Protein Structure Determination using Protein Threading and Sparse NMR Data

(Extended Abstract)

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Abstract

It is well known that the NMR method for protein structure determination applies to small proteins and that its effectiveness decreases very rapidly as the molecular weight increases beyond about 30 kD. We have recently developed a method for protein structure determination that can fully utilize partial NMR data as calculation constraints. The core of the method is a threading algorithm that guarantees to find a globally optimal alignment between a query sequence and a template structure, under distance constraints specified by NMR/NOE data. Our preliminary tests have demonstrated that a small number of NMR/NOE distance restraints can significantly improve threading performance in both fold recognition and threading-alignment accuracy, and can possibly extend threading's scope of applicability from structural homologs to structural analogs. An accurate backbone structure generated by NMR-constrained threading can then provide a significant amount of structural information, equivalent to that provided by the NMR method with many NMR/NOE restraints; and hence can greatly reduce the amount of NMR data typically required for accurate structure determination. Our preliminary study suggests that a small number of NOE restraints may suffice to determine adequately the all-atom structure when those restraints are incorporated in a procedure combining threading, modeling of loops and sidechains, and molecular dynamics simulation. Potentially, this new technique can expand NMR's capability to larger proteins.

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1 Introduction

The NMR method for protein structure determination is mainly based on (i) a network of distance restraints between spatially close hydrogen atoms, derived from nuclear Overhauser effects (NOEs), and (ii) restraints, calculated from scalar coupling constants, on the dihedral angles defined by the positions of atoms separated by three covalent bonds. The NOE restraints are essential to determine the secondary and tertiary structure of a protein, as they relate hydrogen atoms separated by less than about 5Å in amino acid residues that may not be adjacent in the protein sequence. An NMR structure is typically determined through molecular dynamics simulation/energy minimization under the constraints[a] specified by NMR restraints [1, 2, 3, 4]. It typically requires about 15-25 NOE restraints per residue to obtain an accurate (mean) structure (equivalent to a 2-3Å Xray structure).

One problem with the NMR method is that it applies only to "small" proteins. Of the 1558 NMR structures in PDB (release of May 1999) [5], only 25 are larger than 200 amino acids and the largest one has 269 residues (about 30 kD). This limitation is mainly caused by spectral data crowding and line broadening for larger proteins, which result in reduction in the fraction of spectral peaks that can be identified and assigned.

It is often possible to collect some NOE restraints for large proteins. Though these NOE restraints may not

[a] In this paper, a *constraint* is used to refer to optimization algorithms and a *restraint* to NMR data

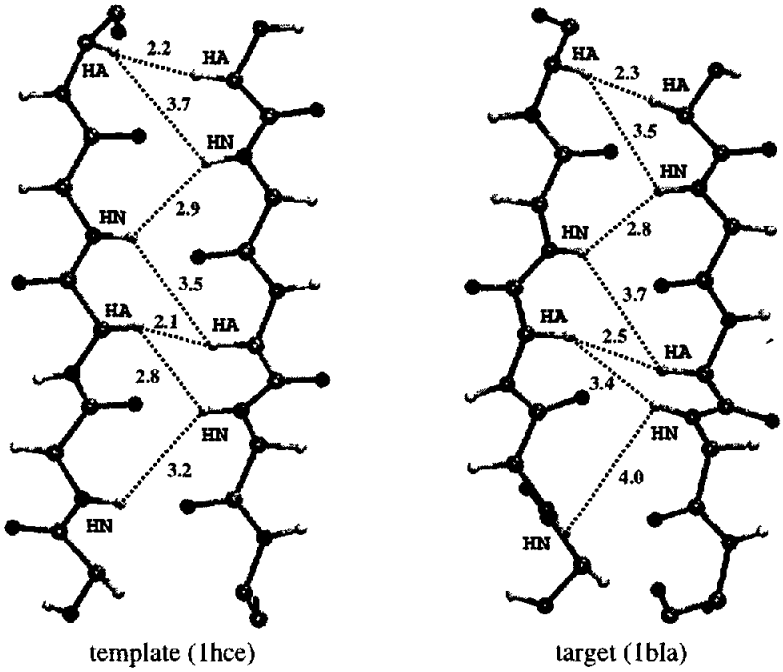


Figure 1: Comparison between the structurally equivalent β-sheets in the two proteins of similar folds. Residues 74-78 and 103-107 for template 1hce, and residues 83-87 and 113-117 for the query protein 1bla, are shown. The dashed lines with the numbers give the distance in Å. This picture was made using VMD [6].

be sufficient to uniquely determine the tertiary structure directly, they can often provide a significant amount of information for accurate fold recognition and alignment. We have observed that the patterns of NOE restraints, particularly the ones that are related to the strong hydrogen bonding across a β sheet, are more conserved than the overall structures of proteins of the similar folds. Effectively utilizing this information can potentially improve the effectiveness of threading. In addition, individual NOEs can be used to quickly rule out certain fold classes and alignments, based on the violations of the NOE restraints, as shown in Figure 2.

The goals of our current research are (i) to develop computational methods to fully utilize partial NMR data for protein structure calculation, and (ii) to expand the scope of the NMR method to larger proteins through the application of structural information obtained by a threading method. Our approach consists of two main steps: (a) *NMR-constrained* threading, and (b) *threading-supported* NMR method. In step (a), we construct a backbone structure of a query protein by using a threading method constrained by the NMR data. In step (b), we build a full-atom (or all heavy-atom) model of the query protein using molecular dynamics/energy minimization under the constraints of NMR data and the backbone structure obtained in step (a).

In the current study, we are focusing on long-range[b] NOE restraints, and have formulated the NMR-constrained threading problem as to find the globally optimal threading under the residue-residue distance constraints specified by NOE data. This constrained threading problem is rigorously solved by a generalized version of our previously-published threading algorithm [7, 8, 9]. By applying this algorithm, we have demonstrated that a small number of NOE restraints can improve threading performance significantly in both fold-recognition and threading-alignment accuracy. In our preliminary tests, we were able to obtain backbone structures with an rmsd of 3-5Å in most cases by using 1-2 NOE restraints per residue.

While a 3-5Å backbone structure may be accurate enough for some functional inferences, it also provides valuable constraints for the full-atom NMR structure determination, and helps to reduce the number of NOE's typically required for a NMR structure. Also, it helps to avoid entrapment in local minima in the NMR energy minimization procedure (as may occur when starting from a random backbone structure), and hence improve both calculation accuracy and efficiency.

We are currently exploring various NMR techniques to obtain as many NOEs as possible for large proteins, and investigating the potential of this new technique in helping to expand the scope of the NMR method to (significantly) larger proteins.

[b] A long-range NOE is an NOE associated with two residues that are not adjacent in the protein sequence

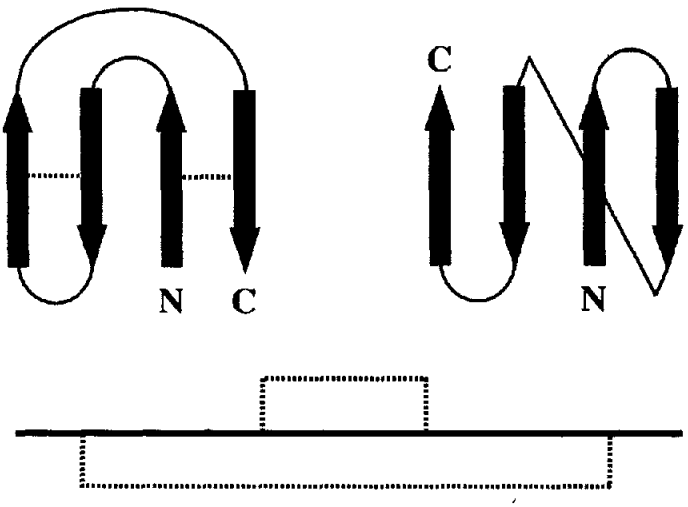


Figure 2: Few pairwise constraints can rule out certain topological classes of proteins. The Greek key topology (shown on the left) satisfies the 2 pairwise constraints on the sequence (shown at the bottom) while the other 4-antiparallel β-strand topology (shown on the right) does not.

2 NMR-Constrained Threading

2.1 An algorithm for NMR-constrained threading

Problem formulation

An *NMR-constrained threading problem* can be defined as to find the optimal[c] alignment between a query sequence and a template structure, which does not violate any distance constraints specified by NOEs. Or more specifically, it is to find an alignment $((\bar{s}_1, \bar{t}_1), ..., (\bar{s}_k, \bar{t}_k))$ between a query sequence $s = s_1...s_n$ and a template structure $t = t_1...t_m$ to minimize the following function[d]:

$$\sum_{1 \le i \le k} E_s(\bar{s}_i, \bar{t}_i) + \sum_{(\bar{t}_i, \bar{t}_j) \in \text{PAIRS}(t)} E_p(\bar{s}_i, \bar{t}_i, \bar{s}_j, \bar{t}_j)$$

$$\textbf{subject to: if an NOE exists between } \bar{s}_i \text{ and } \bar{s}_j \text{ then distance } (\bar{t}_i, \bar{t}_j) \le D. \quad (1)$$

where $\bar{s}_i$ (similarly $\bar{t}_i$) is either an element of s (or t) or an alignment gap; $\max\{n, m\} \le k \le n + m$; $E_s(x, y)$ is the *singleton fitness* term, measuring how well residue x fits the environment of structural position y if neither x nor y is a gap; otherwise it is a gap penalty; $E_p(x_1, y_1, x_2, y_2)$ is the *pair contact* term, measuring how preferable to have residues x_1 and x_2 in nearby structural positions y_1 and y_2; PAIRS(t) denotes all the pairs of template positions between which contact potentials may need to be considered (in our current implementation, we use a distance cutoff, 8Å, between the C_β atoms to define this); D is a cutoff distance, and its default value in our program is 8Å.

In our current implementation, we assume that alignment gaps appear only in loop regions, and consider pair contacts only between core[e] residues. Also we consider only NOEs between residues that are aligned to core elements of the template.

Our threading algorithm

We have previously developed a threading algorithm which guarantees to find an optimal threading as defined in (1) (without the constraints) [8], and have implemented the algorithm as a computer program, called PROSPECT [10]. We now give a brief review on how PROSPECT deals with pair contacts, which provides the basic algorithmic framework for enforcing NOE constraints.

The threading algorithm employs a *divide-and-conquer* strategy for solving the optimal threading problem. It first pre-processes the template structure by repeatedly dividing (bi-partitioning) it into sub-structures until each sub-substructure contains one core secondary structure (see Figure 3). The basic operation of the algorithm is *to calculate the optimal threading score between a sub-structure with links (to the rest of the structure) and a sub-sequence, under the condition that the core secondary structure at the external end of each link is already aligned.* This operation is implemented (recursively) by finding the optimal threading score between even smaller sub-structures and sub-sequences, and combining them optimally. We use Figure 4 (a) to illustrate how to calculate the optimal threading score between $s[k1, k2]$ and $t[i, j]$ with links $\{o1, o2, o3, o4\}$. We have proved [8] that the optimal alignment between

[c] Throughout this paper, the optimal threading means the globally optimal threading

[d] Our actual alignment function uses an affine function to penalize alignment gaps. This simplified version is used here to simplify the description of the algorithm

[e] A core secondary structure is an α-helix or a β-strand

$s[k1, k2]$ and $t[i, j]$ can be constructed by appending the optimal alignments between (i) $t[i, p]$ and $s[k1, k3]$, (ii) $t[q, j]$ and $s[k4, k2]$, and (iii) $t[p+1, q-1]$ and $s[k3+1, k4-1]$, for some $k3, k4 \in [k1, k2]$ and some alignment of the core linked by $o5$. The optimal alignments (i) and (ii) can be calculated recursively using the same operation on their sub-structures and subsequences; and the optimal alignment (iii) can be calculated using a Smith-Waterman type sequence alignment program (note that no pair contacts are considered between loop elements). To determine which of these optimal alignments give the optimal alignment between $t[i, j]$ and $s[k1, k2]$, we need to go through all possible values of $k3, k4 \in [k1, k2]$ and all possible alignments of the core linked by $o5$, and choose the (combined) optimal one. For more details of the algorithm, we refer the reader to [8].

Our threading algorithm applies this basic operation, starting with the whole query sequence and the whole template structure, and continuing until each sub-structure contains one core secondary structure. Pair contact potentials are calculated when links are considered. Since no alignment gaps are allowed within a core, the contact potential between any pair of residues aligned to nearby structural positions can be calculated based on the starting alignment positions of the corresponding cores.

Dealing with NOE restraints

Based on the above discussion, it is not difficult to prove that an alignment between the template and the query sequence does not violate any NOE restraints if and only if (1) no NOEs are aligned to two cores without a link between them, and (2) no pairs of cores, with links between them, are aligned to sequence positions which violate any NOEs. Our NMR-constrained threading algorithm uses the algorithmic framework outlined above with one addition that checks for conditions (1) and (2).

Condition (1) can be checked in the step of combining smaller alignments into a longer alignment (the *conquer* step) as follows. Each sub-structure (as shown in Figure 3) keeps a binary string with the k^{th} bit representing the k^{th} NOE restraint. The k^{th} bit is 1 if one end of the k^{th} NOE is aligned to a core of this sub-structure and the other end is not aligned to any position of this sub-structure nor to any external cores with links to this core. An NOE is aligned to a pair of cores without a link between them if and only if the bitwise AND operation yields a non-zero between the binary strings of the two adjacent sub-structures. A partial alignment with NOE violations will not be further considered. Note that this binary string can be calculated during the *conquer* step by simply doing a bitwise OR operation on the binary strings of its two sub-structures.

We use Figure 4(b) to explain how to check for condition (2) when doing a core alignment. Let c be a core with links $\{o1, o2, o3\}$, and these links connect to cores $c1, c2$, and $c3$, respectively. For each possible alignment of these cores $\{c1, c2, c, c3\}$, we check if any NOEs are violated. That is, we check whether, for any NOE that relates two residues aligned to some of these cores, the corresponding distance is more than D (see the objective function (1)). If a violation is found, this particular alignment will not be further considered when building larger alignments in our divide-and-conquer algorithm.

One way to implement this is to go through the list of all NOEs each time we examine a new arrangement of core alignments. But this simple strategy may not be computationally feasible when there are hundreds of NOEs or more. The running time of our threading algorithm is essentially determined by the number of alignments we have to consider. To examine hundreds to thousands of NOEs for each such alignment may increase the running time of the algorithm by that many times. Our solution to this problem is to examine only the relevant NOEs when examining a particular arrangement of core alignments.

While examining NOEs may increase the computational time, the NOEs can also help to reduce the search space size and hence the computational time, as they will rule out alignments violating NOEs without explicitly examining them. The total effect on computational time of using the NOEs is a rather complicated issue, and will not be addressed further in this abstract. We now present an algorithm for finding all relevant NOEs, given a core and its links.

We can formulate this problem as follows. Let $\mathcal{N} = \{(l_1, r_1), ..., (l_p, r_p)\}$ be the list of NOE restraints with l_i and r_i being the left and right positions (in the query sequence) of the i^{th} NOE. Let $\mathcal{A} = \{(c_l^1, c_r^1), ..., (c_l^q, c_r^q)\}$ be a list of left and right aligned positions of cores[1] $c^1, ..., c^q$. The problem is to find all (l_i, r_i) such that $c_l^j \le l_i \le c_r^j$ and $c_l^k \le r_i \le c_r^k$, for some $j, k \in [1, q]$. To do this fast, we pre-process the NOE restraint list $\mathcal{N}$ to facilitate fast searches. We use a *segment tree* data structure [11] to achieve this.

A segment tree is defined on a list of consecutive integers $[1, p]$ (from 1 to p). A segment tree consists of $p\log(p)$ *standard intervals* of $[1, p]$, e.g., an interval could be $[5, p-3]$. A key property of these specially chosen intervals is that any arbitrary interval $[a, b] \subseteq [1, p]$ can be represented as the union of at most $\log(p)$ non-overlapping standard intervals.

First we sort all NOE restraints in the increasing order of their left positions, i.e., $\{l_i\}$'s, and represent this sorted list as a segment tree data structure. For each standard interval, we sort its NOEs by their right

[1] q is typically a small number (≤ 6) as a core is typically "in contact" with (at most) 4 - 5 other cores

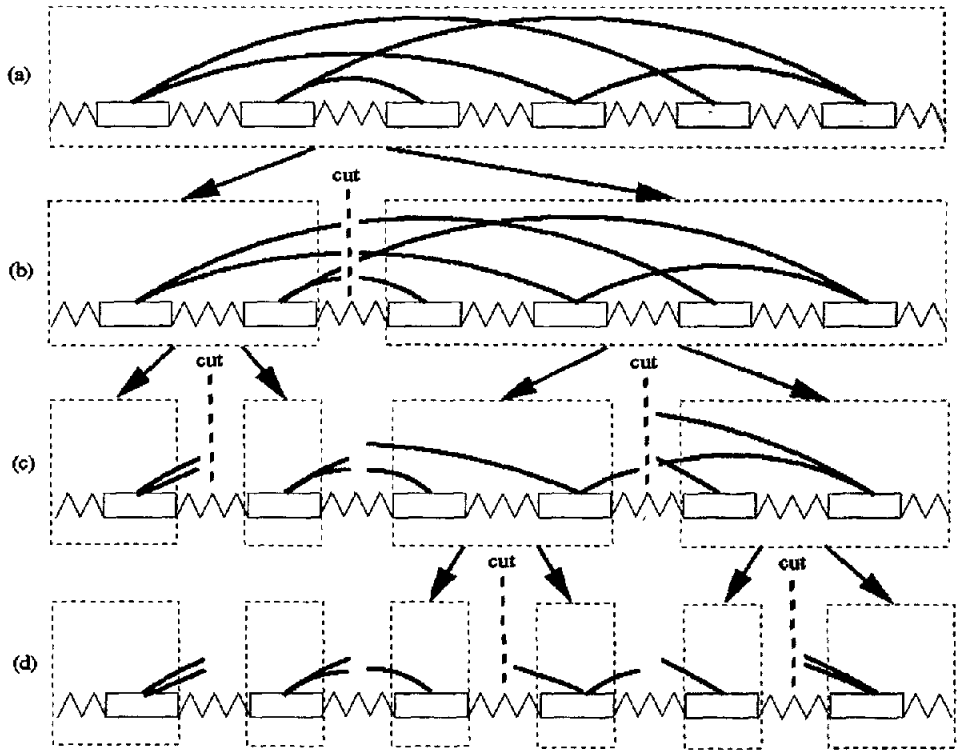


Figure 3: (a) A template with six core secondary structures and connecting loops. A link between two cores represents that there exists at least one pair contact between the two cores. (b) A cut dividing the template into three parts: two sub-templates inside the left and right dotted boxes, respectively, and the connecting loop. (c) and (d) Further partition of the template.

positions, i.e., $\{r_i\}$. We use the following procedure to find all relevant NOEs for a given arrangement, $\mathcal{A}$, of core alignments.

Procedure SEARCH_NOE ($\mathcal{N}, \mathcal{A}$)
1. **for** $1 \le a \le q$ **do**
2. find left and right boundaries, l and r, of $\mathcal{N}$'s sublist whose left positions are within $[c_l^a, c_r^a]$ of $\mathcal{A}$,
3. retrieve the v standard intervals of $[l, r]$;
4. **for** each of the v standard intervals, $\mathcal{J}$ **do**
5. find left and right boundaries of $\mathcal{J}$'s sublist whose right positions are within $[c_l^b, c_r^b]$, for some $b \in [a, q]$
6. "output" all NOEs within the boundary.

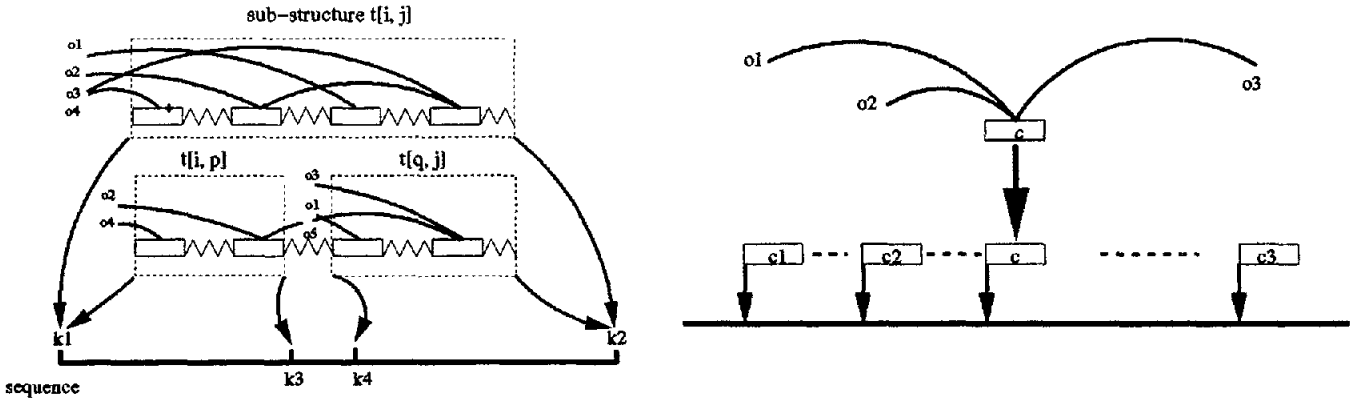
Note that the algorithm spends $O(1)$ time on each relevant NOE, and in addition it spends $O(\log^2(p))$ time to search the segment tree and $O(q)$ time to go through all the involved cores. So the total time spent is $O(q + e\log^2(p))$, compared to $O(q+p)$ time using the straightforward method, where e denotes the number of relevant NOEs for a given arrangement of core alignments.

2.2 A preliminary study on NMR-constrained threading

NMR-constrained threading uses an additional scoring term E_{NMR} (see function (2)) to reward the use of NOEs and to penalize deviations from the NOE-specified distance within the cutoff distance D. We use two types of distance restraints: (a) NOE restraints between backbone hydrogens, and (b) estimated C_β distance restraints based on NOEs involving sidechains. An estimated C_β distance restraint is used only when it is at most 7Å. $E_{\text{NMR}}(x, y)$ will be applied[9] only when an NOE's two ends are aligned to structural positions x and y and satisfy the C_β distance cutoff.

Using function (2), we have conducted preliminary analyses on (i) how NOEs affect the threading-alignment accuracy; and (ii) how NOEs affect the accuracy of fold recognition by our NMR-constrained threading algorithm. For this study, we selected from the FSSP database [12] 17 sequences as the query sequences and 667 proteins as the template structures. The query sequences and templates are selected randomly within the following constraints: (1) the query sequences have NOE data in the PDB database; (2) the query sequences

[9] $E_{\text{NMR}}(x, y)$ may be used twice if both their hydrogen distance and C_β distance satisfy the conditions of (2)



(a) (b)

Figure 4: (a) A schematic of the basic operation of the divide-and-conquer algorithm. (b) Core alignments.

$$E_{\mathrm{NMR}}(x,y) = \begin{cases} -150, & \text{if distance between } x \text{ and } y\text{'s backbone hydrogens} \\ & \leq 3.7\text{Å}, \text{and an NOE aligned to } x, y\text{'s hydrogens,} \\ -300, & \text{if } C_\beta\text{-distance, } D_{cb}(x,y), \text{ between } x \text{ and } y \leq 7\text{Å}, \\ 300 \times (D_{cb}(x,y) - 7)^2, & \text{if } 7\text{Å} < D_{cb}(x,y) \leq 8\text{Å} \end{cases} \quad (2)$$

should evenly represent three classes of proteins: (2a) all-α, (2b) all-β, and (2c) α and β mixed; and (3) each query sequence has an native-like structure in the template set.

Table 1 summarizes how the number of NOEs affects the performance of our algorithm. In this study, NOEs are selected randomly and uniformly from the corresponding PDB files. The alignment accuracy is the highest accuracy over ten runs, and the fold recognition accuracy is based on a single run[h]. When using 2 NOEs per residue in this test, the overall threading-alignment accuracy (the number of residues aligned within a 4-residue shift from the correct positions versus the total number of alignable residues) improved from 70% to 92.7%.

We have also used simulated NOE data to test our method on large proteins. We now outline one such test. The query protein is 1b3ra (an X-ray structure of S-adenosylhomocysteine hydrolase, with 431 residues), and the template is 1t7pa (D-3-phosphoglycerate dehydrogenase with 409 residues). Without using any restraint, PROSPECT aligned 108 residues correctly (within 4-residue shift to the correct positions) among 198 structurally alignable ones. The C_α-RMSD between the model and the experimental structure for the structurally-alignable residues is 21.2 Å. We then generated all hydrogen atoms based on the coordinates of the heavy atoms using X-plor [3], and constructed distance restraints for all the hydrogen pairs within 4.0 Å. We randomly and uniformly selected a subset of NOE restraints, and derived the C_β-C_β pairs with a maximum distance of 7 Å based on the subset of NOE restraints. With only 0.5 and 1.0 (simulated) restraints per residue, our constrained threading program aligned 138 and 172 residues correctly, with the C_α-RMSD of 7.1 Å and 3.6 Å between the model and the experimental structure for the structurally alignable residues, respectively.

[h]We only performed a single run for this abstract due to the tight submission deadline The small sampling size (single run) partially explains the performance fluctuation in our fold recognition test

3 Threading-Supported NMR Method

We have conducted a preliminary study on how an approximate backbone structure predicted by NMR-constrained threading can help reduce the number of NOEs required for accurate structure determination. We now outline our study result on the third IGG-binding domain of protein G (with 61 amino acids).

This protein has both an NMR structure (2igh) and a high-resolution (1.1Å) X-ray crystallographic structure (2igd). The RMSD of all heavy atoms between 2igh and 2igd is 3.6Å. 2ptl is the template structure. The 2ptl and 2igh sequences have 17% identity; and the C_α-RMSD is 4.0Å between their aligned portions. Our threading program finds the alignment between the two correctly. We then applied MODELLER [13] to generate an all-atom structure of 2igh based on the structure of 2ptl and NOE restraints. The NOEs used in this test are selected randomly and uniformly from the whole set of NOEs. Ten runs are performed and the averaged structure accuracy is plotted in Figure 5. The structure is generally becoming more accurate as the number of NOEs increases. The small fluctuations in the averaged RMSD are presumably due to the small sampling size

Table 1. Threading accuracy versus number of NOE restraints.

class	query	nres (a.a.)	temp	iden (%)	rmsd (Å)	RMSD (Å)/rank vs. NOE/a.a.						
						0	0.5	1.0	1.5	2.0	2.5	3.0
α	1bbn	133	1cnt1	8	2.6	**16.2/29**	6.6/-	6.6/1	6.6/-	5.3/1	5.3/-	**5.3/1**
	1itl	130	3inkC	12	2.2	**13.1/7**	4.3/-	4.2/1	4.2/-	3.6/3	3.6/-	**3.6/3**
	1ner	74	1lmb3	14	2.4	3.8/**57**	3.8/-	3.8/57	3.8/-	3.8/49	3.8/-	3.8/**39**
	1il6	166	1bgc	15	2.1	3.0/1	3.0/-	3.0/1	3.0/-	3.0/1	3.0/-	3.0/1
	1ocd	104	1cyj	28	2.0	3.4/1	3.4/-	3.4/1	3.4/-	3.4/1	3.4/-	3.4/1
β	1maj	113	1agdA	7	3.0	**8.8/37**	8.0/-	6.9/1	6.9/-	6.6/1	6.6/-	**6.6/1**
	1nct	98	1bec	12	2.5	**14.7/1**	13.8/-	5.4/1	5.4/-	5.4/1	5.4-/	**5.4/1**
	1bla	155	1hce	14	2.1	**7.7/1**	4.0/-	4.0/1	4.0/-	4.0/1	4.0/-	**4.0/1**
	1vhp	117	1cd8	15	2.9	**4.5/1**	4.4/-	4.4/1	4.4/-	4.4/1	4.4/-	**4.4/1**
	1ghj	79	1iyu	24	1.8	2.0/1	2.0/-	2.0/1	2.0/-	2.0/1	2.0/-	2.0/1
	1a7i	60	1qli	33	2.5	**2.8/1**	2.8/-	2.8/1	2.6/-	2.6/1	2.6/-	**2.6/1**
α/β	1afi	72	2acy	7	2.8	**6.8/376**	5.5/-	5.6/26	5.5/-	3.8/3	3.8/-	**3.8/2**
	3trx	105	1a8y	12	2.7	**4.1/1**	3.1/-	3.1/1	3.1/-	3.2/1	3.0/-	**3.0/1**
	1ikm	69	1dokB	13	2.1	2.1/**2**	2.1/-	2.1/2	2.1/-	2.1/1	2.1/-	2.1/**1**
	3phy	125	1bv6	15	2.3	**12.2/41**	8.8/-	3.6/31	3.6/-	3.6/6	3.6/-	**3.6/4**
	1crp	166	1byuB	24	1.7	2.6/1	2.6/-	2.6/1	2.6/-	2.6/1	2.6/-	2.6/1
	1fht	116	2u1a	34	2.1	**4.5/1**	4.5/-	4.5/1	4.5/-	4.5/1	4.5/-	**2.1/1**

"Query" and "temp" represent the PDB codes of the query and template proteins, respectively. "Nres" is the number of alignable residues between the query and template. "Iden" denotes the sequence identity between the query and template sequences. "Rmsd" is the C_α-RMSD between the structurally equivalent residues of the query and template structures. "RMSD/rank vs. NOE" are the C_α-RMSD between the experimental structure and the predicted structure (alignable portions), and the rank of the correct template structure among 667 templates. 0, 0.5, 1.0, ... represent the averaged number of NOEs used per residue. "-" indicates that no fold recognition test is conducted.

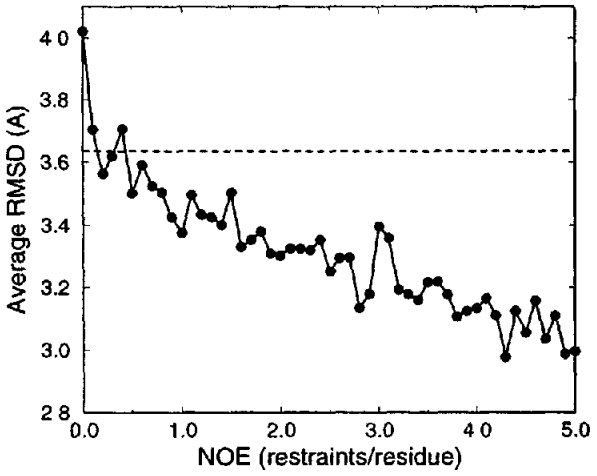


Figure 5: The average RMSD of all heavy atoms between the model and the X-ray structure (2igd) over ten runs versus the number of NOE restraints per residue used. Each solid dot represents the structure accuracy for the corresponding number of NOEs used.

(ten) for averaging.

The above example shows that NMR-constrained threading, followed by modeling of side chains and possibly loops, gives an approximate representation of the true structure of the query protein. We are investigating a method for improving the accuracy of the determined structure by minimizing an objective function consisting of a linear combination of the conformational energy (the CHARMM22 potential [14]) and a pseudo-energy of violation of NMR restraints. Currently we implement this with simulated annealing by molecular dynamics, using the CNS program [15]. In this method, numerous calculations are performed, taking the approximate structure determined above as the starting point, but with different random atomic velocities. We have found that almost every single such calculation performed in our preliminary investigation results in a reduced rms difference between the model and the true structure.

4 Discussion

To fully take advantage of the capability of this new technique, we have considered how to obtain as many NOEs as possible using various NMR techniques. Our ultimate goal is to expand the scope of the NMR method to significantly larger proteins. Currently we are working on a 46 kD enzyme PGK (yeast phosphoglycerate kinase) for its structure determination. We use PGK as an example to briefly explain how we will extract NOE data from this large protein.

We will first conduct multidimensional NMR experiments using uniformly $^{13}C/^{15}N$-labeled enzyme. We expect to make assignments for a limited number of residues. Distance restraints will be derived from ^{15}N and ^{13}C edited ^{1}H-^{1}H NOE experiments. Particular attention will be directed to observe NOEs involving protons of the protein backbone. We believe that this should yield a limited number of distance restraints. If the uniformly labeled PGK fails to provide sufficient number of distance restraints for computation, we will then use selectively isotope-labeled PGK to obtain additional distance restraints by isotope filtered NOE experiments. We have developed procedures for specific labeling of this enzyme at designated residues such as histidines or tyrosines [16]. In addition, we plan to supplement the distance restraints with longer distances determined by paramagnetic probe-T1 method [17]. Earlier, we were able to determine distances up to 14Å using paramagnetic CrATP in selectively isotope labeled PGK [16].

Our prelminary study has strongly suggested that a small number of NOEs can help extend the scope of threading to structural analogs. As in the cases of 1afi-2acy, 3phy-1bv6, and 1bla-1hce (all are analogous pairs with low sequence identities; see Table 1), our program was able to achieve high performance on both fold recognition and threading alignment.

For the further studies, we are planning to integrate other early data obtained in NMR measurements into threading and model building, including (1) chemical shifts as an indicator of local structures, (2) residual dipolar coupling data which characterize the packing between different secondary structures, and (3) scalar coupling constants which can help predict sidechain packing. Using all these early NMR data in a similar fasion as described in this paper is expected to further improve the structure determination.

To summarize, we have demonstrated that (1) a small number of NOEs can significantly improve the threading performance, and (2) the use of threading can greatly reduce the requirement of NOEs for an accurate NMR structure determination. We expect this approach to be extremely useful in cases where experimental procedures can provide only incomplete NMR data. It should also be useful even when structure determination by NMR methods is feasible, by allowing substantial reductions in the number of labeling experiments and the NMR-data collection time – i.e., by achieving equivalent results more rapidly.

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