Engineered oxidase reveals steric control of oxygen

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The PCMH superfamily includes flavoprotein oxidases with bicovalently attached FAD. The model enzyme from this family is berberine bridge enzyme (BBE) which catalyses the C-C bond formation in (S)-reticuline to produce (S)-scoulerine and is regenerated by molecular oxygen (k$_{\text{ox}}$=5·10$^4$ M$^{-1}$s$^{-1}$) [1]. Phlp4 is a grass pollen allergen catalyzing the oxidation of glucose and the first characterized dehydrogenase with a bicovalently attached FAD (k$_{\text{ox}}$=1.2 M$^{-1}$s$^{-1}$) [2]. The reactivity towards oxygen was increased by more than 5 orders of magnitude by a single amino acid exchange (k$_{\text{ox}}$=7·10$^4$ M$^{-1}$s$^{-1}$ in Phlp4 I153V). As a proof of principle, the reverse amino acid exchange in BBE (i.e. variant V169I) decreased the rate of oxygen reduction 500 fold (k$_{\text{ox}}$=1·10$^2$ M$^{-1}$s$^{-1}$). In addition, BBE G164A variants show that a nearby glycine to alanine exchange (the so-called “gate keeper” residue [3]) has the same effect on oxygen reactivity as the V169I variant and the contribution of combined mutations do not accumulate. Enzymatic characterization of other BBE and Phlp4 variants with active site replacements revealed that factors previously discussed, such as redox potential and the occurrence of basic residues (e.g. lysine or histidine) in proximity to the isoalloxazine ring [4-6], can be ruled out as parameters affecting oxygen reactivity in this enzyme family.

X-ray structures of our variants revealed the impact of amino acid replacements on critical spatial requirements for oxygen reactivity. Accessibility of a cavity on the re-side of the isoalloxazine ring in proximity of the reactive C(4a) carbon atom is required for efficient oxygen reduction, while it is freely accessible in all variants from its substrate binding si-side. The structure of NaBr soaked Phlp4 oxidase revealed halide binding in the engineered cavity in a hydrogen bond distance to a backbone amide nitrogen pair that form a proton donor pocket in the proximity of C(4a). Closer inspection of flavoproteins from different structural clans reveals that the proton donor pockets in the proximity of C(4a) are conserved among oxidases but are absent in natural or engineered flavoproteins with decreased oxygen reactivity [4-6].

While our data strongly supports our model for the BBE structural family, we assume that similar parameters are involved in controlling oxygen reactivity in other flavoprotein families.
Figure 1: The Phl p 4 II53V N158H variant (PDB ID 4PWC, collected at Petra III P11, blue color) accommodates a bromide ion in the anion cavity. The surrogate placed into a difference omit map (green mesh) is positioned 2.5 Å above the reactive C(4a) (black atom in yellow FAD). It is in a hydrogen bond distance from backbone nitrogen atoms of C150 and V149, which form the oxygen trap. The ion is only 1.6 Å from I169 and 2.1 Å from A164 side chains of aligned wild type Phl p 4 (PDB ID 4PVE, collected at Petra III P11, pink color) and BBE G164A (PDB ID 4PZF, purple), respectively. The clash-distances are marked with red dashes.

References