DNA-MEDIATED CHARGE TRANSPORT DEVICES FOR PROTEIN DETECTION

Thesis by
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Abstract

Detection of biologically relevant targets, including small molecules, proteins, DNA, and RNA, is vital for fundamental research as well as clinical diagnostics. Sensors with biological elements provide a natural foundation for such devices because of the inherent recognition capabilities of biomolecules. Electrochemical DNA platforms are simple, sensitive, and do not require complex target labeling or expensive instrumentation. Sensitivity and specificity are added to DNA electrochemical platforms when the physical properties of DNA are harnessed. The inherent structure of DNA, with its stacked core of aromatic bases, enables DNA to act as a wire via DNA-mediated charge transport (DNA CT). DNA CT is not only robust over long molecular distances of at least 34 nm, but is also especially sensitive to anything that perturbs proper base stacking, including DNA mismatches, lesions, or DNA-binding proteins that distort the π-stack. Electrochemical sensors based on DNA CT have previously been used for single-nucleotide polymorphism detection, hybridization assays, and DNA-binding protein detection. Here, improvements to (i) the structure of DNA monolayers and (ii) the signal amplification with DNA CT platforms for improved sensitivity and detection are described.

First, improvements to the control over DNA monolayer formation are reported through the incorporation of copper-free click chemistry into DNA monolayer assembly. As opposed to conventional film formation involving the self-assembly of thiolated DNA, copper-free click chemistry enables DNA to be tethered to a pre-formed mixed alkylthiol monolayer. The total amount of DNA in the final film is directly related to the amount of azide in the underlying alkylthiol monolayer. DNA monolayers formed with

this technique are significantly more homogeneous and lower density, with a larger amount of individual helices exposed to the analyte solution. With these improved monolayers, significantly more sensitive detection of the transcription factor TATA binding protein (TBP) is achieved.

Using low-density DNA monolayers, two-electrode DNA arrays were designed and fabricated to enable the placement of multiple DNA sequences onto a single underlying electrode. To pattern DNA onto the primary electrode surface of these arrays, a copper precatalyst for click chemistry was electrochemically activated at the secondary electrode. The location of the secondary electrode relative to the primary electrode enabled the patterning of up to four sequences of DNA onto a single electrode surface. As opposed to conventional electrochemical readout from the primary, DNA-modified electrode, a secondary microelectrode, coupled with electrocatalytic signal amplification, enables more sensitive detection with spatial resolution on the DNA array electrode surface. Using this two-electrode platform, arrays have been formed that facilitate differentiation between well-matched and mismatched sequences, detection of transcription factors, and sequence-selective DNA hybridization, all with the incorporation of internal controls.

For effective clinical detection, the two working electrode platform was multiplexed to contain two complementary arrays, each with fifteen electrodes. This platform, coupled with low density DNA monolayers and electrocatalysis with readout from a secondary electrode, enabled even more sensitive detection from especially small volumes (4 μL per well). This multiplexed platform has enabled the simultaneous

detection of two transcription factors, TBP and CopG, with surface dissociation constants comparable to their solution dissociation constants.

With the sensitivity and selectivity obtained from the multiplexed, two working electrode array, an electrochemical signal-on assay for activity of the human methyltransferase DNMT1 was incorporated. DNMT1 is the most abundant human methyltransferase, and its aberrant methylation has been linked to the development of cancer. However, current methods to monitor methyltransferase activity are either ineffective with crude samples or are impractical to develop for clinical applications due to a reliance on radioactivity. Electrochemical detection of methyltransferase activity, in contrast, circumvents these issues. The signal-on detection assay translates methylation events into electrochemical signals *via* a methylation-specific restriction enzyme. Using the two working electrode platform combined with this assay, DNMT1 activity from tumor and healthy adjacent tissue lysate were evaluated. Our electrochemical measurements revealed significant differences in methyltransferase activity between tumor tissue and healthy adjacent tissue.

As differential activity was observed between colorectal tumor tissue and healthy adjacent tissue, ten tumor sets were subsequently analyzed for DNMT1 activity both electrochemically and by tritium incorporation. These results were compared to expression levels of *DNMT1*, measured by qPCR, and total DNMT1 protein content, measured by Western blot. The only trend detected was that hyperactivity was observed in the tumor samples as compared to the healthy adjacent tissue when measured electrochemically. These advances in DNA CT-based platforms have propelled this class of sensors from the purely academic realm into the realm of clinically relevant detection.

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