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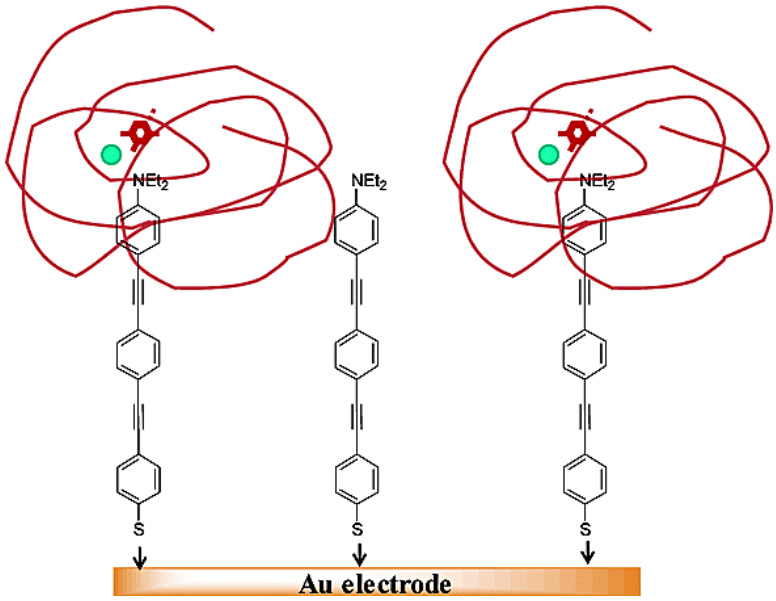

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Gold Electrodes Wired for Coupling with the Deeply Buried Active Site of *Arthrobacter globiformis* Amine Oxidase

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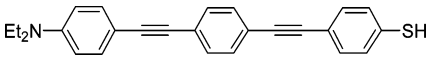
Deeply buried enzyme active sites are difficult to study electrochemically, as the tunneling of electrons to internal redox centers often is too slow to be observed even when redox mediators are employed.[1,2] The amine oxidases are a case in point: AOs catalyze the conversion of amines to aldehydes using copper and an organic cofactor, topaquinone (2,4,6-trihydroxyphenylalanine quinone, TPQ), as redox centers.[3−6] Accurate determination of the cofactor reduction potentials is badly needed, as it is likely that electron transfers are key steps in the AO catalytic mechanism.[7] In the enzyme from *Arthrobacter globiformis* (AGAO), the active center is only accessible to substrates through a hydrophobic channel that is ∼20 Å deep.[8] Because electronic coupling mediated by polypeptide or water at this distance is expected to be very weak,[9,10] the AGAO-electrode kinetics would be sluggish at best.

To enhance electron tunneling to and from the AGAO active site,[11−29] we have synthesized a diethylaniline-terminated oligo-(phenyl-ethynyl)-thiol (DEA-OPE-SH) wire to bind in the substrate channel, thereby allowing TPQ to be coupled more strongly to an electrode surface.



The synthesis, which involves a series of Pd cross-coupling reactions, allows for modification of the headgroup and length of the molecule, to match specific requirements of the protein.[30] Because competitive AGAO inhibition studies have shown that diethylaniline is a strong inhibitor of phenethylamine turnover,[31] the DEA end of the wire will act as the protein-specific functionality. With the opposite (thiol) end adsorbed on a Au surface, electron tunneling through the bridge of repeating phenyl-ethynyl units to the active site of wire-bound AGAO should be rapid.[32,33]

Gold-bead electrodes[34] were soaked in millimolar solutions (1:1 CH_2Cl_2:CH_3OH) of DEA-OPE-SH for ∼24 h to functionalize the surface. Reductive stripping analyses of the resulting films indicated ∼70% coverage of wires on the gold surface.[35] The modified electrodes were subsequently incubated with AGAO for 24−48 h to allow binding to the adsorbed wires. Cyclic voltammetry[36] using electrodes prepared in this manner showed a reversible reduction at −140 mV versus SCE in phosphate buffer, pH 7 (Figure 1), whereas electrodes modified with thiol wire alone gave no response in this potential range. Background-subtracted voltammograms recorded at slow scan rates (<20 mV/s) revealed anodic and cathodic widths from ∼55 to 70 mV, with peak splittings ranging from ∼30 to 50 mV.[37] The wave shapes remained essentially unchanged at scan rates up to 1 V/s, although there was a slight

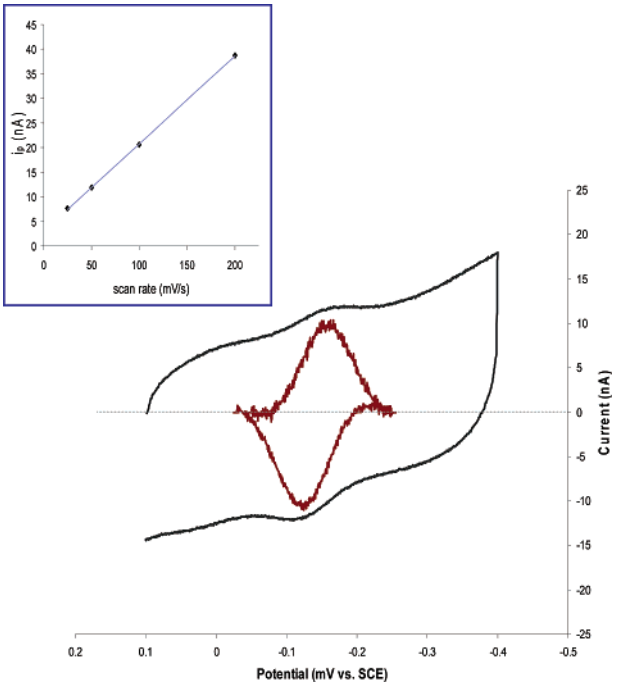
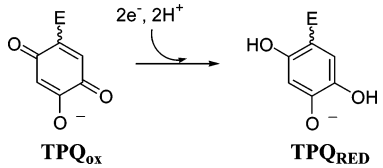


Figure 1. Cyclic voltammogram (black line) and background-subtracted voltammogram (red line) for AGAO on Au-bead electrodes modified with DEA-OPE-SH in 10 mM KPi, pH 7 (scan rate 100 mV/s). The background-subtracted voltammogram is not to scale. Inset: peak current versus scan rate.

broadening of the peaks. The linear dependence of peak current on scan rate (Figure 1, inset) is in accord with expectation for a protein-surface conjugate.

The observed reduction potential, which is close to that reported for quinone model complexes,[38] varies linearly with pH; the slope of ∼ −60 mV/pH indicates a $2e^-$, $2H^+$ TPQ reduction to the hydroxyquinol. A $2e^-$, $3H^+$ reduction of related quinones occurs between pH 4.5 and 8 in the absence of protein.[38] Apparently, the



nearby Cu(II) center stabilizes the anionic form of the product quinol, resulting in a lower pK_a for the 4-hydroxy group in the enzyme.

While AGAO coverages varied somewhat from electrode-to-electrode, integration of the charge under the voltammetric peaks

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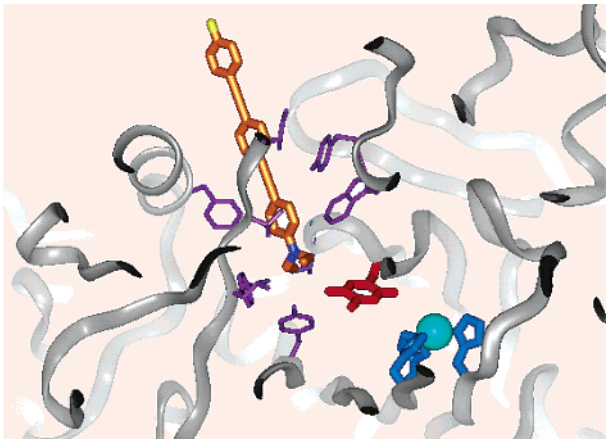

Figure 2. DEA-OPE-SH modeled into the substrate channel of AGAO. Channel residues are purple, TPQ is red, and the Cu site is blue.

gave maximum surface concentrations on the order of ~1 pmol/cm^2. On the basis of crystallographic parameters,[8] this corresponds to ~25% monolayer coverage by the enzyme. Gradual loss of electrochemical signals resulted from prolonged exposure of the AGAO-modified electrodes to buffer solutions, possibly due to slow dissociation of the protein from the wire-modified surface. Substrate inhibition experiments have demonstrated that wires similar to DEA-OPE-SH bind tightly to AGAO, with estimated dissociation constants of ~10 μM.[31] Addition of micromolar phenethylamine solutions completely quenches the electrochemical response in the cell, providing further evidence that the enzyme specifically binds to the electrode by insertion of the adsorbed thiol wire into the substrate channel. As phenethylamine displaces DEA-OPE-SH from this channel, the enzyme is decoupled from the electrode.

AGAO is electroinactive at underivatized gold surfaces, highlighting the importance of wire interactions with the protein in establishing electronic coupling with the active site (Figure 2). Studies of electron tunneling through phenyl-alkynyl bridges in self-assembled monolayers suggest that the distance decay constant is substantially lower (0.4−0.6 Å^{-1})[32,33] than that for tunneling through peptides (1.1 Å^{-1}) or water (1.7 Å^{-1}).[9] Assuming a normal protein reorganization energy (0.8 eV),[39] we estimate $k_o > 4 \times 10^4$ s^{-1} ($\Delta G^\circ = 0$) for tunneling through the 22-Å wire; the corresponding rate through polypeptide would be ~3 s^{-1}, and that through water would be $<10^{-4}$ s^{-1}. Importantly, the CVs obtained at scan rates up to 1 V/s place a lower limit of 10^3 s^{-1} for tunneling to the TPQ, confirming that the DEA-wire is the coupling element at this distance.

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Supporting Information Available: Details of protein preparation, DEA-OPE-SH synthesis, binding studies, and electrochemical measurements (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(37) The voltammetric wave widths are slightly broader than the expected values for a two-electron couple, possibly because of inhomogeneities within the films; the small peak separation at slow scan rates may reflect similar inhomogeneity in electron-transfer rates. See, for example: Rowe, G. K.; Carter, M. T.; Richardson, J. N.; Murray, R. W. *Langmuir* **1995**, *11*, 1797−1806. Alternatively, CV broadening could be due to a breakdown in cooperativity resulting from stabilization of the semiquinone radical in the enzyme.
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