Oligopeptides’ frequencies in the classification of proteins’ primary structures.

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Abbreviations used in the text:
AAs: aminoacids; PCA: Principal Component Analysis;
SOM: Self Organizing Maps; SMH: Set Mean Homology; MMH: Map Mean Homology.

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Abstract

This paper reports about an approach to the classification of proteins’ primary structures taking advantage of the Self Organizing Maps algorithm and of a numerical coding of the aminoacids based upon their physico-chemical properties.

Hydrophobicity, volume, surface area, hydrophilicity, bulkiness, refractivity and polarity were subjected to a Principal Component Analysis and the first two principal components, explaining 84.8 % of the total observed variability, were used to cluster the aminoacids into 4 or 5 classes through a *k-means* algorithm. This leads to an economical representation of the primary structures which, in the construction of the input vectors for the Self Organizing Maps algorithm, allows the consideration of up to tri- and tetrapeptides’ frequency matrices with minimal computational overload.

In comparison with previously explored conditions, namely symbolic coding of aminoacids and dipeptides frequencies, no significant improvement was observed in the classification of 69 cytochromes of the *c* type, characterized by a high degree of structural and functional similarity, while a substantial improvement occurred in the case of a data set including quite heterogeneous primary structures.
1 Introduction

Coding the primary structure of proteins by lists of numbers related to the physico-chemical properties of the amino acids (AAs) in the polypeptide chains should provide substantial help in the study of the correlations between primary and tridimensional structures (Eisenhaber et al. 1995; Rost and Sander 1993; Reyes et al. 1994), and hopefully shade some light on the intricacies of the rules governing proteins’ folding (Fedorov and Baldwin 1997).

Although the issue is in the literature since a long time (Argos 1987; Schneider and Wrede 1993), the vast majority of the software tools devoted to the analysis of the primary structure (Thompson et al. 1994; Wishart et al. 1994) utilize the symbolic coding of AAs, the main reason being the successful drawing of phylogenetic trees on the basis of homologous proteins of different species after proper alignment (Page 1996).

Numerical coding of aminoacidic residues on solid physico-chemical and statistical grounds, however, allows to take advantage of a manifold of numerical multivariate data-analysis techniques and, in particular, to fully exploit the heuristic power of automatic classification based upon Self Organizing Maps (SOMs), introduced by Kohonen several years ago (Kohonen 1984) as a general purpose tool for classifying the elements of a multivariate set. The only strict requirement of their unsupervised learning mechanism, i.e. the same number of variables for each element of the set, can be easily met even if the primary structures to be classified are of different length. To any protein, in fact, can be associated a frequency matrix of \( n^d \) elements, where each element is the number of occurrences of each of the possible oligopeptides of length \( d \) within the primary structure (\( n = 20 \) in the case of the natural AAs). On the basis of this approach, assuming a different symbol for each of the 20 natural amino acids and \( d = 2 \), i.e. generating frequency matrices of \( 20^2 \) elements, it was possible to carry out both fine classifications within sets of structurally similar proteins (Ferrán and Ferrara 1991, 1992), and coarser classifications over much larger sets (Ferrán et al. 1992).

If, on one hand, increasing the length \( d \) of the oligopeptide accounts with higher and higher precision for the fine details of each individual primary structure, the exponential increase in the number of the possible \( d \)-plets in the \( n^d \) matrix poses some practical and theoretical limitations. The former ones obviously refer to the computational load, while the latter are related to the linearly decreasing number of oligopeptides (\( N - d + 1 \)) with which a sequence of length \( N \) may contribute to the non-zero, i.e. significant, elements of the frequency matrix.

In this paper two exemplary cases of proteins’ primary structure classification will be described in which an appropriate balance between the \( n \) and \( d \) values in the frequency matrices feeding the SOM algorithm allows
to: i) use oligopeptides longer than dipeptides as descriptors of the primary structures, and ii) minimize the ensuing computational load by lowering the size of $n$ with no (or minimal) loss of the statistically significant information, through the combined use of principal component and cluster analysis techniques.

2 Methods

2.1 Self Organizing Maps (SOM)

The SOM algorithm, proposed by Teuvo Kohonen in the first 80s (Kohonen 1984), is a fully automatic algorithm that drastically reduces the dimensionality of a highly multivariate data set still preserving the mutual correlations between its elements. The most recent implementation of such algorithm (SOMPAK 3.1, free software available, together with a rich bibliography, at the Web site http://www.cis.hut.fi/nnrc/) has been used throughout the present paper.

In our case the input of the algorithm is a set of numerical vectors obtained by an appropriate recoding of the primary structures of proteins, and the output is a bidimensional map where the mutual locations of the primary structures reflect their intrinsic similarities. An extensive and clear description of the algorithm’s working machinery is available in the literature (Kohonen 1995), where an estimate of the distorsion introduced in the original structure of the data set by reducing their dimensionality is given in the form of a stress factor. As a more specific index of the goodness of the classification obtained in the case of proteins, the Map Mean Homology (MMH) index (see below) has been used throughout this paper.

2.2 Calculation of the MMH (Map Mean Homology) index

To evaluate the goodness of the clustering provided by the SOM algorithm, the Map Mean Homology (MMH) index has been used, along the same line followed by Ferrán and Ferrara (1991). Such index can be defined as

$$MMH = \frac{\sum_{i=1}^{n} QR_{i}^{Clusters}}{n}$$

i.e. the average of the Quality Ratio values ($QR_{i}^{Clusters}$ values) associated to the $n$ clusters present on the map. A cluster is defined by the presence in a cell of at least two elements, and is extended to its first neighbours, counted only once. Thus, the $QR_{i}^{Clusters}$ for the $i^{th}$ cluster is defined as
\[ QR_i^{Clusters} = \frac{\sum_{j=1}^{m} W_j QR_{i,j}^{Couples}}{\sum_{j=1}^{m} W_j} \]  \hspace{1cm} (2)

where \( j \) runs over the \( m \) couples associated to the \( i^{th} \) cluster and to its first neighbours, weighted by \( W_j \) values of 1 and 0.5 in the former and latter case, respectively.

### 2.3 Principal Component Analysis of AAs’ physico-chemical properties.

The Principal Component Analysis (PCA), introduced by Pearson in 1901, is a method of decomposing a correlation or covariance matrix in order to find the best association of points in space (Jolliffe 1986).

The first goal of the principal components is to summarize a multivariate data set as accurately as possible using fewer uncorrelated variables. This can be achieved since the principal components are orthogonal to each other, thus removing any redundancy in the available information. The relation between the original variables and the principal components is expressed in terms of component loadings, i.e. the correlation coefficients of the original variables with the new ones (principal components).

In this paper PCA has been carried out over seven physico-chemical properties of the 20 natural AAs, namely hydrophobicity, volume, surface area, hydrophilicity, bulkiness, refractivity index and polarity which, according to Schneider and Wrede (1993), are relevant in the identification of specific patterns along proteins’ sequences. Among these properties, hydrophobicity has been recently confirmed as by far the most important one in protein folding (Weiss and Herzel 1998). In Table 1 our PCA results are reported in terms of the components’ loadings and of the percent variability explained by each component. The first and second components (PC1, PC2) explain 84.8% of the total variability and hence have been considered as reliable and non-redundant representatives of the whole set of properties.

### 2.4 k-means clusterization of AAs.

The \textit{k-means} algorithm is a semi-automatic procedure to identify classes within a given set of elements described by one or many variables (Everitt 1980). Clusters emerge here from the structural characteristics of the data set, by maximizing the interclass variance and minimizing the intraclass variance. For \( n \) units described by \( m \) variables, the procedure can be schematized as follows:

1. a non-trivial number of classes, \( k \), is defined, being \( 1 < k < n \);
2. \( k \) aggregation points in an \( m \)-dimensional space are arbitrarily chosen;
3. each of the \( n \) units is assigned to the nearest aggregation point;
4. a new set of aggregation points are reckoned as barycentres of the classes defined in the previous step;
5. go back to the 3\(^{rd} \) step until no further change occurs in the classes’ composition.

The external factor which makes the procedure non fully automatic, is the \textit{a priori} definition of \( k \).

In the present case, the algorithm has been used to group into \( k \) classes the 20 AAs on the basis of their hydrophobicity \((m = 1)\), as well as the values of the first and second principal components \((m = 2)\) extracted from their main physico-chemical properties.

The relative optimality of the \( k \) value can be chosen by means of the relation between the fraction of explained variability \((EV)\) relative to the classification, and the value of \( k \): reaching a plateau of \( k \) can be considered the result of a structurally optimal classification (see also the legend to Table 4).

3 Results

3.1 Data sets used in this paper

The leading criterium in the choice of the two data sets used in this work reflects the aim to test the performance of a numerical coding of the AA and of a variable length of the oligopeptides’ describing the primary structures under two different conditions, namely a high and a low value of a global similarity index (see below).

For Data Set I, shown in Table 2, were chosen 69 cytochromes of the \( c \) type, which are known to share a high level of both structural and functional similarity. To impose some rational constraint in the choice of Data Set II, where a high similarity in the primary structures was not a prerequisite, our attention focussed over a group of proteins in which, as shown by Alexandrov and Fisher (1996), a significant similarity in the tridimensional arrangements was unparalleled by any homology in the primary structures. The elements of Data Set II are listed in Table 3.

It is worth stressing that the two data sets should be considered from two complementary viewpoints:
1) since the differences between the elements in Data Set I consist in a number of gaps/point-mutations over essentially the same basic primary structure, any source of variability (information) related to structural and/or functional features, is expected to be minimal. Under these conditions even
the simplest symbolic coding blind to physico-chemical features can be appropriate;

ii) the high heterogeneity within the elements of Data Set II, related to their quite different length, composition, function and primary structure, should be in favour of any classification task based on a numerical coding of the sequences. This introduces, however, new problems about choosing the optimal physico-chemical descriptors of the AAs, or about how to group them into clusters, on which heavily depends the classification’s goodness.

A quantitative estimate of the Set Mean Homology (SMH) among the $n$ elements (in couples) within a set is given by

$$SMH = \frac{\sum_{i=1}^{n-1} \sum_{j=i+1}^{n} QR_{ij}}{n(n-1)/2}$$

where the $QR_{ij}$ are the QualityRatio values, i.e. correspond to the elements of a triangular matrix generated as an intermediate result by the PILEUP program in the GCG suite of programs for the analysis of biosequences (Doelz 1994). More precisely, each $QR_{ij}$ is given by an estimate of the goodness of the alignment between the $i,j$ elements in the data set as provided by: i) the Needleman-Wunsch algorithm (1969), and ii) a substitution matrix of the BLOSUM type (Henikoff and Henikoff 1992), normalized by the number of residues of the shortest sequence between $i$ and $j$. Notice that the procedure used in reckoning $QR_{ij}$ refers to a symbolic coding of the natural AAs, i.e. matches the condition used as a reference (black bars) in Figure 2. However, high-quality classifications of primary structures can also be obtained upon clustering the AAs into 4 or 5 groups through a k-means algorithm, after an appropriate numerical coding provided by PCA.

3.2 Classification of the data sets’ elements.

Figure 1 shows the map generated by the SOM algorithm in the case of Data Set I. This data set, due to the high level of similarity between the primary structures, constitutes a significant benchmark to test the fine discrimination power of the algorithm. A very similar data set has been successfully analyzed by Ferrán and Ferrara (1992) using a symbolic coding of the 20 natural AAs and dipeptide frequencies, i.e. a vector of ($20^2$) components for each primary structure. At difference with these authors, we used a numeric coding for the AAs in the aim to: i) exploit the physico-chemical information characterizing each single residue; ii) increase the length of the oligopeptides; iii) minimize the computational burden by reducing the number of classes in which the residues can be clustered. The main goal was to provide a more direct correlation between primary and tertiary structures’ similarities.
A glance at Figure 1 indicates that even using vectors of 5$^3$ components, corresponding to tripeptides’ frequencies and to clustering the AAs into five groups, in the description of the primary structures, the phylogenetic relationships within cytochromes are very well preserved.

A quantitative estimate of the classification goodness obtained by the SOM algorithm is provided in Figure 2 in terms of the Map Mean Homology (MMH, see methods) score for both Data Sets I and II. In each panel of Figure 2 is also indicated (dotted line) the Set Mean Homology (SMH, see Methods), i.e. an estimate of the overall similarity between all the couples of elements in the set. Under all conditions the bars’ height exceeds the dotted line of an amount indicating the performance of the classifier algorithm. The bars in Figure 2 represent the values of the MMH for various combinations of: i) the coding criteria for the AAs; ii) the number of groups in which the AAs are clusterized; iii) the length of the oligopeptides whose frequencies constitute the vectors associated to each sequence.

The most interesting result provided by our analysis is the striking difference in the efficiency of the adopted coding scheme for the AAs, between the two data sets. Taking as a reference the previously used symbolic coding coupled to dipeptide frequencies (black bars in Figure 2), substantially identical results have been obtained under all conditions when the data set included elements of high SMH (Figure 2A). Upon collapsing the latter constraint, however, a numerical coding based upon a PCA of their main physico-chemical properties (Table 1), and the ensuing techniques of clustering the AAs (Table 4) into 4 or 5 groups, provided a worse performance and a better one in the case of, respectively, dipeptides and tripeptides frequencies (Figure 2B).

To rationalize these results two basic points should be taken into account: first of all, it is quite obvious that, in very general terms, the ability of the SOM algorithm in finding peaks of similarity over a background of globally low similarity in the map is exalted. Such an effect is independent from the coding criteria of the residues and only deals with the specific features of the elements to be classified. It can be described by the expression:

$$\frac{<MMH> - SMH}{SMH}$$

which, for the data shown in Figure 2A and B, gives the average values of 0.19 ± 0.03 and 1.83 ± 0.97, respectively.

Second, the much higher relative variance associated to the results in Figure 2B clearly indicates that the role of the coding criteria, namely i) oligopeptide length, and ii) optimized (through PCA) physico-chemical information, is only emerging in the case of Data Set II.

Finally, special consideration deserves the difference observed between the two data sets when the classification occurs after a random clustering of the AAs in 4, 5 or 10 groups (white columns in Figure 2). Such a condition...
has been included in our analysis to clarify the relative importance of the symbolic coding of AAs (see Discussion).

4 Discussion

In classifying proteins of different length on the basis of their polypeptide sequences a crucial problem consists in the appropriate coding of the AAs, since the appropriate statistical and connectionist procedures usually require as an input numerical vectors of identical dimension. To overcome the problem a ”units-variables” matrix may be worked out, where the rows are associated to the proteins and the columns contain, for example, the relative frequencies of the 20 natural AAs, or of dipeptides, tripeptides, etc., thus providing a more and more accurate (although longer) global description of the primary structures. In particular, such an approach has been applied in the use of a neural classifying algorithm, the SOM (see Materials), endowed with an automatic features’ extraction ability in the absence of any independent information (unsupervised learning), with a minimum number of adjustable parameters.

In this paper we showed that a synergic use of multivariate statistical techniques and of the SOM algorithm is very effective, mainly in the case of heterogeneous data sets, given an appropriate choice of the coding criteria for the AAs and of the length of the oligopeptides used to represent the primary structures. This clearly appears from the comparison of the upper and lower panels in Figure 2, referring to data sets of high and low mean homology, respectively. Under the former condition, as indicated by the high value of the SMH, all the explored criteria for primary structures’s coding look almost equivalent. The improvement obtained with reference to the more traditional symbolic representation of AAs and dipeptides’ frequencies is evident in the lower panel, where the data set includes elements of much lower SMH.

This poses the question whether a further improvement could be obtained by further increasing the oligopeptides length, $d$. For both data sets used in this work this was actually not the case (not reported). The main reason is related to the exponential increase, with increasing $d$, of the size of the frequency matrices, coupled to a linear decrease in the number of oligopeptides associated to each primary structure of length $N$ described over an alphabet of $n$ different symbols ($n = 20$ for an unreduced symbolic representation of the 20 natural AAs). In other words, the ratio

$$\frac{N - d + 1}{n^d}$$

(5)

which represents the fraction of the non-zero elements in the frequency matrix for each polypeptide sequence, tends very rapidly to zero with increasing $d$. Thus, the sparsity of the cumulative matrix related the whole data set,
obtained from the element by element sum of the individual frequency matrices, should be considered as the main factor affecting the efficiency of the SOM classifier. Reducing to more favourable values expression 5 by reducing \( n \), i.e. clustering the AAs residues into relatively homogeneous groups, needs the adoption of a numerical coding for the residues, on the basis of their hydrophobicity (Cid 1982; Reyes 1993) or, even better, of the principal components extracted from a bulk of physico-chemical properties. The optimal number of such groups can be defined, in any case, through the Explained Variability index (see the legend to Table 4). A complementary approach obviously consists in an appropriate filtering of the sparse matrices.

A possible objection to the above sketched strategy could invoke the observed insensitivity to the various coding schemes in the classification of the primary structures included into Data Set I. This focusses our attention on the peculiar features of the elements of this data set, namely on their structural (at the tridimensional level) and functional homogeneity, which seems to pose an intrinsic limit to any substantial improvement in the classification, even by increasing the oligopeptides’ length. It was not possible in fact, under the explored conditions, to outperform the traditional symbolic coding of the residues coupled to dipeptide frequency matrices. A crucial observation in that respect, however, is that even after random grouping the residues into 4 or 5 classes the quality of the classification, as judged by the MMH index, was not decreased. This points to the conclusion that even a relatively poor symbolic coding is able to capture the only relevant source of information in this peculiar data set, which could be associated to a variability of syntactic type, i.e. related to local differences between the elements of the set (relatively) independent from their macroscopic function, since all of them share a common structural and functional backbone (Yockey 1977). In the absence of such common backbone, like in the case of Data Set II, where the substantial differences between the primary structures, give rise to a more semantic (i.e. related to macroscopic functional differences) variability, the numeric coding of AAs should be preferred to the symbolic one. It makes easier, in fact, by getting rid of the redundant information, to increase the length of the oligopeptides describing the primary structures, and hence a more accurate description of their global architecture, with substantial savings in terms of computational requirements.

Up to what extent it is really worth to extend such length remains an open question. On the basis of a symbolic coding of the AAs, Strait and Dewey argued recently (1996) that the Conditional Information Entropy \( (I_k) \) of \( k \)-tuples of AAs, used to estimate the Information Entropy \( (I) \) of proteins’ primary structures through the expression

\[
I = \lim_{k \to \infty} I_k
\]
already reaches a limiting value for \( k \) equal to four. Among other things, these authors are also able to work out a figure for the fraction of the Information Entropy related to the tridimensional structure. Thus, it seem of great interest to check their theoretical conclusions against the results of an empirical approach based on the performance of SOM classifiers and a physico-chemical coding of the AAs.

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Legends to tables and figures

Figure 1: Classification of cytochromes of the c type by a SOM algorithm.

The map is a graphical rearrangement of the output provided by the SOMPAK 1.3 program (see the text) on the cytochromes listed in Table 2. The input vectors, containing $5^3 = 125$ components, have been constructed: i) grouping the AAs into 5 classes by a $k$-means algorithm on the basis of the first and second principal components extracted from 7 physico-chemical properties (see the text), and ii) using the tripeptides' frequency matrices.

The hexagonal lattice of the map and its overall size (6x7 cells) are a compromise between the conditions used by Ferrán and Ferrara (1992) and the Kohonen’s suggestion to use different sizes for the map’s axes. The working parameters of the SOMPAK program are the following:

- Lattice topology: hexagonal; Neighborhood: bubble
- First ordering phase: learning rate = 0.05, 1000 epochs, starting radius 7
- Fine tuning phase: learning rate = 0.02, 10000 epochs, starting radius 2.

The maps refers to the best results obtained, in terms of the internal distortion parameter, over 40 different choices of the random initial conditions (see the Kohonen refs. for details)

Figure 2: Performance of the SOM algorithm for proteins’ classification under various conditions.

Panels A and B refer to the proteins in Data Sets I and II (listed in Table 3 and Table 4) and the histograms represent the MMH (Map Mean Homology) score as defined in the text. The working parameters of the SOMPAK program are the same listed in Figure 1 except that, in the case of Data Set II, the dimension of the maps was 5x4 due to the lower number of elements.

The black, and white, bars refer to the unclustered natural, and randomly clustered AAs, respectively. The darker and lighter grey bars refer to clustering by hydrophobicity and, respectively, the PC1 + PC2 extracted from physico-chemical properties (see the text). In the case of random clustering each bar is the average of ten randomizations and the error bars indicate one standard deviation.
Table 1: PCA on seven physico-chemical properties of the natural AAs.

The table shows the correlations (loadings) between seven physico-chemical properties taken from Schenider and Wrede (1993) and the principal components extracted from them. The first row reports the percent of the total variability (EV%) of the whole set of properties explained by each component.

Table 2: Cytochromes of the c type used as Data Set I.

Column 1 is a numeric identifier for the corresponding entrance, without the cytc prefix, in the SwissProt data-base (column 2). Columns 3 and 4 refer, respectively, to the biological origin and the number of residues of each protein. The used family abbreviations are the following: Amphibia (Am), Angiosperm (Ap), Asteroidea (As), Birds (Av), Gastropoda (Ga), Chlorophyceae (Ch), Euglenoid algae (Eu), Ascomycetes (Fa), Basidiomycetes (Fb), Deuteromycetes (Fd), Gymnosperm (Gp), Insects (In), Mammals (Ma), Oligochaeta (Ol), Agnatha (Pa), Chondrichthyes (Pc), Osteichthyes (Po), Protozoa (Pr), Reptiles (Re).

Table 3: Immunoglobulin-like fold proteins used as Data Set II.

The first four columns contain the same type of information as in Table 2. Notice that the primary structures have been obtained from the PDB data-bank in this case. The full proteins names are listed in column 5.

Table 4: Clustering of the 20 natural AAs according to different criteria.

The first two columns refer to the variable(s) upon which the clustering into 4, 5 or 10 classes has been carried out by the k-means algorithm. In each case the value of the percent of the explained variability (EV%) has been calculated as the following ratio: \( EV\% = \frac{\text{VarBetw}}{\text{VarBetw} + \text{VarWith}} \), where VarBetw and VarWith are, respectively, the variability between the bari-centers of the classes and the mean variability within each class. The last column provides an example of a "random clustering" of the 20 AAs into the same number of classes.
Table 1: PCA on seven physico-chemical properties of the natural AAs.

|                  | PC1    | PC2    | PC3    | PC4    | PC5    | PC6    | PC7    |
|------------------|--------|--------|--------|--------|--------|--------|--------|
| EV%              | 50.04  | 34.73  | 7.43   | 5.29   | 1.90   | 0.47   | 0.14   |
| Hydrophobicity   | 0.231  | 0.953  | 0.865  | -0.560 | 0.857  | 0.863  | -0.047 |
| Volume           | -0.940 | 0.239  | 0.466  | 0.736  | -0.146 | 0.188  | 0.821  |
| Surface Area     | -0.209 | 0.025  | 0.020  | 0.357  | 0.285  | -0.071 | -0.512 |
| Hydrophilicity   | 0.052  | 0.067  | -0.023 | -0.017 | 0.362  | -0.423 | 0.229  |
| Bulkiness        | 0.023  | -0.142 | -0.172 | 0.064  | 0.180  | 0.192  | 0.096  |
| Refractivity     | 0.120  | 0.068  | -0.012 | 0.113  | -0.028 | 0.006  | 0.016  |
| Polarity         | 0.030  | -0.063 | 0.067  | 0.015  | 0.007  | -0.003 | 0.003  |
Table 2: Cytochromes of the \(c\) type used as Data Set I.

| Id  | Code    | Species   | Fam. | Length | Id  | Code    | Species   | Fam. | Length |
|-----|---------|-----------|------|--------|-----|---------|-----------|------|--------|
| 1   | ranca   | Rana Catesb. | Am   | 104    | 36  | schpo  | Schizosac. Pombe | Fa   | 108    |
| 2   | acene   | Acer Negun.  | Ap   | 112    | 37  | hanan  | Hansen. Anom.    | Fa   | 109    |
| 3   | fages   | Fagopyrum Escul. | Ap   | 109    | 38  | isor   | Issatchen. Ori.   | Fa   | 109    |
| 4   | ricco   | Ricinus Comm. | Ap   | 107    | 39  | neucr  | Neurosp. Cr.      | Fa   | 107    |
| 5   | braol   | Brassica Oler. | Ap   | 111    | 40  | torha  | Torulasp. Hans.   | Fa   | 109    |
| 6   | aruma   | Arum Macul.   | Ap   | 109    | 41  | ustsp  | Ustilago Sphaer.  | Fb   | 107    |
| 7   | sanni   | Sambucus Nig. | Ap   | 111    | 42  | thela  | Thermomy. Lan.    | Fd   | 111    |
| 8   | cansa   | Cann. Sativa   | Ap   | 104    | 43  | ginbi  | Ginkgo Biloba      | Gp   | 107    |
| 9   | abuth   | Abutil. Theophr. | Ap   | 108    | 44  | sanycy | Samia Cynthia     | In   | 107    |
| 10  | nigda   | Nigel. Damasc. | Ap   | 101    | 45  | schgr  | Schistoc. Greg.   | In   | 107    |
| 11  | allpo   | Allium Porrum | Ap   | 105    | 46  | boepe  | Boettch. Per.     | In   | 107    |
| 12  | maize   | Zea Mays      | Ap   | 109    | 47  | luccu  | Lucilia Cupr.     | In   | 107    |
| 13  | phaau   | Phaseolus Au. | Ap   | 111    | 48  | apime  | Apis Mell.        | In   | 107    |
| 14  | troma   | Tropaeol. Majus | Ap   | 109    | 49  | haer   | Haematob. Irrit.   | In   | 107    |
| 15  | passa   | Pastin. Sativa | Ap   | 107    | 50  | macma  | Macrobac. Mal.    | In   | 104    |
| 16  | soltu   | Solanum Tuber. | Ap   | 111    | 51  | manse  | Manduca Sexta     | In   | 107    |
| 17  | cucma   | Cucurb. Max.  | Ap   | 111    | 52  | canfa  | Canis Famil.      | Ma   | 104    |
| 18  | orysa   | Oryza Sativa   | Ap   | 111    | 53  | equas  | Equus Asinus       | Ma   | 104    |
| 19  | sesin   | Sesamum Indic. | Ap   | 108    | 54  | horse  | Equus Caball.     | Ma   | 104    |
| 20  | gosba   | Gossypium Barbad. | Ap   | 108    | 55  | human  | Homo Sapiens      | Ma   | 104    |
| 21  | spiol   | Spinacia Oler. | Ap   | 111    | 56  | minsc  | Miniopt. Schreib. | Ma   | 104    |
| 22  | helan   | Helianth. Ann. | Ap   | 111    | 57  | macmu  | Macaca Mulat.     | Ma   | 104    |
| 23  | lyces   | Lycopersicon Escul. | Ap   | 111    | 58  | atesp  | Ateles Sp.        | Ma   | 104    |
| 24  | wheat   | Triticum Aestiv. | Ap   | 112    | 59  | mirle  | Mirounga Leon.     | Ma   | 104    |
| 25  | astru   | Asterias Rub. | As   | 103    | 60  | eisfo  | Eisenia Foetida   | Ol   | 108    |
| 26  | chick   | Gallus Gallus | Av   | 104    | 61  | entr  | Entosphen. Trident. | Pa   | 104    |
| 27  | anapl   | Anas Platyrhyn. | Av   | 104    | 62  | squsu  | Squalus Sucklii    | Pc   | 104    |
| 28  | drono   | Dromains N.-Holl. | Av   | 104    | 63  | cypca  | Cyprin. Carpio     | Po   | 94     |
| 29  | strca   | Struthio Camel. | Av   | 104    | 64  | katpe  | Katsuwon. Pelamis  | Pr   | 103    |
| 30  | aptpa   | Aptonodytos Patag. | Av   | 104    | 65  | cripa  | Crithidia Fasc.    | Pr   | 113    |
| 31  | colli   | Columbia Livia | Av   | 104    | 66  | crion  | Crithidia Oncop.   | Pr   | 112    |
| 32  | helas   | Helix Aspersa | Ga   | 98     | 67  | tetpy  | Tetrabymena Pyr.   | Pr   | 109    |
| 33  | chile   | Chlamydomy. Reinh. | Ch   | 111    | 68  | croat  | Crotaulus Atrix    | Re   | 104    |
| 34  | entin   | Enterom. Intest. | Ch   | 100    | 69  | chese  | Chelydra Serp.     | Re   | 104    |
| 35  | euggr   | Euglena Gracil. | Eu   | 102    |      |        |            |      |        |
Table 3: Immunoglobulin-like fold proteins used as Data Set II.

| No | PDB Id | Source                        | Length | Protein’s name                        |
|----|--------|-------------------------------|--------|--------------------------------------|
| 1  | 1ACX   | Actinomyces globisporus       | 108    | Actinoxanthin                        |
| 2  | 1COB(A)| Bovine erythrocytes          | 151    | Superoxide dismutase                 |
| 3  | 1CTM   | Turnip - Brassica rapa        | 250    | Cytochrome f                         |
| 4  | 1TEN   | Human                         | 90     | Tenascin                             |
| 5  | 3HHR(B)| Human                         | 197    | Human growth hormone                 |
| 6  | 3DPA   | Escherichia coli              | 218    | Pap D                                |
| 7  | 2RHE   | Human                         | 114    | Bence-Jones protein                  |
| 8  | 2MCG(1)| Human                         | 216    | Immunoglobulin lambda                |
| 9  | 1MCO(L)| Human                         | 216    | Immunoglobulin g1                    |
| 10 | 1FAI(L)| Mouse                         | 214    | Fab fragment                         |
| 11 | 2FB4(H)| Human                         | 229    | Immunoglobulin fab                   |
| 12 | 8FAB(B)| Human                         | 215    | Fab fragment                         |
| 13 | 2FB3(H)| Mouse                         | 220    | Ig A fab fragment                    |
| 14 | 1CDB   | Mouse                         | 105    | T lymphocyte adesion glycoprotein    |
| 15 | 1TLK   | Turkey gizzard                | 103    | Telokin                              |
| 16 | 1MCO(H)| Human                         | 428    | Immunoglobulin g1                    |
| 17 | 2IGE(A)| Human                         | 320    | Fc fragment (theoretical model)      |
| 18 | 1PFC   | Guinea pig serum              | 111    | Ig g1 P F c(prime) fragment          |
| 19 | 1CID   | Rat                           | 177    | T-cell surface glycoprotein Cd4      |
| 20 | 3CD4   | Human                         | 178    | T-cell surface glycoprotein Cd4      |
| 21 | 1DLH(A)| Human                         | 180    | Histocompatibility antigen Hla-dr1   |
| 22 | 1DLH(B)| Human                         | 188    | Histocompatibility antigen Hla-dr1   |
| 23 | 3HLA(A)| Human                         | 270    | Histocompatibility antigen Hla-a2    |
Table 4: Clustering of the 20 natural AAs according to different criteria.

| Clusters | Hydrophobicity | PC1 + PC2 | Random |
|----------|----------------|-----------|--------|
| 4 Clusters | | | |
| 1 | A C G I L M F P S T W V | C I L M P T V | R |
| 2 | D E K | R N D Q E H K | N A S Y G H |
| 3 | N Q H Y | A G S | L M D F C W Q E |
| 4 | R | F W Y | I V K P T |
| EV : 94% | 84% | — |
| 5 Clusters | | | |
| 1 | A C I L M F W V | C I L M P T V | M N |
| 2 | D E K | N D Q E H | D F Y P W E |
| 3 | N Q H | A G S | L V A K T H |
| 4 | R | F W Y | S R C |
| 5 | G P S T Y | R K | I G Q |
| EV : 98% | 90% | — |
| 10 Clusters | | | |
| 1 | I L V | I L M V | K |
| 2 | D K | N D | M D P |
| 3 | N Q | A S | Q |
| 4 | R | F Y | L N G |
| 5 | A C W | R K | V T |
| 6 | P Y | C P T | E |
| 7 | H | W | A F |
| 8 | G S T | G | I Y H |
| 9 | M F | Q H | R C W |
| 10 | E | E | S |
| EV : 99.9% | 98% | — |
Figure 1
Figure 2

A

Dipeptides

Tripeptides

# of classes

B

Dipeptides

Tripeptides

# of classes