

## Measurement of the effectiveness of transitive sequence comparison, through a third 'intermediate' sequence

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### Abstract

**Motivation:** Transitive sequence matching expands the scope of sequence comparison by re-running the results of a given query against the databank as a new query. This sometimes results in the initial query sequence ( $Q$ ) being related to a final match ( $M$ ) indirectly, through a third, 'intermediate' sequence ( $Q \rightarrow I \rightarrow M$ ). This approach has often been suggested as providing greater sensitivity in sequence comparison; however, it has not yet been possible to gauge its improvement precisely.

**Results:** Here, this improvement is comprehensively measured by seeing what fraction of the known structural relationships transitive sequence matching can uncover beyond that found by normal pairwise comparison (i.e. direct linkage). The structural relationships are taken from a well-characterized test set, the scop classification of protein structure. Specifically, 2055 known structural similarities (called 'pairs') between distantly related proteins constitute the basic test set. To make the measurement of transitive matching properly, special data sets, called 'baseline sets', are derived from this. They consist of pairs of sequences that have a clear structural relationship that cannot be found by normal sequence comparison (i.e. they cannot be directly linked). Specifically, using standard sequence comparison protocols (FASTA with an  $e$ -value cut-off of 0.001), it is found that the baseline set consists of 1742 pairs. A third intermediate sequence can link 86 of these indirectly (5%), where this third sequence is drawn from the entire, current universe of protein sequences. The number of false positives is minimal. Furthermore, when one considers only the relationships within the test set that correspond to a close structural alignment, the coverage increases considerably. In particular, 862 of the baseline set pairs fit to better than 2.6 Å RMS, and transitive matching can find 62 of these (9%).

**Availability:** All the test data, including precise similarity values calculated from structural alignment, are available in tabular format over the Web from <http://bioinfo.mbb.yale.edu/align>.

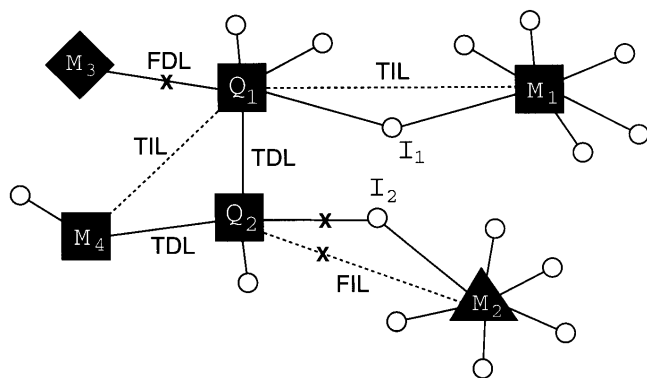
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### Introduction

Transitive sequence matching is an approach taken toward improving sequence comparison. It entails taking the matches found after running a sequence comparison and then re-running them as new queries against the databank. The resulting matches consist of many of the previous matches plus (hopefully) some new ones. These new matches are, in turn, related back to the initial query only indirectly through an intermediate sequence (see TIL in Figure 1). This whole process can be repeated again, iteratively, with these new matches.

The idea of transitive matching has been previously suggested and implemented. In particular, it has been used to improve the sensitivity of single-sequence comparison and to refine templates and hidden Markov models (HMMs) (Gribskov *et al.*, 1990; Tatusov *et al.*, 1994; Yi and Lander, 1994, 1996; Eddy, 1996; Abagyan and Batalov, 1997; Altschul *et al.*, 1997; Park *et al.*, 1997; Pearson, 1997; Sonhammer *et al.*, 1997; Wolf *et al.*, 1997). In the latter application, one forms a template from a small 'seed' alignment, which is then used to find homologues. These are added to the template, and the process is repeated. While this technique has been demonstrated to be effective to varying degrees on specific protein families, it can lead to incorrect assignments.

The objective here is to assess the effectiveness of a simple form of transitive matching, in a comprehensive fashion. For this assessment, the structural classification of proteins (scop) (Murzin *et al.*, 1995), which arranges all the known structures in the protein databank into a few hundred domain-level, fold families, provides a 'gold-standard' reference dataset. Not all the structures in a given fold family are highly similar to each other in terms of sequence, so one can assess the usefulness of a given sequence comparison method by seeing how many of the structural similarities between only marginally similar sequences the method is able to detect.



**Fig. 1.** Schematic illustrating transitive matching via indirect linkage. This schematic illustrates direct and indirect linkage, and how either linkage can result in a true or false positive. The black shapes (square, triangle, diamond) indicate sequences with a known structure (i.e. PDB sequences). For the purposes of the discussion here, one imagines that each of these sequences is a representative sequence drawn from scop-pair test sets and so it stands for a whole sequence family. The four black squares indicate sequences that share a common fold, the 'square fold', while the folds corresponding to the black triangle and diamond are supposed to be different. The line marked TDL ('true direct linkage') indicates a 'true' sequence similarity linking two of these sequences ( $Q_1$  and  $Q_2$ ). In contrast, the line marked FDL indicates a 'false' direct linkage between  $Q_1$  and  $M_3$ , i.e. a linkage that is not a scop pair. False linkages, indicated by 'x' in the figure, give rise to the false positives in Table 1. It is possible for two direct linkages to join two PDB sequences that are not linked directly. This is 'indirect linkage' and is indicated by the dotted line between  $Q_1$  and  $M_4$  in the figure. Here,  $Q_2$  is functioning as the 'intermediate sequence'. One can expand the possible intermediate sequences by considering sequences that do not correspond directly to PDB structures, i.e. sequences from OWL homologous to PDB sequences (the OWL sequences). These are indicated by the small white circles linked to the black shapes. The OWL sequences can function as intermediate sequences (denoted I) linking two PDB sequences (the query Q and the match M) via indirect linkages that are either true or false (i.e. TIL or FIL, 'true or false, indirect linkage').

Brenner *et al.* (1995, 1998; Brenner, 1996) used this approach to assess the effectiveness of the popular FASTA and BLASTP programs and their probabilistic scoring schemes (i.e. the e-value) (Pearson and Lipman, 1988; Altschul *et al.*, 1990, 1994; Karlin and Altschul, 1993; Pearson, 1996). They found that the FASTA e-value closely tracked the number of false positives, i.e. the error rate, and that at a conservative e-value cut-off of 0.001, the FASTA program could detect nearly all the relationships that a full Smith–Waterman comparison would (Smith and Waterman, 1981). Specifically, they found that FASTA with a 0.001 threshold would find 16% more of the structural relationships in scop than would be found by standard sequence comparison with a 40%

identity threshold. Here, a similar approach is taken to assess the effectiveness of transitive matching.

## Methods

### Data

Sequences with known structure were taken from the Protein Databank (PDB) (Bernstein *et al.*, 1977). Fold definitions were taken from scop, Version 1.32 (May 1996) (Brenner, 1996; Murzin *et al.*, 1996; Hubbard *et al.*, 1997). Only the superfamily pairs, as opposed to fold pairs, were used as these have a much clearer structural relationship. It is the intention of the creators of scop that the superfamily pairs represent an evolutionary relationship between proteins with no appreciable sequence similarity, i.e. link proteins that are true homologues (Murzin *et al.*, 1995; Hubbard, 1997). However, this is necessarily speculative, and all one can know for certain is that these pairs have a close structural relationship. Furthermore, the scop pairs have been extensively checked by both manual and automatic methods (Gerstein and Levitt, 1998), and are believed not to contain any false positives.

Based on the scop pairs, Brenner *et al.* (1995, 1998) clustered the PDB into 905 representative sequences at 40% identity (domains split between different chains are omitted from this count), making a list denoted pdb40d, which is distributed through the scop website (<http://scop.mrc-lmb.cam.ac.uk/scop>). The clustering employed a single-linkage approach similar to that in Hobohm *et al.* (1992, 1994), i.e. 'select until done'. For the indirect sequence matching, pdb40d was compared against the 142 737 total sequences in the OWL composite databank (Version 27.1) (Bleasby *et al.*, 1994). Low-complexity sequences were filtered out of OWL using the SEG program (Wootton and Federhen, 1993).

The overall analysis was greatly expedited by using a simple relational database implemented using DBM and perl5 (Wall *et al.*, 1996). A number of detailed tables relevant to this paper will be made available over the Internet at <http://bioinfo.mbb.yale.edu/align>.

### Sequence comparison

All sequence matching was with the FASTA program (Version 2.0) (Lipman and Pearson, 1985; Pearson and Lipman, 1988; Pearson, 1996, 1998; Pearson *et al.*, 1997) with a k-tup value of 1. This program was chosen for a number of reasons.

1. FASTA is commonly used in the comparison of sequences corresponding to structures; for instance, it was used for the original definition of superfolds by Orengo *et al.* (1994) and is the sequence comparison method used by the PDB browser (Stampf *et al.*, 1995).

Consequently, FASTA forms an established standard on which to base this work.

2. FASTA was used in the work of Brenner *et al.* (1998) which forms a necessary background for this work. Brenner *et al.* showed, furthermore, that FASTA performs better than BLAST and essentially the same as Smith–Waterman for the detection of distant similarities between structurally similar proteins, obviating the need to consider these other approaches here.
3. Assessing the improvement in indirect linkage is much more straightforward for single-sequence comparison methods, such as FASTA, than for multiple-sequence methods, such as HMMs or PSI BLAST (Krogh *et al.*, 1994; Altschul *et al.*, 1997), where the performance of the method varies depending on the number of members in the family.

### Structure comparison

Structure matching was by the iterative dynamic programming method from Gerstein and Levitt (1996, 1998). This method aligns protein sequences on the basis of direct comparison of the corresponding three-dimensional structures. Two numbers characterize the alignment: the number of residues aligned ( $N$ ) and the RMS deviation in  $C\alpha$  positions after these atoms are fit onto each other (RMS). Since an alignment with a higher RMS value can be more significant than one with a lower RMS if there are more residues included in the first alignment, Gerstein and Levitt (1998) define a scaled RMS:  $RMS = 225 \text{ RMS} / (N + 135)$ . For an approximately average match of 90 residues, the scaled RMS is nearly the same as RMS (both quantities agree to within 10% for  $N$  between 70 and 110 residues). The distribution of scaled RMS values has a median value of 2.65 Å (with a mean of 2.68 Å and a SD of 0.87 Å), so 2.6 Å marks the approximate halfway point in the range of values and a reasonable division point. Levitt and Gerstein (1998), furthermore, show that this scaled RMS threshold corresponds approximately to a structural similarity  $P$  value of 0.01.

### Overlap on intermediate sequence

In the transitive matching procedure, two sequences corresponding to structures (denoted  $Q$  for the query and  $M$  for the match) are linked through an intermediate sequence  $I$ . One has to take care that the region of match of  $Q$  on  $I$  overlaps with that of  $I$  on  $M$ . The criterion for overlap used here was quite conservative: the overlap region of  $Q$  on  $I$  must share at least 60 residues with that of  $I$  on  $M$ . Other less stringent criteria were tried. These tend to increase the number of matches, both true and false, but not to affect the results greatly, as long as they were reasonable.

As a practical matter, one can deal with the overlap when performing a search as follows. One runs the query against the whole databank, finding a number of direct matches  $I_i$ . Then the precise matching regions of each  $I_i$  are ‘cut out’ and this is re-run against the databank again, producing matches  $M_{i,j}$ , which are then indirectly linked back to the original query  $Q$ . While simple and elegant, this procedure has the effect of changing the scores linking  $I_i$  and  $M_{i,j}$  relative to those found in an all-versus-all of the databank since the length of the intermediate sequence  $I_i$  is different when it is matched by  $Q$  or used as the query to find  $M_{i,j}$ .

## Results

### *The test data: sets of structural relationships*

The analysis begins with the 8330 domains in the PDB indexed by the current version of scop. These are clustered into 905 representative sequences at a 40% identity level in the publicly distributed PDB40D dataset (see Methods). About 400 000 pairs can be formed from these representative sequences (i.e.  $408\,156 = 970 \times 969/2$ ).

*Test set 1.* By definition, all these pairs have a distant (‘twilight-zone’) level of sequence similarity. However, according to the scop classification (Murzin *et al.*, 1995), 2055 (~0.5%) of them have a significant structural relationship, in being joined to one of 171 structural superfamilies (see Methods). These 2055 form the first set of ‘scop pairs’. They are not distributed equally amongst the scop superfamilies, with one superfamily (the Rossmann fold) containing 231 pairs and 70 others with just a single pair (Table 2). Furthermore, not all the structural relationships in these pairs are of equal weight, so it is worthwhile to consider two further ‘selections’ based on somewhat closer structural relationships.

*Test set 2.* Because determining the structural similarity of short sequences is particularly problematic, one can exclude sequences of <60 amino acids. This gives 783 representative sequences, 305 371 possible pairs and 1801 structural relationships, which constitute a selection of 2055 original scop pairs. Gerstein and Levitt (1996, 1998) constructed structural alignments for all the preceding 1801 structural similarities of full-length sequences. These alignments allow one to determine the precise degree of structural similarity by calculating an RMS value from fitting the aligned atoms. There are 862 pairs that align with a scaled RMS of <2.6 Å (see Methods), and these form a second test set of scop pairs. They are (roughly) the more structurally similar half of the scop pairs.

**Table 1.** Overall statistics for sequence matching

FASTA e-value cut-off	Type of pair	Pairs in total: test sets	Linked directly	Pairs remaining: baseline sets	Linked indirectly within dataset	Linked indirectly via OWL sequence
1.0E-05	TPs	2055	220 11%	1835	19 1.0%	67 3.7%
	Low-RMS TPs	862	162 19%	700	15 2.1%	62 8.9%
	FPs		0		0	12
1.0E-04	TPs	2055	271 13%	1784	28 1.6%	73 4.1%
	Low-RMS TPs	862	198 23%	664	17 2.6%	64 9.6%
	FPs		1		0	13
1.0E-03	TPs	2055	313 15%	1742	23 1.3%	86 4.9%
	Low-RMS TPs	862	219 25%	643	18 2.8%	74 12%
	FPs		3		1	13

The table shows how many of the scop pairs can be found by direct linkage (i.e. normal sequence comparison) and indirect linkage (i.e. transitive matching through an intermediate sequence). The rows show the number of true and false positive linkages (TPs and FPs) for various FASTA e-value thresholds. These linkages are computed for two test sets: test set 1, which has 2055 scop pairs, and test set 2, which has 862 scop pairs, corresponding to more closely aligned structures that have a structural alignment with atoms fitting to better than 2.6 Å RMS. The true positives for the latter test set are denoted by 'low-RMS TPs'. There were no false positives for test set 2 data (so there are no 'low-RMS FPs' rows). The first column ('test sets') shows the total number of pairs that one starts with. The next column ('linked directly') shows the number of these pairs that can be found by direct linkage. These are subtracted away to give the baseline sets shown in the third column ('baseline sets'). The final two columns give the number of pairs from the baseline sets that can be found by indirect linkage. The first of these ('indirectly within dataset') lists the transitive matches that can be found purely within a given baseline set. The next column ('indirectly via OWL') lists the larger number of transitive matches that can be found if one allows any sequence in the large OWL database to function as an intermediate sequence. Note that it is possible to have a pair linked directly, but not indirectly, if no suitable intermediate sequence exists. Also, a number of the false-positive linkages were between scop class 8 sequences ('peptides'). (In particular, the pairs d1tiv\_\_-d1tvs\_\_ and d1bba\_\_-d1ppt\_\_) These were excluded from the statistics (but they are still listed, for completeness, in the Web presentation).

**Table 2.** Matching statistics for the various scop superfamilies, divided by family size

Pairs per superfamily	No. of super-families	Total no. of scop pairs	No. linked directly	Frac. linked directly	No. of indirect or dir. links	Frac. linked ind. or dir.
231	1	231	21	9%	26	11%
171	1	171	5	3%	9	5%
153	1	153	3	2%	3	2%
120	2	240	22	9%	47	20%
91	1	91	36	40%	57	63%
78	1	78	4	5%	4	5%
55	3	165	12	7%	12	7%
36	3	108	22	20%	28	26%
28	8	224	21	9%	24	11%
21	6	126	45	36%	53	42%
15	4	60	7	12%	8	13%
10	11	110	15	14%	16	15%
6	17	102	29	28%	33	32%
3	42	126	42	33%	46	37%
1	70	70	29	41%	33	47%
Total	171	2055	313	15%	399	19%

This table shows the statistics for direct and indirect linkage for the 171 scop superfamilies. The statistics are broken down by the size of the superfamily. Details on each column follow. (1) The number of pairs  $P$  in a superfamily, i.e. its size (using PDB40D in scop 1.32 as described in Methods). (2) The number  $N$  of superfamilies of this size in scop. (3) The total number of pairs then follows by multiplication,  $T = NP$ . (4) The number of pairs  $D$  that can be directly linked by sequence comparison with FASTA and an e-value cut-off of 0.001. (5) The fraction of the total number of pairs that the number of directly linked pairs comprises,  $F = D/T$ . (6) The number of pairs  $I$  that can be linked by either indirect or direct linkage. (7) The fraction that  $I$  is of the total ( $I/T$ ).

### Direct linkage: the baseline for comparison

To measure the effectiveness of transitive matching, one can look at how many of the scop pairs in each of the three sets indirect linkage can find, relative to the number of false positives. However, before doing this, one has to determine how many pairs normal sequence comparison can find, in order to establish a baseline upon which transitive matching can improve. Here, pairs found by normal sequence comparison are called direct linkages since they involve no intermediate sequences (TDL; Figure 1).

This is essentially what Brenner *et al.* (1995, 1998; Brenner, 1996) did in asking how many of these real structural relationships could be found using the FASTA and BLASTP programs with their probabilistic scoring schemes. Here, these results are reproduced for the three specific sets of scop pairs discussed above. As Brenner *et al.* found, at a practical e-value threshold of 0.001, FASTA can find ‘direct linkages’ for ~15% of the relationships in the first set of the scop pairs with only a few false positives.

Notice that when one considers just the 862 pairs with a close structural alignment (test set 2), it is possible to find a higher fraction of the pairs (25%). This last result is reasonable, given the established relationship between divergence in structure and sequence (Chothia and Lesk, 1986, 1987; Chothia and Gerstein, 1997). That is, the pairs with greater structural similarity are expected to have more sequence similarity.

As shown in Table 2 and 3, the fraction of pairs found varies considerably amongst the scop superfamilies, with a larger fraction of pairs found in the smaller superfamilies.

Work on direct linkage provides a necessary background against which to examine indirect linkage. Here, the idea is to find out how many additional structurally related pairs can be found by considering a third, intermediate sequence linked to both. These indirect linkages, both true and false, are illustrated schematically in Figure 1. To find them, it was necessary to construct more sets of scop pairs, the same as those described previously, but now with all the pairs found by direct matching removed. These are called baseline sets.

**Baseline set 1–3.** This consists of 1742 pairs taken from the 2055 pairs in test set 1 with direct matches removed. It involves 697 sequences in total. It is based on a FASTA e-value cut-off of  $10e^{-3}$ . If this cut-off is changed, obviously the number of pairs will change, so one also has baseline sets 1–4 and 1–5 for cut-offs of  $10e^{-4}$  and  $10e^{-5}$ , and so on (see Table 1).

**Baseline set 2–3.** This consists of 643 pairs taken from the 862 closely aligned pairs in test set 2 with direct matches removed. It involves 491 sequences in total. It is also derived with a cut-off of  $10e^{-3}$ , and baseline sets 2–4 and 2–5 can be defined in a similar fashion.

### Indirect linkage: the improvement over the baseline

By definition, each of the sequences in the baseline sets has no sequence similarity to any other sequence within the same set. Consequently, it is now readily possible to gauge the improvement provided by transitive matching: any new pairs found constitute the improvement. A transitive match could, in principle, be through the sequences within the baseline sets, i.e. if pair AB and pair BC exist in set 1–3, but not pair AC, there would be an indirect link between A and C. As shown in Table 1, this occurs, but not that frequently. For instance, for baseline set 1–3, one can find 23 of the 1742 pairs.

One can find more transitive matches by considering the entire population of protein sequences as candidate ‘intermediate sequences’. Specifically, one can run the sequences in each of the baseline sets against the OWL composite database (which contains all currently known protein sequences) and determine whether any of the homologues found in OWL linked a scop pair in the baseline sets. Used in this way, the sequences in the baseline sets are better thought of as cluster representatives for whole families than individual sequences. Each indirect link made between them is effectively between all the members of two distant families.

The results are summarized in Table 1. For an e-value threshold of 0.001 (baseline set 1–3), one can find 86 of the 1742 baseline pairs through indirect linkage (5%), with 13 false positives. This means that using both direct and indirect linkage, sequence comparison with FASTA can find about a fifth of the scop pairs (399 of 2055 in test set 1) with 16 total false positives, about one for every 25 true positives.

On the 862 closely aligned scop pairs (test set 2), the coverage improves significantly. In particular, transitive matching can find 74 of the 643 pairs in baseline set 2–3 (12%).

As shown in Table 2, the fraction of extra pairs found by transitive matching varies somewhat among the 171 scop superfamilies, with the larger families having a greater degree of improvement relative to the smaller ones. This is perhaps because direct matching was more successful with the smaller superfamilies and because the larger superfamilies are potentially associated with a larger and more diversified collection of intermediate sequences. For instance, indirect plus direct linkage can find 30% of the 120 pairs in the ‘FAD/NAD(P)-binding domain’ superfamily (representative identifier d2tpa2), whereas direct matching can only find 10% (Table 3). Likewise, for the globin superfamily (d3sdha\_), the comparable statistics are 63% of 91 pairs found, improving on 40%. In contrast, for the 70 scop superfamilies containing only a single pair, indirect plus direct linkage finds 47% of pairs, only a small improvement over the 41% found by direct matching alone.

**Table 3.** Detailed matching statistics for the largest scop superfamilies

Superfamily ID	No. of scop pairs	No. of direct links	Frac. linked directly	No. of indirect or dir. links	Frac. linked ind. or dir.
d1pmy__	55	4	7%	4	7%
d1r69__	28	3	11%	3	11%
d1tssa1	28	0	0%	0	0%
d1yrnb_	55	8	15%	8	15%
d2ebn__	120	10	8%	11	9%
d2olba_	28	1	4%	1	4%
d2pgd_2	231	21	9%	26	11%
d2tgf__	28	13	46%	13	46%
d2tpa2	120	12	10%	36	30%
d2trxa_	36	4	11%	4	11%
d2yhx_2	28	0	0%	1	4%
d3cd4_2	153	3	2%	3	2%
d3dpva_	171	5	3%	9	5%
d3hhrb2	28	1	4%	1	4%
d3inkc_	55	0	0%	0	0%
d3sdha_	91	36	40%	57	63%
d3tgl__	28	1	4%	2	7%
d4icb__	36	8	22%	14	39%
d5cytr_	28	2	7%	3	11%
d5p21__	78	4	5%	4	5%
d5znf__	36	10	28%	10	28%

This table shows the statistics for direct and indirect linkage for the 21 largest scop superfamilies, those with at least 28 pairs. Details on each column follow, many of them being very similar to those in Table 2. (1) The first column gives the identifier for the superfamily. Here this is a scop identifier for a representative domain in this superfamily. Scop identifiers 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 IGGT. (2) The number of pairs  $P$  in the superfamily, i.e. its size (using PDB40D in scop 1.32 as described in Methods). (3) The number of pairs  $D$  that can be directly linked by sequence comparison with FASTA and an e-value cut-off of 0.001. (4) The fraction of the total number of pairs that the number of directly linked pairs comprises,  $F = D/P$ . (5) The number of pairs  $I$  that can be linked by either indirect or direct linkage. (6) The fraction that  $I$  is of the total ( $I/P$ ).

### Qualifications

The specific ‘improvement’ values quoted here for the effect of transitive sequence matching are intended to be representative of the performance of the method on a comprehensive data set using reasonable parameters. They are, nevertheless, contingent upon the selection of proteins in the test set (the scop classification), the particular comparison baselines established (i.e. an e-value cut-off of 0.001 for the direct linkage baseline) and the precise criteria for overlap (as discussed in Methods). These parameters have been selected in a reasonable fashion to exclude highly similar sequences and give a sense of how indirect sequence matching performs near the margin, in the ‘twilight zone’. The scop data set (Murzin *et al.*, 1995), in particular, is a popular and well-documented set of similarities. It has been validated by both

automatic and manual methods (Gerstein and Levitt, 1997), and should give the most comprehensive possible indication of how indirect matching performs on the whole range of known similarities, rather than just on specific families.

Moreover, while the exact improvement values quoted here may change somewhat with different choices for test data, baselines and overlap criteria, with any reasonable choices, transitive matching will be able to find additional real pairs without generating many false positives. That is, while the absolute values may change, the relative improvement will remain. This is shown to some degree in Table 1, where the improvement statistics for a variety of baselines are collected, e.g. 0.0001 and 0.00001. (One could develop this table further to build up a complete analysis of coverage versus error rate.) Furthermore, the entire test set and related data files (including all the precise similarity values from

structural alignment) are available over the Web, thus enabling the analysis to be easily repeated with any parameters of one's choice.

## Conclusion

The results reported here show that transitive sequence matching is an effective technique for improving the sensitivity of standard sequence comparison methods in searching for structural similarities. Specifically, one is able to find considerably more of the known structural similarities in a 'gold-standard' set of test data (scop) by combining indirect linkage via an intermediate sequence with direct linkage than by direct matching alone. Moreover, there are few false positives. One can intuitively rationalize the success of transitive sequence matching as this approach makes use of the (presumably) more diversified outliers of a cluster in a search, instead of searching with the centroid (as is the case, for instance, for profiles).

The effectiveness of transitive sequence matching was measured here for the FASTA program, but a similar analysis could easily be carried out for the other popular sequence comparison approaches, such as profiles and HMMs (Bowie *et al.*, 1991; Johnson *et al.*, 1993; Eddy *et al.*, 1994; Krogh *et al.*, 1994). In fact, such analyses have recently been performed successfully by other groups (C.Chothia and J.Park, personal communication). It is expected that careful measurement of the effectiveness of sequence comparison methods for detecting structural similarities will allow these methods to be used as a baseline for assessing more elaborate fold recognition methods such as threading (Jones *et al.*, 1992; Bryant and Lawrence, 1993; Jones and Thornton, 1996).

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