TROSY in NMR studies of the structure and function of large biological macromolecules

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Transverse relaxation-optimized spectroscopy (TROSY), in combination with various isotope-labeling techniques, has opened avenues to study biomolecules with molecular masses of up to 1 000 000 Da by solution NMR. Important recent applications of TROSY include the structure determination of membrane proteins in detergent micelles, structural and functional studies of large proteins in both monomeric form and macromolecular complexes, and investigations of intermolecular interactions in large complexes. TROSY improves the measurement of residual dipolar couplings and the detection of scalar couplings across hydrogen bonds — techniques that promise to further enhance the determination of solution structures of large proteins and oligonucleotides.

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Abbreviations

CRINEPT	cross-correlated relaxation-enhanced polarization transfer
CSA	chemical shift anisotropy
DD	dipole–dipole
DHPC	dihexanoylphosphatidylcholine
DPC	dodecylphosphocholine
NOE	nuclear Overhauser effect
NOESY	nuclear Overhauser enhancement spectroscopy
TROSY	transverse relaxation-optimized spectroscopy

Introduction

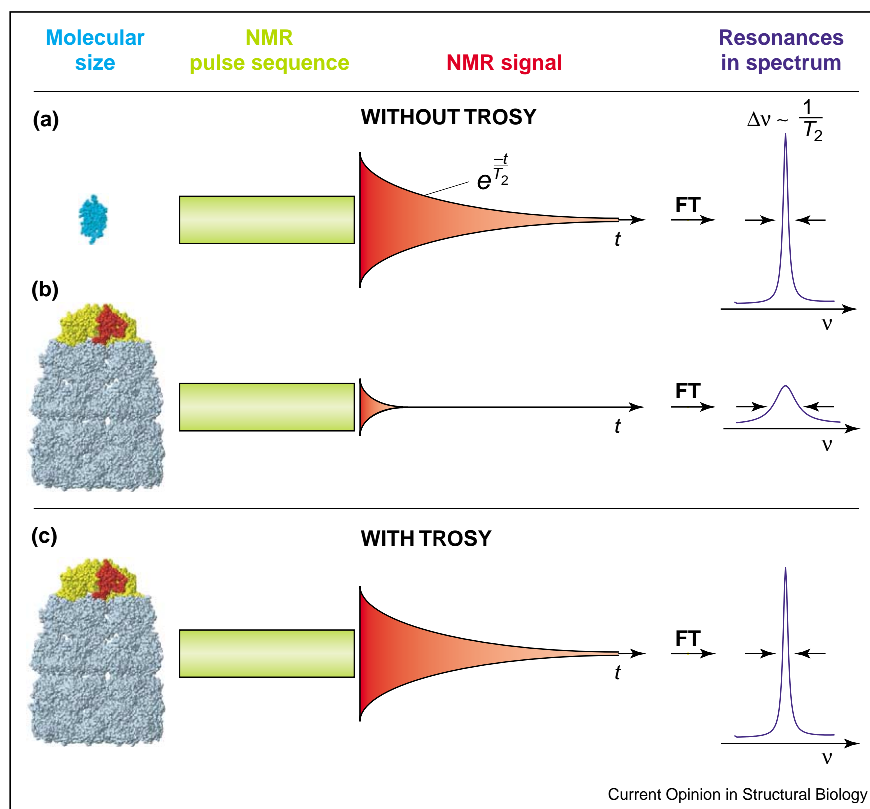
During the past few years, considerable effort has been devoted to extending the applications of NMR spectroscopy in solution to larger molecular systems, for which an alternative technique to X-ray crystallography is highly desirable to obtain structural and dynamic information at atomic resolution. Moreover, many biologically important macromolecules and macromolecular complexes have molecular masses beyond the practical range amenable to traditional NMR spectroscopy in solution. Increasing this size limit allows, for example, structure determinations of proteins that are difficult to crystallize (such as integral membrane proteins), investigations of intermolecular interactions involving large molecules and supramolecular assemblies, and structure determinations of larger oligonucleotides and their complexes with proteins.

When studying large molecules and macromolecular assemblies in solution by conventional NMR methods, two main problems usually arise. First, the large number of resonances causes signal overlap, which can make analysis of the spectra very difficult. Second, NMR signals of larger molecules relax faster, which leads first to line broadening and poor spectral sensitivity, and eventually to no NMR signals at all (Figure 1a,b). The problems that limit NMR studies of larger molecules are directly reflected in the scarcity of NMR structures with molecular masses greater than 25 kDa that have been determined so far. Whereas, in principle, the overlap of signals in the NMR spectra can be overcome by reducing the number of resonance lines by a proper choice of isotope-labeling schemes [1–4], the limitation caused by transverse relaxation poses a more severe technical challenge.

Major sources of relaxation are the omnipresent hydrogen atoms. Their replacement by deuterons [1] substantially reduces transverse relaxation, resulting in increased resolution and significant sensitivity gains. At the same time, however, protons contribute considerably to the structural information and produce the most sensitive NMR signal; thus, measuring totally deuterated proteins is not an option. As a compromise, C–H moieties in macromolecules are often deuterated only to a certain level, such as 70%, and protein samples with either partially or completely deuterated C–H groups are measured in H_2O solution, in which each amino acid residue is protonated at the backbone amide position. Under these measurement conditions, numerous techniques can be applied to obtain complete sequential resonance assignments, and to collect valuable structural and functional information.

Deuteration alone cannot extend the application of solution NMR above the size limit of 50 kDa. Only the introduction of transverse relaxation-optimized spectroscopy (TROSY) [5] reduces relaxation to such an extent that satisfactory line widths and sensitivity can be achieved in NMR experiments with very large molecules. TROSY works best with deuterated proteins and is especially suited for applications to protonated amide groups. TROSY uses spectroscopic means to reduce transverse relaxation (Figures 1b,c and 2) and has greatly extended the size limit of macromolecules that can be studied by

Figure 1



NMR spectroscopy with small and large molecules in solution. **(a)** The NMR signal obtained from small molecules in solution relaxes slowly; it has a long transverse relaxation time (T_2). A large T_2 value translates into narrow line widths ($\Delta\nu$) in the NMR spectrum after Fourier transformation (FT) of the NMR signal. **(b)** By contrast, for larger molecules, the decay of the NMR signal is faster (T_2 is smaller). This results both in a weaker signal measured after the NMR pulse sequence and in broad lines in the spectra. **(c)** Using TROSY, the transverse relaxation can be substantially reduced, which results in improved spectral resolution and improved sensitivity for large molecules.

solution NMR, making possible studies of molecular systems with masses of up to 1 000 000 Da [6••,7].

The introduction of TROSY has made possible a wide range of new applications of solution NMR, in particular in the emerging field of structural and functional genomics. In this review, we discuss important applications of TROSY in structural and functional studies of large biological macromolecules.

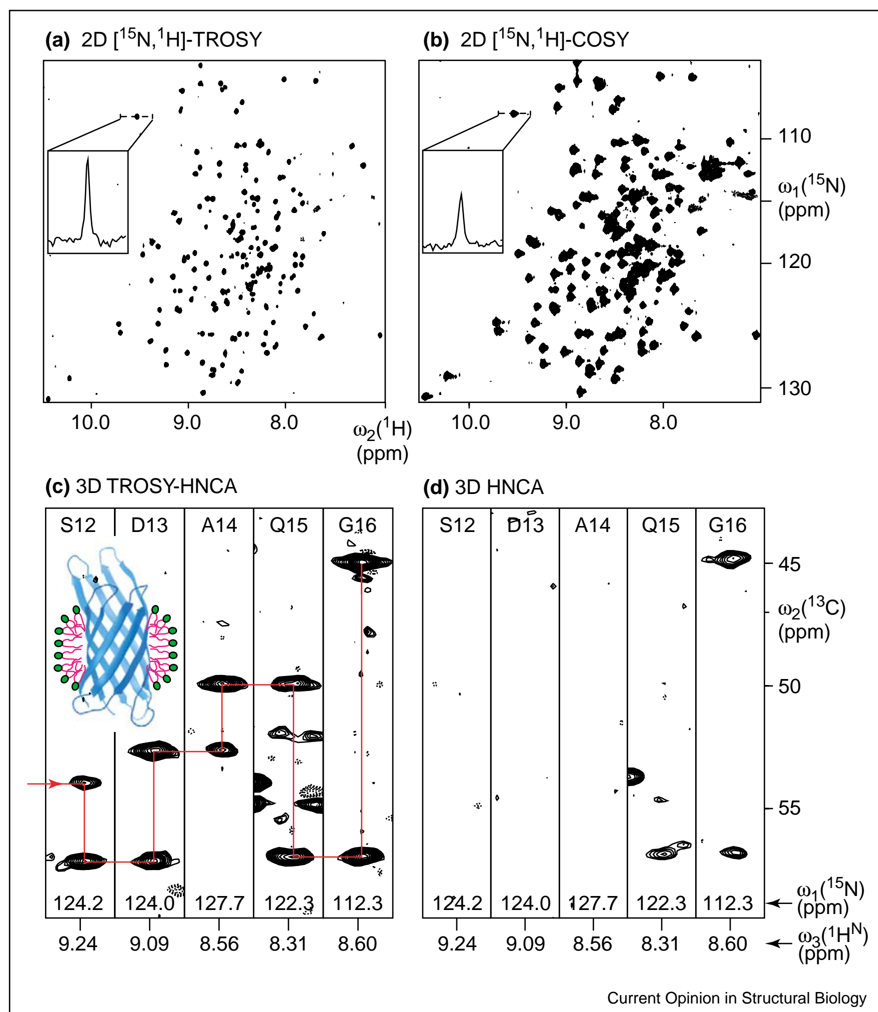
Technical background

Transverse relaxation-optimized spectroscopy

NMR measures the signal of nuclear spins in a large homogenous magnetic field. The signal is the response of the spins to an applied sequence of radio-frequency pulses separated by interpulse time periods — the 'NMR pulse sequence' (Figure 1). The measured signal is the sum of radio-frequencies that have been emitted by the nuclei. The signal decays exponentially with a characteristic time constant — the 'transverse relaxation time', T_2 (Figure 1a). For the analysis, the signal is Fourier transformed into a spectrum containing resonance lines that represent the various emitted radio-frequencies. The width of the resonance lines in the spectrum is inversely proportional to T_2 (Figure 1a), which depends on the size of the molecule: the larger the molecular mass, the shorter T_2 becomes and the broader are the lines in the spectrum (Figure 1b). Relaxation is active not only during data acquisition but also during the pulse sequence, which results in a much weaker and more rapidly decaying NMR signal for large molecules (Figure 1b). With TROSY [5] applied to large molecules, the effective relaxation of the measured signal during the pulse sequence and during data acquisition can be reduced (Figure 1c), enabling the measurement of high-quality spectra for these systems.

To apply the TROSY technique, at least two different interfering relaxation mechanisms must contribute to relaxation. The interference between two relaxation mechanisms can be additive or subtractive; in the latter case, the effective relaxation is reduced. One important example is the amide moiety in a polypeptide chain containing ^{15}N instead of the natural isotope ^{14}N.

Figure 2



Impact of TROSY on NMR spectra. The spectra were measured with a sample of the $^{2}H,^{13}C,^{15}N$-labeled integral membrane protein OmpX in DHPC micelles, a 60 kDa complex that is shown schematically in the inset in (c). **(a,b)** $[^{15}N,^{1}H]$-correlation spectra identically recorded and processed, except that TROSY was used in (a) only. The insets show cross-sections that were taken parallel to the $\omega_2(^{1}H)$ axis at the position indicated by the horizontal broken lines. **(c)** Strips along the ^{13}C dimension from a 3D $[^{15}N,^{1}H]$-TROSY-HNCA spectrum. **(d)** Same spectral region as in (c), but extracted from a conventional 3D HNCA spectrum. The strips were taken at the ^{15}N chemical shifts (indicated at the bottom of the strips) of amino acid residues 12–16, and are centered on the corresponding amide proton chemical shifts, $\omega_3(^{1}H^{N})$. In (c), horizontal and vertical red lines demonstrate the connectivities that can be obtained from such a spectrum. With these connectivities, the complete resonance assignment of backbone $^{1}H^{N}$, ^{15}N and $^{13}C^{\alpha}$ nuclei could be achieved for this protein. All spectra were recorded at a ^{1}H resonance frequency of 750 MHz.

Because ^{1}H nuclei couple to ^{15}N nuclei (scalar coupling), the ^{1}H NMR spectrum of such an amide moiety consists of two lines representing protons attached to ^{15}N nuclei with spin up and protons attached to ^{15}N nuclei with spin down relative to the externally applied magnetic field. In the spectrum of a large protein, the two lines have different line widths, which directly demonstrates the relaxation interference. In conventional NMR experiments, the two lines are collapsed by a technique called 'decoupling', but at the cost of averaging the relaxation rates. For smaller molecules, this is not a problem, but for large molecules the signal may be very much attenuated because of the contribution of the more rapidly relaxing resonance line. The TROSY technique exclusively selects the slowly relaxing resonance line, eliminating the faster relaxing resonance. Thus, TROSY disregards half of the potential signal; in large molecules, however, this is more than compensated for by the slower relaxation during the pulse sequence and the acquisition. Generally, a superior sensitivity is readily achieved with TROSY when working with molecular masses greater than 15–20 kDa at magnetic field strengths corresponding to a proton resonance frequency of at least 700 MHz.

The two interfering relaxation mechanisms in the case of the amide proton are dipole–dipole (DD) relaxation

between the proton and nitrogen spins, and the chemical shift anisotropy (CSA) of the protons. The DD interaction is independent of the static magnetic field, whereas the CSA increases with larger magnetic fields. The optimal TROSY effect can thus be obtained by choosing the appropriate field strength, which, for the amide proton, is about 23.5 T, corresponding to a proton resonance frequency of 1000 MHz. The ^{15}N nuclei in an amide moiety also show interference between DD relaxation and their CSA. Interestingly, this TROSY effect has an optimum at about the same magnetic field strength. In experiments with ^{1}H and ^{15}N nuclei, the line with the slower relaxation rate for both nuclei is selected in a relaxation-optimized experiment.

TROSY is not limited to amide moieties in biological macromolecules; some important applications use C–H groups in aromatic rings [8]. Because N–H groups are strategically located in the polypeptide backbone of proteins and in the bases of nucleotides of DNA and RNA molecules, they are prime targets in the optimization of many NMR experiments with biological macromolecules. The use of TROSY with amide groups in triple-resonance experiments (e.g. [9–13]) allows the measurement of high-quality NMR spectra of $^{2}H,^{13}C,^{15}N$-labeled proteins in molecular systems with a mass well above the size limit of conventional NMR techniques that do not use TROSY. Below, applications of the TROSY technique are discussed, but the technical details of the experiments are beyond the scope of this review and have been considered elsewhere [14,15•,16,17].

Figure 2 illustrates the improvements in spectral quality that can be obtained with TROSY for a molecular complex of about 60 kDa. Figure 2a,b shows correlation spectra between the ^{15}N and ^{1}H nuclei in the amide moieties of the polypeptide backbone. Here, the 2D [$^{15}N,^{1}H$]-TROSY spectrum (Figure 2a) has narrower line widths and higher signal intensities than the corresponding correlation spectrum in Figure 2b, which was measured in a conventional NMR experiment. Figure 2c,d shows a comparison between a 3D TROSY-HNCA spectrum and a conventional 3D HNCA spectrum for the same molecular complex used in Figure 2a,b. The longer pulse sequence with longer time periods that could be optimized with TROSY resulted in a dramatic difference in signal intensities favoring the TROSY-type spectrum. The 3D HNCA spectrum forms the basis for complete assignment of the backbone atoms in a polypeptide chain, because it correlates the ^{1}H and ^{15}N nuclei of an amide moiety with both the intraresidue and the preceding α-carbon nuclei.

Isotope labeling

The full potential of TROSY, manifested in optimal resolution and sensitivity of NMR spectra, is best exploited in combination with deuterium labeling [5]. Typically, larger biomolecules are perdeuterated, or at least deuterated to about 70%, and subsequently dissolved in H_2O solutions to replace deuterons with protons in exchangeable sites. In this way, the strategically important amide groups in the polypeptide backbone of proteins are protonated, a step that is crucial for NMR experiments that yield sequential backbone assignments. The deuteration level and labeling scheme can also be tailored to the system studied; for example, methyl groups can be selectively protonated in an otherwise perdeuterated protein. With such a sample, efficient backbone assignments can be obtained and methyl groups can be sequentially assigned, providing important nuclear Overhauser effect (NOE) data for structure determination [1,18,19•].

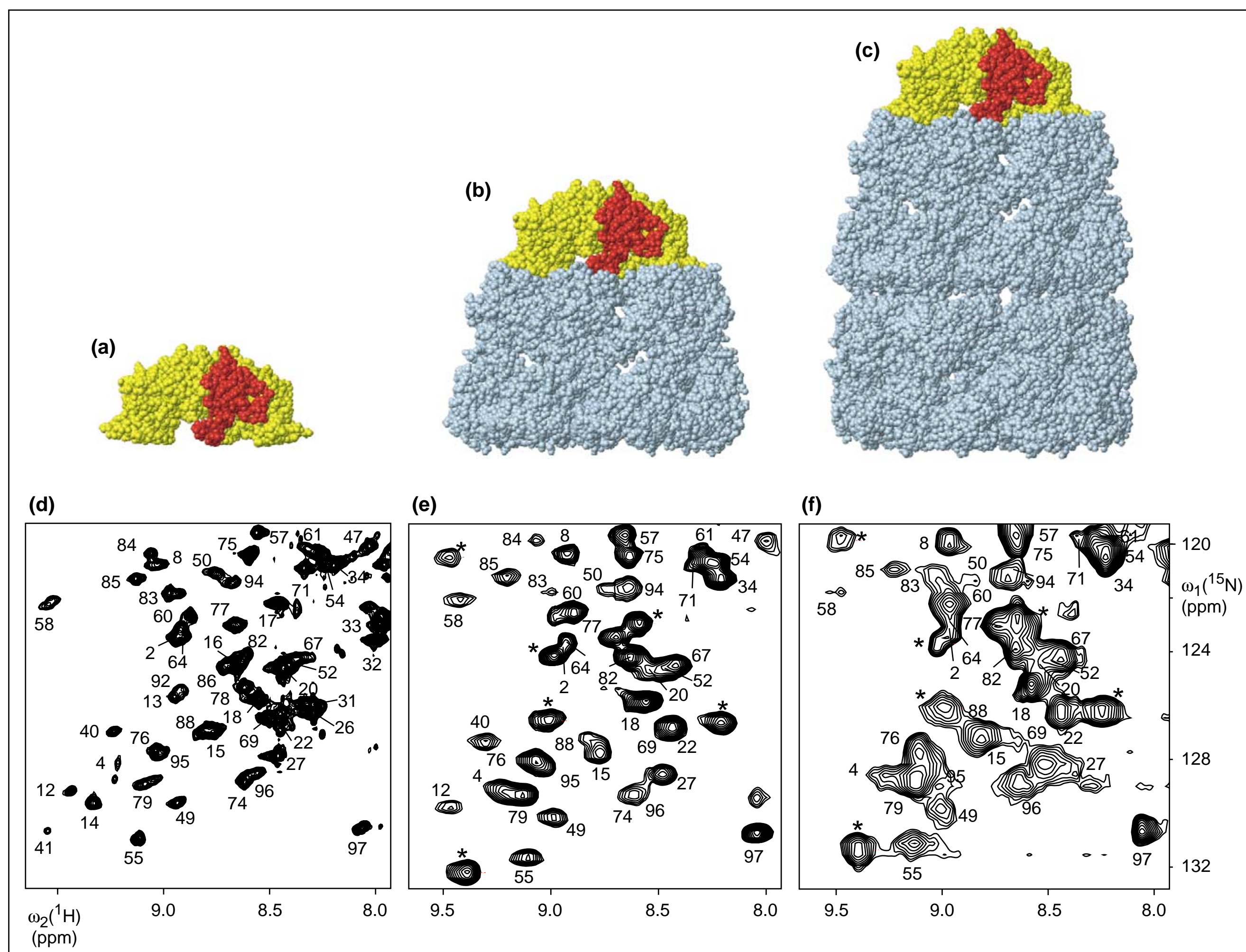
With the increasing size of molecules studied, the number of resonances in the NMR spectra increases and more resonances overlap, complicating the analysis necessary to individually identify all of the resonances. There are, however, systems that do not suffer from this limitation despite their large molecular mass. Homo-oligomeric proteins have identical NMR spectra for all subunits and thus the number of amino acid residues per subunit determines the total number of resonances. Further examples are membrane proteins solubilized in detergent micelles; whereas the detergent molecules contribute considerably to the molecular mass, they usually do not contribute resonances to the spectra of interest. Of course, large monomeric proteins are very interesting targets for structural investigations and for studies of intermolecular interactions or small-ligand binding. In these cases, resonance overlap can be reduced by selective amino acid labeling [1,20••] or by segmental isotope labeling techniques [2–4], in which only a segment of the complete polypeptide chain is labeled with isotopes. Future improvements in the technology for expressing and labeling proteins, in particular *in vitro* expression systems [21–23], should enable site-specific labeling, which will further facilitate analyses of the complex spectra inherent to large biomolecules.

NMR studies of larger biomolecules

Resonance assignments of large proteins

The foundation for extracting information at atomic resolution from NMR data is the resonance assignment, which attributes distinct resonance frequencies to individual nuclei in the biological macromolecule. Using TROSY techniques, resonance assignments can be obtained for proteins with molecular masses well above the size limit of conventional NMR techniques. This was first demonstrated for a homo-octameric protein of 110 kDa. With this protein, 20–50-fold gains in sensitivity were observed when using TROSY-type experiments compared with the corresponding conventional experiments, and the backbone assignment and secondary structure were obtained [24••].

Figure 3



TROSY-based NMR analysis of molecular complexes with masses of up to 900 kDa. (a–c) All-atom representations of the structures of the molecules studied. **(a)** GroES (yellow and red), a 72 kDa homoheptameric protein (one subunit is shown in red). **(b)** GroES in a complex of 470 kDa with unlabeled SR1 (light blue). SR1 is a single-ring variant of GroEL. **(c)** GroES in a complex of ~900 kDa with unlabeled GroEL (light blue). (d–f) 2D [^{15}N,^{1}H]-correlation spectra of uniformly ^{2}H,^{15}N-labeled GroES in the macromolecular complexes shown in (a–c). **(d)** 2D [^{15}N,^{1}H]-TROSY spectrum of free GroES. **(e)** 2D [^{15}N,^{1}H]-CRIPT-TROSY spectrum of GroES bound to SR1 in the presence of ADP. **(f)** 2D [^{15}N,^{1}H]-CRIPT-TROSY spectrum of GroES bound to GroEL. In (e,f), the peaks that shifted significantly upon binding to SR1 or GroEL are marked with an asterisk. The numbers in (d–f) indicate the individual assignments of the resonances. Adapted with permission from [6••].

To date, sequence-specific backbone resonance assignments based on TROSY techniques have been described for numerous proteins. Important examples of the successful application of TROSY to larger molecules include the determination of the chemical shift assignments of the 723-residue monomeric protein malate synthase in 4D TROSY-based triple-resonance experiments [20••], the 91 kDa 11-meric TRAP protein [25] and the 67 kDa dimeric form of the tumor suppression protein p53 [26]. Very recently, it has been demonstrated that it is possible to investigate macromolecular systems with a mass of up to 900 kDa by NMR spectroscopy in solution (Figure 3) [6••,7]. A complex formed by the 72 kDa protein GroES and 800 kDa GroEL was studied by NMR experiments based on TROSY and cross-correlated relaxation-enhanced polarization transfer (CRINEPT) (Figure 3). Like TROSY, CRINEPT makes use of interference effects between different relaxation mechanisms and can be used to supplement TROSY to increase sensitivity in NMR spectra of extremely large molecules [7,27].

In addition to resonance assignments of nuclei in the polypeptide backbone, applications of TROSY for side-chain resonance assignments have been reported. The potential of TROSY has been demonstrated in several NMR experiments with aromatic spin systems in uniformly ^{13}C-labeled proteins, which has enabled improvements in sensitivity of up to one order of magnitude

(reviewed in [15•]). Recently, TROSY-type experiments have been described that allow the assignment of methyl protons and carbons in selectively methyl-protonated and otherwise deuterated proteins. In a comparison with the corresponding conventional NMR schemes, these experiments yielded gains in sensitivity of up to a factor of 2.6 for the membrane protein OmpX in 60 kDa dihexanoylphosphatidylcholine (DHPC) micelles in H_2O solution [19•].

Studies of dynamic processes

Studies of the dynamics of macromolecules by NMR spectroscopy often require considerable measuring time. When working with large proteins, it is therefore highly desirable to incorporate TROSY in the experimental schemes. Key experiments for dynamic studies measure the T_1 and T_2 relaxation times, and the heteronuclear $^{15}N\{^1H\}$ NOEs of the ^{15}N nuclei in amide groups; protocols to measure these parameters by TROSY have been developed [28]. Recently, this type of experiment has been extended to 3D based on a 3D TROSY-HNCO sequence, which can be applied to large molecules that show extensive signal overlap in the 2D [^{15}N,^1H]-TROSY spectrum [29].

Nuclear Overhauser enhancement spectroscopy

Nuclear Overhauser enhancement spectroscopy (NOESY) experiments play a crucial role in NMR studies of macromolecules. Not only are they used to obtain the essential interproton distances for structure determinations, but they can also support and complement the sequential assignment procedure. Moreover, NOESY can be used to detect intermolecular interactions. Several applications of TROSY to NOESY have been reported (reviewed in [15•]). An interesting application of TROSY was presented with the 3D NOESY-[^1H,^{15}N,^1H]-zero quantum-TROSY experiment [30]. A special feature and a great advantage of this experiment lies in the fact that the usually very intense diagonal peaks either are completely suppressed or have small residual negative intensity. In such spectra, the resonances close to the diagonal become amenable for NMR analysis, thereby removing a limitation of conventional NOESY. The utility of this approach has been demonstrated for the 110 kDa protein aldolase [30].

Practical applications of TROSY

Structures of membrane proteins

Membrane proteins constitute a great challenge for structural biologists. One of the major problems for NMR is that such proteins must be solubilized in aqueous solutions by incorporation into a model membrane system, resulting in protein–lipid–detergent supramolecular assemblies that are often too large for conventional NMR studies in solution. Although real membrane systems with lipid bilayers are still too large for liquid-state NMR, model systems in the form of micelles become accessible using TROSY. Membrane proteins in detergent–lipid micelles are interesting targets for TROSY applications, because they yield fewer NMR resonances and thus less signal overlap than a globular protein of the corresponding molecular mass. Even though the detergent molecules may represent a large fraction of the large overall mass of the mixed micelles, proper isotope labeling ensures that protein NMR signals can be detected with little or no interference from the signals of the detergent molecules. To illustrate this, Figure 2 shows spectra of the outer membrane protein OmpX in DHPC micelles, for which TROSY was absolutely vital to obtain sequential resonance assignments [31••,32].

By using TROSY experiments, the first NMR structures of larger integral membrane proteins have been determined during the past two years [31••,32,33••,34,35••]. Up to now, they are all proteins of the β-barrel family [36]. The fold of the outer membrane protein OmpX (148 residues) was obtained in DHPC micelles with a mass of about 60 kDa [31••,32]. More recently, the NMR structure has been refined by the collection of additional NOEs from a sample containing selectively protonated valine, leucine and isoleucine (δ1) methyl groups ([19•]; C Fernández, C Hilty, G Wider, P Güntert, K Wüthrich, unpublished data).

The fold of the outer membrane protein OmpA (177 residues) has been determined in dodecylphosphocholine (DPC) micelles of 45–50 kDa [33••]. TROSY-based NMR relaxation data displayed a dynamic gradient extending from the well-structured central part of the barrel towards the highly mobile loops, which led to speculation that the conformational flexibility of the solution structure may contribute to the membrane channel function of this protein. More recently, the fold of the outer membrane enzyme PagP (164 residues) has been determined both in DPC and in *n*-octyl-β-D-glucoside micelles of 50–60 kDa [35••]. The solution structure obtained with the help of TROSY techniques provided important clues about the mode of action of this enzyme.

The NMR spectral properties of α-helical membrane proteins are less favorable than those of β-barrel proteins and, so far, structure determination has been limited to smaller proteins containing one transmembrane helix (<100 amino acid residues) or to short protein fragments. Furthermore, an appropriate folding protocol is often not available for α-helical membrane proteins. Despite these problems, partial backbone resonance assignments have recently been obtained for native bacteriorhodopsin in dodecylmaltoside micelles [37]. Very promising data have been reported for the 39 kDa homotrimeric protein diacylglycerol kinase in micellar complexes with a molecular mass greater than 100 kDa [38•,39]. These results suggest that, at least from a technical point of view, structural studies of membrane proteins as large and complex as

typical members of the G-protein-coupled receptor family are feasible with TROSY-based NMR [39]. With growing experience and even better technical tools, the first NMR structures of larger, integral α-helical membrane proteins can be expected in the near future.

Intermolecular interactions and drug design

Intermolecular interactions between proteins and nucleic acids, ligands or other proteins often provide clues to the physiological roles of a newly discovered protein, and their investigation is thus of primary interest in structural biology and drug discovery. TROSY provides the basis for a wide range of NMR measurements related to the functional properties of larger macromolecular complexes. Many standard NMR experiments used to study intermolecular interaction can take advantage of TROSY; for example, it can be introduced into experimental schemes used for chemical shift mapping, intermolecular magnetization transfer, spin-relaxation studies and hydrogen–deuterium exchange measurements [40•].

Chemical shift mapping measures the changes in the chemical shifts that occur upon the binding of two molecules. From such mapping studies, putative contact regions in the complex can be identified or, if resonance assignments are not known, at least the binding event can be detected. TROSY was first applied in this context to studies of the protein–protein contacts in the 51 kDa complex formed between the type-1 pilus chaperone FimC and the pilus subunit FimH from *Escherichia coli* [41]. In this work, the sites on FimC that contact FimH were identified. A similar approach has been applied to various macromolecular complexes. The putative Ras interaction sites were identified on the surface of the Ras-binding domain in the protein kinase Byr2 in complexes of 35–40 kDa, constituting a further step towards an understanding of complex formation by Ras and its effectors at atomic resolution [42]. Combined X-ray and TROSY-based NMR studies of the 38 kDa complexin–SNARE complex have provided further insights into complexin function [43]. The contact area of the P-domain of the lectin chaperone calreticulin and Erp57 has been identified in a 66.5 kDa complex [44]. CRINEPT-TROSY NMR studies have shown that the p53 core domain is predominantly unfolded when bound in a complex of ∼200 kDa with Hsp90, shedding light on the nature of the binding interaction, which might be a general feature of substrates of Hsp90 [45]. Furthermore, TROSY-based NMR experiments were used to study the mechanism of ligand-mediated allosteric regulation in the 91 kDa 11-subunit TRAP protein, leading to the hypothesis that allosteric control of this protein is accomplished by ligand-altered protein dynamics [25].

Recently, a novel NMR method for determining the interfaces of large protein–protein complexes has been proposed in which TROSY and isotope-labeling techniques are coupled to perform sequential resonance assignment, chemical shift mapping, measurements of amide proton exchange rates and cross-saturation NMR techniques [46••]. This approach was applied to studies of a 64 kDa immunoglobulin complex with the B domain of protein A (FB), which specifically binds to the Fc fragment of immunoglobulin G. Figure 4 shows the results obtained from different 2D [^{15}N,^{1}H]-TROSY experiments that were used to identify the binding sites of the FB–Fc complex formed between ^{2}H,^{15}N-labeled FB and unlabeled Fc.

When studying intermolecular interactions, often only the surfaces of the molecules are of interest. Based on this assumption, spectral overlap in large complexes can be reduced by using solvent-exposed amides with TROSY (SEA-TROSY), as recently proposed and demonstrated for the 71 kDa protein NADHP cytochrome P450 [47]. This experiment selects solvent-exposed amide groups and, for example, can be incorporated into standard triple-resonance experiments.

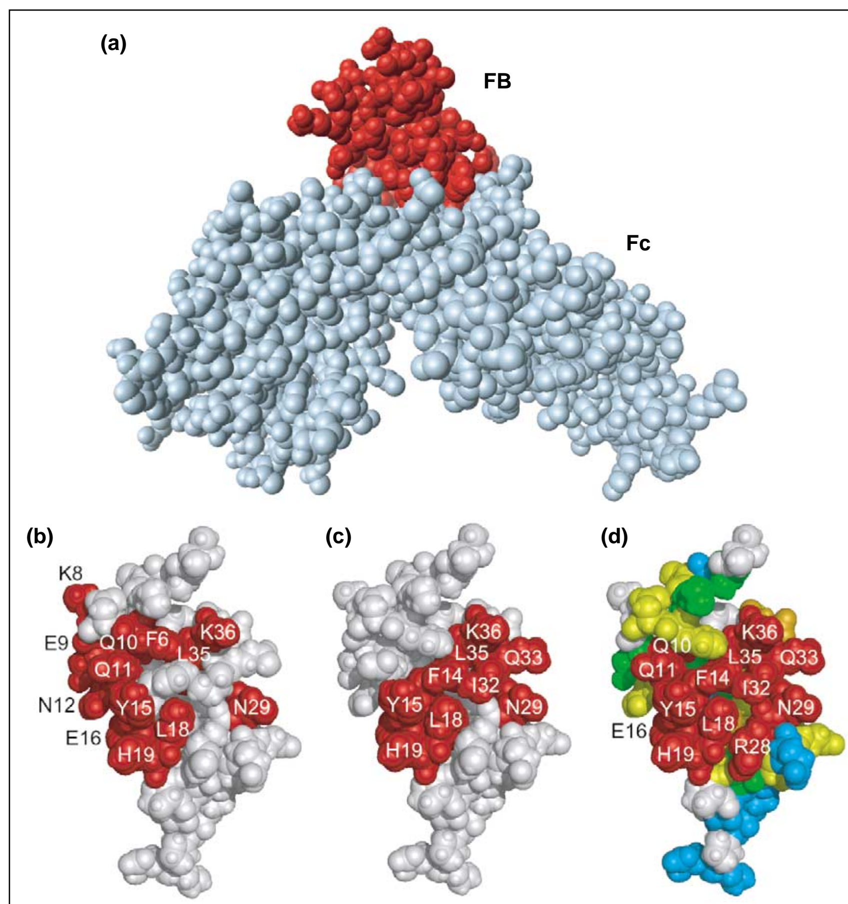
Scalar couplings across hydrogen bonds

Hydrogen bonds play a key role in the structure and function of biomolecules. The direct detection of hydrogen bonds in proteins and oligonucleotides in NMR spectra was facilitated by the recent discovery of scalar spin–spin couplings across hydrogen bonds [48–50]. These couplings not only provide novel insights into the nature of hydrogen bonds but also allow the determination of hydrogen-bond partners, which can be used to refine NMR structures, to study intermolecular interactions at atomic level and to investigate biological mechanisms involving hydrogen-bond interactions.

TROSY provides important improvements in sensitivity for NMR experiments designed to study hydrogen bonds in large biomolecules. The use of TROSY has permitted measurements of one-bond and two-bond scalar couplings across hydrogen bonds in a ^{15}N,^{13}C-labeled DNA duplex tetradecamer, both free in solution and in a 17 kDa complex with the *Antennapedia* homeodomain [49,51•], as well as one-bond couplings in RNA oligonucleotides from a *Bacillus subtilis* $tRNA^{Trp}$ A73 mutant larger than 25 kDa [52].

There have been applications of TROSY to the direct observation of hydrogen bonds in proteins, including the determination of three-bond scalar couplings in the uniformly ^{2}H,^{13}C,^{15}N-labeled, 30 kDa ribosome-inactivating protein MAP30 [53]; the measurement of scalar couplings across NH···OP and OH···OP hydrogen bonds in the 147-residue flavoprotein riboflavin 5′-monophosphate [54]; the detection of several hydrogen bonds in the monomeric, 16 kDa protein superoxide dismutase [55]; and the observation of a hydrogen bond in the active site of the 44 kDa enzyme chorismate mutase, both from

Figure 4



TROSY-based NMR study of the interface of the 64 kDa complex formed by the B domain of protein A (FB) and an Fc fragment of immunoglobulin G (Fc). **(a)** All-atom representation of the 3D structure of the FB–Fc complex. FB is shown in red and Fc in light blue. **(b)** Results from chemical shift mapping studies; residues with large chemical shift differences in the free and bound forms are labeled and highlighted in red on the 3D structure of FB. **(c)** Results from ^{1}H–^{2}H exchange NMR experiments. Residues with slowly exchanging amide protons upon complex formation are labeled and highlighted in red on the 3D structure of FB. **(d)** Intensity changes of the signals from amide protons of FB, caused by irradiation of resonances in Fc. A color code from red, yellow, green to blue identifies large to small intensity changes. Peaks showing large intensity changes were identified as being at the binding interface and are labeled. Reproduced with permission from [46••].

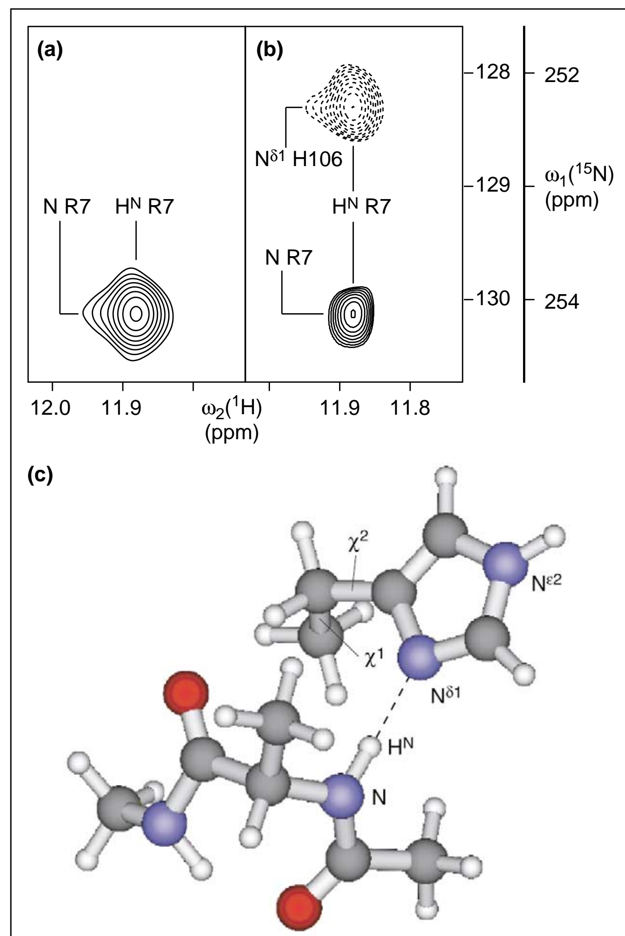
measurement of one-bond and two-bond scalar couplings, and by transfer of nuclear polarization across the hydrogen bond (Figure 5) [56••]. In the last example, the measured scalar couplings provided unique information about the structure of the active site of the enzyme. The approach described presents a general method of detecting hydrogen bonds in large molecules that can be applied to structural refinements of biomolecular structures [56••].

Measurement of residual dipolar couplings

The orientation of interatomic vectors in a molecule can be determined from the measurement of residual dipolar couplings. These orientations can be used as important restraints for obtaining global folds, and for refining the 3D structures of proteins and oligonucleotides, especially in large perdeuterated molecules, where only a very limited number of constraints can be obtained from NOEs.

Measurement of residual dipolar couplings in large molecules can be substantially improved using the TROSY technique. To date, TROSY-based experimental schemes have been developed for measuring dipolar couplings between various nuclei in the polypeptide backbone of ^{2}H,^{13}C,^{15}N-labeled proteins. Applications to the maltose-binding protein in complex with β-cyclodextrin and to carbonic anhydrase II have shown that precise dipolar couplings between various nuclei can be obtained for proteins of 30–40 kDa [57]. Furthermore, dipolar couplings in the amide groups of the protein chymotrypsin inhibitor 2 in lipid bicelles [58] and dipolar couplings between α- and β-carbons in the 41 kDa maltose-binding protein [59] have been measured by TROSY. The latter

Figure 5



TROSY-based NMR study of a hydrogen bond in the active site of the 44 kDa trimeric enzyme chorismate mutase. **(a)** Expansion of the 2D [^{15}N,^{1}H]-TROSY spectrum of the protein around the correlation signal of the amide group of Arg7. **(b)** TROSY spectrum used to correlate the chemical shift of the amide group of Arg7 and the $N^{\delta 1}$ atom of His106 across the hydrogen bond (dotted contour lines, ^{15}N scale is shown on the far right). **(c)** The local geometry of the identified hydrogen-bonding partners is represented in a model and the detected hydrogen bond is shown with a broken line. Reproduced with permission from [56••].

application established the relative orientation of the protein domains in solution.

Applications to nucleic acids

TROSY offers considerable advantages for NMR studies of nucleic acids [60]. The direct detection of hydrogen bonds and the measurement of residual dipolar couplings, topics that are discussed above, are of great importance for the structure determination of nucleic acids because, in comparison to proteins, inherently fewer protons are available as sources of structural information. In addition, TROSY has been widely used to increase the sensitivity of special triple-resonance NMR experiments for ^{13}C,-^{15}N-labeled nucleic acids, increasing the range of their applicability to much larger oligonucleotides. Examples include the use of TROSY in experiments that provided intrabase and sugar-to-base correlations [61–63], and in an experiment that provided correlations among all carbon nuclei in the adenine base [64].

Conclusions

Solution NMR studies of biological macromolecules and macromolecular complexes with molecular masses well above 100 kDa have become a reality with the development of TROSY. This technique has been used in numerous studies that tackle fundamental biological problems, extending from structural studies of large proteins and the structure determination of the first large integral membrane proteins in solution to applications investigating intermolecular interactions and protein function. The ability to obtain resonance assignments for large biomolecules raises the possibility of collecting NOE restraints, including NOE restraints to some sidechain resonances such as those of methyl and aromatic protons. This information, combined with recently developed methods to obtain alternative structural constraints such as residual dipolar couplings and scalar couplings across hydrogen bonds, opens avenues to the determination of much larger 3D structures by NMR. Even in the absence of sufficient information to determine a well-defined 3D structure, the ability to obtain complete backbone and partial sidechain resonance assignments can suffice to perform detailed studies of intermolecular interactions and investigations of dynamic processes. These data will contribute important information to many interesting biological problems. In the near future, we look forward to more applications of the techniques described here, which will answer further challenging questions related to the structure and function of large biological molecules.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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