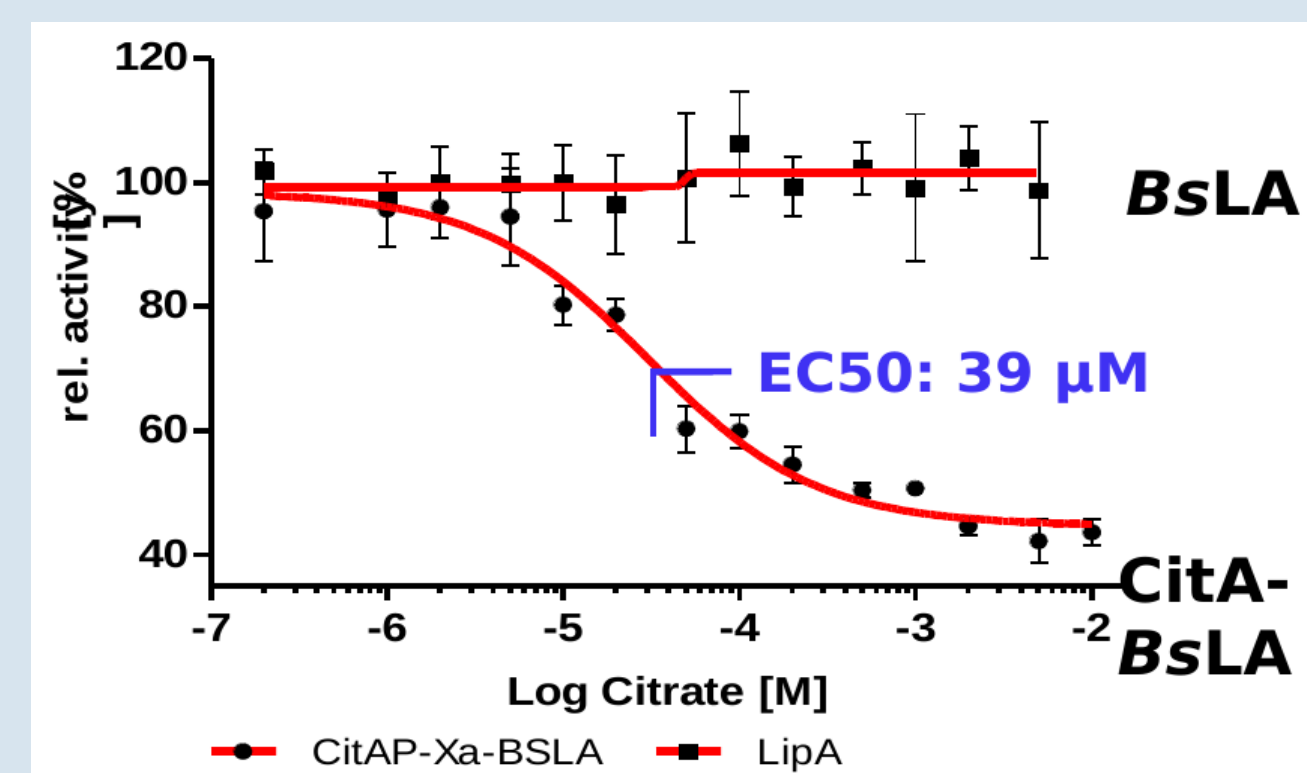# Structure of Lipase-CitAP Fusion Protein
Oliver Schillinger (PhD student)

Motivation

- *Bacillus subtilis* Lipase A
- Citrate receptor CitAP
- Fusion protein: switchable detergent
- **Known:** Individual structures
- **Unknown**: Fusion protein structure

EC50: 39 μM

*Bs*LA

CitA-*Bs*LA

Lipase (BSLA)

- Catalyses hydrolysis and synthesis of **triacylgliycerols**
- Diverse substrate specificity
- Used in industry for
  - Resolution of racemic mixtures
  - Synthesis of esters
  - Additive laundry detergent

oxyanion hole
His156
Asp133
Ser77
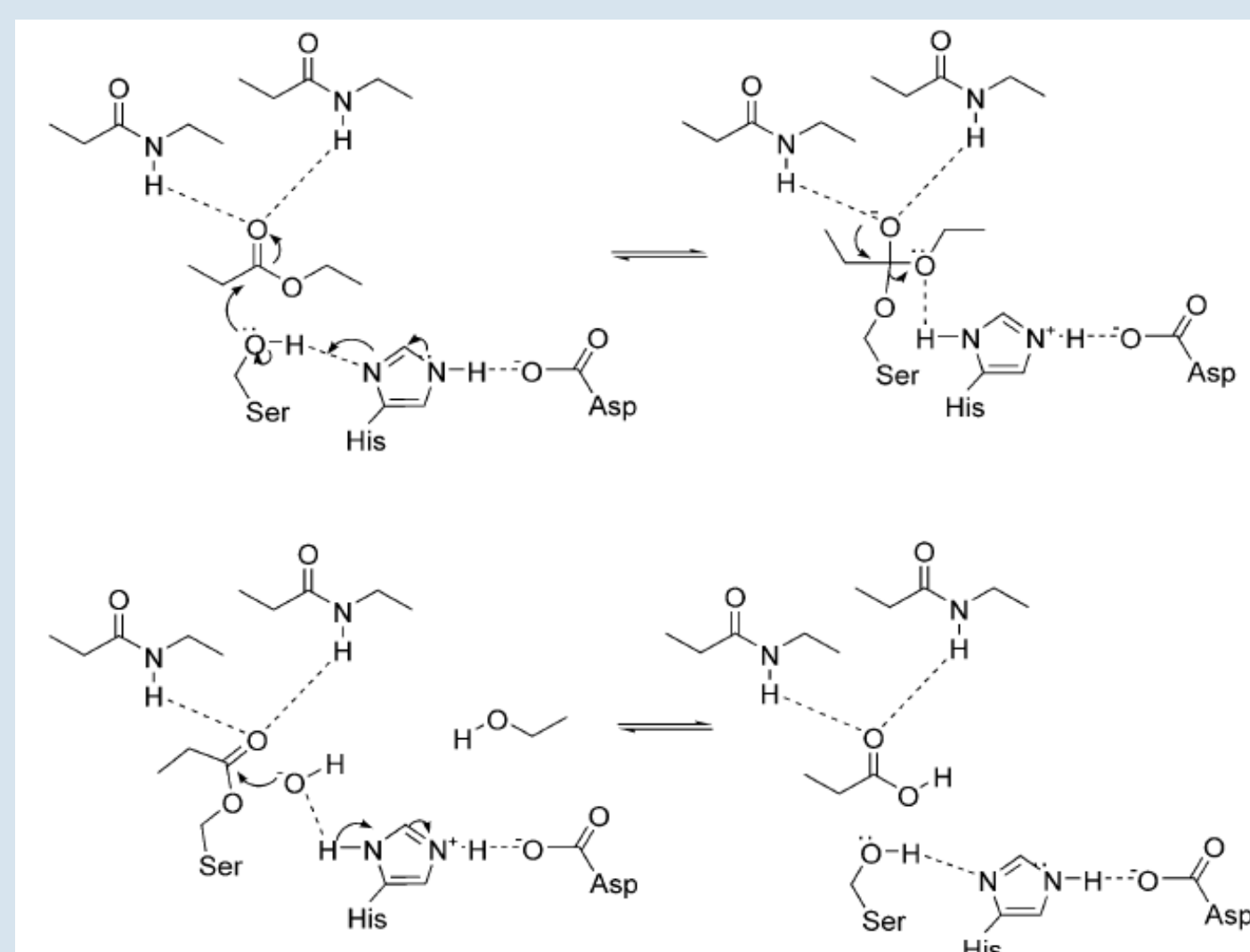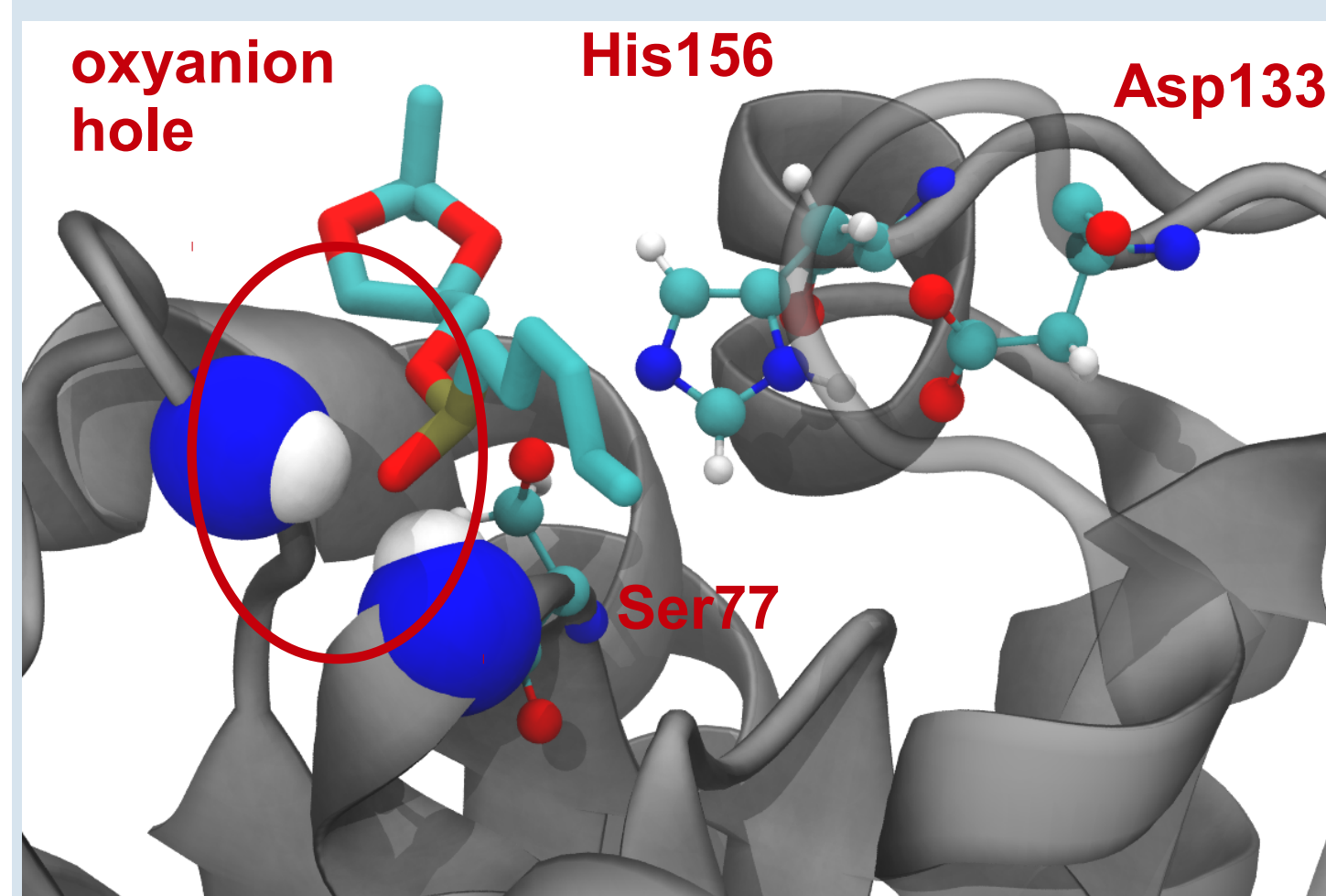
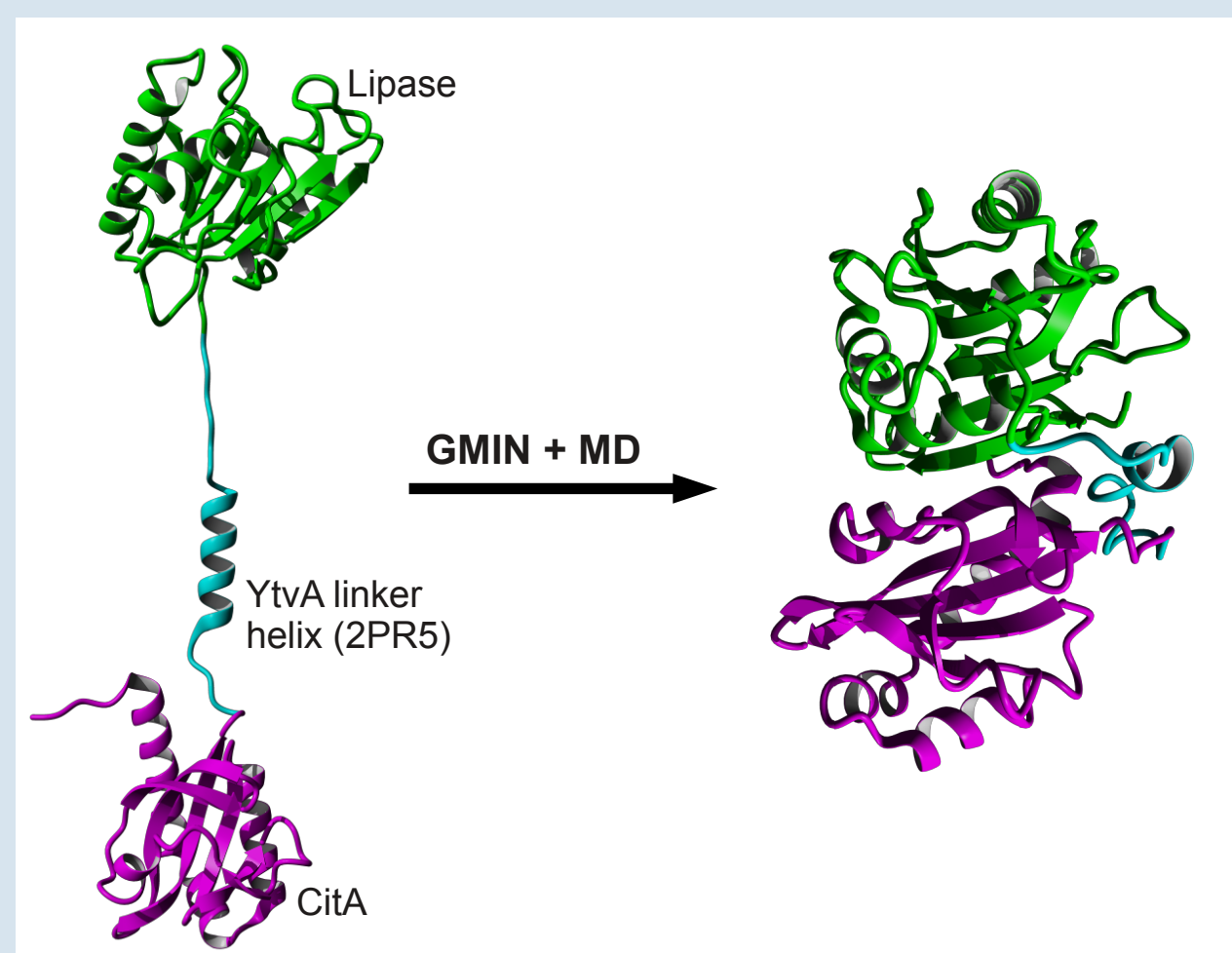His156 – Asp133 distance related to lipase activity

Citrate Receptor (CitAP)

- Periplasmic domain of a two component system of a sensor and a response regulator
- *Klebsiella pneumoniae* two component system is essential for the induction of citrate fermentation genes in the presence of citrate
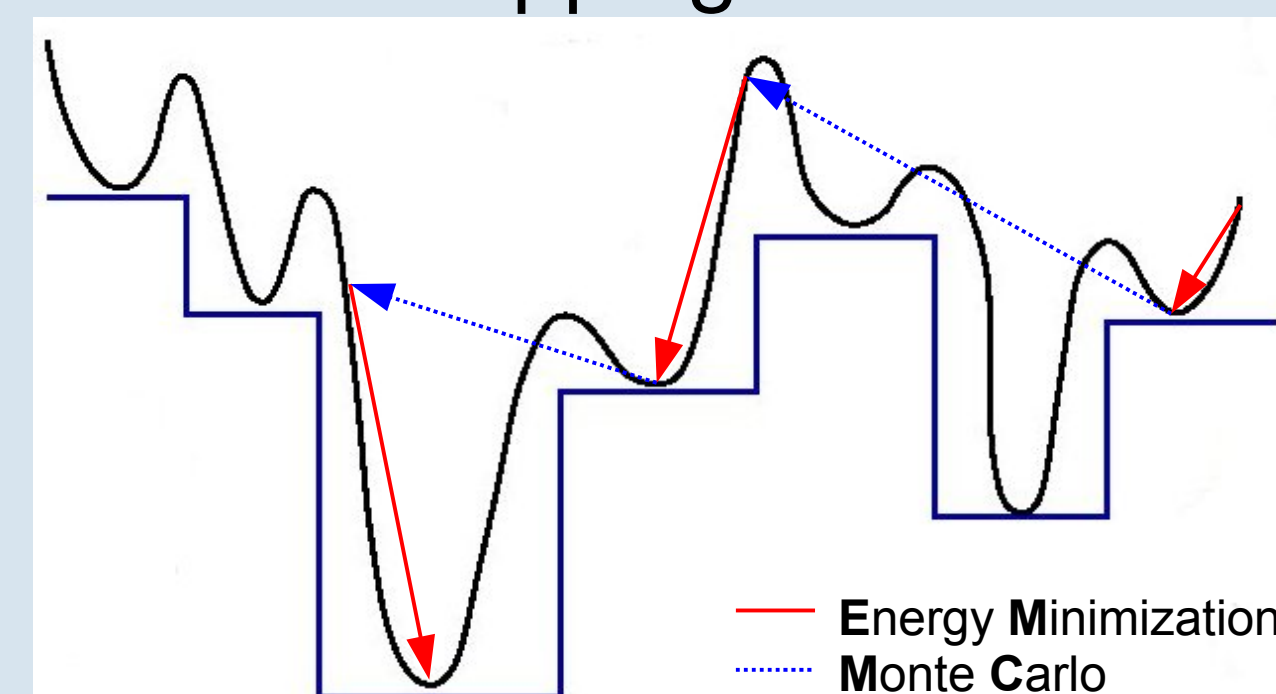

LEU 102 – TYR 56 distance
LEU 102 – GLU 74 distance
GLU 74
LEU 102
TYR 56
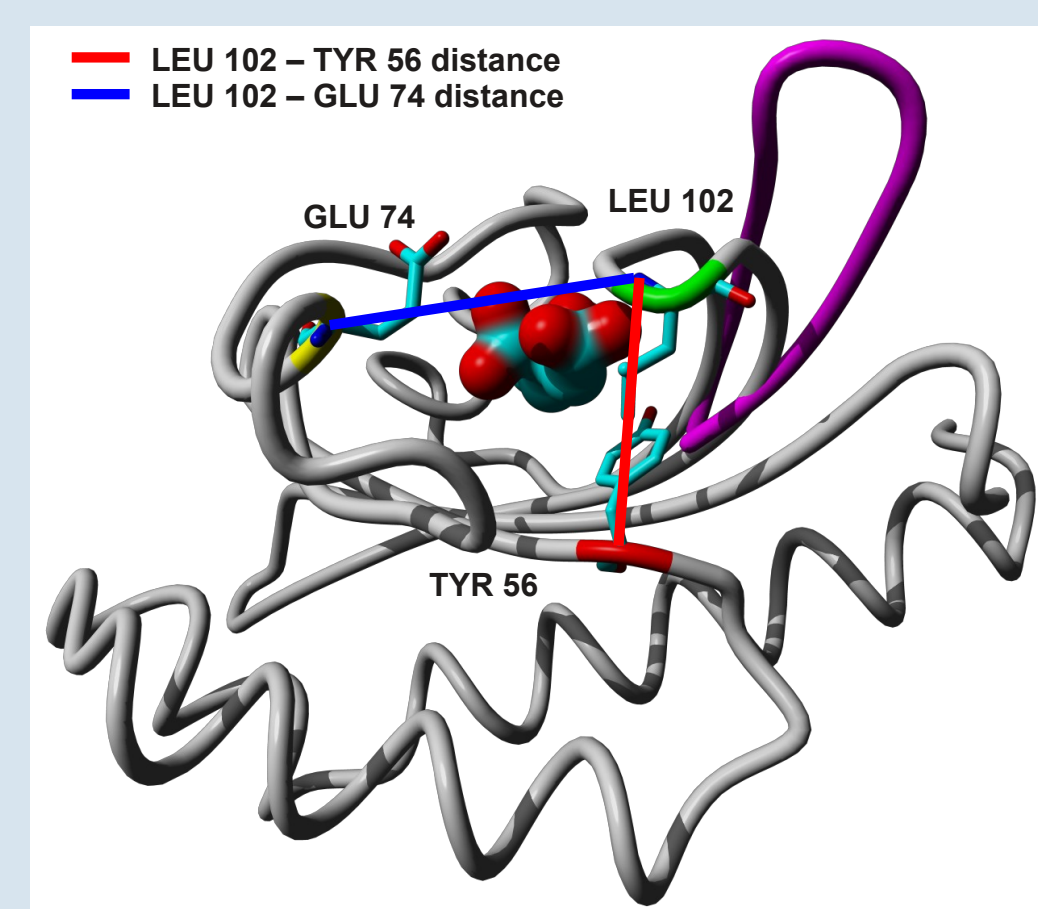
Bacillus subtilis lipase A with covalently bound Rc-IPG-phosphonate inhibitor (PDB: 1R4Z)

Methods

Lipase
GMIN + MD
YtvA linker helix (2PR5)
CitA

Basin Hopping with GMIN

Energy Minimization
Monte Carlo
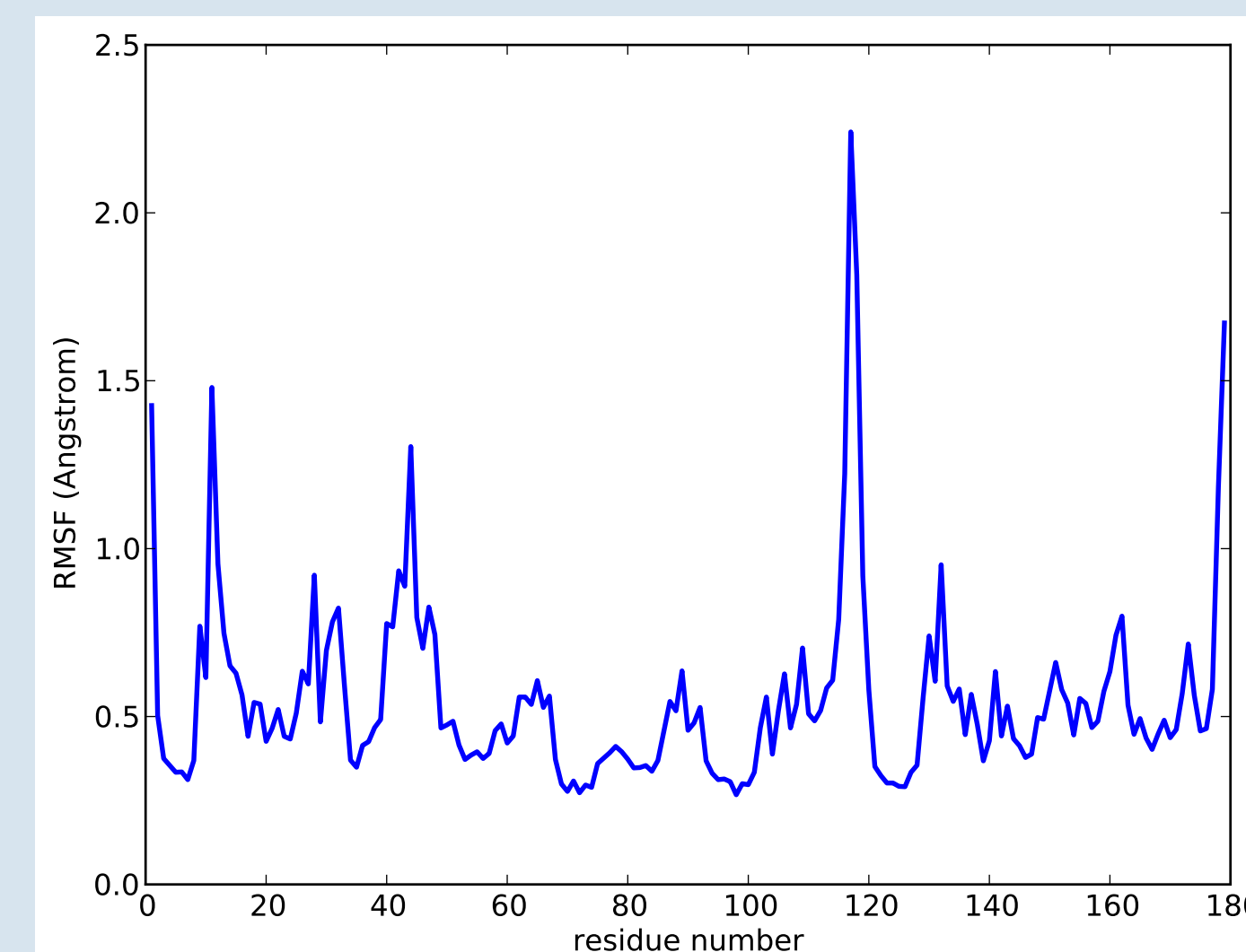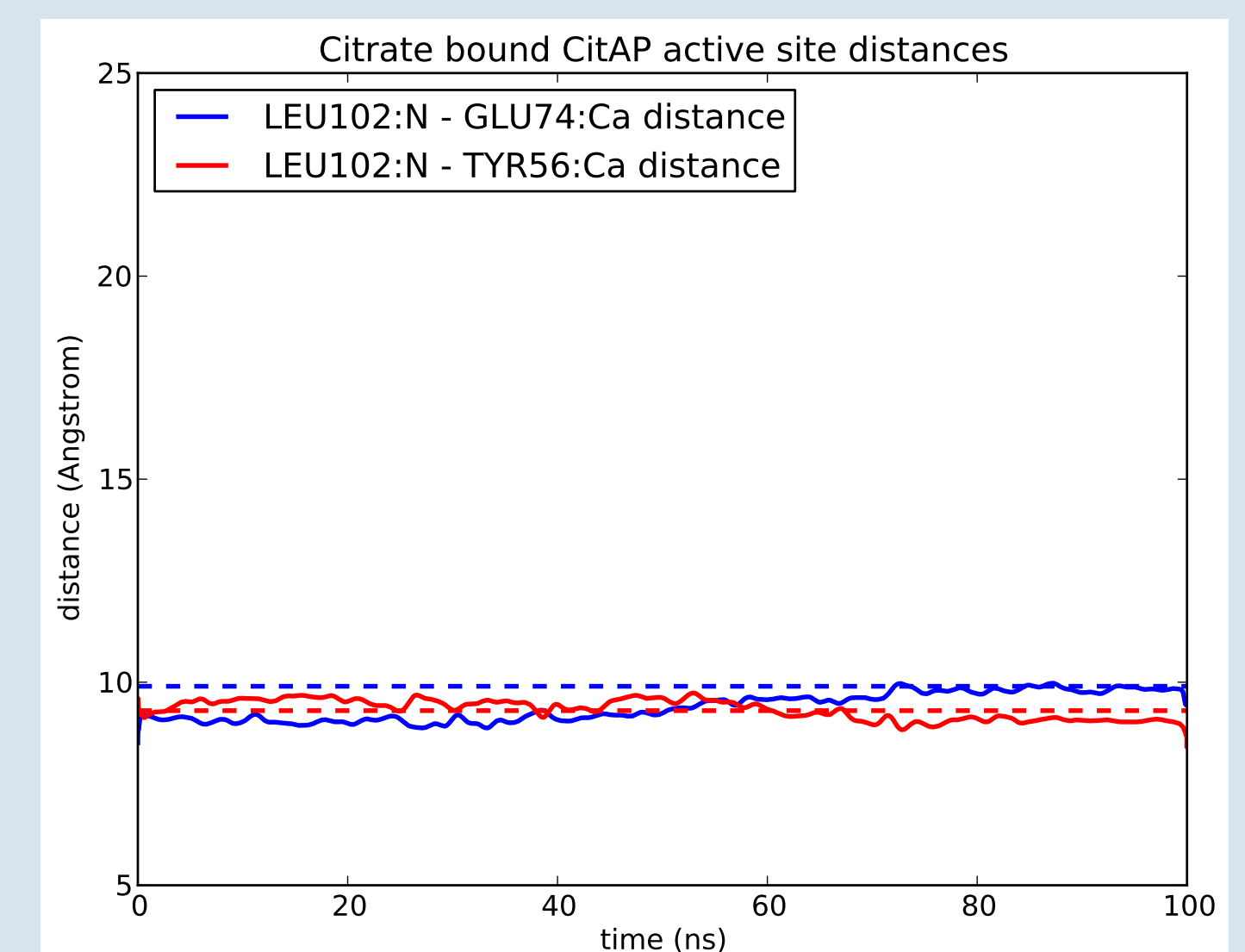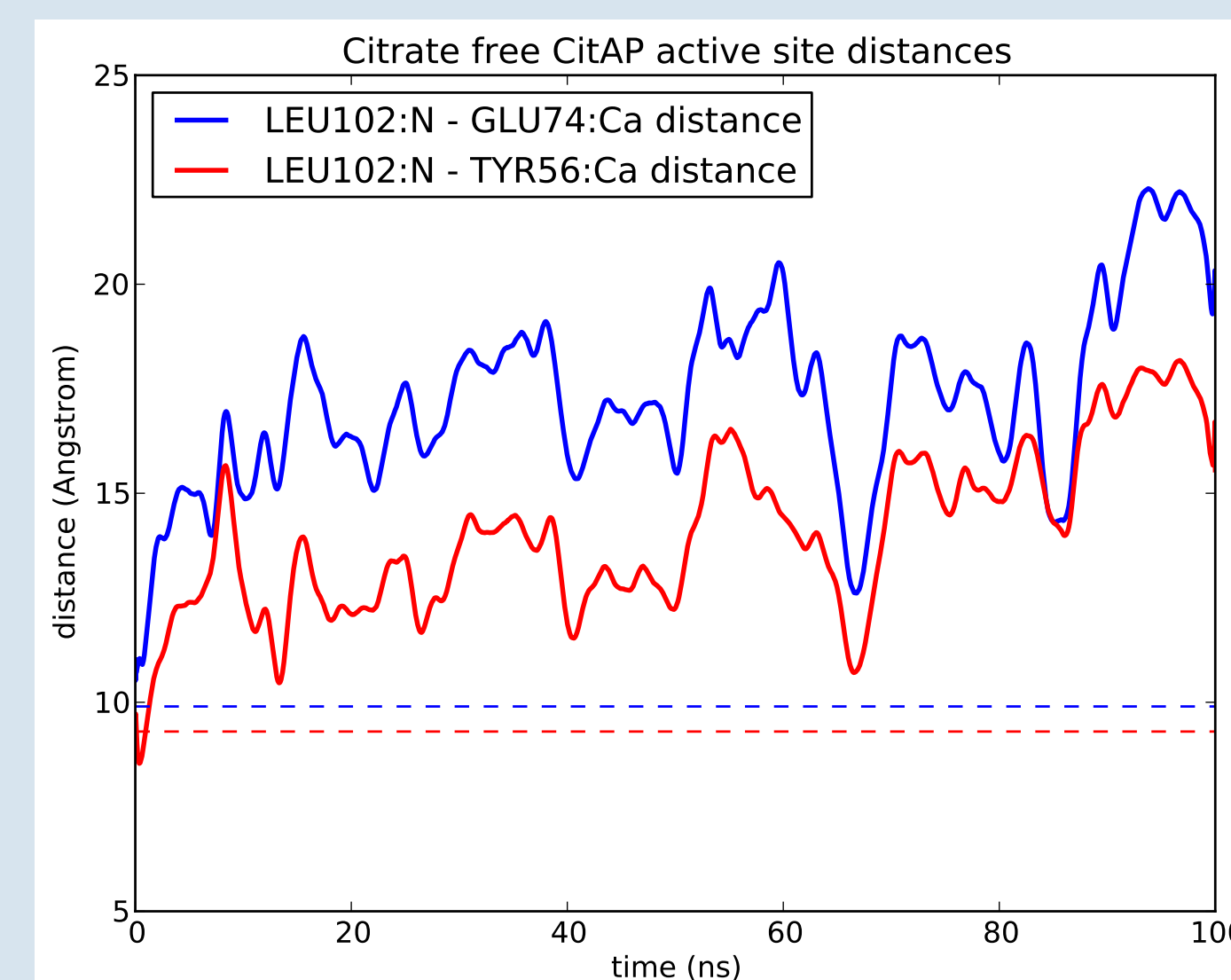
- GROMACS
- **amber99sb-ildn-nmr** force field
- Citrate paremeterization with GAFF
- 10 ns position restrained equilibration
- Gradually decreasing restraining force constant
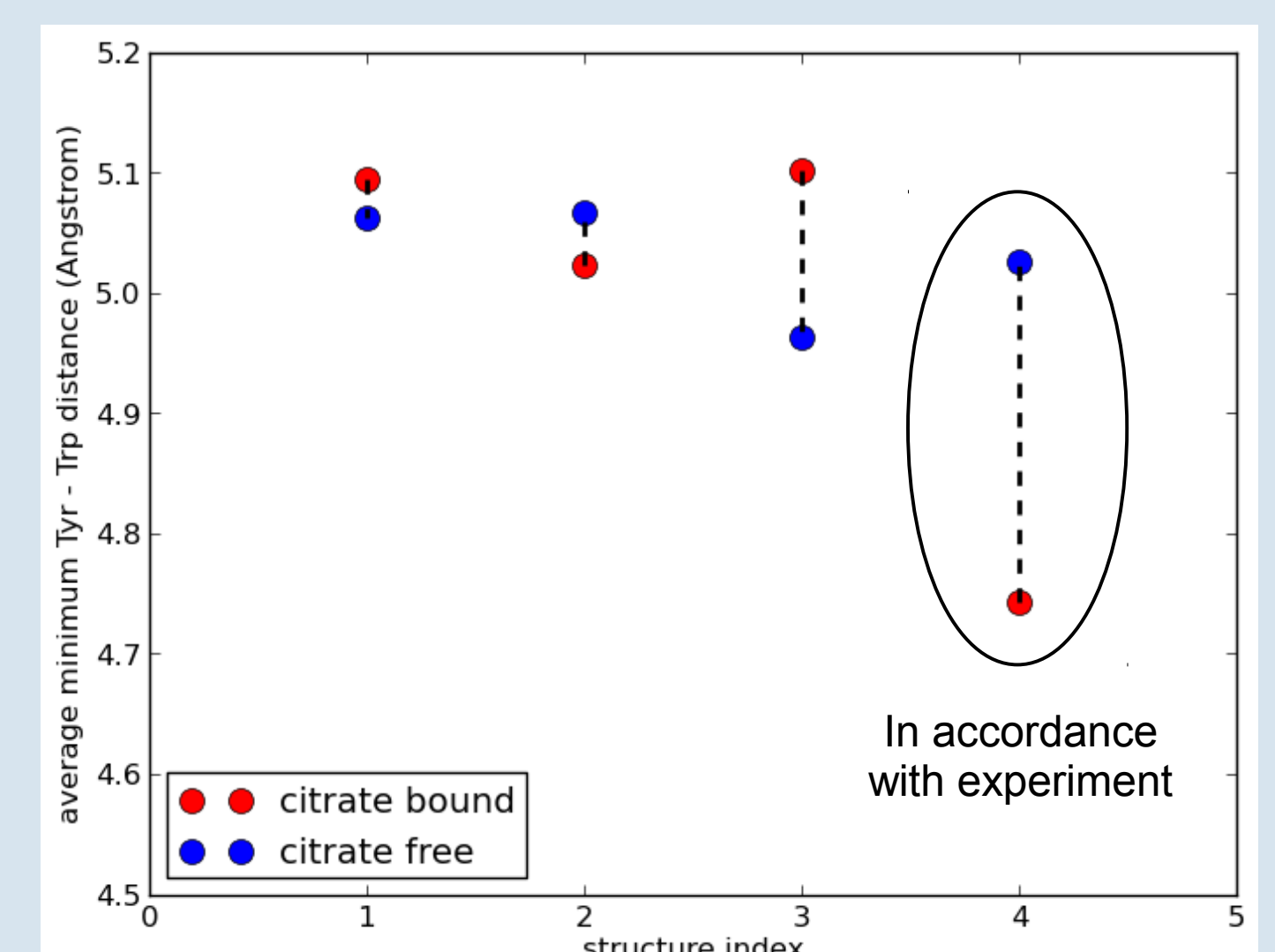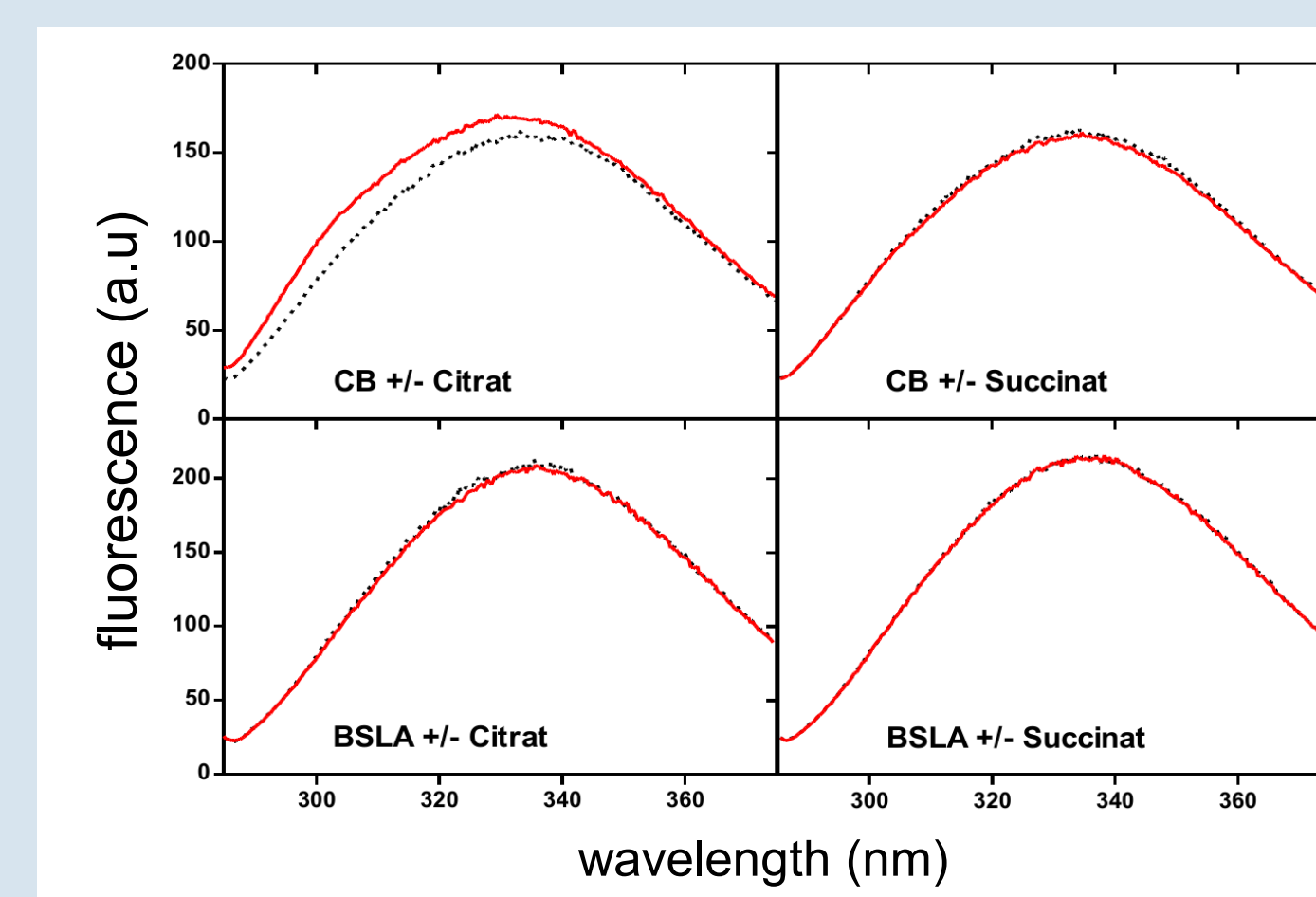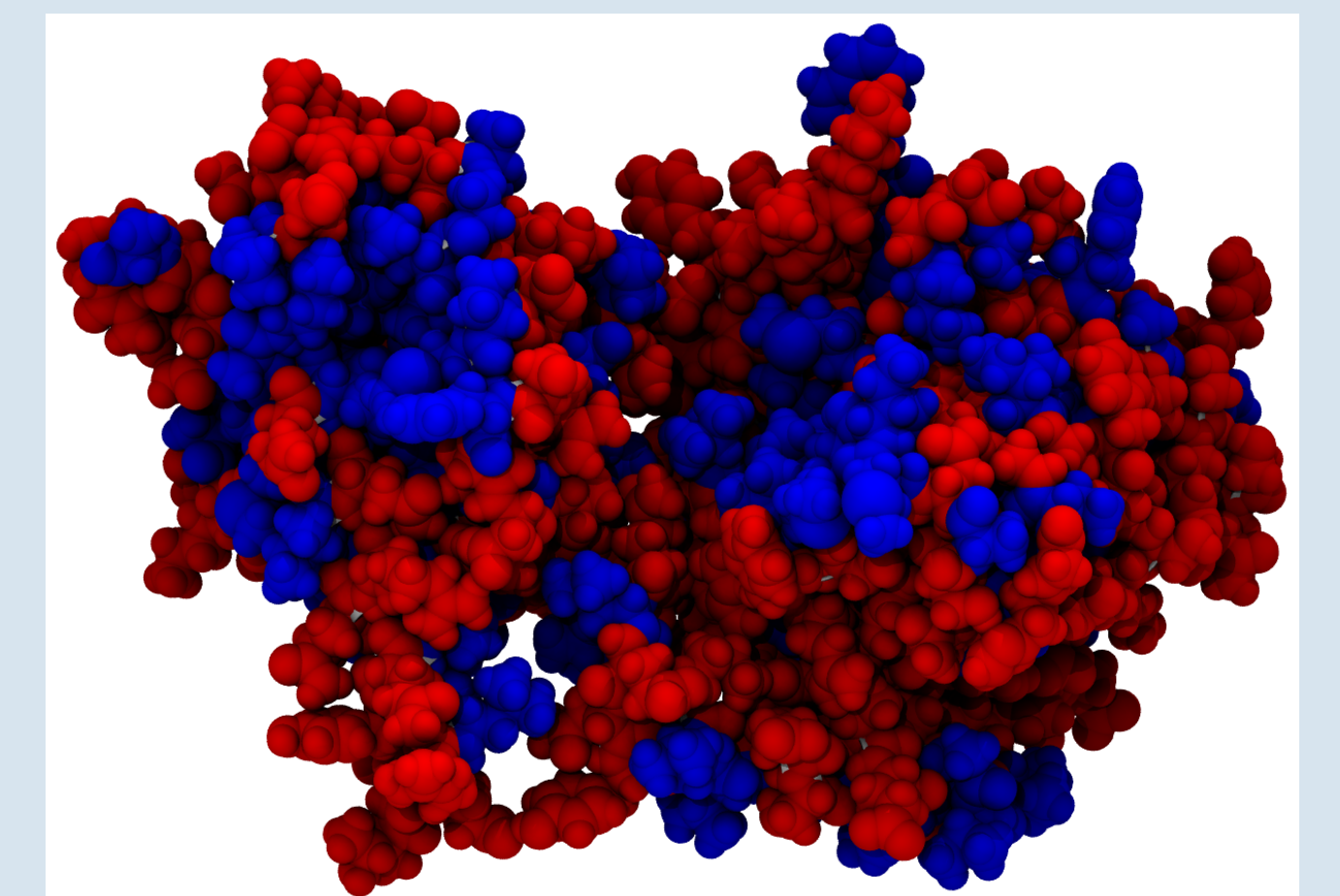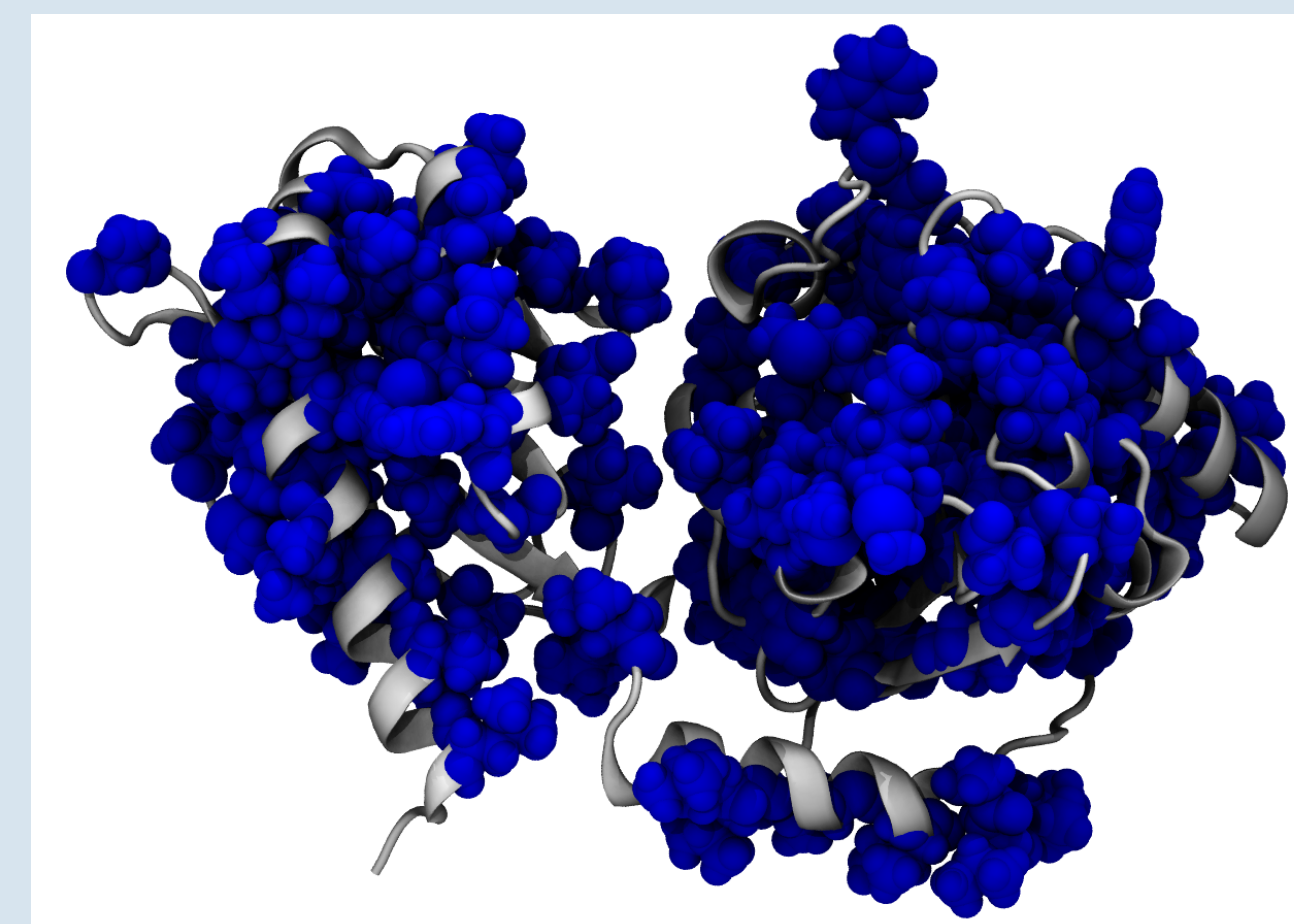- 100 ns production runs
- PBC, NPT, PME

BSLA Results

CitAP Results

Citrate free CitAP active site distances
LEU102:N - GLU74:Ca distance
LEU102:N - TYR56:Ca distance

Citrate bound CitAP active site distances
LEU102:N - GLU74:Ca distance
LEU102:N - TYR56:Ca distance
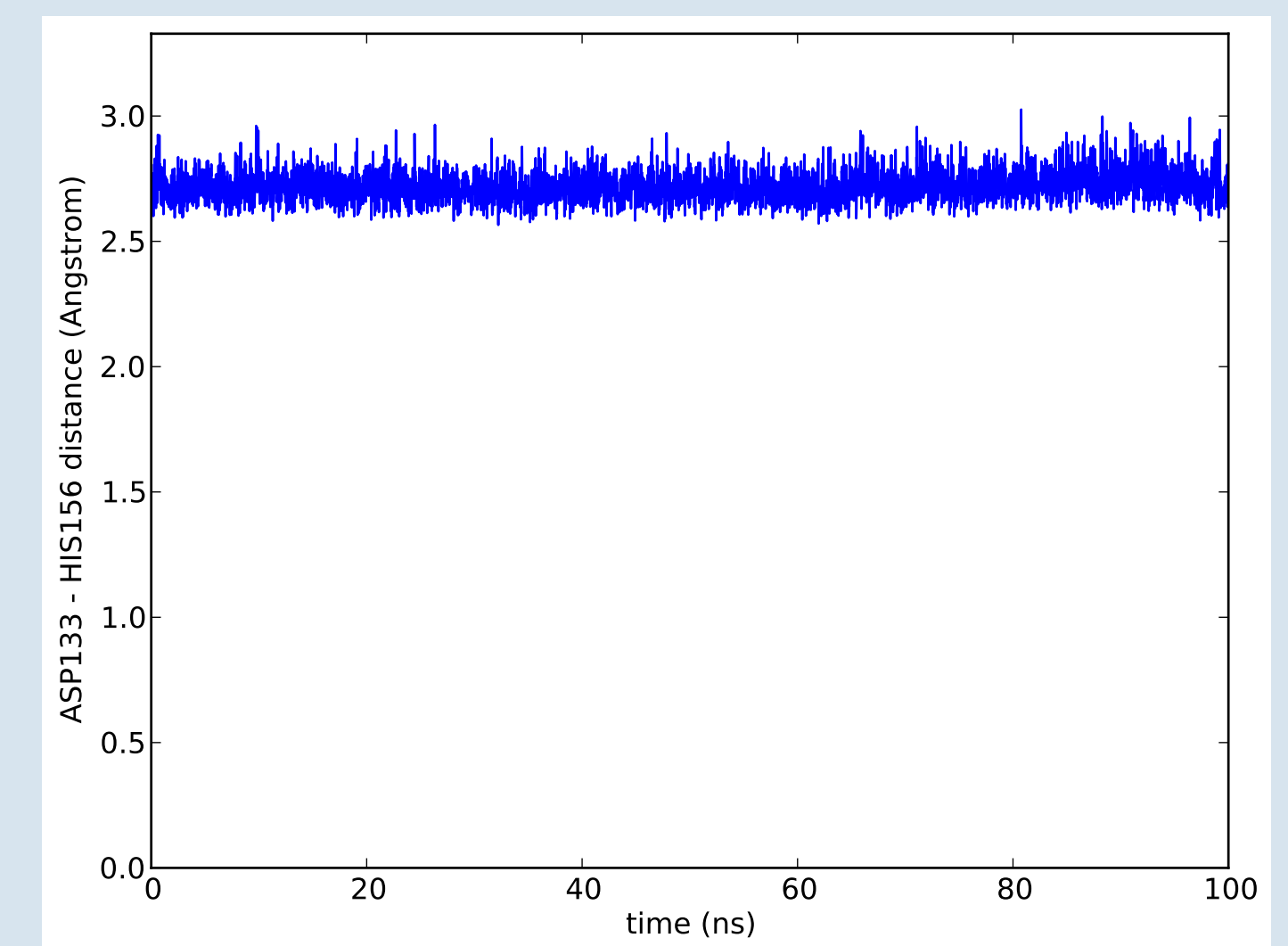
Fusion Protein Results

CB +/- Citrat
CB +/- Succinat
BSLA +/- Citrat
BSLA +/- Succinat

In accordance with experiment

citrate bound
citrate free

Conclusions

- Solo MD simulations gave expected results
- Secondary structure of fusion protein is stable (data not shown)
- 2 hydrophobic cores
- Systems not equilibrated after 100 ns (data not shown)
- Only structure 4 in accordance with TYR/TRP fluorescence
- Binding pocket dynamics different in fusion protein (more flexible, data not shown)
- No unique active site distance correlation between domains (data not shown)

Cooperations

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