Supporting information for: Cytochrome aa_3 oxygen reductase utilizes the tunnel observed in the crystal structures to deliver O_2 for catalysis

Paween Mahinthichaichan,[‡] Robert B. Gennis,[*,§] and Emad Tajkhorshid[*,‡]

‡*Department of Biochemistry, NIH Center for Macromolecular Modeling and Bioinformatics, Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, U.S.A.*

§*Department of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, IL 61801, U.S.A.*

E-mail: r-gennis@illinois.edu; emad@life.illinois.edu

Phone: +1 217 3339075; +1 217 2446914

Running header

O_2 delivery pathway in cytochrome aa_3

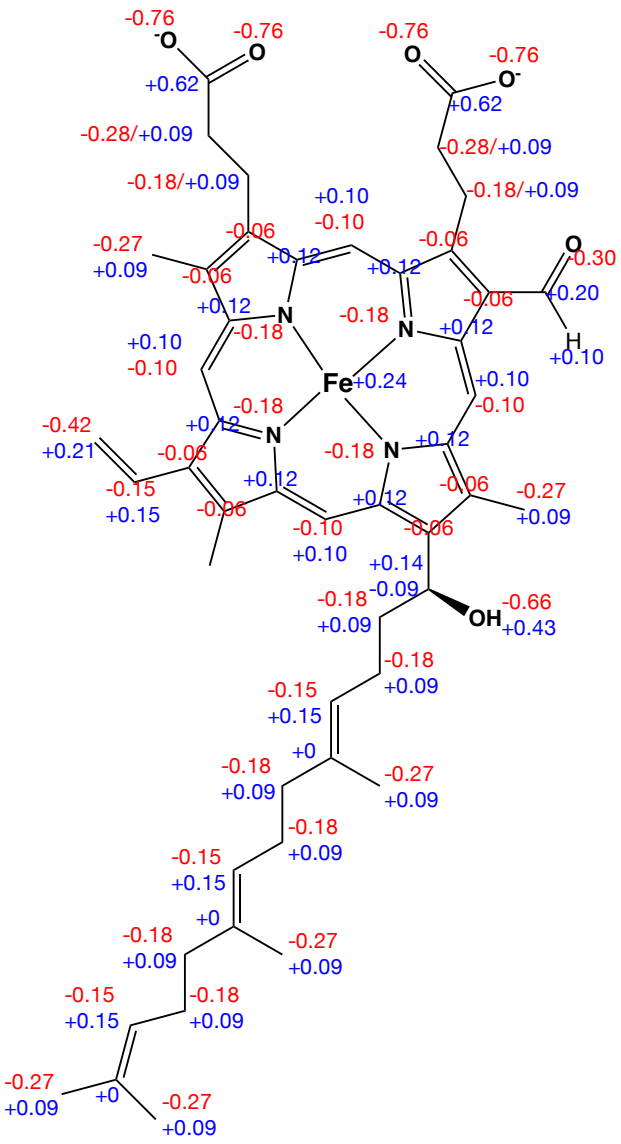

Figure S1: Charge distributions for heme a/a_3 derived from analogy.

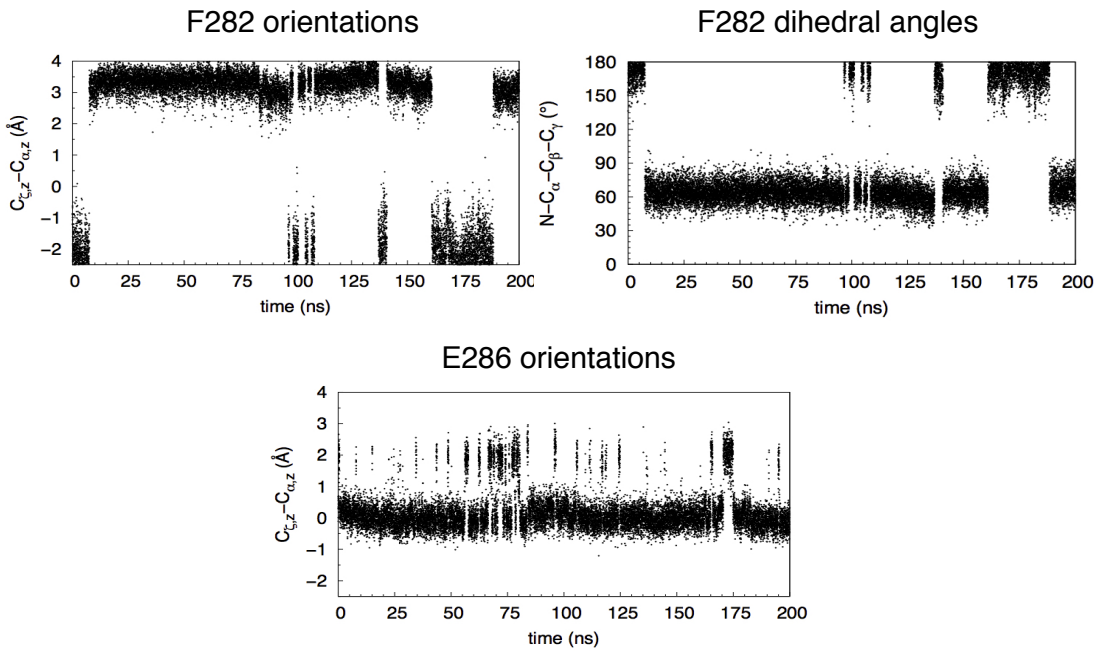

Figure S2: Conformational dynamics of residues F282 and E286. The sidechain of F282 is oriented into the O_2 pathway at N-C_α-C_β-C_γ of $\sim$180° and $C_{\alpha,z}$-$C_{\alpha,z}$ of $\sim$-2 Å. The sidechain of E286 is oriented into the O_2 pathway or in the "up" conformation at $C_{\alpha,z}$-$C_{\alpha,z}$ of $\sim$2 Å. These configurations result in the constriction of O_2 migration across the bottleneck of the pathway.

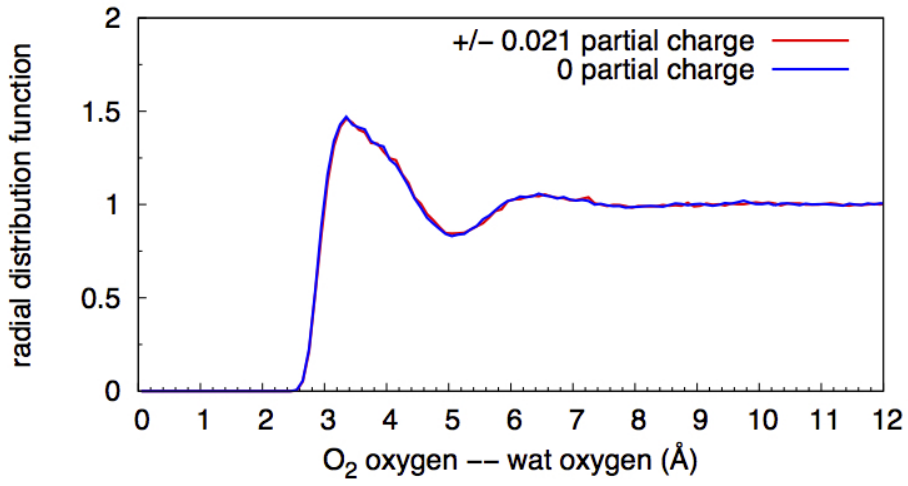

Figure S3: Radial distribution function (RDF) profiles describing contact distance between oxygen atoms of O_2 and water molecules. The profiles were calculated form 5-ns MD simulations in which an O_2 molecule is solvated in a $30\times30\times30\,\text{Å}^3$ water box. The oxygen atoms of the slightly charged O_2 model carry the partial charges of ±0.021. The ones for the apolar model carry the partial charges of 0. Similar RDF profiles indicate negligible effects on O_2-water interactions by the introduction of such small polarizations.

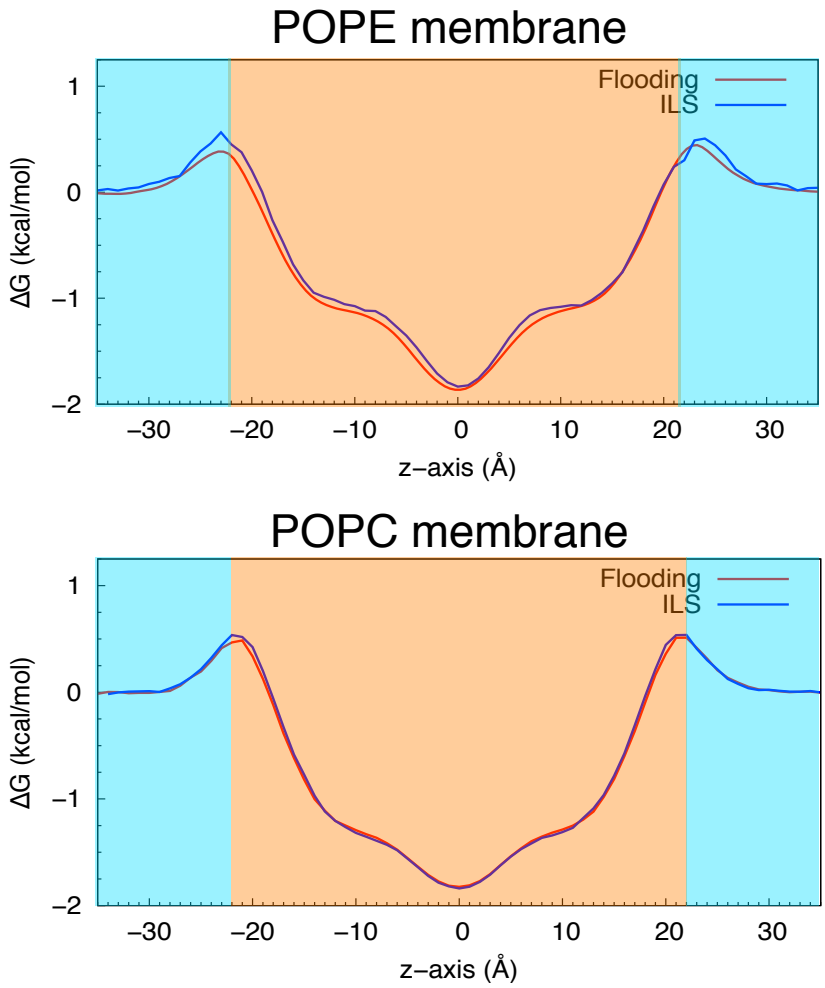

Figure S4: Partitioning free energy profiles of O_2 in membrane (shaded orange) and in the aqueous solution (shaded cyan). The profiles of the flooding simulations were obtained from 200-ns simulations in which 125 O_2 molecules are explicitly incorporated. The slightly charged model of O_2 was used in the flooding simulations. Since no electrostatic terms are included in ILS, the oxygen atoms in the calculations carry the partial charges of 0. These calculated profiles are in close agreement with those reported in other studies, such as by Ghysels et al[1] who used the apolar model, as well as by Hub and de Groot[2] who introduced a quadrupole to the simulated O_2 molecules.

Table S1: Solvation free energy of O_2 from the models used in the present study

O_2 model	Charges on the oxygen atoms	ΔG_{sol} (kcal/mol)
Slightly charged	-0.021/+0.021	2.06±0.05
Apolar	0/0	2.03±0.05

We note that these calculated ΔG_{sol} values are also in agreement with the values of 2.05 kcal/mol) reported by Victor et al[3], who used the apolar model.

References

(1) Ghysels, A., Venable, R.M., Pastor, R.W., Hummer, G. (2017) Position-dependent diffusion tensors in anisotropic media from simulation: Oxygen transport in and through membranes. *Journal of Chemical Theory and Computation*, 13, 2962-2976.

(2) Hub, J.S., de Groot, B.L. (2008) Mechanism of selectivity in aquaporins and aquaglyceroporins. *Proc. Natl. Acad. Sci. U.S.A.*, 105, 1198-1203.

(3) Victor, B.L., Baptista A.M., Soares, C.M. (2009) Dioxygen and nitric oxide pathways and affinity to the catalytic site of rubredoxin:oxygen oxidoreductase from *Desulfovibrio gigas*, *J. Biol. Inorg. Chem.*, 14, 852-862.