Membrane proteins
Porins: FadL

Oriol Solà, Dimitri Ivancic, Daniel Folch, Marc Olivella
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1. INTRODUCTION TO MEMBRANE PROTEINS
2. FADL: OUTER MEMBRANE TRANSPORT PROTEIN
3. MAIN FEATURES OF FADL STRUCTURE
4. MECHANISM OF TRANSPORT
5. SUMMARY
INTRODUCTION
INTRODUCTION

Classification

MEMBRANE PROTEINS

Peripheral membrane proteins

Integral membrane proteins

INTRODUCTION

MEMBRANE PROTEINS

Classification

Peripheral membrane proteins

Figures extracted from class slides BE2.3
INTRODUCTION

MEMBRANE PROTEINS

Classification

Integral membrane proteins

INTRODUCTION

MEMBRANE PROTEINS

- Transport proteins
- Enzymatic activity
- Membrane receptors
- Cell adhesion molecules
INTRODUCTION: IMPORTANCE OF PREDICTION

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

INTRODUCTION: HIDROPATHY PLOT

- Identify protein domains
- Hydrophobic and hydrophilic regions

INTRODUCTION: **DIFFICULTIES OF PURIFICATION**

INTRODUCTION: DIFFICULTIES OF CRYSTALLISATION

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

- Beta barrel proteins → 8-22 β strands
- Alternated polar and nonpolar residues
- Monomers, dimeric and octameric
- “Stopper”
- Homotrimers
INTRODUCTION: **PORINS**

- 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**

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

Le T and Bushan V. *Microbiology* (2016)
INTRODUCTION: FADL Classification

- **Superfamily I**: FadL, GBP, SP, RPP families
- **Superfamily II**: Mycobacterial porin (MBP)
- **Superfamily III**: OEP24 and OEP37 families
- **Superfamily IV**: OEP16 and MPT families
- **Superfamily V**
FADL: OUTER MEMBRANE TRANSPORT PROTEIN
<table>
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<th>Membrane and cell surface proteins and peptides</th>
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<td><strong>Family</strong></td>
<td>Outer membrane transport protein</td>
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<td><strong>Protein</strong></td>
<td>Long-chain fatty acid transport protein FadL</td>
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WHY FADL?

- Outer membrane (OM)
  passive diffusion transport
  (∝-barrel)
- Xenobiotics biodegradation
- Hydrophobic substrates:
  Particular hydrophobic surface
INTRODUCTION: HDROPATHY PLOT

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


Created with ExPASy ProtScale software
FADL STRUCTURE: **MAIN CHARACTERISTICS**

- **PDB id:** 1t16
- **Resolution:** 2.6 Å
- **14 β-strands**
- **Sequence:** 446aa
- **Structure:** 427aa

Sequence and structure obtained with PDB Sum

---

**Key:**
- Green: Family
- Purple: PfamA domain
- Green and purple: Secondary structure
- Green and purple with arrows: CATH domain

**PF03349: Toluene_X - Outer membrane protein transport protein (OMFP1/Fa)**

**CATH domain 2.40.130.80: Mainly Beta, Beta Barrel**

**dL/Tod X**
3  MAIN FEATURES OF FADL STRUCTURE
FADL Structure: **Main Domains**

- Groove
- Pocket
- Kink
- Hatch
FADL STRUCTURE: HATCH
FADL STRUCTURE: KINK (HYDROGEN BONDS)
FADL STRUCTURE: GROOVE

Hydrophobicity surface representation
SEQUENCE ALIGNMENTS: GROOVE

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**LOOP 4**
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

**CLUSTAL 2.1 multiple sequence alignment**

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FADL STRUCTURE: POCKET

L304

K317

A306

I361
MECHANISM OF TRANSPORT
MECHANISM OF TRANSPORT

NanC porin
Sialic acid (acidic sugars)

FadL

Periplasm View
MECHANISM: LATERAL TRANSPORT

B Van den Berg (2009)
MECHANISM: LATERAL TRANSPORT
MECHANISM: LATERAL TRANSPORT

D348
MECHANISM: LATERAL TRANSPORT

WT

ΔS3 MUTANT (kinkless)

A77E/S100R MUTANT
MECHANISM: LATERAL TRANSPORT

- Salt bridge
MECHANISM: LATERAL TRANSPORT

WT

ΔS3 MUTANT (kinkless)
AND--->100SNYG103

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 VAL 79@CA
- ASN 101@N VAL 79@CA

LDA

WT

ΔS3
MUTANT (kinkless)

A77E/S100R
MUTANT
MECHANISM:
LATERAL TRANSPORT

Functional assay

MECHANISM: LATERAL TRANSPORT

Escherichia coli

- PDB id: 1t16
- Resolution: 2.6 Å
- 14 β-strands
- Sequence: 446aa
- Structure: 427aa

Pseudomonas aeruginosa

- PDB id: 3dwo
- Resolution: 2.2 Å
- 14 β-strands
- Sequence: 463aa
- Structure: 444aa

Images extracted from CDC (https://www.cdc.gov)
CHIMERA ALIGNMENT BETWEEN P. AERUGINOSA AND E. COLI

MECHANISM: **LATERAL TRANSPORT**

**Kink (T99-A105)**
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)

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MECHANISM: LATERAL TRANSPORT

Chimera superimposition between E. Coli - P. Aeruginosa
MECHANISM: LATERAL TRANSPORT

Hatch
MECHANISM: LATERAL TRANSPORT

Kink
FadL is not a straight open channel.

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

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


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. β-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 conserved sequence
   e) All of them are correct

8. A specific sequence region conserved 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 β-barrel proteins
   e) All are correct
### Sequence Alignment Between E Coli & Pseudomonas

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References


