NMR can now scrutinize the membrane of a living cell

Nuclear Magnetic Resonance (NMR) is a technique devised by physicists, that has developed into many applications. The most famous one is Magnetic Resonance Imaging that is commonly used in hospitals. In the research lab, NMR is generally used to observe the nuclei of small soluble molecules, each molecule providing a spectrum rather than an image.

Discovered in the late 1940s, NMR spectroscopy first allowed the observation of hydrogen nuclei (also called protons) of ethanol, then went on to study more complex biological molecules such as small proteins or nucleic acids, sometimes allowing to determine their three-dimensional structure. In order to obtain characteristic spectra, these biomolecules had to be synthesized or purified, and studied isolated in water.

Although scientists have attempted to look at biological cells, such as yeast as early as 1955, it is not until 2001 that NMR successfully detected an isolated protein carbon signal from a molecule inside a bacterial cell. This was the beginning of In-cell NMR.

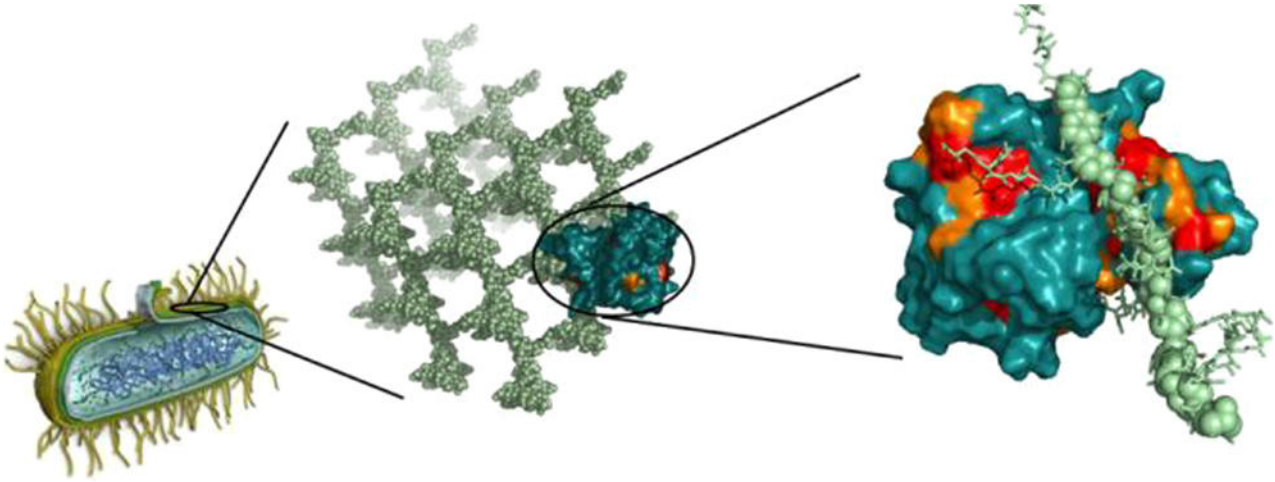

Fig. 1. Cartoon showing the interactions between the cross-linked sugars (in green) in the cell wall of the bacteria Bacillus subtilis, and the enzyme (in blue, red and orange) that performs this cross-linking, as determined by In-cell solid-state NMR (reprint from Schanda et al. (2014) J. Am. Chem. Soc. 136:17852–17860).

However, conventional NMR suffers from a major problem: it can only observe fast tumbling molecules. Some biomolecules tumble fast, but some do not, particularly molecules embedded in membranes, such as lipids and membrane proteins. Alternatively, rigid or “solid” molecules can be studied by solid-state NMR, which uses other tricks such as fast spinning of the sample inside a small rotor. Solid-state NMR was first applied in the 1970s to study isolated purified lipids or

membrane proteins.

It is only in 2011 that solid-state NMR was first successfully applied to the study of a membrane protein embedded in its native *E. coli* bacterial cell membrane. This review is therefore the first of its kind, which covers this 5-years old story of In-cell solid-state NMR, and the years that led to it.

This review covers the study of lipids and sugars that form the membranes and walls of algae, fungi, plants, yeast, bacterial and mammalian living cells, watching their protons, carbons, phosphorous and other nuclei. Such spectra can fill up libraries that can be used to differentiate between “healthy” and “sick” cells, but they can also show how these membranes and cell walls are organized to compartmentalize the cells (see Fig. 1).

But the heroes of this story are membrane proteins, whose functions are complex and involved in many cellular processes and diseases. However, their structures remain mysterious, and In-cell solid-state NMR aims at determining them within their native membranes. Nonetheless, this is only the beginning of the story, and only a handful of such endeavors have been reported, although one of them is quite an impressive challenge: the study of a huge secretion system (T4SScc), within its *E. coli* membrane!

A priori, the ability to determine physical parameters of biomolecules within their native environment is much more relevant than when they are isolated and solubilized in water. This review describes what In-cell solid-state NMR can bring to a better understanding of cellular organization, what kind of objects can be looked at, and the crucial parameters to optimize when dealing with such fragile samples.

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