METHODS AND RESULTS

**Nanosensor fabrication.**

The sodium optode consisted of the following compounds: 30 mg high molecular weight poly(vinyl chloride); 60 mg bis-2-sebacate; 3 mg sodium ionophore X; 0.5 mg chromoionophore III; and, 1 mg of sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate. This was brought up in 500 µl of tetrahydrofuran (all chemicals from Sigma).

The desired amount of surface modifier (either 1,2-Diacyl-*sn*-Glycero-3-Phosphoethanolamine-N - [Methoxy(Polyethyleneglycol)-550] (PEG lipid) or total brain ganglioside, both from Avanti Polar Lipids) was dissolved in 5 ml of distilled water in a 40 ml glass storage container. This solution was sonicated with a probe-tip sonicator (Branson) at 15% amplitude for 30 seconds. 50 µl of the optode solution was diluted in 50 µl of dichloromethane (Sigma) and mixed. This solution was then added drop-wise into the water solution while being sonicated with the probe-tip sonicator. The sonication lasted for a total of 3 minutes. The nanosensor solution was then pushed through a 0.2 µm syringe filter (Acrodisc, Gelman Laboratory) to remove the larger polymer particles.

**TEM**

Transmission electron microscopy images were obtained on a JEOL 200CX General Purpose TEM at the Massachusetts Institute of Technology Center for Materials Science and Engineering. The samples were diluted 1:100,000 prior to analysis.

**Response characterization of Nanosensors**

Following fabrication of the nanosensors they were subjected to a calibration experiment to determine the sodium concentration range at which they respond. The nanosensor solution (200 µl) was added to 800 µl of 10 mM HEPES (Sigma Ultra grade) buffer (pH 7.4) in a low volume cuvette. The fluorescence intensity was then measured in a cuvette reader (Spectramax M2, Molecular Devices) exciting at 570 nm and collecting emission at 680 nm. Subsequent additions of 10 mM HEPES buffer (pH 7.4) containing NaCl were made to increase the concentration of sodium in the solution. The signal was then corrected for dilution. The response is represented as $1 - \alpha$, where $\alpha = (I_{max} - I_{[Na+]})/(I_{max} -$

$I_{min}$), $I_{[Na+]}$ is the intensity at the concentration of sodium being measured and $I_{min}$ and $I_{max}$ are the intensities at zero sodium and 1 M sodium, respectively. This experiment was performed on days 0, 1, 2, 5, and 8 after fabrication to generate a lifetime of the sensors. The sensors were stored at room temperature in the dark and in the aqueous solution in which they were formed.

**Sizing and Solution Stability**

The sizing and zeta potential were performed on a nanosizer (Nano Series ZS90, Malvern). For size measurements, 200 µl of nanosensor solution was added to 800 µl of distilled water in a low volume cuvette. The zeta potential was measured using disposable zeta potential cuvettes (Malvern). The nanosensor solution (500 µl) was added to 500 µl of a solution containing the following; 20 mM HEPES, 274 mM NaCl, 5.4 mM potassium glutamate, 1.8 mM $CaCl_2$, pH 7.4. This was done to establish relevant plasma concentrations of ions upon dilution with the nanosensor solution. After addition the pH remained at 7.4. These conditions allow for measurements under conditions similar to those in cell culture systems. Both the size and zeta potential were measured three times per sample and an average was taken. Batches were made three times for each concentration of surface modifier.

**Biocompatibility**

Biocompatibility was determined by incubating the sensors with HEK 293 cells (ATCC). The cells were trypsonized from normal culture and pipetted into a clear 96-well plate at a concentration of 30,000 cells/well. The cells were grown overnight in 300 µl of media to allow attachment to the plate. 20 µl of aqueous solution containing $10^{11}$ nanoparticles/ml was added to each well. For control experiments 20 µl of distilled water was used. Different nanoparticles were used to compare to the nanosensors. They consisted of the following; gold nanoparticles (colloidal gold 100 nm, SPI) and fluospheres (20 nm and 100 nm carboxylate modified microspheres, Invitrogen). Each group contained 8 wells. The nanoparticles were incubated with the cells overnight and the media was changed the following day.

At 24, 48, and 72 hours following washing an MTT assay was performed (In vitro toxicology assay kit, Sigma). The cells were incubated with MTT for 2 hours. The MTT was then dissolved and the

absorbance of each well was read at 570 and 690 nm. The 690 nm absorbance served as background and was subtracted from the 570 nm value. The data were then averaged and compared to control.