pH-dependent permeability of outer membrane protein G: an *in silico* study

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Outer membrane protein G (OmpG) is a monomeric protein of E. coli outer membrane that mediates a pH-dependent non-specific oligosaccharide transport [1]. Two X-ray structures have been determined at different pH values: a closed conformation at low pH (5.5) that inhibits metabolite transport; and an open one at neutral pH (7.5) that allows it [1,2]. The key structural difference behind the mechanism lies in the position of the external loops, mainly loop 6, that determine the open or closed conformation of the channel.

Given the important function of this protein, a detailed description of the conformation changes that result from varying the pH has enormous biological relevance, possibly contributing in making it a better antibiotic target and more flexible biosensor [3]. Here, we performed constant-pH molecular dynamics (CpHMD) simulations of a membrane-embedded OmpG in order to characterize the conformational/protonation space of both end states (open and closed) and try sample the pH-dependent conformational transitions between them.

Starting from the NMR structures [2], we built two systems, with OmpG in open and closed conformations, inserted in 116 POPC lipid bilayer. The constant-pH molecular dynamics (CpHMD) simulations were performed with GROMACS and the GROMOS 54A7 force field [4]. After equilibration, 4 different setups were explored, based on the combinations of the open/closed conformations and the high (7.5) and low (5.5) pH values. The main conformational difference observed is due to movement in loop 6, which is induced by the pH change.

By following the loop movement, we can map this important conformational transition and correlate it with the pH value. This conformational transition is measured by following a simple distance property. We measured the shortest distance between all Cα in loop 6 and the Cα atom of Gly50, which is in an opposite location in the barrel.

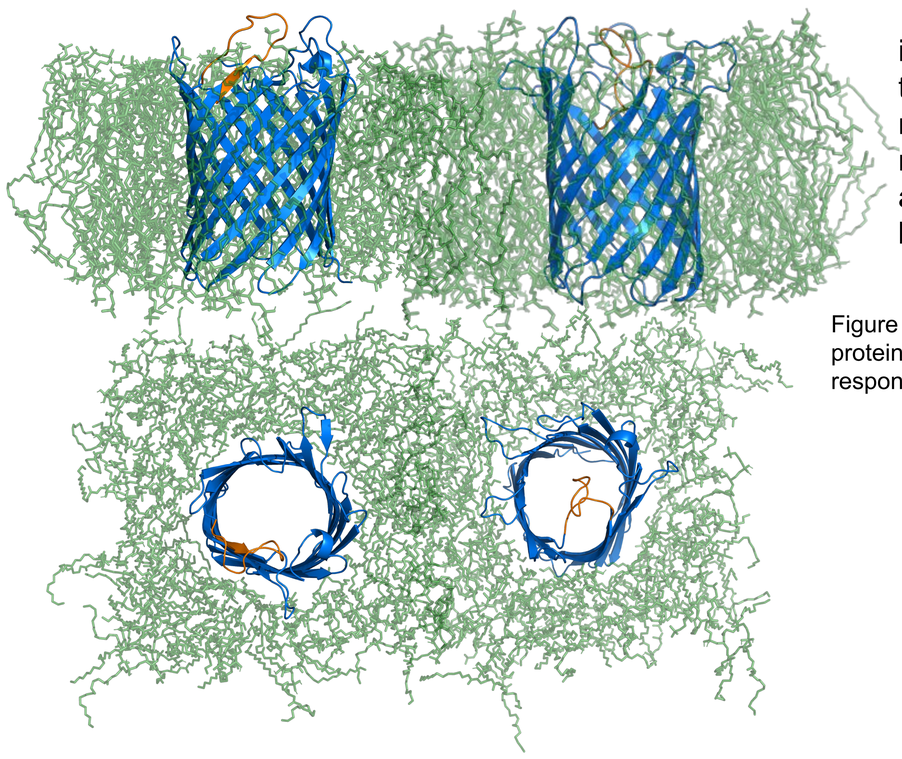
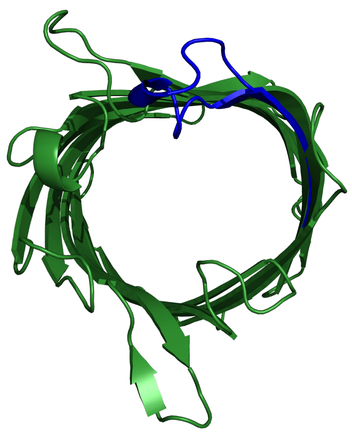
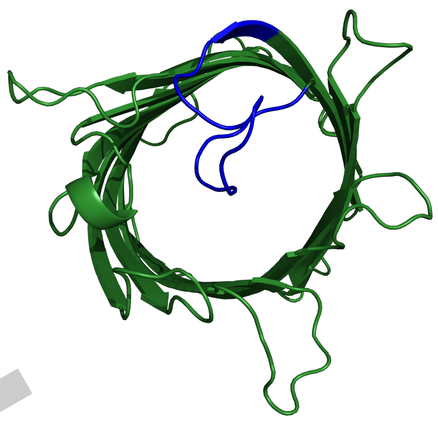


Figure 1: Side and top views of the open and closed conformations of the protein in membrane, respectively. In orange it's highlighted the main loop responsible for the conformational change.



Open conformation



Closed conformation

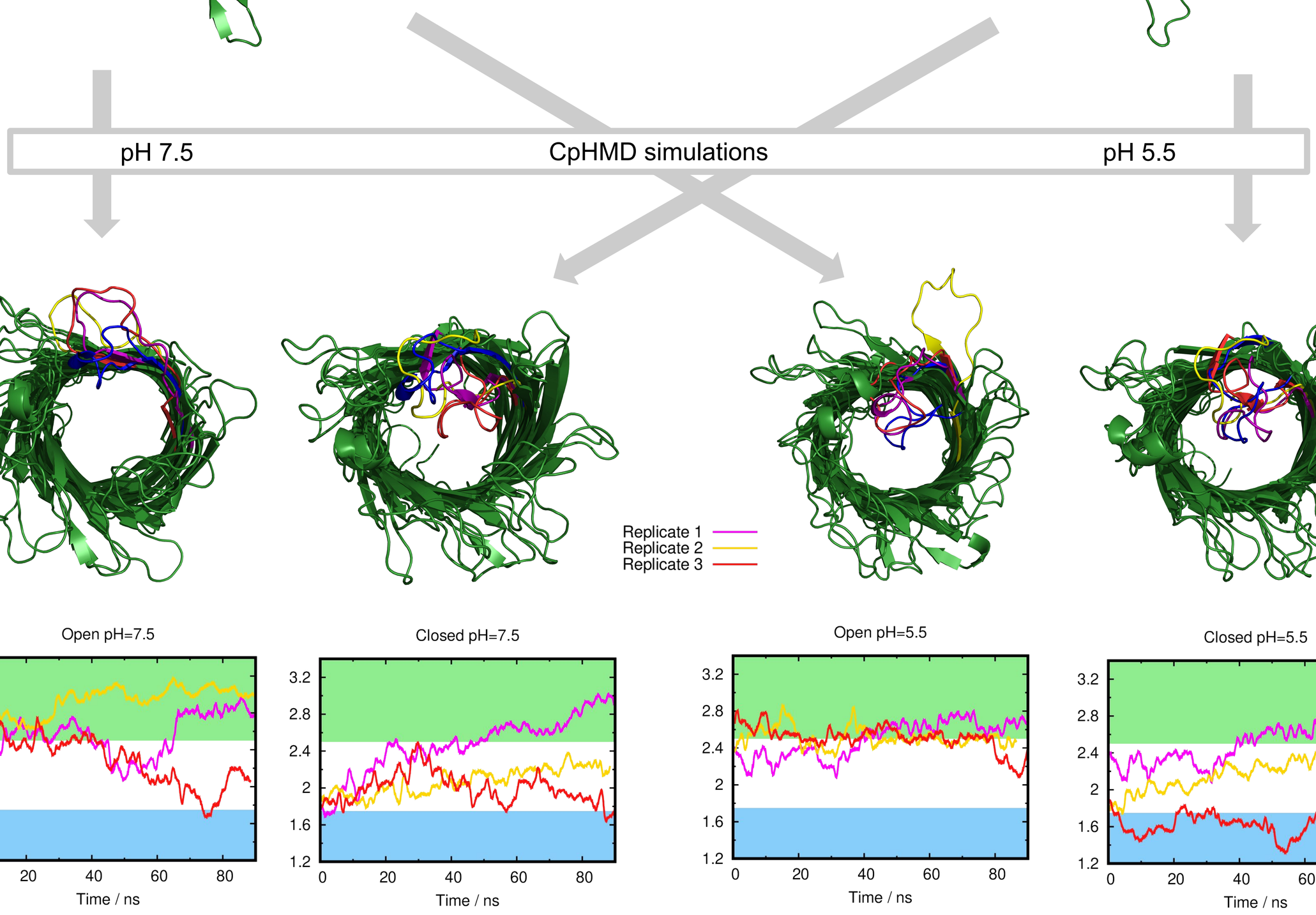


Figure 2: Preliminary CpHMD simulations of the OmpG starting from the open/closed conformations at high/low pH values. Representations of both NMR structures (**Top**), highlighting the position of the loop 6 (blue). The final conformations for all replicates (pink, yellow and red) of each setup are also shown (**Middle**), keeping the starting NMR structure for comparison (blue). The loop distance data is shown for all simulations (**Bottom**). In this property, short distances are typical of closed conformations (light blue shaded region), while longer ones represent the open conformations (light green shaded region).

Concluding Remarks:

It is easier to sample the conformational transition from Closed to Open. It seems that entropy may be playing a role, since there are many ways to open (unfold the loop) while only a few may be available to close it (fold) correctly.

The high pH value stabilizes the open conformation and destabilizes the closed one. At low pH, only one replicate with closed conformation remained correctly folded, while the open ones did not show yet a clear tendency to close.

Ongoing work:

The current simulations, are still too short and we will need to run a few hundreds of nanoseconds to investigate if some transitions are not being observed due to lack of simulation time. Also, if no turnover between end states are observed, we will probably need more replicates to improve our sampling and try estimate the relative stabilities.

We also plan to cover a wider range of pH values to better characterize the conformational transitions and obtain transition-dependent pK_a values.

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FCT Fundação para a Ciência e a Tecnologia
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