

ELECTROCATALYSIS

A fifth copper's in town

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The semi-rational design of improved bioelectrocatalysts requires a deep knowledge of electron transfer and enzyme structure
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Many prokaryotes and eukaryotes express laccase enzymes to oxidize organic substrates. The oxidations are coupled to the reduction $4\text{H}^+ + 4\text{e}^- + \text{O}_2 \rightarrow 2\text{H}_2\text{O}$, a 4e^- half-reaction that aptly occurs at an active site featuring four Cu centres that cycle between +I and +II oxidation states. One Cu^{II} is responsible for oxidizing organics and passing electrons to a nearby Cu^{I} , trio to afford a low-valent $\text{Cu}^{\text{I}}\text{Cu}^{\text{I}}_3$ state that is perfect for O_2 binding. Collectively, the $\text{Cu}^{\text{I}}\text{Cu}^{\text{I}}_3$ cofactors then provide the 4e^- necessary to split O_2 into $2\text{H}_2\text{O}$, which occurs at the Cu_3 subsite and regenerates the oxidized $\text{Cu}^{\text{II}}\text{Cu}^{\text{I}}_3$ form. *Escherichia coli* expresses copper efflux oxidase (CueO), a special regulatory laccase

that does not directly oxidize organics but oxidizes Cu^{I} to Cu^{II} at a fifth binding site. The question of how this site affects electrocatalysis has now been answered by a team led by Lingling Zhang and Ulrich Schwaneberg, who report in *Chemistry — A European Journal* an electrochemical and theoretical study of CueO and its mutants.

Although CueO does not directly oxidize organics, its fifth Cu binding site is labile and close to the surface, such that Cu^{II} can leave the enzyme and independently oxidize a substrate.

Alternatively, Cu^{II} can stay put and accept an electron from a cathode, for example, in a fuel cell. Zhang, Schwaneberg and colleagues expressed and purified CueO with and without supplemental CuSO_4 and found that cyclic voltammograms of the former in air-saturated solution had greater catalytic currents. Thus, Cu-rich conditions enable the growth of the fully metalated, more active form of CueO, while Cu-limiting conditions leave the fifth site vacant and give a slower catalyst.

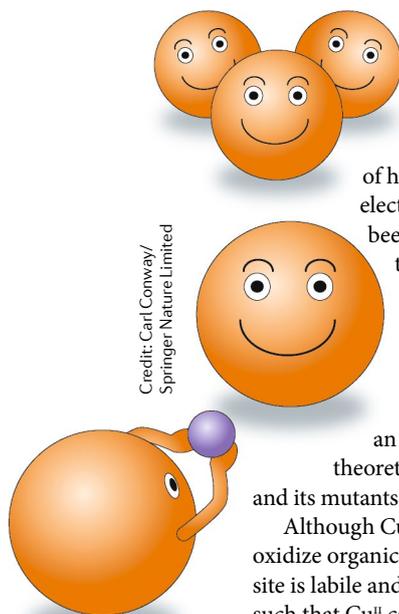
Given the importance of the fifth Cu site, the team sought to determine which properties of the site influence O_2 reduction catalysis. Native CueO binds the fifth Cu through two Met and two Asp residues — four sites that can be mutated to give an array of variants. Solutions of these variants were interrogated eight at a time with a set-up in which eight working electrodes are coupled to the same counter and reference electrodes. The team screened eight 96-well plates and identified 11 variants that gave a higher O_2 reduction current than native CueO at 0 V. The best variants were then immobilized on a rotating disc electrode and subjected to linear sweep voltammetry under O_2 . Each experiment afforded a linear Tafel plot of applied potential versus the logarithm of current density, the slope of which reflects how much potential one has to sacrifice to obtain a desired current increase. Whereas native CueO gives rise to a Tafel slope of $-111 \text{ mV decade}^{-1}$, replacing Asp360 with Lys decreases this to only $-61 \text{ mV decade}^{-1}$.

What makes this mutant a good catalyst? Zhang, Schwaneberg and colleagues turned to molecular dynamics simulations and found that the fourth and fifth Cu sites in the mutant are closer together (5.6 \AA) than they are in native CueO (10.5 \AA), which enhances electron transfer according to Marcus theory. Moreover, the fifth Cu site becomes more rigid, such that the reorganization energy associated with electron transfer decreases. Lastly, switching an anionic Asp ligand for a charge-neutral Lys at the fifth Cu site increases its redox potential and propensity to accept electrons from the electrode.

Decorating cathodes with laccases instead of synthetic catalysts is topical because laccases selectively convert O_2 to $2\text{H}_2\text{O}$ without making any H_2O_2 . However, CueO has a redox potential below that of fungal laccases (and well below the thermodynamic potential of the $\text{O}_2/\text{H}_2\text{O}$ half-reaction) and will need substantial overpotentials to turn over quickly. Towards fixing this, the team is also tuning the fourth Cu site. Moreover, they are furthering their understanding of reorganization energy through computations. “The semi-rational design of improved bioelectrocatalysts requires a deep knowledge of electron transfer and enzyme structure,” says Zhang. In this way, they can optimize the privileged five-Cu platform and perhaps, through ultra-high-throughput screening, apply their methods to enzymes more generally.

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ORIGINAL ARTICLE Zhang, L. et al. Engineering of laccase CueO for improved electron transfer in bioelectrocatalysis by semi-rational design. *Chem. Eur. J.* <https://doi.org/10.1002/chem.201905598> (2020)



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