

Structural studies of DPP III
from *Caldithrix abyssi*

Why *Caldithrix abyssi*?

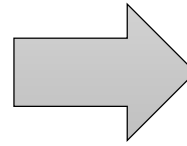
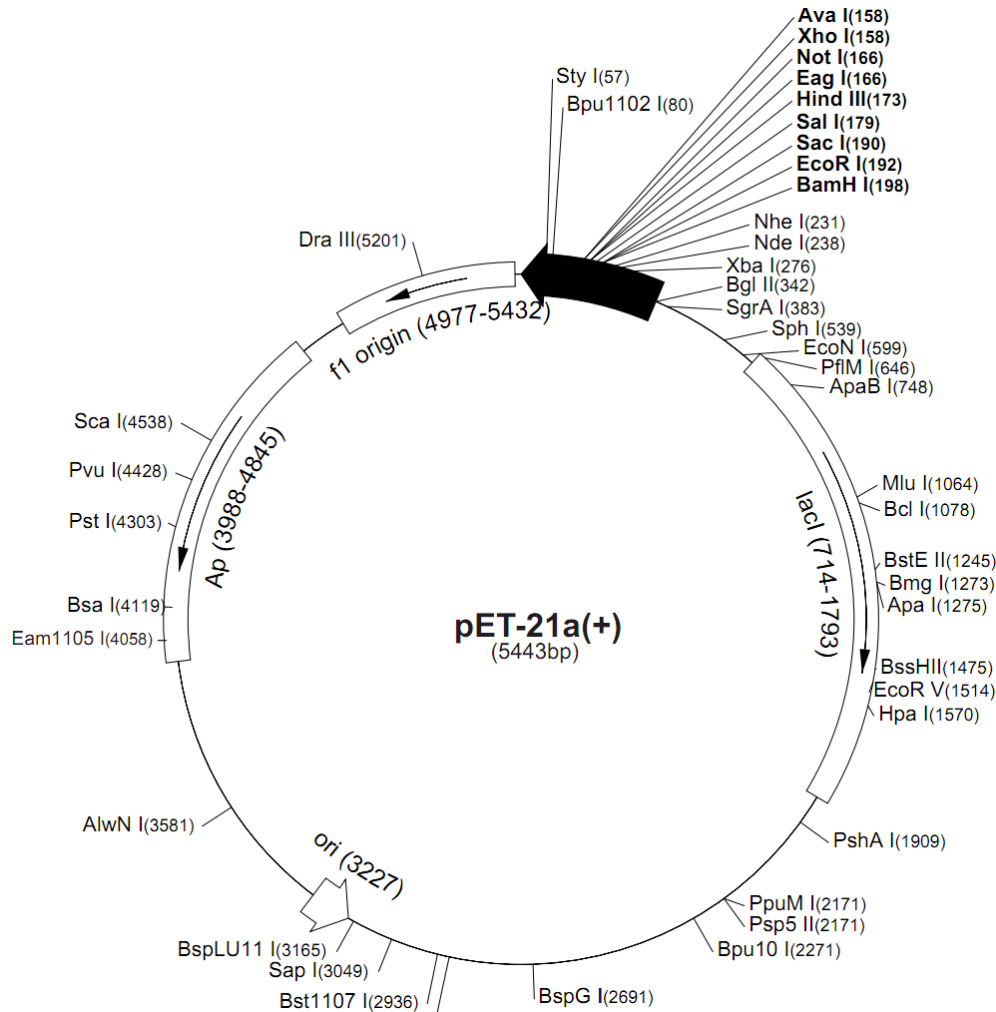
- No structural information on prokaryotic DPP III
- Crystallisation of DPP III from *Bactroides thetaiotaomicron* (BtDPP III) was not going well
- XtalPred – good score for crystallisation
- *Caldithrix abyssi* is an anaerobic organism inhabiting deep-sea hydrothermal vents!
 - Optimal grow temperature 60 °C
- Different active site?

Human	LAVAYATQREK---LT--FLEEDD---KDLYILWKGPSFDVQVGL	HEL-LGH GSGLFVQ	463
Bt	TDAYNKA AHGNGFN EEFVCNDEE-RQRIDQYGDLTG---ELHTDL	HEC-LGH GSGLLLPG	461
Ca	HEAKFDKLLKP---IAEKVLF AEQLPLVTFEGFF-----NHTLM	HEISH GLGPGKIVL-	392
PhyPa	SQAKFDYILLP---IANVCVEASQRGAVDFDSFF-----THTIC	HECCH GIGPHNIVTP	605
	.	.	:: ** * * ::.

Human	DEKGA FNFDQETVINPETGEQIQSWYRSGETWDSKFSTIASSY	EECRAE SVGLYLCLHPQ	523
Bt	--V-----DPDALKAYGSTI	EEARAD LFGLYYVADPK	491
Ca	NGRQT-----EVKKELKETYSI	EECKAD VLGMYNNL--F	425
PhyPa	DGRAS-----TVRLELQEVYSAI	EEAKAD IVGLWALH--F	638
		:: * : ** . : * : . * ::	

- Pentapeptide instead hexapeptide
- Less amino acids between two conserved motives
 - Human – 50 amino acids
 - *Bt* – 20 amino acids
 - *Ca* – 24 amino acids
 - *PhyPa* – 25 amino acids

Preparation of *CaDPP III* with His₆-tag

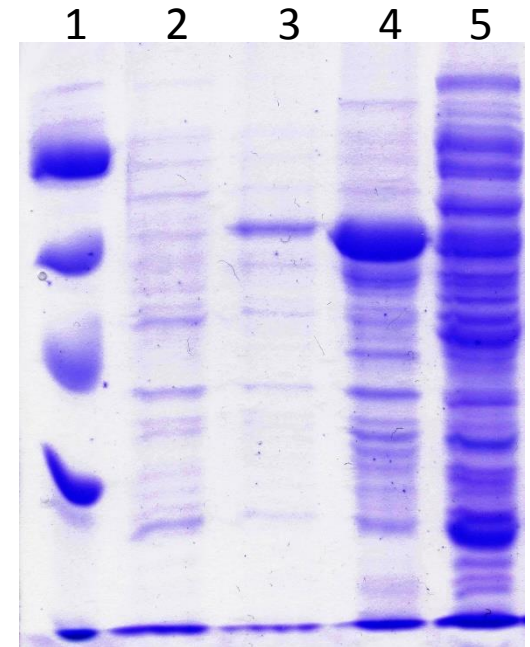


Restriction site:
Nde I
Xho I

Plasmid:
pET-21a

Overexpression

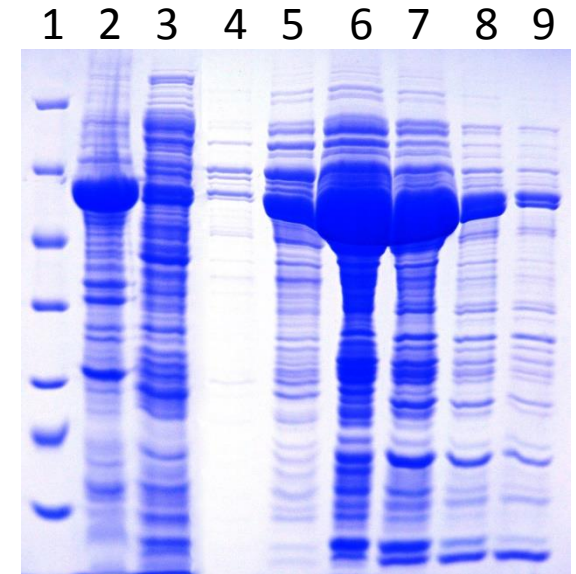
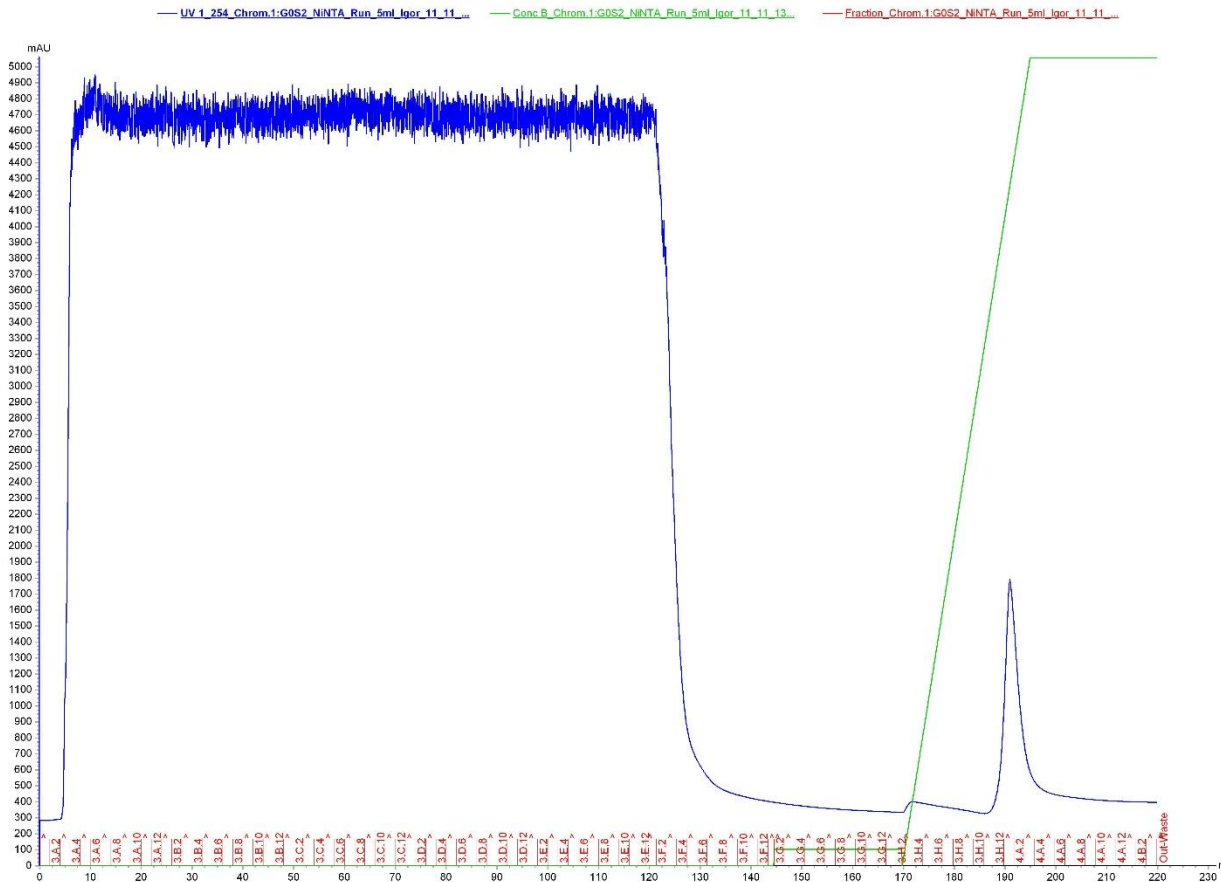
- *E.coli* strain BL21(DE3)RIL⁺.
 - IPTG induced overexpression
 - 4 hours, 37 °C, 250 rpm
- different IPTG concentrations
 - 0.5 mM, 0.25 mM, 0.125mM
- longer overexpression
- lower temperature
- different cells
 - ArcticExpress



1. LMW standards
2. Before inducing with IPTG
3. After inducing with IPTG
4. Pellet
5. Lysate

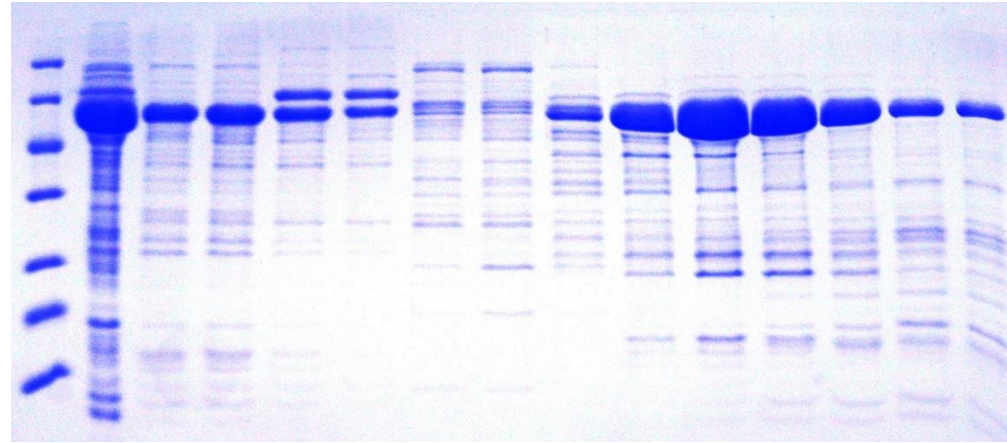
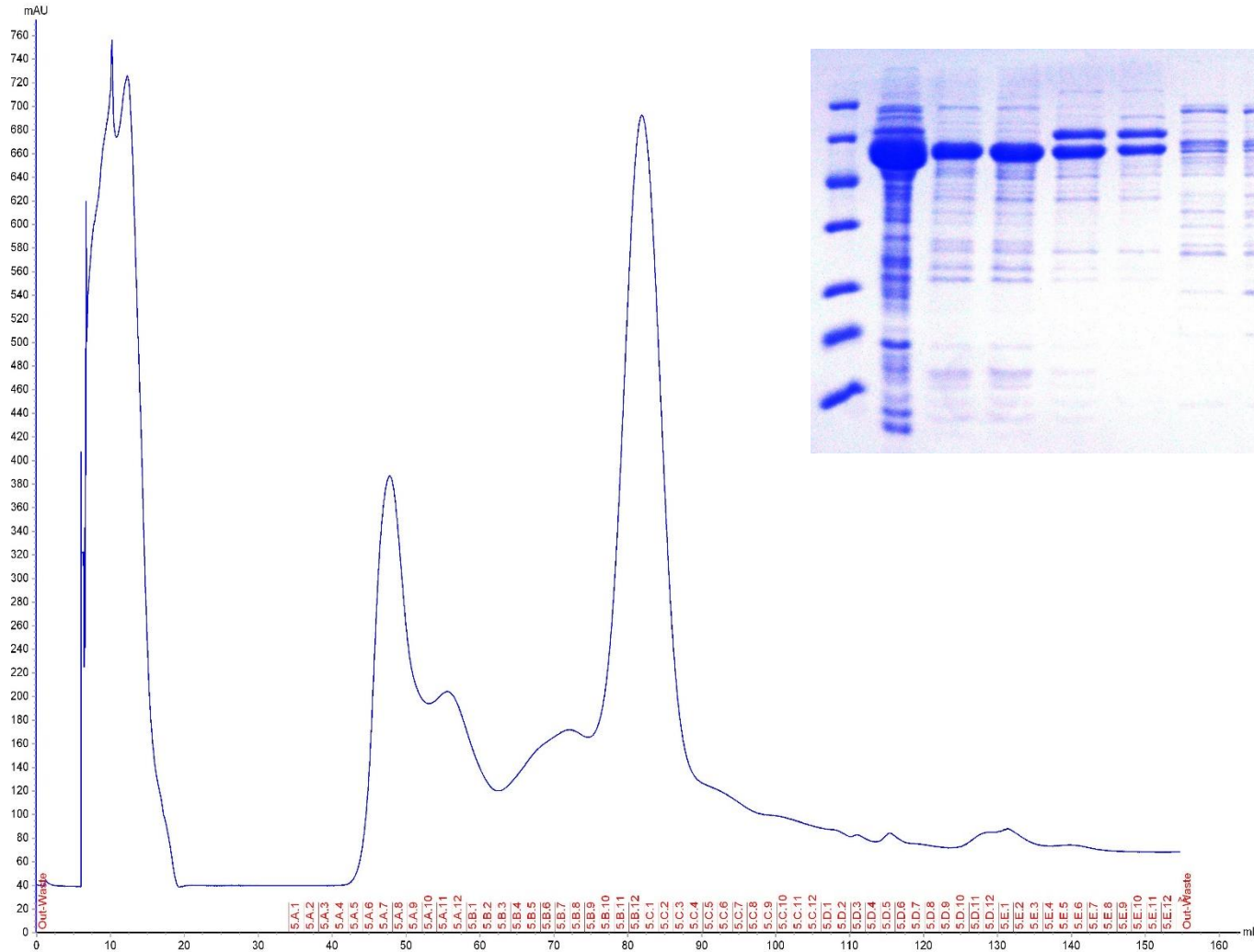
Purification (Graz)

- Affinity chromatography (Ni-NTA)
 - 20 – 300 mM imidazole

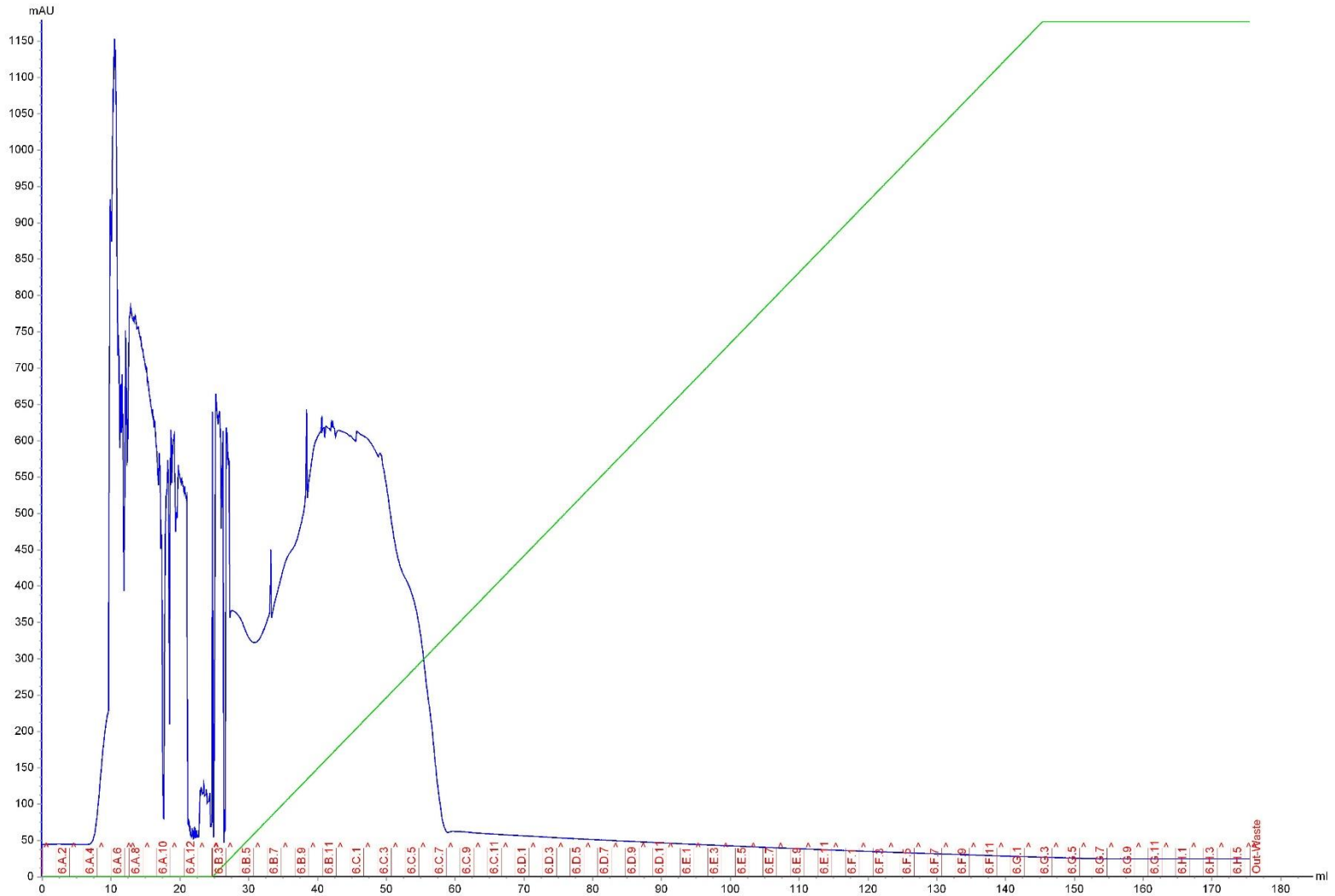


1. LMW standards
2. Pellet
3. Lysate
- 4 - 9. Elution fractions

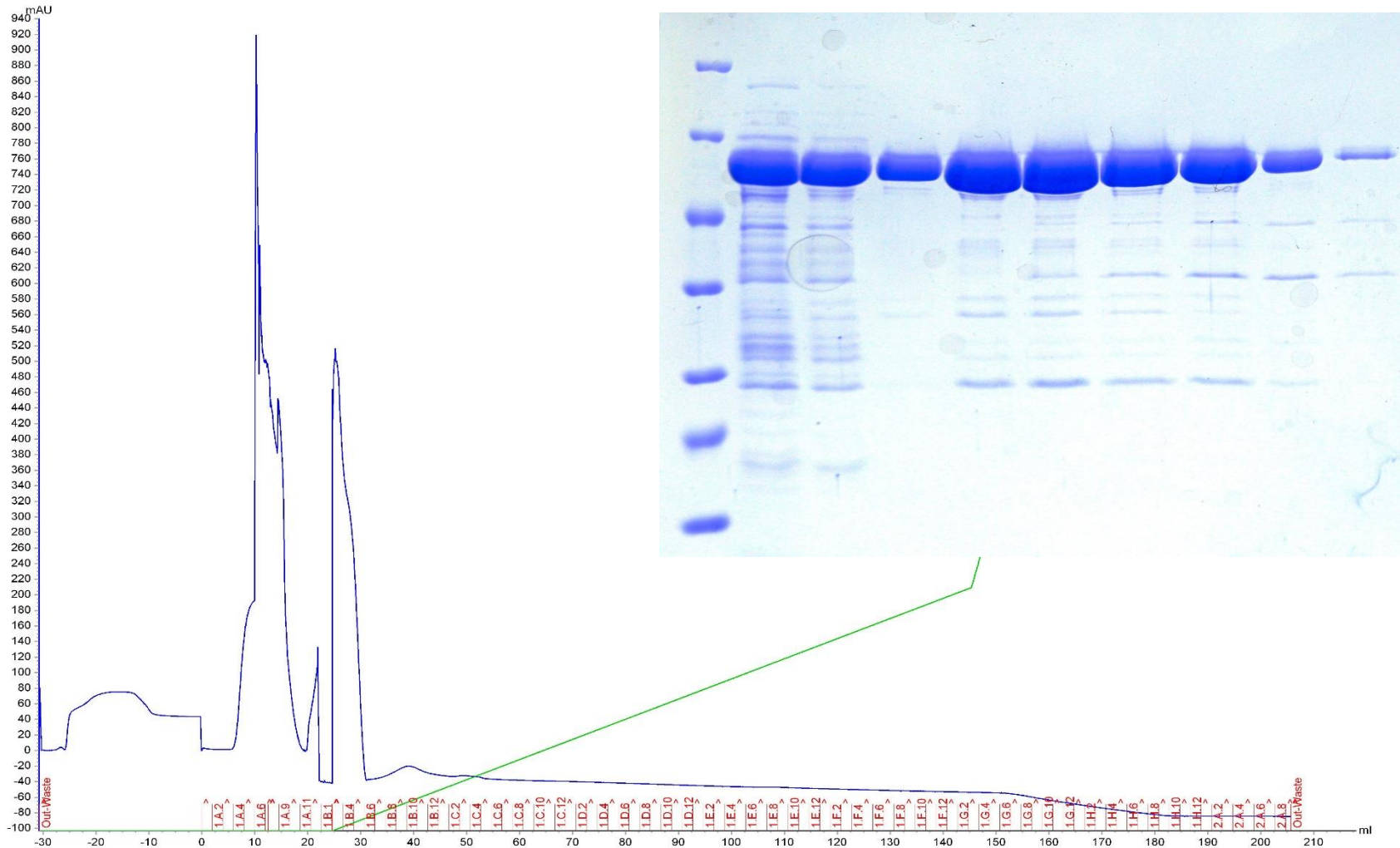
Size-exclusion chromatography



Ion exchange chromatography (Resource Q)



Ion exchange chromatography (Resource Q)



Crystallization (Graz)

- „Pure protein” ~ 120 μ L 14.8 mg/ml
- Screens
 - Index
 - Morphius



Three hits!

Indeks D7 (0.1 M Bis-Tris pH 6.5; 25% PEG 3,335)

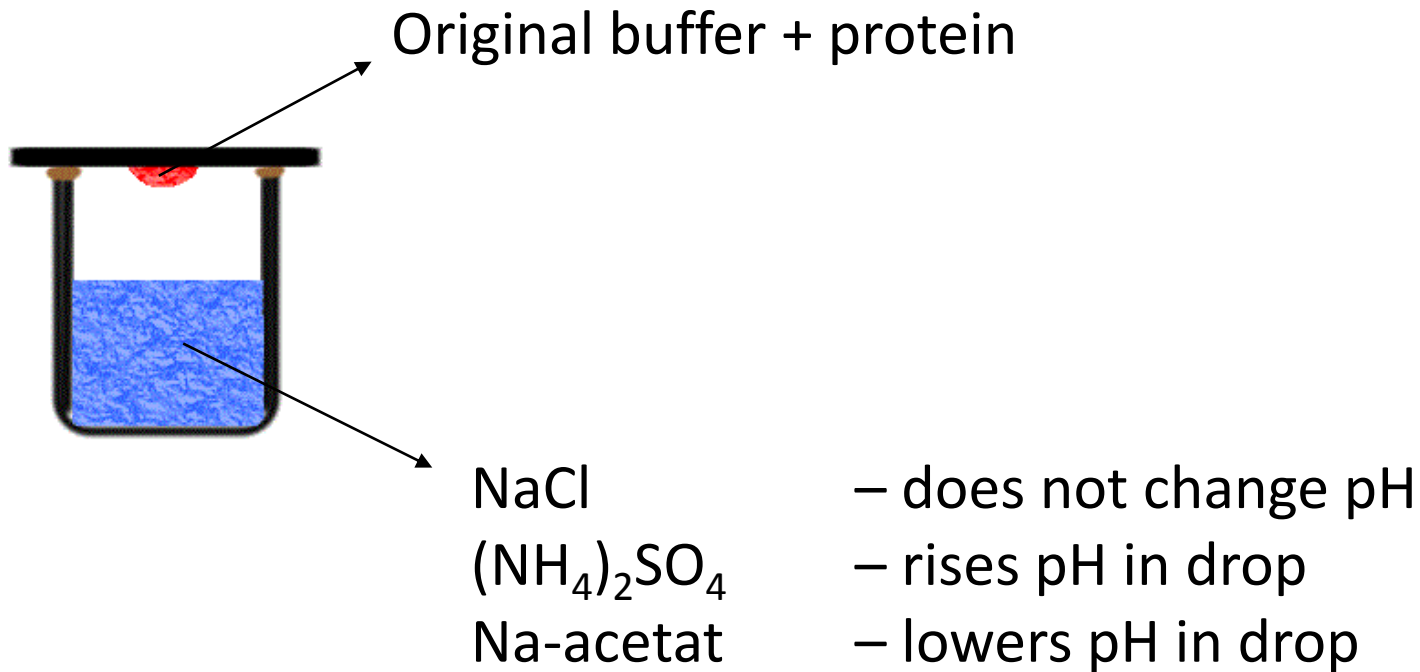
Indeks D9 (0.1 M Tris pH 8.5; 25% PEG 3,335)

Indeks D10 (0.1 M Bis-Tris pH 6.5; 20% PEG 5,000)

Optimization and Scale up

- 24 Well Linbro Plate
 - Hanging drop method
 - Sitting drop method
 - Home-made buffer in reservoir and drop
 - Indeks D7, D9 and D10
 - Home-made buffer in reservoir and original buffer in drop
 - Indeks D7, D9 and D10

Optimization and Scale up



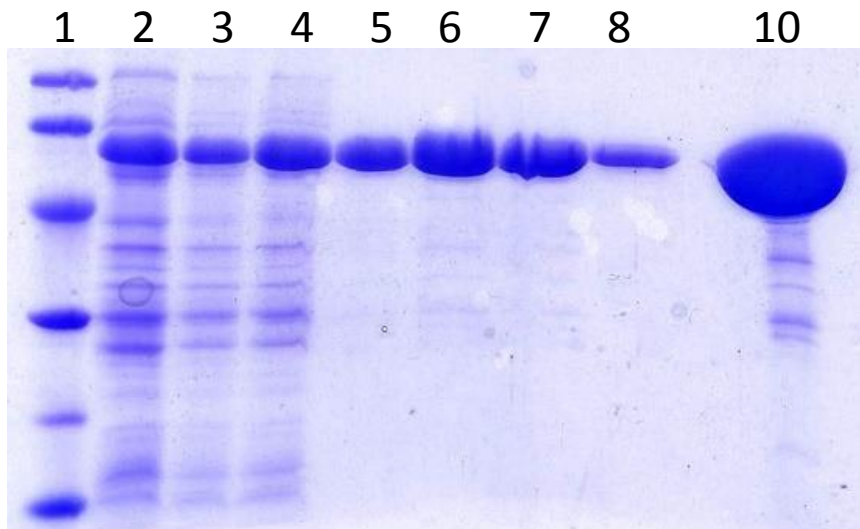
Crystals obtained with (NH₄)₂SO₄ in reservoir!!!

First data collection

- Elettra Sincrotrone Trieste
 - Resolution up to $\sim 2.3 \text{ \AA}$
 - Orthorhombic $P 2_1 2_1 2_1$
 - $a = 51.472$ $b = 90.549$ $c = 132.973$
 - $\lambda = 1.27 \text{ \AA}$
- Single-wavelength anomalous dispersion (SAD) of zinc atom was too weak
- Molecular replacement failed!
 - Sequence identity with:
 - Human 16%
 - Yeast 14%
 - BtDPP III* 19%

Selenomethionine labeled protein (Graz)

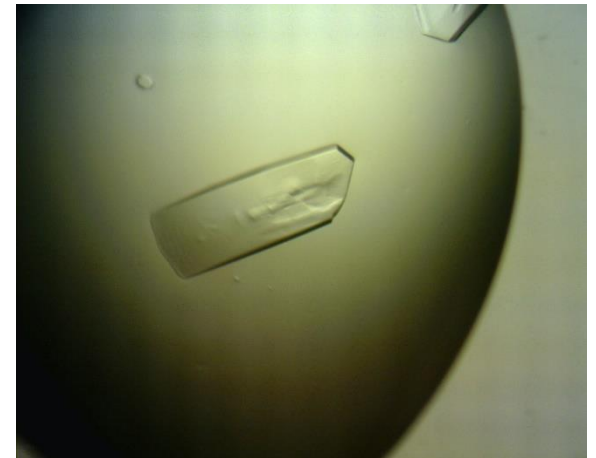
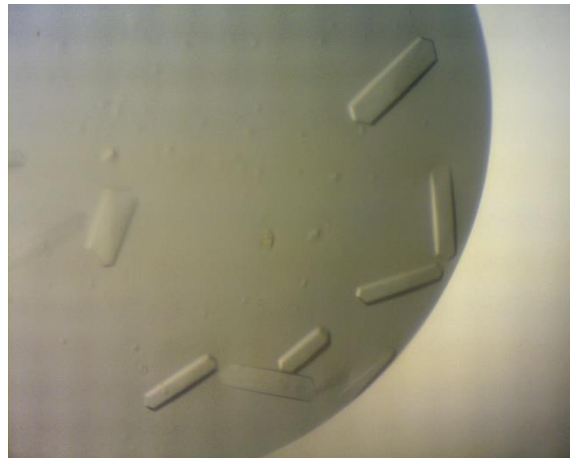
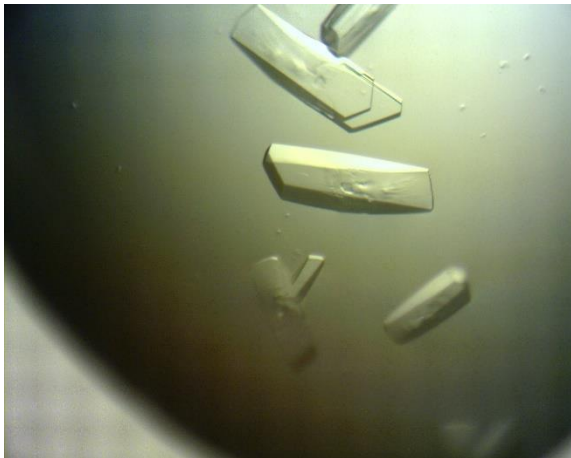
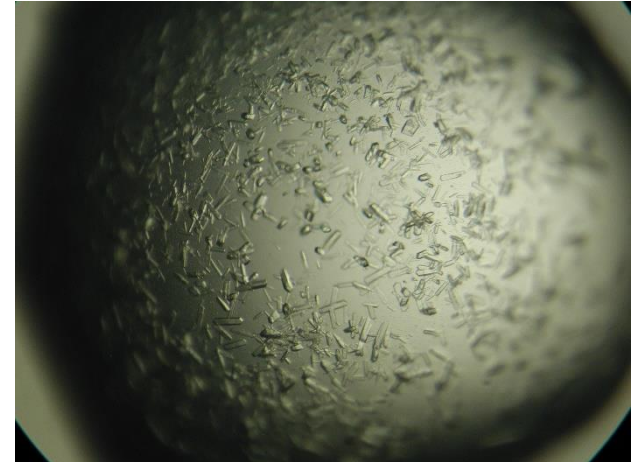
- 16 methionine (558 amino acids)
- Four steps of purification
 - Affinity chromatography
 - MonoQ and MonoS (didn't work well)
 - Size exclusion



1. LMW standards
2. After Ni-NTA
3. After monoQ
4. After monoS
- 5-8. After size-exclusion
10. After concentrating the protein

Crystallization

- 24 Well Linbro Plate
 - Hanging drop method
 - Sitting drop method
 - Home-made buffer in reservoir and drop
 - Indeks D10 (0.1 M Bis-Tris pH 6.5; 20% PEG 5,000)



Data collection

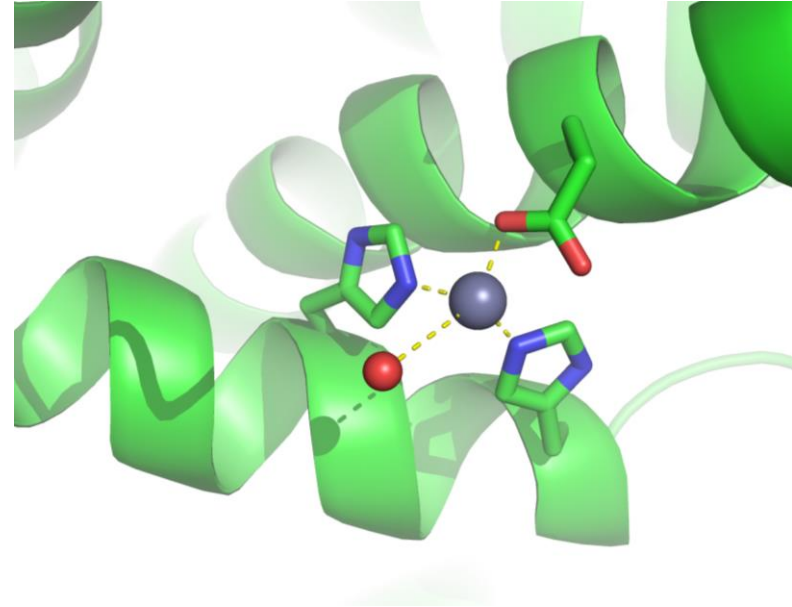
- Elettra Sincrotrone Trieste
 - Resolution up to $\sim 2.9 \text{ \AA}$ (anomalous data up to 3.6 \AA)
 - Monoclinic $P 2_1$
 - $a = 55.48$ $b = 87.76$ $c = 132.4$ $\beta = 92.3$
 - $\lambda = 0.97930/0.97960 \text{ \AA}$

The crystal structure was solved using multi-wavelength anomalous dispersion of selenium atoms

Crystal structure



Active site



- His 379 and His 383; HEISH motif
- Glu 412; EECKAD motif
- Zinc tetrahedral coordination

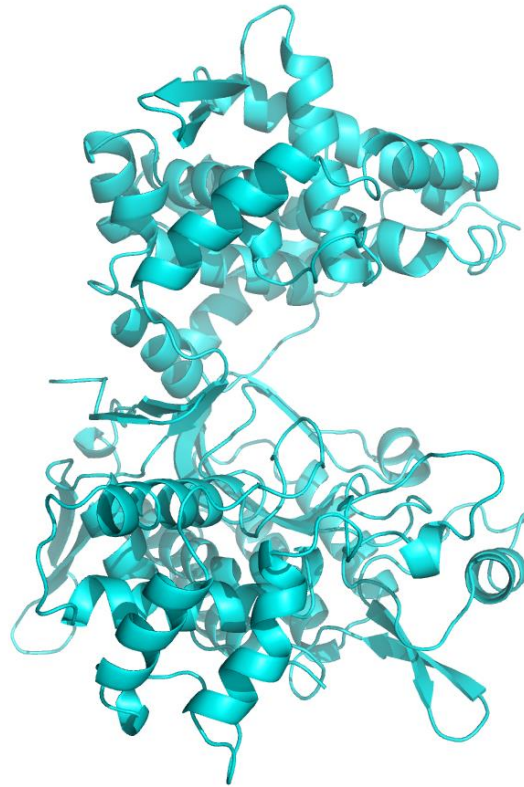
Crystal structure

Human



737
amino acid

*Bacteroides
thetaiotaomicron*



675
amino acid

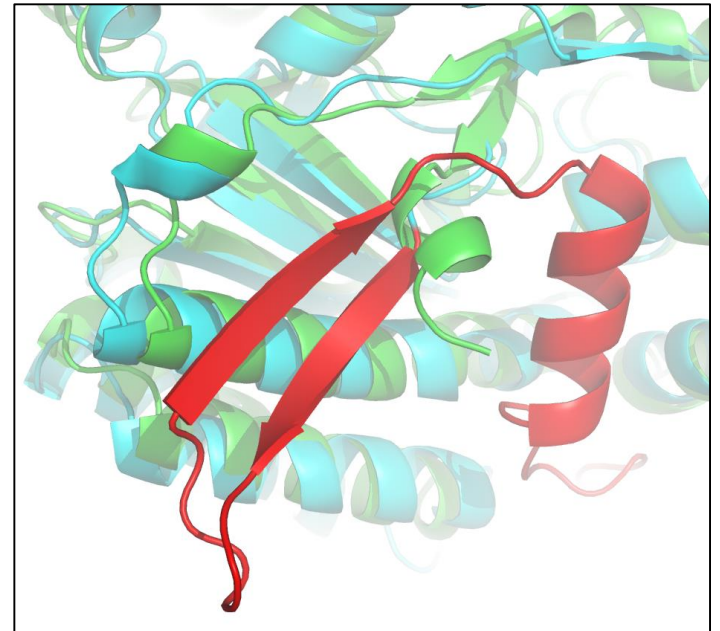
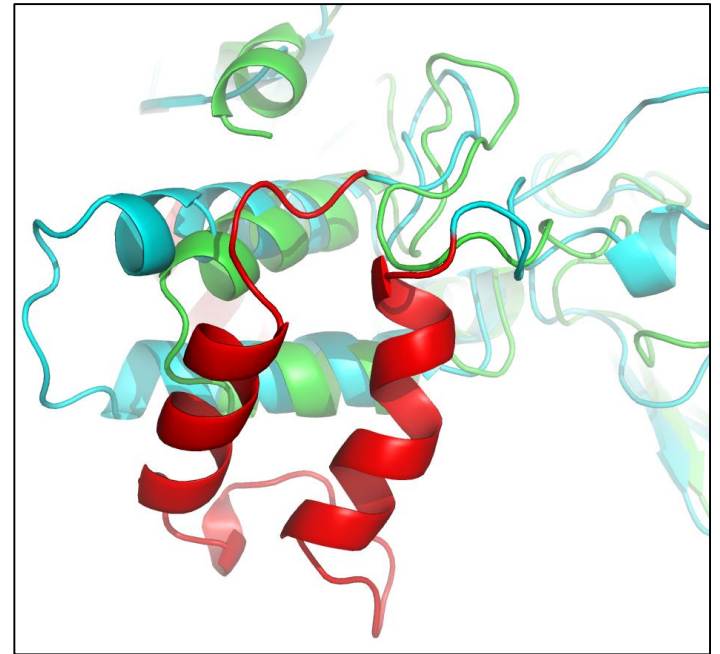
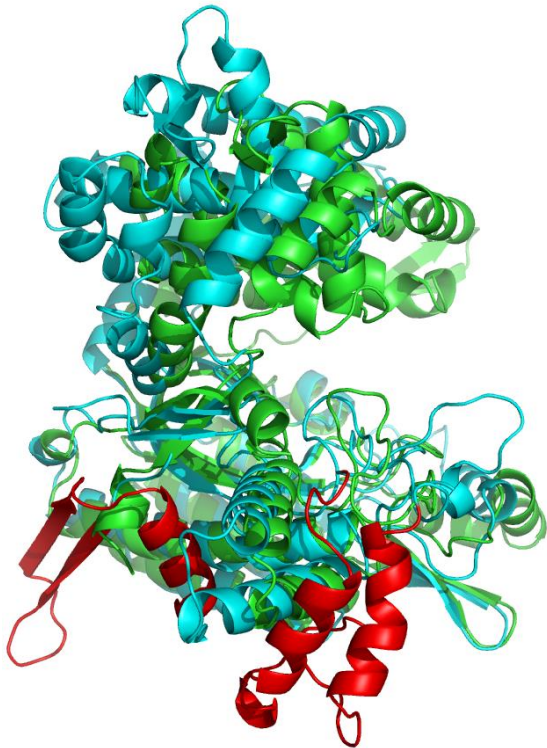
Caldithrix abyssi



558
amino acid

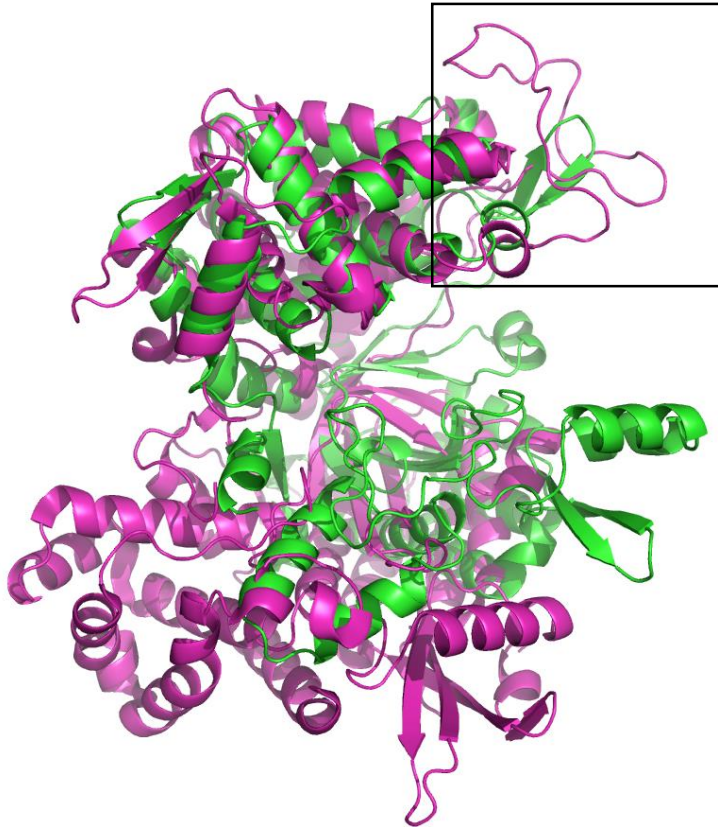
Alignments

- *BtDPP III* and *CaDPP III*
Lower structural domain
- Between 126-166 and last 40 amino acid are missing in *CaDPP III*



Alignments

- Human DPP III and *Ca*DPP III
Upper structural domain
- Loop between two zinc binding motives



Thank you for your attention!