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

Property of the system

Time step

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$_2$O
- 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

\[
\text{imipenem} 
\rightarrow \text{hydrolyzed imipenem}
\]
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

<table>
<thead>
<tr>
<th>strain</th>
<th>( k_2/K_s ), mM(^{-1})s(^{-1} )</th>
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<td>FA19</td>
<td>1710 ± 90</td>
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<td>11.3 ± 0.4</td>
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<tr>
<td>H041</td>
<td>0.74 ± 0.03</td>
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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 $M^{\text{Pro}}$ 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?

\[ nES \overset{K}{\rightleftharpoons} rES \]

✓ Criteria of assignment of conformations to either reactive or nonreactive
Laplacian of electron density

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

\( \nabla^2 \rho(r) > 0 \) – electron density depletion regions

\( \nabla^2 \rho(r) < 0 \) – electron density concentration regions

Criterion to discriminate reactive and nonreactive species

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

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

Red isolines correspond to the ED concentration regions, $\nabla^2 \rho(r) < 0$
Criteria to discriminate reactive and nonreactive species

All three geometry criteria should be satisfied together

\[ R < 2.05 \, \text{Å} \]
\[ R < 2.15 \, \text{Å} \]
\[ R < 3.25 \, \text{Å} \]
Substrate specificity and rES ↔ nES equilibrium

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<tr>
<th>Substrate</th>
<th>(\chi), %</th>
<th>(k_{\text{cat}}) (calc.)</th>
<th>(k_{\text{cat}}) (exp.)</th>
</tr>
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<tr>
<td>S-P2Leu</td>
<td>22.4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>S-P2Ile</td>
<td>10.2</td>
<td>0.46</td>
<td>0.45</td>
</tr>
<tr>
<td>S-P2Ala</td>
<td>0.6</td>
<td>0.03</td>
<td>&lt;0.1</td>
</tr>
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* values relative to S-P2Leu

\[ k_{\text{cat}}(\text{AA}) = k_{\text{cat}}(\text{Leu}) \chi(\text{AA}) / \chi(\text{Leu}) \]

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

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

Nu: oxyanion hole

X = O, NH

CALB
Caprolactam

\( \nabla^2 \rho \)

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
Scientific collaborations

Prof. A.V. Nemukhin and members of Laboratory of Quantum 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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