

# Multi-target ligand binding assay in human cells by real-time in-cell $^{19}\text{F}$ NMR

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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. 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.<sup>1</sup> A major limitation of in-cell NMR is the short lifetime of the cells once they are densely packed in a closed environment. An NMR bioreactor perfuses the cells with fresh nutrients and oxygen, making possible to study intracellular processes in real time over the course of up to 72 hours.<sup>2</sup> Real-time in-cell NMR can provide crucial insights on drug-target interactions, such as target engagement in the intracellular environment, kinetics of cell penetrance and binding stability, which are critical to optimize drug potency.<sup>3,4</sup> Ligand-based in-cell  $^{19}\text{F}$  NMR spectroscopy provides a broadly applicable alternative to target-based approaches, thanks to the high-sensitivity and background-free nature of  $^{19}\text{F}$ . We recently showed that fluorinated ligands can be directly observed as they interact with their intracellular targets, and allow the screening of non-fluorinated compounds by competition binding, where the displacement of a fluorinated spy ligand is used to measure the binding affinity of a second non-fluorinated compound. By exploiting the sensitivity of the  $^{19}\text{F}$  chemical shift to the chemical environment, the approach can in principle be extended to the screening of drugs binding to multiple intracellular targets simultaneously. This multi-target ligand binding assay is demonstrated on a set of cytosolic isoforms of human carbonic anhydrase, with the goal of identifying isoform-selective compounds.

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