Comprehensive Assessment of Automatic Structural Alignment against a Manual Standard, the Scop Classification of Proteins

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Abstract

We apply a simple method for aligning protein sequences on the basis of 3D structure, on a large-scale, to the proteins in the scop classification of fold families. This allows us to assess, understand, and improve our automatic method against an objective, manually derived standard, a type of comprehensive evaluation that has not yet been possible for other structural alignment algorithms. Our basic approach directly matches the backbones of two structures, using repeated cycles of dynamic programming and least-squares fitting to determine an alignment minimizing coordinate difference. Because it is fairly simple, our method can be readily modified to take into account additional features of protein structure such as the orientation of sidechains or the location-dependent cost of opening a gap.

Our basic method, augmented by such modifications, can find reasonable alignments for all but 1.5% of the known structural similarities in scop, i.e. all but 32 of the 2107 superfamily pairs. We discuss the specific protein structural features that make these 32 pairs so difficult to align and show how our procedure effectively partitions the relationships in scop into different categories depending on what aspects of protein structure are involved (e.g. depending on whether or not consideration of sidechain orientation is necessary for proper alignment).

We also show how our pairwise-alignment procedure can be extended to generate a multiple alignment for a group of related structures. We have compared these alignments in detail with corresponding manual ones culled from the literature. We find good agreement (to within 95% for the core regions), and detailed comparison highlights how particular protein structural features (such as certain strands) are problematical to align, giving somewhat ambiguous results. With these improvements and systematic tests, our procedure should be useful for the development of scop and the future classification of protein folds.

Introduction

Reasons for Structural Alignment

Structural alignment consists of establishing equivalences between the residues in two different proteins, as is the case with conventional sequence alignment. However, this equivalence is determined principally on the basis of the three-dimensional coordinates corresponding to each residue, not on basis of the amino-acid “type” of the residue. The general idea of structural alignment has been around since the first comparisons of the structures of myoglobin and hemoglobin (Perutz et al., 1960). Systematic structural alignment began with the analysis of heme binding proteins and dehydrogenases by Rossmann and colleagues (Rossmann et al., 1975; Rossmann & Argos, 1975; Argos & Rossmann, 1979). Currently, there are two basic reasons for wanting to perform this operation:

Firstly, the number of known structures is large and growing rapidly (>8000 domains in the Protein Databank, expected to exceed 10,000 soon) (Holm & Sander, 1997; Murzin et al., 1995; Orengo, 1994; Bernstein et al., 1977). Both for understanding and for applications such as comparative modelling (Sanchez & Sali, 1997), it is advantageous to organize all the structures into fold families. A number of databases currently do this: FSSP and Entrez-MMDB cluster structures purely on the basis of automatic comparison programs (Holm & Sander, 1993, 1994, 1996; Hogue et al., 1996; Gibrat et al., 1996; Schuler et al., 1996). Scop does the same thing manually, based on visual inspection of human experts (Murzin et al., 1995). And CATH and HOMALDB adopt an intermediate approach, using both automatic and manual methods (Sali & Overington, 1994; Overington et al., 1993; Orengo et al., 1994).

Secondly, structural alignment can be used as a "gold standard" for sequence alignment and threading. How does one know if a purely sequence-based alignment is correct? Or which parts of two proteins can be aligned? The current belief is that this is best done by consulting a structural alignment. This second use of structural alignment tends to focus on the accuracy of an alignment given that one already knows that two structures are similar.

Existing Methods for Structural Alignment

Because of their obvious utility, a large number of different procedures for automatic structural alignment and comparison have been developed (Remington & Matthews, 1980; Satow et al., 1986; Taylor & Orengo, 1989; Artymiuk et al., 1989; Sali & Blundell, 1990; Vriend et al., 1991; Russell & Barton, 1992; Holm & Sander, 1993;

Grindley et al., 1993; Godzik & Skolnick, 1994; Gibrat et al., 1996; Falicov & Cohen, 1996; Feng & Sippl, 1996; Cohen, 1997).

To understand these procedures, it is useful to compare structural alignment with the much more thoroughly studied methods for sequence alignment (Doolittle, 1987; Gribskov & Devereux, 1992). Both sequence and structure alignment methods produce an alignment, which can be described as an ordered set of equivalent pairs (i,j) associating residue i in protein A with residue j in protein B. Both methods allow gaps in these alignments which correspond to non-sequential i (or j) values in consecutive pairs — i.e. one has pairs like (10,20) and (11, 22). And both methods reach an alignment by optimizing a function that scores well for good matches and badly for gaps. The major difference between the methods is that the optimization used for sequence alignment is globally convergent whereas that used for structural alignment is not. This is the case for sequence alignment because the optimum match for one part of a sequence is *not* affected by the match for any other part. Structural alignment fails to converge globally because the possible matches for different segments are tightly linked as they are part of the same rigid 3D structure. For this reason, the alignment found by a structural alignment algorithm can depend on the initial equivalences whereas in sequence alignment there is no such dependence.

The lack-of-convergence problem has led to a large number of different approaches to structural alignment, the methods differing in how they attack the problem. However, no current algorithm can find the globally optimum solution all the time; the convergence problem remains unsolved in the general case. The methods also differ in the function they optimize (the equivalent of the amino acid substitution matrix used in sequence alignment) and how they treat gaps.

Some of the methods effectively compare the respective distance matrices of each structure, trying to minimize the difference in *intra*-atomic distances for selected aligned substructures (Taylor & Orengo, 1989; Holm & Sander, 1993; Sali & Blundell, 1990). In contrast, our method, which is derived from that of Cohen (Satow et al., 1986; Cohen, 1997), directly tries to minimize the *inter*-atomic distances between two structures. A similar approach is taken in minimizing the "soap-bubble area" between two structures (Falicov & Cohen, 1996). Other methods involve further techniques, such as geometric hashing or lattice fitting (Artymiuk et al., 1989; Godzik & Skolnick, 1994; Gibrat et al., 1996).

The Importance of Manual Standards

How well do the current structural alignment programs perform? While particular programs have uncovered many interesting similarities in individual cases (e.g. globin-colin, Holm & Sander, 1993; adenylyl cyclase-polymerase, Artymiuk et al., 1997; Bryant et al., 1997), it has not been possible to see how well the programs perform overall, in an aggregate, statistical fashion against a set of objective standards. This is because up to now suitable standards did not exist. However, the recently created scop classification of protein structures provides such a suitable standard (Murzin et al., 1995; Brenner et al., 1996; Hubbard et al., 1997). It consists of thousands of documented similarities between known protein structures based purely on visual inspection. Here we endeavor to test our automatic method of structural comparison against the known similarities in scop. This provides, for the first time, a comprensive sense of how a uniformly applied, automatic procedure does against manual standard. It also allows us to see what type of similarities are especially hard to detect and to optimize our procedure in a systematic fashion.

After a program has found a structural similarity the next question one asks is how correct is the alignment. This is especially important if one wants to use results of structural alignment as a "gold-standard" to evaluate a sequence-alignment or threading algorithm. It is surprisingly difficult to answer this question in detail since many parts of two similar proteins (e.g. loops) may not be alignable at all. Some recent results have highlighted the ambiguities in structural alignment and even suggested that unique alignments do not exist (Feng & Sippl, 1996; Godzik, 1996; Orengo et al., 1995). However, we take the perspective that unique alignments exist for the essential "core" regions of two similar proteins. As was the case with the detection of similarities, it is essential to compare automatic alignments against manual standards in an objective and systematic fashion. Here we test a selection of the alignments derived from scop against corresponding manual alignments from the literature.

Results

Systematic Elaboration of a Simple Procedure (Search then Iterate)

As shown in figure 1, the basic procedure we use for structural alignment is very simple. It is very much like classic Needleman-Wunsch sequence alignment (Needleman & Wunsch, 1971). It consists of building a similarity matrix S_{ij} based on the interatomic distances between each atom i in the first structure and each atom j in the second. Then dynamic programming is applied to this matrix to find the optimal global alignment. If this were sequence alignment, we would be done, as the similarity matrix, which depends only on the two sequences, is constant. However, in structural alignment, the matrix depends on the relative 3D positioning of the two structures, which, in turn, depends on how they have been previously aligned, so the procedure must be iterated until it converges. As we will describe below, this simple procedure is usually able to arrive at the correct alignment. However, there are exceptions. To handle these, we modified our basic procedure in two ways: through an expanded search and through using additional methods to build the similarity matrix. Because of the simplicity of the basic procedure these modifications can be rationalized directly in terms of features of protein structure.

Originally our search consisted of starting at five reasonably chosen points, described in the methods (Subbiah et al., 1993; Laurents et al., 1994; Gerstein & Levitt, 1996). Here we expand the search by allowing additional starting points and, in certain difficult cases, only aligning a section of the bigger of the two proteins. In the basic method, the similarity matrix depended only the distance between alpha carbons (method "Cα"). Here we elaborate on this by taking into account residue exposure and sidechain orientation, specifically by using beta carbons ("Cβ") or weighting according the relative orientation of sidechain vectors ("Cα-Cβ"). A final elaboration allows the gap penalties to vary with position in the structure, so that is more difficult to introduce breaks in helices and strands than in loops ("var. gap").

Using Scop to Assess Our Algorithm: A "Meta-method"

Objective ways for assessing the quality and significance of our alignments are the key point that distinguishes what we do here from previous approaches towards automatic structural alignment. Our attention to validating our procedure against objective external standards is in a sense a "meta method" -- a method for evaluating a method.

To assess sensitivity, we checked our procedure against the entire scop database. This consists hundreds of thousands of relationships between the ~8000 protein domains of known structure. However, many of these relationships are trivial (e.g. same protein in different liganded states) or can readily be derived from sequence homology. The nontrivial relationships are evident only after clustering all the domains on the basis sequence. The current version of scop (1.32) contains 941 unique domains at a 40% identity cutoff (Brenner et al., 1995; Brenner et al., 1997). Of the 441,330 possible pairs of these domains (940 × 939/2), 2107 (~0.5%) are in the same scop superfamily and therefore have a similar 3D structure. These 2107 pairs were what we tested our procedure against.

To check how accurate the alignments produced by our procedure were, we compared them against manual alignments published in the literature (making sure that these alignments were really done by hand and not a product of another computer algorithm). This was done in a most straightforward fashion, by optimally "aligning" the automatically generated alignments *en bloc* against the literature alignments and then counted the mismatches in the "core regions" (see methods). This protocol is a much more objective test than simply inspecting the automatically produced alignments to see whether they "look" reasonable. In that situation it is possible to be either wittingly or unwittingly biased in favor of the program's alignments.

Overall "Sensitivity" in Finding the Scop Pairs

We ran our structural alignment program against all 2107 of the scop pairs. Each comparison gave a value for the number of residues matched (N) and the RMS deviation in alpha-carbon positions after doing a least-squares fit with these N residues (the "RMS"). Our overall results are shown in figure 2 through plotting RMS vs N for each scop pair. There is a fairly wide spread in the values for both RMS (2.66 ± 0.77 Å) and N (98 ± 57) but it is possible to approximately separate the successful matches (low RMS) from the unsuccessful matches (high RMS) by the demarcation line RMS = 4(N + 135)/225. This sloping line indicates that a match with a higher RMS value can be more significant than one with a lower RMS if there are more residues in the first match, as is to be expected. Based on the demarcation line it is convenient to define a normalized RMS: RMS' = 225 RMS / (N + 135). As shown in figure 2b, plotting this quantity against N now gives a flat demarcation line of RMS' = 4 Å (nearly the same as the distance between alpha carbons). Note that for an approximately average match of 90 residues, RMS' is the same as RMS (and that both quantities agree to within 10% for N between 70 and 110 residues).

Figure 3 shows the distributions of RMS and RMS' values. Both distributions have very similar means (2.66 and 2.68 Å) and standard deviations (0.77 and 0.87 Å) The normalized distribution has a sharp fall-off for RMS' greater than 4 Å, justifying this as a criterion for a significant match.

About 15% of the scop pairs (313 of 2107) have some (marginal) sequence similarity (as indicated by a FASTA e-value less than .01, see legend to figure 2). All of these pairs are below the 4 Å RMS' demarcation line, and collectively, they have a lower average RMS (1.9 Å) and a higher average N (129) than the other pairs, indicating that sequence similarity is related to structural similarity even this close to the "twilight zone."

Using a normalized RMS' threshold of 4 Å, we find that only 32 of the 2107 pairs are outliers, less than 2%. These results were obtained using our "optimized" protocol that starts from a number of points and uses a variety of different parameter settings (see methods). However, 1762 of the pairs (~84%) could be found with just a single primary search method (Cβ). Of the remaining pairs, 313 (15%) could be found through application of multiple search strategies, leaving 32 pairs (1.5%) that we could not find at all.

Protein Structure Features that Fooled the Method, and Why

We investigated in detail the 32 outliers that the program missed completely, trying to identify the types of protein structure that were fooling the program. (In this analysis we also looked at an additional 37 pairs where the match was slightly better than our 4 Å RMS' threshold but for which the number of aligned residues was less than 40% of the length of the smaller protein). A number of these "bad pairs" represent unusual residue selections in the scop database. For instance, the scop pair with identifiers d1ggta1 and d1cdcb_ associates, respectively, a full immunoglobulin variable domain with a strangely shaped immunoglobulin fragment (see methods for scop id syntax). Four of the seven worst failures involve the protein with scop identifier d1dhx__, which is an all-β animal virus coat protein.

A number of the difficult to align pairs had circular permutations in their similar structural elements. For instance, the scop pair with identifiers d1scs__ and d1xnb__ consists of two proteins that share the same all-β, concanavalin-A fold but differ in connectivity, having a circular permutation of strands in the central sheet. As our alignment program was not designed to handle circular permutations, its difficulty with this pair is understandable. The complexities of handling topology changes in alignment has been discussed previously (Orengo et al., 1995).

Finally, we found a number of interesting cases where the structures were considered similar in scop because they shared a special structural feature rather than an overall similarity in shape. The scop pair shown in figure 6 (d1dpga2 and d1gd1o2) represents such an instance. Both domains in the pair are considered to share the same superfamily fold, that of the C-terminal domain of glyceraldehyde-3-phosphate dehydrogenase. However, they only have a small amount of common structure, which is only remotely alignable -- in particular, a four-stranded sheet with two helices packed on one face. Thus, in terms of the raw score used by the program (i.e. the average closeness of Cα atoms), the domains could not be matched well. However, they are grouped together in scop because both share a unique type of connectivity between the helices and strands, involving a rare type of loop "cross-over". Moreover, this special cross-over occurs at the "heart" of these proteins, participating in the active site, and occurs in further proteins grouped in the same superfamily (e.g. d1dih_2).

Detailed Accuracy of the Alignments

For the remaining scop pairs that could be aligned with acceptable RMS values, we tried to assess the quality of their alignments in detail. To this end, we compared a set of nine automatically generated multiple alignments, based on portions of the scop superfamilies (involving 40 structures in total), with corresponding manual alignments culled from the literature. Our overall results, in terms of mismatches for each set, are shown in Table 1. Our selection of test cases represents a wide variety of protein structures: all-α (globins), all-β (immunoglobulins, plastocyanin-azurin), α/β (dihydrofolate reductase family), structures with large conformational changes in addition to evolutionary changes (adenylate kinases), and structures with large inserts (Gal6-papain). As was the case with the sensitivity analysis, we found overall that the basic method, minimizing Cα distance, works. However, it has some trouble with beta-sheet proteins.

Our results are shown in greater detail in figure 7, which shows the automatically generated alignments of three well-known protein families: the all-alpha globins, the all-beta immunoglobulins and the alpha/beta dihydrofolate reductase family. Mismatches in the core regions are indicated. The globins and the dihydrofolate reductases are “easy” to align (figure 4). The basic procedure (Cα), as well as any of the variants, was able to generate the correct alignment.

The immunoglobulins are more problematical, especially with regard to aligning the constant and variable domains. As shown in figures 5 and 7, all the variants of our algorithm will generate an alignment with an acceptable RMS, but the alignments differ in detail. In fact, the alignment that minimizes alpha-carbon distance looks deceptively correct and has the best overall RMS. However, it is clearly wrong as it misaligns the conserved disulfide. A variant of our procedure that takes into account sidechain orientation and also uses variable gap penalties is necessary to get the alignment right. Aligning immunoglobulin constant and variable domains has proven difficult with other structural alignment methods (Taylor & Orengo, 1989). The difficulties we found for the immunoglobulins and other all-β proteins suggest that, in general, it is easier to misalign strands than helices unless one takes into account sidechain orientation.

Figure 8 shows how our simple approach toward multiple alignment, align to the “median structure” in the cluster, performs. Clearly as one moves away from the median structure the alignment degrades. Nevertheless, the overall mismatch error rate is very low using this approach (Table 1), indicating it is probably sufficient for the superfamily size currently in scop. (However, in the future this could change, see methods.)

Discussion and Conclusion

We have described how we applied an automatic structural alignment method, on a large-scale, to the proteins in the manually constructed scop classification of fold families. Comparing our automatic alignments against manual standards has allowed us to get a relatively unbiased assessment of how a uniformly applied computer procedure compares with human experts, both in terms of overall sensitivity and detailed accuracy. In a sense our program has acted as foil to expose the subtleties of protein structural similarity.

We find that our method partitions the relationships in scop roughly into three categories: easy to align, hard to align, and impossible to align. In the first category are proteins, such as the globins (figure 4), which can be aligned correctly by our basic method (Cα) or any of the variants. In the next category are proteins, such as the immunoglobulins (figure 5), that need a modified method (e.g. Cβ) for successful alignment. This is necessary either because the basic method can not find an alignment with an acceptable RMS at all or because, even though it finds an alignment with a good RMS, it does not get this alignment completely right. As a rough rule, proteins in this second category tend to have more sheet structure than helical structure, probably because of the greater structural variability allowed in strands than helices and also because (without considering sidechain orientation) it is easier to misalign a strand by one residue.

Finally, in the last category are the ~1.5% of the scop pairs that we could not align at all by the basic methods or any variants (figure 6). Our difficulty with a number of these pairs can be understood because a specific protein-structure “feature,” such as crossed loops, is used as the basis for a resemblance, rather than simply the similarity in backbone structure.

Testing the program against objective standards has allowed us to refine it (taking into account such things as sidechain orientation and exposure, variable gap penalties, and a more comprehensive search), measurably increasing our overall sensitivity (so that we could eventually find 99% of the scop pairs). We have also demonstrated a simple yet effective scheme for generating multiple structural alignments based on our pairwise alignments.

The resulting multiple alignments of the scop superfamilies generated by our procedure are expected to be useful in many applications, ranging from testing sequence alignment and fold recognition algorithms to the construction of profiles and Hidden Markov Models for searching the genome.

Methods

Data

Structures were taken from the Protein Data Bank (PDB, Bernstein et al., 1977). Version 1.32 of the scop fold classification was used (May 96) (Murzin et al., 1995; Brenner et al., 1996; Hubbard et al., 1997). This includes a number of structural similarities that were not in the PDB (i.e. they were taken from the literature). It also has some proteins that have multi-chain "domains". These and other another special cases were removed form the database. Each of the domains classified by scop is associated with a unique identifier, and these are used throughout this text. They have the following syntax: d1pdbcN, where “1pdb” is a PDB id, “c” is a chain identifier, and “N” describes if this is the first, second, or only domain in the chain. Thus, d1ggta1 is the first domain in the A chain of 1GGT.

The creators of scop have clustered the domains in the PDB on the basis of sequence identity (Brenner et al., 1995; Brenner et al., 1997), using a procedure similar to that of Hobohm et al. (1992). At a sequence identity level of 40%, this procedure results in 941 sequences corresponding to the scop domains. These sequences contain 176 different superfamilies, which involve 2107 nontrivial pair relationships between the domains. (Only superfamily pairs were used here as they have considerably closer and more certain relationship than fold pairs.)

The Basic Procedure, Minimize Cα RMS

The basic procedure we use for pairwise structural alignment is based on iterative application of dynamic programming. As such it is a simple generalization of Needleman-Wunsch sequence alignment (Needleman & Wunsch, 1971). The basic method is originally derived from the ALIGN program of G Cohen (Satow et al., 1986; Cohen, 1997) and has been applied to specific cases previously (Subbiah et al., 1993; Laurents et al., 1994; Gerstein & Levitt, 1996). As shown in Figure 1, one starts with two structures in an arbitrary orientation. Then one computes all pairwise distances between each atom in the first structure and every atom in the second structure. This results in an *inter*-protein distance matrix where each entry d_{ij} corresponds to the distance between atom i in the first structure and atom j in the second one. This distance matrix can be converted into a similarity matrix S_{ij}, similar to the one used in sequence alignment, by application of the following formula:

$$s_{ij} = \frac{M}{1+\left(\frac{d_{ij}}{d_o}\right)^2}.$$

Here M is the maximum score of a match, which is arbitrarily chosen to be 20. d_o is the distance at which the similarity falls to half its maximum value (i.e. $d_{ij} = d_o \rightarrow S_{ij} = M/2$). It is taken here to be 2.24 Å - reflecting the intrinsic length-scale of protein structural similarity. This is about midway between the length of a C-C bond (1.54 Å) and the usual distance between Cα atoms (3.8 Å).

One applies dynamic programming to the similarity matrix to get equivalences. If this were normal sequence alignment, one would be finished at this point since dynamic programming gives the optimal equivalences. However, this is not the case for structural alignment. So one takes these equivalences and uses them to least-squares fit the first structure onto the second one (Kabsch, 1976). Then one repeats the procedure over and over, finding all pairwise distances and doing dynamic programming to get new equivalences, until it converges on the same set of equivalences.

Basic Search

In practice, the iteration is tried from a number of different starting points, and the one that gives the best score is taken. This score is calculated as the sum of the S_{ij} values of the selected equivalent pairs (i,j) from the dynamic programming less the penalty for each of the chain breaks or gaps. We use six starting alignments, giving different sets of initial equivalences: (1) align the beginnings of the two sequences, (2) align the mid-points, (3) align the ends, (4) align at a random point, (5) align using sequence identity and (6) align using alpha angles. Most of these starting points were used previously (Subbiah et al., 1993; Laurents et al., 1994; Gerstein & Levitt, 1996). However, in order to correctly match all the scop pairs, we needed to modify our procedure as discussed below.

Sidechain Orientation

An important improvement was taking into account sidechain orientation. This could simply be done by using Cβ rather than Cα atoms for the computation of distances d_{ij}. However, we sometimes used a more elaborate procedure (method CαCβ) where we multiplied each entry in the similarity matrix S_{ij} by a factor representing the relative orientation of the Cα-Cβ (or C=O) bonds (specifically exp(cos A), where A is the angle between the corresponding bond in each structure). Taking into account sidechain orientation makes misalignments by one residue in helices and, especially, in strands more difficult. Misalignments by a single residue are not serious in terms of matching the overall fold but give nonsensical alignments in detail. For instance, in the case of strands they often lead to mismatching of hydrophobic and hydrophilic residues.

Exposure Weighting

Another useful modification was to increase the weight of the aligned residues buried inside the protein relative to those on the surface. This was achieved through the following procedure: the accessible surface area (Lee & Richards, 1971) of each residue was determined (considering an all atom model). These areas (in square Angstroms) were used to assign weights W(i) to each residue i according to the following scheme: 0.5 for the exposed residues (exposed area greater than 100 $Å^2$), 2.0 for the buried residues (exposed area less than 50 $Å^2$), and of 1.0 for the remaining residues. These weights were then used to modify the entries of the similarity matrix (S_{ij}) as follows: $S'_{ij} = W(i) W(j) S_{ij}$.

Secondary Structure Dependent Gap Penalties

In the basic version of the method, the gap penalty is independent of gap size and normally taken to be half the score contribution of a perfectly matched pair (i.e. M/2=10). Because of the similarity between our structural alignment procedure and normal sequence alignment, it is possible to incorporate more complicated variable, position-dependent gap penalties into the alignment in a very straightforward fashion. Since we know the secondary structure of the two proteins we are aligning (e.g. from DSSP, Kabsch & Sander, 1983 or stride, Frishman & Argos, 1995) we can make it more difficult to introduce a gap at a position in a secondary structure (i.e. strand or helix). This is similar to *sequence* alignment methods that make the penalty for opening a gap depend on where it starts (Lesk et al., 1986; Smith & Smith, 1992; Vingron & Waterman, 1994). Other methods for structural alignment have also employed this approach (Zhu et al., 1992).

We derived specific values for the gap penalties by empirically testing them on a number of protein families. We found that as the gap opening penalty is decreased in secondary structure relative to that in loops and coils, one obviously increases the number of spurious gaps in strands and helices. This suggests that very high gap penalties in strands and helices might work well. However, we also found that such high gap penalties make it more difficult to align secondary structural elements (which often vary slightly in size); in fact, a penalty that is too high leads to completely mismatching secondary structures. (For instance, instead of aligning two helices of slightly different size through introducing a gap into the longer helix, the program might introduce many gaps into a loop preceding one helix and align this helix against a loop and the second against the introduced gaps). The specific values we chose are a compromise between these two competing effects. We always set the gap extension penalty to be a small constant value (0.025 M). We arranged the gap opening penalties for each structure into a vector $\alpha(k)$, indexed by the sequence position i or j. Initially, the $\alpha(k)$ values were set to 2 in sheets and helices, and 1, otherwise. $\alpha(k)$ is then smoothed (by convolution with a Gaussian with weights 1,3,8,3,1) and re-scaled so that the overall average gap penalty $<\alpha(k)>$ is half the maximum match score M.

Refinements to the Search

When comparing structures of different size it was sometimes advantageous to split the larger structure into pieces. Here we used three pieces: the first half, the middle half and the second half. Because each of the structures in the set of 941 scop domains was only a single domain, this trimming was only used for 82 pairs out of the total of 2107 (3.9%). Of these 82 comparisons, 50 lead to a successful match. For protein structures that have not been separated into domains, this splitting is most useful for structures with internal symmetry and duplication, such as calmodulin, and for structures that had a small strong similarity in the midst of larger overall similarity that was not as well defined.

The best search strategy consisted of the following five steps: (1) use Cβ atoms; (2) use Cα atoms; (3) use Cβ atoms with exposure weights; (4) use Cα atoms with exposure weights; and (5) try three-way splitting of the longer chain with Cβ atoms and exposure weights. The search is stopped after any step that does not fail, where failure is defined as not being able to get an RMS’ score (defined in the results) less than 4 Å. This strategy is somewhat arbitrary, and other protocols give similar results (e.g. Cα atoms followed by Cα atoms with variable gap penalties, followed by Cβ atoms). The important point is that starting from multiple starting points and using a variety of different definitions for the similarity matrix helped.

Elimination to Determine a Core Structure

After determining an alignment, we “refined” the RMS by eliminating the worst fitting pairs of equivalenced atoms and then refitting to get a new RMS, in a similar fashion to the core-finding procedure in Gerstein & Altman (1995a, 1995b). This refinement is necessary as the dynamic programming tries to match as many residues as possible (i.e. it is a global as opposed to local method). In doing the elimination, we do not change the equivalences but merely eliminate those pairs with the worst individual deviation in atom position.

The threshold for stopping the elimination is somewhat arbitrary. We tried a variety of approaches (absolute threshold, “throwing-out” a given fraction of the atoms, etc.). The scheme we settled on involves eliminating the pair of equivalenced atoms with the largest interatomic distances so long as the following criteria are satisfied: (i) The chosen pair must be adjacent to a chain break (or chain ends), which ensures that the elimination procedure does not increase the number of gaps. (ii) The pair to be eliminated has to have a separation d_{ij} greater than 3.8 Å, which is the distance between adjacent Cα atoms along the polypeptide chain. (iii) Fewer than half the initial pairs have been eliminated. (iv) There are more than remaining 20 pairs. And (v) if there are fewer than 50 matches, the RMS' must exceed 4 Å, which prevents the elimination procedure from generating very short segments that match well.

This elimination scheme performed well in that the lengths of matched regions (N) were not excessively shortened, while at the same time the RMS deviations were reduced considerably. That is, the average RMS drops from 4.64 Å to 2.66 Å, while the average N drops only from 123 to 98. It is also interesting to note that for the 2107 scop pairs, elimination was stopped 82% of the time for criteria (ii) (see above), 5% of the time for criteria (iii), 1% for (iv), and 12% for (v).

Multiple Structural Alignment

We formed multiple structural alignments by combining all possible pairwise alignments for a given collection of structures. From all the pairwise alignments, we picked the structure that is on average closest to all other structures. This is in a sense the "median" structure within the "cluster" of all the structures. We then form a multiple alignment by aligning all the other structures to this median structure and consistently combining their alignments. Tests given show that aligning all the structures to non-median structures works less well (figure 8).

This procedure is somewhat simpler than the usual approach to multiple alignment, for both sequences and structures (Thompson et al., 1994; Taylor & Orengo, 1995; Gototh, 1996; Gussfield, 1997), which proceeds by agglomerative clustering. Often this involves forming a consensus between the closest pairs and then using this in subsequent steps. We felt our simple approach was adequate for the task at hand, as none of our multiple alignments involved a large number of structures (i.e. not more than 15 and usually only around 4). However, it would probably not give optimum results on a much larger number of objects (>100 as is often common in multiple *sequence* alignment).

Comparison of Multiple Alignments (manual vs automatic)

We compared our automatically generated multiple alignments against manual ones one by "aligning" them *en bloc* by dynamic programming so as to minimize the total mismatches. We only count mismatches in structurally conserved, core regions as other regions of the protein structure, particularly some surface loops, are impossible to align correctly. Core regions are often explicitly indicated in the literature alignment. If this is the we used this literature definition. Otherwise, we used the elimination procedure described above and the somewhat more general strategies in Gerstein & Altman (1995) to automatically determine a core.

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