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DNA-Origami Based Biosensor for Assembly of Antibody-Protected Bimetallic Nanoclusters as Biorecognition and Transduction Element

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Extended Abstract

The goal of this work is to develop a non-conventional immunoassay platform based on the assembly and integration of biosensing elements using DNA-origami. Enzymes are usually conjugated to detection antibodies for signal amplification in conventional immunoassays. Natural enzymes suffer from lack of stability due to high susceptibility to changes in environmental conditions, denaturation and digestion and costly and time-consuming preparation and purification. An enzyme-free methodology could overcome these drawbacks. Nanomaterials present several advantages due to their stability in changing environment, ease synthesis, unique optical properties, enzyme-like behaviour, robust detection and compatibility with biomolecules [1]. In the presented biosensor, antibodies with catalytic nanoclusters (NCs) embedded in their structure act as probe, integrating both, the biorecognition and transduction elements [2]. After the introduction of the NCs in their structure, the resulting antibodies retain their affinity for target antigens [3]
A secondary antibody (anti-rabbit IgG) has been chosen for this research providing a broader range of applications. The bimetallic NCs exhibit peroxidase-like activity [4]. The affinity of these antibodies modified with NCs for the target analyte has been tested using a paper-based immunoassay. Detection is based on the catalytic properties of the NCs to oxidize an insoluble chromogenic substrate. The signal is visible to the naked eye and can be further analysed for quantitative results. The use of antibody-protected NCs reveal improvements in terms of stability in comparison with natural enzyme horseradish peroxidase (HRP). Additionally, incorporating the NCs within the antibody structure instead of attaching them via a covalent bond provides enhanced sensitivity.
Transferring the DNA origami-based biosensor to a flexible substrate offers several advantages like suitability for wearable applications, portability and remote controlling. Polystyrene (PS) was tested as flexible substrate due to its chemical stability, flexibility, optical properties and ease of surface modification, allowing integration of functional groups for biomolecule immobilization. Additionally, PS is cost-effective and widely available, making it ideal for large-scale production of flexible biosensors.
The surface modification of PS is achieved by integrating chlorosulfonyl groups, which are chemically stable and allow subsequent reaction with aliphatic amines. This method enables the modification of the PS surface with amino and carboxyl groups. The immobilization of DNA origami on modified PS has been tested through both physisorption and chemisorption strategies. Confirmation of the immobilization was achieved through the hybridization of complementary strands modified for a colorimetric peroxidase reaction.

The nanometric control of the position of all elements of the biosensor due to the use of DNA origami will allow the development of a non-conventional immunoassay. The transduction will be achieved through changes in the SERS signal when the analyte binds to the antibody-protected bimetallic NCs. An enhancement in the SERS signal will be achieved by including nanoparticles (NPs) in strategic locations. This approach will offer significant advantages over traditional immunoassays, such as improved sensitivity, quick turnaround, and suitability for point-of-care (PoC) diagnostics.

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