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Full Length Research Paper

Analyzing influence on the conformation of single-chain antibody with the differential length of linkers

Jianhua Zhang * and Zetao Chen

Faculties of Biomedical Engineering of Zhengzhou University, Zhengzhou 450001, Henan Province, China.

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To analyze the differential length of linkers of peptides on the conformation of single-chain antibody, and seek the length of linkers with which the conformation is the most similar to the wild type antibody. The similarity between the wild type antibody model and the single-chain antibody model was calculated by the comparison method of spherical shell layer; after comparing with conclusions of other algorithms, the length of the linker (connecting peptide) with which does little influence on the conformation of heavy chain variable (VH) and light chain variable (VL) of single-chain antibody was obtained. The similarity value was got when the number of layers range from 2 to 2000 and the length of the linker range from 0 to 9 by the method of layered spherical coordinate-based algorithm for similarity, and the average values were 0.84974, 0.84101, 0.84970, 0.84532, 0.84285, 0.84235, 0.84077, 0.83857, 0.84237 and 0.83905 when n is equal to 0, 1, 2, 3, 4, 5, 6, 7, 8, 9. The VH and VL of the single-chain antibody gain little influence on the conformation when n = 0 (n is even) and n = 3 (n is odd). When the length of G4S linker (n value) is 3, the influence on the conformation of single-chain antibody is least.

Key word: Single-chain antibody, spherical coordinates, similarity, conformation and linker.

INTRODUCTION

Single-chain antibody is a new genetically engineered antibody which is studied most in recent years. The size of the molecule is reduced into only the antigen binding part. The molecule is made by heavy chain variable (VH) and light chain variable (VL) (Krauss et al., 2004); it is a minimum functional molecule which has high affinity to specific antigen (Min and Qunsheng, 2005). Between the VH and the VL of the antibody genes, there is a period of peptide gene about 25 amino acids or some other linkers with which linked the VH and VL into ScFv gene (Shibata et al., 2006; Shunji et al., 1985).

Its expression product composed by single peptide chain can be folded into small molecule antibodies which have the capacity of antigen-binding (Bird et al., 1988; Huston et al., 1988; Huston et al., 1991).

Figure 1 shows a brief structure of single-chain antibody (ScFv). There are many advantages that the single-chain antibody (ScFv) has, such as (1) it can remove the competitive surface protein of the nonspecific reaction, make the background tumor imaging clearly (Karine et al., 1997), (2) the molecule is small and easy to go through the blood vessel wall, penetrating into the tumor tissue easily to increase the concentration of drug treatment (Peter and James, 1996), (3) little immunogenicity, the human anti-mouse's rejection can be eliminated, (4) circulating half-life is short *in vivo*, easy to clean, easy for detoxification and effluvium (Sabine and Andreas, 1997) and (5) easy to connect with the gene of enzyme or toxin, to facilitate direct access to immune toxins or ELISA antibodies, etc (Mallender and Voss 1994). The disadvantages of single-chain antibody mainly include the following three points: (1) less stable, (2) single function and (3) lower affinity.

The antibody is linked by the most widely used flexible peptide G4S. The conformation of antibody is greatly impacted by the length of linkers (Ramesh et al., 1992). The best VH and VL conformation of the single-chain

*Corresponding author. E-mail: zetao_chen@yahoo.com. Tel: 86 158 9067 7530.

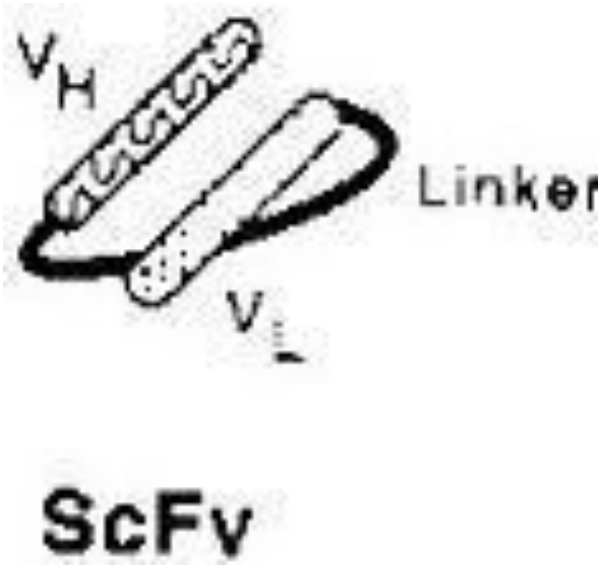

Figure 1. A brief structure of single-chain antibody.

antibody can be achieved with an appropriate length of connecting peptide (linker) (Meike et al., 2006). Thus the single-chain antibody is more suitable for biomedical applications. This paper aims to study the influence of the linker's length of antibody to its structure by a comparison method of similarity (Fang et al., 2009). Figure 2 shows the three-dimensional structure of VH and VL of wild-type model.

METHODS

Modeling

This article defines the atoms of single-chain antibody as single points linked by sorted lines from the Protein Data Bank files (Rodrigo et al., 2008). Every atom was pointed into the spatial spherical polar coordinate by its location from the real structure. Then the whole molecule can be seen as a set of points in the spatial spherical polar coordinate that composed by atoms of it. The study of spatial structure of single-chain antibody can be simulated by studying the structure of this spatial spherical model.

Coordinate and algorithm

There are several ways to study the similarity of protein structure, such as protein structure comparison by sort matrix alignment (Haiyang et al., 2008), comparing 3-D protein structures similarity by using geometry invariants (Shang, 2007), using dihedral angle series of protein for protein structure comparison (Jianzhao et al., 2009), a method for measuring protein structure similarity based on the molecular inner spatial density distribution (Min and Qunsheng, 2005), similarity comparison of protein structures via protein space partition in spherical polar coordinates (Beiji et al., 2009) etc. These methods analyze the protein structure by certain means, and get the degree of similarity of each protein by mathematical algorithm. All methods have certain similarity in essence, namely, the spatial dissection of the structure of protein molecules. The similarity defined by this article is the similarity of the protein structure. The correlation of vectors is gotten by approximate processing of cosine of vector angle formed by the atoms in each spatial layer of the single-chain antibody derived factor, thus, the similarity of two molecules would be determined more accurately.

Compared with the European method of the direct division of coordinate, the advantage of the computation the similarity in single-chain antibodies through the similarity computed by the comparison method of spherical shell layer is that it gives the distance of each atom to the spherical centre in protein molecule. It's easy to layer or divide by using these distances (Huihao et al., 2011). In addition, the even division of spherical coordinate can facilitate the proceeding of structure similarity comparison.

The comparison method of spherical shell layer

The comparison method of spherical shell layer regards the spatial spherical coordinate as being composed by layers of spherical shell with mean thickness of each layer. So different molecules are identically divided under the same coordinate system, and spatial angle does no influence on spatial distributing of every atom. This method can give a more accurate degree of similarity between two molecules.

Algorithm description

(1) Transform the European coordinates into spherical polar coordinates. As molecular structure data provided by the PDB (Protein Data Bank) file are the data of the European coordinates of the atoms spatial distributing, it is necessary to unify the coordinate system of molecule first, by building a coordinate with the focus of the molecule (coordinates center) as its origin of coordinate. All atoms are positioned in the coordinate via the same transfer vector (ensure the accuracy), and united to the same coordinate system. Then the spatial European coordinates are transformed into spherical polar coordinates via formula

$$r = \sqrt{x^2+y^2+z^2},\ r \in [0,+\infty),\ \varphi = \arctan\left(\frac{y}{x}\right),$$

$$\varphi \in [0,2\pi),\ \text{and}\ \theta = \arccos\left(\frac{z}{r}\right),\ \theta \in [0,\pi].$$

The coordinate transformed have the same origin of coordinate with the original coordinate.

(2) Stratify the spherical polar coordinate of the derived factors molecule. Divided each layer by the radius component of the spherical polar coordinates, the shape of each stratification can be regarded as spherical shell with the origin of coordinate for the centre of the sphere, and equidistantly divided into a predetermined number of layers based on D-value of the maximum radius and minimum radius. In this way, the corresponding parallel to the direction of the coordinate system and the unity of division are guaranteed.

(3) The number of the same atom in each spatial layer is calculated separately, with which vector a_i and b_i are built. According to the stratification situation of each derived factor to be compared, the number of the same atom (mainly C, N and O) in different spatial layers is counted, and the summation of the number of certain atom is computed with predetermined weight, final value of the number of atom in each layer is got. Multi-dimensional vector a_i and b_i are

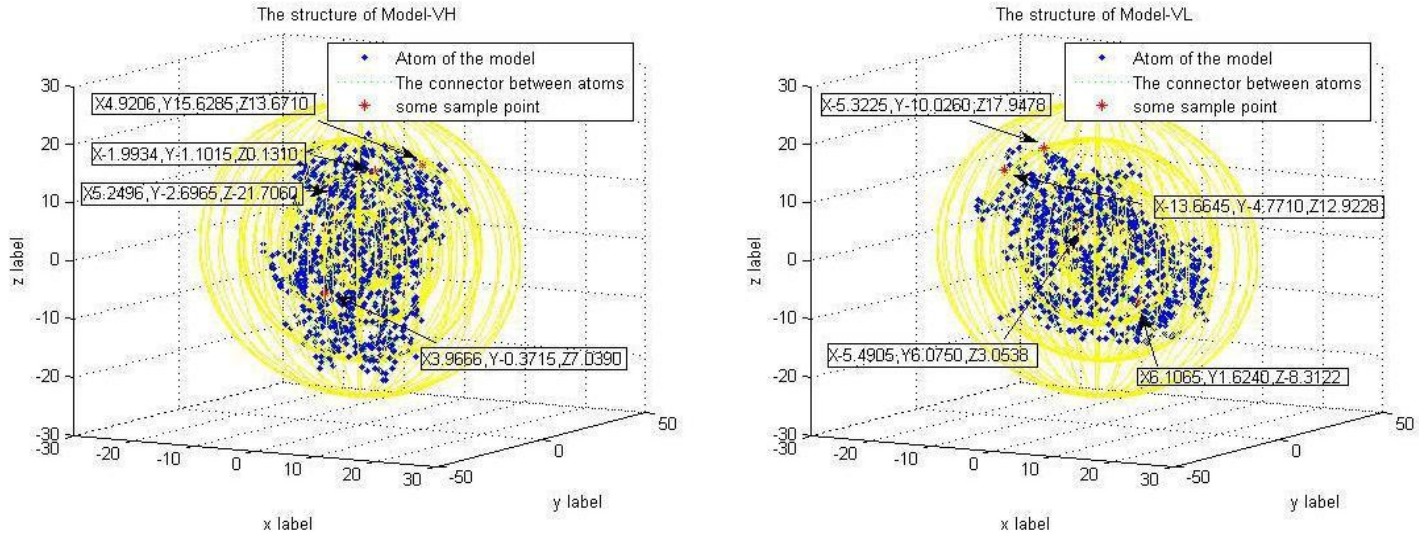

Figure 2. The three-dimensional structure of VH and VL of wild-type model.

built with atom data in order of spatial layers divided. (4) The similarity of each derived factor molecule to be compared. This paper selects the separation angle cosine as approximate function which is in common use of approximate functions, namely:

$$\cos\theta_{a,b} = \frac{\sum_{i=1}^{n} a_i b_i}{\sqrt{\sum_{i=1}^{n} a_i^2 \sum_{i=1}^{n} b_i^2}}$$

Using the vector computed in the third step to the formula above, the similarity value focused on the atom that composed by the weight can be gotten. Then we compute the weighted mean of the similarity value of each atom; finally, the similarity value of each derived factor molecule to be compared is figured out.

The comparison method of spatial partitioning

The comparison method of spatial partitioning is similar to the comparison method of spherical shell layer; the only difference between them is that the comparison method of spatial partitioning also has segments in horizontal and vertical angles component, forming a spatial partitioning dividing like spherical shell debris.

Algorithm description

(1) This algorithm step is the same with that described in the comparison method of spherical shell layer.

(2) Block the derived factor molecule to be compared spatially. The D-value of maximum value and minimum value is divided into m portions based on radius component and the two separation angle component, forming a set of spatial blocks with original point as the centre of sphere, it is presented that the number of portions is m^3 in total.

(3) The number of the same atom in each spatial portion is calculated and a vector is built. According to the portion status of each derived factor, the number of the same atom (mainly C, N and O) is counted in different spatial portions, and the summation of the number of certain atom with predetermined weight is computed, then final value of the number of atom in each portion is got. Multi-dimensional vector a_i and b_i is built with atom data in order of spatial portions blocked.

(4) This algorithm step is the same with that described in the comparison method of spherical shell layer.

RESULTS AND DISCUSSION

Analysis of algorithm

Spatial structure of proteins have uncertainty of rotation in the coordinate system, it is possible that the long axis of different molecules are not in the same longitudinal axis. When conducting the similarity comparison method of spatial partitioning, very certain spatial location is necessary for each spatial block, namely, the similarity comparison method of spatial partitioning has a high degree in precision, but the application here can't ensure its accuracy, then it has effects on the overall fidelity (including precision and accuracy). The comparison method of spherical shell layer did not consider the division on the rotational direction, that is to say, the longitudinal axis of the molecule is not required for this algorithm, therefore, it can avoid the deviation that caused by the different direction of protein molecule in the coordinate system effectively, and the influence caused by the drawback of precision on the fidelity is lower than the comparison method of spatial partitioning does. So the comparison method of spherical shell layer is the ideal choice of this article, expressing the degree of similarity between two single-chain antibody (ScFv) molecules exactly.

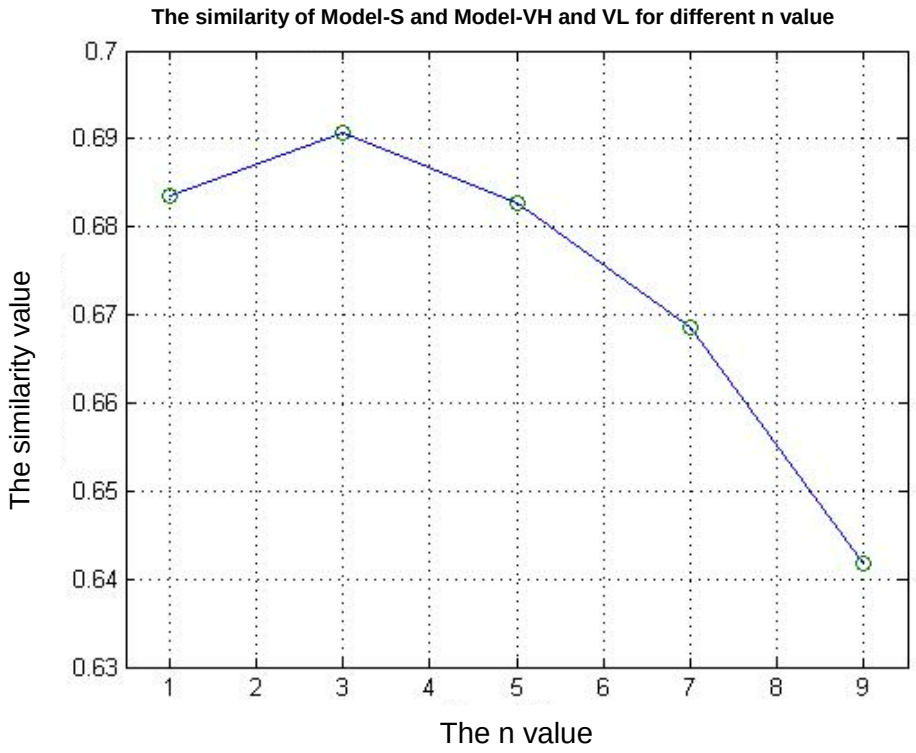

Figure 3. A similarity trend chart which has validated by experiment when n = 1, 3, 5, 7, 9.

Analysis of the results

According to the provided structural characteristics of the single-chain antibody model, the single-chain antibody molecule is divided into two parts from the position of the linker: the heavy chain variable region (VH) and the light chain variable region (VL); the similarity of corresponding portion of the target antibody (wild-type antibody) with the VH and VL is computed, and we define the mean value of these two similarity values which computed above as average similarity. The average value of similarity indicates the influence on the conformation of single-chain antibody with differential length of linker. The bigger the similarity value is, the smaller the influence of the length of linker does on the conformation of the protein.

In early studies, we have tested and verified that when the length of linkers is 3 it shows the most similar conformation of single-chain antibody to wild-type antibody model when the length of linkers is 1, 3, 5, 7, 9 by biological experiments. The data has great deviation can be excluded from results of the comparison method of spherical shell layer by the conclusion of the experiments (Huihao et al., 2011; Huston et al., 1988).

The data of great deviation include results of layers or portions with less reliability. The algorithm of this article shows that the longer length of linker of single-chain antibody, the higher degree of influence on the conformation of VH and VL. It is the most undesirable structure when the length of linker is 8 or 9. Figure 3 is a similarity trend chart which has validated by experiment when n = 1, 3, 5, 7, 9°C

On the process of similarity comparison of single-chain antibody, the similarity of single-chain antibodies is computed via the comparison method of spatial spherical shell layer based on spherical polar coordinate of the method above. Table 1 shows some selected results of the algorithm. The first column data of Table 1 represent the number of layers, and the length of linker for the first line.

It can be seen from data in the table that the tendency of similarity is uncertain when the length of linker is even and 3 is the best length of linker for having a high degree similarity on the conformation of single-chain antibody when the length of linker is odd.

When the length of linker is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, the mean value of similarity computed via the comparison method of spatial spherical shell layer is 0.8497, 0.8410, 0.8497, 0.8453, 0.8428, 0.8424, 0.8408, 0.8386, 0.8424, and 0.8390. Tendency charts in the rectangular coordinate system are showed in Figures 4 and 5. Figure 4 shows changes of similarity with differential length of linker when the number of layers is 4 while Figure 5 shows the relationship between the average similarity of differential number of layers and the length of linkers.

Table 2 shows similarities of differential length of linker computed via comparison method of spatial partitioning.

Table 1. Line for the length of linker, arrow for the number of layers that separated into.

	0	1	2	3	4	5	6	7	8	9
4	0.9980	0.9976	0.9986	0.9987	0.9977	0.9983	0.9984	0.9981	0.9973	0.9980
6	0.9965	0.9959	0.9973	0.9982	0.9975	0.9977	0.9976	0.9977	0.9959	0.9960
9	0.9954	0.9959	0.9973	0.9974	0.9956	0.9967	0.9961	0.9957	0.9966	0.9931
15	0.9899	0.9893	0.9911	0.9945	0.9922	0.9920	0.9941	0.9908	0.9918	0.9917
20	0.9898	0.9865	0.9891	0.9922	0.9897	0.9888	0.9943	0.9901	0.9894	0.9905
30	0.9832	0.9680	0.9778	0.9820	0.9746	0.9750	0.9788	0.9741	0.9708	0.9772

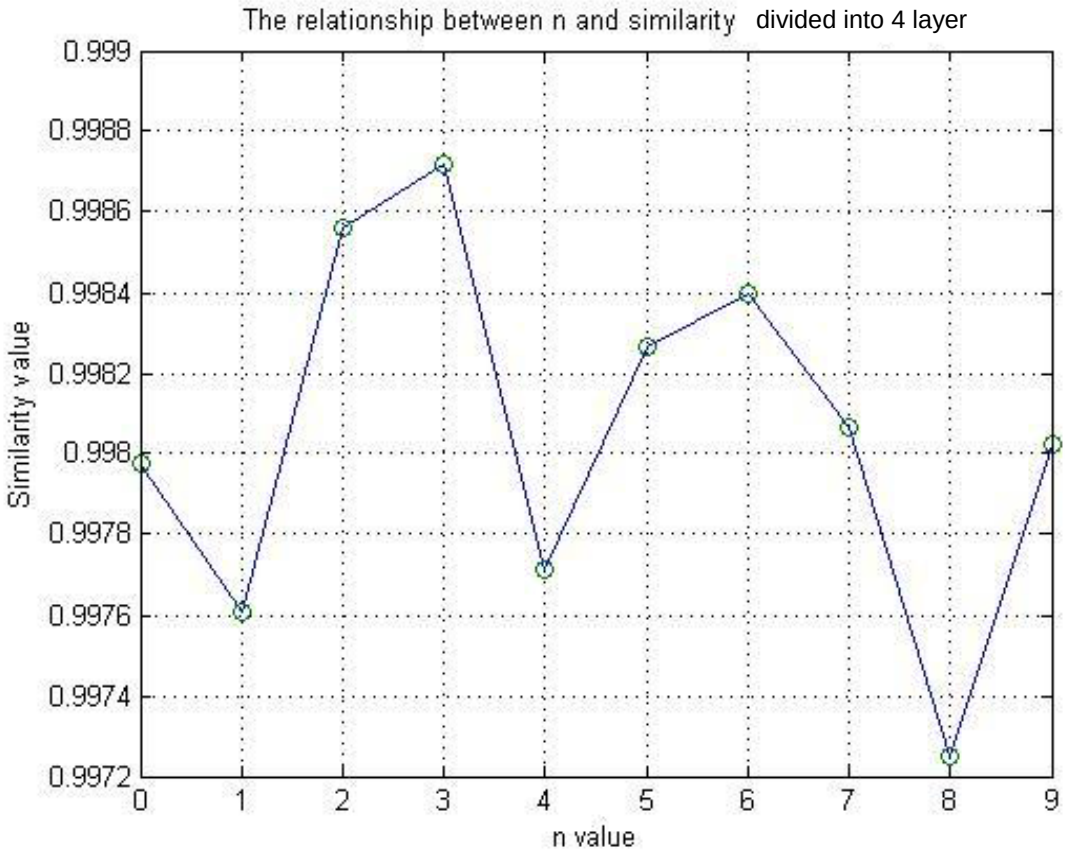

Figure 4. The relationship between the average similarity of differential number of layers and the length of linkers.

The first column of the Table indicates the number of portions and the first line for the length of linker.

When the length of linker is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, the similarity of single-chain antibody computed via the comparison method of spatial partitioning is 0.3616, 0.3645, 0.3635, 0.3608, 0.3660, 0.3576, 0.3576, 0.3649, 0.3662, and 0.3651. Tendency chart in the rectangular coordinate system is shown in Figure 6. This figure shows the trend of similarities for differential length of linker when the number of portions is 18^3. Figure 7 shows the relationship between average similarity of differential number of portions and the length of linkers.

From Figures 6 and 7 and the data of Table 2, it can be seen that the value of similarity computed via the comparison method of spatial partitioning is not ideal, for this algorithm requires a very certain structure of protein, and the direction angle need to be calculated. Therefore, there are some more advantages of the comparison method of spherical shell layer than the comparison method of spatial partitioning. When the length of linker is 3, the conformation of single-chain antibody is the most similar to the wild-type antibody.

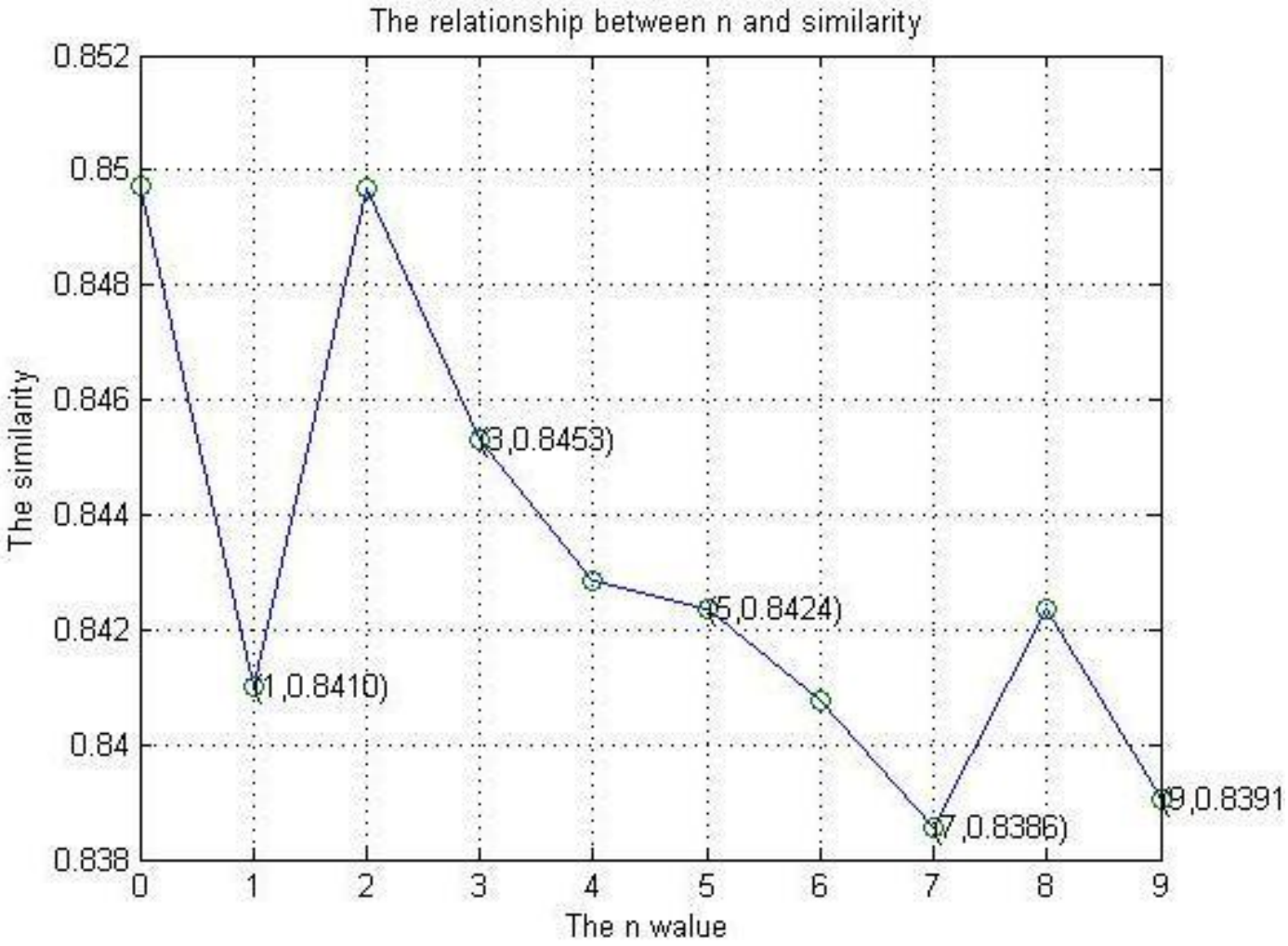

Figure 5. The relationship between the average similarity of differential number of layers and the length of linkers.

Table 2. Line for the length of linker, arrow for the number of parts that divided into.

	0	1	2	3	4	5	6	7	8	9
2^3	0.9487	0.9176	0.9178	0.9202	0.9323	0.9151	0.9234	0.9243	0.9422	0.9216
15^3	0.1567	0.1691	0.1633	0.1697	0.1657	0.1664	0.1729	0.1677	0.1426	0.1712
18^3	0.1094	0.1018	0.1066	0.1166	0.1258	0.1136	0.1091	0.1165	0.1140	0.1119

Conclusion

It is suitable for clinical or biomedical application for analyzing single-chain antibodies with similarity algorithm based on spherical shell layer of spherical polar coordinates. An appropriate number of linkers enable the antibody to be more suitable for the clinical application; the length of linkers have significant influence on the conformation of single-chain antibody, when n = 3, the three-dimensional molecular structure of the single-chain antibody can be considered the most appropriate.

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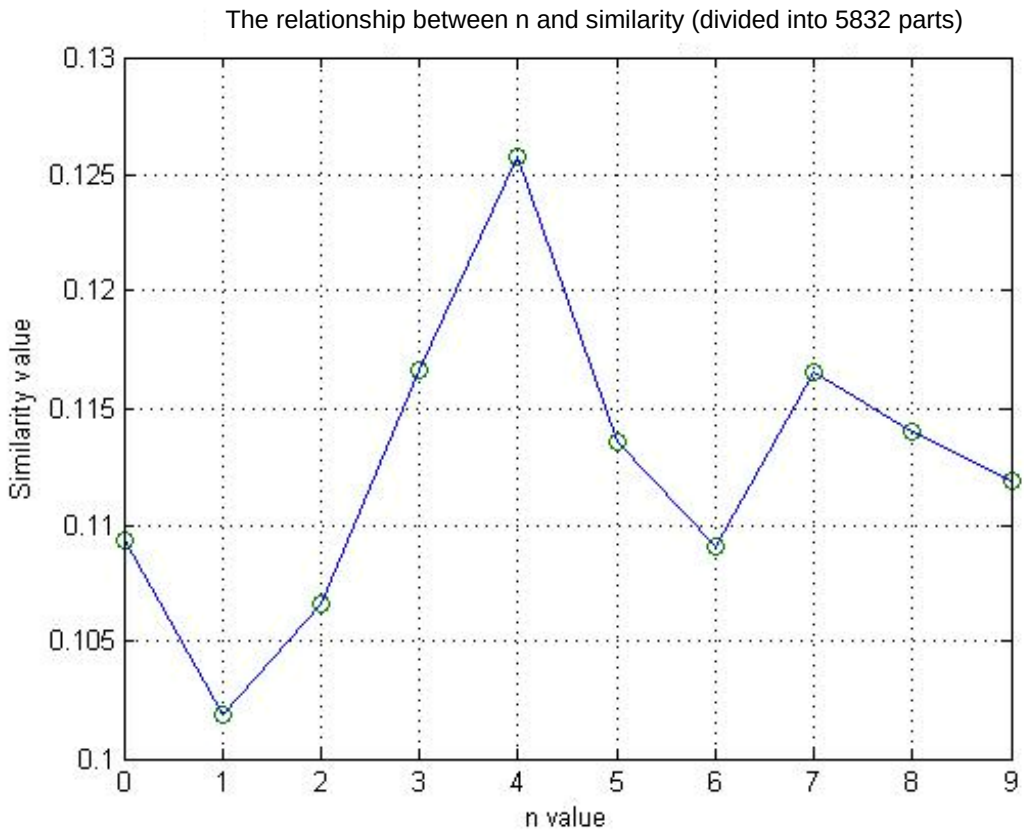

Figure 6. The trend of similarities for differential length of linker when the number of portions is 18^3.

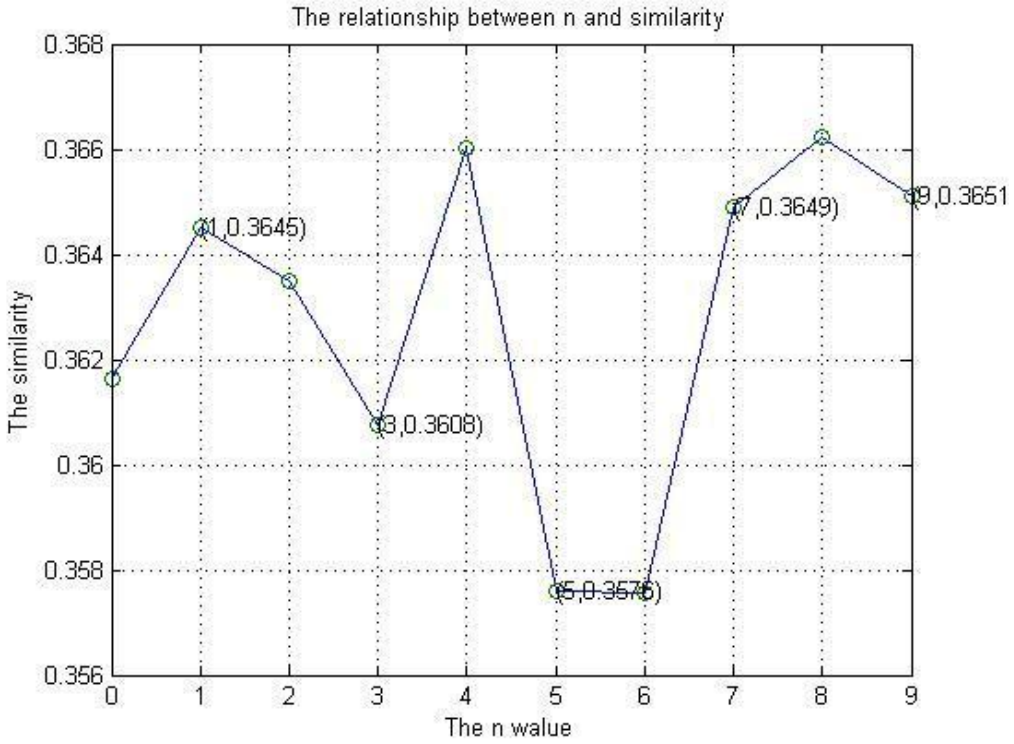

Figure 7. The relationship between average similarity of differential number of portions and the length of linkers.

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