On Design of Optimal Nonlinear Kernel Potential Function for Protein Folding and Protein Design

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Abstract

Potential functions are critical for computational studies of protein structure prediction, folding, and sequence design. A class of widely used potentials for coarse grained models of proteins are contact potentials in the form of weighted linear sum of pairwise contacts. However, these potentials have been shown to be unsuitable choices because they cannot stabilize native proteins against a large number of decoys generated by gapless threading, when the number of native proteins is above 300. We develop an alternative framework for designing protein potential. We describe how finding optimal protein potential can be understood from two geometric viewpoints, and we derive nonlinear potentials using mixture of Gaussian kernel functions for folding and design. In our experiment we use a training set of 440 protein structures representing a major portion of all known protein structures, and about 14 million structure decoys and sequence decoys obtained by gapless threading. The optimization criterion for obtaining parameters of the potential is to minimize bounds on the generalization error of discriminating protein structures and decoys not used in training. We succeeded in obtaining nonlinear potential with perfect discrimination of the 440 native structures and native sequences. For the more challenging task of sequence design when decoys are obtained by gapless threading, we show that there is no linear potential with perfect discrimination of all 440 native sequences. Results on an independent test set of 194 proteins also showed that nonlinear kernel potential performs well, with only 3 structures and 14 sequences misclassified, which compare favorable with the results of 7 structures and 37 sequences misclassified using optimal linear potential. We conclude that more sophisticated formulation other than the simple weighted sum of contact pairs can be useful.

Key words: Contact potential; nonlinear potential; kernel models; protein folding; protein design; optimization; support vector.

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1 Introduction

Potential function plays critical roles in computational studies of protein folding, protein structure prediction, and protein sequence design [1–4]. A variety of empirical potential functions have been developed for coarse-grained models of proteins, where amino acid residues are not represented at atomic details. One prominent class of potentials are knowledge-based potentials derived from statistical analysis of database of protein structures [5–8]. In this class of potentials, the interaction between a pair of residues are estimated from its relative frequency in database when compared with a reference state or a null model. This approach has been successfully applied in fold recognition, in threading, and in many other studies [7–14]. The drawback of this class of potential is that there are several conceptual difficulties. These include the neglect of chain connectivity in the reference state, and the problematic implicit assumption of Boltzmann distribution [15–17]. An alternative approach is to find a set of parameters such that the potential functions are optimized by some criterion, e.g., maximized energy difference between native conformation and a set of alternative (or decoy) conformations [16, 18–25]. This approach has been shown to be very effective in recognizing native structures from alternative conformations [25] and in folding membrane proteins [26]. However, if a large number of native protein structures are to be simultaneously stabilized against a large number of decoy conformations, no such potential functions can be found [20, 22].

There are three key steps in developing effective empirical potential function using optimization: (1) the functional form of the potential, (2) the generation of a large set of decoys for discrimination, and (3) the optimization techniques. The initial step of choosing an appropriate functional form so far has been straightforward. Empirical pairwise potentials are usually all in the form of weighted linear sum of interacting residue pairs (see reference [27] for an exception). In this functional form, the weight coefficients are the parameters of the potential, which are optimized for discrimination. The same functional form is also used in statistical potential, where the weight coefficients are derived from database statistics. For the task of decoy generation, an efficient method to obtain millions of decoys is gapless threading, i.e., a decoy conformation can be obtained by mounting the sequence of the native protein to different parts of the conformation of a larger protein of an unrelated structure [19]. Alternatively, a large number of challenging decoys can be generated by using either a chain growth method [28, 29] or a protein structure refinement method [30], both are technically more complex. The optimization techniques that have been used include perceptron learning and linear programming [20, 22], and analytical solution has also been obtained when assumptions about the distribution of data are made [31]. The objectives of optimization are often maximization of energy gap between native conformation and the average of decoy conformation, or energy gap between native and decoys with lowest energy, or the z-score of the native structure [18, 32–35].

In this study, we develop an alternative formulation of protein potential function in the form of mixture of nonlinear Gaussian kernel functions. We also use a different optimization technique based on quadratic programming. Instead of maximizing the energy gap, here a function related to bounds of expected classification errors is optimized [36–39]. In addition, we explore the relationship between protein folding and protein design. We study a simplified version of the protein folding problem. Our goal is to identify the native protein structure from an ensemble of alternative or decoy structures for a given amino acid sequence. We also study a simplified version of the protein design problem. Our goal of protein sequence design is to identify a protein sequence that is most compatible with a given three-dimensional coarse-grained structure. In this study, we do not address the problem of how to generate candidate conformation or candidate sequence by searching either the conformation space or the sequence space.

Experimentation with the nonlinear function developed shows that it can discriminate simultaneous 440 native proteins against 14 million structure decoys generated by gapless threading. Results of similar experiments with perceptron learning was negative, as reported in literature [22]. We also test our potential function for protein design. Using the same training set of 440 proteins and a corresponding set of 14 millions sequence decoys, we succeed in developing a nonlinear function that correctly classifies all 440 native sequences. In contrast, we cannot obtain a weighted linear sum potential using the state-of-the-art interior point solver of linear programming method as reported in [20, 40], such that it is capable of classifying perfectly all the native sequences. We also perform blind tests for both native structure and native sequence recognition. Taking 194 proteins unrelated to the 440 training set proteins, the nonlinear potential achieves a success rate of 92.8% and 98.4% in sequence design and in structure recognition, respectively. Both results compare favorably with optimal linear potential and statistical potential.
The rest of the paper is organized as follows. We first describe theory and model of linear and nonlinear function, including the kernel model and the optimization technique. We then explain details of computation. We further describe experimental results of learning and results of blind test. We conclude with discussion.

2 Theory and Models

Modeling Protein Folding Potential. To model protein computationally, we first need a method to describe its geometric shape and its sequence of amino acid residues. Frequently, a protein is represented by a $d$-dimensional vector $c \in \mathbb{R}^d$. For example, we can represent a protein as a vector $c \in \mathbb{R}^d$, $d = 20$, by measuring the solvent accessible surface areas of each of the 20 residue types. A method that is widely used is to count nonbonded contacts of various types of amino acid residue pairs in a protein structure. In this case, the count vector $c$ is given, the protein description $f: (s, a) \mapsto \mathbb{R}^d$ will fully determine the $d$-dimensional vector $c$. In the case of contact vector, $f$ corresponds to the mapping provided by specific contact definition, e.g., two residues are in contact if their distance is below a specific cut-off threshold distance.

Based on the classical experiments of Anfinsen [41], a fundamental requirement for protein folding is that the native structure $s_N$ with native amino acid sequence $a_N$ must have an energy $H(f(s_N, a_N))$ that is the lowest among a set of alternative structure called decoys $D = \{(s_D, a_N)\}$, where the native amino acid sequence $a_N$ takes a decoy conformation $s_D$ that is different from the native conformation $s_N$ [19, 42]:

$$H(f(s_N, a_N)) < H(f(s_D, a_N)) \quad \text{for all } (s_D, a_N) \in D$$

Sometimes we can further require that the energy difference must be greater than a constant $b > 0$:

$$H(f(s_N, a_N)) + b < H(f(s_D, a_N)) \quad \text{for all } (s_D, a_N) \in D$$

A widely used functional form for protein potential function $H$ is the weighted linear sum of pairwise contacts [5–8, 20, 21]. The linear sum energy $H$ is then:

$$H(f(s, a)) = H(c) = w \cdot c. \quad (1)$$

As soon as the weight vector $w$ is specified, the potential function is fully defined. For such linear potentials, the basic requirement for protein potential is then:

$$w \cdot (c_N - c_D) < 0$$

or

$$w \cdot (c_N - c_D) + b < 0$$

if we require that the energy difference between a native structure and a decoy must be greater than a real value $b$.

Two Geometric Views of Linear Protein Folding Potentials. There is a natural geometric view of the inequality requirement for weighted linear sum potentials. A useful observation is that each of the inequalities divides the space of $\mathbb{R}^d$ into two halfs separated by a hyperplane. The hyperplane is defined by the normal vector $(c_N - c_D)$ and its distance $b/\|c_N - c_D\|$ from the origin. The weight vector $w$ must be located in the half-space opposite to the direction of the normal vector $(c_N - c_D)$ (Fig 1a). This half-space can be written as $w \cdot (c_N - c_D) + b < 0$.

When there are many inequalities to be satisfied simultaneously, the intersection of the half-spaces forms a convex polyhedron [43]. If the weight vector is located in the polyhedron, all the inequalities are satisfied. Potentials with such weight vector $w$ can discriminate the native protein from the set of all decoys.

For each native protein $i$, there is one convex polyhedron $P_i$ formed by the set of inequalities associated with its decoys. If our potential can discriminate simultaneously $n$ structures from a union of sets of decoys, the weight vector $w$ must be located in a smaller convex polyhedron $P$ that is the intersection of the $n$ convex polyhedra:

$$w \in P = \bigcap_{i=1}^{n} P_i.$$
Figure 1: Geometric views of the inequality requirement for weighted linear protein potential. (a). In the first geometric view, the space \( \mathbb{R}^d \) is divided into two half-spaces by the hyperplane \( \mathbf{w} \cdot (\mathbf{c}_N - \mathbf{c}_D) + b < 0 \). The hyperplane is defined by the normal vector \( (\mathbf{c}_N - \mathbf{c}_D) \) and its distance \( b/\|\mathbf{c}_N - \mathbf{c}_D\| \) from the origin. The weight vector must be located in the half-space opposite to the direction of the normal vector \( (\mathbf{c}_N - \mathbf{c}_D) \). (b). A second geometric view of the inequality requirement for linear protein potential. The space \( \mathbb{R}^d \) is divided into two half-spaces by the hyperplane \( \mathbf{w} \cdot (\mathbf{c}_N - \mathbf{c}_D) + b < 0 \). Here the hyperplane is defined by the normal vector \( \mathbf{w} \) and its distance \( b/\|\mathbf{w}\| \) from the origin. All points \{\( \mathbf{c}_N - \mathbf{c}_D \)\} are located on one side of the hyperplane away from the origin.

There is yet another geometric view of the inequality requirements. The relationship \( \mathbf{w} \cdot (\mathbf{c}_N - \mathbf{c}_D) + b < 0 \) for all decoys and native protein structures can be regarded as a requirement that all points \{\( \mathbf{c}_N - \mathbf{c}_D \)\} are located on one side of a hyperplane, which is defined by its normal vector \( \mathbf{w} \) and its distance \( b/\|\mathbf{w}\| \) to the origin (Fig 1b). We can show that such a hyperplane exists if the origin is not contained within the convex hull of the set of points \{\( \mathbf{c}_N - \mathbf{c}_D \)\} (see Appendix).

The second geometric view is dual and mathematically equivalent to the first geometric view. In the first view, a point \( \mathbf{c}_N - \mathbf{c}_D \) determined by the structure-decoy pair \( \mathbf{c}_N = (\mathbf{s}_N, \mathbf{a}_N) \) and \( \mathbf{c}_D = (\mathbf{s}_D, \mathbf{a}_N) \) corresponds to a hyperplane representing an inequality, a solution weight vector \( \mathbf{w} \) corresponds to a point located in the final convex polyhedron. In the second view, each structure-decoy pair is represented as a point \( \mathbf{c}_N - \mathbf{c}_D \) in \( \mathbb{R}^d \), and the solution weight vector \( \mathbf{w} \) is represented by a hyperplane separating all the points \( \mathcal{C} = \{\mathbf{c}_N - \mathbf{c}_D\} \) from the origin.

**Optimal Linear Potentials.** Several optimization methods have been applied to find the weight vector \( \mathbf{w} \). The Rosenblatt perceptron method works by iteratively updating an initial weight vector \( \mathbf{w}_0 \) [21, 25]. Starting with a random vector, e.g., \( \mathbf{w}_0 = \mathbf{0} \), we test each native protein and its decoy structure. Whenev er the relationship \( \mathbf{w} \cdot (\mathbf{c}_N - \mathbf{c}_D) + b < 0 \) is violated, we update \( \mathbf{w} \) by adding to it a scaled vector \( \eta \cdot (\mathbf{c}_N - \mathbf{c}_D) \). The final weight vector is therefore a linear combination of protein and decoy count vectors:

\[
\mathbf{w} = \sum_{\mathbf{N} \in \mathcal{N}} \eta \cdot (\mathbf{c}_N - \mathbf{c}_D) = \sum_{\mathbf{N} \in \mathcal{N}} \alpha_N \cdot \mathbf{c}_N - \sum_{\mathbf{D} \in \mathcal{D}} \alpha_D \cdot \mathbf{c}_D. \tag{2}
\]

Here \( \mathcal{N} \) is the set of native proteins, and \( \mathcal{D} \) is the set of decoys. The set of coefficients \( \{\alpha_N\} \cup \{\alpha_D\} \) gives a dual form representation of the weight vector \( \mathbf{w} \) as an expansion of the training examples, including both native and decoy structures.

If the final convex polyhedron \( \mathcal{P} \) is non-empty, there can be infinite number of choices of \( \mathbf{w} \), all with perfect discrimination. But how do we find a weight vector \( \mathbf{w} \) that is optimal? This depends on the criterion for optimality. For example, one can choose the weight vector \( \mathbf{w} \) that minimizes the variance of energy gaps between decoys and natives: \( \arg_{\mathbf{w}} \min_{\mathcal{D}} \frac{1}{|\mathcal{D}|} \left( \sum_{\mathbf{D}} (\mathbf{w} \cdot (\mathbf{c}_N - \mathbf{c}_D))^2 - \left[ \frac{1}{|\mathcal{D}|} \sum_{\mathbf{D}} (\mathbf{w} \cdot (\mathbf{c}_N - \mathbf{c}_D))^2 \right]^2 \right) \) as used in reference [20], or minimizing the Z-score of a large set of native proteins, or minimizing the Z-score of the native protein...
The optimal weight vector \( w \) of native proteins and decoys, the optimal weight vector from the protein distribution characterized by the origin. This is related to the novelty detection problem and single-class support vector machine studied in this region such that a new unseen point drawn from the same protein distribution as \( \{c_N - c_D\} \) will have a high probability to fall within the defined region, and non-protein points following a different distribution, which is assumed to be centered around the origin when no \textit{a priori} information is available, will have a high probability to fall outside the defined region. In this case, we are more interested in modeling the region or support of the distribution of protein data, rather than estimating its density distribution function. For linear potential, regions are half-spaces defined by hyperplanes, and the optimal hyperplane \((w, b)\) is the one with maximal distance to the origin. This is related to the novelty detection problem and single-class support vector machine studied in statistical learning theory \([36,39,47]\). In our case, any non-protein points will need to be detected as outliers from the protein distribution characterized by \( \{c_N - c_D\} \). Among all linear functions derived from the same set of native proteins and decoys, the optimal weight vector \( w \) is likely to have the least amount of mislabellings. The optimal weight vector \( w \) can therefore be found by solving the following primal quadratic programming problem:

\[
\begin{align*}
\text{Minimize} \quad & \frac{1}{2} ||w||^2 \\
\text{subject to} \quad & w \cdot (c_N - c_D) + b < 0 \quad \text{for all} \ N \in \mathcal{N} \text{ and } D \in \mathcal{D}.
\end{align*}
\]

The solution maximizes the distance \( b/||w|| \) of the plane \((w, b)\) to the origin. The dual form of the same quadratic programming problem can be written as \([48]\):

\[
\begin{align*}
\text{Minimize} \quad & \sum_{i,j} \alpha_i \alpha_j \cdot x_i \cdot x_j \\
\text{subject to} \quad & \alpha_i \geq 0 \text{ and } \sum_i \alpha_i = 1
\end{align*}
\]

where \( x_i, x_j \in \{c_N - c_D\} \).

**Nonlinear Potential.** However, it is possible that no such weight vector \( w \) exists, \textit{i.e.}, the final convex polyhedron \( \mathcal{P} = \bigcap_{i=1}^{n} \mathcal{P}_i \) may be an empty set. First, for a specific native protein \( i \), there may be severe restriction from some inequality constraints, which makes \( \mathcal{P}_i \) an empty set. Some decoys are very difficult to discriminate due to perhaps deficiency in protein representation. In these cases, it is impossible to adjust the weight vector so the native structure has a lower energy than the decoy. Second, even if a weight vector \( w \) can be found for each native protein, \textit{i.e.}, \( w \) is contained in a nonempty polyhedron, it is still possible that the intersection of \( n \) polyhedra is an empty set, \textit{i.e.}, no weight vector can be found that can stabilize all native proteins against the decoys simultaneously. Computationally, the question whether a solution weight vector \( w \) exists can be answered unambiguously in polynomial time \([49]\), and recent studies using millions of decoys strongly suggest that when the number of native protein structure reaches 300–400, no such weight vector can be found \([20,22]\). When perfect discrimination is impossible, a technique that minimizes the percentage of unsatisfied inequalities or the error rate was developed in reference \([31]\).

A fundamental reason for this failure is that the functional form of linear sum of pairwise interaction is too simplistic. It has been suggested that higher order interactions such as three-body or four-body contacts should be incorporated \([50–52]\). Functions with polynomial terms using up to 6 degree of Chebyshev expansion has also been used to represent pairwise interactions \([27]\).

Here we propose an alternative approach. At this time we still limit ourselves to pairwise contact interactions. We introduce a nonlinear potential function analogous to the dual form of the linear function in Equation (2), which takes the following form:

\[
H(f(s, a)) = H(c) = \sum_{D \in \mathcal{D}} \alpha_D \cdot K(c, c_D) - \sum_{N \in \mathcal{N}} \alpha_N \cdot K(c, c_N)
\]

(3)

where \( \alpha_D \geq 0 \) and \( \alpha_N \geq 0 \) are parameters of the potential function to be determined, and \( c_D \) is a contact vector of decoy \( D \) in the set of decoys \( D = \{s_D, a_N\} \), \( c_N \) a contact vector of native structure \( N \) in the set of native
training proteins $\mathcal{N} = \{(s_N, a_N)\}$. The difference of this functional form from linear function in Equation (2) is that a kernel function $K(x, y)$ replaces the linear term. A convenient kernel function $K$ is:

$$K(x, y) = e^{-||x-y||^2/2\sigma^2}.$$ 

Intuitively, the potential surface has smooth Gaussian hills of height $\alpha_D$ centered on the location $c_D$ of decoy structure $D$, and has smooth Gaussian cones of depth $\alpha_N$ centered on the location $c_N$ of native structures $\mathcal{N}$. Ideally, the value of the potential function will be $-1$ for contact vectors $c_N$ of native proteins, and will be $+1$ for contact vectors $c_D$ of decoys.

**Optimal Nonlinear Potential.** To obtain the nonlinear potential, our goal is to find a set of parameters $\{\alpha_D, \alpha_N\}$ such that $H(f(s_N, a_N))$ has energy value close to $-1$ for native proteins, and the decoys have energy values close to $+1$. There are many different choices of $\{\alpha_D, \alpha_N\}$. We use an optimality criterion originally developed in statistical learning theory [37–39]. First, we note that we have implicitly mapped each structure and decoy from $\mathbb{R}^{210}$ through the kernel function of $K(x, y) = e^{-||x-y||^2/2\sigma^2}$ to another space with dimension as high as tens of millions. Second, we then find the hyperplane of the largest margin distance separating proteins and decoys in the space transformed by the nonlinear kernel. That is, we search for a hyperplane with equal and maximal distance to the closest native proteins and the closest decoys. Such a hyperplane can be found by obtaining the parameters $\{\alpha_D\}$ and $\{\alpha_N\}$ from solving the following Lagrange dual form of quadratic programming problem:

$$\text{Maximize} \quad \sum_{i \in \mathcal{N} \cup \mathcal{D}} \alpha_i - \frac{1}{2} \sum_{i,j \in \mathcal{N} \cup \mathcal{D}} y_i y_j \cdot \alpha_i \alpha_j \cdot e^{-||c_i - c_j||^2/2\sigma^2}$$

subject to $0 \leq \alpha_i \leq C$

where $C$ is a regularizing constant that limits the influence of each misclassified conformation [36–39, 47], and $y_i = +1$ if $i$ is a native protein, and $y_i = -1$ if $i$ is a decoy. These parameters lead to optimal classification of unseen test sets proteins against decoys [36–39, 47].

**Modeling Sequence Design Potential.** For protein sequence design, we assume that native sequence is more stable on the native conformation than on a different structure of another protein with low sequence identity. We use the method of gapless sequence threading to generate sequence decoys by mounting a sequence fragment from a different protein of a larger size of an unrelated structure to the native structure [19]. Because all native contacts are retained, we find that such sequence decoys are quite challenging.

For protein design, we seek potential functions that allows the search and identification of sequences most compatible with a specific given coarse-grain three-dimensional structure. We use a model analogous to the Anfisen experiments. We require that the native amino acid sequence $a_N$ mounted on the native structure $s_N$ has the lowest energy compared to a set of unrelated alternative sequences $\mathcal{D} = \{s_N, a_D\}$ mounted on the same native protein structure $s_N$:

$$H(f(s_N, a_N)) < H(f(s_N, a_D)) \quad \text{for all } a_D \in \mathcal{D}$$

Equivalently, the native sequence will have the highest probability to fit into the specified native structure. This is the same principle described in [53–55]. Much work has been done using linear design function of sum of contact pairs in the form of $H(f(s, a)) = H(c) = w \cdot c$ [53, 54]. The discussion of the two geometric views of the weight vector of linear potential and the optimality criterion also applies to the sequence design problem.

We now explore nonlinear potential function for sequence design using the following functional form:

$$H(f(s, a)) = H(c) = \sum_{D \in \mathcal{D}} \alpha_D \cdot K(c, c_D) - \sum_{N \in \mathcal{N}} \alpha_N \cdot K(c, c_N)$$  \quad (4)

where $\alpha_D \geq 0$ and $\alpha_N \geq 0$ are coefficients to be determined, and $c_D = f(s_N, a_D)$ is the contact vector of a decoy sequence $a_D$ mounted on its native protein structure $s_N$, and $c_N = f(s_N, a_N)$ is the contact vector of a native sequence $a_N$ from the set of native training proteins $N$ mounted on the native structure $s_N$. The only difference
from nonlinear folding potential of Equation (3) is that here \( D \) is a set of sequence decoys mounted on native protein structures, rather than a set of structure decoys. Again, we use kernel function \( K(x, y) = e^{-||x-y||^2/2\sigma^2} \).

The optimal parameters \( \{\alpha_N\} \) and \( \{\alpha_D\} \) are obtained similarly by solving the following Lagrange dual convex quadratic programming problem:

\[
\begin{align*}
\text{Maximize} & \quad \sum_{i \in N \cup D} \alpha_i - \frac{1}{2} \sum_{i,j \in N \cup D} y_i y_j \cdot \alpha_i \alpha_j e^{-||c_i - c_j||^2/2\sigma^2} \\
\text{subject to} & \quad 0 \leq \alpha_i \leq C
\end{align*}
\]

where \( C \) is a regularizing constant [37, 38], and \( y_i = +1 \) if \( i \) is a native protein, and \( y_i = -1 \) if \( i \) is a decoy.

3 Computational Methods

Alpha Contact Maps. Because protein molecules are formed by thousands of atoms, their shapes are complex. In this study we use the count vector of pairwise contact interactions derived from the edge simplices of the alpha shape of a protein structure. Edge simplices in the alpha shape represent nearest neighbor interactions that are in physical contacts. They encode precisely the same contact information as a subset of the edges in the Voronoi diagram of the protein molecule. These Voronoi edges are shared by two interacting atoms from different residues, but intersect with the body of the molecule modeled as the union of atom balls. We refer to references [56, 57] for further theoretical and computational details.

Generating Structure Decoys and Sequence Decoys by Threading. Maiorov and Crippen used the gapless threading method for generating a large number of structure decoys [19]. In this method, the sequence of a smaller protein \( a_N \) is threaded through the structure of an unrelated larger protein and takes the conformation \( s_D \) of a fragment of the larger protein [19]. Along the way, the sequence of the smaller protein can take the conformations of many fragments of the larger protein, each provides a structure decoy. With this approach, we generate for each native protein \( \{s_N, a_N\} \) a set of structure decoys \( \{s_D, a_N\} \) (Fig 2a).

We can generate sequence decoys in an analogous way, as already suggested in [42, 51]. In this case, we thread the sequence of a larger protein through the structure of a smaller protein, and obtain sequence decoys by mounting a fragment of the native sequence from the large protein to the full structure of the small protein. We therefore have for each native protein \( s_N, a_N \) a set of sequence decoys \( s_D, a_D \) (Fig 2b).

Protein Data. Following reference [22], we use protein structures contained in the WHATIF database [58] in this study. WHATIF database contains a representative set of sequence-unique protein structures generated from X-ray crystallography. Structures selected for this study all have pairwise sequence identity < 30\%, R-factor < 0.21, and resolution < 2.1. WHATIF database contains less structures than PDBSELECT because the R-factor and resolution criteria are more stringent [58]. Nevertheless, it provides a good representative set of currently all known protein structures.

We use a list of 456 proteins compiled from the 1998 release (WHATIF98) of the WHATIF database [22], which was kindly provided by Dr. Vendruscolo. There are 192 proteins with multiple chains in this dataset. Some of them have extensive interchain contacts. For these proteins, it is possible that their conformations may be different if there are no interchain contacts present. We use the criterion of Contact Ratio to remove proteins that have extensive interchain contacts. Contact Ratio is defined here as the number of interchain contacts divided by the total number of contacts a chain makes. For example, protein 1ept has four chains A, B, C, and D. The intra chain contact number of chain B is 397. Contacts between chain A and chain B is 178, between B and C is 220, between B and other heteroatoms is 11. The Contact Ratio of chain B is therefore \((178 + 220 + 11)/(397 + 178 + 220 + 11) = 51\%\). Thirteen protein chains are removed because they all have Contact Ratio > 30\%. We further remove three proteins because each has > 10\% of residues missing with no coordinates in the Protein Data Bank file. The remaining set of 440 proteins are then used as training set for developing both folding and design potential functions. Using the sequence and structure threading method described earlier, we generated a set of 14 080 766 sequence decoys and a set of structure decoys of the same size.
Figure 2: Decoy generation by gapless threading. (a). Structure decoys can be generated by threading the sequence of a smaller protein to the structure of an unrelated larger protein. (b). Sequence decoys can be generated by threading the sequence of a larger protein to the structure of an unrelated smaller protein.

**Learning Linear Potential.** For comparison, we develop optimal linear potential following the method and computational procedure described in reference [20]. We apply the interior point method as implemented in BPMD by Mészáros [59] to search for a weight vector $w$. We use two different optimization criteria as described in reference [20]. The first is:

Identify $w$ subject to $w \cdot (c_N - c_D) < \epsilon$ and $|w_i| \leq 10$

where $w_i$ denotes the $i$-th component of weight vector $w$, and $\epsilon = 1 \times 10^{-6}$. Let $\mathcal{C} = \{c_N - c_D\}$, and $|\mathcal{C}|$ the number of decoys. The second optimization criterion is:

Minimize $\min \frac{1}{|\mathcal{C}|} \sum (w \cdot (c_N - c_D))^2 - \left[ \frac{1}{|\mathcal{C}|} \sum (w \cdot (c_N - c_D)) \right]^2$

subject to $w \cdot (c_N - c_D) < \epsilon$
Learning Nonlinear Kernel Potential. We use SVMlight (http://svmlight.joachims.org/) \cite{60} with Gaussian kernels and a training set of 440 native proteins plus 14 080 766 decoys to obtain the optimized parameter \( \{\alpha_N, \alpha_D\} \). The regularization constant \( C \) takes default value, which is estimated from the training set \( N \cup D \) as implemented in SVMlight:

\[
C = |N \cup D|^2 / \left[ \sum_{x \in N \cup D} \sqrt{K(x, x) - 2 \cdot K(x, 0) + K(0, 0)} \right]^2. \tag{5}
\]

Since we cannot load all 14 millions decoys into computer memory simultaneously, we use three heuristic strategies for training. In the first method, the total 14 080 766 decoys are divided randomly into 34 subsets. The training process starts with all native proteins and the first decoy set. After the training process converges, we select the decoy structures that have \( \alpha_i \neq 0 \) (these decoys are called support vectors \cite{37–39}). From the second iteration on, we combined the native proteins, the new decoy set, and all decoys that appeared as support vectors in any of the previous training steps. These form the new training set for the next iteration of learning. This process is continued until all decoys have been exhausted in training.

In the second method, each of the 34 decoy subsets was combined with the native set in turn to form a training set. The decoy support vectors are then selected from each of the 34 training processes. All these decoy support vectors are combined, along with native proteins for a final training process. The results are taken as the optimized protein potential.

The third method is similar to the procedure reported in \cite{20}. We first randomly selected a subset of decoys that fits into the computer memory. For example, we pick every 51st decoy from the list of 14 million decoys. This leads to an initial training set of 276 095 decoys and 440 native proteins. An initial protein potential is then obtained after learning. Next the energies for all 14 million decoys and all 440 native proteins are evaluated. Three decoy sets were collected based on the evaluation results: the first set of decoys contains the violating decoys which have lower energy than the native structures; the second set contains decoys with the lowest absolute energy, and the third set contains decoy support vectors identified in previous training process. The union of these three subsets of decoys are then combined with the 440 native protein as the training set for the next iteration of learning. This process is repeated until the energy difference to native protein for all decoys are greater than 0.0. Using this strategy, the number of iterations typically is between 2 and 10. During the training process, we set the cost factor \( j \) in SVMlight to 120, which is the factor by which training errors on native proteins outweighs errors on decoys.

The value of \( \sigma^2 \) for the Gaussian kernel \( K(x, y) = e^{-||x-y||^2/2\sigma^2} \) is chosen by experimentation. We find that if the value of \( \sigma^2 \) is too large, no \( \{\alpha_N, \alpha_D\} \) can be found that can perfectly classifies the 440 training proteins and their decoys, i.e., the problem is unlearnable. If the value of \( \sigma^2 \) is too small, the performance in blind-test will deteriorate because of overfitting. For the 440 native proteins and the 14 080 766 sequence decoys, the value of \( \sigma^2 \) is chosen between 250.0 (for training method 1) and 138.9 (for training method 3). The final folding potential is obtained with \( \sigma^2 = 227.3 \) and the final design potential is obtained with \( \sigma^2 = 138.9 \), both derived using the third training method.

4 Results

Linear Folding Potentials from Structure Decoys. Using perceptron learning and a large number of decoys generated by gapless threading, Vendruscolo et al showed that if the number of native proteins exceeds 270, it is impossible to find parameters \( w \) for potential function \( H(s, a) = w \cdot c \), such that all native structures have lower energies than decoys \cite{22}. That is, no \( w \) can be found such that \( w \cdot c_N < w \cdot c_D \) holds for all decoys.

Tobi et al. showed that pairwise contact potential \( H(s, a) = w \cdot c \) can be found to distinguish a different set of 572 proteins from a set of 28 213 009 structure decoys generated by gapless threading \cite{20}. In this study, two residues are defined to be in contact if the geometric centers of their side chains are at a distance between 2.0 Å and 6.4 Å. To search for the optimal weight vector \( w \), the authors used linear programming solver based on interior point method as implemented in BPMD by Mészáros \cite{59}.

We succeeded in reproducing the results of Tobi et al \cite{20} and found a \( w \) vector that enables perfect discrimination of the same set of 572 proteins used in \cite{20} against 28 261 307 structure decoys we generated by gapless threading. We used the same contact definition based on Euclidean distance between geometric centers of side
chains, as well as the same optimization criterion described earlier in the Methods section. The values of the 210 pairwise contact potentials (data not shown) are similar although not identical to the values listed in [20].

**Linear Design Potentials from Sequence Decoys.** Structure decoys generated by gapless threading are obtained by taking a fragment of the structure of a large protein such that it contains exactly the same number of amino acid residues as the native protein. However, contacts in the resulting fragment structure often contain only a fraction of the total contacts in native protein.

Design decoys are generated by taking a fragment sequence of a larger protein and thread it onto the native conformation of a smaller protein. As a result, although the contact count vector $c$ may be very different, the number of contacts in the design decoy is exactly the same as that in native protein. For decoys generated by gapless threading, design decoys therefore are far more challenging to discriminate then structure decoys (Fig 3).

Our experimentation confirms this observation. After generating 14 080 766 sequence design decoys for the 440 proteins in the training set, we apply the same interior point method to search for an optimal $w$ that can discriminate native sequences from decoy sequences. That is, we search for parameters $w$ for $H(s, a) = w \cdot c$, such that $w \cdot c_N < w \cdot c_D$ for all sequences. However, we fail to find a feasible solution for the weight vector $w$. This indicates that no $w$ exists capable of discriminating perfectly 440 native sequences from the 14 million decoy sequences. We repeated the same experiment using the set of 572 native proteins from reference [20] and 28 261 307 sequence decoys. The result is also negative.

**Learning Nonlinear Kernel potential.** To overcome the problems associated with linear potentials, we use the same set of 440 native proteins and 14 million decoys to obtain nonlinear kernel folding and design potentials. In both cases, we succeeded in finding a function in the form of Equation (3) that can discriminate all 440 native proteins from 14 million decoys.

Unlike statistical potentials where each native protein structure in the database contribute to the empirical potential, only a subset of native structures contribute and have $\alpha_N \neq 0$. In addition, a small fraction of decoys also contribute to the potential function. Table 1 list the details of the potential, including the numbers of native proteins and decoys that participate in Equation (3). These number represent about 60% of native proteins and < 0.1% of decoys from the original training data.

**Discrimination Tests for Folding Potential.** Blind test in discriminating native proteins from decoys using an independent test set is essential to assess the effectiveness of folding potentials. To construct a test set, we first take the entries in Whatif99 database that are not present in Whatif98. After eliminating proteins with chain length less than 46 residues, we obtain a set of 201 proteins. These proteins all have < 30% sequence identities with any other sequence in either the training set or the test set proteins. Since 139 of the 201 test
proteins have multiple chains, we use the same criteria applied in training set selection to exclude 7 proteins with > 30% Contact Ratio or with > 10% residues missing coordinates in the PDB files. This leaves a smaller set of test proteins of 194 proteins. Using gapless threading, we generate a sets of 3 096 019 structure decoys from the set of 201 proteins. This is a superset of the decoy set generated using 194 proteins.

For comparison, we also test the discrimination results of the optimal linear potential taken as reported in reference [20], as well as the statistical potential developed by Miyazawa and Jernigan. Here we use the contact definition reported in [20], that is, two residues are declared to be in contact if the geometric centers of their side chains are within a distance of 2.0 – 6.4 Å.

To test nonlinear folding potential functions for discriminating native proteins from structure decoys in both the 194 and the 201 test sets, we take the structure $s$ from the conformation-sequence pair $(s, a_N)$ with the lowest energy as the predicted structure of the native sequence. If it is not the native structure $s_N$, the discrimination failed and the folding potential does not work for this protein. The results of discriminating native structures using nonlinear folding potential are summarized in Table 2. There are 3 and 8 misclassified native structures for the 194 set and 201 set, respectively. These correspond to a failure rate of 1.5% and 4.0%, respectively. The optimal nonlinear kernel folding potential performs better than the optimal linear potential based on calculation using potential values taken as reported in reference [20] (failure rates 3.6% and 6.5% for the 194 set and 201 set, respectively). Consistent with previous reports [61], statistical potential has about 43.8% (81 out of 194) and 43.2% (87 out of 201) failure rates for the 194 set and the 201 set, respectively.

### Table 1: Details of learning nonlinear kernel folding and design potentials. The number of native proteins and decoys with non-zero $\alpha_i$ entering the potential function is listed. These native proteins and decoys are called support vectors. The range of the energy values of natives and decoys are also listed, as well as the range of the energy gaps between the native protein and the decoy with the lowest energy.

| Strategy | Design Potential | Folding Potential |
|----------|------------------|-------------------|
|          | $\sigma^2 = 250.0$ | $\sigma^2 = 227.3$ | $\sigma^2 = 138.9$ | $\sigma^2 = 227.3$ |
| Num. of Support Vectors | Natives: 260, Decoys: 2457 | Natives: 258, Decoys: 2877 | Natives: 347, Decoys: 4709 | Natives: 268, Decoys: 2560 |
| Range of Energy Values | Natives: 1.070 – 0.9996, Decoys: -9.495 – 0.7979 | Natives: 0.9993 – 5.762, Decoys: -9.485 – 0.9339 | Natives: 0.9991 – 5.762, Decoys: -6.655 – 0.9882 | Natives: 0.9990 – 5.762, Decoys: -8.379 – 1.039 |
| Range of Smallest Energy Gap | 0.2025 – 14.56, 0.06624 – 14.55 | 0.01226 – 9.237, 0.0610 – 11.36 |

Table 2: The number of misclassified protein structures using nonlinear kernel folding potential, optimal linear potential taken as reported in [20], and Miyazawa-Jernigan statistical potential [9] among the test set of 194 proteins and the set of 201 proteins. The latter include proteins with more than 30% interchain contacts and proteins with > 10% missing coordinates. We also list performance of kernel design potential for structure recognition.

| Potential | Misclassified Natives | Misclassified Natives |
|-----------|-----------------------|-----------------------|
| Kernel Folding Potential | 3/194 | 8/201 |
| Tobi & Elber$^X$ | 7/194 | 13/201 |
| Miyazawa & Jernigan | 85/194 | 92/201 |
| Kernel Design Potential | 7/194 | 13/201 |

**Discrimination Tests for Design Potential.** We use the same 194 set and 201 set of natives proteins and generate a set of 3 096 019 sequence decoys for testing the design potential. We take the sequence $a$ from
the conformation-sequence pair \((s_N, a)\) for a protein with the lowest energy as the predicted sequence. If it is not the native sequence \(a_N\), the discrimination failed and the design potential does not work for this protein.

Sequence decoys obtained by gapless threading are quite challenging, since all native contacts of the protein structures are maintained. No linear design potential function can be found using linear programming method that would succeed in the challenging task of identifying all 440 native sequences in the training set.

We succeeded in obtaining nonlinear design potential capable of discriminating all of the 440 native sequences (Table 4). It also works well in the test set. It succeeded in correctly identifying 92.8% (180 out of 194) of native sequences in the independent test set. This compares favorably with results obtained using optimal linear folding potential taken as reported in [22], which succeeded in identifying 80.9% (157 out of 194) of the test set, and the Miyazawa-Jernigan statistical potential (success rate 58.2%, 113 out of 194).

### Discrimination Test Using Challenging Decoys

For the protein potentials derived with simple decoys generated by gapless threading, a more challenging test is to discriminate native proteins from an ensemble of explicitly generated three dimensional decoy structures with a significant number of near-native conformations [7, 62]. Here we evaluate the performance of nonlinear folding and design potential using three decoy sets from the database “Decoys 'R' Us” [63]: the 4STATE_REDUCED set, the LATTICE_SSFIT set, and the LMSD set. We compare our results in performance with results reported in literature using optimal linear potential [64] and statistical potential [9] (Table 4). For the 4STATE_REDUCED set of decoys, nonlinear design potential has the best performance in terms of identifying the native structure. The only misclassified protein 1sn3 has three disulfide bonds, which are not modeled explicitly in the protein description of a vector in \(\mathbb{R}^{210}\). The correlation of root mean square distance of conformations to the native structure and energy value in the 4STATE set are shown in Fig 4. For proteins 1r69, 2cro, 3ich, and 1ctf, the correlation coefficient between the rmsd values and the energy values are good. We note that the nonlinear potential is obtained by learning from training decoys that are obtained by gapless threading. It is likely that nonlinear kernel potential can be further improved if more realistic structure decoys are included in training.

### Nonlinear Potential Function for Folding and Design

Because nonlinear potential is expressed as a kernel expansion of native protein and decoys, structure decoys and sequence decoys in general lead to different protein functions. For example, the contact count vectors \(c\) can be very different for a sequence decoy of a protein and a structure decoy of the same protein. The potential surface defined by the folding potential and the design potential therefore may be different. There are 268 out of 440 native proteins participating in folding potential function with \(\alpha\) value ranging from 0.01 - 28.04, of which 185 (65%) are between 0.01 and 2.00. There are 347 out of 440 native proteins participating in design potential function, with \(\alpha\) value ranging from 0.02 to 110.64, of which 269 (77%) are between 0.02 and 2.00. Therefore, the majority of the native proteins have similar \(\alpha\) values for both folding and design potentials. Fig 5 shows the difference \(\Delta \alpha_i\) of the coefficient \(\alpha_i\) for protein \(i\) appearing in both folding potential and design potential. For the majority of the native proteins, \(\Delta \alpha_i\) values are small. That is, most native proteins contribute similarly in design potential and in folding potential.

| Kernel Design Potential* | Misclassified Natives | Misclassified Natives |
|--------------------------|-----------------------|-----------------------|
| Tobi & Elber             | 14/194                | 20/201                |
| Miyazawa & Jernigan      | 81/194                | 87/201                |
| Kernel Folding Potential | 24/194                | 30/201                |

Table 3: The number of misclassified protein sequences using nonlinear kernel design potential, optimal linear potential taken as reported in [22], and Miyazawa-Jernigan statistical potential [9] among the set of 194 proteins and the set of 201 proteins. The latter include proteins with more than 30% interchain contacts and proteins with > 10% missing coordinates. The nonlinear kernel design potential is the only function that succeeded in perfect discrimination of the 440 native sequences from a set of 14 million sequence decoys.

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Table 4: Results of discrimination of native structures from decoys using nonlinear kernel potentials. The decoy sets include the 4state\_reduced set, the lattice\_ssfit set, and the lmsd set [63]. The energy rank of the native structure and its z-score are listed. The correlation coefficient $R$ is also listed in parenthesis for the 4state\_reduced set. KFP stands for kernel folding potential, and KDP stands for kernel design potential. TE-13 potential is linear distance based potential optimized by linear programming, taken as reported in [64], and MJ potential is statistical potential as reported in [9]. Results for TE-13 potential and Miyazawa-Jernigan potential are taken from Table II of [64].
Figure 4: The energy values of decoys and native proteins in the 4STATE_REDUCED set by nonlinear design potentials and their correlation with the rmsd values to the native structures.

This is expected, because the main differences between the two potentials are due to differences in decoy sets. The native proteins that contribute most differently to folding potential and design potential are small proteins. For example, there are 34 native proteins whose $|\Delta \alpha| > 5.0$, and they all come from the top 100 smallest protein chains. Table 5 listed 20 proteins with highest $\alpha$ values for both kernel folding and design potentials. They are all small proteins and there are 15 out of the 20 proteins that appear both in folding and design potentials. It is possible that the energy values by kernel folding potential and by kernel design potential may be similar for many structure-sequence pairs $(s, \alpha)$. Figure 6a shows that the test 194 proteins have similar energy values by the kernel folding and kernel design potentials.

We also compare the energy value of the potential functions for each of the 210 unit vector $c_1 = \{1, 0, \ldots, 0\}^T, \ldots$, and $c_{210} = \{0, \ldots, 1\}^T$. We normalize these values so $\max H(c_i) = 1$ for both potentials (Fig 7a). There is strong correlation ($R = 0.91$) for folding and design potentials.

However, other methods reveal that kernel folding and design potentials are different. One method is to compare the energy values of a subset of decoy structures that are challenging. That is, we compare energies of decoys with $\alpha_i \neq 0$. Fig 7b shows that for decoys appearing in the design potentials, there is little correlation in
energy values calculated by design potential and by folding potential. Similarly, there is no correlation between energy values calculated by folding potential and by design potentials for the set of structure decoys entering the design potential function (Fig 7c). It seems that although the values of $\alpha_N$s are similar for the majority of the native proteins, design potential and folding potential can give very different energy values for some conformations. This suggests that the overall fitness for design and folding potential may be somewhat different.

Comparison with Other Potentials. Potential functions derived from kernel models have the form of $H(c) = \sum_{d \in D} \alpha_d \cdot K(c, c_d) - \sum_{N \in N'} \alpha_n \cdot K(c, c_n)$, which cannot be directly compared with other potential functions of the form $H(c) = w \cdot c$. Here we follow the same approach as above and compare the energy values of the unit vectors, the native proteins, and the decoys using different potential functions. The energy values by nonlinear folding and design potentials have very little correlation with energy values evaluated using either optimal linear folding potential [20] or statistical potential [9] (Fig 6c-f). It seems nonlinear potentials developed in this work contains information absent in other potential functions.

5 Discussion

A basic requirement for computational studies of protein folding and protein design is an effective potential function, which allows the search and the identification of native structures and native sequences. Current empirical potential functions are based on weighted linear sum of pairwise interactions. A major flaw of such potentials is that they cannot recognize the native structures of a large number of proteins from alternative decoy conformations [20, 22].

There are several routes towards improving empirical potential functions. One approach is to introduce higher order interactions, where three-body or four-body interactions are explicitly incorporated in the potential function [50–52, 65]. The effectiveness of this approach is likely to be assessed in the future with large scale validation studies similar to those carried out for pairwise potentials [8, 20, 22]. A different approach to improve empirical potential function is to introduce nonlinear terms. Recently, Fain et al uses sums of Chebyshev polynomials up to order 6 for hydrophobic burial and each type of pairwise interactions [27].

In this work, we propose a different framework for developing empirical nonlinear protein potential functions. Instead of using nonlinear function for each term of pairwise interactions, we use a set of simple Gaussian kernel functions located at both native proteins and decoys as the basis set. We regard the decoys as equivalent
Table 5: The 20 proteins with highest $\alpha$ value from both kernel folding potential and kernel design potential. The residue number, and SCOP class also were listed.

to the reference state or null model used in statistical potential. The expansion coefficients $\{\alpha_N\}, N \in N$ and $\{\alpha_D\}, D \in D$ of these Gaussian kernels determines the protein function. As long as the native proteins and decoys are represented as unique vectors $c \in \mathbb{R}^d$, the Gram matrix of the kernel function is full-rank. Therefore, the kernel function effectively maps the protein space into a high dimensional space in which effective discrimination with a hyperplane is easier to obtain. The optimization criterion here is not $Z$-score, rather we search for the hyperplane in the transformed high dimensional space with maximal separation distance between the native protein vectors and the decoy vectors. This choice of optimality criterion is firmly rooted in a large body of studies in statistical learning theory, where expected number of errors in classification of unseen future test data is minimized probabilistically by balancing the minimization of the training error (or empirical risk) and the control of the capacity of specific types of functions of potential function [37–39].

This approach is general and flexible, and can accommodate other protein representation, as long as the final descriptor of protein and decoy is a $d$-dimensional vector $c$. In addition, different forms of nonlinear functions can be designed using different kernel functions, such as polynomial kernel and sigmoidal kernels. It is also possible to adopt different optimality criterion, for example, by minimizing the margin distance expressed in 1-norm instead of the standard 2-norm Euclidean distance.

A useful observation obtained from this study is that sequence decoys obtained from gapless threading are quite challenging. In fact, we found that no linear potential function exists that can discriminate a training set of 440 native sequence from sequence decoys generated by gapless threading. The success of nonlinear potential in perfect discrimination of this training set native sequences and its good performance in identifying the native sequences in an unrelated test set of 194 proteins indicate that nonlinear kernel potential is a general strategy
Figure 6: Comparison of nonlinear kernel folding potential (KFP) and kernel design potentials (KDP) with Tobi-Elber (TE) optimal linear potential and Miyazawa-Jernigan (MJ) statistical potential. The energy values of the 194 proteins in the test set are calculated and scaled to 0.0 – 1.0. (a). The energy values by KFP and by KDP for the 194 proteins are highly correlated. The correlation coefficient is $R = 0.90$. The energy values by (b) MJ and TE are also highly correlated. The correlation coefficient is $R = 0.95$. The energy values by (c) MJ and KDP, (d) by TE and KDP, (e) by MJ and KFP, (f) by TE and KFP are all poorly correlated. This suggests that both the kernel folding and design potential functions are different from MJ and TE potentials.

for developing effective potential function for protein sequence design.

It is informative to examine the three misclassified proteins by the kernel folding potential (1bx7, 1hta, and 3erd). Hirustasin 1bx7 contain five disulfide bonds, which are not modeled explicitly by the protein description. 1hta (histone Hmfa) exists as a tetramer in complex with DNA under the physiological condition. Its nature structure may not be the same as that of a lone chain. The two terminals of this protein are rather flexible, and their conformations are not easy to determine. 3erd.a (estrogen receptor α ligand-binding domain) has extensive contacts with ligand. These unmodeled interactions may alter protein conformation. Among the 14 native sequences misclassified by the kernel design potential (1a73.a, 1bd8, 1bea, 1bm8, 1bn8.a, 1bv.f, 1cku.a, 1dpt.a, 1hta, 1mro.c, 1ops, 1qav.a, 1upb.b, 3ezn.a), several have extensive interchain interactions, although the contact ratio is below the rather arbitrary threshold of 30%: 1a73.a has Contact Ratio of 23%, 1mro.c has 24%, 1upb.b has 19%, and 1qav.a has 13%. It is possible that the substantial contacts with other chains would alter the confirmation of the protein. Amylase inhibitor 1bea contains 5 disulfide bond not explicitly modeled. 1cku.a (electron transfer protein) contains an iron/sulfur cluster, which covalently bind to four Cys residues and prevent them from forming 2 disulfide bonds. These covalent bonds are not reflected in the protein description. 1bv.f (oxidoreductase) is complexed with a heme and an FMN group. The conformations of 1cku.a and 1bv.f may be different upon removing of these functionally important hetero groups. Altogether, there are some rationalization for 10 of the 16 misclassified proteins.
Figure 7: Comparison of nonlinear kernel folding potential and kernel design potential generated by optimized discrimination against decoys from gapless threading. (a). The energy values of the nonlinear folding and design potentials for the 210 unit vectors are strongly correlated ($R = 0.91$). (b). The energy values by both design potentials and by folding potential for decoys that enter the nonlinear design functions are poorly correlated. (c). The energy values for decoys that enter the nonlinear folding functions are also poorly correlated.

Our goal in this study is to explore an alternative formulation of potential function and assess the effectiveness of this new approach with experimental data. The nonlinear potential functions obtained in this study should be further improved. For example, unlike the study of optimal linear potential [20], where explicitly generated three-dimensional decoys structures are used in training, we used only structure decoys generated by threading. The test results using the 4STATE-REDUCED set and the LATTICE-SSFIT are comparable or better with other residue-based potential (see Fig 4 and Table 4). It is likely that further incorporation of explicit three-dimensional decoy structures in the training set would improve the protein potential.

In summary, we show in this study an alternative formulation of protein function using a mixture of Gaussian kernels. We demonstrate that this formulation can lead to effective folding potential and design potential that perform well in independent tests. For protein sequence design where challenging decoys are available from gapless threading, nonlinear kernel potential can have perfect classification in the training set of 440 proteins, while linear potential and statistical potential failed. It also performs better in an independent test set of 194 proteins and reduce the misclassification to 40% of that of optimal linear potential. Our results suggest that more sophisticated functional form other than the simple weighted sum of contact pairs can be useful for studying protein folding and protein design. This approach can be generalized for any other protein representation, e.g., with descriptors for explicit hydrogen bond and higher order interactions.
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7 Appendix

Lemma 1 For a potential function in the form of weighted linear sum of interactions, a decoy always has energy values higher than the native structure by at least an amount of \( b > 0 \), i.e.,

\[
\mathbf{w} \cdot (\mathbf{c}_D - \mathbf{c}_N) > b \quad \text{for all} \quad \{(\mathbf{c}_D - \mathbf{c}_N)|D \in \mathcal{D} \text{ and } N \in \mathcal{N}\}
\]

if and only if the origin \( \mathbf{0} \) is not contained within the convex hull of the set of points \( \{(\mathbf{c}_D - \mathbf{c}_N)|D \in \mathcal{D} \text{ and } N \in \mathcal{N}\} \).

Proof: Suppose that the origin \( \mathbf{0} \) is contained within the convex hull \( \mathcal{A} = \text{conv}(\{\mathbf{c}_D - \mathbf{c}_N\}) \) of \( \{\mathbf{c}_D - \mathbf{c}_N\} \) and Equation (6) holds. By the definition of convexity, any point inside or on the convex hull \( \mathcal{A} \) can be expressed as convex combination of points on the convex hull. Specifically, we have:

\[
\mathbf{0} = \sum_{(\mathbf{c}_D - \mathbf{c}_N) \in \mathcal{A}} \lambda_{\mathbf{c}_D - \mathbf{c}_N} \cdot (\mathbf{c}_D - \mathbf{c}_N), \quad \text{and} \quad \sum_{\mathbf{c}_D - \mathbf{c}_N} \lambda_{\mathbf{c}_D - \mathbf{c}_N} = 1, \lambda_{\mathbf{c}_D - \mathbf{c}_N} > 0.
\]

That is, we have the following contradiction:

\[
0 = \mathbf{w} \cdot \mathbf{0} = \mathbf{w} \cdot \sum_{\mathbf{c}_D - \mathbf{c}_N} \lambda_{\mathbf{c}_D - \mathbf{c}_N} \cdot (\mathbf{c}_D - \mathbf{c}_N) = \sum_{\mathbf{c}_D - \mathbf{c}_N} \lambda_{(\mathbf{c}_D, \mathbf{c}_N)} \cdot \mathbf{w} \cdot (\mathbf{c}_D - \mathbf{c}_N) > \sum_{\mathbf{c}_D - \mathbf{c}_N} \lambda_{\mathbf{c}_D - \mathbf{c}_N} \cdot b = b.
\]

Because the convex hull can be defined as the intersection of half hyperplanes derived from the inequalities, if a half hyperplane has a distance \( b > 0 \) to the origin, all points contained within the convex hull will be on the other side of the hyperplane [43]. Therefore, \( \mathbf{w} \cdot (\mathbf{c}_D - \mathbf{c}_N) > b \) will hold for all \( \{(\mathbf{c}_D - \mathbf{c}_N)\} \).  \( \blacksquare \)
References

[1] Dill K, Bromberg S, Yue K, Fiebig K, Yee D, Thomas P, Chan H. Principles of protein folding—a perspective from simple exact models. Protein Sci 1995;4:561–602.
[2] Levitt M, Gerstein M, Huang E, Subbiah S, Tsai J. Protein folding: the endgame. Annu Rev Biochem 1997;66:549–579.
[3] Bonneau R, Baker D. Ab initio protein structure prediction: progress and prospects. Annu Rev Biophys Biomol Struct 2001;30:173–189.
[4] Saven J. Designing protein energy landscapes. Chemical Reviews 2001;101:453–458.
[5] Tanaka S, Scheraga H. Medium- and long-range interaction parameters between amino acids for predicting three-dimensional structures of proteins. Macromolecules 1976;9:945–950.
[6] Miyazawa S, Jernigan R. Estimation of effective interresidue contact energies from protein crystal structures: quasi-chemical approximation. Macromolecules 1985;18:534–552.
[7] Samudrala R, Moult J. An all-atom distance-dependent conditional probability discriminatory function for protein structure prediction. J Mol Biol 1998;275:895–916.
[8] Lu H, Skolnick J. A distance-dependent atomic knowledge-based potential for improved protein structure selection. Proteins 2001;44:223–232.
[9] Miyazawa S, Jernigan R. Residue-residue potentials with a favorable contact pair term and an unfavorable high packing density term. J Mol Biol 1996;256:623–644.
[10] Wodak S, Rooman M. Generating and testing protein folds. Curr Opin Struct Biol 1993;3:247–259.
[11] Sippl M. Knowledge-based potentials for proteins. Curr Opin Struct Biol 1995;5(2):229–235.
[12] Lemer C, Rooman M, Wodak S. Protein-structure prediction by threading methods - evaluation of current techniques. Proteins 1995;23:337–355.
[13] Jernigan R, Bahar I. Structure-derived potentials and protein simulations. Curr Opin Struct Biol 1996;6:195–209.
[14] Simons KT, Ruczinski I, Kooperberg C, Fox B, Bystroff C, Baker D. Improved recognition of native-like protein structures using a combination of sequence-dependent and sequence-independent features of proteins. Proteins 1999;34:82–95.
[15] Thomas P, Dill K. Statistical potentials extracted from protein structures: How accurate are they? J Mol Biol 1996;257:457–469.
[16] Thomas P, Dill K. An iterative method for extracting energy-like quantities from protein structures. Proc Natl Acad Sci USA 1996;93:11628–11633.
[17] Ben-Naim A. statistical potentials extracted from protein structures: Are these meaningful potentials? J Chem Phys 1997;107:3698–3706.
[18] Goldstein R, Luthey-Schulten Z, Wolynes P. Protein tertiary structure recognition using optimized hamiltonians with local interactions. Proc Natl Acad Sci USA 1992;89:9029–9033.
[19] Maiorov V, Crippen G. Contact potential that recognizes the correct folding of globular proteins. J Mol Biol 1992;227:876–888.
[20] Tobi D, Shafran G, Linial N, Elber R. On the design and analysis of protein folding potentials. Proteins 2000;40:71–85.
[21] Vendruscolo M, Domany E. Pairwise contact potentials are unsuitable for protein folding. J Chem Phys 1998;109:11101–11108.
[22] Vendruscolo M, Najmanovich R, Domany E. Can a pairwise contact potential stabilize native protein folds against decoys obtained by threading? Proteins 2000;38:134–148.
[23] Bastolla U, Farver J, Knapp E, Vendruscolo M. How to guarantee optimal stability for most representative structures in the protein data bank. Proteins 2001;44:79–96.
[24] Dima R, Banavar J, Cieplak M, Maritan A. Scoring functions in protein folding and design. Protein Sci 2000;9:812–819.
[25] Micheletti C, Seno F, Banavar J, Maritan A. Learning effective amino acid interactions through iterative stochastic techniques. Proteins 2000;42(3):422–431.
[26] Dobbs H, Orlandini E, Bonaccini R, Seno F. Optimal potentials for predicting inter-helical packing in transmembrane proteins. Proteins 2002;49(3):342–349.
[27] Fain B, Xia Y, Levitt M. Design of an optimal Chebyshev-expanded discrimination function for globular proteins. Protein Sci 2002;11:2010–2021.
[28] Vendruscolo M, Kussel E, Domany E. Recovery of protein structure from contact maps. Folding Design 1997;2:295–306.
[29] Vendruscolo M, Domany E. Efficient dynamics in the space of contact maps. Folding Design 1998;3:329–336.
[30] Skolni J, Kolinski A, Ortiz A. Monstir: a method for folding globular proteins with a small number of distance constraints. J Mol Biol 1997;265:217–241.
[31] Xia Y, Levitt M. Extracting knowledge-based energy functions from protein structures by error rate minimization: Comparison of methods using lattice model. J Chem Phys 2000;113:9318–9330.
[32] Koretke K, Luthey-Schulten Z, Wolynes P. Self-consistently optimized statistical mechanical energy functions for sequence structure alignment. Protein Sci 1996;5:1043–1059.
[33] Koretke K, Luthey-Schulten Z, Wolynes P. Self-consistently optimized energy functions for protein structure prediction by molecular dynamics. Proc Natl Acad Sci U S A 1998;95(6):2932–2937.
[34] Hao M, Scheraga H. How optimization of potential functions affects protein folding. Proc Natl Acad Sci U S A 1996;93(10):4984–4989.
[35] Mirny L, Shakhnovich E. How to derive a protein folding potential? a new approach to an old problem. J Mol Biol 1996;264:1164–1179.
[36] Vapnik V, Chervonenkis A. Theory of Pattern Recognition. Moscow: Nauka, 1974. (German Translation: W. Vapnik & A. Tschervonenkis, Theorie der Zeichenerkennung, Akademie-Verlag, Berlin, 1979).
[37] Vapnik V. The Nature of Statistical Learning Theory. N.Y.: Springer, 1995.
[38] Burges CJC. A Tutorial on Support Vector Machines for Pattern Recognition. Knowledge Discovery and Data Mining 1998;2.
[39] Schölkopf B, Smola A. Learning with kernels: Support vector machines, regularization, optimization, and beyond. Cambridge, MA: The MIT Press, 2002.
[40] Meller J, Wagner M, Elber R. Maximum feasibility guideline in the design and analysis of protein folding potentials. J Comput Chem 2002;23:111–118.
[41] Anfinsen C. Principles that govern the folding of protein chains. Science 1973;181:223–230.
[42] Jones D, Taylor W, Thornton J. A new approach to protein fold recognition. Nature 1992;358:86–89.
[43] Edelsbrunner H. Algorithms in combinatorial geometry. Berlin: Springer-Verlag, 1987.
[44] Chiu T, Goldstein R. Optimizing energy potentials for success in protein tertiary structure prediction. Folding Des 1998;3:223–228.
[45] Hao MH, Scheraga H. Designing potential energy functions for protein folding. Curr Opinion Structural Biology 1999;9:184–188.
[46] Friedrichs M, Wolynes P. Toward protein tertiary structure recognition by means of associative memory hamiltonians. Science 1989;246:371–373.
[47] Vapnik V, Chervonenkis A. A note on one class of perceptrons. Automation and Remote Control 1964;15.
[48] Mangasarian O. Nonlinear programming. Philadelphia: SIAM Publishers, 1994.
[49] Karmarkar N. A new polynomial-time algorithm for linear programming. Combinatorica 1984;4:373–395.
[50] Betancourt M, Thirumalai D. Pair potentials for protein folding: Choice of reference states and sensitivity of predicted native states to variations in the interaction schemes. Protein Sci 1999;8:361–369.
[51] Munson P, Singh R. Statistical significance of hierarchical multi-body potential based on delaunay tessellation and their application in sequence-structure alignment. Protein Sci 1997;6:1467–1481.

[52] Zheng W, Cho S, Vaisman I, Tropsha A. A new approach to protein fold recognition based on Delaunay tessellation of protein structure. In: Altman R, Dunker A, Hunter L, Klein T, editors, Pacific Symposium on Biocomputing'97. Singapore: World Scientific, pp. 486–497, 1997; pp. 486–497.

[53] Shakhnovich E, Gutin A. Engineering of stable and fast-folding sequences of model proteins. Proc Natl Acad Sci USA 1993;90:7195–7199.

[54] Deutsch J, Kurosky T. New algorithm for protein design. Phys Rev Lett 1996;76:323–326.

[55] Li H, Helling R, Tang C, Wingreen N. Emergence of preferred structures in a simple model of protein folding. Science 1996;273:666–669.

[56] Edelsbrunner H. The union of balls and its dual shape. Discrete Comput Geom 1995;13:415–440.

[57] Liang J, Edelsbrunner H, Fu P, Sudhakar P, Subramaniam S. Analytical shape computing of macromolecules I: Molecular area and volume through alpha-shape. Proteins 1998;33:1–17.

[58] Vriend G, Sander C. Quality control of protein models - directional atomic contact analysis. J Appl Cryst 1993;26:47–60.

[59] Mészáros C. Fast Cholesky factorization for interior point methods of linear programming. Comp Math Appl 1996;31:49 – 51.

[60] Joachims T. Advances in Kernel Methods - Support Vector Learning, MIT Press. 1999; .

[61] Clementi C, Maritan A, Banavar J. Folding, design, and determination of interaction potentials using off-lattice dynamics of model heteropolymers. Phys Rev Lett 1998;81:3287–3290.

[62] Park B, Levitt M. Energy functions that discriminate x-ray and near-native folds from well-constructed decoys. J Mol Biol 1996;258:367–392.

[63] Samudrala R, Levitt M. Decoys ‘R’ us: a database of incorrect conformations to improved protein structure prediction. Protein Sci 2000;9:1399–1401.

[64] Tobi D, Elber R. Distance-dependent, pair potential for protein folding: Results from linear optimization. Proteins 2000;41:40–46.

[65] Rossi A, Micheletti C, Seno F, Maritan A. A self-consistent knowledge-based approach to protein design. Biophys J 2001;80(1):480–490.