

Detecting protein-drug interactions in human cells by real-time ^{19}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.¹ A major limitation of in-cell NMR is the short lifetime of the cells once they are densely packed in a closed environment. NMR flow bioreactors can greatly extend the sample lifetime by providing the cells with fresh nutrients and oxygen. This makes possible to study intracellular processes in real time over the course of up to 72 hours.² Real-time in-cell NMR provides important information on protein-ligand interactions, such as intracellular ligand binding kinetics and thermodynamics, which are critical to optimize drug penetrance and potency.^{3,4} Classical screening by ^1H and ^1H - ^{15}N spectra is often limited by fact that signals from target proteins interacting with cellular components are broadened beyond detection. ^{19}F NMR spectroscopy is ideally suited for the purpose, thanks to the high-sensitivity and background-free nature of ^{19}F . We recently showed that fluorinated amino acids can be incorporated in proteins expressed in human cells.⁵ This allows protein-observed screening on otherwise invisible targets. Finally, fluorinated ligands can be directly observed as they interact with their intracellular targets. We showed that ligand-based in-cell screening can be achieved by competition binding. In this setup, the displacement of a fluorinated spy ligand is monitored by time-resolved NMR in the bioreactor as a function of the concentration of a second non-fluorinated ligand (**Figure 1**). The binding affinity of the latter is then obtained relative to that of the spy ligand.⁶ Such approaches hold great potential in the development of more effective drugs towards pharmacologically relevant targets.

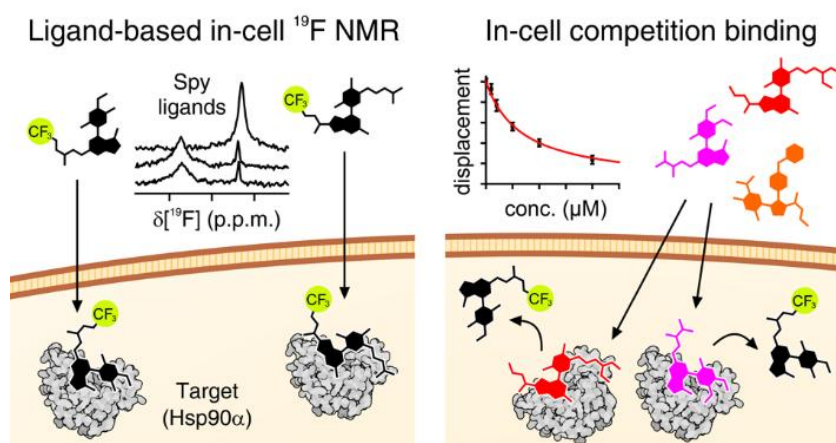


Figure 1. Ligand-based competition binding in human cells by in-cell ^{19}F NMR.

References

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