Structural biology as a versatile tool to understand bacterial proteins: Enzymes and Immune System Regulation

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Research Approach

- In our laboratory we use **molecular biology**, **biochemistry**, **structural bioinformatics** and **structural biology** on two main research fields. We are interested in solving protein structures using **crystallography** and thus access to **synchrotron radiation** facilities is crucial for our ongoing research.

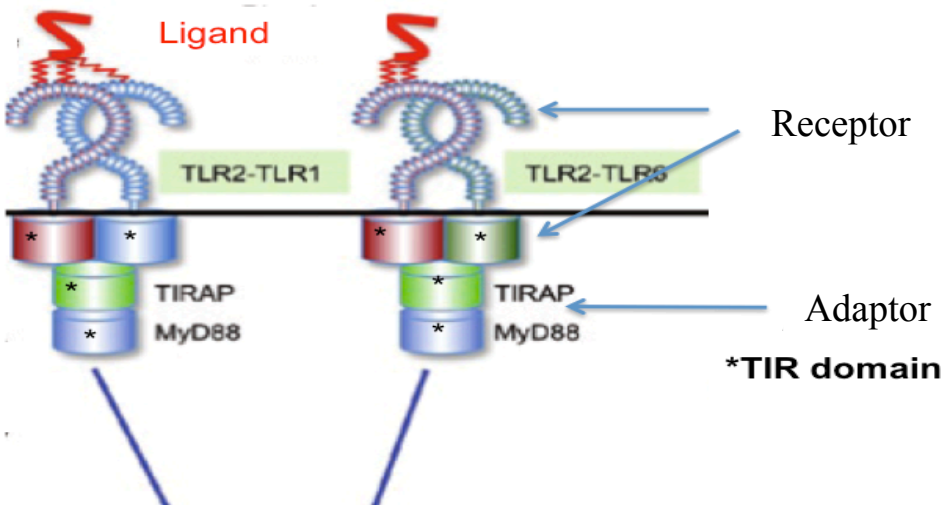
Immune System Regulation

- Innate immune system recognizes pathogens
- Pathogens manages to hide from / supress/ manipulate immune system.

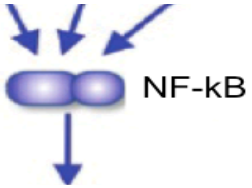
Immune system: TLR Signaling

- First line of defense
- Toll like receptors (TLR) recognize pathogen molecules → immune response.
- The molecular mechanism → interaction between the conserved **structural units**, '**TIR domain**' found in both receptor and adaptor proteins.

TLR2 signal transduction pathway



TIR domain protein interactions is key for the immune response

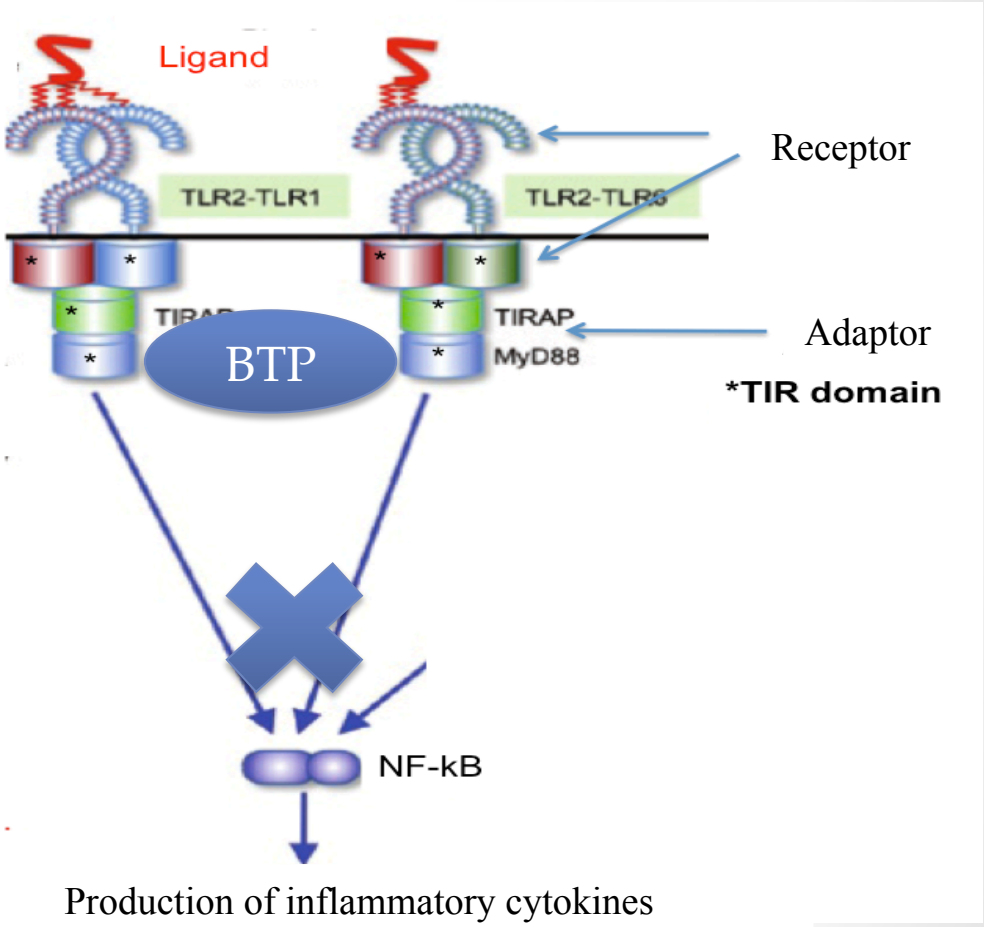


Production of inflammatory cytokines

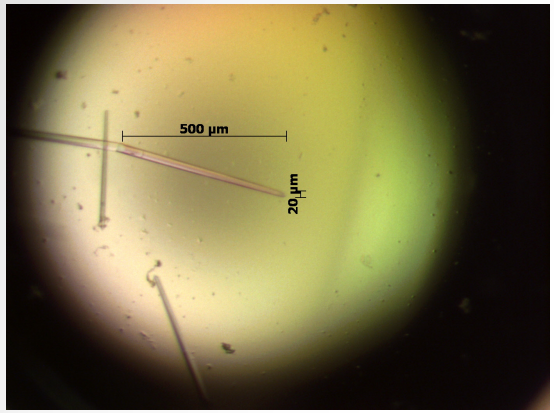
Kawai, T., & Akira, S.,2010, Nature Immunology, 11(5), 373–84)

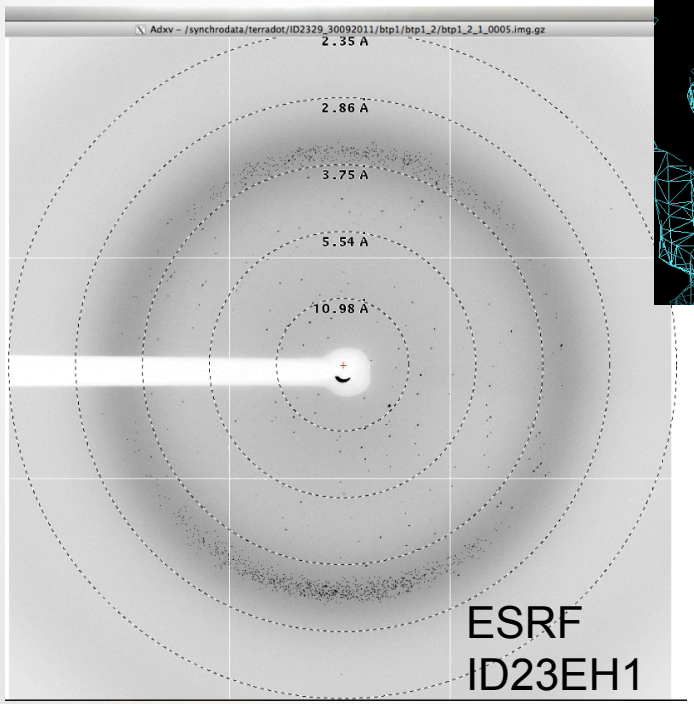
Bacterial Control on Immune System

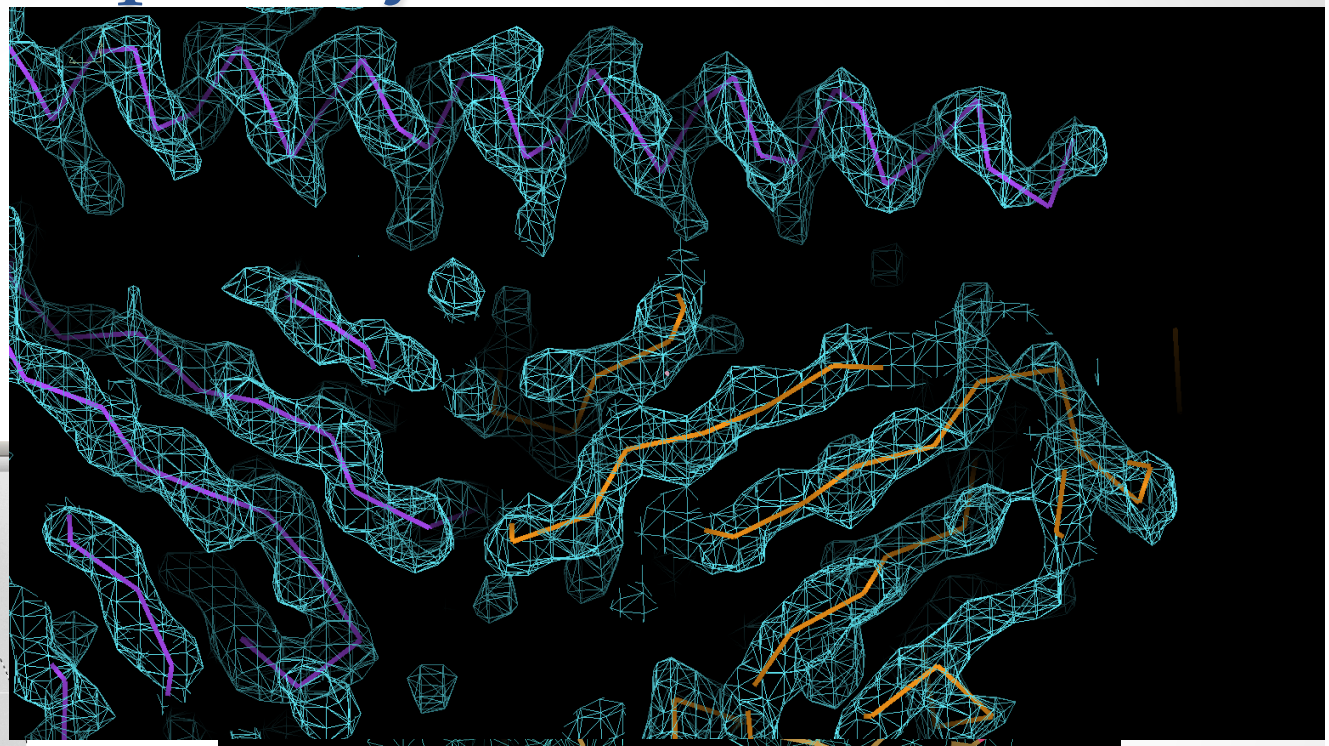
- Family of bacterial TIR domain proteins discovered
- sequence homology to TIR domain
- *Brucella* (BtpA and BtpB), uropathogenic E. coli (TcpC), *Salmonella* (TlpA) and *Yersinia* (YpTIR)
- **can slow down / stop TLR signaling** !

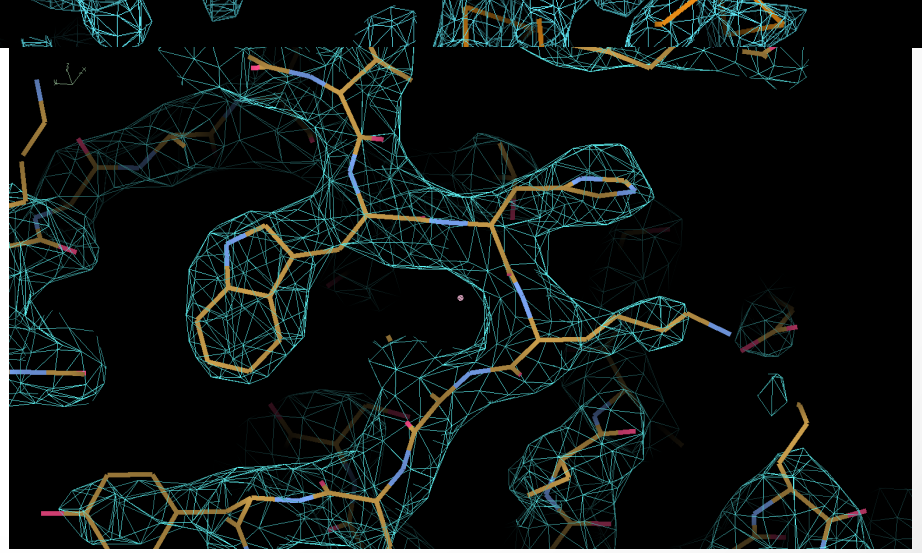


Brucella BtpA Crystal Structure





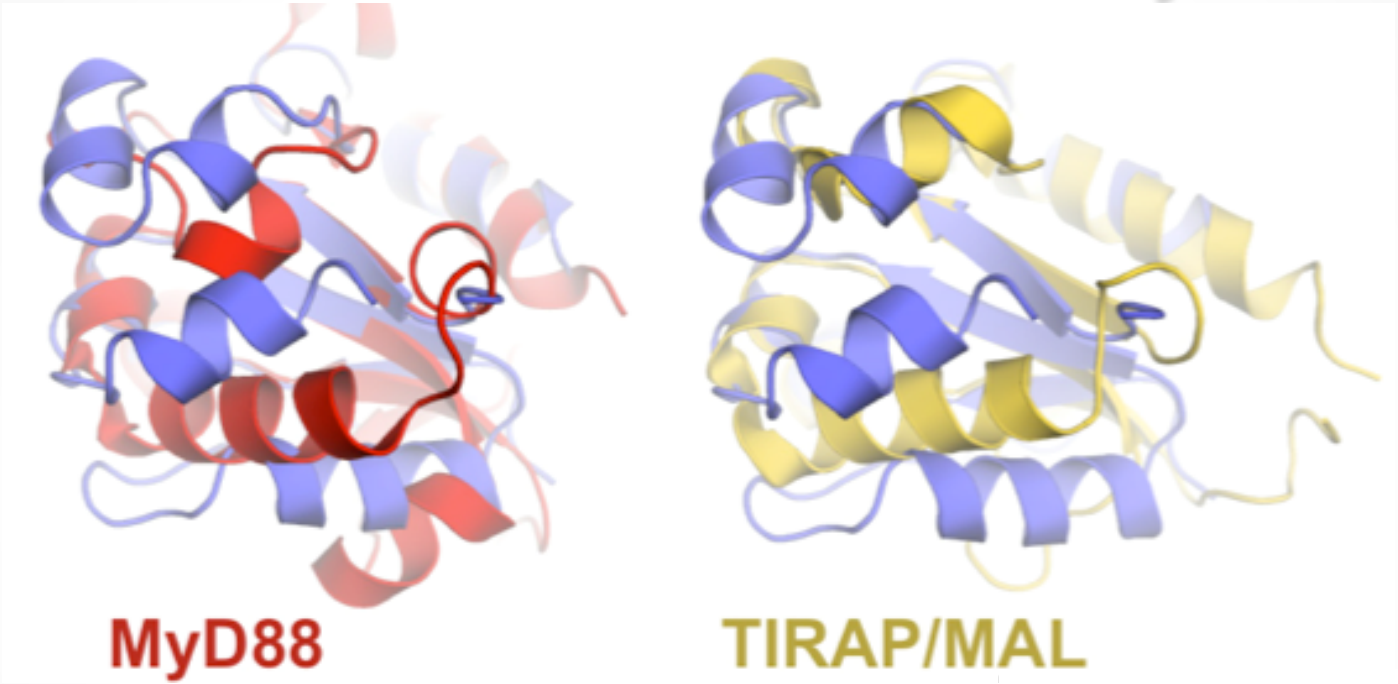




P22121
a=68.14, b=73.93 c= 137.64
4 molecules / asu
Completenes: 98.1%

Kaplan-Türköz, B., et al., FEBS Lett, 587 (21) 3412-16, pdb: 4LZP

Bacterial TIR domain proteins: Structural mimicry



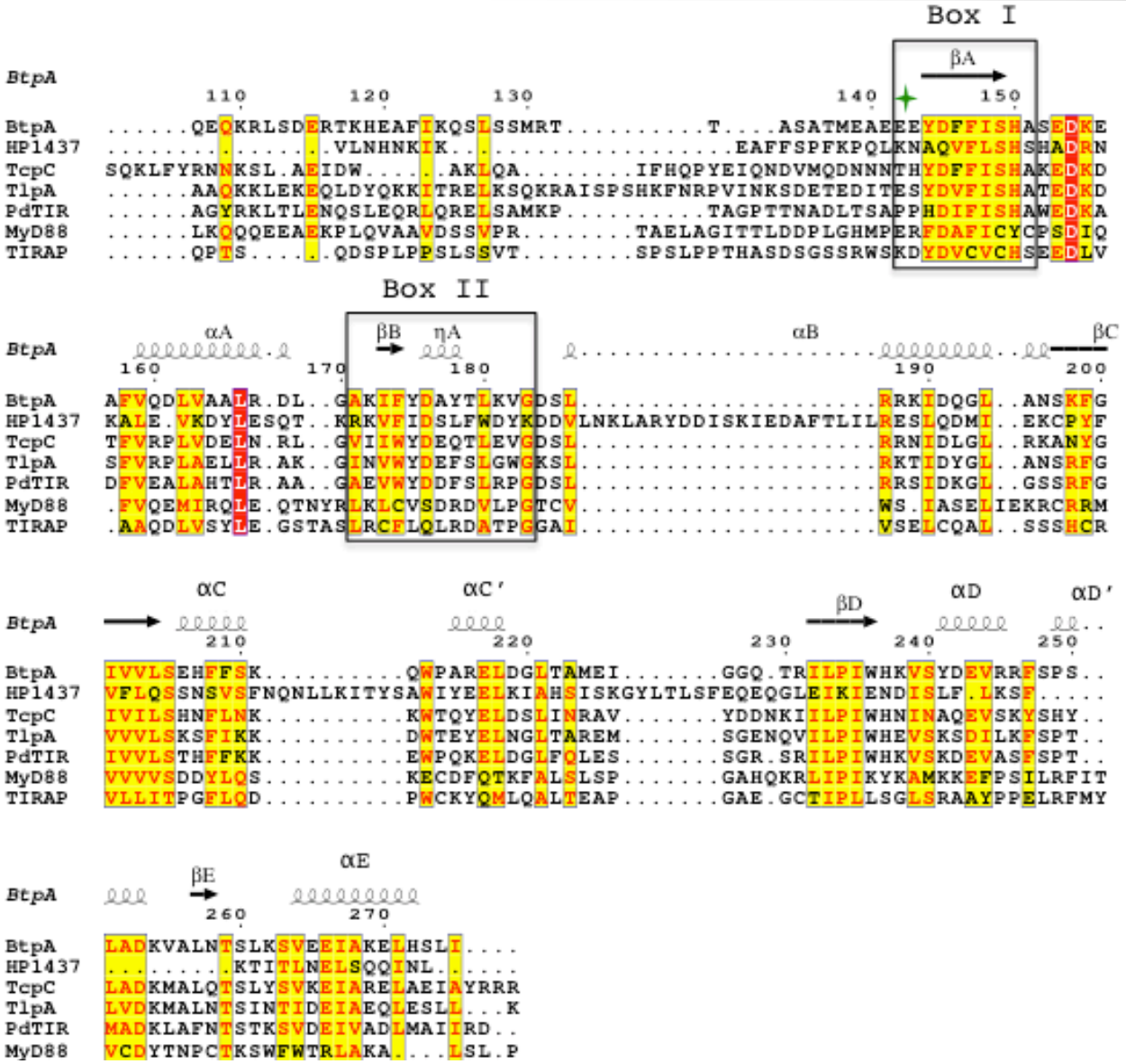
Kaplan-Türköz, B., et al., FEBS Lett, 587 (21) 3412-16

Structure of Brucella TIR domain protein (BtpA) is similar to human TIR domain adaptor proteins MyD88 and TIRAP.

This structural mimicry enables BtpA to replace human proteins and interfere with TIR domain interactions and thus TLR signaling.

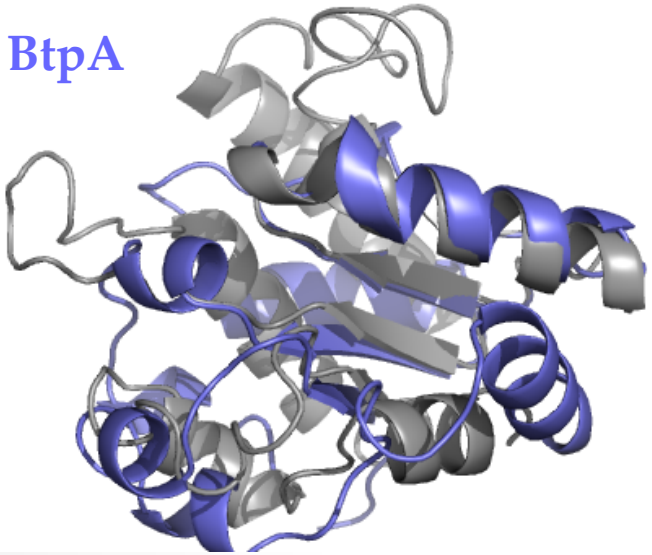
Identification of Bacterial TIR domain Proteins

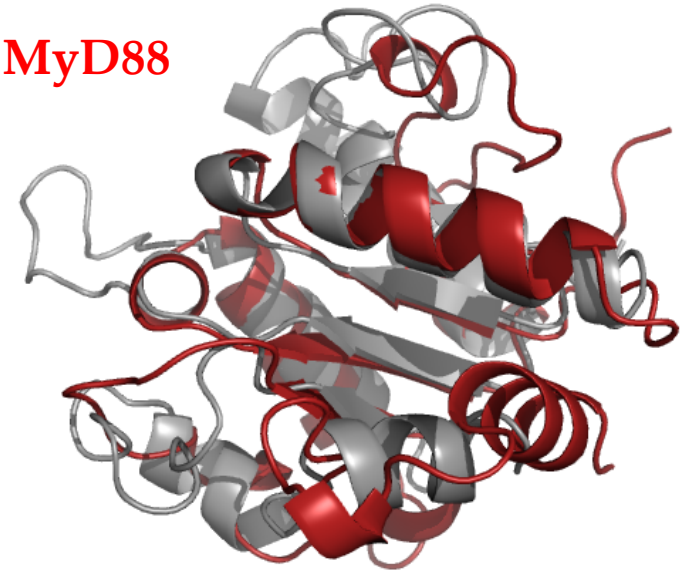
Helicobacter pylori protein HP1437: putative **HpTIR**



Multiple sequence alignment of HP1437 with bacterial (BtpA, TcpC, TlpA, PdTIR) and human adaptor (MyD88, TIRAP) TIR domain proteins. The alignment was done using PSI-Coffee (11) and was formatted using ESPript (12) with secondary structure information from BtpA (pdb: 4lzpB). The predicted start of TIR domain of HP1437 is marked with a star.

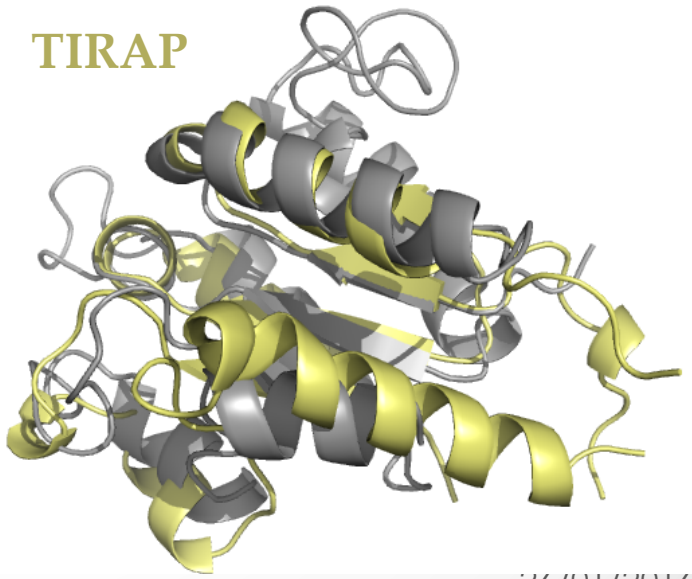
Homology model of HpTIR





Model of *H.pylori* TIR domain protein (HpTIR) is similar to Brucella and human TIR domain adaptor proteins MyD88 and TIRAP.

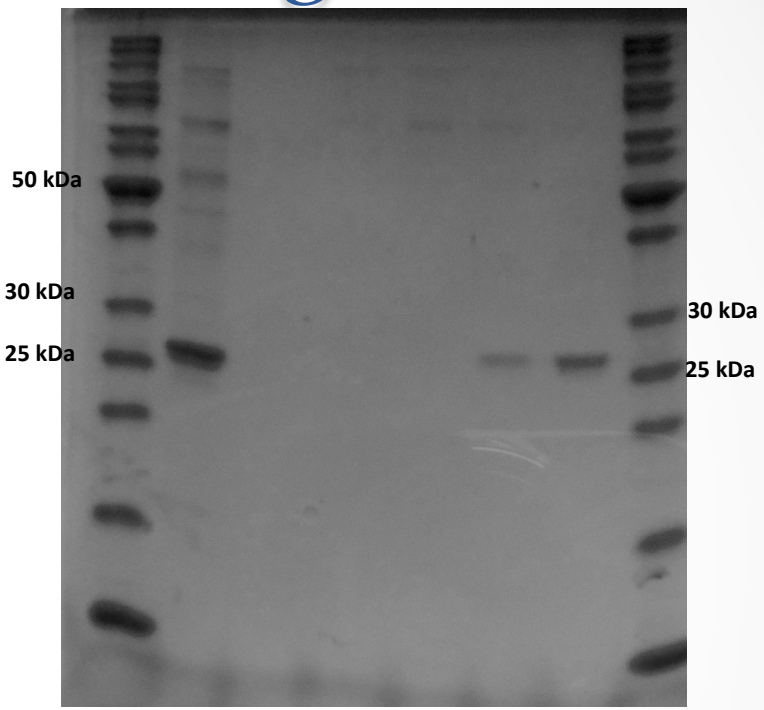
Experimental structure is **necessary to prove** the structural mimicry



B. Kaplan-Türköz (2015)', Acta Crys A, Volume 71, s272

HpTIR Research Progress

- ✓ Purification
- ✓ Oligomeric form: Dimer
- ➢ Crystallization screens
- ➢ Data collection from crystals
- ➢ Structure by Molecular replacement



Acknowledgements

- Funding: TÜBİTAK Returning Scientist Grant and Fellowship, 2014-2015

DESY/ XFEL possibilities ?

Watching proteins change as they interact ?

Bacterial –human TIR domain interactions; conformational changes and interaction surfaces

- Time-resolved X-ray crystallography & SAXS
- Biomolecular Dynamic imaging with XFELS:

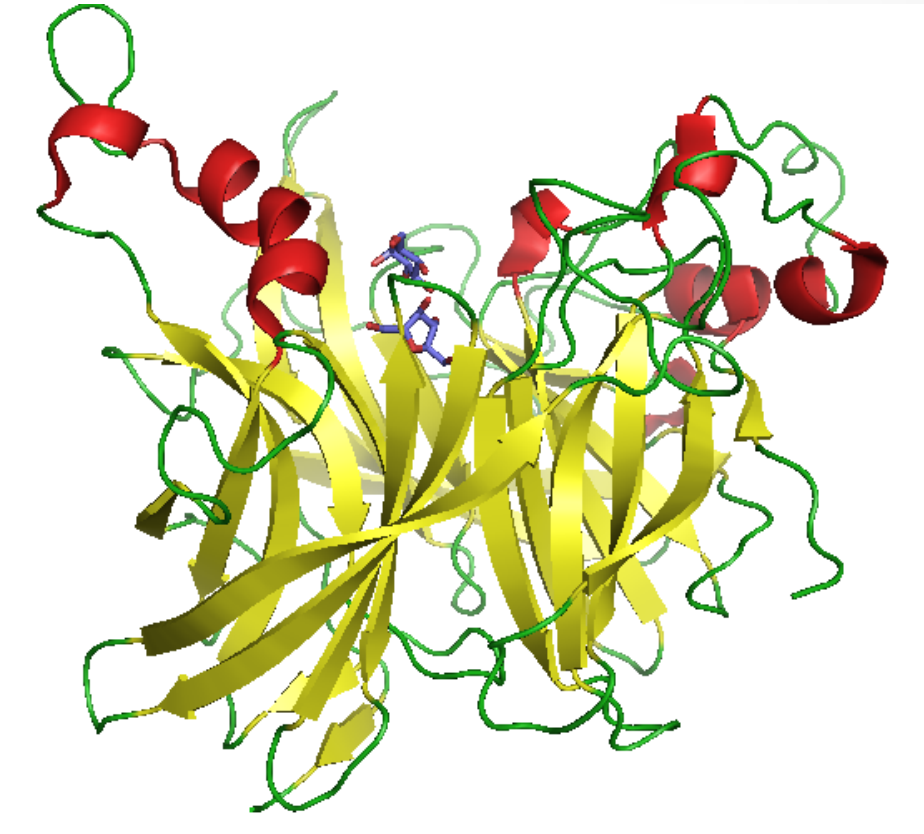
Enzymes: Levansucrase

- Levansucrases are enzymes which catalyze
  - sucrose hydrolysis
  - fructose polymer formation
- Fructose polymers:
  - Levan (Long functional biopolymer)
  - Fructooligosaccharide (FOS) (Short polymer, prebiotic)

Levansucrase Product Selectivity

- *Gluconacetobacter diazotrophicus : FOS*
- *Erwinia amylovora :FOS*
- *Bacillus subtilis*: levan
- *Bacillus megaterium: levan*

Structure of *Erwinia amylovora* levansucrase (PDB: 4D47). Beta-sheets yellow, helices red and glucose & fructose colored in purple.



Wuerges J, Caputi L, Cianci M, Boivin S, Meijers R, Benini S. The crystal structure of Erwinia amylovora levansucrase provides a snapshot of the products of sucrose hydrolysis trapped into the active site. J Struct Biol. 2015 , 191:290

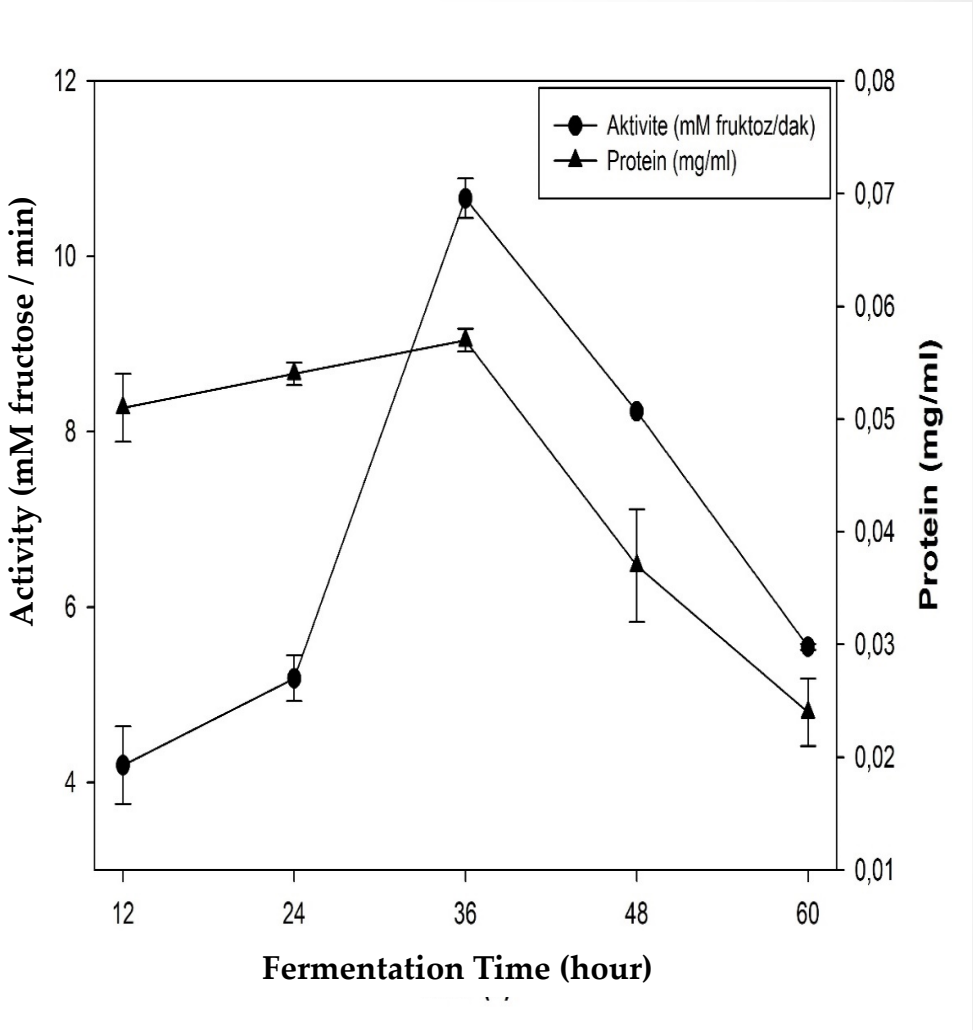
Z. mobilis Levansucrase

- *Zymomonas mobilis* produces extracellular Lsc during sucrose fermentation
- ZmLsc can produce both Levan and FOS polymers with good yields
- Product depends on reaction temperature; low temperature→ Levan

We are interested in solving the atomic structure of the enzyme in order to understand the molecular details of this dual product formation ability.

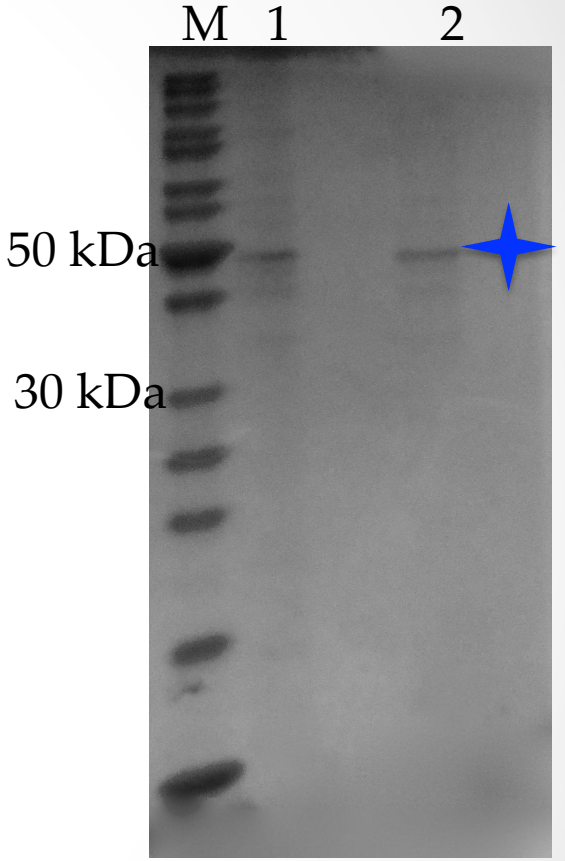
ZmLsc Research Progress

- ✓ Enzyme production
- ✓ Active enzyme



ZmLsc Research Progress

- ✓ Enzyme already partially pure
- ➢ Purification
- ➢ Determination of oligomeric form
- ➢ Crystallization
- ➢ Data collection from crystals
- ➢ Structure by Molecular replacement



DESY/ XFEL possibilities ?

Enzymes in action

- Can we catch & watch each different action of the enzyme;

  binding, hydrolysis and polymerization

- Time-resolved X-ray crystallography
- Biomolecular Dynamic imaging with XFELS

DESY/ XFEL possibilities ?

Serial nanocrystallography

- Lsc is an extracellular enzyme, secreted to the medium
- Can we grow 'in vivo' crystals directly in the fermentation medium bound to its 'natural' substrate/product?

Acknowledgements

Funding: TÜBİTAK Research Project, 2015-2018

Collaborators:

- Prof. Taner Baysal

(Food Processing)

- Prof. Yekta Göksungur

(Food Biotechnology)

- Dicle Akpınar, M.Sc. student
- Özge Taştan, PhD student (Prof. Baysal)
- Özlem Erdal, Filiz Döner MSc. students (Prof .Göksungur)

Thank you for your attention