A protein structural alphabet and its substitution matrix CLESUM

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Abstract

By using a mixture model for the density distribution of the three pseudobond angles formed by C_{α} atoms of four consecutive residues, the local structural states are discretized as 17 conformational letters of a protein structural alphabet. This coarse-graining procedure converts a 3D structure to a 1D code sequence. A substitution matrix between these letters is constructed based on the structural alignments of the FSSP database.

Key words: structure alignment; structural codes; structural substitution matrix.

1 Introduction

Drastic approximations are unavoidable in prediction of protein structure from the amino acid sequence. Most local structure prediction methods use three secondary structure states: helix, strand and loop. However, segments of a single secondary structure may vary significantly in their 3D structures. A refined objective classification of segments may enhance our ability in the prediction of structures, and deepen our understanding of the modular architecture of proteins.

The usual approaches simplify protein structure by modelling proteins as chains of one or two interacting centers representing individual amino acids, and adopt only a small number of discrete conformational states. Many studies to investigate the classification of protein fragments use the backbone (ϕ, ψ) dihedral angles, or angles of C_{α} pseudobonds or distances derived from the positions of C_{α} atoms. Due to the anticorrelation between ϕ and ψ (McCammon et al., 1977; Flocco and Mowbray 1995), there may be instances where a big change in both ϕ and ψ does not represent an obvious change in the C_{α} pseudobond angles, but a reorientation of the peptide in question. Furthermore, the relation between C_{α} coordinates and pseudobond angles is rather straightforward, and pseudobond angles have a more direct geometric meaning than distances. We shall use only pseudobond angles in this paper.

By restricting the local conformations of individual residues to a handful of states, one can discretize protein conformation to convert the 3D structure of a backbone to a 1D sequence of these discrete states akin to the amino acid sequence. Prediction of protein structure depends on the accuracy and complexity of the models used. A model must be as simple as possible to reduce the conformational space to be searched for a correct conformation, while a model of low complexity tends to have a lower accuracy. A model must represent the actual geometry of protein conformations accurately enough, but a complex model is prone to over-fitting the observed data.

Generally, the procedure to deduce finite discrete conformational states from a continuous conformational phase space is a clustering analysis. There have been a variety of different ways of clustering. For example, Park and Levitt (1995) represent the polypeptide chain by a sequence of rigid fragments that are chosen from a library of representative fragments, and concatenated without any degrees of freedom. The average deviation of the global-fit approximations over the training set is taken as the objective function for optimizing the finite representative fragments. The state clusters there are representative points of the phase space. Rooman, Kocher and Wodak (1991) intuitively divide the ϕ - ψ space into 6 regions, which corresponds to

a partitioning based on the Ramachandran plot. Standard methods for clustering analysis have been also used to generate discrete structure states (Bystroff and Baker, 1998).

Hidden Markov models (HMMs; Rabiner, 1989), possessing a rigorous but flexible mathematical structure, have been used in a variety of computational biology problems such as sequence motif recognition (Fujiwara et al., 1994), gene finding (Burge and Karlin, 1997), protein secondary structure prediction (Asai, Hazamizu and Handa, 1993; Zheng, 2004), and multiple sequence alignments (Krogh et al., 1994). The HMMs have been also used for identifying the modular framwork for the protein backbone (Edgoose, Allison and Dowe, 1998; Camproux et al., 1999). In these HMMs conformation states are represented by probability distributions, which is much finer than a simple partition of the phase space. HMMs also take into account the sequential connections between conformational states, hence involve in a large number of parameters, which make the model training a tough task. Furthermore, it is not so convenient to assign structure codes to a short segment with HMMs.

Here we develop a description of protein backbone tertiary structure using psuedobond angles of successive C_{α} atoms. Finite conformational states as structural alphabet are selected according to the density peaks of probability distribution in the phase space spanned by pseudobond angles, and their feasibility of characterizing short segment polypeptide backbone conformation is examined. In order to use the structural codes in the structural comparison, we derive a substitution matrix of these conformational states from a representative pairwise aligned structure set of the FSSP (families of structurally similar proteins) database of Holm and Sander (1994).

2 Methods

Among a variety of abstract representing forms for protein 3D structure, a frequently encountered one is the protein virtual backbone. The C_{α} atom of the residue is chosen as the representative point. In this representation, two adjacent residues in a protein sequence are virtually bonded, forming a pseudobond.

2.1 Pseudo-bond angles

The virtual bond bending angle θ defined for three contiguous points (a, b, c) is the angle between the vectors $\mathbf{r}_{ab} = \mathbf{r}_b - \mathbf{r}_a$ and \mathbf{r}_{bc} , i.e. $\theta = \mathbf{r}_{ab} \cdot \mathbf{r}_{bc}/(|r_{ab}r_{bc}|)$. The range of θ is $[0, 2\pi]$. The virtual bond torsion angle τ defined for four contiguous points (a, b, c, d) is the dihedral angle between the planes abc and bcd. The range of τ is $(-\pi, \pi]$, and its sign is the same as $(\mathbf{r}_{ab} \times \mathbf{r}_{bc}) \cdot \mathbf{r}_{cd}$. In fact, we may adopt a wider range of τ under the equivalence relation that τ_1 and τ_2 are equivalent if $\tau_1 = \tau_2 \pmod{2\pi}$. For the four-residue segment abcd, by takeing a as the origin, and b on the x-axis, and c on the xy-plane, the number of independent relative coordinates are 6. The assumption of the fixed pseudobond length, which is 3.8 Å for the dominating trans peptide, further reduces the number of degrees of freedom to 3. These independent coordinates correspond to the angles $(\theta_{abc}, \tau_{abcd}, \theta_{bcd})$. Elongating the segment by one residue e will add two more angles τ_{bcde} and θ_{cde} . Generally, for a sequence of n residues, we have n-2 bending angles and n-3 torsion angles, 2n-5 in total. We shall assign the angle pair $(\tau_{abcd}, \theta_{bcd}) \equiv (\tau_c, \theta_c)$ to residue e, the third of the four-residue segment.

Bending and torsion angles of a chain correspond to curvature and torsion of a curve. The relative coordinates of the chain $\{\mathbf{r}_0, \mathbf{r}_1, \cdots \mathbf{r}_n\}$ can be recovered from their 2n-5 angles $\{\theta_1; \tau_2, \theta_2; \cdots; \tau_{n-1}, \theta_{n-1}\}$. By convention, we set the origin at \mathbf{r}_0 , put \mathbf{r}_1 along the x-axis, and add $\tau_1 = 0$. Introducing the rotation matrices R_θ and R_τ (with respect to the z- and x-axis, respectively)

$$R_{\theta} = \begin{pmatrix} \cos \theta & -\sin \theta & 0\\ \sin \theta & \cos \theta & 0\\ 0 & 0 & 1 \end{pmatrix}, \qquad R_{\tau} = \begin{pmatrix} 1 & 0 & 0\\ 0 & \cos \tau & -\sin \tau\\ 0 & \sin \tau & \cos \tau \end{pmatrix}, \quad \text{and} \quad \mathbf{d} = \mathbf{r}_1 = \begin{pmatrix} 1\\ 0\\ 0 \end{pmatrix}, \tag{1}$$

position \mathbf{r}_k is determined by

$$T_0 = I$$
, $\mathbf{r}_0 = 0 \cdot \mathbf{d}$, $T_k = T_{k-1} R_{\tau_k} R_{\theta_k}$, $\mathbf{d}_k = T_{k-1} \cdot \mathbf{d}$, $\mathbf{r}_k = \mathbf{r}_{k-1} + \mathbf{d}_k$, $k \ge 1$, (2)

where I is the identity matrix.

Longer fragments will include more correlation than shorter fragments. However, the complexity that can be explored with the longer fragment lengths is limited severely by the relatively small number of known protein structures, and a larger number of discrete states have to be determined for a longer segment. The minimal unit where the relative coordinates fix the angles and vice versa is four contiguous residue segment. We shall concentrate mainly on the structure codes for the four residue unit.

2.2 The mixture model for the angle probability distribution

The three pseudobond angles (θ, τ, θ') of the four-residue unit span the three-dimensional phase space. Our classifiers for conformational states are based on the following mixture model M: The probability distribution of 'points' $\mathbf{x} \equiv (\theta, \tau, \theta')$ is given by the mixture of several normal distributions

$$P(\mathbf{x}|M) = \sum_{i=1}^{c} \pi_i N(\mu_i, \mathbf{\Sigma}_i), \tag{3}$$

where c is the number of the normal distribution categories in the mixture, π_i the prior for category i, and $N(\mu, \Sigma)$ the normal distribution

$$N(\mu, \mathbf{\Sigma}) = (2\pi)^{-3/2} |\mathbf{\Sigma}|^{-1/2} \exp\left[\frac{1}{2}(\mathbf{x} - \mu) \cdot \mathbf{\Sigma}^{-1} \cdot (\mathbf{x} - \mu)\right]. \tag{4}$$

Each normal distribution has 6 parameters for its symmetric covariance matrix Σ and three for its mean μ . Adding one more parameter of the prior for each category, the mixture model has 10c parameters for the total c categories. (The normalization $\sum_i \pi_i = 1$ reduces the number to 10c - 1.) These categories will be translated as the structure codes.

To objectively determine the number c of categories, we investigate density peaks in the phase space with the downhill simplex method of Nelder and Mead (1965). The method requires only function evaluations, not derivatives. It is not very efficient in terms of the number of function evaluations that it requires, but still works well for our problem here. We use counts in a rectangular box as the value of the function for optimization at the center of the box. The box size corresponds to the Parzon window width. A large box size has a low resolution, hence help us to focus on main density peaks in the phase space, and to easily locate them near their real location. Reducing the box size, we can see more peaks which are less conspicuous and then unseen under a larger box size. A too small box size, making local fluctuations visible, is often misleading. Missing out any important modes will affect the model training and the efficacy of the structural codes generated. We first search for maximal points of the one-dimensional marginal probability distributions of θ and τ , and then utilize them to generate a grid in the (θ, τ, θ') space for searching for peaks in the space.

We examine also density peaks in the five-dimensional phase space spanned by $(\theta_b, \tau_c, \theta_c, \tau_d, \theta_d)$ of the five-residue unit *abcde* to investigate the effect of the angle correlation. A five-angle mode $(\theta_b, \tau_c, \theta_c, \tau_d, \theta_d)$ contains two three-angle modes $(\theta_b, \tau_c, \theta_c)$ and $(\theta_c, \tau_d, \theta_d)$. It is demanded that all the important three-angle modes implied by the main density peaks in the five-angle phase space must be included in the modes used for the construction of the mixture model.

The main purpose of searching for density peaks is to estimate the number c of categories and $\{\mu_i\}$ for each category. Once this has been done, we may start with some simple $\{\pi_i\}$ and $\{\Sigma_i\}$, say $\pi_i = 1/c$ and certain diagonal $\{\Sigma_i\}$, and then update the mixture model by the Expectation-Maximization (EM) method as follows. For each point $\mathbf{x}_k = (\theta_{k-1}, \tau_k, \theta_k)$, we calculate the probability for the point to belong to the i-th category C_i according to the Bayes formula as

$$P(C_i|\mathbf{x}_k) \propto \pi_i P(\mathbf{x}_k|C_i)$$

$$\propto \pi_i |\mathbf{\Sigma}_i|^{-1/2} \exp\left[\frac{1}{2}(\mathbf{x}_k - \mu_i) \cdot \mathbf{\Sigma}_i^{-1} \cdot (\mathbf{x}_k - \mu_k)\right], \tag{5}$$

where we always shift τ_k to the interval $[\tau^{(i)} - \pi, \tau^{(i)} + \pi)$ centered at $\tau^{(i)}$ of the τ -component of the mean μ_i . The probability $P(C_i|\mathbf{x}_k)$ satisfies the normalization condition $\sum_{i=1}^c P(C_i|\mathbf{x}_k) = 1$. The updated parameters for the mixture model are estimated by the EM method as

$$n_i = \sum_k P(C_i|\mathbf{x}_k), \qquad \pi_i = n_i/n, \quad n = \sum_i n_i, \tag{6}$$

$$\mu_i = \frac{1}{n_i} \sum_k P(C_i | \mathbf{x}_k) \mathbf{x}_k, \tag{7}$$

$$\Sigma_i = \sum_k P(C_i|\mathbf{x}_k)(\mathbf{x}_k - \mu_k)(\mathbf{x}_k - \mu_k)^T.$$
 (8)

Generally, the objective function for optimizing the mixture model is

$$Prob(\{\mathbf{x}_k\}) = \prod_k \sum_i P(\mathbf{x}_k, C_i) \propto \prod_k \sum_i P(C_i | \mathbf{x}_k).$$
(9)

However, when we convert point \mathbf{x}_k to its structural code i^* , we use

$$i^* = \arg_i \max P(C_i | \mathbf{x}_k). \tag{10}$$

An alternative objective function would be

$$Q(\{\mathbf{x}_k\}) = \prod_k \max_i P(C_i|\mathbf{x}_k). \tag{11}$$

When starting with narrow distributions for Σ_i , a very high value of Q could be seen at the first step. However, by just one step of the EM iteration Q will drop significantly, and then increases at later steps. While $\text{Prob}(\{\mathbf{x}_k\})$ never decreases, Q will decrease after reaching its maximum. We may stop the model training before Q decreases again. Thus, the optimization here is a compromise between $\text{Prob}(\{\mathbf{x}_k\})$ and $Q(\{\mathbf{x}_k\})$.

Once we have the model, we may convert a structure to its conformational code sequence according to (10). Although no effect from the connection of states is directly considered, the model gains the advantage in being able to easily assign codes to short fragments.

	π	$ \Sigma ^{-1/2}$	μ			$\mathbf{\Sigma}^{-1}$							
State			θ	au	θ'	$\theta\theta$	au heta	au au	$\theta'\theta$	heta' au	$\theta'\theta'$		
I	8.2	1881	1.52	0.83	1.52	275.4	-28.3	84.3	106.9	-46.1	214.4		
J	7.3	1797	1.58	1.05	1.55	314.3	-10.3	46.0	37.8	-70.0	332.8		
Η	16.2	10425	1.55	0.88	1.55	706.6	-93.9	245.5	128.9	-171.8	786.1		
K	5.9	254	1.48	0.70	1.43	73.8	-13.7	21.5	15.5	-25.3	75.7		
\mathbf{F}	4.9	105	1.09	-2.72	0.91	24.1	1.9	10.9	-11.2	-8.8	53.0		
\mathbf{E}	11.6	109	1.02	-2.98	0.95	34.3	4.2	15.2	-9.3	-22.5	56.8		
\mathbf{C}	7.5	100	1.01	-1.88	1.14	28.0	4.1	6.2	2.3	-5.1	69.4		
D	5.4	78	0.79	-2.30	1.03	56.2	3.8	4.2	-10.8	-2.1	30.1		
A	4.3	203	1.02	-2.00	1.55	30.5	9.1	8.7	6.0	5.7	228.6		
В	3.9	66	1.06	-2.94	1.34	26.9	4.6	4.9	9.5	-5.0	54.3		
G	5.6	133	1.49	2.09	1.05	163.9	0.6	3.8	2.0	-3.7	32.3		
${ m L}$	5.3	40	1.40	0.75	0.84	43.7	2.5	1.4	-7.0	-2.9	34.5		
	•												

Table 1. The 17 structural states from the mixture model.

3 Result

Μ

Ν

O

Ρ

Q

3.7

3.1

2.1

3.2

1.7

144

74

247

206

25

1.47

1.12

1.54

1.24

0.86

1.64

0.14

-1.89

-2.98

-0.37

1.44

1.49

1.48

1.49

1.01

For establishing the discrete structural states by training the mixture model, we create a nonredundant set of 1544 non-membrane proteins from PDB_SELECT (Hobohm and Sander, 1994) with amino acid identity less than 25% issued on 25 September of 2001. The data of the three-dimensional structures for these proteins

72.9

25.3

170.8

48.0

28.4

2.1

3.2

-0.7

8.2

1.5

4.8

3.1

3.7

7.3

1.2

1.9

9.9

-4.1

-4.9

3.4

-7.9

0.9

3.1

-6.6

0.1

72.9

83.0

98.7

155.6

19.5

are taken from Protein Data Bank (PDB). The secondary structures for these sequences are taken from the DSSP database (Kabsch and Sander, 1983). We consider the reduced 3 secondary structure states $\{h, e, c\}$ generated from the 8 states of the DSSP by the coarse-graining $H, G, I \to h, E \to e$ and $X, T, S, B \to c$. The total number of contiguous fragments is 2248, which gives totally 264,232 points in the three-angle phase space.

Table 2. The percentages of each secondary structure in the structural states.

	I	J	Н	K	F	E	C	D	A	B	G	L	M	N	O	P	Q	Counts
cccc	3	3	1	5	3	7	14	3	7	3	10	9	5	7	4	5	3	25090
ccce	0	1	0	3	3	6	15	2	5	4	24	9	4	4	4	4	3	3272
ccch	2	1	1	6	5	8	22	4	3	2	9	11	7	4	1	3	2	3028
ccee	0	0	0	0	7	20	17	7	0	1	15	24	0	0	0	0	2	4029
cchh	1	3	0	1	0	0	1	0	46	1	0	0	17	4	0	18	1	3664
ceec	0	0	0	0	6	36	26	14	0	8	0	2	0	0	0	0	3	620
ceee	0	0	0	0	7	43	12	22	0	4	0	2	0	0	0	2	3	3676
ceeh	0	0	0	0	3	11	49	19	0	3	0	0	0	0	1	1	7	51
chhh	21	38	28	4	0	0	0	0	0	0	0	0	4	2	0	0	0	4353
eccc	3	3	1	3	2	6	15	3	14	3	5	11	4	7	2	7	4	3007
ecce	3	1	1	6	1	4	5	1	1	1	1	4	1	26	35	0	1	492
ecch	1	1	0	5	1	6	18	5	4	1	9	24	5	6	0	3	2	258
ecee	1	0	0	0	5	18	16	10	1	2	3	33	1	0	0	2	3	80
echh	0	1	0	0	0	0	0	0	52	1	1	0	10	5	0	17	5	256
eecc	0	0	0	0	6	16	19	7	12	6	0	3	0	9	0	11	4	3807
eece	0	0	0	0	6	18	21	11	11	6	0	3	1	11	0	6	2	80
eech	0	0	0	0	4	15	25	16	2	5	0	3	0	6	0	10	10	256
eeec	0	0	0	0	7	36	19	14	1	9	0	6	0	0	0	1	3	3596
eeee	0	0	0	0	5	48	8	17	0	6	0	7	0	0	0	2	1	11418
eeeh	0	0	0	0	4	15	41	17	2	11	0	2	0	0	0	1	4	197
eehh	0	0	0	0	0	0	3	0	57	2	0	0	0	2	1	28	4	248
ehhh	13	43	25	3	0	0	0	0	0	0	0	0	6	5	1	0	0	248
hccc	4	5	2	4	2	4	7	1	4	2	14	6	6	5	18	6	1	3254
hcce	1	2	0	3	5	5	11	0	7	5	22	7	4	4	6	7	3	208
hcch	3	0	1	4	3	5	21	5	4	2	14	9	7	4	4	3	1	328
hcee	0	0	0	0	6	21	18	9	0	4	17	14	1	0	0	0	2	151
hchh	1	2	0	1	0	0	1	0	12	1	0	0	27	18	0	31	0	356
heec	0	0	0	0	5	50	15	10	0	10	0	5	0	0	0	5	0	20
heee	0	0	0	0	4	50	4	17	1	6	0	3	0	0	0	8	1	117
hhcc	9	15	11	9	1	0	0	0	1	0	15	10	9	1	10	2	0	3861
hhce	1	1	0	1	2	0	2	0	1	1	43	15	6	0	14	3	0	151
hhch	8	4	3	19	3	0	1	0	0	0	15	28	7	2	3	0	0	356
hhee	0	0	0	0	0	0	0	0	0	0	52	40	3	0	0	0	0	137
hhhc	23	21	25	20	0	0	0	0	0	0	0	2	2	3	1	0	0	4464
hhhe	29	30	15	11	0	0	0	0	0	0	0	4	4	2	2	0	0	137
hhhh	21	11	60	4	0	0	0	0	0	0	0	0	1	0	0	0	0	31327

3.1 The discrete structural states

The marginal one-dimensional distribution of the pseudobond bending angle has two prominent peaks around $\theta = 1.10$ and 1.55 (radians). Non-zero θ s are in the interval [.4, 1.9]. The marginal one-dimensional distribution of the torsion angle τ has one immediately noticeable peak at $\tau = 0.87$ (corresponding to the helix). Another peak at $\tau = -2.94$ is less prominent. There is a vague peak still recognizable around $\tau = -2.00$. A grid generated with $\theta \in \{1.00, 1.55\}$ and $\tau \in \{-2.80, -2.05, -1.00, 0.00, 0.87\}$ is used to search high dimensional phase space for density peaks by the downhill simplex method. In the box counting, the box size is taken from 0.1 to 0.2 for θ , and the width for τ is twice of that for θ . The helices seen as a single peak in the three-angle phase space are clearly identified as several sub-peaks in the five-angle phase space. Further exploring main peaks in the five-angle phase space, we identify 17 mode centers, which are then used as

the main initial parameters to train the mixture model. Finally, the 17 structural states are obtained for the mixture model by the EM algorithm. They are listed in Table 1. Note that it is the entries of the inverse covariance matrix that are given. The determinant of the matrix is a measure of the divergence of the corresponding mode. The most sharp state is H, while the most vague state is Q, which occupies the least proportion of phase points.

Table 3. The forward transition rates	(multiplied by 100)	between structural states.

T 11 0 T 6

	I	J	Н	K	F	E	C	D	A	B	G	L	M	N	О	P	Q
\overline{I}	28	13	26	12	0	0	0	0	0	0	8	3	6	0	4	0	0
J	19	21	23	12	0	0	0	0	0	0	11	3	5	0	5	0	0
H	11	7	74	2	0	0	0	0	0	0	2	0	1	0	3	0	0
K	8	6	4	23	2	3	2	0	5	2	8	14	11	4	4	6	0
F	0	0	0	0	3	32	16	16	14	8	0	1	0	5	0	1	5
E	0	0	0	0	6	44	12	15	5	7	0	1	0	3	0	4	2
C	0	0	0	3	7	22	22	0	17	5	1	3	1	10	0	8	1
D	0	0	0	0	7	44	14	8	10	6	0	2	0	3	0	4	2
A	12	26	19	8	1	0	0	0	0	0	13	10	5	0	2	2	0
B	2	2	1	5	6	12	5	0	2	4	9	28	8	4	2	10	1
G	0	0	0	1	7	21	28	7	11	7	0	2	1	6	0	7	2
L	0	0	0	0	2	20	17	22	12	9	0	1	0	5	0	2	9
M	14	10	8	11	2	3	2	0	2	2	10	13	9	3	3	8	0
N	3	5	2	4	3	2	2	0	1	1	35	8	13	3	13	4	0
O	1	1	0	2	2	2	1	0	0	3	56	6	17	2	4	4	0
P	12	18	7	7	2	1	1	0	0	0	12	23	9	1	4	3	0
Q	0	0	0	1	3	16	15	8	10	5	1	4	1	20	0	3	13

3.2 The structure alphabet and the secondary structure

Our 17 structural states or letters of the structural alphabet describe the local structure of four-residue segments, and a code is assigned to the third residue of the unit of four residues. The total number of possible four-residue secondary structures is 37. The restriction of the minimal lengths 2 for e and 3 for h removes 44 quartets from the total $3^4 = 81$.

In order to make a detailed comparison between the secondary structures and the discrete structural states, from the training set we extract a subset, which contains 676 fragments and 118,621 residues (hence 116,593 points in the three-angle phase space). We arrange the corresponding counts in Table 2. (Secondary structure heeh has zero count, so it is omitted.) The table shows the percentages of each secondary structure in the structural states. It is clearly seen that there exists a correlation between the two types of structure classifications. For example, from Table. 2 hhhh are mainly attributed to H, I and J, while eeee to E, and D. The mutual information between the conformational codes and the secondary structure states equals 0.731. In Table 2, the row cccc shows rather uniform percentages in different structural states as we would expect.

3.3 Transition between structural states

Any two sequential points $(\theta_{i-1}, \tau_i, \theta_i)$ and $(\theta_i, \tau_{i+1}, \theta_{i+1})$ share the common angle θ_i . The effect of the connection of sequential structural states reflects transition rates between structural states. We first convert the 3D structures of the training set to their structure code sequences, and then determine the transition rates by counting code pairs. The obtained rates are listed in Table 3.

The entries of Table 3 are the forward transition rates as the conditional probability of the (i+1)-th site of a state chain at a given i-th site. Normalized according to the row, the table tells where a row state would like to go. Extended states, e.g. H and E, are characterized by large diagonal elements, while transient states, e.g. A and G, have almost vanishing diagonal rates. From the table, we may trace the capping states

for the helix and β -strand. For example, A is an important mode which leads to the helix, and G is a main leaving mode for the helix.

Table 4. CLESUM: The conformation letter substitution matrix (in the unit of 0.05 bit).

	0.0																
J	38																
Η	15	25															
I	12	14	51														
K	16	8	17	51													
N	-1	-32	-16	28	89												
Q	-43	-87	-69	-24	31	88											
\mathbf{L}	-31	-61	-48	0	5	24	71										
G	-21	-49	-40	-11	-7	8	27	68									
\mathbf{M}	17	-2	-4	14	8	-7	4	21	59								
В	-55	-94	-79	-49	-11	10	-13	12	-14	49							
Р	-33	-58	-55	-35	-4	6	-14	3	7	41	64						
A	-22	-43	-39	-17	10	13	-12	-7	-2	19	34	71					
O	-23	-54	-37	5	14	-13	-5	-2	5	-12	2	23	102				
\mathbf{C}	-42	-75	-59	-32	-5	27	-2	-6	-12	5	4	12	1	51			
\mathbf{E}	-91	-125	-112	-83	-43	-8	-23	-24	-47	13	-6	-27	-49	2	34		
\mathbf{F}	-73	-106	-95	-67	-32	0	-18	-6	-34	4	-2	-22	-31	19	24	48	
D	-87	-122	-105	-81	-45	13	-24	-32	-50	11	-11	-19	-43	19	21	20	49
	J	Η	I	K	N	Q	L	G	M	В	Р	A	Ο	С	Ε	F	D

3.4 Structural substitution matrix

Sequence alignment is the main procedure of comparing sequences. Certain amino acid substitutions commonly occur in related proteins from different or same species. Amino acid substitution matrices, extracted from our knowledge of most and least common changes in a large number of proteins, serve for the purpose of sequence alignment. The popular BLOSUM matrix of Henikoff and Henikoff (1992) is derived from a large set of conserved amino acid patterns without gaps representing various families. The frequency of amino acid substitutions in alignments is counted in sequence alignments. These frequencies are then divided by the expected frequency of finding the amino acids together in an alignment by chance. The ratio of the observed to the expected counts is an odds score. The BLOSUM entries are logarithms of the odds scores with the base 2 and multiplied by a scaling factor of 2.

To use our structural codes directly for the structural comparison, a score matrix similar to BLOSUM is desired. There is a database of aligned structures, the FSSP of Holm and Sander (1997), which is based on exhaustive all-against-all 3D structure comparison of protein structures in the PDB. The proteins in the FSSP are divided into a representative set and sequence homologs of the representative set. The representative set contains no pair which have more than 25% sequence identity. In the version of Oct 2001, there are 2,860 sequence families representing 27,181 protein structures. A tree for the fold classification of the representative set is constructed by a hierarchical clustering method based on the structural similarities. Family indices of the FSSP are obtained by cutting the tree at levels of 2, 4, 8, 16, 32 and 64 standard deviations above database average. We convert the structures of the representative set to their structural code sequences. All the pair alignments of the FSSP for the proteins with the same first three family indices in the representative set are collected for counting aligned pairs of structural codes. The total number of code pairs are 1,143,911. The substitution matrix derived in the same way as the BLOSUM was obtained is shown in Table 4, where a scaling factor of 20 instead of 2 is used to show more details. We call this conformation letter substitution matrix CLESUM. Henikoff and Henikoff (1992) introduced for their BLOSUM the average mutual information per amino acid pair H, which is the Kullback-Leibler distance between the joint model of the alignment and the independent model. The value of H for our CLESUM equals 1.05, which is close to that for BLOSUM83.

4 Discussion

Biologically important modules have been repeatedly employed in protein evolution by gene duplication and rearrangement mechanisms. They form components of fundamental units of structure and function. The presence of modules provides a guide to classify proteins into module-based families, and helps the structure prediction. The existence of such conservative recurrent segments sets a solid foundation for the local analysis. We have discretized the combination of three psuedobond angles formed by four consecutive C_{α} atoms to convert the local geometry to 17 coarse-grained conformational letters according to a mixture model of the angle distribution.

4.1 The precision of the conformational codes

From the correlation between the conformational codes and the secondary structures (Table 2), it is not surprising that there exists a propensity of the codes to amino acids. The coarse-graining would introduce an error. It is then important to examine the precision of the codes. For this purpose, we randomly pick up 1,000 points for each code, and calculate the distance root mean squared deviation (drms) for each of the total 499,500 pairs from their coordinates. The drms of structures a and b, without requiring a structure alignment, is defined as the averaged distance pair difference

$$drms = \left[\frac{2}{n(n-1)} \sum_{i=2}^{n} \sum_{j=1}^{i-1} (|\mathbf{r}_{ai} - \mathbf{r}_{aj}| - |\mathbf{r}_{bi} - \mathbf{r}_{bj}|)^{2}\right]^{1/2},$$
(12)

where \mathbf{r}_{ai} is the coordinate of atom *i* in structure *a*. The averaged coordinate pair difference, i.e. the coordinate root mean squared deviation crms, is about 1.2 times of the drms.

The errors of the conformational codes are listed in Table 5. The most precise code H has an error $0.133 \pm 0.060 \text{Å}$, while the vaguest code L has an error $0.604 \pm 0.365 \text{Å}$. After averaging over the code relative frequencies, the mean error is 0.330 Å.

Conformational code	I	J	Н	K	F	E	С	D
Mean drms (Å)	0.244	0.246	0.133	0.452	0.398	0.307	0.392	0.262
Standard deviation (Å)	0.110	0.124	0.060	0.219	0.287	0.173	0.218	0.149
A	В	G	L	M	N	O	Р	Q
0.347	0.322	0.390	0.604	0.481	0.551	0.538	0.252	0.506
0.163	0.197	0.192	0.365	0.231	0.321	0.318	0.134	0.287

Table 5. The errors of the conformational codes.

4.2 The connection effect of sequential states

Compared with the HMM, the mixture model does not include the connection effect of sequential states. The parameter number increases quadratically with the number of categories for a Markov model, while only linearly for a mixture model. We have to compromise between precision and correlation. A mixture model with fine categories is also promising.

Since the model training involves a global optimization the choice of a good initial trial plays an important role. A careful exploration of the density distribution in the five-angle space corresponding to two consecutive conformational states reveals that the peaks in the five-angle space give a finer picture of the peaks in the three-angle space. That is, subpeaks in the three-angle space are easily recognizable from peaks in the five-angle space. We have identified 17 intense peaks, which survive the later process of model training. Camproux et al. (1999) found 12 modes for the four-residue unit by a HMM. Instead of angles, they used a combination of four distances. Since only three of the four are independent their mode centers need not correspond to a real conformation. However, we still can see the correspondence between their codes and ours: α_1 -H, α_2 -J, α' -K, α' -O, α' +M, γ_1 -N, γ_2 -P, γ_β -Q, $\gamma_{\beta\alpha}$ -A, $\gamma_{\alpha\beta}$ -G, β_2 -D, and β_1 -E.

4.3 Structure alignment via conformational codes

The conversion of a 3D structure of coordinates to its conformational codes requires little computation. To distinguish from the amino acid sequence, we call the converted code sequence the code series, or simply series. Once we transform 3D structures to 1D series, the structure comparison becomes the series comparison. Tools for analyzing ordinary sequences can be directly applied. We have constructed the conformational letter substitution matrix CLESUM from the alignments of the FSSP database. We shall examine the performance of the conformational alphabet derived above.

Table 6. The alignment of 1urnA and 1ha1. The first two lines are their amino acid sequences aligned according to the FSSP, while the last two lines are the global Needleman-Wunsch alignment of the conformational code series. Lowercase letters of amino acids indicate structural nonequivalence.

1urna LKMRGQAFVIFKEVSSATNALRSMqGFPFYDKPMRIQYAKTDSDIIAKM
1ha1b GKKRGFAFVTFDDHDSVDKIVIQ kYHTVNGHNCEVRKAL
...GNGEDBEEALAJHHHHHHIKKGNGCENOGCCEFECCALCCAHIJH
AGCPOLEDEEEALBJHHHHI.IJGALEEENOGBFDEECC......

Holm and Sander (1998) gave an example of the α/β -meander cluster with four members showing different levels of structural similarity. Their PDB-IDs are 1urnA, 1ha1, 2bopA and 1mli. The structure of 1urnA was taken as the frame to superimpose the other structures. The structural similarity to 1urnA from high to low are 1ha1, 2bopA and 1mli. Taking the scaling factor for the CLESUM to be 2, and using -12 for the the gap-opening penalty and -4 for the gap extension, the global Needleman-Wunsch alignment of 1urnA and 1ha1 is shown in Table 6, where, in the first two lines, the amino acid sequences aligned according to the FSSP are also given. It is seen that, except for segment boundaries, the two alignments coincide. The alignment of the FSSP and the code series alignment for 1urnA and 2bopA have three common segments falling in positive score regions of the series alignment. In the alignments for 1urnA and 1mli two common segments longer than 8 are still seen. As for the amino acid sequence alignment, in the case of 1urnA and 1ha1 two segments of lengths 13 and 21 of the sequence alignment coincide with the FSSP, but no coincidence are seen in the other two cases.

The conformational codes are local. Even though a global alignment algorithm is used, this does not guarantee that the found alignment corresponds to the optimal structure superposition. However, the code series alignment does not affected by the domain move, is then good for analyzing the structure evolution. For example, the first helix of 1ha1 is shorter than its counterpart in 1urnA by one turn. The FSSP aligns the N-cap (with codes FA) of the 1ha1 helix to the helix (with codes HH) of 1urnA, but local structure FA is closer to CC (with positive scores) than to HH (with negative scores).

The CLESUM includes only the structural information. When we compare two structures we usually know also their amino acid sequences. Many papers considered a linear combination of structural alignment score and sequence alignment score. This is an approximation of independency. From the FSSP, it is possible to construct a substitution matrix in the joint space of the structure and sequence. However, such a matrix would have about 6×10^4 parameters. When the structure is to be emphasized, we may use a reduced amino acid alphabet (Zheng, 2004). For example, clustering 20 amino acids into 3 groups would reduce the parameter number to about 10^3 . We often want to compare a sequence with unknown structure to a known structure. In this case, a rectangular substitution matrix of the type of (amino acid)×(conformational code) to (amino acid) is useful. The construction of these matrices is our next task.

It is known that the sequence-structure relationships have not always been strong. Bystroff and Baker (1998) have built a library of structure-sequence motifs, which are expected to correspond to functional units recurring in different protein contexts and to be found in different combinations in distantly related or functionally unrelated proteins. To identify the structural features that have strong sequence preferences is

to locate peaks of density distribution in the joint structure-sequence space. Previously, the structure-based clustering was a duty much heavier than the sequence-based clustering, so one had to start with a sequence-based clustering, and was kept constantly to run between the structure and sequence subspaces. It is then interesting to see whether the library can be improved by clustering directly in the joint structure-sequence space with the help of conformational codes. This is under study.

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