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MINI-REVIEW ARTICLE

Searching Protein Structure Databases Has Come of Age

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ABSTRACT The number of protein structures known in atomic detail has increased from one in 1960 (Kendrew, J.C., Strandberg, B.E., Hart, R.G., Davies, D.R., Phillips, D.C., Shore, V.C. *Nature (London)* 185:422-427, 1960) to more than 1000 in 1994. The rate at which new structures are being published exceeds one a day as a result of recent advances in protein engineering, crystallography, and spectroscopy. More and more frequently, a newly determined structure is similar in fold to a known one, even when no sequence similarity is detectable. A new generation of computer algorithms has now been developed that allows routine comparison of a protein structure with the database of all known structures. Such structure database searches are already used daily and they are beginning to rival sequence database searches as a tool for discovering biologically interesting relationships.

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Key words: algorithms, structure alignment, Protein Data Bank, protein superfamilies, structural homology

INTRODUCTION

Attending scientific conferences can lead to exciting revelations. A poster, presenting the three-dimensional structure of the recently solved biotin repressor, immediately caught the eye of the crystallographer R. Wierenga, whose group had just solved the structure of an unrelated small protein domain with homology to protein kinases, called SH3 (*src*-homology 3). Surprisingly, the C-terminus of biotin repressor folds into a six-stranded antiparallel β -barrel with identical topography to that in the SH3 domain (Fig. 1). At about the same time, A.G. Murzin,² using his encyclopedic memory of protein structures, recognized an SH3-like fold in the plasmid-encoded R67 dihydrofolate reductase,³ also an unexpected relationship. On the other side of the globe, M.B. Swindells noted the similarity of the unusual elongated folds of the very distantly related

tumor and nerve growth factors (Fig. 2), which appeared in print within some months.⁴ These are anecdotal illustrations of the dramatic increase in the number of unexpected topological similarities between proteins with little or no sequence similarity over the past year or two, but they also are typical examples of the haphazard fashion in which most of these similarities were discovered.

BOOM OF NEW STRUCTURES

Soon, no single scientist will be able to hold all known protein structures in her memory and scan them for similarities. The world's journals publish experimentally determined protein structures at a rate that exceeds one paper per day (estimate for 1994). Every two or three weeks new and important structures are published. At the end of 1993, there were already 339 different structures in the Protein Data Bank (size of a representative set of protein structures,⁵ using a 30% sequence identity cutoff), compared to only 155 a year earlier. In light of these developments, one may expect that structural analysis by visual inspection will soon be inadequate, in speed and/or precision, as the number of structures rapidly increases. How can we cope with this challenge?

We do not have to look far for a solution. The field of protein sequence analysis faced a similar situation many years ago, when sequence alignment using pencil and paper for all new sequences began to exceed human capacity. Efficient computer alignment algorithms were developed and turned into

Abbreviations: 1D, one-dimensional; 3D, three-dimensional; EGF, epidermal growth factor; HIV, human immunodeficiency virus; PDB, Protein Data Bank; SH2, *src*-homology 2; SH3, *src*-homology 3.

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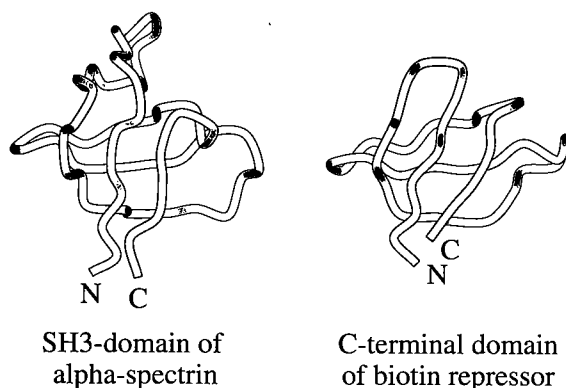


Fig. 1. SH3-fold. Small domains with an SH3-like topology have been identified in protein kinases (1SHG³⁹), myosin subfragment-1, and in bacterial enzymes such as biotin repressor (1BIA⁴⁰) and plasmid-encoded dihydrofolate reductase. Drawn using MolScript.⁴¹

powerful database search tools that are now applied routinely to any newly deduced protein sequence.⁶ This same scenario is now repeating for three-dimensional structures and the solution has the same three essential ingredients: larger databases, more powerful computers, and novel algorithms.

STRUCTURAL ALIGNMENT

Algorithms for structure comparison have to address the full complexity of similarity of shape in 3D space. Early computer methods⁷⁻¹⁰ required manual initial alignment or were very slow or limited to close homologues. The last few years have seen the arrival of a new generation of search algorithms that are general, elegant, and/or fast.¹¹⁻¹⁹ The most efficient of these allow fully automated and rapid similarity searches through the entire database of more than a thousand three-dimensional structures.

The notion of structural equivalence becomes increasingly complex with increasing evolutionary distance. The conformation of a point mutant differs from that of the wildtype protein only locally and only by a few tenths of an Ångström. Much larger deviations are observed in pairs of homologous proteins: with increasing sequence dissimilarity, small shifts in the relative orientations of secondary structure elements accumulate and reach several Ångströms and tens of degrees, as described, e.g., for the globins.²⁰ At the largest evolutionary distances, only the topology of the fold or folding motif is conserved, i.e., the relative location of helices and strands and the loop connections between these. Deviations can be even larger and qualitatively different when structural similarity is the result of convergent rather than divergent evolution. In particular, convergent evolution may result in similar 3D folds that differ in the topology of loop connections.

Just as there is much latitude in the basic formu-

platelet-derived growth factor BB

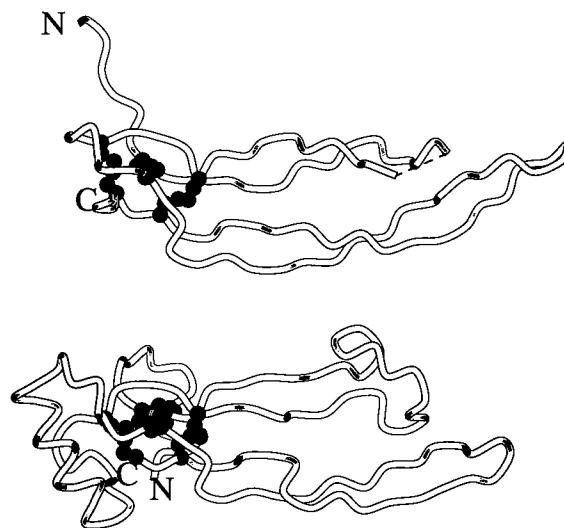


Fig. 2. Cystine-knot fold. The cystine-knot fold lacks a pronounced hydrophobic core. The fold consists of two long, twisted β -hairpins which are held together by three disulphide bridges at the base (black). Conserved half-cystine residues are the principal common sequence feature between known structures with this fold: transforming growth factors $\beta 2$ (1TGI⁴²), nerve growth factor, and platelet-derived growth factor BB (1PDG⁴³). Drawn using MolScript.⁴¹

lation of the structure comparison problem, many different types of optimization algorithm have been employed. These are briefly reviewed here with explicit references to programs that appear in Table I.

DYNAMIC PROGRAMMING ALGORITHMS

Dynamic programming algorithms are a standard tool for finding the global optimum in sequence alignment, a 1D problem. These algorithms have been applied to structure alignment, a 3D problem, by imposing the simplifying assumption that the environment of each individual residue can be adequately summarized in a number or vector or matrix that can then be compared with those of individual residues in the other protein.^{16,19,21,22} The optimal alignment maximizes the sum of similarities of pairs of equivalent residues. An intrinsic limitation of the dynamic programming algorithm is that the order of equivalent pairs along the two protein chains must be sequential, i.e., topological permutations are not detected.

In the Stamp²² and Align¹⁹ programs, comparison is based on the spatial displacement of residue pairs in a 3D superimposition of the structures. The displacements are transformed to similarity scores and an iterative procedure successively refines a non-optimal initial superimposition, e.g., one generated

TABLE I. Recent Examples of Unexpected Topological Similarities*

Structural class, superfamily, or first instance of a fold	New instance	Available in PDB	Detected imme- diately	Method of detection
G-protein like fold	cheY	Yes	No	Protep ⁴⁸
β -Grasp fold (ubiquitin)	Ferredoxin	Yes	No	Whatlf, ¹⁴ Ssap ¹⁶
SH3 domains, R67 dihydrofolate reductase	C-Terminal domain of biotin, repressor	Yes	Yes	Eye ⁴⁹
β -Trefold fold	N-Terminal domain of myosin	No	Yes	Eye ⁵⁰
Globins, phycocyanins	Hisactophilin	No	Yes	Whatlf ⁵¹
	Membrane-insertion domain of colicin A	Yes	No	Dali, ⁵² Ssap ⁵³
Bacteriophage λ repressor	POU-specific domain	No	Yes	Dali, ³⁸ eye ⁵⁴
	Globins	Yes	No	Align, ¹⁹ Ssap ⁵³
$\beta\alpha\beta\alpha\beta$ motif (muconolactone isomerase, ribosomal protein L7/L12, acylphosphatase etc.)	Histidine-containing phosphocarrier protein	Yes	No	Eye, Ssap ⁵⁵
2nd domain of biotin repressor	Ribosomal protein L9	No	Yes	Eye ⁵⁶
	SH2-like folding motif	Yes	No	Stamp ⁵⁷
	Seryl-tRNA synthetase-like folding motif	Yes	No	Dali
Ribonuclease H-like fold	ATPase subdomain 1a of actin, hexokinase, hsp70 and glycerol kinase, and connecting domain of HIV reverse transcriptase	Yes	No	Protep ⁵⁸
Propeller fold	Bacterial sialidase	Yes	Yes	Eye ⁵⁹
Calycins (small hydrophobic ligand binding proteins)	Streptavidin	Yes	No	Eye ³⁶
Barwin	Endoglucanase V	No	Yes	Eye ⁶⁰
Four-helical bundles	C-Terminal domain of kanamycin nucleotidyltransferase	No	Yes	Eye ⁶¹
Transthyretin-like fold	Protocatechuate 3,4-dioxygenase	Yes	No	Dali
TIM (triose phosphate isomerase) barrel	β -Amylase	Yes	Yes	Eye ⁶²
EGF-like modules	N-Terminal domain of prostaglandin H2 synthase-1	No	Yes	Eye ⁶³
Fibronectin type III domains	Chloroplast cytochrome f	No	Yes	Eye ⁶⁴
Domain D of cyclodextrin glycosyltransferase	3rd domain of galactose oxidase	Yes	No	Dali ⁶⁵
Colipase-binding domain of pancreatic lipase	Domain I of arachidonic acid 15-lipoxygenase	No	Yes	Eye ⁶⁶
Phosphoglycerate mutase	Acid phosphatase	No	Yes	Eye ⁶⁷
Carboxypeptidase A	Leucine aminopeptidase	Yes	No	Protep ⁶⁸
Plant lectins	β -Endoglucanase	Yes	Yes	Eye ⁶⁹
	Human serum amyloid component P	Yes	Yes	Eye ⁷⁰
	Animal S-type lectins	Yes	Yes	Eye ⁷¹
	N-Terminal domains of the S2 and S3 subunits of pertussis toxin	No	Yes	Eye ⁷²
Rat mannose-binding protein (C-type eukaryotic lectin)	Methane monooxygenase	No	Yes	Eye ⁷³
Protein R2 of ribonucleotide reductase	Interleukin-5	No	Yes	Eye ⁷⁴
Hematopoietic cytokines	Staphylococcal nuclease	Yes	No	Eye ⁷⁵
Oligonucleotide/oligosaccharide binding fold	Asp-tRNA synthetase	Yes	No	Eye ⁷⁵
	Phage M13 gene 5 protein	Yes	No	Eye ⁷⁵
	β -subunits of heat-labile enterotoxin and verotoxin-1	Yes	No	Eye ⁷⁵
	Cold-shock protein	No	Yes	Whatlf, ³⁷ eye ⁷⁶
	Ribosomal protein S17	No	Yes	Eye ⁷⁷
	Toxic shock syndrome toxin-1	No	Yes	Eye ⁷⁸
	Subunits S2-S5 of pertussis toxin	No	Yes	Eye ⁷²

(continued)

TABLE I. Recent Examples of Unexpected Topological Similarities* (Continued)

Structural class, superfamily, or first instance of a fold	New instance	Available in PDB	Detected immediately	Method of detection
Toxin-agglutinin fold	Complement regulatory protein CD59	No	Yes	Eye ⁷⁹
Astacin, N-terminal domain of thermolysin	Matrix metalloproteinases/collagenases	No	Yes	Eye ^{80,81}
Sweet-tasting protein monellin	Cystatin family of thiol proteinase inhibitors	Yes	No	Eye ⁸²
Glutathione peroxidase, thioredoxin, glutaredoxin, glutathione S-transferase	Thiol:disulfide interchange protein from <i>E. coli</i>	Yes	Yes	Eye ⁸³
DNA-binding domain of catabolite gene activator protein	DNA-binding domain of biotin repressor	Yes	No	Dali, ²⁸ eye ⁸⁴
	LexA repressor	No	Yes	Dali ²⁸
	Histone H5	Yes	Yes	Eye ⁸⁵
	HNF-3/fork head DNA binding domain	No	Yes	Eye ⁸⁴
	DNA-binding domain of heat shock transcription factor	No	Yes	Eye ⁸⁶
DNA polymerase I (Klenow fragment), HIV-1 reverse transcriptase	T7 RNA polymerase	No	Yes	Eye ⁸⁷
Thiamin diphosphate-dependent enzymes	Transketolase	Yes	Yes	Eye ⁸⁸
	Pyruvate oxidase	Yes	Yes	Eye ⁸⁸
	Pyruvate decarboxylase	Yes	Yes	Eye ⁸⁸
Short-chain alcohol dehydrogenases (3 α ,20 β -dihydroxysteroid dehydrogenase)	Dihydropteridine reductase	Yes	No	Eye ⁸⁹
	UDP-galactose 4-epimerase	Yes	No	Dali, eye ²⁹
Cytochrome c peroxidase, myeloperoxidase	Lignin peroxidase	Yes	Yes	Eye ⁹⁰
	Catalytic domain of prostaglandin H2 synthase-1	No	Yes	Eye ⁶³
Methionine aminopeptidase	Creatine amidinohydrolase	No	No	Eye ⁹¹
Animal and phage lysozymes	Plant endochitinase	Yes	No	Dali ³⁰
	Soluble lytic transglycosylase from <i>E. coli</i>	No	Yes	Eye ³¹

*The compilation includes similarities detected by eye (published between March 1993 and March 1994) or by automated database searches.

according to sequence alignment. The Ssap program¹⁶ elegantly eliminates the need for an explicit initial alignment by using dynamic programming at two levels. First, the structural environments of each residue pair are compared in a coordinate frame defined by the trial superimposition of their backbone atoms. The optimal alignment traces are accumulated in a master matrix from which the final alignment is derived. The idea is that only residue pairs with high scores in many trial superimpositions have a chance of being part of the best global match. The original Ssap algorithm⁹ was later improved by various filters to increase speed and reduce background noise.¹⁶

DISTANCE MATRIX ALGORITHMS

The use of intramolecular geometric relationships, such as distances, to describe protein structures has the advantage of being independent of the

coordinate frame.^{12,13,14,18,21,23,24} The Protep program¹³ reduces protein structure to secondary structure elements, i.e., helices and strands, and their geometric relations. The user constructs a search pattern that defines the allowed range of angles and distances between the elements of a structural motif. The complete set of perfect matches to the search pattern can be rapidly retrieved from a prestored database (a single violation is sufficient to discard a potential match).

Several other methods use a more detailed description of internal geometry, in the form of C α -C α distance matrices.^{23,24,18} Instead of search patterns with sharp cutoffs, many of these algorithms use a continuous geometric similarity score summed over all equivalenced intramolecular distances. Optimization of this score as a function of residue equivalences cannot be solved by dynamic programming, as residues as well as all their intramolecular part-

ners have to be equivalenced in an overall consistent fashion. One way out is provided by Monte Carlo algorithms,^{21,23} which efficiently explore the complex search space—although they are not guaranteed to locate the global optimum. The Dali program¹⁴ builds up an optimal alignment using Monte Carlo optimization to combine pairs of matching fragments (matching submatrices of the distance matrices) into larger consistent sets of pairs. In our experience, the method is both sensitive and accurate, although, in its current implementation, not as fast as some pattern matching or 3D clustering algorithms.

3D CLUSTERING ALGORITHMS

Historically, the common structural core is often defined as a self-consistent set of pair equivalences with positional deviations below a user-defined threshold. Finding the largest possible common core that satisfies this condition is a complicated search problem. The problem can be partitioned into subproblems of finding pairs of non-gapped matching fragments, e.g., strands and helices. Consistent sets of fragment pairs can then be assembled using clustering methods.^{11,15} The clustering method in Whatif¹⁴ allows the detection of structural correspondences where the sequential order of equivalent fragments is not preserved (also true for distance matrix methods). In practice, however, several adjustable cutoffs are needed to maintain speed while allowing flexibility in the relative orientations and positions of equivalent fragments.

RECURRENT FOLDS

These efforts at algorithm development are already beginning to yield interesting results. Using the new computer methods for scanning structural databases, a number of nontrivial similarities have been detected some time after the coordinates of the structures were made available (Table I). Nontrivial in this context means similarities that are not easily found by sequence comparison alone. As more and more resemblances and remote evolutionary connections between new and old protein structures are discovered, the growth curve for unique folds rises much less steeply than that for the total number of known structures. The computer methods for structural alignment will be especially useful in creating and developing a classification of the building blocks of protein structure.^{18,25,26}

Two effects limit the total variety of observed protein folds: physical principles and evolutionary history of natural proteins. Sequence database searches are a powerful tool in molecular biology because inferences concerning protein structure and function exploit the biological fact that these can be retained over large evolutionary distances. Structure database searches expand the realm of comparative analysis, and occasionally simplify biochemical clas-

sifications by unifying two or more protein families into one superfamily.²⁷⁻²⁹ We illustrate this point with two examples of bio(techno)logical interest.

FROM STRUCTURAL SIMILARITY TO BIOLOGICAL FUNCTION

The first example sheds some light on the structure of barley endochitinase, an enzyme involved in plant defense reactions against chitin-containing pests (fungi and insects). Several sequences of the barley endochitinase family are known, but site-directed mutagenesis has so far failed to identify the active site. As the result of a database search with the structure of endochitinase, we identified a subtle but unambiguous similarity to lysozymes from animals and phage.³⁰ The structures of three remotely related subclasses of lysozyme have been known for some time. Endochitinase shares with them a structural core of four helices and a small 3-stranded β -sheet. These elements are arranged in topologically identical order in spite of massive peripheral insertions/deletions (Fig. 3). Significantly, the location and composition of the active site and key structural residues, as seen in lysozymes, are also conserved in endochitinase. Recently, the structure determination of a bacterial muramidase has revealed a fifth subclass of lysozymes.³¹ Much of the knowledge about lysozyme can be extended by analogy to these new members and used to guide experiment.

DESIGN OF A VERSATILE BINDING MOTIF

Sometimes biological intuition is ambivalent on the question of evolutionary divergence vs. physical convergence. The second example illustrates this conceptual difficulty in protein taxonomy. Streptavidin, known for its very high affinity for biotin,³² has a similar antiparallel, up-and-down β -barrel topology as a diverse set of tissue-specific carrier proteins that selectively bind a variety of small hydrophobic ligands, called calycons.³³ The two subclasses of calycons (one 8-stranded such as retinol binding protein,³⁴ the other 10-stranded such as P2 myelin protein³⁵) have in common a conserved cluster of hydrophobic residues around an invariant tryptophan that ties together the first and last strands of the β -barrel (Fig. 4). Exactly the same packing pattern is seen in streptavidin, an argument in favor of an evolutionary relationship.³⁶ Two other proteins with similar topologies, catalase and photoactive yellow protein, do not have this packing pattern. The key argument against an evolutionary relationship is based on architectural differences, notably in shear number s (streptavidin $s = 10$; retinol binding protein $s = 12$). In other words, a helical path going around the barrel following the β -sheet hydrogen bonds ends on the starting strand two residues farther in retinol binding protein. In addition, the β -barrel of streptavidin has no central cavity and

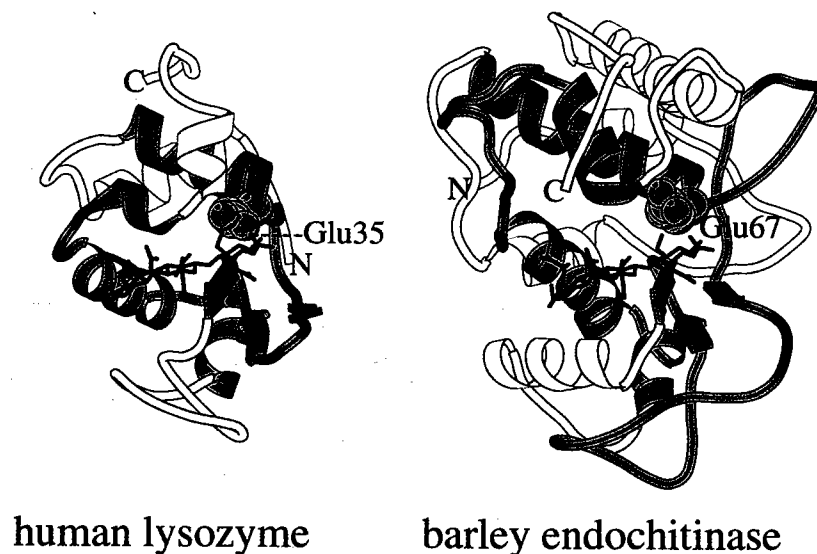


Fig. 3. Active site revealed by structural similarity between plant endochitinase and animal and phage lysozymes. The ribbon diagram⁴¹ highlights structurally equivalent elements of the common core (shaded) between animal and phage lysozymes and barley endochitinase. Regions outside the common structural core are white. The active site of lysozymes is known but that of endochitinase is yet biochemically uncharacterized. A trisaccha-

ride inhibitor of lysozyme (black) was transferred from the cocrystal with human lysozyme (9LYZ) to an equivalent position in the structure of endochitinase. Glu-35 is the principal catalytic residue in human lysozyme (1LHM⁴⁴). By analogy, the structurally equivalent Glu-67 is proposed to be the catalytic residue of barley endochitinase (1BAA⁴⁵).

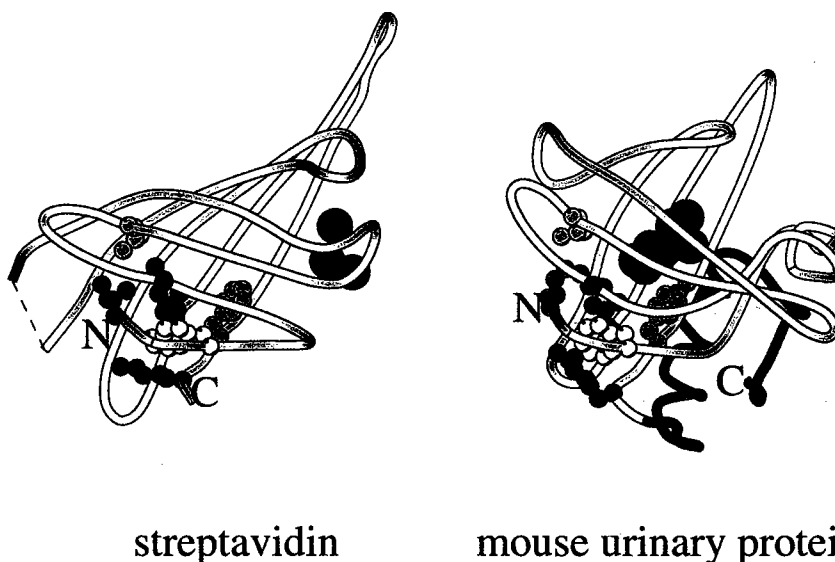


Fig. 4. Is streptavidin distantly related to calycons? Comparison of streptavidin (1PTS⁴⁶) and a member of the calycin superfamily (major urinary protein, 1MUP⁴⁷). The common core¹⁸ consists of residues A13-A25, A27-A36, A38-A44, A51-A58, A76-A79, A88-A91, A93-A98, A101-A111, A121-A133 in 1PTS and 15-27, 45-61, 68-75, 86-93, 96-112, 115-127 in 1MUP, with a

positional root mean square deviation of C α atoms of 2.9 Å over 76 residues. Side chains are shown for a conserved cluster of hydrophobic residues (bold in alignment below, β -strands underlined). The ligand (black) is sequestered inside the barrel in lipocalins and bound by loops in streptavidin. Drawn using MolScript.⁴¹

18 KINGEWH	49 IHVLE-NSL	103 NFLMA	120 LMGLYGRE	major urinary protein
22 NYHGKWE	48 YTPGE-KSV	112 NYIIG	131 FVWVLSRS	bilin binding protein
1 AFDGTWKV	40 ITOEG-NKF	111 NELIO	124 AKRIFKKE	fatty-acid binding protein
19 RFSGTWYA	45 FSVDETGOM	113 TYAVO	133 YSFVFSRD	retinol binding protein
16 GITGTWYN	31 VTAGADGAL	102 ARINT	126 GHDTFTKV	streptavidin

binds the ligand by loops, whereas the barrel of retinol binding protein is wider and more ellipsoidal and sequesters the ligand deep inside the barrel. Classification issues aside, the packing motif of calycin-streptavidin indisputably has been an evolutionary success.

MORE TO COME

The importance of structure comparison will continually increase with the rapid growth of the pool of known structures. At some time in the future, an important part of the protein folding problem will effectively evaporate as structure and sequence comparison tools classify any new protein into an existing family.

We propose that any newly solved protein structure be routinely compared with those in the Protein Data Bank, for the detection of possible topological resemblances. The software required to perform rapid and rigorous searches of structural databases is now sufficiently mature^{14,16-18} and generally accessible to the scientific community.* The experience of the past year (Table I) shows that the chances of finding interesting similarities in the database are greater than those of a fold being unique. The recognition of a distant relationship to a well-characterized protein may reveal possible functional analogies that in turn may lead to considerable time savings in the biochemists' laboratory. The unexpected similarities between the cold-shock protein and several oligonucleotide-binding proteins³⁷ and between the POU-specific domain and λ repressor,³⁸ both identified by automated database searches upon completion of the structure determinations, are motivating examples. Searching protein structure databases has come of age.

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*In particular, the authors offer to assist those with a new structure in attempts at discovering similarities. Send C^α coordinates by e-mail to holm@embl-heidelberg.de. A list of structurally similar proteins, aligned in 3D using Dali, will be returned.

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