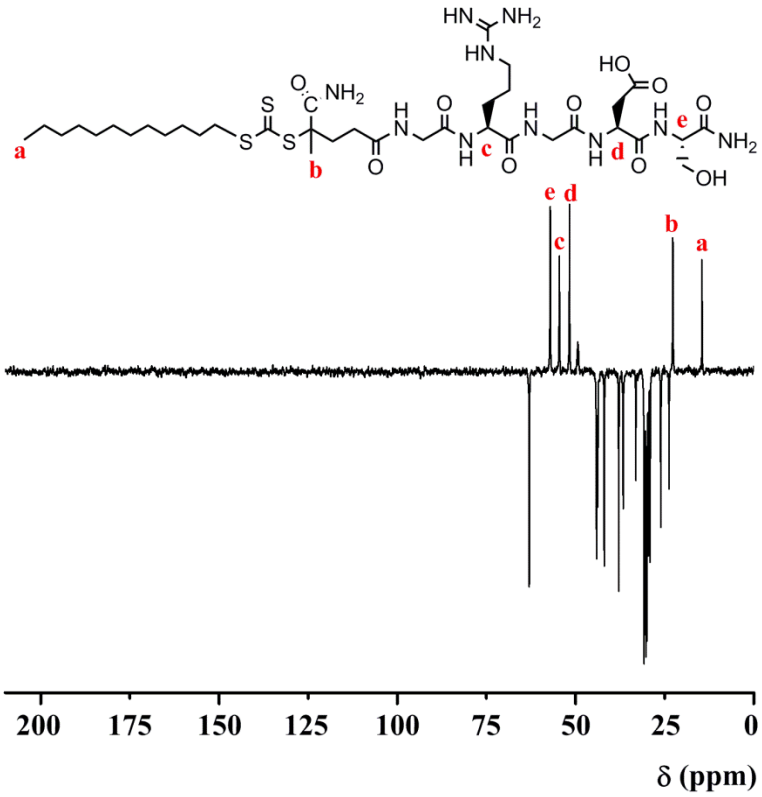

Fig. S2 Distortionless Enhancement by Polarization Transfer (DEPT) spectrum of RGD-based RAFT agent

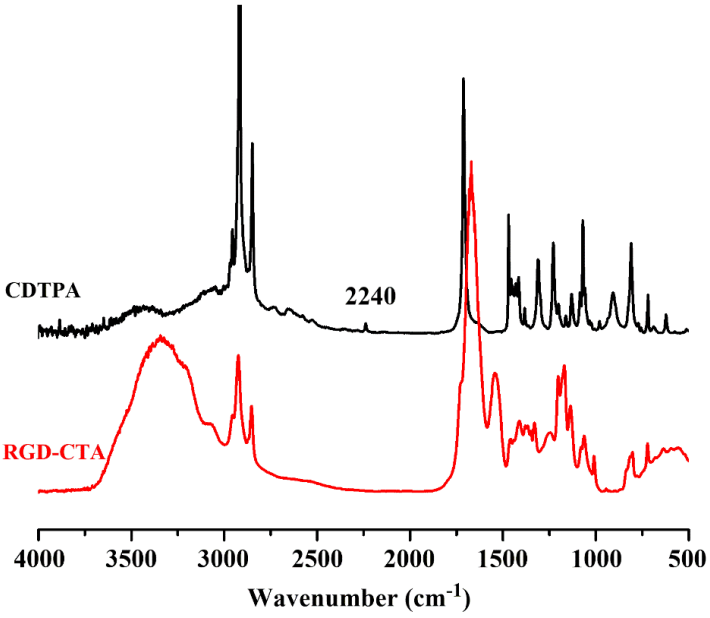

Fig. S3 FT-IR spectra of CDTPA (black) and RGD based RAFT agent (red).

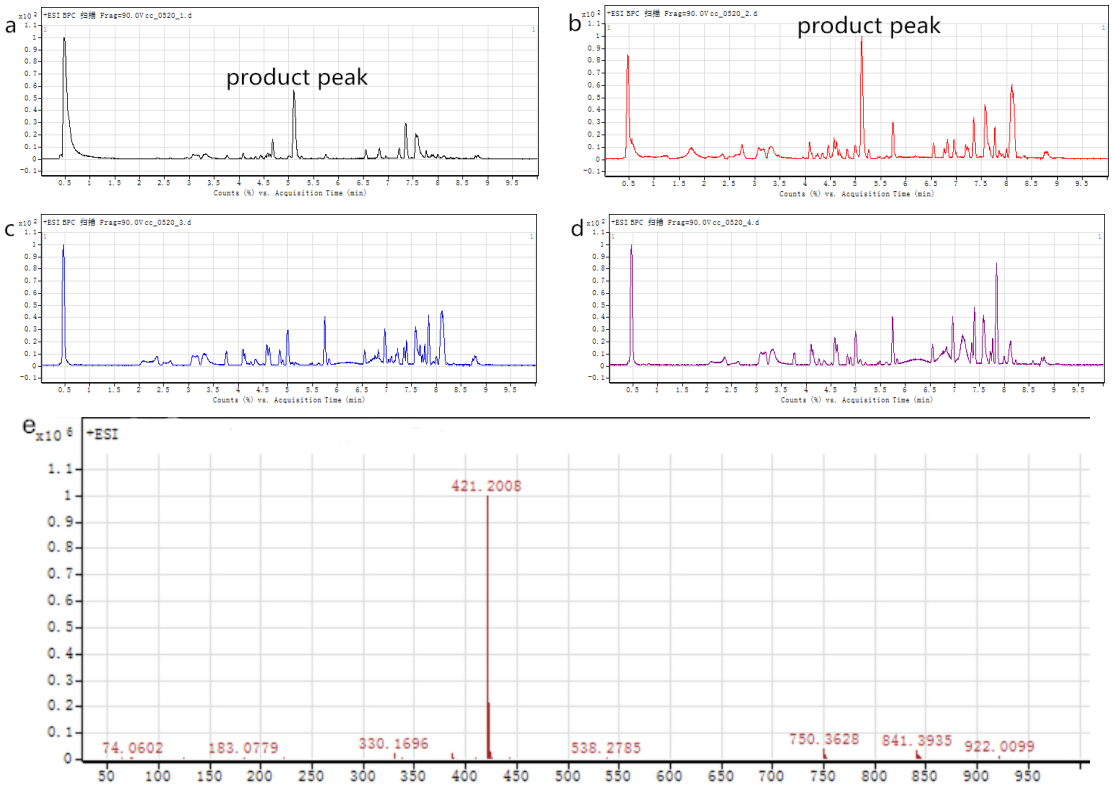

Fig. S4 CDTPA directly conjugated to resin, then the conjugate was cleaved from resin in different time: (a) 1 h, (b) 2 h, (c) 3 h, and (d) 4 h; (e) MS data of CDTPA-NH_2.

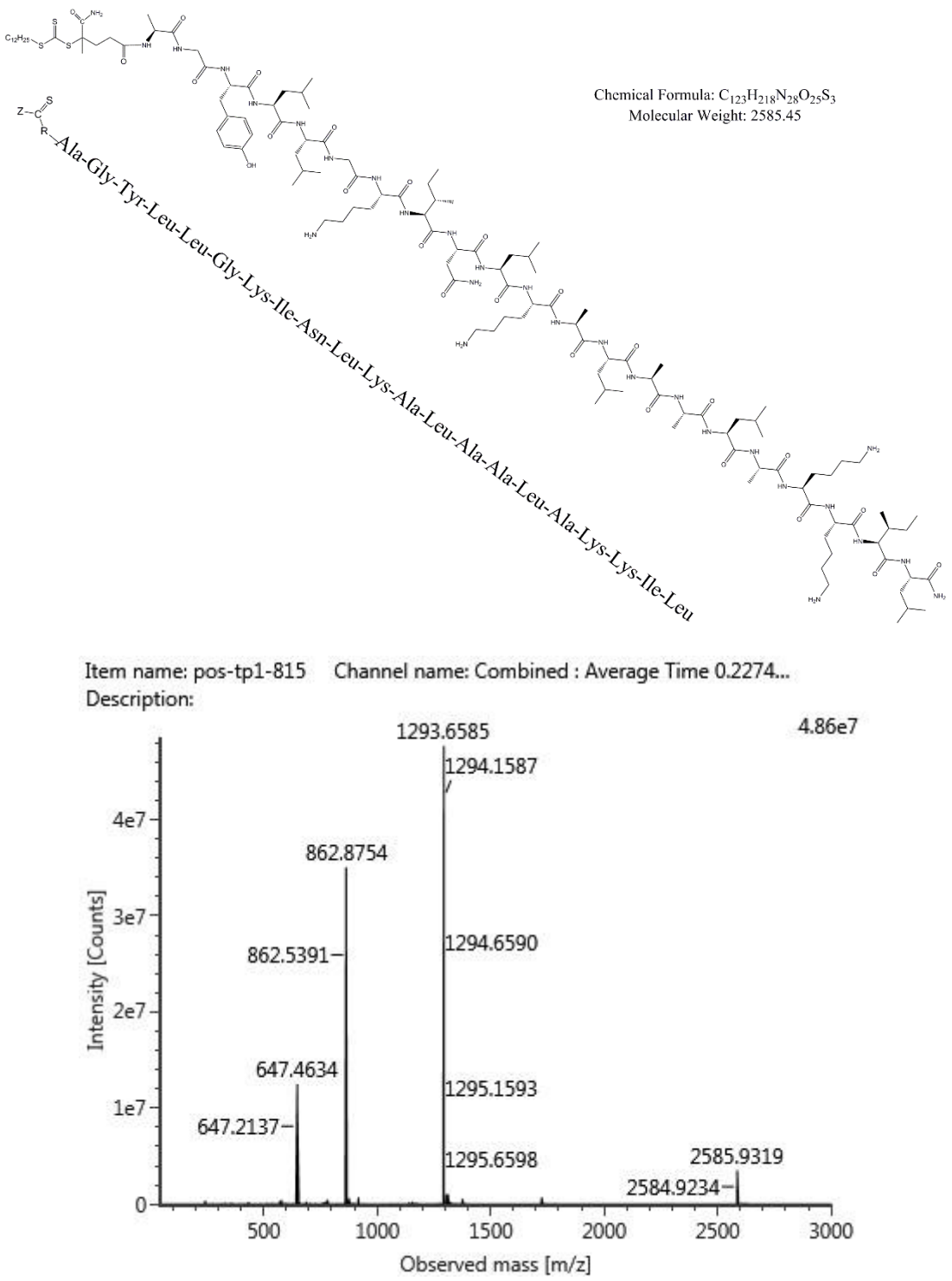

Fig. S5 TP10 based RAFT agent (top); MS data of TP10-based RAFT agent (bottom).

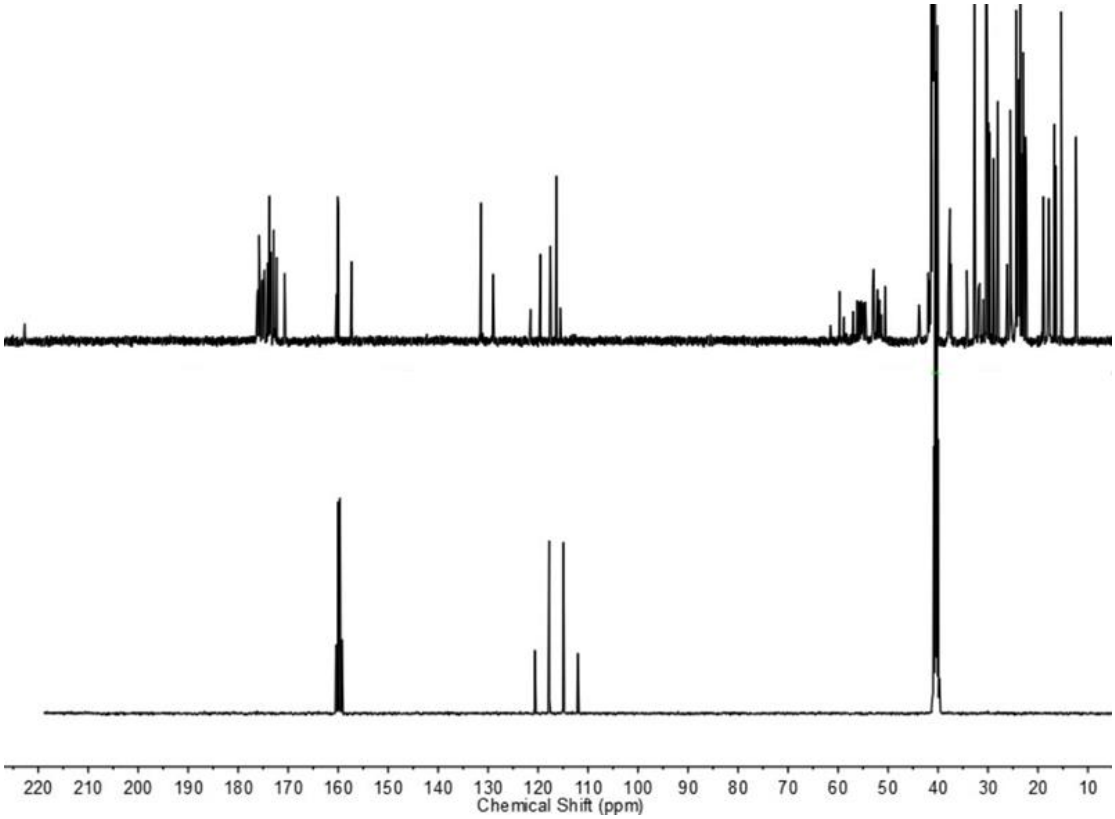

Fig. S6 ^{13}C NMR spectra of TP10-based RAFT agent (top) and pure (Trifluoroacetic acid) (TFA) (bottom).

3.2 Synthesis of PDMAEMA, POEGMA$_{475}$, PBMA by RAFT technique

In a typical procedure, 10.547 mmol (1.50 g, 185 eq) of the monomer BMA, 0.0057 mmol (1.40 mg, 0.1 eq) of ACCN, 0.057 mmol (0.05 g, 1 eq) of RGD-based RAFT agent and DMF (1.5 mL) were added into a sealed Schlenk tube, followed by 3 freeze-pump-thaw cycles. Then the tube was heated at 90 ^{o}C for a specified time as shown in Table S1. The synthesis of PDMAEMA, POEGMA$_{475}$ were the same as PBMA.

Table S1 Summary of RAFT polymerization for the synthesis of PDMAEMA, $POEGMA_{475}$, PBMA.[a]

Polymer	Conversion[b] (%)	M_n[c] (GPC)	M_n[d] (theo.)	$Đ$[e]
PDMAEMA-1h	53.4	15700	14900	1.15
PDMAEMA-2h	75.7	19900	20700	1.17
PDMAEMA-4h	87.8	25000	23900	1.18
PDMAEMA-6h	94.7	29100	25700	1.22
PDMAEMA-8h	96.6	29500	26200	1.23
$POEGMA_{475}$-0.5h	30.0	16100	8800	1.15
$POEGMA_{475}$-1h	47.6	17500	13400	1.16
$POEGMA_{475}$-2h	70.0	20800	19200	1.17
$POEGMA_{475}$-3h	86.1	23200	23500	1.21
$POEGMA_{475}$-4h	94.3	24800	25600	1.20
PBMA-1h	18.0	13100	5600	1.15
PBMA-2h	26.2	16600	7800	1.17
PBMA-4h	55.4	17600	15400	1.18
PBMA-6h	73.0	24500	20000	1.22
PBMA-8h	81.9	23500	22400	1.23

[a] Molar ratio of monomer : CTA : ACCN =185 : 1 : 0.1. [b] Determined by ^{1}H NMR. [c,e]Determined by GPC (DMAc solvent, polystyrene standard). [d]The theoretical molecular weight $M_{n,}$(theo.) = $[([M]_0/[CTA]_0) \times conv. \times M + M_{CTA})]$.

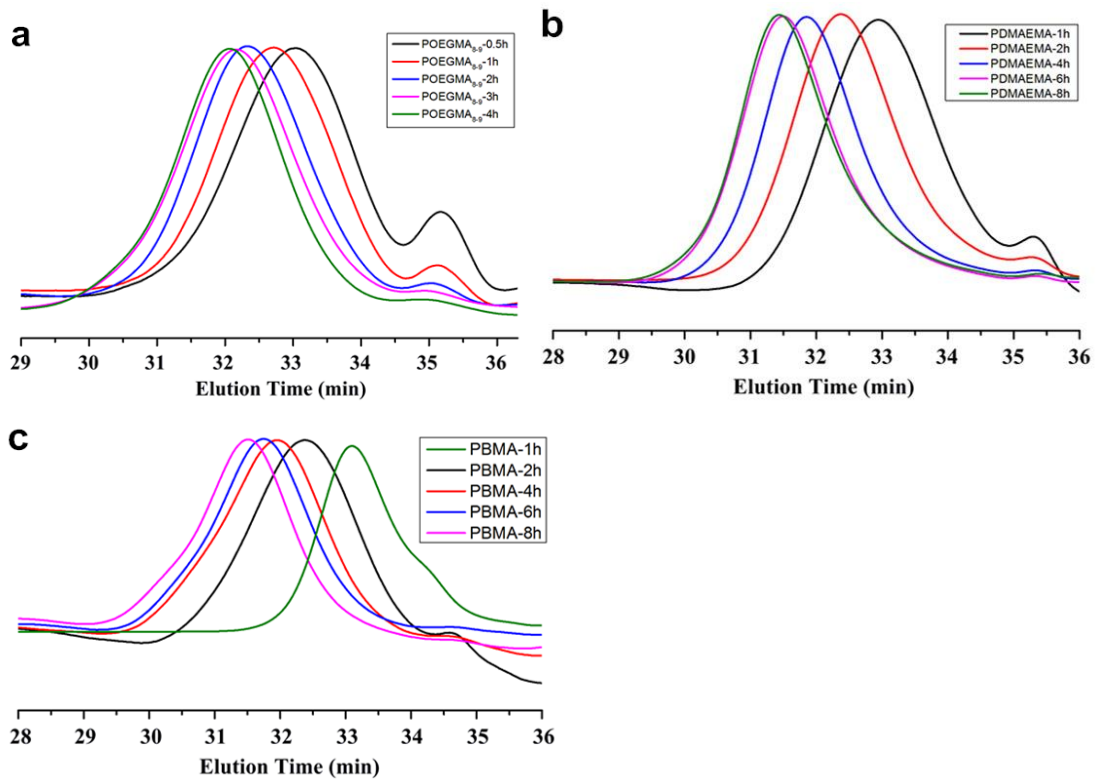

Fig. S7 GPC traces for (a) $POEGMA_{475}$, (b) PDMAEMA, and (c) PBMA at different polymerization times.

Reference

1. G. Moad, Y. K. Chong, E. Rizzardo, A. Postma and S. H. Thang, *Polymer*, 2005, **46**, 8458.