國 立 交 通 大 學

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蛋白質催化位置之結構特性

On the structural characteristics of protein catalytic sites

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摘　　要

大量的未知功能蛋白質結構已經被解出並存放於蛋白質資料銀行(Protein Data Bank-PDB)。因此，藉著蛋白質結構來臆測其功能也變得相當重要。為了達成這個目標，蛋白質結構-功能關係的知識將會成為非常有用。藉著一個有著887個已知酵素結構且有2368個催化殘基的資料集我們提出了一個非常廣泛的分析。我們發現(i) 多數的催化殘基不是有電性(charged)就是有極性(polar); (ii) 催化殘基通常座落於纏繞區(coil region); (iii) 催化位置通常較深層; (iv) 催化殘基具有較低的韌性(flexibility); (v) 催化位置似乎較喜歡座落於接近蛋白質領域(domain)的中心; (vi) 催化位置通常發生在蛋白質中較緊密的地方。我們的結果也提供了蛋白質催化殘基結構特性的資訊，這個資訊和序列保存度具有互補關係。總而言之，我們的結果在了解蛋白質結構-功能關係上也許會有幫助。更進一步而言，這樣的結果也許在未來預測蛋白質催化位置上能提供發展新方法的新視野。

On the structural characteristics of protein catalytic sites

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ABSTRACT

An enormous number of structures of proteins with unknown function has been solved and deposited in PDB. Hence, it becomes increasingly important to infer function directly from protein structures. To do this, the knowledge of protein structure-function relationship will be valuable. We carried out a comprehensive analysis of the dataset consisting of 887 enzymes of known structure with a total of 2368 catalytic sites. We found that (i) most catalytic residues are either charged or polar; (ii) catalytic sites are usually located on coil region; (iii) catalytic sites are usually buried; (iv) catalytic residues appear to be less flexible; (v) catalytic sites seem to prefer to be in the proximity of the centroid of the domains; (vi) catalytic sites usually occur in the compact regions of proteins. Our results also provide information of structural characterization of catalytic residues in protein, which is complementary to that sequence conservation. In summary, our results may be helpful in understanding the protein structure-function relationship. Furthermore, they may give new insight into developing novel methods for the prediction of catalytic sites in protein.

誌謝

謝謝在研究這條路上幫助我，陪伴我，指導我的所有人。還有在生活上支持我，鼓勵我，協助我的所有人。

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1. Introduction

Enzymes play an important role in controlling and performing most life process. Therefore, understanding the characteristics of catalytic sites will help us realize how enzymes work. Most enzymes are proteins and their catalytic machinery occurs in a relatively small region.[1] The regions of an enzyme designed to directly interact with substrates, are defined as catalytic sites. The side-chain of catalytic residues are usually polar or charged[1]: imidazole group of histidine, guauidinium group of arginine, amine group of lysine, carboxylate group of glutamate and aspartate, amide group of glutamine and asparagines, hydroxyl group of serine, threonine and tyrosine, and thiol group of cysteine. We consider the functions of the amino acid's side chain as the overall structure and dynamics of enzymes in catalysis.[2]

The first enzyme structure was the X-ray structure of lysozyme solved in 1965[2]. Since then, a huge number of determined enzyme structures have been stored in database. Because of the Structural Genomics Project, an increasing number of structures with unknown function were deposited in the Protein Data Bank (PDB). The vast number of structures allows us to study the structure-function relationship directly from the protein structures. Then, it is still a challenging task that using only protein structures deduce catalytic sites function.

There are many previous studies identifying and predicting the catalytic sites of enzymes. First, sequence and structural similarity based methods are the most used to recognize catalytic residues in a query enzyme. For example, a number of methods [3-8] based on the observation that most catalytic sites are highly conserved in sequence and structure; Thorton et al. [4,5]developed a methodology, utilizing a library of 3D structural templates

formed from small number of residues to recognize catalytic sites of proteins. However, we do not have to do any sequence or structure alignment in our study. We only utilize the unique structural characteristic of enzymes to analyze catalytic sites. Then, the distinct property of large force constants associated with the catalytic residues is consistent with the recent study [9,10] that the catalytic residues usually have lower B-factors than noncatalytic residues. A residue with lower B-factors will be less flexible (i.e., more rigid). Ben-Shimon and Eisentein[11] then observed that the catalytic residues are usually located in small fractions of the exposed residues closet to the protein centroid. Some literature [12,13] show that the B-factor of the atom is linearly proportional to its squared distance from the protein centriod. In another word, the residues in proximity to the protein centriod will have lower thermal fluctuation or more rigid than those further residues. Besides, a recent study [14] shows that the atom's thermal fluctuations are linear inverse proportion to the protein contact number of this atom. It means that a residue has lower flexibility also more compact in structure. For all of this, catalytic sites may be able to be confirmed through these characteristic information.

According to the previous studies, catalytic sites probably have unusual features to differentiate themse[1]lves from noncatalytic sites in enzyme. With these unique characteristic structural features of catalytic sites, it may enable people to understand the structure function relationship and to identify them. Since 1988, Zvelebil, M. and Sternberg, E[15]formed an analysis of catalytic residues in just 17 enzymes and Thornton et al. in 2002[1] analyzed only 178 structures. However, the completeness of the dataset is a major problem. Hence, in this study we will carry out the most comprehensive analysis of the protein structure properties of the catalytic sites.

2. Materials and Methods

2.1 Dataset of catalytic residues

The enzyme was taken from the Catalytic Site Atlas (CSA)-2.2.10 which original comprised 968 protein structures [16].We do not consider the structures of proteins which situate at the cell membrane, contain inter-chain catalytic residues, and exclude the main-chain functional part. Furthermore, we only consider interactions between charged, hydrophilic residues. The nonpolar residues often not directly participate to the catalysis reaction or only create the hydrophobic environment to help the polar residues to take part in the reaction. Moreover, the nonpolar residues, especially alanine, are usually mutated from the polar charged residues. It would abolish the autoproteolytic activity during protein structure determination. In addition, there has a study also show that only the 11 polar and charged residues of the 20 amino acids are generally observed to occupy directly in catalysis[17]. The residues of dataset in this study are defined by the previous criteria. The final dataset consists of 887 protein structures, including 2368 catalytic residues. The length distribution of our dataset was shown in Table1. The list of our dataset was shown in Appendix I.

2.2 Identification and Classification of catalytic sites

2.2.1 Amino acid type

Different amino acids apparently have various propensities to be catalytic residues. Catalytic residues are classified according to the 11 standard amino acid's three letter abbreviation, i.e., CYS, ASP, GLU, HIS, LYS, ASN, GLN, ARG, SER, THR, and TYR.

2.2.2 Sequence conservation

It is well-known that catalytic residues are highly conserved. In order to know each residue's conservation, we use the Shannon entropy[18] to represent it. Here,

$$H(x) = -\sum_{i=1}^{n} Pi \log_{20} Pi \qquad (1)$$

where *Pi* is taken from the PSSM (position-specific scoring matrices) which generated by the PSI-BLAST, has 20 × L elements , where L is the length of query sequences. The base of *log* is changed to 20 so that we can get the score in a range from 0 to 1, where 0 means strict conserved.

2.2.3 Secondary structure

Secondary structure information may be helpful in realizing catalytic residues. The classification of secondary structures which we use is according to DSSP [19]. DSSP is a database of secondary structure assignments for all protein entries in the Protein Data Bank (PDB). The elements describe the probabilities of each residue in three or eight states of secondary structure. The eight states structural categories followed by DSSPcont include α-helix(H), residue in isolated β-bridge(B), extended strand, participates in β-ladder(E), 3_{10}-helix(G), π-helix(I), hydrogen bonded turn(T), bend(S), and others undefined(U). We further classify these eight states into helix(H)={ H,G,I }, sheet(S)={ B,E }, and coil(C)= { T,S,U}.

2.2.4 Relative Solvent Accessibility (RSA)

The surface area is an important structural characteristic since the protein-protein interaction often happens on the surface. Besides, it has been published that catalytic residues are generally more exposed to solvent than others. Amino acid relative accessibility is the degree to which a residue in a protein is accessible to a solvent module. The relative solvent accessibility is computed by

$$\mathrm{Re}\,lAcc(\%) = \frac{100 \times Acc}{MaxAcc(\%)} \qquad (2)$$

where *Acc* is the solvent accessibility of a residue was assigned by using the program DSSP,

given in $Å^2$ units. *MaxAcc* is the maximal accessibility for the amino acids given by B.Rost et al [20]. We use two models to define the residue's solvent accessibility. One is binary model which distinguish all residues into two states, *RelAcc* <16% means Buried, ≥16% means Exposed. The other one is ternary model which distinguish all residues into three states, *RelAcc* <9% means Buried, 9-36% means Intermediate, ≥36% means Exposed. The thresholds that we selected are the same as those in Rost and Sander [20].

2.2.5 B-factor

The B-factor also called atomic mean-square displacement or temperature factor is used in condensed matter physics to describe the attenuation of x-ray scattering or neutron scattering caused by thermal motion or quenched disorder. It is used to measure residue flexibility. The B-factor of a protein is presented as $b = (b_1, b_2, b_3, ...b_N)$, where b_i is the B-factor of Cα of *i* residue taken from the PDB file; N is the total residue numbers of the protein. We normalize the B-factor by the following formula:

$$Z_i^b = (b_i - \bar{b}) / \sigma_b \qquad (3)$$

where $\bar{b}$ and σ_b are the mean and the standard deviation of the B-factor. Later, we will refer it to zB-factor.

2.2.6 Centroid-model (CM)

Residues contacting with catalytic site usually have more interaction with other residues, so centrality values of catalytic residues of enzyme structures are really important. It is a method which used to compute protein dynamics directly from the static protein geometrical shape without any mechanical models [12]. It based on the observation that the deeper an atom is buried inside a protein structure, the less it will fluctuate around its equilibrium position. It calculates the square of the atomic distance from the protein's center of mass. Let X_0 be the center of mass of the protein, that is, $X_0 = \sum_k m_k X_k \Big/ \sum_k m_k$, where m_k and X_k are the mass and the crystallographic position of atom k, respectively. The distance of atom *i* from the center of mass of the protein is computed by

$$r_i^2 = (X_i - X_0)(X_i - X_0) \qquad (4)$$

where X_i is the coordinate of Cα atoms of the *i* residue, and X_0 is the centroid of the protein. We will refer it as the centroid-model (CM). In order to make it easier to comparison, we normalized r_i^2 by

$$Z_i^{r^2} = \left(r_i^2 - \overline{r^2}\right) / \sigma_{r^2} \qquad (5)$$

where $\overline{r^2}$ and σ_{r^2} are the mean and the standard deviation of r_i^2 .

2.2.7 Weighted contact number model (WCN)

The neighboring atoms would affect function of the catalytic residues. A recent study [21] showed that the atomic mean-square displacement (or B-factor) is closely related to the number of noncovalent neighboring atoms. Here, we will refer to this method as the protein

contact number (CN). This method can be further improved if the protein CN is scaled down by the square of the distance between the contacting pair. To consider the distance factor, a distance-dependent contact number v_i will defined by weighting the integral contact number with the factor $1/r_{ij}^2$ which is the distance between Cα atoms of *i* and *j* residues.

$$v_i = \sum_{j \neq i}^{N} \frac{1}{r_{ij}^2} \tag{6}$$

where N is the total residue numbers of the protein. We will refer is as the weighted CN model (WCN). And, we also normalize v_i to its Z-score:

$$Z_i^v = \left(v_i - \bar{v}\right)/\sigma_v \tag{7}$$

where $\bar{v}$ and σ_n are the mean and the standard deviation of v_i.

3. Results and Discussion

Detailed analyses of these unique properties were illustrated here. It was suggested that catalytic sites and all residues did differ in these characteristics.

3.1 The distribution of amino acid types in catalytic sites

We analyzed the frequency distribution of the 11 polar or charged amino acid types occurring in the catalytic sites compared with all residues in the dataset. Figure 1 shows that the amino acids who have positively or negatively charged R groups (ASP, GLU, HIS, ARG, LYS), they account for 71% of all catalytic residues, while 29% of catalytic residues are provided by the amino acids having polar R groups (SER, THR, CYS, ASN, GLN, TYR). As we already known that catalysis reaction involves the transfer of protons and electrons and charge stabilization, this result is consistent with the previous studies [22] showed that major contribution of enzyme catalysis came from electrostatic interaction.

Aspartate and glutamate residues compose 19% and 14% of all catalytic sites, respectively. It could be that their pKa values far from neutral, around 4.0, provide charges that affect other residues and the substrate. Moreover, aspatate has a shorter side chain than glutamate, making the side-chain less flexible to be easier getting involved with substrate.

We can also see the importance of histidine in enzyme from Figure 1. Histidine constitutes 18% of all catalytic sites. While it has a pKa value of 6, it is really close to neutral and can function as an acid-base or be involved in stabilizing the transition state of catalysis.

The other most commonly observed residues are the positively charged and long side-chain residues, arginine and lysine. Both of them compose about 10% of all catalytic sites. The reason for that might be that both of them have nitrogen groups in the side-chain;

hence, the side-chain of them can make more electrostatic interactions.

The polar residues are used less often, since they are uncharged in proteins. They tend to form covalent intermediates during the catalysis reaction.

3.2 The characteristic feature profiles of catalytic residues

3.2.1 Sequence conservation profile

Catalytic sites are clearly more conserved than other residues; it can be shown in Figure 2(A). The average conservation score of catalytic sites is 0.09. Since the catalytic sites in proteins are all have important function, it would not be easily substituted by other amino acids. Figure 3 shows the conservation score trend of 11 amino acids respectively. The trends of the performance of these amino acids appear to be similar.

3.2.2 Secondary structure profile

Figure 2(B) and Figure 2(C) show the secondary structure distribution of catalytic sites compared with all residues in the dataset. Catalytic sites prefer to locate on the coil regions (about 50%) than other types. On the contrary, catalytic sites not favor to occur in helix regions (only 23%). This is different from the distribution of all residues. When we use the eight states structural categories followed by DSSP, we can found out that catalytic sites are especially prefer to occur in β– ladder (E) and undefined (U) regions (Figure 2(C)).

Make it more clearly, in Figure 4 and Figure 5; we analyze the 11 amino acid individually. The catalytic sites of threonine and tyrosine have different distribution with

others; they do not often locate on β– ladder. It may because both of them have the hydroxyl group on the side-chain. Furthermore, cysteine, whose side-chain has thiol group, also not prefers to occur in β– ladder region. Oppositely, aspartate and glutatmate, who have negatively carboxyl group on the side-chain, are more prefer to locate on β– ladder regions. The remaining six amino acids have similar distribution of secondary structure.

3.2.3 Relative Solvent Accessibility (RSA) profile

Figure 2(D) and Figure 2(E) show the relative solvent accessibilities profiles of catalytic sites compared with all residues in the dataset. As we have mentioned in our method, we use binary and ternary model to analyze the distribution of catalytic sites. The 67% of catalytic sites are more buried residues in protein structures (Figure 2(D)). While in ternary model of relative solvent accessibility, only 11% of all catalytic sites are fully exposed. This result consistent with other study which shows that the catalytic site is often occurred in a large and deep cleft or cavity [23].

We analyzed 11 amino acids respectively, as shown in Figure 6 and Figure 7. The side chain of cysteine is thiol group, surprisingly; its distribution between catalytic sites and all residues is quite different with other amino acids. The RSA trend of catalytic sites or all residues of cysteine are exceptionally similar, both of them tend to have more buried to solvent. The reason for that might because the thiol group of cysteine is the most reactive side chain found amongst the 20 naturally amino acid residues. However, the exposed frequency of catalytic sites of cysteine is unusual higher than all residues. It may due to that the side chain of cysteine is prefer to form disulfide bonds, which is a strong covalent bond and adopted in solution.

3.2.4 Rigidity profile

In this study we use three kind features (i.e., zB-factor, CM, WCN) to represent a protein structural rigidity. The B-factor is often used to measure residue flexibility, the smaller value is, and the less flexibility is. The smaller WCN value a residue is means that it locates on more crowded environment. The CM value represents whether a residue is close to its structural center or not.

Figure 2(F) compares the zB-factor of the catalytic sites with that of all residues. There are around 81% of catalytic sites with zB-factor $\leqq$ 0, compared with 54% of all residues. Figure 2(G) shows that the WCN of catalytic sites compared with all residues. There are around 90% of catalytic sites with WCN $\leqq$ 0, compared with 14% of all residues. Moreover, the CM of catalytic sites compared with all residues is shown in Figure 2(H). It should be noted that there are about 94% of catalytic sites with CM $\leqq$ 0, compared with 13% of all residues. No matter which feature of these three can suggest that catalytic residues tend to be more rigidity, it means catalytic sites often held in fixed place in enzyme than all residues.

However, we further shows that WCN and CM can perform a much better result than zB-factor. If we use a cutoff value $\leqq$ 0, the WCN and CM will contain about more than 90% of the catalytic residues. CM shows that the catalytic sites are usually closet to the protein centroid. The lower WCN means the catalytic sites tend to lie in the more packed regions than other residues do. According to our results, CM and WCN can play an expressive role in determine the catalytic sites, since the refined B-factor easily affecting by factors like temperature, crystallization conditions or structural refinement.

zB-factor, CM, WCN plots for individual amino acid types can be seen in Figure 8,9,10, respectively. The charged side-chain catalytic residues are easier to be differentiated with all residues. Nevertheless, figure 9(F)(G) and figure10 (F)(G) show that the catalytic sites of

serine and threonine, which has polar side chain, especially more prefer the environment which is more crowed and more center than tyrosine. It is reasonable that tyrosi[24]ne needs more space for its larger side chain.

It is worth noting that, our results are consistent with previous study[24]. Since 1894, Emil Fisher proposed that the catalytic site has a specific geometric shape that is complementary to the geometric shape of a substrate molecule. It means that enzymes are very specific, and the catalytic site of enzyme are especially less flexibility than other residues.

3.3 The 2D-profiles of catalytic residues

In order to understand the relationship between each feature, we combine any two features to plot 2D-profiles (Figure 11).

Figure 11(A) shows the relationship between conservation score and relative solvent accessibility. Figure 11(B) shows the relationship between conservation score and normalized B-factor. Figure 11(C) shows the relationship between conservation score and centriod model and figure 11 (D) shows the relationship between conservation score and weighted contact number. The conservation score and RSA in catalytic sites are usually low, but conservation score has no readily observable connection with RSA (Figure 11 (A)). However, using the zB-factor, CM, or WCN may help the conservation score to have a better discrimination between catalytic sites and all residues as shown in Figure 11 (B) (C) (D).

Next, we discuss about the relative solvent accessibility combined with other features, which are zB-factor, CM, and WCN, the values of all features tend to be low as shown in figure 11 (E)(F)(G). In this case, we might say that if a residue's RSA value lower than 0.36 (means intermediate or buried) [20] and CM/WCN value lower than 0, it usually located on the catalytic sites.

It was clear that catalytic residues tend to have lower zB-factor, CM and WCN values according to our characteristics feature profile results. As using any two of them plot a XY chart, we can figure out how correlative they are. We use the same cutoff value with the characteristics feature profile that is zB-factor, CM, WCN values $\leqq$ 0. Figure 11 (H) shows that about 78% of catalytic sites have WCN and zB-factor $\leqq$ 0. When CM and zB-factor $\leqq$ 0, there has around 80% of catalytic sites are included (Figure 11 (I)). One notice that, when we use this threshold value (i.e., CM, WCN $\leqq$ 0) for analyzing catalytic sites, Figure 11 (J) shows close to 90% of catalytic sites can be recognized only uses the CM and WCN features. It is proven that CM and WCN are indeed important characteristics of catalytic sites.

To sum up, sequence conservation score has no significant correlation with CM, or WCN value. The CM, or WCN are used for indicate structural rigidity. However, there are complementary relationship between structural characteristics of catalytic sites and those based on sequence conservation. We can say that using CM or WCN can distinguish catalytic residues from all residues although they are not conserved in sequence.

4. Conclusion

In this work, we represent a structural analysis of enzyme catalytic sites using a dataset of 887 enzymes which was chosen from CSA. This dataset is nonredundant, but Thornton's group does not mention the sequence identity of this dataset. The conclusion that we draw from this analysis is that catalytic sites are highly conserved; they are often found in a coil region, and most of them have very limited exposure to solvent although they are polarity and charged. In addition, catalytic sites have large property to locate on the centroid of a protein and catalytic sites also tend to lie in the crowded regions. It means that catalytic sites are more rigid than other noncatalytic residues according to their low B-factor, CM, and WCN values. Interestingly, the B-factor profile did not perform as well as CM or WCN profiles. It may cause of the refined B-factor easily affecting by factors like temperature, crystallization conditions or structural refinement.

Based on all these distinct characteristics with catalytic sites may enable people to understand the structure-function relationship; furthermore, it will be helpful for predicting catalytic sites in enzymes of unknown function from protein structures.

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TABLE

Table 1. The length distribution of dataset

Length	number
≦100	8
101~200	128
201~300	262
301~400	236
401~500	108
501~600	73
601~700	31
701~800	21
801~900	10
901~1000	2
≧1000	8

FIGURES

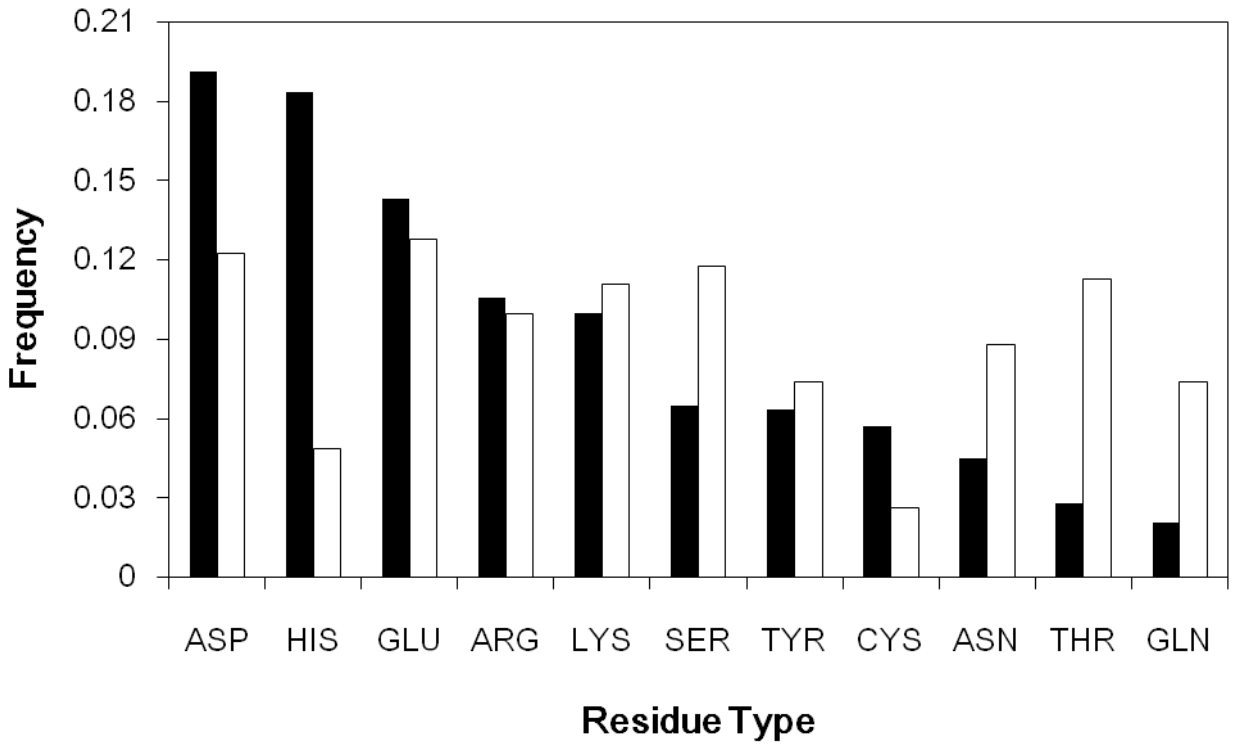


Figure 1. The frequency of polar and charged amino acid type in catalytic sites (black) compared with all residues (white).

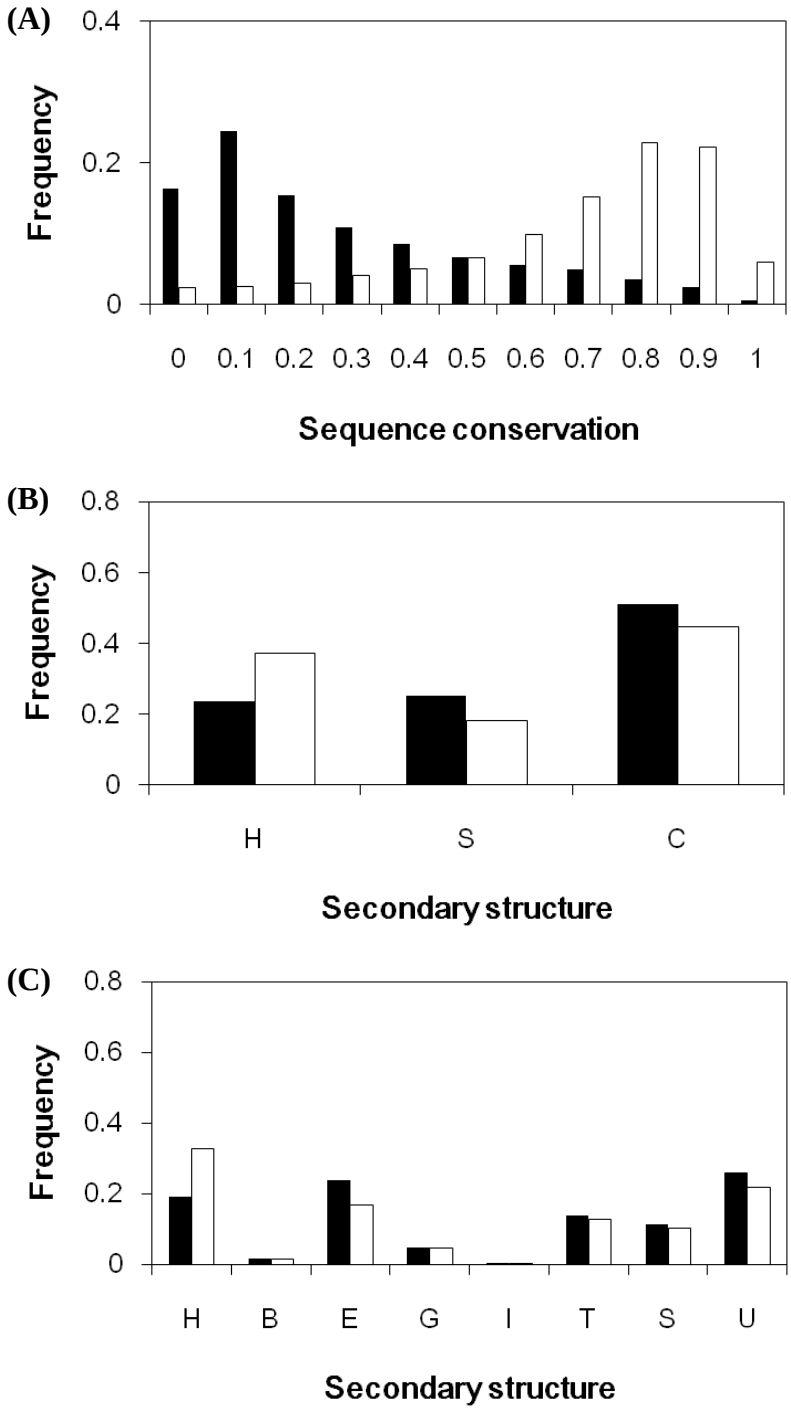
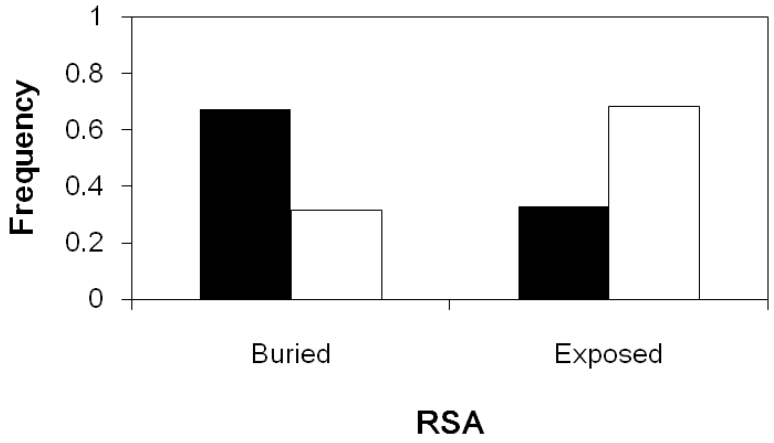
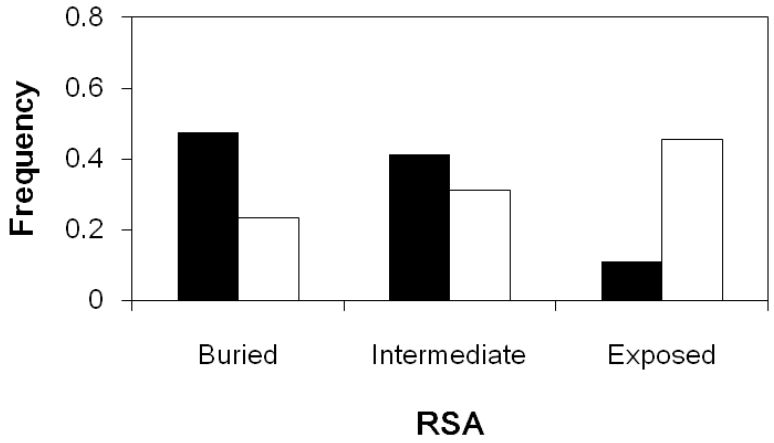


Figure 2. The histograms of the frequency between catalytic sites and all residues (A) Sequence conservation; (B) Secondary structure_ Helix (H), Sheet (S), Coil (C)**; (C) Secondary structure_**α-helix (H), β-bridge(B), β-ladder(E), 3_{10}-helix(G), π-helix(I), hydrogen bonded turn(T), bend(S), and others undefined (U)**; (D) Relative Solvent Accessibility(RSA)_binary model; (E) Relative Solvent Accessibility(RSA)_tertiary model; (F) Normolized B-factor(zB-factor); (G) Weighted contact number model(WCN); (H) Centroid-model(CM).**

(D)



(E)



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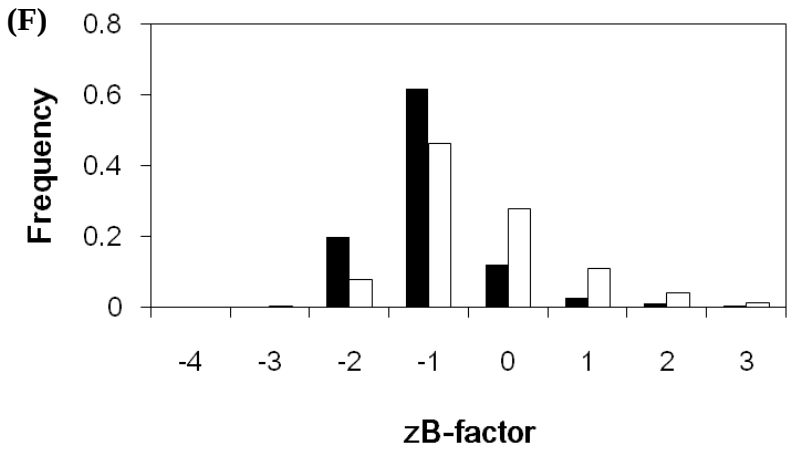
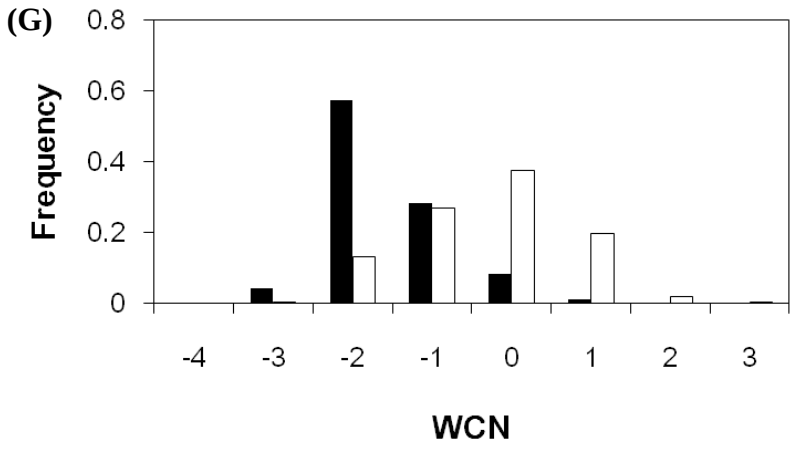




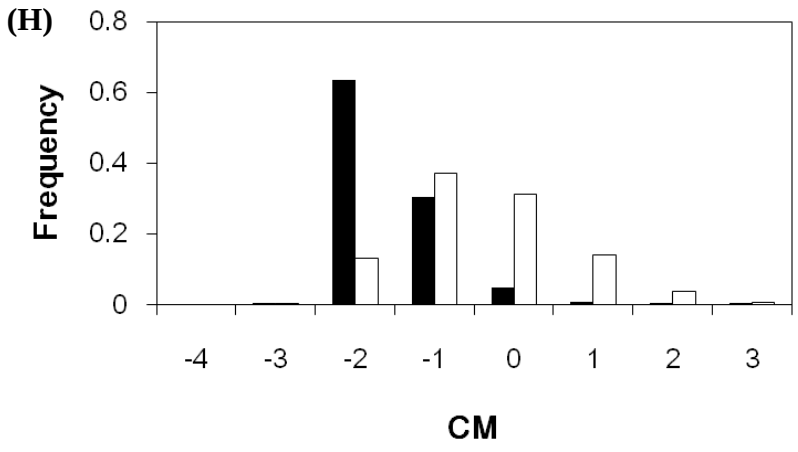


Figure 2. (Continued)

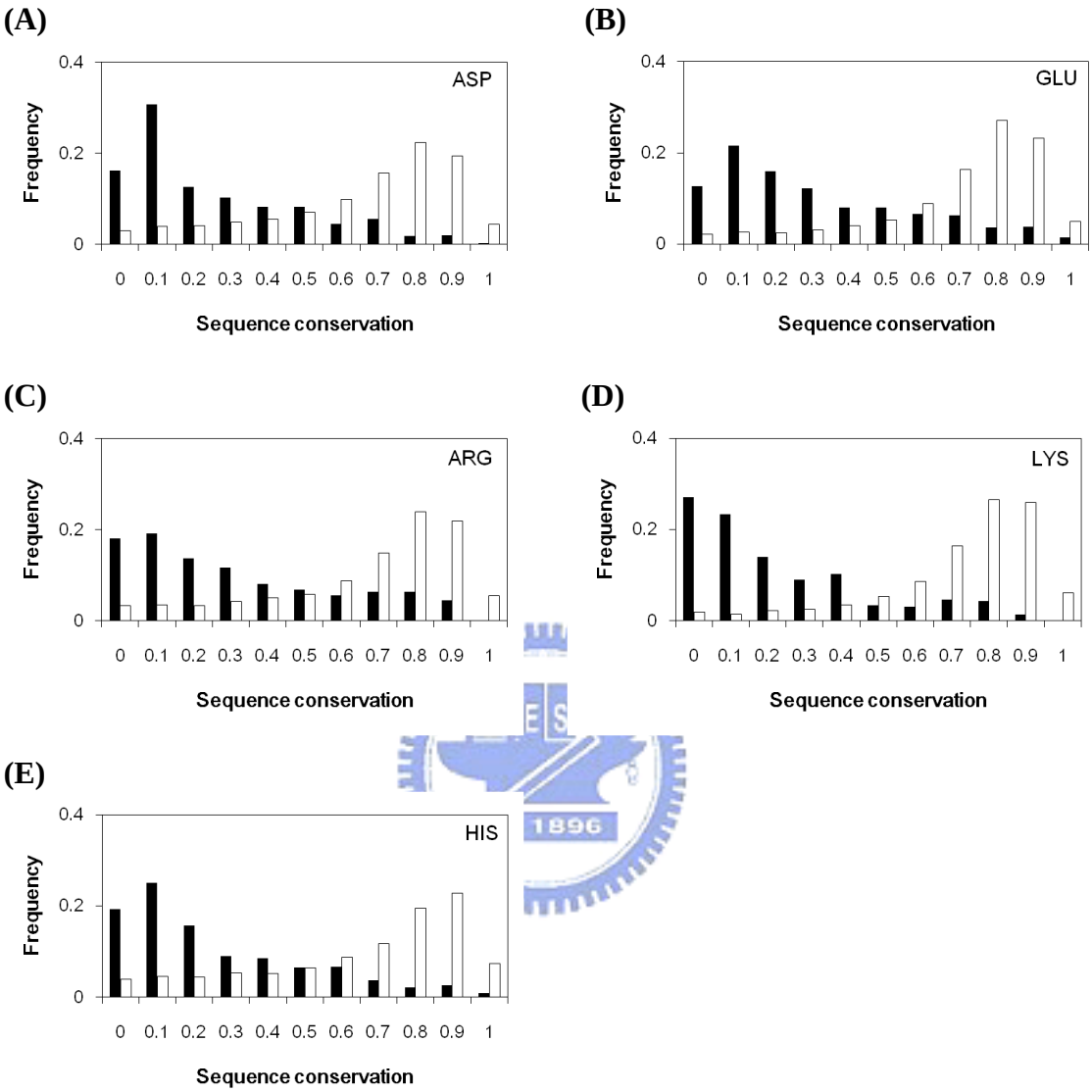


Figure 3. Sequence conservation for individual catalytic residues (ASP, HIS, GLU, ARG, LYS, SER, TYR, CYS, ASN, THR, GLN) compared with all residues of the same type.

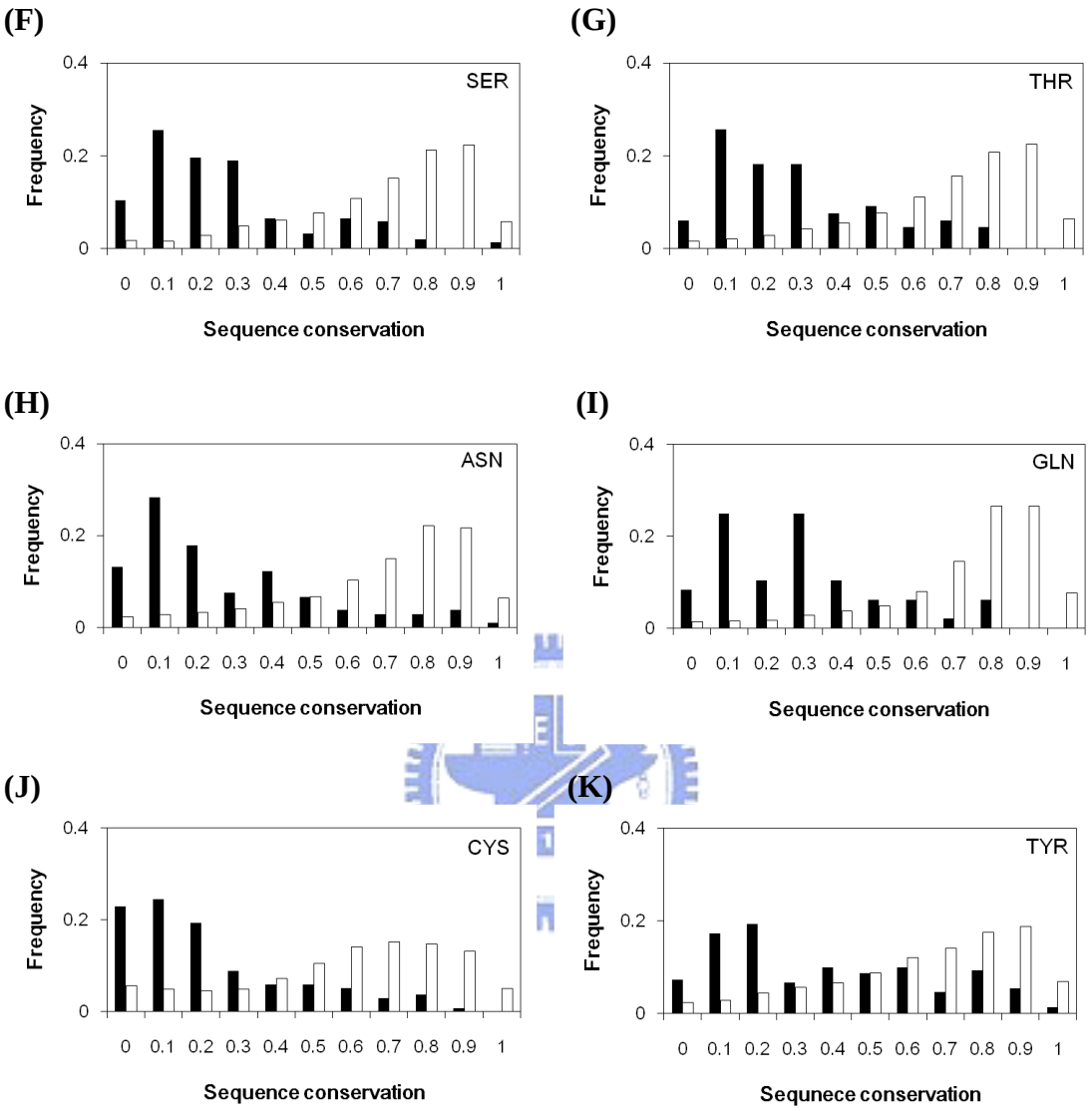


Figure 3. (Continued)

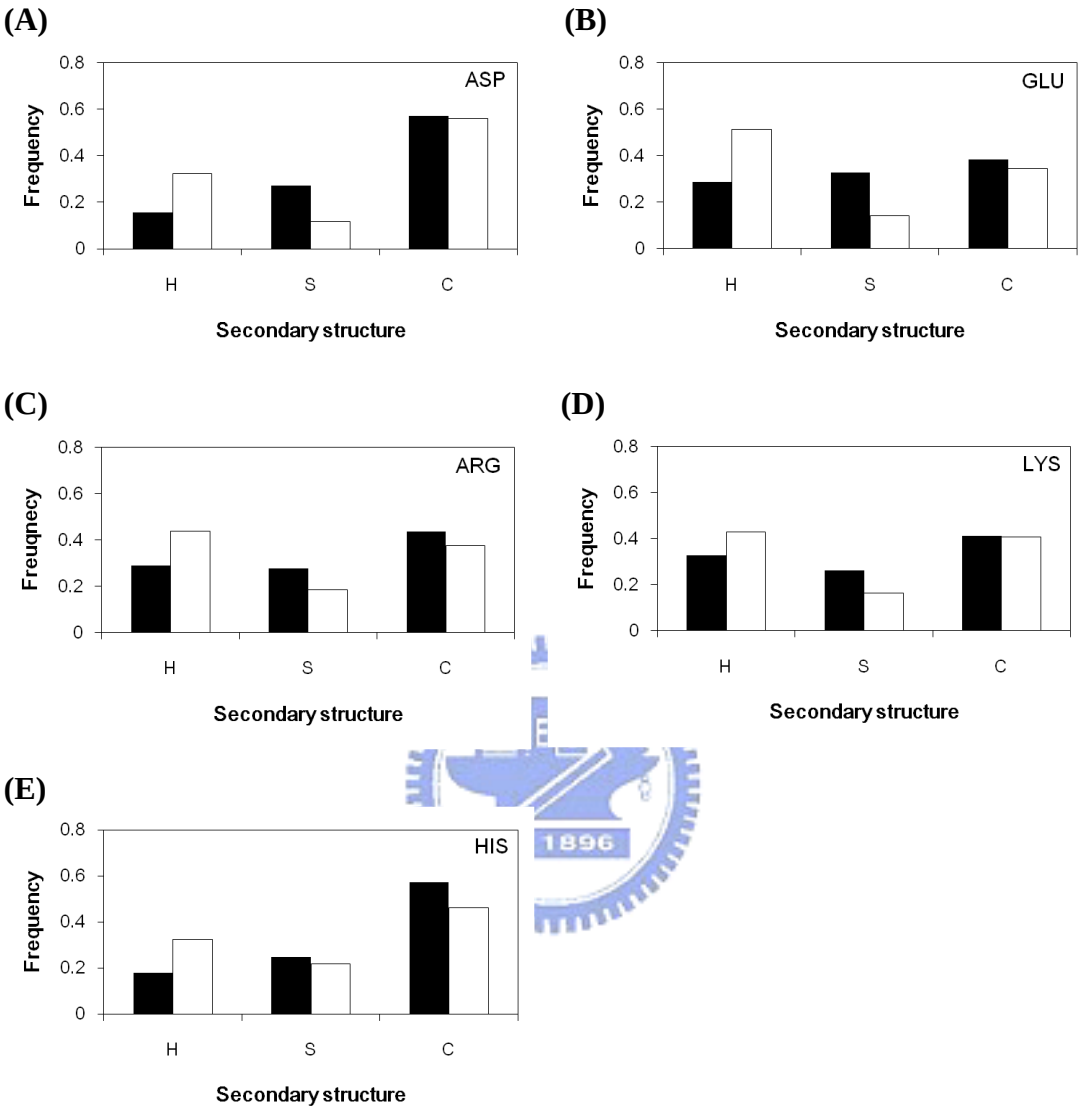


Figure 4. Secondary structure_ Helix (H), Sheet (S), Coil (C) **for individual catalytic residues (ASP, HIS, GLU, ARG, LYS, SER, TYR, CYS, ASN, THR, GLN) compared with all residues of the same type.**

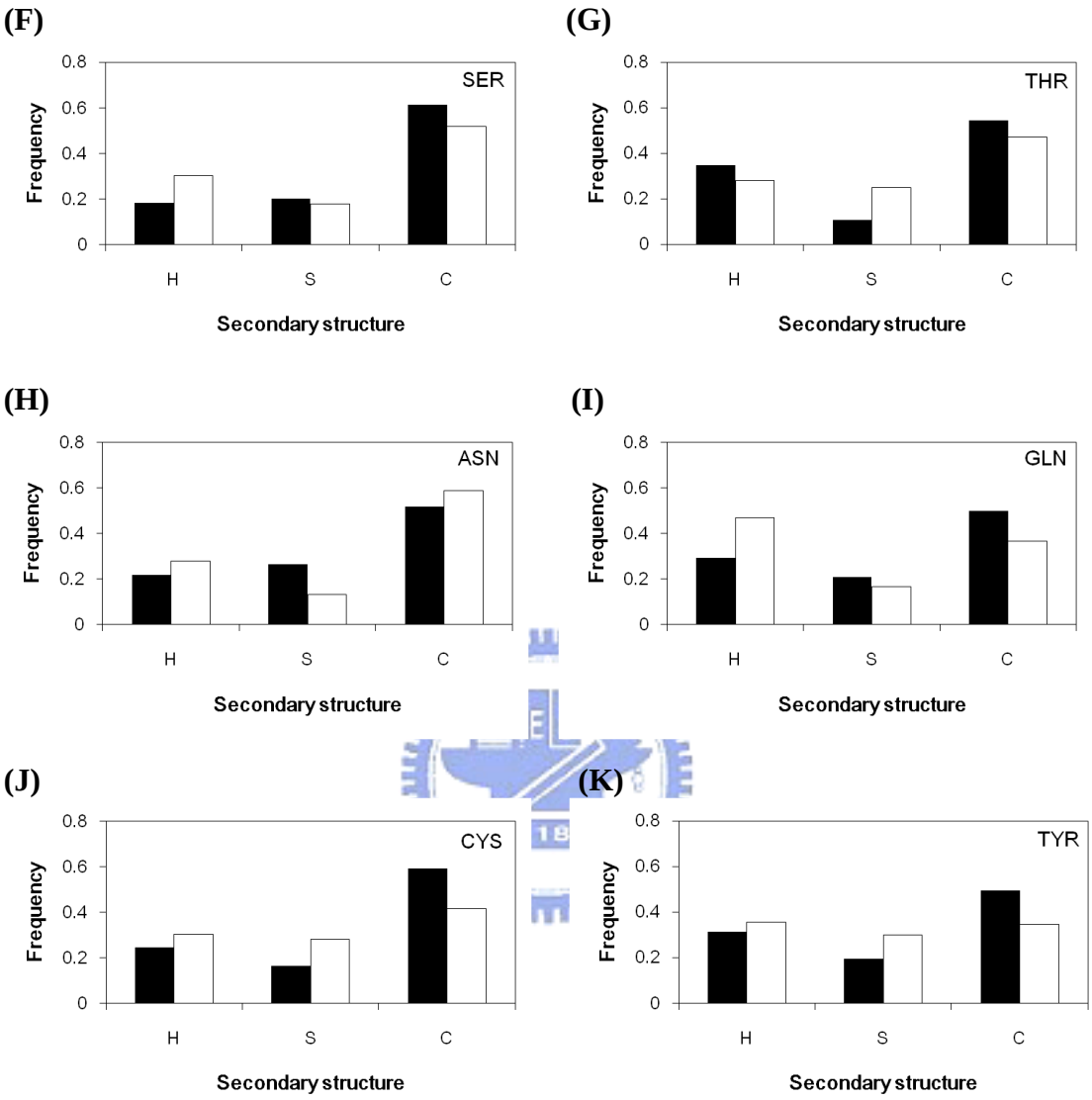


Figure 4. (Continued)

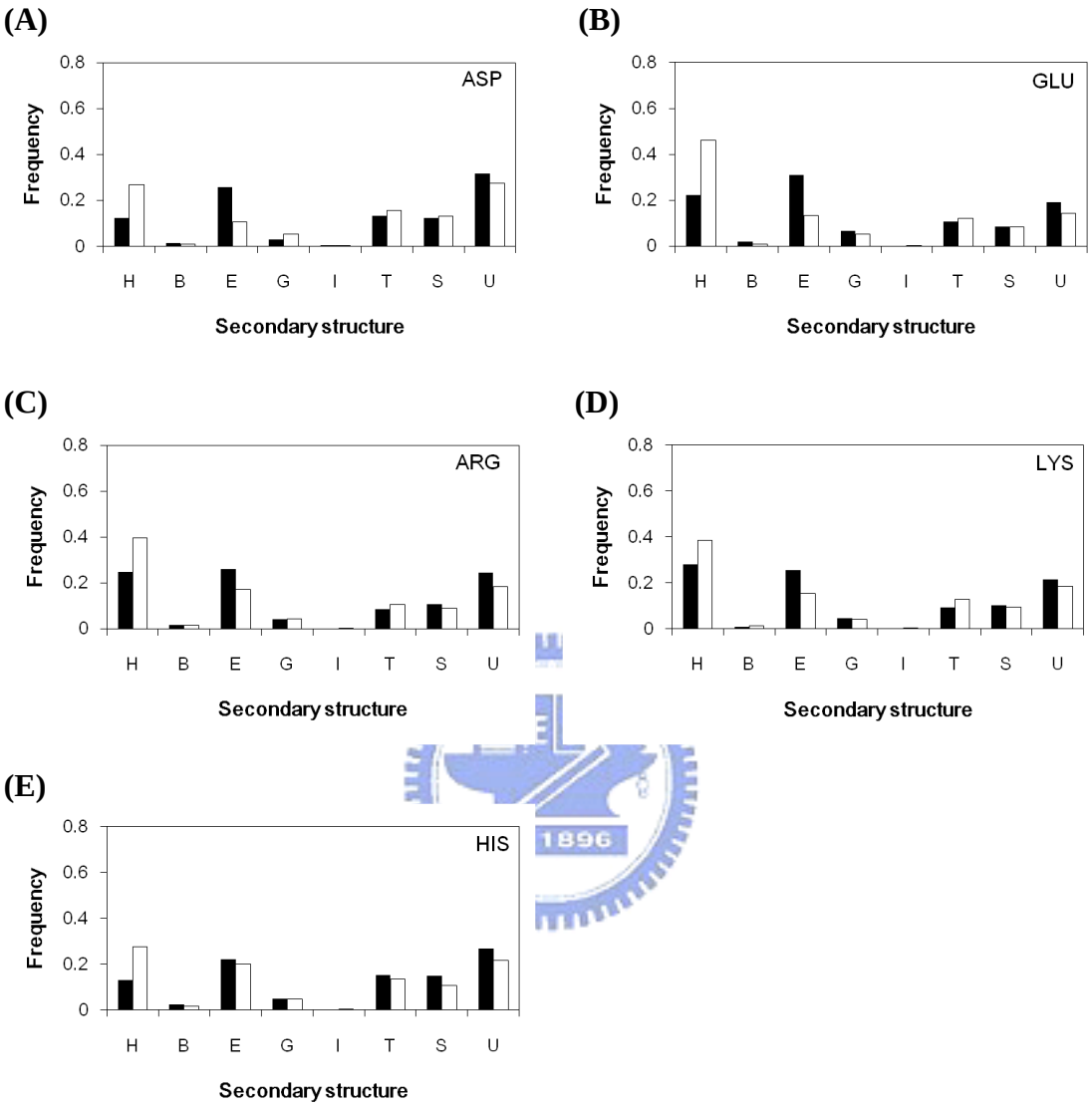


Figure 5. Secondary Structure_α-helix(H), β-bridge(B), β-ladder(E), 3_{10}-helix(G), π-helix(I), hydrogen bonded turn(T), bend(S), and others undefined(U) **for individual catalytic residues (ASP, HIS, GLU, ARG, LYS, SER, TYR, CYS, ASN, THR, GLN) compared with all residues of the same type.**

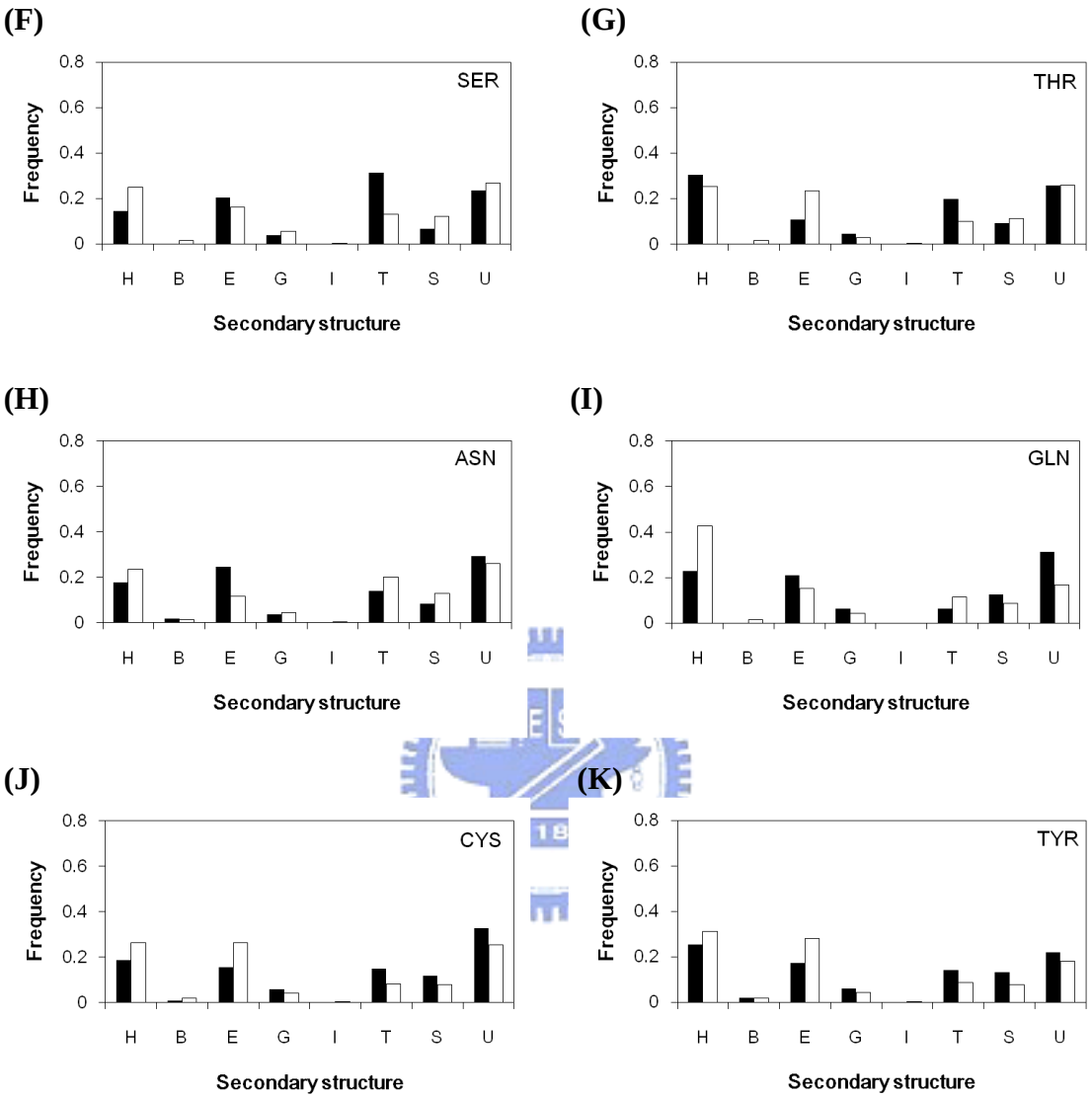


Figure 5. (Continued)

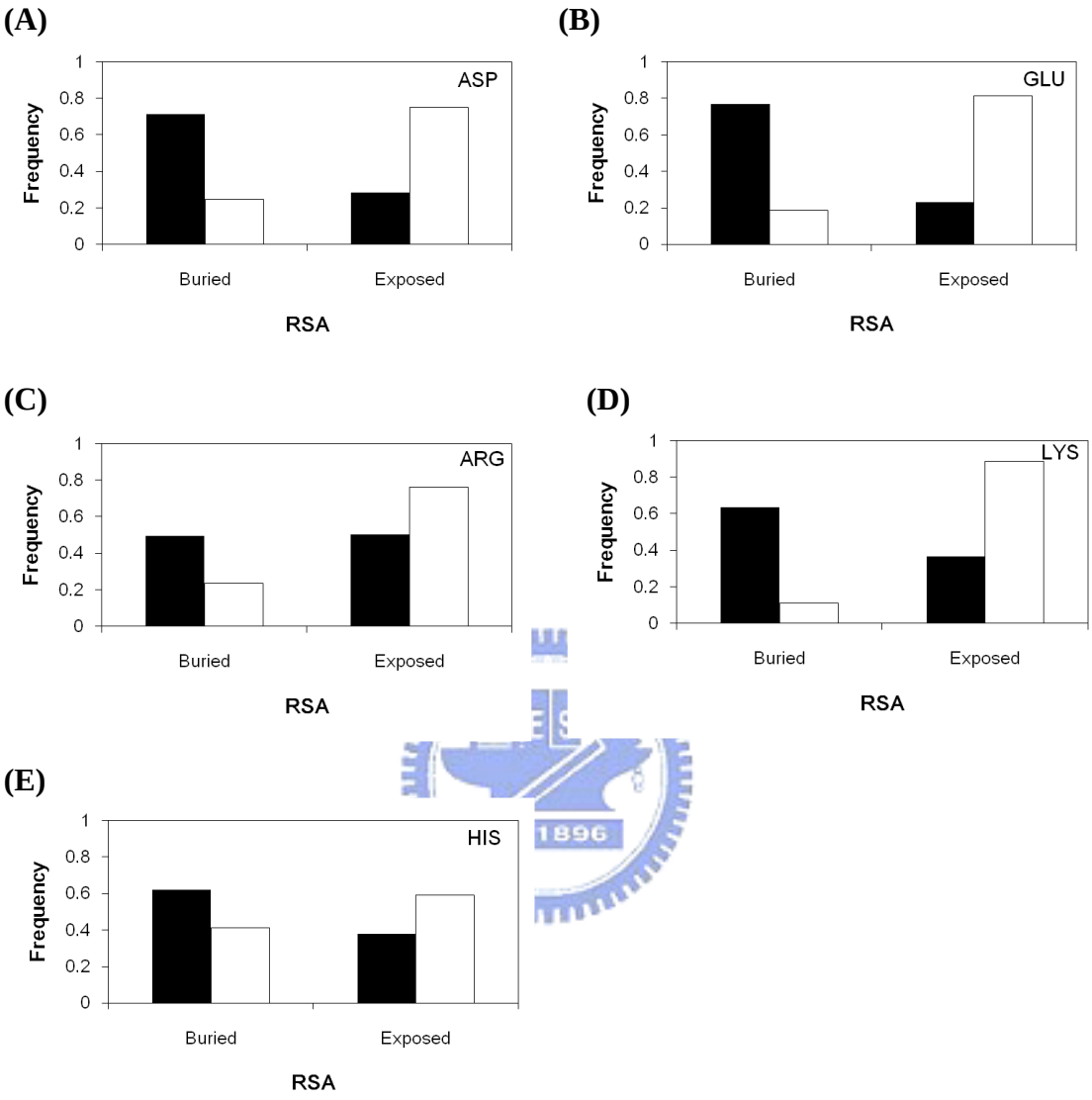


Figure 6. Relative Solvent Accessibility(RSA)_binary model for individual catalytic residues (ASP, HIS, GLU, ARG, LYS, SER, TYR, CYS, ASN, THR, GLN) compared with all residues of the same type.

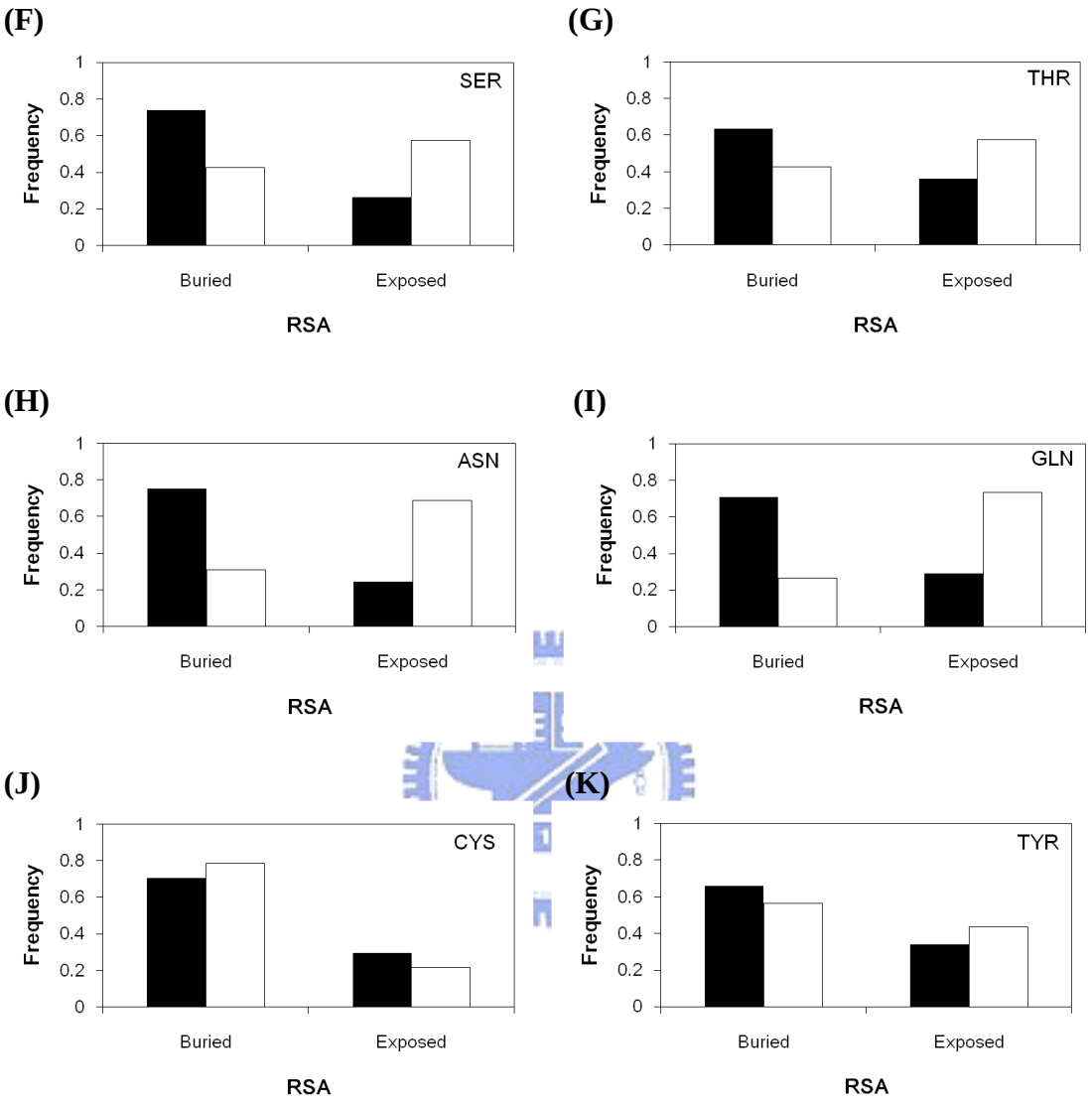


Figure 6. (Continued)

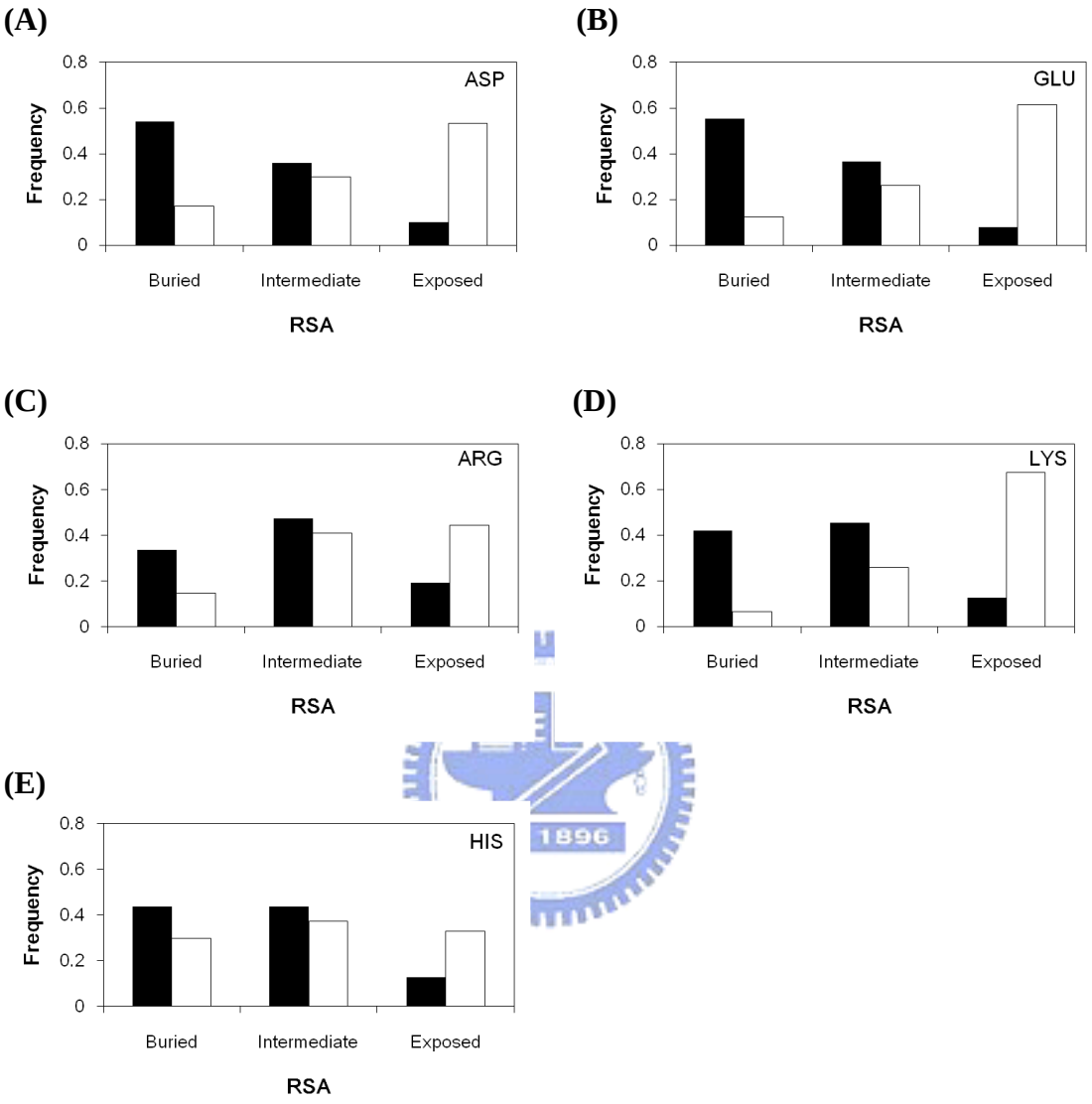


Figure 7. Relative Solvent Accessibility(RSA)_ternary model for individual catalytic residues (ASP, HIS, GLU, ARG, LYS, SER, TYR, CYS, ASN, THR, GLN) compared with all residues of the same type.

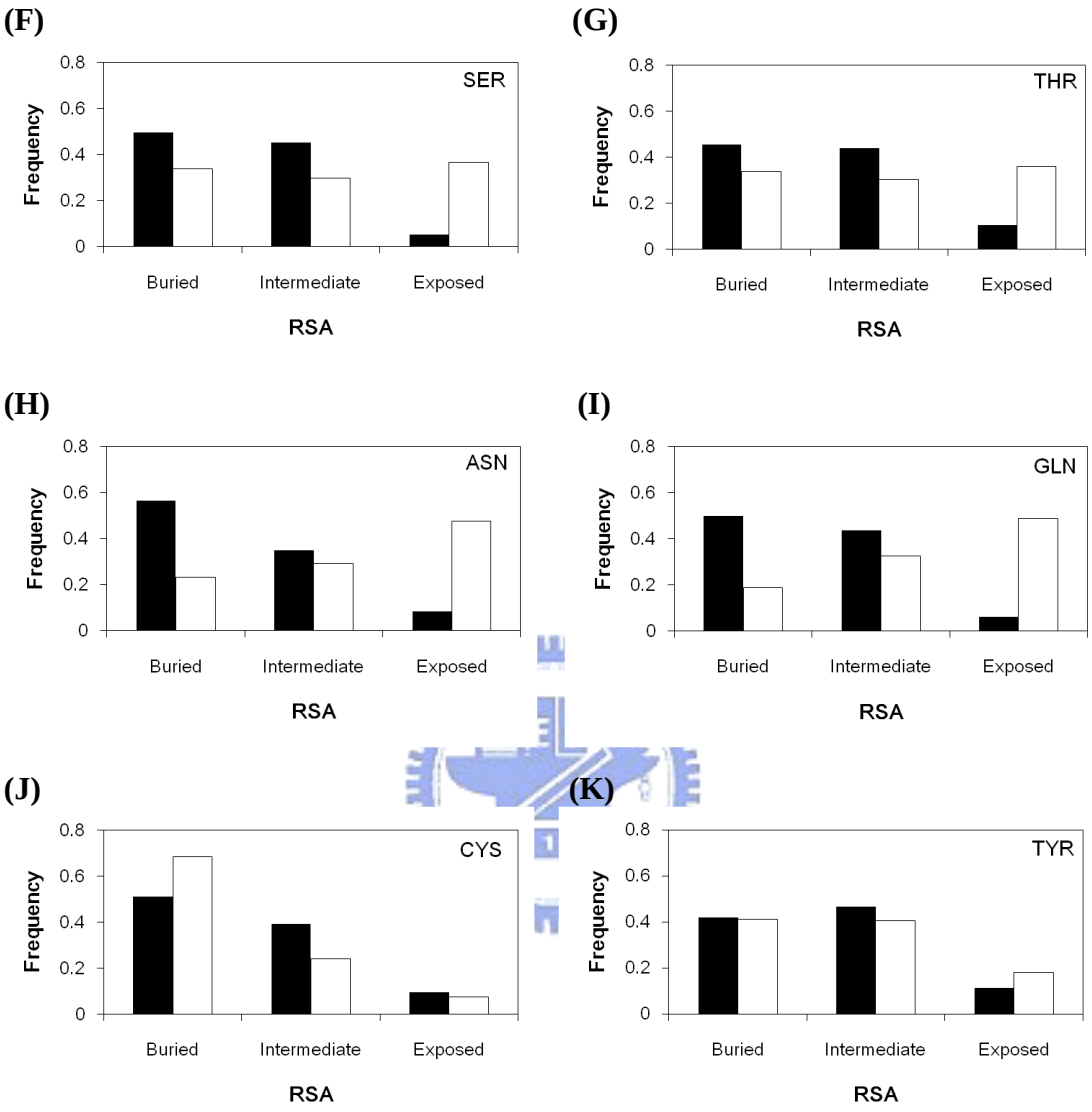


Figure 7. (Continued)

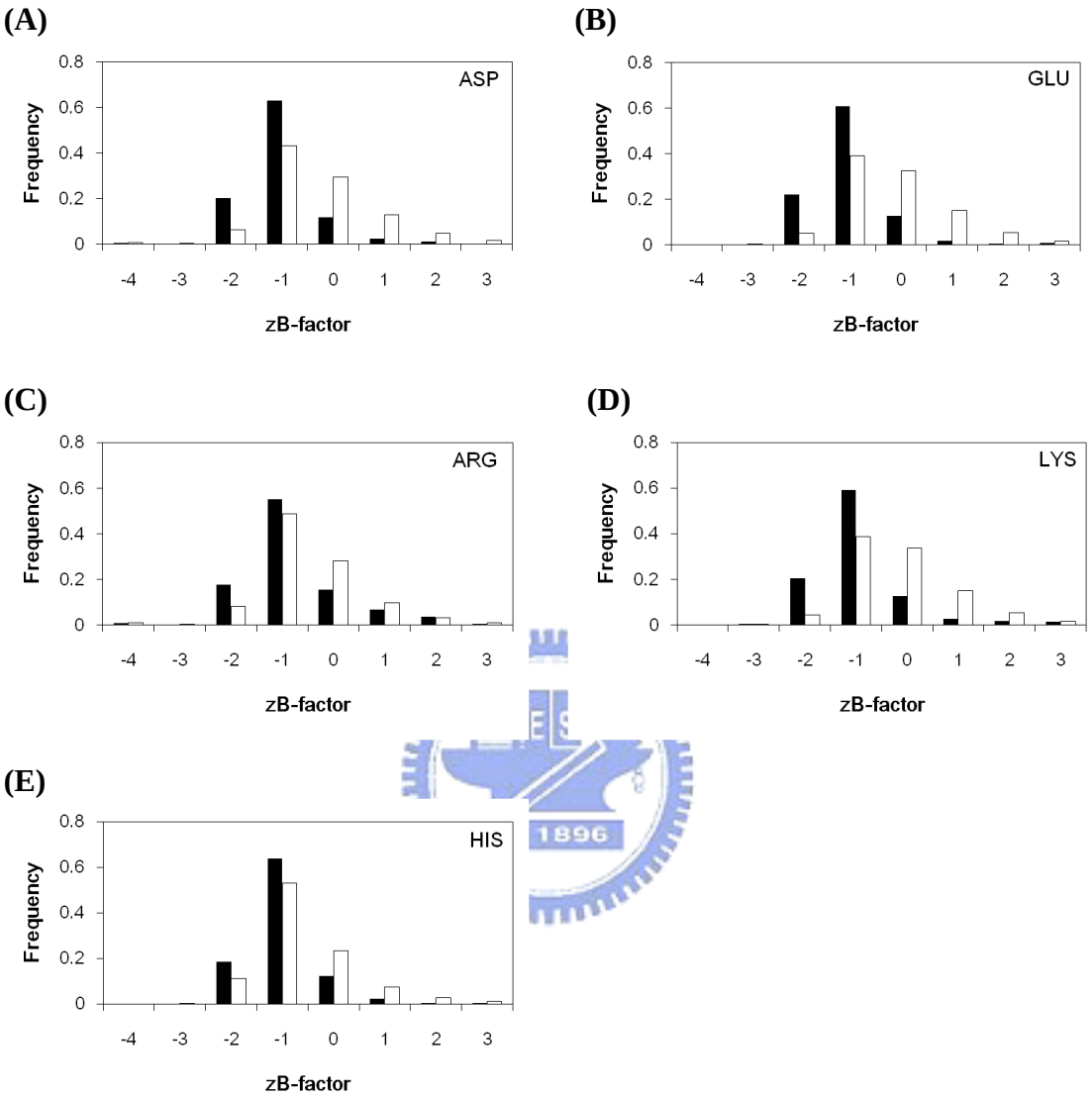


Figure 8. zB-factor for individual catalytic residues (ASP, HIS, GLU, ARG, LYS, SER, TYR, CYS, ASN, THR, GLN) compared with all residues of the same type.

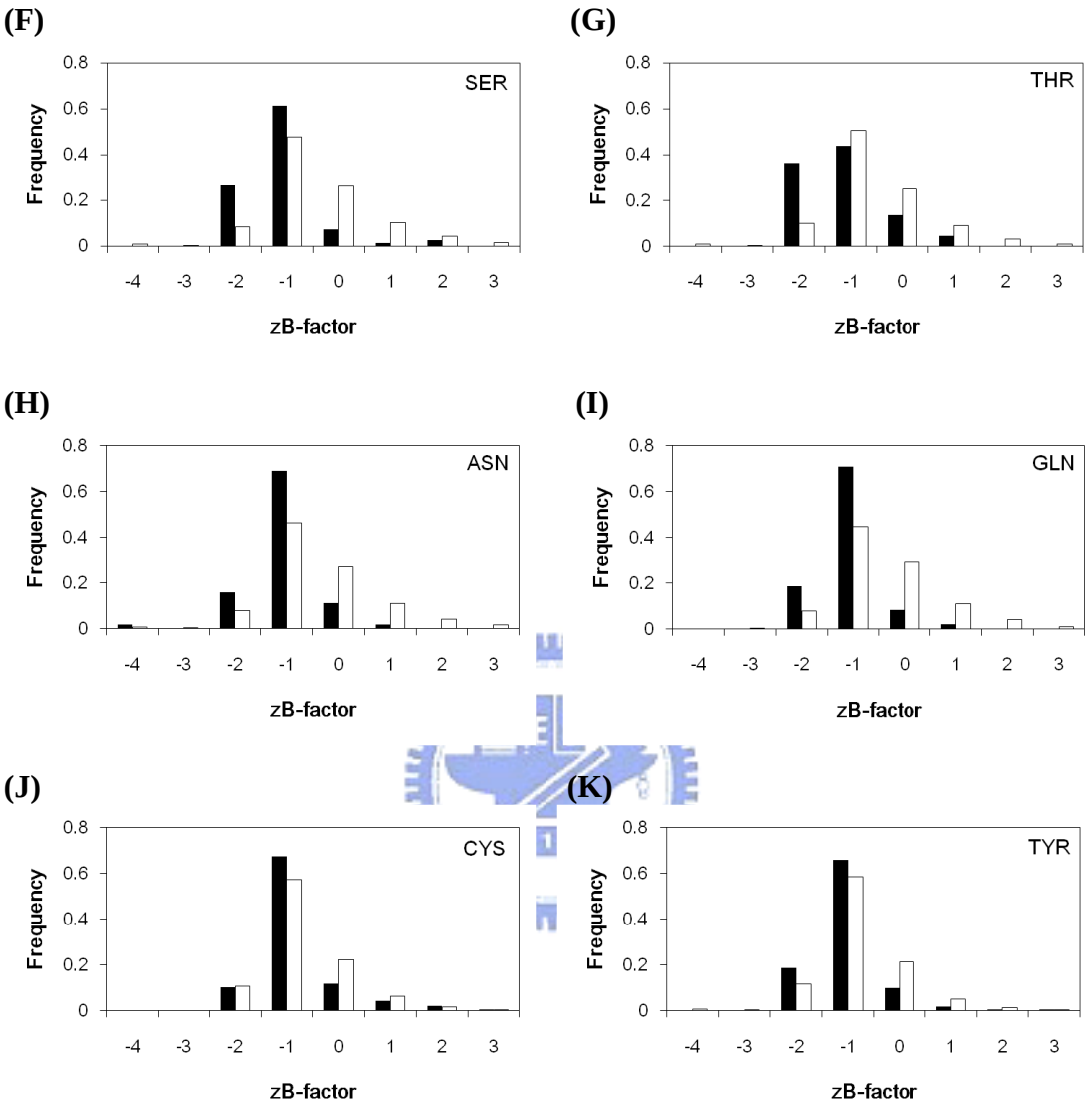


Figure 8.(Continued)

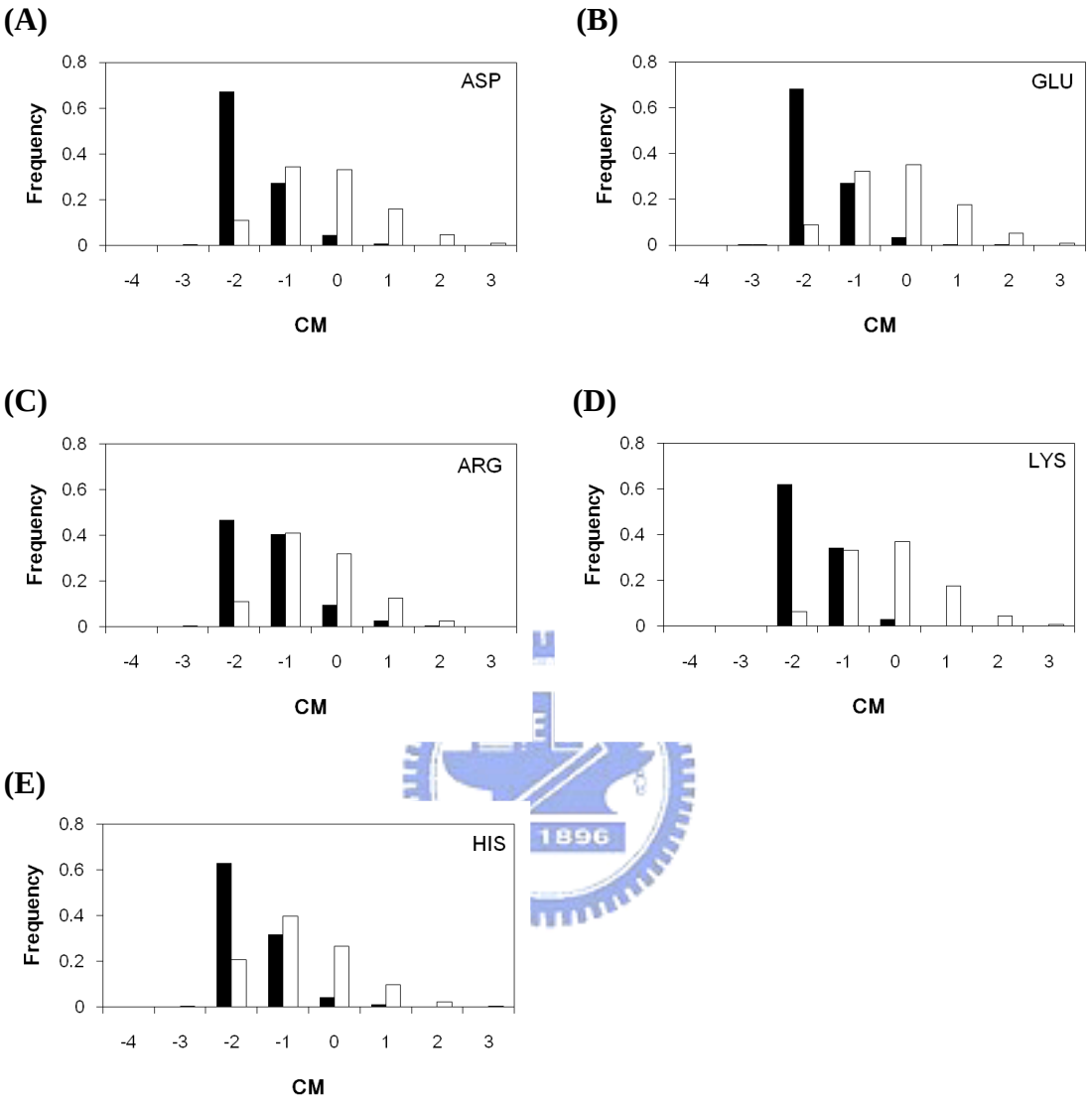


Figure 9. CM for individual catalytic residues (ASP, HIS, GLU, ARG, LYS, SER, TYR, CYS, ASN, THR, GLN) compared with all residues of the same type.

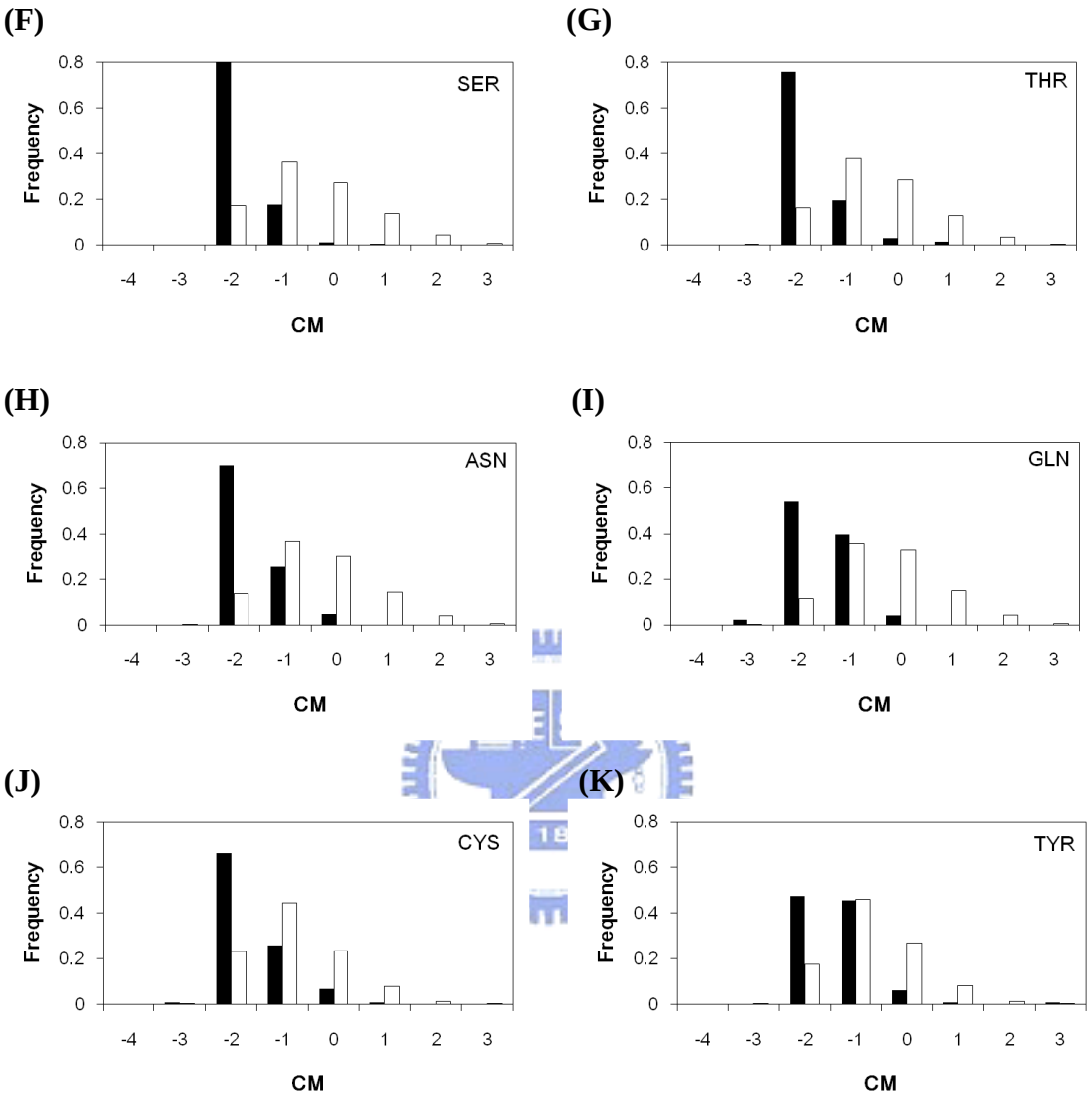


Figure 9. (Continued)

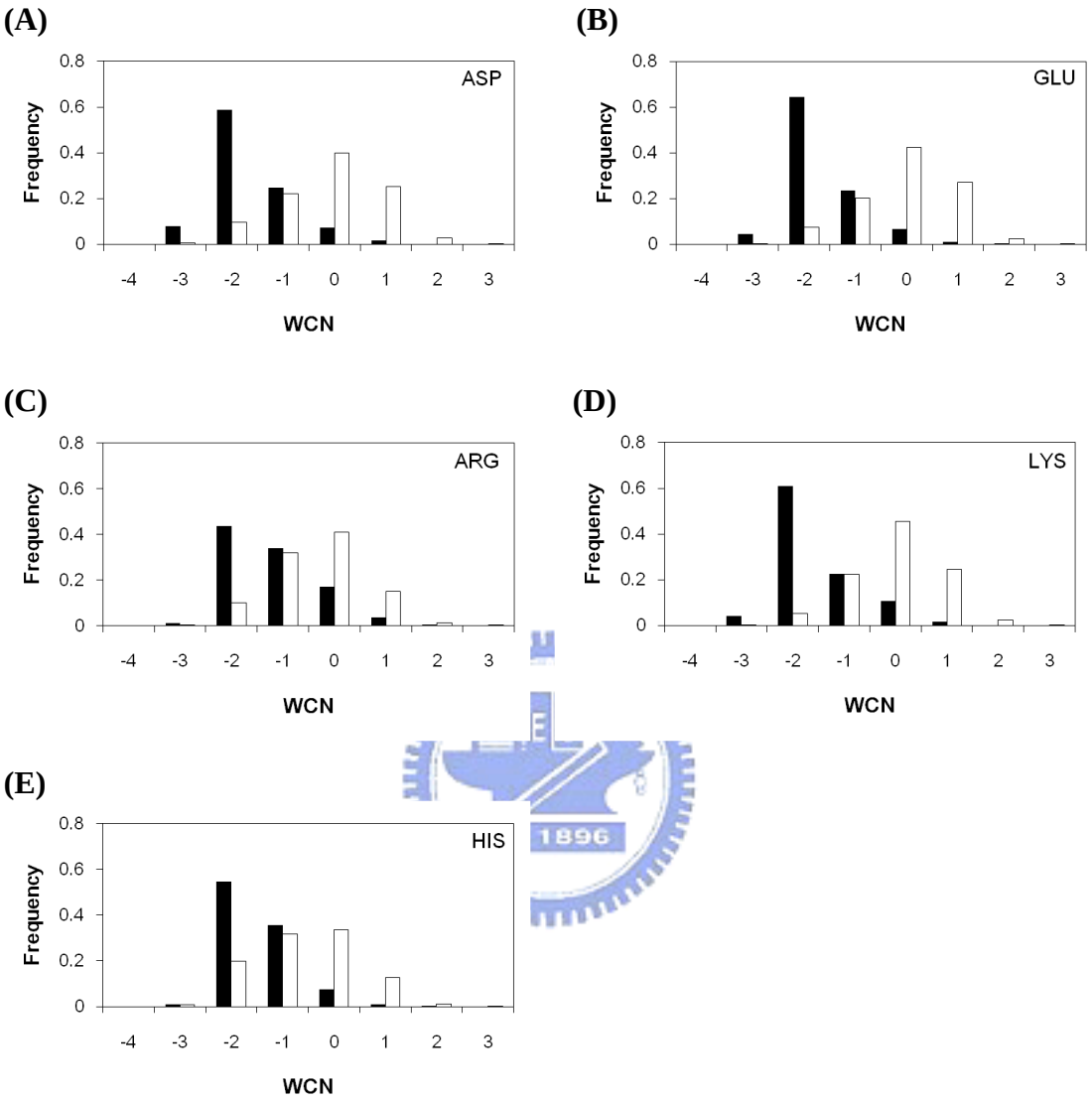


Figure 10. WCN model catalytic residues (ASP, HIS, GLU, ARG, LYS, SER, TYR, CYS, ASN, THR, GLN) compared with all residues of the same type.

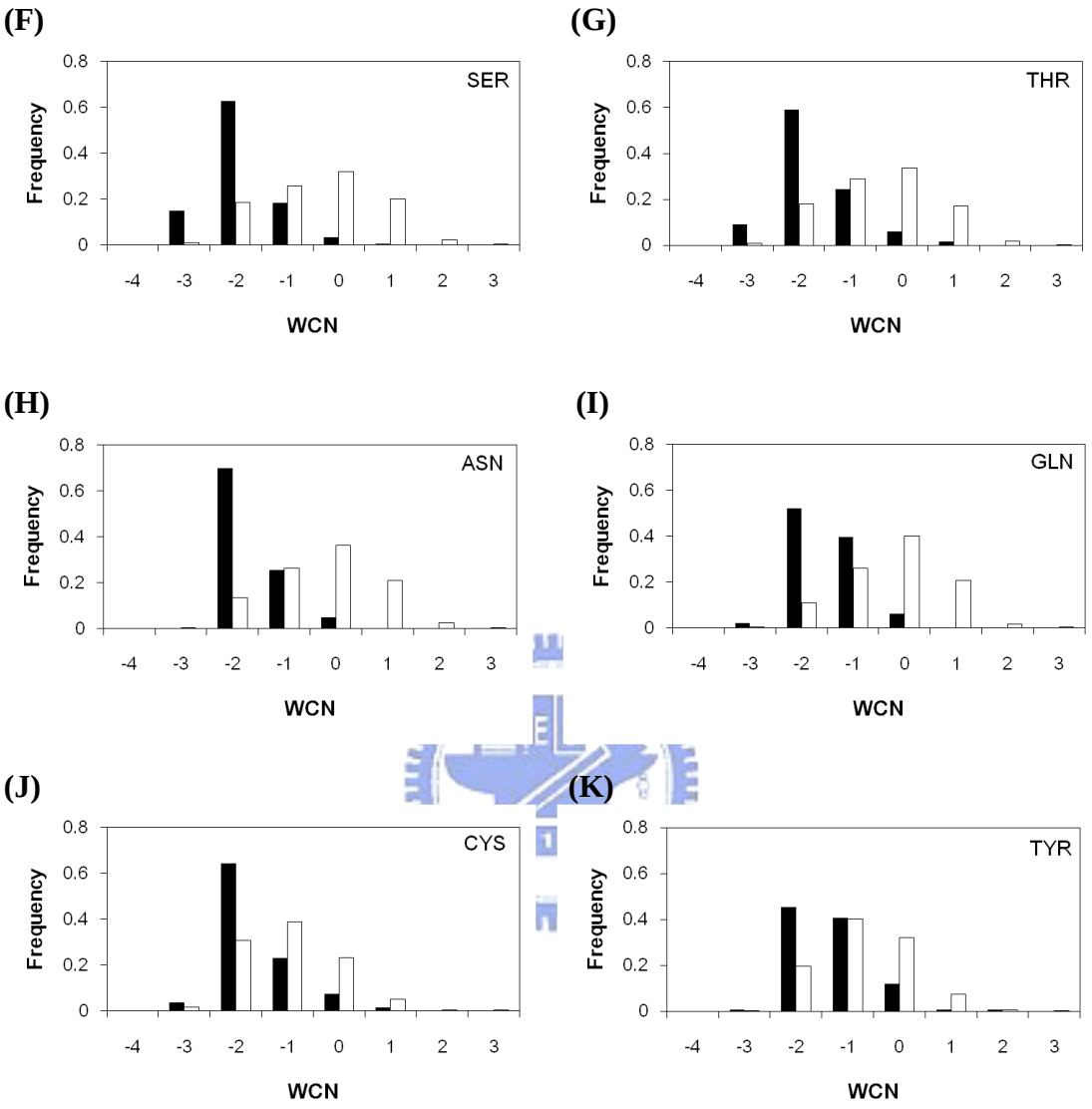


Figure 10. (Continued)

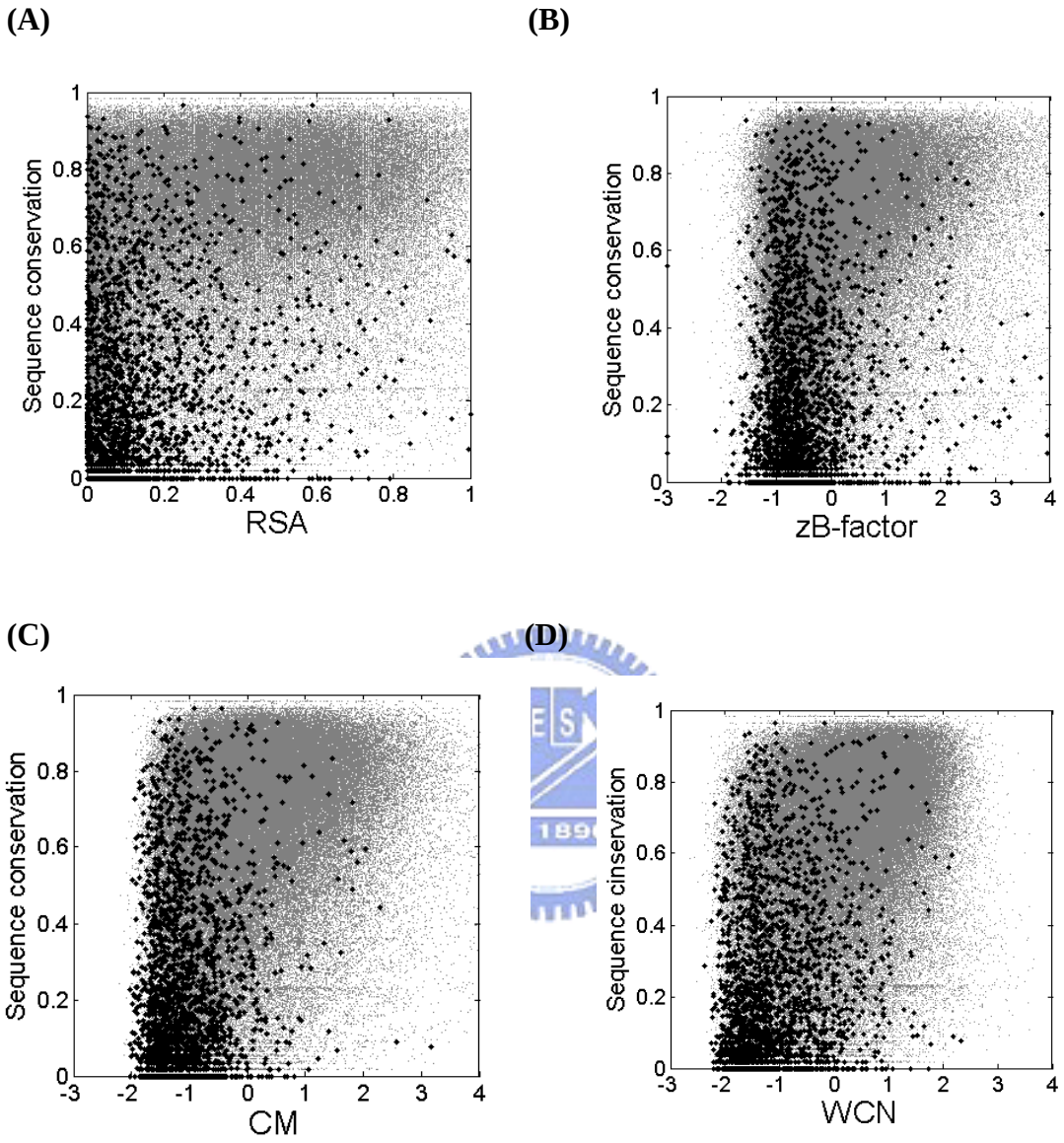
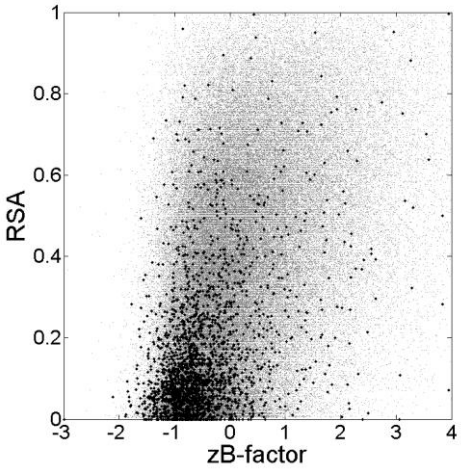
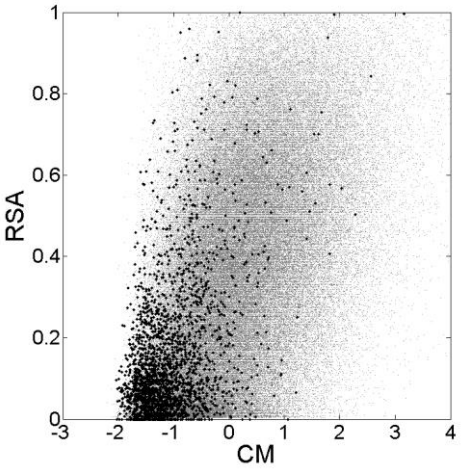


Figure 11. The 2-D profile of catalytic residues (black dots) compare with all residues (grey dots). (A) sequence conservation and relative solvent accessibility properties; (B) sequence conservation and normalized B-factor; (C) sequence conservation and centroid model; (D) sequence conservation and weighted contact number model; (E) relative solvent accessibility properties and normalized B-factor; (F) relative solvent accessibility properties and centroid model; (G) relative solvent accessibility properties and weighted contact number model; (H) normalized B-factor and weighted contact number model; (I) normalized B-factor and centroid model; (J) weighted contact number model and centroid model.

(E)



(F)



(G)

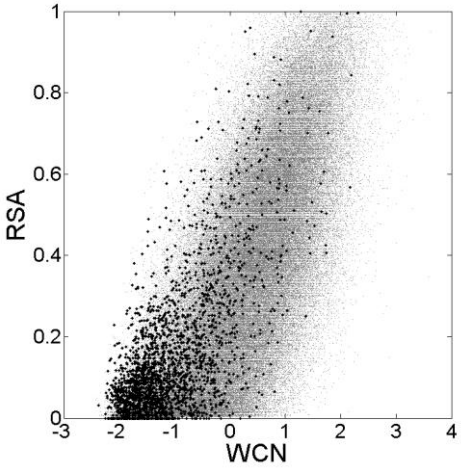
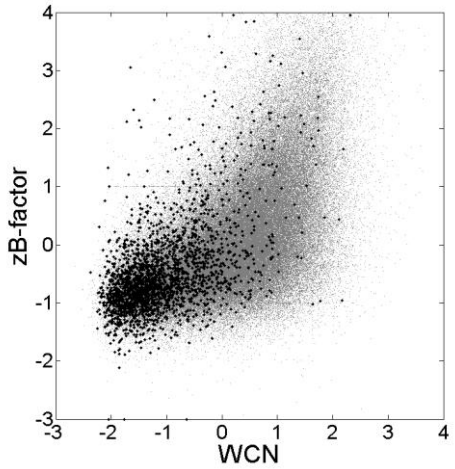
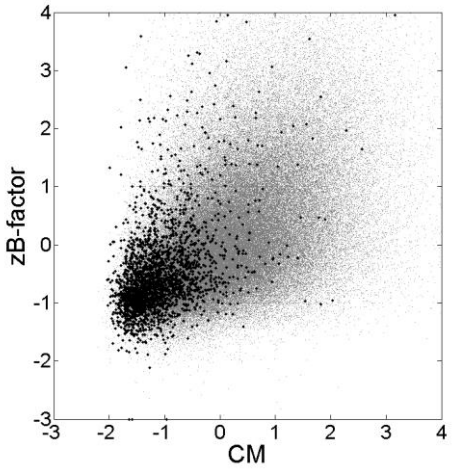


Figure 11. (Continued)

(H)



(I)



(J)

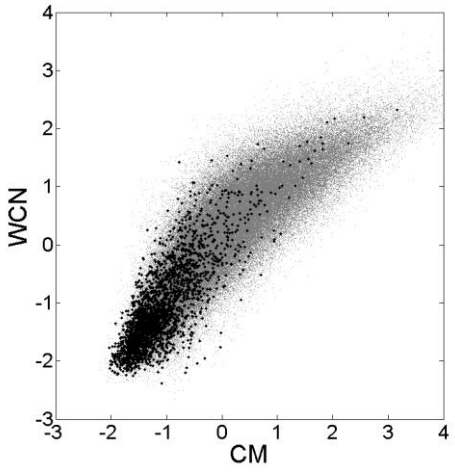


Figure 11. (Continued)

APPENDIX

Appendix I. 887 PDBID[a] IDs

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1a6d_A	1a79_A	1a7u_A	1a8h_A	1a8q_A	1a8s_A	1a95_C	1aa6_A	1aam_A
1ab4_A	1ab8_A	1abr_A	1adn_A	1af7_A	1afr_A	1afw_A	1agm_A	1agy_A
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1alk_A	1am2_A	1am5_A	1amo_A	1amp_A	1amy_A	1aop_A	1apt_E	1apx_A
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1nhx_A	1nid_A	1nir_A	1nkk_A	1nln_A	1nlu_A	1nml_A	1nmw_A	1nn4_A
1nsf_A	1nsj_A	1nsp_A	1nu3_A	1nvm_A	1nvt_A	1nw9_B	1nww_A	1nzy_A
1o04_A	1o98_A	1o9i_A	1oac_A	1oas_A	1oba_A	1odt_C	1oe8_A	1ofd_A
1ofg_A	1og1_A	1ogo_X	1oh9_A	1oj4_A	1ok4_A	1okg_A	1onr_A	1opm_A
1oqz_A	1or8_A	1ord_A	1oro_A	1os7_A	1otg_A	1oxa_A	1oya_A	1oyg_A
1ozh_A	1p1x_A	1p3d_A	1p4n_A	1p4r_A	1p5d_X	1p7m_A	1pa9_A	1pad_A
1pae_X	1pbg_A	1pd2_1	1peg_A	1pfk_A	1pfq_A	1pgs_A	1pii_A	1pj5_A
1pja_A	1pjb_A	1pjh_A	1pjq_A	1pkn_A	1pma_A	1pmi_A	1pnl_B	1pnt_A
1pow_A	1pp4_A	1ps1_A	1ps9_A	1psd_A	1ptd_A	1pud_A	1pvd_A	1pvi_A
1pwh_A	1pwv_A	1pxv_A	1pyl_A	1pym_A	1pz3_A	1q18_A	1q3n_A	1q3q_A
1q6x_A	1q91_A	1qam_A	1qaz_A	1qb4_A	1qba_A	1qcn_A	1qd1_A	1qe3_A
1qf6_A	1qfe_A	1qfl_A	1qfm_A	1qfn_A	1qgn_A	1qgx_A	1qh5_A	1qh9_A
1qhf_A	1qhg_A	1qho_A	1qi9_A	1qib_A	1qj2_B	1qj4_A	1qje_A	1qk2_A

1qlh_A	1qmh_A	1qol_A	1qq5_A	1qrg_A	1qrr_A	1qrz_A	1qsg_A	1qtn_A
1qum_A	1qv0_A	1qwn_A	1qx3_A	1qz9_A	1r16_A	1r1j_A	1r30_A	1r44_A
1r4f_A	1r4z_A	1r6w_A	1r76_A	1ra0_A	1ra2_A	1rba_A	1rbl_A	1rbn_A
1rdd_A	1req_A	1rgq_A	1rhc_A	1rhs_A	1rk2_A	1rne_A	1ro7_A	1roz_A
1rpt_A	1rpx_A	1rql_A	1rtf_B	1rtu_A	1ru4_A	1rvv_A	1s20_A	1s2k_A
1s3i_A	1s76_D	1s95_A	1s9c_A	1sca_A	1ses_B	1sll_A	1slm_A	1sme_A
1sml_A	1smn_A	1snn_A	1snz_A	1sox_A	1ssx_A	1stc_E	1std_A	1szd_A
1szj_G	1t0u_A	1t7d_A	1tah_A	1tde_A	1tdj_A	1teh_A	1thg_A	1tht_A
1ti6_A	1tlp_E	1tml_A	1tmo_A	1tox_A	1tph_1	1trk_A	1tyf_A	1tys_A
1tz3_A	1u3f_A	1u5u_A	1u7u_A	1u8v_A	1uae_A	1uag_A	1uam_A	1uaq_A
1uas_A	1uch_A	1uf7_A	1uk7_A	1ula_A	1un1_A	1uok_A	1uox_A	1uqr_A
1uqt_A	1uro_A	1ush_A	1uw8_A	1v04_A	1v0e_A	1v0y_A	1v25_A	1vao_A
1vas_A	1vid_A	1vie_A	1vlb_A	1vnc_A	1vom_A	1vq1_A	1vr7_A	1vzx_A
1vzz_A	1w0h_A	1w1o_A	1w2n_A	1wd8_A	1wgi_A	1wnw_A	1x7d_A	1x9h_A
1x9y_A	1xa8_A	1xgm_A	1xik_A	1xqd_A	1xqw_A	1xrs_B	1xtc_A	1xva_A
1xvt_A	1xyz_A	1y9m_A	1ybq_A	1ybv_A	1ycf_A	1ygh_A	1ylu_A	1yon_A
1ysc_A	1ytw_A	1yve_I	1ze1_A	1zio_A	1zm2_B	1znv_B	1zoi_A	1zrz_A
1zym_A	206l_A	2a0n_A	2a86_A	2aat_A	2abk_A	2ace_A	2acu_A	2acy_A
2adm_A	2alr_A	2amg_A	2apr_A	2ayh_A	2b3i_A	2bbk_L	2bhg_A	2bif_A
2bkr_A	2blt_A	2bmi_A	2bsx_A	2bx4_A	2c7v_A	2cnd_A	2cpo_A	2cpu_A
2dbt_A	2dhn_A	2dln_A	2dor_A	2dw7_A	2ebn_A	2eng_A	2eql_A	2esd_A
2f61_A	2f9r_A	2f9z_C	2fmn_A	2fok_A	2gsa_A	2hdh_A	2his_A	2hsa_A
2isd_A	2jcw_A	2jxr_A	2lip_A	2lpr_A	2nac_A	2nlr_A	2npx_A	2oat_A
2pda_A	2pec_A	2pfl_A	2pgd_A	2phk_A	2pia_A	2plc_A	2pth_A	2qf7_A
2rnf_A	2tdt_A	2thi_A	2tmd_A	2toh_A	2tpl_A	2tps_A	2ts1_A	2xis_A
2ypn_A	3cla_A	3csm_A	3eca_A	3mdd_A	3nos_A	3pca_M	3pva_A	3r1r_A
4kbp_A	4mdh_A	5cox_A	5cpa_A	5eat_A	5enl_A	5fit_A	5rsa_A	7atj_A
7nn9_A	7odc_A	8pch_A	8tln_E	9pap_A				

[a]12as_A means the A chain of protein PDBID 12as