Supporting Information

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SI Materials and Methods.

Spectral Acquisition. A Perkin-Elmer UV/visible dual path spectrometer with a 1-nm slit width and set to a scan rate of 480 nm/ min was used to acquire the absorption spectra for the Congo red and whole mouse blood. Each was diluted between 100 and 400 × using physiological saline. Sterile water was used as the reference. For each acquisition, 1 mL of each analyte was added to a 1-mL plastic cuvette with a 1-cm path length.

Ex Vivo Bioluminescent Imaging. Three female 6-wk-old BALB/c mice were anesthetized with Ketamine/Xylazine. For each mouse a cardiac puncture was performed to obtain around 1 mL of blood. Prior to the mouse preparation, a glass bottom black 96-well plate was altered so that the base of the plate could rest evenly onto a 10-cm Petri dish. A piece of black paper that had six 1.5-mm-diameter holes in a 3 × 2 orientation was placed in between the 96-well plate and an agar plate such that the pinholes were properly aligned with the wells of the 96-well plate. Five microliter aliquots of bacteria were then pipetted onto the agar directly aligned with the pinholes and the wells. After having time to settle, 2 μL aliquots of quantum dot (QD) 705 were placed onto the second column of bacteria. The setup was then placed into the IVIS100 at 37 °C and bioluminescence confirmed. Then, 50 μL aliquots of blood from each mouse were appropriately distributed and the luminescence observed under the open and QD705 filter sets.

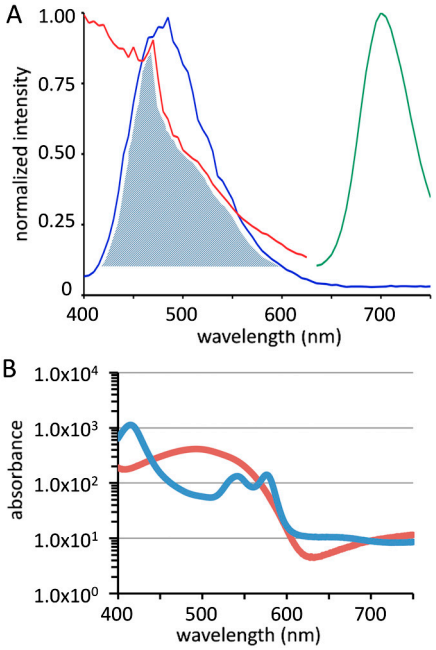

Fig. S1. Spectral information relevant to conditions for fluorescence by unbound excitation from luminescence (FUEL). Emission properties for RT57 (pUC18-mini-Tn7T-Gm-lux) bioluminescence (blue line) were compared against the excitation (red line) and emission (green line) spectra of QD705 (*A*). Data are normalized to their respective maxima. The spectral overlap is indicated (shaded in gray). *B* shows the absorption spectra for mouse whole blood (MWB; blue line) and Congo red (CR; red line). Note how the spectral profiles of MWB and CR are closely aligned and display a sharp diminution at 600–630 nm, consistent with characteristics for strong absorption of blue light and weak absorption for red light.

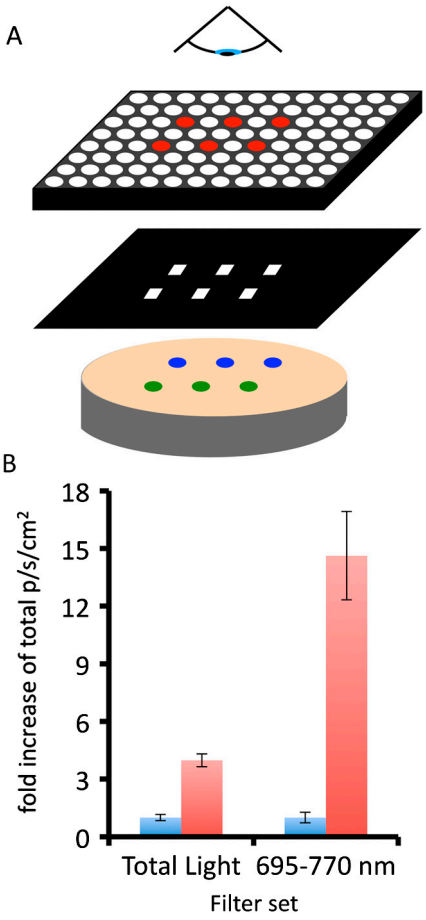

Fig. S2. Demonstration of fluorescence by unbound excitation from luminescence (FUEL) under ex vivo conditions. Prior to attempting FUEL under in vivo conditions, the viability of FUEL was investigated ex vivo. (*A*) Aliquots of RT57 (pUC18-mini-Tn7T-Gm-lux) were distributed onto an agar plate forming two columns of three aliquots each such that each bacterial island would align with individual wells of a glass-bottomed black 96-well plate. After settling, aliquots of the QD705 were placed directly onto each bacterial location of the second column (green), leaving the other row as the control (blue). Mouse whole blood (MWB, red) was evenly distributed into the corresponding wells black 96-well plate, and placed onto the agar dish, separated only by a piece of black paper containing 2 × 2 mm optical windows for each well. (*B*) The total photon flux was observed through the MWB (red) in the IVIS100 both in the absence of an emission filter ("total light") or through a 695–770 nm band-pass emission filter. The photon enhancement was determined by normalizing the FUEL photon flux by the RT57 photon flux. The control (blue) represents the each individual control island compared to their global average in order to further highlight the FUEL effect. The values are reported as the mean fold increase above control ± SEM, $n = 3$.

Table S1. Global average $p \cdot s^{-1} \cdot cm^{-2}$ of subcutaneous pearls

Filter	Control	Layered	Composite
Ventral			
Total light	4.66E+7 ± 0.60E+7	3.4E+7 ± 0.75E+7	4.12E+7 ± 0.28E+7
695–770 nm	3.32E+5 ± 0.35E+5	2.82E+6 ± 0.50E+6	7.11E+6 ± 0.58E+6
Dorsal			
Total light	2.13E+5 ± 0.50E+4	3.95E+5 ± 1.01E+5	7.72E+5 ± 1.81E+5
695–770 nm	1.93E+4 ± 0.27E+4	1.34E+5 ± 0.28E+5	3.25E+5 ± 0.70E+5

Small incisions were made into the inner thighs of five female BALB/c mice before insertion of either the control or layered pearls. The mice were observed through the absence of an emission filter (total light) and a 695–770 nm filter set, from the ventral and then the dorsal side in an IVIS100. Similarly, small incisions were made into the inner thighs of a second set of five female BALB/c mice before the insertion of either control or composite pearls. As before, the mice were observed through the absence of an emission filter (total light) and a 695–770 nm filter set, from the ventral and then the dorsal side in the IVIS100. Values indicate the mean $p \cdot s^{-1} \cdot cm^{-2}$ ± SEM for each pearl; $n = 10$ for the control, and $n = 5$ for the layered and the composite.