NMR STUDIES OF AN ANTIMICROBIAL PEPTIDE: FROM *IN VITRO* TO *IN SITU*

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Antimicrobial peptides (AMPs) have been extensively studied as promising alternatives to traditional antibiotics. Solid-state NMR has been used to characterise their effect on lipid bilayers, their primary target. Such studies are important to provide high-resolution details with a model membrane system but correlation with *in vivo* situations remains speculative, especially in view of the complex modulations observed with slight changes in sample conditions (pH, temperature, lipid composition or peptide concentration). Studying AMPs in live bacteria is, therefore, attractive but presents several challenges, such as sample longevity.

Using dynamic nuclear polarization (DNP) enhanced solid-state nuclear magnetic resonance (ssNMR), we report the impact of an antimicrobial peptide (AMP), on lipid membrane and macromolecular components (i.e., proteins and nucleic acids) of *Escherichia coli*. Global scanning of the cellular components was achieved by monitoring the nitrogen (^{15}N) signals by cross polarization (CP) magic angle spinning (MAS) NMR of whole bacteria grown in isotopically enriched media (^{15}N, ^{13}C and ^{2}H 98% enriched isotopes). The different ^{15}N chemical shifts of cellular components served as an atomic marker for monitoring the action of the AMP maculatin 1.1 on *E. coli* (Fig. 1). The enhanced ^{15}N ssNMR signals from nucleic acids, proteins and lipids identified a number of unanticipated physiological responses to peptide stress, revealing that membrane-active AMPs can have a multi-target impact on bacterial cells.

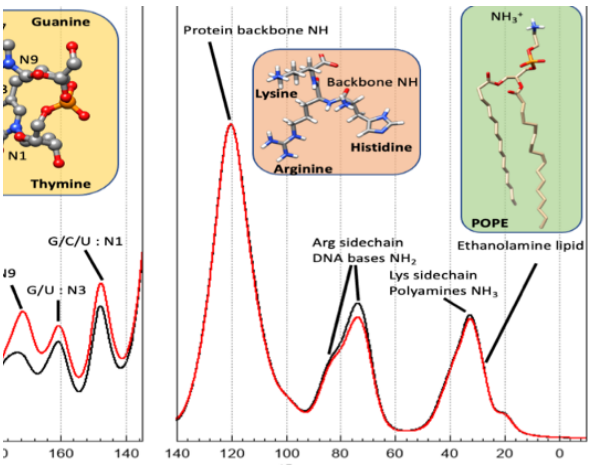

Figure 1 DNP-enhanced ^{15}N CPMAS spectra of untreated *E. coli* cells (black line) and in the presence of Mac1 at 15:1 w/w ratio (red line). The left panel is scaled 4-fold compared to the right panel to increase visibility. The DNA bases, amino acids with nitrogen containing sidechains and the phospholipid palmitoyl-oleoyl-phosphatidyl-ethanolamine (POPE) structures are displayed in the inserts with nitrogen (blue), oxygen (red), phosphorous (orange), carbon (grey) and hydrogen (white) atoms.