

Electronic Supplementary Information

## Thiol Chemistry on Well-Defined Synthetic Peptides

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### Experimental procedures

#### Characterization

For the SEC analysis using HFIP (Biosolve, AR-S from supplier or redistilled) as eluent measurements were done using a Shimadzu LC-10AD pump (flow rate 0.8 ml/min) and a WATERS 2414, differential refractive index detector (at 35 °C). Injections were done by Spark Holland, MIDAS injector, a 50 µL injection volume was used. The column is a PSS, 2\* PFG-lin-XL (7 µm, 8\*300 mm) column at 40°C. Calibration has been done using poly(methyl methacrylate) standards.

For the SEC analysis using DMF (Biosolve) as eluent measurements were done on a Waters Alliance system equipped with a Waters 2695 separation module, a Waters 2414 refractive index detector (40 °C), a Waters 486 UV detector, a PSS GRAM guard column followed by 2 PSS GRAM columns in series of 100 (10 mm particles) and 3000 (10 mm particles) respectively at 60°C. DMF was used as eluent at a flow rate of 1 mL min<sup>-1</sup>. The molecular weights were calculated against polystyrene standards. Before SEC analysis was performed, the samples were filtered through a 0.2 µm PTFE filter (13mm, PP housing, Alltech)

<sup>1</sup>H-NMR analyses were performed on a Mercury 400. For the monomers deuterated chloroform was used. For the polymers DMSO-d<sub>6</sub> and deuterated TFA were used.

Gradient polymer elution chromatography was done using a Zorbax Eclipse XDB-C8 column, 4.6 x 150 mm, 5 µm on an Agilent 1100 series setup. The eluent was initially toluene for 2 minutes, gradually changed to a 1:1 mixture of toluene/DMF over 25. The flow rate was 1

ml/min. The temperature of the column was 50 °C. Detection was done by a Polymer Labs ELSD detector.

## Materials

All materials were purchased from Aldrich and used without any purification, unless otherwise mentioned. Benzylamine 99.5% purified by redistillation, S-*tert*-butylmercapto-L-cysteine, N-(*tert*-butoxycarbonyl)-L-cysteine methyl ester 97%, DL-dithiothreitol  $\geq$ 99%,  $\alpha$ -pinene 98%, bis(trichloromethyl) carbonate (triphosgene) 99%, n-butylacrylate, methylmethacrylate, polyethyleneglycol methyl ether acrylate (Mn= 450 g/mol), were purchased from Aldrich. L-Glutamic acid,  $\gamma$ -benzyl ester was supplied from Bachem. Potassium hydroxide was purchased from VWR. 2,2'-Azobis(2-methylpropionitrile) was purchased from Merck. Cobalt phthalocyanine 90% was purchased from Acros. DMF (extra dry), ethylacetate, n-heptane and diethylether were purchased from Biosolve. DMF and ethylacetate were used directly from the bottle or stored under an inert, dry atmosphere. n-Butylacrylate and methylmethacrylate were purified by passing through a silica column twice before polymerisation.

## Synthesis

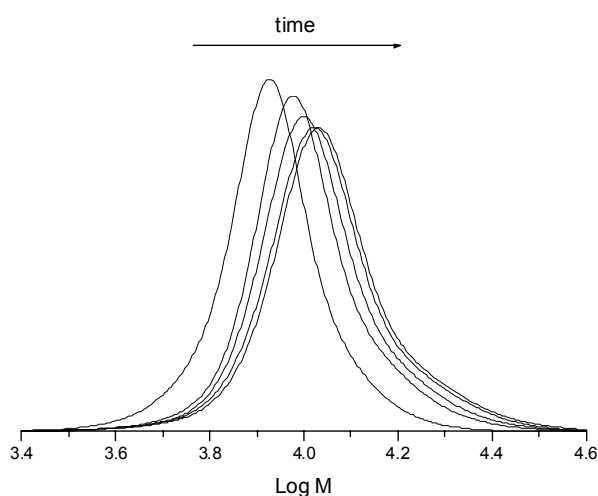
**Synthesis of NCA of  $\gamma$ -benzyl-L-glutamate:** L-Glutamic acid,  $\gamma$ -benzyl ester ( 25.18 g, 106 mmol) and  $\alpha$ -pinene ( 30.1 g, 221 mmol) were dissolved in 240 ml ethylacetate. Triphosgene (13.96 g, 47.04 mmol) was dissolved in 60 ml ethylacetate and added slowly once the reaction was refluxing. The solution became clear and all solids disappeared after 3 hours. 2/3 of the ethylacetate was removed by distillation. 200 ml n-heptane was added and the solution was heated to recrystallize. The NCA was recrystallized twice and subsequently washed with n-heptane, dried under vacuum and stored in a refrigerator over P<sub>2</sub>O<sub>5</sub>. Yield: 23.01 g, 87.4 mmol, 88 %. <sup>1</sup>H-NMR (400 Mhz, CDCl<sub>3</sub>,  $\delta$ , ppm ): 2.20 (m, 2H, CH<sub>2</sub>), 2.59 (t, 2H, CH<sub>2</sub>, J=6.8 Hz), 4.37 (t, H, CH<sub>2</sub>O, J=6.1 Hz), 5.14 (s, 2H, CH<sub>2</sub>O ) 6,57 (s, 1H, NH) 7.36 (m, 5H, ArH), <sup>13</sup>C-NMR (400 Mhz, CDCl<sub>3</sub>,  $\delta$ , ppm ): 26.77 (CH<sub>2</sub>CH), 29.57 (CH<sub>2</sub>CO), 56.81 (CH) 67.03 (CH<sub>2</sub>O), 128.29 (ArH), 128.53(ArH), 128.68(ArH), 135.27(ArH), 152.30 (NHC(O)O), 169.56 (CH<sub>2</sub>C(O)O), 172.36 (CHC(O)O), Melting point: 94 °C.

**Synthesis of NCA of S-*tert*butylmercapto-L-cysteine:** S-*tert*-butylmercapto-L-cysteine (4.97 g, 23.7 mmol) and  $\alpha$ -pinene (7.16 g, 52.6 mmol) were dissolved in 60 ml ethylacetate.

Triphosgene (4.90 g, 16.5 mmol) was dissolved in 20 ml ethylacetate and added slowly once the reaction was refluxing. The solution became clear and all solids disappeared after 2 hours. 2/3 of the ethylacetate was removed by distillation. 70 ml n-heptane was added and the solution was heated to recrystallize. After another recrystallization the NCA was washed with n-heptane, dried under vacuum at 20°C and stored in a refrigerator under P<sub>2</sub>O<sub>5</sub>. Yield: 4.90 g, 20.8 mmol, 88 %. <sup>1</sup>H-NMR (400 Mhz, CDCl<sub>3</sub>, δ, ppm ): 1.36 (d, 9H, ((CH<sub>3</sub>)<sub>3</sub>C)), J=1.0), 2.8 (m, 1H, (CH<sub>2</sub>)), 3.23 (m, 1H, (CH<sub>2</sub>)), 4.71 (dd, 1H, CH, J=3.3 Hz, J=9.1 Hz) 6.57 (s, 1H, NH), <sup>13</sup>C-NMR (400 Mhz, CDCl<sub>3</sub>, δ, ppm ): 29.82 ((CH<sub>3</sub>)<sub>3</sub>C), 40.71 (CH<sub>2</sub>), 49.13 (C(CH<sub>3</sub>)<sub>3</sub>), 57.24 (CH), 151.50 (O(CO)NH), 168.23 (O(CO)CH), Melting point: 107°C.

**Synthesis of P(BLG-co-tBMLC):** The γ-benzyl-L-glutamate NCA (6.00 g, 22.8 mmol) and the tert-butylmercapto-L-cysteine NCA (1.33 g, 5.7 mmol) were dissolved in 45 ml DMF. A solution of benzylamine (123 mg, 1.14 mmol) in 5 ml DMF was added once all NCAs were dissolved. The reaction was maintained for 4 days at 0 °C and precipitated in diethylether, washed with diethylether and dried under vacuum at 20 °C. Yield: 4.70 g, 0.770 mmol (Mn: 6100 g/mol), 76%.

**Synthesis of P(BLG-co-tBMLC) with sampling:** The γ-benzyl-L-glutamate NCA (5.05 g, 19.2 mmol) and the tert-butylmercapto-L-cysteine NCA (1.12 g, 4.8 mmol) were dissolved in 35 ml DMF. A solution of benzylamine (64 mg, 0.60 mmol) in 5 ml DMF was added once all NCAs were dissolved. The reaction was maintained for 4 days at 0°C. Samples were taken and precipitated in diethylether, washed and dried under vacuum.

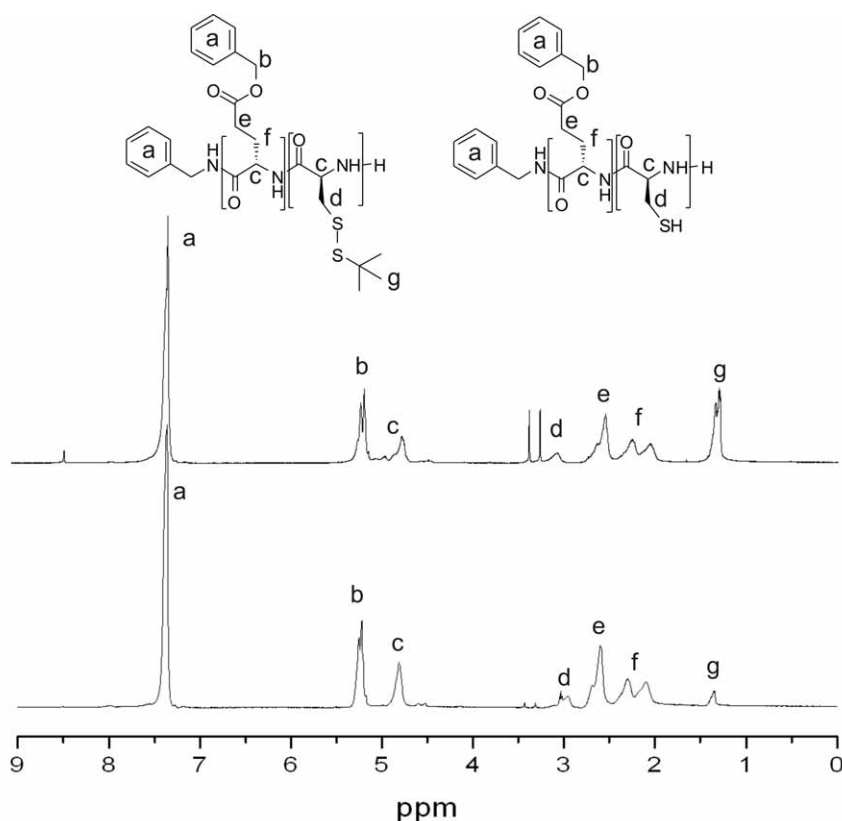


**Figure 1:** SEC traces of sampled NCA ROP reaction of P(BLG-co-tBMLC), t= 3 hours, 1, 2, 3 and 4 days.

**Table 1:**  $^1\text{H-NMR}$  integral ratios of sampled P(BLG-*co*-tBMLC).

#	ppm	3 hours	1 day	2 days	3 days	4 days
H-benzyl	7.5-7.2	21.03	24.67	25.32	23.45	26.52
H-methylbenzyl	5.35-5.10	7.76	9.01	9.27	8.95	9.81
H-Glu	4.9-4.6	3.7	4.33	4.53	4.59	4.68
H-Cys	4.6-4.4	0.64	0.48	0.49	0.92	0.44
CH <sub>2</sub> -S	3.2-3	2	2	2	2	2
CH <sub>2</sub> CH <sub>2</sub> COO	2.9-1.8	15.9	18.79	19.38	18.72	20.86
T-Butyl	1.6-1	12.51	13.13	12.83	11.33	11.94
Ratio(benz:5/t-but:9)		3.025899	3.382026	3.552299	3.725508	3.99799

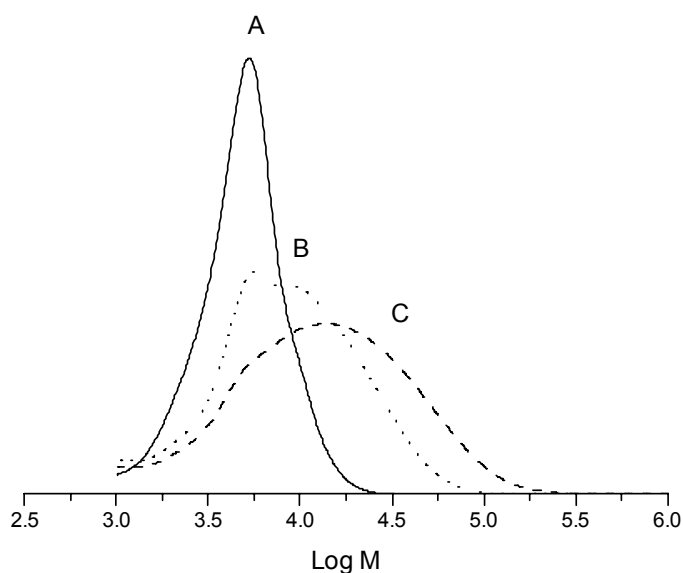
**Synthesis of P(BLG-*co*-Cys) 3:**<sup>2</sup> The P(BLG-*co*-tBMLC) (Mn: 4200 g/mol) (2.25 g, 0.54 mmol) and dithiothreitol (2.15 g, 13.9 mmol) were dissolved in 20 ml DMF. The reaction was maintained for 5 days at 60 °C under a nitrogen atmosphere. After 5 days the polymer was precipitated in diethylether, washed with diethylether and dried under vacuum at 20°C. Yield: 1.16 g, 57.7 wt%.



**Figure 2:**  $^1\text{H-NMR}$  spectra of P(BLG-*co*-tBMLC) before and after deprotection with dithiothreitol.

**Crosslinking of 3:** **3** (54 mg, 13  $\mu\text{mol}$ ) and cobalt phthalocyanine (6.0 mg, 10  $\mu\text{mol}$ ) were dissolved in 0.2 ml of DMF in a 1.5 ml open vial. The reaction was left at room temperature for 24 hours and a sample was taken and dissolved in DMF. After filtration of the sample it was immediately measured by the DMF-SEC.

**Crosslinking of 3 in basic environment:** **3** (53 mg, 13  $\mu\text{mol}$ ), cobalt phthalocyanine (6.0 mg, 10  $\mu\text{mol}$ ) and potassium hydroxide (5.3 mg, 94  $\mu\text{mol}$ ) were dissolved in 0.2 ml of DMF in a 1.5 ml open vial. The reaction was left at room temperature for 24 hours and a sample was taken and dissolved in DMF. After filtration of the sample it was immediately measured by the DMF-SEC.

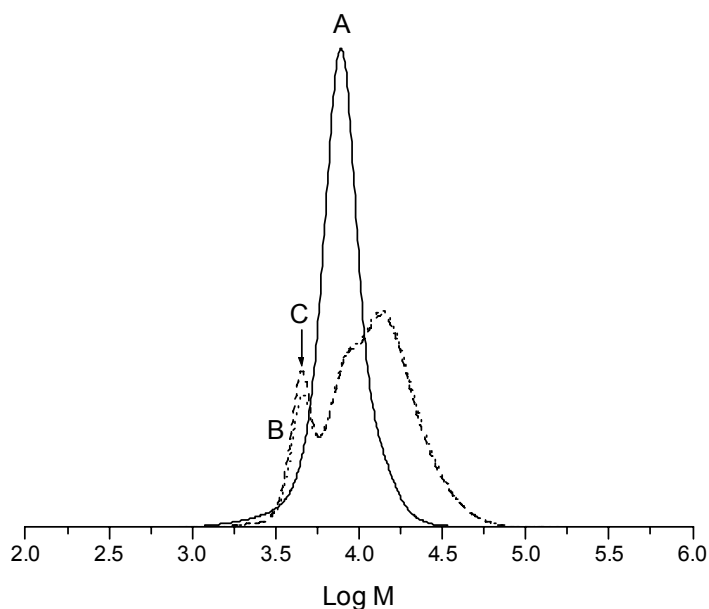


**Figure 3:** SEC-traces of crosslinking reaction of P(BLG-co-Cys). A), P(BLG-co-Cys), B) crosslinking by phthalocyanine, C) crosslinking by phthalocyanine in presence of potassium hydroxide.

**Michael Addition of polyethylene glycol methyl ester acrylate to 3:** **3** (50 mg, 11  $\mu\text{mol}$ ) was dissolved with the poly(ethylene glycol) methyl ether acrylate (Mn: 454 g/mol) (28 mg, 62  $\mu\text{mol}$ ) in 0.7 ml DMF in a 1.5 ml vial. The reaction mixture was shaken for 5 days at room temperature. Afterwards the solvent was evaporated under vacuum.

**Michael Addition of polyethylene glycol methyl ester acrylate to 3 with pyridine:** **3** (50 mg, 11  $\mu\text{mol}$ ) was dissolved with the poly(ethylene glycol) methyl ether acrylate (Mn: 454

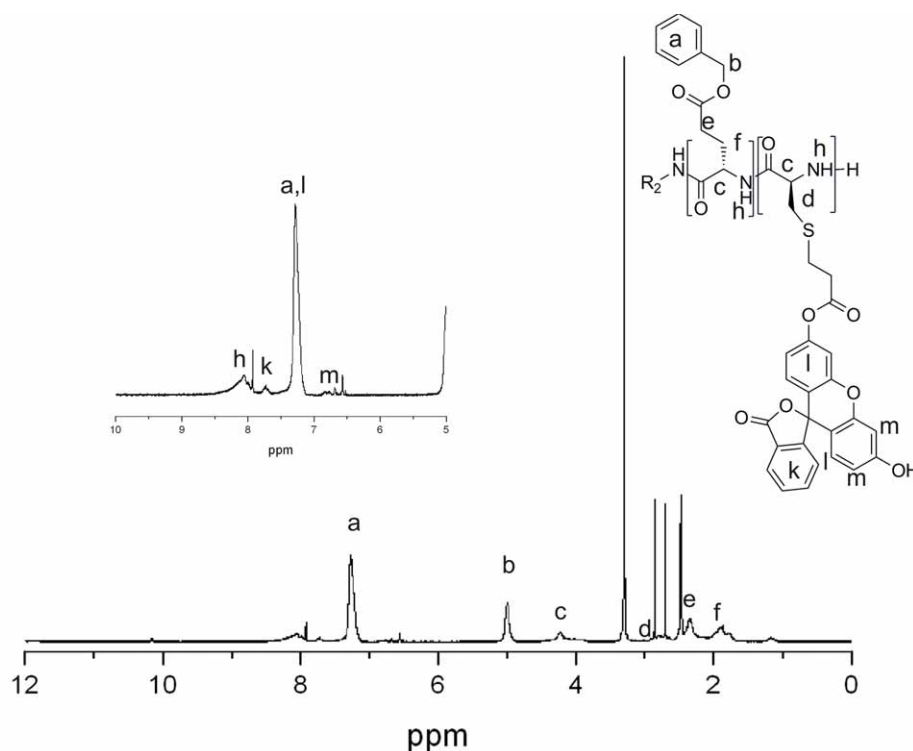
g/mol) (28 mg, 62  $\mu\text{mol}$ ) and pyridine (85 mg, 1.1 mmol) in 0.7 ml DMF. The reaction mixture was shaken for 5 days at room temperature. Afterwards the solvent was evaporated under vacuum. A sample was taken from the dried material for SEC measurements.



**Figure 4:** SEC traces of Michael addition reaction with poly(ethylene glycol) methyl ester acrylate. A) P(BLG-co-Cys), B) P(BLG-co-(Cys-g-PEGA)) (dotted line), C) P(BLG-co-(Cys-g-PEGA)) with pyridine (dashed line).

**Thiol-ene addition of polyethylene glycol methyl ester acrylate to 3. 3** (209 mg, 31  $\mu\text{mol}$ ), poly(ethylene glycol) methyl ether acrylate (Mn: 454 g/mol) ( 50 mg, 0.11 mmol) and  $\alpha,\alpha'$ -azoisobutyronitrile (1.2 mg, 7.3  $\mu\text{mol}$ ) were dissolved in 0.6 ml DMF. The mixture was heated to 70 °C for 1 hour under ambient atmosphere. After the reaction, the solvent was removed under vacuum. A sample was taken from the dried material for SEC measurements.

**Thiol-ene addition of fluorescein O-acrylate to 3. 3** (48 mg, 11  $\mu\text{mol}$ ), fluorescein O-acrylate (22 mg, 57  $\mu\text{mol}$ ) and  $\alpha,\alpha'$ -azoisobutyronitrile (0.2 mg, 1  $\mu\text{mol}$ ) were dissolved in 0.3 ml DMF. The mixture was heated to 60°C for 1 hour under ambient atmosphere. The polymer was purified by two precipitations in diethylether. After the reaction the material was dried under vacuum. Yield: 11 mg, 16 wt%



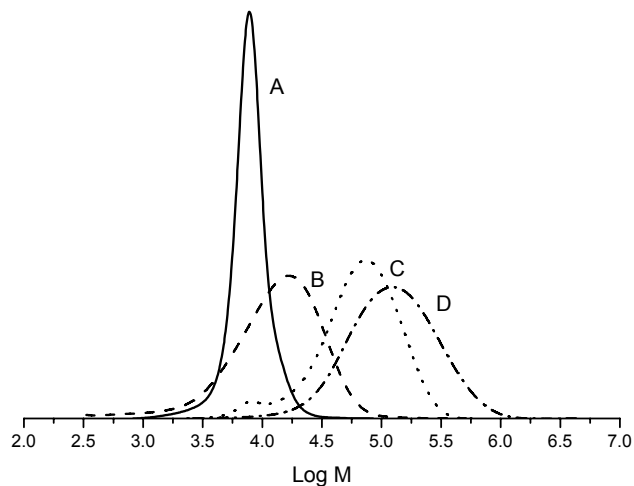
**Figure 5:**  $^1\text{H-NMR}$ : thiol-ene addition of fluorescein O-acrylate to P(BLG-co-Cys) in  $\text{DMSO-d}_6$ .

**Free Radical Chain Transfer of n-butylacrylate with 3. 3** (100 mg, 0.0222 mmol), n-butylacrylate (720 mg, 5.62 mmol) were dissolved in DMF.  $\alpha,\alpha'$ -Azoisobutyronitrile (1.2 mg, 7.3  $\mu\text{mol}$ ) was added from a DMF solution. Total amount of DMF was 2 ml. Oxygen was removed by 3 freeze / thaw cycles. The reaction was maintained at  $60^\circ\text{C}$  for 16 hours under a nitrogen atmosphere. After the reaction the material was precipitated in methanol and dried under vacuum. Yield: 450 mg, 55 wt%.

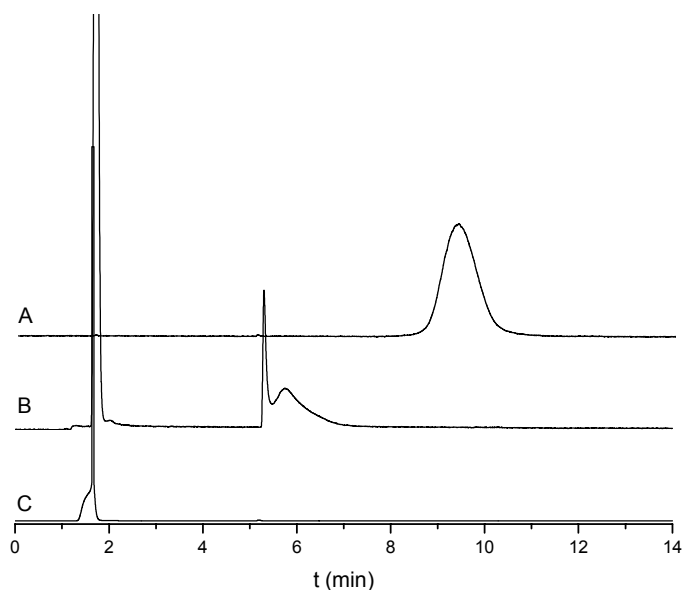
**Free Radical Chain Transfer of n-butylacrylate.** n-Butylacrylate (720 mg, 5.62 mmol) was dissolved in DMF.  $\alpha,\alpha'$ -Azoisobutyronitrile (1.2 mg, 7.3  $\mu\text{mol}$ ) was added from a DMF solution. Total amount of DMF was 2 ml. Oxygen was removed by 3 freeze / thaw cycles. The reaction was maintained at  $60^\circ\text{C}$  for 16 hours under a nitrogen atmosphere. After the reaction the material was precipitated in methanol and dried under vacuum. Yield: 325 mg, 45 wt%.

**Free radical chain transfer of L-Cysteine.** N-t-butyloxycarbonyl-L-cysteine, methyl ester (35 mg, 0.15 mmol) and n-butylacrylate (982 mg, 7.66 mmol) were dissolved in DMF.  $\alpha,\alpha'$ -Azoisobutyronitrile (2.8 mg, 17.4  $\mu\text{mol}$ ) was added from a DMF solution. The total amount of

DMF was 2.7 ml. Oxygen was removed by 3 freeze / thaw cycles. The reaction was maintained at 60°C for 17 hours under a nitrogen atmosphere. After the reaction the polymer did not precipitate in methanol, so solvent and monomer were removed under vacuum. Yield: 562 mg, 55 wt%.

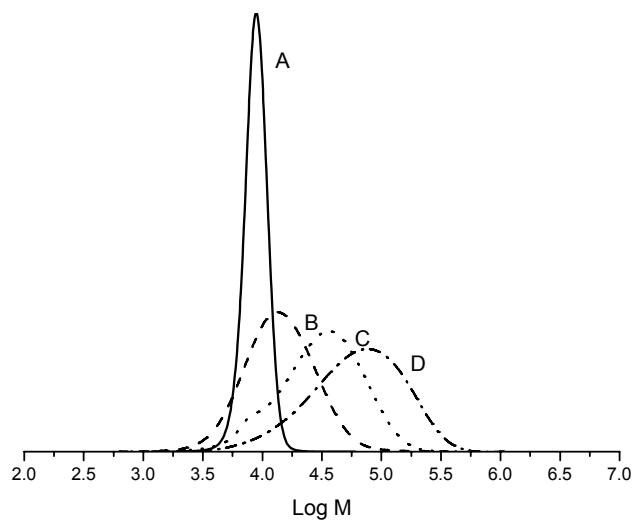


**Figure 6:** SEC traces of all chain transfer related reaction products with n-butylacrylate. (A) P(BLG-co-Cys), (B) polymerization of n-butyl acrylate with N-tert-butyloxycarbonyl-L-cysteine methyl ester as CT agent, (C) P(BLG-co-(Cys-g-PBA)), (D) poly(n-butyl acrylate).



**Figure 7:** GPEC traces of (A) P(BLG-co-Cys), (B) P(BLG-co-(Cys-g-PBA)), (C) poly(n-butyl acrylate).





**Figure 8:** SEC traces of all chain transfer related reaction products with methylmethacrylate. A) P(BLG-co-Cys), B) PMMA with N-tert-butyloxycarbonyl-L-cysteine methyl ester as CT agent, C) P(BLG-co-(Cys-g-PMMA)), D) PMMA

**References:**

- 1) F. Corneille, J-L Copier, Y. Robin, EP 1201659, 2002
- 2) N.V. Tsarevsky, K. Matyjazweski, *Macromolecules* 2002, 35, 9009-9014