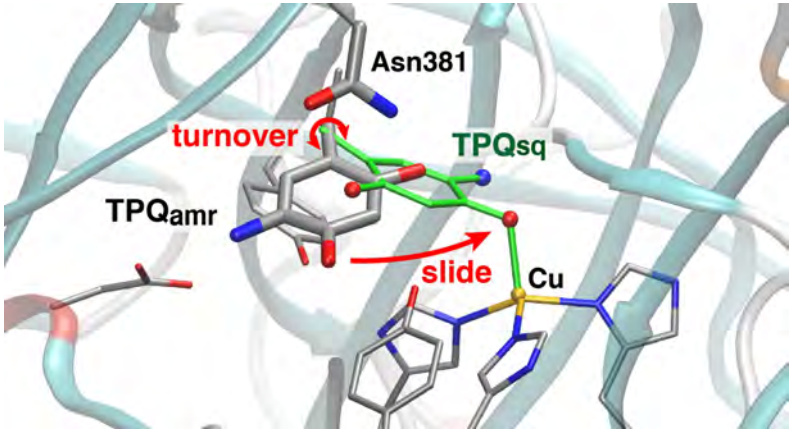
2023/2/22

CCS-KISTI WORKSHOP

Unique reaction mechanism of copper amine oxidase revealed by theoretical QM/MM and experimental approaches



Mitsuo Shoji[1,2]

[1]Biological Function and Information Group, Division of Life Science, CCS, U.Tsukuba、
[2]JST-PRESTO

mshoji@ccs.tsukuba.ac.jp

Acknowledgement

Kenji Mishima (U.Tsukuba)
Yasuteru Shigeta (U.Tsukuba)
Shigenori Nagatomo (U.Tsukuba)
Mauro Boero (Strasbourg U.)
Hideyuki Hayashi (OMPU)
Toshihide Okajima (Osaka U.)
Takashi Ueno (Tokyo Tech)
Eriko Nango (Tohoku U.)

Funds

KAKENHI:
20H05453(representative：Osamu Miyashiya)
16KT0055(representative：Hidetoshi Okajima)
JST-PRESTO:JPMJPR19G6
MEXT Q-LEAP: JPMXS0120330644
20H05088(I4LEC)

Computational resources

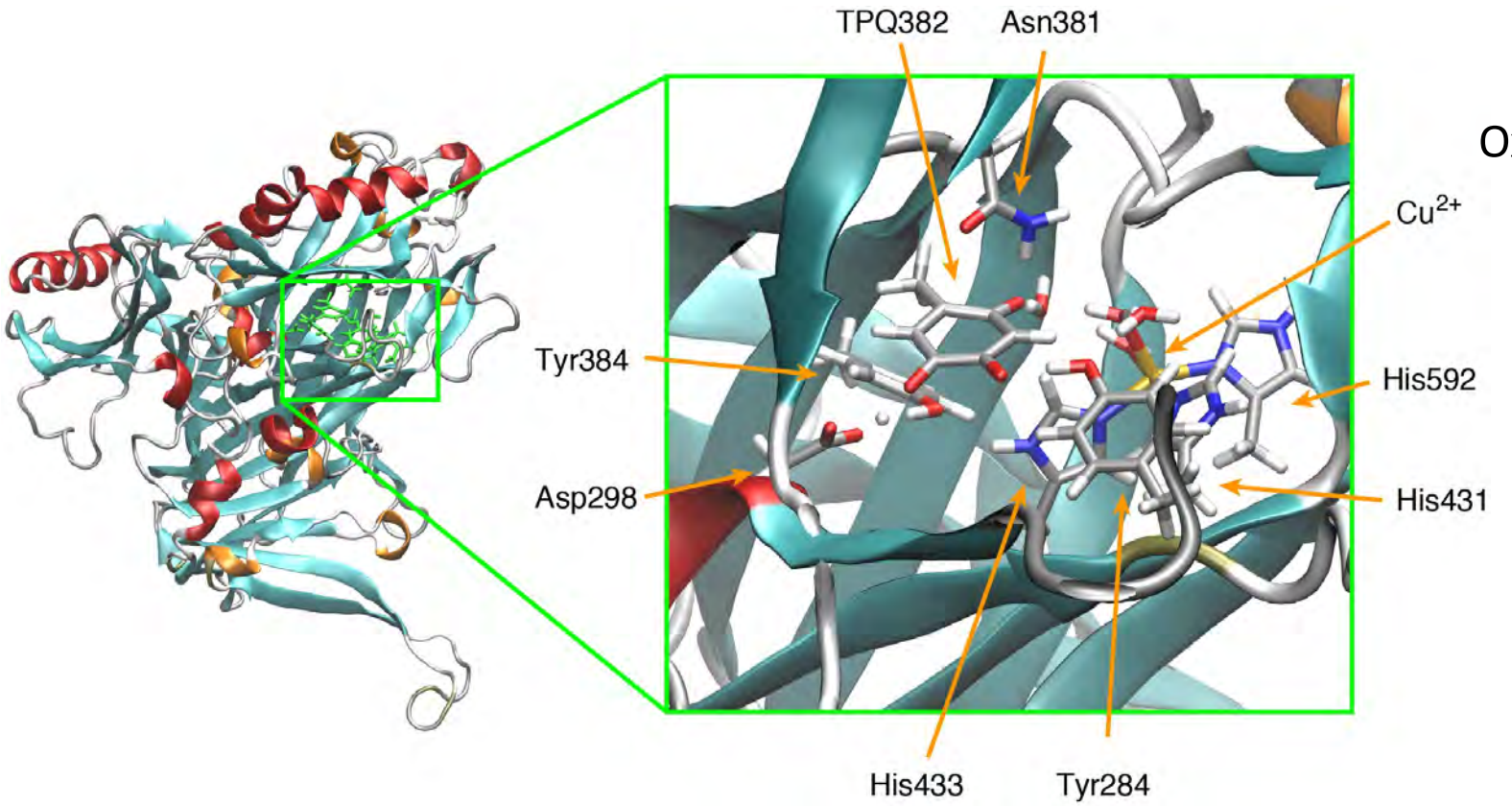
MCRP in CCS, Univ. Tsukuba
HPCI system research project (project ID: hp210115)

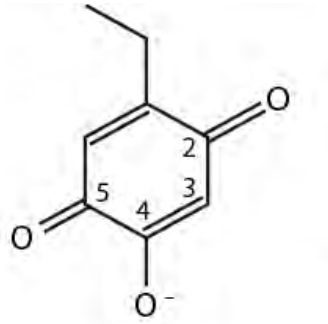
Copper amine oxidases (CAO)

- CAOs catalyze the oxidative deamination of primary amines into their aldehydes.

$$RCH_2NH_3 + O_2 + H_2O \rightarrow RCHO + H_2O_2 + NH_3$$

- CAOs exert fundamental functions in aerobic organisms from bacteria to ye mammals.
- CAOs contain Cu ion and topaquinone (TPQ) cofactor

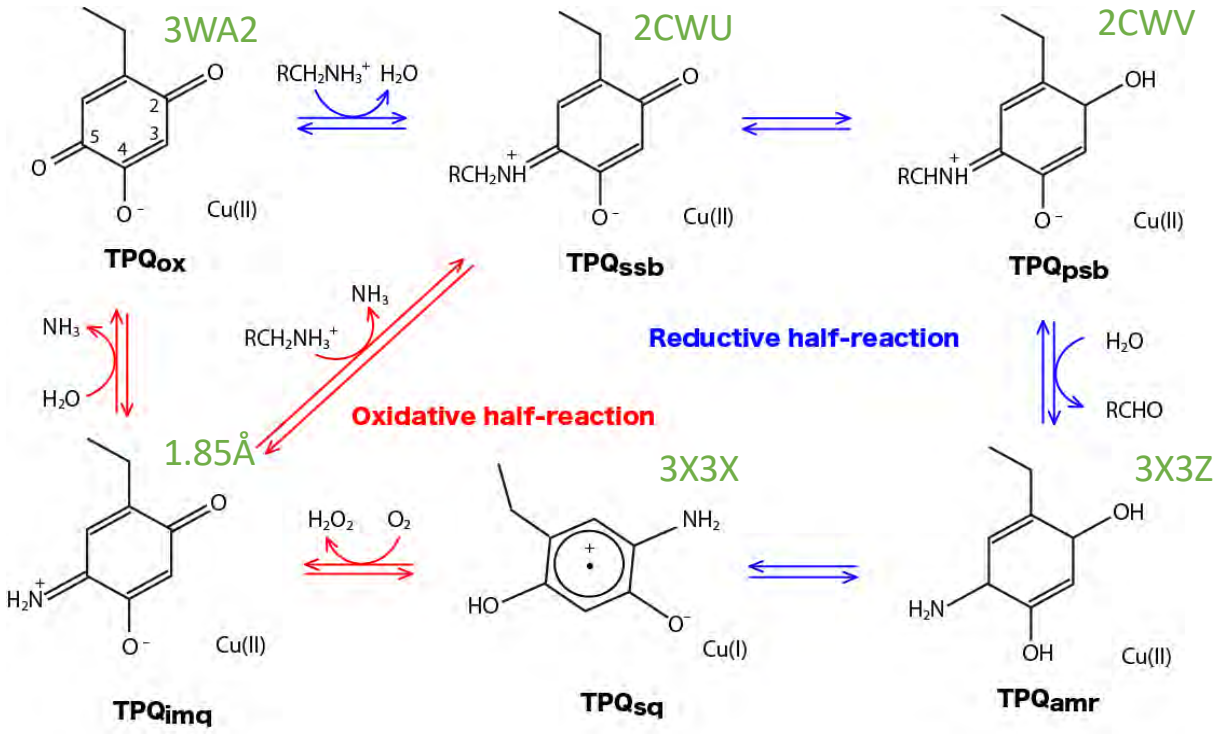




Oxidative form of TPQ (TPQ_{ox})

Catalytic cycle of CAO

- Catalytic cycle of CAO is composed of reductive half-reaction and oxidative half-reaction

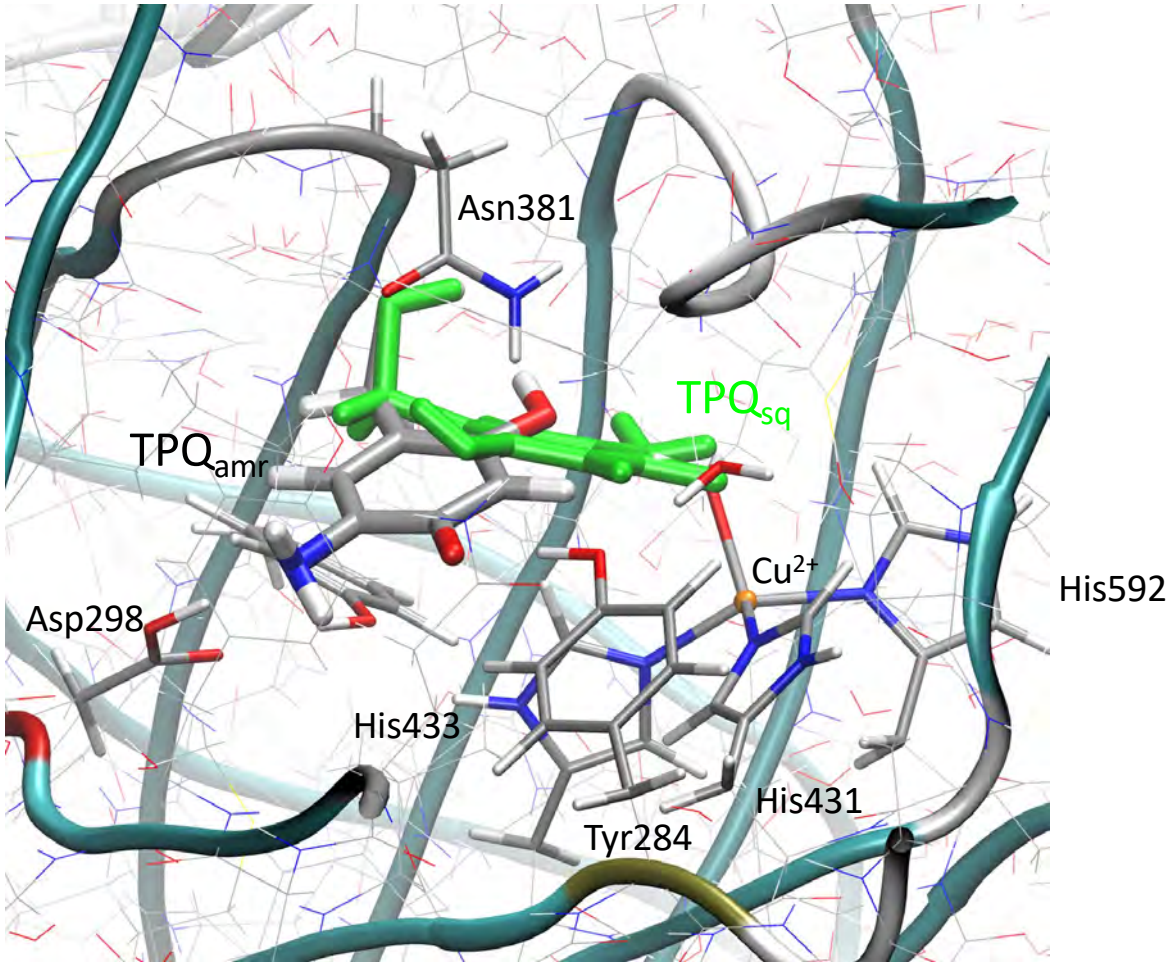


Features

- X-ray structures of all the intermediate states are determined.
- Positions of proton in TPQ_{ox} were determined.
- Spectroscopy can trace the intermediate states.
- Isotope effect of proton transfer (H/D) is observed. (Target for quantum biology)
- Large conformational change during the TPQ_{amr} -> TPQ_{sq} transition (Target for SFX study)

Large conformational change of TPQ

Off-Cu(TPQ_{amr}) → On-Cu (TPQ_{sq})



TPQ_{amr}: 3X3Z

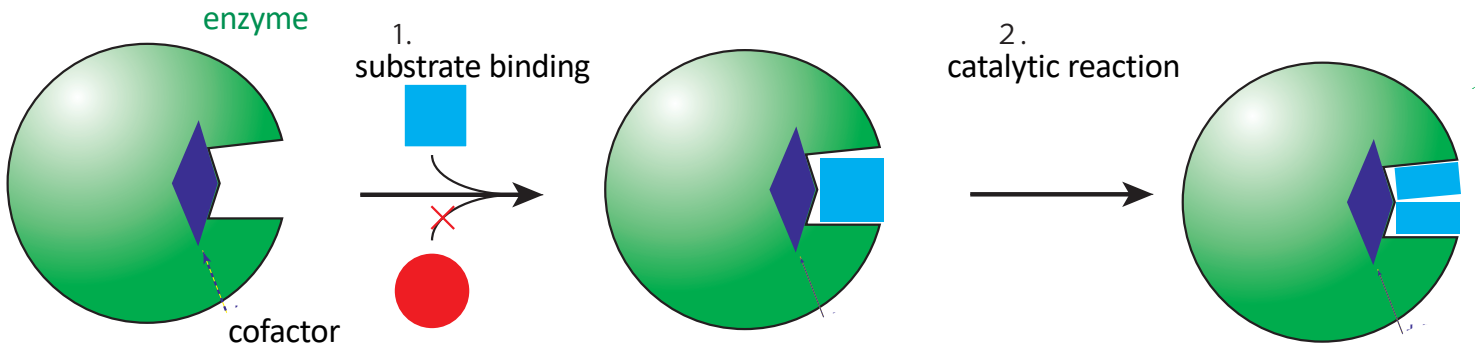
TPQ_{sq}: 3X3X

- Asn381 is a conserved residue
- Reaction mechanism is unsolved （impossible ?）

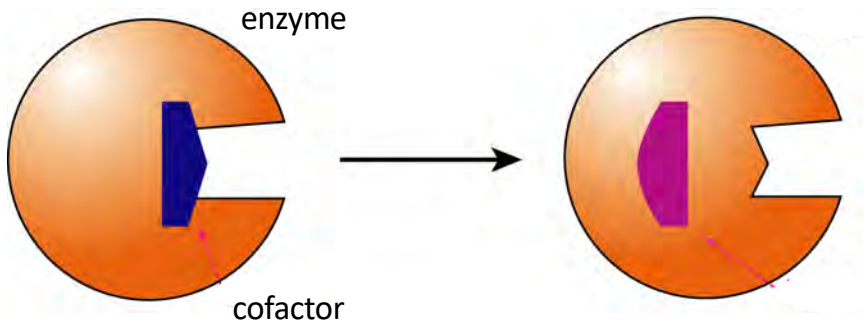
Quite different from a classical picture of enzyme reaction

Structural changes are quite small → substrate specificity： classical enzyme

- Key and keyhole model: E.Fischer(1890)　substrate-binding site is rigid
- Induced fit model: Koshland(1995)　substrate-binding site is more flexible（active site is rigid）



CAO



- Really happen？
- Why and is meaningful？

Purpose of this study

- Elucidate the most difficult reaction step in $TPQ_{amr} \rightarrow TPQ_{sq}$
- Solve the role of Asn381

  Theoretical approach：

  Reaction mechanism of WT by using QM/MM*

  Reaction mechanism of N381A mutant

  Structural change of N381A mutant in TPQ_{ox}

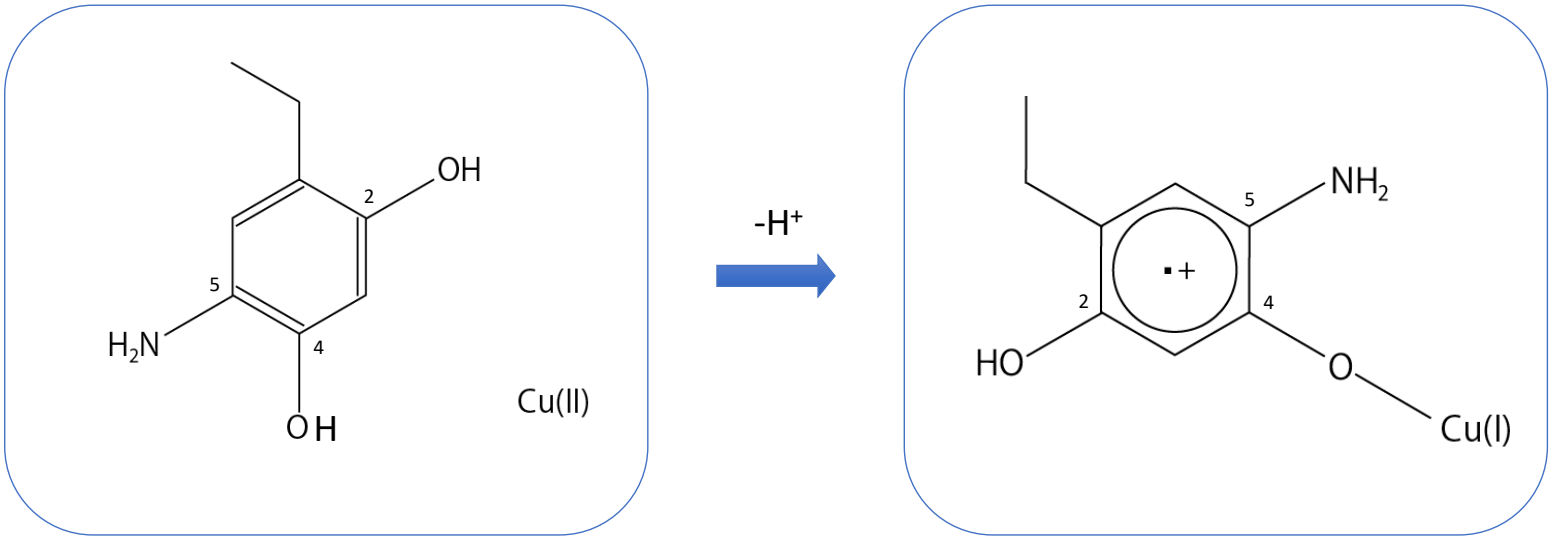
  Experimental approach：

  X-ray crystal structures of N381A and kinetic spectroscopy

*QM/MM: hybrid quantum mechanics and molecular mechanics

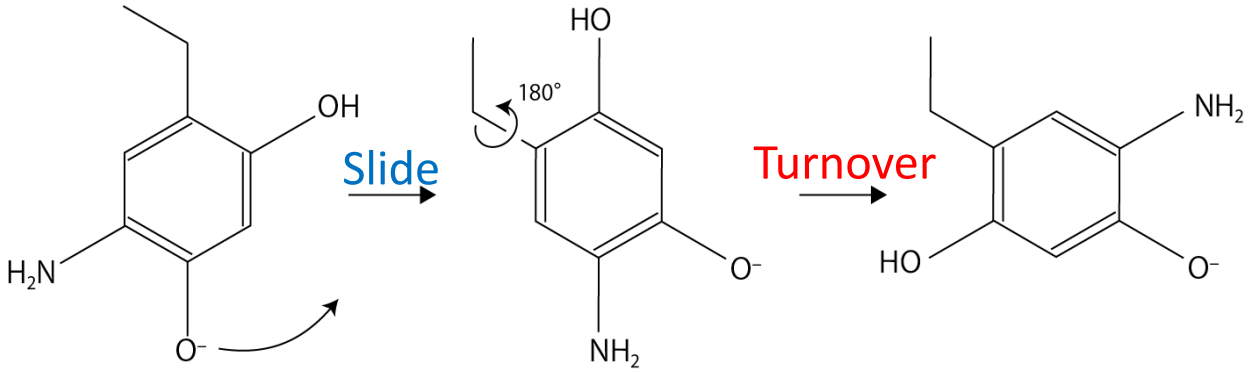
(This study took 2-3 years to find appropriate reaction pathways, because it is a significantly difficult problem)

Off-Cu → On-Cu transition



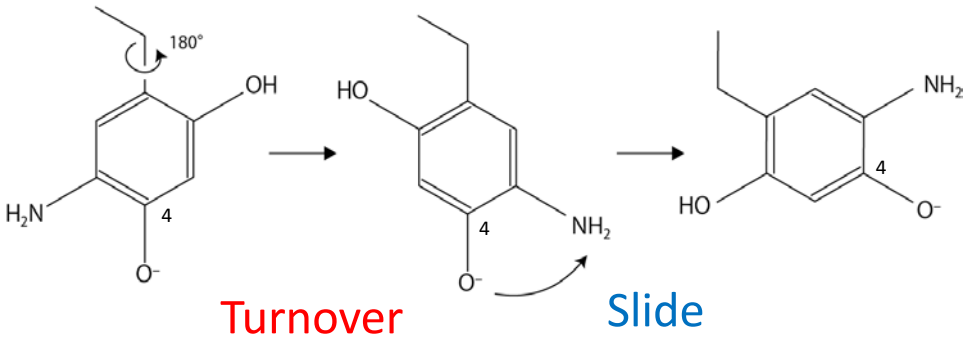
Off-Cu (TPQ_{amr}) On-Cu (TPQ_{sq})

- steps of the TPQ movement



2 x 2 = 4 candidate pathways

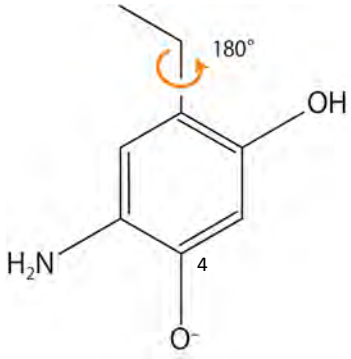
1. Turnover + Slide



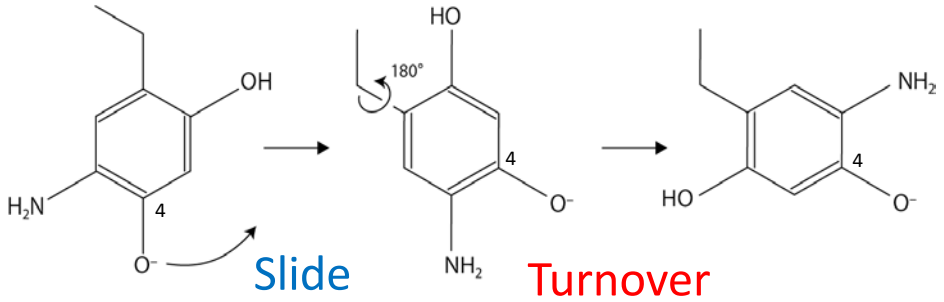
Turnover Slide

Off-Cu (TPQ_{amr}) On-Cu (TPQ_{sq})

A. clockwise-turnover



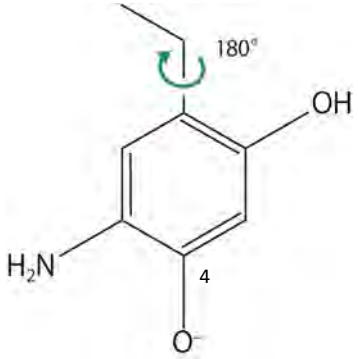
2. Slide + Turnover



Slide Turnover

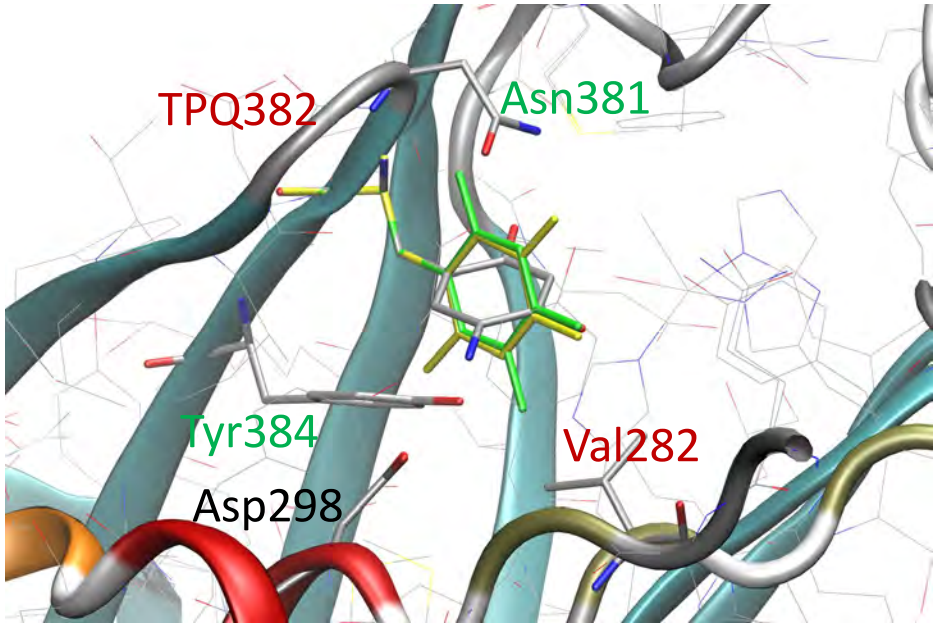
Off-Cu (TPQ_{amr}) On-Cu (TPQ_{sq})

B. counterclockwise-turnover



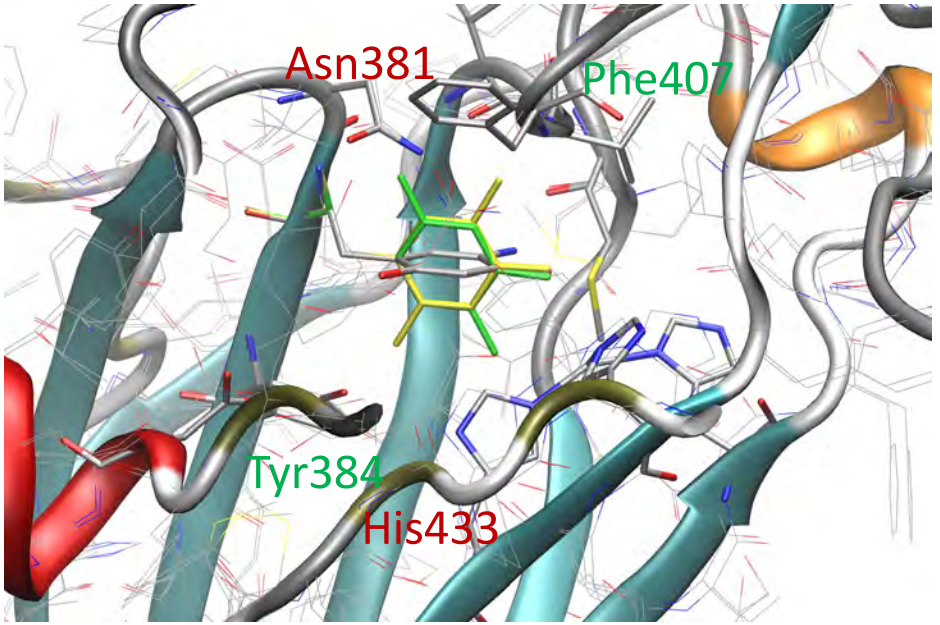
Rotation of TPQ is quit difficult for steric repulsion from surrounding residues

1. Turnover + Slide



- 1A. clockwise turnover: ⊢Asn381, Tyr384
- 1B. counterclockwise turnover: ⊢ main chain of TPQ382, Val282

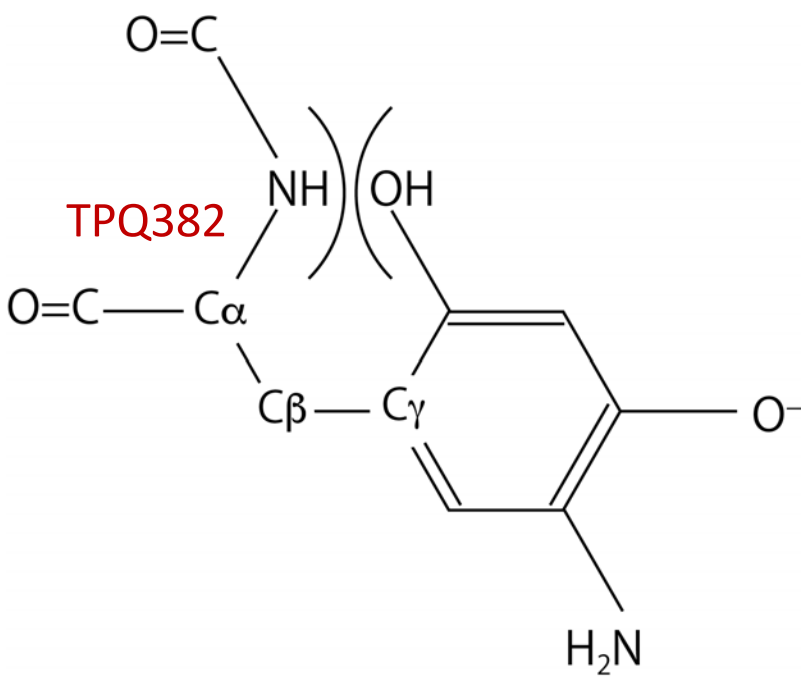
2. Slide + Turnover



- 2A. clockwise turnover: ⊢ main chain of Phe407, Tyr384
- 2B. counterclockwise turnover: ⊢Asn381, His433

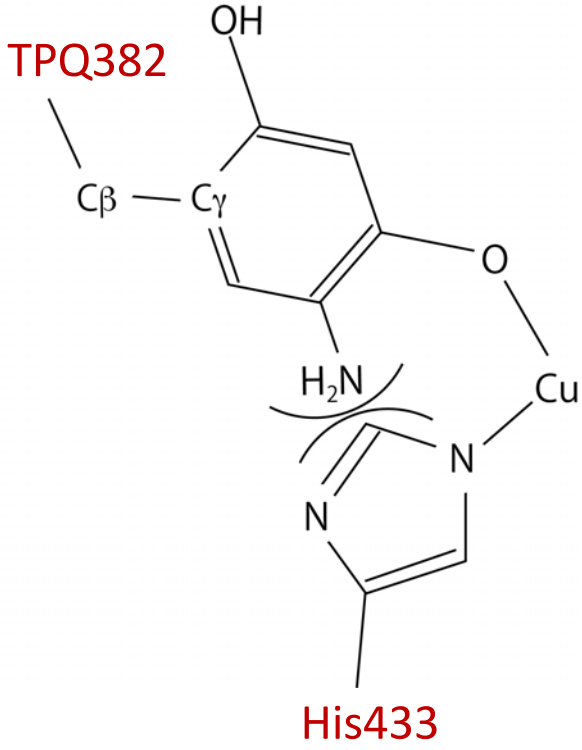
Inevitable reaction step: counterclockwise turnover of TPQ (1B,2B)

1B. Counterclockwise rotation @ Off-Cu



⊢ main chain of TPQ382

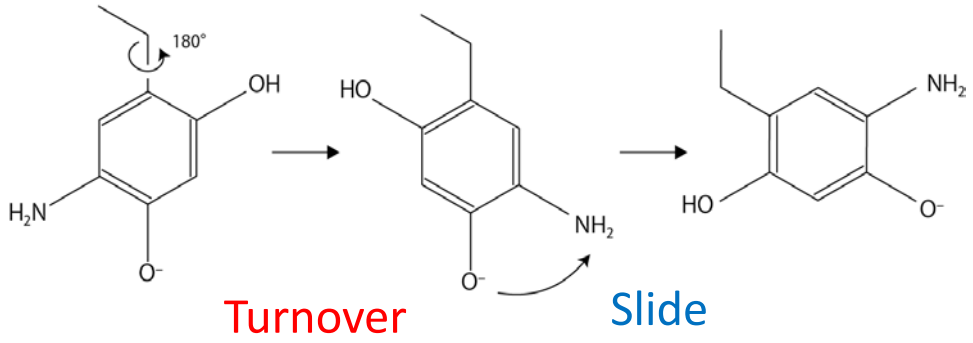
2B. Counterclockwise rotation @ On-Cu



⊢His433

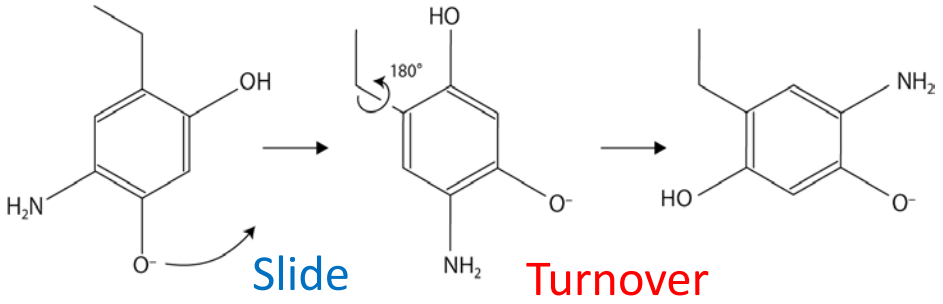
Two candidate pathways: clockwise-turnover of TPQ (1A, 2A)

1A. Turnover (clockwise) + Slide



Off-Cu (TPQ_{amr}) On-Cu (TPQ_{sq})

2A. Slide + Turnover (clockwise)

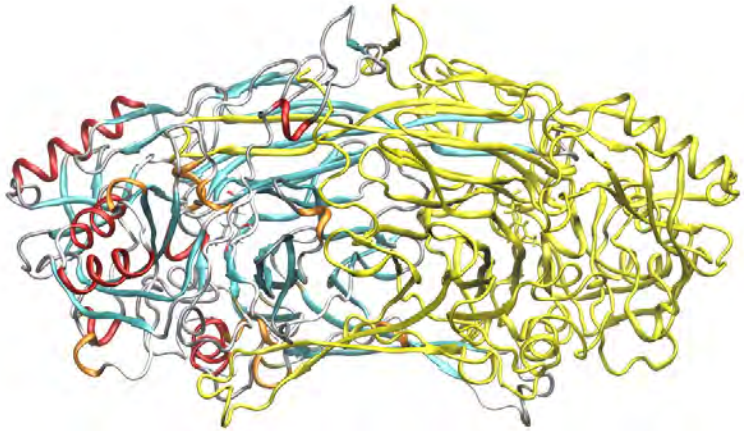


Off-Cu (TPQ_{amr}) On-Cu (TPQ_{sq})

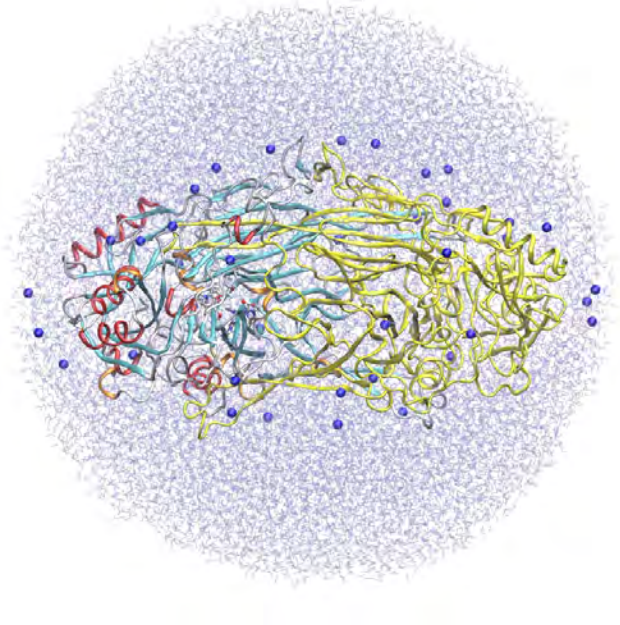
Computational details

Initial settings

- Crystal structure; PDBID: 3X3Z (copper amine oxidase from *Arthrobacter globiformis* (AGAO) in the TPQ_{amr} state)
- Homodimer model
- Protonation state is referred to the neutron structure (PDBID: 6L9C)
- Added 36 Na^+ to charge neutral
- A spherical water droplet of 60 Å radius
- Proton positions are relaxed by 10ps MD (amber99fprce field, 0->250K, 250K->0K).
- Heavy atoms are fixed to the X-ray structure



3X3Z

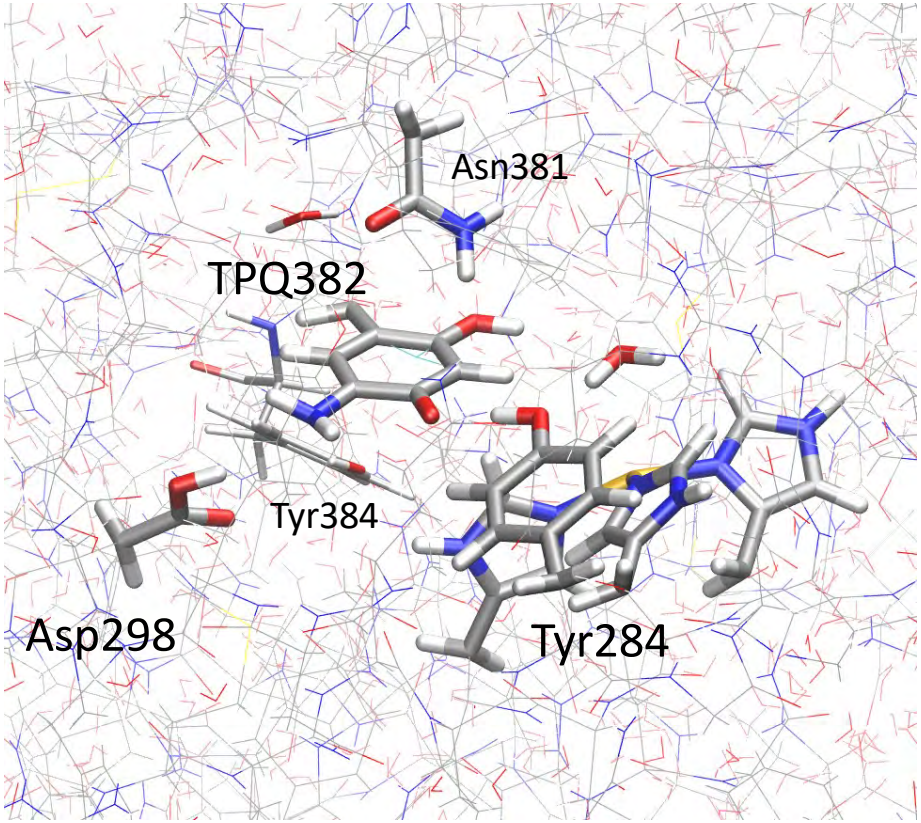


+ 22,000 Waters

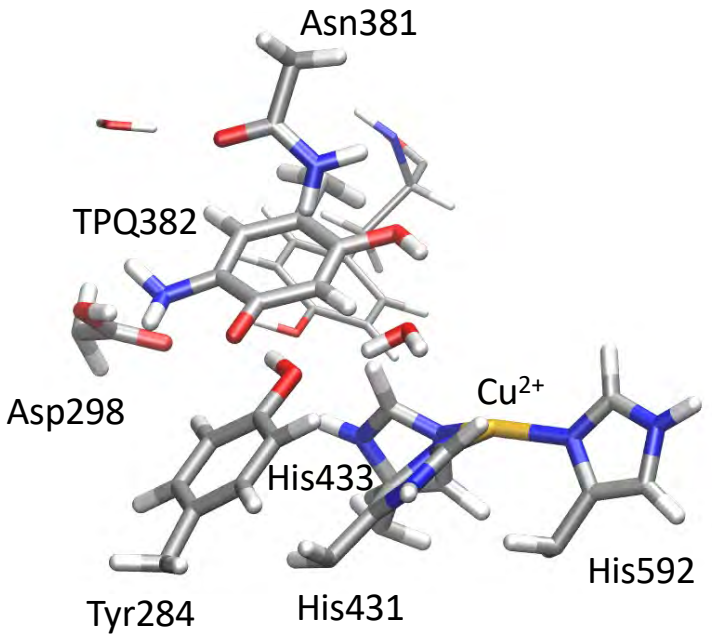
Computational details

QM/MM

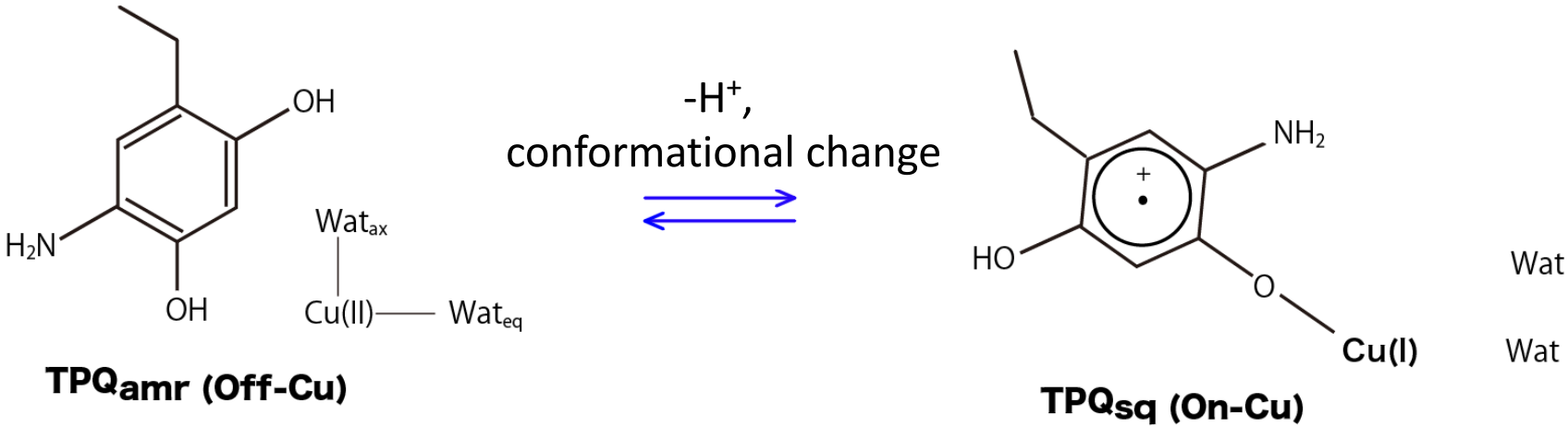
- Program Package: NWChem version 6.8
- Method: UB3LYP/(LANL-2DZ, 6-31G*)| amber 99
- Optimization for 10 Å around the QM center
- #QM=84, #Basis=789



Thick Tube: QM region



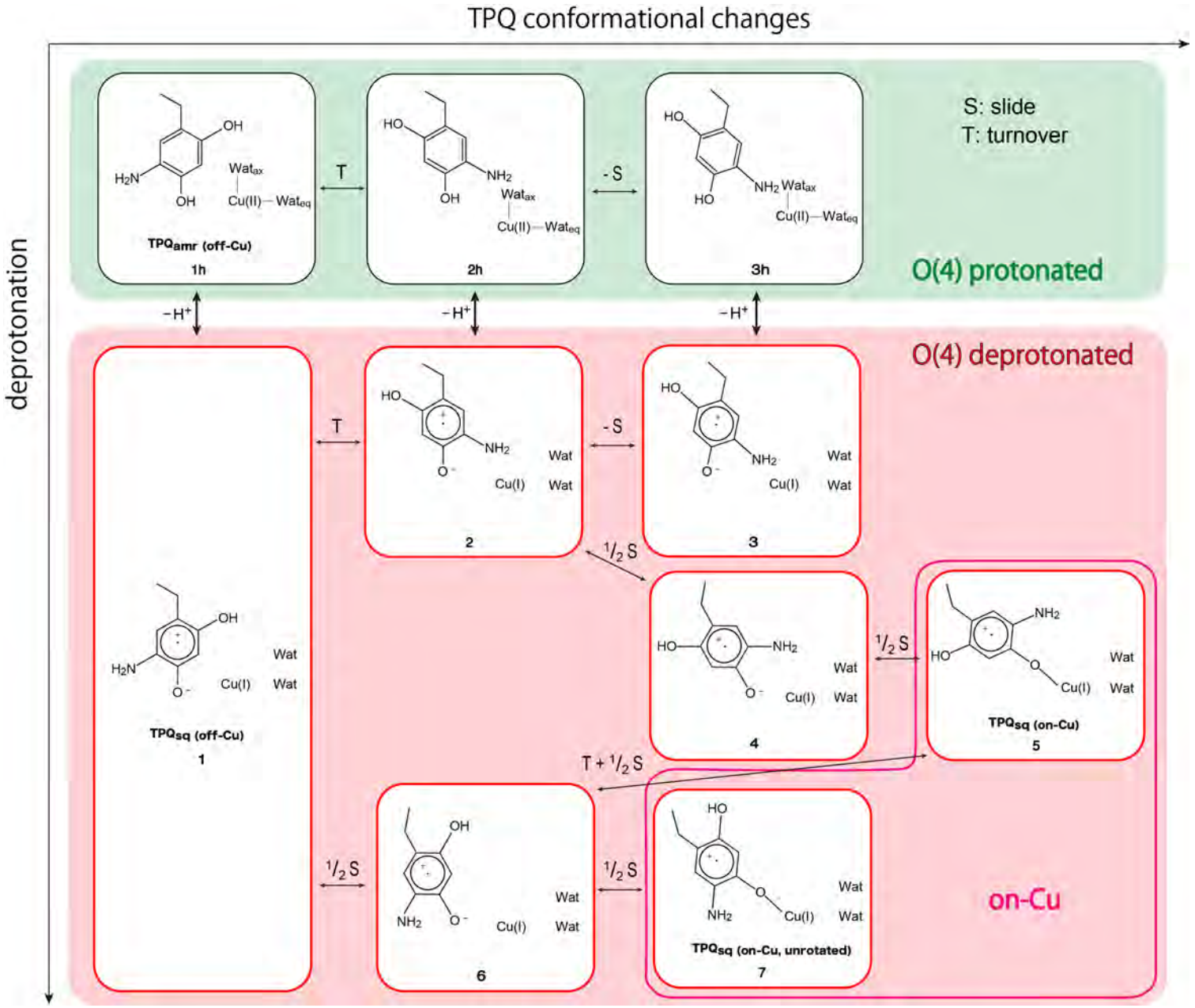
QM atoms (side view)



Calculated results

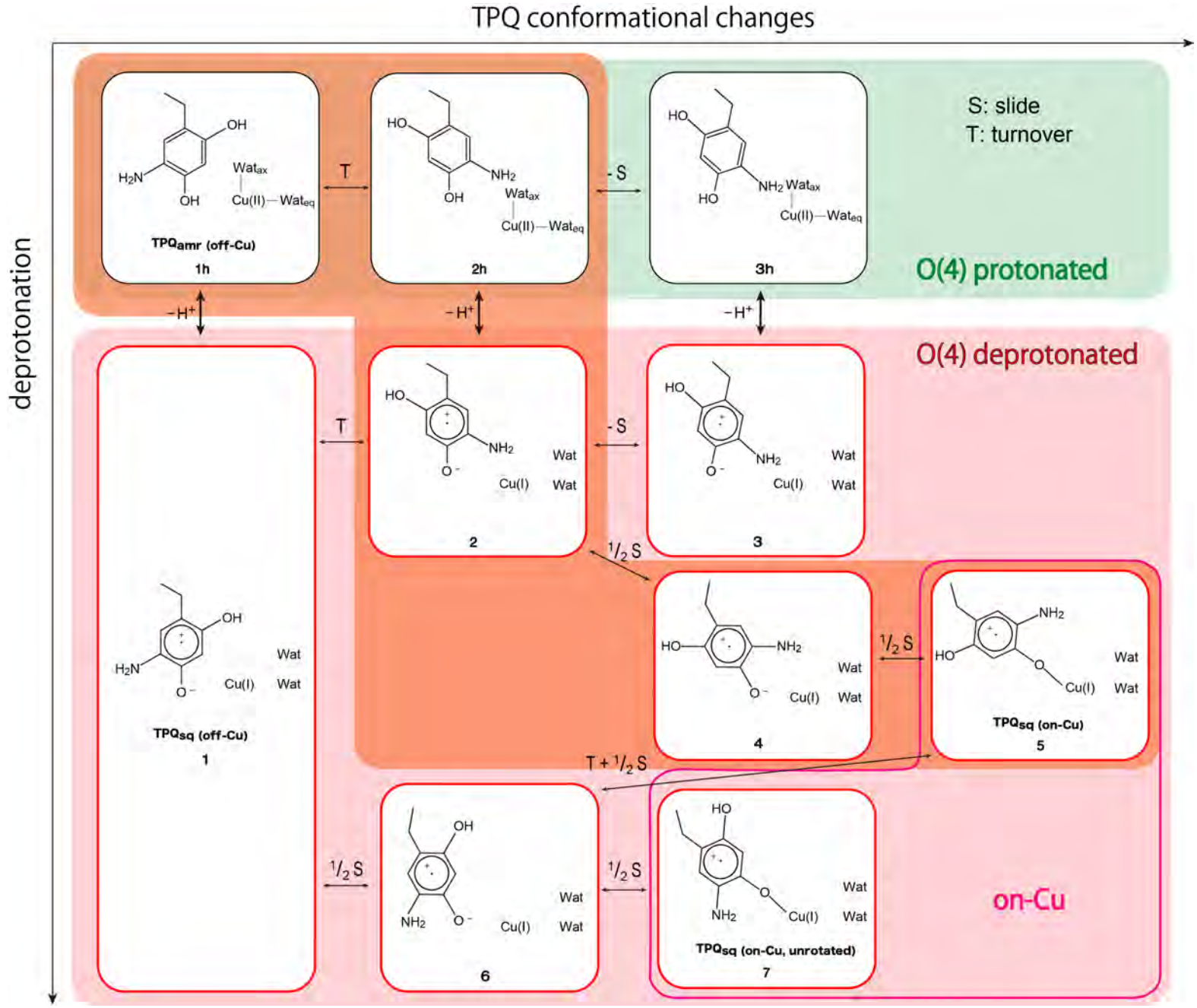
kcal mol^{-1} is simplified as kcal

Intermediate states (IMS) determined by QM/MM



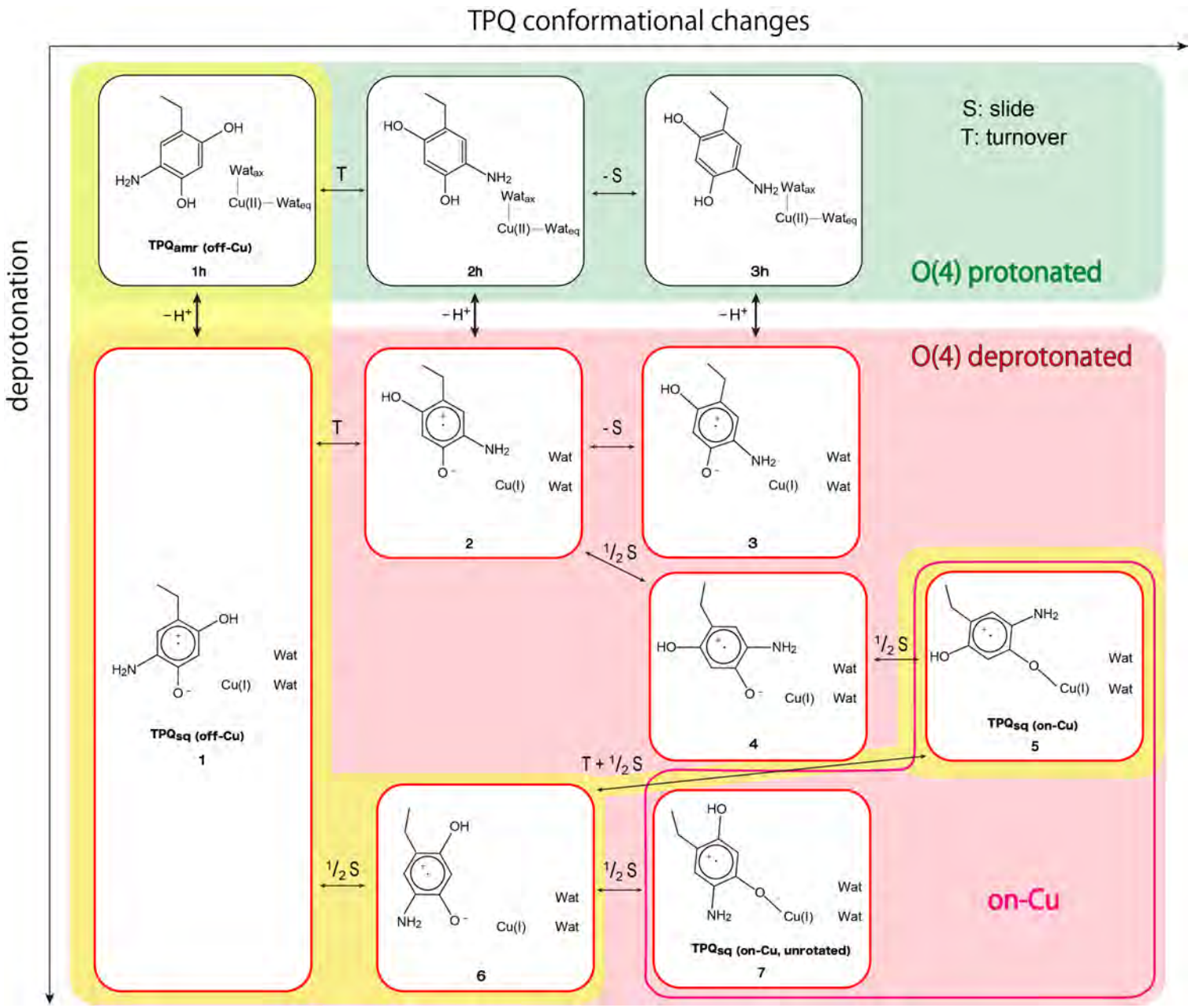
Map of IMS

1A: 1h → 2h → 2 → 4 → 5



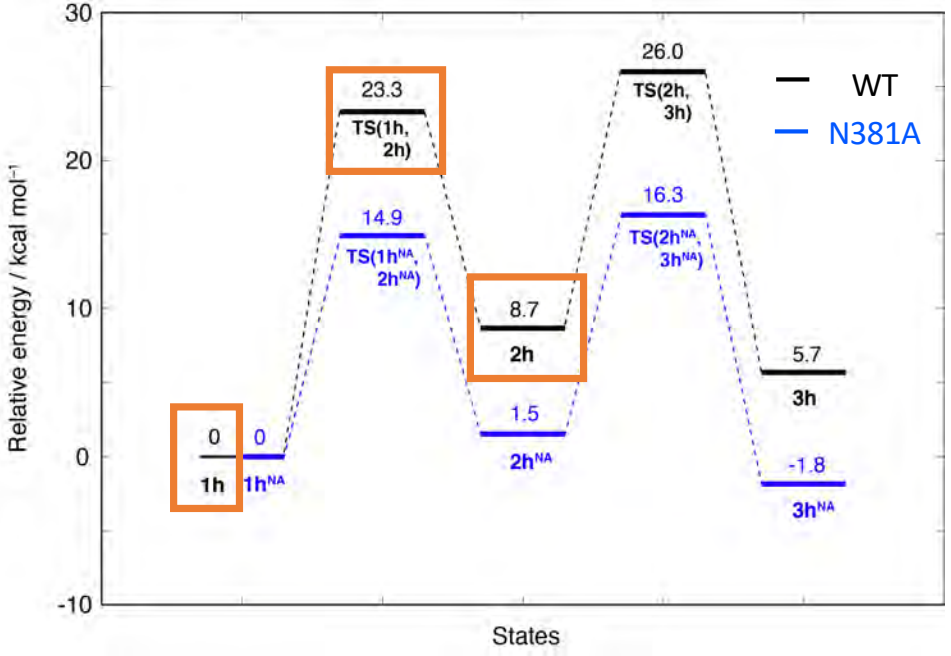
Map of IMS

2A: 1h → 1 → 6 → 5

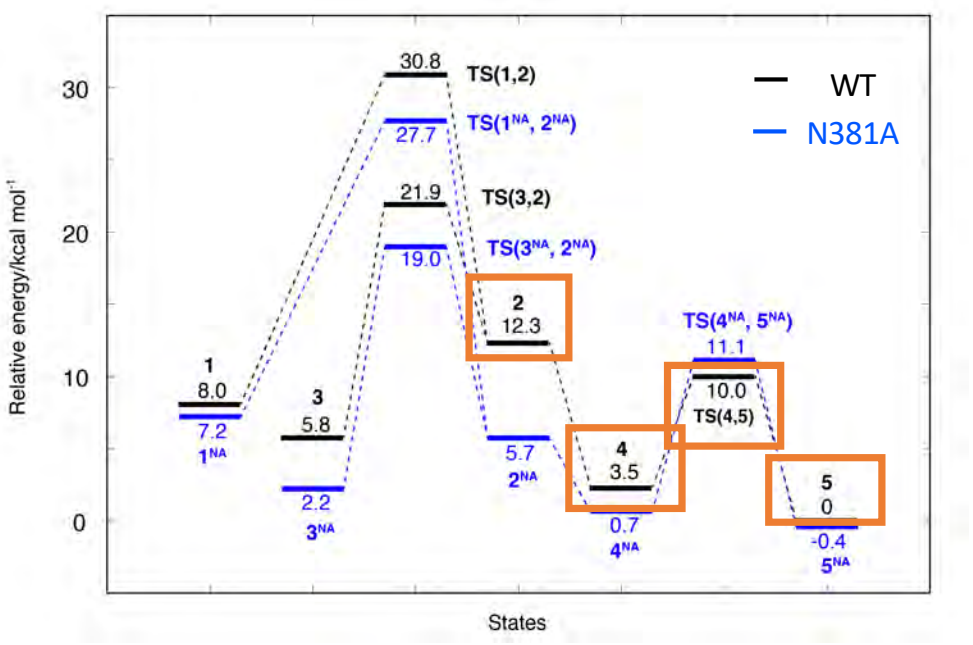


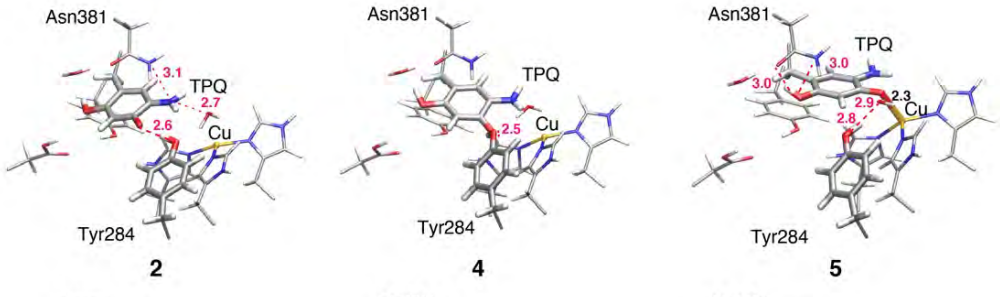
Energy profile of 1A (1h → 2h → 2 → 4 → 5)

O(4) protonated



O(4) deprotonated



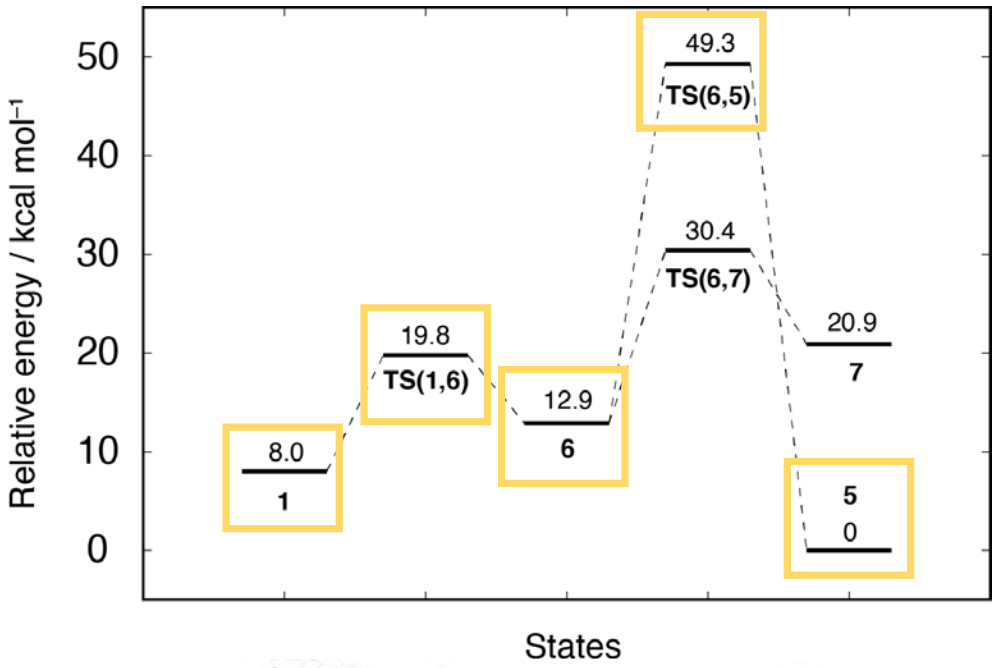


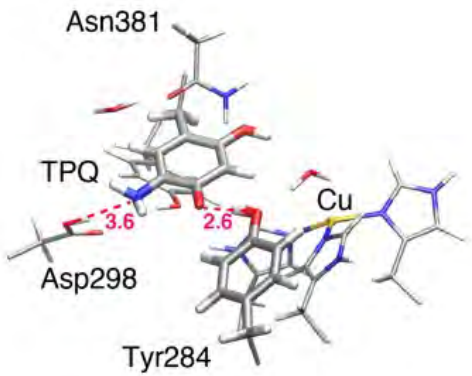
turnover $\Delta E^{\ddagger}$=23.3 kcal (**TS(1h,2h)**)

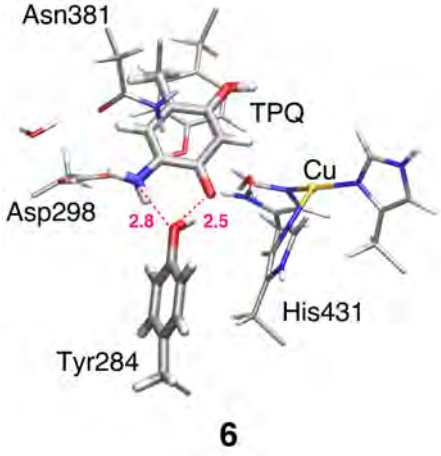
silde $\Delta E^{\ddagger}$=10.0 kcal (**TS(4,5)**))

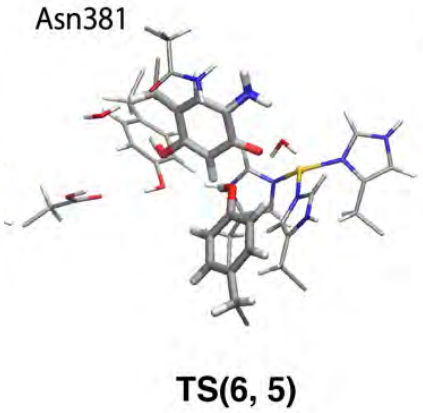
Energy profile of 2A (1h → 1 → 6 → 5)

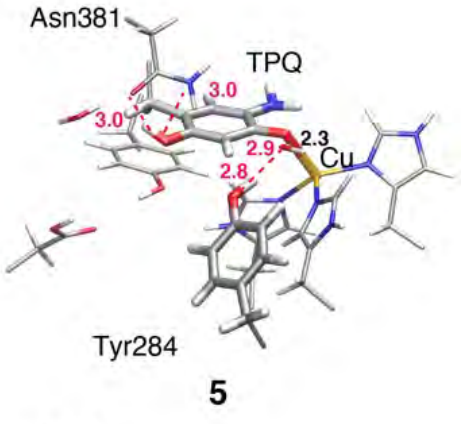
O(4) deprotonated









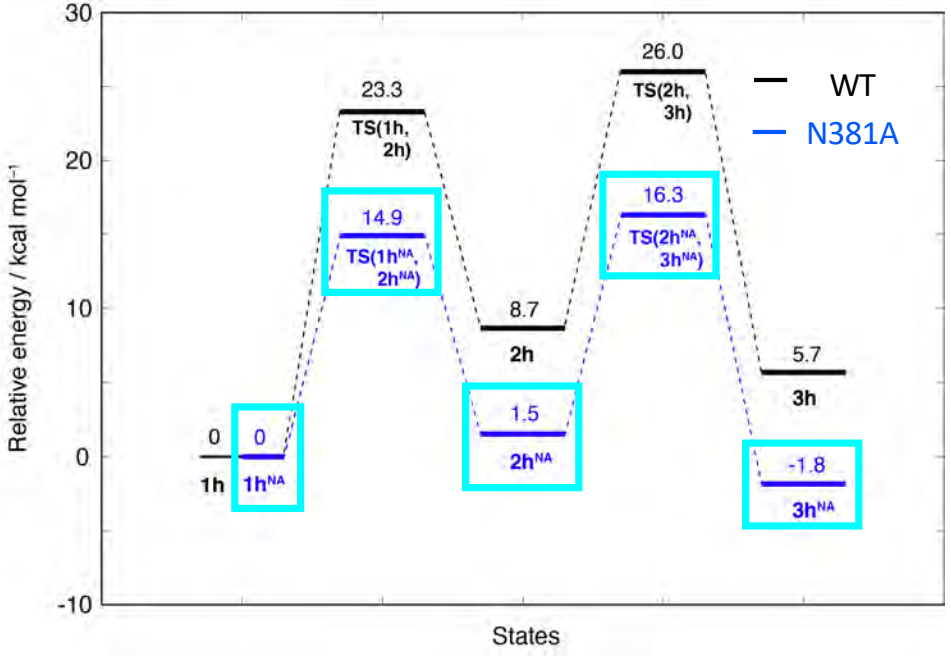


silde $\Delta E^{\ddagger}$=19.8 kcal (**TS(1,6)**)

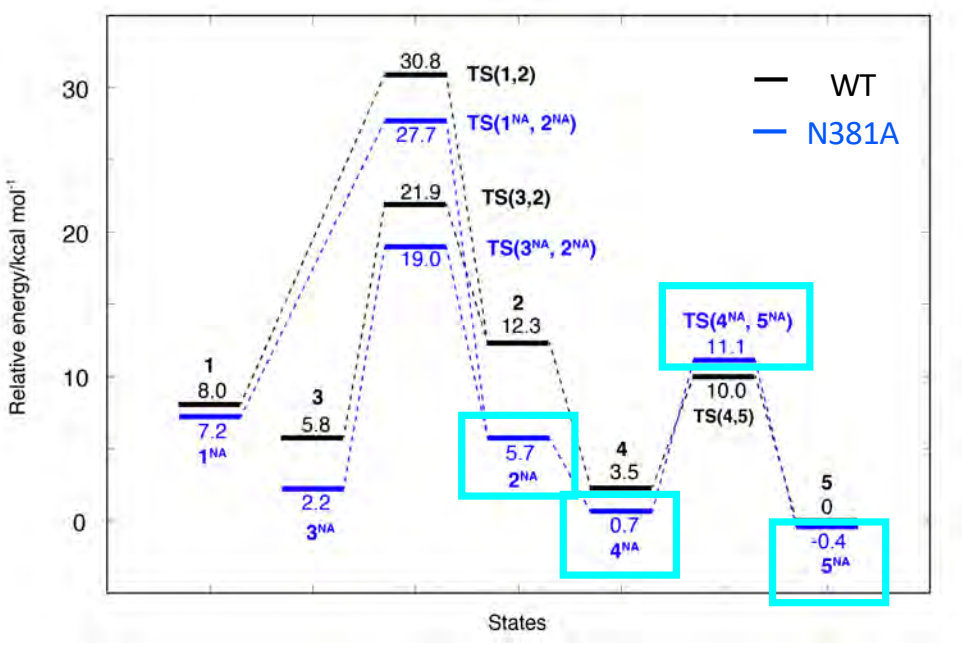
turnover $\Delta E^{\ddagger}$=49.3 kcal (**TS(6,5)**)

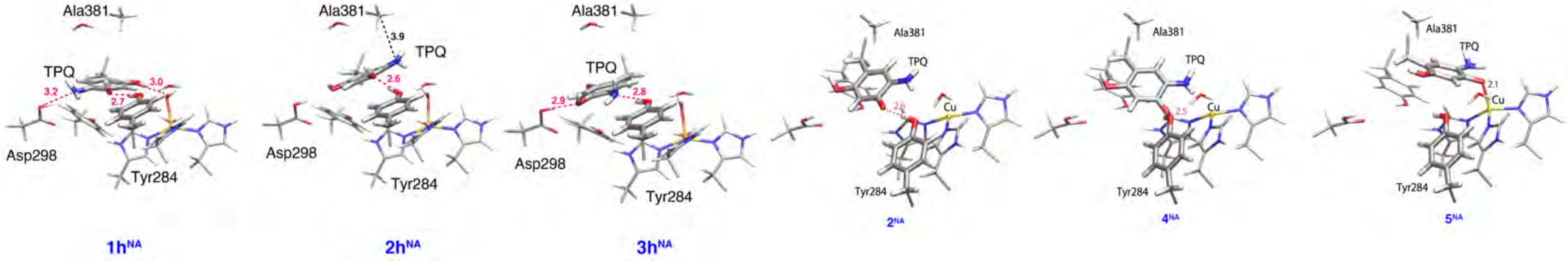
Energy profile of N381A mutant (NA)

O(4) protonated



O(4) deprotonated





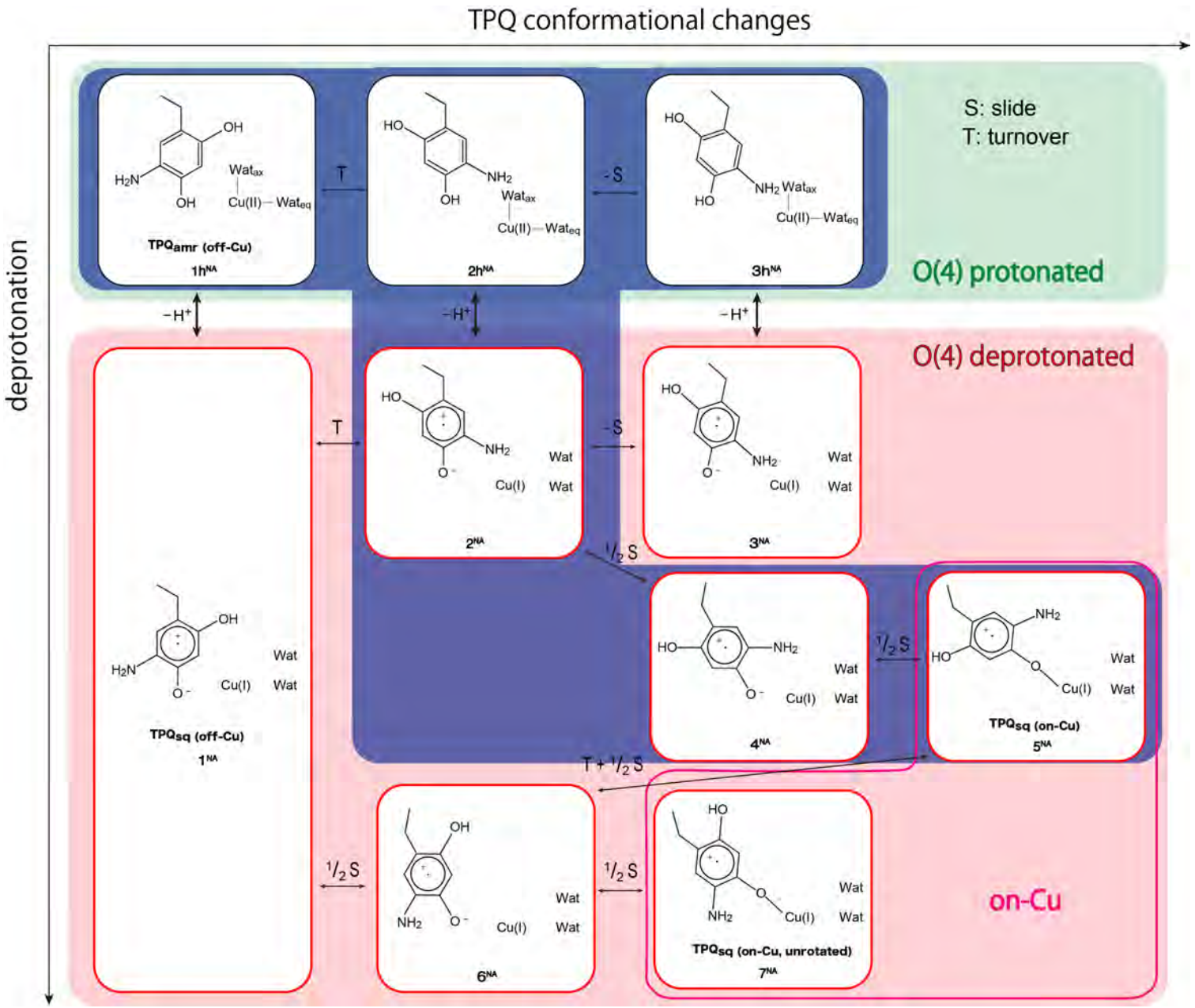
turnover $\Delta E^{\ddagger}$=14.9 kcal

-slide $\Delta E^{\ddagger}$=16.3 kcal

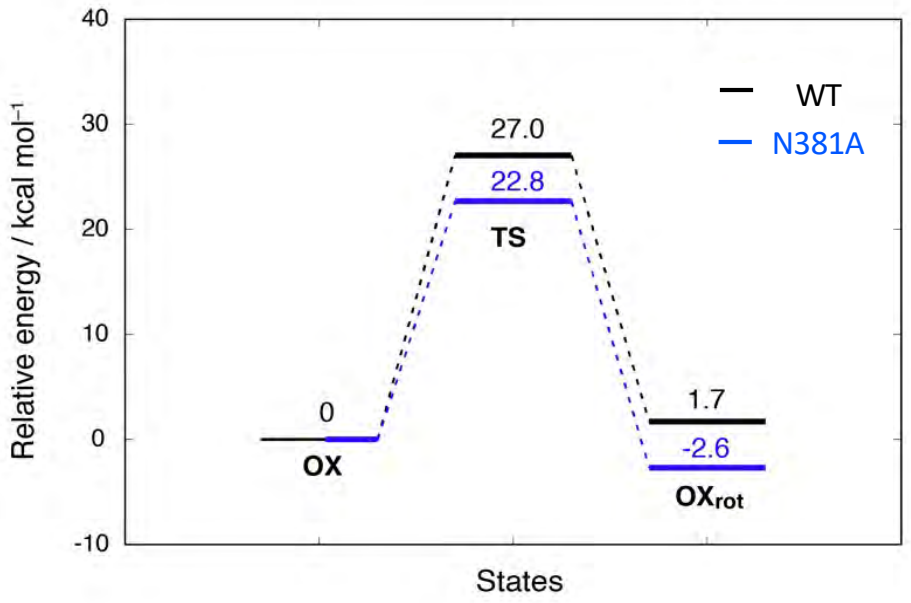
slide $\Delta E^{\ddagger}$=19.0 kcal

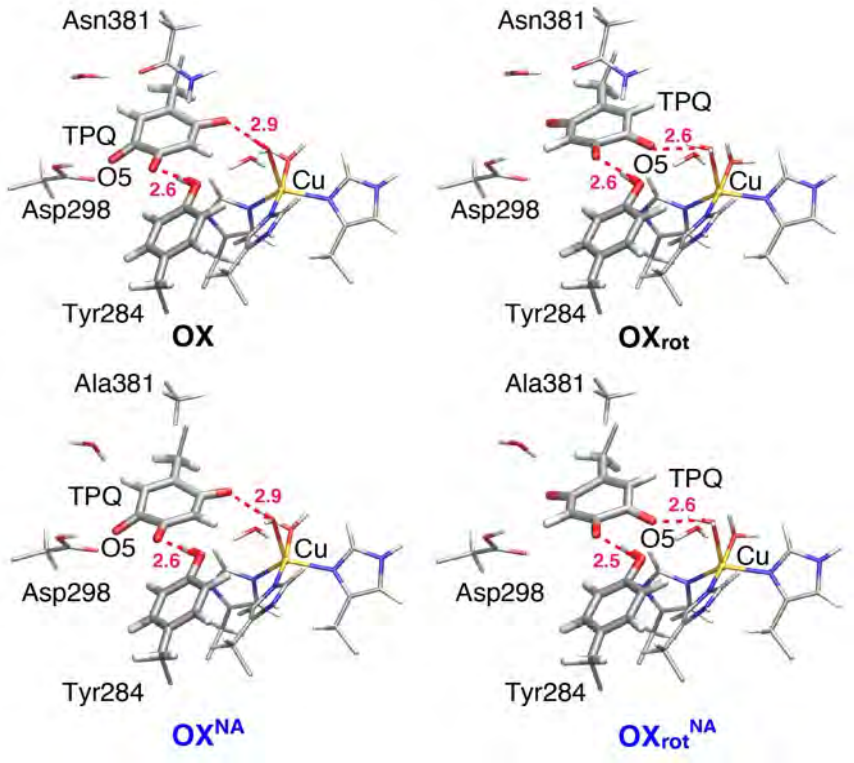
slide $\Delta E^{\ddagger}$=11.1 kcal

Map of IMS Energy profile of N381A ($1h^{NA} \rightarrow 2h^{NA} (\rightleftarrows 3h^{NA}) \rightarrow 2^{NA} \rightarrow 4^{NA} \rightarrow 5^{NA}$)



Possibilities of turnover of TPQ_{ox} in WT and N381A

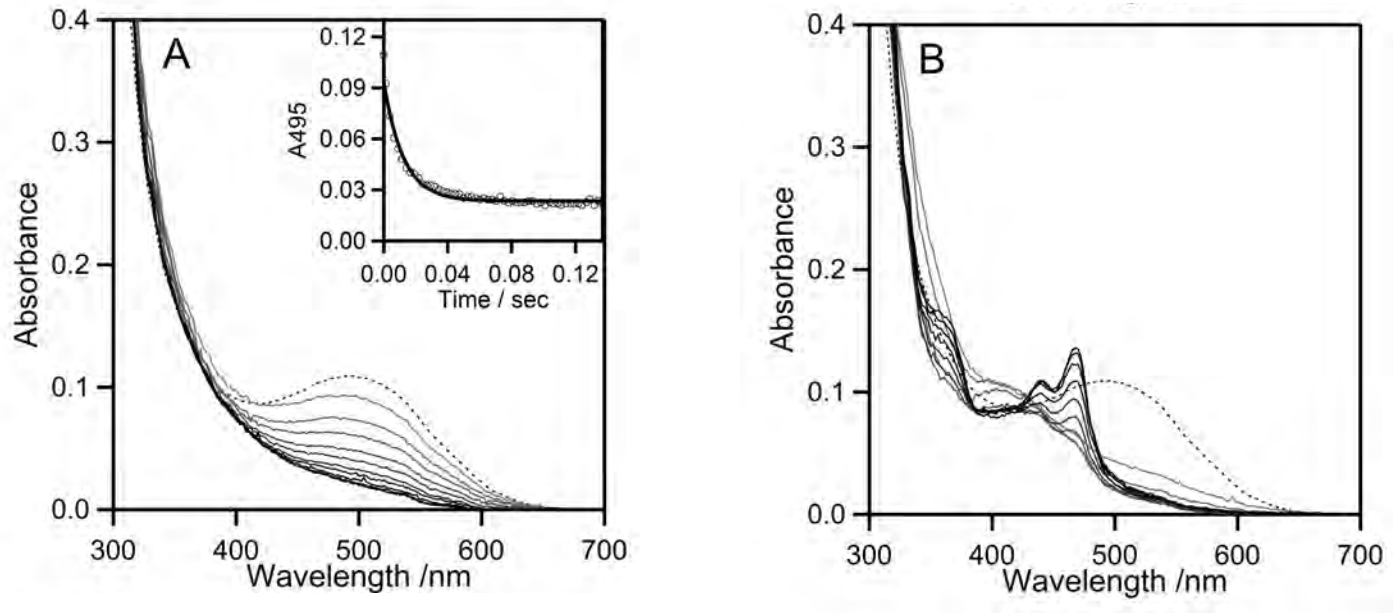




- In WT, turnover of TPQ_{ox} becomes unstable (ΔE =1.7 kcal)
- In N381A, turnover of TPQ_{ox} becomes stabilized (ΔE =-2.6 kcal).
- In N381A, $\Delta E^{\ddagger}$ also becomes low

Kinetics measurements by UV-vis spectroscopy

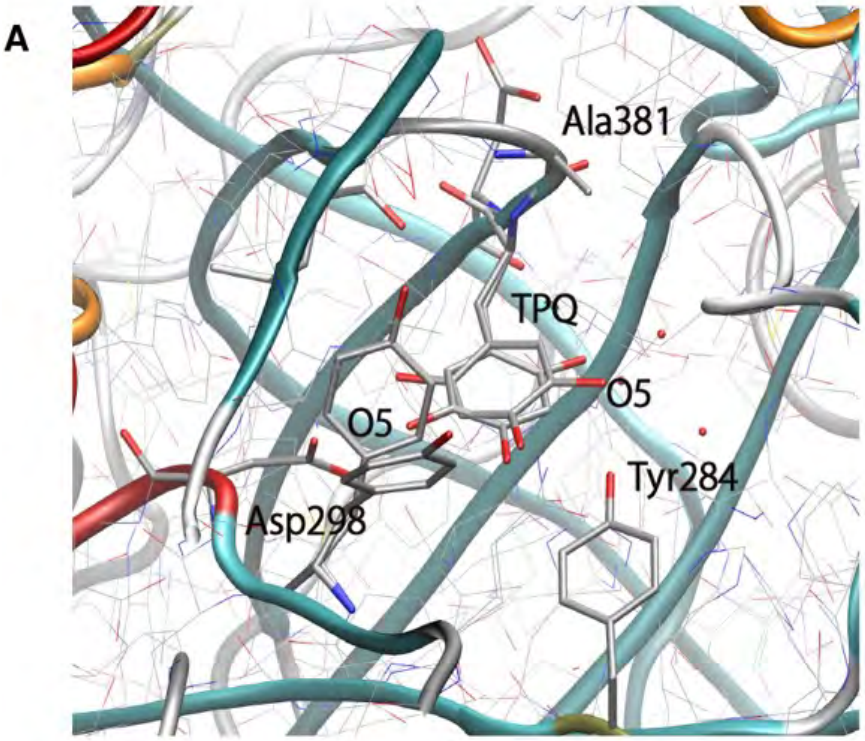
- TPQ formation decreased in N381A (1/180)
- k_{cat} is reduced in N381A (1/160)



UV-vis spectra changes by Stopped-flow spectrometer (A) the N381A mutant and (B) the WT AGAO with 2-PEA.

- Reaction stoped at TPQ_{amr} in N381A
- TPQ_{sq} formed in WT

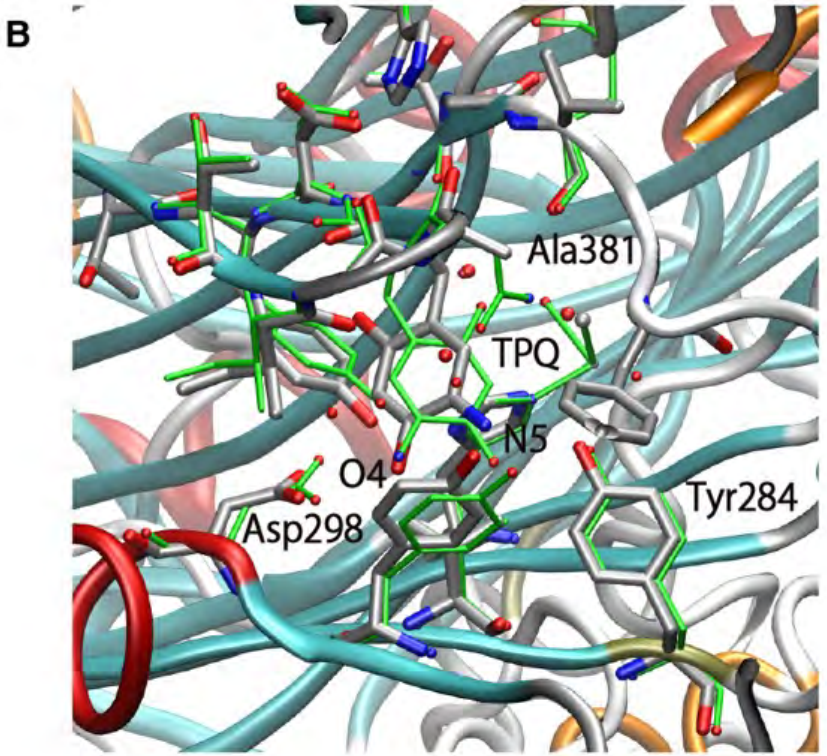
X-ray crystal structures in N381A



N381A$_{holo}$ (TPQox^{N381A})

1.50 Å resolution

TPQ flipped form can be observed (52%)
=> **Ox$_{rot}^{NA}$**



N381A$_{holo/PEA}$ (TPQ$_{amr}^{N381A}$/TPQ$_{amr}$)

1.90 Å resolution

N381A TPQ$_{amr}$ take a TPQ flipped form
=> **3h^{NA}**

Conclusion

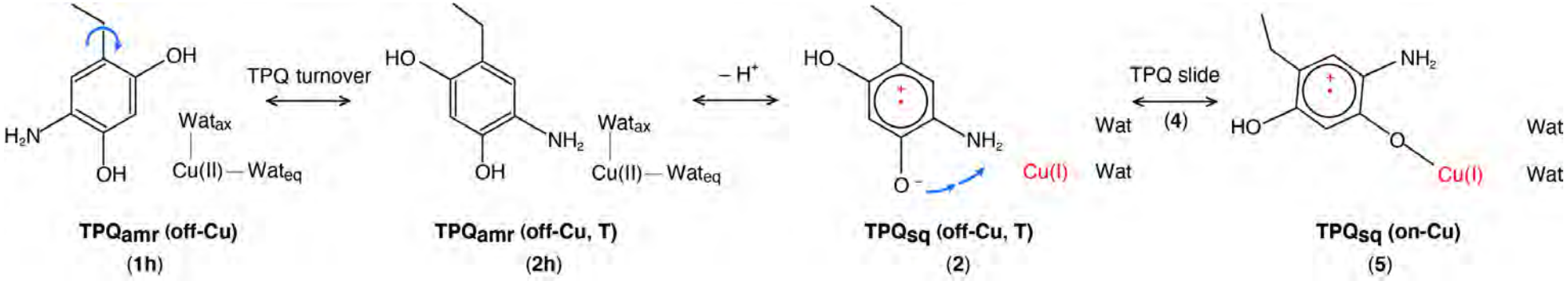
Theoretical study

- In WT, 1A:turnover(clockwise)& slide pathway is possible for TPQ_{amr}→ TPQ_{sq}
- In N381A, reaction barriers of turnover and slide steps become low, and TPQ flipped form, TPQ_{amr}(**3h^{NA}**), is most stabilized even to TPQ_{sq}.
- In N381A, a flipped form of TPQ_{ox} is stabilized. This conformation is inactive for the reductive half reactions.
- N381 controls the conformation of TPQ in TPQ_{ox} and TPQ_{amr} . In the formation of TPQ_{sq}, the conformational change of TPQ contributes to stabilize TPQ_{sq}.

Experimental study

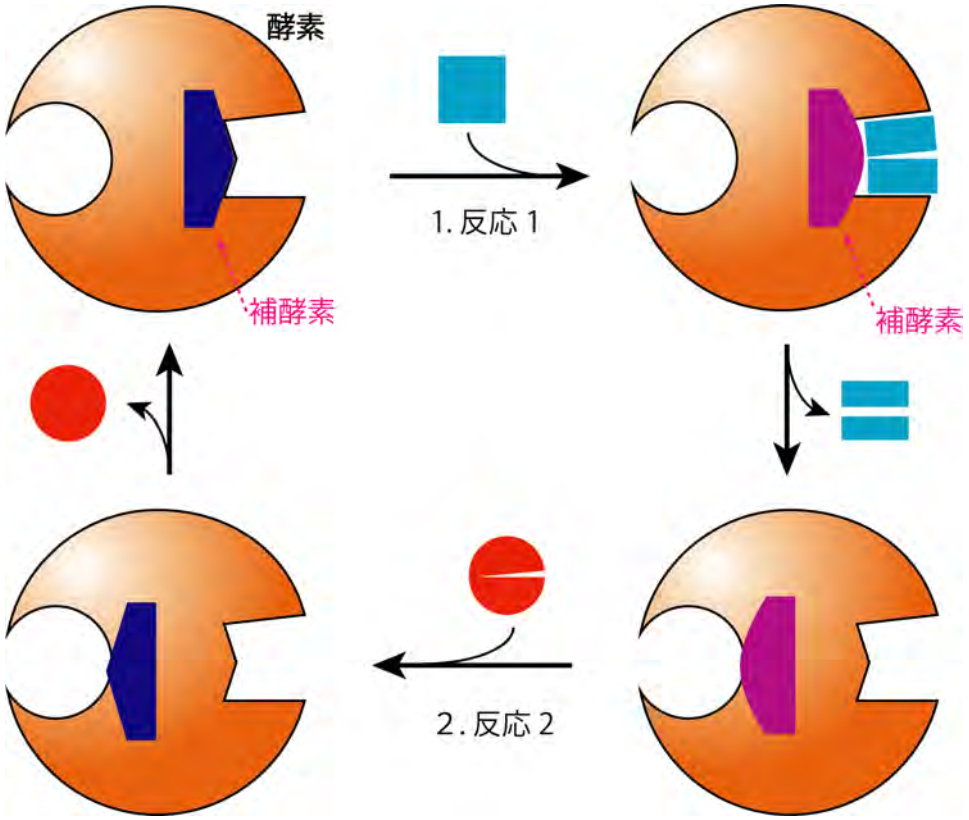
- Detection of intermediate states by Spectroscopy
- X-ray crystal structures of N381A in TPQ_{ox} and TPQ_{amr}

Proposed reaction mechanism for TPQ_{amr}→ TPQ_{sq}



M. Shoji* *et al.*, Molecular mechanism of a large conformational change of the quinone cofactor in the semiquinone intermediate of bacterial copper amine oxidase, *Chem. Sci.* **13**, 10923 (2022).

CAO is a nonclassical enzyme（nonstatic、dynamical、quantum？）

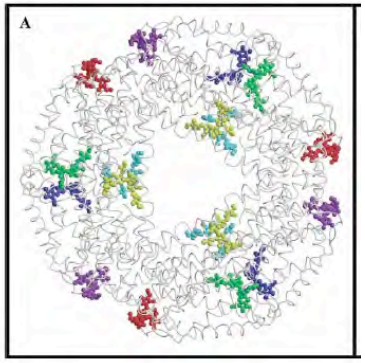


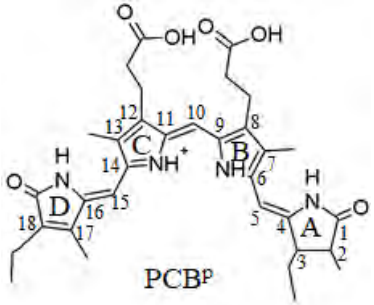
- Reaction really possible？
  There is a pathway
- Why the conformation takes place？
  stabilization of TPQ_{sq}

- Multistep reaction (reaction 1 and 2)
- State specific reaction
- important for highly efficient catalysis

Collaborative researches in a research area, Grant-in-Aid for Scientific Research on Innovative Area, of “Molecular Movies”

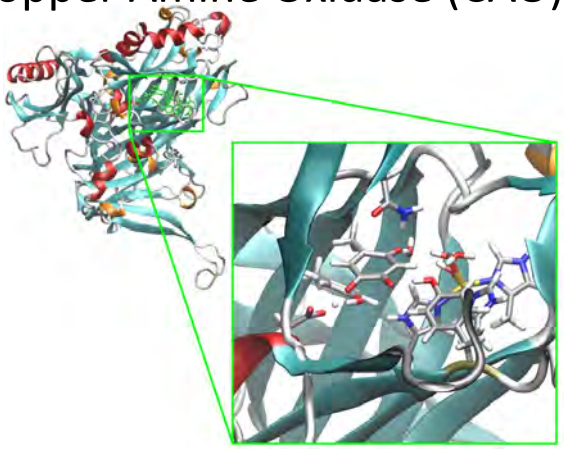
1. Light-harvesting protein, C-Phycocyanin





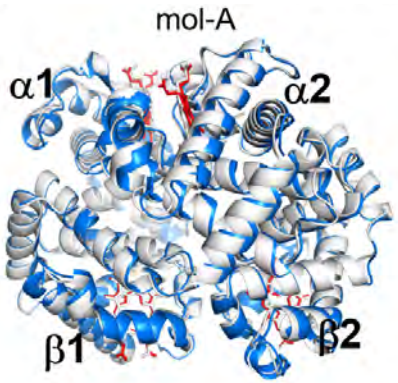
A01: Y. Umena

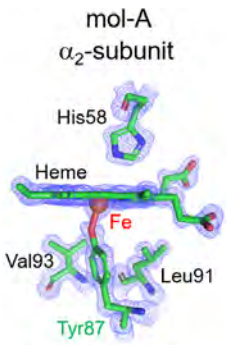
2. Copper Amine Oxidase (CAO)



A01: T. Murakawa

3. Hemoglobin M Iwate (Hb M Iwate)

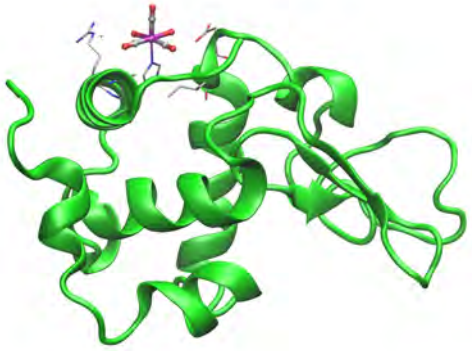


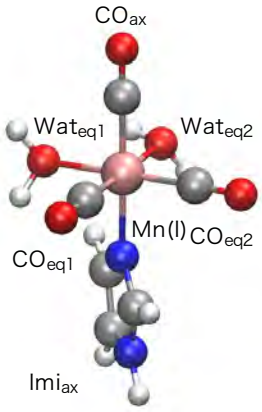


S.Nagatomo, A01: S.-Y. Park, C01: M.Kubo

S.Nagatomo*, M.Shoji, et al., Heme-bound tyrosine vibrations in hemoglobin M: Resonance Raman, crystallography, and DFT calculation, Biophysical Journal, 121(14), 2767-2780, 2022

4. Lysozyme・$Mn(CO_3)$ complex

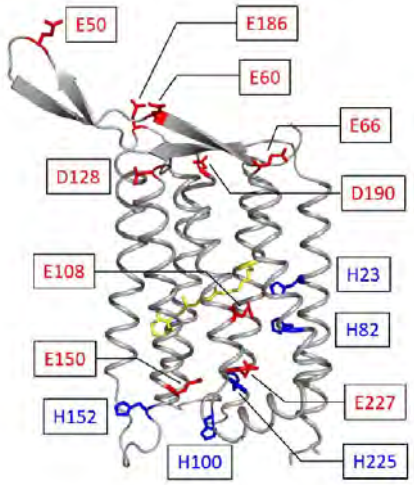




B01: Basudev Maity

Collaborative researches in the research area of "Molecular Movies"

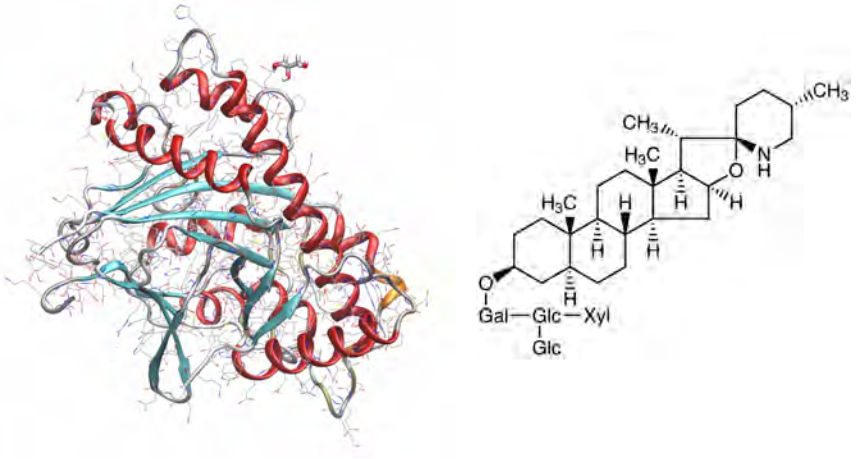
5. Heliorhodopsin (HeR)



M.Hashimoto et al, JPCL 2020

A01: K. Katayama, H. Kandori

6. 2-oxoglutarate-dependent dioxygenase (2OGD)



A01: S. Nagano

M.Hashimoto, K.Miyagawa, M.Singh, K.Katayama, M.Shoji, Y.Furutani, Y. Shigeta, H.Kandori, "Specific zinc binding to heliorhodospin", PCCP, 25, 3535 (2023).