Cognitive Science and Technology
Neuroscience

Quantum Dots Enable Detection of Neuron Activation

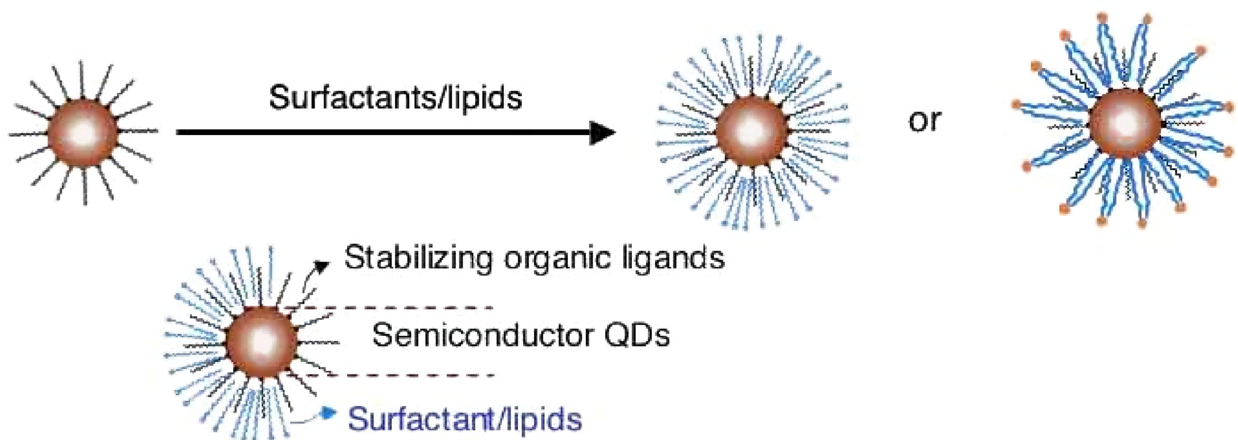

Figure 1: Formation of water-soluble and biocompatible QD-micelles through an interfacially driven micro-emulsion process.

Bioprobes sensitive to membrane potential

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Key processes underlying the operation of individual neurons occur through the binding of neurotransmitter molecules with membrane receptors. Progress in understanding these phenomena requires biocompatible probes that detect neuronal processes within living animals (i.e. *in vivo*). Research conducted through Sandia's Cognitive Science and Technology Initiative has successfully demonstrated that quantum dots (QDs) may be produced that allow fluorescence detection of neuronal voltage changes, and thus exhibit biocompatibility sufficient for their *in vivo* use for many behavioral neuroscience experiments. QDs are generally excellent for fluorescence labeling since they are very bright and stable in comparison with conventional organic dyes.

Monodisperse CdSe and CdSe/CdS core/shell QDs were synthesized through a "hot soap" injection process [1]. Phospholipids were used to encapsulate the QDs within a micelle core to form water-soluble and biocompatible QD micelles (Fig. 1) using an interfacially driven micro-emulsion process developed at Sandia [2,3]. The interdigitated surfactant layers surrounding the QDs resemble a bilayer structure that enable easy fusion of QDs into cell membranes. In this procedure, a concentrated suspension of QDs in chloroform is added to an aqueous solution containing a mixture of surfactants or phospholipids with different functional head groups such as ethylene glycol (-PEG) and amine ($-NH_2$). PEG is used to improve biocompatibility and amine groups provide sites for bioconjugation. Optical characterization of these biocompatible QD micelles indicated they maintained all the optical properies of the original hydrophobic QDs.

Sensing neuronal potential changes cross-membrane is based on the fluorescent intensity changes of those QDs that are close to or within neuronal membranes. Polarization of the local electric field across the neuronal membrane causes electron/hole pairs to be redistributed within each QD, resulting in an increase in fluorescence as detected by fluorescence imaging. Initial

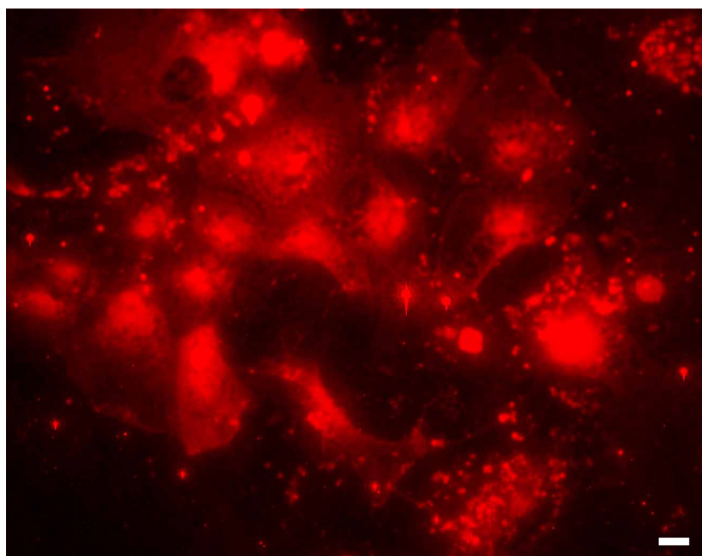

Figure 2: Fluorescence image of cultured hippocampal neurons from mouse brain exposed t QDs (590 nm emission). Scale bar =10 µm.

tests utilizing a QD micelle buffer solution to cultured hippocampal pyramidal neurons demonstrated that the micelle bilayer structure promotes the adherence of nanoparticles to the lipid membrane of the neuron with QDs actually becoming wedged within the membrane (Figure 2). The biocompatibility of these QDs ensures the cells to be alive during measurements. The next step was to evaluate whether the quantum dots were sufficiently inserted into the membrane such that they would respond to a change in membrane voltage.

The resting membrane potential of hippocampal pyramidal neurons is largely determined by the equilibrium potential for K^+ and is typically –75 mV. From the Nernst Equation, adding 40 mM KCl to the solution bathing the neurons should change the membrane potential of the cell from –75mV to approximately –21 mV. Live cell experiments using the QD treated cultured neurons showed a factor of 7 increase in fluorescence intensity in response to this membrane depolarization (Figure 3).

This research demonstrates the use of QDs to detect neuronal voltage changes. QDs have distinct advantages over traditional voltage-indicating fluorescent dyes including high quantum yield and photostability. Further advances should provide the basis for larger scale measurement techniques (e.g., nanoelectrode arrays) essential to gaining an understanding of how nanoscale neural processes aggregate to produce phenomena at the micro (neuron-to-neuron interactions) and millimeter scales (neuronal network interactions).

References:

1. Fan H., et al. *Chem Commun*, *12,* 1383-1394, 2008.
2. Fan, H., et al., *Nano Letters vol. 5*, 645-648, 2005
3. Fan, H., et al., *Science vol. 304*, 567-571, 2004.

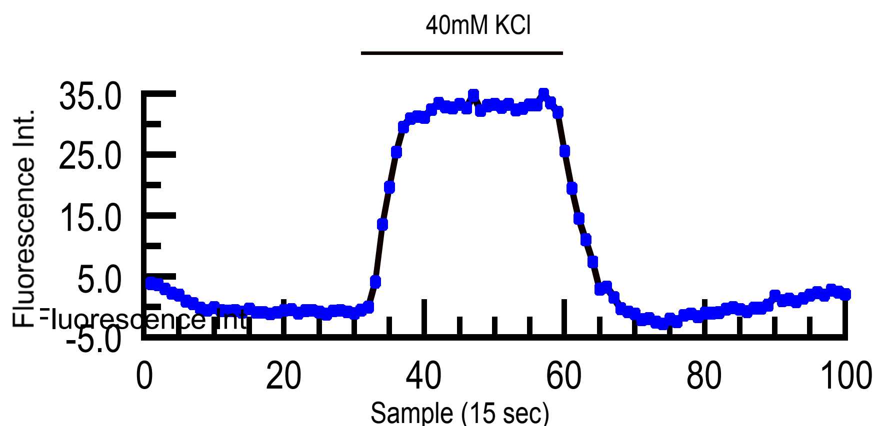

Figure 3: Fluorescence intensity trace of cultured neurons exposed to 590 nm QDs in a flow chamber of the imaging system. A region of interest was picked over a cell body, the average intensity was calculated, then baseline fluorescence was subtracted. The fluorescence intensity was recorded at 15 s time intervals. The fluorescence was allowed to stabilize in normal artificial cerebrospinal fluid (ACSF) for sample numbers 1-30, then 40 mM KCl was applied to the neurons between sample number 30-60, then washed with normal ACSF for sample numbers 60-100 (bar shows application of KCl)