Prediction of protein secondary structure based on residue pairs

Xin Liu¹, Li-Mei Zhang², Wei-Mou Zheng³

¹The Interdisciplinary Center of Theoretical Studies, Chinese Academy of Sciences, Beijing 100080, China

²School of Science at Beijing Jiaotong University, Beijing 100044, China

³Institute of Theoretical Physics, China, Beijing 100080, China

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Abstract

The GOR program for predicting protein secondary structure is extended to include triple correlation. A score system for a residue pair to be at certain conformation state is derived from the conditional weight matrix describing amino acid frequencies at each position of a window flanking the pair under the condition for the pair to be at the fixed state. A program using this score system to predict protein secondary structure is established. After training the model with a learning set created from PDB\_SELECT, the program is tested with two test sets. As a method using single sequence for predicting secondary structures, the approach achieves a high accuracy near 70%.

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1 Introduction

Methods for predicting the secondary structure of a protein from its amino acid sequence have been developed for 3 decades. Besides neural network models and nearest-neighbor methods, the statistically based Chou-Fasman/GOR method is well-established and commonly used. In 1974, assuming an oversimplified independency to cope with the large size 20 of the amino acid alphabets at a small size of database, Chou and Fasman (1974) derived a table of propensity for a particular residue to be in a given secondary structure state. By combining with a set of rules, the protein secondary structure was predicted using this propensity. Later, in the first version of the GOR program (Garnier, Osguthorpe, and Robson, 1978), the state of a single residue $a_i$ was predicted according to a window from $i-8$ to $i+8$ surrounding the residue. Unlike Chou-Fasman which assumes that each amino acid individually influences its own secondary structure state, GOR takes into account the influence of the amino acids flanking the central residue on the central residue state by deriving an information score from the weight matrix describing 17 individual amino acid frequencies at sites $i+k$ with $-8 \leq k \leq +8$. By using a single weight matrix, the correlation among amino acids within the window was still ignored. In the later version GOR III (Gibrat, Garnier, and Robson, 1987), instead of single weight matrix for every structure state, 20 weight matrices, each of which corresponds to a specific type of the central residue, were used. These conditional weight matrices take the pair correlation between the central residue and a flanking one into account. In the most recent version of GOR (GOR IV, Garnier, Gibrat, and Robson, 1996), all pairwise combinations of amino acids in the flanking region were included.
The GOR program maps a local window of residues in the sequence to the structural state of the central residue in the window. Correlations among positions within the window is essential for improvements in prediction accuracy. We give an example to show the importance of high order correlations. For central residue $a_i = K$ being at the extended strand state, the conditional probability for $a_{i-3} = V$ is $P_{e-3;0}(V|K) = 0.088$, while the conditional probability for $a_{i-3} = V$ at $a_i = K$ and $a_{i+1} = E$ is $P_{e-3;0,1}(V|KE) = 0.186$, and $P_{e-3;0,1}(V|KV) = 0.058$. With the growth of protein structure database, now the size of known protein structures allows us to consider correlations higher than pair ones. Here we shall extend the GOR program to include triple correlations, developing a program to predict protein secondary structure based on residue pairs (PSSRP). As a method using single sequence, the computation required is rather light, but its prediction accuracy reaches 70%.

2 Methods

Kabsch and Sander (1983) define eight states of secondary structure according to the hydrogen-bond pattern. As in most methods, we consider 3 states \{h, e, c\} generated from the 8 by the coarse-graining $H, G, I \rightarrow h$, $E \rightarrow e$ and $X, T, S, B \rightarrow c$.

2.1 Window-based scores

A window is a sequence segment $a_i a_{i+1} \ldots a_{i+l-1}$ of the width $l$. Consider two residues $a_{i+j} = x$ and $a_{i+k} = y$ inside the window (with $0 < j < k < l - 1$). Their conformation state are $\alpha$ and $\beta$, respectively. Let us denote by $w$ the set of the sites within the window with $i+j$ and $i+k$ excluded. When discussing probability, we ignore the starting site index $i$.

The Chou-Fasman propensity of residue $x$ to conformation $\alpha$ is defined by

$$CF(x; \alpha) = \frac{P(x|\alpha)}{P(x)},$$

where $P(x)$ is the probability for residue $x$ to appear, and $P(x|\alpha)$ the conditional probability for $x$ to be at conformation $\alpha$. Here we use only a logarithmic propensity, the logarithm of $CF$:

$$LCF(x; \alpha) = \log CF(x; \alpha).$$

As an extension of the Chou-Fasman propensity of residues, the propensity of residue pair $xy$ to conformation
αβ may be defined as

\[ r_d(xy; \alpha\beta) = \frac{P_d(xy|\alpha\beta)}{P_d(xy)}, \quad d = k - j - 1, \]

where \( P_d(xy) \) is the probability for residue pair \( x \) and \( y \) to appear with their site index difference being \( d \), and \( P_d(xy|\alpha\beta) \) the conditional probability on the condition that the conformation states of \( x \) and \( y \) are \( \alpha \) and \( \beta \), respectively.

When a window flanking the pair \( xy \) is examined to infer the conformation \( \alpha\beta \) of \( xy \), a further extension of the Chou-Fasman propensity is

\[ R_d(xy; \alpha\beta) = \frac{P_d(xy, w|\alpha\beta)}{P_d(xy, w)}, \quad d = k - j - 1, \]

where \( P_d(xy, w) \) and \( P_d(xy, w|\alpha\beta) \) are now probability for the whole window, i.e. \( xy \) and \( w \). Making the assumption of independency, we introduce the conditional weight matrix \( Q_{xy} \) of \( l - 2 \) columns and 20 rows, whose entries describe the probability for a specific residue \( z \) to appear at some flanking site, say the \( n \)-th site from the window starting position. We denote the probability by \( Q_{d,n}(z|xy) \), and write

\[ P_d(xy, w) = P_d(xy)P_d(w|xy) = P_d(xy) \prod_{n \in w} Q_{d,n}(a_{i+n}|xy). \]

A similar simplification for \( P_d(xy, w|\alpha\beta) \) is

\[ P_d(xy, w|\alpha\beta) = P_d(xy|\alpha\beta)P_d(w|xy, \alpha\beta) = P_d(xy|\alpha\beta) \prod_{n \in w} Q_{d,n}(a_{i+n}|xy, \alpha\beta), \]

where the meaning of \( Q_{d,n}(z|xy, \alpha\beta) \) is analogous to \( Q_{d,n}(z|xy) \). The window score \( I_d(xy; \alpha\beta) \) for pair \( xy \) to be at conformation \( \alpha\beta \) is then defined as the logarithmic ratio of \( R_d \):

\[ I_d(xy; \alpha\beta) = \log \left[ \frac{P_d(xy|\alpha\beta)}{P_d(xy)} \right] + \sum_{n \in w} \log \left[ \frac{Q_{d,n}(a_{i+n}|xy, \alpha\beta)}{Q_{d,n}(a_{i+n}|xy)} \right]. \]

So far we have not determined the window width \( l \) and the position of the inferred residue pair inside the window, especially the separation \( d \) of the two residues. To do this, we need a measure of the distance between two probability distributions. A well defined measure is the Kullback-Leibler (KL) distance or relative entropy (Kullback et al., 1959; Kullback, 1987; Sakamoto et al., 1986), which, for two distributions \( \{p_i\} \) and \( \{q_i\} \), is given by

\[ KL(\{p_i\}, \{q_i\}) = \sum p_i \log(p_i/q_i). \]
It corresponds a likelihood ratio, and, if \( p_i \) is expanded around \( q_i \), its leading term is the \( \chi^2 \) distance. It is often to use the following symmetrized form

\[
D(\{p_i\}, \{q_i\}) = \frac{1}{2}[KL(\{p_i\}, \{q_i\}) + KL(\{q_i\}, \{p_i\})].
\] (9)

The distance \( D_n(xy, \alpha\beta) \equiv D(\{Q_{d,n}(z|xy, \alpha\beta)\}_z, \{Q_{d,n}(z|xy)\}_z) \) measures the power for site \( i + n \) to infer conformation \( \alpha\beta \). Asymptotically \( D_n(xy, \alpha\beta) \) approaches zero, when \( n \) becomes far away from the sites of pair \( xy \). The power for a window to infer conformation \( \alpha\beta \) of \( xy \) may be measured by \( \langle \sum_{n \in w} D_n(xy, \alpha\beta) \rangle \), the window sum distance averaged with the weight \( P(xy, \alpha\beta) \). We find a reasonable choice is \( l = 16 \) and \( d = 0,1 \) with residue \( y \) being at 9-th site of the window. Detailed discussion will be published elsewhere.

Due to limited samples, we consider only \( \alpha\beta \in \{cc, ee, hh, ce, ch, ec, hc\} \) with \( eh \) and \( he \) excluded.

2.2 Prediction steps

Using scores \( I_d(xy; \alpha\beta) \) and sliding windows, we may calculate scores of true and false windows for each combination of \( xy; \alpha\beta \) and \( d \). The threshold \( T_d(xy; \alpha\beta) \) is determined by the error rate 5% at which non-\( \alpha\beta \) conformations are wrongly predicted as \( \alpha\beta \). For a given window, if its score \( I_d(xy; \alpha\beta) \) is greater than the corresponding threshold \( T_d(xy; \alpha\beta) \), we say that ‘\( x \) at \( \alpha \)’, ‘\( y \) at \( \beta \)’ and ‘\( xy \) at \( \alpha\beta \)’ are evidenced by the event \((d; xy; \alpha\beta)\).

**Step 1:** If ‘\( xy \) at \( \alpha\beta \)’ is evidenced by event \((d; xy; \alpha\beta)\), and, at the same time, both ‘\( x \) at \( \alpha \)’ and ‘\( y \) at \( \beta \)’ are further evidenced by some other events, we say that ‘\( xy \) at \( \alpha\beta \)’ is strongly confirmed. Determine all strongly confirmed pairs.

**Step 2:**

We now consider the case that ‘\( xy \) at \( \alpha\beta \)’ is evidenced, but not strongly confirmed. If either ‘\( zx \) at \( \gamma\alpha \)’ or ‘\( yz \) at \( \beta\gamma \)’ is strongly confirmed, we say that ‘\( xy \) at \( \alpha\beta \)’ is weakly confirmed. Determine all the weakly confirmed pairs.

**Step 3:**

From a confirmed pair, either strongly or weakly, we calculate the score \( I(x; \alpha) \) for single residue \( x \) to be at conformation \( \alpha \) according to

\[
I(x; \alpha) = \max_{d,d',y,z,\beta,\gamma} \{I_d(xy; \alpha\beta) - LCF(y; \beta), I_d'(zx; \gamma\alpha) - LCF(z; \gamma)\},
\] (10)
where only confirmed pairs are searched for maximum. The conformation of $x$ is finally inferred as

$$
\alpha^* = \arg_{\alpha} \max \{ I(x; \alpha) \},
$$

(11)
i.e. $\alpha^*$ is the $\alpha$ corresponding to the maximal $I(x; \alpha)$. Infer residue conformation for all the confirmed pairs.

**Step 4:**

In this step we expand already inferred $h$ and $e$ segments in both direction. Suppose residue $x$ be a candidate for elongating $e$. We calculate score $I(x; e)$ according to (10), but now only pairs fit ‘$x$ at $e$’ are searched for maximum. If $I(x; e)$ is positive, we assign conformation $e$ to $x$. The elongation of $h$ is similar.

**Step 5:**

At both ends of the sequence no full windows are available. The first and last two residues are always assigned to $c$. With the contribution of the missing sites set as zero, scores $I(x; \alpha)$ of some residue $x$ in the end regions are calculated for the elongation of the already determined boundary conformation $\alpha$ in a similar way to the last step. For remaining residues we examine only the cases of four successive residues, say $a_ia_{i+1}a_{i+2}a_{i+3}$, in the same confirmation $\alpha$ by calculating $I_0(a_ia_{i+1}; \alpha\alpha) + I_0(a_{i+2}a_{i+3}; \alpha\alpha)$, and then infer the conformation according to the largest positive score.

**Step 6:**

This final step is filtering. Each single residue $h$ and $e$ segment is discarded. A conformation segment $hh$ is expanded to $hhh$ according to whichever neighbor site has a large score for $h$. Each residue whose conformation cannot be predicted so far is assigned to conformation $c$.

Let us explain the prediction steps in more words by a simple example. Suppose that total of five events are found for segment PDEFGHI of a sequence as shown as follows.
In step 1, EG at ec is strongly confirmed by the first three events. This is the only strongly confirmed pair in the segment. Based on the pair, in Step 2 we find that, all the remaining four events are weakly confirmed. In Step 3, we can easily infer ‘P at e’, ‘E at e’, ‘G at c’, and ‘I at h’. Further calculation of \( I(H; h) \) and \( I(H, c) \) leads to ‘H at h’. The conformation of PDEFGHI is now inferred as e-e-chh. Step 4 then fills up the gaps to get eeeechh.

3 Result

We create a nonredundant set of 1612 non-membrane proteins for training parameters from PDB_SELECT (Hobohm and Sander, 1994) with amino acid identity less than 25% issued on 25 September of 2001. The secondary structure for these sequences are taken from DSSP database (Kabsch and Sander, 1983). As mentioned above, the eight states of DSSP are coarse-grained into 3 states: h, e and c. This learning set contains 268031 residues with known conformations, among which 94415 are h, 56510 are e, and 117106 are c. The size of the learning set is reasonable for training our parameters.

To convert observed amino acid counts into frequencies or probabilities for scoring is a basic problem faced in training. A practical approach is to use pseudocounts (Aitchison and Dunsmore, 1972). We estimate background amino acid frequencies \( \{\rho_z\} \) directly from counts of the whole learning set. Then, we estimate the weight matrix element \( Q_{d,n}(z|xy, \alpha \beta) \) from the count \( N_{d,n}(z|xy, \alpha \beta) \) of amino acid \( z \) at position \( n \) under the condition that residue pair \( xy \) with separation \( d \) is in conformation \( \alpha \beta \) as follows.

\[
Q_{n,z} = \frac{N_{n,z} + \sqrt{N_n \rho_z}}{N_n + \sqrt{N_n}}, \quad N_n = \sum_z N_{n,z},
\]

where \( Q_{n,z} \), indicating specifically only \( n \) and \( z = a_{i+n} \), stands for \( Q_{d,n}(a_{i+n}|xy, \alpha \beta) \), and \( N_{n,z} \) stands for \( N_{d,n}(a_{i+n}|xy, \alpha \beta) \). Here, the conditional probability \( Q_{n,z} \) is estimated by using a pseudocount proportional
to the background distribution $\rho_z$. If $\sum_x N_{d,n}(z|x,y,\alpha\beta)$ is less than 10, the sample size is too small to reliably estimate the score. No such scores will be used for inference. Or, equivalently speaking, they are set to be negative infinity.

In order to assess the accuracy of our approach, we use the following 2 test sets: Sets 1 and 2. A set of 124 nonhomologous proteins is created from the representative database of Rost and Sander (1993) by removing subunits A and B of hemagglutinin 3hmg, which are designated as membrane protein by SCOP (Murzin et al, 1995). The 124 sequences and the learning set are not independent of each other according to HSSP database (Dodge, Schneider and Sander, 1998). That is, some proteins of the 124 sequences and certain proteins in the learning set belong to the same putative homologue family of HSSP. Removing these proteins from the 124 sequences and 5 sequences with unknown amino acid segments longer than 6, we construct Set 1 of 76 proteins, a subset of the 124 sequences. 34 proteins with known structures of the CASP4 database issued in December of 2000 are taken as Set 2.

The predicted counts of each conformation type in the test sets are listed in Table 1. The quantities assessing accuracy on single residue level for a given test set are the total percent correct $Q_3$, percent of types $h$ and $e$ predicted correctly (sensitivity $S_n$) and percent of predictions correct for types $h$ and $e$ (specificity $S_p$). Results obtained on the test sets are listed in Table 2 in comparison with the results of GOR IV and SSP (Solovyev and Salamov, 1991, 1994), another secondary structure predictor based on discriminant analysis using single sequence. The approach PSSRP performs very well. The overall value of $Q_3$ averaged over Sets 1 and 2 is 70.2%. We show $Q_3$ statistics for Sets 1 and 2 in Fig. 1.

Generally, strongly confirmed pairs are the portion with a high confidence in prediction. Strongly confirmed pairs cover 58.4% and 53.8% of Set 1 and 2, respectively. There is a clear correlation between the coverage rate or the percentage of the strongly confirmed pairs in the total length of a sequence and accuracy $Q_3$, which are 79.5% and 77.4% for the coverage portion, respectively. To examine the correlation between the coverage rate $C$ of strongly confirmed pairs and the whole sequence accuracy $Q_3$, we conduct simple linear regression of $Q_3$ on $C$ for Sets 1 and 2, as shown in Fig. 2 with regression line $Q_3 = 0.728C + 0.306$. Correlation coefficient $r$ and standard deviation $\sigma$ are 0.779 and 0.0276 for Set 1, while for Set 2, they are 0.830 and 0.0209, and for the two sets total are 0.795 and 0.0253. Thus, the coverage rate of the strongly confirmed pairs provides us a self-checking confidence level of the prediction accuracy.

$Q_3$ measure gives an overall number of residues predicted correctly. It is well known that single-residue
accuracy sometimes poorly reflects the quality of prediction. Measures concentrating on secondary structure segment prediction accuracy would better reflect the nature of structure. A simple segment overlap measure is: a segment is considered correctly predicted if the predicted and observed segments have at least two amino acids in common (Taylor, 1984). Results of this segment prediction accuracy from our approach are listed in Table 3.

4 Discussions

We have presented an improved approach using single protein sequence to predict secondary structure. The improvement is achieved by including triple correlations. Most recent improvements in accuracy come from methods which are capable to consider correlations nonlocal in sequence. Combining evolutionary information via multiple alignments of homologous sequences is a main way to include such correlations. Although methods using single sequence generally cannot cope with nonlocal correlations easily, their simple nature of requiring least computation in the prediction step is still attractive.

There are rooms for further improvement of PSSRP. The original scores are obtained for residue pairs. The scores are then converted to those for single residues in the prediction Step 3. It is possible to construct a scoring system to directly use pair scores by introducing appropriate weighting. We may tune the thresholds to compromise $S_p$ with $S_n$. We may integrate the segment length statistics into the approach, e.g. by dynamic programming.

The size of the amino acid alphabets is 20. Number of parameters increases drastically with the order of correlations considered. A statistical model containing a tremendous number of parameters will require a huge learning set to train parameters. Furthermore, an over-complicated model can easily result in overfitting. It seems that the overfitting is not too serious. A way to reduce the number of parameters is to coarse-grain the 20 amino acids into a small number of categories. This is under study.

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Table 1. Predicted counts for each conformation type.

|         | Predicted |         |         |
|---------|-----------|---------|---------|
|         | Set 1     | Set 2   |         |
|         | h         | e       | c       | h       | e       | c       |
| Observed| 3296      | 275     | 1097    | 2097    | 205     | 806     |
|         | 271       | 1815    | 912     | 188     | 1030    | 558     |
|         | 749       | 765     | 4999    | 504     | 383     | 2553    |

Table 2. Single residue accuracies.

| Set     | $Q_3$ | $S_n^h$ | $S_p^h$ | $S_n^e$ | $S_p^e$ |
|---------|-------|---------|---------|---------|---------|
| PSSRP   | 1     | 71.3    | 70.6    | 76.4    | 60.5    | 63.6    |
| GOR IV  | 1     | 66.0    | 63.3    | 68.5    | 54.7    | 55.3    |
| SSP     | 1     | 66.8    | 68.1    | 69.0    | 55.3    | 60.0    |
| PSSRP   | 2     | 68.2    | 67.5    | 75.2    | 58.0    | 63.7    |
| GOR IV  | 2     | 63.2    | 67.1    | 64.3    | 43.0    | 54.6    |
| SSP     | 2     | 61.2    | 66.3    | 63.3    | 45.7    | 55.6    |

Table 3. A comparison of segment prediction accuracy for short and long helices and sheets. Here a simple measure of segment overlap is used: a predicted segment is counted as a true positive (TP) if the predicted segment and an observed segment have at least two residues in common. With this definition the sensitivity $S_n$ and specificity $S_p$ are calculated.

|         | Set 1     |         |         | Set 2     |         |         |
|---------|-----------|---------|---------|-----------|---------|---------|
|         | GOR IV    | SSP     | PSSRP   | GOR IV    | SSP     | PSSRP   |
|         | $S_n$    | $S_p$  | $S_n$    | $S_p$    | $S_n$    | $S_p$   |
| All helices | 62.7  | 76.1    | 64.9  | 83.2  | 76.7    | 76.2    | 63.2    | 66.9    | 66.0    | 76.2    | 71.8    | 72.4    |
| Long helices ($l > 8$) | 89.7  | 91.0    | 94.2  | 86.7  | 96.3    | 94.1    | 84.4    | 81.7    | 85.6    | 78.0    | 93.1    | 96.3    |
| Short helices ($l \leq 8$) | 54.7  | 55.3    | 38.9  | 56.5  | 59.3    | 70.0    | 42.3    | 48.4    | 46.6    | 56.5    | 51.0    | 64.2    |
| All sheets | 65.5  | 59.5    | 57.0  | 74.6  | 71.4    | 66.2    | 50.9    | 60.0    | 49.3    | 73.9    | 64.6    | 67.4    |
| Long sheets ($l > 6$) | 84.7  | 75.0    | 82.0  | 80.6  | 86.0    | 88.2    | 64.4    | 75.7    | 58.9    | 80.6    | 86.3    | 86.4    |
| Short sheets ($l \leq 6$) | 59.1  | 56.9    | 48.5  | 67.3  | 66.5    | 63.1    | 47.7    | 57.9    | 47.1    | 66.7    | 59.4    | 63.2    |
Figure 1: $Q_3$ statistics for Sets 1 and 2. $Q_3$ is calculated for each sequence, and the size of the bins is 0.05.

Figure 2: Correlation between whole sequence prediction accuracy $Q_3$ and the coverage rate or the percentage of the strongly confirmed pairs in the total length of a sequence.