Membrane proteins

Porins: FadL

Oriol Solà, Dimitri Ivancic, Daniel Folch, Marc Olivella

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INTRODUCTION

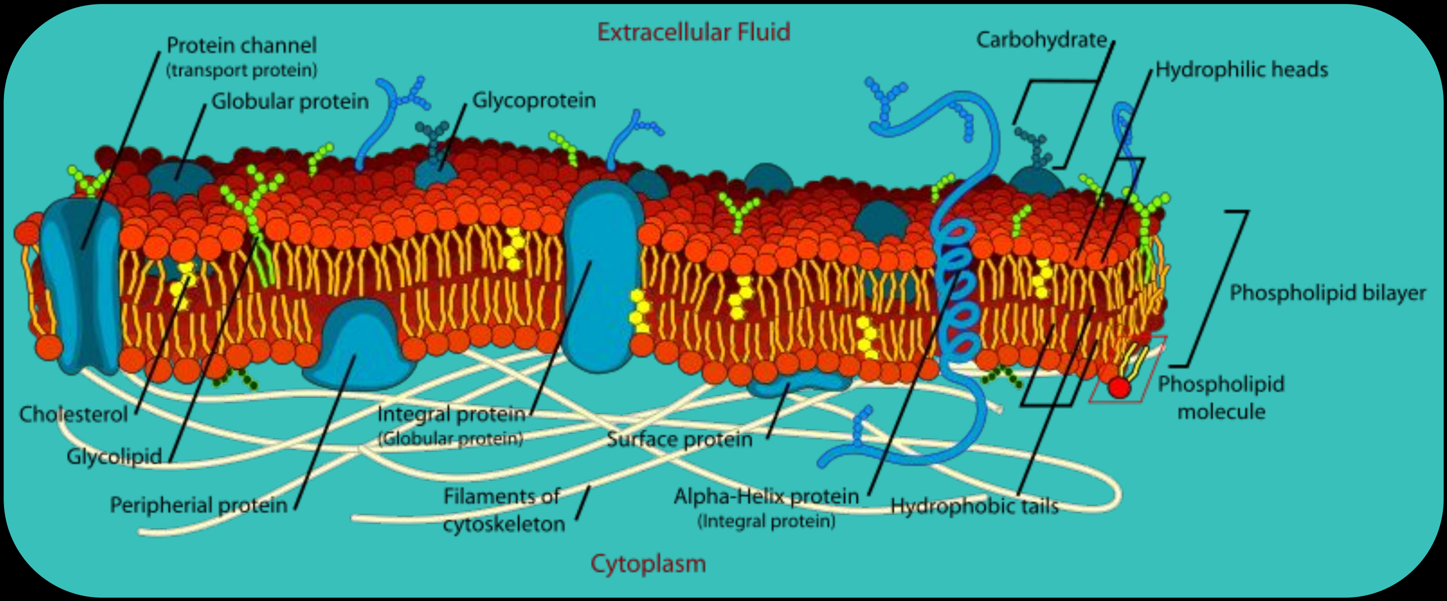
INTRODUCTION

MEMBRANE PROTEINS

Classification

Peripheral membrane proteins

Integral membrane proteins



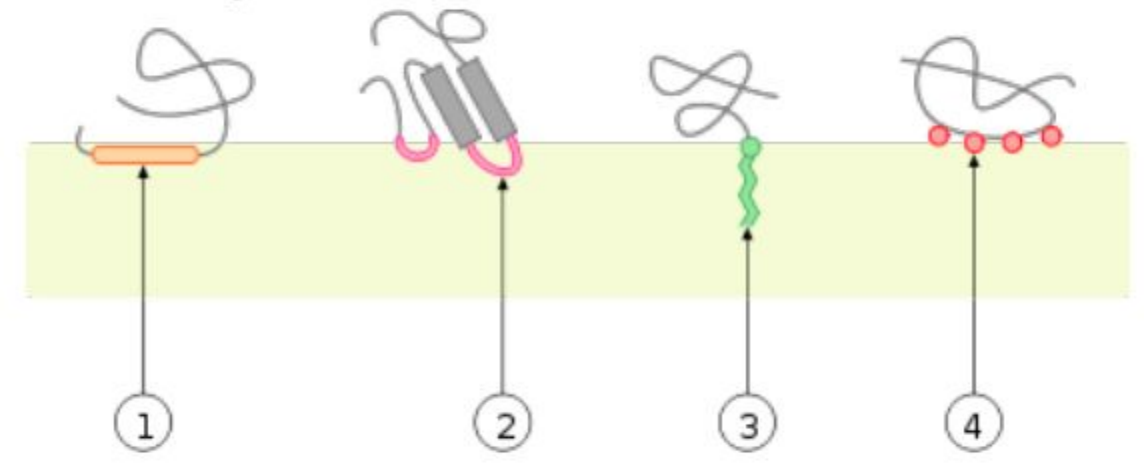
J Singer. *Science.* Vol. 175, pp. 720-731 (1972)

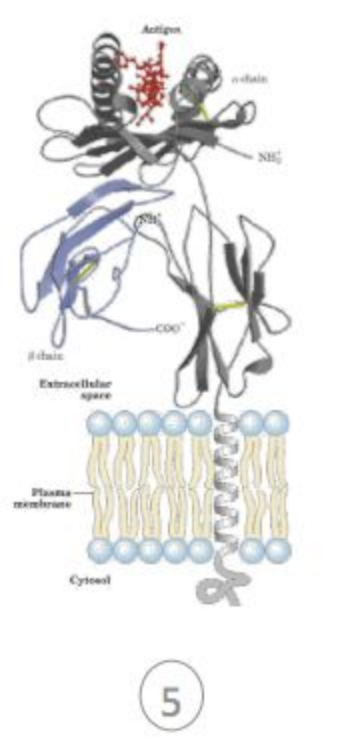
INTRODUCTION

MEMBRANE PROTEINS

Classification

Peripheral membrane proteins



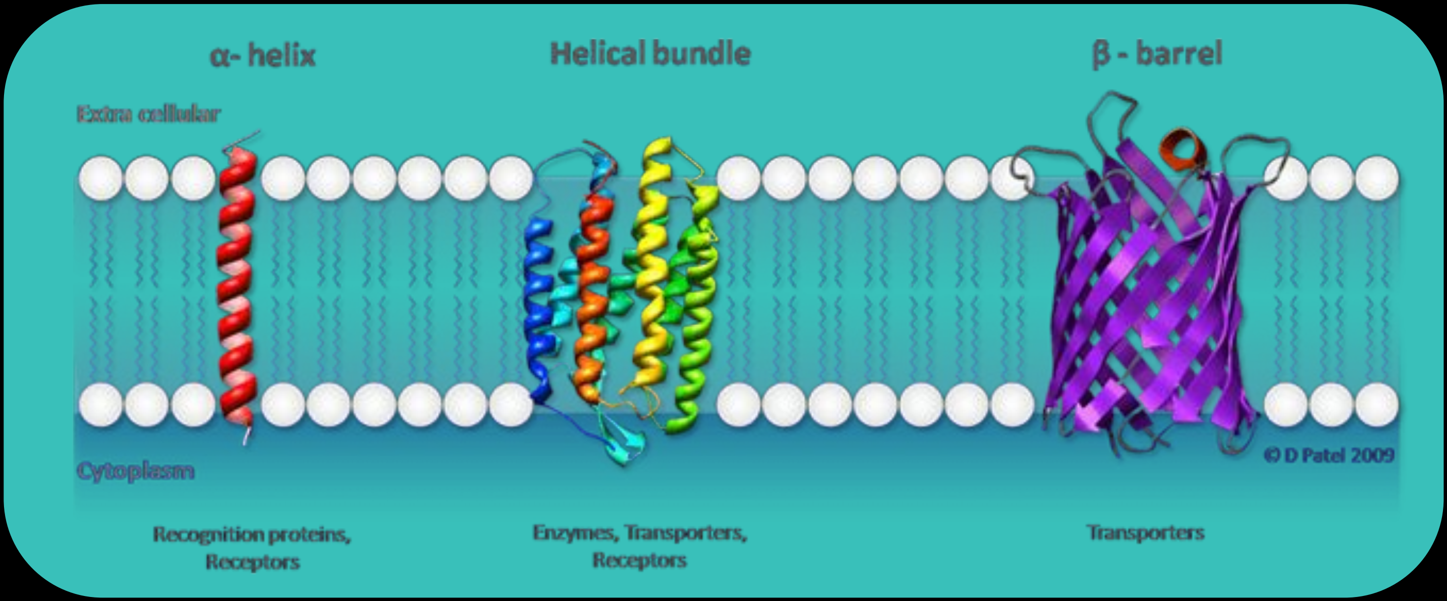


Figures exctracted from class slides BE2.3

MEMBRANE PROTEINS

Classification

Integral membrane proteins



D Patel. *Creative Biostructure* (2006)

INTRODUCTION

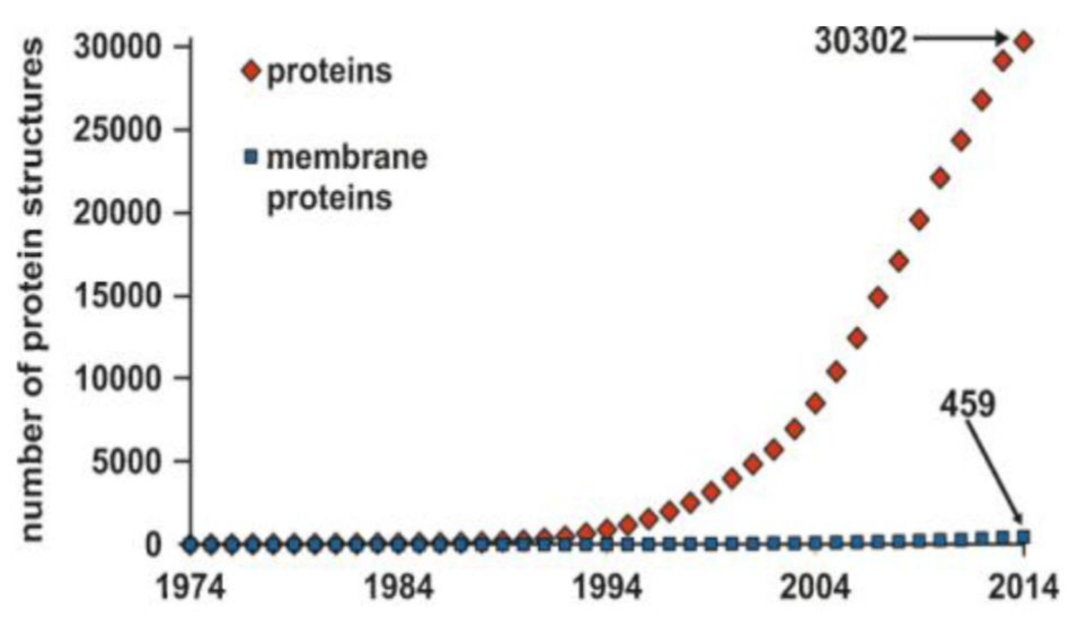
Function

MEMBRANE PROTEINS

- Membrane receptors
- Transport proteins
- Enzymatic ativity
- Cell adhesion molecules

INTRODUCTION: **IMPORTANCE OF PREDICTION**

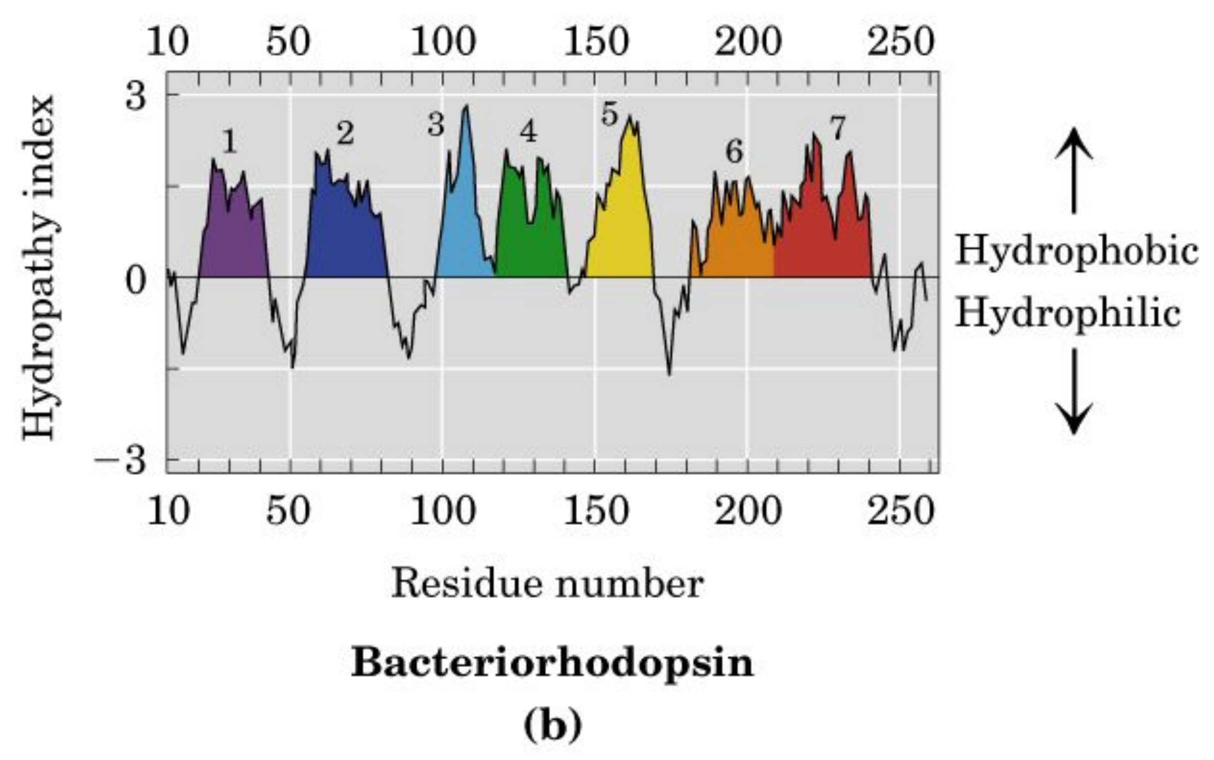
- 30% of the genome encodes for membrane proteins
- Important functions
- Few membrane protein structures solved



Bijelic A. et al (2015)

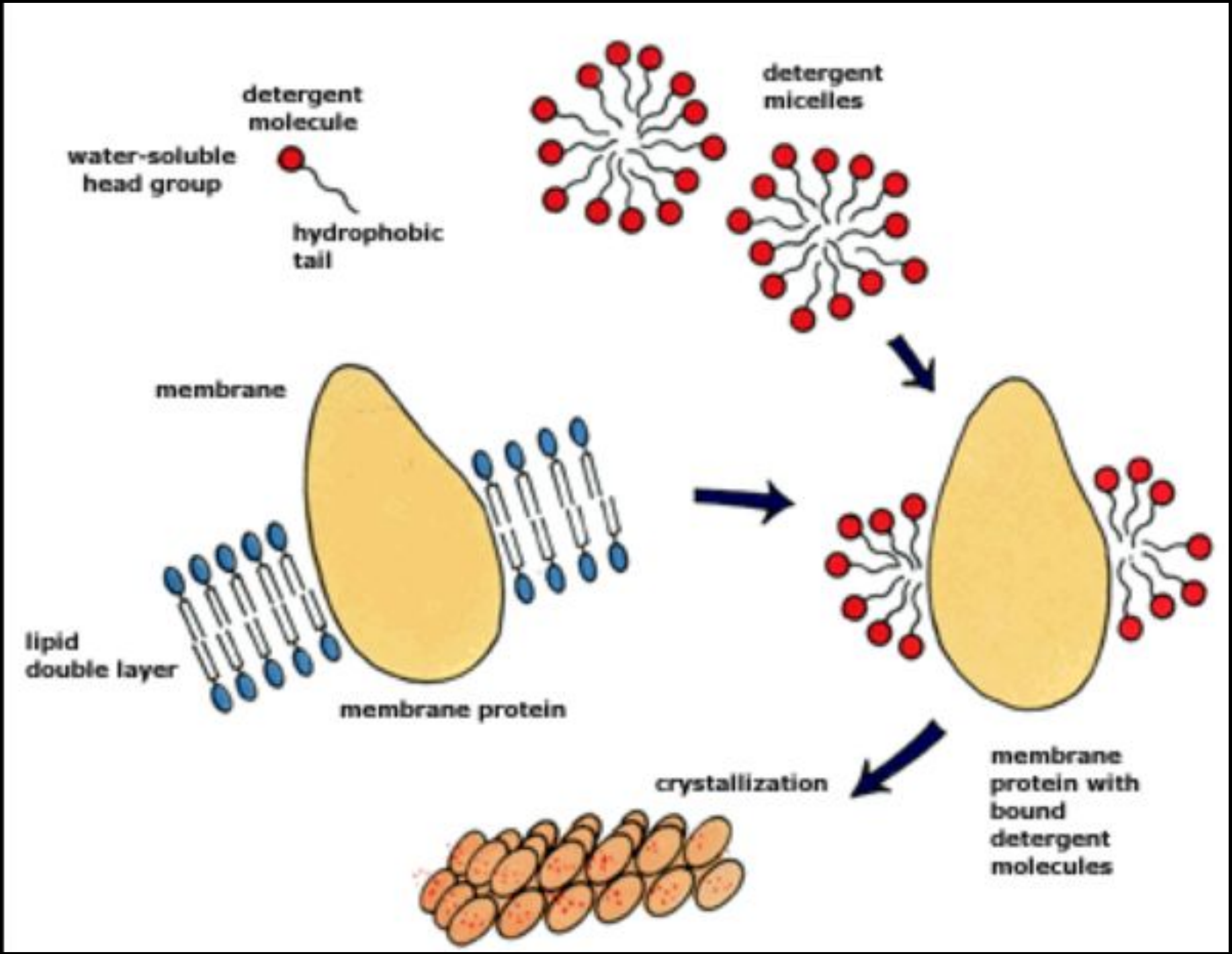
INTRODUCTION: **HIDROPATHY PLOT**

- Identify protein domains
- Hydrophobic and hydrophilic regions



Nelson & Cox. *Lehninger Principles of Biochemistry* (2000)

INTRODUCTION: **DIFFICULTIES OF PURIFICATION**



J. J .Lacapère. *Cell.* Vol 32, pp 259-270 (2007)

INTRODUCTION: **DIFFICULTIES OF CRYSTALLISATION**

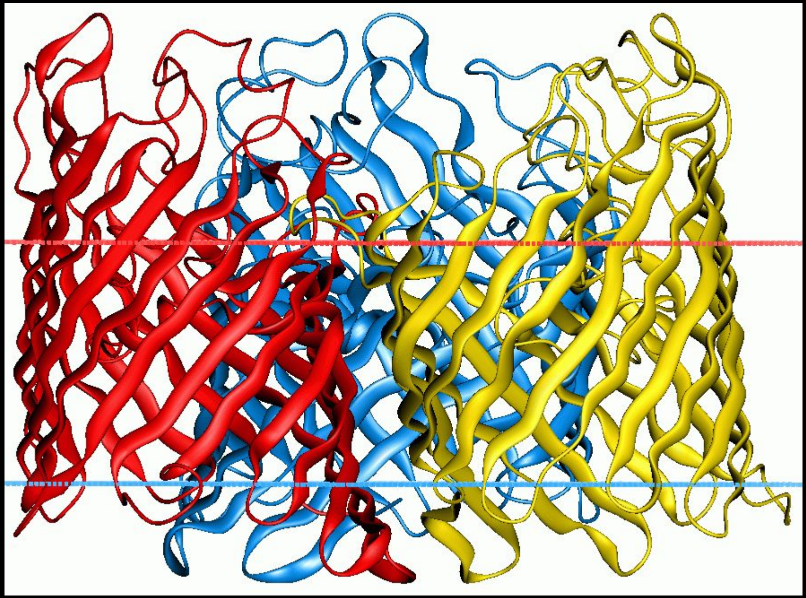
X-ray crystallography

- Partially hydrophobic surfaces
- Flexibility
- Lack of stability
- Expressed in low quantity

INTRODUCTION: **PORINS**

Structure

- Beta barrel proteins → 8-22 ß strands
- Alternated polar and nonpolar residues
- Monomers, dimeric and octameric
- “Stopper”
- Homotrimers



Jap BK, Walian PJ. *Q. Rev. Biophys.* 4. pp 367–403. (1990)

INTRODUCTION: **PORINS**

Function

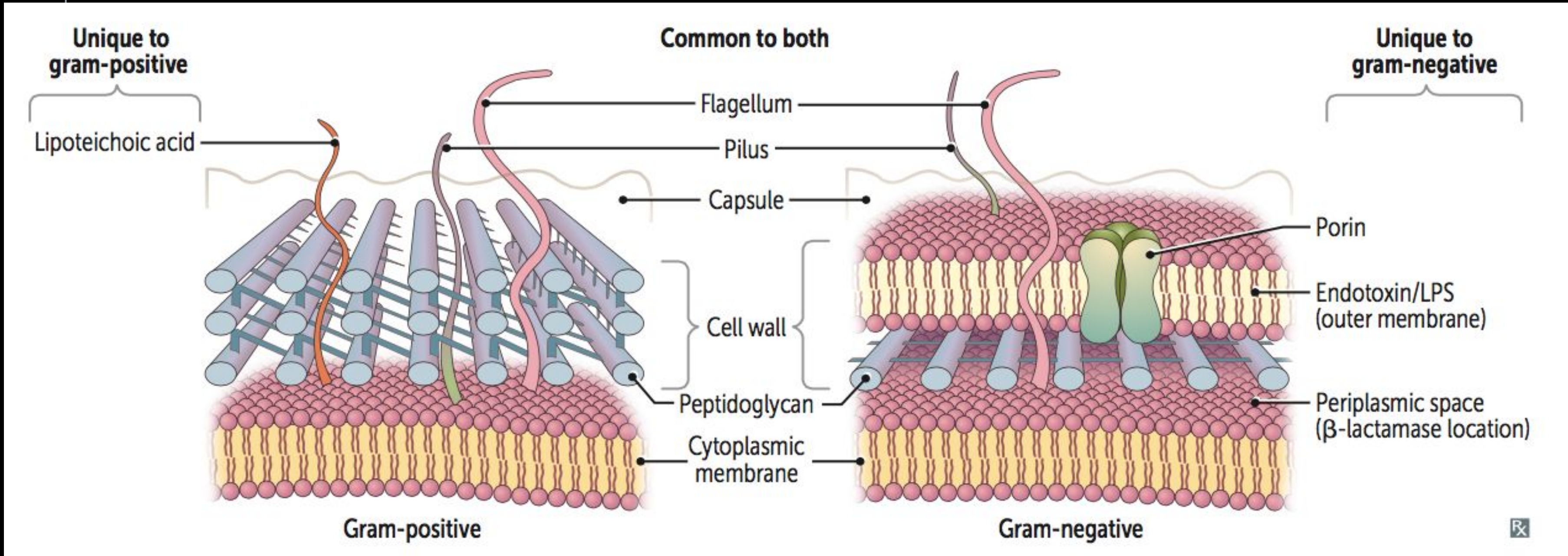
- They act as a pore
- Most of them: Passive diffusion of hydrophilic molecules
- Avoid toxic accumulation
- Regulate permeability
- Prevent lysis
- Types:
  - **General** → No substrate specificities
  - **Selective** → Specific chemical species

INTRODUCTION: **PORINS**

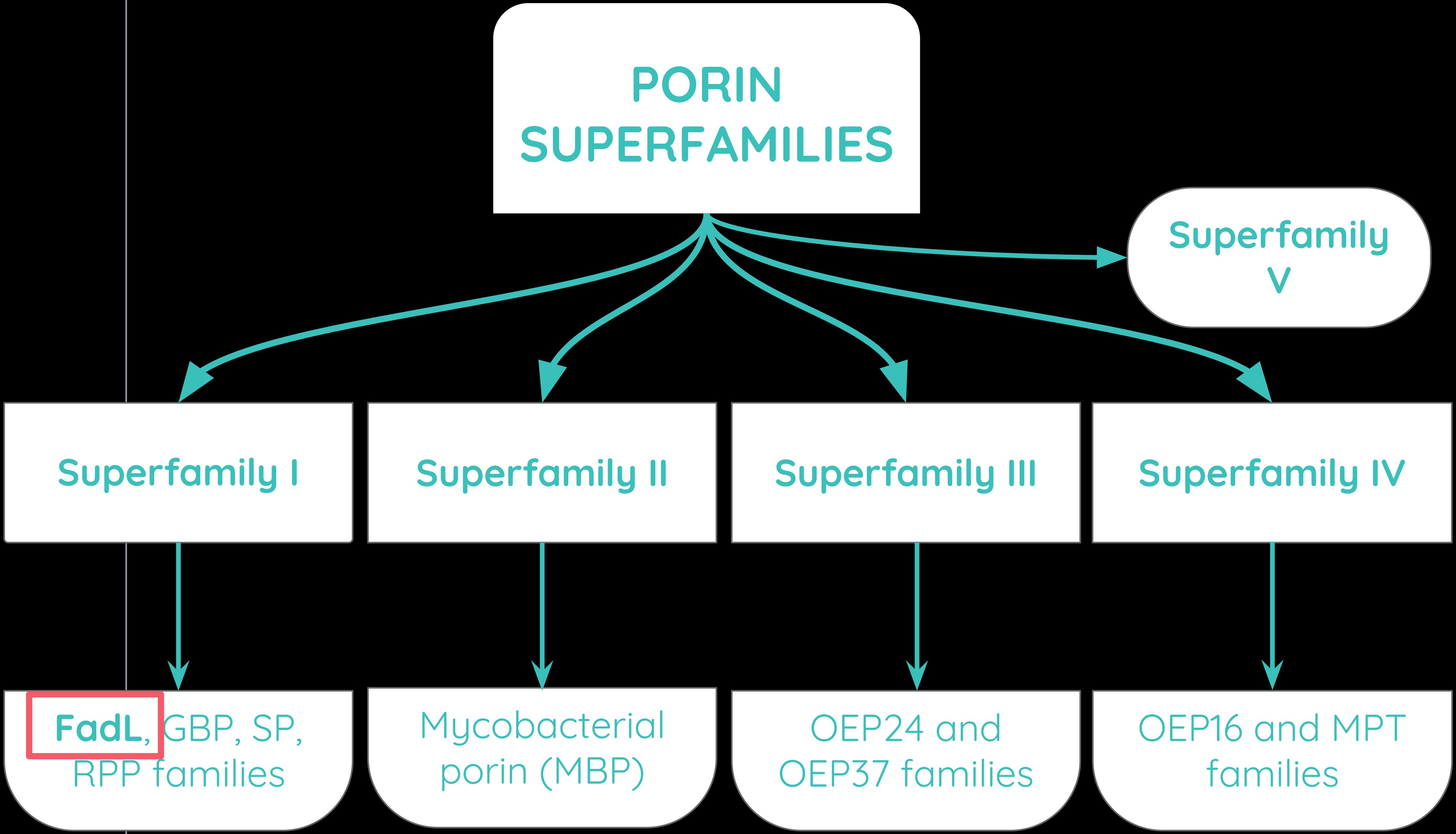
Location

- **Outer membrane** of:
  - Gram-negative bacteria and some gram-positive bacteria (Mycolata)
  - Mitochondria
  - Chloroplast

Le T and Bushan V. *Microbiology* (2016)



INTRODUCTION: **FADL Classification**



PORIN SUPERFAMILIES

Superfamily V

Superfamily I

Superfamily II

Superfamily III

Superfamily IV

FadL, GBP, SP, RPP families

Mycobacterial porin (MBP)

OEP24 and OEP37 families

OEP16 and MPT families

2

FADL: OUTER MEMBRANE TRANSPORT PROTEIN

FADL - SCOP Classification

Class	Membrane and cell surface proteins and peptides
Fold	Transmembrane beta-barrels
Superfamily	Porins
Family	Outer membrane transport protein
Protein	Long-chain fatty acid transport protein FadL

WHY FADL?

ELSEVIER

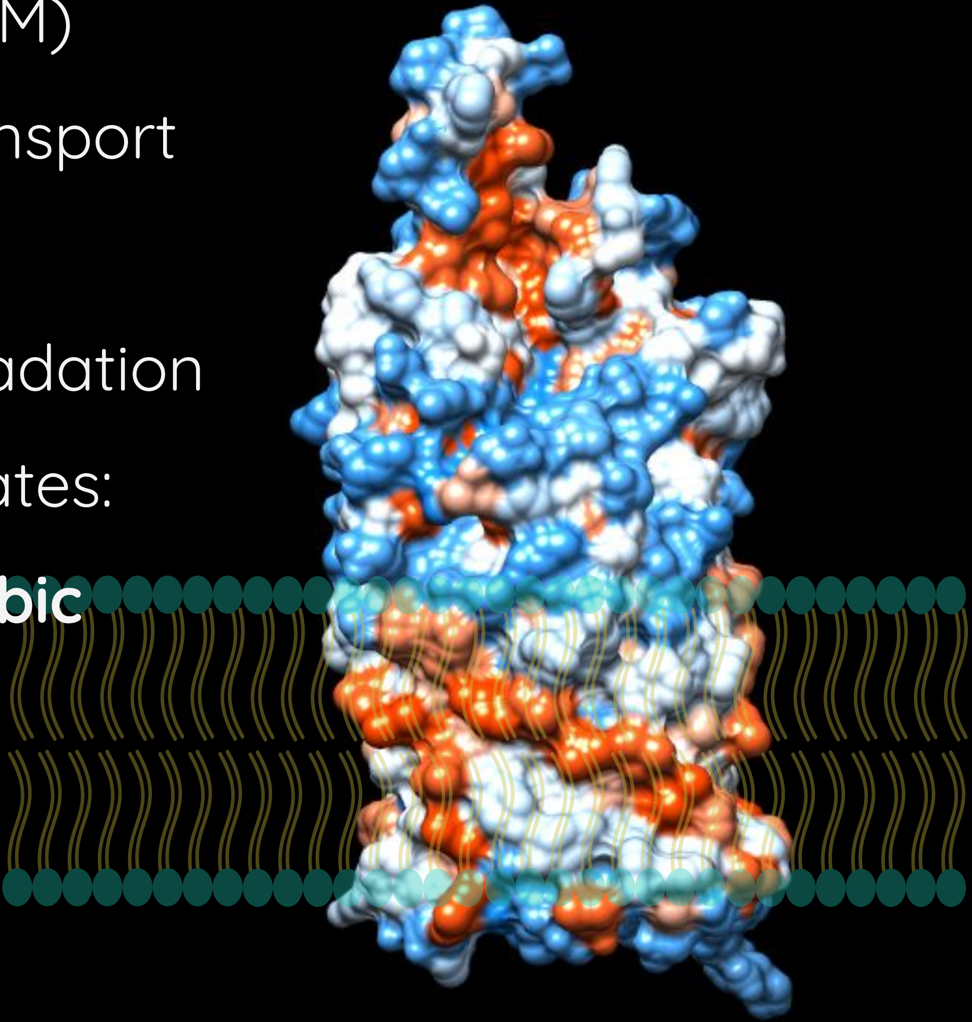
Current Opinion in Structural Biology

Volume 15, Issue 4, August 2005, Pages 401-407

The FadL family: unusual transporters for unusual substrates

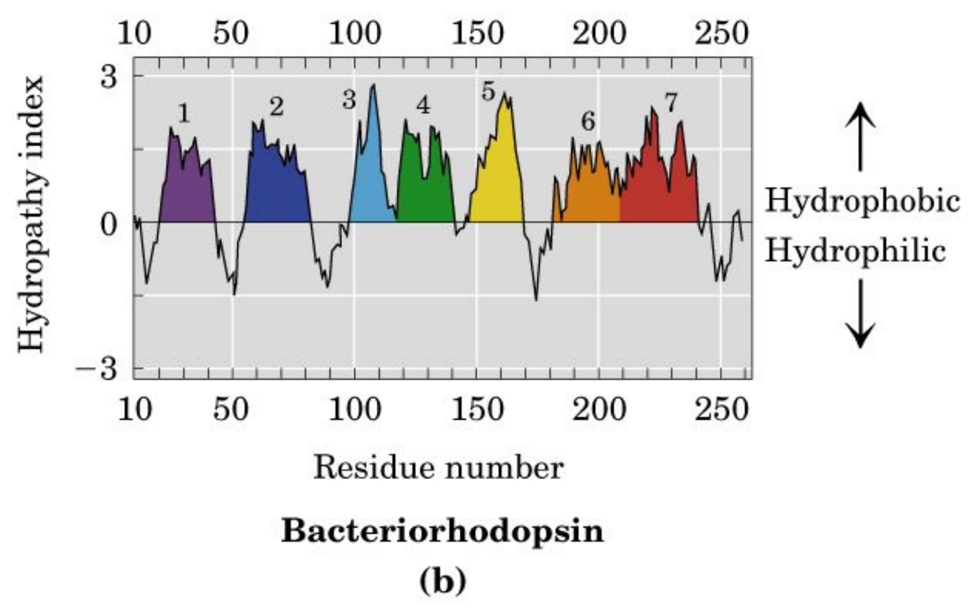
Bert van den Berg

- Outer membrane (OM) passive diffusion transport (ß-barrel)
- Xenobiotics biodegradation
- Hydrophobic substrates: Particular **hydrophobic surface**

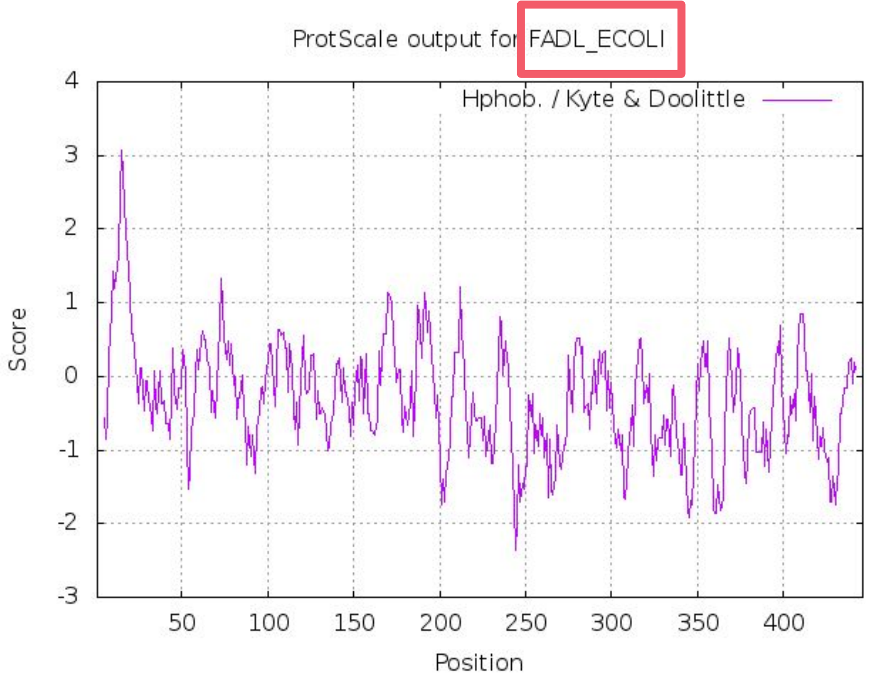


INTRODUCTION: **HIDROPATHY PLOT**

- Not useful for beta-barrel proteins
  - Short transmembrane segments (~10 aa)
  - Aminoacids are hydrophobic and hydrophilic alternately



Nelson & Cox. *Lehninger Principles of Biochemistry* (2000)



Created with ExPASy ProtScale software

PDB id: **1t16**

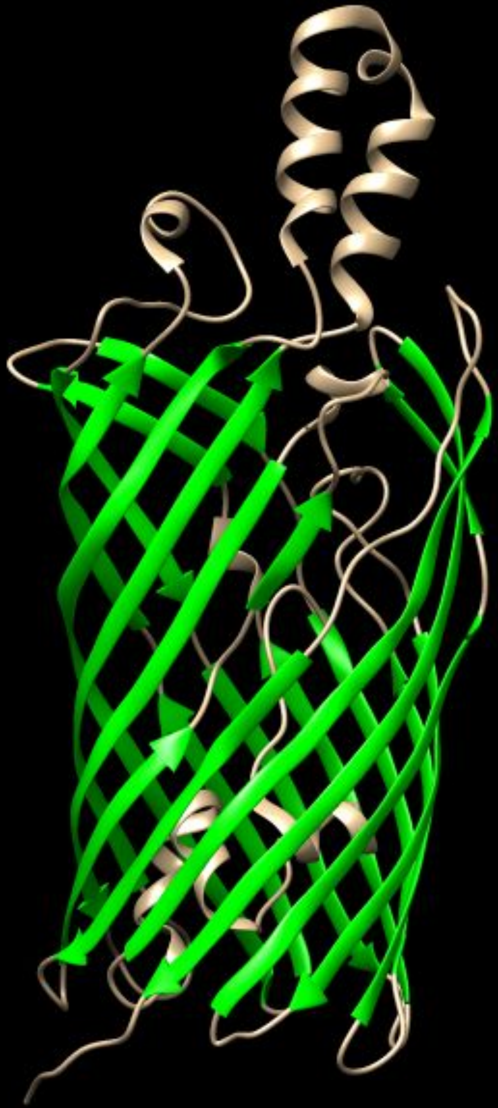
Resolution: 2,6 Å

14 ß-strands

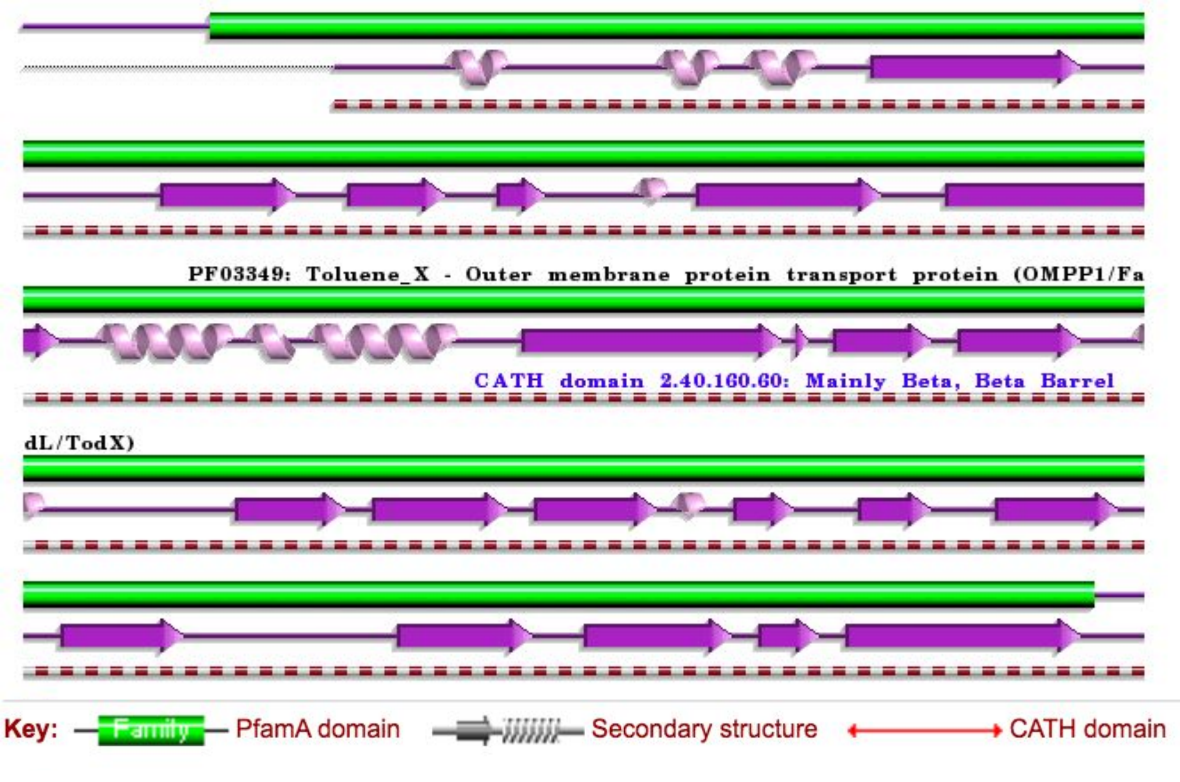
Sequence: 446aa

Structure: 427aa

FADL STRUCTURE: **MAIN CHARACTERISTICS**



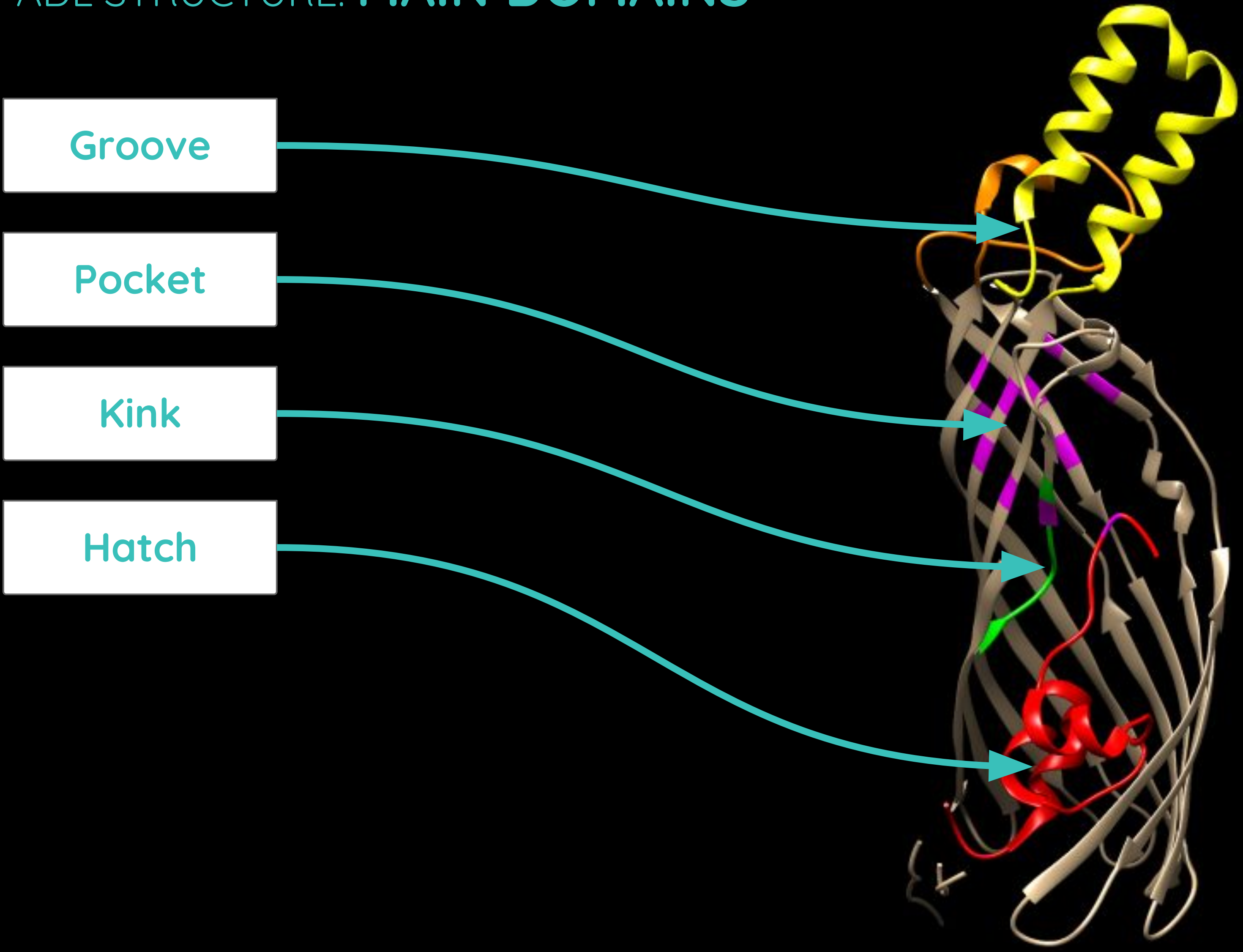
Sequence and
structure obtained
with PDB Sum



3

MAIN FEATURES OF FADL STRUCTURE

FADL STRUCTURE: **MAIN DOMAINS**



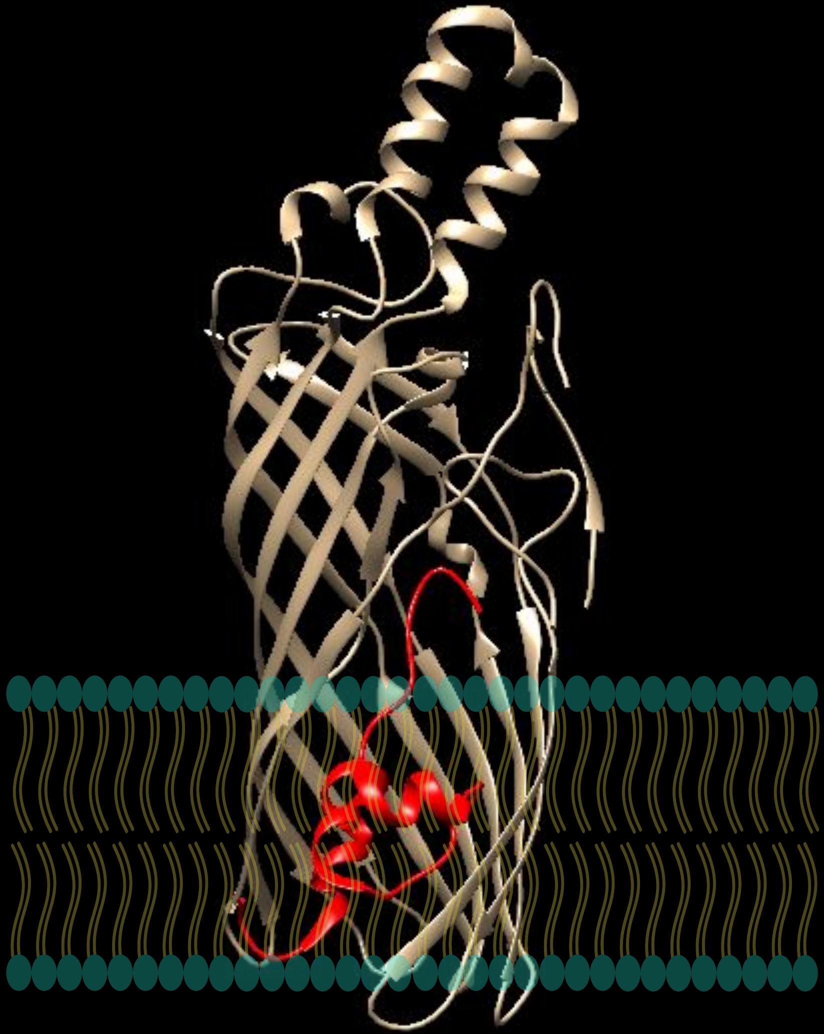
Groove

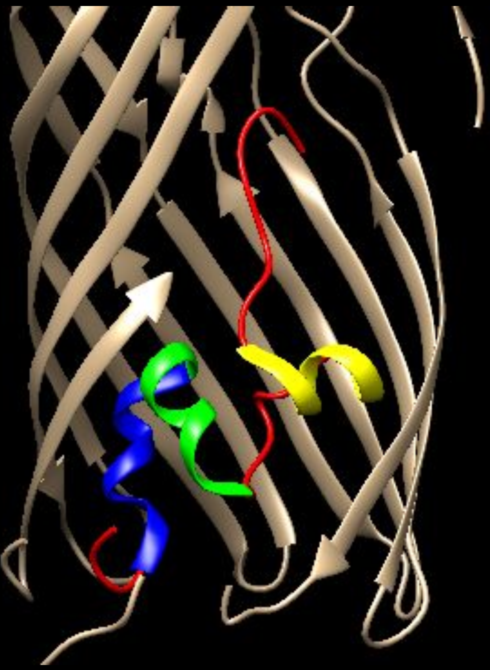
Pocket

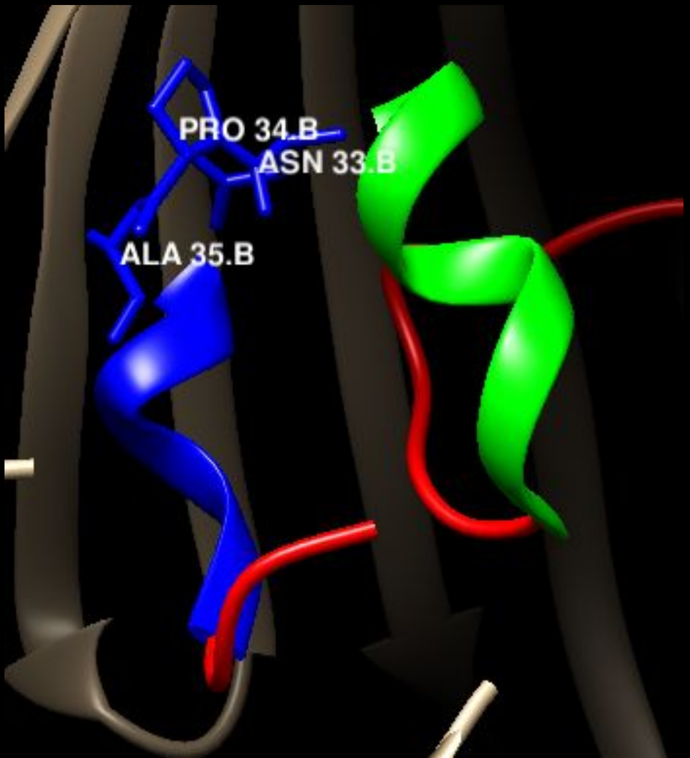
Kink

Hatch

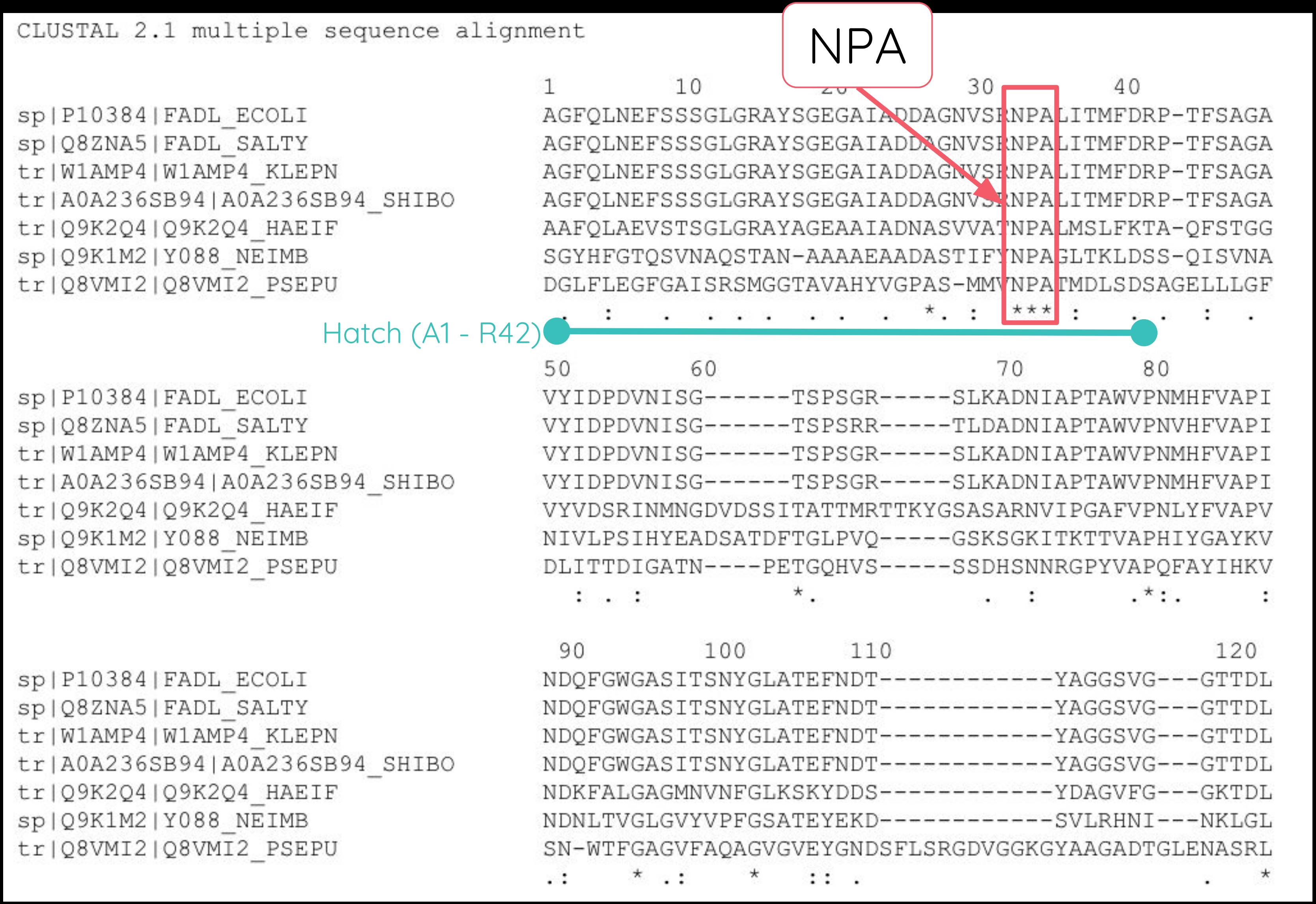
FADL STRUCTURE: **HATCH**







SEQUENCE ALIGNMENTS: **HATCH**



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sp|Q8ZNA5|FADL_SALTY              AGFQLNEFSSSGLGRAYSGEGAIADDAGNVSRNPALITMFDRP-TFSAGA
tr|W1AMP4|W1AMP4_KLEPN            AGFQLNEFSSSGLGRAYSGEGAIADDAGNVSRNPALITMFDRP-TFSAGA
tr|A0A236SB94|A0A236SB94_SHIBO    AGFQLNEFSSSGLGRAYSGEGAIADDAGNVSRNPALITMFDRP-TFSAGA
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                                  50        60                  70        80
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                                   90       100        110                     120
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tr|A0A236SB94|A0A236SB94_SHIBO    NDQFGWGASITSNYGLATEFNDT------------YAGGSVG---GTTDL
tr|Q9K2Q4|Q9K2Q4_HAEIF            NDKFALGAGMNVNFGLKSKYDDS------------YDAGVFG---GKTDL
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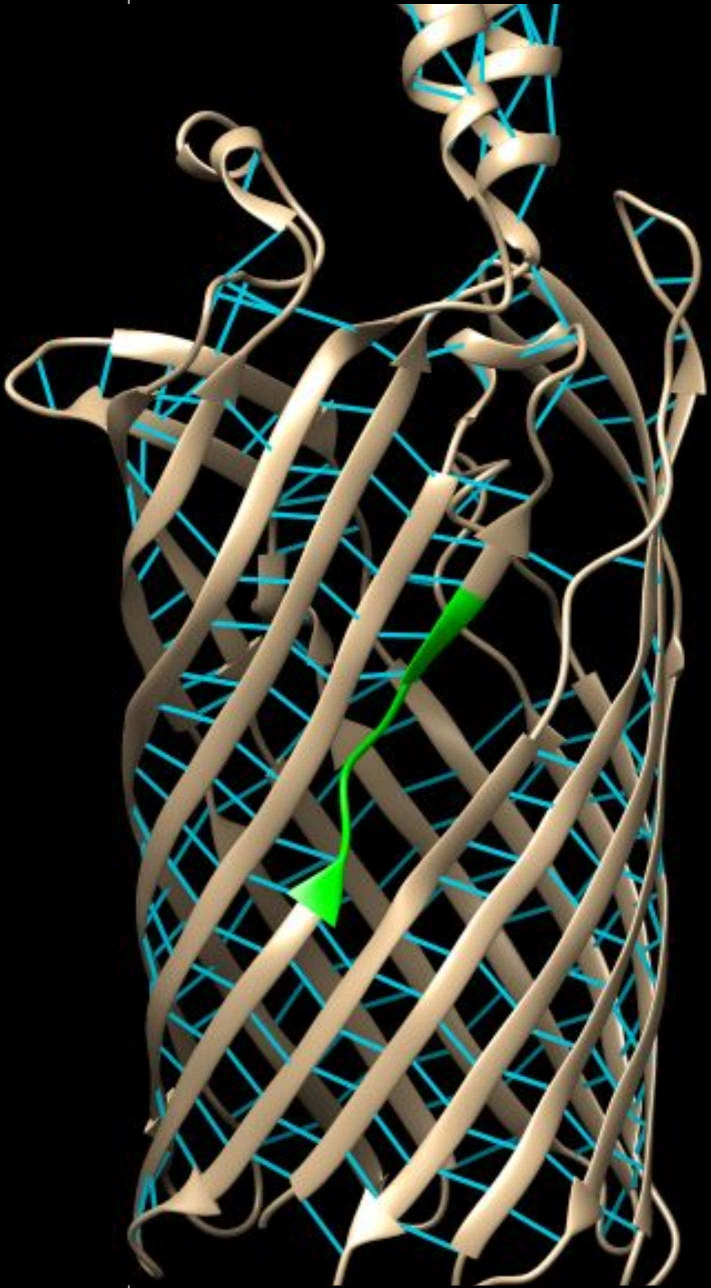
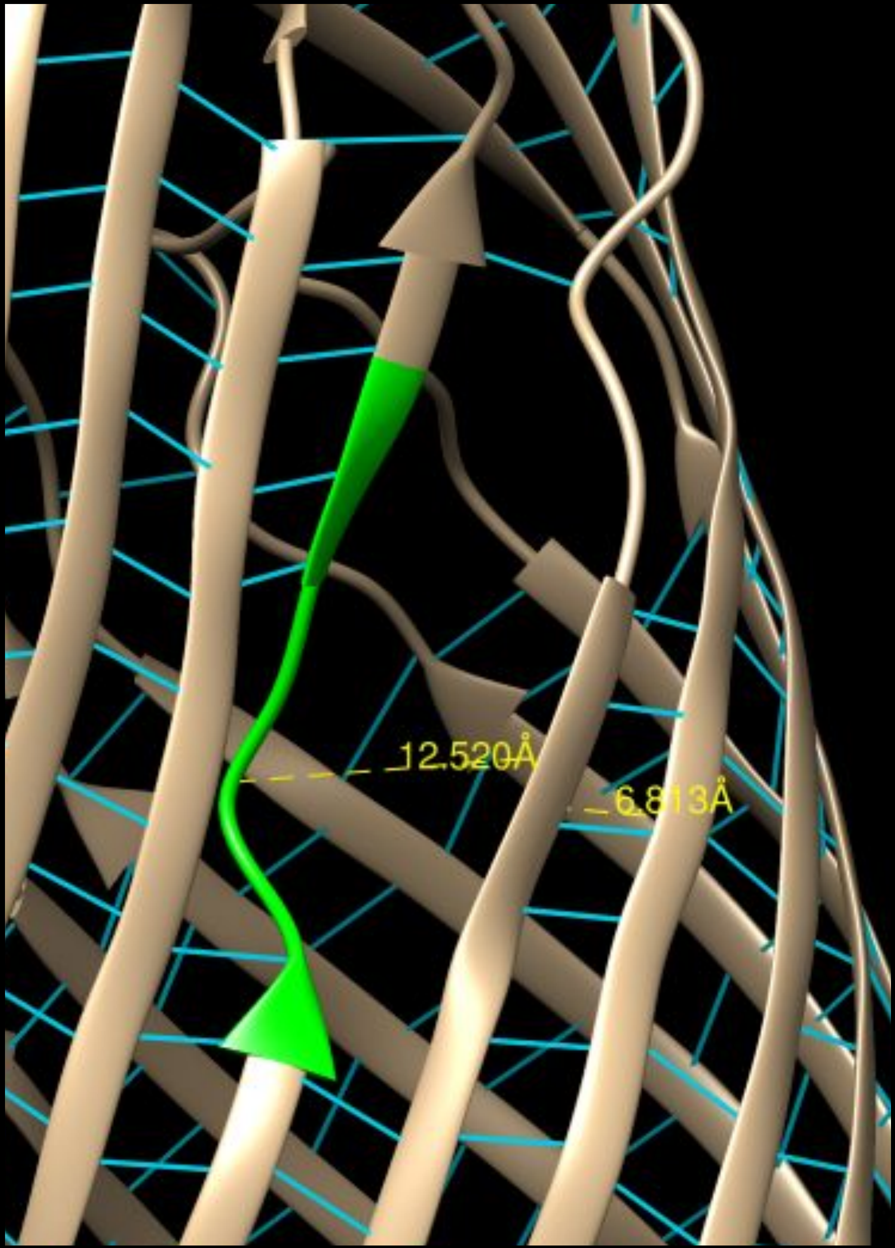
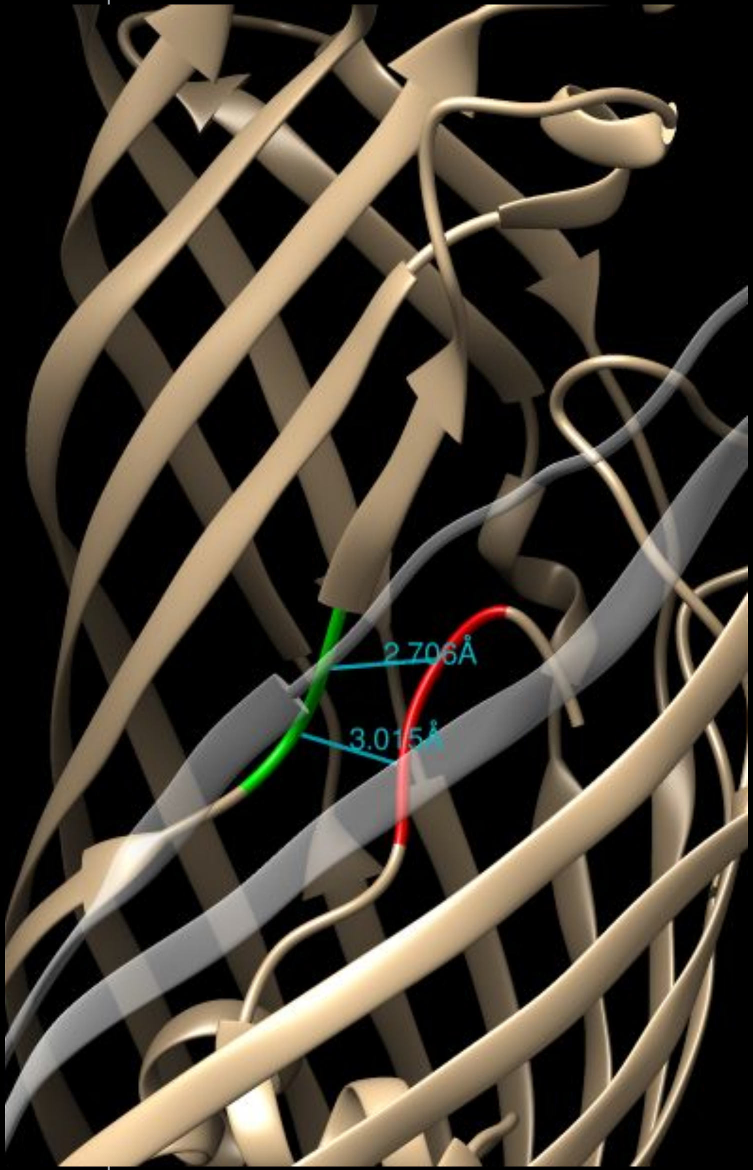
```

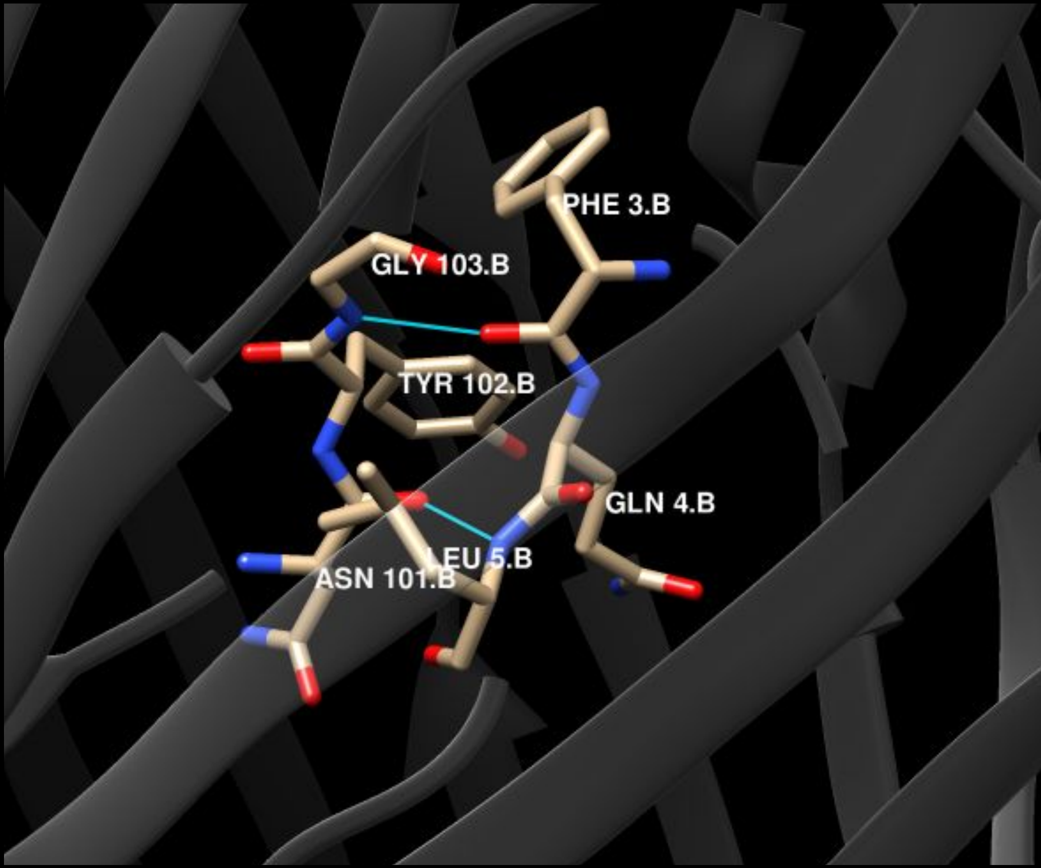
FADL STRUCTURE: **KINK**





FADL STRUCTURE: **KINK (HYDROGEN BONDS)**





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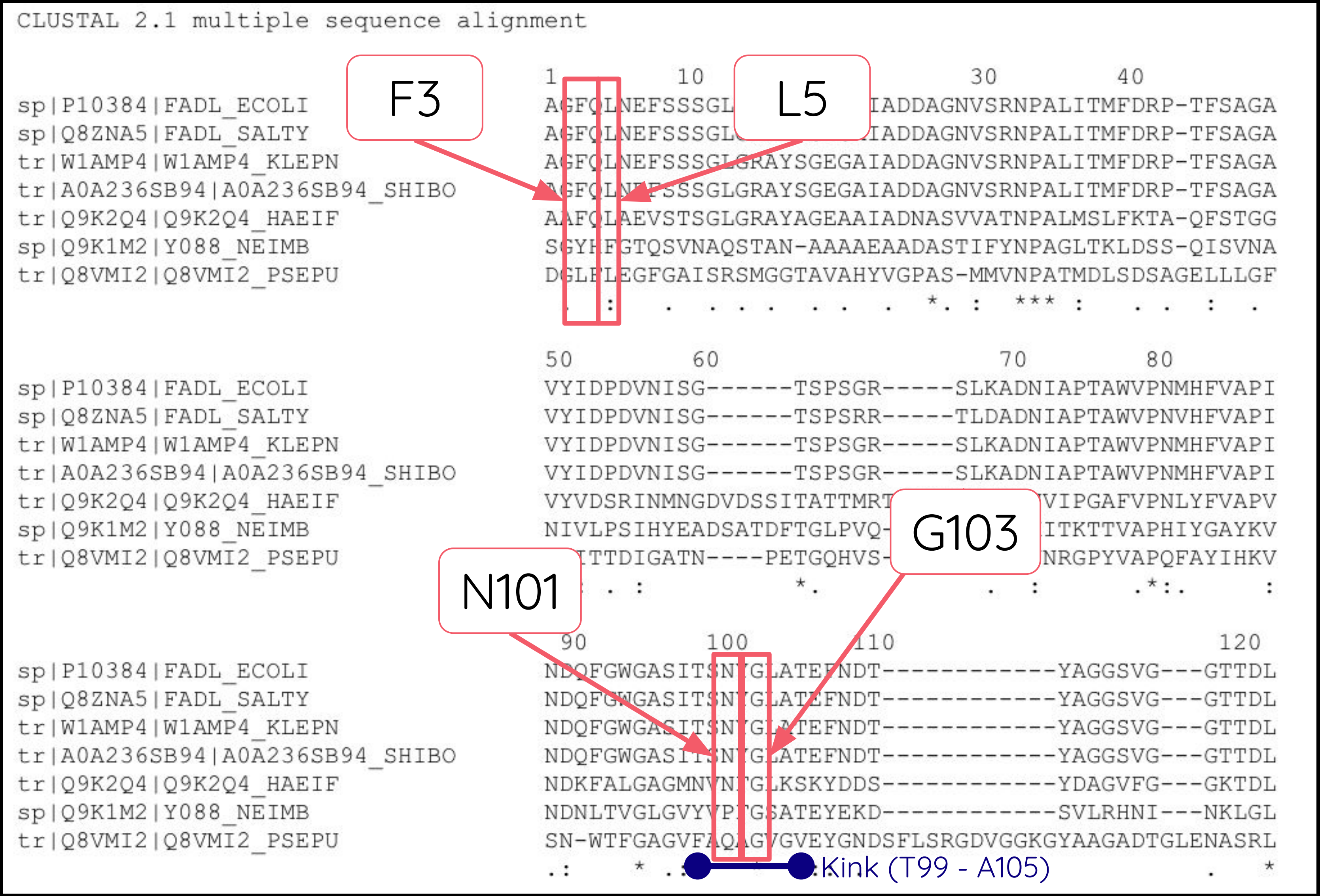

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sp|Q8ZNA5|FADL_SALTY               AGFQLNEFSSSGL         IADDAGNVSRNPALITMFDRP-TFSAGA
tr|W1AMP4|W1AMP4_KLEPN             AGFQLNEFSSSGLGRAYSGEGAIADDAGNVSRNPALITMFDRP-TFSAGA
tr|A0A236SB94|A0A236SB94_SHIBO     AGFQLNEFSSSGLGRAYSGEGAIADDAGNVSRNPALITMFDRP-TFSAGA
tr|Q9K2Q4|Q9K2Q4_HAEIF             AAFQLAEVSTSGLGRAYAGEAAIADNASVVATNPALMSLFKTA-QFSTGG
sp|Q9K1M2|Y088_NEIMB               SGYHFGTQSVNAQSTAN-AAAAEAADASTIFYNPAGLTKLDSS-QISVNA
tr|Q8VMI2|Q8VMI2_PSEPU             DGLFLEGFGAISRSMGGTAVAHYVGPAS-MMVNPATMDLSDSAGELLLGF
                                    .  :   .  . . .  . .  .  *. : *** :   . .  :  .

                                   50        60                  70        80
sp|P10384|FADL_ECOLI               VYIDPDVNISG------TSPSGR-----SLKADNIAPTAWVPNMHFVAPI
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tr|Q8VMI2|Q8VMI2_PSEPU               ITTDIGATN----PETGQHVS-         NRGPYVAPQFAYIHKV
                                     : . :          *.           .  :     .*:.     :

                                     90        100        110                    120
sp|P10384|FADL_ECOLI               NDQFGWGASITSNYGLATEFNDT------------YAGGSVG---GTTDL
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tr|W1AMP4|W1AMP4_KLEPN             NDQFGWGASITSNYGLATEFNDT------------YAGGSVG---GTTDL
tr|A0A236SB94|A0A236SB94_SHIBO     NDQFGWGASITSNYGLATEFNDT------------YAGGSVG---GTTDL
tr|Q9K2Q4|Q9K2Q4_HAEIF             NDKFALGAGMNVNTGLKSKYDDS------------YDAGVFG---GKTDL
sp|Q9K1M2|Y088_NEIMB               NDNLTVGLGVYVPTGSATEYEKD------------SVLRHNI---NKLGL
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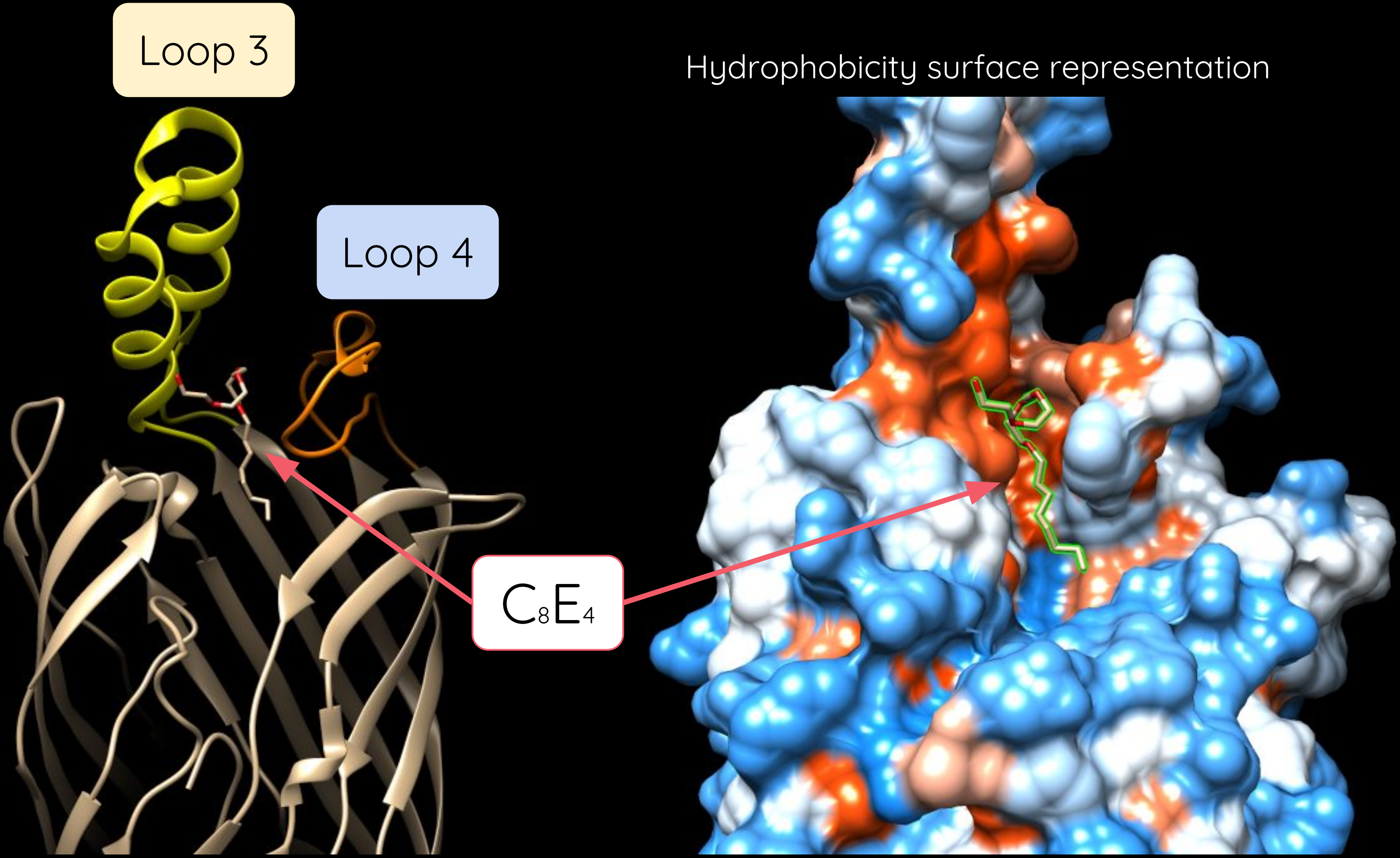
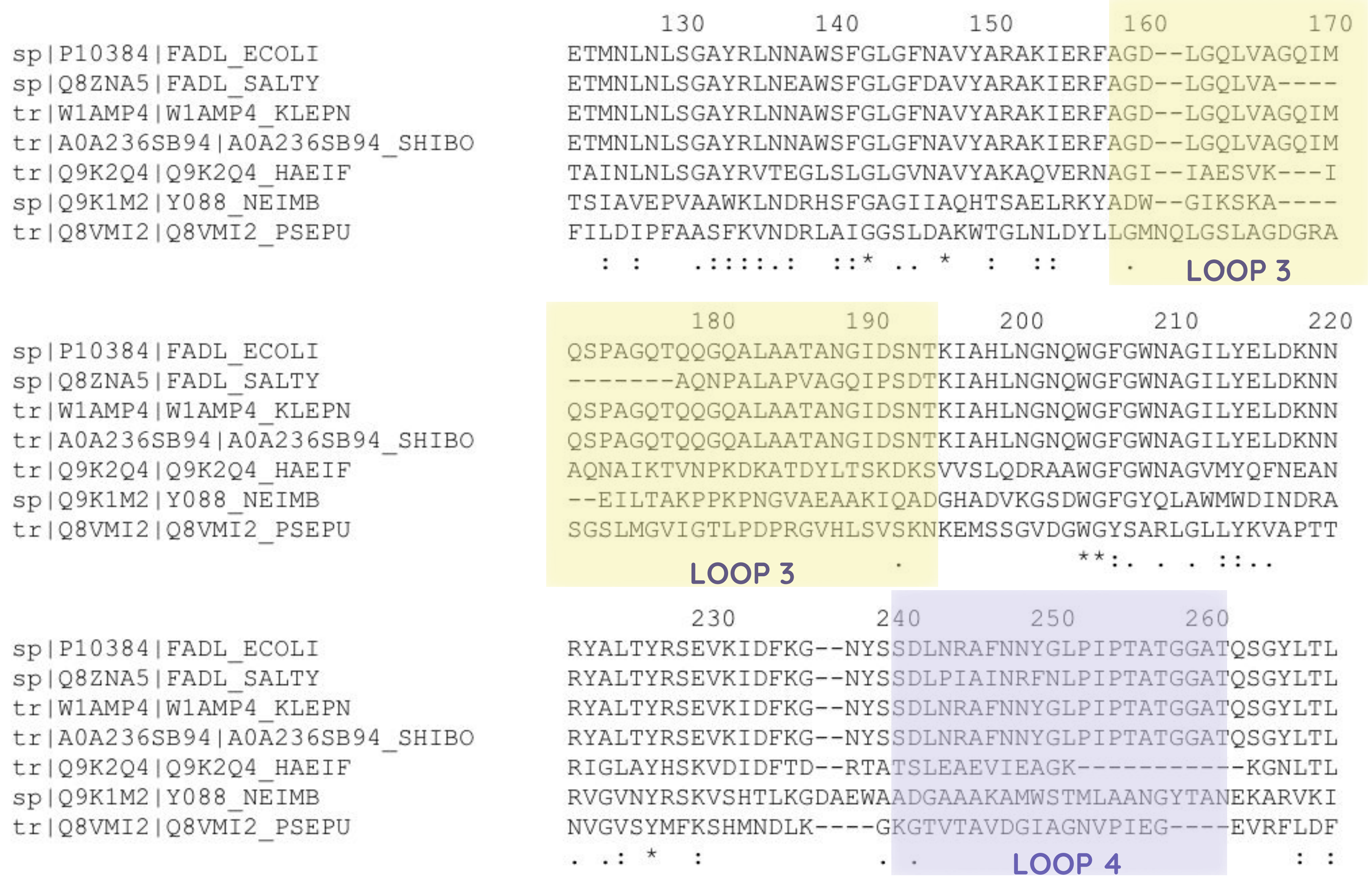
FADL STRUCTURE: **GROOVE**



Hydrophobicity surface representation

SEQUENCE ALIGNMENTS: **GROOVE**



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tr|A0A236SB94|A0A236SB94_SHIBO      ETMNLNLSGAYRLNNAWSFGLGFNAVYARAKIERFAGD--LGQLVAGQIM
tr|Q9K2Q4|Q9K2Q4_HAEIF              TAINLNLSGAYRVTEGLSLGLGVNAVYAKAQVERNAGI--IAESVK---I
sp|Q9K1M2|Y088_NEIMB                TSIAVEPVAAWKLNDRHSFGAGIIAQHTSAELRKYADW--GIKSKA----
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sp|Q9K1M2|Y088_NEIMB                --EILTAKPPKPNGVAEAAKIQADGHADVKGSDWGFGYQLAWMWDINDRA
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                                                         .           **:. . . ::..

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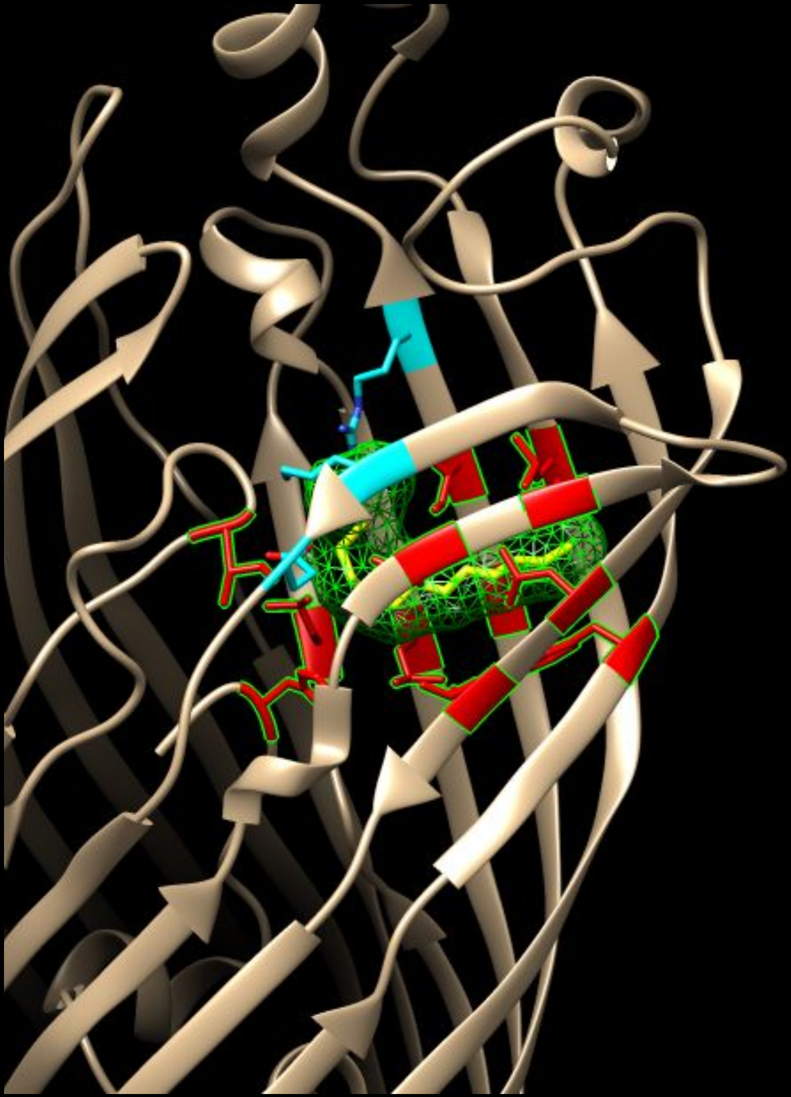
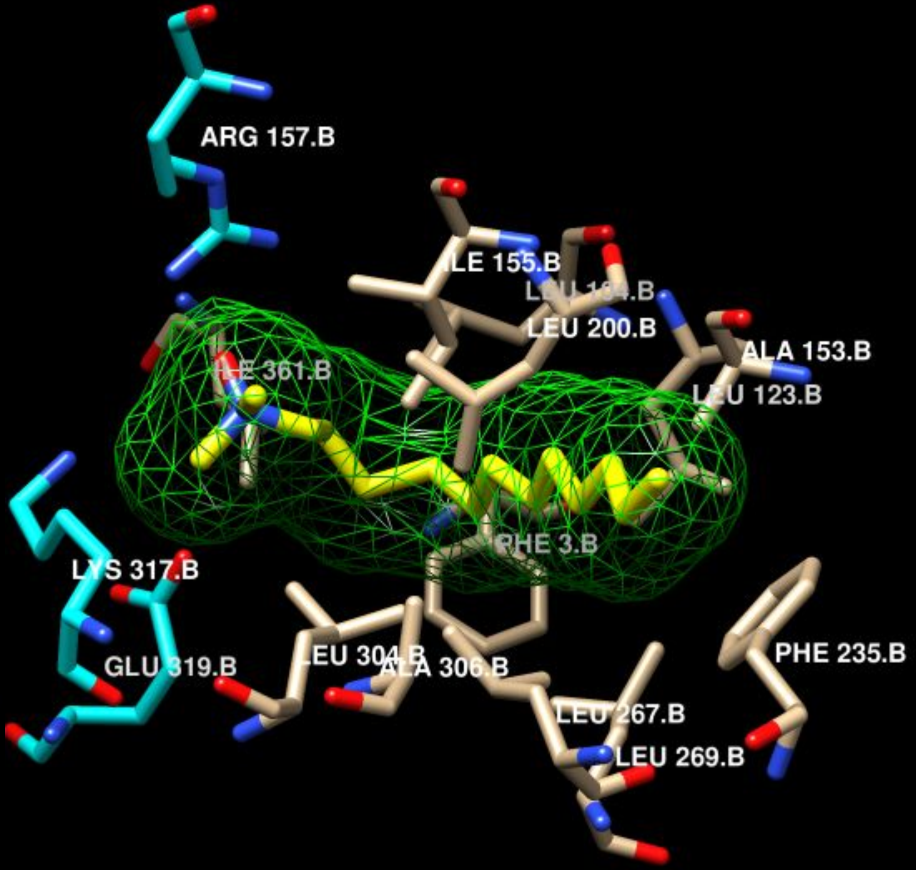
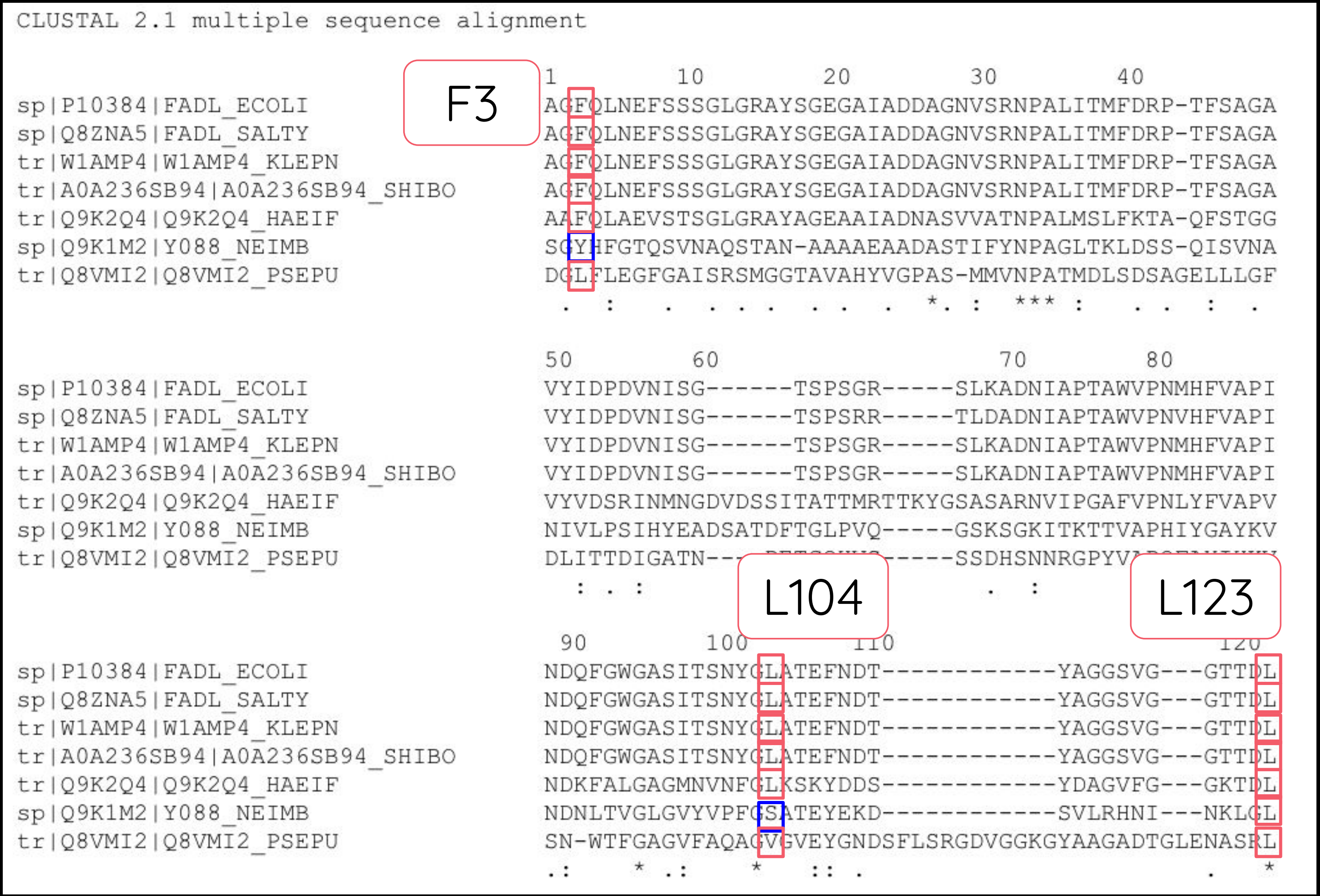
FADL STRUCTURE: **POCKET**





Polar AA	R157; K317; E319
Hydrophobic AA	F3; L104; L123; A153; I155; L200; F235; L267; L269; L304; A306; I361

SEQUENCE ALIGNMENTS: **POCKET**



F3

L104

L123

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CLUSTAL 2.1 multiple sequence alignment

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tr|W1AMP4|W1AMP4_KLEPN              AGFQLNEFSSSGLGRAYSGEGAIADDAGNVSRNPALITMFDRP-TFSAGA
tr|A0A236SB94|A0A236SB94_SHIBO      AGFQLNEFSSSGLGRAYSGEGAIADDAGNVSRNPALITMFDRP-TFSAGA
tr|Q9K2Q4|Q9K2Q4_HAEIF              AAFQLAEVSTSGLGRAYAGEAAIADNASVVATNPALMSLFKTA-QFSTGG
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tr|Q8VMI2|Q8VMI2_PSEPU              DGLFLEGFGAISRSMGGTAVAHYVGPAS-MMVNPATMDLSDSAGELLLGF
                                     .  :   .  . . .  . .  .  *. :  *** :   . .  :  .

                                    50        60                   70        80
sp|P10384|FADL_ECOLI                VYIDPDVNISG------TSPSGR-----SLKADNIAPTAWVPNMHFVAPI
sp|Q8ZNA5|FADL_SALTY                VYIDPDVNISG------TSPSRR-----TLDADNIAPTAWVPNVHFVAPI
tr|W1AMP4|W1AMP4_KLEPN              VYIDPDVNISG------TSPSGR-----SLKADNIAPTAWVPNMHFVAPI
tr|A0A236SB94|A0A236SB94_SHIBO      VYIDPDVNISG------TSPSGR-----SLKADNIAPTAWVPNMHFVAPI
tr|Q9K2Q4|Q9K2Q4_HAEIF              VYVDSRINMNGDVDSSITATTMRTTKYGSASARNVIPGAFVPNLYFVAPV
sp|Q9K1M2|Y088_NEIMB                NIVLPSIHYEADSATDFTGLPVQ-----GSKSGKITKTTVAPHIYGAYKV
tr|Q8VMI2|Q8VMI2_PSEPU              DLITTDIGATN---         -----SSDHSNNRGPYV
                                      : . :                       .  :

                                     90        100       110                      120
sp|P10384|FADL_ECOLI                NDQFGWGASITSNYGLATEFNDT------------YAGGSVG---GTTDL
sp|Q8ZNA5|FADL_SALTY                NDQFGWGASITSNYGLATEFNDT------------YAGGSVG---GTTDL
tr|W1AMP4|W1AMP4_KLEPN              NDQFGWGASITSNYGLATEFNDT------------YAGGSVG---GTTDL
tr|A0A236SB94|A0A236SB94_SHIBO      NDQFGWGASITSNYGLATEFNDT------------YAGGSVG---GTTDL
tr|Q9K2Q4|Q9K2Q4_HAEIF              NDKFALGAGMNVNFGLKSKYDDS------------YDAGVFG---GKTDL
sp|Q9K1M2|Y088_NEIMB                NDNLTVGLGVYVPFGSATEYEKD------------SVLRHNI---NKLGL
tr|Q8VMI2|Q8VMI2_PSEPU              SN-WTFGAGVFAQAGVGVEYGNDSFLSRGDVGGKGYAAGADTGLENASRL
                                    .:    * .:    *   :: .                       .   *
```
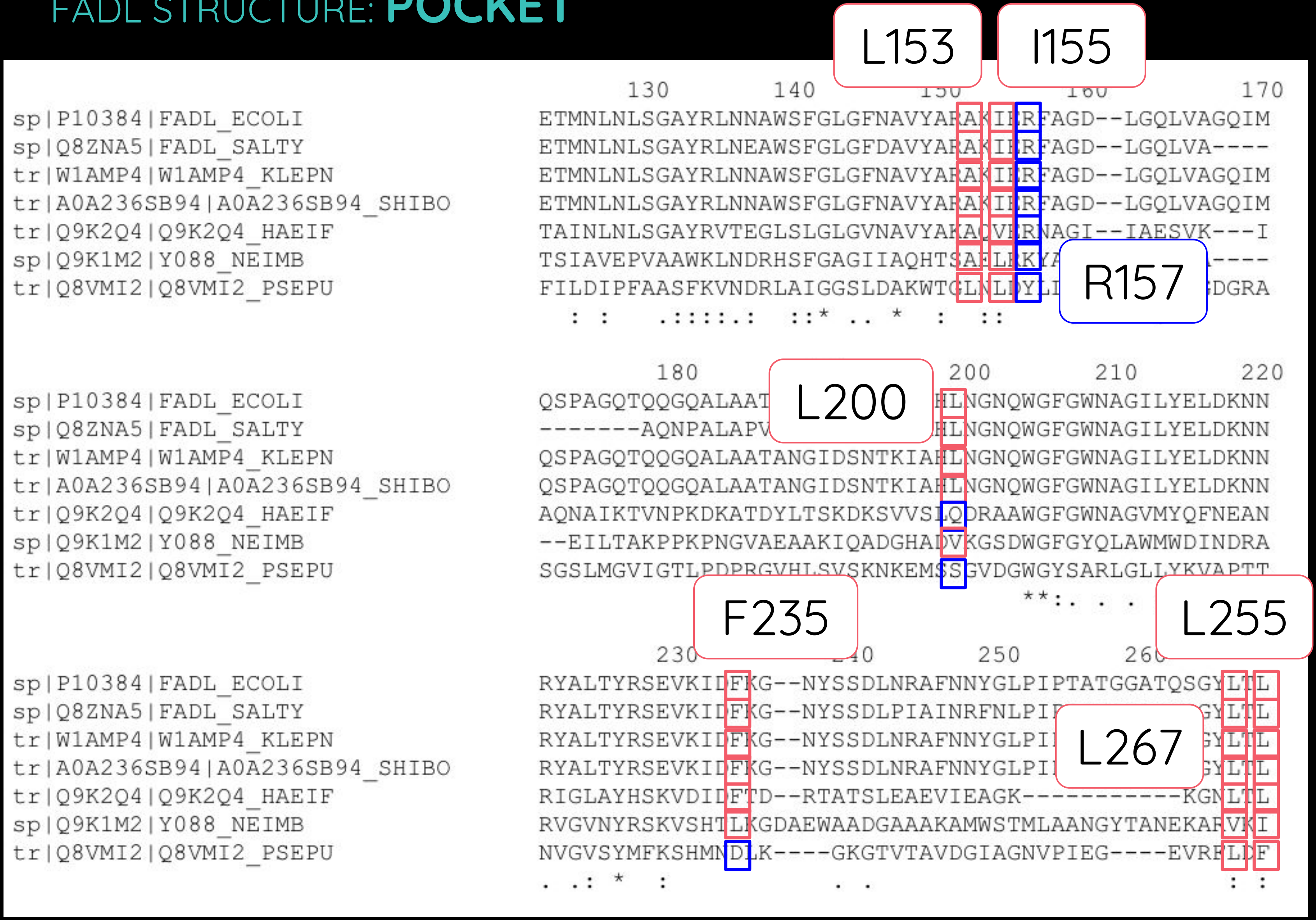

FADL STRUCTURE: **POCKET**



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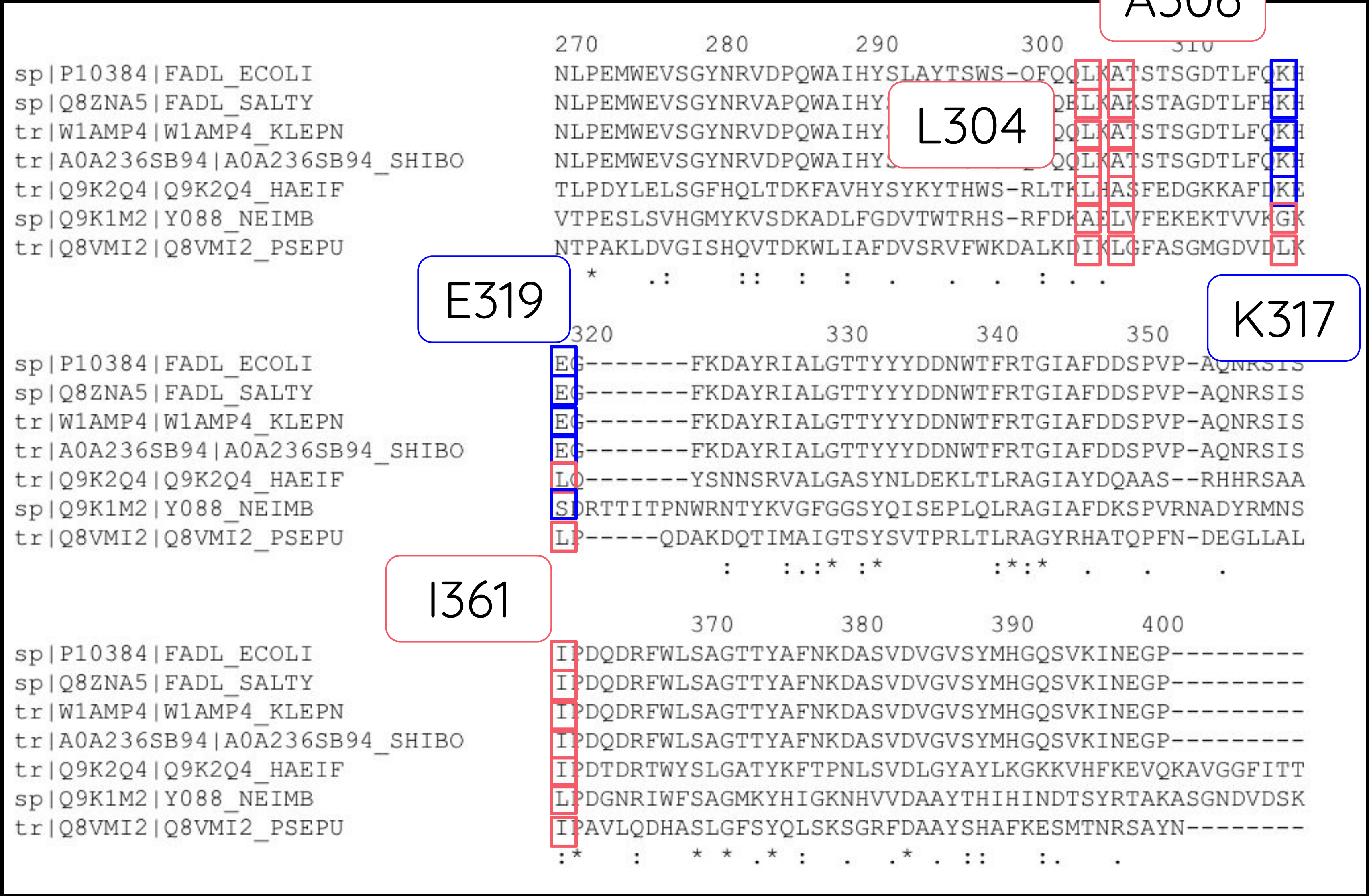
                                          130       140       150       160         170
sp|P10384|FADL_ECOLI                ETMNLNLSGAYRLNNAWSFGLGFNAVYARAKIERFAGD--LGQLVAGQIM
sp|Q8ZNA5|FADL_SALTY                ETMNLNLSGAYRLNEAWSFGLGFDAVYARAKIERFAGD--LGQLVA----
tr|W1AMP4|W1AMP4_KLEPN              ETMNLNLSGAYRLNNAWSFGLGFNAVYARAKIERFAGD--LGQLVAGQIM
tr|A0A236SB94|A0A236SB94_SHIBO      ETMNLNLSGAYRLNNAWSFGLGFNAVYARAKIERFAGD--LGQLVAGQIM
tr|Q9K2Q4|Q9K2Q4_HAEIF              TAINLNLSGAYRVTEGLSLGLGVNAVYAKAQVERNAGI--IAESVK---I
sp|Q9K1M2|Y088_NEIMB                TSIAVEPVAAWKLNDRHSFGAGIIAQHTSAFLRKYA         A----
tr|Q8VMI2|Q8VMI2_PSEPU              FILDIPFAASFKVNDRLAIGGSLDAKWTGLNLDYLI          DGRA
                                      : :   .:::::.: ::* .. *  :  ::

                                            180                 200       210       220
sp|P10384|FADL_ECOLI                QSPAGQTQQGQALAAT           HLNGNQWGFGWNAGILYELDKNN
sp|Q8ZNA5|FADL_SALTY                --------AQNPALAPV          HLNGNQWGFGWNAGILYELDKNN
tr|W1AMP4|W1AMP4_KLEPN              QSPAGQTQQGQALAATANGIDSNTKIAHLNGNQWGFGWNAGILYELDKNN
tr|A0A236SB94|A0A236SB94_SHIBO      QSPAGQTQQGQALAATANGIDSNTKIAHLNGNQWGFGWNAGILYELDKNN
tr|Q9K2Q4|Q9K2Q4_HAEIF              AQNAIKTVNPKDKATDYLTSKDKSVVSLQDRAAWGFGWNAGVMYQFNEAN
sp|Q9K1M2|Y088_NEIMB                --EILTAKPPKPNGVAEAAKIQADGHADVKGSDWGFGYQLAWMWDINDRA
tr|Q8VMI2|Q8VMI2_PSEPU              SGSLMGVIGTLPDPRGVHLSVSKNKEMSSGVDGWGYSARLGLLYKVAPTT
                                                                     **:. . .

                                            230         240       250       260
sp|P10384|FADL_ECOLI                RYALTYRSEVKIDFKG--NYSSDLNRAFNNYGLPIPTATGGATQSGYLTL
sp|Q8ZNA5|FADL_SALTY                RYALTYRSEVKIDFKG--NYSSDLPIAINRFNLPIP         GYLTL
tr|W1AMP4|W1AMP4_KLEPN              RYALTYRSEVKIDFKG--NYSSDLNRAFNNYGLPII         GYLTL
tr|A0A236SB94|A0A236SB94_SHIBO      RYALTYRSEVKIDFKG--NYSSDLNRAFNNYGLPII         GYLTL
tr|Q9K2Q4|Q9K2Q4_HAEIF              RIGLAYHSKVDIDFTD--RTATSLEAEVIEAGK-----------KGNLTL
sp|Q9K1M2|Y088_NEIMB                RVGVNYRSKVSHTLKGDAEWAADGAAAKAMWSTMLAANGYTANEKARVKI
tr|Q8VMI2|Q8VMI2_PSEPU              NVGVSYMFKSHMNDLK----GKGTVTAVDGIAGNVPIEG----EVRFLDF
                                    . .: *  :           . .                        : :
```

FADL STRUCTURE: **POCKET**



```
                                    270       280       290        300       310
sp|P10384|FADL_ECOLI                NLPEMWEVSGYNRVDPQWAIHYSLAYTSWS-QFQQLKATSTSGDTLFQKH
sp|Q8ZNA5|FADL_SALTY                NLPEMWEVSGYNRVAPQWAIHY           QELKAKSTAGDTLFEKH
tr|W1AMP4|W1AMP4_KLEPN              NLPEMWEVSGYNRVDPQWAIHY           QQLKATSTSGDTLFQKH
tr|A0A236SB94|A0A236SB94_SHIBO      NLPEMWEVSGYNRVDPQWAIHY           QQLKATSTSGDTLFQKH
tr|Q9K2Q4|Q9K2Q4_HAEIF              TLPDYLELSGFHQLTDKFAVHYSYKYTHWS-RLTKLHASFEDGKKAFDKE
sp|Q9K1M2|Y088_NEIMB                VTPESLSVHGMYKVSDKADLFGDVTWTRHS-RFDKAELVFEKEKTVVKGK
tr|Q8VMI2|Q8VMI2_PSEPU              NTPAKLDVGISHQVTDKWLIAFDVSRVFWKDALKDIKLGFASGMGDVDLK
                                      *   .:    ::  :  :  .   .  .  : . .

                                     320              330       340       350
sp|P10384|FADL_ECOLI                EG-------FKDAYRIALGTTYYYDDNWTFRTGIAFDDSPVP-AQNRSIS
sp|Q8ZNA5|FADL_SALTY                EG-------FKDAYRIALGTTYYYDDNWTFRTGIAFDDSPVP-AQNRSIS
tr|W1AMP4|W1AMP4_KLEPN              EG-------FKDAYRIALGTTYYYDDNWTFRTGIAFDDSPVP-AQNRSIS
tr|A0A236SB94|A0A236SB94_SHIBO      EG-------FKDAYRIALGTTYYYDDNWTFRTGIAFDDSPVP-AQNRSIS
tr|Q9K2Q4|Q9K2Q4_HAEIF              LQ-------YSNNSRVALGASYNLDEKLTLRAGIAYDQAAS--RHHRSAA
sp|Q9K1M2|Y088_NEIMB                SDRTTITPNWRNTYKVGFGGSYQISEPLQLRAGIAFDKSPVRNADYRMNS
tr|Q8VMI2|Q8VMI2_PSEPU              LP-----QDAKDQTIMAIGTSYSVTPRLTLRAGYRHATQPFN-DEGLLAL
                                               :   :.:* :*      :*:*  .   .   .

                                             370       380       390       400
sp|P10384|FADL_ECOLI                IPDQDRFWLSAGTTYAFNKDASVDVGVSYMHGQSVKINEGP---------
sp|Q8ZNA5|FADL_SALTY                IPDQDRFWLSAGTTYAFNKDASVDVGVSYMHGQSVKINEGP---------
tr|W1AMP4|W1AMP4_KLEPN              IPDQDRFWLSAGTTYAFNKDASVDVGVSYMHGQSVKINEGP---------
tr|A0A236SB94|A0A236SB94_SHIBO      IPDQDRFWLSAGTTYAFNKDASVDVGVSYMHGQSVKINEGP---------
tr|Q9K2Q4|Q9K2Q4_HAEIF              IPDTDRTWYSLGATYKFTPNLSVDLGYAYLKGKKVHFKEVQKAVGGFITT
sp|Q9K1M2|Y088_NEIMB                LPDGNRIWFSAGMKYHIGKNHVVDAAYTHIHINDTSYRTAKASGNDVDSK
tr|Q8VMI2|Q8VMI2_PSEPU              IPAVLQDHASLGFSYQLSKSGRFDAAYSHAFKESMTNRSAYN--------
                                    :*   :   * * .* :  . .* . ::   :.   .
```

A306

L304

E319

K317

I361

4

MECHANISM OF TRANSPORT

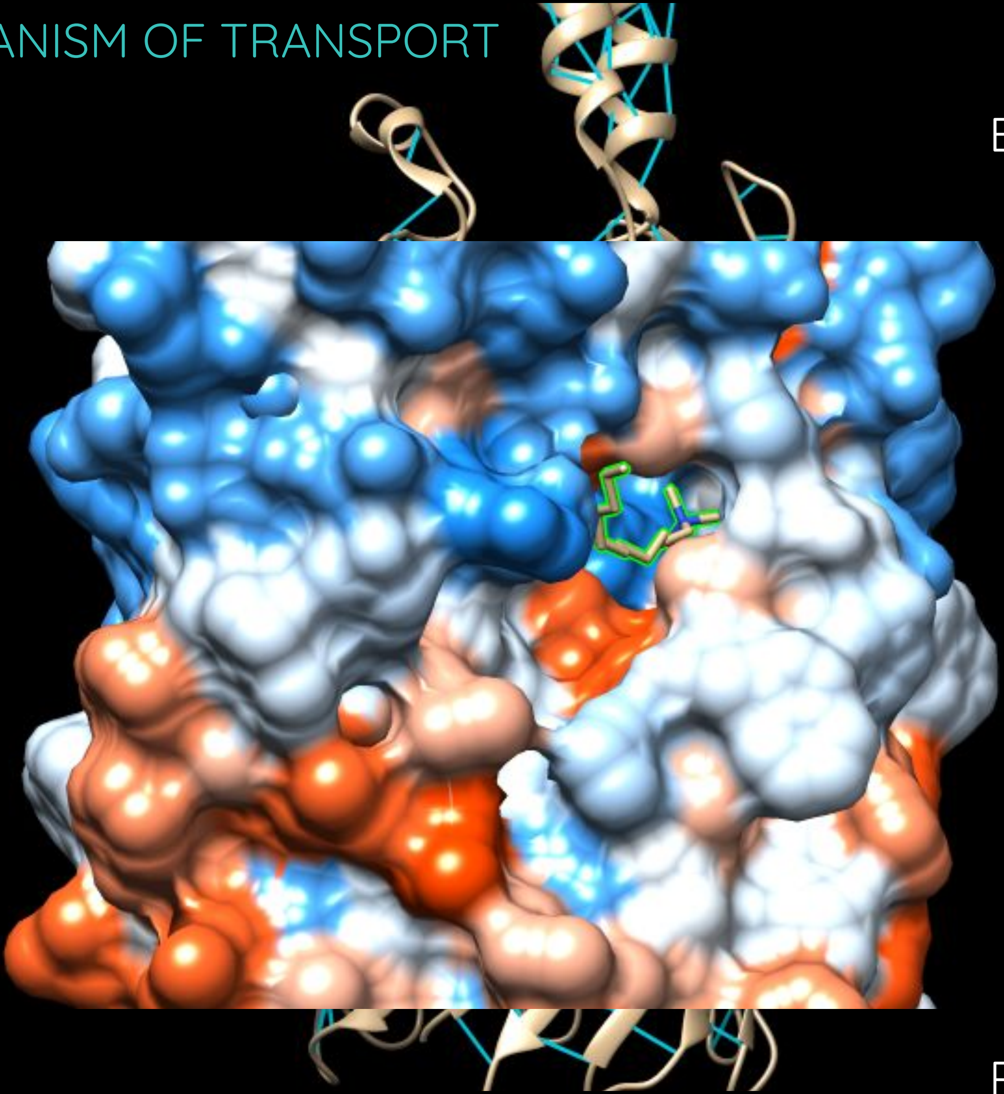
MECHANISM OF TRANSPORT

NanC porin
Sialic acid (acidic sugars)

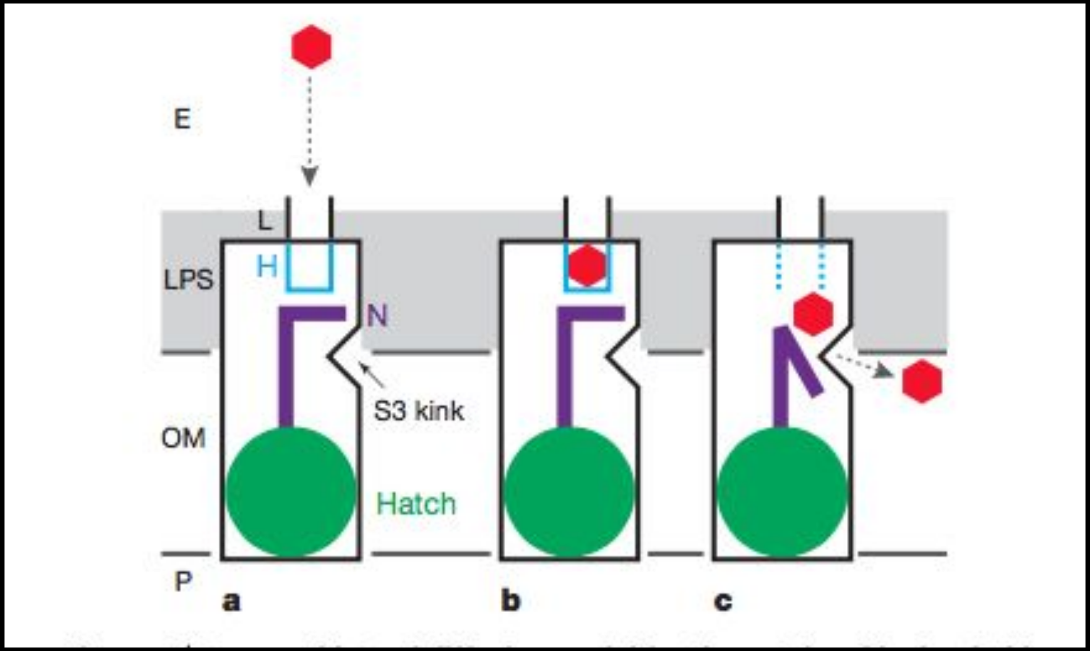
FadL

Periplasm View

MECHANISM OF TRANSPORT



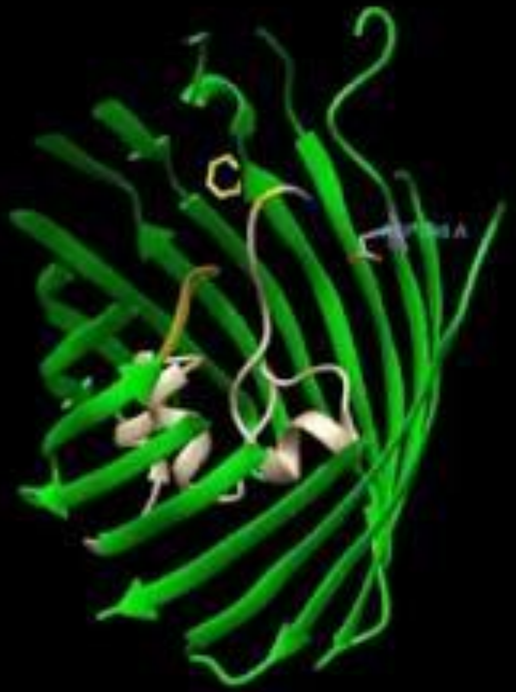
MECHANISM: **LATERAL TRANSPORT**



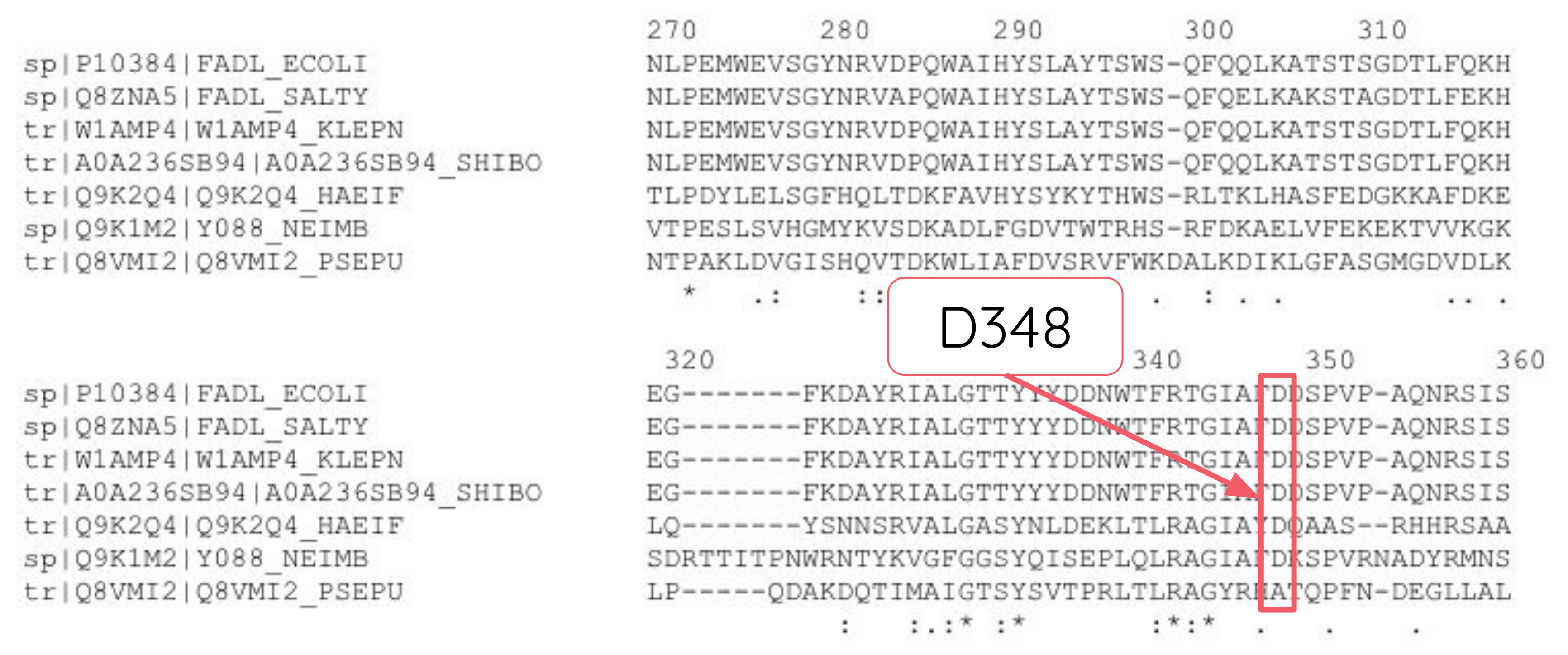
B Van den Berg (2009)


PHE 3.A
ASP 348.A

MECHANISM: **LATERAL TRANSPORT**



MECHANISM: **LATERAL TRANSPORT**



```
                                    270       280       290        300       310
sp|P10384|FADL_ECOLI                NLPEMWEVSGYNRVDPQWAIHYSLAYTSWS-QFQQLKATSTSGDTLFQKH
sp|Q8ZNA5|FADL_SALTY                NLPEMWEVSGYNRVAPQWAIHYSLAYTSWS-QFQELKAKSTAGDTLFEKH
tr|W1AMP4|W1AMP4_KLEPN              NLPEMWEVSGYNRVDPQWAIHYSLAYTSWS-QFQQLKATSTSGDTLFQKH
tr|A0A236SB94|A0A236SB94_SHIBO      NLPEMWEVSGYNRVDPQWAIHYSLAYTSWS-QFQQLKATSTSGDTLFQKH
tr|Q9K2Q4|Q9K2Q4_HAEIF              TLPDYLELSGFHQLTDKFAVHYSYKYTHWS-RLTKLHASFEDGKKAFDKE
sp|Q9K1M2|Y088_NEIMB                VTPESLSVHGMYKVSDKADLFGDVTWTRHS-RFDKAELVFEKEKTVVKGK
tr|Q8VMI2|Q8VMI2_PSEPU              NTPAKLDVGISHQVTDKWLIAFDVSRVFWKDALKDIKLGFASGMGDVDLK
                                      *   .:    ::               .  : . .         .. .

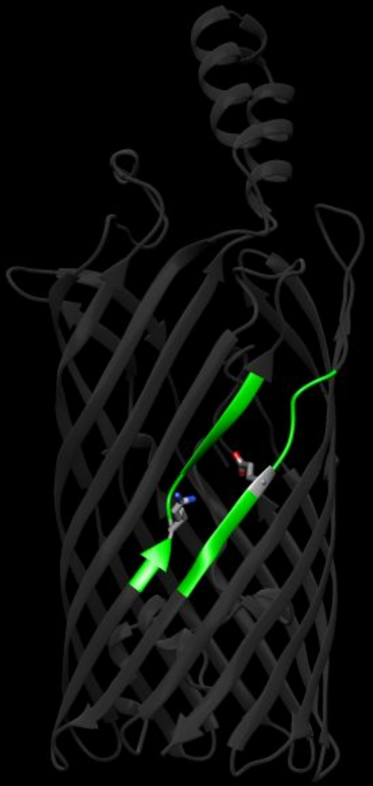
                                     320                       340       350        360
sp|P10384|FADL_ECOLI                EG-------FKDAYRIALGTTYYYDDNWTFRTGIAFDDSPVP-AQNRSIS
sp|Q8ZNA5|FADL_SALTY                EG-------FKDAYRIALGTTYYYDDNWTFRTGIAFDDSPVP-AQNRSIS
tr|W1AMP4|W1AMP4_KLEPN              EG-------FKDAYRIALGTTYYYDDNWTFRTGIAFDDSPVP-AQNRSIS
tr|A0A236SB94|A0A236SB94_SHIBO      EG-------FKDAYRIALGTTYYYDDNWTFRTGIAFDDSPVP-AQNRSIS
tr|Q9K2Q4|Q9K2Q4_HAEIF              LQ-------YSNNSRVALGASYNLDEKLTLRAGIAYDQAAS--RHHRSAA
sp|Q9K1M2|Y088_NEIMB                SDRTTITPNWRNTYKVGFGGSYQISEPLQLRAGIAFDKSPVRNADYRMNS
tr|Q8VMI2|Q8VMI2_PSEPU              LP-----QDAKDQTIMAIGTSYSVTPRLTLRAGYRHATQPFN-DEGLLAL
                                               :   :.:* :*       :*:*  .   .    .
```

D348

MECHANISM: **LATERAL TRANSPORT**

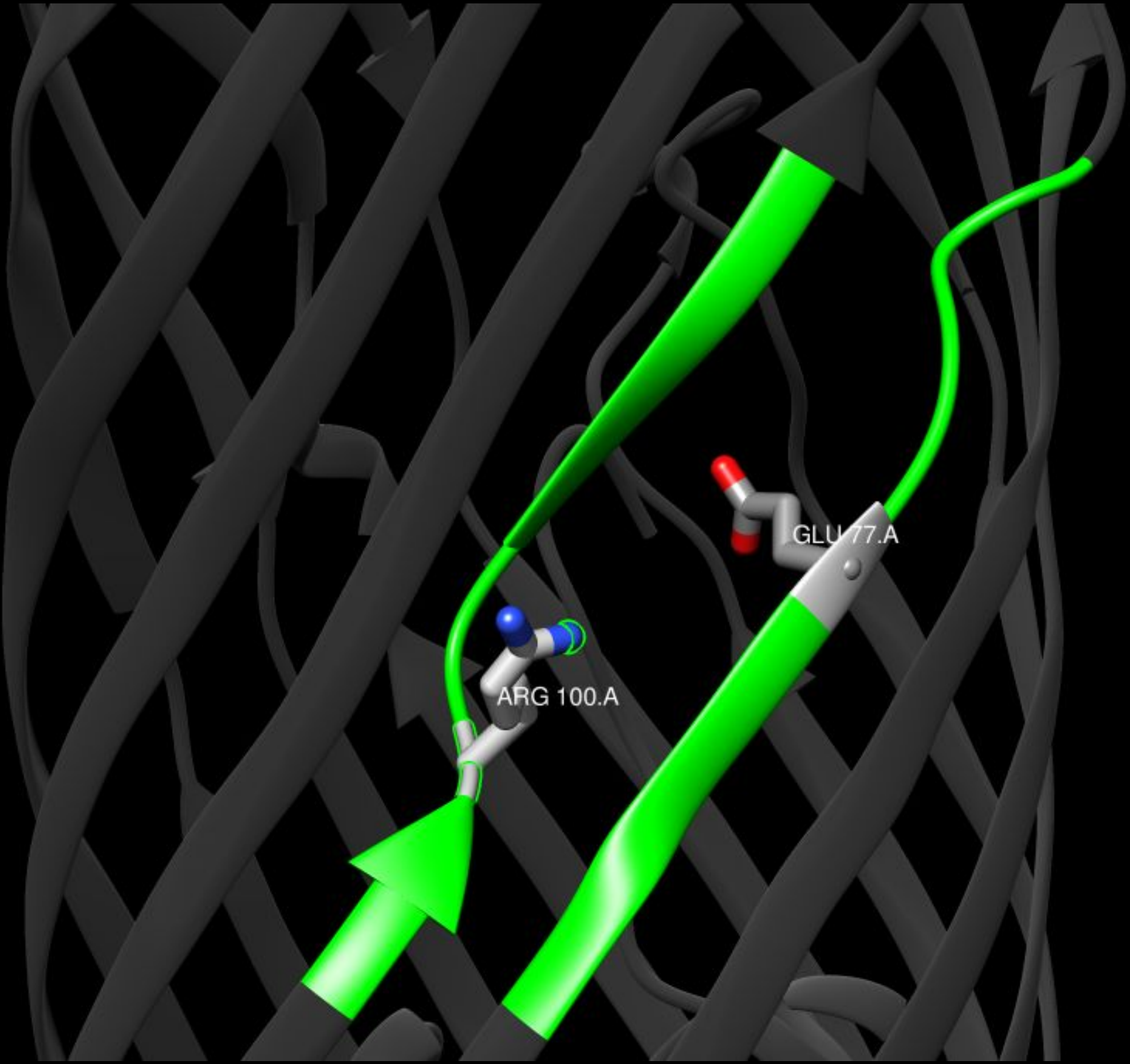
WT

ΔS3
MUTANT
(kinkless)



A77E/S100R
MUTANT

MECHANISM: **LATERAL TRANSPORT**



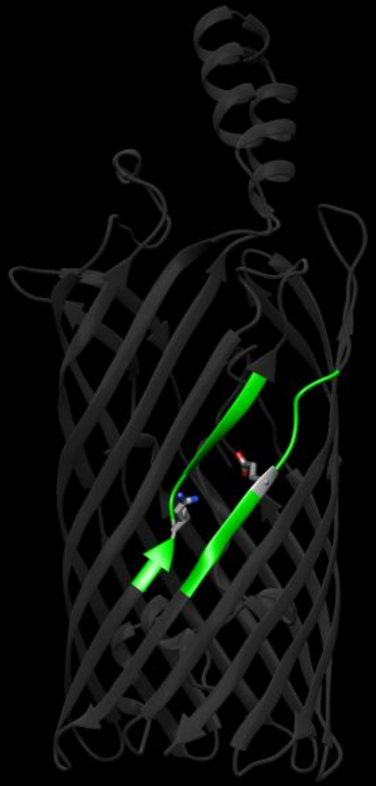
- Salt bridge

A77E/S100R
MUTANT

MECHANISM: **LATERAL TRANSPORT**

WT

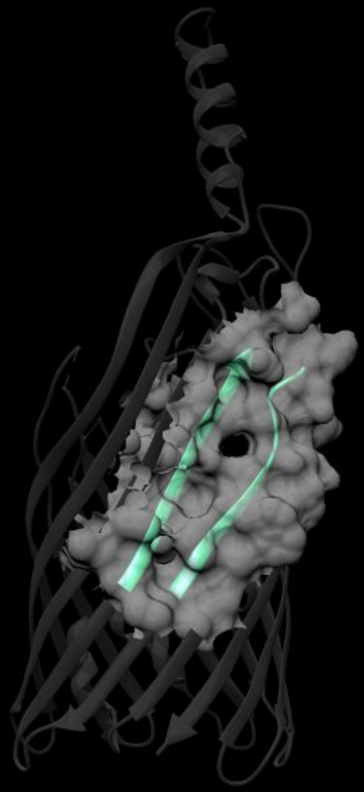
ΔS3
MUTANT
(kinkless)
AND-->^{100}**SNYG**^{103}



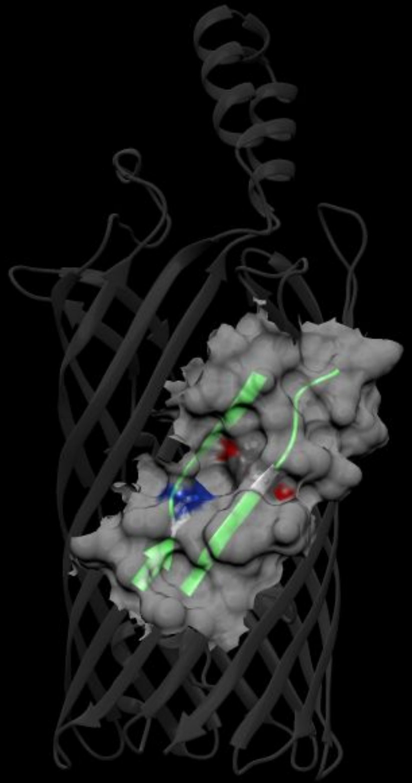
A77E/S100R
MUTANT
alanine -> glutamic;
Serine --> arginine

MECHANISM: **LATERAL TRANSPORT**

WT



ΔS3
MUTANT
(kinkless)

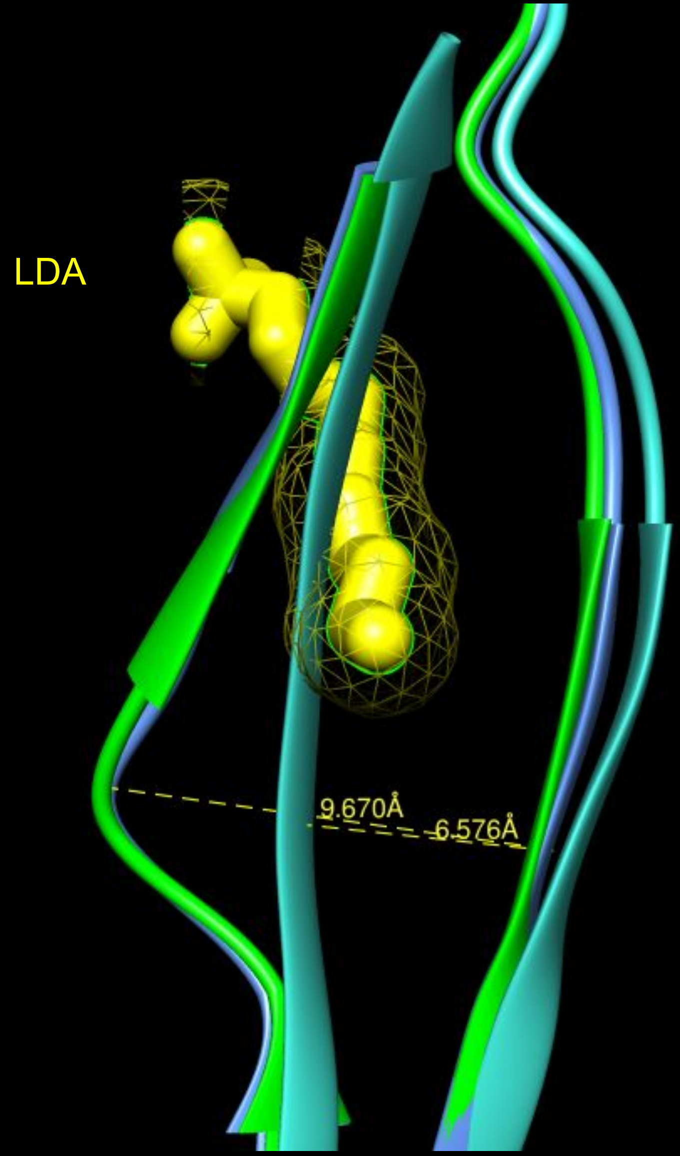


A77E/S100R
MUTANT

alanine -> glutamic;
Serine --> arginine

MECHANISM: **LATERAL TRANSPORT**

- TYR 102@N VAL79@CA
- ASN 101@N VAL 79@CA



WT

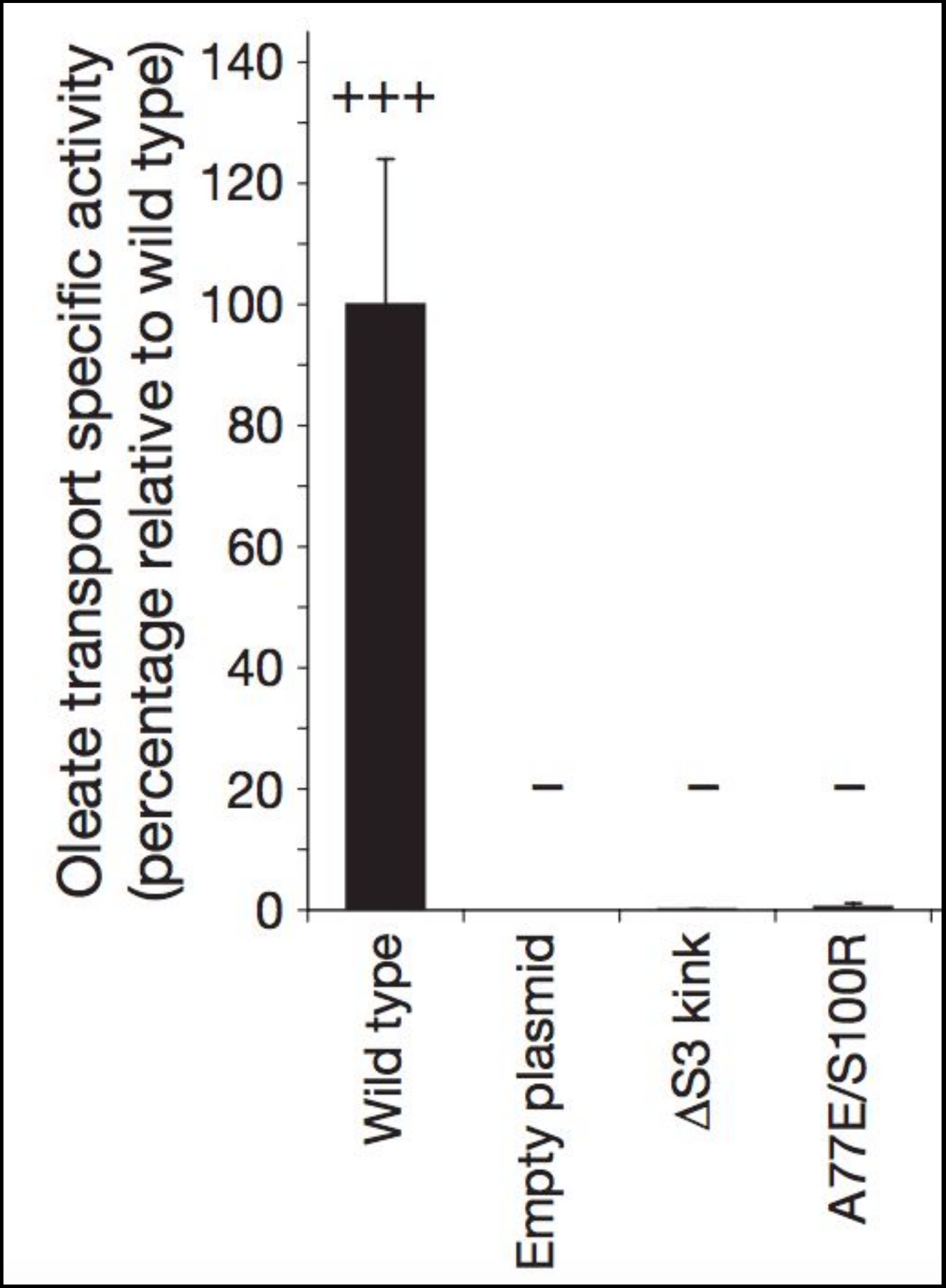
ΔS3
MUTANT (kinkless)

A77E/S100R
MUTANT

MECHANISM:

LATERAL TRANSPORT

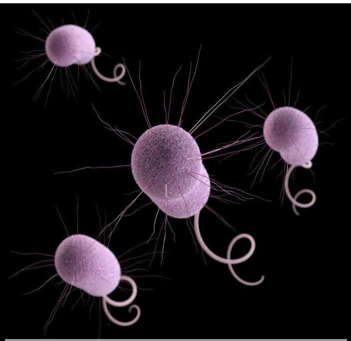
Functional assay



Hearn, E. M., Patel, D. R., Lepore, B. W., Indic, M., & Van Den Berg, B. (2009). Transmembrane passage of hydrophobic compounds through a protein channel wall. *Nature*, *458*(7236), 367–370. https://doi.org/10.1038/nature07678

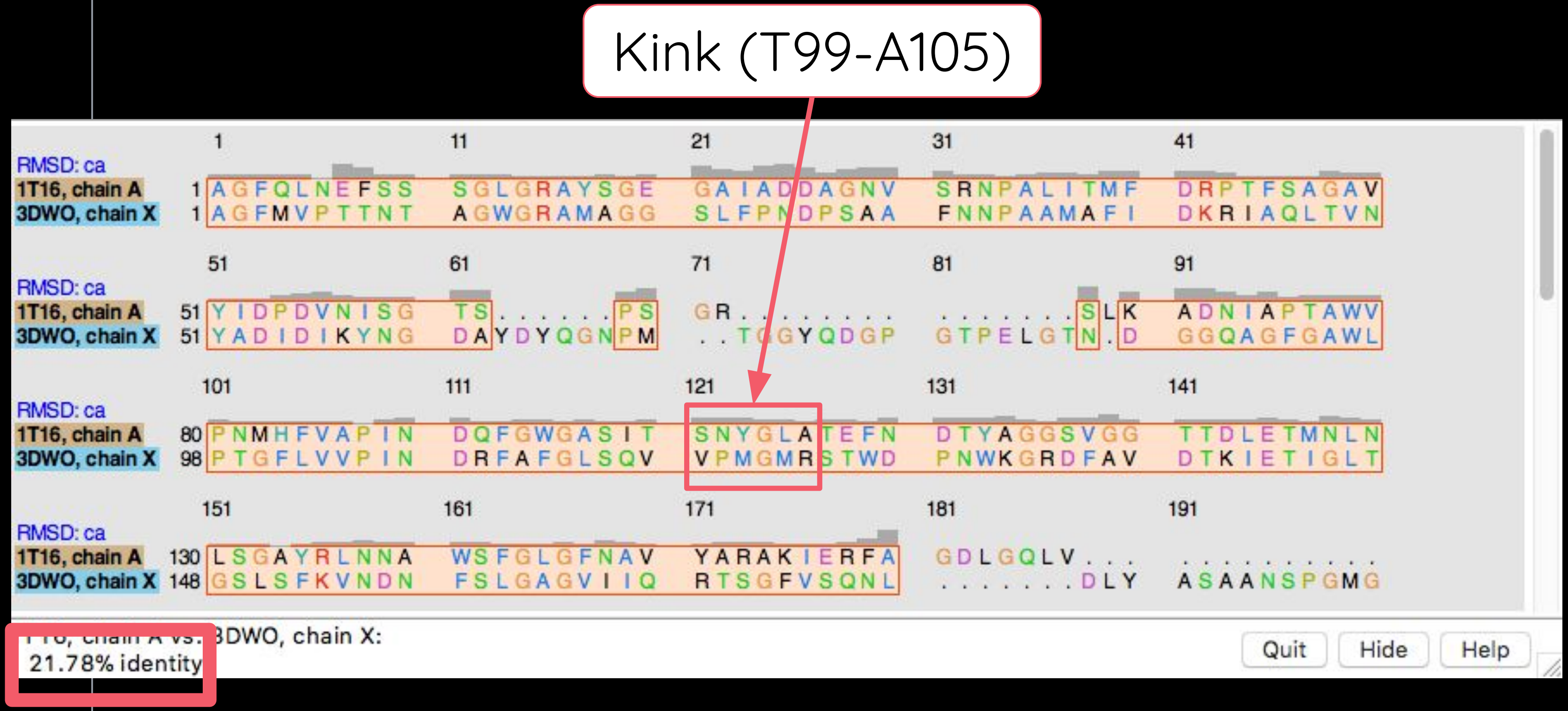
Images extracted from CDC (https://www.cdc.gov)

MECHANISM: **LATERAL TRANSPORT**

Escherichia coli	Pseudomonas aeruginosa
	
PDB id: **1t16**	PDB id: **3dwo**
Resolution: **2,6 Å**	Resolution: **2,2 Å**
14 ß-strands	14 ß-strands
Sequence: **446aa**	Sequence: **463aa**
Structure: **427aa**	Structure: **444aa**

MECHANISM: **LATERAL TRANSPORT**

CHIMERA ALIGNMENT BETWEEN P. AERUGINOSA AND E. COLI



Kink (T99-A105)

```
                         1           11          21          31          41
RMSD: ca
1T16, chain A      1  AGFQLNEFSS  SGLGRAYSGE  GAIADDAGNV  SRNPALITMF  DRPTFSAGAV
3DWO, chain X      1  AGFMVPTTNT  AGWGRAMAGG  SLFPNDPSAA  FNNPAAMAFI  DKRIAQLTVN

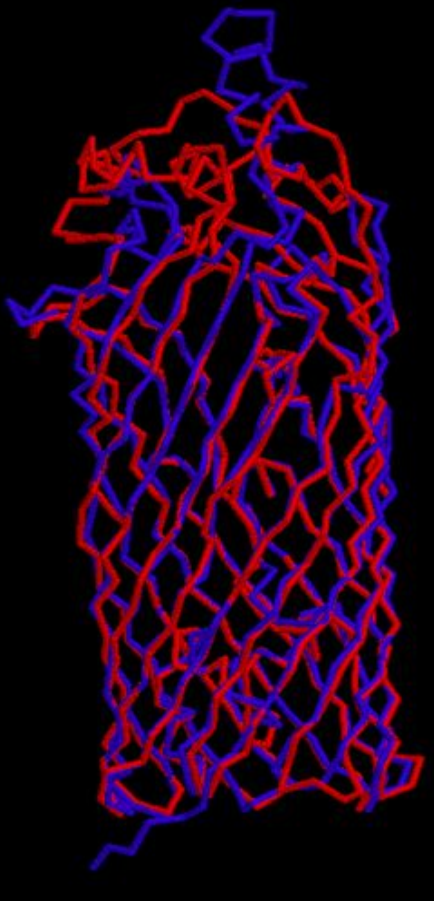
                         51          61          71          81          91
RMSD: ca
1T16, chain A     51  YIDPDVNISG  TS......PS  GR........  .......SLK  ADNIAPTAWV
3DWO, chain X     51  YADIDIKYNG  DAYDYQGNPM  ..TGGYQDGP  GTPELGTN.D  GGQAGFGAWL

                        101         111         121         131         141
RMSD: ca
1T16, chain A     80  PNMHFVAPIN  DQFGWGASIT  SNYGLATEFN  DTYAGGSVGG  TTDLETMNLN
3DWO, chain X     98  PTGFLVVPIN  DRFAFGLSQV  VPMGMRSTWD  PNWKGRDFAV  DTKIETIGLT

                        151         161         171         181         191
RMSD: ca
1T16, chain A    130  LSGAYRLNNA  WSFGLGFNAV  YARAKIERFA  GDLGQLV...  ..........
3DWO, chain X    148  GSLSFKVNDN  FSLGAGVIIQ  RTSGFVSQNL  .......DLY  ASAANSPGMG
```

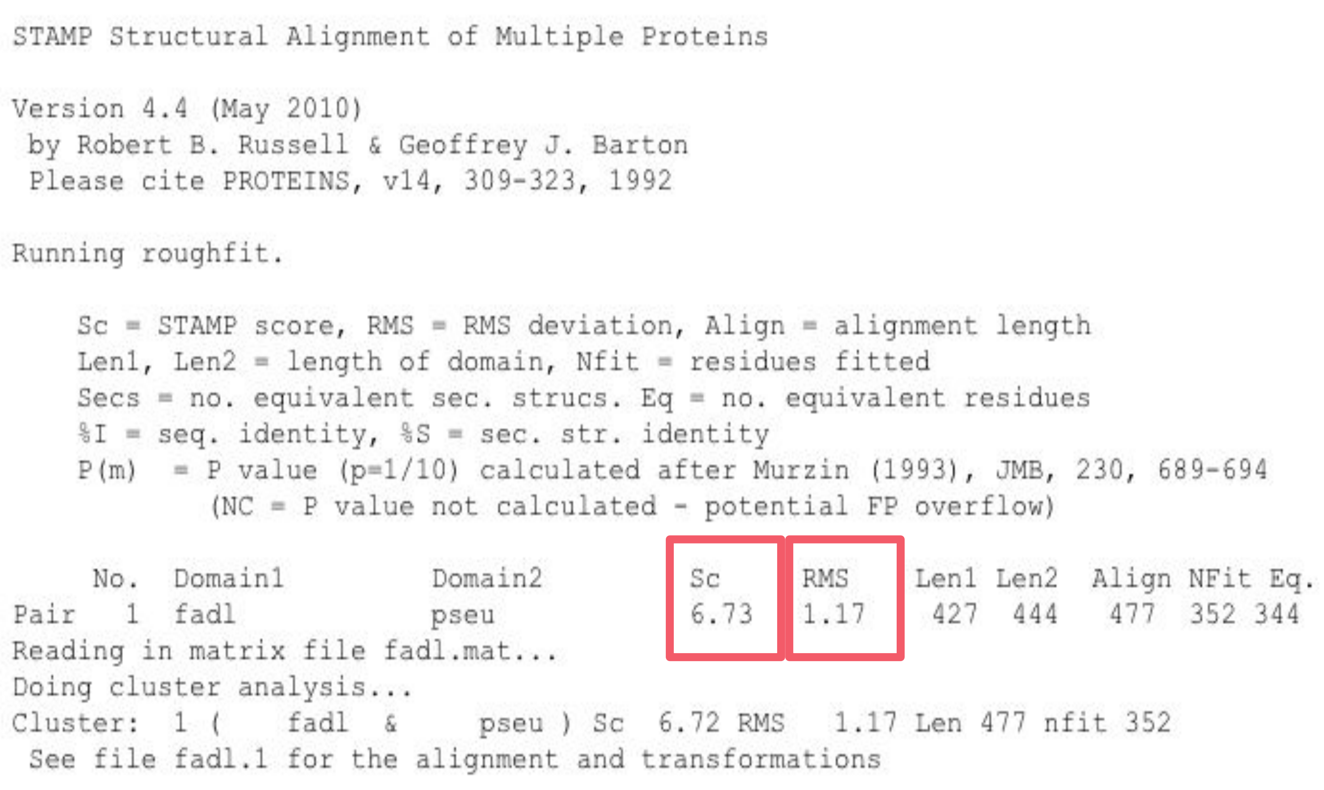
1T16, chain A vs. 3DWO, chain X:
21.78% identity

RasMol superimposition



MECHANISM: **LATERAL TRANSPORT**

STAMP Structural Alignment of Escherichia coli and Pseudomonas aeruginosa FadL protein



```
STAMP Structural Alignment of Multiple Proteins

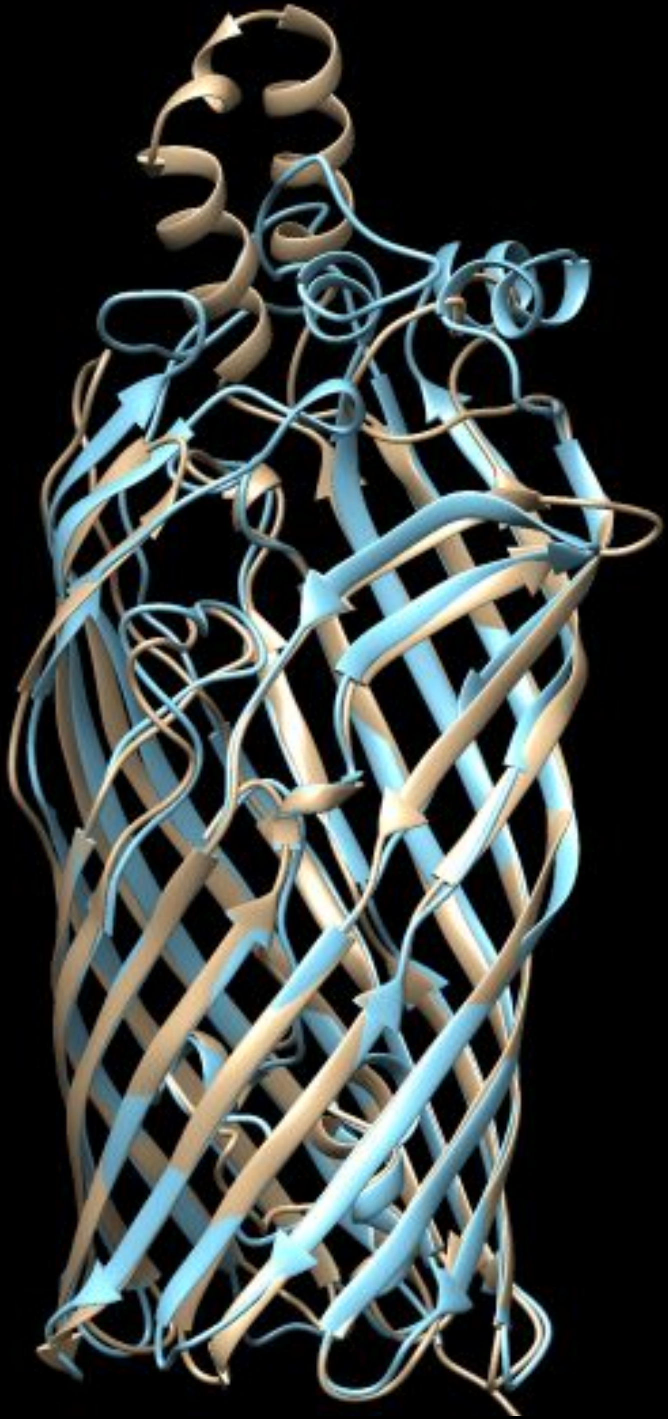
Version 4.4 (May 2010)
 by Robert B. Russell & Geoffrey J. Barton
 Please cite PROTEINS, v14, 309-323, 1992

Running roughfit.

    Sc = STAMP score, RMS = RMS deviation, Align = alignment length
    Len1, Len2 = length of domain, Nfit = residues fitted
    Secs = no. equivalent sec. strucs. Eq = no. equivalent residues
    %I = seq. identity, %S = sec. str. identity
    P(m)   = P value (p=1/10) calculated after Murzin (1993), JMB, 230, 689-694
            (NC = P value not calculated - potential FP overflow)

     No.  Domain1         Domain2          Sc     RMS     Len1 Len2  Align NFit Eq. Secs.    %I    %S    P(m)
Pair   1  fadl            pseu             6.73   1.17     427  444   477  352 344     0  25.87 100.00 2.72e-17
Reading in matrix file fadl.mat...
Doing cluster analysis...
Cluster:  1 (    fadl  &     pseu ) Sc   6.72 RMS   1.17 Len 477 nfit 352
 See file fadl.1 for the alignment and transformations
```

MECHANISM: **LATERAL TRANSPORT**



Chimera superimposition between E. Coli - P. Aeruginosa

MECHANISM: **LATERAL TRANSPORT**

Hatch

MECHANISM: **LATERAL TRANSPORT**

Kink

5

SUMMARY

FadL is not a straight oppen chanel.

It represents one of the very few mechanisms of ligand gated passive diffusion were the ligand is the molecule itself.

Knowing the structure of this receptor can give us insights in how to upgrade it and make bacteria that can have improved functionality on uptaking xenobiotics.

Van den Berg B. Crystal Structure of the Long-Chain Fatty Acid Transporter FadL. *Science*. 2004;304(5676):1506-1509.

Hearn E, Patel D, Lepore B, Indic M, van den Berg B. Transmembrane passage of hydrophobic compounds through a protein channel wall. *Nature*. 2009;458(7236):367-370.

Lepore B, Indic M, Pham H, Hearn E, Patel D, van den Berg B. Ligand-gated diffusion across the bacterial outer membrane. *Proceedings of the National Academy of Sciences*. 2011;108(25):10121-10126.

Thanks!

ANY QUESTIONS?

MULTIPLE CHOICE QUESTIONS

1. In terms of structure, what type of membrane protein is a porin?
   a. Alpha-helix protein
   b. Helical bundle protein
   c. Both previous are correct
   d. ß-barrel protein
   e. All are correct

2. Which is not a main function of membrane proteins?
   a. Receptors
   b. Transcription factors
   c. Transport
   d. Enzymatic activity
   e. Cell adhesion

3. Where do we not find porins?
   a. Chloroplasts
   b. Mitochondria
   c. Eukaryotic cell membrane
   d. Outer membrane of gram-negative bacteria
   e. Outer membrane of gram-positive bacteria (Mycolata)

4. Which is the characteristic transport of porin FadL?

a) Active
b) It requires energy
c) Both previous are correct
[✓ d) Passive diffusion]
e) All of them are correct

5. Which is the structure of FadL involved on the low-affinity binding to the substrate?

[✓ a) Groove]
b) Hatch
c) Kink
d) N-terminus
e) Pocket

6. Which is the structure of FadL involved on the high-affinity binding to the substrate?

a) Groove
b) Hatch
c) Kink
d) N-terminus
[✓ e) Pocket]

7. Which are the most important structures of FadL that allow the lateral diffusion transport?

a) Hatch
b) Kink
c) Both previous are correct
d) NPA conservated sequence
[✓ e) All of them are correct]

8. A specific sequence region conservated in a protein between species shows:

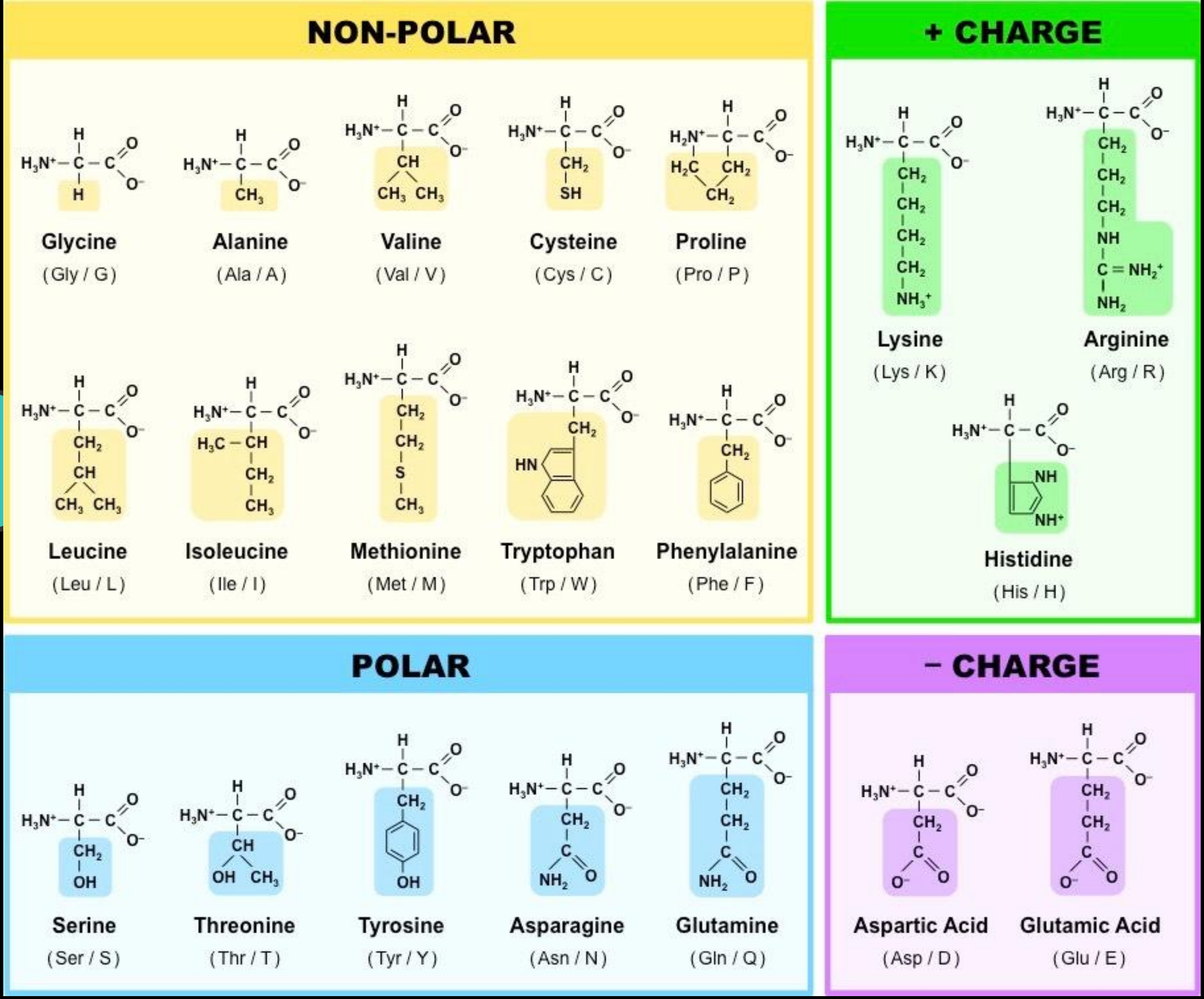
a) The function of that region is important
b) We can ensure that the proteins come from the same ancestor
[✓ c) Both previous are correct]
d) The function of that region is not important
e) All the previous are correct

9. The pocket has high-affinity to the substrate due to:

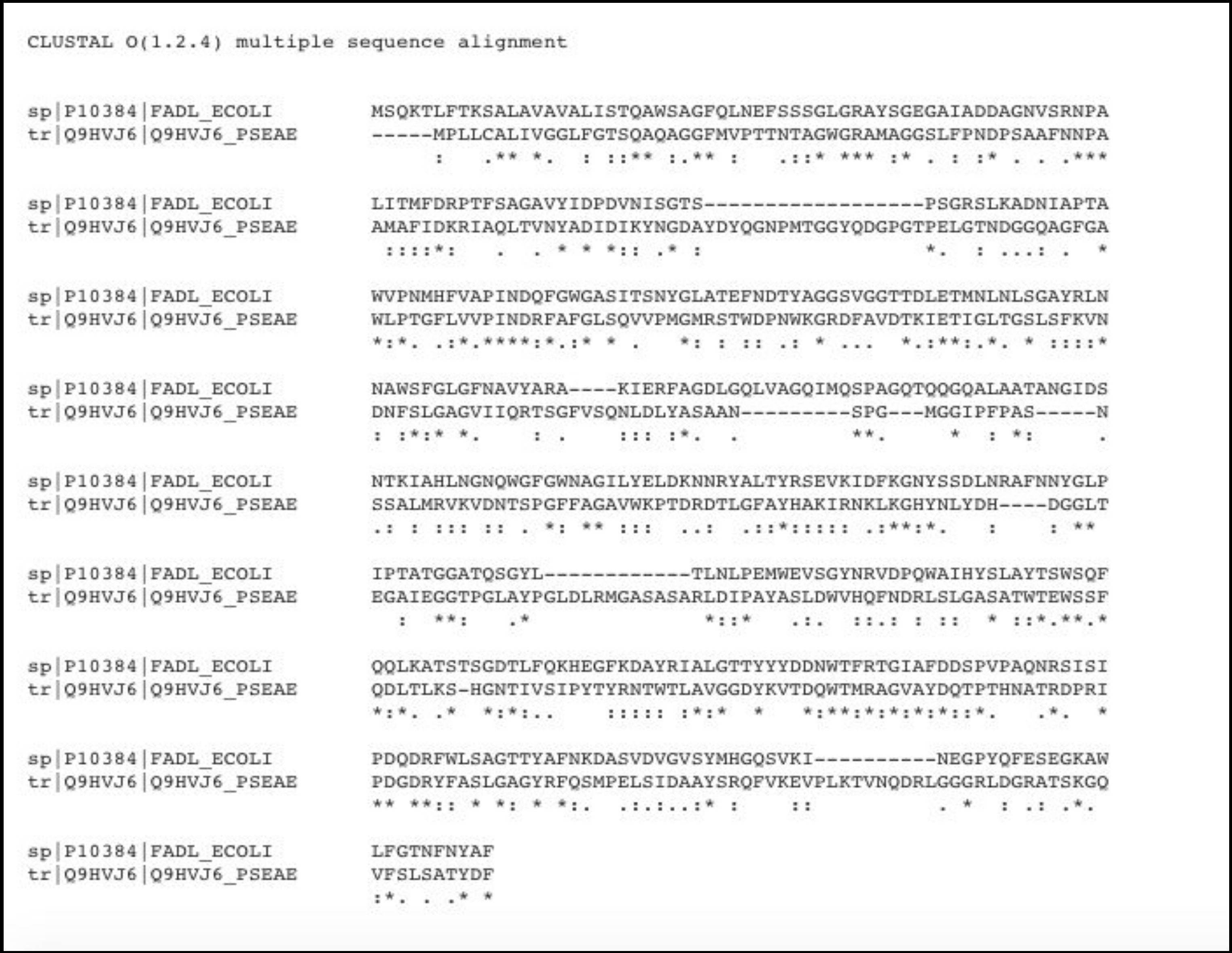
a) 3 positive-charged amino acids that interact with the negative group of the fatty acid
b) 12 hydrophobic amino acids that share the same hydrophobic character of fatty acids
[✓ c) Both previous are correct]
d) 2 negative-charged amino acids that help in the fatty acid location
e) All of them are correct

10. What does an hydropathy plot show?
    a) Hydrophobicity domains of alfa-helix proteins
    b) Hydrophobicity domains of helix bundle proteins
    c) Both previous are correct
    d) Hydrophobicity domains of ß-barrel proteins
    e) All are correct

ANNEX


NON-POLAR
Glycine
(Gly / G)
Alanine
(Ala / A)
Valine
(Val / V)
Cysteine
(Cys / C)
Proline
(Pro / P)
Leucine
(Leu / L)
Isoleucine
(Ile / I)
Methionine
(Met / M)
Tryptophan
(Trp / W)
Phenylalanine
(Phe / F)
+ CHARGE
Lysine
(Lys / K)
Arginine
(Arg / R)
Histidine
(His / H)
POLAR
Serine
(Ser / S)
Threonine
(Thr / T)
Tyrosine
(Tyr / Y)
Asparagine
(Asn / N)
Glutamine
(Gln / Q)
− CHARGE
Aspartic Acid
(Asp / D)
Glutamic Acid
(Glu / E)

SEQUENCE ALIGNMENT BETWEEN E COLI & PSEUDOMONAS



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CLUSTAL O(1.2.4) multiple sequence alignment


sp|P10384|FADL_ECOLI        MSQKTLFTKSALAVAVALISTQAWSAGFQLNEFSSSGLGRAYSGEGAIADDAGNVSRNPA
tr|Q9HVJ6|Q9HVJ6_PSEAE      -----MPLLCALIVGGLFGTSQAQAGGFMVPTTNTAGWGRAMAGGSLFPNDPSAAFNNPA
                                 :   .** *.  : ::** :.** :   .::* *** :* . : :* . . .***

sp|P10384|FADL_ECOLI        LITMFDRPTFSAGAVYIDPDVNISGTS------------------PSGRSLKADNIAPTA
tr|Q9HVJ6|Q9HVJ6_PSEAE      AMAFIDKRIAQLTVNYADIDIKYNGDAYDYQGNPMTGGYQDGPGTPELGTNDGGQAGFGA
                             ::::*:   .  . * * *:: .* :                  *.  : ...: .  *

sp|P10384|FADL_ECOLI        WVPNMHFVAPINDQFGWGASITSNYGLATEFNDTYAGGSVGGTTDLETMNLNLSGAYRLN
tr|Q9HVJ6|Q9HVJ6_PSEAE      WLPTGFLVVPINDRFAFGLSQVVPMGMRSTWDPNWKGRDFAVDTKIETIGLTGSLSFKVN
                            *:*. .:*.****:*.:* * .   *: : :: .: * ...  *.:**:.*. * ::::*

sp|P10384|FADL_ECOLI        NAWSFGLGFNAVYARA----KIERFAGDLGQLVAGQIMQSPAGQTQQGQALAATANGIDS
tr|Q9HVJ6|Q9HVJ6_PSEAE      DNFSLGAGVIIQRTSGFVSQNLDLYASAAN---------SPG---MGGIPFPAS-----N
                            : :*:* *.    : .    ::: :*.  .         **.     *  : *:     .

sp|P10384|FADL_ECOLI        NTKIAHLNGNQWGFGWNAGILYELDKNNRYALTYRSEVKIDFKGNYSSDLNRAFNNYGLP
tr|Q9HVJ6|Q9HVJ6_PSEAE      SSALMRVKVDNTSPGFFAGAVWKPTDRDTLGFAYHAKIRNKLKGHYNLYDH----DGGLT
                            .: : ::: :: . *: ** :::  ..:  .::*::::: .:**:*.   :    : **

sp|P10384|FADL_ECOLI        IPTATGGATQSGYL------------TLNLPEMWEVSGYNRVDPQWAIHYSLAYTSWSQF
tr|Q9HVJ6|Q9HVJ6_PSEAE      EGAIEGGTPGLAYPGLDLRMGASASARLDIPAYASLDWVHQFNDRLSLGASATWTEWSSF
                              :  **:   .*              *::*   .:.  ::.: : ::  * ::*.**.*

sp|P10384|FADL_ECOLI        QQLKATSTSGDTLFQKHEGFKDAYRIALGTTYYYDDNWTFRTGIAFDDSPVPAQNRSISI
tr|Q9HVJ6|Q9HVJ6_PSEAE      QDLTLKS-HGNTIVSIPYTYRNTWTLAVGGDYKVTDQWTMRAGVAYDQTPTHNATRDPRI
                            *:*. .*  *:*:..    ::::: :*:*  *   *:**:*:*:*:*::*.   .*.  *

sp|P10384|FADL_ECOLI        PDQDRFWLSAGTTYAFNKDASVDVGVSYMHGQSVKI----------NEGPYQFESEGKAW
tr|Q9HVJ6|Q9HVJ6_PSEAE      PDGDRYFASLGAGYRFQSMPELSIDAAYSRQFVKEVPLKTVNQDRLGGGRLDGRATSKGQ
                            ** **:: * *: * *:.  .:.:..:* :    ::          . *  : .: .*.

sp|P10384|FADL_ECOLI        LFGTNFNYAF
tr|Q9HVJ6|Q9HVJ6_PSEAE      VFSLSATYDF
                            :*. . .* *
```
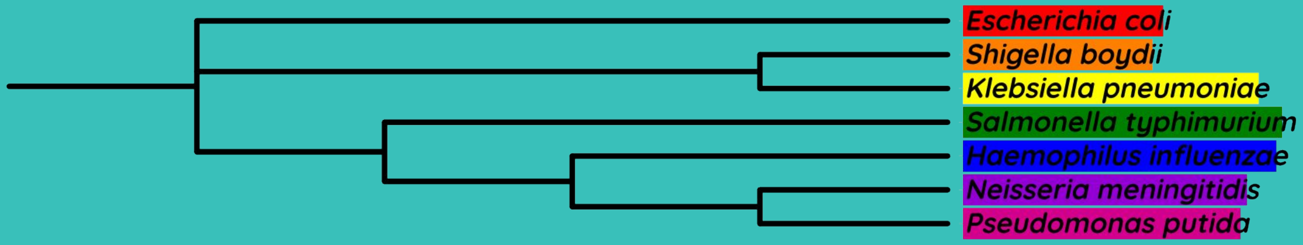

iTOL Phylogenetic Tree



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