國立交通大學

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碩士論文

ATP 作用區域為基之蛋白質分群與交互作用分析

Structural Binding Pocket Clustering and Protein-Ligand Interaction Analysis for ATP-binding Proteins

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碩 士 論 文

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摘　　要

近年來，隨著大規模基因體學與蛋白質體學計畫的發展，人們對生物系統的瞭解也迅速的成長，我們可以透過 PDB 資料庫，取得愈來愈多被結晶出來的蛋白質立體結構。其中，有許多蛋白質的配體也一併被結晶在結構中。這樣大量的蛋白質-配體結構資訊，使得以結構為基之蛋白質-配體間交互作用分析獲得頗大的助益。然而，一些知名的蛋白質分類資料庫，例如 SCOP、CATH 等，由於資料庫更新速度過慢，不能跟上解蛋白質結晶結構的速度，當新的蛋白質結晶結構被解出來後，皆無法儘速將之分類，以致影響研究者對蛋白質的結構、功能、配體結合作用力等重要議題上做深入探討。

在本碩士論文研究中，我們發展一套簡單快速的方法論，用以分析蛋白質-配體結構，並且使用 ATP 結合蛋白作為研究例子。本方法的核心理念乃是根據蛋白質的結構相似度與蛋白質-配體的交互作用側寫，將蛋白質-配體複合物做快速分類。同時也能藉由蛋白質-配體間交互作用的資訊，找出功能性殘基與模版。對於結構相似度，我們同時考慮整個蛋白質或配體結合部位的結構。我們利用快速結構相似度搜尋工具—3D-BLAST，迅速地在整個蛋白質資料庫裡尋找與配體結合蛋白質相似的結構。接著將結合位含有配體的蛋白質結構，以 CE 做詳細的結構比對，檢查全蛋白與配體作用區域的結構相似性，並將蛋白質做初步分群。對於蛋白質-配體間交互作用側寫，我們則是利用軟體辨認出蛋白質-配體間的交互作用。最後，根據結構相似度及功能性交互作用模版，我們將這套分類蛋白質的方法論應用在 ATP 結合蛋白質複合物。

分群結束後，我們比較其結果與 SCOP 資料庫的分類，以每群中佔最多數的 SCOP family 視為該群的正確答案，且同一 SCOP family 可同時為多群的答案。在此比較的依據下，結果獲得了 95%的正確率。接著，我們系統地對每群中的 ATP 結合蛋白進行 ATP 作用區域之交互作用分析，包括氫鍵、π-π 疊合作用與正離子-π 等三種交互作用，將每一群中交互作用所表現的保守性，建立出各群特有的 ATP 結合 motif。結果發現，我們所找出來的 ATP 結合模版不但符合目前研究已發現的模版，甚至也另有發現目前資料庫中所沒有定義，可能是新的 ATP 結合模版。

本論文應用了 3D-BLAST，藉由其結構快速搜尋的特性，大幅降低將相似結構分群的時間，並且針對每一群的蛋白質裡找出包含交互作用資訊的 ATP 結合模版。未來，我們可以利用分群結果及 ATP 結合模版，來對新結晶或未包含 ATP 蛋白質作分析與分類。同時也能輕易地將本方法應用於其他重要的蛋白質-配體複合物的研究上。

Structural Binding Pocket Clustering and Protein-Ligand Interaction Analysis for ATP-binding Proteins

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Abstract

In recent years, information about biological systems has grown rapidly, in particular through large-scale and global approaches addressing DNA sequence (genomics), protein structure (structural genomics) and protein expression and interactions (proteomics). More and more three-dimensional protein structures have been deposited in the Protein Databank. Many of them are protein-ligand complex structures. This enormous increase in the number of known protein-ligand complexes has therefore had a profound effect on structure-based protein-ligand interaction analyses. However, the classification databases, such as SCOP and CATH, are updated too slowly to classify these rapidly increasing complexes. It is hard to classify newly solved protein structure immediately.

In this work, we have developed a very fast method for protein-ligand complexes analysis and used ATP-binding proteins as a study case. The core idea of this method is to cluster protein-ligand complexes based on binding-site structural similarity and protein-ATP interaction profiles. Naturally, this new method is able to analyze the protein-ligand interactions and identify function residues and patterns. For structural similarity, we considered the similarities of both whole proteins and ligand-binding sites. First, we used 3D-BLAST to perform protein-ligand complexes homologous search in whole protein database. Second, CE was used as a detailed structure alignment tool to identify structural similarity of ligand-binding site. Accordingly, we can obtain a preliminary classification for protein complexes. For protein-ligand interaction profiles, the HBPLUS and an in-house software, PiFinder, are used to identify the non-bonded interactions. According to structural similarity and functional protein-ligand interaction patterns, a simple cluster method was applied to group protein-ATP complexes.

To evaluate our clustering results, we compared our results to the SCOP classification. The most popular SCOP family in a cluster is set to the representative family of the cluster. Assigning one SCOP family to multiple clusters is also taken as correct answers. Overall, we got a 95% accuracy of the clustering results. We systematically analyzed the non-bonded interactions, including hydrogen bond, π- π stacking, and cation- π interactions, between ATP and the binding protein chains. We found that the three types of non-bonded interactions show relatively strong conservation within clusters. Not only had the ATP-binding motif discovered in the previous works, some novel potential ATP-binding motifs were also identified in some clusters.

In this work, 3D-BLAST was applied for fast database search and reducing the time consuming of structure clustering. Furthermore, we can identify ATP-binding motif in each cluster results. In the future, we may use cluster result and ATP-binding motif to analyze and classify new crystal structure. Furthermore, this new method is easily applied to fast analyze other protein-ligand complexes.

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謝謝你們。

登凱

九五年，夏，於新竹交大

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Chapter 1

Introduction

1.1 Structural Genomics

During the past few decades, the knowledge about biological systems has grown rapidly, in particular through large scale and global approaches addressing DNA sequences (genomics), protein structures (structural genomics) and protein expression and interactions (proteomics). These developments, including protein sequencing, x-ray crystallography, and NMR, have made primary sequences of several hundred thousand proteins known and over 38000 three-dimensional structures of proteins available *via* the Protein Databank (PDB)[1]. They also raise the expectation that the initial set of basic data will be converted to knowledge resulting in the developments of novel therapies and drugs.

The information in the ligand-binding or catalytic sites is the most interesting issue in drug design. There are great amount of three-dimensional protein structures are crystallized along with heterogen groups. In despite of the solvent or determinants, many of them are binding ligands of proteins. With such great amount of protein-ligand complexed structures, we can learn about how ligands bind to proteins by a systematic analysis on those data.

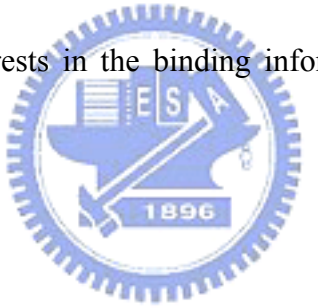
1.2 Protein-ligand Complexes and Drug Design

Arguable the most important application of structural information about proteins lies in the rational design of drugs, which affects proteins in a particular way, i.e. inhibitors causing a particular desired effect. There are numerous examples for structure-based drug design in the literature[2].

Despite the undisputed advances in computer modeling and graphics, a high resolution x-ray structure of a protein-ligand complex is still regarded as the best foundation for structure-based design of biologically active compounds. The more structures there are for any given protein with different ligands or for any given ligand with different proteins, mutant proteins or those from different species, the more convincing the conclusions drawn from the structural data. The enormous increase in the number of known protein-ligand complexes has therefore had a profound effect on structure-based drug design. For some protein classes it is possible to look at a number of such complexes and characterize the binding modes of ligands in great detail. Such detailed analyses of ligand binding may then allow the development of general rules, which can be applied in the design of inhibitors or agonists of other relatively unrelated proteins. In comparison of small compounds, ubiquitous cofactors can be a starting point for protein-ligand binding. Cofactors are important among organisms, and they provide energy to or modify proteins to help proteins function in biological processes, such as ATP, NADP, FAD, and so on. Because of the popularity of cofactors,

protein-cofactor complexes contain important information about protein-ligand interactions.

Some analyses performed on protein-ligand complexes were proposed previously. MuLiSA[3] used the ligand structures in protein-ligand complexes to align these structures and identified some important binding patterns for ATP-, ADP-, and HEM-binding proteins. PDB-Ligand[4] is a database storing ligand-binding site clusters based on the RMSD of the binding sites after superposing them. PRECISE[5] is also a database, while it clustered protein chains according to their EC[6] numbers and the sequence identities then did statistics on the ligand-interacting positions after applying multiple sequential alignment in each cluster. These studies show great interests in the binding information in protein-ligand complexed structures.



1.3 Adenosine 5'-triphosphate

According to a statistics on the protein-ligand complexes in the PDB, adenosine 5'-triphosphate (ATP) is one of the compounds complexed with a large number of protein structures. ATP plays an essential role in all forms of life. It functions as a carrier of energy to fuel biological machines *via* hydrolysis of the high-energy phosphate bonds and participates in the process of cell signaling *via* phosphorylation of proteins, and *etc*. Due to its importance in cellular energy transfer, signal transduction, and protein synthesis, molecular recognition of ATP in proteins has emerged as a subject of great interest in cellular biology[7, 8]. To understand the molecular recognition of ATP, the knowledge of the ATP-binding sites

and specific non-bonded intermolecular interactions between ATP and its surrounding residues in proteins can be a great help.

An ATP molecule is made of the adenine base linked to three phosphate groups *via* ribose. When binding proteins, one or more magnesium ions are often found in coordination with the negatively charged phosphate groups. A study of ribose recognition in ATP-, ADP-, and FAD-protein complexes had appeared recently[9]. Numerous analyses had also been directed at molecular recognition of phosphate groups and their associated magnesium ions[7, 10, 11]. As a matter of fact, several well-know signature sequence motifs, such as the Walker A motif [10] and Kinase-1, Kinase-2 motifs [11] are involved in binding of the adenine moiety of ATP in proteins.

Figure 1a shows the molecular structure and chemical groups of an ATP. Besides the hydrogen bonding to the oxygen atoms of the phosphates and the ribose, the adenine base also has the capacity to form five hydrogen bonds, acting as a donor for two hydrogen bonds at the N6 position and hydrogen bond acceptors at the N1, N3, and N7 positions. (Figure 1b) This hydrogen bonding capacity of ATP is widely accepted as and important intermolecular interaction mode for DNA base-paring and protein-ligand interactions. There are two more equally important intermolecular interaction modes for adenine-protein interactions, i.e. π- π stacking interactions & cation-π interactions[12]. Just as in the case of DNA base-stacking, the conjugated π ring of the adenine base of ATP can interact with surrounding aromatic

residues (Phenylalanine, Tyrosine, and Tryptophan) *via* π-π stacking interactions. (Figure 1c) It can also interact with positively charged residues (Lysine, Arginine, and Histidine) through cation-π interactions. (Figure 1d) A wealth of information has been accumulated displaying the importance of π-π stacking interactions and cation-π interactions in the formation of bio-molecular systems. Typically, π-π stacking interactions and cation-π interactions are of similar or even greater magnitude than the hydrogen bonding energy[13-18].

1.4 3D-BLAST

3D-BLAST[19] has been created as a fast protein structure search tool and that can search >10,000 structures in 1.3 seconds using only an ordinary personal computer. This innovative program dispenses with the need to perform searches for Euclidean distances between corresponding residues; instead, the highly regarded local sequence alignment tool, BLAST, is used to discover homologous proteins and to evaluate the statistical significance of hits by providing *E*-values from structure databases. The core idea of 3D-BLAST is to design a structural alphabet—to be used to encode 3D protein structure databases into structural alphabet sequence databases (SADB)—and a structural alphabet substitution matrix (SASM). The method of 3D-BLAST encodes three-dimensional protein structures into structural alphabet sequences by mapping 5-mer structural segments into corresponding structural letters. These structural alphabet sequences and our new structural alphabet substitution matrix (SASM) enhance the ability of BLAST to search structural homology of a

query sequence to a known protein or family of proteins, often providing clues to the function of a query protein. We then enhanced the sequence alignment tool BLAST, which searches the SADB using the matrix SASM to rapidly determine protein structure homology or evolutionary classification.

3D-BLAST was designed to maintain the advantages of BLAST, including its robust statistical basis, effective and reliable database search capabilities, and established reputation in biology. However, the use of BLAST as a search tool also has several limitations, which are the maximum state (23 states) of the structural alphabet, the need for a new structural alphabet substitution matrix (SASM), and a new *E*-value threshold to indicate the statistical significance of an alignment. Furthermore, 3D-BLAST is slow if the structural alphabet is un-normalized, because the BLAST algorithm searches a statistically significant alignment by two main steps. It first scans the database for hit words that the scores exceed a threshold value if aligned with words in the query sequence. Then, it extends each hit word in both directions to check the alignment score. To reduce the negative effect of un-normalized structural alphabet, we set a maximum number, 16000(~7.0% of total structural segments in the pair database), of segments in a cluster in order to have similar compositions for the 23 structural letters and 20 amino acids.

3D-BLAST has the advantages of BLAST for fast structural database scanning and evolutionary classification. It searches for the longest common substructures, called

SAHSPs (Structural Alphabet High-scoring Segment Pairs), existing between the query structure and every structure in the structural database. The SAHSP is similar to the high-scoring segment pair (HSP) of BLAST, which is used to search amino acid sequences. 3D-BLAST ranks the search homology structures based on both SAHSP and *E*-values, which are calculated from the SASM. 3D-BLAST is much faster than related programs and it is available at http://3d-blast.life.nctu.edu.tw.

1.5 Thesis Overview

In this work, we adopted a structural-based binding pocket clustering scenario on ATP-binding protein chains. To more focus on the information in ATP-binding pockets, we took the binding pocket similarity into account during the clustering process. After clustering the binding pockets, we analyzed the non-bonded interactions between ATP and the binding protein chains systematically. We also calculated the interaction similarity and the interaction-conserved positions for each cluster.

In Chapter 2, we will introduce the materials and the methods, including the dataset preparation, the ATP-binding site extraction, the clustering scenario, and the analysis approaches on the non-bonded interactions. Chapter 3 shows the results and discussions. In that chapter, we will reveal the interaction distributions, the similarity of interactions in all clusters, and the interaction conservations within each cluster. After our clustering process, the ATP-binding pockets show relatively strong conservative properties within each cluster.

The results may contribute to the pattern generation and may help ones discover the structural motifs of the ATP-binding pockets. Therefore, we proposed some applications and the future works in Chapter 4 and drew a conclusion for this study.

Chapter 2

Material and Methods

In this chapter, we are going to introduce the materials used in this research, including the ATP-binding protein chains as the dataset and the ATP-binding SCOP[20] domains used in the verification. Also, we will illustrate the clustering scenario we adopted on those dataset in a step-by-step manner.

The overall framework is shown in Figure 2. In section 2.1, we first fetched the whole list of PDB structures complexed with ATPs and extracted the ATP-binding pockets (contact amino acid residues). Then, in section 2.2, we queried each chain to a protein structural similarity search engine, called 3D-BLAST[19], and the search results were further filtered by structural alignment with CE[21], focusing the structural similarity in the ATP-binding pockets. After that, we applied the simple clustering methods by simply merging clusters with common members.

The non-bonded interactions were identified after the clustering. We identified the non-bonded interactions by HBPLUS and an in-house software, PiFinder. The criteria used in these computer programs are shown in section 2.3. The equations used to calculate interaction similarity and interaction-conserved positions within each cluster are also introduced in the same section. After all, in section 2.4, we bring out the approach of

comparison between the SCOP classifications and our clustering results.

2.1 Preparation of Datasets

Preparation of ATP-binding Protein List

The list of ATP-binding proteins was obtained from the PDBsum[22] database (March 3rd, 2006). After all the obsolete or theoretical models were excluded, we had 246 PDB structures as the material for this research.

Extraction of ATP-binding Sites

After getting the ATP-binding protein list, we extracted the ATP-binding sites and the amino acid residues having contacts with ATPs. To do the job, we processed these PDB structures with an in-house program, which can identify any heterogen group in a PDB structure and every contact amino acid residues to the heterogen group. In our research, the defined contact range is 6 angstrom. If the distance between any atom of an amino acid residue and any atom on an ATP is within 6 angstrom, we considered the amino acid residue having contacts with the ATP and the amino acid residue is a `contact residue' of ATP.

In the extracted ATP-binding sites, we found that there are some poorly bound ATP structures in PDB, ex. 1r8b, 1r9t and 1n5i. The ATPs in these structures bind abnormally due to the missing of some other compounds such as RNA strands[23], the mismatch binding ligand of the protein[24], or the affinity of ATP for this site could be promoted by the

protonation of some hydrophilic residues on the protein surface[25]. Besides, we also found some fragmentary ATP structures in the 246 structures. The ATP-binding sites in these abnormal ATP-binding structures usually are composed of no more than 13 amino acid residues. Therefore, we considered only ATP-binding sites composed of 14 or more amino acid residues as valid binding site structures.

After the extraction, we had 486 protein chains having contacts with ATP. A complete list of the ATP-binding protein chains used in this research comes in Appendix A.

Datasets for Verification

To verify the quality of our clustering results, we compared our results to the SCOP domains involving in ATP-binding sites. Every SCOP domain involving in the binding site was then filtered with the number of contact residues, which also belonging to the SCOP domain. A SCOP domain involving 6 or more amino acid residues were considered as a valid contact SCOP domain. Among the 486 protein chains, 341 of them have records in SCOP and at least one valid contact SCOP domain found.

2.2 The Clustering Scenario

Search for Structurally Similar ATP-binding Protein Chains

As we had the protein chains having contacts to ATPs, we wanted to know that among the 486 protein chains, which chains are structural neighbors to each other in both the whole

protein chain and the ATP-binding pocket view. To do so, we queried each contact protein chain to the 3D-BLAST server[19]. When using 3D-BLAST, we used the whole PDB as the searching database and set the cut-off e-value to 10^{-15}. After querying 486 protein chains to the 3D-BLAST, we had 486 protein chain lists containing structurally similar ATP-binding protein chains (neighbors) to the query chains. However, since our research focused on ATP-binding sites, only PDB structures complexed with ATPs were analyzed in the later steps.

Structural Similarity Filtering

3D-BLAST is a fast structural similarity engine but not a structural alignment tool. It does not actually superpose protein structures. Further, proteins similar in the whole protein structures may not similar in the binding sites. Therefore, we used CE, a popular structural comparison tool of protein chains, to do the further filtering of the 3D-BLAST result lists.

For each 3D-BLAST result list, we did an all-against-query CE comparison. A subject chain in a list survives if the CE results between the query and the subject chains satisfy the following two rules.

One is the whole protein structural similarity. The query and the subject chains must have similar whole protein structures. The whole protein structure similarities were evaluated according to the following criterion.

$$\begin{cases} Z \geq 5.0, \ if \ \ L \geq 200 \\ Z \geq 4.5, \ if \ \ 100 \leq L < 200 \\ Z \geq 4.0, \ otherwise \end{cases} \quad (1),$$

where Z is the CE Z-score and L is the CE alignment length. A subject chain survives if the CE Z-score of the structural alignment to the query chain satisfies the criteria list in (1).

The other is the structural ATP-binding pocket similarity. The query and the subject chains must be similar in the ATP-binding sites after the CE structural alignment. To evaluate this criterion, we introduced the *Binding Site Aligned Coverage* as the following.

$$c_{1,2} = \sqrt{\frac{n_a^{\ 2}}{n_1 n_2}} \quad (2),$$

where n_1 and n_2 are the number of contact residues on the query and the subject chains, respectively, and n_a is the number of amino acid residues that are aligned in the CE results and the residues on both chains are contact residues to ATP. The $c_{1,2}$ represents the structural similarity of two binding sites on chain 1 and 2. The two binding site structures are similar if $c_{1,2} \geq 0.4$. Any subject chain on a 3D-BLAST resulting list having $c_{1,2} \geq 0.4$ to the query chain would be filtered for the dissimilarity of the two binding sites, even if the two protein structures are similar.

Only subject chains satisfying both criteria were considered as structural neighbors to the query chain. We believed this structural similarity filtering process keeps protein chains with similar structures in both whole protein chain and the ATP-binding pocket together.

Merging the 3D-BLAST Result Lists

In this research, we adopt a very simple (or, naïve) clustering concept: if two clusters, A and B, have at least on member in common, A and B are then merged into one cluster. This clustering method may be simple, but somehow performed well.

After applying the structural similarity filtering on the 3D-BLAST result lists, we had 486 "clean" protein chain lists; each contains structurally similar protein chains to the query chain, in both the whole protein chain and the ATP-binding site aspects. We first took the "clean" protein chain lists as a cluster it self. Then, we merged these lists if any two of them have some surviving members in common. The simple clustering resulted in 70 clusters. Appendix A gives the whole list of clustering results and the protein chain information, including the number of contact residues to ATP, the contact SCOP domain family, the EC[6] number of the chain, and the protein name.

2.3 Non-bonded Interaction Analysis

Eliminating Homologues

One of the purposes of this work is to do analysis on the ATP-binding patterns and try to

discover novel ATP-binding motifs. However, the analysis may bias the dominant homologous chains, such as multi-chain PDB structures and highly homologous proteins among various species, presented in a cluster. Therefore, after the clustering, we used the sequence similarity to eliminate the homologues for each cluster. In this step, we adopt ed BLASTCLUST[26], a sequence clustering tool using BLAST[26], to do the job. BLASTCLUST is a DNA/protein sequence clustering tool by using the sequence identity as the clustering features. Chains with 90% or more sequence identity to any other chains in the same cluster were sub-clustered. When analyzing non-bonded interactions, we consider only the longest chain of each sub-cluster in a cluster.

Selecting the Representatives and Multiple Binding Site Alignments

After all the clustering and homologue eliminating steps, we chose a representative chain for each cluster. We selected a chain as the representative if the chain has the highest CE Z-scores to all the other chains in the same cluster.

As the representative chain being selected, we stacked the CE alignments of every chain to the representative (the star alignment). Figure 6b shows an example of structural binding pocket alignment of the cluster 58. The whole list of multiple structural alignments of binding pockets is shown in Appendix B.

Identification of Non-bonded Interactions in ATP-binding Pockets

Non-bonded intermolecular interactions between ATP and surrounding residues in the binding pockets are important to the recognition and binding of ATP. In this work, we focused on hydrogen bond, π-π stacking, and cation-π interactions between ATP and the residues on ATP-binding protein chains. The three types of non-bonded interactions in the 486 chains in PDB structures were identified by HBPLUS[27] and an in-house software, called PiFinder.

HBPLUS[27] identifies all hydrogen bonds in a PDB structure by calculating the distance and the angles between all hydrogen bond donors and acceptors. Then, it outputs the donor-acceptor pairs and their status of the found hydrogen bonds.

The π-π stacking and cation-π interactions between ATP and the residues on ATP-binding protein chains were identified by PiFinder, an in-house software written in C/C++. π-π stacking interactions are formed between the aromatic ring of an ATP and the aromatic rings of a Phenylalanine, Tyrosine, or Tryptophan. While cation-π interactions are formed between the adenine group of an ATP and the positively charged atoms of a Lysine or Arginine. PiFinder identifies a π-π stacking or cation-π interaction by checking the distance between the aromatic ring of an ATP and the aromatic ring or the cation on the amino acid residues. If the aromatic ring of Phe, Tyr, or Trp is in the 5.6 angstrom range of the aromatic ring of an ATP, PiFinder reports the ATP and the residue interact *via* the π-π stacking

interaction. If the cation of Lys or Arg is in the 5.6 angstrom range of the aromatic ring of an ATP, PiFinder will report the ATP and the residue interact *via* the cation-π interaction. The definitions of π-π stacking and cation-π interactions were referred to a previous study in [12].

In the figures showing non-boned interaction profiles for ATP-binding protein chains in this thesis (Figure 3,4,5,6,7), '|' denotes the residues forming a hydrogen bonds to ATP, '=' denotes the residues forming π-π stacking or cation-π interactions to the aromatic ring to ATP, and '+' for combinations of these three types of non-bonded interactions on a residue.

Analysis of Protein-Ligand Interactions

For each cluster, we identified every hydrogen bond, π-π stacking or cation-π interactions to ATP by HBPLUS and PiFinder. Then we encoded the interaction profiles in the binding-pocket to binary strings. For each contact residue, residues that have at least one type of non-bonded interaction to the ATP are marked `1', or `0', otherwise.

After we transformed the hydrogen bond interactions for each chain in the cluster to binary strings, we used the Tanimoto Coefficient (or Jaccard Coefficient) (3) as an interaction similarity index.

$$tanimoto_{1,2} = \frac{|s_1 \wedge s_2|}{|s_1 \vee s_2|} \qquad (3),$$

where s_1 and s_2 are the two binary strings. The closer to 1.0 $tanimoto_{1,2}$ is, the more similar

the non-bonded interaction profiles of the two binding-pockets are.

Beside the interaction similarity, we also identified interaction-conserved positions in each clusters. For each position in a cluster, we calculate the percentage of forming interactions to ATP, $intcon_{c,i}$. (4)

$$intcon_{c,i} = \frac{nInt_{c,i}}{n_c} \qquad (4),$$

where n_c is the number of chains in the cluster c and $nInt_{c,i}$ is the number of chains forming non-bonded interactions to ATP. A position in a cluster is interaction-conserved if $intcon_{c,i} \geq 50\%$.

2.4 Clustering Evaluation

To evaluate the performance of our clustering results, we compared our clustering results to the SCOP classifications. We first extracted the contact SCOP domain(s) for each ATP-binding pockets in the dataset. For each cluster, the most popular SCOP family in the cluster was assigned to the cluster, while the presenting of any other SCOP families was considered as incorrectly clustered. Then we calculated the rate of `correctly clustered' (5) for each cluster and for the whole evaluated dataset.

$$accuracy = \frac{\#\text{corrected clustered protein chains}}{\#\text{protein chains with records in the SCOP}} \qquad (5)$$

To be noticed, one SCOP family may be assigned to two or more clusters, since proteins structurally similar may not function similarly. As our clustering method focused only on

protein structural properties, we consider protein chains under this circumstance as ‘correctly clustered’.

Chapter 3

Results and Discussions

Many works have been proposed to analyze on the ATP-binding proteins. Some of them used the multiple sequence alignment techniques to locate the conserved motifs or domains[21], such as the Walter A motif[10] and the Kinase-1 and the Kinase-2 motifs[11]. Some others adopted the structural alignment tools to find out the structural motifs for binding ATP [28], such as the ATP-grasp family[20]. Some other research groups systematically applied statistics on the distributions of different types of interactions between ATP and the binding proteins[12].

In this work, we adopted 3D-BLAST to search neighbors among ATP-binding protein chains, then used CE to structurally align ATP-binding proteins and used the results, especially the structural similarity in the binding pockets, to do the binding pocket clustering. Then we analyzed the non-bonded interactions, including hydrogen bonding, π-π stacking interactions, and cation-π interactions, between ATP and proteins for every cluster.

3.1 The Overall Results of the Clustering

The clustering resulted in 70 clusters from the 486 ATP-binding protein chains. Appendix A gives the whole clustering results of the 486 ATP-binding protein chains and the information of those protein chains. Among the 70 clusters, 20 of them are singletons and

16 clusters have only two chains. The rest of them, 34 clusters, have three members or more.

In each cluster, there exist many homologous chains, such as mutants or those from different species. The homologous chains may dominate over other chains while analyzing the sequence or the non-bonded interaction conservations in each cluster. Therefore, we applied the non-bonded interaction analyses on the homologue-eliminated clusters (Table 2) rather than the original ones. (Appendix A) The detailed steps for eliminating homologues are shown above in Chapter 2.

After eliminating homologues in each cluster, the number of singletons increased to 50, a relatively large number compared to the total 70 clusters. We compared the contact SCOP domains of those chains in singletons to the chains in the other clusters. We found that among those 50 singletons, except 15 of them with no domain documented in SCOP, the SCOP families of the contact SCOP domains of the 24 singletons are unique in the homologue-eliminated dataset. This somehow explains the large number of singletons that, protein structures in those singletons are structurally unique to the other ATP-binding proteins in the dataset. The rest 11 singletons belong to the same SCOP families as those of some other clusters.

3.2 The Comparison with the SCOP

There are 341 out of the 486 ATP-binding protein chains with domains documented in the SCOP classifications. Currently, they are classified into 50 different SCOP families. We calculated the rate of `correctly clustered‘(5) of those 341 protein chains as the accuracy of our clustering. Protein chains with no records in the SCOP classifications were omitted in the accuracy calculation.

With no surprise, the clustering results got a high correspondence with the SCOP classifications. Most binding pockets belonging with the same SCOP classification were clustered into the same group. The good correspondence was not surprising because we used the structural similarity as the clustering criteria whereas the SCOP classifies protein domains according to their structural components.

Overall, we got 95% accuracy on the original dataset, and 93% accuracy on the homologue-eliminated dataset. The accuracies of all 70 clusters are listed in Table 1.

3.3 The Sequence Identity

When two proteins have 30% or more sequence identity, one can infer that these two proteins have similar function with a high accuracy. To confirm that our clustering can cluster interaction-similar but non-homologous chains together, we checked the sequence identity distributions for all clusters. Table 4 and 5 show the distributions of intra-cluster

sequence identities of non-singleton clusters in the original and the homologue-eliminated datasets.

Before eliminating homologues, many clusters presented high sequence identity (Table 4). In Appendix A, we can see that a cluster with 100% sequence identities is usually made of a single multi-chain PDB structure. Theses clusters therefore would become singleton after the homologue filtering. This shows that, when searching in the 486 ATP-binding protein chains, there was no structurally similar protein chain in both whole protein and the ATP-binding pocket perspectives.

After filtering homologues, as Table 5 shows, only 2 clusters have protein chains with more than 30 percent sequence identity to all the other members, while other clusters present less homology. According to this non-homologous property, our analyses on ATP-binding mode and non-bonded interactions may not be biased by dominant homologous protein chains.

3.4 The Non-bonded Interaction Similarity

Non-bonded interactions play an important role in the ligand recognition of proteins. They also stabilize ligands in the binding pockets. There are three major types of non-bonded interaction between ligands and proteins. They are hydrogen bonding, π-π stacking, and cation-π interactions. There exist plenty of studies about hydrogen bond

interactions[29-32]. Though, there are some studies concerned about the contributions of π-π stacking interactions and cation-π interactions[15, 18, 33, 34]. They reported that π-π stacking interactions and cation-π interactions are of similar or even greater magnitude than the hydrogen bonding energy[13-18]. In this work, we analyzed the profiles of all these three types of non-bonded interactions and try to find out the difference of the interaction patterns between the clusters.

Interaction Similarity by the Tanimoto Coefficient

After the CE structural alignments and identifications of all the three types of non-bonded interactions, we tried to observe the non-bonded interaction profile similarity within each cluster. To achieve that, we adopted the Tanimoto Coefficient (or Jaccard Coefficient) (3) as an interaction similarity index. We encoded the interaction profiles in ATP-binding pockets as binary strings, where `1' denotes the positions forming non-bonded interactions and `0' represents for nothing. Then, we calculated the all-against-all Tanimoto Coefficients in a cluster and did the statistics on them. Table 3 shows the distributions of the interaction similarity of non-singleton clusters.

As the Table 3 shows, we found that many clusters present 25% or more interaction similarity. This shows that our clustering results do conserve on the interaction profile in most of the cases.

However, there are still clusters showing less similarity in their non-bonded interaction profiles, such as the cluster 30. Figure 5 shows the superposition, the CE structural alignments, and the interaction profile of the ATP-binding pockets of the cluster 30. The average interaction similarity of the cluster 30 is 10.5%, which is the lowest among all clusters. But, the 4 hydrolase protein chains are somehow well-aligned by CE. The reason why the interaction profiles are not so similar is the different ATP orientations in 1jknA and 1vc9A, while the proteins do present structural similarity.

3.5 The Interaction-Conserved Positions

For each position *i* a cluster, we calculate the percentage of forming interactions to ATP over a cluster *c*, $intcon_{c,i}$ (4). In Table 3, we also give the counts of interaction-conserved positions in each cluster. As our observation, those interaction-conserved positions are critical for ATP binding and they usually gather up in the regions, which may be potential motifs. We will discuss them in the next section.

3.6 The ATP-binding Motifs

Several well-known signature sequential motifs, such as the Walker A motif[10], Kinase-1, and Kinase-2 motifs[11] involve in binding of phosphate groups and their associated metal ions. In our clustering results, we can also see those well-known sequential motifs showing.

Known Patterns in the Clustering Results

The Walker A motif, G-X{4}-G-K-[TG]-X{6}-[IV], for adenylate kinase, α, β, and myosin. It interacts with the adenine base while an adenylate kinase catalyze an AMP with an ATP[10]. The Walker A motifs show up in the clusters 24, 29, 57, 62, and 64. (Appendix B) The Kinase-1 motif, [GASN]-X{4}-[GACS]-K-[GSTVAP]-[TSADGNM], functions in binding of phosphates of the ligand, which is ATP in our case[11]. It is much frequently found in the clustering results. It shows in the clusters 11, 16, 26, 27, 31, 38, 41, 46, 61, 63, 66, and 67. The Kinase-2 motif is relatively short and less seen in our clustering results. The motif is [VGILNTAYK]-[AFLIGDETCKP]-[ALIGVSPEFHT]-[LGVITDFQMYK]-D. It contains the conserved aspartate that coordinates with the Mg-ATP in the ATP-binding site[11]. In our clustering results, it presents in the cluster 59 only.

Besides those well-known sequence motifs, we found some novel motifs that form hydrogen bonds to ATP.

Potential Patterns in the cluster 29

In the cluster 29, there is a highly conserved region, named C29_PAT in the beginning part of the binding site alignment. (Figure 3) The 4 members are ATP-binding sites from ubiquitin-activating related and adenylyltransferase thiF proteins. Among the 4 chains in the cluster 29, there are several identical positions in both structural and interaction views. As

we query C29_PAT to the PROSITE database by encoding C29_PAT as [IV]G[AL]GG[IL]G-X(17)-[28]-D-[MFLD]-D-[TD]-[IV]-[SDH]-[LV]-SNL-[NQ]RQ-X(11)-K, which is the pattern syntax used in PROSITE, the returned sequences are all related to the for chains in the cluster 29. Besides, the PROSITE reported `no hits' for any documented patterns in the database, while we query the 4 chains to the ScanProsite server[28]. We believed that was the evidence for C29_PAT being a potential novel pattern for ubiquitin-activating related and adenylyltransferase thiF proteins. However, it needs further validation by stronger supports.

Potential Patterns in the cluster 59

The cluster 59 is one of the clusters with most interaction-conserved positions shown. From the multiple structure alignment of the cluster 59 (Figure 4), we identified three potential motifs for interacting to ATP. They are D-[CNL]-G-[ST]-[35]-[MY]-[CST]-[KC], [DS]-[LS]-G-[GDY]-[28]-[FTV]-[TF]-[HGD], and [STV]-G-G-[GST]-[AT]-[KMR]-[IFY]-[PR], ordered by their occurrences in the alignment. We named them as C59_PAT_1, C59_PAT_2, and C59_PAT_3, respectively. The C59_PAT_3 forms non-bonded interaction to the adenine base of ATP while C59_PAT_1 and C59_PAT_2 form hydrogen bonds to the phosphate groups.

The cluster 59 contains chains of α-actin, Arp 2/3, defensin HBD-2, and Hsc70 proteins. Except Arp2 and Arp3, which have no record in the SCOP, they have c.55.1.1 family domains

as the ATP contact SCOP domain. Not only does the SCOP classify these contact domains into a same family, there are literatures support the structural similarity and the genetic relationship among them[36]. As we query the three motifs found in this cluster to PROSITE[28], there is no previous defined pattern matching them. Furthermore, when we queried the whole sequences of each chain to PROSITE to search for known patterns, PROSITE returned `no hit' on the sequences. This tells us that we may have found some novel patterns for Actin/Hsc70 protein families.

There are still other potential motifs interacting with ATP, though, they need to be further validated. They are shown in Appendix B, where we give the overall view of structural alignments and interaction profiles for each cluster.

Chapter 4

Conclusions and Future Works

4.1 Conclusions

The rapid increase of three-dimensional protein-ligand complex structures has made the analysis on protein-ligand binding research. However, the slowly updated classification databases, like SCOP and CATH, make it hard to classify newly solved protein structure immediately.

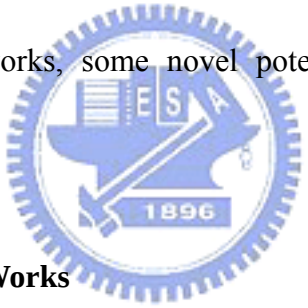
In this work, we adopted a fast protein structural similarity search tool, called 3D-BLAST, to do protein-ligand complexes analysis and used ATP-binding proteins as a study case. We clustered protein-ATP complexes based on the whole protein chain structures, the binding-site structural similarity, and non-bonded interaction profiles. With the clustering, we are able to analyze the protein-ligand interactions and identify functional important residues and potential ATP-binding motifs.

First of all, we used 3D-BLAST to perform protein-ligand complexes homologous search in whole protein database. Secondly, CE was used as a detailed structure alignment tool to identify structural similarity of ligand-binding site. Accordingly, we can obtain a preliminary classification for protein complexes.

For protein-ligand interaction profiles, we adopted the HBPLUS and an in-house

software, PiFinder, to identify the non-bonded interactions including hydrogen bond, π- π stacking, and cation- π interactions. According to structural similarity and functional protein-ligand interaction patterns, a simple cluster method was applied to group protein-ATP complexes.

Overall, we got a 95% accuracy of the clustering results compared to the SCOP classifications. We systematically analyzed the non-bonded interactions, between ATP and the binding protein chains. We found that the three types of non-bonded interactions show relatively strong conservation within clusters. Not only had the ATP-binding motif discovered in the previous works, some novel potential ATP-binding motifs were also identified in some clusters.



4.2 Applications and Future Works

Since the discovered novel motifs are more important to the ATP-binding, the novel motifs can then be used to predict the ATP-binding property of proteins not complexed with ATPs or even protein sequences that the structures are not solved yet.

With the fast protein-ATP complex clustering method and the protein-ligand interaction analyses proposed in this work, we can also apply the same process to protein-ligand complexes of any other ligand. Therefore, we can discover more potential novel ligand-binding motifs that essential for the ligand-binding. Moreover, we can construct a

ligand-binding motif database and provide some services for searching proteins that could be bound by a given ligand or ligands that probably bind to a given protein. However, since the lack of evidence of the novel ligand-binding motifs currently, the newly discovered motifs should be carefully validated in the future days.

Table 1. The accuracy for each cluster

Cid[a]	# Members	# Members having SCOP[b]	# Correct Clustered	Accuracy
1	1	1	1	100%
2	1	0	-	-
3	1	0	-	-
4	1	0	-	-
5	1	1	1	100%
6	1	0	-	-
7	1	0	-	-
8	1	0	-	-
9	1	0	-	-
10	1	0	-	-
11	2	2	2	100%
12	1	1	1	100%
13	1	0	-	-
14	1	0	-	-
15	1	1	1	100%
16	1	1	1	100%
17	1	1	1	100%
18	1	1	1	100%
19	1	1	1	100%
20	1	1	1	100%
21	1	0	-	-
22	1	1	1	100%
23	1	1	1	100%
24	1	1	1	100%
25	1	1	1	100%
26	1	1	1	100%
27	1	1	1	100%
28	1	1	1	100%
29	4	2	1	50%
30	4	2	2	100%
31	2	2	2	100%
32	1	1	1	100%
33	1	1	1	100%
34	1	1	1	100%
35	1	1	1	100%
36	1	1	1	100%
37	2	2	2	100%
38	1	1	1	100%
39	1	0	-	-
40	3	2	2	100%
41	2	1	1	100%
42	1	1	1	100%
43	4	2	2	100%
44	1	1	1	100%
45	1	0	-	-
46	1	1	1	100%
47	1	0	-	-
48	3	1	1	100%
49	1	1	1	100%
50	1	1	1	100%

Cid[a]	# Members	# Members having SCOP[b]	# Correct Clustered	Accuracy
51	1	1	1	100%
52	4	3	1	33%
53	4	4	2	50%
54	1	1	1	100%
55	1	1	1	100%
56	1	1	1	100%
57	3	3	3	100%
58	16	9	7	78%
59	6	4	4	100%
60	7	7	7	100%
61	3	1	1	100%
62	3	2	2	100%
63	11	7	7	100%
64	8	4	4	100%
65	1	0	-	-
66	1	1	1	100%
67	1	1	1	100%
68	5	4	4	100%
69	1	1	1	100%
70	1	1	1	100%

[a] The serial identification of clusters.

[b] The number of cluster members with domain records in SCOP.

Table 2. The cluster results after eliminating homologues

Cid[a]	Rep[b]	Chain	SCOP Families of Contact Domains [c]	EC	Protein Name
1	4at1B	4at1B	d.58.2.1 (21)	2.1.3.2	Aspartate Carbamoyltransferase
2	2c01X	2c01X		3.1.27.5	Nonsecretory Ribonuclease
3	2aruA	2aruA		6.3.2.-	Lipoate-Protein Ligase A
4	2aqxA	2aqxA		2.7.1.127	Inositol 1,4,5-Trisphosphate 3-Kinase B
5	8icnA	8icnA	d.218.1.2 (15)	2.7.7.7	DNA Polymerase Beta
6	1z0sA	1z0sA		2.7.1.23	Polyphosphate/ATP-NAD Kinase
7	1yp3A	1yp3A		2.7.7.27	Glucose-1-Phosphate Adenylyltransferase Small Subunit (ADP-Glucose Synthase)
8	1y56A	1y56A		1.5.99.8	L-Proline Dehydrogenase
9	1xdnA	1xdnA			RNA Editing Ligase Mp52
10	1wklB	1wklB		2.7.4.6	Nucleotide Diphosphate Kinase
11	1vjcA	1vjcA 3pgk_	c.86.1.1 (26) c.86.1.1 (28)	2.7.2.3 2.7.2.3	Phosphoglycerate Kinase Phosphoglycerate Kinase
12	2gnkA	2gnkA	d.58.5.1 (17)		Nitrogen Regulatory Protein
13	1v3sA	1v3sA			Nitrogen Regulatory Protein Pii
14	1twaA	1twaA		2.7.7.6	DNA-Directed RNA Polymerase II Largest Subunit
15	1tc0A	1tc0A	d.122.1.1 (25)		Endoplasmin
16	1qhxA	1qhxA	c.37.1.3 (28)	2.7.1.-	Chloramphenicol Phosphotransferase
17	1obgA	1obgA	d.143.1.1 (13)	6.3.2.6	Phosphoribosylamidoimidazole-Succinocarboxamide Synthase
18	1obdA	1obdA	d.143.1.1 (23)	6.3.2.6	Phosphoribosylamidoimidazole-Succinocarboxamide Synthase
19	1o93B	1o93B	d.130.1.1 (14)	2.5.1.6	S-Adenosylmethionine Synthetase
20	1o93A	1o93A	d.130.1.1 (16)	2.5.1.6	S-Adenosylmethionine Synthetase
21	1yfrA	1yfrA		6.1.1.7	Alanyl-tRNA Synthetase
22	1n48A	1n48A	e.8.1.7 (22)		DNA Polymerase IV
23	1mo8A	1mo8A	d.220.1.1 (24)		Sodium/Potassium-Transporting Atpase Alpha-1
24	1mjhA	1mjhA	c.26.2.4 (32)		(unknown)
25	1miwA	1miwA	d.218.1.4 (17), a.173.1.1 (11)		tRNA Cca-Adding Enzyme
26	1w7aB	1w7aB	c.37.1.12 (28)		DNA Mismatch Repair Protein Muts
27	1ko5A	1ko5A	c.37.1.17 (23)	2.7.1.12	Gluconate Kinase
28	1r8bA	1r8bA	d.218.1.7 (23), a.160.1.3 (6), d.58.16.2 (10)		tRNA Nucleotidyltransferase
29	1jwaB	1jwaB 1r4nB 1y8qB 1zfnA	c.111.1.1 (29) c.111.1.2 (30)	 2.7.7.-	Molybdopterin Biosynthesis MoeB Protein Ubiquitin-Activating Enzyme E1C Ubiquitin-Like 2 Activating Enzyme E1B Adenylyltransferase THIF
30	1xscA	1jknA 1su2A 1vc9A 1xscA	d.113.1.1 (33) d.113.1.1 (21)	3.6.1.17 3.6.1.17	Diadenosine 5',5'''-P1,P4-Tetraphosphate Hydrolase Mutt/Nudix Family Protein HB8 Ap6A Hydrolase Bis(5'-Nucleosyl)-Tetraphosphatase
31	1jjvA	1jjvA 1uf9C	c.37.1.1 (20) c.37.1.1 (24)	2.7.1.24	Dephospho-CoA Kinase (unknown)
32	1jagA	1jagA	c.37.1.1 (33)	2.7.1.113	Deoxyguanosine Kinase
33	3r1rA	3r1rA	a.98.1.1 (19)	1.17.4.1	Ribonucleotide Reductase R1 Protein
34	1hp1A	1hp1A	d.114.1.1 (14)	3.1.3.5, 3.6.1.45	5'-Nucleotidase
35	1hi1A	1hi1A	e.8.1.6 (16)		RNA Polymerase
36	1pj4A	1pj4A	c.2.1.7 (22), c.58.1.3 (7)	1.1.1.39	NAD-Dependent Malic Enzyme, Mitochondrial
37	1n77A	1gtrA 1n77A	c.26.1.1 (29) c.26.1.1 (28)	6.1.1.18 6.1.1.17	Glutaminyl-tRNA Synthetase Glutamyl-tRNA Synthetase

Cid[a]	Rep[b]	Chain	SCOP Families of Contact Domains [c]	EC	Protein Name
38	1g5tA	1g5tA	c.37.1.11 (18)	2.5.1.17	COB(I)Alamin Adenosyltransferase
39	1xdpA	1xdpA		2.7.4.1	Polyphosphate Kinase
40	1gn8A	1f9aA	c.26.1.3 (28)		NMN Adenylyltransferase
		1gn8A	c.26.1.3 (33)	2.7.7.3	Phosphopantetheine Adenylyltransferase
		1yunA		2.7.7.18	Nicotinate-Nucleotide Adenylyltransferase
41	1xexA	1f2uA	c.37.1.12 (21)		RAD50 ABC-Atpase
		1xexA			SMC Protein
42	1kvkA	1kvkA	d.14.1.5 (29)		Mevalonate Kinase
43	1yidB	1h3eA	c.26.1.1 (32)	6.1.1.1	Tyrosyl-tRNA Synthetase
		1m83A	c.26.1.1 (36)	6.1.1.2	Tryptophanyl-tRNA Synthetase
		1yidB		6.1.1.2	Tryptophanyl-tRNA Synthetase
		2a84A		6.3.2.1	Pantoate--Beta-Alanine Synthetase
44	1nsyA	1nsyA	c.26.2.1 (27)	6.3.5.1	NAD Synthetase
45	1r9tB	1r9tB		2.7.7.6	DNA-Directed RNA Polymerase II
46	1fmwA	1fmwA	c.37.1.9 (32)		Myosin II Heavy Chain
47	1sx3A	1sx3A			Groel Protein
48	2bu2A	1tilA	d.122.1.3 (35)	2.7.1.37	Anti-Sigma Factor Spoiiab
		1y8pA		2.7.1.99	[Pyruvate Dehydrogenase [Lipoamide]] Kinase Isozyme 3
		2bu2A		2.7.1.99	Pyruvate Dehydrogensae Kinase Isoenzyme 2
49	1n5iA	1n5iA	c.37.1.1 (10)		Thymidylate Kinase
50	1e2qA	1e2qA	c.37.1.1 (21)	2.7.4.9	Thymidylate Kinase
51	1dy3A	1dy3A	d.58.30.1 (27)	2.7.6.3	7,8-Dihydro-6-Hydroxymethylpterinpyrophosphokinase (Pyrophosphorylase, Pppk)
52	2f02A	1esqA	c.72.1.2 (27)	2.7.1.50	Hydroxyethylthiazole Kinase
		1lhrA	c.72.1.5 (29)	2.7.1.35	Pyridoxal Kinase
		1v1bA	c.72.1.1 (34)		2-Keto-3-Deoxygluconate Kinase
		2f02A		2.7.1.144	Tagatose-6-Phosphate Kinase
53	1dv2A	1dv2A	d.142.1.2 (28)	6.3.4.14	Biotin Carboxylase
		1kj8A	d.142.1.2 (30)	2.1.2.-	Phosphoribosylglycinamide Formyltransferase 2
		1i7lA	d.142.1.3 (32)		Synapsin II
		1pk8A	d.142.1.3 (29)		Synapsin I
54	1d9zA	1d9zA	c.37.1.19 (23)		DNA Repair Protein UVRB
55	1bcpF	1bcpF	b.40.2.1 (9)	2.4.2.-	Pertussis Toxin
56	1bcpE	1bcpE	b.40.2.1 (13)	2.4.2.-	Pertussis Toxin
57	1h8hA	1e79A	c.37.1.11 (17)	3.6.1.34	ATP Synthase Alpha Chain Heart Isoform (Bovine Mitochondrial F1-Atpase)
		1h8hA	c.37.1.11 (19)	3.6.1.34	ATP Synthase Alpha Chain Heart Isoform
		1tf7A	c.37.1.11 (23)		Circadian Clock Protein KAIC
58	1gol_	1atpE	d.144.1.7 (33)	2.7.1.37	cAMP-Depedent Protein Kinase (CAPK)
		1b38A	d.144.1.7 (30)	2.7.1.37	Cell Division Protein Kinase 2
		1ol6A	d.144.1.7 (26)	2.7.1.37	Serine/Threonine Kinase 6
		1csn_	d.144.1.7 (29)	2.7.1.-	Casein Kinase-1
		1phk_	d.144.1.7 (31)	2.7.1.38	Phosphorylase Kinase
		1gol_	d.144.1.7 (22)	2.7.1.-	Extracellular Regulated Kinase 2
		1q97A	d.144.1.7 (29)	2.7.1.-	Sr Protein Kinase
		1e8xA	d.144.1.4 (26)	2.7.1.137	Phosphatidylinositol 3-Kinase Catalytic Subunit
		1tqpA	d.144.1.9 (28)		RIO2 Serine Protein Kinase
		1zp9A			RIO1 Kinase
		1s9iA			Dual Specificity Mitogen-Activated Protein Kinase Kinase 2
		1s9jA			Dual Specificity Mitogen-Activated Protein Kinase Kinase 1
		1u5rA			Serine/Threonine Protein Kinase Tao2
		1ua2A		2.7.1.37	Cell Division Protein Kinase 7
		1zydA		2.7.1.37	Serine/Threonine-Protein Kinase GCN2
		2biyA		2.7.1.37	3-Phosphoinositide Dependent Protein Kinase-1
59	1eqyA	1e4gT	c.55.1.1 (33)		Cell Division Protein FTSA
		1eqyA	c.55.1.1 (36)		Alpha-Actin
		1nge_	c.55.1.1 (36)	3.6.1.3	Heat-Shock Cognate 70Kd Protein
		1yagA	c.55.1.1 (37)		Actin
		1tyqA			Actin-Related Protein 3
		1tyqB			Actin-Related Protein 2

Cid[a]	Rep[b]	Chain	SCOP Families of Contact Domains [c]	EC	Protein Name
60	1b76A	1aszA	d.104.1.1 (22)	6.1.1.12	Aspartyl tRNA Synthetase
		1b76A	d.104.1.1 (29)	6.1.1.14	Glycyl-tRNA Synthetase
		1b8aA	d.104.1.1 (26)	6.1.1.12	Aspartyl-tRNA Synthetase
		1e24A	d.104.1.1 (29)	6.1.1.6	Lysyl-tRNA Synthetase
		1h4qA	d.104.1.1 (27)	6.1.1.15	Prolyl-tRNA Synthetase
		1kmnA	d.104.1.1 (30)	6.1.1.21	Histidyl-tRNA Synthetase
		1nyrA	d.104.1.1 (28)	6.1.1.3	Threonyl-tRNA Synthetase 1
61	1ayl_	1ayl_	c.91.1.1 (37)	4.1.1.49	Phosphoenolpyruvate Carboxykinase
		1xkvA		4.1.1.49	Phosphoenolpyruvate Carboxykinase
		1ytmA		4.1.1.49	Phosphoenolpyruvate Carboxykinase
62	2bekA	1a82_	c.37.1.10 (29)	6.3.3.3	Dethiobiotin Synthetase
		1g21E	c.37.1.10 (31)	1.18.6.1	Nitrogenase Iron Protein
		2bekA			Segregation Protein SOJ
63	1b0uA	1b0uA	c.37.1.12 (19)		ABC Transporter (Histidine Permease)
		1f2uB	c.37.1.12 (19)		RAD50 ABC-Atpase
		1ji0A	c.37.1.12 (21)		ABC Transporter
		1l2tA	c.37.1.12 (22)		ABC Transporter
		1mv5A	c.37.1.12 (17)		Multidrug Resistance ABC Transporter ATP-Binding And Permease Protein
		1q12A	c.37.1.12 (31)		Maltose/Maltodextrin Transport ATP-Binding Protein Malk
		1r0xA	c.37.1.12 (21)		Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)
		1vciA			Sugar-Binding Transport ATP-Binding Protein
		1xefA			Alpha-Hemolysin Translocation ATP-Binding Protein HLYB
		1xexB			SMC Protein
		1xmiA		3.6.3.49	Cystic Fibrosis Transmembrane Conductance Regulator
64	1nsf_	1do0A	c.37.1.20 (29)		Chaperone (Heat Shock Locus U)
		1g3iA	c.37.1.20 (29)		ATP-Dependent HSLU Protease
		1j7kA	c.37.1.20 (31)		Holliday Junction DNA Helicase Ruvb
		1nsf_	c.37.1.20 (26)		N-Ethylmaleimide Sensitive Factor
		1ojlE			Transcriptional Regulatory Protein Zrar
		1svmA			Large T Antigen
		2a5yB			CED-4
		2c96A			PSP Operon Transcriptional Activator
65	1z7eA	1z7eA			Protein ArnA
66	1qhgA	1qhgA	c.37.1.19 (25)		ATP-Dependent Helicase Pcra
67	1ii0A	1ii0A	c.37.1.10 (29)	3.6.3.16	Arsenical Pump-Driving Atpase
68	1xngA	1ee1A	c.26.2.1 (33)	6.3.5.1	NH3-Dependent NAD+ Synthetase
		1j1zA	c.26.2.1 (24)	6.3.4.5	Argininosuccinate Synthetase
		1kp2A	c.26.2.1 (28)	6.3.4.5	Argininosuccinate Synthetase
		1mb9A	c.26.2.1 (33)		Beta-Lactam Synthetase
		1xngA		6.3.1.5	NH(3)-Dependent NAD(+) Synthetase
69	1a49A	1a49A	c.1.12.1 (24), b.58.1.1 (11)	2.7.1.40	Pyruvate Kinase
70	1a0i_	1a0i_	d.142.2.1 (22)	6.5.1.1	DNA Ligase

[a] The serial identification of clusters.

[b] The representative protein chain of the `Cid'-th cluster.

[c] The SCOP families of the contact domains. The numbers in the parentheses are the number of contact residues belonging to the contact SCOP domain.

Table 3. Statistics on interaction similarity of non-singleton clusters

Cid	Interaction Similarity				# ≥ 50% Interactional Conserved Positions
	Average	Std. Dev.	Min	Max	
11	22.20%	0.00%	22.20%	22.20%	2
29	32.50%	10.00%	16.70%	44.40%	4
30	10.50%	11.50%	0.00%	28.60%	5
31	58.30%	0.00%	58.30%	58.30%	7
37	33.30%	0.00%	33.30%	33.30%	3
40	12.80%	3.40%	8.30%	16.70%	5
41	57.10%	0.00%	57.10%	57.10%	8
43	28.60%	13.10%	6.70%	46.20%	8
48	36.70%	6.80%	27.30%	42.90%	7
52	25.00%	8.80%	10.00%	37.50%	6
53	29.10%	20.10%	8.30%	55.60%	11
57	53.90%	11.40%	45.50%	70.00%	8
58	38.90%	16.80%	8.30%	100.00%	5
59	46.60%	21.80%	14.30%	91.70%	11
60	41.00%	11.80%	14.30%	70.00%	6
61	63.90%	13.30%	50.00%	81.80%	9
62	51.90%	14.30%	37.50%	71.40%	11
63	39.10%	31.40%	0.00%	100.00%	6
64	38.50%	14.00%	13.30%	62.50%	8
68	32.70%	17.80%	6.70%	66.70%	6

Table 4. Statistics on sequence identity of non-singleton clusters before eliminating homologues

Cid[a]	Accuracy[b]	Sequence Identity			
		Average	Std. Dev.	Min	Max
1	100%	100%	0%	100%	100%
4	-	100%	0%	100%	100%
6	-	100%	0%	100%	100%
7	-	100%	0%	100%	100%
11	100%	76.00%	16.97%	64.00%	100%
13	-	100%	0%	100%	100%
14	-	100%	0%	100%	100%
15	100%	100%	0%	100%	100%
19	100%	100%	0%	100%	100%
20	100%	100%	0%	100%	100%
21	-	100%	0%	100%	100%
22	100%	100%	0%	100%	100%
24	100%	100%	0%	100%	100%
25	100%	100%	0%	100%	100%
27	100%	100%	0%	100%	100%
28	100%	100%	0%	100%	100%
29	80%	41.14%	35.47%	13.10%	100%
30	100%	38.53%	27.61%	23.00%	100%
31	100%	27.40%	0%	27.40%	27%
32	100%	100%	0%	100%	100%
33	100%	11%	0%	100%	100%
35	100%	100%	0%	100%	100%
36	100%	98.59%	10.50%	97.30%	100%
37	100%	61.00%	38.77%	22.20%	100%
38	100%	100%	0%	100%	100%
39	-	100%	0%	100%	100%
40	100%	55.82%	39.52%	18.70%	100%
41	100%	51.73%	34.13%	27.60%	100%
43	100%	62.26%	38.58%	19.20%	100%
44	100%	100%	0%	100%	100%
45	-	100%	0%	100%	100%
47	-	100%	0%	100%	100%
48	100%	51.55%	40.00%	13.40%	100%
52	44%	37.76%	32.27%	19.80%	100%
53	67%	51.82%	37.03%	17.90%	100%
55	100%	100%	0%	100%	100%
56	100%	100%	0%	100%	100%
57	95%	49.64%	37.61%	18.30%	100%
58	92%	32.71%	23.98%	12.20%	100%
59	100%	73.02%	33.79%	18.40%	100%
60	100%	29.11%	23.29%	18.10%	100%
61	100%	71.35%	22.92%	45.10%	100%
62	100%	47.68%	36.19%	20.50%	100%
63	100%	47.11%	32.88%	13.10%	100%
64	100%	56.08%	38.19%	16.20%	100%
65	-	100%	0%	100%	100%
66	100%	61.55%	38.45%	23.10%	100%
67	100%	100%	0%	100%	100%
68	100%	57.31%	38.58%	16.10%	100%
69	100%	100%	0%	100%	100%

[a] The serial identification of clusters.

[b] The accuracy compared to SCOP. Clusters with no contact SCOP domain found are marked as a dash.

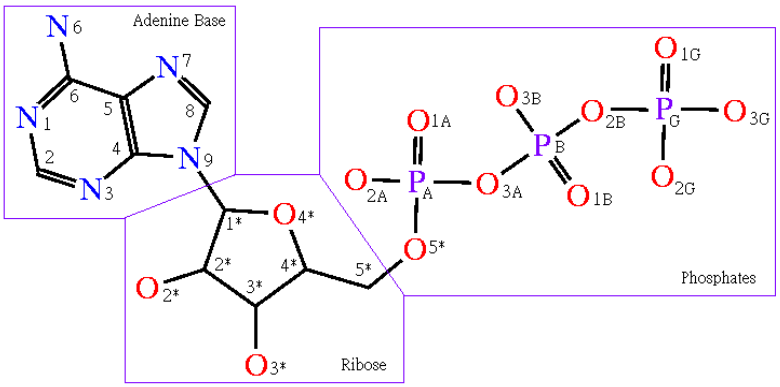
Table 5. Statistics on sequence identity of non-singleton clusters after eliminating homologues

Cid[a]	Accuracy[b]	Sequence Identity			
		Average	Std. Dev.	Min	Max
11	100%	64.00%	0.00%	64.00%	64.00%
29	50%	22.32%	9.92%	13.10%	43.10%
30	100%	26.23%	2.53%	23.00%	30.10%
31	100%	27.40%	0.00%	27.40%	27.40%
37	100%	22.40%	0.00%	22.40%	22.40%
40	100%	19.45%	0.75%	18.70%	20.20%
41	100%	27.60%	0.00%	27.60%	27.60%
43	100%	23.33%	3.84%	18.70%	29.90%
48	100%	30.80%	23.48%	13.60%	64.00%
52	33%	20.95%	0.89%	19.80%	22.30%
53	50%	27.26%	15.07%	18.10%	57.30%
57	100%	20.80%	0.00%	20.80%	20.80%
58	78%	23.08%	7.54%	11.90%	82.50%
59	100%	32.46%	17.68%	18.40%	86.50%
60	100%	21.74%	3.67%	18.10%	35.00%
61	100%	55.60%	10.50%	45.10%	66.10%
62	100%	21.90%	1.07%	20.50%	23.10%
63	100%	23.71%	9.46%	13.10%	78.00%
64	100%	22.71%	12.08%	16.20%	80.20%
68	100%	21.61%	4.61%	16.10%	28.60%

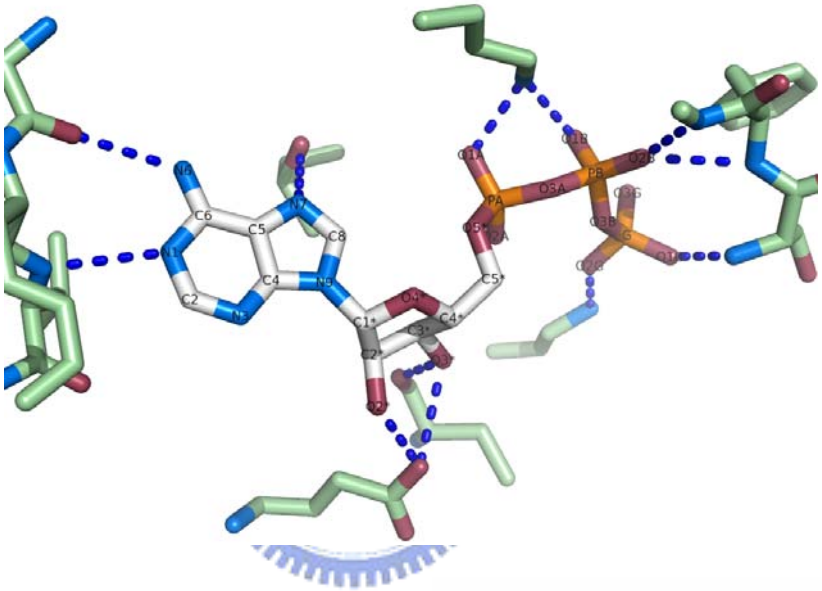
[a] The serial identification of clusters.

[b] The SCOP families of the contact domains.

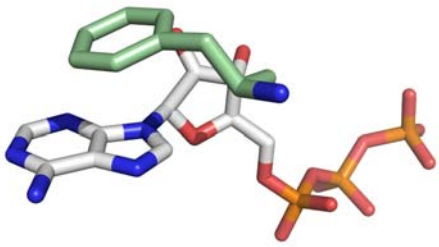
(a)



(b)



(c)



(d)

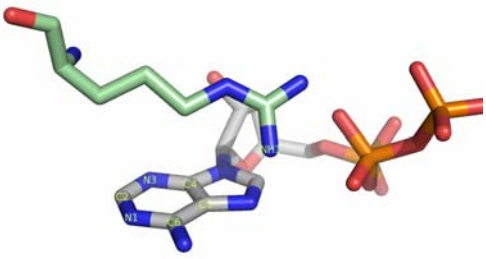


Figure 1. Properties of ATP. (a) Molecular structure and chemical groups of ATP. The atoms are labeled according to the IUPAC_IUB JCBN naming system. (b) The ATP structure and the hydrogen bonds to the surrounding residues in 1atp. ATP acts as a hydrogen bond donor (N6) and a hydrogen bond acceptor (N1, N3, N7, O3*, O4*, O2*, and oxygen atoms on phosphates). (c) The π-π stacking between the π rings of ATP and aromatic amino acids, Phe, Tyr, and Trp. (d) The cation-π interaction between the π ring of ATP and positively charged amino acids, Arg and Lys.

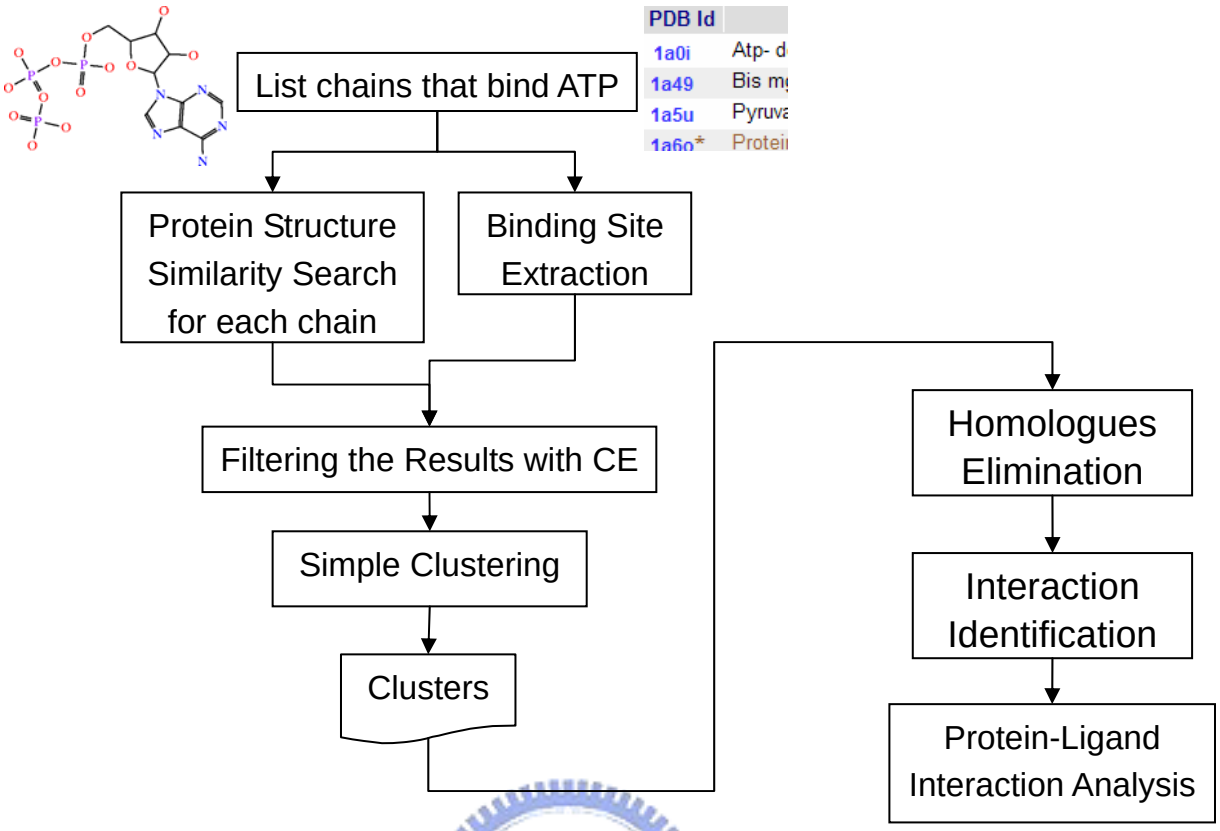
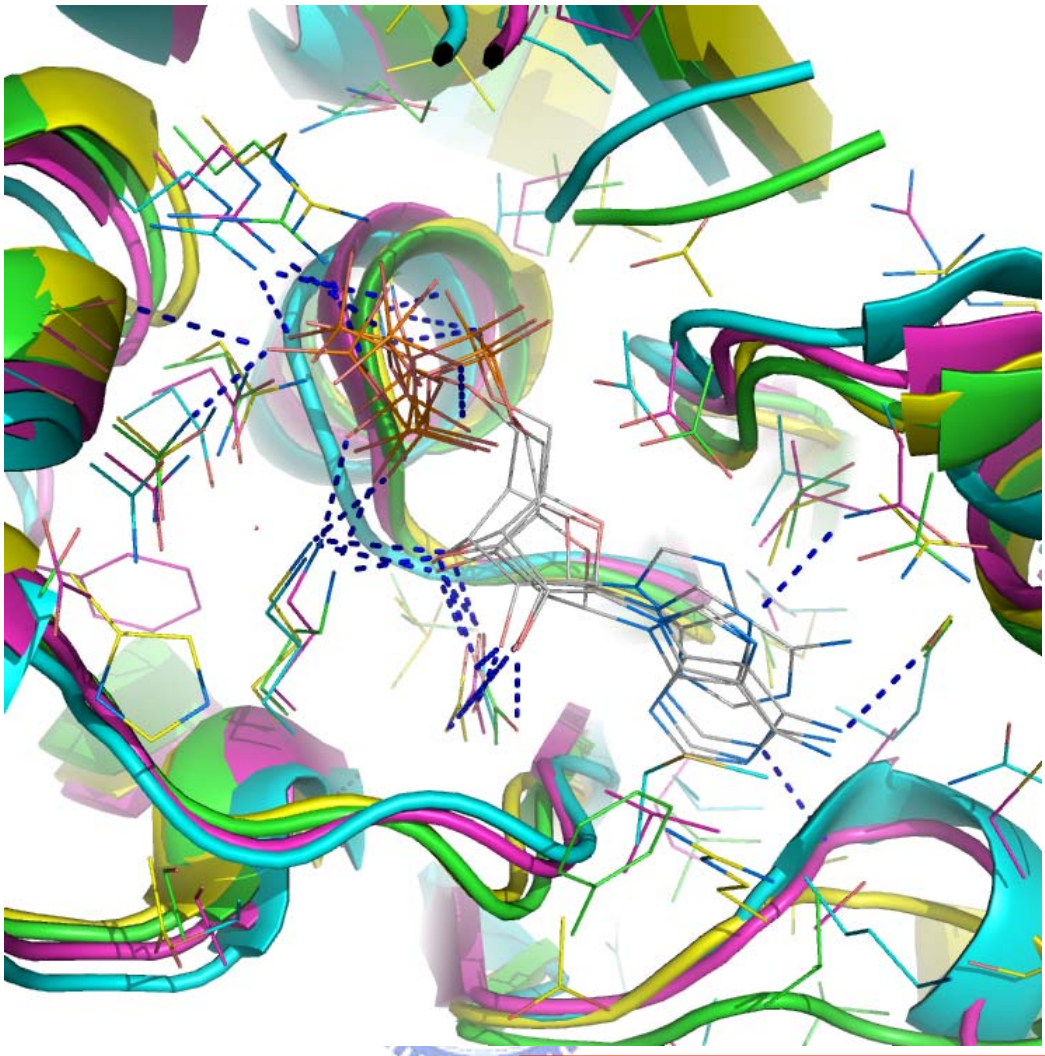


Figure 2. The framework of this research. We first get the whole list of PDB structures complexed with ATPs and extract the binding pockets. Then, we queried each chain to a protein structure similarity search engine, called 3D-BLAST and filtered the results with CE. After that, we applied the simple clustering methods by simply merging clusters with common members. The interactions are identified after the clustering.



(a)

C29_PAT

```
29
                                                  **  * ** *      *   *       * *    *** ** *         *
         1jwaB   rqiilrgfd-------------fdgqealk-dsrvlLVGLGGLGcaasqylasagvgnltlLDFDTvSlSNlqRQtlhsdatvgqpKveeard
                                                     |                        |=     ||  |           |
Z = 5.6, 1r4nB   hvkkf--lersgpfthpdfepsteslqflldtckvlvIGAGGLgcellknlalsgfrqihvIDMDTidvSNlnRQflfrpkdigrpKaevaae
                                                                              | |       |
Z = 5.9, 1y8qB           p-------------relaeava-ggrvlyVGAGGIgcellknlvltgfshidlIDLDtidvSNlnRQflfqkkhvgrsKaqvake
                                                     |                        |           |           |
Z = 6.9, 1zfnA   Rqillddia-------------ldgqqkll-dsqvliIGLGGLgtpaalylagagvgtlvlADDDdvHlSNlqRQilfttedidrpKsqvsqq
                                                     |                        |           |           |
```

(b)

Figure 3. The multiple structure alignment of ATP binding-pockets in the cluster 29. (a) The close view of ATP-binding pockets in the cluster 29. (b) The multiple structure alignment and interaction profile of the cluster 29. The contact residues are shown in uppercases while the others in lowercases. The hydrogen bonds (represented by bars, `|') to the phosphate groups are highly conserved within the cluster. Moreover, we also identified a potential novel motif, [IV]-G-[AL]-G-G-[IL]-G-X(17)-[28]-D-[MFLD]-D-[TD]-[IV]-[SDH]-[LV]-S-N-L-[NQ]-R-Q-X(11)-K (the red box), called C29_PAT, in that area. We believe that C29_PAT can be a signature for ubiquitin-activating related proteins and adenylyltransferases, which are the members of cluster 29.

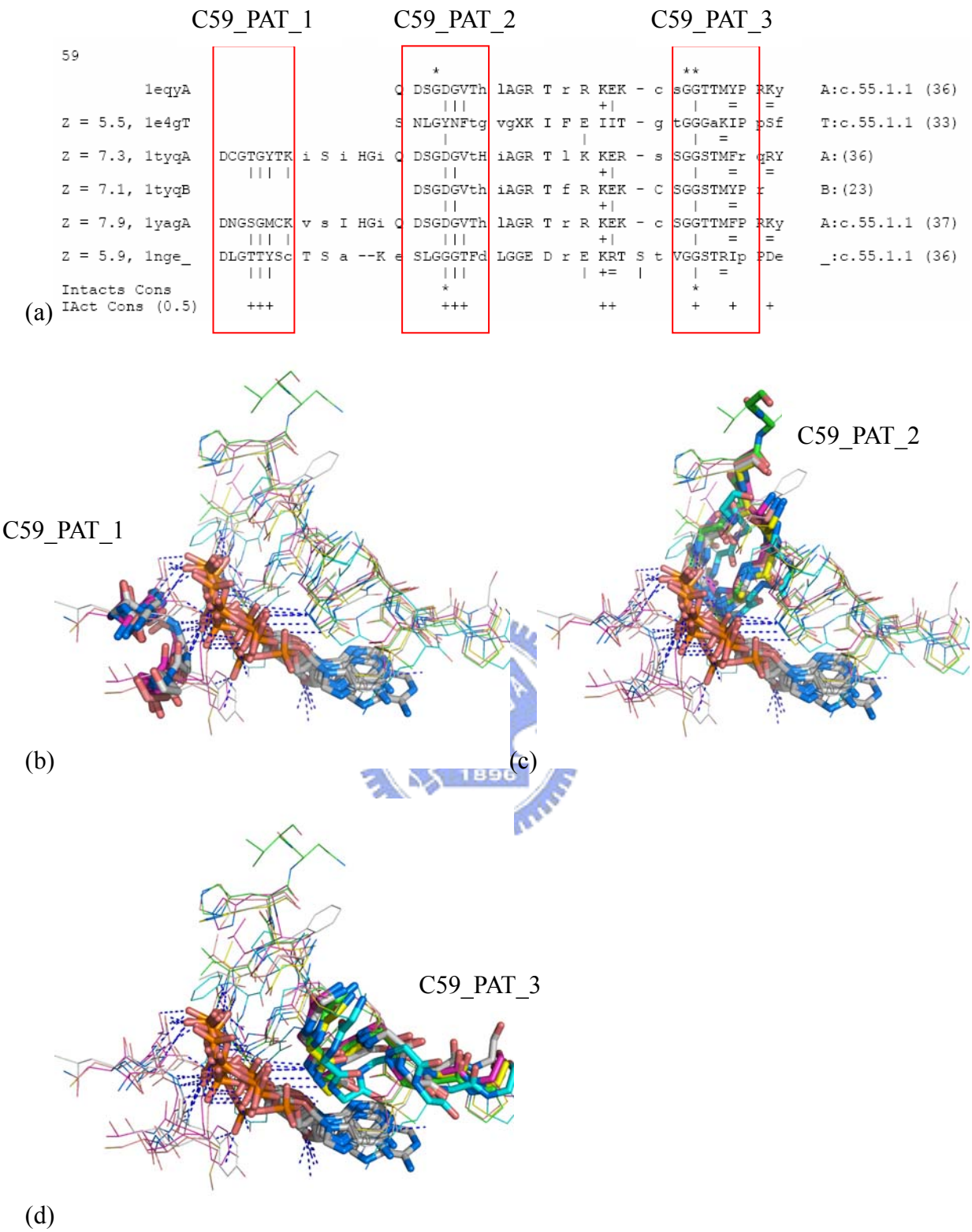
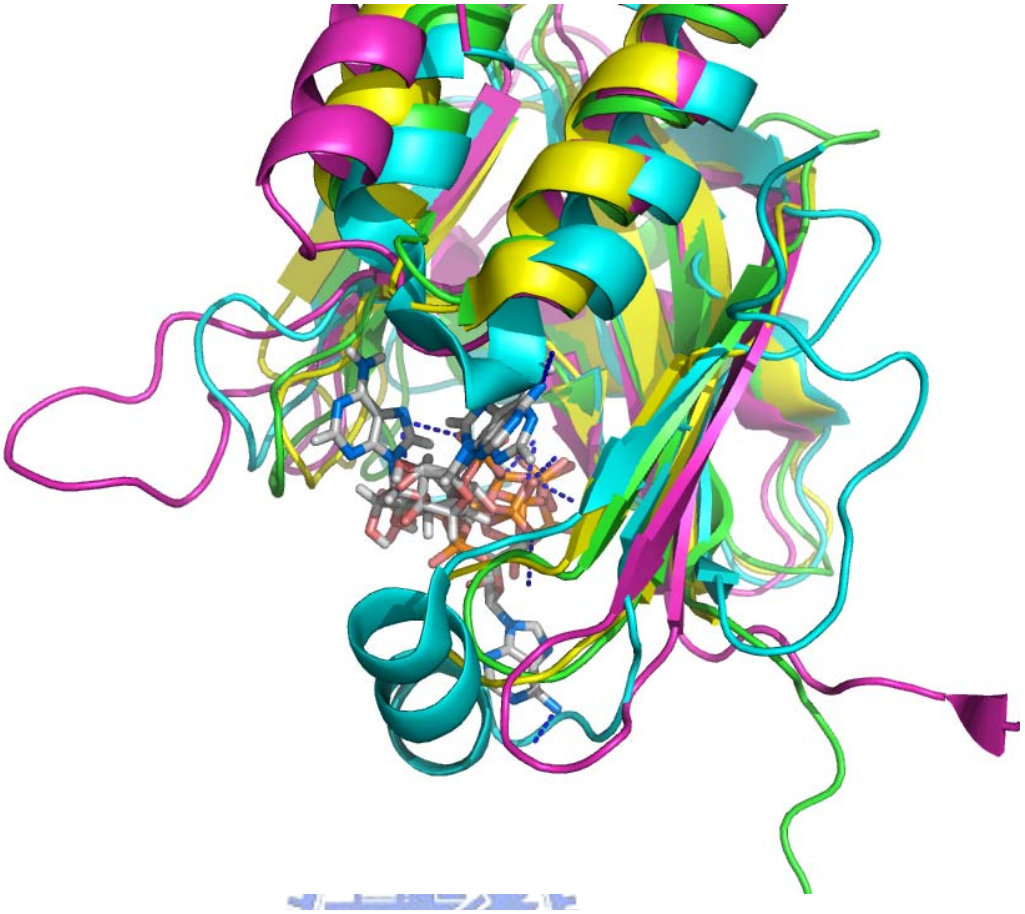


Figure 4. The potential motifs in the cluster 59. (a) The multiple structure alignment of the cluster 59 with showing the potential motifs, C59_PAT_1, C59_PAT_2, and C59_PAT_3. (b) (c) (d) The superposition of the ATP-binding pockets with showing the C59_PAT_1, C59_PAT_2, and C59_PAT_3 as sticks, respectively.

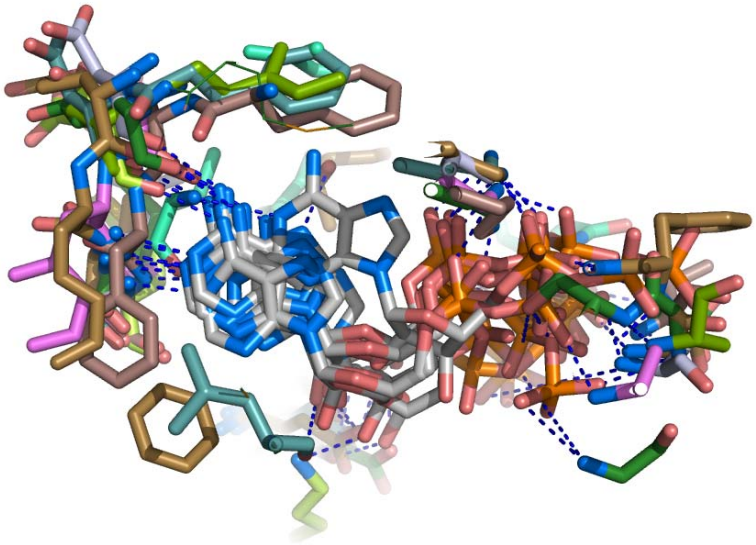
(a)



(b)

```
30
                                 *   *
          1xscA   Lrac ASDGI hHwT Kghv LNYVA- -RN--- -- k K V Y HEh laq---FKEM   A:(25)
                                       =                                   =
Z = 5.6, 1jknA   RrNV RldIp DAWQ QGGi ltYdFP KVReKL QW k Q Q w pEF LTVEFKkpvY   A:d.113.1.1 (33)
                       +                                 |                =|
Z = 5.6, 1su2A   LRAA ekgip glwh SGAV ylgrF- -PDG-- -- V I R v dei qir---Myqt   A:d.113.1.1 (5), A:d.113.1.1 (16)
                                         =    |
Z = 6.0, 1vc9A   Elga DRM-- gFwV KGHp TRYVNP -kg--- -- V R V w egm lla---FPED   A:(22)
                       |           |      + |              |                = |
Intacts Cons
IAct Cons (0.5)        +                 + +              +                +
```

Figure 5. The CE structural alignments and the interaction profile of the ATP-binding pockets of the cluster 30. (a) The superposition of ATP-binding protein chains in the cluster 30. (b) The multiple structure alignment and interaction profile in ATP-binding pockets of the cluster 30. From the figures



(a)

```
58
         1gol_   IGEg--aYgmVc A Rki y r e l I in ivQDLM-E- --T D yK d KpSNlL iC-D fg l - --- - t    _:d.144.1.7 (22)
Z = 6.3, 1ol6A   LGKg--KFGNVy A KVL v q E q L ly liLEYAPl- --G T yr d kpENlL iA-N fg w - --- - t    A:d.144.1.7 (26)
Z = 6.0, 1ua2A   LGEG--QFATVy A Kki a g e l I ll lvFDFM-E- --T D ev D KpNNlL lA-D fg - - KSF X t    A:(30)
Z = 6.6, 1csn_   IGEG--SFGVIf A KfE p q E Y P vy lvIDLL-G- --p S eD D KpDNfL vV-D fg m - --- - t    _:d.144.1.7 (29)
Z = 4.1, 1e8xA   vMaS--KKkPlW g IfK D - d l l YG IEIVKd-A- --T T aK r nDNiMi Fh-I Df g              A:d.144.1.4 (26)
Z = 5.5, 1zp9A   ISTG--kEAnVf A KiY v W E l P py llXEFI-Ge PAp T vE D seYNiX fI-D Xg Q - --- - -    A:(30)
Z = 6.0, 1zydA   LGQG--afgQVV A Kki - t e l V Yy iqMEYC-E- --N t yd D KpMNiF iGDf gl a - sdn - T    A:(25)
Z = 6.8, 1u5rA   IGHG--SFgaVy A KkM k d E l I yr lvMEYC-L- --G S sD D kaGNiL lG-D fG s - --- - t    A:(28)
Z = 6.8, 2biyA   LGEG--SFSTVv A Kil k y E m V ly fgLSYAKn- --G E lk d KpEniL iT-D fg t - --- - t    A:(25)
Z = 6.8, 1atpE   LGTG--SFGRVm A KiL q h E l V le mvMEYV-Ag --g E fs D KpENlL vT-D FG F - --- - T    E:d.144.1.7 (33)
Z = 5.2, 1tqpA   XGeG--KESAVf V Kfh a s E l P vy vlXELI-DA --k E yr D SqYNvL iI-D FP q - --- - -    A:d.144.1.9 (28)
Z = 6.7, 1phk_   LGRG--VSSVVr A Kii l a E l I lk lvFDLM-Kk --G E fD D KpENiL lT-D FG F - --- - t    _:d.144.1.7 (31)
Z = 6.1, 1s9iA   LGAG--NGGVVt A Kli - - e l V fy icMEHM-Dg --G S DQ D KpSNiL lC-D fg a - --- - -    A:(28)
Z = 5.7, 1s9jA   LGAG--NGGVVf A Kli - q e l V fy icMEHM-Dg --G S DQ D KpSNiL lC-D fg n - --- - V    A:(29)
Z = 6.5, 1b38A   kIGEGTYGVVyk A Kki g t e l V ll lvFEFL-H- --Q D kK D KPQNLL lA-D gl a E --- - t    A:d.144.1.7 (30)
Z = 6.8, 1q97A   LGWG--HFSTVw A Kiv y a E l L ll mvFEVL-G- --E N la D KpENvL iA-D lG N - --- - t    A:d.144.1.7 (29)
Intacts Cons
IAct Cons (0.5)
```

(b)

Figure 6. An example of structural binding pocket alignment of the cluster 58. (a) The superposition of ATP-binding protein chains in the cluster 58. (b) The multiple structure alignment and interaction profile in ATP-binding pockets of the cluster 58. The superposition of the ATP-binding pocket structures in 9 protein chains, which have records in the SCOP, of the cluster 58. We can see that the hydrogen bond pattern around the adenine groups is strongly conserved among the cluster.

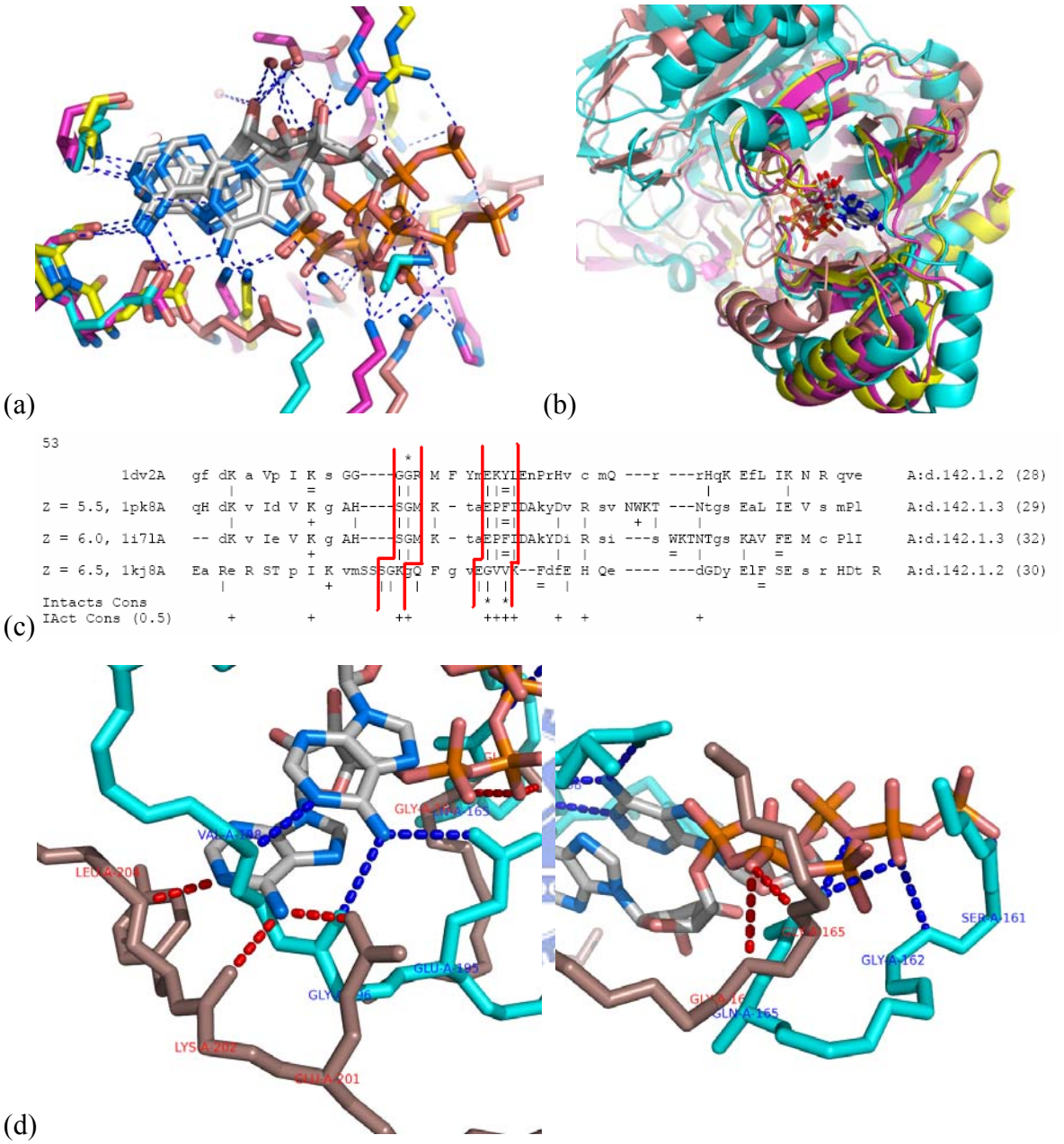


Figure 7. An example of structural binding pocket alignment of the cluster 53. (a) The ATP-binding pockets and the hydrogen bonds in protein chains of the cluster 53. (b) The superposition of the protein chains in the cluster 53. The protein chains colored in cyan, magenta, yellow, and salmon red are 1dv2A, 1pk8A, 1j7lA, and 1kj8A, respectively. (c) The multiple structure alignment and the interaction profile of the cluster 53, with showing the `shifting' region. (d) the superposition of ATP and the residues interacting with ATP in 1dv2A and 1kj8A from two different angles. We can see that the ATP structure is not well superposed to the others but the hydrogen bonds are somehow conserved. The error of superposing 1kj8A causes the shift of the non-bonded interaction pattern in the multiple structure alignment of the cluster.

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Appendix A.

The 486 ATP-binding protein chains and their names, EC numbers, and SCOP families involving in ATP-binding.

Cid	Rep.	Chain	# cRes	Contact SCOP Domain Families	EC	Protein Name
1	4at1B	4at1B	21	d.58.2.1	2.1.3.2	Aspartate Carbamoyltransferase
		4at1D	19	d.58.2.1 (19)	2.1.3.2	Aspartate Carbamoyltransferase
		7at1B	20	d.58.2.1 (20)	2.1.3.2	Aspartate Carbamoyltransferase
2	2c01X	2c01X	24		3.1.27.5	Nonsecretory Ribonuclease
3	2aruA	2aruA	39		6.3.2.-	Lipoate-Protein Ligase A
4	2aqxA	2aqxA	30		2.7.1.127	Inositol 1,4,5-Trisphosphate 3-Kinase B
		2aqxB	31		2.7.1.127	Inositol 1,4,5-Trisphosphate 3-Kinase B
5	8icnA	8icnA	15	d.218.1.2 (15)	2.7.7.7	DNA Polymerase Beta
6	1z0sA	1z0sA	28		2.7.1.23	Polyphosphate/ATP-NAD Kinase
		1z0sB	28		2.7.1.23	Polyphosphate/ATP-NAD Kinase
		1z0sC	28		2.7.1.23	Polyphosphate/ATP-NAD Kinase
		1z0sD	29		2.7.1.23	Polyphosphate/ATP-NAD Kinase
7	1yp3A	1yp3A	33		2.7.7.27	Glucose-1-Phosphate Adenylyltransferase Small Subunit (ADP-Glucose Synthase)
		1yp3C	35		2.7.7.27	Glucose-1-Phosphate Adenylyltransferase Small Subunit (ADP-Glucose Synthase)
8	1y56A	1y56A	41		1.5.99.8	L-Proline Dehydrogenase
9	1xdnA	1xdnA	28			RNA Editing Ligase Mp52
10	1wklB	1wklB	19		2.7.4.6	Nucleotide Diphosphate Kinase
11	1vjcA	1vjcA	26	c.86.1.1 (26)	2.7.2.3	Phosphoglycerate Kinase
		1vjdA	34	c.86.1.1 (34)	2.7.2.3	Phosphoglycerate Kinase
		3pgk_	28	c.86.1.1 (28)	2.7.2.3	Phosphoglycerate Kinase
12	2gnkA	2gnkA	17	d.58.5.1 (17)		Nitrogen Regulatory Protein
13	1v3sA	1v3sA	30			Nitrogen Regulatory Protein Pii
		1v3sB	29			Nitrogen Regulatory Protein Pii
		1v3sC	28			Nitrogen Regulatory Protein Pii
14	1twaA	1twaA	8		2.7.7.6	DNA-Directed RNA Polymerase II Largest Subunit
		1twhA	7		2.7.7.6	DNA-Directed RNA Polymerase II Largest Subunit
15	1tc0A	1tc0A	26	d.122.1.1 (25)		Endoplasmin
		1tc0B	23	d.122.1.1 (23)		Endoplasmin
16	1qhxA	1qhxA	28	c.37.1.3 (28)	2.7.1.-	Chloramphenicol Phosphotransferase
17	1obgA	1obgA	13	d.143.1.1 (13)	6.3.2.6	Phosphoribosylamidoimidazole-Succinocarboxamide Synthase
18	1obdA	1obdA	23	d.143.1.1 (23)	6.3.2.6	Phosphoribosylamidoimidazole-Succinocarboxamide Synthase
19	1o93B	1o93B	14	d.130.1.1 (14)	2.5.1.6	S-Adenosylmethionine Synthetase
		1o9tB	15	d.130.1.1 (15)	2.5.1.6	S-Adenosylmethionine Synthetase

20	1o93A	1o93A	16	d.130.1.1 (16)	2.5.1.6	S-Adenosylmethionine Synthetase
		1o9tA	13	d.130.1.1 (12)	2.5.1.6	S-Adenosylmethionine Synthetase
21	1yfrA	1yfrA	22		6.1.1.7	Alanyl-tRNA Synthetase
		1yfrB	22		6.1.1.7	Alanyl-tRNA Synthetase
22	1s0mB	1n48A	22	e.8.1.7 (22)		DNA Polymerase IV
		1n56A	24	e.8.1.7 (24)		DNA Polymerase IV
		1n56B	23	e.8.1.7 (23)		DNA Polymerase IV
		1ryrA	20	e.8.1.7 (20)		DNA Polymerase IV
		1rysA	18	e.8.1.7 (18)		DNA Polymerase IV
		1rysB	19	e.8.1.7 (19)		DNA Polymerase IV
		1s0mA	22	e.8.1.7 (22)		DNA Polymerase IV
		1s0mB	23	e.8.1.7 (23)		DNA Polymerase IV
23	1mo8A	1mo8A	24	d.220.1.1 (24)		Sodium/Potassium-Transporting Atpase Alpha-1
24	1mjhA	1mjhA	32	c.26.2.4 (32)		(Hypothetical)
		1mjhB	32	c.26.2.4 (32)		(Hypothetical)
25	1miwA	1miwA	28	d.218.1.4 (17), a.173.1.1 (11)		tRNA Cca-Adding Enzyme
		1miwB	28	d.218.1.4 (17), a.173.1.1 (11)		tRNA Cca-Adding Enzyme
26	1w7aB	1w7aB	28	c.37.1.12 (28)		DNA Mismatch Repair Protein Muts
27	1ko5A	1ko5A	23	c.37.1.17 (23)	2.7.1.12	Gluconate Kinase
		1ko5B	25	c.37.1.17 (25)	2.7.1.12	Gluconate Kinase
28	1r8bA	1r8bA	39	d.218.1.7 (23), a.160.1.3 (6), d.58.16.2 (10)		tRNA Nucleotidyltransferase
		1tfwB	27	d.218.1.7 (18), a.160.1.3 (9)	2.7.7.25	tRNA Nucleotidyltransferase
		1tfwD	28	d.218.1.7 (18), a.160.1.3 (10)	2.7.7.25	tRNA Nucleotidyltransferase
		1uevA	29	d.218.1.7 (20), a.160.1.3 (9)	2.7.7.25	tRNA Nucleotidyltransferase
29	1zfnA	1jwaB	29	c.111.1.1 (29)		Molybdopterin Biosynthesis MoeB Protein
		1r4nB	30	c.111.1.2 (30)		Ubiquitin-Activating Enzyme E1C
		1r4nD	29	c.111.1.2 (29)		Ubiquitin-Activating Enzyme E1C
		1r4nF	30	c.111.1.2 (30)		Ubiquitin-Activating Enzyme E1C
		1r4nH	28	c.111.1.2 (28)		Ubiquitin-Activating Enzyme E1C
		1y8qB	30			Ubiquitin-Like 2 Activating Enzyme E1B
		1y8qD	32			Ubiquitin-Like 2 Activating Enzyme E1B
		1y8rB	31			Ubiquitin-Like 2 Activating Enzyme E1B
		1y8rE	31			Ubiquitin-Like 2 Activating Enzyme E1B
		1zfnA	28		2.7.7.-	Adenylyltransferase THIF
		1zfnB	30		2.7.7.-	Adenylyltransferase THIF
		1zfnC	29		2.7.7.-	Adenylyltransferase THIF
		1zfnD	31		2.7.7.-	Adenylyltransferase THIF
30	1vc9A	1jknA	33	d.113.1.1 (33)	3.6.1.17	Diadenosine 5',5'''-P1,P4-Tetraphosphate Hydrolase
		1su2A	21	d.113.1.1 (21)		Mutt/Nudix Family Protein
		1su2B	24	d.113.1.1 (18)		Mutt/Nudix Family Protein
		1vc9A	22			HB8 Ap6A Hydrolase
		1vc9B	21			HB8 Ap6A Hydrolase
		1xscA	25		3.6.1.17	Bis(5'-Nucleosyl)-Tetraphosphatase
31	1jjvA	1jjvA	20	c.37.1.1 (20)	2.7.1.24	Dephospho-CoA Kinase
		1uf9C	24	c.37.1.1 (24)		

32	1jagD	1jagA	33	c.37.1.1 (33)	2.7.1.113	Deoxyguanosine Kinase
		1jagB	33	c.37.1.1 (33)	2.7.1.113	Deoxyguanosine Kinase
		1jagC	33	c.37.1.1 (33)	2.7.1.113	Deoxyguanosine Kinase
		1jagD	32	c.37.1.1 (32)	2.7.1.113	Deoxyguanosine Kinase
		1jagE	33	c.37.1.1 (33)	2.7.1.113	Deoxyguanosine Kinase
		1jagF	33	c.37.1.1 (33)	2.7.1.113	Deoxyguanosine Kinase
		1jagG	33	c.37.1.1 (33)	2.7.1.113	Deoxyguanosine Kinase
		1jagH	33	c.37.1.1 (33)	2.7.1.113	Deoxyguanosine Kinase
33	3r1rA	3r1rA	19	a.98.1.1 (19)	1.17.4.1	Ribonucleotide Reductase R1 Protein
		3r1rB	20	a.98.1.1 (20)	1.17.4.1	Ribonucleotide Reductase R1 Protein
		3r1rC	20	a.98.1.1 (20)	1.17.4.1	Ribonucleotide Reductase R1 Protein
34	1hp1A	1hp1A	14	d.114.1.1 (14)	3.1.3.5, 3.6.1.45	5'-Nucleotidase
35	1hi1A	1hi1A	16	e.8.1.6 (16)		RNA Polymerase
		1hi1B	16	e.8.1.6 (16)		RNA Polymerase
		1hi1C	16	e.8.1.6 (16)		RNA Polymerase
36	1pj4D	1gz3A	30	c.58.1.3 (7), c.2.1.7 (23)	1.1.1.38	NAD-Dependent Malic Enzyme
		1gz3B	29	c.58.1.3 (7), c.2.1.7 (22)	1.1.1.38	NAD-Dependent Malic Enzyme
		1gz3C	29	c.58.1.3 (7), c.2.1.7 (22)	1.1.1.38	NAD-Dependent Malic Enzyme
		1gz3D	29	c.58.1.3 (7), c.2.1.7 (22)	1.1.1.38	NAD-Dependent Malic Enzyme
		1gz4A	33	c.58.1.3 (7), c.2.1.7 (23)	1.1.1.40	NAD-Dependent Malic Enzyme
		1gz4B	27	c.58.1.3 (7), c.2.1.7 (23)	1.1.1.40	NAD-Dependent Malic Enzyme
		1gz4C	33	c.58.1.3 (7), c.2.1.7 (23)	1.1.1.40	NAD-Dependent Malic Enzyme
		1gz4D	27	c.58.1.3 (7), c.2.1.7 (23)	1.1.1.40	NAD-Dependent Malic Enzyme
		1pj4A	32	c.2.1.7 (22), c.58.1.3 (7)	1.1.1.39	NAD-Dependent Malic Enzyme, Mitochondrial
		1pj4B	32	c.2.1.7 (22), c.58.1.3 (7)	1.1.1.39	NAD-Dependent Malic Enzyme, Mitochondrial
		1pj4C	32	c.2.1.7 (22), c.58.1.3 (7)	1.1.1.39	NAD-Dependent Malic Enzyme, Mitochondrial
		1pj4D	32	c.2.1.7 (22), c.58.1.3 (7)	1.1.1.39	NAD-Dependent Malic Enzyme, Mitochondrial
37	1qrsA	1gtrA	29	c.26.1.1 (29)	6.1.1.18	Glutaminyl-tRNA Synthetase
		1n77A	28	c.26.1.1 (28)	6.1.1.17	Glutamyl-tRNA Synthetase
		1n77B	29	c.26.1.1 (29)	6.1.1.17	Glutamyl-tRNA Synthetase
		1qrsA	33	c.26.1.1 (33)	6.1.1.18	Glutaminyl-tRNA Synthetase
		1qrtA	33	c.26.1.1 (33)	6.1.1.18	Glutaminyl-tRNA Synthetase
		1qruA	30	c.26.1.1 (30)	6.1.1.18	Glutaminyl-tRNA Synthetase
38	1g64A	1g5tA	18	c.37.1.11 (18)	2.5.1.17	COB(I)Alamin Adenosyltransferase
		1g64A	26	c.37.1.11 (25)	2.5.1.17	COB(I)Alamin Adenosyltransferase
		1g64B	26	c.37.1.11 (24)	2.5.1.17	COB(I)Alamin Adenosyltransferase
39	1xdpB	1xdpA	33		2.7.4.1	Polyphosphate Kinase
		1xdpB	34		2.7.4.1	Polyphosphate Kinase
40	1yunB	1f9aA	28	c.26.1.3 (28)		NMN Adenylyltransferase
		1f9aB	27	c.26.1.3 (27)		NMN Adenylyltransferase
		1f9aC	28	c.26.1.3 (28)		NMN Adenylyltransferase
		1f9aD	29	c.26.1.3 (29)		NMN Adenylyltransferase
		1f9aE	29	c.26.1.3 (29)		NMN Adenylyltransferase
		1f9aF	28	c.26.1.3 (28)		NMN Adenylyltransferase
		1gn8A	33	c.26.1.3 (33)	2.7.7.3	Phosphopantetheine Adenylyltransferase
		1gn8B	32	c.26.1.3 (32)	2.7.7.3	Phosphopantetheine Adenylyltransferase
		1yunA	25		2.7.7.18	Nicotinate-Nucleotide Adenylyltransferase
		1yunB	27		2.7.7.18	Nicotinate-Nucleotide Adenylyltransferase

41	1f2uA	1f2uA	21	c.37.1.12 (21)		RAD50 ABC-Atpase
		1f2uC	20	c.37.1.12 (20)		RAD50 ABC-Atpase
		1xexA	23			SMC Protein
42	1kvkA	1kvkA	29	d.14.1.5 (29)		Mevalonate Kinase
43	1mauA	1h3eA	32	c.26.1.1 (32)	6.1.1.1	Tyrosyl-tRNA Synthetase
		1m83A	36	c.26.1.1 (36)	6.1.1.2	Tryptophanyl-tRNA Synthetase
		1mauA	36	c.26.1.1 (36)	6.1.1.2	Tryptophanyl-tRNA Synthetase
		1mawA	24	c.26.1.1 (24)	6.1.1.2	Tryptophanyl-tRNA Synthetase
		1mawB	27	c.26.1.1 (27)	6.1.1.2	Tryptophanyl-tRNA Synthetase
		1mawC	27	c.26.1.1 (27)	6.1.1.2	Tryptophanyl-tRNA Synthetase
		1mawD	28	c.26.1.1 (28)	6.1.1.2	Tryptophanyl-tRNA Synthetase
		1mawE	21	c.26.1.1 (21)	6.1.1.2	Tryptophanyl-tRNA Synthetase
		1mawF	23	c.26.1.1 (23)	6.1.1.2	Tryptophanyl-tRNA Synthetase
		1yidB	29		6.1.1.2	Tryptophanyl-tRNA Synthetase
		2a84A	30		6.3.2.1	Pantoate--Beta-Alanine Synthetase
44	1nsyA	1nsyA	27	c.26.2.1 (27)	6.3.5.1	NAD Synthetase
		1nsyB	26	c.26.2.1 (16)	6.3.5.1	NAD Synthetase
45	1twaB	1r9tB	8		2.7.7.6	DNA-Directed RNA Polymerase II
		1twaB	10		2.7.7.6	DNA-Directed RNA Polymerase II 140 Kd Polypeptide
		1twhB	9		2.7.7.6	DNA-Directed RNA Polymerase II 140 Kd Polypeptide
46	1fmwA	1fmwA	32	c.37.1.9 (32)		Myosin II Heavy Chain
47	1sx3A	1sx3A	31			Groel Protein
		1sx3B	31			Groel Protein
		1sx3C	31			Groel Protein
		1sx3D	31			Groel Protein
		1sx3E	31			Groel Protein
		1sx3F	31			Groel Protein
		1sx3G	31			Groel Protein
		1sx3H	31			Groel Protein
		1sx3I	31			Groel Protein
		1sx3J	31			Groel Protein
		1sx3K	31			Groel Protein
		1sx3L	31			Groel Protein
		1sx3M	31			Groel Protein
		1sx3N	31			Groel Protein
48	1tilA	1tidA	29	d.122.1.3 (29)	2.7.1.37	Anti-Sigma Factor Spoiiab
		1tidC	37	d.122.1.3 (37)	2.7.1.37	Anti-Sigma Factor Spoiiab
		1tilA	35	d.122.1.3 (35)	2.7.1.37	Anti-Sigma Factor Spoiiab
		1tilC	35	d.122.1.3 (35)	2.7.1.37	Anti-Sigma Factor Spoiiab
		1tilE	36	d.122.1.3 (36)	2.7.1.37	Anti-Sigma Factor Spoiiab
		1y8pA	30		2.7.1.99	[Pyruvate Dehydrogenase [Lipoamide]] Kinase Isozyme 3
		2bu2A	26		2.7.1.99	Pyruvate Dehydrogensae Kinase Isoenzyme 2
49	1n5iA	1n5iA	10	c.37.1.1 (10)		Thymidylate Kinase
50	1e2qA	1e2qA	21	c.37.1.1 (21)	2.7.4.9	Thymidylate Kinase
51	1dy3A	1dy3A	27	d.58.30.1 (27)	2.7.6.3	7,8-Dihydro-6-Hydroxymethylpterinpyrophosphokinase (Pyrophosphorylase, Pppk)

52	1lhrA	1esqA	27	c.72.1.2 (27)	2.7.1.50	Hydroxyethylthiazole Kinase
		1esqB	27	c.72.1.2 (27)	2.7.1.50	Hydroxyethylthiazole Kinase
		1esqC	27	c.72.1.2 (27)	2.7.1.50	Hydroxyethylthiazole Kinase
		1lhrA	29	c.72.1.5 (29)	2.7.1.35	Pyridoxal Kinase
		1lhrB	31	c.72.1.5 (31)	2.7.1.35	Pyridoxal Kinase
		1v1bA	34	c.72.1.1 (34)		2-Keto-3-Deoxygluconate Kinase
		1v1bB	34	c.72.1.1 (34)		2-Keto-3-Deoxygluconate Kinase
		1v1bC	33	c.72.1.1 (33)		2-Keto-3-Deoxygluconate Kinase
		1v1bD	35	c.72.1.1 (35)		2-Keto-3-Deoxygluconate Kinase
		2f02A	32		2.7.1.144	Tagatose-6-Phosphate Kinase
		2f02B	32		2.7.1.144	Tagatose-6-Phosphate Kinase
53	1kj9B	1dv2A	30	d.142.1.2 (28)	6.3.4.14	Biotin Carboxylase
		1dv2B	30	d.142.1.2 (28)	6.3.4.14	Biotin Carboxylase
		1i7lA	32	d.142.1.3 (32)		Synapsin II
		1i7lB	30	d.142.1.3 (29)		Synapsin II
		1kj8A	32	d.142.1.2 (30)	2.1.2.-	Phosphoribosylglycinamide Formyltransferase 2
		1kj8B	27	d.142.1.2 (26)	2.1.2.-	Phosphoribosylglycinamide Formyltransferase 2
		1kj9A	31	d.142.1.2 (30)	2.1.2.-	Phosphoribosylglycinamide Formyltransferase 2
		1kj9B	29	d.142.1.2 (28)	2.1.2.-	Phosphoribosylglycinamide Formyltransferase 2
		1pk8A	30	d.142.1.3 (29)		Synapsin I
		1pk8B	29	d.142.1.3 (28)		Synapsin I
		1pk8C	27	d.142.1.3 (26)		Synapsin I
		1pk8D	30	d.142.1.3 (29)		Synapsin I
		1pk8E	28	d.142.1.3 (27)		Synapsin I
		1pk8F	28	d.142.1.3 (27)		Synapsin I
		1pk8G	29	d.142.1.3 (28)		Synapsin I
		1pk8H	27	d.142.1.3 (26)		Synapsin I
		1px2A	26	d.142.1.3 (25)		Synapsin I
		1px2B	27	d.142.1.3 (26)		Synapsin I
54	1d9zA	1d9zA	23	c.37.1.19 (23)		DNA Repair Protein UVRB
55	1bcpF	1bcpF	9	b.40.2.1 (9)	2.4.2.-	Pertussis Toxin
		1bcpL	10	b.40.2.1 (10)	2.4.2.-	Pertussis Toxin
56	1bcpE	1bcpE	13	b.40.2.1 (13)	2.4.2.-	Pertussis Toxin
		1bcpK	13	b.40.2.1 (13)	2.4.2.-	Pertussis Toxin
57	1u9iA	1e79A	21	c.37.1.11 (17), a.69.1.1 (4)	3.6.1.34	ATP Synthase Alpha Chain Heart Isoform (Bovine Mitochondrial F1-Atpase)
		1e79C	22	c.37.1.11 (18), a.69.1.1 (4)	3.6.1.34	ATP Synthase Alpha Chain Heart Isoform (Bovine Mitochondrial F1-Atpase)
		1h8hA	23	c.37.1.11 (19), a.69.1.1 (4)	3.6.1.34	ATP Synthase Alpha Chain Heart Isoform
		1h8hB	29	c.37.1.11 (18), a.69.1.1 (4)	3.6.1.34	ATP Synthase Alpha Chain Heart Isoform
		1h8hC	23	c.37.1.11 (19), a.69.1.1 (4)	3.6.1.34	ATP Synthase Alpha Chain Heart Isoform
		1h8hD	7	a.69.1.1 (4)	3.6.1.34	Bovine Mitochondrial F1-Atpase
		1h8hF	34	c.37.1.11 (20), a.69.1.1 (11)	3.6.1.34	Bovine Mitochondrial F1-Atpase
		1mabA	24	c.37.1.11 (20), a.69.1.1 (4)	3.6.1.34	F1-Atpase Alpha Chain
		1mabB	9	c.37.1.11 (5), a.69.1.1 (4)	3.6.1.34	F1-Atpase Beta Chain
		1nbmA	24	c.37.1.11 (20), a.69.1.1 (4)	3.6.1.34	F1-Atpase
		1nbmB	29	c.37.1.11 (18), a.69.1.1 (4)	3.6.1.34	F1-Atpase
		1nbmC	22	c.37.1.11 (18), a.69.1.1 (4)	3.6.1.34	F1-Atpase
		1nbmF	34	c.37.1.11 (20), a.69.1.1 (11)	3.6.1.34	F1-Atpase
		1tf7A	35	c.37.1.11 (23)		Circadian Clock Protein KAIC
		1tf7B	35	c.37.1.11 (23)		Circadian Clock Protein KAIC
		1tf7C	35	c.37.1.11 (24)		Circadian Clock Protein KAIC
		1tf7D	34	c.37.1.11 (23)		Circadian Clock Protein KAIC
		1tf7E	36	c.37.1.11 (24)		Circadian Clock Protein KAIC
		1tf7F	35	c.37.1.11 (24)		Circadian Clock Protein KAIC

57	1u9iA	1u9iA	35			Circadian Clock Protein Kaic
		1u9iB	35			Circadian Clock Protein Kaic
		1u9iC	35			Circadian Clock Protein Kaic
		1u9iD	34			Circadian Clock Protein Kaic
		1u9iE	36			Circadian Clock Protein Kaic
		1u9iF	35			Circadian Clock Protein Kaic
58	2biyA	1atpE	33	d.144.1.7 (33)	2.7.1.37	cAMP-Depedent Protein Kinase (CAPK)
		1b38A	30	d.144.1.7 (30)	2.7.1.37	Cell Division Protein Kinase 2
		1b39A	32	d.144.1.7 (32)	2.7.1.37	Cell Division Protein Kinase 2
		1csn_	29	d.144.1.7 (29)	2.7.1.-	Casein Kinase-1
		1e8xA	26	d.144.1.4 (26)	2.7.1.137	Phosphatidylinositol 3-Kinase Catalytic Subunit
		1finA	25	d.144.1.7 (25)	2.7.1.-	Cyclin-Dependent Kinase 2
		1finC	26	d.144.1.7 (26)	2.7.1.-	Cyclin-Dependent Kinase 2
		1fq1B	27	d.144.1.7 (27)	2.7.1.-	Cell Division Protein Kinase 2
		1gol_	22	d.144.1.7 (22)	2.7.1.-	Extracellular Regulated Kinase 2
		1gy3A	27	d.144.1.7 (27)		Cell Division Protein Kinase 2
		1gy3C	30	d.144.1.7 (30)		Cell Division Protein Kinase 2
		1h1wA	27	d.144.1.7 (27)		3-Phosphoinositide Dependent Protein Kinase-1 (Hpdk1)
		1hck_	30	d.144.1.7 (30)	2.7.1.37	Human Cyclin-Dependent Kinase 2 (Cdk2)
		1jstA	30	d.144.1.7 (30)	2.7.1.-	Cyclin-Dependent Kinase-2
		1jstC	32	d.144.1.7 (32)	2.7.1.-	Cyclin-Dependent Kinase-2
		1ol6A	26	d.144.1.7 (26)	2.7.1.37	Serine/Threonine Kinase 6
		1phk_	31	d.144.1.7 (31)	2.7.1.38	Phosphorylase Kinase
		1q24A	33	d.144.1.7 (33)	2.7.1.37	cAMP-Dependent Protein Kinase, Alpha-Catalytic Subunit
		1q97A	29	d.144.1.7 (29)	2.7.1.-	Sr Protein Kinase
		1ql6A	30	d.144.1.7 (30)	2.7.1.38	Phosphorylase Kinase
		1qmzA	27	d.144.1.7 (27)	2.7.1.-	Cell Division Protein Kinase 2
		1qmzC	29	d.144.1.7 (29)	2.7.1.-	Cell Division Protein Kinase 2
		1rdqE	33	d.144.1.7 (33)	2.7.1.37	cAMP-Dependent Protein Kinase, Alpha-Catalytic Subunit
		1s9iA	28			Dual Specificity Mitogen-Activated Protein Kinase Kinase 2
		1s9iB	29			Dual Specificity Mitogen-Activated Protein Kinase Kinase 2
		1s9jA	29			Dual Specificity Mitogen-Activated Protein Kinase Kinase 1
		1tqpA	28	d.144.1.9 (28)		RIO2 Serine Protein Kinase
		1u5rA	28			Serine/Threonine Protein Kinase Tao2
		1u5rB	28			Serine/Threonine Protein Kinase Tao2
		1ua2A	30		2.7.1.37	Cell Division Protein Kinase 7
		1ua2B	31		2.7.1.37	Cell Division Protein Kinase 7
		1ua2C	31		2.7.1.37	Cell Division Protein Kinase 7
		1ua2D	29		2.7.1.37	Cell Division Protein Kinase 7
		1zaoA	32			RIO2 Serine Kinase
		1zp9A	32			RIO1 Kinase
		1zp9B	31			RIO1 Kinase
		1zp9C	32			RIO1 Kinase
		1zp9D	30			RIO1 Kinase
		1zydA	25		2.7.1.37	Serine/Threonine-Protein Kinase GCN2
		1zydB	26		2.7.1.37	Serine/Threonine-Protein Kinase GCN2
		2biyA	25		2.7.1.37	3-Phosphoinositide Dependent Protein Kinase-1
		2phkA	32	d.144.1.7 (32)	2.7.1.38	Phosphorylase Kinase
59	1c0fA	1atnA	35	c.55.1.1 (35)		Deoxyribonuclease I (Endodeoxyribonuclease)
		1c0fA	35	c.55.1.1 (35)		Dictyostelium CaAtp-Actin
		1c0gA	37	c.55.1.1 (37)		Chimeric Actin
		1d4xA	36	c.55.1.1 (36)		Actin
		1dejA	37	c.55.1.1 (37)		Dictyostelium/Tetrahymena Chimera Actin
		1e4gT	33	c.55.1.1 (33)		Cell Division Protein FTSA

59	1c0fA	1eqyA	36	c.55.1.1 (36)		Alpha-Actin
		1esvA	38	c.55.1.1 (38)		Alpha Actin
		1h1vA	35	c.55.1.1 (35)		Actin
		1hluA	30	c.55.1.1 (30)		Beta-Actin
		1ijjA	40	c.55.1.1 (40)		Actin, Alpha Skeletal Muscle
		1ijjB	39	c.55.1.1 (39)		Actin, Alpha Skeletal Muscle
		1kax_	39	c.55.1.1 (39)	3.6.1.3	70Kd Heat Shock Cognate Protein
		1kay_	39	c.55.1.1 (39)	3.6.1.3	70Kd Heat Shock Cognate Protein
		1kaz_	39	c.55.1.1 (39)	3.6.1.3	70Kd Heat Shock Cognate Protein
		1kxpA	39	c.55.1.1 (39)		Actin,Alpha Skeletal Muscle
		1lcuA	33	c.55.1.1 (33)		Actin, Alpha Skeletal Muscle
		1lcuB	38	c.55.1.1 (38)		Actin, Alpha Skeletal Muscle
		1lotB	38	c.55.1.1 (38)		Actin, Alpha Skeletal Muscle
		1ma9B	38	c.55.1.1 (38)		Actin, Alpha Skeletal Muscle
		1mduB	38	c.55.1.1 (38)		Alpha-Actin
		1mduE	38	c.55.1.1 (38)		Alpha-Actin
		1nge_	36	c.55.1.1 (36)	3.6.1.3	Heat-Shock Cognate 70Kd Protein
		1ngf_	37	c.55.1.1 (37)	3.6.1.3	Heat-Shock Cognate 70Kd Protein
		1ngg_	39	c.55.1.1 (39)	3.6.1.3	Heat-Shock Cognate 70Kd Protein
		1ngh_	36	c.55.1.1 (36)	3.6.1.3	Heat-Shock Cognate 70Kd Protein
		1nlvA	37	c.55.1.1 (37)		Actin
		1nm1A	35	c.55.1.1 (35)		Actin
		1nmdA	37	c.55.1.1 (37)		Actin
		1p8zA	37	c.55.1.1 (37)		Actin, Alpha Skeletal Muscle
		1qz5A	39	c.55.1.1 (39)		Actin, Alpha Skeletal Muscle
		1qz6A	38	c.55.1.1 (38)		Actin, Alpha Skeletal Muscle
		1rdwX	36	c.55.1.1 (36)		Actin, Alpha Skeletal Muscle
		1rfqA	35	c.55.1.1 (35)		Actin, Alpha Skeletal Muscle
		1rfqB	36	c.55.1.1 (36)		Actin, Alpha Skeletal Muscle
		1rgiA	36	c.55.1.1 (36)		Actin, Alpha Skeletal Muscle
		1s22A	38	c.55.1.1 (38)		Actin
		1t44A	38	c.55.1.1 (38)		Actin, Alpha
		1tyqA	36			Actin-Related Protein 3
		1tyqB	23			Actin-Related Protein 2
		1wuaA	45			Actin, Alpha Skeletal Muscle
		1y64A	38			Actin, Alpha Skeletal Muscl
		1yagA	37	c.55.1.1 (37)		Actin
		1yvnA	37	c.55.1.1 (37)		Actin
		1yxqA	35			Actin, Alpha Skeletal Muscle
		1yxqB	38			Actin, Alpha Skeletal Muscle
		2a3zA	38			Actin, Alpha Skeletal Muscle
		2a40A	37			Actin, Alpha Skeletal Muscle
		2a40D	38			Actin, Alpha Skeletal Muscle
		2a41A	38			Actin, Alpha Skeletal Muscle
		2a42A	37			Actin, Alpha Skeletal Muscle
		2asmA	38			Actin, Alpha Skeletal Muscle
		2asoA	39			Actin, Alpha Skeletal Muscle
		2aspA	38			Actin, Alpha Skeletal Muscle
		2btfA	37	c.55.1.1 (37)		Actin
60	1kmnC	1aszA	22	d.104.1.1 (22)	6.1.1.12	Aspartyl tRNA Synthetase
		1aszB	25	d.104.1.1 (25)	6.1.1.12	Aspartyl tRNA Synthetase
		1b76A	29	d.104.1.1 (29)	6.1.1.14	Glycyl-tRNA Synthetase
		1b76B	30	d.104.1.1 (30)	6.1.1.15	Glycyl-tRNA Synthetase
		1b8aA	26	d.104.1.1 (26)	6.1.1.12	Aspartyl-tRNA Synthetase
		1b8aB	28	d.104.1.1 (28)	6.1.1.12	Aspartyl-tRNA Synthetase
		1e24A	29	d.104.1.1 (29)	6.1.1.6	Lysyl-tRNA Synthetase
		1h4qA	29	d.104.1.1 (27)	6.1.1.15	Prolyl-tRNA Synthetase
		1h4qB	26	d.104.1.1 (24)	6.1.1.15	Prolyl-tRNA Synthetase

60	1kmnC	1kmnA	30	d.104.1.1 (30)	6.1.1.21	Histidyl-tRNA Synthetase
		1kmnB	33	d.104.1.1 (33)	6.1.1.21	Histidyl-tRNA Synthetase
		1kmnC	29	d.104.1.1 (29)	6.1.1.21	Histidyl-tRNA Synthetase
		1kmnD	33	d.104.1.1 (33)	6.1.1.21	Histidyl-tRNA Synthetase
		1nyrA	28	d.104.1.1 (28)	6.1.1.3	Threonyl-tRNA Synthetase 1
		1nyrB	27	d.104.1.1 (27)	6.1.1.3	Threonyl-tRNA Synthetase 1
61	1ytmA	1aq2_	32	c.91.1.1 (31)	4.1.1.49	Phosphoenolpyruvate Carboxykinase (ATP-Oxaloacetate Carboxy-Lyase)
		1ayl_	39	c.91.1.1 (37)	4.1.1.49	Phosphoenolpyruvate Carboxykinase
		1os1A	34	c.91.1.1 (33)	4.1.1.49	Phosphoenolpyruvate Carboxykinase
		1xkvA	25		4.1.1.49	Phosphoenolpyruvate Carboxykinase
		1ytmA	32		4.1.1.49	Phosphoenolpyruvate Carboxykinase
		1ytmB	31		4.1.1.49	Phosphoenolpyruvate Carboxykinase
62	1g21F	1a82_	29	c.37.1.10 (29)	6.3.3.3	Dethiobiotin Synthetase
		1g21E	36	c.37.1.10 (31)	1.18.6.1	Nitrogenase Iron Protein
		1g21F	34	c.37.1.10 (31)	1.18.6.1	Nitrogenase Iron Protein
		1g21G	33	c.37.1.10 (28)	1.18.6.1	Nitrogenase Iron Protein
		1g21H	34	c.37.1.10 (34)	1.18.6.1	Nitrogenase Iron Protein
		2bekA	41			Segregation Protein SOJ
		2bekB	41			Segregation Protein SOJ
		2bekC	43			Segregation Protein SOJ
		2bekD	41			Segregation Protein SOJ
63	1q12B	1b0uA	19	c.37.1.12 (19)		ABC Transporter (Histidine Permease)
		1f2uB	19	c.37.1.12 (7), c.37.1.12 (12)		RAD50 ABC-Atpase
		1f2uD	19	c.37.1.12 (12)		RAD50 ABC-Atpase
		1ji0A	21	c.37.1.12 (21)		ABC Transporter
		1l2tA	36	c.37.1.12 (22)		ABC Transporter
		1l2tB	36	c.37.1.12 (36)		ABC Transporter
		1mv5A	18	c.37.1.12 (17)		Multidrug Resistance ABC Transporter ATP-Binding And Permease Protein
		1mv5C	17	c.37.1.12 (17)		Multidrug Resistance ABC Transporter ATP-Binding And Permease Protein
		1q12A	31	c.37.1.12 (31)		Maltose/Maltodextrin Transport ATP-Binding Protein Malk
		1q12B	32	c.37.1.12 (17)		Maltose/Maltodextrin Transport ATP-Binding Protein Malk
		1q12C	31	c.37.1.12 (31)		Maltose/Maltodextrin Transport ATP-Binding Protein Malk
		1q12D	32	c.37.1.12 (17)		Maltose/Maltodextrin Transport ATP-Binding Protein Malk
		1r0xA	25	c.37.1.12 (21)		Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)
		1r0xB	23	c.37.1.12 (23)		Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)
		1r0xC	22	c.37.1.12 (22)		Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)
		1r0xD	23	c.37.1.12 (23)		Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)
		1r0zA	29	c.37.1.12 (26)		Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)
		1r0zB	24	c.37.1.12 (24)		Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)
		1r0zC	26	c.37.1.12 (26)		Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)
		1r0zD	23	c.37.1.12 (23)		Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)
		1r10A	26	c.37.1.12 (22)		Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)
		1r10B	24	c.37.1.12 (24)		Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)
		1vciA	17			Sugar-Binding Transport ATP-Binding Protein

63	1q12B	1xefA	30			Alpha-Hemolysin Translocation ATP-Binding Protein HLYB
		1xefB	30			Alpha-Hemolysin Translocation ATP-Binding Protein HLYB
		1xefC	31			Alpha-Hemolysin Translocation ATP-Binding Protein HLYB
		1xefD	31			Alpha-Hemolysin Translocation ATP-Binding Protein HLYB
		1xexB	8			SMC Protein
		1xf9A	27			Cystic Fibrosis Transmembrane Conductance Regulator
		1xf9B	24			Cystic Fibrosis Transmembrane Conductance Regulator
		1xf9C	23			Cystic Fibrosis Transmembrane Conductance Regulator
		1xf9D	20			Cystic Fibrosis Transmembrane Conductance Regulator
		1xfaA	25			Cystic Fibrosis Transmembrane Conductance Regulator
		1xfaB	26			Cystic Fibrosis Transmembrane Conductance Regulator
		1xmiA	17		3.6.3.49	Cystic Fibrosis Transmembrane Conductance Regulator
		1xmiB	18		3.6.3.49	Cystic Fibrosis Transmembrane Conductance Regulator
		1xmiC	17		3.6.3.49	Cystic Fibrosis Transmembrane Conductance Regulator
		1xmiD	23		3.6.3.49	Cystic Fibrosis Transmembrane Conductance Regulator
		1xmiE	23		3.6.3.49	Cystic Fibrosis Transmembrane Conductance Regulator
		1xmjA	18		3.6.3.49	Cystic Fibrosis Transmembrane Conductance Regulator
		2bboA	20			Cystic Fibrosis Transmembrane Conductance Regulator
		2bbsA	23			Cystic Fibrosis Transmembrane Conductance Regulator
		2bbsB	17			Cystic Fibrosis Transmembrane Conductance Regulator
		2bbtA	17			Cystic Fibrosis Transmembrane Conductance
		2bbtB	25			Cystic Fibrosis Transmembrane Conductance
64	1do0A	1do0A	29	c.37.1.20 (29)		Chaperone (Heat Shock Locus U)
		1do0B	33	c.37.1.20 (30)		Chaperone (Heat Shock Locus U)
		1do0D	29	c.37.1.20 (29)		Chaperone (Heat Shock Locus U)
		1do0E	33	c.37.1.20 (30)		Chaperone (Heat Shock Locus U)
		1g3iA	31	c.37.1.20 (29)		ATP-Dependent HSLU Protease
		1g3iB	28	c.37.1.20 (26)		ATP-Dependent HSLU Protease
		1g3iC	31	c.37.1.20 (29)		ATP-Dependent HSLU Protease
		1g3iD	29	c.37.1.20 (27)		ATP-Dependent HSLU Protease
		1g3iE	31	c.37.1.20 (29)		ATP-Dependent HSLU Protease
		1g3iF	27	c.37.1.20 (25)		ATP-Dependent HSLU Protease
		1g3iS	32	c.37.1.20 (29)		ATP-Dependent HSLU Protease
		1g3iT	31	c.37.1.20 (27)		ATP-Dependent HSLU Protease
		1g3iU	31	c.37.1.20 (28)		ATP-Dependent HSLU Protease
		1g3iV	30	c.37.1.20 (27)		ATP-Dependent HSLU Protease
		1g3iW	31	c.37.1.20 (28)		ATP-Dependent HSLU Protease
		1g3iX	31	c.37.1.20 (28)		ATP-Dependent HSLU Protease
		1j7kA	31	c.37.1.20 (31)		Holliday Junction DNA Helicase Ruvb
		1kyiA	30	c.37.1.20 (28)		ATP-Dependent HSL Protease ATP-Binding Subunit HSLU
		1kyiB	29	c.37.1.20 (27)		ATP-Dependent HSL Protease ATP-Binding Subunit HSLU
		1kyiC	29	c.37.1.20 (27)		ATP-Dependent HSL Protease ATP-Binding Subunit HSLU
		1kyiD	29	c.37.1.20 (27)		ATP-Dependent HSL Protease ATP-Binding Subunit HSLU
		1kyiE	29	c.37.1.20 (27)		ATP-Dependent HSL Protease ATP-Binding Subunit HSLU
		1kyiF	28	c.37.1.20 (26)		ATP-Dependent HSL Protease ATP-Binding Subunit HSLU
		1kyiS	29	c.37.1.20 (27)		ATP-Dependent HSL Protease ATP-Binding Subunit HSLU
		1kyiT	28	c.37.1.20 (26)		ATP-Dependent HSL Protease ATP-Binding Subunit HSLU
		1kyiU	32	c.37.1.20 (30)		ATP-Dependent HSL Protease ATP-Binding Subunit HSLU
		1kyiV	29	c.37.1.20 (27)		ATP-Dependent HSL Protease ATP-Binding Subunit HSLU

64	1do0A	1kyiW	29	c.37.1.20 (27)		ATP-Dependent HSL Protease ATP-Binding Subunit HSLU
		1kyiX	30	c.37.1.20 (28)		ATP-Dependent HSL Protease ATP-Binding Subunit HSLU
		1nsf_	26	c.37.1.20 (26)		N-Ethylmaleimide Sensitive Factor
		1ojlE	24			Transcriptional Regulatory Protein Zrar
		1svmA	37			Large T Antigen
		1svmB	36			Large T Antigen
		1svmC	36			Large T Antigen
		1svmD	36			Large T Antigen
		1svmE	35			Large T Antigen
		1svmF	36			Large T Antigen
		2a5yB	36			CED-4
		2a5yC	36			CED-4
		2c96A	25			PSP Operon Transcriptional Activator
		2c9cA	26			PSP Operon Transcriptional Activator
65	1z7eC	1z7eA	30			Protein ArnA
		1z7eB	30			Protein ArnA
		1z7eC	30			Protein ArnA
		1z7eD	30			Protein ArnA
		1z7eE	30			Protein ArnA
		1z7eF	30			Protein ArnA
66	1qhhA	1qhgA	25	c.37.1.19 (25)		ATP-Dependent Helicase Pcra
		1qhhA	14	c.37.1.19 (14)		PCRA
		3pjrA	27	c.37.1.19 (27)	3.6.1.-	Helicase PCRA
67	1ii0A	1ii0A	29	c.37.1.10 (29)	3.6.3.16	Arsenical Pump-Driving Atpase
		1ii0B	29	c.37.1.10 (29)	3.6.3.16	Arsenical Pump-Driving Atpase
68	1xngA	1ee1A	33	c.26.2.1 (33)	6.3.5.1	NH3-Dependent NAD+ Synthetase
		1j1zA	27	c.26.2.1 (24)	6.3.4.5	Argininosuccinate Synthetase
		1j1zB	31	c.26.2.1 (28)	6.3.4.5	Argininosuccinate Synthetase
		1j1zC	28	c.26.2.1 (25)	6.3.4.5	Argininosuccinate Synthetase
		1j1zD	32	c.26.2.1 (29)	6.3.4.5	Argininosuccinate Synthetase
		1j21A	30	c.26.2.1 (27)	6.3.4.5	Argininosuccinate Synthetase
		1j21B	30	c.26.2.1 (27)	6.3.4.5	Argininosuccinate Synthetase
		1j21C	32	c.26.2.1 (29)	6.3.4.5	Argininosuccinate Synthetase
		1j21D	29	c.26.2.1 (26)	6.3.4.5	Argininosuccinate Synthetase
		1kh2A	31	c.26.2.1 (28)	6.3.4.5	Argininosuccinate Synthetase
		1kh2B	28	c.26.2.1 (26)	6.3.4.5	Argininosuccinate Synthetase
		1kh2C	31	c.26.2.1 (28)	6.3.4.5	Argininosuccinate Synthetase
		1kh2D	29	c.26.2.1 (26)	6.3.4.5	Argininosuccinate Synthetase
		1kp2A	32	c.26.2.1 (28), d.210.1.1 (4)	6.3.4.5	Argininosuccinate Synthetase
		1kp3A	31	c.26.2.1 (29)	6.3.4.5	Argininosuccinate Synthetase
		1mb9A	33	c.26.2.1 (33)		Beta-Lactam Synthetase
		1mb9B	32	c.26.2.1 (32)		Beta-Lactam Synthetase
		1xngA	34		6.3.1.5	NH(3)-Dependent NAD(+) Synthetase
		1xngB	36		6.3.1.5	NH(3)-Dependent NAD(+) Synthetase

69	1a5uA	1a49A	35	c.1.12.1 (24), b.58.1.1 (11)	2.7.1.40	Pyruvate Kinase
		1a49C	35	c.1.12.1 (25), b.58.1.1 (10)	2.7.1.40	Pyruvate Kinase
		1a49D	37	c.1.12.1 (26), b.58.1.1 (11)	2.7.1.40	Pyruvate Kinase
		1a49E	35	c.1.12.1 (25), b.58.1.1 (10)	2.7.1.40	Pyruvate Kinase
		1a49F	33	c.1.12.1 (25), b.58.1.1 (8)	2.7.1.40	Pyruvate Kinase
		1a49G	33	c.1.12.1 (23), b.58.1.1 (10)	2.7.1.40	Pyruvate Kinase
		1a5uA	35	c.1.12.1 (24), b.58.1.1 (11)	2.7.1.40	Pyruvate Kinase
		1a5uC	34	c.1.12.1 (24), b.58.1.1 (10)	2.7.1.40	Pyruvate Kinase
		1a5uD	36	c.1.12.1 (25), b.58.1.1 (11)	2.7.1.40	Pyruvate Kinase
		1a5uE	34	c.1.12.1 (24), b.58.1.1 (10)	2.7.1.40	Pyruvate Kinase
		1a5uF	33	c.1.12.1 (25), b.58.1.1 (8)	2.7.1.40	Pyruvate Kinase
		1a5uG	33	c.1.12.1 (23), b.58.1.1 (10)	2.7.1.40	Pyruvate Kinase
70	1a0i_	1a0i_	23	d.142.2.1 (22)	6.5.1.1	DNA Ligase

Appendix B.

The multiple structure alignment and the non-bonded interaction profile in the ATP-binding sites of all 70 homologue-eliminated clusters. Each section is a multiple structure alignment of binding sites. Except for singletons, the first line shows the cluster id and the structural conservations. The last two lines show the interaction-conserved and partial interaction-conserved positions. Positions with `*'s are conserved over the cluster and `+'s for partial conserved positions. The interactions are shown as `|' for hydrogen bonds, '=' for π-π stacking or cation-π interactions, and '+' for the combinations of the two types above.

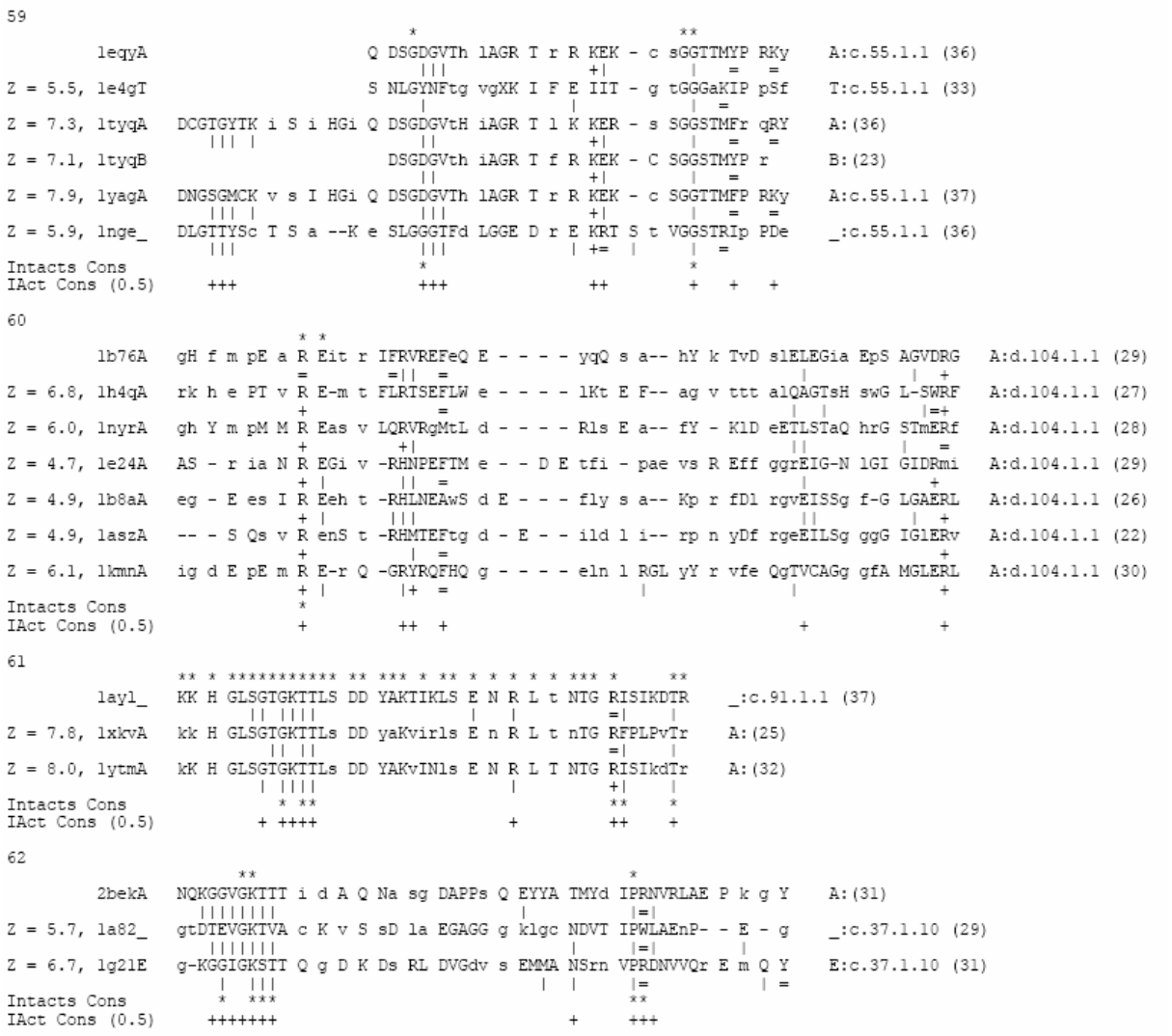
```
1 (singleton)
        4at1B  GVEAIK V D S K L K T N I NYEV GK  B:d.58.2.1
        4at1B   |  |    |       +        +    |

2 (singleton)
        2c01X  M W WF QH Q R K QN C SN R NC ACD VHLD  X:(24)
        2c01X         ||     |         = |       ||

3 (singleton)
        2aruA   IIG R TGGGAVYHD D N VSIPV K TD KIMGAA H AML T L L V T V  A:(39)
        2aruA               |   |         |    +

4 (singleton)
        2aqxA   WIQLAGH SF I K E P MDDLL V D K G S L I IDFGK  A:(30)
        2aqxA   =| ||    =   |      | |    |

5 (singleton)
        8icnA   CGS R DMD D R YFTG D N  A:d.218.1.2
        8icnA     |

6 (singleton)
        1z0sA   DGT LR R NE AKM RCD G TGYA S IAPF ADGQ  A:(18)
        1z0sA        |   ||  |    |     +  |  | =    |

7 (singleton)
        1yp3A   ILGGG R K LTQ QGTADA LAGDH E SMG Y NDFGSE D  A:(33)
        1yp3A          |     | |       |            ||

8 (singleton)
        1y56A   IGGGPAG IEER W GGDM TSALG ATGA R D P AGSA KPHY NY R IK  A:(41)
        1y56A        |   | |     |    |        |       |    |  |     =

9 (singleton)
        1xdnA   YIEID CEKVHGTNF R GE Q F FD A E V R I K R  A:(28)
        1xdnA   =| |   |+|   |  |  |   = =        =   | |

10 (singleton)
        1wklB   K HY H F L F R M T R IDEN IHG A  B:(19)
        1wklB        | =     |   |   |

11
                ***** ** * *        *   ***  *** **** **** **
        1vjcA   lGGAK DK l gGGM F L F -MGLDc NGPVGVFE gGDt gg    A:c.86.1.1 (26)
                      |       =   =   |          |
Z = 7.6, 3pgk_  LGGAK dK L G--- m i F WqGLDN NGPPGVFE GGDT GG    _:c.86.1.1 (28)
                   ||               =    |          |   |
Intacts Cons                        *               *
IAct Cons (0.5)                     +               +

12 (singleton)
        2gnkA   IIK VKGFG L K KIGDGK F  A:d.58.5.1
        2gnkA                   | |+ =

13 (singleton)
        1v3sA   L I GLT VQGHGGET H K EIGV EVGDGK F RIRT  A:(18)
        1v3sA         |      ||        |      |+ =

14 (singleton)
        1twaA   R D DGD Q K T  A:(8)
        1twaA

15 (singleton)
        1tc0A   K EL SNA DA K D GVGM E NL I VGFY S GT I  A:d.122.1.1
        1tc0A   |     |       |        |      |

16 (singleton)
        1qhxA   GGSSAGKSGI R VD D V RE AR R M Q TT KES C  A:c.37.1.3
        1qhxA     || ||||           =   | |        | |

17 (singleton)
        1obgA   K E HLVDI K I SLLV  A:d.143.1.1
        1obgA         |         |
```

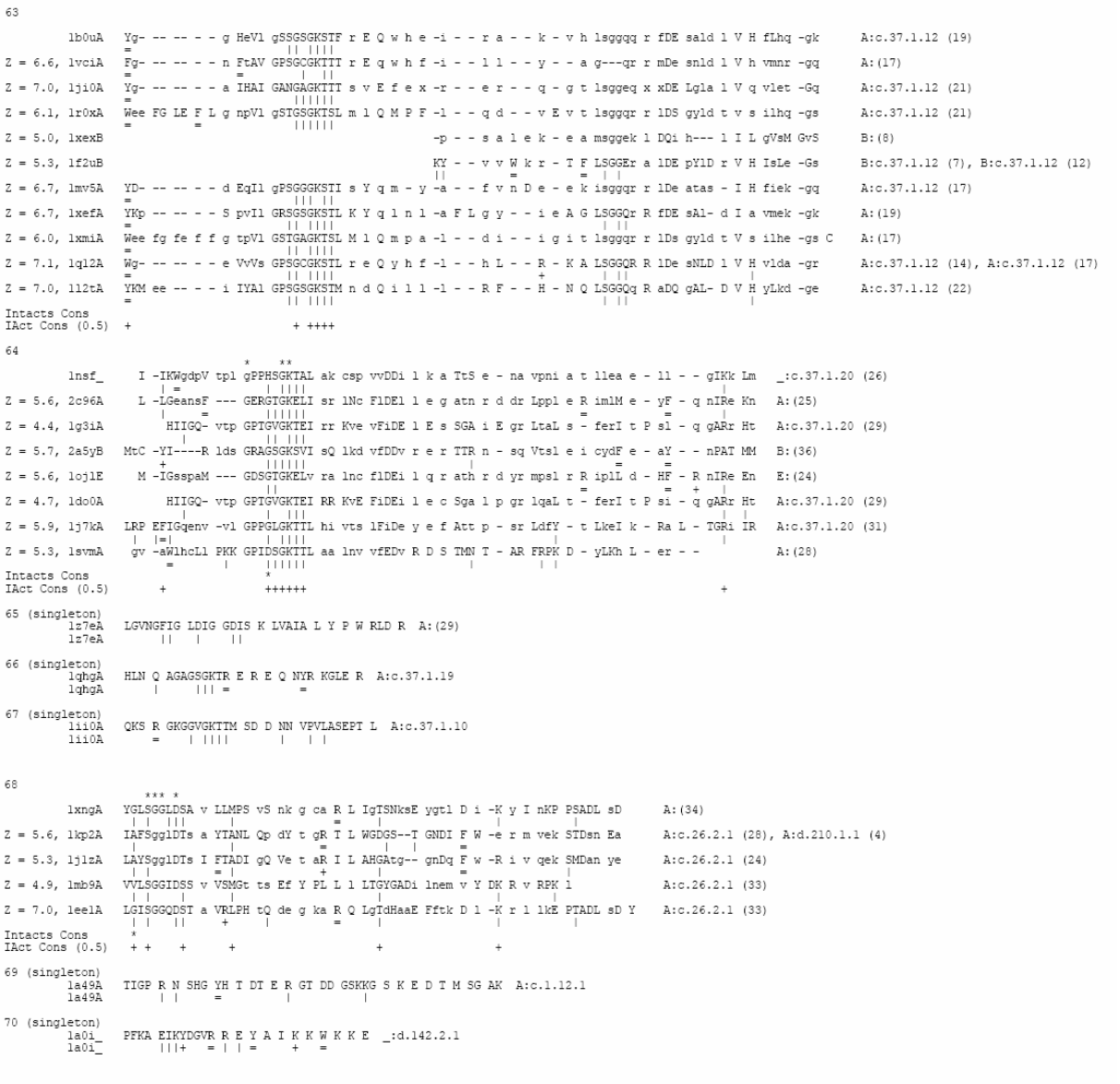
```
18 (singleton)
         1obdA   ARGKVRDI L F H VHKHKL K K E VDE  A:d.143.1.1
         1obdA      | |     = |  | |       |  |

19 (singleton)
         1o93B   KVA E EIT Q D KD TK D  B:d.130.1.1
         1o93B                    =

20 (singleton)
         1o93A   E V HPD DSK F G QGDA RK  A:d.130.1.1
         1o93A       |

21 (singleton)
         1yfrA   R D RHHT F M W E LEIWN GMG ERI N  A:(22)
         1yfrA         |  =        |         +

22 (singleton)
         1n48A   DFDYFY AVATA Y R AGI M DE K D K  A:e.8.1.7
         1n48A      |           |              |

23 (singleton)
         1mo8A   NR DAS FNS NKYQ KGAP RI GERVLG  A:d.220.1.1
         1mo8A    =     =    |   =

24 (singleton)
         1mjhA   YPTD S TA A LHVID P I IMGSHGKTNL I LGSVTE  A:c.26.2.4
         1mjhA    |            |         | || ||      |||

25 (singleton)
         1miwA   VGGA RD R GD D D RRDF NA R D LR R RF E R E K  A:d.218.1.4
         1miwA     |  |           =|   |  = |  + + |

26 (singleton)
         1w7aB   V L EPFIAN GPNMGGKSTYMRQ DE R A L HS  B:c.37.1.12
         1w7aB         =|      | ||||

27 (singleton)
         1ko5A   GVSGSGKSAV D V S L RL R I QPLE V  A:c.37.1.17
         1ko5A     || ||||          =      |

28 (singleton)
         1r8bA   VGSY R TWL S E D F AE Y D V AV RT HH K Y SGY E R K EF R Y TH HT  A:a.160.1.3
         1r8bA     |              =    =           |  | |         |    = |    ||

29
                    * ** *  * *    ** ** *        *
         1jwaB   r VGLGGLG LDFDT S SN RQ K ALLd CTDN V Q a c Ea v I   B:c.111.1.1 (29)
                       |    |=     || |  |
Z = 5.6, 1r4nB   h IGAGGLg IDMDT d SN RQ K NKIQ GLDS A w t K II A N   B:c.111.1.2 (30)
                       | |          |
Z = 5.9, 1y8qB     VGAGGIg IDLDt d SN RQ K DSIM ALDN A h T            B:(30)
                       |    |          |  |   |     |
Z = 6.9, 1zfnA   R IGLGGLg ADDDd H SN RQ K QRLT CTDN T E a c ta v V   A:(27)
                       |    |            |           |
Intacts Cons           *
IAct Cons (0.5)       +    +         +  +

30
                           *   *
         1xscA   Lrac ASDGI hHwT Kghv LNYVA- -RN--- -- k K V Y HEh laq---FKEM   A:(25)
                                    =                                  =
Z = 5.6, 1jknA   RrNV RldIp DAWQ QGGi ltYdFP KVReKL QW k Q Q w pEF LTVEFKkpvY   A:d.113.1.1 (33)
                      +                                 |             =|
Z = 5.6, 1su2A   LRAA ekgip glwh SGAV ylgrF- -PDG-- -- V I R v dei qir---Myqt   A:d.113.1.1 (5), A:d.113.1.1 (16)
                                       =    |
Z = 6.0, 1vc9A   Elga DRM-- gFwV KGHp TRYVNP -kg--- -- V R V w egm lla---FPED   A:(22)
                      |           |      + |              |               = |
Intacts Cons
IAct Cons (0.5)       +                 + +              +               +
```

```
31
                * ***** *    *   *   *    * *
         1jjvA  GGIGSGKTTI v P t RS qrd i q NDAELA nl L    A:c.37.1.1 (20)
                   | ||||        =          | | |
Z = 6.2, 1uf9C  GNIGSGKSTV E p E RV ARS R Q NT---- GS D    C:c.37.1.1 (24)
                   ||||||       =   |      ||
Intacts Cons       * ****       *          *
IAct Cons (0.5)    + ++++       +          +

32 (singleton)
         1jagA  GNIAVGKST E V W L MY FQ S R ER SD FA L R R EE I Y L  A:c.37.1.1
         1jagA     |  ||     =    + =|   +  |    =    | |  |

33 (singleton)
         3rlrA  V KR ERINL KIH V TS IH I F K  A:a.98.1.1
         3rlrA    |  || |  +     |         |

34 (singleton)
         1hplA  R R SGGG R F N SGA F D  A:d.114.1.1
         1hplA  | |      = =       = |

35 (singleton)
         1hilA  Q T K R R R DVSDH D S NN Y  A:e.8.1.6
         1hilA           | =             =

36 (singleton)
         1pj4A  H K R LG GIR R TDRYGR GN F D LGAGEAA FDKY Q GVAGA L LSN E L ILCN R Y RTW  A:c.58.1.3
         1pj4A      |      | +         |       | ||  = |             |          | | |

37
                *  * *   * * *    *   * *     *     *
         1n77A  RIAPSPT pHvGTAYIA n R I Y IRAEEW mPLLr KIsk    A:c.26.1.1 (28)
                   |          =     |         |
Z = 6.3, 1gtrA  RFPPEPN LHIGHaKSi N k i y LCTlEF FSRLN MSKR    A:c.26.1.1 (29)
                   | |       =             |    = =|    |
Intacts Cons       *         *             *
IAct Cons (0.5)    +         +             +

38 (singleton)
         1g5tA  GNGKGKTTA DE Y TGR L P H  A:c.37.1.11
         1g5tA   |  ||||         =

39 (singleton)
         1xdpA  W F V F I NN E R YR R H K N T LY NN R VDRYLEH R S D N R  A:(33)
         1xdpA    =   =              |        =    |       |

40
                  * * *   **        *
         1gn8A  YPGTFDP TNGH DI - IRGLR A E LMPs WSF ISSSLVK   A:c.26.1.3 (33)
                |          |        | +          |    |||
Z = 5.9, 1f9aA  IIGRFQP HKGH eV E YSG-- - n PEMF NRk YSGTEiR   A:c.26.1.3 (28)
                   ||   |                      +  +  | ||  |
Z = 5.5, 1yunA  FGGTFDP HIGH rS - liGw- - f LqR               A:(25)
                =| |    |                      =
Intacts Cons
IAct Cons (0.5) +  +    +                            ++

41
                * *  * ******   *            *
         1xexA  FKSY GANGSGKSNI D R DLIFAG p Q   A:(23)
                   || ||||    | | |=|    |
Z = 5.6, 1f2uA  FRSH GQNGSGKSSL d - EFTKV- G Q   A:c.37.1.12 (21)
                 =      ||||||         =|    |
Intacts Cons            * ****         **    *
IAct Cons (0.5)         + ++++         ++    +

42 (singleton)
         1kvkA  K T L NV NEGLS VWS LPPGAGLGSSA Y E IH D  A:d.14.1.5
         1kvkA  |     |  |   |   |    |    ||  =
```

```
43
                                                          *     *             *
         1yidB   tGDRpT gaLHlGHlAGS qnR a D -H v t E K y pvG---ddQ sr vPRLP AKMSKSL   B:(29)
                  |||     |                                                 |
Z = 6.6, 1h3eA   LGADPT pdLHLGHaV-V rkm g g Rp Y d - - Y MGG---TDQ M- -PLLV eKMSKSl   A:c.26.1.1 (32)
                    |     |  |                          |                 |   +|||
Z = 5.0, 2a84A   VPTMG- -Al----HEGH ALv f f -g d - e r v FFGEKDyqQ VP TVRea AMSSRNr   A:(30)
                    |        |  |                       | +|        |      ||||
Z = 7.5, 1m83A   SGIQPS GviTiGNYIGA rqF V v -w l Q K - Y PVG---EDQ pk GARIM kKMSKSD   A:c.26.1.1 (36)
                    | |       |                  |            |        |   +| ||
Intacts Cons        *
IAct Cons (0.5)     +         +                           +            +   ++++

44 (singleton)
         1nsyA   Y T F N R Y GG LLV TGFFTKY DL E R MT HK W  A:c.26.2.1
         1nsyA   +     | |               +  |         || =

45 (singleton)
         1r9tB   R Y D GQK SR  B:(8)
         1r9tB   |       | |

46 (singleton)
         1fmwA   IY VNPFKRI IY GESGAGKTEN N T RN NSSR D S C  A:c.37.1.9
         1fmwA       |       +   || ||||       |  ||

47 (singleton)
         1sx3A   TLGP KDGV N GDGTITA AGGG I YNAAT M ILD V  A:(31)
         1sx3A     |           ||||    |     ||       |

48
                 *   *       * * * *                                 * *
         2bu2A   EL KNAmRAtv D GGGVp Lfs S aptp tggt LAGFGYGLPisr L S T a   A:(26)
                             |                          |  | |
Z = 4.7, 1tilA   EA TNAIIHGY D GVGIP ARQ - ---- FTIK elERSGMGFTIM v s T V   A:d.122.1.3 (35)
                      |   |   |                    ||      ||||+
Z = 7.5, 1y8pA   EL KNSmRAtv D GGGVp LfN - YSTA slep LAGFGYGLPisr L S T a   A:(30)
                      |  |    |                ||          | ||||
Intacts Cons                 *                             * *
IAct Cons (0.5)       +      +                          + ++++

49 (singleton)
         1n5iA   TLA R Q HT GL Y  A:c.37.1.1
         1n5iA

50 (singleton)
         1e2qA   GVDRAGKSTQ R DR R E ASKSI V  A:c.37.1.1
         1e2qA      | ||||     | =     |

51 (singleton)
         1dy3A   L Q E R RK RWG R DLDIM TERLTVPHYD R M  A:d.58.30.1
         1dy3A         | |   |  |    |    | |   |  |

52
                       *           *                    * *
         2f02A   K n s D s -- - KpN E iSLG KDGA IPTIqAknPVGSGDAT GXAAGX A   A:(32)
                             | |    | |   |                    |
Z = 5.9, 1esqA   v f g D V at - RGN E iTG- -EVD NGHKLLTkVtGAGSlL IssYGv q   A:c.72.1.2 (27)
                             | |    ||     |
Z = 6.8, 1v1bA   A N I D N -- R flS E LKRG AkGA AFAVEAvdPVGAGDAF ANllGA A   A:c.72.1.1 (34)
                               |          |   =        | |     |
Z = 5.7, 1lhrA   g a t D V QR - TPN E iTSS yLma eMHKVDAVFVGIGDlF tVsaMh L   A:c.72.1.5 (29)
                                 |     |          |   =   |
Intacts Cons                     *
IAct Cons (0.5)                + +    ++    +             +
```

```
53
                                      *
         ldv2A   gf dK a Vp I K s GG----GGR M F YmEKYLEnPrHv c mQ ---r ---rHqK EfL IK N R qve     A:d.142.1.2 (28)
                   |       =          ||         ||=|                |       |
Z = 5.5, lpk8A   qH dK v Id V K g AH----SGM K - taEPFIDAkyDv R sv NWKT ---Ntgs EaL IE V s mPl     A:d.142.1.3 (29)
                      |       +    |    ||  |     ||=|    |  |     + |    |
Z = 6.0, li71A   -- dK v Ie V K g AH----SGM K - taEPFIDAkYDi R si ---s WKTNTgs KAV FE M c PlI     A:d.142.1.3 (32)
                               +         ||         ||=|    |  |          =   |    |   =
Z = 6.5, lkj8A   Ea Re R ST p I K vmSSSGKgQ F g vEGVVK--FdfE H Qe ---- ---dGDy ElF SE s r HDt R   A:d.142.1.2 (30)
                    |           +     ||  |      || |   =  |                    =
Intacts Cons                                     * *
IAct Cons (0.5)      +        +         ++       ++++    +  +           +

54 (singleton)
         ld9zA   YEPQG Q GATGTGKTFT E E A P D R R  A:c.37.1.19
         ld9zA   +|    |   ||  | =

55 (singleton)
         lbcpF   DTMLGF S L I  F:b.40.2.1
         lbcpF        = |

56 (singleton)
         lbcpE   V M V K PGS EV LR FM  E:b.40.2.1
         lbcpE             | |   + =

57
                 *   ****            *       * *      *    *
         lh8hA   GDRQTGKTSI D gq - -- D V E is t L t F RPA svsrvgs QGQY   A:a.69.1.1 (4), A:c.37.1.11 (19)
                    ||| ||                    =                |
Z = 8.3, le79A   GDRQTGKTSI D gq - -- D v E is t L t F RPa svsrvgs QGQY   A:a.69.1.1 (4), A:c.37.1.11 (17)
                    ||||||                    = =              | |
Z = 6.0, ltf7A   gAIGTGKTLL r EE N SW D T T ST I L Y R --a FKMRGSW       A:c.37.1.11 (23)
                    | ||||          =               |      | |
Intacts Cons        * * **                          *
IAct Cons (0.5)     ++++++                          +            +

58

         lgol_   IGEg--aYgmVc A Rki y r e l I in ivQDLM-E- --T D yK d KpSNlL iC-D fg l - --- - t    _:d.144.1.7 (22)
                                                   | |   +
Z = 6.3, lol6A   LGKg--KFGNVy A KVL v q E q L ly liLEYAPl- --G T yr d kpENlL iA-N fg w - --- - t    A:d.144.1.7 (26)
                    |       |                      |=|
Z = 6.0, lua2A   LGEG--QFATVy A Kki a g e l I ll lvFDFM-E- --T D ev D KpNNlL lA-D fg - - KSF X t    A:(30)
                 |     |     |                      |=|                                  |
Z = 6.6, lcsn_   IGEG--SFGVIf A KfE p q E Y P vy lvIDLL-G- --p S eD D KpDNfL vV-D fg m - --- - t    _:d.144.1.7 (29)
                    |       |                      | |                |
Z = 4.1, le8xA   vMaS--KKkPlW g IfK D - d l l YG IEIVKd-A- --T T aK r nDNiMi Fh-I Df g              A:d.144.1.4 (26)
                    |     =                      =     |                |    =
Z = 5.5, lzp9A   ISTG--kEAnVf A KiY v W E l P py llXEFI-Ge PAp T vE D seYNiX fI-D Xg Q - --- - -    A:(30)
                    |       |                      |=|
Z = 6.0, lzydA   LGQG--afgQVV A Kki - t e l V Yy iqMEYC-E- --N t yd D KpMNiF iGDf gl a - sdn - T    A:(25)
                                                   |=|                |  | =
Z = 6.8, lu5rA   IGHG--SFgaVy A KkM k d E l I yr lvMEYC-L- --G S sD D kaGNiL lG-D fG s - --- - t    A:(28)
                    |       |                      |=|
Z = 6.8, 2biyA   LGEG--SFSTVv A Kil k y E m V ly fgLSYAKn- --G E lk d KpEniL iT-D fg t - --- - t    A:(25)
                    | |     |                      |=|        |
Z = 6.8, latpE   LGTG--SFGRVm A KiL q h E l V le mvMEYV-Ag --g E fs D KpENlL vT-D FG F - --- - T    E:d.144.1.7 (33)
                    |||     |                      |=|        |     | |    |
Z = 5.2, ltqpA   XGeG--KESAVf V Kfh a s E l P vy vlXELI-DA --k E yr D SqYNvL iI-D FP q - --- - -    A:d.144.1.9 (28)
                    |   ||    |                      | |        |
Z = 6.7, lphk_   LGRG--VSSVVr A Kii l a E l I lk lvFDLM-Kk --G E fD D KpENiL lT-D FG F - --- - t    _:d.144.1.7 (31)
                 |              |                      =| |        |       |
Z = 6.1, ls9iA   LGAG--NGGVVt A Kli - - e l V fy icMEHM-Dg --G S DQ D KpSNiL lC-D fg a - --- - -    A:(28)
                               |                      | |        |  |    |
Z = 5.7, ls9jA   LGAG--NGGVVf A Kli - q e l V fy icMEHM-Dg --G S DQ D KpSNiL lC-D fg n - --- - V    A:(29)
                               |                      | |        |     | |
Z = 6.5, lb38A   kIGEGTYGVVyk A Kki g t e l V ll lvFEFL-H- --Q D kK D KPQNLL lA-D gl a E --- - t    A:d.144.1.7 (30)
                    |          |                      =|=|        |       |
Z = 6.8, lq97A   LGWG--HFSTVw A Kiv y a E l L ll mvFEVL-G- --E N la D KpENvL iA-D lG N - --- - t    A:d.144.1.7 (29)
                    |                                 | |
Intacts Cons                                          *
IAct Cons (0.5)               +                       +++        +
```


59
leqyA Q DSGDGVTh lAGR T r R KEK - c sGGTTMYP RKy A:c.55.1.1 (36)
Z = 5.5, le4gT S NLGYNFtg vgXK I F E IIT - g tGGGaKIP pSf T:c.55.1.1 (33)
Z = 7.3, ltyqA DCGTGYTK i S i HGi Q DSGDGVtH iAGR T l K KER - s SGGSTMFr qRY A:(36)
Z = 7.1, ltyqB DSGDGVth iAGR T f R KEK - C SGGSTMYP r B:(23)
Z = 7.9, lyagA DNGSGMCK v s I HGi Q DSGDGVTh lAGR T r R KEK - c SGGTTMFP RKy A:c.55.1.1 (37)
Z = 5.9, lnge_ DLGTTYSc T S a --K e SLGGGTFd LGGE D r E KRT S t VGGSTRIp PDe _:c.55.1.1 (36)
Intacts Cons
IAct Cons (0.5)
60
lb76A gH f m pE a R Eit r IFRVREFeQ E - - - - yqQ s a-- hY k TvD slELEGia EpS AGVDRG A:d.104.1.1 (29)
Z = 6.8, lh4qA rk h e PT v R E-m t FLRTSEFLW e - - - - lKt E F-- ag v ttt alQAGTsH swG L-SWRF A:d.104.1.1 (27)
Z = 6.0, lnyrA gh Y m pM M R Eas v LQRVRgMtL d - - - - Rls E a-- fY - KlD eETLSTaQ hrG STmERf A:d.104.1.1 (28)
Z = 4.7, le24A AS - r ia N R EGi v -RHNPEFTM e - - D E tfi - pae vs R Eff ggrEIG-N lGI GIDRmi A:d.104.1.1 (29)
Z = 4.9, lb8aA eg - E es I R Eeh t -RHLNEAwS d E - - - fly s a-- Kp r fDl rgvEISSg f-G LGAERL A:d.104.1.1 (26)
Z = 4.9, laszA -- - S Qs v R enS t -RHMTEFtg d - E - - ild l i-- rp n yDf rgeEILSg ggG IGlERv A:d.104.1.1 (22)
Z = 6.1, lkmnA ig d E pE m R E-r Q -GRYRQFHQ g - - - - eln l RGL yY r vfe QgTVCAGg gfA MGLERL A:d.104.1.1 (30)
Intacts Cons
IAct Cons (0.5)
61
layl_ KK H GLSGTGKTTLS DD YAKTIKLS E N R L t NTG RISIKDTR _:c.91.1.1 (37)
Z = 7.8, lxkvA kk H GLSGTGKTTLs DD yaKvirls E n R L t nTG RFPLPvTr A:(25)
Z = 8.0, lytmA kK H GLSGTGKTTLs DD YAKvINls E N R L T NTG RISIkdTr A:(32)
Intacts Cons
IAct Cons (0.5)
62
2bekA NQKGGVGKTTT i d A Q Na sg DAPPs Q EYYA TMYd IPRNVRLAE P k g Y A:(31)
Z = 5.7, la82_ gtDIEVGKTVA c K v S sD la EGAGG g klgc NDVT IPWLAEnP- - E - g _:c.37.1.10 (29)
Z = 6.7, lg21E g-KGGIGKSTT Q g D K Ds RL DVGdv s EMMA NSrn VPRDNVVQr E m Q Y E:c.37.1.10 (31)
Intacts Cons
IAct Cons (0.5)



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