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Structure-function studies of bacterial outer membrane proteins

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* General Introduction/Background

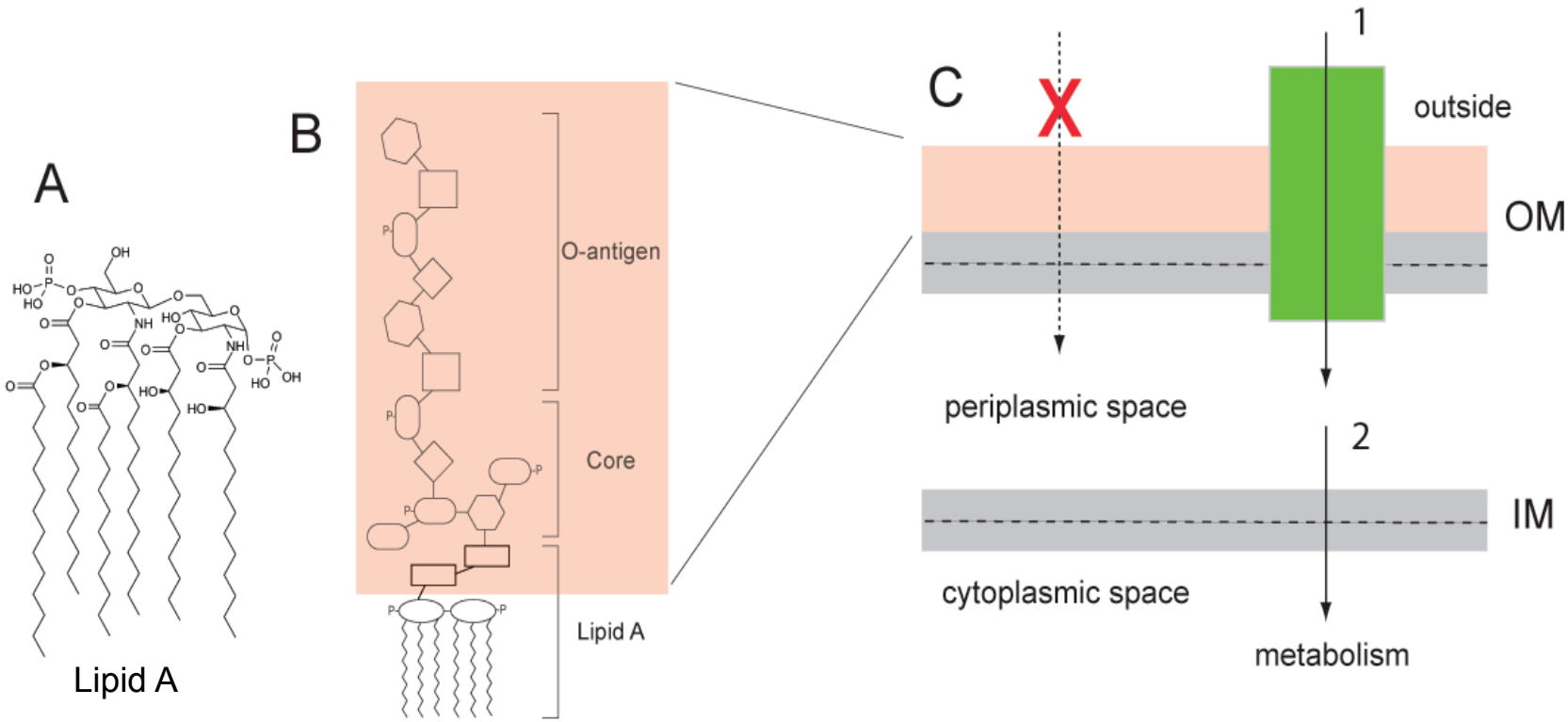
* OM transport of hydrophobic molecules

* Substrate specificity in a family of OM carboxylate channels

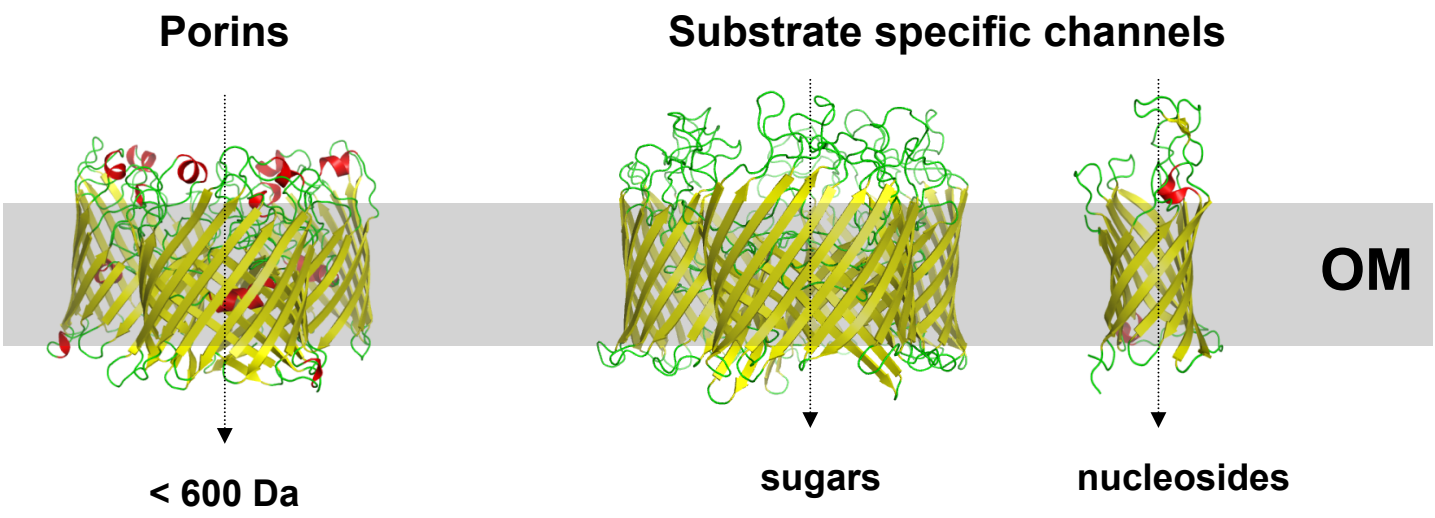
Gram-negative bacteria have 2 membranes:

* **Inner membrane (IM): regular phospholipid bilayer**

* **Outer membrane (OM): polar outer layer of lipopolysaccharide (LPS)**



Very effective barrier for polar and apolar molecules



- Do not bind their substrates (water-filled, aspecific pores)
- Transport substrates <≈ 600 Da
- Usually trimeric
- Efficient at higher substrate concentrations
- Diffusion
- *E. coli* OmpC, OmpF, PhoE

- Have binding sites for substrates (µM-sub mM)
- Similar architecture as porins
- Monomeric or trimeric
- Efficient at low substrate concentrations
- Diffusion
- LamB, FadL, OprP

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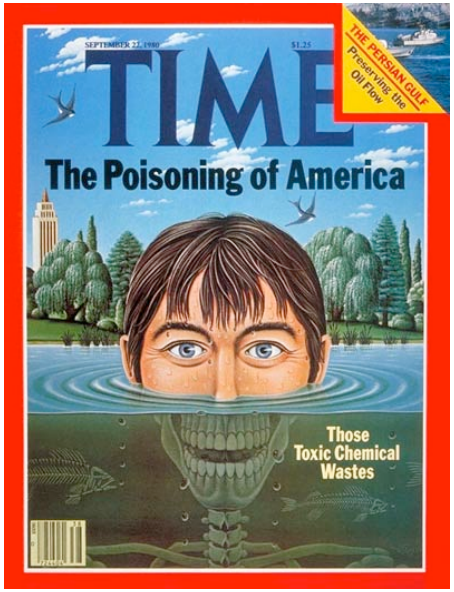
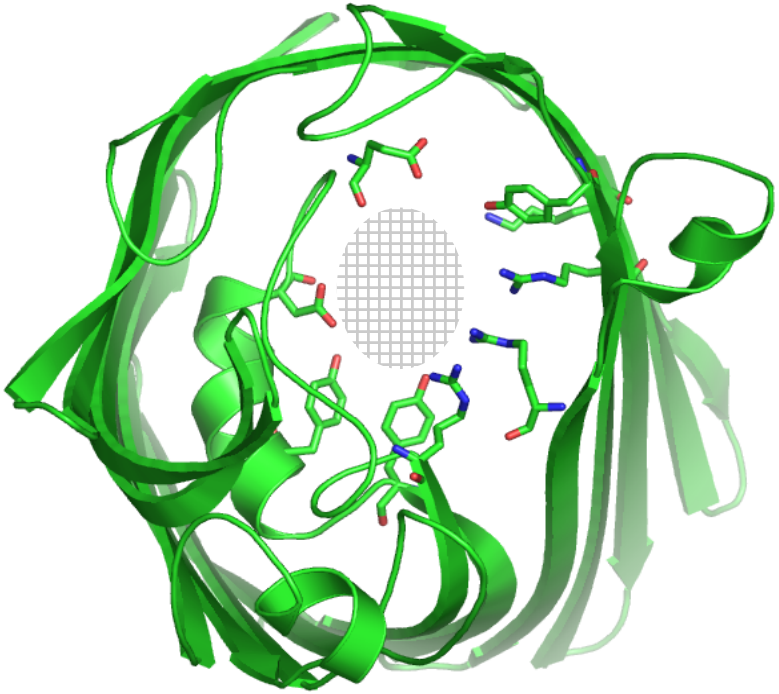
How do greasy molecules enter sugar-coated bacteria?

(aka: Outer membrane transport of hydrophobic compounds)



Most OM channels transport polar molecules

(but not hydrophobic ones!)



Porin pores are highly polar

Structured water molecules in the pore prevent the passage of hydrophobic molecules

Only one protein family has been shown to mediate uptake of hydrophobic molecules across the OM:

FadL family

Model system: *E. coli* FadL: long-chain fatty acid (LCFA) channel

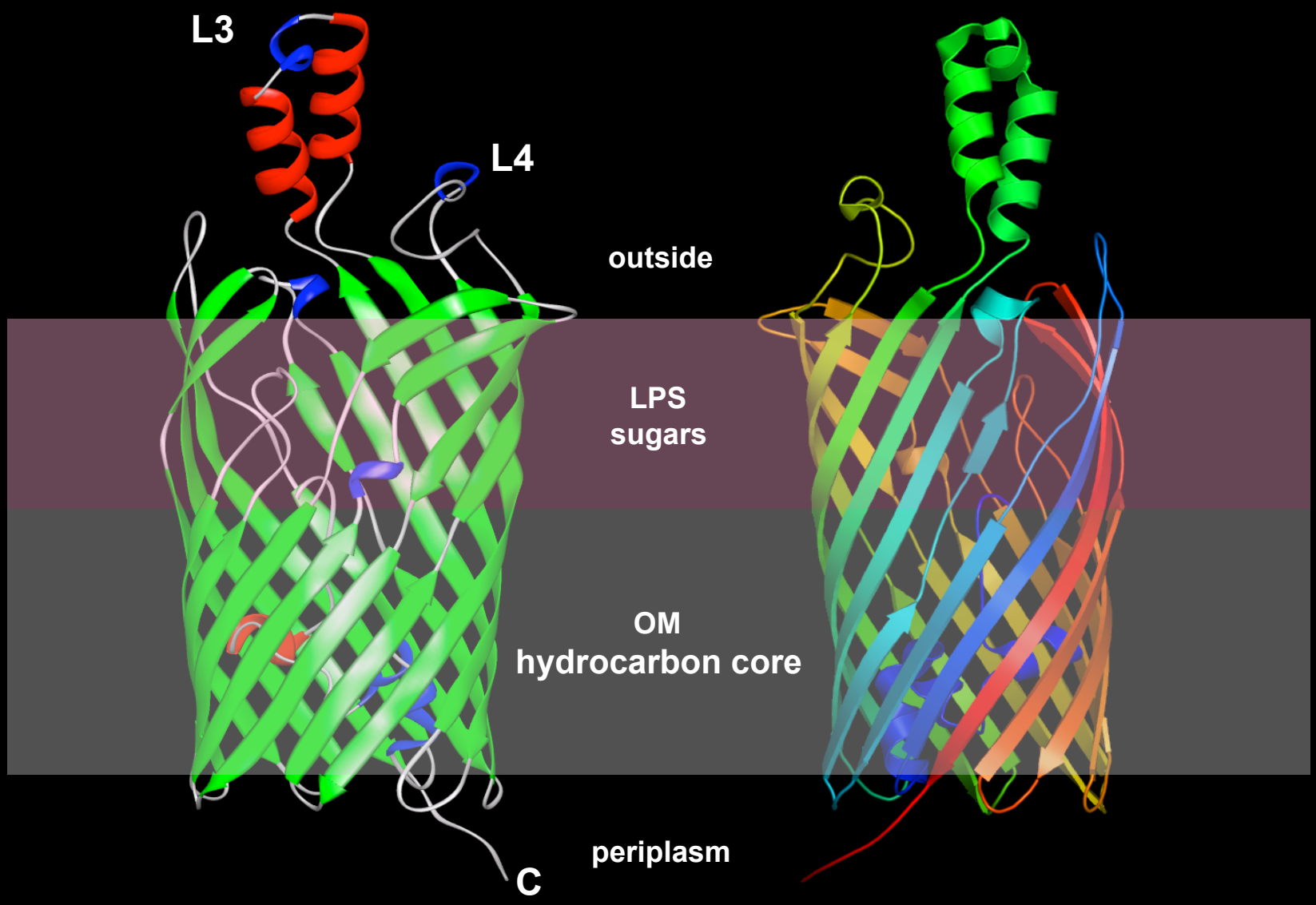
Required for growth on LCFAs

Biodegrading bacteria have FadL orthoologs

* FadL-mediated uptake does not require energy (diffusion)

* FadL is the only OM component

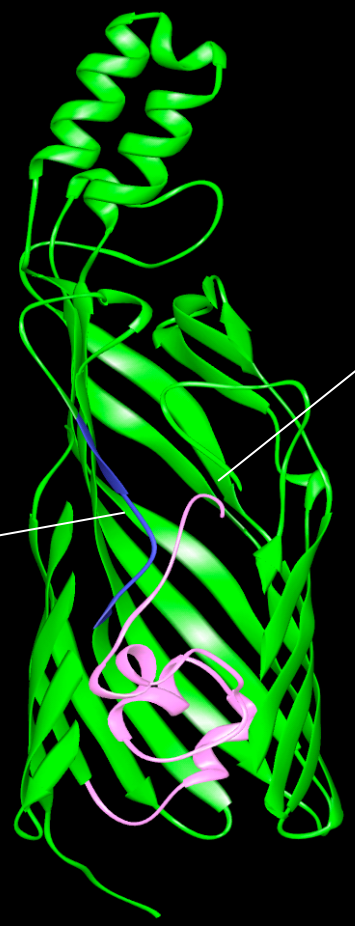
E. Coli FadL structure

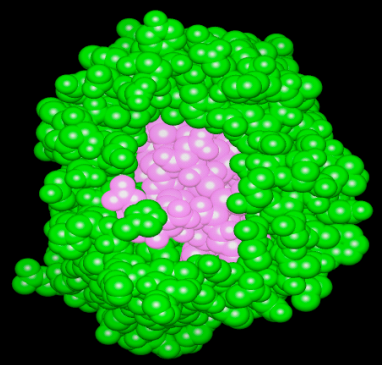


van den Berg *et al*., **Science** 304, 1504 (2004)

The FadL barrel is plugged by a small N-terminal domain

One of the β-strands forms a kink





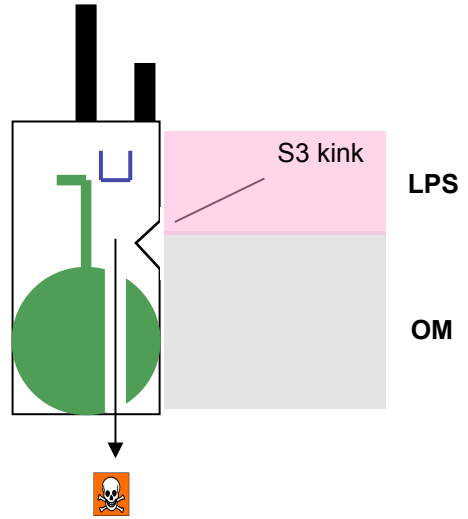
Hatch **plugs**
barrel
No channel!

van den Berg *et al*., **Science** 304**,** 1504 (2004)

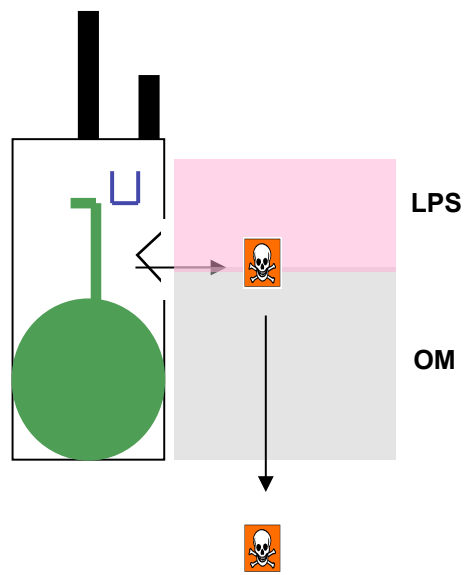
Two possible transport mechanisms

which one is right?

1. Classical model

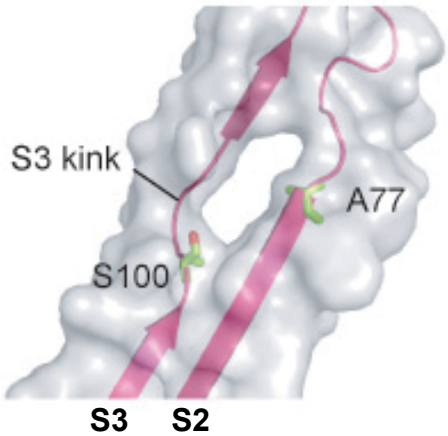


2. Lateral diffusion model



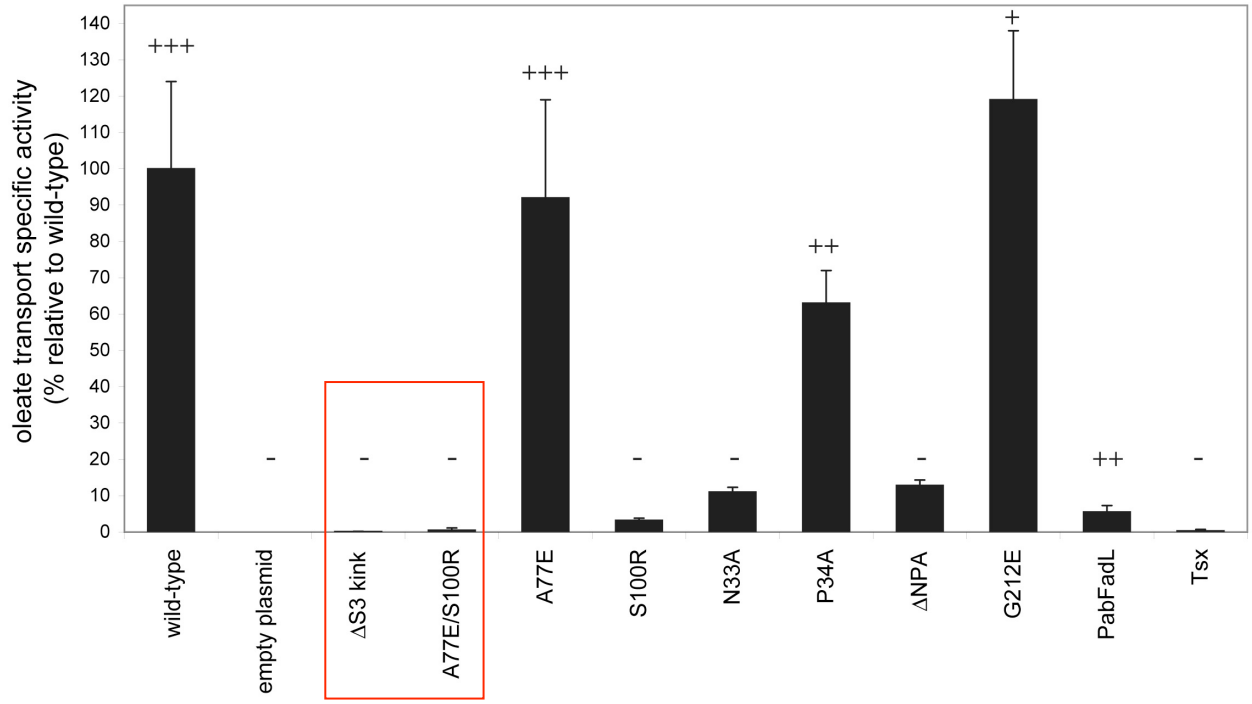
Two mutants made to close the lateral opening

A77E/S100R mutant: forms salt bridge?



WT

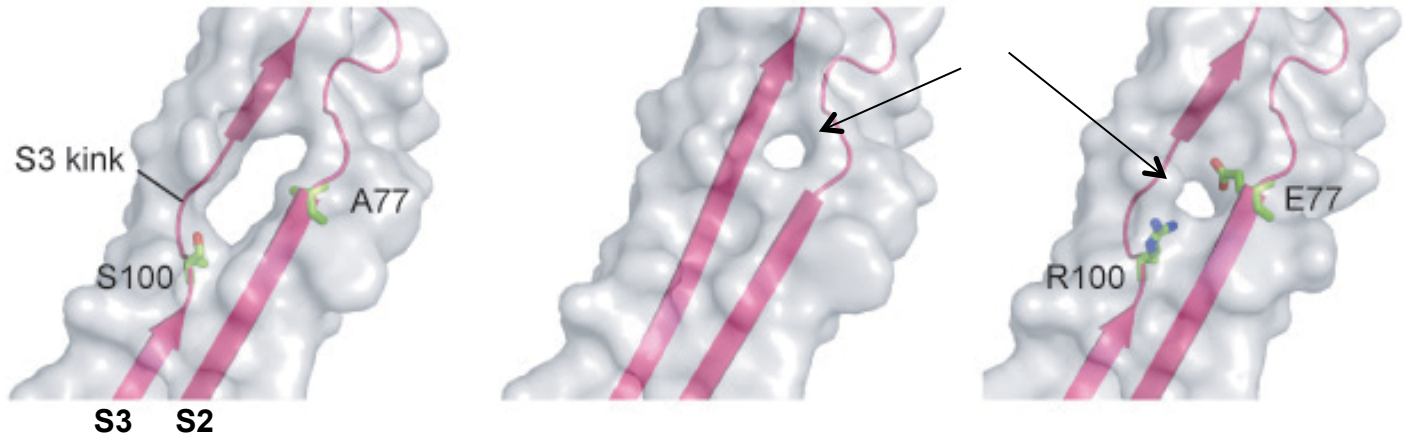
Mutants with a (putative) constricted lateral opening are <u>inactive</u> for ^{3}H-oleate transport



Membrane western

Mutants also do not support growth of a *fadL* knockout strain on palmitate plates

Crystal structures of lateral opening mutants



WT **ΔS3 kink** **A77E/S100R**

Lateral opening is <u>much smaller</u> in the mutants

Constricting the lateral opening blocks LCFA transport: **lateral diffusion**

Hearn *et al*., **Nature** 458, 367 (2009)

A77E/S100R mutant is structurally identical to WT

(except for the smaller lateral opening)

WT
A77E/S100R

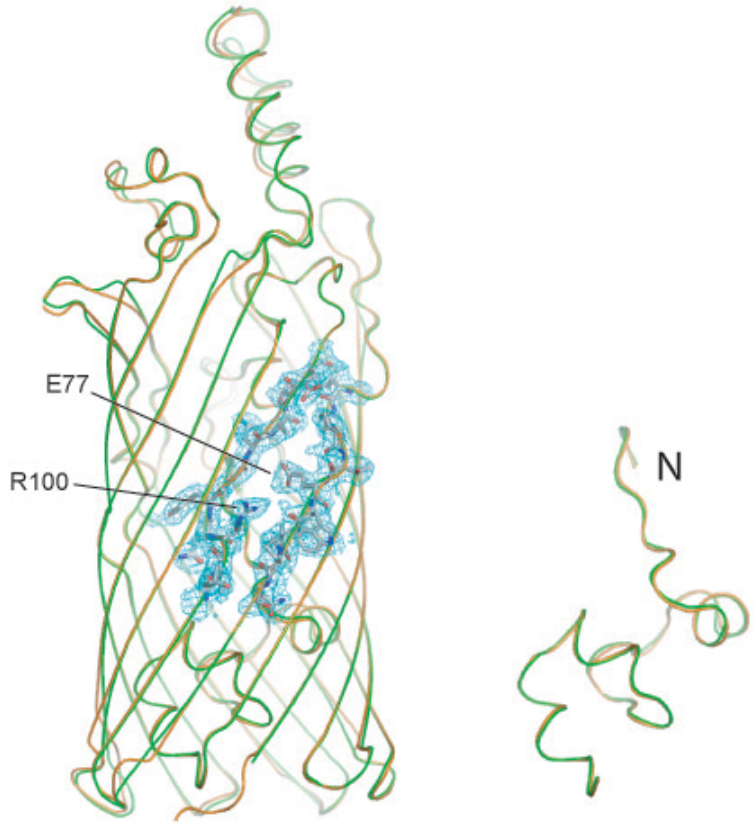
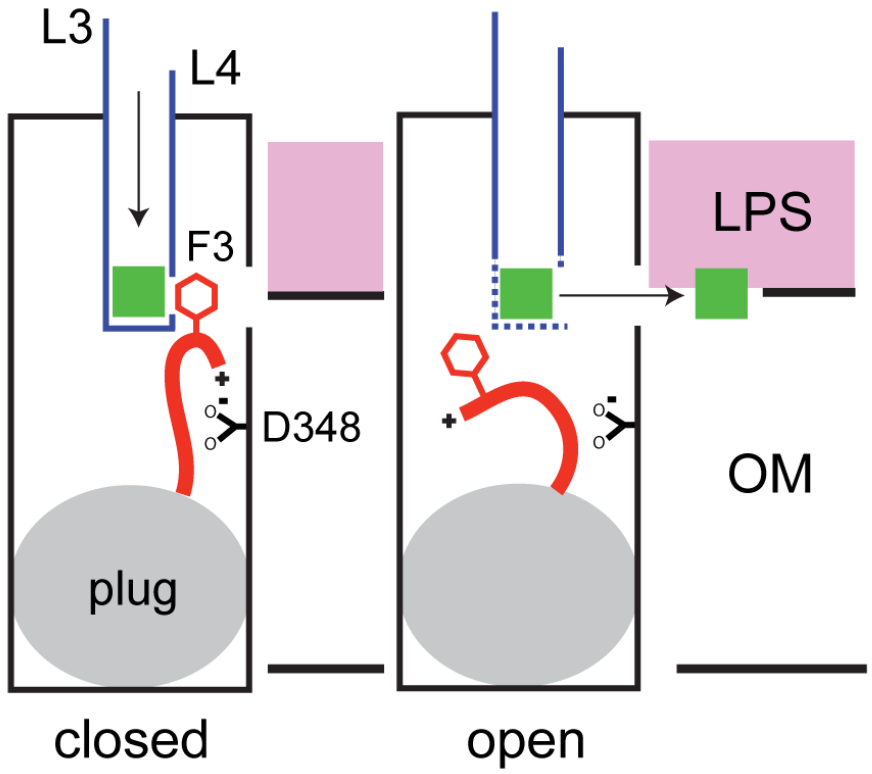


Figure 5

General model for substrate transport by FadL channels

Ligand-gated conformational change of the N-terminus

Lateral substrate diffusion into the OM



Lepore *et al*., **PNAS** 108, 10121 (2011)

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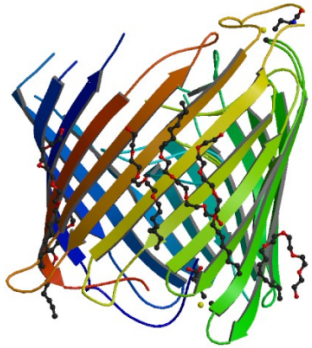
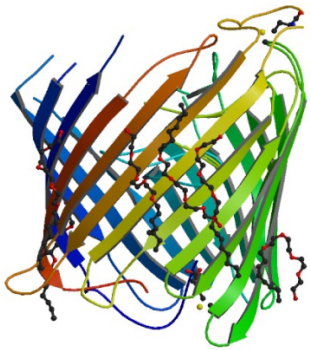
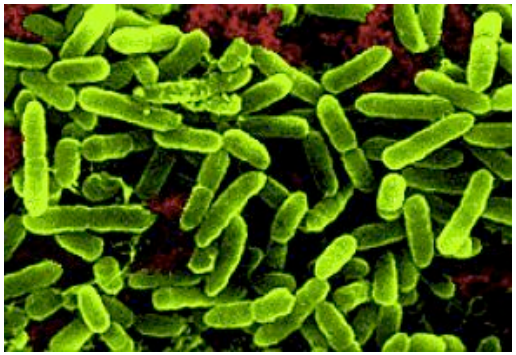


Substrate specificity in a family of OM carboxylate channels from *Pseudomonas aeruginosa*



Pseudomonas aeruginosa



- ***P. aeruginosa*** **is a Gram-negative, rod shaped bacterium commonly found in soil and water.**

- **It possesses a remarkable metabolic versatility.** ***P. aeruginosa*** **can use many (>100) organic compounds for growth.**

- **It is a notorious, opportunistic human pathogen that causes a variety of systemic infections particularly in patients with cancer, cystic fibrosis, AIDS and severe burns.**

- ***P. aeruginosa*** **is the fourth most-commonly isolated nosocomial pathogen accounting for ≈ 10 % of all hospital-acquired infections.**

What does this mean for drug design?

Effective concentration of a drug (*e.g* β-lactams) depends on the balance of

1. (OM) uptake
2. Efflux
3. Degradation

How do small molecules diffuse across the OM via OM channels?

Can drugs be made more effective by optimizing their OM permeation?

P. aeruginosa OprD Family Members

- *P. aeruginosa* OprD family has 19 members.
- division into two subfamilies based on phylogeny:

OprD Subfamily (8 members)

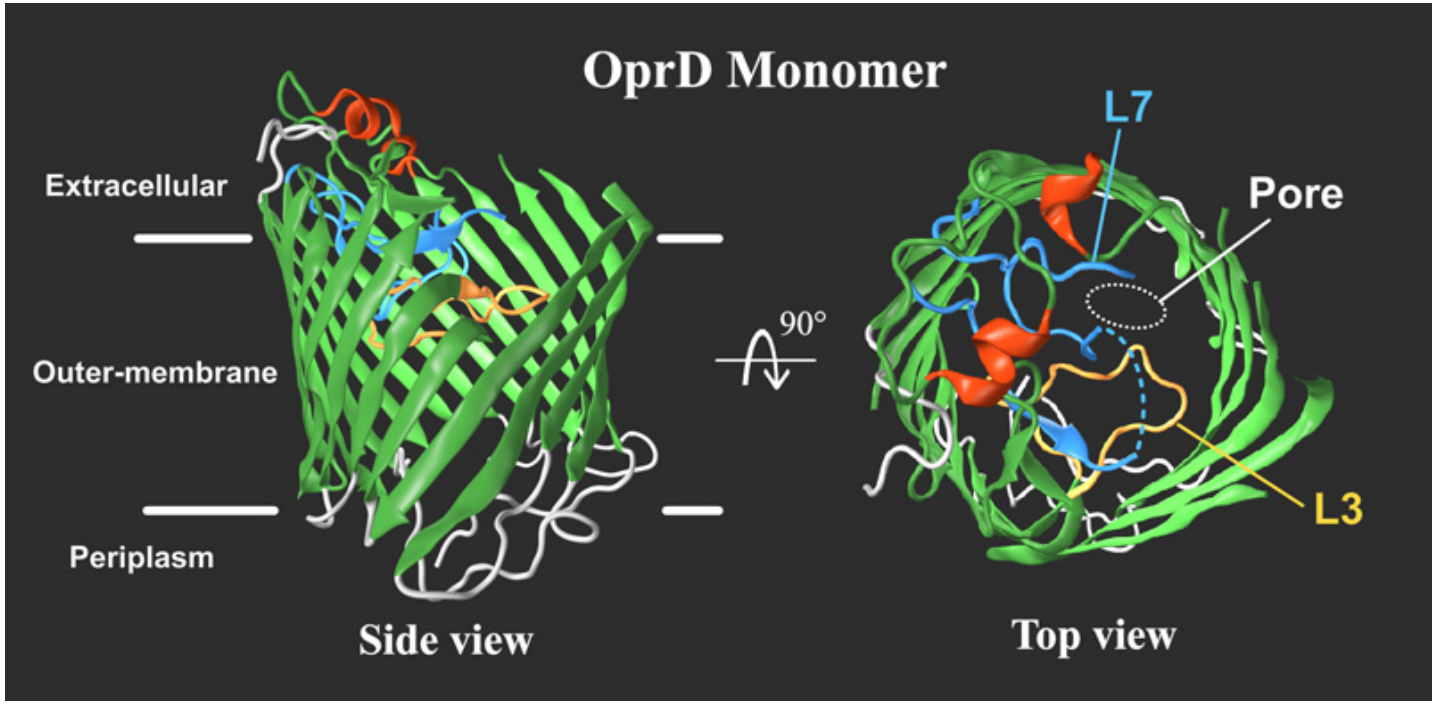
OpdK Subfamily (11 members)

- OprD family channels share high sequence identity (≈ 45-55 %)

- These proteins are widespread in pseudomonads but homologues have been identified in many other Gram-negative bacteria as well (*E. coli* has one homolog).

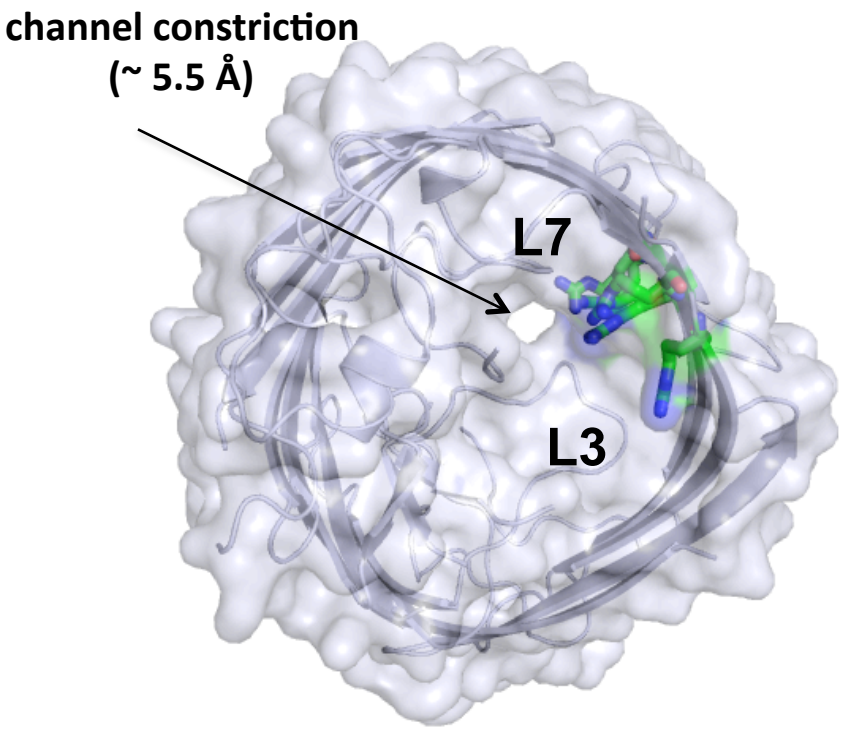
- They are thought to be substrate specific. However, there is no experimental evidence except for OprD (basic amino acids).

OprD Structure

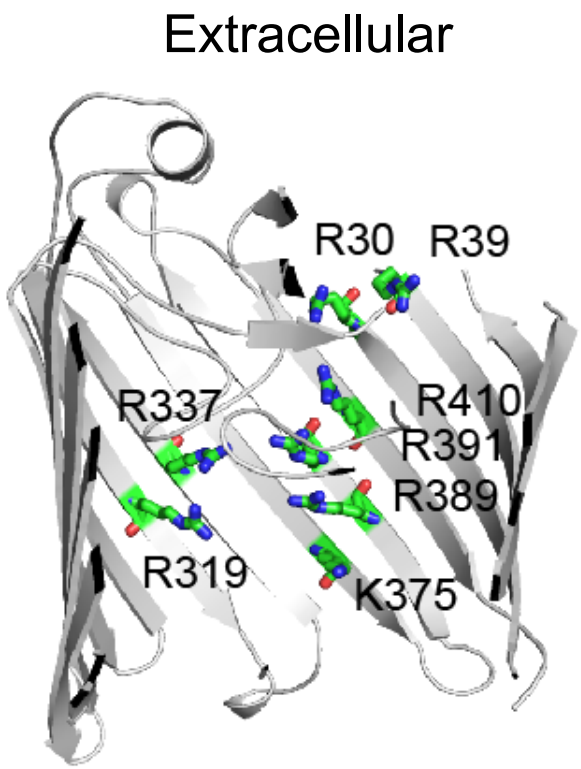


- OprD forms an monomeric 18-stranded β-barrel.
- Two long loops, L3 and L7, fold inward to the interior of barrel and constrict the pore.
- Short periplasmic turns/long extracellular loops

Biswas *et al*, **Nat. Struct. Mol. Biol**. 14, 1108 (2007); Biswas *et al*., **Structure** 16,1027 (2008)



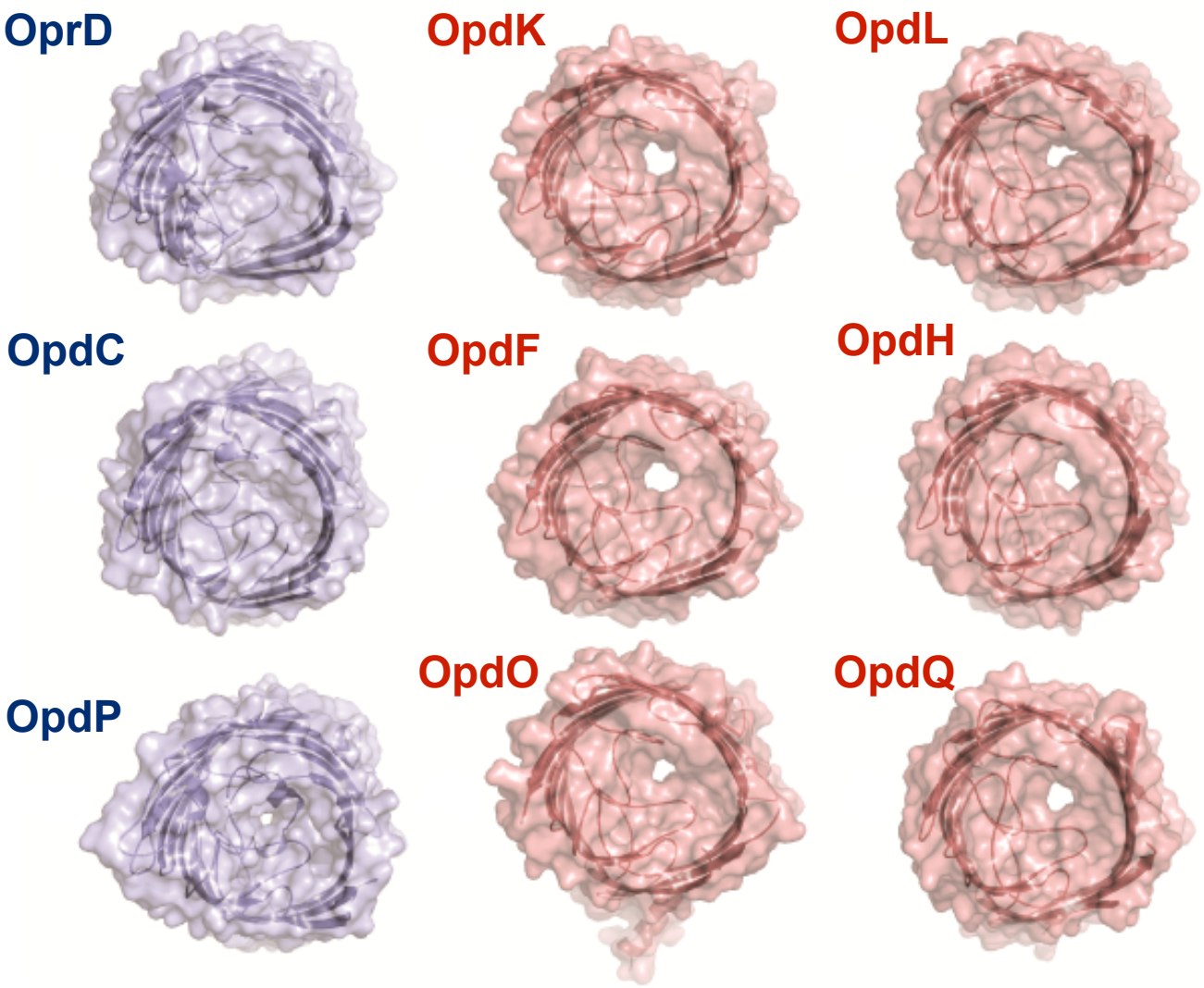
The OprD channel constriction is very narrow.



A basic ladder is present in the OprD structure (a row of arginine and lysine residues in the barrel wall).

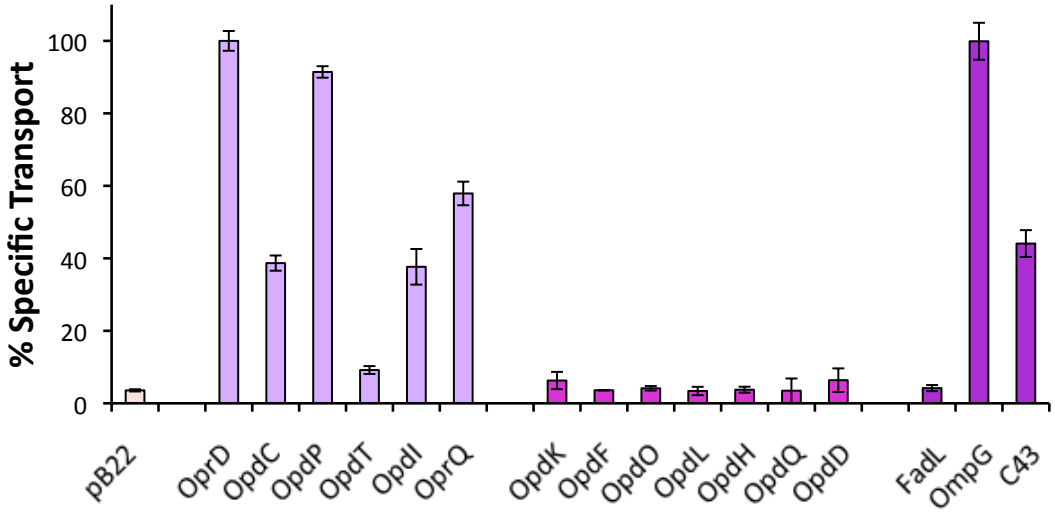
The side chains of these residues point into the lumen of the barrel and may form an electrophoretic conduit that may bind substrate carboxylate groups to guide the substrates towards the constriction.

Crystal Structures of OprD Channels Show a Division Into Two Subfamilies



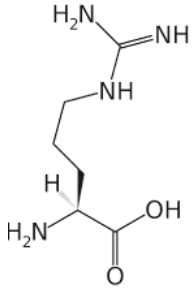
Eren *et al*., **PLOS Biology** 10 (2012)

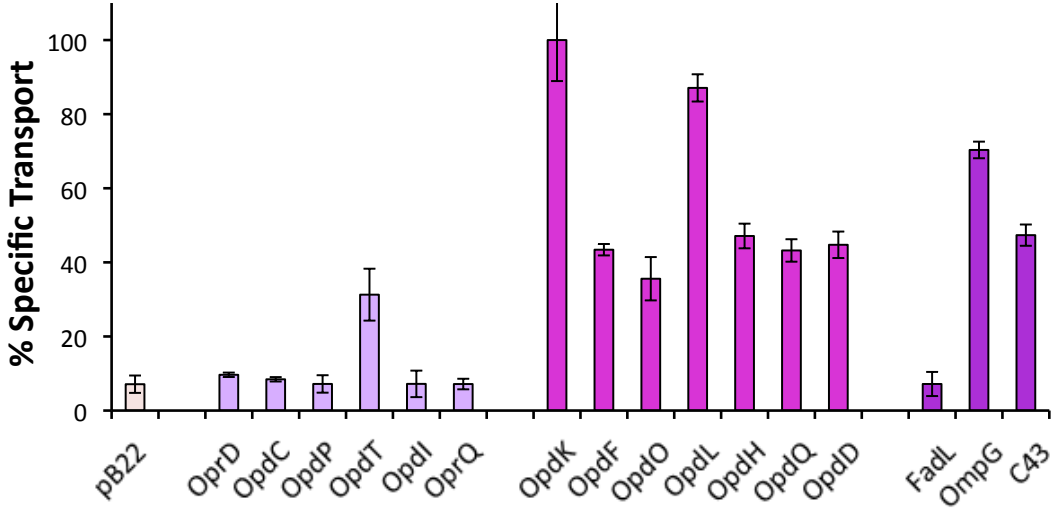
Substrate Specificity of OprD Channels



Arginine

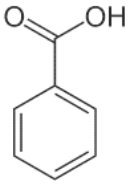
100% = 199.7 ± 5.4 pmol/mg /min





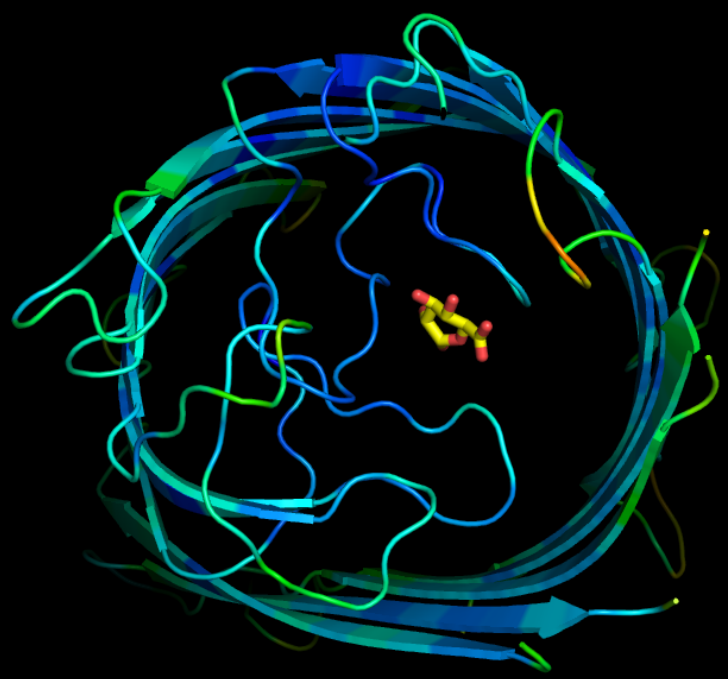
Benzoate

100% = 40.4 ± 0.7 pmol/mg /min

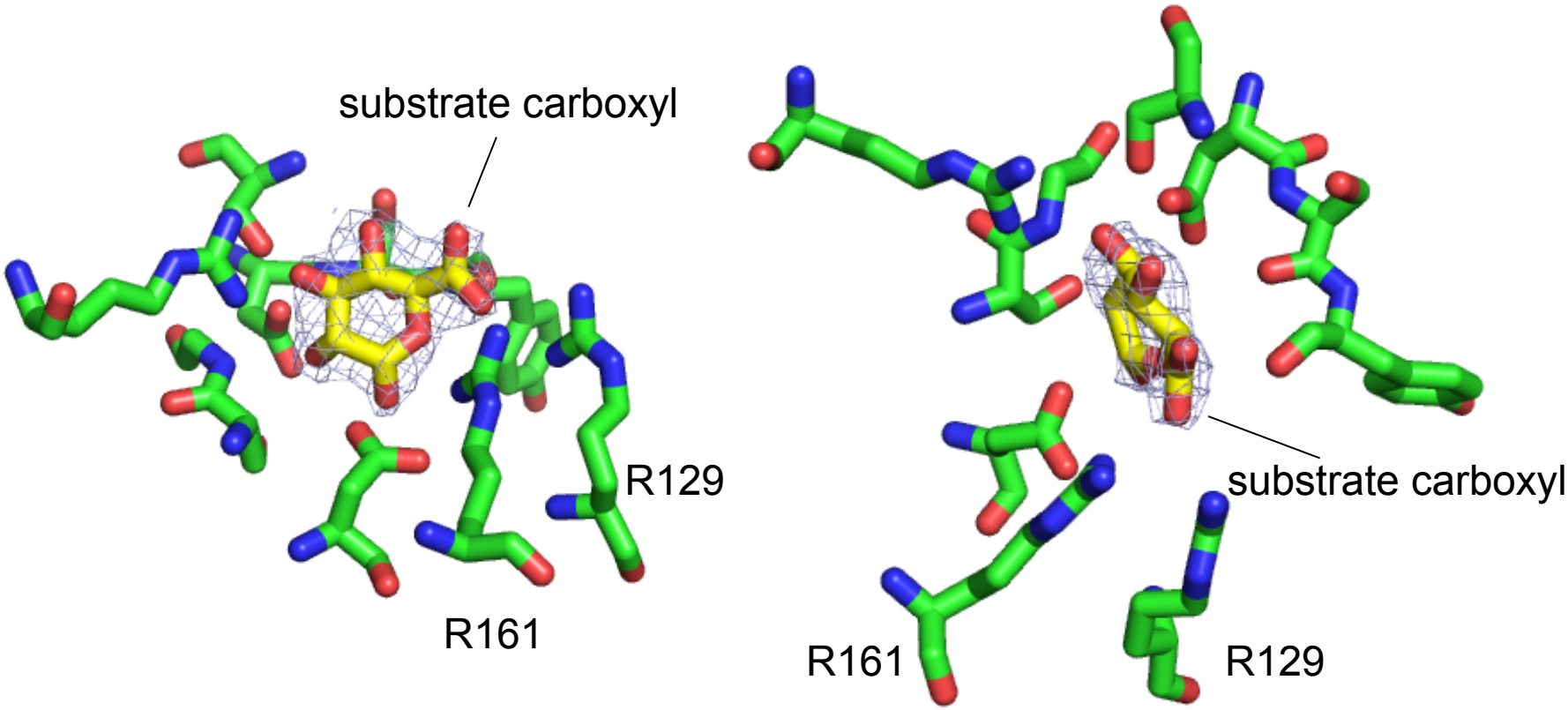


OprD subfamilies have different substrate specificities

Crystal structure of OpdF with glucuronate substrate



OpdF-glucuronate structure



Substrate carboxyl interacts with basic ladder residues
R129 and R161

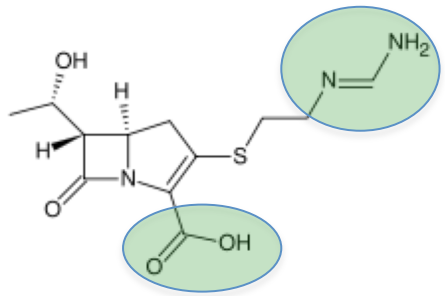
- One of the most worrisome characteristics of *P. aeruginosa* is its low antibiotic susceptibility.

- *P. aeruginosa* is naturally resistant to many antibiotics partly due to the permeability barrier afforded by its outer membrane LPS and the lack of large-channel porins.

One other interesting aspect of OccD1 (OprD)

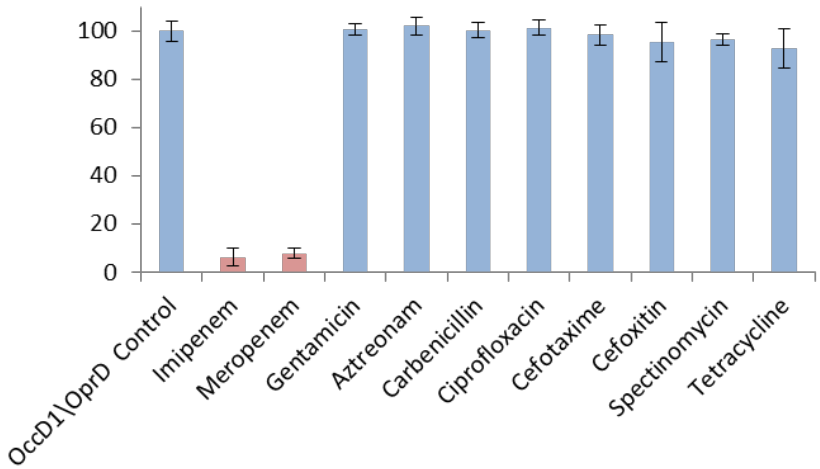
Expression of OccD1 is often reduced/abolished in clinical isolates of *P. aeruginosa* strains resistant to carbapenem antibiotics

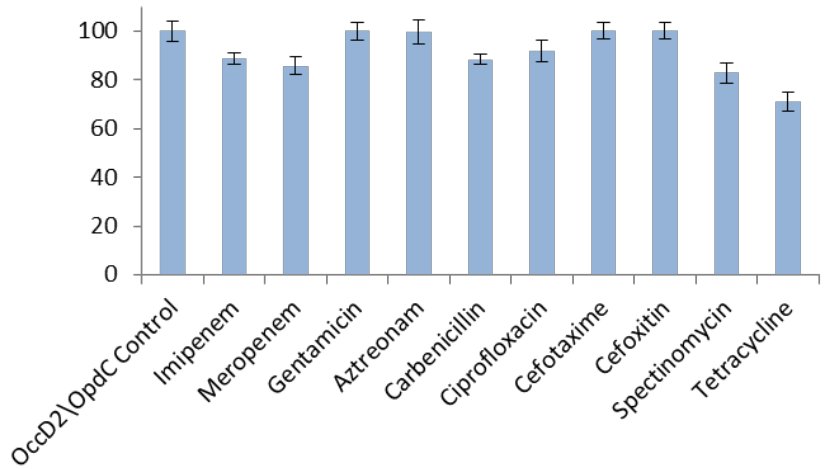
OccD1 is the entryway for carbapenem antibiotics

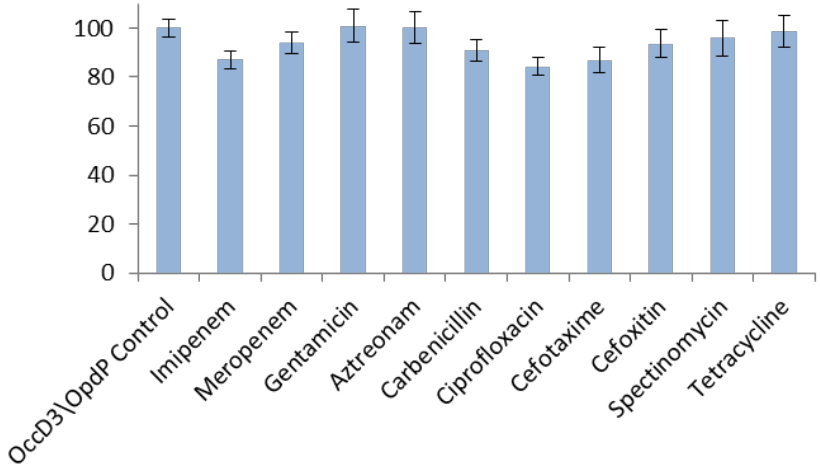


Imipenem (Mw 300 Da): similar to basic amino acids

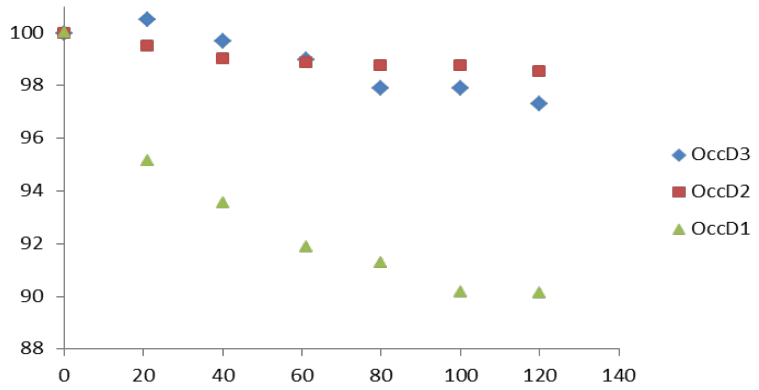
Arginine Transport by OccD1-D3: Inhibition By Antibiotics







Imipenem Transport by Liposome Swelling Assay



Antibiotic binding/transport by OccD proteins is very specific

Deposited PDB coordinates since 2009:

FadL project

3PGR, 2R89, 2R8A, 3PF1, 3PGU, 3PGS
3DWO, 2R88, 3DWN, 2R4L, 2R4N, 2R4O, 2R4P

Occ project

3SY7, 3SY9, 3SYB, 3SYS, 3SZD, 3SZV, 3T0S, 3T20, 3T24

Side projects

3KVN; J.Mol.Biol. 396: 627-633 (2010)

2X4M, 2X55, 2X56; Structure 18, 809-818 (2010)

3PIK; Plos One 6, e15610-e15610 (2011)

2X27; Plos One 5, 15016-e15016 (2010)

Why do we like X6A?

Flexibility and DIY booking of beamtime

E-mail notifications of beamtime available

Automounter

Energy flexibility for SAD/MAD

No worries regarding radiation damage

What could be implemented/improved?

Remote access

Space for data processing/unwinding (coffee!)

Booking schedules X6A/X25 talking to each other

Low-res completeness (small beamstop)

Acknowledgements

FadL:

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Syma Khalid (U. Southampton)

$$$:
NIH (NIGMS)
PEW Scholarship

NSLS Beamlines X6A/X25/X29
APS Beamlines ID23/24