BRANCHING OF PHYSICAL & CHEMICAL PROTONS IN CYTOCHROME C OXIDASE : Insights from QM/MM Simulations

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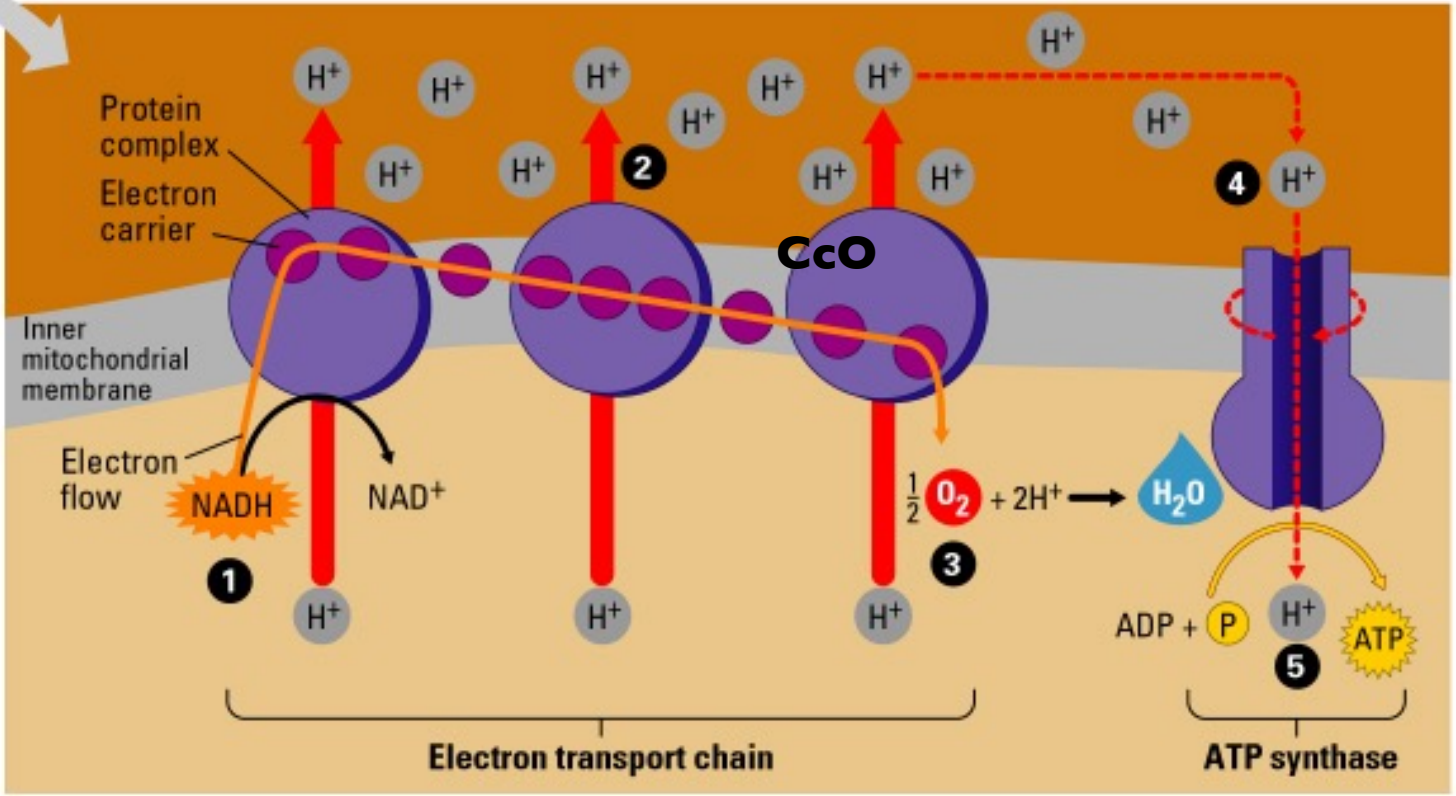
Abstract

Cytochrome c oxidase (CcO) is a heme-copper transmembrane enzyme responsible for the reduction of molecular oxygen to water during respiration. The free energy of oxygen reduction is used to pump protons across mitochondrial membrane. The resulting proton gradient is used for ATP synthesis. Experiments have shown that Glu286 (*Rhodobacter sphaeroids*) residue (pKa ~9.0) serves as a branching point from where the protons are transferred either to the chemical site for oxygen reduction or pumped out of the membrane. It is known that for every pumped proton, one electron and one proton are transferred to the chemical site. However, an understanding of the sequence of proton transfer events and the factors that regulate the coupling between oxygen reduction and proton pumping is lacking. The characterization of the specific pathways and elucidating the molecular mechanism of proton pumping is the goal of our research.

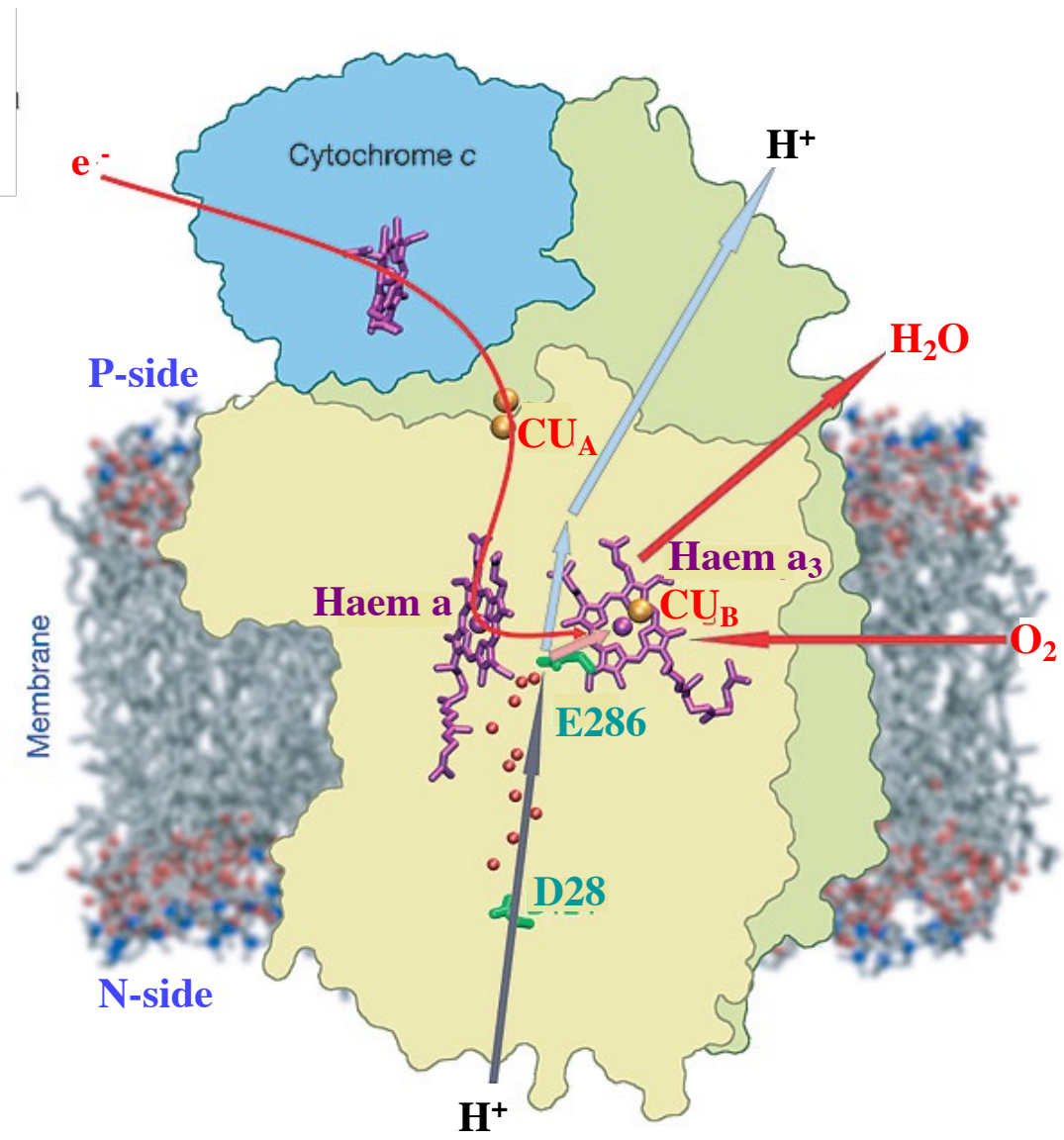
CELLULAR RESPIRATION

MITOCHONDRIA



One of the key problems of molecular bioenergetics is to understand at a molecular level the structure and function of membrane bound proton transporters. Cytochrome c oxidase, the terminal enzyme of the electron transport pathway of cellular respiration is one such proton transporter.

Cytochrome C Oxidase (CcO) : A PROTON PUMP



$O_2 + 4e^- + 8H^+ \rightarrow 2H_2O + 4H^+$

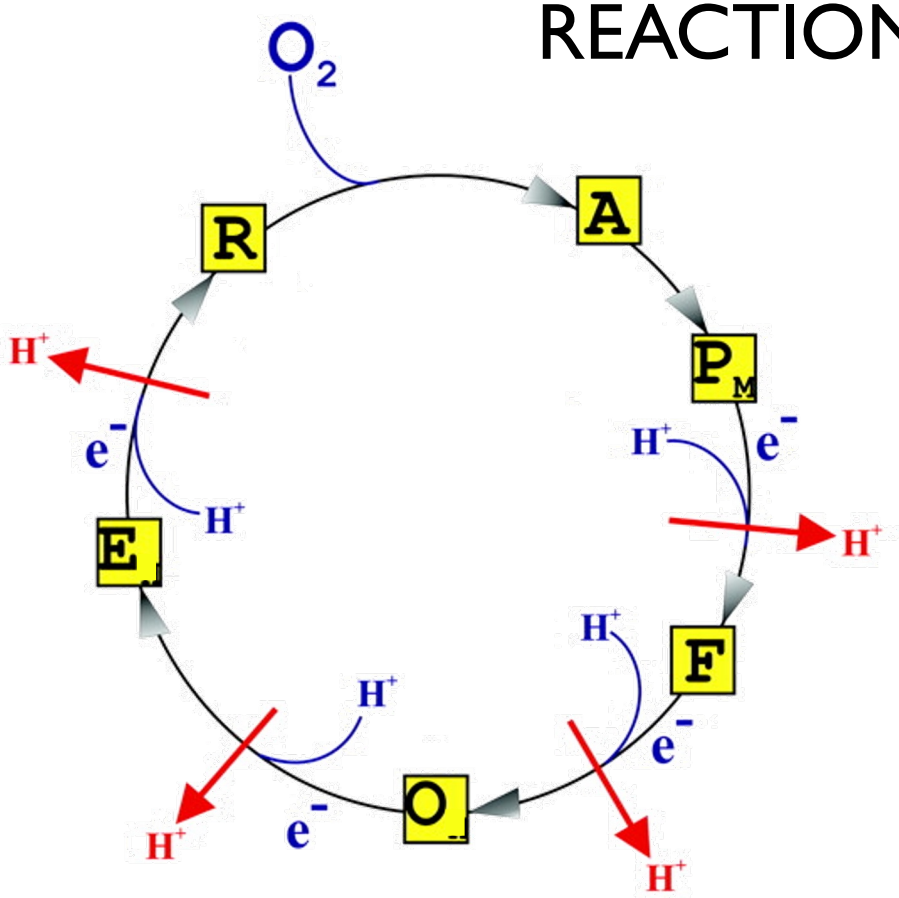
Unidirectional proton pumping

$1e^-$ =14.6 kcal/mol : Pump $2H^+$ =9.2 kcal/mol

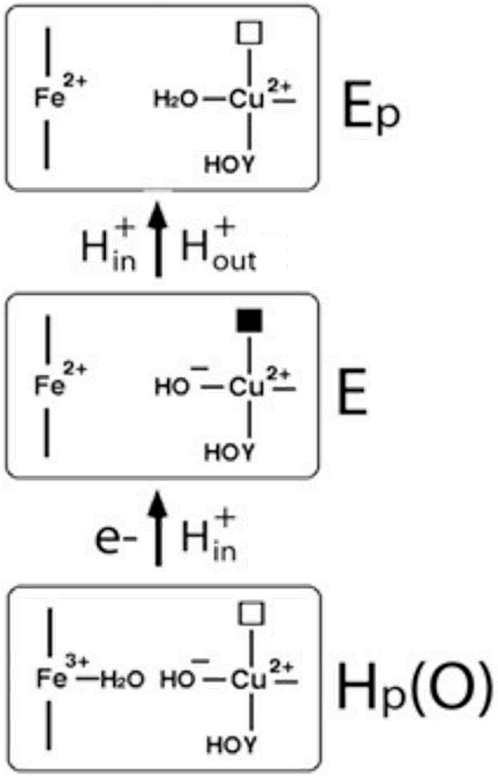
An important feature that distinguishes proton pumps from other enzymes in solution is their "vectorial chemistry" i.e they catalyse reactions that not only have direction in time, but also have well defined direction in space. In a proton pump, transfer of proton takes place without the use of carriers that physically transport the proton. Instead there exist specific proton-transfer pathways involving non-polar residues and water molecules.

Wikstrom et.al. *Nature* **3** 365 (2006)

REACTION CYCLE



Wikstrom et.al. *PNAS* **101** 529 (2004)

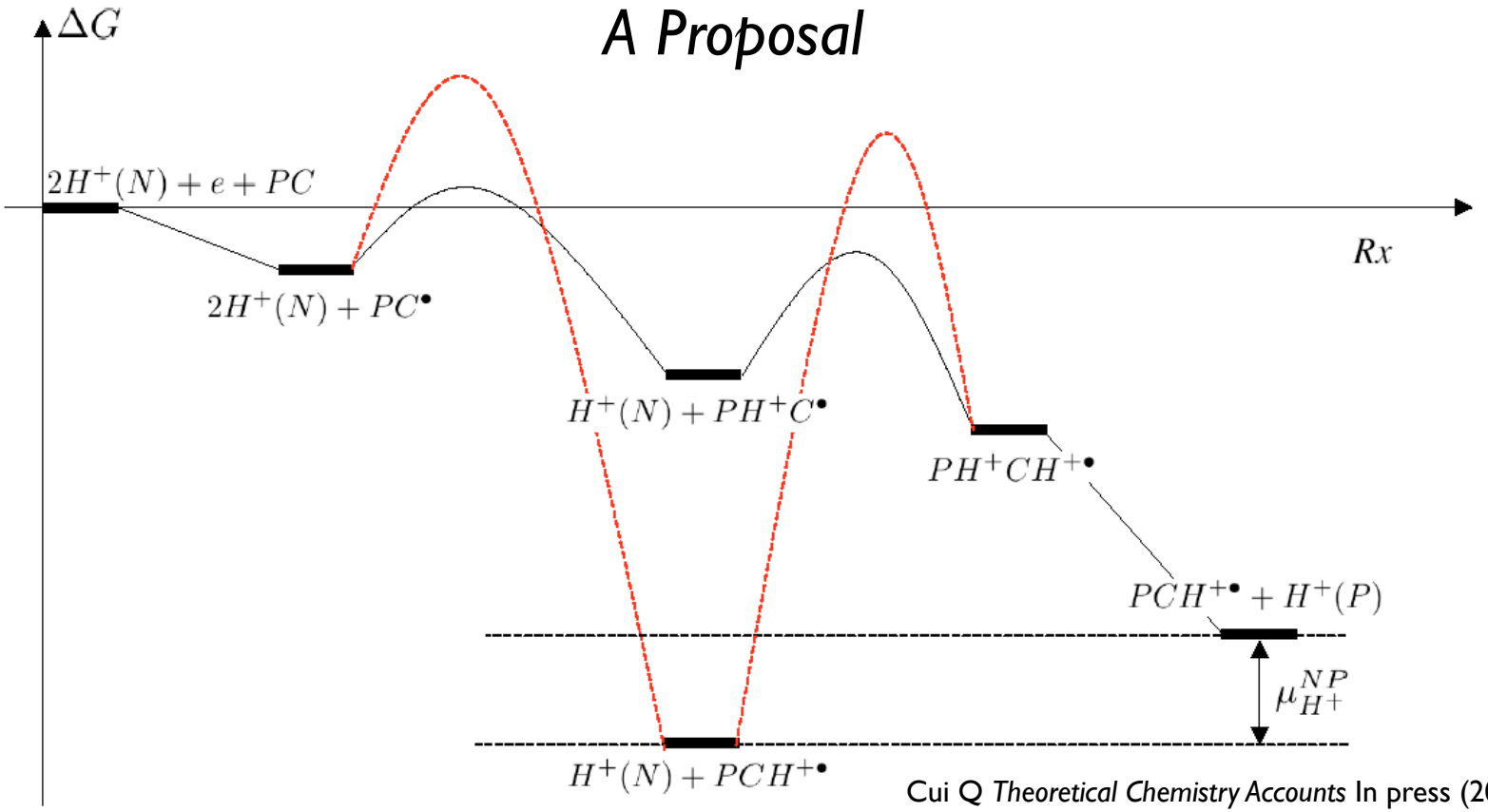


Stuchebrukhov et al *FEBs Lett.* **566** 126 (2004)

Based on extensive experimental studies the complete catalytic/pumping cycle can be seperated into four subcycles, where each subcycle involves the pumping of one (physical) proton, consumption of one (chemical) proton & one electron in the O_2 reduction. For our studies we focus on the O $\rightarrow$ E $\rightarrow$ Ep subcycle, specifically on the E state.

KINETIC GATING to achieve fast & efficient pumping

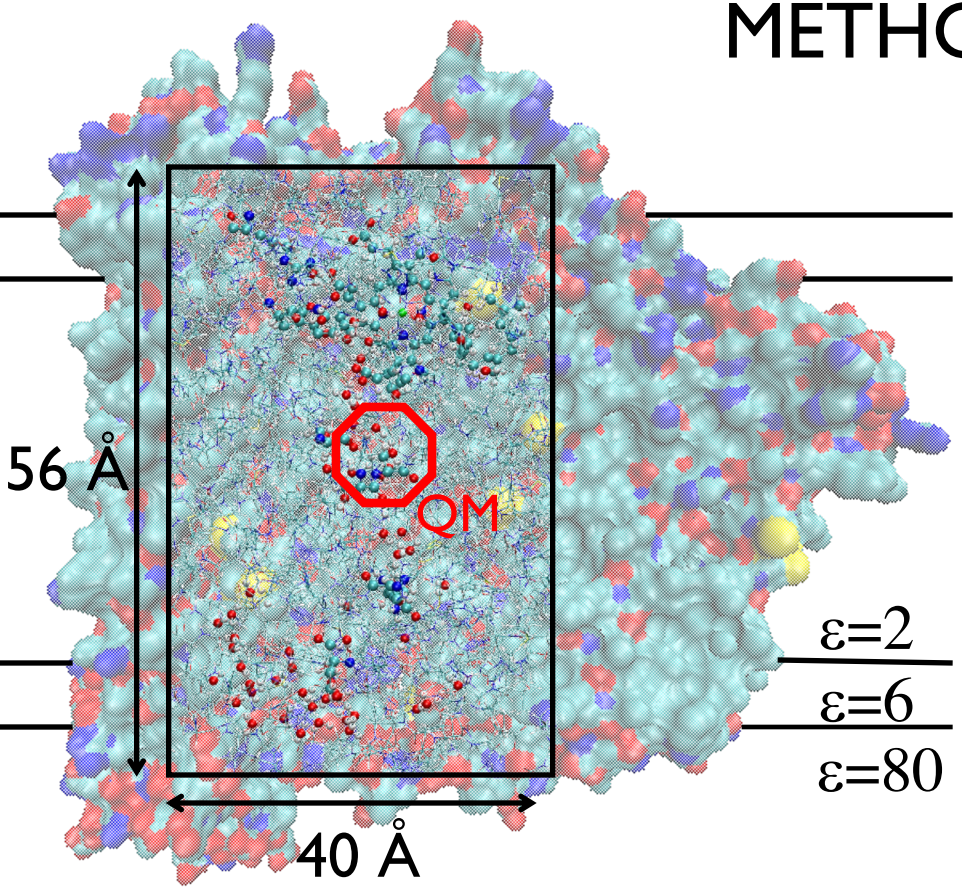
A Proposal



Cui Q *Theoretical Chemistry Accounts* In press (2006)I

Shown above is a qualitative free energy diagram for one sub-cycle. For a pump that is not only efficient (i.e free energy drop associated with the sub-cycle is close to zero), but also fast, the pathway with less stable intermediates has to be chosen. We term this as kinetic gating. The main question however, is how CcO avoids the thermodynamic trap and accomplishes kinetic gating

METHODS



QM/MM:

$H = H_{QM} + H_{MM} + H_{QM/MM}$

GSBP:

$Z = \int d(\mathbf{X})\ 1/N!\ \int d(\mathbf{1}) \ldots d(\mathbf{N}) \exp(-U_{tot}/k_B T)$

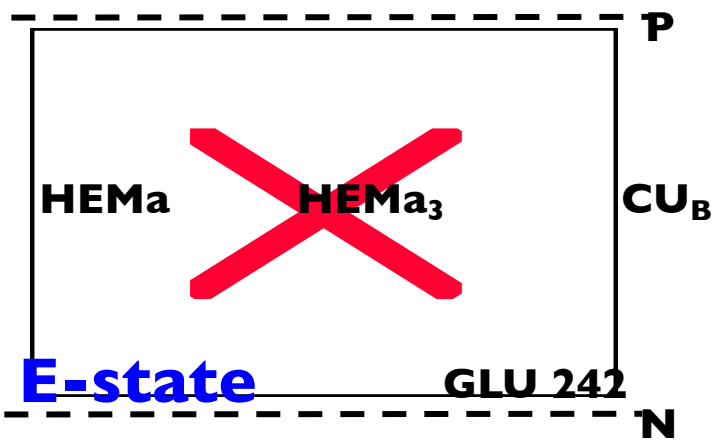
$e^{-W(R,1,\ldots,n)/kBT} = 1/C\ \int d(n+1) \int d(n+2) \ldots e^{-[Utot\ +\ Ucr\ ]/kBT}$

$W(\mathbf{X}) = U(\mathbf{X}) + \Delta W_{cr}(\mathbf{X}) + \Delta W_{np}(\mathbf{X}) + \Delta W_{elec}(\mathbf{X})$

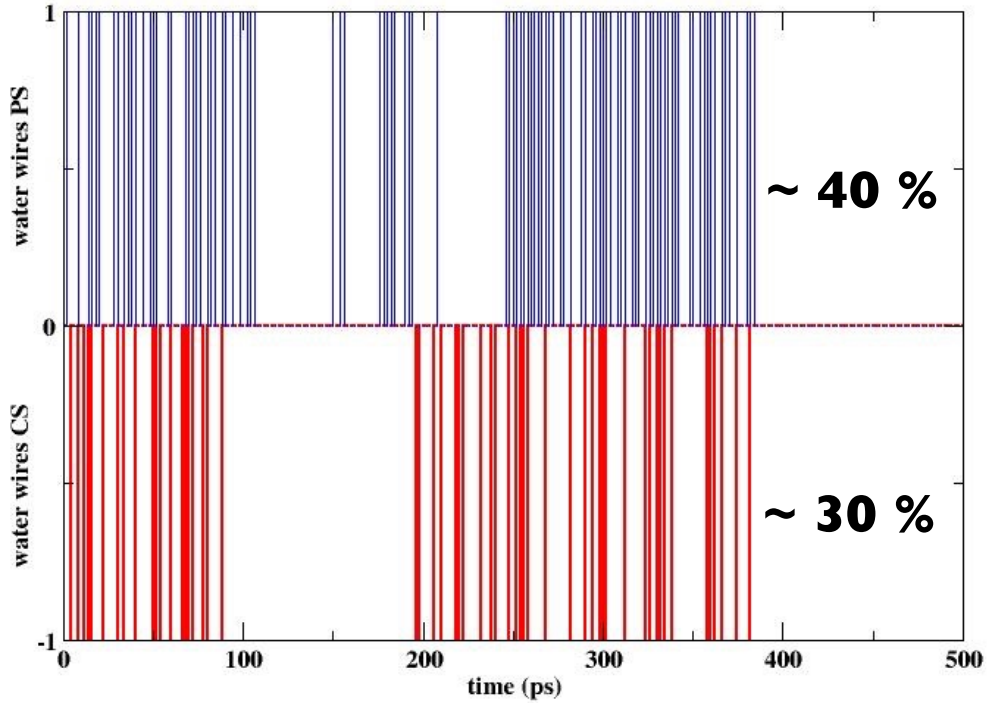
We use the semi-empirical SCC-DFTB as the QM method. The MM treatment is done using the CHARMM force-field. Treatment of long range electrostatics is done using theGeneral Solvent Boundary Potential (GSBP) method. Protonation states of residues,membrane potential and lipid species of the membrane, all contribute significantly to the electrostatics and need to be carefully treated. Water molecules not seen in X-ray structure were modelled in the active site. Molecular Dynamics simulations in the order of ~1 ns were performed. Free Energy Calculations using umbrella sampling (in PMF) and thermodynamic integration (in pKa) is used.

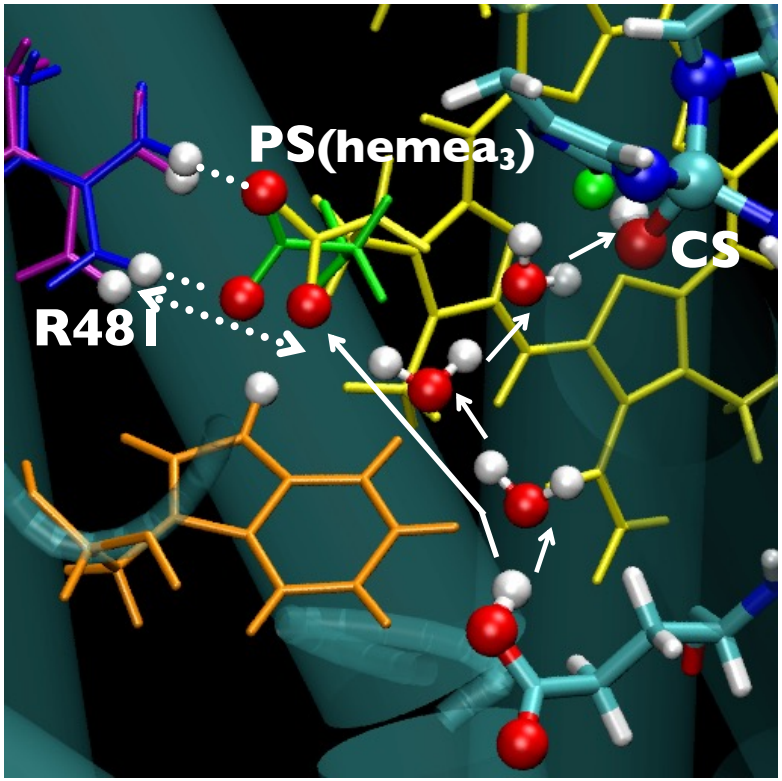
Schaefer et..al. *J. Chem. Phys.* **101** 8643 (2005)

WATER DYNAMICS



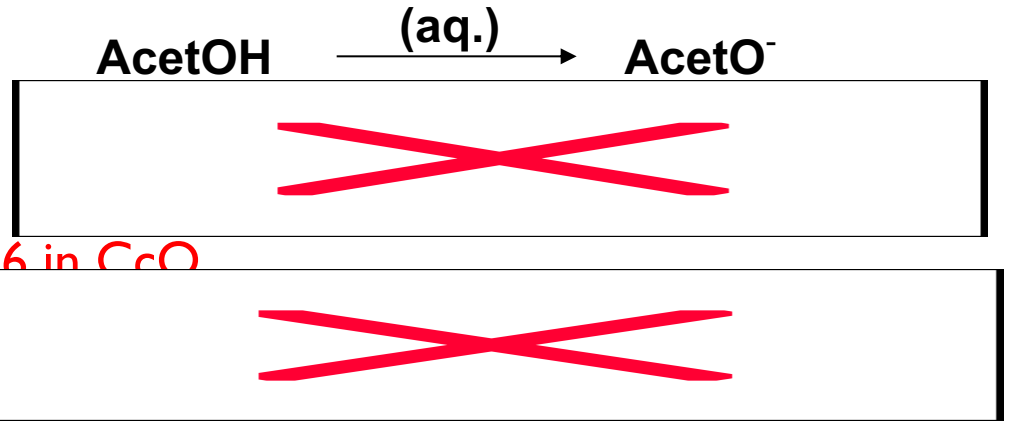
R481- Heme a_3 salt bridge breaks on formation of water wires connecting E286 to the heme a_3 propionate. The propionate could serve as a potential physical site. During dynamics stable water wires connect both the physical (PS) and chemical site(CS) are formed





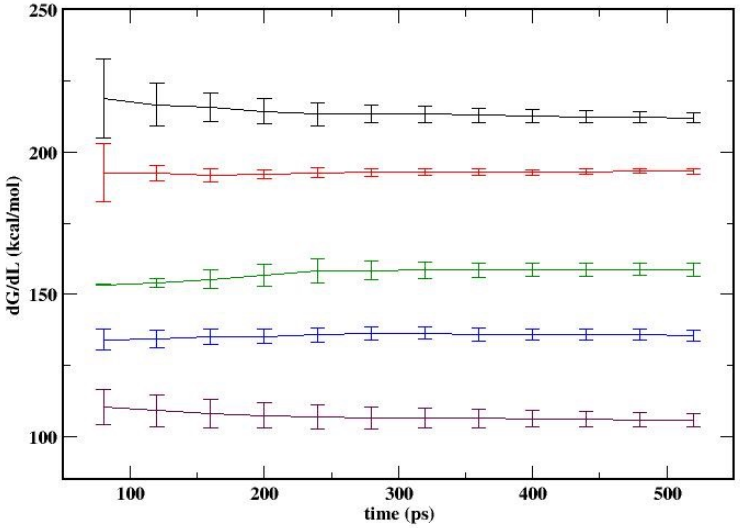
pK_a CALCULATIONs OF E286

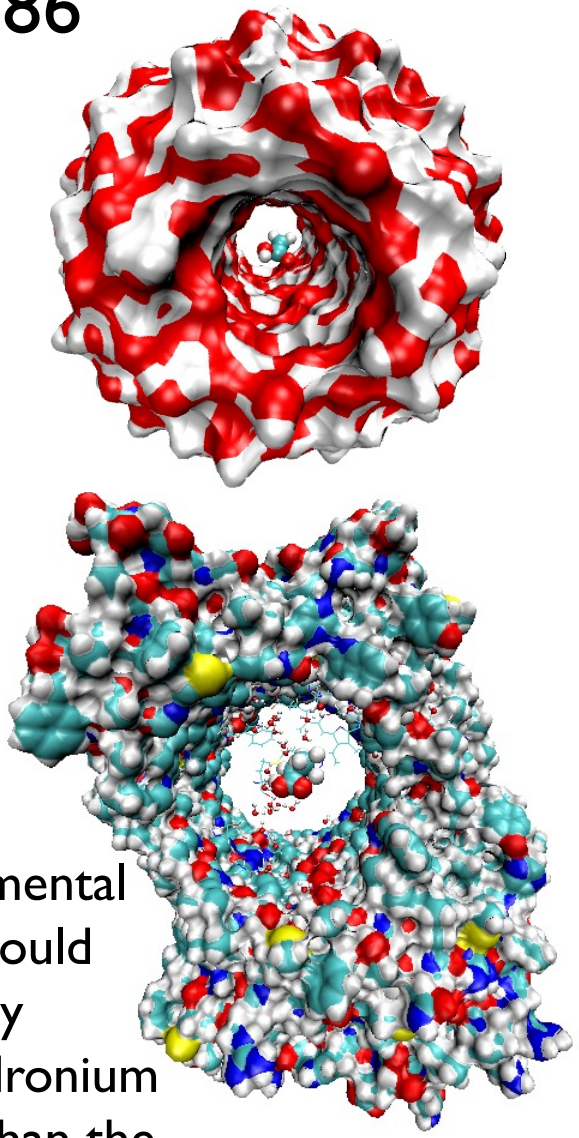
Acetate in bulk water



ΔpK_a(bulk→enzyme) =20.4 !!

Glu286 is **always** protonated



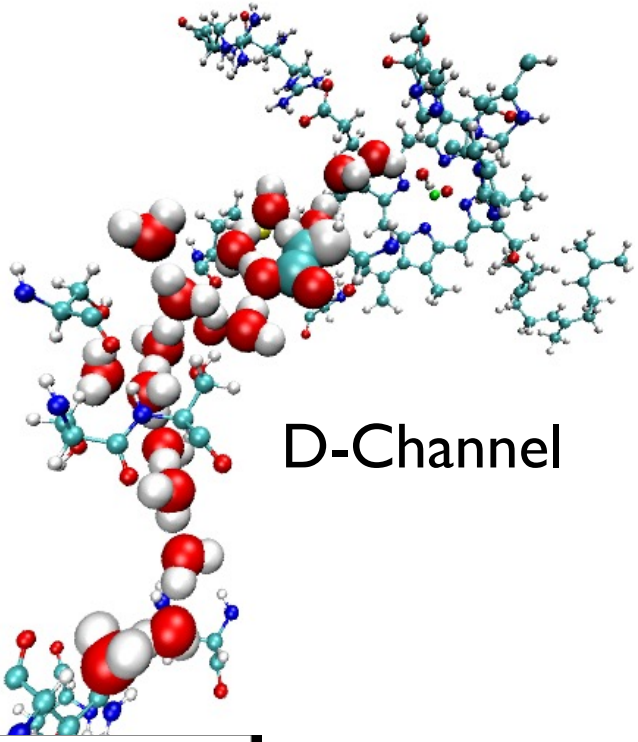


The apparent pK_a experimental value is 9.4, therefore it could be that the experimentally measured pK_a is for a hydronium in the D-channel rather than the glutamic acid residue

Li et.al. *J. Phys Chem. B* **107** 8643 (2003) , Li et.al. *J. Phys Chem. B* **107** 14521 (2003)

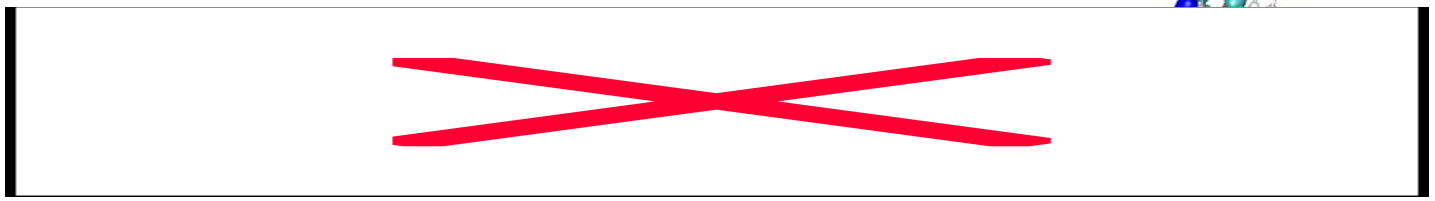
PMF of a proton along the D-channel: METHOD

QM/MM selection: 60/13357 atoms
Proton transfer over a distance of 20 Å
We compute the Potential of Mean Force (PMF) for CcO wild type and ND139 mutant

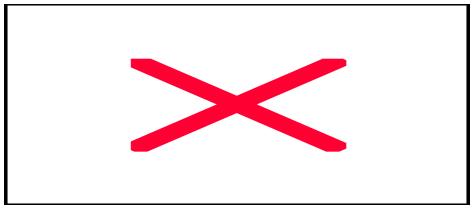


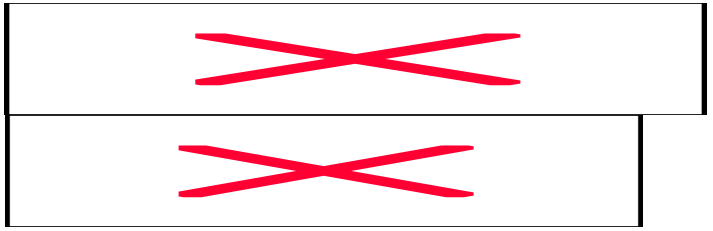
D-Channel

Modified Center of Excess Charge (mCEC):
mCEC is a global and collective coordinate that locates the excess charge (not the proton) in the three-dimensional space

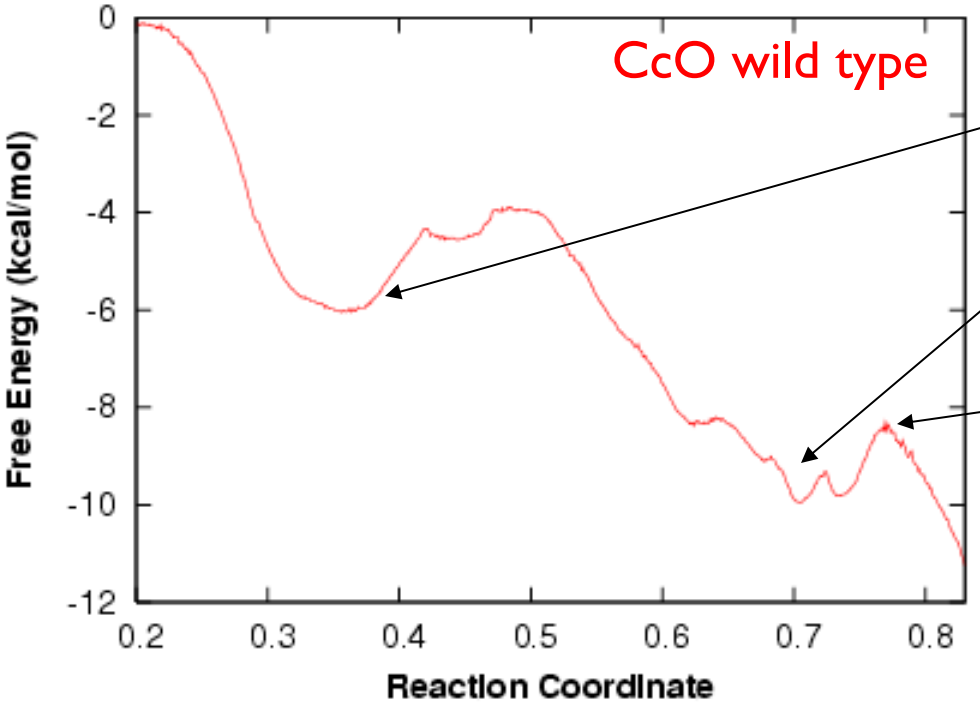


Reaction Coordinate for the PMF





PMF of a proton along the D-channel: RESULTS

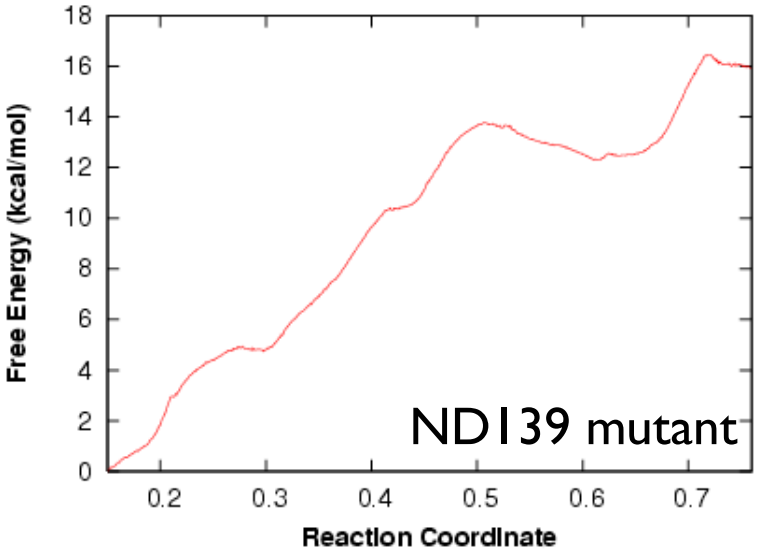


Proton Stabilized at middle of the channel

Minimum at the branching point (Glu286 is always protonated)

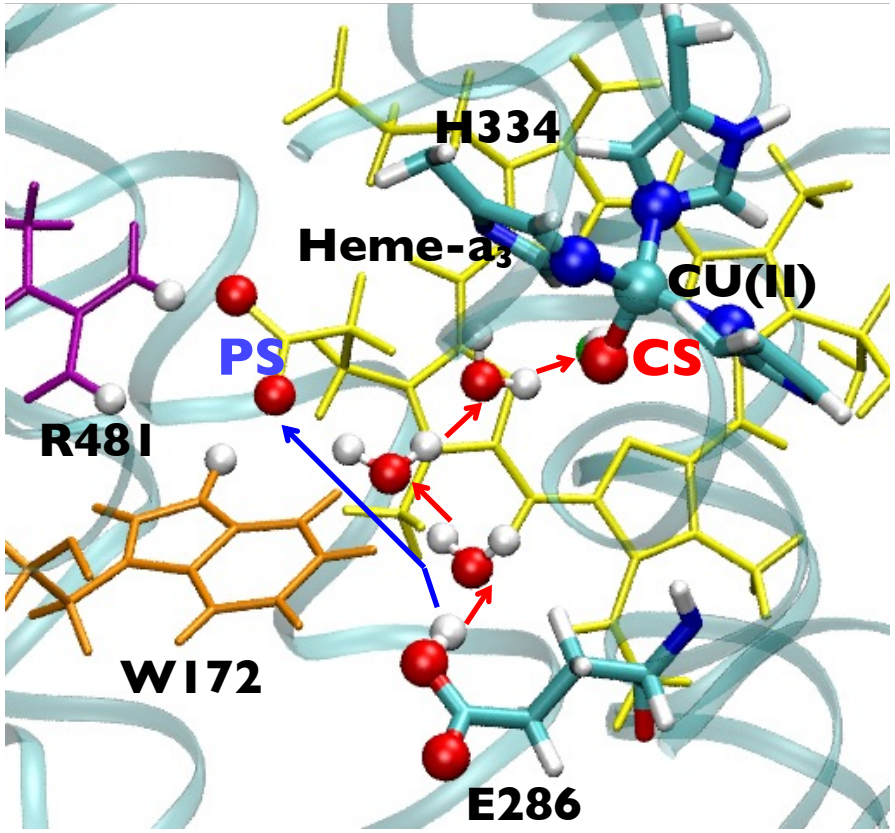
Downhill to protonate the oxygen bound copper (treated as MM)

- Glu286 is always protonated
- Preliminary results shown that if the chemical site contains an additional proton, the PMF is uphill after the first minimum (middle of the channel)

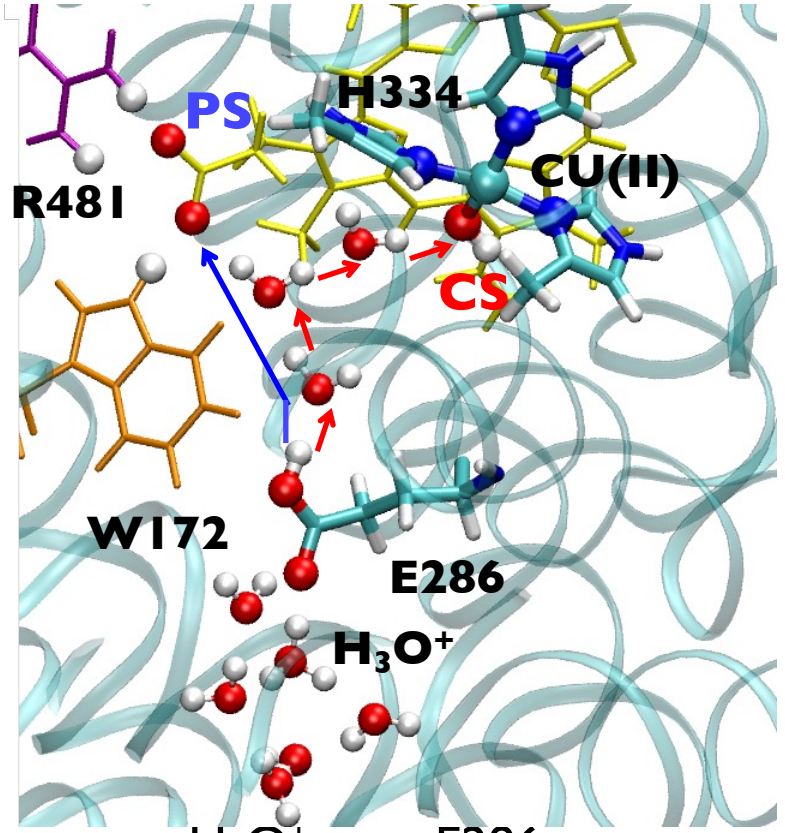


The mutation at the entrance of the channel changes the PT energetics

REACTION PATH STUDIES



PT: CS : uphill ~ 5 kcal/mol
PT: PS : uphill ~10 kcal/mol



H_3O^+ near E286
PT: CS : downhill ~ 20 kcal/mol
PT: PS : downhill ~ 10 kcal/mol

The energetics of proton transfer is sensitive to the protonation state of the H334 and also to the presence of an extra proton in the D-channel. The values (SCC-DFTB) however need to be compared with high-level ab-initio calculations

CONCLUSIONS

A quantitative molecular model:

A molecular model for CcO has been constructed to provide a quantitative analysis of the enzyme reactivity. Important factors such as a QM method that allowslong sampling, long range electrostatics, the protonation states, the number and location of waters have been carefully optimized

Formation of water wires is observed:

In our MD simulations we observe water wires formation in the hydrophobic cavity connecting Glu286 and both chemical and physical site independently of the electronic and protonation state of the active site.

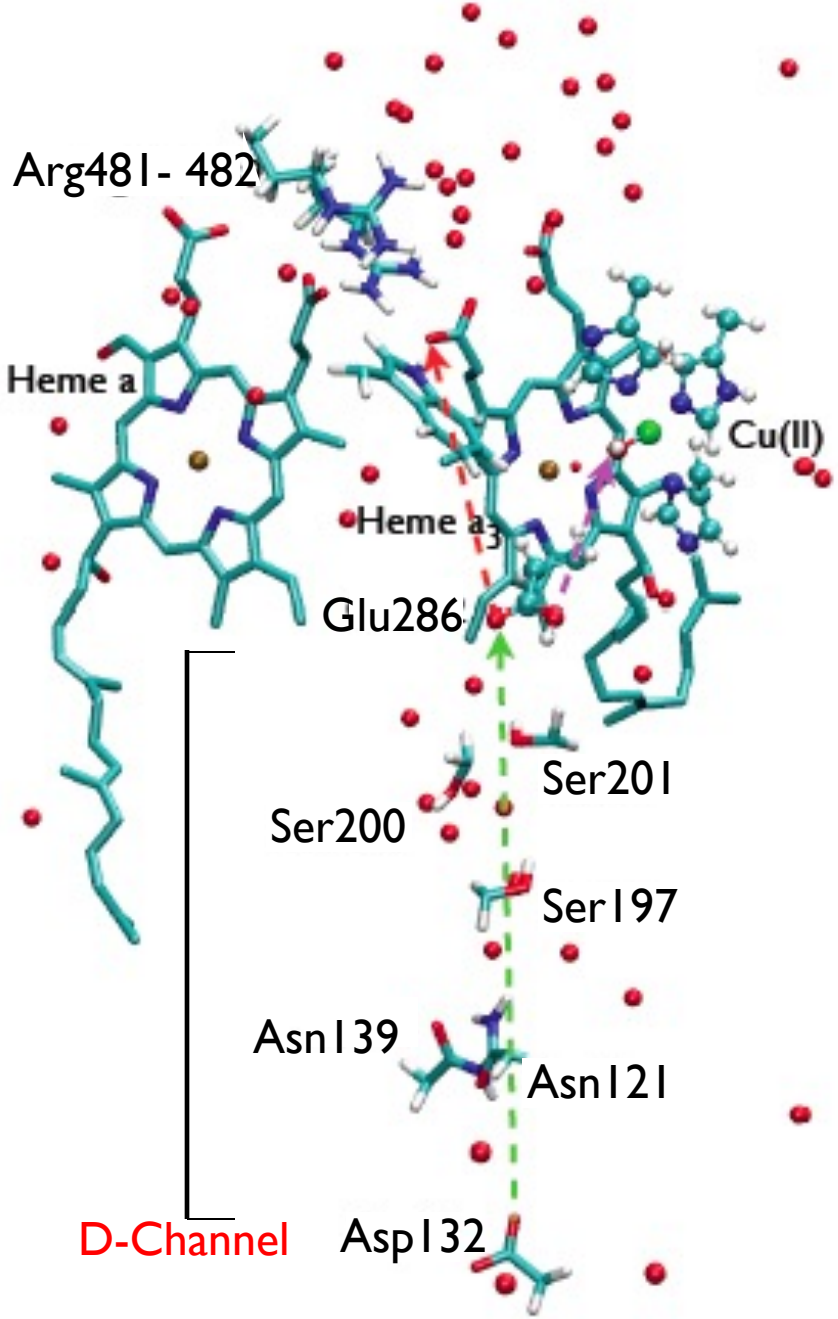
pKa calculations of the branching point Glu286:

pKa calculations using the QM/MM DSTC protocol indicate that Glu286 has a very high pKa and therefore it is always protonated. The pKa calculated in experiments could be of a hydronium in the D-channel. The reaction path studies also favour the presence of an extra proton in the channel.

Proton transfer along the channel:

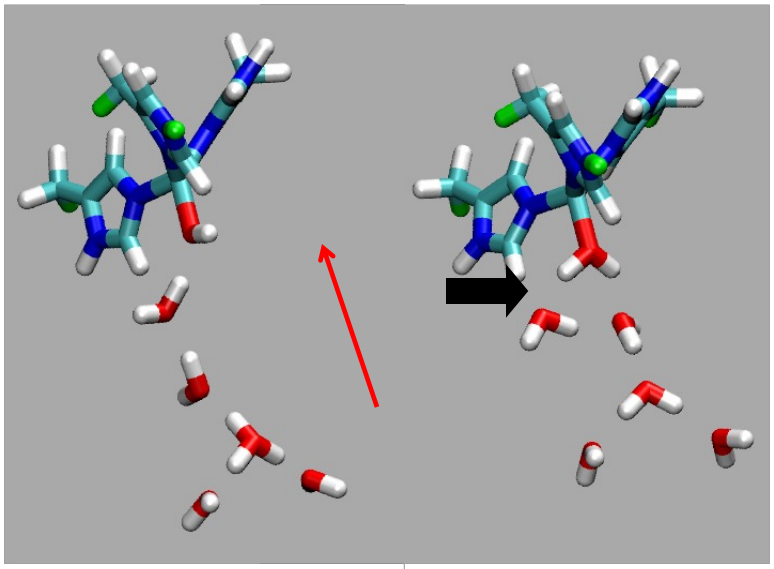
PMF along the channel show that there exists two minima and confirms that Glu286 has to be protonated. Mutation ND139 alters the energetics of the proton channel. However other properties such as protonation state of nearby residues or conformations could undergo change due to the mutation.

HOW DOES CcO WORK ?



At first glance the mechanism of proton pumping does not seem to be profound, the oxidation-reduction reactions in the chemicalcenters alter the electrostatic potential inside the protein, which modulates the pK_a values of certain titratable residues which then facilitate the translocation of proton across membranes. The translocation can occur against concentration gradient because it is coupled to an exothermicprocess (O_2 reduction). More careful considerations at thermodynamic & kinetic level reveals that there is more to it.

Conclusions



	Reaction Energetics
SCC-DFTB + 3rd order B3LYP/6-31G*// SCC+3	4.3 2.1
SCC-DFTB + 3rd + HBOND B3LYP/6-31G*//SCC+3+HB	-3.5 -0.8