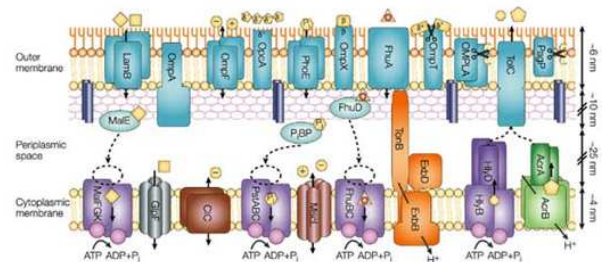


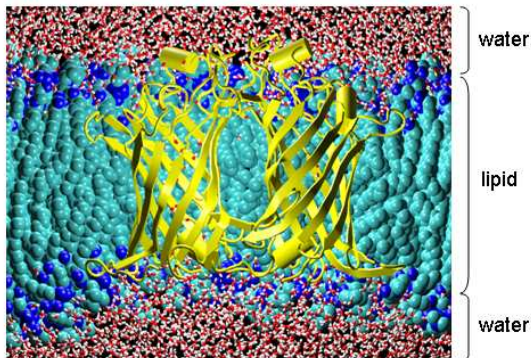
Simulations of Bacterial Outer Membrane Proteins

High resolution structures are known for ca. 20 bacterial outer membrane proteins (OMPs). These OMPs, based upon a β -barrel architecture, embrace a range of functions including: pores, transporters, recognition proteins and enzymes. OMPs are amenable to molecular dynamics (MD) simulations, enabling us to probe their multi-nanosecond timescale dynamics when embedded in lipid bilayers. We have performed MD simulations of a range of OMPs, including: OmpA, OmpX, PagP, OpcA, OmpT, OMPLA, FhuA and FepA. As a case studies we present simulations of OmpA, OpcA, FhuA and OMPLA. These simulations are being used to form a database of OMP simulations, which in turn will be used as the basis of a virtual outer membrane (vOM).



Bacterial outer and inner membranes. From: Faraldo-Gómez & Sansom (2003) *Nature Rev. Mol. Cell Biol.* 4:105

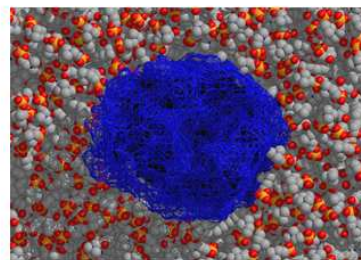
MD Simulations of OMPs



◆ GROMACS; GROMOS forcefield; SPC water

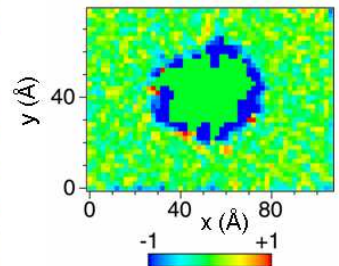
Simulation setup requires insertion of an irregular object (protein) in a bilayer

‘Grow’ a hole in a pre-formed bilayer: Faraldo-Gómez *et al.* (2002) *Eur. Biophys. J.* 31:217



FhuA surface inserted in POPC bilayer

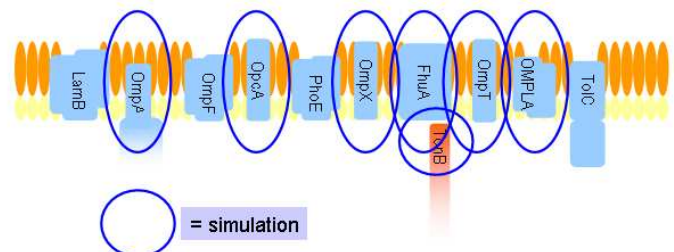
Change in Lipid Density



Towards a Virtual Outer Membrane

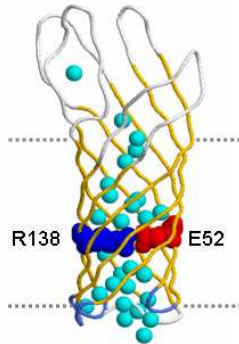
Current Simulations

Protein	Function	Number of β -strands
OmpA	Channel and/or anchor	8 β
OmpX	Recognition	8 β
PagP	Fatty acyl transferase	8 β + N-terminal helix
OpcA	Recognition/adhesion	10 β
OmpT	Protease	10 β
OMPLA	Phospholipase	12 β (dimeric)
FhuA	Ferrichrome transporter	22 β + internal ‘cork’ domain
FecA	Siderophore transporter	22 β + internal ‘cork’ domain



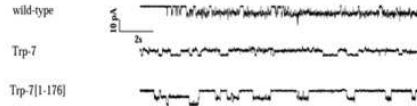
- ◆ First step towards systems biology – select a suitable system...
- ◆ Bacterial OMs – 5 or 6 proteins = 90% of protein content
- ◆ Structures or good homology models of proteins are available
- ◆ Complex lipid – outer leaflet is lipopolysaccharide (LPS)
- ◆ Minimum system size ca. 2.5×10^6 atoms; simulation times ca. 50 ns

OmpA – A Pore or Not a Pore?



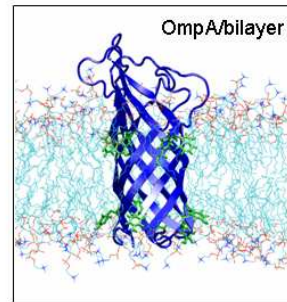
X-ray structure

- no continuous pore – not a channel?
- tightly bound internal waters
- Pautsch & Schulz (1998) *Nature Struct. Biol.* 5:1013



Electrophysiology

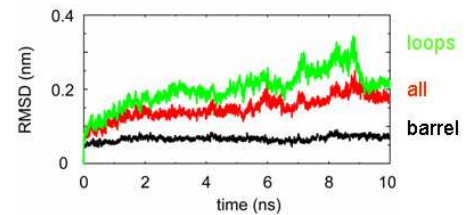
- OmpA forms channels
- Trp mutants change gating
- N-terminal fragment forms channels
- Arora et al. (2000) *J. Biol. Chem.* 275:1594



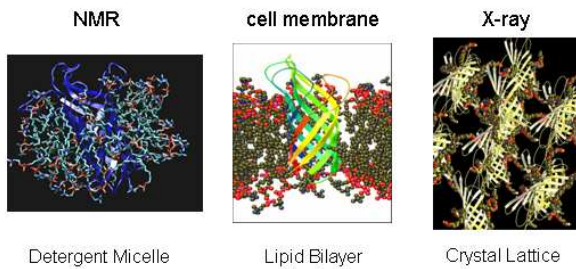
- ◆ DMPC bilayer: 10 ns
- ◆ Rigid barrel; flexible loops
- ◆ Flexibility gradient along barrel axis/bilayer normal

MD & Modelling

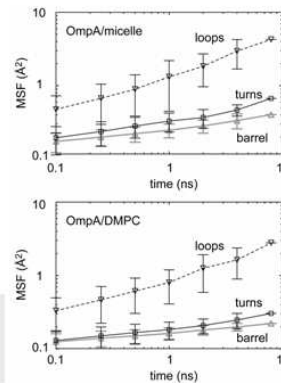
- Simulations in DMPC bilayer
- Explore channel gating models
- Bond et al. (2002) *Biophys J* 83: 763



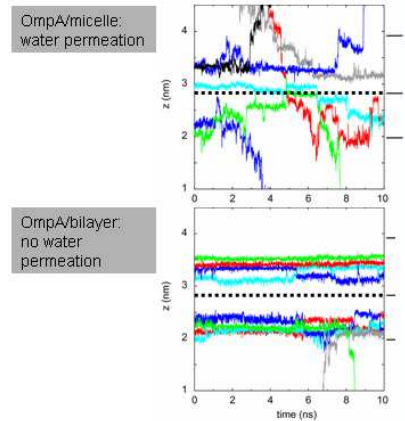
OmpA – Dynamics vs. Environment



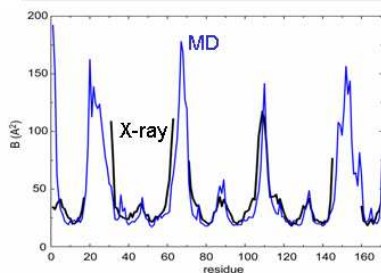
- ◆ Multiple, comparative simulations – on 10 to 50 ns timescale
- ◆ Need to relate dynamics in experiments to *in vivo*
- ◆ Small changes in flexibility – can *open* the central pore



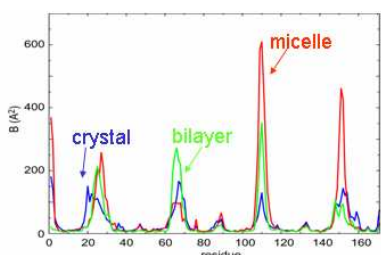
Water Trajectories



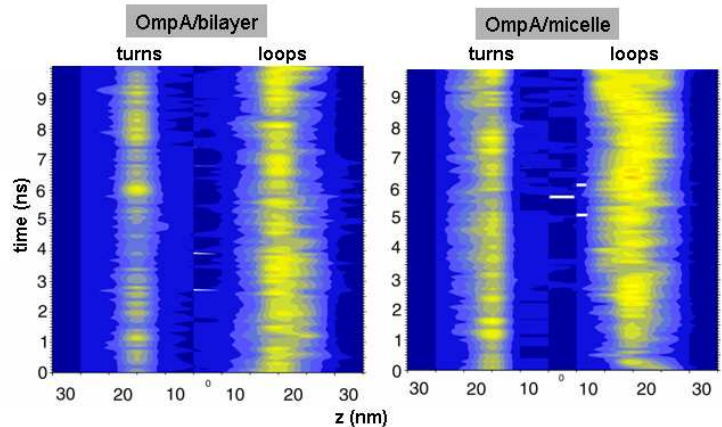
X-ray vs. crystal simulation B-values



Flexibility: micelle > bilayer > crystal

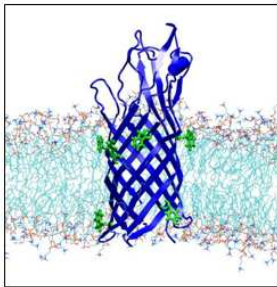


Protein/Lipid Headgroup Interactions vs. Time

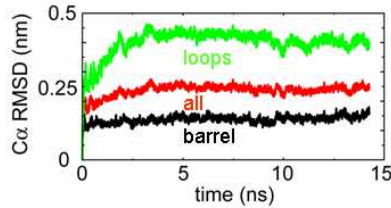
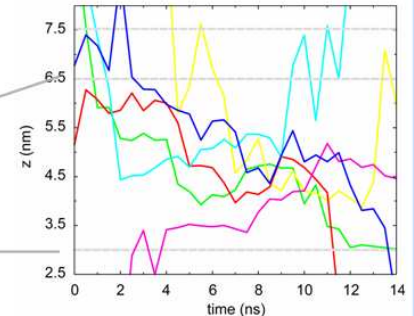
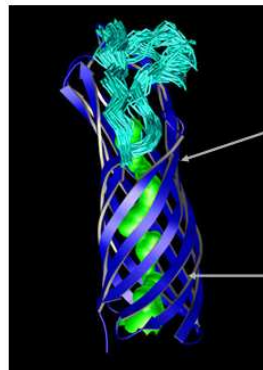


- ◆ Contours = headgroup contacts < 0.35 nm
- ◆ Asymmetry in both environments; loops > turns

OpcA: Predicting Function by MD

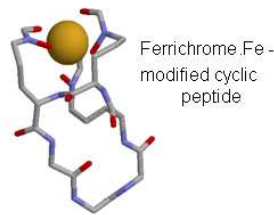
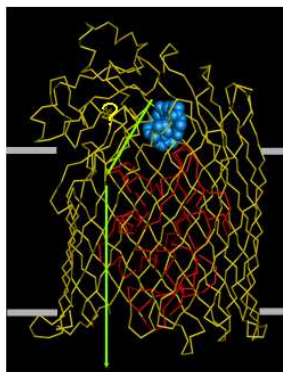


- ◆ OpcA from *Neisseria meningitidis*: a pathogen
- ◆ Recognition protein: binding to target cells
- ◆ Possible ion channel: water filled pore; blocked in crystal by Zn²⁺ and extracellular loops
- ◆ Test pore formation by MD
- ◆ DMPC bilayer, 15 ns

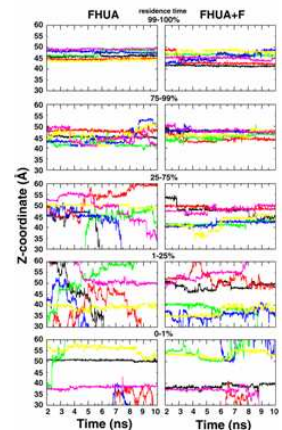
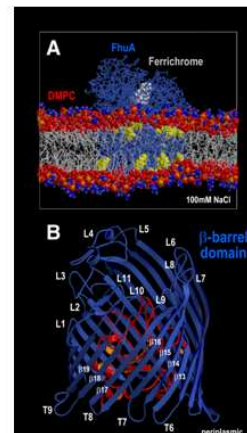


- ◆ Mobile extracellular loop
- ◆ Fluctuations open pore
- ◆ Water permeation
- ◆ Test pore formation by electrophysiology...

FhuA – Searching for a Transport Pathway



FhuA - bacterial Fe^{III} transporter
22 strand β-barrel (yellow) plus N-terminal "plug" domain (red)

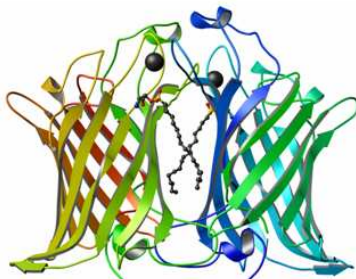


- ◆ Is there a 'protopore' implicit in the FhuA structure?
- ◆ Does "plug" have to be removed for ferrichrome transport?

- ◆ 10 ns simulations in DMPC with & without ferrichrome
- ◆ Water trajectories – support 'plug removal' model of transport

OMPLA: An Outer Membrane Enzyme

- ◆ OMPLA – an outer membrane lipase – dimerises to form active site
- ◆ Ca²⁺ ions stabilize dimer and active site
- ◆ Catalytic triad related to Ser proteases



- ◆ Fluctuations of catalytic triad
- ◆ Stabilisation by Ca²⁺ ions
- ◆ Substrate-induced changes in monomer:monomer interactions

- ◆ MD simulations in DMPC bilayer; 5 ns each
- ◆ Monomer vs. dimer + Ca²⁺ vs. dimer-inhibitor complex + Ca²⁺

