In-Vivo Real-time Biosensors

An In-Depth Discussion of a Seminar by Dr. Kaiyu Fu

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Research Goals and Methods

The goal of Dr. Fu's presented work is easily targetable, highly selective, high sensitivity, multichannel sensors for monitoring, in real-time, inside a living organism. This research incorporates nanopore electrode arrays (NEA) utilizing aptamer-based electrical sensors.

The sensors are created by utilizing methods commonly employed in computer chip manufacturing. The primary constituent of the NEA electrodes is nanoporous gold, using a structural polymeric base. In some cases, layers of silicon nitride and gold are added, with a final nonconductive layer of silicon dioxide. Once the layers are built up, photolithographic techniques, similar to computer chip production, are used to create 100 μm x 100 μm arrays of nanopores, with individual volumes in the attoliter range [1][2][3].

The electrodes, within the wells of the nanopores, are treated with an RNA-based aptamer having a methylene blue terminator. The aptamers undergo a conformal change with the target molecule causing the methylene blue (MB) terminator to get close enough to trigger a small current as it's reduced at the electrode's base. This current is measured to determine the target molecule's concentration. The use of RNA aptamers enables both high selectivity and flexibility in the choice of target molecules. The nanopore structures greatly increase the electric field experienced by the MB terminator.

The following diagram shows the differences in planar vs nanopore electrodes [2].

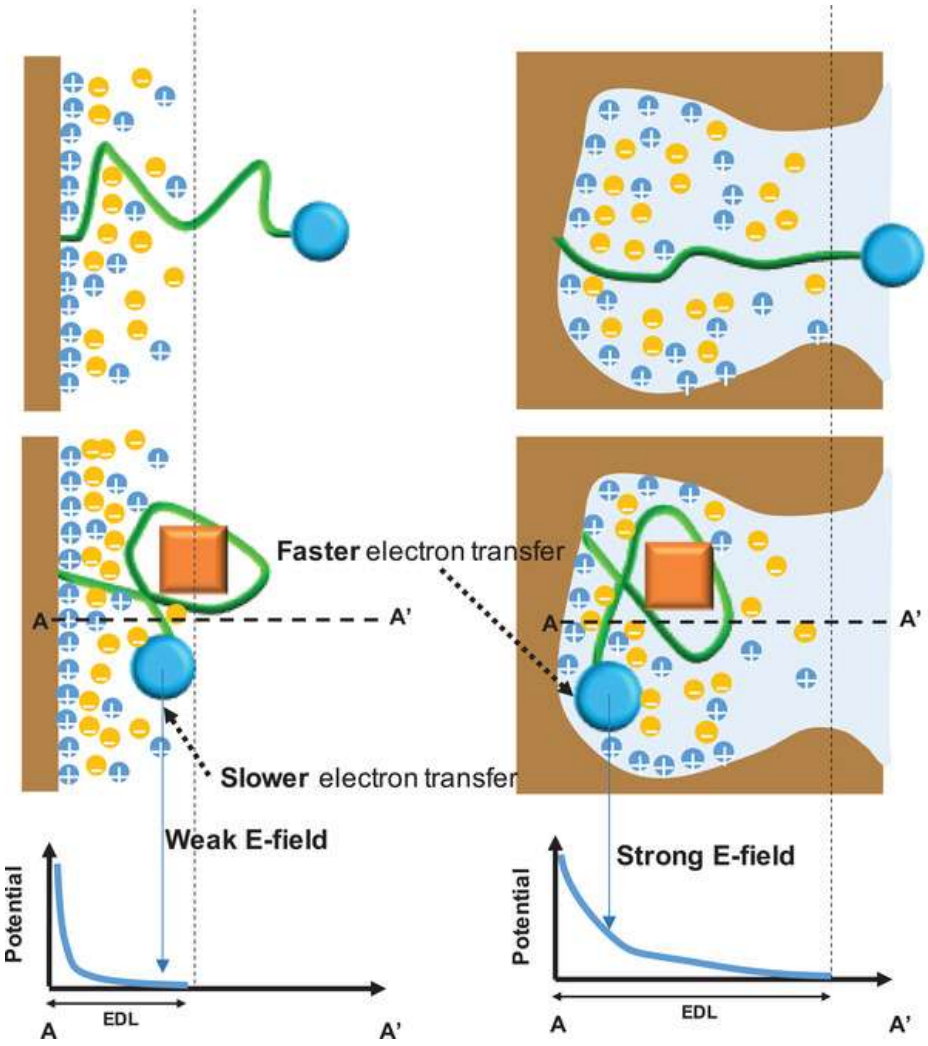

Figure 1
Scheme of how the electric double layer (EDL) from a planar (left) and nanoporous electrode (right) affects electron transfer from a MB reporter (blue circle) tethered to the aptamer (green) before and after target (orange square) binding. During electrochemical measurement, the MB reporter interacts with the EDL (shaded blue region), where a closer distance between the reporter and the electrode surface leads to faster electron transfer. In the nanoporous electrode, the MB group experiences stronger electric fields. A and A' represent the electrode surface and the maximum distance of MB from the electrode, respectively.* [2]

The use of gold and the recessed nature of the electrodes within the nanopores allow for minimal biofouling and mechanical protection of the aptamer molecules. The nanopores allow an increased effective electric field due to the changes in the Debye volume, responsible for a greatly increased level-of-detection (LOD), with LODs in the femtomolar range [2][4][5][6].

** The figure caption is directly from the paper [2], not the words of this paper's author, Glenn Murray*

Extensive testing demonstrated the precision, selectivity, sensitivity, and durability of the probe, allowing it to survive reuse with minimal functional degradation. The first tests were in simple aqueous solutions with various Doxorubicin concentrations, then in plasma to check for potential biofouling and cross-reactivity. The electrodes were checked to see if measurement in living tissue would degrade the measurement capabilities. These tests were run against planar electrodes and NEAs to measure differences in durability, sensitivity, and biofouling. The final set of tests measured the real-time, in-vivo levels of Doxorubicin within a living tumor [1].

Impacts

Having worked in the field of medical technology, I see the potential of this to completely change the world of medical and biochemical research/treatment. With rare exceptions, bodily fluids are required for biochemical measurements. To be able to take measurements in real-time, in-vivo, allows researchers a window into processes typically surmised after the fact as well as the ability of physicians to monitor their patients in real-time.

Learned

I was quite surprised at the level of advance had been made in the few decades since I worked in the field. The specificity and accuracy of aptamers for use in measurements are completely new to me. The effects of nanostructures on the physical and electrochemistry of these systems are fascinating.

References

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