行政院國家科學委員會補助專題研究計畫 ■成果報告 □期中進度報告

核醣核酸二級結構預測與分群分析

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執行期間：　　　97 年　8 月　1 日至　98 年　7 月 31 日

計畫主持人：胡毓志
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計畫參與人員：　鄭家胤，

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執行單位：交通大學資訊工程系

中　華　民　國　98 年　9月　1日

行政院國家科學委員會專題研究計畫成果報告

國科會專題研究計畫成果報告撰寫格式說明

Preparation of NSC Project Reports

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主持人：胡毓志　交通大學資訊工程系

計畫參與人員：鄭家胤，陳柏志，黃全榮 交通大學資訊工程系

一、中文摘要

核醣核酸在後轉譯調控上扮演重要的角色，然而與去氧核醣核酸不同的是，去氧核醣核酸的 motif 大多可在序列間發掘其保留區，而核醣核酸的 motif 則必須在結構間找尋。目前已有部分分析系統工具可以在一群功能性相同的核醣核酸中尋找可能的共通結構元，但是其大多僅能從功能性相同的核醣核酸中搜尋，本計畫提出並完成新的核醣核酸共同結構元預測及分群的系統，且已利用多個已知的核醣核酸家族做系統測試。

關鍵詞: 核醣核酸，二級結構，分群

Abstract

RNA plays a crucial role in post-transcriptional regulation. Unlike DNA binding proteins, which recognize motifs composed of conserved sequences, RNA protein binding sites are more conserved in structures than in sequences. Though some current approaches can now identify common structure motifs from a set of RNAs, they typically assume the given set forms a single family, which is not necessarily correct. We proposed and developed a new adaptive method that conducts structure prediction as well as clustering simultaneously. We demonstrated its performance on several real RNA families.

Keywords: RNA, secondary structures, clustering

Introduction

Like proteins, RNA functions generally depend on their structures. Although structural genomics, the systematic study of all macro-molecular structures in a genome, is currently focused more on proteins, thousands of genes produce transcripts exerting their functions without ever producing protein products [1]. It can be easily argued that the comprehensive understanding of the biology of a cell requires the knowledge of identity of all functional RNAs (both non-coding and protein-coding) and their molecular structures. Since it is often difficult to acquire the 3D spectrum data of RNA molecules for structure determination, versatile and reliable computational methods that can predict RNA structures are highly desirable.

Many computational methods for the prediction of RNA secondary structures have been developed. According to the search strategies used, they can be roughly classified into the following categories: (1) thermodynamics, (2) comparative sequence analysis, (3) stochastic context-free grammars, (4) heuristics and (5) hybrid. By applying dynamic programming, thermodynamic methods are aimed to find the optimal secondary structure for single RNA sequences with the global minimum free energy [2-4]. If homologous sequences are available, from the alignment of these sequences, comparative approaches look for covariance evidence between base pairs to identify consensus structures [5-8]. Some researchers applied stochastic context-free grammars (SCFG) to build a probabilistic model for consensus structures in a family of related RNAs, which is considered the SCFG-based analogue of profile HMMs [9,10]. The tree structure of this SCFG-based analogue allows for a convenient graphical representation that intuitively and compactly reflects the structure of the RNA family being modeled [11]. Due to the fact that the time and space complexity of most methods are still too high to be practical, some approaches adapt useful heuristics to alleviate the problem [12-15]. Although these methods are not guaranteed to fine the optimal structure, they are able to produce the approximately best solutions. There also exist some hybrid approaches that combine the virtue of various strategies such as thermodynamic stability, sequence covariance, phylogenetic analysis, etc. [16-18]. Besides the search strategy that describes *how* to find the solutions, RNA secondary structure prediction methods can also be classified by *what* to find. Some methods focus on finding the optimal structure for an entire single RNA sequence [2,3,12,13,20]; others, the consensus structure elements shared by a family of related RNA sequences [19,21,22].

Many functional RNAs have evolutionarily conserved secondary structures in order to fulfill their roles in a cell. For protein-coding RNAs, some of the functions can be presented by functional motifs. For example, several best-understood structurally conserved RNA motifs are found in viral RNAs, such as the TAR and RRE structures in HIV and the IRES regions in Picornaviridae [23]. Apparently, structural information is very useful in characterizing a class of functional RNAs. Based on characteristic structures, we can likely identify novel functional RNAs or partition given RNAs into biologically meaningful families. Several systems have been developed to find consensus structural elements within a family of functionally related RNAs [9,15,19]; however, there is little work on clustering of unaligned RNAs based on characteristic secondary structures. Given a set of unaligned RNA sequences without prior knowledge of the number or identity of families in the set, our goal is to automate both clustering and secondary structure prediction simultaneously. In this paper, we propose an adaptive approximation approach combined with a genetic programming-based structure prediction method to identify from unaligned RNAs reasonable clusters associated with characteristic secondary structure elements. To demonstrate its performance, we tested it on several real datasets.

System

In order to find a reasonable partition for a given set of unaligned RNAs without knowing beforehand how many clusters actually existing in this set, we assume that each cluster is likely a functional family that contains characteristic structure elements. Based on this assumption, our new method is focused on finding significant consensus structure elements that can be used to characterize the families of RNAs. Since the number of clusters and its size are not known in advance, we adapt a generate-and-test strategy that iteratively adjusts the hypothesized cluster size until some significant consensus structure elements can be found associated with this cluster. After a cluster is obtained, all its members are then removed from the given set of RNAs. We can repeat the same separate-and-conquer strategy to identify other clusters until the set of RNAs is emptied.

Consensus structure element prediction can be considered a supervised learning problem which involves both positive and negative examples [15]. Positive examples are a given set of RNA sequences; negative examples are some number of sequences randomly generated based on the observed frequencies of sequence alphabet in positive examples. The objective here is to learn the structure elements that can be used to distinguish the given functionally related sequences from the random sequences.

We modify GPRM [22], an RNA consensus secondary structure prediction tool, to find significant structure elements from a dataset that may contain multiple variable-sized clusters of unaligned sequences. GPRM has been tested on several real RNA families, including pseudoknots, and shown its effectiveness in predicting conserved structure elements in a given RNA family. To describe the characteristic structure elements for a cluster, we adapt the same representation that is expressive enough to even represent pseudoknots. We also apply the same genetic operators to optimize candidate structure elements during evolutionary process. What is different from the previous work is the fitness function.

The fitness function is used to measure the quality of individuals (i.e. candidate structure elements) in a population. The higher the fitness of an individual, the better its chances of survival to the next generation. In the previous work, the input dataset was assumed to be a single class of functionally related RNA sequences. We were interested in those structure elements that can reflect the characteristics conserved in a family, e.g. the RNA protein binding sites. Derived from the F-score, the fitness function was aimed to balance the importance of two measures, recall (i.e. sensitivity) and precision (i.e. positive predictive value) [15]. It assigns higher values to those structural motifs commonly shared by the given family of RNAs, and rarely contained in random sequences. For a given set of RNA sequences that form a single family only, the fitness function used in [15,22] can effectively guide the evolutionary process in genetic programming. Nevertheless, when the input dataset contains multiple functional classes, the recall measure may dominate the calculation of F-score if the fitness function treats the entire dataset as a single class. This will mislead the system to find over-general elements shared by most sequences. To alleviate the bias, we defined a new measure of recall. By taking cluster size into account, we can better constrain the search space and allow conserved clusters to emerge more likely instead of being buried in bigger but much less coherent clusters.

The GP (Genetic Programming)-based structure prediction method can find the fittest secondary structure elements according to a given range of the cluster size, while the significance of the cluster found along with its characteristic structure elements is highly dependent on the range we choose. With proper adjustment of cluster size through the generate-and-test procedure combined with the GP-based prediction method, we can identify a meaningful cluster and the associated characteristic structure elements.

The adaptive adjustment of cluster size in the generate-and-test procedure is controlled by the consensus structure specificity. It is defined as the Laplace prior precision. The Laplace prior approach has also been applied to inductive leaning to evaluate the significance of inductive rules [24]. We incorporate the Laplace prior into the calculation of precision with the aim to avoid well conserved but too small clusters. Note that the Laplace prior precision is only used to determine the significance of a cluster found, unlike the F-score, which is used to direct the optimization process to find the best structure elements under the constraints of the cluster size. By the comparison of the Laplace prior precision with a pre-specified threshold, we can adjust the range of cluster size accordingly, and then re-run the GP-based method to predict a new structure element and derive the new cluster it characterizes.

Once a significant cluster is found, we separate all its members out of the given dataset of RNA sequences. We then apply the same procedure to those that still remain in the dataset until the entire set is emptied. This separate-and-conquer strategy is effective even when no prior knowledge of the identities of the clusters is given. It can automatically partition the given dataset into meaningful clusters, and also identify their characteristic structure elements.

Experimental Results

Two types of quality were considered to evaluate the performance of our method. One is to measure the agreement between the predicted clusters and the actual cluster identities; the other, to quantify the agreement between the predicted structure elements and the actual structure assignment. We applied the adjusted Rand index [28] and the Matthews correlation coefficient [29] to measure the qualities.

Our algorithm is designed to automatically partition a given set of unaligned RNA sequences into meaningful clusters, each associated with characteristic conserved secondary structure elements. The number of real clusters and the distribution of cluster size may affect the prediction of partitions and characteristic structure elements. To measure their effect on the performance, we tested our method on different datasets with various number and size of clusters. We used three families, including 16S RNA, IRE (Iron Response Element) and viral 3’UTR, to prepare the test datasets. They have been used in previous experiments and published in literature [15,19]. The sequence data and the correct structure elements can be accessed at public databases [26,27]. The 16S RNA dataset contains 34 archaea 16S ribosomal sequences originally derived from a set of 311 sequences extracted from the SSU rRNA database. The archaea set of 311 sequences was further reduced to 34, filtering out the sequences that miss base assignments or are greater than 90% identical. The IRE dataset was constructed by Gorodkin *et al.* [19] from 14 sequences from the UTR database. They modified the IREs and their UTRs to make the search more difficult. By iteratively shuffling the sequences and randomly adding one nucleotide to the IRE conserved region, they built a set of 56 IRE-like sequences from the 14 IRE UTRs. The third data set includes 18 viral 3'UTRs each of which contains a pseudoknot. Seven of the RNA sequences are the soil-borne rye mosaic viruses; the others are the soil-borne wheat mosaic viruses.

With the three real families of RNA sequences, we first tested our method on each possible pair of the families, i.e. 16S RNA/IRE, 16S RNA/viral 3’UTR, and IRE/viral 3’UTR. We then applied our method to the union of all the three families. In each run of the experiment, no information regarding the number of families or the family size was given to the algorithm beforehand. One purpose of this experiment is to analyze the effect incurred by the number of clusters in a dataset. Furthermore, as the real conserved structure elements differ in various families, we can also observe how the interleaving of distinct structure elements within a single dataset may affect the prediction process. The results are presented in Table 1, and some partial predicted secondary structures are shown in Figure 1.

Discussion

In this project, we proposed a new approach that can perform structure prediction and clustering simultaneously for RNA analysis. The predicted results provide biologists with reasonable hypotheses and suggest further biological verifications. The performance of the new strategy has been demonstrated on several real RNA functional families. The system can be extended in the following directions. First, in case domain knowledge is available, we expect the results can be better improved by incorporating the background knowledge into the optimization process to effectively constrain the search space. Second, the discovery of important clusters in data usually goes through a repeated process cycle of finding clusters, interpreting results and augmenting data. No current unsupervised clustering system can produce maximally useful results if operated alone [11]. We plan to design a human-machine interface, so that biologists can easily monitor the system status and adapt the system parameter settings. Third, the algorithm itself is highly modular and most of the modules are independent of each other. This property may lead to a parallel-processing version of the system to significantly reduce its computational time.

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(a)

IRE+viral 3’UTR	Recall	Precision	Matthews
IRE	0.97	0.99	0.97
viral 3’UTR	0.71	0.95	0.79

(b)

16S RNA+viral 3’UTR	Recall	Precision	Matthews
16S RNA	0.97	0.95	0.83
viral 3’UTR	0.77	0.98	0.77

(c)

IRE+16S RNA	Recall	Precision	Matthews
IRE	0.73	0.99	0.85
16S RNA	0.81	0.73	0.67

Table 1. Summary of the experimental results. Table (a), (b) and (c) present the result for the dataset containing IRE and viral 3’UTR, 16S RNA and viral 3’UTR, IRE and 16S RNA, respectively.

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***** IRE *****

> seq_D15071.1

  41    45  47     51          58    62 63     67
t g c g g u c c u g g c c a g u g a g c u g g g c c g c

predicted:
. ( ( ( ( ( . ( ( ( ( ( . . . . . . ) ) ) ) ) ) ) ) ) )

published:
. ( ( ( ( ( . ( ( ( ( ( . . . . . . ) ) ) ) ) ) ) ) ) )

***** 16S RNA *****

> U51469

  13          20   23            31         37             46         52             61
g u u u c a u u g a a g u u u g c u u u u a g u g a g g u g a c g u c u a a u u g g c g u u a u c g

  62       67           75    78          85
  a a c u u g u g g u a a g c g a c a a g g g a a a a

predicted:
. ( ( ( ( ( ( ( ( . . ( ( ( ( ( ( ( ( ( . . . . . ( ( ( ( ( ( ( ( ( ( . . . . . ) ) ) ) ) ) ) ) ) )
  . . . . . ) ) ) ) ) ) ) ) ) . . ) ) ) ) ) ) ) ) . .

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  . ) ) ) ) ) ) ) ) ) ) ) ) ) . . ) ) ) ) ) ) ) ) . .

***** viral 3’UTR *****

> PKB183

  14  16  18        24 25  27       32         38
a c g u c g u g c a g u a c g g u a a a c u g c a c a u

predicted:
. ( ( ( . [ [ [ [ [ [ [ ) ) ) . . . . ] ] ] ] ] ] ] . .

published:
. ( ( ( . [ [ [ [ [ [ [ ) ) ) . . . . ] ] ] ] ] ] ] . .
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Figure 1. A partial result of the predicted RNA motifs. The numbers above the sequences are the indices of the nucleotides. The predicted and the published motifs are both shown for reference.

附件一

行政院國家科學委員會補助國內專家學者出席國際學術會議報告

98年 9月 1日

報告人姓名	胡 毓 志	服務機構及職稱	交通大學資訊工程系 副教授
時間 會議 地點	07/13/2009-07/16/2009 Las Vegas, USA	本會核定補助文號	NSC 97-2221-E-009-131-
會議名稱	（中文）生物資訊暨計算生物學國際研討會 （英文)2009 International Conference on Bioinformatics and Computational Biology		
發表論文題目	1.（中文）利用蛋白質結構字元及一維模組搜尋法分析金屬結合蛋白的結構模組 （英文）Discovery of Structural Motifs in Metalloproteins Using Protein Structural Alphabets and 1D Motif-finding Methods		

報告內容應包括下列各項：

一、參加會議經過

於 07/12 辦理註冊報到，07/13 參加 Opening Remarks by Dr. Arabnia from University of Georgia，於 07/13-07/16 期間，參加與會學者之論文發表，同時，07/13 發表論文。並與多位國外學者討論相關研究議題。會議論文不乏有關基因表現分析，蛋白質結構分析，調控訊號檢視，生物網路分析等等，對於我國內生物資訊的發展，將提供非常多的助益與新的發展方向。

二、與會心得

本次參加人數及國家眾多，其研究領域更包括計算機科學、醫學、生物學等之應用，藉由討論及論文發表，獲得寶貴經驗，對於未來研究提供了新的方向。其中更結識他國友人，經由研討，可明白其他國家的發展經驗。

目前系統生物學已經成為國際重要的研究課題，可預見的是，在不久的將來，大量的基因表現資料以及蛋白質結構將如同 DNA 及蛋白質序列般，不斷地被產生及發表，如何能從這些不同類型的生化資料中發掘有用的訊息將是重要課題。藉由這次與會學習的經驗，我們可以得知國外研究之重點，作為我國在生物科技的發展依據。

三、考察參觀活動(無是項活動者省略)

四、建議

由於生物科技是目前國內新興研究發展之重要產業，懇請國科會及相關單位，能多支持與獎勵國內學者多參與此類國際研討會，除了增加我國在國際相關領域的能見度，同時，提供相互學習之機會，這是直接提昇我國在生技發展地位的最有效做法。

五、攜回資料名稱及內容

The Proceedings of Biocomp2009

六、其他

Discovery of Structural Motifs in Metalloproteins Using Protein Structural Alphabets and 1D Motif-finding Methods

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Abstract

Though the increasing number of available 3D proteins structures has made possible a wide variety of computational protein structure research, yet the success is still hindered by the high 3D computational complexity. Based on 3D information, several 1D protein structural alphabets have been developed, which not only can describe the global folding structure of a protein as a 1D sequence, but can also characterize local structures in proteins. In this paper, we introduce an approach that combines standard 1D motif detection method with structural alphabets to discover metal-binding sites in metalloproteins. We tested our method on different metal-binding proteins. The results show that our combinatorial strategy can efficiently and successfully identify the structural preferences in metal-binding sites.

Keyword: metalloproteins, protein structural alphabets, structural motifs

1. Introduction

As the rapid growth of protein structural information, biologists require accurate classification to understand and rationalize the variety in proteins [1]. To ensure the classification can be more easily constructed and better comprehensible, it is desired we provide only essential characteristic structural descriptions of protein functional parts. With such a classification, we can assign a novel protein to known categories, and thus predict its structures and functions. The task of extracting characteristic structural features for classification becomes more challenging for small proteins, where the characteristic statistics are marginal owing to short protein chains, or for proteins that only share low sequence similarity, e.g. some metal binding proteins. In this paper, we evaluate the feasibility of using structural alphabets and 1D motif detection methods to discover the structural motifs in Mg^{2+}-binding and zinc-binding proteins.

Metalloproteins requires metal cofactors in cellular biochemistry, which play important roles in both intra- and extracellular catalytic activities and structural stabilization [2-4], as metal binding increases thermal and conformational stability of small domains. Among many, here we focus our study on zinc-binding and Mg-binding proteins.

The C2H2 zinc finger is one of the best-studied metal binding domains. It was first observed as a repeated zinc-binding motif with DNA-binding properties in the Xenopus transcription factor IIIA, and the term `zinc finger' is now largely used to denote any compact domain stabilized by a zinc ion [5-7]. Previous studies of zinc fingers include automatic neural network-based numerical taxonomy methods that identify evolutionary relationships among proteins [8-9] or the analysis of sequence and structure similarity using BLAST-based sequence alignment method in combination with DaLiLite followed by visual inspection [10][11]. Although these methods have made significant advances in structural classification, they still left plenty of space for improvement in classification accuracy and efficiency.

Like zinc, magnesium is also a versatile and important metal cofactor. It helps stabilize a variety of protein structures, e.g., the interface of the ribonucleotide reductase subunits [12]. It is also used to stabilize nucleic acids by alleviating electrostatic repulsion between negatively charged phosphates [13]. A few relatively short sequence motifs have been discovered for Mg^{2+} proteins with close sequence homology. Examples include the NA**D**F**D**G**D** motif, found in different RNA polymerases, DNA Pol I and HIV reverse transcriptase, and the YX**DD** or LX**DD** motifs in reverse transcriptase and telomerase, where the residues in bold are the Mg^{2+} ligands [2]. Nevertheless, these Mg^{2+} sequence motifs are sometimes too short to be statistically specific to Mg^{2+}-binding sites, and may easily escape detection. On the other hand, the Mg^{2+}-binding sites share sufficient structural similarity that can characterize Mg^{2+}-proteins.

As the conserved local structural features can be identified in various ways and described in different representations, e.g., the relationships between local sequences and structures [14-16], we took a simpler approach and applied the widely used 1D motif detection algorithms to protein structural alphabet sequences. Representing conserved local structural features by 1D structural alphabets instead of 3D co-ordinates is more efficient in comparison and more economical in storage. The 1D-based approaches can also serve as a pre-processor to filter out remotely related or irrelevant proteins before we apply other more accurate but more computationally intensive structure analysis tool. To demonstrate its applicability, we applied the 1D-based approach to discovering structural motifs in Zn-binding and Mg-binding proteins, and compared the motifs found against those reported in literature.

2. Materials and Methods

We first transformed protein sequences into a 1D representation, and later identified significant motifs from the 1D alphabet sequences that could characterize the local structural features. There are several protein structural alphabets available [17-19]. As these alphabets were derived from different design philosophies, their sizes can vary from a dozen to nearly a hundred. They address different structural characteristics and have various applications. Therefore, in different domains, we can apply an appropriate structural alphabet to transform amino acid sequences or protein 3D structures into 1D structural alphabet sequences as required. Currently, we use the alphabet designed for SA-FAST [20]. It contains 18 letters, five of which represent the helix structure, eight for the sheet, and the rest for the coil.

Given a set of functionally or structurally related proteins, after the conversion into 1D structural alphabet sequences, we can apply a sequence motif detection algorithm to discover significant motifs. There has been significant amount of research on motif discovery with different objective functions, motif representations and search strategies [21-23]. In our study, we used MEME [24] to detect structural motifs, which adopts an expectation maximization approach to find motifs represented as weight matrices. Unlike IUPAC-IUB codes, motifs described in weight matrices are more flexible because a weight matrix can show each alphabet letter preference in every motif position. Besides, a weight matrix can be easily transformed to IUPAC-IUB codes or regular expressions when necessary, but not vice versa.

We call the motifs found by MEME in the structural alphabet sequences *simple motifs*. When the local properties in protein structures are too complicated, e.g. multiple binding sites or sub-domains, to capture in a simple motif, we combine several simple motifs into a *compound motif*. To avoid the computational complexity of combining matrices, we transform simple motifs to regular expressions first, and then combine them to a compound motif. A compound motif example looks like the following.

$M_1[20,50]M_2[0,6]M_3$, where M_1, M_2 and M_3 are simple motifs, and the numbers in the brackets denote the range of residue separation between motifs.

M_1= SP[PS][SN]N[NE]EE,
M_2= [WE][NE]EEACWGQS,
M_3= TTTTTTTTTLK[TG][SH]WNMR[DQ],

where letters in brackets denote the possible structural alphabet letters in the respective motif position.

We illustrate the system flow in Figure 1. We proposed for structural motif discovery a general framework in which the structural alphabet and the motif finding algorithm can be replaced with others when needed in different applications.

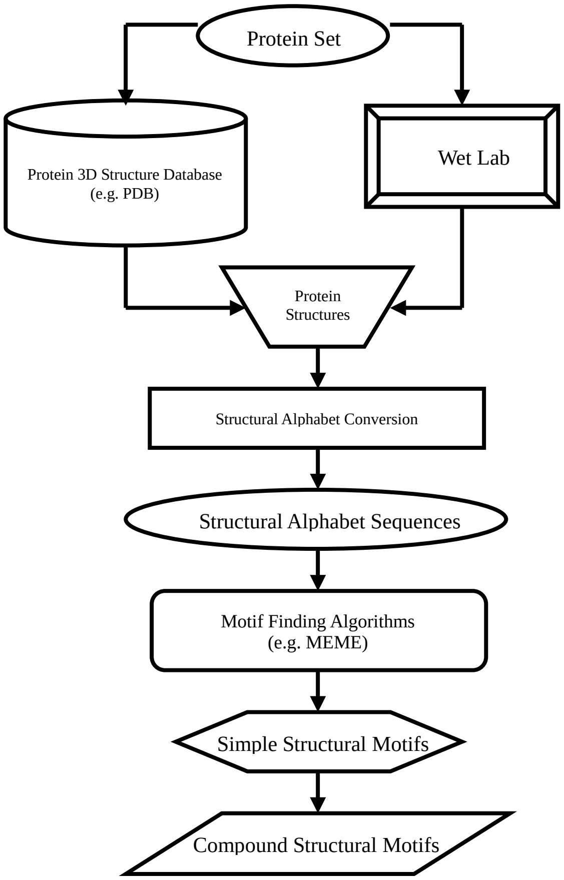

Protein Set
Protein 3D Structure Database
(e.g. PDB)
Wet Lab
Protein
Structures
Structural Alphabet Conversion
Structural Alphabet Sequences
Motif Finding Algorithms
(e.g. MEME)
Simple Structural Motifs
Compound Structural Motifs

Fig 1. System flow of structural motif discovery

3. Experimental Results

The domains from C2H2-like fingers consist of a β-hairpin followed by an α-helix that forms a left-handed ββα-unit, where two zinc ligands are contributed by a zinc knuckle at the end of the β-hairpin and the other two ligands come from the C-terminal end of the α-helix [25,26]. The C2H2 zinc finger motif (classic zinc finger) was first discovered in the Xenopus laevis transcription factor IIIA, and has since been found in many transcription factors and in other DNA-binding proteins.

This classic C2H2 zinc finger typically contains a repeated ~30 amino sequences. To demonstrate that our approach is capable of detecting the structural ββα-unit, we first transformed the 156 zinc finger proteins in SCOP C2H2 zinc finger family into structural alphabet sequences, and then applied standard motif-finding algorithms to these sequences to identify common motifs that can characterize the ββα-unit.

We used 8 as the motif width and ran MEME to find motifs. A motif found was considered as corresponding to a sub-domain correctly if more than half of the residues in the sub-domain were included in the motif. If any simple motif or compound motif correctly corresponded to a sub-domain, we claimed this sub-domain was recovered successfully (i.e. a hit). In Table 1, we present the simple motif or compound motif found to characterize the sub-domains, and its coverage. The results suggest that using standard motif-finding algorithms, e.g. MEME, combined with an appropriate structural alphabet was able to recover the structural sub-domains in C2H2 zinc finger proteins. We show some C2H2 zinc finger proteins with structural motifs highlighted in color in Figure 2.

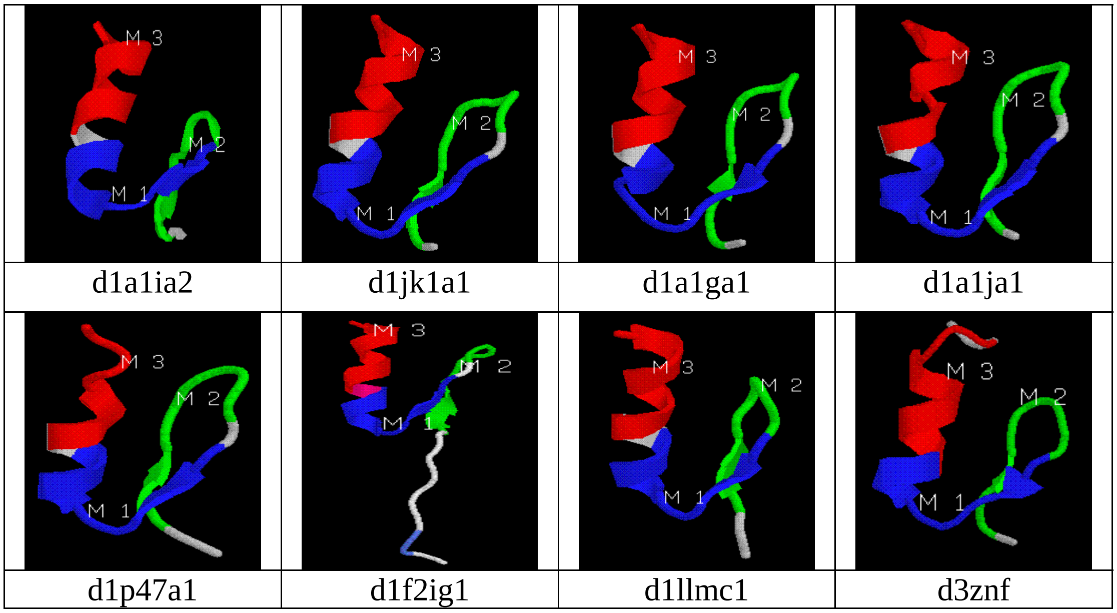
Unlike Zn^{2+} binding sites, Mg^{2+} binding sites have less sequence similarity, but sufficient structural similarity, which make an appropriate test case to verify our motif-finding method's capability of discovering structural motifs with low sequence homology. Previously, Dudev and Lim applied a similar idea to identify the structural motifs in Mg^{2+}-binding proteins [13]. In the study, they successfully discovered four motifs corresponding to the Mg^{2+} binding sites in 16 out of 70 Mg^{2+}-binding proteins. For comparison, we used the same 70 proteins in our experiments. Instead of the structural alphabet in PBE [27], we used the alphabet designed for SA-FAST [20] to represent the 70 Mg^{2+}-binding proteins. Furthermore, we used a widely-used motif-finding system, MEME, rather than a method solely based on motif occurrence frequency [28], to identify common motifs (simple motifs) first, and then combined those significant simple motifs (i.e. with low *E-value*) into compound motifs.

We show in Table 2 some of the compound motifs that cover the Mg^{2+}-binding residues. Each of the simple motifs has an *E-value* lower than 1.9e-015 as presented in Table 3. Based on the compound motifs generated, we noticed that Mg^{2+}-specific structural compound motifs are not commonly shared among the 70 Mg^{2+}-binding proteins. This observation is similar to that by Dudev and Lim [13], who found only four first-shell structural motifs shared by more than three Mg^{2+}-binding proteins, with a total of 16 proteins containing these motifs. Unlike Dudev and Lim, who defined a structural motif based on its occurrence frequency, we constructed compound motifs, more flexible and expressive than Dudev and Lim's, from significant simple motifs found by MEME. By constraining the number of simple motifs, e.g. setting a reasonable *E-value* threshold, we can reduce the search space for compound motif candidates, and still avoid the risk of overlooking less frequent but significant structural motifs.

4. Conclusion

In this paper, we introduced a general framework for structural motif discovery, and applied it to two types of metalloproteins, Mg^{2+}-binding proteins and C2H2 zinc finger proteins. Two major components in our framework are the structural alphabet used to describe protein structures and the motif-finding algorithm used to discover significant local structure features. In our experiments, we used the alphabet designed for SA-FAST, and a widely used motif detection algorithm, MEME. These components can be flexibly replaced with others when necessary to increase the applicability in different domains. The experimental results showed that using structural alphabets combined with standard motif-finding algorithms could successfully identify biologically meaningful sub-domains in proteins.

With the positive results, we plan to carry out the future work as what follows. First, many structural alphabets and quite a few motif detection algorithms have been developed based on different design philosophies and application domains. We intend to incorporate other structural alphabets and motif-finding algorithm into our system. We expect to discover more kinds of motifs in a wider variety of protein structures. Second, we plan to build a structural alphabet motif database. Given the effectiveness and the economy in characterizing and storing structural properties of proteins, a structural alphabet motif database can complement most protein sequence and 3D structure databases. Third, we will design a protein function predictor using structural motifs as important features. Based on the motifs, the functions of novel proteins can be predicted by classifying them to protein groups with known functions. Finally, as there have been many protein structure or function prediction systems available, we also plan to evaluate the feasibility of using structural alphabet-based methods as a preprocessor. Compared with most prediction strategies typically based on 3D information, alphabet-based methods have much lower computational complexity. They can help other predictors constrain the search space efficiently by filtering out irrelevant predictions.



d1a1ia2 d1jk1a1 d1a1ga1 d1a1ja1

d1p47a1 d1f2ig1 d1llmc1 d3znf

Fig 2. Examples of C2H2 zinc finger protein structures. The simple motifs that map to the β-hairpin and the α-helix are highlighted in color, where M_1=[GN][HE][NE]AC[AW]RQ, M_2=[FH]CWNA[RC]QK and M_3= TTTTTT[PL][KPL]. The compound motif mapping to the $\beta\beta\alpha$-unit is [FH]CWNA[RC]QK **(0-2)** [GN][HE][NE]AC[AW]RQ **(0-5)** TTTTTT[PL][KPL].

Table 1. Summary of compound motifs mapping to C2H2 zinc finger ββα-unit that consists of β-hairpin and α-helix.

Structural (sub-)domain	Compound motif	SCOP 1.73 (C2H2 zinc finger) g.37.1.1	
		Hit[a]	Coverage[b]
β-hairpin	[FH]CWNA[RC]QK(0-2) [GN][HE][NE]AC[AW]RQ	131	83.9 %
α-helix	[GN][HE][NE]AC[AW]RQ(0-5)TTTTTT[PL][KPL]	142	91.0%
ββα-unit	[FH]CWNA[RC]QK(0-2) [GN][HE][NE]AC[AW]RQ (0-5) TTTTTT[PL][KPL]	124	79.5%
Total	---	156	100%

[a] We called it a hit for a structural (sub-)domain when more than half of the (sub-)domain residues were contained in a motif. We presented the count of hits of different (sub-)domains.

[b] Coverage was defined as the ratio of the count of hits to the number of zinc finger proteins, e.g., if No.=156 and Hits=131, then Coverage=131/156=83.9%.

Table 2. Structural motifs found in Mg^{2+}-binding proteins.

Compound motif[a]	PDB	Binding residue position	Dudev & Lim's motif[b]	Functional description
m11-**85**-m19	1TW1	254-344-347	b(89)d(2)d	beta-1-4-galactosyltransferase 1
m11-**98**-m22	1JYL	107-216-218	b(115)d(1)b	ctp:phosphocholine cytidylytransferase
m5-**79**-m23-**5**-m23	2BVC	135-219-227	d(83)f(17)d	glutamine synthetase 1
m35-**1**-m1	1IG5	54-56-58-60	m(1)o(1)o(1)a	calbindin d9k
m23-**1**-m1-**0**-m21	1WDC	28-30-32-34-39	m(1)o(1)o(1)a(4)m	scallop myosin
m5-**29**-m5	1OBW	65-70-102	o(4)d(31)d	inorganic pyrophosphatase
m5-**28**-m5	1HUJ	115-120-152	h(4)d(31)d	inorganic pyrophosphatase
m23-**0**-m42	1OFH	157-160-163	m(2)c(2)c	atp-dependent protease hslv
m25-**0**-m8	1XXX	162-164-167	m(1)m(2)n	dihydrodipicolinate synthase
m20-**27**-m6	1IV2	8-10-42	d(1)d(31)m	2-c-methyl-d-erythritol 2-4-cyclodiphosphate synthase
m20-**41**-m11	1WC1	1017-1018-1061	d(0)e(42)b	adenylate cyclase
m8-**27**-m10	1MXG	252-256-292	m(3)k(35)k	N/A
m8-**43**-m11	1KHZ	112-116-164	m(3)n(47)a	adp-ribose pyrophosphatase
m15-**98**-m26-**164**-m5	1ED9	51-155-322	h(103)l(166)d	alkaline phosphatase
m15-**106**-m26-**158**-m5	1SHQ	37-151-310	h(113)l(158)d	alkaline phosphatase

m5-**20**-m20	1ZPD	440-467-469	**k(26)h(1)a**	pyruvate decarboxylase
m5-**23**-m39	1POX	447-474-476	**k(29)h(1)a**	pyruvate oxidase
m5-**27**-m4	1UMD	175-204-206	**k(28)h(1)a**	2-oxo acid dehydrogenase alpha subunit
m5-**25**-m39	2C3M	963-991-993	**k(27h(1)a**	pyruvate-ferredoxin oxidoreductase
m16-**155**-m18	1NUY	1118-1121-1280	**f(1)h(158)m**	fructose-1-6-bisphosphatase
m16-**146**-m18	1KA1	142-145-294	**f(1)h(148)m**	halotolerance protein hal2
m16-**123**-m18	2BJI	1090-1093-1220	**f(1)h(126)m**	inositol-1(or 4)-monophosphatase
m24-**155**-m12	1O08	1008-1010-1170	**f(1)h(159)b**	beta-phosphoglucomutase
m24-**106**-m12	1U7P	11-13-123	**f(1)h(109)b**	magnesium-dependent phosphatase-1
m24-**186**-m12	2C4N	9-11-201	**f(1)h(189)b**	nagd
m24-**118**-m12	2B82	44-46-167	**f(1)h(119)b**	class b acid phosphatase

[a] Compound motifs are composed of significant simple motifs, e.g. m24-161-m12 is a compound motif composed of simple motif m24 and m12, where 161 is the number of residues in between. The significance of a simple motif is determined by its *E-value*. The *E-value* of all simple motifs in table is less than or equal to1.9e-015 (the smaller *E-value*, the more significant).

[b] Dudev & Lim considered a motif significant if the number of its occurrence is greater than or equal to 3. Significance motifs are marked in bold.

Table 3. Summary of simple motifs used in compound motifs.

Simple Motif (regular expression)	**Motif Index**	***E-value***
LKGHN	m1	5.2e-954
M[DA]DHN	m4	9.0e-547
EEARQ	m5	4.8e-531
TLKGH	m6	6.6e-477
ACWNE	m8	8.6e-422
MADWN	m10	1.7e-303
[LM]KGHN	m11	1.2e-287
ACARQ	m12	7.7e-309
EMAD[HQ]	m15	3.9e-231
EEACW	m16	8.5e-204
[FA]RQ[TP]T	m18	1.5e-176
TLKGM	m19	1.6e-163
EEEE[MAE]	m20	3.0e-136
ARQTT	m21	2.8e-095

TTLKG	m22	1.8e-098
TTPP[PS]	m23	5.7e-088
ACW[NF]Q	m24	1.0e-075
SF[RP]CN	m25	7.6e-078
SFRQT	m26	6.9e-055
TT[ST]PP	m35	3.3e-022
MM[DA]DH	m39	1.9e-015
AR[CQW]PP	m42	7.1e-020

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