The reaction mechanism of Cytochrome c Oxidase

Xavier Prat-Resina

Group Meeting

January 27th 2006

Summary

1. Introduction to Cytochrome c Oxidase (CcO) reactivity

2. Description of the setup for simulation

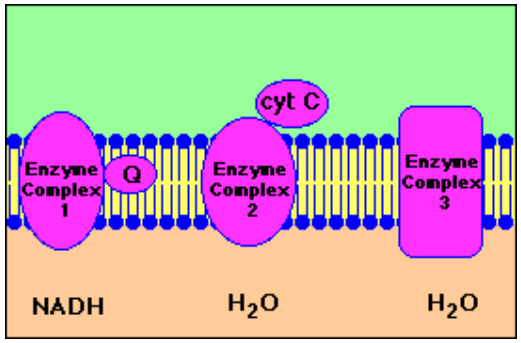
3. Glu242: waters and channels. A qualitative analysis with MD

4. Glu242: pK calculations. A quantitative analysis

5. His291: coupling between physical and chemical site?

6. Conclusions and perspectives

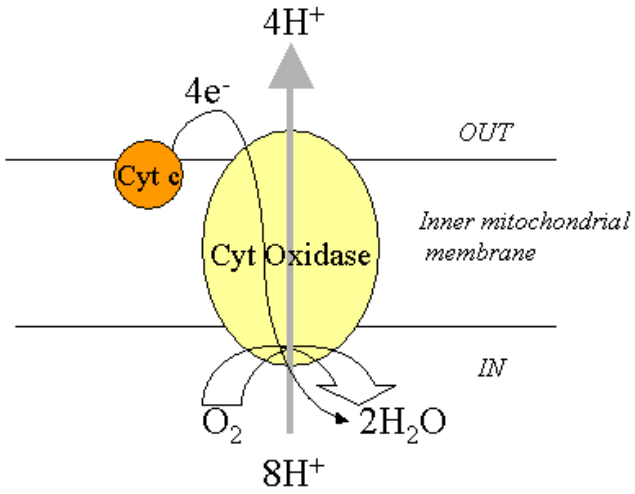
1.Introduction: Cytochrome c Oxidase (CcO)



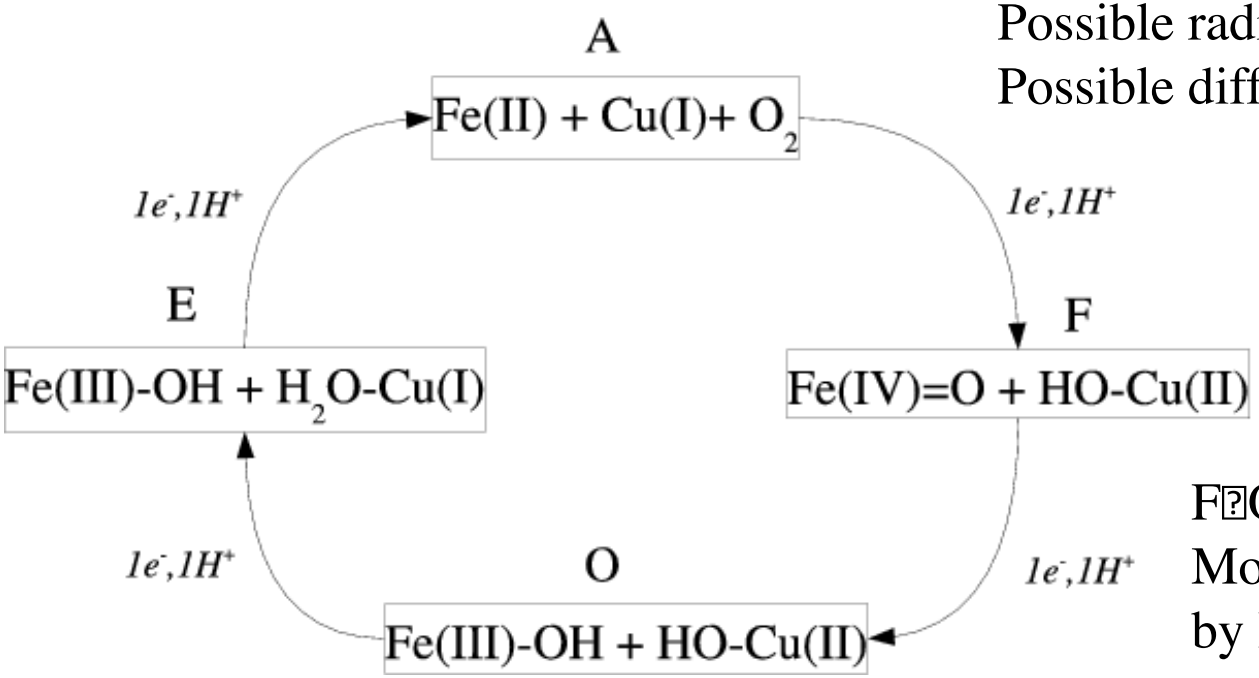
CcO:
The last complex in the Electron transfer chain

$$O_2 + 8H^+_{in} + 4e^- \rightarrow 2H_2O + 4H^+_{out}$$

2 coupled process:
Exergonic Oxigen reduction
Endergonic proton pumping



1.Introduction: Chemical process



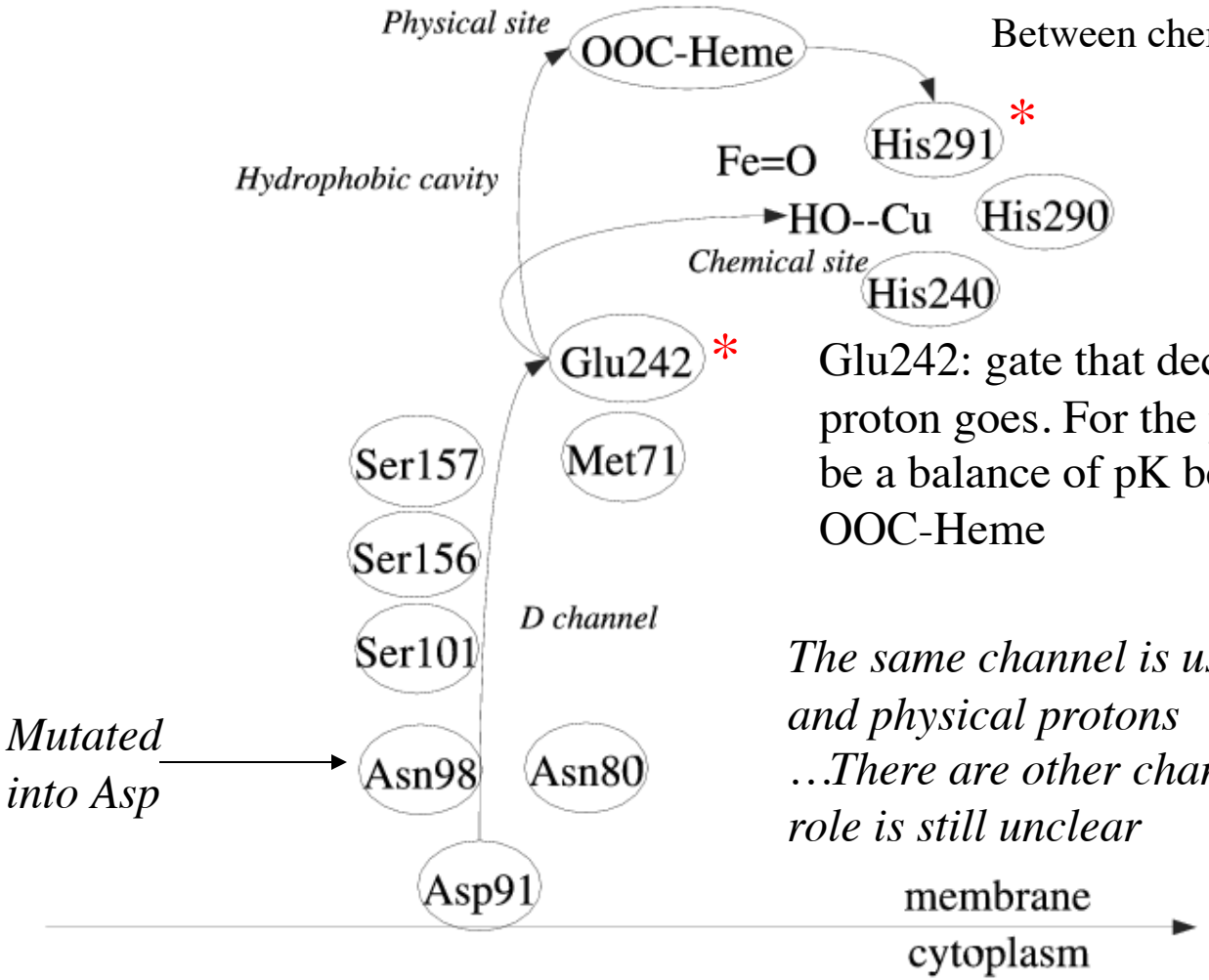
Oxygen bond cleavage
Possible radical intermediates
Possible different sequence 2H/e

F?O
Most studied process
by P. Brzezinski

At every stage one proton is believed to be pumped
In total 4 protons are pumped

Other authors might consider different intermediate species

1.Introduction: Pumping and reduction. A coupled process

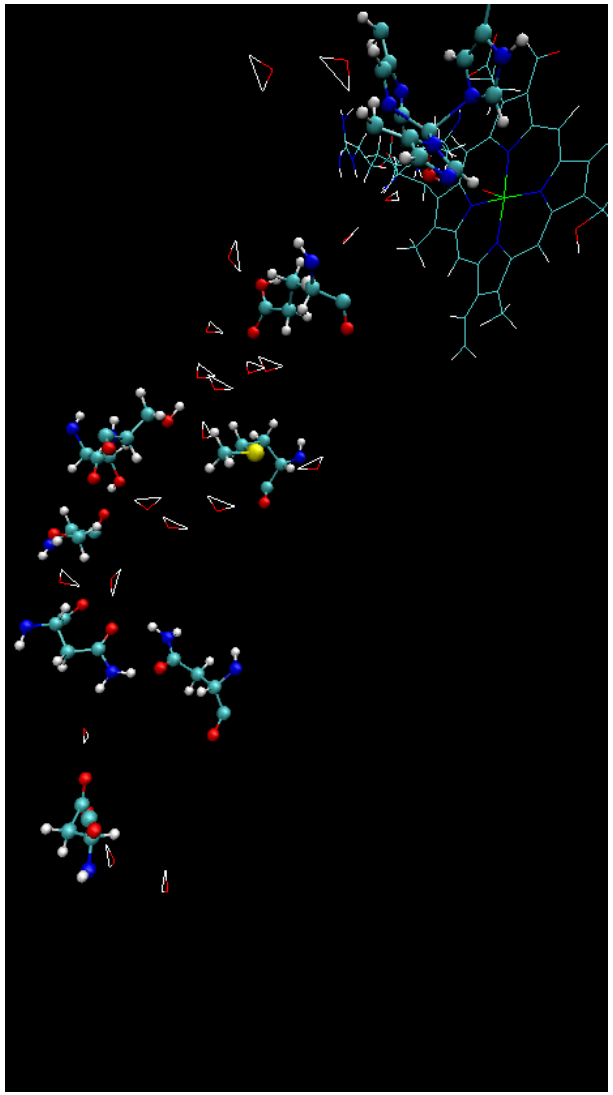


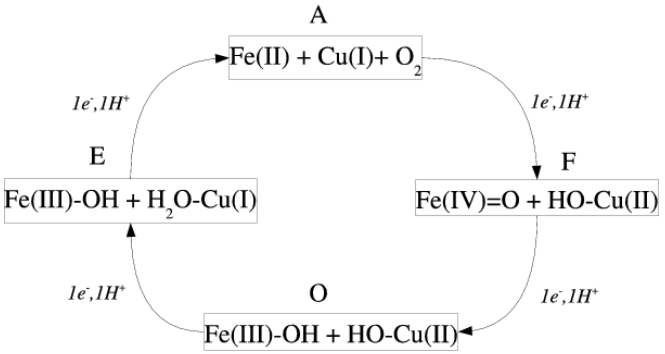
His291: communication
Between chem and phys

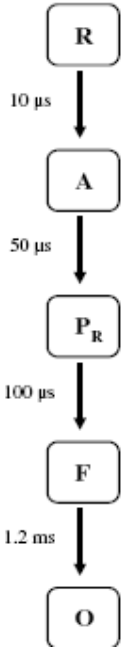
Glu242: gate that decides where the proton goes. For the pumping there must be a balance of pK between Glu242 and OOC-Heme

The same channel is used for chemical and physical protons
…There are other channels which role is still unclear

1.Introduction: Pumping and reduction. A coupled process







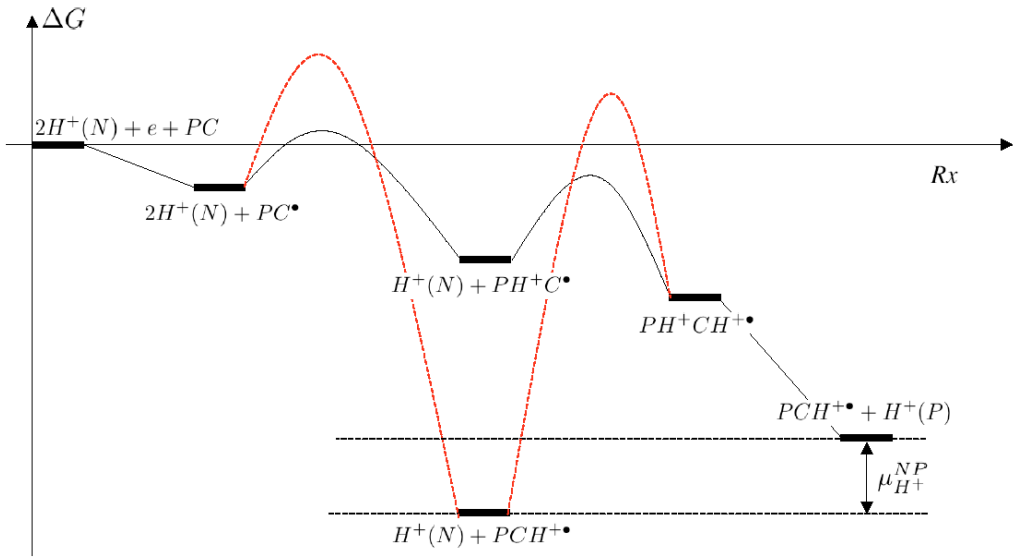
Mutation experiments:

E242A: no pump, chemical step up to Pr

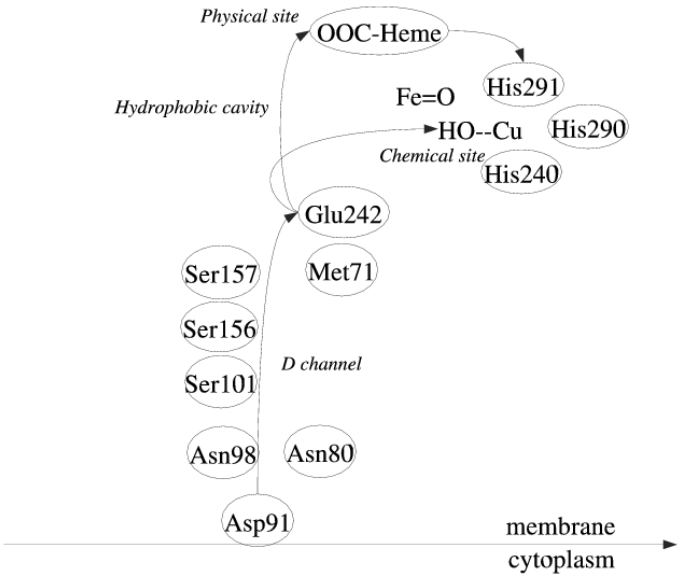
D91N: no pump, chemical step up to F

D98N: oxygen reduction slightly faster, but no pump. pK(E242)=9.4 ⟶ 11.0

1.Introduction: Open Questions



1.The sequence of the two protons and electron:
How OOC-Heme and Glu242 balance their pK (Nilanjan)

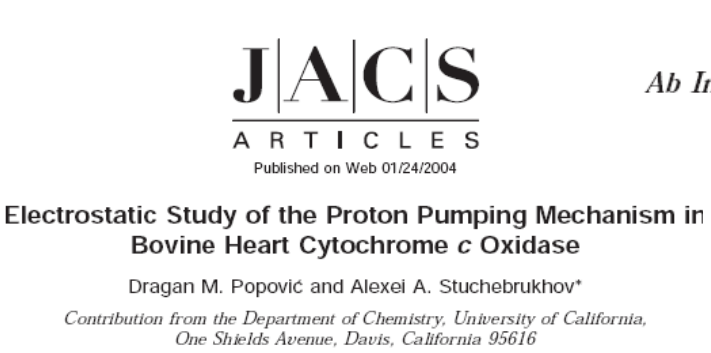


2.The source of protons and the modulation of the pK of Glu242 from the D-channel

Compute the pK of Glu242
For Wild Type CcO and ND98 mutant

1.Introduction: Theoretical studies

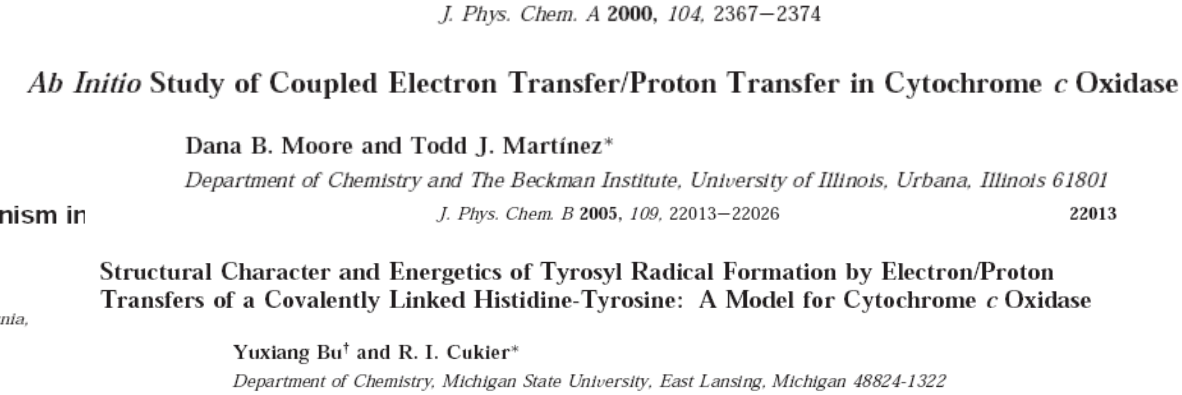
*Strategy 1: *Matar mosques a canonades (killing flies with cannons)*
Accurate method but inadequate model


J|A|C|S
ARTICLES
Published on Web 01/24/2004

Electrostatic Study of the Proton Pumping Mechanism in Bovine Heart Cytochrome *c* Oxidase

Dragan M. Popović and Alexei A. Stuchebrukhov*

Contribution from the Department of Chemistry, University of California, One Shields Avenue, Davis, California 95616


J. Phys. Chem. A **2000**, *104*, 2367−2374 2367

Ab Initio Study of Coupled Electron Transfer/Proton Transfer in Cytochrome *c* Oxidase

Dana B. Moore and Todd J. Martínez*

Department of Chemistry and The Beckman Institute, University of Illinois, Urbana, Illinois 61801

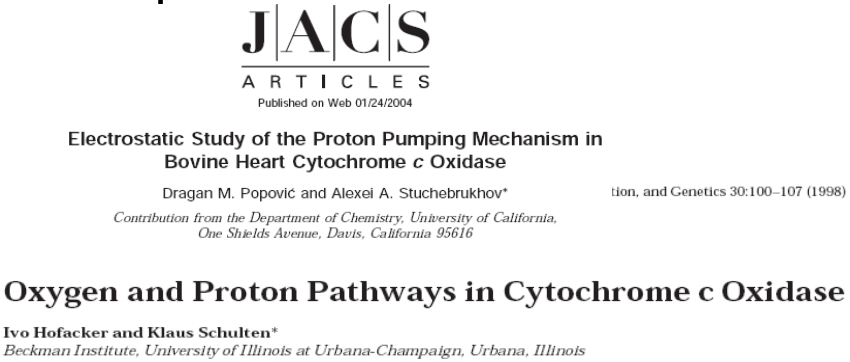
J. Phys. Chem. B **2005**, *109*, 22013−22026 22013

Structural Character and Energetics of Tyrosyl Radical Formation by Electron/Proton Transfers of a Covalently Linked Histidine-Tyrosine: A Model for Cytochrome *c* Oxidase

Yuxiang Bu† and R. I. Cukier*

Department of Chemistry, Michigan State University, East Lansing, Michigan 48824-1322

*Strategy 2: *Matar el burro a pessics (killing a donkey pinching it)*
Adequate model but inaccurate method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tion, and Genetics 30:100–107 (1998)

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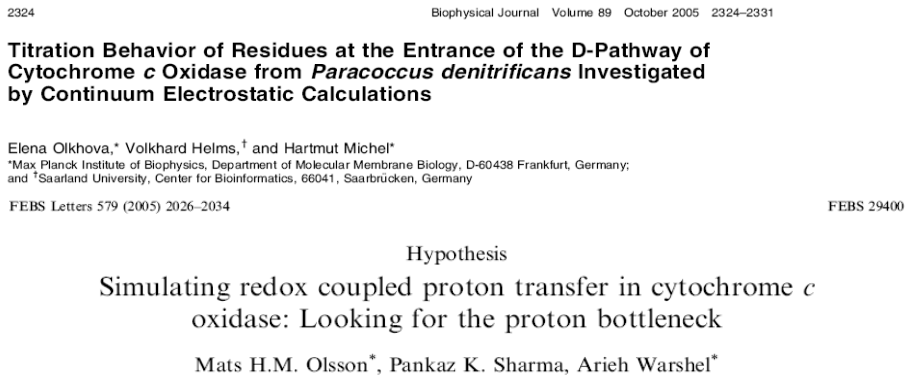
Oxygen and Proton Pathways in Cytochrome c Oxidase

Ivo Hofacker and Klaus Schulten*

Beckman Institute, University of Illinois at Urbana-Champaign, Urbana, Illinois


2324 Biophysical Journal Volume 89 October 2005 2324–2331

Titration Behavior of Residues at the Entrance of the D-Pathway of Cytochrome *c* Oxidase from *Paracoccus denitrificans* Investigated by Continuum Electrostatic Calculations

Elena Olkhova,* Volkhard Helms,† and Hartmut Michel*

*Max Planck Institute of Biophysics, Department of Molecular Membrane Biology, D-60438 Frankfurt, Germany; and †Saarland University, Center for Bioinformatics, 66041, Saarbrücken, Germany

FEBS Letters 579 (2005) 2026–2034 FEBS 29400

Hypothesis

Simulating redox coupled proton transfer in cytochrome *c* oxidase: Looking for the proton bottleneck

Mats H.M. Olsson*, Pankaz K. Sharma, Arieh Warshel*

*Our strategy:
All the system needs to be included but with accurate reactive potentials

2.Setup: metalloenzymes. Why people did not study CcO with QM/MM

1.SCC for copper systems. Comparison with DFT *(ADF in progress)*
Proton affinities

PA(B3lyp/SCC)	*Cu-OH*	*Cu-OH_2*
His291	-282.60/-281.50	-204.80/-203.60

PA(B3lyp/SCC)	*His291(N)*	*His291(NH)*
Cu-OH	-268.40/-287.97	-190.59/-210.07

With NHmod2 parameters

With NH parameters

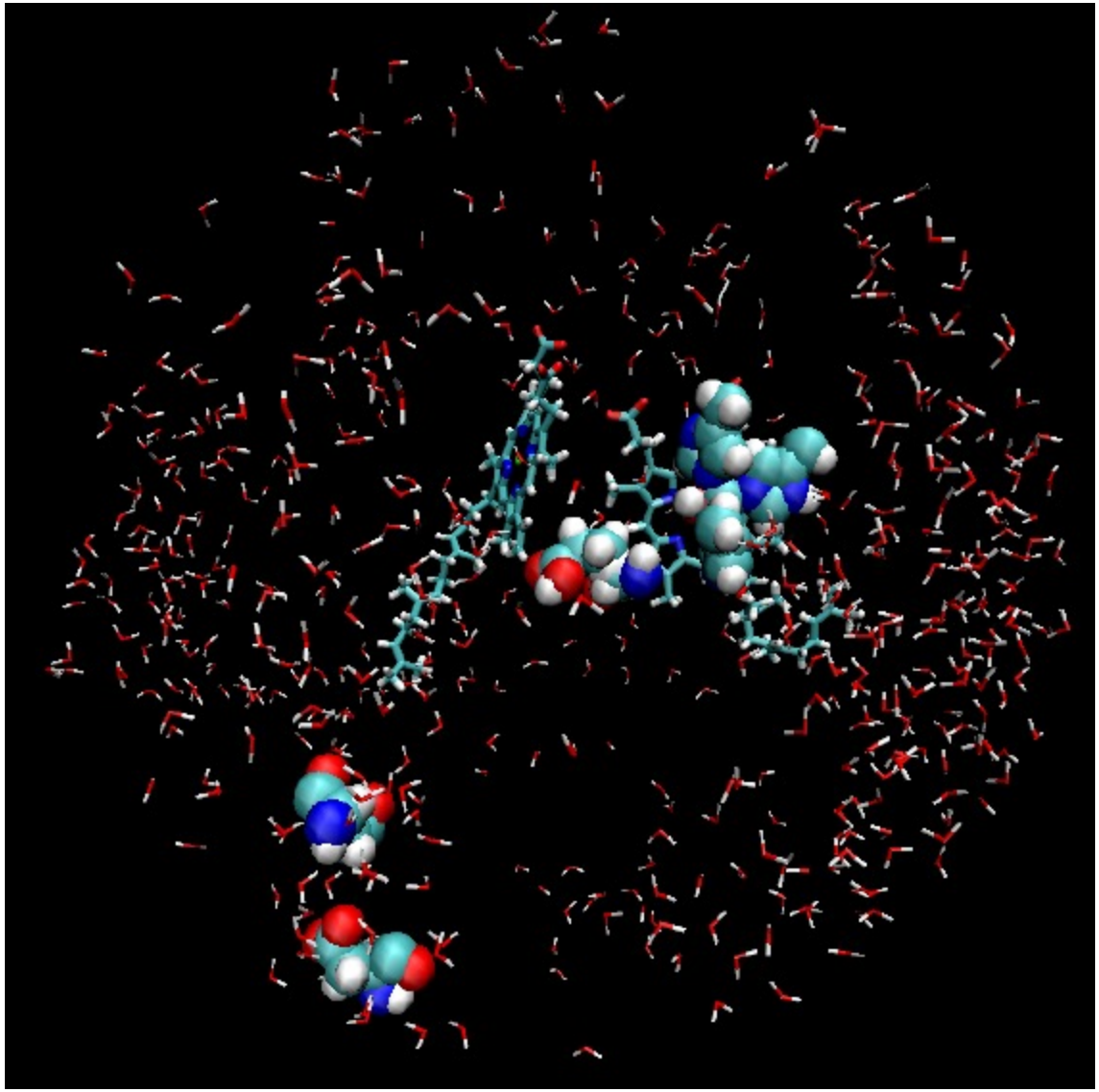
PA(B3lyp/SCC)	*Cu-OH*	*Cu-OH_2*
His291	-282.60/-278.97	-204.80/-201.70

PA(B3lyp/SCC)	*His291(N)*	*His291(NH)*
Cu-OH	-268.40/-282.43	-190.59/-205.17

2. Optimize from *ab initio* data the Van der Waals Parameters for Fe=O and Cu-OH

3.Compute ESP charges for Heme and Cu systems when they are treated with Molecular Mechanics

2.Setup: QM/MM in F state of CcO



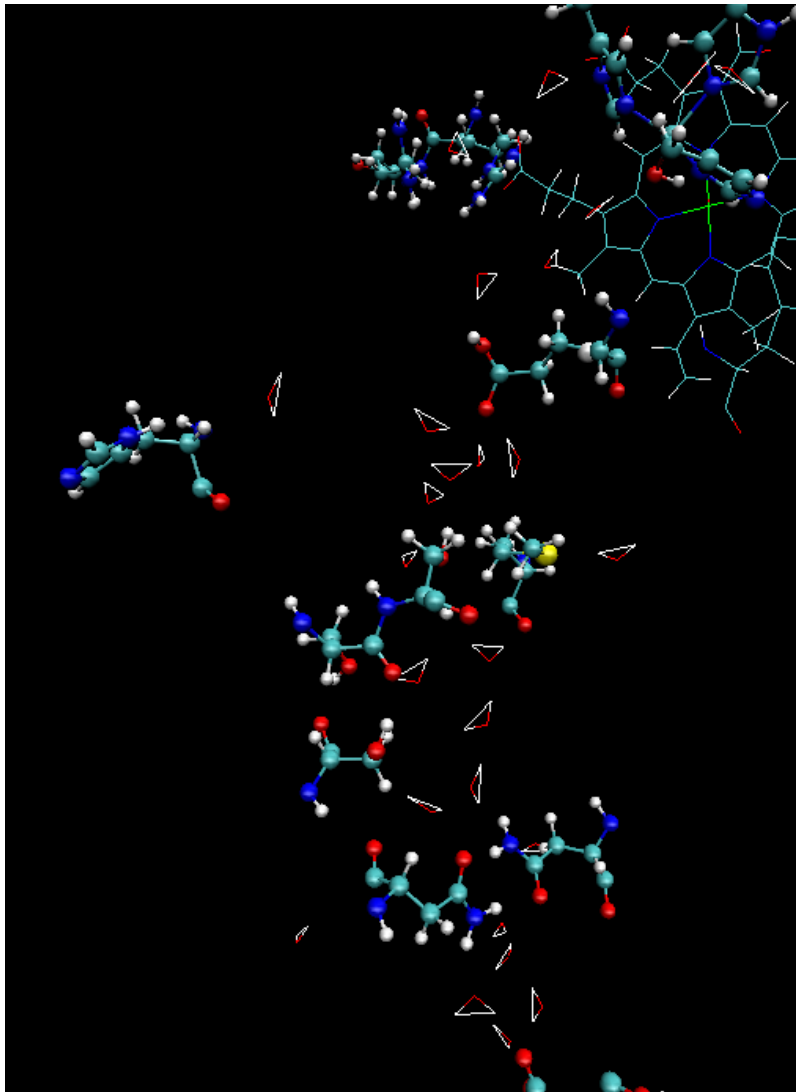
SCC-DFTB/MM/GSBP
Sphere of waters 30Å
~15,500 atoms
Stochastic Boundary MD

QM partitions:
Glu242 (Glu242 pK)
Glu242+13waters (D channel)
HO-Cu-$(His)_3$ (His291 pK)

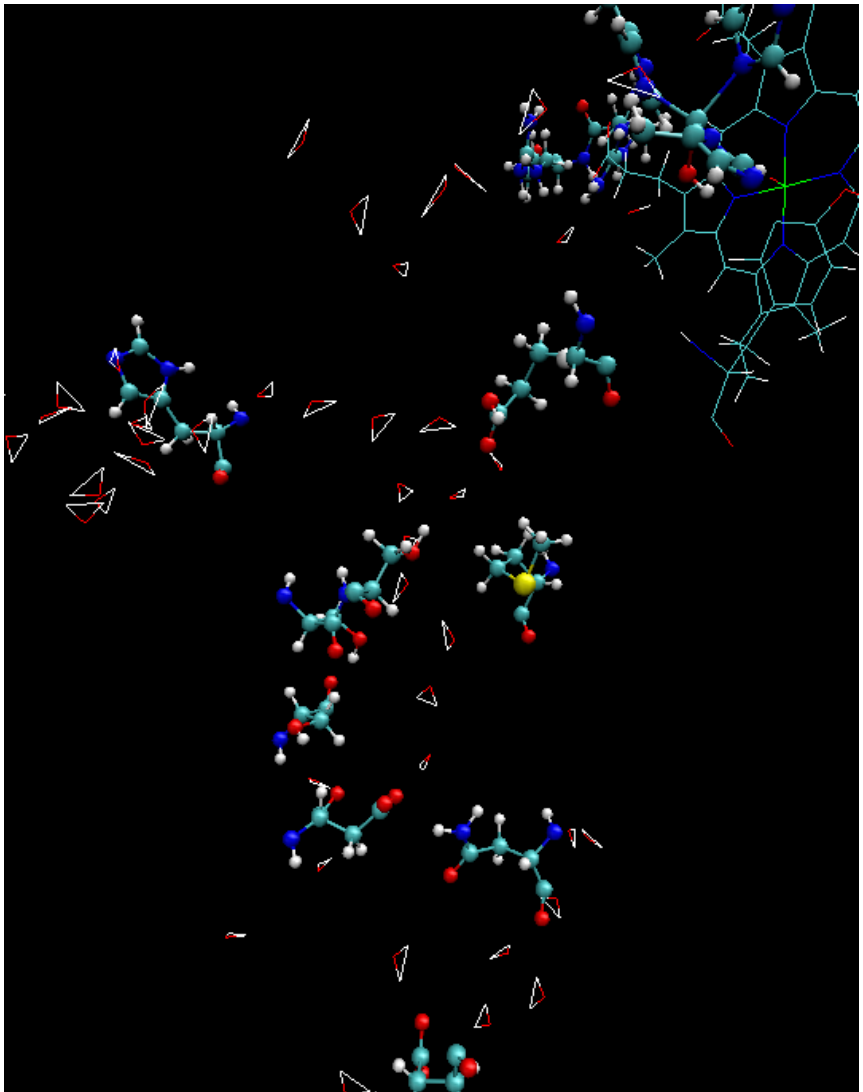
…The rest of protein is not shown

3.Glu242: waters and channels. A qualitative analysis

CcO wild type
(Glu242 is not connected to His151)

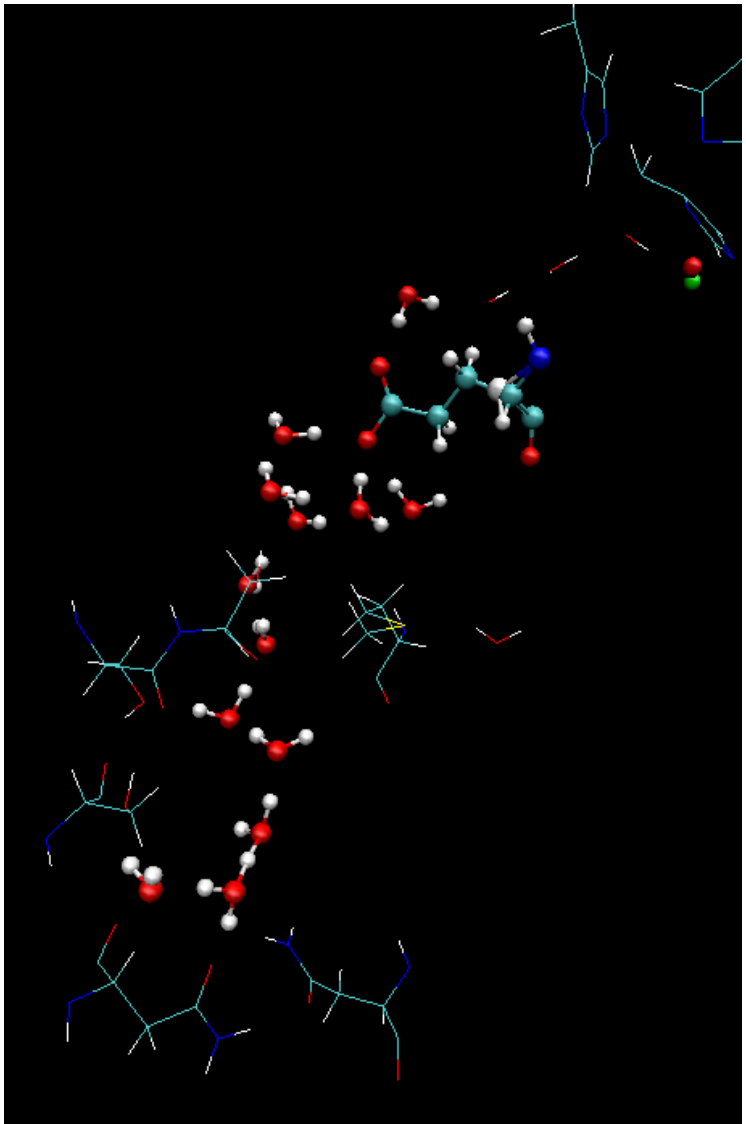


CcO ND98 mutant
(new channel in His151?)

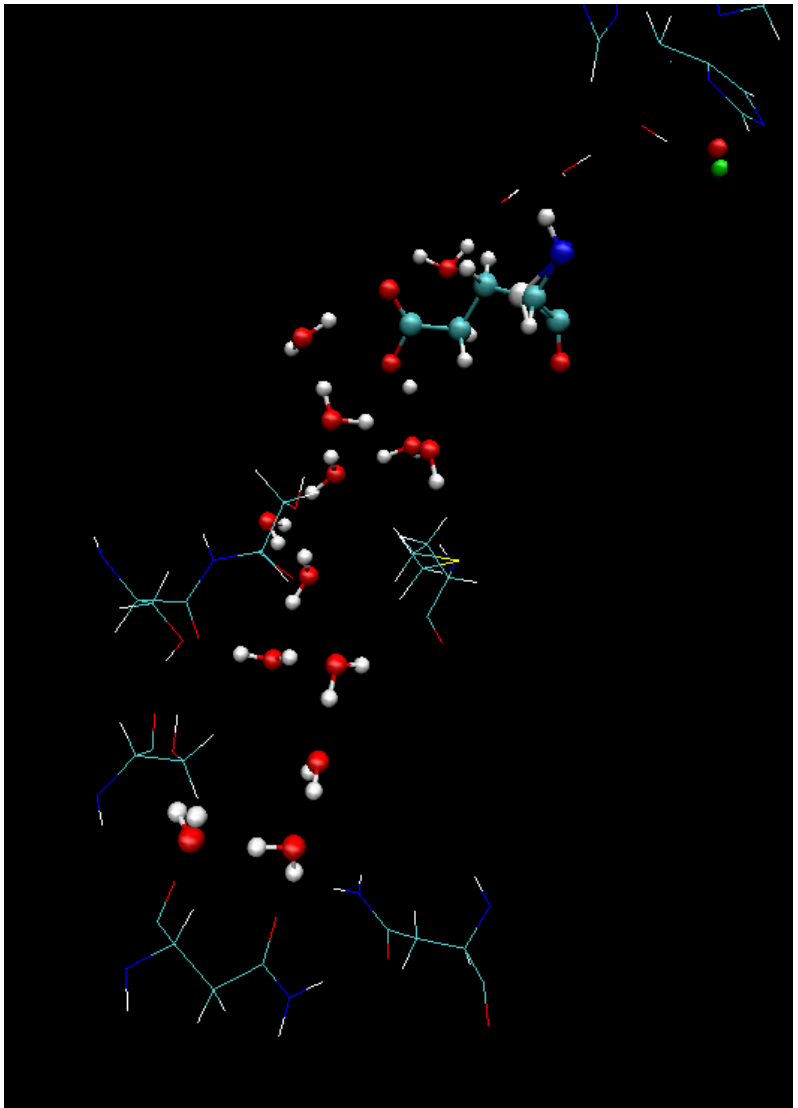


3.Glu242: waters and channels. A qualitative analysis

D-channel in CcO wild type: H^+ protonates Glu242 while in ND98 H^+ stays close to D98

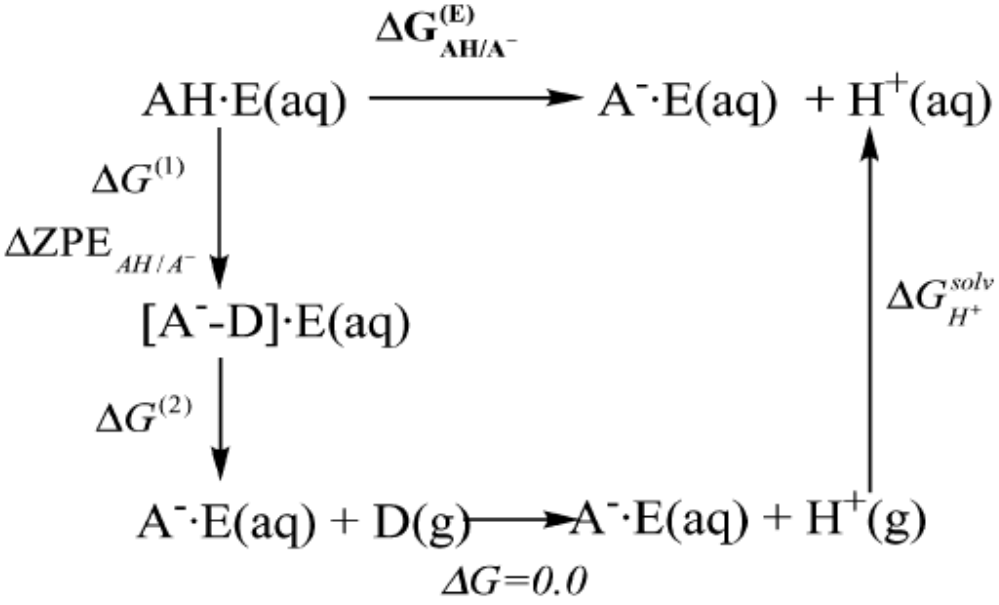


Short MD
At 50 K



4.Glu242: pK calculations. A quantitative analysis

Thermodynamic Integration: Dual Topology Single Coordinate (DTSC)



$$\Delta G = \int < \frac{\delta H}{\delta \lambda} > d\lambda$$

$$H(\lambda_i) = (1 - \lambda_i) H_{AH} + \lambda_i H_{A-D}$$

$$\frac{\delta H(\lambda_i)}{\delta \lambda} = H_{A-D} - H_{AH}$$

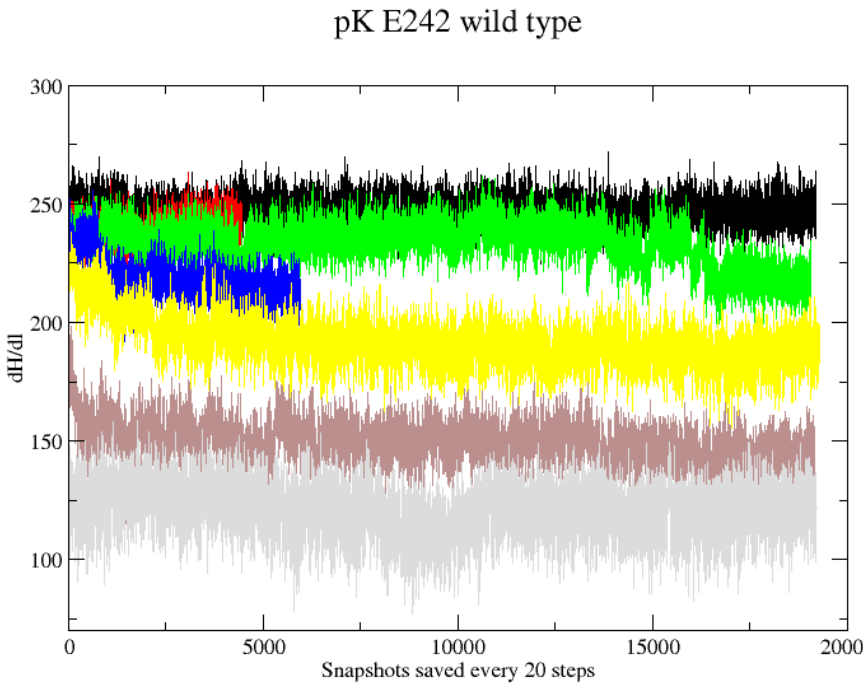
$$\Delta \mathbf{G}^{(E)}_{AH/A^-} = \Delta G^{(1)} + \Delta G^{(2)} + \Delta G^{solv}_{H^+} + \Delta ZPE_{AH/A^-}$$

$$pK_a^{(E)} = \Delta \mathbf{G}^{(E)}_{AH/A^-} / (2.303RT)$$

When do we stop the simulation?

Convergence of simulation: Reverse Cumulative Average (RCA)

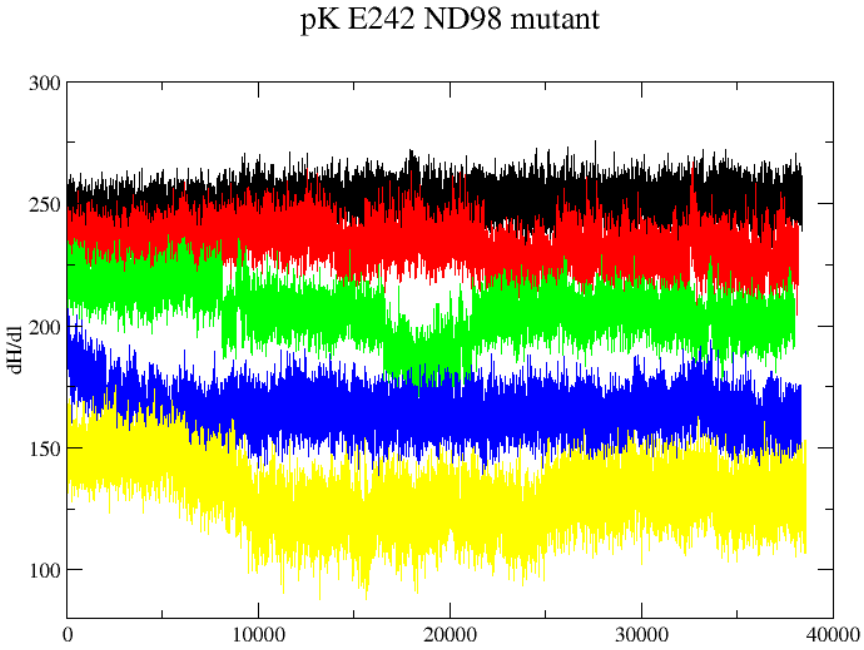
4.Glu242: pK calculations. A quantitative analysis



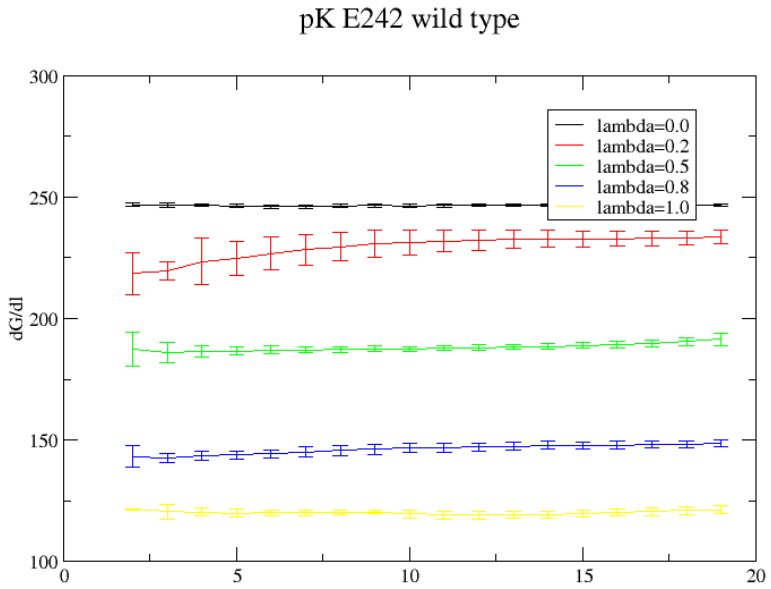
0.8 ns

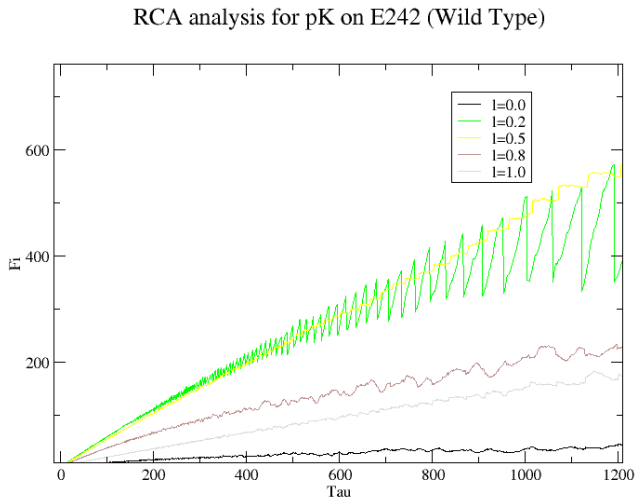
Different windows:
$\lambda = 0.0, 0.2, 0.5, 0.8, 1.0$

1.6 ns



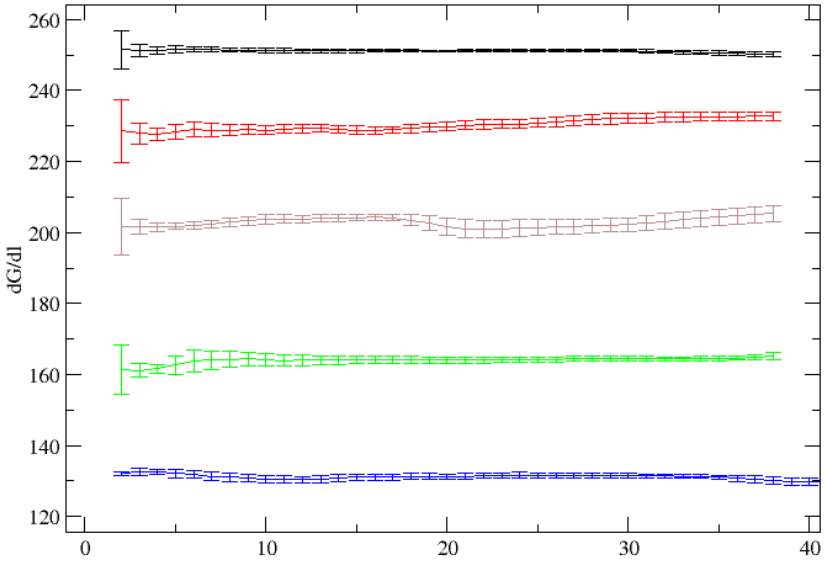
4.Glu242: pK calculations. A quantitative analysis



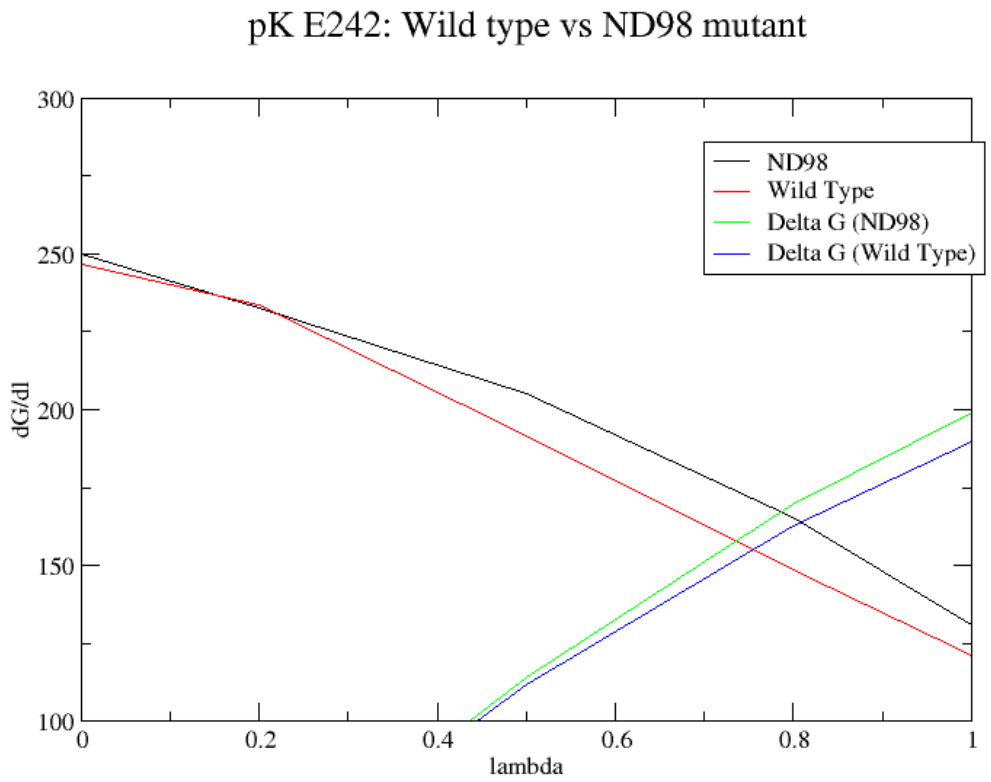


RCA: τ=40 ps

The simulation on every window is converged when ϕ converges at a given value of τ



4.Glu242: pK calculations. A quantitative analysis



Deprotonated Glu242 makes the difference

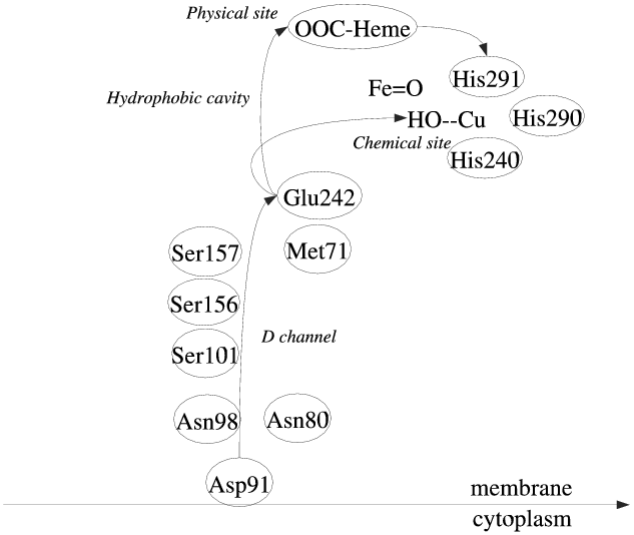
$\Delta G(WT)=189.7$kcal/mol $\Delta\Delta G=9.5$ kcal/mol

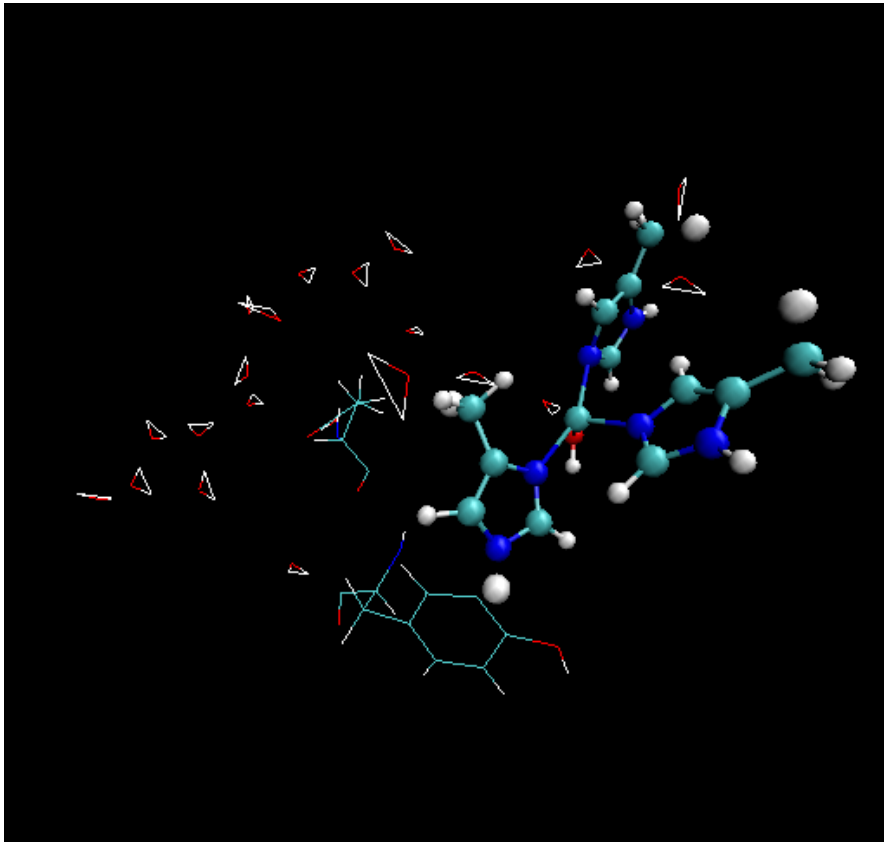
$\Delta G(ND98)=199.2$kcal/mol $\Delta pK=6.8$ (exp. 1.6)

5.His291: coupling between physical and chemical site?

Pomes vs Stuchebrukhov

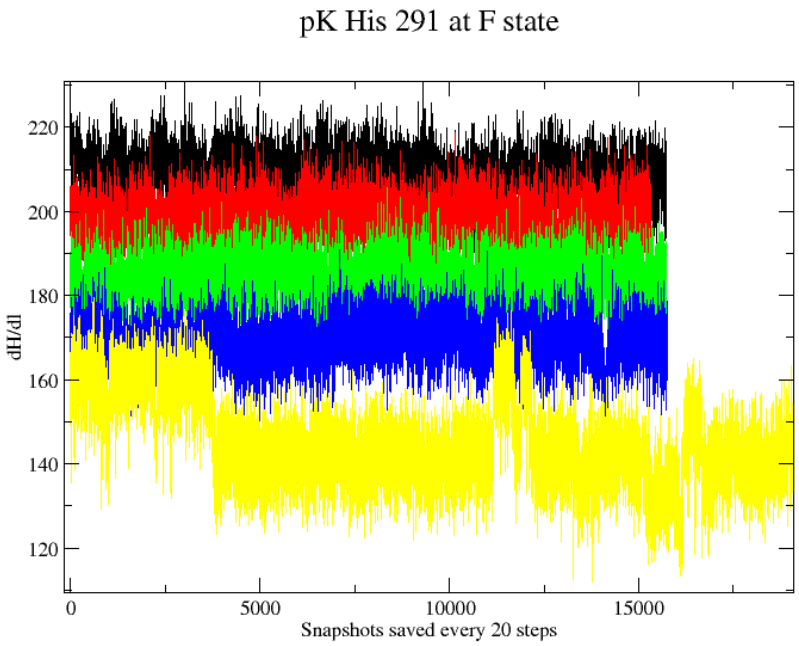
Stuchebrukhov: His291 has a modulated pK depending on the metal ligation. It's responsible for the pumping.
Pomes: His291 is always protonated and its pK doesn't change remarkably at different ligation states of Cu

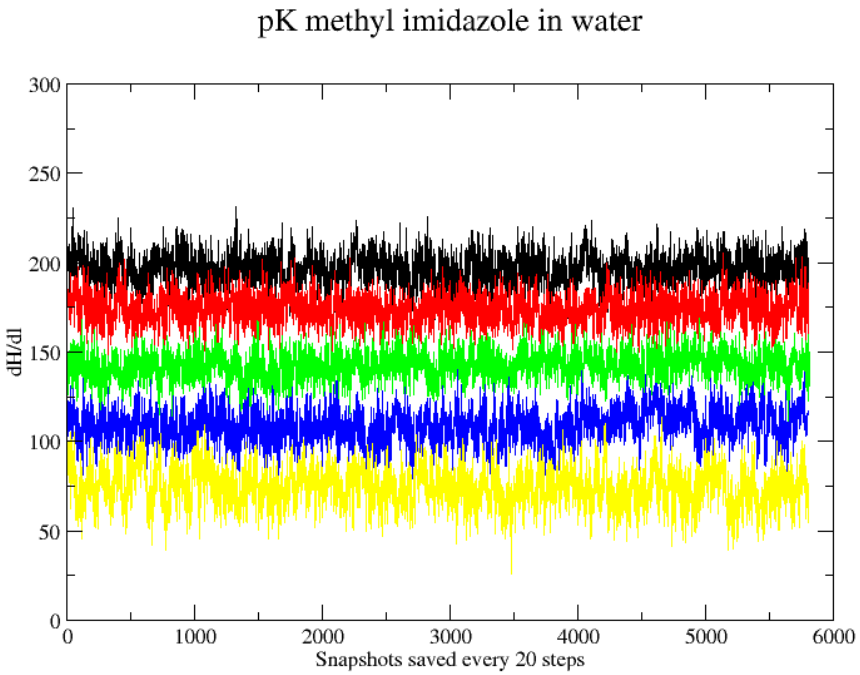




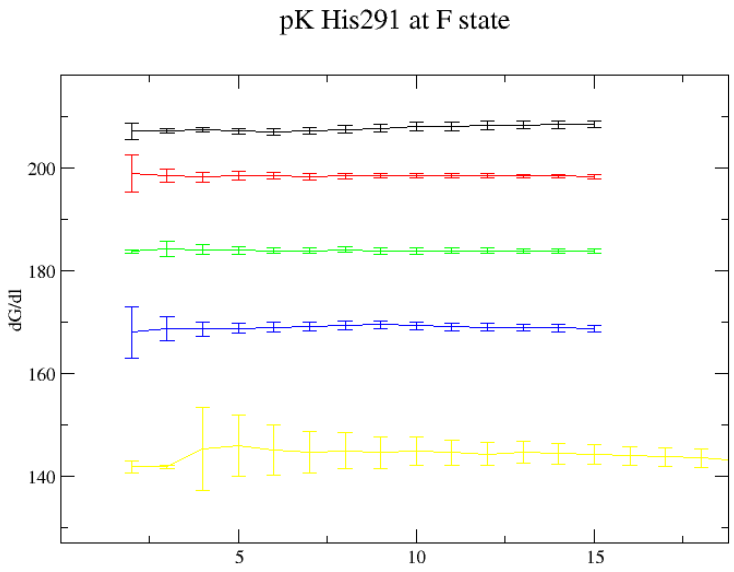
We will compute the pK of His291
And compare it with imidazole and a copper site model in water

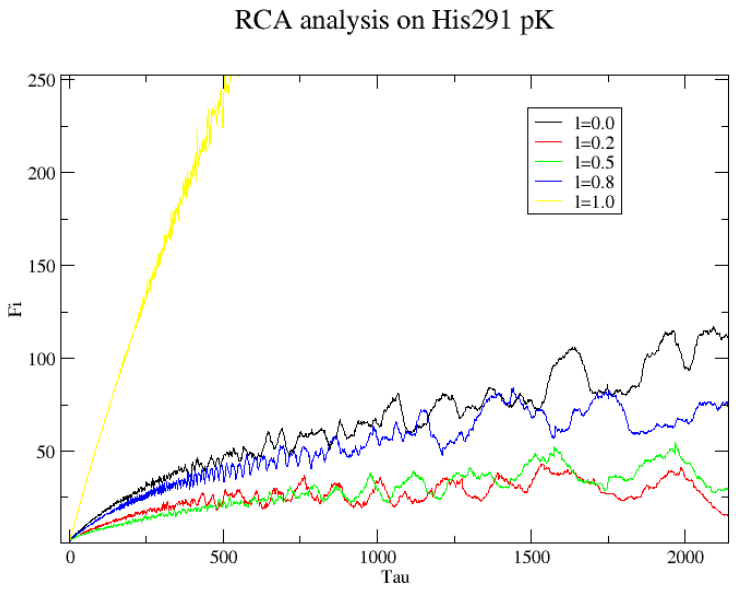
5.His291: coupling between physical and chemical site?



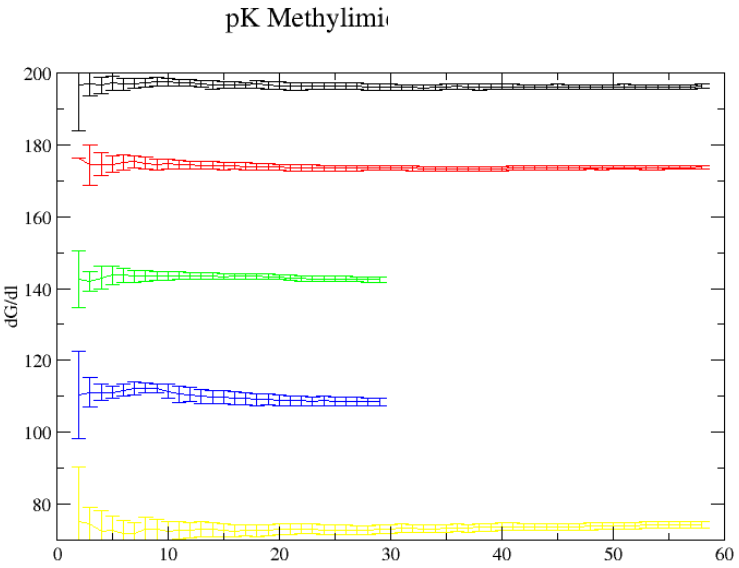


5.His291: coupling between physical and chemical site?





RCA: τ=16 ps



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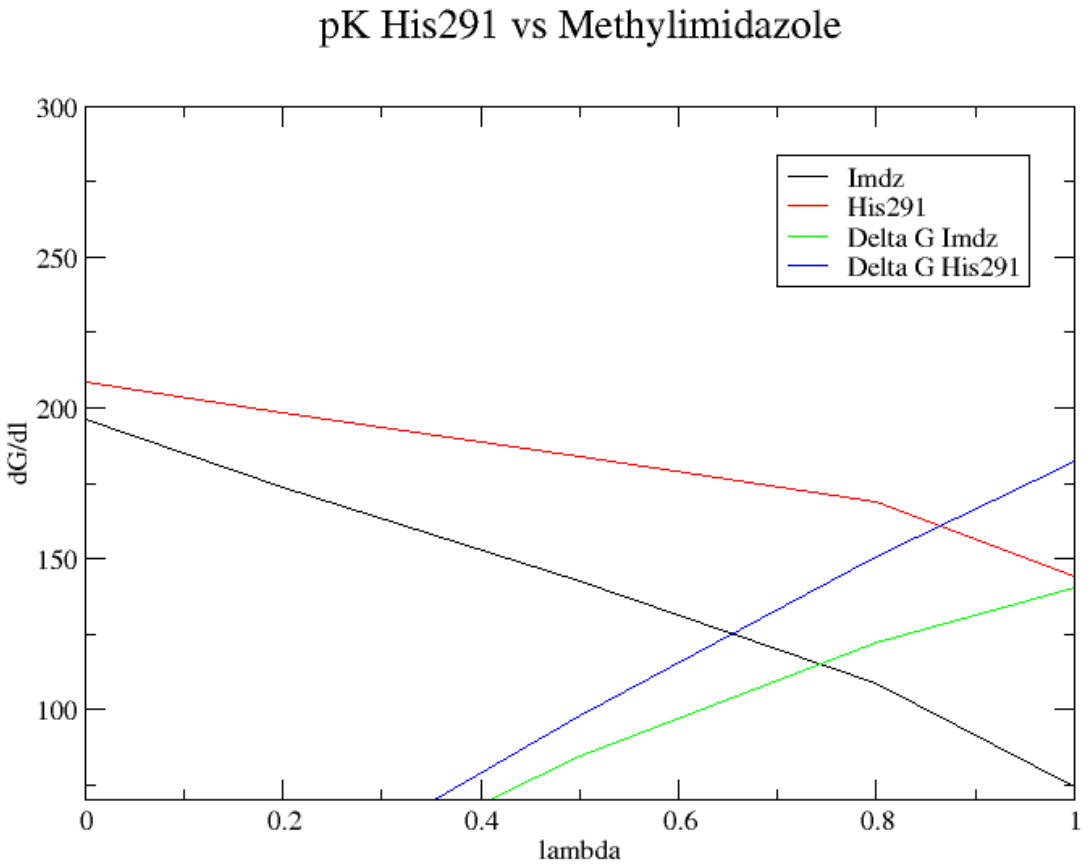

Shift too high

Imidazole is not the adequate system to compare with.
A model of the system in water has problems in SCF convergence
SCC-DFTB for Cu needs to be corrected (ADF)…*work in progress*

6. Conclusions and perspectives

*The number of waters and their mobility are essential for the modulation of pK between the clue residues. Continuum electrostatics calculations might not give an adequate picture of the system

*From plain MD simulations we can explain qualitatively how ND98 mutant has a more basic E242.
Changing the Cu ligation we could see the behaviour of wires

*The quantitative pK shift in E242 requires much more sampling

*Although we can say that His291 has a higher pK than imidazole we still cannot quantify how much. We need a better model and to correct the SCC level. Then compute pK for different Cu states.

A place to relax…

Tossa, Catalonia (Spain) Christmas 2005

4.Glu242: pK calculations. A quantitative analysis

Electrostatic analysis: LRA