Engineered electrode surfaces for biomimetic attachment of oxygen-reducing enzymes

Christopher Blanford

The University of Manchester, School of Materials, Manchester Interdisciplinary Biocentre, Manchester, M1 7DN, U.K.

Fuel cells without precious metals

Fuel cells

- Efficient converters of chemical energy into electrical energy
- Low-temperature fuel cells need platinum-based catalysts
- Reduction of O_2 uses majority platinum in low-temperature fuel cells

Pycnoporus cinnabarinus

Purified laccase

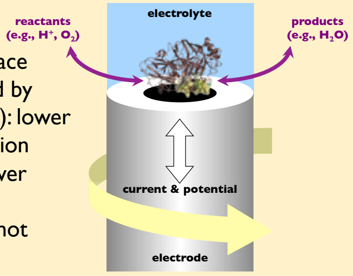
The catalyst that grows on trees

- Blue multicopper oxidases from fungi, e.g., laccases, bilirubin oxidase
- Selective and rapid catalysis: $O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$
- In nature, e^-'s are from oxidation of organics; we use an electrode
- More efficient than platinum
- Need long-lasting attachment to surface and facile e^- transfer

Protein film electrochemistry

Technique

- Redox-active enzyme adsorbed on conductive surface
- Reaction direction controlled by applied potential ($\Delta G = -nF\Delta E$): lower potential drives faster reduction
- Catalytic current gives turnover rate/enzyme activity
- Rapid rotation: reactants do not limit catalysis



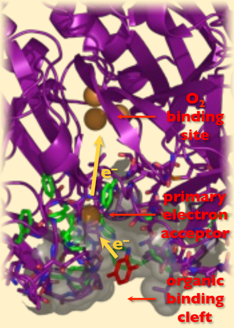
Organic-binding cleft

Wide, rich in aromatic groups

- Binding location for broad range of organic substrates, e.g., xylidine (red)
- Many aromatic side chains (green)
- Electrons extracted from organics transferred to copper atoms (orange)

Varied cleft-surfaces

- Hydrophilicity of binding cleft and substrate specificity vary between families of BMCOs
- Binding cleft may be flexible (*i.e.*, its shape changes for binding)



Matching enzyme and surface

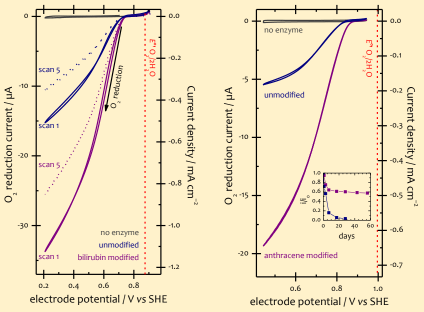
Plug and socket

- Surfaces modified with natural substrates or their analogues greatly increase current per unit area
- Enzyme-specific modifications
- Some surface modifications also increase lifetime

Molecular wire?

- Surface molecules highly conjugated
- Better electronic coupling? Greater coverage? Both?

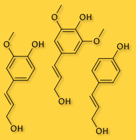
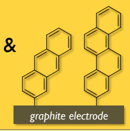
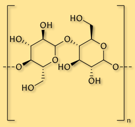
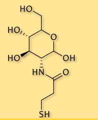
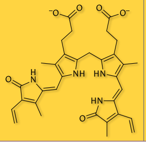
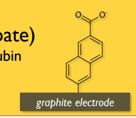
***M. verrucaria* bilirubin oxidase on bilirubin** | ***P. cinnabarinus* laccase on 2-anthracene**



Stability of two BMCO enzymes

Electrodes are electrochemically modified with a substrate or substrate mimic, then enzyme is applied. The electrochemical potential is cycled and the change in activity (current) with time and potential is recorded. More negative currents equal higher activity. Conditions: 10 mV s^{-1}, 25 °C, 100% O_2, 4000 rpm, sodium citrate buffer (left: 0.1 M pH 6.0; right: 0.2 M pH 4.0)

Generalisable to many blue multicopper oxidases

Enzyme	Natural substrate	Surface-bound mimic	Effect
laccases *T. versicolor* & *P. cinnabarinus* (Basidiomycota)	hydrophobic aromatics (esp. lignols) 	2-anthracene & 2-chrysene  graphite electrode	• 2× increase in current • many × increase in lifetime
laccase *M. albomyces* (Ascomycota)	cellulose 	*N*-(3-mercapto-propionyl)-D-glucosamine  gold electrode	• increased stability
bilirubin oxidase *M. verrucaria* (Ascomycota)	bilirubin & hydrophilic aromatics 	6-(2-naphthoate) & adsorbed bilirubin  graphite electrode	• 2–4× increase in current

Further information

Thanks to

- Kulveer Singh
- James Cracknell
- Tom McNamara
- Nina Hakulinen
- Rachel Heath
- UK Engineering and Physical Sciences Research Council

References

- *Dalton Trans.* (2011) 40(25), 6668–6675.
- *Phys. Chem. Chem. Phys.* (2010) 12(42), 13962–13974.
- *Faraday Discuss.* (2009) 140, 319–335.
- *Chem. Commun.* (2007) (17), 1710–1712.

http://blanfordgroup.com