Supporting Information

Controlled release of nitric oxide from liposomes

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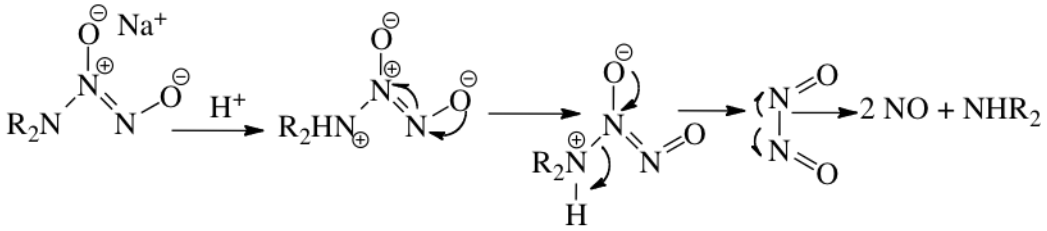
Table of Contents:



Scheme S1. Proton-initiated decomposition mechanism of *N*-diazeniumdiolates to liberate NO.

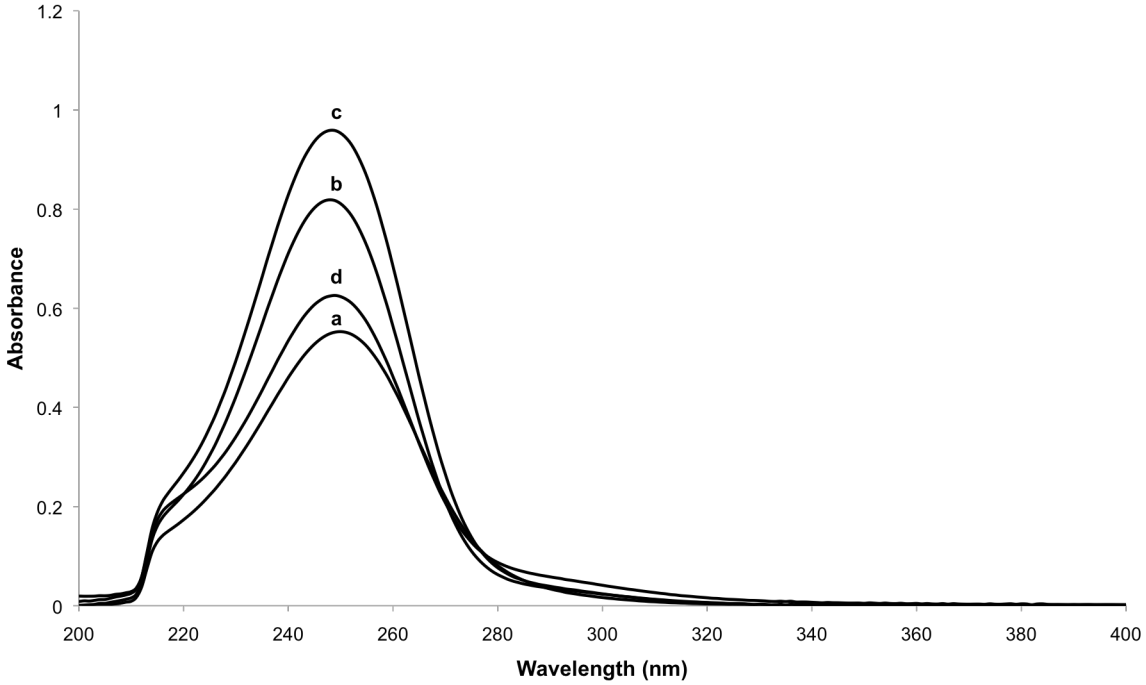
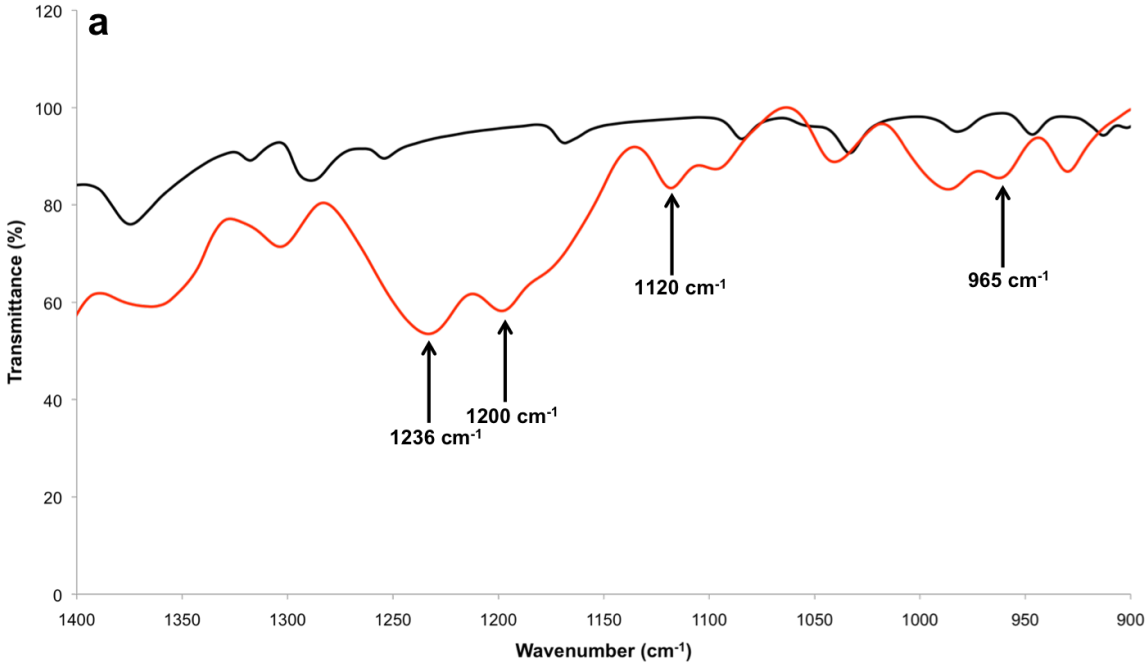
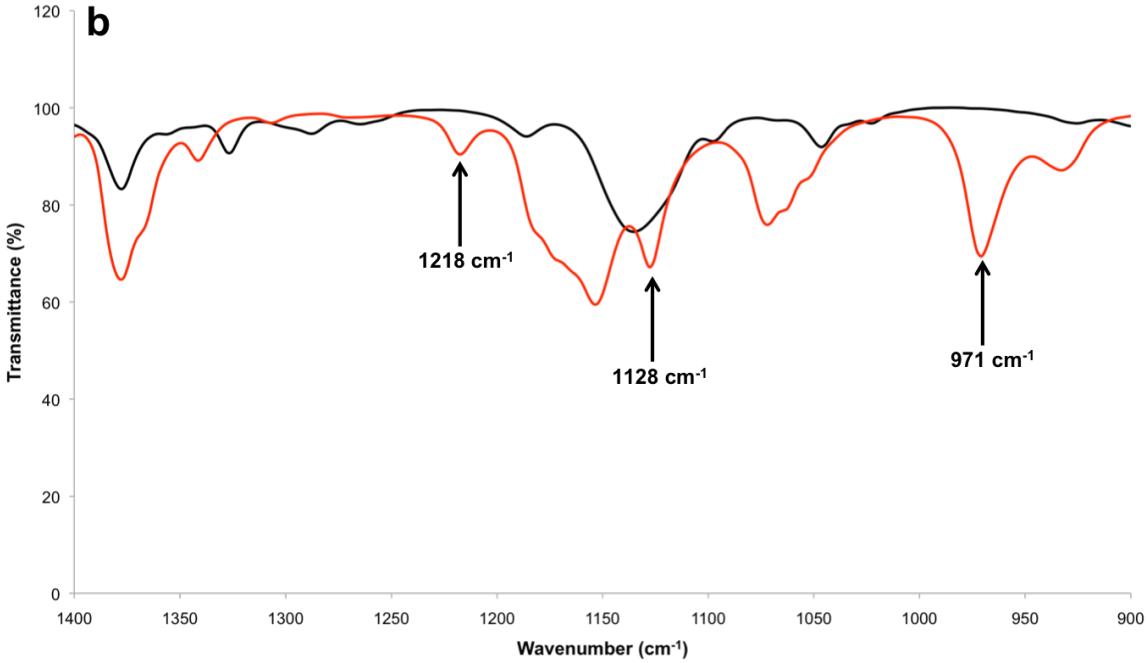


Figure S1. UV-vis spectra of 0.020 mg/mL (a) PROLI/NO, (b) DEA/NO, (c) PAPA/NO, and (d) SPER/NO in 50 mM NaOH. Typical 252 nm absorbance peak associated with the diazeniumdiolate group is observed.[1]


a
Transmittance (%)
Wavenumber (cm-1)
1236 cm-1
1200 cm-1
1120 cm-1
965 cm-1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Transmittance (%)
Wavenumber (cm-1)
1218 cm-1
1128 cm-1
971 cm-1

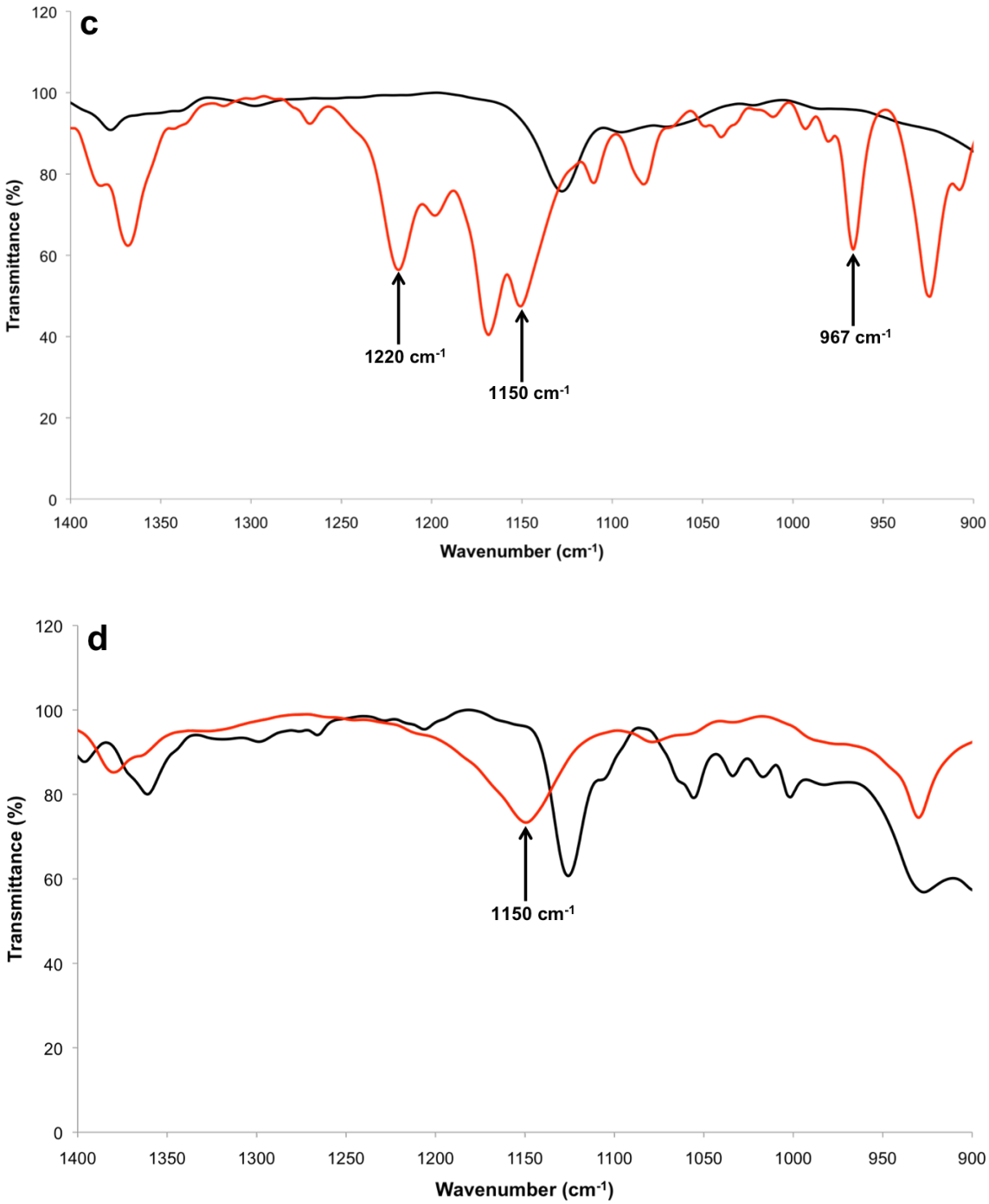

Figure S2. FTIR spectra of (a) PROLI/NO, (b) DEA/NO, (c) PAPA/NO, and (d) SPER/NO. Precursor amine spectra are in black and NO donors are in red. N-O stretches (1236-1210 cm^{-1}), in-plane N2 symmetric stretches (1200-1150 cm^{1} and 965-975 cm^{-1}), and N-N stretches (1128-1120 cm^{-1}) can be observed due to the prescence of the diazeniumdiolate group.[1-2]

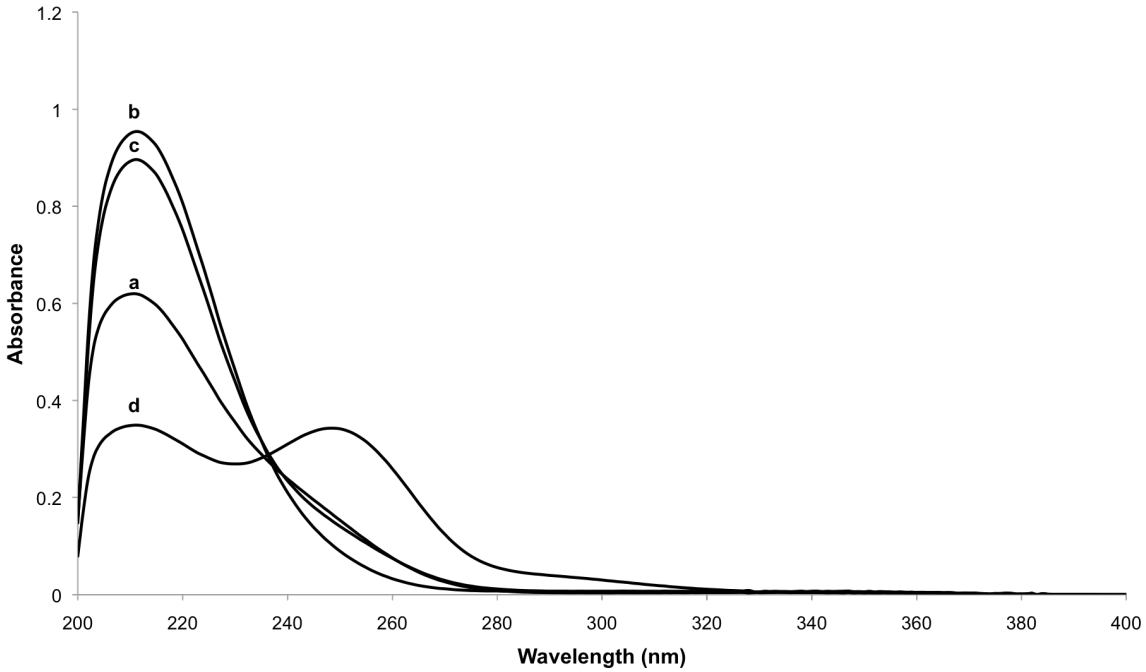

Figure S3. UV-vis spectra of 0.020 mg/mL (a) PROLI/NO, (b) DEA/NO, (c) PAPA/NO, and (d) SPER/NO in 10 mM PBS (pH 7.4) after incubation at 37 °C for 2 h.. Absorbance peak at 210 nm is associated with nitrite/nitrate formation.

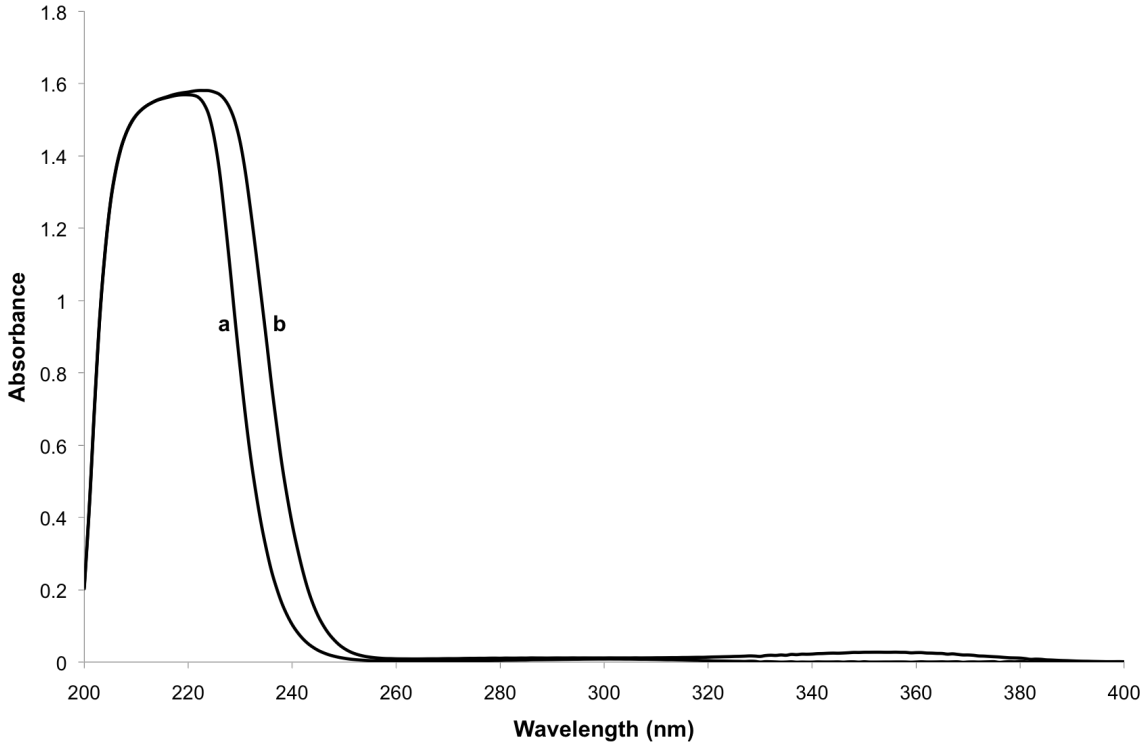

Figure S4. UV-vis spectra of 0.010 mg/mL (a) sodium nitrate and (b) sodium nitrite in 10 mM PBS (pH 7.4, 37 °C). Peak at ~350 nm is from sodium nitrite.

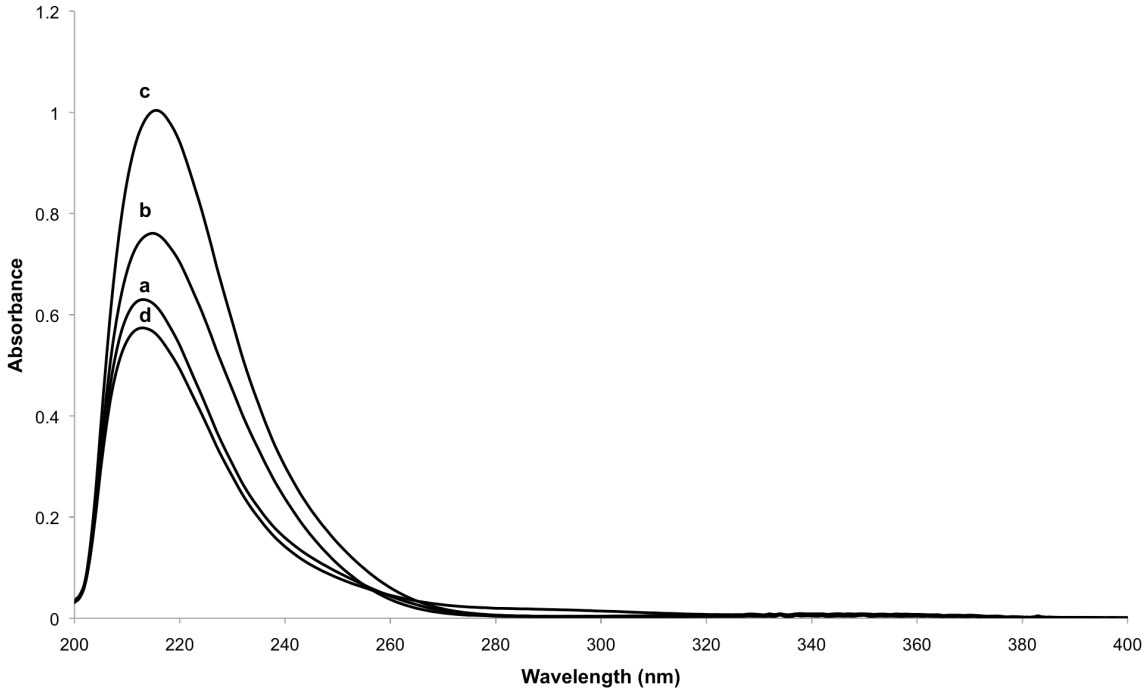

Figure S5. UV-vis spectra of 0.020 mg/mL (a) PROLI/NO, (b) DEA/NO, (c) PAPA/NO, and (d) SPER/NO in 10 mM MES buffer (pH 5.4) after incubation at 37 °C for 2 h.

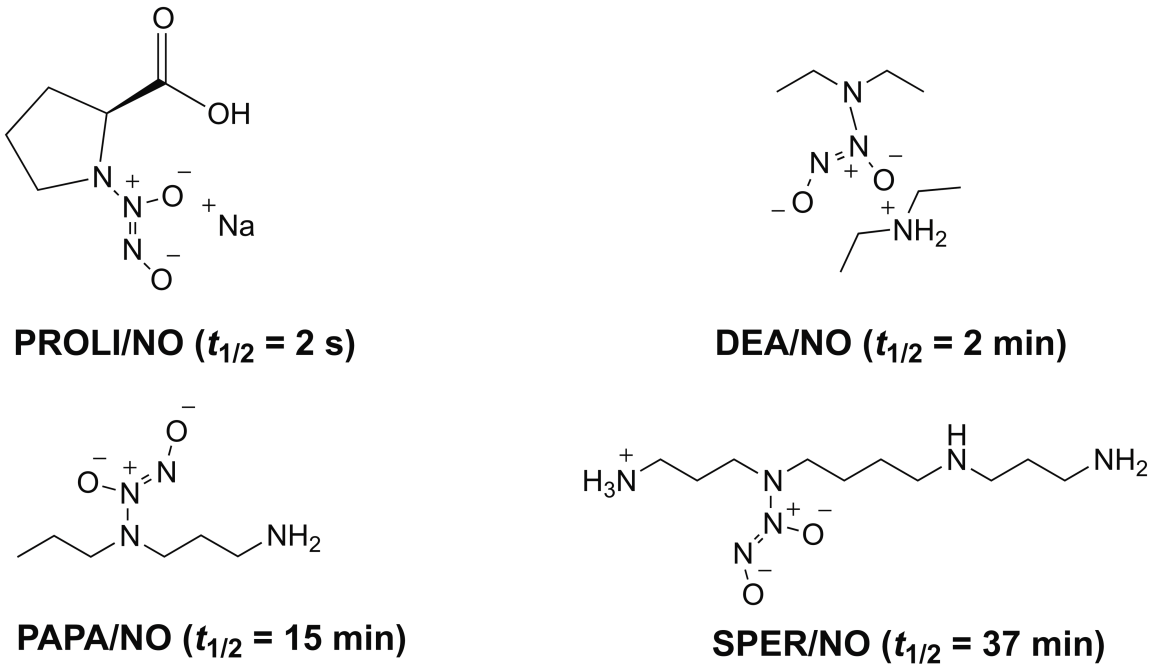

Figure S6. *N*-diazeniumdiolate NO donors with their reported NO-release half-lives in 10 mM PBS (pH 7.4, 37 °C).[3]

Table S1. Nitric oxide-release properties of DPPC liposomes as a function of encapsulated *N*-diazeniumdiolate NO donor in MES (pH = 5.4) at 37 °C.[a]

NO donor	$t_{1/2}$[b] (h)	t_d[c] (h)	$[NO]_{total}$[d] (μmol/mL)
PROLI/NO	0.06 ± 0.02	1.7 ± 0.9	4.71 ± 0.80
DEA/NO	0.12 ± 0.04	5.5 ± 1.6	8.37 ± 0.62
PAPA/NO	0.84 ± 0.24	20.7 ± 4.6	7.75 ± 1.09
SPER/NO	10.4 ± 1.9	44 ± 9.0	6.81 ± 0.78

[a]Error indicates standard deviation from at least 3 different separate liposome preparations. [b]Half-life of NO release. [c]Duration of NO release. [d]Total amount of NO released normalized to the injected volume from the liposome stock solution.

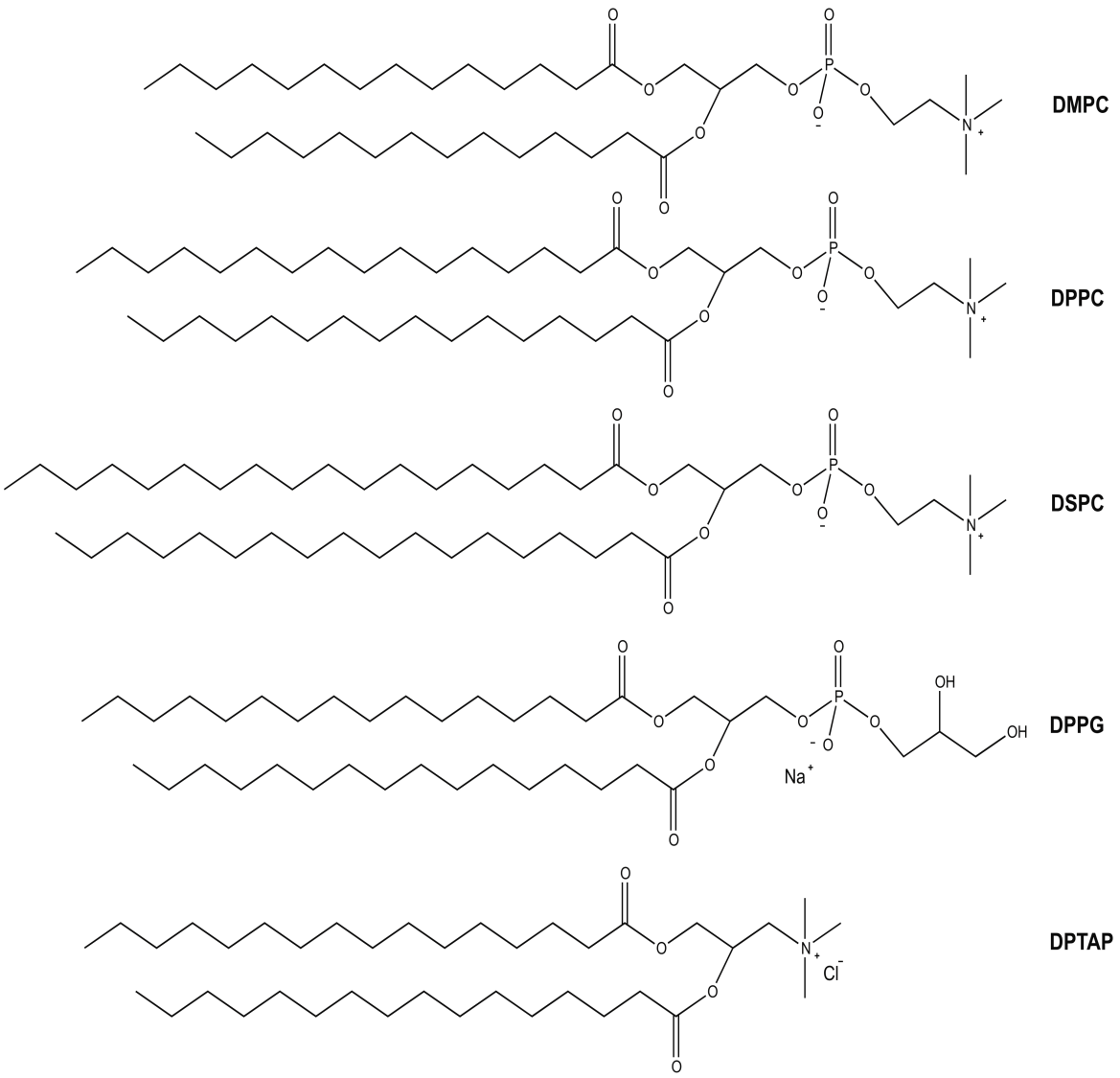

Figure S7. Structures of lipids used to make the liposomes from Table 3.

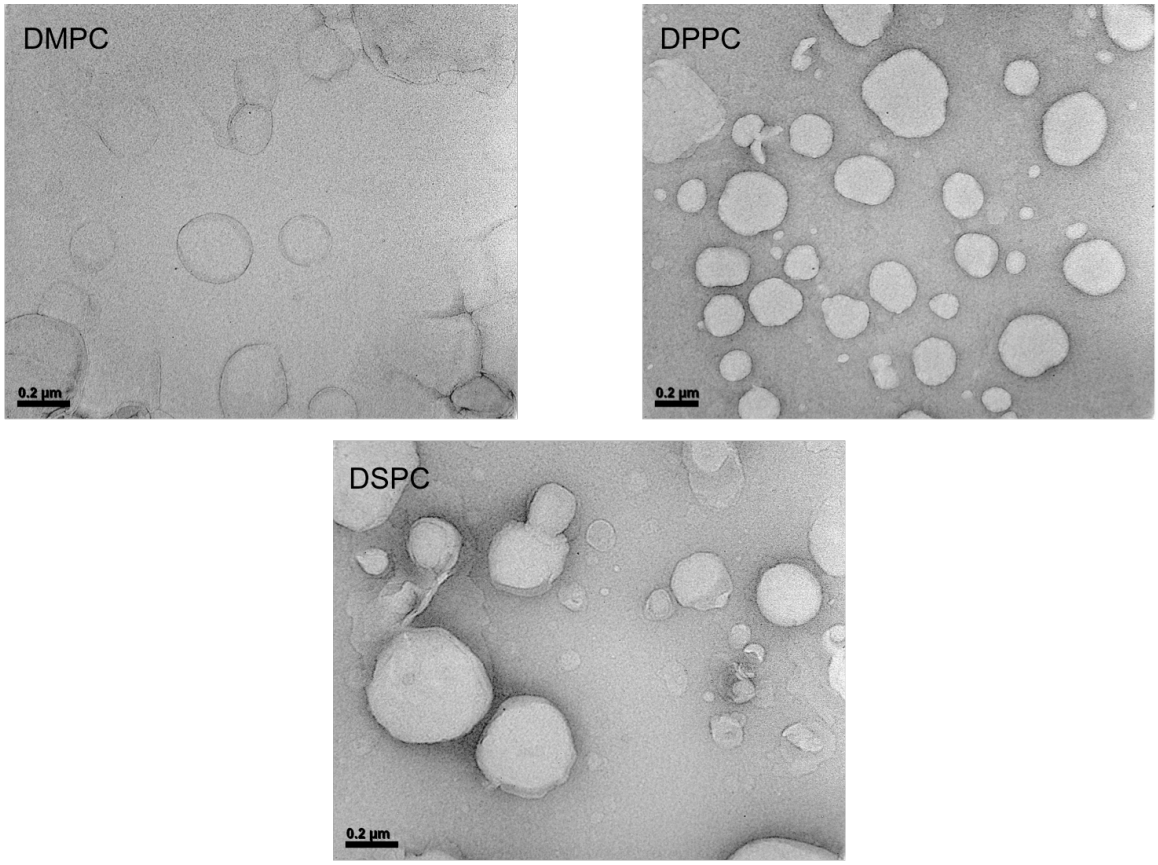

Figure S8. Transmission electron micrographs of DMPC (C_{14}), DPPC (C_{16}), and DSPC (C_{18})-based PAPA/NO liposomes. Scale bar represents 0.2 μm.

Table S2. Fluorophore encapsulation efficiency of DPPC and DPPG liposomes.[a]

Molecule	**DPPC (C_{16}) EE (%)**	**DPPG (- C_{16}) EE (%)**
Coumarin[b]	25.6	26.1
5(6)-carboxyfluorescein[c]	21.7	11.3

[a]Encapsulation efficiency was calculated as the ratio of mol of fluorophore inside liposomes to mol used for synthesis multiplied by 100. A calibration curve was created using each fluorophore for quantification. [b]Excitation at 355 nm and emission measured at 510 nm. [c]Excitation at 495 nm and emission measured at 517 nm.

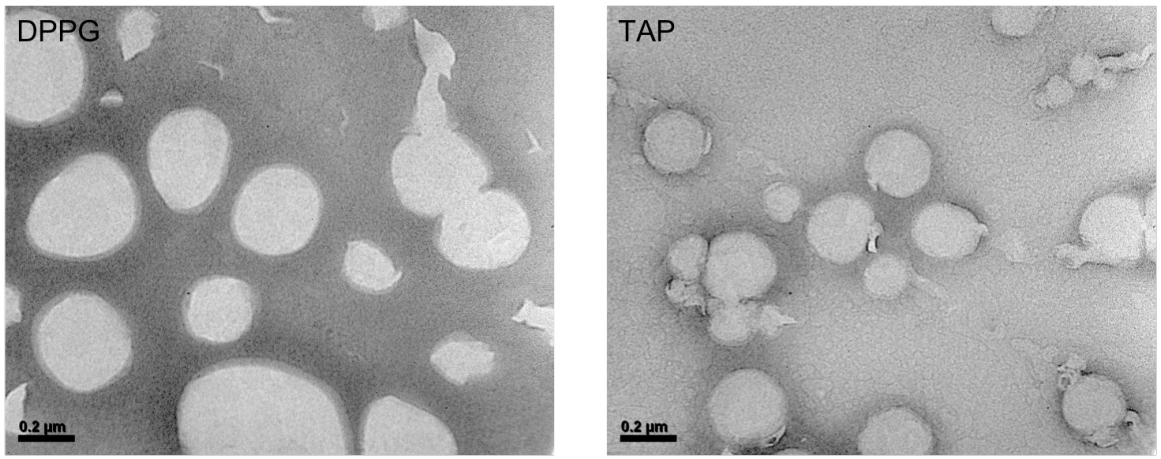

Figure S9. Transmissin electron micrographs of DPPG (- C_{16}) and DPTAP (+ C_{16}) PAPA/NO liposomes. Scale bar represents 0.2 μm.

Table S3. Nitric oxide-release properties of PAPA/NO liposomes as a function of bilayer hydrophobicity and charge in MES (pH = 5.4) at 37 °C

Lipid	$t_{1/2}$[a] (h)	t_d[b] (h)	$[NO]_{total}$[c] (µmol/mL)
DMPC (C_{14})	1.6 ± 1.2	22.3 ± 2.6	7.90 ± 0.29
DPPC (C_{16})	0.84 ± 0.24	20.7 ± 4.6	7.75 ± 1.09
DSPC (C_{18})	12.3 ± 1.3	59.4 ± 7.7	8.91 ± 0.62
DPPG (- C_{16})	1.2 ± 0.3	19.9 ± 6.5	6.13 ± 0.97
DPTAP (+ C_{16})	0.09 ± 0.01	6.5 ± 2.1	7.94 ± 0.96

[a]Half-life of NO release. [b]Duration of NO release. [c]Total amount of NO released normalized to the injected volume from the liposome stock solution.

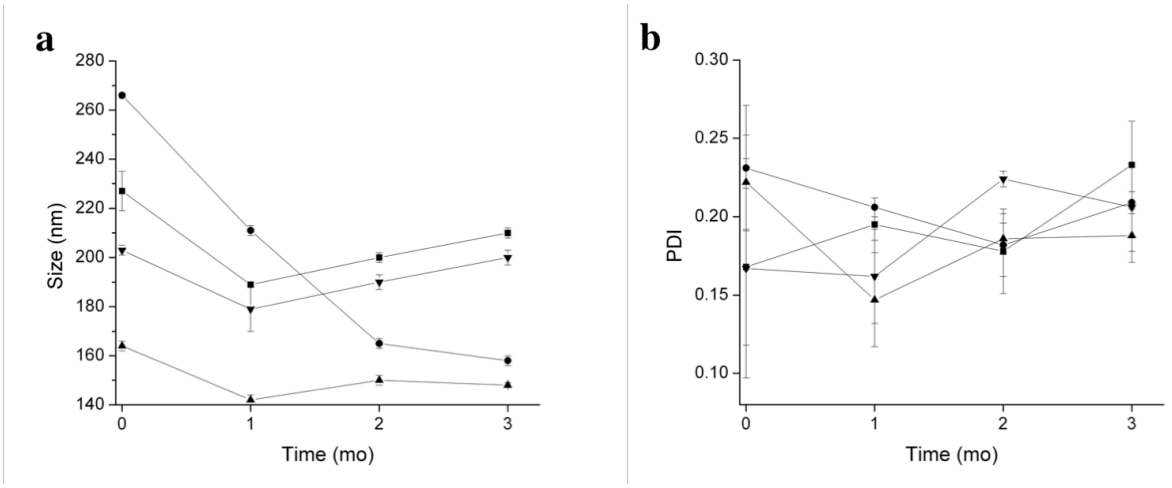

Figure S10. (a) Z-average size and (b) polydispersity index values of PAPA/NO-encapsulated (▼) DPPC, (▲) DPPG, (■) 50:50 DPPC:DPTAP, and (●) 10:90 DPPE-PEG/DPPC liposomes over time as measured by dynamic light scattering. Liposomes were stored at 4 °C between measurements.

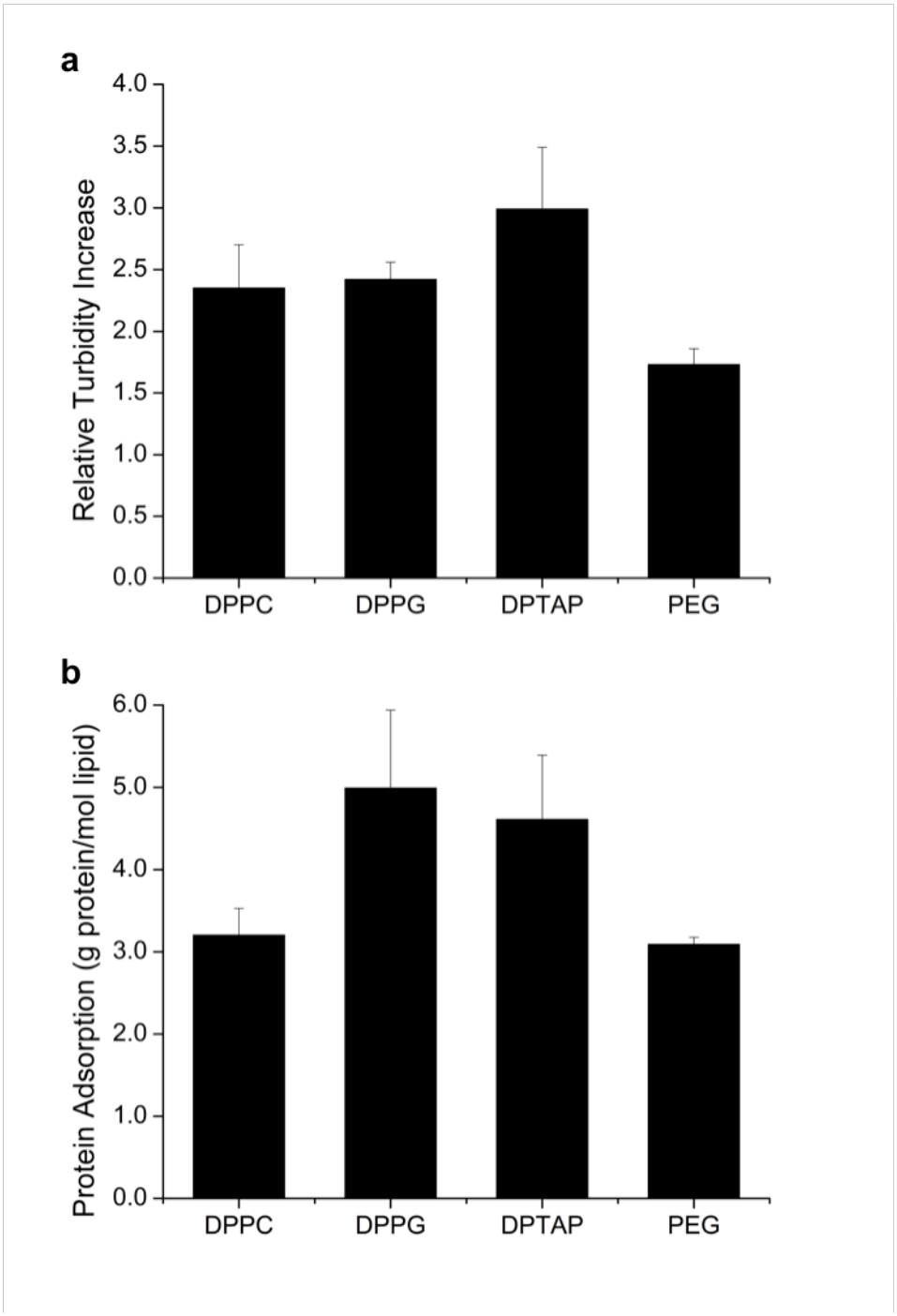

Figure S11 (a) Normalized absorbance measured at 450 nm for 5% (v/v) liposome solutions in serum. (b) Amount of protein adsorbed to the liposomes, normalized to moles of lipid, as measured by the Bradford assay. The DPTAP and PEG liposomes were composed of a 50:50 DPTAP:DPPC and 10:90 DPPE-PEG:DPPC molar ratios, respectively.

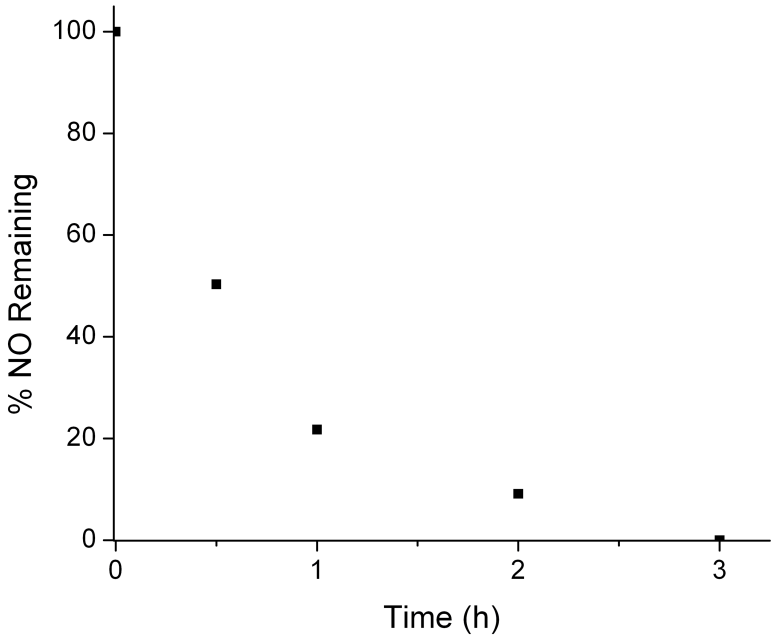

Figure S12. Nitric oxide release from neutral DPPC PAPA/NO liposomes suspended in 10 mM PBS (pH 7.4, 37 °C) containing 157 mg/mL hemoglobin.

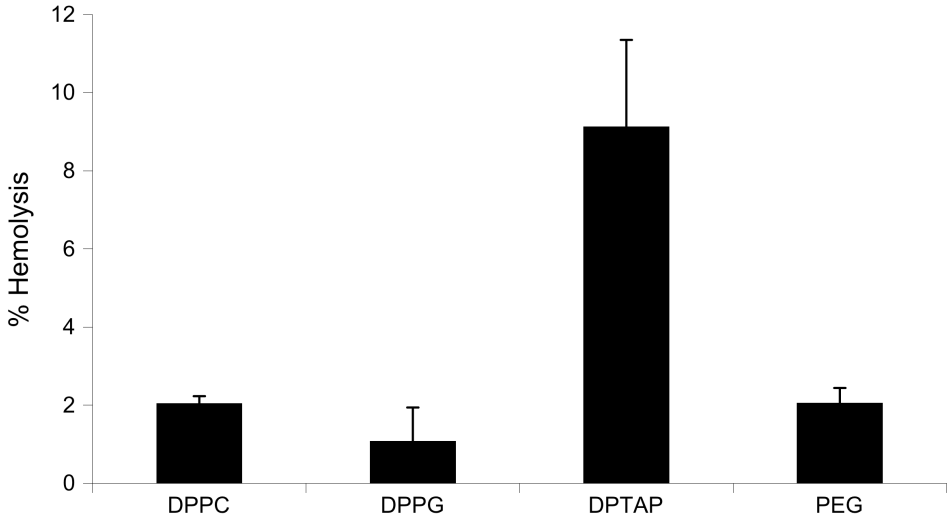

Figure S13. Hemolytic activity of various PAPA/NO-encapsulated liposome systems. The DPTAP liposomes are composed of a 50:50 DPTAP:DPPC molar ratio and PEG liposomes are composed of a 10:90 DPPE-PEG:DPPC molar ratio.

Protocol for hemolysis assay. A standard hemolysis assay was employed for determining the hemolytic activity of the NO-releasing liposomes.[4-6] Red blood cells (RBCs) are harvested from freshly-obtained citrated canine blood and resuspended in 0.9% isotonic saline to a 10% (v/v) concentration. The RBCs (300 µL) are mixed with the stock liposome solution (15 μL) and incubated at 37 °C for 30 min with slight agitation. Afterwards, the RBCs are separated from free hemoglobin by centrifugation at 1,000 × g for 10 min. The supernatant (80 μL) was added to a 96-well plate and the absorbance was measured at 405 nm using a Thermoscientific Multiskan EX plate reader (Waltham, MA). Absorbance values from negative controls (300 μL blood mixed with 15 μL 0.9% saline) were subtracted from all absorbance values. The % hemolysis was determined by dividing the sample absorbance with that of RBCs incubated with 1% triton in saline.

References

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4. Nie, Y.; Ji, L.; Ding, H.; Xie, L.; Li, L.; He, B.; Wu, Y.; Gu, Z. *Theranostics*, **2012,** *2,* 1092-1103.

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