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PROJECT REPORT

प्रोटीन ३डी संरचना की तुलना के लिए ग्राफ थ्योरी आधारित उपकरण का विकास

Development of a Tool for Comparison of Protein 3D Structure using graph theoretic approach

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Project Report

Title of the project

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Submitted by
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आमुख

आज दुनिया भर में आणविक जीव विज्ञान प्रयोगशालाओं और सूचना प्रौद्योगिकी के ठोस प्रयास के कारण आज अनुक्रम और संरचनात्मक आंकड़ों की एक बड़ी मात्रा सार्वजनिक जैविक डेटाबेस में उप्लब्ध हैं । अनुक्रम और संरचनात्मक युक्तिकरण के साथ कुशल साधनों तथा उचच कोटि के विश्लेषणात्मक उपकरणों को भी डिजाइन करने की बायोइनफॉरमैटिक्स में एक केंद्रीय चुनौती हैं । इस तरह के विशेष उपकरण अनुक्रमण डेटा एवं सूचना को जैवरासायनिक और जैवभौतिकी ज्ञान में बदलने तथा इनमें दिये हुए संरचनात्मक, कार्यात्मक और विकास संबंधी संरचनाओ को समझने के लिए आवश्यक है। जैविक अनुक्रम और संरचना डेटा के विश्लेषण में प्रतिरूप सुमेलन (pattern matching) एक महत्वपूर्ण कदम है। प्रतिरूप सुमेलन प्रोटीन 3 डी संरचनाओं का अध्ययन, अन्य प्रोटीन के साथ विकासवादी और संरचनात्मक संबंध ज्ञात करने में एक महत्वपूर्ण भूमिका निभाता है तथा जीवविज्ञानियों को संरचनाओं और विकास से जुड़े विभिन्न पहलुओं को समझने में मदद करता है । प्रोटीन 3 डी संरचनाओं के डेटाबेस अधिक बड़े होने की वजह से हमें नये उन्नत एव कम समय लेने वाले उपकरण और तुलनात्मक तरीकों की आवश्यकता है। 3 डी संरचना की तुलना उपलब्ध डेटाबेस में विविधता, विश्लेषण तथा वैज्ञानिक अंतर्दृष्टि को समझने में एक महत्वपूर्ण भूमिका निभाता हैं। यह ध्यान रखना महत्वपूर्ण है कि इन डेटाबेस कि वृद्धि का मतलब केवल मात्रा नहीं है, बल्कि विविधता, जटिलता, भेद्यता और एकरूपता भी हैं। इसलिए, तुलनात्मक उपकरण को न केवल उच्च शुद्धता तथा विस्तृत आयाम की आवश्यकता होती है, बल्कि संरचनाओं की बढ़ती संख्या से निपटने के लिये कम समय में निकालना भी है। प्रोटीन अनुक्रमण विशिष्ट रूप से अपने मूल परिवेश में एक संरचना को निर्धारित करता है। प्रोटीन के कार्य को समझने में यह संरचनात्मक जानकारी अधिक महत्वपूर्ण है। डेढ़ दशक के बाद भी प्रोटीन संरचना की तुलना आज की प्रमुख शोध प्राथमिकता के रूप में प्रचलित है, तथा बहुत शोध लेख इस् दिशा में प्रकाशित हो रहे है। विगत वर्षों में हुए प्रगति के तरीकों में सुधार जारी है। अतः अभी भी प्रोटीन संरचना की तुलना एक खुली चुनौती है।

साहित्य की समीक्षा के आधार पर, ग्राफ सिद्धांत आधारित तकनीकियों का इस्तेमाल प्रोटीन तुलना के लिए किया जा सकता है। ग्राफ मॉडल को विभिन्न ग्राफ मापदंडों का उपयोग कर बनाया जा सकता है। आम तौर पर, ग्राफ सिद्धांत का उपयोग जटिल स्थानिक संरचना को परिभाषित एवं समझने के लिए किया जाता है जिसके परमाणु आपस में जटिलता से जुड़े हुए है तथा एक दूसरे पर निर्भर है। परमाणु के स्तर पर विश्लेषण ग्राफ सिद्धांतिक विधि 3 डी संरचना विश्लेषण के लिए किसी अन्य विधि की तुलना में बेहतर परिणाम दे सकता है। इन महत्वपूर्ण बिंदुओं को ध्यान में रखते हुए परियोजना प्रोटीन ३डी संरचना की तुलना के लिए ग्राफ थ्योरी आधारित उपकरण का विकास तैयार किया गया है।

हमने 1) ग्राफ विभाजन और 2) ग्राफ गुणों का उपयोग करके प्रोटीन 3 डी संरचना की तुलना करने के लिए दो नयी विधियों को विकसित किया है। दोनों विकसित विधियों को MATLAB में लागू किया गया है। विकसित तरीकों को दो वर्तमान में उप्लब्ध सर्वोत्तम तरीकों जैसे सीई (CE) और जेएफएटीसीएटी (jFATCAT) के साथ 100 प्रोटीन बेंचमार्क डाटासेट पर SCOP डाटाबेस के साथ परीक्षण किया गया है। विकसित की गयी विधियां समय एवं उत्कृष्टता की दृष्टि से उप्लब्ध तकनीकियों से बेहतर साबित सिद्ध हुई।

लेखकगण

PREFACE

Today, large volume of sequence and structural data is publically available in the form of biological databases based on integrated effort of molecular biology laboratories throughout the world and advances in information technology. A global challenge in bioinformatics is the rationalization of the huge amount of sequences and structural data with a view not only to derive efficient and useful meaning from this data, but also for designing sharper analytical tools. The analytical tools are required for conversion of sequence data/information into biochemical and biophysical properties and to decipher the structural, functional and evolutionary clues encoded in the data. Pattern matching is one of main important aspects in the analysis of biological sequence and structure data. The alignment and comparison of protein 3D structures are very important and fundamental task in structural biology to study evolutionary and structural relatedness with other proteins and helps biologists to understand various functions and evolution from these structures to identify its structural neighbors. In addition to this, databases of three-dimensional protein structures became so large that fast search tools and comparison methods are required. The 3D structure comparison play a key role in understanding the diversity of structure space by analyzing and deriving interesting scientific insights in the existing vast structural databases. It is important to note that an increase in deposited structures does not just contain quantity, but also variety, complexity, vulnerability, and singularity. Hence, comparison tools are essentially required not only to improve accuracy and coverage but also reduce time complexity. Protein sequence uniquely determines a structure in its native environment. This structural information is vital in understanding the function of a protein. In last one and an half decade, the research on protein structure comparison has been taken up on priority basis and numbers of research articles were exists in literature. There are incremental advances over previous efforts, and still methods are being development for further improvement.

The graph theory approaches can be used for protein 3D structure comparison. Graph models can be created using various graph parameters. Generally, graph theory is used to represent/decipher complex spatial structures which are mutually connected and dependent. The 3D structure of protein is a complex structure. The atom level analysis may yield better result in 3D structure analysis than any other method. Considering these important points, the project has been formulated to develop a tool for comparison of protein 3D structure using graph theoretic approach.

We have developed two novel methods for comparison of 3D structure based on 1) graph partition and 2) graph properties. Both methods have been implemented in MATLAB by writing codes for various functions. The performance of the developed methodologies is tested with two existing best methods such as CE and jFATCAT on 100 proteins benchmark dataset with SCOP (Structural Classification Of Proteins) database. The proposed methods performed better in terms of classification accuracy and time complexity.

AUTHORS

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Project Report
Development of a Tool for Comparison of Protein 3D Structure using graph theoretic approach

1. Institute Project Code: IRC Proceeding Project No. : AGENIASRISIL201400500024 Project Code No. : IXX10767
2. Project Title : Development of a Tool for Comparison of Protein 3D Structure using graph theoretic approach
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6. Project Team(Name(s) and designation of PI, CC-PI and all project Co-PIs, with time proposed to be spent)

S. No.	Name, designation and institute	Status in the project	Time to be spent (%)	Work components to be assigned to individual scientist
1	U B Angadi, Sr. Scientist	PI	40	Review of relevant literature, Parameters selection, Construction of graph matrix and graph model, Graph Comparison, Evaluation
2	K K Chaturvedi	CPI-1	30	Review of relevant literature, Construction of graph matrix and graph model, Graph Comparison
3	Monendra Grover	CPI-2	25	Parameters selection,
4	Sudhir Srivastava	CPI-3	25	Parameters selection , Graph Comparison, Evaluation(till 1/8/2015)

7. Priority Area to which the project belongs : **DEVELOPMENT OF STATISTICAL TECHNIQUES FOR GENETICS/COMPUTATIONAL BIOLOGY AND APPLICATIONS OF BIOINFORMATICS IN AGRICULTURAL RESEARCH**
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8. Project Duration: Date of Start: **18/03/2014** Likely Date of Completion**: 15/4/2017**
9. (a) Objectives
   - To convert 3D structure of protein to graph model using graph theoretic approach
   - To compare 3D structures using graph theoretic models and data mining techniques

   (b) Practical utility
   - Useful for 3D structure comparison,
   - 3D structure search in the database,
   - Classification/analysis of 3D structures.

CHAPTER –I: INTRODUCTION

GENESIS AND RATIONALE OF THE PROJECT

Protein sequence uniquely determines a structure in its native environment. This structural information is vital in understanding the function of a protein. The structural information of protein is classified into primary, secondary, tertiary and quaternary. The primary structure of a protein refers to the amino acid sequence of the polypeptide chain, which is formed during the process of protein biosynthesis and it is held together by covalent or peptide bonds. The secondary structure refers to highly regular local sub-structures defined by the patterns of hydrogen bonds between the peptide chains. The alpha helix and the beta strand are considered as the major secondary structures, which represent a way of saturating all the hydrogen bond donors and acceptors in the peptide backbone. Tertiary structure is the particular arrangement of secondary structure elements in three dimensional spaces. Quaternary structure is a larger assembly of several protein molecules. Proteins are versatile biological molecules that perform numerous functions in a living organism. These functions are at two levels; one at molecular level (physical/chemical activity) and cellular level (signalling /metabolic pathways activity). In nature, protein 3D structure is more conserved than protein sequence. Hence, 3D structure can provide significant insights about protein function. The intimate relationship between protein structure and function has been well established (Perutz 1960).

The quantitative comparison of protein 3D structures is an important and fundamental task in structural biology to study evolutionary and structural relatedness with other proteins and helps biologists to understand various aspects of function, evolution from these structures and identify its structural neighbours. In addition to this, databases of three-dimensional protein structures are very large and increasing day by day. Hence, fast search tools and comparison methods are needed. The 3D structure comparison play a key role in understanding the diversity of structure space by analysing and deriving interesting scientific insights from the existing vast structural databases. It is important to note that an increase in deposited structures does not just imply quantity, but also variety, complexity, and singularity. Hence, efficient methods require for protein structure comparison for not only high accuracy but also fast execution to cope up with the increasing number of structures.

KNOWLEDGE/TECHNOLOGY GAPS

Since one and an half decade, the research on protein structure comparison has been taken up on priority and numbers of research articles were published. There are incremental advances over previous efforts, and still methods continue to improve. Hence there is still an open challenge for protein structure comparison. Despite of extensive research, the accuracy of their alignments has not been benchmarked or compared. They are not capable to report whether the computed similarity is optimal according to the corresponding scoring function used in structure comparison.

The alignment of protein structures is a difficult task, and its accuracy may depend on the method or program used. The major approaches to structure comparisons are based on Cα and Secondary Structure Elements (SSEs) alignments, Comparing intramolecular & inter-residue distances (SSAP, DALI), Matching main-chain fragments by CE (Combinatorial Extension) & dynamic programming by representing proteins as a set of Cα distances for octamers (i.e., between eight consecutive residues in the structure) and each pair of octameric fragments that can be aligned within a given threshold is considered in an Aligned Fragment Pair (AFP). Secondary Structure Elements methods (VAST, SARF, MATRAS) use the Cα atoms to generate a set of vectors of connecting residues. Such vectors effectively represent the structure in two dimensions providing both position and directionality.

One of the disadvantages of using the SSEs is that active sites are frequently small and contained in the coiled regions, and it is particularly important to align these correctly. Methods based on decomposition of protein structures to smaller blocks are most likely to suffer from combinatorial complexity. Another method of curbing combinatorial complexity is by using the scoring function based on the rigid-body superposition, possibly allowing for “hinges” between superposable rigid parts.

Root Mean Square Deviation (RMSD) values are considered as reliable indicators of variability when applied to very similar proteins, like alternative conformations of the same protein. On the other hand, RMSD data calculated for structure pairs of different sizes cannot be directly compared, because the RMSD value obviously depends on the number of atoms included in the structural alignment. RMSD is a good indicator for structural identity, but less so for structural divergence.

Well-designed method or tool is needed to address the below mentioned issues while comparing 3D protein structures.

- **Accurate and Fast Methods for Multiple Structure Alignment:** Existing methods for multiple structure alignment are reaching unprecedented levels of coverage and accuracy. Currently, the PDB contains 93,788 proteins. A full set of comparisons approximately requires K^2x10^9 (K is no of SSEs) comparisons to be computed and stored. Faster and more biologically meaningful clustering and classification algorithms are needed.
- **Flexible Structure Alignment:** Biological features that depend on flexibility have yet to be considered as part of the alignment procedure.
- **Biologically Relevant Alignments:** Existing methods usually focus on optimizing geometrical similarities between two or more structures and not contemplate with biological information. Few methods are able to account for additional biological (chemical, physical, or evolutional) information that might lead to more accurate alignments.
- **Biologically Relevant Division of the Structural Space:** Defining and identifying unique structural units that are recurrent between protein structures remains an unresolved issue.

LITERATURE REVIEW

National level

Bhattacharya *et al.* (2007) proposed a protein structure comparison scheme, which is capable of detecting correct alignments even in difficult cases, *e.g.*, non-topological similarities. This method computes protein structure alignments by comparing, small substructures, called neighbourhoods.

Deshmukh *et al.* (2008) proposed GIPSCo (Geometric Invariant based Protein Structure Comparison) that compares a protein pair by using geometric invariants of local geometry of the backbone structures. The method first generates a list of aligned fragment pairs (AFPs) using the geometric invariants of the local geometry and then these structurally similar AFPs are assembled using a graph theoretic approach to maximum weighted clique to obtain global structural alignment between two proteins.

Shivashankar *et al.* (2011) and group have proposed an improved representation of protein structures using latent dirichlet allocation (LDA) topic model. In this, they compared the proposed representations and retrieval framework on the benchmark dataset developed by Kolodny and co-workers (2002). Further, they also demonstrated that LDA indeed models relationships between fragments in protein structures effectively and another important contribution of this work is stated that proposed multi-viewpoint homology detection framework is able to effectively find close, as well as, remote homologous proteins for a query protein structure.

International Level

Holm and Sander (1993) developed (DALI) pair wise structural alignment using residues-residues distance from protein co-ordinates to form distance matrix (DM). This DM decomposed into elementary contact patterns. Subsequently, submatrices formed and decomposed into large consistent set of pairs. Furter, Monte Carlo procedure have been used to optimize similarity. Finally, alignment or superimposition has been obtained.

The SARF (Spatial ARangement of backbone Fragments) (Alexandrov 1996) is arrangements of fragments in a pair of proteins; it measures similarity using RMSD between Cα atoms and statistical significance of the similarities. Then take searching common spatial arrangement of backbone fragments in a pair of proteins.

Vector Alignment Search Tool (VAST) (Gibrat *et al.* 1996) calculates a p-value for the best substructure superposition as the probability; this score would be seen by chance in drawing SSE pairs at random, Generates the possible number of alternative substructure alignments of SSEs in the protein pair. The p-value calculation makes use of an empirical distribution of superposition scores for randomly aligned fragment pairs and the search space is determined by a combinatorial formula giving the number of possible SSE alignments, Here is used the statistical theory of BLAST (Basic Local Alignment Search Tool).

Singh and Brutlag (1997) have proposed hierarchical protein structure superposition using both Secondary Structure and atomic representations.

- **Local Secondary Structure Superposition**: Compare pairs of vectors from target and query protein using orientation independent scoring functions. Select the pair that results in the best local secondary structure alignment and transform the query protein to minimize the RMSD between this pair of vectors. Using dynamic programming, compare all vectors from the target and query proteins based on orientation independent and orientation dependent scores. Transform the query protein to minimize the RMSD between the atoms of the aligned secondary structure elements.
- **Atomic Superposition**: For every atom in the query protein, find the nearest atom (within a threshold distance) on the target protein. Transform the query protein to minimize the RMSD between these pairs of atoms. Iterate until the RMSD converges.
- **Core Superposition**: Find the best core of correctly aligned and sequentially ordered atoms and minimize the RMSD between them. Iterate until the RMSD converges.

Taylor (1999) has developed a tool using by incorporating a random element into an iterative double dynamic programming algorithm. The maximum scores from repeated comparisons from a pair of structures converged on a value that was taken as the global maximum. Finally, this has been characterized the alignment by their alignment length and root-mean-square deviation (RMSD).

Shindyalov and Bourne (1998) have developed protein structure alignment by incremental combinatorial extension (CE) of the optimal path. This involves a combinatorial extension of an alignment path defined by aligned fragment pairs (AFP). AFPs are pairs of confer similar fragments based on local geometry and one from each protein. Combinations of AFPs that represented possible continuous alignment paths are selectively extended or discarded, leading to a single optimal alignment.

Carugol and Pongor (2001) have employed normalized root-mean-square distance for comparing protein three-dimensional structures. A very popular quantity RMSD used to express the structural similarity between equivalent atoms in two structures, defined as where *d* is the distance between each of the *n* pairs of equivalent atoms in two optimally superposed structures. The RMSD is 0 for identical structures, and its value increases as the two structures become more different.

Kawabata (2003) has developed server MATRAS (MArkov TRAnsition of protein Structure evolution) for protein 3D structure comparison based on transition matrix and Markov transition probability for calculating environment, distance and SSE scores then finally calculating similarity between two proteins using these scores. The server has three main services. The first one is a pairwise 3D alignment, which is simply align two structures. The second service is a multiple 3D alignment, which compares several protein structures. In which pairwise 3D alignments are assembled in the proper order. The third service is a 3D library search, which compares one query structure against a large number of library structures.

Zemla (2003) has proposed LGA (Local-Global Alignment) method to facilitate the comparison of protein structures or fragments of protein structures in sequence dependent and sequence independent modes.

Zotenko *et al.*, (2007) employed *Structural foot printing* methods (SEGF); In this method, first is selection of a representative set of structural fragments as models and then map a protein structure to a vector in which each dimension corresponds to a particular model and "counts" the number of times the model appears in the structure. It used contiguous segments (thirty-two residues long) of protein backbone as structural fragments. The conformation of a backbone segment is captured by a set of fourteen shape descriptors introduced by Rogen *et al* (1996). This method measures structural similarity based on the presence/absence of common structural fragments.

Wohlers *et.al*. (2010) and his team introduced a general mathematical model for optimal alignment of inter-residue distance matrices. The proposed model is based on an integer linear programming (ILP) formulation of Caprara *et al.* (2004). This computes a pair wise alignment of two protein structures that maximizes the number of common contacts. Two residues are in contact if they are in some sort of chemical interaction, e.g. by hydrogen bonding and whenever the distance between two residues is below a predefined distance threshold, the residues are considered to be in contact. Lagrangian relaxation uses to compute alignments, which leads to an iterative double dynamic programming (DP) algorithm and every optimal solution of the relaxed problem provides an upper bound on the optimal score of the original problem.

Nguyen and Madhusudhan (2011) have developed the algorithm consists of four sequential steps, as follows.

- **Extracting features**: Residues in a protein are represented by the Cartesian coordinates of one representative atom (typically the Ca), side-chain solvent accessibility and secondary structure.
- **Forming cliques**: all possible internal pair-wise distances between the representative atoms are computed and defined a clique as a subset of *n* points, where the Euclidean distance between any pair within the clique is within a predefined threshold.
- **Clique matching**: The objective is to compute a one to one mapping between amino acid residues of the two structures.

Based on a formalism for representing and comparing local structure called Local Descriptors of Protein Structure (LDPS) (Daniluk and Lesyng 2011). All of the local descriptors in each structure are identified as they are compared against each other. Pairs of similar descriptors are then used as building blocks for the alignment. Further it has Identified all residues in contact with the descriptor’s central residue. Elements are then built by including two additional residues along the main-chain, both upstream and downstream of each contact residue.

BRIEF ABOUT THE PROJECT

Based on the review of literature, the graph theory approaches can be used for protein comparison. Many graph models can be created using various graph parameters. Generally, graph theory is used to represent/decipher complex spatial structure which mutual connected and depended. As we know that 3D structure of protein is a complex structure. The atoms level analysis may yield better result for 3D structure analysis than any other method. Considering these important points, the project is formulated for comparison of 3D protein structure using graph theoretic approach. This research will also leads to application of graph theory to other bioinformatics area such as biological networks (PPI, protein function prediction, disease network, drug-drug relation etc.

The above discussed methods are based on alignment of SSEs, Cα coordinates and geometry of residues. None of the these methods used graphical methods for quantification of 3D structure of protein. Further, based on extensive literature survey given below, graph theoretic approach can be employed for comparison of 3D structures.

- Demonstrated calculation of free energy for all-atom models of protein structures using GBP (Generalized Belief Propagation) and Markov Random Field model for protein structure (Kamisetty *et al.,* 2008).
- A chain graph model built on a causally connected series of segmentation conditional random fields (SCRFs) (Liu *et a.,* 2009) has been proposed to predict protein folds with structural repeats using segmentation conditional random field and position weight matrix.
- A graph theoretical algorithm to identify backbone clusters of residues in proteins (Patra and Vishveshwara, 2000) to cluster protein sites with the highest degree of interactions. This based on adjacency matrix of 3D structure and eigenvectors.
- Described graph-theoretic (Artymiuk *et al.* 1994) by subgraph-isomorphism methods for the representation and searching of three-dimensional patterns of side-chains in protein structures.
- Razavian and his team (2010) developed Time-Varying Gaussian Graphical Models for Molecular Dynamics Data. Based learning sparse, maximum aposteriori (MAP) estimate to learn structure using topology, parameters of the model, L1-regularization of the negative log-likelihood to ensure sparsity (density), and a kernel to ensure smoothly varying topology and parameters over time.
- A novel methodology presented to track a simple 3D biological event/structures and quantitatively analyse the underlying structural change over time using graph theory (Lund 2009). Raland and his team (Luth *et al.,* 1992) has explained for assessing quality of protein model with 3D profiles.
- Graph theoretical (Frommel *et al.,* 2003) approach for screening hierarchy of the protein. The approach encompass Molecular Surface Patches (MSP) and defined similarity matrix, considered the similarity matrix as weighted graph (similarity Graph) and performed random permutation of the edges and then hierarchy screening the protein.

In view of above point from the published literature, the project was taken on comparison of 3D protein structure using graph theory, graph properties and machine learning techniques.

GRAPH THEORY

A graph is a symbolic representation of a network or connected components. It implies an abstraction of the reality so it can be simplified as a set of linked nodes or components.

Graph theory is a branch of mathematics concerned about how networks can be encoded and their properties measured. It has been enriched in the last decades by growing influences from studies of social and complex networks.

The origins of graph theory can be traced to Leonhard Euler who devised it in 1735 a problem that came to be known as the "Seven Bridges of Konigsberg". In this problem, someone had to cross each of these bridges only once and in a continuous sequence. A problem the Euler proved to have no solution by representing it as a set of nodes and links. This led the foundation of graph theory and its subsequent improvements. Initially graph theory applications on most networks have spatial basic, namely road, transit and rail networks. This it is not necessarily the case for all transportation networks. Later, this has been extended to telecommunication system such as Mobile telephone networks or the Internet. Now, this is extended to bioinformatics areas such as gene regulatory network, gene interaction, protein-protein interactions, and many more.

A graph G=[V,E] in context of protein 3D structure is defined as an ordered pair consisting of two sets V and E, where V represents a set of vertices or atoms and E is a set of edges and weighted distances in set V. The edges of the graph are discriminated from each other by giving different weights for each of them for calculating Euclidian distance between atoms. Some of the important terms are defined as below:

- **Graph:** A graph *G* is a set of vertex (nodes) *v* connected by edges (links) *e*. Thus *G=(v , e)*.
- **Vertex (Node).** A node *v* is a terminal point or an intersection point of a graph.
- **Edge (Link)**: An edge *e* is a link between two nodes. The link (*i* , *j*) is of initial extremity *i* and of terminal extremity *j*. A link is the abstraction of a transport infrastructure supporting movements between nodes. It has a direction that is commonly represented as an arrow. When an arrow is not used, it is assumed the link is bi-directional.
- **Sub-Graph:** A sub-graph is a subset of a graph *G* where *p* is the number of sub-graphs. For instance *G'* = (*v'*, *e'*) can be a distinct sub-graph of *G*.
- **Simple graph:** A graph that includes only one type of link between its nodes. In proposed work we have taken simple graph means it has only one connection.
- **Multigraph**: A graph that includes several types of links between its nodes.
- **Connection:** A set of two nodes as every node is linked to the other. Considers if a movement between two nodes is possible, whatever its direction. Knowing connections makes it possible to find if it is possible to reach a node from another node within a graph.
- **Path:** A sequence of links that are traveled in the same direction. For a path to exist between two nodes, it must be possible to travel an uninterrupted sequence of links. Finding all the possible paths in a graph is a fundamental attribute in measuring accessibility and traffic flows.
- **Chain:** A sequence of links having a connection in common with the other. Direction does not matter.
- **Length of a Link, Connection or Path:** Refers to the label associated with a link, a connection or a path. This label can be distance, the amount of traffic, the capacity or any attribute of that link. The length of a path is the number of links (or connections) in this path.
- **Cycle:** Refers to a chain where the initial and terminal node is the same and that does not use the same link more than once is a cycle.
- **Circuit:** A path where the initial and terminal node corresponds. It is a cycle where all the links are traveled in the same direction. Circuits are very important in transportation because several

distribution systems are using circuits to cover as much territory as possible in one direction (delivery route).

- **Clique**: A clique is a maximal complete subgraph where all vertices are connected.
- **Cluster**: Also called community, it refers to a group of nodes having denser relations with each other than with the rest of the network. A wide range of methods are used to reveal clusters in a network, notably they are based on modularity measures (intra- versus inter-cluster variance).
- **Symmetry and Asymmetry**: A graph is symmetrical if each pair of nodes linked in one direction is also linked in the other direction. By convention, a line without an arrow represents a link where it is possible to move in both directions. However, both directions have to be defined in the graph. Most transport systems are symmetrical but asymmetry can often occur as it is the case for maritime (pendulum) and air services. Asymmetry is rare on road transportation networks, unless one-way streets are considered.
- **Assortativity and disassortativity**: Assortative networks are those characterized by relations among similar nodes, while disassortative networks are found when structurally different nodes are often connected. Transport (or technological) networks are often disassortative when they are non-planar, due to the higher probability for the network to be centralized into a few large hubs.
- **Completeness:** A graph is complete if two nodes are linked in at least one direction. A complete graph has no sub-graph and all its nodes are interconnected.
- **Connectivity:** A complete graph is described as connected if for all its distinct pairs of nodes there is a linking chain. Direction does not have importance for a graph to be connected, but may be a factor for the *level* of connectivity. If p>1 the graph is not connected because it has more than one sub-graph (or component). There are various levels of connectivity, depending on the degree at which each pair of nodes is connected.
- **Complementarity:** Two sub graphs are complementary if their union results in a complete graph. Multimodal transportation networks are complementary as each sub-graph (modal network) benefits from the connectivity of other sub-graphs.

MACHINE LEARNING TECHNIQUES FOR CLUSTERING AND CLASSIFICATION

Biological research world over has generated a vast quantity of bioinformatics data both sequences and structural. One of the challenge in bioinformatics is developing effective computational methods that can recognize the patterns which are leads to decipher functional, structural and evolutionary relatedness of data. A most promising approach for these challenges is pattern recognition. Brief descriptions of pattern recognition by machine learning approaches, similarity metrics and validation techniques are presented below

Pattern recognition can be defined as "the act of taking raw data and making an action, based on the category of the pattern (Duda *et al.,* 2007). Human beings are good at recognizing/distinguishing patterns but it is difficult to recognize patterns correctly when there is high complexity in patterns and a large number of predefined classes are present. Reliable, fast and accurate pattern recognition by machine would be immensely useful from the practical point of view. Pattern recognition deals with the design of systems that recognize patterns in data. Important application areas are image analysis, character recognition, fingerprint identification, speech analysis, DNA and protein sequence analysis, person identification, etc.

The goal of pattern recognition research is to devise ways and means of automating certain decision-making processes based on supervised learning or classification and unsupervised learning or clustering. This process generally has steps of acquisition of the data, pre-processing to remove noise or normalization of the data, feature extraction, classification or supervised learning / clustering or unsupervised learning and finally evaluation.

Pattern recognition scheme employs two learning paradigms namely, supervised and unsupervised learning. In supervised learning, a teacher provides a category label for each pattern in a training set. The objective is to use the learnt abstraction to assign a label to the given new pattern. In unsupervised learning, there is no explicit teacher and training patterns are not labeled. Clustering is a unsupervised learning technique. The technique forms clusters or natural groupings of the input patterns (*Duda et al.*, 2007). In this work, we have employed clustering techniques for pattern recognition and to develop methodology for protein structure comparison and clusters analysis is used to evaluate the proposed methods.

Clustering is a grouping procedure accomplished by finding similarities between data according to the characteristics found in the given dataset. Clustering is a collection of data objects which are similar to one another within the same cluster but dissimilar to the objects in other clusters. Clustering is an unsupervised learning technique to divide a collection of patterns into groups of similar objects. The main objective of this learning technique is to find a natural grouping or meaningful partition by using distance or similarity measures. Some basic features of clustering are:

- The number of clusters is not known
- There may not be any prior knowledge concerning the clusters
- Cluster results are dynamic.

For a given dataset $D= \{t_1, t_2, \ldots\ldots t_n\}$ of n tuples and integer value k as number of clusters, the clustering problem is to define a mapping $f : D \rightarrow [1, 2, \ldots. k]$ where each tuple t_i is assigned to one cluster $K_j, 1 \leq j \leq k$. A cluster, K_j , contains precisely those tuples mapped to it: that is, $K_j = \{t_i \mid f(t_i)=K_j , 1 \leq i \leq n \text{ and } t_i \in D\}$

Categorization of clustering algorithms: Clustering methods are broadly classified into three main categories namely, 1) Sequential, 2) Hierarchical, 3) Partitional and 4) Hybrid clustering.

- **Sequential Clustering:** These algorithms produce clusters in a single loop or few loops. They are quite straight forward and fast. In these, all the feature vectors are presented to the algorithms once or a few times (less than 5). These schemes produce compact and hyperspherically or hyperellipsoidally shaped dynamic clusters using threshold. Examples of sequential clustering techniques including Neural Network as a leader.

- **Hierarchy Clustering:** With hierarchical clustering, a nested set of clusters are created. Each level in the hierarchy has a separate set of clusters. At the highest level, all items belong to the same cluster and at the lower level, each item is in its own unique cluster. The output of the algorithm is called as *dendrogram.* There are two approaches for hierarchical clustering:

    - Top-down (Divisive/splitting) approach: Starts with the entire data in one cluster and then hierarchically splits the dataset into smaller blocks successively until all items are in their own cluster.
    - Bottom-up (Agglomerative/merging) approach: Starts with each individual item in its own cluster and iteratively merges clusters until all items belong in one cluster.

- **Partitional Clustering:** Partitional clustering creates the clusters in one step. Only one set of clusters is created, although several different sets of clusters may be created internally. Since only one set of clusters is outputted, the user must provide initially k, the desired number of clusters. In addition, some metric or criterion function is used to determine the goodness of any such solution. This measure of quality could be the average distance between clusters or some other metric. Examples of partitional clustering algorithm are K-Means, K-Centroids, K-Medians, K-Mediods and PAM (Partitioning Around Mediods).

- **Hybrid Clustering (Combination of different methods):** The idea of design a hybrid classifier is combining the merits of various techniques. The hybrid algorithm is a choice at a high level between at least two distinct algorithms and each of which can solve the same problem. The choice is motivated by an improved performance. Fuzzy-neural networks and Fuzzy C-means clustering are commonly used hybrid methods for pattern classification in a variety of applications.

SIMILARITY AND DISTANCE MEASURES

The concept of dissimilarity (or distance) or dual similarity is the essential component of any form of clustering and classification that help us to navigate through the data space and form clusters/classes. Thus, clustering and classification methods require an index of proximity, or alikeness, or association between pairs of patterns such as distance or similarity measures.

The similarity between two tuples t_i and t_j with *h* dimension, $sim(t_i, t_j)$, in a dataset *D*, is a mapping from *DxD* to the range *[0,1]*. Thus, it can be represented as $sim(t_i,t_j) \in [0,1]$. The objective is to define the similarity mapping so that the documents that are more alike and have a higher similarity value. Thus, the following are desirable characteristics of a good similarity measure:

- $\forall t_i \in D, sim(t_i, t_i) = 1$
- $\forall t_i, t_j \in D, sim(t_i, t_i) = 0$ *if* t_i *and* t_j *are not alike at all*
- $\forall t_i, t_j, t_k \in D, sim(t_i, t_j) < sim(t_i, t_k)$ *if* t_i *is more like* t_k *than it is like* t_j

Some of the similarity measures commonly used in classification and clustering are given below.

Euclidean distance : $$dist(t_i, t_j) = \sum_{h=1}^{k} (t_{ih} - t_{jh})^2$$

Manhattan distance: $$dist(t_i, t_j) = \sum_{h=1}^{k} | t_{ih} - t_{jh} |$$

Minkowski distance: $$dist(t_i, t_j) = \sum_{h=1}^{k} (|t_{ih} - t_{jh}|^p)^{1/p}$$

Mahalanobis distance: $$dist(t_i, t_j) = (t_i - t_j)^T \Sigma^{-1} (t_i - t_j)$$

Distance measures are often used instead of similarity measures. The following are desirable properties of distance measures:

- Nonnegative: $Dist(t_i, t_j) \geq 0$
- Reflexivity: $Dist(t_i, t_j) = 0$ *if and only if* $t_i = t_j$
- Symmetry: $Dist(t_i,t_j) = Dist(t_j,t_i)$
- Triangle inequality: $Dist(t_i,t_j) + Dist(t_j, t_k) \geq Dist(t_{i,\ k})$

Similarity using Jaccard co-efficient for binary data is defined as follows

$$Dissimilarity(t_i, t_j) = (b+c/(a+b+c)$$
$$Similarity(t_i, t_j) = 1\text{-}dissimilarity(t_i, t_j)$$

where *a* is the number of attributes (or features) equal to 1 for both tuples, *b* is the number of attributes that is equal to 1 for tuple t_i and 0 for tuple t_j, *c* is the number of attributes that is equal to 0 for tuple t_i and 1for tuple t_j, d is number of attributes that are equals to 0 for both.

VALIDATION AND EVALUATION TECHNIQUES

Evaluation is important measure to assess the performance of technique and to identify the need for improvements in its components. To compare the proposed techniques with existing techniques, we have employed following benchmark data and performance matrices.

Benchmark data

A datasets of protein structures from SCOP (Structural Classification of Proteins) (Murzin *et al* 1995) database have been selected as benchmark data. This data were also used by Liu *et al. (2010)* and compared their techniques for classification of proteins. A dataset consists of 100 proteins structures in three classes and having 45 proteins from class I, 40 from class II and 15 from class III and details is given in Table -1.

Table-1 Benchmark data set used for evaluation of proposed method.

	Class	Fold	SF	No. of Proteins
a.1.1.1 1DLW,1S69,1IDR,1NGK,1UX8 1-5	1	1	1	5
a.1.1.2 1B0B,1H97,1A6M,1MBA,1ASH 6-10	1	1	2	5
a.1.1.3 1JBO,1ALL,1B8D,1XG0 11-14	1	1	3	4
a.2.3.1 1XBL,1NZ6,1IUR,1FAF,1GH6,1WJZ 15-20	1	2	4	6
a.3.1.1 1C75,1CTJ,1C52,1QL3,1E29,1YCC,1I8O 21-27	1	3	5	7
a.3.1.4 1M70,1H1O,1FCD 28-30	1	3	6	3
a.4.1.1 1P7I,1LE8,1K61,1LFB,1PUF 31-35	1	4	7	5
a.4.1.2 1IJW,1GDT,1TC3,1U78,2EZL,2EZI 36-41	1	4	8	6
a.4.1.3 1GV2,1GVD,1FEX,1UG2 42-45	1	4	9	4
b.1.1.1 1QFO,1DQT,1NEU,1PKO,1EAJ,1JMA,1XED 46-52	2	5	10	7
b.1.1.2 1DN0,1L6X,1FP5,1HXM,1K5N,1HDM,1UVQ 53-59	2	5	11	7
b.1.1.3 1VCA,1IAM,2OZ4,1ZXQ,1CID,1CCZ 60-65	2	5	12	6
b.6.1.1 1PLC,1KDJ,2Q5B,1BQK,1F56 66-70	2	6	13	5
b.6.1.3 2BW4,1KBV,1KV7,1GSK,1AOZ 71-75	2	6	14	5
b.7.1.1 1QAS,1RLW,1BDY,1GMI,2ZKM 76-80	2	7	15	5
b.7.1.2 1RSY,1UOW,1UGK,1RH8,1A25 81-85	2	7	16	5
c.2.1.1 2JHF,1JVB,1H2B,1RJW,1VJ0 86-90	3	8	17	5
c.3.1.1 1DJQ,1PS9,1LQT,1GTE 91-94	3	9	18	4
c.3.1.5 1ONF,1GES,1FEC,1H6V,1TRB,1M6I 95-100	3	9	19	6

Classification Accuracy

The simplest measure of classifier performance is the Classification Accuracy (Duda *et al.*, 2007). The *Classification Accuracy* (*CA)* depends on the number of samples correctly classified is evaluated by the formula:

CA= (No. correctly classified sample) /(Total number of samples)

***Precision*, *Recall* and f-measure**

Generally, most of the clustering/classification methods suffer from overfitting problem, therefore evaluation is needed to improve the performance by adjusting the parameters, or changing the algorithm or changing the training set. Therefore, we use *f-measure* as validation in our experiment. *f-measure* combines *Precision* and *Recall*. The traditional *f-measure* or balanced *f-score* is the harmonic mean of *precision* and *recall*. According to Yang and Liu (Yang & Liu, 1999), this measure was first introduced by van Rijsbergen (van Rijsbergen, 1979), which combines both *precision* (*p*) and *recall*(*r*) with equal weights and is defined as

$$F = 2.\frac{precision.recall}{precision + recall}$$

Precision and *Recall* are two widely-used evaluation measures in classification and clustering. *Precision* can be seen as a measure of exactness, whereas *Recall* is a measure of completeness.

In a classification task, a *Precision* score of 1.0 for a class *C* means that every item labelled as belonging to class *C* does indeed belong to class *C*, but gives no information about the number of items from class *C* that were not labelled correctly, whereas a *Recall* of 1.0 means that every item from class *C* was labelled as belonging to class *C*, but has no information about how many other items were incorrectly labelled as belonging to class *C*).

The *Precision* for a class is the number of true positives (i.e.) the number of items correctly labelled as belonging to the positive class divided by the total number of elements labelled as belonging to that particular class (i.e.) the sum of true positives and false positives, which are items incorrectly labelled as belonging to the class. *Recall*, in this context, is defined as the number of true positives divided by the total number of elements that actually belong to the positive class (i.e.) the sum of true positives and false negatives, which are items that were not labelled as belonging to the positive class but should have been.

The terms true positives, true negatives, false positives and false negatives (statistical *Type I* and *Type II* errors) are used to compare the given classification of an item (i.e.) the class label assigned to the item by a classifier with the desired correct classification (i.e.) the class the item actually belongs to. This is illustrated in Table 1.

Table 1. Confusion matrix table

	True classification	
Obtained classification	***tp*** (true positive)	***fp*** (false positive)
	fn (false negative)	***tn*** (true negative)

Precision and *Recall* are calculated by:

$$\text{Precision} = \frac{tp}{tp + fp} \qquad \text{Recall} = \frac{tp}{tp + fn}$$

Receiver Operating Characteristic (*ROC*) is used to evaluate the method followed in this study with other methods. The *ROC* is a comparison of two operating characteristics, True Positive Rate (*TPR*) and False Positive Rate (*FPR*). *TPR=tp/(tp+fp)* and *FPR=fp/(fp+tn).*

Here, we also use Accuracy (*ACC*) to measure the degree of closeness between the observed and true classes and Matthews Correlation Coefficient (*MCC*) for correlation between experimental classes and actual classes.

ACC= (tp+tn)/(tp+fn+fp+tn)
*MCC = ((tp*tn)-(fn*fp))/ ((tp+fn)(fp+tn)(tp+fp)(fn+tn)).*

The report is organized as follows

Chapter-I is about introduction and basic existing knowledge employed in the proposed methods, which contains genesis and rationale of the project, knowledge/technology gaps, literature review, existing study and status at national and international level, brief about the proposed project, basic of graph theory, machine learning techniques, similarity and distance measures, benchmark data and evaluation procedure and metrics. Based on existing knowledge discussed in this, we have developed two methods using graph partitioning and graph properties techniques, and illustrated in detail in following chapters.

In Chapter- II, we have described a novel method based on graph partitioning and alignment of graph partitions to derive global alignment of 3D structure of protein. In this chapter, we illustrated in detail about all steps such as converting 3D structure to graph model, partitioning the graph model into sub-graphs, and then aligning subgraphs to complete graphs, final computing similarity from aligned pair of graphs in detail. Here, we presented results about evaluation of the proposed method in results and discussion section and final concluded in the conclusion section.

Chapter- III is about another method using graph properties and machine learning techniques. This demonstrates extracting graph properties from 3D structure of proteins, use of graph properties to compare protein 3D structures and its application in structural classification of proteins.

Summarised the conclusion of both proposed methods in Chapter-IV -Summery and Conclusion.

The proposed both methods have been implemented in MATLAB. All functions such as PDB file reading, selection for protein structure model and chain, reading x, y, z co-ordinates, calculating distance & adjacency matrix, graph partition, graph properties extraction, clustering algorithms, similarity measures and graphical presentations codes are presented in Annexure –I.

CHAPTER- II: GRAPH PARTITIONING/CLUSTERING AND ALIGNMENT

INTRODUCTION

In this chapter, a detail about novel method for comparison of 3D structure using graph partition and alignment method has been illustrated. A novel methodology has been developed for comparing protein structure by employing conversion of 3D graph into 2D graph, partitioning of 2D graph into sub-graphs and then finally aligning sub-graphs to structure. Also, structure similarity has been calculated by identifying local structural similarities to global structural similarity. The proposed method has been implemented in MATLAB by writing codes for various functions. The performance of the developed methodology is tested with two existing best methods such as CE and jFATCAT on 100 proteins benchmark dataset with SCOP (Structural Classification Of Proteins) database. The proposed method has shown significant improvement over jFATCAT and accuracy has increased up to 12-15%.

MATERIAL AND METHODS

An algorithm has been developed to calculate the similarity between two protein structure (Figure 1) based on i) Representing 3D protein structure into 2D graph model, ii) Partitioning the graph models into sub-graphs and iii) aligning the sub-graphs between pair of proteins. The information in 2D model is comprehensive and can be perceived to a 3D protein model. It is also observed that information about geometric and molecular properties in 3D are lost upon representation of a protein structure from 3D to 2D, but favourable interactions between atoms are carried (Pietal *et al.* 2015). The favourable interactions tend to be preserved in physico-chemical and evolutionary evolution reflections. Also these interactions are covalent bonds, Ionic bonds, Hydrogen bonds and Hydrophobic interactions, Van der Waals forces that represent transient and weak electrical attraction of one atom to another and common contact due to chemical interactions. 2D graph model is an adjacency or distance matrix of all atoms means NXN matrix, where N is number of atoms in the 3D structure. The matrix is an undirected weighted graph model. This graph has been decomposed into subgraphs using graph partitioning/clustering algorithm. These partitioning on principal of retaining connection between atoms having strong interaction and discard connections of weak interaction atoms. Further, it is identified isomorphism of sub-graphs of two proteins and finally alignment has been done based on similarity between sub-graphs (Figure-1).

a) <u>Basic concepts</u>

A graph G=[V,E] in context of protein 3D structure is defined as an ordered pair consisting of two sets V and E, where V represents a set of vertices or atoms and E is a set of edges and weighted distances in set V. The edges of the graph are discriminated from each other by giving different weights for each of them for calculating Euclidian distance between atoms.

In this, the protein 3D structure is represented as a graph models in terms of 2D distance matrices. The distance matrix of a protein D= d_{ij} is the Euclidean distances between all pairs (i, j) of its atoms. The matrix provides a 2D representation of a 3D structure, and contains enough information for retrieving the actual structure, except for overall chirality (Havel et al.1983, Holm and Sander 1993). The idea underlying DALI (Holm 1993) is that if two structures are similar, then their distance matrices must be similar too. An analogous idea is used to compare structures via their contact maps and is well described (Havel et al., 1983; Holm and Sander, 1993) in literature.

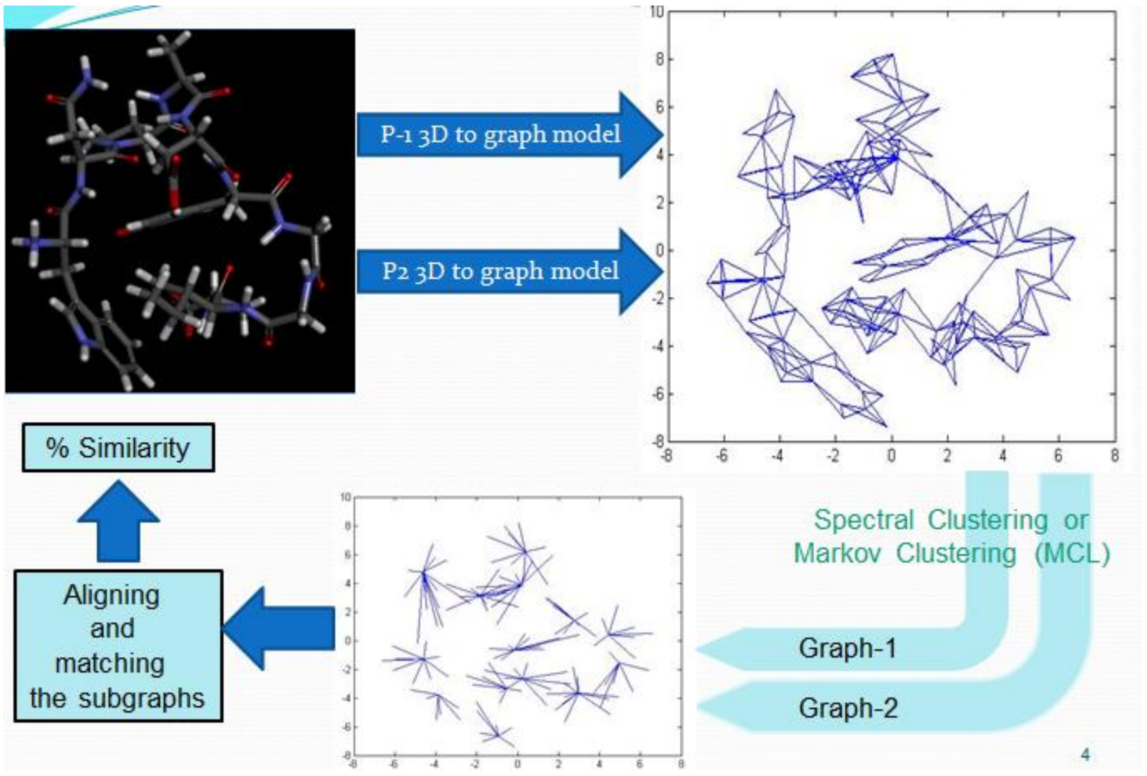

P-1 3D to graph model
P2 3D to graph model
% Similarity
Aligning and matching the subgraphs
Spectral Clustering or Markov Clustering (MCL)
Graph-1
Graph-2
4

Fig. 1. Overall schematic data and work flow diagram

b) Conversion of protein 3D structure to 2D graph model

In PDB file, all atoms are labelled with coordinates and these coordinates are based on Ångström. The 2D graph and corresponding distance matrix is derived from the distance (in Ångström) between all pair-wise atoms in the conformation. This 2D graph is fully connected weighted graph and weight is distance between pair of atoms. This fully connected graph intricate all bonded and non-bonded interactions. If a structure contains N atoms, the matrix will have size N×N. In graph-theoretic applications the atoms are referred to as vertices and weighted connections are referred to as edges.

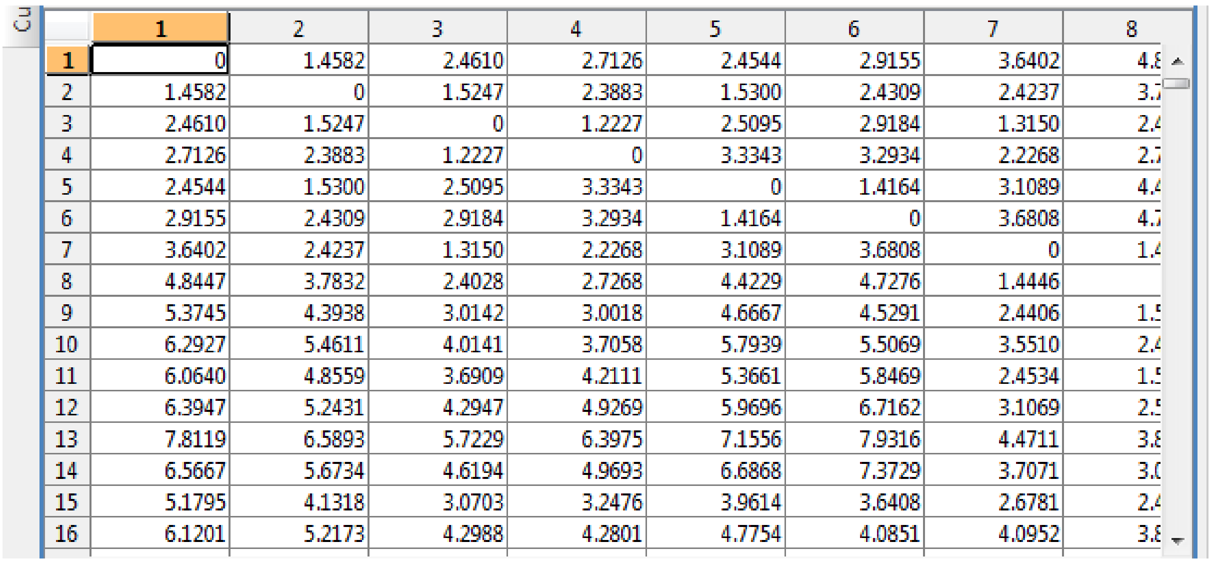
$$D_{NxN} = d_{ij} = (|x_i-x_j| + |y_i-y_j| + |z_i-z_j|) \text{ for } i,j= 1 \text{ to } N$$

where x, y and z are 3D co-ordinated of protein.

- The entries of diagonal are all zero, i.e. $d_{ii} = 0$ for all i=1 to N.
- All the off-diagonal entries are positive ($d_{ij} > 0$ if $i \neq j$),
- The matrix is a symmetric matrix ($d_{ij} = d_{ji}$), and
- $d_{ij} \leq d_{ik} + d_{kj}$ for all i, j, k.

The distance matrix represents all atoms as weighted connectedness of graph. In this graph, each vertex is a representation of an atom (all atoms except hydrogen atoms). The graph thus represents the mathematical relation of spatial proximity for all atoms pairs in 3D space. The proposed distance function is simple Euclidean distance as real values.

For a typical protein of length 200 amino acids, these 200 amino acids of protein 3D structures are converted to corresponding distance matrix. This conversion using MATLAB usually takes less than one second. A visualization of a protein distance matrix is shown in Figure 2.



	1	2	3	4	5	6	7	8
1	0	1.4582	2.4610	2.7126	2.4544	2.9155	3.6402	4.8
2	1.4582	0	1.5247	2.3883	1.5300	2.4309	2.4237	3.7
3	2.4610	1.5247	0	1.2227	2.5095	2.9184	1.3150	2.4
4	2.7126	2.3883	1.2227	0	3.3343	3.2934	2.2268	2.7
5	2.4544	1.5300	2.5095	3.3343	0	1.4164	3.1089	4.4
6	2.9155	2.4309	2.9184	3.2934	1.4164	0	3.6808	4.7
7	3.6402	2.4237	1.3150	2.2268	3.1089	3.6808	0	1.4
8	4.8447	3.7832	2.4028	2.7268	4.4229	4.7276	1.4446	
9	5.3745	4.3938	3.0142	3.0018	4.6667	4.5291	2.4406	1.5
10	6.2927	5.4611	4.0141	3.7058	5.7939	5.5069	3.5510	2.4
11	6.0640	4.8559	3.6909	4.2111	5.3661	5.8469	2.4534	1.5
12	6.3947	5.2431	4.2947	4.9269	5.9696	6.7162	3.1069	2.5
13	7.8119	6.5893	5.7229	6.3975	7.1556	7.9316	4.4711	3.8
14	6.5667	5.6734	4.6194	4.9693	6.6868	7.3729	3.7071	3.0
15	5.1795	4.1318	3.0703	3.2476	3.9614	3.6408	2.6781	2.4
16	6.1201	5.2173	4.2988	4.2801	4.7754	4.0851	4.0952	3.8

Figure 2. Example distance matrix

c) Partitioning of the graph model into sub-graphs

Partitioning of graph into subgraphs is done by clustering of nodes /atoms. Subgraph isomorphism are leads to fold, pattern identification, structural motif recognition. Subgraph is important for function as binding site, structure, folding and identification of similar folds. This will finally help to build or generate knowledge between graph topological and physical properties to protein function.

Identification of clusters is an important operation carried out in the field of unsupervised classification and it requires several iterative steps to complete clustering. Spectral and MCL (Markov Clustering) techniques are popular and used as a general techniques for graph partitioning. Graph spectral method is an important technique and yield unique results by a single numeric computation (Hagen and Kahng, 1992). Further, it can also be used to get clustering information on weighted graphs. These concepts are adopted to obtain non-bonded clusters in protein structures (Kannan and Vishveshwara, 1999; Patra and Vishveshwara, 2000)

The structure of proteins is governed to a large extent by non-covalent, but non-bonded interactions are conferring unique three-dimensional structures of proteins. Analysis of the topological details of atoms in proteins using the clustering analysis, specifically non-bonded atoms in a cluster leads to decipher knowledge of structural confirmation, fold and function.

Detection of clusters/subgraph and isomorphism among them is influential procedure in protein structure comparison. Since graph partitioning is a hard problem and one of the main research areas in clustering. As discussed in disadvantages for optimal size of decomposition of protein structure in aligning SSE approach, we have introduced automatic decomposition of structure into sub-structures. There are two broad categories of methods namely local and global methods. Local methods are the random initial partitioning of the vertex set and rely on properties of initial partitions, which can affect the quality of final solution. Global methods rely on properties of the entire graph. The most common algorithms on global properties are spectral partitioning and Markov Chain Clustering (MCL) as Algorithm-2 (Stijn van Dongen, 2000).

Two algorithms Spectral (Algorithm-1) and MCL (Algorithm-2) have been used in the proposed method. In view of time complexity and accuracy, MCL performed well compared to Spectral as mentioned in the literature. In spectral clustering, number of clusters and size of clusters are influenced by length of the protein, but MCL produces clusters in small range of variation in cluster size and this variation indicates class of proteins.

The eigen values and eigen vectors of adjacent or distance matrix associated with a graph are most important graph spectral parameters, which provide information on the structure and topology of the graph and analysis. Graph spectra are also extensively used in chemical graph theory to derive topological indices such as the resonance energy, molecular orbital energy and topology of electron systems. There is no unique way of identifying graph isomorphism. Graph spectral analysis is one of prime technique in isomorphism. Graph spectral analysis gives information on isomorphism and isomorphic graphs have the same spectra (Vishveshwara *et al.* 2002).

In view of above points, we have employed two graph partitioning or clustering algorithm to extrication a graph into subgraphs. These clusters exhibit properties of folding, non-bonded interaction and conserved motifs, which are more frequently observed as properties for 3D structure confirmation.

Algorithms of Spectral and MCL

<u>Algorithm 1. Automatic spectral clustering (</u>Sanguinetti *et. al.*, 2005)

Given a dataset consisting of n x n symmetric similarity matrix.

1. *Form an affinity matrix of the order nxn .*
2. *Normalize (Laplacian matrix)* $L=D^{-½} S\, D^{-½.}$
3. *Compute k eigen vectors with the largest eigen values of the matrix L and form a matrix X of order nxk.*
4. *Initialize q =2 and two centers from rows of matrix X on maximum value in the 1st and 2nd column.*
5. *Select the first q columns from matrix X and assemble them in n x q as Matrix Y and initialize (q+1)th center at the origin.*
6. *Perform automated k-means clustering with q+1 centers on Y (Use Weighted Mahalanobis Distance procedure).*
7. *If the (q+1)th cluster contains any data points, then there must be at least one extra cluster and (q+1)<given maximum no. of clusters then set q=q+1 and go back to step 5. Otherwise, end the algorithm.*

Procedure. Weighted Mahalanobis Distance (Khaled and Younis 1995; Mao and Jain 1996)

1. *For each c_i, compute the distance of all points x from it as follows:*
   If $c_i c_i^T > 0$ if the centre is not the origin
   $e\text{-}dist(x,c_i)=(x-c_i)\, M\, (x-c_i)^T$
   where $M=(I-\lambda)\,(c_i^T c_i + I\lambda)^{-1}$
   Here λ is the sharpness parameter that controls the elongation (if greater, the clusters are more elongated)
   If the centre is very near the origin, $c_i c_i^T < e$, the distances are measured using the Euclidean distance.
2. *Using this distance measure, assign each point x to the nearest centre. Update the location for each centre by taking the mean of all the data assigned to it.*
3. *Return to step 1 and repeat until there is no change in the location.*

Algorithm 2. Markov Chain clustering (Stijn van Dongen 2000; Enright *et al 2002*)

```
1. G is a graph and create the adjacent matrix
2. set Γ to some value for granularity
3. set M_1 to be the matrix of random walks on G
4. Normalize the matrix
5. Initialize change=1
6. while (change)
   {
      M_2 = M_1 * M_1  //expansion
      M_1 = Γ(M_2)  // inflation
      change = difference(M_1, M_2)
   }
7. Interpret resulting matrix to discover clusters of M_1
```

d) Aligning the sub-graphs between pair of proteins and similarity measure

Two protein graphs can be compared by graph isomorphism detection method, which leads sharing common confirmation. A number of heuristic methods are available for searching a subgraph in the given graph. Tree searching algorithm compares successive subgraph isomorphism by superimposing the graph one over another. The superimpose areas are structurally similar, which may involve structural/functional commonalities.

Two sub-graphs of the same number of nodes (N), they represent two sets of pairwise interaction matrix of size NxN such as if there a connectivity, the value is set as 1(one) otherwise 0 (zero). The comparison of two graphs by comparing the induced set of 0 or 1 showed the connectivity of nodes among subgraphs. Let the clusters of protein-1 and protein-2 are represented by graph G1 and graph G2 respectively. We construct the graph connectivity/interaction matrix G1, by computing the connectivity between each pair of elements i, j $\in C_k$. The connectivity between the two elements i, j, denoted by 1 if these elements are in the same kth cluster (C_k) otherwise 0. It means that, ith will interact with jth if i and j are in the same cluster. Similarly, the G2 will be constructed for protein-2. Once interaction matrix for graph G1 and G2 for all pairs of nodes have been constructed, it can find the similarity/distance between them by using any distance or similarity measures such as Jaccard coefficients, Kullback - Leibler distance and Tanimoto similarity measures etc. This is superimposing of two graphs with best matching by sliding the matrices with window size minimum (M,N). Here, f-measure calculated as harmonic mean of precision and recall. The f-measure value shows the percentage similarity between two proteins.

Given two binary matrices, G1 and G2, the Jaccard coefficient is one of the useful measures for overlapping of G1 and G2 with their attributes. Each attribute of G1 and G2 can either be 0 or 1. The total number of each combination of attributes for both G1 and G2 are specified as follows:

Algorithm 3: Aligning subgraphs to complete graph and similarity measure

- M_{11} represents the total number of attributes where G1 and G2 both are having value 1.
- M_{01} represents the total number of attributes where the attribute of G1 is 0 and the attribute of G2 is 1.
- M_{01} represents the total number of attributes where the attribute of G1 is 1 and the attribute of G2 is 0.
- M_{00} represents the total number of attributes where G1 and G2 both have a value of 0.
- The Jaccard similarity coefficient, $J = M_{11}/(M_{11}+M_{10}+M_{01})$
- The jaccard distance $d_j = 1\text{-}J = (M_{10}+M_{01}) / (M_{11}+M_{10}+M_{01})$
- Precision (p) = $M_{11}/(M_{11}+M_{10})$
- Recall (r) = $M_{11}/(M_{11}+M_{01})$
- f-measure = $2^* p^*r /(p+r)$

RESULTS AND DISCUSSION

The above algorithms of the proposed method including reading of pdb file and generating distance matrix for comparing protein structures are implemented in MATLAB. The performance of the developed methodology has been tested and compared with two existing best methods such as CE and jFATCAT on 100 proteins benchmark dataset with SCOP (Structural Classification Of Proteins) (Murzin *et al.*, 1995).

Interaction matrix represents small clusters of atoms that detect local structure similarity. This reveals bonded and non-bonded interaction of atoms between clusters and within the clusters. Superpositioning of two binary matrices (interaction matrix) shows the performance with global similarity. These clusters have intrinsic topological characteristics of the structures. The proposed method maps cluster to cluster and compares two structures by taking consideration of position of atom in the clusters over set of residue level structural alignment. Majority of 3D structure comparison methods are rely on only C_α atoms, but here all atoms are considered except hydrogen atoms.

The decomposition of SSEs to scaffolds and interfaces rather than single residues could be observed as basic interaction units in the arrangement of structure elements within a protein structure. Similarly, the decomposition of structure to sub-structure/clusters than single residues and SSE, could be favourable interaction of non-bonded residues within a structure as discussed above. Atom positions and associations with other atoms within sub-structure are considered and complete alignment of the structure was used to identify similar geometry and similarity of the two structures.

The proposed method showed improved performance over the existing techniques based on classification accuracy. The reason for the improvement in the accuracy is due to inclusion of all atoms but CE and jFATCAT used only backbone Cα atoms. Non-bonded interaction in a cluster divulge Van der Waals forces and play important role in inclusion of folding information of protein while comparing the 3D structures.

Clusters of bonded and non-bonded atoms

It is important to note that sub-graphs in a graph would result in cluster of atoms composed nearest bonded and non-bonded atoms and disconnection of weak bonded atoms. The Non-bonded interactions are confirming unique three-dimensional structures to proteins. Analysis of the topological details of atoms in proteins using the clustering analysis specifically non-bonded atoms in a cluster leads to decipher knowledge of structural confirmation and folding of protein. The specific interactions between different non-bonded atoms constitute the structural basis for protein stability. The figure 3(a) sows 3D structure of protein 1tos and 3(b) represents graphical representation of 1tos. Figure 3(c) and 3(d) depict clusters where some atoms of residues 1, 7, 8 and 10 are belong to same cluster (5th cluster in figure 3(c) and magenta color in figure 3(d)). This indicates some non-bonded interaction between them.

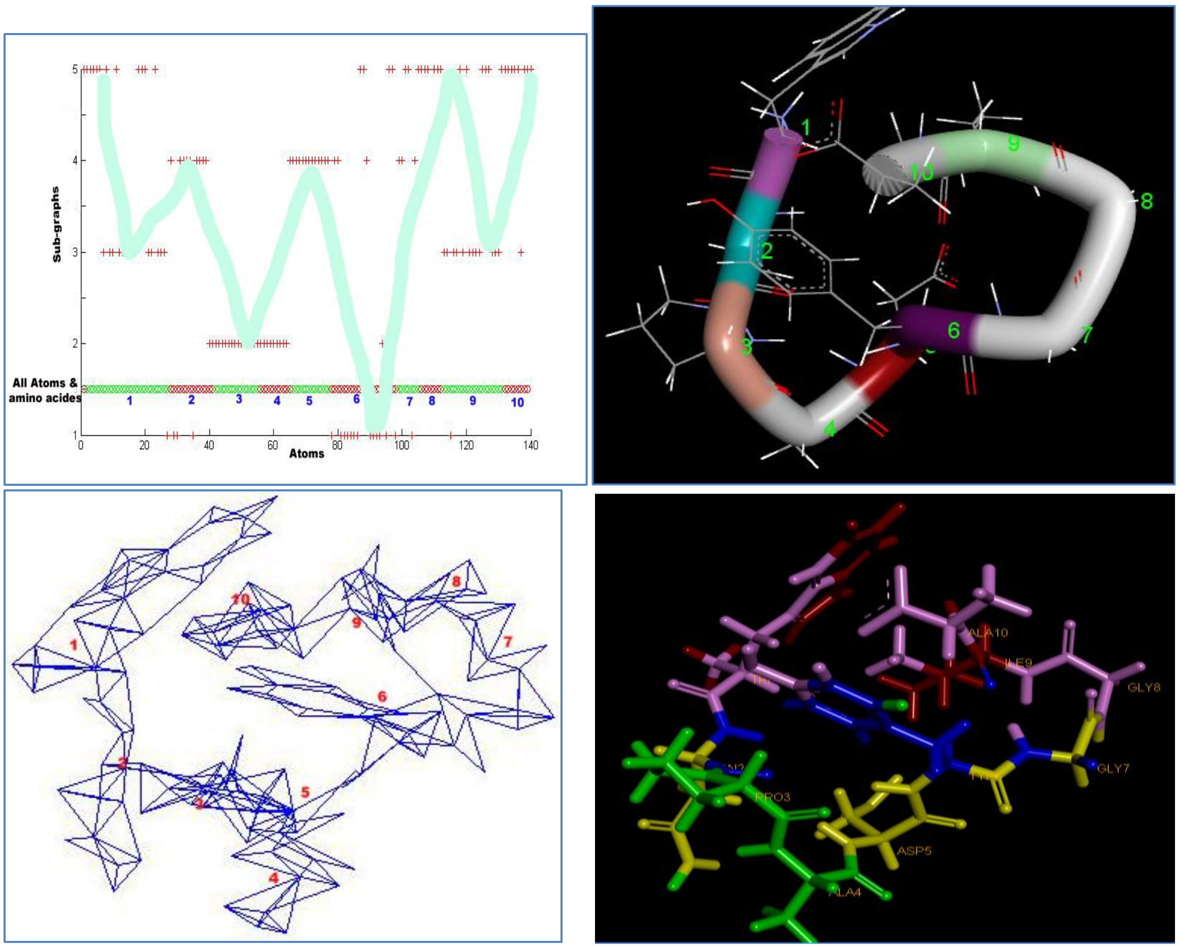


Figure 3. 3D structure of 1tos protein, Graph model, clusters of atoms and cluster presentation.

Similarly, later part of atoms of residues 2 are identified with atoms of residues 4 and 5. Atoms of residues 3 and 6 belong to same cluster. Similarly some atoms of 1th and 10th shares same clusters. These patterns are converted into binary matrix with locations using algorithm-3. Then similar patterns searched in another protein binary matrix. Such patterns convey interaction and 3D confirmation of atoms in structure. Small clusters of atoms detect local structure similarity as well as get global similarity by aligning all sub-graphs. The method maps sub-graph to sub-graph and compares two structures by taking into account the position of atom in the clusters over residue-level (C_α) structural alignment of existing methods. In existing methods, the quality of 3D superposition is often measured by the number of matched C_α atoms. Automatic decomposition using graph portioning is unique from existing fragment decomposition methods and is able to identify local structural similarities and lead to global similarity. Folded polypeptide chain enable to even residues at precisely different position in amino acid sequences to come into contact one another and vice-versa.

Relation between number of clusters and number of SSEs

We observed that number of amino acid and atoms in clusters range 2.5 to 3.0 and 15 to 30 respectively and significant bifurcating level of alpha-helix and beta-sheet combination in the structures (Table 1).

As shown in Table 1, number of atoms and residues per cluster are clearly indicated by main class of protein structure. Number of atoms per cluster is varying with range from 16 to 20 and this range indicated structures have influenced by β-sheet (Class B in SCOP). Numbers of atoms per cluster are in range from 21 to 23 and this range indicated structures have influenced by α-helix and β-sheet (Class C & D in SCOP). Similarly, α-Helix (Class –A of SCOP) are belongs to a range from 23 to 25 atoms per cluster.

Table 1. Benchmark proteins data with number of atoms and residues and sub-graphs by clustering

PDB	pdb total atoms	No. of atoms in A chain	No of C alpha in A Chain	No of subgraphs	Ration of no. of C-alpha in subgraph	Ration of no. of atoms in subgraph	helix	beta	coil
1F56.pdb	2070	690	91	41	2.22	16.83	1	7	9
1FP5.pdb	1618	1617	208	92	2.26	17.58	5	17	29
1VCA.pdb	3106	1553	199	88	2.26	17.65	3	17	19
2OZ4.pdb	5245	2022	266	114	2.33	17.74	5	20	46
1DQT.pdb	3624	906	117	51	2.29	17.76	1	11	13
1IAM.pdb	1436	1435	185	79	2.34	18.16	2	19	48
1L6X.pdb	1949	1658	207	91	2.27	18.22	5	18	37
1ZXQ.pdb	1500	1499	192	82	2.34	18.28	3	18	21
1AOZ.pdb	8732	4366	552	237	2.33	18.42	11	32	43
1BDY.pdb	2354	960	123	52	2.37	18.46	2	8	10
1KDJ.pdb	758	757	102	41	2.49	18.46	2	8	11
UGK.pdb	2190	1090	138	59	2.34	18.47	2	8	10
1EAJ.pdb	1962	1010	124	54	2.30	18.70	2	13	23

HXM.pdb	13756	1611	206	86	2.40	18.73	4	18	9
1KBV.pdb	13746	2291	302	122	2.48	18.78	6	21	27
1XED.pdb	4988	865	111	46	2.41	18.80	3	8	12
1NEU.pdb	931	930	115	49	2.35	18.98	2	11	15
1JMA.pdb	2783	2053	261	108	2.42	19.01	8	14	12
1QFO.pdb	2702	901	115	47	2.45	19.17	3	11	11
1GMI.pdb	1057	1056	135	55	2.45	19.20	2	8	8
HDM.pdb	2927	1482	184	77	2.39	19.25	2	12	17
1GSK.pdb	4044	4043	502	210	2.39	19.25	10	31	40
1CCZ.pdb	1411	1410	171	73	2.34	19.32	2	16	12
2Q5B.pdb	2432	812	105	42	2.50	19.33	4	9	14
UVQ.pdb	3075	1456	182	75	2.43	19.41	2	13	16
1DN0.pdb	6594	1638	215	84	2.56	19.50	4	19	23
RLW.pdb	1001	1000	126	51	2.47	19.61	2	8	49
2ZKM.pdb	1098	986	986	50	2.47	19.61	2	8	8
1KV7.pdb	3561	3560	463	181	2.56	19.67	10	30	39
1RH8.pdb	2317	1162	142	59	2.41	19.69	4	8	5
1A25.pdb	2174	1087	132	55	2.40	19.76	8	20	26
1IUR.pdb	1511	754	88	38	2.32	19.84	3	0	3
1CID.pdb	1381	1380	177	68	2.60	20.29	1	15	16
UOW.pdb	1248	1247	156	61	2.56	20.44	4	8	22
1M6I.pdb	3525	3524	459	171	2.68	20.61	18	28	41
1DJQ.pdb	11480	5740	729	278	2.62	20.65	29	29	54
1P7I.pdb	1721	434	53	21	2.52	20.67	3	0	4
1RSY.pdb	1066	1065	135	51	2.65	20.88	3	8	22
1FCD.pdb	8724	3018	401	144	2.78	20.96	11	25	35
1FEC.pdb	7453	3743	490	178	2.75	21.03	18	25	41
1TRB.pdb	2394	2393	316	113	2.80	21.18	11	19	30
1K5N.pdb	3362	2404	285	113	2.52	21.27	7	17	24
GVD.pdb	491	490	56	23	2.43	21.30	3	0	13
1QAS.pdb	7969	3990	505	187	2.70	21.34	22	27	39
1GES.pdb	6832	3417	450	160	2.81	21.36	18	27	40
1H6V.pdb	22514	3764	490	176	2.78	21.39	19	29	37
1GTE.pdb	30878	7683	1005	359	2.80	21.40	46	54	77
1PUF.pdb	2082	664	79	31	2.55	21.42	3	0	4

2BW4.pdb	2875	2874	374	134	2.79	21.45	6	30	28
1H2B.pdb	5331	2668	343	124	2.77	21.52	17	23	29
1PS9.pdb	5097	5096	671	235	2.86	21.69	26	36	47
1IJW.pdb	915	287	52	13	0.00	22.08	3	0	5
1VJ0.pdb	11302	2830	366	128	2.86	22.11	15	16	30
1RJW.pdb	10216	2554	339	115	2.95	22.21	15	23	28
1PLC.pdb	1546	784	102	35	2.91	22.40	2	10	11
1JVB.pdb	2555	2554	339	113	3.00	22.60	16	23	32
1U78.pdb	1876	815	103	36	2.86	22.64	7	0	8
1BQK.pdb	913	912	124	40	3.10	22.80	3	9	12
1TC3.pdb	1238	435	51	19	0.00	22.89	3	0	8
1GH6.pdb	3627	941	114	41	2.78	22.95	4	0	40
2EZI.pdb	1225	623	75	27	2.78	23.07	4	0	43
2JHF.pdb	5866	2954	392	128	3.06	23.08	18	18	30
1XBL.pdb	1230	624	75	27	2.78	23.11	3	0	14
1ONF.pdb	3457	3456	439	149	2.95	23.19	17	20	37
1YCC.pdb	838	837	107	36	2.97	23.25	5	0	6
1GDT.pdb	4255	1424	183	61	3.00	23.34	9	4	13
1NZ6.pdb	1552	773	94	33	2.85	23.42	7	0	19
1FEX.pdb	938	469	59	20	2.95	23.45	3	0	14
1WJZ.pdb	1453	730	94	31	3.03	23.55	4	0	5
1E29.pdb	1073	1072	135	45	3.00	23.82	7	2	9
1UG2.pdb	1381	698	95	29	3.28	24.07	3	0	3
1M70.pdb	5548	1390	190	57	3.33	24.39	12	0	13
1LQT.pdb	14287	3585	473	147	3.22	24.39	23	19	37
1GV2.pdb	879	878	103	36	2.86	24.39	6	0	13
1QL3.pdb	2928	732	99	30	3.30	24.40	6	0	8
1B0B.pdb	1051	1050	141	43	3.28	24.42	13	15	7
1LFB.pdb	641	640	77	26	2.96	24.62	4	0	5
1IDR.pdb	1910	961	127	39	3.26	24.64	10	0	9
1PKO.pdb	1040	1039	130	42	3.10	24.74	3	10	15
1XG0.pdb	3699	569	76	23	3.30	24.74	6	15	4
1I8O.pdb	848	847	113	34	3.32	24.91	11	9	7
1CTJ.pdb	724	723	91	29	3.14	24.93	6	0	6
1DLW.pdb	837	836	116	33	3.52	25.33	8	0	9

1JBO.pdb	2541	1244	162	49	3.31	25.39	9	0	10
2EZL.pdb	1553	789	99	31	3.19	25.45	5	0	42
1H1O.pdb	2618	1318	172	51	3.37	25.84	12	0	13
1LE8.pdb	1807	414	53	16	3.31	25.88	3	0	4
1ALL.pdb	2399	1198	160	46	3.48	26.04	10	0	11
1C75.pdb	985	527	73	20	3.65	26.35	5	0	6
1FAF.pdb	1297	644	79	24	3.29	26.83	4	0	12
1S69.pdb	969	968	123	36	3.42	26.89	8	0	9
1UX8.pdb	971	970	118	36	3.28	26.94	8	0	9
1C52.pdb	1228	997	131	37	3.54	26.95	8	2	11
MBA.pdb	1083	1082	146	40	3.65	27.05	8	0	9
1H97.pdb	2343	1171	147	43	3.42	27.23	10	0	10
1ASH.pdb	1239	1238	147	45	3.27	27.51	9	0	9
1B8D.pdb	6255	1240	164	45	3.64	27.56	8	0	8
1K61.pdb	2749	486	60	17	3.53	28.59	3	0	7
1A6M.pdb	1336	1335	151	46	3.28	29.02	10	0	9
NGK.pdb	12856	1078	131	37	3.54	29.14	8	0	9

Cluster Analysis

The above analysis confirmed that by observation of non-bonded interaction found in clusters and shows clear indication about association between secondary structures in 3D protein structure and number clusters. Cluster analysis of benchmark SCOP data set of 100 proteins has been carried out to compare the proposed method with CE and jFATCAT. The benchmark data has proteins belongs to all major classes and number of residues are ranging from 51 to 1005. For cluster analysis, a number of clustering techniques such as Hierarchical clustering, K-Means, C-Means, Spectral K-Means have been used and various accuracy metrics are calculated. In this analysis, k-means clustering is found suitable over other clustering techniques. Recall, Precision and f-measure are considered as accuracy metrics. The percentage of the precise clusters indicates the performance of protein 3D structure comparison algorithm.

To find out the potential roles of clusters in aligning and comparing two structures with alignment location, a set of 100 proteins benchmark data has been used. Detailed analysis has been done and compared with existing tools CE and FATCAT. Under this analysis, similarity matrix of 100x100 using proposed method has been constructed. The example data has shown in table 2. Similarly, we have calculated similarity matrix of 100x 100 for best existing method CE and jFATCAT. The K-means cluster technique applied to these three similarity matrices. The clustering results are evaluated with exact classification obtained from SCOP database of benchmark data. The cluster analysis results are presented in table 3 with recall, precision, f-measure and random index as accuracy metrics. Significantly 97% accuracy with CE, 86% similarity with jFATCAT and proposed method performed at 91% accuracy. The proposed algorithm is computationally expensive since it involves up to (|M-N|+1)* Min(M,N) comparisons, where M and N are nodes of the two graphs.

Table 2. Example values from 100X100 similarity scores and alignment position between pair of structures from MCL Graph partitioning method.

Protein-1	Protein-2	Similarity	Aligned position
1DLW.pdb	1DLW.pdb	1	1
1DLW.pdb	1S69.pdb	0.5097	133
1DLW.pdb	1NGK.pdb	0.539	243
1DLW.pdb	1UX8.pdb	0.47	135
1DLW.pdb	1B0B.pdb	0.531	215
1DLW.pdb	1H97.pdb	0.4697	336
1DLW.pdb	1A6M.pdb	0.4043	500
1DLW.pdb	1MBA.pdb	0.5133	247
1DLW.pdb	1ASH.pdb	0.5	403
1DLW.pdb	1JBO.pdb	0.5435	409
1DLW.pdb	1ALL.pdb	0.4792	363
1DLW.pdb	1B8D.pdb	0.5364	405
1DLW.pdb	1XG0.pdb	0.4819	268
1DLW.pdb	1XBL.pdb	0.498	213

Table 3. Performance metrics obtained from proposed method and CE and jFATCAT.

Method	Time (hrs)	Metric	Values
CE	126.18	Precision	0.9600
		Recall	0.9333
		F measure	0.9465 [1]
		R-index	**0.9694**
jFACTCAT	019.14	Precision	0.6653
		Recall	0.6043
		F measure	0.6333 [3]
		R-index	**0.8554**
GT Method (proposed method)	500.00	Precision	0.7252
		Recall	0.7979
		F measure	0.7598 [2]
		R-index	**0.9054**

CONCLUSIONS

In this study, a novel methodology/algorithm has been developed based on graph partitioning technique by including 3D co-ordinates of all atoms of protein. The proposed algorithm has been implemented in MATLAB to assess the performance of the algorithm. The methods encompass converting 3D structure to 2D graph, partitioning of the graph into sub-graph and alignment of sub-graphs. Prime notion of the method is the decomposition of structure to clusters than single residues and SSE. There could be a base interaction of non-bonded residues in the arrangement of structure elements within a structure. These interaction leads protein fold space and arrangement of SSE elements in 3D space. The aligned structures are based on atoms positions and association with other atoms within clusters and between clusters to identify similar geometry and similarity of the two structures. The proposed method performed better in terms of classification accuracy due to inclusion of all atoms but CE and jFATCAT uses only backbone C-α atoms and non-bonded atoms. This may play an important role in inclusion of folding information of protein while comparing the 3D structures.

CHAPTER –III: GRAPH PROPERTIES AND MACHINE LEARNING TECHNIQUES.

INTRODUCTION

Construction of graph, database of graph, graph mining, pattern recognition are rich fields of computational techniques to study structures, topologies and properties of graphs. These techniques provide a good asset in bioinformatics for transforming biological data into graphs and decipher biological knowledge hidden in biological structure data. In this chapter, we have demonstrated the way of converting a protein 3D structure into graphs using various graph properties such as total degree, maximum degree, no of adjacencies, average number of degree, cluster coefficient, graph energy, spectrum and number of components etc. Further, we have exploited the graph properties and data mining technique to perform complex studies on protein 3D structure. The proposed method is fast in terms of computational time complexity. As far as accuracy is concerned, there is an improved in proposed method over jFATCAT method. In spite of gaining a small amount of improvement in accuracy, his method has further scope to improve accuracy by including more graph properties.

MATERIAL AND METHODS

Basic Graph Definition

A graph is a symbolic representation of a network or connected components. It implies an abstraction of the reality so it can be simplified as a set of linked nodes or components.

Graph theory is a branch of mathematics concerned about how networks can be encoded and their properties are measured. It has been enriched in the last decades by growing influences of social networks and other complex networks.

The origins of graph theory can be traced to Leonhard Euler who devised it in 1735 as a problem that known as the "Seven Bridges of Konigsberg". In this problem, someone had to cross all the bridges by visiting each bridge only once in a continuous sequence, a problem the Euler proved to have no solution by representing it as a set of nodes and links. This led the foundation of graph theory and its subsequent improvements. Initially, graph theory applications on most networks have spatial basic, namely road, transit and rail networks. This it is not necessarily the case for all transportation networks. Later, this has been extended to telecommunication system such as Mobile telephone networks or the Internet. In the current time, this is being extended in bioinformatics to study the hidden phenomenon of gene regulatory network, gene interaction, protein-protein interactions, and etc.

The transformation of protein 3D structures into graphs 2D model, it is straight forward to consider the relevant chemical interactions of the proteins. Chemical interactions are the electrostatic forces that hold atoms and residues together, stabilizing proteins and forming molecules that give them their 3D confirmation. These interactions are mainly:

- Covalent bonds between two atoms sharing a pair of valence electrons.
- Ionic bonds between oppositely charged components.
- Hydrogen bonds between two partially negative charged atoms sharing partially positive charged hydrogen.

- Hydrophobic interactions where hydrophobic amino acids in the protein closely link their side chains together.
- Van der Waals forces that represent transient and weak electrical attraction of one atom for another when electrons are fluctuating.

The 2D graph model is abstraction of these properties and indeed these properties are transferred into graph properties (Holm and Sander 1993).

A novel method has been developed to study complexity of 3D structure of protein and calculate the similarity between two protein structures using graph properties of protein 3D structures. Figure 1 represents the workflow for the proposed method consisting of four steps namely, i) Representing 3D protein structure into 2D graph model, ii) Calculating graph properties iii) Calculate similarity and iv) Evaluation by cluster/classification.

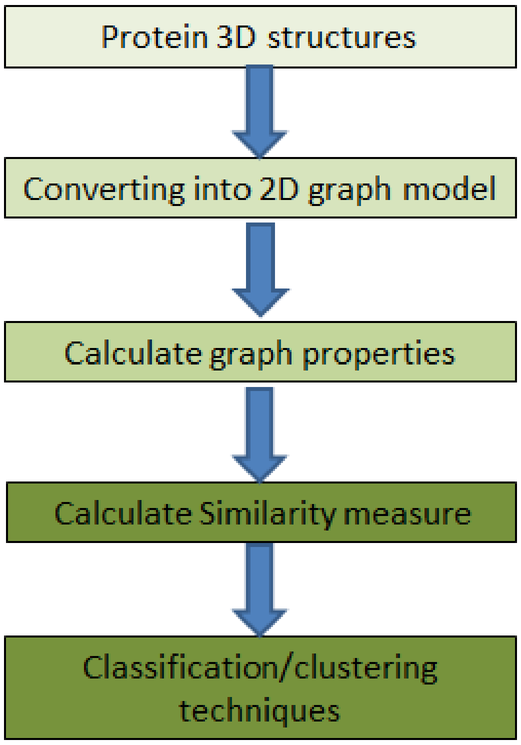

Protein 3D structures
Converting into 2D graph model
Calculate graph properties
Calculate Similarity measure
Classification/clustering techniques

Fig. 1: Workflow of proposed method

All functions regarding reading PDB file, selection of module, selection of chain and reading of X, Y and Z coordinates, converting 3D (X, Y and Z) co-ordinates to 2D graph model, calculating all above mentioned properties and final calculation of similarity measures are implemented in MATLAB. For evaluating the proposed 3D structure comparison method, we have taken 100 proteins benchmark SCOP dataset (as mentioned in Chapter-I).

Construction of Graph models

In PDB file, all atoms are labelled with coordinates and these coordinates are based on Ångström. The 2D graph and corresponding distance matrix is derived from the distance (in Ångström) between all pair wise atoms in the conformation. This 2D graph is fully connected weighted graph and weight is denoted as distance between pair of atoms. This fully connected graph intricate all bonded and non-bonded interactions. If a structure contains N atoms, the matrix will have size N×N. In graph-theoretic applications, the atoms are referred to as vertices and weighted connections are referred as edges.

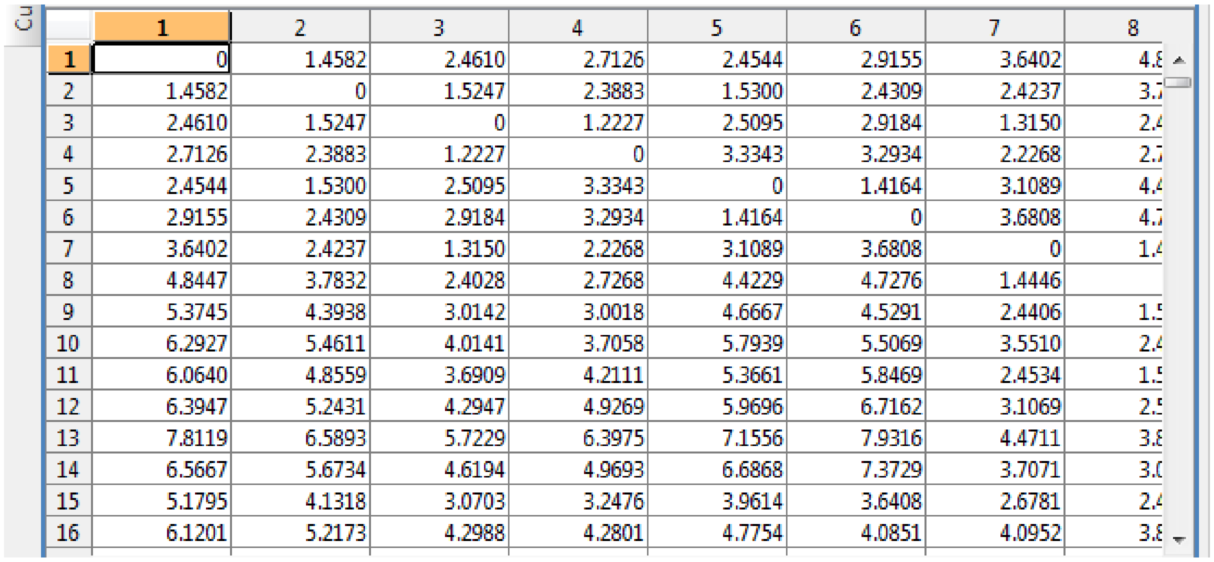
$$D_{NxN} = d_{ij} = (|x_i\text{-}x_j| + |y_i\text{-}y_j| + |z_i\text{-}z_j|) \text{ for } i,j = 1 \text{ to } N \text{ or}$$

$$D_{NxN} = d_{ij} = \|x_i - x_j\|_2^2 = \sqrt{(x_i - x_j)^2 + (y_i - y_j)^2 + (z_i - z_j)^2}$$

- The entries of diagonal are all zero, i.e. $d_{ii} = 0$ for all i=1 to N.
- All the off-diagonal entries are positive ($d_{ij} > 0$ if $i \neq j$),
- The matrix is a symmetric matrix ($d_{ij} = d_{ji}$), and
- $d_{ij} \leq d_{ik} + d_{kj}$ for all i, j, k.
- Adjacency matrix d_{ij}<threshold $a_{ij}=1$ otherwise $a_{ij}=0$

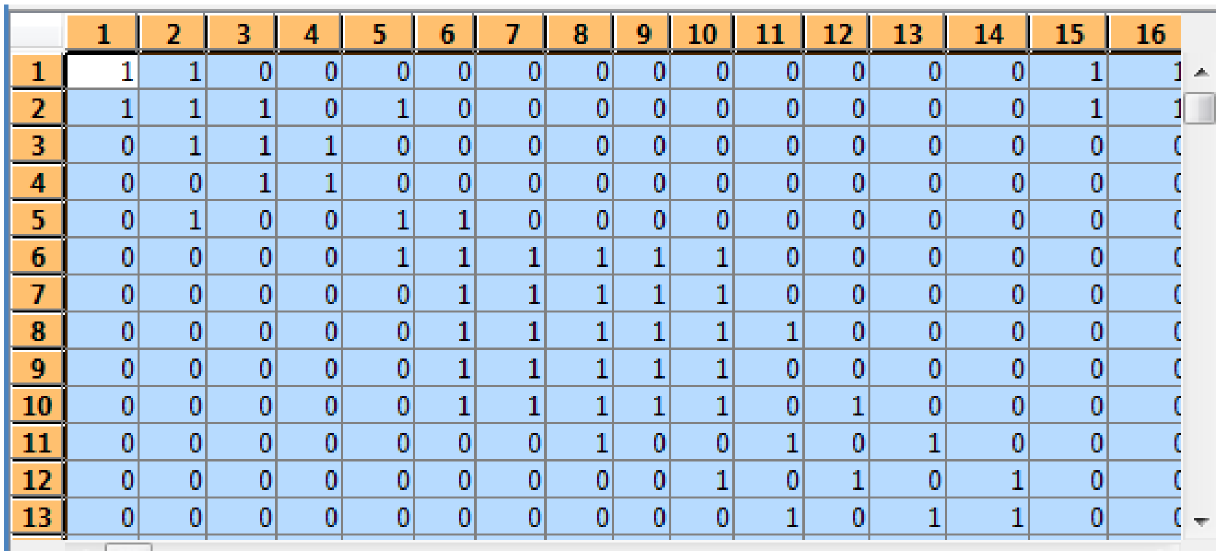
The distance matrix represents all atoms as weighted connectedness of graph. In this graph, each vertex is a representation of an atom (all atoms except hydrogen atoms). The graph thus represents the mathematical relation of spatial proximity for all atoms pairs in 3D space. The distance function used in the study is simple Euclidean distance by considering its real values.

For a typical protein of length 200 amino acids, these 200 amino acids of protein 3D structure is converted to corresponding distance matrix. This conversion using MATLAB usually takes less than one second. A visualization of a protein distance matrix is shown in Figure 2. A visualization of adjacency matrix shown in Figure 3.



	1	2	3	4	5	6	7	8
1	0	1.4582	2.4610	2.7126	2.4544	2.9155	3.6402	4.8
2	1.4582	0	1.5247	2.3883	1.5300	2.4309	2.4237	3.7
3	2.4610	1.5247	0	1.2227	2.5095	2.9184	1.3150	2.4
4	2.7126	2.3883	1.2227	0	3.3343	3.2934	2.2268	2.7
5	2.4544	1.5300	2.5095	3.3343	0	1.4164	3.1089	4.4
6	2.9155	2.4309	2.9184	3.2934	1.4164	0	3.6808	4.7
7	3.6402	2.4237	1.3150	2.2268	3.1089	3.6808	0	1.4
8	4.8447	3.7832	2.4028	2.7268	4.4229	4.7276	1.4446	
9	5.3745	4.3938	3.0142	3.0018	4.6667	4.5291	2.4406	1.5
10	6.2927	5.4611	4.0141	3.7058	5.7939	5.5069	3.5510	2.4
11	6.0640	4.8559	3.6909	4.2111	5.3661	5.8469	2.4534	1.5
12	6.3947	5.2431	4.2947	4.9269	5.9696	6.7162	3.1069	2.5
13	7.8119	6.5893	5.7229	6.3975	7.1556	7.9316	4.4711	3.8
14	6.5667	5.6734	4.6194	4.9693	6.6868	7.3729	3.7071	3.0
15	5.1795	4.1318	3.0703	3.2476	3.9614	3.6408	2.6781	2.4
16	6.1201	5.2173	4.2988	4.2801	4.7754	4.0851	4.0952	3.8

Fig. 2: Example distance matrix



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1
2	1	1	1	0	1	0	0	0	0	0	0	0	0	0	1	1
3	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
5	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0
7	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0
8	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0
9	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0
10	0	0	0	0	0	1	1	1	1	1	0	1	0	0	0	0
11	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0
12	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0
13	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0

Figure 3: Example adjacency matrix

Graph properties

We have considered both above graph matrices for calculation of following properties to study the complexity of 3D structure

1. Average degree deg(G): Minimum and maximum by degrees of all nodes.

$$\deg(G) = \frac{1}{n}\sum_{i=1}^{n}(\deg(u_i))$$

deg(u_i) is degree of node u_i and n is total number of nodes in the graph G. Similarly, minimum and maximum degree in the graph G can also be calculated

2. Algebraic connectivity of a graph: the second smallest eigenvalue of the Laplacian.
3. Average path length for a network (The average shortest path): Calculate length of the shortest paths between u and every other node v in G and then calculate average path by summation of all shortest path and divide by nxn.
4. Clustering coefficient: This is based on triangle motifs count and local clustering.

$$c(u) = \frac{2e_u}{k_u(k_u - 1)} \qquad C(G) = \frac{1}{n}\sum_{i=1}^{n} c(u_i)$$

where k_u is the number of neighbors of u and e_u is the number of connected pairs of neighbors

5. The ith component of the eigenvector corresponding to the greatest eigen value.
6. Graph energy defined as: the sum of the absolute values of the real components of the eigenvalues.
7. The eigenvalues of the Laplacian of the graph (2nd to 6th eigenvalues).
8. Closeness centrality: Tracks how close a given node is to any other node. The closeness centrality measures how fast information spreads from a given node to other reachable nodes in the graph. For a node u, it represents the reciprocal of the average shortest path length between u and every other reachable node in the graph.

$$C_c(u) = \frac{(n-1)}{\sum_{vs(V|u)} d(u,v)}$$

where *d(u, v)* is the length of the shortest path between the nodes u and v.

$$C_c(G) = \frac{1}{n}\sum_{i=1}^{n} c(u_i) \qquad\qquad C_c(G) = \frac{1}{n}\sum_{k=1}^{n} C_c(u_k)$$

9. Pi Index: The relationship between the total length of the graph L(G) and the distance along its diameter, an indicator of the shape of a graph.
10. Beta Index: Measures the level of connectivity in a graph and is expressed by the relationship between the number of links (e) over the number of nodes (v). =e/v
11. Graph Density: A *"potential connection"* is a connection that could potentially exist between two "nodes", regardless of whether or not it actually does. GD=Actual connection/potential connection.
12. Synchronizability (S) describes the graph's capacity to synchronize and is calculated from the eigen values of the graph's Laplacian matrix L, L = D -A, where D represents a diagonal matrix containing the nodal degrees. The synchronizability is defined as the ratio between the first non-zero eigenvalue and the largest eigenvalue (S= EV_2/EV_max)

Using above properties a features table for all benchmark proteins has been prepared (example table- Table-1). All graph properties mentioned in above are calculated and represented in the table (Table-2). In this calculation, all properties are not significantly different. Based on visual screening and manual selection, we have dropped some of properties which are not important to differentiate characteristics of proteins. Results shown in table (Table-3) are based on selected properties which are ominously influence to measure similarity between pair of proteins.

Table -1:Example protein graph features/properties table.

Features →	F1,	F2,	……	Fj	…………	Fk
P1						
P2						
:						
Pi						
:						
Pn						

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Table 2. All graph properties as described above of all benchmark data

	Total deg	Max deg	Min deg	no of adjances	algebric connectivity	Ave_no Deg totaldeg1	Max deg1	Min deg1	Ave path length	Ave degree	cluster coeff1	cluster coeff	diameter	max eigencentrality	Graph energy	Radius	spectrum	no of connect comps
1BRF.pdb	1836	8	3	1424	0.0002	1447.411	5.6667	2.3333	56.6069	4.4563	1.2422	0.8502	161	0.4672	578.9776	81	7.1221	1
1BC9.pdb	23500	14	4	20239	0.0018	20938.04	8.8	3.5	21.3405	7.2064	0.5711	0.763	64	0.2707	5733.5	33	13.2302	1
1DI1.pdb	20886	7	3	16195	0	16460.73	5.5	2.3333	Inf	4.4524	1.2867	0.8539	Inf	0.4462	6540.889	Inf	6.4555	5
1dly.pdb	4148	7	3	3214	0	3269.469	5.5	2.3333	122.39	4.4411	1.2834	0.8548	366	0.4462	1301.86	183	6.3701	1
1ecd.pdb	4698	8	3	3651	0	3706.316	5.6667	2.3333	137.4245	4.4871	1.2611	0.8513	410	0	1471.192	205	7.1221	1
1EHS.pdb	6360	14	3	5313	0.0001	5524.963	7.875	2.3333	45.8564	6.0745	0.6806	0.8161	156	0.2386	1648.759	78	13.0974	1
1FQT.pdb	8226	11	3	6469	0	6587.333	8.3333	2.3333	Inf	4.6818	1.1123	0.852	Inf	0.372	2481.342	Inf	10.3512	2
1gvh.pdb	13794	7	3	10698	0	10871.88	5.5	2.3333	399.9704	4.4554	1.2859	0.8532	1191	0.4462	4329.277	596	6.2547	2
1H3E.pdb	23494	8	3	18414	0	18884.76	6	2.3333	Inf	4.6248	1.0919	0.8471	Inf	0.4265	7295.912	Inf	7.6006	5
1hbi.pdb	23428	9	3	18348	0	18804.33	6.4286	2.3333	Inf	4.6118	1.0863	0.8459	Inf	0	7301.391	Inf	8.3593	6
1IAA.pdb	23470	8	3	18390	0	18847.25	6.25	2.3333	Inf	4.6201	1.0897	0.8458	Inf	0.4163	7296.313	Inf	7.7878	5
1IAB.pdb	23462	8	3	18382	0	18837.55	6.25	2.3333	Inf	4.6185	1.0898	0.8457	Inf	0.4163	7297.86	Inf	7.7878	5
1IAC.pdb	23488	8	3	18408	0	18868.05	6.25	2.3333	Inf	4.6236	1.0867	0.8457	Inf	0.4163	7302.569	Inf	7.7878	5
1IQW.pdb	23334	8	3	18254	0	18713.54	6	2.3333	Inf	4.5933	1.0737	0.8436	Inf	0.4265	7318.565	Inf	7.6006	5
1JHK.pdb	23380	8	3	18300	0	18765.76	6	2.3333	Inf	4.6024	1.0746	0.8442	Inf	0.4265	7315.21	Inf	7.6006	7
1K9O.pdb	22720	8	3	17640	0	17978.15	6	2.3333	Inf	4.4724	1.207	0.8511	Inf	0.4294	7160.792	Inf	7.5995	5
1KA8.pdb	22632	8	3	17552	0	17863.14	6	2.3333	Inf	4.4551	1.2209	0.8479	Inf	0	7173.011	Inf	7.5995	3
1mbd.pdb	22776	8	3	17696	0	18017.1	6	2.3333	Inf	4.4835	1.2228	0.8497	Inf	0	7173.021	Inf	7.5995	3

1MCN.pdb	22622	8	3	17542	0	17854.57	6	2.3333	Inf	4.4531	1.2195	0.8487	Inf	0	7162.108	Inf	7.5995	4
1MLB.pdb	22874	12	3	17794	0	18123.94	9.3333	2.3333	Inf	4.5028	1.1939	0.8488	Inf	0	7180.06	Inf	11.0926	4
1MN8.pdb	23158	9	3	18078	0	18437.33	6.1667	2.3333	Inf	4.5587	1.1957	0.8503	Inf	0.4304	7182.738	Inf	8.1749	4

Table 3. Selected graph properties as described above of all benchmark data

	Total Degree	Max Degree	No. of adjances	Aveerage No. of deg (totaldeg1)	Maximum Degree (maxdeg1)	Average degree	cluster coeff1	cluster coeff2	max eigen centrality	Graph energy	spectrum	no of connect comps
1BRF.pdb	1836	8	1424	1447.411	5.6667	4.4563	1.2422	0.8502	0.4672	578.9776	7.1221	1
1BC9.pdb	23500	14	20239	20938.04	8.8	7.2064	0.5711	0.763	0.2707	5733.5	13.2302	1
1DI1.pdb	20886	7	16195	16460.73	5.5	4.4524	1.2867	0.8539	0.4462	6540.889	6.4555	5
1dly.pdb	4148	7	3214	3269.469	5.5	4.4411	1.2834	0.8548	0.4462	1301.86	6.3701	1
1ecd.pdb	4698	8	3651	3706.316	5.6667	4.4871	1.2611	0.8513	0	1471.192	7.1221	1
1EHS.pdb	6360	14	5313	5524.963	7.875	6.0745	0.6806	0.8161	0.2386	1648.759	13.0974	1
1FQT.pdb	8226	11	6469	6587.333	8.3333	4.6818	1.1123	0.852	0.372	2481.342	10.3512	2
1gvh.pdb	13794	7	10698	10871.88	5.5	4.4554	1.2859	0.8532	0.4462	4329.277	6.2547	2
1H3E.pdb	23494	8	18414	18884.76	6	4.6248	1.0919	0.8471	0.4265	7295.912	7.6006	5
1hbi.pdb	23428	9	18348	18804.33	6.4286	4.6118	1.0863	0.8459	0	7301.391	8.3593	6
1IAA.pdb	23470	8	18390	18847.25	6.25	4.6201	1.0897	0.8458	0.4163	7296.313	7.7878	5
1IAB.pdb	23462	8	18382	18837.55	6.25	4.6185	1.0898	0.8457	0.4163	7297.86	7.7878	5
1IAC.pdb	23488	8	18408	18868.05	6.25	4.6236	1.0867	0.8457	0.4163	7302.569	7.7878	5
1IQW.pdb	23334	8	18254	18713.54	6	4.5933	1.0737	0.8436	0.4265	7318.565	7.6006	5
1JHK.pdb	23380	8	18300	18765.76	6	4.6024	1.0746	0.8442	0.4265	7315.21	7.6006	7
1K9O.pdb	22720	8	17640	17978.15	6	4.4724	1.207	0.8511	0.4294	7160.792	7.5995	5
1KA8.pdb	22632	8	17552	17863.14	6	4.4551	1.2209	0.8479	0	7173.011	7.5995	3
1mbd.pdb	22776	8	17696	18017.1	6	4.4835	1.2228	0.8497	0	7173.021	7.5995	3

1MCN.pdb	22622	8	17542	17854.57	6	4.4531	1.2195	0.8487	0	7162.108	7.5995	4
1MLB.pdb	22874	12	17794	18123.94	9.3333	4.5028	1.1939	0.8488	0	7180.06	11.0926	4
1MN8.pdb	23158	9	18078	18437.33	6.1667	4.5587	1.1957	0.8503	0.4304	7182.738	8.1749	4
1oj6.pdb	22708	8	17628	17944.05	6	4.4701	1.2457	0.8516	0	7131.944	7.5995	3
1P4X.pdb	22644	8	17564	17868.16	6	4.4575	1.2574	0.8522	0	7119.181	7.5995	3
1PSR.pdb	22922	9	17842	18147.06	6.75	4.5122	1.2386	0.8521	0.4215	7132.165	8.5654	3
1PZ8.pdb	24598	8	19114	19446.47	5.8333	4.4854	1.2856	0.8567	0	7638.606	7.3923	8
1PZ9.pdb	24696	8	19212	19549.95	5.8333	4.5033	1.2779	0.857	0	7644.012	7.3923	6
1QGJ.pdb	25724	10	20240	20625.08	7.7778	4.6907	1.0913	0.8532	0.3357	7660.732	10.144	3
1QN2.pdb	25176	10	19692	20051.9	7.7778	4.5908	1.142	0.8527	0.3357	7677.794	10.144	4
1QUU.pdb	25300	10	19816	20165.28	7.7778	4.6134	1.1622	0.8551	0.3357	7643.699	10.144	3
1RIE.pdb	25298	10	19814	20168.53	7.7778	4.6131	1.1525	0.854	0.3357	7658.223	10.144	2
1RQI.pdb	24518	9	19034	19351.2	6.4286	4.4708	1.2866	0.8565	0.4088	7635.934	8.4238	3
1rte.pdb	24916	11	19432	19773.44	7.9	4.5434	1.2082	0.8555	0.3576	7657.405	10.2365	3
1TOS.pdb	25208	12	19724	20093.07	7.9	4.5966	1.1552	0.8544	0.3576	7688.755	11.2812	3
1urv.pdb	25080	12	19596	19939.4	9.7273	4.5733	1.1554	0.8537	0.302	7690.002	12.2145	3
1ux8.pdb	25168	12	19684	20032.65	9.7273	4.5894	1.1512	0.8537	0.302	7692.64	12.2145	3
1VCK.pdb	25102	12	19618	19967.82	9.7273	4.5773	1.1526	0.8538	0.302	7691.909	12.2145	3
1WOV.pdb	24474	8	18990	19308.61	5.8333	4.4628	1.2808	0.855	0	7655.166	7.3923	4
1WOW.pdb	24390	8	18906	19230.68	5.8333	4.4475	1.2902	0.8555	0	7642.503	7.3923	5
1WOX.pdb	24404	8	18920	19244.7	5.8333	4.45	1.2893	0.8555	0	7640.233	7.3923	5
1X3A.pdb	28568	14	23084	23674.4	8.5	5.2093	0.7899	0.8321	0.3108	8163.846	7.3923	5
1XG7.pdb	17234	8	13352	13578.19	5.5	4.4395	1.2667	0.8526	0	5430.895	7.0947	11
1Y2O.pdb	17054	7	13172	13379.18	5.5	4.3931	1.3228	0.8557	0.4462	5392.577	6.3078	16
1YLL.pdb	28898	11	22788	23214.12	8.4	4.7296	1.1276	0.8557	0.3538	8548.02	10.7331	12
1YV0.pdb	28120	10	22010	22388.49	7.7778	4.6023	1.1933	0.8549	0.3844	8541.531	9.6247	8
1Z77.pdb	28012	10	21902	22284.98	7.7778	4.5846	1.1902	0.8542	0.3844	8547.656	9.6247	14
2A1K.pdb	27852	10	21742	22120.06	7.7778	4.5584	1.1969	0.8535	0.3844	8544.077	9.6247	6

2B0Z.pdb	27982	10	21872	22250.99	7.7778	4.5797	1.2034	0.8543	0.3844	8534.36	9.6247	6
2BBA.pdb	28058	10	21948	22340.2	7.7778	4.5921	1.1906	0.8541	0.3844	8549.307	9.6247	6
2CJ8.pdb	27876	10	21766	22157.26	7.7778	4.5624	1.1937	0.8544	0.3844	8546.543	9.6247	6
2EFK.pdb	27892	10	21782	22149.08	7.7778	4.565	1.2185	0.8555	0.3844	8511.593	9.6247	11
2gdm.pdb	28000	10	21890	22274.18	7.7778	4.5827	1.2009	0.855	0.3844	8535.311	9.6247	9
2GH2.pdb	27992	10	21882	22263.11	7.7778	4.5813	1.2082	0.8551	0.3844	8525.556	9.6247	8
2H6B.pdb	27886	10	21776	22160.51	7.7778	4.564	1.185	0.8531	0.3844	8558.437	9.6247	5
2A1K.pdb	15184	7	11751	11938.99	5.5	4.423	1.2689	0.8515	0.4461	4803.38	6.4339	2
2B0Z.pdb	15314	8	11881	12069.92	5.5	4.4608	1.2807	0.8529	0.4461	4793.663	7.1148	3
2BBA.pdb	15390	10	11957	12159.13	7	4.483	1.2517	0.8525	0	4808.609	9.5105	3
2CJ8.pdb	15208	8	11775	11976.19	5.7143	4.4299	1.2612	0.8531	0.4003	4805.846	7.2328	3
2EFK.pdb	15224	8	11791	11968.01	5.5	4.4346	1.3161	0.855	0.4461	4770.896	7.0862	8
2gdm.pdb	15332	9	11899	12093.11	7	4.4661	1.2748	0.8541	0.4104	4794.614	8.2818	6
2GH2.pdb	15324	8	11891	12082.05	5.5	4.4637	1.2909	0.8543	0	4784.858	7.0862	5
2H6B.pdb	16850	10	13052	13267.75	6.8571	4.4365	1.2486	0.8513	0.4181	5324.909	9.4636	3
2H6B.pdb	16850	10	13052	13267.75	6.8571	4.4365	1.2486	0.8513	0.4181	5324.909	9.4636	3
2HVC.pdb	16910	10	13112	13328.32	6.8571	4.4523	1.2574	0.8521	0.4181	5310.174	9.4636	2
2HYJ.pdb	16922	10	13124	13345.5	6.8571	4.4555	1.2651	0.8536	0.4181	5304.897	9.4636	2
2IBN.pdb	17234	10	13432	13667.17	7.1111	4.5329	1.23	0.8526	0.4084	5320.878	9.6136	26
2IEK.pdb	17012	9	13210	13432.41	7.1111	4.4745	1.2545	0.8526	0.2989	5315.439	9.3568	23
2IOL.pdb	17014	9	13212	13437.02	7.1111	4.475	1.2753	0.8544	0.3733	5305.335	9.3568	20
2J0N.pdb	16892	9	13090	13310.06	7.1111	4.4429	1.256	0.8524	0.3733	5327.032	9.3568	9
2NXN.pdb	17070	9	13268	13511.13	7.1111	4.4897	1.2468	0.853	0.3733	5338.253	9.3568	10
2P8T.pdb	16924	9	13122	13351.98	7.1111	4.4513	1.2578	0.853	0.3733	5325.32	9.3568	10
2Q0A.pdb	17086	9	13284	13497.2	7.1111	4.494	1.2387	0.8514	0.3733	5324.444	9.3568	5
2RD6.pdb	16928	9	13126	13346.42	7.1111	4.4524	1.2606	0.8528	0.3733	5316.998	9.3568	5
2VOD.pdb	16956	9	13154	13392.57	7.1111	4.4598	1.2226	0.8505	0.3733	5345.501	9.3568	5
2ZP0.pdb	20270	8	15726	16002.89	5.5	4.4608	1.2629	0.8533	0.4462	6357.017	7.1113	11

3BGE.pdb	20358	10	15814	16097.03	7.1111	4.4802	1.2227	0.8517	0	6369.463	9.5143	7
3CJR.pdb	20224	8	15680	15968.26	5.5	4.4507	1.2522	0.8527	0	6373.501	7.0807	7
3ELN.pdb	20614	13	16070	16361.93	9.8	4.5365	1.1919	0.8521	0.3526	6380.827	12.0674	6
3MDS.pdb	20374	8	15830	16102.99	5.6667	4.4837	1.2674	0.8531	0	6363.934	7.1221	5
3SGO.pdb	20362	8	15818	16091.1	5.6667	4.4811	1.2698	0.8534	0	6360.107	7.1221	5
4hhb.pdb	21670	9	17126	17583.55	5.7143	4.7689	1.0598	0.8544	0	6553.378	8.2732	23
1GMI.pdb	4716	7	3660	3725.217	5.5	4.4659	1.2582	0.8541	0	1476.916	6.5457	2
2ZKM.pdb	25242	7	19539	19856.47	5.5	4.4261	1.2742	0.8517	0.4462	7972.134	6.3099	7
1RSY.pdb	25256	8	19553	19871.47	5.5	4.4285	1.2734	0.8518	0	7974.414	7.1561	6
1UOW.pdb	25304	8	19601	19924.36	5.5	4.437	1.2705	0.8522	0	7973.845	7.2531	6
1UGK.pdb	31614	14	25911	26556.5	8.8889	5.5434	0.689	0.8142	0.344	8808.687	13.2684	4
1RH8.pdb	31854	14	26151	26851.56	9.1818	5.5855	0.6804	0.8136	0.2372	8834.991	13.2903	4
1A25.pdb	25770	14	20067	20414.55	7.9	4.5187	1.166	0.8496	0.3717	8027.881	13.1192	4
2JHF.pdb	27464	14	21599	22015.97	10	4.6827	1.0677	0.8503	0.3476	8295.283	13.7352	4
1JVB.pdb	26620	12	20755	21136.9	8.6364	4.5388	1.1673	0.8525	0.3416	8240.255	11.8887	10
1H2B.pdb	26238	9	20373	20727.43	6.8889	4.4737	1.2739	0.8553	0.4071	8185.585	8.6772	4
1RJW.pdb	45296	7	35081	35698.71	5.5	4.4343	1.2692	0.8541	0	14279.89	6.4567	8
1VJ0.pdb	50438	9	39137	39800.71	7.125	4.4631	1.2746	0.8545	0	15780.67	8.663	8
1DJQ.pdb	51504	8	40025	40701.35	5.5	4.4868	1.2819	0.855	-0.2714	20343.8	7.9181	20
1ONF.pdb	15348	8	11892	12087.11	5.1667	4.441	1.2742	0.8538	0.4627	4828.047	7.0854	3
1GES.pdb	30514	11	23683	24101.5	7.7778	4.467	1.2565	0.8542	0.372	9571.008	10.4101	4
1FEC.pdb	33466	10	26014	26463.28	8.3333	4.4909	1.2497	0.8548	0.3033	10406.25	10.3194	6

Similarity measures

A similarity matrix has been generated by 1/ (1+e-dist), is an $n \times n$ matrix representing the spacing of a set of n points in Euclidean space. where e-dist is an **Euclidean distance** and the points are defined on m-dimensional proteins graph properties.

$$\text{e-dist (i,j)} = \|p_i - p_j\|_2^2 = \sqrt{(p_{i1} - p_{j1})^2 + (p_{i2} - p_{j2})^2 + (p_{i3} - p_{j3})^2 \ldots.(p_{ik} - p_{jk})^2}$$

WHERE P_{I1}, P_{I2}, P_{I3} .....P_{IK} ARE PROPERTY 1, 2 ,3 … K OF ITH PROTEIN AND SIMILARLY P_{J1}, P_{J2}, P_{J3} .....P_{JK} ARE PROPERTY 1, 2 ,3 … K OF JTH PROTEIN

Cluster analysis

In this, the proposed method is compared with commonly used best available techniques. Cluster analysis has been done to evaluate the performance of the proposed method. Cluster analysis requires calculation of similarity matrix of NxN by calculating the pair wise similarity measure where N is number proteins as mentioned in the above paragraph. The clustering technique has been applied for this similarity matrix and clustering results compared with original SCOP classes. Number of clustering techniques such as K-Means, C-Means, Spectral K-Means clustering techniques have been tried with respect to commonly used clustering accuracy measures such as Random Index (RI), Recall (r), Precision (p) and F-measure. Result of these cluster analysis clearly indicates that the K-Means clustering technique is found better and hence K-mean clustering has been further taken for evaluation of the proposed method. The percentage of the precise clusters indicates the performance of the proposed protein 3D structure comparison algorithms.

Table 3. Precision, recall and F-measure results obtained from proposed method and CE and jFATCAT.

Method	Time (hrs)	Metric	Values
CE	126.18	Precision	0.9600
		Recall	0.9333
		F measure	0.9465 [1]
		R-index	0.9694
jFACTCAT	019.14	Precision	0.6653
		Recall	0.6043
		F measure	0.6333[4]
		R-index	0.8554
Graph properties Method (threshold =0.1)	51.25	Precision	0.5416
		Recall	0.6069
		F measure	0.5724
		R-index	0.8300
Graph properties Method (threshold =0.2)	13.50	Precision	0.5464
		Recall	0.6507
		F measure	0.5940
		R-index	0.8332
Graph properties Method (threshold =0.3)	03.03	Precision	0.5886
		Recall	0.6853
		F measure	0.6333
		R-index	0.8512
Graph properties Method	03.10	Precision	0.5967
		Recall	0.7259

(threshold =0.4)		F measure	0.6550
		R-index	0.8566
Graph properties	03.00	Precision	0.5967
Method		Recall	0.7259
(threshold =0.5)		F measure	0.6550
		R-index	0.8566

Results and Discussion

In this study, the proposed method resulted with accuracy level of 65.50 % for the benchmark dataset. The analysis was performed using various combinations of threshold values to consider a connectivity of pairs between atoms distance less than 0.1, 0.2, 0.3, 0.4 and 0.5. The f-measure is found as 57.24 %, 59.40%, 63.33 %, 65.50 % and 65.50 % respectively at each class level clustering with values as 0.1, 0.2, 0.3, 0.4 and 0.5 respectively(Table 3). The comparative values of f-measure for proposed method (Best value has been taken with respect to various threshold levels), CE and jFATCAT are 65.50, 94.50, 63.33 respectably (Table-3).

These methods were also evaluated with respect to proposed algorithm in terms of computational running time in performing the analysis of protein structure comparison (Table 3). The earlier methods, CE and jFATCAT consumed 126 hours and 19 hours respectively to perform the experiment of 100 protein structures comparison was recorded on a desktop computer of 8 GB RAM having 64-bit Windows 7 OS and MATLAB version 2010. Under the same setup, the proposed algorithm takes 51 hours, 13 hours, 3 hours and 3 hours for distance threshold 0.1, 0.2, 0.3, 0.4 and 0.5 respectively. The proposed method in this article performed better in terms of classification accuracy over jFATCAT and in terms of computation time over CE and jFATCAT.

Conclusion

Comparison of protein structures has fundamental of tasks in structural biology to understand the evolutionary and functional relationships among proteins. With the advent of high-throughput methods for generation of 3D protein structure by X-ray, NMR and even in-silico, the availability of structural information of proteins is increasing at a much accelerated pace. Hence, there is a requirement of automatic annotation and classification of proteins using 3D structural information in order to save resources in terms of time. Therefore, the development of fast and efficient algorithm is required to find out best identity and similarity between two protein structures.

In this study, an efficient methodology/algorithm has been developed in terms of accuracy and computational running time for comparing protein structures based on graph properties. The 3D coordinates of protein with all atoms have been used to build graph models and then exploited to graph properties for comparison. The proposed method has been implemented MATLAB programs for protein structure comparison. The proposed method in this project performed better in terms of classification accuracy over jFATCAT and in terms of computation time over both i.e., CE and jFATCAT. In future, this work can be extended to include more number of graph properties and more research can be done to find out the best structural identity or similarity amount protein structures.

CHAPTER- IV: SUMMERY AND CONCLUSION

We have illustrated in detail about two novel methods for comparison of 3D structure using 1) graph partition and 2) graph properties. The both proposed methods have been implemented in MATLAB by writing codes for various functions. The performance of the developed methodologies are tested with two existing best methods such as CE and jFATCAT on 100 proteins benchmark dataset with SCOP (Structural Classification Of Proteins) database.

First method “graph partitioning” method is comprises conversion of 3D graph into 2D graph, partitioning of 2D graph into sub-graphs and then aligning sub-graphs. Finally structure similarity has been calculated by identify local structural similarities between sub-graphs to global similarity of the whole graph. The proposed method has shown significant improvement over jFATCAT and accuracy has increased up to 12-15%. Prime notion of the method is the decomposition of structure to clusters than single residues and SSE, this could be basic interaction of non-bonded residues in the arrangement of structure elements within a structure. These interaction leads protein fold space and arrangement of SSE elements in 3D space. Then, aligned the structures based on atoms positions and association with other atoms within clusters and between clusters to identify similar geometry and similarity of the two structures. The proposed method performed better in terms of classification accuracy due to the inclusion of all atoms but CE and jFATCAT uses only backbone C-α atoms and non-bonded atoms in a cluster may plays important role to inclusion of folding information of protein while comparing the 3D structures.

In second method, is based on concepts of construction of graph from real world problems, database for graph and graph mining, pattern recognition are rich fields of computational techniques to study structures, topologies and properties of graphs. In this method, we have demonstrated that converting protein 3Dstructures into graphs and into properties such as total degree, maximum degree, no of adjacencies, average number of degree, cluster coefficient, graph energy, spectrum and number of components etc. Exploitation of the graph properties and data mining technique to perform complex studies on protein 3D structure. The proposed method is fast in terms of computation time complexity. Regarding accuracy is little improved over jFATCAT method. In future, this work can be extended for including more number of graph properties and more research can be done to find out the best structural identity or similarity among protein structures.

सारांश

हमने इस परियोजना के अंतर्गत दो नई विधियों का विकास किया है । यह विधियां 1) ग्राफ विभाजन और 2) ग्राफ गुणों का उपयोग करके प्रोटीन के 3 डी संरचना की तुलना करने के लिए बनाई गई हैं । इन विधियों को MATLAB में कोड लिखकर लागू किया गया है । विकसित तरीकों को दो मौजूदा सर्वोत्तम तरीकों (CE और jFACTCAT) के साथ 100 प्रोटीन बेंचमार्क डाटासेट पर SCOP (स्ट्रक्चरल वर्गीकरण ऑफ प्रोटींस) डाटाबेस के साथ तुलन भी की गयी है । इस रिपोर्ट में इन विधियों को एक सरल तरीके से परिभाषित कर उदहारण के माध्यम से समझने का प्रयास भी किया गया है ।

ग्राफ विभाजन विधि में 3–डी ग्राफ का 2 –डी ग्राफ में रूपांतरण, 2 डी ग्राफ का उप–ग्राफों में विभाजन और फिर इन उप–ग्राफों को संरेखित करता है। तत्पश्चात पूरे ग्राफ को उप–ग्राफों में वैश्विक समानता और स्थानीय संरचनात्मक समानताओं के आधार पर समानता की गणना की गई है। प्रस्तावित विधि ने jFATCAT की तुलना में महत्वपूर्ण सुधार दिखाया है और सटीकता 12–15% तक बढ़ी है। यह विधि प्रधान विचार, एकल अवशेषों और एसएसई (SSE) की तुलना में समूहों के लिए संरचना का एक अंग है । यह संरचना के तत्वों की व्यवस्था में नॉनबॉण्डेड अवशेषों की बुनियादी रिश्तों को खोजने में सहायक सिद्ध हो सकता है। परमाणुओं की स्थितियों और समूहों के बीच अन्य परमाणुओं के साथ उनके संबंधों और समूहों के बीच स्थितियों और दो संरचनाओं की समानता की पहचान करने के लिए भी किया गया है । प्रस्तावित विधि सभी परमाणुओं को शामिल करने के कारण वर्गीकरण की सटीकता के मामले में बेहतर प्रदर्शन करती है, लेकिन सीई (CE) और जेएफएटीएसीएटी (jFATCAT) केवल सी–अल्फा (Cα) परमाणुओं का उपयोग करती है और एक क्लस्टर में गैर–बंधित परमाणुओं की तुलना करते हुए प्रोटीन की जानकारी को शामिल करने की महत्वपूर्ण भूमिका निभा सकती है ।

दूसरी विधि ग्राफ के विभिन्न गुणों पर आधारित है । ग्राफ निर्माण और ग्राफ खनन के लिए डेटाबेस, पैटर्न मान्यता संरचनाओं, टोपोलॉजी और आलेखों के गुणों का अध्ययन करने के लिए कम्प्यूटेशनल तकनीकों के समृद्ध क्षेत्रों में प्रचलित हैं। इस पद्धति में हमने प्रोटीन 3 डी स्ट्रक्चर को ग्राफ में बदलने, ग्राफ के विभिन्न गुणों जैसे कुल डिग्री, अधिकतम डिग्री, कोई औसत संख्या, क्लस्टर गुणांक, ग्राफ ऊर्जा, स्पेक्ट्रम और घटकों की संख्या आदि के गुणों में परिवर्तन का प्रयोग किया है । प्रोटीन 3 डी संरचना पर जटिल अध्ययन करने के लिए गुण और डेटा खनन तकनीक का प्रयोग से पुरानी विधियों से समय की तुलना में श्रेष्ठ साबित हुई है जबकि सटीकता के बारे में जेएफएटीसीएटी (jFATCAT) विधि से थोड़ा सुधार हुआ है। भविष्य में यह कार्य और अधिक ग्राफ गुणों को शामिल करने के लिए किया जा सकता है और प्रोटीन संरचनाओं में सबसे अच्छी संरचनात्मक पहचान या समानता जानने के लिए अधिक शोध किया जा सकता है।

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Annexure – I : MATLAB codes of all functions used in the proposed methods

Converting protein 3D structure to graph model

```
function [B,t,xy, yx]=pdb_model_to_cord_matrix(pdbmodel,  cutoffdist)

[m1,n1]=size(pdbmodel.Model(1,1).Atom);
j=1;
k=1;

for i=1 : n1-1
    if strcmp(pdbmodel.Model(1,1).Atom(1,i).chainID,'A')
    if strcmp(pdbmodel.Model(1,1).Atom(1,i).AtomName,'CA')
      k=k+1;
    %  t1(k)=i;
    end
        x(j)= pdbmodel.Model(1,1).Atom(1,i).X;
        y(j)= pdbmodel.Model(1,1).Atom(1,i).Y;
        z(j)= pdbmodel.Model(1,1).Atom(1,i).Z;
        xy(j,1)=y(j);
        xy(j,2)=z(j);
        yx(k,1)=y(j);
        yx(k,2)=z(j);
        j=j+1;
        t(i)=k;
    end
end
n2=j;
for i=1:n2-1
    for j=1:n2-1
    %m(i,j)= abs(x(i)-x(j))+abs(y(i)-y(j))+abs(z(i)-z(j)) ;
    m(i,j)= sqrt((x(i)-x(j))^2+(y(i)-y(j))^2+(z(i)-z(j))^2) ;
   if((1/(1+m(i,j))) >cutoffdist)
    % m(i,j)= (1/(1+m(i,j)));
      B(i,j)=1;
   else
       B(i,j)=0;
   end

    end

end
```

Similarity measure calculation of 100 protein (100x100) using spectral clustering

```
clc
 clear all;
 close all;
i=1;
  fid=fopen('ce1.csv');
        while 1
            tline = fgetl(fid);
            if ~ischar(tline), break, end
            filename(i,:)=tline;
            i=i+1;
        end
        fclose(fid);

  fid = fopen('BMData_fvalue100','a');

  for fi=1 : 100
    filename1=strcat('E:/work/benchmark/',filename(fi,:));
    PDBStruct1 = pdbread(filename1);
    [x1,t1]=pdb_madel_to_cord_matrix(PDBStruct1,.2);
    [e1, nc1] =SpectralClusterAuto(x1, .005,100);

      for fi1=1:100
          disp( [' fi=' num2str(fi), ' fj=' num2str(fi1)]);
          t11=now();
          filename2=strcat('E:/work/benchmark/',filename(fi1,:));
          PDBStruct2 = pdbread(filename2);
          [x2,t2]=pdb_madel_to_cord_matrix(PDBStruct2,.2);
          [e2, nc2] =SpectralClusterAuto(x2, .05,  20);
            [p,r,f,pos] =f_measure_two_graphs(e1,e2);
            t22=now()-t11;
            fprintf(fid,'%s \t%d \t %s \t %d \t %10.4f \t %d \t %10.5f
\n',filename(fi,:), nc1, filename(fi1,:), nc2, f, pos, t22);
      end
  end
```

Cluster analysis code for C-means,K-means, spectral K-means

```
% reading data from file
% format %d %d  %e
  clc;
 clear all;
 close all;
 filename1= 'ESA100_ESAO.csv';
 filename2='ClassSCP100C3.txt';
i=1;
j=1;
 fid = fopen(filename1,'r');
  s = textscan(fid, '%f');
  [n,m]=size(s{1})
  n=sqrt(n);
 cnt=1;
      for i=1: n
          for j=1:n
         B(i,j)=1/(1+ s{1}(cnt));
         cnt=cnt+1;
          end
      end

        fclose(fid);

  for i=1:n
     B(i,i)=1;
     for j=1:m
       if B(i,j)>B(j,i);
       B(j,i)=B(i,j);
       else
       B(i,j)=B(j,i);
       end
     end
 end

 fid = fopen(filename2,'r');
        while 1
            tline = fgetl(fid);
            if ~ischar(tline), break, end
            % disp(tline);
            ls=size(tline);
            Qlabel=str2num(tline(1:8));
            Slabel=str2num(tline(9:ls(2)));
            t(Qlabel)=Slabel;
        end
 fclose(fid);


 ac=[0 45 85 100];
 ac=[0 14 20 30 45 65 75 85 90 100];
 ac=[0 5 10 14 20 30 35 41 45 52 59 65 70 75 80 85 90 94 100];
maxdim=3;
noc=3;

t0=clock;
```

```
D = (sum(B,2));
L = inv(diag(sqrt(D)))*(B/diag(sqrt(D)));


OPTS.disp = 0;
[u, eigvals] = eig(L);
[eigvals, eigvalIndices] = dsort(diag(eigvals));
%  eigvalIndices
eigvalindex=eigvalIndices(1:maxdim);
u = u(:,eigvalindex);
ui=sum(u.^2,2);

for i=1:n
y(i,:)=u(i,:)/sqrt(ui(i));
end

% for j=1:maxdim
%     [cv,ci]=max(y(:,j));
%      disp(['val=' num2str(cv) '  indx=' int2str(ci)]);
% end


[cidx, ctrs] = kmeans(B, noc);
PcEig=u;
disp(['Time = ' num2str(etime(clock,t0))]);


[n,m]=size(u);
  x1=1:.0001:1.1;
  rtold=1;
%   figure(1);
% hold on;
% for i=1:21-1
%      rt=abs(eigvals(i)/eigvals(i+1));
%      uy(i)=rt;
%      irt=1/100;
%      jrt=abs(rtold-rt)/100.;
%      xi=i:irt:i+1;
%         if rtold<rt
%         xj=rtold:jrt:rt;
%         else
%         xj=rtold:-jrt:rt;
%         end
%      % plot(i,rt, 'r+');
%      plot(i,x1, 'b-');
%      plot(xi, xj, 'g.');
%      rtold=rt;
% %      disp(['i=' num2str(i) ' gap = ' num2str(rt)]);
% end
% ux=[1:1:20];
% line(ux, uy);

figure(2);
for i=1:noc
 index1 = find(cidx==i);
 disp(['Cluster-' int2str(i) ' = ' int2str(index1')]);
 m(index1')=i;
[um,un]=size(index1);
if(un>0)
  plot(index1,i, 'r+');
end
hold on;
end
grid;
ylabel('Clusters');
xlabel('Sequences');
x1=0:.01:noc;

r=n;
nac=size(ac);
for i=2:nac(2)
 plot(ac(i),x1,'g-');
end

mm=zeros(r,r);
tt=zeros(r,r);
cm=0;
ct=0;
for i=1:r-1
    for j=i+1:r
        if m(i)==m(j)
            mm(i,j)=1;
            cm=cm+1;
        end
        if t(i)==t(j)
            tt(i,j)=1;
            ct=ct+1;
        end
    end
end
aa=0;
bb=0;
cc=0;
dd=0;
for i=1:r
    for j=1:r
     if mm(i,j)==1 & tt(i,j)==1
         aa=aa+1;
     end
     if mm(i,j)==0 & tt(i,j)==1
         bb=bb+1;
     end
     if mm(i,j)==1 & tt(i,j)==0
         cc=cc+1;
     end
     if mm(i,j)==0 & tt(i,j)==0
         dd=dd+1;
         %    disp( [num2str(i) ',' num2str(j) '= d']);
     end

 end
```

```
end
 disp( ['a=' num2str(aa) ',b=' num2str(bb) 'c=' num2str(cc)  'd='
num2str(dd)]);
  disp( ['cm=' num2str(cm) ',ct=' num2str(ct)])

pp=aa/(aa+cc);
rr=aa/(aa+bb);
ff=2*rr*pp/(rr+pp);

disp([' p=' num2str(pp) ' r1=' num2str(rr) ' f=' num2str(ff)])
fid = fopen('pscresult010716-fcm.txt','a');
fprintf(fid,'%s %12.4f  %12.4f  %12.4f  %12.4f %12.8f\n',filename1,pp,rr,ff,
noc);
fclose(fid);

color = ['k', 'r', 'g', 'b', 'm'];
sym = ['>', 'o', '+', 'x', 's', 'd', 'v', '^', '<'];
symbol = zeros(noc, 2);
for i = 1:noc;
    symbol(i, :) = [color(mod(i, length(color))+1) sym(mod(i,
length(sym))+1)];
end

nac=size(ac);
for i=2:nac(2)
    plot(ac(i),x1,'g-');
end
```

Alignment and comparison of two graph models

```
function [pp,rr,maxf, maxpos,am] = f_measure_two_graphs(e,t)
[r1,q1]=size(e);
[r2,q2]=size(t);
maxf=0;

rr=0;
pp=0;
ff=0;

if (r1<r2)
    r=r1;
    e1=e;
    t1=t;
else
    r=r2;
    e1=t;
    t1=e;
end

mm=zeros(r,r);
tt=zeros(r,r);


for i=1:r-1
    for j=i+1:r
        if e1(i)==e1(j)
            mm(i,j)=1;
        end

    end
end

for d=1:abs(r1-r2)+1
    maxf=0;
    maxpos=0;
    tt=zeros(r,r);
    i1=1;
for i=d:r+d-1
    j1=i-d+2;
    for j=i+1:r+d-1
         if t1(i)==t1(j)
            tt(i1,j1)=1;
         end
        j1=j1+1;
    end
    i1=i1+1;
end

aa=0;
bb=0;
cc=0;
dd=0;
```

```
for i=1:r
    for j=1:r
     if mm(i,j)==1 & tt(i,j)==1
         aa=aa+1;
     end
     if mm(i,j)==0 & tt(i,j)==1
         bb=bb+1;
     end
     if mm(i,j)==1 & tt(i,j)==0
         cc=cc+1;
     end
     if mm(i,j)==0 & tt(i,j)==0
         dd=dd+1;
     end
    end

end
pp=aa/(aa+cc);
rr=aa/(aa+bb);
ff=2*rr*pp/(rr+pp);
ri=(aa+dd)/(aa+bb+cc+dd);
am=(aa+dd)/(aa+bb+cc+dd);

%disp( [' r1=' num2str(r1) ', r2=' num2str(r2) ', d=' num2str(d)  ', r='
num2str(r)]);
%disp( [' a=' num2str(aa) ', b=' num2str(bb) ', c= ' num2str(cc)  ', d='
num2str(dd)]);
%disp([' p=' num2str(pp) ' r=' num2str(rr) ', f=' num2str(ff) ', ri='
num2str(ri)])

if (maxf<ff)
    maxf=ff;
    maxpos=d;
end

end
```

MCL clustering of a Graph

```
function [ma]=mcl_cluster(B)
% gplot (mm,xy,'r-');
%
% figure(2);
 mm=mcl(B);
[n2,m2]=size(B)
k1=1;
for i=1:m2
 index1 = find(mm(i,:)>=1);
     k=mod(i,6)+1;
     [um,un]=size(index1);
    if(un>0)
   %  disp(['Cluster-' int2str(k1) ' = ' int2str(index1)]);
     ma(index1')=k1;
    % plot(index1,k1, 'r+');
     k1=k1+1;
    end
  %  hold on;
end
ma=ma';
end
```

MLC clustering algorithm

```
function m = mcl(m)
% test the explanations in stijn van dongens thesis.
% @author gregor :: arbylon . net

if nargin < 1
    % m contains T(G3 + I) as stochastic matrix
    load -ascii m.txt
end

p = 2;
minval = 0.0000001;
e = 1;
emax = 0.0000001;
i=1;
while e > emax
    fprintf('iteration %i before expansion:\n', i);
    fprintf('iteration %i after expansion/before inflation:\n', i);
    m2 = expand(m);
    fprintf('inflation:\n')
    [m, e] = inflate(m2, p, minval);
    fprintf('residual energy: %f\n', e);
    i=i+1;
end % while e
end % mcl

% expand by multiplying m * m
% this preserves column (or row) normalisation
function m2 = expand(m)
    m2 = m * m;
end

% inflate by Hadamard potentiation
% and column re-normalisation
% prune elements of m that are below minval
function [m2, energy] = inflate(m, p, minval)
    % inflation
    m2 = m .^ p;
    % pruning
    m2(find(m2 < minval)) = 0;
    % normalisation
%     if sum(m2)>0
     dinv = diag(1./sum(m2));
%      else
%      dinv=1;
%     end
    m2 = m2 * dinv;
    % calculate residual energy
    maxs = max(m2);
    sqsums = sum(m2 .^ 2);
    energy = max(maxs - sqsums);
end
```

Spectral clustering Algorithm

```
function [elist, noc, PcEig, Centres] = SpectralClusterAuto(x, sigma, numk)

% SPECTRALCLUSTER Clusters a dataset automatically determining the number of
clusters.
%
% [labels, PcEig, Centres] = SpectralCluster(x, sigma)

% Copyright (c) 2005 Guido Sanguinetti and Jonathan Laidler
% SPECTRAL toolbox version 0.1
%
% Clusters an arbitrary dataset using the spectral clustering algorithm
% outlined in "Automatic Determination of the Number of Clusters using
% Spectral Algorithms" by Sanguinetti, Lawrence and Laidler.
%
% SPECTRALCLUSTER(x, sigma, lambda)
% x is the input data.
% sigma is the variance measure used when computing the affinity matrix.
% lambda is a measure of the elongation of a centre's variance when doing
% a Mahalanobis k-means clustering. i.e. Lambda = 1 gives a circular
% covariance, Lambda < 1 gives preference to points lying along the vector
% normal between the centre and the origin.
%
% Written by Guido Sanguinetti and Jonathan Laidler, April 2005

% initialisations
npts=size(x,1);
options=foptions;
ExtraCluster=0;
Dim=numk;
lambda = 0.2;

% compute the similarity matrix A and the matrix L
A=x;
% A=zeros(npts,npts);
% for i=1:npts
%     for j=1:npts
%         A(i,j) = exp(-norm(x(i,:)-x(j,:))^2/sigma);
%     end
% end
D = (sum(A,2));
L = inv(diag(sqrt(D)))*(A/diag(sqrt(D)));

maxdim=numk;
% find the eigenvectors associated with the 10 largest eigenvalues of L
OPTS.disp = 0;
 % [Y, eigvals] = eigs(L, 50, 'LM', OPTS);
   [Y, eigvals] = eig(L);
 % [void,Y,eigvals]= ppca(L,10); %ppca gives an alternative eigen-
decomposition
 % [u, eigvals, v]=svd(L);

[eigvals, eigvalIndices] = dsort(diag(eigvals));
% eigvals
```

```
eigvalindex=eigvalIndices(1:maxdim);
u = Y(:,eigvalindex);

clear Y;
ui=sum(u.^2,2);
[n,m]=size(u);
for i=1:n
Y(i,:)=u(i,:)/sqrt(ui(i));
end

for j=1:maxdim
    [cv,ci]=max(Y(:,j));
     %disp(['val=' num2str(cv) '  indx=' int2str(ci)]);
     % disp(ci)
end

PcEig = Y(:,(1:Dim));

% the first Centre is initialised
norms = diag(PcEig*PcEig');
[void, index(1)] =  max(PcEig(:,1));  % max(norms);
Centres = PcEig(index(1), :);

% the second Centre is initialised
S = ((PcEig*Centres(1, :)').^2)./(norms);
for ii=1:Dim
[void, index(ii)] =  max(PcEig(:,ii));  % min(S);
end
Centres = [PcEig(index, :)];

% [void, index3] = max(PcEig(:,Dim+1));

while  ExtraCluster<=3  %& Dim <=maxdim
sumdist=0;
    % introduce a new centre at the origin and do k-means

%     Centres = [Centres; PcEig(index3,:)];
    [Centres, options, labels] = mahKmeans(Centres, PcEig, lambda, options,
.05);
   % [W,iter,Sw,Sb,Cova]=kmeansf(PcEig,Centres,.01,100,0)
    for i = 1:Dim
        for j = 1:npts
            CentrDist(i,j) = norm(PcEig(j,:)-Centres(i,:));
            sumdist=sumdist+CentrDist(i,j);
        end
    end
%     sumdist
    % Centres
    % if anything is assigned to this new centre, there is an extra cluster
%     if max(labels(:,Dim)) == 1
%         ExtraCluster = 0;
%         Dim = Dim+1;
%         % take the next eigenvector from Y
%         PcEig = Y(:,(1:Dim));
%         % re-initialise the centres
```

```
        Centres=zeros(Dim,Dim);
        for i=1:Dim
            [void,point] = min(CentrDist(i,:));
            Centres(i,:)=PcEig(point,:);
        end
%     else
        ExtraCluster = ExtraCluster+1;
%     end
end

% erase the last column of labels, as it is empty
labels = labels(:,1:Dim);

[r,noc]=size(labels);
elist(1)=0;
for i=1:noc
    for j=1:r
     if labels(j,i)==1
         elist(j)=i;
     end
   end
end
elist=elist';
```

Mahalanobis distance calculation for spectral K-means algorithm

```
function [centres, options, post, errlog] = mahKmeans(centres, data, lambda,
options, egv)
% MAHKMEANS Trains a k means cluster model, using Mahalanobis distance (based
on Netlab k-means).
%
% [centres, options, post, errlog] = mahKmeans(centres, data, lambda,
options)

% Copyright (c) 2005 Guido Sanguinetti and Jonathan Laidler
% SPECTRAL toolbox version 0.1

% Additional Copyright (c) Ian T Nabney (1996-2001)


[ndata, data_dim] = size(data);
[ncentres, dim] = size(centres);

if dim ~= data_dim
  error('Data dimension does not match dimension of centres')
end

if (ncentres > ndata)
  error('More centres than data')
end

% Sort out the options
if (options(14))
 niters = options(14);
else
  niters = 100;
 end

store = 0;
if (nargout > 3)
  store = 1;
  errlog = zeros(1, niters);
end

% Check if centres and posteriors need to be initialised from data
 if (options(5) == 1)
  % Do the initialisation
  perm = randperm(ndata);
  perm = perm(1:ncentres);

  % Assign first ncentres (permuted) data points as centres
  centres = data(perm, :);
 end
% Matrix to make unit vectors easy to construct
id = eye(ncentres);

% Main loop of algorithm
```

```
for n = 1:niters

  % Save old centres to check for termination
  old_centres = centres;

  % Calculate posteriors based on Mahalanobis distance from the existing
  % centres. (GS)
  d2 = mahDist2(data, centres, lambda, egv);
  % Assign each point to nearest centre
  [minvals, index] = min(d2', [], 1);
  post = id(index,:);


  num_points = sum(post, 1);
  % Adjust the centres based on new posteriors
  for j = 1:ncentres
    if (num_points(j) > 0)
      centres(j,:) = sum(data(find(post(:,j)),:), 1)/num_points(j);
    end
  end

  % Error value is total squared distance from cluster centres
  e = sum(minvals);
  if store
    errlog(n) = e;
  end
  if options(1) > 0
    fprintf(1, 'Cycle %4d  Error %11.6f\n', n, e);
  end

  if n > 1
    % Test for termination
    if max(max(abs(centres - old_centres))) < options(2) & ...
        abs(old_e - e) < options(3)
      options(8) = e;
      return;
    end
  end
  old_e = e;
end

% If we get here, then we haven't terminated in the given number of
% iterations.
options(8) = e;
if (options(1) >= 0)
  %disp(maxitmess);
end
```

Codes for Calculation of graph properties

```
clc
 clear all;
 close all;
 fi=9;
 filename=char('1A0F.pdb', '1AR4.pdb', '1AST.pdb', '1AUE.pdb', '1AVX.pdb' ,
'1BC9.pdb',    '1BP3.pdb' ,    '1BRF.pdb' ,     '1DGP.pdb', '1DI1.pdb' ,
'1dly.pdb',    '1ecd.pdb' ,    '1EHS.pdb' ,    '1FQT.pdb',    '1gvh.pdb',
'1H3E.pdb' ,   '1hbi.pdb' ,    '1IAA.pdb' ,    '1IAB.pdb' ,    '1IAC.pdb' ,
'1IQW.pdb' ,   '1JHK.pdb',     '1K9O.pdb' ,    '1KA8.pdb' ,    '1mbd.pdb',
'1MCN.pdb', '1MLB.pdb',     '1MN8.pdb' ,    '1oj6.pdb',     '1P4X.pdb',
'1PSR.pdb',    '1PZ8.pdb', '1PZ9.pdb',     '1QGJ.pdb',     '1QN2.pdb',
'1QUU.pdb' ,   '1RIE.pdb' , '1RQI.pdb',     '1rte.pdb' ,    '1TOS.pdb',
'1urv.pdb'  ,  '1ux8.pdb',     '1VCK.pdb', '1WOV.pdb',     '1WOW.pdb',
'1WOX.pdb' ,   '1X3A.pdb' ,    '1XG7.pdb' ,    '1Y2O.pdb' , '1YLL.pdb',
'1YV0.pdb',    '1Z77.pdb' ,  '2A1K.pdb' ,   '2B0Z.pdb' ,    '2BBA.pdb',
'2CJ8.pdb' ,   '2EFK.pdb',     '2gdm.pdb' ,    '2GH2.pdb' ,        '2H6B.pdb',
'2HVC.pdb',    '2HYJ.pdb' ,    '2IBN.pdb',     '2IEK.pdb',     '2IOL.pdb' ,
'2J0N.pdb',    '2NXN.pdb',     '2P8T.pdb',     '2Q0A.pdb',     '2RD6.pdb',
'2VOD.pdb',    '2ZP0.pdb',     '3BGE.pdb' ,   '3CJR.pdb', '3ELN.pdb' ,
'3MDS.pdb' ,   '3SGO.pdb' ,    '4hhb.pdb');
% filename1='1EHS.PDB' % '1tos.PDB' %'1h3e.pdb', '1C7M.pdb',  '1Q5W.pdb',
'2GTT.pdb' , '2JA7.pdb',    '2LFB.pdb',    '2MLI.pdb', '2Q4S.pdb' ,;
  % PDBStruct2 = pdbread(filename1);
  filename1=strcat('E:/work/pdbs/',filename(fi,:))
  PDBStruct2 = pdbread(filename1);
PDBStruct2.Model
% atoms = fastPDBRead('1TOS.pdb')
% plot3(atoms.X, atoms.Y, atoms.Z, '.');
% atoms.atomNum
[m1,n1]=size(PDBStruct2.Model(1,1).Atom);
%x=zeros(100);
j=1;
k=0;
for i=1 : n1-1
    if strcmp(PDBStruct2.Model(1,1).Atom(1,i).AtomName,'CA')
      k=k+1;
      t1(k)=i;
    end
        x(j)= PDBStruct2.Model(1,1).Atom(1,i).X;
        y(j)= PDBStruct2.Model(1,1).Atom(1,i).Y;
        z(j)= PDBStruct2.Model(1,1).Atom(1,i).Z;
        j=j+1;
        t(i)=k;
  %  end
end
n2=j;
m=zeros(j-1);
for i=1:n2-1
    for j=1:n2-1
    %m(i,j)= abs(x(i)-x(j))+abs(y(i)-y(j))+abs(z(i)-z(j)) ;
    m(i,j)= sqrt((x(i)-x(j))^2+(y(i)-y(j))^2+(z(i)-z(j))^2) ;
   if((1/(1+m(i,j))) >.3)
    % m(i,j)= (1/(1+m(i,j)));
    B(i,j)=1;
   else
       B(i,j)=0;
   end
    end

end
% B=m;
[n,m]=size(B);
k=20;
% C:\Program Files\MATLAB\R2010a\toolbox\mnr
fid = fopen('structparm1-2-16.txt','a');
fid1 = fopen('structparm_header.txt','a');
fprintf(fid,'%s ',filename(fi,:));

%1 calculate degrees of all nodes
[deg, ideg,odeg]=degrees(B);
totaldeg=sum(deg);
maxdeg=max(deg);
mindeg=min(deg);
fprintf(fid,'%12.4f %12.4f %12.4f ',totaldeg, maxdeg, mindeg);
fprintf(fid1,'%s %s %s ','totaldeg,', 'maxdeg,', 'mindeg,');

%2 List out all connected nodes to all nodes
% L1 = adj2adjL(B);

%3 Converts an adjacency graph representation to an adjacency list
% L = adj2adjL(B);

%4 function to convert adjacency matrix to UCINET dl format
% adj2dl(B,'filename.UCINET');

%5 adjacency matrix to edges list;
el=adj2edgeL(B);
[n1,n2]=size(el);

fprintf(fid,'%12.4f  ',n1);
fprintf(fid1,'%s  ','no of adjances,');

%6 Convert adjacency matrix to an incidence matrix
% inc = adj2inc(B);

%7 Converts an adjacency matrix representation to a Pajek .net read format
% adj2pajek(B,'filename.txt',x,y,z);

%8 The algebraic connectivity of a graph: the second smallest eigenvalue of
the Laplacian
 a=algebraic_connectivity(B);
fprintf(fid,'%12.4f  ',a);
fprintf(fid1,'%s  ','algebric connectivity,');

%9 Computes the average degree of neighboring nodes for every vertex
 ave_n_deg=ave_neighbor_deg(B);

totaldeg1=sum( ave_n_deg);
```

```
maxdeg1=max( ave_n_deg);
mindeg1=min( ave_n_deg);
fprintf(fid,'%12.4f %12.4f %12.4f ',totaldeg1, maxdeg1, mindeg1);
fprintf(fid1,'%s %s %s ','ave_n_deg totaldeg1,','maxdeg1,', 'mindeg1,');

 %10 Compute average path length for a network - the average shortest path
 ll = ave_path_length(B);

fprintf(fid,'%12.4f ',ll);
fprintf(fid1,'%s ','ave path length,');

 %11 Computes the average degree of a node in a graph, defined as 2*num_edges
k=average_degree(B);

fprintf(fid,'%12.4f ',k);
fprintf(fid1,'%s ','ave degree,');

%12 Implementation of breadth-first-search of a graph
%T=BFS(L,1);
%T
%13 Computes clustering coefficient, based on triangle motifs count and local
clustering
 [C1,C2,C] = clust_coeff(B);
fprintf(fid,'%12.4f %12.4f  ',C1, C2);
fprintf(fid1,'%s %s  ',' cluster coeff1,','cluster coeff,');

% The longest shortest path between any two nodes nodes in the network
diam = diameter(B);
fprintf(fid,'%12.4f ', diam);
fprintf(fid1,'%s ', 'diameter,');

%15 Draws the matrix as a column/row sorted square dot-matrix pattern
%dot_matrix_plot(B)
% model1=simple_spectral_partitioning(B,k);

%16 Draw a circular graph with links and nodes in order of degree
%draw_circ_graph(B)

% 17 The ith component of the eigenvector corresponding to the greatest
 x=eigencentrality(B);
 fprintf(fid,'%12.4f ', max(x));
 fprintf(fid1,'%s ', 'max eigencentrality,');

 % Plot geometry based on extended edgelist
% el2geom(el)

%18 Algorithm for finding connected components in a graph but I got no
meaning
%comp_mat = find_conn_comp(B);

%19 Graph energy defined as: the sum of the absolute values of the real
components of the eigenvalues
 G=graph_energy(B);
 fprintf(fid,'%12.4f ', G);
 fprintf(fid1,'%s ', 'Graph energy,');

 %20 The minimum vertex eccentricity is the graph radius
 Rg=graph_radius(B);
 fprintf(fid,'%12.4f ', Rg);
 fprintf(fid1,'%s ', 'Radius,');

 %21 Computes the similarity matrix between two graphs
 %S=graph_similarity(B,B);

 %22 The eigenvalues of the Laplacian of the graph
 s=graph_spectrum(B);
 fprintf(fid,'%12.4f ', max(s));
 fprintf(fid1,'%s ', 'spectrum, ');

 %23 Test whether a graph is bipartite, if yes, return the two vertex sets
 L = adj2adjL(B);
 [isit,A1,B2]=isbipartite(L);
 fprintf(fid,'%12.4f ', isit);
 fprintf(fid1,'%s ', 'isbiparate,');

  %24 Check if a graph is Eulerian, i.e. it has an Eulerian circuit
% "A connected undirected graph is Eulerian if and only if every graph vertex
has an even degree."
% "A connected directed graph is Eulerian if and only if every graph vertex
has equal in- and out- degree."
 S=iseulerian(B);
fprintf(fid,'%12.4f ', S);
fprintf(fid1,'%s ', 'is eulerian,');

 %25 Checks whether a graph is regular, i.e. every node has the same degree.
 Sr=isregular(B);
 fprintf(fid,'%12.4f ', Sr);
 fprintf(fid1,'%s ', 'is regular,');

 %26 Find the "optimal" number of communities given a network using an
eigenvector method
 modules=newman_eigenvector_method(B);
 [n2,m2]=size(modules);
 fprintf(fid,'%12.4f ', m2);
 fprintf(fid1,'%s ', 'no of modules,');

 %27 Calculate the number of connected components using the Laplacian
% eigenvalues - counting the number of zeros
 nc=num_conn_comp(B);
fprintf(fid,'%12.4f \n', nc);
fprintf(fid1,'%s \n', 'no of connect comps,');

 %28 Uses the fiedler vector to assign nodes to groups
% modules = simple_spectral_partitioning(B,10)

% 29 Weighted clustering coefficient
 % wc=weighted_clust_coeff(B);
```

```
%30  Number of hubs (NHUBS) as hubs when their nodal degree exceeded the
average degree of the graph

%31 Degree centrality: for node i =di(g)/n -1

%32  Closeness centrality: Tracks how close a given node is to any other
node: for node i, one such measure is =n – 1/dij  j<>i

%33 Betweenness centrality (or shortest-path between-ness). A measure of
accessibility that is the number of times a node is crossed by shortest paths
in the graph.

%34 Pi Index: The relationship between the total length of the graph L(G) and
the distance along its diameter, an indicator of the shape of a graph.

%35 Alpha Index: A measure of connectivity which evaluates the number of
% cycles in a graph in comparison with the maximum number of cycles

%36 Eta Index: Average length per link. Adding new nodes will cause a
decrease of Eta as the average length per link declines.

%37 Beta Index: Measures the level of connectivity in a graph and is
expressed by the relationship between the number of links (e) over the number
of nodes (v). =e/v

%38 Theta Index: Measures the function of a node, which is the average amount
of traffic per intersection. The measure can also be applied to the number of
links (edges).

%39 Graph Density

%40  Synchronizability (S) describes the graph’s capacity to synchronize and
is calculated from the eigen values of the graph’s Laplacian matrix L
% L = D -A , where D represents a diagonal matrix containing the nodal
degrees. The synchronizability is defined as the ratio between the first non-
zero eigenvalue and the largest eigenvalue (S= EV_2/EV_max)

fclose(fid);
fclose(fid1);
```