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EDITORIAL

A topical issue: production and labeling of biological macromolecules for NMR investigations

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Producing samples of biological macromolecules in a form that is suitable for structural and functional studies by NMR is still a major obstacle. In the past, many advances in biomolecular NMR were possible due to new methods for sample production, most prominently recombinant protein expression, ^{15}N and ^{13}C labeling, deuteration, or methyl labeling. New ways of producing labeled proteins and nucleotides have indeed stimulated the development of novel experiments. Luckily, there is still much progress being made, which promises the development of new technologies that will expand the scope of NMR with biological macromolecules.

The manuscripts assembled in this special issue cover several crucial aspects of producing labeled samples or using specially labeled samples for extending the capabilities of biomolecular NMR. The first four articles are primarily concerned with issues of expressing proteins. Most samples used for NMR are still produced in *E. coli*, which has limitations for proteins that have posttranslational modifications. Thus, the perspective by Takahashi and Shimada (2010) describes the current art of producing proteins in non-*E. coli* prokaryotic and eukaryotic cells. This is still a challenging topic, and procedures have to be developed case by case. Hopefully new methods will be forthcoming that will be accessible to the broad biological NMR community. Currently, *E. coli* expression is still the dominant approach and is almost exclusively used in high-throughput efforts. The quality of the spectra depends crucially on finding good sample conditions. Montelione and coworkers describe high-throughput methods for screening optimal solution conditions of *E. coli* expressed proteins as used in the Northeast Structural Genomics Consortium (Rossi et al. 2010).

Unfortunately, many proteins are marginally soluble and rather unstable since they may have to be present in cells only at very low concentrations and are designed to have limited life times. Thus, optimizing solution conditions may never lead to samples suitable for NMR structure determination. Thus, other approaches have been developed to cope with this problem. One approach to achieve this is described by Zhou and Wagner (2010) who summarize efforts to enhance protein solubility and stability by attaching solubility enhancement tags. This approach has enabled NMR studies on many difficult proteins and is constantly expanded by various laboratories.

As an alternative to expression in life bacteria, cell free expression is an important new development for protein production. It can overcome problems of product toxicity and enables new labeling procedures. Dötsch and coworkers describe progress in cell-free expression of membrane proteins using *E. coli* extracts (Sobhanifar et al. 2010).

A second group of three articles is concerned with specific labeling issues. A prominent example is the stereo-array isotope labeling (SAIL) method. Kainosho and coworkers describe the use of SAIL for simplifying the spectral signatures and NOESY connectivities of tyrosines and phenylalanines (Takeda et al. 2010). These residues are typically found in the interior of proteins, sample a large fraction of the core NOEs but typically their resonances are heavily overlapped and difficult to resolve in conventionally labeled samples. The authors demonstrate the simplification of spectra achievable with SAIL labeling and provide an overview of the extent of NOEs observable

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from alternate labeling schemes. Another approach to simplify protein spectra is by segmental labeling. Allain and coworkers describe recent progress with this approach (Skrisovska et al. 2010). They discuss developments and current state of the art of native chemical ligation, trans-splicing using inteins and ligation by enzymes, such as subtiligase and sortase. They also provide an overview of strategies for labeling carbohydrate moieties in glycoproteins and describe successful applications in their own work.

Optimal isotope labeling of proteins has been crucial for recent successes in solid state NMR of proteins. In the past, however, solid state NMR of proteins has primarily relied on detecting nuclei other than hydrogen avoiding problems of severe line broadening due to the strong dipole interactions. Oschkinat and coworkers have shown recently that detection of exchangeable protons is feasible in solid state NMR of perdeuterated proteins. Here they analyze the optimum deuteration levels and report the results on small proteins (Akbey et al. 2010).

A next group of articles is about the use of specialized labels. Ruschak and Kay (2010) describe methods for labeling methyl groups and discuss applications for investigation large molecular machines with NMR. They provide an overview of what questions can be addressed with this approach and what might be expected in the future. The NMR spectroscopists' wish list could easily be extended to more exotic probes that could facilitate structural or functional studies. Unnatural amino acids can be introduced using an orthogonal tRNA/aminoacyl-tRNA synthetase pair. Geierstanger and coworkers describe methods for incorporation of $^{15}N/^{13}C$-labeled, fluorinated or photocaged and UV-activatable unnatural amino acids. This also includes incorporation of amino-acid derivatives that can be activated to bind paramagnetic ions (Jones et al. 2010). The use of paramagnetic labels is increasingly being recognized as source of structural information. Applications have gone well beyond the initial spin label approaches, such as attachment of special tags that bind paramagnetic ions. The article by Su and Otting (2010) describes currently available approaches and applications for their use in structural biology, both for proteins and nucleic acids.

Isotope labeling has transformed the field of nucleic acid NMR as well. Summers and coworkers describe the current state of the art for labeling RNA (Lu et al. 2010). This includes procedures for RNA synthesis, isotope labeling and segmental labeling. In particular, it is shown that advanced deuteration schemes can enable high-resolution NMR studies of RNAs beyond 700 nucleotides, such as the complete 5'UTR of HIV-1.

This thematic issue of the journal provides samples of recent advances in production and labeling methods for proteins and nucleic acids. It is incomplete, and sophisticated new expression, optimization and labeling procedures will certainly emerge in the future. As the past has shown new developments in this field will undoubtedly lead to significant and unexpected enhancements of NMR spectroscopy with biological macromolecules, yield new biological insights and advance applications to improve human health.

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