Supplemental Information for:

Photoexcited quantum dots as efficacious and nontoxic antibiotics in an animal model

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Supplementary Methods

Characterization of CdTe-2.4 QDs

Cysteine-coated CdTe-2.4 QDs, synthesized and filtered as described in Materials and Methods, were diluted to a concentration of 800 nM in pH 7 deionized water or pH 7 deionized water with 10% fetal bovine serum (Biowest LLC, Riverside, MO). QDs were then added to an Omega cuvette (Anton-Paar) and characterized using an Anton-Paar Litesizer 500 particle size analyzer. Hydrodynamic radius and zeta potential were measured.

Antibiotic Resistance Screening of Clinical Isolates:

The clinical isolates were provided by the lab of Nancy Madinger at the University of Colorado Anschutz campus and maintained as freezer stocks in 90% CAMHB, 10% glycerol at -80°C. Clinical isolates were grown on solid CAMHB with 1.5% agar at 37°C. Freezer stocks were streaked out onto solid CAMHB with 1.5% agar and incubated for 16 h to produce single colonies. For each biological replicate, a single colony was picked from solid media and grown for 16 h in liquid CAMHB at 37°C with 225 rpm shaking prior to experiments. Multidrug-resistant *Escherichia coli*, *Salmonella typhimurium*, and extended-spectrum beta-lactamase producing *Klebsiella pneumoniae* were tested against the following antibiotics: ampicillin, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, meropenem, nalidixic acid, and tetracycline. Liquid cultures of the clinical strains were diluted to a 0.5 McFarland standard and added to the respective antibiotic test condition. The antibiotic minimum inhibitory concentration (MIC) for each clinical isolate was determined as the lowest antibiotic concentration which prevented visible cell growth for 24 h, as determined by optical density measurement. Sensitive/resistant breakpoints were taken from the 2020 Clinical & Laboratory Standards Institute report[1] (Table S1). Strains were "sensitive" if the MIC was equal to or below the sensitive-

breakpoint concentration, “resistant” if the MIC was greater than or equal to the resistant-breakpoint concentration, and “intermediate” if the MIC was in between.

Toxicity of CdTe-2.4 QDs in HeLa Cells in vitro:

HeLa cells (American Type Culture Collection, Manassas, VA) were brought up from long-term storage, 10% dimethyl sulfoxide and full growth media in liquid nitrogen, and maintained at sub-confluent density in full growth media, Dulbecco's Modified Eagle Medium (DMEM, Thermo Fisher Scientific, Waltham, MA) supplemented with 10% fetal bovine serum (Biowest LLC, Riverside, MO) and 1% penicillin and streptomycin, at 37℃, 5% CO_2, and 100% humidity. Once recovered from freezer stock, cells were passaged into three separate flasks and maintained as separate biological replicates. HeLa cells were seeded at 1.0×10^4 cells per well in 96-well tissue culture plates and grown for 24 hours. Cells were then washed with Dulbecco’s PBS (DPBS, Thermo Fisher Scientific, Waltham, MA) and incubated with media containing appropriate concentrations of CdTe-2.4 QDs under white light for 18 hours. Cells were then assessed for viability using a resazurin colorimetric assay. After washing with DPBS, cells were incubated in a 44 μM solution of resazurin sodium salt (Sigma Life Sciences, St. Louis, MO) dissolved in media for three hours at 37℃ while protected from light. Fluorescence was measured at 560 nm excitation, 590 nm emission.

Pilot Studies for Subcutaneous Infection in Mice

Twenty-one female mice (age 8-11 weeks, Charles River Laboratories) were infected with MDR *E. coli* and monitored for seven days. The mice were kept together, three to five to a cage, in standard cages.

The injection site on the dorsal region of each mouse was shaved, then mice were injected subcutaneously with 50 μl *E. coli* at 1.0×10^8 to 1.0×10^{10} CFU/ml per mouse under the dorsal skin.

The bacteria were allowed to grow without any treatment for a period of one hour. Three mice then received 50 µl 4mg/kg gentamicin (Lonza, Walkersville, MD) in PBS. Dosing of gentamicin occurred every 24 hours.

On the sixth day after initial injection, mice were euthanized by CO_2 exposure. Abscess or skin samples were excised and gently homogenized in 1 ml PBS per sample. The resulting solution was serially diluted and spread onto solid CAMHB agar plates with 50 ug/ml ampicillin for selectivity toward MDR *E. coli*. Plates were left to grow for 12 hours in 5% CO_2 at 37°C to determine CFU counts.

(1) Clinical and Laboratory Standards Institute. *M100 Perfomance Standards for Antimicrobial Susceptibility Testing*, 30th ed.; 2020.

Supplementary Tables and Figures

Table S1: Resistance profiles of clinical isolates.

	AMP	CRO	CHL	CIP	GEN	KAN	MER	NXA	TET
MDR E. coli	R	R	S	R	S	S	S	R	R
S Typh	R	R	S	I	S	S	S	R	R
ESBL KPN	R	R	R	R	S	R	R	R	R

Table S1. Resistance profiles of clinical isolates were measured and compared to CLSI breakpoints. Multidrug-resistant *Escherichia coli*, *Salmonella typhimurium*, and extended-spectrum beta-lactamase producing *Klebsiella pneumoniae* were tested against the following antibiotics: ampicillin, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, meropenem, nalidixic acid, and tetracycline. “S” indicates susceptible, “I” indicates intermediate, and “R” indicates resistance, based on CLSI breakpoints.

Table S2: Pilot Studies for Subcutaneous Abscess Formation

Group	Initial Injection CFU/ml	Final Average CFU/abscess	Sample Size
Gentamicin	$2.5\text{-}7.3\times10^{8}$	0	3
Pilot 1 Low CFU	2.5×10^{8}	9.6×10^{7}	1
Pilot 1 Medium CFU	5.8×10^{8}	1.2×10^{10}	1
Pilot 1 High CFU	7.3×10^{8}	1.4×10^{10}	1
Pilot 2 Low CFU	8.8×10^{8}	$9.4\times10^{7} \pm 7.6\times10^{7}$	5
Pilot 2 Medium CFU	4.0×10^{9}	$1.5\times10^{8} \pm 6.0\times10^{7}$	5
Pilot 2 High CFU	1.3×10^{10}	$9.7\times10^{8} \pm 4.1\times10^{8}$	5

Table S2: Mice were injected subcutaneously with MDR *E. coli* and observed for six days. At time of euthanasia, abscesses or skin at injection site were excised and tested for CFU.

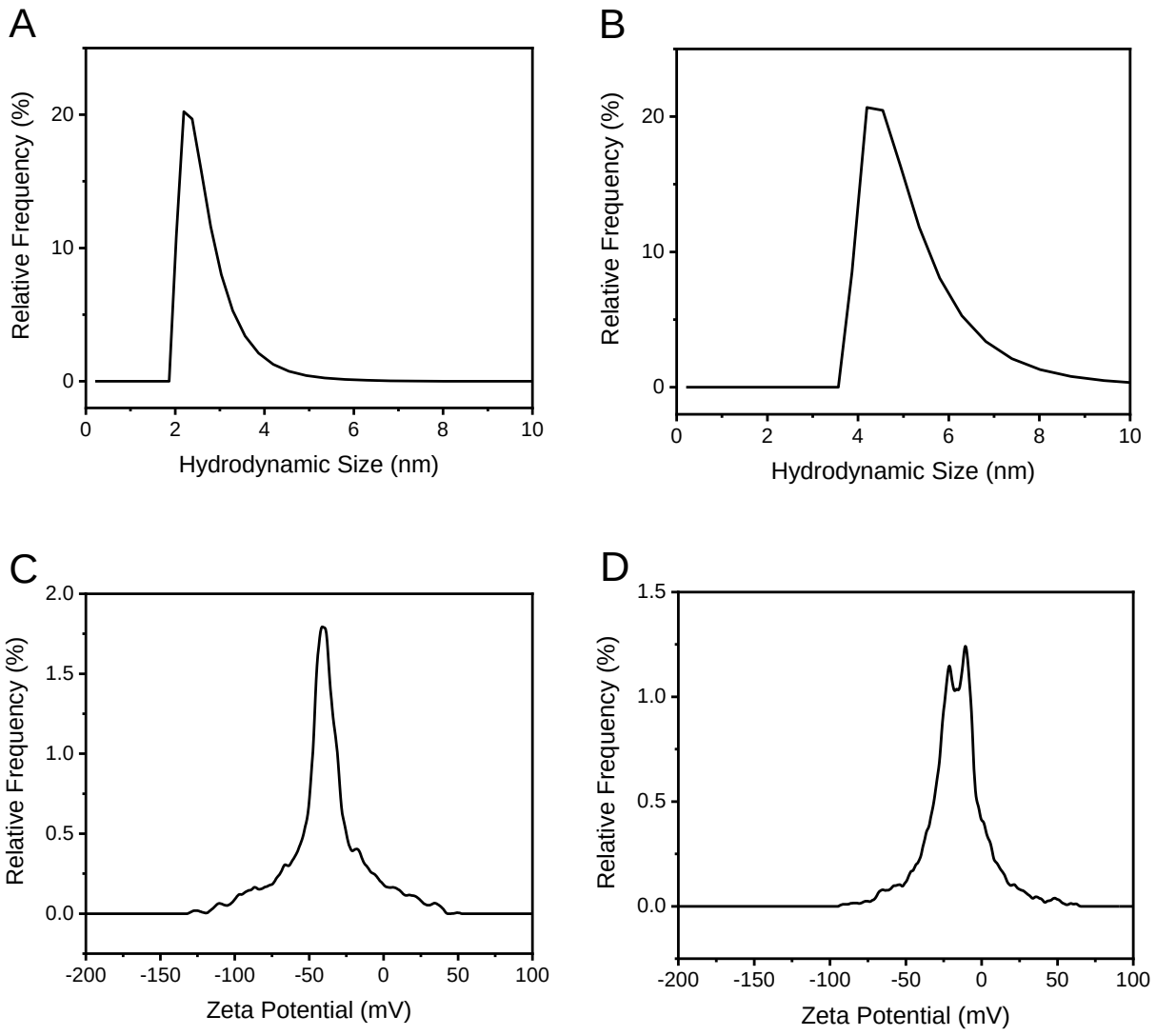

Figure S1. Characterization of cysteine-coated CdTe-2.4 QDs. Hydrodynamic radius and zeta potential of cysteine-coated CdTe-2.4 QDs were measured. Hydrodynamic radius was found to be 2.62 nm (± 0.42) for QDs alone (A) and 5.06 nm (± 0.82) with inclusion of 10% fetal bovine serum (B). Zeta potentials for the QDs were -44.99 mV (± 1.22) (C) and -11.06 mV (± 0.51) (D), respectively.

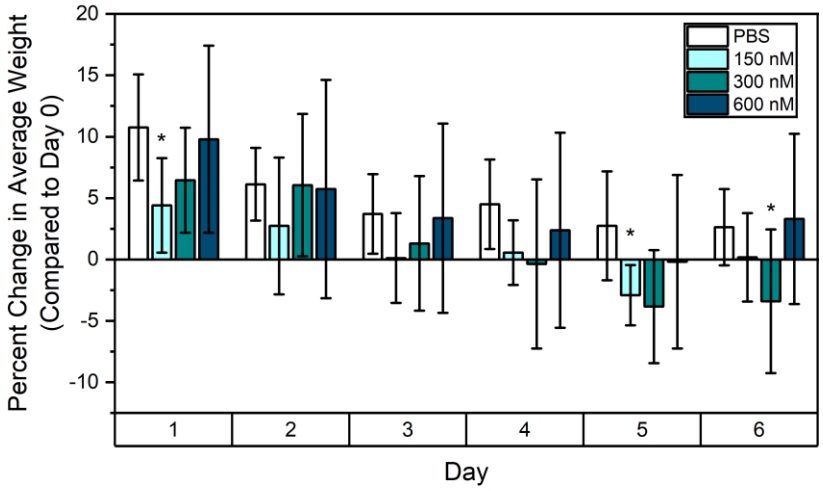

Figure S2. Body weight change of mice in dark after 6 consecutive days of daily QD treatment. Mice equipped with battery packs but not connected to LED lights were weighed daily, and daily weight was compared to initial weight at start of the study. Error bars represent standard deviation for each group, n = 8. When compared to PBS control mice in a two-tailed student's T-test, no groups differed significantly from PBS control ($p > 0.13$), except the 150 nM treatment group on day 1 and 5, and 300 nM treatment on day 6 ($p < 0.05$).

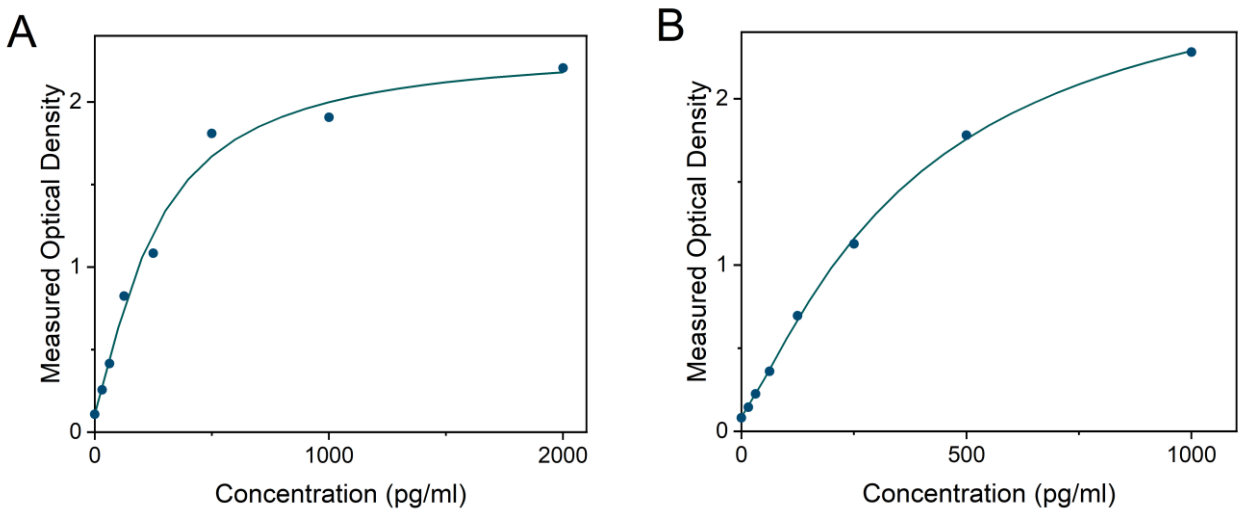

Figure S3. TNF-alpha and IL-6 levels were below limit of detection for all samples. Standard curves of ELISA results for serum TNF-alpha (A) and IL-6 (B) show limits of detection to be 31.25 pg/ml and 15.6 pg/ml, respectively. R-squared values are 0.9893 and 0.9995, respectively. Mouse serum from all groups showed values below 31.25 pg/ml for TNF-alpha and below 15.6 pg/ml for IL-6.

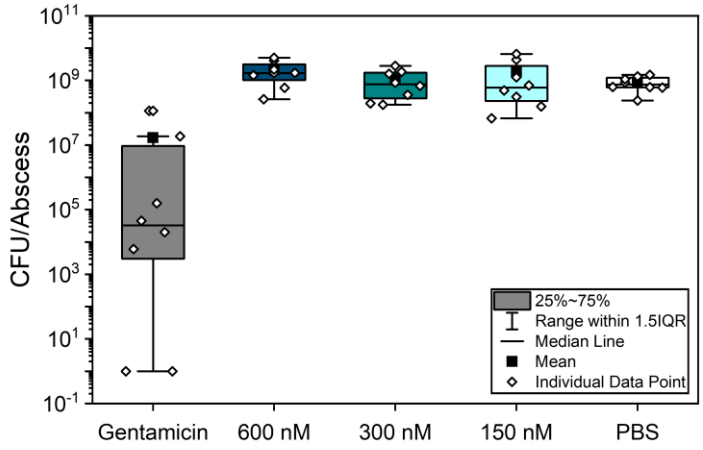

Figure S4. CdTe-2.4 QDs are unable to reduce CFU count in high-CFU abscesses. CFU/abscess of individual mice indicated with white diamonds (n = 8 per group). Mice infected with 3.5×10^{9} CFU/ml MDR *E. coli* were treated daily with 600, 300, or 150 nM QDs and compared to positive control mice treated with gentamicin or negative control mice treated with PBS. No difference was seen in final abscess CFU between mice treated with QDs and mice treated with PBS.

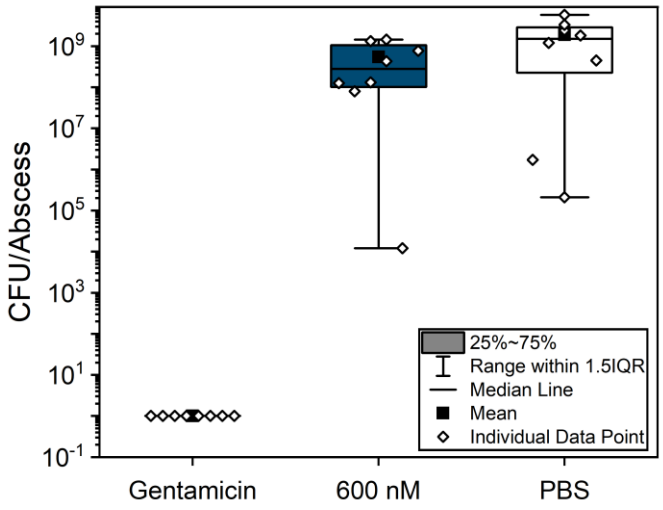

Figure S5. CdTe-2.4 QDs are unable to reduce CFU count in moderate-CFU abscesses. CFU/abscess of individual mice indicated with white diamonds (n = 8 per group). Mice infected with 5×10^8 CFU/ml MDR *E. coli* were treated daily with 600 nM QDs and compared to positive control mice treated with gentamicin or negative control mice treated with PBS. No difference was seen in final abscess CFU between mice treated with QDs and mice treated with PBS.

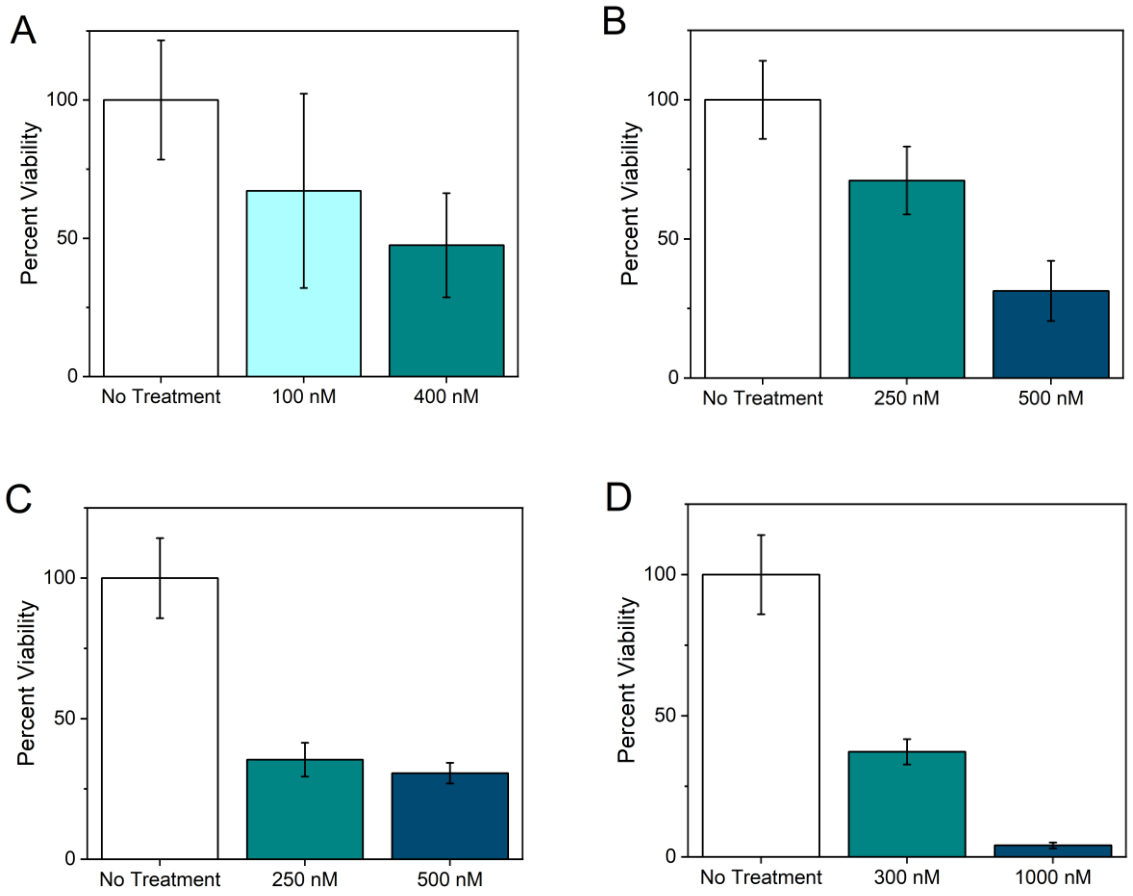

Figure S6. Toxicity of HeLa cells by incubation with CdTe-2.4 QDs is dose-dependent. In four separate experiments, HeLa cells were incubated with CdTe-2.4 QDs at varying concentrations for 18 hours under light and assessed for viability via resazurin assay, normalized to a no-treatment control. N = 3 biological replicates for each treatment group.

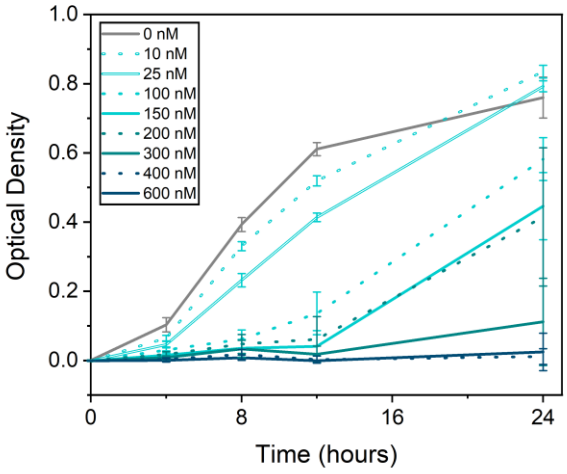

Figure S7. Growth of MDR *E. coli* inhibited in presence of CdTe-2.4 QDs at 100 nM and above. Growth of MDR *E. coli* incubated with CdTe QDs in presence of 3V battery-powered blue LEDs was measured over 24 hours. Blank reading was subtracted from optical density measurements. Bacteria were initially loaded at a 1:10,000 dilution. 150 nM QD treatment shows inhibition of bacterial growth but falls short of the IC50 compared to 0 nM control. 300 nM QD treatment shows significant growth inhibition, while no bacterial growth is observed for the 600 nM QD treatment.