HOW DO ENZYMES RECOGNIZE SUBSTRATES AND INHIBITORS: STRUCTURAL AND ELECTRON DENSITY ASPECTS

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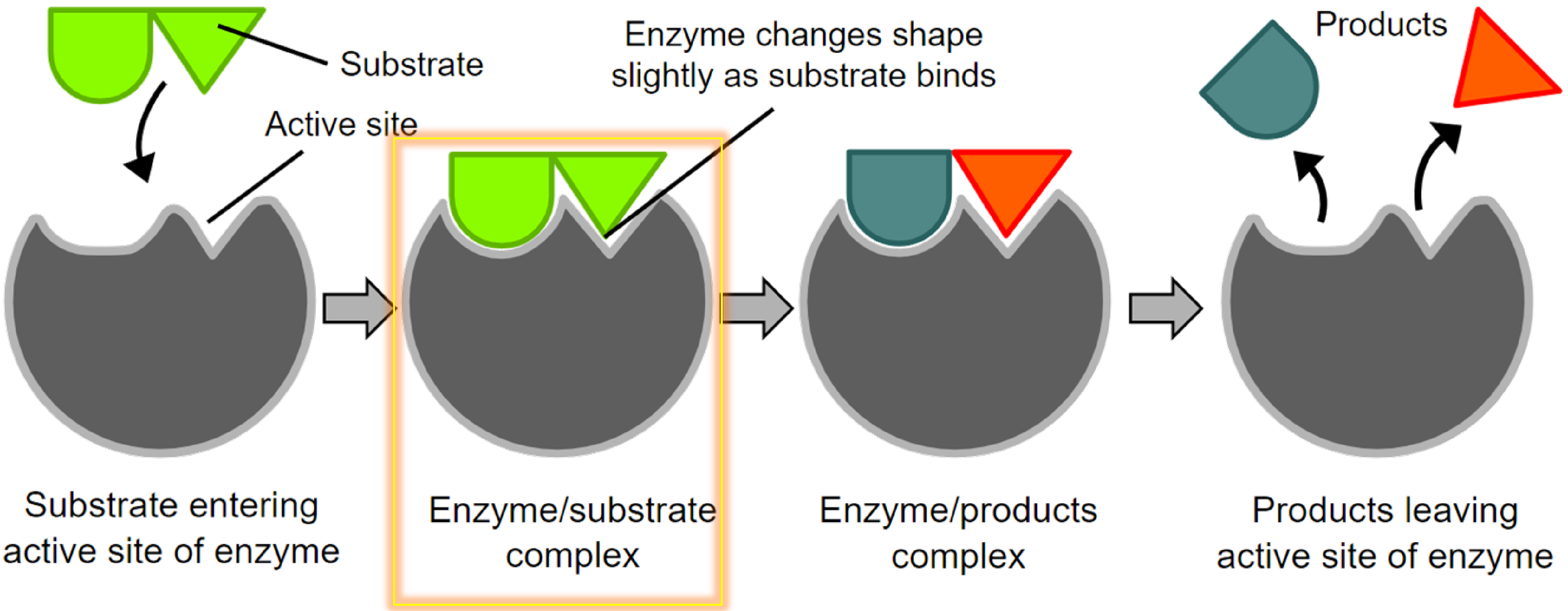
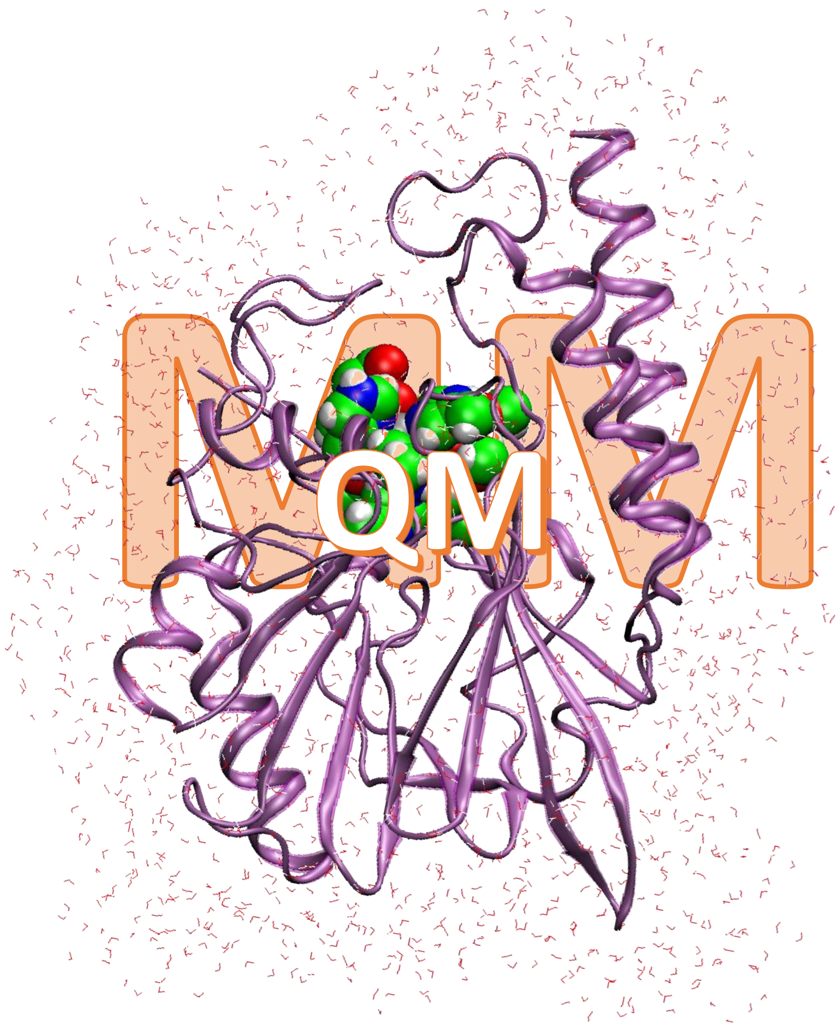
Enzyme-substrate interactions



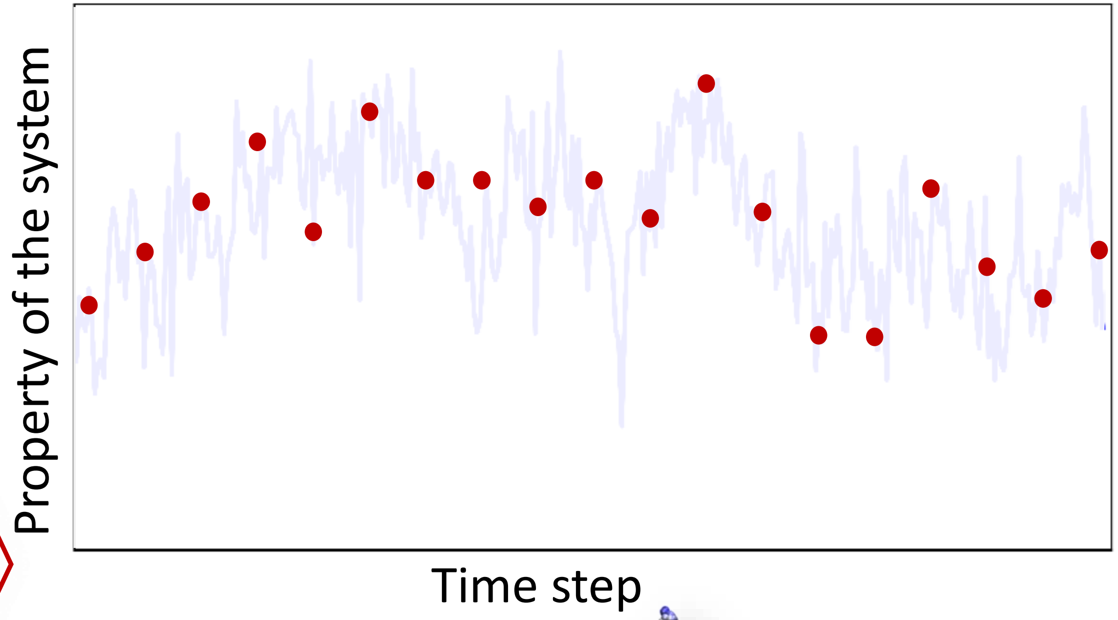
What is the substrate activation in the active site of the enzyme?

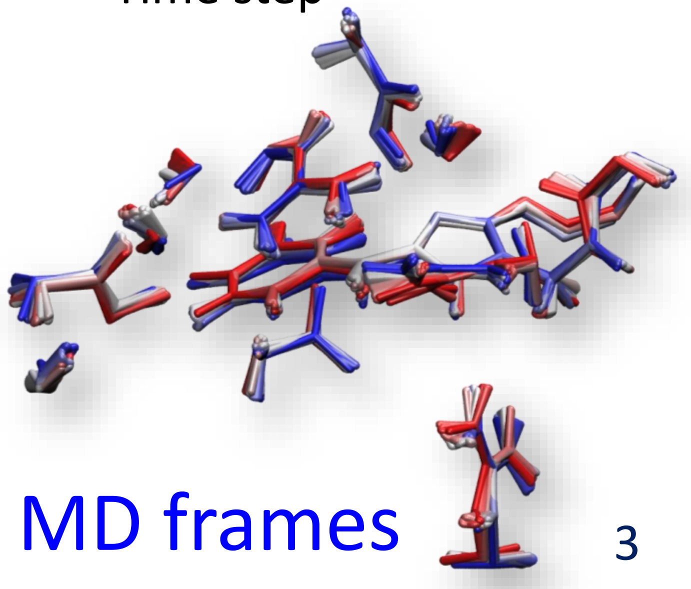
https://en.wikipedia.org/wiki/Enzyme_catalysis
https://www.thesciencehive.co.uk/enzymes-alevel

From local minima to ensembles of states



Molecular dynamics

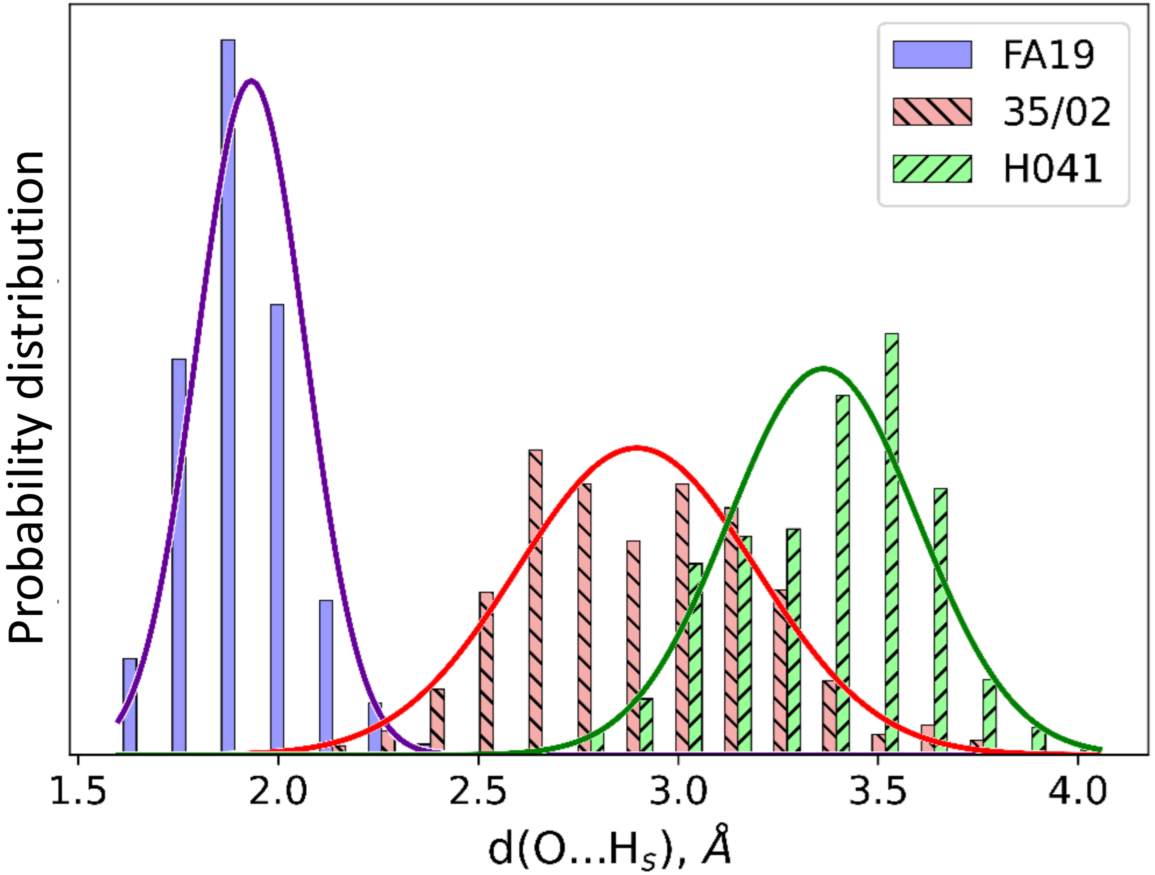


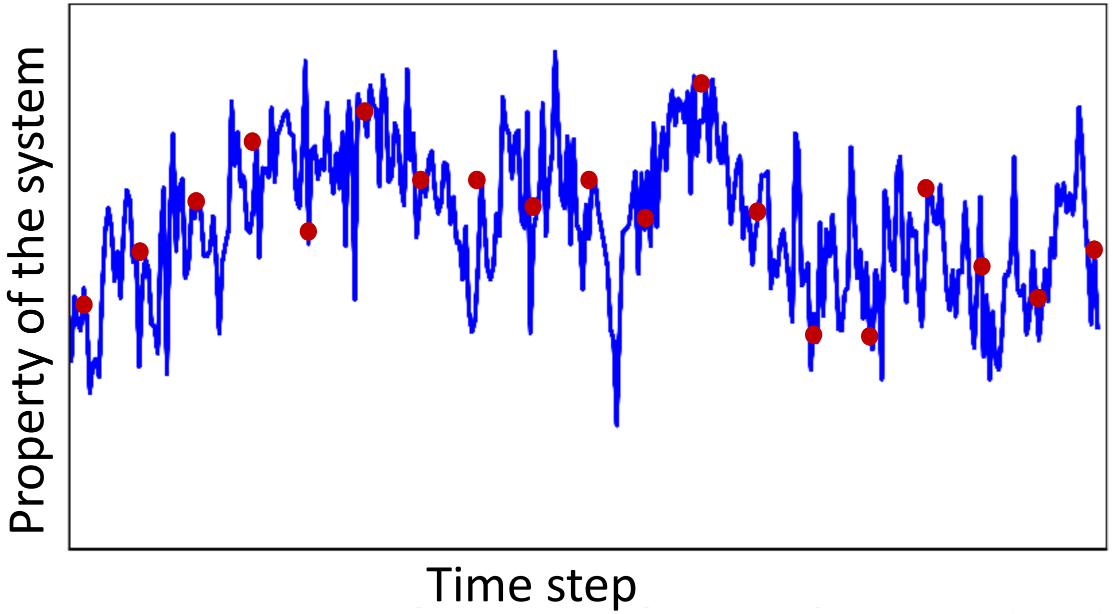


Set of MD frames

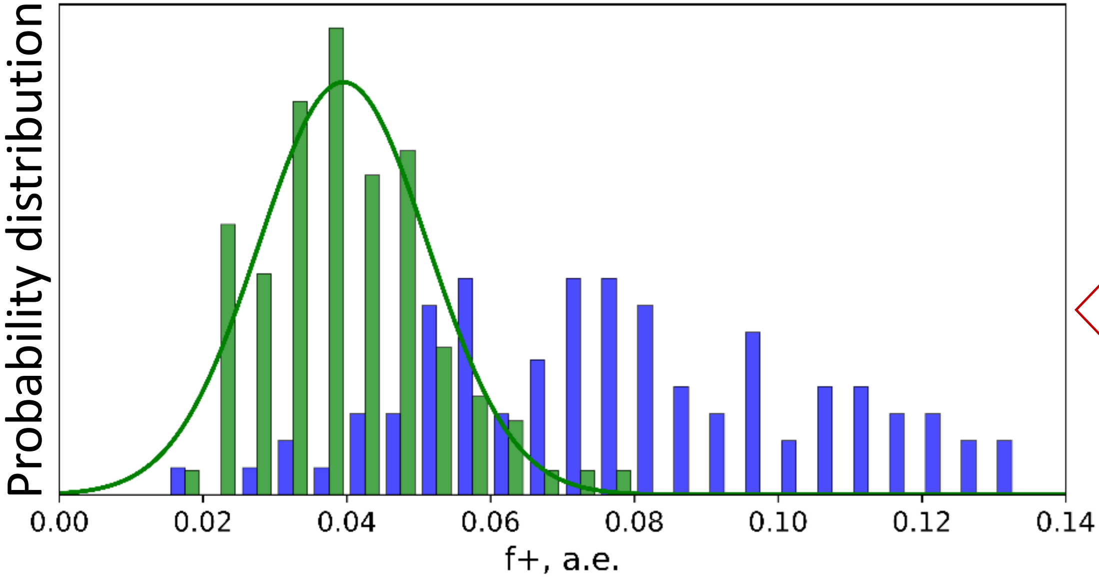
Distributions and average values

Geometry parameter





Electron density based descriptors



Breakout: GPU-based DFT code

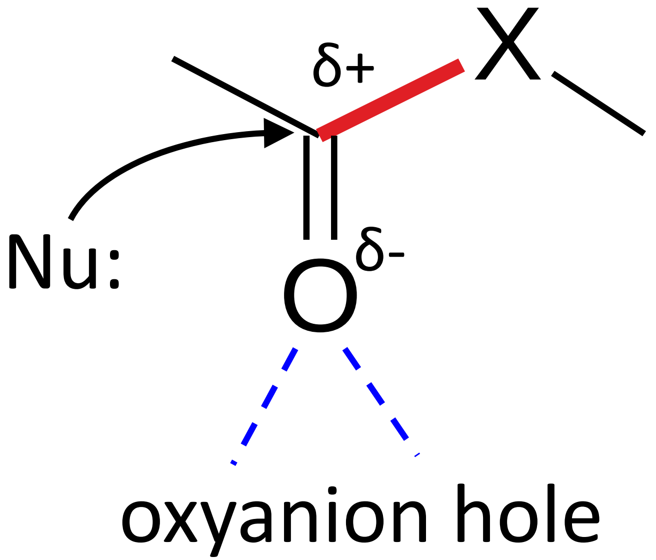
Terachem:

- QM subsystem: DFT(hybrid functional/6-31G**), ~100 atoms
- Benchmark (energy + gradient)
  - NVIDIA 1070 TI – 2 min
  - NVIDIA 3070 TI – 1 min.

Nucleophilic attack in enzymatic reactions

EC 3 Hydrolases:

- EC 3.1 Acting on ester bonds;
- EC 3.4 Acting on peptide bonds;
- EC 3.5 Acting on C-N bonds, other than peptide bonds;
- EC 3.7 Acting on C-C bonds.

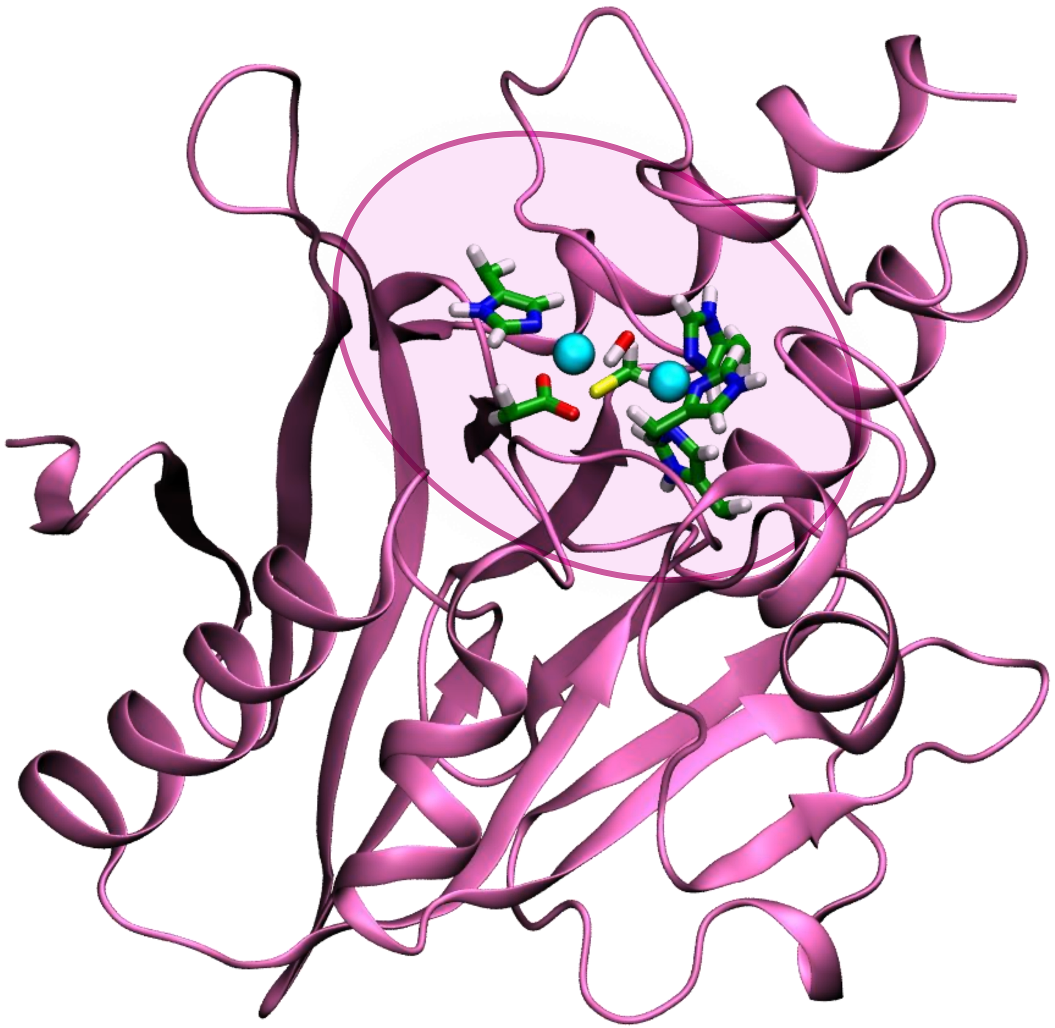


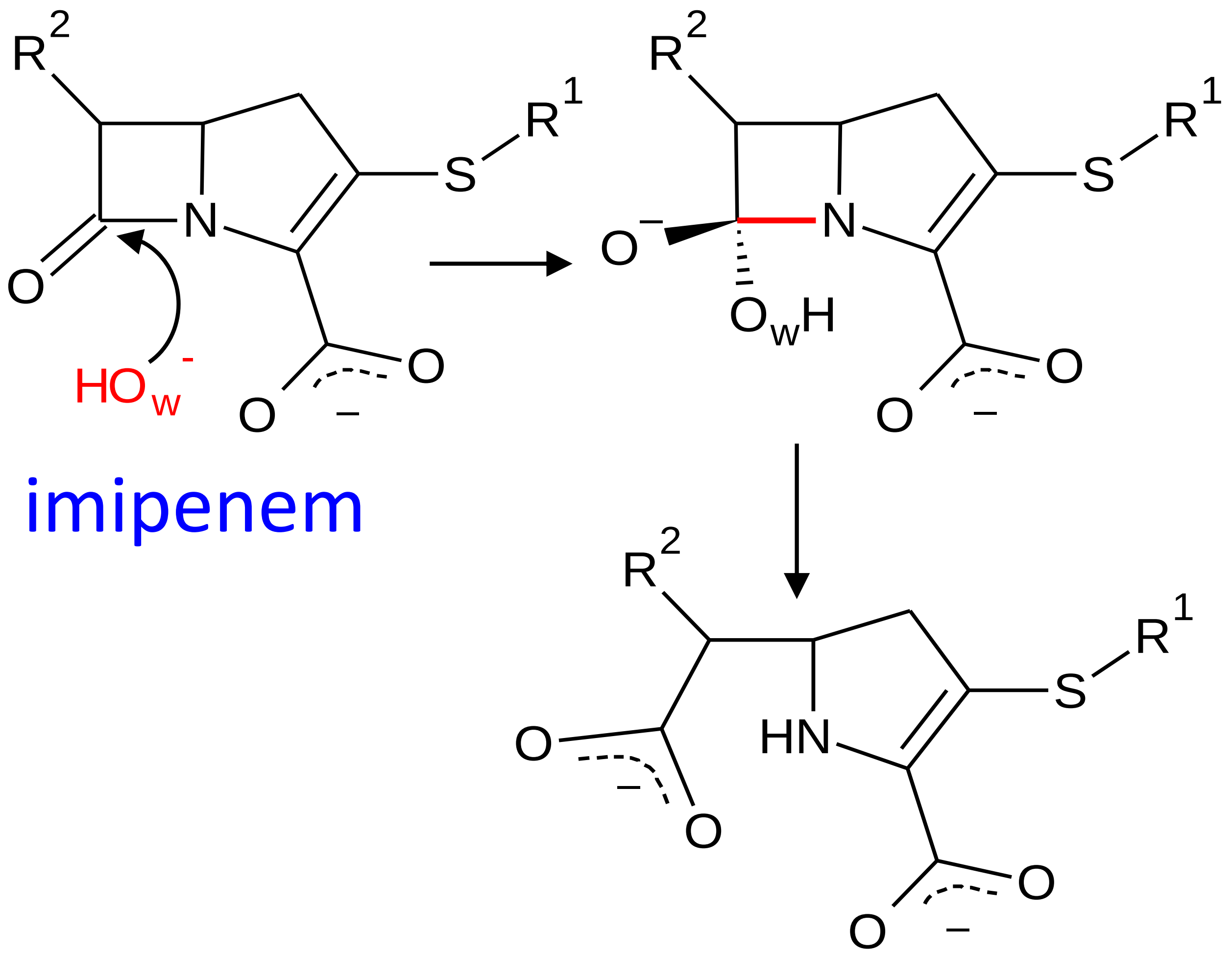
Nucleophile:

- H_2O
- OH^-
- OH of Ser
- OH of Thr
- SH of Cys

Case 1: NDM-1 metallo-β-lactamase

Hydrolysis of antibiotics related to the drug resistance

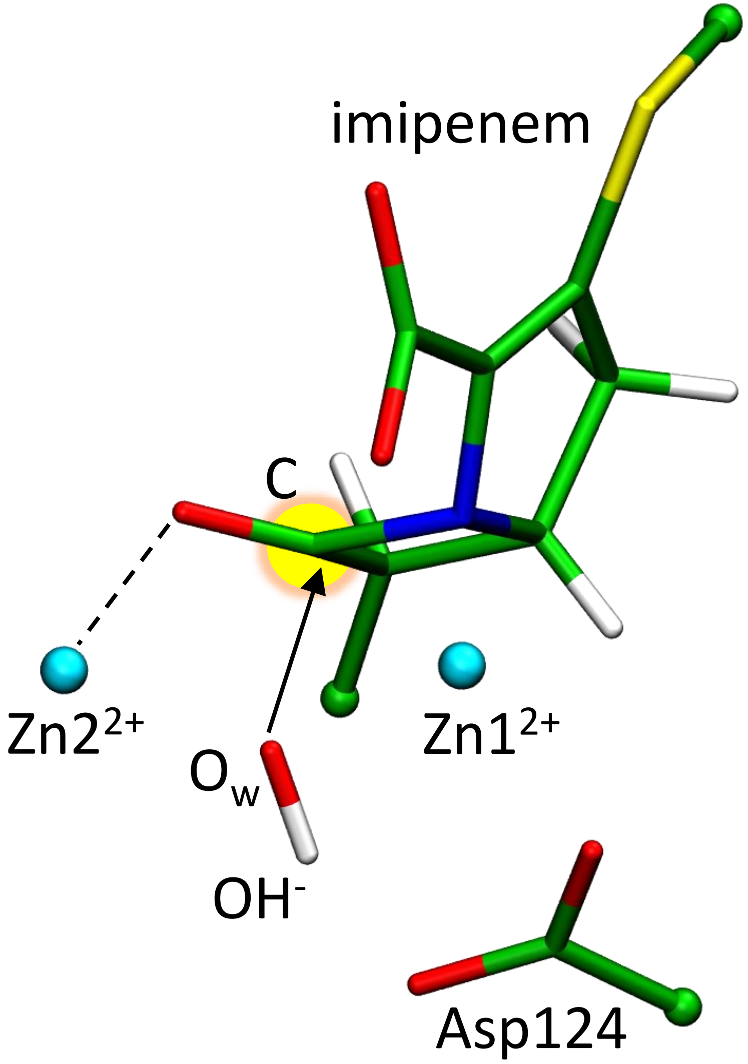




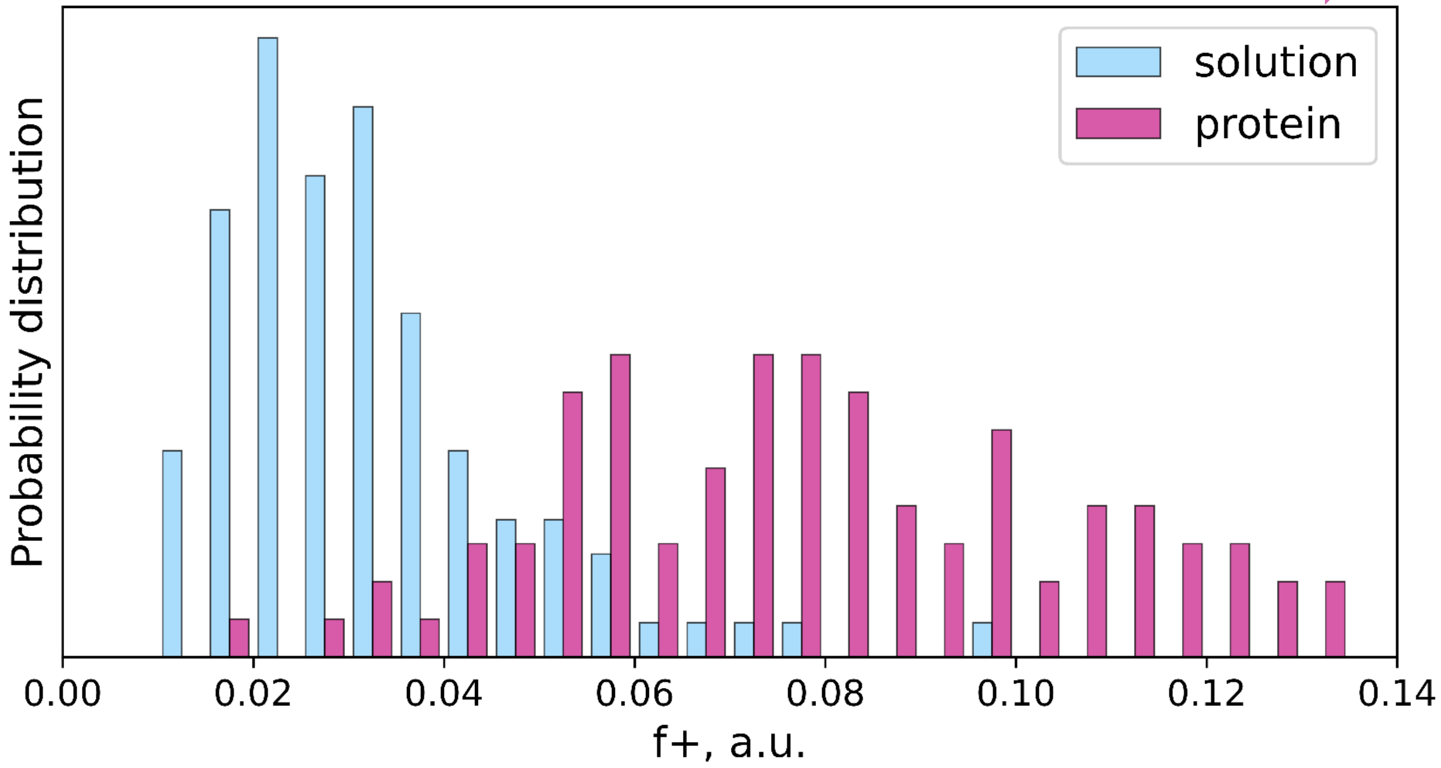
imipenem

hydrolyzed imipenem

Case 1: NDM-1 metallo-beta lactamase



Protein activates a substrate



Fukui electrophilicity index of a carbonyl carbon atom

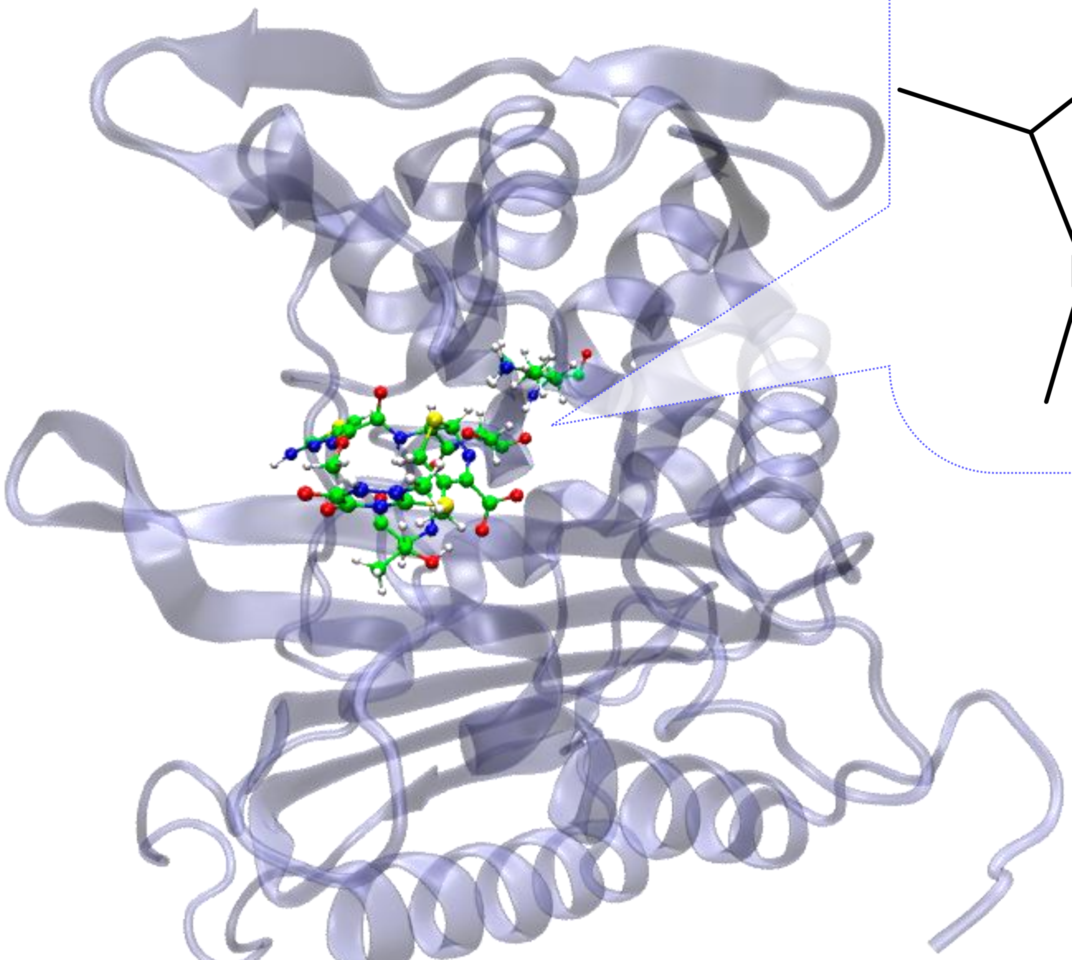
$$f+(C) = q_{N+1}(C) - q_N(C)$$

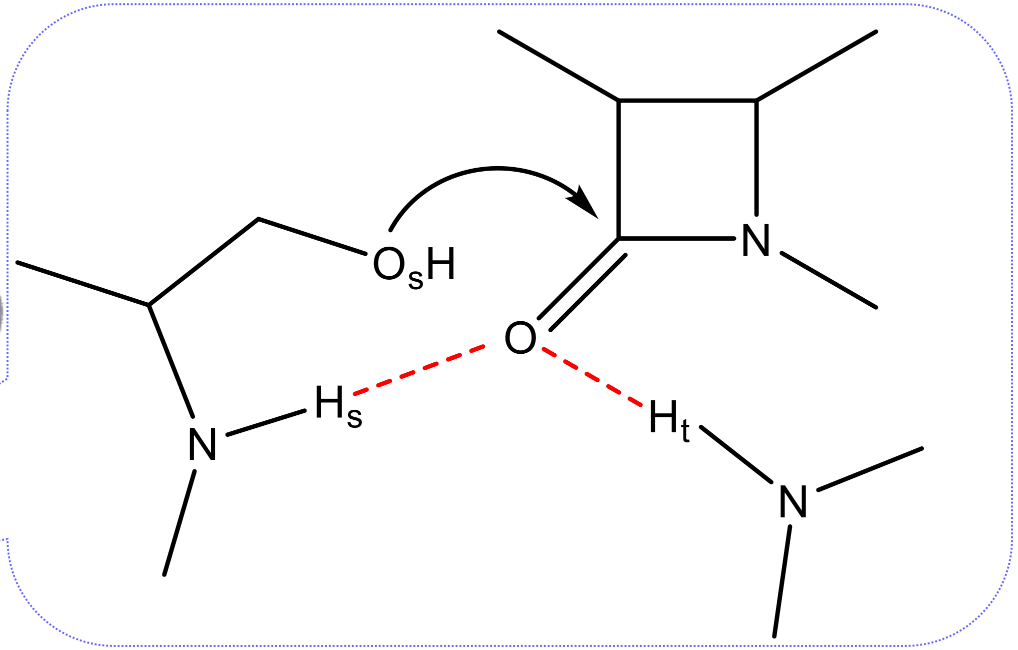
$q_N(C)$ – Hirshfeld charge of C atom

$q_{N+1}(C)$ – Hirshfeld charge of C atom in a system with an extra electron

QM(PBE0-D3/6-31G**)/MM MD

Case 2: Penicillin binding protein 2



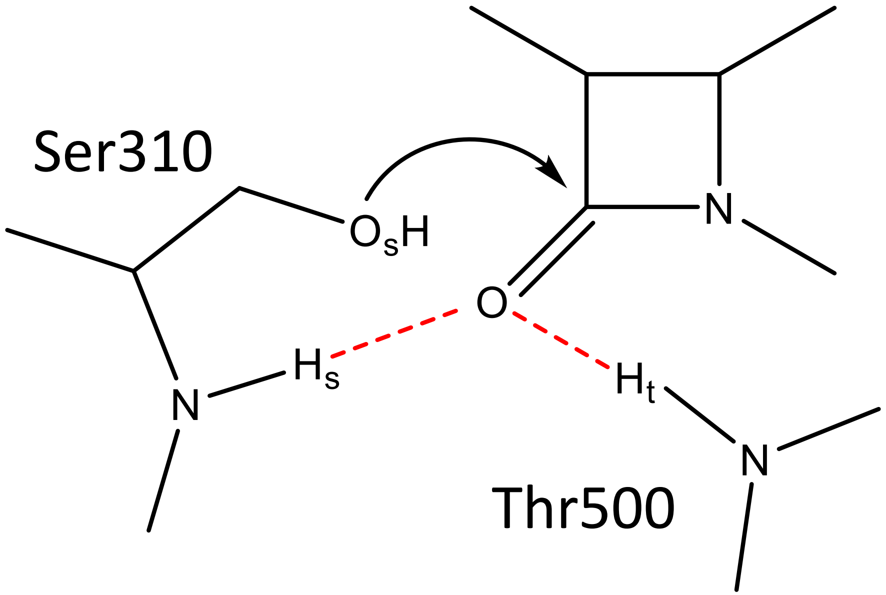


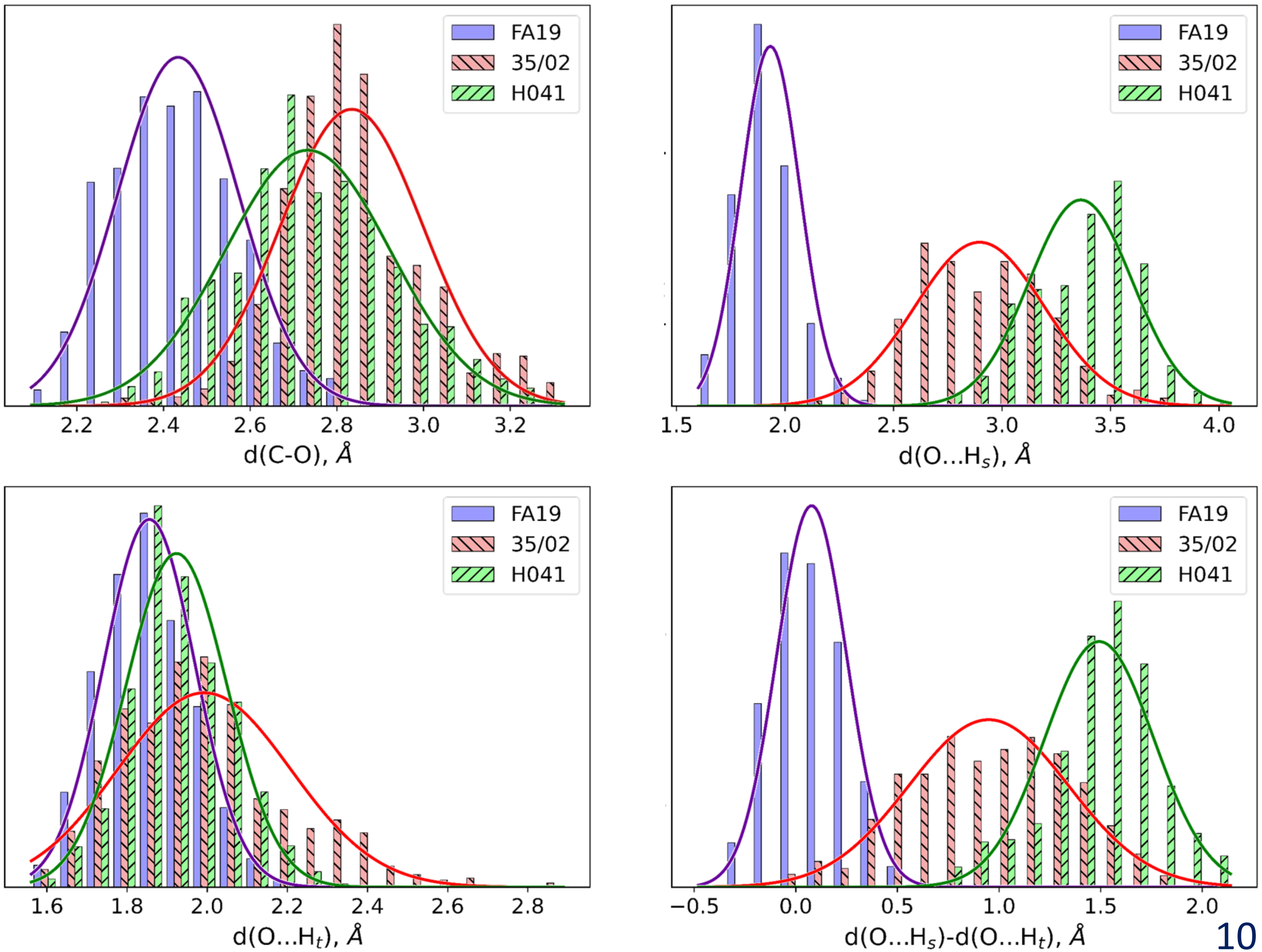
k_2/K_s for PBP2 from different *Nisseria gonorrhoeae* strains for ceftriaxone

strain	k_2/K_S , $mM^{-1}s^{-1}$
FA19	1710 ± 90
35/02	11.3 ± 0.4
H041	0.74 ± 0.03

J. Tomberg et. al, *Antimicrob. Agents Chemother.*, 2013

QM/MM MD simulations of the ES complexes

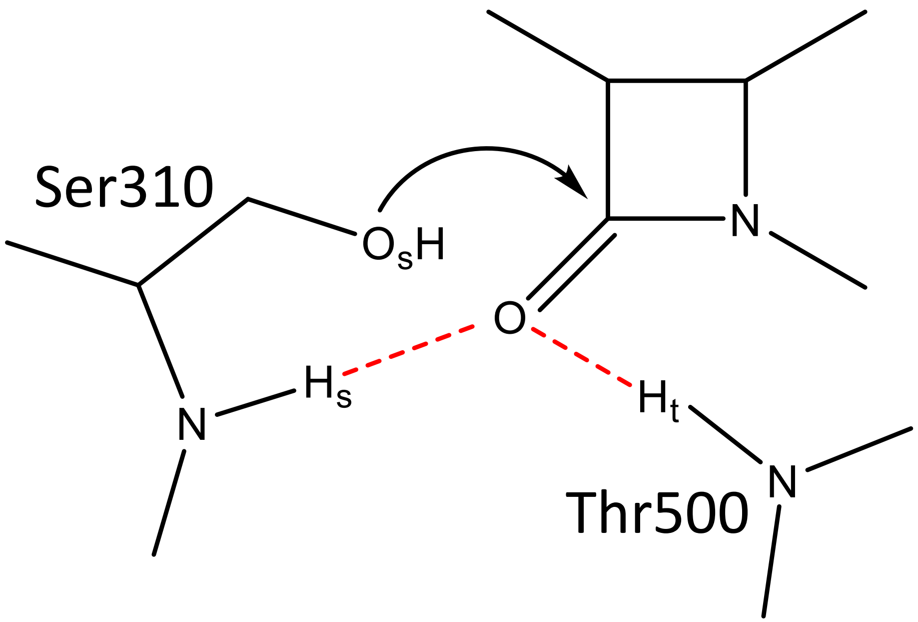




- Hydrogen bonds in the oxyanion hole are responsible for the substrate activation

QM(PBE0-D3/6-31G**)/MM MD

QM/MM MD simulations of the ES complexes



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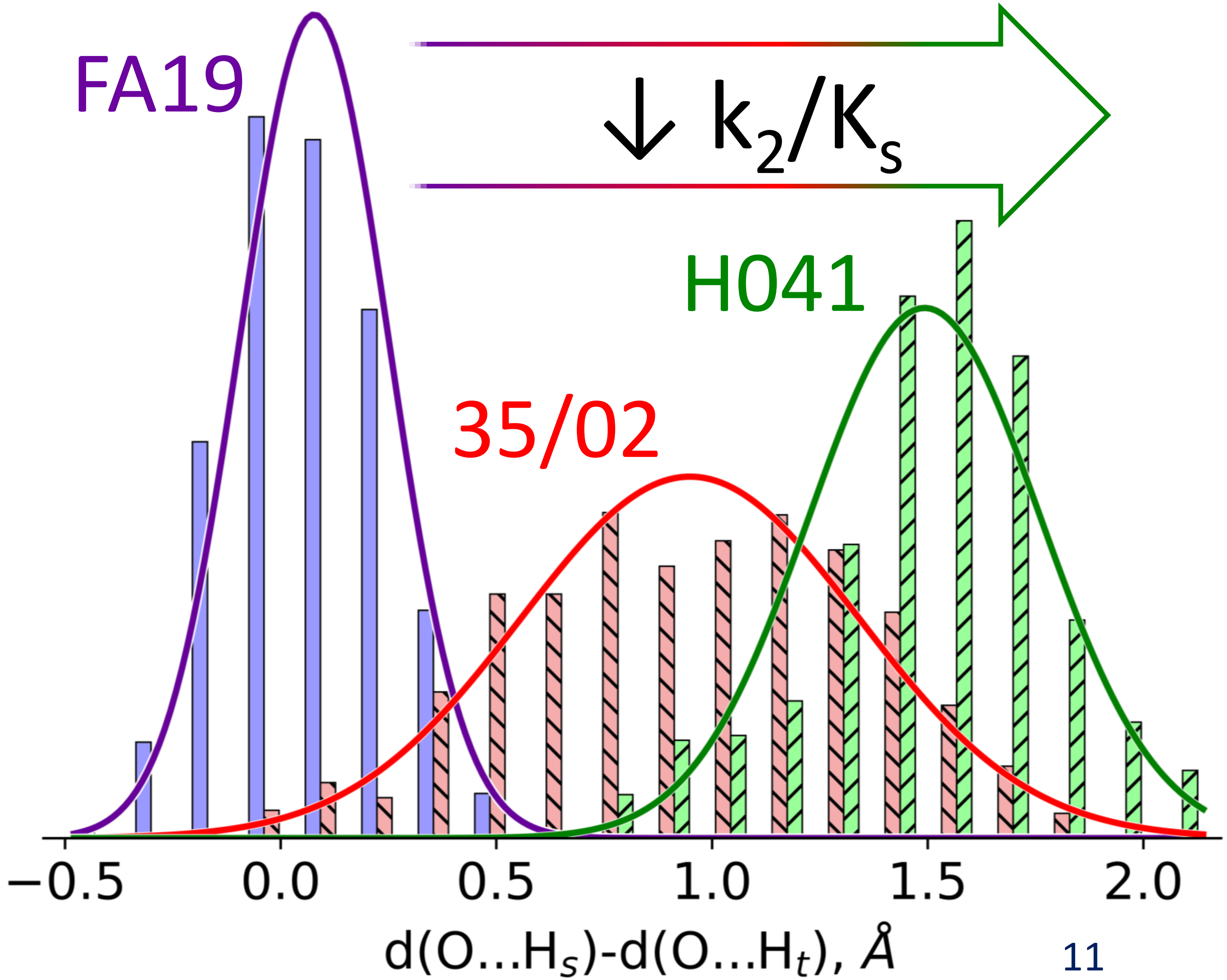
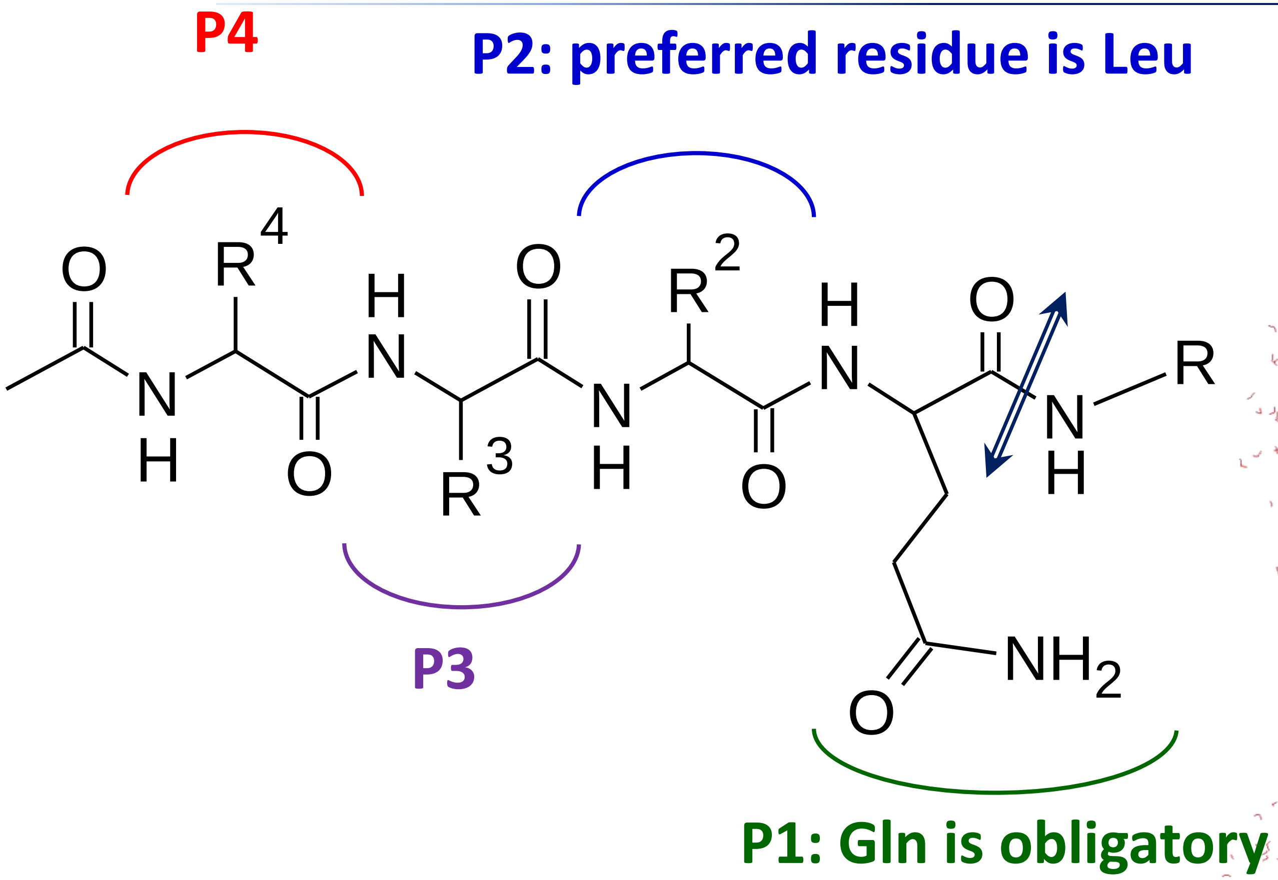
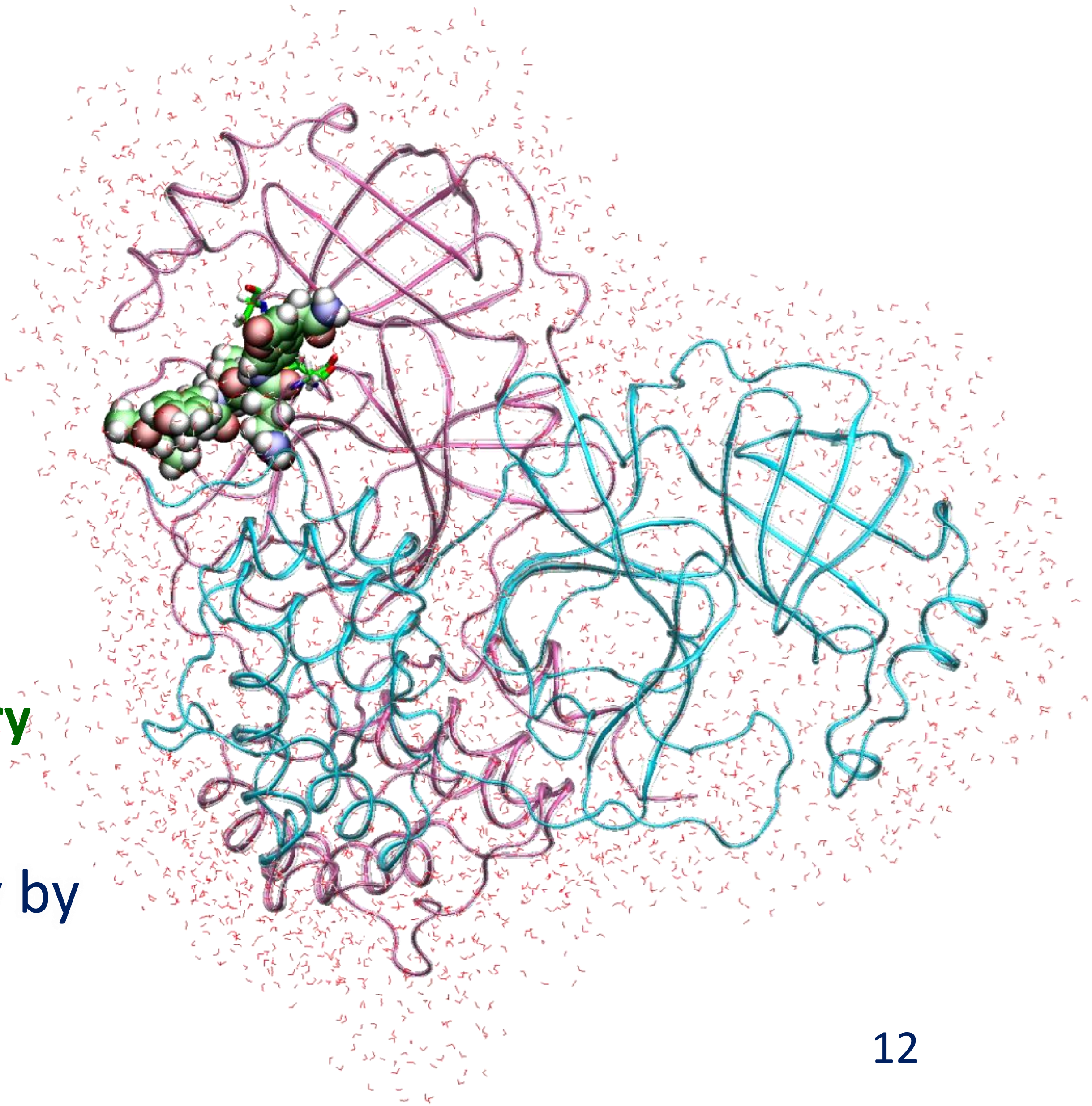


Case 3: Main protease M^{Pro} from SARS-CoV-2

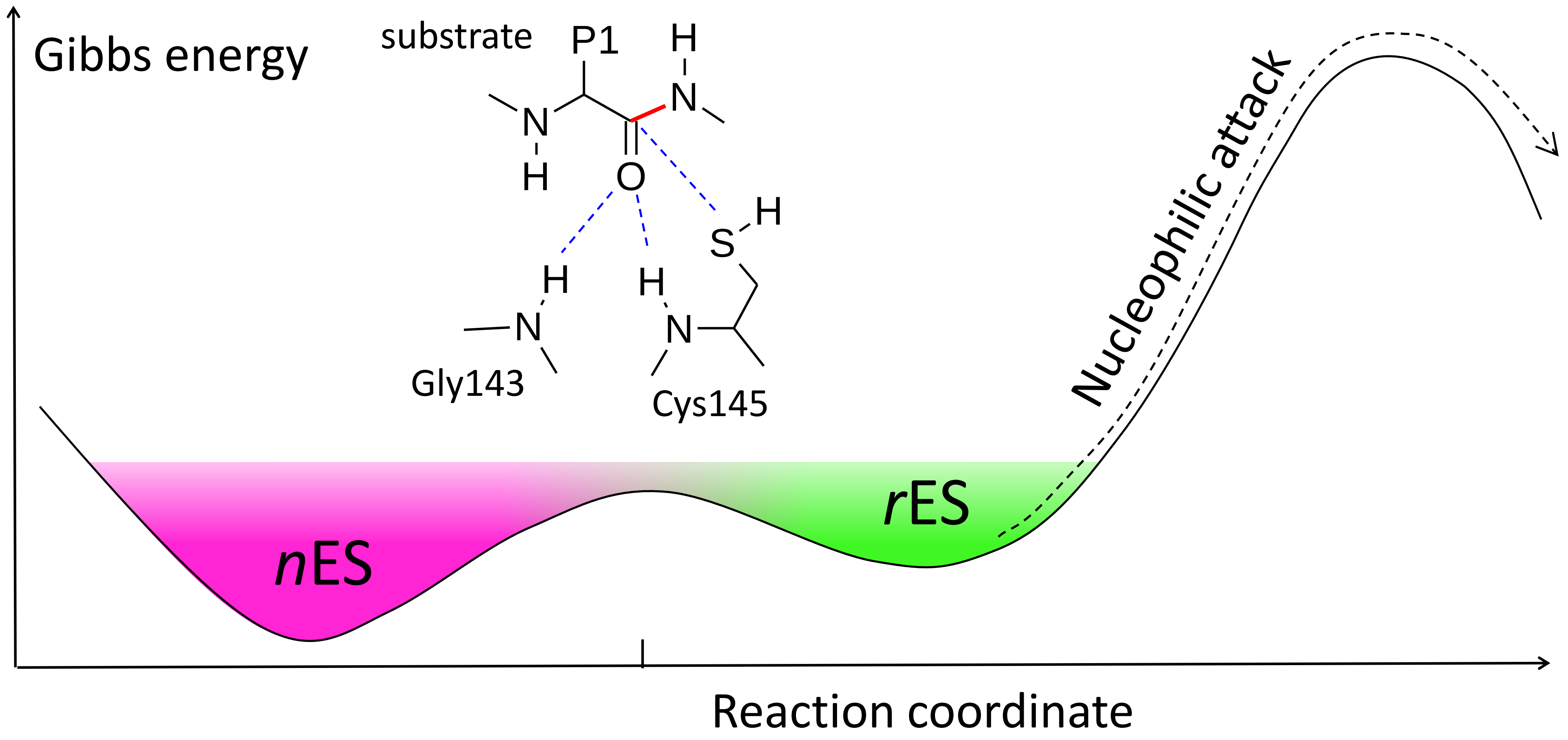




High substrate specificity:
Replacement of Leu at P2 decreases reactivity by 2 – 50 fold.

What is the origin of substrate specificity?

Efficiency of the substrate activation might be the reason



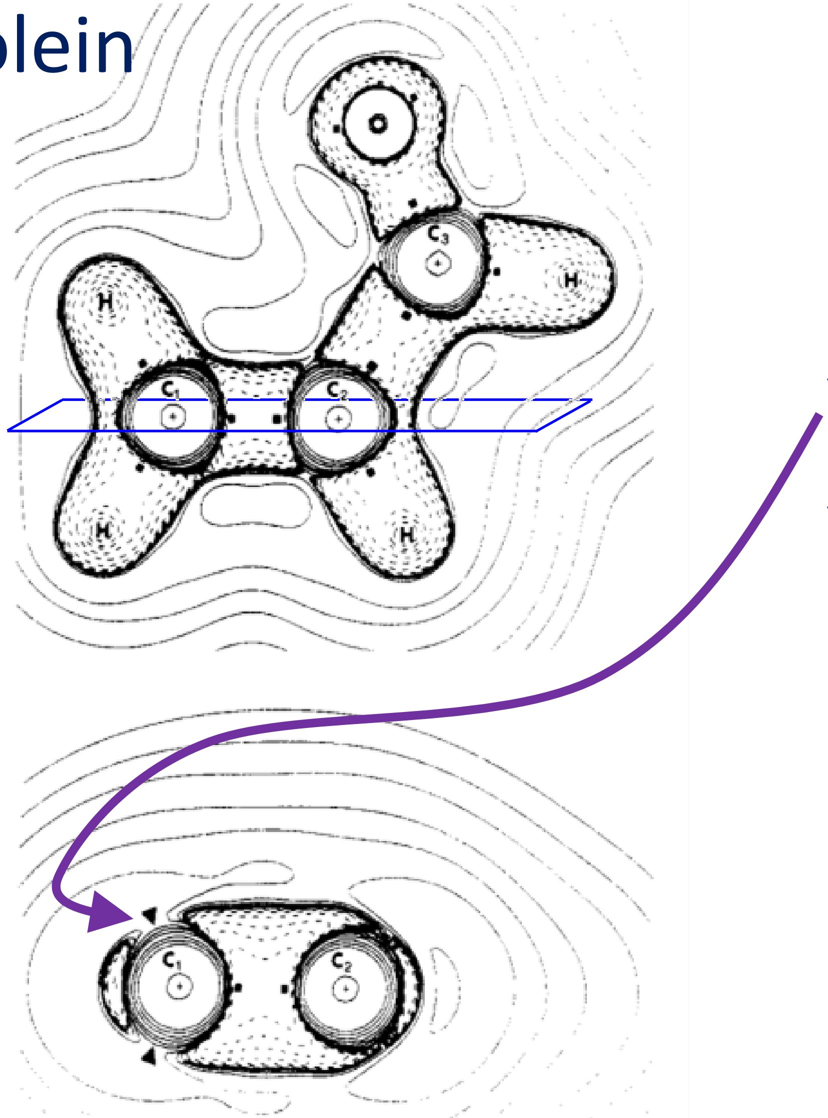
How to evaluate substrate activation?

$$n\mathrm{ES} \overset{K}{\rightleftarrows} r\mathrm{ES}$$

- ✓ Criteria of assignment of conformations to either reactive or nonreactive

Laplacian of electron density

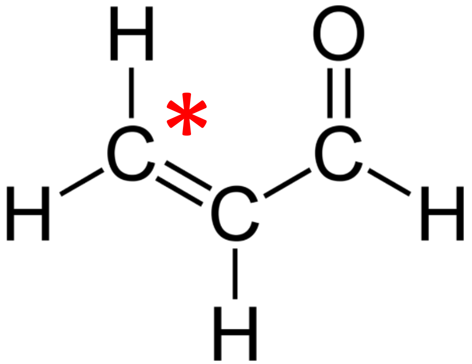
Acrolein



$$\nabla^2 \rho(\mathbf{r}) = \frac{\partial^2 \rho(\mathbf{r})}{\partial x^2} + \frac{\partial^2 \rho(\mathbf{r})}{\partial y^2} + \frac{\partial^2 \rho(\mathbf{r})}{\partial z^2}$$

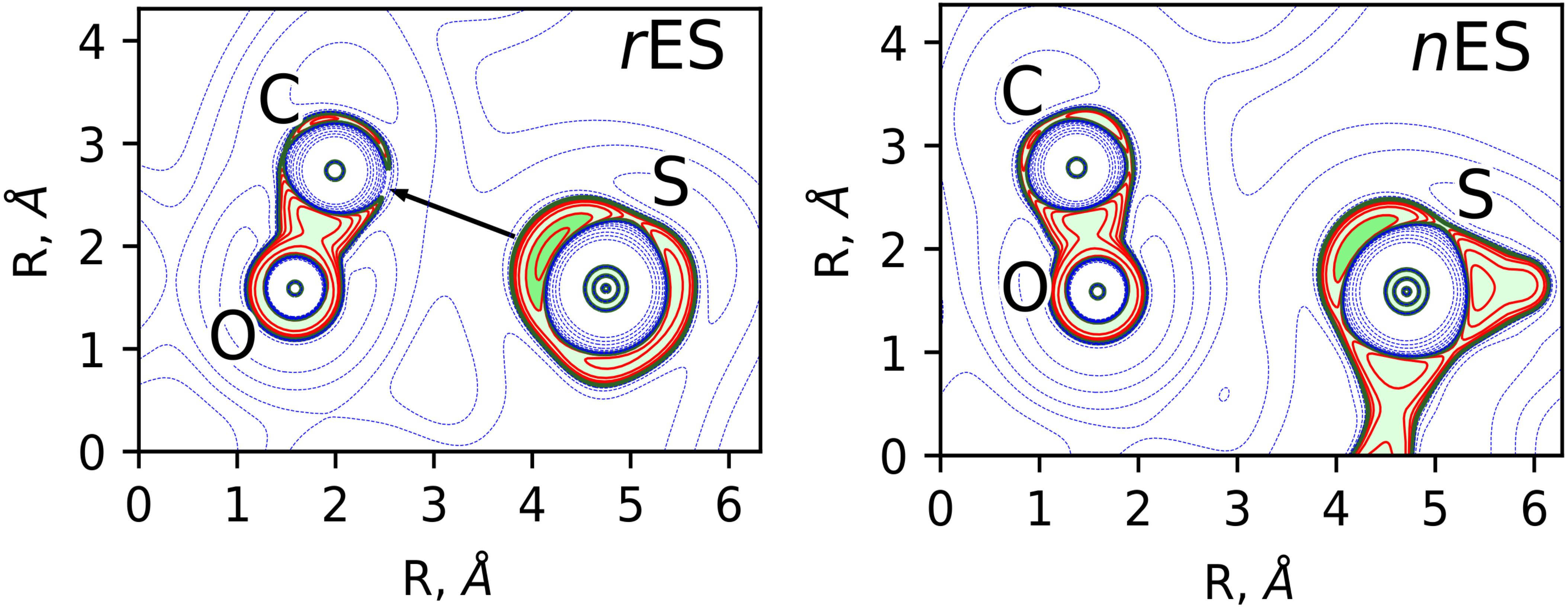
$\nabla^2 \rho(\mathbf{r}) > 0$ – electron density depletion regions

$\nabla^2 \rho(\mathbf{r}) < 0$ – electron density concentration regions



* J. Phys. Chem. (1989) V. 93. P. 5120-5123

Criterion to discriminate reactive and nonreactive species



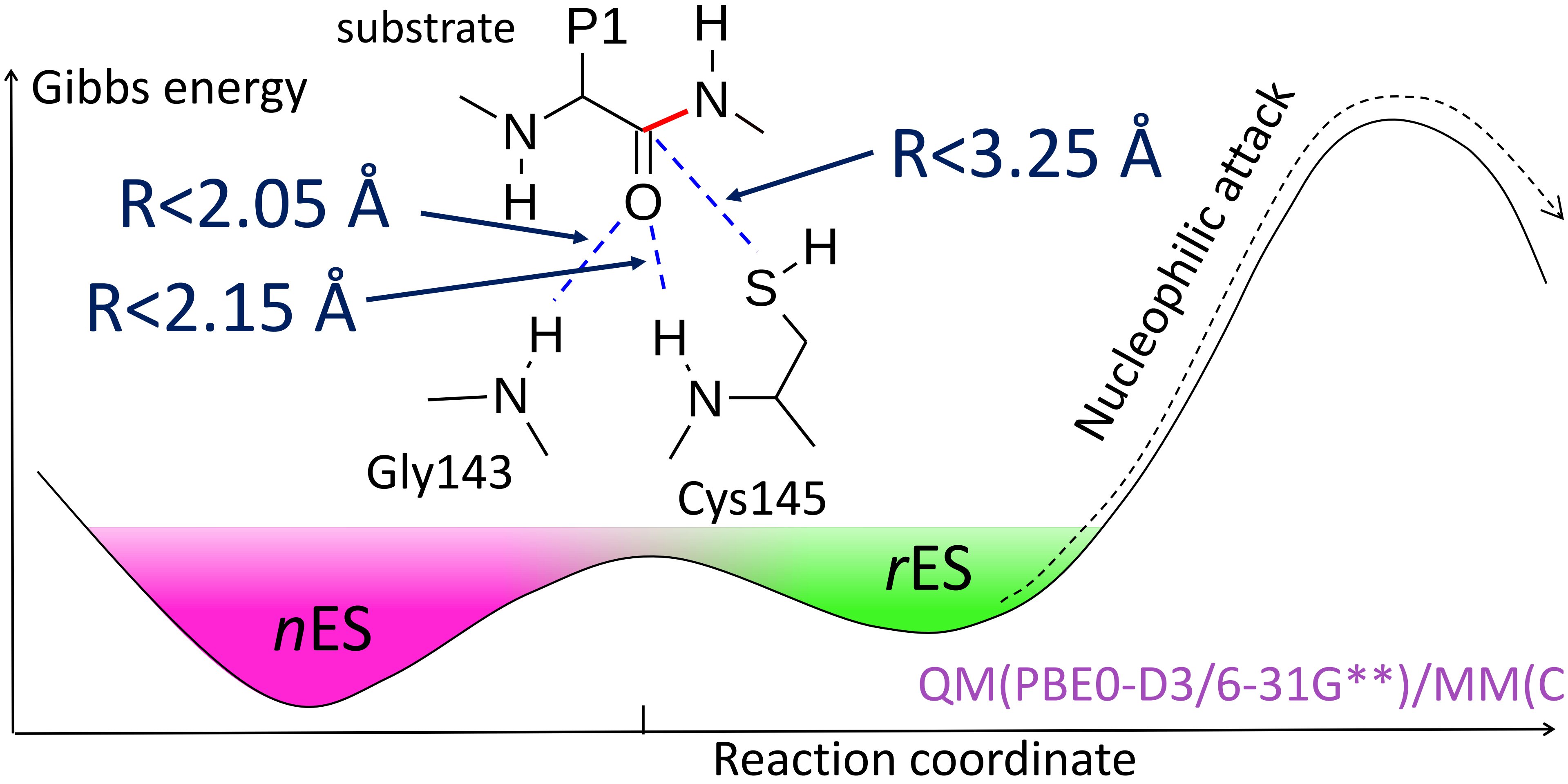
$\nabla^2\rho(\mathbf{r})$ maps in the S (Cys145) and C=O (substrate) plane

Blue isolines correspond to the ED depletion regions, $\nabla^2\rho(\mathbf{r})>0$

Red isolines correspond to the ED concentration regions, $\nabla^2\rho(\mathbf{r})<0$

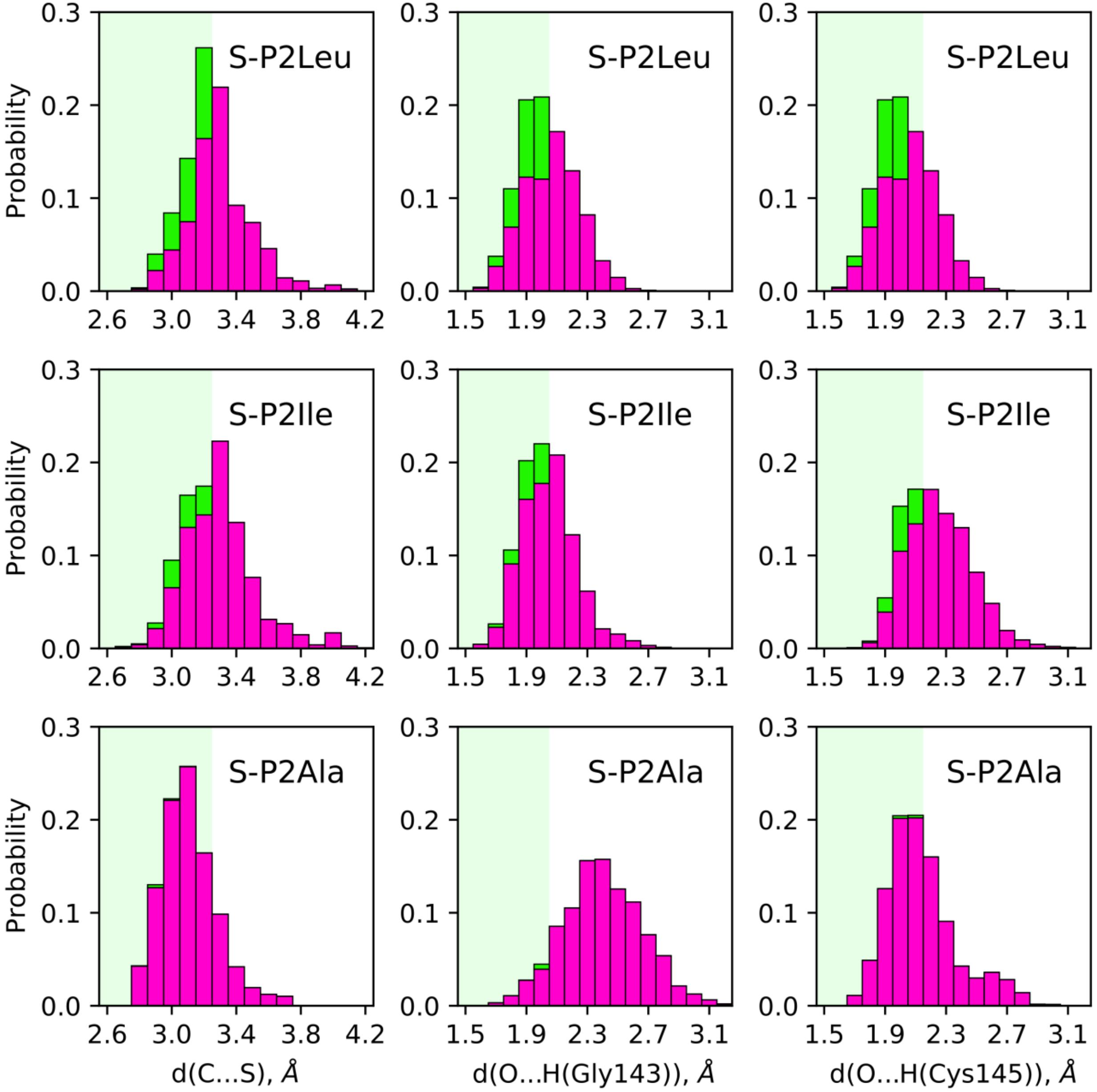
Criteria to discriminate reactive and nonreactive species

All three geometry criteria should be satisfied together



QM(PBE0-D3/6-31G**)/MM(CHARMM)

Substrate specificity and rES ↔ nES equilibrium



Substrate	χ, %	k_{cat}(calc.)	k_{cat}(exp.)
S-P2Leu	22.4	1	1
S-P2Ile	10.2	0.46	0.45
S-P2Ala	0.6	0.03	<0.1

* values relative to S-P2Leu

$$k_{cat}(AA)=k_{cat}(Leu)\chi(AA)/\chi(Leu)$$

Results obtained at the QM(PBE0-D3/6-31G**)/MM(CHARMM)

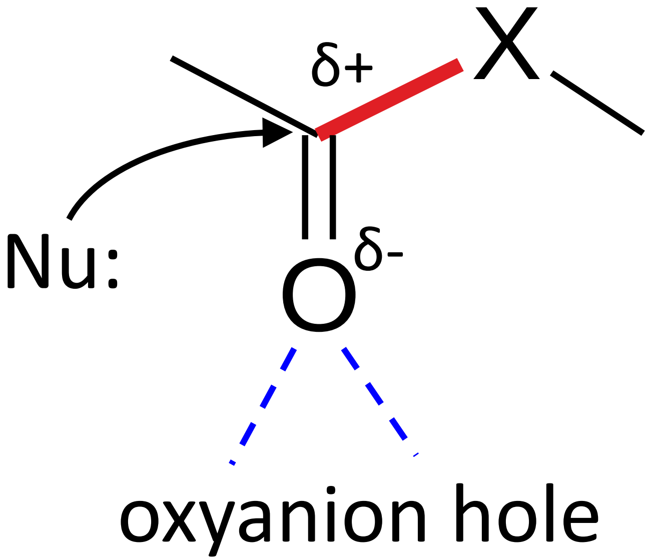
Example from the literature data

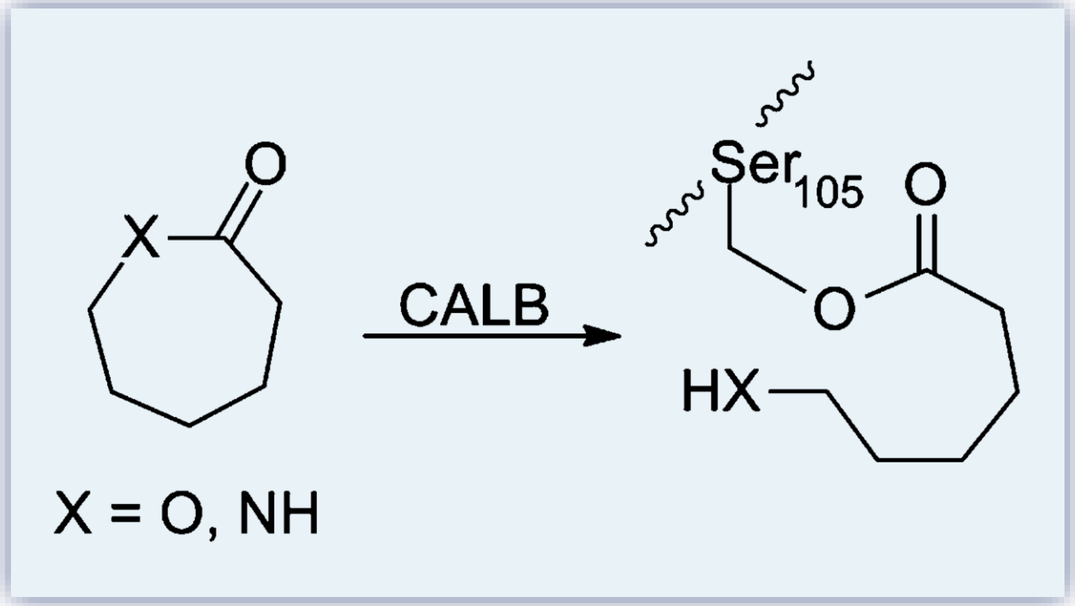
ACS Catalysis
Research Article
pubs.acs.org/acscatalysis

Comparative Theoretical Study of the Ring-Opening Polymerization of Caprolactam vs Caprolactone Using QM/MM Methods

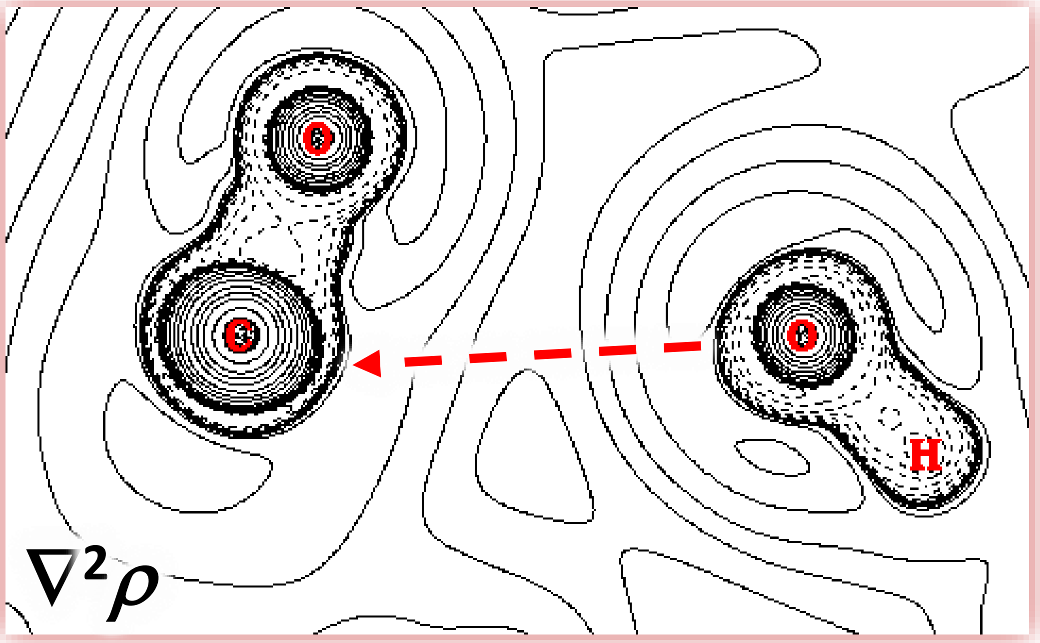
Brigitta Elsässer,* Iris Schoenen, and Gregor Fels

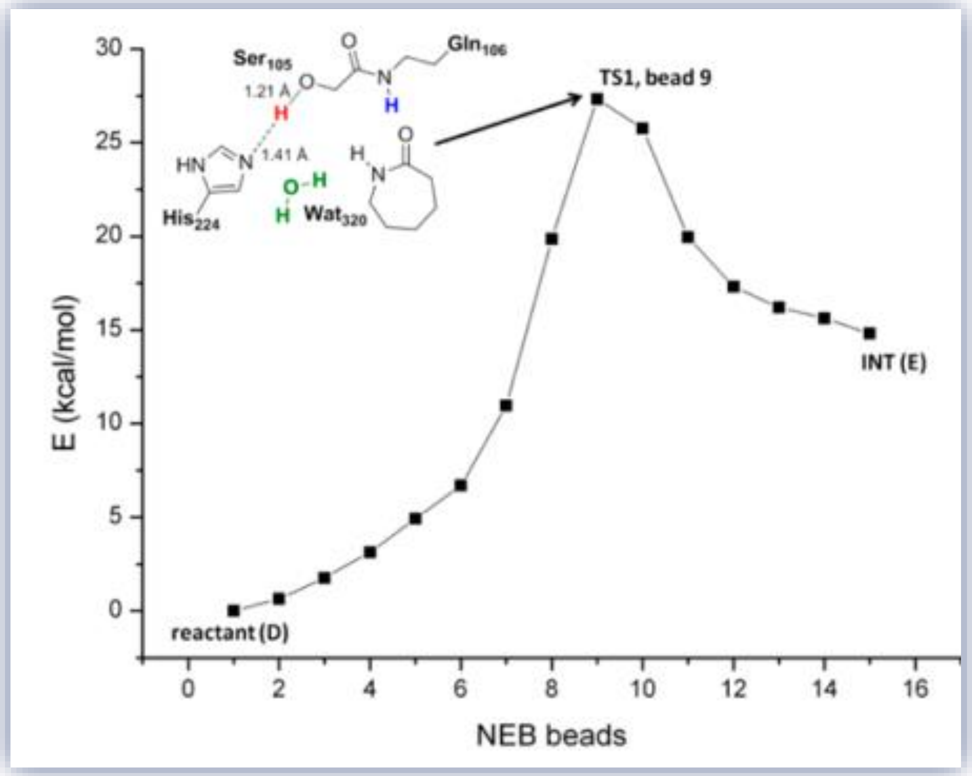
Department of Chemistry, University of Paderborn, Warburger Strasse 100, D-33098 Paderborn, Germany





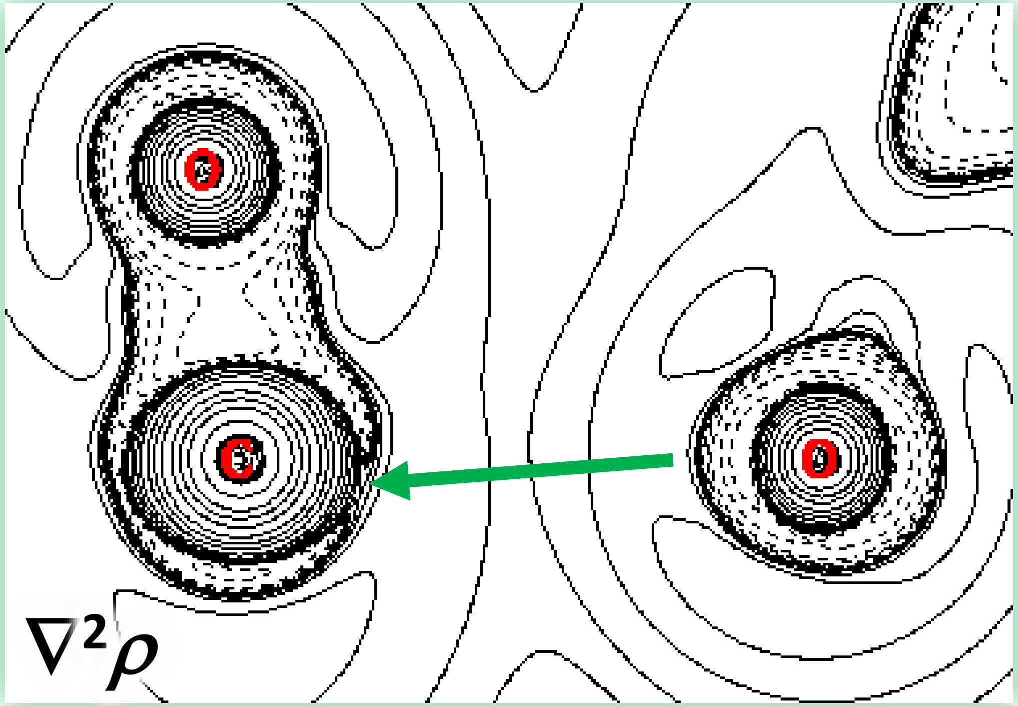
Caprolactam

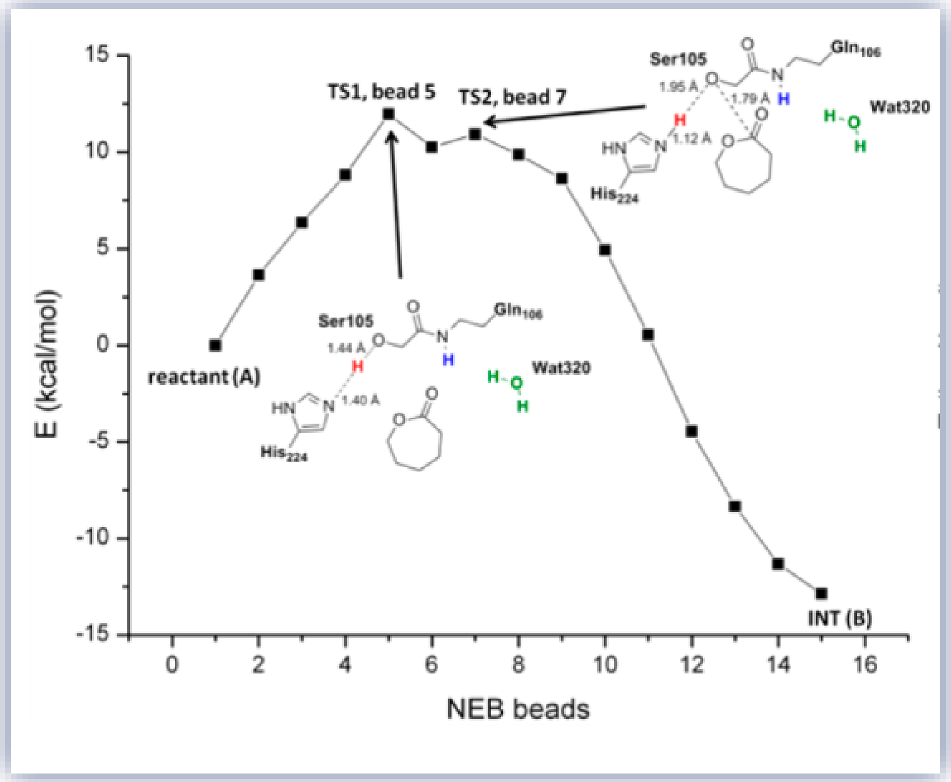




QM(PBE0/cc-pvdz)/MM

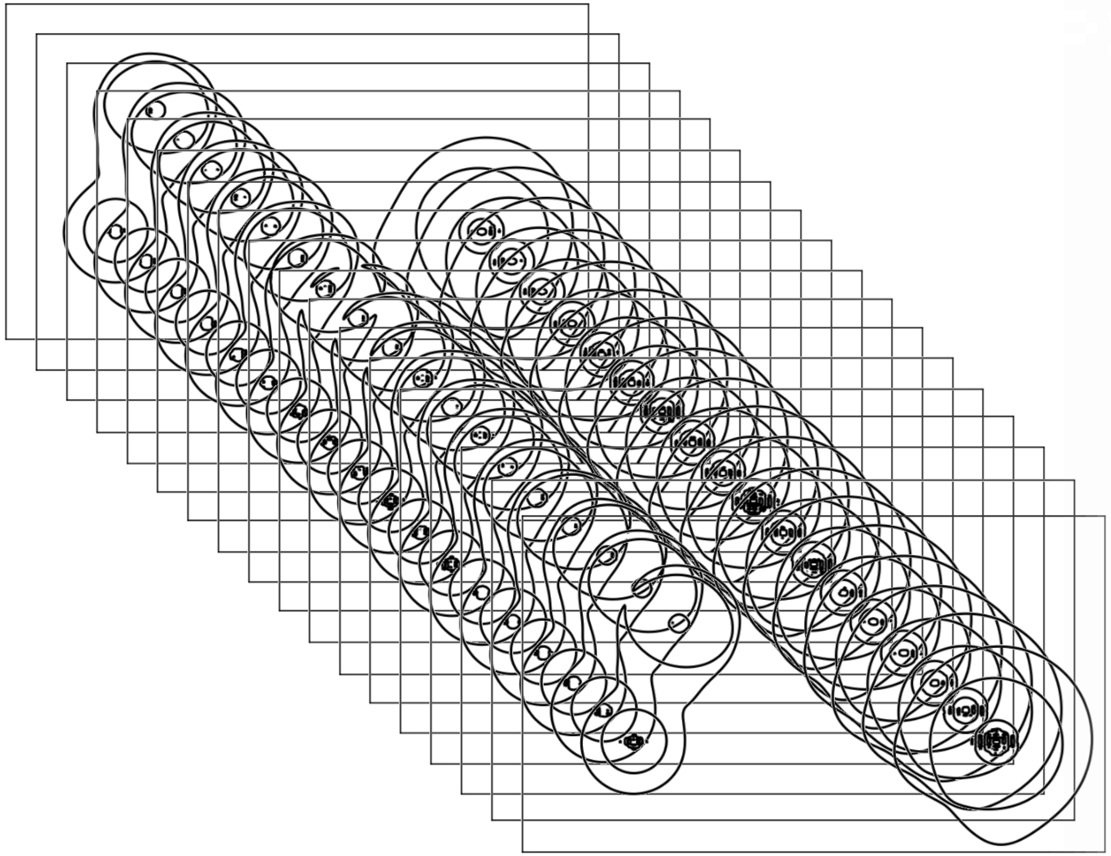
Caprolactone





QM(PBE0/cc-pvdz)/MM

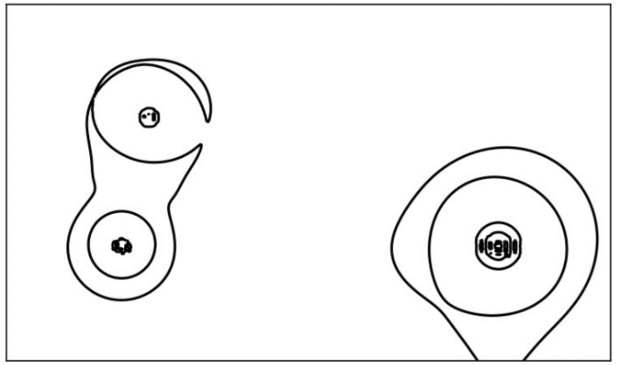
On-the-fly identification of the reactive and non-reactive species from MD trajectories



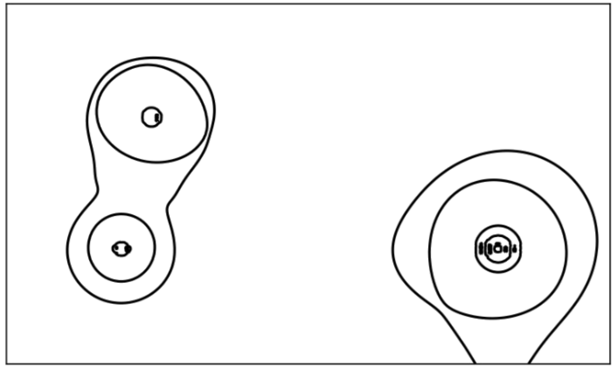
or

neural network

reactive



nonreactive



Scientific collaborations

Prof. A.V. Nemukhin
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Chemistry and Molecular
Modeling

Prof. V.G. Tsirelson
and members of
Quantum Chemistry
Department

Prof. M.G. Khrenova
and members of
Group of Molecular
Modeling

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