CryoEM Skeleton Length Estimation using a Decimated Curve

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Abstract - Cryo-electron Microscopy (cryoEM) is an important biophysical technique that produces 3-dimensional (3D) images at different resolutions. De novo modeling is becoming a promising approach to derive the atomic structure of proteins from the cryoEM 3D images at medium resolutions. Distance measurement along a thin skeleton in the 3D image is an important step in de novo modeling. In spite of the need of such measurement, little has been investigated about the accuracy of the measurement in searching for an effective method. We propose a new computational geometric approach to estimate the distance along the skeleton. Our preliminary test results show that the method was able to estimate fairly well in eleven cases.

Skeleton, computational geometry, electron microscopy, protein, length

I. INTRODUCTION

Electron cryo-microscopy (cryoEM) continues to produce 3-dimensional (3D) images of large protein complexes with a wide range of resolutions, from about 3 Å to over 80 Å. However, only an extremely small portion of such 3D images has been resolved to the atomic structures of the samples [1]. De novo modeling has been demonstrated as a promising method to derive atomic protein structures from 3D images at medium resolution, such as 5-10 Å [2-4], without relying on the availability of a template structure such as those found in the Protein Data Bank (PDB). Figure 1 illustrates the major steps in de novo modeling to generate an initial atomic structure. The input information includes the 3D image, called the protein density map, and the amino acid sequence of the protein. First, image processing methods help detect secondary structures such as α-helices (red sticks in Figure 1) and β-sheets in the 3D image [5-10], and secondary structure prediction tools determine their location on the protein sequence [11-14]. Next, skeletonization methods detect the skeleton connection (green in Figure 1) in the 3D image [4, 15]. The topology of the secondary structures can then be inferred by combining the two sources of information about the secondary structures, one from the 3D image and the other from the protein sequence [2, 16]. Once the topology of the secondary structures is determined, the atomic structure will be modeled.

Figure 1: The de novo modelling approach. The two inputs are the protein SSEs on the amino acid sequence and the 3D image (density map) at the medium resolution. The helices (red sticks) were detected using SSELearner [10] and the skeleton (green) was detected using Gorgon [4]. The native protein structure is shown as a ribbon (purple).

A protein sequence has a direction, from the N-terminal to the C-terminal. When the protein sequence is threaded in the 3D image, it visits the detected helix sticks in a unique order. The determination of this order is the topology problem. The
topology of the Secondary Structure Elements (SSEs) is a critical piece of information in protein structure prediction. It has been demonstrated that the topology of the helix sticks can be derived using graph-matching approaches [2, 16]. Our previous work translated the graph-matching problem into one in which we find a constrained shortest path in a topology graph, for which we presented an $O(N^2 2^N)$ dynamic programming algorithm [2]. The idea of topology determination is to use the distances between the SSEs as a metric in matching. For example, two helices 5 Å apart in the 3D image should be matched to two helices of similar distance in the protein sequence. The distance between two helices on the amino acid sequence can be translated into the distance in 3D space by assuming a 3.8 Å separation between two adjacent amino acids on the sequence. The assumption is that the correct topology results in the overall best match when all pairs of helices are considered.

Skeletonization of the 3D image has been shown to be an important technique to extract the connections between the SSEs. The skeleton can be derived using thinning and pruning methods [17]. It roughly represents the major paths in the 3D image. The skeleton is a set of grid points, or voxels, along the paths that appears to zigzag. Ideally, the distance between two specific ends of two helices should be measured along the skeleton connecting the two ends. If we simply add the length of the line segments along the path, there is a danger of overestimation due to the potential zigzag nature of the path. Moreover, the skeleton is expected to contain errors, since the 3D image often contain errors. It is not clear if the skeleton length estimation methods are accurate enough for topology determination.

This paper introduces a new method to approximate the distances between the detected SSEs from a density map, for use in the matching algorithm. In particular, we measure the length along the skeleton using a combination of graph-theoretic and computational geometric methods. We tested the method using a small dataset consisting of the experimentally derived 3D images from the Electron Microscopy Data Bank (EMDB). The measured length appears to agree with the expected length when the atomic structure of the turn aligns well with the skeleton. In our future work, we expect to perform an extensive evaluation of the algorithm and fine-tune it accordingly.

II. METHODOLOGY

**Problem:** estimate the length along a skeleton connecting two $\alpha$-helices in the 3D image of a protein.

The solution, which is an approximation, is described in the flow chart in Figure 2. There are three basic steps: (1) pre-process the skeleton, (2) construction of the trees and paths, and (3) decimation of the paths derived from step (2).

### A. Processing the Skeleton

Initially, we needed to isolate specific instances of the helix-turn-helix motif in available protein cryoEM images. In an actual molecule, there are many helices, and therefore many segments of backbone that fall between adjacent pairs of helices. For the purpose of demonstration, we extracted regions from the 3D images containing a single pair of helices and the backbone turn in between.

![Flow Chart](image)

### B. Graph Theoretic Approach

Virtually every approximating method requires that the input data points be ordered in some way. However, the skeleton voxels are grid points in 3D without any order. Our first step is to construct a connected graph of the skeleton voxels. Then we construct the minimal spanning tree (MST) using Cormen’s implementation of Prim’s algorithm [18]. Without loss of generality, we use arbitrary edges of the MST to describe the path of the turn, so we must throw out the outlying branches and create a piecewise linear curve. In doing so, we need to eliminate the minimum amount of data points to preserve as much information as possible. To find such a path, we used the Floyd-Warshall algorithm (again implemented by Cormen in [18]) to compute all-pairs shortest
paths in the MST, and reconstruct the longest such path, which we refer to as the all-pairs longest [simple] path (APLP). Conveniently, the APLP implies an order on the points it contains for use in the actual approximating step.

C. Computational Geometric Approach

An artifact directly related to the initial skeleton construction is that our APLP contains right angles at the skeleton voxels, giving it the undesired zigzag appearance. This introduces a margin of error in length when compared to the relatively smooth curve of the protein backbone. To overcome this, we simplify the line by removing certain points using the Douglas-Peucker line simplification, generalized to three dimensions by modifying de Halleux’s implementation given in [19].

The Douglas–Peucker line decimation algorithm [6] allows us to remove points from a piecewise-linear three-dimensional line (referred here as polyline), such that the resulting polyline remains within some tolerance epsilon ε from the original one. Consider a two-dimensional example in Figure 3. The top drawing shows an initial polyline a...b. Its points are chosen from a rectilinear grid, and therefore the total length of the polyline a...b overestimates a smoother line that could connect points a and b and pass through the same geometrical neighborhood. The algorithm is recursive, and takes as parameters the tolerance ε and a multi-point segment of a polyline (which is initially the original polyline). At each recursive call it finds an interior point of the current segment which is the most distant from the straight line connecting the end points of the segment. If the most distant point is within ε from the straight line, the segment is replaced by the straight line, and all interior points are removed. Otherwise, the segment is split into two sub-segments by this most distant interior point, and the algorithm proceeds recursively on each of the sub-segments. The example in the Figure shows how the initial polyline a...b is simplified into polyline aceb. Figure 4 shows the result of decimating an APLP from a test case in three dimensions with ε = 1.0.

Figure 3: Illustration of the Douglas-Peucker polyline decimation algorithm at work in a 2D case.

Figure 4: The MST (blue), APLP (green) and decimate curve approximation (red) for a 3-residue turn in EMDB_5001.

III. COMPLEXITY

Masking the skeleton voxels near helices during data preprocessing is linear in the number of skeleton voxels to test, as it simply looks at each in turn and calculates a distance. However, constructing the initial graph of skeleton voxels is an Θ(n^2) operation, because it must compare each pair of voxels and decide whether or not to place an edge connecting them based on their distance.

Prim’s algorithm to construct the MST of a graph is known to run in O(|E| + |V| log |V|) time [20], where |E| is the number of edges in the graph and |V| is the number of vertices. An additional speedup is achieved by our decision to construct the local graphs in the beginning of step 2 in the preceding section with a maximum edge length, which reduces E. The Floyd-Warshall algorithm we use to find APLPs runs in Θ(n^3) time, where n is the number of voxels in the MST. This, along with graph construction, imposes undesirable bounds on the efficiency of our overall method; we have left matters of optimization for future works. Our final step, the Douglas-Peucker line simplification algorithm has been shown to run in O(n log n) time [20], where n is the number of points on the APLP.
IV. RESULTS

We used 3D images from EMDB and their corresponding atomic structures from PDB to test our method. The density maps are experimentally derived, therefore providing real world test cases. We selected five density maps from EMDB with different resolutions:

- EMDB 5030 (6.4 Å),
- EMDB 1733 (6.8 Å),
- EMDB 5001 (4.2 Å),
- EMDB 1740 (6.8 Å), and
- EMDB 5168 (6.6 Å).

Each of these 3D images is aligned with their PDB structures at download. We extracted turns less than seven amino acids in length from the PDB file and extracted from the density map the corresponding local region around the turn connected by two helices. We obtained the skeletons using Gorgon and processed the skeleton so its voxels inside the helix cylinders are deleted leaving those belonging to the turns. Each processed skeleton was measured and its decimated APLP length compared with the expected length of the turn. The expected length of the turn is calculated by the number of the amino acids on the turn with the consideration of 3.8 Å in between two amino acids.

A. \( \varepsilon \) Threshold

\( \varepsilon \) is one of the major parameters in the Douglas-Peucker algorithm affecting the approximated skeleton length between two helices. In general, the smaller the \( \varepsilon \) value, the less change in the decimated curve compare to the APLP. Figure 5 shows the approximated length of the 3-residue turn in EMDB 1733 measured using different \( \varepsilon \) values in the range [0.5, 3.5]. In this case, \( \varepsilon = 0.75 \) produces the closest approximation to the actual loop length (see case 4 in Table 1). For all the cases in our current test, a value of \( \varepsilon \) between 0.5 and 1.0 produces an approximated length very near to the expected length of the turn, as illustrated in Figures 6 and 7.

<table>
<thead>
<tr>
<th>Decimated curve length</th>
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<tr>
<td>12.5</td>
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**Table 5**: \( \varepsilon \) values of the Douglas-Peucker method.

Figure 6 shows an example of a decimated curve for the 3-residue turn (row 4 of Table 1). The skeleton derived from Gorgon is shown as the surface representation using Chimera [21]. The skeleton is superimposed on the backbone C\(_\alpha\) trace of the protein, obtained from the PDB file. In this case, the backbone chain appears to fit in the skeleton fairly well at the turn region (green). The central end point (yellow) of the helix was estimated using the geometrical center of the last three C\(_\alpha\) atoms on the helix. After the removal of the helix portion of the skeleton, the MST was built to find the APLP (purple). The turn length was estimated to be 11.11 Å using the decimated curve (dark blue). It is fairly close to the estimated distance of 11.4 Å.

Table 1 summarizes the testing results. The length-3 turns dominate with eight out of eleven test cases. In our observations, length-3 turns are more prevalent than other lengths of turns among the helix-turn-helix motif. If we use the \( \varepsilon \) value (column 4 of Table 1) that produces the closest estimation with respect to the true length, the approximated skeleton length (column 5) is fairly close to the expected length with the difference between 0.14 Å to 1.32 Å (column 7). This result suggests that it is possible to estimate the length of the turn using the skeleton length at least for the length-3 turns.

**Table 1: Results of approximation.**

<table>
<thead>
<tr>
<th>No</th>
<th>ID(^{a})</th>
<th>AA(^{b})</th>
<th>( \varepsilon )</th>
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<th>Reale</th>
<th>Diff(^{f})</th>
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</tr>
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<td>21.89</td>
<td>22.8</td>
<td>-0.91</td>
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</table>

\( ^{a}\) EMDB ID.
\( ^{b}\) Number of amino acids in the turn.
\( ^{c}\) Douglas-Peucker epsilon value used to derive the approximating curve.
\( ^{d}\) Actual length of the turn. For a turn with \( n \) residues, the length \( l \) is assumed to be \( l = n \times 3.8 \) Å.
\( ^{e}\) Approximated skeleton length
\( ^{f}\) Difference between actual and approximated lengths.
These results support the previous finding of our group and other groups in terms of the use of graph matching for topology determination. Our preliminary test here shows that there is a \( \epsilon \) value that produces close estimation of the skeleton length for certain type of turns. When the turn is even shorter, having one amino acid, our current estimation still gives reasonable accuracy (row 1 and 2 of Table 1). However, the \( \epsilon \) value varies more in the case of length-3 turns. We need more test cases for the other lengths to make a conclusion.

The cases in Table 1 (except case 10) all have a skeleton that aligns well with the actual protein backbone throughout the loop. However, in some cases we see that the skeleton is not aligned well with the backbone chain of the loop. Sometimes it lies inside the loop, producing a shorter approximated length than the expected length. The skeleton for case 10 lies inside the actual protein backbone (Figure 7), leading to the large approximation error. Further study on the relationship between distances of skeletons to backbones and approximated length than the expected length is needed, as well as investigation of the skeleton length for certain type of turns. When the turn is even further investigation in the approximated lengths is needed, as well as investigation of the relationship between distances of skeletons to backbones and approximated length than the expected length. The skeleton sometimes lies inside the loop, producing a shorter approximated length than the expected length. We plan to carry further investigation in the approximation algorithm.

**Figure 7:** An example of misaligned skeleton and the backbone of the turn (case 10 from Table 1), producing an erroneously short approximation of the loop length.

**V. SUMMARY**

We have investigated the question how accurate it can be to estimate the skeleton length between two helices. Although the skeleton length has been used in topology determination, there has not been a detailed study in the computation of a 3D curve that closely approximates the skeleton of the image. We propose an effective method in estimating the skeleton length using a decimated curve. A test of eleven cases using the experimentally derived data shows that the estimation can be potentially accurate to a fair degree if the backbone of the protein chain fits in the skeleton. This was demonstrated well for the helix-turn-helix motif with three amino acids on the turn. Our method can detect the turns in which the turn is outside the skeleton. We plan to carry further investigation in this direction.

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