



**Tackling the cellular drug resistance of
thymidylate synthase
– Disruption of an obligate dimer?**

**ACS 234th National Meeting&Exposition
Boston, August 23, 2007**

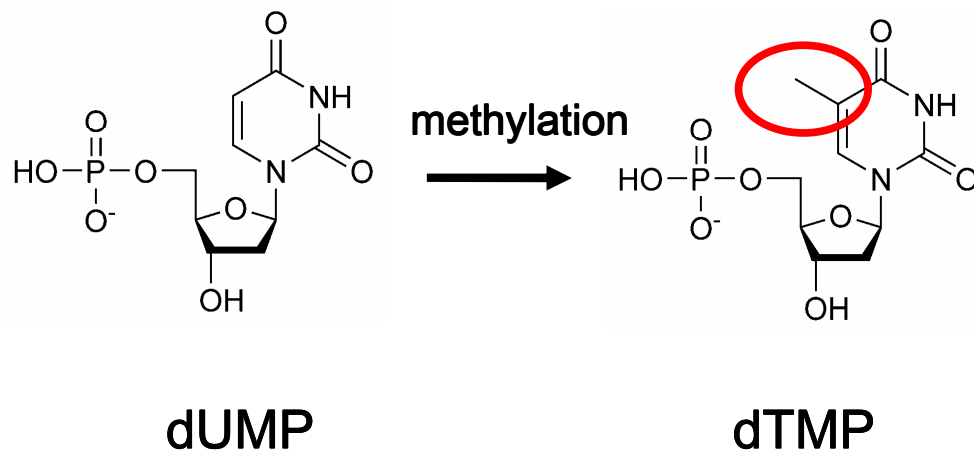
Outi Salo-Ahen

Overview

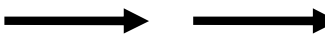
- Background
- Analysis of the dimer interface
 - Hot spots
 - Interface crevices in the X-ray structure
 - Transient interface pockets?
- Summary and future work

Thymidylate synthase (TS) – Essential enzyme

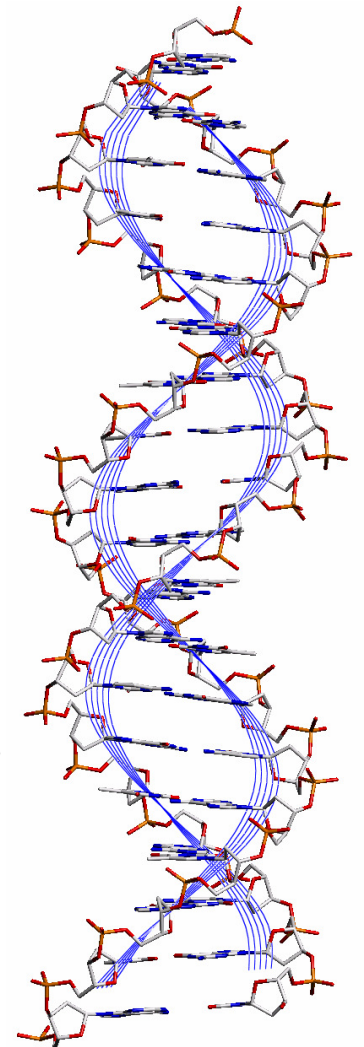
Catalytic activity:



phosphorylation



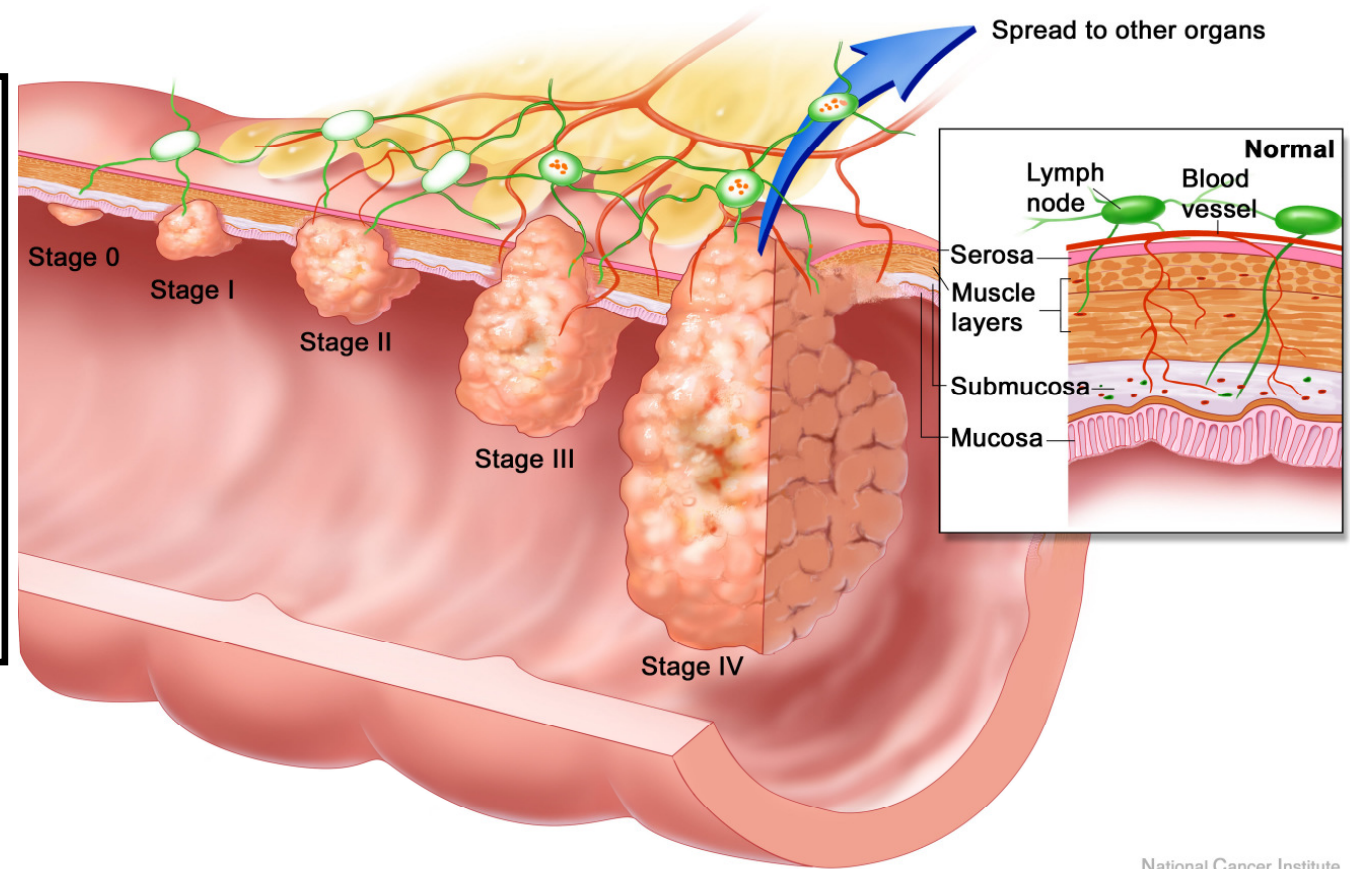
dTDP → dTTP



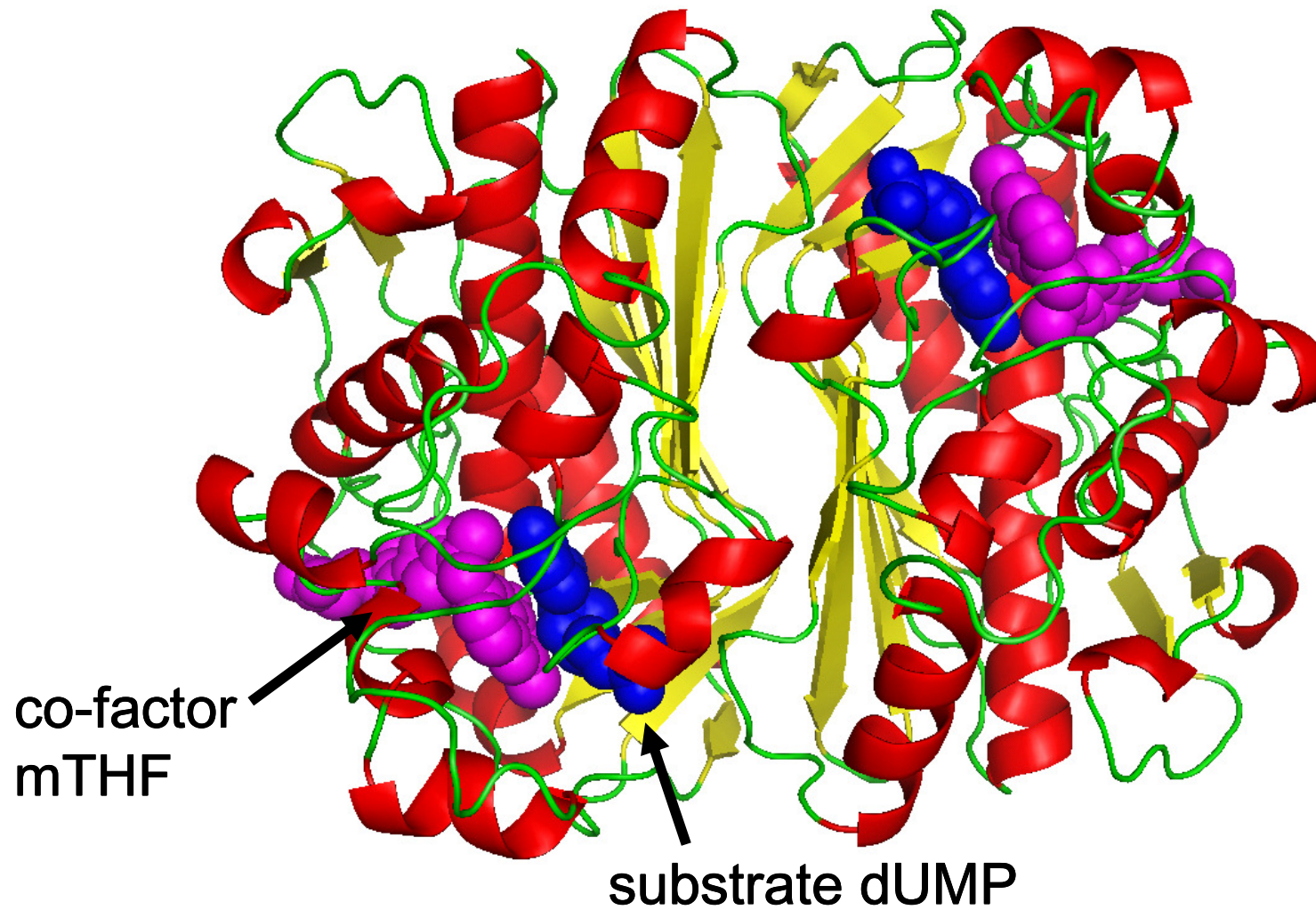
Critical target in cancer therapy

Cancer types:

- ovarian
- colorectal
- breast
- head and neck
- pancreas
- gastric



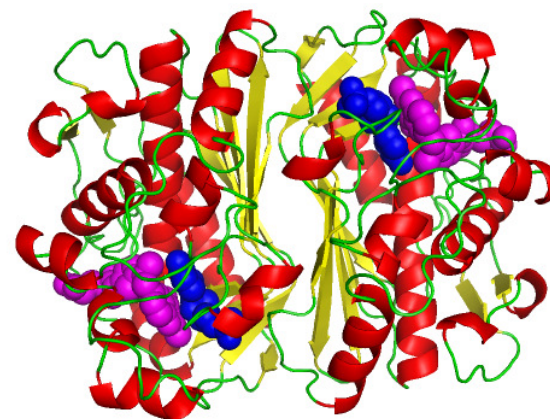
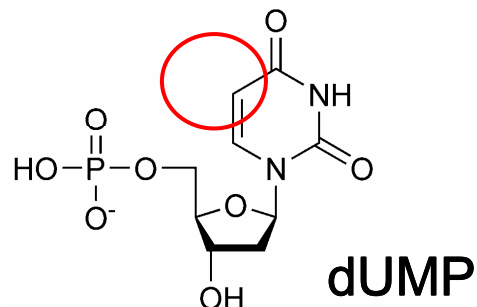
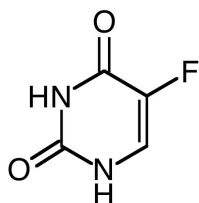
Homodimer - Two active sites



Current TS enzyme inhibitors:

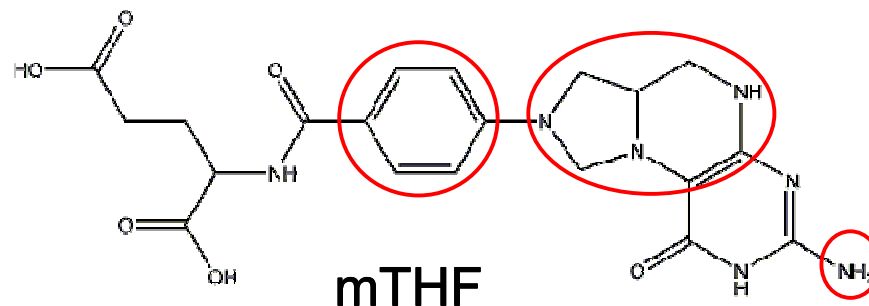
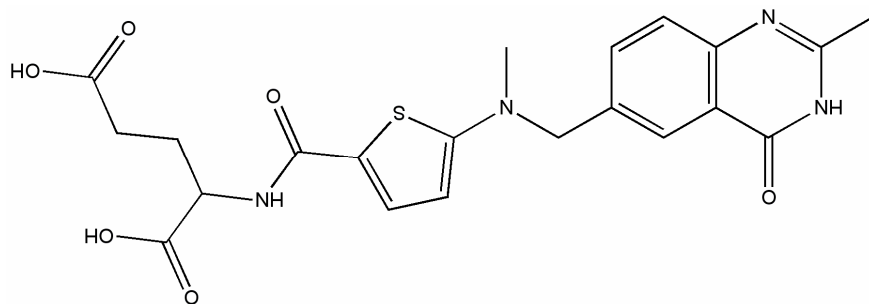
a) Substrate (**dUMP**) analogs:

* e.g. 5-fluorouracil



b) Cofactor (**5,10-methylenetetrahydrofolate**) analogs:

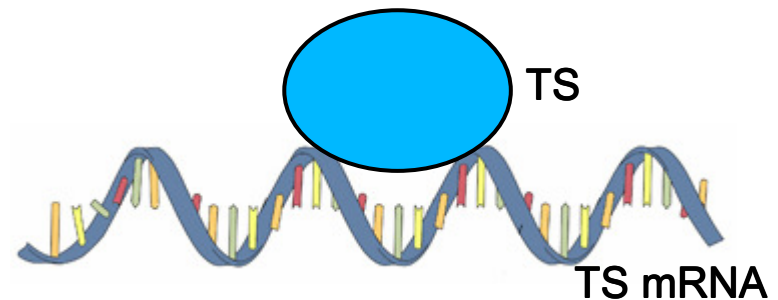
* e.g. raltitrexed



Autoregulation of TS synthesis – Mechanism of drug resistance

Regulatory activity:

→ Blocks the translation of
TS mRNA to TS protein

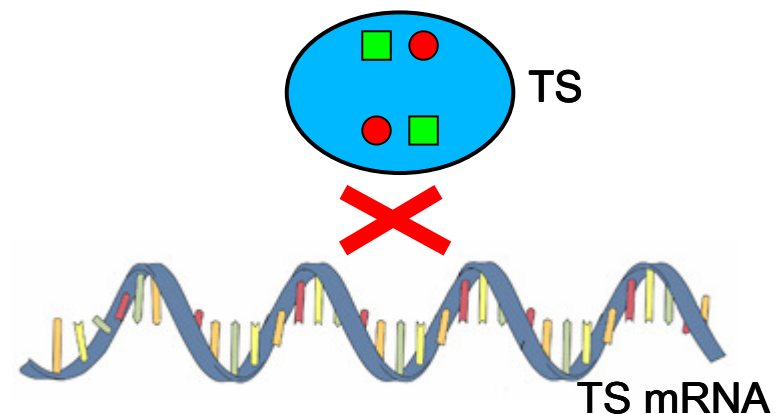
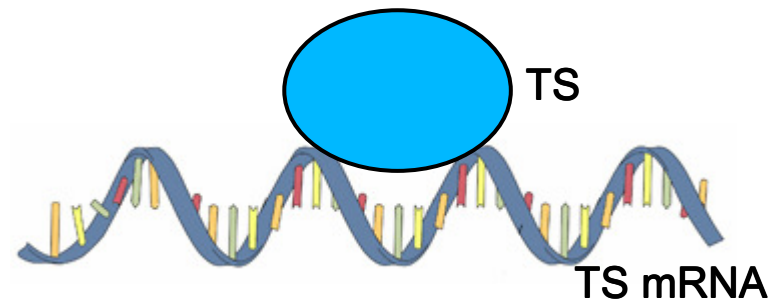


Autoregulation of TS synthesis – Mechanism of drug resistance

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regulation



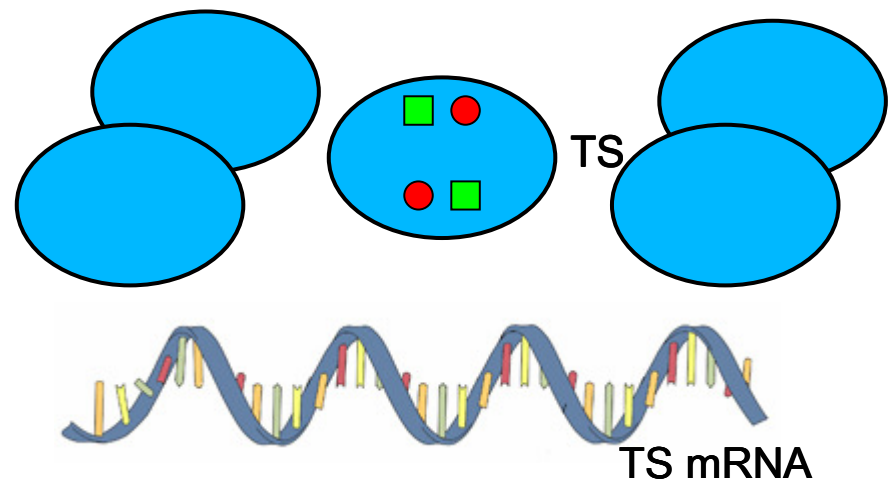
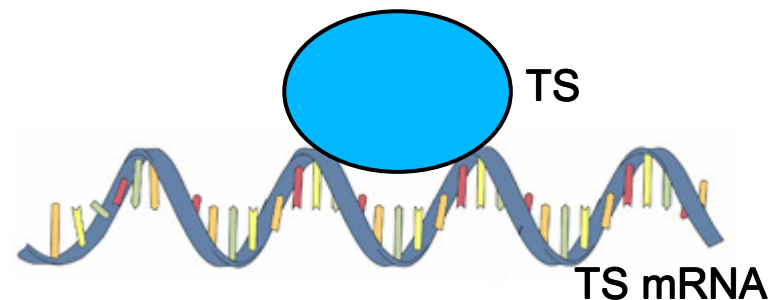
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→ Drug resistance

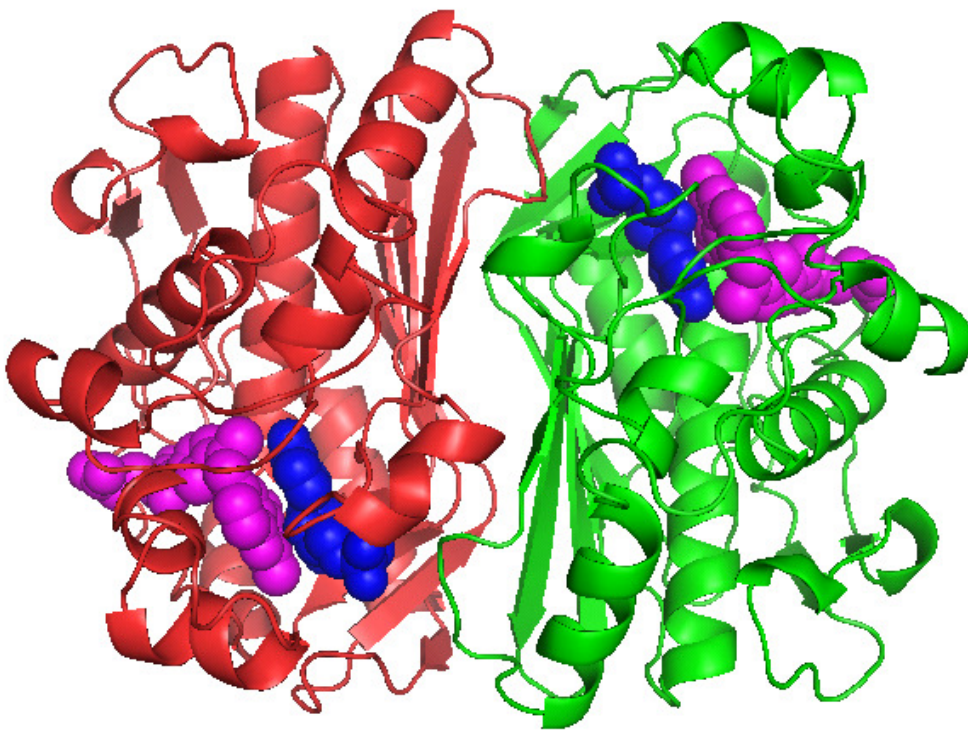


Aim of the project

- Development of better TS inhibitors –

Obligate dimer...

Disrupting the dimer – novel way of inhibiting the enzyme?



1) Disrupt the dimer

OR

2) Inhibit dimerization

* **With** peptidic or small molecules

* **Without** causing drug resistance

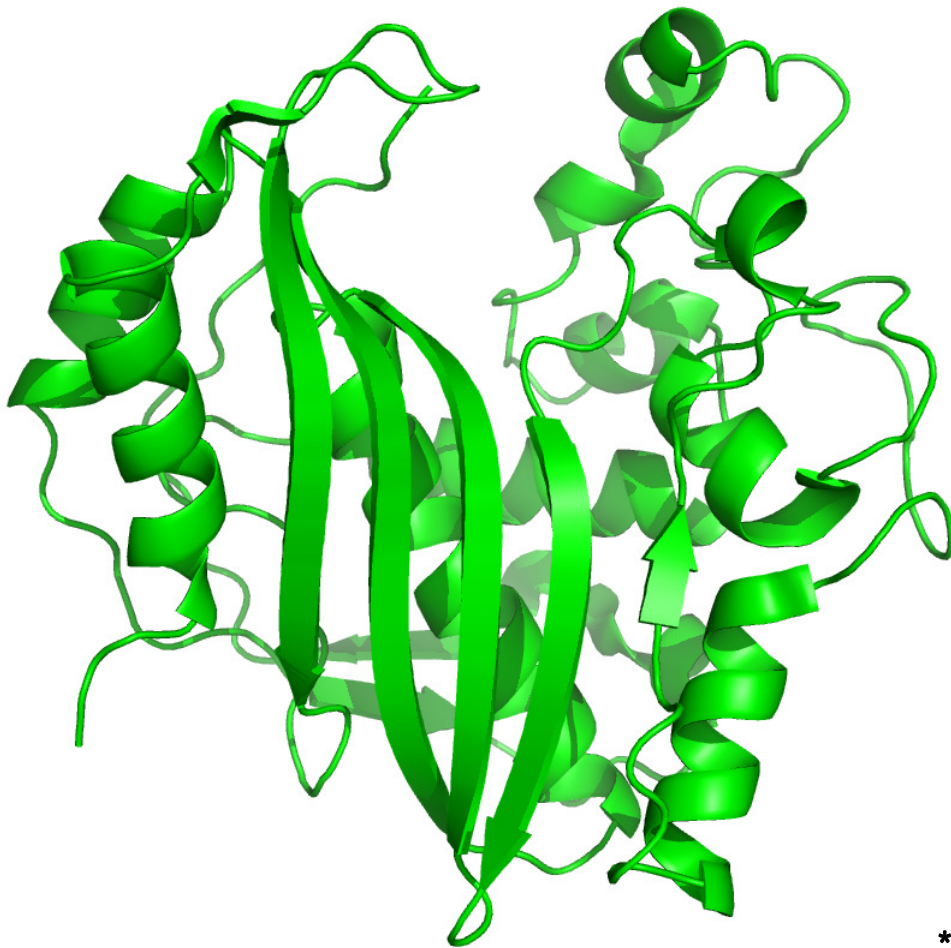


Analysis of the dimer interface

- Hot Spots –

Hot Spots

Predicting hot spots – residues important for dimerization



FoldX* / Robetta**

Web servers:

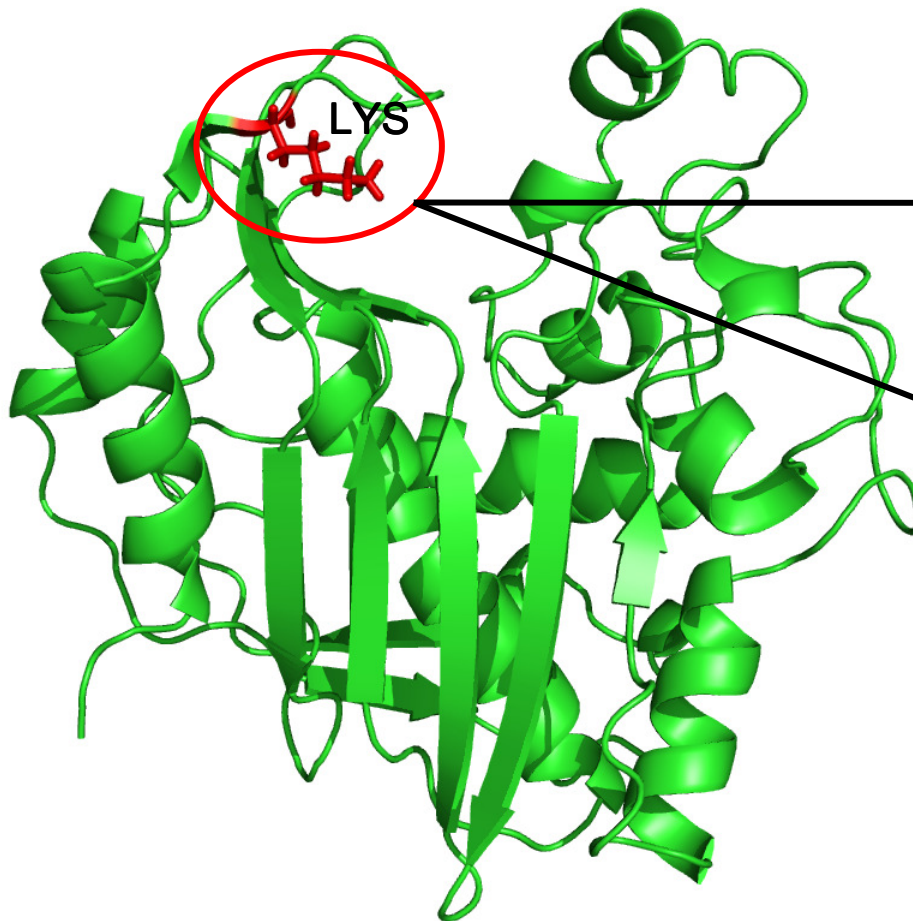
→ Free energy change
(ddG) upon alanine mutation

Hot spot: ≥ 1 kcal/mol

Neutral residue: < 1 kcal/mol

* <http://foldx.embl.de>; ** <http://robetta.bakerlab.org/>

Hot Spots



LYS to ALA ddG (kcal/mol)

Dimer

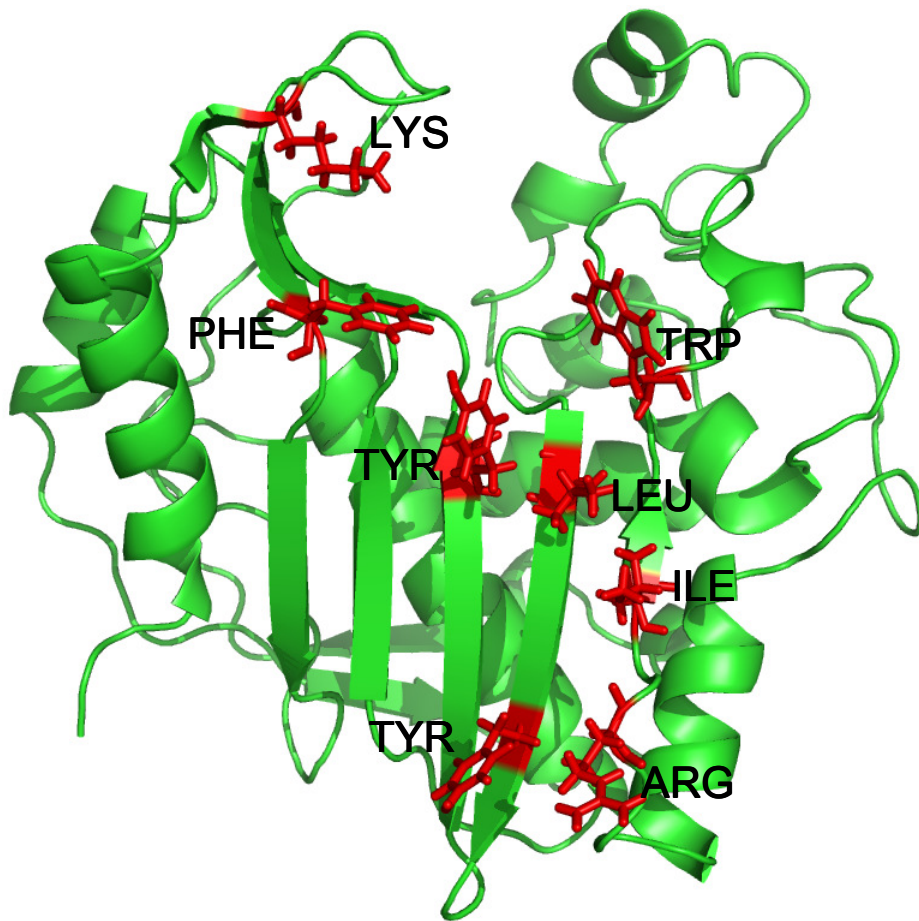
2.2* / 2.3**

Monomer

0.7* / 0.3**

FoldX* / Robetta**:

Hot Spots



→ **Mutations** to test the hot spots: can we disrupt the dimer?

If Yes:

→ **Design ligands** that bind in the proximity of the hot spots

* **Range of ddG (dimer)** for the predicted hot spots: 1.5-5.5 kcal/mol

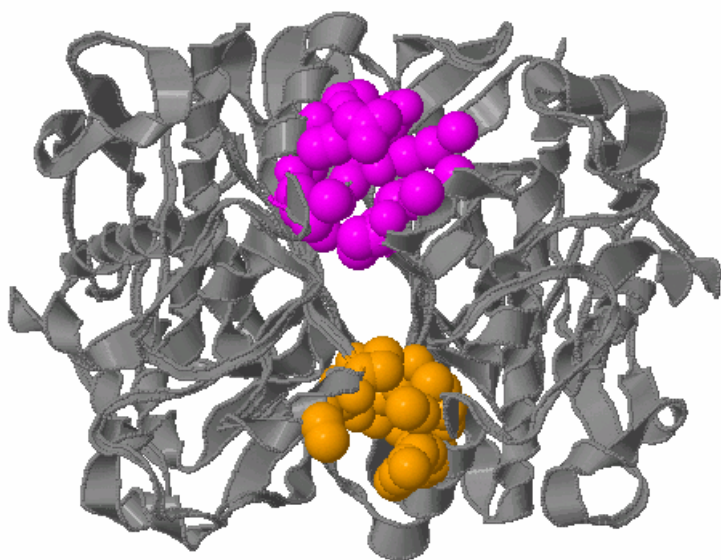
Analysis of the dimer interface

- Interface crevices in X-ray structure –

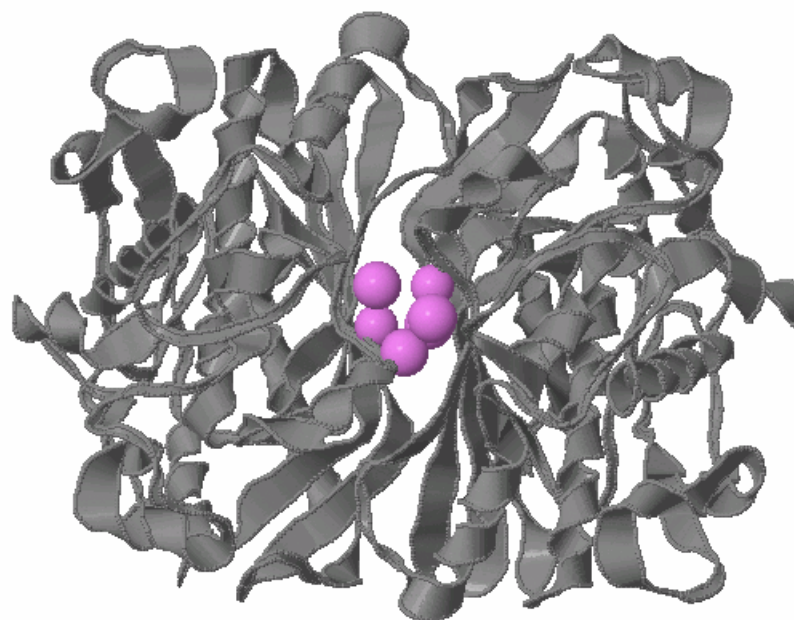
Interface crevices in the hTS dimer

→ Cavities at the edges of the dimer interface

Software: PASS 1.1 / SITE-ID (Sybyl 7.3) / CASTp



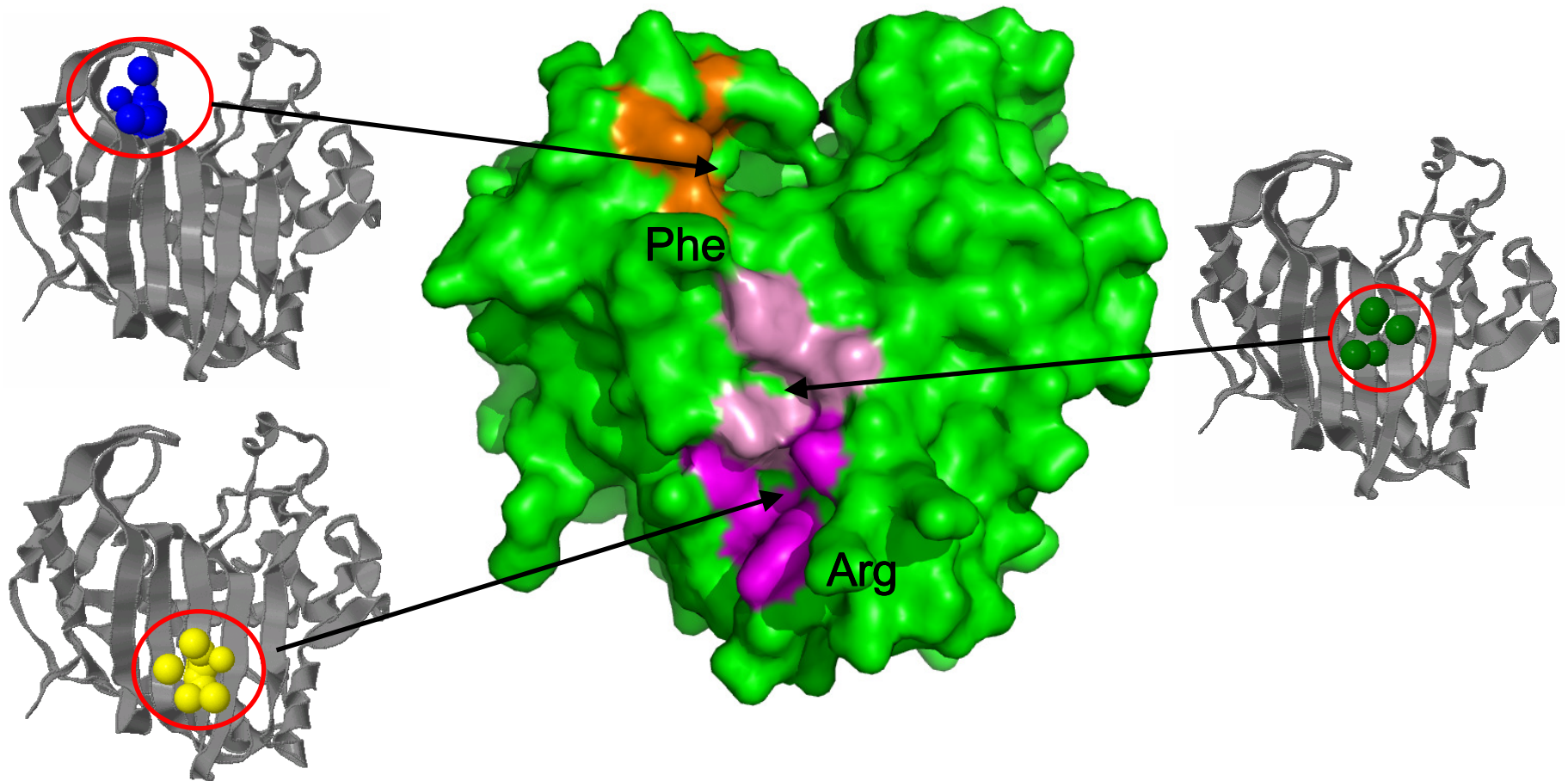
232 / 362 Å³ (I, W)



25 Å³ (W)

Interface crevices in the hTS monomer

- Two relatively deep cavities and one shallow pocket
- not present in dimer



Analysis of the dimer interface

- Transient interface pockets by MD –

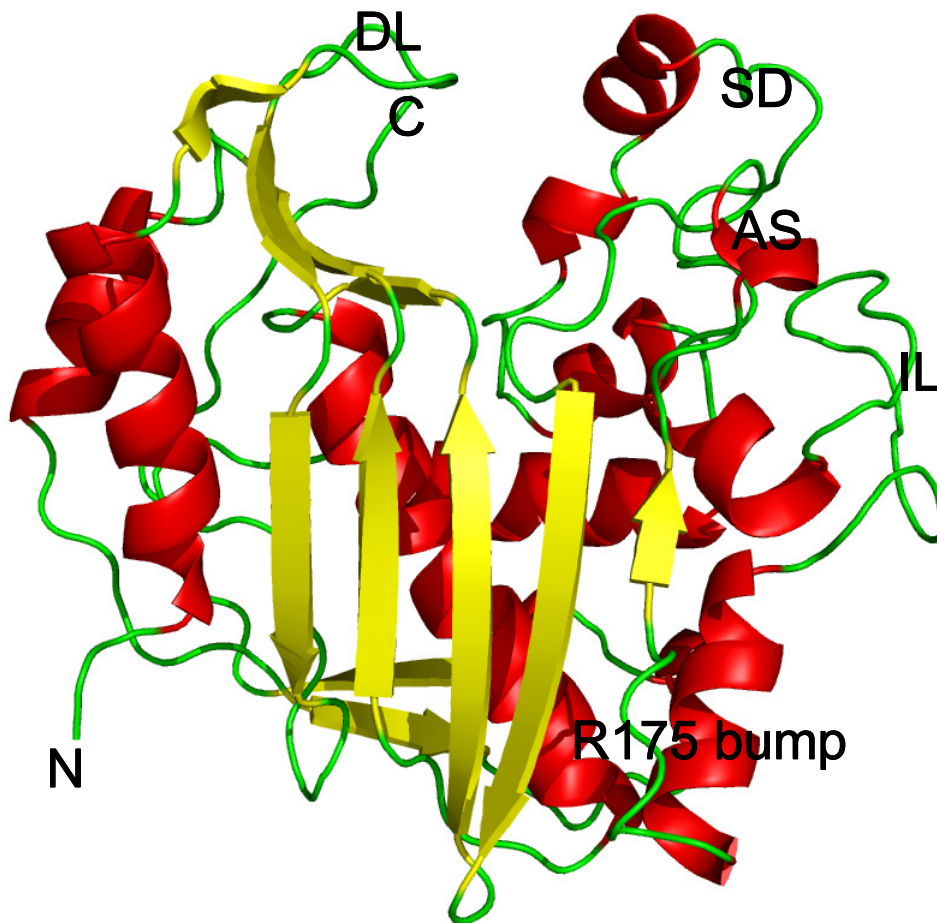
See for example:

Wong et al., Proteins, 61, 850, 2005

Eyrisch and Helms, J. Med. Chem. 50, 3457, 2007

Transient interface pockets?

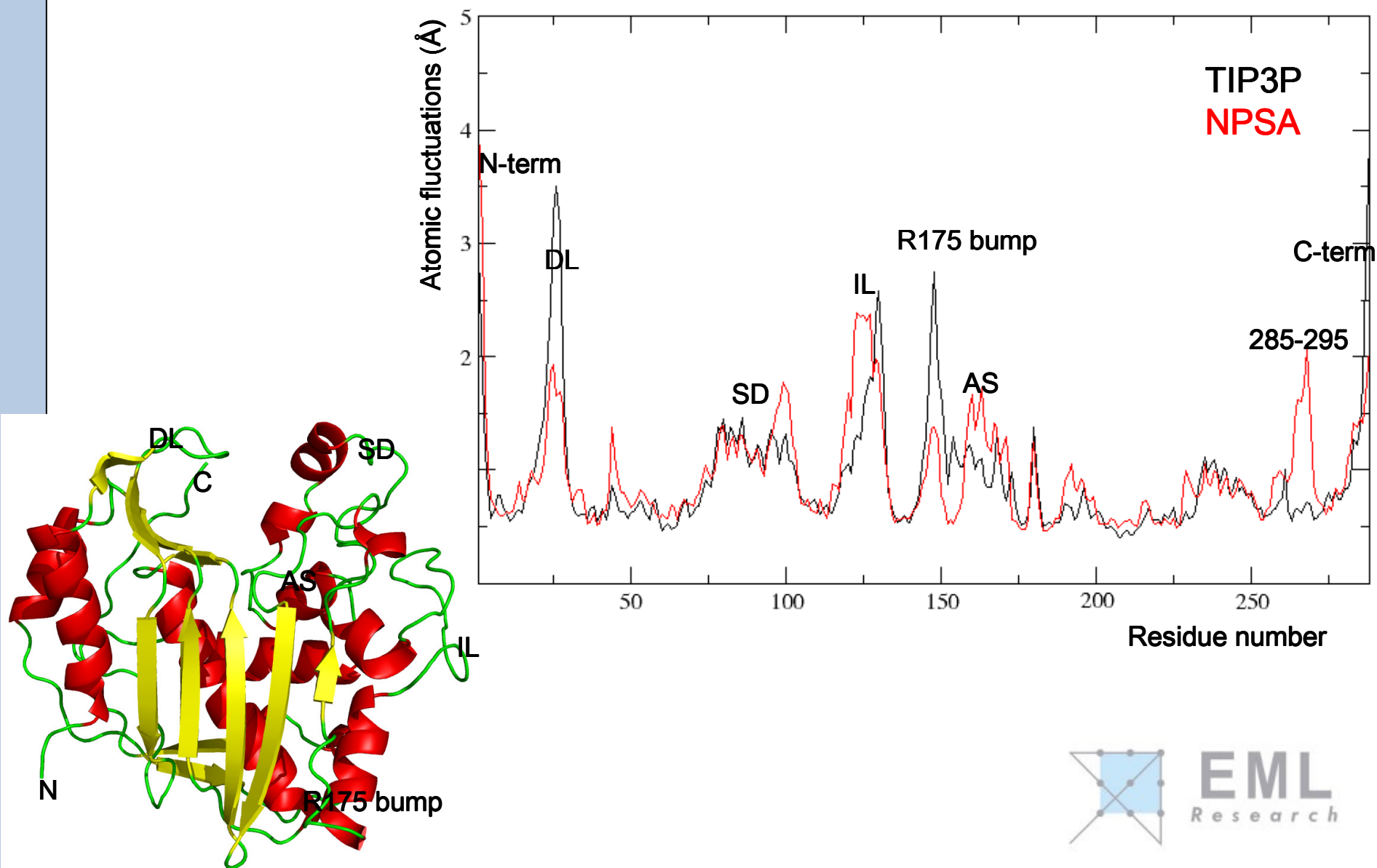
→ MD simulations of the TS monomer



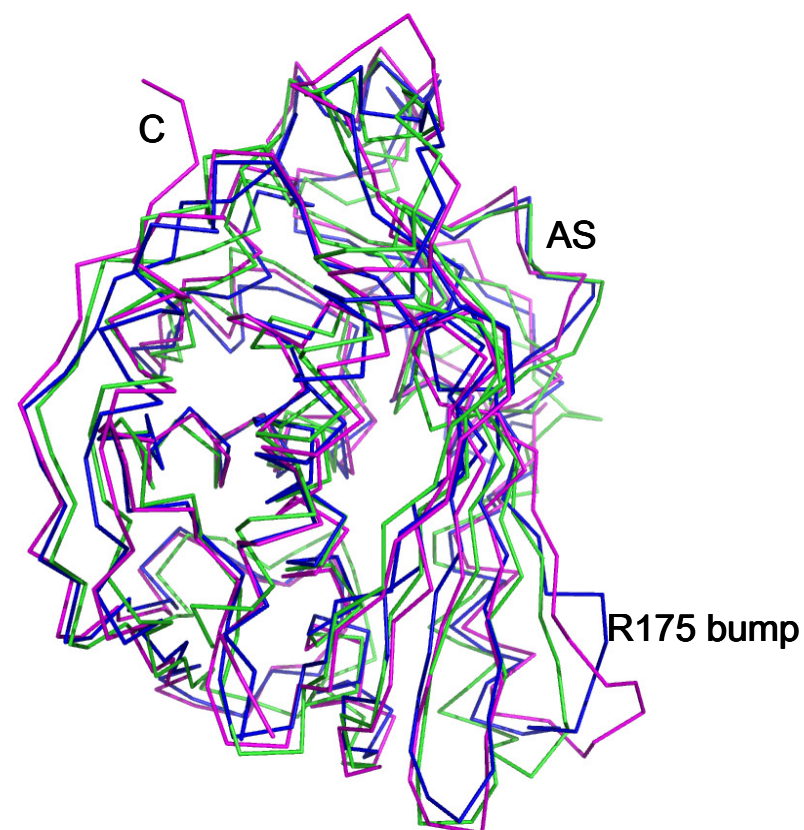
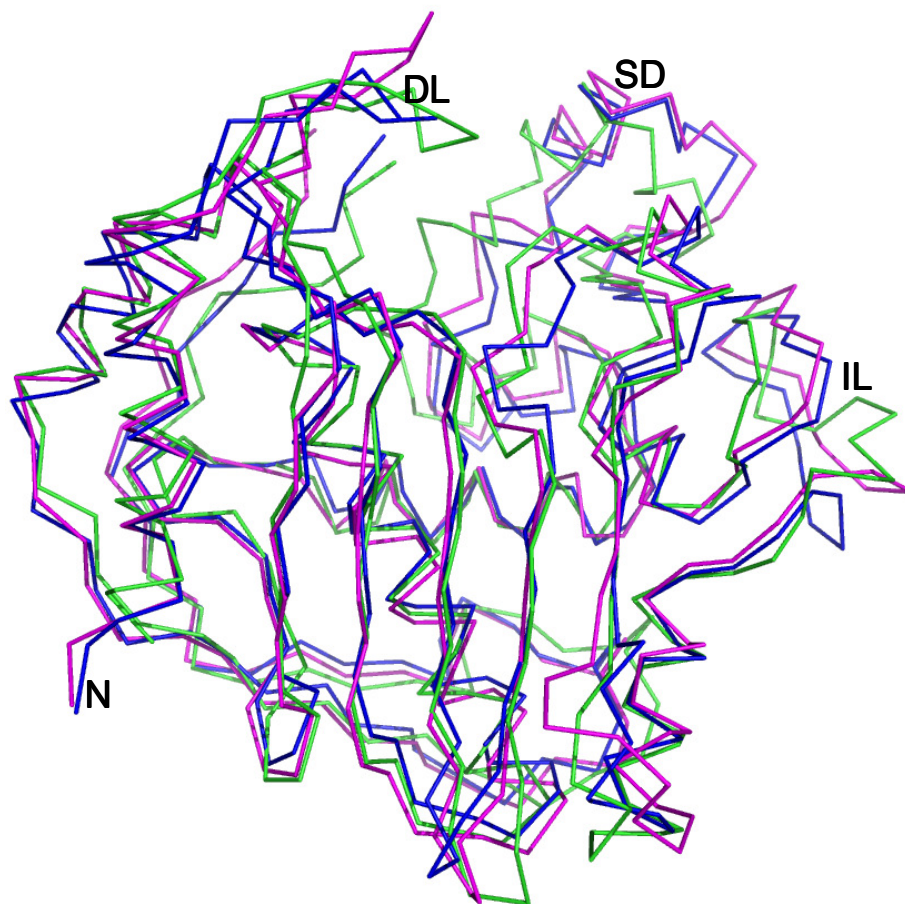
- AMBER 8, ff03
- 1HVY.pdb (A chain)
- No ligands
- NPSA* implicit water model
- 300 K, 7 ns
- heating in 3 steps
- reference MD with TIP3P water

* Wang and Wade, Proteins 50, 158, 2003

MD Trajectory analysis: Atomic fluctuations



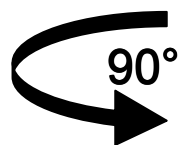
MD Trajectory analysis: Conformational changes



Initial structure

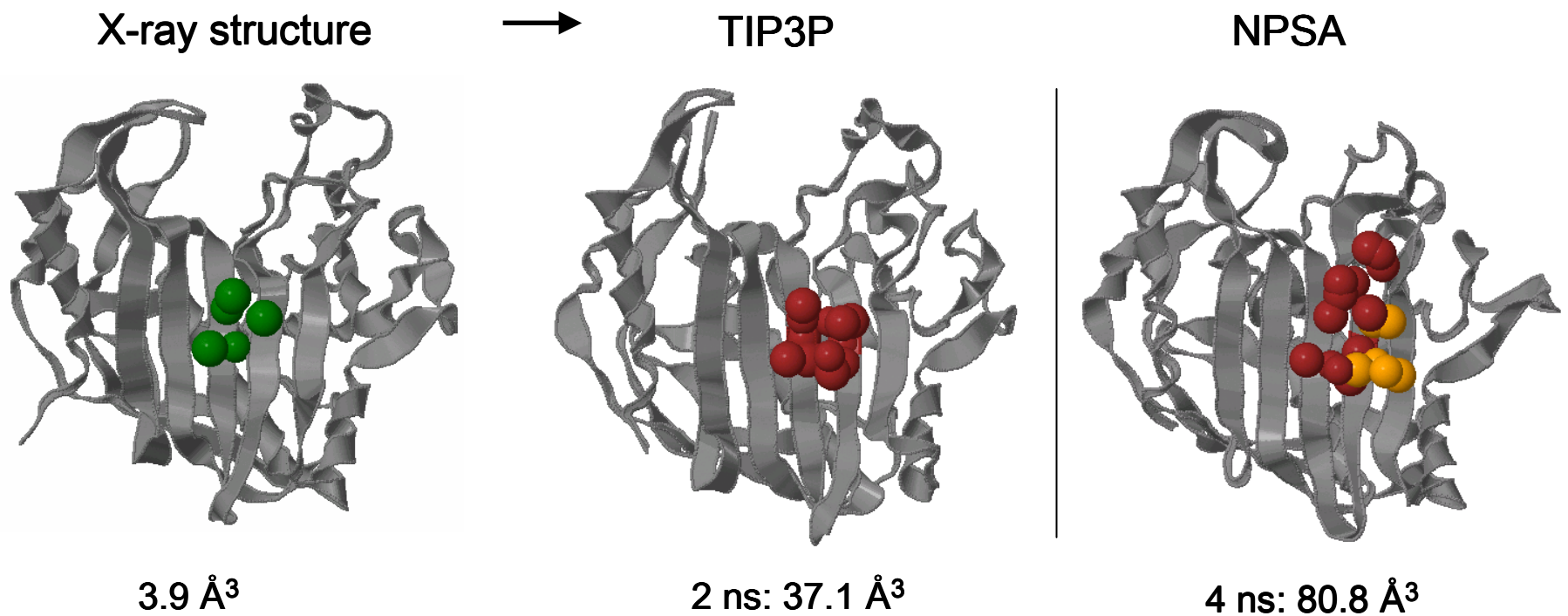
Final frame NPSA

Final frame TIP3P



Interface pocket dynamics

1) Pockets changing size



Interface pocket dynamics

2) New pockets appearing

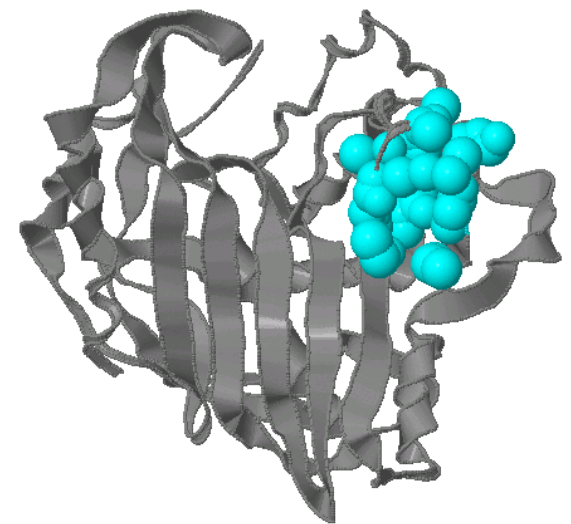
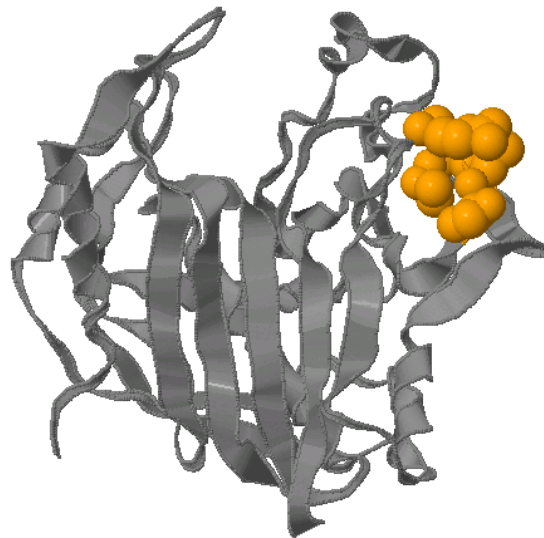
X-ray structure



TIP3P

NPSA

Non-existent

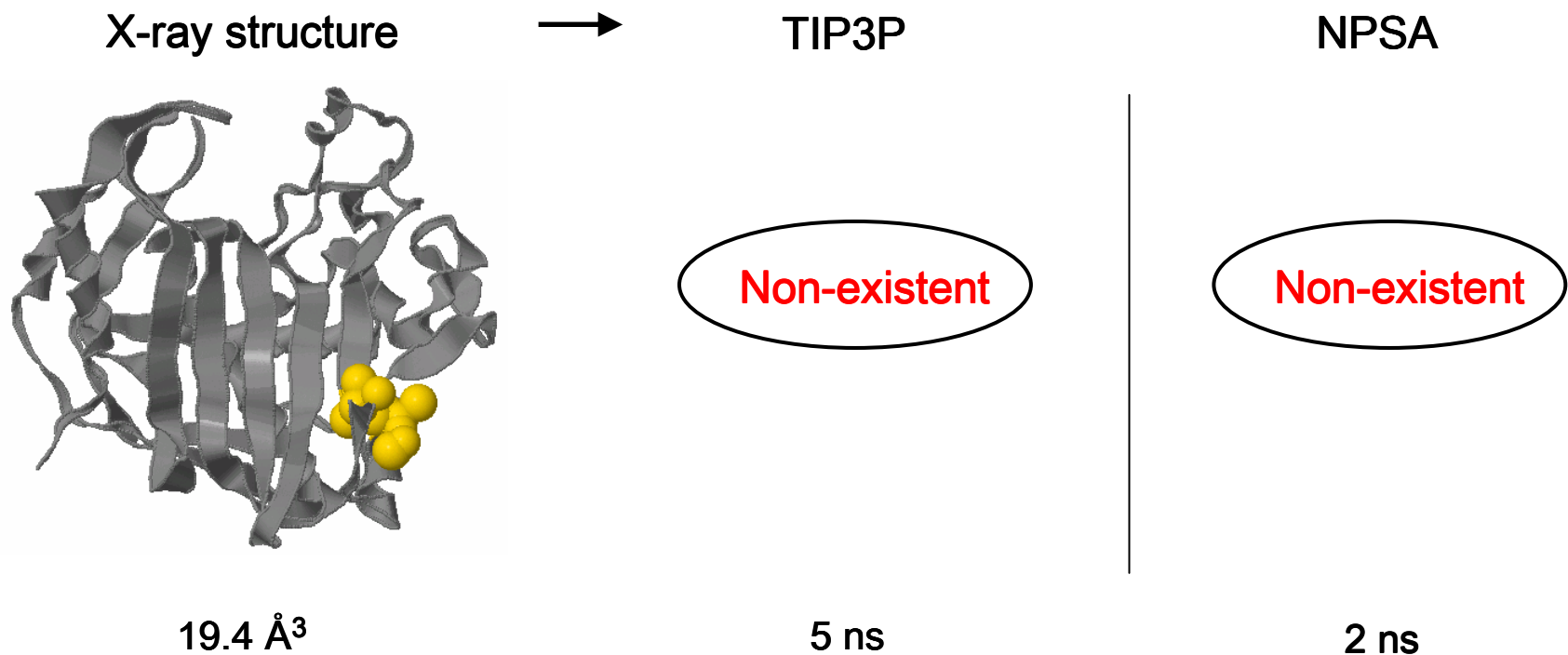


7 ns: 167.9 Å³

5 ns: 259.6 Å³

Interface pocket dynamics

3) Pockets disappearing (reappearing)



Summary and future work

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- 4) Performed MD simulations to find additional **transient interface pockets** at the monomer interface.

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Future:

- Use the identified pockets for **virtual screening** of ligand libraries.
- Test the ligands against hTS.

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