

Supporting Information

Poly(oxanorbornene)-Coated CdTe Quantum Dots as Antibacterial Agents

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Butyl Poly(oxanorbornene) Polymers (PONs) Synthesis and Characterization

Materials. All chemicals and solvents were used as received from Sigma Aldrich, Fluka, or Acros Organic.

Instrumentation. Gel permeation chromatography (GPC) was performed on a Polymers Standards Service styrene-divinylbenzene (SDV) copolymer network column using chloroform as the solvent system at a 1.0 mL/min flow rate and 30 °C. GPC was calibrated with poly (methyl methacrylate) standards. Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker 250 MHz spectrometer. Deuterated methanol and chloroform were used as solvents, and tetramethylsilane was used as the internal reference.

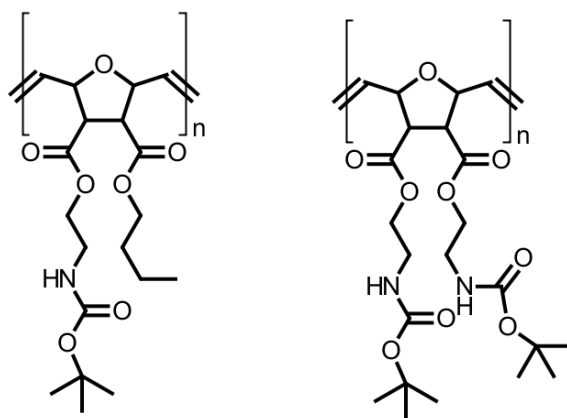
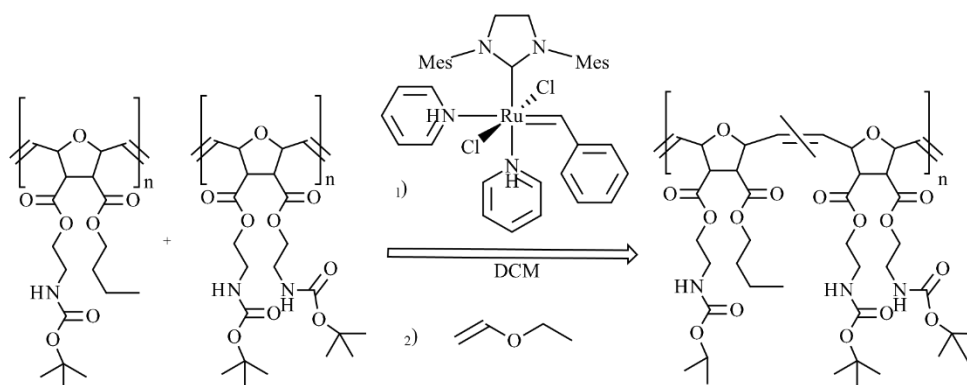


Figure S1. Structure of the two BOC-protected oxanorbornene monomers **B** (left) and **D** (right).

Polymer Synthesis and Characterization. The polymers in this study are the same as reported in the publication by Zheng *et al.* on the membrane disruption activity of PONs-covered gold nanoparticles.¹ Figure S1 shows the two monomers, one with a butyl and an amine side chain (**B**) and the other with two diamine side chains (**D**), synthesized and polymerized according to previously reported protocols.^{2,3} The monomers were synthesized with tert-butyloxycarbonyl(BOC) protection on the amine functional groups. For polymerization (Scheme S1), dichloromethane mixtures of the monomers and Grubbs' third generation catalyst, synthesized as described by Love *et al.*,⁴ were allowed to react for 30 minutes under inert N₂ gas. The ratio of **B** to **D** monomers was varied in order to obtain polymers with varying amine content (Table S1). The reaction was stopped with the addition of excess ethyl vinyl ether. BOC-protected polymers were precipitated with cold hexane and dried under vacuum. To remove the BOC protecting groups, polymers were dissolved in a 1:1 mixture of dry chloroform and 4M HCl in dioxane. The precipitated, deprotected polymers were dissolved in dry methanol and finally re-precipitated in cold diethyl ether under vacuum. NMR and GPC were used to confirm the polymer structure and mass according to previous literature.



Scheme S1. Grubbs' third generation catalysts initiated polymerization of BOC-protected **B** and **D** monomers via ring opening metathesis.

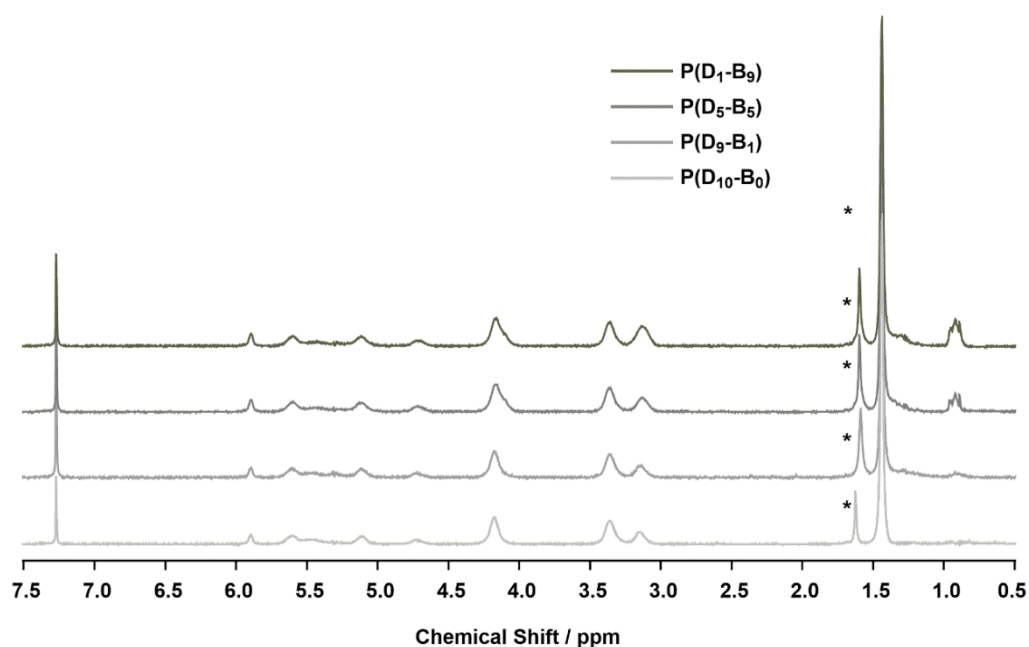


Figure S2. NMR data of BOC-protected PONs in deuterated chloroform. ¹H-NMR (250 MHz, CDCl₃) δ = 5.85 - 5.93 (br m, C=CH, trans), 5.55 - 5.66 (br m, C=CH, cis), 5.32 - 5.46 (br s, NH), 5.07 - 5.19 (br m, C=CH-CH, cis), 4.65 - 4.76 (br m, C=CH-CH, trans), 4.04 - 4.26 (m, O-CH), 3.27 - 3.45 (br m, N-CH₂), 3.05 - 3.21 (br m, CH), 1.55 - 1.63 (m, CH₂), 1.23 - 1.49 (m, B-CH₂ & 3 \times boc-CH₃), 0.93 (t, B-CH₃). Note: * Indicates water peak.

Table S1. The properties of the BOC-protected PONs, which were synthesized to have 10 repeat units of varying amine content. *Determined via GPC analysis.

PONs Sample	n _D : n _B	Average MW _{Monomer} (g/mol)	Average MW _{Polymer} * (g/mol)
55% Amine	1:9	391.89	3900
75% Amine	5:5	426.71	4000
95% Amine	9:1	461.53	4700
100% Amine	10:0	470.23	4700

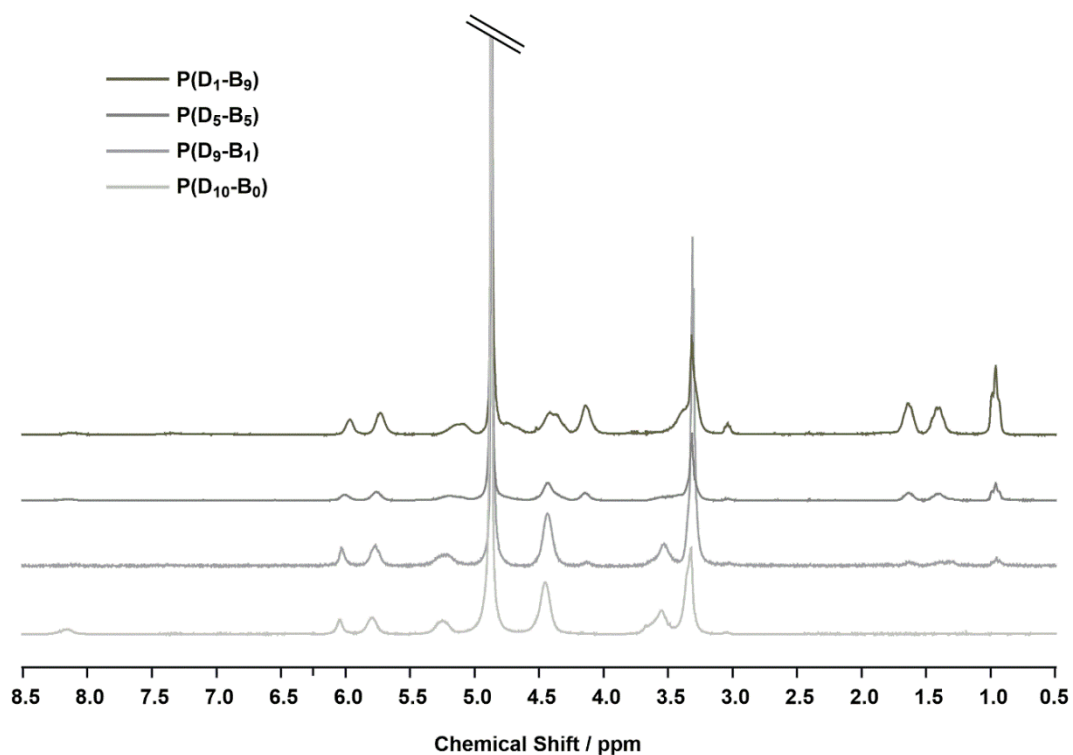


Figure S3. NMR data for deprotected PONs. ^1H -NMR (250 MHz, MeOD- d_4 , peaks corresponding to $\text{C}=\text{CH}-\text{CH}$ (trans) and CH overlapped with the solvent signals) $\delta = 8.05 - 8.20$ (br. m, NH_3^+), $5.89 - 6.05$ (br m, $\text{C}=\text{CH}$, trans), $5.63 - 5.84$ (br m, $\text{C}=\text{CH}$, cis), $5.01 - 5.29$ (br m, $\text{C}=\text{CH}-\text{CH}$, cis), $4.01 - 4.53$ (m, $\text{O}-\text{CH}$), $3.30 - 3.70$ (m, $\text{N}-\text{CH}_2$), $1.55 - 1.73$ (m, CH_2), $1.30 - 1.50$ (m, $\text{B}-\text{CH}_2$), 0.97 (t, $\text{B}-\text{CH}_3$).

Table S2. The properties of the deprotected PONs, which were synthesized to have 10 repeat units of varying amine content.

PONs Sample	$n_D : n_B$	Average $\text{MW}_{\text{Monomer}}$ (g/mol)	Average $\text{MW}_{\text{Polymer}}$ (g/mol)
55% Amine	1:9	281.74	2800
75% Amine	5:5	276.52	2800
95% Amine	9:1	271.30	2700
100% Amine	10:0	270.00	2700

Additional Characterization of the MPA-QDs

In the main text, we report the size of the QDs as determined by calculations from the absorbance measurements and DLS measurements in Millipore water (pH11) as this is the system in which the MPA-QDs were synthesized and stored until use. Here we characterize the size via DLS at pH 7, to capture the size distribution at physiological pH, and via HRTEM.

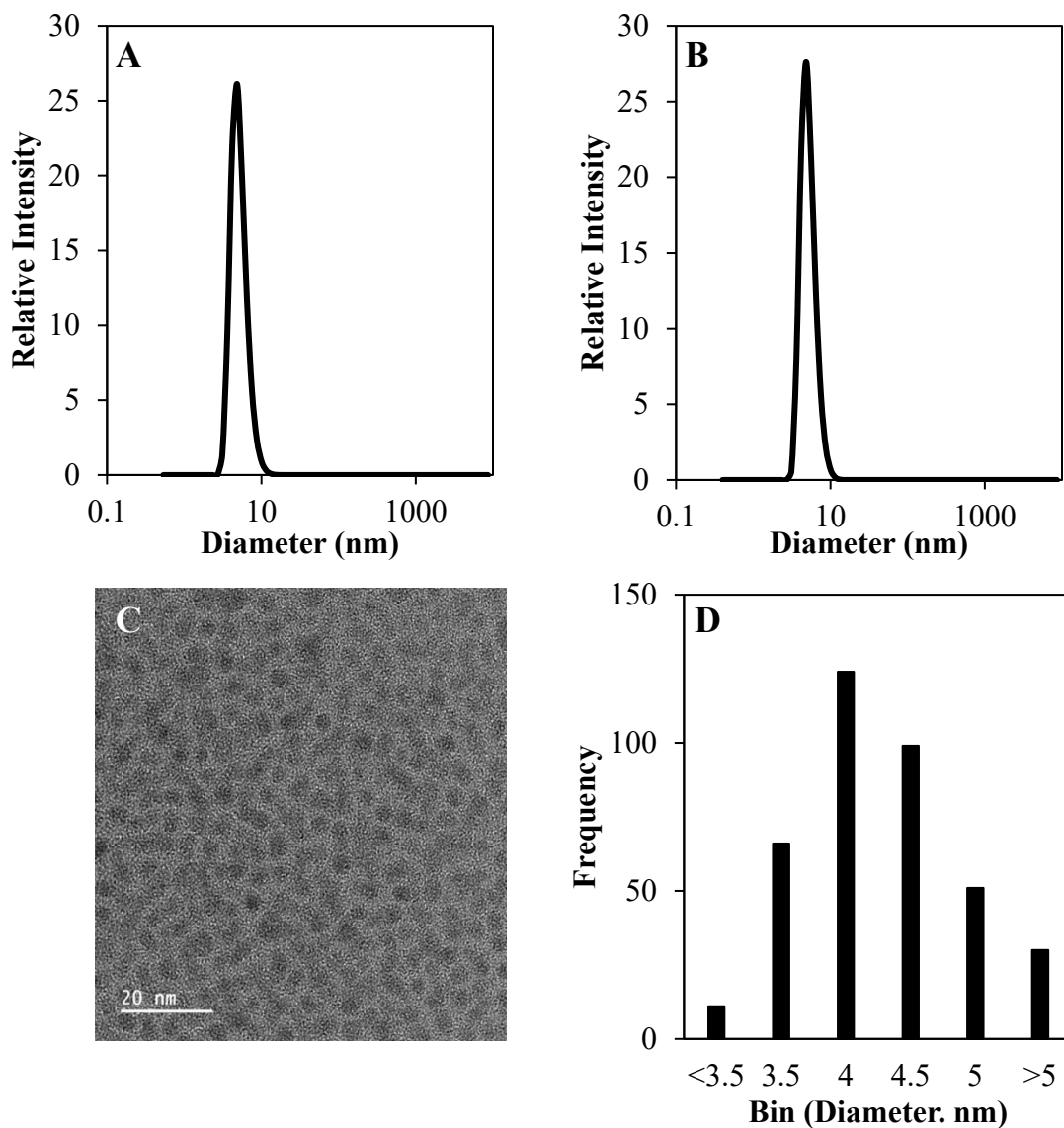


Figure S4. (A) DLS-measured number size distribution of MPA-QDs in Tris buffer (pH 7) determined their hydrodynamic diameter to be 5.2 ± 1.3 nm; N=3. (B) DLS-measured number size distribution of MPA-QDs in water (pH 7) determined their hydrodynamic diameter to be 5.2 ± 1.2 nm; N=3. (C) A representative HRTEM image of MPA-QDs. (D) Analysis of HRTEM images, using ImageJ, of MPA-QDs determined their diameter to be 4.0 ± 0.6 nm; N=381.

LED Panel Characterization

An Environmental Lights Ultra Thin LED Light Panel (Neutral White) was used to irradiate MPA-QDs and PONs-QDs during cell incubation and ROS assays. The exposure procedure for LED irradiated incubations was similar to those without incubation, except the well plates were incubated while sitting on top of the panel for two hours at the beginning of the MIC incubation or the entire duration of the hemolysis incubation. The panel was measured to have an average output of 3mW using the Newport Corporation 843-R Optical Power Meter, with the 919P-030-18 thermopile sensor placed on the opposite side of an empty 96 well plate from the LED panel.

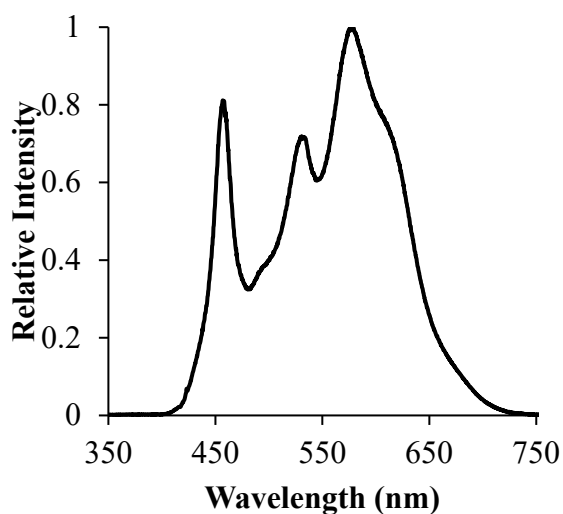


Figure S5. Characterization of the LED panel's emission spectrum using an EO Edmund CCD Spectrometer(D).

Minimum Inhibitory Concentration (MIC) Assay with *E. coli*

The MIC procedure was conducted with methods similar to those reported in the literature.⁵ The assay, with controls and varying concentrations of the substrates, was completed in 96 well plates. Duplicates of each sample were averaged together to equal one biological replicate. At least three biological replicates were completed for each sample and concentration. The OD₅₉₅ of the cells was measured after overnight incubation with the substrates. The percent cell growths reported in Figure 3 and Table S3 were calculated via the equation: Percent Cell Growth = $[(OD_{595, \text{Substrate}} - OD_{595, \text{Medium control}}) / (OD_{595, \text{Growth control}} - OD_{595, \text{Medium control}})] * 100$.

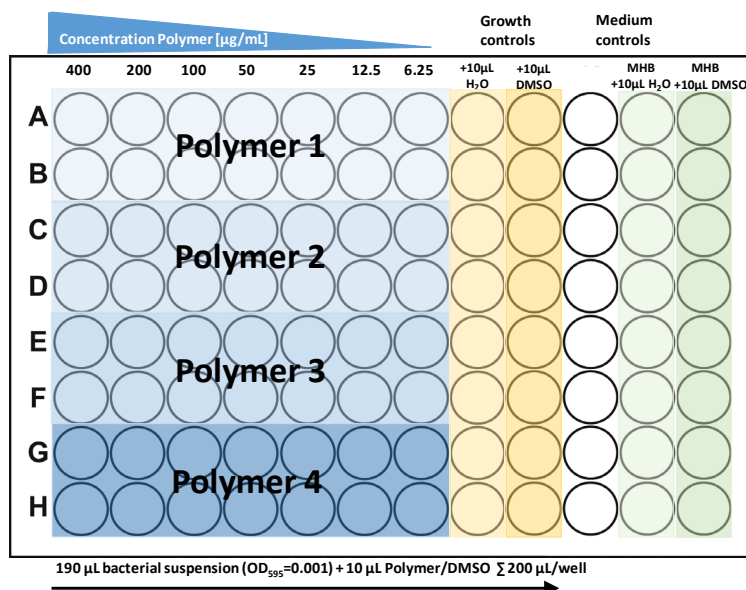


Figure S6. Example 96 well plate layout for one biological replicate of the bacteria MIC assay. Note: Empty wells were filled with 200uL of sterile water.

Table S3. Antibacterial activity in µg/mL of the PONs-QDs and free PONs

Sample	Free PONs		PONs-QDs			
	MIC optical	MIC ₉₀	MIC optical		MIC ₉₀	
			No Irradiation	With Irradiation	No Irradiation	With Irradiation
55% Amine	25	12.5	100	200	100	100
75% Amine	200	100	>400	100	200	100
95% Amine	200	>400	200	200	200	200
100% Amine	200	>400	200	100	100	50
MPA-QDs	-	-	0.3 µM	0.25µM	0.3 µM	0.25µM

MIC optical values = the concentration at which no bacterial growth is visible to the naked eye
MIC₉₀ = the concentration at which the bacterial growth is below 10% according to the optical density.

Human Red Blood Cell Lysis (Hemolysis) Assay

The hemolysis assay was conducted as previously described.⁵ The assay was completed in 96 well plates, with duplicates of each control and substrate sample averaged together to equal one biological replicate. At least three biological replicates were completed for each sample concentration. The OD₄₁₄ was measured of the red blood cell supernatant after 30 minutes of cell incubation with the substrates. Triton X 100 was used as positive control. The percent hemolysis values reported in Figures 4 and S8 were calculated via the equation:

$$\text{Percent Hemolysis} = \frac{[(\text{OD}_{414, \text{Substrate}} - \text{OD}_{414, \text{Solvent Blank}}) / (\text{OD}_{414, \text{Positive control}} - \text{OD}_{414, \text{Solvent Blank}})] * 100}.$$

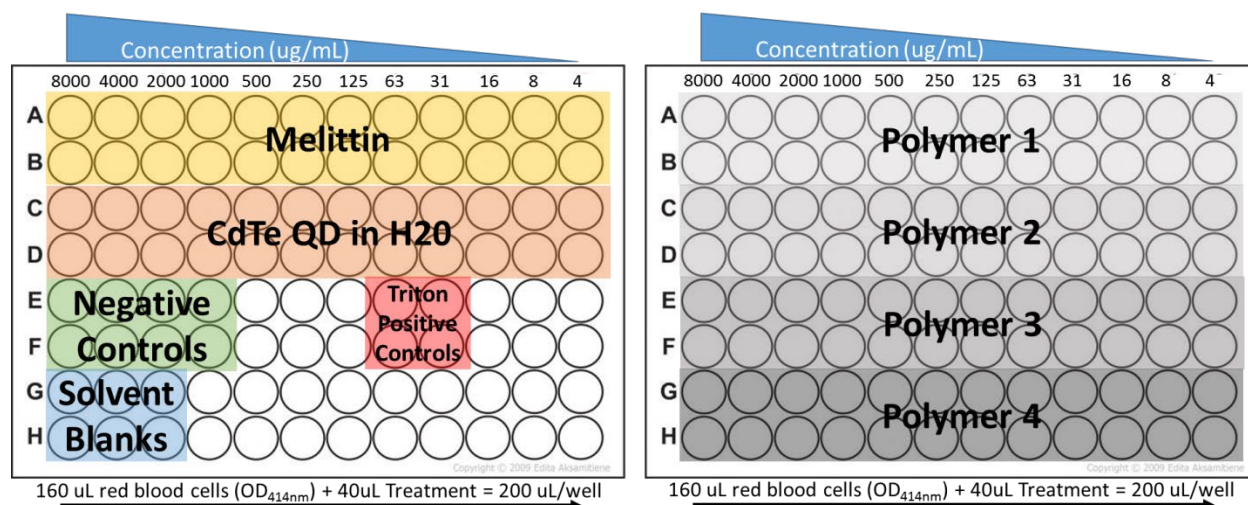


Figure S7. Example plate layouts for one biological replicate of the hemolysis assay.

Note: Empty wells were filled with water. Melittin and Triton were both tested as positive controls. Triton caused the greatest hemolysis in this study and was thus used as the positive control in the calculations.

Hemolytic Activity of Free PONs, MPA-QDs, and PONs-QDs

The hemolytic activity of the PONs-QDs was investigated parallel to that of the free PONs' and MPA-QDs conjugates' activity in the hemolysis assays. Incubations were done with and without LED panel irradiation during incubation. Irradiated incubation did not change the hemolytic activity of the MPA-QDs or PONs-QDs samples (data not shown).

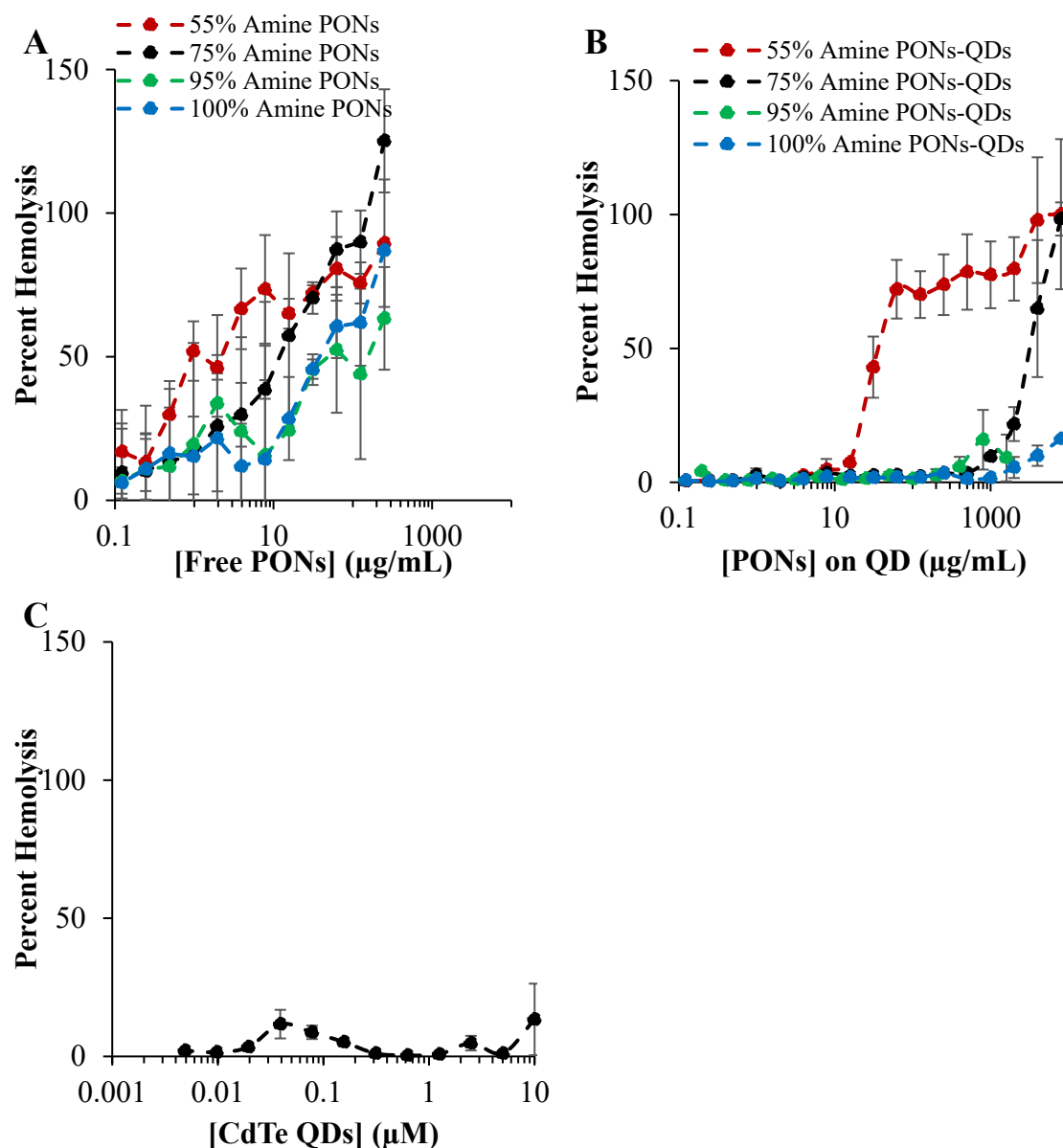


Figure S8. Hemolytic activity of PONs-QDs compared to free PONs molecules and MPA-QDs. All PONs-QDs (B) have lower hemolytic activity than free PONs molecules (A). (C) MPA-QDs had no hemolytic activity at tested concentrations. ($N \geq 3$ biological replicates.)

Therapeutic Indices of Free PONs, PONs-QDs and MPA-QDs

Table S4. Therapeutic Indices (HC_{50}/MIC_{90}) for *E. coli*.

PONs Sample	Free PONs	PONs-QDs (With Irradiation)
55% Amine	0.08	0.63
75% Amine	0.12	40
95% Amine	Inconclusive	>40
100% Amine	Inconclusive	>160
MPA-QDs	>40	

Note: *E. coli* strain ATCC 25922 was used in the literature source⁵ and this study.

If,

‘>’: samples did not have hemolytic activity at measured concentrations

‘Inconclusive’: samples did not have antibacterial activity at measure concentrations

References

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