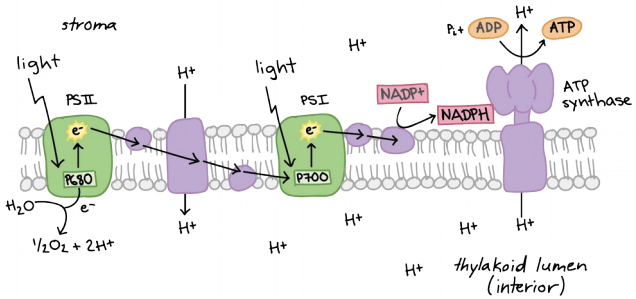
Multiscale modeling of charge transfer reactions in biological systems

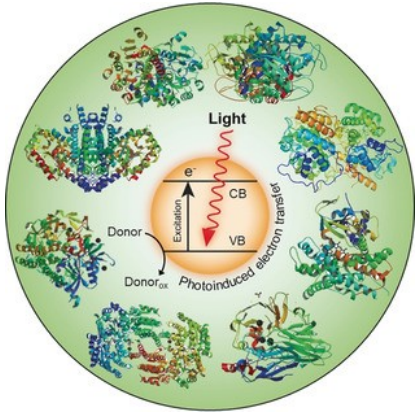
Laura Zanetti Polzi

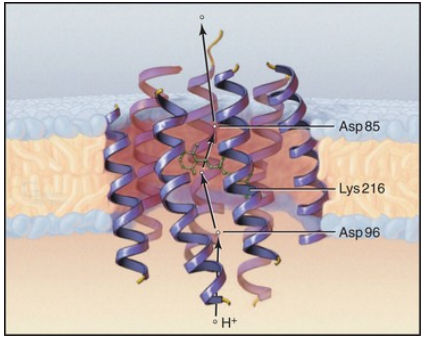
Charge Transfer in Biological Systems

"Life on earth is energized by the stepwise vectorial transport of individual electrons and protons."*

- Photosynthesis
- Respiration
- Proton pumping
- Catalysis
- ...







*Beratan et al. Acc. Chem. Res. 2015, 48.2, 474-481.

Hybrid quantum/classical approaches

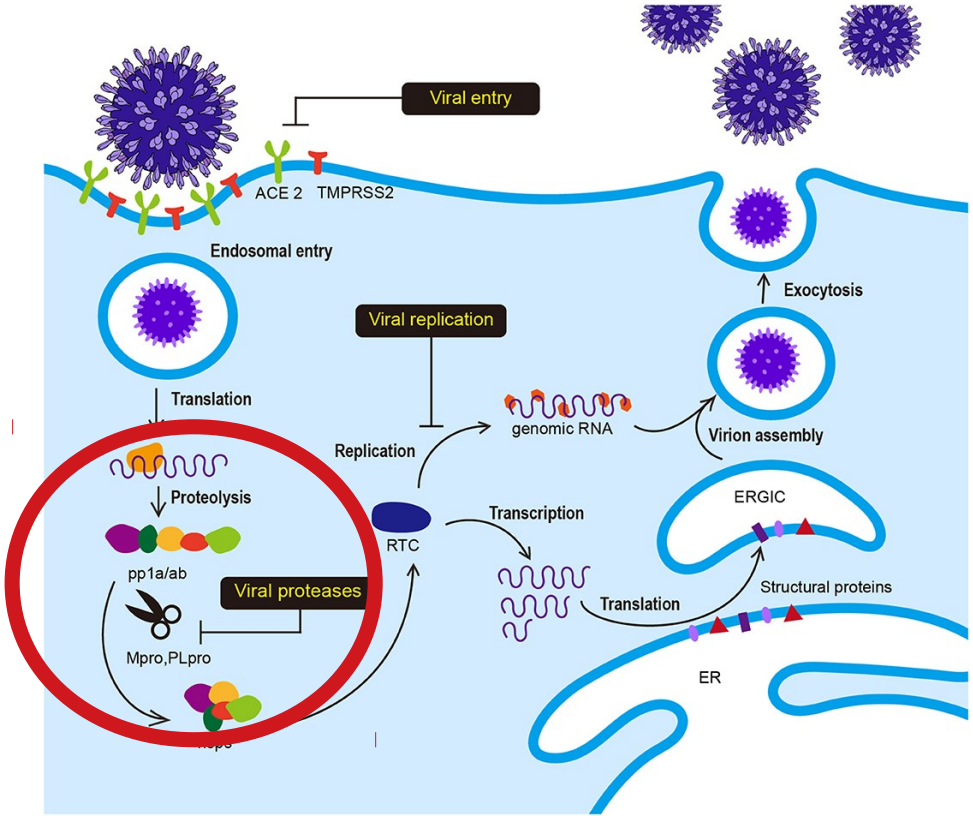
- Proper treatment of the reaction event at the quantum level
- Proper description of the complexity of the system
- Interplay between reactants and environment considered

Interpretation at the molecular level of the reaction/process of interest

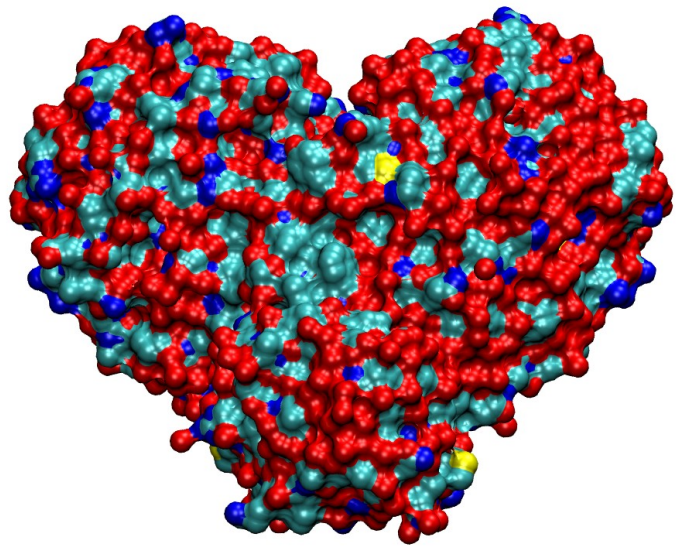
Two charge transfer reactions

- The catalytic proton transfer (PT) reaction in SARS-CoV-2 main protease (Mpro)
- The light-induced electron transfer (ET) reaction in lactate monooxygenase (LMO)

SARS-Cov-2 main protease (Mpro)



Mpro plays a key role in viral replication and transcription

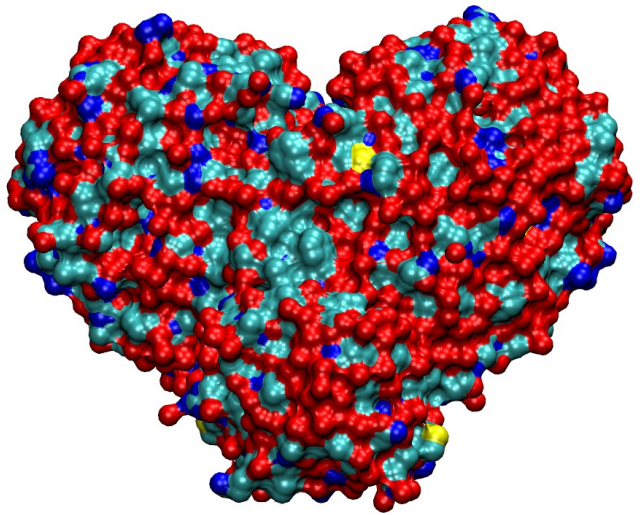


Jeong et al. *Front. Microbiol*. 11, 2020, 1723.

SARS-Cov-2 main protease (Mpro)

Mpro is one of the most promising targets for drug development

- Inhibition of its cleaving activity would block the viral replication cycle
- Its recognition sequence is different from that of all human proteases
- Mpro structure is very similar among the coronaviruses family

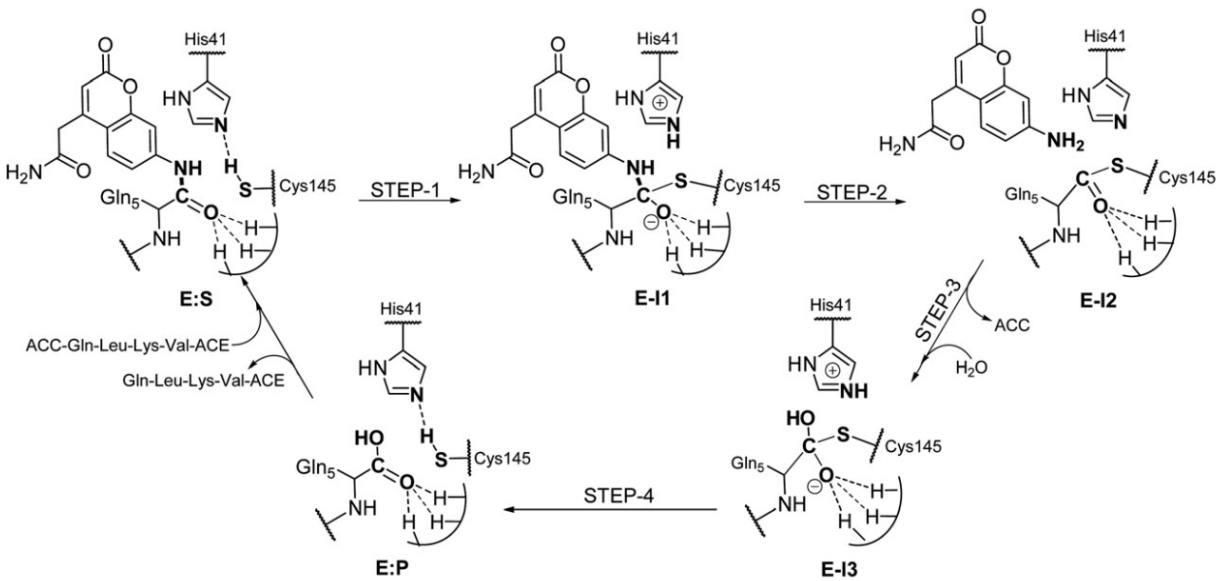


H. Su et al., *Angew. Chem.* **2021**, 133, 2 – 16.

SARS-Cov-2 main protease (Mpro)

Cysteine-histidine catalytic dyad (Cys145/His41)

Protein hydrolysis mediated by Cys145 that binds to the carbonyl carbon of a susceptible peptide bond



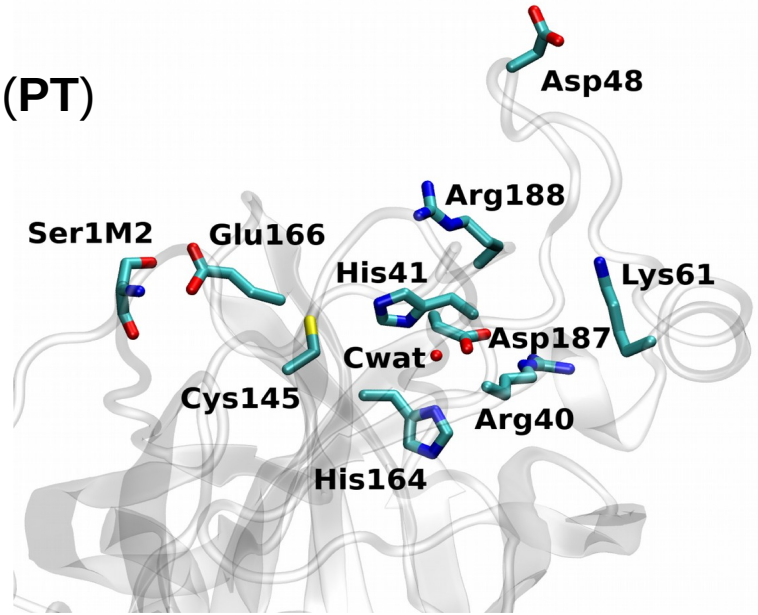
Moliner V. et al., *Chem. Sci.* **2020**, 11, 10626–10630; Tunon, I. et al., *ACS Catal.* **2020**, 10, 12544–12554; Warshel, A. et al, *Biochem.* **2020**, 59, 4601–4608; Paasche, A. et al., *Biochem*. **2014**, 53, 5930–5946.

SARS-Cov-2 main protease (Mpro)

Cysteine-histidine catalytic dyad (Cys145/His41)

The imidazole of **His41** is the base of the proton transfer (**PT**) reaction leading to a highly reactive zwitterionic couple:

$$Cys145H + His41 \rightleftharpoons Cys145^{-} + His41H^{+}$$



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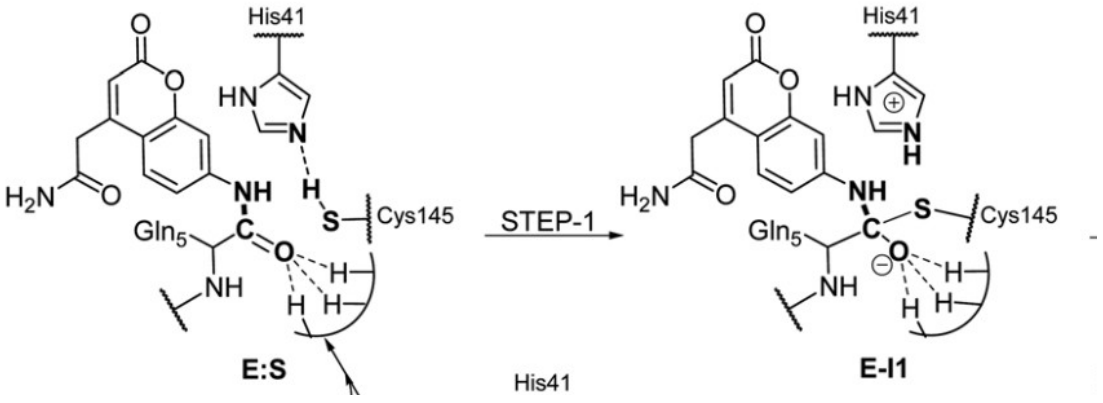
SARS-Cov-2 main protease (Mpro)

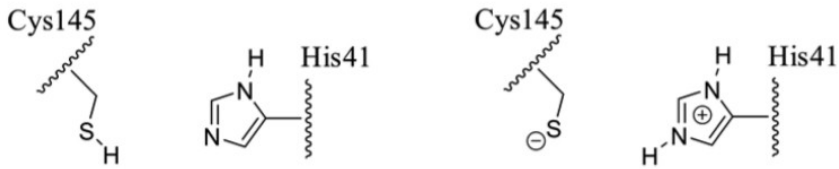
- The covalent binding of some classes of inhibitors (such as Michael acceptors and ketoamides) also requires **deprotonated Cys145** through a **PT** to **His41**.



SARS-Cov-2 main protease (Mpro)

- The covalent binding of some classes of inhibitors (such as Michael acceptors and ketoamides) also requires **deprotonated Cys145** through a **PT** to **His41**.

- The inability to efficiently promote the **PT** reaction has been suggested to determine the low inhibition potencies of some known inhibitors*.



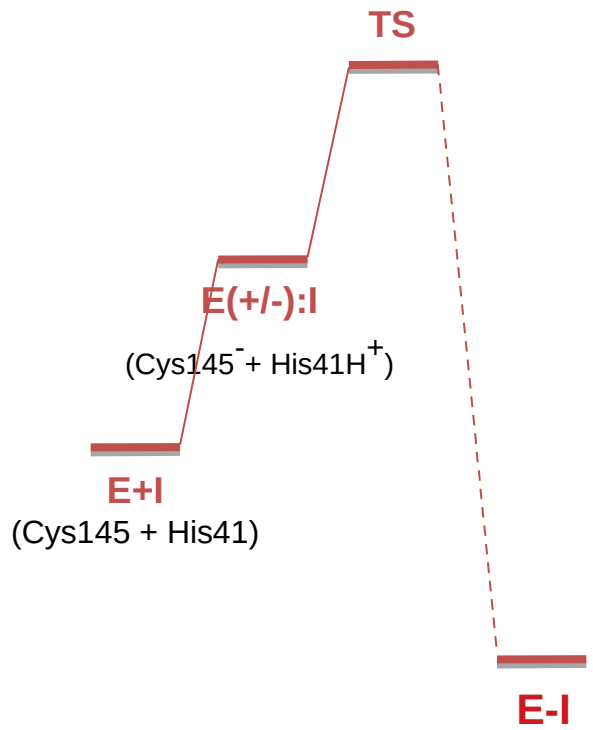
*Paasche, A. et al., *Biochem*. **2014**, 53, 5930–5946.

SARS-Cov-2 main protease (Mpro)

- The covalent binding of some classes of inhibitors (such as Michael acceptors and ketoamides) also requires **deprotonated Cys145** through a **PT** to **His41**.

- The inability to efficiently promote the **PT** reaction has been suggested to determine the low inhibition potencies of some known inhibitors*.

- The PT reaction free energy in the presence of two inhibitors (**N3** and a-ketoamide **13b**) accounts for **40-50%** of the total activation free energy for the formation of the covalent complex**.

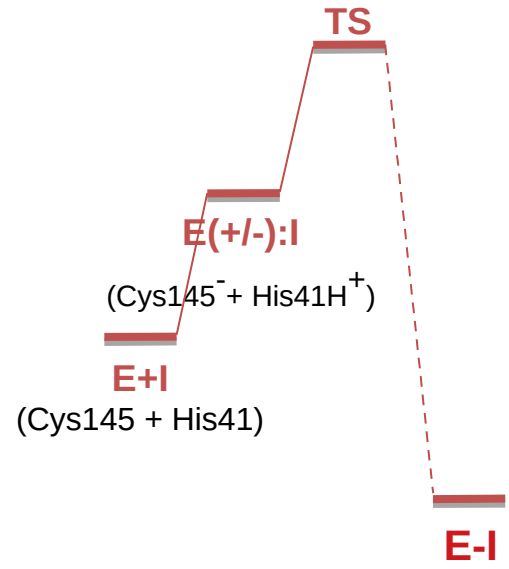


*Paasche, A. et al., *Biochem*. **2014**, 53, 5930–5946; **Tunon, I. et al., *ACS Catal.* **2020**, 10, 12544–12554; Warshel, A. et al, *Biochem.* **2020**, 59, 4601–4608.

SARS-Cov-2 main protease (Mpro)

The improvement of the stability of the charged catalytic dyad by the inhibitor binding could be a strategy to promote inhibition

Knowledge of the protein regions capable of affecting the PT reaction is a crucial point for the design and screening of potential inhibitors



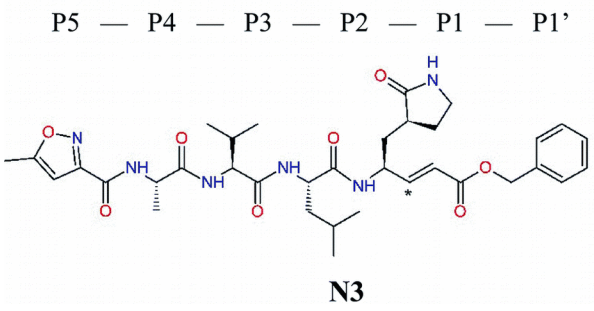
SARS-Cov-2 main protease (Mpro)

Here we focus on the investigation of the thermodynamics of the PT reaction in the apo enzyme and in complex with two inhibitors

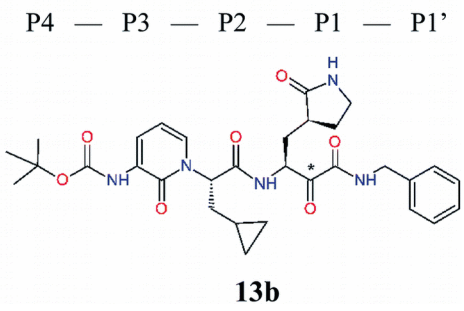
The main aim is to identify the enzyme regions and specific water molecules that control the activation of the catalytic PT reaction

Protonation state of key residues

MD simulations to investigate the effects
of different protonation states for crucial residues in Mpro
in both the **apo** form as well as **ligand-bound complexes**

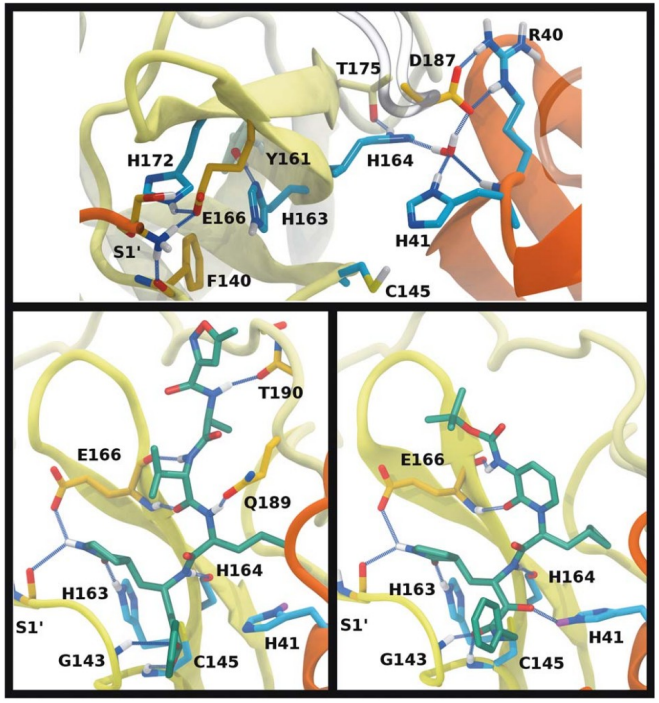


N3



13b

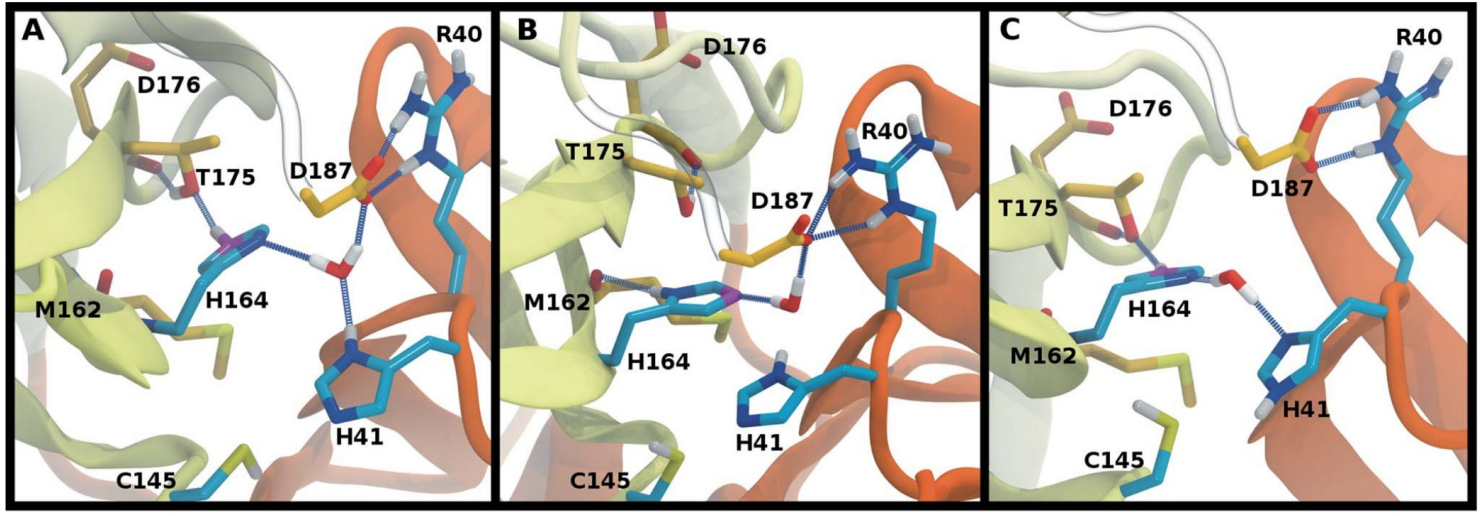
Peptidomimetic covalent inhibitors **N3** and **13b**



Pavlova, et al., *Chem. Sci.* 2021, 12, 1513–1527.

Protonation state of key residues

The combination of protonation states for histidines in or near the catalytic site can have a profound impact on Mpro's structural stability



Pavlova, et al., *Chem. Sci.* 2021, 12, 1513–1527.

Modelling the PT reaction

We treat at the QM level a small portion of the system (the **QC**, here **Cys/His sidechains**) while the rest of the system (the **environment**) exerts a **classical electrostatic perturbation** on the **QC***

$$\mathrm{Cys145H} + \mathrm{His41} \rightleftharpoons \mathrm{Cys145^-} + \mathrm{His41H^+}$$

Reactant ensemble — Product ensemble

MD simulations* of the **apo** enzyme: in the reactant ensemble and in the product ensemble

*Zanetti-Polzi et al., *Phys. Chem. Chem. Phys.* 2020, 22, 19975–19981; *Pavlova, et al., *Chem. Sci.* 2021, 12, 1513–1527.

Modelling the PT reaction

$$\mathrm{Cys145H} + \mathrm{His41} \rightleftharpoons \mathrm{Cys145^-} + \mathrm{His41H^+}$$

Reactant ensemble

Product ensemble

QM calculations of gas-phase electronic Hamiltonian eigenstates for the quantum centers (QCs):

⇒ Unperturbed energies and dipoles for C/H

⇒ Unperturbed energies and dipoles for C-/H+

$$H^{\circ}_{QC} = \begin{pmatrix} E^{\circ}_{S0,QC} & 0 & 0 & \cdots \\ 0 & E^{\circ}_{S1,QC} & 0 & \cdots \\ 0 & 0 & E^{\circ}_{S2,QC} & \cdots \\ & \vdots & & \end{pmatrix}$$

Modelling the PT reaction

Construction and **diagonalization** of the perturbed Hamiltonian matrix in order to get the **perturbed** energies at each step of the **MD simulation**:

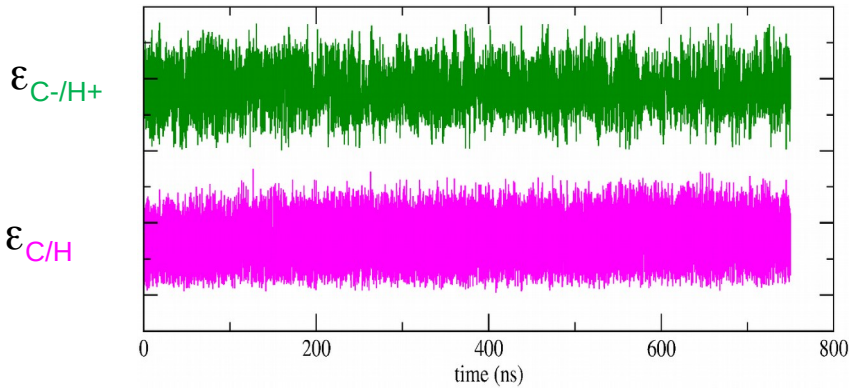
$$V_{i,j} = q_{qc}\mathcal{V}(r_0) - \boldsymbol{E}(r_0) \cdot \langle \Phi_i^0 | \hat{\boldsymbol{\mu}}_{i,j} | \Phi_j^0 \rangle$$

environment electrostatic potential

environment electric field

$$H_{QC}^{P} = \begin{pmatrix} E^{\circ}_{S0,QC} + V_{00,QC} & V_{01} & V_{02} \cdots \cdots \\ V_{10} & E^{\circ}_{S1,QC} + V_{11} & V_{12} \cdots \cdots \\ V_{20} & V_{21} & E^{\circ}_{S2,QC} + V_{22} \cdots \\ & \vdots & \end{pmatrix}$$

Instantaneous perturbed eigevalues

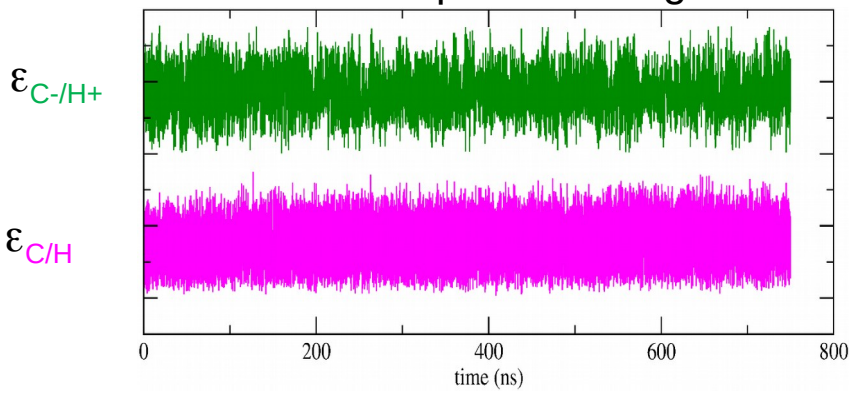


Product ensemble $Cys145^{-} + His41H^{+}$

Reactant ensemble $Cys145H + His41$

Modelling the PT reaction

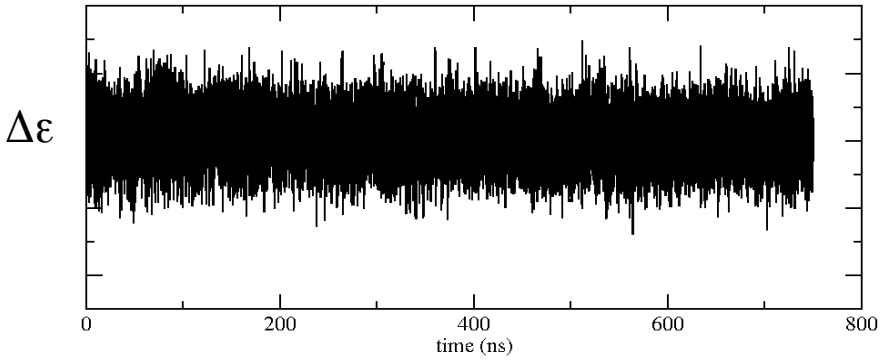
Instantaneous perturbed eigevalues



Product ensemble $Cys145^{-} + His41H^{+}$

Reactant ensemble $Cys145H + His41$

Calculation of the PT energy variation



$\Delta\varepsilon = \varepsilon_{C-/H+} - \varepsilon_{C/H}$

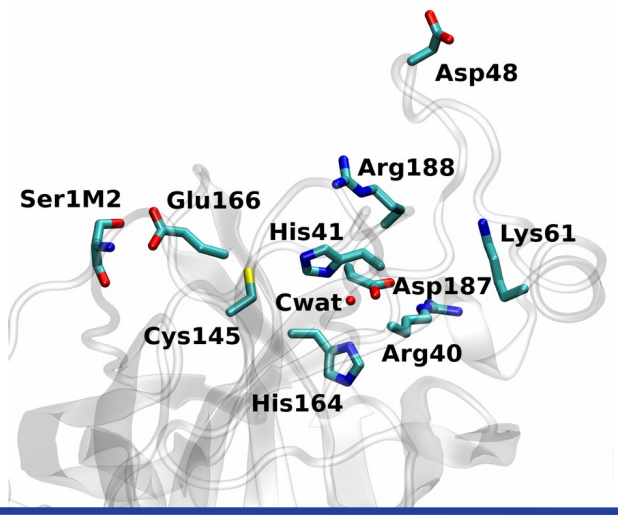
Calculation of the free energy change, $\Delta G°$, from the $\Delta\varepsilon$:

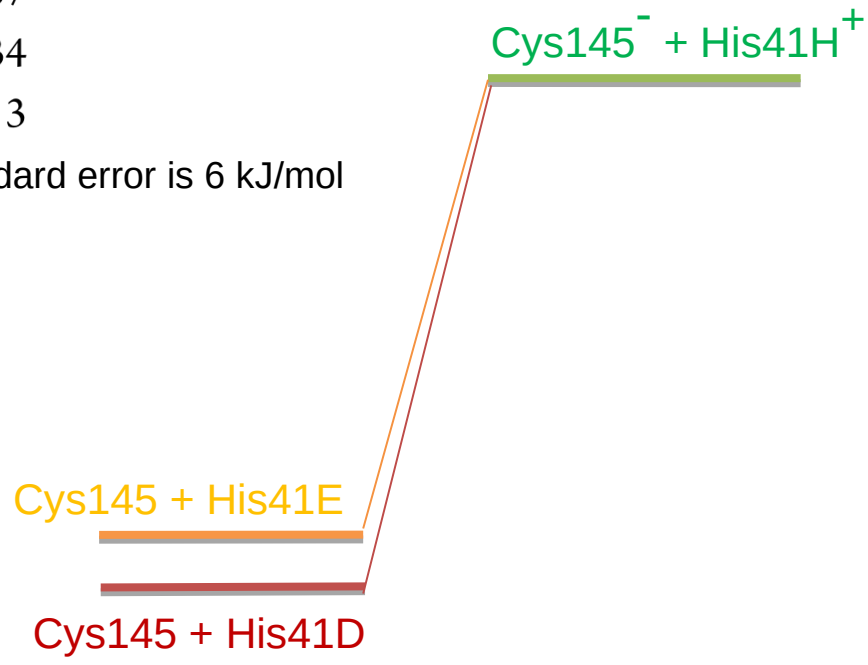
$$\Delta G^0 \cong \frac{k_B T}{2} \ln \frac{\langle e^{\beta \Delta \varepsilon} \rangle_P}{\langle e^{-\beta \Delta \varepsilon} \rangle_R}$$

PT reaction in the apo state

	ΔG^0 *
$(\text{Cys145H} + \text{His41E} \rightleftharpoons \text{Cys145}^- + \text{His41H}^+)_{\text{apo}}$	37
$(\text{Cys145H} + \text{His41D} \rightleftharpoons \text{Cys145}^- + \text{His41H}^+)_{\text{apo}}$	34
$(\text{His41E} \rightleftharpoons \text{His41D})_{\text{apo}}$	3

*kJ/mol, mean standard error is 6 kJ/mol





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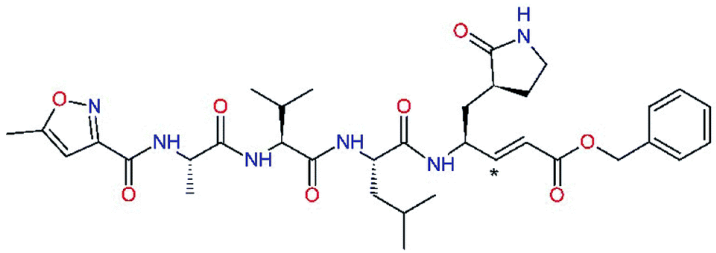
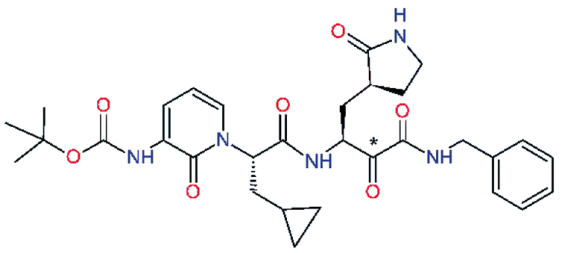
PT reaction with inhibitors

P5 — P4 — P3 — P2 — P1 — P1’



N3

P4 — P3 — P2 — P1 — P1’



13b

Peptidomimetic covalent inhibitors **N3** and **13b**

Stars (*) indicate the sites of nucleophilic attack of anionic sulphur of cysteine of the catalytic dyad

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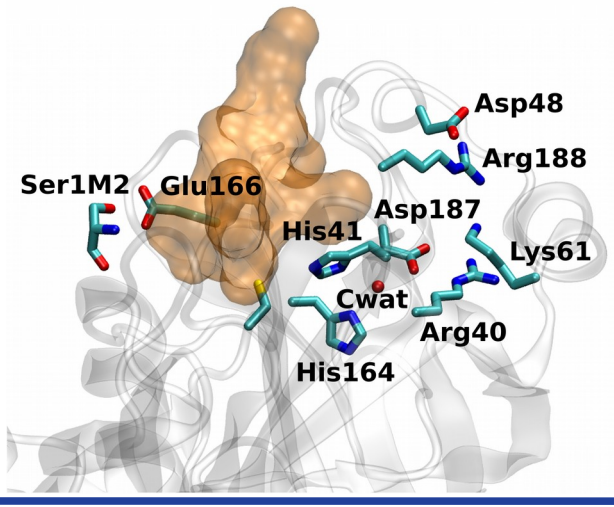
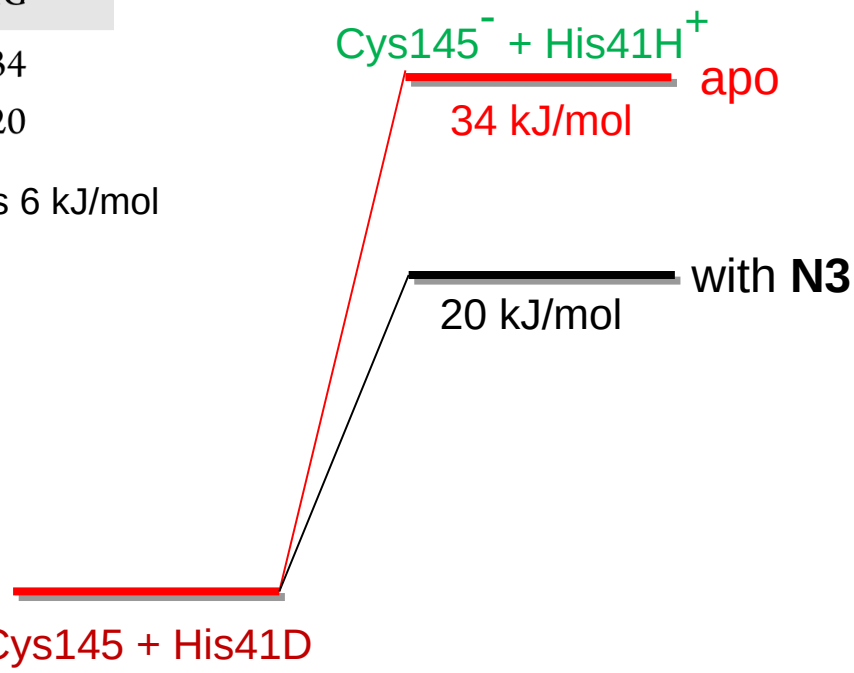
PT reaction with inhibitor N3

	ΔG^0 *
$(\mathrm{Cys145H + His41D \rightleftharpoons Cys145^- + His41H^+})_{\mathrm{apo}}$	34
$(\mathrm{Cys145H + His41D \rightleftharpoons Cys145^- + His41H^+})_{\mathrm{N3}}$	20

*kJ/mol, mean standard error is 6 kJ/mol





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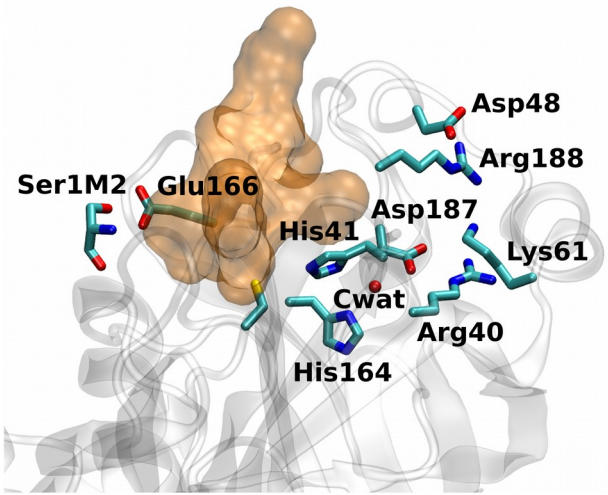

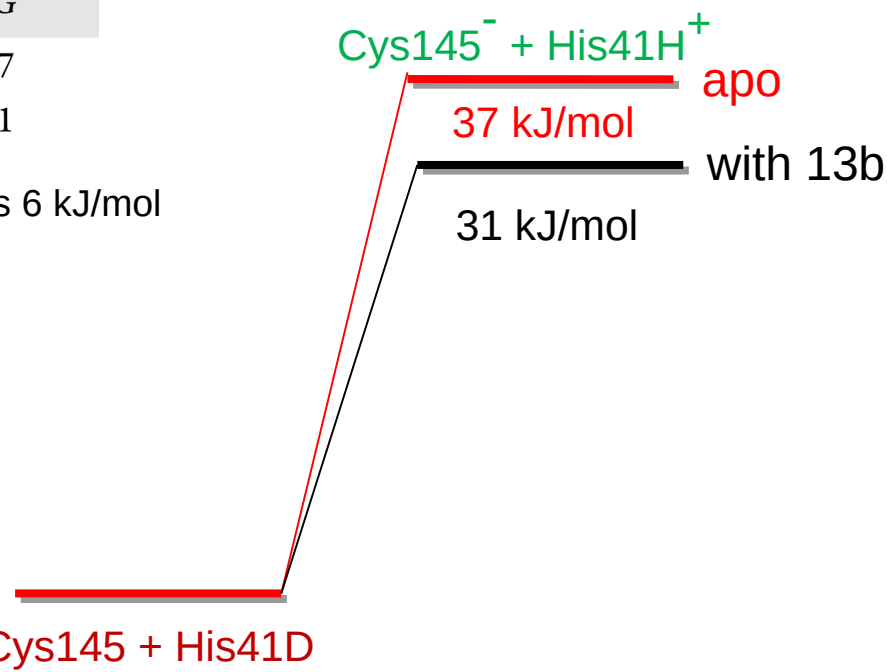

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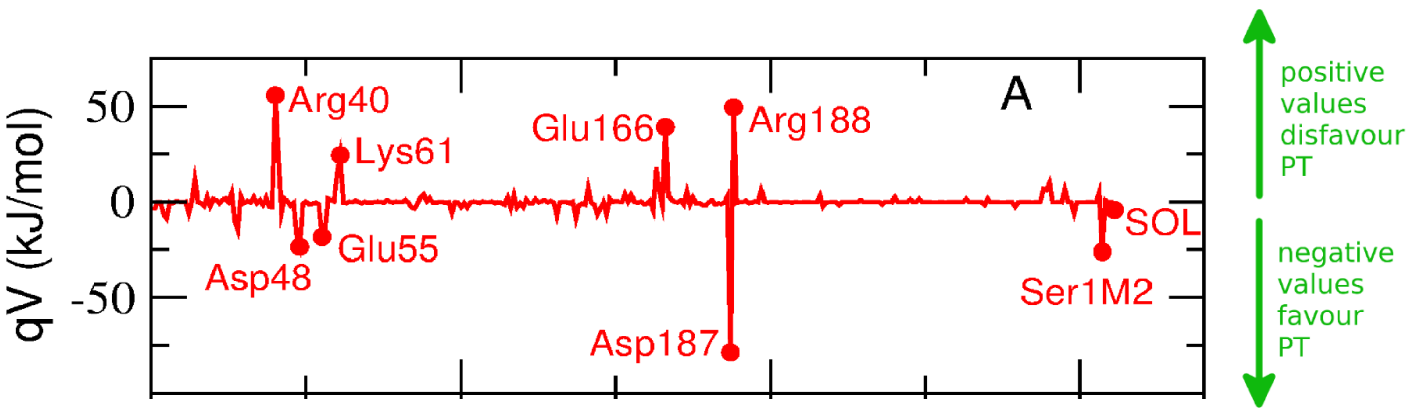
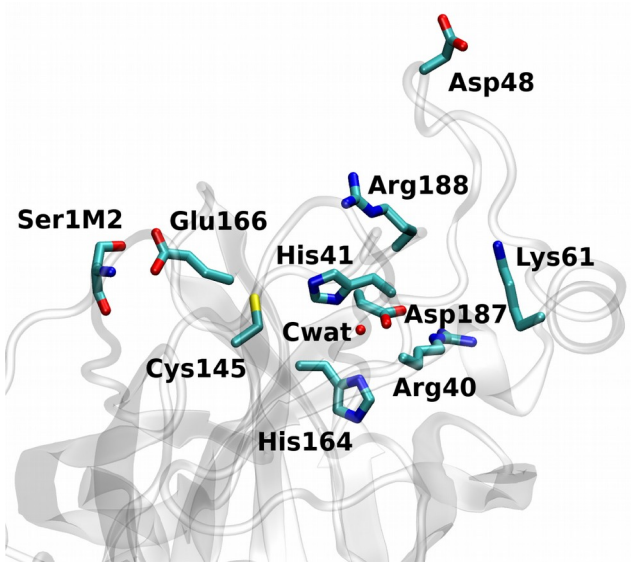
Molecular contributions to the PT energetics

Analysis of the contribution of each protein residue to the **electrostatic potential** to understand which protein regions contribute the most to the **PT** energy

APO state





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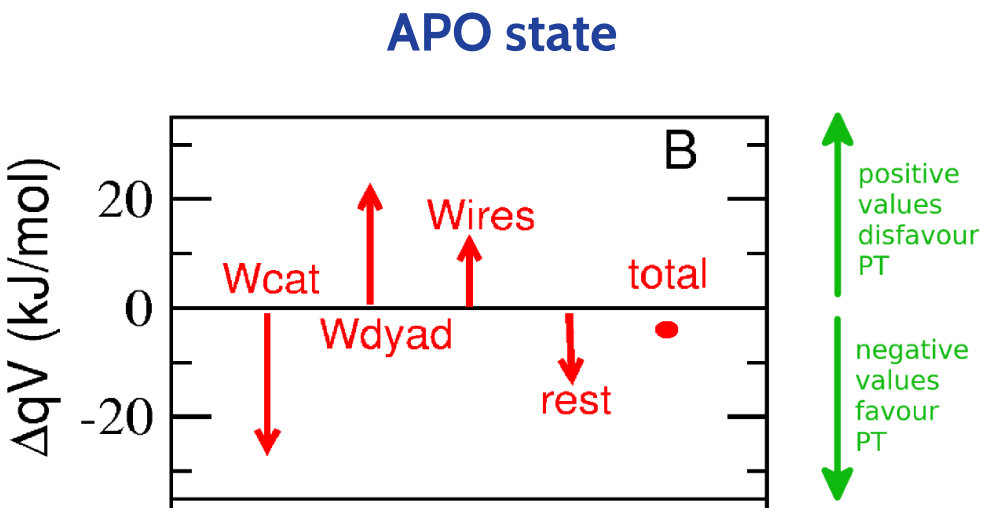

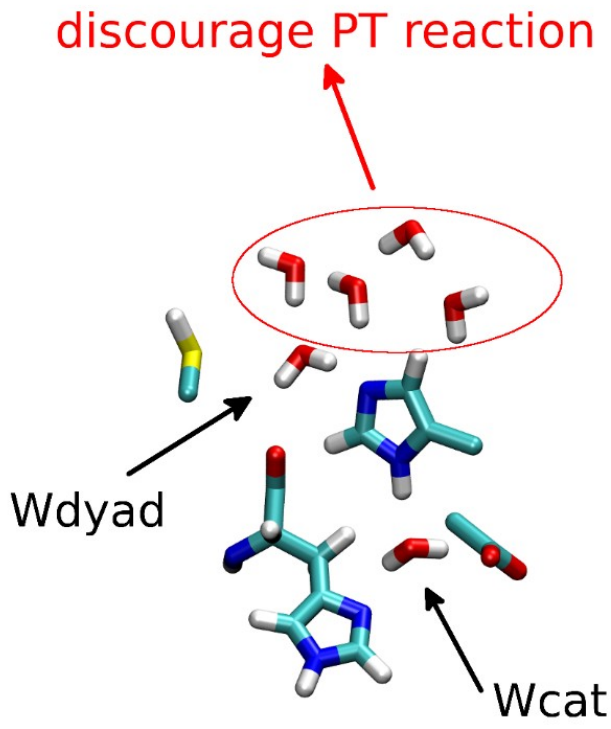

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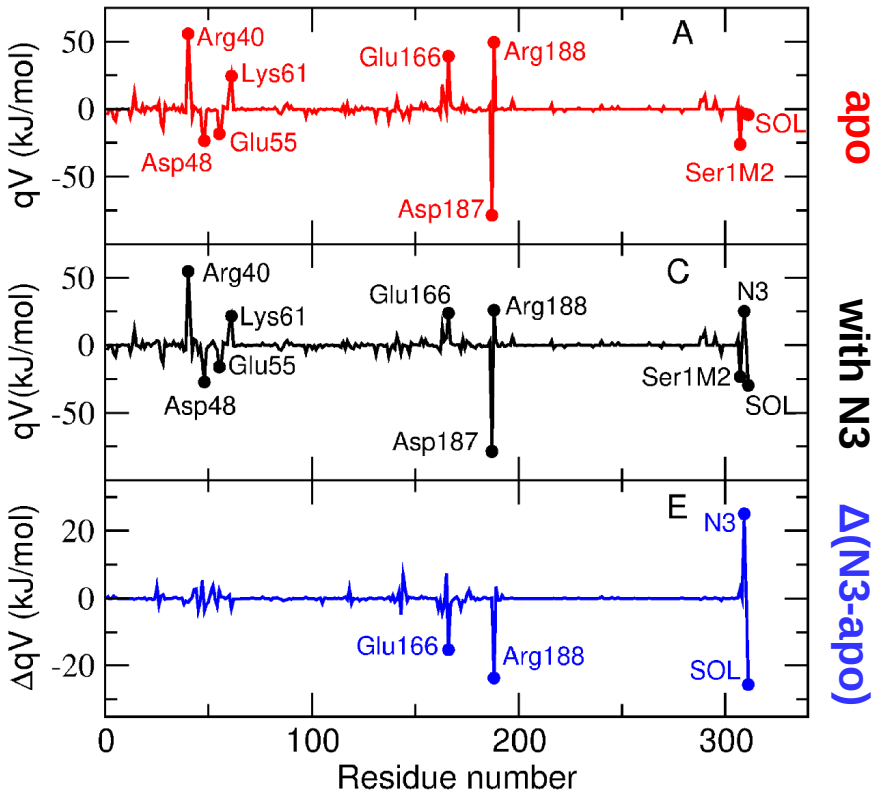
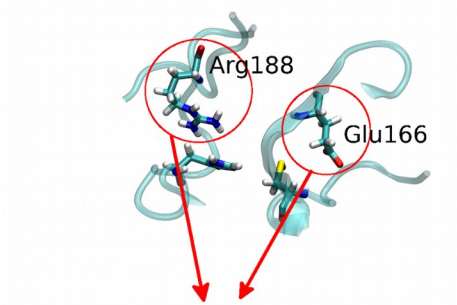


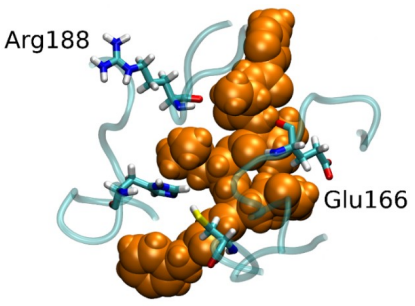
positive values disfavour PT

negative values favour PT



discourage PT reaction

APO state



unfavourable contribution reduced

with N3

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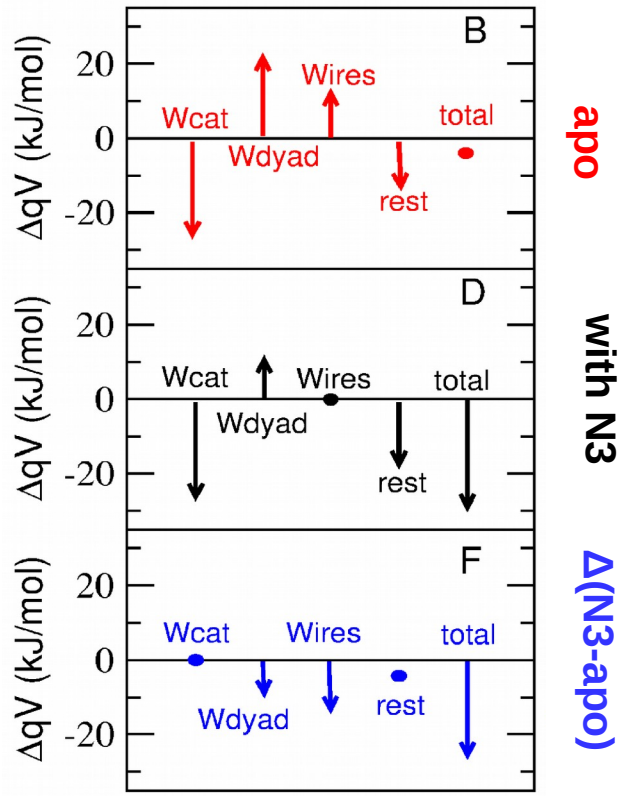
Molecular contributions to the PT energetics



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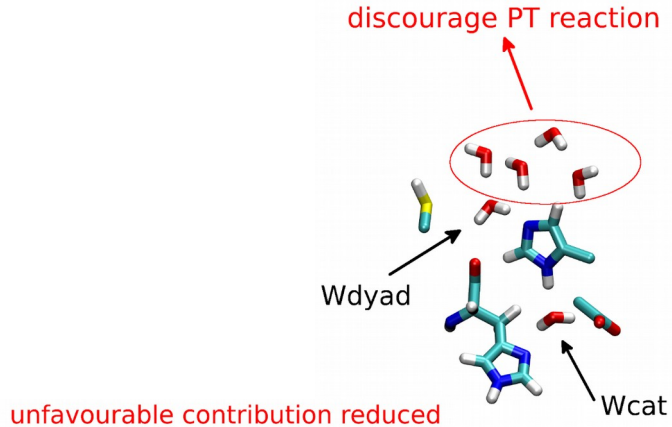
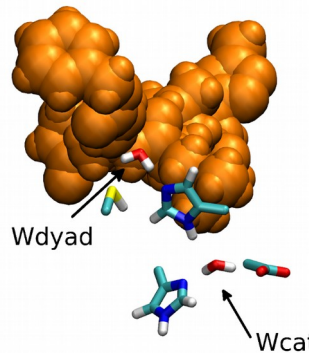


APO state

unfavourable contribution reduced



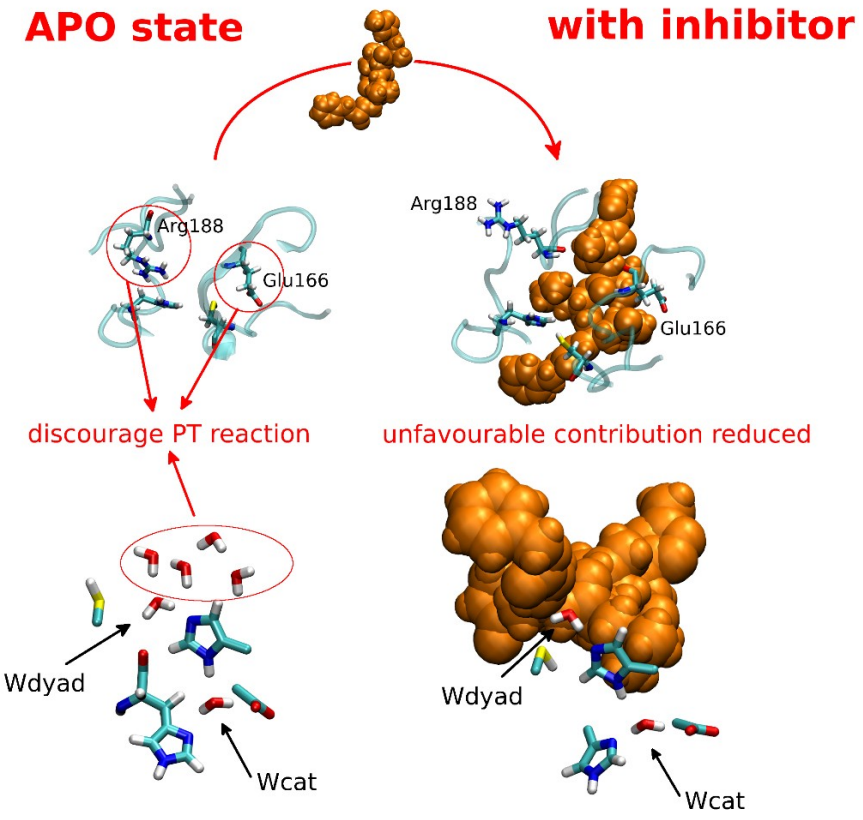
with N3

Laura Zanetti-Polzi et al. J. Phys. Chem. Lett. 2021, 12(17), 4195-4202

In Summary

The present results can help identify:

- **compounds** that can **promote** the catalytic **PT reaction** and, therefore, be good candidates as covalent inhibitors;
- specific **water molecules** able to affect the PT energetics and that could be explicitly included in docking procedures;
- **key sites** that can be targeted with ligands, in the framework of **allosteric inhibition**, to suppress the enzymatic activity.



Two charge transfer reactions

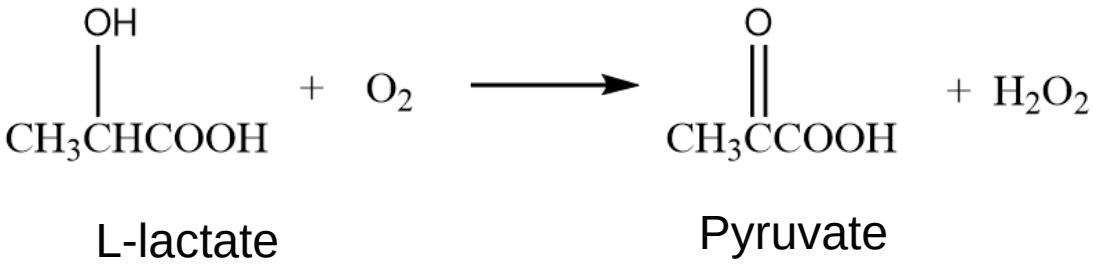
- The catalytic proton transfer (PT) reaction in SARS-CoV-2 main protease (Mpro)

- The light-induced electron transfer (ET) reaction in lactate monooxygenase (LMO)

An ongoing work

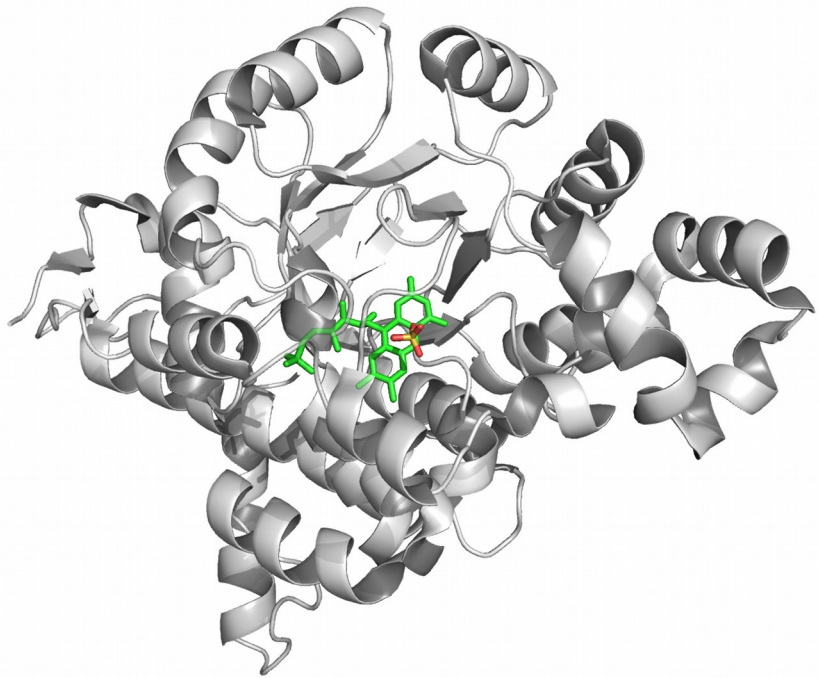
Lactate Monooxygenase (LMO)

Biological function:



L-lactate

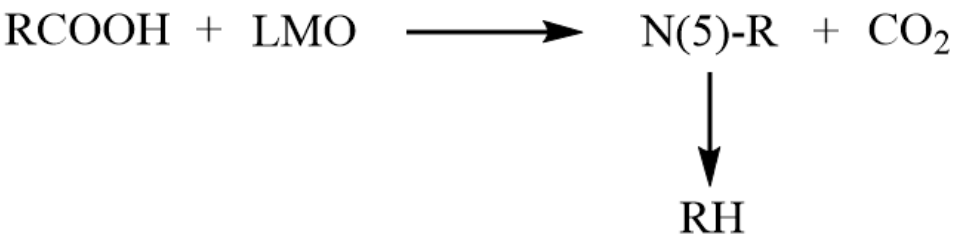
Pyruvate



Kean and Karplus, Protein Sci., 2019, **28**, 135-149

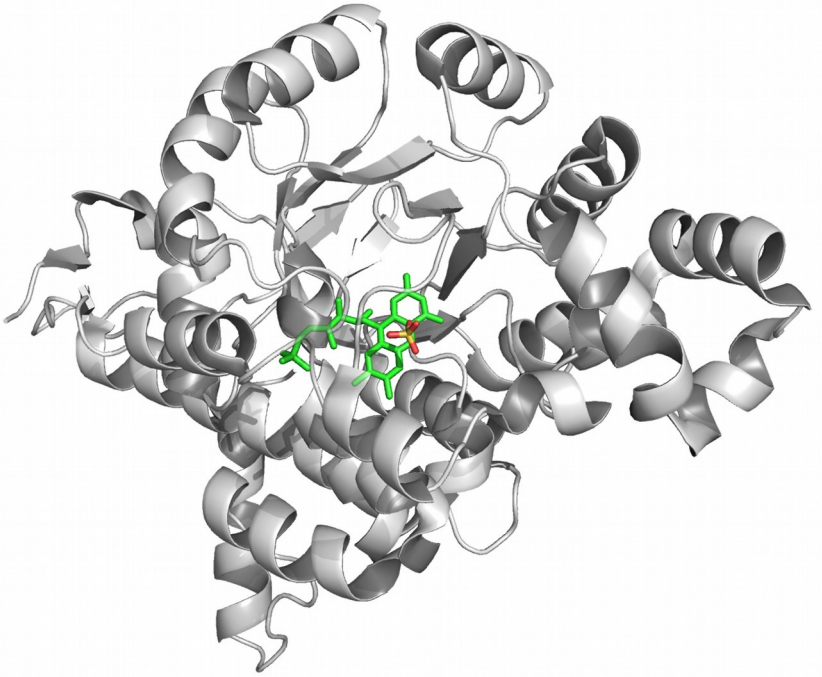
Lactate Monooxygenase (LMO)

Photoreaction:



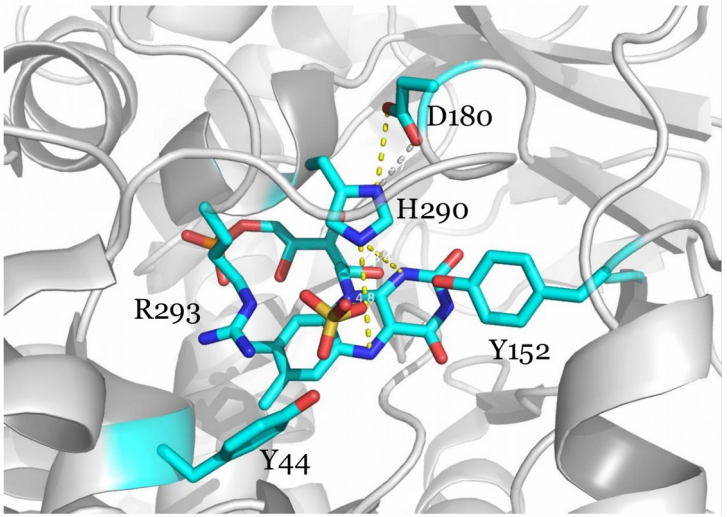
R = -COOH -$CONH_2$ -$COCH_3$ -COCOOH
-CH_2COOH -CHOHCOOH .....

Mechanism?

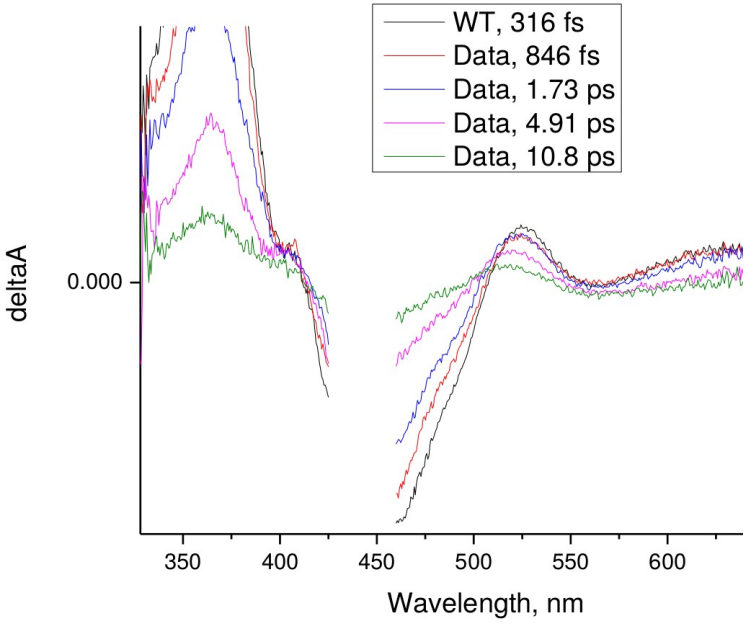


Experimental data

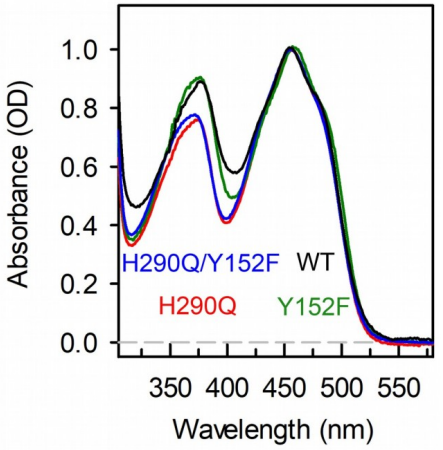
Intrinsic ET (apo state)



Pump-probe spectroscopy

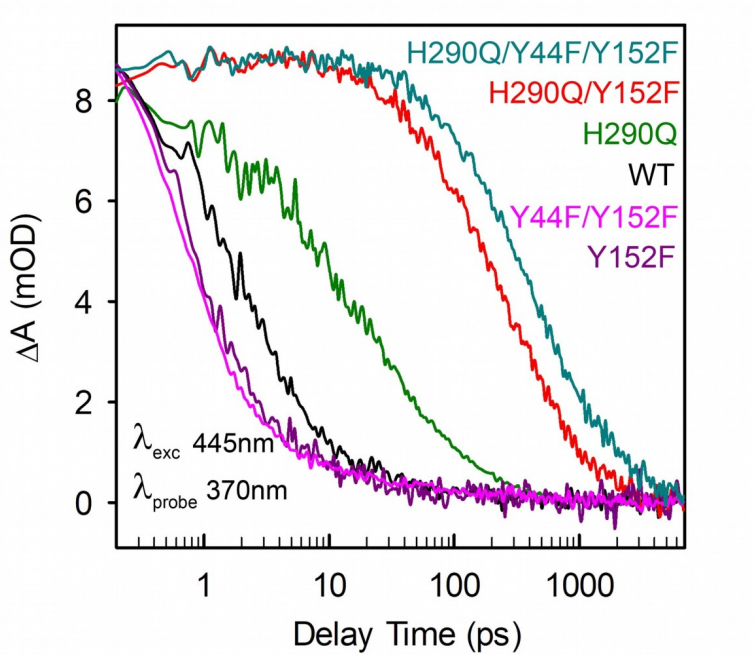


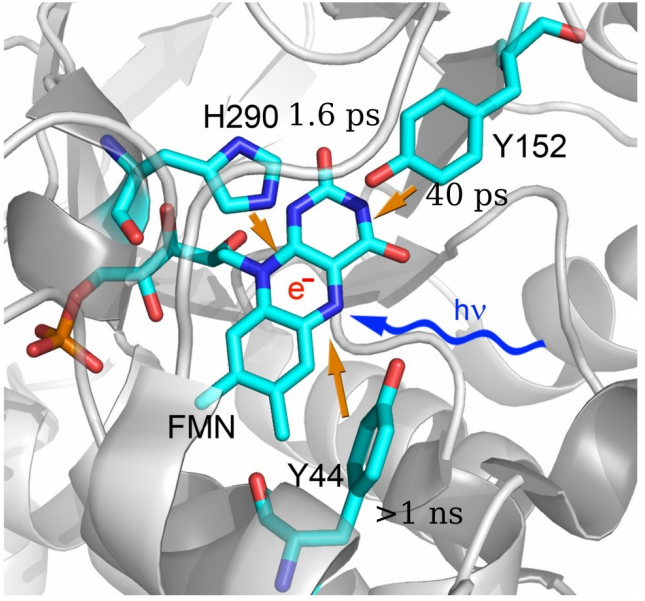
Pump @ 445 nm



Experimental data

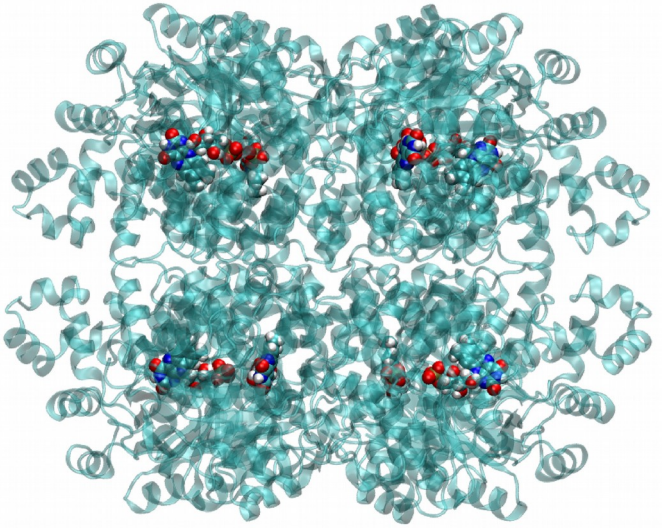
Intrinsic ET (apo state)





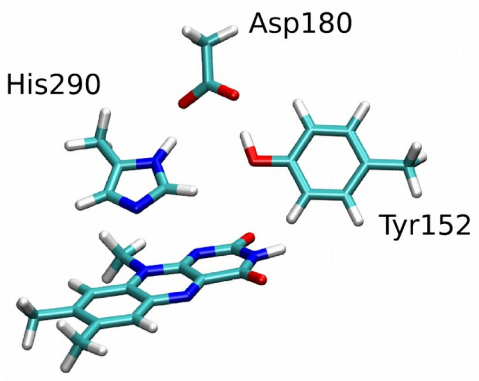
Computational data: intrinsic ET

75 ns-long MD simulation of
LMO octamer in solution
in the first excited state

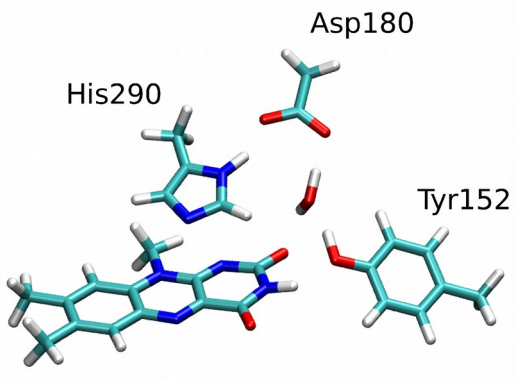


Computational data: intrinsic ET

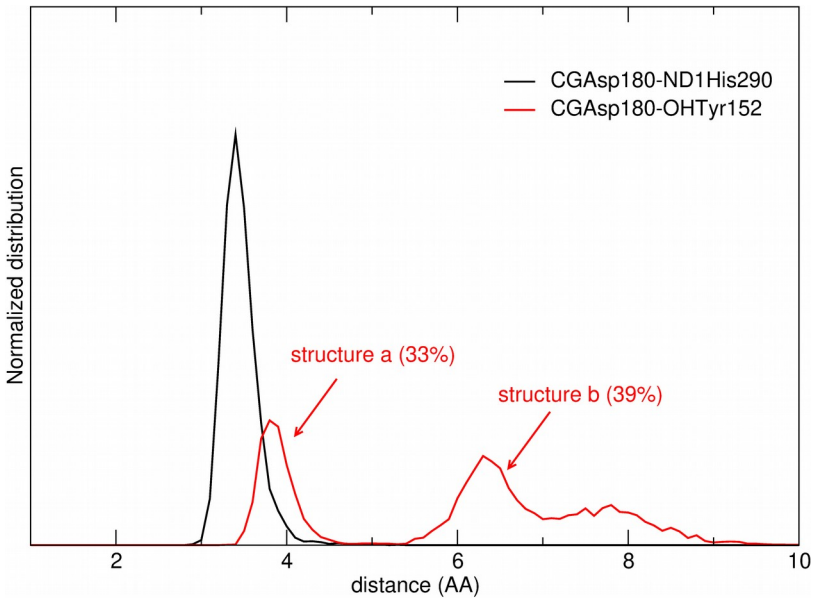
75 ns-long MD simulation of LMO octamer in solution in the first excited state



Structure A

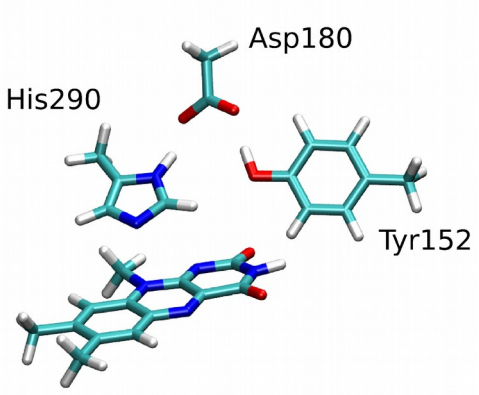


Structure B

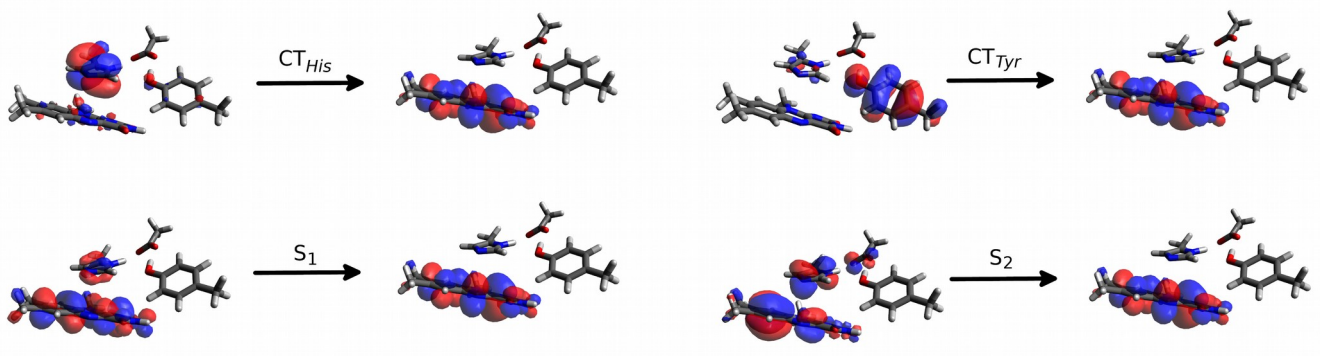


Computational data: intrinsic ET

Gas phase QM calculations on structure A

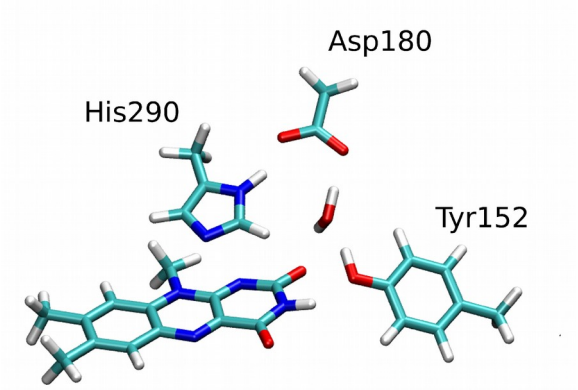


	Excitation Energy (eV)	Oscillator Strength
(1) CT_{His}	3.02	0.0090
(2) CT_{Tyr}	3.16	0.0006
(3) S_1	3.39	0.2861
(7) S_2	4.37	0.0843

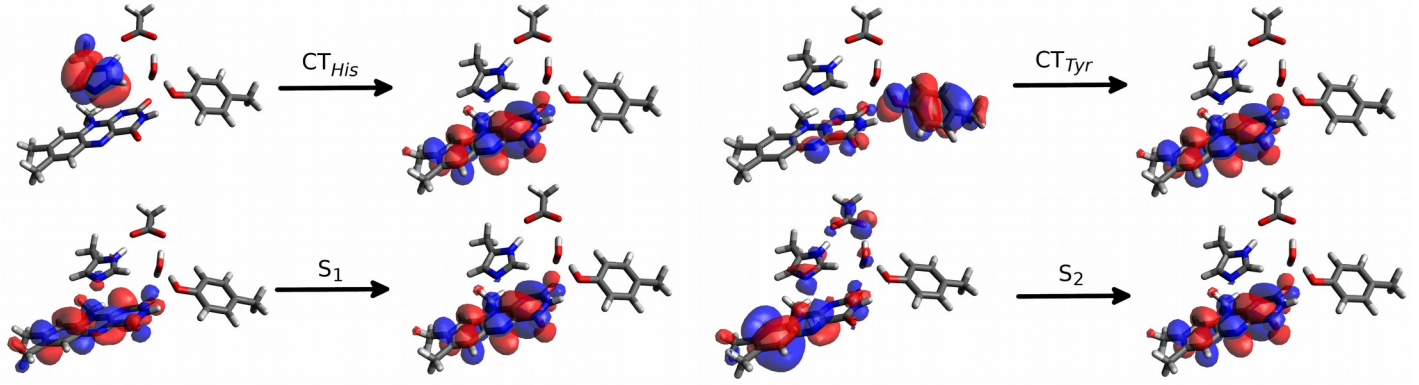


Computational data: intrinsic ET

Gas phase QM calculations on structure B

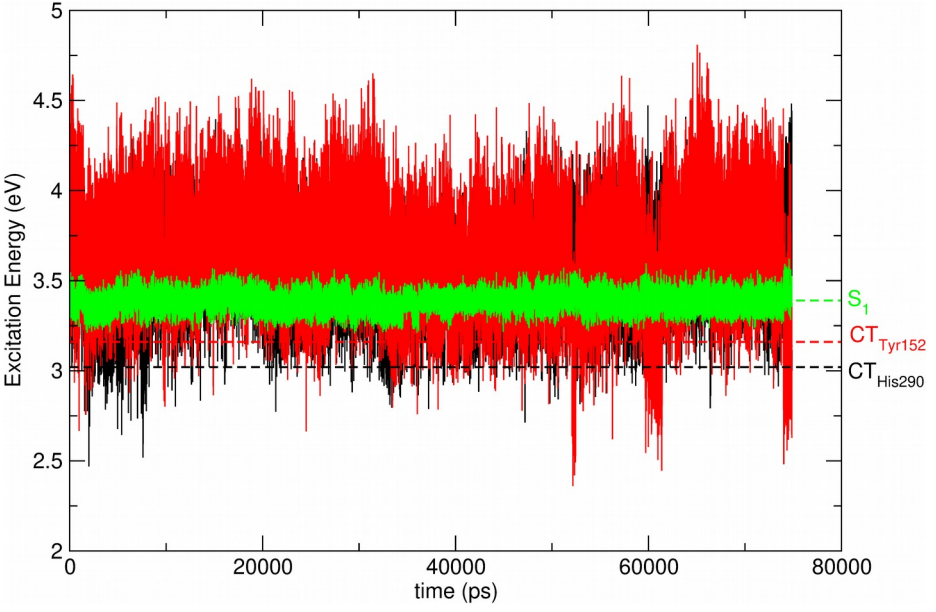


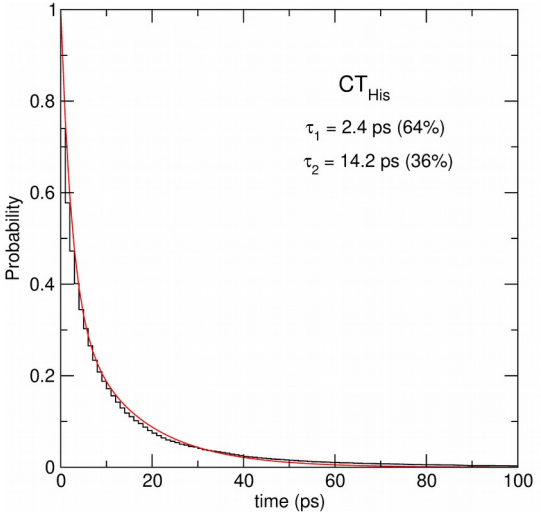
	Excitation Energy (eV)	Oscillator Strength
(1) CT_{His}	3.00	0.0035
(2) S_1	3.44	0.3163
(4) CT_{Tyr}	3.79	0.0005
(9) S_2	4.53	0.0684

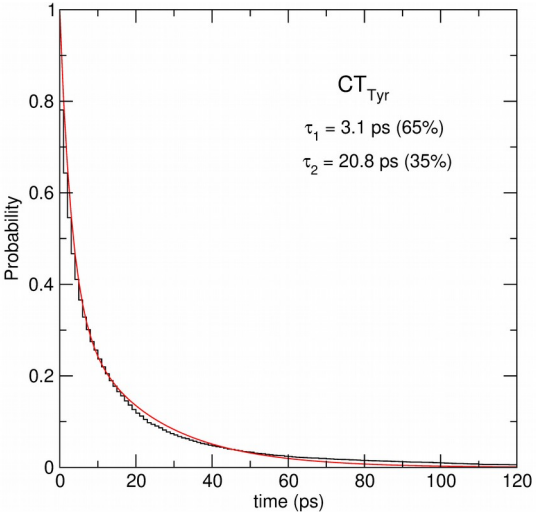


Computational data: intrinsic ET

Protein electrostatic effect inclusion for structure A

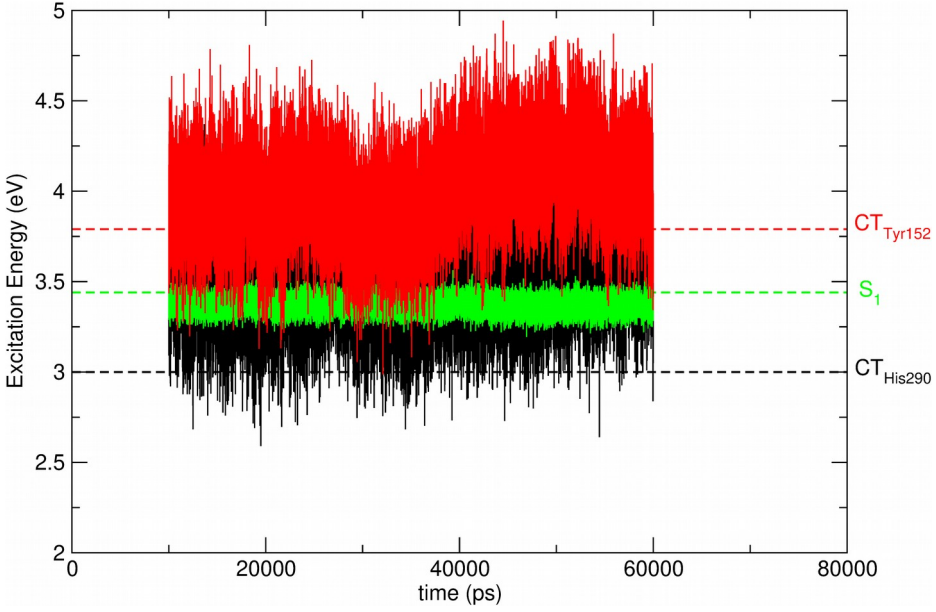


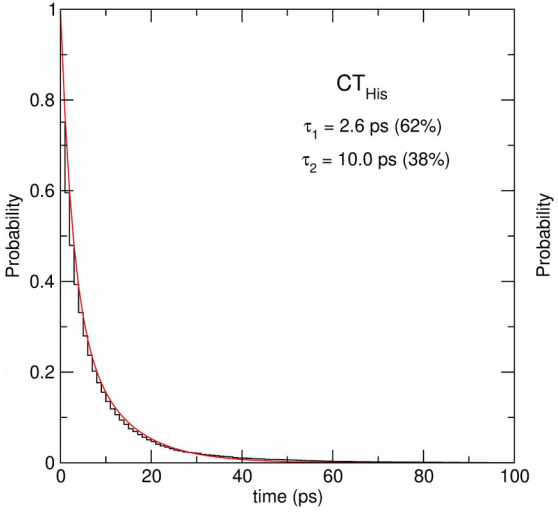


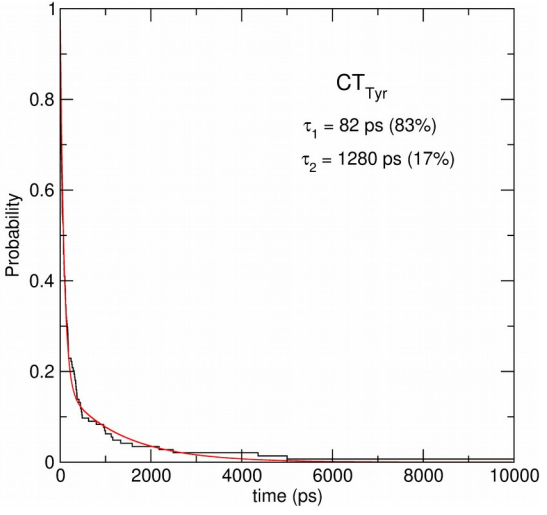


Computational data: intrinsic ET

Protein electrostatic effect inclusion for structure B





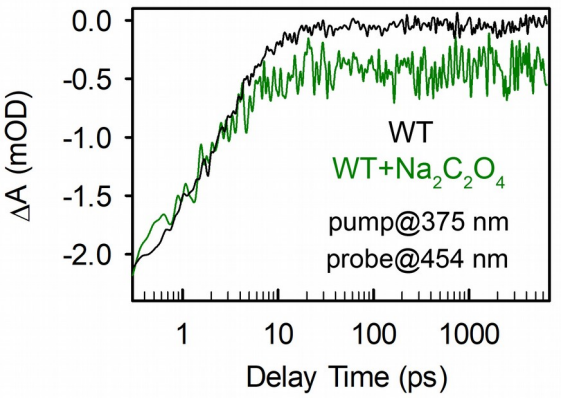


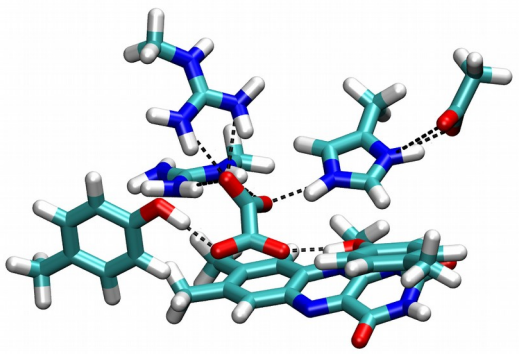
In summary

- **WT protein:** efficient ET from His290, less efficient ET from Tyr152. Subsequent PT from His/Tyr to Asp180
- **Y152F:** ET with His similar to WT but His/Asp PT more efficient as Tyr no longer competes
- **H290Q:** less efficient ET from Tyr152

Next:

Investigation of LMO
in complex with oxalate





Acknowledgents

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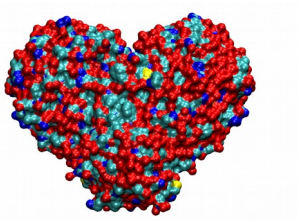
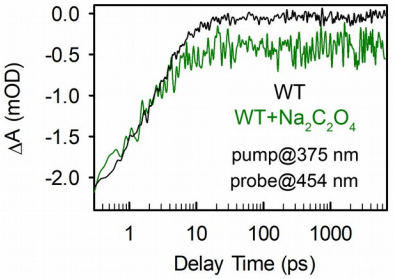
J.C. Gumbart

Xiankun Li

Gregory Scholes

PRINCETON UNIVERSITY





Thank you for your attention