

## In-Cell Quantification of Drugs

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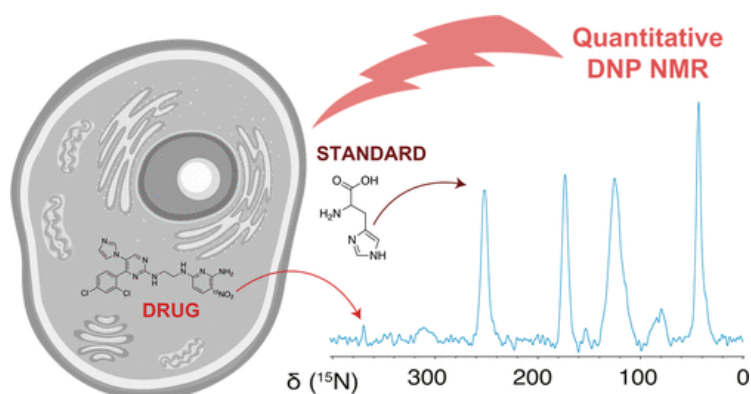
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In pharmacology, the quantity of drug inside cells is key to understand the activity of the molecules. The quantification of intracellular drug concentrations would provide a better understanding of the drug function and efficacy. The most accurate quantification methods for drugs in-cells should be performed without modification of either the drug or the target, and with the capability to detect low amounts of the molecule of interest. Here, typically, the relevant range of detection should be in many cases in the  $\mu\text{M}$  to nM (pmol to fmol per million cells) range. Thus, it is currently challenging to provide direct quantitative measurements of intracellular drug concentrations that simultaneously satisfy these requirements. Here, we show that Dynamic Nuclear Polarization enhanced solid-state NMR spectroscopy (DNP NMR) can satisfy all these requirements. We develop a quantitative  $^{15}\text{N}$  DNP NMR approach combined with  $^{15}\text{N}$  labeling in order to quantify the intracellular amount of the drug [ $^{15}\text{N}$ ]CHIR-98014, an activator of the Wnt and Int-1 signalling pathway. We determine intracellular drug amounts in the range of tens to hundreds of picomoles per million cells.<sup>1</sup> This is, to our knowledge, the first time that DNP NMR has been used to successfully estimate intracellular drug amounts. The method should pave the way to access in-cell pharmacokinetics data.



**Figure 1.** In-cell quantification of drugs by DNP MAS

[1] Bertarello, A.; Berruyer, P.; Artelsmair, M.; Elmore, C. S.; Heydarkhan-Hagvall, S.; Schade, M.; Chiarparin, E.; Schantz, S.; Emsley, L. In-Cell Quantification of Drugs by Magic-Angle Spinning Dynamic Nuclear Polarization NMR. *J. Am. Chem. Soc.* **2022**, *144*, 6734–6741.