行政院國家科學委員會補助專題研究計畫期中進度報告

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※　　　　基因調控網路之組合型重建 (1/3)　　　　※

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計畫主持人：胡毓志

共同主持人：

計畫參與人員：吳秉蔚，賴昀君，張巽昌

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執行單位：交通大學 資訊工程系

中　華　民　國　95 年　4　月　27　日

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一、中文摘要

在本計劃執行的第一年中，我們針對多數已常被應用於基因網路重建研究之方法做了詳細的探討，這其中包含布林網路，貝氏網路，線性網路等。本年度的目標在於評估各個方法的優劣，希望藉由各系統的研究與探討，奠定我們新系統的基礎，在透過仔細的分析評估之後，我們決定採用多策略結合方式，整合轉錄子結合區與基因表現資訊做為基因調節模組的預測依據。

關鍵詞：專題計畫、報告格式、國科會

Abstract

During the first year for this project, we investigated those approaches that have been widely applied to genetic network research, including Boolean Networks, Bayesian Networks, Linear Models and Non-linear Models, etc. The goal of our first-year project is to evaluate the pros and the cons of these various methods on which our network reconstruction system can be based. According to the evaluation, we have concluded that our new approach will be built under a multi-strategy framework that combines motif binding sites and gene expression profiles.

Keywords: genetic networks, Boolean networks, Bayesian networks, motif binding sites

二、緣由與目的

藉由分析大量的基因體實驗資料來瞭解所有基因(gene)及其產物(gene product，如蛋白質, rRNA, tRNA 等)在種種刺激下所產生的交互作用，是功能基因體學(functional genomics)重要目的之一。為了達成此一目的，我們必須以系統化的方式、全面性的角度對基因及其產物進行分析，以建構出這些分子在細胞進行各種活動、接受外在刺激時的交互關係，亦即基因調控網路(genetic networks, or gene regulatory networks)。基因調控網路的建立，可以幫助研究人員瞭解參與細胞內各種生化反應的因素，讓製藥、醫學、生化研究人員將實驗資源集中於重要的基因上；也可以作為研究人員的虛擬實驗平臺，由電腦來模擬他們所提出的假設。

準確地預測轉錄調控模組，才能重建正確的轉錄調控網路。因此，我們的研究目的就是提升轉錄控模組的預測能力，並以此建構具參考價值的轉錄調控網路。

目前已有許多不同的方法被應用於基因調控網路的重建，我們針對其優缺點做了評估，希望藉由已知的方法中尋找靈感以作為我們新系統的基礎。

三、結果與討論

我們特別針對一些常使用於基因調控網路重建的方法做了比較：

布林網路是以邏輯關係來描述基因調控網路。其核心假設為：

(1) 每一基因的表現程度可以分為高(表現)、低(不表現)兩種層次。

(2) 每一段基因的表現程度可以由其他基因的表現程度以及外在條件所形成的布林函式來決定。

這種表式法的優點是易於藉由邏輯關係解釋基因間的交互作用，而缺點則是資料需求量高。在一個 M 個節點的布林網路中，我們需要 2^M 個觀察點來求得正確的布林關係。Liang et al. 提出演算法試圖解決此一問題(Liang et al. 1998)，他們假設每一個基因的基因表現至多由 k 個輸入所決定，則解出 M 個節點的布林關係所需要的資料量的估計值為 $2^{k\log(M)}$。除了資料需求量過高外，布林網路的假設與在實際狀況也有所出入。因此，Silvescu 等人(Silvescu et al. 2001)及 Soinov 等人(Soinov et al. 2001)都以 Decision Tree 來建立布林網路，並且試圖修正一般的布林網路所忽略的時間因素。此外，Ilya Shmulevich 等人則為為布林網路加入機率模式，來模擬細胞中生化反應的不確定性(Shmulevich et al. 2002)。

線性模式是研究許多物理現象常用的數學模式，而以線性模式來表示基因調控網路的假設如下：

(1) 每一段基因的表現程度是連續的，而非離散狀態。

(2) 每一段基因的表現程度可以由其他基因的表現程度以及外在條件以線性方程式表達。

這種表示法優於布林網路的地方在於保留了基因表現的原始資訊，但缺點則是此方法並不如布林網路般易於解釋基因之間的交互作用。在一個 M 變數的線性模式中，所需的資料量為 M 個觀察點，但在目前的應用中，我們仍沒有那麼多的生物實驗資料對整個基因組求解。因此，D’haeseleer P. 等人以內插法方式來增加資料數目(D’haeseleer P. et al. 1999b)；而 van Someren E. P. 等人則利用分群法將基因表現相似者視為同一個節點，以減少整個基因調控網路的複雜度(van Someren E. P. et al. 2000)。

貝氏網路(Bayesia Networks)是 graphical models 之一，它以條件機率來表示基因調控網路中各基因間的相關性。其核心假設為：

(1)每一個節點可代表可觀察變數(information variables)，如基因表現；或者是不可觀察變數(latent variables)，如外在刺激、蛋白質表現、蛋白質結構、實驗誤差等。變數可以是連續或離散狀態。

(2)變數間的關係分為相關(dependency)以及條件獨立(conditional independence)。

(3) 給定資料 D 與假設的網路結構 S，若 S 的事後機率(posterior probability)p(S|D)越大，代表在以資料 D 為證據下，網路結構 S 成立的機率越大。

貝氏網路的優點在於其模式能夠包含不可觀察變數，並以事後機率來衡量可能的網路結構，這些都有助於模擬真實世界中的不確定性與變異性。其缺點是貝氏網路必須先提出可能的網路結構，才能以實驗資料來評估該結構的事後機率。而 M 個結點的網路，有 2^M 種可能的結構，因此計算出 global optimal solution 所需計算量過大，其時間複雜度為 NP-complete(Fisher et al. 1996)。Hartemink 等人則利用 AI 中常見的幾種 heuristic search 方法來找出 local optimal solution(Hartemink et al. 2002)。

根據以上的結論，我們決定採行整合型的系統以重建基因網路，整合多種資訊，包含轉錄調控區間、基因表現實驗，以及已知的轉錄因子結合區，其目的是預測被這些轉錄因子所調控的基因，也就是轉錄調控模組，並將預測的轉錄調控模組整合成轉錄調控網路。而預測轉錄調控模組的核心假設為：

(1)在其轉錄調控區間含有特定轉錄因子結合區的基因，可能會受到該轉錄因子的調控。

(2)基因產物的行為，大部份決定於基因表現程度。因此，產生轉錄因子所需的基因，其基因表現程度與轉錄因子的行為相關。

(3)轉錄因子所調控的基因，其基因表現程度會受轉錄因子影響；間接的，也會與構成轉錄因子的基因之表現程度相關。

Fujibuchi 等人曾經以酵母菌(Saccharomyces cerevisiae)為研究對象，整合酵母菌基因組的轉錄調控區序列、基因表現實驗、轉錄因子結合區等資

訊，定義出新的評分方式來對調控關係進行篩選(Fujibuchi W. et al. 2001)，其研究結果顯示在整合了多種資訊後，可以更準確地預測轉錄調控模組。Fujibuchi 等人的核心假設是受到共同轉錄因子所調控的基因，其表現行為相似。由於我們的假設與其有所不同，我們也將整合兩方的評方分式，希望以更完整假設來預測轉錄調控模組。

我們的整合型系統之重點在於結合 motif binding sites 與 expression profiles 的資訊，如此可加強基因調控模組的預測準確性，此外，即便在 motif binding sites 資訊欠缺的情況下，亦可藉由 expression profiles 的資訊找到逼近的較佳解，這樣便可提升我們系統的實用性。

為求將來實驗比較的統一性，我們已蒐集了與 Fujibuchi 等人的研究相同的實驗資料，其為 121 組在酵母菌上的基因表現實驗所得，此外，我們也依循 Spellman 等人做了資料的前置處理，我們計畫於第二年實做系統，並設計評估實驗。實驗目的將包含基因調控模組的預測準確性評估，以及利用已知的基因網路作為 benchmark 檢測系統的網路重建能力。

四、參考文獻

Cho R. J., Campbell M. J., Winzerler E. A., Steinmetz L., Conway A., Wodicka L., Wolfsberg T. G., Gabielian A. E., Landsman D., Lockhart D. J., and Davis R. W. (1998). A Genome-Wide Transcriptional Analysis of The Mitotic Cell Cycle. Molecular Cell, 2(1): 65-73.

Chu S., DeRisi J., Eisen M., Mulholland J., Botstein D., Brown P. O., and Herskowitz I. (1998). The Transcriptional Program of Sporulation in Budding Yeast. Science, 282(5389): 699-705.

DeRisi J. L., Iyer V. R., and Brown P. O. (1997). Exploring the Metabolic and Genetic Control of Gene Expression on A Genomic Scale. Science, 278(5338): 680-686.

D'haeseleer P., Wen X., Fuhrman S., and Somogyi R. (1997). Mining the Gene Expression Matrix: Inferring Gene Relationships from Large Scale Gene Expression Data. In: M.Holcombe, R.Paton(eds) Information Processing in Cells and Tissues. Plenum, 203-212.

D'haeseleer P., Liang S., and Somogyi R. (1999a). Gene Expression Analysis and Genetic Network Modeling. Pacific Symposium on Bioinformatics, Tutorial session on Gene Expression and Genetic Networks.

D'haeseleer P., Wen X., Fuhrman S., and Somogyi R. (1999b). Linear Modeling of mRNA Expression Levels during CNS Development and Injury. Pacific Symposium on Bioinformatics, 4: 41-52.

Eisen M. B., Spellman P. T., Brown P. O., and Bostein D. (1998). Cluster Analysis and Display of Genome-Wide Expression Patterns. Proceedings of National Academy of Sciences, 95(25): 14863-14868.

Fujibuchi W., Anderson J. S., and Landsman D. (2001). PROSPECT Improves Cis-Acting Regulatory Element Prediction by Integrating Expression Profile Data with Consensus Pattern Searches. Nucleic Acids Res., 29(19): 3988-3996.

Hartemink A. J., Gifford D. K., Jaakkola T. S., and Young R. A. (2001). Using Graphical Models and Genomic Expression Data to Statistically Validate Models of Genetic Regulatory Networks. Pacific Symposium on Bioinformatics, 6: 422-433.

Hartemink A. J., Gifford D. K., Jaakkola T. S., and Young R. A. (2002).

Combining Location and Expression Data for Principled Discovery of Genetic Regulatory Networks. Pacific Symposium on Bioinformatics, 6: 437-449.

Hertz, G. Z., Hartzell G. W. 3rd, and Stormo G. D. (1990). Identification of Consensus Patterns in Unaligned DNA Sequences Known to Be Functionally Related. Computer Applications in the Biosciences (now Bioinformatics), 6(2): 81-92.

Lashkari D. A., DeRisi J. L., McCusker J. H., Namath A. F., Gentile C., Hwang S. Y., Brown P. O., and Davis R. W. (1997). Yeast Microarrays for Genome Wide Parallel Genetic and Gene Expression Analysis. Proc. Natl. Acad. Sci. USA, 94(24): 13057-13062.

Lee T. I., Rinaldi N. J., Robert F., Qdom D. T., Bar-J. Z., Gerber G. K., Hannett N. M., Harbison C. T., Thompson C. M., Simon I., Zeitlinger J., Jennings E. G., Murray H. L., Gordon D. B., Ren B., Wyrick J. J., Tagne J.-B., Volkert T. L., Fraenkel E., Gifford K. D., and Richard A. (2002). Transcriptional Regulatory Networks in Saccharomyces cerevisiae. Science, 298(5594): 799-804.

Liang S., Fuhrman S., and Somogyi R. (1998). Reveal, A General Reverse Engineering Algorithm for Inference of Genetic Network Architectures. Pacific Symposium on Bioinformatics, 3: 18-29.

Mendenhall M. D., and Hodge A. E. (1998). Regulation of Cdc28 Cyclin-Dependent Protin Kinase Activity during the Cell Cycle of the Yeast Saccharomyces cerevisiae. Microbiol. Mol. Biol. Rev. 62(4): 1191-1243.

Shmulevich I., Dougherty E. R., Kim S., and Zhang W. (2002). Probabilistic Boolean Networks: A Rule-based Uncertainty Model for Gene Regulatory Networks. Bioinformatics, 18(2): 261-274

Silvescu A., and Honavar V. (2001). Temporal Boolean Network Models of Genetic Networks and Their Inference from Gene Expression Time Series. Complex Systems., 13(1): 54-.

Simon I., Barnett J., Hannett N., Harbison C. T., Rinaldi N. J., Volkert T. L., Wyrick J. J., Zeitlinger J., Gifford D. K., Jaakkola T. S., and Young R. A. (2001). Serial Regulation of Transcriptional Regulators in the Yeast Cell Cycle. Cell 106(6): 697-708.

Soinov L. A., Krestyaninova M. A., and Brazma A. (2003). Towards Reconstruction of Gene Networks from Expression Data by Supervised Learning. Genome Biology, 4(1): R6.

Spellman P. T., Sherlock G., Zhang M. Q., Iyver V.R., Anders K., Eisen M. B., Brown P. O., Bostein D., and Futcher B. (1998). Comprehensive Identification of Cell Cycle-Regulated Genes of the Yeast Saccharomyces Cerevisiae by Microarray Hybridization. Molecular Biology of the Cell, 9(12): 3273-3297.

Tamayo P., Slonim D., Mesirov J., Zhu O. Kitareewan S., Dmitrovsky E., and Golub T. R. (1999). Interpreting Patterns of Gene Expression with Self-Organizing Maps: Methods and Application to Hematopoietic Differentiation. Proc. Natl. Acad. Sci. USA, 96:2907-2912.

Tavazoie S., Hughes J. D. Campbell M. J., Cho R. J., and Church G. M. (1999). Systematic Determination of Genetic Network Architecture.

Nature Genet., 22:281-285.

van Someren E. P., Wessels L. F. A. and Reinders M. J. T. (2000). Linear Modeling of Genetic Networks from Experimental Data. Intelligent Systems for Molecular Biology, 8: 355-366.

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

95 年　4 月　27 日

附件一

報告人姓名	胡　毓　志	服務機構及職稱	交通大學資訊科學系 副教授
時間 會議 地點	06/26/2006-06/29/2006 Las Vegas, USA	本會核定補助文號	NSC 94-2213-E-009-143-
會議名稱	（中文）生物資訊暨計算生物學國際研討會 （英文)2006 International Conference on Bioinformatics and Computational Biology		
發表論文題目	1.（中文）以多策略方法設計蛋白質結構字元 （英文）A Multi-Strategy Approach to Protein Structural Alphabet Design		
	因期中進度報告繳交期限在研討會舉行之前，因此，僅附上會議論文，請參考。		

A Multi-strategy Approach to Protein Structural Alphabet Design

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Abstract

The search for structural similarity among proteins can provide valuable insights into their functional mechanisms and their functional relationships. Though the protein 1D sequence contains the information of protein folding, the performance of predicting the 3D-structure directly from the sequence is still limited. As the increase of available protein structures, we can now conduct more precise and thorough studies of protein structures. Among many is the design of protein structural alphabet that can characterize protein local structures. We use the self-organizing map combined with the minimum spanning tree algorithm for visualization to determine the alphabet size and then apply the k-means algorithm to group protein fragments into clusters corresponding to the structural alphabet. The intra-cluster and inter-cluster analyses show the significant structural cohesiveness. A comparative study of our alphabet with one of the recently developed structural alphabets also demonstrated a competitive result.

1. Introduction

Various genome sequencing projects have been producing numerous linear amino acid sequences; however, complete understanding of the biological roles played by these proteins requires knowledge of their structures and functions [1]. Despite that experimental structure determination methods provide reasonable structure information regarding subsets of proteins, computational methods are still required to provide valuable information for a large fraction of proteins whose statures may not be experimentally determined. Even though the primary sequence implies the whole information guiding the protein folding, yet the performance of predicting the 3D-structure directly from the sequence is still limited. The complexity and the number of physicochemical, kinetic and dynamic parameters involved in protein folding prohibit an efficient 3D-structure prediction without first knowing the 3D-structures of closely related proteins [2]. Some ab initio methods do not directly use 3D-structures, but their applications are often limited to small proteins [3].

Early analysis of protein structures has shown the importance of repetitive secondary structures, i.e. α-helix and β-sheet. With variable coils, they constituted a basic standard 3-letter alphabet, and this has led to early secondary structure prediction algorithms, e.g. GOR [4], and more recent ones that apply neural networks and homology sequences [5-8] with prediction accuracy approaching 80%. In spite of the increase of predictive accuracy, the approximation of 3D-structures with only a 3-letter alphabet is apparently too crude for meaningful 3D reconstruction. All the predictions are highly dependent on the definitions of periodic structures, but unfortunately the structure description is incomplete. As the increase of available protein structures, it allows more precise and thorough studies of protein structures. Various more complex structural alphabets have been developed by taking into account the heterogeneity of backbone protein structures through sets of small protein fragments frequently observed in different protein structure databases [2][9]. The alphabet size can vary from several to around 100. For example, Unger *et al.* [10] and Schuchhardt *et al.* [11] used k-means method and self-organizing maps respectively to identify the most common folds, but the large number of clusters (about 100) is not appropriate for prediction. Rooman *et al.* found 16 recurrent folding motifs, ranging from 4 to 7 residues and categorized into four classes corresponding to α-helix, β-strand, turn and coil [12]. By applying autoassociative neural networks, Fetrow *et al.* defined six clusters representing supersecondary structures that subsume the classic secondary structures [13]. Bystroff and Baker produced similar short folds of different lengths and grouped them into 13 clusters for prediction [14]. Taking into account the Markovian dependence, Camproux *et al.* developed an HMM approach to lean the geometry of the structural alphabet letters and the local rules for assembly process [15].

In this paper, we propose a multi-strategy approach to identifying structural alphabet that can characterize protein local structures. Instead of applying cross-validation [14] or shrinking procedures [16] to refine the clusters directly, we use self-organizing maps as a visualization tool to determine the size of structural alphabet. Given the alphabet size, we later apply the k-means algorithm [17] to group protein fragments into clusters that correspond to a structural alphabet. The analysis of structural similarities between proteins not only provides significant insight into functional mechanisms and biological relationships, but also offers the basis for protein fold classification. An expressive structural alphabet can allow us to quantify the similarities among proteins encoded in appropriate letters. It also enables us to work with a primary representation of 3D structures, simply using standard 1D amino acid sequence alignment methods. To demonstrate the performance of our new method, we tested it on the all-α proteins in SCOP. The experimental results show that using our structural alphabet rather than the standard amino acid letters can outperform BLAST in finding the best hit for a protein query. This suggests that our structural alphabet can successfully reflect protein structural characteristics which are implied in protein fragments. Besides, in order to make a consistent and fair comparison, we also compared our alphabet with others that are also developed by the SOM, but in a different design methodology [9][19]. Our structural alphabet shows competitive performance in protein matching.

2. Material and methods

The use of frequent local structural motifs embedded in polypeptide backbone has recently shown improvement in protein structure prediction [1][14][18]. Its success has shed some light on further studies of structural alphabet. We used the proteins classified to all-α fold within the SCOP database (version 1.65) in our study with the aim to build the structural alphabet suitable for all-α proteins. The same approach can be easily applied to other databanks as well.

There are three issues addressed in our study. They are: (1) protein fragment representation, (2) alphabet size determination and (3) structural alphabet definition. Like others, we transform each protein backbone into a series of the dihedral angles (ϕ and ψ, neglecting ω) [14][16]. Adapted from [16], the analysis is limited to fragments of five residues since they are adequate for describing a short α helix and a minimal β structure. With the fixed window size of five residues, we slid the window along each all-α protein in SCOP, advancing one position in the sequence for each fragment, and collected a set of overlapped 5-residue fragments. As the relation between two successive carbons, C_{α_i} and $C_{\alpha_{i+1}}$, located at the *i*th and (*i+1*)th positions, can be defined by the dihedral angles ψ_i of C_{α_i} and ϕ_{i+1} of $C_{\alpha_{i+1}}$, a fragment of *L* residues can then be defined as a vector of *2*(*L-1*) elements. Thus, in our study, each protein fragment, associated with α-carbons $C_{\alpha_{i-2}}$, $C_{\alpha_{i-1}}$, C_{α_i}, $C_{\alpha_{i+1}}$ and $C_{\alpha_{i+2}}$, is represented by a vector of eight dihedral angles, i.e. $[\psi_{i-2}, \phi_{i-1}, \psi_{i-1}, \phi_i, \psi_i, \phi_{i+1}, \psi_{i+1}, \phi_{i+2}]$. Based on this representation, we totally gathered 1,143,072 fragment vectors.

Self-organizing maps (SOM) are widely used as a data mining and visualization tool for complex data sets. A self-organizing map usually consists of a regular 2D grid of so-called map units, each of which is described by a reference vector $m_i = [m_{i1}, m_{i2}, m_{i3}, \ldots, m_{id}]$, where *d* is the input vector dimension, e.g., $d = 8$, in our case of fragment vectors. The map units are usually arranged in a rectangular or hexagonal configuration. The number of units affects the generalization capabilities of the SOM, and thus is often specified by the researcher/user. It can vary from a few dozen to several thousands. An SOM is a mapping from the ensemble of input data vectors ($X_i=[x_{i1}, x_{i2}, x_{i3}, \ldots, x_{id}] \in R^d$) to a 2D array of map units. During training, data points near each other in input space are mapped onto nearby map units to preserve the topology of the input space [19][20]. The SOM is trained iteratively. In each training step *t*, distances between a randomly picked input vector x_j and all the reference vectors are computed. The unit with the least distance is then selected as the winner unit and denoted by *w*. The winner unit and its topological neighbors are updated to move closer to input vector x_j in the input space by the following rule:

$$m_i(t+1) = m_i(t) + \alpha(t)h_{wi}(t)\left|x_j - m_i(t)\right|$$

where *t* is time, $\alpha(t)$ is the adaptation coefficient, $|x_j - m_i(t)|$ is the component-wise difference between the input vector and the *i*th reference vector, and $h_{wi}(t)$ is the neighborhood function acting on the array of units, whose form includes bubble kernel, Gaussian kernel and other more complicated ones. In our study, we used the bubble kernel [20][21]. Unlike previous works that directly apply SOM to obtain clusters of backbone fragments as the basis to define the structural alphabet, our approach instead uses SOM only for the visualization purpose to predetermine the number of letters in the alphabet.

By visual inspection of the trained SOM, we can get a preliminary idea of the number of clusters on the map. The unified distance matrix (U-matrix) is one of the most widely used methods for visualizing the clustering result on the SOM. It shows distances between neighboring reference vectors, and can be efficiently visualized using grey shade [22], as shown in Figure 1(a). In spite of the initial idea of the cluster structure provided by the U-matrix, a systematic method to determine the number of clusters on the map is still desired. We implement a post-process on the U-matrix that is based on the minimum-spanning-tree algorithm. Given the grey levels in the U-matrix, we can build the minimum spanning tree for all the map units, e.g., in Figure 1(b), all map unit are linked in the spanning tree. Based on a threshold of the grey level, we can partition the entire tree into several disconnected subtrees, by removing the links between map units with grey levels below the threshold, as shown in Figure 1(c). Conceptually, it means that we break the links of a distance longer than some threshold.

Furthermore, those relatively smaller subtrees left can be also deleted later such that the remaining clusters can maintain a reasonable size, as presented in Figure 1(d). The number of the subtrees finally kept becomes the structural alphabet size. As the SOM can be viewed as a topology preserving mapping from input space onto the 2D grid of map units [19], the number of map units can affect the clustering result. We systematically increase the number of units, and repeat the above process till the alphabet size stabilizes.

Rather than adapt the two-level approach that first trains the SOM, then performs clustering of the trained SOM [19], after determining the alphabet size, we apply the k-means algorithm to the input data vectors directly to obtain the clusters. The SOM established a local order among the set of reference vectors in such a way that the closeness between two reference vectors in the R^d space is dependent on how close the corresponding map units are in the 2D array. Nevertheless, an inductive bias of this kind may not be appropriate for structural alphabets since the local order does not always faithfully characterize the relation between structural building blocks, and can sometimes be misleading, e.g. forcing the topology to preserve mapping from the input space of α-helix and β-strand to a 2D grid of units could be harmful to clustering. As a result, we use the SOM only for visualization the alphabet size, and rely on the k-mean algorithm to extract the local features from the input data that can actually reflect the characteristics of the clusters respectively. The centroid of each cluster forms the prototypical representation of each alphabet letter. Given the clustering result by the k-means algorithm as the basis of the structural alphabet, we can transform a protein into a series of the alphabet letters by matching each of its fragments against our alphabet prototypes. The control flow of our system named SMK is illustrated in Figure 2.

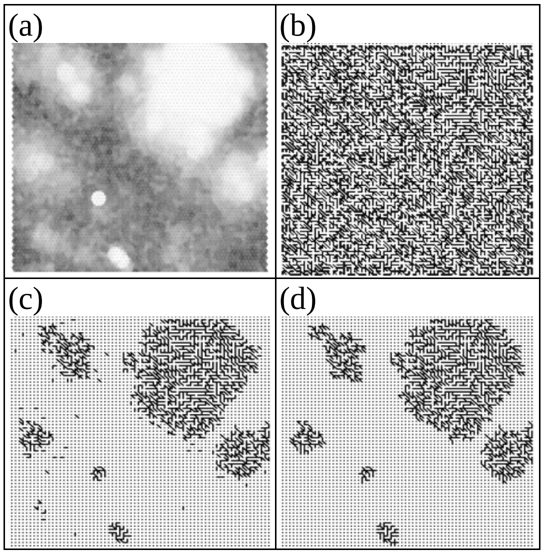


Figure 1. Visualization of the trained SOM. (a) the grey shade of the trained SOM, where darker areas mean larger distances, (b) the minimum spanning tree for the map units, (c) the disconnected subtrees after removing the links below some threshold and (d) the final disconnected subtrees after discarding those relatively small ones.

3. Experimental results

We tested our approach on the all-α proteins in SCOP. By this experiment, we show that our method can produce an appropriate structural alphabet for describing these all-α proteins. After transforming protein backbones into dihedral angles and extracting protein fragments, we trained the SOM on these dihedral angle vectors.

Three issues were addressed in the experiments. First, the meaningfulness of the structural alphabet size in terms of the number of clusters was presented by showing the size stability given various parameters. Second, we demonstrated cluster cohesiveness by visual superimpositions of protein fragments as well as computed the intra-cluster and inter-cluster distance. Third, we proved the fragment clusters found were not arbitrary by comparing our result with that from a random background.

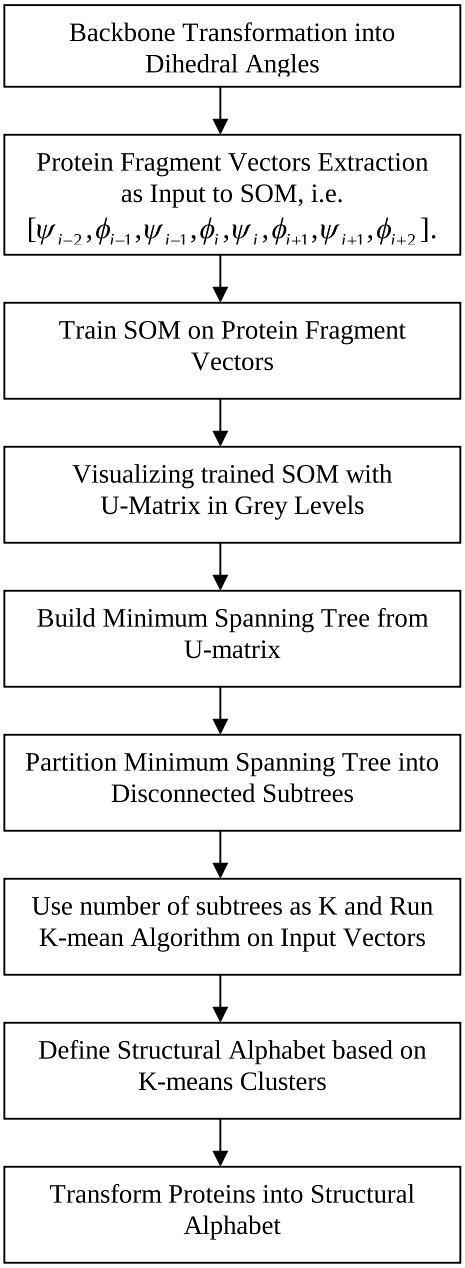

Backbone Transformation into Dihedral Angles
Protein Fragment Vectors Extraction as Input to SOM, i.e. $[\psi_{i-2},\phi_{i-1},\psi_{i-1},\phi_i,\psi_i,\phi_{i+1},\psi_{i+1},\phi_{i+2}]$.
Train SOM on Protein Fragment Vectors
Visualizing trained SOM with U-Matrix in Grey Levels
Build Minimum Spanning Tree from U-matrix
Partition Minimum Spanning Tree into Disconnected Subtrees
Use number of subtrees as K and Run K-mean Algorithm on Input Vectors
Define Structural Alphabet based on K-means Clusters
Transform Proteins into Structural Alphabet

Figure 2. The system control flow of SMK

Since the number of map units has influence over the SOM's clustering behavior, to obtain the optimal number of clusters, we varied the number of units on the map until the number of clusters found became steady. The results are shown in Figure 3, which indicates a distinctive plateau within the range between nine and twelve. Because eleven is the most frequent number of clusters on the plateau, as shown in Figure 4, it is designated as the structural alphabet size.

To further confirm the general geometric regularities characterized by the structural alphabet, we also built a negative all-α protein fragment set for comparison. The negative set was derived from the real all-α protein fragment vectors prepared earlier by rotating the dihedral angles at random (increase or decrease) within a certain degree, e.g. 30° in our analysis. We compared the clusters produced by clustering on the real vector set and on the negative control set. Insignificant difference suggests that the alphabet we found could be arbitrary. Our experiments (see Figure 5) show that clustering on the negative control set cannot even produce consistent clusters, which supports our hypothesis that the clusters found from the real fragment vectors reflect the classes of local protein structures; otherwise, these clustering results would have been similar.

Given the size, we ran the k-means algorithm on the input fragment vectors to find the twelve clusters by which to define the structural alphabet. Figure 6(a) and (b) shows the fragment superimpositions for the alphabet. Even though the fragment structures do not superimpose perfectly, yet the general structural cohesiveness of each category is quite evident. In addition, we computed the Euclidean distances from each fragment in a given cluster to its centroid. The average of these within-cluster distances was then compared with the center-to-center distances between clusters as presented in Table 1. It shows that in most cases, the center-to-center distance between any two clusters is greater than the mean distance of all vectors in that cluster from its center plus one standard deviation. The result indicates that the individual clusters are fairly well separated from each other.

The detection and analysis of structural similarities between proteins allows deeper insight into their functional mechanisms and relationships. To search for structural similarities, the structural alphabet provides a good basis on which to work with a 1D representation. As a result, numerous 1D alignment algorithms can be used, with minor modifications, to detect structural similarities. In our experiments, we first transformed the 3D structures of proteins into a 1D sequence of the letters in our structural alphabet. To demonstrate the applicability of the alphabet, we used FASTA to search for structural similarities between a query protein and a bank of proteins, using an identify matrix of our alphabet letters to find maximal exact matches. For comparison, we also conducted the same tests also using FASTA but based on different structural alphabets, one developed by de Brevern *et al.* [9], the other by the two-level SOM approach [19]. As the baseline reference, we used BLAST with the standard 20 amino acid letters to find the best sequence hit.

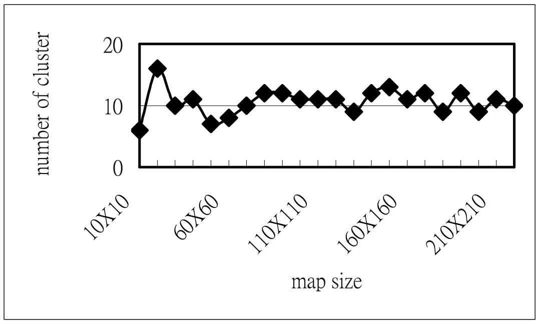


Figure 3. The variance in the number of clusters produced by the SOMs of varying sizes. There exists a distinctive plateau that suggests the cluster number has stabilized.

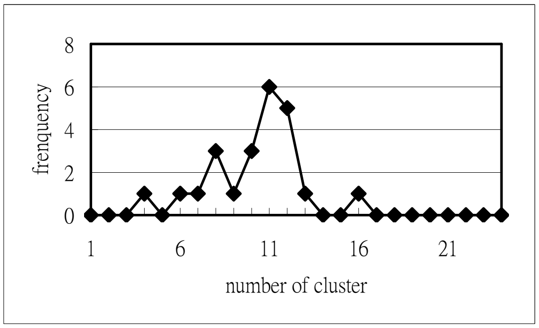


Figure 4. The frequencies of cluster numbers. It shows 11 is the most frequent number of clusters.

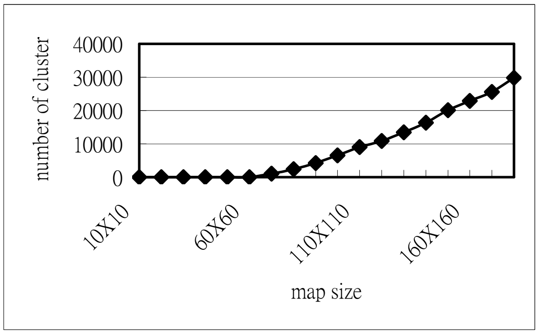


Figure 5. The variance in the number of clusters produced by the SOMs of varying sizes trained on a negative fragment set. It shows no sign of convergent cluster number.

The proteins used in the experiments were selected from the all-α proteins in SCOP. After filtering out those with more than 30% sequence similarity, we have totally 1055 proteins. For each run of the experiment, we randomly picked one protein as the query, and then matched it against the rest, using FASTA or BLAST with different alphabets. Given the best hit, we computed the RMSD between the query and the hit,

Table 1. Summary of within-cluster distances and center-to-center distances.

	within-cluster	center-to-center										
	mean±sd	***A***	***B***	***C***	***D***	***E***	***F***	***G***	***H***	***I***	***J***	***K***
A	186.10±68.07	0	282.3	205.27	216.75	226.93	236.72	399.53	246.5	325.94	197.44	245.81
B	192.84±74.97		0	284.59	203.41	202.8	275.08	414.99	169.3	321.03	208.28	264.69
C	173.58±77.59			0	250.31	251.6	197.76	383.86	243.02	333.41	188	226.52
D	193.67±69.19				0	234.31	252.05	388.9	261.9	323.81	183.77	233.33
E	150.41±71.53					0	302.93	511.04	284.51	343.02	282.19	358.48
F	143.62±90.84						0	346.14	220.63	346.98	161.11	177.48
G	220.52±87.79							0	343.07	276.03	341.22	278.5
H	155.02±77.8								0	335.84	136.63	164.87
I	196.75±97.2									0	358.58	360.95
J	88.77±53.33										0	86.711
K	43.15±50.13											0

and recorded the lowest level in the SCOP hierarchy at which the query and the hit are both located, i.e. class, fold, superfamily or family. Smaller RMSD and lower common level in SCOP hierarchy indicates higher structural similarity. We repeated the same experiment for 100 times and the results are summarized in Table 2 and 3. According to Table 2, we notice that our method SMK and de Brevern *et al.*'s both produced higher frequencies at lower common levels than the other two methods. This suggests that our structural alphabet and de Brevern *et al.*'s can better characterize the SCOP hierarchy. Table 3 shows that SMK has the lowest mean RMSD and standard deviation among all.

Table 2. Summary of frequencies at the lowest common level. The first column shows the methods used in the experiments. The remaining columns present the frequency for different levels at which the query and the best hit are both located.

	frequency at different level			
Method	**class**	**fold**	**super family**	**family**
BLAST	71	4	5	20
SMK	55	11	5	29
de Brevern	58	4	11	27
2-level SOM	73	6	14	7

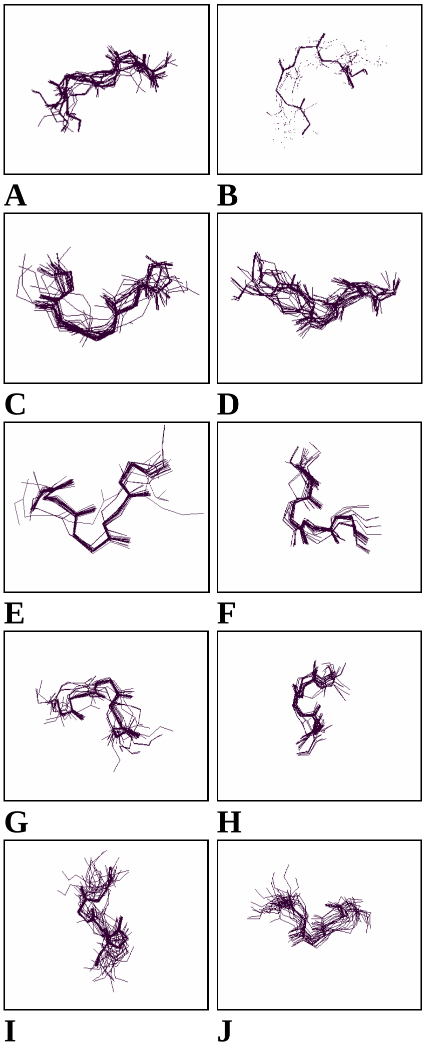
Table 3. Summary of average RMSD and standard deviation between the queries and the best hits.

method	**mean (RMSD)**	**sd (RMSD)**
BLAST	8.953744	4.764597
SMK	7.290972	3.934283
de Brevern	8.076746	4.819178
2-level SOM	10.38624	5.217078

4. Discussion

In this paper, we propose a multi-strategy approach to designing the structural alphabet which allows local approximation of protein

3D structures as well as enables the applications of 1D alignment algorithms to search for 3D structural similarities. The success of the alphabet design depends on three crucial factors. First, it is the protein fragment representation, which determines what and how 3D structural characteristics to be approximated, e.g. thermodynamic stability, amino acid physicochemical properties, amino acid usage in known proteins, distances, dihedral angles, bond lengths, bond angles, etc.



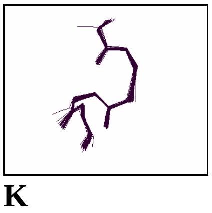


Figure 6(a). The superimposition in wireframe format for the structures of each structural cluster found by SMK.

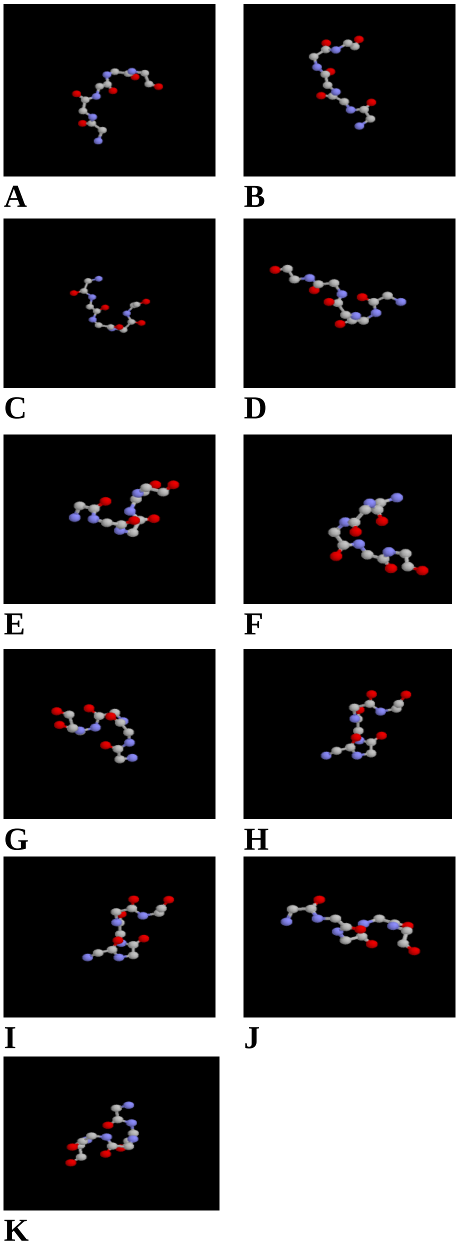


Figure 6(b). The superimposition of the structures of each structural cluster found by SMK in the ball-and-stick form.

The effects of the representation selected are entangled with the performance of the learning approach we apply to develop the structural alphabet. Overcomplicated representations can sometimes lead to overfitting. To avoid this problem, we currently focus on the dihedral angles. Other features can be easily included in the representation if proved necessary.

The second factor is the size of the alphabet. We took advantage of the SOM as a visualization tool that helps determine the alphabet size. By systematically varying the number of map units on the map, we visualized the clustering behavior of the SOM. Our experiments showed a distinct plateau corresponding to the convergent number of clusters, compared with the increasing number of clusters in the results of clustering on the random negative control dataset. This suggests that the structural alphabet size we found is not arbitrary.

Various types of algorithms have been applied to clustering local protein 3D fragments into a limited set of fold patters, e.g. self-organizing maps (SOM), hidden Markov models (HMM), neural networks, hierarchical clustering, k-means clustering, etc. Each has its own learning bias and inherent limitations. For example, the topology (e.g. number of layers or map units) of neural networks, the SOM and the HMM strongly affect the performance. The value of k in k-means algorithm determines the clusters. As a consequence, the third factor is the learning algorithm. In our study, we took a multi-strategy approach. We first used the SOM and the minimum-spanning tree algorithm to determine the alphabet size, and then applied the k-means algorithm to group fragments into meaningful clusters. The number of map units in the SOM and the value of k in k-means are not pre-specified in advance, but instead determined systematically. To verify the correspondence of our structural alphabet letter to the fold patterns, we computed the average within-cluster distance for each alphabet cluster as well as the distance across clusters. The small average within-cluster distance and the relatively large between-cluster distance demonstrate the significance of the structural alphabet we found. Furthermore, the visualized superimposition of protein fragments in each cluster also justifies the structural cohesiveness.

The objective of the paper is to propose a new approach to developing the structural alphabet. To verify its usefulness, we tested it on the all-α proteins in SCOP, and the experimental results show its promising applicability. After the success on the all-α proteins in SCOP, we plan to test our method on different data banks to further verify its feasibility and generality. Also as mentioned above, the representation is a crucial factor in the alphabet design. We will consider other structural features besides dihedral angles, add more useful features to enhance our structural alphabet, and test the new approach on other families in SCOP.

5. References

[1] D. Baker and A. Sali, “Protein Structure Prediction and Structural Genomics”, *Science*, vol. 294, 2001, pp. 93-96.

[2] A.G. de Brevern and S.A. Hazout, “Hybrid Protein Model(HPM): a method to compact protein 3D-structure information and physicochemical properties”, *IEEE Comp. Soc*. S1, 2000, pp. 49-54.

[3] C.A. Orengo, J.E. Bray, T. Hubbard, L. LoConte and I. Sillitoe “Analysis and

assessment of ab initio three-dimensional prediction, secondary structure, and contacts prediction", *Protein*, vol. 37, 1999, pp. 149-170.

[4] J. Garnier, D. Osguthorpe and B. Bobson, "Analysis of the accuracy and implications of simple methods for predicting the secondary structure of globular protein", *Journal of Molecular Biology*, vol. 120, 1978, pp. 97-120.

[5] B. Rost and C. Snader, "Prediction of protein secondary structure at better than 70% accuracy", *Journal of Molecular Biology*, vol. 232, 1993, pp. 584-599.

[6] A. Salamov and V. Solovyev, "Protein secondary structure prediction using local alignments", *Journal of Molecular Biology*, vol. 268, 1997, pp. 31-36.

[7] T.N. Petersen, C. Lundegaard, M. Nielsen, H. Bohr, J. Bohr, S. Brunak, G.P. Gippert and O. Lund, "Prediction of protein secondary structure at 80% accuracy", *Proteins*, vol. 41, 2000, pp. 17-20.

[8] B. Rost, "Review: Protein secondary structure prediction continues to rise," *Journal of Structural Biology*, vol. 134, 2001, pp. 204-218.

[9] A.G. de Brevern, H. Valadie, S.A. Hazout and C. Etchebest, "Extension of a local backbone description using a structural alphabet: A new approach to the sequence-structure relationship," *Protein Science*, vol. 11, 2002, pp. 2871-2886.

[10] R. Unger, D. Harel, S. Wherland and J.L. Sussman, "A 3D building blocks approach to analyzing and predicting structure of proteins", *Proteins*, vol. 5, 1989, pp. 355-373.

[11] J. Schuchhardt, G. Schneider, J. Reichelt, D. Schomburg and P. Wrede, "Local structural motifs of protein backbones are classified by self-organizing neural networks", *Protein Engineering*, vol. 9, 1996, pp. 833-842.

[12] M.J. Rooman, J. Rodriguez and S.J. Wodak, "Automatic definition of recurrent local structure motifs in proteins", *Journal of Molecular Biology*, vol. 213, 1990, pp. 327-336.

[13] J.S. Fetrow, M.J. Palumbo and G. Berg, "Patterns, structures, and amino acid frequencies in structural building blocks, a protein secondary structure classification scheme", *Proteins*, vol. 27, 1997, pp. 249-271.

[14] C. Bystroff and D. Baker, "Prediction of local structure in proteins using a library of sequence-structure motif", *Journal of Molecular Biology*, vol. 281, 1998, pp. 565-577.

[15] A.C. Camproux, R. Gautier and P. Tuffery, "A hidden Markov model derived structural alphabet for proteins", *Journal of Molecular Biology*, doi: 10.1016/j.jmb.2004.04.005.

[16] A.G. de Brevern, C. Etchebest and S. Hazout, "Bayesian probabilistic approach for predicting backbone structures in terms of protein blocks", *Proteins*, vol. 41, 2000, pp. 271-287.

[17] J.A. Hartigam and M.A. Wong, "A k-means clustering algorithm", *Applied Statistics*, vol. 28, 1975, pp. 100-108.

[18] K.T. Simons, I. Ruczinski, C. Kooperberg, B.A. Fox, C. Bystroff and D. Baker, "Improved recognition of native-like protein structures using a combination of sequence-dependent and sequence-independent features of proteins", *Proteins*, vol. 34, 1999, pp. 82-95.

[19] J. Vesanto and E. Alhoniemi, "Cluster of the self-organizing map", *IEEE trans. Neural Networks*, vol. 11, 2000, pp. 586-600.

[20] T. Kohonen, "Self-organizing Maps", Berlin/Heidelberg, Germany; *Springer*, Vol. 30, 1995.

[21] P. Tamayo, D. Slonim, J. Mesirov, Q. Zhu, S. Kitareewan, E. Dmitrovsky, E.S. Lander and T.R. Golub, "Interpreting patterns

of gene expression with self-organizing maps: Methods and applications to hematopoietic differentiation", *PNAS*, vol. 96, 1999, pp. 2907-2912.

[22] J. Iivarinen, T. Kohonen, J. Kangas and S. Kaski, ”Visualizing the clusters on the self-organizing map", in Proc. Conf. Artificial Intelligence Research Finland, 1994, pp. 122-126.