國　立　交　通　大　學

生物資訊及系統生物研究所

碩士論文

分析纖維酵素蛋白質結構了解內切及外切纖維酵素

結合位置

Analysis of the cellulase protein structure to understanding the binding site of endo- and exo-glucanase

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摘要

纖維素為地球存在量最豐富的有機物並且在工商業產品用途上扮演重要的角色。然而纖維分解酵素是一群水解酵素，可將不具溶解性纖維素的β-1, 4鍵水解而分解成單糖。近年纖維分解酵素陸續依蛋白質序列相似度被分成118個家族，主要的酵素包括內切型纖維素分解酵素（endo-β-1, 4-glucanase) EC 3.2.1.4和外切型纖維素分解酵素（exo-β-1, 4-glucanase ）EC 3.2.1.91。這兩類酵素若在同家族中作用機制和三級結構上據有相似性。到目前為止，被發現的纖維素分解酵素大都被分離且純化，且除了酵素的氨基酸序列，生化特性和作用機轉大部份已被分析討論出來，但卻還沒有可以利用三級結構來分辨內切型級外切型纖維素分解酵素的方法。所以為了達成辨認纖維素分解酵素的目的，我們思考是否能利用結合位置來作區分的橋梁，以至於必須先分析纖維素分解酵素結合位置。我們應用了已被開發的方法“蛋白質接觸數目模型”來分析是否能預測酵素的結合位置，然而我們的結果提供了利用蛋白質接觸數目模型對於預測結合位置是有幫助的。更進一步而言，我們也許在未來預測結合位置上提供發展新而有效方法並朝著能辨別纖維素分解酵素的目的。

Analysis of the cellulase protein structure to understanding the binding site of endo- and exo-glucanase

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Abstract

Cellulose is a well-known biomass energy and plays an important role due to its abundance source to be used for producing commercially industrial products in the world. However, cellulase (Glycoside hydrolases) is referring to a class of enzymes that hydrolyses the β-1, 4-glycosidic linkages of cellulose. And it has already been classified more than 118 families on amino acid sequence similarities that have been proposed so far, and traditionally divided into two classes denominated "endo-glucanase" (EC 3.2.1.4) and "exo-glucanase" (EC 3.2.1.91). Both of them utilize the same catalytic mechanisms, and have overall similar structures. In spite of considerable structure of cellulase − function information, mechanistic studies, and catalytic site simulations seems to be well understood in recent works, there is still way to distinguish the structures between endo- and exo-glucanase being researched up to now. Therefore, in order to identify the structures of cellulase, we apply a computational method that had been developed, WCN (Weighted Contact Number) model to analyze the dynamic properties of cellulase. It means that the atom's thermal fluctuations are in reverse proportion to the protein contact number of this atom, and a residue with lower flexibility is also more compact in proteins. Thus, through WCN model, would be helpful in binding site prediction. In summary, the fundamental

purpose in this work is to realize and analyze binding site of cellulase then maybe we can recognize endo-glucanase and exo-glucanase in further. Our results provide information of cellulase protein binding site prediction. Furthermore, we can get more useful and powerful studies by using the method that we applied in the future.

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1. Introduction

Annually, plants produce about 180 billion tons of cellulose making it the largest reservoir of organic carbon on Earth.[1] Owing to the population growth goes with energy crisis that there is not infinite and sufficient resources in our planet progressively, the idea that utilize and convert cellulose effectively into renewable biomass resources for the production of alternative fuels has become significant and attractive. Cellulose itself is a very simple polymer of glucose units joined together by β-1, 4-glycosidic linkages.[2] Coupling of adjacent cellulose molecules by hydrogen bonds and van der Waal's forces results in a parallel alignment and a crystalline structure in order to form homopolysaccharide.[3] Two different ending groups are found in each cellulose chain edge. At one end of each chains, a non-reducing group is show where a closed ring structure. A reducing group with both an aliphatic structure and a carbonyl group is at the other end of the chains. And according to the difference of hydrogen bond pattern, there are two forms discriminated from cellulose: amorphous domain and crystalline domain. The natural consequence of this difference in the crystalline structure is that the hydrolysis rate is much faster for amorphous cellulose than crystalline one.

Cellulase, hydrolysis of cellulose requires the co-operative actions of three classes, namely endo-β-1, 4-glucanases (EC 3.2.1.4), exo-β-1, 4-glucanases (EC 3.2.1.91) and β-glucosidases (EC 3.2.1.21). The CAZy (carbohydrate active enzymes) classification system collates glycosyl hydrolase (GH) enzymes into families according to sequence similarity, which have been shown to reflect shared structural features.[4] Endo-glucansaes cut randomly at internal amorphous sites in the cellulose polysaccharide chain, generating oligosaccharides of various lengths and

consequently new chain ends. Exo-glucanase act in a processive manner on the reducing or nonreducing ends of cellulose polysaccharide chains, liberating either glucose or cellobiose as major product. And β -glucosidases hydrolyze soluble cellobiose to glucose.[5]

According to the classification that allows the identification of cellulases with common folds and a similar catalytic mechanism within the same family, a rational method of telling cellulase endo-glucanases and exo-glucanases from structures isn't established yet. However before accomplish this hard work, the main issue of our present work is to understand the cellulases protein structures and their binding site. We apply the method, WCN (Weighted Contact Number) model, which may provide more information from the binding site of cellulase. Due to a recent study shows that the protein B-factor of the atom is linearly proportional to its squared distance from the protein centroid. [6,7] In other words, the residues in proximity to the protein centroid will have lower thermal fluctuation or more rigid than those further residues. Besides, the atom's thermal fluctuations are linear inverse proportion to the protein contact number of this atom, it account for that a residue has lower flexibility also more compact in structure.[8] Moreover, we add the filter Relative Solvent Accessibility (RSA) to increase the degree of accuracy. Therefore, binding sites may be able to be confirmed through the characteristic information above mentioned. Consequently, in further work, the purpose we would like to deal with is that whether endo-glucanases and exo-glucanases could be distinguished from their binding site or not, and that would be an interesting issue and great challenge to carry out.

2. Material and Methods

2.1 Dataset of cellulase proteins

The enzyme of cellulase was taken from the National Center for Biotechnology Information (NCBI), we search all endo-glucanases (EC 3.2.1.4) and exo-glucanases (EC 3.2.1.91) by their EC number. There are 154 endo-glucanase proteins, 65 exo-glucanases, and the grand total number of enzyme we obtained is 219, the detail was shown in Table 1, 2. However, we only selected 4 proteins that have their own active site residues literature information from the Catalytic Site Atlas (CSA)-2.2.10 and binding site residues for each glucanases, the list of our dataset informatin was shown in Table 3. [2,9-14]

2.2 Comprehend characteristics of cellulase sturcture

Cellulose is a nature polymer composed of repeating glucose units, and each glucose unit is rotated 180° relative to its neighbors along the main axis.[15] Cellulose exits in a highly crystalline form, therefore, hydrolysis of cellulose requires co-operative activities of three classes of enzymes:

i. Endo-glucanase or 1,4-β-D-glucanhydrolase (EC 3.2.1.4)

ii. Exo-glucanase or 1,4-β-D-glucan cellobiohydrolases (EC 3.2.1.91)

iii. β-glucosidases or β-glucoside glucohydrolases (EC 3.2.1.21)

The structures of endo-glucanases are commonly characterized by a groove or a cleft to bind a linear cellulose chain in order to fit in a random manner at amorphous sites. Generally, exo-glucanases or cellobiohydrolases (CBH) possess tunnels-like active sites, which can only accept a substrate chain via its terminal regions.[16] These tunnels proved to be essential to the cellobiohydrolases for the processive cleavage of cellulose chains from the reducing or nonreducing ends. The Cellulose degradation flow is shown in Figure 1.[17]

2.3 Analysis and Classification of binding sites

2.3.1 Amino acid type

Different amino acids apparently have various propensities to be binding site residues. Binding site residues are classified according to the 20 standard amino acid one letter abbreviation from hydrophobic group, hydrophilic one to Charged type as follows, i.e., G, A, V, L, I, M, P, F, W, Y, C, S, T, N, Q, D, E, H, R and K.

2.3.2 Weighted contact number model (WCN)

It has recently been shown that in proteins the atomic mean-square displacement (or B-factor) can be related to the number of the neighboring atoms (or protein contact number) and the square distance from the center of mass of a protein.[18] Here, we will refer this method as the 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 $1/r_{ij}$ which is the distance between Cα atoms of i and j residues.

$$v_i = \sum_{j \neq 1}^{N} \frac{1}{r_{ij}^2} \qquad (1)$$

where N is total residue numbers of the protein, and we refer is as the weighted CN model (WCN). The CN (or WCN) profile of protein of *N* residues is defined as

$$w = (\omega_1, \omega_2, \ldots \omega_N) \tag{2}$$

where ω_1 is defined as the reciprocal contact number, i. e., $\omega_i = 1/\nu_i$.

For the purpose of easy comparison, we will normalize ν_i to its Z-scores:

$$z_{xi} = \left(x_i - \bar{x}\right)/\sigma_x \tag{3}$$

where ν_i and σ_x are the mean and the standard deviation of x. Here x designates ω.

2.3.3 Relative Solvent Accessibility (RSA)

The surface area is an important structure characteristic in binding a non-protein molecule (such as the substrate or cofactor) and in protein-protein complexes interaction.[19] Thus, the binding site 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

$$\mathrm{Re}\,lAcc(\%) = \frac{100 \times Acc}{MaxAcc(\%)} \tag{4}$$

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] A residue is considered as accessible if its relative accessible

surface area (RSA) ≥ 5%, a cut-off devised and optimized by Miller et al.[21] If a residue is accessible in the protomer it is in the protein surface, otherwise it is core. Re$lAcc$i < 5% means Buried, ≥ 5% means Exposed. Therefore, the thresholds that we selected are the same as those in Rost and Miller.[20,21]

2.3.4 Performance measures

The performance measurements of sensitivity and specificity are measured by true positive rate (TPR) and false positive rate (FPR). The TPR is given by

$$TPR = \frac{TP}{TP + FN} \tag{5}$$

And the FPR is given by

$$FPR = \frac{FP}{FP + TN} \tag{6}$$

Where TP is the number of true positives, TN is the number of true negatives, FP is the number of false positives, and FN is the number of false negatives. The sensitivity value is equal to TPR, and the specificity value is given by

$$Specificity = 1 - FPR \tag{6}$$

In order to reconfirm the WCN (z-score) threshold to predict binding site, our calculation is in the process of drawing a diagram of TPR and FPR. For testing case, the WCN (z-score) outputs the probabilities of endo-glucanase and exo-glucanase. Consequently, the decision threshold we selected for endo-glucanase is less than -0.5,

for exo-glucanase is smaller then -0.8, thus the class with higher specificity is the predicted of the WCN.

3. Results and Discussion

3.1 The dataset

We analyzed the WCN (z-score) distribution of cellulase binding site residues that we can find from literature. Figure 2 shows the frequency of endo-glucanase binding residues (black) compared exo-glucanase binding residues (white). From the distribution, we can see WCN (z-score) of cellulase that most of the binding residues are between -1.6 ~ 0.9. After that, making statistics to measure sensitivity and specificity in order to decide the proper WCN threshold to predict binding site as follow.

3.2 The prediction performance

In this statistic, we calculate various WCN z- score threshold values to verify the sensitivity and specificity with respect to each threshold value. Besides, the threshold ranges from -1.6 to 0.9, increasing by 0.1 each time. If the residues we selected under threshold and also match the literature binding site residues, it is considered as “positive”; otherwise, it is considered as “negative”. Therefore each threshold value will produce a group of TPR and FPR, which decides a point on the diagram in Figure 3 and the list in Table 5. Figure 3(A)(B)(C) from top to the bottom shows all dataset cellulase diagram, endo-glucanase group diagram and exo-glucanase group diagram. Based on the measurement of sensitivity, specificity and the diagram

of a relationship TPR and FPR, we decide the suitable WCN z- score binding site threshold for endo-glucanases is < – 0.5 and for exo-glucanase is < -0.8.

3.3 Comprehend analysis of endo- and exo-glucanases

Despite the good prediction performance of WCN model, the sensitivity and specificity for predicting cellulase binding sites are need to improve, so we add a filter RSA to raise the values of specificity. The RSA threshold we selected (≥ 5%) based on Rost and Miller.[20,21]

3.3.1 Endo-glucanases

In this study, the endo-glucaase dataset we selected is as follows, PDB id 1TML, 2ENG, 1JS4 and 2NLR. The Figure 4(A) shows the WCN model of enzyme 1TML structure, Figure 4(B) shows the WCN z- score distribution of 1TML, Figure 4(C) compares Figure (D)(E) shows the experimental binding site residues colored in red, the residues under the WCN threshold (< -0.5) colored in orange and then selected residues that are exposed colored in orange, all of them are surface form and Figure (F) compares Figure (G)(H) shows the cartoon protein structure form, Figure 4(E)(H) means that we pick the residues that conform to WCN and RSA at the same time. And the comparison method WCN with WCN included RSA of sensitivity and specificity is shown in Table 6. Figure 5(A) to (H) shows the information of enzyme 2ENG structure like as Figure 4, the comparison method WCN with WCN included RSA of sensitivity and specificity is shown in Table 7. Figure 6(A) to (H) shows the information of enzyme 1JS4 structure like as Figure 4 enzyme 1TML, the comparison

method WCN with WCN included RSA of sensitivity and specificity is shown in Table 8. Figure 7 (A) to (H) shows the information of enzyme 2NLR structure like as Figure 4 enzyme 1TML, the comparison profile of endo-glucanase 2NLR method WCN with WCN included RSA of sensitivity and specificity is shown in Table 9. Above-mentioned the relationship of performance, we combine two methods WCN and WCN include RSA, we can figure out the residues under WCN z-score threshold of enzymes we selected are much more than the method include RSA, although the sensitivity value will decrease, however we can lower the false positive value and enhance the true negative value then our specificity value will increase much more. It is clear that the binding site residues tend to have lower WCN z-score value and exposed according to our comparison with performance profile results.

3.3.2 Exo-glucanases

In this study next to the endo-glucanases, the exo-glucaase dataset we selected is as follows, PDB id 1CEL, 1QK2, 2HIS and 1EXP. Because of the enzyme 2HIS and 1EXP are in the same family, we select 1EXP for discussing only. The Figure 8(A) shows the WCN model of enzyme 1CEL structure, Figure 8(B) shows the WCN z- score distribution of 1CEL, Figure 8(C) compares Figure (D)(E) shows the experimental binding site residues colored in red, the residues under the WCN threshold (< -0.8) colored in orange and then selected residues that are exposed colored in orange, all of them are cartoon form, Figure 8(D)(E) means that we pick the residues that conform to WCN and RSA at the same time. And the comparison method WCN with WCN included RSA of sensitivity and specificity is shown in Table 10. Figure 9(A) to (E) shows the information of enzyme 1QK2 structure like as

Figure 8, the comparison method WCN with WCN included RSA of sensitivity and specificity is shown in Table 11. Figure 10(A) to (H) shows the information of enzyme 1EXP structure like as Figure 8 enzyme 1CEL, the comparison method WCN with WCN included RSA of sensitivity and specificity is shown in Table 12. Above-mentioned the relationship of performance, we also combine two methods WCN and WCN include RSA, we can figure out the residues under WCN z-score threshold (≤ -0.8) of enzymes we selected are much more than the method include RSA, although the sensitivity value will decrease, however we can lower the false positive value and enhance the true negative value then our specificity value will increase much more. It is clear that the binding site residues tend to have lower WCN z-score value and exposed according to our comparison with performance profile results.

Figure 11 shows the frequency of amino acid type in cellulase experimental binding site compared with our method of binding site prediction base on WCN including RSA, we expect that experimental and our work would be have similar amino acid type on binding substrates. However, the experimental frequency of hydrophobic, hydrophilic and charged amino acid type in endo-glucanases binding site are 35%, 35% and 31%; in exo-glucanases are 23%, 38% and 38%. And in our work, the frequency of hydrophobic, hydrophilic and charged amino acid type in endo-glucanases binding site are 41%, 34% and 25%; in exo-glucanases are 45%, 24% and 31%. There is no significant correlation with the frequency of amino acid type in binding substrate between experimental and our work. However, we still can figure out the performance values of endo-glucanases and exo-glucanases show in Table 12, that values are increasing in specificity, through different WCN z- score threshold and RSA included. It means that enzymes are very specific, and the binding sites of enzyme are especially less flexible than other residues. The WCN is used for indicate

structure rigidity. However, there are complementary relationships between structural characteristics of binding sites and based on the method WCN. The WCN z- score threshold for endo-glucanases is larger than exo-glucanases, it means that most of endo-glucanases binding substrate structure are flexible, and exo-glucanases are more rigid for cellulose hydrolyze. It is reasonable that endo-glucanses need more space for hydrolyzing cellulose. Thus, using WCN and RSA may help understanding and finding binding site residues of cellulase as more as possible.

4. Conclusion

In this work, we present a structural analysis of cellulase binding sites using a dataset of 219 enzymes which was chosen from NCBI and CSA. This dataset is nonredundant, and we selected 4 enzymes for each endo-glucanase and exo-glucanase, total 8 enzymes that have their own experimental catalytic residues data form literature. The conclusion that we analysis is that through our methods, the WCN (z- score) threshold for endo-glucanases is larger than exo-glucanases, it is reasonable that endo-glucanses need more space for hydrolyzing cellulose. Besides, the performance value of predicting binding sites let us know that we can increase the specificity values based on WCN and RSA. It means that most of binding sites are rigid than other residues and exposed although they are hydrophobic.

Based on all these characteristics with binding sites may enable people to understand more information for structure- function relationships; furthermore, it will be helpful for predicting binding sites in cellulase of unknown function from protein structures and maybe we could tell endo-glucanase and exo-glucanase by their binding sites in the further work.

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TABLES

Table1. The dataset of Exo-glucanases from NCBI, CSA

PDB	GH	Catalytic Residues				
1BVW	6	Y174	R179	D180	D226	align 1qk2
1CB2	6	F169	R174	D175	D221	align 1qk2
1GZ1	6	Y174	R179	D180	D226	align 1qk2
1HGW	6	Y169	R174	A175	D221	align 1qk2
1HGY	6	Y169	R174	A175	D221	align 1qk2
1OC5	6	Y174	R179	D180	D226	align 1qk2
1OC6	6	Y174	R179	D180	D226	align 1qk2
1OC7	6	Y174	R179	D180	D226	align 1qk2
1OC8	6	Y174	R179	D180	D226	align 1qk2
1OCJ	6	Y174	R179	D180	D226	align 1qk2
1OCN	6	Y174	R179	D180	D226	align 1qk2
1QJW	6	F169	R174	D175	D221	align 1qk2
1QK0	6	Y169	R174	D175	D221	align 1qk2
1QK2	6	Y169	R174	D92	D139	
2BVW	6	Y174	R179	D180	D226	align 1qk2
3A64	6	Y96	R101	D102	D150	align 1qk2
3A9B	6	Y96	R101	D102	D150	align 1qk2
3ABX	6	Y96	R101	D102	D150	align 1qk2
3CBH	6	Y169	R174	D175	D221	align 1qk2
1CEL	7	E212	D214	E217	H228	
1DY4	7	E212	D214	E217	H228	align 1cel
1EGN	7	E212	D214	E217	H228	align 1cel
1GPI	7	E207	D209	E212	H223	align 1cel
1H46	7	E207	D209	E212	H223	align 1cel
1Q2B	7	E212	D214	E217	H228	align 1cel
1Q2E	7	E212	D214	E217	H228	align 1cel
1Q9H	7	E209	D211	E214	H225	align 1cel
1Z3T	7	E207	D209	E212	H223	align 1cel
1Z3V	7	E207	D209	E212	H223	align 1cel
1Z3W	7	E207	D209	E212	H223	align 1cel
2CEL	7	E212	D214	E217	H228	align 1cel
2RFW	7	E212	D214	E217	H228	align 1cel
2RFY	7	E212	D214	E217	H228	align 1cel
2RFZ	7	E212	D214	E217	H228	align 1cel
2RG0	7	E212	D214	E217	H228	align 1cel
2V3I	7	E212	D214	E217	H228	align 1cel
3CEL	7	E212	D214	E217	H228	align 1cel
4CEL	7	E212	D214	E217	H228	align 1cel
5CEL	7	E212	D214	E217	H228	align 1cel
6CEL	7	E212	D214	E217	H228	align 1cel
7CEL	7	E212	D214	E217	H228	align 1cel
1RQ5	9	D383	D386	Q795		align 1js4
1EXP	10	E127	H205	E233	D235	
1FH7	10	E127	H205	E233	D235	align 1exp
1FH8	10	E127	H205	E233	D235	align 1exp
1FH9	10	E127	H205	E233	D235	align 1exp
1FHD	10	E127	H205	E233	D235	align 1exp
1J01	10	E127	H205	E233	D235	align 1exp
2EXO	10	E127	H205	E233	D235	align 1exp
2HIS	10	A127				
2XYL	10	E127	H205	E233	D235	align 1exp
3CUF	10	E127	H205	E233	D235	align 1exp
3CUG	10	E127	H205	E233	D235	align 1exp
3CUH	10	E127	H205	E233	D235	align 1exp
3CUI	10	E127	H205	E233	D235	align 1exp

Table2. The dataset of Endo-glucanases from NCBI, CSA

PDB	GH	Catalytic Residues					
1A3H	5	N138	E139	H200	Y202	E228	align 1bqc
1CEC	5	N139	E140	H198	Y200	E280	align 1bqc
1CEO	5	E140	E247				align 1cz1
1E5J	5	N138	E139	H200	Y202	E228	align 1bqc
1ECE	5	N161	E162	H238	Y240	E282	align 1bqc
1EDG	5	N169	E170	H254	Y256	E307	align 1bqc
1EGZ	5	N132	E133	H192	Y194	E220	align 1bqc
1G01	5	N372	E373	H442	Y444	E485	align 1bqc
1G0C	5	N372	E373	H442	Y444	E485	align 1bqc
1GZJ	5	N132	E133	H198	Y200	E240	align 1bqc
1H11	5	N138	E139	H200	Y202	E228	align 1bqc
1H1N	5	N132	E133	H198	Y200	E240	align 1bqc
1H2J	5	N138	E139	H200	Y202	E228	align 1bqc
1H5V	5	N138	E139	H200	Y202	E228	align 1bqc
1HF6	5	N138	E139	H200	Y202	E228	align 1bqc
1LF1	5	N138	E139	H200	Y202	E228	align 1bqc
1OCQ	5	N138	E139	H200	Y202	E228	align 1bqc
1QHZ	5	N138	E139	H200	Y202	E228	align 1bqc
1QI0	5	N138	E139	H200	Y202	E228	align 1bqc
1QI2	5	N138	E139	H200	Y202	E228	align 1bqc
1TVN	5	N134	E135	H194	Y196	E222	align 1bqc
1TVP	5	N134	E135	H194	Y196	E222	align 1bqc
1VJZ	5	R42	E139	E259			align 1fhl
1VRX	5	N161	E162	H238	Y240	E282	align 1bqc
1W3K	5	N138	E139	H200	Y202	E228	align 1bqc
1W3L	5	N138	E139	H200	Y202	E228	align 1bqc
2A3H	5	N138	E139	H200	Y202	E228	align 1bqc
2CKR	5	R185	E263	Q355			align 1fhl
2CKS	5	N262	E263	H328	Y330	E355	align 1bqc
2V38	5	N138	E139	H200	Y202	E228	align 1bqc
2ZUM	5	N200	E201	H297	Y299	E342	align 1bqc
2ZUN	5	N200	E201	H297	Y299	E342	align 1bqc
3A3H	5	N138	E139	H200	Y202	E228	align 1bqc
4A3H	5	N138	E139	H200	Y202	E228	align 1bqc
5A3H	5	N138	E139	H200	Y202	E228	align 1bqc
6A3H	5	N138	E139	H200	Y202	E228	align 1bqc
7A3H	5	N138	E139	H200	Y202	E228	align 1bqc
8A3H	5	N138	E139	H200	Y202	E228	align 1bqc
1DYS	6	Y86	R91	D92	D139		align 1qk2
1TML	6	D117	D265				
2BOD	6	Y73	R78	D110	D117		align 1qk2
2BOE	6	D117	D265				align 1tml
2BOF	6	D117	D265				align 1tml
2BOG	6	D117	D265				align 1tml
1A39	7	E197	D199	E202	H213		align 1cel
1DYM	7	A197	D199	E202	H213		align 1cel
1EG1	7	E196	D198	E201	H212		align 1cel
1OJI	7	S197	D199	E202	H213		align 1cel
1OJJ	7	S197	D199	E202	H213		align 1cel
1OJK	7	S197	D199	E202	H213		align 1cel
1OVW	7	E197	D199	E202	H213		align 1cel
2A39	7	E197	D199	E202	H213		align 1cel
2OVW	7	E197	D199	E202	H213		align 1cel
3OVW	7	E197	D199	E202	H213		align 1cel
4OVW	7	E197	D199	E202	H213		align 1cel
1CLC	9	D198	D201	E555			align 1js4
1G87	9	D55	D58	E420			align 1js4
1GA2	9	D55	D58	E420			align 1js4

1IA6	9	D56	D59	E410		align 1js4
1IA7	9	D86	D89	E410		align 1js4
1JS4	9	D55	D58	E424		
1K72	9	D55	D58	E420		align 1js4
1KFG	9	D55	D58	E420		align 1js4
1KS8	9	D54	D57	E412		align 1js4
1KSC	9	D54	D57	E412		align 1js4
1KSD	9	D54	D57	E412		align 1js4
1TF4	9	D55	D58	E424		align 1js4
1UT9	9	D383	D386	E555		align 1js4
3EZ8	9	D143	D146	E515		align 1js4
3GZK	9	D143	D146	E515		align 1js4
3H2W	9	D143	D146	E515		align 1js4
3H3K	9	D143	D146	E515		align 1js4
4TF4	9	D55	D58	E424		align 1js4
2DEP	10	E185	H264	E293	D295	align 1exp
1H8V	12	E116				align 2nlr
1KS4	12	E116				align 2nlr
1KS5	12	E116				align 2nlr
1NLR	12	E120				align 2nlr
1OA2	12	E116				align 2nlr
1OA3	12	E116				align 2nlr
1OLQ	12	E116				align 2nlr
1OLR	12	E120				align 2nlr
1QA3	12	E116				align 2nlr
1QA4	12	E120				align 2nlr
1UU4	12	E120				align 2nlr
1UU5	12	E120				align 2nlr
1UU6	12	E120				align 2nlr
1W2U	12	E120				align 2nlr
2BW8	12	E124				align 2nlr
2BWA	12	E124				align 2nlr
2BWC	12	E124				align 2nlr
2JEM	12	Q155				align 2nlr
2JEN	12	A155				align 2nlr
2NLR	12	E120				
3B7M	12	E114				align 2nlr
2UWA	16	E94	D96	E98		align 2ayh
2UWB	16	E94	D96	E98		align 2ayh
2UWC	16	E94	D96	E98		align 2ayh
2VH9	16	E94	D96	E98		align 2ayh
2E0P	44	E186				align 1pz3
2E4T	44	E186				align 1pz3
2EEX	44	E186				align 1pz3
2EJ1	44	E186				align 1pz3
2EO7	44	E186				align 1pz3
2EQD	44	E186				align 1pz3
3FW6	44	E221				align 1pz3
3II1	44	E221				align 1pz3
1HD5	45	D10	D122			align 2eng
1L8F	45	D10	D120			align 2eng
1QA7	45	D10	D120			align 2eng
1QA9	45	D10	D120			align 2eng
2ENG	45	D10	D121			
3ENG	45	D10	D121			align 2eng
4ENG	45	D10	D121			align 2eng

Table3. Proteins have own catalytic residues data from literature

PDB	GH	Catalytic Residues			
1JS4	9	D55	D58		
1TML	6	D117	D256		
2ENG	45	D10	D121		
2NLR	12	E120			
1CEL	7	E212	D214	E217	H228
1EXP	10	E127	H205	E233	D235
1QK2	6	Y169	R174	D175	D221
2HIS	10	A127			

Table4. Bindings site residues of each protein from literatures

	PDB	Binding Residues
Endo-glucanase	1JS4[9]	H125 W128 F205 W209 W256 D261 W313 R317 R378 Y429
	1TML[10]	W41 Y73 D117 D155 H159 W162 S189 W231 E263 D265 A271
	2ENG[11]	T6 R7 Y8 D10 K13 W18 A19 K21 S45 E82 S110 H119 D121 G127 G128 V129 Y147 G148 D178 N179
	2NLR[12]	F8 N22 W24 H65 Y66 N100 D104 W106 E120 M122 N155 S157 Q199 E203
Exo-glucanase	1CEL[15]	N141 Y145 E217 H228 W367
	1EXP[15]	W84 N126 Y171 H205 E233
	1QK2[16]	W135 D137 Y169 R174 D175 D221 H266 W269 N305 R353 W376 K395 E399 D401
	2HIS[17]	A127 H205

Table 5.1. The measurement of all dataset

Threshold	TPR	FPR
wcn<-1.6	0.01	0.00
wcn<-1.5	0.03	0.02
wcn<-1.4	0.10	0.05
wcn<-1.3	0.23	0.07
wcn<-1.2	0.28	0.10
wcn<-1.1	0.34	0.12
wcn<-1	0.40	0.15
wcn<-0.9	0.53	0.19
wcn<-0.8	0.64	0.23
wcn<-0.7	0.72	0.27
wcn<-0.6	0.76	0.31
wcn<-0.5	0.77	0.34
wcn<-0.4	0.82	0.38
wcn<-0.3	0.86	0.42
wcn<-0.2	0.87	0.46
wcn<-0.1	0.83	0.50
wcn<0	0.89	0.54
wcn<0.1	0.89	0.57
wcn<0.2	0.87	0.58
wcn<0.3	0.93	0.65
wcn<0.4	0.95	0.68
wcn<0.5	0.95	0.71
wcn<0.6	0.95	0.74
wcn<0.7	0.96	0.76
wcn<0.8	0.98	0.79
wcn<0.9	0.99	0.81

TPR=TP/(TP+FN), FPR=FP/(FP+TN)
TP: true positive, TN: true negative, FN: false negative, FP: false positive.

Table 5.2. The measurement of endo-glucanase

Threshold	TPR	FPR
wcn<-1.6	0.02	0.00
wcn<-1.5	0.02	0.02
wcn<-1.4	0.08	0.05
wcn<-1.3	0.11	0.08
wcn<-1.2	0.20	0.11
wcn<-1.1	0.29	0.13
wcn<-1	0.32	0.15
wcn<-0.9	0.47	0.18
wcn<-0.8	0.49	0.22
wcn<-0.7	0.51	0.26
wcn<-0.6	0.54	0.30
wcn<-0.5	0.54	0.33
wcn<-0.4	0.63	0.38
wcn<-0.3	0.72	0.42
wcn<-0.2	0.74	0.46
wcn<-0.1	0.66	0.50
wcn<0	0.78	0.54
wcn<0.1	0.78	0.57
wcn<0.2	0.80	0.61
wcn<0.3	0.87	0.65
wcn<0.4	0.89	0.68
wcn<0.5	0.89	0.72
wcn<0.6	0.89	0.74
wcn<0.7	0.93	0.76
wcn<0.8	0.96	0.78
wcn<0.9	0.98	0.81

Table 5.3. The measurement of exo-glucanases

Threshold	TPR	FPR
wcn<-1.6	0.00	0.00
wcn<-1.5	0.05	0.02
wcn<-1.4	0.12	0.05
wcn<-1.3	0.35	0.08
wcn<-1.2	0.37	0.11
wcn<-1.1	0.39	0.13
wcn<-1	0.48	0.15
wcn<-0.9	0.59	0.18
wcn<-0.8	0.79	0.22
wcn<-0.7	0.93	0.26
wcn<-0.6	1.00	0.30
wcn<-0.5	1.00	0.33
wcn<-0.4	1.00	0.38
wcn<-0.3	1.00	0.42
wcn<-0.2	1.00	0.46
wcn<-0.1	1.00	0.50
wcn<0	1.00	0.54
wcn<0.1	1.00	0.57
wcn<0.2	1.00	0.56
wcn<0.3	1.00	0.65
wcn<0.4	1.00	0.68
wcn<0.5	1.00	0.71
wcn<0.6	1.00	0.74
wcn<0.7	1.00	0.76
wcn<0.8	1.00	0.79
wcn<0.9	1.00	0.82

Table 6. Endo-glucanase 1TML. The comparison with WCN and WCN & RSA included.

1TML	TP	FP	FN	TN	Sensitivity (%)	Specificity (%)
WCN (< -0.5)	7	94	4	181	64	66
WCN (< -0.5) & RSA (≥ 0.05)	5	24	6	251	45	91

TP: true positive, FP: false positive, FN: false negative, TN: true negative, Sensitivity: TP/(TP+FN), Specificity: 1-(FP/(FP+TN)),

All statistical measures are percentage value (%).

Table 7. Endo-glucanase 2ENG. The comparison with WCN and WCN & RSA included.

2ENG	TP	FP	FN	TN	Sensitivity (%)	Specificity (%)
WCN (< -0.5)	10	63	10	122	50	66
WCN (< -0.5) & RSA (≥ 0.05)	9	19	11	167	47	90

TP: true positive, FP: false positive, FN: false negative, TN: true negative, Sensitivity: TP/(TP+FN), Specificity: 1-(FP/(FP+TN)),
All statistical measures are percentage value (%).

Table 8. Endo-glucanase 1JS4. The comparison with WCN and WCN & RSA included.

1JS4	TP	FP	FN	TN	Sensitivity (%)	Specificity (%)
WCN (< -0.5)	4	220	6	375	40	63
WCN (< -0.5) & RSA (≥ 0.05)	3	52	7	543	30	91

TP: true positive, FP: false positive, FN: false negative, TN: true negative, Sensitivity: TP/(TP+FN), Specificity: 1-(FP/(FP+TN)),
All statistical measures are percentage value (%).

Table 9. Endo-glucanase 2NLR. The comparison with WCN and WCN & RSA included.

2NLR	TP	FP	FN	TN	Sensitivity (%)	Specificity (%)
WCN (< -0.5)	9	71	5	137	64	66
WCN (< -0.5) & RSA (≥ 0.05)	5	12	9	196	36	94

TP: true positive, FP: false positive, FN: false negative, TN: true negative, Sensitivity: TP/(TP+FN), Specificity: 1-(FP/(FP+TN)), All statistical measures are percentage value (%).

Table 10. Exo-glucanase 1CEL. The comparison with WCN and WCN & RSA included.

1CEL	TP	FP	FN	TN	Sensitivity (%)	Specificity (%)
WCN (< -0.8)	5	96	0	333	100	78
WCN (< -0.8) & RSA (≥ 0.08)	3	19	2	410	60	96

TP: true positive, FP: false positive, FN: false negative, TN: true negative, Sensitivity: TP/(TP+FN), Specificity: 1-(FP/(FP+TN)), All statistical measures are percentage value (%).

Table 11. Exo-glucanase 1QK2. The comparison with WCN and WCN & RSA included.

1QK2	TP	FP	FN	TN	Sensitivity (%)	Specificity (%)
WCN (< -0.8)	9	74	5	275	64	79
WCN (< -0.8) & RSA (≥ 0.08)	8	8	6	314	57	97

TP: true positive, FP: false positive, FN: false negative, TN: true negative, Sensitivity: TP/(TP+FN), Specificity: 1-(FP/(FP+TN)), All statistical measures are percentage value (%).

Table 12. Exo-glucanase 1EXP. The comparison with WCN and WCN & RSA included.

1EXP	**TP**	**FP**	**FN**	**TN**	**Sensitivity (%)**	**Specificity (%)**
WCN <-0.8	5	67	0	240	100	78
WCN (< -0.8) & RSA (≥ 0.08)	4	6	1	301	80	98

TP: true positive, FP: false positive, FN: false negative, TN: true negative, Sensitivity: TP/(TP+FN), Specificity: 1-(FP/(FP+TN)), All statistical measures are percentage value (%).

Table 13. The comparison of WCN with WCN include RSA

		Sensitivity (%)	Specificity (%)
WCN	Endo-glucanase (< -0.5)	79	78
	Exo-glucanase (< -0.8)	54	65
	Average	67	72
WCN & RSA	Endo-glucanase (< -0.5)	40	92
	Exo-glucanase (< -0.8)	65	97
	Average	51	94

FIGURES

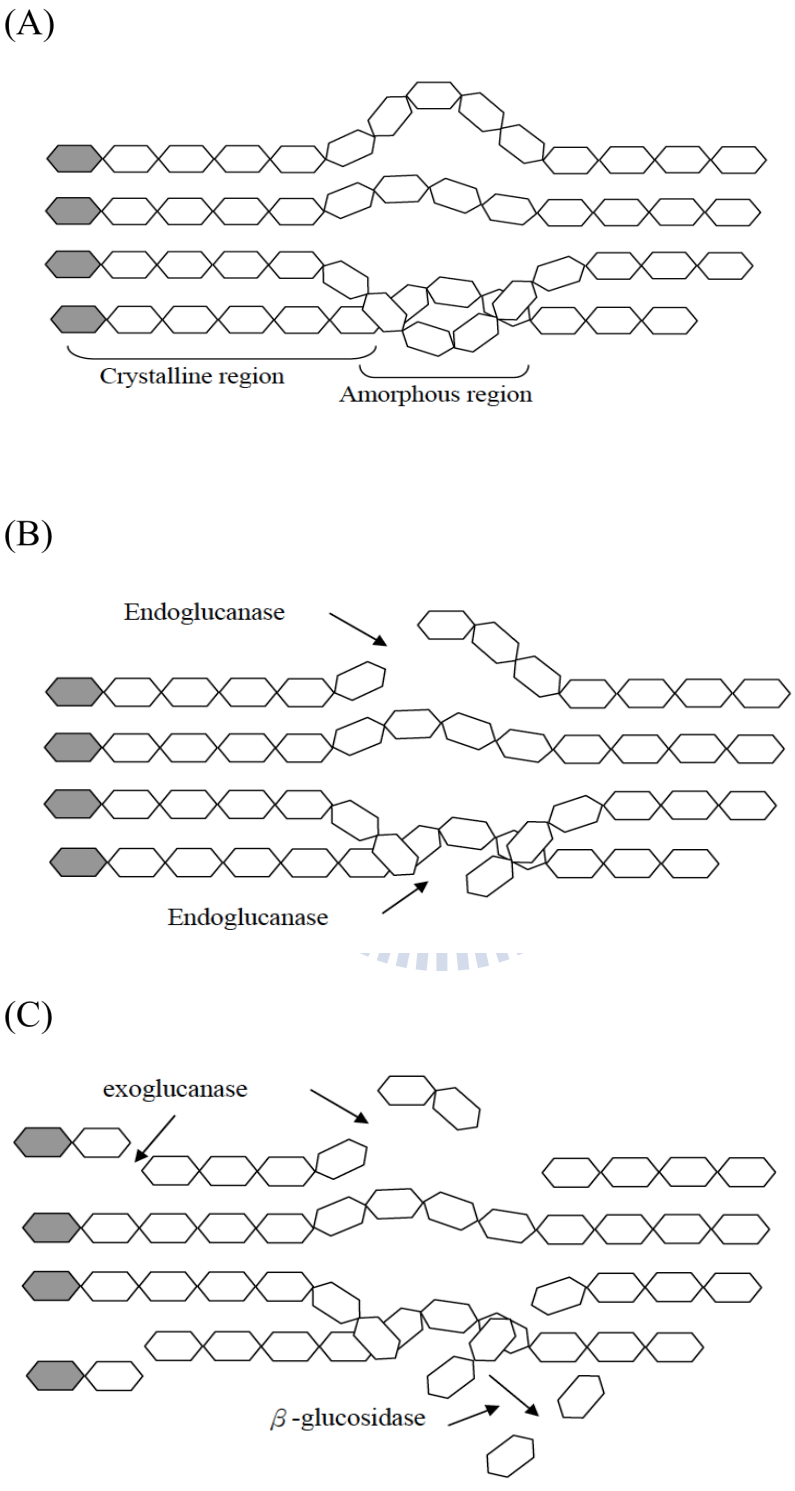
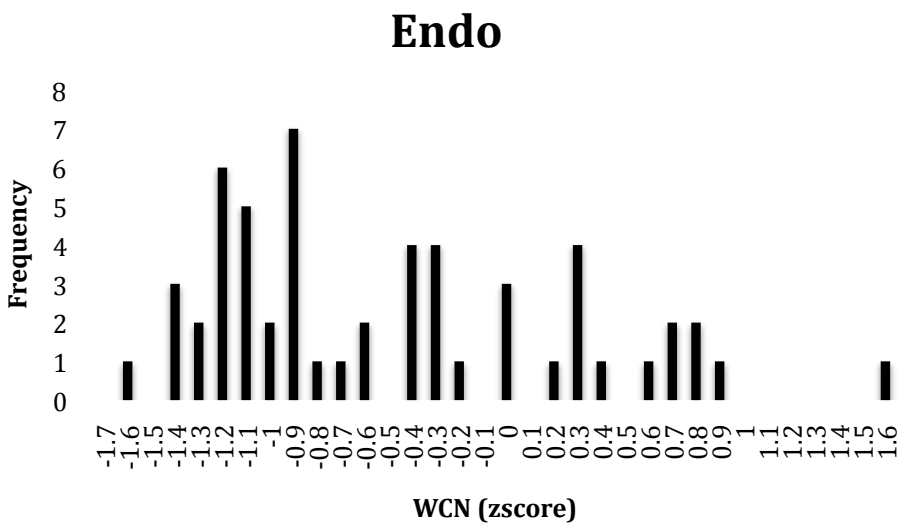


Figure 1. The processive synergy mechnism of cellulose hydrolysis. (A) Cellulose consist crystalline region and amorphous region. (B) Endo-glucanase cut at the internal amorphous sites. (C) Exo-glucanase acts on the reducing or nonreducing ends of chains. β-glucosidases hydrolyze soluble cellodextrins and cellobiose to glucose.

(A)



(B)

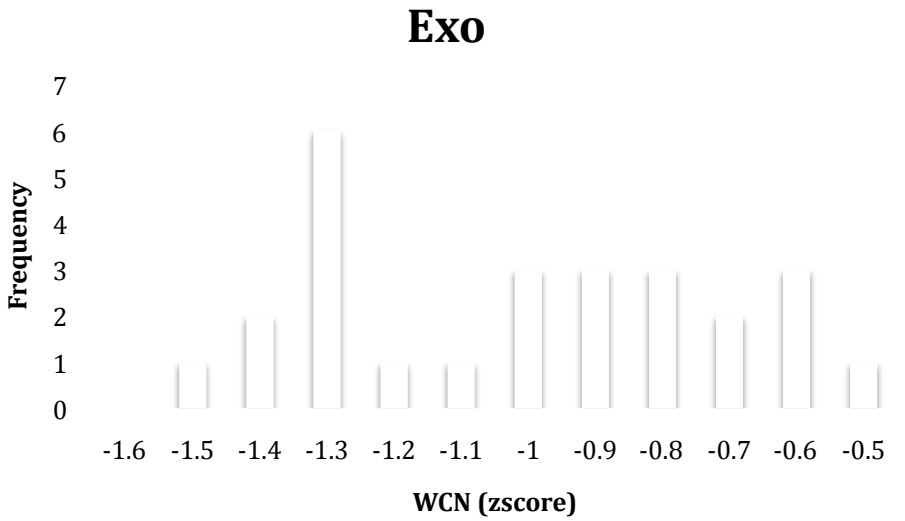
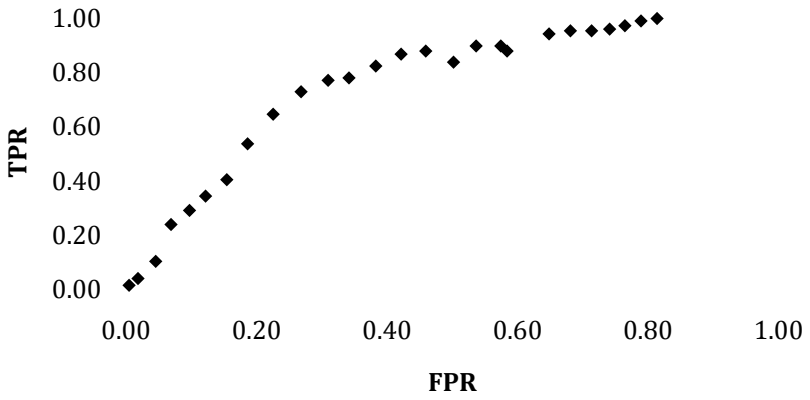
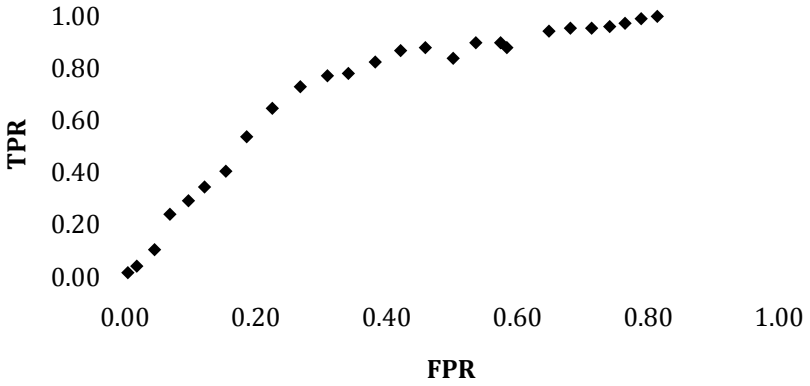


Figure 2.WCN z-score distribution of literature binding site residues. The frequency of endo-glucanase (A) binding residues colored in black compared with exo-glucanase (B) binding residues colored in white.

(A)



(B)



(C)

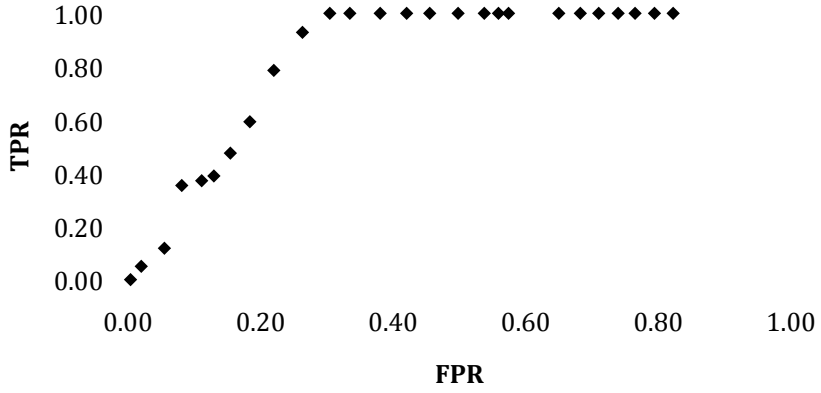
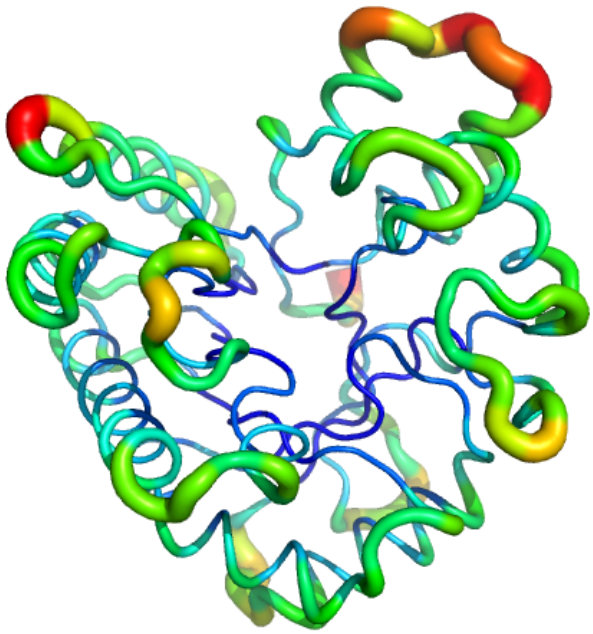


Figure3. The diagram of relationship TPR and FPR from top to the bottom are (A) all selected cellulase dataset, (B) endo-glucanase group, (C) exo-glucanase group.

(A)



(B)

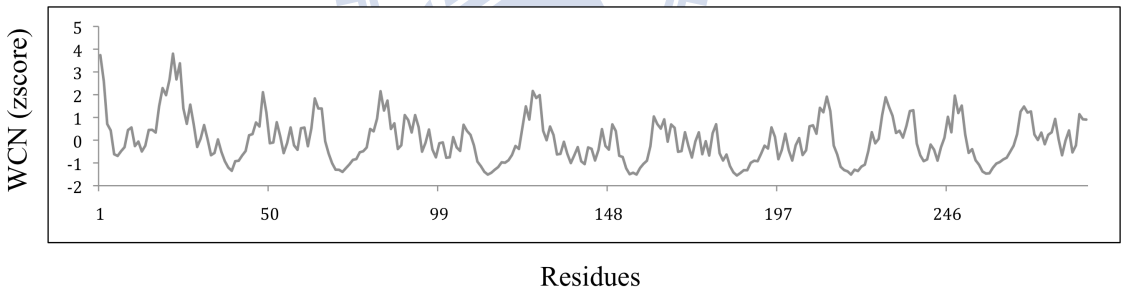
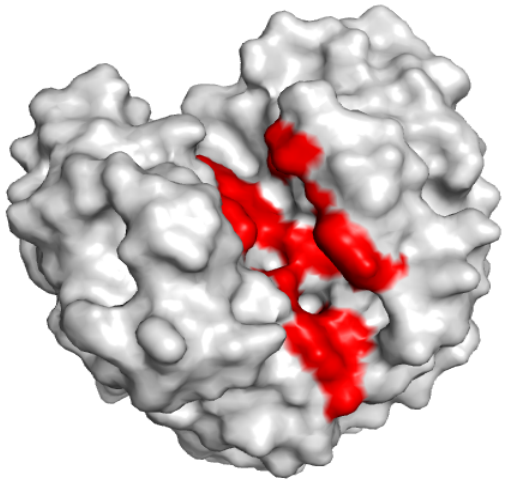
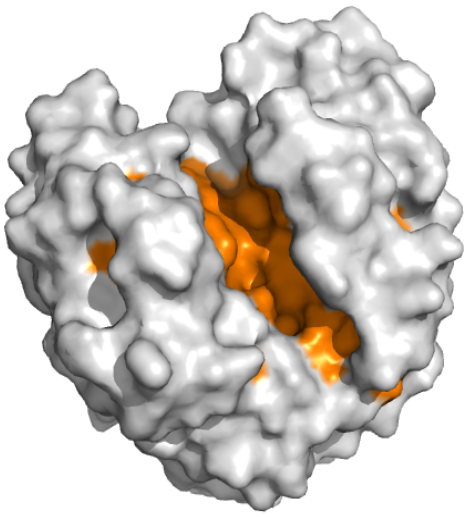


Figure 4. (A) 1TML protein WCN model in putty form. (B) The WCN z- score distribution of protein 1TML.

(C)



(D)



(E)

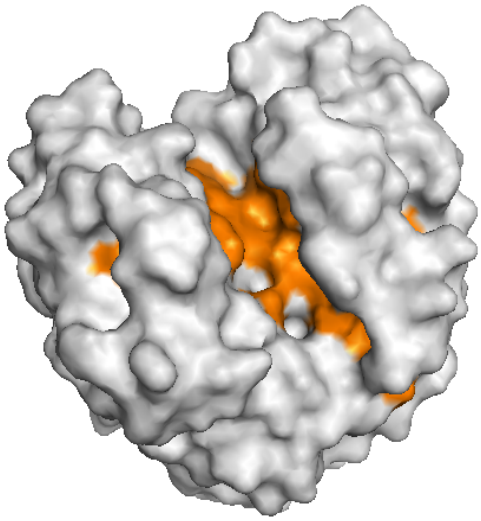
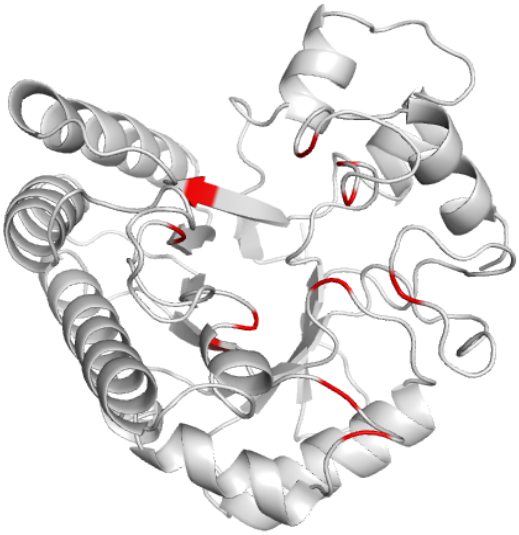


Figure 4. Proteins are surface form. (C) 1TML experimental binding site residues colored in red. (D) The residues under WCN threshold (< -0.5) are colored in orange. (E) The residues selected include WCN and RSA threshold are also colored in orange.

(F)



(G) (H)

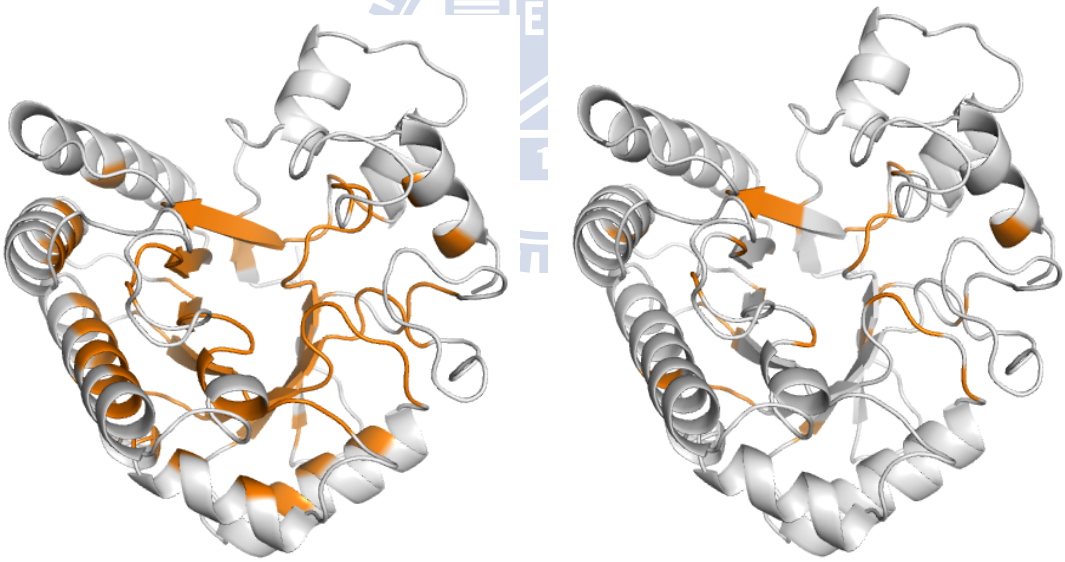
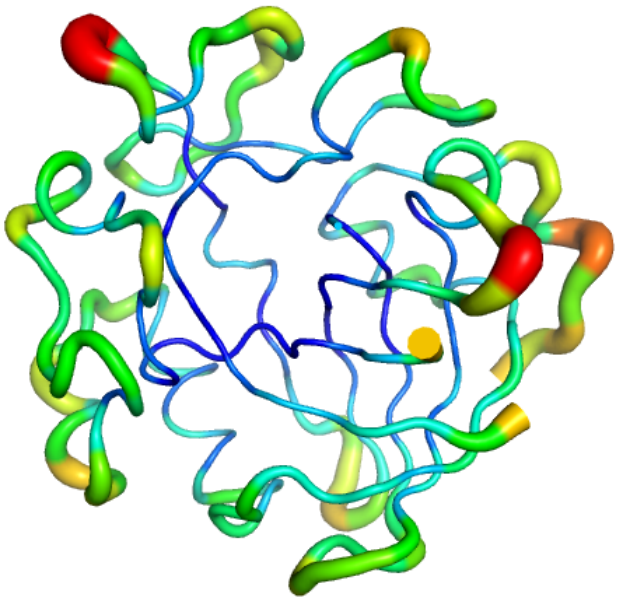


Figure 4. Proteins are cartoon form. (F) 1TML experimental binding site residues colored in red. (G) The residues under WCN threshold (< -0.5) are colored in orange. (H) The residues selected include WCN and RSA threshold are also colored in orange.

(A)



(B)

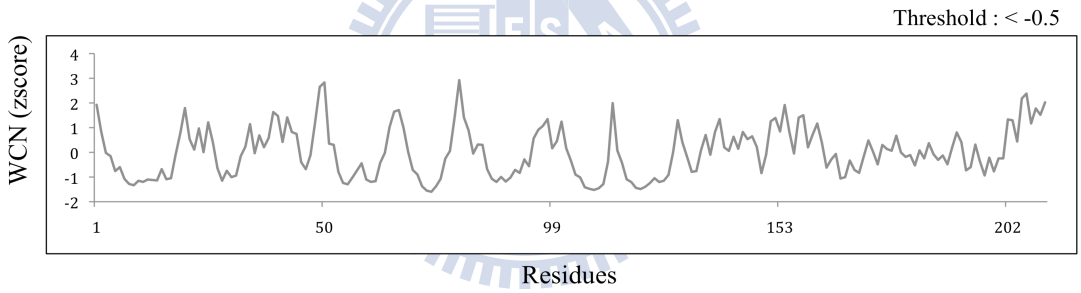
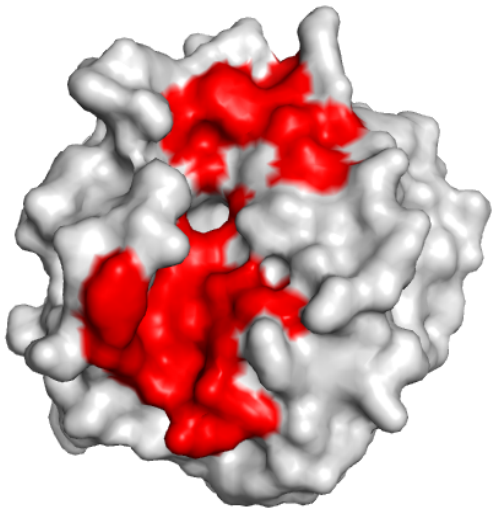
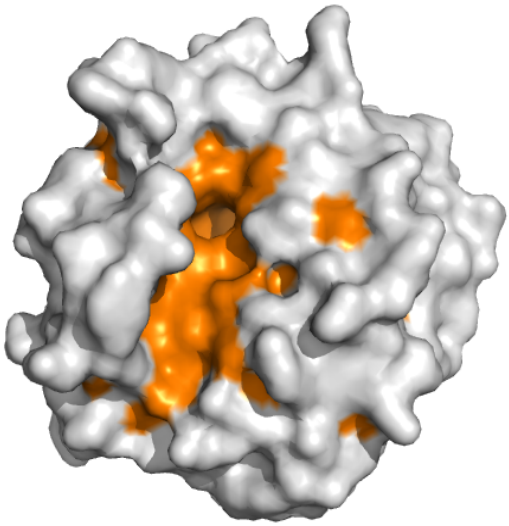


Figure 5. (A) 2ENG protein WCN model in putty form. (B) The WCN z- score distribution of protein 2ENG.

(C)



(D)



(E)

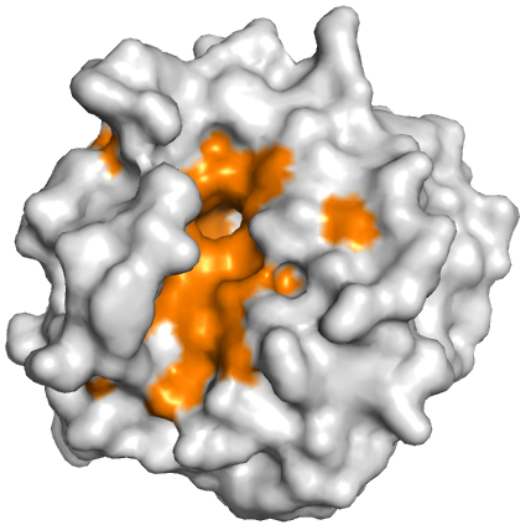
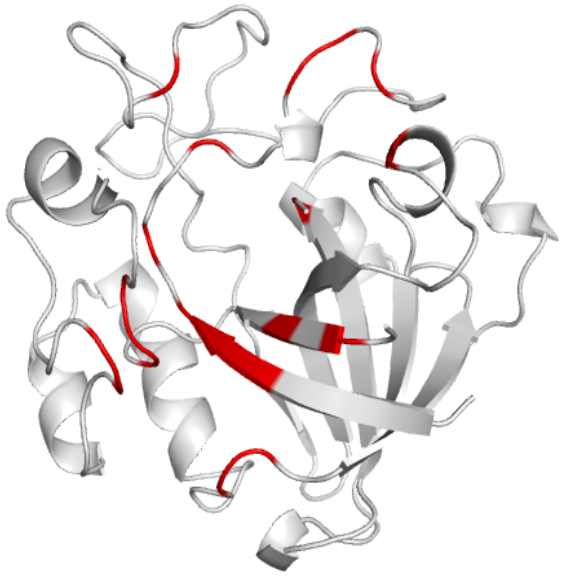
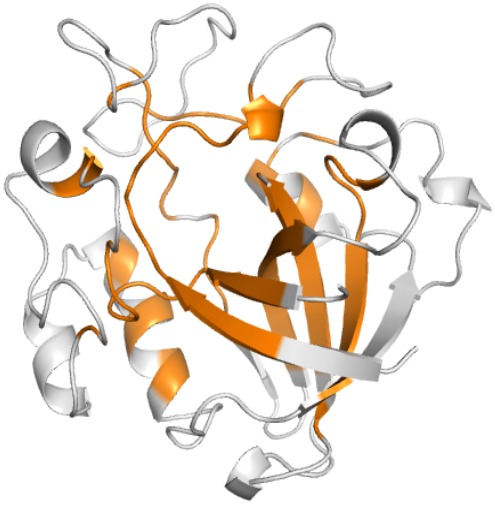


Figure 5. Proteins are surface form. (C) 2ENG experimental binding site residues colored in red. (D) The residues under WCN threshold (< -0.5) are colored in orange. (E) The residues selected include WCN and RSA threshold are also colored in orange.

(F)



(G)



(H)

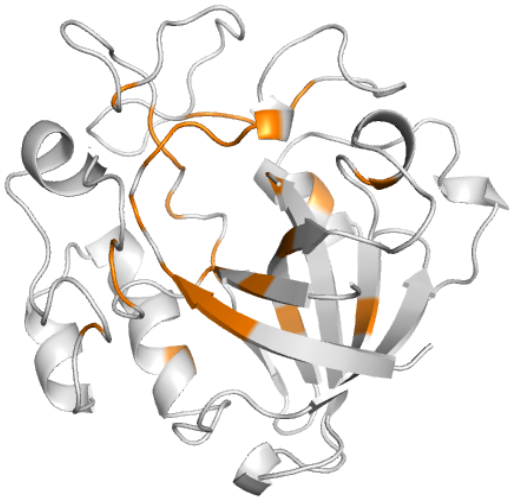
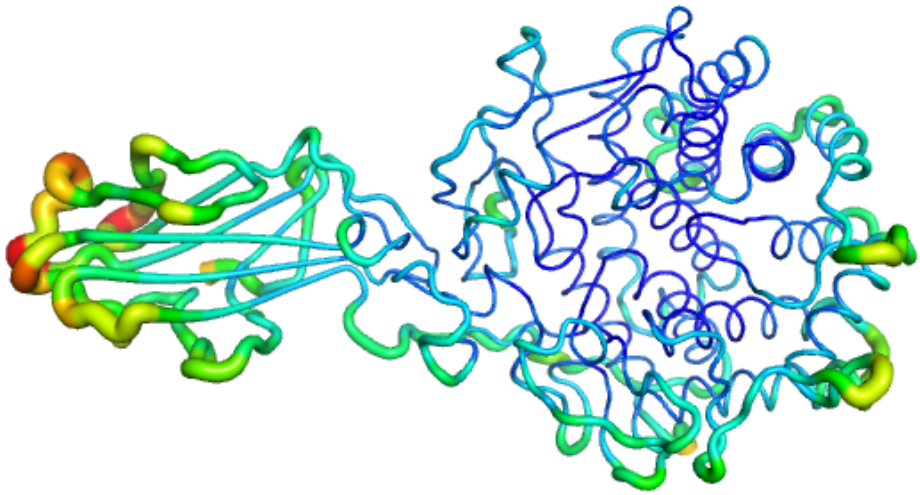


Figure 5. Proteins are cartoon form. (F) 2ENG experimental binding site residues colored in red. (G) The residues under WCN threshold (< -0.5) are colored in orange. (H) The residues selected include WCN and RSA threshold are also colored in orange.

(A)



(B)

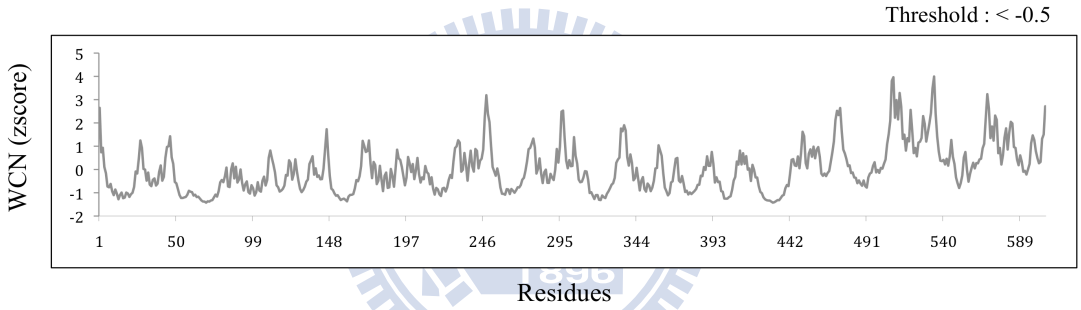
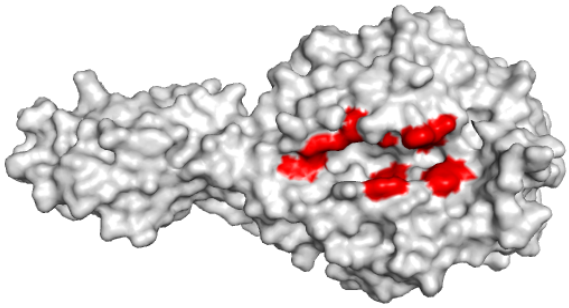
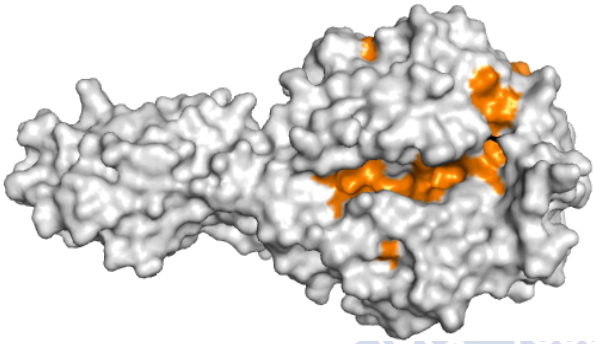


Figure 6. (A) 1JS4 protein WCN model in putty form. (B) The WCN z- score distribution of protein 1JS4.

(C)



(D)



(E)

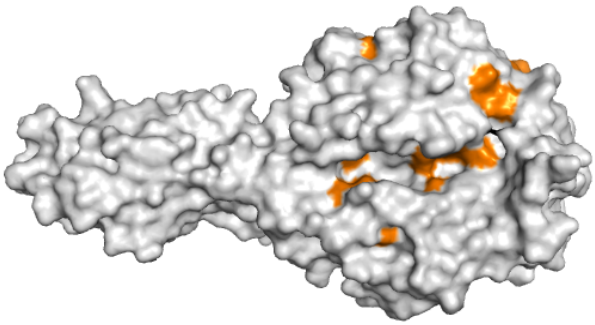
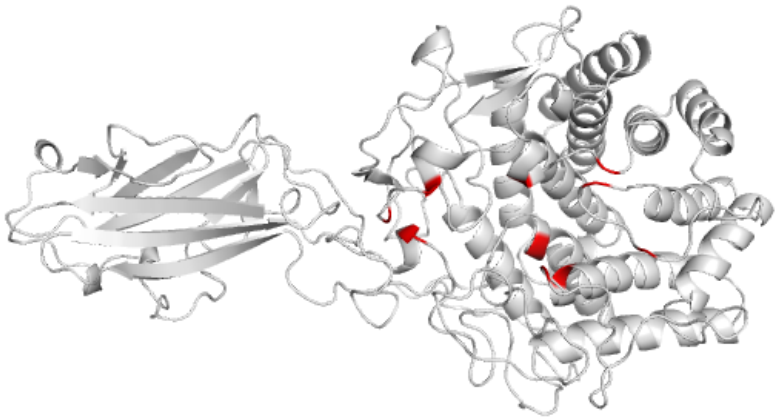
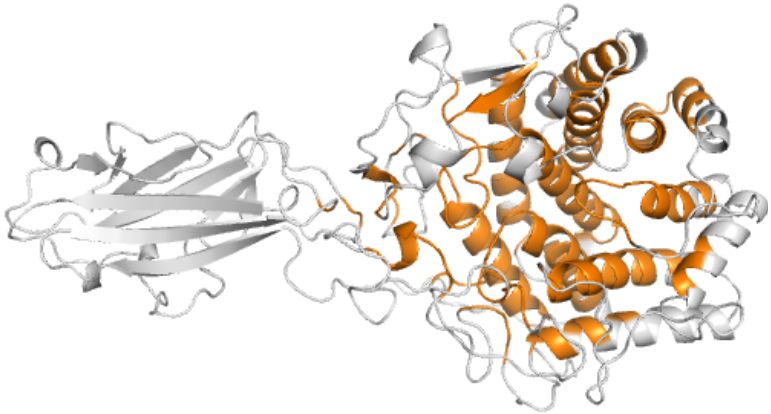


Figure 6. Proteins are surface form. (C) 1JS4 experimental binding site residues colored in red. (D) The residues under WCN threshold (< -0.5) are colored in orange. (E) The residues selected include WCN and RSA threshold are also colored in orange.

(F)



(G)



(H)

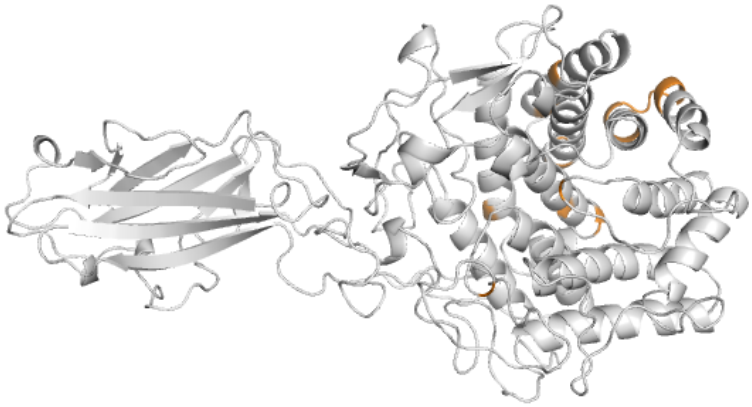
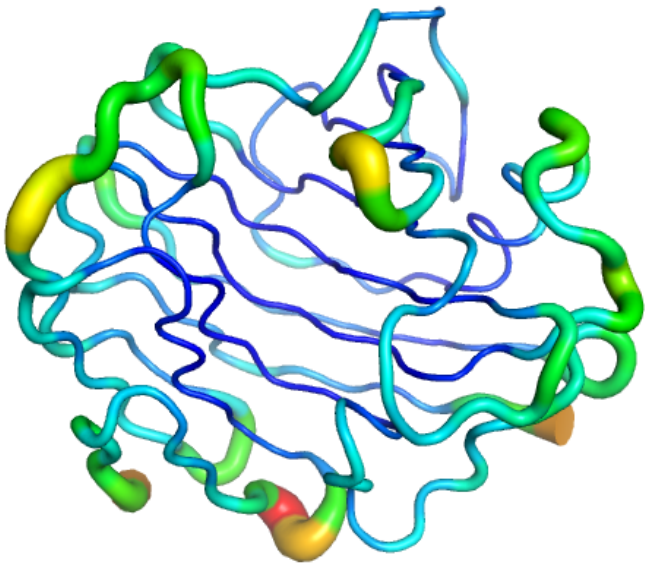


Figure 6. Proteins are cartoon form. (F) 1CEL experimental binding site residues colored in red. (G) The residues under WCN threshold (< -0.5) are colored in orange. (H) The residues selected include WCN and RSA threshold are also colored in orange.

(A)



(B)

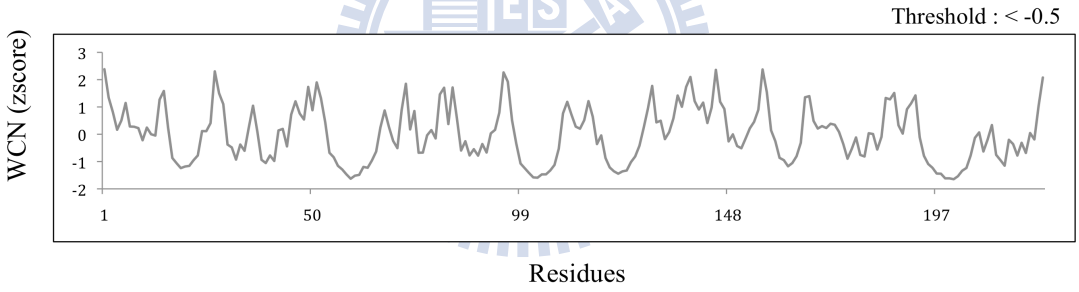
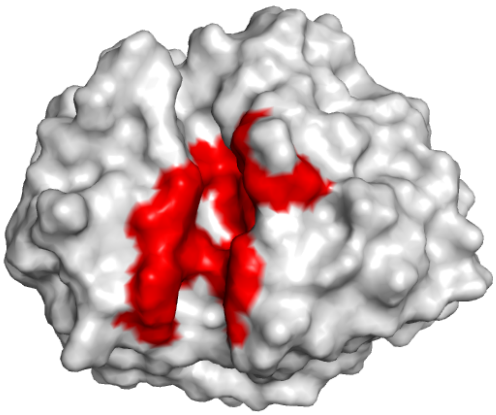
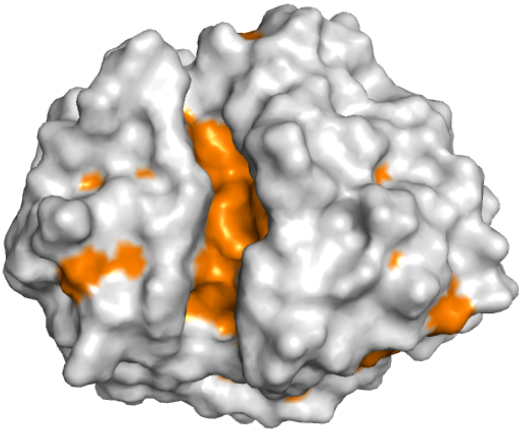


Figure 7. (A) 2NLR protein WCN model in putty form. (B) The WCN z- score distribution of protein 2NLR.

(C)



(D)



(E)

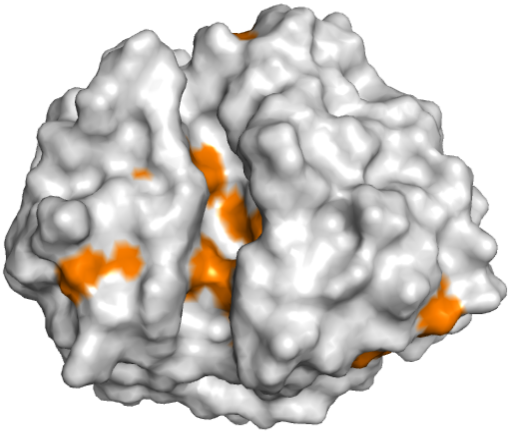
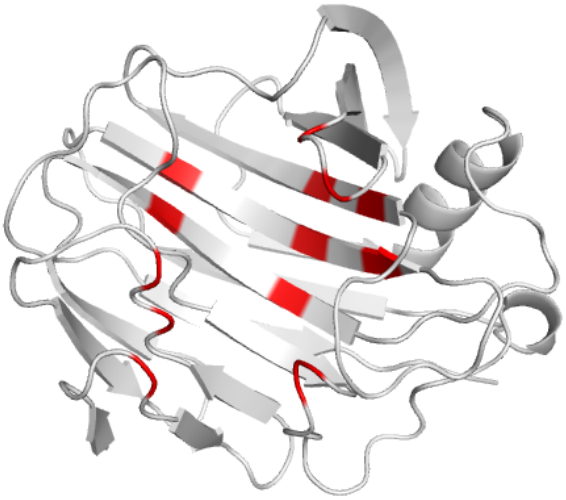


Figure 7. Proteins are surface form. (C) 2NLR experimental binding site residues colored in red. (D) The residues under WCN threshold (< -0.5) are colored in orange. (E) The residues selected include WCN and RSA threshold are also colored in orange.

(F)



(G)

(H)

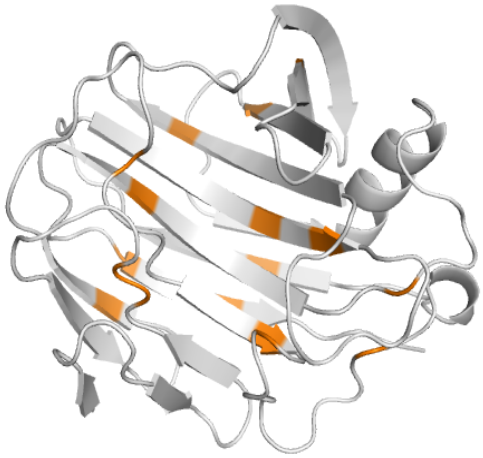
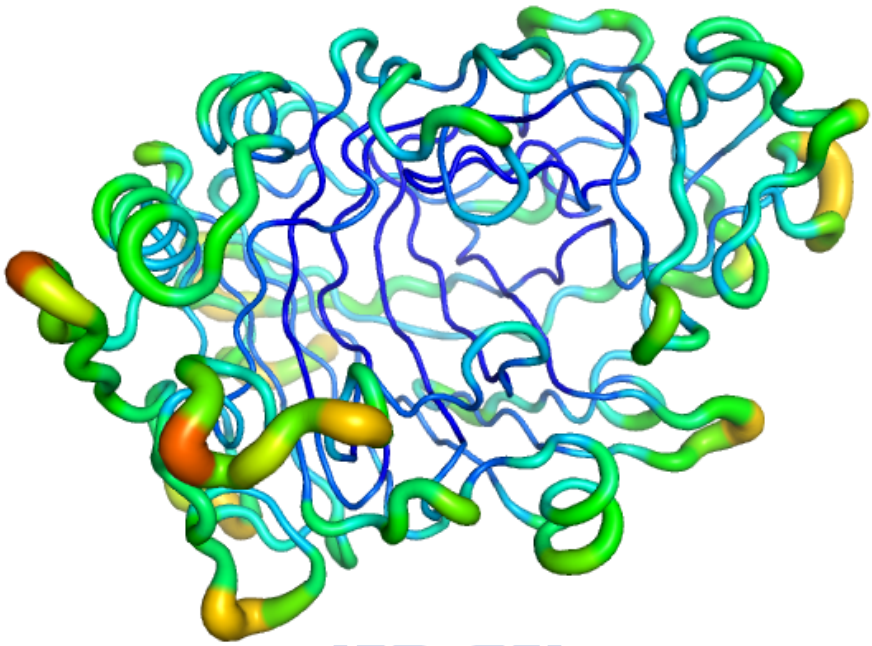


Figure 7. Proteins are cartoon form. (F) 2NLR experimental binding site residues colored in red. (G) The residues under WCN threshold (< -0.5) are colored in orange. (H) The residues selected include WCN and RSA threshold are also colored in orange.

(A)



(B)

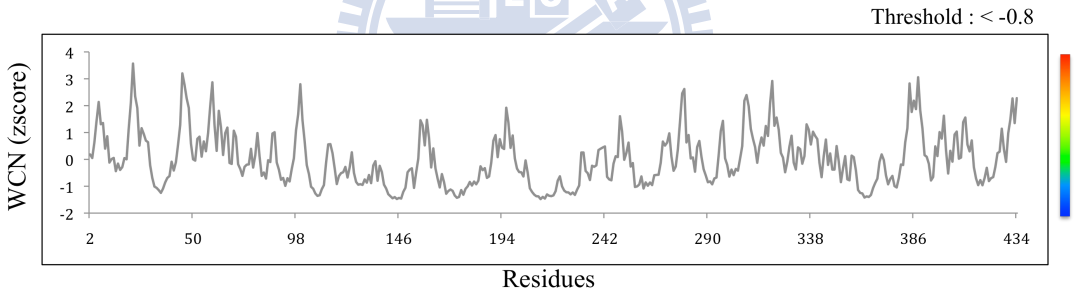
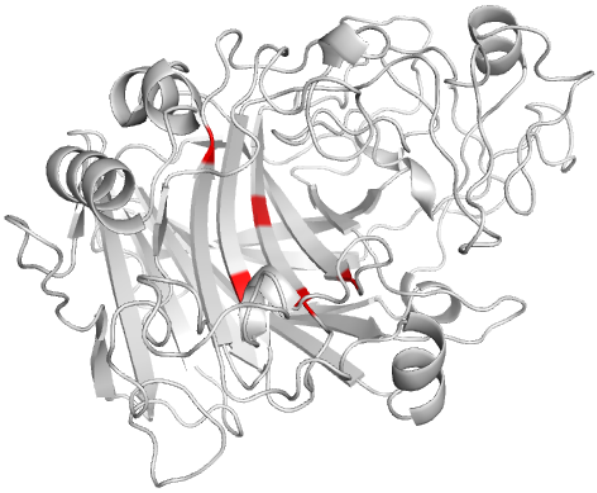
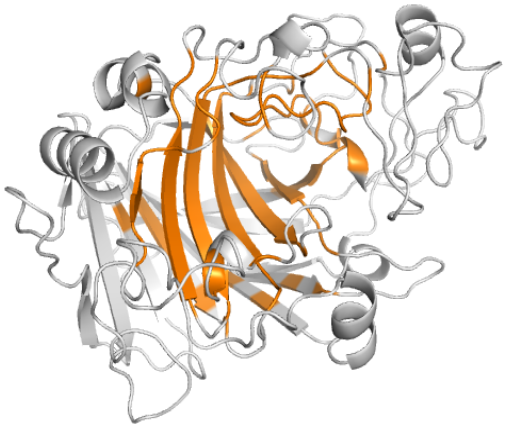


Figure 8. (A) 1CEL protein WCN model in putty form. (B) The WCN z- score distribution of protein 1CEL.

(C)



(D)



(E)

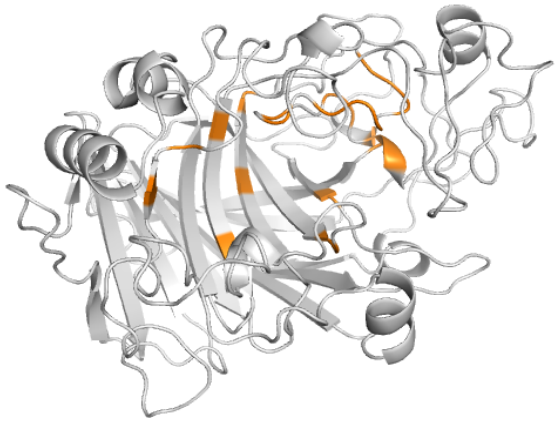
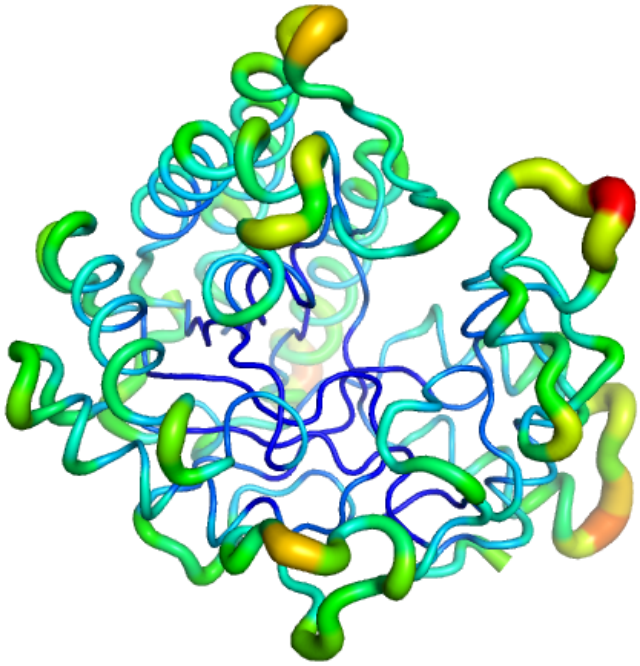


Figure 8. Proteins are cartoon form. (C) 1CEL experimental binding site residues colored in red. (G) The residues under WCN threshold (< -0.8) are colored in orange. (H) The residues selected include WCN and RSA threshold are also colored in orange.

(A)



(B)

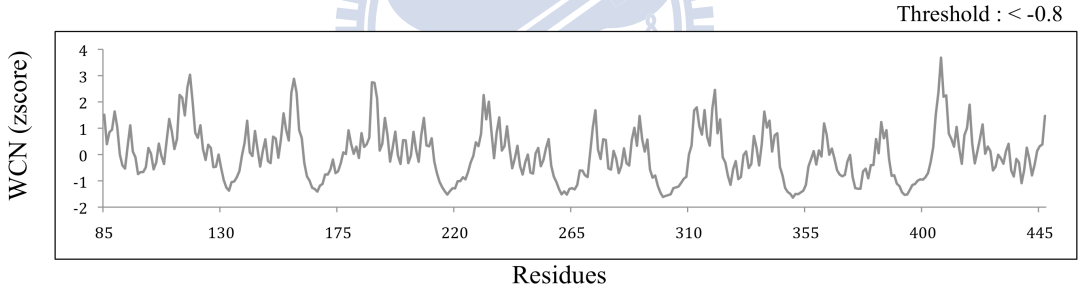
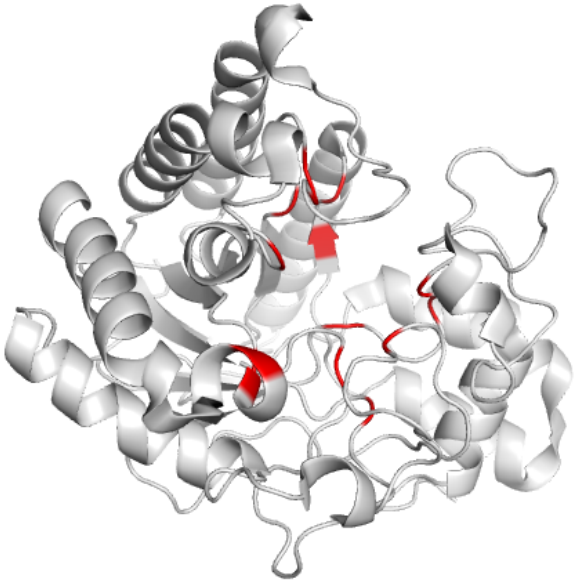
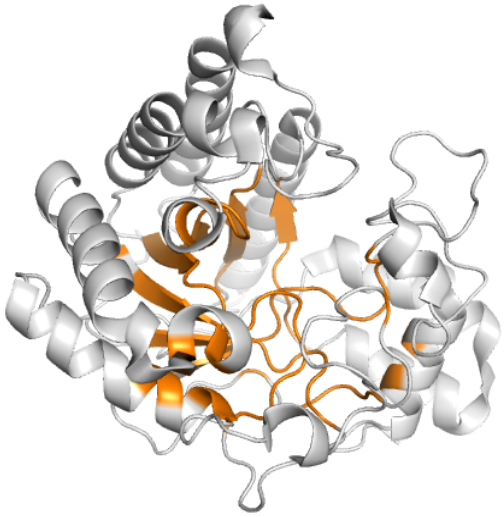


Figure 9. (A) 1QK2 protein WCN model in putty form. (B) The WCN z- score distribution of protein 1QK2.

(C)



(D)



(E)

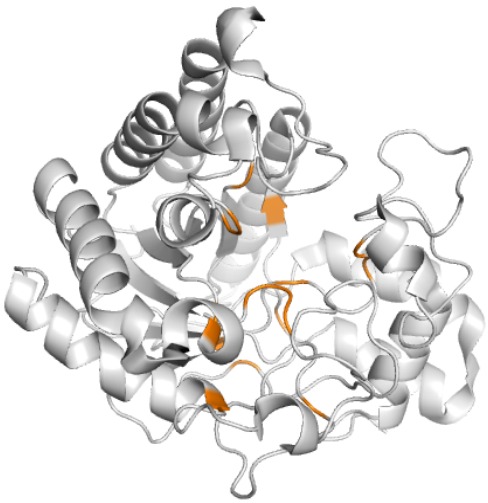
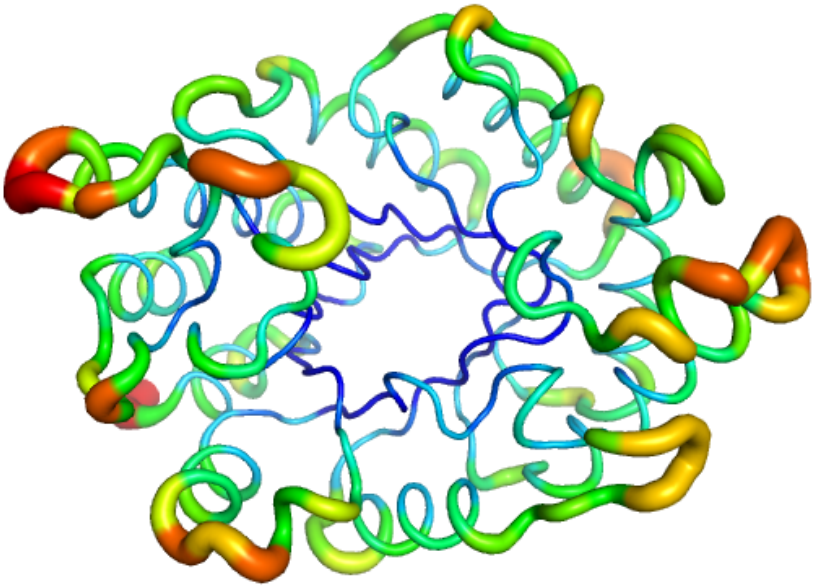


Figure 9. Proteins are cartoon form. (C) 1QK2 experimental binding site residues colored in red. (G) The residues under WCN threshold (< -0.8) are colored in orange. (H) The residues selected include WCN and RSA threshold are also colored in orange.

(A)



(B)

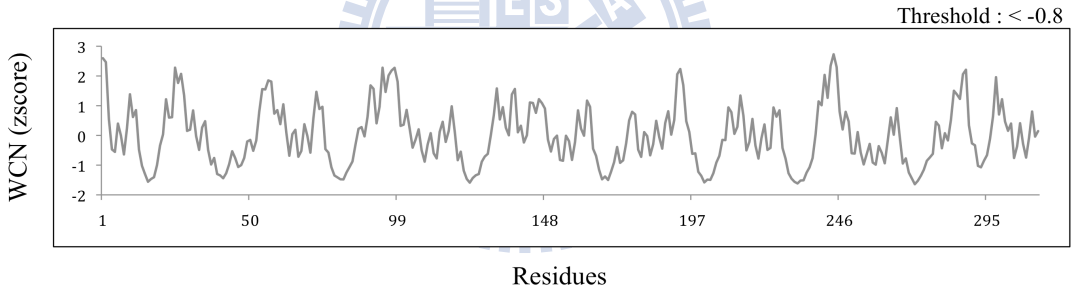
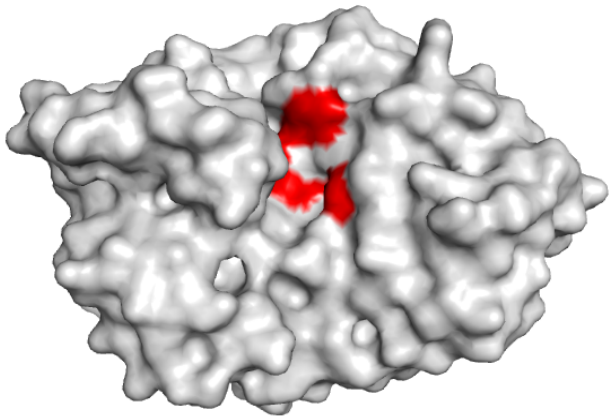
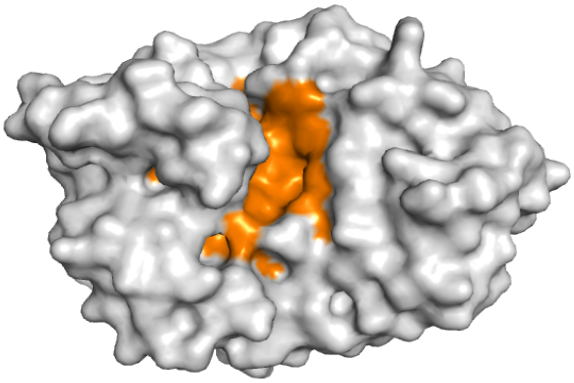


Figure 10. (A) 1EXP protein WCN model in putty form. (B) The WCN z- score distribution of protein 1EXP.

(C)



(D)



(E)

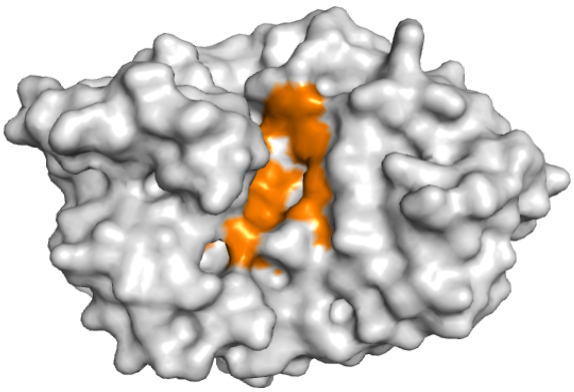
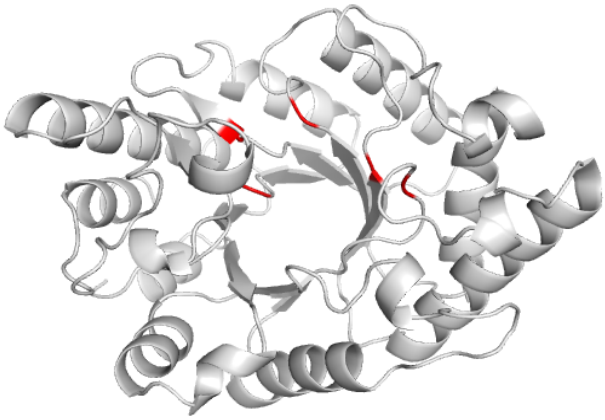
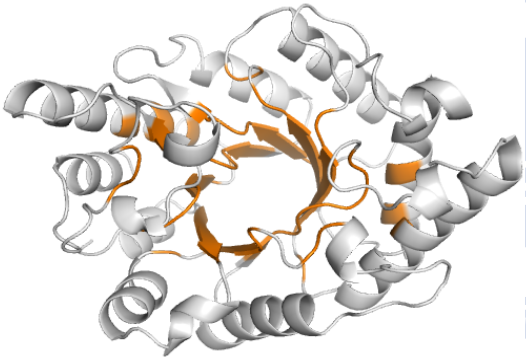


Figure 10. Proteins are surface form. (C) 1EXP experimental binding site residues colored in red. (D) The residues under WCN threshold (< -0.8) are colored in orange. (E) The residues selected include WCN and RSA threshold are also colored in orange.

(F)



(G)



(H)

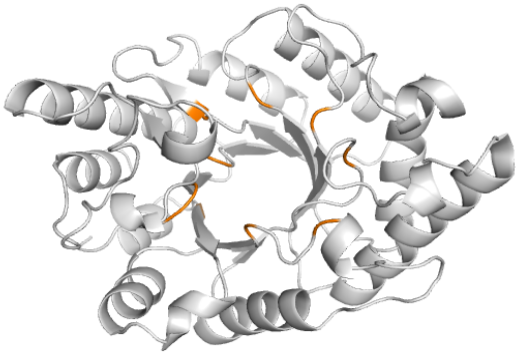
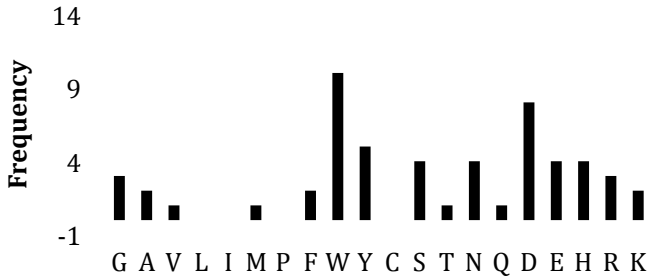
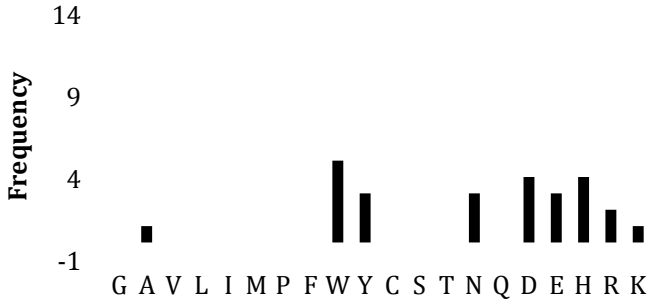


Figure 10. Proteins are cartoon form. (C) 1EXP experimental binding site residues colored in red. (G) The residues under WCN threshold (< -0.8) are colored in orange. (H) The residues selected include WCN and RSA threshold are also colored in orange.

(A)



(B)



(C)

(D)

Figure 11. The frequency of amino acid type in cellulase experimental binding site compared with our method of binding site prediction base on WCN including RSA. Figures (A) to (D) show as follows, Endo-glucanases, Exo-glucanases in experimental and Endo-glucanases, Exo-glucanases based on our method.