Nature of Driving Force for Protein Folding –
A Result From Analyzing the Statistical Potential

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In a statistical approach to protein structure analysis, Miyazawa and Jernigan (MJ) derived a
20 × 20 matrix of inter-residue contact energies between different types of amino acids. Using
the method of eigenvalue decomposition, we find that the MJ matrix can be accurately reconstructed
from its first two principal component vectors as $M_{ij} = C_0 + C_1 (q_i + q_j) + C_2 q_i q_j$, with constant
$C$’s, and 20 $q$ values associated with the 20 amino acids. This regularity is due to hydrophobic
interactions and a force of demixing, the latter obeying Hildebrand’s solubility theory of simple
liquids.

Proteins fold into specific three dimensional structures to perform their diverse biological functions. It is now
well established that for small proteins the information contained in the amino acid sequence is sufficient to
determine the folded structure, which is the structure with minimum free energy $\beta$. Thus the native structure is
dictated by the physical interactions between amino acids in the sequence, and understanding the nature of such in-
teractions is crucial for protein structure prediction.

As a protein contains thousands of atoms and interacts with a huge number of water molecules, it is not feasible to calculate the free energy function from first principles. An often adapted practical approach is to
derive a coarse grained potential (often on the level of protein data banks. In such an approach, the energy of
a particular substructure in proteins is derived from the number of its appearances in the structure data bank via
a Boltzmann factor $\gamma$. A classic example of such a statistical potential is the Miyazawa-Jernigan (MJ)
matrix, a 20 × 20 inter-residue contact-energy matrix derived by Miyazawa and Jernigan $\delta$.$\delta$. This matrix tabulates
the interaction strength between any two types of amino acids in proteins, and has been widely applied in protein
design and folding simulations $\delta$.$\delta$.$\delta$.$\delta$.

In this letter, we apply a general method of matrix analysis, namely, eigenvalue decomposition, to the MJ
matrix $\delta$.$\delta$. The analysis reveals an intrinsic regularity of the MJ matrix, which yields basic information about
the nature of the driving force for protein folding. We show that despite the complicated interactions in pro-
teins, the major driving force is hydrophobic interaction and a force of demixing, the latter obeying Hildebrand’s
solubility theory of simple liquids $\delta$. The result allows us to attribute the interactions responsible for folding to
quantifiable properties of individual amino acids. These properties suggest further experimental tests, and can be
used for analyzing sequence-structure relation.

Eigenvalue decomposition is a general approach to analyzing matrices. A given $N \times N$ real symmetric matrix
$M$ can be reconstructed by the following formula

$$M_{ij} = \sum_{\alpha=1}^{N} \lambda_{\alpha} V_{\alpha,i} V_{\alpha,j},$$  

where $M_{ij}$ is the element of the matrix in row $i$ and column $j$, $\lambda_{\alpha}$ is the $\alpha$th eigenvalue, and $V_{\alpha,i}$ is the $i$th
component of the corresponding eigenvector. We have analyzed the MJ matrix using eigenvalue decomposition.
First, we subtract the mean $\bar{M}_{ij}$ from each element and then analyze the eigenvalue spectrum of the remaining
matrix. We find that the eigenvalue spectrum has two dominant eigenvalues which are much larger in magnitude than the rest. Specifically, we find $\lambda_1 = -22.49$, $\lambda_2 = 18.62$, while the rest of the eigenvalues have absolute values between 2.17 and 0.013. This suggests (as
we shall demonstrate below) that the matrix can be accurately reconstructed using only the first two eigenvectors,
$\tilde{M}_{ij} = < M_{ij} > + \lambda_1 V_{1,i} V_{1,j} + \lambda_2 V_{2,i} V_{2,j}$. Further analysis shows that the second eigenvector is related to the
first one by a shift and rescaling, i.e., $V_{2,i} = \beta + \gamma V_{1,i}$, with $\beta = -0.30$, $\gamma = -0.90$, and a correlation coefficient
0.986. Using this relation, the expression for $\tilde{M}_{ij}$ can be written simply as

$$\tilde{M}_{ij} = C_0 + C_1 (q_i + q_j) + C_2 q_i q_j,$$  

where $q_i \equiv V_{1,i}$, and the $C$’s are constants, $C_0 = -1.492$, $C_1 = 5.030$, $C_2 = -7.400$. Thus we can reconstruct the
MJ matrix (which in principle could have 210 independent elements) by using only twenty parameters $q_i$, associated with the twenty amino acids, and three interaction coefficients. Such a simple interaction form is often the starting point for theoretical modeling of proteins $\delta$.$\delta$.$\delta$.$\delta$.

The spectrum of the MJ matrix (two large eigenvalues
with corresponding eigenvectors related to each other) reflects the specific physical interaction between the amino
acids. The connection between the interaction and the spectrum can be understood in the following general way: Consider a pairwise interaction matrix $M_{ij}$ which is determined by certain properties of two species $i$ and $j$, denoted by $q_i$ and $q_j$. Assume, on physical grounds, that
$M_{ij}$ can be expressed as an analytical function $f(q_i,q_j)$ with a well defined converging power series, $f(q_i,q_j) = C_0 + C_1(q_i+q_j) + C_2q_iq_j + C_3(q_i^2 + q_j^2) + C_4(q_iq_j^2 + q_jq_i^2) + \ldots$, where the $C$'s are constants. Take first the example where the expansion ends at the $C_2$ term, i.e., $M_{ij} = C_0 + C_1(q_i+q_j) + C_2q_iq_j$. Since any row of the matrix $M$ is given by a vector $\mathbf{U}_i = (C_0 + C_1q_i)\mathbf{I} + (C_1 + C_2q_i)\mathbf{Q}$, which is a linear combination of $\mathbf{I}$ and $\mathbf{Q}$, where $\mathbf{I} = \{1,1,\ldots,1\}$, and $\mathbf{Q} = \{q_1,q_2,\ldots,q_n\}$, one can decompose the vector space $\mathcal{G}$ into the subspace $\mathcal{G}_{\perp}$ spanned by $\mathbf{I}$ and $\mathbf{Q}$, and its perpendicular compliment $\mathcal{G}_{\parallel}$. It is obvious that $\mathcal{G}_{\perp}$ gives rise to $n - 2$ zero eigenvalues, as $M\mathbf{V}_{\perp} = 0$ for any vector $\mathbf{V}_{\perp}$ in the subspace $\mathcal{G}_{\perp}$. Furthermore, the two eigenvectors with nonzero eigenvalues must be expressible as a linear combination of $\mathbf{I}$ and $\mathbf{Q}$, therefore they are related to each other by a shift and rescaling. Similarly, if the expansion ends at the $C_4$ term, there will be three nonzero eigenvalues, and the corresponding eigenvectors will lie in a subspace spanned by $\mathbf{I}$, $\mathbf{Q}$, and $\mathbf{Q}^2$, where $\mathbf{Q}^2 = \{q_1^2, q_2^2, \ldots, q_n^2\}$. The same argument applies to all higher order expansions. This analysis applies to the ideal case where there is no noise in the matrix. Introducing noise leads to a slight mixing of $\mathcal{G}_{\perp}$ and $\mathcal{G}_{\parallel}$ and therefore to small nonzero values for the rest of the eigenvalue spectrum.

![FIG. 1. Correlation between $M_{ij}$, the original matrix elements and $\tilde{M}_{ij}$, the matrix elements reconstructed from Eq. (3). The regression line is $y = 0.999x - 0.008$. The correlation coefficient is 0.989. Insert: the distribution of the MJ matrix elements. The unit of energy is $k_BT$.](image)

The reconstructed matrix in Eq. (3) reproduces the original MJ matrix to a high accuracy. Fig. 1 shows the correlation between the original MJ matrix and the reconstructed one. The regression line is $y = 0.999x + 0.008$, and the correlation coefficient is 0.989. On average Eq. (3) gives matrix elements with only 5% error compared to the original matrix.

Notice that one can redefine the $q$’s in Eq. (3) by a shift and rescaling while leaving the interaction form unchanged. Therefore any transformation $q \rightarrow Aq + B$ with a corresponding change in the $C$'s yields an identical matrix. To better understand the physical meaning of Eq. (3), we rewrite it in the following form,

$$\tilde{M}_{ij} = h_i + h_j - C_2(q_i - q_j)^2 / 2,$$  \hspace{0.5cm} (3)

where

$$h_i = C_0/2 + C_1q_i + (C_2/2)q_i^2.$$  \hspace{0.5cm} (4)

Now each term in Eq. (3) above is invariant under the transformation discussed above.

What is the physical basis for the simple interaction form in Eq. (3)? Consider the quantity $\chi_{ij} \equiv 2M_{ij} - M_{ii} - M_{jj}$. Since $M_{ij}$ is the energy of forming a contact between type $i$ and type $j$ amino acids in water, $\chi_{ij}$ gives the energy of breaking one $i$-$i$ contact and one $j$-$j$ contact and forming two pairs of $i$-$j$ contacts; thus $\chi_{ij}$ is the energy change due to the mixing of the two types of amino acids. According to Eq. (3), $\chi_{ij} = -C_2(q_i - q_j)^2$. This form has a striking similarity to the mixing energy of two simple liquids as given by Hildebrand’s solubility theory (HST) [9]. In his 1933 classic paper, Hildebrand derived the energy of mixing of two simple liquids by summing over the pairwise interactions throughout the mixture. Assuming that the mixing is random and that the potentials between molecules are of the Lennard-Jones type due to the London dispersion force [11], Hildebrand arrived at a formula which expresses the energy of mixing of liquids $A$ and $B$ as $E_{\text{mixing}} \propto (\delta_{A} - \delta_{B})^2$, where $\delta_{A,B}$ are pure component properties related to the square root of the vaporization energies of liquid $A$ and $B$, traditionally called the “solubility parameter”.

Now we can imagine the formation of 2 $i$-$j$ contacts in water by two steps, formation of an $i$-$i$ contact and a $j$-$j$ contact followed by a mixing. The energy change for the first step is $2h_i + 2h_j$, and that for the second step $\chi_{ij}$. As the formation of an $i$-$i$ contact in water is related to the segregation of amino acids of type $i$ in water, we expect that $h_i$ is related to the hydrophobicity of amino acid $i$. Indeed, we find that $h_i$ correlates very well with the hydrophobicity scales published in the literature [12] (see Fig. 2). Thus despite the complicated interactions in proteins, we find that the pairwise inter-residue interactions responsible for folding can be attributed to the hydrophobic force and a force of demixing, the latter obeying HST (Although HST was derived for simple non-polar molecules, it was found previously that the theory describes well the behavior of polymer blends [13]. The application to proteins is another example of the more general scope of HST.).
FIG. 2. Calculated $h_i$ and measured hydrophobicities [12] of the 20 amino acids. The type of amino acid is indicated using the standard one letter code. The straight line is a linear fit (excluding the charged amino acids) with slope 1.314 and intercept 0.759. The correlation coefficient is 0.769.

The above analysis presents a simple picture of the nature of interactions between amino acids. It also provides experimentally testable predictions. Comparison with HST indicates that the $q_i$ we derive should be linearly related to the solubility parameter of amino acid $i$, which can be measured. Furthermore, we predict from Eq. (4) that hydrophobicity can be expressed as a quadratic function of the solubility parameter. Since the solubility parameter and the hydrophobicity of an amino acid can be measured independently, this prediction can also be tested.

Comparison of the terms in Eq. (3) shows that the linear term $h_i + h_j$ is the dominant one in selecting the native structure. This is because the typical difference of the linear term $\delta h$ (among different types of contacts) is much larger than the typical difference of the square term $\delta \chi/2$, specifically, $\delta h = 6.52(\delta \chi/2)$. Therefore the energy difference between different compact structures (due to different arrangements of the contacts) is mainly due to the linear term. Thus, through a quantitative analysis of the MJ matrix we arrive at the conclusion that the hydrophobic force is the dominant driving force for protein folding [14].

The term $-C_2(q_i - q_j)^2/2$ has an important consequence, however. This term favors demixing of amino acids ($C_2$ is negative). The microscopic basis for such a demixing force is the dissimilar polarizability of the two monomers [1]. Since the interior of a protein is composed of various types of amino acids which tend to segregate, an amino acid buried in the interior of a protein will experience an environment which is quite different from a uniform non-polar environment. It has been controversial whether one can model the interior of a protein as a uniform non-polar environment [13]. This study suggests that in general it is not adequate to do so.

Notice that in Fig. 2 the charged amino acids (E, D, R, K) fall into a distinct group. Since Eq. (2) also gives accurate values for the matrix elements involving charged amino acids, we believe our $q$ scale captures more information regarding folding than a simple hydrophobicity scale. Other noticeable exceptions are the cyclic amino acid Proline, and the two amino acids with aromatic residues Tryptophan and Tyrosine.

The $q$ values we obtain can be used to characterize amino acids. The distribution of the $q$ values is bimodal (see Fig. 3), which supports the notion that amino acids naturally fall into two distinct groups: "polar" (P) and "hydrophobic" (H). This division also accounts for the three different regions in the distribution of the MJ matrix elements (see the insert to Fig. 1), which reflect the three possible combinations of the two groups: polar–polar, polar–hydrophobic, hydrophobic–hydrophobic. The sharp division between the two groups as indicated in Fig. 3 also suggests that amino acids in the same group may play similar roles in structure determination. There is experimental evidence to this effect insofar as certain proteins can be designed by specifying only the HP pattern of the sequence [15]. For the purpose of protein design, the $q$ values can serve as a useful scale for selecting amino acids.

The $q$ values can also be used to analyze the relation
between sequence and structure. In previous studies, hydrophobicity scales have been used to analyze sequences and locate helical segments [17]. However, there exist many different hydrophobicity scales. Our $q$ scale has the advantage of being more closely related to the interactions which determine structure. We find that for a given sequence, segments with alternating large and small $q$ values usually correspond to $\alpha$ helices (consistent with the previous findings using hydrophobic scales), segments with long stretches of large $q$ values usually correspond to reverse turns, and segments with long stretches of small $q$ values usually correspond to $\beta$ strands. An example is shown in Fig. 4 of the 3D structure of the protein flavodoxin [18], with amino acids color coded according to their $q$ values.

To summarize, we were able to extract the regularity of the Miyazawa-Jernigan matrix of inter-residue contact energies between amino acids using the method of eigenvalue decomposition. The analysis reveals that the driving force for protein folding is the hydrophobic force and a force of demixing between amino acids. We were able to construct a solubility scale for amino acids which can be tested experimentally. This scale can also be used for selecting amino acids for the purpose of protein design, and for analyzing sequence-structure relation.

FIG. 4. 3D structure of the protein flavodoxin with only the main chain atoms plotted. Amino acids are color coded according to their $q$ values.

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