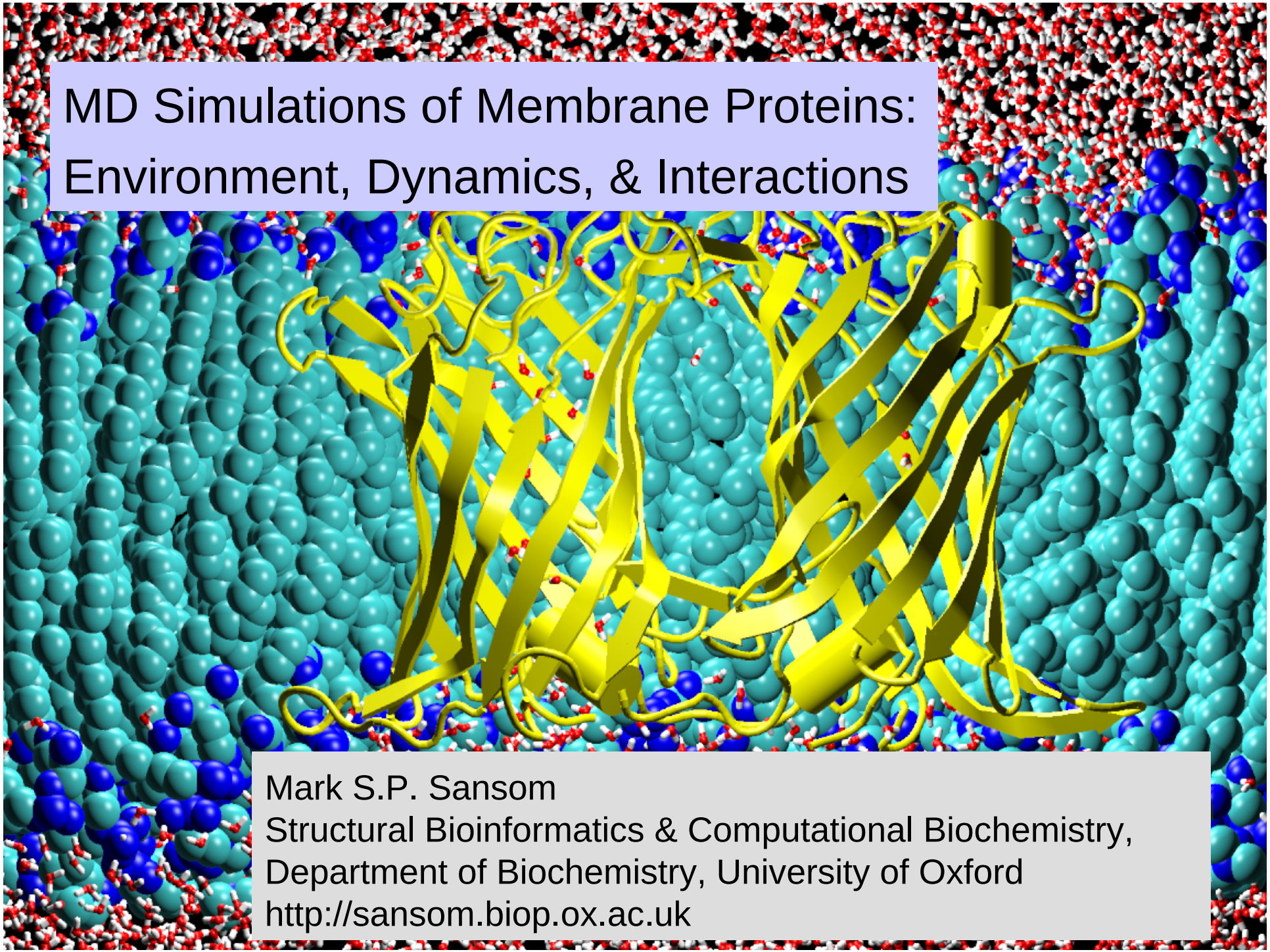
MD Simulations of Membrane Proteins: Environment, Dynamics, & Interactions



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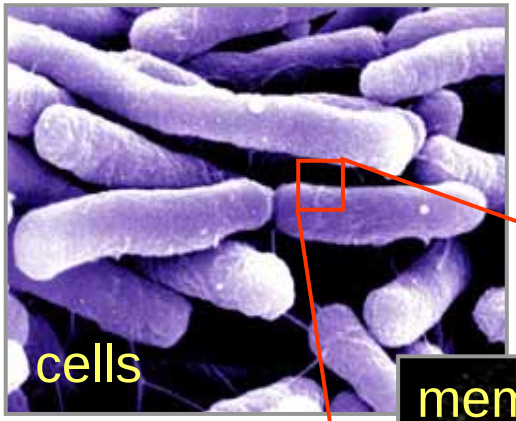
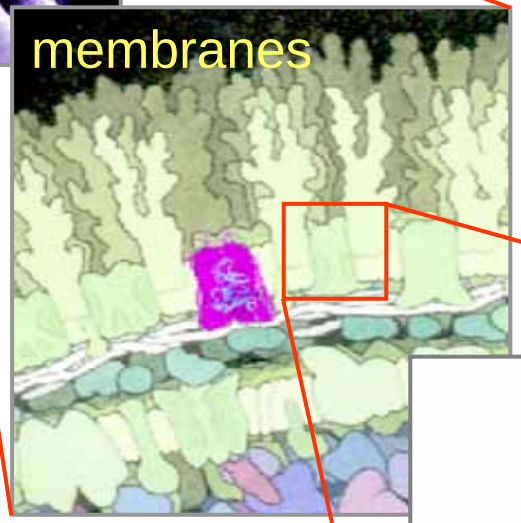
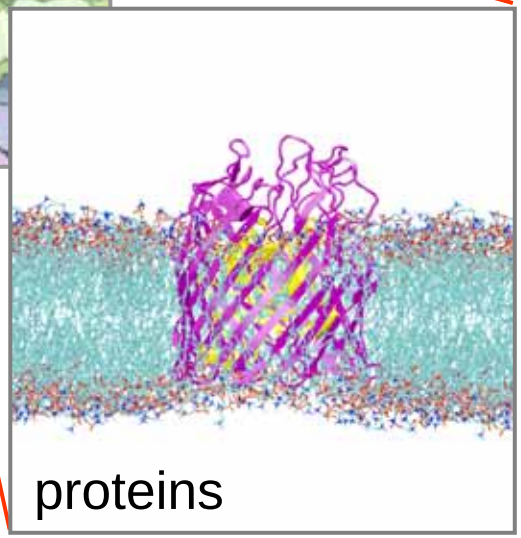
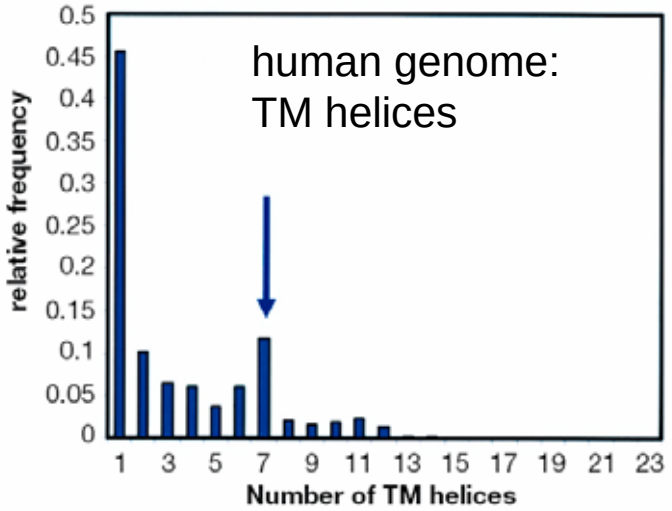
Cells, Membranes & Proteins









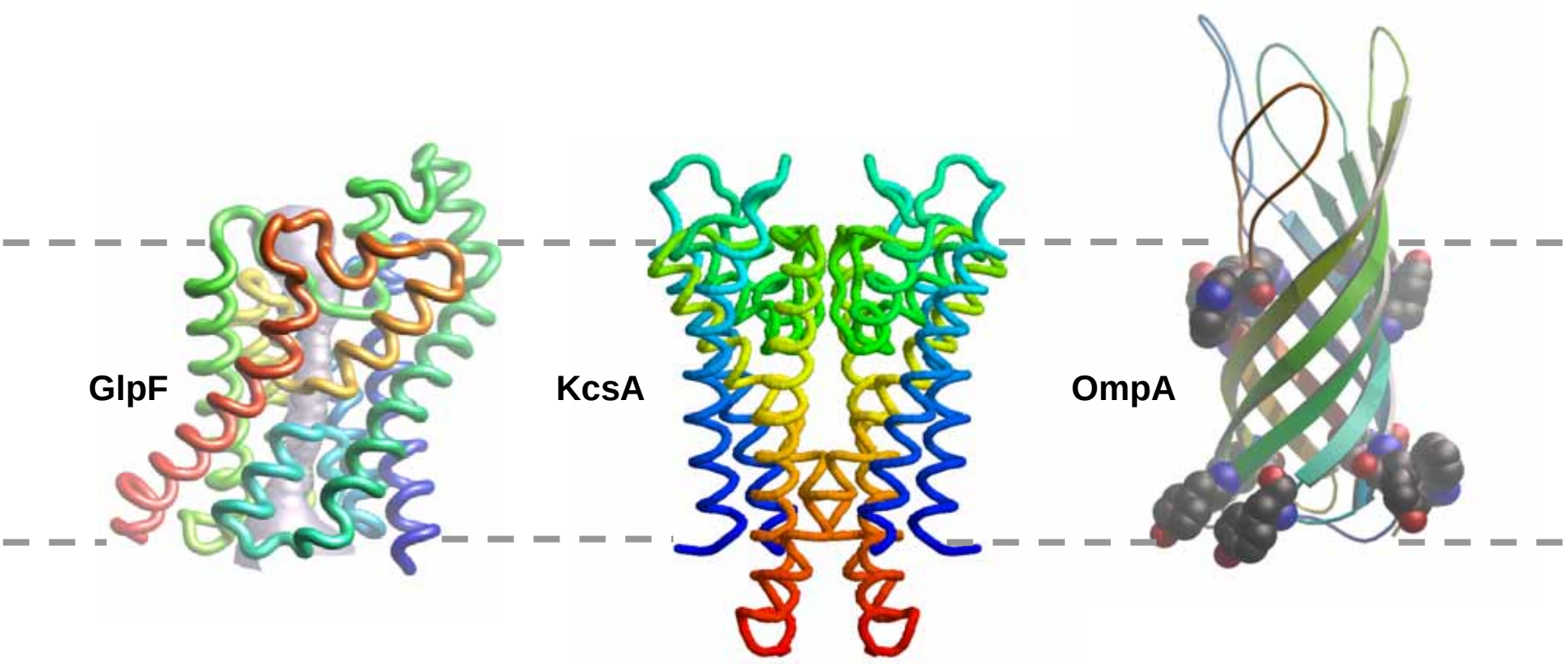
- biological membrane: lipid bilayer + proteins
- membrane proteins: ~25% of genes
- drug targets: ion channels & receptors

Membrane Proteins: Simulations

- Membrane proteins: structures, environments, simulations
- Outer Membrane Proteins
  - OmpA – dynamics & function *vs.* environment
  - OpcA – prediction of function
- Protein/lipid interactions:
  - KcsA *vs.* OmpA – protein interactions with a PC membrane
  - OmpT & KcsA – specific interaction sites revealed
- Protein/detergent micelles:
  - GlpF – detergent vs. lipid interactions
  - OmpA & GpA – self-assembly of detergent/protein micelles

MD Simulations: Why?

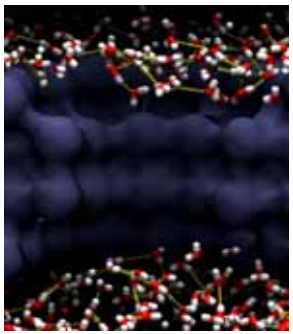
- X-ray structure: static, average structure at **100 K**
  crystal = protein (+ detergent) + water + ions
- MD simulation: multi-nanosecond dynamics at **300 K**
  system = protein + membrane + water + ions
- The challenge: to relate dynamics to biological function –
  e.g. conformational dynamics & interactions *vs.* environment



An Overview of Research

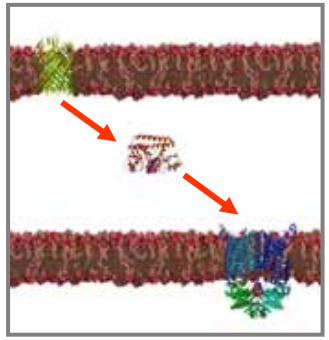
Bionanoscience:
nanopores &
TM helix nanoswitches

Membrane proteins:
bioinformatics
structure & stability



Channels & receptors:
modelling, simulations

Transporters:
MFSs, ABCs, AcrAB, etc.



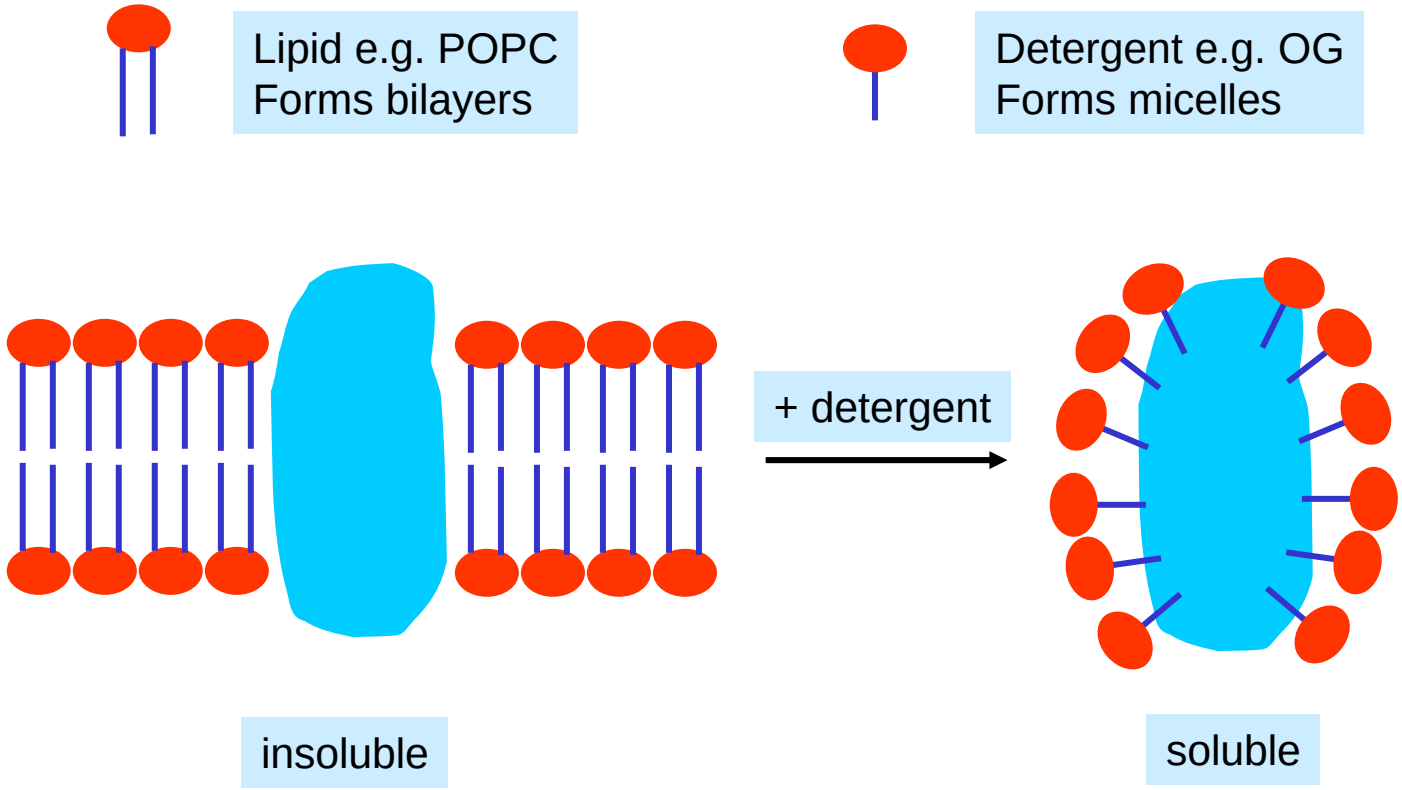
Bacterial OMPs:
a virtual outer
membrane

BioSimGrid & IntBioSim:
towards systems biology

biophysics

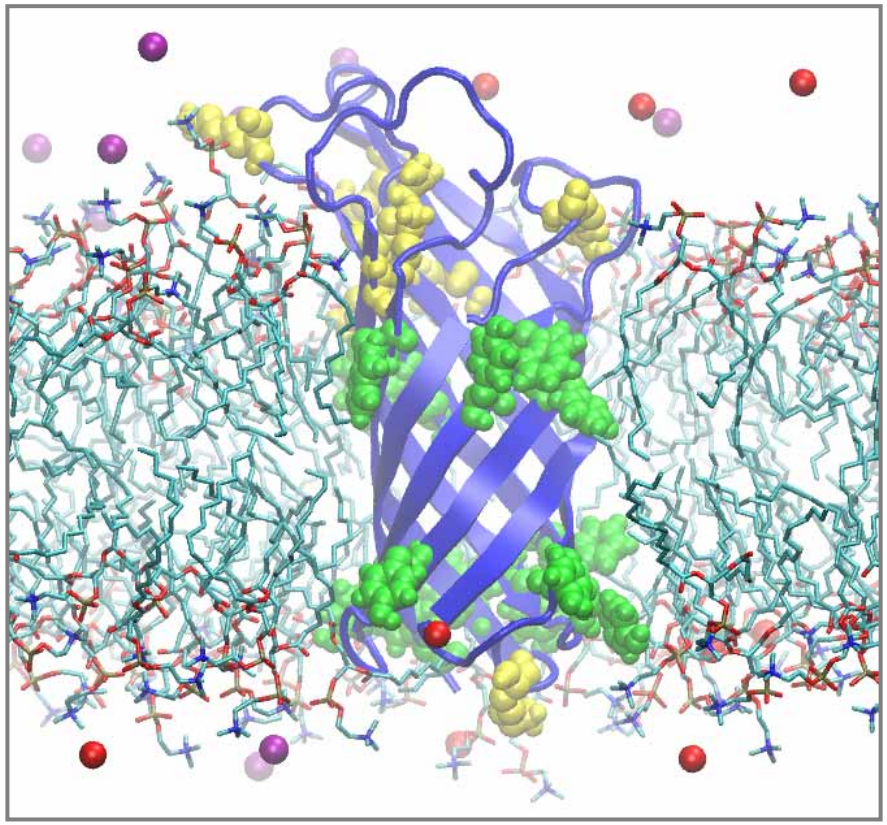
biology

Detergents & Membrane Proteins



- Membrane proteins are isolated and purified (and crystallised) using detergents
- Need for non-denaturing detergents e.g. octyl glucoside
- Important to compare conformational dynamics in lipid bilayer *vs.* detergent micelles

MD Simulations of Membrane Proteins



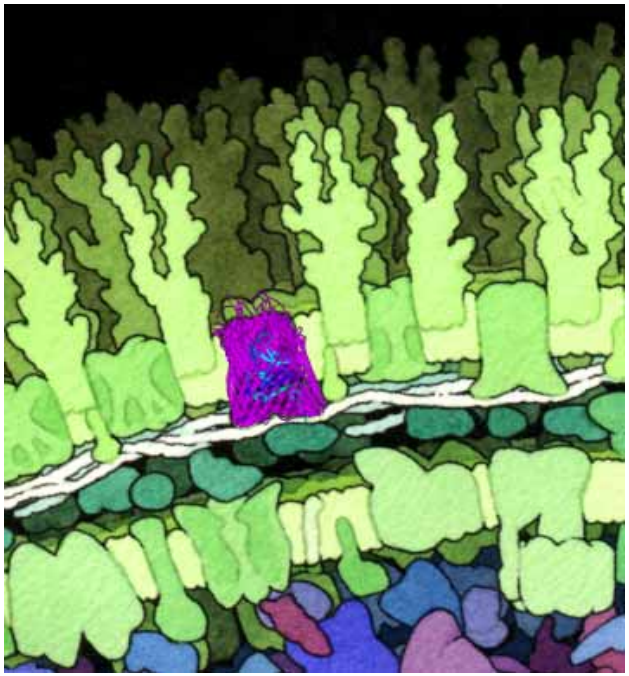
water, ions

proteins & phospholipids

minimum system

50,000 to 100,000 atoms

- Describe the forces on all atoms: $F = -dU(x)/dx$
- Integrate: $F = ma$ (a few million times…)
- Result: positions of all atoms for ~10 ns
- Experimental (static) structure → *in vivo* dynamics



OM

IM

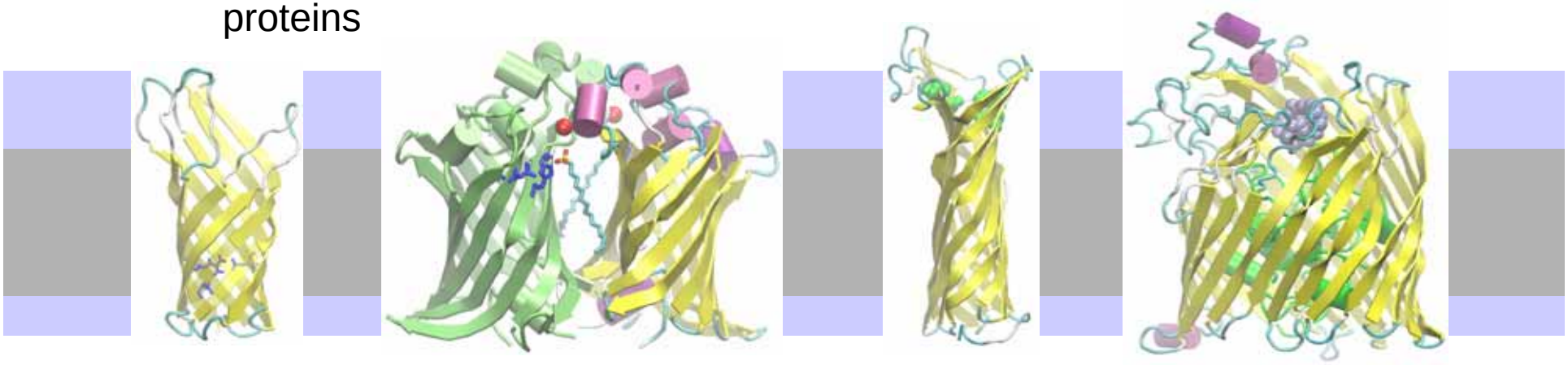
Bacterial Outer Membranes

- Bacterial outer membrane proteins: numerous structures known
- Potential antibiotic and vaccine targets
- Functional diversity: pores; recognition proteins; enzymes; transporters
- Aim: to build a library of OMP simulations
- Bond & Sansom (2004) *Molec. Membr. Biol.* 21:151

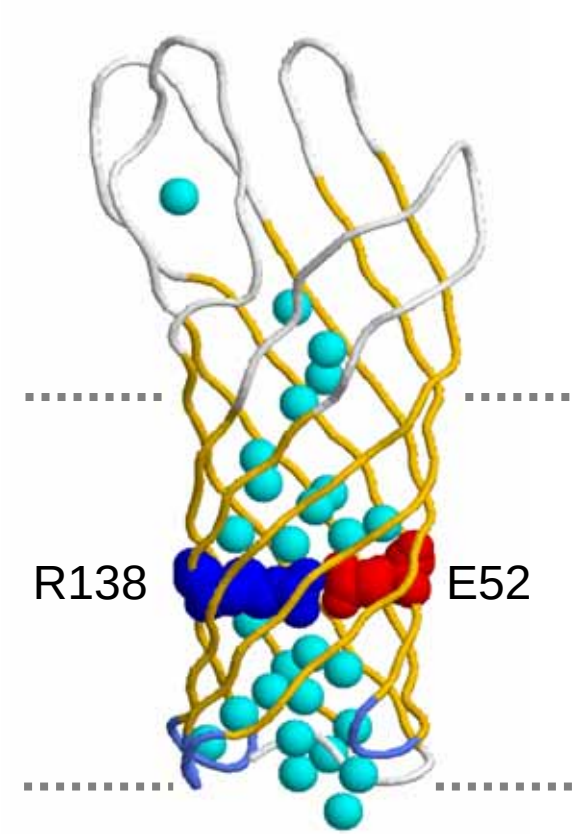
recognition proteins

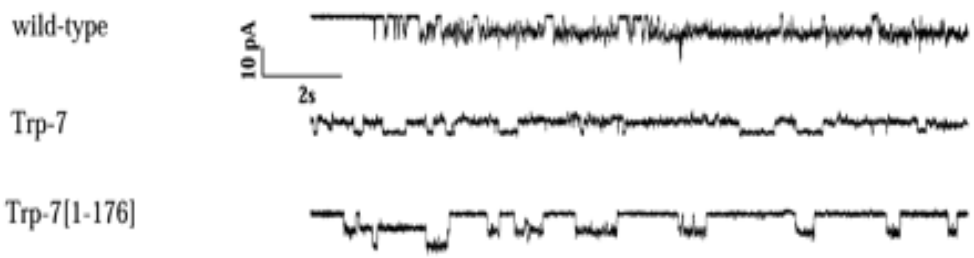
enzymes

transporters



OmpA - A Closed Channel?





Electrophysiology
- OmpA forms channels
- Trp mutants change gating
- N-terminal fragment forms channels
  Arora *et al.* (2000)

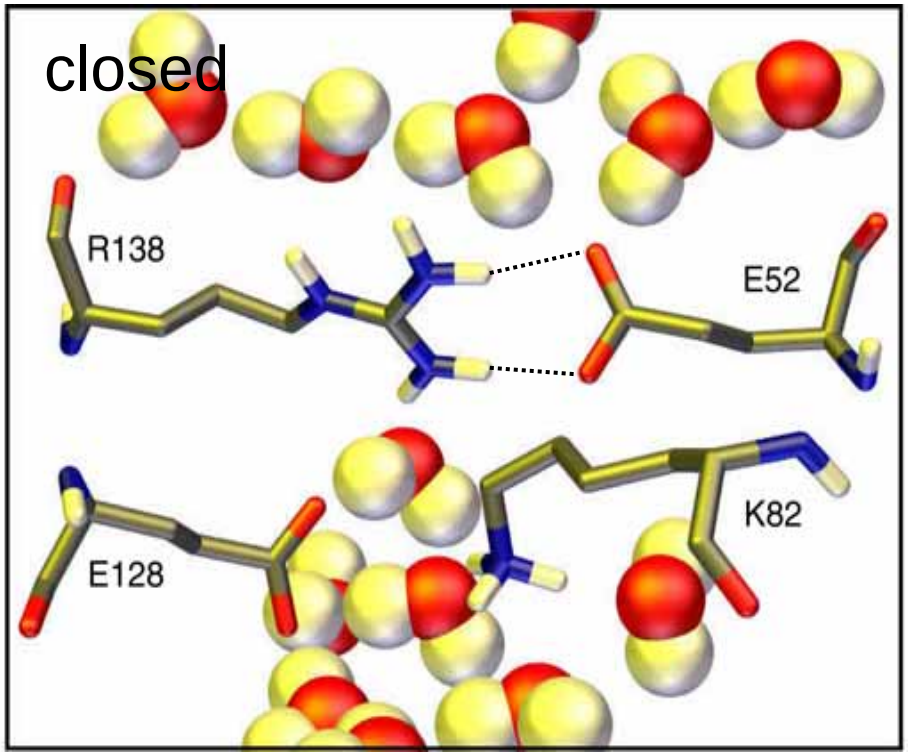
X-ray structure Pautsch & Schulz
- a closed channel?
- tightly bound internal waters

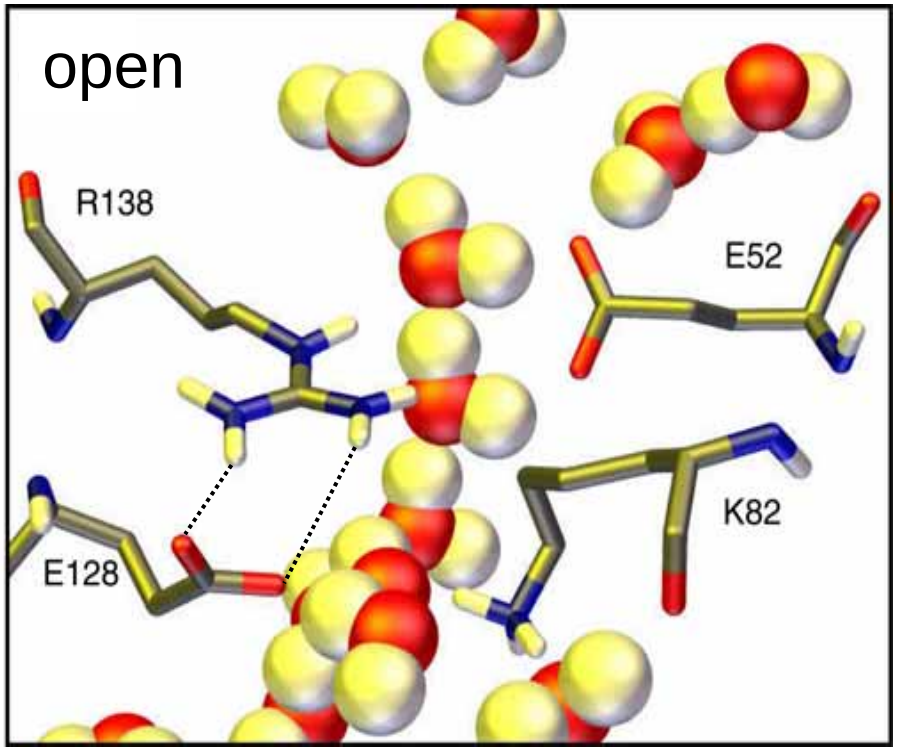
NMR structures Tamm; Wüthrich
- DPC micelle
- flexibility gradient

MD & Modelling
- Simulations in DMPC bilayer & DPC micelle
- Explore channel gating models

Bond et al. (2002) *Biophys. J.* 83:763
Bond & Sansom (2003) *J. Mol. Biol.* 329:1035

OmpA – Closed vs. Open Gates



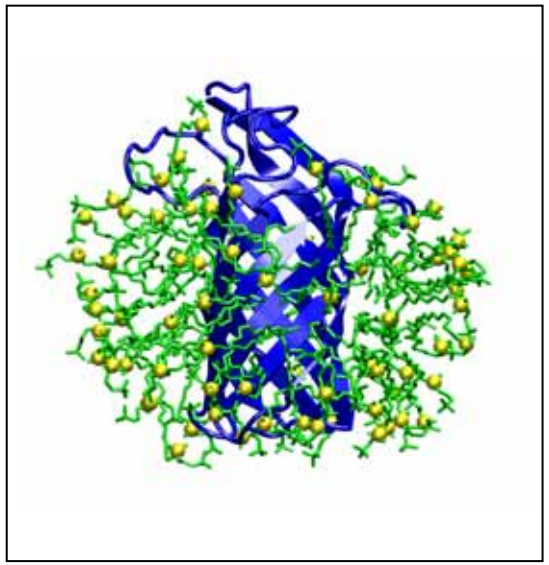


- X-ray structure
- R138-E52 salt bridge
- Gate closed – water molecules do *not* pass in MD simulations (up to 10 ns)

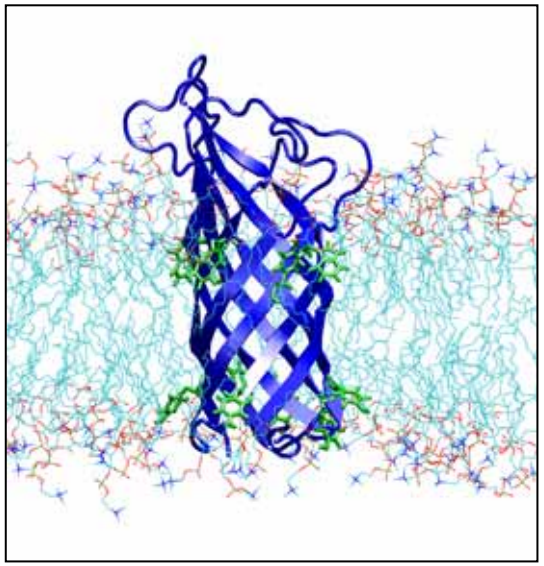
- Model
- R138-E128 salt bridge
- Gate open – water molecules can pass freely on 10 ns timescale

OmpA: Dynamics *vs.* Environment

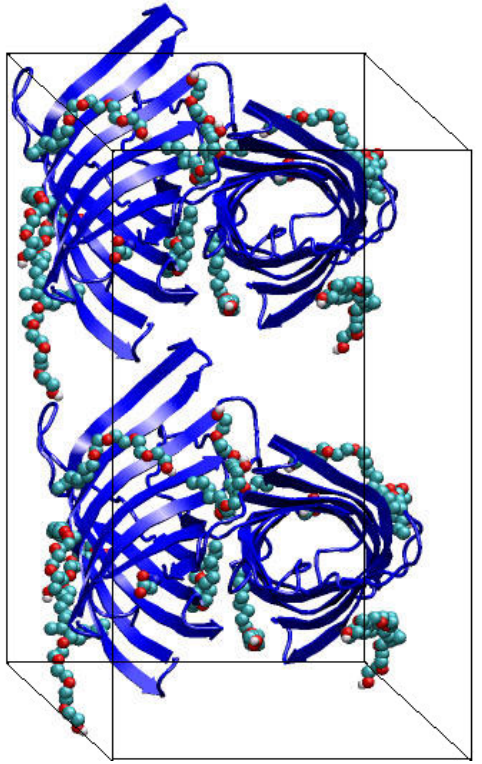
NMR: detergent micelle
~25 ns



In vivo: lipid bilayer
~25 ns

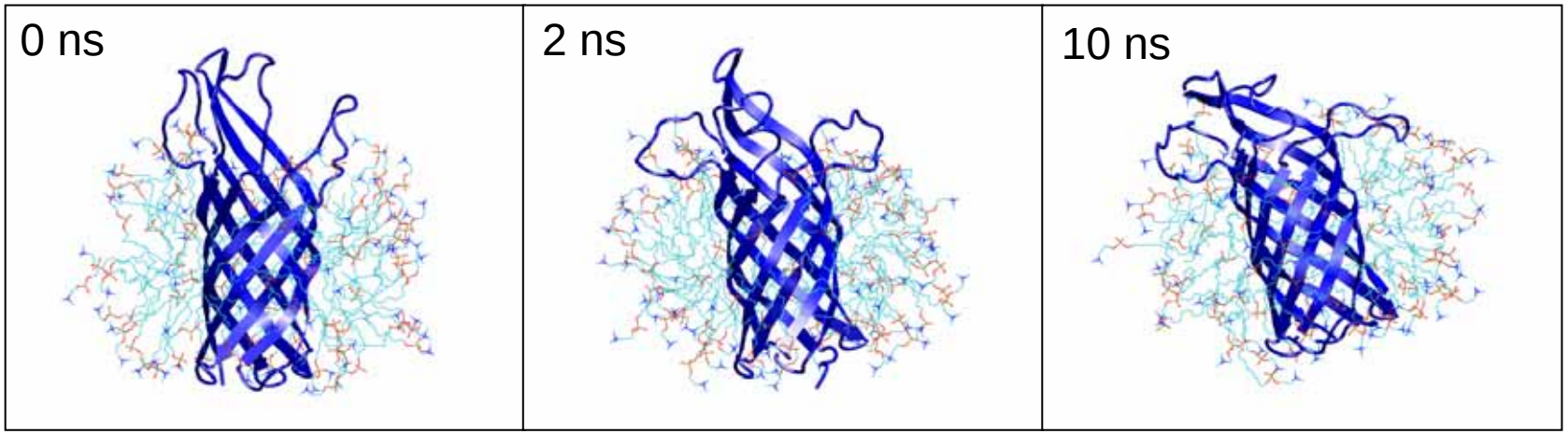


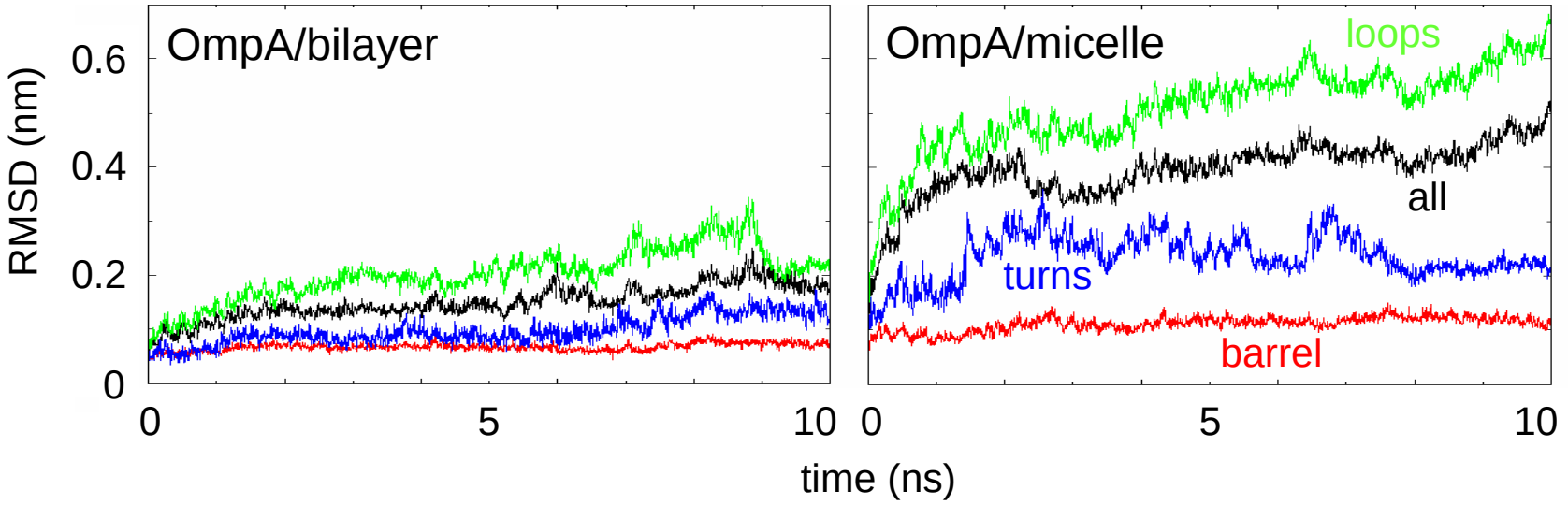
X-ray: crystal lattice
~50 ns



- Micelle: 80 dodecyl phosphocholines
- Bilayer: 111 DMPCs
- Crystal: 4 OmpAs & 24 C8E4 detergents per unit cell
- GROMACS; PME; GROMOS parameters

Micelle *vs.* Bilayer

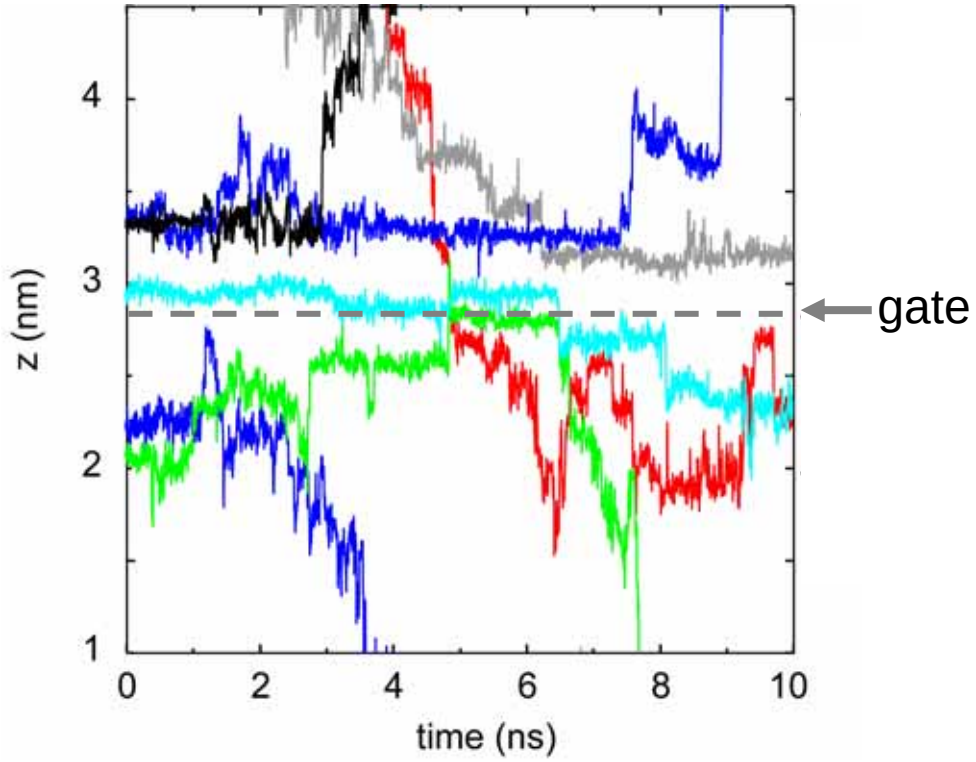


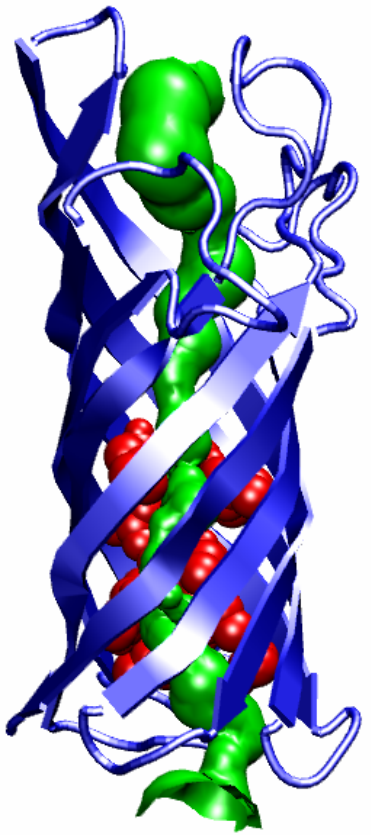


- Greater structural drift in micelle than bilayer

  Bond & Sansom (2003) *J. Mol. Biol.* 329:1035-1053

OmpA: Functionally Open in Micelle MD

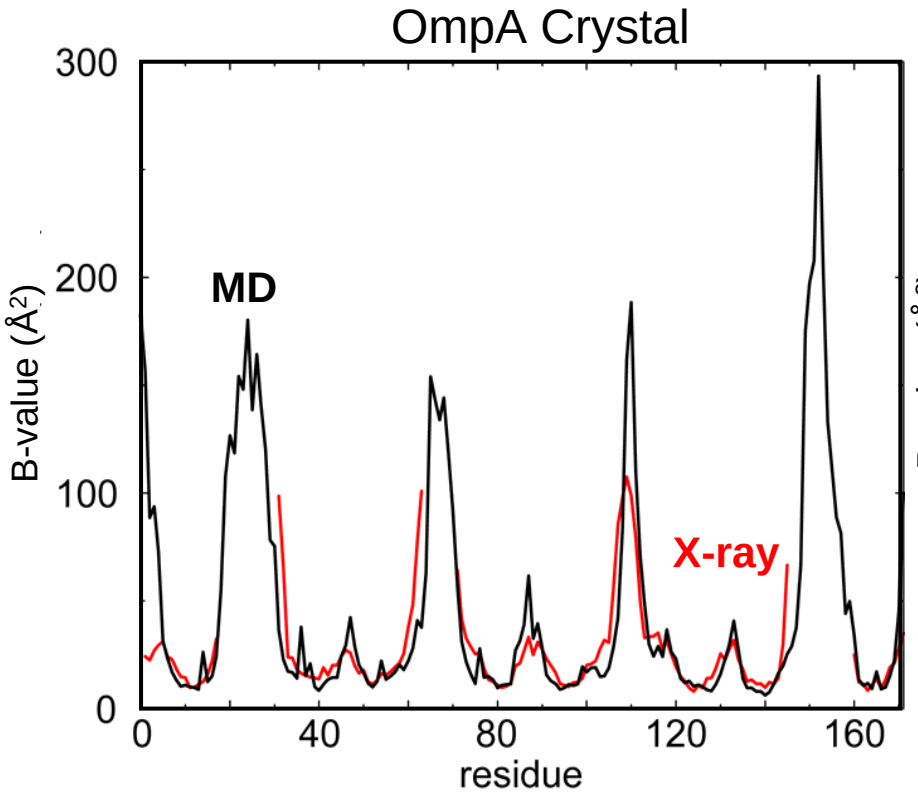


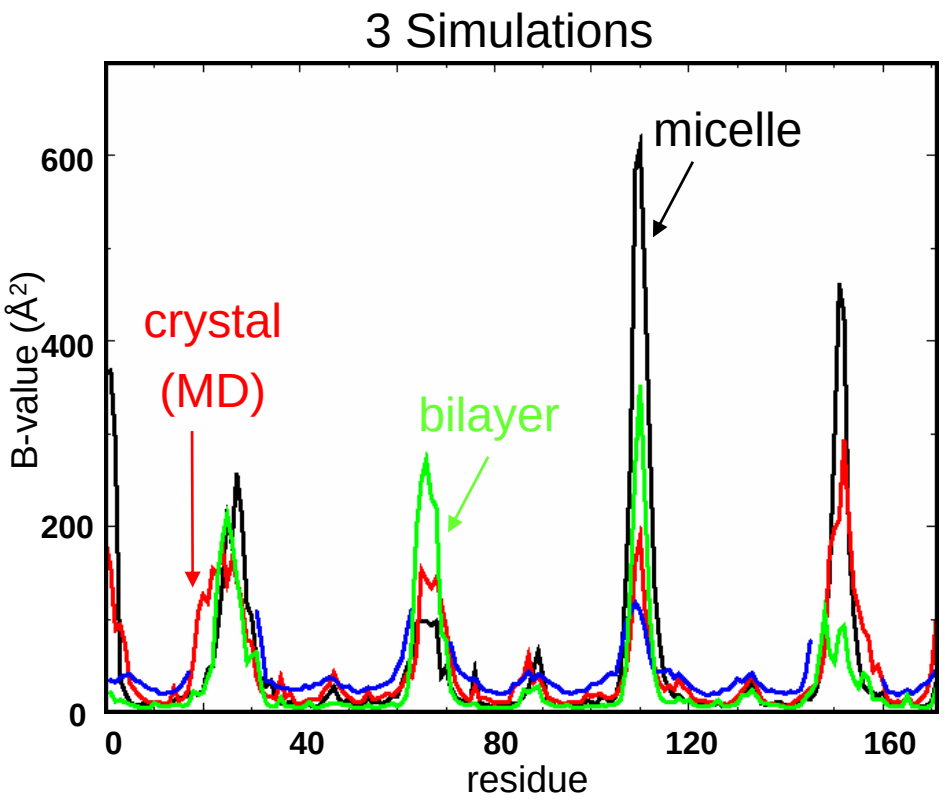


- Water trajectories – projected onto pore axis
- Opening of R138/E52 gate
- Suggests: crystal = closed *vs.* micelle = open
- Bond & Sansom (2003) *J. Mol. Biol.* 329:1035

OmpA: Simulated *vs.* Experimental B-Values

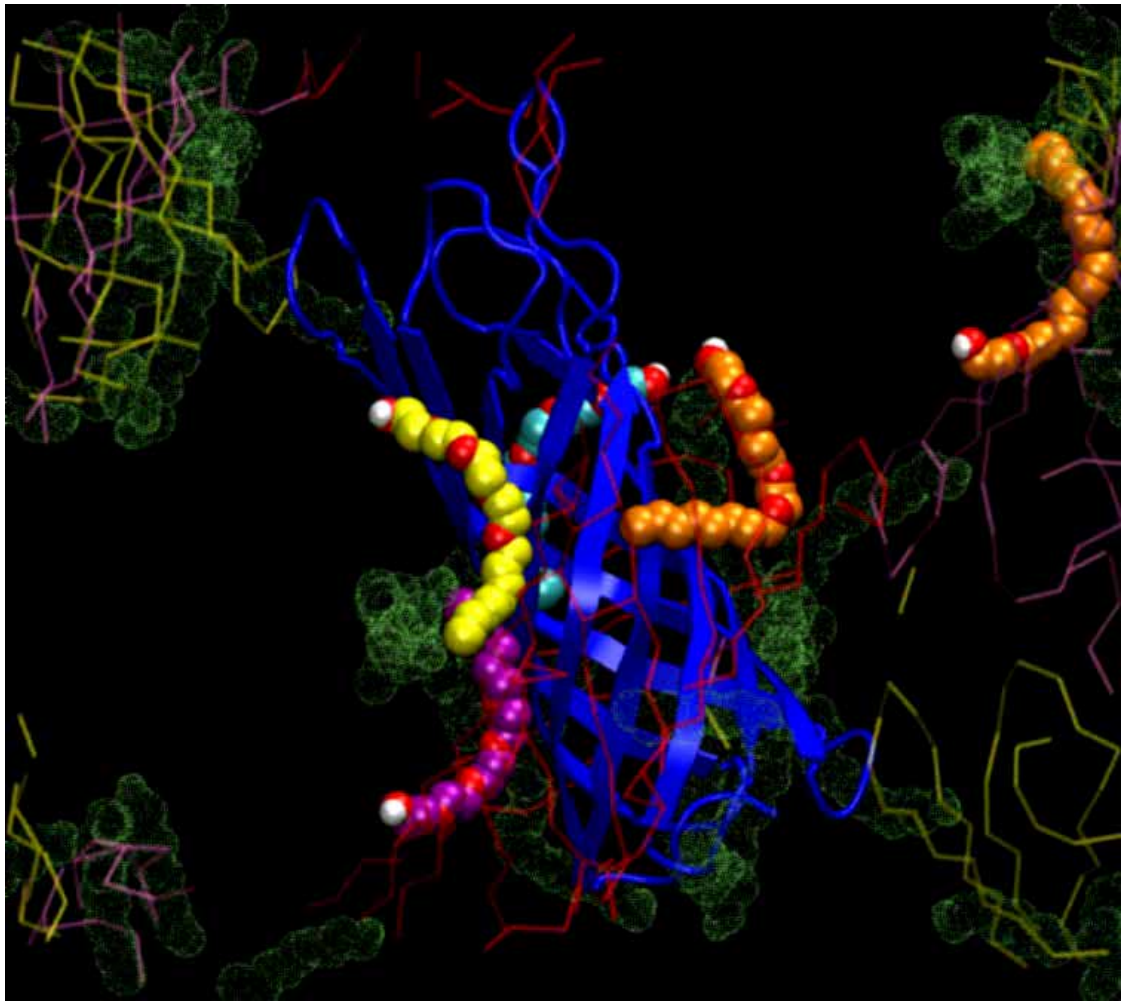
- OmpA crystal MD – good agreement with X-ray data
- Flexibility: micelle (nmr) > bilayer (~*in vivo*) > crystal
- Small changes in flexibility – can *open* the central pore



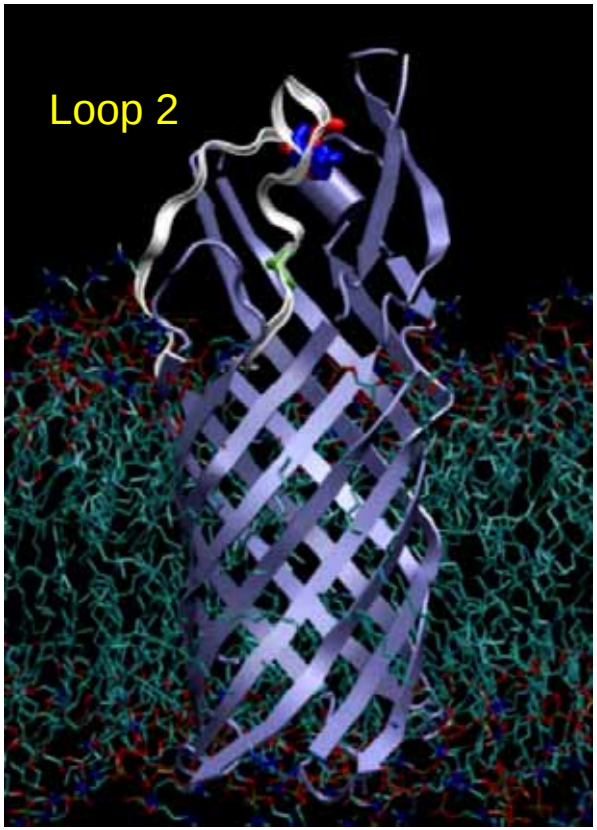


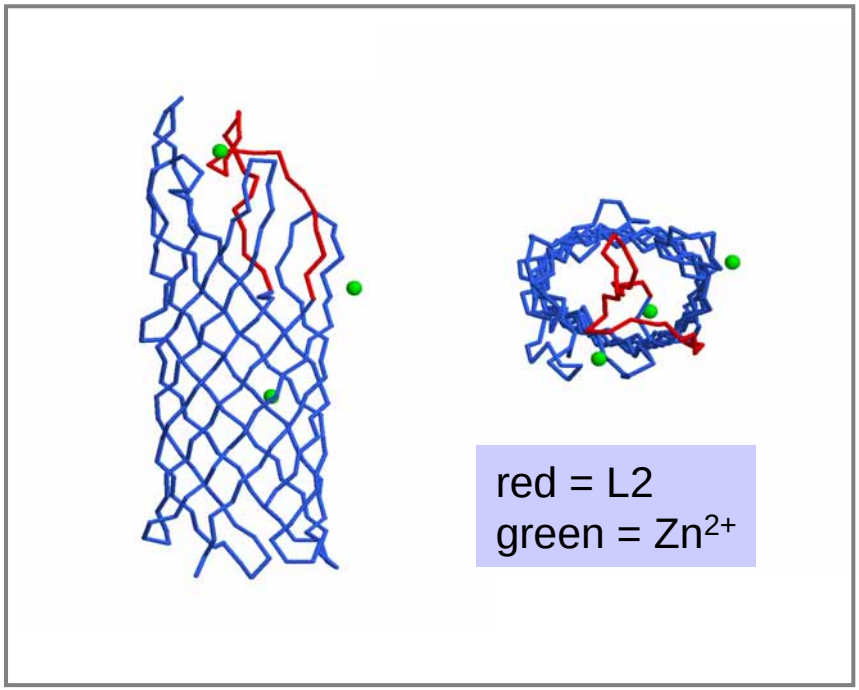
Dynamics of Detergent in a Membrane Protein Crystal

- OmpA – 4 monomers/unit cell
- MD of crystal - 50 ns
- Selected (bound) detergents highlighted
- Dynamic nature of detergent/protein interactions (at room temperature)



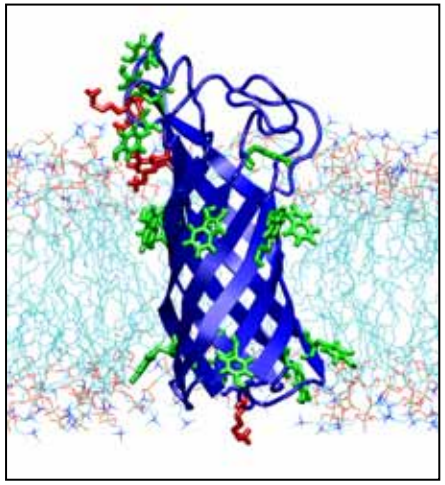
OpcA: Predicting Function by MD



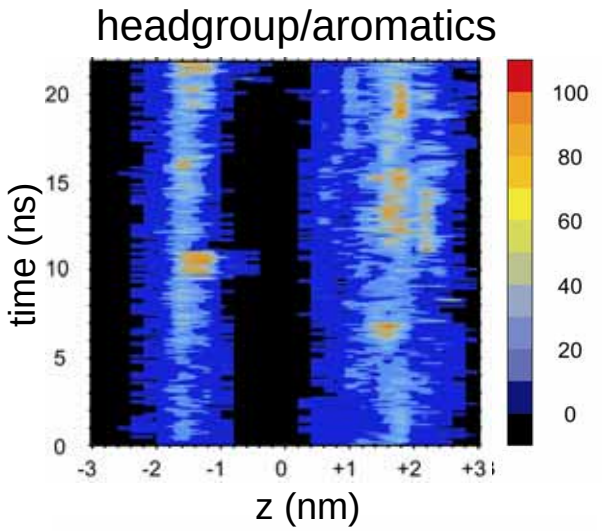


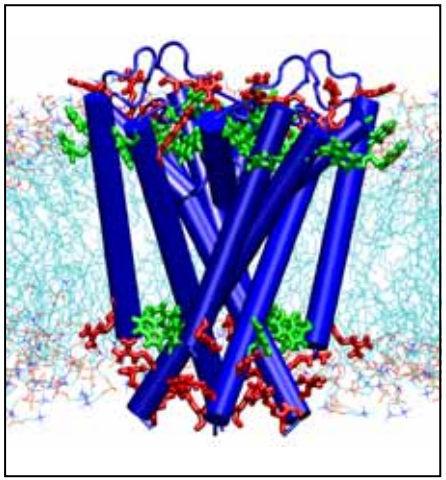
- OpcA from *Neisseria meningitidis*: a pathogen*;* mediates interactions with target cells
- MD: 2 x 20 ns complete in DMPC bilayer: 0.1M and 1M NaCl
- Possible pore: water-filled; blocked in crystal by Zn^{2+} and extracellular loops (L2)
- Fluctuations in loop 2 **functionally open** the pore: enable water & ion permeation

Lipid/Protein Interactions in Membranes

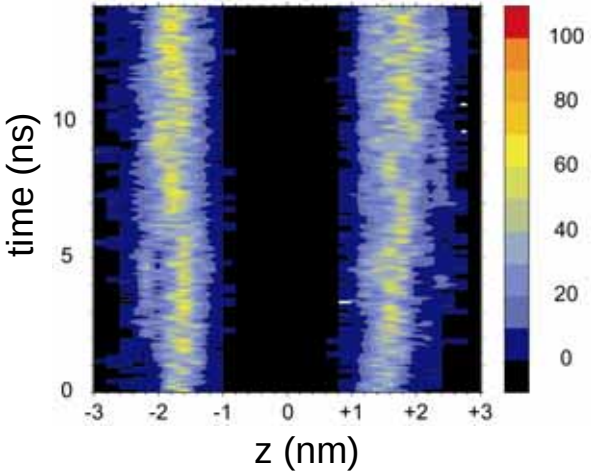


OmpA/DMPC





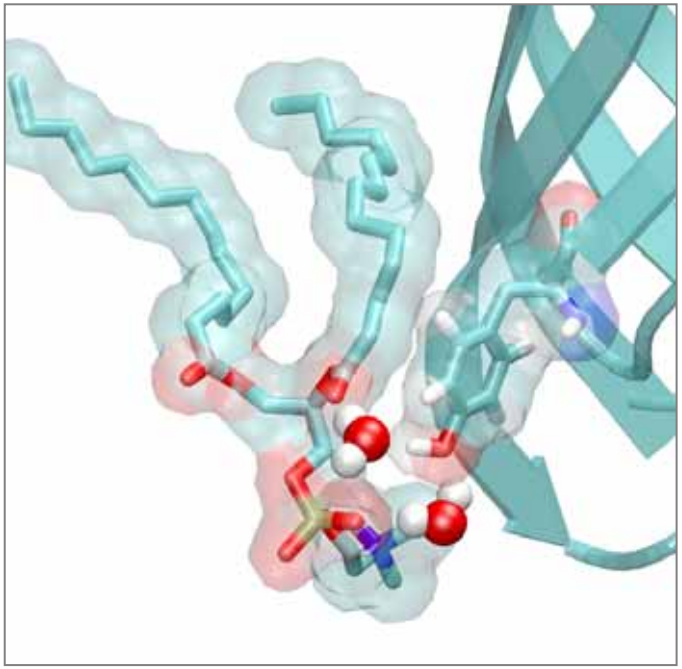
KcsA/POPC



- Fluctuations on ~5 ns timescale
- Headgroup/aromatic (Trp, Tyr) and phosphate/basic (Lys, Arg) interactions
- Domene, Bond, Deol & Sansom (2003) *J. Amer. Chem. Soc.* 125:14966

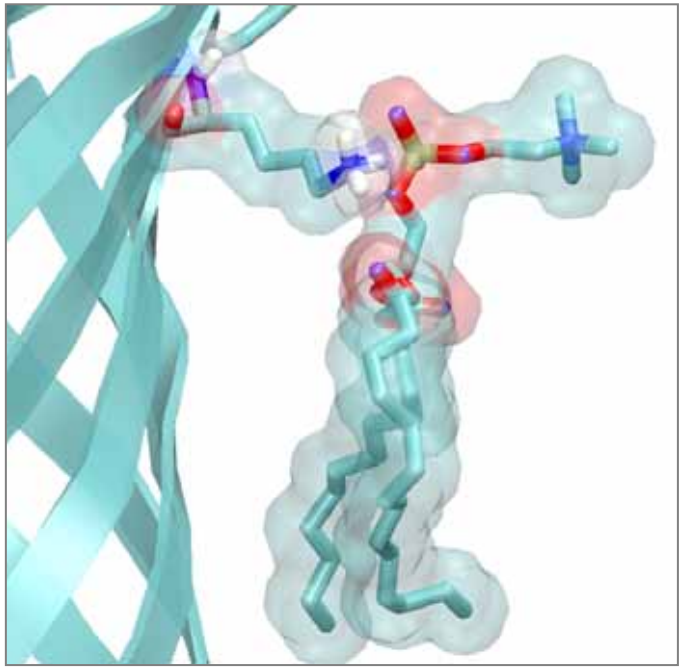
Examples of OmpA/DMPC Interactions

Aromatic Belt



Tyr – water - carbonyl

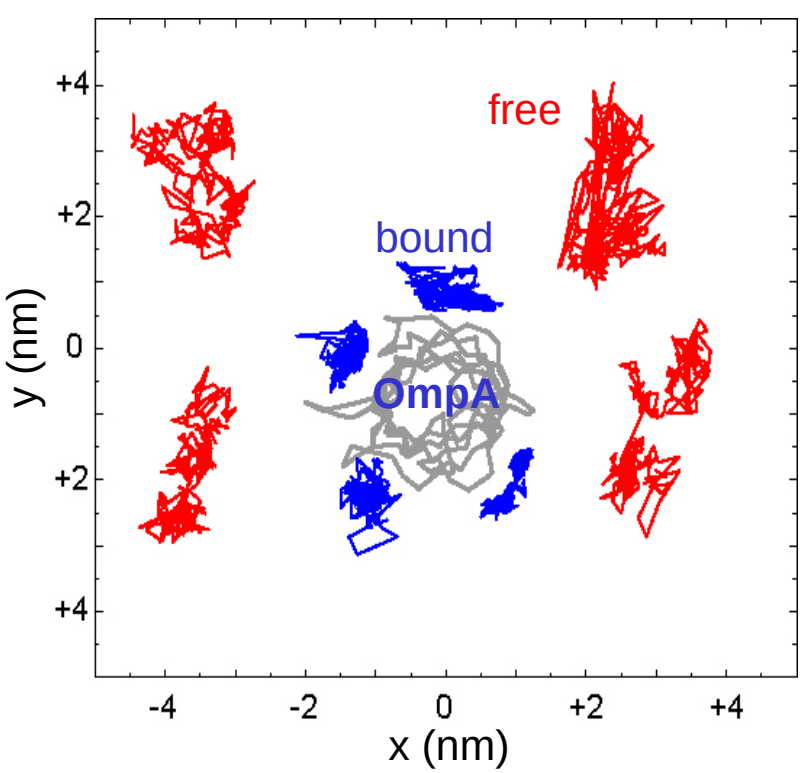
Lysine ‘Snorkels’

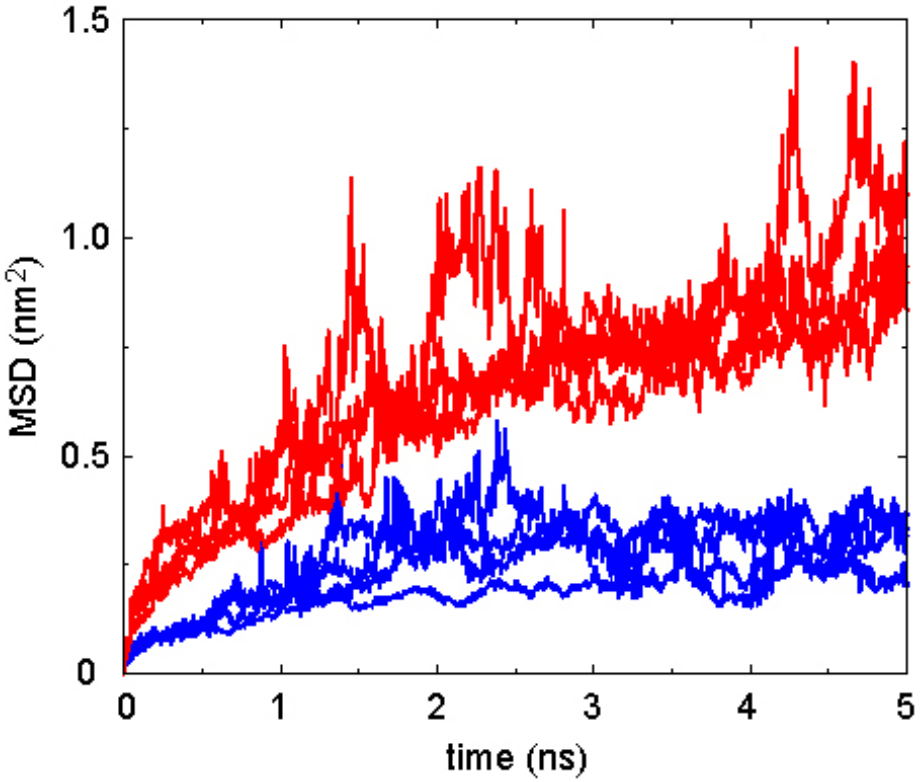


Lys - phosphate

- >10 ns MD can provide details of lipid-protein interactions
- Deol et al. (2004) *Biophys. J.* (in press)

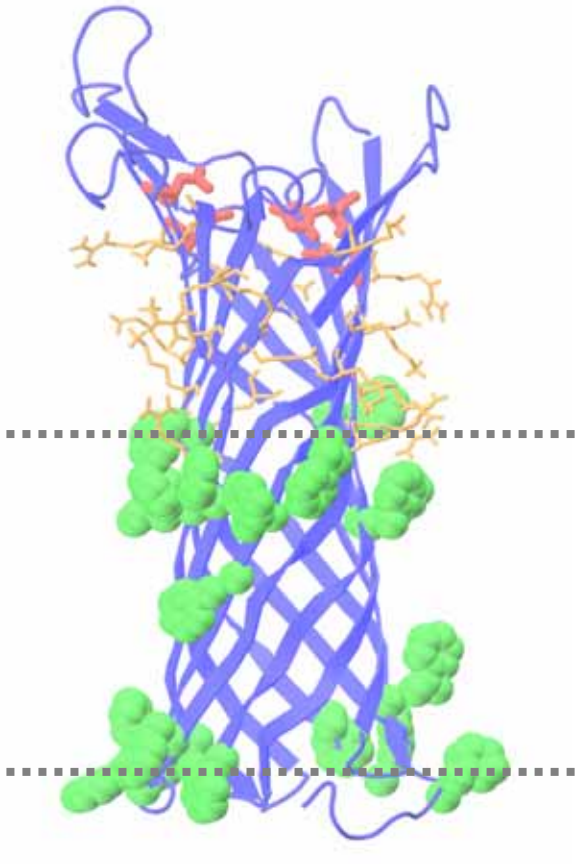
Boundary Lipid: OmpA

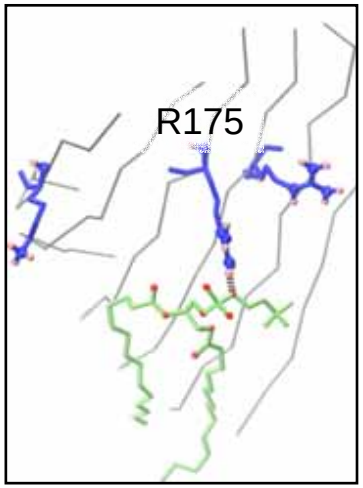


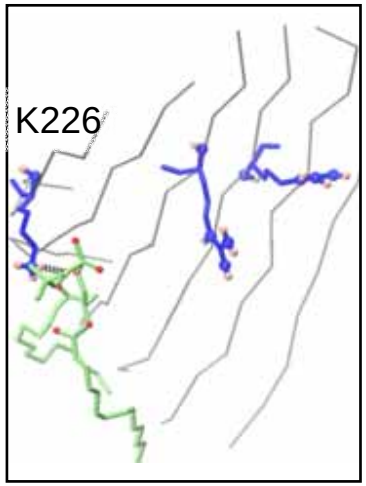


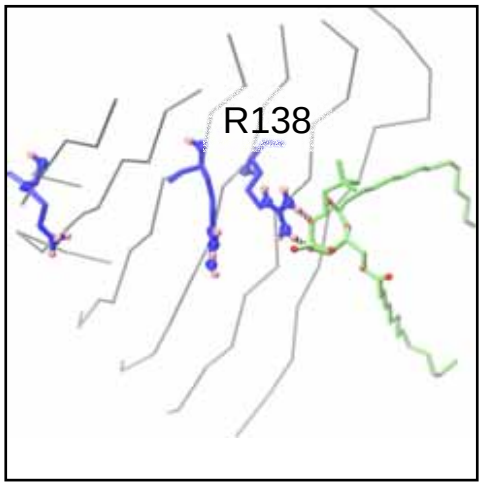
- Lateral diffusion coefficients: D (units = 10^{-5} cm^2 s^{-1})
- 'Bound' lipids: D = 0.028 (± 0.007)
- 'Free' lipids: D = 0.056 (± 0.045)
- ~14 'bound' DMPCs; cf. 11 DMPGs by EPR (Marsh et al.)

Specific Lipid/Protein Interactions: OmpT



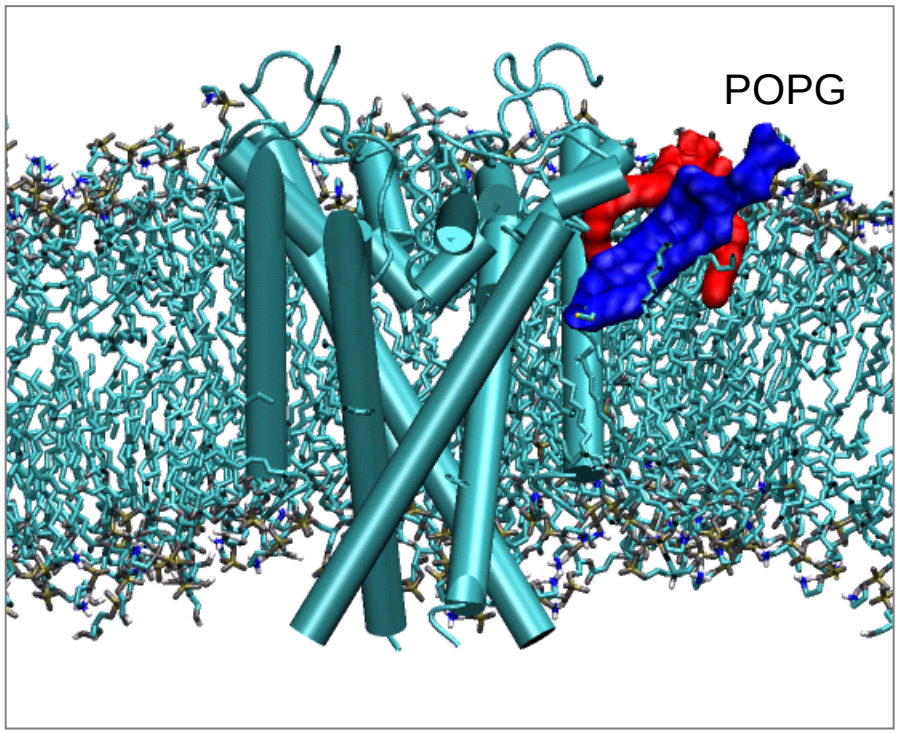




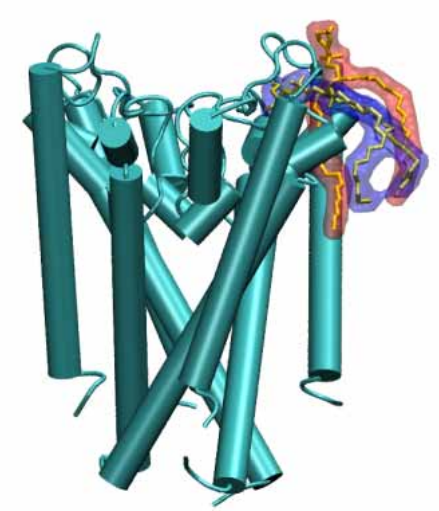


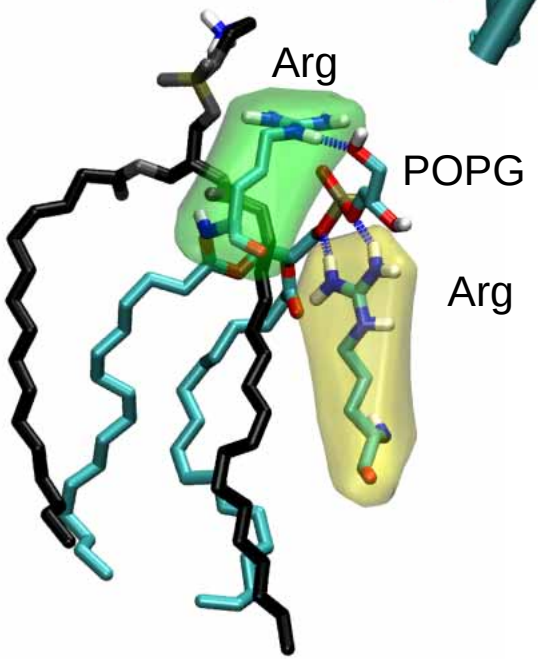
- OmpT: an outer membrane protease, activated by Lipid A
- Crystal structure: suggests Lipid A binding site (by comparison with FhuA)
- MD simulation in DMPC: phosphate/basic interactions at binding site
- Baaden & Sansom (2004) *Biophys. J.* 87:2942

KcsA: A Specific Lipid Binding Site



20 ns

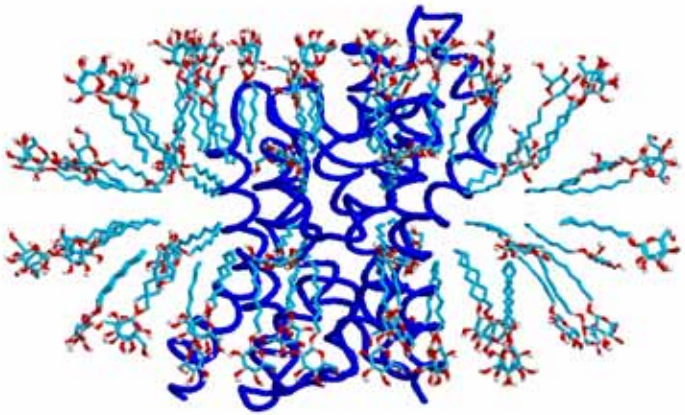




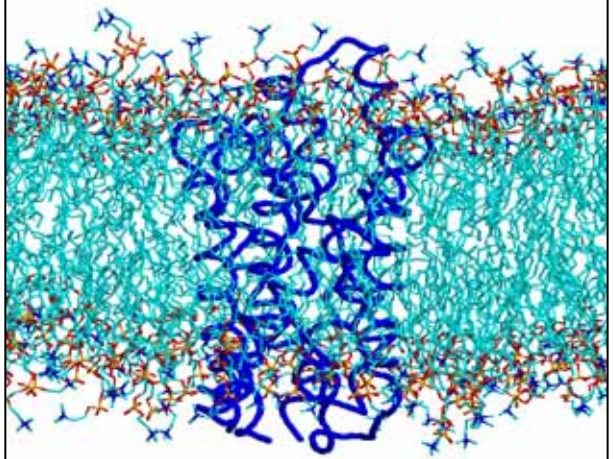
- PX structure of KcsA: bound lipid, essential for stability and function
- POPG: POPE bilayer, 20 ns MD
- Specific interactions of PG at P-helix/M2-helix binding site

GlpF: Lipid Bilayer *vs.* Detergent Micelle

GlpF-Octyl Glucoside micelle



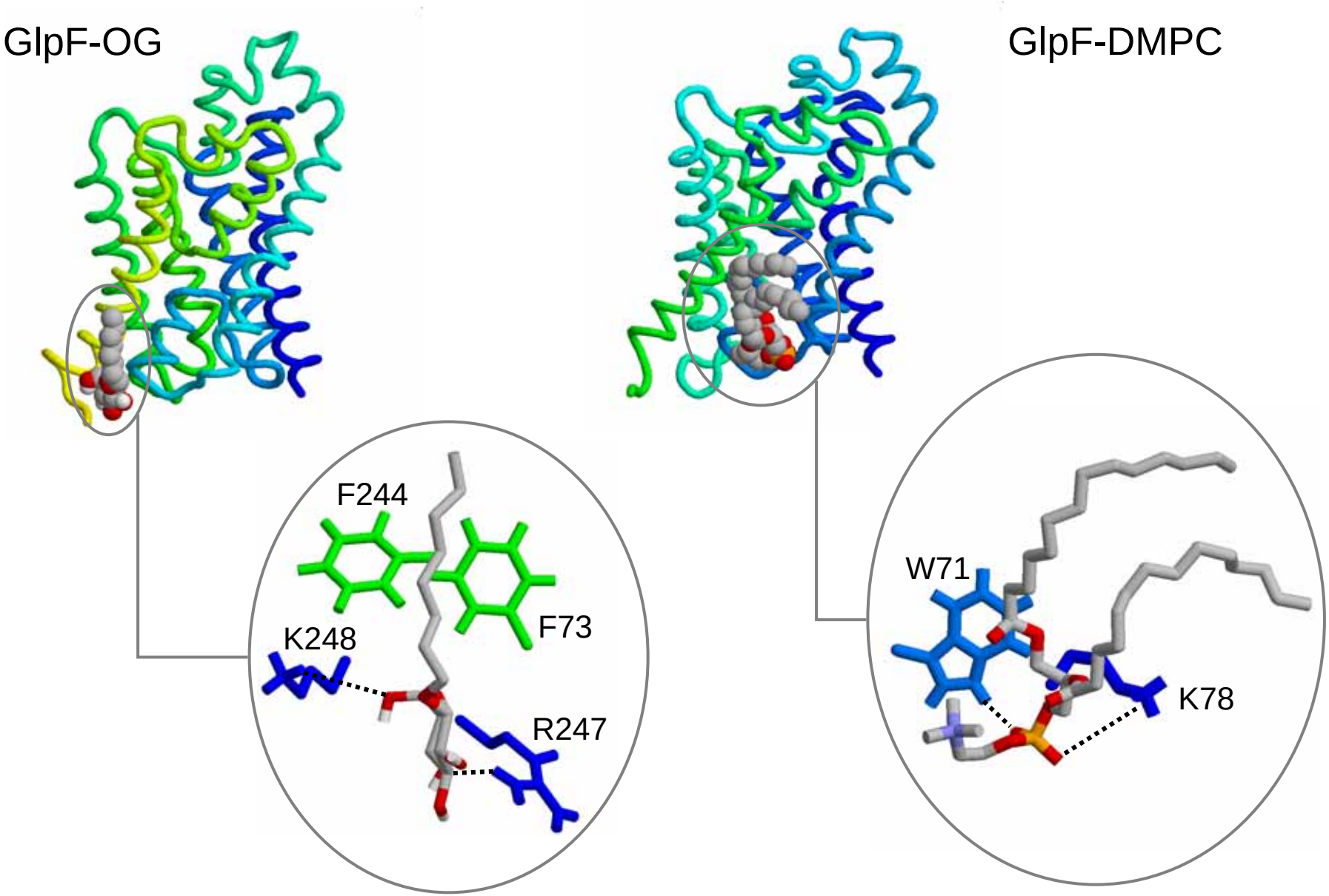
GlpF-DMPC bilayer



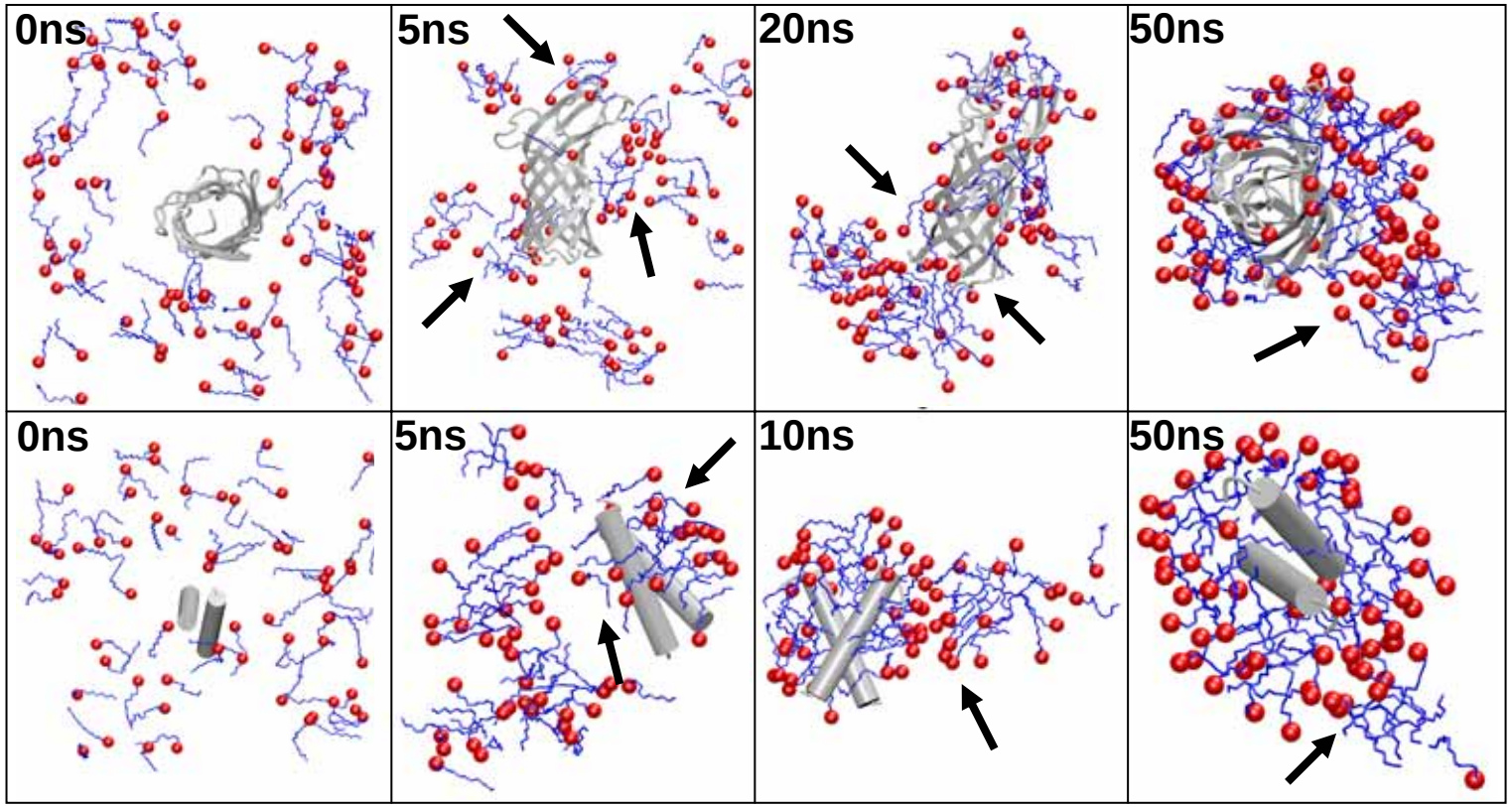
- α-Helical membrane protein – bacterial aquaporin
- Dynamics vs. environment – micelle *vs.* bilayer
- Compare protein-lipid vs. protein-detergent interactions
- Both simulations 10 ns; GROMACS; PME

Patargias et al. (2004) *J. Phys. Chem. B* (in press)

GlpF: Interactions with OG *vs.* DMPC

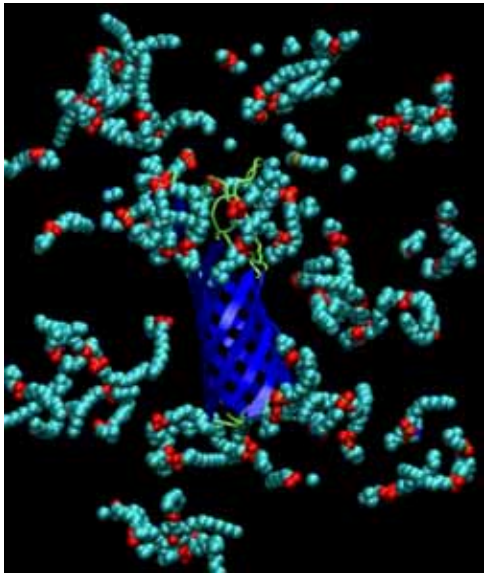


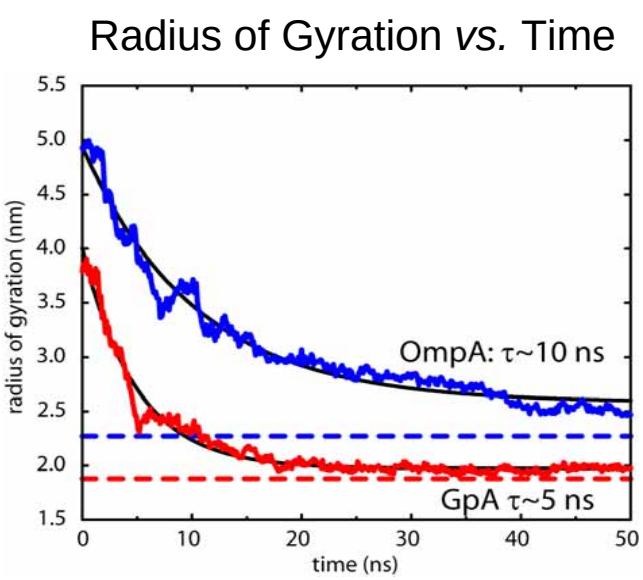
Glycophorin & OmpA: Self Assembly of Micelles

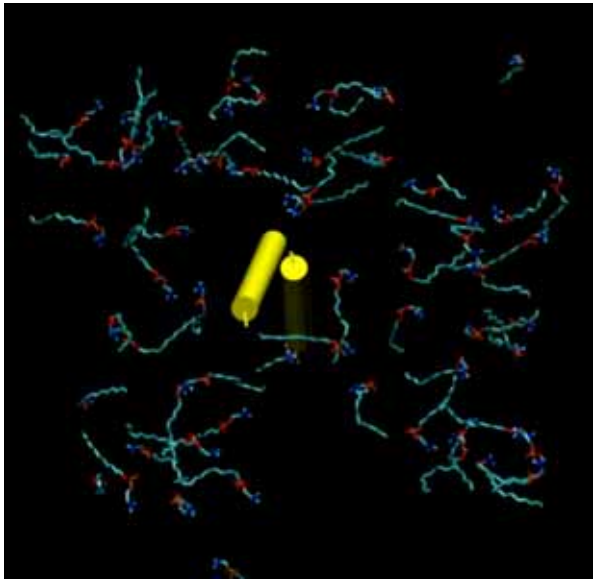


- OmpA *vs.* GpA; both with dodecylphosphocholine
- Initial configuration: protein + randomly placed DPCs + water
- Self-assembly on ~10 ns timescale

Kinetics of Self-Assembly







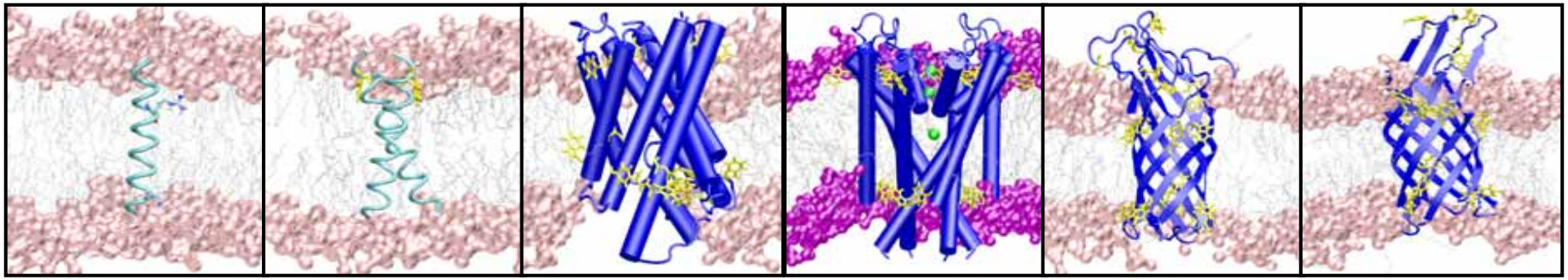
- From 50 ns simulations of OmpA/DPC and of GpA/DPC micelle self-assembly
- Comparison with pre-assembled micelles

- Kinetics: suggest a “diffusion + adsorption” model
- Towards “brute force” simulations of dynamics of membrane transitions

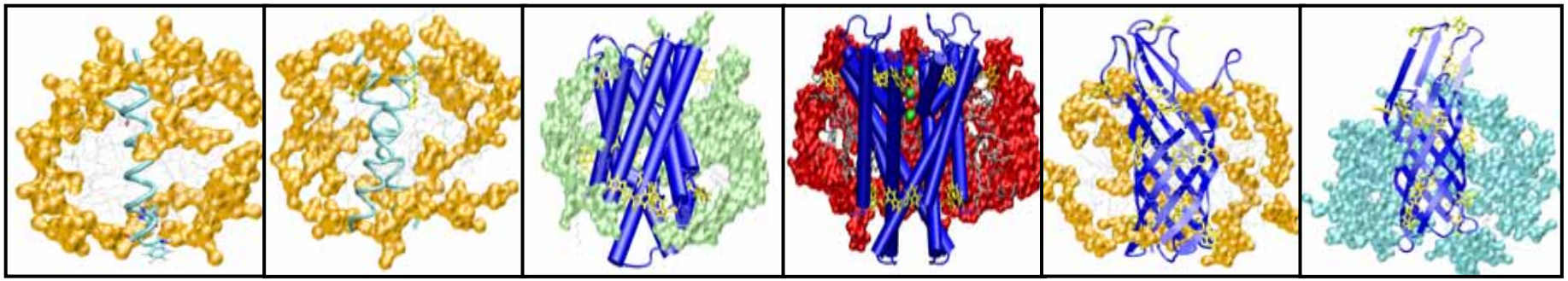
More MD Data: Comparative Simulations

Lipid bilayers

M2Gly | glycophorin | GlpF | KcsA | OmpA | OmpX



Detergent micelles



- 6 proteins; α-helix bundles *vs.* β-barrels; bilayers *vs.* micelles

Conclusions

- Membrane protein simulations reveal conformational dynamics *vs.* environment
- Enhanced flexibility in detergent micelles relative to lipid bilayers
- Functional importance of small changes in dynamics
- Both non-specific & specific lipid-protein interactions can be revealed
- ‘Brute force’ simulations can sample assembly of protein/detergent micelles
- Towards more complex dynamical transitions…

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