Exploring the Limits of Precision and Accuracy of Protein Structures Determined by Nuclear Magnetic Resonance Spectroscopy

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The effects of the number, precision and accuracy of interproton distance restraints, of direct refinement against nuclear Overhauser enhancement (NOE) intensities and of the description of the non-bonded contacts on the precision and accuracy of a nuclear magnetic resonance (NMR) protein structure determination have been investigated. The model system employed is the 58 residue immunoglobulin G binding domain of streptococcal protein G. This choice was based on the availability of a very high resolution NMR structure (atomic root-mean-square distribution of the ensemble of 60 calculated structures about the mean co-ordinate positions of 0.35 Å for the backbone atoms, 0.65 Å for all atoms and 0.39 Å for all atoms excluding disordered surface side-chains). The experimental NMR data set for this structure determination comprised a total of 1058 experimental restraints of which 854 were approximate interproton distance restraints corresponding to all the structurally useful NOEs observable for this protein. The calculations presented in this paper reveal the following. (1) The number of interproton distance restraints constitutes the single most important determinant of both precision and accuracy. The ensemble precision and accuracy improves significantly as the number of interproton distance restraints is increased to an average of ~15 per residue, of which ~60% involve unique proton pairs; subsequent additions of interproton distance restraints, however, lead to less dramatic improvements as information redundancy sets in. (2) The ratio of ensemble precision to ensemble accuracy (which ranges from 0.5 to 0.7 for the backbone atoms) is approximately independent both of the number, precision and accuracy of the interproton distance restraints, and of whether the structures are refined against interproton distance restraints or directly against NOE intensities. (3) In an ensemble of structures generated from a large number of loose approximate interproton distance restraints (an average of ~15 restraints per residue with ~60% involving unique proton pairs), the interproton distance vectors corresponding to the restraints are very well defined with ~60% of vectors between unique proton pairs having a standard deviation of ~0.1 Å. (4) The accuracy of the mean co-ordinates of an ensemble of structures is significantly higher than the average accuracy of the individual structures comprising the ensemble. For an average ensemble precision of ~0.6 Å, the dependence of the accuracy of the mean co-ordinates on ensemble precision is approximately linear. As the ensemble precision, however, increases beyond ~0.6 Å, the dependence is asymptotic and the accuracy of the mean co-ordinates reaches a limiting value of ~0.1 Å. (5) There is no significant difference in the results obtained with precise interproton distance restraints versus those with loose approximate interproton distance restraints. (6) The ensemble precision of a structure determination can potentially be improved by the use of either accurate (as opposed to simply precise) interproton distance restraints or by direct refinement against NOE intensities; the former, however, cannot be obtained experimentally, while the latter can potentially introduce systematic errors unless effects of internal motions are taken into account appropriately. This increase in precision, however, is not necessarily reflected by a corresponding increase in the accuracy of the mean co-ordinates. Hence, in practice, direct refinement against NOE intensities is unlikely to have a significant impact in terms of attainable accuracy over the use of the much simpler and computationally much more efficient loose approximate interproton distance restraints for all but a very few exceptional cases. (7) Neglecting the effects of the non-bonded contacts, the maximum ensemble accuracy that can be achieved in practice is ~0.25 to
0.30 Å for the backbone atoms. The description of the non-bonded contacts can have a profound effect on both the precision and accuracy of the resulting structures, and when these effects are taken into account, the likely practical limit on the attainable ensemble accuracy is 0.4 to 0.6 Å for the backbone atoms, 0.8 to 1.1 Å for all atoms and 0.5 to 0.8 Å for all ordered atoms, with the corresponding accuracy of the mean co-ordinates being 0.1 to 0.2 Å better.

Keywords: protein structure; NMR; accuracy; precision; NOE

1. Introduction

Over the past few years significant advances have been made in increasing both the precision and molecular weight range of protein structures determined by nuclear magnetic resonance (NMR) spectroscopy (for reviews, see Clore & Gronenborn, 1991a,b). For example, it has been shown in the case of interleukin-1β, a protein of 133 residues, that the positional errors in the atomic co-ordinates of the high resolution NMR structure (Clore et al., 1991), determined on the basis of 3146 approximate experimental restraints, are comparable to those of three independent X-ray structures solved at 2 Å (1 Å = 0.1 nm) resolution (Clore & Gronenborn, 1991c). This high degree of precision was achieved by making use of three- and four-dimensional heteronuclear NMR spectroscopy to obtain the maximum number of structurally useful interproton distance restraints from NOE data, and systematic conformational grid search procedures to obtain stereospecific assignments and φ, ψ and χ₁ torsion angle restraints from NOE and coupling constant data. All these experimental restraints were specified as loose ranges. Thus, the interproton distance restraints were classified into three ranges, 1.8 to 2.7 Å, 1.8 to 3.3 Å and 1.8 to 5.0 Å, corresponding to strong, medium and weak NOEs; likewise, the minimum ranges employed for the φ, ψ and χ₁ restraints were ±30°, ±50° and ±20°, respectively. In an effort to further increase the precision of NMR structures it has been suggested that either more accurate interproton distance restraints derived from an interactive relaxation matrix analysis of the NOE intensities (Borgias & James, 1988, 1990; Boelens et al., 1988, 1989; Post et al., 1990; Borgias et al., 1990; Thomas et al., 1991) or direct refinement against the NOE intensities (Yip & Case, 1989; Nilges et al., 1991) should be employed.

In the present paper, we set out to analyze the practical limits of precision and accuracy that are attainable in NMR protein structure determinations and to delineate the factors that have the greatest bearing on them. To this end, we have carried out a series of model calculations on the IgG binding domain of streptococcal protein G, a protein of 56 residues, for which a very high resolution NMR structure is available (Gronenborn et al., 1991). The structure published originally was based on 1058 experimental restraints comprising 854 NOE derived approximate interproton distance restraints, 60 distance restraints for 30 backbone hydrogen bonds and 144 torsion angle restraints. The atomic r.m.s. distribution of the ensemble of 60 simulated annealing structures about the mean co-ordinate positions was 0.25 Å for the backbone atoms, 0.65 Å for all atoms, and 0.39 Å for all atoms excluding those of disordered surface side-chains. To our knowledge, this structure represents the most precise experimental NMR structure determination published to date using conservative approximate experimental restraints. Further, the NOE restraints are complete in so far that every single cross-peak in the NOESY spectra was assigned. Hence, the collection of 854 NOE restraints used in the structure calculations comprises all the structurally useful NOEs that are observable for this protein (i.e. they do not include, for example, intra-residue interproton distance restraints between protons whose separation is fixed by covalent geometry or between non-stereospecifically assigned protons separated by three bonds). Thus, this experimental data set provides an ideal system for investigating the effects of the (1) number, (2) precision and (3) accuracy of the interproton distance restraints, of (4) direct refinement against model NOE intensities, and of (5) alterations in the description of the non-bonded contacts on the precision and accuracy of the resulting ensemble of calculated structures.

2. Calculational Strategy

(a) The source data

The data for the present investigation comprises the 60 simulated annealing structures (PDB accession code 2GB1) and the restrained minimized mean structure, (SA)₃₅₄ (PDB accession code 1GB1) of the IgG binding domain of protein G published by Gronenborn et al. (1991), together with the set of 1058 experimental restraints (PDB accession code R1GB1MR) used to calculate these structures. The latter comprise 854 structurally useful, approximate NOE interproton distance restraints which are subdivided into 146 sequential (|i−j| = 1), 108 short range (1 < |i−j| ≤ 5) and 291 long range (|i−j| > 5) interresidue restraints, and 309 intraresidue restraints. In addition, there were 60 distance restraints (r_MHO ≤ 2.3 Å and r_NO = 2.5 to 3.3 Å) for

† Abbreviations used: NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; NOESY, nuclear Overhauser enhancement spectroscopy; r.m.s., root-mean-square; SA, simulated annealing; Ig, immunoglobulin; PDB, Protein Data Bank; 3D, 4D, 3-dimensional, 4-dimensional, respectively.
30 hydrogen bonds involving slowly exchanging backbone amide protons, and 144 torsion angle restraints (54 \( \phi \), 31 \( \psi \), and 39 \( \chi_1 \) angles with minimum ranges of \( \pm 30^\circ \), \( \pm 50^\circ \) and \( \pm 20^\circ \), respectively, derived from the experimental NOE and three bond coupling constant data by means of a systematic conformational grid search of \( \phi \), \( \psi \), \( \chi_1 \) space). The 854 NOE interproton distance restraints in the original publication were classified into three ranges, 1.8 to 2.7 Å, 1.8 to 3.3 Å (1.8 to 3.5 Å for NOEs involving NH protons) and 1.8 to 5.0 Å, corresponding to strong, medium and weak NOEs, respectively, with appropriate corrections for center averaging added to the upper bounds of distance restraints involving methyl protons and non-stereo specifically assigned protons. The NOE target function was represented by a square-well quadratic potential, \( F_{\text{NOE}} \), with center averaging given by Clore et al. (1986a):

\[
F_{\text{NOE}} = \sum \begin{cases} k_{\text{NOE}} (R_{ij} - r_{ij}^2), & \text{if } R_{ij} > r_{ij}^2 \\ 0, & \text{if } r_{ij}^2 \leq R_{ij} \leq r_{ij}^2 \\ k_{\text{NOE}} (R_{ij} - r_{ij}^2)^2, & \text{if } R_{ij} < r_{ij}^2, \end{cases}
\]

where \( R_{ij} \) is the calculated distance between protons \( i \) and \( j \), \( r_{ij}^2 \) and \( r_{ij}^2 \) are the values of the upper and lower limits of the target distances, respectively, and \( k_{\text{NOE}} \) is the NOE force constant. The NOE data set included stereospecific assignments for 24 of the 30 \( \beta \) methylene protons, for the \( \alpha \)-methylene protons of all four Gly residues, and for the methyl groups of three of the four Val residues and two of the three Leu residues. In addition, although the \( ^1H \) chemical shifts of the methyl groups of Val21 and Leu7 and of the aromatic ring protons on either side of the Tyr and Phe rings were degenerate, many of the NOEs involving these protons could be stereo specifically assigned on the basis of the initial structures. Finally, it should be noted that the \( \chi_1 \) side-chain torsion angles for the six residues whose \( \beta \)-methylene protons were not stereo assigned are conformationally disordered on the basis of the \( ^1J_{\text{HH}} \) coupling constants of 6 to 7 Hz.

(b) Generation of data sets with accurate or precise NOE distance restraints

To simulate the effect of highly accurate or precise NOE distance restraints, we used the 60 simulated annealing (SA) structures of Gronenborn et al. (1991) (PDB accession code 2GB1) to calculate the mean and standard deviation (\( \sigma \)) of the interproton distances corresponding to the 854 structurally useful, experimentally assigned NOEs. We then replaced the conservative approximate NOE distance restraints with restraints based on the calculated means and moments of the corresponding interproton distances in the 60 SA structures. For NOE restraints involving two uniquely identified protons (e.g. methine, stereospecifically assigned methylene or backbone amide protons), the calculation of the mean and standard deviation of the interproton distance is straightforward. For other protons (e.g. methyl protons, non-stereospecifically assigned methylene and aromatic ring protons), it is necessary to correct the means and standard deviations appropriately. This was accomplished by using the \( <r^{-6}>^{-1/6} \) mean of all the interproton distances corresponding to the protons specified in the NOE restraints file. The resulting standard deviation thus contained a contribution from the variance of the means. The means and standard deviations of the interproton distances were calculated to the nearest 0.001 Å, and the NOE restraints files recalculated using the resulting mean interproton distance as the target distance and twice the standard deviation (2\( \sigma \)) as the characteristic half-width for the interproton distance potential in the simulated annealing protocol.

The large majority of these interproton distances are, in fact, very well defined in the 60 SA structures, as evidenced by very small standard deviations in their values (see Fig. 1 and Table 1). Of the 543 distances between unique proton pairs (even including those with values between 5 and 6 Å), 78% and 94% had a standard deviation of less than 0.1 Å and 0.2 Å, respectively (Fig. 1A and Table 1). Further, 23 of the 103 long-range (5 to 6 Å) distances had a standard deviation of less than 0.1 Å. Not surprisingly, the values of the standard deviations are larger for the 311 distances involving methyl groups or non-stereospecifically assigned protons (Fig. 1B), with the largest deviations being restricted to distances involving disordered surface side-chains. However, even for these distances, 87% had a standard deviation of less than 1.0 Å (Table 1). Thus, the standard deviations of the interproton distance vectors for the 60 SA structures that correspond to the approximate interproton

### Table 1

<table>
<thead>
<tr>
<th>Number of distances (percentage of total)</th>
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<tr>
<td>Standard (Å)</td>
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<tr>
<td>----------------</td>
</tr>
<tr>
<td>1-9</td>
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<tr>
<td>0-1-10</td>
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<tr>
<td>0-10-90</td>
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<td>0-20-50</td>
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<td>0-50-100</td>
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<tr>
<td>100-200</td>
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The 854 interproton distance restraints comprise 146 sequential \( |i-j|=1 \), 108 short range \( |i-j|<5 \), 5) and 291 long range \( |i-j|>8 \) intramolecular restraints and 308 intramolecular restraints.
distance restraints used in the calculation of these structures are, in fact, significantly smaller than the likely errors arising from attempts to obtain accurate or precise interproton distance restraints experimentally through such procedures as interactive relaxation matrix analysis of the NOE intensities (Borgias & James, 1990; Borgias et al., 1990; Post et al., 1990).

This result may at first seem a little surprising, but goes hand in hand with the high precision of the atomic co-ordinates of the structures calculated with the approximate interproton distance restraints. This stems from the fact that although the distance restraints are approximate, taken as a whole they are conformationally restrictive owing to two factors acting in concert: (1) many of the vectors corresponding to the interproton distance restraints are highly correlated, and (2) short distance restraints (<5 Å) between residues far apart in the sequence severely limit the conformational space accessible to the polypeptide chain (Clore & Gronenborn, 1989a).

To simulate the effect of "accurate" interproton distance restraints we employed a harmonic quadratic NOE potential with \( r^{-5} y^{-1.6} \) averaging given by Clore et al. (1985):

\[
F_{\text{NOE}} = \sum \frac{S_{\text{NOE}} k_B T}{2 \sigma_{ij}^2} (R_{ij} - d_{ij})^2.
\]

where \( S_{\text{NOE}} \) is a scale factor, \( k_B \) the Boltzmann constant, \( T \) the temperature in the simulated annealing schedule, \( R_{ij} \) the calculated interproton distance, \( d_{ij} \) the "equilibrium" target distance (set to the mean interproton distance found in the 60 SA structures calculated using all 854 approximate interproton distance restraints), and \( \sigma_{ij} \) the characteristic distance for the harmonic potential set to 2\( \sigma \) of the interproton distances in the 60 SA structures. Thus, \( F_{\text{NOE}} = 0 \) only when the calculated distance \( R \) is equal to the target distance \( d \).

To assess the effect of precise distance restraints we employed a restrictive square-well quadratic potential (Clore et al., 1986a) which assigns no contribution to \( F_{\text{NOE}} \) whenever the interproton distance is within two standard deviations (\( \sigma \)) of the mean value:

\[
F_{\text{NOE}} = \sum \begin{cases} 0, & \text{if } |R_{ij} - d_{ij}| \leq 2\sigma_{ij} \\ k_{\text{NOE}} (R_{ij} - d_{ij} - 2\sigma_{ij})^2, & \text{if } |R_{ij} - d_{ij}| > 2\sigma_{ij} \end{cases}
\]

(c) Generation of data sets with different numbers of NOE restraints

To evaluate the effect of the number of NOE restraints on the precision and accuracy of the ensemble of resulting simulated annealing structures, ten randomly shuffled sets of all 854 NOE restraints were generated; 64% of the 854 restraints are between unique proton pairs, while the remaining 36% involve either methyl and/or non-stereospecifically assigned protons. Because of random sampling, this distribution, as well as that between intra- and interresidue restraints, and between sequential (|i−j| = 1), short-range (1 < |i−j| ≤ 5) and long-range (|i−j| > 5) interresidue restraints, is approximately preserved in all the smaller subsets of restraints. Subsets of four different sizes comprising 76, 107, 153 and 300
restraints, corresponding to an average of 1.36, 1.91, 2.73 and 5.36 restraints per residue, respectively, were then collected by selecting the appropriate number of restraints from the shuffled restraint files, and used in a series of simulated annealing calculations with the three different interproton distance potentials (i.e., accurate harmonic, precise square-well and approximate square-well). Thus, a total of 40 different subsets of NOE interproton distance restraints were used, in addition to the complete set comprising all 834 interproton distance restraints. The number of structures calculated for the different types of interproton distance restraints were as follows. For both the accurate and precise sets of restraints, a structure corresponding to each subset of restraints was calculated, in addition to ten structures calculated for the complete set of restraints, making a total of 50 structures with ten structures for each size category. (Note that in the ensemble of structures calculated with 76 and 107 precise interproton distance restraints, one calculation in each case failed to converge and was therefore excluded from subsequent analysis.) For the approximate set of restraints, two structures corresponding to each subset of restraints were calculated, yielding 20 structures for each size category ranging from 76 to 300 restraints, in addition to the 60 original structures for the complete set of restraints, making a total of 140 structures.

(d) Calculation of structures with interproton distance restraints

Ensemble of structures with the different number and types of interproton distance restraints were calculated using the simulated annealing protocol of Nilges et al. (1988) with the program XPLOR (Brünger et al., 1986, 1987; Brünger, 1992a). In addition to a potential term for the NOE interproton distance restraints, this protocol employs the following terms. Quadratic harmonic potential terms (with force constants of 500 kcal·mol⁻¹·Å⁻² and 500 kcal·mol⁻¹·rad⁻² for bond and angular terms, respectively; 1 kcal = 4.184 J) are used to maintain the covalent geometry very close to ideality. The non-bonded contacts are described by a quartic van der Waals repulsion term, E_repel, given by:

\[ E_{\text{repel}} = \begin{cases} 0, & \text{if } r \geq s_{\text{vdw}}(r_{\text{vdw},i} + r_{\text{vdw},j}) \\ k_{\text{vdw}}[(s_{\text{vdw}}(r_{\text{vdw},i} + r_{\text{vdw},j}))^2 - r^2]^2, & \text{if } r < s_{\text{vdw}}(r_{\text{vdw},i} + r_{\text{vdw},j}). \end{cases} \]

where \( k_{\text{vdw}} \) is a variable force constant, \( r \) is the distance between two atoms, \( r_{\text{vdw},i} \) and \( r_{\text{vdw},j} \) are the standard values of the van der Waals radii of atoms \( i \) and \( j \), respectively, as represented by the Lennard-Jones potential used in the CHARMM PARAM19 and PARAM20 empirical energy function (Brooks et al., 1983; Reider, 1985) (a list of the hard sphere van der Waals radii is provided in Table 2), and \( s_{\text{vdw}} \) is a van der Waals radius scale factor to account for the fact that interatomic separations slightly smaller than the sum of the hard sphere radii can easily occur owing to the attractive component of the van der Waals interaction. In computing equation (4), 1-2 and 1-3 interactions are excluded from the non-bonded list. A square-well quadratic potential is used for the experimental torsion (\( \phi, \psi \) and \( \chi_1 \)) angle restraints and is given by Clore et al. (1986a):

\[ F_{\text{tor}} = \begin{cases} k_{\text{tor}}(\Delta \lambda)^2, & \text{if } \Delta \lambda > \Delta \lambda^* \\ 0, & \text{if } \Delta \lambda \leq \Delta \lambda \leq \Delta \lambda^* \\ k_{\text{tor}}(\Delta \lambda)^2, & \text{if } \Delta \lambda < \Delta \lambda^* \end{cases} \]

where \( \Delta \lambda \) and \( \Delta \lambda^* \) are the upper and lower limits of the target range of a particular torsion angle, respectively. \( \Delta \lambda \) is its calculated value, and \( k_{\text{tor}} \) a force constant. Finally, a square-well quadratic potential (given by eqn (1)) is employed for the hydrogen bonding distance restraints derived experimentally on the basis of the NOE and NH exchange data.

To simplify the analysis, all the simulated annealing calculations included the 60 hydrogen bonding distance restraints and the 144 torsion angle restraints employed in the original structure determination of Gronenborn et al. (1991). The protocol employed proceeded in four phases: (1) 200 cycles of Powell minimization with \( k_{\text{NOE}} = 0 \) kcal·mol⁻¹·Å⁻² (eqns (1) or (3)) or \( S_{\text{NOE}} = 0 \) (eqn (2)), \( k_{\text{tor}} = 0 \) kcal·mol⁻¹·rad⁻², \( s_{\text{vdw}} = 1.0 \) and \( k_{\text{vdw}} = 0.001 \) kcal·mol⁻¹·Å⁻², (2) 75 ps of dynamics at 1000 K (with a time step of 1 fs and coupling to a heat bath) during which time \( k_{\text{NOE}}, S_{\text{NOE}} \) (if applicable) and \( k_{\text{tor}} \) were increased from 0.5 to 30 kcal·mol⁻¹·Å⁻², 0.5 to 30, and 0.5 to 200 kcal·mol⁻¹·rad⁻², respectively, by doubling their values every 75 fs, and \( k_{\text{vdw}} \) was increased from 0.001 to 0.25 kcal·mol⁻¹·Å⁻² by multiplying its value by 1.25 every 75 fs with the van der Waals radius scale factor \( s_{\text{vdw}} \) maintained at a constant value of 1.0; (3) 1.5 ps of dynamics during which time the

| Table 2

| Value of hard sphere van der Waals radii (\( r_{\text{vdw}} \)) used in the present work |
|-----------------|-----------------|-----------------|
| \( r_{\text{vdw}} \) (Å) | \( r_{\text{vdw}(\text{eff})} \) (Å) for \( s_{\text{vdw}} = 0.8 \)† | \( r_{\text{vdw(Dis)}} \) (Å)‡ |
| H polar | 0.90 | 0.94 | 0.95 |
| H non-polar | 1.46 | 1.17 | 1.00 |
| N | 1.35 | 1.24 | 1.20 |
| C | 1.80 | 1.44 | 1.40 |
| (1.35 for aromatic C) |
| O | 1.48 | 1.19 | 1.20 |
| S | 1.90 | 1.52 | 1.60 |

The hard sphere radii are those used in the CHARMM PARAM19/PARAM20 energy parameters (Brooks et al., 1983; Reider, 1985).
† The effective hard sphere van der Waals radius \( r_{\text{vdw(eff)}} \) is given by \( s_{\text{vdw}} \cdot r_{\text{vdw}} \) (cf. eqn (4)).
‡ \( r_{\text{vdw(Dis)}} \) are the hard sphere van der Waals radii employed by the programs DISMAN (Brünger & Go, 1985) and DIANA (Güntert et al., 1991).
system was cooled to 300 K in steps of 25 deg. K every 50 fs, with the van der Waals repulsion terms $k_{vdw}$ and $\phi_{vdw}$ set to 4 kcal mole$^{-1}$ Å$^{-4}$ and 0.8, respectively, and the force constants for the torsion and angle terms set to the same values reached at the end of phase (2); and finally 500 steps of Powell minimization with the same force constants used in phase (3).

To facilitate the calculations and ensure high convergence, the starting structures for the simulated annealing were chosen from the ensemble of the original set of 60 calculated structures published by Gronenborn et al. (1991) and different random number seeds were used in each case for the assignment of initial velocities to a Boltzmann distribution. While this choice of initial structures may at first appear to limit sampling, extensive experience with the present protocol indicates that the use of high temperature and an initially very low value for the van der Waals repulsion force constant $k_{NOE}$ during phase (2) of the protocol (which, for example, enables interpenetration of peptide chains) permits very efficient sampling of the conformational space consistent with the experimental restraints (Nilges et al., 1988; Clore & Gronenborn, 1989a). To verify this assertion, some trial simulated annealing calculations with the subsets of 76, 107, 153 and 200 approximate restraints were carried out using ten different substructures (comprising about one-third of the atoms) obtained by projection from a multidimensional distance space into cartesian co-ordinate space with one of the sets of 76 approximate interproton distance restraints, by means of the distance geometry module of XPLOR (Kuszewski et al., 1992). The resulting accuracy and precision of the trial structures obtained in this manner was not different, within experimental error, from those obtained using starting structures from the original ensemble of 60 simulated annealing structures.

(e) Assessment of the effect of the van der Waals radius scale factor

To assess the effect of the van der Waals radius scale factor, $\alpha_{vdw}$, on the precision and accuracy of the resulting structures, a series of simulated annealing calculations were carried out with the full set of 854 approximate interproton distance restraints, together with the other experimental restraints, using various values of $\alpha_{vdw}$ in phases (3) and (4) of the simulated annealing protocol. All other aspects of the simulated annealing protocol were as described above. Twenty structures were calculated for each of four different values of $\alpha_{vdw}$ (0.7, 0.75, 0.85 and 0.9) and compared to the original ensemble of 60 structures calculated with $\alpha_{vdw} = 0.8$. In addition, a set of 20 structures was calculated using the hard sphere van der Waals radii employed by the programs DISMAN (Braun & Go, 1985) and DIANA (Güntert et al., 1991). The values of these van der Waals radii are listed in Table 2 for comparison with those employed in the CHARMM PARAM19/PARAM20 empirical energy function (Brooks et al., 1983; Reiher, 1985).

(f) Evaluation of relaxation matrix refinement

To evaluate the performance of relaxation matrix refinement on the accuracy and precision of the resulting ensemble of structures, a series of simulated annealing calculations using XPLOR 3.0 (Brünger, 1992a; Nilges et al., 1991) were carried out in which the interproton distance restraints term in the target function was replaced by one directly describing the NOE intensities using the method of Yip & Case (1991). The NOE relaxation target function, $F_{rel}$, is given by:

$$F_{rel} = \sum \left[ \frac{k_{rel}(s_{rel}(I_{obs} - \Delta))^{1/6} - I_{calc}^{1/6}}{2} \right] + \frac{k_{rel}(I_{calc} - \Delta)^{1/6}}{2},$$

where $I_{obs}$ and $I_{calc}$ are the observed and calculated NOE intensities, respectively, $\Delta$ is the error in the observed NOE intensity, $s_{rel}$ is a calibration factor to scale the observed NOE intensities to the calculated ones and is simply determined by the ratio of all the calculated and observed intensities, and $k_{rel}$ is the NOE relaxation force constant.

Model NOE intensities corresponding to the 854 structurally useful NOEs assigned experimentally (i.e. the NOE intensities corresponding to the 854 interproton distance restraints used in the preceding calculations), were calculated for the restrained minimized average structure (SA)$_{854}$ and for one of the SA structures, denoted SA$_{i}$, from the original ensemble of 60 SA structures, using a mixing time of 150 ms, $\langle r^{-2}\rangle^{-1/2}$ averaging for methyl groups, uniform isotropic tumbling with a rotational correlation time of 3.3 ns, and no distance cutoff for the computation of relaxation pathways. The errors specified for the NOE intensities were 5%, 20% and 50% for strong (intensity >0.1, normalized to the theoretical diagonal intensity), medium (0.1 > intensity >0.01) and weak (intensity <0.01) NOEs, respectively. A series of simulated annealing refinements starting from the SA structures were carried out against these model NOE intensity data sets (ten for the data set derived from (SA)$_{854}$ and six for the data set derived from one of the SA structures). The protocol employed comprised three steps: (1) 0.25 ps dynamics (with a time step of 1 fs) at 1000 K in which the force constant $k_{rel}$ was increased from 512 to a maximum value of 312 by doubling its value every 50 fs; (2) 1 ps of simulated annealing with $k_{rel}$ set to 512 during which time the temperature was gradually decreased from 1000 K to 100 K; and (3) 125 cycles of Powell minimization with $k_{rel}$ set to 512. The values of the force constants for the other terms in the target
function (i.e. covalent geometry, van der Waals repulsion, and torsion angle and hydrogen bonding restraints) were held constant at the same final values used in the simulated annealing protocol with interproton distance restraints described above; similarly, the van der Waals radius scale factor $s_{vdw}$ for the quartic van der Waals repulsion term (eqn (4)) was set to 0.8 throughout. A cutoff of 4.5 Å was used in the computation of the relaxation matrix, and $F_{rel}$ was recalculated every time a proton in the NOE restraints list moved by more than 0.03 Å.

To assess the agreement of the calculated and target NOE intensities, two $R$ factors, $R_1$ and $R_{ij/6}$, given by:

$$ R_n = \sum \left[ \frac{\{|s_{rel}(I_{10} - \Delta I)|^n - |I_{10}|^n \}}{\{ |s_{rel}I_{10}|^n \}} \right], $$

were computed with $n = 1$ and $n = 1/6$. The latter corresponds to the $R$-factor suggested by Thomas et al. (1991).

(g) Definition and accuracy of calculated structures

A key aim of the present analysis is to examine the effects of a variety of parameters on both the precision and accuracy of the resulting ensemble of structures. While it is tempting to regard precision and accuracy as identical, they are, in fact, quite distinct, even if to some extent they are interdependent. The precision of an NMR structure determination indicates how close the calculated structures are to each other and is given by the value of the average atomic r.m.s. deviation of the individual structures in a given ensemble from the overall mean co-ordinate positions for the ensemble. The accuracy of an NMR structure determination, on the other hand, represents how close the calculated structures are to the "true" mean structure and is therefore given by the average atomic r.m.s. deviation of the structures in a given ensemble from the "true" mean structure. In an experimental structure determination, the true mean structure is, of course, unknown so that it is impossible to obtain a direct measure of accuracy. In the present case, however, the model interproton distance data sets are derived from an ensemble of 60 simulated annealing structures whose average, $S\Delta A_{554}$, therefore represents the true mean structure. In all the calculations presented below using interproton distance restraints, this mean structure is used as the reference structure. In the calculations using model NOE intensities and relaxation matrix refinement, the structure used to calculate the model NOE intensities represents the true structure.

In addition to the accuracy and precision of an ensemble of structures, we also consider the accuracy of the calculated mean co-ordinates which is defined by the r.m.s. difference between the mean co-ordinates of a given ensemble of structures and the true mean.

One other concept, namely that of intrinsic accuracy, should also be discussed. In real life, the structure of a protein, either in solution or in the crystal state, is not static but dynamic. Consequently, it can only be represented by an ensemble of structures which can be described by mean and atom-based standard deviations. Hence, non-mobile regions of the structure will have a high intrinsic accuracy, whereas mobile regions will be of low intrinsic accuracy. This concept imposes an absolute limit on the attainable accuracy and precision of an experimental structure determination, as

$$ \text{if } l_{10}^* < |s_{rel}(I_{10} - \Delta I)| \text{ then } l_{10}^* \leq |s_{rel}(I_{10} + \Delta I)|, $$

$$ \text{if } |s_{rel}(I_{10} - \Delta I)| \leq l_{10}^* \leq |s_{rel}(I_{10} + \Delta I)|, $$

it is evident that accuracies or precisions that are higher than the intrinsic accuracy have no physical significance.

3. Results

(a) General structural statistics

A key aspect of any NMR structure determination is that the ensemble of calculated structures should satisfy the experimental NMR restraints, exhibit good non-bonded contacts and display very small deviations from idealized covalent geometry. All the calculated structures satisfy the above criteria, indicating that they have located the global minimum region of the target function employed in their computation. Thus, the average r.m.s. deviations from ideality are $\leq 0.003$ Å for bonds, $\leq 2^\circ$ for angles and $\leq 0.5^\circ$ for improper torsions (i.e. planarity and chirality restraints); the average r.m.s. deviations from the experimental torsion ($\phi$, $\psi$ and $\chi$) and hydrogen-bonding restraints range from $0^\circ$ to $0.4^\circ$ and from $0.001$ Å to $0.05$ Å, respectively; and none of the structures exhibit violations $>0.5$ Å with respect to the experimental interproton distance restraints used in their calculation. Finally, for all structures calculated with the van der Waals radius scale factor, $s_{vdw}$, set to 0.8 (cf. eqn (4)), the values of the quartic van der Waals repulsion term $F_{repel}$ and the Lennard–Jones energy (calculated after the fact using the CHARMM PARAM19/PARAM20 empirical energy function) range from 4 to 11 kcal mol$^{-1}$ and from $-186$ to $-225$ kcal mol$^{-1}$, respectively.

(b) Effect of number, precision and accuracy of interproton distance restraints on the precision and accuracy of the resulting structures

Superpositions of calculated structures obtained with the different number, precision and accuracy of interproton distance restraints, are shown in Figure 2, and the corresponding values for their precision
and accuracy are plotted in Figure 3 and summarized in Table 3. These data permit one to draw the following conclusions.

(1) The approximate global fold can be determined, albeit with both low precision and accuracy, with as few as an average of ∼1.4 restraints per residue. Thus, for example, the precision and accuracy of the structures calculated with 76 approximate interproton distance restraints, have values of 1.2 Å and 20 Å, respectively (Table 3). In practice, however, at least twice as many restraints would probably be required. The reason for this is that the ten subsets of 76 interproton distance restraints originate from a much larger set of 854 restraints, and therefore comprise NOE restraints that could not be unambiguously assigned at such an early stage of a structure determination. Further, the restraints list includes stereospecific assignments, torsion angle restraints and hydrogen bonding restraints which, likewise, would not be available at such an early stage.

(2) For a given number of interproton distance restraints, there is only a small difference in either the precision or accuracy of the resulting structures computed with approximate or precise restraints (Table 3). The precision of the structures calculated with accurate restraints, on the other hand, is significantly higher than those calculated with approximate restraints (Table 3). However, it should be borne in mind that, in a real experimental case, irrespective of the accuracy of the measured NOE intensities, it is not feasible to obtain accurate interproton distance restraints, owing to ill-determinacy of spin-diffusion pathways, as well as systematic errors arising from internal motion (Clore & Gronenborn, 1989). The former arises when a particular NOE is dominated by indirect cross-relaxation so that the direct cross-relaxation
rate is ill-determined; consequently, an upper limit of the distance is determined purely by the limits imposed by covalent geometry, while the lower limit is determined by the balance between the direct and indirect cross-relaxation rates.) Further, as discussed in Calculation Strategy, section (b), the precise restraints employed in these calculations are significantly more precise than the highest precision that can possibly be achieved using, for example, iterative relaxation matrix analysis.

(3) The most dramatic improvement in both precision and accuracy of the calculated structures results from increasing the number, rather than either the precision or accuracy, of the interproton distance restraints (Figs 2 and 3; Table 3). The reason for this is that the extent of redundancy in a data set consisting of less than about ten restraints per residue is rather small. Hence, it is not surprising to find that while the structures calculated with a subset of the interproton distance restraints satisfy the restraints used to calculate these structures (i.e. no violations >0.5 Å), they do not satisfy the remainder of the interproton distance restraints, as evidenced by numerous viola-

Figure 3. Variation of the precision, the accuracy, the ratio of precision to accuracy and the relative accuracy for the backbone (N, Cα, C) atoms of the ensemble of structures calculated with approximate (●), precise (■) and accurate (▲) interproton distance restraints as a function of the number of interproton distance restraints. The symbols represent the mean values and the vertical bars are their standard deviations. As the ensemble of 60 structures calculated with the complete set of 854 approximate restraints is used as a reference in the current study, the ratio of precision to accuracy and the relative accuracy of this ensemble of structure is by definition 1. Hence, the use of broken lines to connect the values of the ratio of precision to accuracy and of the relative accuracy for the ensemble of structures calculated with 300 approximate interproton distance restraints to those calculated with 854 approximate interproton distance restraints.
Table 3
Effect of number, precision and accuracy of interproton distance restraints on the precision and accuracy of the ensemble of calculated structures for the 56 residue IgG binding domain of streptococcal protein G

<table>
<thead>
<tr>
<th>Number of interproton distance restraints</th>
<th>Approximate restraints</th>
<th>Precise restraints</th>
<th>Accurate restraints</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Atomic r.m.s. deviation: precision/accuracy (Å)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Backbone atoms†</td>
<td>1.9 ± 0.4/1.9 ± 0.90</td>
<td>1.8 ± 0.2/1.9 ± 0.57</td>
<td>0.82 ± 0.3/0.4 ± 0.45</td>
</tr>
<tr>
<td>76</td>
<td>0.95 ± 0.3/1.0 ± 0.10</td>
<td>0.85 ± 0.3/1.0/0.10</td>
<td></td>
</tr>
<tr>
<td>107</td>
<td>0.72 ± 0.1/0.8 ± 0.23</td>
<td>0.84 ± 0.3/0.9 ± 0.21</td>
<td></td>
</tr>
<tr>
<td>153</td>
<td>0.59 ± 0.2/0.6/0.2 ± 0.17</td>
<td>0.59 ± 0.7/0.6 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>0.39 ± 0.0/0.4 ± 0.10</td>
<td>0.39 ± 0.3/0.4 ± 0.04</td>
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</tr>
<tr>
<td>854</td>
<td>0.25 ± 0.0/0.3 ± 0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All atoms</td>
<td>1.56 ± 0.4/1.58 ± 0.90</td>
<td>1.50 ± 0.2/1.58 ± 0.48</td>
<td>1.18 ± 0.2/1.78 ± 0.42</td>
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<tr>
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<td>1.24 ± 0.1/1.28 ± 0.39</td>
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<td>107</td>
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<td>0.84 ± 0.9/0.9 ± 0.09</td>
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<tr>
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</tr>
<tr>
<td>854</td>
<td>0.14 ± 0.4/0.6 ± 0.5 ± 0.5</td>
<td>1.35 ± 0.2/0.2 ± 0.53</td>
<td>0.41 ± 0.2/0.4 ± 0.34</td>
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<td>107</td>
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<td>0.82 ± 0.1/0.13 ± 0.22</td>
<td>0.62 ± 0.8/0.9 ± 0.17</td>
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<tr>
<td>153</td>
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<td>0.64 ± 0.0/0.6 ± 0.11</td>
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<tr>
<td>300</td>
<td>0.39 ± 0.0/0.39 ± 0.03</td>
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</tr>
<tr>
<td>854</td>
<td>0.39 ± 0.0/0.39 ± 0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All ordered atoms‡</td>
<td>0.64 ± 0.3/0.72 ± 0.25 ± 0.06 ± 0.28$‡</td>
<td>0.12 ± 0.0/0.17 ± 0.09 ± 0.07 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>76</td>
<td>0.14 ± 0.1/0.17 ± 0.09 ± 0.07 ± 0.06</td>
<td></td>
<td></td>
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<tr>
<td>107</td>
<td>0.14 ± 0.1/0.17 ± 0.09 ± 0.07 ± 0.06</td>
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<tr>
<td>153</td>
<td>0.14 ± 0.1/0.17 ± 0.09 ± 0.07 ± 0.06</td>
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<tr>
<td>300</td>
<td>0.14 ± 0.1/0.17 ± 0.09 ± 0.07 ± 0.06</td>
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<td></td>
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<tr>
<td>854</td>
<td>0.14 ± 0.1/0.17 ± 0.09 ± 0.07 ± 0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td>0.14 ± 0.1/0.17 ± 0.09 ± 0.07 ± 0.06</td>
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<td></td>
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<tr>
<td>B. Ratio of precision to accuracy</td>
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<tr>
<td>backbone‡/all atoms/all ordered atoms‡</td>
<td>backbone‡/all atoms/all ordered atoms‡</td>
<td>backbone‡/all atoms/all ordered atoms‡</td>
<td></td>
</tr>
<tr>
<td>76</td>
<td>0.59 ± 0.0/0.66 ± 0.02 ± 0.25</td>
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<tr>
<td>107</td>
<td>0.59 ± 0.0/0.66 ± 0.02 ± 0.25</td>
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<td>153</td>
<td>0.67 ± 0.0/0.72 ± 0.02 ± 0.27</td>
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<tr>
<td>mean</td>
<td>0.66 ± 0.0/0.72 ± 0.02 ± 0.27</td>
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<tr>
<td>C. Relative accuracy</td>
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<td>backbone‡/all atoms/all ordered atoms‡</td>
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<td>backbone‡/all atoms/all ordered atoms‡</td>
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<tr>
<td>76</td>
<td>0.12 ± 0.0/0.17 ± 0.09 ± 0.07 ± 0.06</td>
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<td>mean</td>
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<tr>
<td>mean</td>
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<td>D. Accuracy of mean co-ordinates (r.m.s. difference between ensemble mean and SAx(x))</td>
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<tr>
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</tr>
<tr>
<td>854</td>
<td>0.82</td>
<td>0.82</td>
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</tbody>
</table>

The precision is given by the average atomic r.m.s. deviation of the individual structures of a given ensemble from the ensemble average co-ordinates; the accuracy is given by the average atomic r.m.s. deviation of the individual structures within a given ensemble to the reference mean structure, SAx(x), obtained from the 60 individual simulated annealing structures calculated with the 854 approximate interproton distance restraints and 8≈ = 0.80. The relative accuracy is given by the ratio of the accuracy of the structures in a given ensemble to that of the reference ensemble of 60 structures calculated with the 854 approximate interproton distance restraints and 8≈ = 0.80 (i.e. to PDB accession code number 2BG1).† The atoms included for the backbone atoms are the N, Cα and C atoms.‡ The disordered surface side-chains that are excluded are: Met1 from the C' position onward, Lys4 from C', Lys10 from C', Lys13 from C', Glu15 from C', Glu19 from C', Lys28 from C', Lys31 from C', Glu32 from C', Glu42 from C' and Glu56 from C' (Gronenborn et al., 1991).§ The atomic r.m.s. deviations for the 60 structures calculated with the 894 approximate interproton distance restraints and 8≈ = 0.8 represent the reference ensemble whose mean structure is SAx(x). Hence, their precision and accuracy are by definition identical.
tions >1 Å, including a number >4 Å. Naturally, the larger the subset of restraints used in a given calculation, the smaller the number of violations with respect to the unused restraints, be they approximate, precise or accurate.
(4) The ratio of precision to accuracy for an ensemble of structures appears, in general, to be approximately independent of either the number, the precision or the accuracy of the interproton distance restraints (Fig. 3 and Table 3B). This ratio, however, does depend on the intrinsic accuracy of the true structure. In this series of model calculations, the intrinsic accuracy is by definition equal to both the precision and accuracy of the 60 structures calculated with the 854 approximate interproton distance restraints, as these comprise the basis set for all the model interproton distance restraints. In this particular case, and in all likelihood in general, the intrinsic accuracy of the backbone is greater than that for all atoms, as the latter comprises, for example, disordered surface side-chains, which necessarily have low intrinsic accuracy. The value of the ratio of precision to accuracy is inversely related to the intrinsic accuracy. Hence, the higher the intrinsic accuracy, the lower the ratio of ensemble precision to ensemble accuracy of the calculated structures. This is manifested by a lower value of the ratio of precision to accuracy for the backbone (∼0-06) compared to that for all atoms (∼0-75), with an intermediate value for all atoms excluding those of disordered side-chains (∼0-7).

(5) The accuracy of the mean co-ordinates is invariably better than that of any of the individual structures in a given ensemble (cf. compare Tables 3A and D). In other words, the r.m.s. difference between the mean co-ordinates of a given ensemble and the true mean is always smaller than the r.m.s. difference between the individual structures of a given ensemble and the true mean. This parallels previous observations made in both model calculations (Clore et al., 1986b) and in comparisons between NMR and X-ray structures (Clore et al., 1987a,b; Clore & Gronenborn, 1991c,d,e; Billeter et al., 1989, 1992). When the ratio of precision to accuracy of the ensemble of structures exceeds a value of ∼0-75, the r.m.s. difference between the ensemble mean and the true mean is smaller than the r.m.s. difference between the individual structures of the ensemble and the ensemble mean. Hence, under these conditions, the true mean lies within the ensemble of calculated structures. The dependence of the accuracy of the mean co-ordinates as a function of the precision of the ensemble is shown in Figure 4. For an average ensemble precision >0-6 Å, the dependence of the mean co-ordinates on the ensemble precision is approximately linear. As the ensemble precision is increased beyond ∼0-6 Å, the dependence is asymptotic, reaching a limiting value of ∼0-1 Å for backbone atoms, all atoms and all ordered atoms. The curves obtained for the structures calculated with the approximate (excluding the reference ensemble) and precise interproton distance restraints are completely superimposable. The curves obtained for the structures calculated with the accurate interproton distance restraints, on the other hand, are displaced to the left. Hence, for a given level of ensemble precision, the accuracy of the mean co-ordinates obtained for an ensemble of structures calculated with approximate or precise interproton distance restraints is actually more accurate than that of the mean co-ordinates obtained from an ensemble of structures calculated with accurate interproton distance restraints. This is presumably due to a slightly different balance of forces in the target function for these three cases. Further, for a given level of ensemble precision, the accuracy of the mean co-ordinates is greater for all atoms than for all ordered atoms which, in turn, is greater than that for the backbone atoms. This is related to the concept of intrinsic accuracy discussed above.

(6) Both the precision and accuracy of the structures is directly correlated to their radii of gyration (Fig. 5). Thus, the low precision, low accuracy structures calculated with a small number of restraints are expanded relative to the high precision, high accuracy structures calculated with the full complement of restraints. This can also be appreciated visually from the various superpositions of structures shown in Figure 2. This observation is not too surprising as the only attractive forces present in the target function are those associated with the potential for the interproton distance restraints. Hence, the fewer the number of interproton distance restraints, the smaller the contribution of the attractive forces to the target function.

(7) The maximum accuracy achievable is about 0-25 Å for the backbone atoms, 0-6 Å for all atoms and 0-4 Å for ordered side-chains (Table 3). Thus, for example, even though the precision of the backbone co-ordinates for the structures calculated with the 854 accurate interproton distance restraints (∼0-12 Å) is almost double that of the reference ensemble calculated with the 854 approximate restraints (0-25 Å), the accuracy of the two sets of structures is identical (0-25 Å). (Note that as the structures calculated with the 854 approximate interproton distance restraints comprise the reference ensemble, their precision and accuracy are by definition identical.) Similar results are observed for all atoms, as well as all ordered atoms. It is also noteworthy that despite the fact that the precision of the ensemble of structures calculated with the 854 precise interproton distance restraints is approximately the same as that calculated with the 854 approximate interproton distance restraints, the accuracy of the former is a little lower than that of the latter. Nevertheless, with the exception of the backbone atoms for the ensemble of structures calculated with the 854 accurate restraints, the true mean lies within the ensemble of structures calculated with either 854 precise or accurate restraints. These results, in all likelihood, represent the absolute limits on both accuracy and precision that can be obtained in practice as this data set
comprises all the structurally useful NOEs that are observed experimentally. Moreover, analysis of the data indicates that both the accuracy and precision follow an approximately \( x^{-n} \) dependence on the number of distances \( x \), where \( n \) varies from \( \sim 0.6 \) to \( \sim 0.8 \). Hence, any significant improvement over the results obtained with the set of 854 interproton distance restraints (corresponding to \( \sim 15 \) restraints per residue with \( \sim 9 \) restraints per residue involving unique proton pairs) would require an approximate doubling of the number of interproton distance restraints.

(c) Effect of full NOE relaxation matrix refinement on the precision and accuracy of the calculated structures

Although it is not feasible to obtain accurate interproton distances from NOE data, it is potentially possible to obtain accurate measurements of NOE intensities. Consequently, it is of interest to assess the effect of direct refinement against NOE intensities on the accuracy and precision of the resulting structures. Two series of calculations were carried out, using model NOE intensities corre-
sponding to the 854 interproton distance restraints. In the first series the NOE intensities were derived from the restrained minimized average structure (SA)$_{854}$, while in the second they were obtained from one of the SA structures, denoted as SA$_x$, calculated with the 854 approximate interproton distance restraints. In both cases the NOE intensities represent idealized intensities as they are calculated assuming a rigid molecule and hence do not include any complications that could arise in practice from internal motions. They do, however, include likely experimental errors in peak integration as they are represented by square-well potentials with errors of 5, 20 and 50% for strong, medium and weak NOEs, respectively (see Calculational Strategy, section (e)). A superposition of the backbone atoms of the refined structures from the first series of calculations is shown in Figure 6, together with a superposition of the mean structure after relaxation matrix refinement with the restrained minimized average structure (SA)$_{854}$. A summary of the $R$-factors and r.m.s. differences before and after relaxation matrix refinement is provided in Tables 4 and 5, respectively.

In both sets of calculations, the structures generated by the full relaxation matrix refinement

![Graphs showing precision and accuracy](image)

**Figure 5.** Variation of the precision and accuracy for the backbone (N, C$^\alpha$, C) atoms of the ensemble of structures calculated with approximate (■), precise (■) and accurate (▲) interproton distance restraints as a function of their radii of gyration.

**Table 4**

<table>
<thead>
<tr>
<th>Structure</th>
<th>$R_{1%}$</th>
<th>$R_1$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Model NOE intensities from (SA)$_{854}$</strong>&lt;br&gt;Before relaxation matrix refinement&lt;br&gt;⟨SA⟩ ($N = 10$)</td>
<td>0.0051 ± 0.0011</td>
<td>0.070 ± 0.0021</td>
</tr>
<tr>
<td>After relaxation matrix refinement&lt;br&gt;⟨RR⟩ ($N = 10$)</td>
<td>0.0013 ± 0.0003</td>
<td>0.020 ± 0.0004</td>
</tr>
<tr>
<td><strong>B. Model NOE intensities from structure SA$_x$</strong>&lt;br&gt;Before relaxation refinement&lt;br&gt;⟨SA⟩$_{854}$ ($N = 9$)</td>
<td>0.0005 ± 0.0010</td>
<td>0.007 ± 0.0017</td>
</tr>
<tr>
<td>After relaxation refinement&lt;br&gt;⟨RR2⟩ ($N = 6$)</td>
<td>0.0031 ± 0.0006</td>
<td>0.029 ± 0.0005</td>
</tr>
</tbody>
</table>

The structures are obtained by simulated annealing relaxation matrix refinement against model NOE intensities corresponding to the 854 approximate interproton distance restraints (PDB accession code no. 1GB1MR). (RR) represent the 10 structures calculated using model NOE intensities derived from (SA)$_{854}$ (PDB accession code no. 1GB1). (RR2) represent the 6 structures calculated using model NOE intensities derived from one of the 60 structures, denoted as SA$_x$, obtained with the 854 approximate interproton distance restraints (PDB accession code no. 2GB1). The definition of the two $R$-factors, $R_{1\%}$ and $R_1$, is given by eqn (7). The model NOE intensities are represented by square-well potentials (eqn. (6)) with errors of 5, 20 and 50% for strong medium and weak NOEs, respectively (see Calculational Strategy, section (e)).
Figure 6. A. Superposition of the backbone (N, Cα, C) atoms of the 10 structures obtained by simulated annealing relaxation matrix refinement against the calculated NOE intensities (corresponding to the 854 structurally useful NOEs assigned experimentally) derived from the restrained minimized mean structure (SA)_{854}. B. Superposition of the backbone (N, Cα, C) atoms of the mean co-ordinates of the 10 relaxation matrix refined structures (thin line) and the restrained minimized mean structure (SA)_{854} (thick line).

display good covalent geometry and non-bonded contacts, as well as very small deviations from the torsion angle and hydrogen bonding restraints (cf. section (a), above). In addition, the agreement with the model NOE intensities improved significantly. Thus, in the case of the calculations with the model NOE intensities derived from (SA)_{854}, the R_{16} and R_{1} R-factors decreased from values of 0.5% and 7%, respectively, before refinement to 0.13% and 2%, respectively, after refinement (Table 4). Interestingly, however, the number of interproton distance violations with respect to the complete set of 854 approximate or precise interproton distance restraints is larger for the structures (〈RR〉) after relaxation matrix refinement than for either the ten SA structures before refinement or the restrained minimized average structure (SA)_{854}. Thus, whereas the ten SA structures prior to relaxation matrix refinement exhibit no interproton distance violations >0.3 Å, the ten RR structures after relaxation matrix refinement display an average of 14.4±3.1, 4.9±1.5 and 0.2±0.4 violations in the range 0.3 to 0.5 Å, 0.5 to 1.0 Å and 1.0 to 2.0 Å, respectively. This reflects the ill-determinacy of some of the relaxation pathways, particularly with regard to the balance between direct and indirect cross-relaxation (Clore & Gronenborn, 1989). Moreover, it should be noted that the starting SA structures in both cases, show very good agreement with the model NOE intensities, providing further proof of the small standard deviations in the vectors corresponding to the 854 interproton restraints (cf. Fig. 1 and Table 1).

The calculations carried out with the model NOE intensities derived from (SA)_{854} reveal a surprising observation. Namely, while the precision of the structures following relaxation matrix refinement is increased significantly, there is no improvement in accuracy (Table 5A). Thus, for example, the precision of the backbone co-ordinates improves from 0.27 Å before refinement to 0.15 Å following relaxation matrix refinement, while the accuracy of the backbone co-ordinates actually decreases slightly from 0.27 Å to 0.31 Å. Further, a comparison of the values for the precision of the ensemble with the accuracy of the mean co-ordinates, indicates that in the case of the backbone atoms, the true structure (i.e. (SA)_{854}) actually lies outside the calculated ensemble of relaxation matrix refined structures (cf. Table 5A and 5C). In the case of the co-ordinates for either all atoms or all ordered atoms, the precision of the co-ordinates is also improved, but their accuracy remains unchanged (Table 5A).

For the calculations carried out with the model NOE intensities derived from one of the SA structures (denoted SA_{x}), the starting co-ordinates are, of
Table 5
Effect of relaxation matrix refinement on the precision and accuracy of the ensemble of calculated structures for the 56 residue IgG binding domain of streptococcal protein G

<table>
<thead>
<tr>
<th>Structure</th>
<th>Backbone atoms†</th>
<th>All atoms</th>
<th>All ordered atoms‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Model NOE intensities from (SA)$_{185a}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before relaxation matrix refinement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(SA) (N = 10)</td>
<td>0.25 ± 0.03/0.27 ± 0.04</td>
<td>0.67 ± 0.05/0.77 ± 0.05</td>
<td>0.32 ± 0.03/0.48 ± 0.04</td>
</tr>
<tr>
<td>(93 ± 25)</td>
<td>(987 ± 912)</td>
<td>(985 ± 914)</td>
<td></td>
</tr>
<tr>
<td>After relaxation matrix refinement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(RR) (N = 10)</td>
<td>0.15 ± 0.02/0.31 ± 0.02</td>
<td>0.48 ± 0.05/0.68 ± 0.07</td>
<td>0.27 ± 0.01/0.45 ± 0.03</td>
</tr>
<tr>
<td>(048 ± 0.10/0.87 ± 0.19)</td>
<td>(971 ± 041/1.3 ± 0.19)</td>
<td>(969 ± 0.06/1.02 ± 0.16)</td>
<td></td>
</tr>
<tr>
<td>B. Model NOE intensities from structure SA$_2$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before relaxation matrix refinement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(SA)$_{28}$ (N = 6)</td>
<td>0.25 ± 0.02/0.33 ± 0.06</td>
<td>0.68 ± 0.05/0.78 ± 0.04</td>
<td>0.38 ± 0.03/0.54 ± 0.03</td>
</tr>
<tr>
<td>(048 ± 0.15)</td>
<td>(985 ± 0.11)</td>
<td>(970 ± 0.09)</td>
<td></td>
</tr>
<tr>
<td>After relaxation matrix refinement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(RR2) (N = 6)</td>
<td>0.16 ± 0.03/0.30 ± 0.03</td>
<td>0.49 ± 0.03/0.79 ± 0.12</td>
<td>0.27 ± 0.02/0.49 ± 0.06</td>
</tr>
<tr>
<td>(033 ± 0.15)</td>
<td>(969 ± 0.15)</td>
<td>(955 ± 0.11)</td>
<td></td>
</tr>
<tr>
<td>C. Accuracy of mean co-ordinates after relaxation matrix refinement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RR vs (SA)$_{185a}$</td>
<td>0.28</td>
<td>0.49</td>
<td>0.36</td>
</tr>
<tr>
<td>RR vs SA$_2$</td>
<td>0.26</td>
<td>0.64</td>
<td>0.41</td>
</tr>
</tbody>
</table>

The precision of the ensemble of (RR) and (RR2) structures is defined as the average atomic r.m.s. difference of the individual structures about their respective ensemble means. The accuracy of the (RR) and (RR2) ensemble of structures is defined as the average atomic r.m.s. difference of the individual structures from (SA)$_{185a}$ and SA$_2$, respectively, which represent the 2 structures, respectively, from which the target NOE intensities were calculated. The relative accuracy is only given for the (RR) set of structures and is defined as the ratio of the accuracy of the (RR) structures to that of the corresponding 10 (SA) structures prior to relaxation matrix refinement. Hence, the relative accuracy of the 10 (SA) structures is by definition 1.† The atoms included for the backbone atoms are the N, C$^\alpha$, and C atoms.‡ The disordered surface side-chains that are excluded are given in footnote † to Table 3.

d) Effect of the non-bonded contact term on the precision and accuracy of the resulting structures

Because the main source of structural information derived from NMR resides in short interproton distance restraints, other information in the form of restraints on covalent geometry and non-bonded contacts are absolutely essential to an NMR structure determination. While there is general agreement on the values of standard bond lengths and angles which vary little from one molecule to another, the representation of non-bonded contacts is more problematic. The non-bonded contributions may be partitioned into three categories: van der Waals, electrostatic, and hydrogen bonding. It is precisely these terms that differ most from one empirical energy parameter set to another (Momany et al., 1974; Brooks et al., 1983; Reiher, 1985; Weiner et al., 1984, 1986; Nilsson & Karplus, 1986; Dauber-Osguthorpe et al., 1988). In the case of an NMR structure determination, the simplest approach is to represent the non-bonded contacts solely by a repulsion term which prevents atoms from coming too close together (e.g. eqn (4)). To test the effect of this term on the resulting structures, we carried out a series of calculations in which the van der Waals radius scale factor $\sigma_{dW}$ of the quartic van der Waals repulsion potential given by equation (4) was varied from 0.70 to 0.90 for the CHARMM PARAM19/PARAM20 van der Waals radii (Brooks et al., 1983; Reiher, 1985) given in Table 2. The results are summarized for the backbone in Figure 7 and a similar trend is observed for all atoms and all ordered atoms.

While the precision of the co-ordinates remain essentially unchanged as $\sigma_{dW}$ is increased from 0.80 to 0.90, there is a significant decrease in accuracy, and the true mean lies outside the ensemble of calculated structures. In addition, the van der Waals Lennard–Jones energy (calculated with the CHARMM PARAM19/PARAM20 empirical energy parameters) becomes more negative, decreasing
from $-210(\pm 7)$ kcal\cdot mol$^{-1}$ for $s_{vdw} = 0.8$ to $-284(\pm 5)$ kcal\cdot mol$^{-1}$ for $s_{vdw} = 0.9$, as the atoms are forced to be further apart.

When $s_{vdw}$ is decreased from 0.80 to 0.70, there is both a decrease in precision and accuracy, but in contrast to the results with $s_{vdw} \geq 0.85$, the true mean still lies within the ensemble of calculated structures. The reason for the decrease in precision with decreasing values of $s_{vdw}$ can be attributed to the increase in conformational space compatible with the target function as the effective hard sphere van der Waals radii are decreased. Also, in contrast to the results with $s_{vdw} \geq 0.85$, the Lennard–Jones van der Waals energy becomes more positive ($-144(\pm 19)$ kcal\cdot mol$^{-1}$ for $s_{vdw} = 0.75$ and $-19(\pm 34)$ kcal\cdot mol$^{-1}$ for $s_{vdw} = 0.70$) as the average interatomic distances between contacting atoms decreases. Perhaps more significant is that a change in the effective hard sphere van der Waals radii by as little as 6% from their values with $s_{vdw} = 0.8$ to those with $s_{vdw} = 0.75$ or 0.85, results in a decrease of about 70% in the accuracy of the backbone co-ordinates. This is of considerable practical significance as variations in the van der Waals radii of this magnitude and larger are seen from one empirical energy parameter set to another. Also of interest is that the radius of gyration is proportional to $s_{vdw}$.

To test the effect of a set of van der Waals radii from another empirical energy parameter set, we also carried out a series of calculations using the hard sphere van der Waals radii employed by the programs DISMAN (Braun & Go, 1985) and DIANA (Güntert et al., 1991). These effective radii
were derived from the ECEPP energy parameters (Momany et al., 1974) and were set to values resulting in a calculated Lennard–Jones energy of 3 kcal·mol⁻¹ between atom pairs (Braun & Go, 1985). The main difference between these radii and those of the PARAM19/PARAM20 energy parameters of CHARMM with \( s_{ab} = 0.8 \) lies in the values of the radii of the proton (Table 1). Thus, the effective hard sphere van der Waals radii of polar hydrogens (e.g., backbone and side-chain amide protons) are ~30% larger in the DISMAN/DIANA parameter set, while those of aliphatic and aromatic protons are approximately 15% smaller. It is worthwhile noting, in this regard, that the uncertainty in the values of the hard sphere van der Waals radii is largest for hydrogens. In addition, there are still sizeable differences of 5 to 6% in the effective hard sphere van der Waals radii employed for the nitrogen and aromatic carbon atoms. Despite these differences, the decrease in precision of the resulting structures relative to the reference structures obtained with the CHARMM PARAM19/PARAM20 radii and \( s_{ab} = 0.8 \) is small (30%, 10%, and 20%, for backbone atoms, all atoms, and all ordered atoms, respectively). The accuracy, however, is reduced significantly by 60% for backbone atoms, 20%, for all atoms, and 40% for all ordered atoms. Nevertheless, the true mean still lies within the ensemble of calculated structures as the r.m.s.d differences between the ensemble mean and the true mean, for backbone atoms, all atoms and all ordered atoms, are smaller than the corresponding r.m.s.d values for the precision of the calculated structures. It is also worth noting that the Lennard–Jones van der Waals energy calculated with the CHARMM PARAM19/PARAM20 energy parameters is more positive for the DISMAN ensemble of structures (\(-172(±7)\) kcal·mol⁻¹) than for the reference structures calculated with \( s_{ab} = 0.8 \) (\(-210(±7)\) kcal·mol⁻¹).

It must be emphasized that these results do not in any way imply that the DISMAN radii provide a worse representation of the non-bonded interactions than the CHARMM PARAM19/PARAM20 radii with \( s_{ab} = 0.8 \), as the true and exact description of the non-bonded interactions of a protein in solution is, of course, unknown. Rather, these results provide a measure of the uncertainties in the calculated coordinates engendered by the uncertainties in the description of the non-bonded contacts.

4. Discussion

The results presented in this paper provide a benchmark for assessing the limits of precision and accuracy that are attainable in NMR protein structure determinations.

The main conclusions that can be derived are as follows:

1. The most crucial determinant of both precision and accuracy is the number of interproton distance restraints (Table 3; Figs 2 and 3). Further, while large increases in precision and accuracy are observed as the number of restraints is increased to an average of ~15 per residue (of which ~9 per residue, corresponding to ~60% of the total number, are between unique proton pairs), subsequent increases in the number of restraints lead to less dramatic improvements (Fig. 3). This is due to the fact that a significant degree of information redundancy sets in above this limit.

2. As a corollary to (1), the interproton distance vectors corresponding to the restraints in an ensemble of structures generated from a large number of loose approximate interproton distance restraints (i.e., an average of >15 per residue) are very well defined with ~80% of the vectors between unique proton pairs having a standard deviation of ≤0.1 Å (Table 1 and Fig. 1).

3. The use of precise interproton distance restraints (represented by a restrictive square-well potential; eqn (3)), as opposed to loose approximate ones (cf. eqn (1)) has almost no effect on the attainable precision or accuracy (cf. Table 3; Figs 2 and 3).

4. The precision of an NMR structure determination can be improved by either the use of accurate (as opposed to simply precise) interproton distance restraints (represented by a harmonic potential; eqn (2)) or by direct refinement against the NOE intensities (cf. eqn (6)). Interestingly, however, the structures obtained with the complete set of 854 accurate interproton distance restraints or with direct refinement against the corresponding 854 NOE intensities are no more accurate than those of the reference ensemble obtained with the 854 approximate interproton distance restraints (cf. Tables 3 and 5). Moreover, for a given level of ensemble precision, the accuracy of the mean coordinates for an ensemble of structures calculated with accurate interproton distance restraints is actually less than that of the mean coordinates for an ensemble of structures calculated with either approximate or precise interproton distance restraints (Fig. 4). Indeed, there is no difference in the accuracy of the mean coordinates for the ensemble of structures calculated with the complete set of 854 approximate or accurate interproton distance restraints (Table 3D), despite the fact that the precision of the former is less than that of the latter (Table 3A).

5. The description of the non-bonded interactions, as evidenced, for example, by small variations in the effective hard sphere van der Waals radii used in the van der Waals repulsion term (cf. eqn (4)), has a significant effect on both precision and accuracy (Fig. 7). This imposes very real limits on the accuracy of an NMR structure determination and presents a major source of uncertainty, irrespective of the manner in which the geometric information of the NOE is incorporated into the target function. Indeed, even an alteration in the balance of forces within the target function, and in particular between the van der Waals term and the experimental NMR terms, is sufficient to have a significant effect on accuracy. This is
evidenced by the results obtained with accurate interproton distance restraints and full relaxation matrix refinement. In both these cases the "true" structure actually lies outside the ensemble of calculated structures, despite the fact that the latter are highly precise (cf. Tables 3 and 5).

What do these results imply in practice, particularly with regard to assessing the accuracy of a protein structure determination? It has been suggested (Thomas et al., 1991; Nilges et al., 1991) that the computation of an NMR R-factor between calculated and observed NOE intensities (cf. eqn (7)) would provide a more reliable method of assessment than the r.m.s. difference and violation count between calculated interproton distances and their corresponding target upper bounds for a set of approximate interproton distance restraints (in which the lower bound is set to 1.8 Å). The results presented suggest that this is not the case. Several criteria have to be met in an NMR structure determination, namely, the calculated structures must satisfy the experimental restraints as well as the restraints imposed by covalent geometry and stereochemistry (i.e. non-bonded contacts). However, these are not sufficient in themselves for assessing the quality of the NMR structure determination. For example, it is quite clear that if the average number of NOE restraints per residue is small, the structures generated will be of low accuracy despite the fact that they may exhibit very good agreement with the NOE restraints, be it in terms of violations or NOE intensities, as well as with the other restraints in the target function. Moreover, it is also clear that owing to the limited redundancy of the data, the structures will be unable to satisfy many of the remaining unassigned NOE in the spectrum. Hence, one can only begin to talk seriously about an accurate structure determination when at least 90% of the structurally useful NOEs have been assigned; that is to say when a significant degree of redundancy has set in. (This can be evaluated by carrying out a series of refinements using different subsets of interproton distance restraints or NOE intensities comprising, say, 90 to 95% of the total number, and calculating the agreement with the remaining 5 to 10%, which are not included in the target function, a procedure similar in spirit to that of the free R-factor in X-ray crystallography (Brüning, 1992).) Further, it is only under these circumstances that potential errors in NOE assignments can be assessed and corrected.

The results summarized in Tables 4 and 5 indicate that at this stage of a protein NMR structure determination, any potential improvement that may be obtained by switching from a target function based on approximate interproton distance restraints to one based on direct refinement against NOE intensities, is likely to be insignificant. Moreover, given possibly large systematic errors in NOE intensities (up to 50 to 100%, see below), it seems likely in practice that any increased precision obtained by relaxation matrix refinement may be achieved at the expense of accuracy.

While relaxation matrix refinement can clearly account for multiple spin-relaxation pathways, inherent problems remain in terms of its practical applications, particularly with respect to the goal of obtaining accurate structures. For example, in a recent analysis of a 100 ps dynamics simulation of lysozyme, it has been shown that the rigid but correct structure would yield an R_{1s}-factor of 22% for non-methyl internal proton pairs and 30% for pairs involving methyl protons, purely due to picosecond motions ≤ 10 ps (Post, 1992). Even larger discrepancies can be expected from internal motions which lie outside the extreme narrowing limit (i.e. motions with an internal correlation time τ_{i} in the range 0.5 ps < τ_{i} < τ_{s}, where τ_{s} is the rotational correlation time of the protein). Examples of such transitions have been seen as rare events in a recent 500 ps molecular dynamics simulation of interleukin-1β in water (Chandrasekhar et al., 1992), and direct experimental evidence for such motions has been obtained from 15N relaxation measurements (Clore et al., 1990a,b; Stone et al., 1992; Kördel et al., 1992; Barbato et al., 1992; Nicholson et al., 1992, Powers et al., 1992). These studies have also revealed the existence of heterogeneity arising from chemical exchange which will give rise to systematic errors in NOE intensities. While these two effects may generally be expected to involve exposed regions of the protein (e.g. loops and surface residues), this need not always be the case. Indeed, the recent joint NMR/X-ray refinement of the structure of interleukin-1β reveals that the side-chains of two completely buried leucine residues exist in two distinct X_{2} rotamer conformations (Shaanan et al., 1992). Another source of error arises from practical considerations involved in the actual implementation of the NOE experiment. For example, it may not be feasible to allow sufficient time for complete relaxation back to equilibrium to occur between successive scans. This is particularly a problem in 3D and 4D NMR where the relaxation delay between scans has to be limited to a maximum of ~1 s in order to ensure that the experiment can be completed in a reasonable time frame. In addition, differential efficiency of transfer of magnetization from NH to 15N and/or CH to 13C via one-bond heteronuclear couplings in 3D and 4D experiments, arising from variations in linewidths, will introduce significant errors in NOE intensities. In contrast to relaxation matrix refinement, the use of loose approximate interproton distance restraints (e.g. 1.8 to 2.7 Å, 1.8 to 3.3 Å and 1.8 to 5 Å for strong, medium and weak NOE intensities, respectively, with additional corrections to the upper bounds in the case of methyl groups and non-stereospecifically assigned protons) avoids these problems (Withrich, 1988; Clore & Gronenborn, 1989d). The distance ranges are sufficiently generous to take into account the effects of spin diffusion. At the same time, internal motions (whose effect is simply to reduce the observed NOE intensity of a given proton-proton interaction) will not introduce errors, but rather simply increase the estimated range for a
particular interproton distance. The use of distinct
cut-offs, on the other hand, can result in systematic
errors for interproton distances whose values lie at
the boundary of two distance ranges. This, however,
can be corrected by examining the distribution of
violations in the ensemble of calculated structures.
If a particular interproton distance restraint is
violated in nearly all the structures, even by as little
as 0.1 Å, it is likely that it should either be reclassi-
fied into the next class (i.e., strong to medium,
medium to weak) or that errors in NOE assignments
are present.

The results presented in this paper also permit
one to obtain an approximate estimate of accuracy
from the precision of the ensemble of calculated
structures. For a given non-bonded potential in the
target function, the ratio of ensemble precision to
ensemble accuracy is approximately independent of
the number, precision or accuracy of the interproton
distance restraints with an average value of 0.65,
0.75 and 0.7 for the backbone atoms, all atoms and
all ordered atoms, respectively (Table 3).
Independently, uncertainties in the values of the
hard sphere van der Waals radii will also reduce the
ratio of precision to accuracy (cf. Fig. 7).
Examination of the van der Waals parameters for a
variety of empirical force fields suggests that the
values of the effective hard sphere van der Waals
radii given by \( r_{vdw} \) can vary within the
range of 0.75 to 0.85 using the van der Waals radii of
the CHARM PARAM19/PARAM20
force field for \( r_{vdw} \). This, for example, encompasses
the hard sphere van der Waals radii used in the
programs DISMAN (Braun & Go, 1985) and
DIANA (Güntert et al., 1991; cf. Table 2).
The results with \( r_{vdw} = 0.85 \) have the lowest ratio of
precision to accuracy (~0.6, ~0.8 and ~0.7 for
backbone atoms, all atoms and all ordered atoms)
and therefore probably represent an upper limit of the
effect of uncertainties in the van der Waals radii
on these values. Hence, the overall ratio of ensemble
precision to ensemble accuracy is the product of
these two factors which yields average values of
~0.4, ~0.6 and ~0.5 for backbone atoms, all atoms
and all ordered atoms. If we consider that the
precision of an ensemble of structures attainable in
the relaxation matrix refinement calculations with
the 854 NOE restraints represents the best that can
be achieved in practice, then the likely best upper
limits of ensemble accuracy that can be achieved in
an NMR structure determination are ~0.4 Å for
backbone atoms, ~0.8 Å for all atoms and ~0.5 Å
for all ordered atoms. These values are comparable
to those of a 2 Å resolution crystal structure, as
judged by the r.m.s. differences between independ-
ently solved crystal structures of the same protein
in the same crystal form (Clore & Gronenborn,
1991e). However, these estimates do not take into
account the potential systematic errors in relaxation
matrix refinement arising from internal
motions. Hence, if one considers that the precision
of the ensemble of structures calculated with the
854 approximate interproton restraints represents a
more realistic estimate for an upper limit on
ensemble precision, then the corresponding limits on
ensemble accuracy would be ~0.6 Å for backbone
atoms, ~1.1 Å for all atoms and ~0.8 Å for all
ordered atoms. The accuracy of the mean co-
ordinates of a given ensemble is significantly higher
than the average accuracy of the individual struc-
tures within an ensemble. From the plots shown in
Figure 4, the accuracy of the mean co-ordinates
reaches a limiting value of ~0.1 Å. Adding in an
uncertainty of 0.2 to 0.3 Å (cf. Fig. 7) attributable
to the non-bonded contacts suggests that the actual
limiting accuracy of the mean co-ordinates is 0.3 to
0.4 Å. Given that there are a finite number of short
interproton distance contacts that can be observed
experimentally, this translates to a likely upper
limit on the attainable accuracy of the mean co-
ordinates of 0.4 to 0.5 Å for backbone atoms, 0.6 to
0.7 Å for all atoms and 0.5 to 0.6 Å for all ordered
atoms. In this light, it is perhaps not surprising that
even for the most precise NMR structures deter-
tained to date, the backbone atomic r.m.s. differ-
ences between them and the corresponding X-ray
structure(s) range from 0.7 to 1.2 Å (Billiet et al.,
1989, 1992; Clore & Gronenborn, 1991d; Moore
et al., 1991).

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