Supporting Information

Mode of nitric oxide delivery affects antibacterial action against cystic fibrosis-relevant pathogens

*Jackson R. Hall [a,†], Kaitlyn R. Rouillard [a,†], Dakota J. Suchyta [a,†], Micah D. Brown [a], Mona Jasmine R. Ahonen [a], and Mark H. Schoenfisch [a,b]**

[a] Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, 27599, USA
[b] Division of Pharmacoengineering and Molecular Pharmaceutics, UNC Eshelman School of Pharmacy, Chapel Hill, NC 27599, USA

[†] Authors contributed equally to this work

*To whom correspondence should be addressed: schoenfisch@unc.edu

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Synthesis of NO-releasing chitosan

Synthesis of chitosan oligosaccharides (COS). Chitosan oligosaccharides were prepared as described previously.[1] Briefly, medium molecular weight chitosan (2.5 g) was suspended in hydrogen peroxide (50 mL, 15% v/v) and stirred at 85 ˚C for 1 h. Insoluble, undegraded chitosan was pelleted via centrifugation while filtering the supernatant. Water-soluble oligosaccharides were collected via precipitation in acetone and dried in vacuo. An Ubbelohde viscometer was used to measure the viscosity of chitosan oligosaccharide solutions prepared in sodium chloride (0.20 M) and acetic acid (0.10 M) at 25 °C. The molecular weight was calculated to be 4.41 ± 0.04 kDa using the classic Mark-Houwink equation ($\eta = 1.81 \times 10^{-3} M^{0.93}$).[2]

Synthesis of tosylated ethanolamine Schiff base (TES). Ethanolamine (34.0 mL) and *p*-anisaldehyde (68.1 mL) were stirred for 24 h. Remaining solvent was removed under vacuum to give product in quantitative yield. The Schiff base intermediate (2-(benzylideneamino)ethan-1-ol, 7.1 g) was dissolved in anhydrous dichloromethane (25 mL) and anhydrous pyridine (6.5 mL). The solution was cooled to 0 ˚C and *p*-tolunesulfonyl chloride (10 g) was added to the stirred solution over 1 h. The solution was stirred overnight at 0 ˚C and the resulting precipitate was filtered, washed with diethyl ether (3 x 25 mL), and dried in vacuo overnight.

Synthesis of ethanolamine-modified chitosan oligosaccharides (COS-EA). Water soluble COS were subsequently modified with TES. Briefly, COS (1.0 g) were dissolved in deionized water (20 mL) in a 100 mL round bottom flask equipped with a magnetic stir bar. The solution was stirred at 80 ˚C, and TES (1.0 g) dissolved in dimethyl sulfoxide (25 mL) was added dropwise to the

stirring solution. The resulting suspension was stirred for 1 h until the suspension became a homogenous solution. To this, 5 M HCl (5 mL) was added dropwise and the solution was stirred overnight (18 h) at 80 ˚C. The solution was cooled to room temperature and the pH was adjusted to 7.0 by addition of 1 M NaOH. The product was precipitated with ethanol and collected by centrifugation. The solid was washed with ethanol (2 x 40 mL), collected by centrifugation, and dried in vacuo overnight.

Synthesis of N-diazeniumdiolate-modified COS-EA (COS-EA/NO). Nitric oxide-releasing chitosan oligosaccharides synthesis was adapted from a previous protocol.[3,4] Briefly, in a 450 mL stainless steel Parr bottle equipped with a PTFE liner and a magnetic stir bar, 1 g of COS-EA was dissolved in Milli-Q water (10 mL). To this solution, methanol (56.7 mL) and sodium methoxide (1.67 mL, 5.4 M (30 wt%) in methanol) was added. The resulting solution was stirred for 15 min. The stainless-steel reactor was purged with argon (10 s at 7 bar) three times followed by three long purges (10 min at 7 bar). The vessel was pressurized with NO gas (20 bar) and stirred for 3 d at room temperature. The purging protocol was repeated and the product was precipitated in acetone. The precipitate was collected by centrifugation, washed with cold ethanol, and dried in vacuo overnight. The dried product was stored in a vacuum-sealed container at -20 ˚C. The total NO storage for COS-EA/NO is 0.44 ± 0.02 μmol NO/mg (Figure S1).

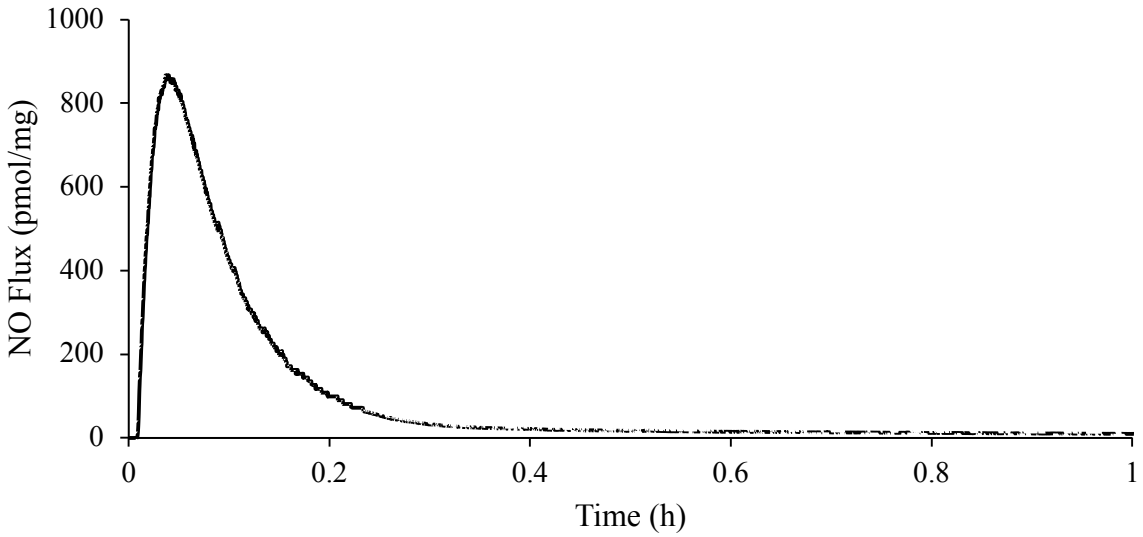

Figure S1. Real-time NO-release profile for the first 1 h from COS-EA/NO in PBS (pH 6.5) measured using a chemiluminescence NO analyzer.

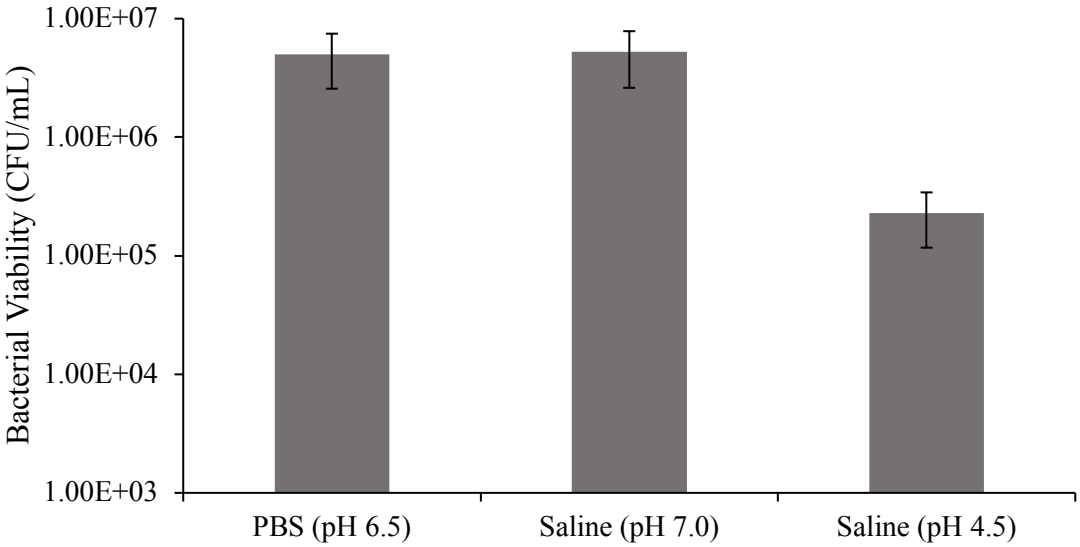

Figure S2. Bactericidal efficacy of saline and PBS against *Pseudomonas aeruginosa* after a 5 h exposure in 96 well plate with a working volume of 200 µL. Solution pH of saline (pH 4.5) and PBS (pH 6.5) were altered with HCl. All solutions contained 1% TSB. Saline concentration was 0.9% (w/v).

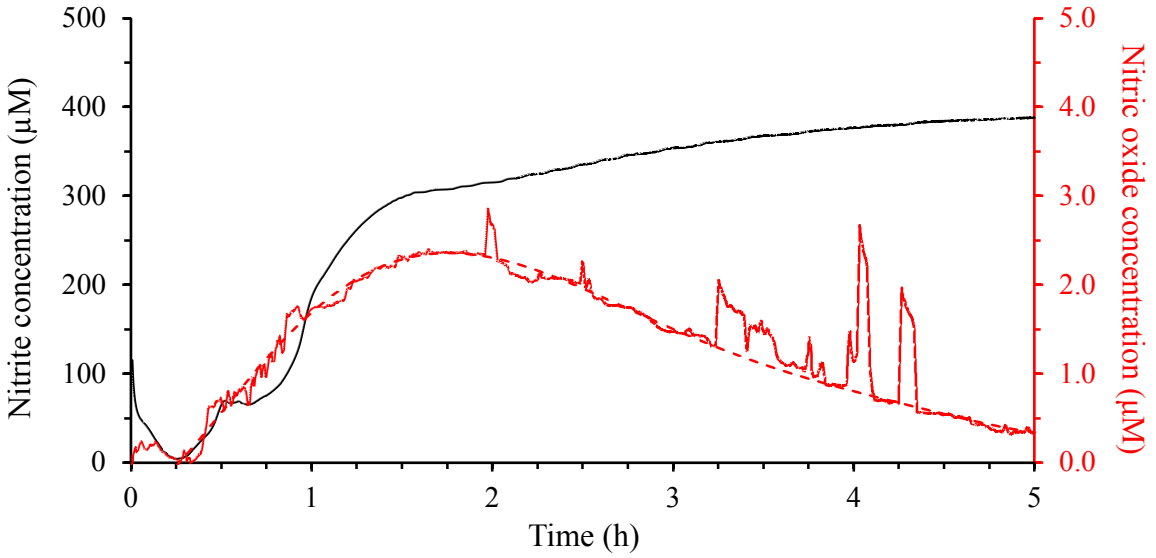

Figure S3. Simultaneous electrochemical detection of nitrite and NO in 10 mM PBS (pH 6.5, 37 °C) containing 1% (v/v) TSB over a 5 h exposure to 160 ppm gNO. Red trace represents NO, while the black trace represents nitrite.

References

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