双精氨酸转运Tat体系研究背景初探

Tat: twin arginine translocation

小组成员

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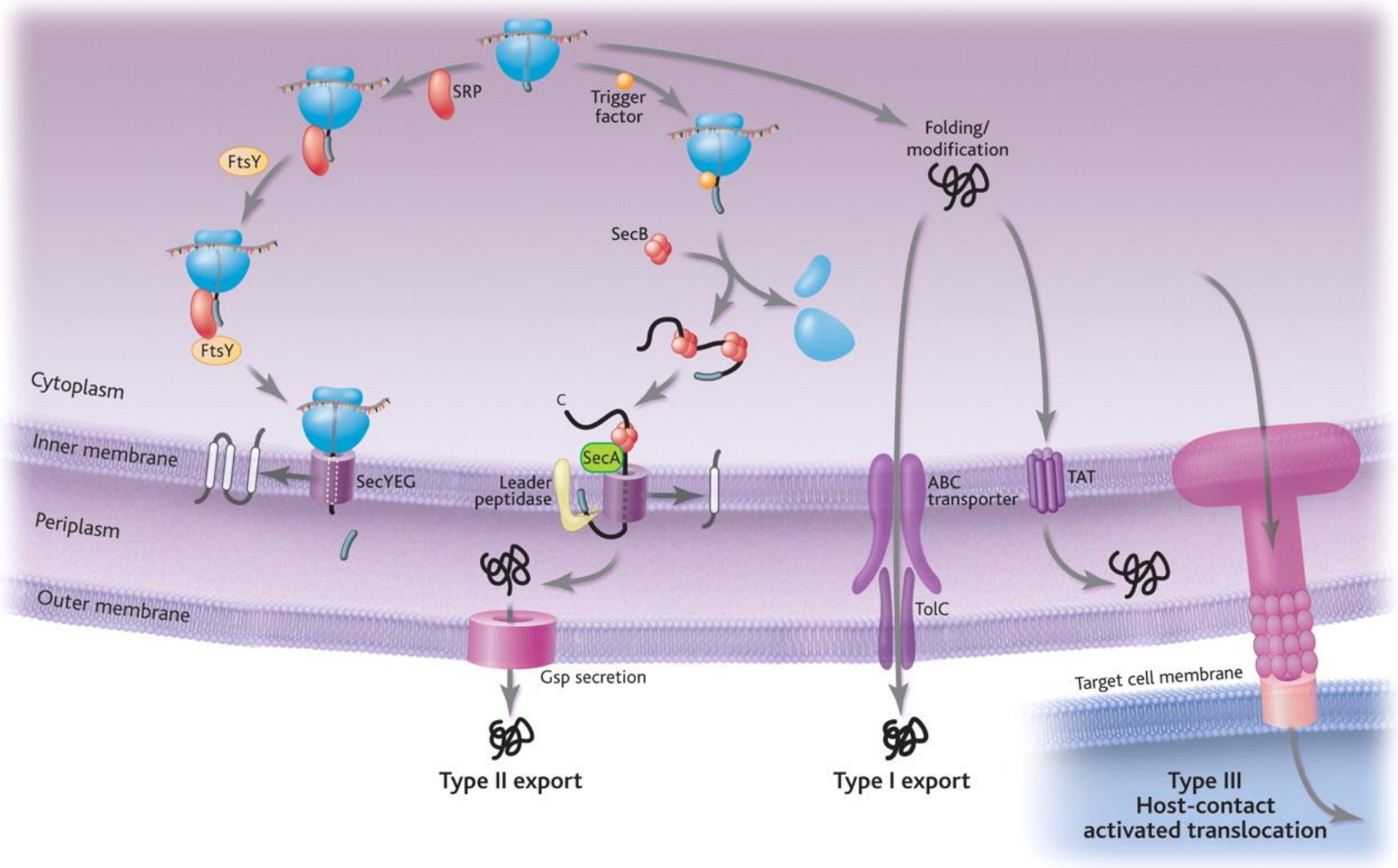
Diversity of bacterial translocation pathways

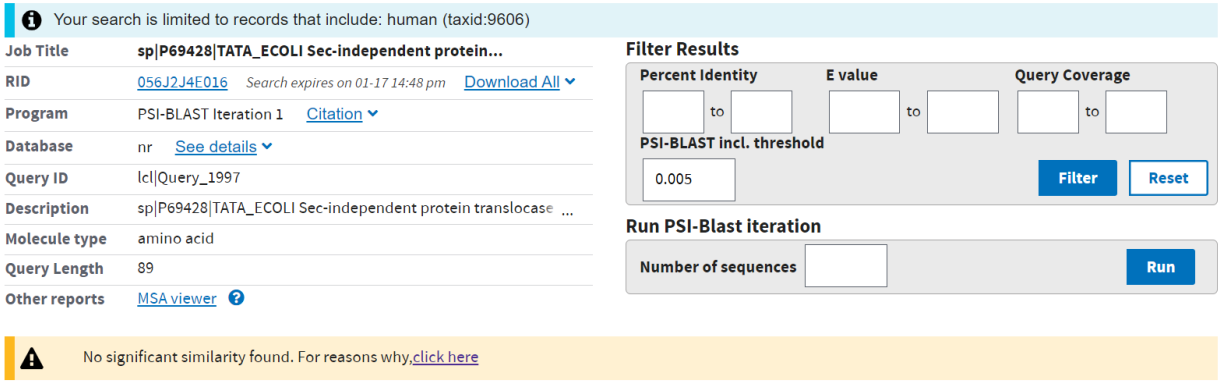


Wickner, William, and Randy Schekman. "Protein translocation across biological membranes." *science* 310.5753 (2005): 1452-1456.

Tat exists in Chloroplast but not in human cells

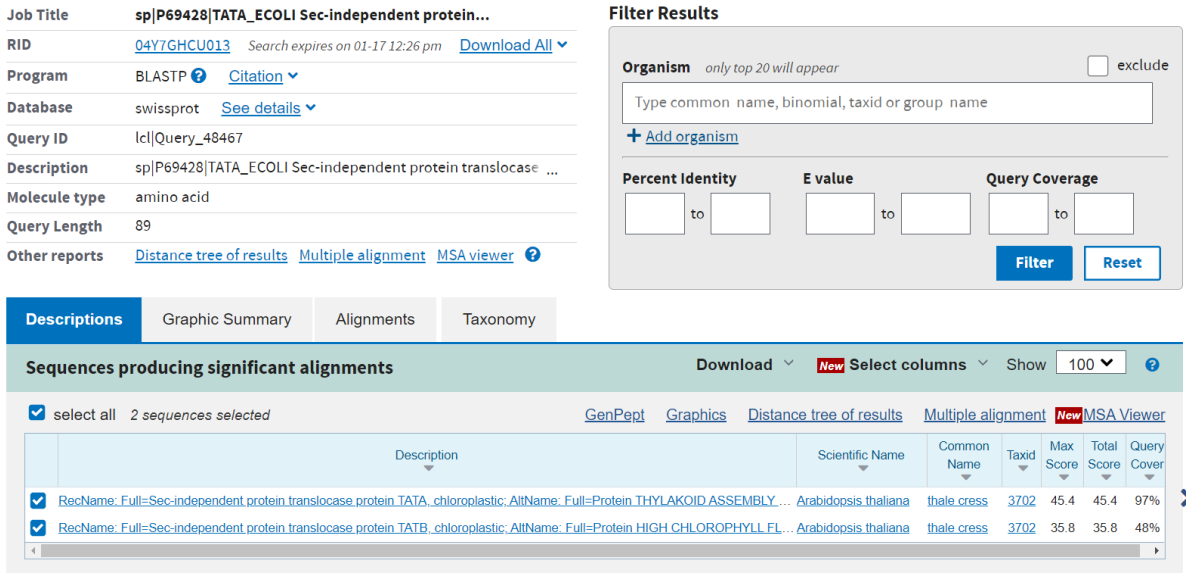
BLAST TatA

Human



BLOSUM 45
word size 2
PSI-BLAST
Non-redundant protein sequences (nr)

Arabidopsis



BLOSUM 60
word size 3
BLASTP
Swiss-Prot

The finding of Tat

Prof Ben Berks

Protein transport across bacterial cell membranes

We study the molecular machines involved in forming the bacteria cell envelope, with a special emphasis on those machines that transport macromolecules across the cell membranes. These nanomachines have crucial roles in pathogenesis, motility, and antibiotic resistance, and are amongst the most mechanistically interesting proteins in the cell. The most established systems under study in our laboratory are:

The Tat (twin-arginine translocation) protein transport system which exports folded proteins across the bacterial inner membrane. The Tat system is involved in a wide range of fundamental cellular processes in bacteria and is essential for the virulence of bacterial pathogens. The mechanism of Tat transport is radically different from that employed by other protein transporters, enabling it to translocate folded proteins without compromising the ion permeability barrier of the membrane.

The Type IX secretion system (T9SS) which is involved in severe dental disease and in bacterial gliding motility. This multi-component system exports proteins through a huge pore in the outer membrane using the energy of the inner membrane proton electrochemical gradient.

Our work is grounded in protein biochemistry and bacterial cell biology to which we add a full range of cutting edge molecular techniques. In particular, we collaborate with local colleagues on the structural analysis of the transporter complexes by cryoEM, X-ray crystallography, and other approaches, and we dissect the transport processes using live cell single molecule fluorescence imaging.

> We take a multi-disciplinary approach to understanding bacterial protein transport

https://www.bioch.ox.ac.uk/research/berks

I. Periplasmic proteins binding iron-sulfur clusters

			evidence	length	ref.
A. MauM family ferredoxins					
P. denitrificans	MauM	MEARMTGRRKV**TRR**DAMADAARAVGVACLGGFSLAALVRTASPVDA	VH	46	[1]
E. coli	NapG	MSRSAKPQNG**RRRFL**RDVVRTAGGLAAVGVALGLQQQTARA	VH	41	[2]
B. `16Fe' ferredoxin superfamily					
E. coli	NrfC	MTW**SRR**Q**FL**TGVGVLAAVSGTAGRVVA	VH	27	[3]
D. vulgaris	Hmc2	MD**RRRFL**TLLGSAGLTATVATAGTAKA	VH	27	[4]
C. High potential iron protein (HiPIP)					
T. ferrooxidans	Iro	MSEKDKMI**TRR**DA**L**RNIAVVVGSVATTTMMGVGVADA	EX	37	[5]
D. Periplasmically-located [Fe] hydrogenase small subunits					
D. vulgaris	HydB	MQIVNL**TRRG**F**LK**AACVVTGGALISIRMTGKAVA	VH	34	[6]
E. Periplasmically-located [NiFe] hydrogenase small subunits					
E. coli	HyaA	MNNEETFYQAMRRQGV**TRRS**F**LK**YCSLAATSLGLGAGMAPKIAWA	EX	45	[7]
+*M. mazei*	VhoG	MSTGTTNLVRTLDSMDFLKMD**RRT**F**MK**AVSALGATAFLGTYQTEIVNA	EX	48	[8]
D. gigas	HynB	MKCYIGRGKNQVEERLERRGV**SRR**D**FMK**FCTAVNVAMGMGPAFAPKVAEA	EX	50	[9]
E. coli	HybA	M**NRRN**F**IK**AASCGALLTGALPSVSHA	VH	26	[10]
F. Membrane-anchored Rieske proteins					
P. denitrificans	FbcF	MSHADEHAGDHGA**TRRD**F**L**YYATAGAGTVAAGAAAWTLVNQMNP			[11]
+*Synechocystis*	PetC	MTQISGSPDVPDLG**RRQF**M**N**LLTFGTITGVAAGALYPAVKYLIP			[12]
+*S. acidocaldarius*	SoxF	M**DRRT**F**L**RLYLLVGAAIAVAPVIKPALDYVGY			[13]

II.Periplasmic proteins binding the molybdopterin cofactor

A. Molybdopterin guanine dinucleotide-binding proteins, some of which also bind an iron-sulfur cluster					
R. sphaeroides	DmsA	MTKLSGQELHAEL**SRRA**F**L**SYTAAVGALGLCGTSLLAQGARA	EX	42	[14]
E. coli	BisZ	MTL**TRREF**I**K**HSGIAAGALVVTSAAPLPAWA	VH	31	[15]
T. pantotropha	NapA	MTI**SRR**D**LLK**AQAAGIAAMAANIPLSSQAPA	VH	31	[16]
W. succinogenes	FdhA	MSEALSGRGND**RRK**F**LK**MSALAGVAGVSQAVG	EX	32	[17]
E. coli	DmsA	MKTKIPDAVLAAEV**SRR**GLVKTTAIGGLAMASSALTLPFSRIAHA	EX	45	[18]
H. influenzae	DmsA	MSNFNQI**SRR**D**FVK**ASSAGAALAVSNLTLPFNVMA	VH	35	[19]
S. typhimurium	PhsA	MSI**SRRSFL**QGVGIGCSACALGAFPPGALA	VH	30	[20]
B. Molybdopterin cytosine dinucleotide-binding proteins					
P. diminuta	IorB	MKTVLPSVPETVRL**SRR**G**FL**VQAGTITCSVAFGSVPA	VH	37	[21]
A. polyoxogenes	Ald	MGRLNRFRLGKDGRREQASL**SRR**G**FL**VTSLGAGVMFGFARPSSA	EX	44	[22]

III.Periplasmic enzymes with polynuclear copper sites

A. Nitrous oxide reductases					
P. stutzeri	NosZ	MSDKDSKNTPQVPEKLGL**SRR**G**FL**GASAVTGAAVAATALGGAVMTRESWA	EX	50	[23]
B. Multicopper oxidase superfamily					
P. syringae	CopA	MESRT**SRR**T**FVK**GLAAAGVLGGLGLWRSPSWA	VH	32	[24]
E. coli	SufI	MSL**SRR**QFIQASGIALCAGAVPLKASA	VH	27	[25]

IV.Methylamine dehydrogenase small subunits (tryptophan tryptophylquinone cofactor)

M. extorquens	MauA	MLGKSQFDDLFEKMSRKVAGHT**SRR**G**F**IGRVGTAVAGVALVPLLPVDRRGRVSRANA	EX	57	[26]

V.Periplasmic proteins binding flavin adenine dinucleotide

C. vinosum	FccB	MTLN**RRD**F**IK**TSGAAVAAVGILGFPHLAFG	EX	30	[27]
+*B. sterolicum*	ChoB	MTDSRANRADATRGVASV**SRRR**F**L**AGAGLTAGAIALSSMSTSASA	EX	45	[28]

Periplasmic proteins

Berks, B.C. (1996), **A common export pathway for proteins binding complex redox cofactors?**. Molecular Microbiology, 22: 393-404.

Entry	Entry name		Protein names	Gene names	Length	Organism
P46923	TORZ_ECOLI		**Trimethylamine-N-oxide reductase 2**	**torZ** bisZ, b1872, JW1861	809	Escherichia coli (strain K12)
P69739	MBHS_ECOLI		**Hydrogenase-1 small chain**	**hyaA** b0972, JW0954	372	Escherichia coli (strain K12)
P0AAL3	NAPG_ECOLI		**Ferredoxin-type protein NapG**	**napG** yojA, yojB, b2205, JW2193	231	Escherichia coli (strain K12)
P0AAK7	NRFC_ECOLI		**Protein NrfC**	**nrfC** yjcJ, b4072, JW4033	223	Escherichia coli (strain K12)
P0AAJ8	HYBA_ECOLI		**Hydrogenase-2 operon protein HybA**	**hybA** b2996, JW2964	328	Escherichia coli (strain K12)
P18775	DMSA_ECOLI		**Dimethyl sulfoxide reductase DmsA**	**dmsA** b0894, JW5118	814	Escherichia coli (strain K12)

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CLUSTAL multiple sequence alignment by MUSCLE (3.8)


sp|P69739|MBHS_ECOLI      MNNEETFYQAMRRQGVTRRSFLKYCSLAATSLGLGAGM--APKIAWALENKPRIP-----
sp|P0AAJ8|HYBA_ECOLI      ---------------MNRRNFIK--AASCGALLTGALP---SVSHAAAENRPPIP-----
sp|P0AAK7|NRFC_ECOLI      -------------MTWSRRQFLT--GV--GVLAAVSGT-----------AGRVVAK---
sp|P0AAL3|NAPG_ECOLI      -------MSRSAKPQNGRRRFLRDVVRTAGGLAAVGVA-------LGLQQQ---------
sp|P18775|DMSA_ECOLI      --MKTKIPDAVLAAEVSRRGLVKTTAI--GGLAMASSALTLPFSRIAHAVDSAIPTKSDE
sp|P46923|TORZ_ECOLI      -------------MTLTRREFIKHSGIAAGALVVTSAA---PLPAWAEEKGGKILTAGR-
                                        ** ::        *  .

sp|P69739|MBHS_ECOLI      ------------------------------------------------------------
sp|P0AAJ8|HYBA_ECOLI      ------------------------------------------------------------
sp|P0AAK7|NRFC_ECOLI      ------------------------------------------------------------
sp|P0AAL3|NAPG_ECOLI      ------------------------------------------------------------
sp|P18775|DMSA_ECOLI      KVIWSACTVNCGSRCPLRMHVVDGEIKYVETDNTGDDNYDGLHQVRACLRGRSMRRRVYN
sp|P46923|TORZ_ECOLI      ---WGAMNVEVKDG---KIVSSTGALAKTIPNSLQSTAADQVHTTARI--QHPMVRKSY-


sp|P69739|MBHS_ECOLI      --------------------VVWIHGLE----------------------CTCCTESF
sp|P0AAJ8|HYBA_ECOLI      ------------------------------------------------------G-S
sp|P0AAK7|NRFC_ECOLI      -----------------------------------------------------TLNINGVR
sp|P0AAL3|NAPG_ECOLI      ----------------------------------------------------TARASGVR
sp|P18775|DMSA_ECOLI      PDRLKYPMKRVGARGEGKFERISWEEAYDIIATNMQRLIKEYGNESIYLNYGTGTLGGTM
sp|P46923|TORZ_ECOLI      ---LDNPLQPAKGRGEDTYVQVSWEQALKLIHEQHDRIRKANGPSAIF----AGSYGWRS
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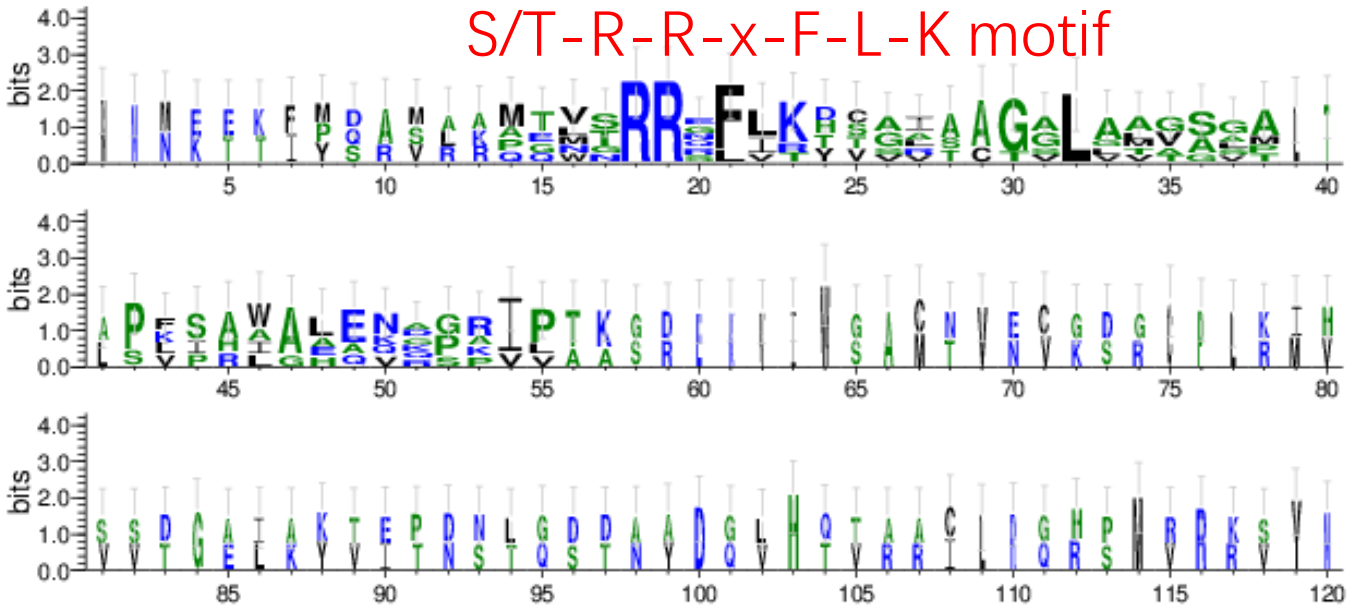
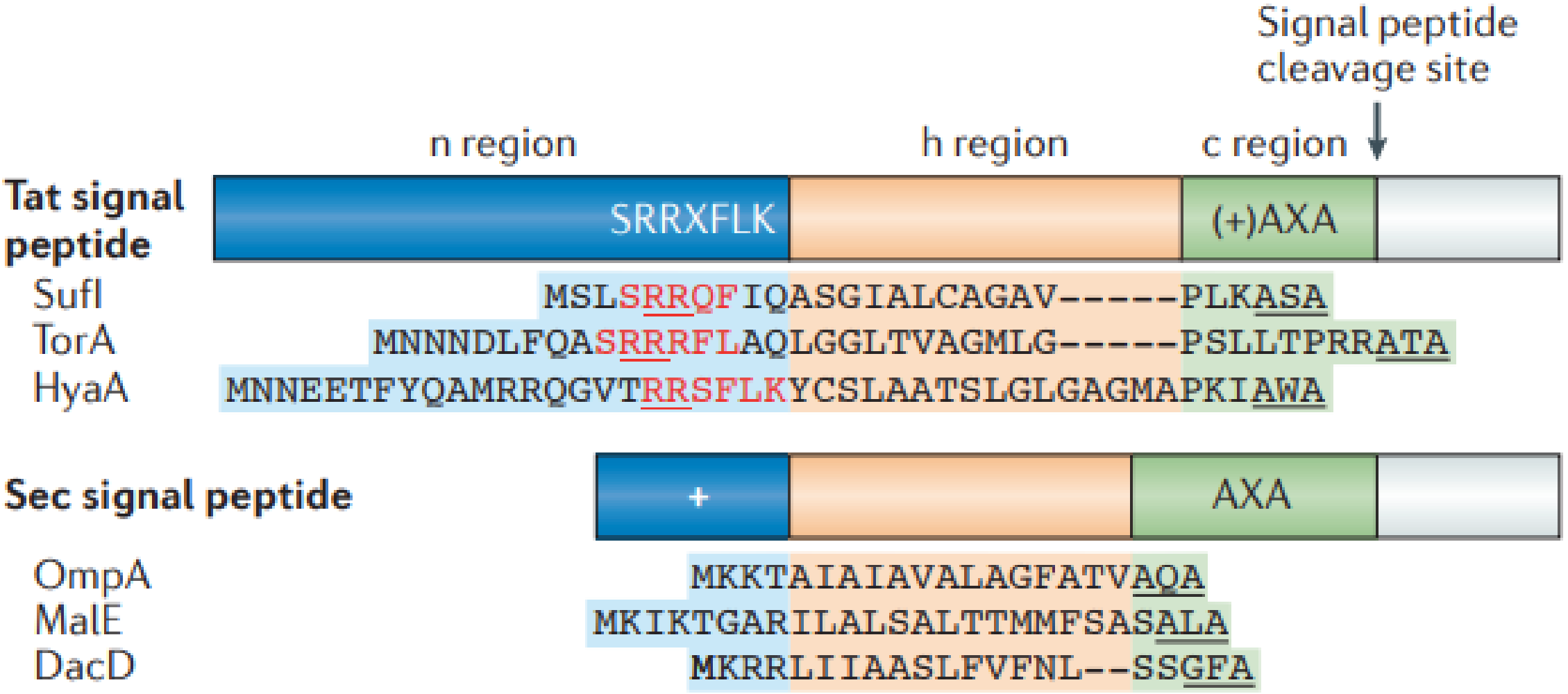


https://www.ebi.ac.uk/Tools/msa/muscle/

http://weblogo.threeplusone.com/create.cgi

The difference between Tat and Sec signal peptide

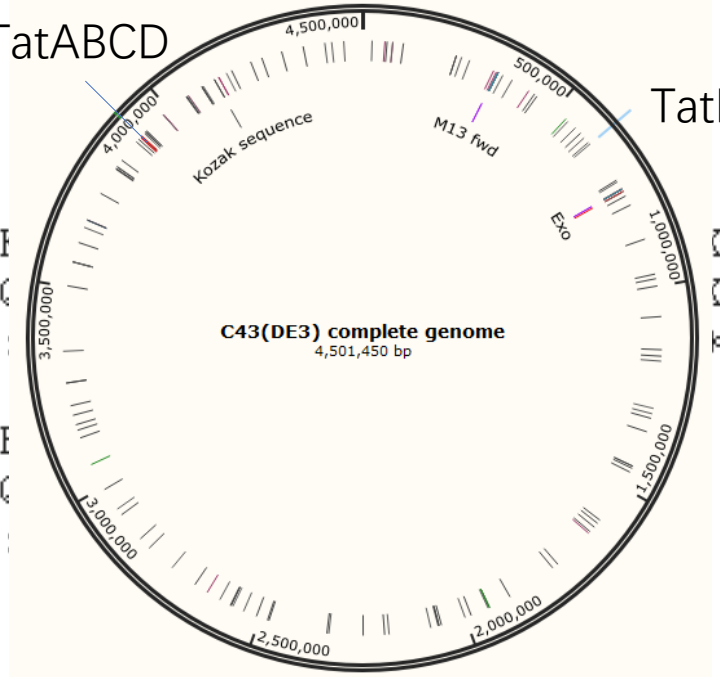


Tat components

☐	P69428	TATA_ECOLI	**Sec-independent protein translocase...**	**tatA** mttA1, yigT, b3836, JW3813	89	Escherichia coli (strain K12)
☐	P69423	TATC_ECOLI	**Sec-independent protein translocase...**	**tatC** mttB, yigU, yigV, b3839, JW3815	258	Escherichia coli (strain K12)
☐	P69425	TATB_ECOLI	**Sec-independent protein translocase...**	**tatB** mttA2, ysgB, b3838, JW5580	171	Escherichia coli (strain K12)
☐	P0A843	TATE_ECOLI	**Sec-independent protein translocase...**	**tatE** ybeC, b0627, JW0622	67	Escherichia coli (strain K12)

```
TATE_ECOLI      MGEISITI                                  KAMNDDDAAAKKGA-DVDLQ
TATA_ECOLI      MGGISIWG                                  KAMSDDEPKQDKTSQDADFT
                ** ***                                    ****.**:.   .* : *.*:

TATE_ECOLI      AEKLSHKI
TATA_ECOLI      AKTIADKG
                *:.:: *
```



NCBI Reference Sequence: NZ_CP011938.1

tatA sequence alignment

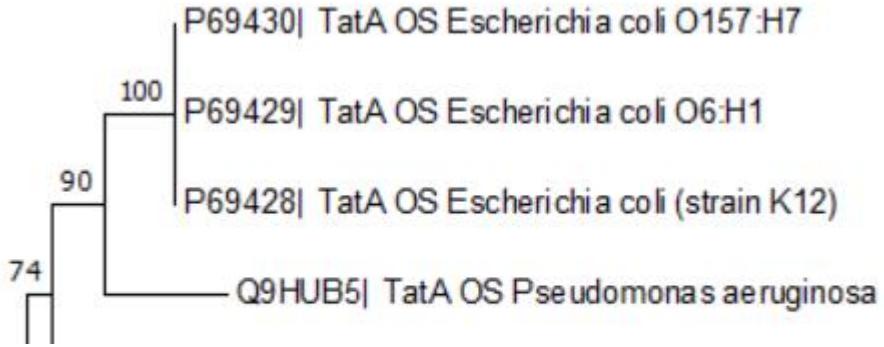
E.coli (strain K12) tatA
Escherichia coli O157:H7 tatA
Escherichia coli O6:H1 tatA
Pseudomonas aeruginosa tatA

Mycobacterium tuberculosis ta

Bacillus subtilis Ta

Haloferax volcanii T

Arabidopsis thaliana T
Pisum sativum T
Zea mays T




```
TATA_ECOLI  ----------------------------------------MG----------
TATA_ARATH  MATSVATLSSPPPVSLPLLSSSRSSFFSNCFTVTTRPNTRSLVAIGRRIRQEPTRKPLTC
                                                    :*

TATA_ECOLI  ----GISIWQLLIIAVIVVLLFGTKKLGSIGSDLGASIKGFKKAMSDDEPKQDKTSQDAD
TATA_ARATH  NALFGLGVPELAVIAGVAALLFGPKKLPEIGKSIGKTVKSFQQAAKEFESELKTEPEESV
             *:.: :* :** :..****.*** .**..:* ::*.*::* .: *.: .. .:::

TATA_ECOLI  FTAKTIADKQADTNQEQAKTEDAKRHDKEQV
TATA_ARATH  AESSQV----ATSNKEEEKKTEVSSSSKENV
              :. :    * :*:*: *. :..  .**:*
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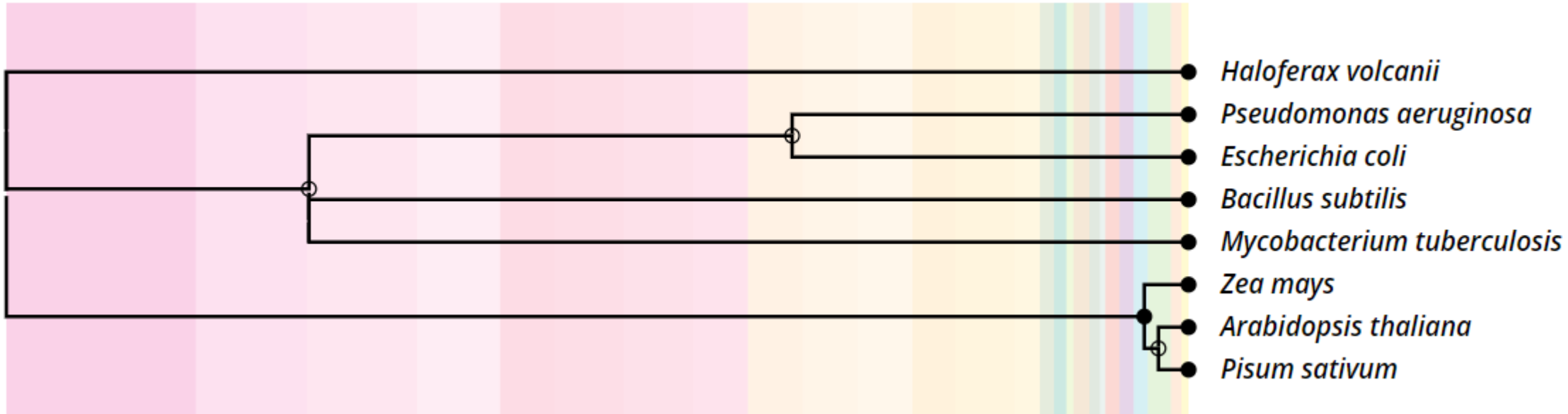
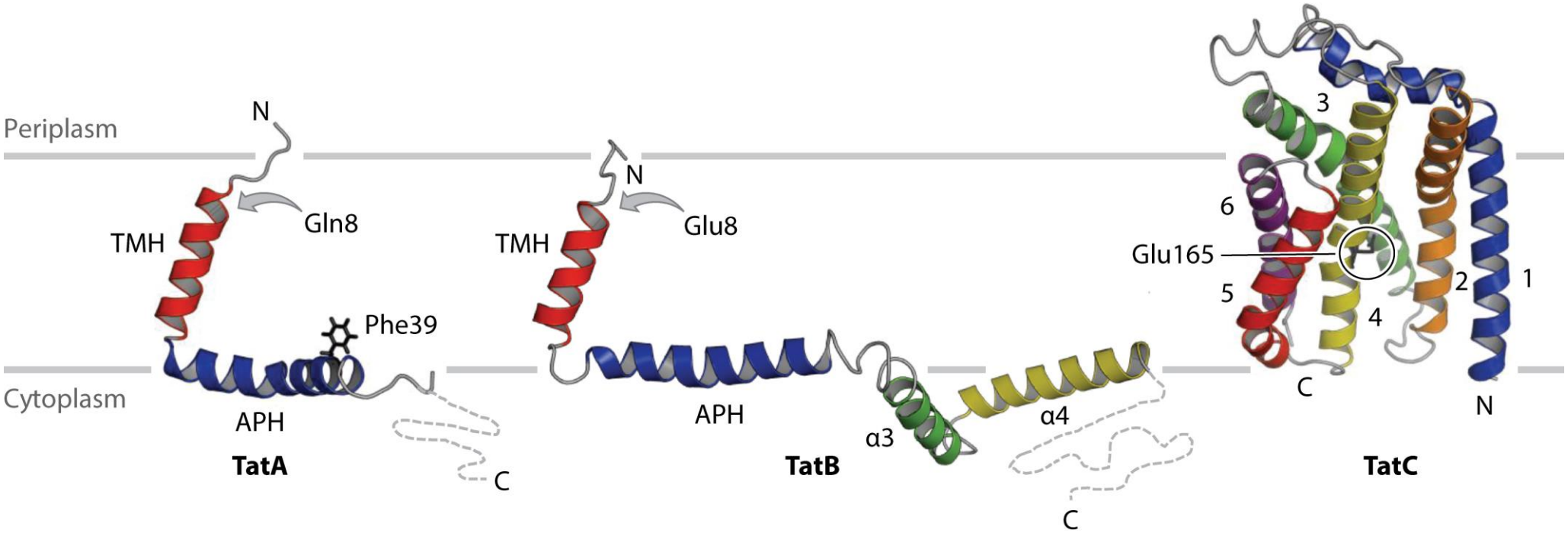
0.10



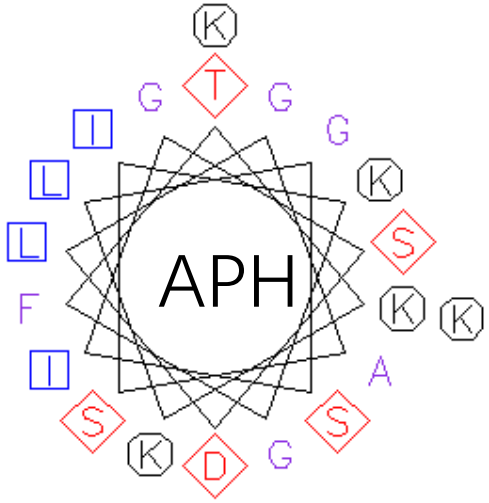
http://www.timetree.org/



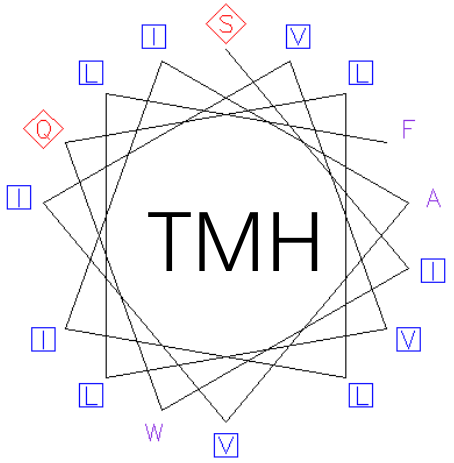
Solution NMR structures of the Escherichia coli TatA and TatB proteins and the highest resolution X-ray structure of Aquifex aeolicus TatC

```
TATB_ECOLI      -------------------------MFDIGFSELLLVFIIGLVVLGPQRLPVAVKTVAGW
TATAT_HALVD     ----------------MFETITPLFPGLPGGPELLVVLLIVVLLFGANKIPKLARSSGQA
TATA_PSEAE      -------------------------MGIFDWKHWIVILIVVVLVFGTKRLKNLGSDVGEA
TATA_ECOLI      -------------------------MGGISIWQLLIIAVIVVLLFGTKKLGSIGSDLGAS
TATA_ECO57      -------------------------MGGISIWQLLIIAVIVVLLFGTKKLGSIGSDLGAS
TATA_ECOL6      -------------------------MGGISIWQLLIIAVIVVLLFGTKKLGSIGSDLGAS
TATE_ECOLI      -------------------------MGEISITKLLVVAALVVLLFGTKKLRTLGGDLGAA
TATA_MYCTU      -------------------------MGSLSPWHWAILAVVVIVLFGAKKLPDAARSLGKS
TATAD_BACSU     -------------------------MFSNIGIPGLILIFVIALIIFGPSKLPEIGRAAGRT
TATA_MAIZE      AVAASVAARPRRAGSGGGGALGCKCLFGLGVPELAVIAGVAALVFGPKQLPEIGRSIGKT
TATA_PEA        LKKARIKTRTT-KG------FTCNAFFGLGVPELVVIAGVAALVFGPKKLPEVGRSIGQT
TATA_ARATH      AIGRRIRQEPTRKP------LTCNALFGLGVPELAVIAGVAALLFGPKKLPEIGKSIGKT
                                              .     ::  :  :::*...:      .
```

HELICAL WHEEL

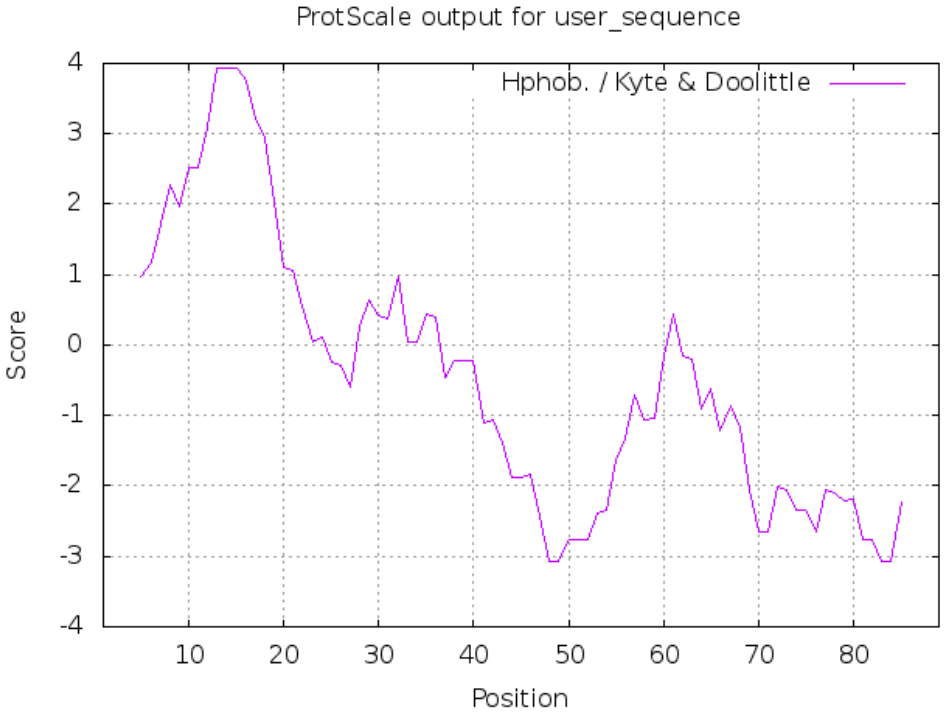


TKKLGSIGS DLGASIKGFK K



SIWQLL IIAVIVVLLF

aliphatic residues are marked with squares

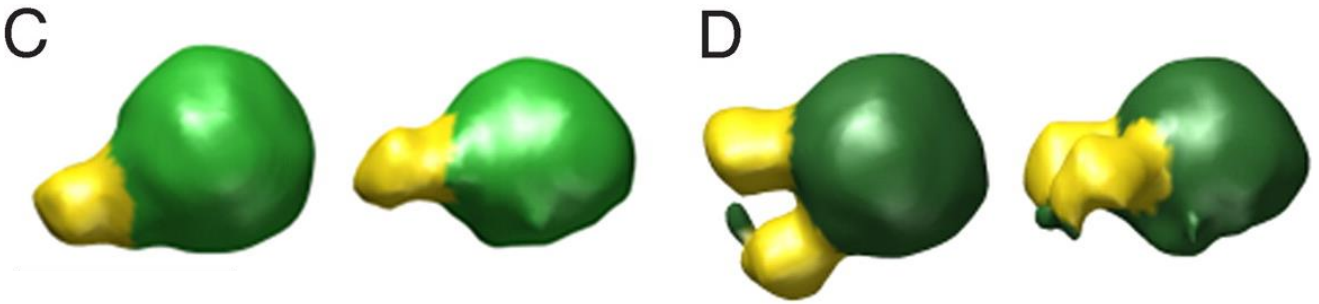


http://www.bioinformatics.nl/cgi-bin/emboss/pepwheel

https://web.expasy.org/protscale/

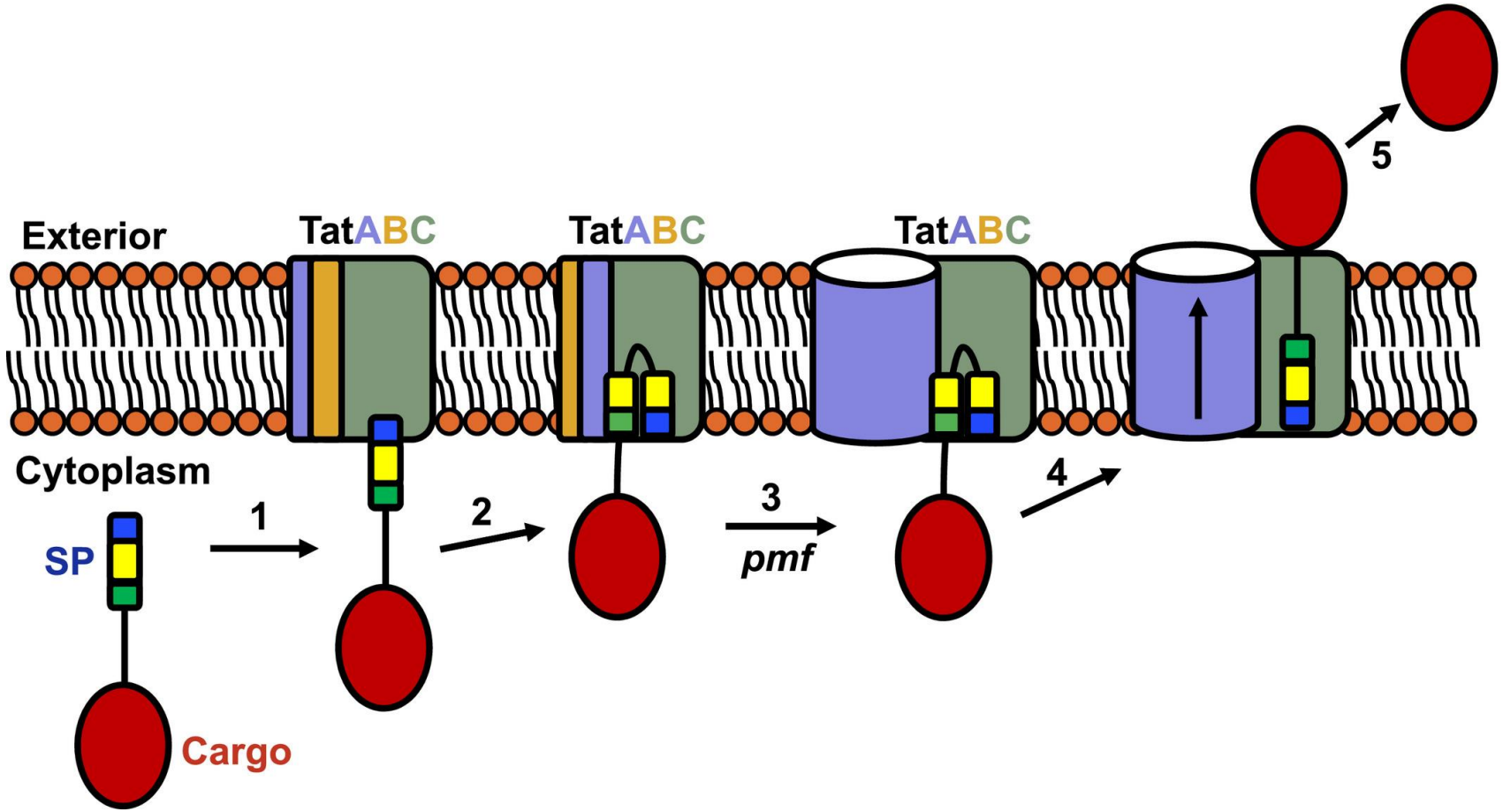
Tat complex

TatA oligomer
PDB: 2LZS



TatBC-
$CueO_{His}$ structure

Translocation model



Palmer T, Stansfeld PJ. Targeting of proteins to the twin-arginine translocation pathway. Mol Microbiol. 2020 May;113(5):861-871.

Thanks for listening