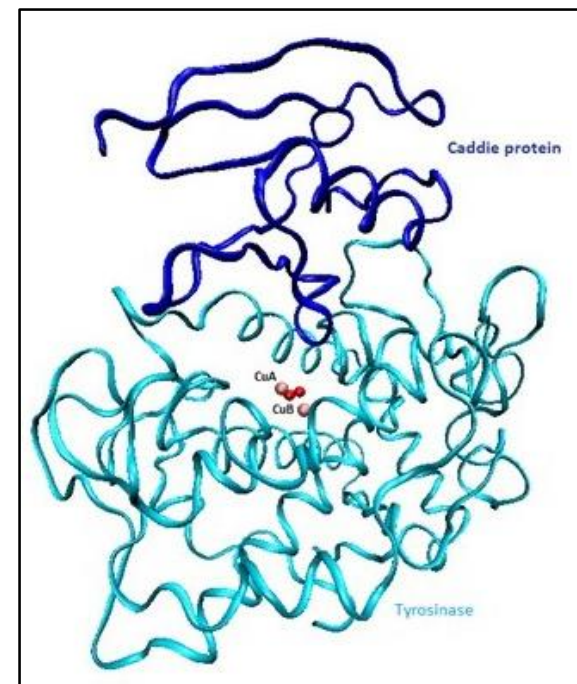# Theoretical studies of di-copper centres

Océane MANGEL

Supervisor: Dr. Hélène JAMET

Di-copper centres

- Essential systems in biology, present in enzymes

- Important for bio-inspired chemistry

- Large variety of systems from small complexes to large enzymes

- Active sites with similar properties and reactivity

      -Coupling between the two coppers

      -Redox reaction

- Usually large systems

➤ Need appropriate methods and adequate resources to study them
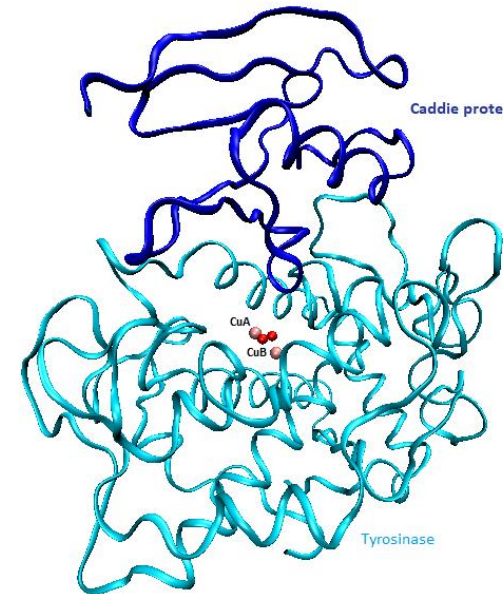
Tyrosinase, a di-copper enzyme
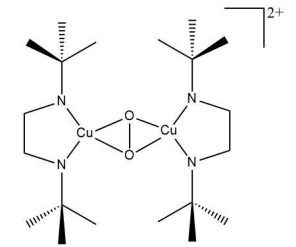
DBED complex
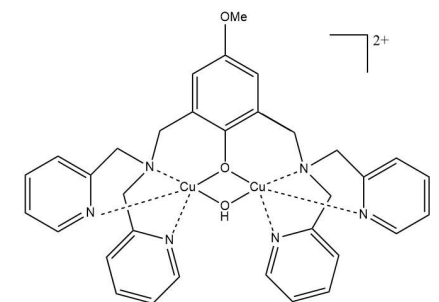
BPMP complex

Theoretical chemistry approaches

- **Molecular mechanics** (MM): classical mechanics, electrons not explicitly described, fast

- **Quantum mechanics** (QM): description of the electrons, longer calculation time

- Different QM approaches:

  -Wavefunction based methods, get expensive quickly

  -Electronic density based methods (DFT), not too expensive, requires several approximations

- Dynamic and static approaches, depending on the size of the system

Resources and softwares

Resources used:

- Ceciccluster

- DAHU cluster: use of regular and fat nodes
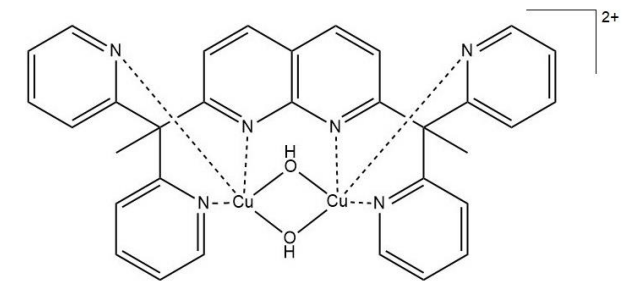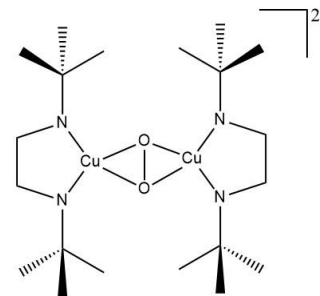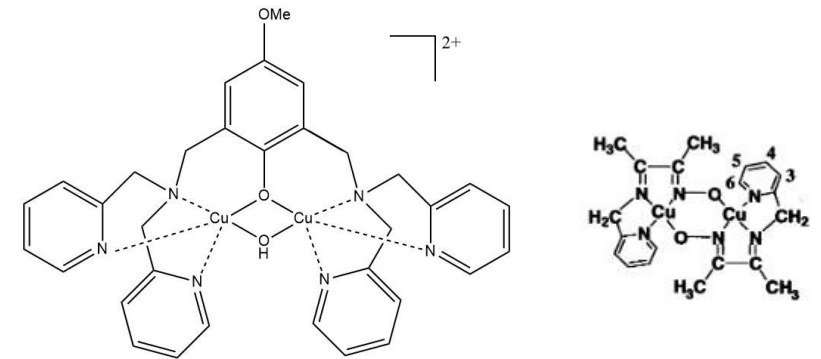
- Access to national cluster Jean-Zay

Softwares used:

- ORCA 5.0.4

- Gaussian 16

- CP2K 6.1

- ASH

- IBOView
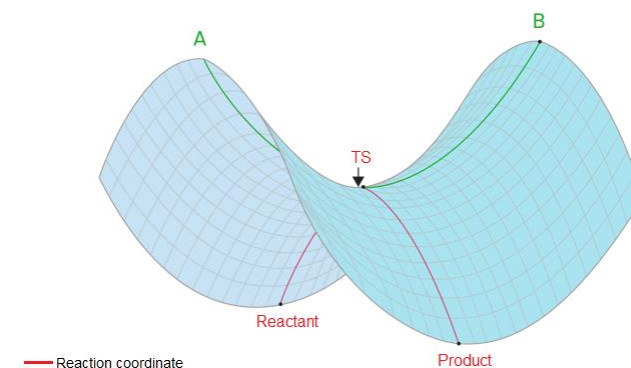
Part 1: study of di-copper complexes

- Di-copper complexes: diverse systems

- Need appropriate methodologies to study them

    -Electronic structure

    -Reactivity

    -Redox potentials

- Focus on different systems

Method of work

- Use of static QM approaches

- Analysis of electronic structure and properties of the system by DFT

- Localisation of Transition State (TS) by DFT

- IRC (Intrinsic reaction coordinate): Method to connect the TS to the reactant and the product by following the reaction coordinate on the potential energy surface

- IBO (Intrinsic Bonding Orbital): Method of defining localised orbitals allowing to follow the electrons along the reaction path

Knizia G. , Chem. Theory Comput. 2013, 9 (11), 4834–4843.
Knizia G. , and Klein J. E. M. N. Angew. Chem. Int. Ed. 2015, 54 (18), 5518–5522

Method of work: DFT

- DFT approaches requires approximations

- Exchange and correlation functional to chose accordingly
    - Benchmarks: around 15 possibilities tested for each calculation type

- Description of orbitals using basis sets

- Need proper calibration of the methodology
    - Optimisation: TPSSH/CNOH:def2SVP, Cu:def2TZVP
    - Redox potential calculations: M11L/def2TZVP

- Take both accuracy and calculation time into account
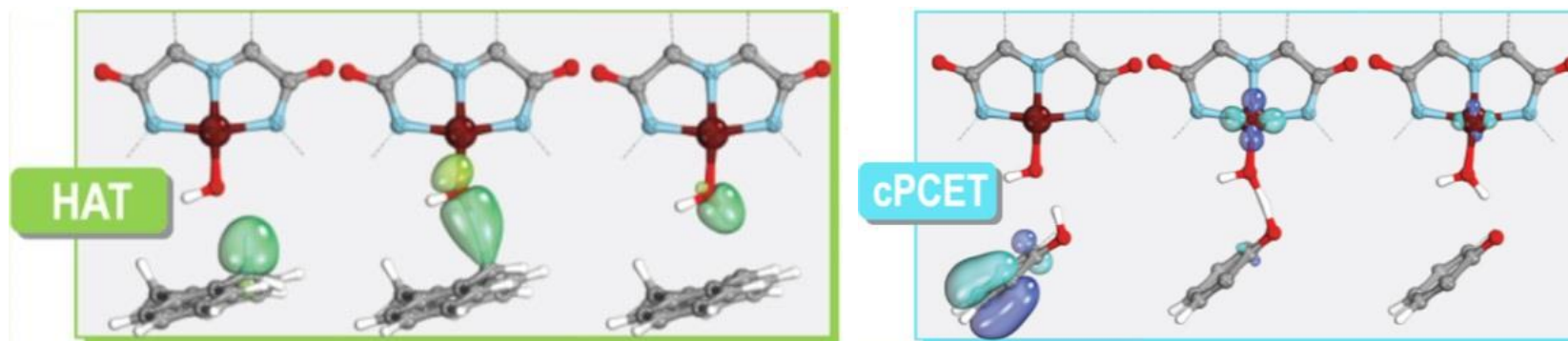
Method of work: IBO

- Two mechanisms possible for C-H activation

    -HAT: H Atom Transfer

    -cPCET: concerted Proton Coupled Electron Transfer
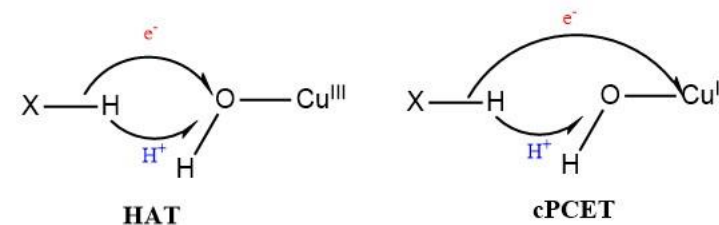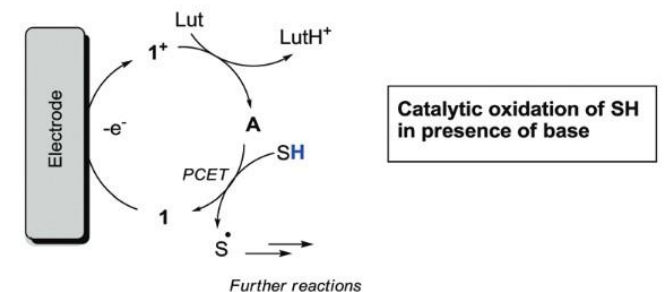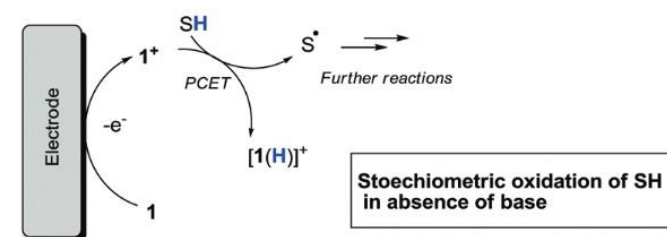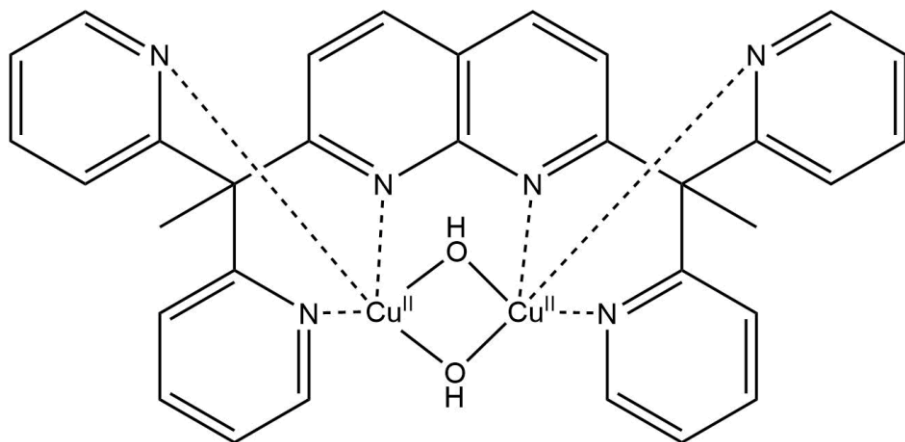

- IBO visualisation of the mechanism

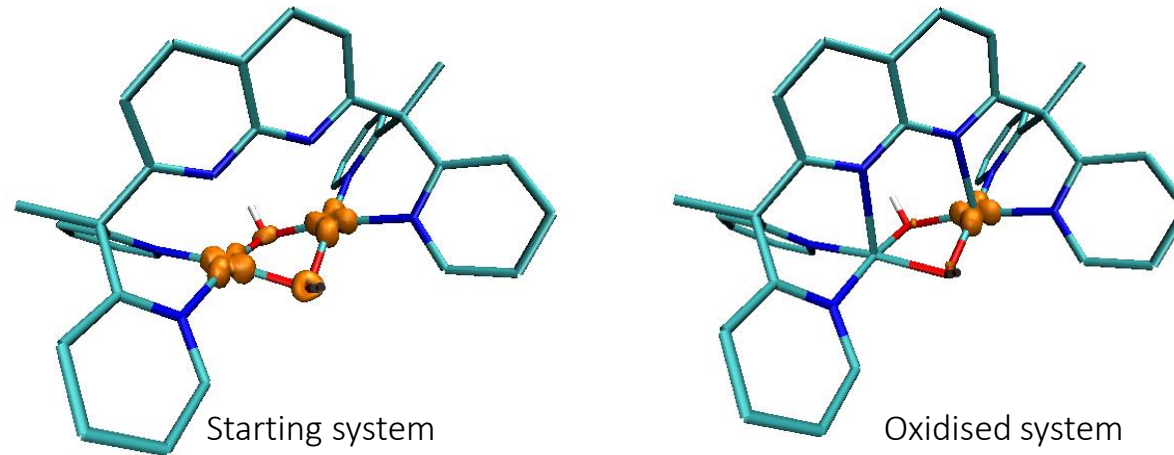Taken from: Mandal M. *et al*, J. Am. Chem. Soc. 2019, (141), 17236–17244

The DPEN complex

- Cu^{II}-Cu^{II} system, will form a Cu^{II}-Cu^{III} upon electrolysis

- Cu^{II}-Cu^{III} system able to activate the C-H bond

- Redox potential: E^0 = 1.12V vs FC

- Importance of the reactivity of the system

Isaac, J *et al*, Inorg. Chem. 2016, 55, 17, 8263–8266
Isaac, J *et al,* Chem. Commun., 2019, 55, 12711--12714 | 12711

System in absence of a base
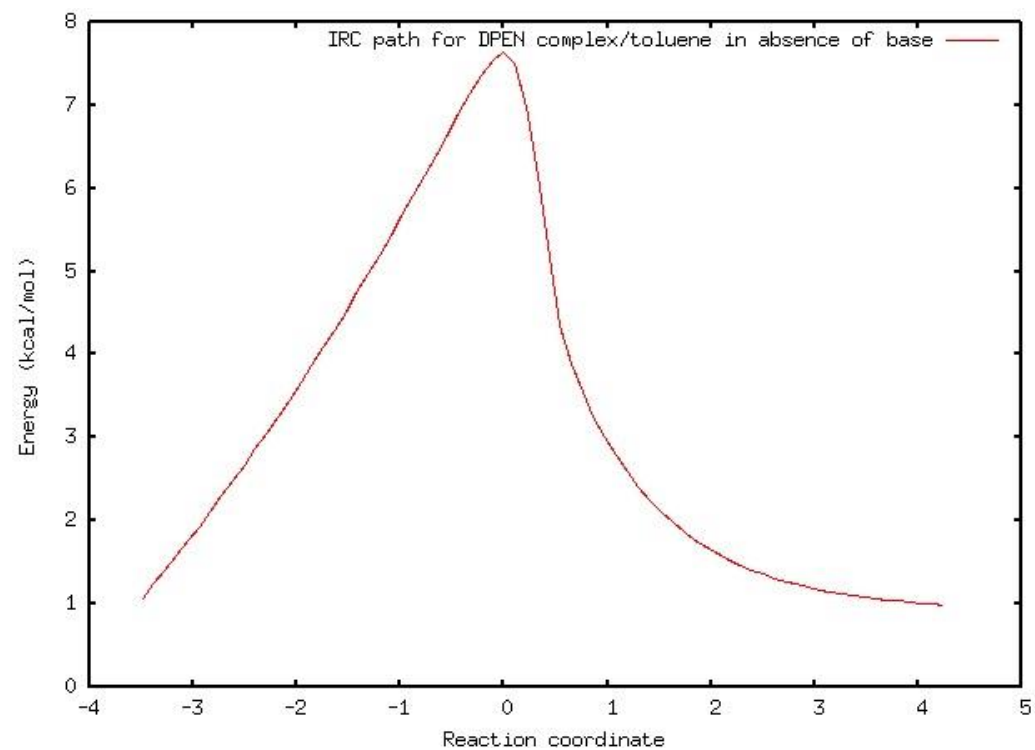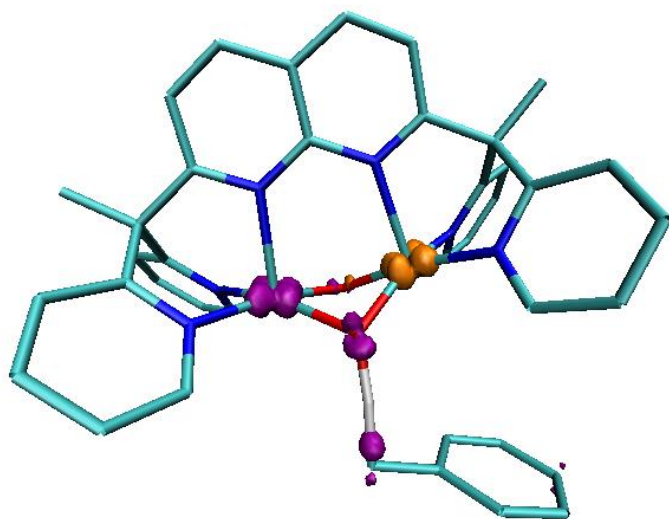
Starting system

Oxidised system

Spin density plots for the two states

➢ Oxidation to a CuII-CuIII as experimentally described
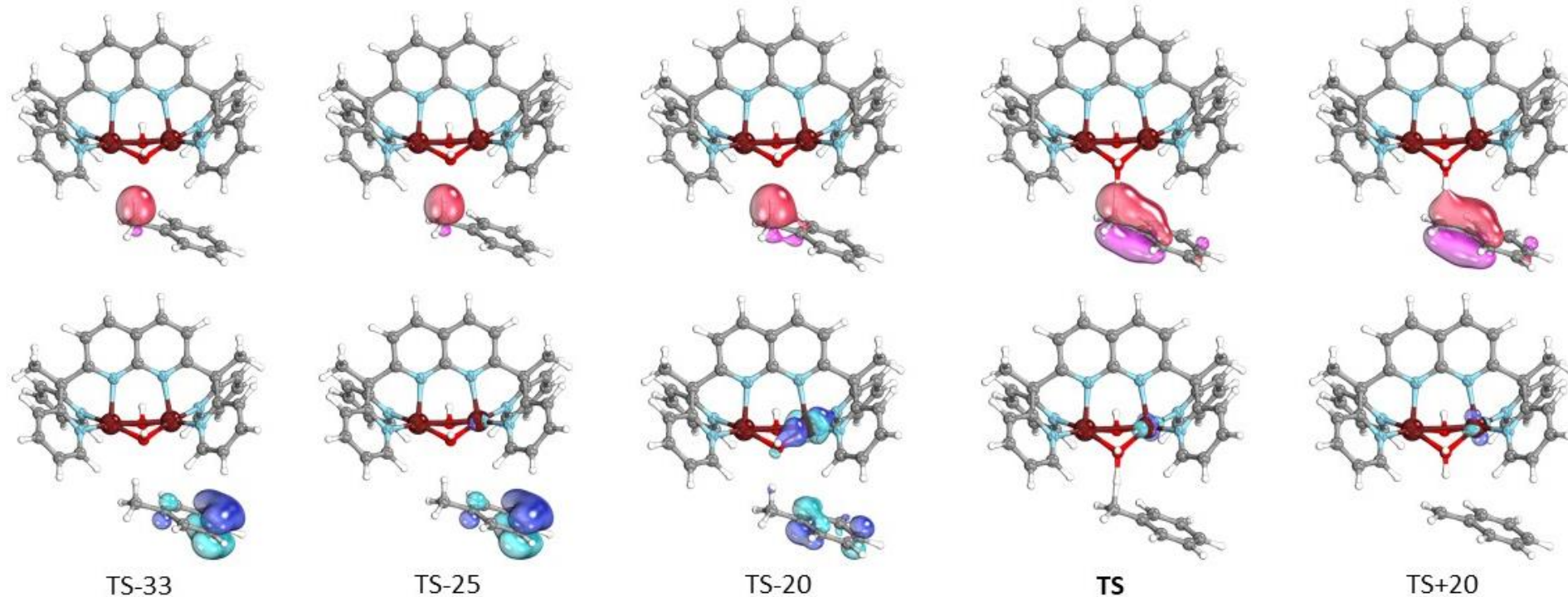   ➢ Good description of the system

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Transition state

- Toluene substrate

IRC path for DPEN complex/toluene in absence of base

Energy barrier (recalculated to take the approach of the substrate into account): 26 kcal/mol

Reaction mechanism

TS-33   TS-25   TS-20   **TS**   TS+20

➢ Proton and electron transfered to two different sites at different times
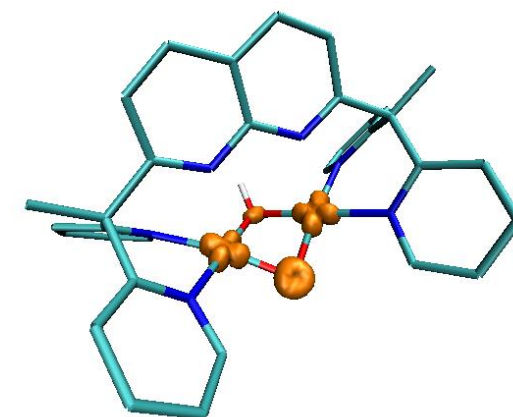➢ Oxidatively asynchonous cPCET mechanism

System in presence of a base

- Small energy difference between the quartet Cu^{II}-$O°$-Cu^{II} and the doublet Cu^{II}-Cu^{III} for the optimised system, smaller than the accuracy of the method

➢ Calculation of the stability of different states with different functionals

Functional	TPSSH	PBE0	B3LYP	MN15	CAM-B3LYP
Cu^{III}-Cu^{II} doublet	-1.2	4.5	2.1	2.3	5.5
Quartet (Cu^{II}-$O°$-Cu^{II})	0.0	0.0	0.0	0.0	0.0

Single point energies for the different functionals taking the quartet as a reference

Spin density plot for the Cu^{II}-$O°$-Cu^{II} system

- Different results depending on the functionals

➢ Hard to conclude, need to use another approach

CASSCF approach

- Multiconfigurational method, selection of an active space of orbitals

- Reasonable calculation time but energies not always good

- Need corrections, e.g. DDCI3

➤ Corrections expensive in calculation time and memory (requires fat nodes, for more than 48h in some cases)

Level of theory	CASSCF CAS(3,3)	CASSCF CAS(7,5)	CASSCF-NEVPT2	CASSCF-DDCI3
Cu^{III}-Cu^{II} doublet	10.5	10.7	-4.4	-30.8
Quartet (Cu^{II}-$O°$-Cu^{II})	0.0	0.0	0.0	0.0

Energies for the different levels of theory taking the quartet as a reference (kcal/mol)

- CASSCF-DDCI3 in favour of a Cu^{II}-Cu^{III} configuration

➤ DLPNO-CCSD(T) calculations will be considered (requires large memory as well)
➤ Both paths will be investigated

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Reaction mechanism: Cu^{II}-$O°$-Cu^{II}

- Energy barrier: 4kcal/mol

TS-60   TS-20   TS   TS+3   TS+60
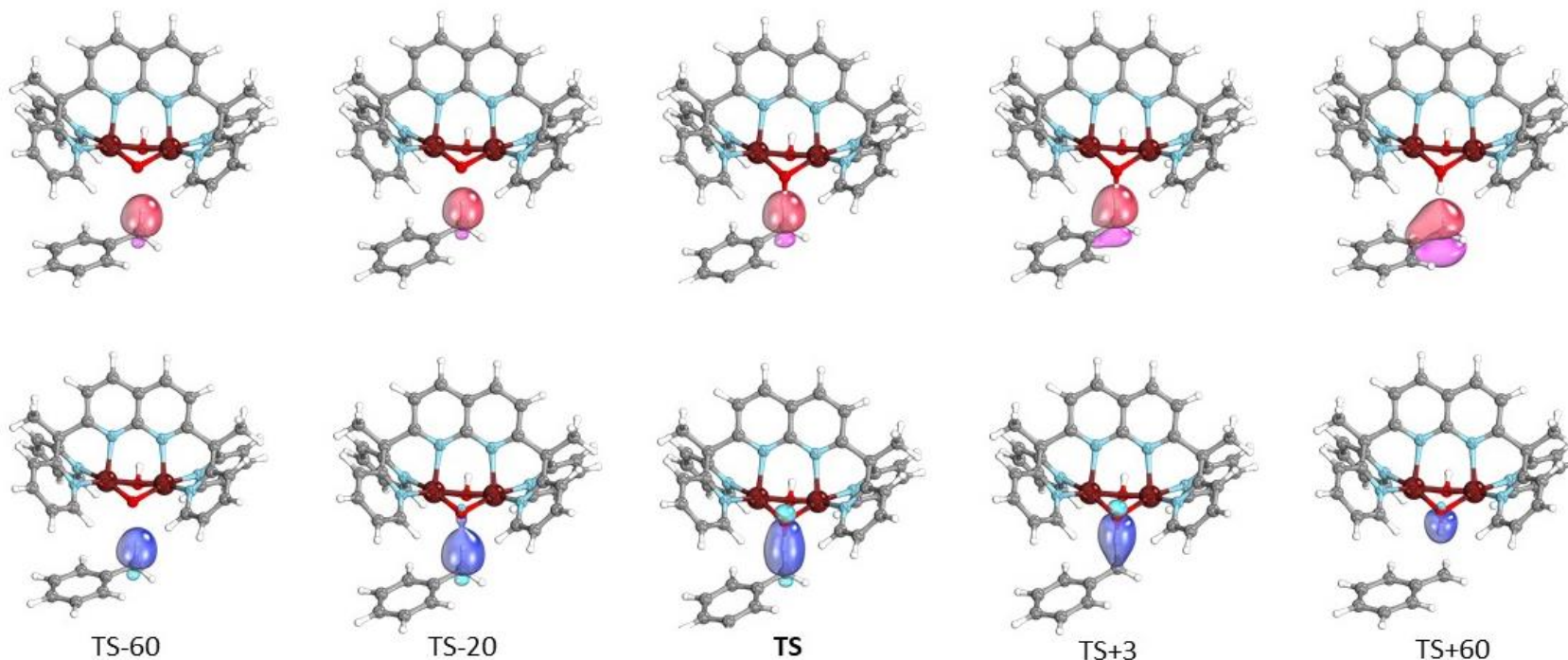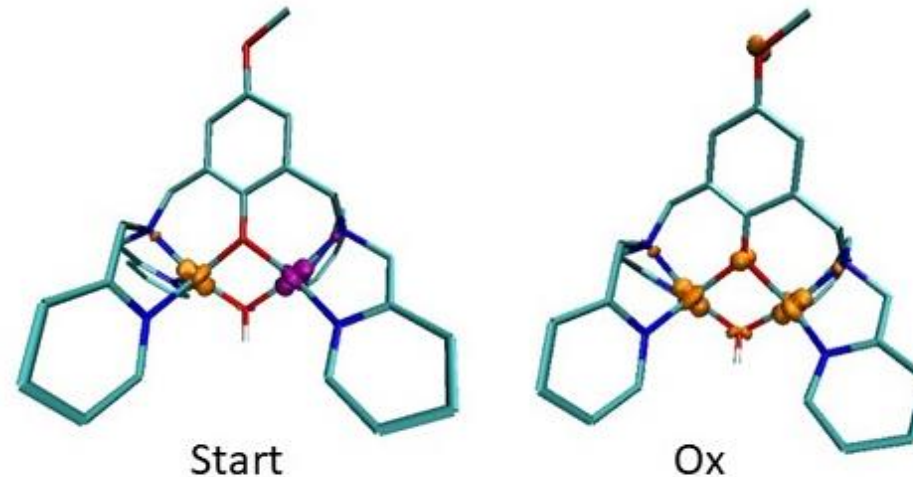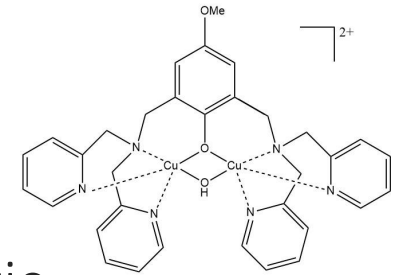
➢ Electron and proton transferred to the same site, HAT mechanism
➢ Cu^{II}-Cu^{III} system currently studied

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The OMe-BPMP system

- Another complex for C-H activation when oxidised

- Starting system: antiferromagnetic coupling

- Use of a Broken Symmetry approach to model the antiferromagnetic coupling
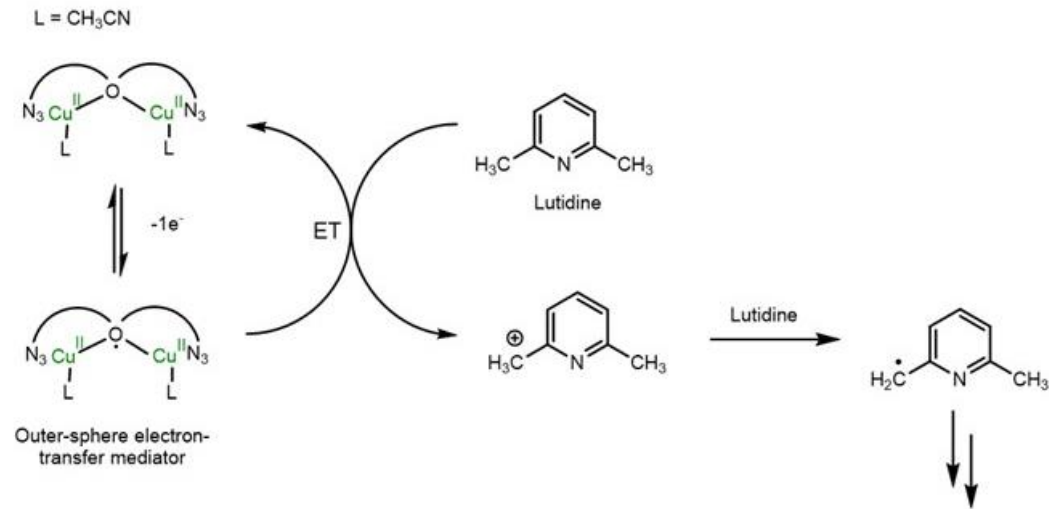
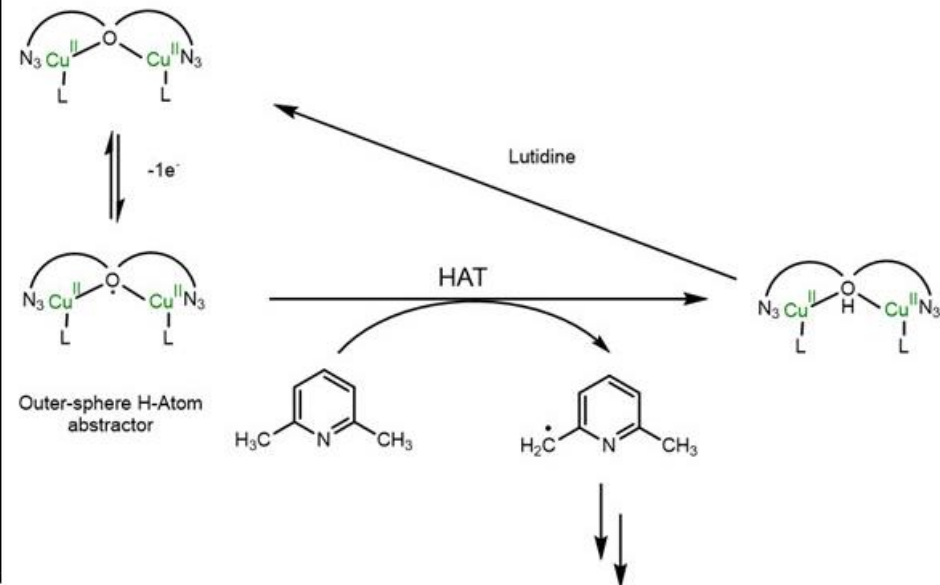- Oxidation on the ligand

Start   Ox

Reaction mechanism

- Two proposed mechanisms
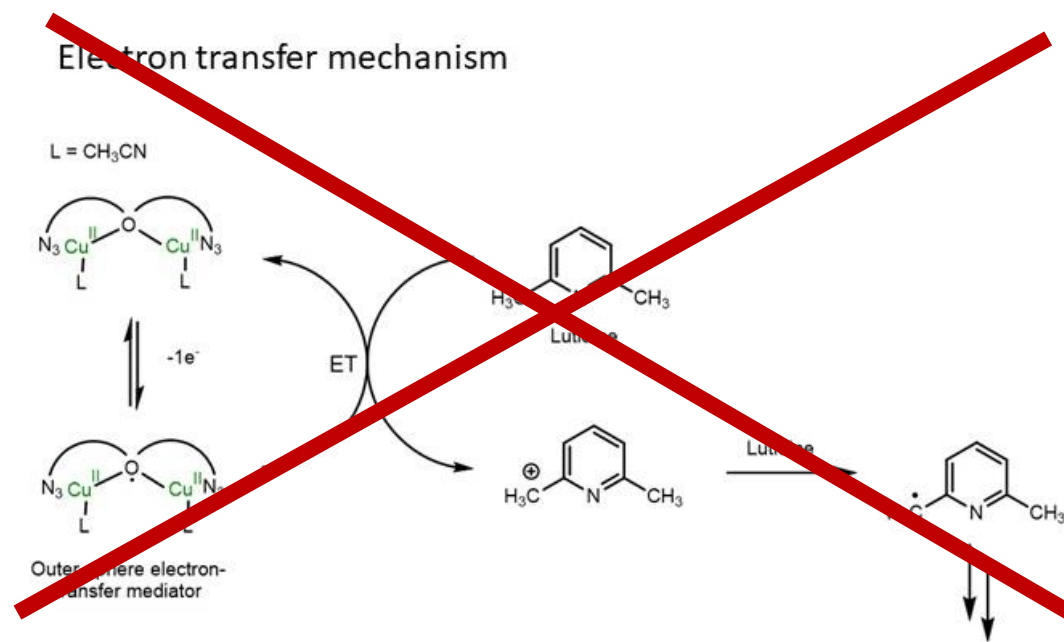
Reaction mechanism

Electron transfer mechanism

- Calculated redox potentials of the systems

- BPMP-OMe: 0.53V vs FC$^+$/FC
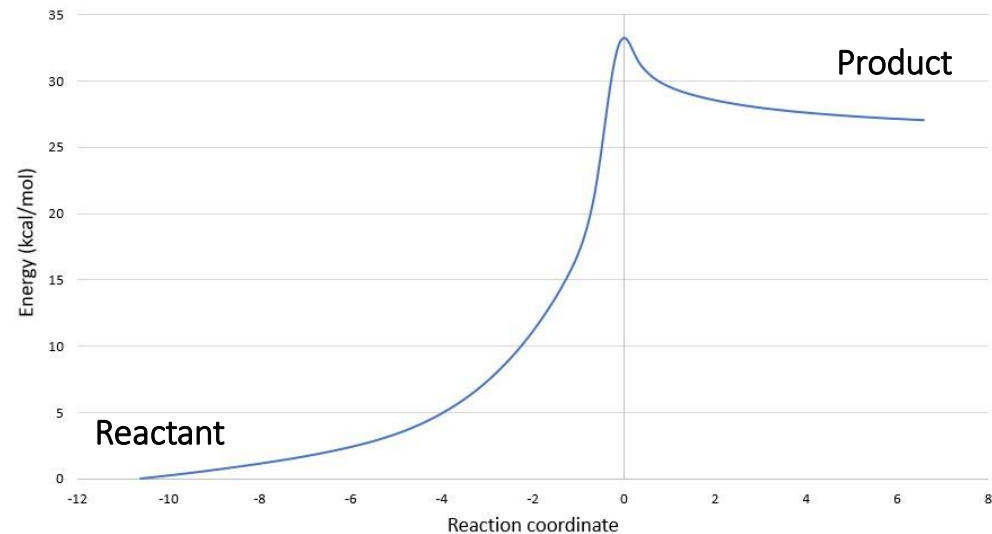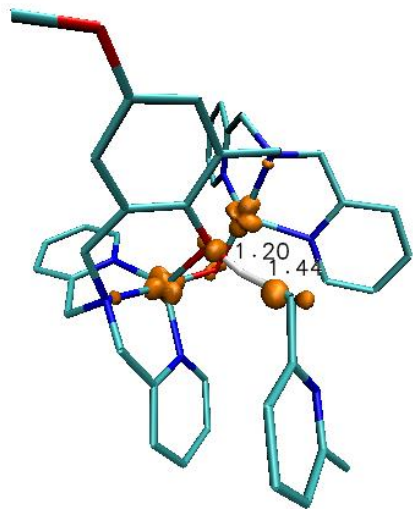- Lutidine: 1.75 V vs FC$^+$/FC

➢ Electron transfer not favourable, HAT mechanism must be considered

Reaction mechanism

H atom transfer mechanism

- BPMP-OMe system: TS localised, energy barrier 33 kcal/mol

IRC path for the BPMP-OMe reaction mechanism

➢ Reaction possible, will need an IBO analysis for proper characterisation of the mechanism

Part 1: Conclusion

- Appropriate method for the description of the reactivity

➢ Will be extended to similar systems, e.g. OMe-BPMP with a different bridging group

- Methodology for the description of the electronic structure not always appropriate

➢ Higher level of theory often requires long calculation time and a lot of memory

➢ Will be atempted on smaller models of our systems
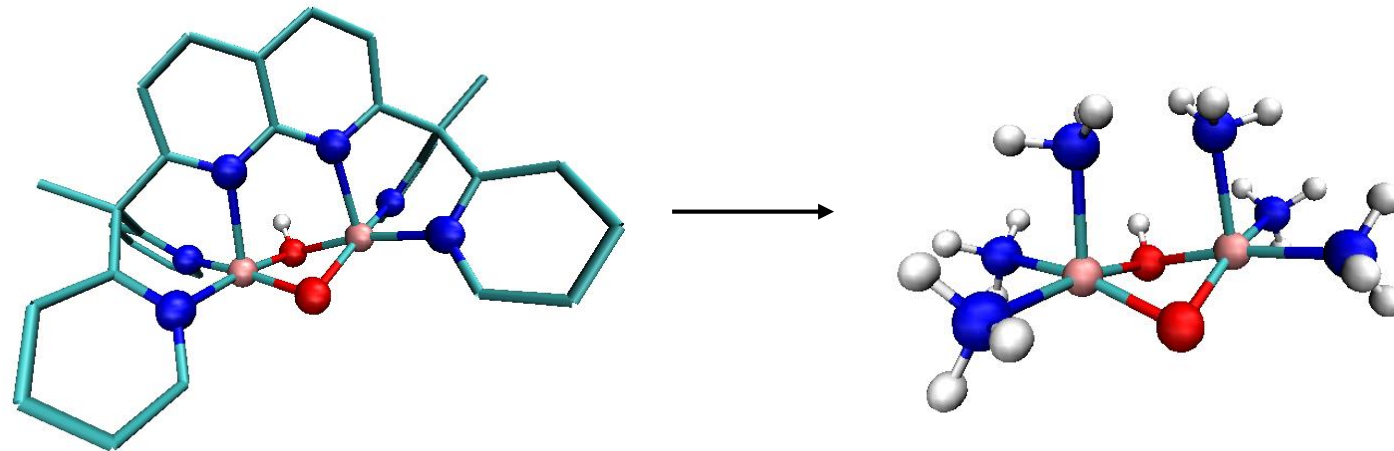
Part 2: study of the tyrosinase mechanism

• Tyrosinase: Metalloenzyme with a di-copper centre that participates in the biosynthesis of melanin by converting the tyrosine into dopaquinone

• Crystallographic study performed

Caddie protein
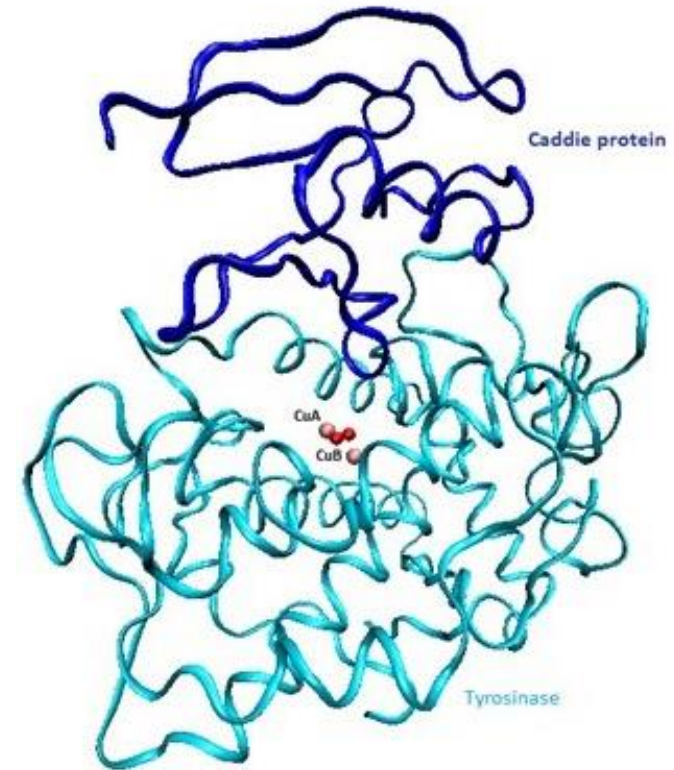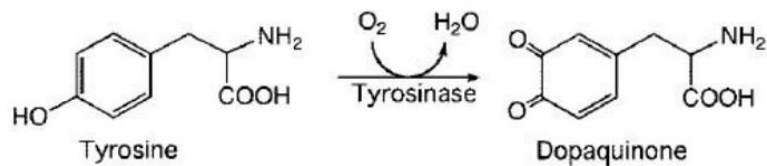
Tyrosinase

• Bacterial (Streptomyces) tyrosinase in complex with a caddie protein

Methodology

- System too large to model the system using only QM approaches

- MM approaches do not allow the description of the reactivity

- Use of mixed QM/MM approaches
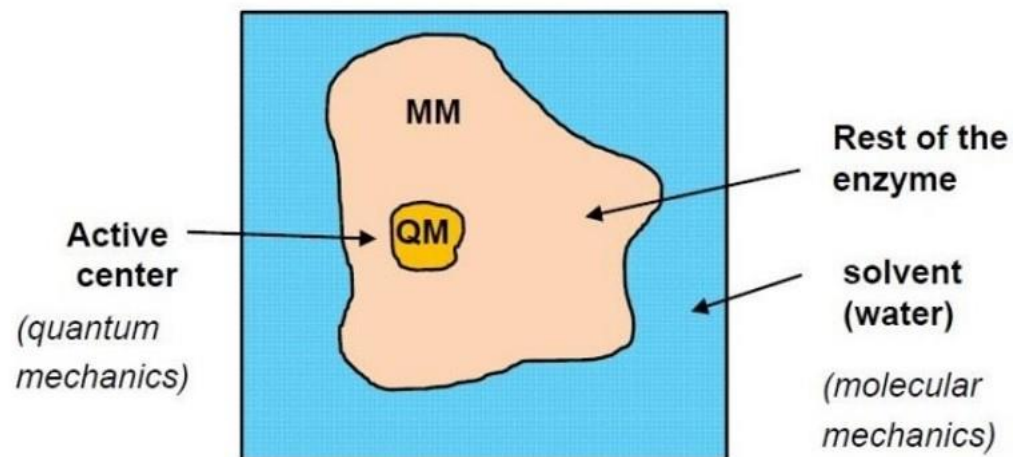
Methodology

- Use of dynamic approaches

- QM/MM dynamics (simulation): cannot see rare events (reactivity) in a reasonable time

- Metadynamics approach: enhanced sampling of the energy surface by adding repulsive gaussians on a set of collective variables (CV)

- Thermodynamic integration (TI) to get an estimation of the energy barrier
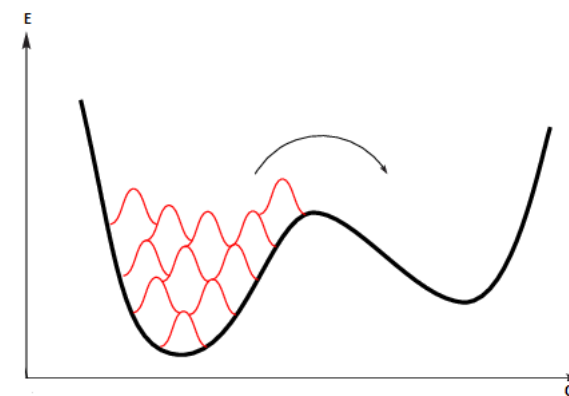
Methodology

- Long calculation time: between 7 and 30 days for a dynamic

- TI: requires several dynamics (~8) -> 8 jobs for around a week

- Use of 128 cores per job

➤ The restart system (Pierre Girard)

    -Allow to resubmit automatically jobs using the OAR idempotent system

    -Takes results from the previous calculation into account

    - Restart management: define what is the important limit (calculation time, number of steps…)

Methodology

- Start with crystallographic structure

- MM equilibration of the system

- Active site selected as QM site, addition of other amino acids when necessary

- QM/MM equilibration of the system

- Metadynamic exploration of the reaction path

- Estimation of the energy barrier by thermodynamic integration (TI)
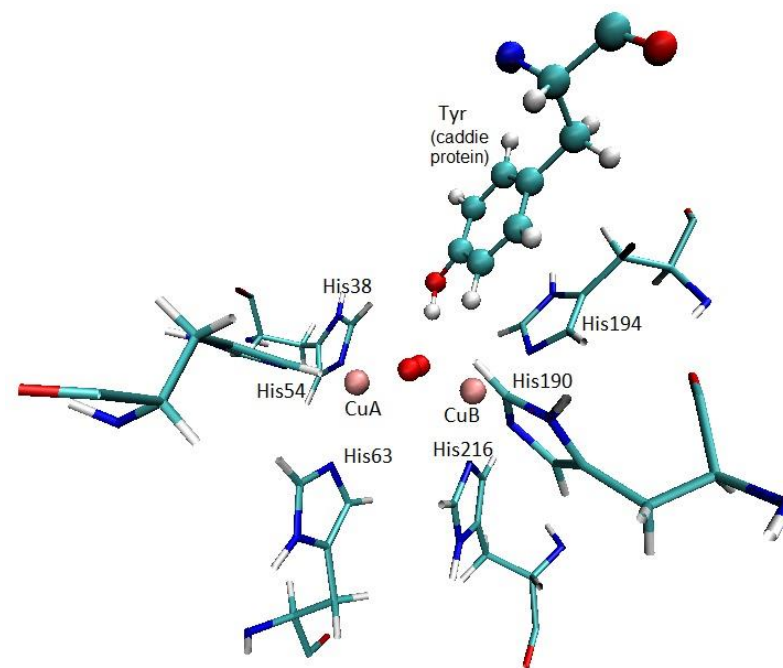

Active site of the tyrosinase

Methodology

- Start with crystallographic structure

- MM equilibration of the system

- Active site selected as QM site, addition of other amino acids when necessary

- QM/MM equilibration of the system

- Metadynamic exploration of the reaction path

- Estimation of the energy barrier by thermodynamic integration (TI)
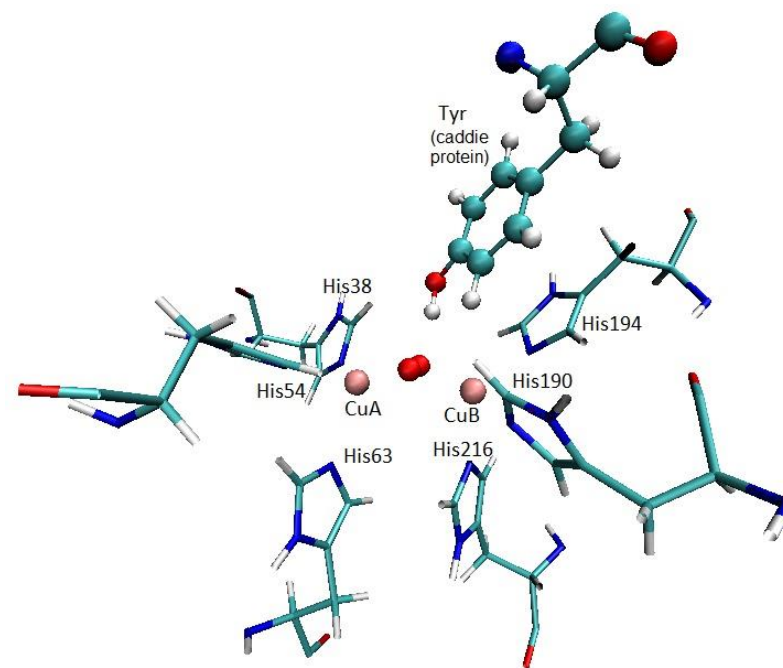

Active site of the tyrosinase
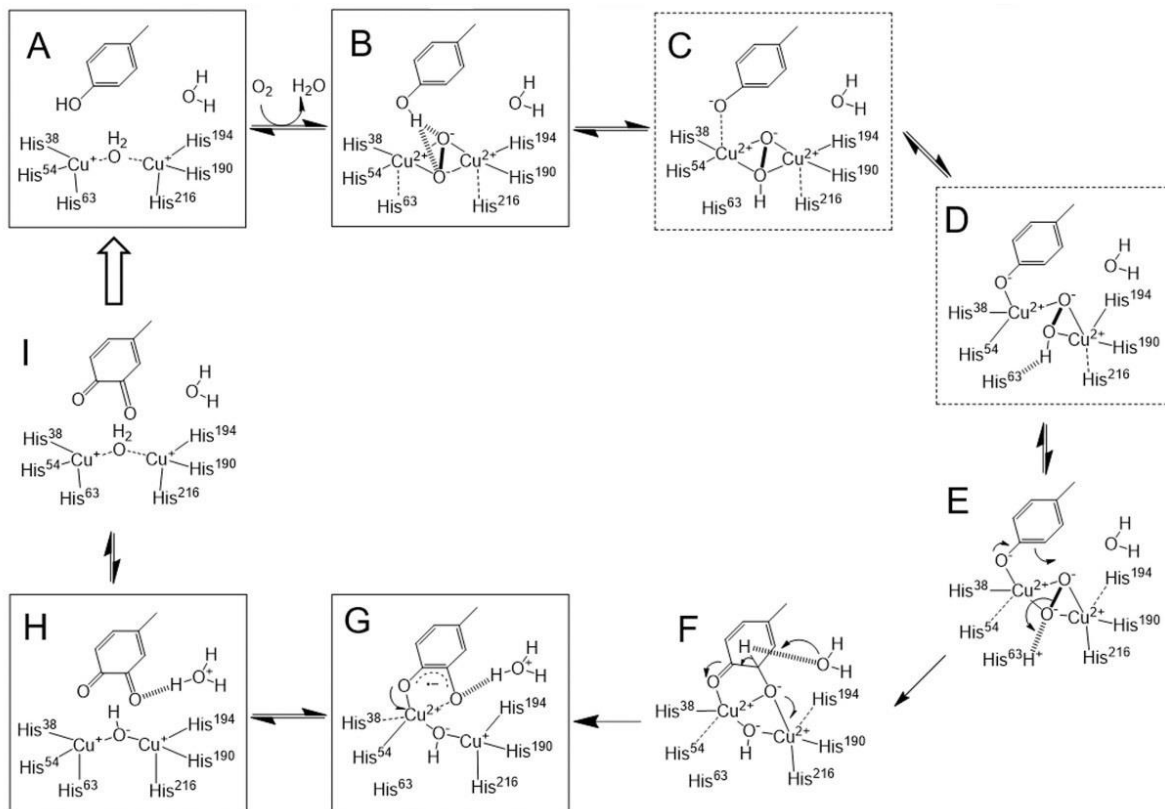
- 1st step: deprotonation of tyrosine

- 2nd step: movement of the copper and coordination with the tyrosine

- 3rd step: coordination of one of the oxygens to the ortho carbon of the tyrosine and opening of the peroxo group

- 4th step: release of dopaquinone
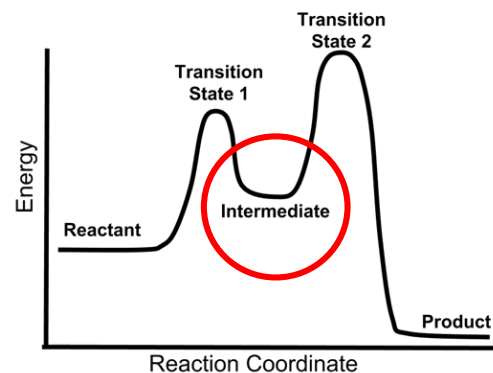
Matoba Y. *et al*, PLoS Biol. 2018, 16, e3000077

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First step: deprotonation of the tyrosine

- Calculated by metadynamics
- Addition of a serine in the active site
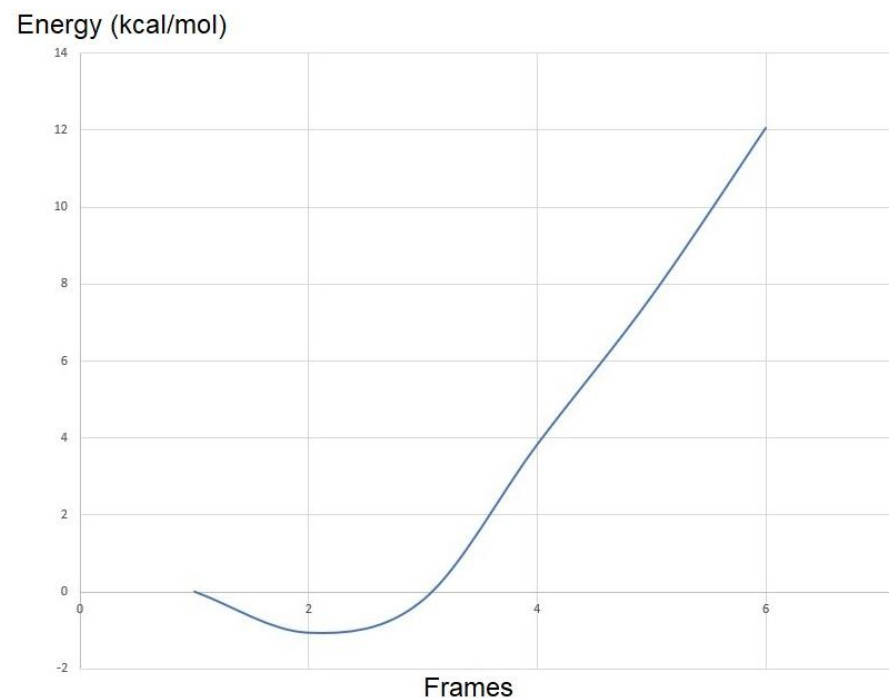- Leads to a spontaneous opening of the peroxo group
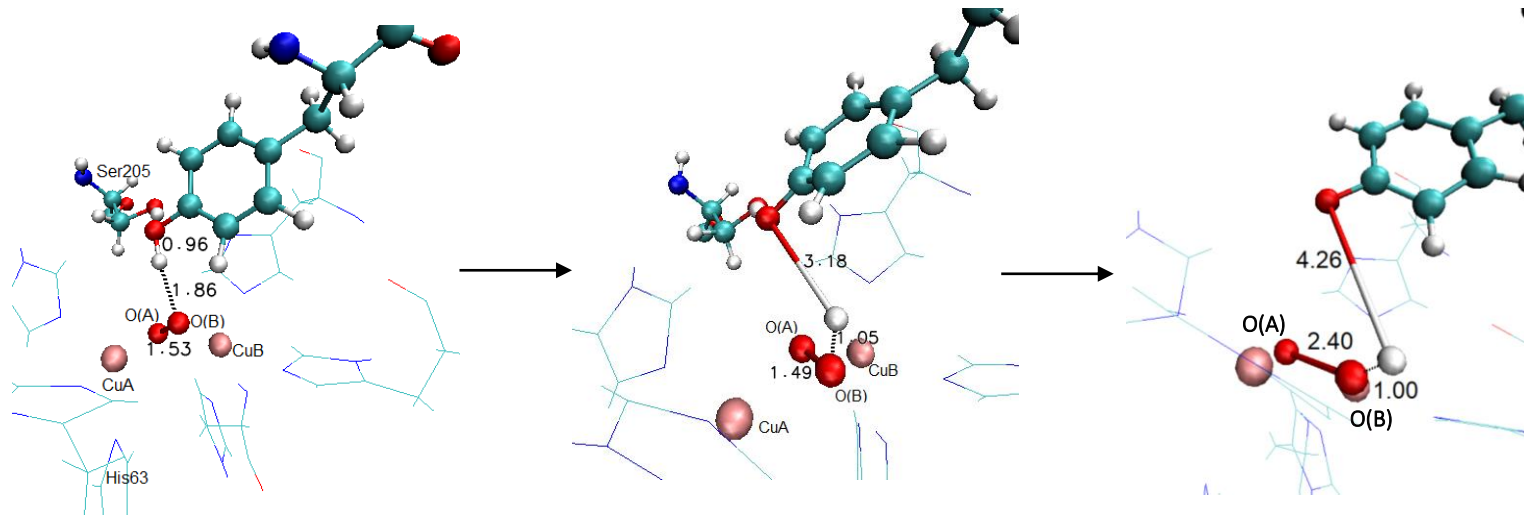
- One or two step mechanism?
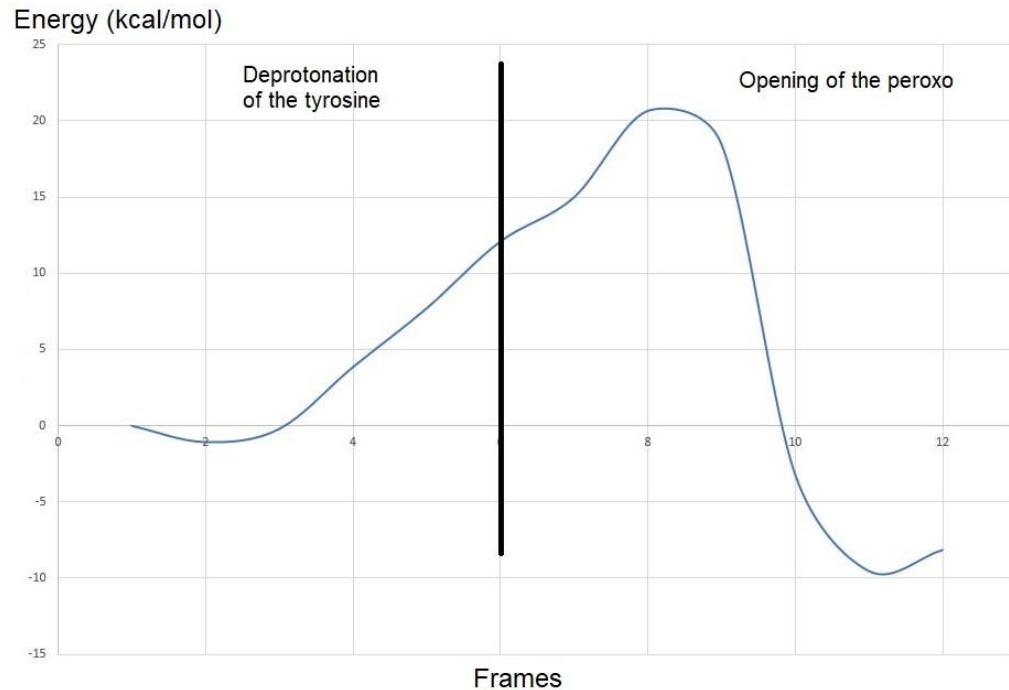
Thermodynamics integration

Expected reaction path for a two reaction step mechanism

- Thermodynamic integration on the first step, deprotonation on the O(B)

- No stabilisation, no intermediate found

- One reaction step mechanism?

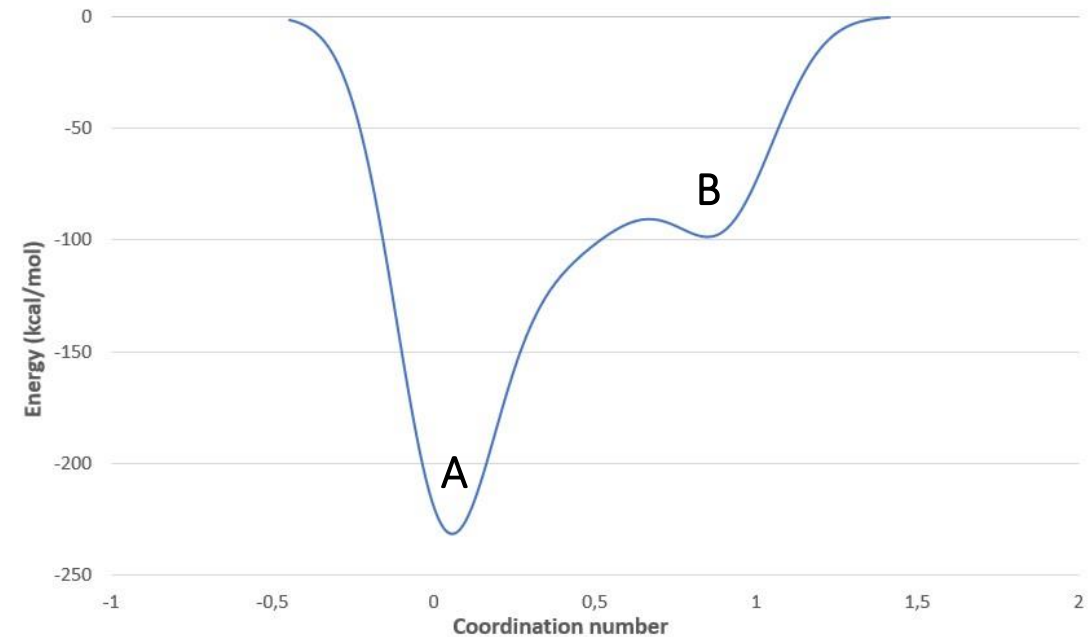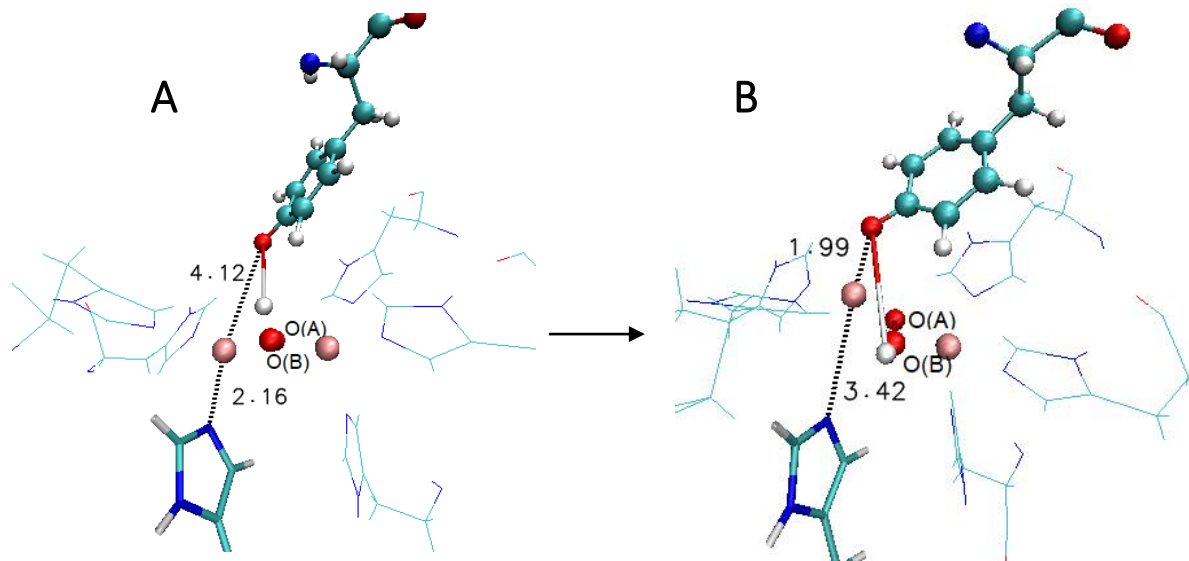Reaction path for the first step of the mechanism

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Thermodynamics integration

- When combining the two, actual reaction path

- TS upon the opening of the peroxo

- Energy barrier around 20 kcal/mol
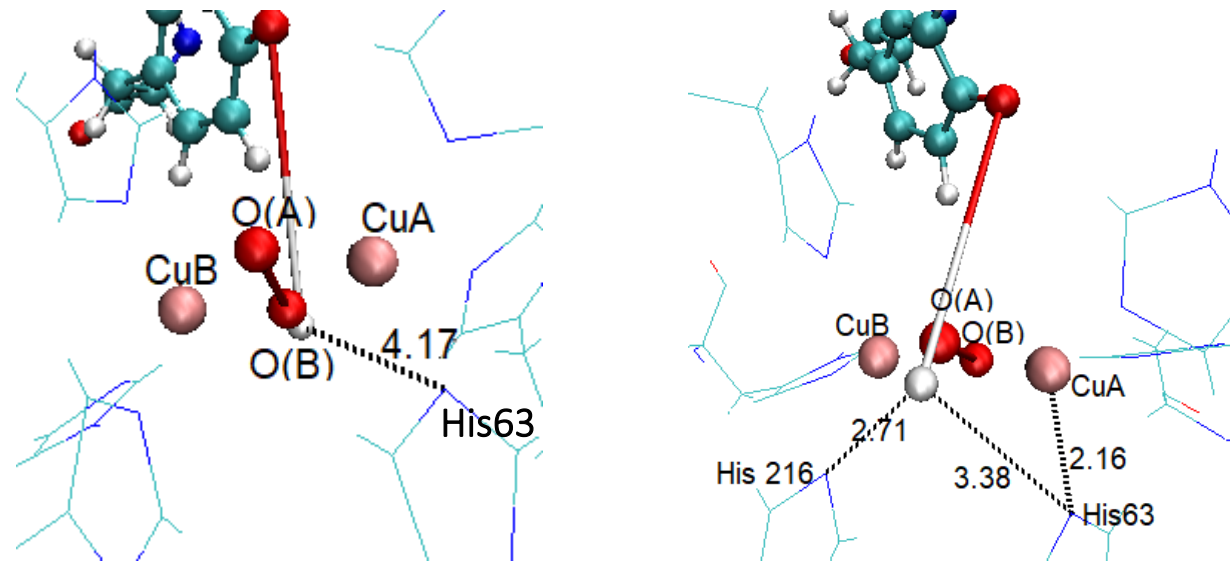
30

Second step: movement of the copper

- Metadynamic study
- CV chosen: coordination between CuA and O (tyr)

> Large energy barrier, do not retrieve the flexibility of the copper

Question of the environment

- Two possibilities for the protonation on the peroxo: O(A) or O(B)

- On O(A), more influence of the environment with the proximity of the His63
- Could have an influence on the energy barrier for the movement of the copper

Protonation on the O(A)

- TI performed on the two steps

- Constraints added on the first TI to prevent a breaking of the coupling between the peroxo group and the two coppers
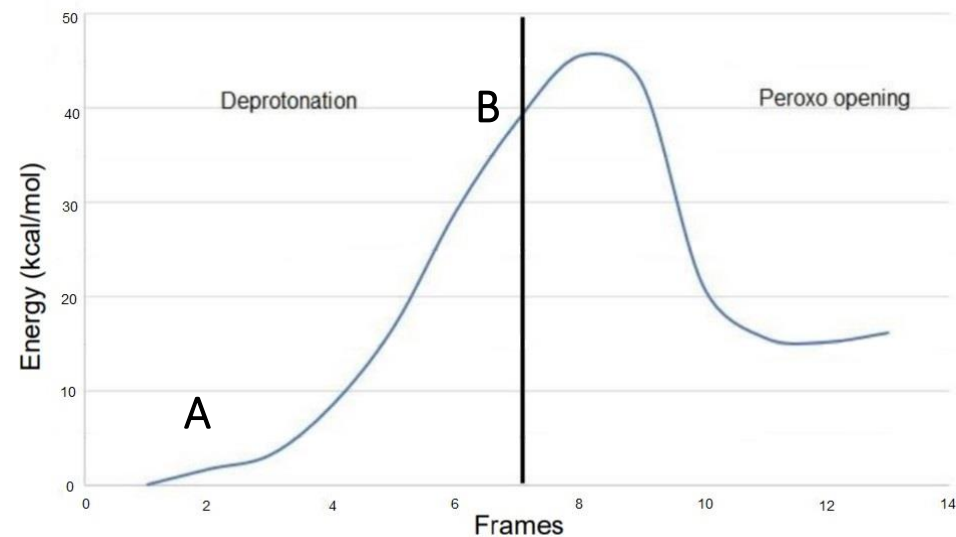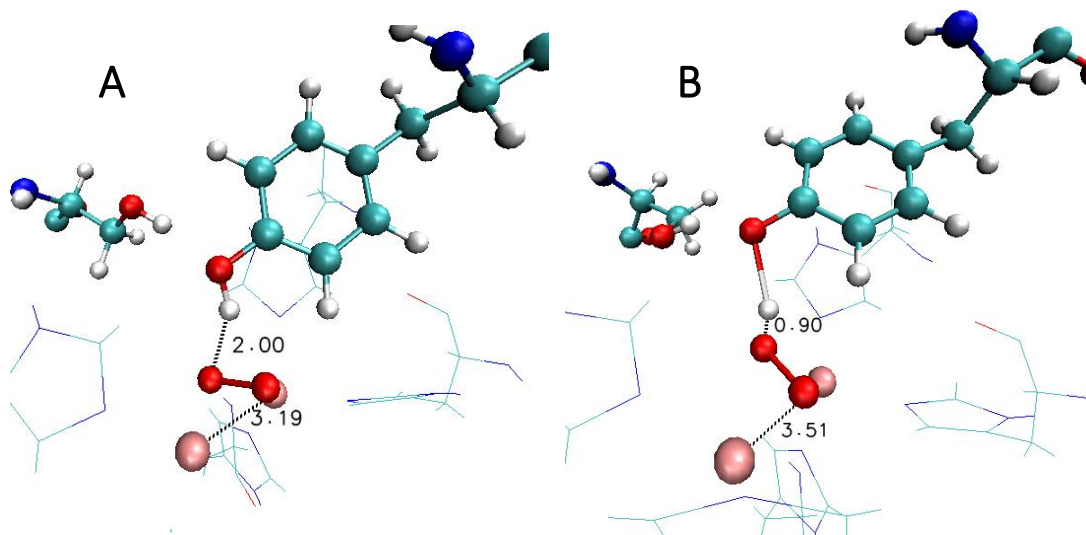
- Higher energy barrier, 45 kcal/mol, maybe due to the addition of constraints?

Second step: movement of the copper

- QM/MM equilibration of the system, no energy added

- Spontaneous movement of the copper (not observed when protonation on O(B))

QM/MM dynamic of the system after protonation on O(A)
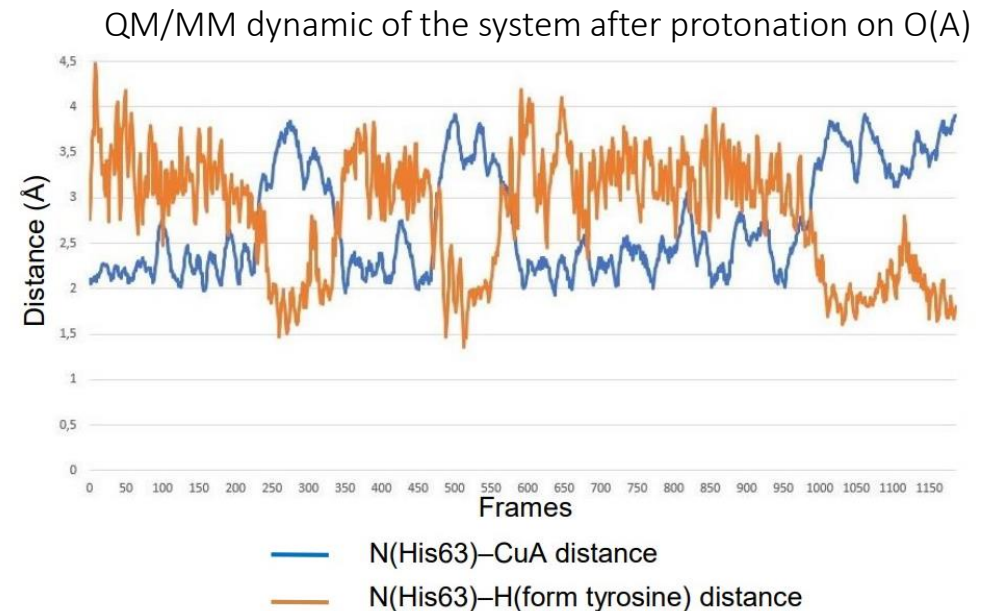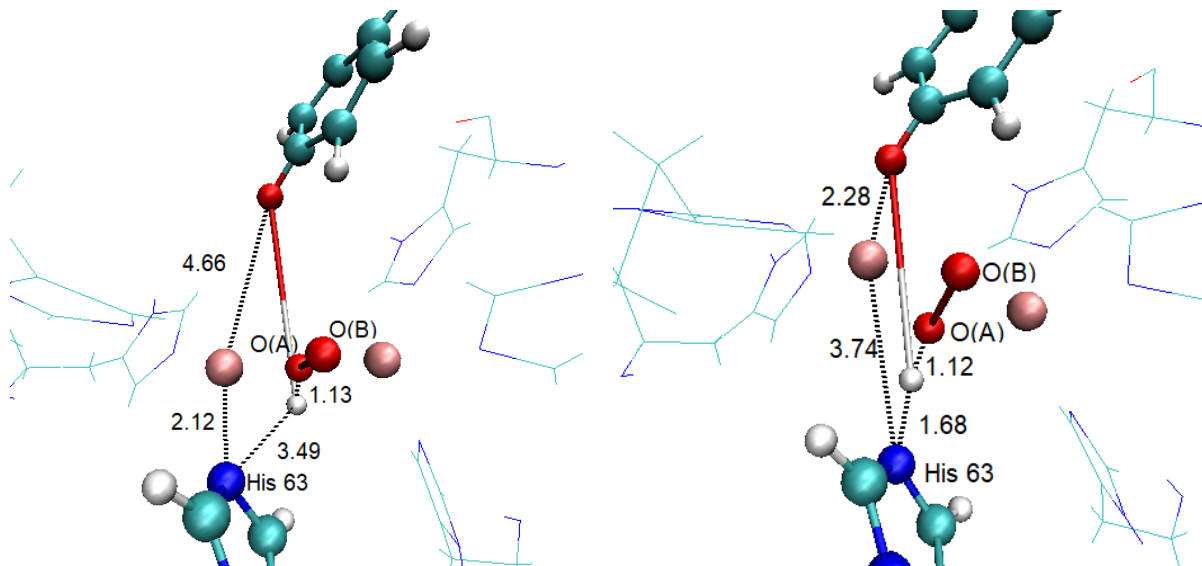
➤ Reproduces well the flexibility of the copper experimentally observed
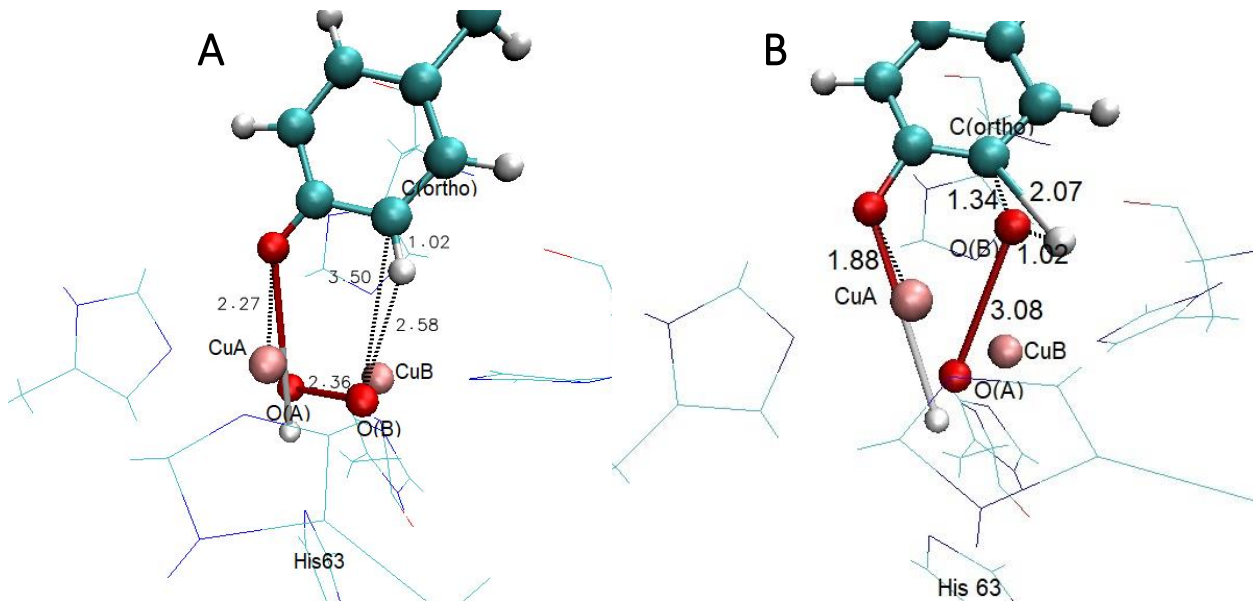
Third step: Formation of the C-O bond

- Importance of the environment

- Addition of the water molecules into the environment along with an asparagine and a glutamate, participating in the basicity of the system

- Water molecules described to be important for the reactivity
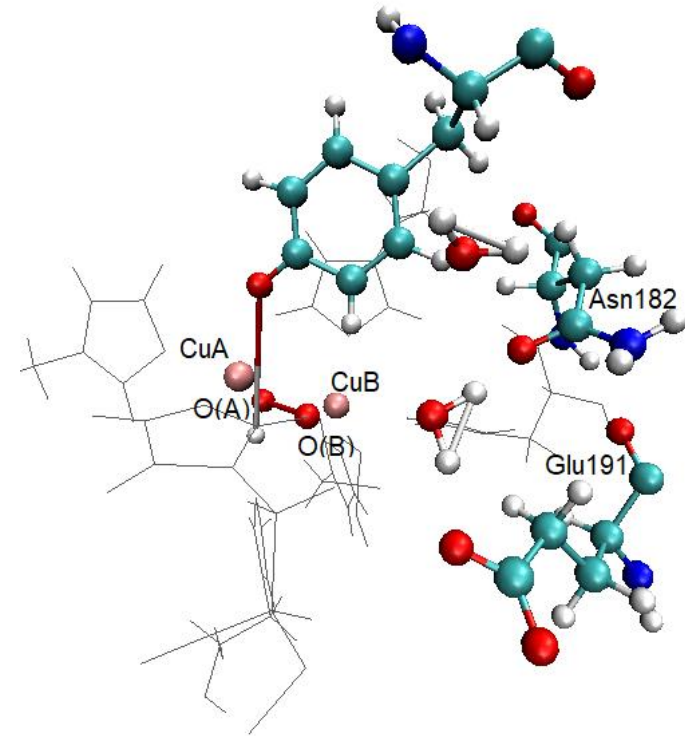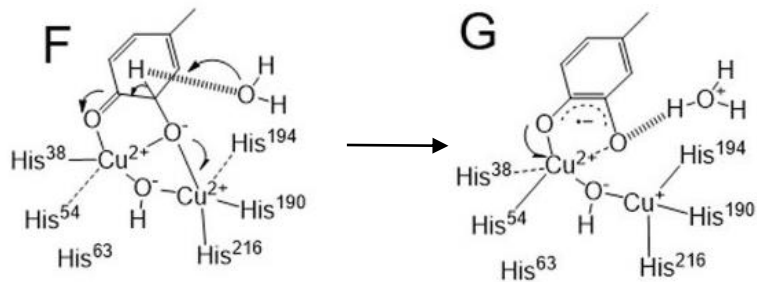
Third step: Formation of the C-O bond

- Metadynamics approach first
- Small energy barrier, ~10kcal/mol, stable product
- Protonation on the peroxo group not on one of the water molecules
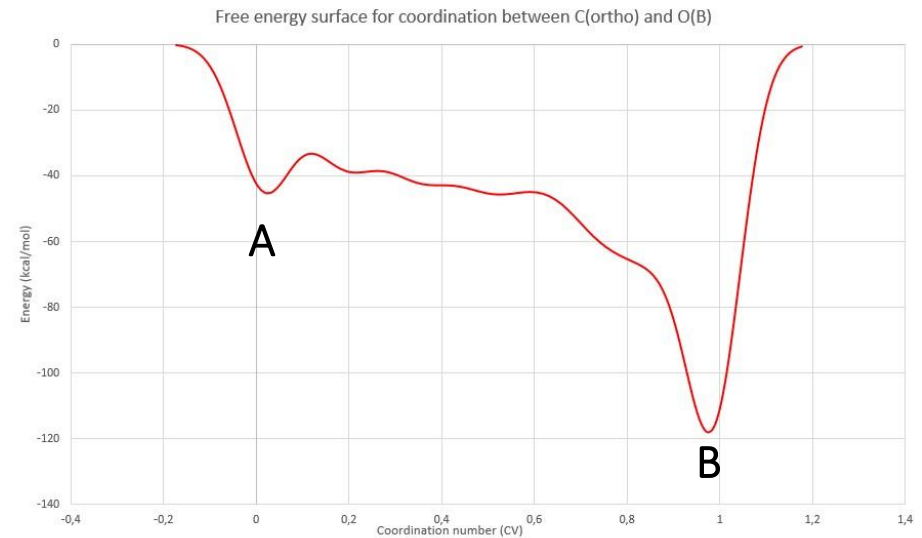
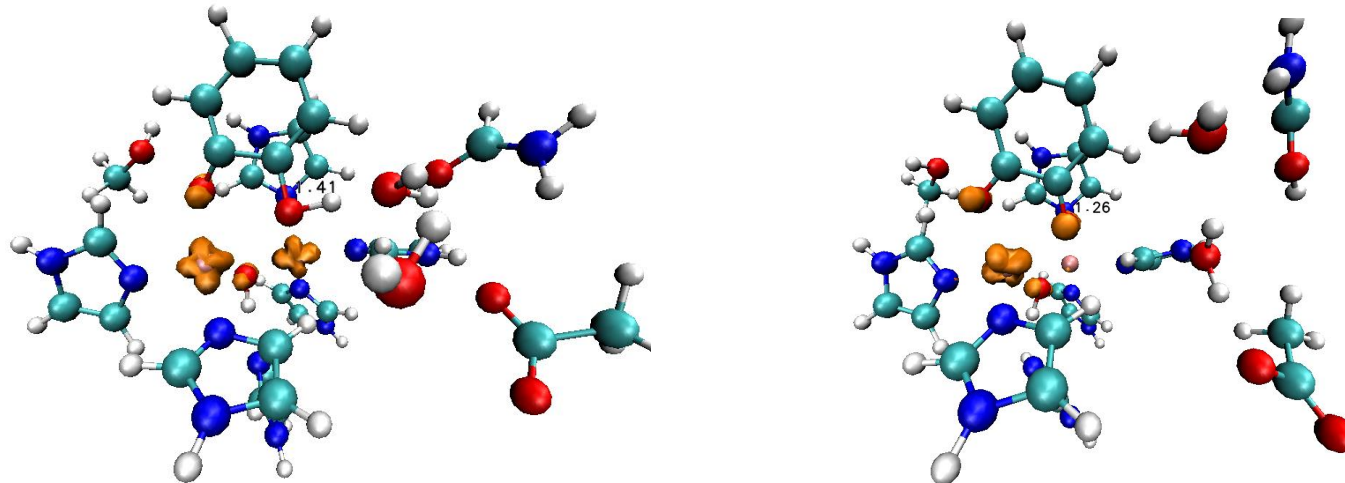# Third step: Formation of the C-O bond

- Estimation of the energy barrier calculated by TI (CV: coordination between C(ortho) and O(B)): 7 kcal/mol
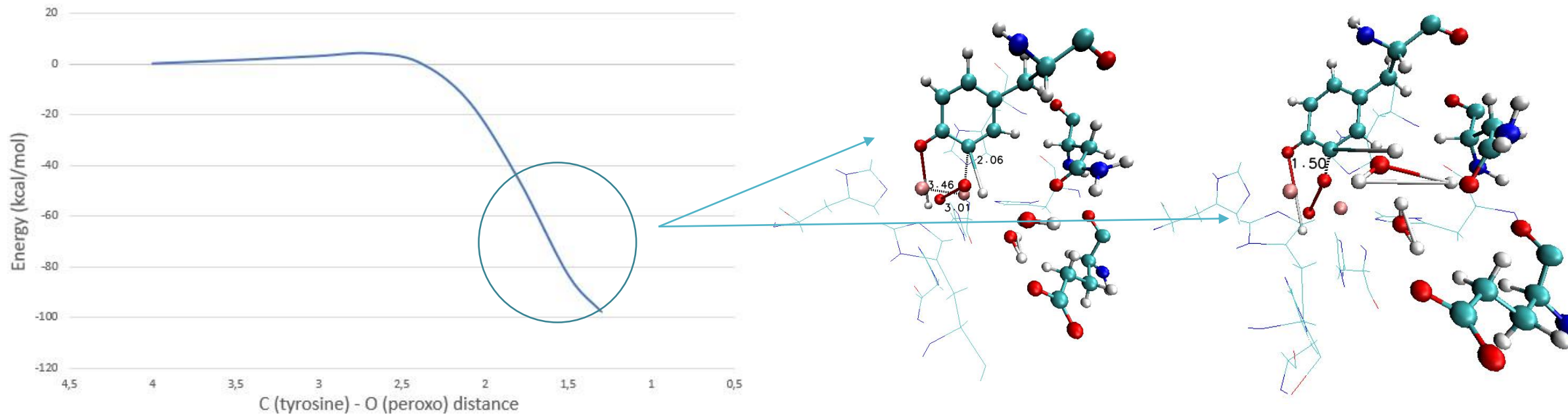
- Several structures observed for the deprotonation of the C(ortho)

Electronic structure

- Cu^{II}-Cu^{I}-$O°$ structure proposed in the mechanism, not found with CP2K

- Optimisation of the different structures with ASH to obtain a better description of the electronic structure (larger basis set)

- Impossible to obtain the Cu^{II}-Cu^{I} system when H still on the oxygen, only when delocalised on the water molecules
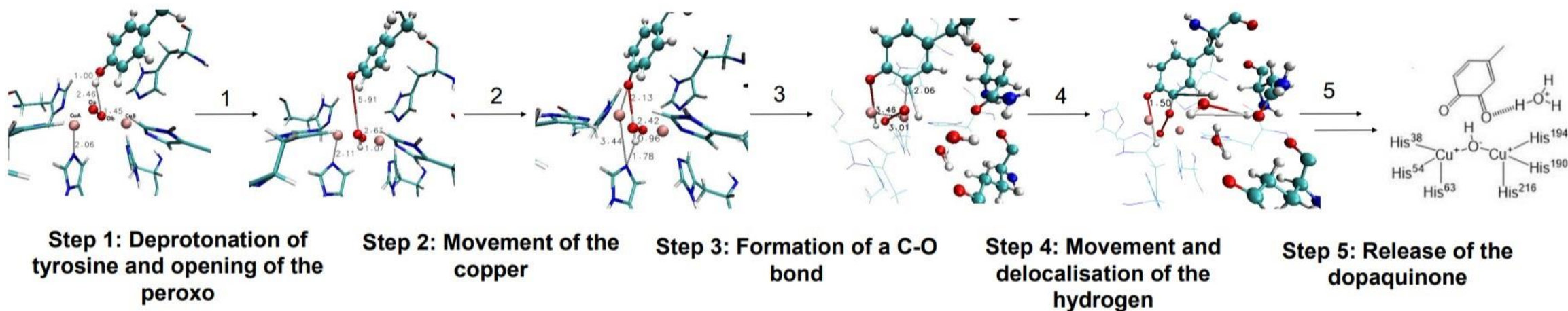

Spin density plots for the two structures after optimisation

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Part 2 : Conclusion

- Good understanding of the first steps of the mechanism

- Characterisation of the electronic structure for the formation of the C-O bond

- Need better description of that step

- Next step of the reaction (release of the dopaquinone) of interest

Step 1: Deprotonation of tyrosine and opening of the peroxo

Step 2: Movement of the copper

Step 3: Formation of a C-O bond

Step 4: Movement and delocalisation of the hydrogen

Step 5: Release of the dopaquinone

Acknowledgments

- COSACH team

- SITh team
  Dr. Pierre Girard

And thank you for your attention!