

Expression of fluorinated proteins in human cells for ^{19}F in-cell NMR

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ABSTRACT

In-cell NMR spectroscopy is a unique approach to study the structure and function of biological macromolecules in their native cellular environment at atomic resolution.¹ At CERM/CIRMMMP, we developed an approach for expressing and labelling proteins directly in human cells, which is ideally applied to monitor functional processes such as protein folding and maturation, metal binding, chemical modifications, and interactions with ligands or with specific partners.² Such approach provides important insights on protein-ligand interactions, which are critical to optimize drug penetrance and potency.³ However, protein-observe drug screening by ^1H and ^1H - ^{15}N spectra is limited by fact that signals from proteins interacting with cellular components are broadened beyond detection. ^{19}F NMR spectroscopy is an attractive alternative, thanks to the high-sensitivity and background-free nature of ^{19}F . We have shown that fluorinated amino acids incorporated in proteins expressed in human cells allow protein-observed screening on otherwise invisible targets.⁴ Furthermore, the incorporation efficiency of fluorinated amino acids can be controlled by changing the media composition, and tuned to either maximise ^{19}F signal intensity or to minimize structural perturbations.⁵ This approach holds great potential to study “invisible” protein targets in cells.

REFERENCES:

1. Luchinat E., Cremonini M., Banci L. *Radio signals from live cells: the coming of age of in-cell solution NMR*. Chemical reviews, **122**, 9267-9306 (2022).
2. Barbieri L., Luchinat E., Banci L. *Characterization of proteins by in-cell NMR spectroscopy in cultured mammalian cells*. Nature Protocols **11**, 1101–1111 (2016).
3. Luchinat E., Barbieri L., Cremonini M., Nocentini A., Supuran C. T., Banci L. *Drug screening in human cells by NMR spectroscopy allows the early assessment of drug potency*. Angewandte Chemie, **132**, 6597-6601 (2020).
4. Pham L. B. T., Costantino A., Barbieri L., Calderone V., Luchinat E., Banci L., *Direct Expression of Fluorinated Proteins in Human Cells for ^{19}F In-Cell NMR Spectroscopy*. J. Am. Chem. Soc. **145**, 1389–1399 (2023).
5. Costantino A., Pham L.B., Barbieri L., Calderone V., Ben-Nissan G., Sharon M., Banci L. Luchinat E., *Controlling the incorporation of fluorinated amino acids in human cells and its structural impact*. Protein Science, **33**, p.e4910 (2024).