

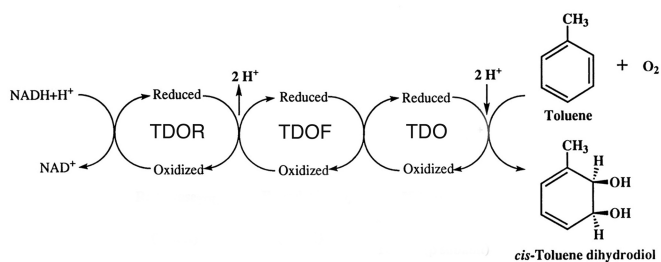
Structural Basis for Electron Transfer in the Multicomponent Rieske Non-Heme Iron Toluene Dioxygenase Enzyme System

Supplementary Material

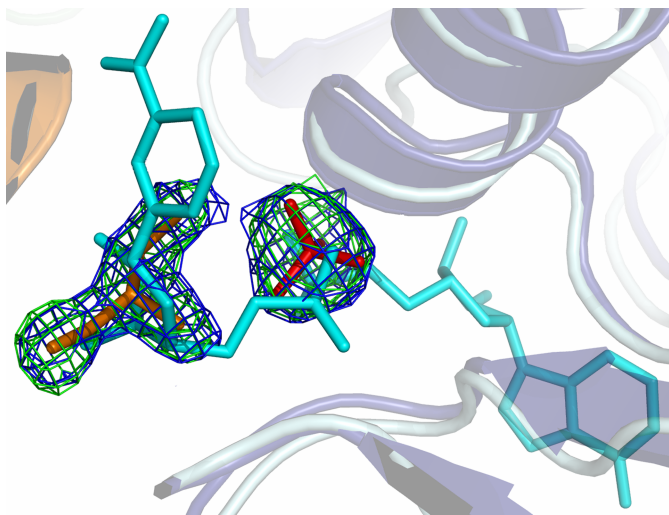
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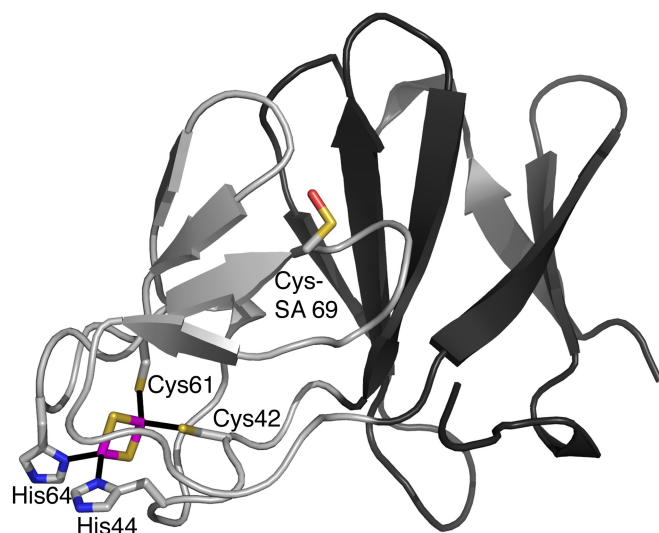
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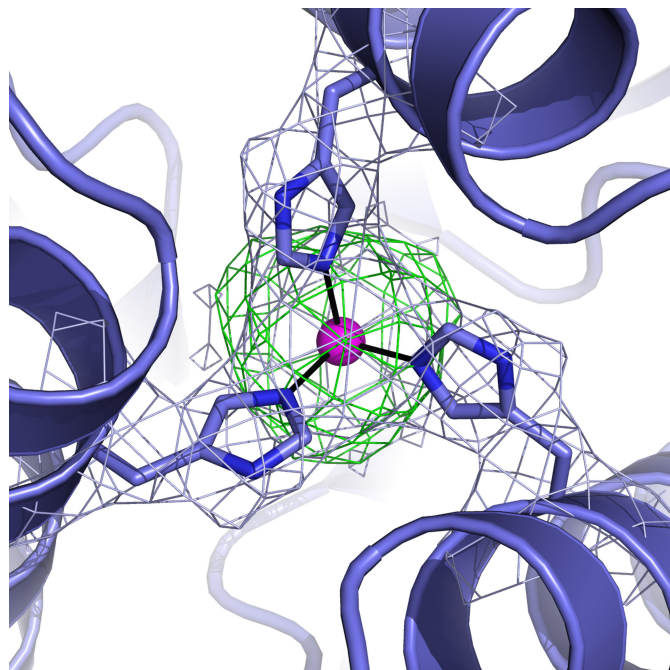
Supplementary Figure 1 Dihydroxylation of toluene to *cis*-toluene dihydrodiol catalyzed by the three-component TDO enzyme system.



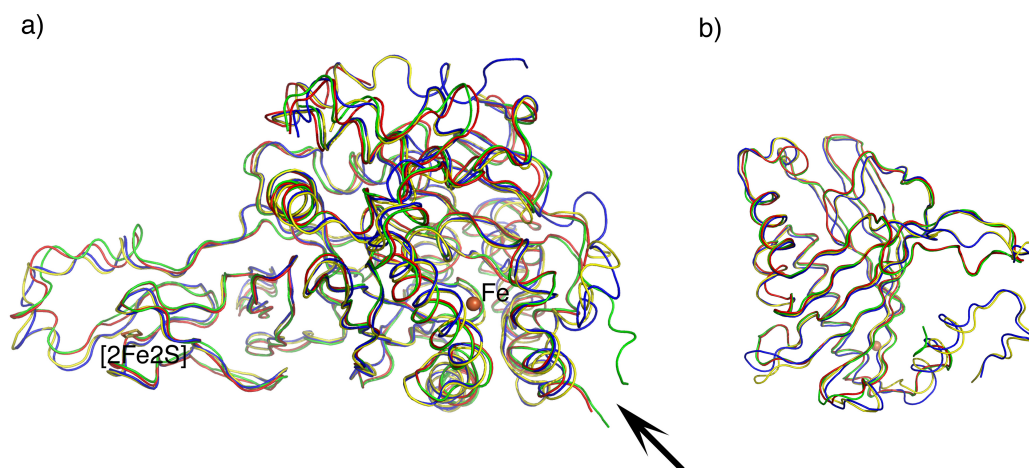
Supplementary Figure 2 Superposition of the NADH binding domains of TDO-R (dark blue) and BPDO-R_{KKS102} (light blue). NADH bound in the NADH binding domain of BPDO-R_{KKS102} is shown in cyan sticks. One sulfate ion (red sticks) and one glycerol molecule (orange sticks) occupy the binding cleft of TDO-R. The 2F_{obs}-2F_{calc} (blue) contoured at 1σ and F_{obs}-F_{calc} (green) maps are computed before the sulfate ion and glycerol molecule were modeled. The figure was made using PyMOL (<http://www.pymol.org>).



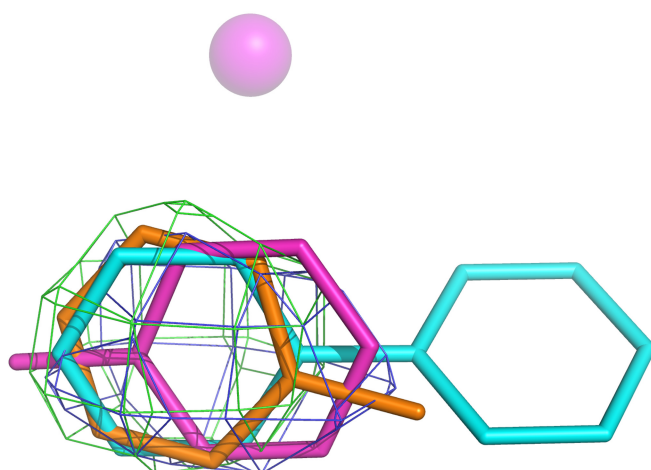
Supplementary Figure 3 Overall structure of TDO-F showing the location of Cys69 sulfenic acid (Cys-SA 69) in sticks. The figure was made using PyMOL (<http://www.pymol.org>).



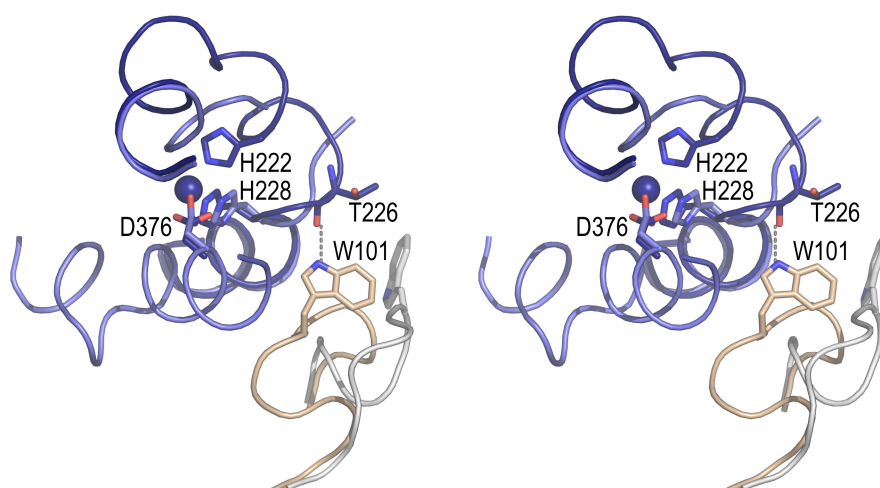
Supplementary Figure 4 The metal atom bound between the three β -subunits (magenta coloured sphere) is coordinated by the side chain of His24 from each β -subunit. The $2F_{\text{obs}}-2F_{\text{calc}}$ (light blue) contoured at 1σ and $F_{\text{obs}}-F_{\text{calc}}$ (green) maps are computed before the metal ion was modelled. The figure was made using PyMOL (<http://www.pymol.org>).



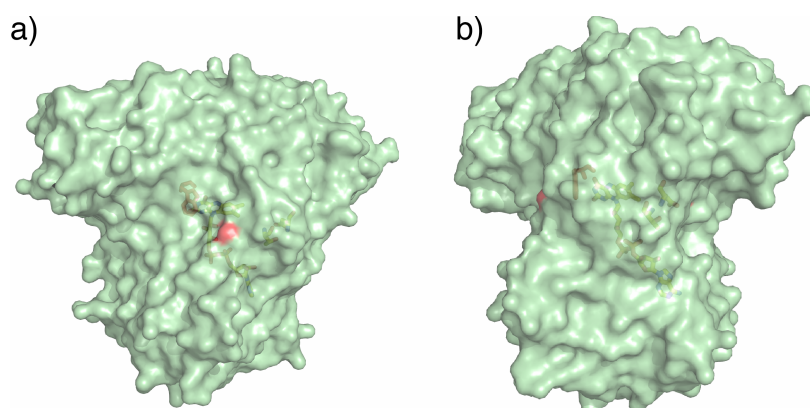
Supplementary Figure 5 Superposition of TDO-O (green), NBDO-O (yellow), NDO-O (blue) and BPDO-KKS102 (red). a) show the α subunits, where the TDO-O mononuclear iron and Rieske [2Fe-2S] cluster are shown as a sphere and in sticks, respectively. The loops at the entrance of the active site are marked with an arrow. b) illustrate the β subunits, where the TDO-O metal ion is shown as a sphere. The figure was made using PyMOL (<http://www.pymol.org>).



Supplementary Figure 6 The active site of TDO-O with toluene bound. The $2F_{\text{obs}} - 2F_{\text{calc}}$ (blue) contoured at 1σ and $F_{\text{obs}} - F_{\text{calc}}$ (green) maps are computed before toluene was modelled. The two possible orientations of toluene are shown in sticks (orange and magenta). An overlap of BPDO-KKS102 shows the position of biphenyl (cyan sticks) in the active site. The mononuclear iron is illustrated as a magenta-coloured sphere. The figure was made using PyMOL (<http://www.pymol.org>).



Supplementary Figure 7 Stereo representation of the conformational differences of TDO-O and apo-TDO-O. The α subunits of TDO-O and apo-TDO-O are coloured dark- and light blue, respectively. The β subunits of TDO-O and apo-TDO-O are coloured beige and white, respectively. The hydrogen bond between the β subunit Trp101 of TDO-O and the main chain oxygen of Thr226 of the α subunit are shown as a gray dotted line. The figure was made using PyMOL (<http://www.pymol.org>).



Supplementary Figure 8 Surface representation of TDOR; a) shows the depression on the side of Thr129 and Asp132. The FAD, Thr129 and Asp132 are shown in sticks. Trp320 is shown in red. b). Illustration of the depression on the side of Trp320. The figure was made using PyMOL (<http://www.pymol.org>).