Scientists use neutrons to understand the secrets of extremophile bacteria like the ones decomposing the RMS Titanic

- **Understanding microbe adaptation to extreme environments remains a challenge of high biotechnological potential—in bioremediation and waste management, for example.**
- ***Halomonas* microorganisms isolated from the oceans or salt marshes, reversibly accumulate high concentrations of the molecule ectoine, within their cells, to counterbalance fluctuating external salt concentrations. It has been estimated that a rust-producing *Halomonas* species could bring about the total deterioration of the sunken RMS Titanic by 2030.**
- **A range of specialised neutron scattering experiments were designed in order to understand how ectoine permits *Halomonas* to survive in their extreme environment. They revealed that within the microbe cells, ectoine acts by enhancing the remarkable dynamic properties of water that are essential to life processes.**

Microorganisms represent the most numerous life on earth and the understanding of how they behave is integral to our own survival and well-being. Microbial life has an amazing flexibility for adapting to extreme environments — niches that are extraordinarily hot or cold, acidic or basic, salty as in the Dead Sea or at high pressure in great ocean depths — that would be detrimental to complex organisms. These organisms are known as extremophiles.

Among the extremophiles, bacteria, isolated from salt marshes or marine environments include a variety of interesting species of high biotechnological potential such as the recently discovered *Halomonas titanicae* in the hull of the sunken RMS Titanic ship. It has been estimated that the action of this rust-producing *Halomonas* may bring about the total deterioration of the Titanic by 2030; in the same way it has been identified as a potential danger to oil rigs and other man-made objects in the deep sea. But the rusting property could also be harnessed in bioremediation or waste management, for example to accelerate the decomposition of shipwrecks littering the ocean floor.

A range of experiments conducted at the world's leading neutron research centre, the Institut Laue-Langevin (ILL), in collaboration with Max Planck Institute of Biochemistry (MPIB) and the Institut de Biologie Structurale (IBS, CNRS/CEA/UGA), concentrated on the interaction of ectoine with water and protein.

Ectoine is a natural compound found in many organisms, including *Halomonas*. It serves as a protective substance by acting as an osmolyte – a molecule that plays a role in fluid balance and cell volume maintenance and thus helps organisms survive extreme environmental stress. Ectoine is called a *compatible solute* in the sense that its occurrence within the internal material of the cell does not interfere with cellular biochemistry and metabolism. *Halomonas* can produce ectoine up to an intracellular concentration of 20% of the cellular dry mass. By this adaptive regulatory process, the microorganism is said to be *halotolerant* over a broad range of salt concentration, from 0.5 to 25% NaCl (on average, sea water has a salt concentration of 3.5%). Ectoine itself, which displays a stabilising effect on proteins and

membranes and a related inhibitory effect on inflammation in mammalian cells, has found broad cosmetic and clinical applications through its hydration, stabilisation and anti-inflammatory properties.

Neutrons illustrated how ectoine acts by being excluded from a shell of pure water around protein and membrane surfaces. H_2O molecules in liquid water interact with each other through a highly dynamic fluid network of hydrogen bonds (H-bonds) between the O and H atoms of adjacent molecules. The presence of other substances in the water can hamper this organisation. The neutron experiments described the effects of ectoine on water H-bond dynamics to reveal how ectoine's protective characteristics do not interfere with bacterial metabolism. In fact, ectoine, rather than hindering, enhances the remarkable dynamic properties of H-bonds in water—properties that are essential for water's unique solvent capabilities, and vital for the proper organisation, stabilisation and function of proteins, membranes, RNA and DNA.

Dr Joe Zaccai, Emeritus Scientist of the CNRS at the ILL says: "Pure liquid water has remarkable properties, based on dynamic H-bond networks that play vital roles in macromolecular folding and interactions, which in turn determine their biological functions. The results in this study illustrate how the osmolyte behind the halotolerance response in microorganisms induces compensating effects on water H-bonding that respect these essential biological properties. Neutrons are the ideal tool for investigating structure and dynamics in water and biological molecules– they have a number of unique advantages, including amongst others their high penetrative power with no radiation damage to the sample. Like a 'giant microscope', they allow details of the crucial hydrogen-bonding interactions to be observed. Although much spectroscopic and thermodynamic investigations have been done before on ectoine, we are proud that through the use of neutrons this is the first study that has allowed a direct experimental structural characterisation of ectoine-water-protein and membrane interactions."

Notes to Editors:

- Ectoine purification from *Halomonas and* deuterium labelled samples were prepared at the MPI Martinsried and ILL. Neutron scattering data at ILL were collected on the D4C liquids diffractometer to observe H- bonds in ectoine solutions in water, on the D22 small angle scattering camera to observe hydration around a protein in the presence of ectoine, on the D16 diffractometer to compare hydration around a membrane in the presence and absence of ectoine, and on the IN16 spectrometer to compare membrane dynamics in the presence and absence of ectoine. For more information, please visit http://www.ill.fr.

About ILL – the Institut Laue-Langevin (ILL) is an international research centre based in Grenoble, France. It has led the world in neutron-scattering science and technology for almost 40 years, since experiments began in 1972. ILL operates one of the most intense neutron sources in the world, feeding beams of neutrons to a suite of 40 high-performance instruments that are constantly upgraded. Each year 1,200 researchers from over 40 countries visit ILL to conduct research into condensed matter physics, (green) chemistry, biology, nuclear physics, and materials science. The UK, along with France and Germany is an associate and major funder of the ILL.

About MPI Biochemie – Proteins are the molecular building blocks and engines of the cell, and are involved in almost all processes of life. The scientists at the Max Planck Institute of Biochemistry (MPIB) investigate the structure of proteins and how they function – from individual molecules up to whole

organisms. With about 850 employees coming from 45 nations, the MPIB is one of the largest institutes within the Max Planck Society. In currently eight departments and about 25 research groups, scientists contribute to the newest findings in the areas of biochemistry, cell biology, structural biology, biophysics and molecular science. They are supported by several scientific, administrative and technical service facilities. For more information please visit http://www.biochem.mpg.de/en.

About IBS – The Institut de Biologie Structurale (IBS) is a research center for integrated structural biology funded by the CEA, the CNRS and the University Grenoble Alpes (Unite Mixte de Recherche, UMR 5075). The Institute performs interdisciplinary research at the interface of biology, physics and chemistry, and combines basic and applied science and technical innovation. The IBS employs approximately 270 people and comprises eighteen groups. IBS scientists are active in training of undergraduate, master and PhD students and comprise more than 20 professors and assistant professors who teach at the University Grenoble Alpes. For more information, please visit http//:www.ibs.fr.