Order independent structural alignment of circularly permuted proteins

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Abstract—Circular permutation connects the N and C termini of a protein and concurrently cleaves elsewhere in the chain, providing an important mechanism for generating novel protein fold and functions. However, their in genomes is unknown because current detection methods can miss many occurrences, mistaking random repeats as circular permutation. Here we develop a method for detecting circularly permuted proteins from structural comparison. Sequence order independent alignment of protein structures can be regarded as a special case of the maximum-weight independent set problem, which is known to be computationally hard. We develop an efficient approximation algorithm by repeatedly solving relaxations of an appropriate intermediate integer programming formulation, we show that the approximation ratio is much better than the theoretical worst case ratio of $r = 1/4$. Circularly permuted proteins reported in literature can be identified rapidly with our method, while they escape the detection by publicly available servers for structural alignment.

Keywords—circular permutations, integer programming, linear programming, protein structure alignment

INTRODUCTION

A circularly permuted protein arises from ligation of the N and C termini of a protein and concurrent cleavage elsewhere in the chain [1, 2]. In nature, circular permutation often originate from tandem repeats via duplication of the C-terminal of one repeat together with the N-terminal of the next repeat, as is the case of swaposin. Another mechanism is ligation and cleavage of peptide chains during post-translational modification, as is the case of concanavalin A. The full extent of circular permutation and the biological consequences are currently unknown. Discovery of circular permutation at genome wide scale will enable systematic studies of its contribution to the generation of novel protein function and novel protein fold.

Currently, sequence alignment based methods are the only available tools for detecting circular permutations. These include postprocessing output from standard dynamic programming methods, as well as customized algorithms [3]. Sequence based methods can miss many circularly permuted proteins, because either one or both fragments may escape detection by local alignment if the two proteins are distantly related.

Protein structures are far more conserved than sequences, and they can reveal very distant evolutionary relationship [4]. Jung et. al. [5] showed that there is potential to discover remotely related protein domains by artifically permuting proteins and superimposing them on native domains in the protein structure database. Detecting circular permutation from structures has the promise to uncover many more ancient permutation events that escape sequence methods.

In this study, we describe a new algorithm for detecting circular permutation in protein structures. We show with examples that our algorithm can align circular permutations reported in literature. Our work introduces an efficient approximate method for protein substructure comparison. Two protein structures are first cut into pieces exhaustively of varying lengths and then compared. An approximation algorithm is used to search for optimal combination of peptide pieces from both structures. With the special nature of spatial distribution of proteins, a fractional version of local-ratio approach for scheduling split-interval graphs works well for detecting very similar spatial substructures. Our experimentation showed that the approximation ratio is excellent, with an average value of at least $1/2.53$.

STRUCTURAL ALIGNMENT OF CIRCULARLY PERMUTED PROTEINS

Three simplified protein structures that are related by circular permutation are shown in Figure 1. The three corresponding domains (labeled A, B, and C, respectively) are all very similar across proteins. The global spatial arrangements of these three domains are also very similar. However, the orderings of these domains in primary sequence are completely different.

The discontinuity and different ordering of spatially neighboring domains in primary sequence makes the detect-
tion of global structural similarity. Most structural alignment methods are unable to connect the break where circular permutation occurs, and would prematurely terminates the alignment. In addition, the reverse ordering of residues within a domain between two proteins also poses challenge for such methods. For example, the A domain in Figure 1a is ordered from N to C. The same domain in Figure 1b is ordered from C to N. The algorithm outlined below is well-suited to solve these types of challenging problems.

**Basic Methodology** We first introduce some notations: A substructure $\lambda_a$ of a protein structure $S_a$ is a continuous fragment $\lambda_a = (a_1, a_2) \in \Lambda_a$, where $a_1, a_2$ are the residue numbers of the beginning and end of the substructure, respectively, and $1 \leq a_1 < a_2 \leq |S_a|$. A residue $t \in \lambda_a$ is part of substructure $\lambda_a$ if $a_1 \leq t \leq a_2$. $\Lambda_a$ is the set of all possible continuous substructures or fragments of protein structure $S_a$, i.e., $\Lambda_a = \{(a_1, a_2)\}$. $S_b, \lambda_b, b_1, b_2$, and $\Lambda_b$ are similarly defined.

The problem of protein substructure comparison is captured in the following *Basic Substructure Similarity Identification* (BSSI$_{\Lambda,\sigma}$) problem.

**Definition 1 Instance**: a set $\Lambda \subseteq \Lambda_a \times \Lambda_b$ of ordered pairs of substructures of $S_a$ and $S_b$ and a similarity function $\sigma : \Lambda \to \mathbb{R}_+$ mapping these pairs of substructures to similarity values (non-negative real numbers).

**Valid solutions**: a set $\{\lambda_1, \lambda_2, \ldots, \lambda_k\} = \{(\lambda_{a1,1}, \lambda_{b1,1}), (\lambda_{a2,2}, \lambda_{b2,2}), \ldots, (\lambda_{ak,k}, \lambda_{bk,k})\}$ of pairs of substructures such that $\lambda_{i,j} \in \Lambda_{i}$ and $\lambda_{i,j} \in \Lambda_{j}$ are disjoint for $\ell \in \{a, b\}$ and $i \neq j$.

**Objective**: maximize the total similarity of the selection \[ \sum_{i=1}^{n} \sigma(\lambda_{a,i}, \lambda_{b,i}). \]

The BSSI$_{\Lambda,\sigma}$ problem is a special case of the famous maximum-weight independent set (MWIS) problem in graph theory [6]. BSSI$_{\Lambda,\sigma}$ is MAX-SNP-hard even when the substructures are restricted to short lengths [7]. Our approach is to adopt the approximation algorithm for scheduling split-interval graphs [7], which is based on a fractional version of the local-ratio approach. We introduce the following definitions:

- The conflict graph $G_\Delta = (V_\Delta, E_\Delta)$ for any subset $\Delta \subseteq \Lambda$, is a graph in which $V_\Delta = \Delta$ and $E_\Delta$ consists of all distinct pairs of vertices $\{(\lambda_i, \lambda_j), (\lambda'_i, \lambda'_j)\}$ from $V_\Delta$ such that $\lambda_i$ and $\lambda'_i$ are not disjoint for some $i \in \{a, b\}$.

\[ \frac{1}{1-\epsilon} \] A maximization problem being MAX-SNP-hard implies that there exists a constant $0 < \epsilon < 1$ such that no polynomial time algorithm can return a solution with a value of at least $(1 - \epsilon)$ times the optimum value.

| Table I: The LP formulation of the BSSI problem. |
| --- |
| **Maximize** \[ \sum_{\lambda \in \Lambda} \sigma(\lambda) \cdot x_\lambda \] |
| subject to:
| \[ \sum_{\lambda, \lambda \in \Lambda_a \& \lambda = (\lambda_a, \lambda_b) \in \Lambda} \gamma_{\lambda, \lambda} \leq 1 \quad \forall \lambda \in \Lambda_a \] (1) |
| \[ \sum_{\lambda, \lambda \in \Lambda_b \& \lambda = (\lambda_a, \lambda_b) \in \Lambda} \gamma_{\lambda, \lambda} \leq 1 \quad \forall \lambda \in \Lambda_b \] (2) |
| \[ \gamma_{\lambda_a, \lambda_b} - x_\lambda \geq 0 \quad \forall \lambda \in \Lambda_a \] (3) |
| \[ \gamma_{\lambda_a, \lambda_b} - x_\lambda \geq 0 \quad \forall \lambda \in \Lambda_b \] (4) |
| \[ x_\lambda, \gamma_{\lambda_a, \lambda_b}, \lambda, \lambda \geq 0 \quad \forall \lambda \in \Lambda \] (5) |

- The closed neighborhood of a vertex $v$ of $G_\Delta$, denoted by $Nbr_\Delta[v]$, is defined as $\{u \mid \{u, v\} \in E_\Delta\} \cup \{v\}$.

The recursive algorithm for solving BSSI$_{\Lambda,\sigma}$ is as follows:

- Remove every substructure pairs $\lambda = (\lambda_a, \lambda_b) \in \Lambda$ such that $\sigma(\lambda_a, \lambda_b) \leq 0$. If $\Lambda = \emptyset$ after these removals, then return $\emptyset$ as the solution.

- Solve a linear programming (LP) formulation of the BSSI$_{\Lambda,\sigma}$ problem by relaxing a corresponding integer programming version of the BSSI$_{\Lambda,\sigma}$ problem. For every $\lambda = (\lambda_a, \lambda_b) \in \Lambda$, introduce three indicator variables $x_\lambda, \gamma_{\lambda_a, \lambda_b}$ and $\gamma_{\lambda_a, \lambda_b}$ in $\{0, 1\}$, but relaxed to real numbers. The LP formulation is shown in Table I.

- For every vertex $\lambda \in \Lambda$ of $G_\Lambda$, compute its local conflict number $\alpha_{\Lambda,\sigma} = \sum_{\mu \in Nbr_\Lambda[\lambda]} x_\mu$. Let $\lambda_{min}$ be a vertex with the minimum local conflict number $r_{\Lambda,\sigma} = \min_{\lambda} \{\alpha_{\Lambda,\sigma}\}$. Define a new similarity function $\sigma_{new}$ from $\sigma$ as follows:

\[ \sigma_{new}(\lambda) = \begin{cases} \sigma(\lambda), & \text{if } v \notin Nbr_\Lambda[\lambda_{min}] \\ \sigma(\lambda) - \sigma(\lambda_{min}), & \text{otherwise} \end{cases} \] .

- Recursively solve the BSSI$_{\Lambda,\sigma_{new}}$ problem using the same approach. Let $\Lambda' \subseteq \Lambda$ be the solution returned.

- If the substructure pair $\lambda_{min}$ can be selected together with all the pairs in $\Lambda'$ then return $\Lambda' \cup \{\lambda_{min}\}$ as the solution, else return $\Lambda'$ as the solution.

A brief explanation of the various inequalities in the LP formulation as described above is as follows:

- The *interval clique inequalities* in (1) (resp. (2)) ensures that the various substructures of $S_a$ (resp. $S_b$) in the selected pairs of substructures from $\Lambda$ are mutually disjoint.

- Inequalities in (3) and (4) ensure consistencies between the indicator variable for substructure pair $\lambda$ and its two substructures $\lambda_a$ and $\lambda_b$.

- Inequalities in (5) ensure non-negativity of the indicator variables.
Implementation and Computational Details. We present a simplified example for illustration two protein structures \( S_a \) and \( S_b \) (Figure 2a) and \( S_b \) (Figure 2a) are selected for alignment. Here \( S_b \) is the structure to be aligned to the reference structure \( S_a \). We systematically cut \( S_b \) into fragments of length 7–25 and exhaustively compute a similarity score of each fragment from \( S_b \) to all possible fragments of equal length in \( S_a \). Each fragment pair can be thought of as a vertex in a graph, as shown in Figure 2b.

Suppose we have the following similarity scores for aligned substructures: \( \sigma(\lambda_1) = \sigma((1,3)(1',3')) = 8, \sigma(\lambda_2) = \sigma((4,5)(4',5')) = 5, \sigma(\lambda_3) = \sigma((4,7)(4',7')) = 7, \sigma(\lambda_4) = \sigma((6,8)(6',8')) = 3, \) and \( \sigma(\lambda_5) = \sigma((9,10)(9',10')) = 6. \)

We can describe the problem of selecting the best structural fragment pairs as to maximize \( 8x_{\lambda_1} + 5x_{\lambda_2} + 7x_{\lambda_3} + 3x_{\lambda_4} + 6x_{\lambda_5}. \)

Figure 2b shows the conflict graph for the set of fragments. A sweep line (shown as dashed lines in Figure 2c) is implicitly constructed \( (O(n) \) time after sorting) to determine which vertices of fragment pair overlap. A conflict is shown in Figure 2b as edges between vertices. Vertices \( \lambda_1 \) and \( \lambda_5 \) do not conflict with any other fragments, while \( \lambda_2 \) and \( \lambda_3 \) conflict with \( \lambda_3 \). For this graph, the constraints in the linear programming formulation are shown in Table II. The linear programming problem is solved using the Benders package [8].

The algorithm guarantees that there is a vertex \( \lambda_i \) that satisfies \( x_{\lambda_i} + \sum_{j \in \text{Nbr}[\lambda_i]} x_{\lambda_j} \leq 4 \), and all vertices are searched to find such a vertex. We then update \( \sigma(\lambda_i) \) for vertices that are neighbors of \( \lambda_i \): \( \sigma_{\text{new}}(\lambda_i) = \sigma(\lambda_i) - \sigma(\lambda_j) \) if \( \lambda_j \in \text{Nbr}[\lambda_i] \) Vertices that have no neighbors, such as \( \lambda_1 \) and \( \lambda_5 \) in our example, are included in our final solution. Using the updated values of \( \sigma(\lambda_i) \), we remove vertices of substructure pairs that have score less than 0, and recursively solve the (smaller) LP problem again. The selected final set of fragments are then used to construct a substructure of \( S_b \). They are then aligned structurally by minimizing cRMSD.

Table II: The constraints of the conflict graph for the set of fragments in Figure 2c.

| Interval Clique inequality: | Line sweep at 1 |
|----------------------------|-----------------|
| \( y_{\lambda_1, \lambda_6} \leq 1 \) | Line sweep at 1 |
| \( y_{\lambda_2, \lambda_6} + y_{\lambda_3, \lambda_6} \leq 1 \) | Line sweep at 4, 5 |
| \( y_{\lambda_2, \lambda_6} + y_{\lambda_4, \lambda_6} \leq 1 \) | Line sweep at 6, 7 |
| \( y_{\lambda_2, \lambda_6} \leq 1 \) | Line sweep at 8 |
| \( y_{\lambda_3, \lambda_6} \leq 1 \) | Line sweep at 9 |

| Interval Clique inequality: | Line sweep at 1' |
|----------------------------|-----------------|
| \( y_{\lambda_1, \lambda_6} \leq 1 \) | Line sweep at 4', 5' |
| \( y_{\lambda_2, \lambda_6} + y_{\lambda_3, \lambda_6} \leq 1 \) | Line sweep at 6', 7' |
| \( y_{\lambda_2, \lambda_6} \leq 1 \) | Line sweep at 8' |
| \( y_{\lambda_3, \lambda_6} \leq 1 \) | Line sweep at 9' |

| Interval Clique inequalities: | (3, 4) |
|--------------------------------|-------|
| \( y_{\lambda_1, \lambda_3} - y_{\lambda_1} \geq 0 \) | \( y_{\lambda_1, \lambda_6} - y_{\lambda_1} \geq 0 \) |
| \( y_{\lambda_2, \lambda_3} - y_{\lambda_2} \geq 0 \) | \( y_{\lambda_2, \lambda_6} - y_{\lambda_2} \geq 0 \) |
| \( y_{\lambda_3, \lambda_3} - y_{\lambda_3} \geq 0 \) | \( y_{\lambda_3, \lambda_6} - y_{\lambda_3} \geq 0 \) |
| \( y_{\lambda_4, \lambda_3} - y_{\lambda_4} \geq 0 \) | \( y_{\lambda_4, \lambda_6} - y_{\lambda_4} \geq 0 \) |
| \( y_{\lambda_5, \lambda_3} - y_{\lambda_5} \geq 0 \) | \( y_{\lambda_5, \lambda_6} - y_{\lambda_5} \geq 0 \) |

Similarity score. The similarity score \( \sigma(\lambda) = \sigma(\lambda_a, \lambda_b) \) between two aligned substructures \( \lambda_a \) and \( \lambda_b \) is a weighted sum of a shape similarity value derived from cRMSD value and a sequence composition score (SCS): \( \text{SCS} = \sum_{i=1}^{N} B(A_{a,i}, A_{b,i}) \), where \( A_{a,i} \) and \( A_{b,i} \) are the amino acid residue type at aligned position \( i \), \( N \) the number of residues in the aligned fragment, and \( B(A_{a,i}, A_{b,i}) \) the similarity score between \( A_{a,i} \) and \( A_{b,i} \) by the modified BLOSUM50 matrix, in which a constant is added to all entries such that the smallest entry is 1.0. The similarity
score $\sigma(\lambda_a, \lambda_b)$ is calculated as follows:

$$\sigma(\lambda_a, \lambda_b) = \frac{\alpha(C - \text{cRMSD}) + \beta(\text{SCS})}{N}.$$  

For the current implementation, $C = 60, \alpha = 6, \text{ and } \beta = 1$.

To improve computational efficiency, a threshold measure is introduced to immediately exclude low scoring fragment pairs from further consideration. Only fragment pairs scoring above the threshold will be candidates to be included in the final solution. In the above example, we assume that only fragment pairs represented by vertices $\lambda_1, \lambda_2, \lambda_3, \lambda_4, \text{ and } \lambda_5$ (Figure 2b) are above the threshold.

**RESULTS**

We discuss the structural alignments of several well known examples of naturally occurring circularly permuted proteins.

The results including other examples are summarized in Table III, which includes two additional examples of experimentally constructed human-made permuted proteins. None of them are found by existing servers.

The first naturally occurring circular permutation was found in concanavalin A and lectin favin. The structures of lectin from garden pea (1rin) and concanavalin A (2cna) (Figure 3a,b). In the structural alignment, three fragments align over 45 residues with a root mean square distance of 0.82Å. The superposition based on the aligned fragments is shown in Figure 3b. The residues spanning the break due to the circular permutation are highlighted in red.

**DISCUSSION**

The approximation algorithm introduced in this work can find good solutions for the problem of detecting circular permuted proteins. In contrast to methods based on sequence alignment alone, the ability to incorporate both structural and sequence similarity is critical. An experimentation in the alignment of 1rin and 2cna illustrates this point. After turning off the contribution from cRMSD in the similarity function $\sigma(\lambda_a, \lambda_b)$ by setting $\alpha = 0$, we find altogether 452 fragments that score in the top 10% of the set of all SCS scores. This number is too large as the size of the conflict graph will be large. This makes subsequent comparison very difficult. Similarly, cRMSD measurement alone is inadequate for comparison. When matching fragments from 1rin and 2cna, we find there are 287 fragments that can be aligned with an cRMSD value < 3.0 Å after setting $\beta = 0$. The use of composite similarity function is therefore essential to reduce false positives in substructure alignments.

In our method, scoring function plays pivotal role in detecting substructure similarity of proteins. Much experiments are needed to optimize the similarity scoring system for general sequence order independent structural alignment. A necessary ingredient for a fully automated search of circular permuted proteins in database is a statistical measurement of significance of matched substructures. Since cRMSD measurement and therefore $\sigma(\lambda_a, \lambda_b)$ strongly depends on the length of matched parts and the gaps of the sequence break, a statistical model assessing the $p$-value of a measured $\sigma(\lambda)$ against a null model needs to be developed. Circular permutations that are likely to be biologically significant can then be automatically declared [4].

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