Electronic Supplementary Information

Selective Nitrosation of Modified Dextran Polymers†

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S1a. ^{1}H NMR characterization

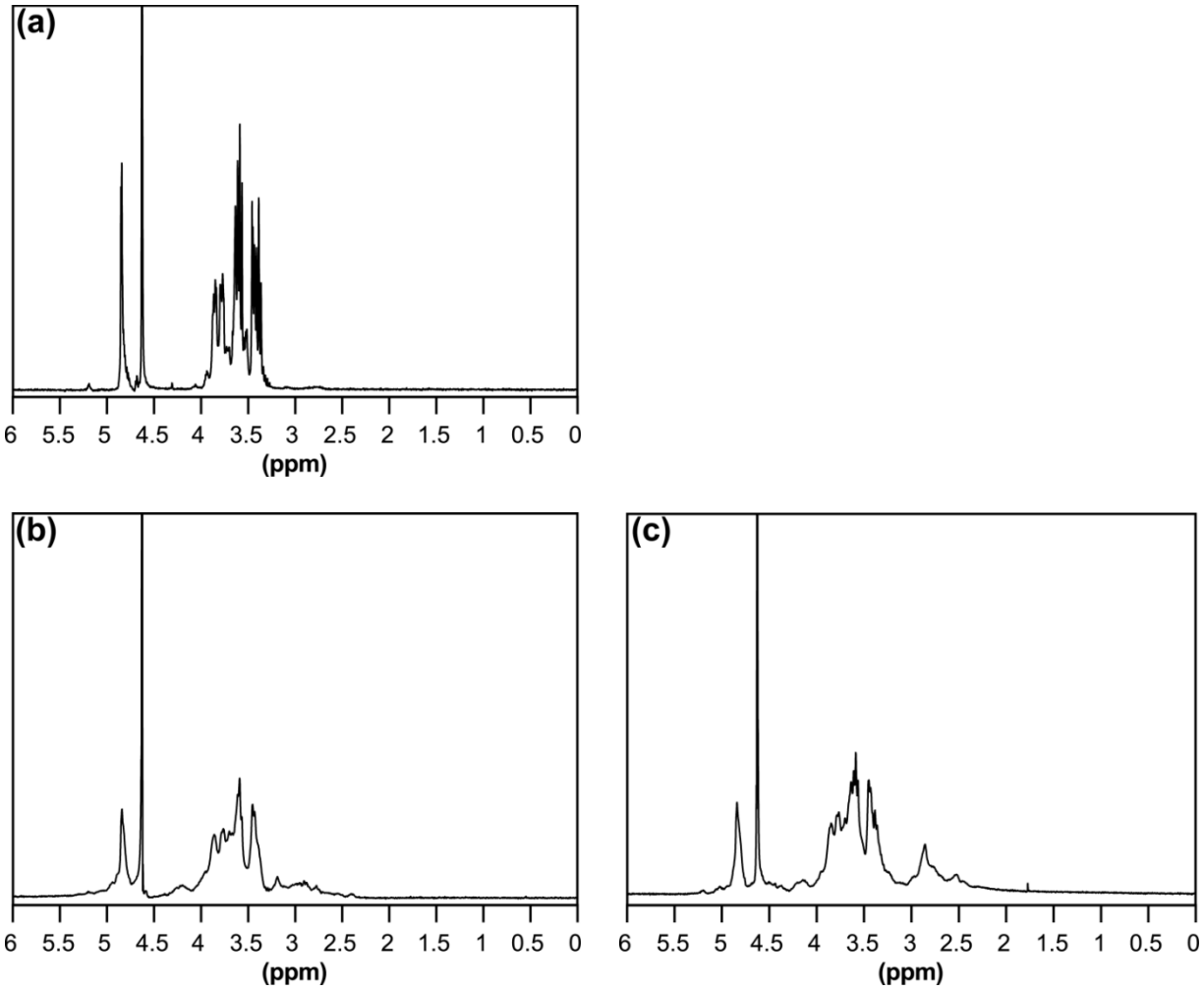

Figure S1. ^{1}H NMR spectra of (a) dextran, (b) dextran-cysteine and (c) dextran-cysteamine prepared via reductive amination.

S1b. IR characterization

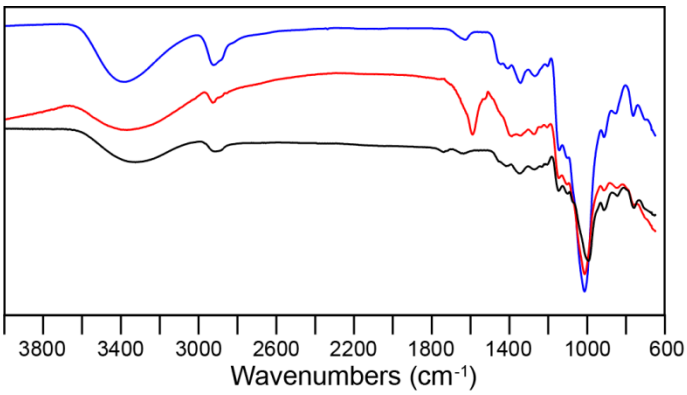

Figure S2. FTIR-ATR spectra of dextran (black), dextran-cysteine (red) and dextran-cysteamine (blue) prepared via reductive amination.

S2. *Spectroscopic characterization of nitrosated dextran derivatives prepared via reductive amination (**1a**, **1b**)*

S2a. UV-vis analysis of nitrosated cysteine derivative (1b) before and after NO release

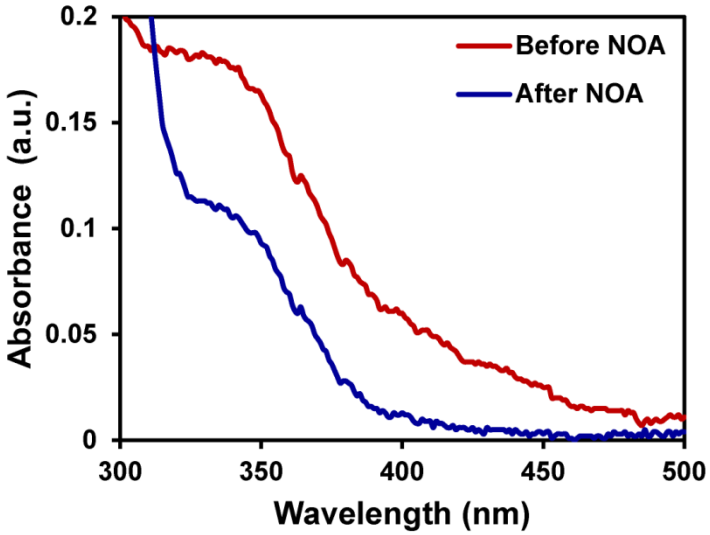

Figure S3. UV-vis spectra of nitrosated dextran-cysteine (**1b**) prepared via reductive amination in PBS (0.75 mg mL^{-1}) before and after NO analysis.

S2b. IR analysis of nitrosated cysteamine derivative (1a) before and after nitrosation

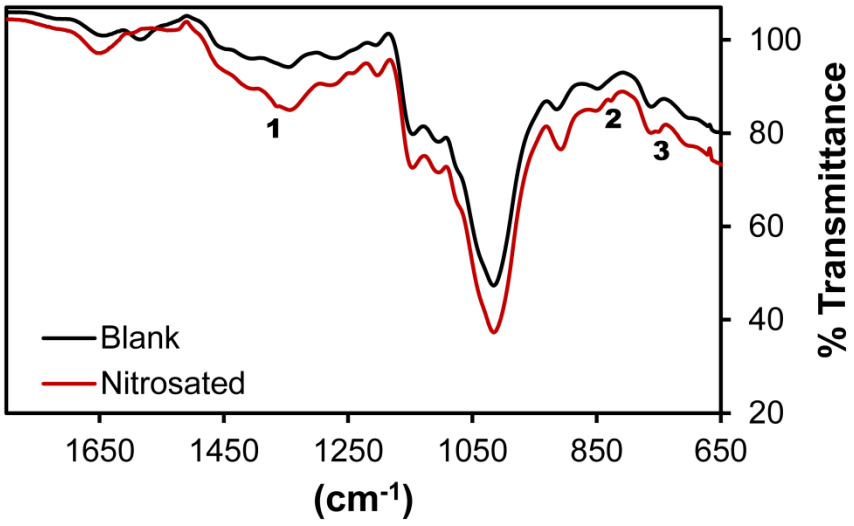

Figure S4. Full ATR-IR spectra of dextran-cysteamine (**1a**) prepared via reductive amination before and after nitrosation.

S2c. IR analysis of nitrosated cysteine derivative (1b) before and after nitrosation

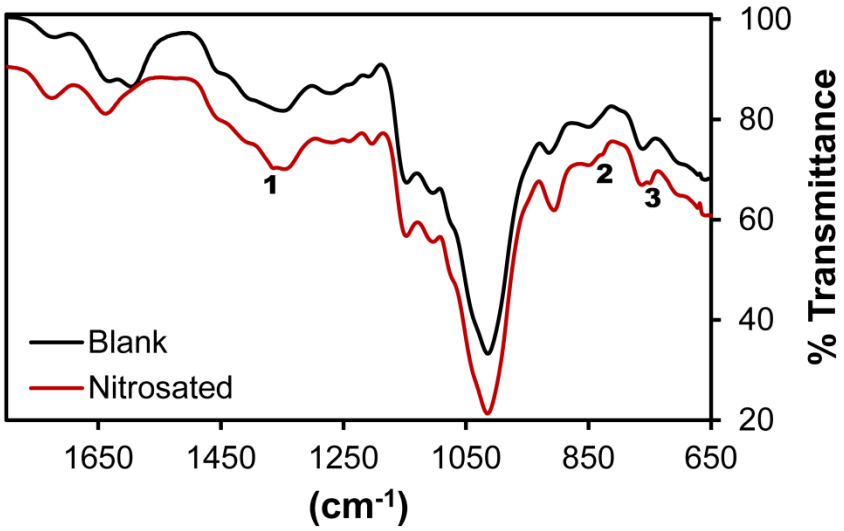

Figure S5. Full ATR-IR spectra of dextran-cysteine (**1b**) prepared via reductive amination before and after nitrosation.

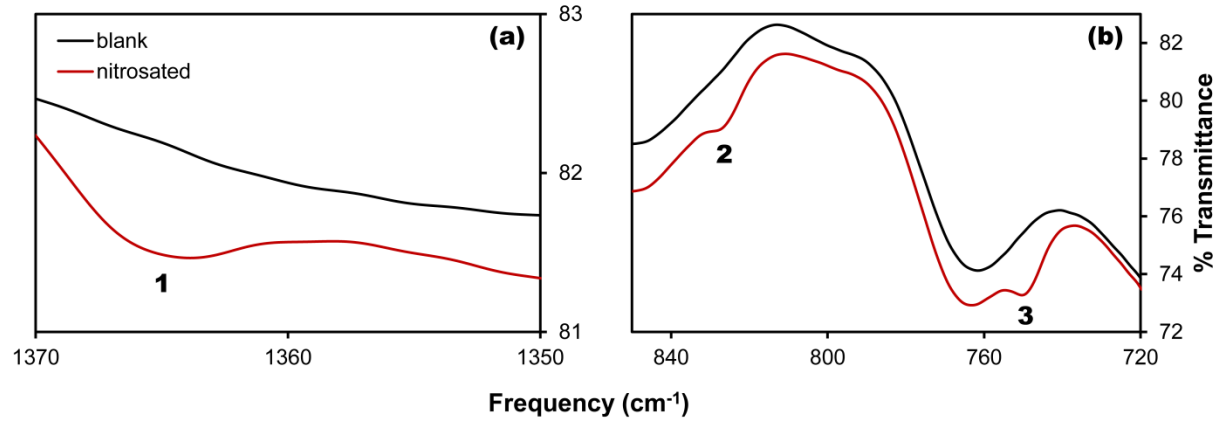

Figure S6. Highlighted IR regions for dextran-cysteine (**1b**) prepared via reductive amination before and after nitrosation where **(a)** the 1370-1350 cm^{-1} region indicates an absorbance feature **(1)** corresponding to a N=O stretch and **(b)** the 850-720 cm^{-1} region indicates absorbance features **(2)** corresponding to a N-N stretch and **(3)** corresponding to a S-N stretch.

S3. *NO release profiles for nitrosated dextran derivatives prepared via reductive amination (**1a**, **1b**)*

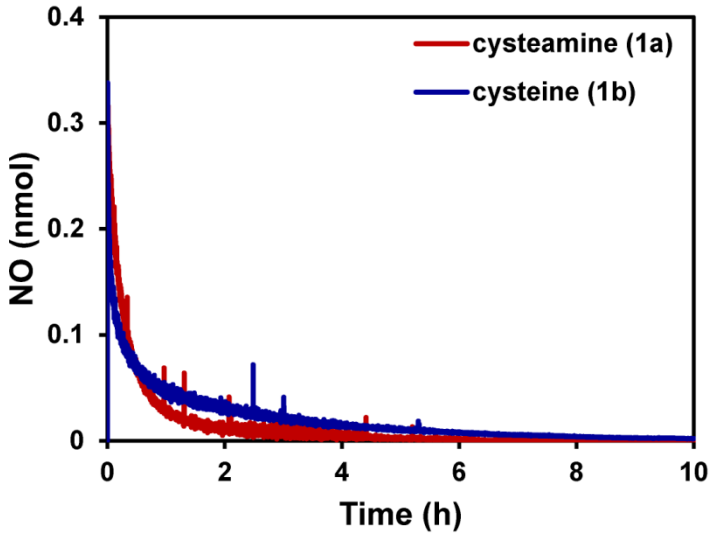

Figure S7. Real-time NO release profiles associated with solutions (0.75 mg mL^{-1} in PBS, room temperature) of the cysteamine (**1a**) and cysteine (**1b**) dextran derivatives prepared via reductive amination.

S4. *Control experiments for potential interferences in the UV-vis (~350 nm)*

The 350 nm peak that remains (**Figures 2, S3**) after NO analysis of the nitrosated dextran derivatives (**1a**, **1b**) prepared via reductive amination when no more detectable NO release occurs could be due to a few different species.

S4a. Nitrosated dextran blank

To account for any detectable residual *t*-butyl nitrite reagent in the system as well as any potential *C*- or *O*- nitrosation of the dextran backbone, a non-thiolated dextran blank was subjected to the same nitrosation procedure as all the other thiolated dextran derivatives. The dextran product was isolated from the methanol/*t*-butyl nitrite under vacuum and analyzed via solution-phase UV-visible spectroscopy (0.75 mg mL^{-1} in PBS). The dextran product recovered after exposure to *t*-butyl nitrite exhibited no features in the 300-500 nm range (**Figure S8a**) and did not release any detectable NO. Different *O*-nitroso groups, such as those due to nitrosation of alcohols and carbohydrates, have been demonstrated to result in an absorbance in the region of 300-400 nm.[1] The lack of an absorbance feature demonstrates that no detectable nitrosation occurs due to reaction of *t*-butyl nitrite with the dextran backbone, which in turn does not contribute to the recovered NO. Additionally, the lack of any absorbance features after dextran exposure to *t*-butyl nitrite indicates that no detectable residual nitrosating agent is trapped in the isolated dextran product. The presence of detectable levels of *t*-butyl nitrite would result in a multiplet absorbance feature, as shown in **Figure S8b**.

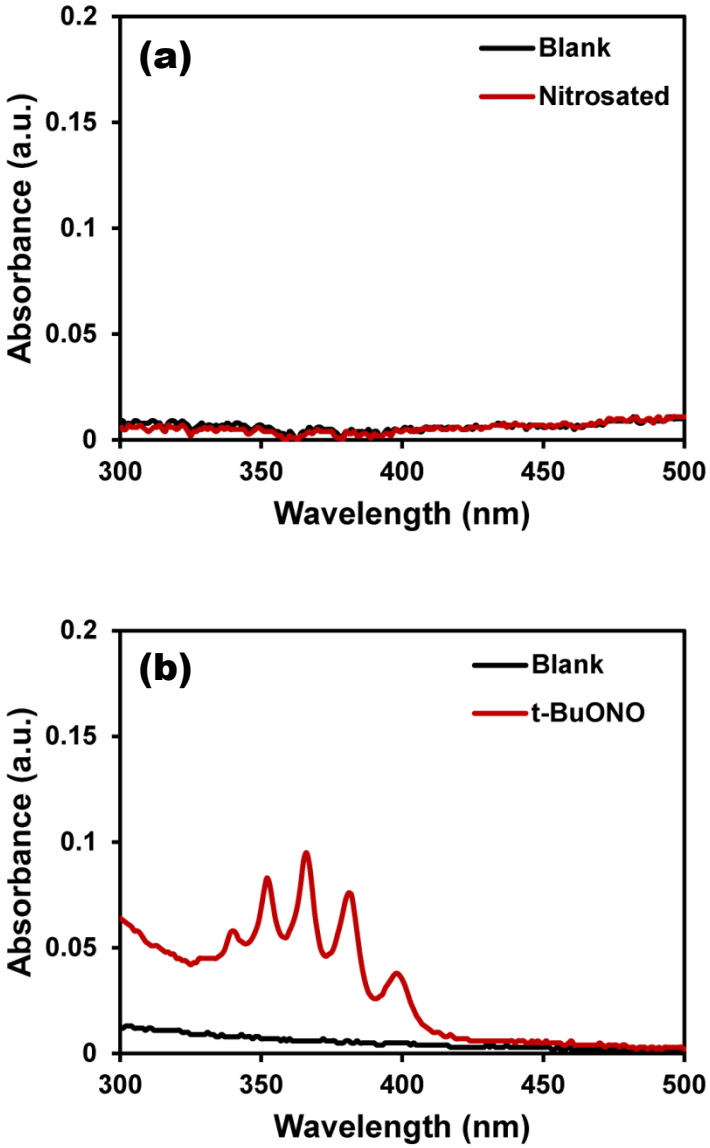

Figure S8. (a) 0.75 mg mL^{-1} isolated dextran in PBS with and without exposure to *t*-butyl nitrite nitrosating conditions and **(b)** 0.75 mg mL^{-1} dextran in PBS with and without the addition of 3 v/v% *t*-butyl nitrite.

S4b. Nitrite interference

During the preparation of the nitrosated dextran solution in PBS, the cysteine (**1a**) and cysteamine (**1b**) derivatives were sonicated 15-20 minutes to allow the dextran to solubilize. Any NO released during solution preparation occurred under oxygenated conditions, which would have resulted in the formation of nitrite. Nitrite has an absorbance maximum at 354 nm which could serve as an interfering feature at high enough nitrite concentrations. The molar extinction coefficient for nitrite (ε_{max}) was determined to be 22.8 M^{-1} cm^{-1} from the slope of the Beer's law plot shown in **Figure S9**. The Griess assay was performed on the resulting polymer solutions after NO analysis to determine if the concentration of nitrite was high enough to allow for interference around 350 nm. Indeed, the nitrite concentrations were determined to be on the order of 5-10 μM, which would result in <0.0005 absorbance contribution, which is below the sensitivity of the spectrometer measurements. Therefore, the amount of nitrite formed during solution preparation is not significant enough to contribute to any absorbance at 350 nm.

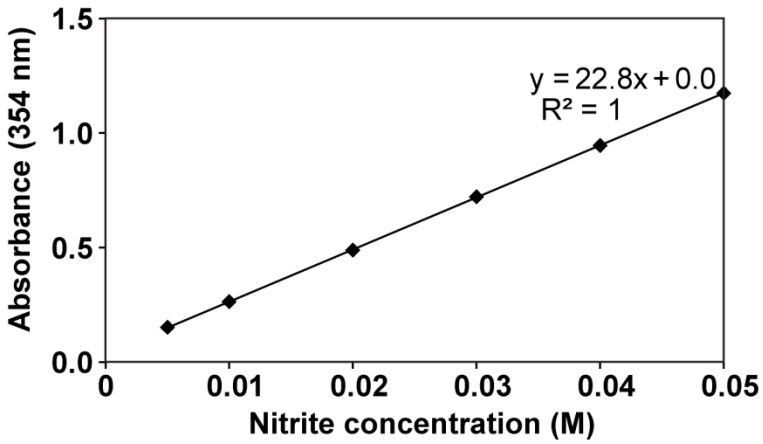

Figure S9. The Beer's law plot associated with nitrite in solution where the slope indicates a molar extinction coefficient of 22.8 M^{-1} cm^{-1} associated with the 354 nm absorbance.

S4c. Baseline shift for thiolated dextran derivatives (1a, 1b) after NO analysis

It is possible that the polymer behavior in soaking solution during the duration of the analysis could lead to a shift in the UV-vis baseline. To account for this, the non-nitrosated thiolated dextran derivatives (**1a**, **1b**) were analyzed in solution (0.75 mg mL^{-1} in PBS) via UV-vis and NOA on the same timescales as reported for the nitrosated derivatives, where no significant shift was seen in the baselines (**Figure S10**). Since dextran degradation is not expected under the analysis conditions employed, we would not expect a shift in the baseline.

Overall, the persistent absorbance feature at ~350 nm exhibited by the nitrosated derivatives **1a** and **1b** after NO analysis is not due to nitrite formation, a shift in polymer baseline, or any features that could occur due to nitrosation of the polymer backbone or residual nitrosating agent.

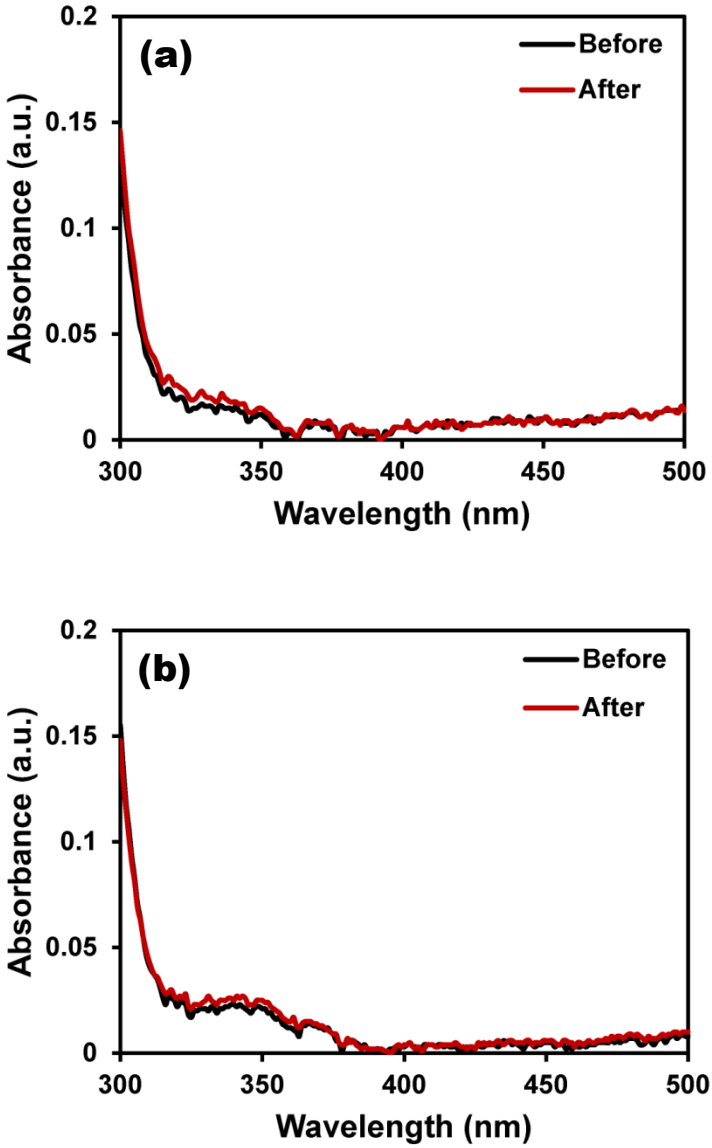

Figure S10. Representative UV-vis spectra for the **(a)** cysteamine and **(b)** cysteine dextran derivatives prepared via reductive amination before and after NO analysis of each derivative in solution (0.75 mg mL^{-1} in PBS).

S5. *N-nitrosoproline control*

To consider the formation of the *N*-nitrosamine moiety during nitrosation of dextran derivatives (**1a**, **1b**) prepared via reductive amination with *t*-butyl nitrite, a model amine-containing substrate, proline, was nitrosated. *N*-nitrosoproline serves as a good model *N*-nitroso compound because, compared to other carcinogenic *N*-nitrosamines, it has demonstrated no carcinogenic acitivity.[2, 3] Its use in the laboratory is therefore safe as toxic products are not unnecessarily formed. The resulting *N*-nitrosoproline was prepared at 5 mg mL^{-1} in a 0.75 mg mL^{-1} dextran solution, which yielded the spectrum shown in **Figure S11**. The absorbance at 345 nm due to the *N*-nitroso group matches the absorbance location of the nitrosated dextran derivatives **1a** and **1b** after NO analysis. Of further note is that the *N*-nitrosoproline solution did not release any detectable NO for up to 5 h, after which time there was no significant change in the absorbance spectrum. This indicates that *N*-nitrosamine formation is feasible under *t*-butyl nitrite nitrosation conditions in methanol, resulting in RNNO formation. Further, this suggests that the RNNO moiety is stable under the conditions reported herein, so the RNNO is not a significant source of NO under these conditions. Due to this stability factor, any remaining RNNO species on the polymer backbone could be available after biodegradation of the material. Due to the toxic and carcinogenic nature of *N*-nitrosamines in general, this is undesirable.

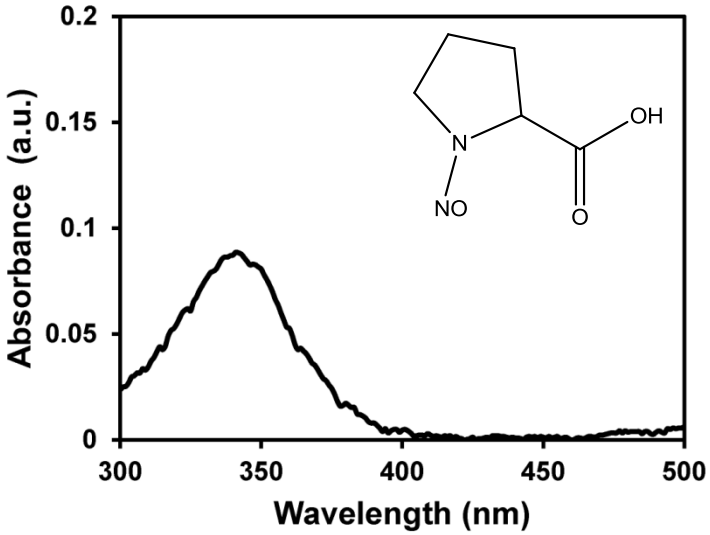

Figure S11. Representative absorbance spectrum for *N*-nitrosoproline (structure shown) in 0.75 mg mL^{-1} dextran solution.

S6. *Remaining RSNO control*

To ensure that the remaining absorbance feature after NO analysis of the nitrosated dextran derivatives prepared via reductive amination was not due to remaining RSNOs in the system, the recovered dextran solution for the cysteamine (**1a**) derivative was exposed to intense UV light (Blak-Ray B-100AP High Intensity UV lamp; 100 Watt, 365 nm) for 30 min. **Figure S12** demonstrates no significant decrease in the ~350 nm feature, indicating that the feature is not due to residual RSNOs. If RSNOs were present in the system, near complete decomposition of the RSNOs and corresponding UV-vis feature would be expected. **Figure S13** shows the decrease in absorbance (335 nm) for a representative small molecule RSNO, *S*-nitrosoglutathione, in PBS which experiences 100% decomposition after 30 min UV exposure.

S6a. UV exposure of dextran cysteamine (1a) derivative

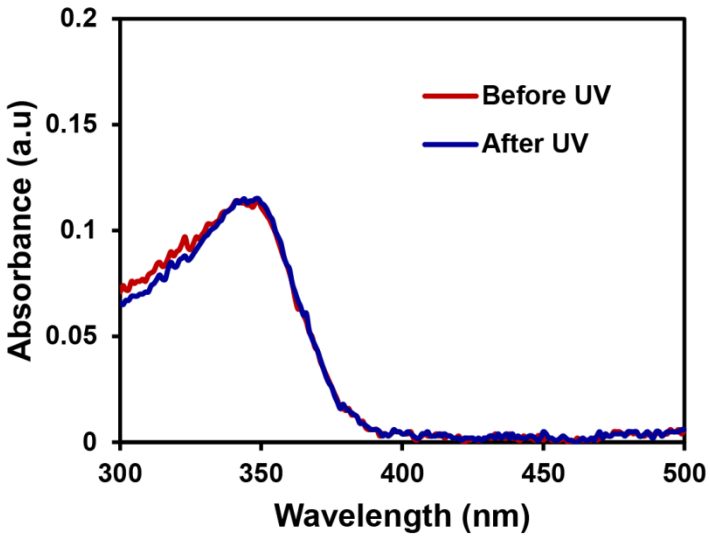

Figure S12. Representative absorbance spectra for dextran cysteamine (**1a**) derivative after NO analysis before and after exposure to intense UV light.

S6b. UV exposure of *S*-nitrosoglutathione

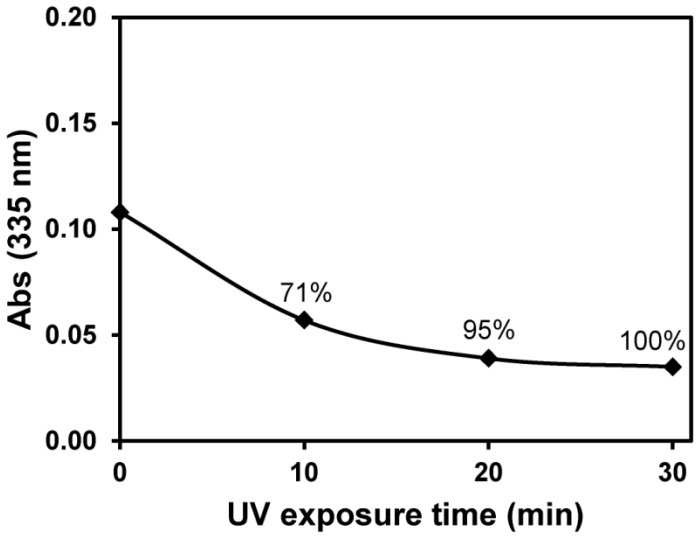

Figure S13. Upon exposure of a 0.1 mM *S*-nitrosoglutathione solution to intense UV light, the RSNO experiences 71% decomposition at 10 min, 95% decomposition at 20 min, and 100% decomposition at 30 min exposure.

S7. *Spectroscopic characterization of nitrosated cysteine dextran derivative (**2b**) prepared via carboxymethyl intermediate approach*

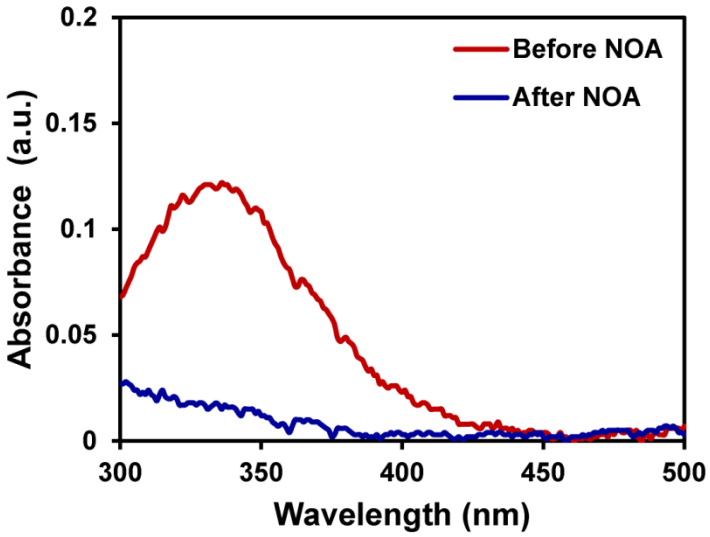

Figure S14. UV-vis spectra of nitrosated dextran-cysteine (**2b**) prepared via carboxymethyl intermediate approach in PBS (0.75 mg mL^{-1}) before and after NO analysis.

S8. *Molar extinction coefficient determination for nitrosated dextran derivatives (**2a**, **2b**) prepared via carboxymethyl intermediate approach*

Solutions of each nitrosated dextran derivative were prepared in PBS via serial dilution of a 1.0 mg mL^{-1} stock solution to yield 0.05, 0.25, 0.50, 0.75 and 1.0 mg polymer mL^{-1}. The amount of NO recovered during the NOA analysis period was normalized by the mass of polymer present in the 0.75 mg mL^{-1} solution. This value was further corrected to account for the fact that the RSNO moiety experienced around 90% decomposition (based upon UV-vis analysis) to yield a value that allowed for the conversion of the mg mL^{-1} polymer concentrations to mol RSNO L^{-1} (assuming a 1 NO: 1 RSNO molar ratio). **Figure S15** shows the representative Beer's law plots associated with the cysteamine **(2a**) and cysteine (**2b**) dextran derivatives. The slope of each plot corresponds to the molar extinction coefficient (M^{-1} cm^{-1}) of each nitrosated material, where ε_{max} corresponding to 335 nm was determined to be 1115±42 M^{-1} cm^{-1} for the cysteamine derivative (**2a**) and 869±12 M^{-1} cm^{-1} for the cysteine derivative **(2b**).

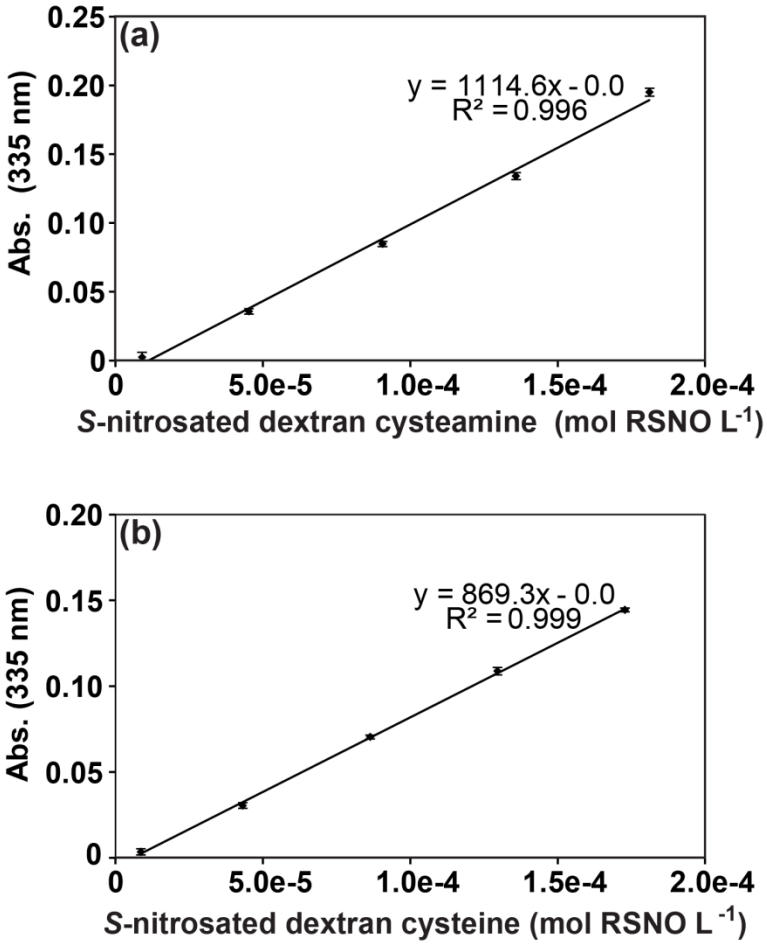

Figure S15. Beer's law plots associated (a) cysteamine and (b) cysteine derivatives prepared via carboxymethyl intermediate approach in PBS where the slope of each plot indicates molar extinction coefficients of 1115 M^{-1} cm^{-1} and 869 M^{-1} cm^{-1}, respectively, associated with the 335 nm absorbance.

S9. *References*

1. S. E. Aldred, D. L. H. Williams and M. Garley, *J. Chem. Soc., Perkin Trans. 2*, 1982, 777-782.
2. H. T. Nagasawa, P. S. Fraser and D. L. Yuzon, *J. Med. Chem.*, 1973, **16**, 583-585.
3. J. E. Nixon, J. H. Wales, R. A. Scanlan, D. D. Bills and R. O. Sinnhuber, *Food Cosmet. Toxicol.*, 1976, **14**, 133-135.