The first 3D model of Omp2a: a porin from *Brucella melitensis*

Maximilien Lopes Rodrigues, Emmanuel Tinti, Eric A. Perpète, Catherine Michaux

Laboratoire de Chimie Physique des Biomolécules (CPB), UCPTS, UNamur, rue de Bruxelles 61, 5000 Namur, Belgium

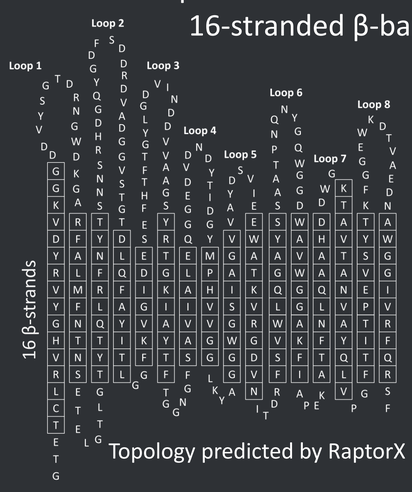
Introduction

Brucella melitensis is a pathogenic bacteria responsible for brucellosis, an infectious disease of mammals and humans [1]. The outer membrane proteins of *B. melitensis* control the diffusion of solutes through the cell and have a potential role in the resistance of the bacteria against drugs. The study of those particular proteins can therefore lead to the design of new diagnostics and vaccines. In this work, a 3D model of Omp2a is built by threading [2]. The model is characterized and then embedded in three phospholipid membranes (palmitoyl-oleoyl-glycerophosphocholine [POPC], PO-phosphoethanolamine [POPE] and a mixture of both) to assess the effect of the membrane composition on the protein. It is demonstrated that the proposed model shares the main characteristics of other known general porins such as phosphoporin E (PhoE) and OmpF, thus proving the relevance of the predicted structure. The molecular dynamics simulations monitor the stability of the barrel and its inner channel over time in an environment mimicking its natural membrane. This work provides essential information to understand the specific features of Omp2a and its function.

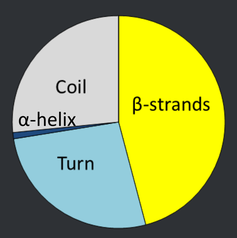
Construction of the Omp2a model by threading

Template-based prediction by RaptorX [2]

Best templates: PhoE and OmpF (*E. coli*)
16-stranded β-barrel

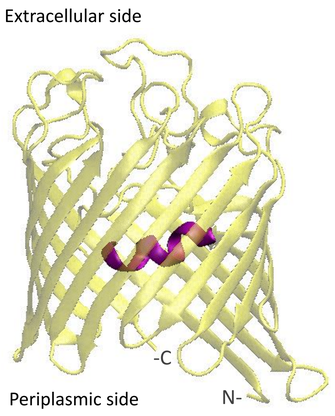


Topology predicted by RaptorX

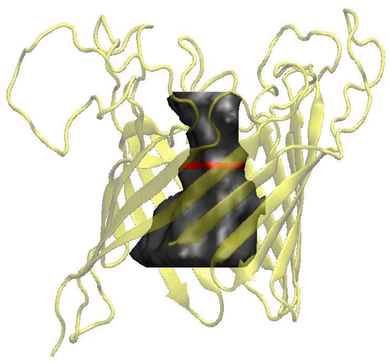


Secondary structure

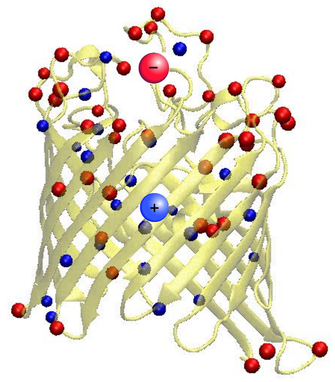
Features of the first 3D Omp2a model



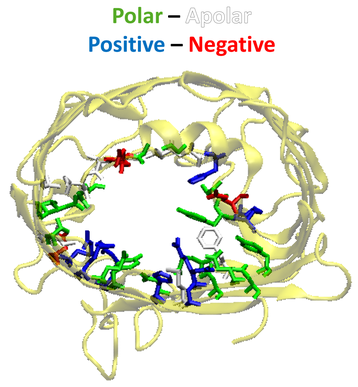
- Long extracellular loops
- α-helix (L3) inside the barrel



- Inner channel
- Constriction zone (red)
  R_{min} = 0.38 nm



- Asymetric charge localization
- Excess of ⊖ on the loops
- Accumulation of ⊕

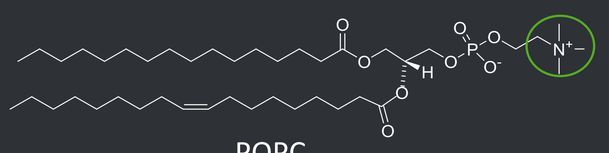


- Polar residues in the channel
- Majority of positive residues

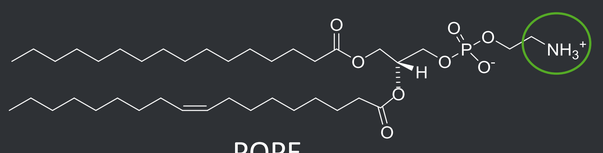
Simulation of Omp2a trimer in phospholipid bilayers by Molecular Dynamics

The trimer was assembled and embedded in three bilayers for 200 ns:

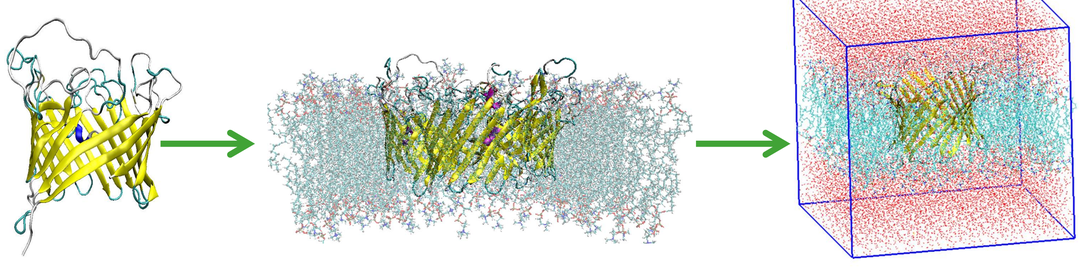
- POPC (*B. melitensis*)
- POPE (*B. melitensis* and *E. coli*)
- POPC:POPE (1:1)



POPC



POPE

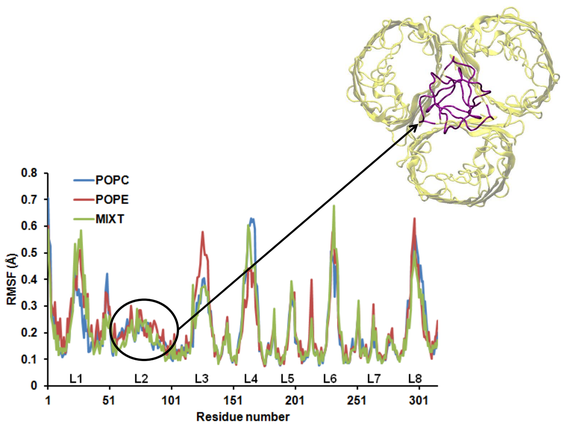


Omp2a model | Insertion in lipid bilayers | Molecular dynamics with GROMACS

	Area per lipid (nm²)	Thickness (nm)	Ref. area per lipid (nm²)	Ref. thickness (nm)
POPC	0.61	4.03	0.62	3.88
POPE	0.52	4.33	0.53	4.31
POPC/POPE	0.57	4.40	0.58	4.10
	Simulations		Experiments	

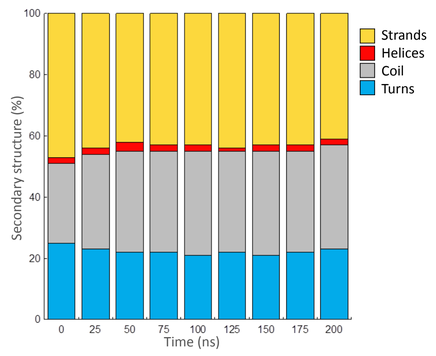
Data describing the quality of the bilayers and comparison with litterature [3]

Root Mean Square Fluctuation of Omp2a residues



- Versatile loops and stable strands
- L2 interacts with other monomers

Evolution of the secondary structure content over time



- Consant average content
- Slight variations on the L3 loop (α-helix)

Conclusions and perspectives

The present work demonstrates the validity of the first 3D structure of *Brucella melitensis* Omp2a built by threading. The model shares the major features of known general porins such as PhosE or OmpF. The typical L3 loop folds inside the barrel as an α-helix to constrict the channel. The key residues defining its potential selectivity are highlighted and suppose a different pathway for the cations and anions crossing the barrel. The trimer inserted in lipid membranes undergoes few structural changes proving its stability. The most versatile parts are the extracellular loops and the unique α-helix. The latter affects the radius of the channel over time (data not shown). Neither POPC nor POPE shows a better capacity to maintain the structure.
The next steps plan to simulate the movement of ions/solutes inside the protein and to experimentally reconstitute Omp2a in POPC/E membranes.

References:

[1] Pappas, G. *et al*., (2006). The new global map of human brucellosis, *Lancet Infect Dis.*, *6*:91–99.
[2] Källberg, M. *et al*., (2012). Template-based protein structure modeling using the RaptorX web server. *Nat. Prot.*, *7(8)*:1511–1522.
[3] Leekumjorn, S. *et al*., (2007). Molecular characterization of gel and liquid-crystalline structures of fully hydrated POPC and POPE bilayers. *J. of Phys. Chem. B*, *111(21)*:6026–6033.
[4] Roussel, G. *et al*. (2012). Purification, refolding and characterization of the trimeric Omp2a outer membrane porin from *Brucella melitensis*. *Prot. Expr. Purif.*, *83(2)*:198–204.
[5] Puiggalí-Jou, A. *et al*. (2016). Confinement of a β-barrel protein in nanoperforated free-standing nanomembranes for ion transport. *Nanoscale*, *8*:16922–16935.

maximilien.lopesrodrigues@unamur.be

www.unamur.be