Molecular Dynamics Simulations of Outer Membrane Phospholipase A

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INTRODUCTION

Ompla, a phospholipase located in the bacterial outer membrane of *E. coli*, degrades a wide variety of phospholipids in cells with a perturbed envelope. It is a ***12-stranded b-barrel*** and exists in monomeric and dimeric forms.
Enzymatic activity is regulated by reversible dimerisation in conjunction with Ca^{2+}-binding, leading to 2 active sites at the monomer-monomer interface.

Recently, crystal structures of monomeric and dimeric Ompla with and without Ca^{2+} were solved (Snijder *et al.*, 1999 and 2001) including apo- and inhibitor-bound forms.
An improved understanding of the ***structure-function relationship*** in terms of dimerisation, the role of Ca^{2+} and structural impact of the cell envelope can be gained from computational approaches.

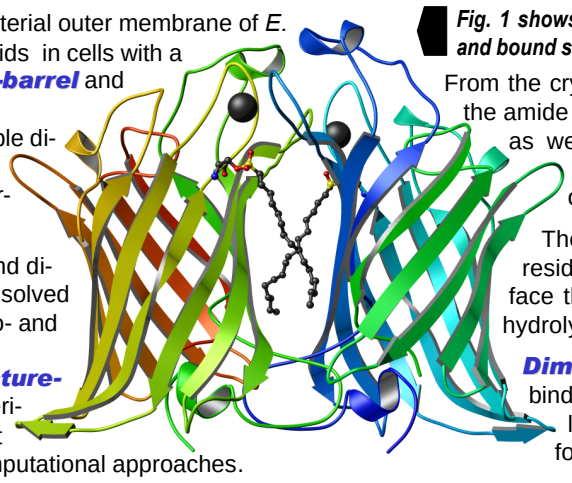


Fig. 1 shows the Ompla dimer with its two calcium binding sites and bound substrate analogues.

From the crystal structures it appears that ***Ca^{2+}-binding*** occurs via the amide oxygens of S106, R147 and the hydroxyl oxygen of S152, as well as 3 water molecules with an octahedral geometry. S106 and S152 belong to one monomer and R147 to the other.

The nearby ***active site*** consists of two highly conserved residues H142 and S144 and one less conserved, N156. They face the outer leaflet of the lipid bilayer. Ompla is believed to hydrolyse its substrate through a nucleophilic attack by S144.

Dimerisation results in formation of two extended substrate binding clefts and is partially substrate dependent. There is little structural difference between monomeric and dimeric forms (RMSD 0.63 Å).

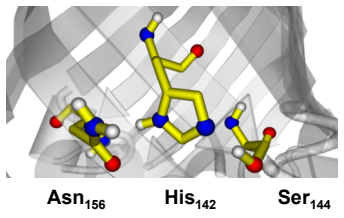


Fig. 2 shows the arrangement of active site residues in OMPLA, which resembles that of serine proteases.

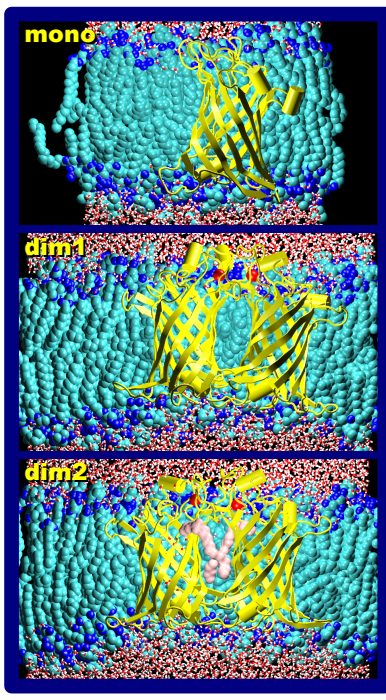


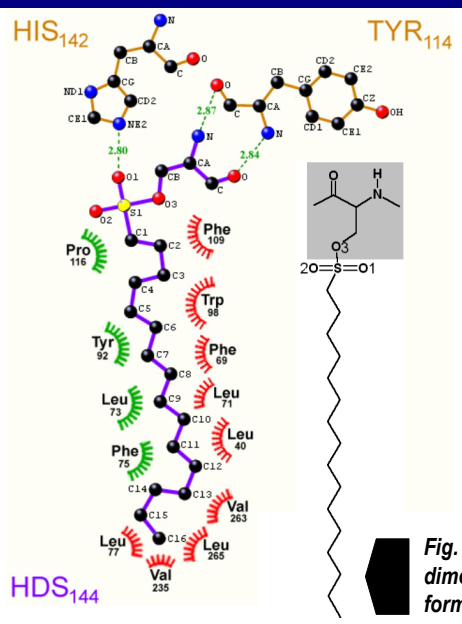
Fig. 3 highlights the membrane part of the 3 simulation systems. The protein is shown in yellow, calcium in red and the inhibitor in pink. The bilayer consists of POPC.

SIMULATION SETUP

Starting structures for the protein in its monomeric and dimeric forms were taken from the `1qd5` and `1qd6` entries of the PDB respectively and missing parts repaired. Residues 1-12 were replaced by an acetyl group.

Protonation states were assigned from finite-difference solutions to the Poisson-Boltzmann equation at pH 7 taking into account the influence of the environment by comparing the bare protein (in a water-like environment) and the protein within a low dielectric medium. ***Active site*** protonation states were assigned manually.

Three systems were considered for molecular dynamics simulations in a membrane-like environment: the Ca^{2+}-free monomer, the Ca^{2+} bound dimer and the dimer with inhibitor bound to S144.

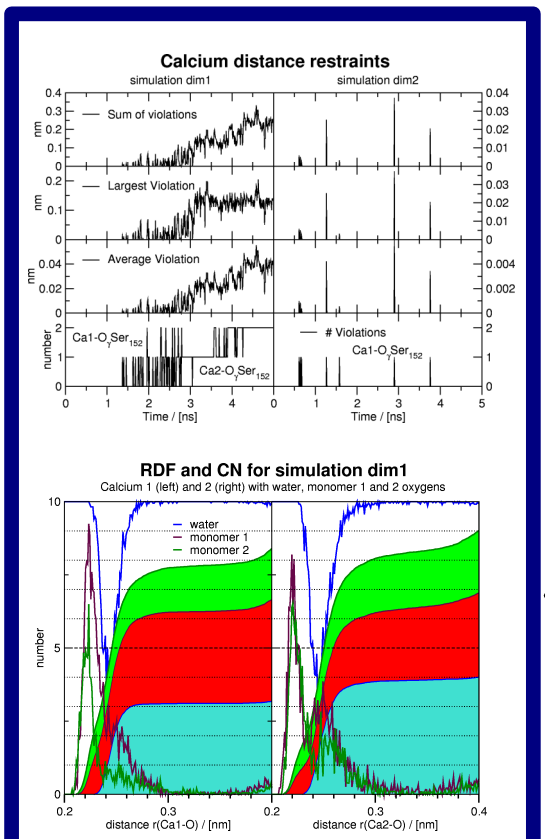


Calcium interactions were implemented using a modified forcefield for Ca^{2+} itself and the surrounding residues S152, R147 and S106. Partial atomic charges and Lennard-Jones parameters were adapted from Shiratori & Nakagawa, 1991. In addition, distance restraints of 200 kJ $mol^{-1}nm^{-2}$ were placed between calcium and these residues.

The ***hexadecanesulphonyl inhibitor*** was modelled by modifying S144, leading to a hexadecanesulphonyl serine (HDS) residue generated via PRODRG (van Aalten, 1996).

The system was then inserted in a ***lipid bilayer*** consisting of palmitoyl-oleyl-phosphatidylcholine (POPC) and hydrated with an aqueous phase of about 0.1M in NaCl.

Fig. 4 shows the modified residue 144 (HDS) and a two-dimensional representation of its interactions with residues forming the surface exposed binding cleft.



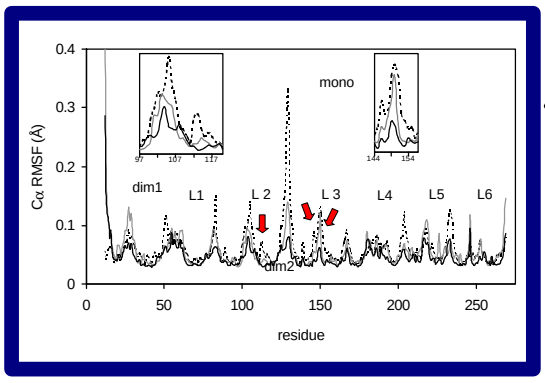


Fig. 6. Root mean square fluctuations for simulations mono, dim1 and dim2 with inlays focusing on the residues near the calcium-binding site. Coordinating residues S106, R147 and S152 are indicated by red arrows.

MOLECULAR DYNAMICS IN A LIPID BILAYER

Molecular dynamics simulations in full atomic detail have been initiated in order to study the conformational dynamics of mono- and dimeric Ompla in a membrane-like environment.

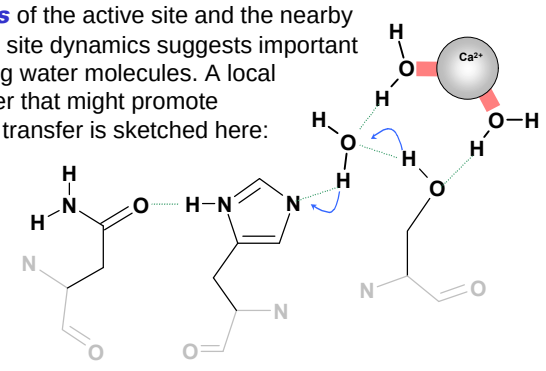
- Timescale ~5 ns per system
- System size ~ 8 x 10 x 10 nm
- Membrane mimetics: octane, POPC
- Twin-cutoff 14/18 Å

One point of interest and concern is the behavior of calcium in these simulations. As can be seen on the left, the distance restraints that were applied, were necessary for system dim1, but not for dim2. The radial distribution functions of Fig. 5 show that calcium is coordinated by 3 to 4 water molecules and 2 to 3 residues from each monomer, totalling an ***average coordination number*** (CN) of 8.

Fig. 5. Top: Violation of imposed distance restraints in the dimer simulations. Bottom: radial distribution functions of calcium in simulation dim1 separated in contributions from monomer1, monomer2 and water.

The comparison of the ***root mean square fluctuations*** (RMSF) in the mono, dim1 and dim2 systems shows that dimerisation leads to reduced fluctuations, in particular near the residues involved in calcium binding. This agrees well with the overall root mean square deviation which is also lower for the dimer simulations. As a general trend beta-barrel carbon alpha RMSDs range from 1.0 to 1.2 Å and are 0.8-1.1 Å lower than values observed for loops and turns.

First analysis of the active site and the nearby calcium binding site dynamics suggests important roles for bridging water molecules. A local ordering of water that might promote catalytic proton transfer is sketched here:



Another important feature seems to be the collapse of the ***substrate-binding cleft*** in the dim1 simulation as compared to dim2 (with inhibitor bound). This can *eg* be seen by an increase of the monomer-monomer contact area or the change of accessible surface of the substrate binding cleft.

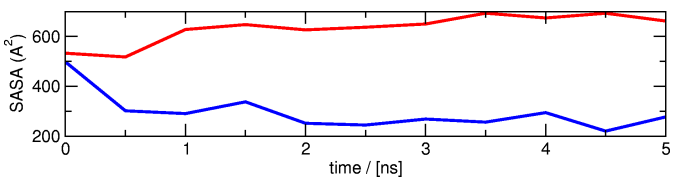


Fig. 7. Solvent accessible surface area of one substrate binding cleft for simulations dim1 and dim2 as a function of time (ignoring the bound inhibitor for simulation dim2).

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Which link to BioNanoTech ?

Ompla as potential security valve for membrane based devices …

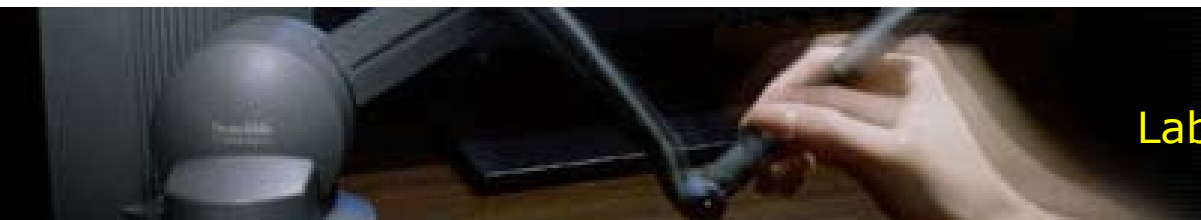
.. in order to maintain the membrane integrity

Many biological nano-devices are based on membranes (e.g. liposomes and nanosomes). To maintain membrane integrity under various conditions one might add specific proteins, like anti-freeze (glyco)proteins. In this context the Ompla enzyme could help to develop a security valve with respect to mechanical stress.

The Ompla enzyme functions as a kind of security valve in the bacterial outer membrane. It's enzymatic cycle is activated by a mechanical deformation of the membrane, which triggers lysis of phospholipids. The mechanical trigger may be caused by an imbalance in membrane composition or by physical stress. Lysis and hence removal of phospholipids from the membrane helps to restore it's integrity.

If the activity of Ompla could be controlled and fine-tuned, one might be able to develop self-regulating devices, with a capacity of dealing with a certain amount of environmental stress and membrane distorsion.

A first step is to fully uncover the enzymatic mechanism of Ompla, which is related to the presence of calcium ions, a specific hydration shell, dimerization and membrane distorsion.



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