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Visualizing Single Molecule Electrochemistry with TIRF Microscopy

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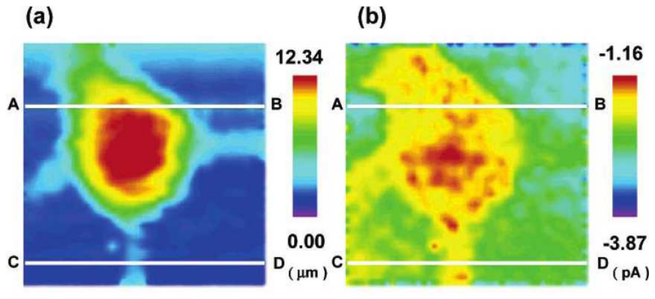
Introduction

Single molecule electrochemical measurements have recently been demonstrated,[1-2] observing the redox cycling between electrodes separated by a nanogap. Combining total internal reflection fluorescence microscopy (TIRF) with the electrochemical measurements offers the potential for ultrasensitive devices capable of selectively detecting and monitoring single redox molecules. These devices represent a new class of sensors and will facilitate the measurement of extracellular signaling events, however the single molecule behavior within the nanogap is not fully understood. Here, we use TIRF microscopy to examine the dynamics of single molecules on substrates enabling simultaneous electrochemical measurements. The microscopy requires either imaging through a transparent electrode such as indium tin oxide (ITO) or employing a zero-mode waveguide geometry. Our initial efforts employ TIRF microscopy through an ITO-coated substrate to understand single molecule diffusion, including absorption on the electrodes and the influence of the substrates upon the fluorescence dynamics.

Scanning Electrochemical Microscopy Cell Imaging

- Scanning electrochemical microscopy (SECM) measures the Faradaic current due to redox cycling
- Provides information about reactivity and identity of chemicals in cellular microenvironments
- Measurements have included imaging neurotransmitter release, hydrogen peroxide levels, glucose uptake, and cytosolic enzyme activity[3]



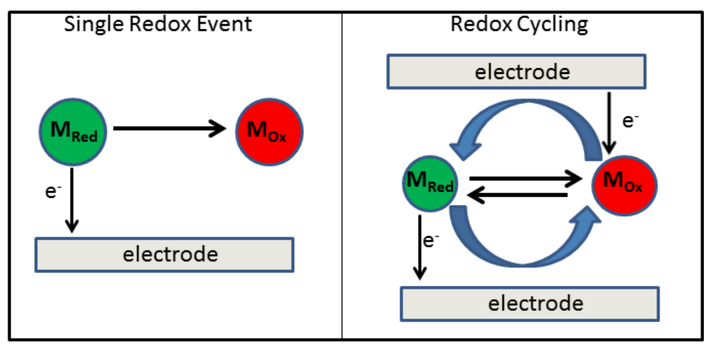
(a) Topographic and (b) electrochemical images of neuron-like PC12 cells in PBS solution. The electron probe potential of -0.50 V is sensitive to Oxygen concentration. The field of view is 47 x 47 µm.

Takahashi, Y., et al. (2006). "Topographic, electrochemical, and optical images captured using standing approach mode scanning electrochemical/optical microscopy." Langmuir **22**(25): 10299-10306.

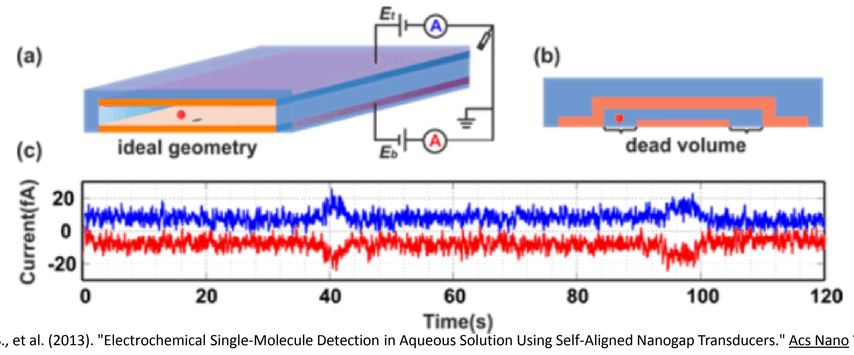
- Unfortunately, SECM **lacks single molecule sensitivity**

Redox Cycling Between Nanogap Electrodes

- Current from a single redox event is insufficient for detection
- Multiple redox cycles between reduced and excited states result in current accumulation



- Current can be detected from redox cycling of single molecules confined to nanogaps

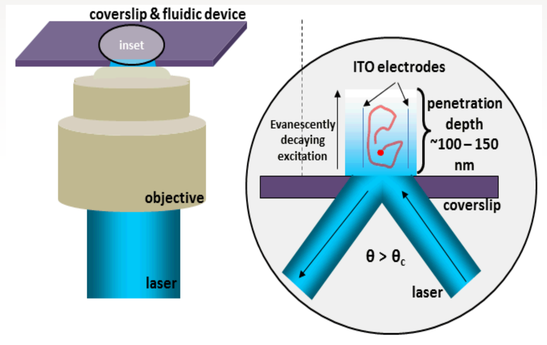


Kang, S., et al. (2013). "Electrochemical Single-Molecule Detection in Aqueous Solution Using Self-Aligned Nanogap Transducers." Acs Nano **7**(12): 10931-10937.

- **First demonstration of aqueous single molecule electrochemical measurements**

Integrating Total Internal Fluorescence Microscopy

- Data interpretation for single molecule electrochemical measurements in nanogaps is complicated by a number of factors
  - Inability to know whether only a single molecule is present
  - Signal suppression due to adsorption to the substrate (often 80% of total time[1])
  - Varying diffusion paths which may or may not traverse the channel
- Simultaneous observation with total internal fluorescence microscopy (TIRF) through transparent indium tin oxide (ITO) channels provides an orthogonal measurement
- TIRF observations allow monitoring of the number and position of redox molecules within the channel.
  - Will eliminate ambiguity about whether observations are truly single molecule
  - In combination with molecular modeling, will provide insight into the diffusive behavior of redox molecules undergoing electron transfer



- TIRF allows simultaneous measurement of redox molecule spatial position
- Two electrode geometries considered, TIRF through electrode and zero-mode waveguide geometry

Potential Impact

- SECM has proved an invaluable research tool for biology
  - Redox processes are fundamental to cellular respiration
  - Numerous bioactive molecules lend themselves to electrochemical analysis
  - Oxidative stress is thought to be involved in numerous diseases including cancer, Parkinson's, Alzheimer's, and artheriosclerosis
- Extending the sensitivity of electrochemical measurements to the single molecule level opens new posibilities
  - A valuable alternative single molecule analysis method when fluorescent labeling is invasive or impossible
  - Potential for ultrasensitive devices to detect single enzymes or proteins
  - Compatible with integrated electronics and microfabrication, which may enable inexpensive diagnostic and clinical tools

Conclusions

Single molecule electrochemical measurements have only recently been demonstrated for the first time, a technique clearly in its initial development. However, electrochemical measurements of lesser sensitivity such as SECM have already proven a valuable tool for biologists, allowing localized measurements of cellular processes such as respiration and neurotransmitter release. Therefore, extending electrochemical measurement capabilities down to single molecule sensitivity shows great potential as a research technique. At the moment, greater fundamental understanding of the single molecule interactions within the nanogap electrodes, including the diffusion and adsorption of the redox molecules undergoing electron transfer, is needed. Integrating an orthogonal observation method, TIRF, provides a direct picture into the underlying processes.

References

1. Kang, S., et al. (2013). "Electrochemical Single-Molecule Detection in Aqueous Solution Using Self-Aligned Nanogap Transducers." Acs Nano **7**(12): 10931-10937.
2. Zevenbergen, M. A. G., et al. (2011). "Stochastic Sensing of Single Molecules in a Nanofluidic Electrochemical Device." Nano Letters **11**(7): 2881-2886.
3. Schulte, A., et al. (2010). "Scanning Electrochemical Microscopy in Neuroscience." Annual Review of Analytical Chemistry, Vol 3 **3**: 299-318.
4. Takahashi, Y., et al. (2006). "Topographic, electrochemical, and optical images captured using standing approach mode scanning electrochemical/optical microscopy." Langmuir **22**(25): 10299-10306.

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