

Designing bioreactors for in-cell NMR spectroscopy

E. Luchinat, T. Campbell, L. Barbieri, E. Ravera, C. Luchinat, L. Banci

Magnetic Resonance Center - CERM, University of Florence, Via Luigi Sacconi 6, 50019 Sesto Fiorentino, Italy, eluchinat@cerm.unifi.it

In-cell NMR is a powerful approach to obtain structural and functional information on macromolecules in their native cellular environment at atomic resolution. Usually, in-cell NMR samples are kept in closed tubes, where oxygen consumption, depletion of nutrients and buildup of toxic byproducts result in short sample lifetimes. To overcome this limit, a continuous flow of fresh nutrients and oxygen has to be applied to the sample. Existing NMR bioreactor designs are either focused on metabolic flow analysis, requiring low cell densities in the NMR active volume, or are custom made flow systems optimized for specific applications. We are developing a modular in-flow system based on the commercially available Bruker InsightMR flow unit. One design exploits a coaxial microdialysis membrane to provide continuous exchange of nutrients, and can be applied on both bacteria and mammalian cells. The same setup also allows for *in vitro* applications such as protein-based ligand screening. Alternative membrane-free designs are also being developed, in which mammalian cells are embedded in a gel matrix and directly exposed to the flow of nutrients. These designs allow for high cell density and ensure cell viability for up to 72 h.