Functional Neighbors: Inferring Relationships between Nonhomologous Protein Families Using Family-Specific Packing Motifs

Deepak Bandyopadhyay, Jun Huan, Jinze Liu, Jan Prins, Jack Snoeyink, Wei Wang, and Alexander Tropsha

***Abstract*—We describe a new approach for inferring the functional relationships between nonhomologous protein families by looking at statistical enrichment of alternative function predictions in classification hierarchies such as Gene Ontology (GO) and Structural Classification of Proteins (SCOP). Protein structures are represented by robust graph representations, and the fast frequent subgraph mining algorithm is applied to protein families to generate sets of family-specific packing motifs, i.e., amino acid residue-packing patterns shared by most family members but infrequent in other proteins. The function of a protein is inferred by identifying in it motifs characteristic of a known family. We employ these family-specific motifs to elucidate functional relationships between families in the GO and SCOP hierarchies. Specifically, we postulate that two families are functionally related if one family is statistically enriched by motifs characteristic of another family, i.e., if the number of proteins in a family containing a motif from another family is greater than expected by chance. This function-inference method can help annotate proteins of unknown function, establish functional neighbors of existing families, and help specify alternate functions for known proteins.**

***Index Terms*—Delaunay tessellation, enrichment evaluation, frequent subgraph mining, functional neighbors, Gene Ontology (GO), protein structure, remote homology, Structural Classification of Proteins (SCOP).**

I. Introduction

STRUCTURAL genomics projects generate many new protein structures, including hypothetical proteins from fully sequenced genomes with unknown function. These developments underlie a need for powerful and reliable function-inference methods. Earlier [1], [2], we described a method for inferring protein function using *family-specific packing motifs*, i.e., residue clusters in folded proteins. The motifs are identified automatically by mining protein families, with each member structure represented as a labeled graph, for frequent common subgraphs. In contrast to traditional function-inference methods that rely on comparison of the entire sequence or fold, our approach relies on these local packing motifs as possible determinants of protein function.

In this paper, we describe how family-specific motifs can be used to discover hidden connections between protein families with no apparent fold and sequence similarity, i.e., remote homologs. We introduce a new measure of functional similarity between families based on statistically significant enrichment of one family with motifs characteristic of another family. We present several case studies demonstrating that our approach correctly predicts functional similarity between remotely homologous families.

A. Related Work

Protein function annotation using local structural features is known to be more accurate [3] than using only sequence alignments/patterns (e.g., SMART [4]) or global fold similarity (e.g., DALI [5]). The following methods have been proposed to find local structural motifs and known functional sites in protein structures or families.

1) *Depth-first search* starts at simple geometric patterns (triangles), progressively finding larger patterns [6]–[8].
2) *Geometric hashing* can compare two protein structures [9] or a structure to a database [10].
3) *Functional site template* methods represent functional sites as pockets [11], clefts [12], or patches [13], and match them with new protein structures using geometry, conserved residues and electrostatic/chemical properties.
4) *String pattern matching* uses string search algorithms on encoded local structure/sequence [6], [14].
5) *Graph matching* methods have been developed to compare protein structures modeled as graphs, usually with clique detection techniques [15]–[19].
6) *Other methods* like inductive programming language [20], fuzzy functional forms [21], computed protonation properties [22] and geometric depth potentials [23].
7) *Hybrid methods*, e.g., clique hashing [24].

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The problem of frequent subgraph mining is to identify all frequent subgraphs for a set of graphs G, where a subgraph must occur in more than some fraction of G called the support (σ) to be considered frequent. Our recent work [25] explored Delaunay tessellation as a means to generate a sparse graph representation of protein structure. Later, we introduced and employed *almost-Delaunay* edges to account for imprecision in atomic coordinates by using a parameter ϵ [26]. Our mining method [27] builds frequent subgraphs directly using a tree representation, and thus is faster and applicable to larger structures and databases than exhaustive subgraph enumeration by depth-first search [28].

There have been recent efforts toward annotation of protein structures (and homology models built from sequences) using functional signatures derived from structural alignments [29], overlapping sphere representations of functional sites [19], and clusters of functionally important residues determined by predicted protonation properties [22] or a geometric depth potential [23], [30], to name just a few. Our method, unlike the first [29], does not depend on sequence/structure alignment, thus finding motifs not conserved in the sequence. It differs from the second [19] in that functionally important residues in graph patterns are inferred from protein families rather than chosen manually from literature or bound ligand positions. It distinguishes itself from the other methods mentioned [22], [23] by insisting that the motifs found and used for annotation be unique to each family. Remote similarities found using family-specific motifs are thus more significant than binding sites matched by other methods, since the same site can be involved in multiple functions [31].

Lastly, we study the problem of automatically finding functional relationships between families unrelated by sequence or structure, i.e., finding remote function similarity or functional neighbors. This has been reported for pairs of known families [32], for pairs of individual proteins [33], for a predefined set of structural patterns and a protein [19], and recently, for a computed set of functional sites and the Protein Data Bank [30]. Our method is critically different and more robust in that it compares and relates protein families, rather than pairs of proteins or a protein database and a functional site database, using local structure patterns specific to those families.

II. Materials and Methods

Our method initially finds and calibrates motifs using the fast frequent subgraph mining (FFSM) program (http://www.cs.unc.edu/∼huan/FFSM/). We briefly describe below five steps of the procedure discussed in detail in our previous papers [1], [25], followed by an in-depth discussion of the additional step of enrichment evaluation which is the major new development reported in this paper.

1) Select families of nonredundant proteins from a classification database such as Structural Classification of Proteins (SCOP), Enzyme Commission (EC), or as defined by the user. Also, define *background* dataset to represent all protein structures. We chose 29 EC families and 125 families from SCOP [34] version 1.65, which was current at the time we initiated these studies. Our background dataset used PISCES [35] with sequence identity $\leq$90%, resolution $\leq$3 Å , and R-factor $\leq$1.0, which led to 6625 valid chains.
2) Represent protein structures as graphs, with nodes at each residue, and contact between residues defined using the *almost-Delaunay* [26] edges. It is possible to merge two or more node types to create a reduced set of node labels. We add length-dependent edge labels and distance constraint edges between noncontacting residues to ensure consistent geometry in patterns [1].
3) Mine family-specific motifs using the FFSM method [25]. Subgraphs are defined as family-specific motifs if they occur in at least 80% of the family (support), and in at most 5% of the background (background occurrence). If the background check step is omitted, the patterns are merely called frequent subgraphs or spatial motifs.
4) Search for motifs in a new structure, using a graph similarity index to speed up subgraph isomorphism.
5) Assign a significance to the function inference, by counting family motifs found in step 4 and examining their distribution in background proteins.
6) Calculate statistical enrichment of motifs in nodes of the SCOP and GO hierarchies, using the hypergeometric distribution with a p-value cutoff of 10^{-6}.

A. Enrichment Evaluation in SCOP and GO

SCOP enrichment evaluation aims to determine if the set of background proteins containing a large fraction of motifs from a SCOP/EC family is enriched with proteins from another SCOP family, i.e., if there are more proteins in the set from that other family than would be expected by chance. While checking in the background for the occurrence of some family-specific motifs, proteins containing each motif are extracted into a list, and these lists are used to evaluate enrichment in the SCOP hierarchy. A geometric distribution is used to model the probability that from n proteins sharing the same motif by chance, at least k proteins will belong to a category (i.e., SCOP family) containing f proteins, from a total protein data bank size of g. The p-value is given by

$$P = 1 - \sum_{i=0}^{k} \frac{\binom{f}{i}\binom{g-f}{n-i}}{\binom{g}{n}}. \tag{1}$$

The hypergeometric distribution [36] is also commonly used: given a collection of representative proteins M, a subset of proteins $T_{\mathrm{motif}} \subseteq M$ sharing one common spatial motif, and a subset of proteins $T_{\mathrm{class}} \subseteq M$ of a predefined category, the probability of observing a subset of proteins $K \subseteq T_{\mathrm{motif}} \wedge T_{\mathrm{class}}$ with at least size k is given by

$$p\text{-value} = 1 - \sum_{i=0}^{k-1} \frac{\binom{|T_{\mathrm{motif}}|}{i}\binom{|M|-|T_{\mathrm{class}}|}{|T_{\mathrm{motif}}|-i}}{\binom{M}{T_{\mathrm{motif}}}}. \tag{2}$$

For example, it is unlikely that most of the group of proteins sharing a motif come from a single SCOP superfamily;

therefore, such a category would be statistically significant, with p-value close to zero.

We adopt the Bonferroni correction for multiple independent hypotheses [37], $0.001/|C|$, where C is the set of categories, as the default threshold for significance of individual test p-values. With $|C| \approx 1300$ SCOP superfamilies, we chose the p-value threshold for significant function similarity from 10^{-6} to 10^{-8}.

Enrichment evaluation helps verify that motifs from a SCOP family do not occur in too many other families, find related families and superfamilies on the basis of shared motifs, and correlate EC classes to SCOP structural families that share their motifs. This analysis enables the characterization of false positive motif matches—proteins not in the same functional family but inferred with high confidence—to determine if they are random or perform a related or unrelated function. Families that are highly enriched with another family's motifs but are not near that family in the SCOP hierarchy are denoted *functional neighbors*. This novel functional similarity relationship is defined by conservation of 3-D residue-packing patterns, or motifs, between families. We hypothesize that such families may have related functions, or they share some aspects of function.

The Gene Ontology (GO, [38]) provides a controlled vocabulary for describing protein function. GO terms form directed acyclic graphs (DAGs) connected by relationships such as "is-a" and "part-of." Terms at lower depth in the DAGs describe more general functions; the greater the depth, the more specific is the function. Enrichment evaluation on the GO hierarchy is similar to that described for SCOP; it aims to determine whether the set of proteins sharing the motifs of a SCOP/EC family is enriched with proteins from a particular functional category (as defined by GO) to a greater extent than would be expected by chance. Combining GO and SCOP enrichment evaluations, we can test the hypothesis that two SCOP families marked as functional neighbors have related functions.

III. Results: Enrichment and Functional Neighbors

To characterize the hits for EC family-specific motifs in SCOP, and to evaluate the functional roles of proteins returned as positives by our annotation method based on local structure patterns, we study the enrichment of all motif hits in the background within the SCOP and GO hierarchies. As an example, we have looked at the distribution of GO functions for proteins containing motifs for the serine protease family in SCOP. We extracted all background proteins containing each of the 72 serine protease motifs, and evaluated these lists for GO enrichment. The number of background hits per motif ranged from 60 to 97. The GO categories related to peptidase activity (see Fig. 1), which is functionally most similar to protease activity, were consistently enriched in the protein lists for all 72 motifs, with p-value $<10^{-15}$.

The above result suggests that proteins sharing the same motif may be expected to have similar functions. It follows that any positives, i.e., the background proteins that contain multiple motifs, belong to related families that share functional similarity with serine proteases. This leads to the definition of a *functional neighbor* relation between families based on the observation that they share motifs, with the strength (or probability) of functional similarity possibly proportional to the number of motifs shared.

```
GO:0008233 : peptidase activity (k:55/f:377)
        GO:0004175 : endopeptidase activity (k:55/f:297)
                GO:0004252 : serine-type endopeptidase activity (k:55/f:130)
                        GO:0004263 : chymotrypsin activity (k:52/f:73)
                        GO:0004295 : trypsin activity (k:55/f:85)
        GO:0008236 : serine-type peptidase activity (k:55/f142)
                GO:0004252 : serine-type endopeptidase activity (k:55/f:130)
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Fig. 1. Significantly enriched GO categories for the 62 background hits for one motif of serine proteases. In each GO category, k is the number from the 62 hits and f is the number of background proteins.

Note that the functional neighbor relation as defined is not symmetric, i.e., if family A's motifs are enriched in family B, it does not imply that B's motifs are enriched in A. Most families share some motifs with their subfamilies, superfamilies, or siblings in SCOP, and these are structural as well as functional neighbors. Some other families share motifs with families in a different branch of the SCOP hierarchy, and thus, not obviously related to them; such families are functional neighbors but not structural neighbors, at least in SCOP.

Table I shows some SCOP families from our current dataset that share motifs and are functional neighbors, but are not structural neighbors. We calculate these family pairs (motifs of family F, enriched in families E_i) by finding all proteins in the background dataset having enough motifs of F that their function can be inferred with 99% specificity, and using them as input to the SCOP enrichment method. We report that families E_i:

1) have p-values $<10^{-7}$ for the enrichment;
2) are superfamily or family level nodes of SCOP;
3) do not share a parent/child or sibling relationship with F;
4) for $>20\%$ of their members the function F is inferred with 99% specificity.

The choice of 10^{-7} for p-value cutoff highlights strong relationships, though some known functional neighbors with larger p-values are hidden. Also, the restriction to use SCOP families rather than EC families hides the functional neighbor relationship between alcohol dehydrogenases (EC family in our dataset) and flavin/nicotinamide adenine dinucleotide (FAD/NAD) reductases (SCOP). This relationship can be inferred from the many families that have both alcohol dehydrogenases and FAD/NAD reductases or FAD/NAD(P) binding domains as functional neighbors.

In Table I, we removed seven families[1] whose functional neighbors were two molybdenum-related protein families: CO-dehydrogenase molybprotein like (SCOP: 54666) and molybdenum-cofactor binding domain (SCOP: 56004). These two families show local structure similarity to many diverse families, which seems an artifact of either the motifs or the enrichment evaluation; we ignore them for now.

The remaining families in Table I show many plausible functional neighbor associations, based on comparing the family names and not assuming biological knowledge.

[1]ARM repeat, β-carbonic anhydrase, carbohydrate phosphatase, CutA divalent ion tolerance, enolase superfamily, nucleotidyltransferase, and WD40 repeat.

TABLE I
EXAMPLES OF FUNCTIONAL NEIGHBOR FAMILIES WITHOUT OVERALL STRUCTURAL SIMILARITY, FOUND BY ENRICHMENT EVALUATION IN SCOP

Motifs of family (F)	**Enriched in family(-ies) (E_i)**	**p-value exponent**
ABC transporter ATPase domain (52686)	Elongation factors (50448)	-10
6-phosphogluconate dehydrogenase (48179)	Succinate dehydrogenase/fumarate reductase flavoprotein (46978, 51934, 56426)	-11
	Alcohol dehydrogenase-like (50136, 51736)	-11
	FAD/NAD linked reductase (51943, 55425)	-15
Adenine nucleotide α-hydrolase (52402)	Alcohol dehydrogenase-like (50136, 51736)	-7
	FAD/NAD(P) binding domain (51905)	-7
Adenylyltransferase (52397)	Formate-dehydrogenase/DMSO-reductase (53707)	-8
Amino acid de-hydrogenase-like (51883)	Succinate dehydrogenase/fumarate reductase flavoprotein (46978, 51934, 56426)	-10
	Subtilase (52744)	-7
	FAD/NAD linked reductase (51943, 55425)	-13
Alkaline phosphatase (53649)	FAD/NAD linked reductase (51943, 55425)	-7
Bacterial lipase (53570)	Cu,Zn superoxide dismutase-like (49330)	-8
	Lipase/lipooxygenase (PLAT/LH2) domain (49723)	-7
β-Glycanase (51487)	Starch-binding domain (49453)	-7
	E-set domains of sugar-utilizing enzymes (81282)	-8
Carbon-nitrogen hydrolase (56317)	Subtilase (52744)	-10
Carboxylesterase (53487)	Phosphoglycerate kinase (53749)	-7
	FAD/NAD(P) binding domain (51905)	-8
	Colipase-binding domain (49730)	-8
CheY-related (SCOP1.67)(52173) CheY-like (sf, SCOP1.67)(52172)	Haloacid dehalogenase (HAD) like (56784)	-12
	NAD(P)-binding Rossmann fold domain (51735)	-10
Cupredoxin, multidomain (49550)	Cu,Zn superoxide dismutase-like (49330) ($\rightleftarrows$)	-13
	Fe,Mn superoxide dismutase-like (46610, 54720)	-15
	RuBisCo (51650)	-9
	Matrix metalloprotease (55528)	-13
DHS-like NAD/FAD binding domain (52467)	Lactate and malate dehydrogenases (56328)	-10
	Subtilase (52744)	-8
	FAD/NAD(P) binding domain (51905)	-11
dsRNA-binding domain (54768)	Thiolase-related (53902)	-12
EGF/Laminin (57196)	Vertebrate phospholipase A2 (48623)	-7
	Periplasmic binding-like II (53850)	-8
	Snake venom toxin (57303)	-15
	Cystine-knot cytokine (57501)	-11
Endonuclease, His-Me finger (54060)	Formate-dehydrogenase/DMSO-reductase (53707)	-11
	Galactose mutarotase-like (74650)	-8
ETFP subunit (52432)	Alcohol-dehydrogenase-like (50136, 51736)	-8
	FAD/NAD linked reductase (51943, 55425)	-14
Extended AAA-ATPase (81269)	Elongation factors (50448)	-8
FMN-linked oxidoreductase (51396)	Alcohol dehydrogenase-like (50136, 51736)	-9
	Succinate dehydrogenase/fumarate reductase flavoprotein (46978, 51934, 56426)	-8
	FAD/NAD-linked reductase (51943, 55425)	-7
Fungal lipase (53558)	Lipase/lipooxygenase (PLAT/LH2) domain (49723)	-7
Gln-amidotransferase cls I (52318)	Thiamin diphosphate binding (52518)	-10
Glycosyl hydrolase family 1 (51521)	Starch-binding domain (49453)	-7
	E-set domains of sugar-utilizing enzymes (81282)	-8
HAD-like (SCOP1.67)(56784)	DnaQ-like 3'-5' exonuclease (53118)	-7
Inosine Monophosphate dehydrogenase (51413)	Alcohol dehydrogenase-like (50136, 51736)	-11
	FMN-linked oxidoreductase (51396)	-9
	Aldolase, Class I (51570)	-7
Integrin A(I)(53301)	Amino-acid dehydrogenase like (51883)	-8
Metallo-dependent phosphatase (56300)	Cu,Zn superoxide dismutase-like (49330)	-10
Metallohydrolase/ oxidoreductase (56281)	Pectin lyase-like (51126)	-7
	Metallodependent hydrolase (51556)	-7
Metalloprotease "zincin" (55486)	Cu,Zn superoxide dismutase-like (49330)	-7
	Peptide deformylase (56421)	-11
N-type ATP pyrophosphatase (52403)	ALDH-like (53721)	-7
NADH Oxidase/flavin reductase (55468)	Formate-dehydrogenase/DMSO-reductase (53707)	-7
p53-like transcription factor (49417)	Galactose mutarotase-like (74650)	-7
PDZ domain (50157)	Alcohol dehydrogenase-like (50136, 51736)	-8
	Glyceraldehyde-3-phosphate dehydrogenase (51800, 55347)	-8
Phospholipase C/P1 (48537)	Galactose mutarotase-like (74650)	-7
Pyruvate ox/decase (52475)	Thiamin diphosphate binding (52518)	-7
Ribonuclease H (53099)	Thiamin diphosphate binding (52518)	-8
	Subtilase (52744)	-7
Ribulose phosphate binding barrel (51366)	α-Amylase (51012, 51446)	-10
	Aldolase, Class I (51570)	-11
	Cystathionine synthase like (53402)	-8
RuvA C-terminal domain (46928)	DNA polymerase I (56673)	-7
SGNH hydrolase (52266)	FMN-linked oxidoreductase (51396)	-12
	FAD/NAD(P) binding domain (51905)	-17
SIS domain (53697)	FAD/NAD(P) binding domain (51905)	-9
	Thiamin diphosphate binding (52518)	-8
	Subtilisin-like (52743)	-7
Succinyl-CoA synthetase (52210)	Succinate dehydrogenase/fumarate reductase flavoprotein (46978, 51934, 56426)	-8
	FAD/NAD-linked reductase (51943, 55425)	-7
Trp biosynthesis (51381)	Aldolase (sf) (51569)	-7

1) Many (oxido)reductase and dehydrogenase families are functional neighbors of each other.
2) Multidomain cupredoxins and Cu,Zn superoxide dismutases are mutually functional neighbors, and it would seem that a copper-binding site or some elements of function are shared. The bidirectional similarity with high p-values reinforces the functional neighbor relationship.
3) Lipase/lipooxygenase is a functional neighbor of both bacterial and fungal lipases but has a different fold.
4) Starch-binding domains and E-set sugar-binding domains bind carbohydrates, similar to β-glycanases and glycosyl hydrolase family 1 that have them as functional neighbors, but with different folds.
5) The CheY-related family has the Haloacid Dehalogenase (HAD)-like family as functional neighbors; these two families are known to share similarity in the Mg^{2+}-ion binding site [32].
6) Succinyl-CoA synthetase of flavodoxin fold is a neighbor of a family of flavoproteins that oxidize succinate.
7) Metallohydrolase/oxidoreductase of $\alpha+\beta$-fold is a neighbor of metallodependent hydrolase of triosephosphate isomerase (TIM) barrel (α/β)-fold.

Undoubtedly, there are many more valid functional neighbor associations in Table I that may be confirmed and elucidated by further computational and biological analysis of common local structures.

A. Case Study: NADPH Binding Proteins

Nicotinamide adenine dinucleotide phosphate (NADPH) is a large ligand found in many enzymes. In SCOP [34], there are two superfamilies of NADPH-binding proteins: FAD/NAD(P)-binding domains (SCOPID: 51905) and NAD(P)-binding Rossmann-fold domains (SCOPID: 51735), which share no sequence or fold similarity.

In order to test our method further, we have applied it to the SCOP superfamily FAD/NAD(P)-binding domain (SCOPID: 51905) to: 1) obtain recurring spatial motifs (frequent subgraphs/cliques); 2) search for the occurrences of each identified motif in all representative protein structures; and 3) report those SCOP (super)families in which a particular motif is significantly enriched.

1) Remote Superfamilies Identified: The superfamilies enriched in spatial motifs are listed in Table II. As expected, we detect the other SCOP superfamily: NAD(P)-binding Rossmann-fold domains, which has no sequence or fold similarity yet shares several NADPH-binding motifs with the original SCOP family. In Fig. 2, we show all significant spatial motifs shared between the two NADP binding families in a protein from the FAD/NAD binding domain superfamily. Most residues covered by the motifs are located near the NAD ligand.

In Fig. 3, we show a motif that is statistically enriched in both families; it has conserved geometry and is adjacent to NADPH in two proteins that belong to the two families.

We emphasize that we did not include any information from NADPH during our search process, yet were still able to identify the motifs since they represent local residue patterns conserved

TABLE II
ELEVEN MOTIFS OBTAINED FROM THE SCOP SUPERFAMILY: FAD/NAD(P)-BINDING DOMAIN PROTEINS

Motif	S	F	p	f
1	5	51905	10^{-10}	0.37
ILVVV		51735	10^{-10}	0.19
2	4	51905	10^{-15}	0.72
GVVV		51735	10^{-12}	0.33
3	4	51905	10^{-14}	0.70
GGGG		50494	10^{-15}	0.58
4	4	51905	10^{-14}	0.70
GGGS		51735	10^{-13}	0.36
		50494	10^{-15}	0.69
		53383	10^{-13}	0.60
5	4	51905	10^{-14}	0.72
GGGT		51735	10^{-15}	0.49
		50494	10^{-14}	0.57
6	4	51905	10^{-11}	0.40
CGGG		50494	10^{-14}	0.56
7	4	51905	10^{-14}	0.60
GGII		51735	10^{-13}	0.33
8	4	51905	10^{-10}	0.46
CGGL		50494	10^{-14}	0.60
9	4	51905	10^{-15}	0.65
GGGL		51735	10^{-13}	0.35
10	4	51905	10^{-15}	0.60
GGGI		51735	10^{-12}	0.30
11	5	51905	10^{-12}	0.40
AGIIV		56235	10^{-10}	0.33

We used σ value of 15/43. No new significantly enriched (super) families were discovered using lower support thresholds such as10. S: the number of residues included in a motif, F: SCOP (super) family ID: 51905: FAD/NAD(P)-binding domain, 51735: NAD(P)-binding Rossmann-fold domains, 50494: Trypsin-like serine proteases, 53383: Pyridoxal phosphate (PLP) PLP-dependent transferases, 56235: N-terminal nucleophile aminohydrolases (Ntn hydrolases). p, the p-value of the motif's occurrences in the related SCOP family. f: the support of the motif in the related family.

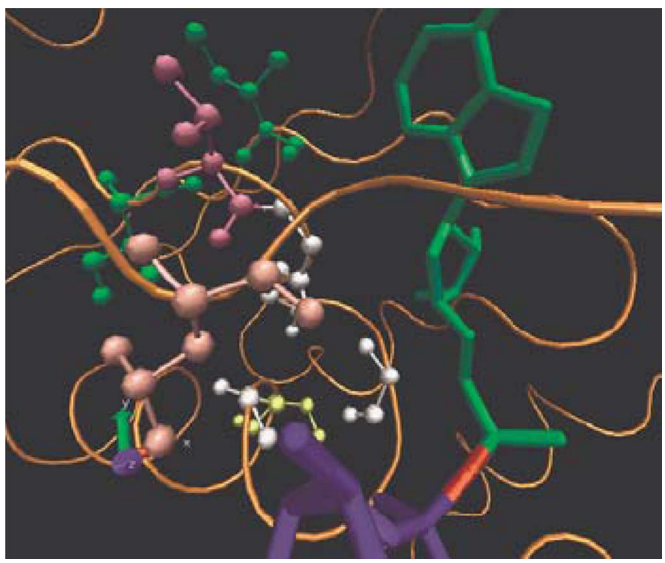

Fig. 2. Examples of all motifs which are significantly enriched in SCOP superfamily FAD/NAD(P)-binding domain in protein 1kew (chain a). Only residues are shown in this figure; their interactions are omitted for clarity.

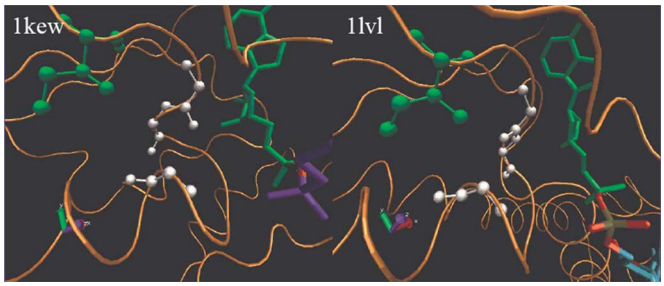

Fig. 3. Example of an NADPH binding motif that is significantly enriched in two SCOP superfamilies. The four involved residues are ILE5, GLY7, GLY8, GLY 13 (PDB: 1kew A), and ILE10, GLY12, GLY13, GLY17 (PDB: 1lvl). The DALI z-score of the two protein structures is 4.5, pairwise sequence identity is 16%, and sequences are dissimilar in the region of the motif.

among proteins in both SCOP superfamilies. This remarkable local commonality and clear interaction between motifs and the ligand show that our method helps reveal hidden biologically significant patterns.

IV. Conclusion

Our approach affords automated identification of protein family-specific packing motifs that are used to annotate protein structures or even families enriched by such motifs. Consequently, this method helps establish functional similarity and functional neighbor relationships defined by sharing of conserved 3-D residue-packing patterns, or motifs, between protein families, even if they are unrelated in sequence or structure. These relationships are deduced using statistical enrichment evaluation of family-specific motifs within hierarchical structure classifications such as SCOP and GO.

This method presents an unconventional means of identifying similarity between nonhomologous (sequence/structure) protein families. The meaning of this similarity (defined by shared packing motifs) in each particular observational case is yet to be established; possible implications include functional similarity (as indicated by the NADPH example), or evolutionary relationships, or conservation of thermodynamically stable motifs. Future studies will examine specific cases of shared motifs between families as well as look into possible clusters (or networks) formed by similar protein families, where similarity is defined by shared motifs.

The enrichment characterized by the p-value (see Eq. 1) represents a novel function similarity measure for protein families that could help annotate families of proteins with unknown or not well- understood function. We believe that studies described in this paper represent a promising new direction in the area of local similarity-based protein function inference.

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