Supporting Information

Optical Drug Monitoring: Photoacoustic Imaging of Nanosensors to Monitor Therapeutic Lithium In Vivo

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Supporting Figure 1.
Photoacoustic Spectrum Ratio. The ratio of photoacoustic intensity at 515 nm divided by that at 660 nm from the spectral data presented in Figure 2A.
Tuning Nanosensor Formulation

Supporting Figure 2.

Chromoionophore (CH) Selection. The working range for these lithium sensors is primarily determined by the selection of the chromoionophore. Chromoionophore VII yields a response in the physiologically relevant lithium range, whereas Chromoionphore II (with a higher pKa) responds only at supraphysiological levels. The remainder of the formulation experiments were performed with Chromionophore VII.
Supporting Figure 3

Lithium Ionophore (LiI) Selection. The selectivity of sensors is primarily determined by the selection of ionophore. For *in vivo* lithium monitoring sodium is the potential interferant as the concentration (140 mM) is several orders of magnitude larger than that of lithium. Selectivity is tested by calibrating nanosensors against both lithium and sodium in the absence of the other ion the separate solution method. The selectivity coefficient ($K_{Li,Na}$) is determined as the difference between the logEC50s of the two ions. A lower value indicates better selectivity. For these sensors Lithium ionophore VI performs better than Lithium ionophore III. This matches with previous experiments on macroscale optodes. The remainder of the formulation experiments were performed with Lithium ionophore VI.
Supporting Figure 4
Addition of additives for selectivity. The selectivity can also be improved through the use of additives such as TOPO\textsuperscript{3}. At large amounts of TOPO (12\%) selectivity is increased, but the sensors suffer from a shift in response parameters and a decrease in fluorescence response amplitude. An optimum value of 4\% TOPO retains the selectivity of the 12\% TOPO condition while maintaining a large sensor response.
Supporting Figure 5
Plasticizer selection. The effect of plasticizer selection on the nanosensors was examined through the use of four different plasticizers. DOS and NPOE had the same selectivity ($p=0.6$), whereas NPPE and CFA6 were not as selective for lithium. DOS was used for all other formulations due to previous experience with DOS based nanosensors.\(^{4,6}\)
Fluorescent Nanosensor Characterization

Supporting Figure 6
Calibration of the lithium nanosensors. The final calibration for lithium nanosensors in the presence of physiological sodium (140 mM, tested in PBS). The EC50 for these sensors is 3.7 mM with a sensitivity of 45%/log unit. This calibration was measured with the IVIS animal imager.
Supporting Figure 7
Absorption spectrum of the lithium sensitive nanosensors. Arrows indicate the direction of increasing lithium concentration.
Supporting Figure 8
Fluorescence spectrums of the lithium sensitive nanosensors. The left shows the spectrum of the signal when chromoionophore is excited and transfers energy to DiR. The right shows the DiR directly excited.
Supporting Figure 9
DLS particle sizing. The nanosensors are 27 nm diameter (number average) and 166 nm (effective diameter) by DLS. Particles were sized on a Brookhaven 90Plus ($n=3$).
Supporting Figure 10
SEM particle sizing. The nanosensors are ~50 nm diameter by SEM.
Supporting Figure 11
Response time. The nanosensors were mixed rapidly with lithium chloride solution to a final volume of 10 mM. The response of the nanosensors was faster than the time to mix the solutions and acquire the next measurement (15 seconds; at t~125 seconds). Based on previous reports the response time is likely in the sub-millisecond regime.\textsuperscript{7}
Supporting Figure 12

*In vitro* reversibility. The nanosensors were encapsulated in microdialysis tubing as described previously, and imaged using the IVIS Lumina with settings used for animal imaging. Solutions of either lithium chloride or PBS were washed over the dialysis tubing before each measurement.
Supporting Figure 13
Photobleaching control. The nanosensors do not experience any appreciable photobleaching over 2 hours in the IVIS imager with the same settings as used for animal imaging.
Supporting Figure 14

*In vivo* calibration. Nanosensors were injected in six spots on the back of a mouse mixed with varying concentrations of lithium for each injection. The concentrations were 0, 0.1, 0.5, 1, 2, and 10 mM. The raw intensity (bottom left) for each channel changes with concentration as well as other factors (site of injection, depth of injection etc.), but the fluorescence ratio (bottom right) calibrates with lithium concentration similar to *in vitro* calibrations. The leftmost point on both graphs is the 0 mM Lithium point (not included in the fit on the right).
Supporting Figure 15
Full dataset for in vivo experiment from Figure 4 – 38 mg/kg Li dataset. The left column shows the raw fluorescent intensities for the FRET intensity (note that the signal decreases for increasing lithium concentrations). The middle column shows the fluorescence ratio between the reference channel and the FRET channel (increases with increasing lithium concentrations). The right column shows the ratio normalized to the first point after the lithium injection. Mice were imaged in pairs as the imager could not image all six mice simultaneously. Data for 12 mg/kg Li+ is similar.
Supporting Figure 16
Combination of normalized ratios. The red datasets are the three experimental mice and the black datasets are for the three control mice. The left panel is the fluorescence ratio (center column in Supporting Figure 15) and the right panel shows the normalized ratios (right column in Supporting Figure 15).
Supporting Figure 17

Full dataset for *in vivo* experiment from Figure 3. The left column shows the raw photoacoustic signals for the two interrogated wavelengths. The middle column shows the ratios between the photoacoustic signals acquired at the 515 nm wavelength and the 660 nm wavelength (increases with increasing lithium concentrations). The right column shows the ratios normalized to the first point after the lithium injection. Mice 1-3 are all experimental mice (lithium injection) and mouse 4 is a control mouse (vehicle injection only).
Supporting Figure 18
Combination of normalized ratios for photoacoustics. The red datasets are the normalized photoacoustic signal ratios for the three experimental mice (shown in the right column in Supporting Figure 17). The black dataset is for the control mouse.
Supporting Video 1
This video is a three dimensional scan of the nanosensor injection detailed in Figure 3 of the manuscript.

Supporting References