Label-free electrochemical detection of enzymatic activity using microarrays

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Introduction

Recently, the immobilisation of peptide aptamers on gold electrode microarrays and the detection of their binding to proteins in cell lysates by label-free electrochemical spectroscopy have been demonstrated. We are currently investigating the use of this technology to construct substrate microarrays for the detection of activity and substrate specificity of enzymes responsible for post-translational modification of proteins. The concept is illustrated in Figure 1: Individual electrodes will be selectively functionalised with substrates via a self-assembled monolayer (SAM), and the conversion of the immobilised substrates will be detected by label-free electrochemical spectroscopy.

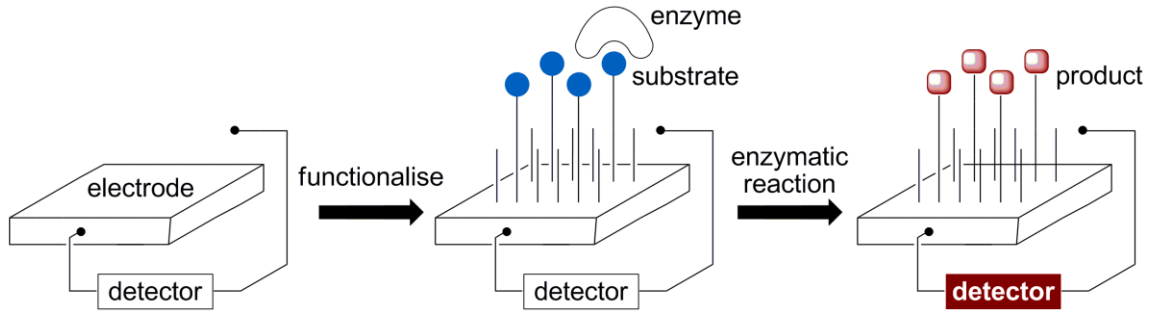

Figure 1: label-free electro-chemical detection of enzymatic activity. Substrates will be chemo-selectively immobilised on electrodes. Upon enzymatic processing, the change of their size and/or charge will be measured using label-free electrochemical spectroscopy.

Results

A collection of protease substrates labelled with an alkanethiol (for formation of SAMs) via a PEG linker (**1**) was synthesised using a combination of solution phase and solid phase methods (Figure 2). The synthesis protocols were used to prepare additional analogues of **1** incorporating a ferrocenyl group – a redox label – (**2**) and PEGylated peptides incorporating a fluorogenic aminocoumarin (**3**) for solution phase protease assays.

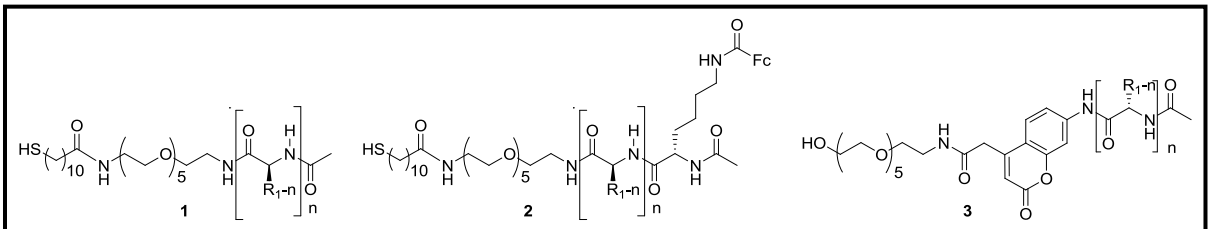

Figure 2: alkanethiol-labelled peptides **1** and **2** and PEGylated peptide **3**. Fc = ferrocenyl.

The efficiency of proteolysis of peptides **3** is currently being studied in solution. So far, Electrochemical Impedance Spectroscopy (EIS) and Cyclic Voltammetry (CV) have been used to characterise electrodes functionalised with peptides **1** and **2** and to evaluate the quality of the SAMs formed by these molecules. All electrochemical experiments were carried out in a bespoke three-electrode cell, including an Ag/AgCl reference electrode, a platinum wire counter electrode and a SAM-modified Au surface working electrode. Figure 3 shows a characteristic Bode plot (magnitude of impedance (log |Z|) and phase (Z) plotted as a function of frequency) for mixed SAMs assembled from different ratios of peptide **1a** and a

shorter dilutant. The magnitude of |Z| at 100 mHZ and the minimum phase angle of -88˚ are commensurate with the formation of a well-packed, insulating monolayer.

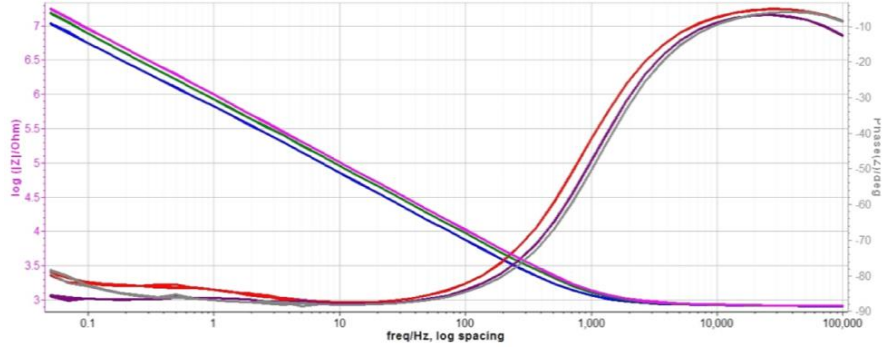

Figure 3: EIS (Bode plot) of a gold electrode modified with 0.5 mM mixed SAM (dilutant:peptide **1**). Red line – 9:1 ratio, purple line – 1:1 ratio and gray line – 1:9 ratio.

Figure 4 shows the redox process of $Fe(CN)_6^{3-/4-}$ using a SAM-modified electrode before and after SAM desorption. We observe significant suppression of redox activity following SAM assembly. This is observed for all SAMs investigated and independent of the ratio of the dilutant and peptide **1a** integrated within the SAM. These results are consistent with the formation of a well-packed, insulating molecular monolayer.

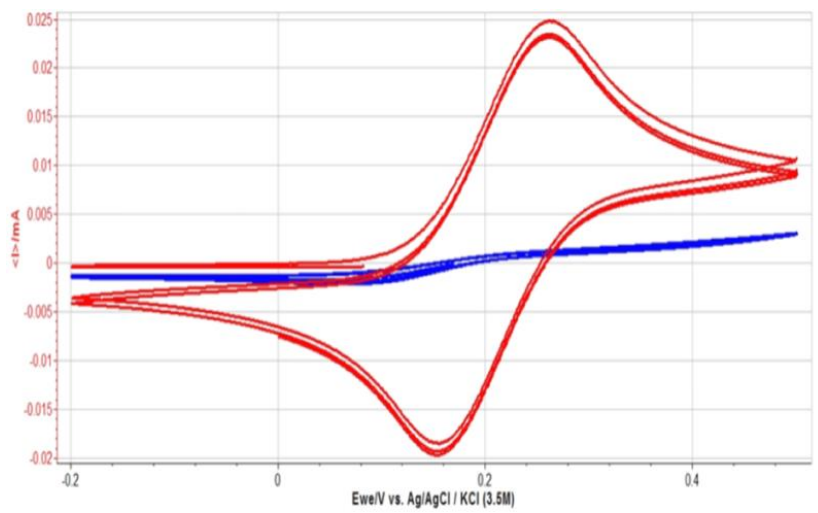

Figure 4: CV voltammograms of an electrode modified with 0.5 mM mixed SAM (dilutant:peptide **1** 1:1) (blue line) and a bare electrode after desorption of SAM from the gold surface (red line).

Unfortunately, peptide **2** proved unsuitable for CV measurements, most likely because of the instability of the ferrocenyl moiety during the measurements. Alternative redox labels are currently under investigation. We are also in the process of determining the suitability of peptides **1** as SAM-bound protease substrates using SPR.

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