Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supplementary Information

Electron Beam Lithography of Poly(glycidol) Nanogels for Immobilization of Three-Enzyme Cascade

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1. NMR Inverse-Gated ^{13}C Characterization for Semi-Branched Polyglycidol

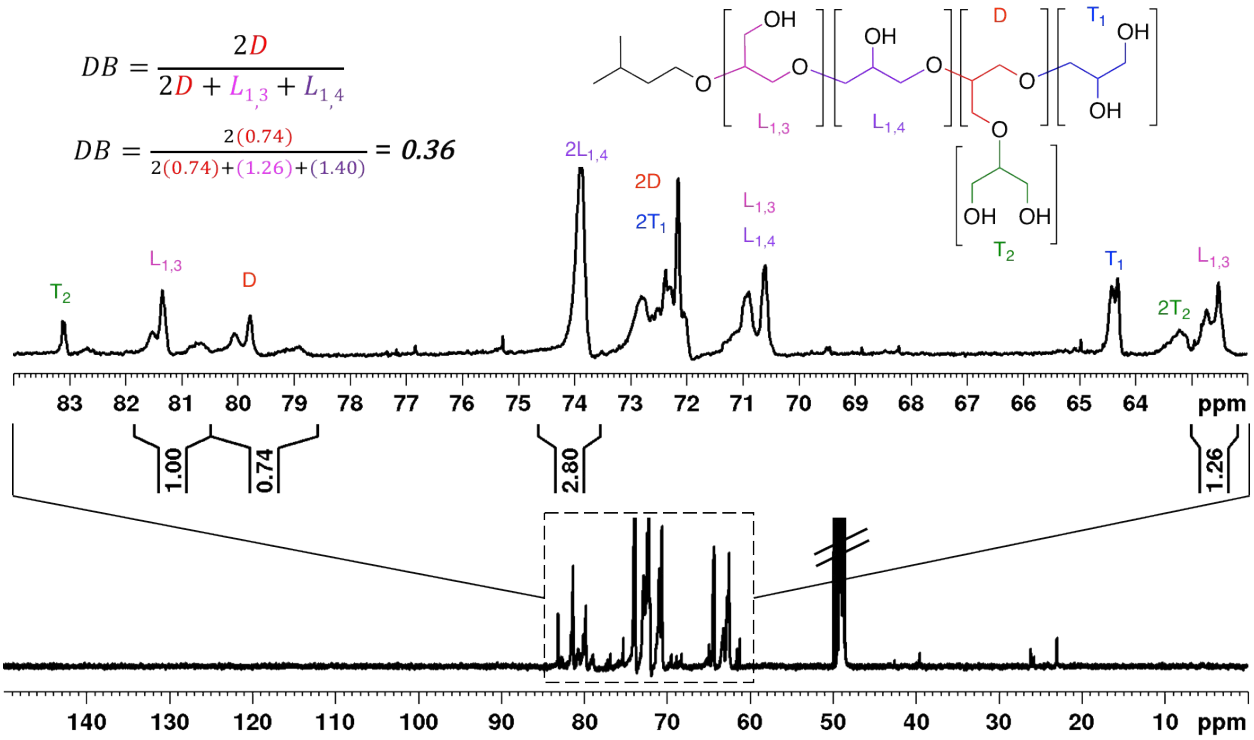

Figure S1: Full labeled inverse-gated ^{13}C-NMR (600 MHz) spectrum of semi-branched polyglycidol homopolymer in deuterated methanol with inset (top) of 62-84 ppm region. Relaxation time (D_1) was 10 sec, and number of scans (NS) was 1024. Degree of branching was calculated as 0.36 (semi-branched) based on relative integration values of dendritic units (D) compared with linear backbone units ($L_{1,3}$, $L_{1,4}$) as described in the literature.[1 2 3 4]

2. Film Thickness Determination from Spin-coat Parameters for Semi-branched Polyglycidol

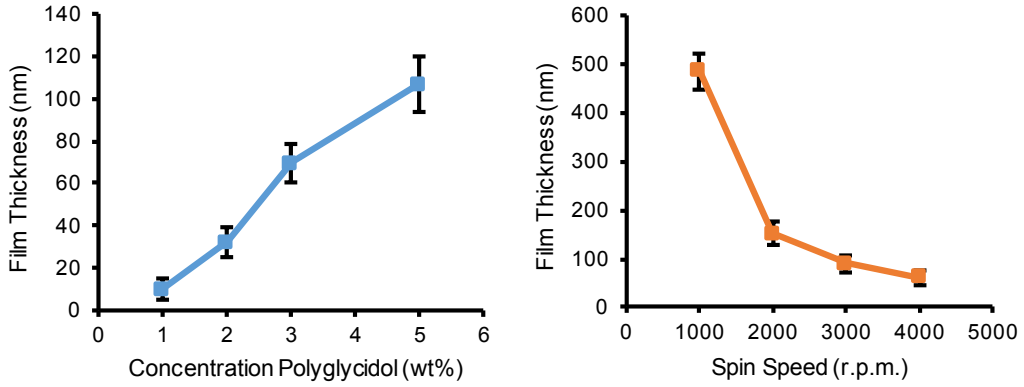

Figure S2: Left plot represents dry film thickness resulting from various concentrations of semi-branched polyglycidol (wt% in DI water) after spin-coating 50 µL on to piranha clean 10 x 10 mm silicon chips at 3000 rpm for 60 s, followed by drying overnight. The bottom plot represents film thickness as a result of spin-coating 50 µL of 5 wt% polyglycidol in DI water onto clean silicon at varying spin speeds (1000-4000 r.p.m. for 60 s) followed by drying overnight. Film thickness was measured by ellipsometry, and all experiments were performed in triplicate. Error bars represent standard deviation (n=3).

3. Dose Test Pattern of Semi-branched Polyglycidol via Electron Beam Lithography

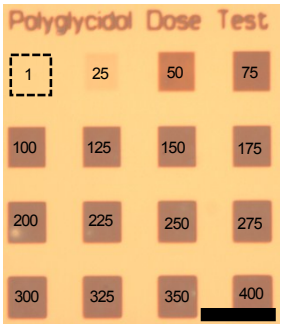

Figure S3. Left: Bright field microscopy image of semi-branched polyglycidol dose test from 1-400 μC/cm^2 captured at 20 x magnification. black bar represents 20 μm.

4. Crosslinked Polyglycidol Thickness (nm) vs. Electron Beam Dose (μC/cm^2)

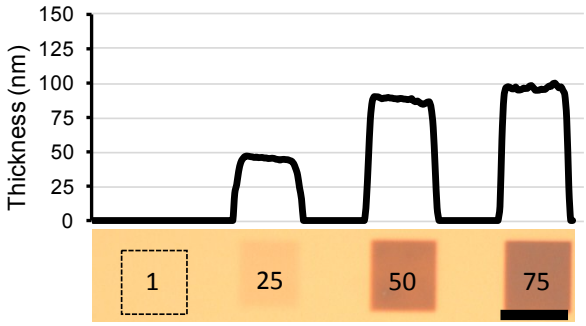

Figure S4. Atomic force microscopy plot (top) which indicates crosslinked polyglycidol film thickness post-development at varying electron beam irradiation doses (μC/cm^2). Corresponding dose squares (bottom) were captured with a bright field microscope equipped with a camera, and the black bar represents 10 μm.

5. Optimization of Enzyme Ratio for Cascade Reaction in Solution

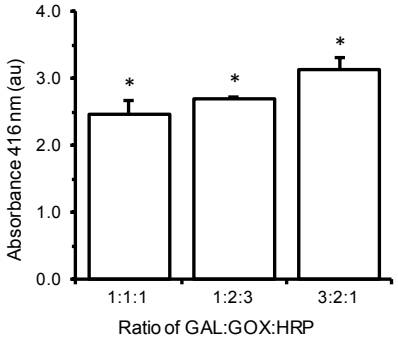

Figure S5. Relative absorbance measurements of 2,3-diaminophenazine at 416 nm after 30 min incubations in three different enzyme ratio concentrations (equivalent to 5 μm) in free solution. Error bars are standard deviation (n=3), and asterisk (*) denotes significance ($p < 0.05$) between all sets as determined via ANOVA single factor analysis.

References

1. Spears, B. R.; Marin, M. A.; Montenegro-Burke, J. R.; Evans, B. C.; McLean, J.; Harth, E., Aqueous Epoxide Ring-Opening Polymerization (AEROP): Green Synthesis of Polyglycidol with Ultralow Branching. *Macromolecules* **2016,** *49* (6), 2022-2027.
2. Spears, B. R.; Waksal, J.; McQuade, C.; Lanier, L.; Harth, E., Controlled branching of polyglycidol and formation of protein-glycidol bioconjugates via a graft-from approach with "PEG-like" arms. *Chemical communications (Cambridge, England)* **2013,** *49* (24), 2394-6.
3. Schull, C.; Gieshoff, T.; Frey, H., One-step synthesis of multi-alkyne functional hyperbranched polyglycerols by copolymerization of glycidyl propargyl ether and glycidol. *Polymer Chemistry* **2013,** *4* (17), 4730-4736.
4. Wilms, D.; Stiriba, S. E.; Frey, H., Hyperbranched Polyglycerols: From the Controlled Synthesis of Biocompatible Polyether Polyols to Multipurpose Applications. *Accounts Chem Res* **2010,** *43* (1), 129-141.