

## Electronic Supplementary Information

### Preparation and characterization of an improved Cu<sup>2+</sup>-cyclen polyurethane material that catalyzes generation of nitric oxide from *S*-nitrosothiols

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## 1. Experimental Section

### 1.1 Materials

Thermoplastic polyurethane (TPU) Tecophilic<sup>®</sup> SP-93A-100 was a gift from Lubrizol Advanced Materials Inc. (Cleveland, OH). 1,4,7,10-tetraazacyclododecane (cyclen) was purchased from Strem Chemicals (Newburyport, MA). Hexamethylene diisocyanate (HMDI), tetrahydrofuran (THF), and pyridine were freshly distilled prior to use. Dibutyltin dilaurate (DBTDL), trifluoroacetic acid (TFA), ethylenediaminetetraacetic acid (EDTA), *S*-nitroso-*N*-acetyl-DL-penicillamine (SNAP), and the anhydrous solvents including *N,N*-dimethylacetamide (DMAc), *N,N*-dimethylformamide (DMF), acetonitrile, and toluene, and other chemical reagents or solvents were used as received from Sigma-Aldrich (Milwaukee, MI) or Fisher Scientific (Fair Lawn, NJ). Phosphate buffered saline (PBS) used in NO generation measurements was prepared to contain 140 mM NaCl, 8.1 mM Na<sub>2</sub>HPO<sub>4</sub>, and 1.9 mM KH<sub>2</sub>PO<sub>4</sub> (pH 7.4). *S*-Nitrosoglutathione (GSNO) and *S*-nitrosocysteine (CySNO) were freshly prepared from reduced L-glutathione (GSH) or cysteine (CySH), separately, by methods reported previously<sup>1,2</sup>. Deionized water was provided by a Milli-Q filter system (18 MΩ cm<sup>-1</sup>; Millipore Corp., Billerica, MA). The platelet-rich sheep plasma was obtained by centrifuging (1300 rpm for 18 min at 4 °C) the whole blood from sheep (purchased from Lampire Biological Lab, Pipersville, PA).

### 1.2 Characterization

<sup>1</sup>H-NMR spectra were obtained on a Varian 400 MHz spectrometer. Mass spectra were collected on a micromass LCT Time-of-Flight mass spectrometer with electrospray and APCI ionization modes. FTIR spectra were collected from a Perkin-Elmer spectrum BX FT-IR system. UV

spectra were recorded by a Perkin-Elmer Lambda 35 UV/VIS spectrometer. Copper contents of the polymers were measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES) following an established method<sup>3</sup>: a given small piece of polymer (~ 5 mg) was dissolved in concentrated nitric acid (2 mL) at RT, which was then diluted with DI water (13 mL). The resultant clear solution was passed through a syringe filter (0.45 µm PTFE, a National Scientific Company product), and then the copper content in the filtrate was analyzed by ICP-AES. The quantitatively plasma copper concentration assay was carried out by use of inductively coupled plasma – high resolution mass spectrometry (ICP-HRMS). In brief, 50 µL of plasma sample was digested with 200 µL of concentrated nitric acid, and the resultant clear yellow solution was diluted with water (250 µL). Any insoluble matter was removed by microcentrifuging at 14K rpm for 5 min. The supernatant was used for ICP-HRMS analysis. A similar analytical method via ICP-AES was employed to the copper leaching study. The whole sheep blood was centrifuged at 2600 rpm for 10 min to provide the plasma samples. 2 mL of plasma sample was analyzed after digestion with concentrated nitric acid (3 mL), dilution with water (1 mL), and filtration via microcentrifugal separation. The concentration of pendant isocyanate groups on TPU-NCO (**6**) was determined *via* a modified titration method<sup>4</sup>. All NO measurements were made using a chemiluminescence Sievers Nitric Oxide Analyzer (NOA), model 280. Each measurement was pre-calibrated by an internal two-point calibration (the nitrogen flow as zero gas and 45 ppm of NO gas flow). The sample solution for the NOA measurement was continuously bubbled with a nitrogen flow (50 mL/min), and thus the generated NO was purged into the detector cell in the NOA by vacuum (200 mL/min). The *in vitro* fibrinogen adsorption immunofluorescence assay was performed as described previously<sup>5</sup>. The NO generating polymer TPU-PEG-cyclen-Cu<sup>II</sup> (**1**) and the control polymer TPU were used for parallel study. The blank

polypropylene was used as an internal control to normalize the fluorescence signals within different plates. All measurements were done in triplicate and performed with a Synergy 2 fluorescence reader.

### 1.3 Synthesis

**1,14-Dichloro-3,6,9,12-tetraoxatetradecane (9).** To a solution of pentaethylene glycol (2.43 g, 10 mmol) and dry pyridine (2.06 mL, 25 mmol) in anhydrous toluene (15 mL) in ice bath was slowly added dropwise a solution of thionyl chloride (1.86 mL, 25 mmol) in 2 mL of dry toluene. After addition, the reaction mixture was stirred and heated at reflux for 3~4 h, as monitored by TLC (methanol/ethyl acetate 1:19). The toluene mixture was washed successively with 2M HCl and water. After drying over anhydrous sodium sulfate, the mixture was concentrated by rotary evaporation. The residue was purified by column chromatography (eluent: ethyl acetate/petroleum ether 1:2) to provide an oily product 2.15 g (78%). IR (neat) = 2868  $\text{cm}^{-1}$  ( $\text{CH}_2$ ), 1148  $\text{cm}^{-1}$  (C-O), 664  $\text{cm}^{-1}$  (C-Cl).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  (ppm) 3.72 (4H, t,  $\text{OCH}_2\text{CH}_2\text{Cl}$ ), 3.65 (12H, t,  $\text{OCH}_2\text{CH}_2\text{O}$ ), 3.60 (4H, t,  $\text{OCH}_2\text{CH}_2\text{Cl}$ ).

**2,2'-(3,6,9,12-Tetraoxatetradecane-1,14-diyl)bis(isoindoline-1,3-dione) (10).** A mixture of **9** (2.2 g, 8 mmol) and phthalimide potassium (4.4 g, 24 mmol) in dry DMF (25 mL) was stirred and heated at 120 °C for 24 h under an inert atmosphere, as monitored by TLC (ethyl acetate/petroleum ether 1:2). The reaction mixture was poured into an ice-water mixture, that was then extracted with plenty of ethyl acetate. The combined organic layers were washed with water, and dried, and then concentrated under reduced pressure. The residue was purified by column chromatography (eluent: acetone/hexane 1:2) to afford a white solid 3.21 g (81%). IR

(neat) = 3061  $\text{cm}^{-1}$  (ArC-H), 2869  $\text{cm}^{-1}$  ( $\text{CH}_2$ ), 1772 & 1717  $\text{cm}^{-1}$  (phthalimide C=O), 1613  $\text{cm}^{-1}$  (C=C), 1119  $\text{cm}^{-1}$  (C-O), 1026  $\text{cm}^{-1}$  (C-N), 834 & 721  $\text{cm}^{-1}$  (Ar-H).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ):  $\delta$ (ppm) 7.80 (4H, dd, ArH), 7.69 (4H, dd, ArH), 3.86 (4H, t,  $\text{CH}_2\text{N}$ ), 3.69 (4H, t,  $\text{OCH}_2\text{CH}_2\text{N}$ ), 3.59 & 3.52 (4H each, t,  $\text{OCH}_2\text{CH}_2\text{O}$ ), 3.48 (4H, s,  $\text{OCH}_2\text{CH}_2\text{O}$ ). MS (ESI)  $m/z$ : 519 ( $\text{M}+\text{Na}^+$ , 100).

**3,6,9,12-Tetraoxatetradecane-1,14-diamine (4). 10** (2 g, 4 mmol) was stirred in boiling absolute ethanol (50 mL) until fully dissolved. And then 7 mL (0.12 mol) of hydrazine hydrate was added. The reaction mixture was stirred and heated under reflux for 8 h. The resultant precipitate was filtered off and the filtrate was concentrated *via* a rotary evaporator. The residue was extracted with methylene chloride by stirring their mixture for 1 h. After filtration, a yellow oily product 0.86 g (90%) was obtained after removal of  $\text{CH}_2\text{Cl}_2$  and drying under vacuum. IR (neat) = 3367  $\text{cm}^{-1}$  (N-H), 2866  $\text{cm}^{-1}$  ( $\text{CH}_2$ ), 1599  $\text{cm}^{-1}$  (N-H), 1456 ( $\text{CH}_2$ ), 1114  $\text{cm}^{-1}$  (C-O), 1050  $\text{cm}^{-1}$  (C-N).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ):  $\delta$ (ppm) 3.59~3.54 (12H, m,  $\text{OCH}_2\text{CH}_2\text{O}$ ), 3.46 (4H, t,  $\text{OCH}_2\text{CH}_2\text{NH}_2$ ), 2.81 (4H, t,  $\text{OCH}_2\text{CH}_2\text{NH}_2$ ), 2.46 (4H, br,  $\text{NH}_2$ ). MS (ESI)  $m/z$ : 237 ( $\text{M}+\text{H}^+$ , 100), 297 ( $\text{M}-\text{H}^++\text{Na}^++\text{K}^+$ , 100).

**1,4,7-Tris(*tert*-butyloxycarbonyl)-1,4,7,10-tetraazacyclododecane (3Boc-cyclen) (11)** was synthesized according to literature<sup>6</sup>. Yield: 91%. IR (neat) = 3311  $\text{cm}^{-1}$  (N-H), 2975  $\text{cm}^{-1}$  ( $\text{CH}_3$ ), 2931 & 2815  $\text{cm}^{-1}$  ( $\text{CH}_2$ ), 1690 (C=O), 1171  $\text{cm}^{-1}$  (C-O), 1088  $\text{cm}^{-1}$  (C-N).  $^1\text{H-NMR}$  (Acetone- $\text{D}_6$ ):  $\delta$ (ppm) 3.68 (2H, m,  $\text{CH}_2$ ), 3.39~3.28 (8H, m,  $\text{CH}_2$ ), 2.82~2.78 (6H, m,  $\text{CH}_2$ ), 1.47~1.44 (27H, brs,  $\text{C}(\text{CH}_3)_3$ ). MS (ESI)  $m/z$ : 473 ( $\text{M}+\text{H}^+$ , 100), 495 ( $\text{M}+\text{Na}^+$ , 100).

**1-(3-Bromo)propyl-4,7,10-tris(*tert*-butyloxycarbonyl)-1,4,7,10-tetraazacyclododecane (12).** Under an inert atmosphere, a solution of **11** (2 g, 4.2 mmol) in 20 mL of acetonitrile (anhydrous) was slowly added dropwise into a mixture of 1,3-dibromopropane (4 mL, 39.4 mmol) and sodium carbonate (1 g, 8.7 mmol) in acetonitrile (180 mL). The reaction mixture was then stirred and heated at 80 °C for 3~4 d, as monitored by TLC (acetone/hexane 1:4). The insoluble inorganic salts were removed and the filtrate was concentrated *via* rotary evaporator. The residue was purified by silica gel column chromatography (eluent: acetone/hexane 1:4) to afford 1.9 g (75%) of white solid. <sup>1</sup>H-NMR (CDCl<sub>3</sub>):  $\delta$ (ppm) 3.54 (2H, t, BrCH<sub>2</sub>), 3.48~3.26 (14H, m, cyclen-CH<sub>2</sub>), 2.69~2.61 (4H, m, cyclen-CH<sub>2</sub> + NCH<sub>2</sub>), 2.33 (2H, q, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Br), 1.44 (27H, brs, C(CH<sub>3</sub>)<sub>3</sub>). MS (ESI) *m/z*: 593 (M<sup>+</sup>, 100), 595 (M+2, 100).

**1-(1-Amino-3,6,9,12-tetraoxa-15-azaoctadecan-18-yl)- 4,7,10-tris(*tert*-butyloxycarbonyl)-1,4,7,10-tetraazacyclododecane (5).** Sodium hydride (60% dispersed in mineral oil, 447 mg, 11.2 mmol) was added to a solution of **4** (1.3 g, 5.6 mmol) in 15 mL of dry THF at 0 °C and the mixture was allowed to stir for 15 min. A solution of **12** (680 mg, 1.2 mmol) in dry THF (11 mL) was slowly added dropwise into the above mixture and the temperature was maintained at 0 °C. After addition, the temperature of the reaction mixture was allowed to rise to RT and the mixture was stirred for 6 d under an inert atmosphere. Then the reaction mixture was neutralized by 2M HCl until excess of sodium hydride was completely quenched. The insoluble salts were removed and the filtrate was employed to separate and purify *via* column chromatography (gradient eluent: ethyl acetate/hexane 1:1 to 3:1, methylene chloride/methanol/triethylamine 10:1:0.3) to yield compound **5** with a yield of 68% (580 mg). MS (ESI) *m/z*: 749 (M+H<sup>+</sup>, 100), 750 (M+2H<sup>+</sup>, 100), 787 (M+K<sup>+</sup>, 100).

**TPU-PEG-cyclen-3Boc (7).** A procedure adapted from a previous report<sup>7</sup> was employed to prepare the isocyanated polymer (TPU-NCO, **6**) by treatment of the urethane groups of TPU with freshly distilled HMDI in presence of a tin catalyst (DBTDL). The amount of free isocyanate sites created on the isocyanated TPU (**6**) was determined by a titration method<sup>4</sup> to afford 1.05 mmol of isocyanate sites per one gram of polymer. IR (neat) = 3325 cm<sup>-1</sup> (N-H), 2923 & 2862 cm<sup>-1</sup> (CH<sub>2</sub>), 2268 cm<sup>-1</sup> (NCO), 1715 & 1692 (C=O), 1615 cm<sup>-1</sup> (urea), 1530 cm<sup>-1</sup> (N-H bending), 1247 cm<sup>-1</sup> (C-N), 1106 cm<sup>-1</sup> (C-O).

The polymer with pendant isocyanate groups was used to synthesize the cyclen moiety tethered TPU (TPU-PEG-cyclen-3Boc, **7**) by means of a modified urea-forming reaction<sup>8,9</sup>. The polymer **6** (640 mg, 0.67 mmol of isocyanate groups) was dissolved in anhydrous DMAc (50 mL), and then mixed with a solution of **5** (860 mg, 1.15 mmol) in anhydrous DMAc (15 mL). The reaction mixture was stirred for 5 d at 40 °C under argon atmosphere. The resultant polymer solid was precipitated out using ethyl ether, and then filtered and washed with ether. The filter cake was dried under vacuum for 2 d to yield the desired polymer **7** (930 mg). IR (neat) = 3327 cm<sup>-1</sup> (N-H), 2920 & 2864 cm<sup>-1</sup> (CH<sub>2</sub>), 1715 & 1691 (C=O), 1616 cm<sup>-1</sup> (urea), 1530 cm<sup>-1</sup> (N-H bending), 1247 cm<sup>-1</sup> (C-N), 1104 cm<sup>-1</sup> (C-O). <sup>1</sup>H-NMR (CDCl<sub>3</sub>): δ(ppm) 1.434~1.471 (Boc-CH<sub>3</sub>).

**TPU-PEG-cyclen-Cu<sup>II</sup> (1).** After the cyclen moiety was appended to the modified TPU, TFA/CHCl<sub>3</sub> was employed to remove Boc groups from the cyclen moieties at low temperature as reported previously<sup>10</sup>, and the polymer TPU-PEG-cyclen (**8**) was obtained with a yield of 580 mg, starting from 930 mg of TPU-PEG-cyclen-3Boc (**7**). IR (neat) = 3327 cm<sup>-1</sup> (N-H), 2918 &

2863  $\text{cm}^{-1}$  ( $\text{CH}_2$ ), 1715 & 1692 ( $\text{C}=\text{O}$ ), 1615  $\text{cm}^{-1}$  (urea), 1531  $\text{cm}^{-1}$  (N-H bending), 1247  $\text{cm}^{-1}$  (C-N), 1103  $\text{cm}^{-1}$  (C-O).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ): the bands for the Boc- $\text{CH}_3$  groups disappeared.

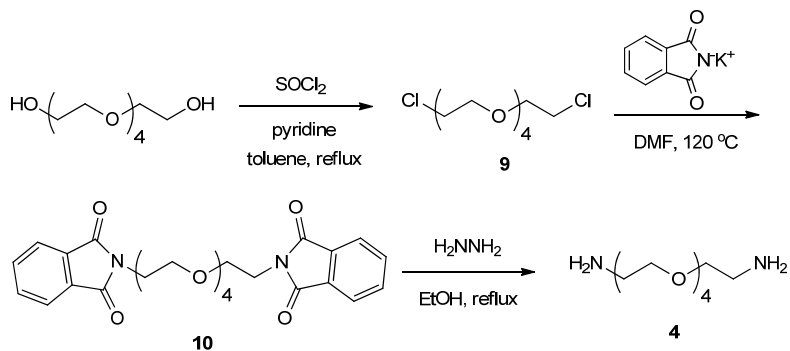
At last, copper ions were incorporated into the deprotected cyclen by treatment of the polymer **8** (580 mg) with cupric chloride dihydrate in absolute ethanol at 50  $^\circ\text{C}$ <sup>10, 11</sup> to produce a slightly bluish polymer TPU-PEG-cyclen- $\text{Cu}^{\text{II}}$  (**1**) (500 mg). IR (neat) = 3325  $\text{cm}^{-1}$  (N-H), 2927 & 2888  $\text{cm}^{-1}$  ( $\text{CH}_2$ ), 1715 & 1693 ( $\text{C}=\text{O}$ ), 1617  $\text{cm}^{-1}$  (urea), 1530  $\text{cm}^{-1}$  (N-H bending), 1249  $\text{cm}^{-1}$  (C-N), 1100  $\text{cm}^{-1}$  (C-O).

#### **1.4 Preparation of the polymeric films used in NO generating measurements.**

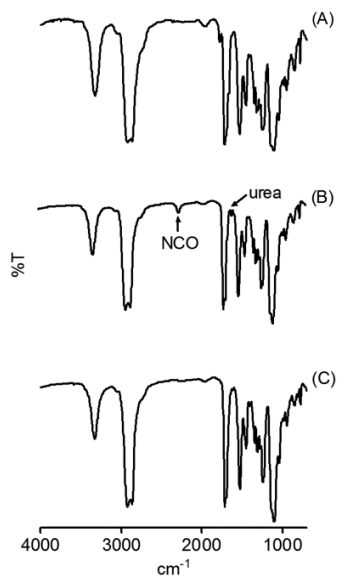
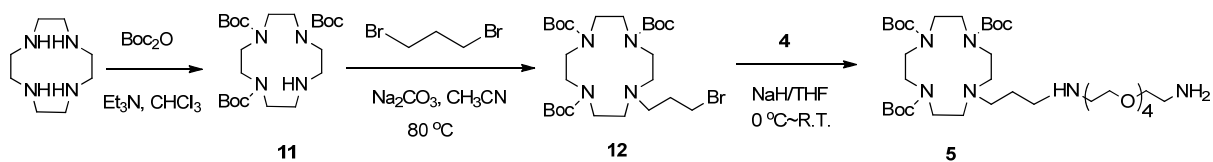
All the polymeric films used in NO generating measurements were prepared following an established method<sup>12</sup>: the polymer was dissolved in freshly distilled THF to make a 4% (g/mL) solution. The polymer solution was then cast into a 2.8 cm diameter glass ring fixed on a Teflon base. Depending on the thickness of the film needed, the volume of the polymer solution was calculated and applied accordingly. The films were allowed to cure overnight while covering. A small film was cut from the parent film and evaluated for the NO generation *via* the chemiluminescence NOA.



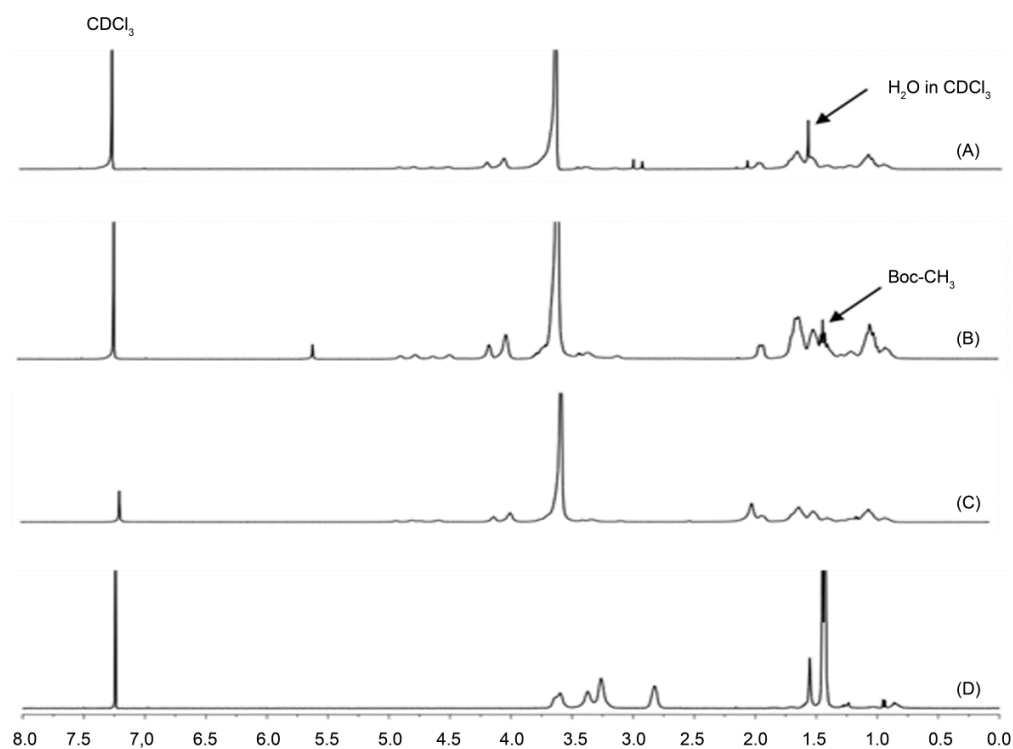
**Scheme S1.** Synthesis of an amino-terminated PEG linker (**4**).



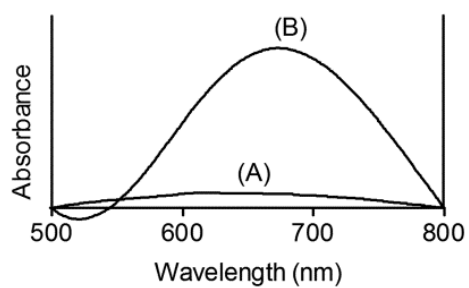
**Scheme S2.** Synthesis of a cyclen derivative: 3Boc-cyclen-PEG-amine (**5**).



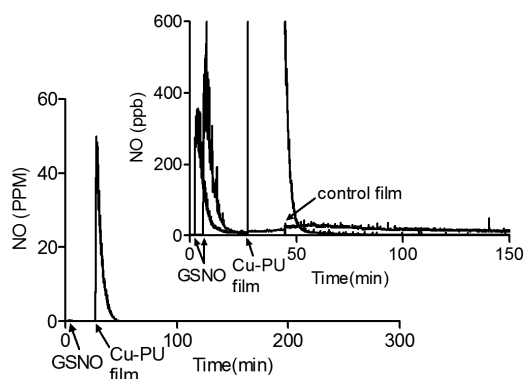
**Figure S1.** Typical IR spectra of (A) TPU (Tecophilic, SP-93A-100), (B) TPU-NCO, and (C) TPU-PEG-cyclen-3Boc.



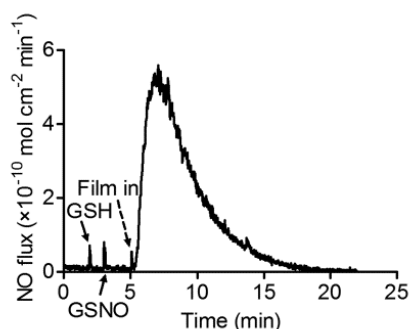
**Figure S2.** Typical  $^1\text{H}$  NMR spectra of (A) TPU-NCO, (B) TPU-PEG-cyclen-3Boc, (C) TPU-PEG-cyclen, and (D) 3Boc-cyclen as measured in  $\text{CDCl}_3$ .



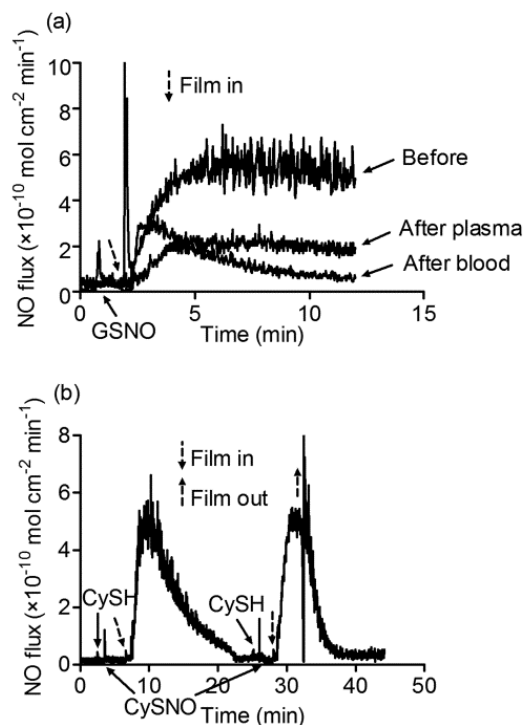
**Figure S3.** UV-Vis spectra of (A) TPU-PEG-cyclen and (B) TPU-PEG-cyclen- $\text{Cu}^{\text{II}}$  as measured in THF.



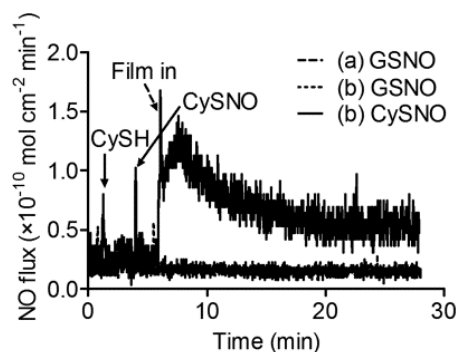
**Figure S4.** The measurements of NO generation catalyzed by a film of TPU-PEG-cyclen-Cu<sup>II</sup> (**1**) (thickness = 30  $\mu\text{m}$ , Cu content = 0.47 wt %, weight = 0.86 mg) and a corresponding control film of TPU-PEG-cyclen (**8**) (weight = 0.89 mg) (see inset figure data with expanded scale) in a solution of 1 mM GSNO/CySH in 10 mM PBS buffer (pH 7.4) containing 3  $\mu\text{M}$  EDTA *via* a chemiluminescence NOA.



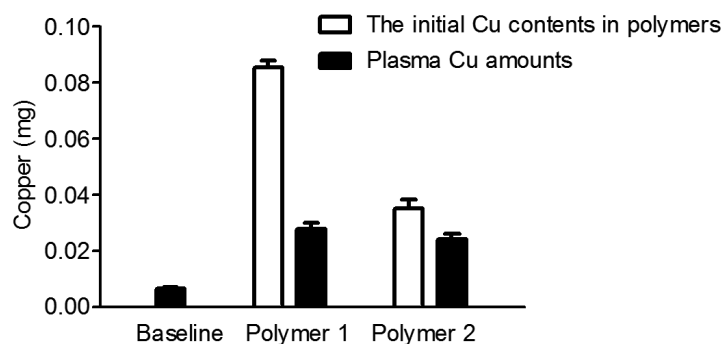
**Figure S5.** *In vitro* generation of NO from 1  $\mu\text{M}$  GSNO, 30  $\mu\text{M}$  GSH and 5  $\mu\text{M}$  EDTA in PBS (10 mM, pH 7.4) at 37  $^{\circ}\text{C}$  catalyzed by a small film of TPU-PEG-cyclen-Cu<sup>II</sup> (**1**) (area = 0.38  $\text{cm}^2$ , thickness = 30  $\mu\text{m}$ , Cu content = 0.47 wt %).



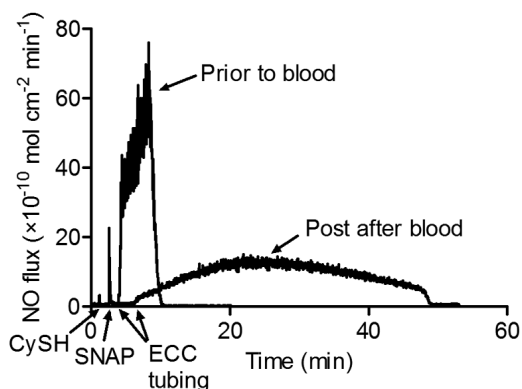
**Figure S6.** The measurements of NO generation catalyzed by the films of TPU-PEG-cyclen- $\text{Cu}^{\text{II}}$  (**1**) (thickness = 30  $\mu\text{m}$ , Cu content = 0.47 wt %) in a solution of (a) 5  $\mu\text{M}$  GSNO/GSH (area = 0.29  $\text{cm}^2$ ), or (b) 0.5  $\mu\text{M}$  CySNO/CySH (area = 0.23  $\text{cm}^2$ ) in 10 mM PBS buffer (pH 7.4, 3  $\mu\text{M}$  EDTA) before and after soaking in a platelet-rich sheep plasma or whole sheep blood at 4  $^{\circ}\text{C}$  for 24 h *via* a chemiluminescence NOA.



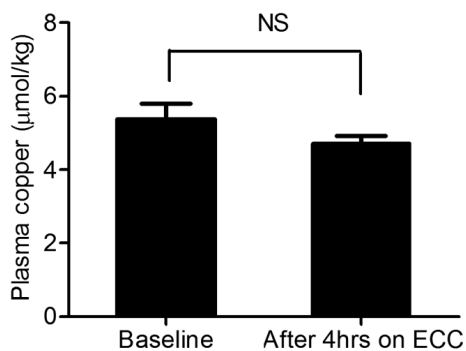
**Figure S7.** The evaluation of NO generation catalyzed by the polymer **2** (Cu content = 0.11 wt %) using the same sized films in a solution of 5  $\mu\text{M}$  GSNO/GSH or 0.5  $\mu\text{M}$  CySNO/CySH in 10 mM PBS buffer (pH 7.4, 3  $\mu\text{M}$  EDTA) after contacting with (a) whole sheep blood or (b) platelet-rich sheep plasma at 4  $^{\circ}\text{C}$  for 24 h measured by a chemiluminescence NOA.



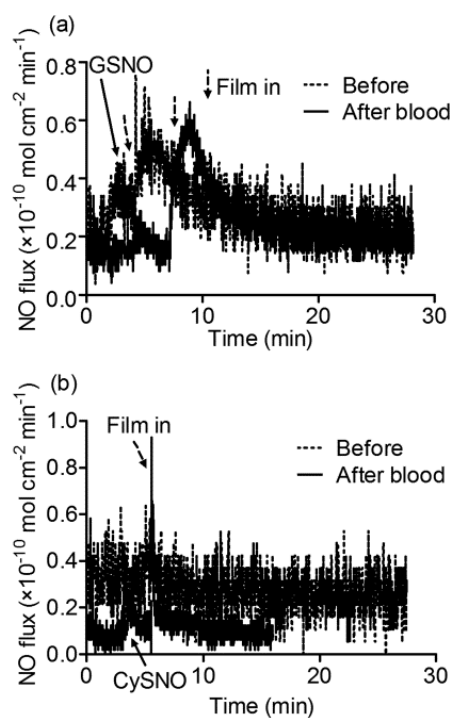
**Figure S8.** Plasma copper concentration assay *via* ICP-AES. Quantitation of the total copper amounts leached out of the TPU-PEG-cyclen-Cu<sup>II</sup> (**1**) material and polymer **2** into plasma after soaking with fresh whole sheep blood at 4 °C for 2 days.



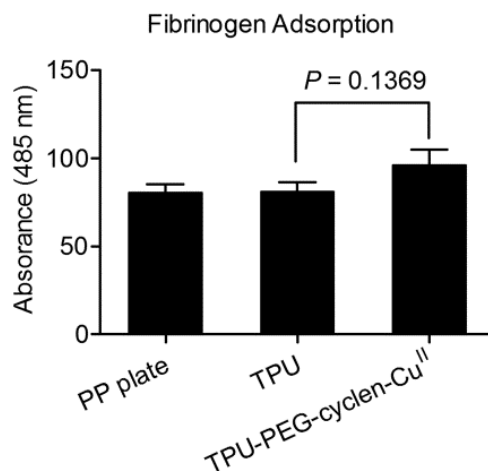
**Figure S9.** The evaluation of NO generation catalyzed by ECC tubing coated with TPU-PEG-cyclen-Cu<sup>II</sup> (**1**) (Cu content = 0.38 wt %) in a solution of 2  $\mu$ M SNAP and 30  $\mu$ M CySH in 10 mM PBS buffer (pH 7.4, 5  $\mu$ M EDTA) at 37 °C prior to and 4 h after ECC blood exposure, as measured using the chemiluminescence NOA.



**Figure S10.** Plasma copper concentration assay *via* ICP-HRMS. Quantitation of the total copper concentration in rabbit plasma prior to and 4 h after the polymer TPU-PEG-cyclen-Cu<sup>II</sup> (**1**) based ECC blood exposure. NS = No Significance.



**Figure S11.** The measurements of NO generation on the control polyurethane (SP-93A-100) using the same thickness films in a solution of (a) 5 µM GSNO/GSH (area = 0.19 cm<sup>2</sup>) or (b) 0.5 µM CySNO/CySH (area = 0.38 cm<sup>2</sup>) in 10 mM PBS buffer (pH 7.4, 3 µM EDTA) before and after soaking with whole sheep blood for 24 h at 4 °C *via* chemiluminescence NOA.



**Figure S12.** *In vitro* fibrinogen adsorption immunofluorescence assay with fluorescein-labeled goat IgG (polyclonal) antibody for TPU-PEG-cyclen-Cu<sup>II</sup> (**1**) and TPU (SP-93A-100) precoated on the polypropylene (PP) plate (thickness = 32  $\mu$ m, Cu content = 0.1 wt %) utilizing 3 mg/mL of human fibrinogen ( $P > 0.1$ ). The blank polypropylene was used as an internal control to normalize the fluorescence signals within different plates. All measurements were recorded at 485 nm (excitation) and 530 nm (emission) on a Synergy 2 fluorescence microplate reader.

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