Supplementary Material

Binding of FAD and tryptophan to the tryptophan 6-halogenase Thal are negatively coupled

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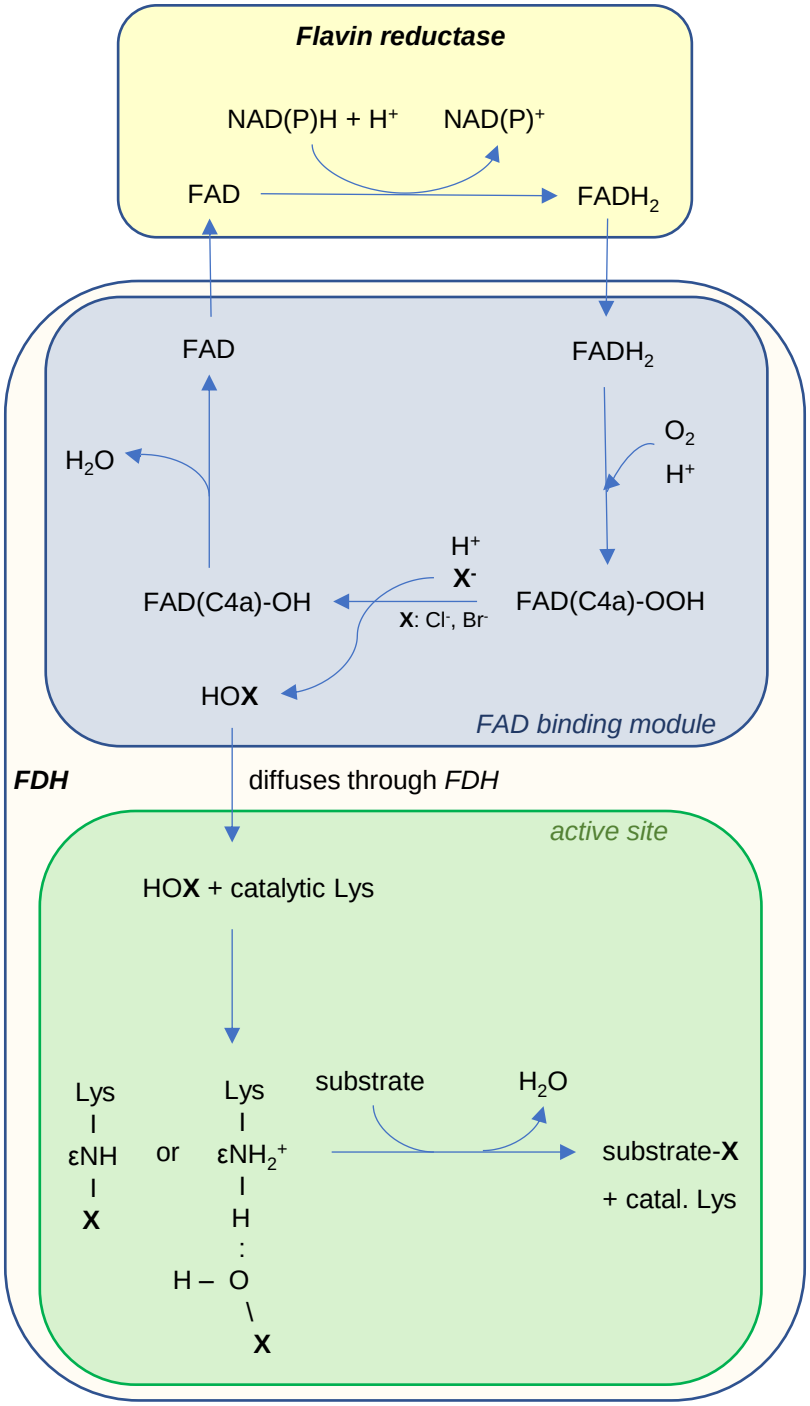

Supplementary Figure S1. Schematic reaction mechanism of two-component flavin-dependent halogenases (FDHs). A separate flavin reductase (yellow) reduces FAD to $FADH_2$. Once bound to the FDH (beige) within the FAD binding module (blue), $FADH_2$ reacts with molecular oxygen to a flavin hydroperoxide. The FAD(C4a)-OOH then reacts with a halide anion forming hypohalous acid (HOX) and FAD(C4a)-OH. The hypohalous acid diffuses through the enzyme towards the active site (green), where halogenation of the substrate takes place. Whether the conserved catalytic lysine forms a hydrogen bond with HOX to orient and activate it or whether it forms a covalent lysine haloamine (e.g. lysine chloramine) is still under debate.

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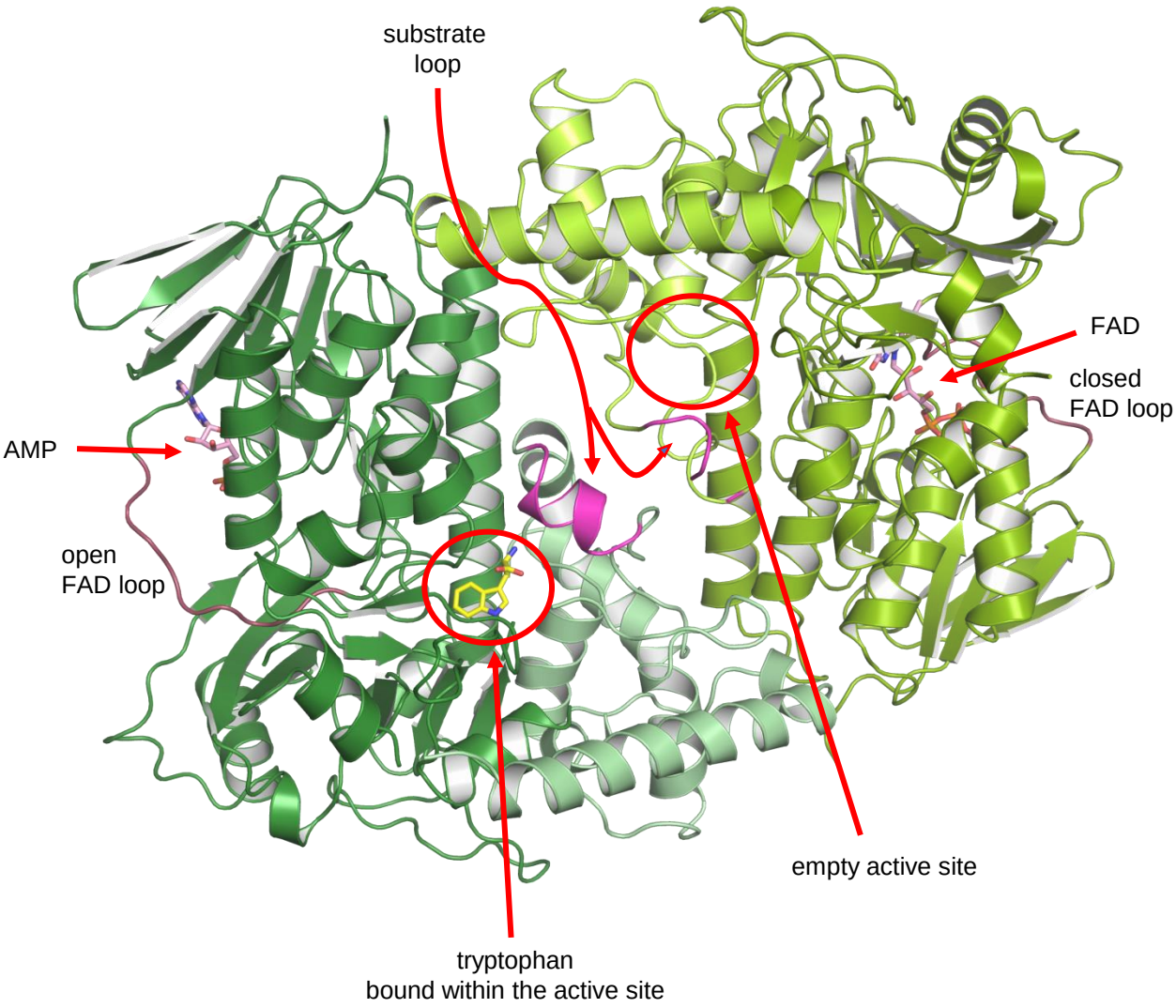
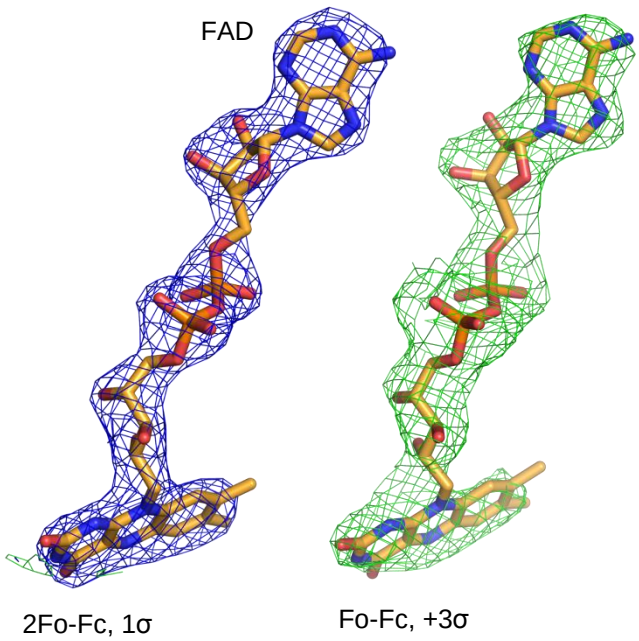


Supplementary Figure S2. Trp-FAD-Thal dimer with regions of interest labeled. In chain A (dark green) the substrate tryptophan (yellow) is bound within the active site, the substrate loop (magenta) is ordered forming a short helix, AMP (pink) is bound within the FAD binding module and the FAD loop (darkpink) adopts an open conformation. In chain B (green) the active site is empty, the substrate loop is not resolved (start and end of the disordered region are shown in magenta), FAD (pink) is bound within the FAD binding module and, therefore, the FAD loop adopts a closed conformation.



Supplementary Figure S3. Electron density of the cofactor FAD in the FAD-Thal structure. Fo-Fc electron density before building FAD contoured at +3 σ (green) and the final 2Fo-Fc electron density contoured at 1 σ (blue).

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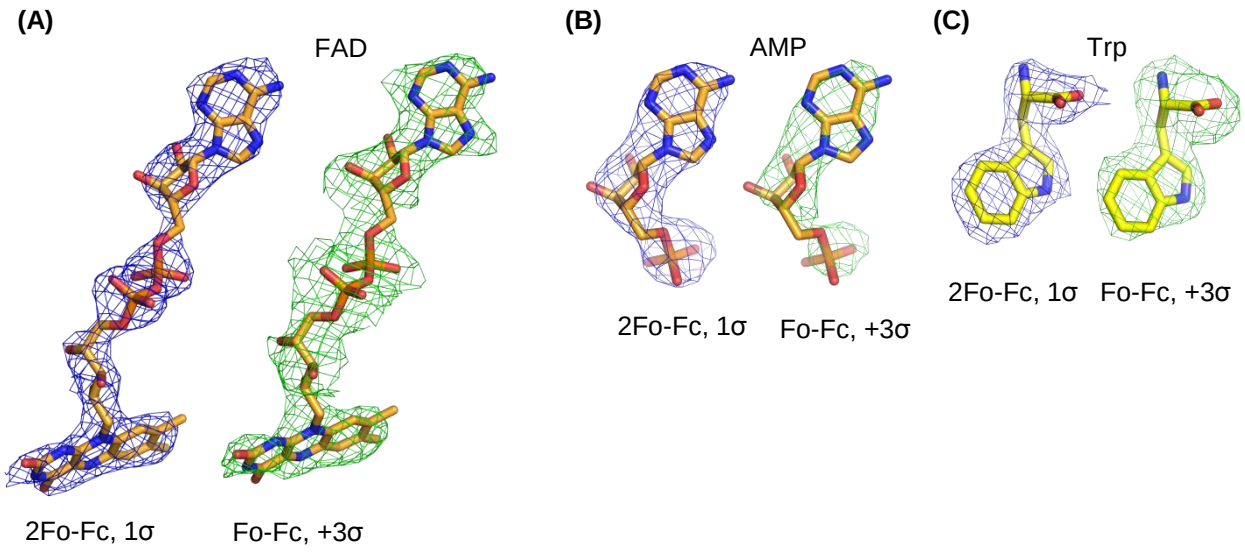

Supplementary Figure S4. Electron density of the ligands in the Trp-FAD-Thal structure. **(A)** Fo-Fc electron density before building the cofactor FAD contoured at +3 σ (green) and the final 2Fo-Fc electron density contoured at 1 σ (blue). **(B)** Fo-Fc electron density before building AMP contoured at +3 σ (green) and the final 2Fo-Fc electron density contoured at 1 σ (blue). **(C)** Fo-Fc electron density before building tryptophan contoured at +3 σ (green) and the final 2Fo-Fc electron density contoured at 1 σ (blue).

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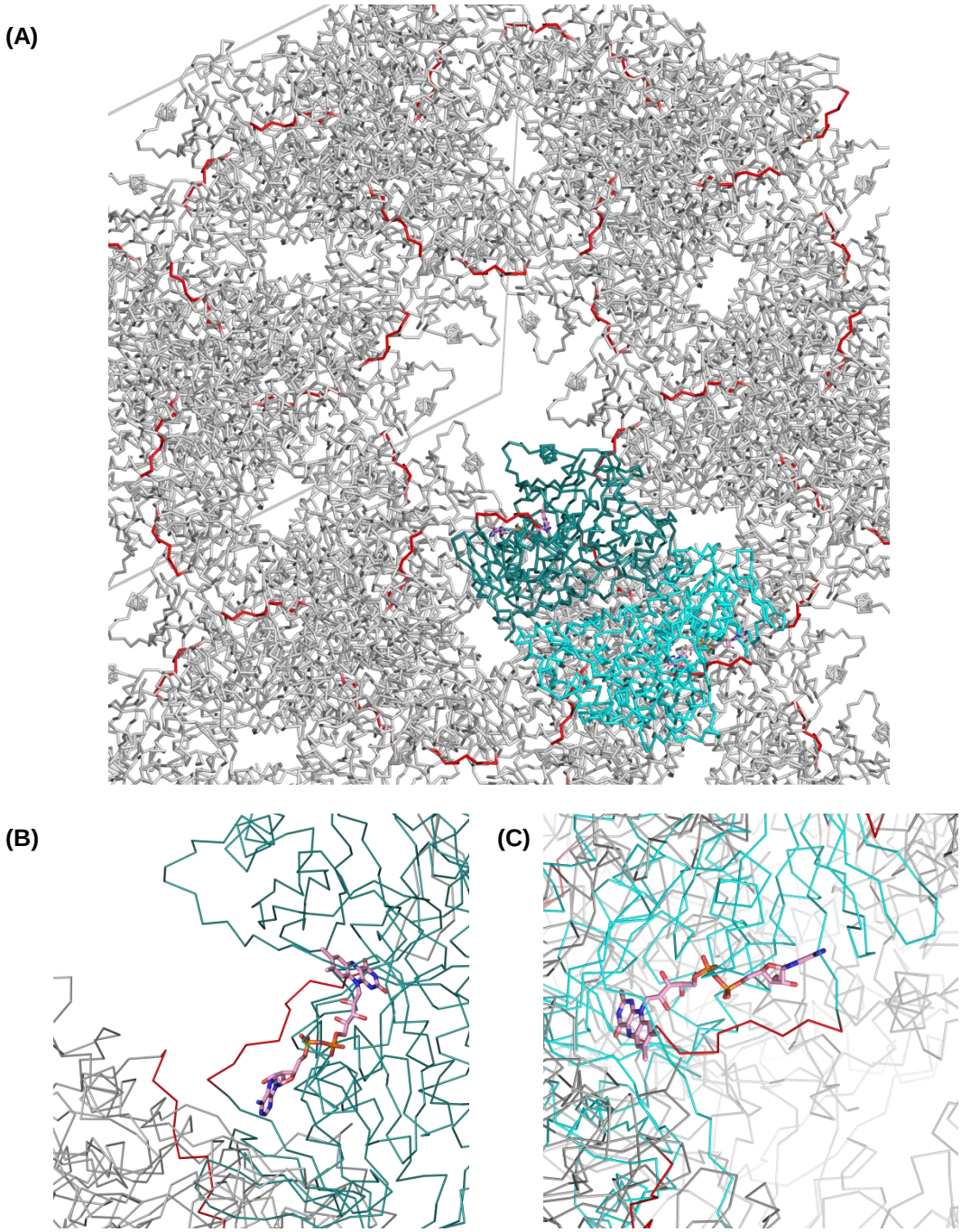

Supplementary Figure S5. Crystal packing of FAD-Thal. **(A)** View along the c axis. The FAD-Thal dimer is colored in cyan and deepteal , symmetry mates are shown in gray and the FAD loops in red. Both FAD loops show no crystal contacts to symmetry mates. **(B, C)** The FAD loop is colored in red. Bound FAD is shown as stick model in pink. **(B)** Zoom in on the FAD loop of chain B of the FAD-Thal dimer. **(C)** Zoom in on the FAD loop of chain A of the FAD-Thal dimer.