Published in final edited form as:

J Struct Biol. 2011 March ; 173(3): 515–529. doi:10.1016/j.jsb.2010.11.005.

Structural NMR of Protein Oligomers using Hybrid Methods

Xu Wang, Hsiao-Wei Lee, Yizhou Liu, and James H. Prestegard*

Complex Carbohydrate Research Center, University of Georgia, Athens, GA 30602. USA

Abstract

Solving structures of native oligomeric protein complexes using traditional high resolution NMR techniques remains challenging. However, increased utilization of computational platforms, and integration of information from less traditional NMR techniques with data from other complementary biophysical methods, promises to extend the boundary of NMR-applicable targets. This article reviews several of the techniques capable of providing less traditional and complementary structural information. In particular, the use of orientational constraints coming from residual dipolar couplings and residual chemical shift anisotropy offsets are shown to simplify the construction of models for oligomeric complexes, especially in cases of weak homodimers. Combining this orientational information with interaction site information supplied by computation, chemical shift perturbation, paramagnetic surface perturbation, cross-saturation and mass spectrometry allows high resolution models of the complexes to be constructed with relative ease. Non-NMR techniques, such as mass spectrometry, EPR and small angle X-ray scattering, are also expected to play increasingly important roles by offering alternative methods of probing the overall shape of the complex. Computational platforms capable of integrating information from multiple sources in the modeling process are also discussed in the article. And finally a new, detailed example on the determination of a chemokine tetramer structure will be used to illustrate how a non-traditional approach to oligomeric structure determination works in practice.

Keywords

residual dipolar coupling; SAXS; CCL5; chemokines; dimers; protein structure

Introduction

In recent years, the focus of structural biology has shifted from the study of single molecule structures to the study of molecular interactions and the structure of the complexes formed. This is an important step in improving the functional relevance of structural biology since biological macromolecules, proteins in particular, rarely function as independent entities; they more commonly interact with numerous partners to modulate and enhance activities of each. However, along with new opportunities in functional understanding come new challenges. Complexes with high association constants are inherently larger, pushing the size limitations of traditional NMR techniques. Complexes can also exhibit weak association requiring analysis of dynamically averaged NMR data. NMR is fortunately a versatile technique allowing integration of traditional NOE-based approaches with other NMR data types, such as residual dipolar couplings (RDCs) and residual chemical shift anisotropy

*Corresponding Author: James H. Prestegard, jpresteg@ccrc.uga.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

offsets (RCSAs), that are more suited to structural analysis of complexes and their functional properties. NMR data also integrate well with data from other technologies, X-ray crystallography, small angle X-ray scattering (SAXS), and mass spectrometry, for example. Integrated approaches using versatile computational platforms have also allowed new routes of attack on systems dependent on molecular interactions. It is these integrated approaches that we wish to highlight in this presentation. We will begin with a review of the theoretical basis of non-traditional and complementary technologies along with citations to recent examples of application. We will end with a detailed application to oligomer formation by the chemokine CCL5, an important process in the regulation of these pervasive cell signaling molecules.

It is actually no surprise that molecular interactions are prevalent and that a significant portion of all proteins will form stable homo or hetero-oligomers. For example, from an evolutionary perspective, non-covalent oligomers carry an expression advantage over the equivalent single-chain multidomain proteins, as short monomers can be produced more efficiently than a single long one due to the effects of transcriptional or translational errors on longer coding sequences. Likewise, it may be easier to tailor the properties of oligomeric complexes to the demands of evolution by mutation of an oligomeric subunit rather than domains within a single chain protein. Several modeling studies over the years have also shown that homo-oligomers are substantially more thermodynamically stable than their respective monomers, (Andre et al., 2008; Goodsell and Olson, 2000; Lukatsky et al., 2007; Wolynes, 1996) and associations between subunits of an oligomeric complex are known to provide additional means of regulating different biological functions and enhance ligand affinity in multimeric lectin receptors. (Rini, 1995; Weis and Drickamer, 1996) Even the impact of weak oligomer formation in biology is being increasingly recognized; in the oligomerization of chemokines, the example we highlight at the end of this presentation, higher order oligomers are formed at high concentrations or in the presence of the sulfated polysaccharide glycosaminoglycan, a process essential in the controlled trans-epithelial migration of activated lymphocytes. (Appay et al., 1999; Proudfoot et al., 2003)

Traditional high-resolution techniques such as X-ray crystallography and solution NMR have served structural biology admirably for decades. The accumulated knowledge obtained through these techniques has greatly enhanced our understanding of the structures of biological macromolecules, especially in the area of oligomer formation. However, as the frontiers of structural biology are pushed to the levels of large molecular systems and membranous environments, the limitations of the techniques are beginning to hinder investigations of meaningful targets. Even though X-ray crystallography has had enormous success in studying structures of large complexes (ribosome, viral capsids), the inherent flexibility and transient nature of many other macromolecule complexes can work against their ability to crystallize or produce quality diffracting crystals. (Robinson et al., 2007) In systems where dynamics serve an important biological role, there is also the danger of over interpreting conformations that may be trapped by crystal packing effects. In solution NMR the traditional NOE-centric method of solving oligomer structures also has limitations. Although capable of characterizing macromolecular structure in a more natural environment, straight-forward applications normally assume a static representation appropriate only for tight binding complexes ($K_d < 10 \mu\text{M}$), and applications are still limited to smaller systems ($\sim 70 \text{ kDa}$).

Even where application of traditional NMR techniques is appropriate, NOE interpretation for homo-oligomers is plagued by chemical shift degeneracy and the resulting ambiguity in assignment. (Nabuurs et al., 2006) These limitations are addressed to a certain extent through the use of 3D isotope filtered/edited experiments. (Folkers et al., 1994; Ikura and Bax, 1992; Zwahlen et al., 1997) These experiments rely on obtaining intermolecular

contacts from samples in which only one partner in a complex is isotopically labeled. Unfortunately, these experiments can be quite insensitive, particularly for larger complexes because of severe signal attenuation during the isotope filter periods. In the case of homo-oligomers, the requirement of selective isotopic labeling of one partner within the structures observed requires dilution with unlabeled material and reduces sensitivity by an additional 50 %. When used on higher order homo-oligomers involving three or more subunits, results of these isotope filtered/edited experiments become even more ambiguous due to their inability to distinguish intermolecular NOEs between the labeled partner and multiple unlabeled components.

In view of the limitations of traditional approaches, many researchers are beginning to build on high resolution structures of monomeric units by collecting data on complexes using a variety of lower resolution techniques, and combining these with alternate types of NMR data that can be acquired on larger complexes and their components. Unlike the traditional NMR approach, most alternate NMR methods do not require insensitive 3D experiments and, with the exception of some cross-saturation experiments, do not require selective isotopic labeling. It is also worth pointing out that many of the approaches do not require uniform labeling, as sparse labeling is sufficient to extract required information; this allows the techniques to be applied to proteins expressed in eukaryotic systems, where uniform-labeling is often prohibitively expensive. The aim of this article is to present a clear and brief description of alternate NMR techniques and complementary biophysical methods, along with an emphasis on their strengths and weaknesses (a summary of the methods discussed in this review can be found in table 1). Although citations to applications of each method are not meant to be comprehensive, they hopefully provide a source of practical advice on the finer points of each technique. The detailed description of a study on the oligomeric structure of the chemokine CCL5 provided at the end utilizes several of the methods described and will hopefully serve as a good illustration of the power and complementarity of the techniques presented.

Orientational Constraints through Weak Alignment

Building Oligomeric Structures Using RDCs

RDCs, along with closely related RCSA offsets, provide a powerful source of orientational constraints on assembly of subunits into a complex when the structures of isolated subunits have been determined previously. RDCs originate from incomplete averaging of the through-space dipolar interaction between a pair of magnetic nuclei, ^1H - ^{15}N , ^1H - ^{13}C , ^{15}N - ^{13}C , ^{13}C - ^{13}C , or ^1H - ^1H , for example. When a molecule samples orientations uniformly, as it does in normal solution NMR, dipolar couplings average to zero and are not observable. However, if a molecule is dissolved in an orienting medium, such as a dilute liquid crystalline medium or polyacrylamide gel, or has a sufficiently large anisotropic magnetic susceptibility to self-orient in high magnetic fields, it becomes partially aligned, and the dipolar couplings are not completely averaged to zero. This leads to a small addition to the normal scalar couplings seen in NMR spectra. This additional contribution is dependent on the length of the inter-nuclear vector, r , and the angle this vector makes with the magnetic field, θ . This angular dependence for a pair of spin $\frac{1}{2}$ nuclei, i and j , can be expressed more precisely as follows (Tjandra and Bax, 1997; Tolman et al., 1995):

$$D_{ij} = \langle D_{\max} / r^3 \rangle (3 \cos^2(\theta) - 1) / 2 \quad (1)$$

Here the angular brackets denote averaging over molecular orientations and $D_{\max}$ is a constant representing the contribution that would occur for a particular pair of nuclei

separated by unit distance (1 Å) and lying on a direction parallel to the magnetic field. Contributions to couplings of directly bonded ^{15}N - ^1H pairs of nuclei in the amide bonds of a polypeptide are most easily measured. The amide bond length is assumed to be fixed at 1.025 Å, making the additional coupling a direct indicator of the average orientation of H-N bonds relative to the magnetic field.

The nature of the averaging that occurs is conveniently described in the elements of a 3 by 3 symmetric and traceless order matrix, S_{kl} .

$$D_{ij} = (D_{\text{max}ij} / r^3) \sum_{kl} S_{kl} \cos(\theta_k) \cos(\theta_l) \quad (2)$$

Here the $\theta_{k,l}$ are angles between the internuclear vector and the axes of a molecular frame. These are assumed to be defined by the known molecular geometry of the particular subunit and the molecular frame chosen for that subunit. Averaging of angular functions relating the magnetic field direction to a particular frame are now contained in the order matrix elements (Prestegard et al., 2004).

With a sufficient number of measured RDCs in a single subunit (≥ 5 if truly independent, but in practice >20), one can solve for the order matrix elements in the above equations using methods such as singular value decomposition (Losonczi et al., 1999). Finding the transformation that diagonalizes this matrix also provides a means of transforming the coordinates of a subunit to a principal alignment frame. For a stable complex, all subunits must share the same alignment frame and this provides a powerful constraint on assembly of a model for a multi-subunit complex.

There are some complexities in this process. The $\cos^2(\theta)$ dependencies of the order matrix elements means that the principal frame axes can only be determined to a level where a 180° rotation about any axis is allowed. This leads to a four-fold degeneracy in the way any two subunits can be assembled. However, this type of degeneracy can be removed by collecting data in two or more independent alignment media (Al-Hashimi et al., 2000b). Beginning with data from one alignment medium, a particular alignment frame is chosen for one subunit and four models are constructed using the four possible frames for the second subunit. The process is repeated using data from the second alignment medium and all models are superimposed using coordinates of the first subunit from each set. The orientation of the second subunit is common to both sets only when the proper frame is selected.

Translation of subunits is not addressed in the orientation process and usually requires additional distance constraints. However, small numbers of NOEs, PREs, or even knowledge of contact surfaces from chemical shift perturbations or computational prediction often suffice. (see Interaction Interface Determination section below) These same constraints also can allow removal of the degeneracy in alignment frame of choice using just a single alignment medium and set of RDCs (Bewley and Clore, 2000; Clore, 2000; Clore and Schwieters, 2003; McCoy and Wyss, 2002; Rumpel et al., 2008).

Symmetric homo-oligomers provide a special case in which data need not be collected separately for each subunit; a single set of data pertains equally to both subunits. In this case a restriction on the geometry of an assembled model comes from the fact that for a dimer with C_{2v} symmetry, one of the alignment axes must lie along the two-fold rotation axis. Despite the four-fold degeneracy of alignment axis systems, the symmetry axis can be identified by the preservation of the orientation for one axis when axis directions for two

alignment media are displayed on a single Sauson-Flamsteed plot. (Wang et al., 2008) It is also worth noting that a rotationally symmetric trimer, and other higher order rotationally symmetric oligomers, such as a square planar tetramer, would have axially symmetric alignment tensors with the unique axis along the rotational symmetry axis. (Al-Hashimi et al., 2000a) Jain et al. have utilized this property to study the orientation of the acyl carrier protein on the LpxA trimer and that of trimanoside on the mannose-binding protein trimer. (Jain et al., 2003; Jain et al., 2004) There are several assumptions underlying applications to these symmetric systems, including the oligomer being the species making the dominant contribution to the observed RDCs where multiple oligomeric species are in equilibrium, and the complex being sufficiently rigid to exclude internal motion contributions to RDCs.

One of the pre-requisites for building oligomeric structures using RDC orientational restraints is the availability of a high-quality protein structure for each component of an oligomer; these may come from either X-Ray or NMR spectroscopy. It is important that at least parts of these structures are known to be rigid and do not experience a conformational change upon interaction with a binding partner. If the protein structure is to be determined by NMR, NOE constraints must be used with care. If a significant population of an oligomer exists under data collection conditions, inter-subunit NOEs at the interface can be misinterpreted as intra-subunit NOEs, and this can lead to errors in derived monomer structures (Nabuurs et al., 2006). The quality of the initial structure from either technique can often be validated with RDC data. Erroneous monomer structures are often inconsistent with the RDC data and can be identified through outliers in back-calculated values or using the Q-factor (quality score) as a metric (Cornilescu and Bax, 2000). Large Q-factors (> 0.4) would be an indication of a low quality structure or change in protein conformation on oligomerization or alignment.

There are now numerous examples in the literature illustrating variations in implementation of the general procedure outlined above (Al-Hashimi et al., 2000a; Bolon et al., 1999; Fischer et al., 1999; Jain et al., 2003; Losonczi et al., 1999). A convenient approach is to roughly align the two subunits using the orientation procedure described above, and then translate the oriented subunits into an approximate interfacial position using NOE, chemical shift perturbation, or PRE derived restraints. This is followed by geometry optimization with a simulated annealing program. If ambiguity in alignment exists, as in the case of a single RDC set, all degenerate models need to be examined and scored using supplementary data. An RDC-assisted approach using just computational predictions of alignment and interface preferences has also been demonstrated for a high affinity symmetrical dimer (Wang et al., 2008). The structure of a monomer unit in this case was taken from the deposited crystal structure of the Northeast Structural Genomics (NESG) Target Sr360. The crystal structure actually shows a tetramer with two different dimer interfaces. A unique two fold axis direction was first determined using RDC data from two alignment media; then a two dimensional grid search was performed to generate dimer models (Figure 1A). These were subsequently scored based on agreement of experimental RDCs with RDCs predicted using the steric alignment program PALES (Zweckstetter and Bax, 2000) and minimization of a residue pairing score for residues at the dimerization interface (Moont et al., 1999; Zweckstetter and Bax, 2000). The RDC-assisted approach was able to construct a dimer model that is in good agreement with one of the dimer structures seen in the X-Ray data.

When interactions between subunits are weak, and mixtures of monomeric and oligomeric species exist, constraints for use in oligomer structure determination must be determined by proper extrapolation of experimental data. Recently a procedure similar to that described above was used by Lee et al. to generate a dimer model for a symmetrical homo-dimer with a large dissociation constant (3.4 mM). In this case RDCs for each of two alignment media were measured at different protein concentrations, and these measured dipolar couplings,

along with the dissociation constant, were used to extrapolate to RDCs for the dimeric state. (Lee et al., 2010) A similar extrapolation procedure was also used in the work of Ortega-Roldan et al. (Ortega-Roldan et al., 2009) In the example of Lee et al. the symmetry axis of the dimer was determined unambiguously from the two sets of RDCs and the dimeric models were constructed based the same grid search algorithm described above.

Orientation constraints can also be integrated more directly with traditional distance-based and back-bone torsional angle data during the simulated annealing process. Here, both RDC and distance data are represented as pseudo potentials added to the energy landscape of the simulation (Clore, 2000). When integrating data in this fashion it is convenient to assume that one knows the orientation of the principal alignment frame in the coordinates of the final complex and recast equation (2) in this frame (Bax et al., 2001):

$$D_{ij}=(D_a/r^3)[(3\cos^2(\theta)-1)+(3/2)R\sin^2(\theta)\cos(2\varphi)] \quad (4)$$

Here θ and φ are the polar angles of the ij nuclear vector in the principal alignment frame. D_a and R are the axial and rhombic components of the alignment tensor with D_a representing the maximum splitting in Hz for a ^{15}N - ^1H amide pair. These can be related to $D_{\max}$ for a ^{15}N - ^1H amide pair and the principal frame order parameters, (S_{kk}), of equation (2) as:

$$D_a=(S_{zz} \cdot 24350)/2; \quad R=(2/3)(S_{xx} - S_{yy})/S_{zz} \quad (5)$$

A radius of gyration term can also be applied to keep the subunits in close contact (Kuszewski et al., 1999), and the procedure can be executed using a rigid body protocol. The side chains of the interfacial residues, however, are more ideally treated with a conjoined rigid body/torsion angle dynamic protocol that allows side chains to freely rotate and optimize contacts in the interfacial area (Clore and Bewley, 2002; Clore and Schwieters, 2003).

Building Oligomeric Structures Using RCSA Offsets

It is generally the asymmetrical character of a chemical shielding tensor that leads to the orientation dependence of chemical shifts and the changes in observed chemical shifts on partial orientation. These changes, which can add to the information normally available from RDCs, are referred to as residual chemical shift anisotropy (RCSA) offsets. In solution the random rotational motion of a molecule averages chemical shift to a unique value, i.e., the isotropic chemical shift δ_{iso} . The difference between the chemical shift observed under partially aligned conditions, δ_{align} , and δ_{iso} is the RCSA offset. (Sanders and Landis, 1994) RCSAs have similar angular dependent properties to RDCs. This dependence is illustrated in equation 3.

$$\delta^{\text{CSA}} = \delta_{\text{align}} - \delta_{\text{iso}} = \frac{2}{3} \sum_{ijk} S_{ij} \cos(\theta_{ik}) \cos(\theta_{jk}) \delta_{kk} \quad (3)$$

Here the δ_{kk} are the principal elements of the chemical shift tensor and the θ_{ik} are the angles between the chosen molecular frame and the principal axes of a spin's chemical shift tensor. The additional summation index is necessary because, while the RDC equation depends on the orientation of an inter-atom vector in a molecular frame, the RCSA equation depends on the orientation of the CSA principal frame relative to the molecular frame. Therefore in

order to use RCSAs in the structure determination of oligomeric complexes, it is prerequisite to know the eigenvalues of the CSA tensor and the tensor orientation with respect to a relatively “fixed” structural entity, such as a peptide plane. Substantial work has been done in this area, and the CSA tensors of protein backbone nitrogen and carbonyl carbon atoms have been extensively described in the literature (Hartzell et al., 1987; Hiyama et al., 1988; Loth et al., 2005; Oas et al., 1987a; Oas et al., 1987b; Sitkoff and Case, 1998; Teng et al., 1992; Yao et al., 2010).

RCSAs are not quite as convenient to measure as RDCs; shifts are small and accuracy depends on maintaining an environment under isotropic conditions that is identical to anisotropic conditions, so that chemical shift differences reflect only RCSA contributions. Existing studies have made use of the temperature-dependent phase transition of certain liquid crystals (Bryce et al., 2005; Cornilescu and Bax, 2000; Cornilescu et al., 1998; Hansen and Al-Hashimi, 2006; Lipsitz and Tjandra, 2001; Wu et al., 2001; Ying et al., 2006; Yu et al., 2007), concentration-dependent alignment amplitude (Burton and Tjandra, 2006), field-dependent alignment amplitude (Ottiger et al., 1997), and magic angle spinning (Grishaev et al., 2009) to move between aligned and isotropic conditions. However, it is important to correct for temperature or solvent effects for the first two methods; the RCSA from the third method tends to be small and molecules possessing the required field dependent alignment properties are limited; and the fourth method requires special hardware that is not common in high-resolution NMR laboratories. A recent addition to the arsenal is a modified two-stage NMR tube specifically designed for the measurement of RCSAs in stretched polyacrylamide gels, a widely used alignment medium (Liu and Prestegard, 2010). This device also allows for the measurement of RDCs as well as acquisition of reference and aligned spectra for measurement of RCSA offsets under near identical sample conditions. The use of polyacrylamide gels as the source of alignment also extends the utility of RCSAs and RDCs to membrane containing systems. RCSAs for carbonyl carbons in proteins are particularly large, and they are highly complementary to most RDCs because of their sensitivity to vectors oriented perpendicular to the peptide plane.

Since RDCs and RCSAs result from the same anisotropic molecular tumbling process, both are averaged through the same order tensor. Therefore, they can be combined in the SVD analysis for a more robust order matrix determination. This combination can be particularly helpful for small proteins or selectively labeled proteins in which the number of reporter sites is small; it can also be useful in cases where the ^1H - ^{15}N vectors are nearly parallel, as in protein structures having near parallel α -helices.

Interaction Interface Determination

Chemical Shift Surface Perturbation Mapping

As discussed above, the use of orientational constraints from RDCs or RCSAs in the construction of oligomer models usually requires some form of supplemental distance information. The chemical shifts of amide protons and amide nitrogens on the protein backbone are exquisitely sensitive to changes in the magnetic environment, and they are easily measured from standard HSQC or TROSY spectra. Because of this, they have been used as indicators of inter-molecular interactions for some time. (Zuiderweg, 2002) The method has a broad K_d range of effectiveness. In fact, it is capable of detecting both strong interactions and weak interactions. For strong interactions the exchange between bound and free forms is likely to be slow on the NMR time scale and signals from both forms will be observed for sites near the interface. For weak interactions (K_d s in the 10 μM to 10 mM range) exchange is likely to be fast on the NMR time scale with interface signals shifting in response to changes in the proportion of bound and free states. If the system of interest forms heterodimers, the chemical shift changes can be easily extracted by

comparing ^{15}N - ^1H HSQC spectra of the one component, enriched in ^{15}N , in the presence and absence of the other unlabeled component. In the case of homo-dimers, spectra of the protein at a series of different concentrations can be acquired to determine which residues are most affected by the change in the monomer-oligomer proportionality. K_d s for oligomerization can also be determined by plotting intensity loss in the slow exchange case, or chemical shift change in the fast exchange case, versus subunit concentration. As changes showing most sensitivity to K_d take place at concentrations close to the K_d , this method is most applicable to weak dimers with K_d s greater than 10 μM .

The major caveat in chemical shift perturbation mapping is the assumption that chemical shift changes are mainly due to surface perturbation, not propagated structural changes. This is true in most weak associations. Nevertheless, it is always prudent to validate the perturbation results with other techniques. The observed shifts are usually evaluated by the magnitude of residue specific shifts relative to the root-mean square chemical shift deviations (RMSDs) over the entire protein (Farmer, 1996; Otting, 1993). As a rule of thumb, shifts over two standard deviations larger than RMSD are selected, and if perturbed residues form a contiguous surface on the three dimensional structure of the molecule, the reliability of the observation is high.

Information revealed by chemical shift mapping, even in the best of situations, is low-resolution; however, there are procedures for systematically including these types of data in structure refinement. The program HADDOCK in particular has tools for including surface perturbation information as highly ambiguous distance restraints in structure refinements (see the Computational Platform section on details of HADDOCK). In any case, the combination of chemical shift-derived interface information and orientation information from RDCs or RCSA offsets is often sufficient to produce an accurate model of a complex.

A particularly nice example using RDCs as orientational restraints in combination with distance restraints derived from chemical shift perturbations is given by Ortega-Roldan and coworkers in their determination of the structure for the weak complex of the CD2AP SH3 domain and ubiquitin (Ortega-Roldan et al., 2009). The chemical shift perturbations and RDCs for the SH3:ubiquitin complex were obtained by titrating differentially isotopically labeled fragments. The RDCs for the bound form were extracted using dissociation constants and RDCs were collected on samples containing different distributions of oligomeric species. The preliminary dimer structure of the SH3:ubiquitin complex was in this case generated using the HADDOCK program (Dominguez et al., 2003) and interaction restraints derived from chemical shift perturbation. The proposed dimer models from HADDOCK were then refined with CNS by restraining the two components to a common alignment tensor. (Ortega-Roldan et al., 2009)

Paramagnetic surface perturbations and long-range constraints

Paramagnetic effects, which stem from the presence of unpaired electrons in a molecular fragment, provide both qualitative and quantitative sources of distance constraints on oligomeric structures. Electrons have large magnetic moments and, among other things, the variations in vectors (length or orientation) connecting unpaired electron centers with NMR active nuclear sites, induce distance-dependent nuclear magnetic relaxation. Measurement of relaxation effects, often referred to as Paramagnetic Relaxation Enhancements (PREs), has been used extensively in NMR to obtain long range distance restraints between paramagnetic centers and atoms in the same molecular complex. While most structural applications have been to macromolecules naturally containing such centers (Banci et al., 2002; Bertini et al., 2002; Lee and Sykes, 1983), similar paramagnetic perturbations occur when small soluble paramagnetic species are utilized to probe the binding interface of

complexes (Arumugam et al., 1998; Fesik et al., 1991; Niccolai et al., 2001; Petros et al., 1990).

Soluble paramagnetic molecules, such as TEMPO or Gd-DTPA and their variants, produce significant relaxation enhancement in surface residues at low millimolar concentrations. In most instances, oligomerization results in a decrease in total solvent-accessible surface area, protecting the residues in the interface from paramagnetic perturbation. By comparing residue-specific relaxation time changes produced by paramagnetic compounds on oligomeric and non-oligomeric states, surface residues can be identified. For homo-oligomers the most practical way of altering percentages of oligomeric and non-oligomeric states is by dilution. This limits application to complexes with weak to moderate association constants.

The paramagnetic experiments for probing binding surfaces can also be plagued by artifacts. For instance, the net charge of Gd-DTPA at neutral pH conditions (-2) can result in preferential interaction between Gd-DTPA and basic residues on the surface. The possibility of enhanced relaxation being reversed by changes in protein dynamics upon complex formation, even at some distance from the interface, also exists, creating false positives in the analysis. However, an uncharged Gd complex, in the form of Gd-DTPA-BMA, is available. (Pintacuda and Otting, 2001) The use of this complex, which also has reduced hydrophobicity compared to other neutral adducts, is highly recommended. One must also be aware that under circumstances where effective correlation times for the paramagnetic interaction are short, enhancements of both longitudinal and transverse relaxation can be significant. This can have opposing effects on signal intensity if magnetization is not allowed to relax fully between acquisitions, requiring caution in experimental setup and quantitative interpretation. Some of the shortcomings mentioned might be avoided by the use of alternate reagents or observables. For example, pseudo contact shifts might be useful, as might the observation of short range TEMPO induced contact shifts on solvent-exposed ^{13}C atoms reported in a recent article by Moriya et al. (Moriya et al., 2009)

Paramagnetic compounds can also be used to obtain accurate constraints at longer distances by selectively labeling one component in the complex with a paramagnetic label while observing changes in signals from other components. Calcium binding proteins will usually also bind paramagnetic lanthanides, allowing the calcium sites to be converted into natural paramagnetic centers. For non-metal binding proteins, the most popular method for specific placement of a paramagnetic center is introduction of surface cysteines, followed by attachment of the radical MTSL (S-(2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-yl)methyl methanesulfonothioate) through a disulfide bond. (Berliner et al., 1982; Bolin et al., 1998) For proteins containing native disulfide bonds that might be compromised by such treatment, an option may be incorporation of a lanthanide binding peptide sequence in the expression construct for one of the proteins of interest (Nitz et al., 2004; Su et al., 2006; Zhuang et al., 2008). Theoretically, a single paramagnetic center is sufficient to determine or limit choices in contact geometry; however, owing to the error in distance estimates from PRE using flexible adducts, paramagnetic labeling at multiple sites maybe necessary to unambiguously determine the interaction geometry. In the case of homodimers, uniform ^{15}N labeling should be incorporated only in the non-paramagnetically labeled species to avoid confusion in data interpretation. If precise relaxation rates can be measured, simulated annealing in XPLOR-NIH allows the PRE rates to be used as structural constraints directly in oligomeric structural determination.

There is a wealth of studies incorporating both specific long-range and non-specific short-range PRE perturbations in structural investigations of complexes. (Bernini et al., 2006; Eichmueller and Skrynnikov, 2007; Peterson et al., 2008; Wu et al., 2007; Yang et al., 1996)

As a particular example of non-specific short-range PRE perturbation, we cite the work of Lee et al. on the weakly associating NESG target, SeR13. (Lee et al., 2010) This study compared Gd-DTPA induced relaxation perturbations at higher and lower protein concentrations to identify the dimer interface. The region identified overlapped well with the region identified by chemical shift perturbation. Interestingly, besides the surface protection effect seen for residues in the binding site, several residues actually exhibited enhanced relaxation upon dimerization. Examination of the protein sequence revealed that these residues are basic residues that formed a positively charged patch that might have experienced enhanced concentration of Gd-DTPA upon dimerization.

As an example of the use of specific longer-range PRE effects to derive distance constraints and determine dimer structures in NMR we cite a recent study by Sattler et al. This application determined the complex structure of two RNA binding tandem repeats from the human splicing factor U2AF65. The structure of each tandem repeat was solved individually, and through the placement of spin labels at three different locations on each tandem repeat, a network of intermolecular distance constraints connecting the two tandem repeats was obtained. These distance constraints, along with an extensive set of RDCs, allowed the assembly of the dimer. (Simon et al., 2010)

Interface identification from Cross Saturation

Yet another means of interface identification comes from magnetization transfer between spins on different subunits across an oligomer interface. Magnetization transfer, in response to non-equilibrium polarization created in one subunit (by saturation, for example), usually occurs via dipolar interactions among closely proximate spins. In NMR, the network of protons in a macromolecule allows the polarization to be transferred quickly throughout a given subunit and then to a molecule at the subunit's interface. This is the basis for the popular saturation transfer difference (STD) experiment for detecting binding motifs on small ligands using only a small amount of protein. (Mayer and Meyer, 1999) In the cross-saturation case the ligand is a second subunit at the interface. (Takahashi et al., 2000) The experiment requires one partner to be fully protonated while the other is deuterated and ^{15}N -labeled. By saturating protons on the protonated partner, signals from amide protons in the binding interface of the deuterated partner can be attenuated in a manner dependent on the amide proton's distance from the saturated partner. Ideally, tabulating the differences in signal intensity with and without saturation would reveal residues involved in the interface. A refinement of the technique uses protonated methyl protons in deuterated proteins as reporters of cross saturation. (Takahashi et al., 2006) This increases the sensitivity of detection both by reducing the need for a protonated solvent and by placing the reporter closer to the binding interface. The technique is applicable to complexes with a range of binding affinities and molecular weights. So far, the technique has been used to study a variety of hetero dimeric complexes (see (Shimada et al., 2009) for a detailed review) and it has proven most useful in the study of complexes between receptors and their targets. (Kofuku et al., 2009; Nakamura et al.)

Constructing Oligomer Models using Distance Information from EPR

EPR is a high sensitivity technique that can provide unique distance information for various types of biological systems (Banham et al., 2006; Hilger et al., 2005; Jeschke et al., 2004b; Jeschke et al., 2005; Nakamura et al., 2005; Zhou et al., 2005). In EPR, long range distance information between subunits in an oligomeric complex is usually derived from dipolar coupling between two electron spins. The two spins, on nitroxide spin-labels, for example, can be introduced into a protein using methods similar to those mentioned in the paramagnetic surface perturbation section of this review. The most commonly used EPR experiment for measuring long distances ($> 20 \text{ \AA}$) in biomolecules is the four pulse double

electron-electron resonance experiment (DEER) (Jeschke, 2002; Pannier et al., 2000). In principle, the DEER experiment can extract the distance between two spin labels that are less than 8.0 nm apart (Borbat et al., 2004; Godt et al., 2006; Jeschke et al., 2004a). At these longer distances protein samples are often deuterated to remove the influence of proton spin diffusion effects, thus increasing the total dipolar evolution time. The experiment is also usually carried out at a temperature of less than 70 K to reduce the motional averaging of the dipolar interactions (Jeschke and Polyhach, 2007; Steinhoff, 2004). Distances between two spin labels that are less than 2 nm apart can be measured using a CW EPR measurement (Rabenstein and Shin, 1995).

A recent example combining distance information from DEER measurements with NMR structure determination is the dimeric structure determination of the Northeast Structural Genomic Target, DhR8C (PDB accession code 2KYI). (Yang et al., 2010) This 62 amino acid protein exists as a 13.6 kDa homodimer in solution. Spin labels were introduced at S16C and S32C using MTSL chemistry. In this case, structure determination of the dimer using conventional ^{13}C -filtering/editing experiments was challenging due to factors such as the slow dimer dissociation kinetics that limit the formation of isotopically mixed dimers and the limited number of NOE-based side chain interactions seen between subunits.

Protein Footprinting & Mass Spectrometry

Mass spectrometry can also be used in the identification of interaction surfaces. Compared to the NMR-based methods mass spectrometry has an enormous sensitivity advantage. Most of the mass-spec based methods depend on alteration of the mass for exposed protein residues, and protection of those residues from alteration on complex formation. In early studies, mass alteration was accomplished using proton-deuterium exchange of moderately labile surface exposed amide protons (Englander et al., 1979; Englander and Kallenbach, 1983; Gregory and Rosenberg, 1986; Woodward et al., 1982). However, the rate of exchange of these protons is more a function of the stability of secondary structure elements than surface exposure, and the interpretation of results can be complex. More recent studies have relied on highly reactive chemical reagents to differentially modify exposed side chains in complexed and uncomplexed states, thus mapping protein interaction surfaces. One example is the use of highly reactive free radicals to randomly label surface exposed residues. (Takamoto and Chance, 2006) The advantage of the free radical footprinting technique lies in the rapid nature of the radical reaction and the ease of detection of the modified segments. Nevertheless, reactivity toward each amino acid depends on both side chain type and solvent exposure, complicating analysis. Also, the final analysis is often carried out, not at the residue level, but at the level of the peptide fragments, lowering resolution. However, Xu et al. have tabulated the most common hydroxyl modification and reactivity of each amino acid side chain, allowing solvent exposure to be estimated more quantitatively. Several techniques have also been used to generate radicals for the reaction. Chance et al., for example, pioneered the generation of hydroxyl radicals through γ -ray and X-ray induced water radiolysis. (Maleknia et al., 1999; Sclavi et al., 1998) Recent work by several groups has explored the possibility of using high energy UV laser induced hydrogen peroxide photolysis and high energy electron beam water radiolysis to generate the same radicals (Aye et al., 2005; Hambly and Gross, 2005; Watson et al., 2009). The latter approaches, when used in the presence of quenching agents, are capable of labeling proteins at a sub- μs time scale, thus avoiding the detection of protein structure changes associated with an initial radical labeling event.

The use of hydroxyl radical footprinting to analyze structures of macromolecular complexes is still a fledgling area under active development, yet because of its high sensitivity, the approach has already been successfully utilized in a number of investigations to probe protein structure changes as well as protein-protein and protein-DNA interactions. (Gupta et

al., 2007; Kamal et al., 2007; Orban et al.; Takamoto et al., 2007) When combined with computational modeling techniques, unambiguous high resolution models can be generated from the MS data. The utility of MS data in structural biology is well exemplified in the study of the G-actin to F-actin transition for which Mg^{2+} induced polymerization is a key step. Using the footprinting technique, Chance and coworkers were able to study the difference between Mg^{2+} G-actin and Ca^{2+} G-actin, a slower polymerizing variant of G-actin. Their data showed that Mg^{2+} G-actin possesses a closed cleft that was open in Ca^{2+} bound G-actin, establishing a first step in understanding the fast ATP hydrolysis of Mg^{2+} G-actin. (Takamoto et al., 2007) While applications to this point have been minimal, MS footprinting data can clearly be combined with NMR orientational data to generate models of oligomeric complexes.

Computational Approaches to Interface Identification

Even with the variety of experimental techniques available, there will be cases where the ability to acquire experimental data is limited. For example, sparse isotopic labeling of proteins may be the only option for proteins requiring expression in mammalian cell culture. (Bruggert et al., 2003; Meng et al., 2006) The resulting limited number of sites may be enough to obtain orientational constraints from RDC data, but may not be enough to ensure detection of paramagnetic surface perturbation or chemical shift perturbation. Fortunately, purely computational methods may ultimately provide a reliable route to identification of oligomer interfaces. The protein-protein docking field is well established and has been reviewed thoroughly elsewhere (Andrusier et al., 2008; de Vries and Bonvin, 2008; Vakser and Kundrotas, 2008). While it is fair to say that accurate prediction of interaction surfaces from first principles continues to be challenging, small amounts of experimental data clearly help. For example, knowledge of the relative orientation between the units greatly reduces the number of possible candidates, thus boosting the likelihood of success. (De Vries et al., 2007) Montalvao et al. recently also demonstrated that chemical shifts can be a good aide to computationally predicting interaction interfaces. (Montalvao et al., 2008) Knowledge-based scoring functions for evaluating interfaces can also be of value in combination with experimental data. In the study of Sr360, Wang et al. utilized a simple scoring matrix, based on likelihood of intermolecular residue level contact, derived from known PDB structures. (Moont et al., 1999; Wang et al., 2008) Similar scoring matrices have been developed based on sophisticated machine learning algorithms and incorporate a much wider array of parameters. (de Vries and Bonvin, 2008) The accuracy of the prediction is also enhanced by optimization of side chain geometries at the proposed interaction site. Wang et al. used the molecular dynamics program NAMD (Phillips et al., 2005) to relax the side chains after the dimer models have been constructed. However, the use of RosettaDock (Gray et al., 2003), a docking program that specializes in local geometry optimization, may improve the accuracy even more. This combination of experimental data and the use of a knowledge based scoring function can reduce the need for high resolution, unambiguous structural information.

Global Shape from Scattering & Hydrodynamic measurements

Shape information from SAXS

Small angle X-ray scattering (SAXS), very much like long-range paramagnetic distance constraints, can provide more than an identification of an interaction interface; it can provide data on the actual shape of a complex and can do so in solution. SAXS has been utilized productively by structural biologists for some time. However, increased desire to study macromolecular complexes in solution has triggered a resurgence of interest in combining SAXS with high resolution techniques such as X-ray crystallography and solution NMR (Grishaev et al., 2005; Putnam et al., 2007; Wang et al., 2009). In SAXS, structural information is embodied in the pair-wise distance distribution function, obtained from the

reverse Fourier transform of an X-ray scattering curve. (Koch et al., 2003) In general this is more discriminating than techniques that depend on hydrodynamic parameters, including dynamic light scattering, analytical ultracentrifugation, and anisotropic spin relaxation analysis of NMR data. Like any scattering technique, the sensitivity of SAXS is dependent on both the size of the complex and the concentration. In general, 5 to 10 mg/ml is required for good sensitivity with non-synchrotron X-ray sources as opposed to concentrations on the order of 1 mg/ml for synchrotron sources. The scattering curves of homogeneous samples are most easily interpreted. Scattering curves from inhomogeneous samples, such as samples of weakly associating complexes, are still interpretable if scattering curves at several different concentrations are acquired. If the oligomer species distribution is known, a system of linear equations can be used to calculate the scattering curve for each species based on the populations of each at a particular concentration. If the K_d of interaction is not known, given data of sufficient quality and quantity, it may also be possible to extract a K_d as well as the component scattering curves from concentration dependent data. (Bilgin et al., 1998; Fetter et al., 1995; Fowler et al., 1983; Perez et al., 2001) However, optimization of the approach for direct structure determination, aided by NMR data, is still evolving and performance will have to be evaluated as applications appear.

Using SAXS data to score or test models of complexes is more advanced. Since the theory of solution X-ray scattering is quite well developed and the scattering factors of chemical functional groups in biomolecules have been extensively tabulated, it is not difficult to calculate the scattering profile of a protein complex from a proposed atomic resolution model. Currently, this allows SAXS information to be combined with data from NMR and be incorporated into various computational platforms using a pseudo potential. (Schwieters et al., 2003; Wang et al., 2009) However, because of the low resolution of the information, it often carries less weight in the scoring function than high resolution data, or is included only late in the structure refinement process.

SAXS has always been a useful biophysical tool for studying macromolecule oligomeric distributions (Carvalho-Alves et al., 2000; Crennell et al., 2006; Ferreira-da-Silva et al., 2006; Fujisawa et al., 1997; Harada et al., 1991; Segel et al., 1999; Shilton et al., 2002; Wang et al., 1989). Its recent adoption by the solution NMR community has proven especially fruitful. This is in part due to the experimental convenience of having data from both techniques collected at similar concentrations and in similar solutions. A typical example of SAXS's utility as a primary source of structural information was demonstrated by Sousa et al. in their study of the protease-chaperone complex HslUV. (Sousa et al., 2000) Using SAXS combined with X-ray crystallography, Sousa et al. were able to determine that the solution conformation of the HslUV complex is in fact much more compact than indicated by an earlier crystal structure.

The first published example of a dimeric protein structure determined using NMR and SAXS data came from a recent study by Parsons et al., where the authors utilized both RDC and SAXS data to assemble the dimer model from a monomer structure determined using traditional solution NMR techniques. (Parsons et al., 2008) Wang and coworkers more recently demonstrated the use of RDC orientational restraints along with SAXS shape restraints in the construction of oligomeric structures for both homo- and hetero- multi-domain proteins (Wang et al., 2009). The GASR program was devised for this purpose (Wang et al., 2009). In the GASR algorithm, both fragments of a complex or multi-domain protein are first placed in a common frame as defined by alignment tensors determined for individual subunits. A grid search is employed by translating one of the fragments around an orientationally fixed partner in the four allowed degenerate orientations to generate models with all possible binding interfaces. The calculated models are then assessed based on the agreement between the back-predicted SAXS and experimental SAXS data.

An extremely impressive example of the complementation between X-ray or neutron scattering and solution NMR has just been published by Schwieters et al. (Schwieters et al., 2010) In this study, the authors investigated the conformation adopted by the 128 kDa dimer of the bacterial protein Enzyme I. Enzyme I is a two domain protein with the N-terminal domain attached flexibly to the C-terminal dimerization domain. To find out the solution conformation of the entire protein, RDCs were first used to determine the orientation of the N-terminal domain relative to the C-terminal domain; small and wide angle X-ray scattering data were then used as shape constraints in a simulated annealing search for the best position of the N-terminal domain relative to the C-terminus. The resulting best-fit conformation was further validated independently with small angle neutron scattering data.

Shape from Ion Mobility Mass Spectrometry

Another MS technique that might be of interest to structural biologists is ion mobility MS. (Ruotolo et al., 2005) In this technique, the intact complex is ionized (by electrospray) and passed through a drift chamber filled with inert gas. The speed with which the ions can traverse the column is determined by the collisional cross-section of the complex. When coupled with MS, ion mobility MS allows the separation of heterogeneous oligomers, along with determination of their respective masses and collisional cross-sections. So far, the technique has been used to analyze several large protein aggregates including RNA binding proteins, chaperones and intact viral capsids. (Ruotolo et al., 2005; Uetrecht et al., 2008; van Duijn et al., 2009) Cross sectional areas provide very low resolution structural information, very much along the lines of a hydrodynamic measurement. However, it does this with very small sample requirements and a ready identification of species via direct analysis of subunit masses. The utility of the information will ultimately rely on the complex retaining its native shape during drift in the gas phase. Whether or not retention of shape is a general phenomenon will require a systematic examination of numerous data sets.

Computational platforms for integrating NMR and other structural data

Data from complementary techniques yielding specific atom-based structural information, can often be incorporated into existing NMR-based structure refinement programs. (Brunger et al., 1998; Schwieters et al., 2003) This is particularly easy for distance based constraints such as those that come from PRE effects. Distance-dependent penalty function, or pseudo functions, already exist as a means of incorporating NOE constraints, and longer-range constraints on the distance separation of a paramagnetic center and a particular nuclear site can use the same functions. However, refinement against functions that directly treat relaxation enhancement data may be preferred; this procedure has been implemented in XPLOR-NIH. (Schwieters et al., 2003) Multiple subunits are easily incorporated in NMR-based structure refinement programs, and parts of subunits can often be designated for movement as a rigid body.

Improved data handling from techniques that produce global structural information, such as shape from SAXS, and intermolecular orientation from RDC & CSA is continually evolving in structure refinement programs like XPLOR-NIH. (Schwieters et al., 2003) XPLOR-NIH provides specific pseudo potentials for these data types, allowing comparison of model-based predicted data with experimental data during search processes. XPLOR-NIH also provides facilities for rigid body docking and ensemble fitting in cases where two or more conformations maybe present simultaneously. Automated structure determination programs such as ARIA & CYANA are also incorporating RDC and PRE data into their algorithms for NOE-based structure determination. (Herrmann et al., 2002; Nilges et al., 1997)

Ambiguous information produced by either chemical shift or paramagnetic surface perturbation should always be treated with care. The program HADDOCK contains an

ambiguous interaction restraint module that allows highly ambiguous information to be included as distance constraints between multiple atoms. (Dominguez et al., 2003) The module takes a comprehensive approach to the constraints by including distances between all possible atom pairings in its scoring function. The model with the smallest sum of inter-atomic distances between all pairs of atom in the ambiguous restraint will be assigned the least energetic penalty by the ambiguous interaction restraint module. As the module uses the ambiguous distance constraint feature in CNS, its energetic weighting can be controlled independently of non-ambiguous NOEs and it can be used in combination with all other pseudo potentials.

In many cases where component structures are known and RDCs provide definitive orientation information between the components in a complex, it can be advantageous to reduce a structural search to movement on a translational grid while scoring the binding interface and the shape of the complex. This scoring approach easily accommodates the types of ambiguous restraints that come from interface identification as well as shape evaluations and molecular interaction potentials on atomic or residues levels. Neither XPLOR-NIH nor CNS provides a default protocol for conducting translational grid searches. However, both programs contain a full complement of built-in functions for manipulating coordinates, making the implementation of translational grid searches within the framework of these programs a possibility. A number of authors have also devised custom programs or scripts to implement these procedures. (Wang et al., 2009; Wang et al., 2008). Criteria for evaluating the validity of the models generated by the grid search will depend mostly on the experimental data available.

A new breed of computational platforms for dealing with low resolution data is also in development. The most prominent example among them is the Integrated Modeling Platform (IMP) created by Sali and co-workers. (Alber et al., 2007) IMP is designed to study large complexes that have a high number of components and are transient in nature. It is capable of handling ambiguous data from biochemical studies, such as immuno-precipitation assays that identify protein-protein interactions and immuno-EM data that specify the placement of a component in the complex. Instead of high resolution structures, low-resolution shape information of the components from SAXS or analytical ultracentrifugation is used. The program can also easily take into consideration stoichiometry and symmetry information to greatly restrict the configuration space available. This approach of combining powerful computational techniques with highly ambiguous data from multiple sources can be applied to a large number of biological systems that have been beyond capabilities of conventional structural biology techniques.

Oligomer of CCL5: integrating diverse structural information

As a final example, we present a previously unpublished application that incorporates several of the above approaches. The application is to CCL5, or RANTES, a member of a family of small signaling proteins known as chemokines. The members of the family are secreted by endothelial cells and their accumulation helps to build up a gradient that directs the migration of lymphocytes from the blood stream to sites of injury. Like many chemokines, wild type CCL5 oligomerizes prodigiously at physiological pH, and although monomeric forms of CCL5 are found bound to the receptor CCR5 *in vitro*, *in vivo* assays show that the formation of the CCL5 oligomer is crucial in the migration of lymphocytes and that a tetramer is the minimal oligomer size capable of activating the CCR5 receptor on the lymphocyte surface. (Appay et al., 1999; Czaplewski et al., 1999) Interest in this particular system is heightened by the fact that CCR5 is the co-receptor, along with CD4, for HIV during the HIV infection process. (Alkhatib et al., 1996; Dragic et al., 1996) Mutants of

CCL5 have been designed to compete with HIV for CCR5 (Appay et al., 1999; Czaplewski et al., 1999), and oligomerization properties of these mutants may also be of interest.

The existence of non aggregating mutants of CCL5 (E66S) has aided the determination of CCL5 dimer structures using both X-ray crystallography and solution NMR.(Chung et al., 1995; Shaw et al., 2004; Skelton et al., 1995) These high resolution structures reveal that CCL5 forms a typical CC-type dimer with the N-terminal β -strand acting as the dimerization element. However, the high aggregation propensity of wild type CCL5 at physiological pH has made the application of conventional structure determination techniques to higher oligomers impractical. A tetramer structure could potentially provide important information on the mechanism of higher order oligomer formation. It may also provide a glimpse into the structural basis for glycosaminoglycan-promoted CCL5 oligomerization, something that is also of physiological interest. However, the crystal and NMR structures of E66S-CCL5 solved so far, provide no indication of a possible tetramerization interface. Our approach is to study the oligomer using wild type CCL5 at pH 4.5 rather than the dimeric mutant. Acidic pH greatly reduces the oligomeric tendency of wild type CCL5, allowing us to study the protein in predominantly tetramer form at millimolar concentrations using solution NMR and small angle X-ray scattering.

Both NMR translational diffusion studies and measurement of transverse relaxation rates confirmed that at pH 4.5, wild type CCL5 at millimolar concentrations exists mostly as a tetramer. In particular, the diffusion coefficient of wild type CCL5 was 32% less than that of the dimeric E66S mutant of the protein. As diffusion coefficients are inversely proportional to the radius of gyration, this decrease signals roughly a doubling in the volume of wild type CCL5 compared to the mutant. The tetrameric state was also confirmed by the observation that the ^{15}N transverse relaxation constant of the wild type is twice as high as that of the E66S mutant. The NMR evidence was further corroborated by ion mobility MS, which detected the tetramer as the predominant oligomer form at pH 4.5. However, small amounts of monomer, dimer and species up to octamer were also detected. The data were collected at 20 μM in 50 mM acetate, pH 4.5, buffer as opposed to 200 μM in 50 mM acetate, pH 4.5, buffer for the NMR data. The lower protein concentration may explain the significant monomer and dimer populations, or they may be the result of disruption of oligomer structures in the gas phase experiments. The MS data do, however, provide a cautionary note regarding the possible existence of several different oligomer forms.

To determine the orientational properties of the wild type CCL5 subunits, RDCs of both the mutant and the wild type CCL5 in stretched 6% polyacrylamide gel were measured. The data were collected using interleaved ^{15}N -HSQC & TROSY experiments on uniformly ^{15}N labeled wild type and E66S CCL5. Surprisingly, both forms showed a similar degree of alignment in the gel and corresponding residues in the two forms possessed similar RDC values. SVD fitting of the RDCs to the structured regions of the wild type CCL5 dimer gave principal order tensor components of 0.87×10^{-4} (S_{xx}), 1.62×10^{-3} (S_{yy}) and -1.71×10^{-3} (S_{zz}). This compares to principal order tensor elements of 2.33×10^{-4} (S_{xx}), 1.36×10^{-3} (S_{yy}) and -1.59×10^{-3} (S_{zz}) for RDCs from the E66S mutant in a similar gel. Both sets of RDCs fit the known dimer structure well: fitting the E66S RDCs to the structure produced a quality factor of 0.20 while the wild type RDCs produced a quality factor of 0.24. Correlation plots of experimental RDCs and their back calculated values for both wild type and E66S mutants are shown in Figure 2. As RDC values of symmetric oligomers reflect the orientation of the symmetry tensor, the similarity in RDCs between the proteins suggests that the symmetry axis, as well as the structural detail of the dimer structure, is preserved in the tetramer. Figure 3 displays the dimer structure with the symmetry axis and the orientation of the alignment tensor as determined by the E66S RDCs. The orientation of

the symmetry axis, as seen in the crystal structure of the mutant, differs by only 11 degrees from the X-axis of the alignment tensor principle axis frame.

Small angle X-ray scattering of the wild type protein was carried out at a protein concentration of 10 mg per ml of 50 mM acetate, pH 4.5, buffer. The data were collected using a Bruker Nanostar U X-ray system equipped with a 1.54 Å X-ray source. The sample was irradiated for one hour in a 1.8 mm quartz capillary tube, which was also used to collect the background scattering on a buffer sample. The 2D scattering patterns of both the background and the sample were reduced to 1D scattering curves using Bruker's SAXS software and the scattering profile of the protein was determined as the difference between them. Analysis of the SAXS data showed that the complex has an average radius of gyration (R_g) of 32 Å. The molecular mass obtained by extrapolating the intensity to a scattering angle of zero and comparing it to calmodulin indicates the average weight of the complex in solution is approximately 40 kDa. This once again points to the tetramer as being the predominant species in the solution, possibly with a small amount of higher oligomer also present. The *ab initio* fitting of the oligomer shape based on the scattering curve showed that the oligomer possesses an irregular, elongated shape that does not correspond to the spherical tetramer seen with some other types of chemokines. (Lau et al., 2004; Swaminathan et al., 2003) Figure 4 shows the back-calculated scattering curves for four distinct models. They all employ the dimer structure of the E66S mutant, as justified by the similarity of ^{15}N -HSQCs of the mutant and wild type protein and the fit of wild type RDCs to the mutant structure. For the first two models the placements of the dimers within the tetramer are based on observed structures of other chemokines: one is based on the MCP-1 tetramer (Lau et al., 2004) and the other is based on the M-form of IP-10 tetramer. (Swaminathan et al., 2003) Best fit curves calculated from both of these models have unreasonably high χ values when compared to experimental data. The third and fourth models are derived as described below using a set of in-house scripts. However, the procedure parallels the recently published GASR program. (Wang et al., 2009)

Using the symmetry axis of the dimer as a constraint, a simple grid search was devised to generate unbiased tetramer models that best fit the SAXS scattering curve. In the grid search, one dimer unit was moved in a plane having another dimer unit fixed at the origin and having the dimer symmetry axis as its normal. Movements were constrained by insisting the units share the full alignment axis system (Figure 5). All possible grid locations were taken into consideration except those exhibiting high steric clashes or the lack of any van der Waals contacts. The remaining tetramer models were then ranked according to the agreement between the experimental scattering curve and the theoretical scattering curve of the model calculated using the program CRY SOL. (Svergun et al., 1995) Figure 6A shows the contour plot of the χ values for the models generated in the grid search. Four locations are identified as having good fits to the scattering curve (points 13×12, 54×45, 4×43, 61×18). Due to the symmetrical nature of the tetramer, the models at the four locations are in fact rotationally degenerate. Figure 7 shows the two non-degenerate configurations that fit the scattering curve best (χ values of 1.4 and 1.9 respectively). In addition to the models generated by translation in the plane, two more models satisfying the symmetry axis constraint can be produced by placing one dimer directly above or below the other. The χ values for these models are significantly higher, 3.9 and 2.9 respectively. The two models shown in Figure 7 exhibit remarkable resemblance to the shape derived from *ab initio* calculation (Figure 8) carried out using the DAMMIF program. (Franke and Svergun, 2009)

To unambiguously distinguish between the two tetramer models, some independent information on the interface would be useful. The residue pairing potential as described in the computational interface identification section above was calculated for the two models (Figure 6B). Tetramer model A has a score of 3.9 and tetramer B has a score of -2.67.

Examination of the models shows that tetramer A possesses a more favorable interface owing to the existence of possible electrostatic interactions connecting E66 of one dimeric subunit with K25 of the second dimeric unit. In tetramer B, however, the interaction surface is dominated by an aggregation of basic residues, creating an unlikely union.

The selected structure has a significant precedent in the recent crystal structure of the chemokine CXCL12. (Murphy et al., 2010) This crystal structure shows a decamer assembly of five dimers. Although CXCL12 belongs to the CXC, rather than CC family, and has only a 26% sequence identity with CCL5, CC-like dimer units are recognizable, and the interface between dimers is very similar to that proposed above. A comparison of ribbon diagrams for the CXCL12 tetramer and our derived tetramer for CCL5 is presented in Figure 9. Thus, our study of the CCL5 transient oligomer produces a reasonable structure for the next stage in CCL5 oligomerization. However, the selected structure should not be taken as a final statement on the structure of the CCL5 tetramer. Ideally one would supplement the residue pairing scoring function with some experimental data on interface identification, and given the indication of some higher order oligomers in the preparations, one would collect SAXS data at several concentrations to eliminate contributions from these oligomers. The preliminary structure is presented here more as an illustration of the potential of combining non-traditional NMR data with data from other biophysical techniques to generate structures normally beyond reach of traditional NMR methods. A refined model will be presented when additional data are available.

Acknowledgments

We are grateful to Bruker AXS for use of their NANOSTAR system and to Brian Jones for help with collecting and evaluating the bioSAXS data on wild type CCL5, to Joshua Sharp & Caroline Watson of University of Georgia for performing the ion mobility MS experiment on wild type CCL5, and to Michael Kennedy of the Miami University for sharing his experiences with DEER data. We also acknowledge the National Center for Research Resources (a part of the NIH) for financial support of the Resource for Integrated Glycotechnology and the CCL5 project (P41-RR005351), the National Institute of General Medical Sciences Protein Structure Initiative for support of H-W. L. and Y. L. as a part of the NESG consortium (U54-GM074958, G.T. Montelione, PI), and the National Institute of General Medical Sciences K99 program for support of X.W. (K99GM088483). The content of this work is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Abbreviations

RDC	residual dipolar coupling
RCSA	residual chemical shift anisotropy
PRE	paramagnetic relaxation enhancement
SAXS	small angle X-ray scattering
EPR	electron paramagnetic resonance
DEER	double electron-electron resonance
HSQC	heteronuclear single quantum coherence
TROSY	transverse relaxation optimized spectroscopy

References

- Al-Hashimi HM, Bolon PJ, Prestegard JH. Molecular symmetry as an aid to geometry determination in ligand protein complexes. *J Magn Reson* 2000a;142:153–8. [PubMed: 10617446]
- Al-Hashimi HM, Valafar H, Terrell M, Zartler ER, Eidsness MK, Prestegard JH. Variation of molecular alignment as a means of resolving orientational ambiguities in protein structures from dipolar couplings. *Journal of Magnetic Resonance* 2000b;143:402–406. [PubMed: 10729267]

- Alber F, Dokudovskaya S, Veenhoff LM, Zhang WZ, Kipper J, Devos D, Suprpto A, Karni-Schmidt O, Williams R, Chait BT, Rout MP, Sali A. Determining the architectures of macromolecular assemblies. *Nature* 2007;450:683–694. [PubMed: 18046405]
- Alkhatib G, Combadiere C, Broder CC, Feng Y, Kennedy PE, Murphy PM, Berger EA. CC CKRS: A RANTES, MIP-1 alpha, MIP-1 beta receptor as a fusion cofactor for macrophage-tropic HIV-1. *Science* 1996;272:1955–1958. [PubMed: 8658171]
- Andre I, Strauss CEM, Kaplan DB, Bradley P, Baker D. Emergence of symmetry in homooligomeric biological assemblies. *Proceedings of the National Academy of Sciences of the United States of America* 2008;105:16148–16152. [PubMed: 18849473]
- Andrusier N, Mashiah E, Nussinov R, Wolfson HJ. Principles of flexible protein-protein docking. *Proteins-Structure Function and Bioinformatics* 2008;73:271–289.
- Appay V, Brown A, Cribbes S, Randle E, Czaplowski LG. Aggregation of RANTES is responsible for its inflammatory properties - Characterization of nonaggregating, noninflammatory RANTES mutants. *Journal of Biological Chemistry* 1999;274:27505–27512. [PubMed: 10488085]
- Arumugam S, Hemme CL, Yoshida N, Suzuki K, Nagase H, Bejanskii M, Wu B, Van Doren SR. TIMP-1 contact sites and perturbations of stromelysin 1 mapped by NMR and a paramagnetic surface probe. *Biochemistry* 1998;37:9650–9657. [PubMed: 9657677]
- Aye TT, Low TY, Sze SK. Nanosecond laser-induced photochemical oxidation method for protein surface mapping with mass spectrometry. *Anal Chem* 2005;77:5814–22. [PubMed: 16159110]
- Banci L, Bertini I, Cavallaro G, Luchinat C. Chemical shift-based constraints for solution structure determination of paramagnetic low-spin heme proteins with bis-His and His-CN axial ligands: the cases of oxidized cytochrome b(5) and Met80Ala cyano-cytochrome c. *Journal of Biological Inorganic Chemistry* 2002;7:416–426. [PubMed: 11941499]
- Banham JE, Timmel CR, Abbott RJ, Lea SM, Jeschke G. The characterization of weak protein-protein interactions: evidence from DEER for the trimerization of a von Willebrand Factor A domain in solution. *Angew Chem Int Ed Engl* 2006;45:1058–61. [PubMed: 16402415]
- Bax, A.; Kontaxis, G.; Tjandra, N. *Nuclear Magnetic Resonance of Biological Macromolecules*. Academic Press Inc; San Diego: 2001. Dipolar couplings in macromolecular structure determination; p. 127-174.
- Berliner LJ, Grunwald J, Hankovszky HO, Hideg K. A Novel Reversible Thiol-Specific Spin Label - Papain Active-Site Labeling and Inhibition. *Analytical Biochemistry* 1982;119:450–455. [PubMed: 6280514]
- Bernini A, Spiga O, Clutti A, Venditti V, Prischi F, Governatori M, Bracci L, Lelli B, Pileri S, Botta M, Barge A, Laschi F, Niccolai N. NMR studies of BPTI aggregation by using paramagnetic relaxation reagents. *Biochimica Et Biophysica Acta-Proteins and Proteomics* 2006;1764:856–862.
- Bertini I, Luchinat C, Parigi G. Paramagnetic constraints: An aid for quick solution structure determination of paramagnetic metalloproteins. *Concepts in Magnetic Resonance* 2002;14:259–286.
- Bewley CA, Clore GM. Determination of the relative orientation of the two halves of the domain-swapped dimer of cyanovirin-N in solution using dipolar couplings and rigid body minimization. *Journal of the American Chemical Society* 2000;122:6009–6016.
- Bilgin N, Ehrenberg M, Ebel C, Zaccari G, Sayers Z, Koch MHJ, Svergun DI, Barberato C, Volkov V, Nissen P, Nyborg J. Solution structure of the ternary complex between aminoacyl-tRNA, elongation factor Tu, and guanosine triphosphate. *Biochemistry* 1998;37:8163–8172. [PubMed: 9609712]
- Bolin KA, Hanson P, Wright SJ, Millhauser GL. An NMR investigation of the conformational effect of nitroxide spin labels on Ala-rich helical peptides. *Journal of Magnetic Resonance* 1998;131:248–253. [PubMed: 9571100]
- Bolon PJ, Al-Hashimi HM, Prestegard JH. Residual dipolar coupling derived orientational constraints on ligand geometry in a 53 kDa protein-ligand complex. *J Mol Biol* 1999;293:107–15. [PubMed: 10512719]
- Borbat PP, Davis JH, Butcher SE, Freed JH. Measurement of large distances in biomolecules using double-quantum filtered refocused electron spin-echoes. *J Am Chem Soc* 2004;126:7746–7. [PubMed: 15212500]

- Bruggert M, Rehm T, Shanker S, Georgescu J, Holak TA. A novel medium for expression of proteins selectively labeled with N-15-amino acids in *Spodoptera frugiperda* (Sf9) insect cells. *Journal of Biomolecular NMR* 2003;25:335–348. [PubMed: 12766395]
- Brunger AT, Adams PD, Clore GM, DeLano WL, Gros P, Grosse-Kunstleve RW, Jiang JS, Kuszewski J, Nilges M, Pannu NS, Read RJ, Rice LM, Simonson T, Warren GL. Crystallography & NMR system: A new software suite for macromolecular structure determination. *Acta Crystallographica Section D-Biological Crystallography* 1998;54:905–921.
- Bryce DL, Grishaev A, Bax A. Measurement of ribose carbon chemical shift tensors for A-form RNA by liquid crystal NMR spectroscopy. *J Am Chem Soc* 2005;127:7387–7396. [PubMed: 15898787]
- Burton RA, Tjandra N. Determination of the residue-specific N-15 CSA tensor principal components using multiple alignment media. *J Biomol NMR* 2006;35:249–259. [PubMed: 16823597]
- Carvalho-Alves PC V, Hering R, Oliveira JMS, Salinas RK, Verjovski-Almeida S. Requirement of the hinge domain for dimerization of Ca²⁺-ATPase large cytoplasmic portion expressed in bacteria. *Biochimica Et Biophysica Acta-Biomembranes* 2000;1467:73–84.
- Chung CW, Cooke RM, Proudfoot AE, Wells TN. The three-dimensional solution structure of RANTES. *Biochemistry* 1995;34:9307–14. [PubMed: 7542919]
- Clore GM. Accurate and rapid docking of protein-protein complexes on the basis of intermolecular nuclear overhauser enhancement data and dipolar couplings by rigid body minimization. *Proc Natl Acad Sci U S A* 2000;97:9021–5. [PubMed: 10922057]
- Clore GM, Bewley CA. Using conjoined rigid body/torsion angle simulated annealing to determine the relative orientation of covalently linked protein domains from dipolar couplings. *J Magn Reson* 2002;154:329–35. [PubMed: 11846592]
- Clore GM, Schwieters CD. Docking of protein-protein complexes on the basis of highly ambiguous intermolecular distance restraints derived from 1H/15N chemical shift mapping and backbone 15N-1H residual dipolar couplings using conjoined rigid body/torsion angle dynamics. *J Am Chem Soc* 2003;125:2902–12. [PubMed: 12617657]
- Cornilescu G, Bax A. Measurement of proton, nitrogen, and carbonyl chemical shielding anisotropies in a protein dissolved in a dilute liquid crystalline phase. *Journal of the American Chemical Society* 2000;122:10143–10154.
- Cornilescu G, Marquardt JL, Ottiger M, Bax A. Validation of protein structure from anisotropic carbonyl chemical shifts in a dilute liquid crystalline phase. *J Am Chem Soc* 1998;120:6836–6837.
- Crennell SJ, Cook D, Minns A, Svergun D, Andersen RL, Karlsson EN. Dimerisation and an increase in active site aromatic groups as adaptations to high temperatures: X-ray solution scattering and substrate-bound crystal structures of *Rhodothermus marinus* endoglucanase Cel12A. *Journal of Molecular Biology* 2006;356:57–71. [PubMed: 16343530]
- Czaplewski LG, McKeating J, Craven CJ, Higgins LD, Appay V, Brown A, Dudgeon T, Howard LA, Meyers T, Owen J, Palan SR, Tan P, Wilson G, Woods NR, Heyworth CM, Lord BI, Brotherton D, Christison R, Craig S, Cribbes S, Edwards RM, Evans SJ, Gilbert R, Morgan P, Randle E, Schofield N, Varley PG, Fisher J, Waltho JP, Hunter MG. Identification of amino acid residues critical for aggregation of human CC chemokines macrophage inflammatory protein (MIP)-1 alpha, MIP-1 beta, and RANTES - Characterization of active disaggregated chemokine variants. *Journal of Biological Chemistry* 1999;274:16077–16084. [PubMed: 10347159]
- de Vries SJ, Bonvin A. How proteins get in touch: Interface prediction in the study of biomolecular complexes. *Current Protein & Peptide Science* 2008;9:394–406. [PubMed: 18691126]
- De Vries SJ, van Dijk ADJ, Krzeminski M, van Dijk M, Thureau A, Hsu V, Wassenaar T, Bonvin AMJJ. HADDOCK versus HADDOCK: New features and performance of HADDOCK2.0 on the CAPRI targets. *Proteins-Structure Function and Bioinformatics* 2007;69:726–733.
- Dominguez C, Boelens R, Bonvin AMJJ. HADDOCK: A protein-protein docking approach based on biochemical or biophysical information. *Journal of the American Chemical Society* 2003;125:1731–1737. [PubMed: 12580598]
- Dragic T, Litwin V, Allaway GP, Martin SR, Huang YX, Nagashima KA, Cayanan C, Maddon PJ, Koup RA, Moore JP, Paxton WA. HIV-1 entry into CD4(+) cells is mediated by the chemokine receptor CC-CKR-5. *Nature* 1996;381:667–673. [PubMed: 8649512]

- Eichmueller C, Skrynnikov NR. Observation of μ s time-scale protein dynamics in the presence of $\text{Ln}(3+)\text{stop}$ ions: application to the N-terminal domain of cardiac troponin C. *Journal of Biomolecular Nmr* 2007;37:79–95. [PubMed: 17180551]
- Englander JJ, Calhoun DB, Englander SW. Measurement and Calibration of Peptide Group Hydrogen-Deuterium Exchange by Ultraviolet Spectrophotometry. *Analytical Biochemistry* 1979;92:517–524. [PubMed: 443552]
- Englander SW, Kallenbach NR. Hydrogen-Exchange and Structural Dynamics of Proteins and Nucleic-Acids. *Quarterly Reviews of Biophysics* 1983;16:521–655. [PubMed: 6204354]
- Farmer BT. Localizing the NADP(+) binding site on the MurB enzyme by NMR. *Nature Structural Biology* 1996;3:995–997.
- Ferreira-da-Silva F, Pereira PJB, Gales L, Roessle M, Svergun DI, Moradas-Ferreira P, Damas AM. The crystal and solution structures of glyceraldehyde-3-phosphate dehydrogenase reveal different quaternary structures. *Journal of Biological Chemistry* 2006;281:33433–33440. [PubMed: 16963457]
- Fesik SW, Gemmecker G, Olejniczak ET, Petros AM. Identification of Solvent-Exposed Regions of Enzyme-Bound Ligands by Nuclear-Magnetic-Resonance. *Journal of the American Chemical Society* 1991;113:7080–7081.
- Fetler L, Tauc P, Herve G, Moody MF, Vachette P. X-Ray-Scattering Titration of the Quaternary Structure Transition of Aspartate-Transcarbamylase with a Bisubstrate Analog - Influence of Nucleotide Effectors. *Journal of Molecular Biology* 1995;251:243–255. [PubMed: 7643401]
- Fischer MW, Losonczi JA, Weaver JL, Prestegard JH. Domain orientation and dynamics in multidomain proteins from residual dipolar couplings. *Biochemistry* 1999;38:9013–22. [PubMed: 10413474]
- Folkers PJM, Nilges M, Folmer RHA, Konings RNH, Hilbers CW. The Solution Structure of the Tyr41-JHis Mutant of the Single-Stranded-DNA Binding-Protein Encoded by Gene-V of the Filamentous Bacteriophage M13. *Journal of Molecular Biology* 1994;236:229–246. [PubMed: 8107108]
- Fowler AG, Foote AM, Moody MF, Vachette P, Provencher SW, Gabriel A, Bordas J, Koch MHJ. Stopped-Flow Solution Scattering Using Synchrotron Radiation - Apparatus, Data-Collection and Data-Analysis. *Journal of Biochemical and Biophysical Methods* 1983;7:317–329. [PubMed: 6619508]
- Franke D, Svergun DI. DAMMIF, a program for rapid ab-initio shape determination in small-angle scattering. *Journal of Applied Crystallography* 2009;42:342–346.
- Fujisawa T, Kuwahara H, Hiromasa Y, Niidome T, Aoyagi H, Hatakeyama T. Small-angle X-ray scattering study on CEL-III, a hemolytic lectin from Holothuroidea *Cucumaria echinata*, and its oligomer induced by the binding of specific carbohydrate. *Febs Letters* 1997;414:79–83. [PubMed: 9305736]
- Godt A, Schulte M, Zimmermann H, Jeschke G. How flexible are poly(para-phenyleneethynylene)s? *Angew Chem Int Ed Engl* 2006;45:7560–4. [PubMed: 17051574]
- Goodsell DS, Olson AJ. Structural symmetry and protein function. *Annual Review of Biophysics and Biomolecular Structure* 2000;29:105–153.
- Gray JJ, Moughon S, Wang C, Schueler-Furman O, Kuhlman B, Rohl CA, Baker D. Protein-protein docking with simultaneous optimization of rigid-body displacement and side-chain conformations. *Journal of Molecular Biology* 2003;331:281–299. [PubMed: 12875852]
- Gregory RB, Rosenberg A. Protein conformational dynamics measured by hydrogen isotope exchange techniques. *Methods Enzymol* 1986;131:448–508. [PubMed: 3022109]
- Grishaev A, Wu J, Trewella J, Bax A. Refinement of multidomain protein structures by combination of solution small-angle X-ray scattering and NMR data. *Journal of the American Chemical Society* 2005;127:16621–16628. [PubMed: 16305251]
- Grishaev A, Yao L, Ying J, Pardi A, Bax A. Chemical shift anisotropy of imino ^{15}N nuclei in Watson-Crick base pairs from magic angle spinning liquid crystal NMR and nuclear spin relaxation. *J Am Chem Soc* 2009;131:9490–1. [PubMed: 19537719]
- Gupta S, Cheng H, Mollah A, Jamison E, Morris S, Chance MR, Khrapunov S, Brenowitz M. DNA and protein footprinting analysis of the modulation of DNA binding by the N-terminal domain of

- the *Saccharomyces cerevisiae* TATA binding protein. *Biochemistry* 2007;46:9886–9898. [PubMed: 17683121]
- Hambly DM, Gross ML. Laser flash photolysis of hydrogen peroxide to oxidize protein solvent-accessible residues on the microsecond timescale. *J Am Soc Mass Spectrom* 2005;16:2057–63. [PubMed: 16263307]
- Hansen AL, Al-Hashimi HM. Insight into the CSA tensors of nucleobase carbons in RNA polynucleotides from solution measurements of residual CSA: towards new long-range orientational constraints. *J Magn Reson* 2006;179:299–307. [PubMed: 16431143]
- Harada M, Ito Y, Sato M, Aono O, Ohta S, Kagawa Y. SMALL-ANGLE X-RAY-SCATTERING STUDIES OF MG.AT(D)P-INDUCED HEXAMER TO DIMER DISSOCIATION IN THE RECONSTITUTED ALPHA-3-BETA-3-COMPLEX OF ATP SYNTHASE FROM THERMOPHILIC BACTERIUM PS3. *Journal of Biological Chemistry* 1991;266:11455–11460. [PubMed: 1828800]
- Hartzell CJ, Whitfield M, Oas TG, Drobny GP. Determination of the N-15 and C-13 Chemical-Shift Tensors of L-[C-13]Alanyl-L-[N-15]Alanine from the Dipole-Coupled Powder Patterns. *J Am Chem Soc* 1987;109:5966–5969.
- Herrmann T, Guntert P, Wuthrich K. Protein NMR structure determination with automated NOE assignment using the new software CANDID and the torsion angle dynamics algorithm DYANA. *J Mol Biol* 2002;319:209–27. [PubMed: 12051947]
- Hilger D, Jung H, Padan E, Wegener C, Vogel KP, Steinhoff HJ, Jeschke G. Assessing oligomerization of membrane proteins by four-pulse DEER: pH-dependent dimerization of NhaA Na⁺/H⁺ antiporter of *E. coli*. *Biophys J* 2005;89:1328–38. [PubMed: 15894644]
- Hiyama Y, Niu CH, Silverton JV, Bavoso A, Torchia DA. Determination of N-15 Chemical-Shift Tensor Via N-15-H-2 Dipolar Coupling in Boc-Glycylglycyl[N-15]Glycine Benzyl Ester. *J Am Chem Soc* 1988;110:2378–2383.
- Ikura M, Bax A. Isotope-Filtered 2d Nmr of a Protein Peptide Complex - Study of a Skeletal-Muscle Myosin Light Chain Kinase Fragment Bound to Calmodulin. *Journal of the American Chemical Society* 1992;114:2433–2440.
- Jain NU, Noble S, Prestegard JH. Structural characterization of a mannose-binding protein-trimannoside complex using residual dipolar couplings. *J Mol Biol* 2003;328:451–62. [PubMed: 12691753]
- Jain NU, Wyckoff TJ, Raetz CR, Prestegard JH. Rapid analysis of large protein-protein complexes using NMR-derived orientational constraints: the 95 kDa complex of LpxA with acyl carrier protein. *J Mol Biol* 2004;343:1379–89. [PubMed: 15491619]
- Jeschke G. Distance measurements in the nanometer range by pulse EPR. *Chemphyschem* 2002;3:927–32. [PubMed: 12503132]
- Jeschke G, Polyhach Y. Distance measurements on spin-labelled biomacromolecules by pulsed electron paramagnetic resonance. *Phys Chem Chem Phys* 2007;9:1895–910. [PubMed: 17431518]
- Jeschke G, Bender A, Paulsen H, Zimmermann H, Godt A. Sensitivity enhancement in pulse EPR distance measurements. *J Magn Reson* 2004a;169:1–12. [PubMed: 15183350]
- Jeschke G, Wegener C, Nietschke M, Jung H, Steinhoff HJ. Interresidual distance determination by four-pulse double electron-electron resonance in an integral membrane protein: the Na⁺/proline transporter PutP of *Escherichia coli*. *Biophys J* 2004b;86:2551–7. [PubMed: 15041691]
- Jeschke G, Bender A, Schweikardt T, Panek G, Decker H, Paulsen H. Localization of the N-terminal domain in light-harvesting chlorophyll a/b protein by EPR measurements. *J Biol Chem* 2005;280:18623–30. [PubMed: 15755729]
- Kamal JKA, Benchaar SA, Takamoto K, Reisler E, Chance MR. Three-dimensional structure of cofilin bound to monomeric actin derived by structural mass spectrometry data. *Proceedings of the National Academy of Sciences of the United States of America* 2007;104:7910–7915. [PubMed: 17470807]
- Koch MHJ, Vachette P, Svergun DI. Small-angle scattering: a view on the properties, structures and structural changes of biological macromolecules in solution. *Quarterly Reviews of Biophysics* 2003;36:147–227. [PubMed: 14686102]

- Kofuku Y, Yoshiura C, Ueda T, Terasawa H, Hirai T, Tominaga S, Hirose M, Maeda Y, Takahashi H, Terashima Y, Matsushima K, Shimada I. Structural Basis of the Interaction between Chemokine Stromal Cell-derived Factor-1/CXCL12 and Its G-protein-coupled Receptor CXCR4. *Journal of Biological Chemistry* 2009;284:35240–35250. [PubMed: 19837984]
- Kuszewski J, Gronenborn AM, Clore GM. Improving the packing and accuracy of NMR structures with a pseudopotential for the radius of gyration. *Journal of the American Chemical Society* 1999;121:2337–2338.
- Lau EK, Paavola CD, Johnson Z, Gaudry JP, Geretti E, Borlat F, Kungl AJ, Proudfoot AE, Handel TM. Identification of the glycosaminoglycan binding site of the CC chemokine, MCP-1 - Implications for structure and function in vivo. *Journal of Biological Chemistry* 2004;279:22294–22305. [PubMed: 15033992]
- Lee HW, Wylie G, Bansal S, Wang X, Barb AW, Macnaughtan MA, Ertekin A, Montelione GT, Prestegard JH. Three-dimensional structure of the weakly associated protein homodimer SeR13 using RDCs and paramagnetic surface mapping. *Protein Science* 2010;19:1673–1685. [PubMed: 20589905]
- Lee L, Sykes BD. Use of Lanthanide-Induced Nuclear Magnetic-Resonance Shifts for Determination of Protein-Structure in Solution - Ef Calcium-Binding Site of Carp Parvalbumin. *Biochemistry* 1983;22:4366–4373. [PubMed: 6626506]
- Lipsitz RS, Tjandra N. Carbonyl CSA restraints from solution NMR for protein structure refinement. *J Am Chem Soc* 2001;123:11065–11066. [PubMed: 11686713]
- Liu Y, Prestegard JH. A device for the measurement of residual chemical shift anisotropy and residual dipolar coupling in soluble and membrane-associated proteins. *J Biomol NMR* 2010;47:249–58. [PubMed: 20506033]
- Losonczi JA, Andrec M, Fischer MW, Prestegard JH. Order matrix analysis of residual dipolar couplings using singular value decomposition. *J Magn Reson* 1999;138:334–42. [PubMed: 10341140]
- Loth K, Pelupessy P, Bodenhausen G. Chemical shift anisotropy tensors of carbonyl, nitrogen, and amide proton nuclei in proteins through cross-correlated relaxation in NMR spectroscopy. *J Am Chem Soc* 2005;127:6062–8. [PubMed: 15839707]
- Lukatsky DB, Shakhnovich BE, Mintseris J, Shakhnovich EI. Structural similarity enhances interaction propensity of proteins. *Journal of Molecular Biology* 2007;365:1596–1606. [PubMed: 17141268]
- Maleknia SD, Brenowitz M, Chance MR. Millisecond radiolytic modification of peptides by synchrotron X-rays identified by mass spectrometry. *Analytical Chemistry* 1999;71:3965–3973. [PubMed: 10500483]
- Mayer M, Meyer B. Characterization of ligand binding by saturation transfer difference NMR spectroscopy. *Angewandte Chemie-International Edition* 1999;38:1784–1788.
- McCoy MA, Wyss DF. Structures of protein-protein complexes are docked using only NMR restraints from residual dipolar coupling and chemical shift perturbations. *J Am Chem Soc* 2002;124:2104–5. [PubMed: 11878950]
- Meng L, Glushka J, Stanton L, Fang T, Collins R, Carey G, Wiley G, Gao Z, Prestegard J, Moremen KW. Expression and isotope labeling of ST6Gal1 - Enabling NMR characterization of glycosylated proteins. *Glycobiology* 2006;16:1127–1128.
- Montalvao RW, Cavalli A, Salvatella X, Blundell TL, Vendruscolo M. Structure Determination of Protein-Protein Complexes Using NMR Chemical Shifts: Case of an Endonuclease Colicin-Immunity Protein Complex. *Journal of the American Chemical Society* 2008;130:15990–15996. [PubMed: 18980319]
- Moont G, Gabb HA, Sternberg MJ. Use of pair potentials across protein interfaces in screening predicted docked complexes. *Proteins* 1999;35:364–73. [PubMed: 10328272]
- Moriya J, Sakakura M, Tokunaga Y, Prosser RS, Shimada I. An NMR method for the determination of protein binding interfaces using TEMPOL-induced chemical shift perturbations. *Biochimica et Biophysica Acta (BBA) - General Subjects* 2009;1790:1368–1376.

- Murphy JW, Yuan H, Kong Y, Xiong Y, Lolis EJ. Heterologous quaternary structure of CXCL 12 and its relationship to the CC chemokine family. *Proteins-Structure Function and Bioinformatics* 2010;78:1331–1337.
- Nabuurs SB, Spronk CAEM, Vuister GW, Vriend G. Traditional biomolecular structure determination by NMR spectroscopy allows for major errors. *Plos Computational Biology* 2006;2:71–79.
- Nakamura M, Ueki S, Hara H, Arata T. Calcium structural transition of human cardiac troponin C in reconstituted muscle fibres as studied by site-directed spin labelling. *J Mol Biol* 2005;348:127–37. [PubMed: 15808858]
- Nakamura T, Takahashi H, Takahashi M, Shimba N, Suzuki E, Shimada I. Direct Determination of the Insulin-Insulin Receptor Interface Using Transferred Cross-Saturation Experiments. *Journal of Medicinal Chemistry* 53:1917–1922. [PubMed: 20158183]
- Niccolai N, Ciutti A, Spiga O, Scarselli M, Bernini A, Bracci L, Di Maro D, Dalvit C, Molinari H, Esposito G, Temussi PA. NMR studies of protein surface accessibility. *Journal of Biological Chemistry* 2001;276:42455–42461. [PubMed: 11546818]
- Nilges M, Macias MJ, O'Donoghue SI, Oschkinat H. Automated NOESY interpretation with ambiguous distance restraints: the refined NMR solution structure of the pleckstrin homology domain from beta-spectrin. *J Mol Biol* 1997;269:408–22. [PubMed: 9199409]
- Nitz M, Sherawat M, Franz KJ, Peisach E, Allen KN, Imperiali B. Structural origin of the high affinity of a chemically evolved lanthanide-binding peptide. *Angewandte Chemie-International Edition* 2004;43:3682–3685.
- Oas TG, Hartzell CJ, Dahlquist FW, Drobny GP. The Amide N-15 Chemical-Shift Tensors of 4 Peptides Determined from C-13 Dipole-Coupled Chemical-Shift Powder Patterns. *J Am Chem Soc* 1987a;109:5962–5966.
- Oas TG, Hartzell CJ, McMahon TJ, Drobny GP, Dahlquist FW. The Carbonyl C-13 Chemical-Shift Tensors of 5 Peptides Determined from N-15 Dipole-Coupled Chemical-Shift Powder Patterns. *J Am Chem Soc* 1987b;109:5956–5962.
- Orban T, Bereta G, Miyagi M, Wang BL, Chance MR, Sousa MC, Palczewski K. Conformational Changes in Guanylate Cyclase-Activating Protein 1 Induced by Ca²⁺ and N-Terminal Fatty Acid Acylation. *Structure* 18:116–126. [PubMed: 20152158]
- Ortega-Roldan JL, Jensen MR, Brutscher B, Azuaga AI, Blackledge M, van Nuland NAJ. Accurate characterization of weak macromolecular interactions by titration of NMR residual dipolar couplings: application to the CD2AP SH3-C:ubiquitin complex. *Nucleic Acids Research* 2009;37.
- Ottiger M, Tjandra N, Bax A. Magnetic field dependent amide N-15 chemical shifts in a protein-DNA complex resulting from magnetic ordering in solution. *J Am Chem Soc* 1997;119:9825–9830.
- Otting G. Experimental NMR techniques for studies of protein-ligand interactions. *Current Opinion in Structural Biology* 1993;3:760–768.
- Pannier M, Veit S, Godt A, Jeschke G, Spiess HW. Dead-time free measurement of dipole-dipole interactions between electron spins. *J Magn Reson* 2000;142:331–40. [PubMed: 10648151]
- Parsons LM, Grishaev A, Bax A. The periplasmic domain of To1R from haemophilus influenzae forms a dimer with a large hydrophobic groove: NMR solution structure and comparison to SAXS data. *Biochemistry* 2008;47:3131–3142. [PubMed: 18269247]
- Perez J, Vachette P, Russo D, Desmadril M, Durand D. Heat-induced unfolding of neocarzinostatin, a small all-beta protein investigated by small-angle X-ray scattering. *Journal of Molecular Biology* 2001;308:721–743. [PubMed: 11350171]
- Peterson DW, Zhou HJ, Dahlquist FW, Lew J. A soluble oligomer of tau associated with fiber formation analyzed by NMR. *Biochemistry* 2008;47:7393–7404. [PubMed: 18558718]
- Petros AM, Mueller L, Kopple KD. Nmr Identification of Protein Surfaces Using Paramagnetic Probes. *Biochemistry* 1990;29:10041–10048. [PubMed: 2176860]
- Phillips JC, Braun R, Wang W, Gumbart J, Tajkhorshid E, Villa E, Chipot C, Skeel RD, Kalé L, Schulten K. Scalable molecular dynamics with NAMD. *Journal of Computational Chemistry* 2005;26:1781–1802. [PubMed: 16222654]

- Pintacuda G, Otting G. Identification of Protein Surfaces by NMR Measurements with a Paramagnetic Gd(III) Chelate. *Journal of the American Chemical Society* 2001;124:372–373. [PubMed: 11792196]
- Prestegard JH, Bougault CM, Kishore AI. Residual dipolar couplings in structure determination of biomolecules. *Chemical Reviews* 2004;104:3519–3540. [PubMed: 15303825]
- Proudfoot AEI, Handel TM, Johnson Z, Lau EK, LiWang P, Clark-Lewis I, Borlat F, Wells TNC, Kosco-Vilbois MH. Glycosaminoglycan binding and oligomerization are essential for the in vivo activity of certain chemokines. *Proceedings of the National Academy of Sciences of the United States of America* 2003;100:1885–1890. [PubMed: 12571364]
- Putnam CD, Hammel M, Hura GL, Tainer JA. X-ray solution scattering (SAXS) combined with crystallography and computation: defining accurate macromolecular structures, conformations and assemblies in solution. *Q Rev Biophys* 2007;40:191–285. [PubMed: 18078545]
- Rabenstein MD, Shin YK. Determination of the distance between two spin labels attached to a macromolecule. *Proc Natl Acad Sci U S A* 1995;92:8239–43. [PubMed: 7667275]
- Rini JM. Lectin Structure. *Annual Review of Biophysics and Biomolecular Structure* 1995;24:551–577.
- Robinson CV, Sali A, Baumeister W. The molecular sociology of the cell. *Nature* 2007;450:973–982. [PubMed: 18075576]
- Rumpel S, Becker S, Zweckstetter M. High-resolution structure determination of the CylR2 homodimer using paramagnetic relaxation enhancement and structure-based prediction of molecular alignment. *J Biomol NMR* 2008;40:1–13. [PubMed: 18026911]
- Ruotolo BT, Giles K, Campuzano I, Sandercock AM, Bateman RH, Robinson CV. Evidence for macromolecular protein rings in the absence of bulk water. *Science* 2005;310:1658–1661. [PubMed: 16293722]
- Sanders CR, Landis GC. Facile Acquisition and Assignment of Oriented Sample Nmr-Spectra for Bilayer Surface-Associated Proteins. *Journal of the American Chemical Society* 1994;116:6470–6471.
- Schwieters CD, Kuszewski JJ, Tjandra N, Clore GM. The Xplor-NIH NMR molecular structure determination package. *Journal of Magnetic Resonance* 2003;160:65–73. [PubMed: 12565051]
- Schwieters CD, Suh JY, Grishaev A, Ghirlando R, Takayama Y, Clore GM. Solution Structure of the 128 kDa Enzyme I Dimer from *Escherichia coli* and Its 146 kDa Complex with HPr Using Residual Dipolar Couplings and Small- and Wide-Angle X-ray Scattering. *Journal of the American Chemical Society*. 2010
- Sclavi B, Sullivan M, Chance MR, Brenowitz M, Woodson SA. RNA folding at millisecond intervals by synchrotron hydroxyl radical footprinting. *Science* 1998;279:1940–1943. [PubMed: 9506944]
- Segel DJ, Eliezer D, Uversky V, Fink AL, Hodgson KO, Doniach S. Transient dimer in the refolding kinetics of cytochrome c characterized by small-angle X-ray scattering. *Biochemistry* 1999;38:15352–15359. [PubMed: 10563821]
- Shaw JP, Johnson Z, Borlat F, Zwahlen C, Kungl A, Roulin K, Harrenga A, Wells TN, Proudfoot AE. The X-ray structure of RANTES: heparin-derived disaccharides allows the rational design of chemokine inhibitors. *Structure* 2004;12:2081–93. [PubMed: 15530372]
- Shilton BH, McDowell JH, Smith WC, Hargrave PA. The solution structure and activation of visual arrestin studied by small-angle X-ray scattering. *European Journal of Biochemistry* 2002;269:3801–3809. [PubMed: 12153577]
- Shimada I, Ueda T, Matsumoto M, Sakakura M, Osawa M, Takeuchi K, Nishida N, Takahashi H. Cross-saturation and transferred cross-saturation experiments. *Progress in Nuclear Magnetic Resonance Spectroscopy* 2009;54:123–140.
- Simon B, Madl T, Mackereth CD, Nilges M, Sattler M. An Efficient Protocol for NMR-Spectroscopy-Based Structure Determination of Protein Complexes in Solution. *Angewandte Chemie-International Edition* 2010;49:1967–1970.
- Sitkoff D, Case DA. Theories of chemical shift anisotropies in proteins and nucleic acids. *Progress in Nuclear Magnetic Resonance Spectroscopy* 1998;32:165–190.
- Skelton NJ, Aspiras F, Ogez J, Schall TJ. Proton NMR assignments and solution conformation of RANTES, a chemokine of the C-C type. *Biochemistry* 1995;34:5329–42. [PubMed: 7537088]

- Sousa MC, Trame CB, Tsuruta H, Wilbanks SM, Reddy VS, McKay DB. Crystal and solution structures of an HsIUUV protease-chaperone complex. *Cell* 2000;103:633–643. [PubMed: 11106733]
- Steinhoff HJ. Inter- and intra-molecular distances determined by EPR spectroscopy and site-directed spin labeling reveal protein-protein and protein-oligonucleotide interaction. *Biol Chem* 2004;385:913–20. [PubMed: 15551865]
- Su XC, Huber T, Dixon NE, Otting G. Site-specific labelling of proteins with a rigid lanthanide-binding tag. *Chembiochem* 2006;7:1599–1604. [PubMed: 16927254]
- Svergun D, Barberato C, Koch MHJ. CRY SOL - A program to evaluate x-ray solution scattering of biological macromolecules from atomic coordinates. *Journal of Applied Crystallography* 1995;28:768–773.
- Swaminathan GJ, Holloway DE, Colvin RA, Campanella GK, Papageorgiou AC, Luster AD, Acharya KR. Crystal structures of oligomeric forms of the IP-10/CXCL10 chemokine. *Structure* 2003;11:521–532. [PubMed: 12737818]
- Takahashi H, Nakanishi T, Kami K, Arata Y, Shimada I. A novel NMR method for determining the interfaces of large protein-protein complexes. *Nature Structural Biology* 2000;7:220–223.
- Takahashi H, Miyazawa M, Ina Y, Fukunishi Y, Mizukoshi Y, Nakamura H, Shimada I. Utilization of methyl proton resonances in cross-saturation measurement for determining the interfaces of large protein-protein complexes. *Journal of Biomolecular Nmr* 2006;34:167–177. [PubMed: 16604425]
- Takamoto K, Chance MR. Radiolytic protein footprinting with mass Spectrometry to probe the structure of macromolecular complexes. *Annual Review of Biophysics and Biomolecular Structure* 2006;35:251–276.
- Takamoto K, Kamal JKA, Chance MR. Biochemical implications of a three-dimensional model of monomeric actin bound to magnesium-chelated ATP. *Structure* 2007;15:39–51. [PubMed: 17223531]
- Teng Q, Iqbal M, Cross TA. Determination of the C-13 Chemical-Shift and N-14 Electric-Field Gradient Tensor Orientations with Respect to the Molecular Frame in a Polypeptide. *J Am Chem Soc* 1992;114:5312–5321.
- Tjandra N, Bax A. Direct measurement of distances and angles in biomolecules by NMR in a dilute liquid crystalline medium. *Science* 1997;278:1111–4. [PubMed: 9353189]
- Tolman JR, Flanagan JM, Kennedy MA, Prestegard JH. Nuclear magnetic dipole interactions in field-oriented proteins: information for structure determination in solution. *Proc Natl Acad Sci U S A* 1995;92:9279–83. [PubMed: 7568117]
- Utrecht C, Versluis C, Watts NR, Wingfield PT, Steven AC, Heck AJR. Stability and shape of hepatitis B virus capsids in vacuo. *Angewandte Chemie-International Edition* 2008;47:6247–6251.
- Vakser IA, Kundrotas P. Predicting 3D structures of protein-protein complexes. *Current Pharmaceutical Biotechnology* 2008;9:57–66. [PubMed: 18393862]
- van Duijn E, Barendregt A, Synowsky S, Versluis C, Heck AJR. Chaperonin Complexes Monitored by Ion Mobility Mass Spectrometry. *Journal of the American Chemical Society* 2009;131:1452–1459. [PubMed: 19138114]
- Wang J, Zuo X, Yu P, Byeon IJ, Jung J, Wang X, Dyba M, Seifert S, Schwieters CD, Qin J, Gronenborn AM, Wang YX. Determination of multicomponent protein structures in solution using global orientation and shape restraints. *J Am Chem Soc* 2009;131:10507–15. [PubMed: 19722627]
- Wang X, Bansal S, Jiang M, Prestegard JH. RDC-assisted modeling of symmetric protein homooligomers. *Protein Sci* 2008;17:899–907. [PubMed: 18436958]
- Wang ZX, Tsuruta H, Honda Y, Tachiiri Y, Wakabayashi K, Amemiya Y, Kihara H. KINETIC-STUDY ON THE DIMER-TETRAMER INTERCONVERSION OF PHOSPHORYLASE-B BY A STOPPED-FLOW X-RAY-SCATTERING METHOD. *Biophysical Chemistry* 1989;33:153–160. [PubMed: 2502199]

- Watson C, Janik I, Zhuang T, Charvatova O, Woods RJ, Sharp JS. Pulsed electron beam water radiolysis for submicrosecond hydroxyl radical protein footprinting. *Anal Chem* 2009;81:2496–505. [PubMed: 19265387]
- Weis WI, Drickamer K. Structural basis of lectin-carbohydrate recognition. *Annual Review of Biochemistry* 1996;65:441–473.
- Wolynes PG. Symmetry and the energy landscapes of biomolecules. *Proceedings of the National Academy of Sciences of the United States of America* 1996;93:14249–14255. [PubMed: 8962034]
- Woodward C, Simon I, Tuchsén E. Hydrogen-Exchange and the Dynamic Structure of Proteins. *Molecular and Cellular Biochemistry* 1982;48:135–160. [PubMed: 6757714]
- Wu YQ, Shih SCC, Goto NK. Probing the structure of the Ff bacteriophage major coat protein transmembrane helix dimer by solution NMR. *Biochimica Et Biophysica Acta-Biomembranes* 2007;1768:3206–3215.
- Wu ZR, Tjandra N, Bax A. P-31 chemical shift anisotropy as an aid in determining nucleic acid structure in liquid crystals. *J Am Chem Soc* 2001;123:3617–3618. [PubMed: 11472143]
- Yang DW, Yamamoto K, Kanaya E, Kanaya S, Nagayama K. Characterization of an artificial dimer of ribonuclease H using H-1 NMR spectroscopy. *Journal of Biomolecular Nmr* 1996;7:29–34. [PubMed: 8720829]
- Yang Y, Ramelot TA, McCarrick RM, Ni S, Feldmann EA, Cort JR, Wang H, Ciccocanti C, Jiang M, Janjua H, Acton TB, Xiao R, Everett JK, Montelione GT, Kennedy MA. Combining NMR and EPR methods for homodimer protein structure determination. *Journal of the American Chemical Society* 2010;132:11910–3. [PubMed: 20698532]
- Yao LS, Grishaev A, Cornilescu G, Bax A. Site-Specific Backbone Amide N-15 Chemical Shift Anisotropy Tensors in a Small Protein from Liquid Crystal and Cross-Correlated Relaxation Measurements. *J Am Chem Soc* 2010;132:4295–4309. [PubMed: 20199098]
- Ying JF, Grishaev A, Bryce DL, Bax A. Chemical shift tensors of protonated base carbons in helical RNA and DNA from NMR relaxation and liquid crystal measurements. *J Am Chem Soc* 2006;128:11443–11454. [PubMed: 16939267]
- Yu F, Wolff JJ, Amster IJ, Prestegard JH. Conformational preferences of chondroitin sulfate oligomers using partially oriented NMR spectroscopy of C-13-labeled acetyl groups. *J Am Chem Soc* 2007;129:13288–13297. [PubMed: 17924631]
- Zhou Z, DeSensi SC, Stein RA, Brandon S, Dixit M, McArdle EJ, Warren EM, Kroh HK, Song L, Cobb CE, Hustedt EJ, Beth AH. Solution structure of the cytoplasmic domain of erythrocyte membrane band 3 determined by site-directed spin labeling. *Biochemistry* 2005;44:15115–28. [PubMed: 16285715]
- Zhuang TD, Lee HS, Imperiali B, Prestegard JH. Structure determination of a Galectin-3-carbohydrate complex using paramagnetism-based NMR constraints. *Protein Science* 2008;17:1220–1231. [PubMed: 18413860]
- Zuiderweg ERP. Mapping protein-protein interactions in solution by NMR Spectroscopy. *Biochemistry* 2002;41:1–7. [PubMed: 11771996]
- Zwahlen C, Legault P, Vincent SJF, Greenblatt J, Konrat R, Kay LE. Methods for measurement of intermolecular NOEs by multinuclear NMR spectroscopy: Application to a bacteriophage lambda N-peptide/boxB RNA complex. *Journal of the American Chemical Society* 1997;119:6711–6721.
- Zweckstetter M, Bax A. Prediction of sterically induced alignment in a dilute liquid crystalline phase: Aid to protein structure determination by NMR. *Journal of the American Chemical Society* 2000;122:3791–3792.

Figure 1.

Steps of the grid search used to produce dimer models of Sr360 & SeR13. (a) Place monomer in the alignment tensor frame. (b) Create 2nd monomer by rotating the structure 180 degrees around one of the principal axes. (c) Translate the 2nd monomer in the plane perpendicular to the rotation axis. Discard models that have monomers too far apart or too close. (d) Perform energy minimization and MD to relax the interface and create better complementarity. (e) Evaluate the model based on experimental/simulated RDC correlation, RP score and VDW energy. Adapted from (Wang et al., 2008).

Figure 2. Correlation between experimental RDCs for both the wild type and E66S CCL5 and those calculated from the CCL5 dimer structure (PDB accession code 1U4L).

Figure 3. Orientation of the alignment tensor principal axes and the symmetry axis of the dimer. The symmetry axis is shown in gold; the S_{xx} axis of the tensor is shown in red; the S_{yy} axis is shown in blue and the S_{zz} axis is shown in green. The angle between the symmetry axis and the S_{xx} axis is 11 degrees.

Figure 4.

Experimental scattering curve for wild type CCL5 and the calculated scattering curves for the MCP-1 tetramer, the IP-10 tetramer, and a tretramer based on RDC and SAXS data. q is the magnitude of the momentum transfer vector, $4\pi\sin(\theta)/\lambda$, where 2θ is the scattering angle and λ is the X-ray wavelength.

Figure 5.

Steps in the grid search used in constructing the tetramer models of CCL5. (a) Place one dimeric unit in the alignment tensor frame. (b) Create 2nd dimer by rotating the structure 180 degrees around the dimeric symmetry axis. (c) Translate the 2nd dimer in the plane perpendicular to the rotation axis. Discard models that have the dimers too far apart or too close. (d) Perform energy minimization and MD to relax the interface and create better complementarity. (e) Evaluate the model based agreement between theoretical and experimental scattering curve and residue pairing score.

Figure 6.

A) Contour plot of the scattering curve fitting χ values for models generated by the grid search. The four locations on the grid that produced better fitting models are 3×12 , 54×45 , 4×43 , 61×18 . B) Contour plot of the residue-pairing score of the models generated by the grid search.

Figure 7.

Two models of the tetramer whose scattering profile showed good agreement with the experimental scattering curve of wild type CCL5. Model A is from grid 3×12 and model B is from grid 4×43 .

Figure 8. Comparison of the grid search models with the ab initio shape generated from the scattering curve. The tetramer model A superimposed onto the dummy atom model calculated from the scattering curve (green spheres).

Figure 9. Comparison of the tetrameric interface of CXCL12 (PDB accession code 3HP3, upper panel) and Model A of the CCL5 tetramer model found by grid search.

Table 1

Summary of the techniques discussed in the review.

Techniques		Information content
Solution NMR based	RDC	Subunit orientation in symmetric oligomers
	RCSA	Subunit orientation in symmetric oligomers
	Chemical shift mapping	Interaction surface
	Paramagnetic surface perturbation	Interaction surface
	PRE from specific paramagnetic label	Long range intermolecular distances.
	Cross-saturation	Interaction surface
Non-NMR	EPR	Long range intermolecular distances
	MS footprinting	Interaction surface
	MS ion mobility measurements	Hydrodynamic radius/ complex shape
	SAXS/SANS/WAXS	Overall complex shape