Stability, structure and transport features in membrane-embedded artificial waterchannels

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Design of future artificial water channels:

The increase of clean water have boosted the development of new membrane technologies for desalination towards synthetical materials like carbon nanotubes, unimolecular transmembrane artificial molecules and artificial self-assemblies. The aim of this project is the modelling of such self-assemblies in lipid membranes to understand the relation between the structure of channels when inserted into membranes and their transport properties.

Experimental insights:

We are currently working with a experimental team (*Barboiu et al.*, IEM Montpellier, France) that synthetize new families of compound forming channels with high water permeability and high ion selectivity. For a majority of molecules, experimentalists have access to the structure of the assemblies using crystallisation and X-ray measurements. We currently focus on compounds that present both an hydrophilic part capable of forming hydrogen bonds with water molecules and also an hydrophobic part which favour the insertion in the lipid bilayer (Figure 1).

Simulation methods:

From the knowledge of the crystal structure, we are able to identify possible orientation of the crystal patch in the bilayer membrane. The channel structure is build by duplicating the crystal unit in the three dimension space and in order to fit with the height of the lipid bilayer. Using online CharmmGUI tools, we set up the system by inserting the patch in a lipid composition corresponding to the experimental ones (mixture of POPS,POPC and cholesterol molecules in the ratio 4:1:5). The membrane is solvated using an explicit water model and a concentration of 0.15M of choride and sodium ions is added.

We use molecular dynamics to know if a nanoconstruct stay stable in the membrane environment. The simulations are performed on Gromacs software and the interactions are modeled by a potential function that model bonded and non-bonded interactions between atoms in the system. The input Lennard-Jones coefficients and partial charges for electrostatics comes from Charmm36 force field for lipids, TIP3 or SPC/E parameters for water and CgenFF and GAFF force field parameters for the compound. This latter provides a way to parametrize each new molecules using similarity with other molecules and chemical functions that have already been parametrized in the original Charmm36 force field.

Water transport have been measured counting the number of translocation events, which are defined when a molecule cross the membrane from one solvant compartment to the opposite one.

The ion permeation occuring at larger timecales have been probed for a few systems using non-equilibrium conditions with unbalanced charge in a double-membrane setup.

When channel structure are well-delimited with single-file water structures, we were able to compute the permeation distribution along channels, the mean pore radius and the mean number of hydrogen bonds that water form in the channels.

Figure 1: Chemical composition and crystal structure of channels

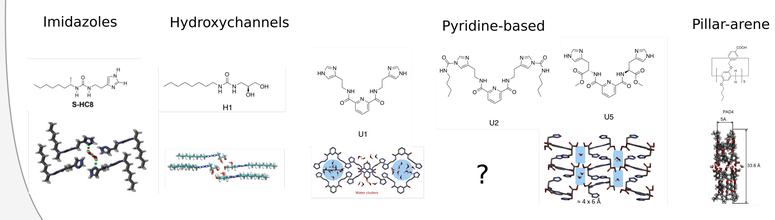


Figure 2: Modelling Nanoconstructs in MD simulations

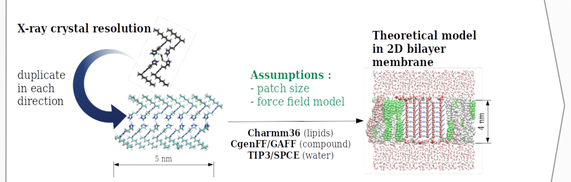
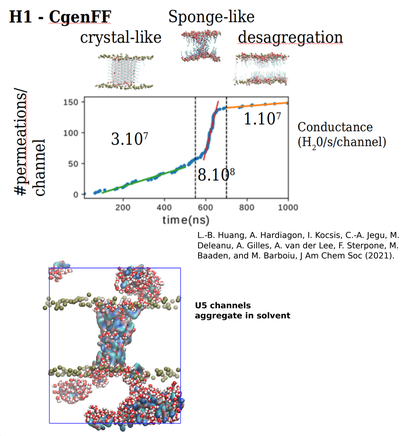
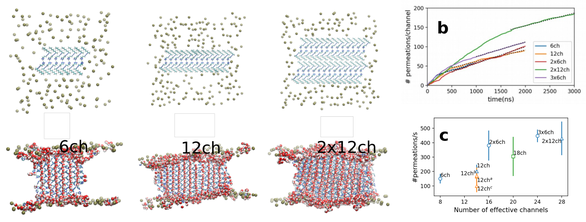


Figure 3: Struture of waterchannel models in lipid bilayers



L.-B. Huang, A. Hardiagon, I. Kocsis, C.-A. Jegu, M. Deleanu, A. Gilles, A. van der Lee, F. Sterpone, M. Baaden, and M. Barboiu, J Am Chem Soc (2021).

Figure 4: **(a)** Initial and final configurations of crystal-like channels in the scaling study . **(b)** Permeation time-series **(c)** Water permeation with respect to number of channels



A. Hardiagon, S. Murail, L. Huang, M. Barboiu, F. Sterpone, and M. Baaden, in New Trends in Macromolecular and Supramolecular Chemistry for Biological Applications (Springer, 2020).

Figure 5: (a) Permeation distribution in crystal-like large assemblies. Correlation between water conductance and **(b)** Mean pore radius **(c)** Mean Number of hydrogen bonds

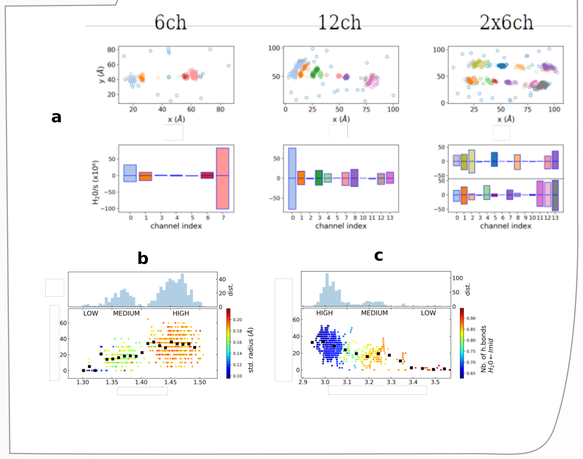


Figure 6: (a) RMSD distribution of the imidazole channels lining a central channel for different replica in REST2 simulation **(b)** Water conductance in MD simulations initialized in each cluster configuration **(c)** Time-series of mean pore radius

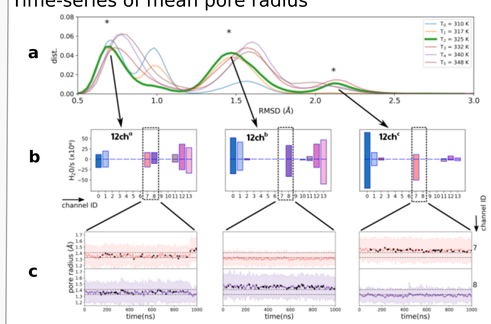
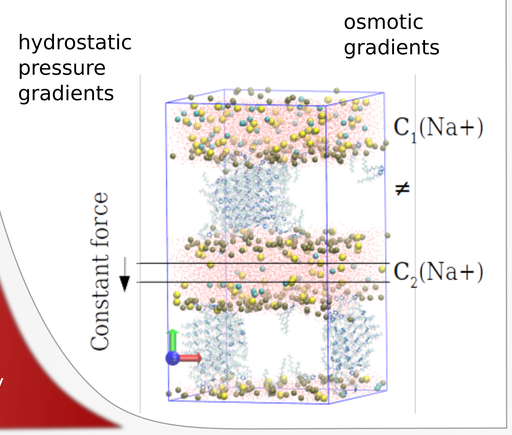


Figure 7: Double-membrane setup for non-equilibrium simulations



Stability and structure

In order to test the robustness of the model, we performed several simulations varying the type of compounds, **the water model**, the method to derive **force field paramaters** and the use of **non-equilibrium conditions** using a assymetric concentration of ions in a double-membrane setup (Figure 7). The following points summarises our results :

- Crystal structure is not stable for some systems (HC8,H1,U1,U5) using CgenFF froce field (Figure 3)

- GAFF parameters always stabilizes the system if the initial construction is crystal-like.

- In the case of S-HC8, the crystal phase is stable.

- Under use of an osmotic pressure comparable to the experimental one, we have not observed structural changes, neither measurable osmotic flow.

- water model factor : in crystal-like systems, conductance(TIP3P)≃ 2 x conductance (SPC/E)

Conclusion

- Structural Disorder in self-assembly water channels favour water permeation
- Empirical models and MD simulations allow to access stability and allow to hypothetize permeation mechanisms
- Simulations of small systems helps experimentalists to design future molecules for synthesis

Perspectives and future work

- Collect experimental data and optimize force field parameters by studing the phases of the aggregates in organic solvents.
- Extend non-equilibrium simulations (osmotic pressure, hydrostatic pressure, computationnal electrophysiology) to channel structures far from crystal-like structure (Figure 7).
- Design and empirical equilibration of crystal-like structures favoured by hydrophobic interaction with lipids.
- Test of other force field for lipids for a better compatibility with GAFF parameters.

Enhance sampling for central activation channel

Replica exchange with Solute Scaling (REST2) was used to improve the quality of the sampling. By using 12 replicas and scaling the hamiltonian terms for the S-HC8 molecules resulting to a effective temperature range between 310K and 400K, we were able to select configuration of central channels that contributes to high permeaton levels in an additional MD simulation (Figure 6).

Statistical analysis in crystal-like channels

The whole set of simulations with different sizes for the patch reveals interesting features that relates the single-permeabilty of channels and geometric features like mean pore radius and mean number of hydrogen bonds.

We denote some "inactive" channels with an almost perfect water single-file and "active channels" with local breaks of the single-file structure. The "active" channels shared both higher mean pore radius and lower mean number of hydrogen bonds formed by water molecules than the "inactive" channels (Figure 5)

Size scaling

Because of the lack of experimental data on the size of the aggregates, we performed a set of MD simulations to probe the scalability of a system, S-HC8 by increasing the size of the intial patch.

- The permeation rate scales linearly with the size of the system for the range of size we focus on (Figure 4)
- Large assemblies display non-uniform spatial permeation patterns (Figure 5)