Does enterobactin prepare iron transport by remodeling the water network through FepA?

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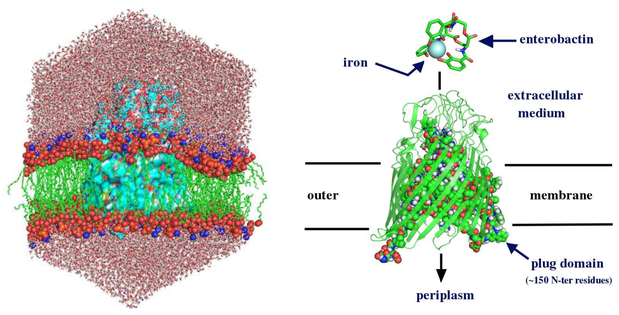
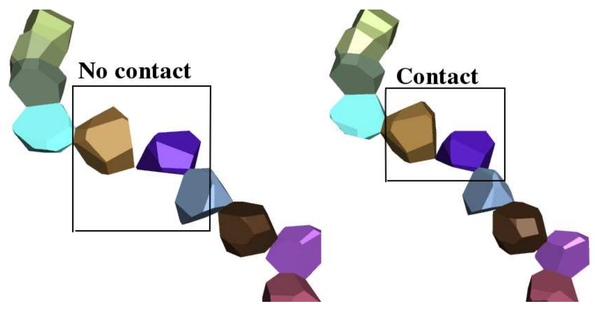
1 FepA protein, Molecular dynamics and VLDP

FepA is an outer membrane protein of bacteria E. *coli* involved in the iron transport. The extracellular iron is captured and uptaken by the enterobactin, a small organic molecule and privileged ligand of FepA protein. Currently, the transport mechanism of enterobactin-iron through FepA is not solved [Chakraborty *et al.*, 2007, Biometals **20**].
The entire system (protein, lipids, water, ions) has been simulated by molecular dynamics [M. Baaden], both in presence (holo) and in absence (apo) of enterobactin. To probe the transport mechanism, we have analyzed about ten snapshots for each form. The topology of the water network has been investigated by means of VLDP, a computer program based on weighted Voronoi diagrams (Laguerre diagrams). These diagrams are space partitions into polyhedra around the atomic positions

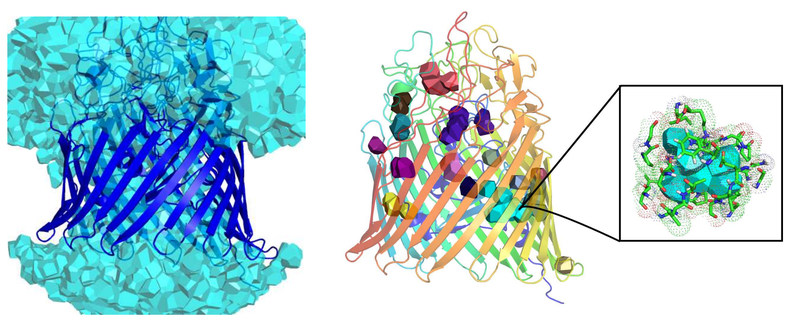


2 Method : Laguerre diagrams



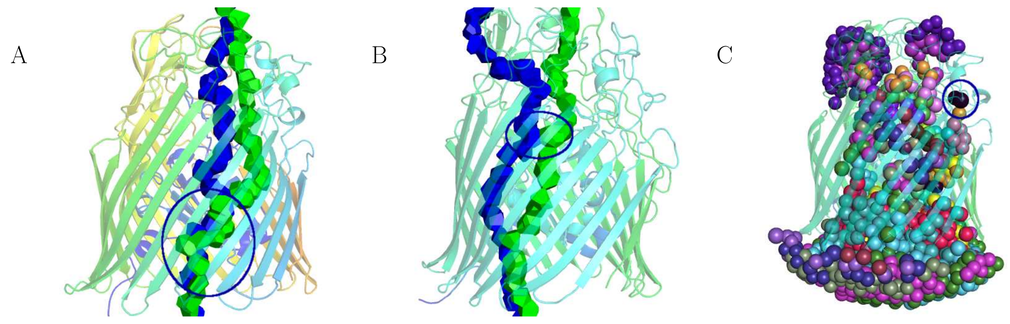
The Laguerre diagrams (weighted Voronoi) achieve accurate results if the weights attributed to each atoms are optimized. The specific weight of water molecules compensates for the discarded hydrogen atoms. Varying the weights can be used to probe the path stability, as shown above. On the left, the path is disconnected with a standard weight w_{ref} and a tiny contact appears when the weight is 40% larger (on the right). Water paths are contiguous series of Laguerre polyhedra surrounding water oxygen (all hydrogen atoms have been discarded) and are determined using Dijkstra's algorithm, either standard or as modified by Petrek [Petrek *et al.*, 2007, Structure **15**].

3 Main water connected component and inclusions



Overall (in apo and holo cases), a main connected component forms a water bridge between the extracellular and periplasmic media ; at least one water path crosses the FepA protein (on the left). Other smaller connected components correspond to water inclusions in the protein (on the right). Inclusions are found in all snapshots (apo and holo) but their number varies by orders of ten.

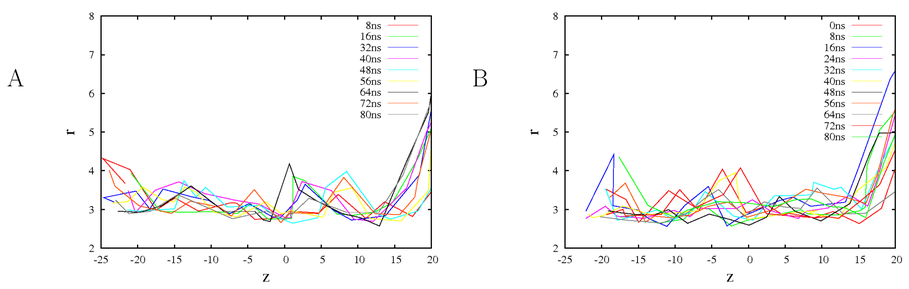
4 Disjoint water paths through FepA and stratification



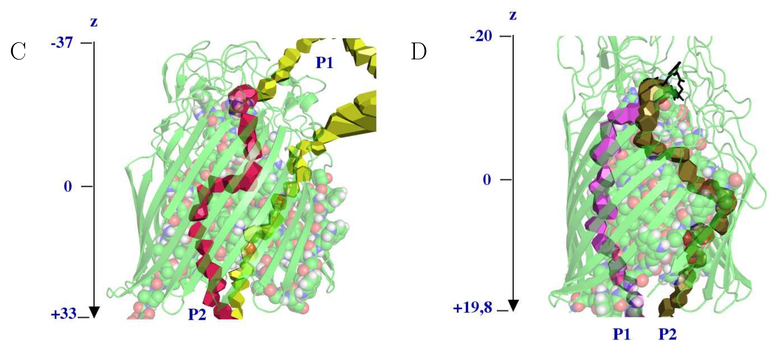
The maximum number of disjoint paths never exceeds 3, indicating that water paths must share common segments. These paths either gather into common channels (A) or overlap in constricted passages (B). In all snapshots, the water network is ramified ; a bottleneck is shown in (C).

5 Main water channels (transport precursors ?)

Here, Dijkstra's algorithm is modified in order to maximize the water-protein distance. The start point is chosen close to amino acids in contact with the enterobactin.



The mean radius of the widest water channels is about 3Å without (A) or with the ferric complex (B), negligibly influenced by the presence of enterobactin.



In the last snapshots, two direct channels appear quite stable in the presence of enterobactin (D) ; whereas mostly indirect channels find their way through the apo form (C). Above, the pymol images show the protein (cartoon), plug domain (balls) and enterobactin (black sticks). The paths are ranked by the cost function : P1 < P2.

6 Conclusions and outlook

Although the plug domain folds up inside the barrel and obstructs it, this study shows that several channels can cross the FepA protein, forming a water bridge between extracellular and periplasmic media. These channels are ramified and dynamic in time. The water network connects to the exterior by a number of small openings but, on the periplasmic side, there is a single wide conical opening (flared shape). The presence of the enterobactin seems to stabilize one or two channel(s) directed to periplasm ; however the mean radius is too small to allow a passage without deforming the protein.
In the future, we plan to run longer molecular dynamics simulations (80 ns is short for conformational changes). Along the discovered channels, we would like to list amino acids playing a key role in the transport. Mutations could be so proposed, such as substituting polar to aliphatic residues or building disulphide bridges. For example, we can target amino acid pairs, localized in some constrictions as shown below for two channels in the holo snapshot at 80 ns. The displayed couples are polar (SER96/ASN314) or charged (ARG50/ASP676) amino acids, in agreement with favorable interactions with water.