Enhanced Glucose-Responsivity of PBA–Diol Hydrogel Networks by Reducing Crosslink Affinity

Sijie Xian[1], Yuanhui Xiang[1], Svenja Deichmann[1], Matthew J. Webber[1,*]

1- University of Notre Dame, Department of Chemical & Biomolecular Engineering, Notre Dame, IN 46556 USA

*- mwebber@nd.edu

Table of Contents:

Detailed Synthetical Methods

Materials

Diacetone L-sorbose, 4-Methylbenzylsulfonyl chloride, Triethylamine (TEA), D-gluconolactone (99%), sodium sulfate (99%), dichloromethane (DCM), dimethyl sulfoxide (DMSO), *N*,*N*′-dimethylformamide (DMF), fluorescein isothiocyanate isomer I (FITC, 90%), *(R)*- (+)-glycidol, (*S*)- (-)-glycidol and (*racemic mixture*)- (±)-glycidol were purchased from Sigma-Aldrich. 4-Carboxy-3-fluorophenylboronic acid (98%) was purchased from Combi-Blocks, Tetrahydrofuran (THF), D-Glucose, 4-Dimethylaminopyridine (DMAP, 98%), *N*-(3-(dimethylamino)propyl)-*N*′-ethylcarbodiimide hydrochloride (EDC·HCL, 97%), *N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine (PMDETA, 99%), methanol (MeOH), copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$, 98%), sodium sulfate anhydrous and ascorbic acid (99%) were purchased from VWR. The 4-arm polyethylene glycol-NH_2·HCL (10 kDa) and 4-arm-polyethyleneglycol-chloride (10 kDa) were purchased from Creative PEGWorks. Gibco recombinant Human Insulin AOF (rHu Insulin) was purchased from Fisher Scientific.

Synthesis of 4a-PEG-Glucose like diol (4aPEG-GLD)

4aPEG-NH_2·HCL (10 kDa, 0.1 mmol) and D-gluconolactone (0.8 mmol) were dissolved in methanol (MeOH, 25 mL). Triethylamine (TEA, 0.1 mL) was added to the reaction mixture. 4aPEG-Glucose-like diol (4aPEG-GLD) was synthesized through ring-opening of the lactone group of D-gluconolactone by reacting with the amine groups of the 10 kDa 4aPEG-NH_2 macromers. Reaction was left at room temperature for 3 d with continuous stirring. MeOH was then evaporated off under reduced pressure. The residue was dissolved in deionized water, loaded into regenerated cellulose dialysis tubing (MWCO= 3,500 Da) and dialyzed for 1 d. The final product was lyophilized yielding a white powder (1 g, 91% yield). ^{1}H NMR (400Hz, D_2O, δ): 4.3 (m, 4H), 4.0 (m, 4H), 3.6 (bs, PEG, 909H)

Synthesis of 4aPEG-alkyne

4aPEG-NH_2·HCL (10 kDa, 0.1 mmol) and 4-pentynoic acid (0.8 mmol) were dissolved in DMF (20 mL). 4-dimethylaminopyridine (4-DMAP, g/mol, 0.04 mmol) were added to reaction mixture separately. The reaction mixture was stirred at room temperature for 10 min. Then N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC·HCl, 2 mmol) was added to the mixture slowly drop-wise. The reaction mixture was stirred at room temperature for 2 d. The reaction mixture was then evaporated under reduced pressure to remove DMF. Residue was dissolved in deionized water, loaded into regenerated cellulose dialysis tubing (MWCO= 3,500 Da) and dialyzed for 1 d against DI water. The final product was lyophilized yielding a white powder (0.98 g, 89% yield). ^{1}H NMR (400Hz, D_2O, δ): 3.6 (bs, PEG, 909H), 2.46 (m, J = 2.96 Hz, 8H), 2.44 (m, J = 3.52 Hz, 8H), 2.3 (s, 4H),

Synthesis of protected fructose-N_3 precursor

Diacetone L-sorbose (4 mmol), 4-Methylbenzylsulfonyl chloride (8 mmol) were dissolved in DCM (40 mL). 4-Dimethylaminopyridine (4-DMAP, 0.2 mmol) and triethylamine (TEA, 8mmol) were added to the reaction mixture. Reaction was left at room temperature for one day. The reaction mixture was then washed with water and saturated brine for three times. The organic layer was dried over sodium sulfate anhydrous (Na_2SO_4) and was then evaporated off. The residue was purified through silica column with a mobile phase gradient from 100% to 65% of hexane in ethyl

acetate. The protected fructose-sulfonyl precursor (2.4 mmol) was dissolved in DMF (20 mL). Sodium azide (NaN_3, 14.4 mmol) was added to the reaction mixture slowly. The reaction mixture was stirred at 100 °C for 3 d and was then condensed under reduced pressure. The residue was then diluted with ethyl acetate (40 mL) and washed with water and saturated brine for 3 times. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure to remove solvent. The crude was purified through silica column with a mobile phase gradient from 100% to 75% of hexane in ethyl acetate. The final product was a yellow oil. ^{1}H NMR (400Hz, $CDCl_3$, δ): 4.4 (s, 1H), 4.3 (s, 1H), 4.1 (s, 1H), 4 (d, J = 8.64 Hz, 2H), 3.7-3.4 (dd, J= 12.96 Hz, 2H), 1.52 (s, 3H), 1.46(s, 3H), 1.42 (s, 3H), 1.36 (s, 3H).

Synthesis of 4a-PEG-Frucose like diol (4aPEG-FLD)

The protected fructose-N_3 precursor (0.14 mmol) and 4a-PEG-alkyne (0.033 mmol) were dissolved in DMF (15 mL) in a Schlenk flask. Copper Sulfate Pentahydrate ($CuSO_4 \cdot 5H_2O$, 0.028 mmol) and N,N,N′,N″,N″-Pentamethyldiethylenetriamine (PMDETA, 0.014 mmol) were added to the reaction mixture. The Schlenk flask was degassed with three freeze-pump-thaw cycles. On the last cycle, the reaction mixture was added with ascorbic acid (0.15 mmol) and was stirred at 50ºC for 2 d in oil bath. 4aPEG-Frucose-like diol (4aPEG-FLD) was synthesized *via* a click reaction between the alkyne group of the 4aPEG-alkyne macromer and the azide group of the fructose-N_3 small molecule. The reaction mixture was then evaporated under reduced pressure to remove DMF. The crude was then treated with 90% trifluoroacetic acid (TFA) in water for 4 h to remove the protecting group. The crude was then concentrated under reduced pressure, dissolved in deionized water, loaded into regenerated cellulose dialysis tubing (MWCO= 3,500 Da) and dialyzed for 1 day. The final product was lyophilized yielding a white powder (0.91 g, 93% yield). ^{1}H NMR (400Hz, D_2O, δ): 7.7 (s, 4H), 4.6 (d, J = 14.1 Hz, 4H), 4.5 (d, J = 12.1 Hz, 4H), 3.6 (bs, PEG, 909H), 3.3 (t, J = 5.4 Hz, 8 H), 3.1 (d, J = 9.28 Hz, 4 H), 2.9 (t, J = 7.32 Hz, 8H), 2.6 (t, J = 7.2 Hz, 8H).

Synthesis of 4a-PEG-cis1,3-diol (4aPEG-D-1,3)

4aPEG-$NH_2 \cdot$HCL (10 kDa, 0.1 mmol) and 3-hydroxy-2-(hydroxymethyl)-2-methylpropanoic acid (0.8 mmol) were dissolved in in DMF (20 mL). 4-dimethylaminopyridine (4-DMAP, g/mol, 0.04 mmol) were added to reaction mixture separately. The reaction mixture was stirred at room temperature for 10 min. Then N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC·HCl, 2 mmol) was added to the mixture slowly and drop-wise. 4a-PEG-cis1,3-diol (4aPEG-D-1,3) was synthesized through amide bond formation by coupling the amine group of the PEG macromers with the carboxylate of 3-hydroxy-2-(hydroxymethyl)-2-methylpropanoic acid. The reaction mixture was stirred at room temperature for 2 d. The reaction mixture was then evaporated under reduced pressure to remove DMF. Residue was dissolved in deionized water, loaded into regenerated cellulose dialysis tubing (MWCO= 3,500 Da) and dialyzed for 1 day. The final product was lyophilized yielding a white powder (0.43 g, 85% yield). ^{1}H NMR (400Hz, D_2O, δ): 7.5 (s, 4H), 4.7 (s, 8H), 3.2 (m, J = 5.64 Hz, 8H), 3.6 (bs, PEG, 909H), 0.96 (s, 12H).

Synthesis of 4a-PEG-methylamine

4aPEG-chloride (10 kDa, 0.1 mmol) was dissolved in methanol (4 mL). Methylamine (30 mmol) was added into pressure tube in an acetone/dry ice bath under stirring. The polymer solution was added into the pressure tube drop-wise. The pressure tube was then flushed with nitrogen and

sealed. The reaction mixture was then warmed to room temperature in a warm water bath and then heated to 80 °C in an oil bath for 16 h. The reaction mixture was then evaporated under reduced pressure to remove MeOH. Residue was dissolved in DI water, loaded into regenerated cellulose dialysis tubing (MWCO= 3,500 Da) and dialyzed for 1 d. The final product was lyophilized yielding a white powder (1.05 g, 95% yield). ^{1}H NMR (400Hz, D_2O, δ): 3.6 (bs, PEG), 2.3 (s, 12H).

Synthesis of 4a-PEG-cis1,2-diol-*S* or -*R* (4aPEG-D-1,2(*S*) or 4aPEG-D-1,2(*R*))
4aPEG-methylamine (10 kDa, 0.1 mmol) was dissolved in THF (20 mL). (*R*)- (+)-glycidol or (*S*)-(-)-glycidol (0.88 mmol) was added to the reaction mixture. The desired products were synthesized through ring-opening of the epoxy group of the glycidol by reaction with the amine group of the 4a-PEG macromer. The reaction was left at room temperature for 1 d under stirring. The reaction mixture was then evaporated under reduced pressure to remove THF. Residue was dissolved in DI water, loaded into regenerated cellulose dialysis tubing (MWCO= 3,500 Da) and dialyzed for 1 d. The final product was lyophilized yielding a white powder (0.27 g, 92% yield for 4aPEG-D-1,2(*S*); 0.26 g, 89% yield for 4aPEG-D-1,2(*R*)). ^{1}H NMR (400Hz, D_2O, δ): 3.6 (bs, PEG, 909H), 3.4 (m, 16H), 3.2 (t, J = 5.24 Hz, 8H), 2.3 (s, 12H)

Synthesis of 4a-PEG-cis1,2-diol-du-*S, R,* or *racemic mixture* (4aPEG-Du-1,2(*S*), 4aPEG-Du-1,2(*R*), or 4aPEG-Du-1,2(*SR*))
4aPEG-NH_2 (10 kDa, 0.1 mmol) was dissolved in THF (20 mL). (*R*)- (+)-glycidol, (*S*)- (-)-glycidol or (*racemic mixture, SR*)-(±)-glycidol (10 mmol) was added to the reaction mixture. The desired products were synthesized through ring-opening of the epoxy group of glycidols by reacting with the primary amino groups of the 4aPEG macromers; each primary amine can ring-open two epoxy groups. The reaction was left at room temperature for 1 d under stirring. The reaction mixture was then evaporated under reduced pressure to remove THF. Residue was dissolved in DI water, loaded into regenerated cellulose dialysis tubing (MWCO= 3,500 Da) and dialyzed for 1 d. The final product was lyophilized yielding a white powder (0.56 g, 94% yield for 4aPEG-Du-1,2(*S*); 0.54 g, 91% yield for 4aPEG-Du-1,2(*R*); 0.58 g, 96% yield for 4aPEG-Du-1,2(*SR*)). ^{1}H NMR (400Hz, D_2O, δ): 3.6 (bs, PEG, 909H), 2.8-2.4 (bs, 24H).

Synthesis of Benzylamine-GLD_{sm}
Benzylamine (5 mmol) and D-gluconolactone (10 mmol) were dissolved in MeOH (20 mL). The reaction mixture was then heated to reflux and stirred for 3 h. The solvent was evaporated under reduced pressure and the residue was recrystallized with EtOH to obtain the target product as a white solid (2 g, 80% yield). ^{1}H NMR (400 MHz, D_2O, δ) 7.32 – 7.19 (m, 5H), 4.34 (d, J = 1.7 Hz, 2H), 4.24 (d, J = 3.7 Hz, 1H), 3.98 (t, J = 3.2 Hz, 1H), 3.68 (dd, J = 11.5, 2.3 Hz, 1H), 3.62 (dd, J = 5.6, 2.5 Hz, 2H), 3.55 – 3.47 (m, 1H).

Synthesis of Dimethylamine-FLD_{sm}
The protected fructose-N_3 precursor (0.2 mmol) and N,N-dimethylpropargylamine (0.24 mmol) were dissolved in DMF (15 mL) in a Schlenk flask. Copper Sulfate Pentahydrate ($CuSO_4 \cdot 5H_2O$, 0.02 mmol) and N,N,N′,N″,N″-Pentamethyldiethylenetriamine (PMDETA, 0.02 mmol) were added to the reaction mixture. The Schlenk flask was degassed with three freeze-pump-thaw cycles. On the last cycle, the reaction mixture was added with ascorbic acid (0.2 mmol) and was stirred at 50ºC for 2 d in oil bath. The reaction mixture was then evaporated under reduced pressure to

remove DMF. The crude was then treated with 90% trifluoroacetic acid (TFA) in water for 4 hours to remove the protecting group. The crude was then concentrated under reduced pressure and ran through silica column with a mobile phase gradient of 0% to 15% MeOH in DCM. The final product was a yellow oil (0.091 g, 61% yield). ^{1}H NMR (400Hz, D_2O, δ): 8.2 (s, 1H), 4.4 (S, 2H), 3.7-3.4 (m, 7H) 2.8 (S, 6H)

Synthesis of Benzylamine-Du-S_{sm}, Du-R_{sm} and Du-SR_{sm}

Benzylamine (0.9 mmol) were dissolved in ethanol (EtOH, 10 mL). (*R*)- (+)-glycidol, (*S*)- (-)-glycidol or (*SR*)- (±)-glycidol (2.7 mmol) was added to the reaction mixture. The reaction was left at room temperature for 1 d under stirring. The reaction mixture was then evaporated under reduced pressure to remove THF. Residue was run through silica column with a mobile phase gradient of 0% to 15% MeOH in DCM. The final was a transparent oil (68%, 74%, and 79% yield for Du-S_{sm}, Du-R_{sm} and Du-SR_{sm}, respectively). ^{1}H NMR (400Hz, D_2O, δ): 7.2 (m, J = 7.6 Hz, 4H), 7.1 (t, J = 7.2 Hz, 1H), 4.4 (m, J = 4.5 Hz, 4H), 3.57 (s, 2H), 3.52 (m, J = 5.76 Hz, 2H), 3.2 (m, J = 5.2 Hz, 4H), 2.3 (m, J = 7.48 Hz, 4H).

Synthesis of FITC-Insulin

Recombinant Human Insulin (0.017 mmol) was dissolved in sodium carbonate solution (0.1M, pH~9, 5mL). Fluorescein isothiocyanate isomer I (FITC, 0.022 mmol) was dissolved in dimethyl sulfoxide (DMSO, 1mL). The pH of both insulin solution and FITC solution were titrated to ~11 with sodium hydroxide solution (NaOH, 1M). Then the FITC solution was added to insulin solution drop-wise in the dark. The reaction mixture was kept in dark for 12 h. Then, the reaction mixture was titrated to pH ~5.3 with hydrochloric acid (HCl, 1N), followed with centrifugation (4000 rpm, 30 min, 4 °C). The pellet was then resuspended in water and dialysis in DI water for three times in dark. Finally, product was frozen and lyophilized, yielding a yellow powder.

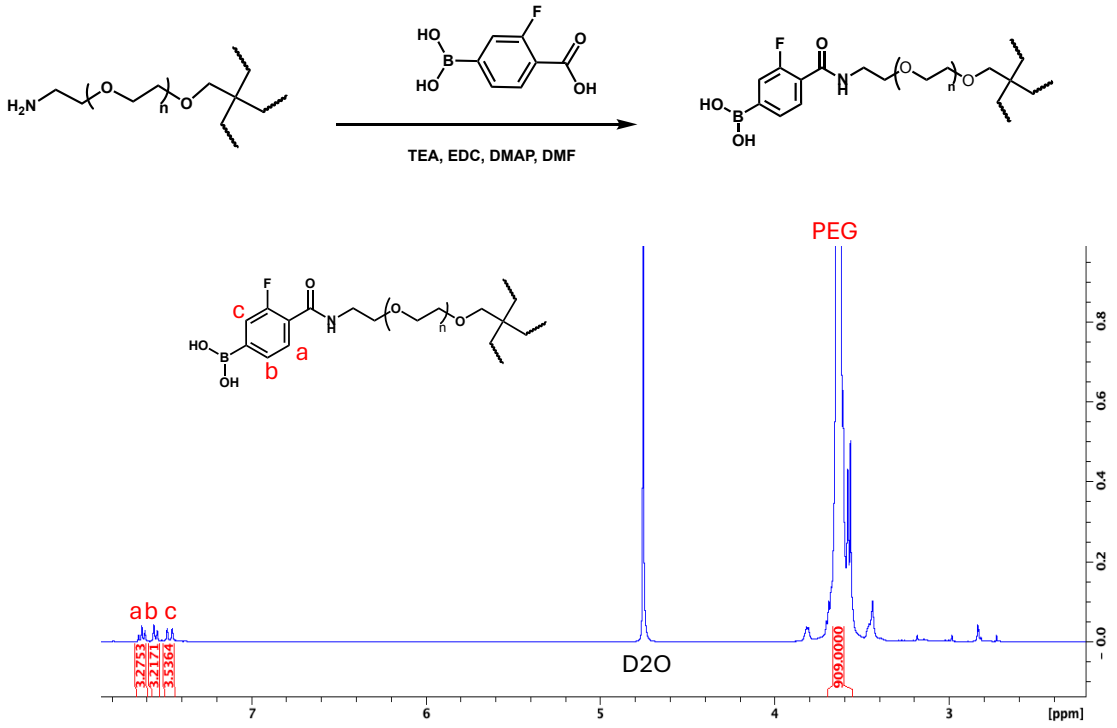

Figure S1. ^{1}H NMR spectrum of 4aPEG-FPBA

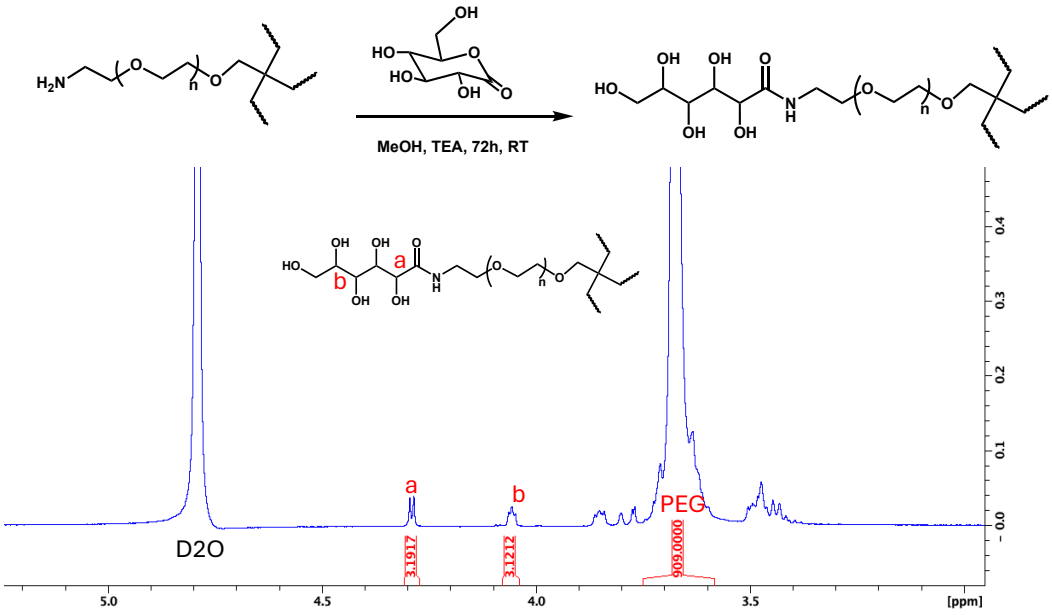

Figure S2. ^{1}H NMR spectrum of 4aPEG-GLD

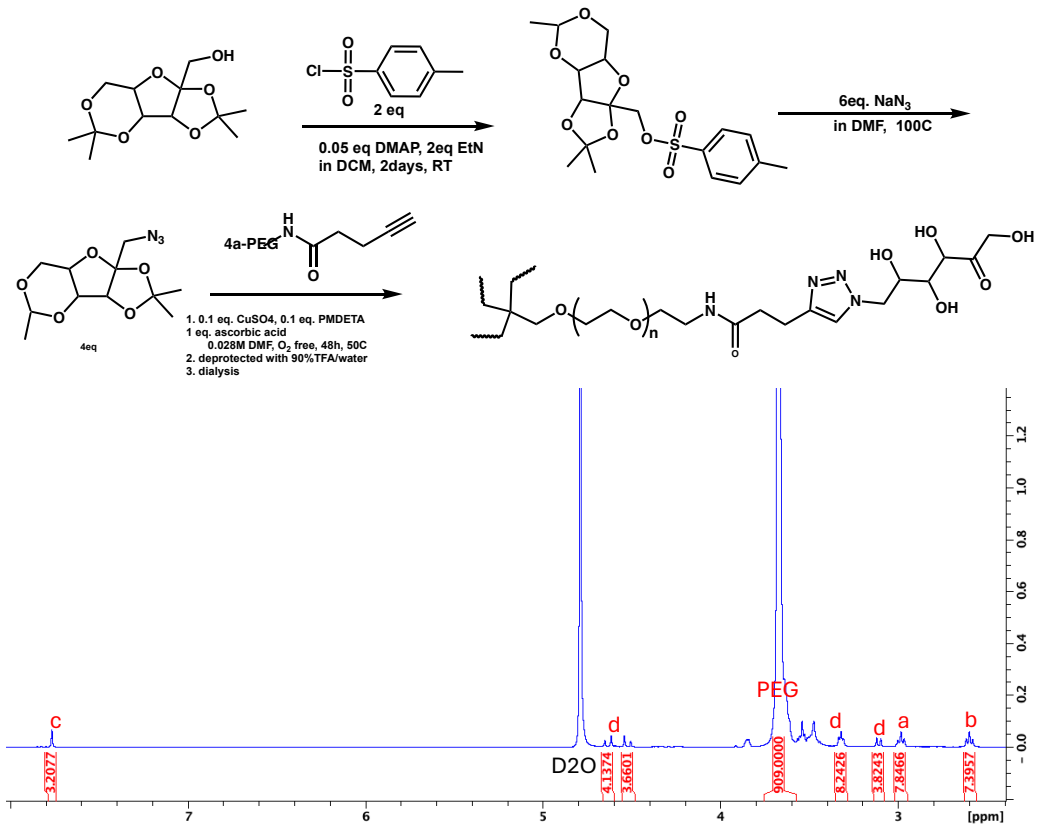

Figure S3. ^{1}H NMR spectrum of 4aPEG-FLD

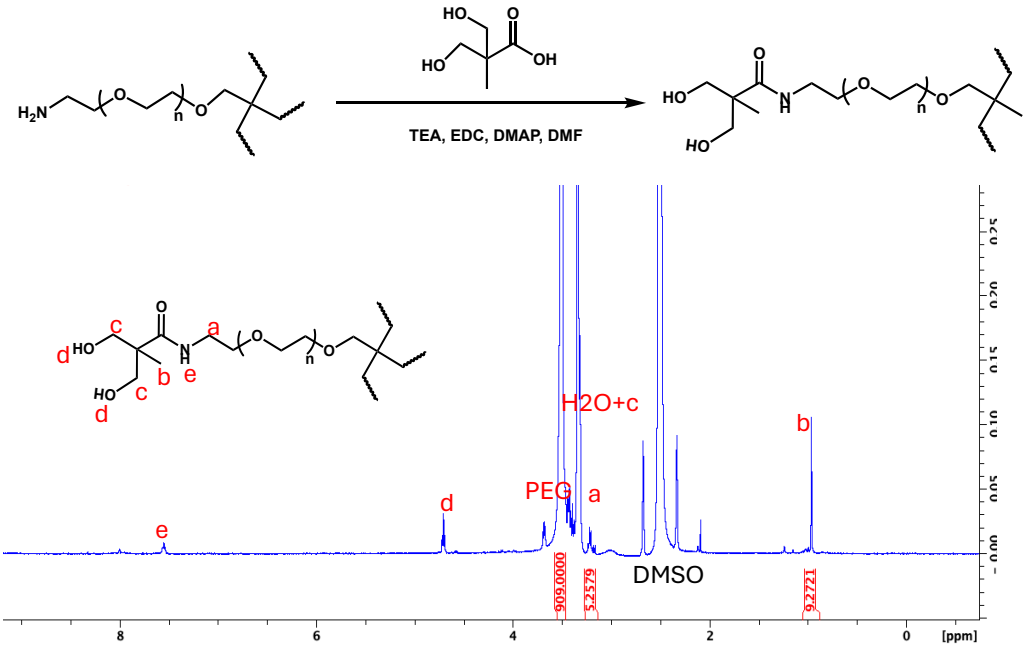

Figure S4. ^{1}H NMR spectrum of 4aPEG-D-1,3

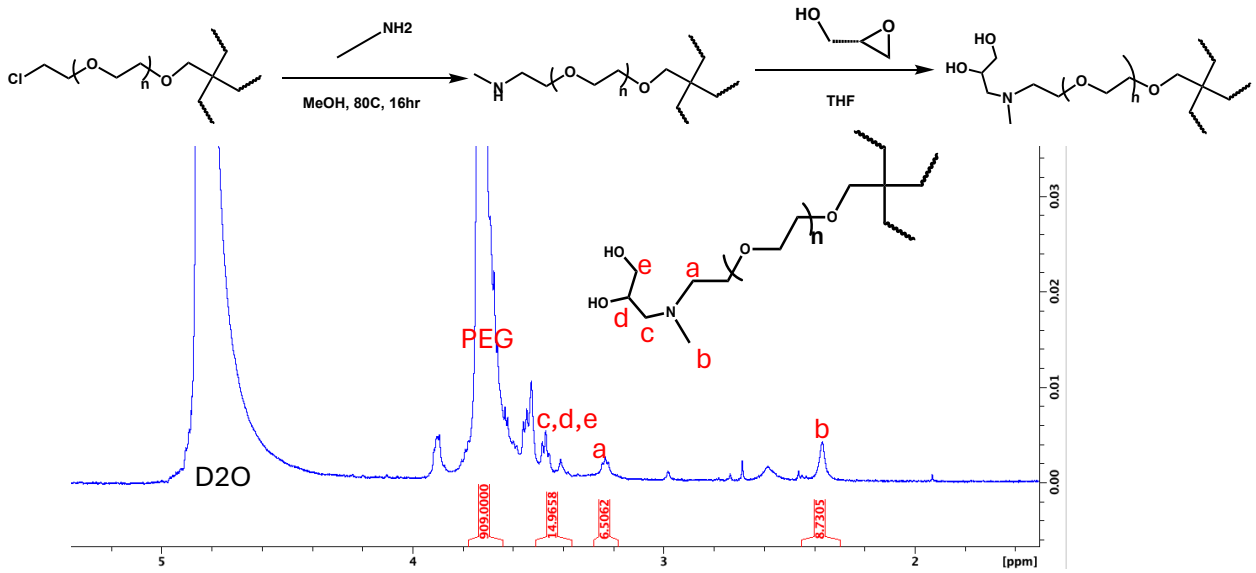

Figure S5. ^{1}H NMR spectrum of 4aPEG-D-1,2(S)

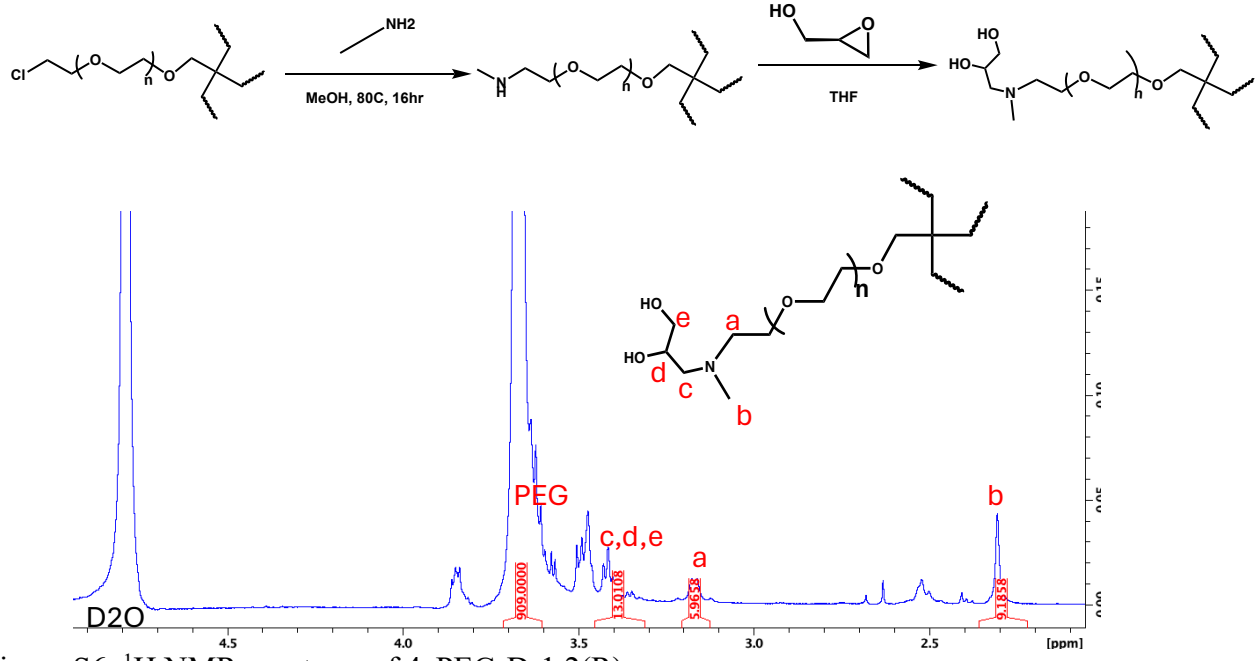

Figure S6. ^{1}H NMR spectrum of 4aPEG-D-1,2(R)

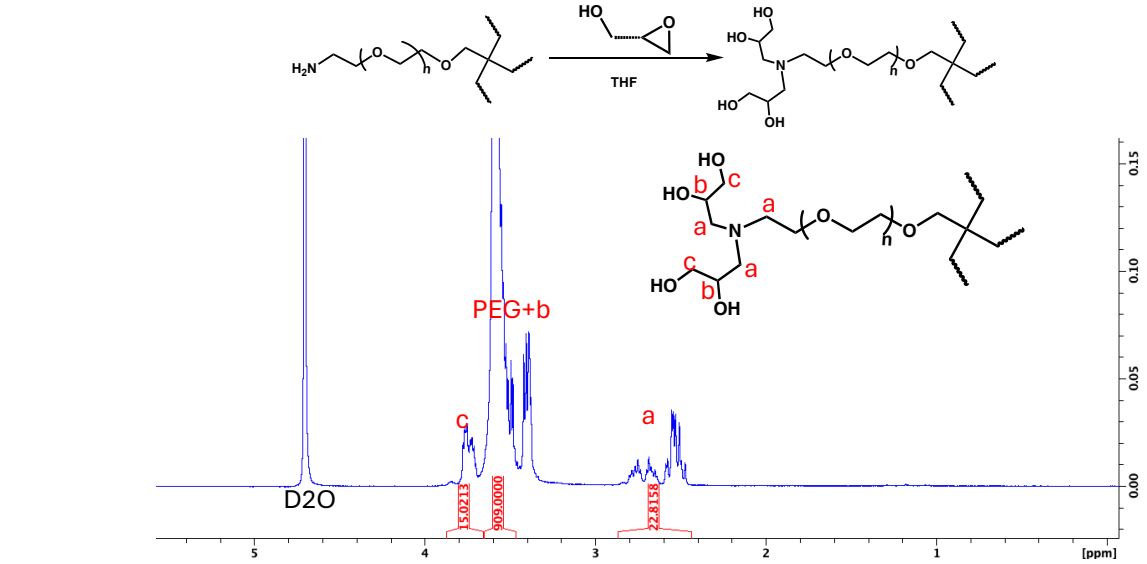

Figure S7. ^{1}H NMR spectrum of 4aPEG-Du-1,2(*S*)

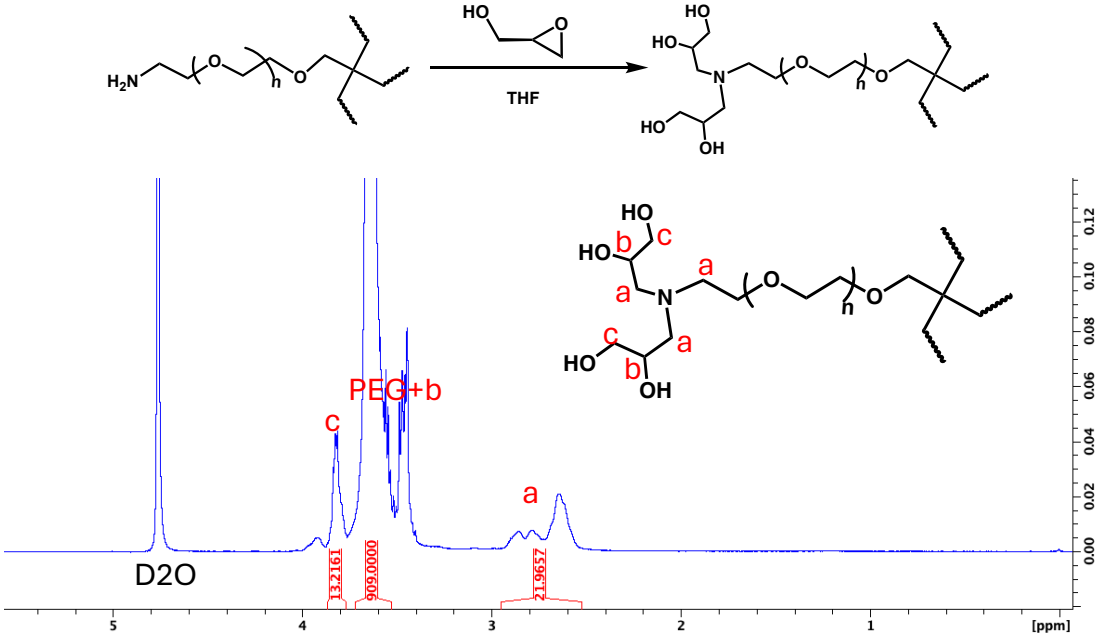

Figure S8. ^{1}H NMR spectrum of 4aPEG-Du-1,2(*R*)

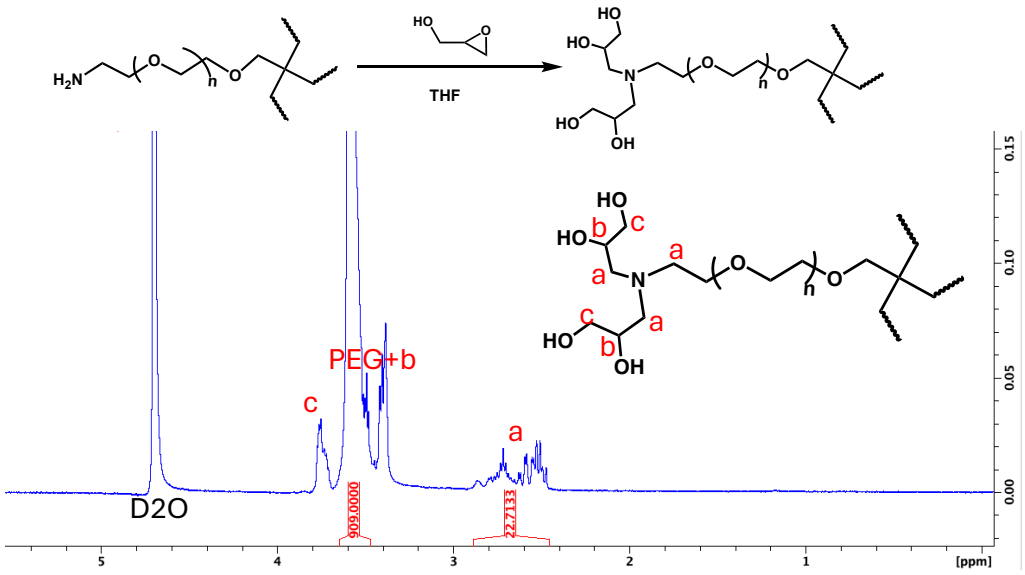

Figure S9. ^{1}H NMR spectrum of 4aPEG-Du-1,2(S*R*)

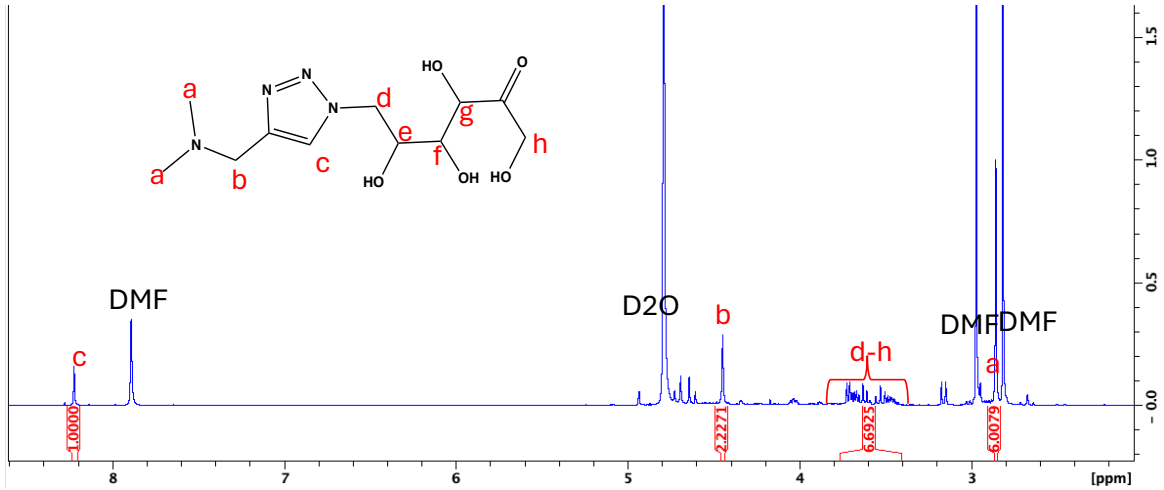

Figure S10. ^{1}H NMR spectrum of FLD_{sm}

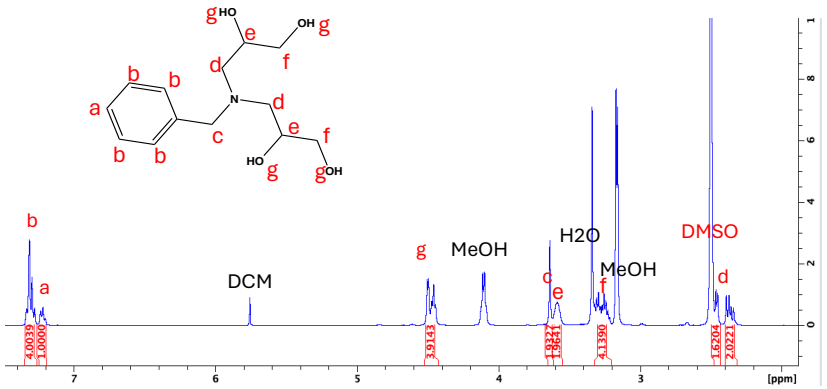

Figure S11. ^{1}H NMR spectrum of Du-S_{sm}

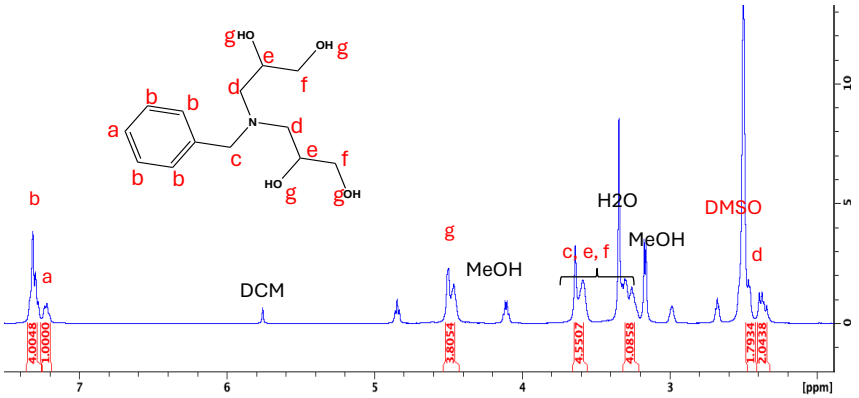

Figure S12. ^{1}H NMR spectrum of Du-R_{sm}

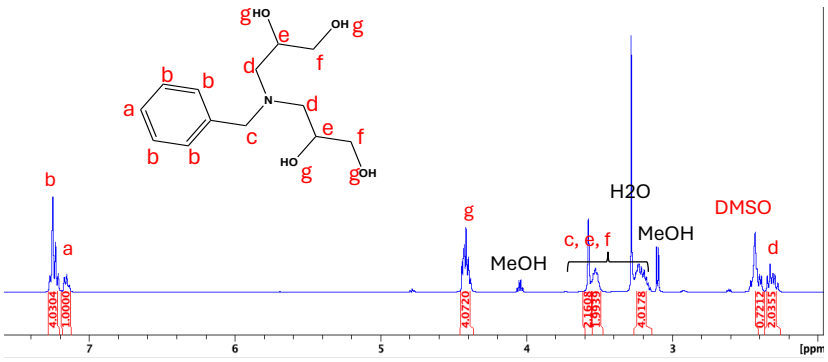

Figure S13. ^{1}H NMR spectrum of Du-SR_{sm}

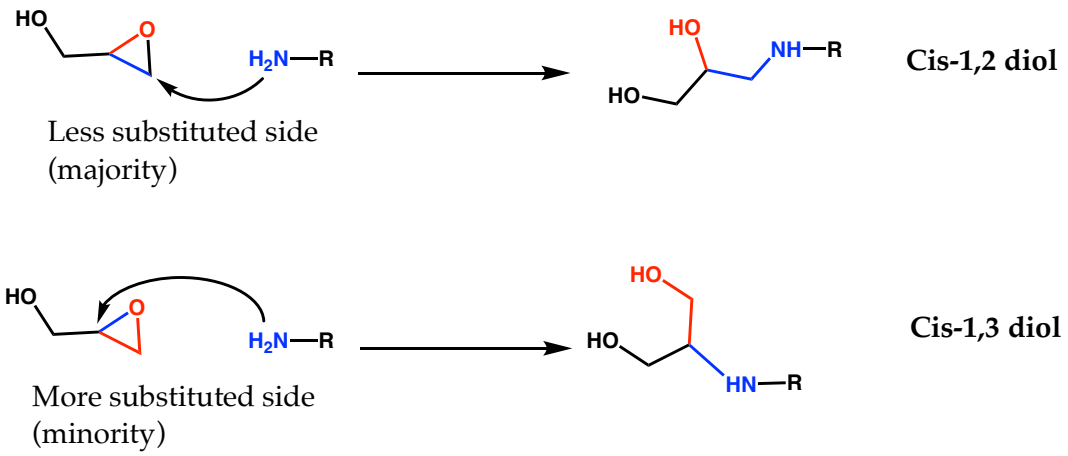

Figure S14. Illustration of epoxy-ring opening reaction with primary amines. The epoxy ring of glycidol can be opened by a primary amine from either the less substituted side or the more substituted side, leading to the formation of *cis*-1,2-diol or *cis*-1,3-diol, respectively.

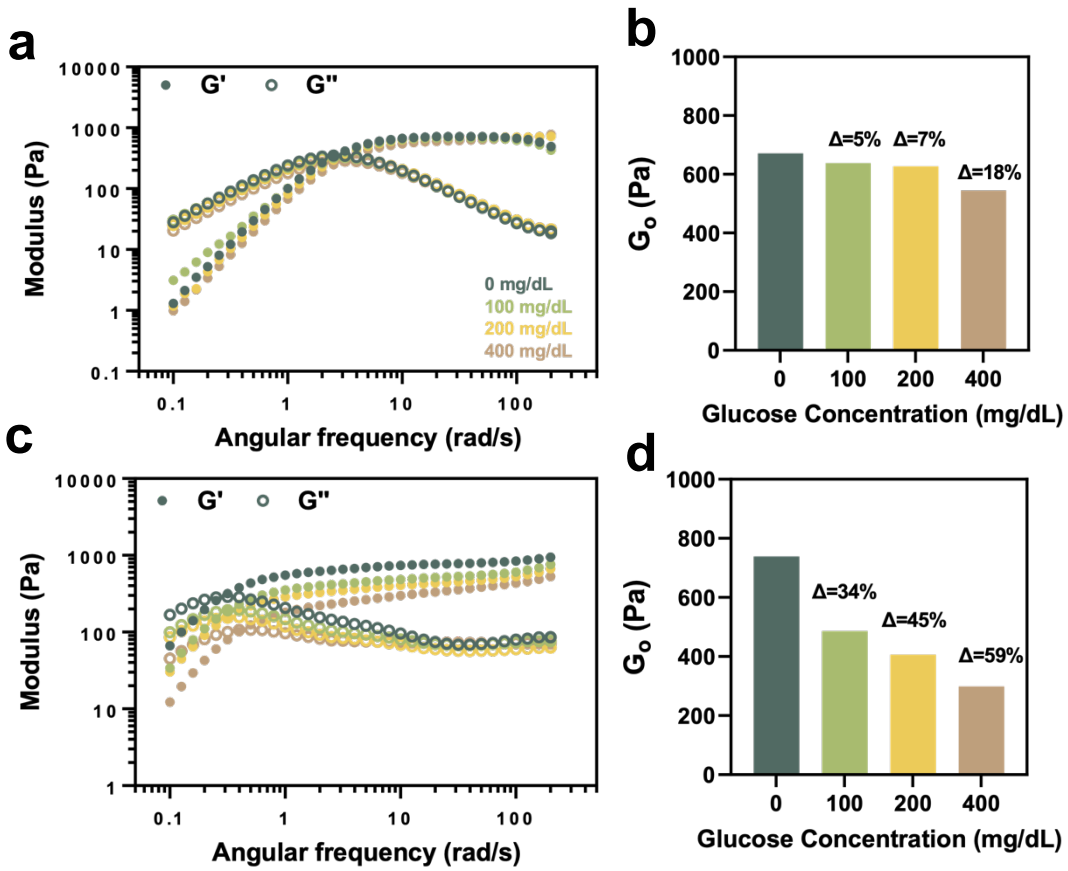

Figure S15. Glucose Dependent Rheology study at gel with similar stiffness. (a)&(b): Oscillatory rheology frequency sweep performed for FPBA–GLD hydrogel at 3wt% at various glucose concentrations. (c)&(d): Oscillatory rheology frequency sweep performed for FPBA-FLD hydrogel at 5wt% at various glucose concentrations.

Table S1. Specific concentrations of each compound for ITC experiments.

	GLD_{sm}	**FLD_{sm}**	**Du-*S*$_{sm}$**	**Du-*R*$_{sm}$**	**Du-*SR*$_{sm}$**
	Concentration (M)				
Syringe (Diols)	6.00E-03	1.00E-02	7.00E-02	7.00E-02	7.00E-02
Cell (FPBA)	8.00E-04	1.00E-03	8.00E-03	8.00E-03	8.00E-03

Table S2. Dynamic rheological properties for each hydrogel formulation.

		Glucose concentration (mg/dL)			
		0	**100**	**200**	**400**
4aPEG-GLD (10%)	**ω_c (rad/s)**	5.01	5.01	5.01	5.01
	τ_r (s)	1.25	1.25	1.25	1.25
4aPEG-FLD (10%)	**ω_c (rad/s)**	0.32	0.32	0.40	0.50
	τ_r (s)	19.87	19.87	15.78	12.54
4aPEG-Du-1,2(*S*) (10%)	**ω_c (rad/s)**	1.00	1.00	1.58	10.00
	τ_r (s)	6.28	6.28	3.96	0.63
4aPEG-Du-1,2(*R*) (10%)	**ω_c (rad/s)**	1.00	1.26	1.26	7.94
	τ_r (s)	6.28	4.99	4.99	0.79
4aPEG-Du-1,2(*SR*) (10%)	**ω_c (rad/s)**	0.79	1.26	3.16	6.31
	τ_r (s)	7.91	4.99	1.99	1.00
4aPEG-GLD (3%)	**ω_c (rad/s)**	3.16	3.16	3.16	3.16
	τ_r (s)	1.99	1.99	1.99	1.99
4aPEG-FLD (5%)	**ω_c (rad/s)**	0.32	0.40	0.40	0.50
	τ_r (s)	19.87	15.78	15.78	12.54