



CEITEC



Central European Institute of Technology
BRNO | CZECH REPUBLIC

Návrhy nových léčiv s použitím výpočetních nástrojů

Jaroslav Koča

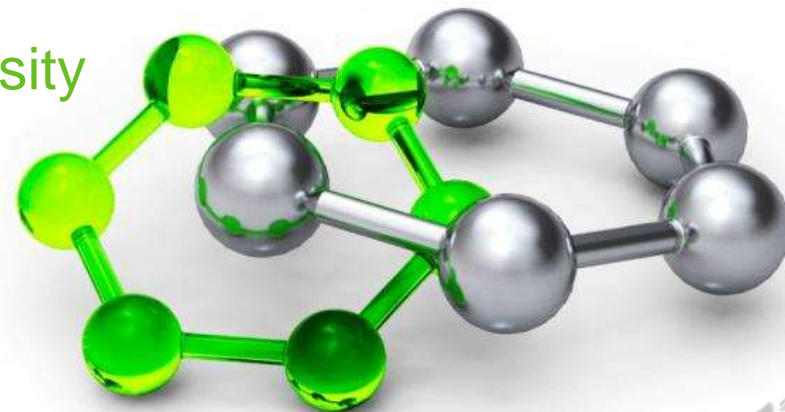
CEITEC and NCBR, Fac Sci, Masaryk University



EUROPEAN UNION
EUROPEAN REGIONAL DEVELOPMENT FUND
INVESTING IN YOUR FUTURE



OP Research and
Development for Innovation



Computational chemistry group at CEITEC/NCBR

Protein/carbohydrate interaction with various techniques
(docking, QM, TI, ...)

Reaction mechanism of enzymatic reactions using QM/MM molecular
dynamics

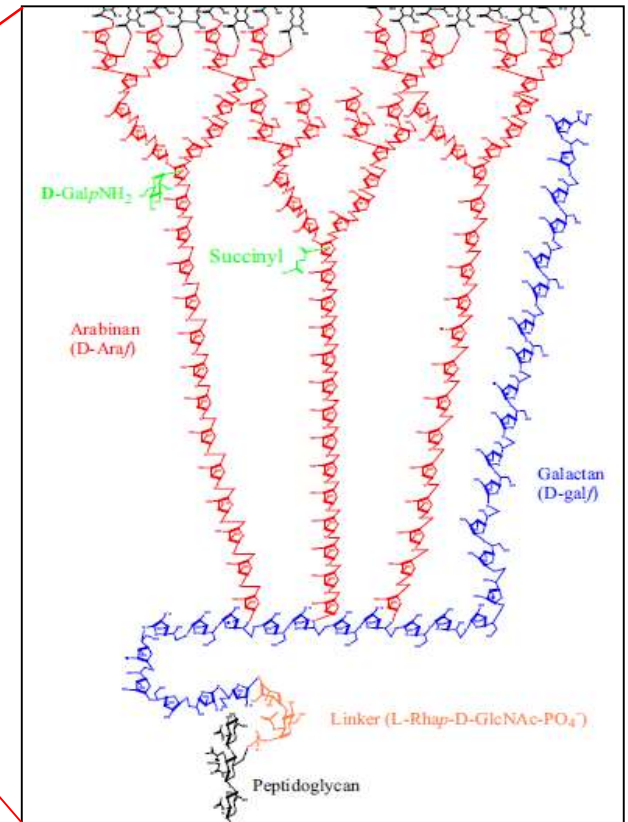
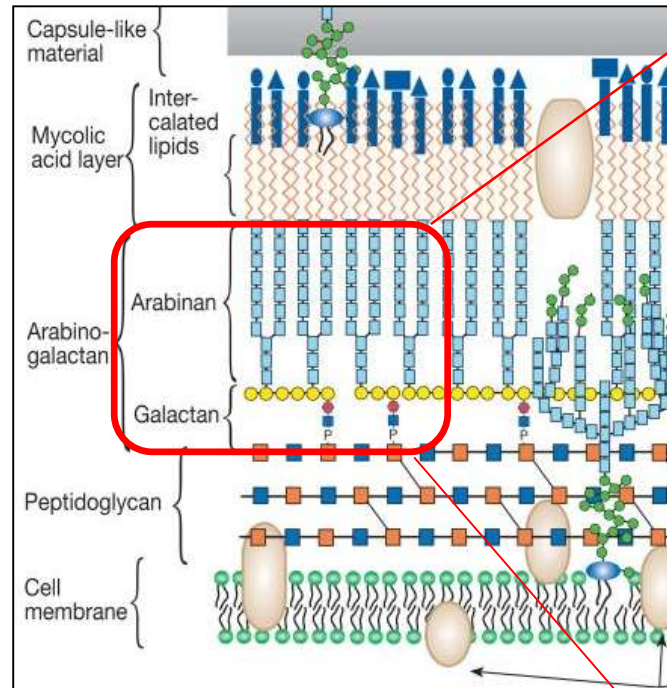
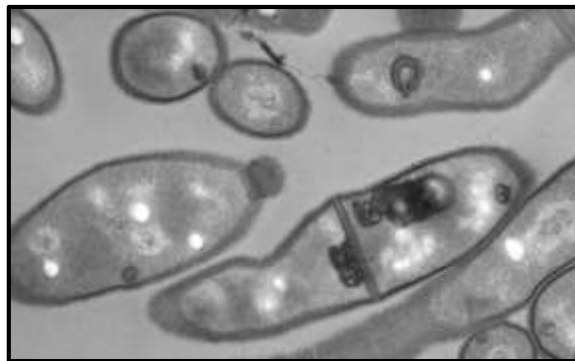
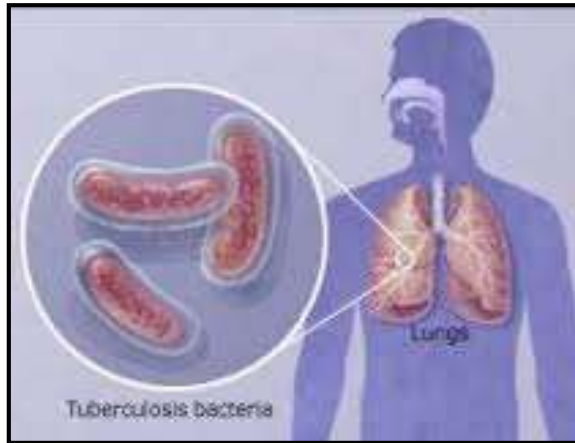
Structural bioinformatics (Radka Svobodová)

“Classical” bioinformatics (Crina Maria Ionescu)



Tuberculosis come back !

Carbohydrate-active enzymes – *Mycobacterial glycosyl transferases*

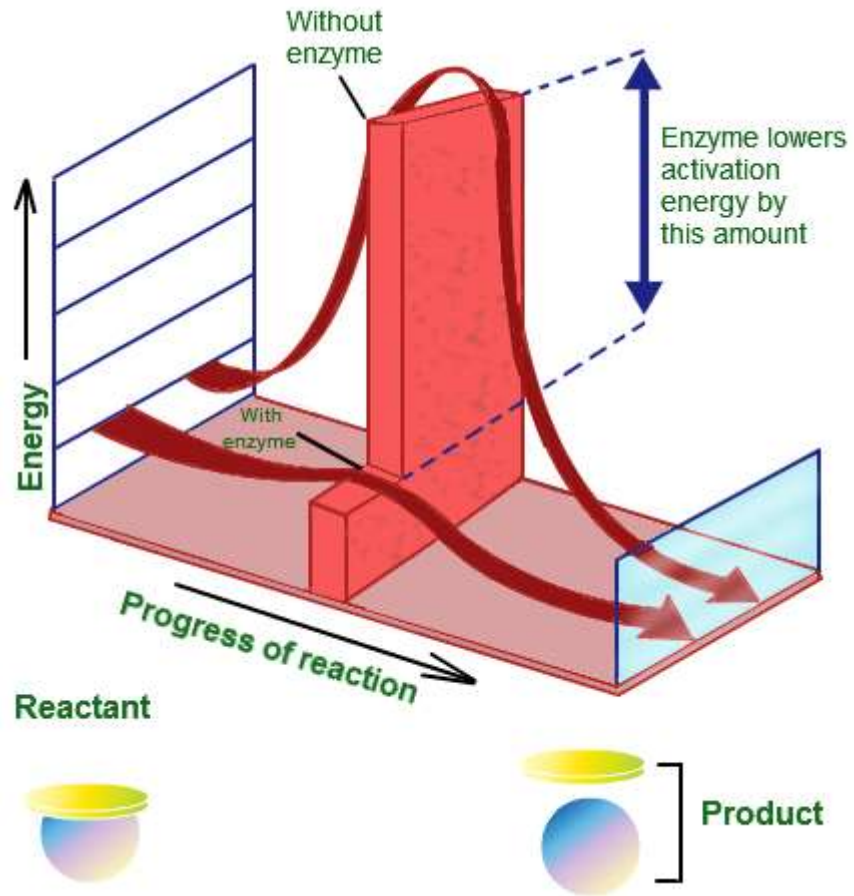


Thick, hydrophobic cell wall
⇒ resistance of *Mycobacterium*
against disinfectants, antibiotics,
dehydration. Supports the survival in
the macrophages.

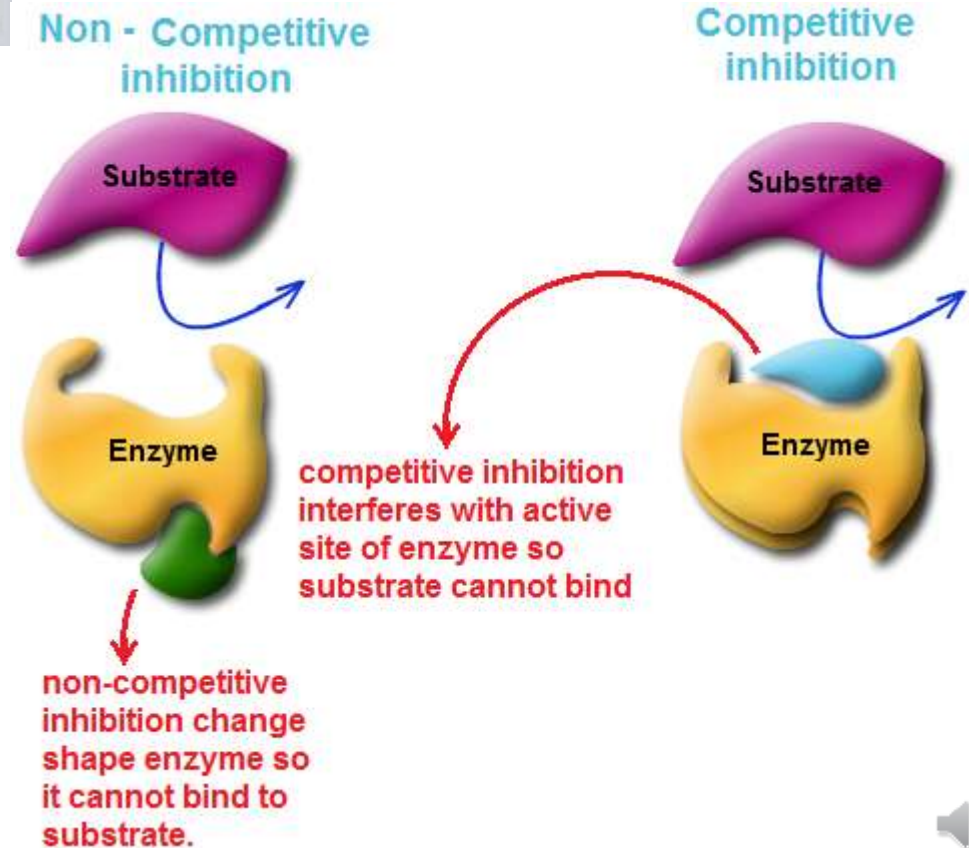
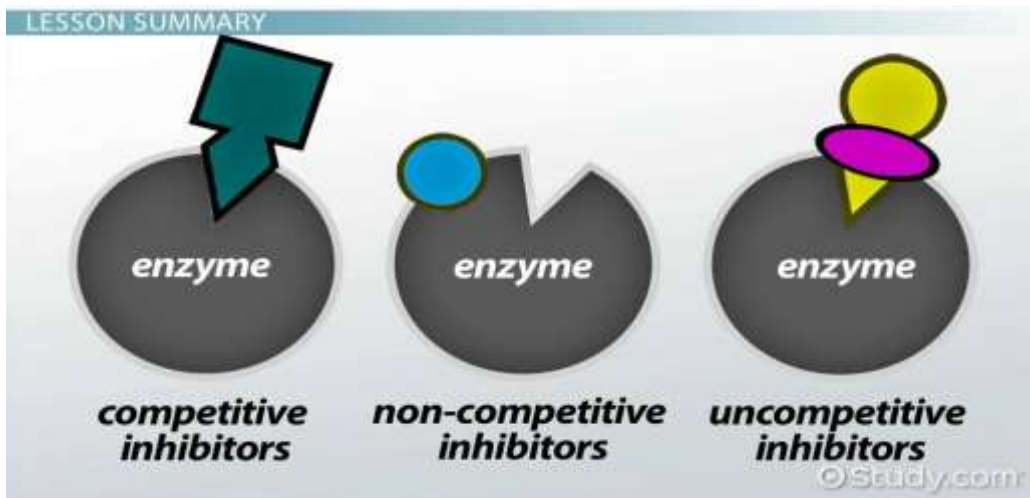
- 1.3 million deaths caused by tuberculosis in 2012, 1.8 million deaths in 2015 (out of 10.4 million cases)
- multidrug-resistance (MDR)

Arabinogalactan
Synthesised by complex
of glycosyltransferases.
**Attractive target for
drug design.**

Enzymatic reaction



Enzyme inhibition



Transition State Analogue Inhibitors (TSAI)

DESIGNING TRANSITION-STATE INHIBITORS

A transition-state mimic has the power to bind an enzyme at its tipping point as strongly as any available inhibitor and more strongly than most, preventing enzymatic activity. In order to replicate the structure of an enzyme's transition state, which only lasts a few femtoseconds, we use computational and experimental methods to reveal the shape, atom by atom.

THE REACTION

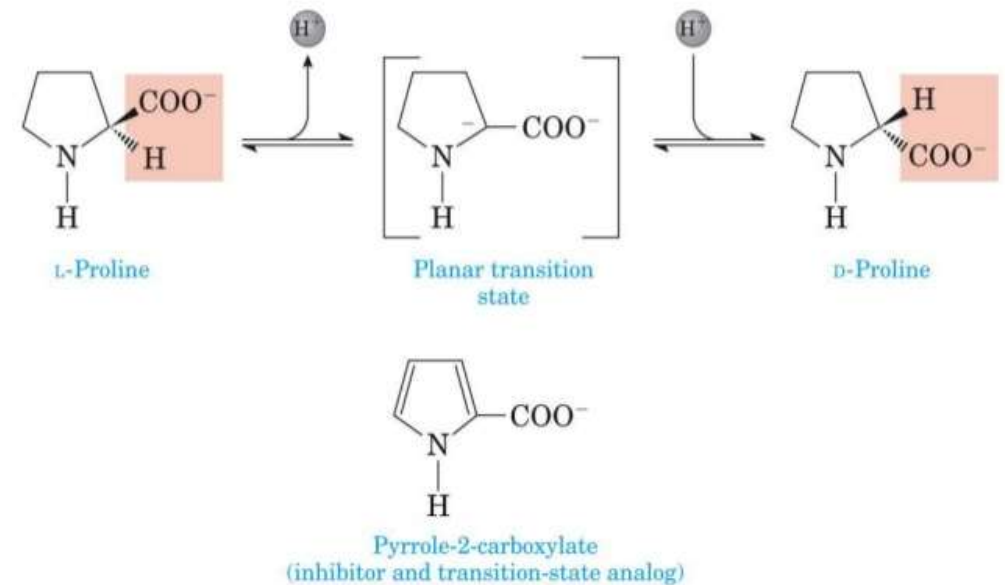
An enzyme normally binds a reactant in its active site **1** to accelerate the reactant's conversion into a product **3**. That timescale during which bonds are broken to form a product—the so-called transition state—occurs over the span of several femtoseconds **2**. We decided to design a mimic for the transition state of the enzyme purine nucleoside phosphorylase (PNP), because it is involved in keeping T cells alive. If we could inhibit this enzyme using a transition-state analog, we could perhaps halt the runaway replication of T cells that occurs in leukemia **4**.

INHIBITING THE REACTION

We first defined the transition-state structures of PNP, and then created a mimic **1** of that structure that would be able to bind the enzyme millions of times more strongly than the reactant, because bond-breaking forces are converted to binding energy **2**. With help from our collaborators in New Zealand who synthesized the transition-state analog called imuzicidin-H, we were able to successfully block PNP's activity for the lifetime of the cell **3**. The transition-state mimic is now in clinical trials for the treatment of several forms of leukemia.

LARRY RICHARDS, UNIVERSITY OF THE SAUTHERN CROSS

Transition-State Analog



"Freezing Time" by Vern L. Schramm (Scientist 26 (5), 30-35, May 1, 2012)



Why Transition State Analogue Inhibitors (TSAI) ?

Plus:

Much lower amount/dose needed (could be like 1,000,000 (or even more) lower)

Minus:

Difficult to obtain TS structure

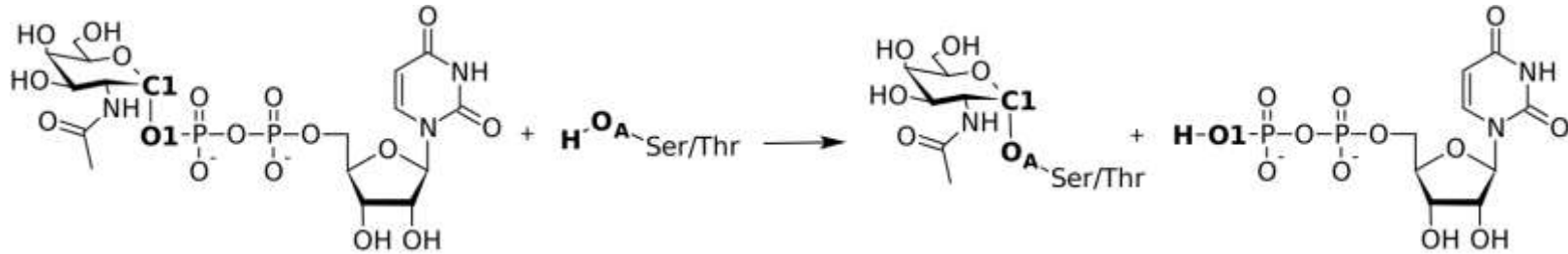
Works only for enzymes

For TSAI see, for example, a paper "Freezing Time" by Vern L. Schramm (Scientist 26 (5), 30-35, May 1, 2012)

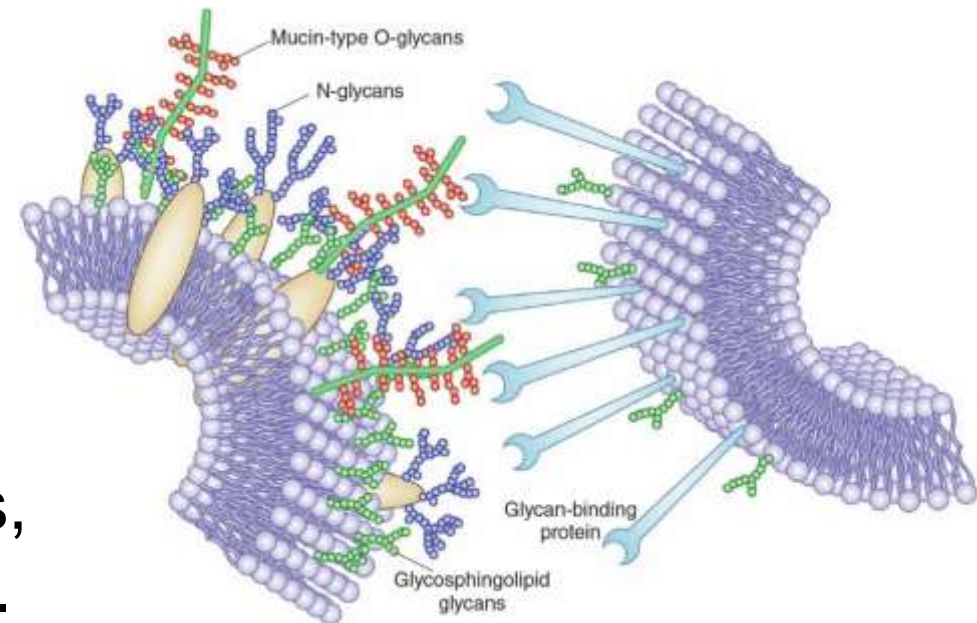


Chemical and Biological Background

- **Glycosyltransferases** - catalyze the transfer of saccharide **from activated nucleotide sugar to nucleophilic glycosyl acceptor molecule**



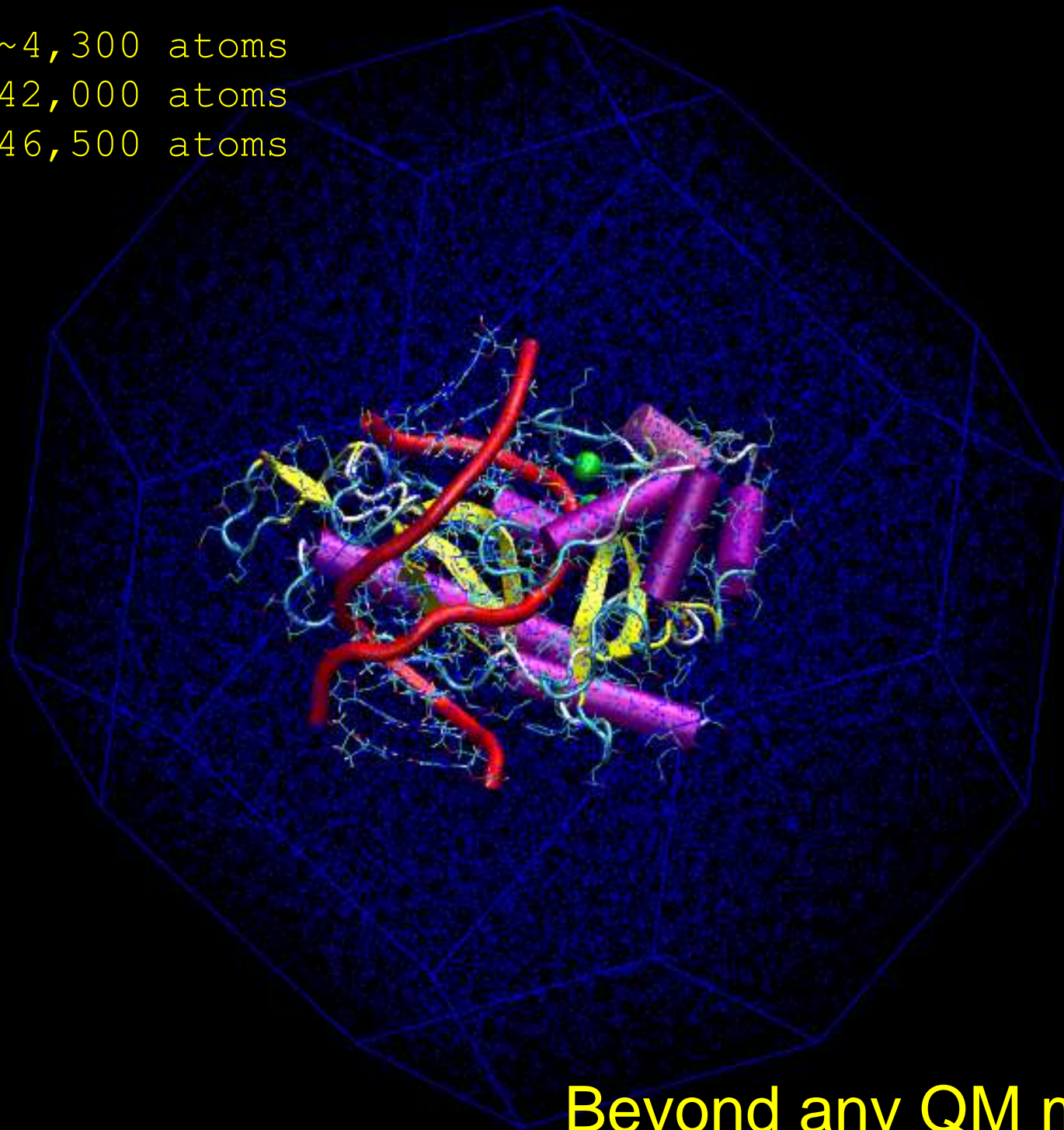
- Glycoconjugates:
 - one of the fundamental biopolymers found in cells
 - Glycoproteins, glycolipids, ...
 - involved in cell–cell interactions, signaling, folding, pathogenesis, bacterial cell wall formation, ...



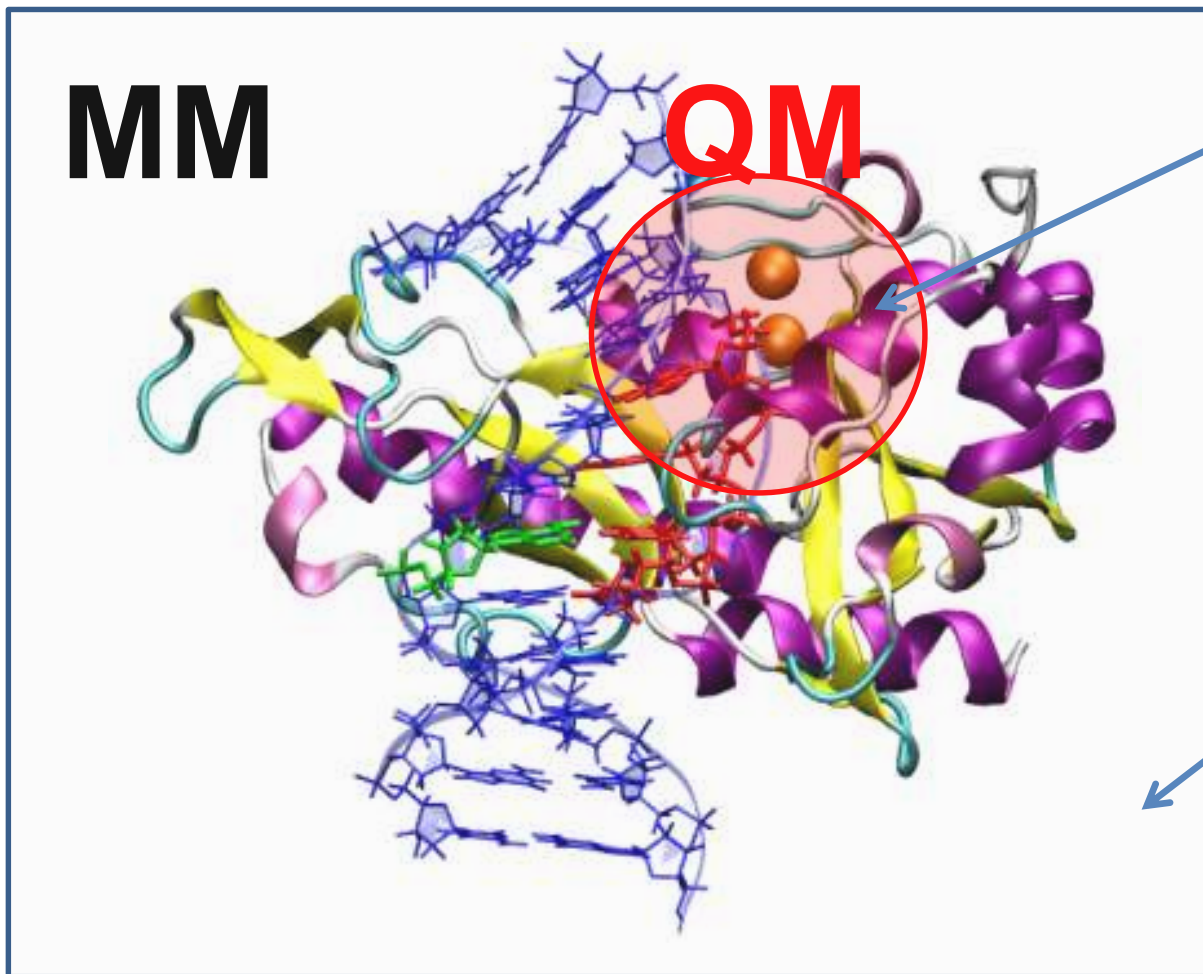
Zaia, Joseph. "At Last, Functional Glycomics." *Nat Meth* 8, 1. 2011.



Enzyme: ~4,300 atoms
Water: ~42,000 atoms
Total: ~46,500 atoms



Beyond any QM method!



MM

QM

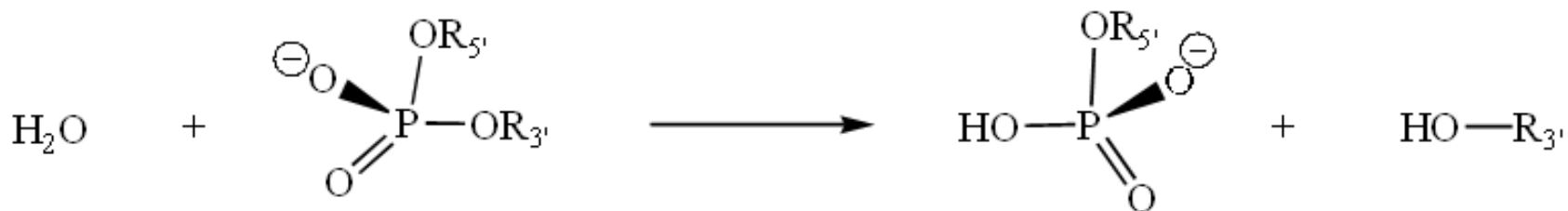
QM

active site only
tens to hundreds atoms

MM

rest of enzyme
solvent (water)

Catalyzed reaction – hydrolysis of phosphodiester bond

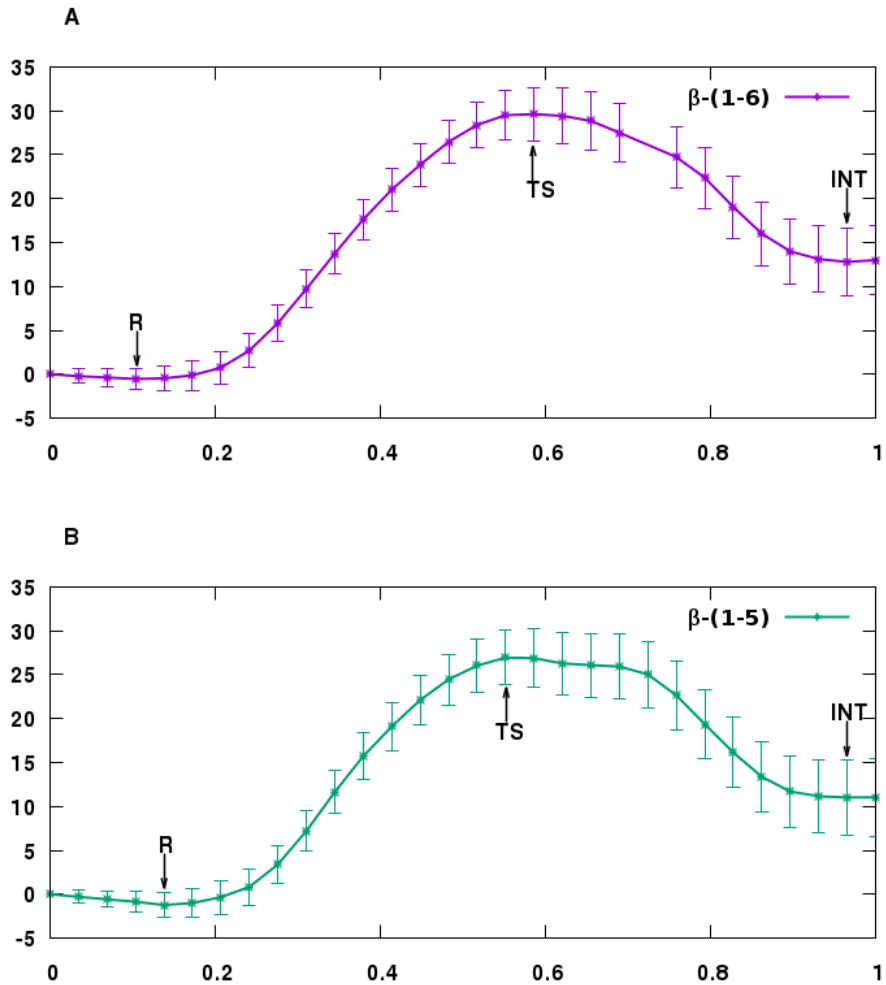


How to Study Mechanisms of Enzymatic Reactions

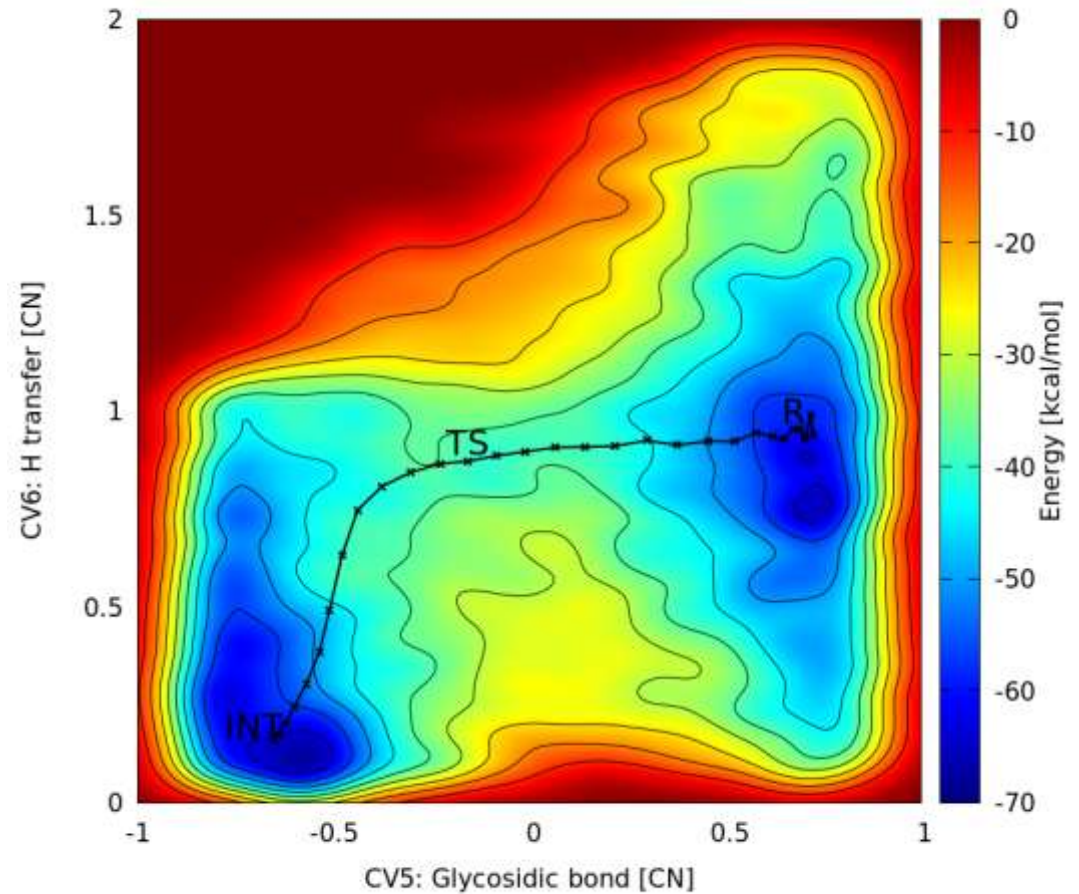
- MD based methods (CPMD and others)
 - ❖ Proper statistical sampling of states (in theory)
 - ❖ Extremely computationally demanding, hard to reach converged results
- Analysis of the Potential Energy Surface
 - ❖ Overview of the whole energetic landscape
 - ❖ Selecting suitable scan coordinates is absolutely crucial, errors hard to detect
- Optimization of Minimum Energy Reaction Paths
 - ❖ Guaranteed continuous smooth reaction path
 - ❖ Impossible to tell if the path is physically relevant



Results



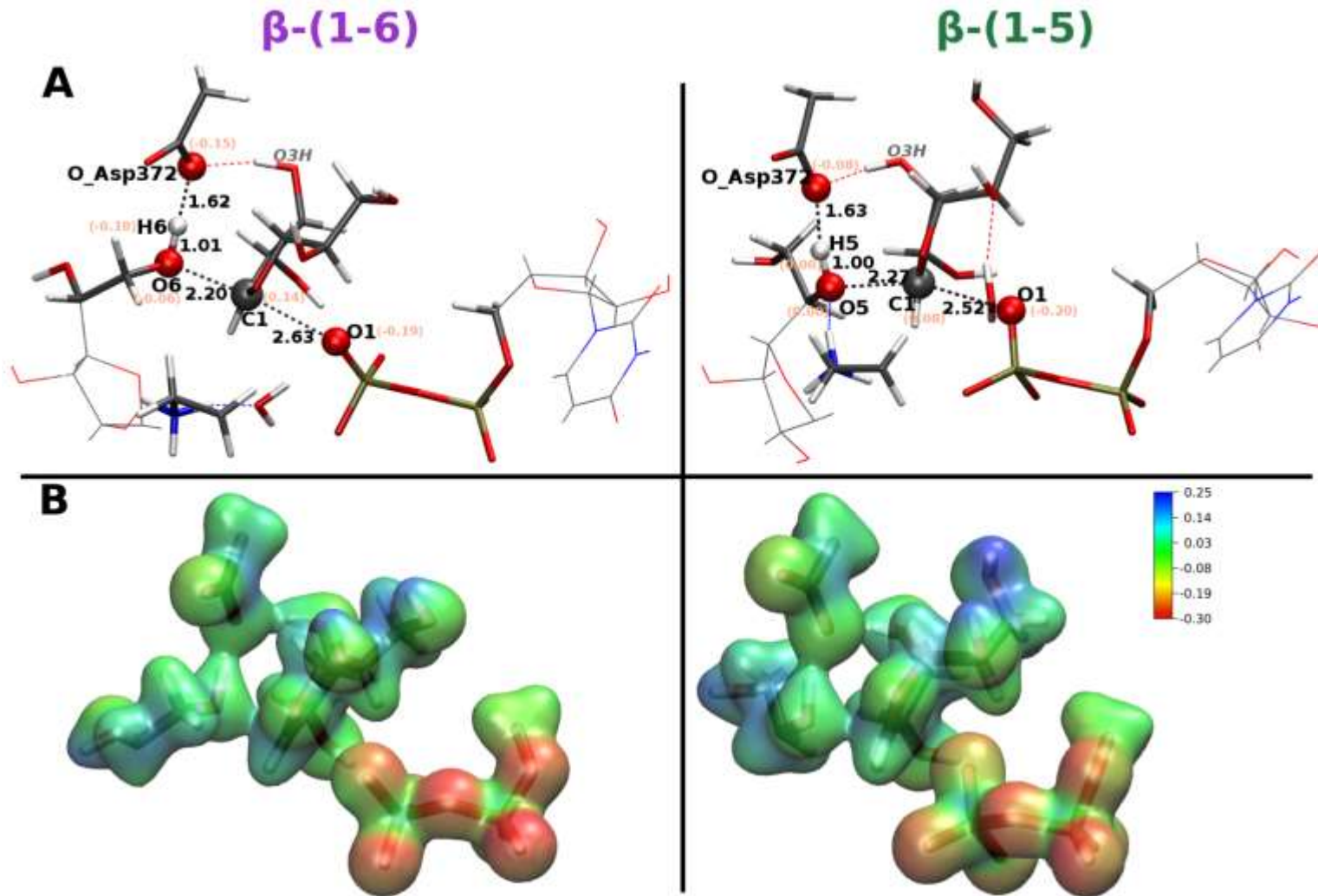
STM free energy profiles of β -(1-6) and β -(1-5) reactions.



MTD free energy surface of β -(1-6) reaction.



Results



Average transition state structures and the electrostatic potential from STM.

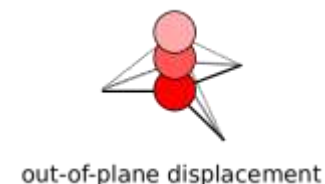
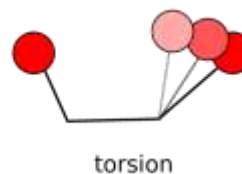
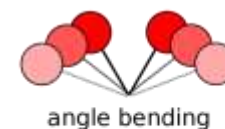
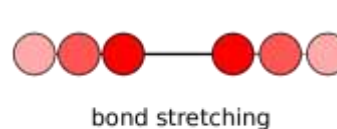
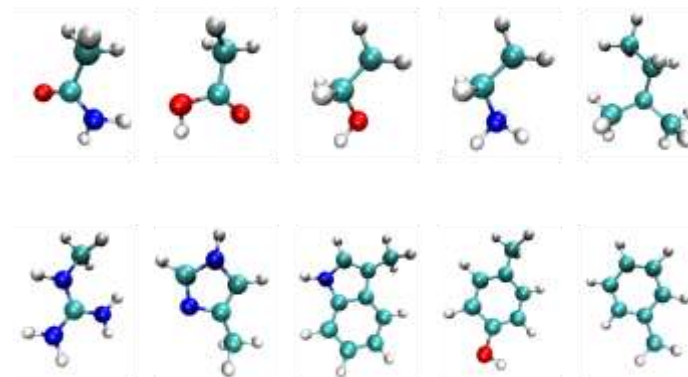
Reactive Molecular Dynamics

- Ordinary QM/MM calculation is very time consuming !!!
- Ordinary MM models (force fields) cannot handle reactions
 - Predefined bonding topology
- ReaxFF reactive force field:
 - Determines bonds on the fly
 - Able to handle diverse systems (explosives, hydrocarbons, geochemistry, catalysis etc.)
- Suitable parameter set for enzymes missing



Parametrizing ReaxFF


- Hundreds of empirical parameters need to be tuned
 - Strong dependencies between parameters – simultaneous optimization necessary
 - State-of-the-art numerical optimization algorithm (VD-CMA-ES)
- Training set: over 7600 geometries of 31 small model molecules
 - Reference data calculated using accurate QM (M06-2X)
 - Fully automated generation by perturbing bonds lengths, valence and torsion angles



Summary

- Parameters of the ReaxFF models optimized for enzymatic reactivity
 - Advanced numerical optimizer
 - Automated generation of the training set with QM data
- Real-life performance compared with QM/MM on ppGalNAcT2 glycosyltransferase
 - Qualitative match
 - **ReaxFF is a million times faster !**
(i.e. 1hr compared to 114 years)





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