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Integrated BioNMR – "getting by with a little help from my friends"

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ABSTRACT

Single types of methodologies are insufficient to adequately describe complex biological structures. As a result, integrated approaches that combine complementary data are being developed. Here, I describe the benefits of integrating solution and magic angle spinning BioNMR approaches to characterize structure and dynamics of protein assemblies.

Not too long ago, NMR was primarily applied as an analytical method for characterizing novel compounds, whether *de novo* synthesized or isolated from natural sources. This changed on the heels of the molecular biology revolution. Spurred by the advent and ease of cloning DNA and by the creation of protein expression vectors, site-specific mutagenesis techniques, and robust protein purification strategies, NMR moved firmly and successfully into the biological arena. Proteins and nucleic acids were generated recombinantly and became the subject of NMR studies. Those of us lucky enough to have been part of the revolutionary changes of the late 60s and early 70s remember the year 1969 well, not only as the birth year of JMR.

The virtues of NMR cannot be overemphasized: NMR is non-destructive, quantitative and highly reproducible. However, it also has its shortcomings: it is relatively insensitive, compared to mass spectrometry, often requires several different experiments or samples, compared to crystallography, and ... well, just listen to the cynics. Yet, over the last 50 years, NMR has come a long way, and, as it stands today, NMR is still the only structural methodology that can provide spatial and dynamics information at the atomic level in liquids as well as in the solid state.

Notably, long before descriptive terms such as "multidisciplinary" or "integrative" were fashionable among scientists, biological NMR (BioNMR), from the very beginning, was, and remains, multidisciplinary in and by itself. From its origins in physics, via its rise to prominence in analytical chemistry, to its more recent uses for characterizing macromolecules with important cellular functions, practitioners of BioNMR have come from diverse backgrounds, crossing the boundaries of their original traditional disciplines. Such adventurous border crossings are necessary for innovation and evolution, since disciplinary barriers can be confining and constitute obstacles to addressing important and complex problems.

BioNMR has always been good at embracing supplemental and enhancing methods, and integration of complementary data has been a constant thread throughout its development, in part driven by methodological pragmatism but also by an inherent cumulative rise of technological advances. As a reminder, several examples of such technological integration by BioNMR practitioners can readily be cited: some of the first algorithms for 3D structure determination involved combining NMR-derived distance restraints with molecular dynamics (MD) simulations [1]; isotope labeling of recombinant materials enabled 3- and 4D heteronuclear NMR [2]; residual dipolar coupling (RDC)-derived orientational restraints borrowed the anisotropy from aligned samples [3], a common procedure in solid-state NMR; paramagnetic relaxation enhancements (PRE) [4] make use of tags first employed in electron paramagnetic resonance (EPR) spectroscopy; and integration of sequence and structure information in databases with carbon chemical shifts of proteins resulted in robust secondary structure predictions [5]. Furthermore, combinations of NMR data with data obtained by other structural techniques is becoming more and more widespread, such as seen in joint refinement of protein structures based on NMR and X-ray data [6], and in characterization of multidomain proteins and protein complexes, in solution by integration of small angle X-ray scattering (SAXS) and NMR data [7]

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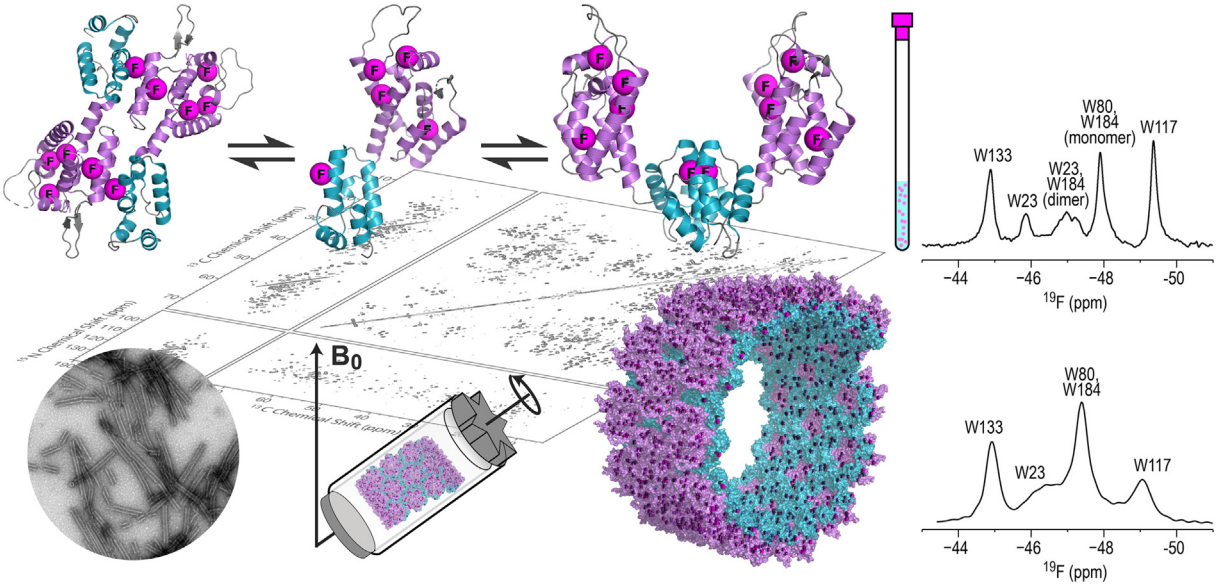

Fig. 1. Schematic illustration of the combined use of solution and MAS NMR for HIV-1 CA protein studies. Top, equilibrium between monomeric and dimeric CA protein species in solution and the solution ^{19}F spectrum of 5F-Trp CA. Bottom, tubular assemblies of CA and ^{19}F MAS spectrum of the helical assembly. The structure of the CA polypeptide chain is depicted in ribbon representation with N- and C-terminal domains colored magenta and cyan, respectively. The positions of the five Trp residues are shown as purple balls. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

or in the solid state by integration of cryo-electron microscopy (cryoEM) and magic-angle spinning (MAS) NMR data [8].

This report focusses on the complementarity of solution and MAS solid state NMR methods and illustrates how integration of data from these sister methodologies allows big and complex systems to be tackled and to obtain answers to questions that would be nearly impossible to achieve using either technique alone. I will use the HIV-1 capsid as an example (see Fig. 1), since over the last decade members of the Pittsburgh Center for HIV Protein Interactions (PCHPI) have studied this system by integrating a large number of techniques. The HIV-1 capsid is the protein shell which encloses the HIV genome in the virion. Conical capsids are pleomorphic and exhibit variable shapes. They contain large and varying numbers of a single protein, the CA protein. CA is a helical, two domain protein, and the linker that connects the N-and C-terminal domains (NTD and CTD) is associated with the observed varied capsid morphologies. Solution NMR permitted structure determination of the dimeric CTD [9], which together with the crystal structure of the NTD [10] and with cryoEM data allowed the generation of a pseudo-atomic model for a tubular in-vitro assembly and a native conical capsid structure [11]. In addition, combining a 5 Å resolution cryoEM density map of a tubular HIV-1 capsid assembly with MAS backbone chemical shift data from the same assembly was used for joint structure refinement by MD [12].

Naturally, obtaining such structures that contain >10^7 atoms was a feat, but what did we learn about the biology? For this, we needed to turn our attention to the dynamic behavior of the capsid and the influence of dynamics on the interactions between capsid and important cellular modulators of infectivity. Cyclophilin A (CypA) is such a crucial species-specific cofactor for HIV-1. Cellular CypA interacts with a surface-exposed loop on the capsid, and binding is necessary for full viral infectivity. Intriguingly, several CA mutants have been described that lead to CypA independence, such as A92E and G94D. The locations of these amino acid changes map to CA's CypA binding loop, and mutant viruses, in contrast to wild-type, are fully infectious when the CA-CypA interaction is inhibited by cyclosporin. In solution, the CypA binding loop of wild-type CA-NTD is flexible and exhibits conformational heterogeneity, with both the trans- (86%) and cis-Pro90 (14%) conformations observed by solution NMR, while in the assembled state only the trans conformation is detected by MAS NMR. All surrounding loop residues exhibit motions on the micro- to nanosecond timescales. In the MAS spectra, the NH resonances of G89 and A92 displayed essentially isotropic line shapes, consistent with extensive and broad conformational sampling of the bond vectors, demonstrating that these loop residues are extremely mobile, contrasting the results for the CA/CypA complex, in which the motions are significantly reduced. This, not surprisingly, implies that the interaction rigidifies the loop. Most unexpectedly, however, when the CypA escape mutants of CA were investigated, their dynamics profiles were markedly different and resembled that of the CA/CypA complex, rather than wild-type CA assemblies [13]. Therefore, it is not a structural change that causes the altered phenotype of these mutants, but a change in the motional signatures of the residues in the CypA-binding loop. Indeed, this dynamics change mimics the effect of CypA binding, and it is motional rather than structural mimicry which lies at the root of escape from CypA-dependence. Such a conclusion was only possible after integration of solution and MAS NMR findings.

Where are we next going with this joint venture between solution and MAS NMR? While most experiments in BioNMR are performed using ^{1}H, ^{13}C, and ^{15}N nuclei, I would like to refocus the attention of the community to the 100%-abundant, magnetically active ^{19}F isotope (see Fig. 1). As an NMR probe, ^{19}F is uniquely attractive, possessing the third highest gyromagnetic ratio after tritium and hydrogen and, hence, very high sensitivity. Further, it can be readily incorporated into biomolecules [14], and, given that fluorine chemical shifts are exquisitely responsive to changes in the local environment, it should be easy to take advantage of its tremendous chemical shift range. For liquids, a widespread notion is that high-field ^{19}F NMR spectroscopy is impractical, because of the large ^{19}F chemical shift anisotropy, and a specific challenge to ^{19}F solid-state NMR is posed by very broad lines. However, challenges ask for solutions to be devised, and novel and exciting approaches using fluorine NMR are likely to emerge. Indeed, through the integration of solution and solid-state ^{19}F NMR, a range of systems, inaccessible if each individual method is used in isolation, will become amenable to analysis. I anticipate that

judiciously placing a small number of ^{19}F probes onto proteins, novel solution and MAS NMR experiments can be devised for cleanly mapping long-range distances and interaction sites in proteins, such as those accessible via ^{19}F paramagnetic relaxation enhancements (PRE). While we have demonstrated the initial proof-of-principle for using ^{19}F PRE experiments in solution [15], equivalent experiments in solid-state NMR are currently not available. However, it should be possible to develop ^{19}F-based homonuclear MAS NMR correlation experiments for long-range distance measurements in large proteins and multiprotein complexes, and initial steps along those lines have been made [16]. Furthermore, given that fluorine is virtually absent from all biological materials, fluorine NMR may be the method of choice to propel in-cell NMR into the next powerful in-situ technology.

Let me finish on a philosophical note. While it would be foolish to predict where NMR will stand in 50 years, addressing important scientific questions undoubtedly will benefit from an integrative transdisciplinary approach. In complex biological phenomena, numerous features are involved in causal behavior, often at multiple scales or levels of organization. What is relegated to the undescribed context in some models (as derived by NMR, for example) is explicitly represented in other models (as derived by crystallography or cryoEM, for example), and many compatible models are necessary to reach an accurate description of the investigated object or phenomenon. Inherent to the plurality of methods and models and the partiality of representation obtained by any one methodological approach alone, integration of multiple empirically adequate models is a necessity. In this sense, BioNMR can play a special role in catalyzing the merging of methods and models, aimed at providing a more complete picture of a biological object.

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