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SUPPORTING INFORMATION

Self-Deoxygenating Glassware

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MATERIALS

Glucose Oxidase from *Aspergillus niger* (GOx, Sigma Aldrich, 170 U/mg) was stored under Ar at -80°C and used as received. (3-Aminopropyl)triethoxysilane (98%, Alfa Aesar), glutaraldehyde (25% Aq. Solution, Ajax Finechem), D-glucose (Merck), L-ascorbic acid (99%, Sigma Aldrich), 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AMPD, 97%, Sigma Aldrich), acetone (A.R., ChemSupply), methanol (MeOH, A.R., ChemSupply), dimethyl sulfoxide (DMSO, A.R., ChemSupply), *N,N*-dimethylformamide (DMF, Emsure, Merck), tetrahydrofuran (THF, HPLC-grade, VWR) and RAFT agent 4-cyano-4-[(dodecylsulfanylthiocarbonyl) sulfanyl]pentanoic acid (TTC-1, Dulux Group Australia Pty. Ltd.) were all used as received. The monomer *N,N*-dimethylacrylamide (DMA, 99%, Sigma Aldrich) was filtered over a column of basic alumina to remove the stabilizer and stored at 4°C prior to use. Deuterium oxide (D_2O, was purchased from Sigma Aldrich and deuterated chloroform ($CDCl_3$, 99.9%) was purchased from Cambridge Isotope Laboratories Inc. All water used was bulk laboratory deionized (DI) water.

CHARACTERIZATION

Nuclear Magnetic Resonance (NMR) Spectroscopy. NMR spectroscopy was conducted on a Varian Unity 400 MHz spectrometer operating at 400 MHz. Deuterated solvent (D_2O or $CDCl_3$) was employed as reference and sample concentrations were approximately 2 mg/mL. Monomer conversion was calculated by comparing the integration of the vinyl peaks of monomer (DMA: δ = 5.5–6.6 ppm) with the broad peak of the polymer backbone protons of p(DMA) (δ = 1.1–1.8 ppm).

UV-Vis Spectroscopy

UV−vis absorbance spectra were obtained using a Shimadzu UV-1800 spectrophotometer and UVProbe software package. Samples were loaded into 1.5 mL "semi-micro" cuvettes at room temperature (23 °C $\pm$ 1.5 °C) and the relevant wavelength range assessed with a slow scan speed and sampling interval of 1 nm.

Gel Permeation Chromatography (GPC). The polymerization samples were analyzed directly by GPC eluting with DMF conducted on a Shimadzu liquid chromatography system equipped with a Shimadzu RID-10 refractometer (λ = 633 nm) and Shimadzu SPD-20A UV-Vis detector using three identical Jordi columns (5 µm bead size, Jordi Gel Fluorinated DVB Mixed Bed) in series operating at 70°C. DMF with 0.05 mol·L^{-1} LiBr (> 99%, Aldrich) was employed as the mobile phase at a flow rate of 1 mL·min^{-1}. The system was calibrated using low dispersity poly(methyl methacrylate) standards as reference. All samples were filtered through 0.45 µm nylon filters prior to injection.

EXPERIMENTAL SECTION

General procedure for preparing glucose oxidase-functionalized glass

All glassware was initially washed thoroughly with hot water, followed by an acetone rinse and dried in an oven at 100 °C. As per Tzanov *et al*.,[1] the glassware was filled with (or soaked in) a solution of (3-Aminopropyl)triethoxysilane) in acetone (4 v/v%) at 45 °C for 24 hours. The glassware was then rinsed thoroughly several times with acetone and left to dry. The amine-functionalized glassware was then exposed to a solution of glutaraldehyde (2 v/v%) in water at room temperature for 2 hours, followed by extensive rinsing with water. The glutaraldehyde glassware was then exposed to a solution of *glucose oxidase* (GOx, 0.05–1.0 mg/mL) dissolved in a 0.15 M NaCl solution for 12–16 hours. The resulting GOx-

functionalized glass was rinsed 3 times with 0.15 M NaCl solution, followed by several rinses with water, and dried with compressed air before storage.

Protein Quantification

The quantity of GOx immobilized onto the surface of glassware was determined via Bradford's assay.[2] This procedure uses the linear absorbance shift at 595 nm of Coomassie Brilliant Blue dye when reacting with proteins in solution. A standard curve was first plotted measuring the absorbance at 595 nm, with samples consisting of 100 μL GOx in 0.15 M NaCl solution (0.01–1.5 mg/mL) diluted with 900 μL Bradford's reagent and left to react for 20 mins. GOx loading was determined by the difference in GOx in the supernatant prior to loading, and after steeping overnight, combined with 3 x NaCl solution washes to remove adsorbed protein. Negligible desorbed active GOx in a 4th wash with NaCl solution was confirmed by addition of D-glucose to the wash solution and assaying for H_2O_2 production after 2 hours. Where necessary, solutions with greater than 0.1 mg/mL GOx were diluted with 0.15 M NaCl solution prior to assaying. GOx loading experiments were conducted in triplicate and are reported as the mean of three independent assays, with the standard error of the mean calculated using the software GraphPad Prism 8. GOx loading in 4 mL glass vials is shown in Figure S1.

Hydrogen peroxide Assay

To quantify the production of H_2O_2 within the GOx glassware and infer O_2 consumption, the spectroscopic method described by Hochanadel was used.[3] In this method, I^- is oxidized to I^{3-} by hydrogen peroxide in a ratio 1:1, under the catalytic activity of ammonium molybdite. In practice, 0.5 ml of each of the two solutions, A (0.4 M KI, 0.1 M NaOH, and 0.02 mM $(NH_4)_6Mo_7O_{24}$) and B (0.1 M $C_8H_5KO_4$) were mixed with 0.5 mL of solution sampled directly from the GOx glassware. The mixture was left to react for 10 mins before being assessed by UV-Vis spectroscopy at 353 nm, with the D-glucose solution used to load the GOx glassware assessed as a baseline for the assay. H_2O_2 concentration was determined by comparison with a standard curve of known concentrations (standard curve H_2O_2: 0.1–2 mM). H_2O_2 assay experiments were conducted in triplicate and are reported as the mean of three independent assays, with the standard error of the mean calculated using the software GraphPad Prism 8 illustrated as error bars.

General Polymerization Procedure

Thermal Initiation

In a typical experiment, a reaction mixture containing 1.03 mL DMA (2.5 M, 200 eq.), 20.2 mg TTC-1 (15 mM, 1 eq.), 72 mg D-glucose (100 mM, 8 eq.) and 2.92 mL PBS buffer (1x, pH 6.0) was prepared and transferred into a 4 mL GOx-functionalized vial. A small magnetic stirrer bar was inserted, and the vial was sealed with a rubber septum and left for an incubation period of approximately 30 mins at room temperature. After incubation, the vial was brought to 45 °C in an oil bath and a 50 μL aliquot of a PBS solution containing AMPD (final AMPD conc. = 2.5 mM, 0.2 eq.) was injected into the reaction mixture to initiate polymerization. For kinetics experiments, samples were taken periodically via a degassed syringe and immediately diluted with deuterated solvent for NMR and GPC analysis. To maintain internal O_2 composition within the vial headspace, backfilling during sampling was done with Ar. For the non-functionalized control reactions, the method was identical except for a 30 min degassing stage in place of incubation, composed of sparging with Ar for 30 minutes.

Ascorbic acid Initiation

In a typical experiment, a reaction mixture containing 1.03 mL DMA (2.5 M, 200 eq.), 20.2 mg TTC-1 (15 mM, 1 eq.), 72 mg D-glucose (100 mM, 8 eq.) and 2.92 mL PBS buffer (1x, pH 6.0) was prepared and transferred into a 4 mL GOx-functionalized vial. A small magnetic stirrer bar was inserted, and the vial was sealed with a rubber septum and left for an incubation period of approximately 30 mins at room temperature. After incubation, a 50 μL aliquot of a PBS solution containing L-ascorbic acid (final L-ascorbic acid conc. = 1.3 mM, 0.1 eq.) was injected into the reaction mixture to begin polymerization, and the vial placed in the dark. For kinetics experiments, samples were taken periodically via a degassed syringe and immediately diluted with deuterated solvent for NMR and GPC analysis. To maintain internal O_2 composition within the vial headspace, backfilling during sampling was done with Ar.

SUPPLEMENTARY RESULTS

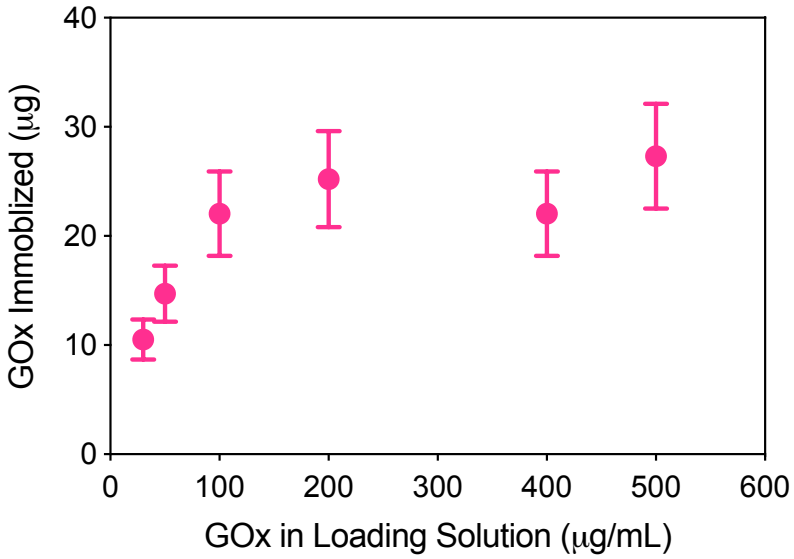

Figure S1: GOx loading in 4 mL (48 mm H x 12.5 D, internal surface area ≈ 14.7 cm^2) glass vials steeped in aqueous solutions of GOx with varied enzyme concentration, as determined by the Bradford assay. GOx attachment appears to plateau with a loading solution of 0.2 mg/mL at around 25 μg, which equates to a GOx surface coverage of 1.7 μg/cm^2.

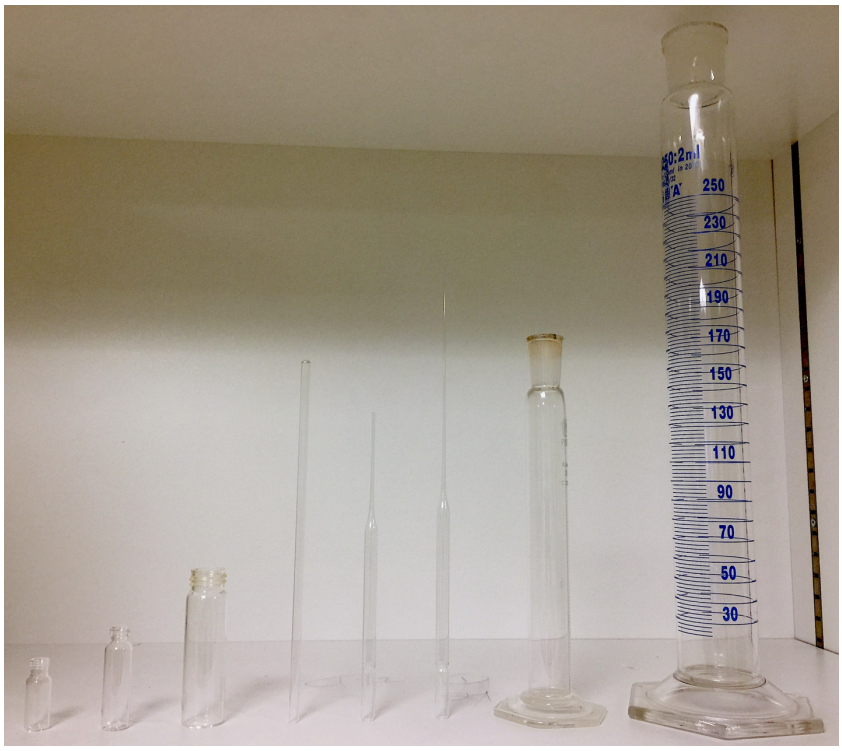

Figure S2: A variety of glassware functionalized with GOx according to the current procedure. (*left to right*) 1.5 mL autosampler vial; 4 mL glass vial (used for illustrative experiments reported in this work); 20 mL vial; standard 5 mm O.D. NMR tube; short

Pasteur pipette; long Pasteur pipette; 25 mL measuring cylinder; 250 mL measuring cylinder. Also functionalized: 0.25 mm diameter glass beads.

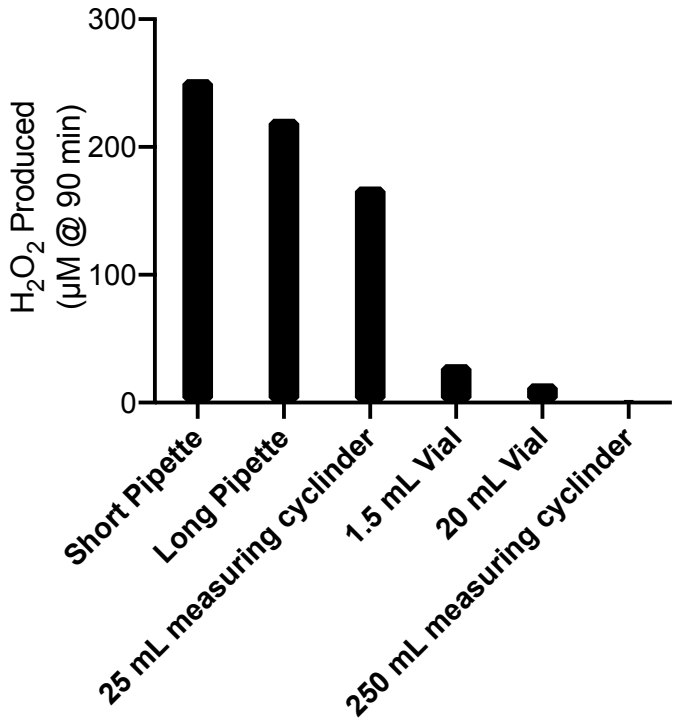

Figure S3: Deoxygenation activity of various GOx-functionalized glassware after 90 minutes incubation time without stirring. While no H_2O_2 was observed in the 250 mL measuring cylinder after 90 mins, over 300 μM H_2O_2 was observed after vigorous stirring for 24 hours.

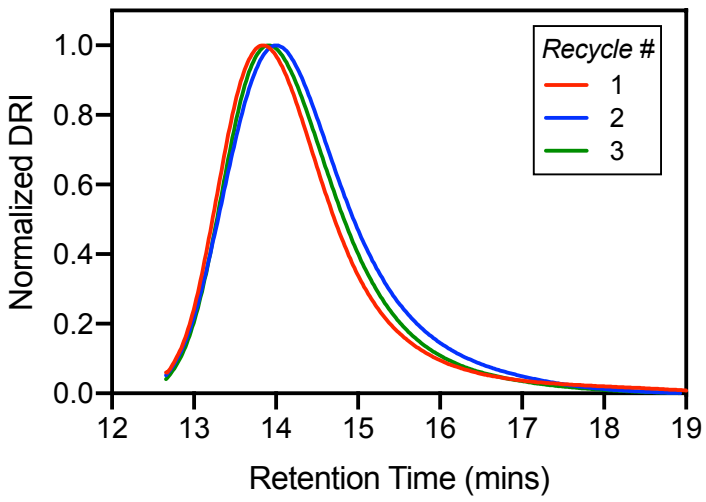

Figure S4: GPC traces for p(DMA) formed in the same GOx vial recycled 3 times, highlighting the similar material achievable with sequential reactions.

Table S1: Polymerization results for reactions within GOx-functionalized 4 mL vials[a]

Initiation	Conditions	Time (h)	Conv. (%)	$Mn_{Theo.}$ (g/mol)[b]	$\ln([M]_0/[M]_t)$	Mn_{GPC} (g/mol)[c]	PDI
Ascorbic Acid	Lid + Stir	2	33	7404	0.400	26100	1.8
		4	43	9526	0.562	24100	1.7
		6	58	12708	0.868	29800	1.63
		8	72	15678	1.273	35400	1.56
		21	99	21405	4.605	45200	1.46
	No Lid + No Stir	2	33	7404	0.400	22500	1.83
		4	51	11223	0.713	30200	1.64
		6	64	13981	1.022	35800	1.57
		8	73	15890	1.309	38900	1.56
		21	94	20345	2.813	45800	1.45
	No Lid + Stir	2	30	6768	0.357	21600	1.69
		4	45	9950	0.598	27500	1.62
		6	58	12708	0.868	-	-
		8	70	15253	1.204	36100	1.52
		21	97	20981	3.507	47000	1.42
Thermal	Lid + Stir	2	1	602	0.010	-	-
		4	12	2783	0.128	-	-
		6	75	15273	1.386	31000	1.4
		7	93	18842	2.659	38100	1.36
		8	97	19635	3.507	39900	1.35
		8[d]	95	19238	2.996	36509	1.39
		8[e]	97	19635	3.507	40014	1.40
	No Lid + No Stir	2	0	404	0.000	-	-
		4	10	2386	0.105	-	-
		6	75	15273	1.386	26500	1.48
		7	86	17454	1.966	31400	1.44
		8	93	18842	2.659	34500	1.41
	No Lid + Stir	2	0	404	0.000	-	-
		4	4	1197	0.041	-	-
		6	29	6153	0.342	12900	2.12
		7	64	13092	1.022	25400	1.58
		8	80	16264	1.609	31000	1.49
	Lid + Stir (Non GOx vial, Ar Degassing)	0.67	15	3378	0.163	-	-
		1	27	5757	0.315	8387	2.30
		1.33	50	10317	0.693	17403	1.56
		1.67	67	13687	1.109	23302	1.45
		2	85	17256	1.897	32407	1.40
		3	97	19635	3.507	36851	1.38

(a) In all experiments solvent is PBS 1x pH 6.0 (~2.97 mL). Target degree of polymerization (DP) is 200 in all experiments, with molar concentration of DMA fixed at 2.5 M and TTC-1 concentration fixed at 12.5 mM. **(b)** The difference between theoretical and observed MWs is ascribed to the different column behaviour of the poly(DMA) and the poly(MMA) standards **(c)** Theoretical molecular weights are calculated from ^{1}H NMR spectra as per the procedure described above and using the formula: $Mn_{Theo} = [monomer]_0/[TTC]_0 \times conversion \times (Mn)_{monomer}/100 + (Mn)_{TTC}$. **(d)** Recycled vial, second use. **(e)** Recycled vial, third use.

Supplementary References

1. Tzanov, T.; Costa, S. A.; Gübitz, G. M.; Cavaco-Paulo, A., Hydrogen peroxide generation with immobilized glucose oxidase for textile bleaching. *J. Biotechnol.* **2002,** *93* (1), 87-94.
2. Bradford, M. M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976,** *72* (1), 248-254.
3. Hochanadel, C. J., Effects of Cobalt γ-Radiation on Water and Aqueous Solutions. *The Journal of Physical Chemistry* **1952,** *56* (5), 587-594.