SIMILARITIES/DISSIMILARITIES ANALYSIS OF PROTEIN SEQUENCES BASED ON PCA-FFT

PENGYAO PING,^{*,†} XIANYOU ZHU[‡] and LEI WANG^{*,†,§}

^{}College of Information Engineering, Xiangtan University
Yuhu District, Xiangtan, Hunan 411105, P. R. China*

[†]Key Laboratory of Intelligent Computing and Information Processing

*[‡]Department of Computer Science
Hengyang Normal University, 421008, P. R. China*

[§]364715358@qq.com

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In this paper, a novel method to analyze the similarity/dissimilarity of protein sequences based on Principal Component Analysis-Fast Fourier Transformation (PCA-FFT) is proposed, in which, the PCA is utilized to transform protein sequences into time series and the FFT is utilized to analyze the time series while considering them as signals. To test the effectiveness of our newly proposed method, it is applied to analyze the similarity/dissimilarity of 16 different ND5 protein sequences and 29 different spike protein sequences, respectively. Furthermore, the correlation analysis is presented for comparing with others methods, and the simulation results show that it has better performances in the aspects of computation complexity and recognition degree than some existing methods.

Keywords: Time Series; Similarity/Dissimilarity Analysis; Principal Component Analysis; Fast Fourier Transformation; Protein Sequence.

1. Introduction

Homology is the relationship between biological structures or sequences that derived from a common ancestor. Homology among proteins or DNAs is typically inferred from their sequences similarity. Doing homology analysis is helpful for scientists' study on the function of DNA, proteins and cells. Alignments of sequences are used to discover the homologous regions. There are a variety of reasons why protein alignment is almost always preferable to DNA alignment.¹ Firstly, redundant codons mean $\sim 1/3$ of DNA mutations often do not matter. Secondly, protein sequences are easier to achieve statistically significant alignment by comparing with DNA sequences, which are made of a larger alphabet of characters of four nucleotides

[§]Corresponding author.

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because you are much less likely to get a match by chance. Thirdly, the DNA database is cluttered with non-coding sequences, and not all amino acid mutations are equally harmful to protein structure. Homology analysis is one of the hot topics in the area of protein sequences analysis. Up till now, lots of methods have been proposed for the homology analysis of protein sequences.^{2,3} The graphical technique which was first proposed by Hamori⁴ over 30 years ago was mainly used for the graphical representation of DNA. Currently, many researchers have proposed different methods for the graphical representation of protein sequences, which is a very powerful tool for visual comparison of protein sequences. For example, Hou *et al.*⁵ outlined a 3D graphical representation based on the classification of amino acids by different physicochemical properties, and then computed the similarities among different protein sequences. Elmaatv *et al.*⁶ proposed a 3D graphical representation of protein sequences based on three physicochemical properties of amino acid side chains and calculated similarities among nine ND5 protein sequences. Wen *et al.*⁷ raised a 2D graphical representation of protein sequence according to the physicochemical properties of amino acids and considered the numerical characterization of mutations and analyzed the similarities of nine ND5 proteins. Zhang *et al.*⁸ used the occurrence frequency of 20 amino acids and took the new numerical characteristic of 2D graphical representation to compute the similarities of ND5 proteins of nine species based on the Euclidean distance. Wu *et al.*⁹ proposed a two-dimensional graph, called “2D-MH” to compare the evolutionary distances between any number of protein sequences. Yao *et al.*¹⁰ introduced a dynamic 2D graphical representation based on physicochemical properties of amino acids and compared the sequences of nine ND5 proteins. Mu *et al.*¹¹ compared the similarity/dissimilarity of protein sequences according to the distance frequency of amino acids. Yao *et al.*¹² outlined a 2D graphical representation of protein sequences based on two classifications of amino acids. Liu *et al.*¹³ proposed an alignment-free method based on the relative Lempel–Ziv complexity to compare biological sequences. Liu *et al.*¹⁴ constructed a PH Curve based on two kinds of physicochemical properties of amino acids to analyze the similarity/dissimilarity of protein sequences. Lakkani *et al.*¹⁵ proposed a numerical method to measure the degree of similarity sequences of protein sequences based on 2D and 3D adjacency matrices. Ma *et al.*¹⁶ introduced a graphical representation of protein based on a novel iterated function system. Yao *et al.*¹⁷ proposed a 3D graphical representation of protein sequences based on three kinds of physical-chemical properties of amino acids.

For those existing graphical representations of protein sequences given above, the main drawbacks are that, the higher the dimension of the protein sequence graphs, the heavier the computation complexity, or the lower the recognition degree of the protein sequence graphs. Principal Component Analysis (PCA) is a standard tool in multivariate data analysis to reduce the number of dimensions, which has been proven effective in the process of protein sequence analysis.^{18–20} Therefore, in order to overcome the main drawbacks of existing graphical representation methods given above, in this paper, we proposed a novel graphical representation of protein

sequences, in which, the PCA is utilized to transform protein sequences into time series and the Fast Fourier Transformation (FFT),^{21,22} a common used signal analysis tool, is utilized to analyze the time series while considering them as signals. Additionally, a novel method based on PCA-FFT is designed to analyze the similarity/dissimilarity of protein sequences, which has lower computation complexity and higher recognition degree of protein sequence graphs than the existing methods. To validate the effectiveness of our method, we utilize it to analyze the similarity/dissimilarity of the 16 ND5 proteins spike proteins respectively. Moreover, comparing with existing methods, our new method is better at the same time, since it doesn't need to construct any complex matrixes when it is utilized to analyze the similarity/dissimilarity of protein sequences.

2. Material and Methods

2.1. Amino acids and their properties

Proteins are composed of 20 different amino acids. In order to analyze the similarity/dissimilarity of the protein sequences, the properties of the amino acids are considered first. Amino acids have many different physicochemical properties and biological characteristics including the molecular weight (mW), Hydropathy index (*hI*), solubility (S), van der Waals radius of side chains (vR), the *pKa* value for terminal amino acid groups COOH (*Pk1*), the *pKa* value for terminal amino acid groups NH_3^+ (*pK2*), isoelectric point (*pI*), the number of triplet codons (*cN*), frequency of human proteins (*F*) and so on. The nine properties of these amino acids are collected and illustrated in Table 1.^{23–25}

Table 1. The full list of 20 amino acids and their different properties.

Amino acid	Symbol	mW	<i>hI</i>	<i>pk1</i>	<i>pK2</i>	<i>pI</i>	S	<i>cN</i>	<i>F</i> (%)	vR
Alanine	A	89.079	1.8	2.34	9.69	6.01	167.2	4	7.8	67
Cysteine	C	121.145	2.5	1.96	10.28	5.07	0	2	1.9	86
Aspartic acid	D	133.089	−3.5	1.88	9.6	2.77	5	2	5.3	91
Glutamic acid	E	147.116	−3.5	2.19	9.67	3.22	8.5	2	6.3	109
Phenylalanine	F	165.177	2.8	1.83	9.13	5.48	27.6	2	3.9	135
Glycine	G	75.052	−0.4	2.34	9.6	5.97	249.9	4	7.2	48
Histidine	H	155.141	−3.2	1.82	9.17	7.59	0	2	2.3	118
Isoleucine	I	131.16	4.5	2.36	9.68	6.02	34.5	3	5.3	124
Lysine	K	146.17	−3.9	2.18	8.95	9.74	739	2	5.9	135
Leucine	L	131.16	3.8	2.36	9.6	5.98	21.7	6	9.1	124
Methionine	M	149.199	1.9	2.28	9.21	5.74	56.2	1	2.3	124
Asparagine	N	132.104	−3.5	2.02	8.8	5.41	28.5	2	4.3	96
Proline	P	115.117	1.6	1.99	10.96	6.48	1620	4	5.2	90
Glutamine	Q	146.131	−3.5	2.17	9.13	5.65	7.2	2	4.2	114
Arginine	R	174.188	−4.5	2.17	9.04	10.76	855.6	6	5.1	148
Serine	S	105.078	−0.8	2.21	9.15	5.68	422	6	6.8	73
Tyrosine	T	119.105	−0.7	2.11	9.62	5.87	13.2	4	5.9	93
Valine	V	117.133	4.2	2.32	9.62	5.97	58.1	4	6.6	105
Tryptophan	W	204.213	−0.9	2.38	9.39	5.89	13.6	1	1.4	163
Threonine	Y	181.176	−1.3	2.2	9.11	5.66	0.4	2	3.2	141

2.2. Principal component analysis

PCA is a common technique for dimensionality reduction and finding patterns in high dimension data. Proposed by Pearson in 1901, it is based on computation of low-dimensional representation of a high-dimensional dataset that maximizes the total scatter, which is optimal in reconstruction.²⁶ The following describes the description in steps:

Step 1. The m samples can be represented by a set $\Psi = \{X_1, X_2, \dots, X_m\}$. For each sample, which has n indicators, it can be described as $X_i = \{x_{i1}x_{i2} \dots, x_{in}\}$ for $i \in \{1, 2, \dots, m\}$, and then a $m \times n$ matrix X can be constructed with all the observations as follows:

$$X = \begin{bmatrix} X_1 \\ X_2 \\ \vdots \\ X_m \end{bmatrix} = \begin{bmatrix} x_{11} & x_{12} & \dots & x_{1n} \\ x_{21} & x_{22} & \dots & x_{2n} \\ \vdots & \vdots & \vdots & \vdots \\ x_{m1} & x_{m2} & \dots & x_{mn} \end{bmatrix}. \quad (2.1)$$

Then, based on the matrix X , the standardized matrix X^* can be obtained by the following procedure.

$$X^* = \begin{bmatrix} X_1^* \\ X_2^* \\ \vdots \\ X_m^* \end{bmatrix} = \begin{bmatrix} x_{11}^* & x_{12}^* & \dots & x_{1n}^* \\ x_{21}^* & x_{22}^* & \dots & x_{2n}^* \\ \vdots & \vdots & \vdots & \vdots \\ x_{m1}^* & x_{m2}^* & \dots & x_{mn}^* \end{bmatrix}, \quad (2.2)$$

where $X_i^* = (x_{i1}^*, x_{i2}^*, \dots, x_{in}^*)$, $x_{ij}^* = (x_{ij} - \bar{x}_j) / \sqrt{\text{var}(x_j)}$ is the value of the element in the X^* after standardizing, $\bar{x}_j = (1/n) \sum_{i=1}^n x_{ij}$ and $\text{var}(x_j) = (1/(n-1)) \sum_{i=1}^n (x_{ij} - \bar{x}_j)^2$ is the average value and the variance of the j th column of matrix X respectively, for $i \in \{1, 2, \dots, m\}$ and $j \in \{1, 2, \dots, n\}$.

Step 2. Then we can obtain the correlation matrix R based on the matrix X^*

$$R = \begin{bmatrix} r_{11} & r_{12} & \dots & r_{1n} \\ r_{21} & r_{22} & \dots & r_{2n} \\ \vdots & \vdots & \vdots & \vdots \\ r_{n1} & r_{n2} & \dots & r_{nn} \end{bmatrix}, \quad (2.3)$$

where the value of element r_{ij} in the matrix R can be calculated as follows:

$$r_{ij} = \frac{\sum_{k=1}^m (x_{ki}^* - \bar{x}_i^*)(x_{kj}^* - \bar{x}_j^*)}{\sqrt{\sum_{k=1}^m (x_{ki}^* - \bar{x}_i^*)^2 \sum_{k=1}^m (x_{kj}^* - \bar{x}_j^*)^2}}, \quad (2.4)$$

where $\bar{x}_i^* = (1/n) \sum_{j=1}^n x_{ij}^*$, for $i \in \{1, 2, \dots, n\}$ and $j \in \{1, 2, \dots, n\}$.

Step 3. The n eigenvalues $\lambda_1 \geq \lambda_2 \geq \dots \geq \lambda_n > 0$ of matrix R and the corresponding n eigenvectors $\mathbf{a}_1, \mathbf{a}_2, \dots, \mathbf{a}_n$ can be obtained,

$$\mathbf{a}_1 = \begin{bmatrix} a_{11} \\ a_{21} \\ \vdots \\ a_{n1} \end{bmatrix}, \quad \mathbf{a}_2 = \begin{bmatrix} a_{12} \\ a_{22} \\ \vdots \\ a_{n2} \end{bmatrix}, \dots, \mathbf{a}_n = \begin{bmatrix} a_{1n} \\ a_{2n} \\ \vdots \\ a_{nn} \end{bmatrix}. \quad (2.5)$$

Then we can get n principal components as follows:

$$F_i = \mathbf{a}_{1i} \times X_1^* + \mathbf{a}_{2i} \times X_2^* + \dots + \mathbf{a}_{ni} \times X_n^*, \quad (i = 1, 2, \dots, n) \quad (2.6)$$

Step 4. Compute the contribution rate CR_i and the accumulative contribution rate ACR_i of each principal component F_i ($i = 1, 2, \dots, n$), respectively:

$$CR_i = \frac{\lambda_i}{\sum_{k=1}^n \lambda_k}, \quad (2.7)$$

$$ACR_i = \sum_{k=1}^i CR_k \quad (2.8)$$

Usually, we just retain the top t principal components $F_1, F_2, \dots, F_t$, which correspond to the eigenvalues $\lambda_1, \lambda_2, \dots, \lambda_t$, and the corresponding accumulated contribution rate should satisfy that $ACR_t \geq 85\%$.

Step 5. Through plugging the elements in the matrix X^* into the expression of t principal components, we can obtain the score matrix F .

$$F = \begin{bmatrix} F_{11} & F_{12} & \dots & F_{1t} \\ F_{21} & F_{22} & \dots & F_{2t} \\ \vdots & \vdots & \vdots & \vdots \\ F_{m1} & F_{m2} & \dots & F_{mt} \end{bmatrix}, \quad (2.9)$$

where $F_{ij} = \mathbf{a}_{1i}x_{j1}^* + \mathbf{a}_{2i}x_{j2}^* + \dots + \mathbf{a}_{ni}x_{jn}^*$ for $i \in \{1, 2, \dots, m\}$ and $j \in \{1, 2, \dots, t\}$.

Therefore, we transform the original matrix X into matrix F that has reduced dimensionality, while not losing much information of the original data.

Step 6. The score matrix $F = [F_{ij}]_{m \times t}$ is a $m \times t$ matrix, we can obtain the i th sample's total points (TP), $TP_i = \sum_{j=1}^t F_{ij} \times CR_j$, where CR_j is the j th eigenvalue's contribution rate.

2.3. PCA of the amino acids

As mentioned above, proteins are composed of 20 different amino acids, and each of the amino acid has many different properties. If we use all of the properties to

analyze the protein sequences, we will have to handle the high-dimension matrices, and the computational effort will also be very large. PCA is an applicable algorithm for us to reduce the dimension of the amino acids' properties matrices. Here, we will apply the PCA algorithm to the nine properties of the 20 amino acids in Table 1.

According to the procedures in Sec. 2.2, the 20 amino acids are the 20 samples, we can get their nine properties as the observations, and then, we obtain the matrix X . We can use the functions in the software Matlab to help us for PCA. We list the eigenvalues and the contribution rate CR and the accumulative contribution rate ACR in Table 2.

Table 2 shows that the accumulative contribution rate of first four eigenvalues is $0.8588 > 0.85$, so we can just retain the first four principal components. The eigenvectors corresponding to the four eigenvalues are listed in Table 3.

Then, the four principal components are described as $F_i = \mathbf{a}_{1i}X_1^* + \mathbf{a}_{2i}X_2^* + \cdots + \mathbf{a}_{9i}X_9^*$, for example, the first principal components is presented as follows:

$$F_1 = 0.5036X_1^* - 0.2454X_2^* - 0.1634X_3^* - 0.3101X_4^* + 0.0702X_5^* \\ - 0.1665X_6^* - 0.3872X_7^* - 0.4377X_8^* + 0.4349X_9^*$$

Then the score matrix F and the total points of the 20 amino acids are obtained and listed in Table 4.

Table 2. The eigenvalues, contribution rates and the accumulative contribution rates.

Number	λ	CR	ACR
1	3.2237	0.3582	0.3582
2	1.9132	0.2126	0.5708
3	1.4048	0.1561	0.7269
4	1.1876	0.1320	0.8588
5	0.4959	0.0551	0.9139
6	0.4467	0.0496	0.9635
7	0.1992	0.0221	0.9857
8	0.1218	0.0135	0.9992
9	0.0071	0.0008	1.0000

Table 3. The elements of the eigenvectors.

a_1	a_2	a_3	a_4
0.5036	0.1436	0.0571	0.2158
-0.2454	-0.1875	0.2304	0.6547
-0.1634	0.1820	0.6298	0.2288
-0.3101	-0.1883	-0.3964	0.5071
0.0702	0.6464	-0.0786	0.0532
-0.1665	0.4465	-0.5280	0.1877
-0.3872	0.3931	0.1003	-0.0532
-0.4377	0.1844	0.2544	-0.2273
0.4349	0.2643	0.1738	0.3495

Table 4. The elements of the score matrix and the total points of the amino acids.

Symbol	S1	S2	S3	S4	TP
A	-2.6914	-0.2241	0.7502	-0.2868	-0.9324
C	-0.2117	-2.0889	-1.4026	1.0634	-0.5985
D	0.2342	-1.9671	-1.0269	-1.3364	-0.6709
E	0.2161	-1.1982	0.2191	-0.6557	-0.2296
F	1.6369	-0.7903	-0.3388	0.4879	0.4298
G	-2.8770	-0.2695	0.3369	-1.0823	-1.1780
H	2.1245	-0.0444	-1.2399	-0.8369	0.4476
I	-0.5598	-0.2612	1.2524	1.5462	0.1435
K	1.1324	2.4599	-0.3867	-0.6164	0.7868
L	-1.9755	0.8575	1.9353	0.7785	-0.1205
M	1.4097	-0.7517	0.6791	0.9274	0.5735
N	0.9764	-0.5803	-0.1437	-1.7290	-0.0242
P	-2.4070	0.7955	-3.5031	1.9525	-0.9822
Q	1.1724	-0.2717	0.2473	-0.8789	0.2848
R	0.9832	4.1089	-0.4523	-0.2891	1.1169
S	-2.0600	0.6987	0.2926	-1.2430	-0.7077
T	-0.9005	-0.2815	0.0018	-0.5338	-0.4525
V	-1.5026	-0.1298	1.1941	0.8726	-0.2643
W	3.1302	-0.0067	0.9381	1.5664	1.4729
Y	2.1692	-0.0551	0.6471	0.2936	0.9050

2.4. Graphical representation of protein sequences

Let $\Omega = \{A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y\}$, $\Psi = p_1 p_2 p_3 \dots p_N (p_i \in \Omega, \forall i \in \{1, 2, \dots, N\})$ represent a protein sequence with N amino acids, then, we can obtain a time series S_Ψ as we replace each letter p_i in the Ψ with its corresponding value of TP. Since the length of the time series is not the same, we will use fast Fourier transform convert the time series into sequences of complex numbers with the same length.

For the first 10 amino acids of the ND5 protein sequences of human, gorilla and opossum, we can get three time sequences and their corresponding spectrums,

Fig. 1. (a) The time series for the first 10 amino acids of the ND5 proteins of human, opossum and gorilla. (b) The corresponding part of their FFT spectrums.

which are shown in Fig. 1

$$Hu = \text{MTMHTTMTTL}; \quad Gor = \text{MTMYATMTTL}; \quad Opo = \text{MKVINISNTM}.$$

$$S_{Hu} = \{0.5735, -0.4525, 0.5735, 0.4476, \\ -0.4525, -0.4525, 0.5735, -0.4525, -0.4525, -0.1205\};$$

$$S_{Gor} = \{0.5735, -0.4525, 0.5735, 0.9050, \\ -0.9324, -0.4525, 0.5735, -0.4525, -0.4525, -0.1205\};$$

$$S_{Opo} = \{0.5735, 0.7868, -0.2643, 0.1435, \\ -0.0242, 0.1435, -0.7077, -0.0242, -0.4525, 0.5735\}.$$

Fig. 2. The time series of human and opossum and their spectrums after the FFT method.

From Fig. 1(a), we can visualize the similarity/dissimilarity between the three time sequences, and the result shows that protein sequences of human and gorilla are similar and protein sequences of human and opossum are dissimilar when we observe the curves carefully. After using the FFT, it shows the similarity between human and gorilla and the dissimilarity between human and opossum vividly and obviously, which is described in Fig. 1(b). It is obvious that the result in Fig. 1(b) is more visual and clear than that in Fig. 1(a).

The full sequences and their corresponding FFT spectrum of the ND5 protein sequences of human and opossum are plotted in Fig. 2. Apparently from Fig. 2, the similarity/dissimilarity between the two protein sequences are not distinguished easily. In the next section, we utilize the Euclidean distance between the curves and the cumulative difference between the spectrums of the corresponding protein sequences to compute the similarity/dissimilarity between the sequences respectively.

3. Results and Discussion

3.1. Method for similarity analysis of protein sequences based on the PCA-FFT method

To quantify comparisons of the protein sequences, in this section, we propose a new method for similarity analysis based on the distance between the time series and the complex sequences after the utilizing the FFT on them.

Firstly, for any two protein sequences Ψ_1 and Ψ_2 , let $\{x_1, x_2, \dots, x_{n1}\}$ and $\{y_1, y_2, \dots, x_{n2}\}$ be their original time series respectively, then, we can define the distance between Ψ_1 and Ψ_2 as follows:

$$d(\Psi_1, \Psi_2) = \sqrt{\sum_{i=1}^N (x_i - y_i)^2}, \quad (3.1)$$

where $N = \max(\text{length}(\Psi_1), \text{length}(\Psi_2))$

Obviously, the distance between two protein sequences can be used as an index for comparison of these two protein sequences, and the shorter the distance between them, the more similar these two protein sequences will be.

Based on the distance between two protein sequences defined above, the basic information of the 16 ND5 proteins is presented in Table 5, and the similarity/dissimilarity matrix is listed in Table 6.

Observe Table 6, it is easy to find that there are some similar pairs such as Fin Whale–Blue Whale (with distance of 3.3013), Pigmy Chimpanzee–Common Chimpanzee (with distance of 3.4477), Human–Common Chimpanzee (with distance of 4.4890), Sheep–Goat (with distance of 4.5737), Human–Pigmy Chimpanzee (with distance of 4.7036), hare–rabbit (with distance of 4.9311). Specially, the Opossum and gallus seem to be two peculiar animals since the shortest distance between them and the remaining mammals is more than 15.00, which is consistent with the fact

Table 5. The basic information of 16 ND5 protein sequences.

No.	Name	Abbreviation	Access No.	Length
1	Human	human	ADT80430.1	603
2	Gorilla	gorilla	NP_008222	603
3	Pigmy Chimpanzee	pi-chim	NP_008209	603
4	Common Chimpanzee	c-chim	NP_008196	603
5	Fin Whale	f-whale	NP_006899	606
6	Blue Whale	b-whale	NP_007066	606
7	Rat	rat	AP_006899	610
8	Mouse	mouse	NP_904338	607
9	Opossum	opossum	NP_007105	602
10	Sheep	sheep	ABW22903.1	606
11	Goat	goat	BAN59258.1	606
12	Lemur	lemur	CAD13431.1	603
13	Cattle	cattle	ADN11902.1	606
14	Hare	hare	CAD13291.1	603
15	Gallus	gallus	BAE16036.1	605
16	Rabbit	rabbit	NP_007559.1	603

that Opossum is the most remote species from the remaining mammals and the Gallus is not a mammal.

If we use the complex sequences of the time series after applying FFT on them, let $\{c_{11}, c_{12}, \dots, c_{1N}\}$ and $\{c_{21}, c_{22}, \dots, c_{2N}\}$ be the two complex sequences of the protein sequences Ψ_1 and Ψ_2 then we can compute the distance between the proteins in the following way.

$$d(\Psi_1, \Psi_2) = \sum_{i=1}^N abs(c_{1i} - c_{2i}), \tag{3.2}$$

where c_{1i} and c_{2i} are complex numbers and $abs(c_{1i} - c_{2i})$ computes the module of the difference between c_{1i} and c_{2i} . We list the similarity/dissimilarity matrix for the 16 ND5 proteins based on the distance between the complex sequences of protein sequences in Table 7.

Obviously, the same similar pairs are shown in Table 7, so the FFT will not change the inner structure of the original time series, and we can consider the protein sequences as the signal sequences for our research.

3.2. The phylogenetic tree of the protein sequences based on the PCA-FFT method

A phylogenetic tree or evolutionary tree is a branching diagram or “tree” showing the inferred evolutionary relationships among various biological species or other entities and their phylogeny based upon similarities and differences in their physical and/or genetic characteristics. It is a commonly used tool by researchers in some fields to help and analyze the clustering of different species. We construct a phylogenetic tree of the above 16 ND5 proteins based on our PCA-FFT method. We use the time series-based similarity matrix and the complex

Table 6. The similarity/dissimilarity matrix for the nine ND5 proteins based on the distance between the time series of protein sequences.

	Human	Gorilla	pi-chim	c-chim	f-whale	b-whale	Rat	Mouse	Opossum	Sheep	Goat	Lemur	Cattle	Hare	Gallus	Rabbit
Human	0															
Gorilla	5.500923	0														
pi-chim	4.703619	5.385455	0													
c-chim	4.48896	5.259163	3.4477	0												
f-whale	9.235933	9.085764	8.899921	9.050157	0											
b-whale	9.065265	8.92513	8.80428	8.913356	3.301302	0										
Rat	10.27774	10.81451	10.41969	10.48337	9.716409	9.5153142	0									
Mouse	9.873205	10.2674	10.1567	10.09308	9.372206	9.2236507	7.684268	0								
Opossum	16.50134	16.71323	16.49918	16.49224	15.82737	15.66074	16.15151	16.08221	0							
Sheep	8.582139	8.468802	8.287619	8.511169	7.526585	7.3567862	9.642779	8.757651	16.0312	0						
Goat	8.797962	8.792936	8.814971	8.908065	7.247185	7.4072911	9.61131	8.822475	16.304	4.573707	0					
Lemur	9.16356	9.184774	8.628009	9.018554	8.3093931	8.309077	10.34968	9.597126	16.22414	7.787294	7.882462	0				
Cattle	9.117992	9.050178	8.914896	9.355155	7.293226	7.1417665	9.934493	9.152396	16.19664	5.438314	5.536721	8.080172	0			
Hare	8.733429	8.95493	8.416004	8.404698	8.440079	8.0419346	9.585414	9.132389	15.69136	7.66273	8.241252	8.155104	8.320874	0		
Gallus	16.10484	16.1814	16.37469	16.36731	15.65169	15.569448	16.1698	15.78115	16.81071	15.70863	15.99996	16.06106	16.21761	15.7812	0	
Rabbit	8.984907	9.060652	8.864646	8.75227	8.807788	8.5351111	9.801457	9.052151	15.83072	7.884322	8.215288	8.014057	8.503256	4.931132	15.67941	0

Table 7. The similarity/dissimilarity matrix for the nine ND5 proteins based on the distance between the time series of protein sequences.

	Human	Gorilla	pi-chim	c-chim	f-whale	b-whale	Rat	Mouse	Opossum	Sheep	Goat	Lemur	Cattle	Hare	Gallus	Rabbit
Human	0															
Gorilla	2981.258	0														
pi-chim	2588.019	2915.8	0													
c-chim	2409.91	2825.331	1861.989	0												
f-whale	4983.93	4895.911	4827.771	4928.243	0											
b-whale	4851.253	4767.245	4766.777	4851.621	1767.544	0										
Rat	5525.081	5841.619	5653.435	5633.736	5200.332	5029.0002	0									
Mouse	5319.267	5554.139	5513.707	5444.948	5120.269	5001.058	4172.16	0								
Opossum	8707.729	8784.911	8756.717	8756.362	8271.891	8185.2298	8653.226	8594.21	0							
Sheep	4640.349	4547.743	4524.675	4569.375	4093.521	3992.4965	5150.633	4718.811	8504.373	0						
Goat	4722.742	4701.679	4748.725	4769.493	3983.214	4034.9342	5120.118	4838.206	8649.148	2462.512	0					
Lemur	4889.712	4848.676	4598.65	4826.458	4512.196	4531.889	5544.324	5237.505	8680.948	4160.213	4210.138	0				
Cattle	4947.452	4855.012	4820.787	5027.681	3900.558	3834.188	5305.958	5003.189	8545.737	2930.599	3039.352	4358.573	0			
Hare	4761.344	4857.071	4536.894	4510.254	4533.822	4350.7163	5184.895	4964.674	8319.127	4153.277	4429.338	4484.493	4508.157	0		
Gallus	8535.1	8526.681	8652.828	8676.832	8292.252	8267.6087	8509.391	8334.253	8834.389	8366.897	8547.139	8501.893	8639.767	8371.985	0	
Rabbit	4925.531	4902.064	4828.638	4735.793	4760.704	4629.3622	5299.299	4871.211	8456.629	4263.431	4388.914	4426.524	4564.441	2677.03	8342.092	0

Fig. 3. The phylogenetic tree of the 16 species based on the PCA-FFT analysis of their ND5 protein sequences.

sequence-based similarity matrix as input data, respectively, and we got the same phylogenetic trees applying the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method,²⁷ which is a simple agglomerative hierarchical clustering method that can be used to construct a phylogenetic tree. The phylogenetic tree based our method is presented in Fig. 3.

From Fig. 3, we can find that our method successfully clusters these 16 species based on the PCA-FFT analysis of their ND5 protein sequences. Specifically, the result shows that the gallus is peculiar, since the distance between it and the remaining animals is shortest. Obviously, the result is consistent with the fact that gallus is not a kind of mammal.

To validate the effectiveness of our method, we applied our method to another group of proteins that exist in a virus called spike protein (S protein), which is a large type I transmembrane protein ranging from 1160 amino acids for avian infectious bronchitis virus (IBV) and up to 1400 amino acids for feline coronavirus (FCoV). In addition, spike protein is highly glycosylated as it contains 21–35 N-glycosylation sites. The basic information of these proteins is presented in Table 8.

According to the above method, we construct the phylogenetic tree of these 29 spike proteins in Fig. 4.

The viruses in Table 8 are Coronavirus, they are always classified into four classes, where Group I includes the Canine coronavirus (CCoV), the Feline coronavirus (FCoV), the Human coronavirus 229E (HCoV-229E), the Porcine epidemic diarrhea virus (PEDV), the Transmissible gastroenteritis virus (TGEV) and so

on. Group II includes the Bovine coronavirus (BCoV), Human coronavirus OC43 (HCoV-OC43), the Murine hepatitis virus (MHV), the Porcine hemagglutinating encephalomyelitis virus (HEV), the Rat coronavirus (RtCoV) and so on. Group III contains the Infectious bronchitis virus (IBV), the Turkey coronavirus (TCoV) and so on. The others are Severe Acute Respiratory Syndrome Coronaviruses (SARS-CoV). In Fig. 4, we can observe that the SARS-CoVs, Groups II and III can be separated correctly, however, Group I has been broken into two parts.

Table 8. The basic information of 29 spike proteins.

No.	Access No.	Abbreviation	Length
1	CAB91145	TGEVG	1447
2	NP_058424	TGEV	1447
3	AAK38656	PEDVC	1383
4	NP_598310	PEDV	1383
5	NP_937950	HCoVOC43	1361
6	AAK83356	BCoVE	1363
7	AAL57308	BCoVL	1363
8	AAA66399	BCoVM	1363
9	AAL40400	BCoVQ	1363
10	AAB86819	MHVA	1324
11	YP_209233	MHVJHM	1376
12	AAF69334	MHVP	1321
13	AAF69344	MHVM	1324
14	AAP92675	IBVBJ	1169
15	AAS00080	IBVC	1169
16	NP_040831	IBV	1162
17	AAS10463	GD03T0013	1255
18	AAU93318	PC4127	1255
19	AAV49720	PC4137	1255
20	AAU93319	PC4205	1255
21	AAU04646	civet007	1255
22	AAU04649	civet010	1255
23	AAV91631	A022	1255
24	AAP51227	GD01	1255
25	AAS00003	GZ02	1255
26	AAP30030	BJ01	1255
27	AAP50485	FRA	1255
28	AAP41037	TOR2	1255
29	AAQ01597	TaiwanTC1	1255

Moreover, to demonstrate the effectiveness of our method, the similarities of ND5 proteins of nine species are compared. Clustal W is one of most popular multiple sequence alignment methods which uses sequence weighting, position specific gap penalties and weight matrix choice to improve the sensitivity of progressive multiple sequence alignment.²⁸ For comparing with our results, the scatter diagrams are plotted to obtain the results of other graphical representation correlation with Clustal W's results in Fig. 5. In these scatter diagrams, the linear lines are given. If the points are all around the trend line, this shows that the correlation is better. Observing the figure, our method presented higher correlation with Clustal W than the methods of Hou *et al.*,⁵ Elmaatv *et al.*,⁶ Wen *et al.* method,⁷ Zhang *et al.* method,⁸ Wu *et al.*,⁹ Yao *et al.*,¹⁰ and He *et al.*¹⁴ So in this paper, a new approach is provided to analyze the similarities/dissimilarities of protein sequence, which is comparatively more efficient.

Fig. 5. Comparison of different methods by correlation analysis.

4. Conclusions

In this paper, we apply the PCA to transform the protein sequences into time series. By this way, we can easily find the differences between protein sequences if we plot them in a same coordinate system. We consider the time series as signals and utilize the FFT to produce them, and then we get the information of their time-frequency features. We make use of the time series and their time-frequency features to analyze the similarity/dissimilarity of protein sequences, which not only contain more information of about the proteins, but also do not need to build the high dimensional matrixes which require complex computation. To observe the similarity more conveniently, we construct the phylogenetic tree of the similarity/dissimilarity matrix using the UPGMA method. Then two groups of sequences are chosen to validate our method, and we get the satisfying results. Finally, the scatter diagrams are constructed for comparing our method with some existing methods to validate the effectiveness of the method presented in this paper.

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