# DIFFUSION OF WATER IN HALOPHILIC ARCHAEA

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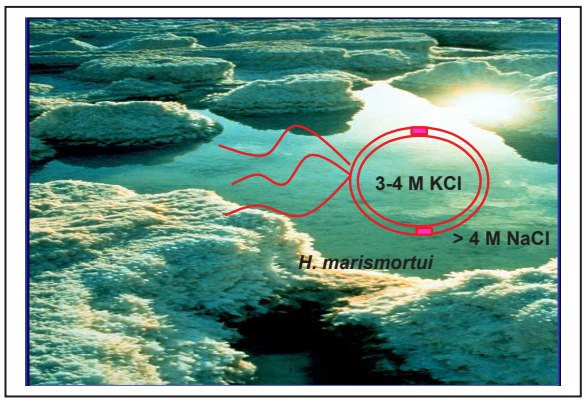
Although acknowledged as an essential part of all cells, very little is known about the properties of intra-cellular water. Most cell functions require the diffusion of either macromolecules or smaller solutes through the highly crowded aqueous cell compartments. What are the characteristics of this intra-cellular water? In what way does its interactions with many surfaces modify its dynamics?
These questions have mostly been studied by NMR and dielectric measurements and the values found by different groups have been subject to much controversy. Previous work by Ginzburg and collaborators concluded on a very different cytosolic environment for *Haloarcula marismortui* (*Hm)* compared to other organisms [1].




The "Dead" sea, where Haloarcula marismortui thrive in 4 molar salt

Neutron scattering has been used to investigate water dynamics since the early days of the method [2]. More recently it has been used to probe confined water [3], water associated with proteins [4], and the average internal dynamics of proteins in whole cells [5]. Here it provides a particularly well-adapted tool to probe the dynamics of water in the highly crowded cytoplasm 'in vivo'.
Spin echo spectroscopy is unique in providing information directly in the time domain, as compared to other types of neutron spectroscopy where the signal is measured in energy. The instrument resolution is not convoluted with the signal, but simply multiplied and we can directly observe the distribution of characteristic times in the sample.
In spin echo spectroscopy the polarisation measured is proportional to the coherent contribution minus one third of the incoherent contribution. Using a completely deuterated system is therefore of great interest. Since the signal is dominated by coherent scattering, it is possible to clearly identify the water peak around 1.9 $Å^{-1}$. In such a complex system as a biological cell, it is important to be sure the signal measured is in fact dominated by water, and by choosing Q values on this peak means that the signal mainly arises from the diffusion of $D_2O$ molecules.
*Hm* are halophilic archae (organisms which are neither eukaryotes, nor bacteria, but share characteristics with both of these organisms) living in almost saturated salt. Mechanisms for adaptation to such salt concentrations have created much interest [6]; *the aim of this project is to study the dynamics of water in this almost saturated in salt cytoplasm.*
To take full advantage of the spin echo technique, we have succeeded in cultivating deuterated *Hm* cells. These were then harvested, washed in a deuterated buffer, and layered in an aluminium sample holder. This fully deuterated system was then measured on g1bis at the LLB, to determine the dynamics of water in the cytoplasm. The spectra were compared to that of the deuterated buffer in which the cells are washed, to check that the effect observed is not only due to the high salt concentration.
We first checked that the $D_2O$ peak is present in the cell samples (see figure 1) and then performed quasi-elastic spectra at Q values on the $D_2O$ peak.

The buffer, which is almost saturated in NaCl, is slightly slowed down in comparaison to pure $D_2O$ (see figure 2). The intracellular water is however slowed considerably more : a fit with a stretched exponential yields a characteristic diffusion time which is a factor of ten longer than for that of bulk.

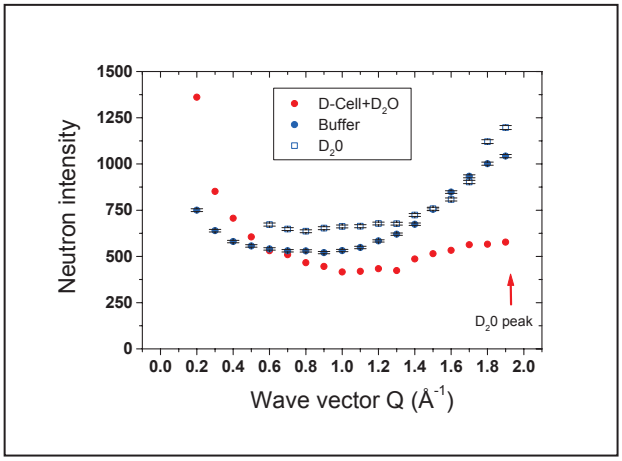



Figure 1. scattering function measured on g1bis. (pure $D_2O$ in empty blue squares, buffer ($D_2O$ with 4M NaCl) in full blue diamonds, and Hm cells in red.)

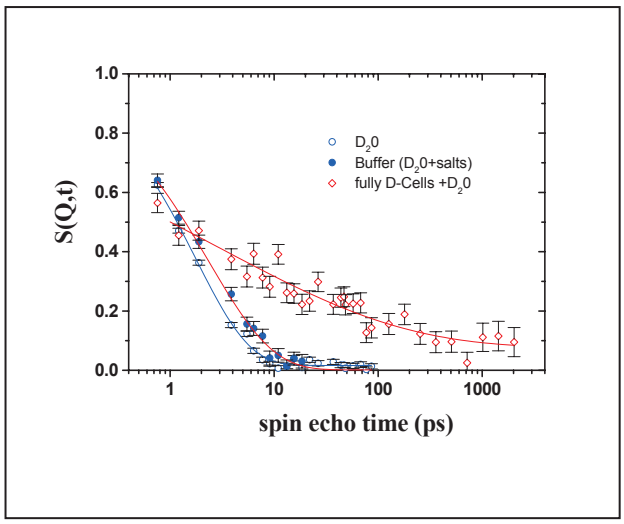



Figure 2 : spectra at Q = 1.7 Å$^{-1}$ on g1bis, pure $D_2O$ in empty blue circles, buffer ($D_2O$ with 4M NaCl) in full blue circles, and Hm cells in red.

The feasability of this study has been shown in this experiment, and we now expect to improve statistics with a larger quantity of deuterated cells in future beam time, and also to examine the Q dependence of the characteristic diffusion time.

Our next aim is to determine if this slowing down of water is specific to these archaic halophiles, or if it is a general property of intracellular water.

## References

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