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NMR analysis of protein interactions

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Recent technological advances in NMR spectroscopy have alleviated the size limitations for the determination of biomolecular structures in solution. At the same time, novel NMR parameters such as residual dipolar couplings are providing greater accuracy. As this review shows, the structures of protein–protein and protein–nucleic acid complexes up to 50 kDa can now be accurately determined. Although *de novo* structure determination still requires considerable effort, information on interaction surfaces from chemical shift perturbations is much easier to obtain. Advances in modelling and data-driven docking procedures allow this information to be used for determining approximate structures of biomolecular complexes. As a result, a wealth of information has become available on the way in which proteins interact with other biomolecules. Of particular interest is the fact that these NMR-based methods can be applied to weak and transient protein–protein complexes that are difficult to study by other structural methods.

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Introduction

In recent years, we have seen great improvements in NMR spectroscopy as a tool for the study of biomolecular interactions. The sensitivity of the technique has been enhanced by the advent of high-field spectrometers (900 MHz) and cryogenic probes. Novel methodologies (transverse relaxation-optimized spectroscopy (TROSY) [1]; residual dipolar couplings [2,3]) have enabled the structural analysis of larger molecules and complexes. Also, better use can now be made of spectral information such as chemical shift perturbations (CSPs) through improved modelling and docking procedures. Consequently, it is possible to characterize larger and biologically more relevant biomolecular complexes with higher accuracy. In this review, we discuss recent advances in NMR structural studies of the interactions of proteins with other proteins, RNA and DNA. Interactions with small molecules and peptides is not included, as these have been adequately covered in other reviews [4,5].

Biomolecular complexes reported since 2003 range from full *de novo* determined structures calculated from NMR-derived restraints (such as nuclear Overhauser effects (NOEs) and residual dipolar couplings (RDCs)) to models obtained by docking individual components based on knowledge of the interface from chemical shift perturbations. Although in the latter case the coordinate accuracy is necessarily lower, the information on the interaction gained is often quite important in guiding subsequent research.

Methodological developments

Next to the 'classical' approach based on the use of intermolecular NOEs, in combination with RDCs when available [6], the characterization of protein interactions has greatly benefited from the incorporation of interface mapping information in the computational modelling of complexes. NMR is particularly powerful in mapping interfaces, allowing the study of weak and transient complexes that can be very difficult to study by other experimental techniques.

The use of CSP data obtained from NMR titration experiments as structural restraints for solving the structure of protein complexes was first demonstrated by McCoy and Wyss [7]: RDC data were first introduced to orient the complex and the solutions were then optimized by a grid search and back calculation of chemical shift perturbations with the SHIFTS software [8]. In the HADDOCK approach [9••], CSP data are translated into ambiguous interaction restraints to drive the docking process. The CSP interaction restraints can also be combined with RDC data allowing a better definition of the relative orientation of the components [10•,11]. Note that CSP data have also been combined with docking methods to filter *a posteriori* the docking solutions [12], also in combination with RDC data [13], or to provide anchor points to start the search [14]. New chemical shift-based methods relying on amino-acid specific labeling have also been developed to map interfaces [15,16]. These methods no longer require resonance assignment provided the 3D structure of the unbound protein is known.

Several other NMR techniques can provide information on interfaces. In cross-saturation or saturation transfer (SAT) experiments [17], the observed protein is perdeuterated and ^{15}N-labeled with its amide deuterons

exchanged back to protons, whereas the other 'donating' partner protein is unlabeled. Saturation of the unlabeled protein leads by cross-relaxation mechanisms to signal attenuation (typically monitored by ^{15}N-HSQC spectra) of those residues in the labeled protein that are in close proximity. Deuteration here is a requisite. Cross-saturation experiments are believed to give a more reliable picture of the interface than CSP data, which can suffer from 'false positives' due to conformational changes. Experimental intensity changes from SAT have been used as restraints in a docking procedure in combination with RDC data [18]. For paramagnetic systems, another useful NMR parameter is the pseudocontact shift, a long range effect that results from electron–nuclei dipolar interactions. The use of paramagnetic tags attached to a protein can also induce this phenomenon. Pseudocontact shifts have been used to study transient redox complexes [19] and recently to break the symmetry in symmetrical complexes [20]. It is also possible to use paramagnetic ions as probes, using the line broadening effect for the residues they contact. In a complex, the interface residues will be protected from such effects allowing identification of the interface [21,22]. Finally, orientational information similar to that provided by RDCs can also be extracted from relaxation experiments in the case of rotational diffusion anisotropy [23].

Protein–protein interactions

NMR studies of protein–protein complexes have varied from full structure determination to NMR-filtered docking and modeling using interface information. We limit our discussion mainly to complexes for which the atomic coordinates have been deposited into the Protein Data Bank (PDB; http://www.rcsb.org) (see Table 1).

Since 2003, the structures of 14 protein–protein complexes have been solved using intermolecular NOEs detected from ^{13}C/^{15}N-filtered NOESY experiments in combination with RDCs when available. From these, 12 were determined completely *de novo* by NMR [24•,

Table 1

Overview of protein–protein, protein–RNA and protein–DNA complexes solved by NMR and deposited in the Protein Data Bank (http://www.rcsb.org) since 2003.

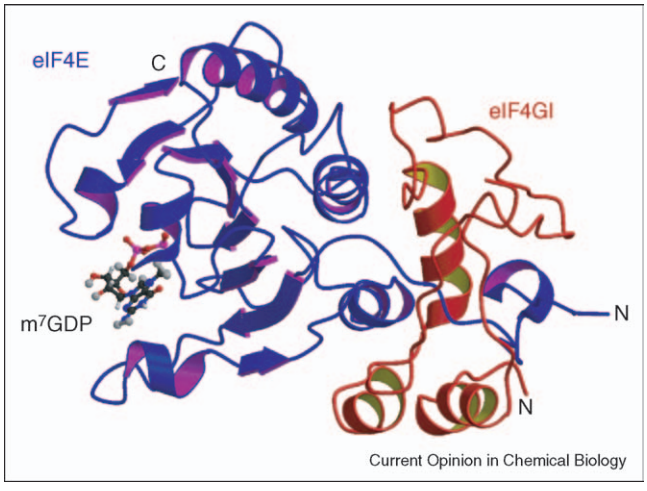
Complex name	Size[a] (aa/bp/nt)	PDB ID	Reference
Protein–protein complexes			
NOE-based complexes			
Enzyme IIA^{Mannose}–HPr	2 × (129 + 85)	1VRC	[36•]
Nck-2 SH3 domain–PINCH-1 LIM4 domain	71 + 66	1U5S	[24•]
CD3t–CD3δ ectodomains	178	1XMW	[25]
Ubiquitin–Npl4 zinc finger	76 + 31	1Q5W	[26]
HP1 chromo domain–PXVXL motif of CAF-1	2 × 75 + 30	1S4Z	[27]
ThKaiA108C–KaiC peptide	2 × 107 + 34	1SUY	[28]
CPB TAZ1–CITED2	50 + 100	1R8U	[30]
EIF4Ecap–eIF4G	213 + 100	1RF8	[31••]
Enzyme IIA^{Glc}–Enzyme IICB^{Glc}	168 + 90	1O2F	[32••]
HHR23A Ubl–S5a-UIM-2	45 + 78	1P9D	[34]
CUE–Ubiquitin	49 + 76	1OTR	[33]
CITED2 TAD–p300 CH1	52 + 101	1P4Q	[35]
MMP-3–N-TIMP-1	168 + 126	1OO9	[21]
Chemical shift mapping-based complexes			
RPA32 C domain–SV40 T antigen domain	105 + 132	1Z1D	[40•]
Plastocyanin–cytochrome *f*	105 + 254	1TU2	[38•]
UbcH5B–CNOT4	147 + 52	1UR6	[39•]
Atx1–Ccc2 copper transporting ATPases	73 + 72	1UV1/2	[37]
Protein–RNA complexes			
Tandem zinc finger domain of TIS11d–AU-rich ssRNA	70 + 9 nt	1RGO	[49••]
Nucleocapsid protein–Mlv encapsidation signal RNA	56 + 101nt	1U6P	[50]
Nucleocapsid protein–AACAGU	56 + 6 nt	1WWD1WW	[51]
Nucleocapsid protein–UUUUGCU	56 + 7 nt	E1WWF	
Nucleocapsid protein–CCUCCGU	56 + 7 nt	1WWG	
Nucleocapsid protein–UAUCUG	56 + 6 nt		
RNase III (Rnt1P)–dsRNA	90 + 32 nt	1T4L	[53•]
Protein–DNA complexes			
Oct1–Sox2–Hoxb1 DNA (ternary complex)	167 + 88 + 19 bp	1O4X	[56••]
MarA–α-CTD RNAP–DNA (ternary complex)	132 + 84 + 20 bp	1XS9	[41•]
Cdc13 CTD–ssDNA	199 + 11 nt	1S40	[60•]
Dimeric lac headpiece–non-specific DNA	2 × 62 + 18 bp	1OSL	[57••]

[a] The size is expressed in amino acid residues (aa) unless otherwise specified with bp (base pairs) or nt (nucleotides).

25–30,31•,32••,33–35] while the remaining two [21,36•] were solved starting from the known 3D structures of the unbound components or from parts of other complexes. Only five exceed 200 amino acid residues, indicating that the structure determination of large protein complexes by NMR remains challenging. Three of these were obtained by including RDCs to properly define the relative orientation of the molecules [21,32••,36•]. In particular, the 258 amino acid complex between the signal-transducing Enzyme $IIA^{Glucose}$ and the cytoplasmic domain of the glucose transporter Enzyme IICB [32••] provides a good illustration of the power of the combination of NOE and RDC for *de novo* NMR structure determination. One of the largest (313 amino acids) complexes solved by NMR was the one between eIF4G and eIF4E [31••] (Figure 1). The NOE information was supplemented in this case by restraints derived from paramagnetic broadening effects. The recently published 48 kDa Enzyme $IIA^{Mannose}$–HPr complex [36•] is the largest NMR complex solved on the basis of intermolecular NOEs and RDCs. An ultraweak complex with a K_d in the millimolar range was solved using a combination of intermolecular NOEs and RDCs, illustrating the power of NMR when it comes to studying weak interactions [24•].

Next to the NOE-based structures, an increasing number of complexes are being reported that have been solved by (data-driven) docking using mainly CSP data, often in combination with additional information to either generate or validate the resulting structures. Coordinates for a few of these (see Table 1) have been deposited into the PDB. These structures are the direct result of recent methodological development to make use of CSP data [9••,10•] and include weak and transient complexes [37,38•]. Most structural models obtained in this way were validated using independent data. They often provide a starting point for site-directed mutagenesis in combination with binding assays [39•–41•]. The 95 kDa complex of the acyltransferase protein with the acyl carrier protein was modeled based on NMR (CSP + RDCs) and mutagenesis data [42•]. That chemical shifts perturbations alone can lead to reliable models was shown for the RPA32–SV40 complex [40•]: the CSP-based model was validated using RDC data. Finally, the structure of plastocyanin and cytochrome *f* complex [38•] illustrates the great promise of paramagnetic NMR in characterizing transient complexes by a combination of CSP data with pseudo-contact shifts providing long-range distance and angular information.

Figure 1



NMR solution structure of the complex of the eukaryotic initiation factors 4E (eIF4E) (in blue) and 4G (eIF4G) (in red) (PDB entry 1RF8) [31••]. The complex is formed by a coupled folding transition of eIF4G and the N-terminus of eIF4E.

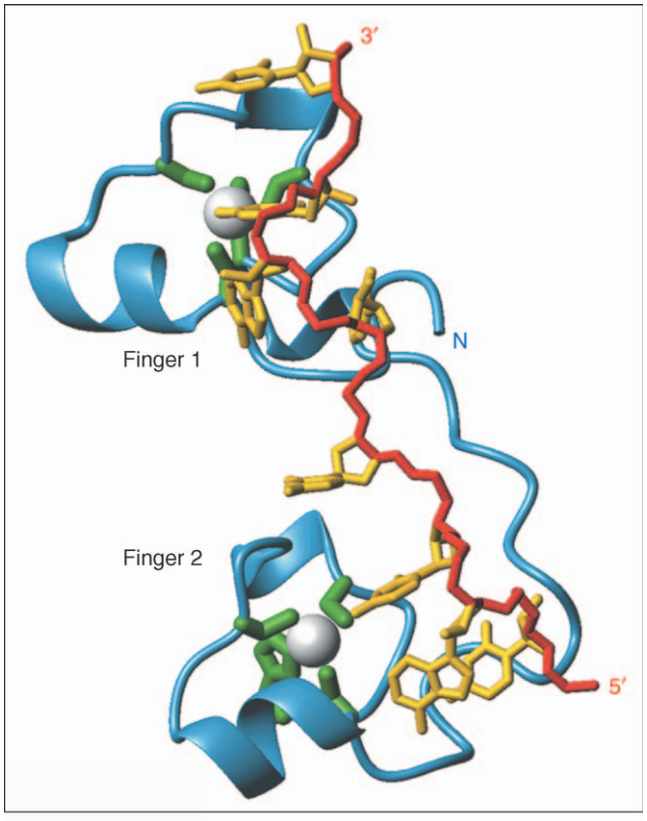
Protein–RNA interactions

One of the most abundant RNA binding motifs with an occurrence of 1.5–2% in the human genome is the RNA recognition motif (RRM) recently reviewed in [43,44]. RRMs are often found as tandem repeats within a protein together with other domains. Specificity mainly comes from direct interactions between the RNA bases and the protein side chains and main chains. Recently, the complex of the two N-terminal domains RRM1 and RRM2 of nucleolin with the nucleolin responsive element (b2NRE) was solved by NMR [45•]. The higher stability of an *in-vitro* selected pre-rRNA [46] is accounted for by additional contacts with the double-stranded RNA stem. Not all RRM motifs, however, are involved in RNA binding; interactions with protein have been observed as well [44].

Another abundant single-stranded RNA binding motif is the KH domain, reviewed in [43]. A solution structure has been determined for the KH domain of splicing factor SF1 in complex with an 11-mer RNA containing a 7 nucleotide branchpoint sequence [47].

The most abundant eukaryotic nucleic acid binding motif (with 3% of the genes in the human genome) is the CCHH-type zinc-finger (ZF) motif. Although this motif is mainly associated with DNA regulatory proteins such as transcription factors, it has been found to bind RNA as well. The X-ray structure of the complex of a triple ZF domain of TFIIIA with a truncated 5S RNA shows how the α-helices of the different ZFs interact with the phosphate backbone and the 5S RNA grooves [48]. The solution structure of the tandem zinc finger domain of the protein TIS11d in complex with an AU-rich single-stranded mRNA element is very different [49••] (Figure 2). It shows two compact CCCH-type zinc-fingers structures in a novel fold with the mRNA along the surface of the two ZF motifs. The NMR structures of several CCHC knuckles of the nucleocapsid (NC) domains of retroviral Gap polyproteins in complex with RNA packaging signals have been determined over the

Figure 2



NMR solution structure of the tandem zinc-finger domain of the protein TIS11d bound to the class II AU-rich element (PDB entry 1RGO) [49••]. Only ordered regions of TIS11d (residues 153–217) (blue) and RNA bases U2–U9 (red and yellow) are shown. The location and coordination of the zinc atom in each finger is indicated. Reprinted with the permission from [49••]. Copyright 2004, *Nature Structural Biology*, http://www.nature.com.

past few years. The NC protein has a compact and globular structure that interacts with unpaired 4nt RNA sequences, as noted in the various complexes of Moloney murine leukaemia virus NC [50,51] (see Table 1). The high affinity for such sequences causes significant RNA secondary structure rearrangements that are implicated in viral packaging and encapsidation.

Double-stranded-RNA binding domains (dsRBDs) are found in a large number of RNA regulatory proteins, recently reviewed in [52]. The NMR structure of the dsRBD of Rnt1p RNase III in complex with a dsRNA fragment showed that the AGNN tetraloop is recognized in a structure-specific manner [53•].

Proteins involved in RNA interference show a conserved domain involved in recognition of small interfering RNA (siRNA) called the PAZ domain. The solution structure of the Argonaute2 PAZ domain bound to short single-stranded RNA and single-stranded DNA oligonucleotides shows how the two-nucleotide 3′ ssRNA overhang of an siRNA is recognized [54••].

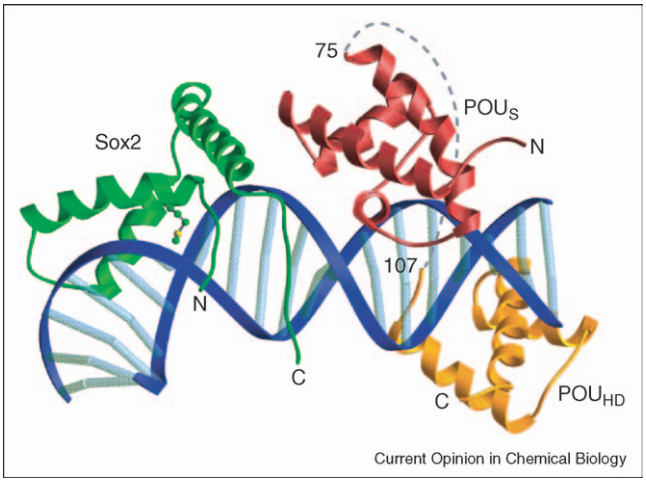
A common observation in protein–RNA complex formation is a significant adaptation of protein and RNA structure to make an optimal complex. Examples are found in the structure of the complex of the Jembrana virus Tat protein and HIV TAR RNA. The unstructured peptide folds into a stable β-hairpin in the complex and stabilizes an RNA conformation with a bulged-out uridine [55].

Protein–DNA interactions

A structure has been reported for the ternary complex of the POU domain of Oct1, Sox2 and the Hoxb1-DNA regulatory element [56••]. A modular approach was used to build the structure based on the binary DNA complexes of POU and Sox2 (or rather the homologous SRY). The structure (shown in Figure 3) was refined by extensive use of RDCs. Both transcription factors co-interact while bound to adjacent sites at the DNA. Comparison with various other regulatory sites sheds light on the mechanism of cell-specific transcription regulation [56••].

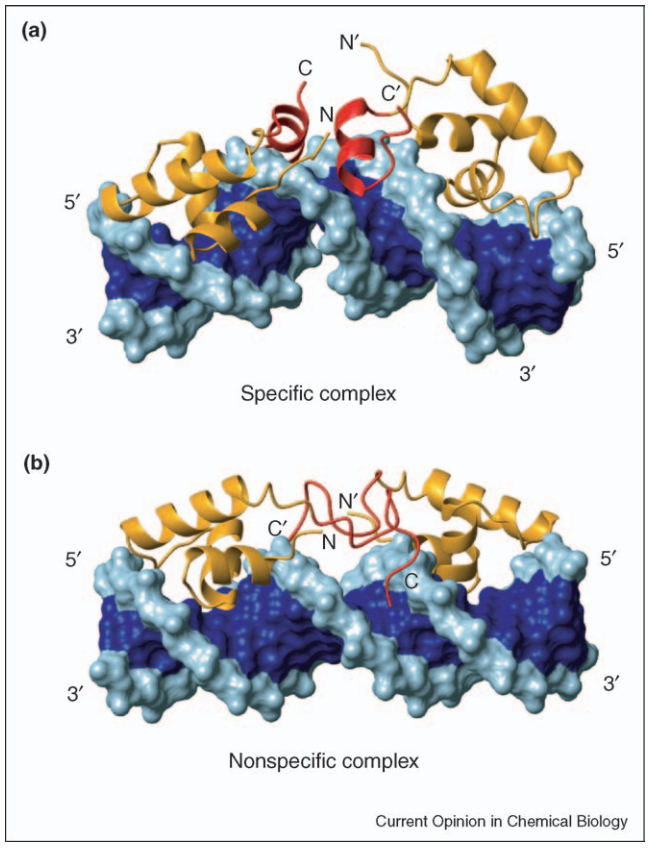
Gene regulatory proteins that bind DNA in a sequence-specific manner usually also interact non-specifically. This non-specific binding allows a much faster search for the specific target site by one-dimensional diffusion along the DNA. For the *Escherichia coli* lac repressor, the NMR structure of a complex between a covalent lac headpiece dimer and non-operator DNA has given detailed information about the non-specific binding mode [57••]. The structure revealed many differences with that of the complex with lac operator (Figure 4). It accounts for

Figure 3



NMR solution structure of the ternary Oct1–Sox2–Hoxb1 DNA complex (PDB entry 1O4X), solved using a combination of intermolecular NOEs and RDCs [56••].

Figure 4



Comparison of the NMR solution structures of the **(a)** specific and **(b)** non-specific dimeric lac headpiece–DNA complexes. The specific complex (PBD entry 1L1M) was solved using the 22 bp natural O1 operator sequence, whereas for the non-specific complex (PDB entry 1OSL) [57••] an 18 bp non-operator DNA sequence was used. In the specific complex, the hinge helices are formed leading to DNA bending whereas these helices are unstructured in the non-specific complex. A large difference in dynamics was noted: the protein in the non-specific complex displayed extensive mobility in the microsecond to millisecond range, whereas all motions are frozen in the specific complex.

long-standing thermodynamic data and, for instance, the fact that the affinity towards random DNA is much more salt dependent than that towards lac operators.

Another approach to the study of non-specific DNA binding makes use of paramagnetic relaxation enhancement [58,59]. A paramagnetic tag (Mn^{2+} chelated by EDTA) is attached to deoxythymidine in a DNA duplex. The relaxation enhancement of protons on the bound protein yields long-range distance information. Combined with RDCs and limited NOE information, this data improves the accuracy with which the structures of protein–DNA complexes can be determined. Applications were reported for the HMG box proteins SRY and HMGB-1 [58,59].

The telomere binding protein Cdc13 of budding yeast binds specifically to telomeric single-stranded DNA. A complex was solved for the DNA binding of Cdc13 with cognate single-stranded DNA [60•]. The structure provides a rationale for the high affinity and high specificity towards GT sequences.

In several reports, protein–DNA interactions were studied by CSP mapping using protein structures obtained from NMR or X-ray crystallography [61–64]. The ternary complex consisting of the transcriptional activator MarA, its DNA target site and the C-terminal domain of the α-subunit of RNA polymerase (α-CTD RNAP) was studied by CSP mapping from a titration of the MarA–DNA complex with α-CTD RNAP [41•]. The structure of the ternary complex was obtained by data-driven computational docking.

The human replication protein A (RPA) has received considerable attention. The protein plays a crucial role in DNA replication, recombination and repair by interacting with single-stranded DNA. It is a heterotrimer consisting of three subunits of 70, 32 and 14 kDa. In the context of DNA repair, RPA together with the exclusion repair protein XPA forms a complex with the damaged DNA site. The interaction of RPA with a DNA decamer, containing a cyclobutane thymine dimer lesion, was studied using CSP mapping [65]. Addition of XPA displaces RPA to the undamaged strand, whereas XPA prefers binding to the lesion. It was also shown that single-stranded DNA and XPA compete for the same binding site on RPA [66]. These studies shed light on the role of RPA and XPA in recognition and verification of DNA damage. Interaction surfaces of RPA were determined for a variety of protein–protein interactions. These include the interaction with the large T antigen (Tag) of SV40 [40•], with Rad 51 and its role in homologous recombination [67], and with the P53 transactivation domain [68].

Conclusions

It is clear that NMR has become a powerful and versatile method for the study of biomolecular interactions. Several spectacular protein–protein and protein–nucleic acid complexes in the molecular weight rang 40–50 kDa have been solved, and larger assemblies are within reach. The identification of interaction surfaces by relatively simple NMR experiments such as ^{15}N-HSQC is becoming very popular. The resulting chemical shift perturbations (or any information related to the interaction) can now be used as restraints in data-driven docking algorithms to produce approximate structures for the complexes. Thus, NMR appears to be ready for the structural characterization of biomolecular complexes, an area that is often viewed as the next phase in structural proteomics.

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