Prediction of Protein B-factor Profiles

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ABSTRACT **The polypeptide backbones and side chains of proteins are constantly moving due to thermal motion and the kinetic energy of the atoms. The B-factors of protein crystal structures reflect the fluctuation of atoms about their average positions and provide important information about protein dynamics. Computational approaches to predict thermal motion are useful for analyzing the dynamic properties of proteins with unknown structures. In this article, we utilize a novel support vector regression (SVR) approach to predict the B-factor distribution (B-factor profile) of a protein from its sequence. We explore schemes for encoding sequences and various settings for the parameters used in SVR. Based on a large dataset of high-resolution proteins, our method predicts the B-factor distribution with a Pearson correlation coefficient (CC) of 0.53. In addition, our method predicts the B-factor profile with a CC of at least 0.56 for more than half of the proteins. Our method also performs well for classifying residues (rigid vs. flexible). For almost all predicted B-factor thresholds, prediction accuracies (percent of correctly predicted residues) are greater than 70%. These results exceed the best results of other sequence-based prediction methods. Proteins 2005;58:905–912.**

Key words: B-factor; flexibility; support vector regression; evolutionary information; ROC analysis; protein sequence

INTRODUCTION

Protein structures are not static and rigid. The polypeptide backbones, and especially the side chains, are constantly moving due to thermal motion and the kinetic energy of the atoms (Brownian motion). Protein internal motion or flexibility is highly correlated with protein functions such as catalysis and allostery.[1] Thermal motion (displacement of functional groups) is one of the most important motions within protein structures.

The B-factor (atomic displacement parameter) in protein crystal structures reflects the fluctuation of an atom about its average position. The distribution of B-factors along a protein sequence is regarded as an important indicator of the protein's structure, reflecting its flexibility and dynamics. A large B-factor indicates high mobility of individual atoms and side chains. B-factors provide a solid experimental source of information about the dynamics of a protein. B-factors have a variety of applications, such as predicting protein flexibility,[2,3] studying protein thermal stability,[4,5] analyzing active sites,[6–8] correlating side-chain mobility with conformation,[9,10] analyzing protein disordered regions[11,12] and investigating protein dynamics.[13] B-factors have been predicted from protein sequences,[2,3,12] protein atomic coordinates[14] and protein electron density maps.[15]

Measured B-factors in different known structures may be on different scales owing to the application of different refinement procedures.[16] Instead of raw data, normalized data is usually used to compare B-factors of different protein chains and structures.[2,3,17,18]

Since high-throughput experiments have rapidly generated a large number of protein sequences, fast and accurate computational methods to annotate their structural, functional and dynamic properties are in demand. Numerous prediction methods have been developed during the last few decades to predict secondary structure, solvent accessibility and subcellular localization. In contrast, few prediction methods for protein flexibility or mobility exist.

In this study, we put forward a new method to predict B-factor distributions based on a support vector regression (SVR)[19–21] approach. This approach is often effective when the input data is of high dimension and the function to be predicted is highly non-linear. SVR has been successfully applied to some biological problems such as simulating the age of *Drosophila* embryos,[22] analyzing microarray data[23] and predicting protein solvent accessible areas.[24]

Most previous work in the prediction of B-factors has been on the so-called "classification problem." The classification problem assigns residues to one of two states, rigid or flexible, with arbitrary B-factor cutoff thresholds.[2,3,12] The selection of thresholds is neither objective nor optimal, and the decomposition of residues into two classes decreases the prediction accuracy. To overcome these disadvantages, we predict the B-factor for each residue. That is, our method predicts a series of real values representing a protein sequence (also regarded as the B-factor profile).

Our new method has been examined on a well-prepared non-redundant dataset by cross-validation tests. Our method achieves a Pearson's correlation coefficient (CC) of

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0.53, which exceeds all previously reported results. In the two-class problem, our method achieves classification accuracy greater than 70% regardless of the B-factor threshold used to define the two classes.

MATERIALS AND METHODS

Support Vector Regression (SVR)

The objective of the regression problem is to estimate an unknown continuous-valued function, $y = f(\mathbf{X})$, based on a finite number of samples. In our problem, the training samples were represented by the set of paired values $\{(\mathbf{X_i},y_i)\}$ $(i = 1, \ldots M)$, where the feature vector $\mathbf{X_i}$ characterizes a residue in a protein sequence and y_i represents its associated normalized B-factor value. We estimated the true function, $f(\mathbf{X})$, by the following function, using support vector regression to find optimum weights (W) and bias b

$$f(\mathbf{X_i}) = \langle W,\Phi(\mathbf{X_i})\rangle + b. \quad (1)$$

Since $\Phi(\mathbf{X_i})$ may be a non-linear mapping from the input space to a (possibly higher dimensional) feature space, SVR is able to perform non-linear regression.

The regression parameters (W and b) were optimized to minimize an objective function that combines the norm of the weights, $\|W\|^2$, and an empirical risk function. The risk function is based on Vapnik's ε-insensitive loss function,

$$L_\varepsilon[y_i - f(\mathbf{X_i})] = \begin{cases} 0 & \text{if } |y_i - f(\mathbf{X_i})| \le \varepsilon \\ |y_i - f(\mathbf{X_i})| - \varepsilon, & \text{otherwise} \end{cases} \quad (2)$$

Only those errors larger than ε contribute to the loss function. The risk function is defined as

$$R_{\text{emp}}(W,b) = \frac{1}{M}\sum_{i=1}^{M} L_\varepsilon(y_i - f(\mathbf{X_i})) \quad (3)$$

Slack variables ξ and ξ* are introduced to measure the deviation of samples outside the ε-insensitive zone. Using the slack variables, the objective function for SVR is defined as

$$F(W,b) = \frac{1}{2}\|W\|^2 + C\sum_{i=1}^{M}(\xi_i + \xi_i^*), \quad (4)$$

where C is a regularization constant that determines the trade-off between training errors and model complexity.

With the foregoing definitions in place, we formulated the SVR problem as the following constrained optimization problem:

$$\text{minimize } F(W,b)$$

$$\text{subject to } \begin{cases} f(\mathbf{X_i}) - y_i \le \varepsilon + \xi_i \\ y_i - f(\mathbf{X_i}) \le \varepsilon + \xi_i^* \\ \xi_i,\xi_i^* \ge 0 \text{ for } i = 1, \ldots, M \end{cases} \quad (5)$$

To solve the optimization problem, Lagrange multipliers α_i and α_i^* were added to the condition equations and the above problem was written as its dual form:

$$\text{Maximize } -\varepsilon\sum_{i=1}^{M}(\alpha_i + \alpha_i^*) + \sum_{i=1}^{M}(\alpha_i - \alpha_i^*)y_i - \frac{1}{2}\sum_{i,j=1}^{M}(\alpha_i - \alpha_i^*)(\alpha_j - \alpha_j^*)\langle\Phi(\mathbf{X_i}),\Phi(\mathbf{X_j})\rangle$$

$$\text{subject to } 0 \le \alpha_i,\alpha_i^* \le C \text{ and } \sum_{i=1}^{M}(\alpha_i - \alpha_i^*) = 0 \quad (6)$$

Only the non-zero values of Lagrange multipliers are useful in predicting the regression line, and their corresponding samples are known as support vectors.

Notice that in this form, the solution only requires that the dot product of the transformed input vectors be known. So the user of SVR does not choose the mapping function $\Phi(\mathbf{X})$ explicitly. Instead, the user chooses a so-called kernel function whose value computed on two input points equals the dot product of the transformed points in the feature space. A suitable function

$$K(\mathbf{X_i},\mathbf{X_j}) = \langle\Phi(\mathbf{X_i}),\Phi(\mathbf{X_j})\rangle, \quad (7)$$

can be found under general conditions. Choices for the kernel function include polynomial functions,

$$K(\mathbf{X_i},\mathbf{X_j}) = (\langle\mathbf{X_i},\mathbf{X_j}\rangle + 1)^n, \quad (8)$$

and, radial basis functions (RBF),

$$K(\mathbf{X_i},\mathbf{X_j}) = \exp(-\gamma\|\mathbf{X_i} - \mathbf{X_j}\|^2). \quad (9)$$

The regression function in eq. (1) can be rewritten in terms of the kernel function, the Lagrange multipliers and the bias term as

$$f(\mathbf{X}) = \sum_{i=1}^{M}(\alpha_i - \alpha_i^*)K(\mathbf{X_i},\mathbf{X}) + b. \quad (10)$$

Once the Lagrange multipliers and the bias (b) are determined from the training samples, eq. (10) can be used to predict the B-factor values of a novel protein.

Residue Encoding Scheme and Normalization of B-factors

We encoded the feature vector in our regression problem $\mathbf{X_i}$, representing a single residue in a protein, using sequence information in a window of width 15 centered on the residue.[25] We used two different encodings for protein residues, single-sequence and multiple-sequence. Each of these encodings represents a residue by a vector of length 21. The feature vector for a residue in a protein is composed by concatenating the 15 vectors representing the residues in the window centered on it. In both encodings, the feature vector encoding a residue in a protein is a (21*15) or 315-dimensional vector.

In the single-sequence encoding, a residue is encoded as a vector of length 21. One vector element, corresponding to the residue type, is set to one, and the other 20 elements are set to zero. The first 20 elements in the vector each

represent one of the 20 standard amino acids. The twenty-first element represents gaps, positions beyond the end of the sequence and non-standard amino acids.

The multiple-sequence encoding incorporates evolutionary information by encoding residues via their columns in a PSI-BLAST position-specific scoring matrix.[26] The matrices were obtained by querying the input protein using PSI-BLAST against the non-redundant protein sequence database (ftp://ftp.ncbi.nlm.nih.gov/blast/db/) with three rounds, masking coiled-coil and low-complexity regions.[27] The elements in PSI-BLAST position-specific scoring matrices are the log-odds values, which represent the log-likelihood of that particular residue substitution at that position in the template. To normalize the log-odds values, we divided them by 10 so that nearly all of the values fell between -1.0 and 1.0. For masked residues, we encoded the residue in the single-sequence format. For non-masked residues, the first 20 elements in the vector were the normalized log-odds values and the last one was set to zero.

The predicted variable in our regression problem, y_i, represents the normalized B-factor of the C_α atom of the corresponding residue. We extracted raw B-factor values from the protein's Protein Data Bank (PDB) entry[28] and normalized them using the method of Smith et al.[17] First, outliers were detected by a median-based method and removed from the input. Then, the sample mean ($\bar{y}$) and sample standard deviation (S) of the data points were calculated. Finally, the raw B-factors (y_i') were normalized according to the following equation:

$$y_i = \frac{y_i' - \bar{y}}{S},\ i = 1, \ldots, M. \tag{11}$$

Dataset Preparation and Accuracy Measurement

We prepared 766 protein chains using PDB–REPRDB[29] and selected only the structures solved by x-ray crystallography with resolution ≤ 2.0 Å^2 and R-factors ≤ 0.2. We excluded protein chains shorter than 60 amino acids. No two chains were included that have pair-wise identity of more than 25%. The protein names can be found in Table II (supplementary material).

To perform five-fold cross-validation tests, we divided this dataset into five groups, with groups having roughly equal numbers of protein sequences. Each group in turn was chosen as the testing set, while the proteins in other groups were merged to form a training set.

To measure the performance of SVR methods, we calculated the CC between predicted and observed B-factors as given by

$$\rho = \frac{\sum_{i=1}^{M}(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\left[\sum_{i=1}^{M}(x_i - \bar{x})^2\right]\left[\sum_{i=1}^{M}(y_i - \bar{y})^2\right]}} \tag{12}$$

where x_i and y_i are the experimental and predicted values of the B-factor of the ith C_α atom, and x and y are their corresponding sample means.

To further examine the SVR performance, we chose a series of cutoff thresholds to classify B-factor values. Residues were classified as flexible (or rigid) if their B-factors were greater than (or less than or equal to) the threshold. The overall accuracy was defined as the number of correctly predicted residues over the total number of residues. The sensitivity was defined as the ratio between the number of correctly predicted flexible residues and the number of flexible residues. Specificity is the ratio between the number of correctly predicted rigid residues and the number of rigid residues.

We also measured classification accuracy using Receiver Operating Characteristic (ROC) analysis.[30,31] ROC is a classic method in Signal Detection Theory[31] and has also been used in medical diagnosis.[30] For every possible cutoff threshold, ROC plots classification sensitivity as a function of one minus specificity. Since higher sensitivity and specificity equates with better classification performance, the highest and leftmost ROC curve represents the best-performing classification method.

RESULTS AND DISCUSSION

The distribution of normalized B-factors for the 191,474 C_α atoms in our dataset was similar that of datasets used by other authors in related work.[17,18] As shown in Figure 1, the distribution is skewed with mean and median values 0.03 and -0.23, respectively.

We tried four different combinations of input encoding, kernel function and regularization. These four "models" are described in detail in Table I. Model 1 used the single-sequence input encoding and a fourth-order polynomial kernel function. Models 2 and 3 used the single-sequence input encoding and radial basis function kernels. The RBF kernel was found to perform better than the polynomial kernel, as observed in our previous studies in the prediction of solvent accessible surface area.[24] Based on RBF kernel, the multiple-sequence input encoding was used to design Model 4. We set the value of ε [eq. (2)] to 0.01 when training each model using SVR.

Among the four models, Model 4 provided the most accurate predictions of B-factors. For Model 4, the cross-validated CC was 0.53. The equivalent results for Models 1, 2 and 3 were 0.40, 0.43 and 0.44, respectively. As expected, the evolutionary information contained in the multiple-sequence input encoding significantly improved the prediction of B-factors.

Figure 2 shows the distribution of the CC values for the individual protein chains (computed using Model 4). More than half of the proteins were found to have CC values greater than 0.56. The correlations for individual proteins ranged from -0.20 to 0.89, and their values are given in Table II (supplementary material). Among them, only four protein chains were predicted to have negative CCs.

Each of the four models can also be used to classify residues into one of two classes (rigid or flexible). We selected the threshold values of -2.0 to 2.0 with a step size

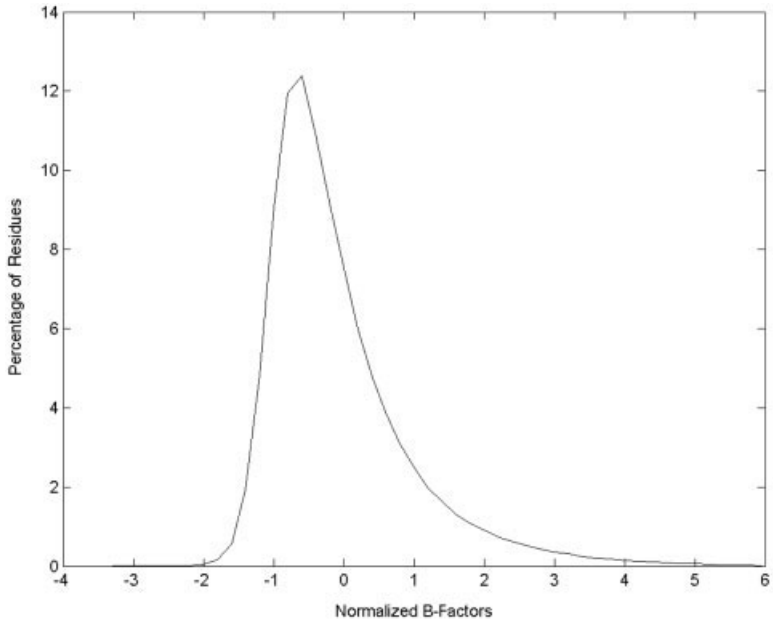

Fig. 1. Distribution of normalized B-factors. Each point shows the percentage of residues with B-factors in a bin of size of 0.2 among the total residues in the 766 protein chains in the dataset.

TABLE I. Models: Sequence Coding Schemes, Kernel Functions and Control Parameters

Model	Encoding scheme	Kernel function	Parameters
1	Single-sequence	$K(X_i, X_j) = (X_i \cdot X_j + 1)^n$	$n = 4; C = 0.001$
2	Single-sequence	$K(X_i, X_j) = \exp(-\gamma \|X_i - X_j\|^2)$	$\gamma = 0.1; C = 0.7$
3	Single-sequence	$K(X_i, X_j) = \exp(-\gamma \|X_i - X_j\|^2)$	$\gamma = 0.01; C = 2.0$
4	Multiple-sequence	$K(X_i, X_j) = \exp(-\gamma \|X_i - X_j\|^2)$	$\gamma = 0.01; C = 2.0$

of 0.1 and calculated the corresponding accuracies. Figure 3 shows that Model 4 achieves equal or better classification accuracy than the other models regardless of the B-factor threshold used to define the two classes. For all choices of threshold, the relative classification accuracies of the models are always the same (from best to worst): Model 4, Model 3, Model 2 and Model 1. It is also evident from Figure 3 that the B-factor threshold used to define the two classes strongly affects the absolute classification accuracy. If the threshold is set at the mean value (0.03) or the median value (-0.23), the corresponding Model 4 classification accuracies are 71.3% and 69.5%, respectively.

As can be seen from the ROC plots in Figure 4, when we set the B-factor threshold to its mean value (0.03), Model 4 surpasses the other models. That is to say, with this definition of flexible/rigid residues, Model 4 has better sensitivity for any choice of specificity compared to the other models. The same result holds for all choices of threshold we tried (data not shown).

Figure 5 illustrates the meanings of different CC values. It shows the predicted and experimental B-factor profiles for two particular proteins, cytokine 1KXG (chain A) and binding protein 1PUC. The CCs for 1KXG and 1PUC were found to be 0.54 and 0.78, respectively.

The largest (absolute) errors in the representative B-factor profiles shown in Figure 5 occur for residues with the largest (absolute value) experimental B-factors. This is a general characteristic of our method, as can be seen in Figure 6. In that figure we plot the absolute value of the difference between predicted and experimental B-factors for each residue in our test sets versus the experimental B-factor of the residue.

A probable explanation for the lower accuracy of the method on residues with very large and very small B-factors is the fact that these types of residues are much less common in the training sets. It is a well-known property of machine learning algorithms (such as SVR) that predictive accuracy is often strongly correlated with the amount of training data available.

Discussion

The accuracy of the prediction of B-factors based on protein sequences or structures has an upper limit due to the noise in B-factor experimental data. Measured B-factors not only reflect the authentic fluctuation and static, dynamic and lattice disorders, but they also depend on refinement methods, stages and temperatures.[32–34] The presence of ligands may also impact the B-factor values of active sites. Some non-biological contributions such as crystal contacts are also the sources of noise. However, a careful examination by Radivojac and coworkers[12] has shown that the B-factor distributions of homologous pro-

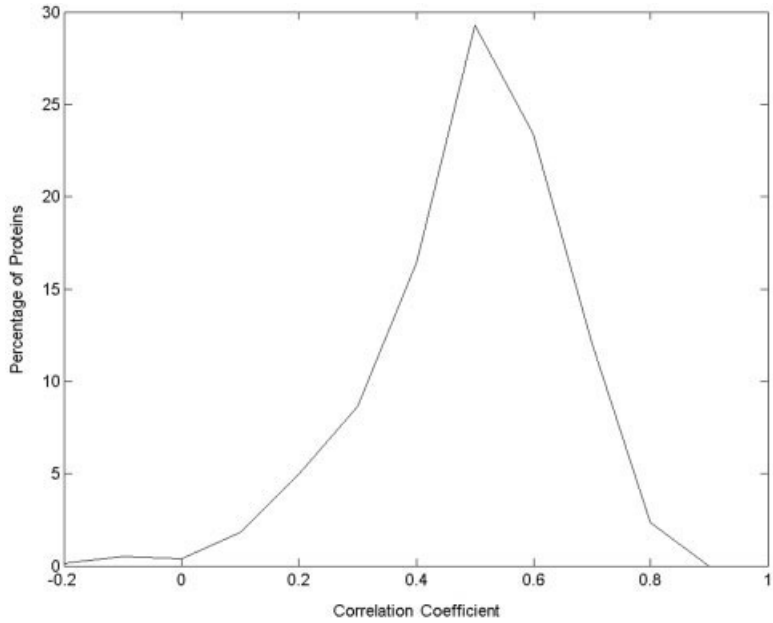

Fig. 2. The distribution of CC values between predicted and experimental B-factors. Each point shows the percentage of proteins in the dataset with CCs in a bin of size of 0.1.

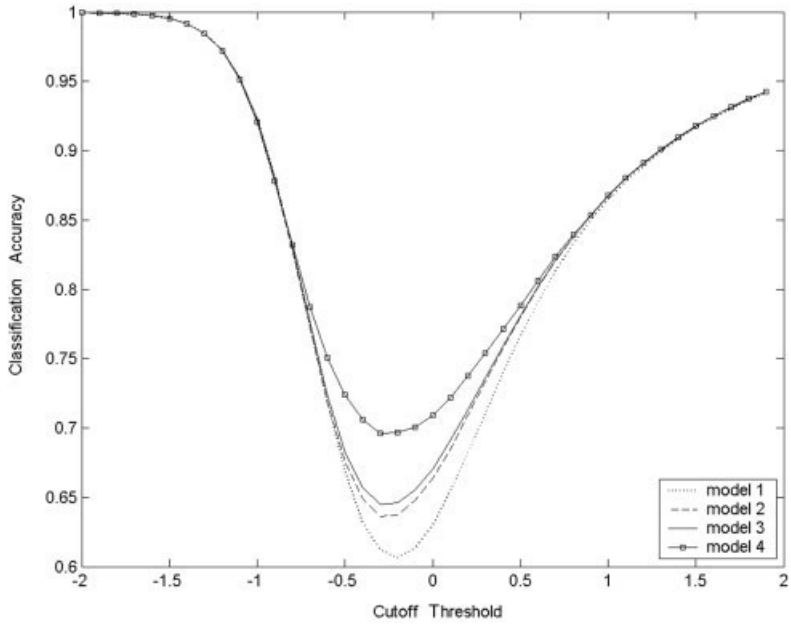

Fig. 3. Prediction accuracies versus cutoff thresholds for different models.

tein sequences are highly correlated (average CC of 0.80). This coefficient may be regarded as a prediction limit.

Several sequence-based B-factor prediction methods were compared by Radivojac et al.[12] using a smaller training dataset (290 protein chains). The method of Vihinen el al.[3] achieved a CC of 0.32. The best method based on neural networks and using multiple-sequence encoding as input, achieved a CC of 0.43.[12] Based on our large dataset of high-resolution proteins, our best method has a CC of 0.53, and more than half of the proteins are predicted with CCs greater than 0.56. This improvement may be partially due to our larger dataset (766 protein chains). Radivojac et al.[12] also reported classification accuracies, but these are not directly comparable with our results because they used a different B-factor normalization method.

Different models have been used to predict B-factor distribution based on protein atomic coordinates. The translation liberation screw model[35–37] simplifies the protein as a rigid body with movement along translation, liberation and screw axes. Considering only the liberation movement and defining B-factors as the squared distances of C_α atoms from the protein mass

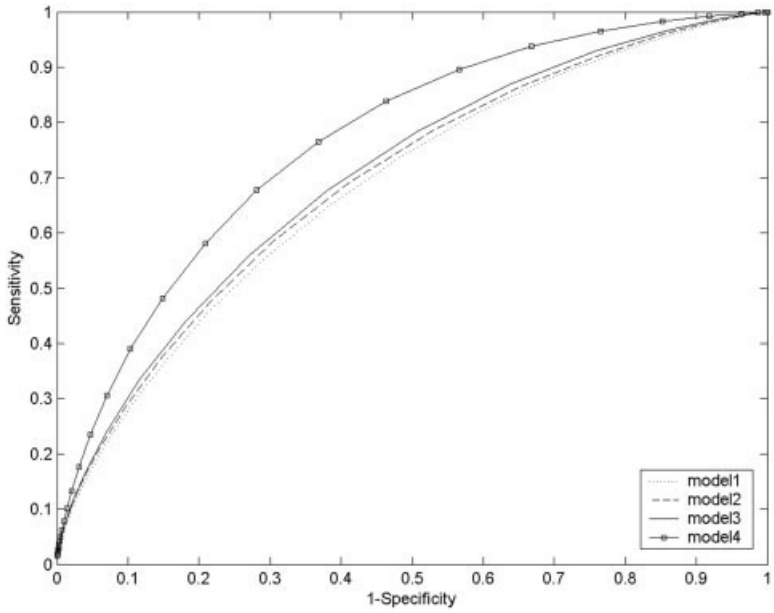

Fig. 4. ROC analysis of different models when the B-factor classification threshold is set at 0.03.

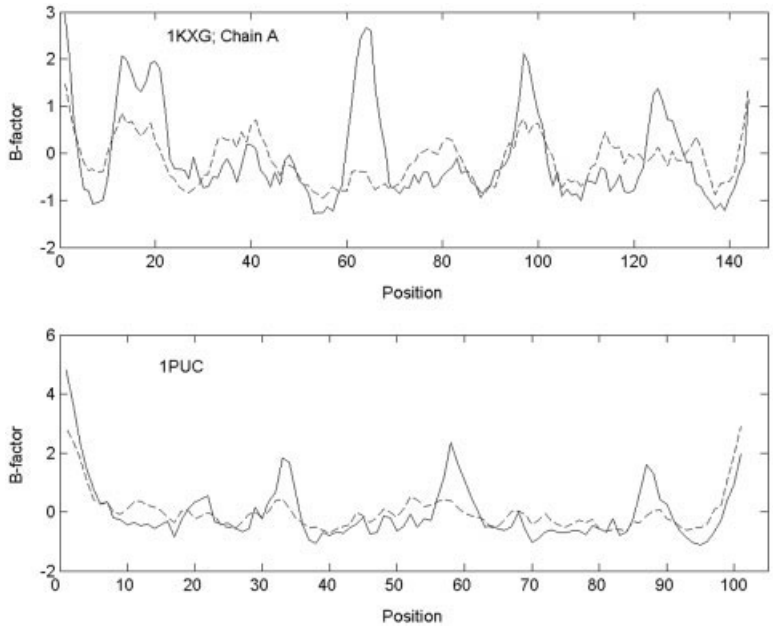

Fig. 5. Prediction results for protein 1KXG (chain A) and 1PUC. The dashed lines represent B-factors predicted by SVR, and the solid lines represent the measured B-factors. The CCs for 1KXG and 1PUC are 0.54 and 0.78, respectively.

centroid, Kundu et al.[14] achieved an average CC of 0.52 on a dataset of 113 high-resolution proteins. This result is comparable with our result (0.53) obtained from protein sequences.

More complicated structure-based analytical methods may predict B-factor distribution more accurately. The Gaussian network model (GNM)[38–41] regards a protein as an elastic network of C_α atoms that fluctuate about their mean positions. The fluctuations are assumed to be isotropic (without directional preferences) and also to obey Gaussian distributions. The mean-squared displacements of C_α atoms are used to define their B-factor values. Environmental parameters such as ligands and crystal contacts may be fed into the model to yield different B-factors values. Kundu et al.[14] found that GNM using C_α atom coordinates in an isolated molecule could achieve a CC of 0.59 with the experimental B-factors. This CC was improved to 0.66 if the atoms involved in crystal contacts were excluded from calculation. In addition, a CC value of 0.66 could also be achieved if all neighboring molecules

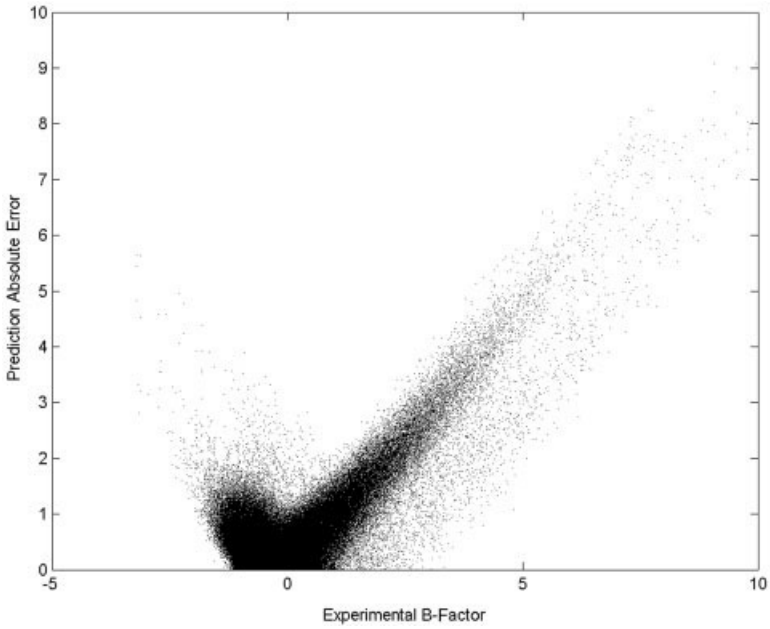

Fig. 6. Absolute prediction error versus experimental B-factors. Lower and higher B-factors are predicted with greater (absolute) errors.

capable of making crystal contacts were considered in the model.

Our SVR approach was trained and tested on primary protein chains only. Therefore, some aspects of B-factors, which are impacted by protein chain–chain contacts, protein–ligand contacts and molecule crystal contacts (non-biological contacts), cannot be well predicted by our method. The purpose of this study is to find a mapping function between protein sequences and their B-factor distributions using a machine learning approach. In this work, our method was trained using B-factor data of molecules in crystals, which may not reflect the real situation of molecules in solution. If adequate training data were available, we could train our method using biological B-factor data. This is a good topic for future work.

The advantage of our method is that it does not require any structural information about the input protein. This is very useful in obtaining information about protein flexibility when only its sequence is known. This information is useful for investigating protein function, since active sites are often located in flexible loop regions. Furthermore, prediction of protein B-factor profiles complements the prediction of other protein properties such as disordered regions[12,42,43] and surface residues.[25,44,45]

CONCLUSIONS

We provide an SVR approach for prediction of protein B-factor distributions (B-factor profiles) from sequence data alone. Because our method requires only the sequence of the protein as input, it is applicable to the large numbers of proteins whose structures are not yet known. Our best method, using multiple sequence alignment input encoding and a radial basis function kernel, greatly improves on the prediction accuracy of previous methods (CC of 0.53 compared to 0.43). In addition, our method achieves a CC of 0.56 or better more than half of the time.

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