The living cell is a complex system that contains many biological macromolecules in a relatively small volume. It is the ultimate goal of many biochemical researchers to understand the nature of these molecules and how their interactions regulate the biological activities within the living system. However, during their studies, there are formidable challenges due to the complexity of living matters and the relative scarcity of appropriate \textit{in vivo} methods. In terms of protein science, most of the studies are performed under \textit{in vitro} conditions, which consist mostly of water. With the growing awareness of this deficiency, there is a drive towards studying proteins under their physiological conditions, including the application of spectroscopic techniques into the proteins inside living cells.

Nuclear magnetic resonance (NMR) spectroscopy is a particularly attractive method for in-cell studies of proteins since it can provide atomic-level data in a noninvasive and nondisruptive way. Additionally, NMR has undergone significant advances in instrumentation and methods to increase the sensitivity and reduce the data acquisition time.\textsuperscript{1,2} In-cell NMR experiments do not focus on \textit{de novo} determining protein structures directly in the cellular environment, but utilize the sensitivity of the chemical shift towards changes in the environment to obtain information about the native states of the proteins and their interactions with other cellular components. Changes in the environment caused by post-translational modifications, conformational changes or binding events, lead to changes in the resonance frequencies of the affected nuclei and can thus be detected in the in-cell NMR experiments (\textbf{Figure 1}).\textsuperscript{1,3}

\textbf{Figure 1.} Applications for in-cell NMR experiments: Changes in the chemical environment of a protein’s nuclei caused by post-translational modifications (A), conformational changes (B) or binding events (C) can be detected by differences in chemical shifts in in-cell NMR experiments. Schematic heteronuclear single quantum correlation (HSQC) spectra indicating the sensitivity of the chemical shift to the changes described above are shown next to each cell.\textsuperscript{1}
The investigation of proteins in living cells has to overcome some difficulties. Firstly, the NMR signals of the protein of interest should be distinguished from the resonances of all other cellular components. This distinction can be achieved by labeling of the protein of interest with NMR-active isotopes $^{15}$N or $^{13}$C. In principle, as for prokaryotic cells, the protein of interest can be overexpressed and labeled simultaneously in living cells while for eukaryotic cells such as Xenopus laevis oocytes and HeLa cells, the current protocols are based on the injection of isotopically labeled proteins into cells or the delivery of labeled proteins via cell-penetrating peptides.

Secondly, the viscosity of the cytoplasm and the binding of the proteins to larger cellular components may cause long rotational correlation time, which lead to line broadening. Fortunately, the introduction of transverse relaxation-optimized spectroscopy (TROSY) and other techniques has extended the applicability of NMR spectroscopy to observe large macromolecules. Thirdly, the cells need to survive the conditions in NMR tubes for the time period of detection without significant changes of their metabolic state. This can be achieved by encapsulation of the cells and the exchange of the media.

In-cell NMR has been applied into several fields of protein study for the last ten years. Structural changes of proteins and the chemical environment in which the protein resides are usually observed by monitoring the protein 2D $^1$H–$^{15}$N (or $^1$H–$^{13}$C) heteronuclear single quantum correlation (HSQC) “fingerprint” spectra. After the detection of the differences between the in vitro and in-cell spectra, further in vitro experiments are performed to identify the cause of these differences. The suspected interaction partners will be added into the protein solutions to see if the same chemical shift differences can be produced. Using this method, Pielak and his co-workers studied the conformational changes of intrinsically disordered proteins in crowding cytoplasm. In-cell NMR also provides the means to detect protein-protein interactions such as mapping the binding interface between two proteins, which are involved into biological function of the proteins. Furthermore, in terms of the interactions between proteins and small molecules, for instance, protein-drug interactions, in-cell NMR has its own advantages that can reveal some interactions not occurring in vitro, thus making a potential screening tool in the pharmaceutical industry in the future. Last but not least, protein resonance signals are also sensitive to post-translational modifications. The induced chemical shift changes of these modified substrate residues enable the direct detection of protein phosphorylation during defined cellular events, such as the progression through different stages of the cell cycle, or upon the stimulation of intracellular signaling pathways by external factors.

The main focus of this presentation is around the applications of in-cell NMR into several areas of protein study such as conformational changes, protein-protein interactions, protein-drug interactions and protein processing. The technical aspects of in-cell NMR will be outlined and the limit and future perspective of in-cell NMR will also be discussed.
Reference