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Structure–function relationships affecting the sensing mechanism of monolayer-protected cluster doped xerogel amperometric glucose biosensors

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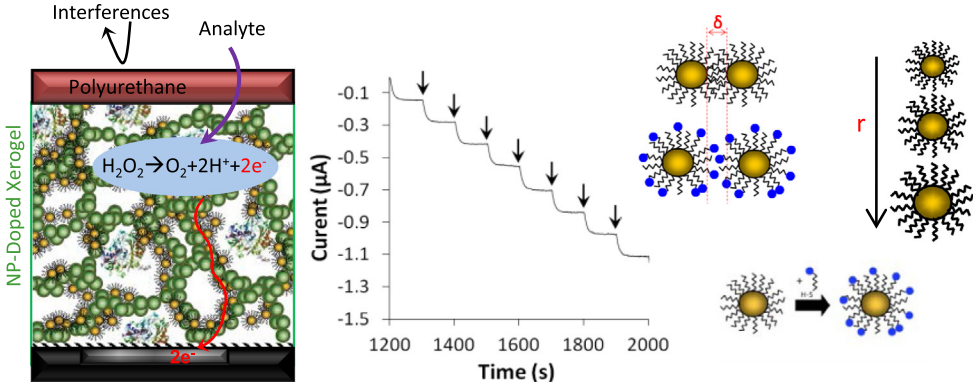
GRAPHICAL ABSTRACT



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ABSTRACT

A systematic study of the structure–function relationships critical to understanding the sensing mechanism of 1st generation amperometric glucose biosensors with an embedded nanoparticle (NP) network is presented. Xerogel-based films featuring embedded glucose oxidase enzyme and doped with alkanethiolate-protected gold NPs, known as monolayer protected clusters (MPCs), exhibit significantly enhanced performance compared to analogous systems without NPs including higher sensitivity, faster response time, and extended linear/dynamic ranges. The proposed mechanism involves diffusion of the glucose to glucose oxidase within the xerogel, enzymatic reaction production of H_2O_2 with subsequent diffusion to the embedded network of MPCs where it is oxidized, an event immediately reported via fast electron transfer (ET) through the MPC system to the working electrode. Various aspects of the film construct and strategy are systematically probed using amperometry, voltammetry, and solid-state electronic conductivity measurements, including the effects of MPC peripheral chain length, MPC functionalization via place-exchange reaction, MPC core size, and the MPC density or concentration within the xerogel composite films. The collective results of these experiments support the proposed mechanism and identify interparticle spacing and the electronic communication through the MPC network is the most significant factor in the sensing scheme with the diffusional aspects of the mechanism that may be affected by film/

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MPC hydrophobicity and functionality (i.e., glucose and H_2O_2 diffusion) shown to be less substantial contributors to the overall enhanced performance. Understanding the structure–function relationships of effective sensing schemes allows for the employment of the strategy for future biosensor design toward clinically relevant targets.

1. Introduction

Biosensor research represents an important and expanding sector of materials and interfacial chemistry due to their many and varied applications across disciplines. Electrochemical sensor development continues to draw a significant level of interest as it remains relatively simple and affordable while still maintaining the potential for adaptability to different analytes relevant for both clinical and industrial applications [1–3]. In particular, 1st generation amperometric biosensors, which utilize immobilized enzymes to catalyze an analyte to hydrogen peroxide (H_2O_2) that is then subsequently oxidized at the working electrode to produce a current response representative of analyte concentration, continue to receive attention due to their ability to provide increased sensitivity, analyte selectivity and quick response times with the potential of miniaturization leading to *in vitro* remote sensing as well as *in vivo* applications [1].

In a 1st generation biosensing model, the enzyme must be immobilized without compromising structural integrity of the sensor or the inhibiting enzyme function. Sol–gels represent an appealing method of immobilization because they easily and without harsh conditions form a rigid silicate network that is both chemically inert and resistant to significant swelling in the presence of water while maintaining enzyme activity [4]. When cast from solvent and allowed to age under controlled humidity and temperature, the sol–gels formed are known as xerogels [5,6]. The expansive catalogue of silanes allows for a multitude of xerogels to be constructed for specific chemistry. For example, 3-mercaptopropyltrimethoxy silane (3-MPTMS) introduces thiol functionality into the gels where it has been used to bind certain metallic nanomaterials (NMs) [7,8]. Among their other properties, the ability of NMs to aid in electron transfer (ET) processes has made NP-assisted biosensor designs increasingly prevalent in the field. The multitude of different nanomaterials allows for possible specialization of a biosensor depending on their specific size, shape and composition [3].

Literature reports suggest that the use of certain NMs, specifically metallic nanoparticles (NPs), allow for greater microenvironment control through the creation of a NP network that enhances the ET from reaction site to the electrode [9,10]. A major focus of that body of work is on the use of colloidal gold NPs as a component of electrochemical biosensors. Most of these reports focus on proof-of-concept rather than exploring the role of NP structure in the sensor performance and functionality [11–16]. Few, if any, of these reports adequately focus on mechanistic understanding the NPs within their sensing schemes.

In 2013, a 1st generation amperometric glucose biosensor model system featuring a MPTMS xerogel embedded with glucose oxidase (GO_x) and doped with gold NPs known as monolayer protected clusters (MPC) was demonstrated [8]. The composite MPC-doped xerogel film was deposited on a platinum electrode and coated with a semi-permeable polyurethane (PU) layer to assist in interferent discrimination (Scheme 1). The sensor was found to have an order of magnitude increase in sensitivity, doubled linear range, and a 4-fold decrease in response times compared to similar sensors without MPCs (Fig. 1). While the enhanced performance and characterization of this model system

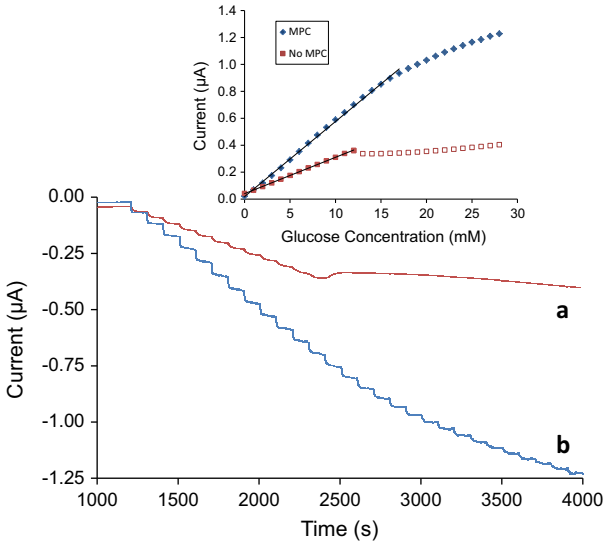

Fig. 1. Examples of amperometric *I–t* curves during successive 1 mM injections of glucose at platinum electrodes modified with (a) GO_x embedded 3-MPTMS xerogels and (b) GO_x embedded MPTMS xerogel doped with C6-MPCs, each coated with PU (Scheme 1). Inset: Calibration curves for glucose biosensors constructed with platinum electrodes modified with GO_x embedded MPTMS xerogels with and without C6-MPC doping where solid markers indicate a step-like response to glucose concentration increases whereas open markers indicate a non-step response (dynamic range). Linear regression has been performed with the linear range shown as a solid black line. Error bars have been omitted for clarity [8].

was well-documented and compared with other literature reports of similar sensors with and without NMs, a mechanistic understanding of the structure–function relationships involving the embedded MPCs was not a major focus of the study. More recent work [17] investigated the functionality of the layered approach, including the specific role of the silane precursor material and polyurethane, but did not connect the findings to the MPC-doped xerogels. In this paper, we follow up that important finding with the systematic interrogation of MPC characteristics within the doped xerogels including core size, ligand chain length, and ligand functionality, with an aim of establishing the critical structure–function relationships in these composite materials that are responsible for the observed sensing enhancement [8] and that may help shape a proposed sensing mechanism (*vide infra*).

2. Experimental

2.1. Materials and instrumentation

All chemicals were purchased from Sigma Aldrich unless otherwise stated. Tecoflex SG-80A polyurethane (TPU) and Hydrothane AL25-80A polyurethane (HPU) was obtained from Lubrizol and AdvanSource Biomaterials, respectively. Solutions were prepared using 18.2 MΩ cm ultra-purified water. Sensors were constructed as described below on platinum (2 mm dia.) working electrodes (CH Instruments) with the analytical performance of the composite films evaluated via amperometric current–time (*I–t*) curves recorded on eight channel potentiostat (CH Instruments, 1000B). Electrochemistry was measured versus common Ag/AgCl