FFP: joint Fast Fourier transform and fractal dimension in amino acid property-aware phylogenetic analysis

Wei Li¹, Lina Yang¹*, Yu Qiu¹, Yujian Yuan¹, Xichun Li² and Zuqiang Meng¹

Abstract

Background: Amino acid property-aware phylogenetic analysis (APPA) refers to the phylogenetic analysis method based on amino acid property encoding, which is used for understanding and inferring evolutionary relationships between species from the molecular perspective. Fast Fourier transform (FFT) and Higuchi's fractal dimension (HFD) have excellent performance in describing sequences' structural and complexity information for APPA. However, with the exponential growth of protein sequence data, it is very important to develop a reliable APPA method for protein sequence analysis.

Results: Consequently, we propose a new method named FFP, it joints FFT and HFD. Firstly, FFP is used to encode protein sequences on the basis of the important physicochemical properties of amino acids, the dissociation constant, which determines acidity and basicity of protein molecules. Secondly, FFT and HFD are used to generate the feature vectors of encoded sequences, whereafter, the distance matrix is calculated from the cosine function, which describes the degree of similarity between species. The smaller the distance between them, the more similar they are. Finally, the phylogenetic tree is constructed. When FFP is tested for phylogenetic analysis on four groups of protein sequences, the results are obviously better than other comparisons, with the highest accuracy up to more than 97%.

Conclusion: FFP has higher accuracy in APPA and multi-sequence alignment. It also can measure the protein sequence similarity effectively. And it is hoped to play a role in APPA's related research.

Keywords: Phylogenetic analysis, Amino acid property, Fast Fourier transform, Dissociation constant, Higuchi's fractal dimension, Protein sequence similarity, Multi-sequence alignment

Background

Proteins perform vital roles in countless biological processes, they help to build the structure of living organisms. Generally, proteins’ three-dimensional structure depends on primary amino acid sequence and determines their biological function [1]. Sequence analysis based on biomolecular data can reduce the time and cost of traditional laboratory experiments for protein family identification, function prediction and gene...
annotation [2]. Due to the explosive growth of genome sequence data, it is necessary to find a reliable algorithm for sequence analysis [3].

Detecting similar fragments between sequences is the core idea of multi-sequence alignment (MSA) [4, 5], whose reliability directly affects protein phylogenetic analysis in revealing the distance relationship among different species [6]. Existing MSA algorithms can be divided into two categories: alignment-based and alignment-free algorithms. Compared with the former algorithms [7, 8], alignment-free has lower computing complexity and better visualization. Among these alignment-free algorithms, the graphic representation of protein is one of the most effective and commonly used ways. Hamori and Ruskin first applied it to biomolecular sequences data [9]. After that, many different graphical representation methods of protein sequences have been proposed for further sequence analysis. El-Lakkani [10] represent protein sequences using 3D adjacency matrix, which is an improvement based on 2D adjacency matrix representation [11]. Gupta et al. [12], Wu [13], Yang [14] represent protein sequences and carried out similarity analysis based on hydrophobicity values of the amino acid.

In addition, the physical and chemical properties of amino acids play a significant role in the functional and structural formation of proteins. Thus, there are some methods based on properties have been proposed. The literature [15–20] reduced 20 amino acids to 4–12, and they divided the amino acids into 4–12 groups based on amino acids hydrophobicity and isoelectric points. This simplification may result in the loss of biological information. Yu [21] used the hydrophobicity, dissociation constant and accessible surface area of amino acids to combine with spherical coordinates to represent protein sequences. Mu [22] transformed sequences into 578 numerical vectors for protein phylogenetic analysis. Rout et al. [23] proposed EightyDVec for protein phylogenetic analysis based on the physicochemical properties of amino acids.

Moreover, some signal processing algorithms (Discrete Fourier Transform, Fast Fourier Transform (FFT), Higuchi’s fractal dimension (HFD)) have also been introduced into protein sequence analysis. Hou et al. [24] proposed a sequence similarity analysis method based on Discrete Fourier Transform and Dynamic Time Warping that has a high time calculation cost and it can only compare time domain sequence, not in the frequency domain [25]. Compared with Discrete Fourier Transform, FFT can save exponential computing time. FFT is good at capturing the frequency content of the signal, which may contain the essence of the data. Guo proposed a method to classify G-protein coupled receptors based on FFT [26]. Chen proposed a random projection method based on FFT for self-interacting proteins prediction [27]. Fractal dimension describes the complexity of geometric objects. Smits used HFD to monitor the complexity of brain activity [28]. There exists similarity between the whole and part of the protein sequence, so they can be represented by fractal curve. Hu [29] calculated the similarity between protein sequences based on box-counting dimension.

Although FFT and HFD have been widely used, no one used them together for Amino acid property-aware phylogenetic analysis (APPA), which refers to the phylogenetic analysis based on amino acid property encoding, and it is an effective method to study the similarity and functional relationships between protein sequences [30]. The primary sequence is represented by 20 amino acid letters, and this representation cannot be processed directly and needs to be converted to numbers [31]. Effective
amino acid digital coding is related to the overall performance of the model, which is usually called feature extraction or amino acid coding scheme [32]. The property of amino acids plays a decisive role in the formation of protein structure and function. Therefore, amino acid property encoding is used in this paper, and we aim to discuss the application of FFT and HFD in APPA.

In this paper, we present FFP, it is a hybrid method for APPA. Above all, the primary amino acid sequence is converted into digital sequence using the pH(COOH) value, which is critical for the dissociation constant. In previous works, the hydrophobicity of amino acids is the most used, as an equally important dissociation constant, it is rarely used. Next, the feature vector of each protein is generated by integrating FFT and HFD. Then the distance matrix is obtained by the cosine function, the shorter the distance between two species, the more similar they are. (Details are shown in Fig. 1 and Materials and Methods). Finally, FFP is applied to the phylogenetic analysis of a set of ND6 proteins and three sets of β-globin proteins with different sizes.

![Fig. 1 Overall steps of sequence comparison algorithm. Step 1 The primary amino acid sequences (Protein 1,...,Protein N) are queried from the NCBI database according to the Accession ID of proteins. Step 2 Each amino acid letter in P_j is mapped to its attribute value and obtain the curvilinear representation of P_j. Step 3 Calculate the Discrete Fourier transform of P_j using the FFT. Step 4 Calculate the feature vectors of Step 3 based on HFD. Step 5 Calculate the distance between pairwise protein sequences using Cosine function. Finally, phylogenetic trees can be constructed based on single linkage.](image-url)
respectively. And the results are also compared with previous works to demonstrate the effectiveness of our method.

Results

To demonstrate the accuracy of our method, we used FFP for phylogenetic analysis on four groups of frequently-used protein sequences. The protein data information used in this section is given in Table 1. We use trial and error to set the FFT level to 2, the sliding window width to 9 by observing the phylogenetic tree, which is obtained by the linkage and dendrogram function in Matlab. For comparison, we also chose the same data set with some existing distance-based phylogenetic algorithms, they are based on Neighbor-Joining algorithm ([33]), UPGMA algorithm ([34]) ([19, 20] and [35]), Euclidean distance algorithm ([18]) and Jeffrey’s and Matusita distance algorithm ([29]). All of these methods are alignment-free. In order to illustrate the performance of our method more effectively, we also compare with ClustalW, the representative of alignment-based methods. The phylogenetic tree built by ClustalW is implemented using UPGMA algorithm in the

| Species/Set | Accession ID   | Length | Species/Set | Accession ID   | Length |
|-------------|---------------|--------|-------------|---------------|--------|
| 1. ND6Set   |               |        | 2. 10-BetaSet |               |        |
| Human       | YP_003024037  | 174    | Human       | AAA16334      | 147    |
| Gorilla     | NP_008223     | 174    | Gorilla     | P02024        | 147    |
| C.Chimp     | NP_008197     | 174    | Gibbon      | P02025        | 146    |
| Wallaroo    | NP_007405     | 167    | G.Panda     | P18983        | 147    |
| Harbor Seal(H.Seal) | NP_006939 | 175    | Goose       | P02117        | 146    |
| Gray Seal(G.Seal) | NP_007080  | 175    | Swan        | P68945        | 146    |
| Rat         | AP_004903     | 172    | Goat        | AAA30913      | 145    |
| Mouse       | NP_904339     | 172    | Sheep       | NP_001091117  | 145    |
|             |               |        | Bovine      | CAA25111      | 145    |
|             |               |        | Bison       | P09422        | 145    |
|             |               |        |             |               |        |
| 3. 11-BetaSet |          |        | 4. 17-BetaSet |               |        |
| Human       | AAA16334      | 147    | Human       | ALLJ64020     | 147    |
| Lemur       | AAA36822      | 147    | Gorilla     | P02024        | 147    |
| Mouse       | ADD52696      | 147    | Chimpanzee (Chimp) | P68873      | 147    |
| Goat        | AAA30913      | 145    | Cattle      | CAA25111      | 145    |
| Rabbit      | CAA24251      | 147    | Bantering   | BAU05126      | 145    |
| Chimpanzee  | P68873        | 147    | Goat        | AAA30913      | 145    |
| Gorilla     | P02024        | 147    | Sheep       | ABC36525      | 145    |
| Rat         | CAA33250      | 147    | European hare (E.Hare) | CAA68429   | 147    |
| Bovine      | CAA25111      | 145    | Rabbit      | CAA24251      | 147    |
| Opossum     | AAA30976      | 147    | House mouse (H.Mouse) | ADD52660   | 147    |
| Gallus      | CAA23700      | 147    | Western wild mouse | ACY03394   | 147    |
|             |               |        | Spiny mouse (S.Mouse) | ACY03377 | 147    |
|             |               |        | Norway Rat (N.Rat) | CAA29887    | 147    |
|             |               |        | Opossum     | AAA30976      | 147    |
|             |               |        | Guttata     | CH46399       | 147    |
|             |               |        | Gallus      | CAA23700      | 147    |
|             |               |        | Muscovy duck (M.Duck) | CAA33756   | 147    |
MEGA [36]. All protein data used in the experiment are obtained from NCBI database [37].

Phylogenetic analysis of 8 ND6 protein sequences

This dataset contains 8 ND6 protein sequences from different species, the sequence details are given in Table 1: ND6Set. A 159 × 8 feature vector was obtained by FFT and HFD.

The cosine function was used to calculate the distance matrix of eight ND6 protein sequences of mammals, the matrix is filled in Table 2. The smaller the value in the matrix, the smaller the distance between species and the more similar they are. And the phylogenetic tree was constructed by single linkage, as shown in the Fig. 2. The horizontal axis (branch) is the similarity between species, and the vertical axis is eight different species. The shorter the branches, the smaller the distance between sequences and the closer the two species.

As shown in Fig. 2a, these proteins were correctly divided into four groups, and each category was highlighted in a different color: they are Hominidae (Human, Gorilla and C.Chimp), Phocidae (Harbor seal and Gray seal), Muridae (Rat and Mouse) and Macropus (Wallaroo). In terms of molecular evolution, Human and Gorilla shared the common ancestor millions of years ago. The closer the species are to each other, the shorter their evolutionary distance. From the biochemical point of view, there are minimal different sites in the primary amino acid sequence between them, so they are clustered firstly. The same is true for other species. Moreover, chimpanzees are more closely related to humans than are gorillas [38], Wallaroo is the most distant from the other seven mammals. These results are consistent with known evolutionary facts.

Phylogenetic trees constructed by previous studies [29, 33], and ClustalW are shown in Fig. 2b–d, respectively. Figure 2b also correctly classifies eight species into four groups, but incorrectly connects Wallaroo to the Seal branch. Wallaroo is the farthest from the other seven species. In Fig. 2c, the phylogenetic tree given by Hu shows Muridae (Rat and Mouse) are the most distant of the eight species, they are closer to Hominidae (Human, Gorilla and C.Chimp) than Wallaroo. Figure 2d is the phylogenetic tree constructed by ClustalW [7] using Mega [36] package, which constructs the phylogenetic tree by UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method, it is one of the most recognized methods in protein MSA [39], the difference between it and

### Table 2 The distance matrix of ND6Set by FFP

|       | Human | Gorilla | C.Chimp | Wallaroo | H.Seal | G.Seal | Rat   | Mouse |
|-------|-------|---------|---------|----------|--------|--------|-------|-------|
| Human | 0     | 0.0002  | 0.0017  | 0.0122   | 0.0061 | 0.0065 | 0.0078| 0.0085|
| Gorilla| 0.0002| 0       | 0.0019  | 0.0128   | 0.0070 | 0.0071 | 0.0080| 0.0091|
| C.Chimp| 0.0017| 0.0019  | 0       | 0.0098   | 0.0032 | 0.0037 | 0.0054| 0.0065|
| Wallaroo| 0.0122| 0.0128  | 0.0098  | 0        | 0.0070 | 0.0066 | 0.0064| 0.0062|
| H.Seal | 0.0061| 0.0070  | 0.0032  | 0.0070   | 0      | 0.0006 | 0.0051| 0.0048|
| G.Seal | 0.0065| 0.0071  | 0.0037  | 0.0066   | 0.0006 | 0      | 0.0046| 0.0041|
| Rat    | 0.0078| 0.0080  | 0.0054  | 0.0064   | 0.0051 | 0.0046 | 0     | 0.0016|
| Mouse  | 0.0085| 0.0091  | 0.0065  | 0.0062   | 0.0048 | 0.0041 | 0.0016| 0     |
Fig. 2a is which family is closer to Phocidae (H.Seal and G.Seal), Hominidae or Muridae (Rat and Mouse). According to the Encyclopedia Britannica [40], Rat and Mouse are insectivores, G.Heal and H.Seal are carnivores, Human, Gorilla and C.Chimp are omnivorous, thus, Muridae is closer to Phocidae than Hominidae. And Wallaroo is herbivorous, so it is the most distant from the other seven mammals. He’s [18] result showed that Muridae branch is closer to Hominidae than Phocidae.

We also calculated the Correlation coefficient (CC) between existing works (including ours, Ref. [29, 33]) with ClustalW’s result. The CC of Human is calculated by the first row of our distance matrix in Table 2 and the first row of the matrix obtained by ClustalW and so on. In statistical analysis, if CC c between variable A and variable B satisfies $c_0.05(n-2) < |c| \leq c_0.01(n-2)$ (n is the number of variables), this is to say that A and B in linear correlation. In this part, n=8, so when 0.707 < |c| ≤ 0.834, it’s in linear correlation, and when |c| > 0.834, it’s in strongly linear correlation. The calculated CC results are filled in Table 3. It can be seen that our results are all strongly linear correlation with ClustalW except Wallaroo, but it’s still in linear correlation, and our result’s correlation coefficients with ClustalW’s are all higher than Ref. [33]. However, some of [29]’s CCs with ClustalW’s are higher than ours, his clustering of Wallaroo was inaccurate.

**Phylogenetic analysis of 10 β-globin protein sequences**

This dataset used 10 β-globin from different species (see Table 1: 10-BetaSet for details). The distance matrix using cosine function is shown in Table 4. The smaller the value
between them, the more similar the protein sequences are, and the more closely related the species are. To more intuitively describe this relationship, we constructed the phylogenetic tree (Fig. 3a) of these 10 species using the single linkage.

Table 3  The correlation coefficients for ND6Set between our, Saw’s [33] and Hu’s [29] method with ClustalW

|              | Human | Gorilla | C.Chimp | Wallaroo | H.Seal | G.Seal | Rat   | Mouse |
|--------------|-------|---------|---------|----------|--------|--------|-------|-------|
| Our CC       | 0.9754| 0.9715  | 0.8881  | 0.7431   | 0.9016 | 0.8866 | 0.9107| 0.8544|
| [33]'s CC    | 0.8469| 0.8832  | 0.8554  | 0.8653   | 0.7602 | 0.7847 | 0.8112| 0.7536|
| [29]'s CC    | 0.9707| 0.9700  | 0.9648  | 0.9806   | 0.9436 | 0.9481 | 0.8890| 0.9197|

Table 4  The distance matrix of 10-BetaSet by FFP

|              | Human  | Gorilla | Gibbon | G.Panda | Goose | Swan | Goat | Sheep | Bovine | Bison |
|--------------|--------|---------|--------|---------|-------|------|------|-------|--------|-------|
| Human        | 0      | 0.0000  | 0.0001 | 0.0004  | 0.0032| 0.0028| 0.0028| 0.0018| 0.0034 |
| Gorilla      | 0.0000 | 0       | 0.0001 | 0.0004  | 0.0032| 0.0028| 0.0028| 0.0018| 0.0034 |
| Gibbon       | 0.0001 | 0.0001  | 0      | 0.0003  | 0.0031| 0.0028| 0.0027| 0.0019| 0.0035 |
| G.Panda      | 0.0004 | 0.0004  | 0.0003 | 0       | 0.0035| 0.0024| 0.0023| 0.0016| 0.0032 |
| Goose        | 0.0032 | 0.0032  | 0.0031 | 0.0035  | 0     | 0.0000| 0.0000| 0.0000| 0.0008 |
| Swan         | 0.0032 | 0.0032  | 0.0031 | 0.0036  | 0.0000| 0     | 0.0038| 0.0037| 0.0030|
| Goat         | 0.0028 | 0.0028  | 0.0028 | 0.0024  | 0.0038| 0.0038| 0     | 0.0000| 0.0011|
| Sheep        | 0.0028 | 0.0028  | 0.0027 | 0.0023  | 0.0037| 0.0037| 0.0000| 0     | 0.0011|
| Bovine       | 0.0018 | 0.0018  | 0.0019 | 0.0016  | 0.0029| 0.0030| 0.0011| 0     | 0.0011|
| Bison        | 0.0034 | 0.0034  | 0.0035 | 0.0032  | 0.0041| 0.0041| 0.0008| 0.0008| 0.0011|

Fig. 3  Phylogenetic trees of 10-BetaSet constructed by a Our method, b ClustalW and c Hu’s method
As shown in Fig. 3a, these species are divided into two main groups: mammals and non-mammals. Among the mammals, they are classified into **Primate**: Human (Hominidae) and Gorilla (Hominidae) and Gibbon (Hylobatidae), **Carnivora**: Giant panda and **Hoofed**: Sheep, Goat, Bison and Bovine. Non-mammals include **Anatidae**: Swan and Goose.

In terms of molecular evolution, Swan and Goose are non-mammals, so they are the most evolutionarily distant from the other mammals. And they have the minimal different sites in their amino acid sequences, so their distance is near to 0. Among them, Human and Gorilla are the most similar, they are belong to Hominidae. Gibbon is similar in size to apes (Gorillas, Chimpanzees, etc.) and with no tail, just longer arms and thicker hair. In addition, Human, Gorilla and Gibbon are belong to the primate group of Mammals. In terms of eating habits, Human, Gorilla and Gibbon are omnivorous. In accordance evolution aspect, G.Panda’s ancestors were carnivores millions of years ago and gradually became omnivorous over the course of biological evolution, although its main diet is bamboo. Furthermore, Sheep, Goat, Bison and Bovine are herbivores. Given that, G.panda is closer to Human than Hoofed. These conclusions are almost consistent with ClustalW (Fig. 3b). The only difference is that our phylogenetic tree didn’t cluster Bison and Bovine together preferentially. In Fig. 3c, the phylogenetic tree constructed by Ref. [29], G.Panda is the farthest species from the other seven mammals, which could be due to the loss of biological information.

The CC of our method with ClustalW’s and Hu’s [29] with ClustalW’s can be found in Table 5. In this part, n=10, so when \(0.632 < |c| \leq 0.735\), it’s in linear correlation, and when \(|c| > 0.765\), it’s in strongly linear correlation. It can be seen that our results are all strongly linear correlation with ClustalW. Half of the results are higher than Hu’s, the CC of G.Panda of Hu’s is only about 0.6, which is considered to be low correlated.

**Phylogenetic analysis of 11 β-globin protein sequences**

In this experiment, we choose β-globin protein sequences from 11 different species, and their detailed information is shown in Table 1: **11-BetaSet**. The distance matrix obtained by cosine function is filled in Table 6. It can be seen in Table 6, the distance between Human and C.Chimp is near to 0, which means they are the most similar of these species. The next smallest distance is Gorilla and Human and so on. According to these, the constructed phylogenetic tree is shown in Fig. 4a.

Figure 4a shows that Human, Chimpanzee and Gorilla are the closest among 11 species because they all belong Hominidae. Next are Goat and Bovine (Hoofed), Lemur (Lemuridae) and Rabbit (Leporidae), they are clustered together since they are herbivorous. The next branch is Muridae: Rat and Mouse. Last is Opossum (Didelphidae) and Gallus (Phasianidae). It seems that Opossum and Gallus should not be grouped together because Gallus is non-mammal. Figure 4b is the phylogenetic tree of ClustalW, which

|          | Human | Gorilla | Gibbon | G.Panda | Goose | Swan | Goat | Sheep | Bovine | Bison |
|----------|-------|---------|--------|---------|-------|------|------|-------|--------|-------|
| Our CC   | 0.8942| 0.8828  | 0.8788 | 0.9070  | 0.9675| 0.9701| 0.9397| 0.9402| 0.9383 | 0.8436|
| [29]’s CC| 0.8721| 0.8908  | 0.8504 | 0.6484  | 0.9439| 0.9458| 0.9597| 0.9610| 0.9502 | 0.9586|
Table 6  The distance matrix of 11-BetaSet by FFP.

|          | Human | Lemur | Mouse | Goat   | Rabbit | C.Chimp | Gorilla | Rat    | Bovine | Opossum | Gallus |
|----------|-------|-------|-------|--------|--------|---------|---------|--------|--------|---------|--------|
| Human    | 0     | 0.0052| 0.0075| 0.0054 | 0.0046 | 0       | 0.0000  | 0.0084 | 0.0050 | 0.0077  | 0.0107 |
| Lemur    | 0.0052| 0     | 0.0058| 0.0032 | 0.0039 | 0.0052  | 0.0052  | 0.0054 | 0.0028 | 0.0076  | 0.0081 |
| Mouse    | 0.0075| 0.0058| 0     | 0.0044 | 0.0070 | 0.0075  | 0.0075  | 0.0038 | 0.0073 | 0.0121  | 0.0107 |
| Goat     | 0.0054| 0.0032| 0.0044| 0      | 0.0031 | 0.0054  | 0.0054  | 0.0062 | 0.0020 | 0.0084  | 0.0076 |
| Rabbit   | 0.0046| 0.0039| 0.0070| 0.0031 | 0      | 0.0046  | 0.0046  | 0.0076 | 0.0027 | 0.0106  | 0.0085 |
| C.Chimp  | 0     | 0.0052| 0.0075| 0.0054 | 0.0046 | 0       | 0.0000  | 0.0084 | 0.0050 | 0.0077  | 0.0107 |
| Gorilla  | 0.0000| 0.0052| 0.0075| 0.0054 | 0.0046 | 0.0000  | 0       | 0.0084 | 0.0050 | 0.0077  | 0.0107 |
| Rat      | 0.0084| 0.0054| 0.0038| 0.0062 | 0.0076 | 0.0084  | 0.0084  | 0       | 0.0065 | 0.0108  | 0.0113 |
| Bovine   | 0.0050| 0.0028| 0.0073| 0.0020 | 0.0027 | 0.0050  | 0.0050  | 0.0065 | 0      | 0.0074  | 0.0079 |
| Opossum  | 0.0077| 0.0076| 0.0121| 0.0084 | 0.0106 | 0.0077  | 0.0077  | 0.0108 | 0.0074 | 0       | 0.0093 |
| Gallus   | 0.0107| 0.0081| 0.0107| 0.0076 | 0.0085 | 0.0107  | 0.0107  | 0.0113 | 0.0079 | 0       | 0       |
clustered Rabbit and Lemur to the human branch. In Fig. 4c, the result in Ref. [35], didn’t cluster Human and Chimpanzee firstly, which didn’t fit the biochemical and molecular evolution facts and it indicated that the Muridae (Rat and Mouse) is closer to Opossum and Gallus.

**Phylogenetic analysis of 17 β-globin protein sequences**

The data set for the final set of experiments was β-globin sequences from 17 different species. The accession ID is filled in Table 1: 17-BetaSet. After calculating of FFP, a 137 × 17 feature vector was obtained. The choice of distance function is cosine, the distance matrix is shown in Table 7.

It is clear from Table 7 that the distance between Human, Chimp and Gorilla is the shortest. After four decimal places, the distance between Human and Chimp is 0, which means they are the most similar. The same and more precise information can be obtained from the phylogenetic tree constructed using the single method in Fig. 5a.

In Fig. 5a, it clusters Human, Gorilla and Chimpanzee firstly. The second branch is Banteng, Cattle, Sheep and Goat, they are Hoofed. Next is family Leporida, Rabbit and European hare. And Rodent: House mouse, Western wild mouse, Spiny mouse and Norway Rat. Finally is family Phasianidae: Guttata and Gallus and family Anatidae: MuscovyDuck. It shows that our results are basically consistent with ClustalW (Fig. 5b) and Ref. [20] (Figure 5c). Nevertheless, Fig. 5c thought that Opossum are closer to Rodent than Human and other species. Opossum is the most distant species from the other thirteen mammals.
### Table 7: The distance matrix of 17-BetaSet by FFP.

|       | Human | Gorilla | Chimp | Cattle | Banteng | Goat   | Sheep  | E.Hare | Rabbit | H.Mouse | W.Mouse | S.Mouse | N.Rat | Opossum | Guttata | Gallus | M.Duck |
|-------|-------|---------|-------|--------|---------|--------|--------|--------|--------|---------|---------|---------|-------|---------|---------|--------|--------|
| Human | 0     | 0.0000  | 0.0050| 0.0051 | 0.0054  | 0.0049 | 0.0057 | 0.0046 | 0.0075 | 0.0083  | 0.0092  | 0.0084  | 0.0077 | 0.0102  | 0.0107  | 0.0121 |
| Gorilla| 0     | 0.0000  | 0.0050| 0.0051 | 0.0054  | 0.0049 | 0.0057 | 0.0046 | 0.0075 | 0.0083  | 0.0092  | 0.0084  | 0.0077 | 0.0102  | 0.0107  | 0.0121 |
| Chimp | 0     | 0.0050  | 0.0051| 0.0054 | 0.0049  | 0.0057 | 0.0046 | 0.0075 | 0.0083 | 0.0092  | 0.0084  | 0.0077 | 0.0102 | 0.0107 | 0.0121 |
| Cattle| 0     | 0.0002  | 0.0005| 0.0044 | 0.0027  | 0.0073 | 0.0077 | 0.0083 | 0.0065 | 0.0074  | 0.0072  | 0.0079  | 0.0082 |
| Banteng| 0    | 0.0023  | 0.0005| 0.0045 | 0.0028  | 0.0074 | 0.0079 | 0.0085 | 0.0061 | 0.0069  | 0.0071  | 0.0080  | 0.0076 |
| Goat  | 0     | 0.0015  | 0.0042| 0.0031 | 0.0044  | 0.0048 | 0.0053 | 0.0062 | 0.0084 | 0.0076  | 0.0076  | 0.0076  | 0.0089 |
| Sheep | 0     | 0.0042  | 0.0023| 0.0073 | 0.0076  | 0.0081 | 0.0063 | 0.0079 | 0.0070 | 0.0075  | 0.0070  | 0.0075  | 0.0079 |
| E.Hare| 0     | 0.0023  | 0.0072| 0.0078 | 0.0078  | 0.0080 | 0.0080 | 0.0078 | 0.0080 | 0.0078  | 0.0078  | 0.0078  | 0.0092 |
| Rabbit| 0     | 0.0070  | 0.0075| 0.0085 | 0.0076  | 0.0076 | 0.0086 | 0.0085 | 0.0102 | 0.0111  | 0.0107  | 0.0128 |
| H.Mouse| 0    | 0.0010  | 0.0019| 0.0038 | 0.0121  | 0.0111 | 0.0117 | 0.0134 | 0.0134 |
| W.Mouse| 0   | 0.0014  | 0.0054| 0.0133 | 0.0115  | 0.0111 | 0.0111 | 0.0134 |
| S.Mouse| 0   | 0.0052  | 0.0121| 0.0086 | 0.0082  | 0.0102 | 0.0082 | 0.0102 |
| N.Rat | 0     | 0.0108  | 0.0115 | 0.0113 | 0.0126  | 0.0083 |
| Opossum| 0  | 0.0081  | 0.0093 | 0.0083 |
| Guttata| 0    | 0.0006  | 0.0009 |
| Gallus| 0     | 0.0016  | 0.0016 |
| M.Duck| 0     | 0       | 0        |
Extended experiments
In this part, the hydrophobic value, basicity coefficient and relative molecular weight of amino acids were used to encode the primary amino acid sequences in four data sets, respectively. After applying FFP to each data set, the constructed phylogenetic trees are shown in Figs 6, 7 and 8, which are also highly similar to our previous tree in Results. Hence, it can be concluded that FFP we proposed in this paper is robust.

Discussion
In this paper, a hybrid method called FFP for APPA was proposed. The differences between FFP and existing works are as follows: (1) In the step of drawing protein sequence curve, we choose dissociation constant among the rich physical and chemical properties of amino acids to encode the protein sequence, which determines the acidity and basicity, making the constructed protein sequence curves more reliable. (2) When extracting the numerical features of protein curves, we use FFT to decompose the initial N-point sequence into a series of short sequences to obtain the potential information in the sequence. (3) To extract more accurate features, we use HFD as the next step of the FFT, which can get information about the geometrical structure.

We tested FFP on one group of ND6 sequences and three groups of globin sequences with different sizes in the experimental part. The results show that FFP is effective for APPA. This method can play a powerful role in the protein classification and the prediction of functional structure. In the meanwhile, FFP still has some improvements to make. For instance, the current FFP algorithm describes protein sequences only based
Fig. 6  Phylogenetic trees of four test sets using hydrophobicity encoding based on FFP

Fig. 7  Phylogenetic trees of four test sets using basicity encoding based on FFP
on the properties of amino acids, which may not be comprehensive. Our next research topic will be how to effectively utilize the structural information of proteins and combine it with their properties. In addition, our subsequent work will improve FFP so that it can be more accurate when analyzing protein families with a more significant number.

**Conclusions**

Based on the dissociation constant of amino acids, we proposed a hybrid algorithm named FFP for APPA. We tested one group of ND6 sequences and three groups of globin sequences with different sizes in the experimental part. The results show that FFP is effective for proteins phylogenetic analysis. This method can play a powerful role in protein sequences similarity analysis and functional structure prediction. In addition, our subsequent work will improve the algorithm so that it can be more accurate when analyzing protein families with a more significant number.

**Methods**

**Data selection and feature extraction**

The four different data sets used in the experiment are as follows:

(i)  *ND6Set*: NADH Dehydrogenase 6 (ND6) protein sequences of 8 species.
(ii)  *10-BetaSet*: β-globin protein sequences of 10 species.
(iii)  *11-BetaSet*: β-globin protein sequences of 11 species.
(iv)  *17-BetaSet*: β-globin protein sequences of 17 species.
All sequence information are obtained from the NCBI (National Center for Biotechnology Information) database [37], including amino acid sequence, definition, accession ID, sequence length and source.

As the primary structure of protein, amino acid sequence has an important influence on the structure and function of protein. In general, each amino acid is represented by a corresponding letter: A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y. The rich properties of amino acids play a decisive role in the structure formation and function of proteins [41]. Isoelectric point (pI) is one of the most important and commonly used properties of amino acids, and the dissociation constant of –COOH (pK$_a$(COOH)) is closely related to pI, it reflects the ionized state of –COOH in solutions. So pK$_a$(COOH) values are used as features to represent amino acids and vectorial protein sequence is obtained. Detailed mappings of each amino acid and their pK$_a$(COOH) values are listed in Table 8.

Take two short sequences of Saccharomyces cerevisiae as an example, and their sequences are:

Protein I (P1): WTFESRNDPAKDPVILWLNGGPGCSSLTGL
Protein II (P2): WFFESRNDPAMDPILWLNGGPGCSSFTGL

Their feature curves are shown in Fig. 9. The four positions of the yellow circle are where the two sequences differ.

**Fast Fourier transform**

As a widely used tool in signal analysis, Discrete Fourier Transform (DFT) and its extension has also been applied to biological sequence analysis [42–47]. Using DFT can discover hidden signal information without loss in the time domain. Fast Fourier Transform (FFT) is a fast algorithm for DFT. The time complexity of DFT is $\Theta(n^2)$, however, the time complexity of FFT is only $\Theta(n \log n)$. After feature extraction in the previous section, protein sequence $S = \{s_1, s_2 \ldots s_N\}$ can be represented by $P = \{p_1, p_2 \ldots p_N\}$, and $N$ is the length of protein $S$, $s_i$ is the i-th amino acid of $S$ and $p_i$ is pK$_a$(COOH) value corresponding to $s_i$, $i = 1..N$.

The DFT of sequence $P = \{p_1, p_2 \ldots p_N\}$ at frequency $k$ is

### Table 8 Information and feature values of 20 amino acids

|    | code | pK$_a$(COOH) | code | pK$_a$(COOH) |
|----|------|--------------|------|--------------|
| Ala| A    | 2.34         | Met  | M            | 2.28 |
| Cys| C    | 1.71         | Asp  | N            | 2.02 |
| Asp| D    | 2.09         | Pro  | P            | 1.99 |
| Glu| E    | 2.19         | Glu  | Q            | 2.17 |
| Phe| F    | 1.83         | Arg  | R            | 2.17 |
| Gly| G    | 2.34         | Ser  | S            | 2.21 |
| His| H    | 1.82         | Thr  | T            | 2.63 |
| Ile| I    | 2.36         | Val  | V            | 2.32 |
| Lys| K    | 2.18         | Trp  | W            | 2.38 |
| Leu| L    | 2.36         | Tyr  | Y            | 2.2  |
Higuchi’s fractal dimension

The concept of fractal [48] is very important for the study of non-linear objects. Fractal dimension is an important approach to study fractal, which includes information about the complexity of fractal objects [49]. Hausdorff dimension is one of the oldest and most important fractal dimensions, it gave a new form to the usual concepts of length and area, and it formed the basic theoretical model of other fractal dimensions. However, in practical application, Hausdorff dimension is difficult to calculate or estimate by general calculation method [14]. In contrast, Box counting dimension [50] is more practical and convenient because it is the only dimension that can be computed with a limited
range of scales [49]. In order to apply Box counting dimension to digital image processing more conveniently, scholars also put forward Minkowski dimension [50].

However, in some signal and image processing applications, the calculation of Box counting dimension is time-consuming. Thus, some approximate algorithms for fractal dimension were proposed. Higuchi’s fractal dimension (HFD) [51] can provide a better measure of signal complexity when there are few data points available [52]. Therefore, HFD has been widely used in biomedical signal and image processing [53–55]. HFD can be calculated as follows. Suppose that $Z = \{z_1, z_2, \ldots, z_M\}$ is a M sample data sequence, and its sub-sequence can be represented as [56]:

$$Z_{mn}^n : \{z(n), z(n + m), z(n + 2m), \ldots, z\left(n + \left\lfloor \frac{M - n}{m} \right\rfloor m\}\}, n = 1 \ldots m,$$

and symbol $\lfloor \ast \rfloor$ is floor operation, $n$ is initial position, $m$ means the number of sub-sequences. Now, set $M = 6$ and $m = 2$, then two sub-sequences are obtained:

$Z_1^2 : \{z(1), z(3), z(5)\}, Z_2^2 : \{z(2), z(4), z(6)\}.$

The length of each sub-sequence is:

$$H_n^m = \sum_{i=1}^{\left\lfloor \frac{M - n}{m} \right\rfloor} |z(n + im) - z(n + (i - 1)m)(M - 1) ÷ \left\lfloor \frac{M - n}{m} \right\rfloor m^2$$

In addition, we also choose sliding window combine with HFD, a feature vector of length $M - d + 1$ can be obtained. $H_n^m$ can be rewritten to:

$$H_n^m(m) = \sum_{i=1}^{\left\lfloor \frac{d - n}{m} \right\rfloor} |z(n + im + j - 1) - z(n + (i - 1)m + j - 1)(d - 1) ÷ \left\lfloor \frac{d - n}{m} \right\rfloor m^2$$

where $d$ means the window width, $j = 1 \ldots M - d + 1$ and $n = 1 \ldots m$. Then the average length is:

$$H^l(m) = \frac{1}{m} \sum_{n=1}^{m} H_n^l(m).$$

Finally, the HFD of window $j$ is:

$$f^* = \arg\min_{m} \sum_{m=1}^{M} (\log(\frac{1}{m}) - \log(H^l(m)) + b)^2,$$

where $b$ is the bias, and the final vector could be represented as $F^* = \{f^{1*}, f^{2*}, \ldots, f^{(M-d+1)*}\}$. Fig. 11 is the HFD of Fig. 10 with window width 9.

**Similarity function**

Phylogenetic tree construction depends heavily on the selection of similarity function. After experimental comparison, cosine similarity is selected in this paper. It evaluates
the similarity of two vectors by calculating the cosine of the angle between them [14]. Its calculation formula is as follows:

\[ C = \cos(\theta) = \frac{A \cdot B}{\|A\|\|B\|} = \frac{\sum_{i=1}^{n} A_i \times B_i}{\sqrt{\sum_{i=1}^{n} (A_i)^2} \times \sqrt{\sum_{i=1}^{n} (B_i)^2}}, -1 \leq C \leq 1 \]  

where \( A_i \) and \( B_i \) represent the components of vectors \( A \) and \( B \). Finally, the method for linkage is single, it clusters samples according to the distance from near to far.

**Algorithm 1: Hybrid method of FFT and HFD for Phylogenetic Analysis based on p\(K_a\) value**

*Input*: L protein sequences \( \{S_j, j = 1 \ldots L\} \).

*Output*: Similarity matrix \( D_m \) and phylogenetic tree \( R_p \).

1. **for each amino acid \( s_i \) in \( S_j \)** do
2. \( \quad \text{Map} \ s_i \text{ to the } pK_a(COOH) \text{ value shown in Table 8 to generate the feature vectors} \)
3. \( \quad \text{Calculate the Discrete Fourier transform of } P_j \text{ using the FFT} \)
4. \( \quad T_j = \{t_i\} = \{fft(p_i)\}, i = 1 \ldots N, j = 1 \ldots L. \)  
5. \( \quad \text{Calculate the Higuchi’s fractal dimension } F_j \text{ in each window by} \)
6. \( \quad H_j(n) = \text{hfd}([T_j(n), T_j(n+1) \ldots T_j(n-d+1)]), \)  
7. \( \quad \text{where } n \in \{1, \text{length}(T_j) - d + 1\}, \text{ and} \)
8. \( \quad F_j = [H_j(1), H_j(2) \ldots H_j(\text{length}(T_j) - d + 1)], j = 1 \ldots L. \)
9. **Calculate the similarity matrix \( D_m \) and build the phylogenetic tree \( R_p \):**
10. \( \quad D_m = \text{pdist}(F_j, \cosine), \)  
11. \( \quad R_p = \text{dendrogram(linkage}(D_m, \text{single})). \)

**Algorithm summary**

The specific algorithm of FFP is shown in Algorithm 1, it is the concrete implementation of the overall step diagram (Fig. 1).
Abbreviations
FFP  Joint fast Fourier transform and fractal dimension in amino acid property-aware phylogenetic analysis
APPA  Amino acid property-aware phylogenetic analysis
FFT  Fast Fourier transform
HFD  Higuchi’s fractal dimension
MSA  Multi-sequence alignment
UPGMA  Unweighted pair group method with arithmetic mean
CC  Correlation coefficient
ND6  NADH dehydrogenase 6
DFT  Discrete Fourier transform

Acknowledgements
Not applicable.

Author Contributions
We clarify that all the contributors currently listed do meet the ICMJE guidelines for authorship. Conceptualization, WL; methodology, WL and LY; formal analysis, LY; investigation, WL and LY; resources, LY and XL; data curation, WL; writing—original draft preparation, WL; writing—review and editing, YY and WL.; visualization, YQ; funding acquisition, LY and ZM. All authors read and approved the final manuscript.

Funding
This work was financially supported by the National Natural Science Foundation of China under No. 61862005 and No. 61762009. No. 61862005 supported the design and analysis of this study, and No. 61762009 supported the data collection.

Availability of data and materials
All data generated or analysed during this study are included in this published article and its supplementary information files.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Received: 30 May 2022   Accepted: 11 August 2022

References
1. Mu Z, Yu T, Qi E, Liu J, Li G. Dcgr: feature extractions from protein sequences based on cgr via remodeling multiple information. BMC Bioinf. 2019;20(1):1–10.
2. Cong Q, Grishin NV. Messa: Meta-server for protein sequence analysis. BMC Biol. 2012;10(1):1–12.
3. Terwilliger TC, Stuart D, Yokoyama S. Lessons from structural genomics. Ann Rev Biophys. 2009;38:371–83.
4. Rigden DJ. From protein structure to function with bioinformatics. Berlin: Springer; 2009.
5. Hew B, Tan QW, Goh W, Ng JW, Mutwil M. Lstrap-crowd: prediction of novel components of bacterial ribosomes with crowd-sourced analysis of ma sequencing data. BMC Biol. 2020;18(1):1–13.
6. Kapli P, Yang Z, Telford MJ. Phylogenetic tree building in the genomic age. Nat Rev Genet. 2020;21(7):428–44.
7. Thompson JD, Higgins DG, Gibson TJ. Clustal w: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucl Acids Res. 1994;22(22):4673–80.
8. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990;215(3):403–10.
9. Harbord E, Ruskin J. H-curves, a novel method of representation of nucleotide series especially suited for long DNA sequences. J Biol Chem. 1983;258(2):1318–27.
10. Ei-Lakkani A, El-Sherif S. Similarity analysis of protein sequences based on 2d and 3d amino acid adjacency matrices. Chem Phys Lett. 2013;590:192–5.
11. Randić M, Novič M, Vracko M. On novel representation of proteins based on amino acid adjacency matrix. SAR QSAR Environ Res. 2008;19(3–4):339–49.
12. Gupta K, Thomas D, Vidya S, Venkatesh K, Ramakumar S. Detailed protein sequence alignment based on spectral similarity score (sss). BMC Bioinform. 2005;6(1):1–16.
13. Wu Z-C, Xiao X, Chou K-C. A fractal dimension and wavelet transform based method for protein sequence similarity analysis. IEEE/ACM Trans Comput Biol Bioinf. 2015;12(2):348–59. https://doi.org/10.1109/TCBB.2014.2363480.
et al. BMC Bioinformatics          (2022) 23:347

52. Al-Nuaimi, A.H., Jammeh, E., Sun, L., Ifeachor, E.: Higuchi fractal dimension of the electroencephalogram as a
51. Higuchi T. Approach to an irregular time series on the basis of the fractal theory. Phys D: Nonlinear Phenomena.
50. Robert S, Fractals C. Power Laws: Minutes from an Infinite Paradise. New York: NY, Dover; 2012.
49. Fernández-Martínez M, Sánchez-Granero M. Fractal dimension for fractal structures. Topology Appli.
48. Mandelbrot, B.B., Mandelbrot, B.B.: The fractal geometry of nature
46. Lichtblau D. Alignment-free genomic sequence comparison using fcgr and signal processing. BMC Bioinf.
45. Pei S, Dong R, He RL, Yau SS-T. Large-scale genome comparison based on cumulative fourier power and phase
44. Yin C, Yau SS-T. A coevolution analysis for identifying protein-protein interactions by fourier transform. PLoS One.
43. Hoang T, Yin C, Zheng H, Yu C, He RL, Yau SS-T. A new method to cluster DNA sequences using Fourier power spec-
42. Yin C, Yau SS-T. An improved model for whole genome phylogenetic analysis by fourier transform. J Theor Biol.
41. Xia X, Li W-H. What amino acid properties affect protein evolution? J Mol Evol. 1998;47(5):557–64.
40. Rat. https://www.britannica.com/animal/rat. Accessed 1 May 2022.
39. Saw AK, Tripathy BC, Nandi S. Alignment-free similarity analysis for protein sequences based on fuzzy integral. Sci
38. Human Being. https://www.britannica.com/topic/human-being. Accessed 1 May 2022.
37. Protein Database. https://www.ncbi.nlm.nih.gov/protein. Accessed 16 Jan 2022.
36. Sokal RR. A statistical method for evaluating systematic relationships. Univ Kansas Sci Bull. 1958;38:1409–38.
35. Das JK, Sengupta A, Choudhury PP, Roy S. Mapping sequence to feature vector using numerical representation of
codons targeted to amino acids for alignment-free sequence analysis. Gene. 2021;766:145096.
34. Manikandakumar K, Gokulraj K, Muthukumaran S, Srikan R. Graphical representation of protein sequences by cgr:
33. Manikandakumar K, Gokulraj K, Muthukumaran S, Srikan R. Graphical representation of protein sequences by cgr:
32. Jing X, Dong Q, Hong D, Lu R. Amino acid encoding methods for protein sequences: A comprehensive review and
31. Jing X, Dong Q, Hong D, Lu R. Amino acid encoding methods for protein sequences: A comprehensive review and
30. Song, L., Wu, S., Tsang, A.: Phylogenetic analysis of protein family, 267–275 (2018)
29. Hu H, Li Z, Dong H, Zhou T. Graphical representation and similarity analysis of protein sequences based on fractal
28. Hu H, Li Z, Dong H, Zhou T. Graphical representation and similarity analysis of protein sequences based on fractal
27. Chen Z-H, You Z-H, Li L-P, Wang Y-B, Wong L, Yi H-C. Prediction of self-interacting proteins from protein sequence
26. Xia X, Li W-H. What amino acid properties affect protein evolution? J Mol Evol. 1998;31(2):277–83.
25. Manikandakumar K, Gokulraj K, Muthukumaran S, Srikan R. Graphical representation of protein sequences by cgr:
24. Hou W, Pan Q, Peng Q, He M. A new method to analyze protein sequence similarity using dynamic time warping.
23. Liu J-F, Qu A, Tang H-C, Wang F-H, Wang C-L, Wang H-M, Wang J-H, Zhu H-Q. A novel numerical model for protein
22. Yu J-F, Qu A, Tang H-C, Wang F-H, Wang C-L, Wang H-M, Wang J-H, Zhu H-Q. A novel numerical model for protein
21. Sun L, Deng C, et al. A novel descriptor of protein sequences and its application. J Theor Biol. 2014;347:109–17.
20. He P-A, Xu S, Dai Q, Yao Y. A generalization of cgr representation for analyzing and comparing protein sequences. Int
19. Li C, Liu Y. Numerical characterization of protein sequences based on the generalized chuous pseudo amino acid
18. He P-A, Xu S, Dai Q, Yao Y. A novel descriptor of protein sequences and its application. J Theor Biol. 2014;347:109–17.
17. Yao Y, Yan S, Han J, Dai Q. He P. A novel descriptor of protein sequences and its application. J Theor Biol. 2014;347:109–17.
53. Shamsi E, Ahmadi-Pajouh MA, Ala TS. Higuchi fractal dimension: an efficient approach to detection of brain entrain-
ment to theta binaural beats. Biomed Signal Process Control. 2021;68: 102580.
54. Spasic S, Kesic S, Kalauzi A, Saponjic J. Different anesthesia in rat induces distinct inter-structure brain dynamic
detected by higuchi fractal dimension. Fractals. 2011;19(01):113–23.
55. Doyle TL, Dugan EL, Humphries B, Newton RJ. Discriminating between elderly and young using a fractal dimension
analysis of centre of pressure. Int J Med Sci. 2004;1(1):11.
56. Hame BP. Higuchi fractal dimension analysis of EEG signal before and after om chanting to observe overall effect on
brain. Int J Elect Comput Eng. 2014;4(4):585.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.