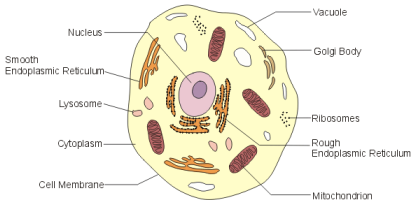
Insights into catalytic mechanism of an enzyme

Ivana Leščić Ašler, Zoran Štefanić, Marija Luić

Ruđer Bošković Institute, Bijenička cesta 54, HR-10002 Zagreb

Enzymes

- Macromolecular biological catalysts
- 6 classes, according to reaction they catalyse
- Industrial applications, inhibitors-drugs, …
- Mechanism of action?



→ *in vivo*, *in vitro*, *in silico*

– Localization, physiological role

– Reaction conditions

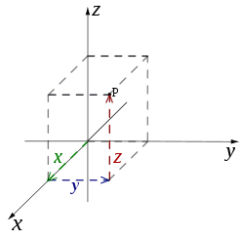
– Prediction of properties, analysis of big data

Mechanism of enzyme action

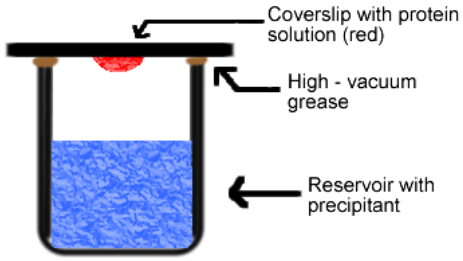
- Plethora of methods
  - How does the active site of an enzyme look like?
  - What will bind into the active site?
  - What will undergo chemical change (substrates)?
  - What will this change be (reaction type)?
  - What are the steps in this change (reaction course)?
  - In what time will it occur (reaction kinetics)?
  - In what conditions?
  - How can this information be used?

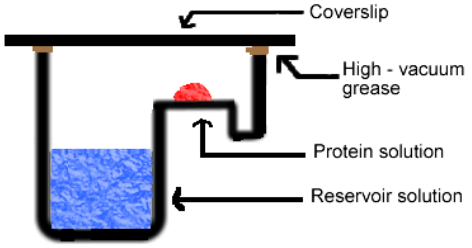
X-ray crystallography

- 3D-structure, positions of all the atoms in an enzyme molecule, fold
- Size and shape of the active site



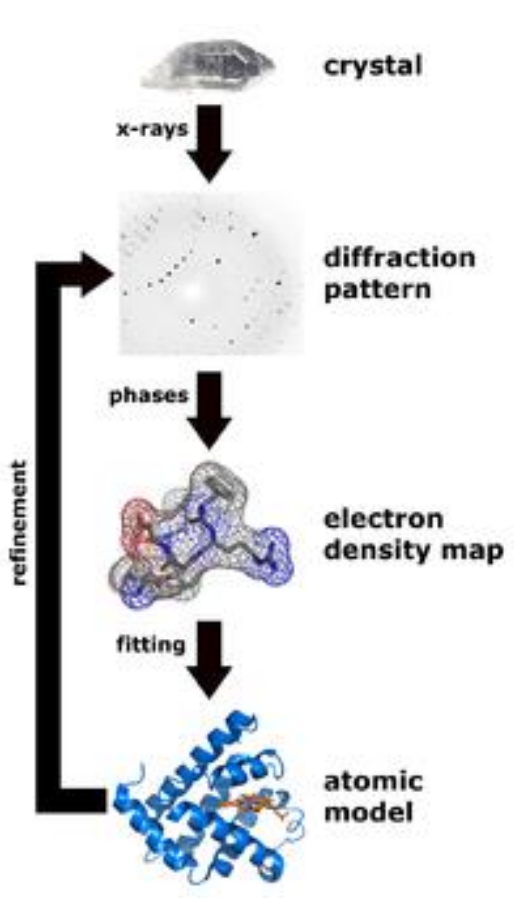
- Structures with bound substrate analogues – connections in the active site → suggestion of mode of action
- Higher resolution → more details → more accurate model
- Crystallization (purified protein)!

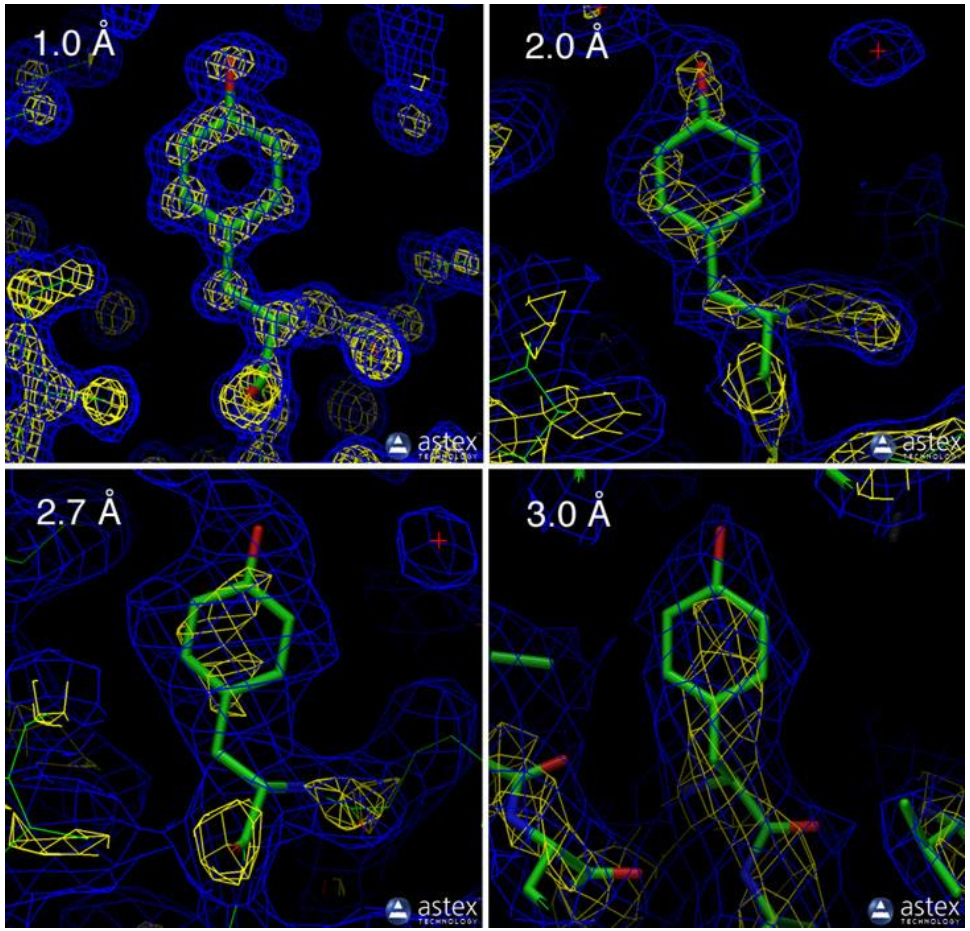




X-ray crystallography

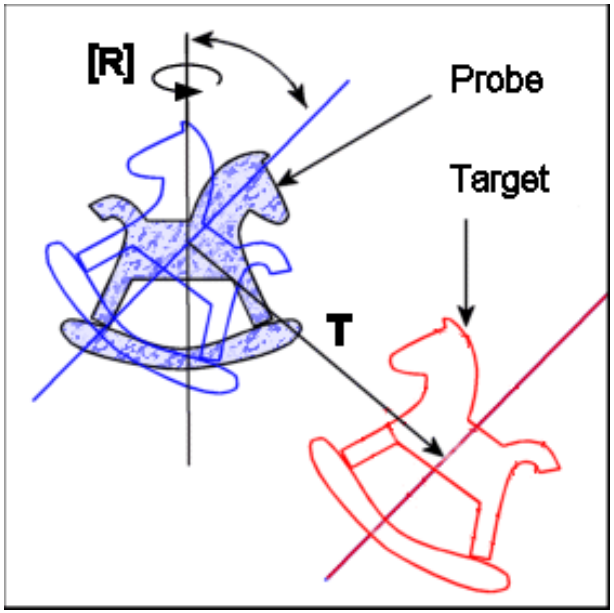
- Importance of resolution

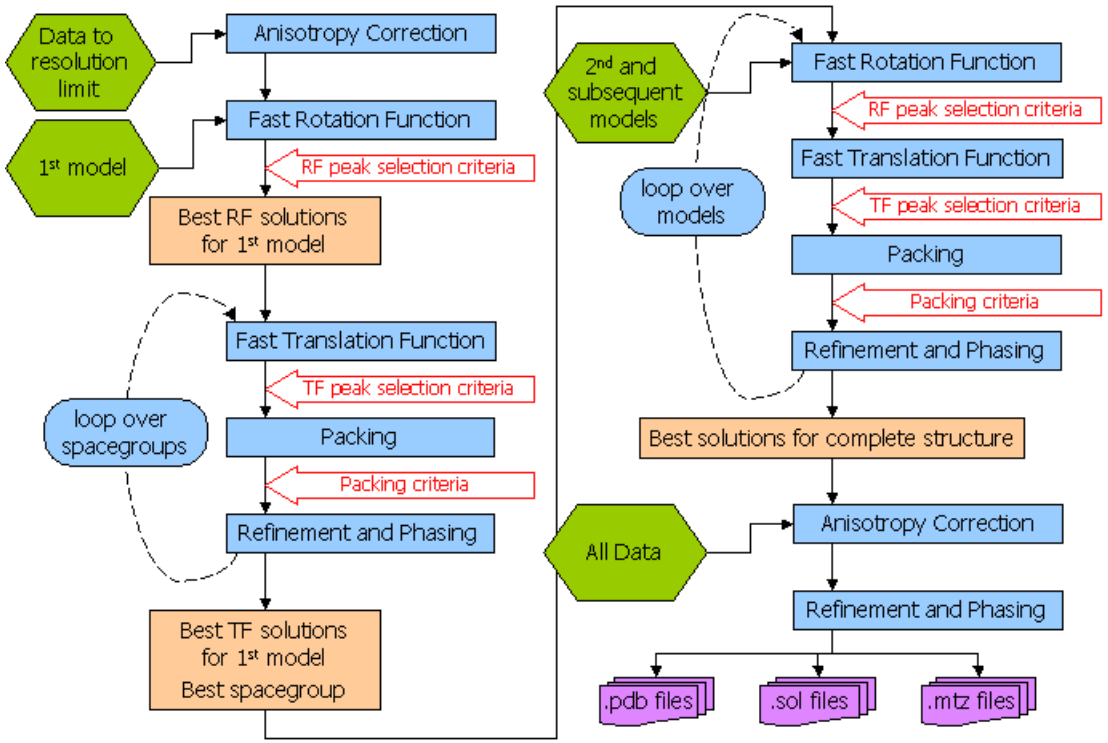




X-ray crystallography

- Molecular replacement





Purine nucleoside phosphorylase

- One of the key enzymes in purine metabolism

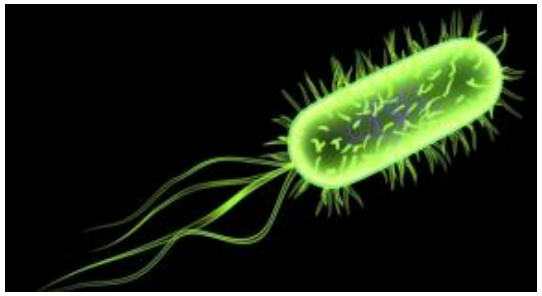
- Reaction:

β-purine nucleoside + P_i $\rightleftharpoons$ purine base + α-D-pentose-1-phosphate

- Present in both eucaryotes and procaryotes
- 302 protein sequences in SwissProt, 169 structures in PDB
- LCBC: PNPs from *Escherichia coli* and *Helicobacter pylori*

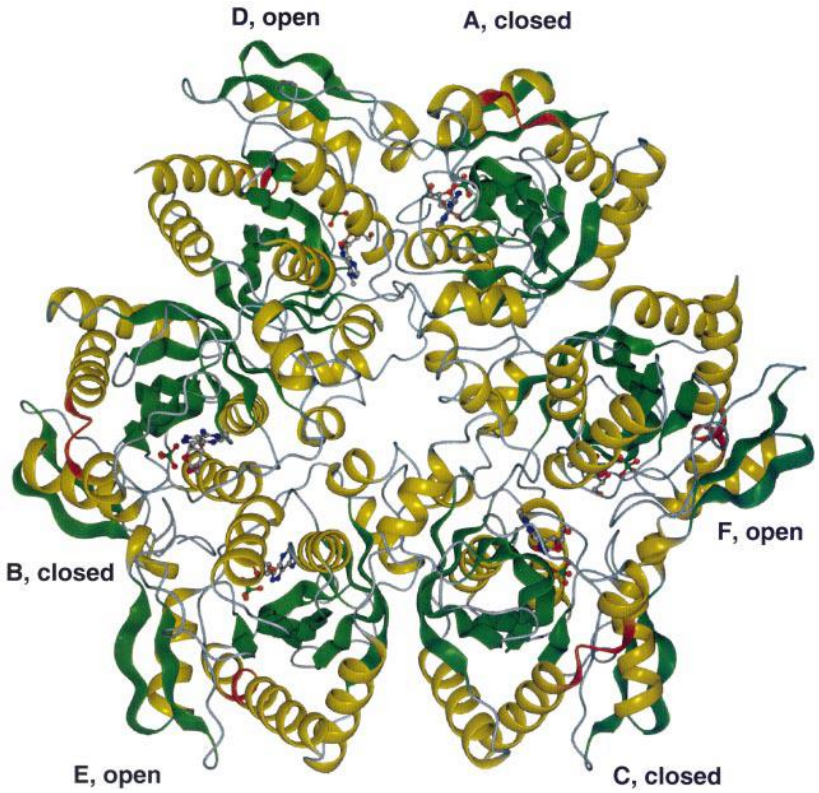
E. coli PNP

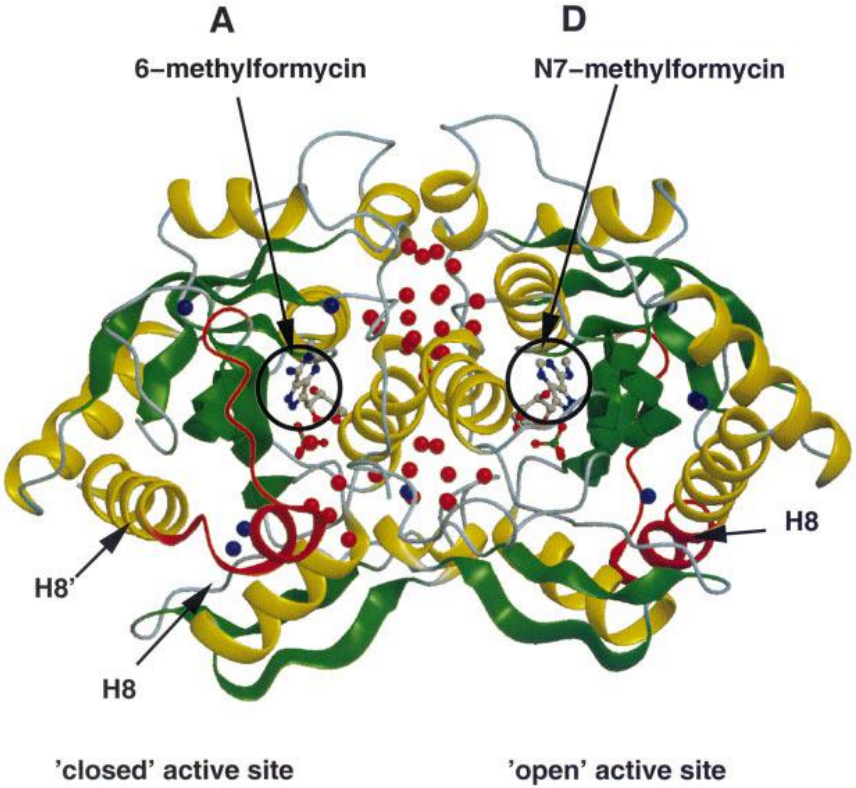
- *E. coli* – pathogen, enzyme-activating prodrug gene therapy enabling selective killing of tumour cells expressing the *E. coli P*NP gene



- 21 sequence in SwissProt (different strains) – 238 amino acids (25.8 kDa)
- 30 structures in PDB (mutants, ligands...)
- Important – different substrate specificity than human PNP (different structure)

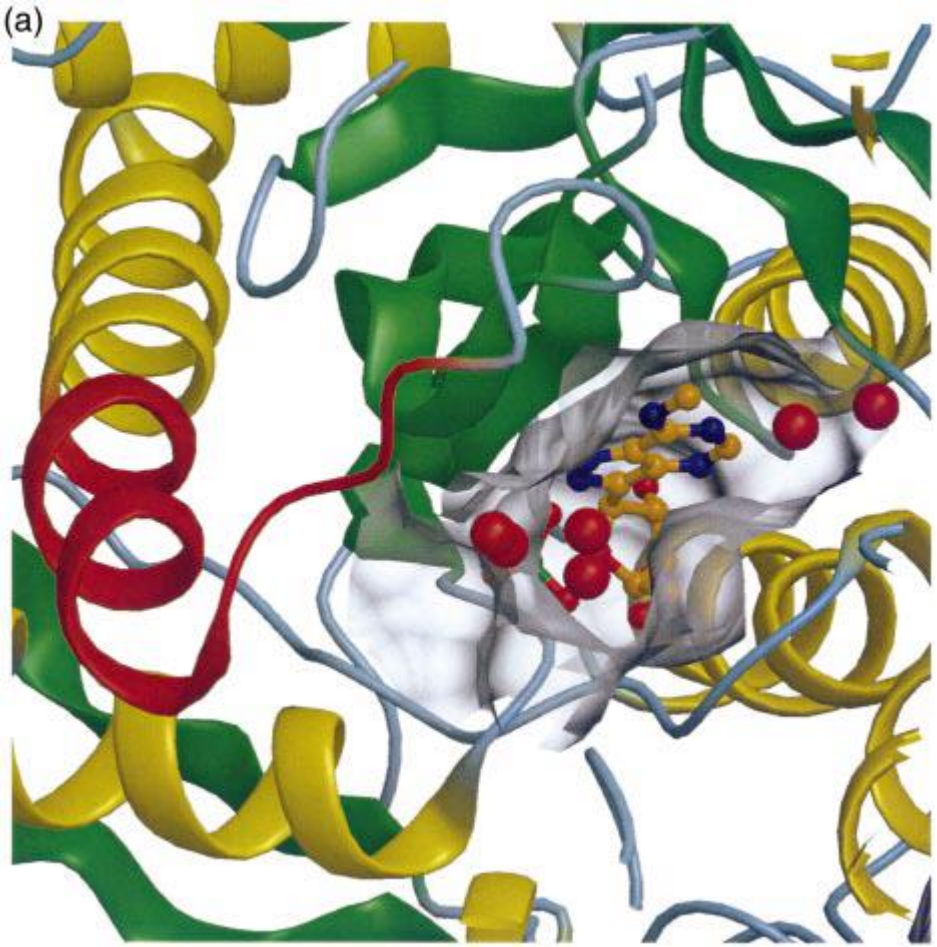
E. coli PNP

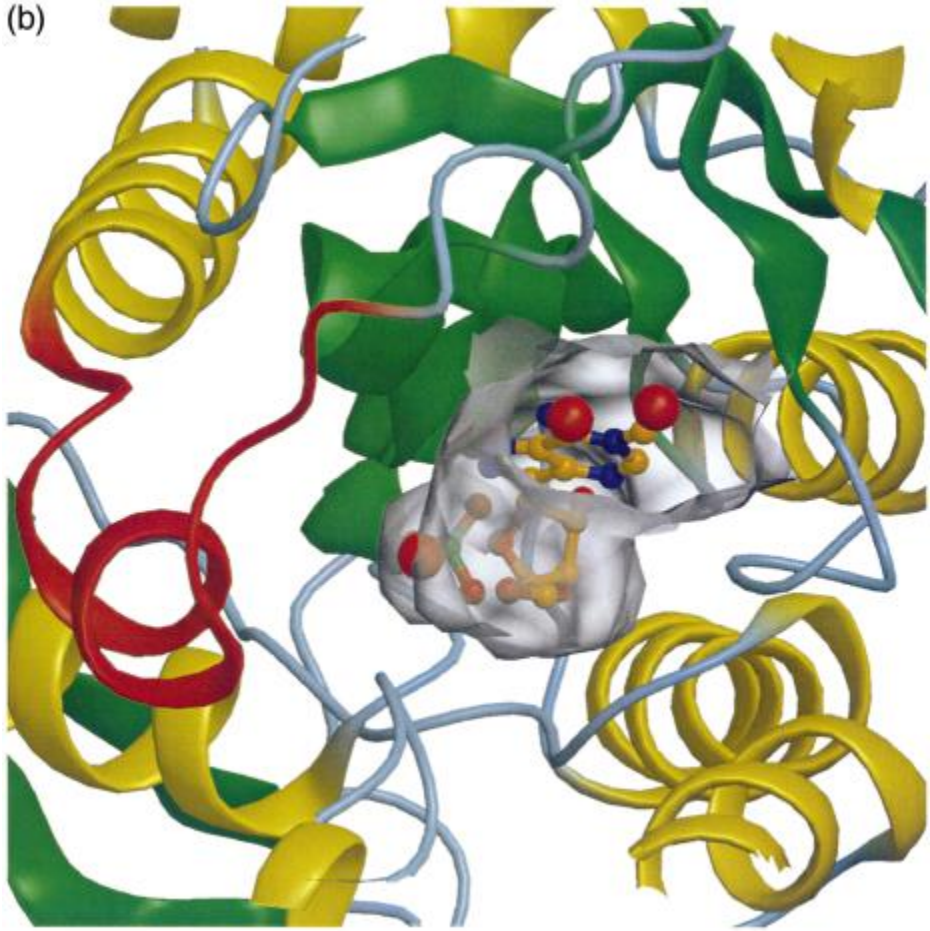




Koellner et al. (2002) J. Mol. Biol. 315: 351±371

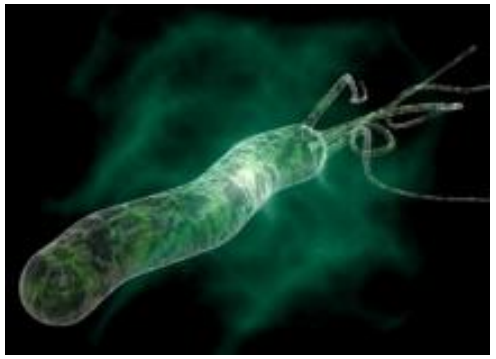
E. coli PNP





Koellner et al. (2002) J. Mol. Biol. 315: 351±371

H. pylori PNP



- *H. pylori* – pathogen, inhibitors for eradication of the bacteria
- 6 sequences in SwissProt (different strains) – 232 amino acids (25.7 kDa)
- 0 structures in PDB
- 52% identity with *E. coli* PNP
- Important aminoacids in the active site conserved → similar structures and mechanisms

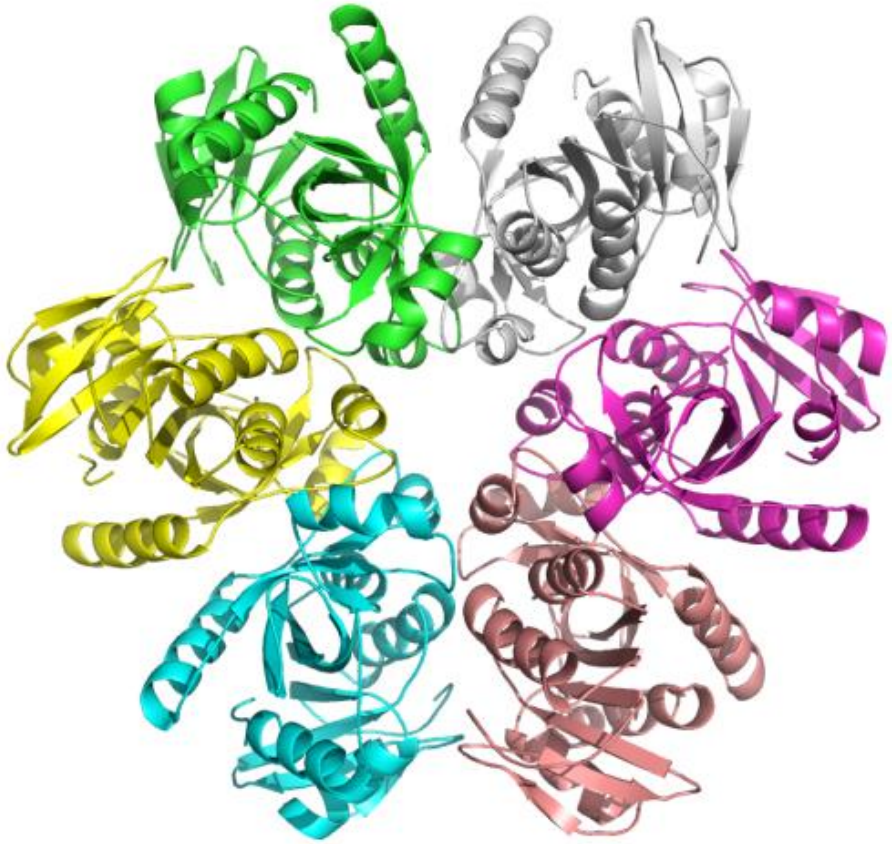
H. pylori PNP

- Crystallization conditions: 0.2M $MgCl_2$, 0.1M Tris pH 7.0, 10% (w/v) PEG 8000; 18°C; 1-2 weeks (or shorter with „seeding”)
- Structures of apo enzyme; complexes with P_i, Formycin A (inhibitor), both
- in-house instrument (Oxford Diffraction Xcalibur Nova R) and synchrotron (XRD1, Elettra, Trieste)

H. pylori PNP

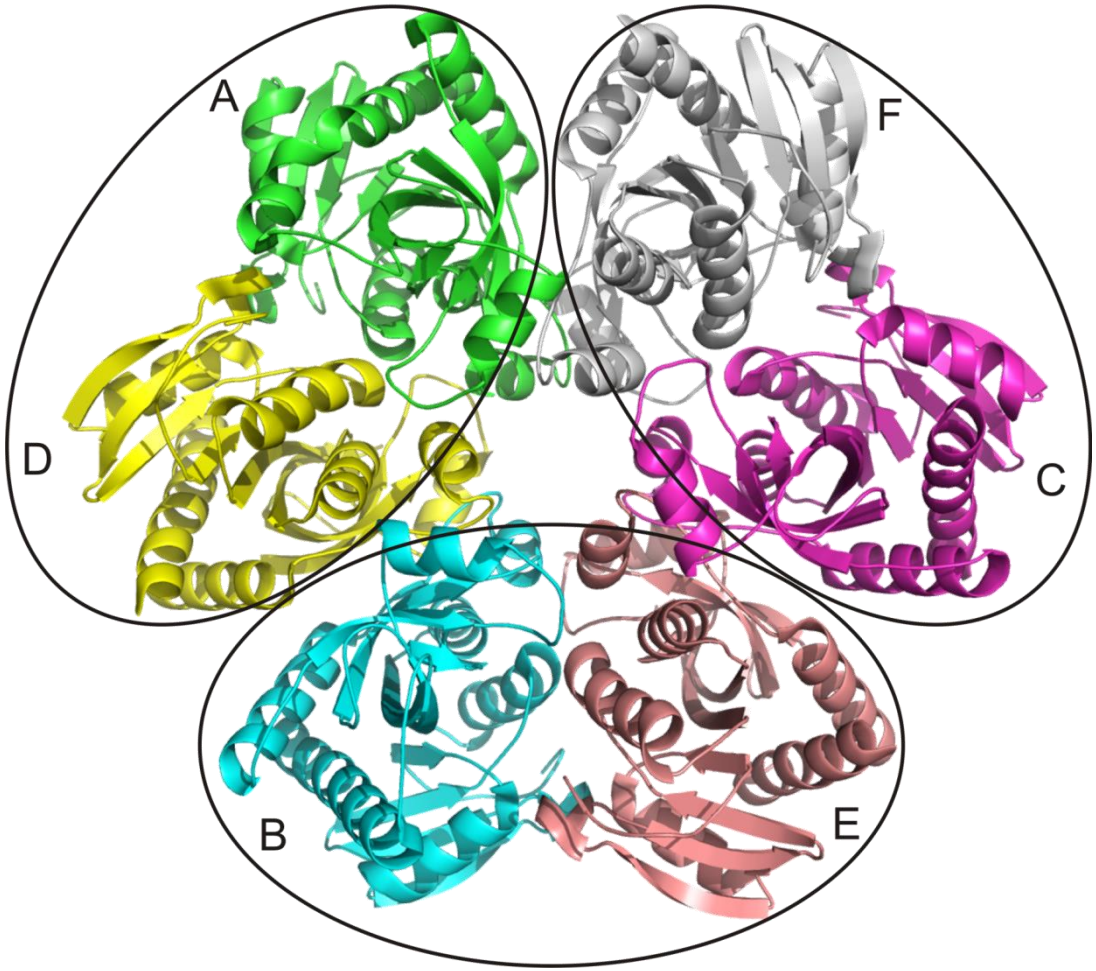
- Molecular replacement

  with *E. coli* PNP structure as a starting model



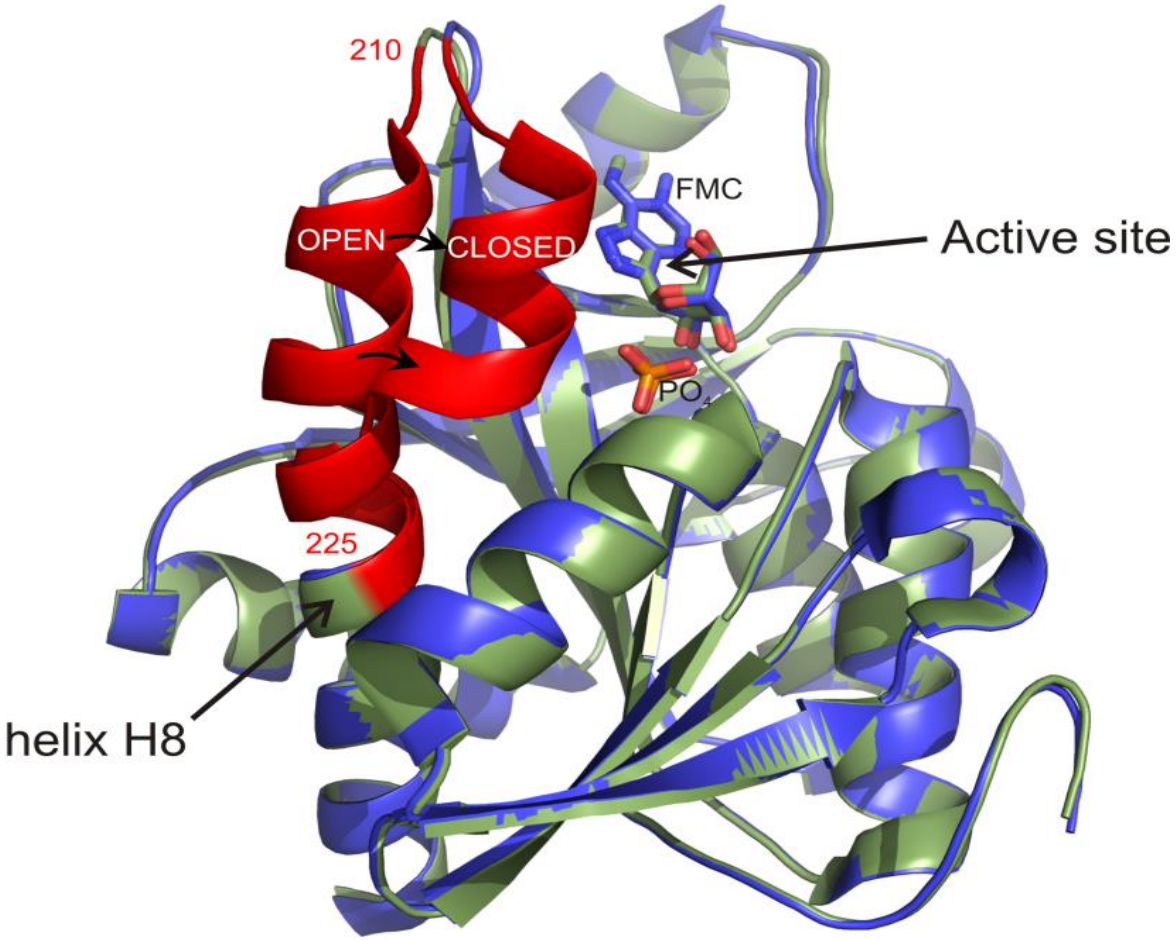
H. pylori PNP

- Trimer of dimers



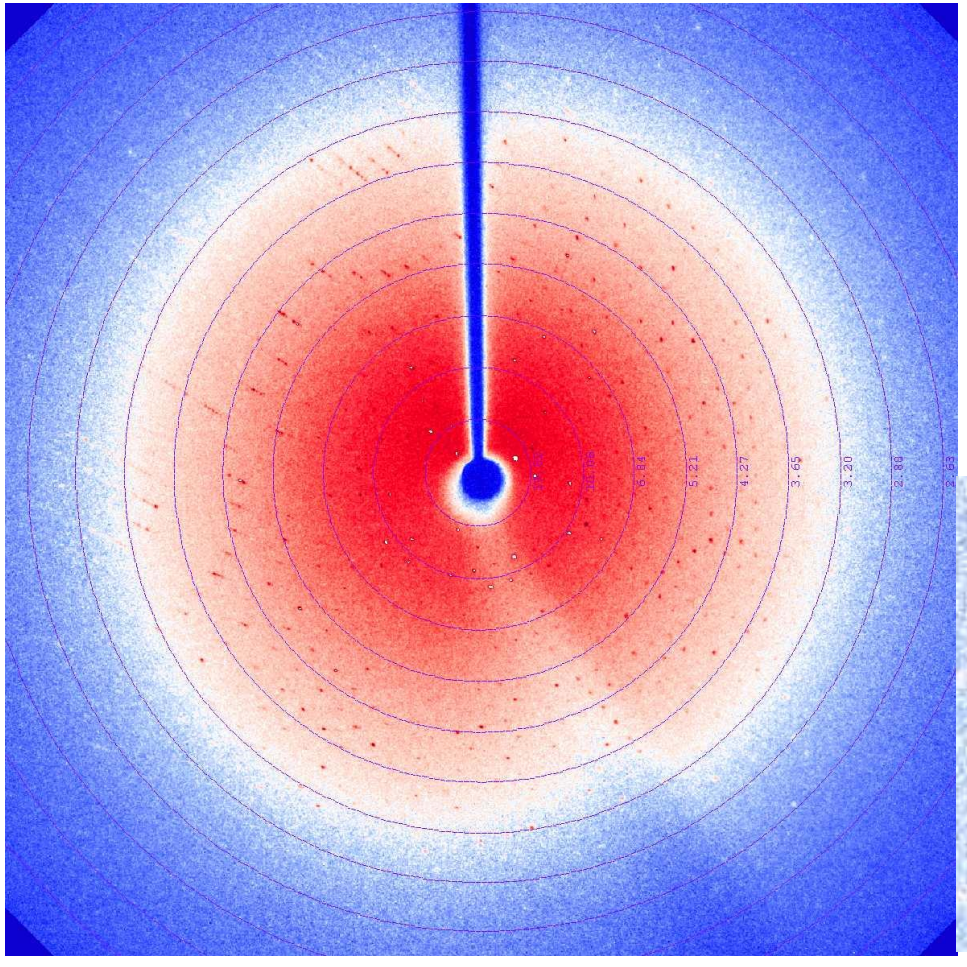
H. pylori PNP

- Open and closed active site

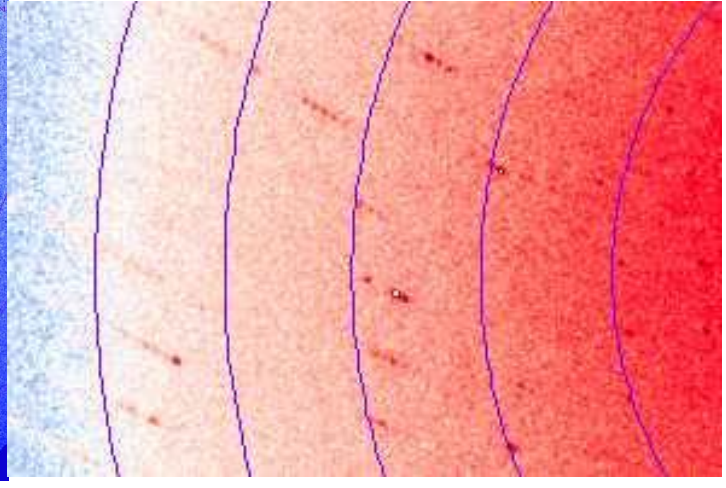


H. pylori PNP

- Diffraction image from in-house instrument

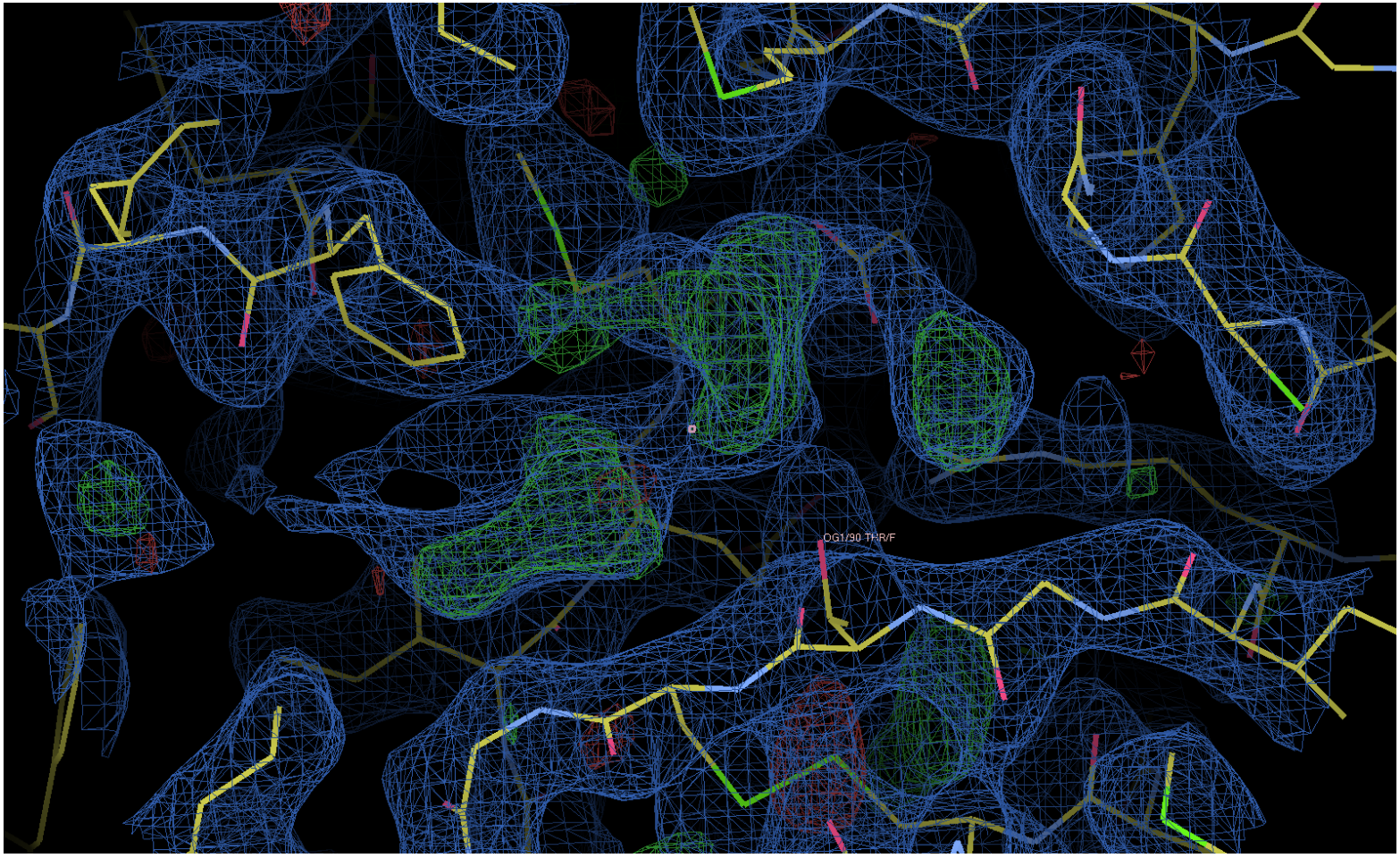


Diffraction up
to ~2.6 Å



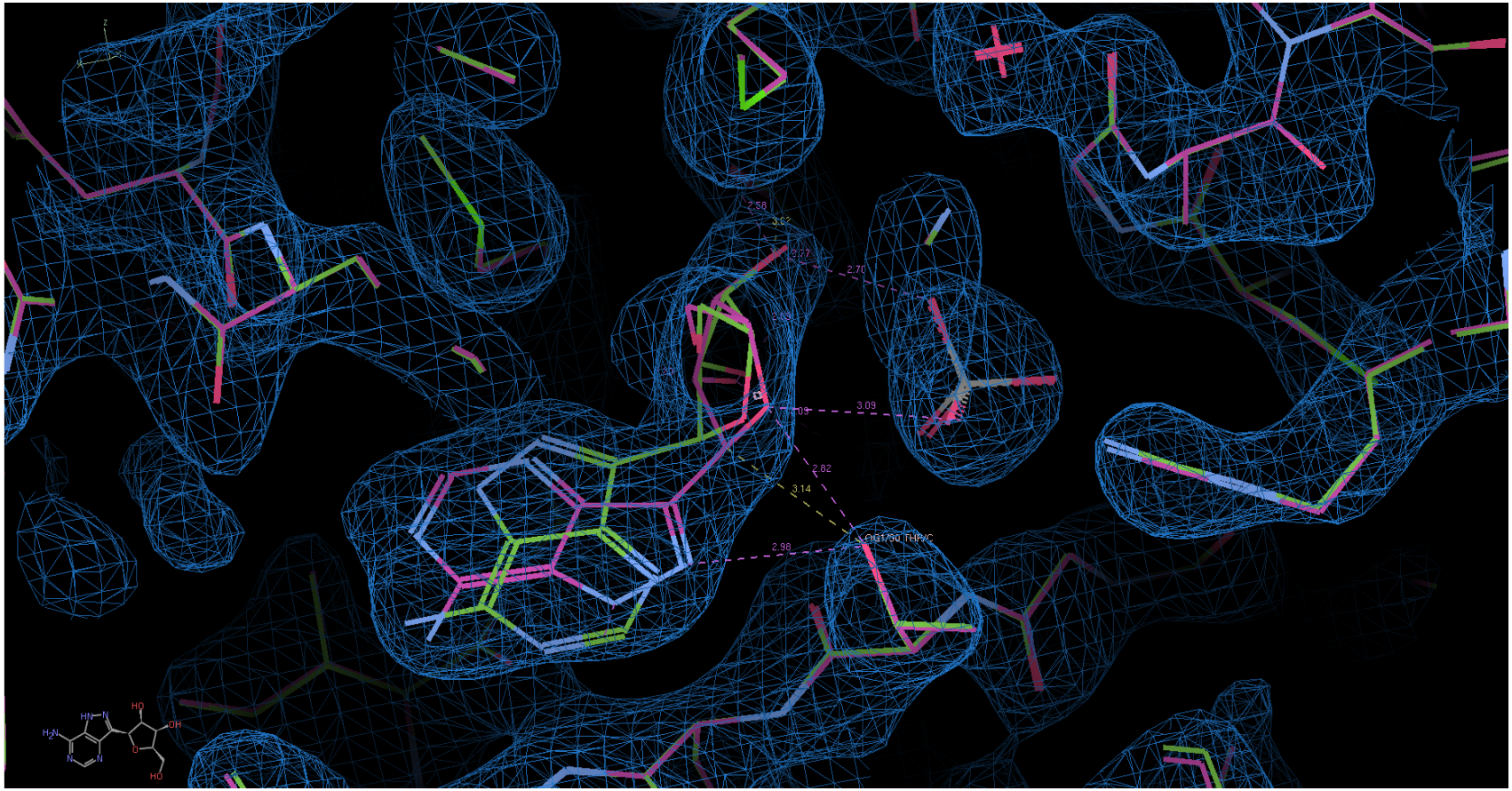
H. pylori PNP

- Electron density map from in-house instr. data



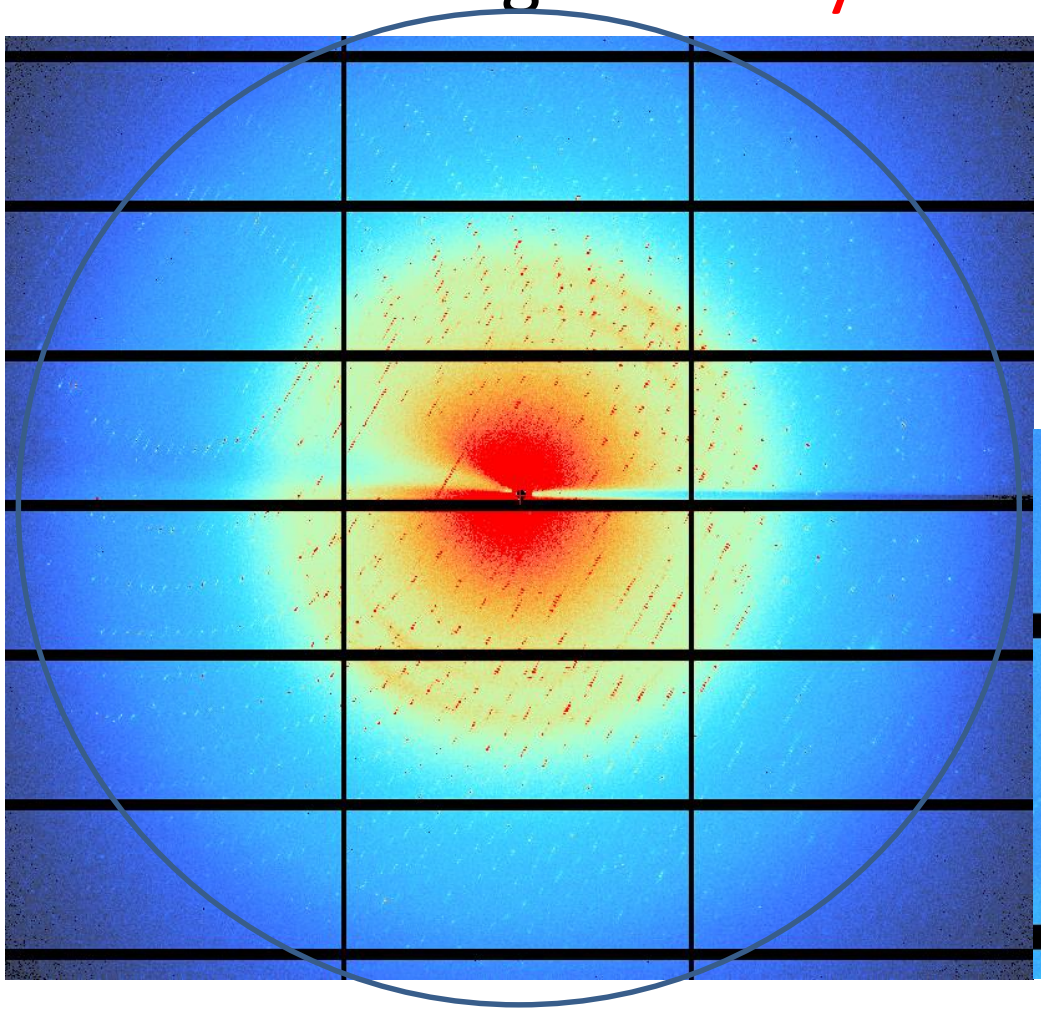
H. pylori PNP

- Electron density map from in-house instr. data

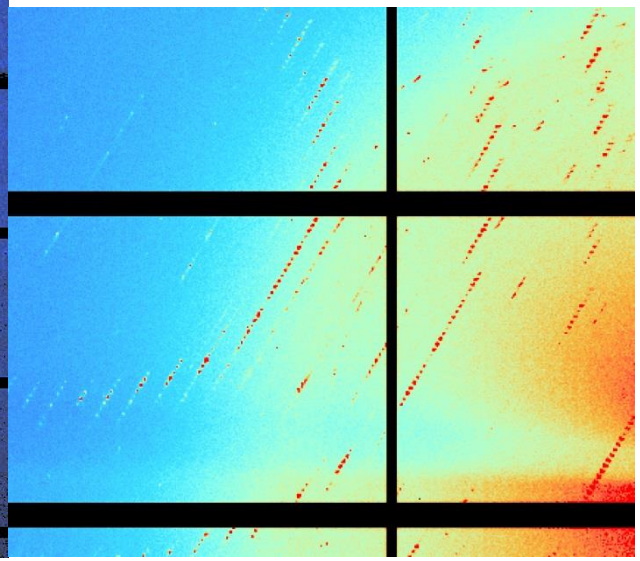


H. pylori PNP

- Diffraction image from synchrotron

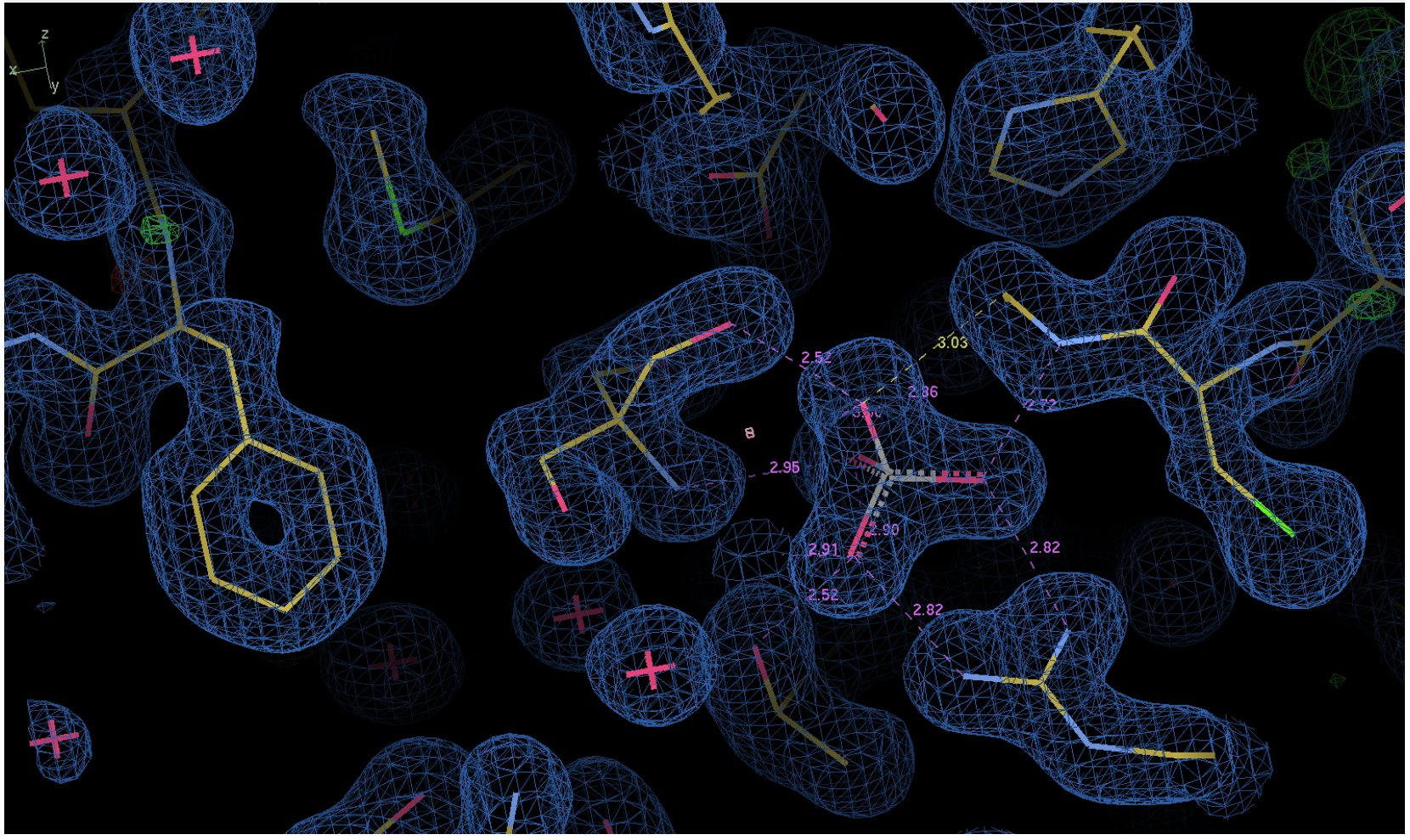


Diffraction up
to 1.7 Å



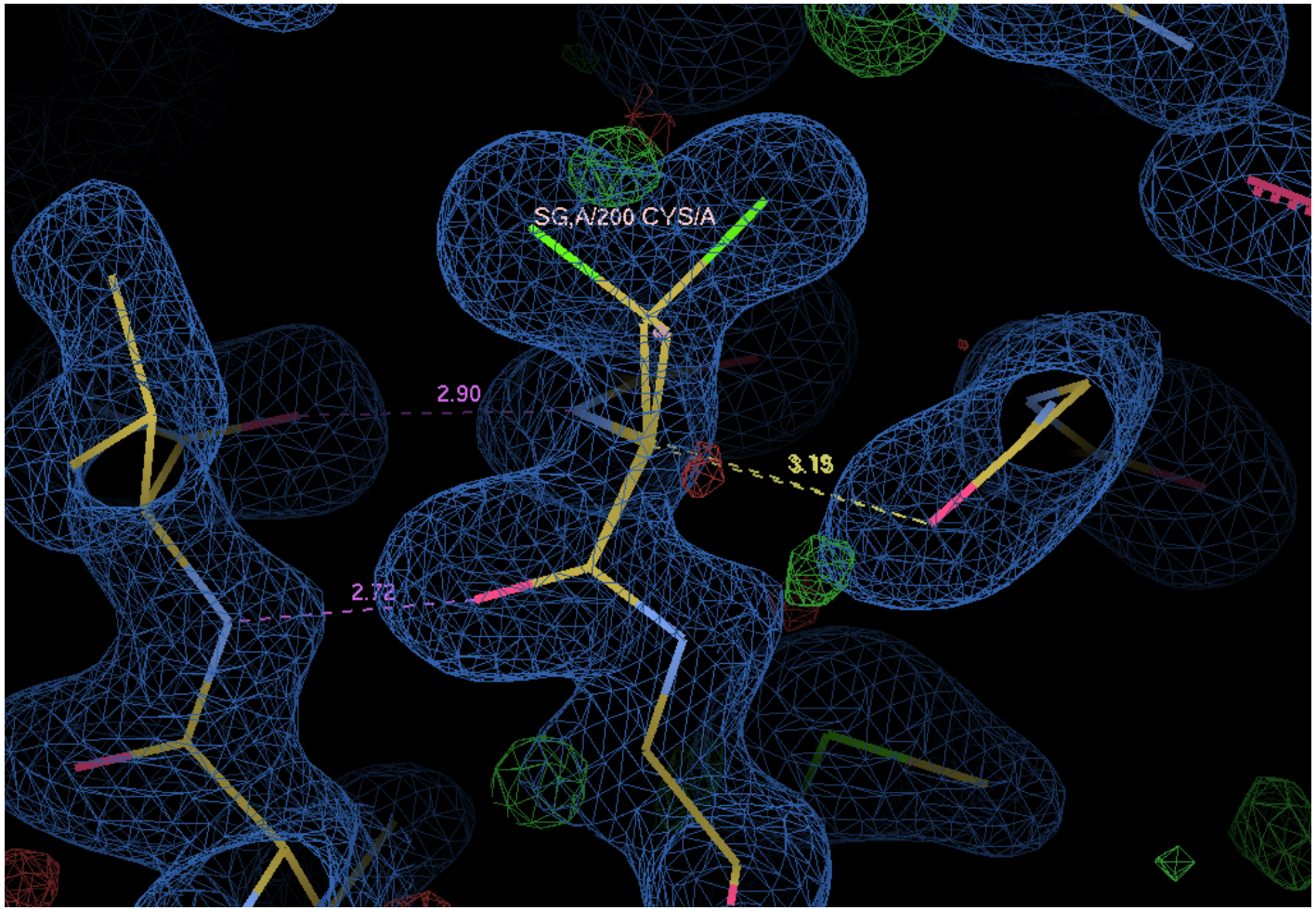
H. pylori PNP

- Electron density map from synchrotron data



H. pylori PNP

- Electron density map from synchrotron data



H. pylori PNP

- Conclusions:
  - Structures of apo-protein, binary complex and ternary complex give insight into interactions in the active site
  - In lower-resolution structures, electron density is not clear to define ligands bound, particularly not their conformation
  - Higher-resolution structures enable to see ligands clearly
  - However, new questions arise: is the mechanism of action proposed for *E. coli* PNP also valid for *H. pylori* PNP? (problem of open and closed sites)

Lipase

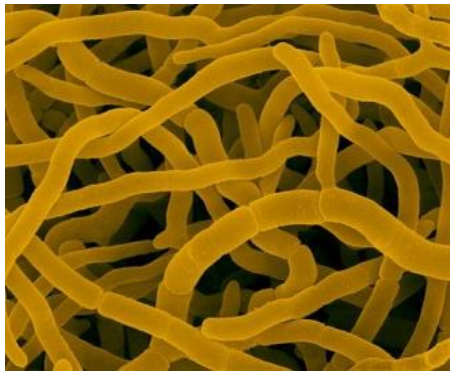
- Diverse and ubiquitous family of enzymes, in biological systems initiate the catabolism of fats and oils
- Reaction:

$$\text{ester} \rightleftharpoons \text{alcohol} + \text{fatty acid}$$

- α/β-hydrolase fold, catalytic triad (Ser-His-Asp)
- Used in organic sythesis, many branches of industry, inhibitors – treatment of obesity
- LCBC: lipase from *Streptomyces rimosus*

S. rimosus lipase

- *S. rimosus* – soil bacterium, produces large amount of secondary metabolites (antibiotics-Pliva)



- Lipase: Q93MW7 in SwissProt

  234 amino acids (24.2 kDa)

  monomer (SDS-PAGE/gel-filtration)

  catalytic promiscuity

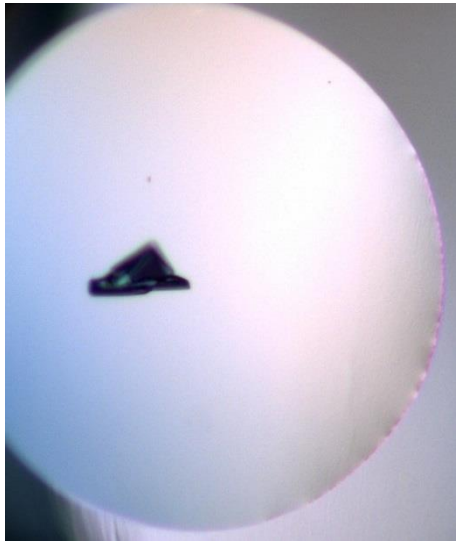
  predicted amino acids of catalytic triad:

  Ser10 (confirmed by MS), Asn213, His216

  three disulfide bridges identified by MS

S. rimosus lipase

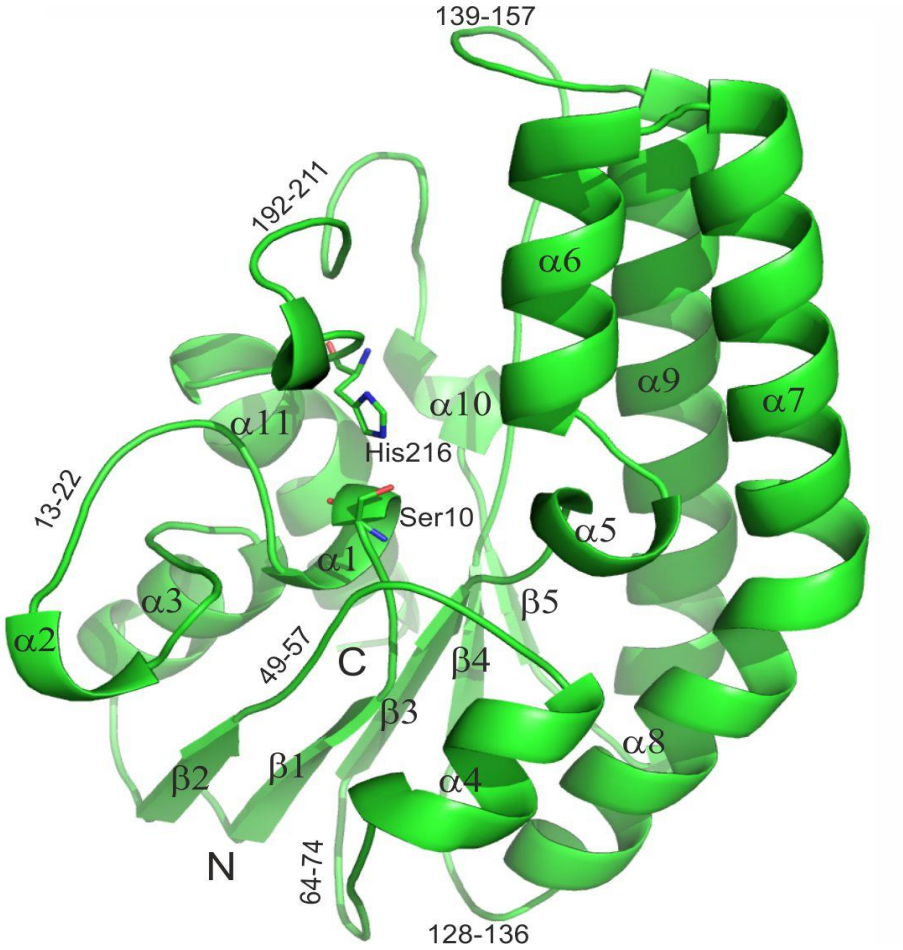
- Structure – one (1) crystal from screening with inhibitor (conditions: 0.1 M MES pH 6.5, 25% PEG 2000 MME), on synchrotron (XRD1, Elettra, Trieste), MR with structure of *Streptomyces albidoflavus* phospholipase A_1 (4HYQ)



- Resolution: 1.7 Å
- Rossmann-like 3-layer αβα sandwich fold typical of the SGNH family
- Active site: catalytic dyad!

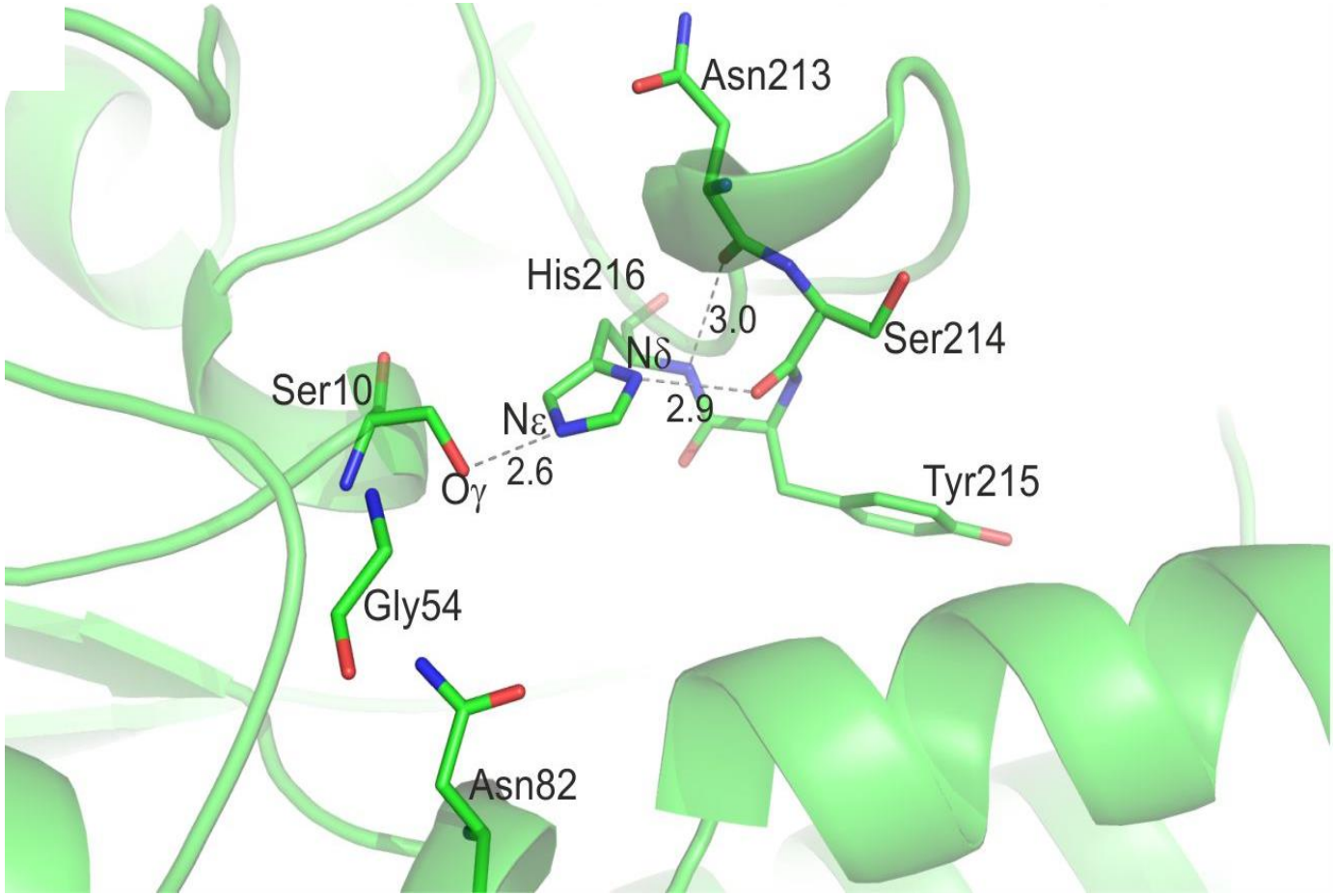
S. rimosus lipase

- Structure



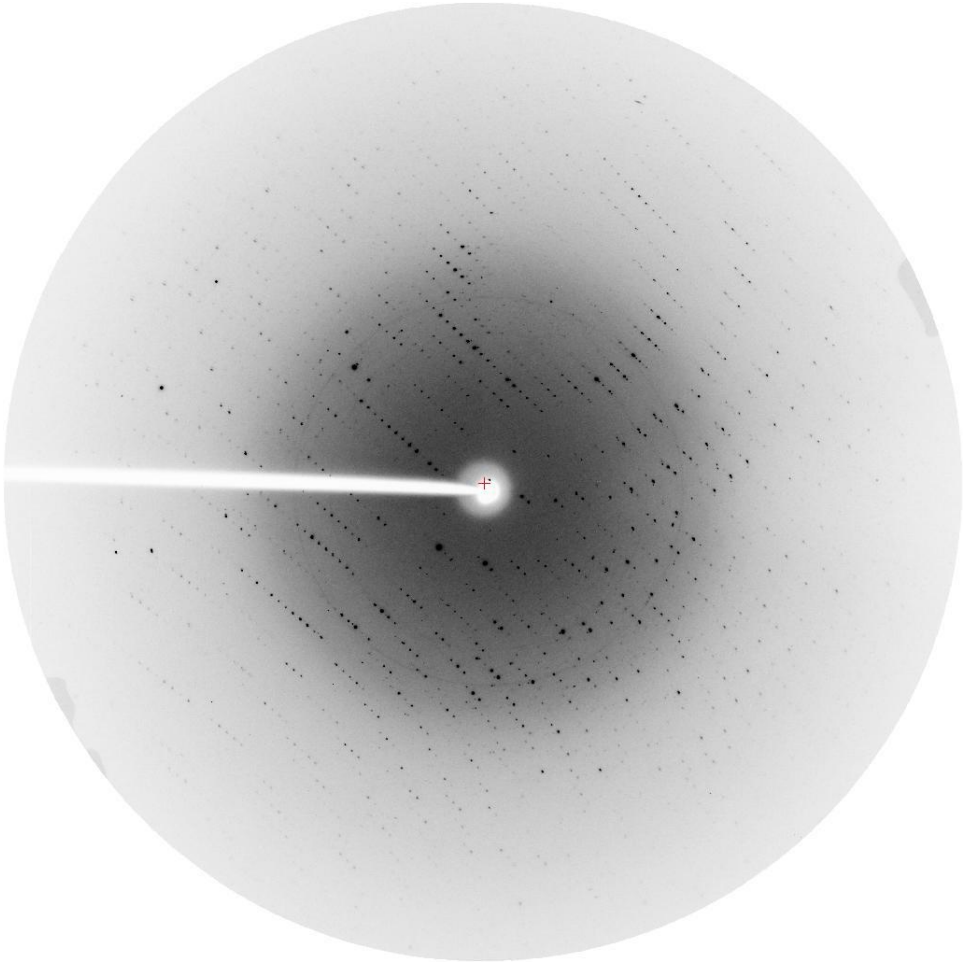
S. rimosus lipase

- Active site



S. rimosus lipase

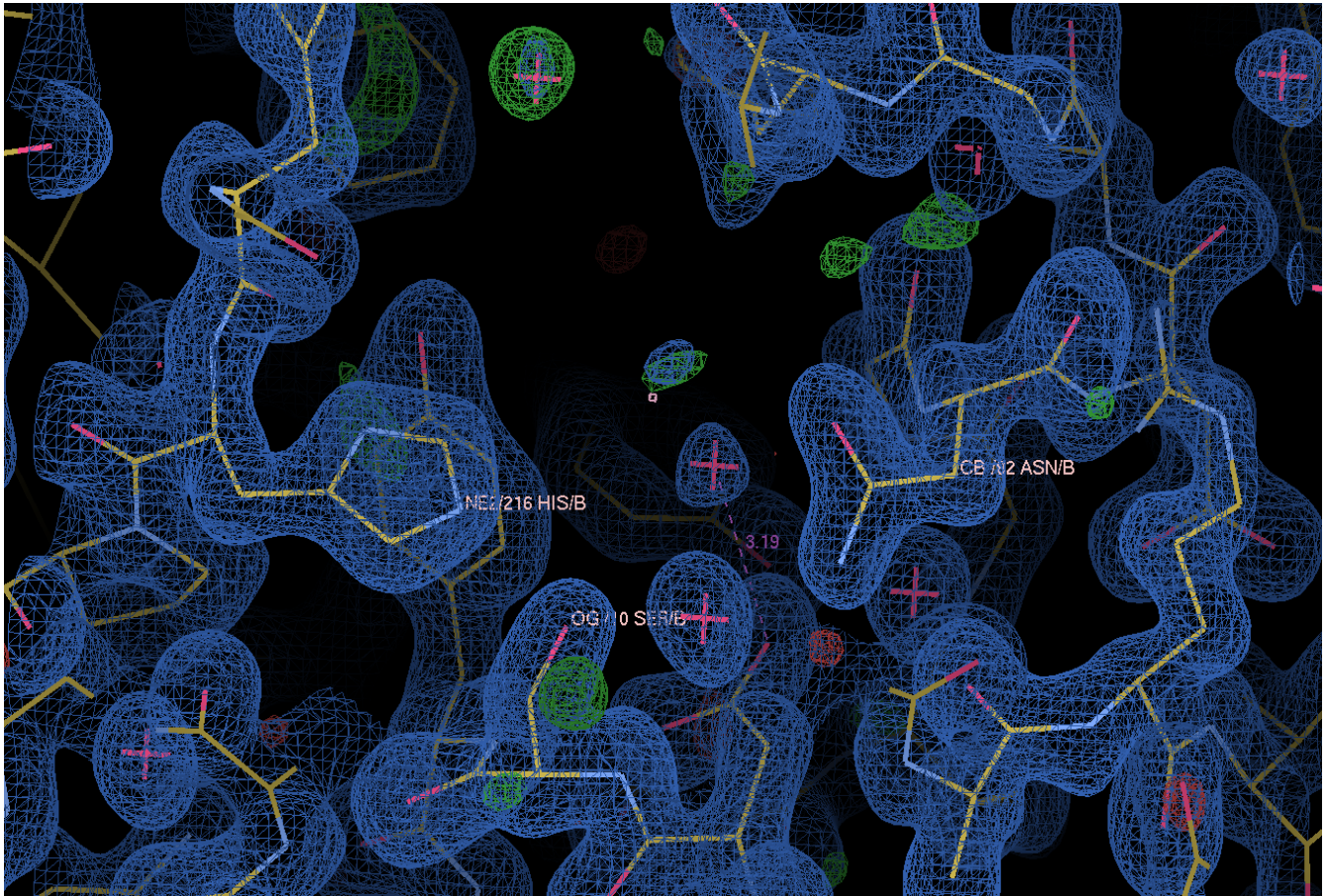
- Diffraction image



Diffraction up to 1.7 Å

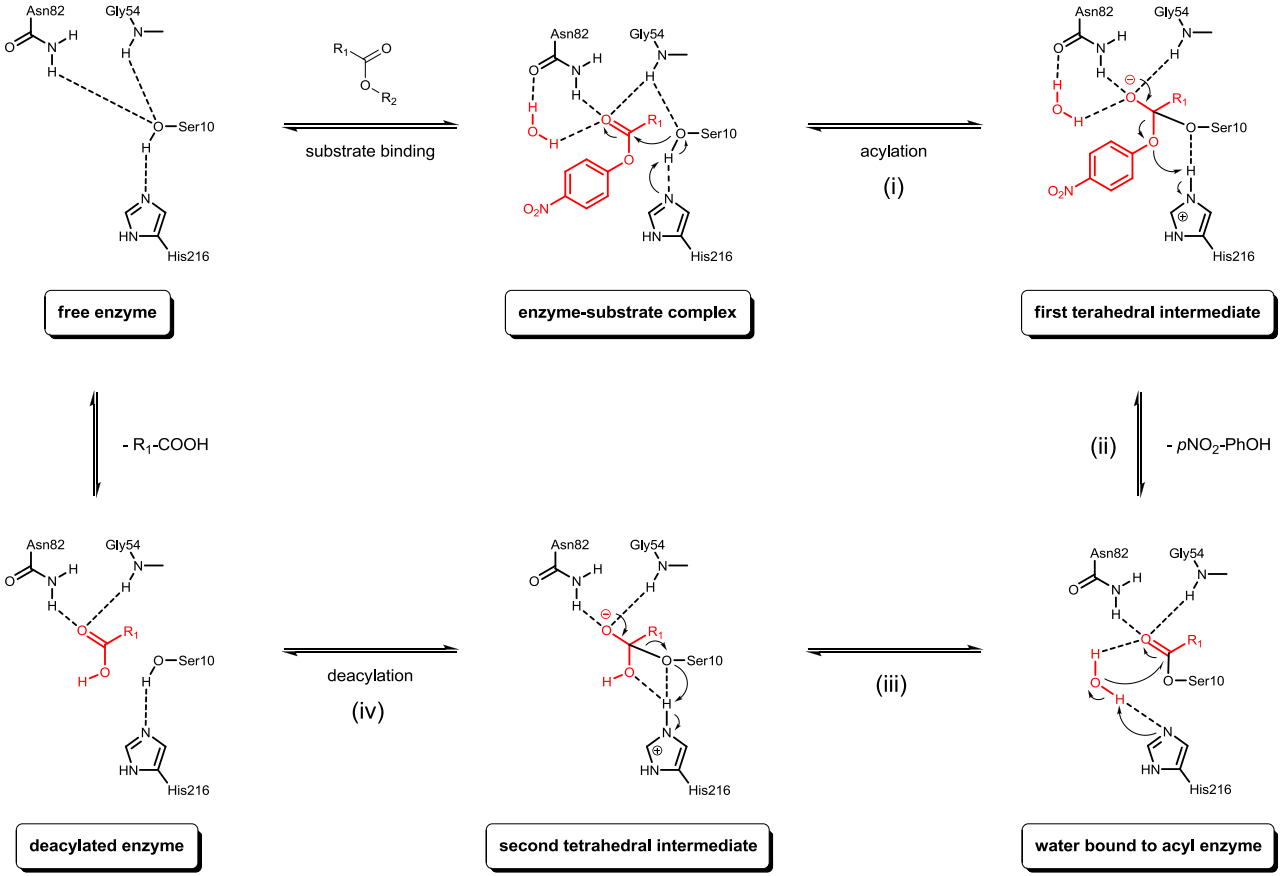
S. rimosus lipase

- Electron density map from synchrotron data – no inhibitor!



S. rimosus lipase

- Mechanism (modelling):



S. rimosus lipase

- Conclusions:
  - High-resolution structure enabled to define network of H-bonds within the active site → catalytic dyad
  - No inhibitor → cleaved
  - Shape of the active site explains promiscuity
  - High-resolution structure enabled confident modelling with substrate (*p*-nitrophenyl caprylate) → mechanism of hydrolysis reaction

Acknowledgements

- Laboratory for Chemical and Biological Crystallography, especially dr. Marija Luić, dr. Biserka Kojić-Prodić, dr. Zoran Štefanić
- Elettra Synchrotron, Trieste
- Laboratory for Protein Biochemistry and Molecular Modelling

- Funding: Ministry of Science and Technology
  projects 098-1191344-2943 and 0098036
  Croatian Science Foundation project 7423