CEITEC

Central European Institute of Technology

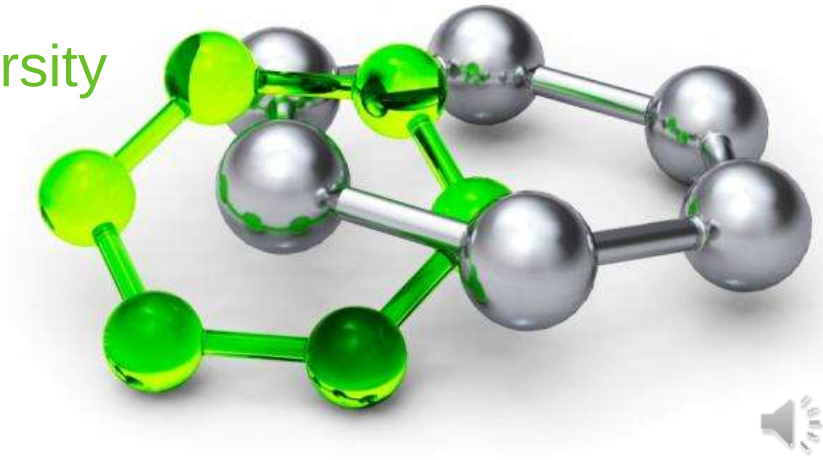
BRNO | CZECH REPUBLIC

UNIVERSITAS MASARYKIANA BRUNENSIS

# Návrhy nových léčiv s použitím výpočetních nástrojů

Jaroslav Koča

CEITEC and NCBR, Fac Sci, Masaryk University



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OP Research and Development for Innovation

# Computational chemistry group at CEITEC/NCBR

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

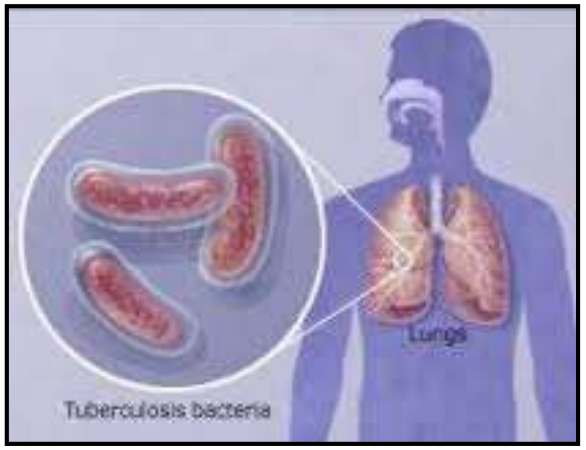
Reaction mechanism of enzymatic reactions using QM/MM molecular dynamics

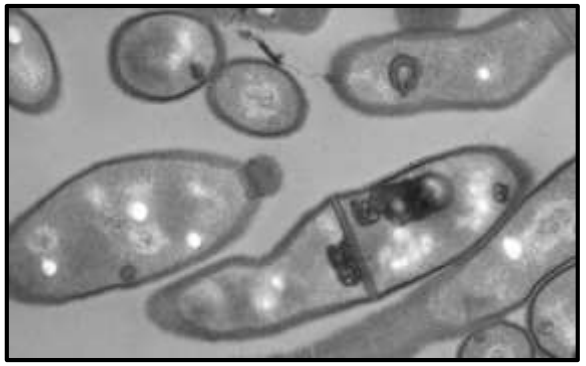
Structural bioinformatics (Radka Svobodová)

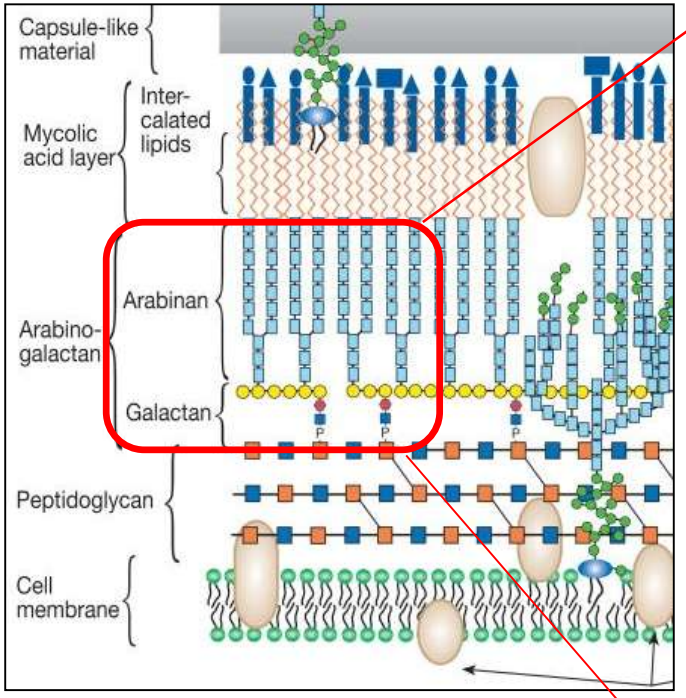
“Classical” bioinformatics (Crina Maria Ionescu)

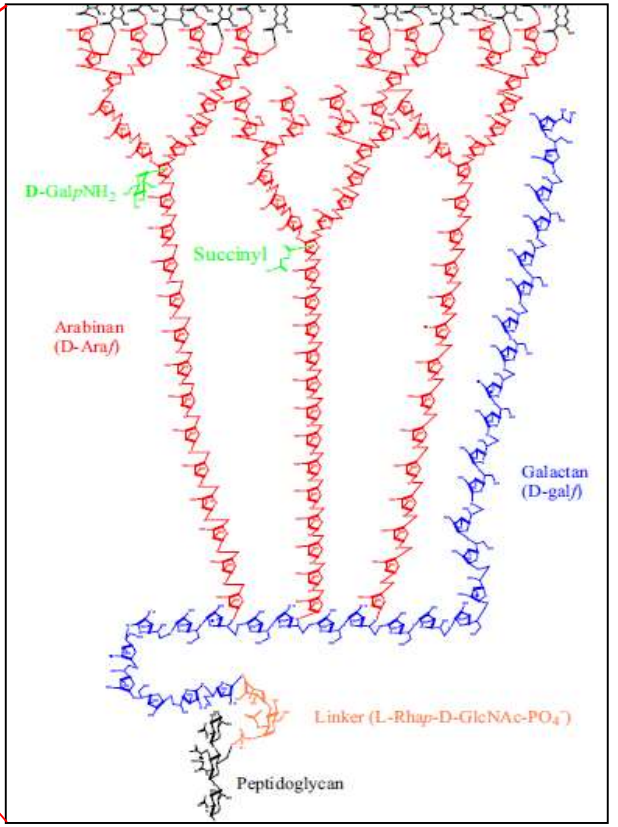
# Tuberculosis come back !

## Carbohydrate-active enzymes – *Mycobacterial glycosyl transferases*






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

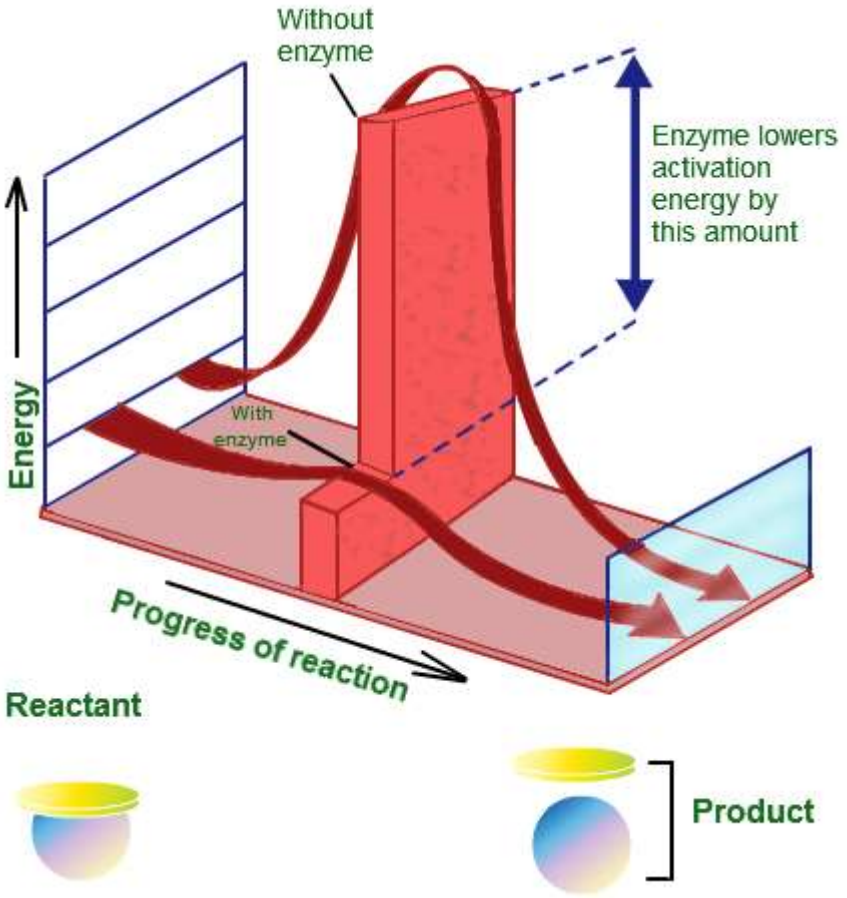
- 1.3 million deaths caused by tuberculosis in 2012, 1.8 million deaths in 2015 (out of 10.4 million cases)
- multidrug-resistance (MDR)

**Arabinogalactan**

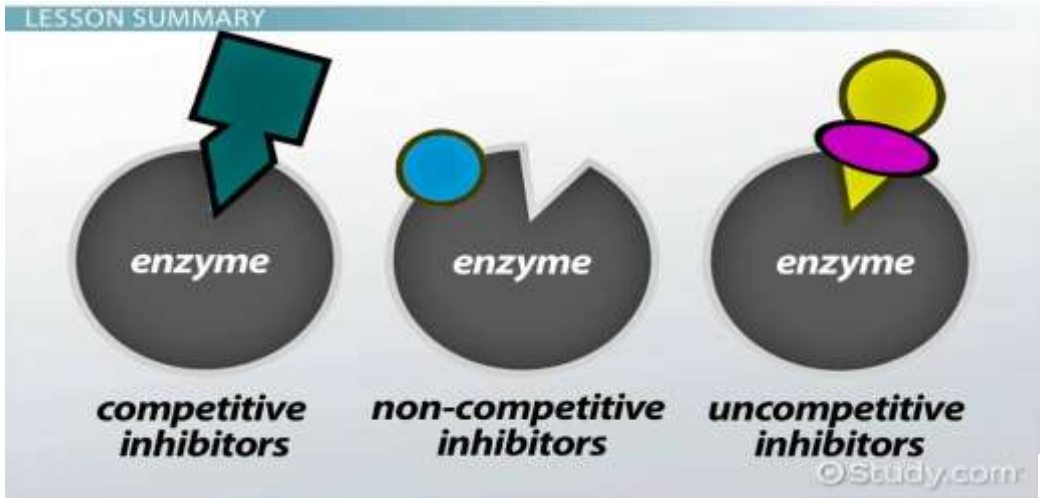
Synthetised by complex of glycosyltransferases.

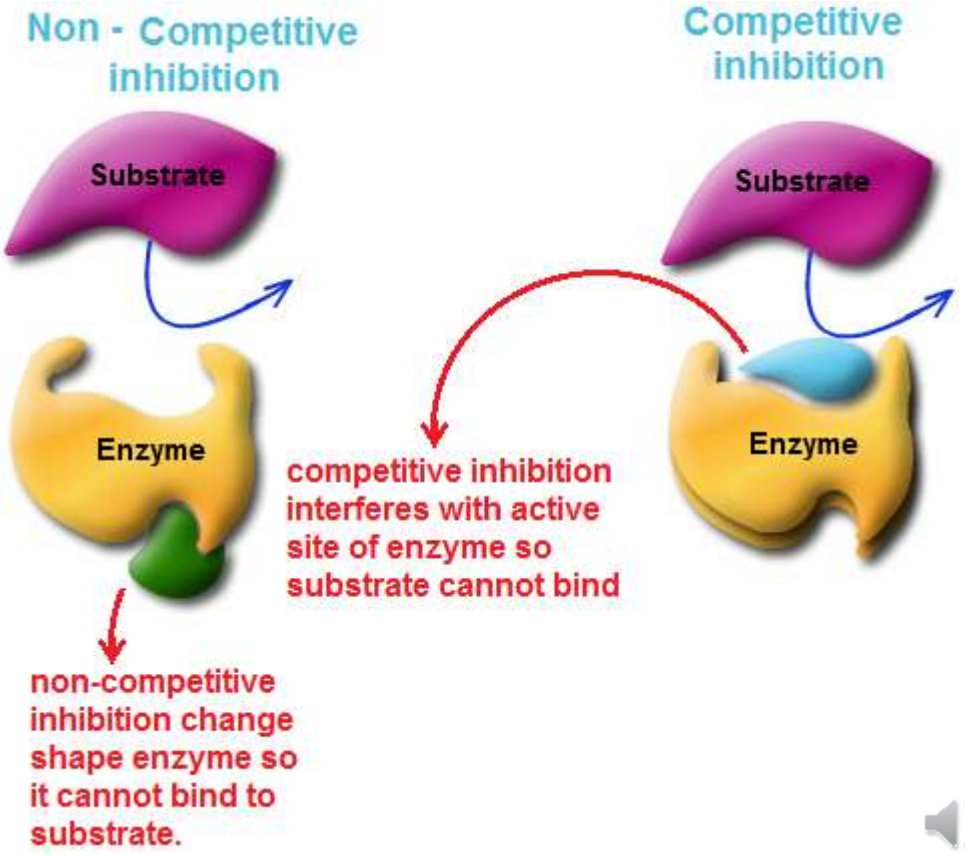
**Atractive target for drug design.**

# Enzymatic reaction

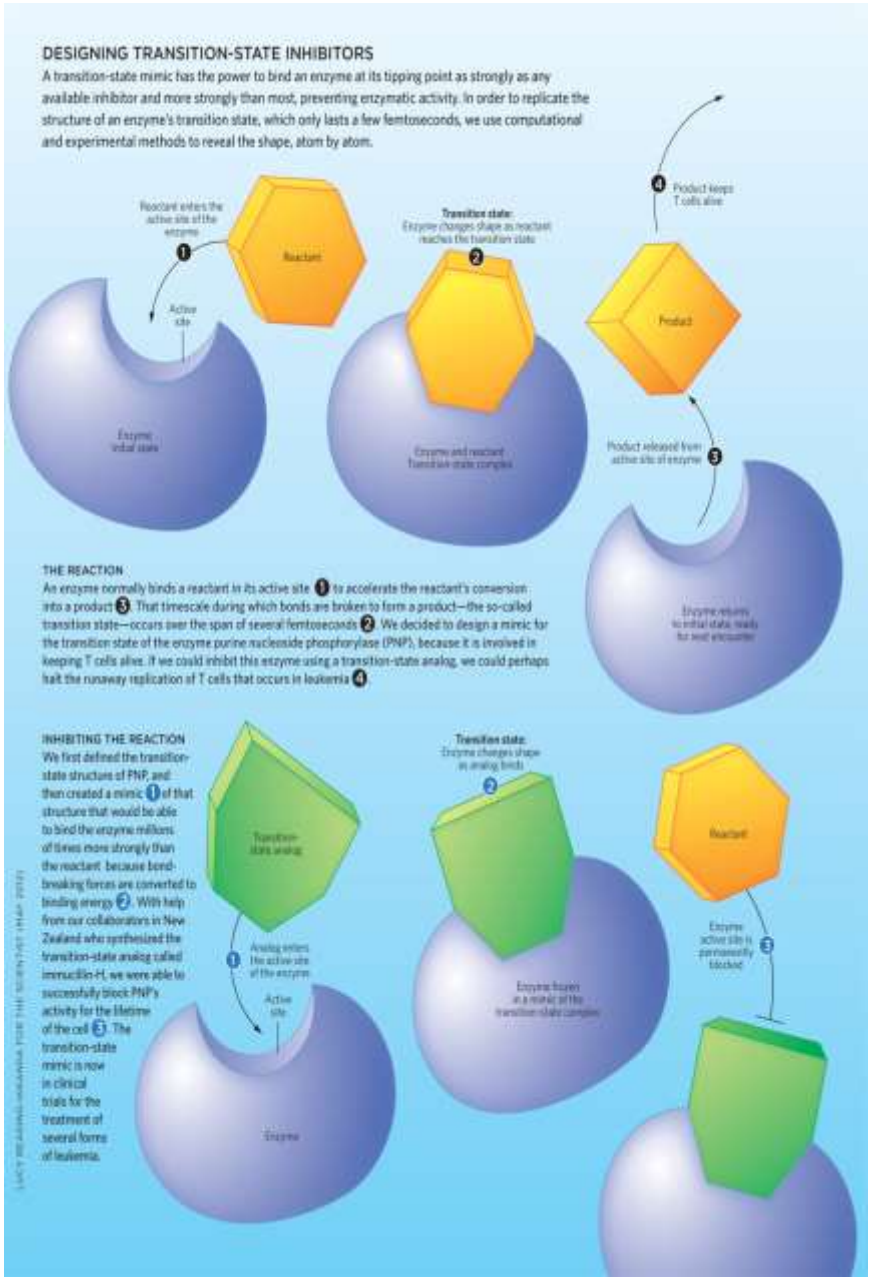


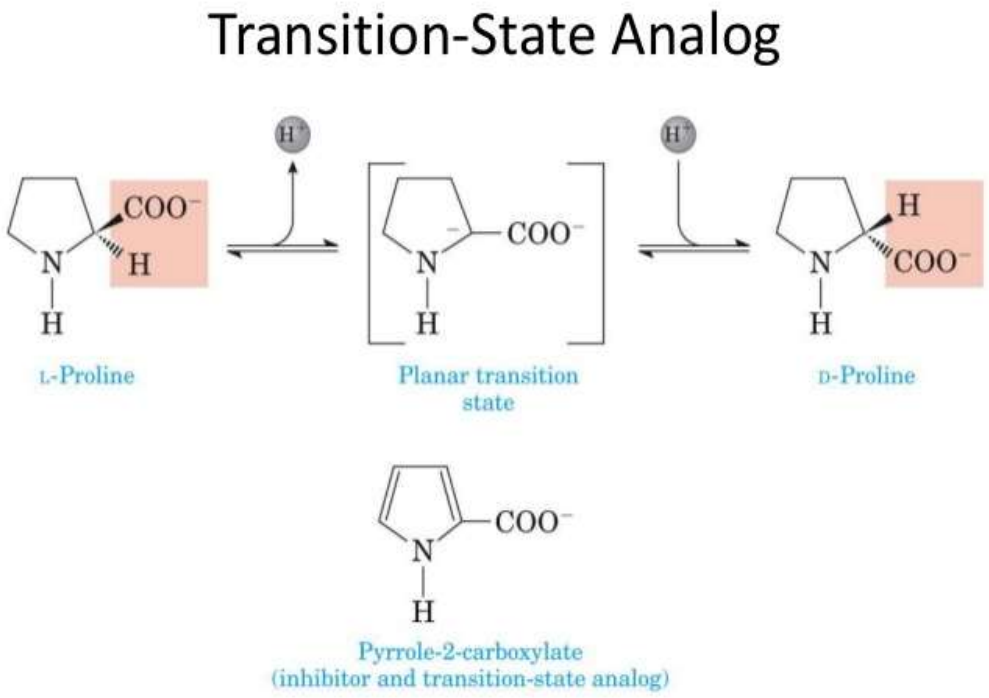
# Enzyme inhibition



# Transition State Analogue Inhibitors (TSAI)




"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)

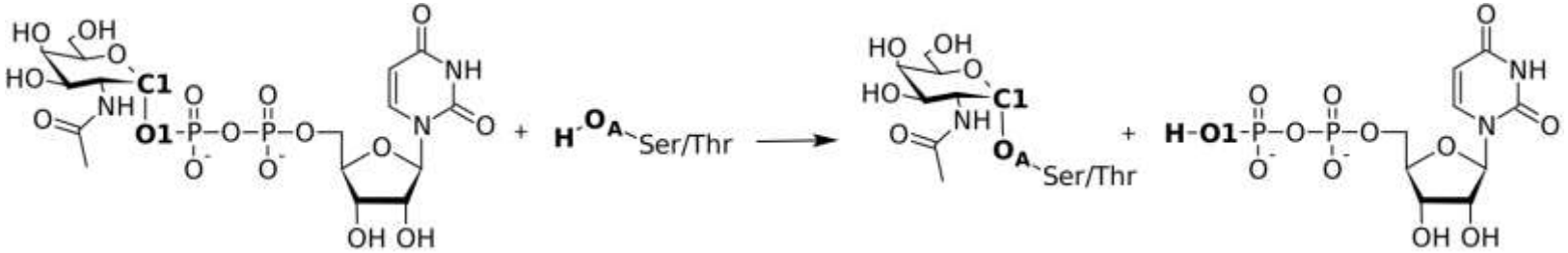
Minus:

Difficult to obtain TS structure
Works only for enzymes

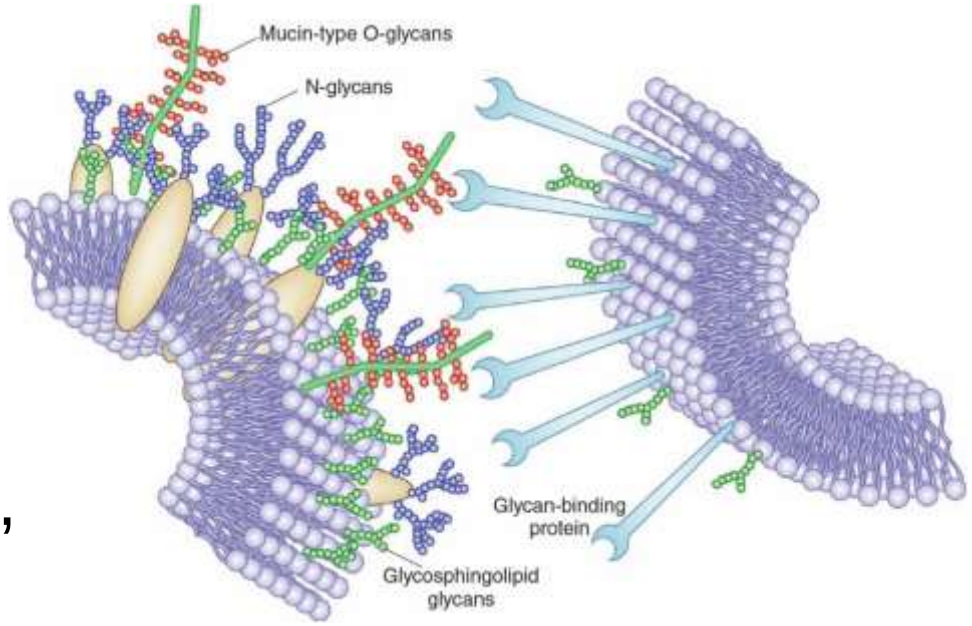
For TSAI see, for example, a paper ”Freezing Time” by Vern L. Schramm (Scientist 26 (5), 30-35, May 1, 2012)

# Chemical and Biological Background

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

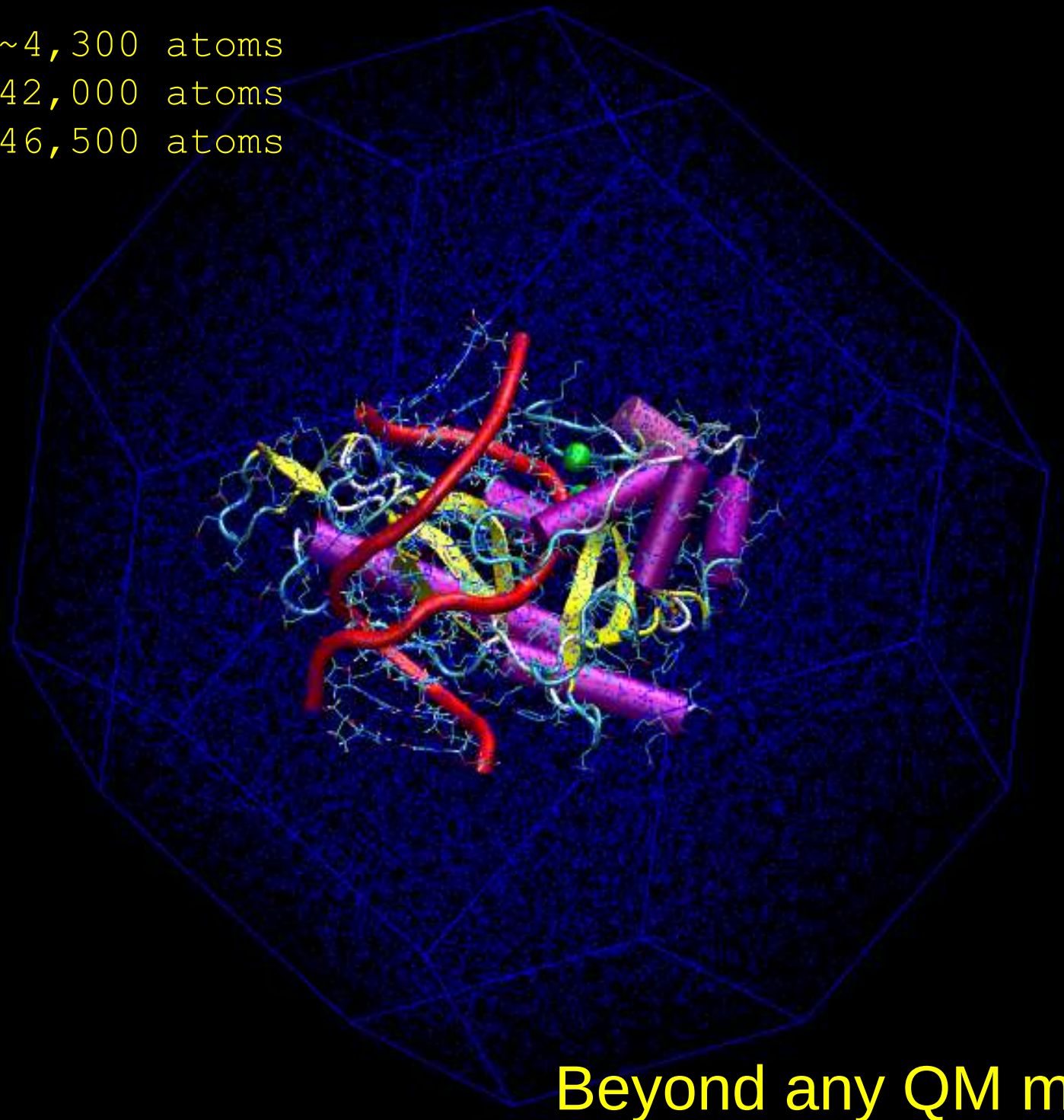



- Glycoconjugates:
  - one of the fundamental biopolymers found in cells
  - Glycoproteins, glycolipids, ...
  - involved in cell–cell interactions, signaling, folding, pathogenesis, bacterial cell wall formation, ...

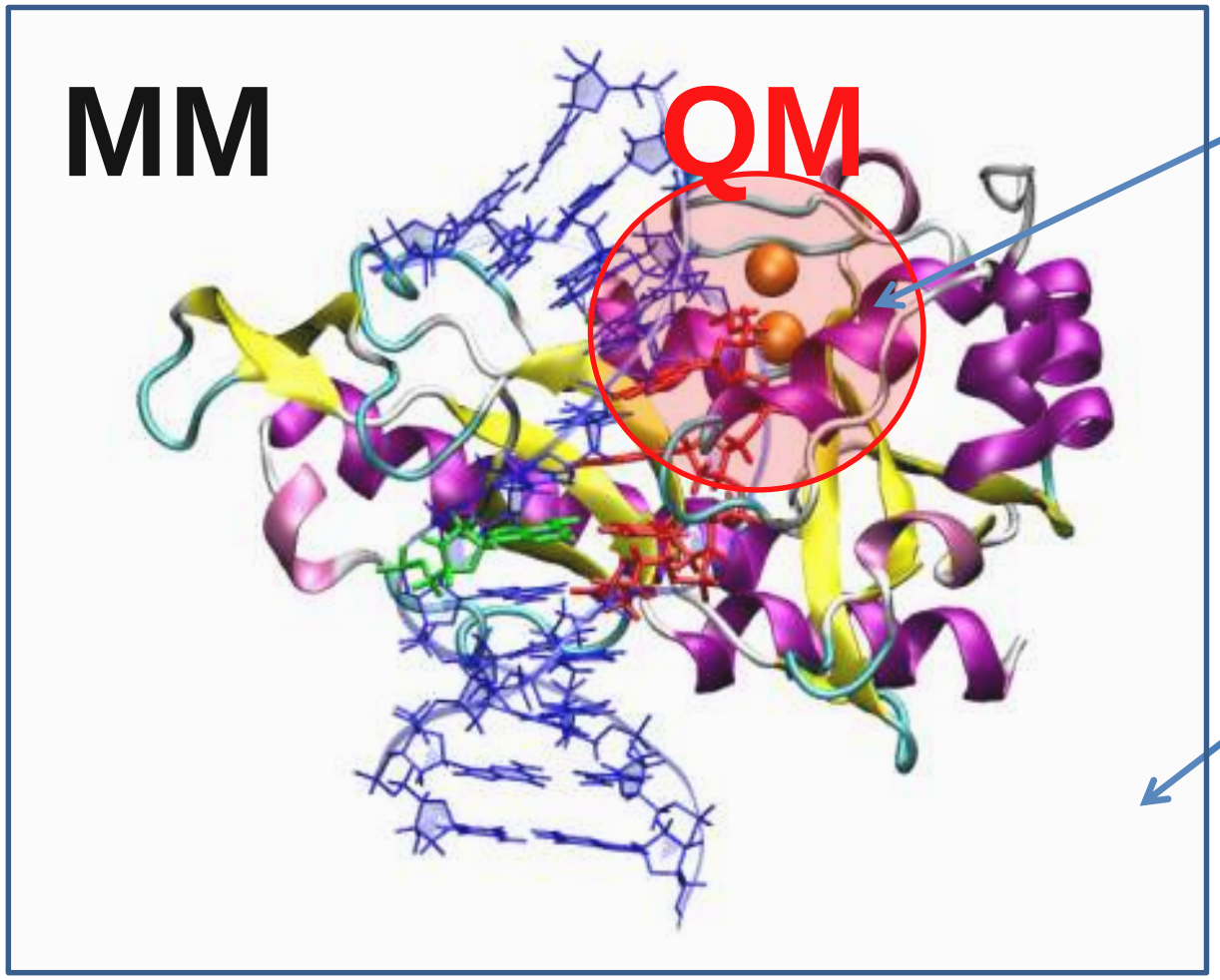



Zaia, Joseph. “At Last, Functional Glycomics.” Nat Meth 8, 1. 2011.

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



Beyond any QM method!



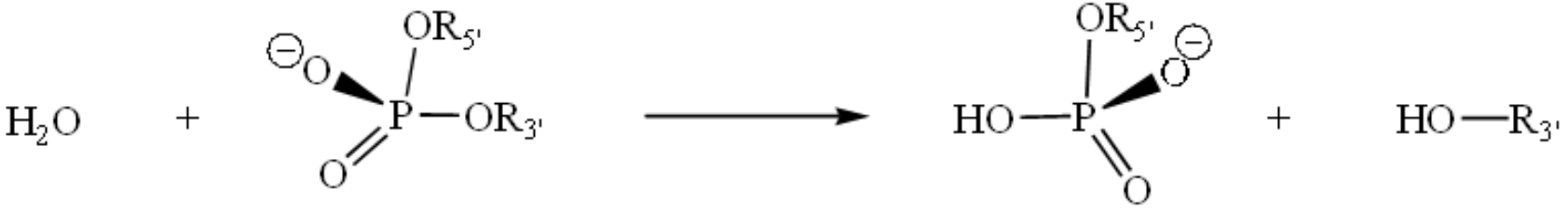

**QM**

**active site only**
**tens to hundreds atoms**

**MM**

**rest of enzyme**
**solvent (water)**

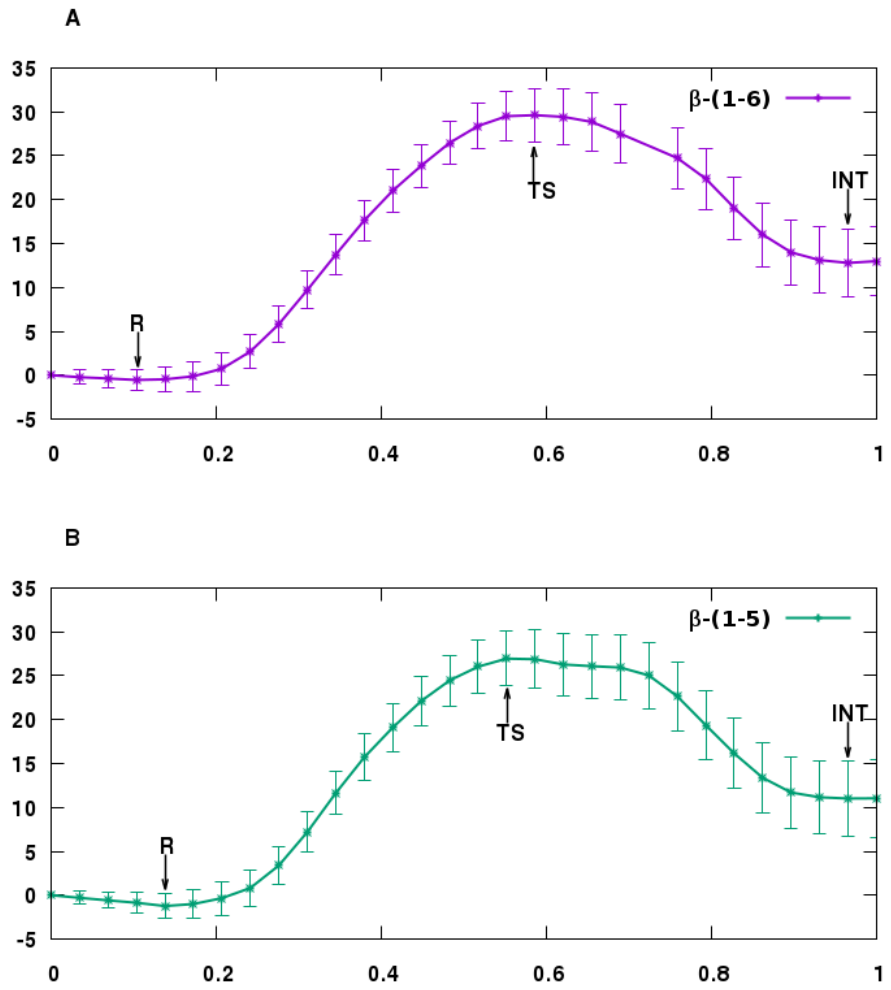
**Catalyzed reaction – hydrolysis of phosphodiester bond**



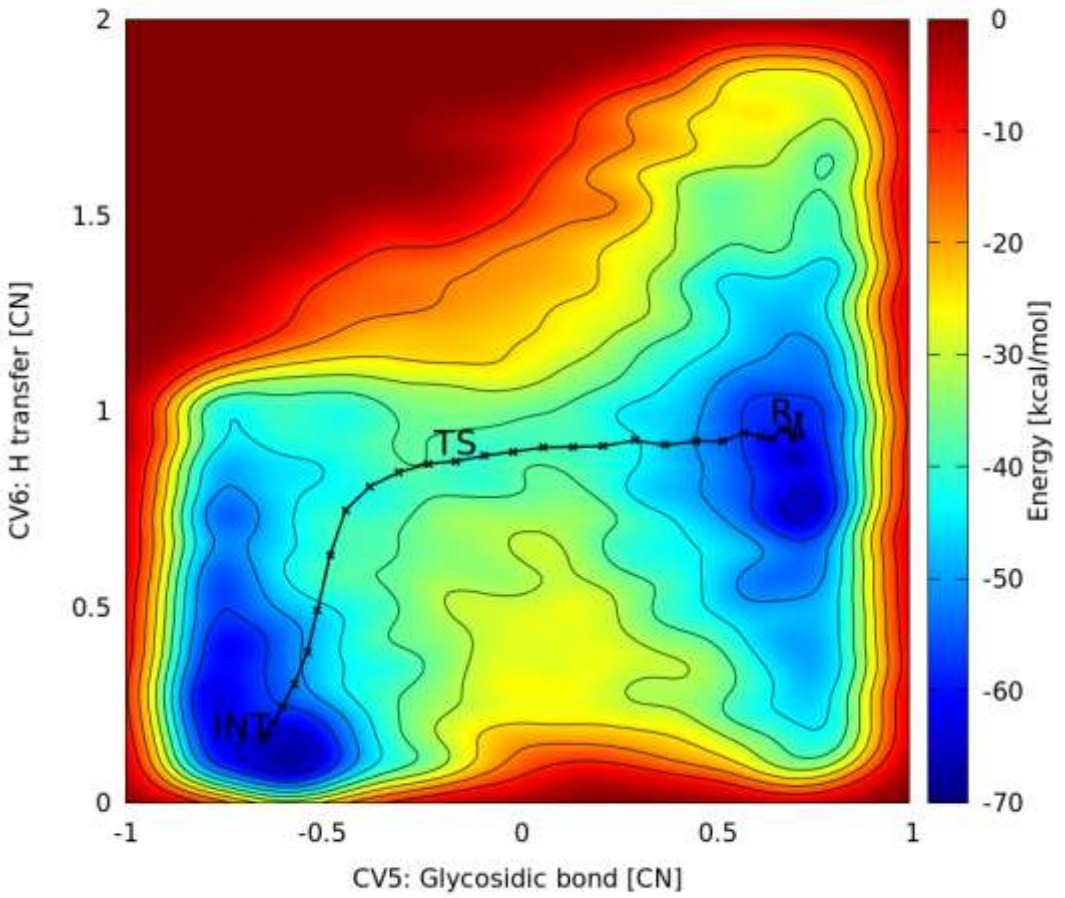
# How to Study Mechanisms of Enzymatic Reactions

- 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

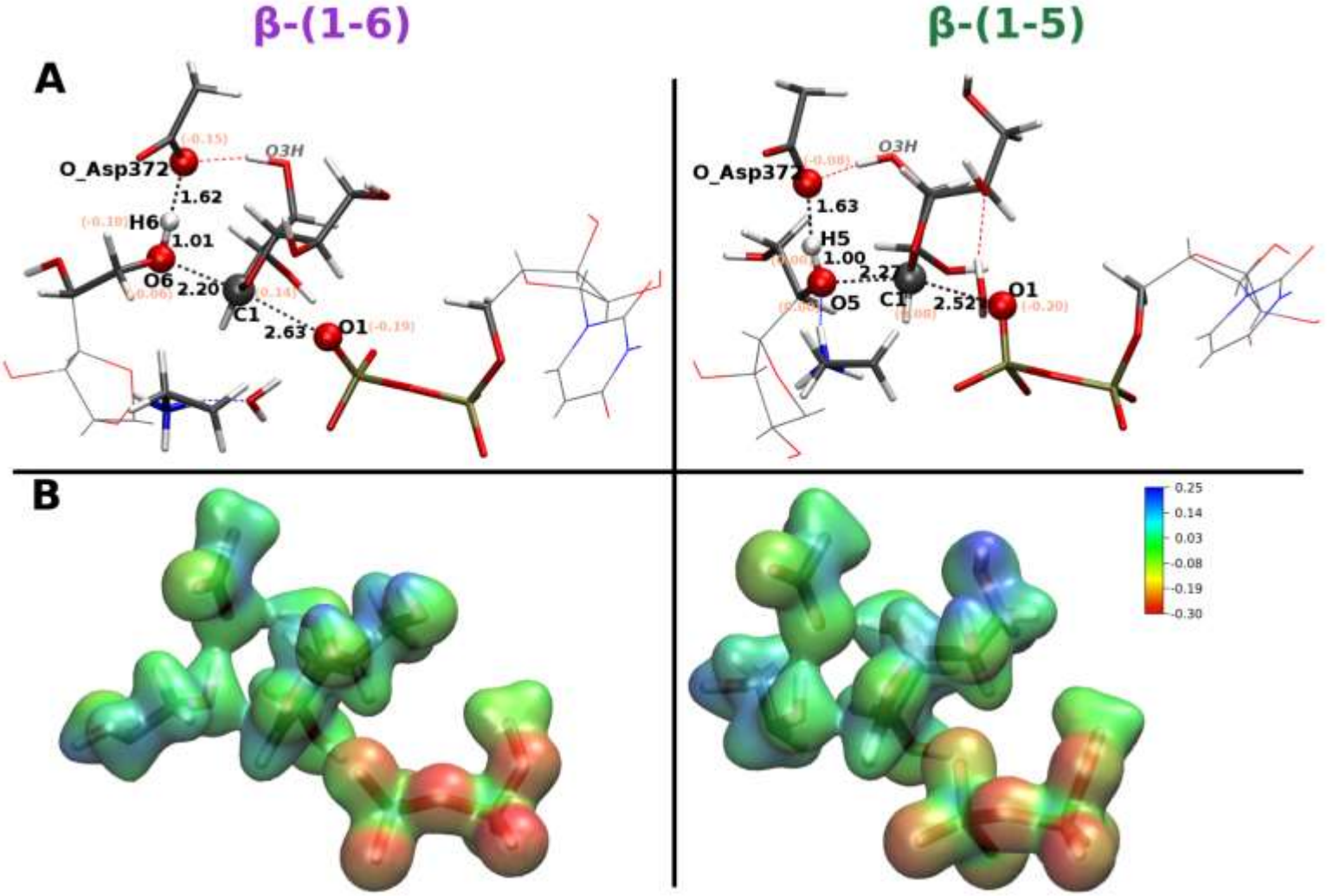



STM free energy profiles of β-(1-6) and β-(1-5) reactions.




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

# Results



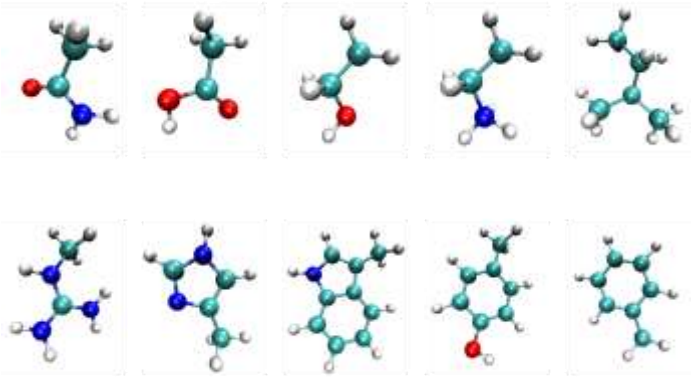

Average transition state structures and the electrostatic potential from STM.

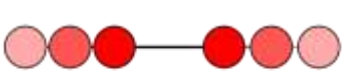
# Reactive Molecular Dynamics

- 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

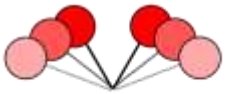
# Parametrizing ReaxFF

- 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

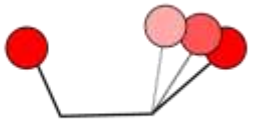




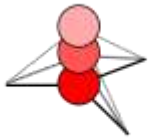
bond stretching



angle bending



torsion



out-of-plane displacement

# 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)

*Published in J. Chem. Theory and Computation*

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