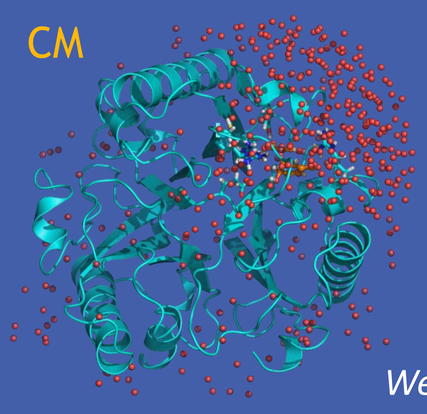
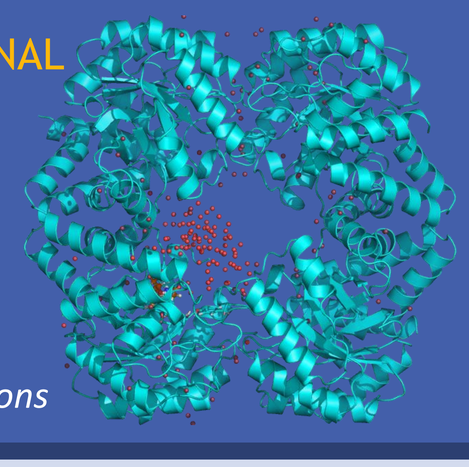


Bridging the gap between enzymologists and state-of-the-art simulation



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Enzyme-ligand simulation: automated protocols

- Automate the setup and simulation of enzyme-ligand complexes: require *only* a PDB file with a ligand (*incl*. ligand net charge and hydrogens)
- Meant to compare enzyme and/or ligand variants by *non-experts* on *standard PCs*
- Ideally free (& open source) software, and run on Mac OS, Windows, Linux

PREP for ligand parameterization, adding hydrogens & solvent, topology files
- Ligand parameterization: antechamber (sqm, AmberTools14[1]) , am1-bcc charges
- Protein prep (SS-bonds, alt. conformations etc.): pdb4amber (AmberTools14[1])
- Adding hydrogens, residue flips, histidine tautomers: reduce (AmberTools14[1])
- Check ASP/GLU(/LYS/TYR) protonation in presence of ligand: propka3.1[2]
- Solvation and amber ff14SB topology files: tleap (AmberTools14[1])

STRUCT for structural effects, through brief simulated annealing MD & minimization
- sander (now free, part of AmberTools14[1]) or NAMD
- Solvent sphere (e.g. 20 Å radius) with outer & outside sphere fixed

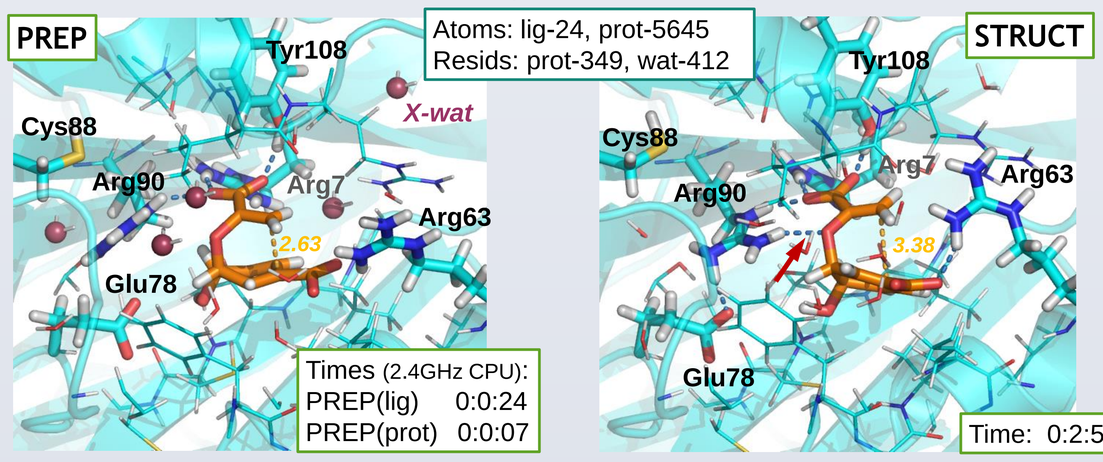
DYNAM for further molecular dynamics: fluctuations (in active site)
- Either ~100ps MD with solvent sphere (on single CPU, with sander/NAMD), or
- ~1ns MD in periodic box (on GPU, with pmemd.cuda, openMM) - *to be implemented*

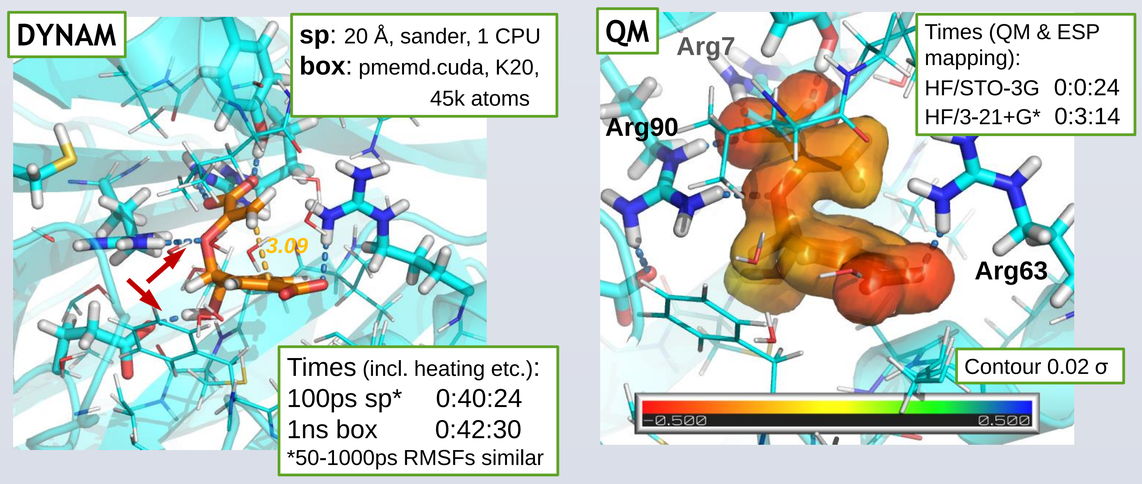
QM for electronic structure of ligand in enzyme (single point QM/MM, with Gaussian or NWChem or …)

Follow progress on: https://github.com/marcvanderkamp/enzlig_tools

Test case 1: Chorismate mutase (CM)

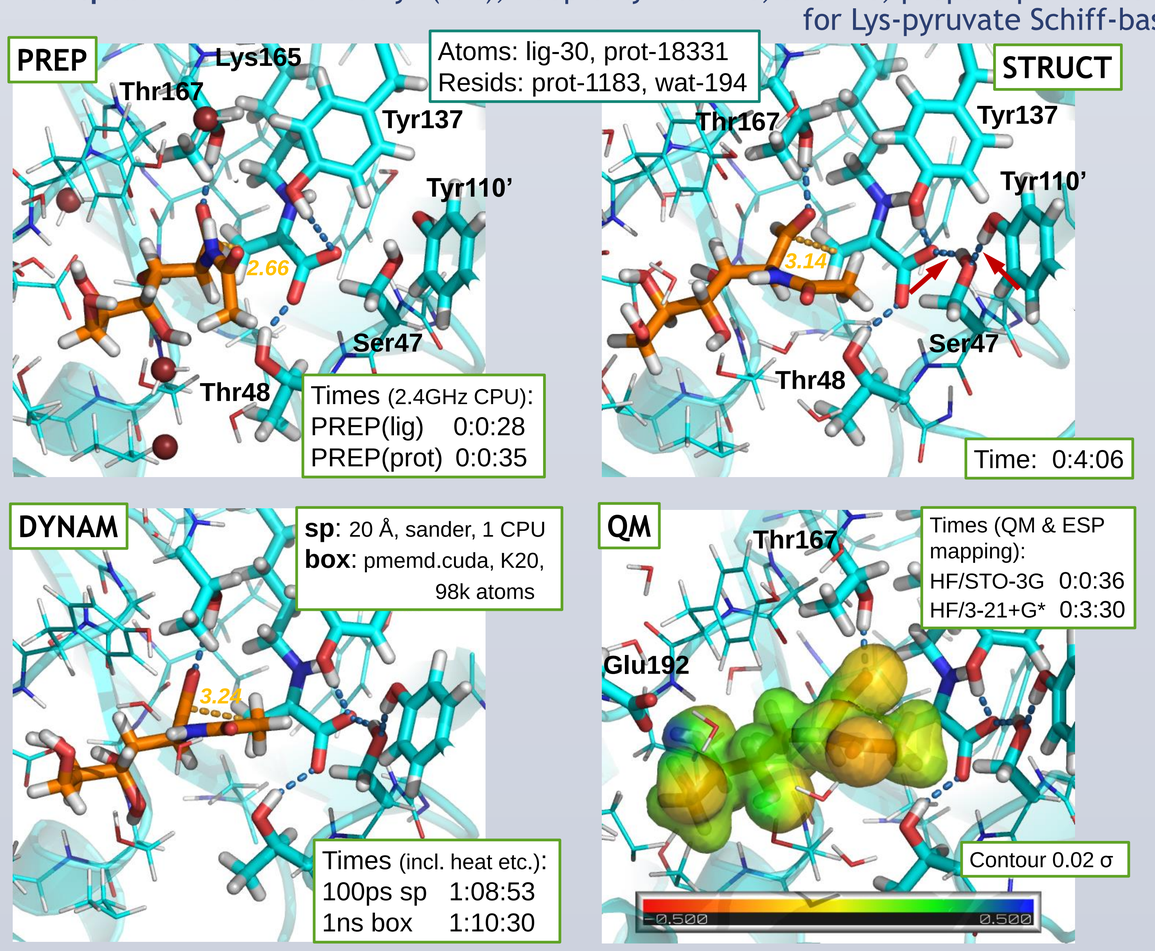
2CHT.pdb : select 1st trimer, keep only one TSA & change to chorismate with H's





Test case 2: Neuraminic acid lyase (NAL)

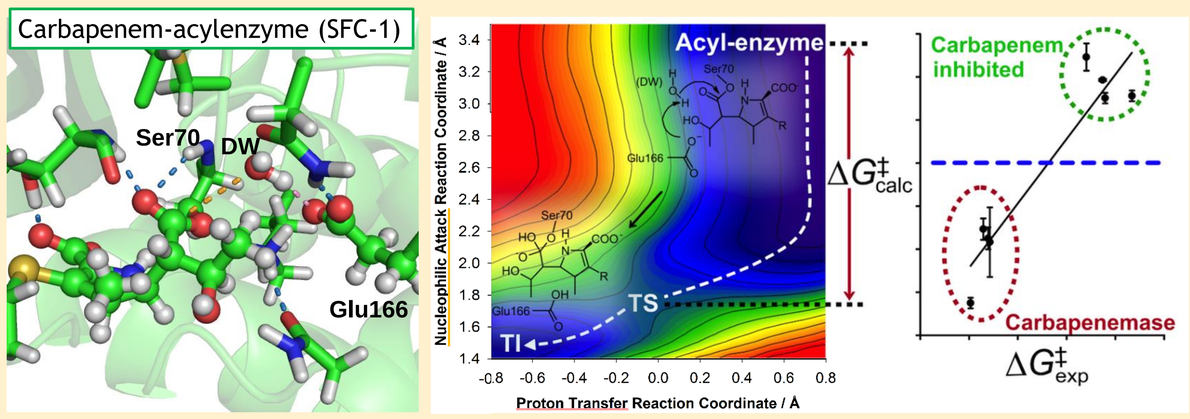
4BWL.pdb[3]: Ala137 back to Tyr (WT), keep only ManNAc, add H's, prepare parameters for Lys-pyruvate Schiff-base



Efficient QM/MM activity screening

QM/MM modelling as an assay for carbapenemase activity[4]

- *Challenge*: Given a 3D structure of a class A ß-lactamase, calculate the barrier for the rate-limiting step (*acyl-enzyme deacylation*)
- Take structure, model in carbapenem in acylenzyme form (MM or QM/MM MD)
- Follow deacylation reaction with QM/MM umbrella sampling and extract barrier



- Full profile:[4] 340 windows, 20ps QM/MM MD each, using SCC-DFTB (41+3 QM atoms)
- Minimal energy path only (28 windows, 20 or 2ps MD per window): MEP-20 takes 38 hrs, MEP-2 <4 hrs (16 CPUs) or <19 hrs (1 CPU) → *Barriers still predictive*

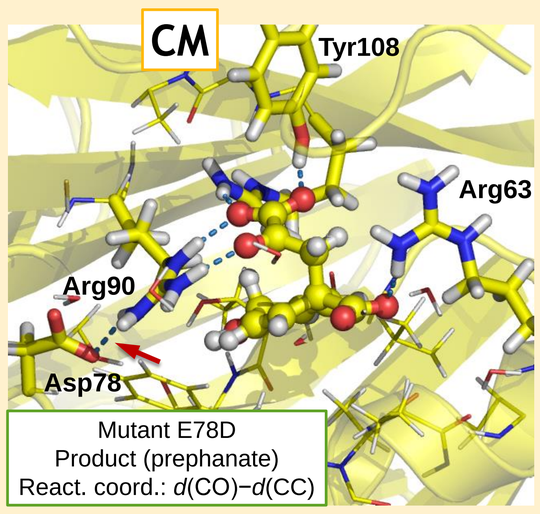
enzyme	k_{cat} (s^{-1})	$\Delta G^{\ddagger}_{exp}$ (kcal/mol)	$\Delta G^{\ddagger}_{calc}$ FULL (kcal/mol)[4a]	$\Delta G^{\ddagger}_{calc}$ MEP-20 (kcal/mol)[4b]	$\Delta G^{\ddagger}_{calc}$ MEP-2 (kcal/mol)[4b]
BlaC	1.7×10^{-3}	21.5	17.9 (0.08)	17.6 (2.3)	21.8 (1.8)
CTX-M	4.2×10^{-3}	20.8	18.9 (1.1)	17.3 (1.9)	20.3 (3.5)
SHV-1	1.3×10^{-3}	21.6	17.0 (0.43)	18.0 (0.7)	20.8 (1.0)
TEM-1	2.3×10^{-3}	22.7	17.1 (0.43)	16.4 (1.4)	20.6 (1.3)
KPC-2	3.6	16.8	10.5 (0.88)	10.5 (1.9)	11.1 (2.3)
NMC-A	12.0	16.1	7.5 (0.43)	7.5 (1.7)	10.5 (1.3)
SFC-1	6.5	16.6	10.9 (0.86)	8.7 (1.1)	11.5 (0.8)
SME-1	3.2	16.9	10.3 (2.80)	10.7 (2.9)	12.9 (0.5)

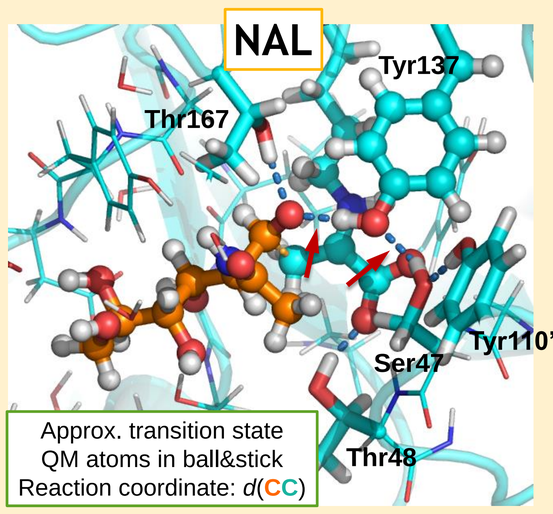
Values are averaged from 3 profiles; standard deviations in parentheses

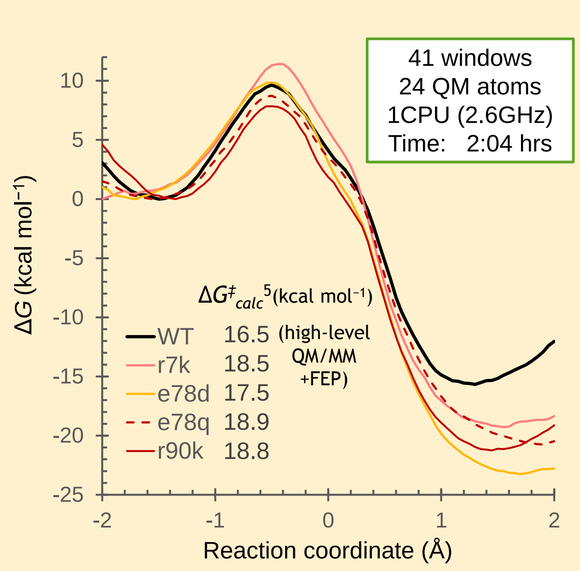
- Protocol valid for other ß-lactam antibiotics also;[4b] tested on *cephalosporin*

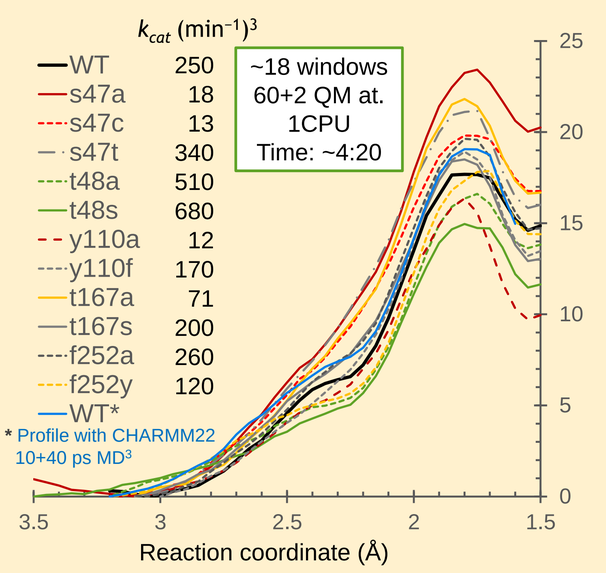
Initial ('blind') QM/MM screens for CM & NAL variants

- Use automated setup (**PREP** with Scwrl4 for rotamer selection, **STRUCT**, **DYNAM**)
- Define QM region & reaction coordinate; perform 2ps QM/MM MD per window (SCC-DFTB/ff14SB); select profile with lowest barrier from 25, 50, 75, 100 ps MM MD









- Despite high degree of automation and inexpensive protocols, reasonable reaction profiles are obtained ➔ *potential for high-throughput activity screening*
- Useful for: *reaction visualization, reaction coord. testing, hypothesis generation*

Footnotes & References

1. See www.ambermd.org and the Amber14.pdb manual for further information & references
2. See propka.ki.ku.dk; Søndergaard et al. *J. Chem. Theory Comput. 7*, 2284, **2011.**
3. Daniels AD, Campeotto I, Van der Kamp MW, Bolt AH, Trinh CH, Phillips SEV, Pearson AR, Nelson A, Mulholland AJ, Berry A. *ACS Chem. Biol.* 9, 1025, **2014**.
4. a) Chudyk EI, Limb MAL, Jones C, Spencer J, Van der Kamp MW; Mulholland AJ. *Chem. Commun. 50*, 14736, **2014**. b) New data, with thanks to Kate Hammond & Mike Limb.
5. Ishida T. *J. Am. Chem. Soc. 132*, 7104, **2010**.