Supporting Information for

Design Considerations for Silica Particle-Doped Nitric Oxide-Releasing Polyurethane Glucose Biosensor Membranes

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Synthesis of *N*-diazeniumdiolate-modified silica nanoparticles

Secondary amine-modified nanoparticles were first synthesized by variants of the Stöber method. Ultimately, secondary amines introduced to the SNPs were converted to *N*-diazeniumdiolate NO donors by reaction with NO gas. Mesoporous silica nanoparticles of different sizes (~150, 450, and 800 nm) were synthesized via a previously published aminosilane-surfactant ion exchange approach.[1] Bare mesoporous silica particles were synthesized by addition of TEOS to a stirred solution of water, ethanol, ammonia, and CTAB (the template surfactant). After forming the SNPs (2 h after TEOS addition), the particles were reacted overnight (~16 h) with secondary amine-containing organosilanes (either MAP, AHAP, or DET) by dropwise addition of the silane to the TEOS precursor solution. Reactant concentrations and chemical structures of the aminosilanes are provided in Figure S1 and Table S1. Particles were recovered by centrifugation and purified as described previously.[1]

Bare (i.e., not functionalized with secondary amines) 150 nm mesoporous silica particles were synthesized according to the procedure above but were isolated/purified without further aminosilane ion exchange reaction. Nonporous ~150 nm silica particles were synthesized by adding 3.795 mL TEOS to a solution of EtOH (88.4 mL), water (1.10 mL), and aqueous NH_4OH (6.8 mL). The solution was stirred for 2 h to allow for particle nucleation and growth. The particle sol was centrifuged (6540*g*, 10 min, 4 ^{o}C) and the resulting pellet washed thrice with ethanol to remove residual ammonia/TEOS. The bare silica particles were recovered by drying the particle pellet under vacuum. For both bare particle systems (i.e., 150 nm mesoporous and nonporous particles), amine modification was carried out under identical conditions using a surface grafting approach. The bare SiO_2 particles (~200 mg) were exposed to ozone in a BioForce Tip Cleaner (Ames, IA) for 30 min to generate additional, modifiable surface silanols. Subsequently, the particles (100 mg) were reacted with DET3 (207 µL) and trimethylamine (50 µL) for 14 h at 90 ^{o}C in toluene. The reaction was carried out under nitrogen atmosphere and with an attached reflux condenser. Similarly to the procedures above, the particles were isolated via centrifugation, washed with EtOH to remove solvent and unreacted reagents, and dried under vacuum to yield the amine-based particles.

After particle functionalization, *N*-diazeniumdiolates were formed on the secondary amines by reaction with NO gas. The particles were dispersed via sonication at 5 mg mL^{-1} in a 9:1 mixture of DMF:MeOH and 25 µL 5.4 M methanolic NaOMe added to catalyze *N*-diazeniumdiolate formation. Glass vials containing the particle

dispersions were transferred to a stainless steel Parr bottle and connected to an in-house NO reactor. The Parr bottle was flushed with 8 bar Ar gas six times (three brief, three 10 min) to remove atmospheric oxygen and minimize formation of NO byproducts. The reaction vessel was then pressurized with 10 bar 99.99% NO gas (purified over solid potassium hydroxide for at least 4 h) and the particle solutions stirred for 3 d. Subsequently, the NO gas was vented from the Parr bottle and the vessel was again flushed with Ar gas (6 ×). The *N*-diazeniumdiolate-modified particles were collected via centrifugation, washed three times with EtOH, and dried under vacuum. The resulting NO-releasing particles were stored in a vacuum-sealed Mylar bag at -20 ^{o}C.

Synthesis of *S*-nitrosothiol-modified silica nanoparticles

Thiol-modified SNPs were synthesized by co-condensation of MPTMS with either TEOS or TMOS (tetraalkoxy backbone silanes).[2] The particles were formed by dropwise addition (0.5 mL min^{-1} using a syringe pump) of a silane mixture (MPTMS and the backbone silane) to stirred solutions of water, ethanol, and ammonia and allowed to react for 2 h. The MPTMS (i.e., alkanethiol) content of the product SNPs was controlled by altering the molar ratio of MPTMS to TEOS or TMOS in the silane precursor solution. Reactant and solvent amounts for each particle type are provided in the Supporting Information (Table S2). After particle formation, the particles were collected by centrifugation (6540*g*, 10 min, 4 ^{o}C), washed thrice with EtOH, and dried in vacuo.

S-nitrosothiol (RSNO) formation was carried out under identical conditions for all thiol-modified particle systems. Initially, 200 mg MPTMS-based particles were suspended in 4.00 mL MeOH via sonication and stirred. The mixture was acidified with the addition of excess HCl (2.00 mL 5 M HCl) and cooled on ice. Sodium nitrite (2.3 M in water; 2.00 mL) was added dropwise to the MPTMS particle solution and stirred on ice for 1 h in the dark. Of note, the sodium nitrite solution was supplemented with 500 μM diethylenetriaminepentaacetic acid (DTPA) to chelate trace metal ions in solution and prevent copper-mediated RSNO decomposition during the *S*-nitrosation reaction. The resulting pink RSNO-modified particles were collected by centrifugation (6540*g*, 10 min, 4 ^{o}C), washed thrice with cold MeOH, and dried under vacuum for 1 h. All experiments involving RSNO particles were carried out immediately following the *S*-nitrosation reaction.

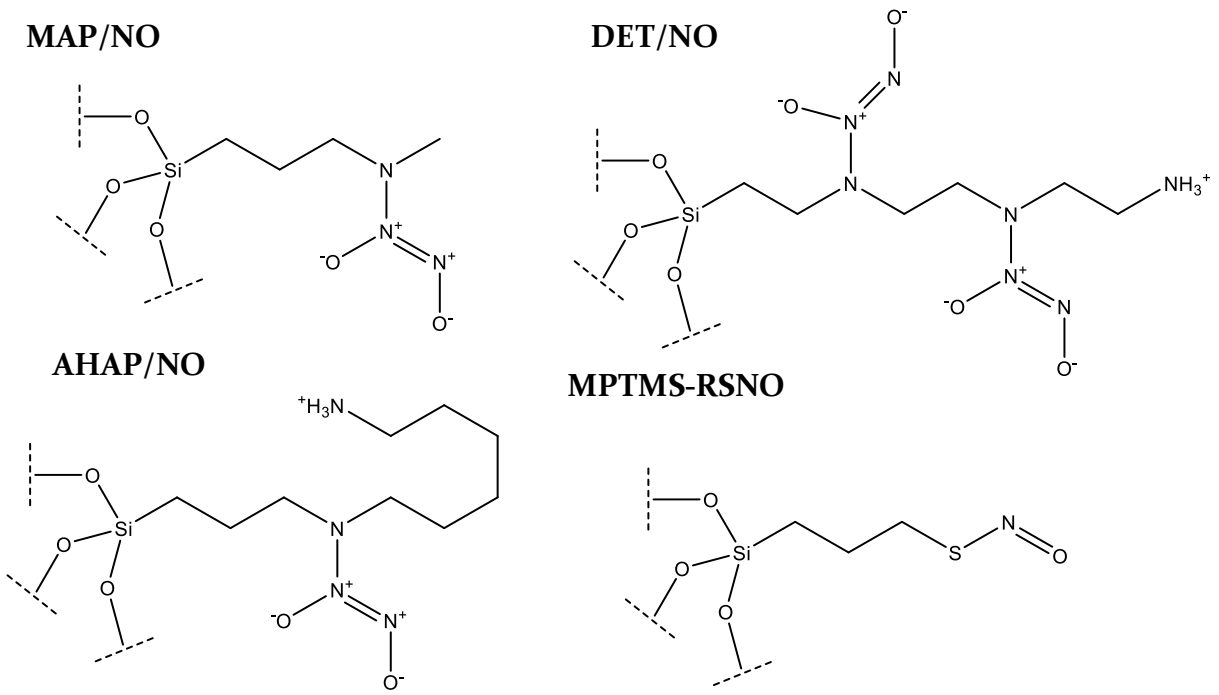

Figure S1. Proposed chemical structures of selected silanes modified with either *N*-diazeniumdiolate (MAP, AHAP, DET) or *S*-nitrosothiol (MPTMS) NO donors.

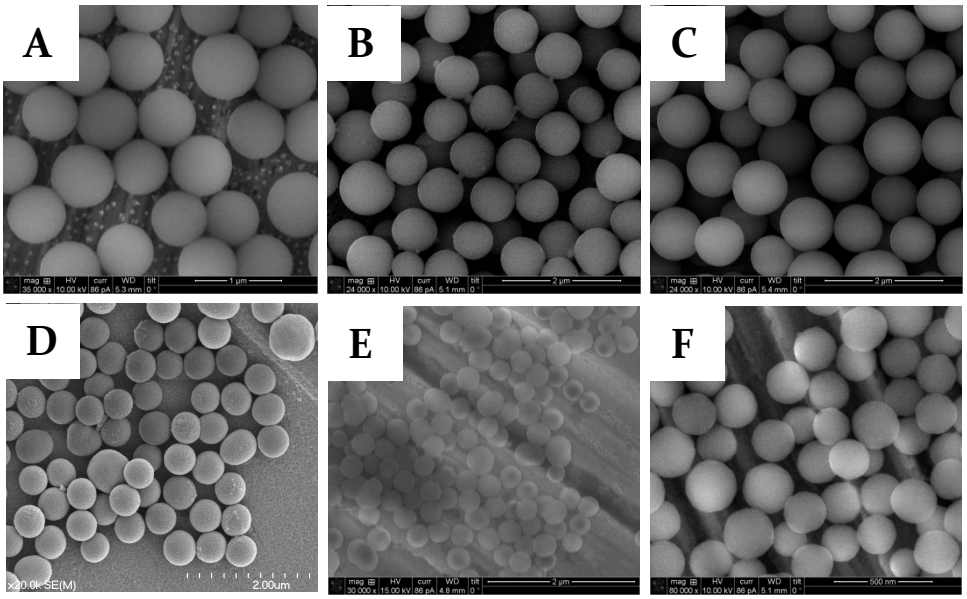

Figure S2. Scanning electron micrographs of 800 nm (A) DET, (B) AHAP, and (C) MAP mesoporous SNPs. (D) and (E) are SEM micrographs for smaller DET-modified mesoporous particles (450 and 150 nm, respectively), and (F) are non-porous DET-modified SNPs.

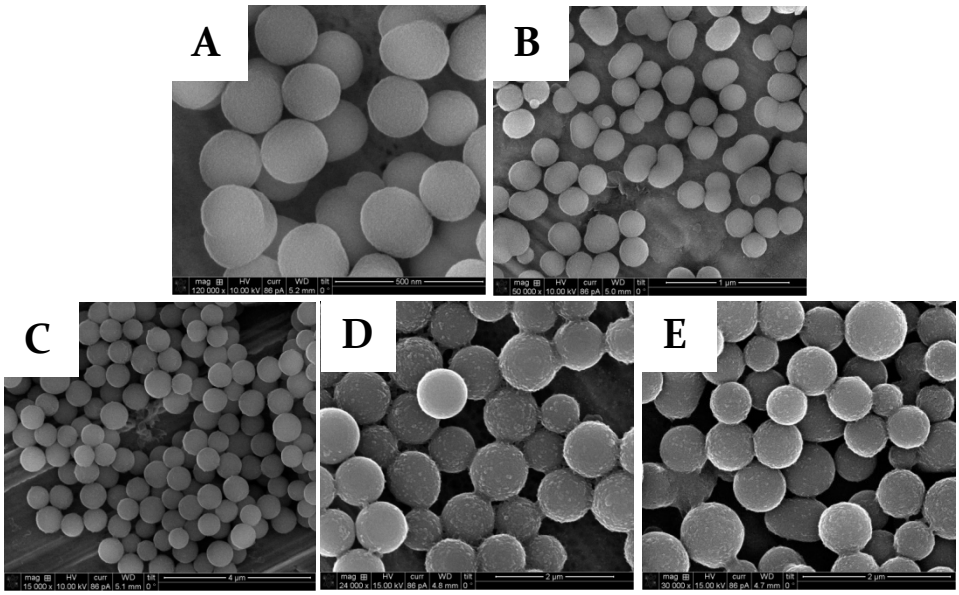

Figure S3. Scanning electron micrographs of (A) 25% MPTMS/TMOS, (B) 40% MPTMS/TMOS, (C) 75% MPTMS/TEOS, (D) 85% MPTMS/TEOS, and (E) 85% MPTMS/TMOS particles.

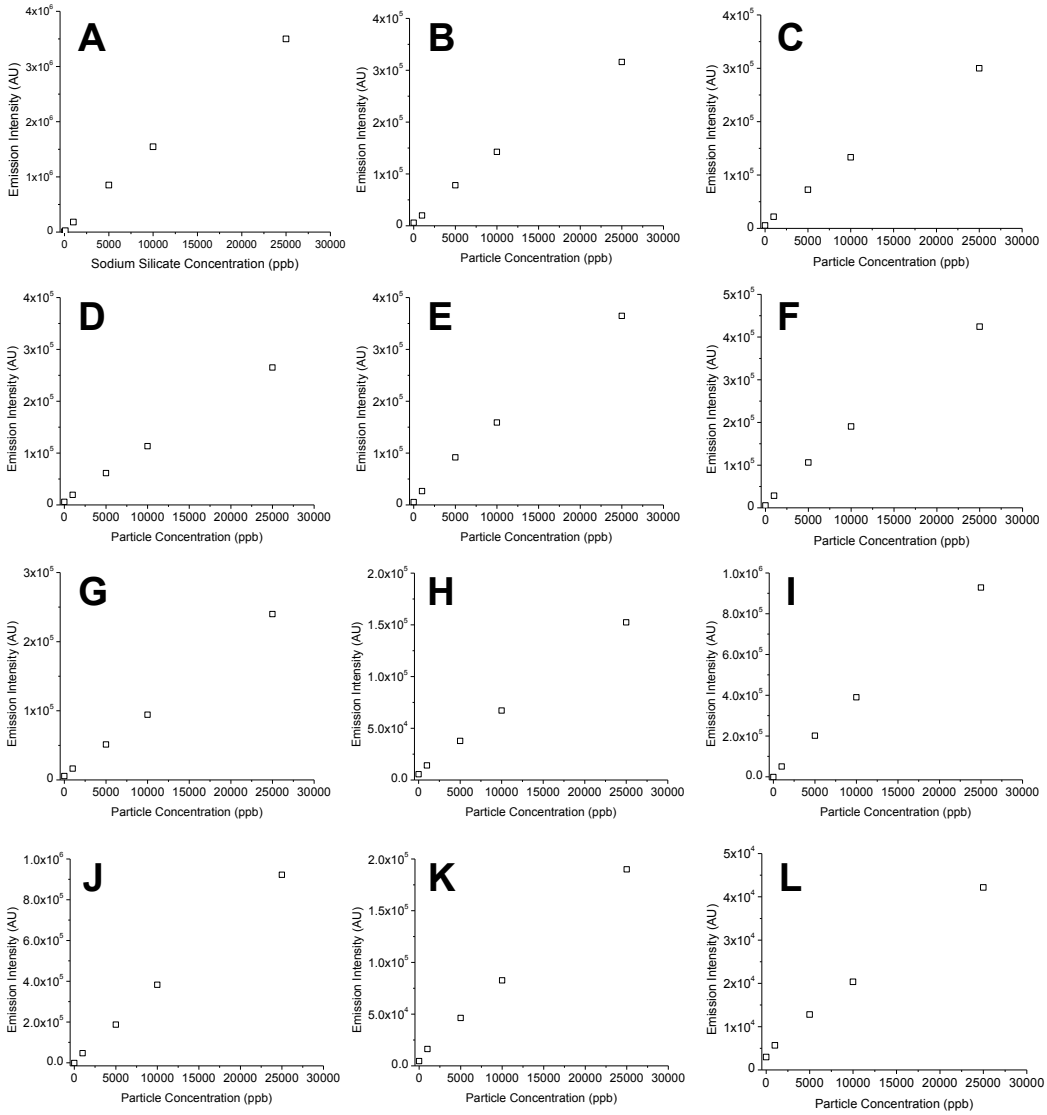

Figure S4. Inductively coupled plasma optical emission spectrometer instrumental response to sodium silicate standard solution (A) and silica particle standard solutions (B–L) prepared in pH 7.4 PBS. The graphs in B–L represent calibration curves for 800 nm AHAP (B), 800 nm DET (C), 800 nm MAP (D), 450 nm DET (E), 150 nm DET (F), nonporous 150 nm DET (G), 150 nm DET functionalized via surface grafting (H), 25% MPTMS/TMOS (I), 40% MPTMS/TMOS (J), 75% MPTMS/TEOS (K), and 85% MPTMS/TEOS (L).

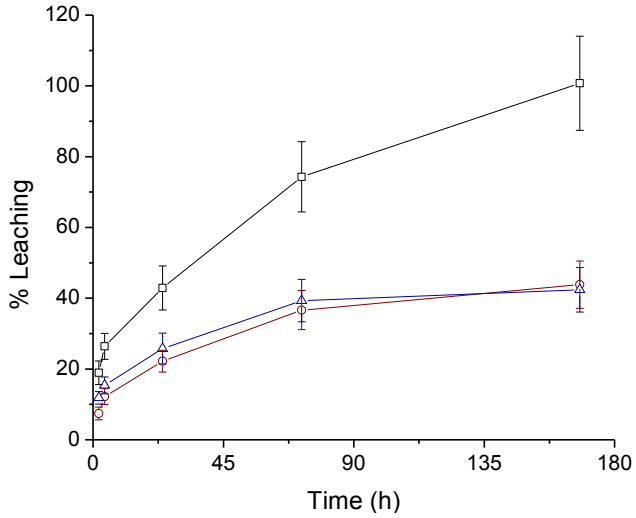

Figure S5. Leaching measurements over 1 wk incubation in PBS at 37 °C for 800 nm particles modified with MAP/NO (black), AHAP/NO (green), or DET/NO (blue). Particles were incorporated at 20 wt% (relative to PU mass) in AL-25-80A PU.

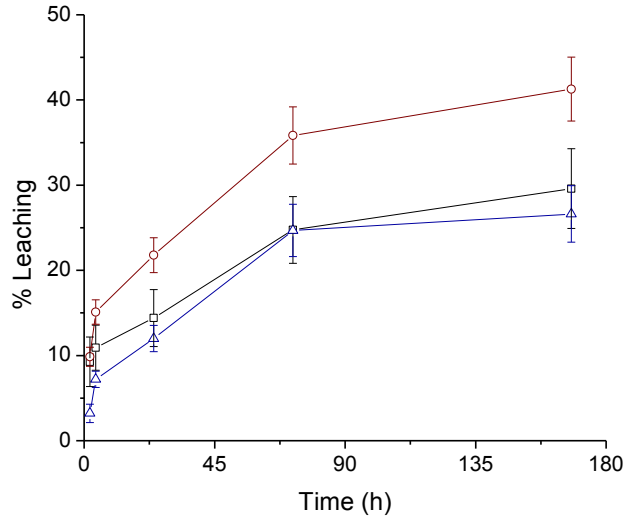

Figure S6. Leaching measurements over 1 wk incubation in PBS at 37 °C for 150 nm (black), 450 nm (green), and 800 nm DET/NO-modified SNPs. Particles were incorporated at 20 wt% (relative to PU mass) in a 1:1 mixture of AL-25-80A:SG-85A PUs.

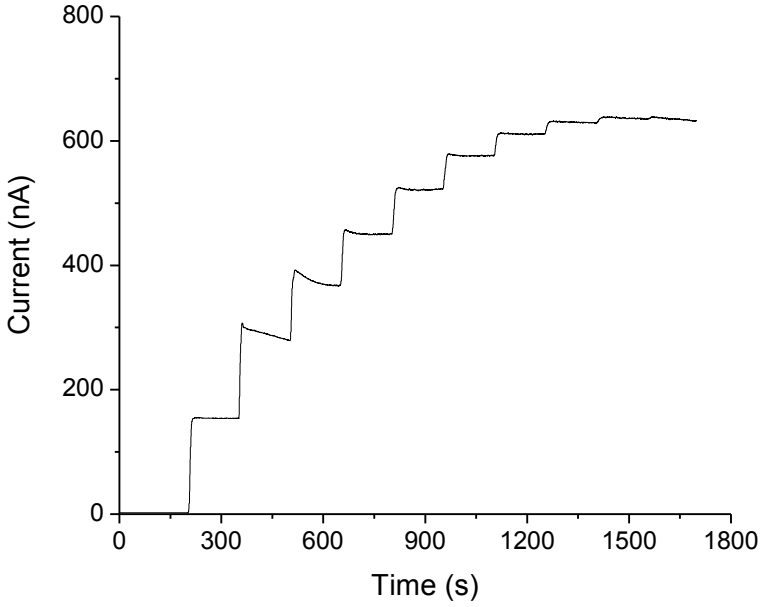

Figure S7. Amperometric glucose response of biosensors coated with the 75% MPTMS (33.3 wt%) HP-93A-100 NO-releasing layer in PBS at 37 °C. The glucose concentration was increased in ~3 mM increments (3–30 mM).

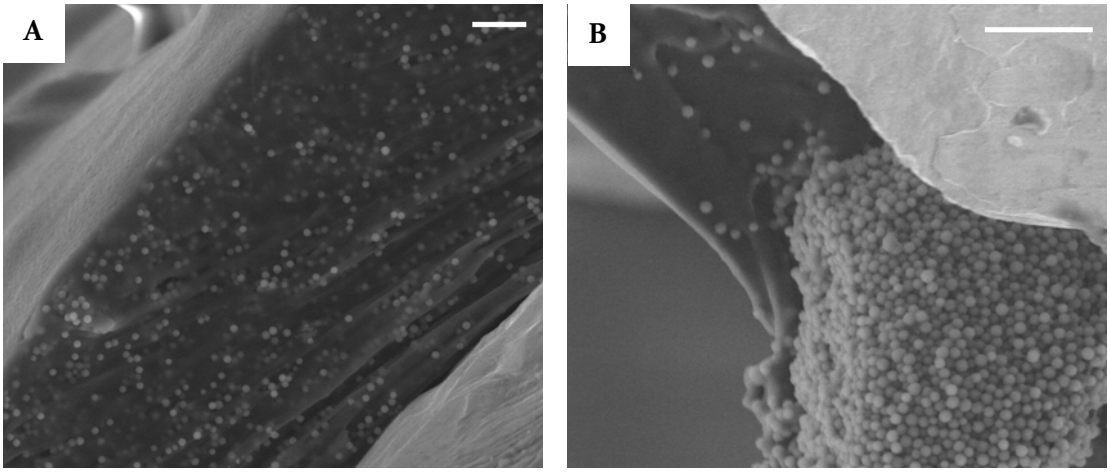

Figure S8. Scanning electron micrographs of glucose biosensors coated with both the 75% MPTMS (33.3 wt%) HP-93A-100 NO-releasing layer and the PC-3585A topcoat. Cross-sections of the sensors (A) as prepared and (B) after 2 wk immersion in PBS revealed the particles aggregate over time. The scale bar in the electron micrographs represents 5 μm.

MPTMS mol%	85	85	75	40	25
Backbone Silane	TMOS	TEOS	TEOS	TMOS	TMOS

Figure S9. Solutions of alkanethiol-modified SNPs (10 mg mL^{-1}) prepared in phosphate buffered saline (PBS; pH 7.4). Aggregates of particles with elevated MPTMS content (≥75%) repel water, whereas materials with lower alkanethiol composition readily interact with water.

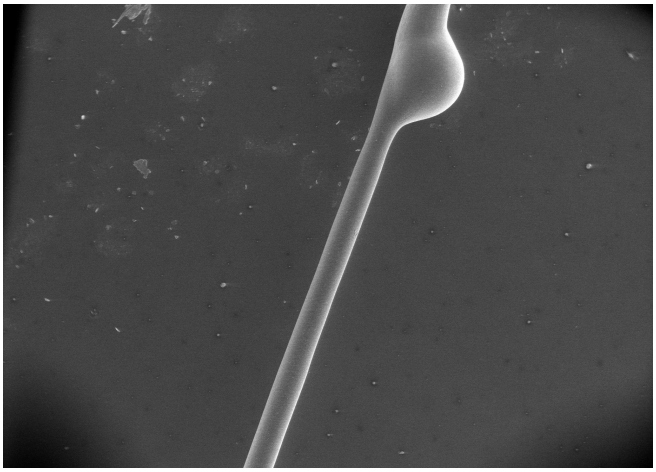

Figure S10. Scanning electron micrograph of glucose sensor working electrode coated with NO-releasing PU/SNP composite. The beading irregularity is formed over the PFA-insulated portion of the Pt-Ir wire from removal of the insulation and does not originate from the coating process.

Table S1. Reaction parameters for synthesis of amine-modified mesoporous silica nanoparticles.

Particle Size (nm)	Reagent/Solvent Volume (mL)				Aminosilane	
	H_2O	Aq. NH_4OH	EtOH	TEOS	Type	Volume (mL)
150 nm	107	2.6	41	0.554	DET3	0.520
450 nm	95	2.6	53	0.698	DET3	0.654
800 nm	162	11.8	175	1.395	DET3	1.310
800 nm	162	11.8	175	1.395	AHAP3	1.342
800 nm	162	11.8	175	1.395	MAP3	0.955

Table S2. Reaction parameters for synthesis of thiol-modified silica nanoparticles.

Mol % MPTMS	Reagent/Solvent Volume (mL)				Tetraalkoxysilane	
	H_2O	Aq. NH_4OH	EtOH	MPTMS	Type	Volume (mL)
25%	1.40	11.0	16.2	0.170	TMOS	0.404
40%	1.40	11.0	16.2	0.271	TMOS	0.324
75%	1.40	11.0	16.2	0.424	TEOS	0.169
85%	8.9	11.0	8.8	0.476	TMOS	0.066
85%	8.9	11.0	8.8	0.476	TEOS	0.099

Table S3. Nitric oxide-release properties for selected NO donor-modified silica particles.

Particle Size	NO Donor Modification	Porosity	Synthesis Strategy	$t_{1/2}$ (min)[a]	$[NO]_{total}$ ($\mu mol\ mg^{-1}$)[b]	t_d (h)[c]
800 nm	DET/NO	Mesoporous	Ion Exchange	35.6±7.5	1.61±0.17	38.6±4.2
800 nm	AHAP/NO	Mesoporous	Ion Exchange	2.7±1.0	1.30±0.11	7.8±2.0
800 nm	MAP/NO	Mesoporous	Ion Exchange	1.5±0.4	2.20±0.20	2.5±0.2
150 nm	DET/NO	Mesoporous	Ion Exchange	38.8±7.9	1.88±0.05	34.2±3.1
500 nm	DET/NO	Mesoporous	Ion Exchange	23.9	2.41	46.2
200 nm	DET/NO	Mesoporous	Surface Grafting	169.5	1.13	42.8
200 nm	DET/NO	Nonporous	Surface Grafting	91.2	0.22	11.0
620 nm	75% MPTMS-	Nonporous	Co-condensation	193.4±23.6	4.29±0.59	51.4±7.2

[a]Half-life of NO release. [b]Total NO storage. [c]Duration of NO release above a threshold value of 10 ppb/mg.

Table S4. Analytical performance merits of glucose biosensors coated with different PUs.[a,b]

PU Type	PU Water uptake (mg mg^{-1})[d]	Glucose Linear Dynamic Range[e]	S (nA mM^{-1} mm^{-2})[f]	Sensitivity Retention (%)[g]		
				3 d	5 d	14 d
HP-93A-100	2.6±0.3[c]	1–3 mM	398±41	64.1±0.0	57.5±14.6	30.9±6.6
AL-25-80A	0.6±0.3[c]	1–6 mM	85.6±13.4	71.0±12.8	56.0±4.3	42.9±3.9
SG-85A	0.2±0.2[c]	1–15 mM	12.4±1.6	92.9±11.2	91.3±16.5	53.5±6.9
PC-3585A	0.0±0.0	1–15 mM	9.6±4.2	99.7±4.7	86.8±3.2	57.1±2.4

[a]Error bars represent standard deviation for n≥3 separate experiments. [b]PU concentration in the loop-casting solution was 80 mg mL^{-1}. [c]Water uptake measurements described in Koh et al., *Biosensors and Bioelectronics* **2011**, *28*, 17–24. [d]Water uptake expressed as mg_{water} per mg_{PU}. [e]Linear dynamic range determined from glucose sensor calibration curves as the concentration range over which the associated linear trendline had an R^2 value >0.99. [f]Determined as the slope of the trendline fit to the sensor current-glucose response over the linear dynamic range on the first day of testing. [g]Glucose sensitivity after soaking sensors in PBS at 37 °C (relative to the sensitivity on the first day of testing).

REFERENCES

1. Soto, R. J.; Yang, L.; Schoenfisch, M. H. Functionalized Mesoporous Silica Via an Aminosilane Surfactant Ion Exchange Reaction: Controlled Scaffold Design and Nitric Oxide Release *ACS Appl. Mater. Interfaces* **2016**, *8*, 2220-2231.
2. Riccio, D. A.; Nugent, J. L.; Schoenfisch, M. H. Stöber Synthesis of Nitric Oxide-Releasing *S*-Nitrosothiol-Modified Silica Particles *Chem. Mater.* **2011**, *23*, 1727-1735.