The Structure and Function Analysis of *Pseudomonas aeruginosa* ClpV1
铜绿假单胞菌ClpV1的结构和功能分析

深圳研究生院

小组成员：蔡晓丹 邓绮雯 王莉娜 余敏

报告人:邓绮雯

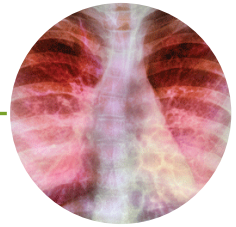
2014-06-17

Outline

1. Background
2. Sequence analysis
3. Secondary structure prediction
4. Domains analysis
5. 3D structure prediction
6. Discussion

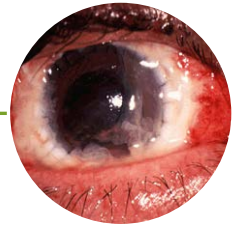
Pseudomonas aeruginosa

- a Gram-negative bacterium
- a remarkable capacity to cause disease in susceptible hosts
- locomotion, attachment, transport and utilization of nutrients, antibiotic efflux, and systems involved in sensing and responding to environmental changes



Cystic Fibrosis

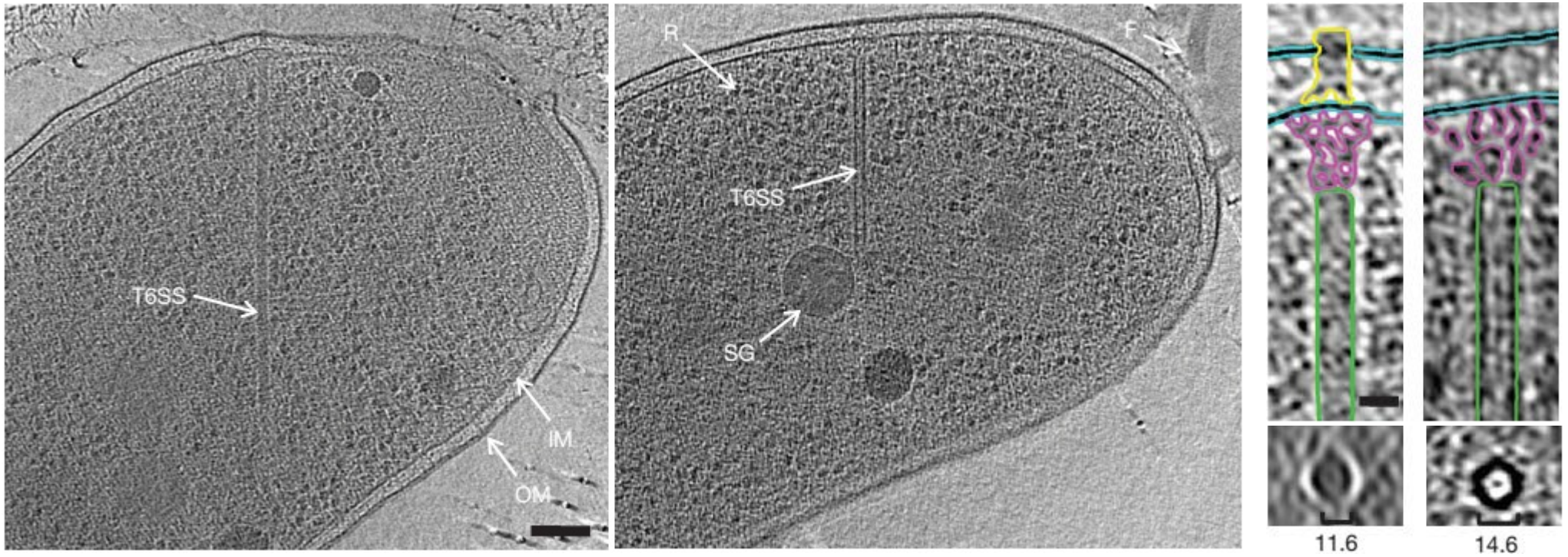
Green Nail



Infection

T6SS(Type VI Secretion System)

- First described in Pseudomonas aeruginosa and Vibrio cholerae in 2006
- Is present in about 25% of all sequenced Gram-negative bacteria



Electron cryotomographic imaging of T6SS structures inside intact cells, an extended and a contracted structure

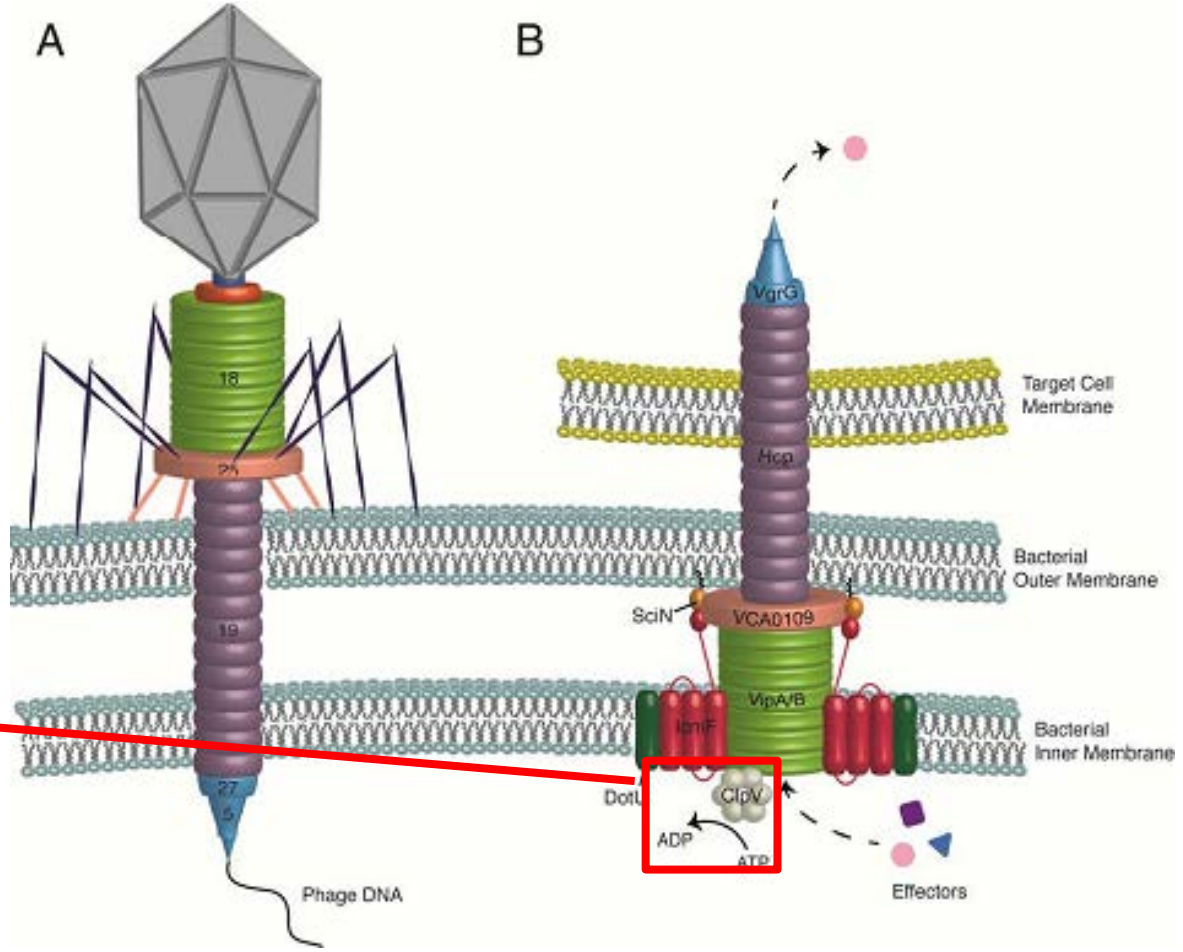
- M. Basler. 2012. Type VI secretion requires a dynamic contractile phage tail-like structure. Nature.Vol. 483,182-186

T6SS(Type VI Secretion System)

A. Bacteriophage T4 molecular architecture
B. The T6SS model

gp19 → Hcp1(tube)
gp27 & gp5 → VgrG(tip)
gp25 → VCA0109(baseplate)
gp18 → VipA/VipB(sheath)

IcmF, DotU, and SciN: structural components
ClpV : provide energy

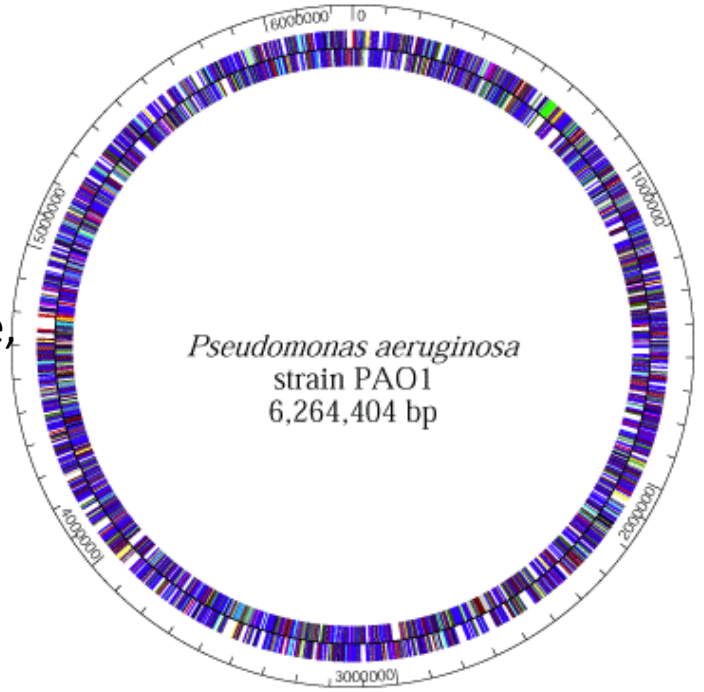


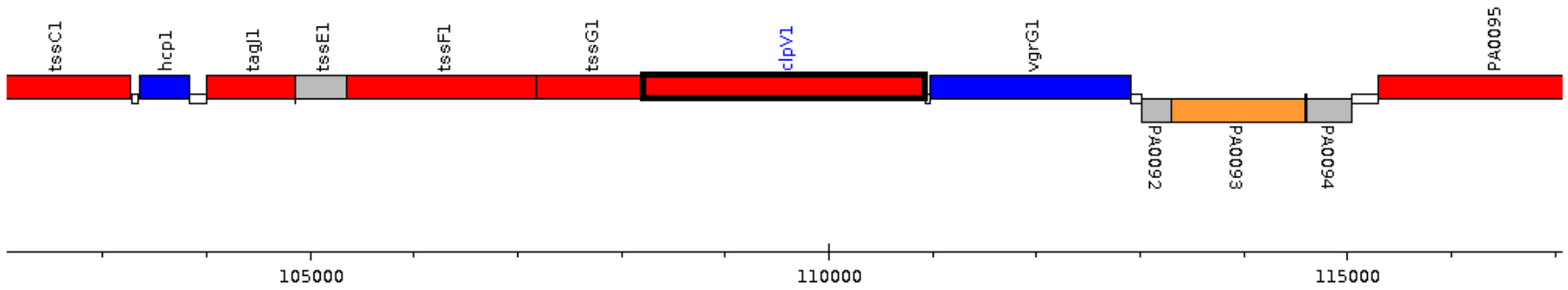
- Angela R. Records. 2011. The Type VI Secretion System: A Multipurpose Delivery System with a Phage-Like Machinery. MPMI Vol. 24, No. 7. pp. 751–757.

ClpV1

PSEUDOMONAS GENOME DATABASE
Improving Disease Treatment Through Genome Research

- Replicon: Pseudomonas aeruginosa PAO1 chromosome, complete genome(5681 genes, 6264404 bp)
- Genomic location: 108221 - 110929 (+ strand)
- Subcellular location: cytoplasm
- Sequence similarities: belongs to the ClpA/ClpB family
- Molecular function: Chaperone

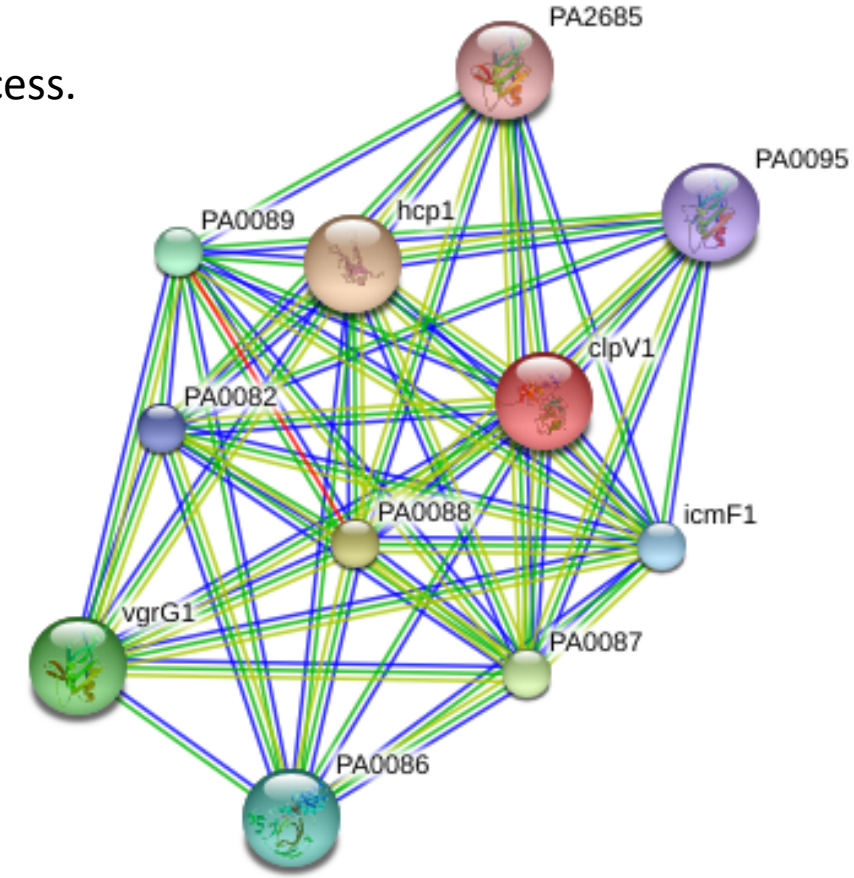




ClpV1

STRING 9.1

- is responsible for energizing the effector transport process.
- may sit at the base of the T6SS apparatus and deliver unfolded protein effectors to the Hcp nanotube.
- is responsible for ATP-driven remodeling of VipA/VipB tubules and may be required to ensure their proper assembly into a phage tail sheath–like structure.
- Predicted functional partners: Hcp1, vgrG1,icmF1



- Bönemann, G. 2010. Tubules and donuts: A type VI secretion story. Mol. Microbiol. 76:815-821.
- Bönemann, G. 2009. Remodelling of VipA/VipB tubules by ClpV-mediated threading is crucial for type VI protein secretion. EMBO J. 28:315-325.
- Mougous, J. 2006. A virulence locus of Pseudomonas aeruginosa encodes a protein secretion apparatus. Science 312:1526-1530.

Sequence analysis

UniProt: Q9I742 (CLPV1_PSEAE)

>sp|Q9I742|CLPV1_PSEAE Protein ClpV1 OS=Pseudomonas aeruginosa

MSEISRVALFGKLNSLAYKAIEAATVFCKLRGNPYVELVHWFHQILQLPDSDLHQIVRQS
GIDPARLAKDLTEALDRLPRGSTSITDLSSHVEEAVERGWVYGSLMFGESQVRTGYLVIG
ILKTPSLRHALTGLSAEFAKLKVEALTERFDEYVGASPENGLSASDGFNAGAAPGEASGA
LAPSAMGKQEALKRFTVDLTEQARSGKLDPIVGRDEEIRQLVDILMRRRQNNPILTGEAG
VGKTAVVEGFALRIVAGDVPPALKDVELRALDVGLLQAGASMKGEFEQRLRQVIEDVQSS
EKPIILFIDEAHTLVGAGGAAGTGDAANLLKPALARGTLRTVAATTWAEYKKHIEKDPAL
TRRFQVVQVDEPSEHKAILMMRGVASTMEKHHQVQILDEALEAAVRLSHRYIPARQLPDK
SVSLLDTACARTAISLHAVPAEVDDSRRRIEALETELAIIRRESAIGVATAERQRNAETL
LAEERERLAALEQRWAEEKRLVDELLETRARLRAAAEAVDAGGVPLGEGEVRLDEEQRQA
LHARLAELQAQLSALQGEEPLILPTVDYQAVASVVADWTGIPVGRMARNEIETVLNLDRH
LKKRIIGQDHALEMIAKRIQTSRAGLDNPSKPIGVFMLAGTSGVGKTETALALAEAMYGG
EQNVITINMSEFQEAHTVSTLKGAPPGYIGYGEGGVLTEAVRRKPYSVVLLDEVEKAHPD
VHEIFFQVFDKGVMEDGEGRVIDFKNTLILLTTNAGTEMIASLCADPELMPEPEAIAKSL
REPLLKIFPPALLGRLVTIPYYPLSDDMLKAISRLQLGRIKKRVEATHKVPFEFDEGVVD
LIVSRCTETESGGRMIDAILTNTLLPDMSREFLTRMLEGKPLAGVRISSRDNQFHYDFAE
AE

Sequence analysis

Cell, Vol. 115, 229–240, October 17, 2003, Copyright ©2003 by Cell Press

The Structure of ClpB: A Molecular Chaperone that Rescues Proteins from an Aggregated State

involved in the recovery of the cell from heat-induced damage, in cooperation with DnaK, DnaJ and GrpE.

Needle: CLPV1_PSEAE(902aa) & CLPB_THET8(854aa)

LENGTH	SCORE	IDENTITY	SIMILARITY	GAPS
936	1424.5	341/936 (36.4%)	506/936 (54.1%)	116/936 (12.4%)

Amino acids composition

ProtParam

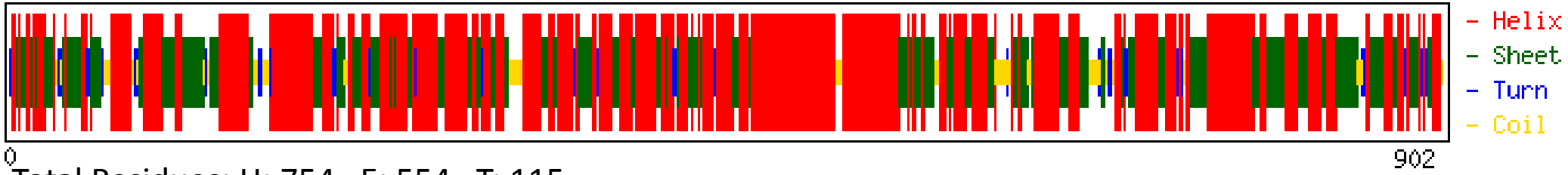
Amino acid	No.(ClpV1/ClpB)	Pct(ClpV1/ClpB)	Amino acid	No.(ClpV1/ClpB)	Pct(ClpV1/ClpB)
Ala (A)	104/91	11.5%/10.7%	Leu (L)	104/112	11.5%/13.1%
Arg (R)	67/84	7.4%/9.8%	Lys (K)	39/49	4.3%/5.7%
Asn (N)	17/12	1.9%/1.4%	Met (M)	20/9	2.2%/1.1%
Asp (D)	47/41	5.2%/4.8%	Phe (F)	24/17	2.7%/2.0%
Cys (C)	4/0	0.4%/0.0%	Pro (P)	40/34	4.4%/4.0%
Gln (Q)	33/32	3.7%/3.7%	Ser (S)	47/24	5.2%/2.8%
Glu (E)	85/108	9.4%/12.6%	Thr (T)	48/34	5.3%/4.0%
Gly (G)	67/57	7.4%/6.7%	Trp (W)	5/6	0.6%/0.7%
His (H)	20/13	2.2%/1.5%	Tyr (Y)	15/18	1.7%/2.1%
Ile (I)	50/54	5.5%/6.3%	Val (V)	66/59	7.3%/6.9%

Total number of negatively charged residues (Asp + Glu): 132/149
Total number of positively charged residues (Arg + Lys): 106/133

Secondary structure prediction

CFSSP: Chou & Fasman Secondary Structure Prediction Server

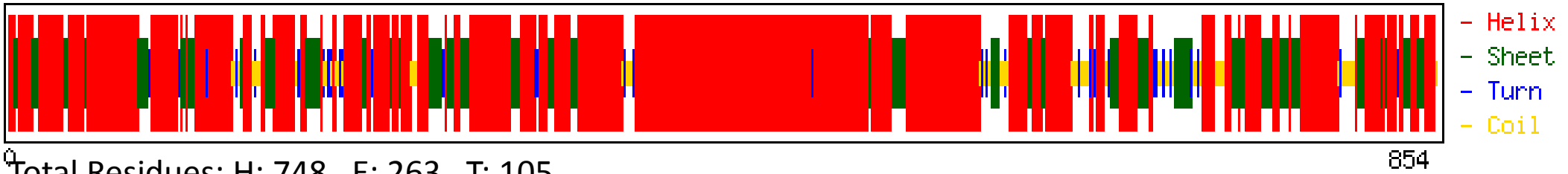
CLPV1_PSEAE



Total Residues: H: 754 E: 554 T: 115

Percent: H: 83.6 E: 61.4 T: 12.7

CLPB_THET8



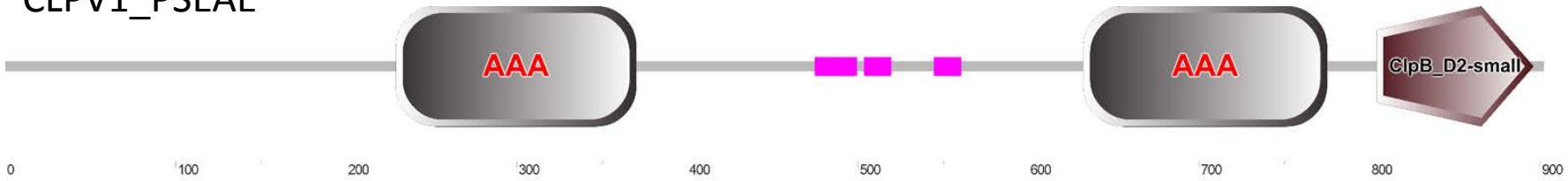
Total Residues: H: 748 E: 263 T: 105

Percent: H: 87.6 E: 30.8 T: 12.3

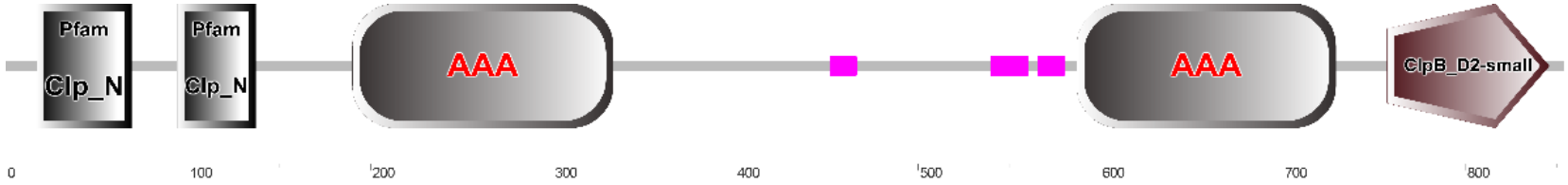
- Peter Y. Chou, and Gerald D. Fasman. Prediction of protein conformation. Biochemistry. 1974 Jan; 13(2), pp 222–245.
- Peter Y. Chou, and Gerald D. Fasman. Conformational parameters for amino acids in helical, β-sheet, and random coil regions calculated from proteins. Biochemistry. 1974 Jan; 13(2): pp 211–222.

Domains analysis

CLPV1_PSEAE



CLPB_THET8



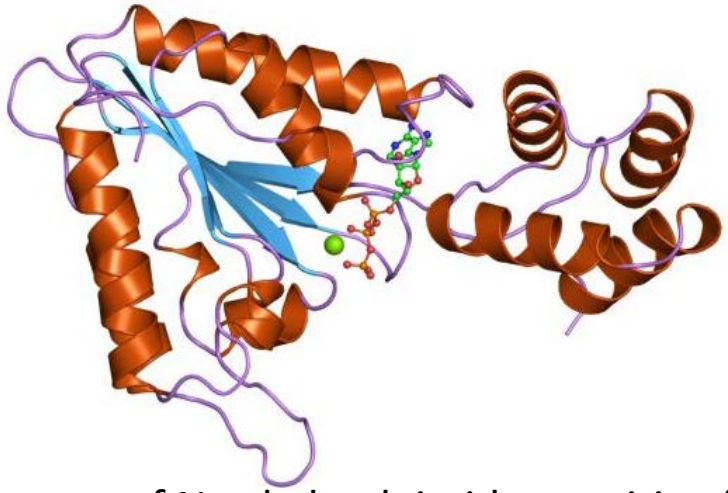
- **Clp_N domain**: is found in one or two copies at the amino terminus of ClpA and ClpB proteins
- **AAA domain**: ATPases associated with a variety of cellular activities
- **ClpB_D2-small domain**: It is the C-terminal domain of ClpB protein

- Schultz et al. (1998) Proc. Natl. Acad. Sci. USA 95, 5857-5864
- Letunic et al. (2012) Nucleic Acids Res , doi:10.1093/nar/gkr931

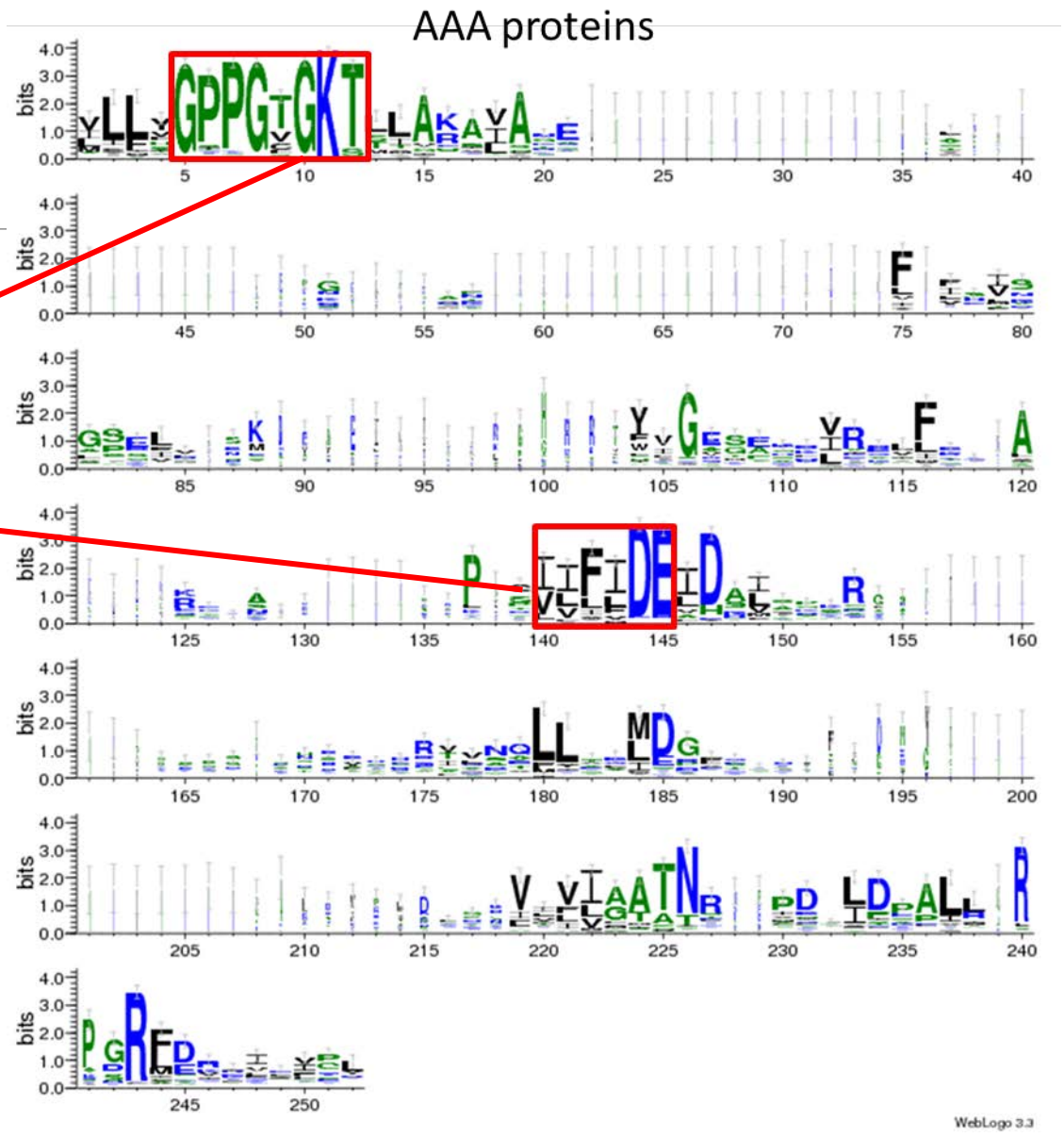
Domains analysis

WEBLOGO Pfam

- N-terminal alpha/beta domain
  Walker A motif: GXXXXGK(T/S)
  Walker B motif: hhhhDE
  binds and hydrolyzes nucleotides
- C-terminal alpha-helical domain



Structure of N-ethylmaleimide-sensitive factor

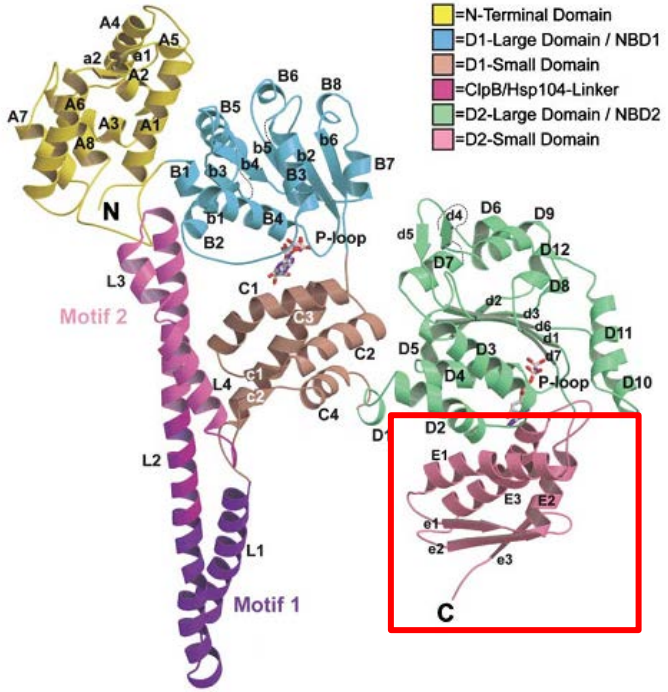


- Crooks GE, Hon G, Chandonia JM, Brenner SE. 2004. WebLogo: A sequence logo generator, Genome Research, 14:1188-1190.
- M. Punta, P.C. 2014. The Pfam protein families database. Nucleic Acids Research. Database Issue 42:D222-D230.

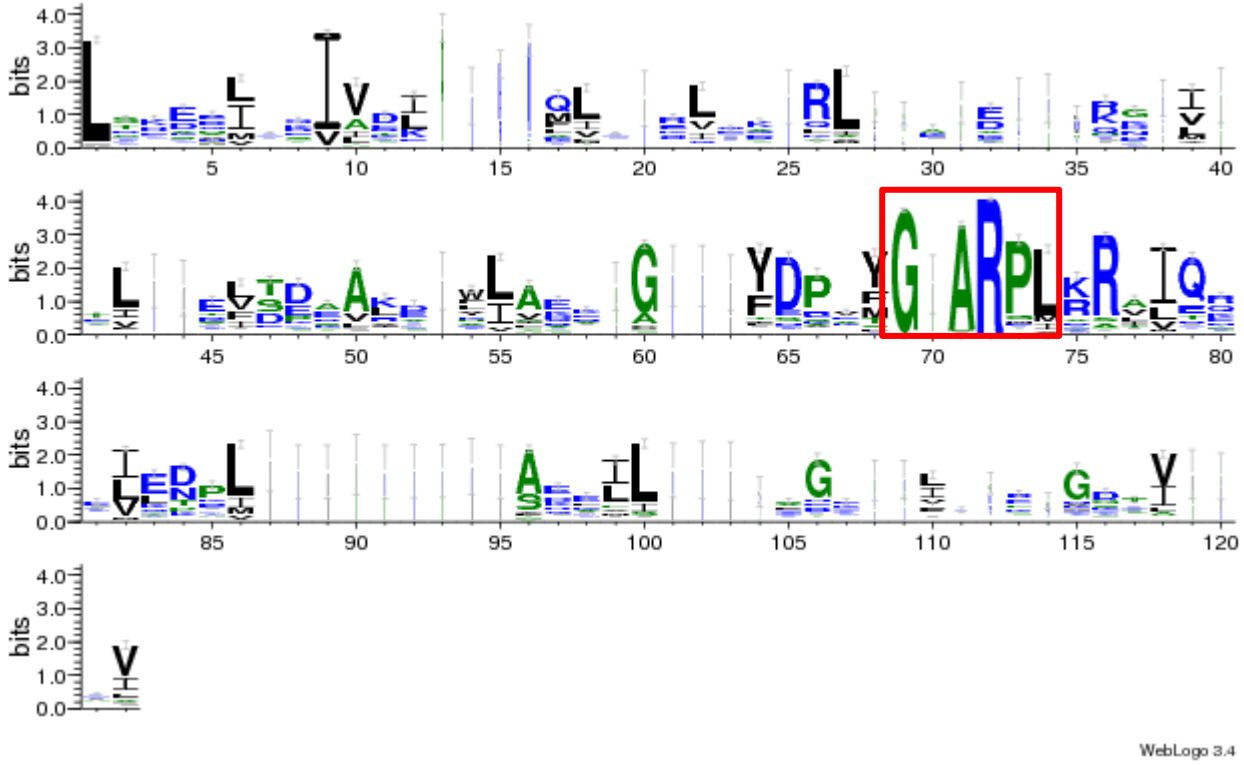
Domains analysis

WEBLOGO Pfam

1QVR



ClpB_D2-small domain



This is the C-terminal domain of ClpB protein. It is a mixed alpha-beta structure essential for oligomerisation.

- Crooks GE, Hon G, Chandonia JM, Brenner SE. 2004. WebLogo: A sequence logo generator, Genome Research, 14:1188-1190.
- M. Punta, P.C. 2014. The Pfam protein families database. Nucleic Acids Research. Database Issue 42:D222-D230.

Domains analysis

UniProt › UniProtKB

CLPV1_PSEAE

Regions

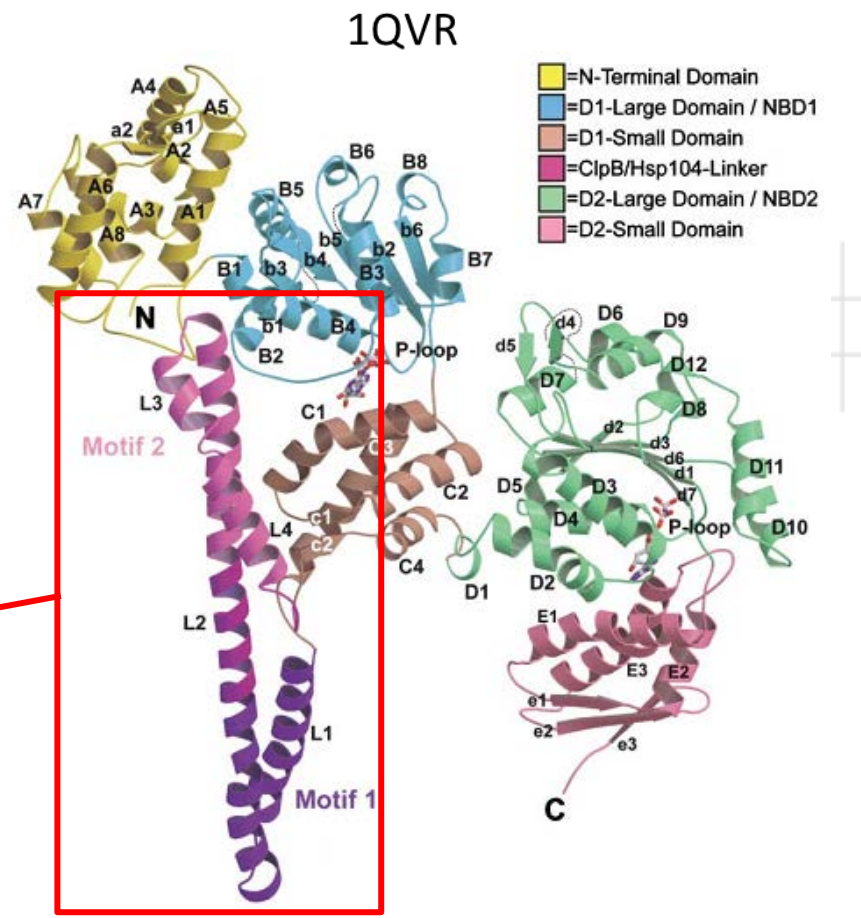
☐	Nucleotide binding	237 – 244	8	ATP 1 By similarity
☐	Nucleotide binding	640 – 647	8	ATP 2 By similarity
☐	Coiled coil	441 – 559	119	Potential

?

Identity: 19.58%

mobile

protein disaggregation



Structure of theTClpB Monomer

- Sukyeong Lee. 2003. The Structure of ClpB: A Molecular Chaperone that Rescues Proteins from an Aggregated State.Cell, Vol. 115, 229–240

3D structure prediction

Phyre²

Top template information

PDB header:chaperone
Chain: B: **PDB Molecule:**clpb protein;
PDBTitle: crystal structure analysis of clpb

Confidence and coverage

Confidence:	100.0%	Coverage:	89%

800 residues (89% of your sequence) have been modelled with 100.0% confidence by the single highest scoring template.

Additional confident templates have been detected (see Domain analysis) which cover other regions of your sequence.

883 residues (98%) could be modelled at >90% confidence using multiple-templates.

Disordered (19%)
Alpha helix (57%)
Beta strand (9%)

% Identity: 36%
Resolution: 3.00 Å

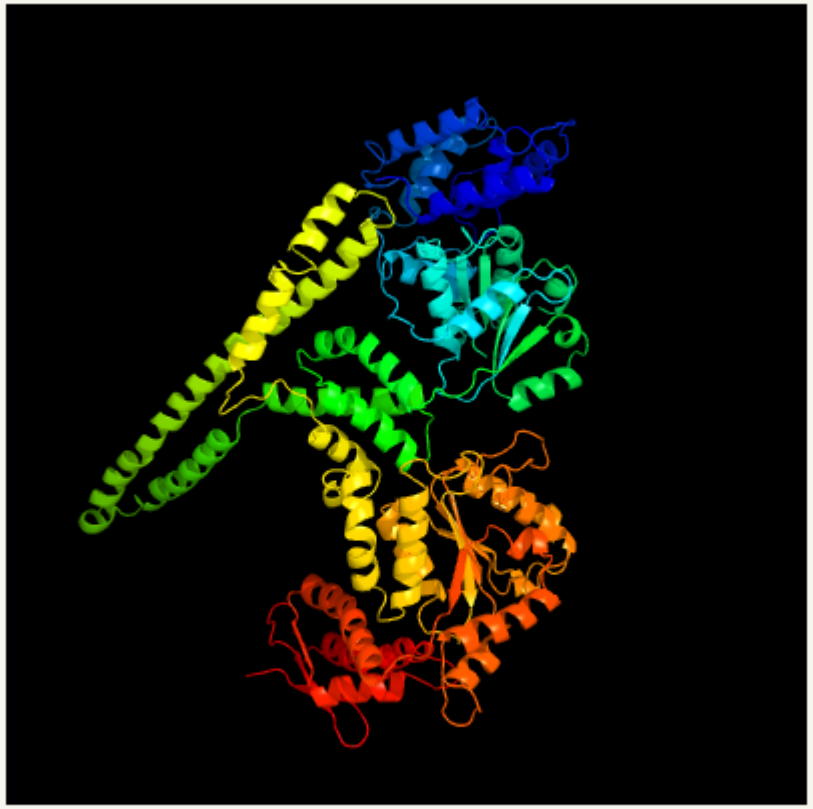


Image coloured by rainbow N → C terminus

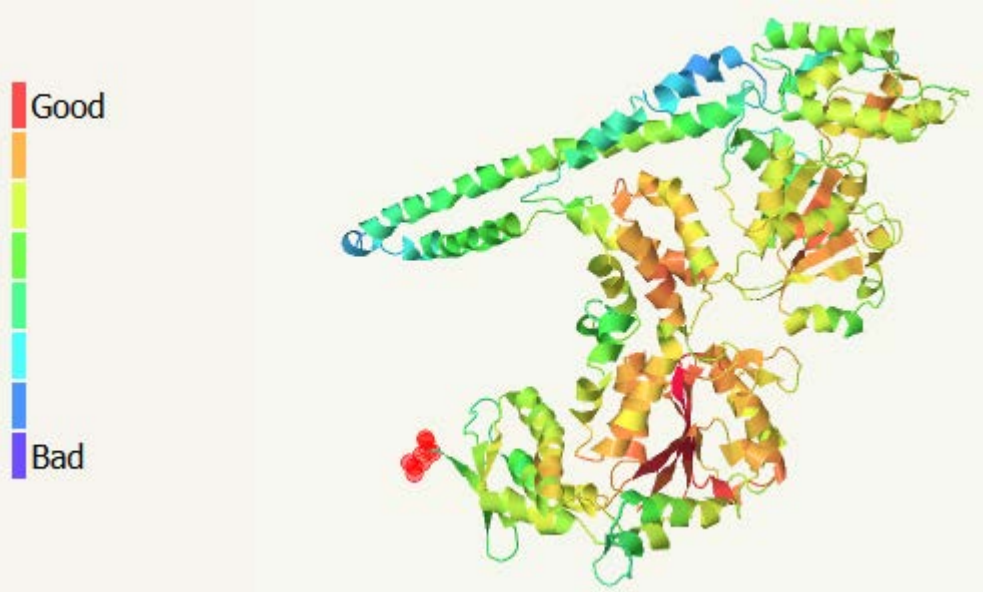
Model dimensions (Å): **X**:113.402 **Y**:73.689 **Z**:114.477

- Kelley LA and Sternberg MJE. 2009. Protein structure prediction on the web: a case study using the Phyre server. Nature Protocols 4, 363 – 371.

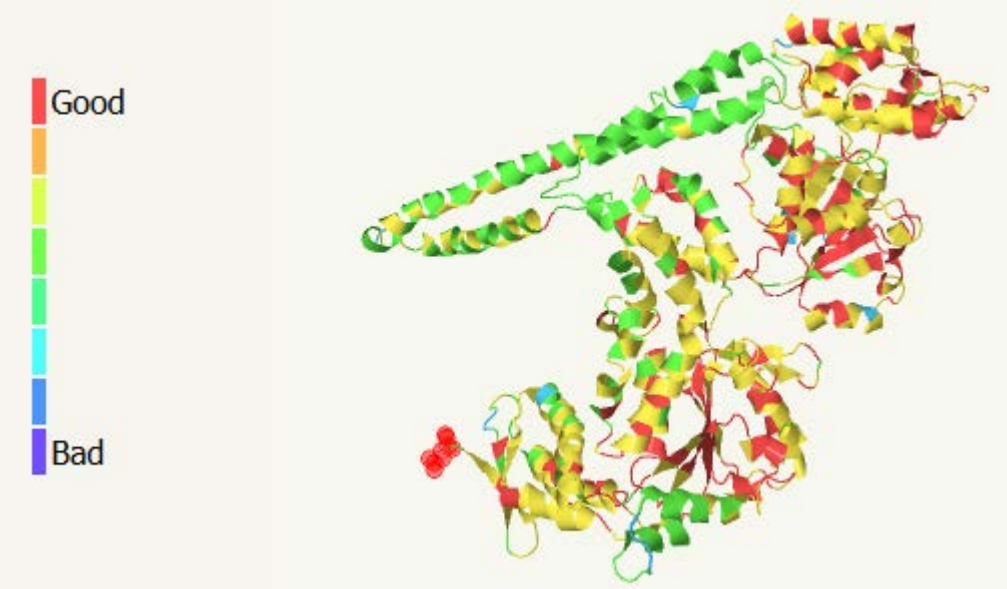
Model quality analysis

Phyre²

ProQ2 quality assessment



Alignment confidence

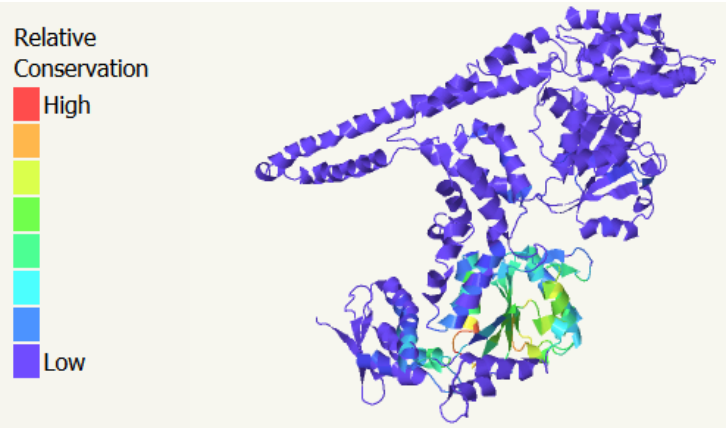


- Kelley LA and Sternberg MJE. 2009. Protein structure prediction on the web: a case study using the Phyre server. Nature Protocols 4, 363 – 371.

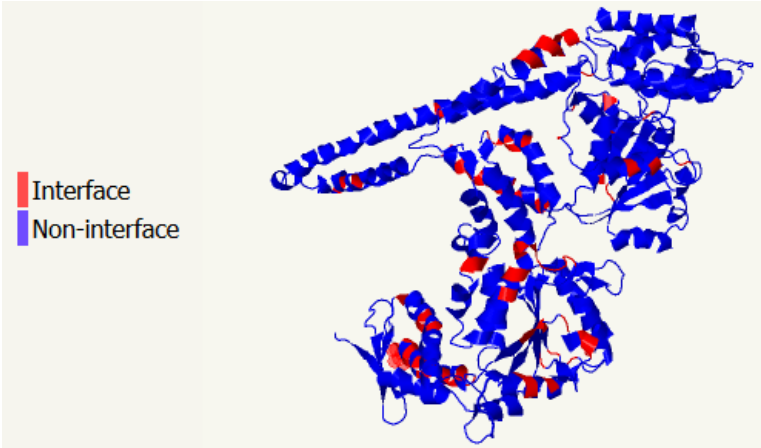
Model function analysis

Phyre²

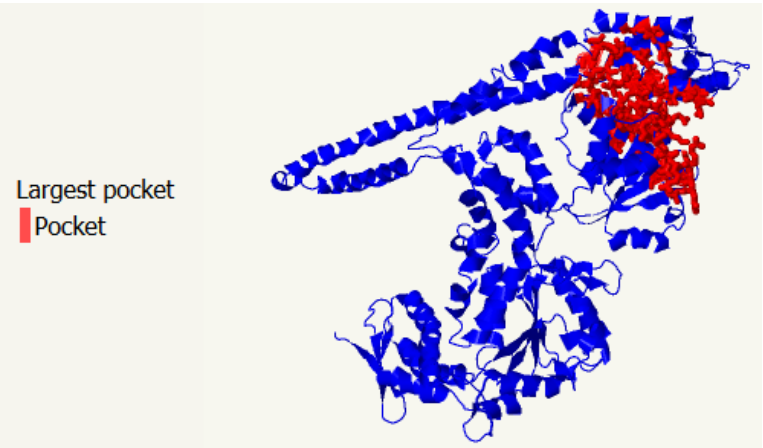
Conservation



ProtinDB interface



Pocket detection

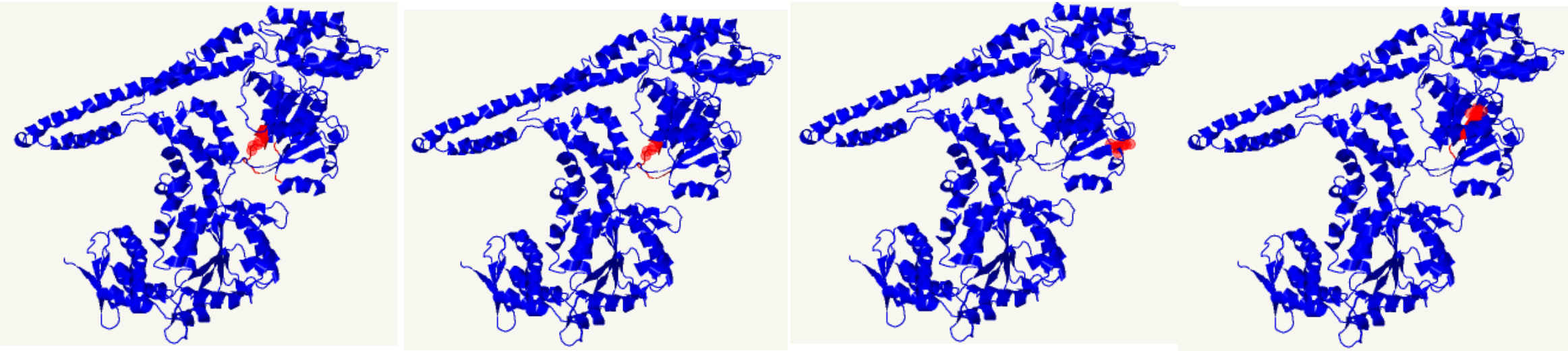


- Kelley LA and Sternberg MJE. 2009. Protein structure prediction on the web: a case study using the Phyre server. Nature Protocols 4, 363 – 371.

Model conserved domain analysis

Phyre²

ATP binding site | **Walkers A motif** | **Arginine finger** | **Walkers B motif**



- ATP binding site: E238,A239,G240,V241,G242,K243,T244,A245,D309,T346
- arginine finger: R363
- Walker A motif: G237,E238,A239,G240,V241,G242,K243,T244
- Walker B motif: I305,L306,F307,I308,D309,E310

- Kelley LA and Sternberg MJE. 2009. Protein structure prediction on the web: a case study using the Phyre server. Nature Protocols 4, 363 – 371.

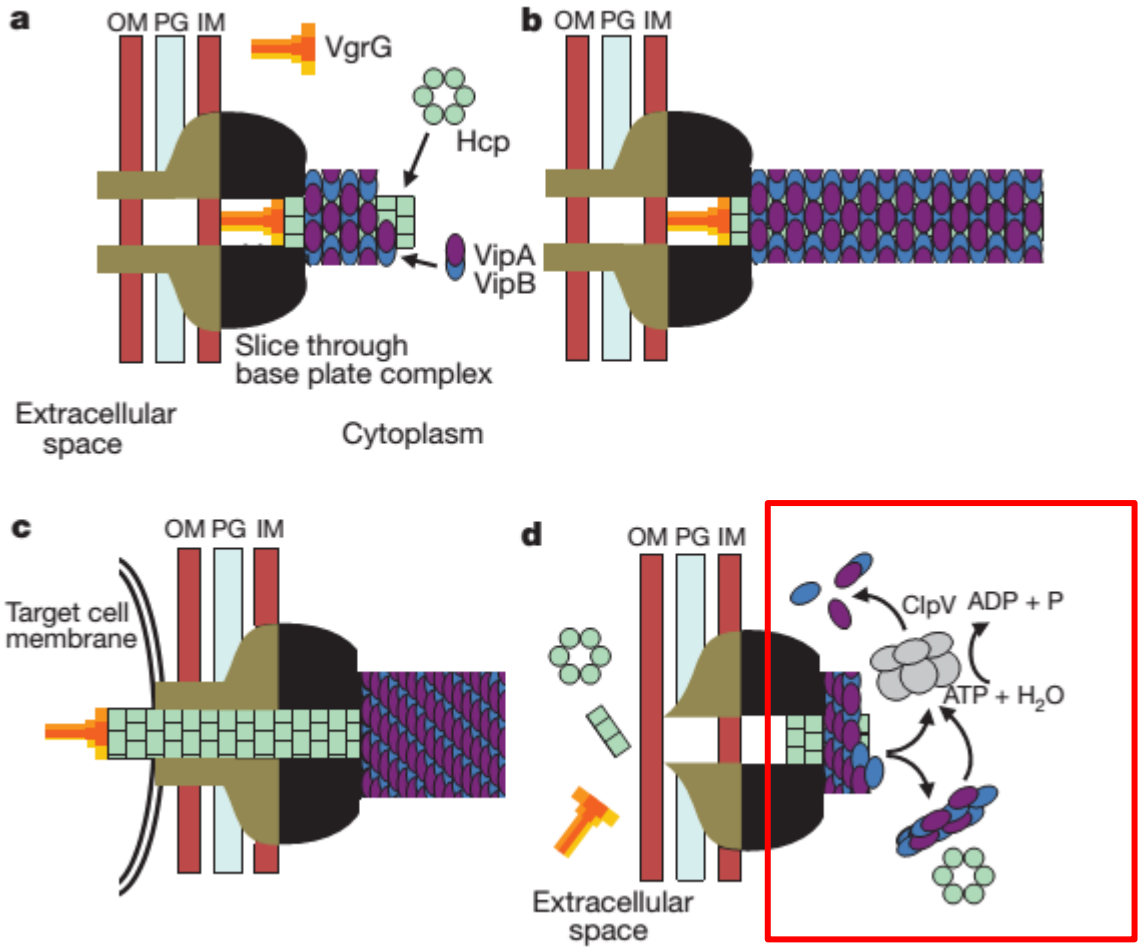
Discussion

- a. Assembly ⟶ b. “ready to fire” conformation

  ⟶ c. Contraction ⟶ d. Disassembly

- ClpV1 is an ATPase with classic conserved domains. AAA domain may be important for ATP binding and hydrolysis, while ClpB_D2-small domain may be essential for oligomerisation.

- The coiled coil may be critical for chaperone activity



Model of T6SS action.OM, outer membrane; PG, peptidoglycan; IM, inner membrane.

- M. Basler. 2012. Type VI secretion requires a dynamic contractile phage tail-like structure. Nature.Vol. 483,182-186

Thank you!