



Multiscale simulations reveal the proton pumping mechanism in cytochrome c oxidase



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Abstract

Cytochrome c oxidase (CcO) reduces oxygen to water and uses the released free energy to pump protons across the membrane, contributing to the transmembrane proton electrochemical gradient that drives ATP synthesis. Herein, we provide a complete atomic level description of the key steps of the proton pumping mechanism in aa₃-type CcO. We have used multiscale reactive molecular dynamics simulations to explicitly characterize (with free energy profiles and calculated rates) the internal proton transport events that enable pumping and chemistry during a reaction step that involves proton transport to the pump loading site (PLS) and to the catalytic site (binuclear center, BNC) (the A→P_R→F transition). Our results show that both proton transport events are thermodynamically driven by electron transfer from heme a to the BNC, but that pumping (amino acid residue E286 to the PLS) is kinetically favored, while transfer of the chemical proton (E286 to the BNC) is rate-limiting. The calculated rates are in quantitative agreement with experimental measurement. The back flow of the pumped proton from the PLS to E286 is prevented by the fast reprotonation of E286 through the D-channel and a large free energy barrier for the back flow reaction. Proton transport through the D-channel is not rate-limiting during the A→P_R→F transition, but is strongly coupled to solvation changes across the N121-N139 asparagine gate. Our results also show how the D-channel biases unidirectional proton transport from the inner to outer side of the membrane.

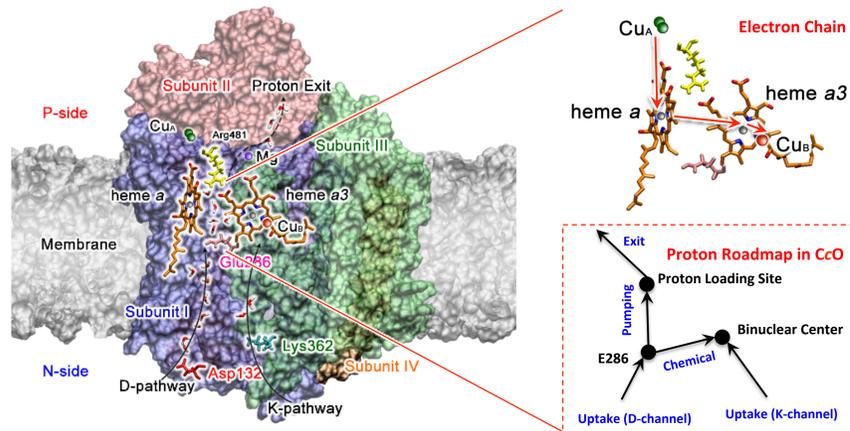


Fig. 1 Left: cytochrome c oxidase embedded in lipid membrane. Right: electron transfer and proton transport pathways in the proton pumping cycle.

Objectives

A complete atomic level description of the key steps of the proton pumping mechanism during the A→F transition in aa₃-type CcO.

Methodology

- Model the hydrated excess proton by multiscale reactive molecular dynamic (MS-RMD) simulation.
- Parameterize MS-RMD models for protonatable groups in protein by FitEVB¹.
- Identify proton transport pathway by metadynamics simulation with MS-RMD models.
- Calculate 1-D or 2-D free energy profiles by umbrella sampling with MS-RMD models.
- Evaluate reaction rates by transition state theory.

Results

Transport of the Pumped and Chemical Protons

- Electron transfer provides thermodynamic driving force for proton pumping and chemical reaction.
- Proton pumping is more kinetically favorable over chemical reaction and therefore is not short-circuited by the latter.
- After electron transfer, the large proton back flow barrier prevents proton back leakage.

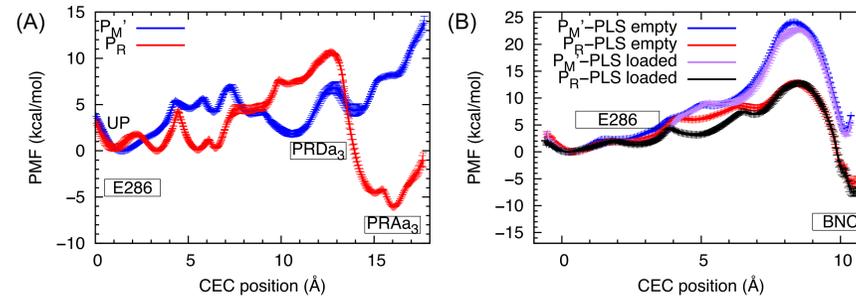


Fig. 2 Free energy profiles (PMFs) for (A) proton pumping and (B) chemical reaction before (P_M' blue) and after (P_R red) electron transfer.

Table 1. Calculated rates for proton transport of the proton pumping, chemical reaction and back leakage before (P_M') and after electron transfer (P_R), compared with experimental rates for A→F transition.

State	Pumping (μs)	Chemical reaction (μs)		Back leakage (μs)
		Deprotonated PLS	Protonated PLS	
P _M '	0.11	1.8 × 10 ¹⁰	2.5 × 10 ⁹	5.9 × 10 ⁻⁵
P _R	4.7	130	160	5.0 × 10 ⁴
Experiment ²		200		

Proton transport through the D-channel

- Proton transport in D-channel is strongly coupled to hydration level change across N139-N121 gate.
- Proton transport in D-channel is not rate limiting during the A→F transition.
- The fast forward proton transport in D-channel prevents proton back leakage in hydrophobic cavity.
- The large back flow barrier prevent proton back leakage through D-channel to the N-side bulk.

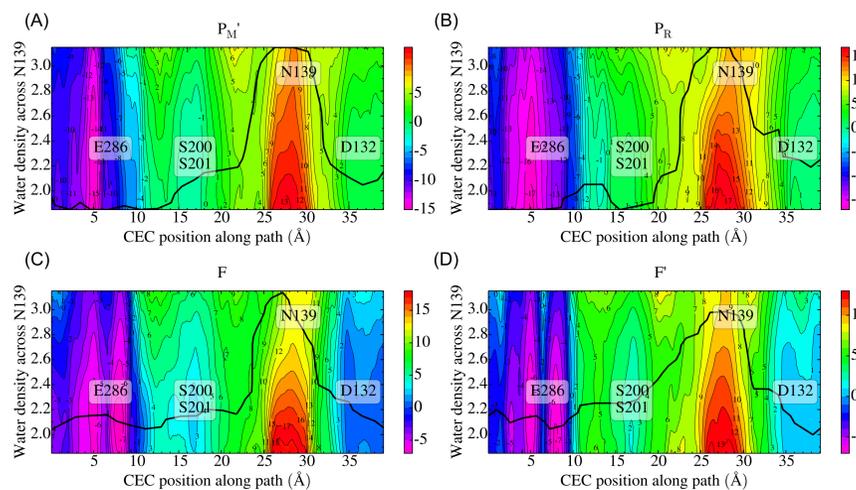


Fig. 3. Two-dimensional free energy profiles (PMFs) in the D-channel for the (A) P_M', (B) P_R, (C) F, and (D) F' states.

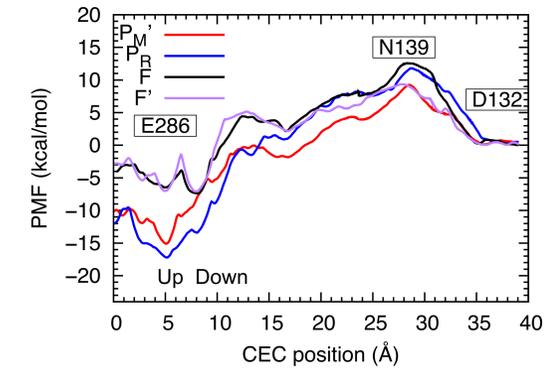


Fig. 4. Free energy profiles (PMFs) for proton transport in the D-channel along the minimum free energy pathway for P_M' (red), P_R (blue), F (black), and F' (purple) states.

Table 2. Calculated rates for proton transport in the D-channel for forward and backward proton transport in the P_M', P_R, F, and F' states, compared with the experimental rate for A→F transition.

State	Forward (μs)	Backward (μs)
P _M '	8.3 × 10 ⁻²	1.6 × 10 ¹⁰
P _R	6.7	5.0 × 10 ¹³
F	40	1.8 × 10 ⁷
F'	0.21	5.6 × 10 ⁴
Experiment ²	200	

Conclusions

- Electron transfer from heme a to binuclear center makes proton pumping and chemical reaction more favorable both thermodynamically and kinetically.
- Chemical reaction at binuclear center is the rate limiting step during the A→F transition.
- Possible proton leakage pathways during the enzyme pumping cycle are blocked by kinetic gating in three ways:
 1. Faster proton pumping over chemical reaction.
 2. Faster E286 reprotonation through D-channel than proton back leakage from pump loading site.
 3. Faster chemical reaction than back leakage through D-channel.

References

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ACKNOWLEDGMENTS

This research was supported by the National Institutes of Health (NIH grant R01 GM053148).