Supporting Information For

Near-Infrared Emitting Dual-Stimuli-Responsive Carbon Dots from Endogenous Bile Pigments

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BR-CDot



BV-CDot



Figure S1. TEM images of BR-CDots and BV-CDots confirm successful nanoparticle synthesis. Yellow arrows and dashed outlines indicate some examples of nanoparticles. BV-Cdots are smaller than BR-CDots.



Figure S2. FT-IR Spectra of (A) Bilirubin (BR) and BR-CDot and (B) Biliverdin (BV) and BV-CDot. Dashed lines depict peaks that are seen in both the precursor and the nanoparticle. Retention of some FT-IR peaks in the carbon dots suggests that some imidolic structures from the precursors are retained by the carbon dots.



Figure S3. Fluorescence stability of BR-CDots incubated at 37 °C under different pH conditions for 8 days. Data is presented is a percentage of the initial fluorescence intensity. Each data point represents an average of two trials, and error bars represent standard deviation. N.S. indicates there is not a statistically significant difference from the initial fluorescence intensity. * indicates statistical significance (P<0.05) as determined by a Student's T test.



Figure S4. Fluorescence stability of BV-CDots incubated at 37 °C under different pH conditions for 8 days. Data is presented is a percentage of the initial fluorescence intensity. Each data point represents an average of two trials, and error bars represent standard deviation. N.S. indicates there is not a statistically significant difference from the initial fluorescence intensity. * indicates statistical significance (P<0.05) as determined by a Student's T test.



Figure S5. Fluorescence spectra of BR-CDots for excitation wavelengths of 465 nm (A), 565 nm (B), and 600 nm.



Figure S6. Fluorescence images of drop-casted solutions of methylene blue and BR-CDots using different filters. Scale bar represents 50 μ m. The scale is the same for all images.

Excitation filter : 465	Excitation filter : 465	Excitation filter : 465	Excitation filter : 465	Excitation filter : 465
Emission filter : 500	Emission filter : 520	Emission filter : 540	Emission filter : 560	Emission filter : 580
Min= 1.10e8	Min= 1.40e8	Min= 1.35e8	Min= 1.16e8	Min= 8.71e7
1 Max= 2.06e9	2 Max= 2.63e9	3 Max= 2.57e9	4 Max= 2.18e9	5 Max= 1.67e9
Excitation filter : 465	Excitation filter : 465	Excitation filter : 465	Excitation filter : 465	Excitation filter : 465
Emission filter : 580	Emission filter : 600	Emission filter : 620	Emission filter : 640	Emission filter : 660
Min= 8.78e7	Min= 6.05e7	Min= 3.83e7	Min= 2.65e7	Min= 1.74e7
6 Max= 1.69e9	7 Max= 1.17e9	8 Max= 7.25e8	9 Max= 5.09e8	10 Max= 3.36e8
Excitation filter : 465	Excitation filter : 465	Excitation filter : 465	Excitation filter : 465	Excitation filter : 465
Emission filter : 680	Emission filter : 700	Emission filter : 720	Emission filter : 740	Emission filter : 780
Min= 1.08e7	Min= 6.96e6	Min= 6.37e6	Min= 7.04e6	Min= 7.69e6
11 Max= 2.05e8	12 Max= 1.36e8	13 Max= 8.36e7	14 Max= 5.85e7	15 Max= 2.72e7
Excitation filter : 465 Emission filter : 800	Excitation filter : 465 Emission filter : 820	Excitation filter : 465 Emission filter : 840		
Min= 6.59e6	Min= 8.51e6	Min= 8.92e6		

Figure S7. IVIS tissue phantom images of surface-injected BR-CDots for excitation wavelength of 465 nm and emissions in the range of 500 nm-840 nm.



Figure S8. IVIS tissue phantom images of surface-injected BR-CDots for excitation wavelength of 500 nm and emissions in the range of 540 nm-840 nm.



Figure S9. IVIS tissue phantom images of surface-injected BR-CDots for excitation wavelength of 605 nm and emission wavelengths in the range of 660 nm-840 nm.



Figure S10. IVIS tissue phantom images of surface-injected BR-CDots for excitation wavelength of 640 nm and emission wavelengths in the range of 680 nm-840 nm.



Figure S11. IVIS tissue phantom images of surface-injected BR-CDots for excitation wavelength of 675 nm and emission wavelengths in the range of 720 nm-840 nm.

Excitation filter : 465	Excitation filter : 465	Excitation filter : 465	Excitation filter : 465	Excitation filter : 465
Emission filter : 500	Emission filter : 520	Emission filter : 540	Emission filter : 560	Emission filter : 580
Min= 2.39e7	Min= 2.25e7	Min= 2.13e7	Min= 1.83e7	Min= 1.40e7
1 Max= 3.01e8	2 Max= 4.42e8	3 Max= 4.18e8	4 Max= 3.63e8	5 Max= 2.78e8
Excitation filter : 465	Excitation filter : 465	Excitation filter : 465	Excitation filter : 465	Excitation filter : 465
Emission filter : 580	Emission filter : 600	Emission filter : 620	Emission filter : 640	Emission filter : 660
Min= 1.44e7	Min= 1.10e7	Min= 7.25e6	Min= 6.05e6	Min= 5.93e6
6 Max= 2.78e8	7 Max= 2.15e8	8 Max= 1.40e8	9 Max= 9.73e7	10 Max= 6.86e7
Excitation filter : 465	Excitation filter : 465	Excitation filter : 465	Excitation filter : 465	Excitation filter : 465
Emission filter : 680	Emission filter : 700	Emission filter : 720	Emission filter : 740	Emission filter : 780
Min= 6.09e6	Min= 5.93e6	Min= 5.17e6	Min= 7.04e6	Min= 6.64e6
11 Max= 4.43e7	12 Max= 3.06e7	13 Max= 2.19e7	14 Max= 1.87e7	15 Max= 1.99e7
Excitation filter : 465				
Emission filter : 800	Excitation filter : 465 Emission filter : 820	Excitation filter : 465 Emission filter : 840		

Figure S12. IVIS tissue phantom images of 8.5-mm deep BR-CDots for excitation wavelength of 465 nm and emission wavelengths in the range of 500 nm-840 nm.

Excitation filter : 500	Excitation filter : 500	Excitation filter : 500	Excitation filter : 500	Excitation filter : 500
Emission filter : 540	Emission filter : 560	Emission filter : 580	Emission filter : 600	Emission filter : 620
Min= 6.50e6	Min= 6.52e6	Min= 5.77e6	Min= 5.30e6	Min= 4.95e6
2 Max= 1.25e8	3 Max= 1.23e8	4 Max= 1.03e8	5 Max= 8.93e7	6 Max= 6.77e7
Excitation filter : 500	Excitation filter : 500	Excitation filter : 500	Excitation filter : 500	Excitation filter : 500
Emission filter : 640	Emission filter : 660	Emission filter : 680	Emission filter : 700	Emission filter : 720
Min= 4.77e6	Min= 4.69e6	Min= 4.00e6	Min= 4.43e6	Min= 3.87e6
7 Max= 4.94e7	8 Max= 3.68e7	9 Max= 2.68e7	10 Max= 1.97e7	11 Max= 1.23e7
Excitation filter : 500	Excitation filter : 500	Excitation filter : 500	Excitation filter : 500	Excitation filter : 500
Emission filter : 740	Emission filter : 760	Emission filter : 780	Emission filter : 800	Emission filter : 820
Min= 3.95e6	Min= 4.19e6	Min= 4.59e6	Min= 4.93e6	Min= 5.28e6
12 Max= 9.83e6	13 Max= 9.88e6	14 Max= 1.04e7	15 Max= 1.09e7	16 Max= 1.22e7
Excitation filter : 500 Emission filter : 840 Min= 6.78e6 17 Max= 1.43e7				

Figure S13. IVIS tissue phantom images of 8.5-mm deep BR-CDots for excitation wavelength of 500 nm and emission wavelengths in the range of 540 nm-840 nm.



Figure S14. IVIS tissue phantom images of 8.5-mm deep BR-CDots for excitation wavelength of 605 nm and emission wavelengths in the range of 660 nm-840 nm.



Figure S15. IVIS tissue phantom images of 8.5-mm deep BR-CDots for excitation wavelength of 640 nm and emission wavelengths in the range of 680 nm-840 nm.



Figure S16. IVIS tissue phantom images of 8.5-mm deep BR-CDots for excitation wavelength of 675 nm and emission wavelengths in the range of 720 nm-840 nm.



Figure S17. ROI analysis for BR-CDots in chicken tissue at excitation wavelengths of 465 nm and 500 nm.



Figure S18. Percentage of signal transmitted through 8.5 mm tissue. Data is presented as a percentage ratio of the background-subtracted average radiant efficiency at 8.5 mm depth to the background-subtracted average radiant efficiency at the surface level. The percentage of nanoparticle signal transmitted increases with an increase in emission wavelength. This trend holds true across excitation wavelengths of 605 nm, 640 nm, and 675 nm.



Figure S19. Example ROIs for tissue phantom image. Yellow oval outlines BR-CDot ROI and blue rectangle outlines background ROI.



Figure S20. Example ROIs for *ex vivo* mouse imaging. White oval outlines BR-CDot ROI and yellow rectangle outlines background ROI.