Nanoparticle-based sensing voltage change across a lipid bilayer

YungChia Chen*†, Thomas J. O'Shaughnessy#, Alexander L. Efros#, Alan Huston&, Kimihiro Susumu&, Michael H. Stewart, Igor L. Medintz' and James. B. Delehanty*

* Center for Bio/Molecular Science and Engineering, Code 6900,
#Materials Science Division, Code 6300
&Optical Sciences Division, Code 5600
*† American Society for Engineering Education Washington, D.C. 20036, U.S. Naval Research Laboratory, 4555 Overlook Ave, S.W. Washington, DC 20375

ABSTRACT

Developing nanoscale probes capable of real-time sensing and imaging of neuronal action potentials would be useful in understanding neuronal communication. However, currently available tools for imaging action potentials lack either the spatial or temporal sensitivity to understand neuronal networks with single cell resolution. Two methods were used here to determine how voltage-induced changes in nanoparticles' emission spectra could be employed to image membrane action potentials. First. voltage was applied to a polymeric matrix loaded with quantum dots (QDs) was used to assess luminescence responses to voltage. Second, QDs were loaded into cultured cells coupled with the controlled induction of action potentials.

Keywords: quantum dots, voltage sensing, action potential

1. INTRODUCTION

Understanding neuronal network activity with single cell resolution is critical to understanding how the brain works. Taking advantage of the inherent photophysical and electronic properties of various nanoparticle (NP) materials (e.g., semiconductor quantum dots (QDs), gold NPs) and NP-peptide hybrid constructs that allow for specific localization to cell plasma membrane [1]–[4], voltage changes due to action potentials can be directly detected. QDs can be engineered such that their spectral properties can be discretely tied to changes in a surrounding electric field and do not require genetic manipulation of the cell for function. This latter attribute makes them attractive alternatives to fluorescent methods such as genetically-encoded calcium indicators (GECI) which uses calcium signaling as a proxy for action potentials [5].

Neurons communicate through action potentials – the modulation of the electrical potential across the 5-7 nm expanse of the neuronal cell plasma membrane. Action potentials that traverse down the axon result in neurotransmitter release at the synapse which results in activation of nearby neurons resulting in action potentials in these cells.

Much is know about how an action potential occurs. But to understand whole brain activity and behavior it is necessary to understand neuronal network behavior – the activity of hundreds to thousands of neurons at single cell resolution. This goal is currently hindered by several critical technological limitations. There are a number of methods available to understand brain functionality from the synapse level to the whole brain level, but the limitations of each make it difficult draw concrete conclusions.

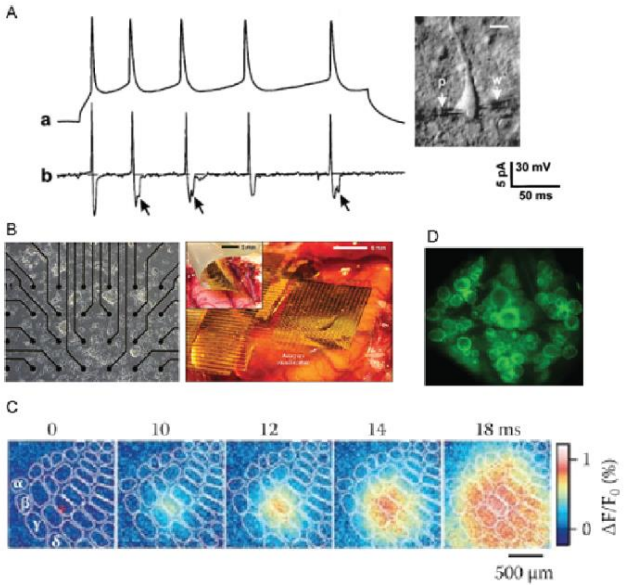

Figure 1. Examples of current methods for detecting neuronal activity. (A) Patch clamp detects single cells activity (image from [6]). (B) Typical microelectrode array for an *in vitro* preparation is along with a new generation of flexible electrodes for *in vivo* implantation (image from [7]). (C) Example of neuronal activity in the barrel cortex of an anesthetized mouse as detected with commercial VSD (image from [8]). (D) Labeling with a new type of VSD that has better spatial labeling *in vitro* (image from [9]).

Currently neuronal electrical activity can be measured through electrophysiology, voltage sensing dyes (VSD) or electrode arrays. Electrophysiology, especially patch clamp can fully delineate the electrical activity of a single neuron but is hampered by low throughput, high degree of difficulty, and invasiveness. Furthermore, if conducting

these experiments *in vivo*, animals need to be anesthetized, which has shown to alter brain activity [10].

VSDs can measure the activity of whole neuronal populations but currently suffer from spatial resolution and cause significant toxicity. Other optical imaging techniques typically use calcium indicators in lieu of reading electrical activity but the dynamics of calcium imaging is much slower than that of action potentials [11]. Furthermore, disease states may cause uncoupling of second messenger systems from action potentials.

Electrode arrays combat the low throughput of electrophysiology by employing electrodes that can measure the fast spiking dynamics of neurons but the nature of the electrode means that one cannot differentiate the origin of the signal to distinguish between type of neuron and thus lack single cell resolution [11].

2. MATERIALS AND METHODS

2.1 QD synthesis

CdSe–ZnS core–shell QDs with emission maxima centered at 530, or 550 nm were synthesized and made with the hydrophobic ligands in Table 1.

Table 1. Hydrophobic ligand and QD combinations

QD	Ligand
550nm	Hexylphosphonic acid (HPA), Trioctyl phosphine oxide (TOPO), Trioctyl phosphine (TOP)
530nm	zwitterionic d-penicillamine (DPA), Trioctyl phosphine oxide (TOPO), Trioctyl phosphine (TOP), Oleylamine
530nm	Hexane Thiol

2.2 Cell culture

Cell culture was performed using PC12 cells maintained in RPMI-1640 (ATCC) supplemented with 10% HS (ATCC), 5% FBS (ATCC) and 1% (v/v) antibiotic/antimycotic (Sigma). Cultures were grown in T25 flasks in 5%CO2 at 37C and passaged every seven days. PC12 cells were plated on collagen coated MatTek dishes with 2/3 media replacement every 2-3 days.

3. RESULTS

3.1 QD fluorescence to changes in voltage

QDs have been theorized and shown experimentally to be sensitive to changes in voltage/electric field strengths [12], [13]. Preliminary data collected in our laboratory show that QDs' emission intensity decreases in a voltage-dependent fashion (Figure 2). The higher the voltage the lower the number of photons emitted per second with an almost linear relationship. Based on signal detection theory calculations, QDs have been shown to be capable of detecting neuronal membrane voltage dynamics with millisecond precision (Figure 3) and with greater sensitivity than with traditional methods such as VSDs and genetically encoded calcium indicators [13]. The QDs display reproducible photoluminescence changes with respect to electrical fields that do not suffer from hysteresis effects.

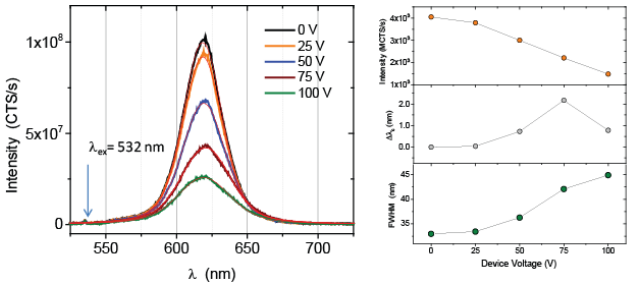

Figure 2. Preliminary data showing the voltage-dependent fluorescence quenching of quantum dots as measured in photon counts/second. The quantum dots yielded specific intensities that decreased linearly with increasing voltage. The full width half maximum (FWHM) also changed with voltage.

4. CONCLUSION

Potentiometric nanoparticles can be a useful tool in the field of neuroscience for measuring neuronal activity. Their fast response time, small size, and resistance to photobleaching make them ideal for delivery to and measuring voltage changes across a plasma membrane (Figure 3).

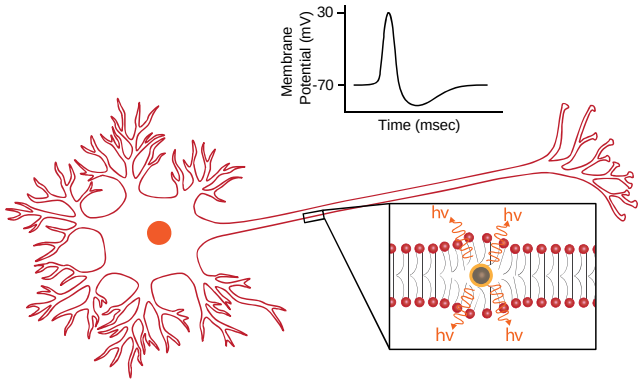

Figure 3. QDs can have altered fluorescence behavior in the presence of an electric field and this effect can be used to measure the change in electric field in the plasma membrane during an action potential.

REFERENCES

[1] J. B. Delehanty, J. B. Blanco-Canosa, C. E. Bradburne, K. Susumu, M. H. Stewart, D. E. Prasuhn, P. E. Dawson, and I. L. Medintz, "Site-specific cellular delivery of quantum dots with chemoselectively-assembled modular peptides,"

Chem. Commun., vol. 49, no. 72, pp. 7878–7880, Aug. 2013.
[2] G. Gopalakrishnan, C. Danelon, P. Izewska, M. Prummer, P.-Y. Bolinger, I. Geissbühler, D. Demurtas, J. Dubochet, and H. Vogel, "Multifunctional Lipid/Quantum Dot Hybrid Nanocontainers for Controlled Targeting of Live Cells," *Angew. Chem. Int. Ed.*, vol. 45, no. 33, pp. 5478–5483, Aug. 2006.
[3] J. B. Delehanty, K. Boeneman, C. E. Bradburne, K. Robertson, J. E. Bongard, and I. L. Medintz, "Peptides for specific intracellular delivery and targeting of nanoparticles: implications for developing nanoparticle-mediated drug delivery," *Ther. Deliv.*, vol. 1, no. 3, pp. 411–433, Sep. 2010.
[4] J. B. Delehanty, I. L. Medintz, T. Pons, F. M. Brunel, P. E. Dawson, and H. Mattoussi, "Self-Assembled Quantum Dot−Peptide Bioconjugates for Selective Intracellular Delivery," *Bioconjug. Chem.*, vol. 17, no. 4, pp. 920–927, Jul. 2006.
[5] C. Grienberger and A. Konnerth, "Imaging Calcium in Neurons," *Neuron*, vol. 73, no. 5, pp. 862–885, Mar. 2012.
[6] J. Kang, J. R. Huguenard, and D. A. Prince, "Voltage-gated potassium channels activated during action potentials in layer V neocortical pyramidal neurons," *J. Neurophysiol.*, vol. 83, no. 1, pp. 70–80, 2000.
[7] J. Viventi, D.-H. Kim, L. Vigeland, E. S. Frechette, J. A. Blanco, Y.-S. Kim, A. E. Avrin, V. R. Tiruvadi, S.-W. Hwang, A. C. Vanleer, D. F. Wulsin, K. Davis, C. E. Gelber, L. Palmer, J. Van der Spiegel, J. Wu, J. Xiao, Y. Huang, D. Contreras, J. A. Rogers, and B. Litt, "Flexible, foldable, actively multiplexed, high-density electrode array for mapping brain activity in vivo," *Nat. Neurosci.*, vol. 14, no. 12, pp. 1599–1605, Dec. 2011.
[8] I. Ferezou, F. Matyas, and C. C. H. Petersen, "Imaging the Brain in Action: Real-Time Voltage-Sensitive Dye Imaging of Sensorimotor Cortex of Awake Behaving Mice," in *In vivo optical imaging of brain function.*, 2nd ed., 2009.
[9] E. W. Miller, J. Y. Lin, E. P. Frady, P. A. Steinbach, W. B. Kristan, and R. Y. Tsien, "Optically monitoring voltage in neurons by photo-induced electron transfer through molecular wires," *Proc. Natl. Acad. Sci.*, vol. 109, no. 6, pp. 2114–2119, Feb. 2012.
[10] D. S. Greenberg, A. R. Houweling, and J. N. D. Kerr, "Population imaging of ongoing neuronal activity in the visual cortex of awake rats," *Nat. Neurosci.*, vol. 11, no. 7, pp. 749–751, Jul. 2008.
[11] A. P. Alivisatos, A. M. Andrews, E. S. Boyden, M. Chun, G. M. Church, K. Deisseroth, J. P. Donoghue, S. E. Fraser, J. Lippincott-Schwartz, L. L. Looger, S. Masmanidis, P. L. McEuen, A. V. Nurmikko, H. Park, D. S. Peterka, C. Reid, M. L. Roukes, A. Scherer, M. Schnitzer, T. J. Sejnowski, K. L. Shepard, D. Tsao, G. Turrigiano, P. S. Weiss, C. Xu, R. Yuste, and X. Zhuang, "Nanotools for Neuroscience and Brain Activity Mapping," *ACS Nano*, vol. 7, no. 3, pp. 1850–1866, 2013.
[12] J. D. Marshall and M. J. Schnitzer, "Optical Strategies for Sensing Neuronal Voltage Using Quantum Dots and Other Semiconductor Nanocrystals," *ACS Nano*, vol. 7, no. 5, pp. 4601–4609, 2013.
[13] K. Park, Z. Deutsch, J. J. Li, D. Oron, and S. Weiss, "Single Molecule Quantum-Confined Stark Effect Measurements of Semiconductor Nanoparticles at Room Temperature," *ACS Nano*, vol. 6, no. 11, pp. 10013–10023, Nov. 2012.