Fabrication of Vesicle-like Dual-responsive Click Capsules by Direct Covalent Layer-by-Layer Assembly

Cheng-Jyun Huang, Chia-Wei Hong, Fu-Hsiang Ko, Feng-Chih Chang*

Institute of Applied Chemistry, National Chiao Tung University, Hsinchu 30050, Taiwan

E-mail: changfc@mail.nctu.edu.tw
Scheme S1. Synthesis of the $N$-(2-(2-(2-azidoethoxy)ethoxy)ethyl)acrylamide monomer, (1).

Synthesis of 2-(2-(2-azidoethoxy)ethoxy)ethanol, (2). 2-(2-(2-chloroethoxy)ethoxy)ethanol (25.0 g, 148.3 mmol) and sodium azide (14.46 g, 222.3 mmol) were added in 400 mL DMF. The mixture was stirred at 100 °C for 15 h and then cooled to RT. After the precipitate was filtered off, the solvent was evaporated under vacuum to afford the product 2, (24.2 g, 93 %) as a colorless oil.

Synthesis of 2-(2-(2-azidoethoxy)ethoxy)ethyl methanesulfonate, (3). A solution of 20.0 g (114 mmol) of compound 2 and 25 mL of Et$_3$N in 500 mL of dry CH$_2$Cl$_2$ was cooled to 0 °C under a nitrogen atmosphere. A solution of methanesulfonyl chloride (13.7 g, 120 mmol) in CH$_2$Cl$_2$ (25 mL) was added dropwise to this mixture over a 30-min period, and the solution was warmed to room temperature and stirred for 1.5 h. After the precipitate was filtered off, the solvent was evaporated and the crude product was purified by column chromatography eluting with a 2:1 mixture of $n$-hexane and ethyl acetate to give 3 as a pale yellow oil (21.8 g, 75.5 %). $^1$H NMR (500MHz, CDCl$_3$): δ 4.33 (m, CH$_2$OSO$_2$CH$_3$, 2H), 3.74 (m, CH$_2$OCH$_2$CH$_2$N$_3$, 2H), 3.66-3.61 (br, CH$_2$OCH$_2$CH$_2$O, 6H), 3.35 (m, CH$_2$N$_3$, 2H). 3.03 (s, OSO$_2$CH$_3$, 3H). $^{13}$C NMR: δ 70.55 (CH$_2$CH$_2$OCH$_2$CH$_2$N$_3$, 1C), 70.50 (CH$_2$OCH$_2$CH$_2$N$_3$, 1C), 69.94 (OCH$_2$CH$_2$N$_3$, 1C), 69.13 (CH$_2$CH$_2$OSO$_2$CH$_3$, 1C), 68.97 CH$_2$OSO$_2$CH$_3$, 1C), 50.56 (CH$_2$N$_3$, 1C), 37.52 (OSO$_2$CH$_3$, 1C).
**Synthesis of 2-(2-(2-azidoethoxy)ethoxy)ethyl isoindoline-1,3-dione, (4).** A solution of 20.0 g (79 mmol) of compound 3 and 22.0 g (118.5 mol) of potassium phthalimide in 400 mL of dry DME was heat at reflux for 18 h under a nitrogen atmosphere. After cooling to room temperature and concentration in vacuo, the resulting residue was diluted with ethyl acetate and the solids were filtered. Concentration of the filtrate in vacuo followed by purification of the crude product by column chromatography eluting with a 4:1 mixture of n-hexane and ethyl acetate to give 4 as a colorless oil (15.3 g, 63.5 %). $^1$H NMR (500MHz, CDCl3): $\delta$ 7.78 (m, phthalyl aromatic, 2H), 7.66 7.78 (m, phthalyl aromatic, 2H), 3.85 (m, CH$_2$N, 2H), 3.70 (m, CH$_2$CH$_2$N, 2H), 3.60 (m, CH$_2$CH$_2$N$_3$, 2H), 3.55 (br, OCH$_2$CH$_2$O, 4H), 3.25 (m, CH$_2$N$_3$, 2H). $^{13}$C NMR: $\delta$ 168.12 (C$_O$, 2C), 133.81 (aryl-C, 2C), 131.98 (aryl-C, 2C), 123.08 (aryl-C, 2C), 123.08 (aryl-C, 2C), 70.48 (CH$_2$OCH$_2$CH$_2$N, 1C), 70.04 (CH$_2$OCH$_2$CH$_2$N$_3$, 1C), 69.87 (CH$_2$CH$_2$N$_3$, 1C), 67.87 (CH$_2$CH$_2$N, 1C), 50.48 (CH$_2$N$_3$, 1C), 37.15 (CH$_2$N, 1C).

**Synthesis of 2-(2-(2-azidoethoxy)ethoxy)ethanamine, (5).** A solution of 10.0 g (32.9 mmol) of compound 4 and 4.0 mL of 80% hydrazine hydrate in 100 mL of absolute ethanol was heated at 55 °C for 2 h, during which time a white precipitate formed. The mixture was cooled to room temperature and concentrated in vacuo, after which the crude residue was diluted with xxx mL of dry CH$_2$Cl$_2$. After the precipitate was filtered off, the solvent was dried and concentrated in vacuo to afford pale yellow oil, which was used in the next step without further purification (5.04 g, 88 %). $^1$H NMR (500MHz, CDCl$_3$): $\delta$ 3.66-3.60 (br, CH$_2$OCH$_2$CH$_2$O, 6H), 3.50 (m, CH$_2$CH$_2$NH$_2$, 2H), 3.37 (m, CH$_2$N$_3$, 2H), 2.86 (m, CH$_2$NH$_2$, 2H). $^{13}$C NMR: $\delta73.12$ (CH$_2$CH$_2$NH$_2$, 1C), 70.62 (CH$_2$CH$_2$N$_3$, 1C), 70.26 (CH$_2$CH$_2$OCH$_2$CH$_2$NH$_2$, 1C), 70.02 (CH$_2$OCH$_2$CH$_2$NH$_2$, 1C), 50.63 (CH$_2$N$_3$, 1C), 41.59 (CH$_2$NH$_2$, 1C).

**Synthesis of N-(2-(2-(2-azidoethoxy)ethoxy)ethyl)acrylamide, (1).** A mixture of triethylamine (5.2 mL, 37.3 mmol) and compound 5 (5.0 g, 28.7 mmol) dissolved in dry CH$_2$Cl$_2$ (250 mL) were fed into a 250 mL two-necked round-bottomed flask fitted with a Ar inlet and a rubber septum and were cooled in an ice bath. The acryloyl chloride (3.12 g, 34.4 mmol) was added into the mixture dropwise over 30 mins. White precipitate was observed and the reaction was allowed to stir at room temperature
overnight. After the precipitate was filtered off, the solvent was evaporated and the crude product was purified by column chromatography eluting with a 1:2 mixture of n-hexane and ethyl acetate to give 1 as a pale yellow oil (3.84 g, 58.5 %). 1H NMR (500MHz, CDCl3): δ 6.27 (dd, J = 16.99, 1.48 Hz, CH₂CHNH, 1H), 6.21 (br s, NH, 1H), 6.10 (dd, J = 16.99, 10.28 Hz, trans CH₂CH, 1H), 5.62 (dd, J = 10.28, 1.48 Hz, cis CH₂CH, 1H), 3.67 (m, NHCH₂CH₂O, 2H), 3.63 (m, OCH₂CH₂O, 4H), 3.58 (m, OCH₂CH₂N₃, 2H), 3.53 (m, NHCH₂, 2H), 3.38 (m, CH₂N₃, 2H). 13C NMR: δ 165.51 (C=O, 1C), 130.76 (CH₂=CH, 1C), 126.36 (CH₂=CH, 1C), 70.43 (NHCH₂CH₂O, 1C), 70.16 (NH(CH₂)₂OCH₂, 1C), 70.06 (NH(CH₂)₂OCH₂CH₂, 1C), 69.76 (OCH₂CH₂N₃, 1C), 50.56 (CH₂N₃, 1C), 39.02 (NHCH₂, 1C).


Preparation of Alkyne-functionalized Acrylamide Random polymer, Poly[NIPAm-r-propargyl acrylamide] (PNIPAm-r-PPAm), (6). The initial reactants were mixed at a 80: 20: 1 molar ratio of NIPAm (2.32 g, 2.05 mmol), (trimethylsilyl)propargyl acrylamide (0.93 g, 0.51 mmol), dansyl-labeled ATRP initiator (0.064 g, 0.119 mmol), and 2-propanol (2.5 mL). The solution was deoxygenated by performing freeze-pump-thaw cycle. Upon equilibration at 20 °C after the third cycle, the flask was immersed into an ice bath. To allow the buildup of the complex between the metal and ligand, an oxygen free solution of 2-propanol (0.5 mL) containing CuBr (0.0184 g, 1.28 μmol) and Me₆TREN (29.65 mg, 35.00 μL) was prepared separately. This solution was then added to the monomers and
initiator mixture via an argon-washed syringe to start polymerization (6 hr). The reaction mixture was exposure to air to stop polymerization, then evaporated to dryness and the residue was dissolved in 50mL of THF, and the copper catalyst was removed by passing through a neutral alumina column. Excess tetrabutylammonium fluoride was added to the solution of poly[NIPAm-r-(trimethylsilyl)propargyl acrylamide] in dry THF to 0 °C. The mixture was then stirred for 2 h at 0 °C. The solution was dialyzed extensively against DI water and the product was isolated via lyophilization to give 6 as a pale yellow powder. $^1$H NMR (500 MHz, D$_2$O), δ 1.08 (br, CH(CH$_3$)$_2$), 1.25–1.77 (br, CH$_2$CH, copolymer backbone), 1.78–2.22 (br, CH$_2$CH polymer backbone and C≡CH) 3.71–3.97 (br, CH(CH$_3$)$_2$ and NHCH$_2$C≡CH).

**Preparation of Azido-functionalized Acrylamide Random polymer, Poly[NIPAm-r-N-(2-(2-azidoethoxy)ethoxy)ethyl] acrylamide] (PNIPAm-r-PEOAm), (7).** The initial reactants were mixed at an approximate 80: 20: 1 molar ratio of NIPAm (2.0 g, 17.7 mmol), N-(2-(2-azidoethoxy)ethoxy)ethyl) acrylamide (1.0 g, 4.4 mmol), dansyl-labeled ATRP initiator (0.064 g, 0.119 mmol), and 2-propanol (0.5 mL). The solution was deoxygenated by performing freeze-pump-thaw cycle. Upon equilibration at 20 °C after the third cycle, the flask was immersed into an ice bath. To allow the buildup of the complex between the metal and ligand, an oxygen free solution of 2-propanol (0.5 mL) containing CuBr (0.0184 g, 0.129 mmol) and Me$_6$TREN (38.8 mg, 46 μL) was prepared separately. This solution was then added to the monomers and initiator mixture via an argon-washed syringe to start polymerization (6 h). The reaction mixture was exposure to air to stop polymerization, then evaporated to dryness and the residue was dissolved in 30 mL of THF, and the copper catalyst was removed by passing through a neutral alumina column. The solution was concentrated and precipitated in n-hexane to give 7 as a pale yellow powder. $^1$H NMR (500 MHz, D$_2$O), δ 1.00 (br, CH(CH$_3$)$_2$), 1.15-1.67 (br, CH$_2$CH, copolymer backbone), 1.70–2.07 (br, CH$_2$CH polymer backbone), 2.76 (br, N(CH$_3$)$_2$), 3.25 (br, NHCH$_2$CH$_2$), 3.36 (br, NHCH$_2$CH$_2$), 3.48 (br, CH$_2$CHN), 3.57 (br, NHCH$_2$CH$_2$OCH$_2$), 3.75 (br, CH(CH$_3$)$_2$).
**Figure S1.** $^1$H NMR (500 MHz, D$_2$O) spectrum of PNIPAm-$r$-PEOAm.

**Scheme S3.** Synthetic Pathway for the Aqueous Reversible Addition Fragmentation Chain Transfer (RAFT) Polymerization of Alkyne- and Azido-functionalized Poly(N-acryloylalanine)
Random Copolymer with and 4,4-Azobis(4-cyanopentanoic acid) V-501 as the Free Radical Initiator.

\[
\begin{align*}
1. & \quad \text{HNCO} + \text{HNCO} + \text{COOH} \\
2. & \quad \text{HNCO} + \text{HNCO} + \text{COOH}
\end{align*}
\]

Synthesis of Poly(N-acryloylalanine)-r-Poly(propargyl acrylamide) (PAAL-r-PPAm), (8). The initial reactants were mixed at an approximate 60:15:1:0.2 molar ratio of N-acryloylalanine (1.5 g, 10.5 mmol), propargyl acrylamide (0.286 g, 2.63 mmol), chain transfer agent CMP (49.25 mg, 0.1744 mmol) and V-501 (9.8 mg) were mixed together in deionized water. After the pH was adjusted to 6.5, the mixture was purged with nitrogen at 5 °C for 30 min prior to the reaction. The polymerization was allowed to proceed for 3 h at 45 °C and then was quenched by immersion in liquid nitrogen. The product was purified by dialysis against deionized water and isolated by lyophilization (1.49 g, 83.5 %, \(M_n\) = 8,650 g/mol, PDI = 1.60). \(^1\)H NMR (500MHz, D_{2}O): \(\delta\) 0.93 (br, SC(CH\textsubscript{3})\textsubscript{2}), 1.23 (br, CH(CH\textsubscript{3})\textsubscript{2}), 1.32-1.78 (br, C\textsubscript{H}2CH, copolymer backbone), 1.78-2.36 (br, CH\textsubscript{2}CH, copolymer backbone), 2.44-2.71 (br, C≡CH), 3.60-4.22 (br, CH\textsubscript{2}C≡CH and CH(CH\textsubscript{3})\textsubscript{2}).

Synthesis of poly(N-acryloylalanine)-r-Poly[N-(2-(2-(2-azidoethoxy)ethoxy)ethyl) acrylamide] (PAAL-r-PEOAm), (9). The initial reactants were mixed at an approximate 60:15:1:0.2 molar ratio of N-acryloylalanine (1.5 g, 10.5 mmol), N-(2-(2-(2-azidoethoxy)ethoxy)ethyl) acrylamide (0.6 g, 2.63
mmol), chain transfer agent CMP (49.25 mg, 0.1744 mmol) and V-501 (9.8 mg) were mixed together in deionized water. After the pH was adjusted to 6.5, the mixture was purged with nitrogen at 5 °C for 30 min prior to the reaction. The polymerization was allowed to proceed for 3 h at 45 °C and then was quenched by immersion in liquid nitrogen. The product was purified by dialysis against deionized water and isolated by lyophilization (1.79 g, 85.4 %, $M_n$ PEO = 10,280 g/mol, PDI = 1.83). $^1$H NMR (500MHz, D$_2$O): $\delta$ 0.93 (br, SC(CH$_3$)$_3$), 1.23 (br, CH(CH$_3$)$_3$), 1.32-1.78 (br, CH$_2$CH, copolymer backbone), 1.78-2.36 (br, CH$_2$CH, copolymer backbone), 3.40 (br, CH$_2$N$_3$), 3.52 (br, NHCH$_2$), 3.61 (br, CH$_2$CH$_2$N$_3$), 3.61 (br, NHCH$_2$CH$_2$OCH$_2$CH$_2$O), 3.98 (br, CH(CH$_3$)$_2$).

![Chemical structures](image)

**Figure S2.** $^1$H NMR (500 MHz, D$_2$O) spectra of (a) PAAL-r-PAAm and (b) PAAL-r-PEOAm.