Solid-state NMR applied to bacterial and human cells: Concepts & applications

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Increasing evidence suggests that the highly complex and dynamic environment of bacterial and human cells imposes critical control on cellular functions which are difficult to mimic under *in vitro* conditions. Complementary to high-resolution light microscopy and electron tomography, in-cell solution-state NMR can track such structural and dynamical interactions at the most detailed, i.e., atomic level, provided that the molecular units tumble rapidly.

Cellular solid-state NMR (ssNMR), on the other hand, provides increasing possibilities to probe molecular structure in bacterial and human cell preparations largely irrespective of molecular size. In our contribution we discuss novel preparative (Figure) as well as NMR-based hybrid concepts that allow for the study of proteins and (membrane)protein complexes *in-situ*.

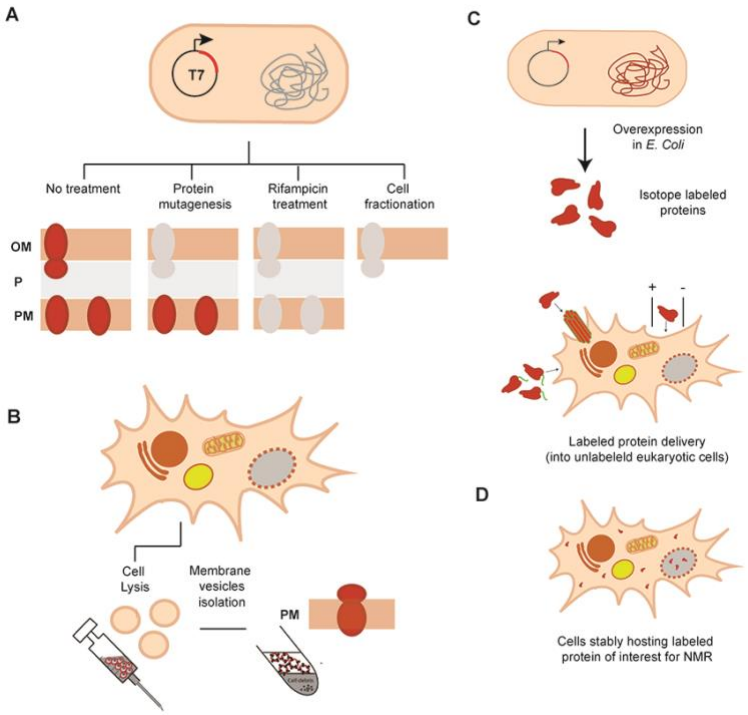

Figure: Preparation strategies for ssNMR-based studies of prokaryotic and eukaryotic cellular systems. (A) Isotope-labeled membrane proteins can be produced in *Escherichia coli* using the bacteriophage T7 promoter-driven overexpression. The quality of cellular ssNMR spectra of cellular envelope (CE) and whole cell (WC) preparations can be significantly improved by the deletion of abundant *E. coli* proteins, rifampicin treatment during protein production and the isolation of the subcellular compartment of interest after cell fractionation. (B) Preparation of protein-rich membrane vesicles from human cells cultured in suitable growth media. (C) Protocol to study isotope-labeled soluble proteins produced in bacteria after using dedicated delivery methods in mammalian cells. (D) Transient transfection of host cells can be used to directly study soluble proteins in mammalian cells.