Faster is better: improving the sensitivity of solid-state NMR

Stanley J Opella

A method to improve the sensitivity of solid-state nuclear magnetic resonance spectroscopy promises to extend this technology to larger and more biologically interesting systems than previously feasible.

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As the most powerful form of spectroscopy, nuclear magnetic resonance (NMR) spectroscopy is widely applied to study proteins and other biomolecules. With its roots in physics, the frequencies, splittings and correlations of the signals reveal fundamental chemical and structural properties of molecules. High-resolution NMR spectra can contain separate signals from each atom in the protein, even from the same type of atom, for instance, the alpha carbons of the leucine residues, distributed throughout the polypeptide backbone. No other form of spectroscopy has these attributes. NMR spectroscopy can be applied to proteins in solution as well as crystals or insoluble proteins in supramolecular complexes such as membranes, virus particles or amyloid fibrils through the use of a variation of the technology called solid-state NMR. In this issue, Ishii and colleagues[1] present an approach to increase the sensitivity of solid-state NMR spectroscopy by allowing shorter data acquisition times, which should expand the reach of the technology to more demanding protein targets.

Solution NMR spectroscopy methods can be applied to soluble proteins because proteins reorient rapidly enough in aqueous solution to average-out the most severe line broadenings owing to internuclear interactions. In solid-state NMR spectroscopy, radiofrequency irradiations substitute for motional averaging as a mechanism for attenuating the effects of the line-broadening interactions.

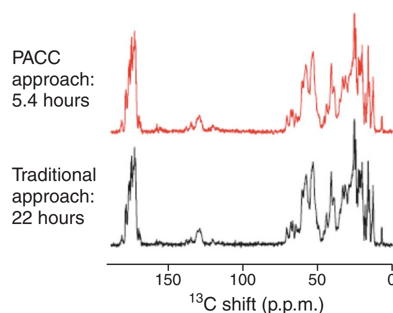

Figure 1 | The ^{13}C spectrum of uniformly ^{13}C-labeled ubiquitin in microcrystals collected using the PACC approach is nearly identical to the ^{13}C spectrum collected using the traditional solid-state NMR spectroscopy approach, but in one-quarter of the time. Figure modified from Supplementary Figure 2a of reference 1.

The era of high-resolution solid-state NMR started with the demonstration that precisely applied pulses of radiofrequency irradiations could permit the observation of signals with different chemical shifts[2]. However, it was the subsequent implementation of proton-enhanced nuclear induction spectroscopy[3] that opened up many applications in chemistry and biochemistry by providing access to ^{13}C and ^{15}N with both high sensitivity and spectral resolution. Before this, the observation of ^{13}C signals in crystal was forbidding because of their very long relaxation times in crystalline solids[4]. The ^{1}H nuclei in many compounds, including amino acids, have somewhat shorter relaxation times owing to the effects of local large-amplitude and rapid molecular motions, such as the 3-site hops of methyl groups in alanine or the flips of aromatic rings in phenylalanine. Proton-enhanced nuclear induction spectroscopy uses cross-polarization from the ^{1}H nuclei to the ^{13}C nuclei so that the recycle time of the experiment becomes dependent on the more favorable ^{1}H relaxation times. There are several additional advantages to using the ^{1}H nuclei as the initial source of magnetization: they have a large gyromagnetic ratio; they have nearly 100% natural isotopic abundance and most compounds have many more hydrogens than carbons or nitrogens; and all the ^{1}H nuclei are tightly coupled. Unfortunately, the most elegant feature of proton-enhanced nuclear induction spectroscopy—that the keg of ^{1}H magnetization can be tapped many times in a single experiment, allowing ten or more signals to be obtained for each recycle delay—is generally not practical in protein applications because of their relatively short rotating frame relaxation times and the undesirable effects of sample heating during the long periods of continuous radiofrequency irradiation.

After the ^{13}C (or ^{15}N) magnetization is generated by cross-polarization, an additional step is necessary to obtain high-resolution solid-state NMR spectra of proteins, and that is to either mechanically rotate an unoriented (powder) sample at 55° relative to the field (the magic angle)[5] or to align the protein molecules parallel to the field in a stationary sample[6]. Since the initial spectra were first obtained, advances have been made through the use of high-field magnets, improved instrumentation and computers, and a wealth of prescriptions for pulsed radiofrequency irradiations for multidimensional experiments. It is now feasible to determine the three-dimensional structures of both polycrystalline[7] and aligned proteins[8] at atomic resolution by solid-state NMR spectroscopy, albeit for small well-behaved examples with less than 100 residues.

Stanley J. Opella is in the Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, California 92093, USA.
e-mail: sopella@ucsd.edu

Many important protein systems have been difficult or impossible to tackle by solid-state NMR spectroscopy because of the low intrinsic sensitivity of the experiments. The nuclei are only weakly polarized by even the highest-field magnets that are available. The signal averaging required to obtain acceptable signal-to-noise ratios can be extremely time-consuming because the rate of repetition of the experiments is restricted by the long longitudinal (T_1) relaxation times of the nuclei and sample heating from radiofrequency irradiations. The chief limitation to applying solid-state NMR spectroscopy to larger and more interesting biological systems is sensitivity.

Thus, it is notable that Ishii and coworkers[1] present an approach to reducing the total experimental time by 5–20-fold by decreasing T_1, without affecting spectral quality (**Fig. 1**). They call this method paramagnetic-relaxation-assisted condensed data collection (PACC) for solid-state NMR spectroscopy analysis of proteins[1]. Their approach uses a combination of paramagnetic doping that reduces the T_1, very fast magic angle spinning and fast recycling of the experiments. The addition of the paramagnetic compound to the sample reduces the ^{1}H T_1 to 50–100 ms (compared to 1–4 s for the traditional solid-state NMR spectroscopy approach). Notably, the sample is not subjected to excessive heating because low-radiofrequency-power irradiations are effective when the sample spinning is fast enough to attenuate the bulk of the internuclear interactions responsible for line broadening.

The PACC method has the potential to have a major impact on our understanding of proteins, especially those resistant to conventional X-ray crystallography and solution NMR spectroscopy. For such proteins, solid-state NMR spectroscopy is the only method capable of providing atomic resolution structures by improving the feasibility of the requisite experimental measurements.

Sensitivity problems can be pernicious. Not only do they limit the number of measurements that can be made because access to high-field NMR spectrometers is always at a premium, but more importantly they place limits on the imagination of the investigators. Other efforts to improve sensitivity range from the use of higher magnetic fields, the construction of probes that reduce sample heating[9] and the implementation of dynamic nuclear polarization[10]. The value of the large sensitivity increases will be realized in the form of innovative new experiments and ambitious new applications.

COMPETING INTERESTS STATEMENT
The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturemethods/.

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