J Biosens Bioelectron 2016, 7:2 (Suppl)
http://dx.doi.org/10.4172/2155-6210.C1.028

Molecular self-assembly for signal enhancement in nucleic acids biosensors

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Some of the possible perspective advantages of the uptake of nucleic acids biosensor technology are already within reach, still, sometimes, limited sensitivity can seriously inhibit the application of biosensor-based detection methods when these could be useful towards detection of nucleic acids variants present only at a very low concentration. While in a research lab, this objective is achieved using complex but sensitive amplification techniques, such as PCR, or the deployment of sophisticated sensitive detection techniques, this goal might prove a prohibitive objective for point-of-need biosensors. Recently, through knowledge and capabilities developed within the field of DNA nanotechnology, means to implement very specific molecular recognition and signal enhancement methods have been presented that could prove of direct application for biosensor detection methods. We have adapted and attempted at using the hybridization chain reaction (HCR) towards enhancing the signal due to the specific recognition and binding of soluble nucleic acids to a surface-bound probe. The enhancement strategy consists in a triggered supramolecular polymerization of DNA sequences or nanostructures at the location of specific nucleic acids recognition. We have showed that the method can be used towards the detection of an arbitrary DNA target through proper design of the sequences of the components. Preliminary experimental evidence shows a significant enhancement of the signal, which could prove useful in some applications. We also proved that HCR can have single-nucleotide sensitivity for the detection and signal enhancement. We recently extended the application of HCR towards the detection of miRNA targets. This strategy is compatible with several detection techniques that can be implemented on a lab-on-chip, such as electrochemistry or surface plasmon resonance, while it can also be measured via fluorescence and luminescence, achieving higher detection sensitivity..

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Highly sensitive biomarker detection via stimuli-responsive reagents

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Clinical diagnostic tests such as immunoassay have been utilized for patient diagnosis to significantly improve health care and reduce costs by detecting trace amounts of certain proteins in patients for identifying harmful cells and troublesome cellular processes. The mainstream immunoassays utilize antibodies immobilized on solid supports for biomarker recognition and separation, which result in long assay time and compromise assay detection limit. In order to achieve higher assay sensitivity, our group has developed stimuli-responsive affinity reagents to address some of the biomarker separation challenges. The reagents such as antibodies conjugated with stimuli-responsive polymers respond sharply and reversibly to physical or chemical stimuli by changing their conformation and physical-chemical properties, i.e., changing from a hydrophilic state to a more hydrophobic state. Stimuli-responsive reagents can replace the antibodies immobilized at solid supports to overcome the mass transport limitations associated with heterogeneous immunoassays because the biomarker binding occurs in a homogeneous solution where molecular diffusion of the reagents facilitates rapid mass transport equilibration. The conjugates can interface with different diagnostic devices to enable rapid immunoassay by facilitating simple and effective biomarkers (or full sandwich immunocomplexes) separation and detection. Additionally, the rapid assay system is scalable to larger starting volumes, which provides opportunities to concentrate dilute biomarkers, thus improving detection ranges and expanding diagnostic options in immunoassays. In this presentation, I will discuss the utilities of stimuli-responsive affinity reagents for microfluidic immunoassay to enable sensitive detection for prostate specific antigen in human plasma as well as rapid biomarker purification and enrichment for infectious disease such as malaria.

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