Mining Conserved Local Structure from Functional Hierarchical Classification via Local Structure Comparison

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ABSTRACT

Local region conservation has been observed in recent years and become more and more important in structure biology. Recent researches point out that local conservation regions are correlated to protein functional sites and functions and studies show that some local conservation on sequence or structure are close to binding area. Hence, in order to realize how function works, we can discover local structure region to understand protein function via observation in local conservation. Furthermore, many researches show that function would be activate on the surface of protein structure, but not whole structure and local region conservation can be discovered from sequence, structure or both in current status.

Sequence conservation has been discovered in recent researches. There are existing examples which show that structure conservation can be mapped from sequence conservation; however, it is still a problem to mining structure conservation via structure comparison. Structure conservation has become a hot topic to be discussed. Protein function needs to take place in local region to activate the biochemical reaction. Therefore, our motivation is to apply protein structure comparison algorithm to mining local structure conservation. Because these local structure conservations would be used to support structure or provide function, we use functional site to connect the relationship between local structure conservation and protein function. Given functional hierarchical classification, we can easily identify protein function and using proteins with the same EC number to mining or discover conservation which may be related to function. Furthermore, we try to extract local structure region associated to its protein functional site.

1: INTRODUCTIONS

With the growth of Protein Data Bank (PDB) [1], protein functional analysis has become more important. Moreover, protein structure comparison among mass protein structure data is widely applied on protein structure analysis. Hence, similarity between proteins can be measured by structure comparison and RMSD (Root Mean Square Deviation) is evaluation function for the quality of structural alignment. Global/local structure comparison can be used to distinguish global/local structure.

Global similarity can help us to identify global structure conformation. Local structure similarity [12] can tell us similar local structure which may highly relate to protein function. According to researches and observation from biologists, protein function is highly correlated to its three-dimensional (3D) structure and researches are especially focused on special structure fragments which may connect to protein function or overall framework support [2, 3, 4]. Some of them are used to support the core of protein structure; some are used to contact with small molecule or protein to provide function or speedup chemical reaction.

As protein function is activated in special protein structure in 3D space and also local structure particularly, local structure comparison plays an important role in detecting local structure similarity. Proteins with the same function should share similar local structure and provide binding area to contact with small molecule in order to activate their functions and these local structures are functional areas. Therefore, we try to detect or discover similar local structure via local structure comparison and find the relationship between local structure and functional areas. Beyond that, we will discuss the discovery of local structure conservation and relationships between local structures and functional areas.

2: LOCAL REGION CONSERVATION

In protein sequence analysis, sequence conservations can be discovered by evolutionary method. As found by Campbell and Jackson [5, 6], Src homology 2 (SH2) family can be divided into two groups on the basis of similarity of binding site residues. From this research, it showed that proteins with the same family share similar local sequences and local structures [9, 10] closed to its bind area. The result also showed that sequence conservation would fall on whole sequence diversely but compact in 3D space. In this case, they observed that there are existing conservation on local sequence which can be mapping to local structure and has relationship between local structure and binding area.

In addition, the functions of proteins are mainly affected by their structures, especially in local structure. The functions often occur in cavity, packets or voids of proteins. Therefore, the study of protein local structures is helpful in understanding the protein function. Besides, enzyme classification provides a good environment to realize protein structures and functions. Each EC number symbolizes the proteins have same function or activate the same reaction would be grouped together. Enzyme active sites commonly occur in large and deep clefts on the protein surface, and they need significant favorable interactions between ligand and protein, which usually means that other small molecule ligand are also in surface depressions. It is also a trend to discovery relationship between functions and structures, especially local structures. Protein structure comparison algorithm is one of analysis tools on discovering structure conservations in protein structure research.

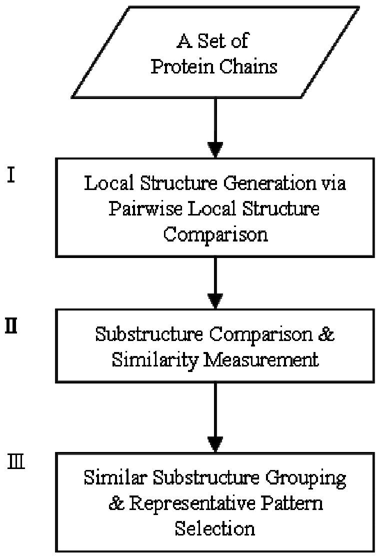


Figure 1. The flow chart for mining conserved structural patterns.

3: MINING LOCAL STRUCTURE PATTERNS

In order to mining local structure patterns related to protein function or closed to protein binding area. In previous researches, proteins with the same function share similar local structure. Hence, to mining local structure region that have biochemical meaning will be very useful for identifying protein function. In this section, we will introduce the method of mining local structure patterns. Given a set of protein chains, our goal is to extract local structure patterns shared among those protein chains which have the same function. As shown in Figure 1, the overall framework contains three major parts: (I) local structure generation via pair-wise local structure comparison, (II) substructure comparison and similarity measurement, (III) similar substructure grouping and representative pattern selection, and will be illustrated in detail in the following sections.

3.1: LOCAL STRUCTURE GENERATION via PAIRWISE LOCAL STRUCTURE COMPARISON

In the first step, we approach a pair-wise protein structure comparison instead of multiple structure comparison. The reason is that multiple structure alignment will only report common part of substructure shared with proteins, but we want to have substructures shared with a subset of whole proteins. Our purpose is to detect all possible conserved substructures among a group of protein structures. In addition, we also want to detect substructures related to function or structure support via local structure detection. Therefore, we apply EMPSC algorithm [14] of rough alignment to detect similar local structure between two protein structures. EMPSC is one of the global structure comparison algorithm based on protein secondary structure elements (SSE) information. The kernel of EMPSC is to decompose protein structure into ellipsoidal representation of secondary structure of α-helix and β-sheet recognized by DSSP program and remaining segments, coils. Because of restricted parameter setting, EMPCS can use restricted RMSD value to become a local structure comparison algorithm. The EMPSC can perform both global and local protein structure comparison via parameter adjustment. If the parameter is restricted below the threshold, EMPSC will perform as local structure comparison.

In order to keep sequence information, we make continuous amino acids of aligned points into subsequences. For the spherical conformation, we cluster subsequences with distance of 5Å between subsequences, and we call them substructure. Hence, we will extract all possible substructures via pair-wisely local structure comparison on protein chains with the same EC number. Furthermore, we can compare all pair-wise local structure comparison within a set of protein chains.

$$score = \frac{\min(S_1, S_2)}{\max(S_1, S_2)} \quad (1)$$

, where S_1 and S_2 are the size of substructures.

$$GH - Score = RMSD \times matched\ resides\ number \quad (2)$$

3.2: SUBSTRUCTURE COMPARISON AND SIMILARITY MEASUREMENT

In the second step, geometric hashing is used to compare two substructures extracted from in first step and calculate similarity score for a pair of substructures. The similarity score is used to define how similar between two substructures in their 3D structures. If the ratio of the size of these two substructures, score, as shown in Eq 1 is larger than 80%, the similarity score will be calculated; otherwise the similarity score will be zero. The reason is that comparison on substructures with the wide gap between sizes of two structures won't make sense. Therefore, the gap of the size of two substructures is oversize; we will discard the comparison to speedup pair-wise comparison time. If the comparison passes the filter criterion, the similarity score between two substructures will be calculated as defined in Eq 2 called GH-Score.

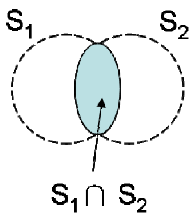
$$matchedrelation = \frac{S_1 \cap S_2}{S_1 \cup S_2} \quad (3)$$

, where S_1 and S_2 are the size of substructures.

(a) $S_1 \cap S_2$ is the number of matched residues.

(b) $S_1 \cup S_2$ is the number of total residues of two substructures.

The below diagram will show this relationship.



(a)

(b)

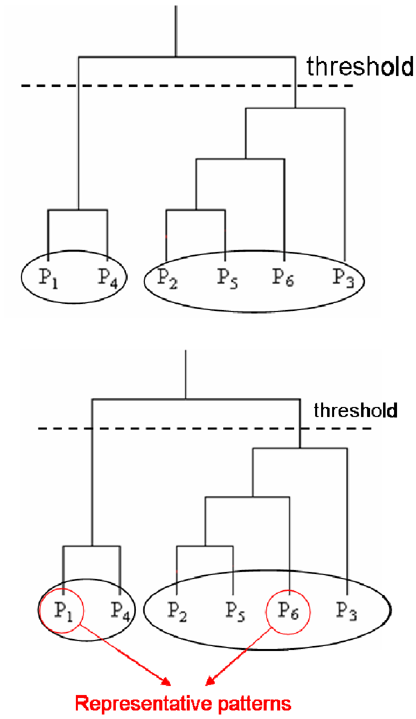


Figure 2. The procedure of clustering and representative patterns selection. (a) Applying complete link clustering algorithm to obtain cluster tree, and using threshold cut-off to obtain clusters. (b) For each cluster, we select a substructure as the representative and the one that covers most of common structure within a cluster. In this example, P1 and P6 are representative patterns for a cluster of P1 and P4 and a cluster of P2, P5, P6, P3 respectively.

3.3: SIMILAR SUBSTRUCTURE GROUPING AND REPRESENTATIVE PATTERN SELECTION

In the third step, we apply hierarchical clustering algorithm [8] to cluster similar substructures according to pair-wise scores calculated in the second step. Before similar substructure grouping, we filter out pairs via machedrelation in Eq 3. In Eq 3, we try to reserve pairs that are much similar between two substructures to take into account. We use complete linkage algorithm to cluster similar substructures and score is calculated by GH-Score. In this step, our idea is to group similar substructures together which share the common part of structures. After that, we select a substructure within a cluster as representative pattern for further pattern reorganization. We select a substructure as representative from a cluster which the representative with a cluster has highly similarity to others in the cluster, in other words, the representative shares most common part of substructures within a cluster. Therefore, the substructure is selected as representative pattern which is similar to others within a cluster. The diagram for substructure grouping and representative selection are showed in Figure 2.

4: EXPERIMENTS

This experiment is to evaluate the idea of discovering conserved local structure among protein chains with the same function. As we know, enzyme classification is one kind of functional hierarchical classification, and proteins with the same EC number have the same function or reaction, which comes from different species. Therefore, it would be a good example to observe conserved local structure under functional classification because these proteins provide the same function and have local structures which involves in protein function. The experiment is designed as follows: (I) randomly select 10 protein chains from a set of protein chains with the same EC number, (II) run the procedure of mining local structure patterns, (III) repeat I and II until all pairs are selected and verified.

As the experimental result shown in Table 1, randomly select 10 EC numbers from about 600 EC numbers to test our goal. The map between protein structure and EC is generated by PDBSprotEC [11]. In this experiment, randomly select 10 protein chains from each EC group and we set 5Å as threshold for substructure conformation and 0.8 as threshold for substructure similarity assessment. In this table, we still have no patterns in few EC numbers, and we guess that too similar global structures can't generate conserved patterns even we use restrict threshold on comparing them.

As the experimental result shown in Table 2, we take EC 1.6.2.4 as example to discover conserved local structure patterns. We randomly select 10 protein chains among 18 protein chains to extract patterns. We use 5 Å as criterion for substructure conformation and then we can obtain 497 substructures and 3 structure patterns after substructure grouping and representative pattern selection. The coverage of these three patterns covers all training data. In Figure 3, it marks the locations of mined structure patterns of protein PDB ID (1AMO:A) by JMol (http://jmol.sourceforge.net/), and the areas colored in green, blue and red are patterns.

Table 1. Experimental results for 10 EC numbers.

EC	Protein Chains	# of Protein Chains for Train	# of Substructure		#of Pattern		Coverage	
			5 Å	10 Å	5 Å	10 Å	5 Å	10 Å
1.1.1.2	18	10	252	299	2	4	8	10
1.1.1.37	38	10	473	217	4	2	10	10
1.2.99.2	15	10	100	256	0	3	n/a	9
1.8.1.2	15	10	336	493	3	4	8	10
1.12.2.1	16	10	61	188	0	2	n/a	9
1.14.13.2 5	33	10	429	497	7	4	10	10
1.14.99.3	39	10	236	162	4	3	8	10
1.18.6.1	30	10	312	420	4	4	9	10
2.3.1.74	16	10	244	121	3	0	10	n/a
2.7.2.3	15	10	546	356	4	4	10	10

Table 2. The experimental result of EC number 1.6.2.4.

EC number	1.6.2.4 (18)
Training Data	10
# of Substructures	497
# of Patterns	3
Coverage of Patterns	10

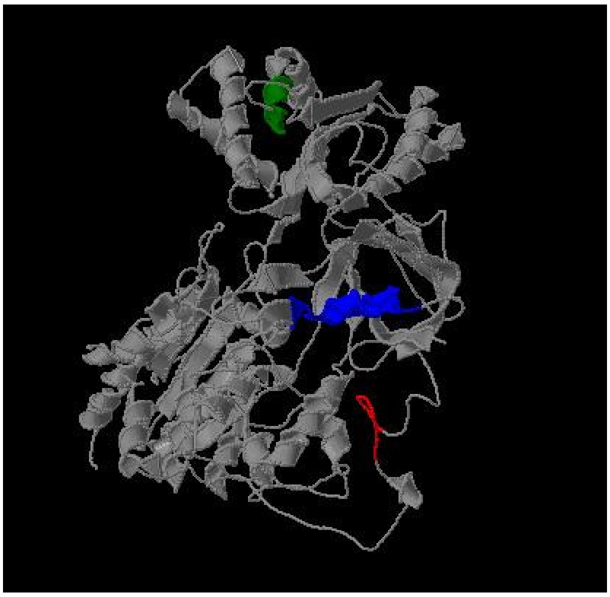


Figure 3. This is a protein of PDB ID 1AMO:A. The 3D substructure colored in green, blue and red are mined local structures.

5: RESULTS AND DISCUSSIONS

Although we only test few cases on discovering conserved structure patterns of proteins with same function, the result shows that there exists local structure conservation region under functional classification. In order to verify the threshold value for substructure conformation, we use 5 Å and 10 Å to understand the relationship between conserved patterns and coverage rate, as shown in Table 3. In coverage rate, the result shows no difference except in size of patterns. We only use 5 Å as threshold value for substructure conformation due to keeping spherical conformation of substructure.

Table 3. Comparison on different threshold value for substructure conformation.

	Threshold of Substructure Conformation	
	5 Å	10 Å
Pattern Length	Shorter	Longer
# of Structures / # of Patterns	More	Fewer
Coverage of Patterns	The same	

In addition, we know that enzymes bind substrates to speeds up biochemical reactions. Therefore, we select all possible substrates information from PDBSum (http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/) related to protein chains within EC 1.6.2.4. In Figure 4, 5, and 6, the pictures show the relationships between conserved patterns and substrates. In Figure 4, (a) is protein 1BVY:A, (b) is protein 1J9Z:A and substructures are areas colored in aqua, midnight-blue, and tan, and the ball colored in red and yellow are substrates (Yellow: EDO, Red: HEM in (a) and Yellow: FAD, Red: NAP in (b)).

In Figure 5, (a) is protein 1AMO:A, (b) is protein 1BU7:A and substructures are areas colored in aqua, midnight-blue, and tan, and the ball colored in red, yellow and green are substrates (Yellow: FAD, Red: NAP, Green: FMN in (a) and Red: HEM, Green: EDO in (b)). In Figure 6, (a) is protein 1SMI:A, (b) is protein 1B1C:A and substructures are areas colored in aqua, midnight-blue, and tan, and the ball colored in red is a substrate (Red: HEM (a) and Red: FMN in (b)). From these observations, we can find that some substructures are close to substrates but some are not. The circles on the pictures show the contact areas between substructures and substrates.

6: CONCLUSIONS AND FUTURE WORKS

This work tries to identify relationships between local structures and functional areas. In the experiment, conserved local structure can be discovered and the observations show contact areas but not all elements of substrate contact with a substructure. As we know, the binding site in enzyme is determined by few key residues but not all of contact points will get involved into protein function [7, 13]. Therefore, our conserved patterns correspond with this point. We also find that our approach suffers from too similar global structures within the EC number because the conservation will be the whole protein structure. Therefore, the good situation for this approach is that protein chains share some common substructures and their global structures are a little bit dissimilar.

Although this work is an incomplete study, the work of discovering conserved local structure from functional hierarchical classification, is still a beginning to realize relationships between local structures and substrates via local structure comparison based on global structure comparison with parameter restricted for local structure comparison. We can discover conserved local structure region from functional hierarchical classification because proteins have the same function will share some attributes reflect on their structures. Furthermore, we should discover all possible conserved local structure patterns for all EC numbers in the future. The computation time of geometric hashing on comparing with substructures will be a major problem to be improved. The reason is that mass substructures will be generated by pair-wise local structure comparison.

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(a) (b)

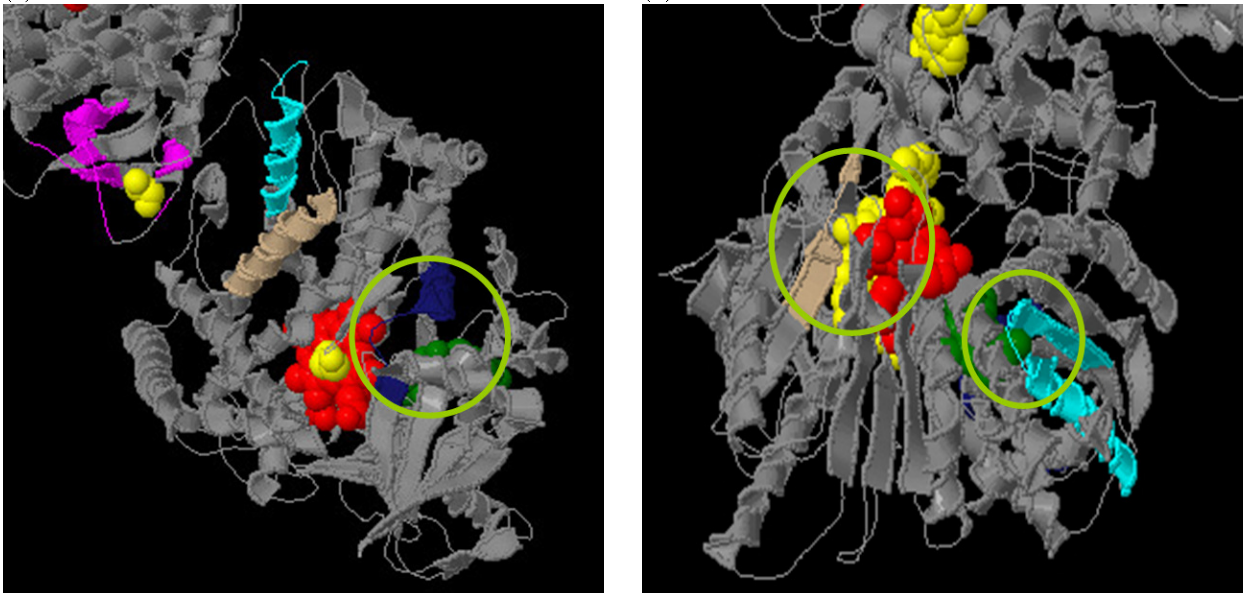

Figure 4. (a) PDB ID: 1BVY:A. (b) PDB ID: 1J9Z:A.

(a) (b)

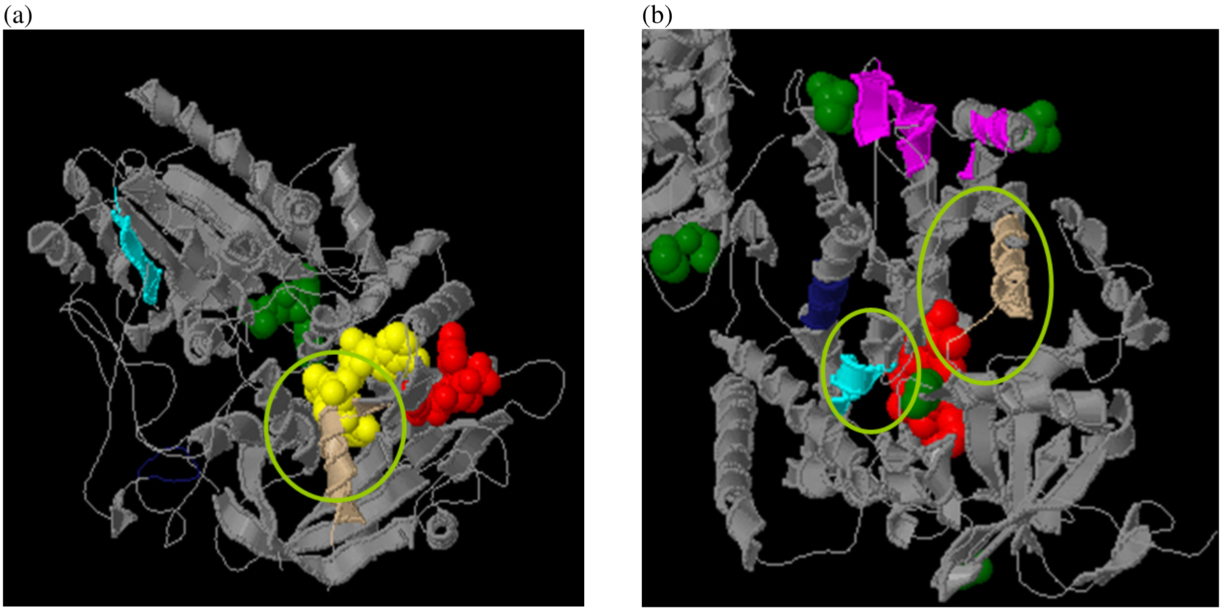

Figure 5. (a) PDB ID: 1AMO:A. (b) PDB ID: 1BU7:A.

(a) (b)

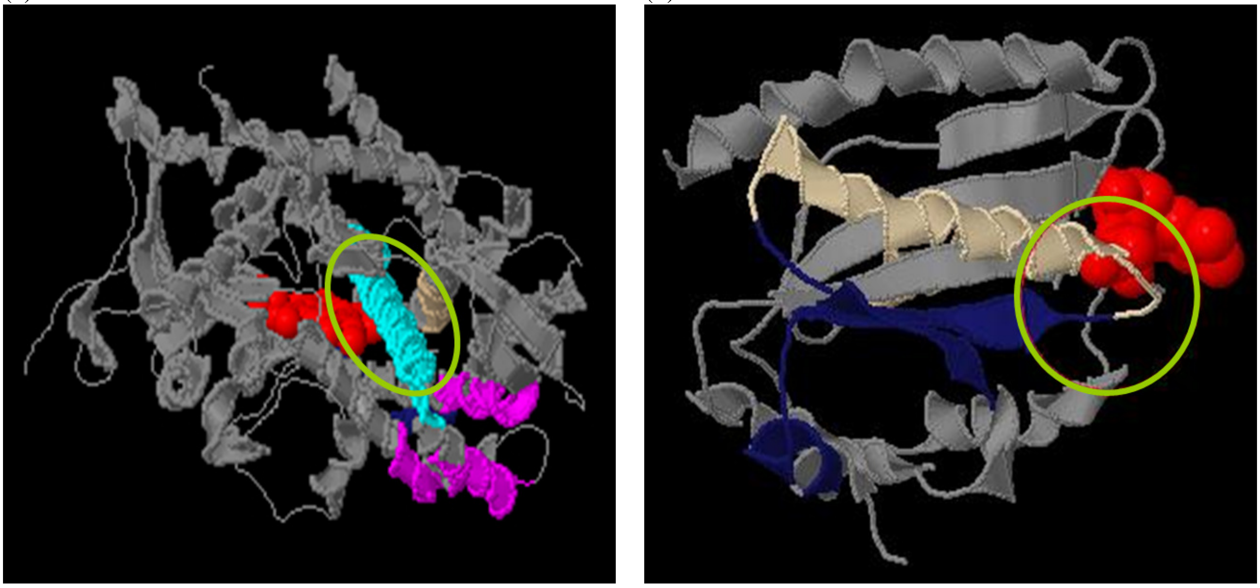

Figure 6. (a) PDB ID: 1SMI:A. (b) PDB ID: 1B1C:A.