

Control of nanoparticle penetration into biofilms through surface design

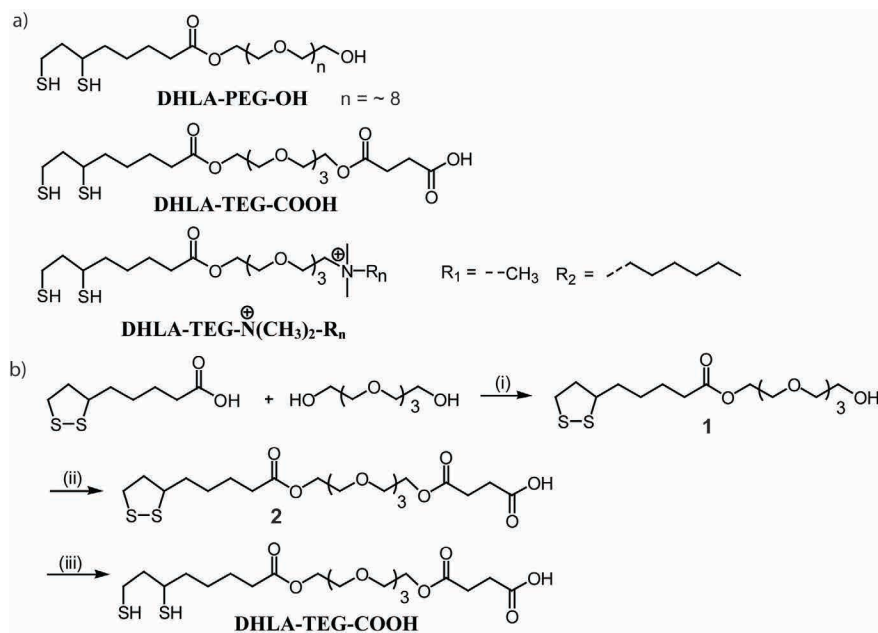
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Supporting Information

Materials

All chemicals were purchased from Sigma-Aldrich unless otherwise stated. The organic solvents were bought from Fisher and used as received while dichloromethane (DCM) was distilled in the presence of calcium hydride. Flash column chromatography was performed for purification using silica gel (SiO₂, particle size 40-63 μm). Milli-Q water from a Millipore Simplicity 185 system (Millipore Corp., Billerica, MA) was also used to prepare samples.

Synthesis of water soluble ligands



Scheme S1. (a) Structures of the ligands used in the studies. (b) Synthetic route of **DHLA-TEG-COOH** ligand. Reagents and conditions: (i) EDC, HOBt, DIPEA, DCM, r.t., 24 h; (ii) DMAP, DIPEA, succinic anhydride, THF, r.t., 24 h; (iii) NaBH₄, EtOH/ H₂O, r.t., 1 h.

Abbreviations: dihydrolipoic acid (DHLA), tri-ethylene glycol (TEG), poly-ethylene glycol (PEG), dichloromethane (DCM), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), hydroxybenzotriazole (HOBt), N,N-diisopropylethylamine (DIPEA), THF (tetrahydrofuran) and DMAP (4-Dimethylaminopyridine).

The syntheses of **DHLA-PEG-OH** and **DHLA-TEG-N(CH₃)₂-R_n** ligands have been reported in the published literature.^{1,2} **DHLA-TEG-COOH** ligands were synthesized in a stepwise

procedure (Scheme. S1b).³ Briefly, Compound **1** (2 g, 5.2 mmol) was dissolved in 30 ml dry THF (30 ml). DMAP (0.64 g, 5.2 mmol) and DIPEA (0.67 g, 5.2 mmol) were added to the solution and stirred for 5 mins. Succinic anhydride (1.57 g, 15.6 mmol) was added to the solution and stirred for 24 h at room temperature. The reaction mixture was evaporated under reduced pressure to remove the solvent, and further diluted with water and extracted with DCM. Compound **2** was obtained by using column chromatography with 100 % EtOAc as eluent. NaBH₄ was used to reduce the disulfide bond to obtain the final product **DHLA-TEG-COOH**.

¹H NMR (400 MHz, CDCl₃) of **DHLA-TEG-COOH**: δ 4.24 (t, 4H, COO-CH₂-CH₂ and CH₂-CH₂-OOC-) 3.74-3.62 (m, 12H, -CH₂-TEG-), 2.59-2.51 (brm, 2H, COO-CH₂-CH₂-COOH), 2.47-2.40 (brm, 2H, COO-CH₂-CH₂-COOH), 2.36 (t, 2H, CH₂-CH₂-COO), 1.75-1.62 (m, 4H), 1.58-1.39 (m, 2H).

Preparation of water soluble QDs

CdSe/ZnS core-shell QDs were synthesized according to the reported procedure.² The preparations of PEG-QDs and COOH-QDs were through a ligand exchange process.^{1,3} DHLA-PEG-OH or DHLA-TEG-COOH ligands have placed in DCM under ligand to hydrophobic trioctylphosphine oxide/trioctylphosphine (TOPO/TOP)-capped QDs ratio by weight (3: 1), and the solution was stirred at 35 °C for 24 h. After 24 h of stirring, DCM was evaporated and the resultant QDs were dispersed in Milli-Q water and purified by dialysis. The preparation of cationic QD (i.e., TTMA-QDs and Hexyl-QDs) was through a two-step ligand exchange reaction.² Briefly, hydrophobic TOPO/TOP-capped QDs were mixed with amphiphilic HS-(CH₂)₅-TEG-OH ligands in MeOH and the reaction mixture was stirred at 35 °C for 24 h. Next step involved the purification of amphiphilic QDs with hexane and the addition of DHLA-TEG-N(CH₃)₂-R_n ligands to the amphiphilic QDs in MeOH. After 24 h of stirring, MeOH was evaporated and the dithiolate cationic QDs were dispersed in Milli-Q water and purified by dialysis.

DLS and zeta potential

DLS experiments and zeta potential measurements were performed using a Malvern Zetasizer (Nano series, Malvern Instruments Inc, USA). Samples were sonicated and filtered with 0.2 μm syringe filter before measurement.

Biofilm culture

Bacteria were inoculated in 3 mL LB broth and grown to stationary phase at 37 °C. The cultures were then diluted 1:100 in a 1/10 strength LB broth supplemented with 100 μM IPTG (isopropyl β-D-1-thiogalactopyranoside). 25 mL of the dilution was transferred into a petri dish containing six sterile glass cover slips (22 × 22 mm). The petri dish was kept at 25 °C and the biofilms were allowed to grow for three days. In general, the cover slips with biofilms were rinsed in deionized water for three times before placed in QD solutions. The biofilms were incubated in 300 nM QD solutions and free QDs were washed away by rinsing in deionized water for three times.

CLSM image

The CLSM images were obtained using Zeiss510 META. The green fluorescent channel was collected at 488/505-550 band pass and the red fluorescent channel was collected at 543/560 longpass.

Cytotoxicity assay of QDs

Human Airway smooth muscle (ASM) cells were derived from dissociation of lung samples which were available following pneumonectomies or lobectomies under protocol approved by the Mayo Clinic Institutional Review Board. These non-infected lung specimens were collected from surgical pathology at Saint Mary's Hospital, Rochester, MN. ASM cells were isolated from 3rd-6th generation airway branches after separation from adventitia and the epithelium and placed in Hank's balanced salt solution (HBSS; Invitrogen). Collagenase and palpain was used to enzymatically dissociate the ASM layer and cells were seeded onto cell cultured flasks containing DMEM/F-12 medium (Invitrogen) supplemented with 10% FBS and 1% penicillin/streptomycin. ASM cell lines from three different patients were used were seeded to chambered coverglass (Thermo Scientific). When cells reached confluence, the media was replaced with fresh media that contained 300 nM TTMA or Hexyl QD NPs. Cells were incubated with NPs for 24 hrs. NPs were sonicated in a water bath for 5 minutes prior to application. Toxicity of the NPs was examined by staining cells with LIVE/DEAD Viability/Cytotoxicity Kit (Molecular Probes) according to manufacturer's instruction. Cells that served as positive dead control were treated with 70% methanol for 30 minutes at 37°C prior to staining.

Supporting Figures

Table S1. Physicochemical properties of the functionalized CdSe/ZnS QDs.

QDs	Absorption peak (nm)	Emission peak (nm)	Hydrodynamic diameter (nm)	Zeta potential (mV)
PEG-QD	536	550	24.4 ± 8.8	-8.0 ± 7.3
COOH-QD	540	562	11.7 ± 4.2	-29.5 ± 16.3
TTMA-QD	536	550	8.7 ± 3.0	+26.8 ± 3.2
Hexyl-QD	538	558	7.5 ± 2.0	+26.0 ± 3.8

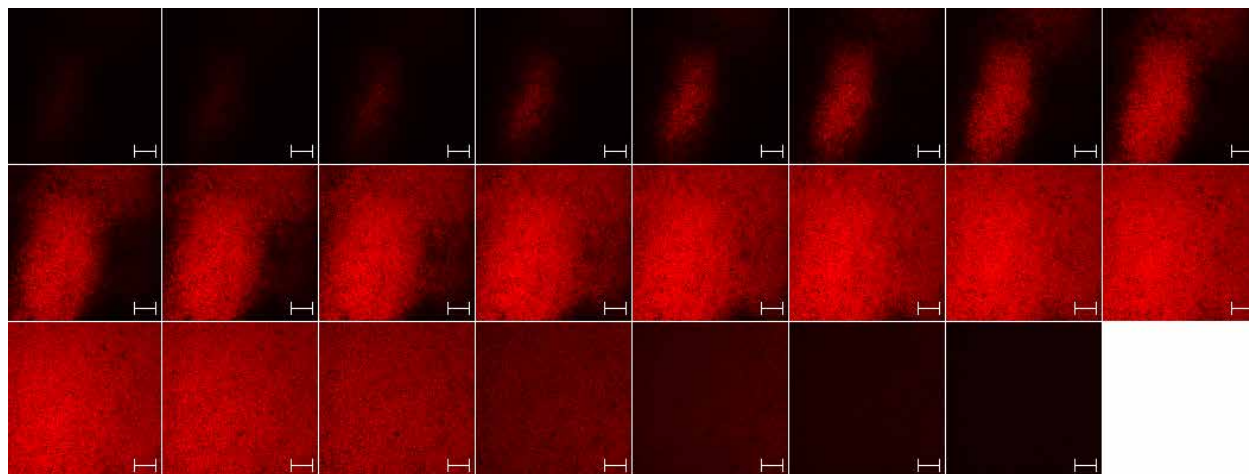


Fig. S1 Representative CLSM image slices of z-stack with a 0.4 μm interval showing bacterial cells (red) in *E. coli* biofilms and distribution of PEG-QD.

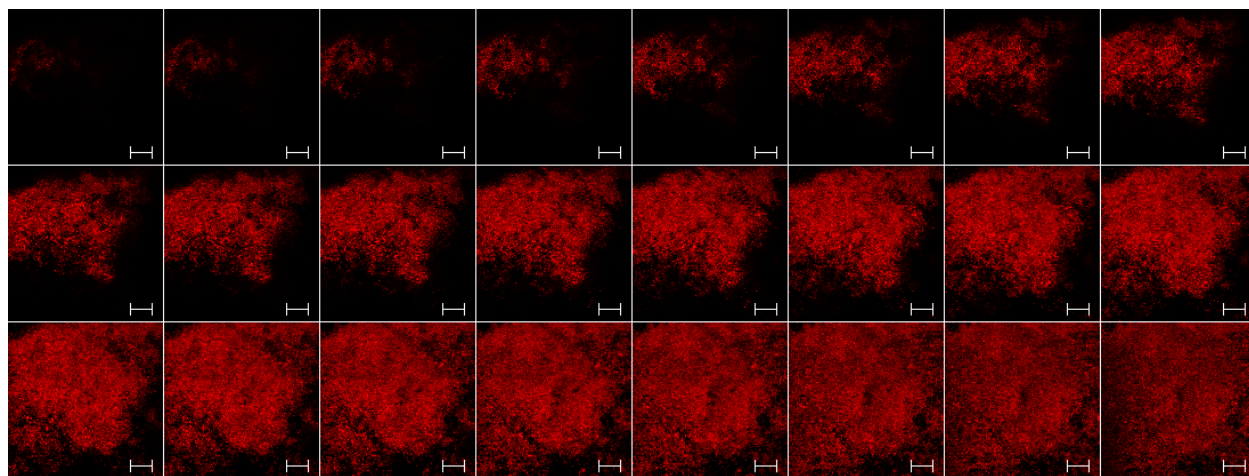


Fig. S2 Representative CLSM image slices of z-stack with a 0.4 μm interval showing bacterial cells (red) in *E. coli* biofilms and distribution of COOH-QD.

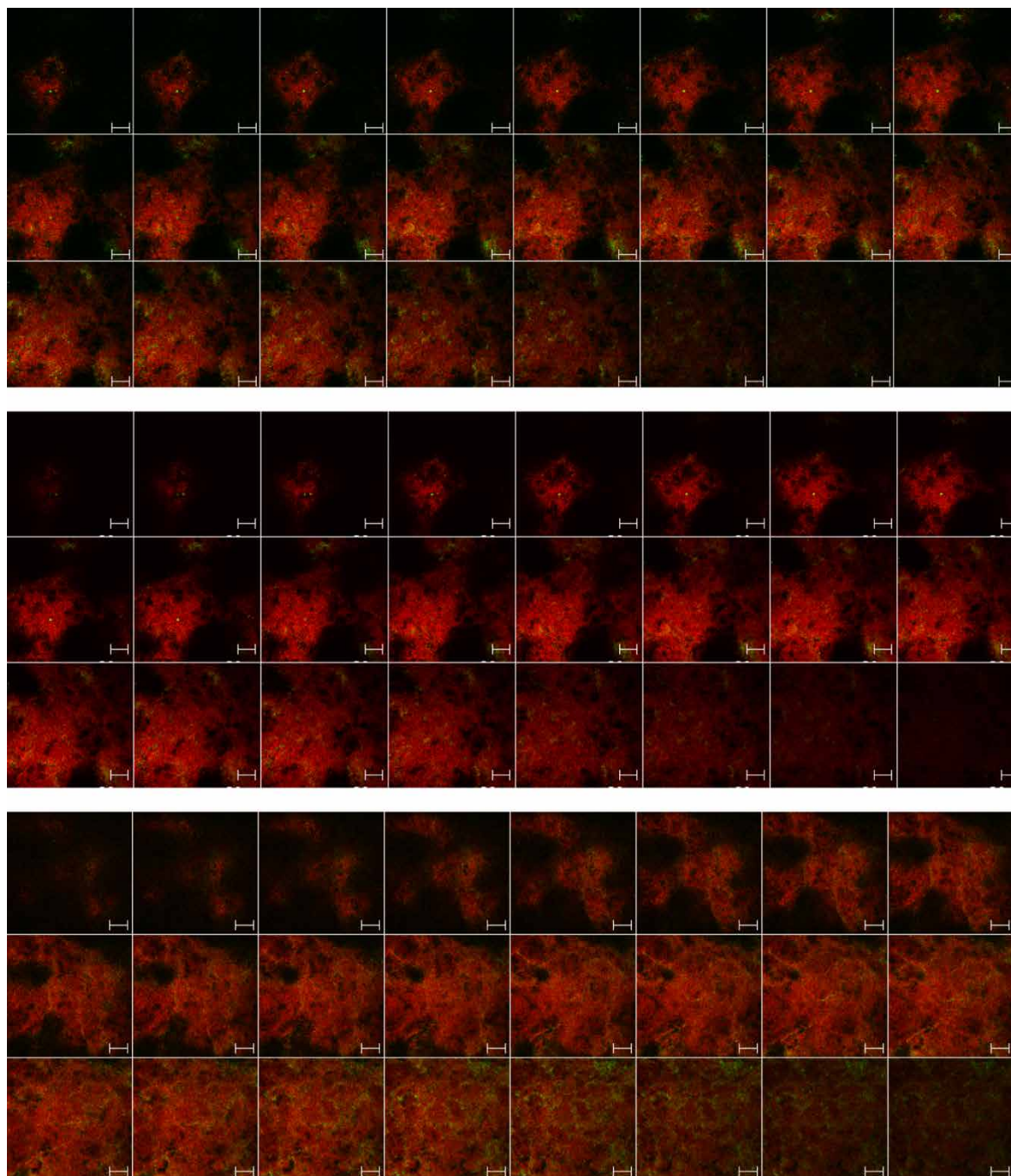


Fig. S3 CLSM image stacks used for analysis of TTMA-QD penetration profile after 1 hr incubation.

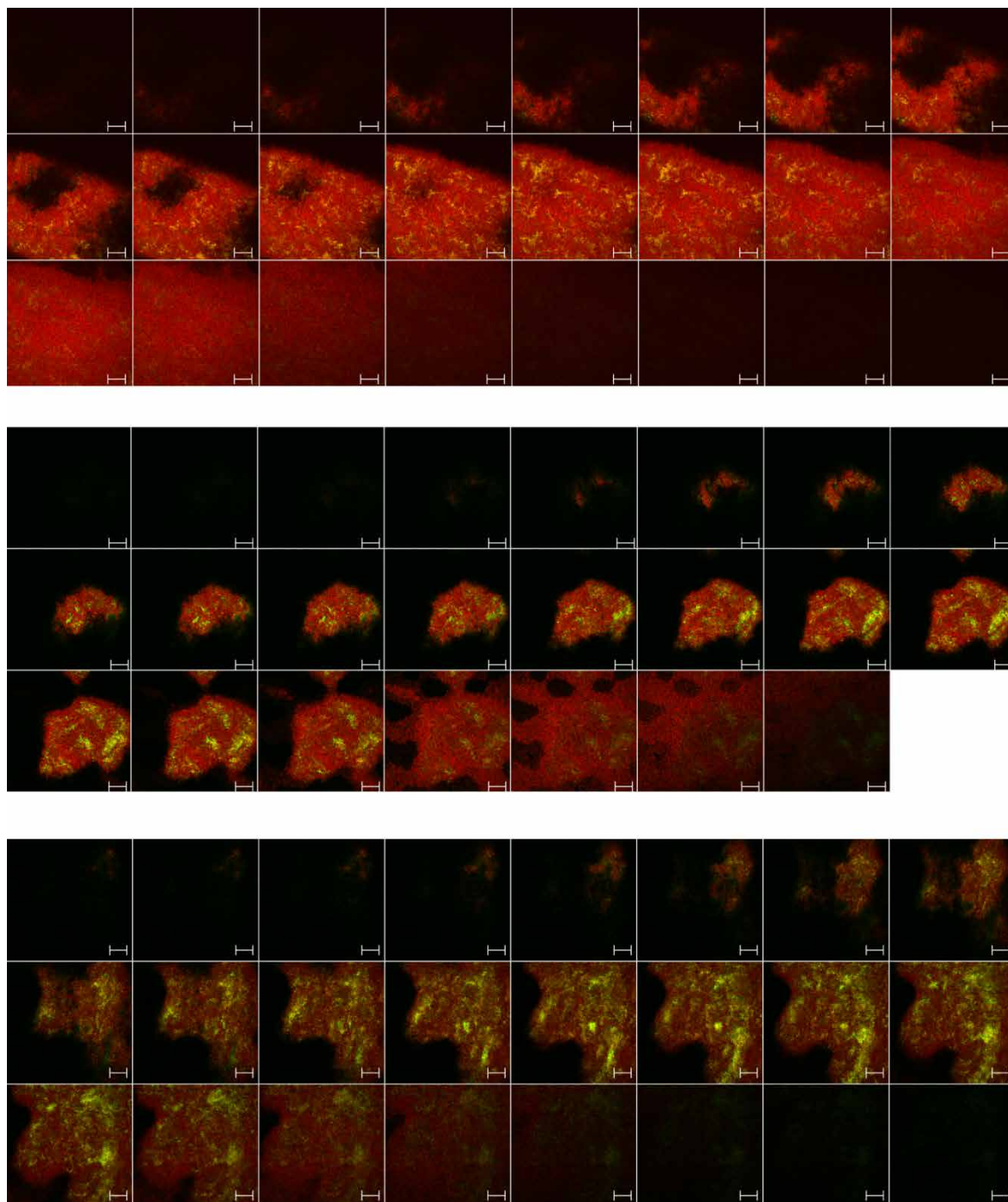


Fig. S4 CLSM image stacks used for analysis of Hexyl-QD penetration profile after 1 hr incubation.

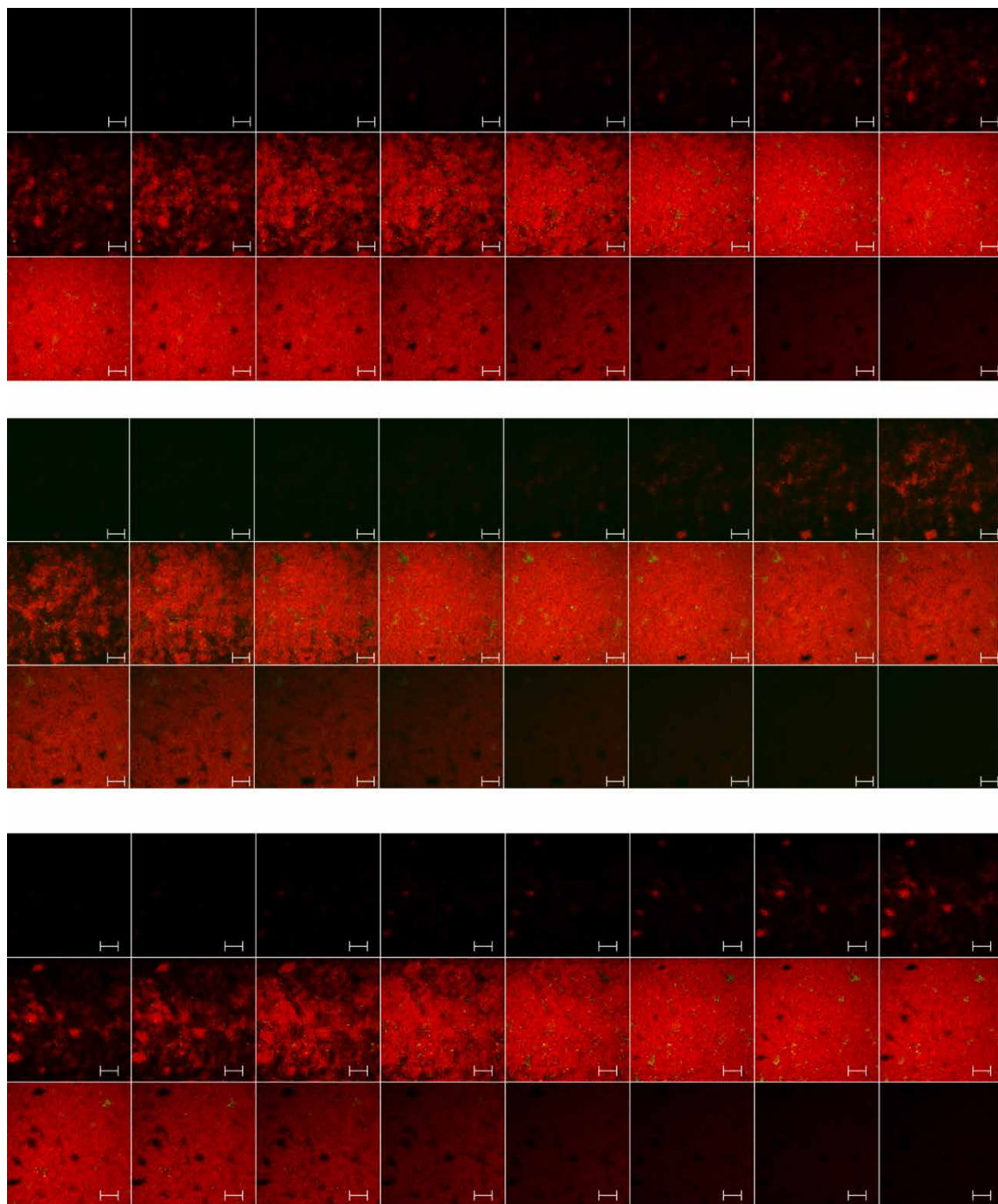


Fig. S5 CLSM image stacks used for analysis of TTMA-QD penetration profile after 3 hr incubation.

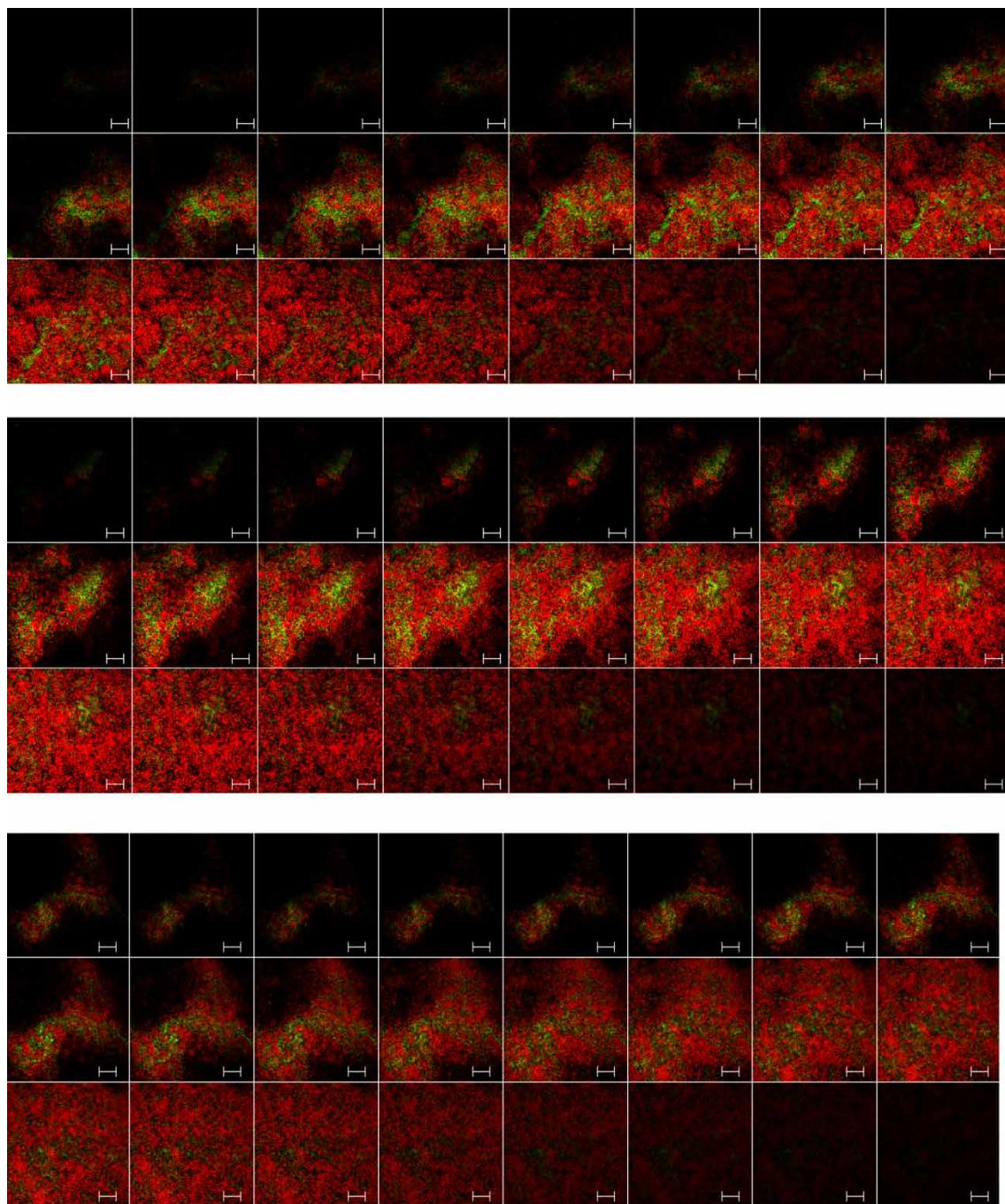


Fig. S6 CLSM image stacks used for analysis of Hexyl-QD penetration profile after 3 hr incubation.

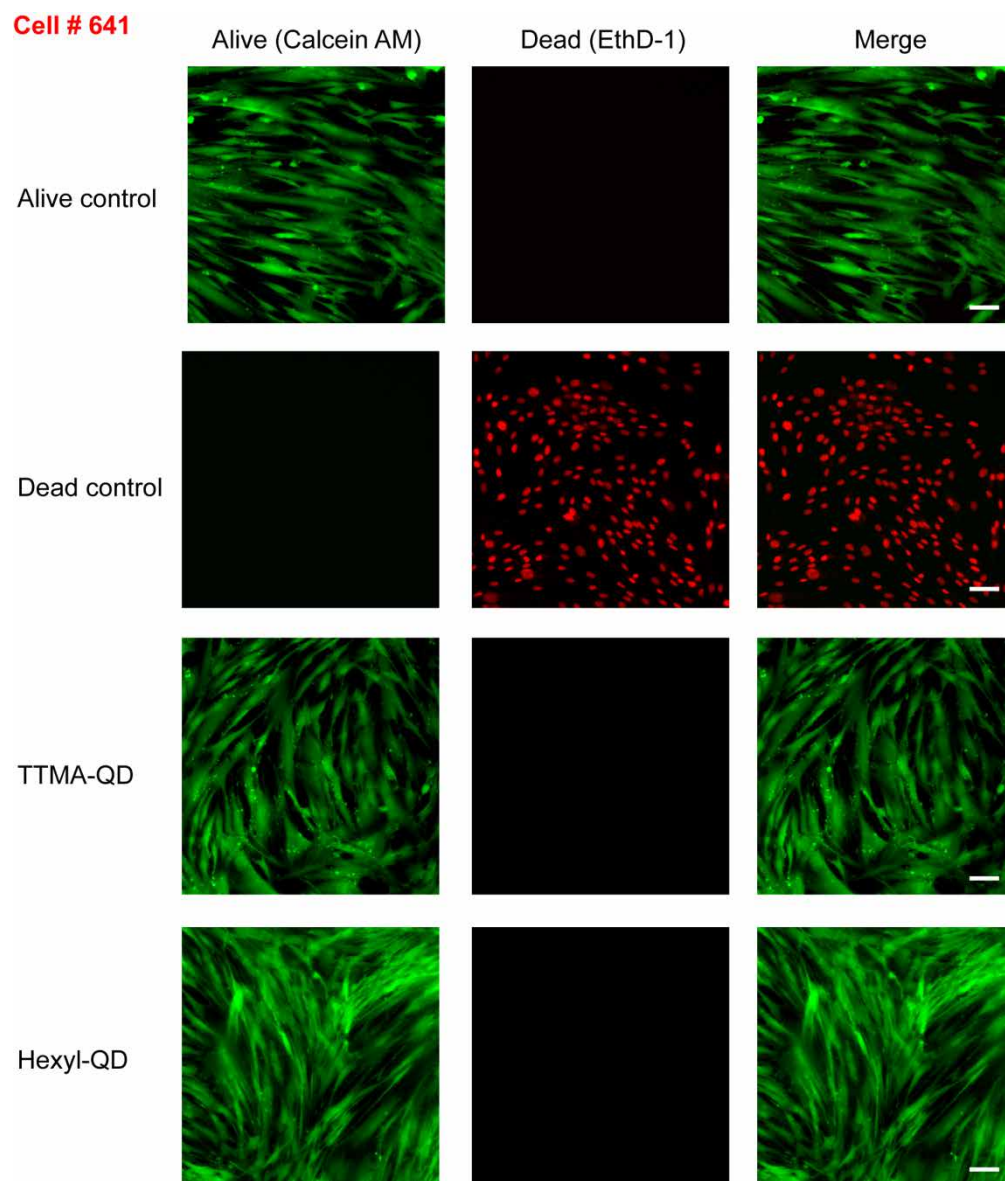


Fig. S7 Cytotoxicity assay results of TTMA-QD and Hexyl-QD at 300 nM after 24 hours incubation with airway smooth muscle cells (cell line number 641). Scale bars are 100 μ m.

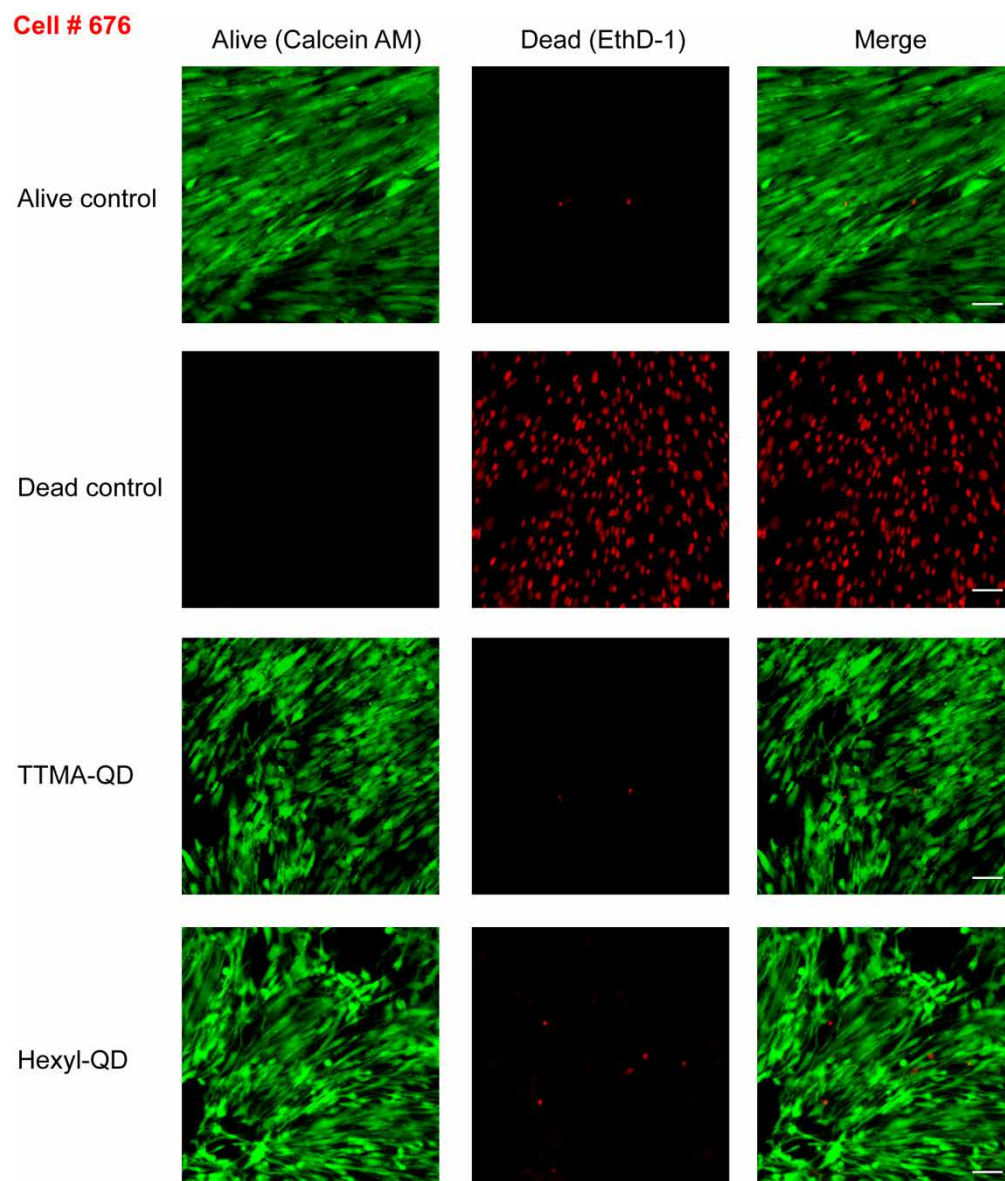


Fig. S8 Cytotoxicity assay results of TTMA-QD and Hexyl-QD at 300 nM after 24 hours incubation with airway smooth muscle cells (cell line number 676). Scale bars are 100 μ m.

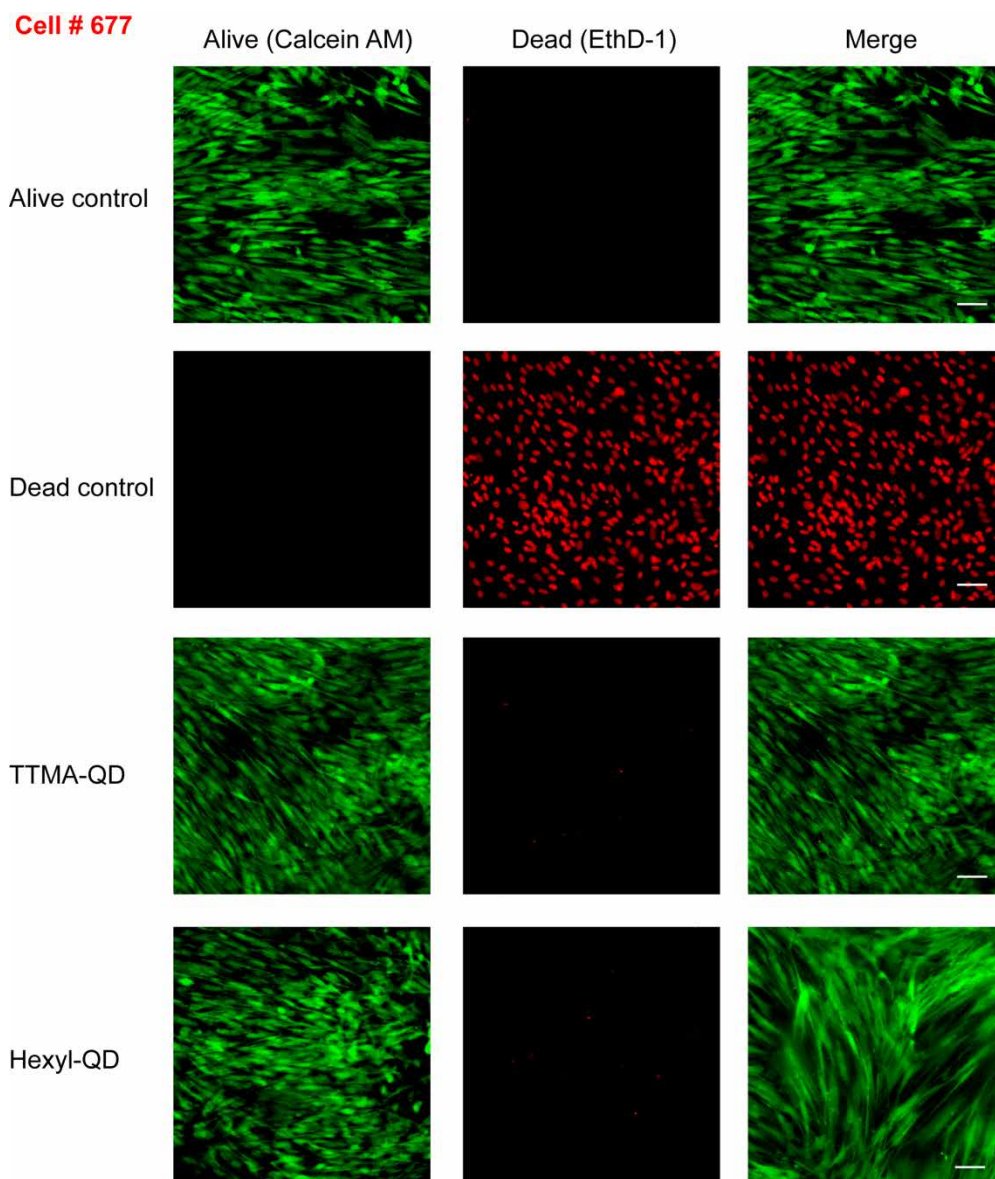


Fig. S9 Cytotoxicity assay results of TTMA-QD and Hexyl-QD at 300 nM after 24 hours incubation with airway smooth muscle cells (cell line number 677). Scale bars are 100 μm .

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- 1 H. T. Uyeda, I. L. Medintz, J. K. Jaiswal, S. M. Simon, H. Mattoussi, *J. Am. Chem. Soc.* 2005, **127**, 3870-3878.
 - 2 Y.-C. Yeh, D. Patra, B. Yan, K. Saha, O. R. Miranda, C. K. Kim, V. M. Rotello, *Chem. Commun.*, **2011**, 47, 3069-3071.
 - 3 K. Susumu, B. C. Mei, H. Mattoussi, *Nat. Protocols*, **2009**, 4, 424-436.