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# Probing of Enzyme Reactions by the Biocatalyst-Induced Association or Dissociation of Redox Labels Linked to Monolayer-Functionalized Electrodes

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Materials:

Dithiobis(succinimidyl propionate), tyramine, ferrocene boronic acid, ascorbic acid tyrosinase, thrombin and α-chymotrypsin were purchased from Sigma-Aldrich and used as supplied. The tyramine functionalized peptide was synthesized in-house, by a conventional stepwise solid-phase peptide synthesis method using Fmoc chemistry.

Modification of Electrodes:

Au wire electrodes (0.5 mm diameter, geometrical area ca. 0.16 $cm^2$, roughness factor ca. 1.4) were used in the experiments. Prior to modification, the electrodes were soaked in concentrated $HNO_3$ for 5 min, rinsed with water. Then the electrodes were rinsed again with boiling ethanol and water, dried with $N_2$. The Au electrodes were reacted in 1 mM dithiobis(succinimidyl propionate) in dry DMSO for 1 hour, rinsed with DMSO and then briefly with water. The active ester modified electrodes were further reacted with 1 mM tyramine, or 1 mM tyramine functionalized peptide in 0.1 M HEPES buffer, pH=7.4 for 2 h, washed with water. Then the tyramine or tyramine functionalized peptide modified electrodes were incubated in different concentration of tyrosinase solution in 50 mM $KH_2PO_4$, pH = 6.5 for 20 min in the presence of 1 mM ascorbic acid. It should be noted that here ascorbic acid prevents the further oxidation of dopamine to quinine. These dopamine modified electrodes were reacted with 1 mM ferrocene boronic acid in 0.1 M phosphate buffer, pH = 5.0 for 1 h, and then rinsed carefully with water.

For the peptide modified electrode, after reacted with ferrocene boronic acid, these electrodes were further incubated in $10^{-7}$ M thrombin to cleave off the peptide.

Electrochemical measurements:

A conventional three-electrode cell, consisting of a modified Au wire working electrode, a glassy carbon auxiliary electrode isolated by a glass frit, and a saturated calomel reference electrode (SCE) connected to the working volume with a Luggin capillary, was used for the electrochemical measurement. All potentials are reported with respect to the SCE. The cell was placed in a ground Faraday cage. Differential pulse voltammetry and cyclic voltammetry were performed using an Autolab electrochemical system (Eco chemie, Netherlands). All measurements were done in 0.1 M phosphate buffer, pH = 7.4. Differential pulse voltammetry was scanned between 0V and 0.3V with a step potential of 0.01V and an amplitude potential of 0.02V.