Efficient resonance assignment and global fold determination of backbone labelled proteins

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Introduction

As the result of a massive effort, several genomes have been sequenced and soon the sequence of all human genes will be known. In most cases it will be impossible to devise the structure of newly discovered proteins from their sequence alone. However, the number of experimentally determined structures is small compared to the number of known protein sequences. This is due to the fact that determining a structure by X-ray crystallography or NMR spectroscopy is a long and tedious process. A rapid determination of the protein fold, even at low resolution, will be useful for characterizing the structure of proteins and finally their function.

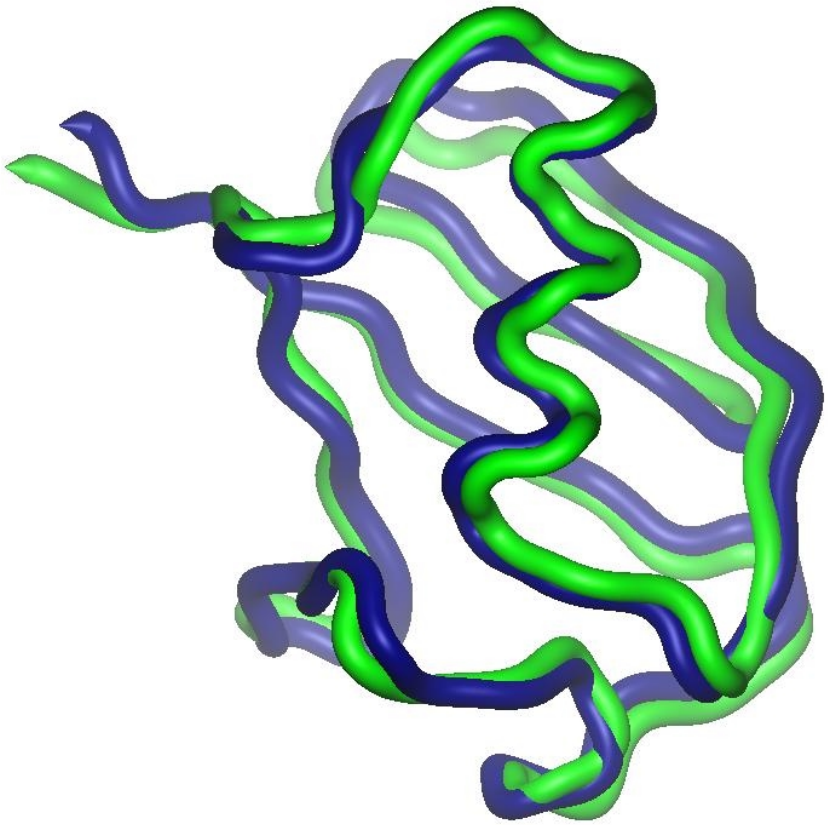
Multidimensional NMR

With the development of multidimensional triple resonance NMR methods, the structure of proteins of significant size and spectral complexity can now be determined. A fundamental limitation on the size of proteins whose resonances can be assigned by triple resonance methods that transfer magnetisation through alpha-carbon nuclei (e.g. HNCA, HN(CO)CA) is the rapid transverse relaxation of these nuclei. The relaxation time of these carbons can be increased by substitution of deuterons for protons. A second problem is the homonuclear C-alpha-C-beta coupling. For fully ^{13}C-labelled proteins one works either with limited resolution in the ^{13}C dimension to prevent the resolution of this coupling, or a constant time (CT) period is applied during ^{13}C frequency labelling. The first approach frequently does not allow unambiguous identification of the connectivities, whereas a CT experiment increases the time period during which transverse C-alpha magnetisation is present to approximately 27 msec. This means that even for deuterated proteins a significant loss of magnetisation will occur. In principle, selective decoupling of C-beta overcomes this limitations. However this approach introduces other undesirable complications. Firstly, it is in general impossible to selectively decouple serine C-beta atoms, and secondly, the composite pulse train introduces an undesirable Bloch-Siegert shift on C-alpha resonances.

New isotopic enrichment strategies

An alternative approach, which does not suffer from these disadvantages, involves a protein that is isotopically enriched exclusively in the backbone N, C-alpha and C-carbonyl atoms and deuterated in the C-alpha position. This permits optimal resolution and sensitivity to be obtained in triple resonance experiments, since the undesirable C-alpha-C-beta scalar coupling is absent. We synthesised backbone ^{13}C, ^{15}N, ^{2}H(50%)-labelled amino acids by chemical means starting from ^{13}C, ^{15}N-labelled glycine. A 32 kDa protein was then obtained from these selectively labelled amino acids. This protein sample allows the acquisition of high resolution NMR spectra which facilitate the resonance assignment process.

Recently devised NMR methods of measuring residual dipolar couplings in liquid crystalline media provide a new rapid route to the determination of protein fold. For proteins that are isotopically enriched exclusively in their backbone nuclei, residual dipolar couplings can be measured effectively with HSQC-based and HNCA-based techniques. Optimal sensitivity is maintained due to the absence of the constant-time building blocks that are required for fully labelled proteins. The structural information available from backbone labelled proteins allows the determination of the global fold.



Global fold of ubiquitin determined by i) X-Ray crystallography (blue) and ii) backbone labelling NMR strategy (green). Giesen *et al*., submitted.

Collaborators

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References

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