

Supporting information for:

Quantum Dot Probes for Bacteria Distinguish *Escherichia coli* Mutants and Permit In Vivo Imaging

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Cell Labeling: Compound **1** (4 μM) was incubated in TES buffer (5 mM TES, 145 mM NaCl, pH = 7.4) for 10 minutes with either streptavidin-coated **GQD** (Qdot[®] 565 Streptavidin Conjugate, em:565 nm, Invitrogen, Q10101MP) or streptavidin-coated **RQD** (Qdot[®] 655 Streptavidin Conjugate, em: 665 nm, Invitrogen, Q10121MP) or streptavidin-coated **NIRQD** (Qdot[®] 800 Streptavidin Conjugate, em: 800 nm, Invitrogen, MP10171MP). The pellet resulting from centrifugation of 0.5 mL of bacteria (OD = 0.5) was resuspended in 50 μL of TES buffer and treated with **1-QDG** or **1-RQD**. After 10 minutes, the labeled cells were washed twice (centrifugation and resuspension in 100 μL TES buffer) then imaged. In the case of **1-NIRQD**, a 100 μL solution of **NIRQD** (160 nM) and **1** (640 nM) was sterile filtered (0.2 μm pore size) and added to a pellet from 1 mL of *E. coli* K12 (OD = 1). After 10 minutes, the labeled cells were washed twice (centrifugation and resuspension in 50 μL TES buffer) and used for in vivo imaging.

Microscopy: Images were acquired on a Nikon Eclipse TE2000-U epifluorescence microscope using a 100X objective, 10X ocular and 1.5X optivar to obtain images at 1500X. **1-GQD** was imaged using a “green” filter set (Exciter: D480/30X, Dichroic: 400DCLP, Emitter: HQ535/50m), **1-RQD** was imaged using a “red” filter set (Exciter: HQ545/30x, Dichroic: Q570LP, Emitter: HQ610/75m) **1-NIRQD** was imaged using a “near infrared” filter set (Exciter HQ710/75x, Dichroic Q750LP, Emitter HQ810/90m).

In Vivo Imaging: A nude mouse (strain nu/nu, Taconic Inc.) was anesthetized under isoflurane and a 50 μL suspension of **1-NIRQD** bound to *E. coli* JM83 cells was injected in the left rear leg muscle. After 5 minutes, a 16-bit fluorescence image of the mouse was obtained using an IVIS Lumina in vivo imaging station, with the Cy 5.5 exciter (635 \pm 20 nm) and ICG (840 \pm 30 nm) emitter using a 10 s acquisition time, Fstop =1, and low binning (2x2). The image was then background subtracted and set to a “Fire” fluorescence intensity scale using the ImageJ v1.37 software suite available for free download at the NIH website <http://rsb.info.nih.gov/ij/>.

QD Photophysics: The Figure below was taken from the Invitrogen web-site at www.invitrogen.com.

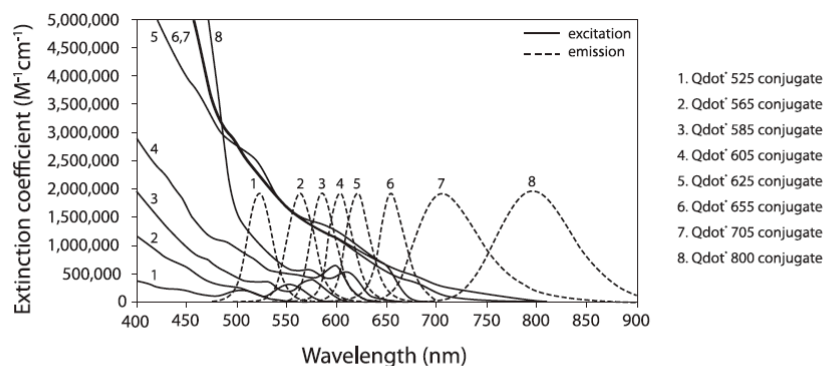


Figure 3. Typical absorption and emission spectra of Qdot[®] 525 streptavidin conjugate (1), Qdot[®] 565 streptavidin conjugate (2), Qdot[®] 585 streptavidin conjugate (3), Qdot[®] 605 streptavidin conjugate (4), Qdot[®] 625 streptavidin conjugate (5), Qdot[®] 655 streptavidin conjugate (6), Qdot[®] 705 streptavidin conjugate (7), Qdot[®] 800 streptavidin conjugate (8).