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Photoswitchable peptide-based 'on-off' biosensor for electrochemical detection and control of protein-protein interactions

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ABSTRACT

Neuronal nitric oxide synthase (nNOS) is an enzyme responsible for catalyzing the production of the crucial cellular signalling molecule, nitric oxide (NO), through its interaction with the PDZ domain of α-syntrophin protein. In this study, a novel light-driven photoswitchable peptide-based biosensor, modelled on the nNOS β-finger, is used to detect and control its interaction with α-syntrophin. An azobenzene photoswitch incorporated into the peptide backbone allows reversible switching between a *trans* photostationary state devoid of secondary structure, and a *cis* photostationary state possessing a well-defined antiparallel β-strand geometry, as revealed by molecular modelling. Electrochemical impedance spectroscopy (EIS) is used to successfully detect the interaction between the gold electrode bound peptide in its *cis* photostationary state and a wide range of concentrations of α-syntrophin protein, highlighting both the qualitative and quantitative properties of the sensor. Furthermore, EIS demonstrates that the probe in its random *trans* photostationary state does not bind to the target protein. The effectiveness of the biosensor is further endorsed by the high thermal stability of the photostationary state of the *cis*-isomer, and the ability to actively control biomolecular interactions using light. This approach allows detection and control of binding to yield a regenerable on-off biosensor.

1. Introduction

The detection of protein-protein interactions and other biomolecular interactions is commonly carried out using optical based sensors such as fluorescent probes and FRET based sensors. These optical methods use fluorescence or chemiluminescence to detect the interaction of the probe with its biological target (Silva et al., 2018), however this process can be limited by photobleaching and interference from background autofluorescence (Nakamura et al., 2016). Such probes also require labelling with a fluorophore for signal detection, which can significantly alter binding to the biological target (Daniels and Pourmand, 2007; Hushegyi et al., 2015). New approaches for studying and exploiting protein-protein interactions are needed in order to address these shortcomings, while recognizing that these interactions are predominantly transient, temporary and reversible in nature. Label-free, affinity-based techniques based on electrochemical signals present as an attractive option to allow direct detection of real-time events (Silva et al., 2018) with high selectivity and sensitivity (Mannoor et al., 2010) and with potential for future use in point-of-care applications (Chaudhary et al., 2016; Kang et al., 2017). Among these, electrochemical impedance spectroscopy (EIS) has become a powerful and informative technique for studying biorecognition processes, for example in the detection of antibodies (Afkhami et al., 2017), antibiotics (Hassani et al., 2017) and pathogens (Hou et al., 2018). In this approach, the electrical impedance of the probe and that of the probe/biological target complex, is measured as a function of the frequency of an applied electrical current to enable detection of a particular interaction. Impedance is the measure of opposition to the flow of electrical current (AC) when an external voltage is applied, and it possesses both magnitude and phase (cf. resistance, which possesses only magnitude). Biomolecular interactions between the ligand-modified sensor and a protein of interest, can be precisely correlated to changes in the impedance spectrum, providing a basis for rapid, non-destructive, robust, low-cost, and low-limits of detection (LOD) of biomolecules (Dibao-Dina et al., 2015; Fusco et al., 2017; Lim et al., 2017). Importantly, electrochemical transduction circumvents the need for labelling in

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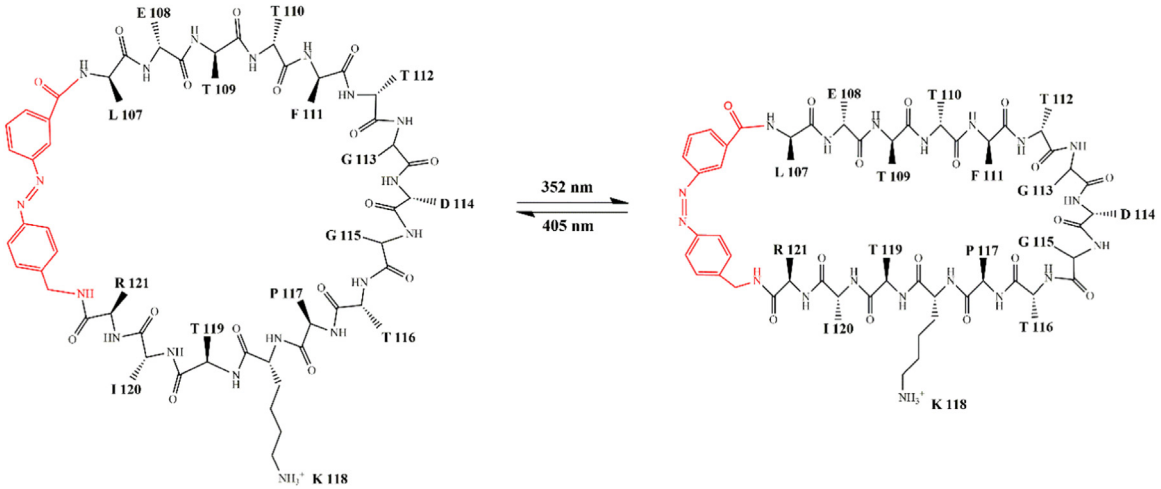


Fig. 1. Schematic representation of photoswitchable cyclic peptide **1** with residues L107 to R121 retained from the native nNOS β-finger sequence. left: *trans* conformation, and right: *cis* conformation. The azobenzene photoswitch is highlighted in red. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

optical-based methods, affording real-time measurement of the interaction while using less time and reagents than label-based methods (Templier et al., 2016), thus making EIS an ideal candidate for protein detection (Daniels and Pourmand, 2007).

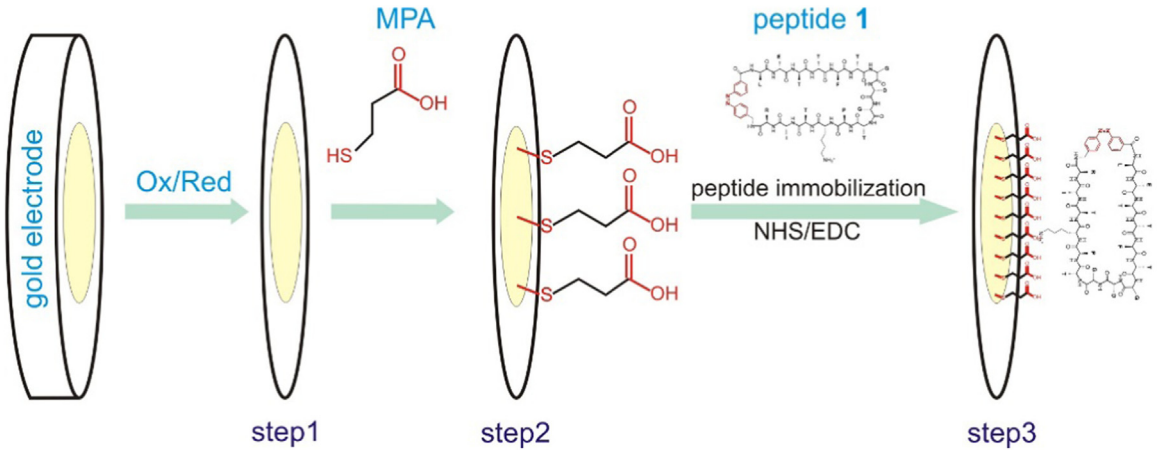
Here, we present an EIS-based probe that allows detection of α-1-syntrophin protein binding to a gold electrode bound azobenzene-containing peptide (**1**), derived from the β-finger of neuronal nitric oxide synthase (nNOS). Peptide **1** represents a truncated form of the native β-finger peptide that is known to bind to α-1-syntrophin via a well-defined protein-protein interaction. The azobenzene component allows reversible switching between a *trans* photostationary state (PSS) possessing an ill-defined conformation, and a *cis* PSS containing a well-defined secondary structure (see Fig. 1) that mimics the bound form of the native nNOS β-finger ligand (Hoppmann et al., 2009). This photoswitching occurs with high spatiotemporal precision (Kumeria et al., 2015; Mourot et al., 2018). Attachment of **1** to a gold electrode (see Scheme 1) then allows detection of its interaction with the α-syntrophin protein by way of electrochemical impedance spectroscopy.

Neuronal nitric oxide synthase (nNOS) was chosen for this study because of its well-defined interaction with α-syntrophin protein and its important role in catalyzing the production of the crucial cellular signalling molecule nitric oxide (NO) (Nichols et al., 2015). We know that the β-finger ligand from the PDZ domain of nNOS forms an extended antiparallel β-sheet which interacts with the binding groove on the α-syntrophin PDZ domain to form a heterodimer (Hillier et al., 1999). PDZ protein interaction domains are among the most common protein modules, and in recent years have emerged as novel drug targets for disease (Pedersen et al., 2016), thus highlighting their potential in the development of tailored interfaces at the nanoscale.

2. Experimental

2.1. Electrode modification

The stepwise modification of gold electrodes is outlined in Scheme 1. Specifically, gold disk electrodes (Φ = 2 mm) were polished with Metadi diamond pastes of 1 μm, and 0.25 μm particle sizes, and cleaned with sonication in Milli-Q water for 2 min. The polished electrodes were chemically treated in 25% v/v H_2O_2 / KOH (50 mM) for 10 min, and then electrochemically cleaned by potential cycling between −400 mV and 1400 mV (vs. Ag/AgCl, 100 mV s^{-1}) for 50 cycles in 1.0 mM aqueous $HClO_4$ (Johari-Ahar et al., 2015). The electrodes were further subjected to potential cycling (step 1) between −200 mV and −1200 mV (vs. Ag/AgCl, 50 mV s^{-1}) for 50 cycles in 50 mM KOH (Kitagawa et al., 2008). The cleaned electrodes were immediately immersed in a 20 mM solution of 3-mercaptopropionic acid (MPA) in absolute ethanol at room temperature in the dark overnight, resulting in exposed carboxylic acid groups (step 2). Peptide **1** was then coupled to the electrode according to literature methodology (Hoppmann et al., 2009). After the surface groups were activated to *N*-



Scheme 1. Schematic representation of stepwise fabrication of electrochemical biosensor.