IMMOBILIZATION OF A CATALYTIC DNA FOR ADVANCED MATERIALS AND SENSORS

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Functional DNA molecules, such as catalytic DNAzymes, are gaining momentum in the field of biosensors. Such molecules are identified by a method of *in vitro* selection, a combinatorial methodology for high-throughput analysis of biomolecular analyte binding. The capacity of these molecules for specific recognition of analytes allows implementation of various signal transduction methods to generate highly specific and sensitive sensors. Here, DNAzymes are immobilized on bulk surfaces for fluorescent and electrochemical sensors and advanced materials.

Immobilization of a Pb^{2+}-specific DNAzyme with cleavage activity was accomplished on gold using thiol-Au self-assembly procedures. The thiolated-enzyme strand of the DNAzyme complex was self-assembled, and the monolayer was backfilled with a mercaptohexanol monolayer to displace non-specific DNA adsorption. A fluorophore labeled substrate strand was hybridized for assembly of the active DNAzyme complex. Immobilization allowed multiple regenerations and fully-assembled sensor storage. Detection limits of the DNAzyme for Pb^{2+} on planar gold and Au-coated nanocapillary membranes were 1 nM and 17 nM, respectively. A UO_2^{2+}-specific DNAzyme was also immobilized following the established immobilization protocol with over 10-fold increase in fluorescence observed with addition of 1 μM UO_2^{2+}, demonstrating the immobilization protocol is a general methodology for catalytic DNA.

Additionally, a non-cleavable substrate with a different fluorophore was hybridized to provide ratiometric fluorophore analysis of DNAzyme activity. Ratiometric analysis monitored non-specific release of substrate from the DNAzyme complex, standardized variability in sensor size and monolayer density and provided real-time observation of Pb^{2+} activity. Finally, a ferrocene-labeled substrate strand was assembled into the DNAzyme complex on Au electrodes for electrochemical detection of DNAzyme activity.

The DNAzyme was also immobilized on patterned glass surfaces via covalent attachment. Substrate-functionalized 100 nm polystyrene particles were then hybridized in the absence and presence of Pb^{2+}. The result was the Pb^{2+}-mediated tethering of particles onto patterned grids, where Pb^{2+} induced the cleavage of the DNAzyme complex and prevented assembly of the particles. The assembly of these advanced materials was monitored in real-time using laser diffraction and was characterized via scanning electron microscopy. Addition of 10 μM Pb^{2+} showed >99% inhibition of assembly.