

Supplemental Information

Controlled Branching of Polyglycidol and Formation of Protein-Glycidol Bioconjugates via a Grafting-from Approach with “PEG-like” Arms

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Materials. Glycidol (Sigma Aldrich, 96%) was vacuum distilled prior to use. 3-Buten-1-ol (Sigma Aldrich Inc.), Tin(II) trifluoromethanesulfonate (Strem Chemicals Inc., 99%), N,N-Dimethylformamide (DMF, Sigma Aldrich 99.8%), N,N,N',N'-ethylenediaminetetraacetic acid (EDTA, Sigma Aldrich, 99.4-100.6%), triethylamine (Sigma Aldrich, ≥99%), bovine serum albumin (BSA, Aldrich, Approx. 99%), 5,5'- dithio-bis-(2-nitrobenzenzoic acid) (Ellman's reagent, Aldrich, 99%), tris(2-carboxyethyl)phosphine hydrochloride (Sigma Aldrich, TCEP, ≥98 %), and 4-nitrophenyl acetate (Fluka, ≥99%) were used as received. Dialysis membranes (Spectra/Por® 7, molecular weight cut-off (MWCO): 25,000 and 50,000 Da) were obtained from Spectrum Laboratories, Inc. Precise Tris-glycine 12% pre-cast PAGE gels were obtained from Fisher Scientific Company.

Synthesis of GLY Homopolymer at a Range of Temperatures. In order to maintain a consistent temperature throughout the entirety of the polymerization, a specialized round bottom flask and cooling system were employed. The round bottom flask was formed with a 25mL reaction vessel enveloped by a glass "jacket" with an inlet at the bottom and an outlet at the top. This allowed for a temperature controlled liquid to constantly surround the reaction vessel, thus allowing for sustained reaction temperature. The pump system used was a 7 Liter Refrigerated Circulator obtained from PolyScience with a temperature range of -40°C to 200°C and a temperature stability of ±0.01°C.

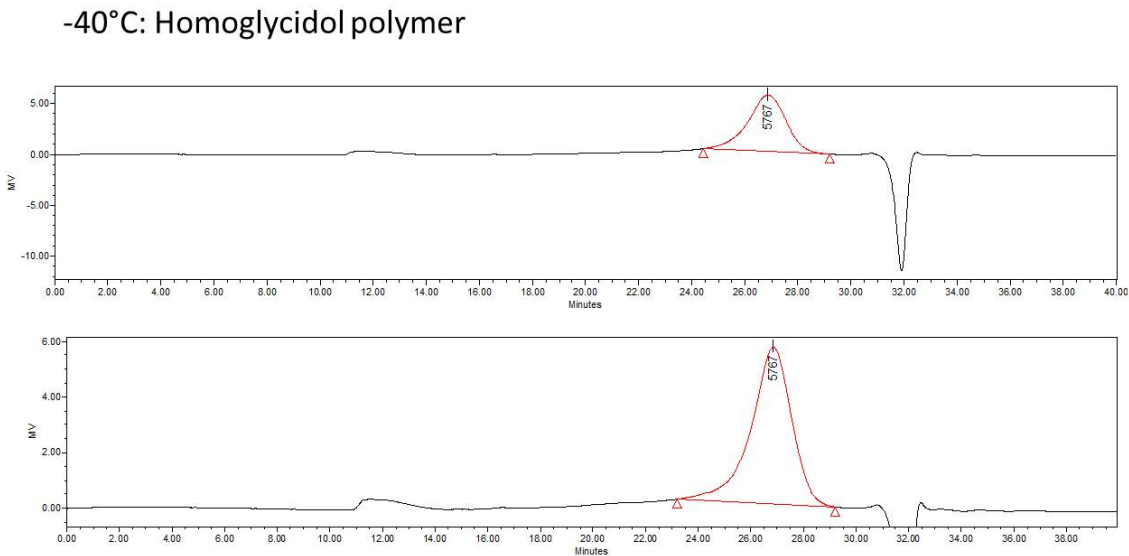
Two 1-dram vials were fitted with septa and flame dried under N₂(g). The first 1-dram vial was used to make a 1.7M Iso-Amyl alcohol (IAOH) stock solution was formed using dry THF, while the second was used to create a 3.7x10⁻²M tin triflate stock solution, also using dry THF. Stock IAOH (0.20mL, 3.33x10⁻⁴ mol, 0.066eq) and Sn(OTf)₂ (0.26mL, 9.45x10⁻⁶ mol, 0.00035 eq) were added to the jacketed reaction flask, equipped with a magnetic stir bar, at the chosen reaction temperature, and the reaction solution was allowed to stir for 30 minutes to allow for the alcohol/catalyst conjugation. After the activation step, the glycidol monomer (2.00g, 27mmol, 1.0eq) was added drop wise. After stirring was completely impeded (Reaction time varied with temperature), the crude viscous polymer product was dissolved in a minimal amount of methanol and precipitated into vigorously stirring

hexanes, which were then decanted to afford the pure glycidol homopolymer product as translucent viscous material. ¹H-NMR (600MHz, CDCl₃) δ: 3.31-3.94 (6H). ¹³C-NMR (150MHz, CDCl₃) δ: 81.37, 79.81, 75.12, 73.88, 72.01-72.94, 70.42-71.17, 64.41, 62.53, 62.06.

CAUTION: When the scale of the reaction is above 1g, the reaction can become very exothermic during monomer addition and a cooling of the reaction vessel with an ice bath is recommended to avoid overheating.

Polymer	Temperature	Reaction Time	Yield
Glycidol Homopolymer	40°C	1h	92.3%
Glycidol Homopolymer	20°C	2.5h	90.7%
Glycidol Homopolymer	0°C	42h	93.5%
Glycidol Homopolymer	-20°C	4d	94.8%

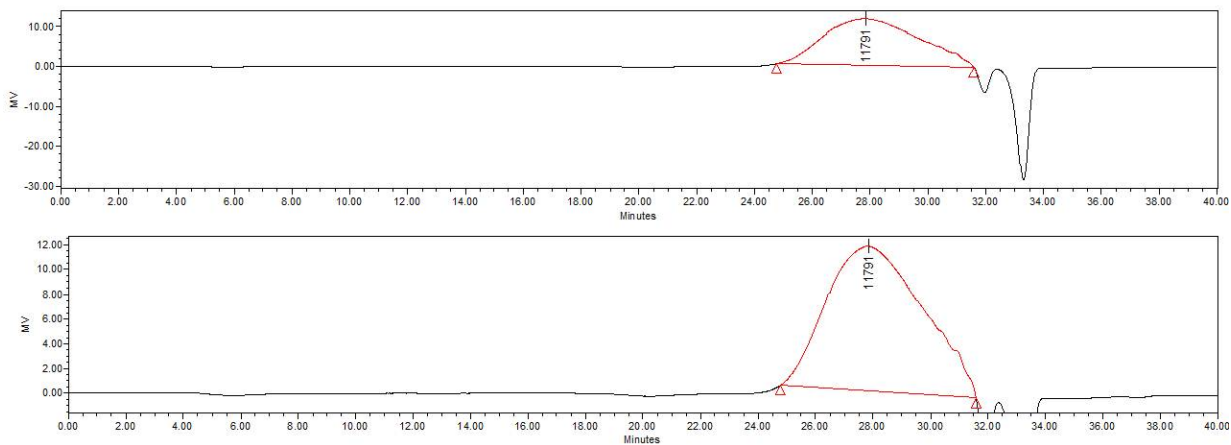
GPC – Data:



	Name	Retention Time	Peak Cuts	Det Name	Mn	Mw	MP	UV	Mz	Mz+1	Polydispersity	PP	K	alpha	Mz/Mw	Mz+1/Mw	Id	Peak Sigma	System Sigma	Area	% Area	Height	Int Type	MW at 10 pct	MW at 50 pct	Mn above 90 pct of dist	Mn below 10 pct of dist	Mw above 90 pct of dist	Mw below 10 pct of dist	Mz above 90 pct of dist	Mz below 10 pct of dist
1	Blank	26.63			5762	7242	5767		998	1479	1.25807				1.37202	2.04036	0			60890	100.00	564.96		11913	3712	3075	12991	3151	17792	3213	19885

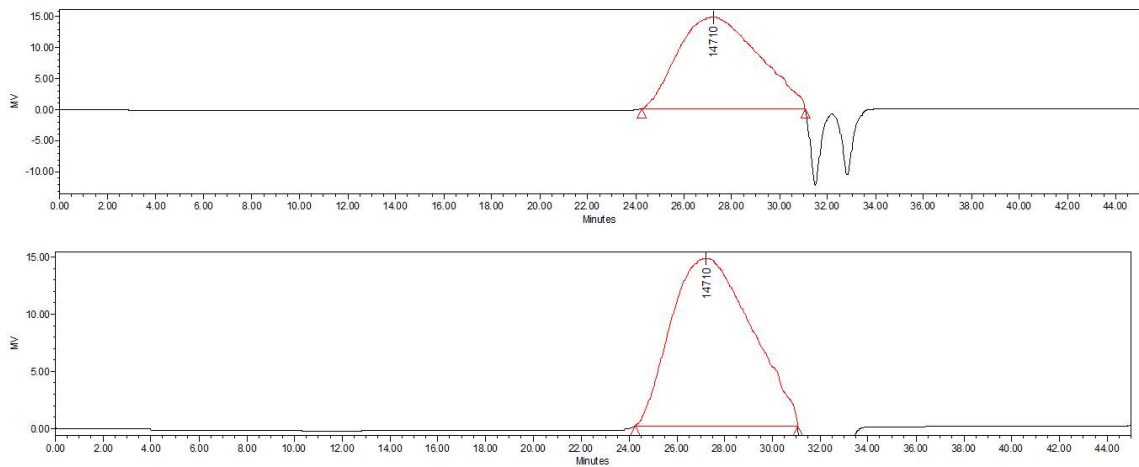
Mn: 5762; Mw: 7242, PDI 1.25

0°C: Homoglycidol polymer



Mn: 8566, Mw: 11645, PDI: 1.35

20°C: Homoglycidol polymer



	Name	Retention Time	Peak Codes	Det Name	Mn	Mw	MP	Mv	Mz	Mz+1	Polydispersity	[η]	K	alpha	Mn/Mw	Mz+1/Mw	Id	Peak Signa	System Signa	Area	% Area	Height	IntType	MW_at_10 pd	MW_at_90 pd	Mn_above_90wt_of_d at	Mn_below_10wt_of_d at	Mn_above_90wt_of_d at	Mn_below_10wt_of_d at	Mz_above_90wt_of_d at	Mz_below_10wt_of_d at
1	Bead	27.267	C06		10976	14589		14710	18245	21472	1.329218				1.292640	1.471737	0			3421578	100.00	14837	8s		5947	4618	28969	4756	29394	4882	28777

Mn: 10976, Mw: 14589, PDI: 1.32

Conjugation of BSA with Maleimide Alcohol to Obtain BSA Alcohol Conjugate. BSA (0.5g, 7.5 μmol , 1 eq) was dissolved in phosphate buffer (PB) solution (37.5mL, pH 7.2) in a 100mL round bottom flask equipped with a magnetic stir bar and was then purged under $\text{N}_2(\text{g})$ for 1h. 1-(2-hydroxyethyl)-1H-pyrrole-2,5-dione (21.23mg, 6×10^{-5} mol, 20 eq) was solubilized in nitrogen-purged DMF (2.5mL). The maleimide solution was then added drop wise to the stirring BSA solution and the reaction was allowed to run for 20 @ 25°C. The resulting reaction mixture was dialyzed against deionized (DI) water for 36h using a MWCO of 25,000 Da, and then lyophilized to isolate the BSA Alcohol Conjugate (BSA-OH).

General Procedure for the Polymerization of GLY using BSA-OH in Different Solvent Systems. $\text{Sn}(\text{OTf})_2$ (0.87mg, 2.087 μmol), BSA-OH (80mg, 1.198 μmol), and PB pH 6.0 (3.6mL) were added to a 25mL round bottom flask equipped with a magnetic stir bar and purged with $\text{N}_2(\text{g})$ for 30 minutes in an ice bath. The glycidol monomer (1.194mmol, 79.4 μL) was then added by syringe and the reaction was allowed to run for 24h at 25°C. The resulting reaction mixture was dialyzed against DI water for 36h using a MWCO of 25,000 Da, and then lyophilized to isolate the BSA Glycidol Conjugate (BSA-GLY).

This reaction was repeated with the PB replaced with either DMF or DMSO in order to ascertain the optimal reaction conditions. The products obtained from these three solvent systems were visibly different with a seeming decrease in solubility in the trend: PB>DMF>DMSO. However, apart from this caveat, the reaction procedure remained unchanged.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (PAGE). PAGE was accomplished using an Invitrogen Novex Mini-Cell system. Precast 12% Precise™ Protein Gels (Thermo Scientific) were run at 100V and 120mA for 1.75 hours. Solutions were prepared with a BSA concentration of 6g/L in DI water. PAGE samples were formed by mixing 7.5 μL of BSA solution with 2.5 μL 4x LDS sample buffer (Invitrogen) before loading the 10 μL solutions onto the gel plate. Staining of the gels was accomplished using coomassie blue.

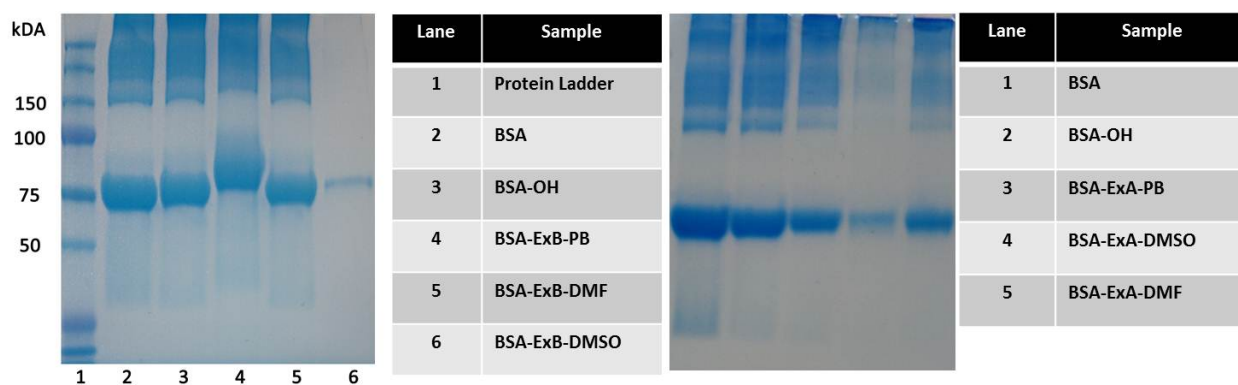


Figure 1. (left), Experiment B (ExB) higher concentration of glycidol monomer 1.33M (4 fold): PAGE of BSA and the synthesized BSA-polymer conjugates. Lane1: Protein Ladder. Lane 2: BSA. Lane 3: BSA-OH initiator. Lane 4: BSA-polymer reaction in PB. Lane 5: BSA-polymer reaction in DMF. Lane 6: BSA-polymer in DMSO. (right), Experiment A (ExA) lower concentration of glycidol monomer of 0.33M. Lane1: Native BSA protein. Lane 2: BSA-OH. Lane 3: BSA-polymer reaction in PB. Lane 5: BSA-polymer reaction in DMSO. Lane 6: BSA-polymer in DMF.

Bioactivity assay. The bioactivity of the synthesized BSA-OH conjugates and subsequent polymer coated species was determined and compared to the native BSA protein by measuring the absorbance related to the hydrolysis product with 4-nitrophenyl acetate as previously published by Sumerlin.¹ Sample preparation was performed by combining a BSA or BSA conjugate solution (50 μ L, [BSA]=0.27mM) in PB (pH=8.0) with a 10mM solution of 4-nitrophenyl acetate dissolved in acetonitrile (10 μ L) and PB (0.94mL, pH=8.0) and centrifuging the samples for 5 minutes at 6,000 rpm. The samples were allowed to incubate for 30 minutes at room temperature before measuring the absorbance at $\lambda = 405\text{nm}$ to determine the bioactivity of each sample.

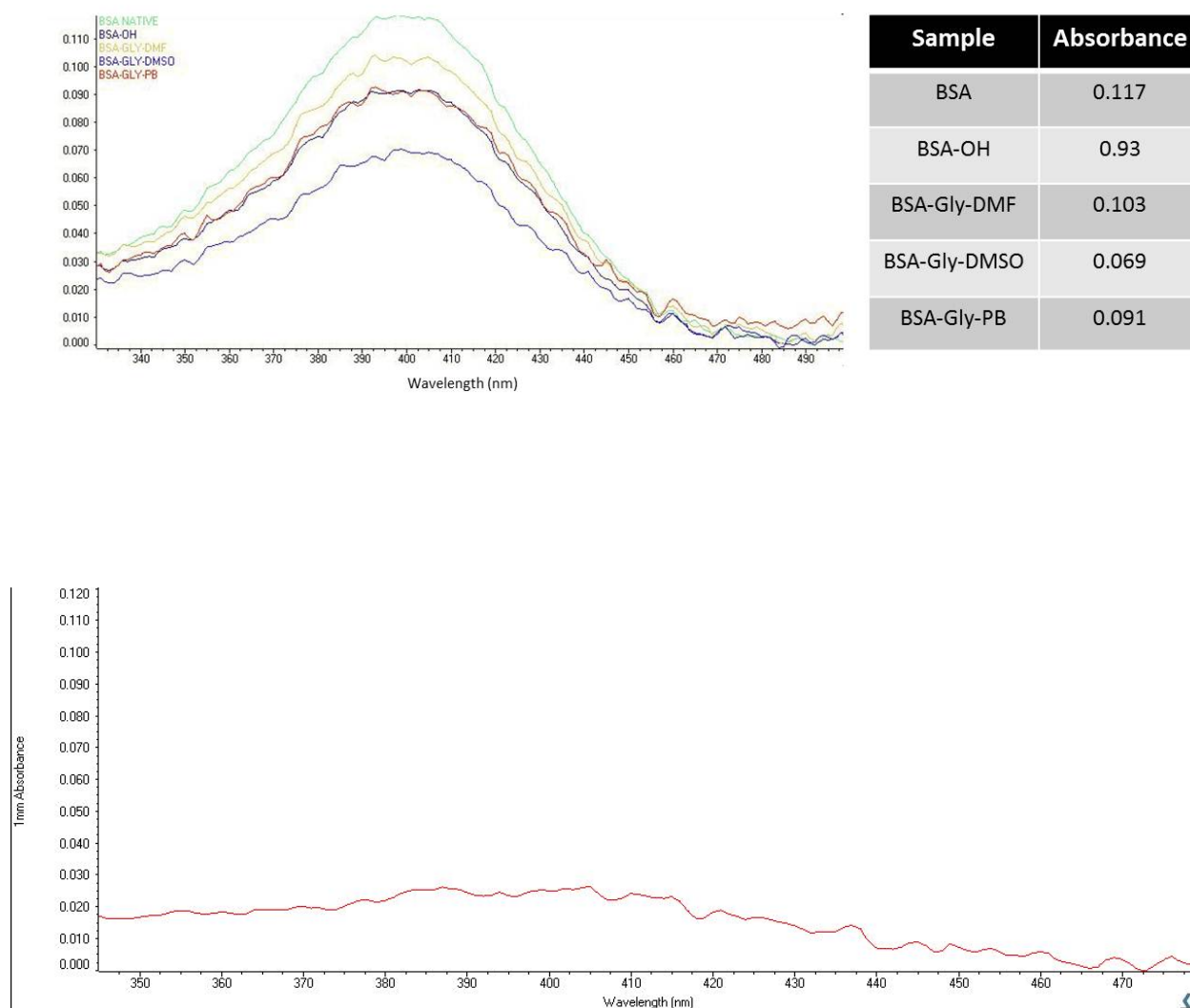


Figure 2a. Bioactivity assay performed using UV-vis spectroscopy. Data presented includes BSA-polyglycidol conjugate run in PB at lower (top) and 4- fold higher glycidol concentration (bottom). Sample in the bottom trace represented the PB only with 10 K molecular weight increase (MALDI) since the molecular weight increase is not as significant in the other samples run in organic solvents.

Gel Permeation Chromatography (GPC). Size exclusion chromatography of the glycidol homopolymers was performed in DMF at 70°C with a flow rate of 1.0mL/min (Waters 1525 binary HPLC pump; columns: 7.8x300mm; Styragel HR 5 DMF, Styragel HR 4E, and Styragel HR 3: molecular weight range 50,000 to 4x10⁶, 50 to 100,000, and 500 to 30,000 g/mol,

respectively). Detection was accomplished using a Waters 2414 refractive index detector. Molecular weights were determined using polystyrene standards.

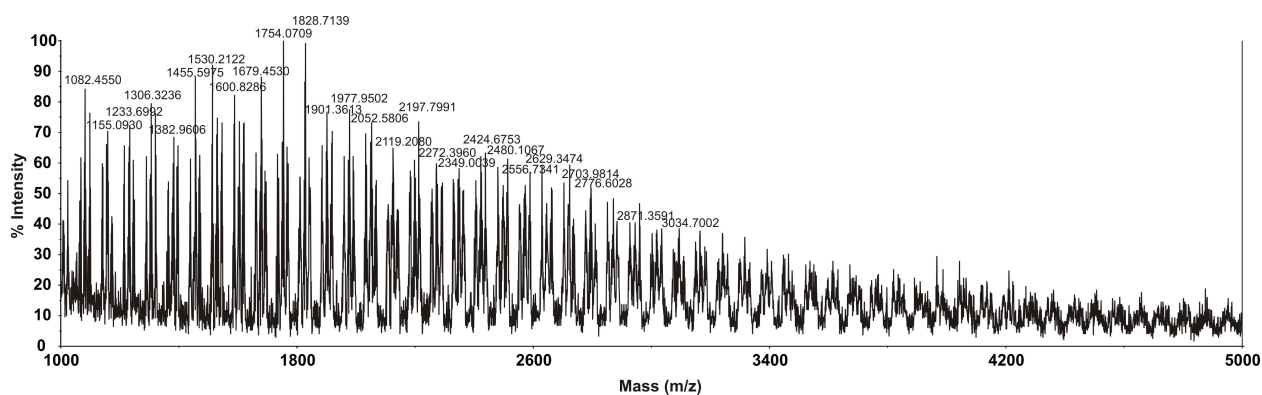
UV-vis Spectroscopy. UV-vis spectra were obtained using a Thermo Scientific Nanodrop 2000

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectroscopy (MALDI-TOF). MALDI-TOF MS of glycidol homopolymers was performed using product samples of 3mg/mL and a α -cyano-4-hydroxycinnamic acid matrix solution of 20mg/mL, both in methanol. To confirm the addition of the maleimide alcohol initiator to the BSA protein and the subsequent glycidol polymer growth from the BSA-OH conjugate similar parameters were used. The MALDI samples were formed using 6mg/mL product samples in DI water and a 5mg/mL matrix solution of sinapic acid dissolved in 2 parts 0.1% formic acid, 1 part acetonitrile.

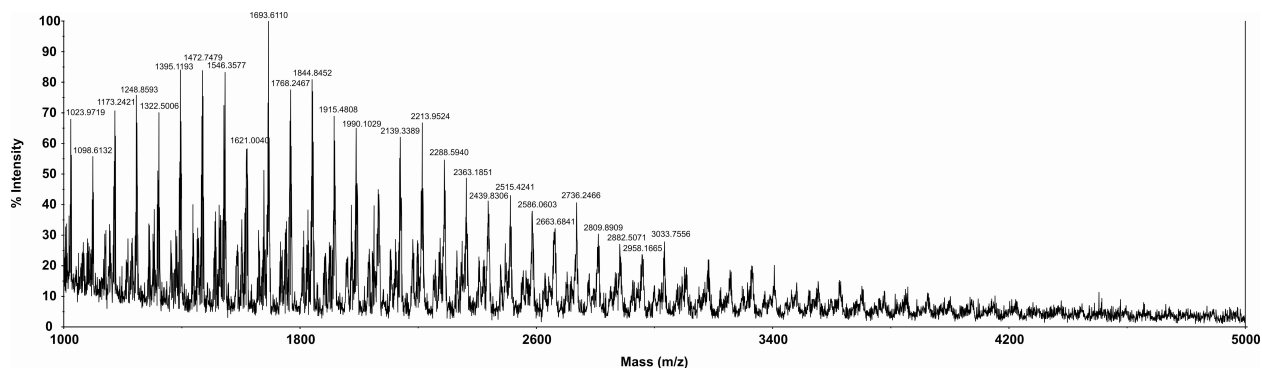
The sample and matrix were mixed in a 1:1 ratio and 1.4 μ L was spotted onto the MALDI plate for characterization. The instrument used was a Voyager DE-STR MS in linear TOF mode equipped with a nitrogen gas laser at λ = 337nm with external calibration. The instrument parameters were set to: 25,000V; 90% grid; 600ns delay; 1000 shots per spectrum.

Homopolymers:

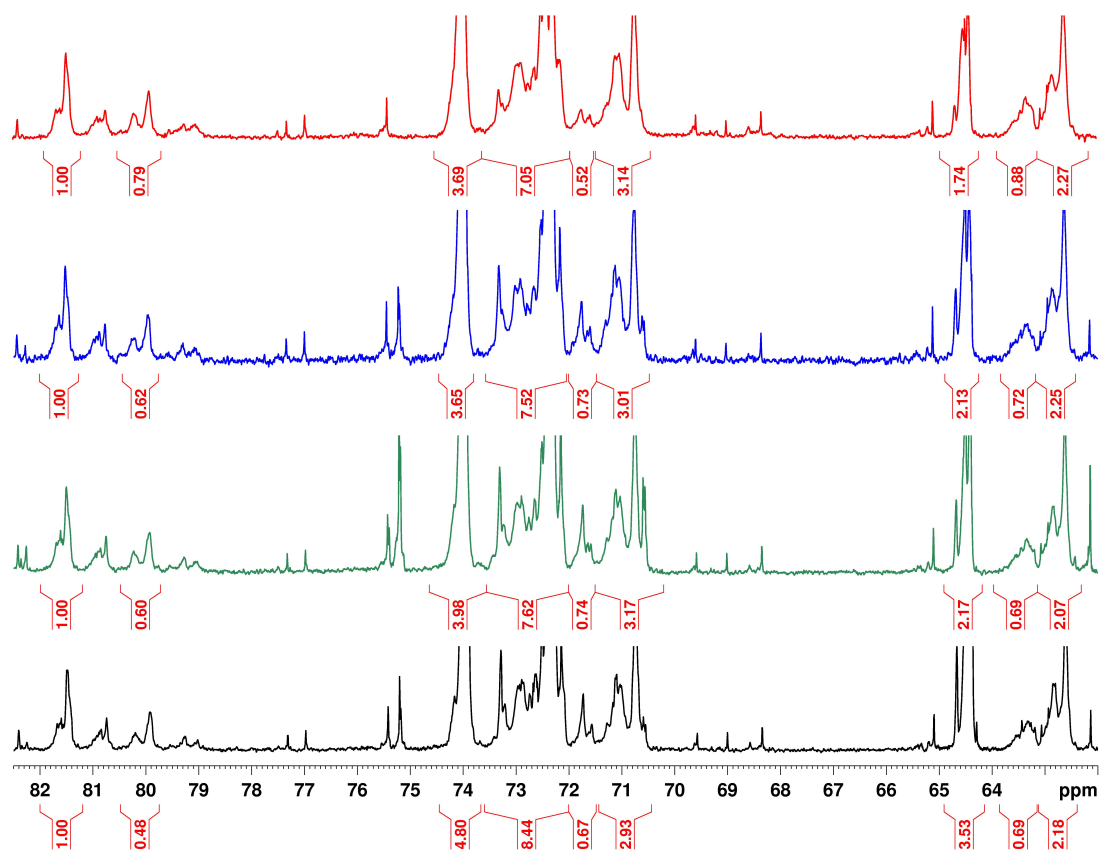
40°C experiment



0°C experiment



NMR Spectroscopy. ^1H and ^{13}C NMR spectroscopy was performed using a Bruker 600 spectrometer operating at 600 and 150 MHz, respectively. The instrument is equipped with a 14.1 Tesla Bruker magnet, which is controlled by a Bruker AV-II console, and a 5mm Z-gradient TCI Cryo-probe. A 20 second recycle delay was used to insure full relaxation for quantitative measurements, which allowed for the elucidation of specific monomer unit peaks and subsequent calculation of the degree of branching present in each glycidol homopolymer.

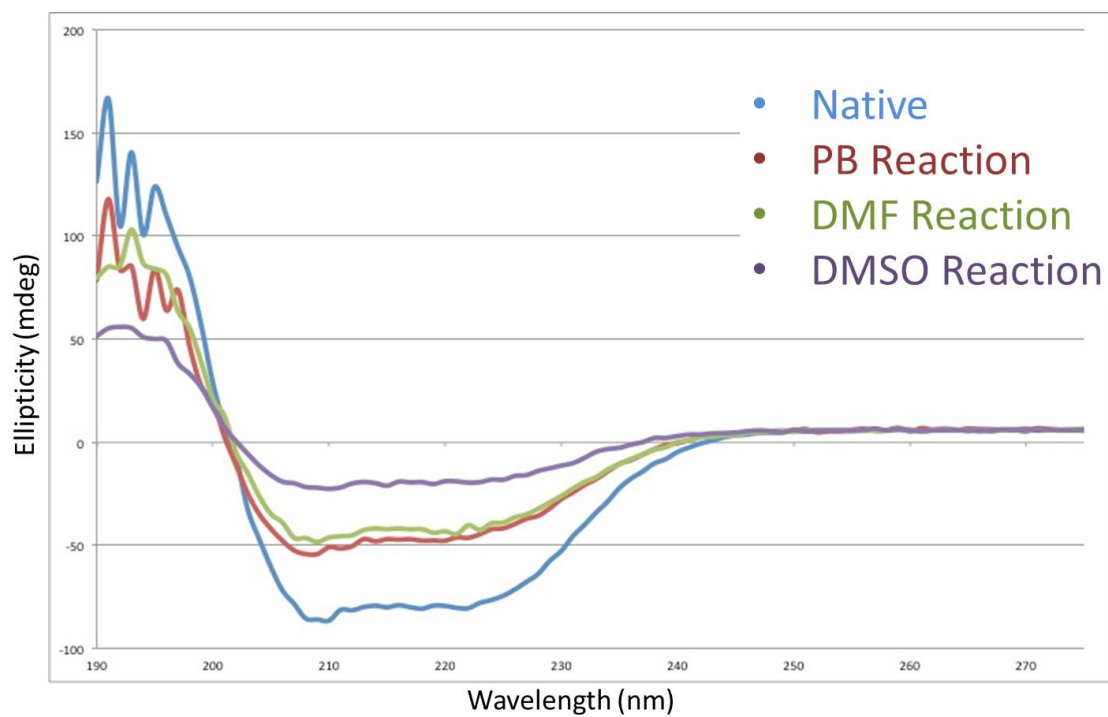


From 40°C (top, see also Figure 1 in article), 20°C, 0°C, -20°C (bottom), basis for data of table in Figure 1.

Calculation of the Degree of Branching (DB) for data of table in Figure 1 reported by Frey².

$$DB = \frac{2D}{2D+L13+L14}$$

Circular Dichroism (CD): The use of CD spectrophotometry was employed to verify the retention of the secondary structure of the BSA protein. Spectra were obtained with a Jasco J-720 at 25 ± 1 °C in DI water (1.5x10⁻⁵ M) in a cell with a path length of 1cm (bandwidth=1 nm; step resolution = 0.5 nm; scan speed = 50nm/min; response time = 0.5s). Spectral data was collected from 190-275 nm to ascertain information about the α-helix structure of the BSA protein as well as the structure of the BSA conjugates formed.



1. P. De, M. Li, S. R. Gondi and B. S. Sumerlin, *J Am Chem Soc*, 2008, **130**, 11288-+.
2. A. Sunder, R. Hanselmann, H. Frey and R. Mulhaupt, *Macromolecules*, 1999, **32**, 4240-4246.