Experimental report

29/09/2017

Proposal: 8-04-785 **Council:** 4/2016

Title: Intracellular and transmembrane water dynamics of Shewanella oneidensis bacteria at 500 and 1500 MPa

Research area: Physics

This proposal is a new proposal

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Samples: H/D substituted Shewanella oneidensis bacteria (no known physiological/medical hazard) suspended in PBS (phosphate/saline) aqueous buffer solution

Instrument	Requested days	Allocated days	From	To
IN6	4	4	28/09/2016	03/10/2016
IN5	5	0		

Abstract:

We propose to measure intracellular and transmembrane water dynamics in microbes at extremely high pressure. We will study S. oneidenisis that provides a model for extremophile behaviour and that we have previously adapted to survive into the GigaPascal pressure range. QENS experiments on H- vs D-labelled cells suspended in H/D media using specially developed HP devices at IN5 or IN6 will allow us to observe intracellular water mobility under extremely high pressure for the first time, and examine pressure effects on water transport through transmembrane Aquaporin channels. The results will give a unique view on microbial functioning under extreme conditions.

Intracellular and transmembrane water dynamics of pressure-adapted Shewanella oneidensis bacteria at 200 and 500 MPa

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Experiment 8-04-785: Instrument IN6 : 28.9.16 - 3.10.16

Our project used quasi-elastic neutron scattering to measure intracellular and transmembrane water dynamics in microbes at extremely high pressure. We studied *Shewanella oneidenisis* that provides a model for extremophile behaviour. We used a population that had been selected for survival in previous laboratory experiments at extremely high pressure, extending into the GigaPascal (GPa) pressure range. QENS experiments were carried out on H- vs D-labelled cells prepared in the Deuteration Lab (PSB/ILL) suspended in H/D media at the IN6 instrument. The results were compared with data obtained previously at the TOFTOF instrument (FRM-II, Garching) for wild-type samples that had not been previously exposed to high pressure conditions. The QENS datasets allow us to study intracellular water mobility and transmembrane dynamics for the 1 atm and P-adapted populations, measured *in situ* at pressures extending up to 500 MPa (5 kbar, 5,000 atm). The results are leading to a new understanding of microbial functioning under extreme environmental conditions.

Understanding the adaptation and functioning of extremophile bacteria under high pressure conditions is essential for answering critical questions concerning Earth's deep biosphere as well as the behaviour of microbes exposed to hyperbaric food sterilisation processes. Recently we observed the survival and adaptation of organisms including *E. coli* [1] and *S. oneidensis* [2] at pressures extending into the Gigapascal (GPa) range. The experiments were carried out using either wild type (WT) species, or "pressure-adapted" (PA) specimens cultured from survivors following exposure to increasingly higher P conditions. The results established a new baseline for microbial survival under extreme pressure conditions. A key aspect of cellular functioning involves mobility of water both inside the cell and its transport across the cell envelope. We first examined intracellular and transmembrane water dynamics for WT Shewanella oneidensis using quasi-elastic neutron scattering (QENS) at 1 atm and 200 MPa at the TOFTOF instrument at the FRM-II reactor source [3]. The experiments were carried out using normal (H-) and perdeuterated (D-) bacteria prepared in the Deuteration Facility within ILL's Life Sciences Group in Grenoble, then re-suspended in H- vs D-buffer solutions, togenerate isotopically contrasted QENS datasets that were used to highlight the dynamics within specific cellular regions. These initial data revealed several interesting results: (1) that intracellular water dynamics were indistinguishable from bulk aqueous media at 1 atm but slowdown due to macromolecular crowding was present at high P; (2) that we could identify a region of anomalously long correlation times for molecular H_2O/D_2O motions through the cell membrane, for $Q = 0.7–1.1$ Å^{-1} corresponding to the real space length scale (6–9 Å) of the central "neck" region of Aquaporin water transport channels. The intracellular dynamics showed a small but significant slowdown as P was increased between 1 atm and 200 MPa, but the transmembrane diffusion appeared to be only slightly affected by pressure. The new data obtained during the ILL (IN6) experiment now allow us to (a) conduct a comparative study between WT and PA species of S. oneidensis at 1 atm and 200 and 500 MPa to observe changes changes in transmembrane dynamics between the WT and PA populations, as well as (b) observing further changes in intracellular water diffusion that are accentuated at the higher pressure.

In advance of the QENS experiments, PA *S. oneidensis* were cultured (by feeding them D-enriched nutrients) using the Deuteration Facility at ILL. The techniques used previously for WT samples were applied to these samples [3]. A proposal was submitted and approved to carry out this work alongside the ILL experiment. Bacterial cell suspensions were prepared at a constant concentration of 50 mg/mL in PBS buffer and loaded into a piston cylinder cell that could be pressurized to 500 MPa. Experiments were carried out on (i) D-cells in H-buffer at 200 and (ii) 500 MPa; (iii) H-cells in D-buffer at 200 and (iv) 500 MPa; (v) D-cells in D-buffer at 200 and (vi) 500 MPa. Additional

QENS runs were also carried out using a flat plat cell at 1 atm.

We are now analyzing the QENS results to obtain information from the H/D contrast data. However the data analyses are more challenging than those we obtained previously from TOFTOF because of (a) a higher background of instrumental noise and (b) reduction in the accessibly Q range at the IN6 instrument. However the IN6 high-P sample environment enabled our study to significantly higher pressures than were available at TOFTOF. We are also combining the data analysis with some results obtained at the ISIS IRIS instrument at ambient and 500 MPa. We show some of our initial results and data analyses showing a comparison of the raw QENS data for pressure-adapted H- and D-bacteria in D- and H-buffers at 200 and 500 MPa, for selected Q values. The strong central feature is due to the elastic scattering line, that is determined by the instrument resolution and incident neutron wavelength (5.12 Å). The most obvious feature is that we clearly observe a QENS signal, due to H_2O dynamics in the bacteria. The strongest signal is for H-bacteria in D-buffer. There is apparently no change in QENS linewidth between 200 and 500 MPA signals, although there is a clear reduction in signal width (hence increased correlation time) between 1 atm and the high P condition. That supports and extends our previous discussion about intracellular water dynamics in the bacteria at high pressure. We are now studying and interpreting the full set of QENS data in detail to extract information from the H/D contrast studies and build a complete picture of intracellular *vs* transmembrane water dynamics for WT *vs* PA *S. oneidensis* at pressures extending up to 500 MPa (Fig. 1).

References: [1] M. Haertlein et al, Meth Enzym 566 (2016) 133; [1] D. Vanlint et al, mBio 2, e00130-10 (2011); [2] R. Hazael et al, Frontiers Microbiol 5, 612 (2014)**;** [3] F. Foglia et al, Sci. Rep. 6, 18862 (2016)

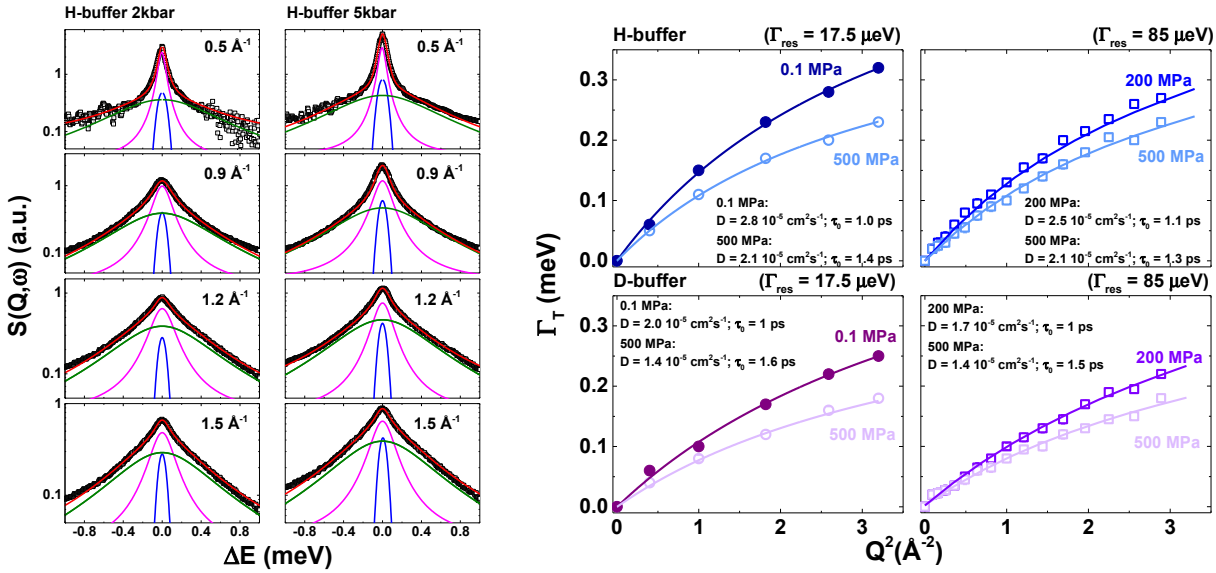

Figure 1. Preliminary analyses of QENS data collected at IN6 for pressure-adapted *S. oneidensis* at 200 MPa (2 kbar) and 500 MPa (5 kbar). At left are shown representative data for the H-buffer at different Q values. The FWHM of the central (Gaussian) elastic line represents the instrumental energy resolution. The QENS signal is fit by one or two Lorentzian functions representing translational (diffusion) and rotational relaxation of the H_2O molecules. At right $\Gamma_T(Q^2)$ plots show the large reduction in intracellular and transmembrane H_2O diffusion from isotopic contrast experiments for PA bacteria previously surviving exposure to 500-750 MPa.