

# FINDING PROTEIN FAMILY SIMILARITIES IN REAL TIME THROUGH MULTIPLE 3D AND 2D REPRESENTATIONS, INDEXING AND EXHAUSTIVE SEARCHING

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**Abstract:** Research suggests that the complex geometric shapes of amino-acid sequence folds often determine their functions. In order to aid domain experts to classify new protein structures, and to be able to identify the functions of such new discoveries, accurate shape-related algorithms for locating similar protein structures are thus needed. To this end, we present our Content-based Analysis of Protein Structure for Retrieval and Indexing system, which locates protein families, and identifies similarities between families, based on the 2D and 3D signatures of protein structures. Our approach is novel in that we utilize five different representations, using a query by prototype approach. These diverse representations provide us with the ability to view a particular protein structure, and the family it belongs to, focusing on (1) the C- $\alpha$  chain, (2) the atomic position, (3) the secondary structure, based on (4) residue type or (5) residue name. Our experimental results indicate that our method is able to accurately locate protein families, when evaluated against the 53.000 entries located within the Protein Data Bank performing an exhaustive search in less than a fraction of a second.

## 1 INTRODUCTION

Currently, there are more than 53.000 protein structures contained in the Protein Data Bank, the primary repository for experimentally determined 3D protein structures, with the number of structures being added growing exponentially (Ber et al. 00, 08). This is due, mainly, to the advent of high throughput systems. This current explosion of the number of known 3D protein structures, created by x-ray crystallography, theoretical prediction or NMR techniques, brings an urgent need for fast, accurate approaches to find protein structure families, to identify similarities between families, to locate outliers or structural surprises, and to determine possible mutations.

In recent years, a number of researchers have investigated finding similar 3D protein structures, mainly using structure alignment (YCO05, AKW06, CS04). Research includes the work of (PPP05,

ONI04, CSS04, Hua et al. 06), who use local approaches to calculate the similarities of protein structures, thus possibly accumulating error and potentially overlooking semantic information about the interrelationships of the structures. Other work includes shape-based approaches such as (AKW06, Dar et al. 06, YKY08, CZ08, Abe et al. 08, ZB08) which typically employ a sphere, grid, pie or spherical trace transform to compare structures.

This paper described a system for protein structure description and retrieval. Our system, in contrast to other approaches, utilizes a number of different representations to create both 3D and 2D signatures of the protein structures. Furthermore, our method is scale, rotation and translation invariant and eliminates the need for prior structure alignment. It employs the global 3D shape as well as the 2D colour, texture and composition of a protein structure, thus avoiding accumulating possible error. By using the different representations, our system is able to provide the domain expert with diverse

representations of the same protein structure and its family members. Also, our system is able to identify related protein structures in between families. An advantage of our algorithms, when compared to that of others, is that they are very fast. Our system searches for similar protein structures, against 53.000 proteins, in less than a second while performing an exhaustive search.

This paper is organized as follows. Section 2 describes the different representations, the algorithms for finding the 3D and 2D signatures and well as our similarity search method. This is followed, in Section 3, with our experimental results, when applying the system to seven diverse families within the Protein Data Bank.

## 2 METHODS

This section discusses the approach we employ to locate protein families within the Protein Data Bank. Firstly, a number of depictions are used in order to create diverse representations of each protein structure. Secondly, for each of the representations, 3D and 2D signatures are calculated offline (i.e. during preprocessing), and placed in a database. Lastly, a Euclidian distance-based similarity search algorithm is used to locate the family to which a query protein structure belongs.

### 2.1 Representing the Protein Structures

We first focus our attention on the 3D representations employed. Figure 1 illustrates the 1by5 protein structure from the ligand-gated protein channel family, when employing the various representations. The first representation, as shown in Figure 1 (left), is the so-called tube representation, where the protein is represented using a smooth cylindrical tube through the C- $\alpha$  atoms. This representation is useful for the following reason. The C- $\alpha$  chain is the backbone of the protein. There might be more than one, if the protein is made up of many chains. As such, it is a fundamental representation which is also visually very intuitive. Its fundamental character comes from the fact that the folding of the chain is determined by the sequence of amino-acids (and of course the milieu in which the protein is located) which are attached to the chain; the so-called residues. Secondly, the van der Waal representation is depicted in Figure 1 (right), which associates a van der Waal spheres to each atom, without displaying the chemical bounds. This

“low level” representation allows us to show the atoms and the range of their van der Waal interaction; without assuming any relationship between them. The position of the constituent atoms corresponds to the centre of the van der Waal sphere and the range of the van der Waal interaction corresponds to its radius. Such a representation is not as visually intuitive as the tube, but it does not require as much “understanding” of the data, because no structure labelling is involved.

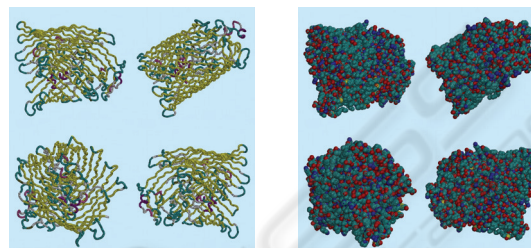


Figure 1: The 3D tube and Van der Waal Representations.

We depict the 2D representations in Figure 2, again showing the 1by5 protein structure. Let us first consider the 2D representations associated with the tube representation. In the first representation, as shown in Figure 2 (top-left), the different secondary structures are depicted by one of seven colours. For example, the  $\alpha$ -helix is purple, the 3-10 helix is mauve and the extended  $\beta$  is yellow. This representation was chosen, due to the fundamental importance of the secondary structures in structural proteomics in order to establish their classification.

The second representation, in Figure 2 (top-right), encodes the amino acid (residue) name, using 20 different colours to distinguish between them. Here, for example, the ALA is encoded in blue, the LEU in pink and the HSD in cyan. Not only are the main chains displayed, but also the topological relation in between the amino-acids. Proteins which have similar amino-acid sequences have, in general, a similar shape. Proteins with relatively different amino-acid sequences might also have similar shape. This is usually the case when a protein has a specific functionality. For example, consider the Haemoglobin protein structures for different species. Here, the shape is determined by the fact that Haemoglobin must carry oxygen. In this case, one may want to explore shapes having very similar amino-acid content, as indicated by this representation.

The representation shown in Figure 2 (bottom-left) denotes the residue type, which determines the interactions with its surrounding. For example, a solvent is coded in yellow, an acid in red, a polar in

green and an ion in tan. This representation is especially useful for drug design. For instance, consider the situation where two distinct amino acid sequences might have the same type of interaction, where one combination is toxic while the other is not. The residue type representation is more adapted to this particular problem, since the coding is related to the function of the residues, not their nature “per se”.

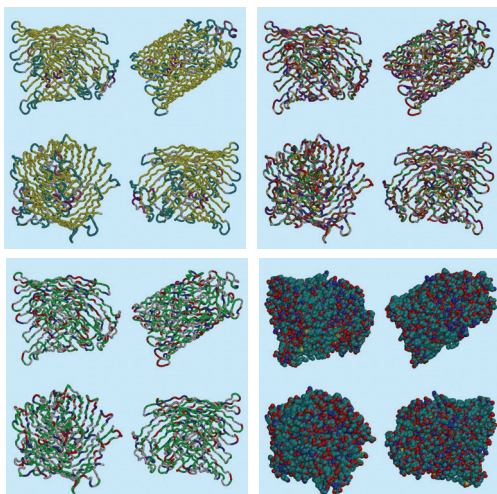


Figure 2: The four 2D Representations.

We also show, in Figure 2 (bottom-right), the 2D representation when considering the van der Waal representation, where the colour corresponds to the atom names. In this case, a colour is associated with each atom. That allows labelling the atomic content of the protein. Note that the discrimination of this representation is poor, since a small set of atoms tend to appear repeatedly in the structure, which then tends to form a repetitive pattern.

## 2.2 Creating the 3D Protein Structure Index

This section describes the algorithm to create the 3D signatures of protein structures. Our algorithm creates a protein structure signature that is translation, scale and rotation invariant, in order to facilitate for the protein’s arbitrary location and pose in space. It is important to understand that, in our algorithm, the proteins are not aligned relative to one another. Instead, the most natural orientation, in terms of its spatial distribution, is found through the tensor of inertial. This reduces processing time significantly, and increases discrimination. The algorithm describes a protein either as a whole, the so-called global approach, or focuses on a subpart of

its structure, the so-called local approach. Only the first approach is considered in the present paper. In order to apply the local approach, it suffices to divide the protein into smaller substructures as required by a given application.

Our algorithm proceeds as follows [PV07, PV08]. Firstly, the protein is triangulated into a mesh, the centre of mass of the object is computed and the coordinates of the vertices are normalized. The tension of inertia is subsequently calculated and, in order to achieve rotation invariance, the Eigen vectors are determined, resulting in a 3x3 matrix. The normalized signature is based on the concept of a cord, which refers to a vector that originates from the barycentre of the protein and terminates on a given barycentre of a triangle (assuming a triangular mesh representation of the protein surface). The statistical distribution of this cord is represented in terms of three histograms, to depict the radial and angular distributions thereof. These three histograms thus present the shape signature of the corresponding protein structure, which is placed in a database for future querying [PV07, PV08].

## 2.3 Creating the 2D Protein Structure Signature

This section describes the algorithm which produces the 2D protein structure signatures. Our motivation for employing 2D as well as 3D signatures is as follows. While the 3D signatures are based on shape, it does not take the chemical substructures of the protein into account. Analysing chemical substructures are of especial importance when, for example, analyzing the docking of two proteins in which the interaction of the related amino-acids plays a key role. In our system, this is achieved by viewing the structures as 2D images and then by attributing a colour code to each chemical structure. Here, a feature-based image signature, based on only four (4) views of the protein structure (as opposed to typically hundred in most current approaches) [YCO05], is created using the colour, texture and interrelationship between components (the so-called composition). The patterns created by the substructures are associated with the texture, while the local spatial organization is associated with the composition. The advantages of describing the 3D shape by only four views, as opposed to many, are twofold. Firstly, the calculation of the descriptor is much faster since it involves less views and the corresponding index is more compact since less views are described, which allows faster searching and retrieval. Secondly, because the description



associated with each view represents the information thereof more efficiently (this is why less views are needed), it is possible to describe and retrieve, efficiently, sub-regions and to search for local features.

Our 2D protein structure signature creation algorithm employs a triangular mesh representation and principal component analysis (PCA) is used to obtain a reference frame that is translation and orientation invariant, from which four 2D views are obtained. Next, the Sobol sequence is used to sample image points with a window in a quasi-random manner. For each point, two bi-dimensional histograms are computed based on hue, saturation and the relative proportion thereof. These histograms are accumulated for each point of the sequence. The displacement of the window over the image ensures that not only global information about the later is accumulated, but structural information is extracted as well. Finally, the two histograms are converted into a signature (or index), which provides us with an abstract description of the composition of the 2D protein structure image [PV07, PV08].

## 2.4 Measuring Signature Similarity

The resultant 3D and 2D signatures for the six representations as described Section 2.1, which have been calculated offline, is subsequently placed in a database. This database thus contains 14 different sets of signatures (or tables), for the 53.000 member Protein Data Bank.

The next step involves finding the family to which a query protein structures belongs. To this end, a similarity search algorithm is used, in order to find the structures which are the most similar, for each representation. For example, let us assume that we want to calculate the similarity of all proteins in  $PB_r$ , i.e. all proteins presented using representation “r” against a query protein  $Pq_1$ . We calculate the similarity measure between  $Pq_1$  and each other protein structure in  $PB_r$ . This distance is calculated using the Euclidian metric. With our present system, an exhaustive search, for a given representation, is performed and all 53.000 protein structures are ranked in a fraction of a second.

## 3 RESULTS

This section describes our experimental results when evaluating our system against the 53.000 proteins structures, as contained in the Protein Data Bank (PDB) [Ber et al. 00]. We verified our finding against the SCOP (Structural Classification of

Proteins) system, which describes the structural relationships of proteins of known structure (And et al. 08). In the SCOP classification system, proteins are grouped into families, based on experts' experience (Dar et al. 06). More specifically, proteins are classified into (from large to small) folds, super-families and families. Our aim is to find the family that a structure belongs to, and those family members that it is closest too. Furthermore, we wish to explore the “usefulness” of the various representations, and to distinguish between the applicability of the 3D versus 2D representations. Our experiments were conducted using workstations with two Xeon™ 3.4 GHz CPUs and 2.8 GB of RAM and our system was implemented using Java/Java3D.

Table 1: Protein Families used in Experiments.

Family	Members	Species	Query	Class
L-aspartase/fumara se	10	Anas platyrhynchos (Domestic duck)	1k7w	$\alpha$
Pyridoxine 5'-Phosphate synthase	7	Escherichia coli	1ho1	$\alpha + \beta$
Pyridoxal dependent decarboxylase	2	Sus scrofa (Pig)	1js3	$\alpha + \beta$
Bacterial AB5 toxins, B-subunits	3	Bordetella pertussis	1prt	$\beta$
Fluorescent proteins	2	Coral (Discosoma sp.)	1ggx	$\alpha + \beta$
$\beta$ -catenin-interacting ICAT	3	Homo sapiens	1t08	$\alpha$
Ligand-gated protein channel	11	Escherichia coli	1by5	Membrane and cell surface

All the queries were executed in less than a second, using an exhaustive search against all 53.000 of the structures as contained in the Protein Data Bank. Table 1 shows the seven protein families we used in our experiments, together with the query (or seed) structure. These families were chosen so as to illustrate our system against a variety of families in terms of size, species, SCOP classification and structure.

In order to analyze our results, we evaluate the efficiency of the different (3D and 2D) representations, by determining whether the query protein and the closest matches belong to the same family. For illustration purposes, the result of the search for the ligand-gated protein channel family, using the 1by5 structure as seed query and employing the tube representation, is shown in Figure 3. To evaluate our results, we retrieved the most similar protein structures and then calculate the precision and recall of our system. Here, precision is defined as the proportion of the retrieved protein

structures that was been classified (by SCOP) as to belong to the same family as the query and the recall is the proportion of family members in the entire 53.000 member database that are retrieved as a result of the query.

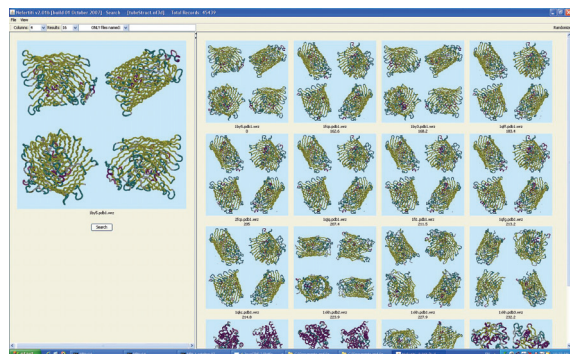


Figure 3: Results when retrieving the ligand-gated protein channel family, using the 1by5 structure as query and the 3D tube representation.

Formally, Precision and Recall are defined as

$$P = \frac{N_{family}}{N_{family} + N_{false}} \quad (1)$$

and

$$R = \frac{N_{family}}{N_{family} + N_{miss}} \quad (2)$$

where  $N_{family}$  refers to the number of relevant family members retrieved,  $N_{false}$  denotes the number of structures retrieved that did not belong to the family; and  $N_{miss}$  refers to the family members that were not retrieved. Figure 4 shows the Precision-Recall curve for all seven protein structure families used in this experiment, when employing the various representations described in Section 2.1. The figure shows that our system is able to locate family members accurately and precisely.

We first discuss the results obtained by the 3D protein structure signature component of our system. For the 3D tube representation, the results indicate that our algorithm was able successfully locate the family members in all cases, when searching a database of 53.000 structures. In the case of the red fluorescent and  $\beta$ -catenin interacting ICAT protein structures, our system also found additional family members which have not yet been classified in the SCOP system. For the l-aspartase/fumarase, our system was able to locate the eight similarly shaped structures, while two other structures (1hy0 and 1u16) were not retrieved due to their distinct shape.

Interestingly, the protein structure of the Western graylag goose (1xwo) is found in position 4, which has a shape very similar to that of the domestic duck. Also, the results show that, with the van der Waal representation, our system provides good results.

Next, we consider the results when using the 2D protein structure signature, considering the four different representations as introduces in Section 2, again against the above-mentioned seven protein families. The results, as shown in Figure 4, indicate that the 2D algorithm is also able to find family members accurately, for the first three representations employed, but with a slightly lower precision and recall than the 3D component. The reason for this lies in the fact that, the 2D representation is more ambiguous than the 3D representation, in the sense that the later is based on the geometrical 3D shape, while the former is based on four 2D projections of the associated 3D shape. Why, then, include the 2D representations? The strength of the 2D representation lies in the fact that it may be employed when the chemical structure is of particular interest or relevance. The colours of protein structures provide us with a semantic key to the functionality thereof: therefore, the 2D image retrieval provides us with a complementary view, in contrast to when we apply a shape-based description. In this way, the results of 2D indexing and similarity search may be utilised in order to refine the results of a 3D query. The 2D approach may be used in a generic way (four standard views) or for analysing the docking view from a chemical perspective.

Our experimental evaluation of the 2D representation of the van der Waal representation yielded inferior results. Our analysis indicates the following reason for this. When using the 2D representation, the inside of the protein is occluded by the outer van der Waal spheres. That is, one is only able to access the outer spheres, which creates very similar patterns in terms of texture. In contrast, for the 3D van der Waal representation, the entire volume of the protein (inner as well as outer regions) is analysed. Therefore, in the case of the 3D van der Waal representation enough discrimination is obtained.

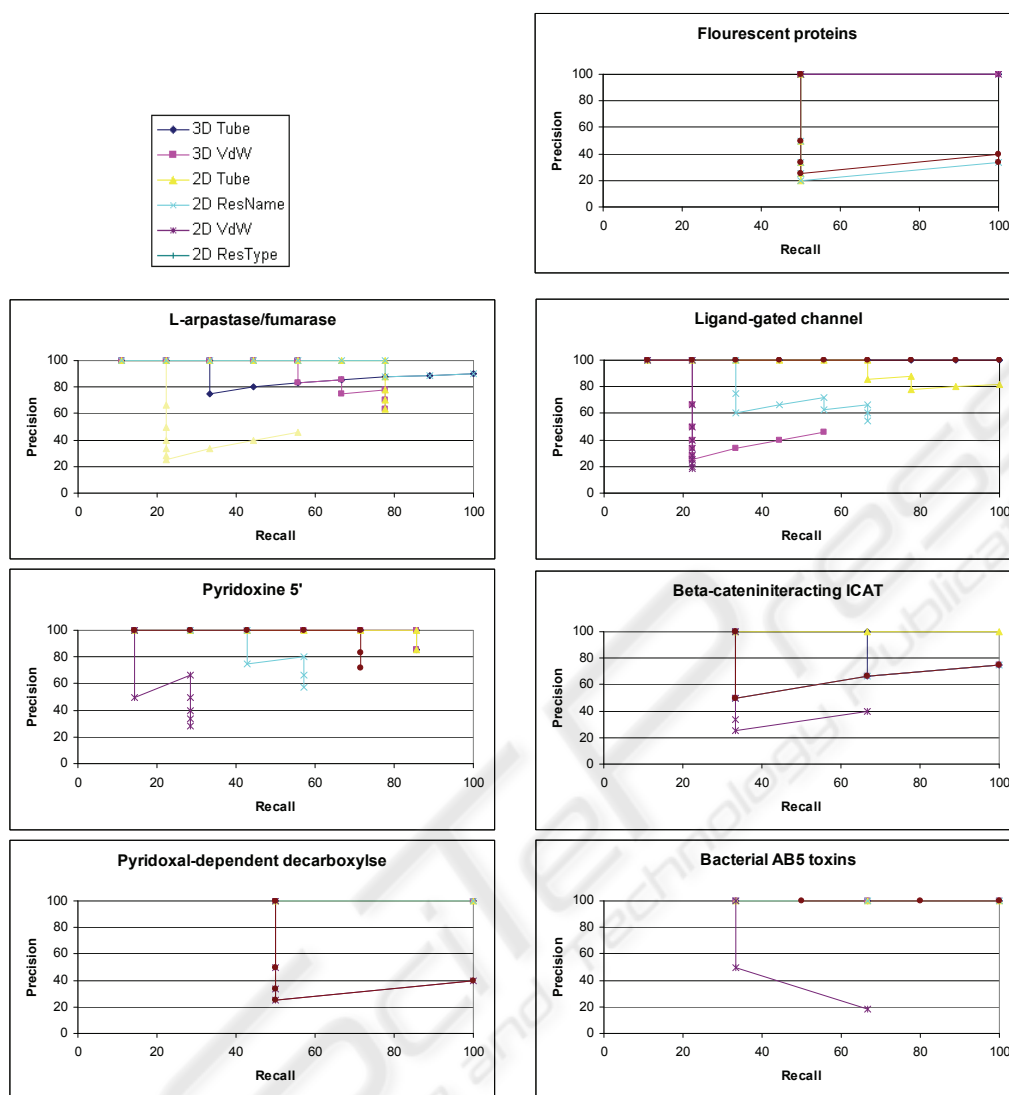


Figure 4: Precision-recall curves for the Seven (7) families with Six (6) Representations.

## 4 CONCLUSIONS

The number of known protein structures is increasing rapidly. A total of 5.000 of the 53.000 proteins currently in the Protein Data Bank have been added in the last year. With the foreseen introduction of fast throughput systems, an explosion in terms of the number of structures is expected. There is an urgent need for systems to aid the domain expert to classify such new structures, and, prior to performing actual synthesis and biological studies, computationally screen candidate structures. The accurate recognition of relevant protein structures is pertinent to unlock this

potentially rich source of information, for applications such as drug design, studying protein-protein interaction, the prediction of protein function, and so on.

This paper presents our system, which employs various 3D and 2D representations, to locate families of protein structures, out of the 53.000 members of the Protein Data Bank. Not only were we able to find most members of the family, but we also found members of other families that are very similar. This implies that, in addition to locating families, we were able to recognize inter-family similarities. The later results may prove itself useful in order to replace a toxic or expensive protein by

another one presenting a similar functionality. Furthermore, it was possible to divide some families into two subgroups. This indicates that, despite the fact that some structures belong to the same family, their appearance are dissimilar enough to be grouped in two distinct sub-families. In addition, an exhaustive search and the ranking of proteins against the entire Protein Data Bank may be performed in under a second, as opposed to the approaches of others, as introduced in Section 1. In these algorithms, the search is either performed using a very small subset, typically a few hundred. Alternatively, these methods employ a non-exhaustive search and rely on heuristic assumptions. In comparison, our method performs a search on the entire Protein Data Bank database without any such a priori assumptions or query size constraints.

In future, we plan to conduct a robust empirical comparative study contrasting our system with other approaches in the field. We are also interested in investigating whether using other similarity measures will have an impact on our results [CS04] and addressing the automatic classification of very large databases of protein structures.

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