Reversible siRNA-Polymer Conjugates by RAFT Polymerization

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Electronic Supplementary Information

Experimental Section

Materials

3-(2-pyridinyldisulfanyl)propanol[1] and 2-(ethyl trithiocarbonate)propionic acid[2] were synthesized as previously reported. Dichloromethane was distilled over CaH_2 and stored under argon prior to use. 2,2'-Azobisisobutyronitrile (AIBN) was recrystallized twice from ethanol. Merck 60 (230-400 mesh) silica gel was used for column chromatography. Complementary 5'-thiol modified sense siRNA (5'ThioMC6-D-GCU GAC CCU GAA GUU CAU CUU-3') and antisense siRNA (5'-GAU GAA CUU CAG GGU CAG CUU-3') specific for enhanced green fluorescent (EGF) protein mRNA were purchased from Integrated DNA Technologies and annealed following the manufacturer's protocol. SYBR-Safe nucleic acid staining dye was purchased from Invitrogen. All other chemicals were purchased from Sigma-Aldrich of Fisher Scientific and used as received.

Analytical Techniques

^{1}H and ^{13}C NMR spectra were obtained on an Avance DRX 400 or 500 MHz spectrometer. UV-Vis spectroscopy was performed using a Biomate 5 Thermo Spectronic spectrometer. High resolution electrospray ionization mass spectrum (HRESI-MS) was obtained on an IonSpec Ultima 7T ICR, (Varian Inc.) in the MIC center at UCLA. Infrared spectroscopy was performed using a PerkinElmer FT-IR equipped with an ATR accessory. Gel permeation chromatography was conducted on a Shimadzu HPLC system equipped with a refractive index detector RID-10A, one Polymer Laboratories PLgel guard column, and two Polymer Laboratories PLgel 5 μm mixed D columns. DMF containing 0.10 M LiBr at 40 °C was used as the eluent (flow rate: 0.80 mL/min) and near-monodisperse poly(methyl methacrylate) standards

from Polymer Laboratories were used for calibration. Chromatograms were processed using the EZStart 7.2 chromatography software. Gel electrophoresis was undertaken using 13% polyacrylamide gels. Samples were loaded with 40% glycerol in water, and run at a constant voltage of 115 V for 1.5 h using 1X TBE buffer (tris-borate EDTA, pH 8.0). The gel was stained with SYBR Safe stain (Invitrogen) in 1X TBE buffer for 30 minutes. Visualization was conducted using a Versadoc Imaging System Model 500 located in the DOE-Biochemistry Facility at UCLA. The gel was irradiated with UV light and fluorescence was measured at 520 nm.

Methods

*Synthesis of 3-(pyridin-2-yldisulfanyl)propyl-2-(ethylthiocarbonothioylthio) propanoate (**1**).* 3-(2-pyridinyldisulfanyl)propanol (124.3 mg, 0.618 mmol) and 2-ethyl trithiocarbonate propionic acid (137 mg, 0.680 mmol) were dissolved in dichloromethane (25 mL) and then cooled to 0 °C. EDC (130.7 mg, 0.680 mmol) and DMAP (7.80 mg, 0.0618 mmol) were then added in one portion. The reaction was allowed to warm to 23 °C with constant stirring. After 12 h, the crude product was concentrated and purified by silica gel chromatography (2:1 hexane:ethyl acetate) yielding **1** as a yellow oil (184 mg, 76%). δ ^{1}H NMR 400 MHz ($CDCl_3$): 8.47-8.45 (m, 1H, C**H**N), 7.69-7.62 (m, 2H, C**H**C**H**CN), 7.10-7.07 (m, 1H, C**H**CHN), 4.77 (q, *J* = 7.4 Hz, 1H, C**H**(CH_3)S), 4.29-4.18 (m, 2H, $CH_2C\mathbf{H_2}O$), 3.34 (q, *J* = 7.4 Hz, 2H, $SC\mathbf{H_2}CH_3$), 2.85 (t, *J* = 7.1 Hz, 2H, $SC\mathbf{H_2}CH_2$), 2.08-2.02 (m, 2H, $CH_2C\mathbf{H_2}CH_2$), 1.57 (d, *J* = 7.4 Hz, 3H, CHC**H$_3$**), 1.34 (t, *J* = 7.4 Hz, 3H, $SCH_2C\mathbf{H_3}$); δ ^{13}C NMR 400 MHz ($CDCl_3$): 222.15, 171.18, 160.19, 149.87, 137.29, 120.87, 119.95, 64.08, 47.93, 35.32, 31.77, 28.07, 16.95, 13.24; IR

(cm^{-1}): 1729, 1573, 1445, 1416, 1246, 1157, 1116, 1080, 1043, 817, 760; HRESI-MS expected (observed) [MH+]: 394.0092 (394.0072); UV-Vis (MeOH) λ_{max} (SC=SS) = 307 nm.

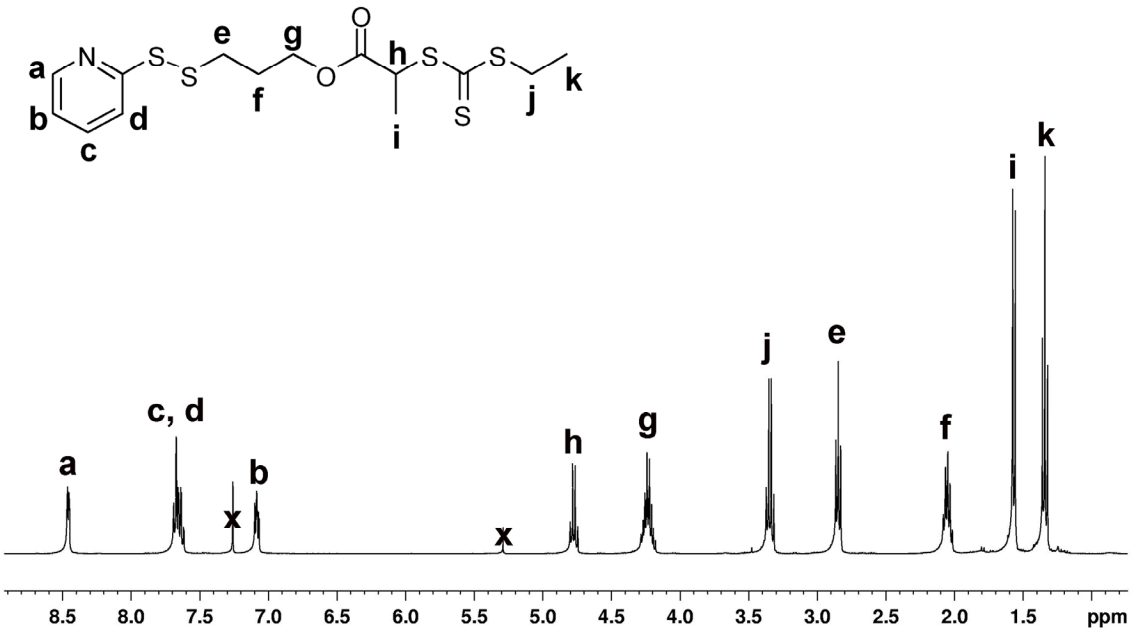

Fig. S1. ^{1}H NMR spectrum ($CDCl_3$) of pyridyl disulfide chain transfer agent. x = solvent.

Typical RAFT polymerization of polyethylene glycol ethyl methyl ether acrylate (PEGA): Polymerization of PEGA was conducted using molar ratio of 1:30:0.1 of **1**:PEGA:AIBN and 50:50 v/v ratio of DMF:PEGA using standard Schlenk techniques. The pyridyl disulfide functional CTA **1** (47.94 mg, 0.12 mmol), PEGA (1.660 g, 3.66 mmol) and AIBN (2.0 mg, 0.012 mmol) were dissolved in 1.52 mL of DMF in a Schlenk tube. The solution was then subjected to five freeze-pump-thaw cycles and the tube was sealed and immersed into a 60 °C oil bath to begin the polymerization. For kinetic analysis, aliquots were removed periodically and diluted

into $CDCl_3$ or DMF for ^1H NMR and GPC analysis, respectively. Conversions were calculated by ^1H NMR using the following equation: $1-M_t/M_0$ where M_t was determined from the integral value of the peak for the vinylic protons of the monomer at 6.14 ppm and M_0 from the integral of the peak obtained from monomer and polymer overlap at 4.23 ppm. The polymer was purified by dialysis against methanol (MWCO 6-8000). δ ^1H NMR 500 MHz ($CDCl_3$, assignments in text): 8.39-8.38, 7.64-7.57, 7.04-7.02, 4.77-4.73, 4.10, 3.58-3.44, 3.34-3.27, 2.80-2.77, 2.28-2.41, 1.98-1.95, 1.40-1.84, 1.28-1.26, 1.04.

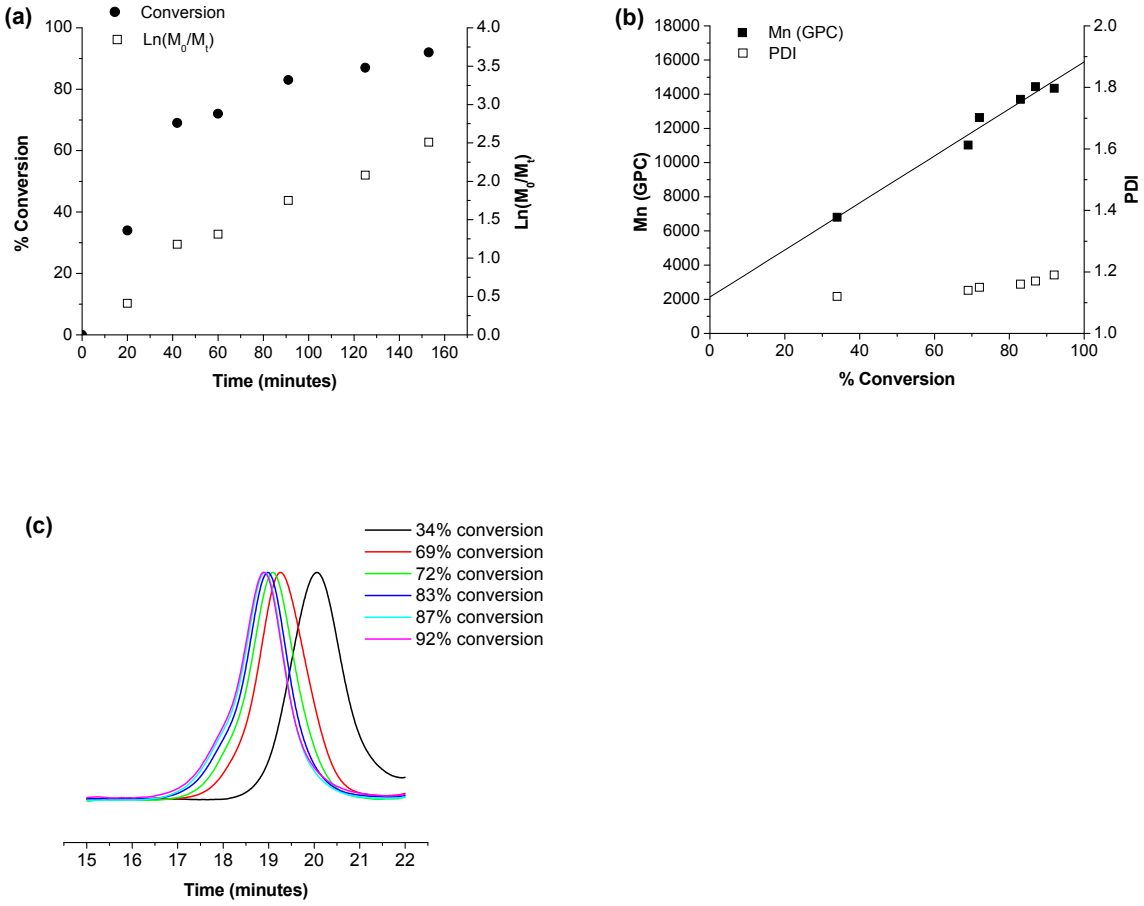

Fig. S2. RAFT of PEGA: kinetic study at 70 °C. (a) Kinetic trace and conversions determined by ^1H NMR spectroscopy. (b) Molecular weight and PDI were determined by GPC. (c) GPC traces of resultant polymers. GPC conditions: DMF (0.1 M LiBr) at 40 °C, 0.80 mL/min.

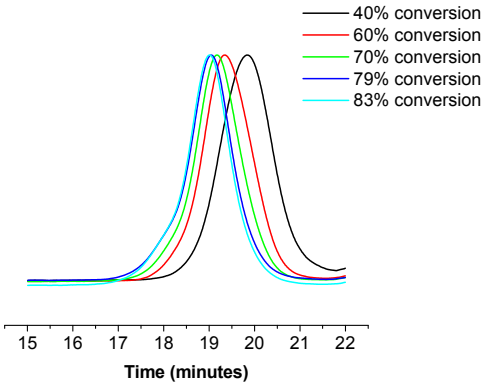

Fig. S3. RAFT of PEGA: kinetic study at 60 °C. GPC traces of resultant polymers. GPC conditions: DMF (0.1 M LiBr) at 40 °C, 0.80 mL/min.

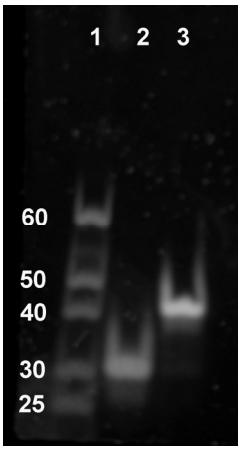

Fig. S4. Polyacrylamide gel electrophoresis of single and double stranded siRNA. Lane 1: DNA ladder; lane 2: single stranded siRNA; lane 3: siRNA duplex.

Conjugation of siRNA to pyridyl-disulfide end-functional poly(PEGA): 30 µl of a 0.0383 mM solution of double stranded (ds)-siRNA was mixed with 5 µl of 200 mM DTT solution and kept for 3 h at 24 °C. To remove unreacted DTT, ds-siRNA was precipitated using ethanol. The siRNA pellet was completely resuspended in 30 µL of poly(PEGA) solution (3.5 mg in 500 µl of

a 100 mM sodium bicarbonate buffer at pH 8.5) and left at 23 °C for 20 h before assessing the conjugation by polyacrylamide gel electrophoresis (PAGE). ds-siRNA was stained by incubating the gel in 1 X SYBR-Safe nucleic acid dye/TBE buffer. Conjugation efficiency was quantified from five sample replicates using the Quanti-One program (BioRad) and the results are provided in Table 1.

Table 1. Quantification of conjugation of poly(PEGA) to siRNA.

Replicate number	Conjugated siRNA (%)	Unconjugated siRNA (%)
1.0	94.1	5.9
2.0	79.4	20.6
3.0	95.2	4.8
4.0	86.4	13.7
5.0	86.6	13.4
Average	**88.3**	**11.7**
Standard deviation	6.5	6.5

Reduction of poly(PEGacrylate) from siRNA. Unmodified siRNA or siRNA-poly(PEGA) conjugate was incubated in 200 mM DTT solution for 2 h at 24 °C prior to characterization by PAGE.

References

1. N. Murthy, J. Campbell, N. Fausto, A. S. Hoffman and P. S. Stayton, *Bioconjugate Chemistry* 2003**, 14**, 412-419.
2. M. R. Wood, D. J. Duncalf, S. P. Rannard and S. Perrier, *Organic Letters* 2006**, 8**, 553-556.