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APRIL

WEEK1

Date	04/01/2020-04/11/2020

Aim	Determine the research topic
Participant	All
Content	1. The topics discussed in the meeting focused on *The Guidelines for the Application of Key Projects of "Synthetic Biology" in 2020 (draft for comments)* and *The Guidelines for the Application of Key Projects of "Synthetic Biology" in 2019 (draft for comments)*； 2. Divided into four groups for discussion, group one has no results, group two, group three, and group four considered the related content of protein engineering, modification of E. coli metabolic pathway, environmental heavy metal detection and other topics of interest.
Result	We unanimously believed that the environment is a hot topic at the moment, and it is also a topic of great significance related to human survival, and we planned to explore interesting and meaningful topics in the direction of the environment.

WEEK2

Date	04/12/2020-04/18/2020
Aim	Determine the research topic
Participant	All
Content	Since last week's topic discussion was inconclusive, the instructor suggested a possible direction: PET and PETase. We checked relevant literature and news on the Internet and learned about PET and PETase. The superiority of PETase. Compared with its homologous enzymes, PETase not only has the ability to degrade PET with low crystallinity, but more importantly, this enzyme also has a strong ability to degrade PET with high crystallinity, which is far superior to other homologous enzymes.
Result	By consulting the information, we learned the following: The severity and urgency of the plastic pollution problem. A large amount of PET is manufactured and used every year, but only a small part of it can be recycled. There are a large number of PET products remaining in the environment, which poses a huge threat to the ecological environment and will eventually be fed back to human beings. In contrast, biological enzymatic hydrolysis has outstanding advantages. Among them, PETase has shown great advantages. Compared with its homologous enzymes, PETase not only has the ability to degrade PET with low crystallinity, but more importantly, this enzyme also has a strong ability to degrade PET with high crystallinity. We are aware of the research potential and significance of this subject, and we are determined to carry out research on PETase.

WEEK3

Date	04/19/2020-04/25/2020
Aim	Choose specific transformation directions
Participant	All

Content	1. Reading the literature *Enzymatic Degradation of Plant Biomass and Synthetic Polymers* etc., combined with daily life, it is found that there are great problems in the recycling and degradation of plastic bottles (PET bottles). A large number of them are manufactured and used, and only a small amount can be recycled. Moreover, the recovery means are physical and chemical recovery, which wastes energy, causes pollution and is inefficient, so it is difficult to achieve the purpose of recycling; 2. It is mentioned in the literature that the degradation efficiency of PETase is very low and cannot meet the needs of actual production and life, so we decided to modify it to improve its degradation efficiency.
Result	The problems in the plastic recycling process, physical and chemical recycling, high cost and low recovery rate. We have also put forward many ideas: transform the substrate binding pocket, stabilize the catalytic triad, improve the stability of PETase and substrate binding, and inhibit contact products and so on. But no specific transformation direction was finalized.

WEEK4

Date	04/26/2020-05/02/2020
Aim	Explore the direction of transformation
Participant	All
Content	1. Read the literature *An Engineered PET Depolymerase to Break Down and Recycle Plastic Bottles*, study its subject background, transformation ideas, technical details, etc., to prepare for our own research of PETase and its substrate PET； 2. Focused on the research on the modification methods of LCC in the literature, the calcium ion binding site in the enzyme was mutated to introduce disulfide bonds, and multiple mutations were combined at the same time； 3. Discussed and exchanged about this document, and started the process of document research.
Result	1. We have gained a lot of background knowledge related to PET； 2. Understand the research status of PET degrading enzymes such as LCC and PETase, as well as some of the advantages of these enzymes, such as the high thermal stability of LCC, and the degradation ability of PETase for high crystallinity PET, etc..

MAY

WEEK1

Date	05/03/2020-05/09/2020
Aim	Determine the transformation direction
Participant	All
Content	Deepen the understanding of the literature last week, especially the predecessors' understanding of the modification of the thermal stability of LCC

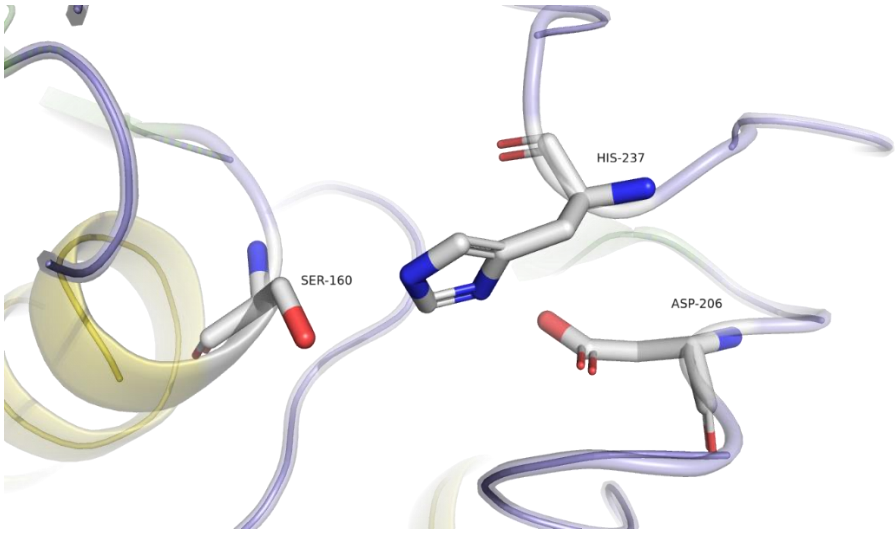
	and the limitation of PETase that only maintains activity at room temperature and degrades PET with low crystallinity.
Result	LCC is limited by its own thermal stability to the degradation efficiency of PET. Improving the thermal stability of LCC will be of great significance to the improvement of the degradation efficiency of low crystallinity PET. At the same time, PETase also has similar problems, so we analogize PETase to improve the thermal stability of PETase, so that it can remain stable in the high temperature environment in vitro. If the Tm value can be increased above the glass transition temperature of PET, the degradation activity of PETase will produce a qualitative leap. Therefore, the PETase transformation plan was finally finalized: by improving the thermal stability of PETase, improving its catalytic degradation ability of high crystallinity PET.

WEEK2

Date	05/10/2020-05/16/2020
Aim	Seek ways to improve enzyme thermal stability
Participant	All
Content	1. Learn about a molecular visualization software pymol from our instructor, and start to organize everyone to learn this software； 2. Read the literature Rational Protein Engineering of Thermo-Stable PETase from Ideonella sakaiensis for Highly Efficient PET Degradation, and understand the previous research ideas and transformation methods of PETase; 3. For the need to modify the protein, we have learned the physical and chemical properties of amino acids, such as hydrophobicity, polarity, charging status, etc., in order to select the best alternative amino acid when performing site mutations.
Result	It is known from the literature that the stability of a protein is mainly determined by the internal forces of the protein, such as hydrogen bonds, disulfide bonds, hydrophobic forces, etc. The predecessors started with these forces to improve thermal stability. So we decided on the direction of transformation from different molecular forces, and initially divided the teams into hydrogen bond group, salt bridge group, disulfide bond group, glycosylation group, proline group, hydrophobic force group, etc. The group, each is mainly responsible for the research of one kind of force, different people choose different forces as the transformation direction, and start the initial transformation.

WEEK3

Date	05/17/2020-05/23/2020
Aim	Research from different transformation directions
Participant	Shuying Wu, Yanjiao Lu, Yuan Wang, Guosheng Gao, Yuhan Li, Xujia Liu, Yufei Zhou, Dewu Liu, Xinyi Zhang, Tao Fu, Haoyang Shi, He Zhang, Duo Zhang
Content	1. In order to evaluate the local thermal stability better, we introduced B factor

	as one of the preliminary evaluation methods； 2. Read more literature about PETase research, such as Structural insight into catalytic mechanism of PET hydrolase, etc., and learn the reform ideas in the literature; 3. Check the literature Computationally designed libraries for rapid enzyme stabilization to find computational prediction software (dry experiment tools) to test the mutation results and overcome the difficulty of entering the laboratory for actual wet experiments due to the epidemic.
Result	1. Have a further understanding of the structure of PETase, clarify the catalytic triad, pocket region, and some residues that play a key role in the catalytic process, such as W159, and study the reform ideas in the literature;. 2. Found a batch of protein thermal stability prediction software such as FoldX, Rosetta and a series of MD software, as well as auto dock vina for molecular docking, etc., and determined the result test plan (the basic idea of dry experiment) using protein thermal stability prediction software FoldX in combination with MD to predict the mutation results.  Figure 1 The catalytic triad of PETase.

WEEK4

Date	05/24/2020-05/30/2020
Aim	Research from different transformation directions: hydrogen bond, pocket hydrophobicity
Participant	Guosheng Gao, Yuhan Li, Xujia Liu, Dewu Liu, Xinyi Zhang, Duo Zhang
Content	1. Hydrogen bond: Reading the literature *Enhanced catalytic site thermal stability of cold-adapted esterase EstK by a W208Y mutation etc.,* get ideas; 2. Pocket hydrophobicity: The substrate PET itself is hydrophobic. While enhancing the hydrophobicity of the PETase substrate binding pocket, it can not only increase the enzyme activity by promoting the binding of the substrate and the enzyme, but also enhance the hydrophobic interaction and improve the thermal stability of the enzyme.
Result	1. Knowing that the lipase mutation W208Y would produce increased

	hydrogen bonds, we searched for similar sites in PETase and found the S238D+W159Y mutant. After PyMOL mutation, the result was similar to W208Y, and both produced Additional hydrogen bonds; 2. The transformation of the hydrophobic direction has great potential and needs to be further explored.

JUNE

WEEK1

Date	06/01/2020-06/06/2020
Aim	Research from different directions: π-π interaction, glycosylation
Participant	Guosheng Gao, Yuhan Li, Xujia Liu, Xinyi Zhang
Content	1. Glycosylation：After reading *Stabilizing leaf and branch compost cutinase (LCC) with glycosylation: Mechanism and effect on PET hydrolysis*, it is concluded that LCC has a high aggregation tendency, which is the main reason for its thermal inactivation. Glycosylation can prevent aggregation by improving protein conformational stability or by imposing steric constraints that inhibit protein-protein interactions. 2. π - π interaction：Reading the literature *A conserved and buried edge-to-face aromatic interaction in small ubiquitin-like modifier (SUMO) has a role in SUMO stability and function* and *Carbohydrate – Protein aromatic ring interactions beyond CH/π interactions: A Protein Data Bank survey and quantum chemical calculations etc.*，learn and understand π-π interaction and find ideas.
Result	1. Inspiration from the literature: study whether PETase also has thermal aggregation phenomenon, introduce glycosylation at natural sites or artificially introduce sites, and detect PETase activity at high temperature. Since this is a protein modification work, and there is no related literature that reports PETase thermal aggregation, we temporarily abandoned this research direction. 2. Understand the essence of π-π interaction, which is a kind of weak hydrogen bonding. The reason for its formation is the mutual attraction of electron clouds between aromatic systems. There are three main types of aromatic interactions, namely F-type accumulation, edge-to-face accumulation and T-type accumulation. The F-type accumulation will adversely affect the system due to the strong repulsion of the electron cloud and reduce the stability. There is no positive repulsion between the latter two electron clouds, and the electron clouds with opposite electrical properties attract each other, making the system more stable. Understand the geometrical requirements for the formation of π-π interaction, and proceed to carry out related mutations in PETase. Finally, the π-π interaction was selected for in-depth research, and the research direction of glycosylation was abandoned.

WEEK2

Date	06/07/2020-06/13/2020
Aim	Research from different transformation directions: proline, disulfide bond
Participant	Shuying Wu, Yuan Wang, He Zhang
Content	1.Proline： Looking at the literature *Review: Engineering of thermostable enzymes for industrial applications*, it is found that proline substitution can improve the thermal stability of the enzyme: Pro (proline) is more special, containing an imino group, a carboxyl group and a pyrrolidine ring. The unique cyclic structure of the side chain gives Pro a special conformational rigidity relative to other amino acids, and has a special role in protein thermal stability.Use the protein stability prediction website http://kazlab.umn.edu/ to find a suitable proline substitution site, use the modeling website https://web.expasy.org/protparam/ to model the mutant, use The thermal stability prediction website https://servicesn.mbi.ucla.edu/SAVES/ evaluates the stability of the mutant, and uses the software PyMOL to conduct in-depth research on the structure of the mutant. 2.Disulfide bond：We found that the introduction of disulfide bonds into the calcium binding sites in the homologous LCC and Tfcut2 has achieved good results. For Tm, Tfcut2 (D238C/S283C) increased by about 24.9°C (69.8 to 94.7), and LCC (D204C/E253C) increased by about 9.5° C (84.7 to 94.2). We analyzed the structure of calcium ion binding sites in LCC and Tfcut2.
Result	1. A suitable proline substitution site (E246P) was found, and it was found that the proline mutation site predicted by the website could not meet the requirements for improving stability. On the one hand, the b-factor value of the site selected by the website was low. It belongs to the region with relatively stable structure; on the other hand, the stability of the protein after mutation decreases, which is contrary to our original intention. Need to continue research. 2. The introduction of disulfide bonds into calcium ion binding sites in LCC and Tfcut2 was successful. It is found that these positions do meet part of the geometric criteria of the disulfide bond structure (mainly the distance between Cα-Cα and Cβ-Cβ). These results indicate that it is feasible to introduce disulfide bonds at the calcium ion binding site. A homology comparison was performed to speculate the calcium binding site of PETase (E175-N204-S253), and the group of mutations N233C/S282C was finally determined from a geometric perspective.

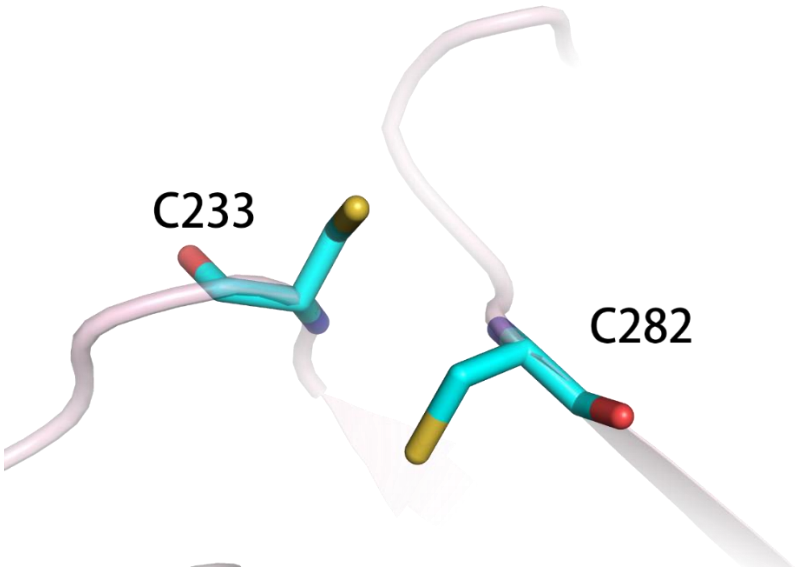
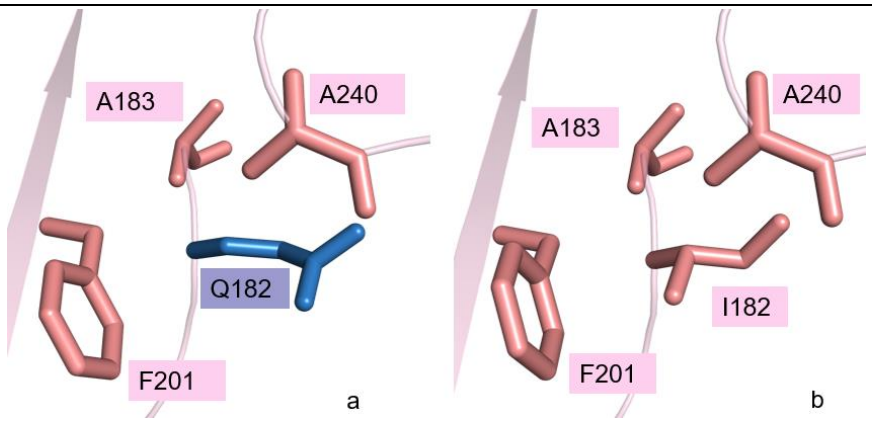


Figure 2 Schematic diagram of N233C/S282C disulfide bond. And it can be seen that the construction of the disulfide bond failed.

WEEK3

Date	06/14/2020-06/20/2020
Aim	Research from different transformation directions: salt bridge, hydrophobic core
Participant	Guosheng Gao, Yuhan Li, Xujia Liu, Tao Fu, Duo Zhang
Content	1. Salt bridge：Check information to learn about salt bridge. Learn about basic mutation methods by reading the literature *Evolutionary Stability of Salt Bridges Hints Its Contribution to Stability of Proteins*； 2. hydrophobic core：Read the literature *Enthalpic and entropic contributions to hydrophobicity*, etc. to learn related knowledge, explore and analyze ideas.
Result	1. Understand the basic mutation method: single point mutation of endogenous amino acid, select an endogenous amino acid, find the mutation point within 5 amino acids nearby, and try our idea through PyMOL; 2. It is understood that the amino acids in the main hydrophobic binding sites of the protein have larger hydrophobic side chains, forming a tighter hydrophobic core, which can enhance the thermal stability of the enzyme; the amino acids with larger side chains on the β-sheet may reduce the thermal stability , but it can increase enzyme activity and lower the optimal pH (glucose isomerase); the non-polar amino acid Leu on the α-helix may enhance thermal stability (glucose isomerase). We believe that the stability improvement of the hydrophobic core region has great potential and will continue to be studied in the future.

	 Figure 3 One of the hydrophobic cores we structured successfully. The Polar residue Q182 is showed in blue(a), which is mutated to the hydrophobic residue Ile(b).

WEEK4

Date	06/21/2020-06/30/2020
Aim	Combine mutations within a single force and set the target DDG≤-10kcal/mol
Participant	Shuying Wu, Yanjiao Lu, Guosheng Gao, Yuhan Li, Xujia Liu, Xinyi Zhang, Tao Fu, Haoyang Shi, He Zhang, Duo Zhang
Content	1. Hydrogen bond: Reading the literature *分子动力学模拟研究 CGTase 酶活性区域的热稳定性 and Extended protein/water Hbond networks in photosynthetic water oxidation*, etc. gave great inspiration for the construction of our hydrogen bond network. We mutated the A152N/N173R/S193R/S214H site in an attempt to construct a hydrogen bond network; 2. Salt bridge: Use homologous enzyme sequence alignment to observe the salt bridges that exist in the enzymes with high thermostability such as LCC, CUT190, TFCUT2 but not in PETase, and introduce mutation points in PETase;
Result	1. Hydrogen bond：Mutation was made at the A152N/N173R/S193R/S214H sites, but calculated by FoldX, its DDG was -5.43591kJ/mol, which was far lower than expected. For this reason, we conducted visual screening and found that many new hydrogen bonds in single point mutations did not appear in combined mutations. In this regard, we believe that hydrogen bonds are greatly affected by conformational changes, and other site mutations will have a greater impact on the formation of hydrogen bonds, so the expected hydrogen bond network is not formed when combining mutations. In the future, we will continue to analyze the hydrogen bond network in the combined mutants in order to make a greater contribution to the improvement of thermal stability. Have a clear understanding of the level of thermal stability of single-point mutations and single-force combination mutations, and hope to find more excellent mutation sites to achieve the goal of DDG. 2. Salt bridge: Single point mutations such as A40D, T116D/R, and D220E have been made, and new salt bridges have been successfully introduced.

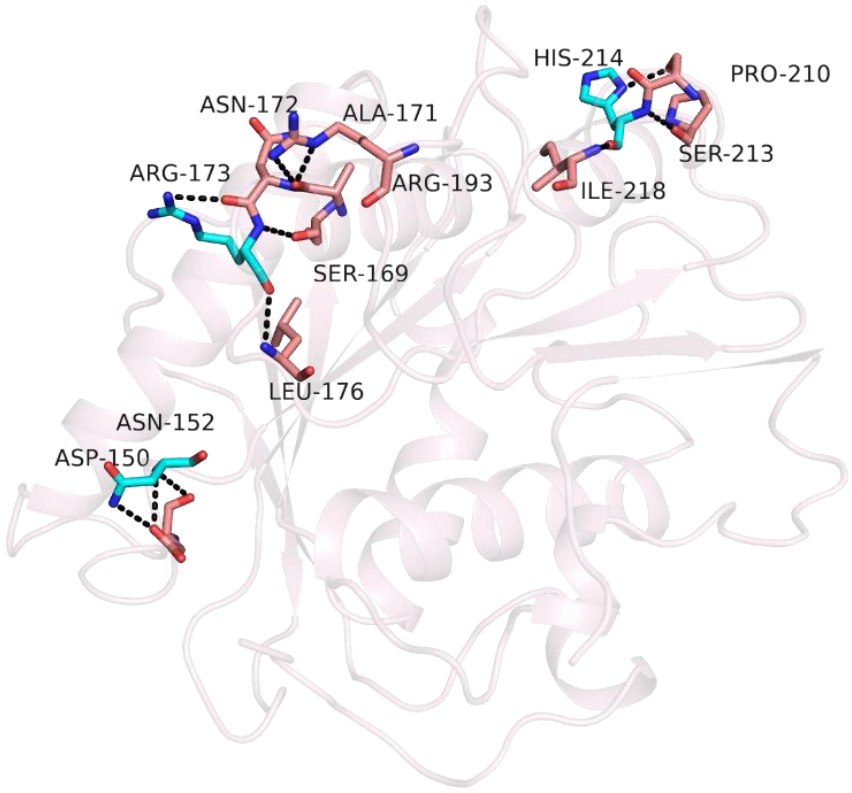


Figure 4 Schematic diagram of A152N/N173R/S193R/S214H mutation. The mutant builds a small hydrogen bond network.

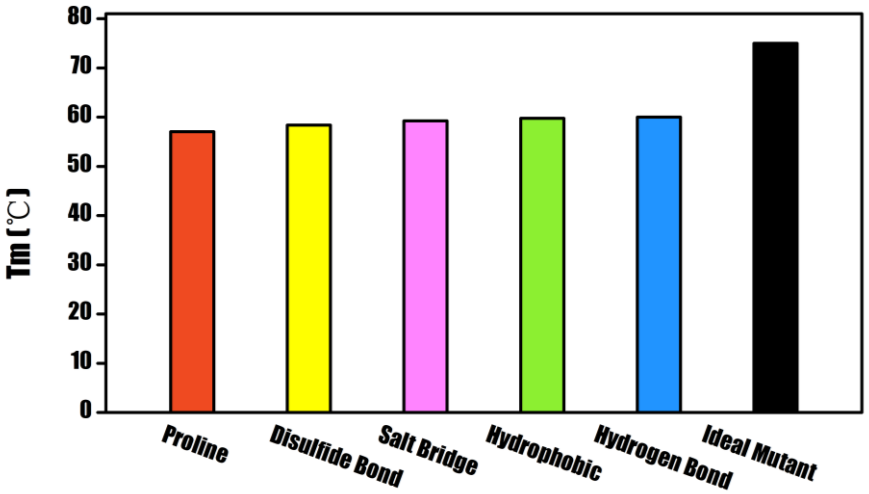


Figure 5 Each single force cannot reach the temprature we demand.

JULY

WEEK1

Date	07/01/2020-07/11/2020
Aim	1. Further study single points and provide more single points;

	2. Further analysis of mutants by homology comparison.
Participant	Shuying Wu, Yanjiao Lu, Yuan Wang, Guosheng Gao, Yuhan Li, Xujia Liu, Dewu Liu, Xinyi Zhang, Tao Fu, Haoyang Shi, He Zhang, Duo Zhang
Content	Observe and study the unstable regions in PETase, that is, the sites with high B factor values. Check the corresponding structure on the PDB website, and hope to add molecular forces of proteins in these regions, such as mutations adding hydrogen bonds, salt bridges, hydrophobicity, etc. 1. Hydrogen Bond：Find single-point mutations based on homology alignment and website predictions, and read the literature on mutating hydrogen bonds to improve PETase thermal stability: *Rational Protein Engineering of Thermo-Stable PETase from Ideonella sakaiensis for Highly Efficient*; at the same time, use homologous enzyme sequence comparisons, using b-factor as a measure, introduce stable sites in Tfcut2, LCC, Cut190 into PETase; 2. Proline：Read the literature *Increasing protein stability by improving Beta-turn* Continue to study proline substitution; 3. Hydrophobic：Through the comparison of homologous enzyme sequences, observe the enzymes with high thermostability such as LCC, CUT190, TFCUT2, expand the hydrophobic site mutation library.
Result	1. Successfully constructed new hydrogen bonds at S46D, A47R, T56R, T72R, R90D, S92D, T116R, S136N, G139R, Y146R, K148R, A152N/S, N173R, S188N, I208Q, D220N, S278D and other sites, especially K148R stabilizes two loops, Y146R increases hydrophilicity and stabilizes the connection between loop and beta sheet; 2. It is understood that there is a proline residue preference at position i+1 in the structure of β-turn, and mutations such as S58P, S141P, S207P, and S223P have been determined; 3. The stable hydrophobic interaction contribution sites in LCC, CUT190, TFCUT2 and other enzymes were introduced into PETase, and effective sites with reasonable structure, data and empirical explanation were determined, such as V68I, A80V, S124A, Q127L, V134L, V149I, S166A, S169I, A179V, Q182L, T198V, N246I, K252L, N275I, etc., to further expand and determine the hydrophobic site mutation library.

WEEK2

Date	07/12/2020-07/18/2020
Aim	1. Improve the level of PyMOL mastery and unify the drawing standards; 2. Determine calculation software and basic plan.
Participant	All
Content	1. 1. Learn to use PyMOL and PyMOL to draw; 2. 3d the substrate to facilitate docking. Use autodock software for molecular docking; 3. A protein visualization software YASARA based on FoldX is installed to realize the visualization of FoldX operation and improve the computational efficiency.

Result	1. The PyMOL drawing standard is determined, and details such as color and angle when drawing are uniformly specified; 2. In the process of setting flexible residues, if the number of settings exceeds three, an error will occur. After consulting the data, it is found that this is a bug of the software. Since it is difficult to repair in a short time, I decided to change to another backup software auto dock vina. We started to try to connect the substrate to the pocket and observe on PyMOL, but the effect is not ideal; 3. A new experimental protocol was determined: using FoldX to perform saturation mutations at all sites of PETase, and adopt a reverse analysis method that uses data to guide visual screening to screen out all mutations that significantly improve stability, and perform visual screening on them. Outstanding mutations under double standards.

WEEK3

Date	07/19/2020-07/25/2020
Aim	1. Determine the molecular docking experiment protocol; 2. Use FoldX to make a full-point saturation mutation library.
Participant	Shuying Wu, Yanjiao Lu, Dewu Liu, Haoyang Shi, Kegen Chen, Yansong Bai
Content	1. Looking for software that can construct molecular docking model, tried various software such as discovery studio, sybyl, autodock, etc. 2. After continuous attempts, we began to use FoldX to initially evaluate the thermal stability of the mutants designed by the experiment. After that, we think it is better to carry out large-scale predictions to screen out the single mutations with the most significant improvement in thermal stability, and to guide the site selection together with visual structure screening.
Result	1. Finally, autodock was determined as the modeling software; but when testing the stability of autodock modeling, it was found that the setting of flexible residues would cause the software to report an error docking instance has no attribute 'ligMol'. In the end, I chose autodock vina with more complete software optimization to do the docking work, and at the same time learned the molecular docking tutorial and docking site knowledge. Try to dock PETase and BETH small molecules, the docking site is in the active pocket, the effect is very good; 2. We directly performed saturation mutations on 260 sites of PETase, constructed a full-site saturation single mutation stability database, and finally obtained a total of 4940 single-point mutations, and screened out 514 DDG drops within the error range (Stable mutations other than 0.46kcal/mol).

	 Figure Group 6 The library of saturated mutation on every site. The values on different levels are showed in different colors. The color darker, the stability level higher.

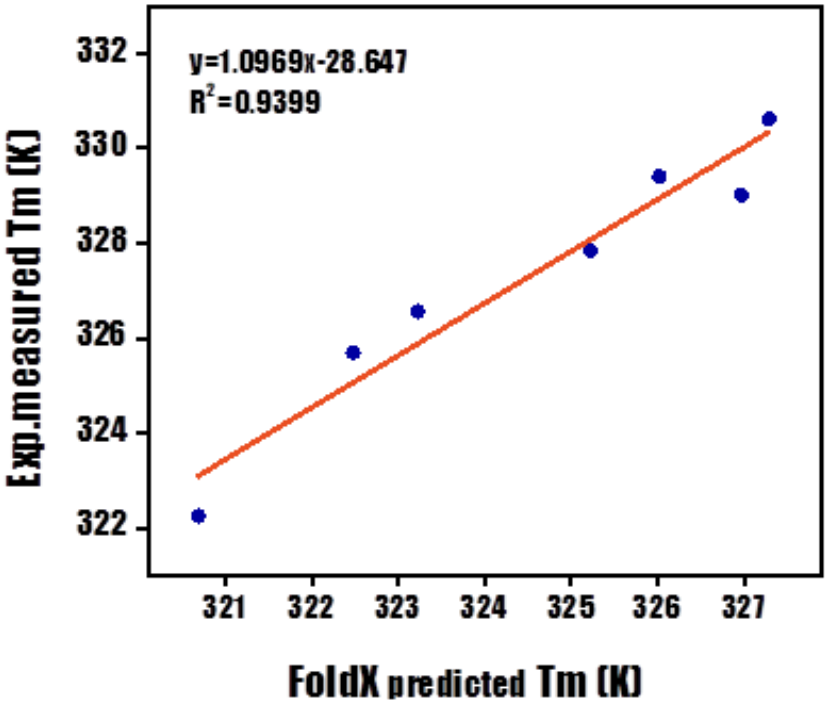
WEEK4

Date	07/26/2020-07/31/2020
Aim	Promote the mastery and experimental progress of Molecular Docking and Molecular Dynamics
Participant	Shuying Wu, Yanjiao Lu, Kegen Chen, Yansong Bai
Content	1. Learn MD theory process, install and learn to use MD software amber and GROMACS; 2. Learned the design points of gridbox parameters and literature such as *Structural insight into molecular mechanism of poly (ethylene terephthalate) degradation* to determine the setting of flexible residues, and read papers on the binding scores output by autodock vina such as *Can we use docking and scoring for hit-to-lead optimization?* and *Rescoring ligand docking poses* to further improve the accuracy of molecular docking.
Result	1. We found that there was a problem with amber, a software used for MD. the command pdb4amber could not be used. After asking for help from many parties and collecting a lot of data from the network, we still could not solve the problem, so we gave up amber and successfully installed and used GROMACS; 2. In the next few molecular dockings, the accuracy of the theoretical docking results that have been learned has been improved significantly.

AUGUST

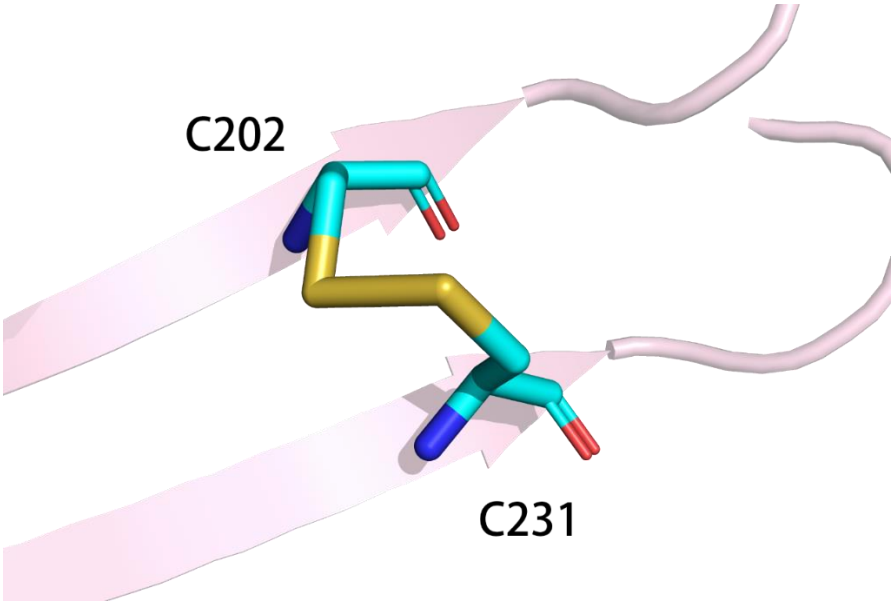
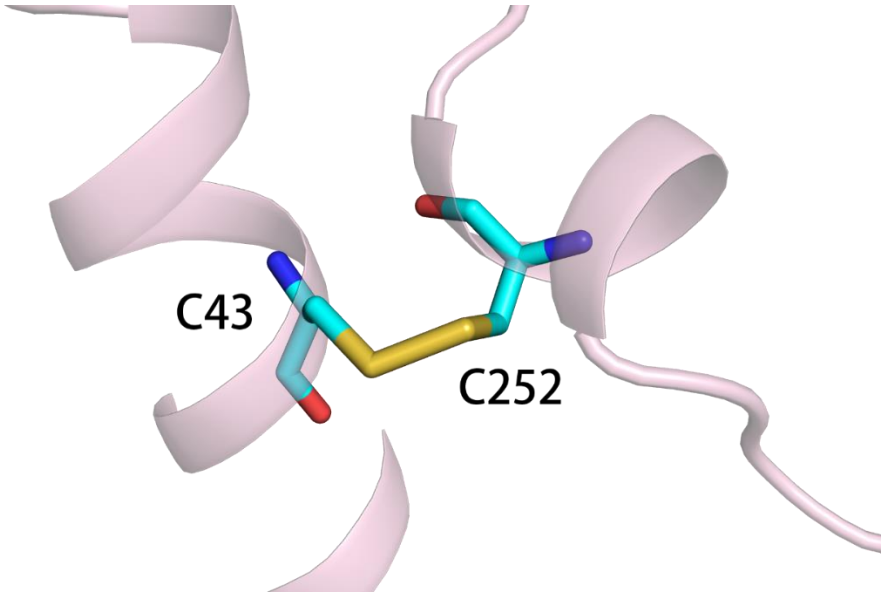
WEEK1

Date	08/01/2020-08/08/2020
Aim	Calculate the relationship between DDG and Tm
Participant	Yanjiao Lu, Dewu Liu, He Zhang, Yansong Bai
Content	1. In order to predict and process data, learn how to use Matlab to perform numerical fitting calculations; 2. Through reading literature and mathematical derivation, the PETase and homologous enzyme mutant DDG measured by FoldX are fitted to their Tm literature values to solve the relationship between the two.
Result	1. Use Matlab to successfully solve the problem of data prediction, and make related charts and PPT; 2. According to ΔG = -RTlnK, the Tm value corresponding to FoldX predicted DDG

	was derived and fitted with the literature value, and then the DDG predicted value was fitted with the Tm literature value. It was found that there is a linear relationship between DDG and Tm, and the correlation coefficient R=0.9399.  Figure 7 The relationship fitted between DDG and Tm.

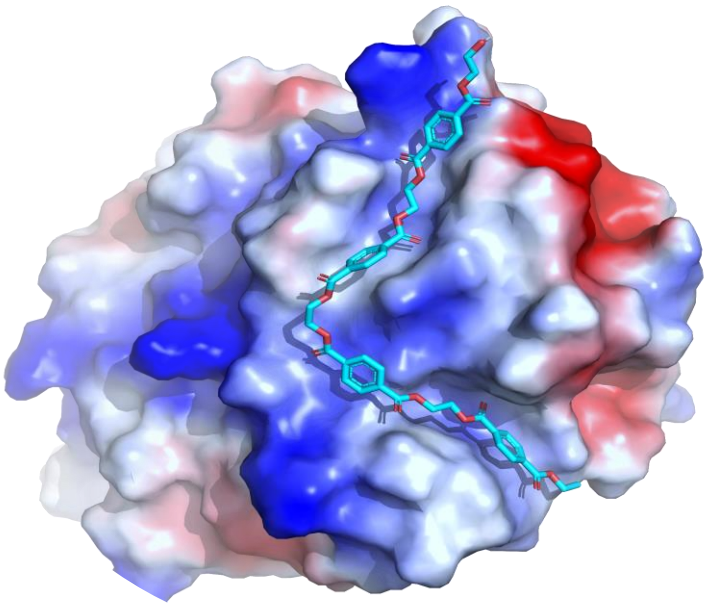
WEEK2

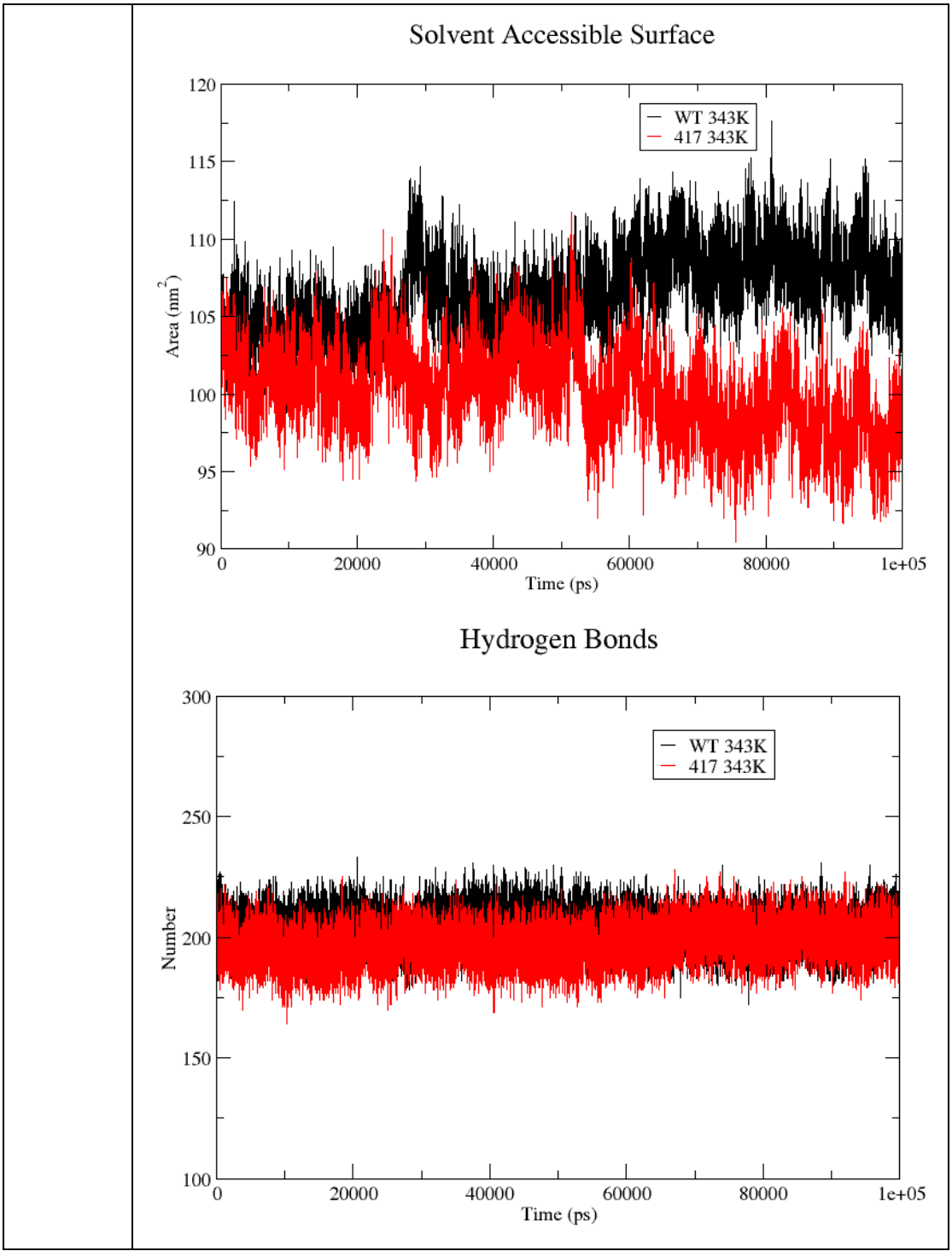
Date	08/09/2020-08/15/2020
Aim	Combine FoldX and multiple other forecasting websites to conduct in-depth analysis of their respective visual screening results
Participant	Shuying Wu, Yanjiao Lu, Yuan Wang, Guosheng Gao, Yuhan Li, Xujia Liu, Dewu Liu, Xinyi Zhang, Tao Fu, Haoyang Shi, He Zhang, Duo Zhang
Content	Introduce iMutant, Fireprot and other web pages to assist in the design of salt bridges and hydrogen bonds. For disulfide bonds, we searched on PudMed for the methods and current research status of artificial disulfide bonds in the past 15 years. Study the literature *Disulfide by Design 2.0: a web-based tool for disulfide engineering in proteins, Multi-Template Approach to Modeling Engineered Disulfide Bonds*, etc.. Use Biosynthesis software to perform geometric evaluation on all sites of the whole enzyme, and select the sites that may form bonds; study and research Biosynthesis software such as DbD2, SSBOUND, MODIP, Bridge-D ; conduct homology modeling of possible bond sites, observe bond formation, and measure each bond with PyMOL; FoldX energy evaluation. Study and analyze the thermodynamic explanation of the hydrophobic mechanism, the mechanism of the hydrophobic interaction between aromatic rings on the atomic surface, the analysis of the hydrophobic force between aliphatic chains at the atomic level, and the integration of different hydrophobic single point mutations.
Result	1. Design salt bridges and hydrogen bond networks.

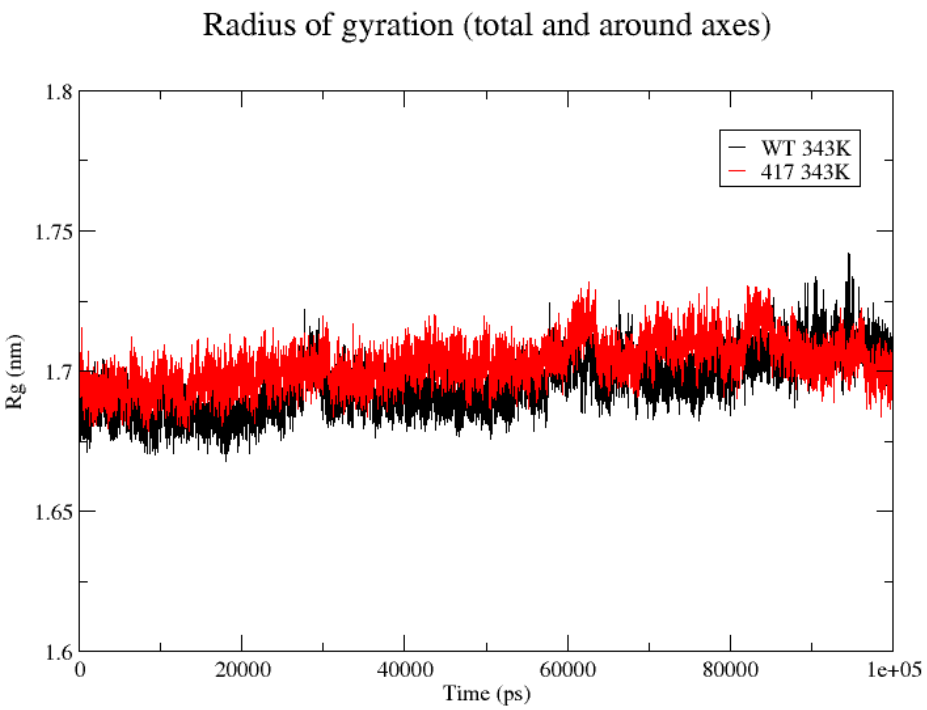
	2. The introduction of disulfide bonds in the homology region failed, and the mutant predicted by FoldX did not form disulfide bonds. There are 7 intersections between 10 MODIP Grade A sites and 40 DbD prediction sites: L43C-K252C, Y69C-A102C, V156C-S166C, A170C-V196C, P197-F261C, A202-E231C, A256C-F271C. Visually screen out two unreasonable mutations located in the protein, Y69C-A102C, V156C-S166C. FoldX screened out 3 Y69C-A102C, A170C-V196C, P197-F261C. Finally, successfully 2 L43C-K252C, A202-E231C.  Figure 8 A202-E231C disulfide bond. The disulfide bond formed is showed in yellow.  Figure 9 L43C-K252C disulfide bond. The disulfide bond formed is showed in yellow.

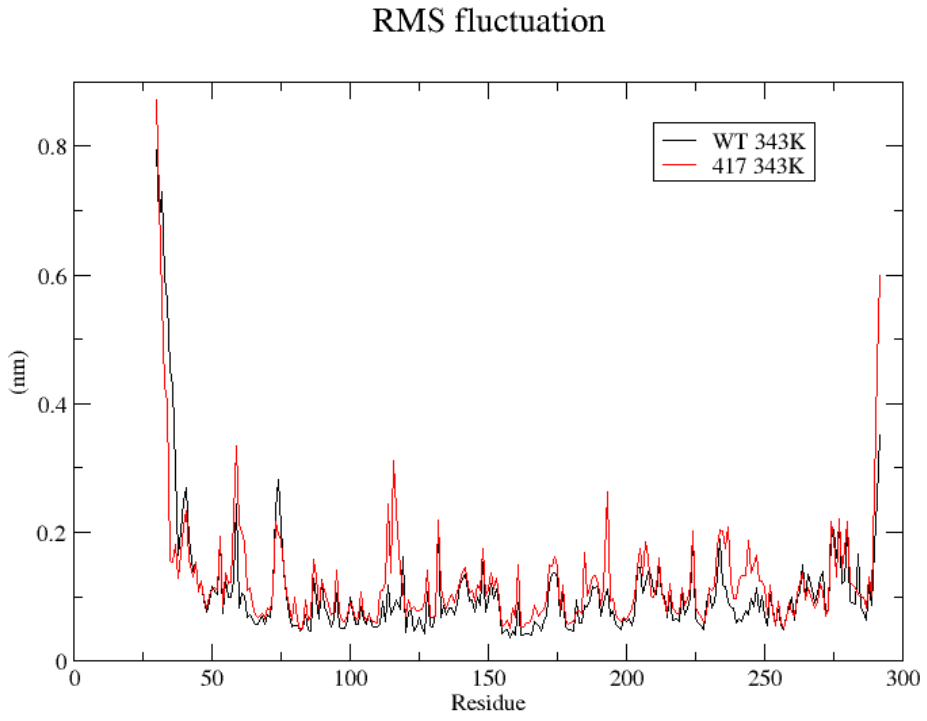
WEEK3

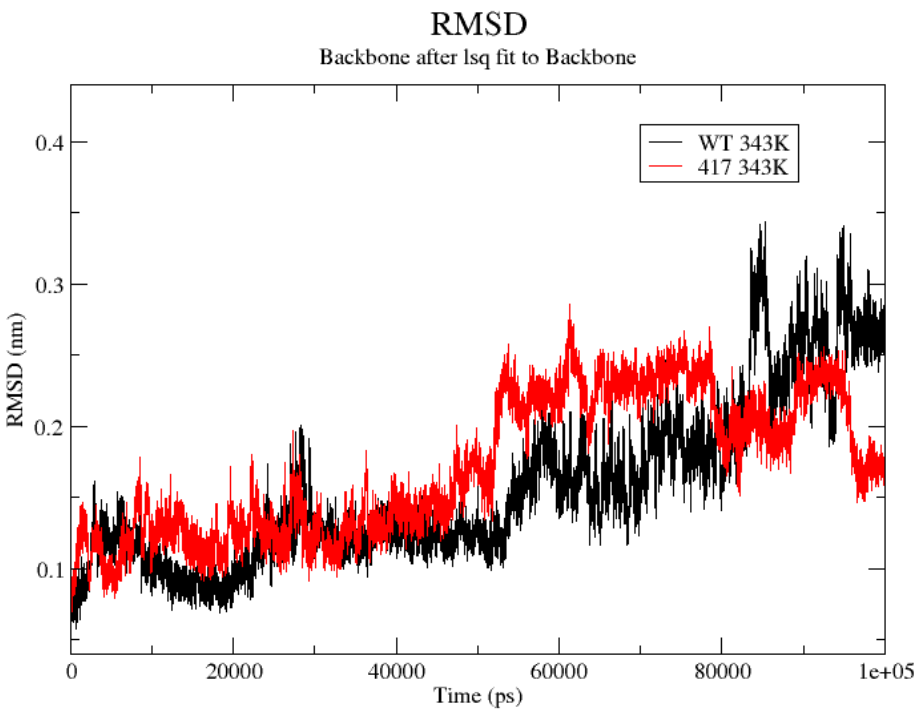
Date	08/16/2020-08/22/2020
Aim	1. Speed up MD operation;

	2. Determine MD parameters and analysis methods, and put MD into practice.
Participant	Yanjiao Lu, Kegen Chen, Yansong Bai
Content	1. Try to run Gromacs on a high-configuration computer to increase computing power; 2. Read the literature on the use of MD to study the thermostability of enzymes, and make preliminary attempts on software and analytical methods.
Result	Using software such as VMD xmgrace, we successfully analyzed the RMSD, RMSF, gyration radius, solvent accessible surface area, hydrogen bond, salt bridge, free energy topography, motion trajectory animation and other related content of small molecule proteins at different temperatures. The MD simulation process is very smooth. First, the protein structure energy is minimized, followed by protein position restriction simulation (NVT and NPT), and finally simulation. Molecular docking has been mastered and matured and can be directly applied.  Figure 10 Connection diagram of substrate binding pocket and PET


Solvent Accessible Surface
WT 343K
417 343K
Area (nm^2)
Time (ps)
Hydrogen Bonds
WT 343K
417 343K
Number
Time (ps)


Radius of gyration (total and around axes)
WT 343K
417 343K
Rg (nm)
Time (ps)
1.8
1.75
1.7
1.65
1.6
0
20000
40000
60000
80000
1e+05


RMS fluctuation
WT 343K
417 343K
(nm)
Residue
0.8
0.6
0.4
0.2
0
0
50
100
150
200
250
300



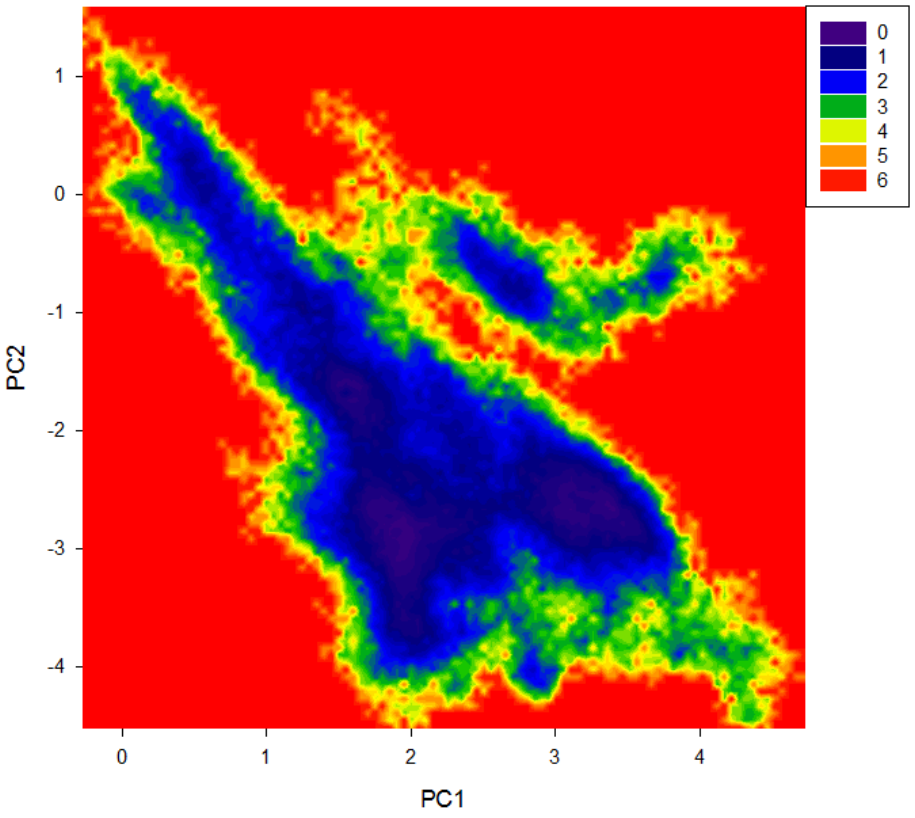
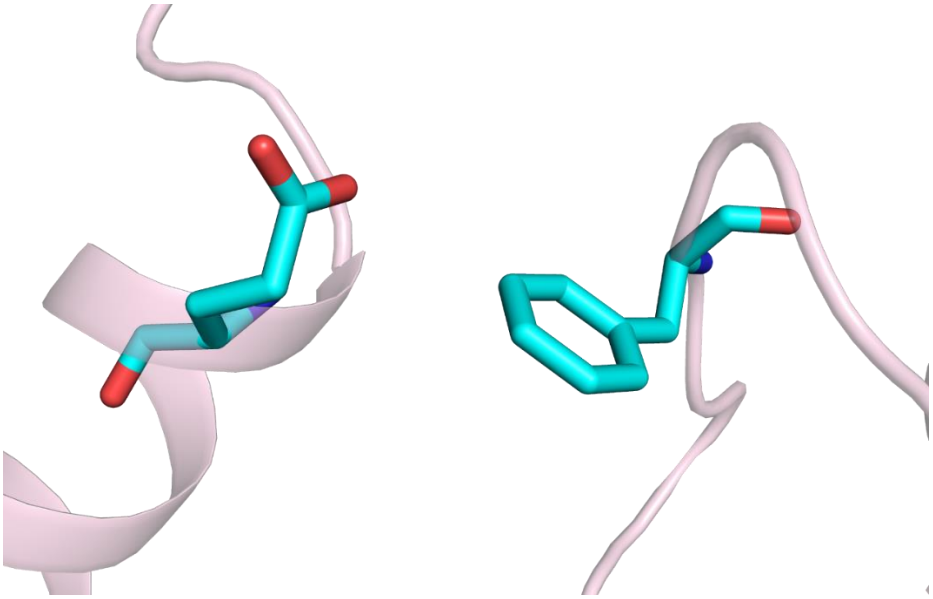


Figure Group 11 eg. The The consequence of MD analysis on the mutant NO.417.

WEEK4

Date	08/23/2020-08/31/2020
Aim	1. Explore the feasibility of reducing DDG to a lower level by combining different

	force mutation points.； 2. Prepare the preliminary model for modeling.
Participant	All
Content	1. A combination of mutations with a single force cannot achieve the expected stabilization effect, try a combination of mutations with multiple forces. Salt bridges, hydrogen bonds, disulfide bonds, proline, and hydrophobic parts begin to integrate their mutations, and use FoldX to predict their DDG; 2. Investigate all Best Models in the IGEM team in the past four years. Considering that our team's project is not suitable for biochemical reaction modeling, the modeling team collected relevant data on molecular docking, FoldX, Rosetta, and MD, and supplemented the prediction of TM based on FoldX.
Result	1. Hundreds of multi-point combination mutants containing multiple forces were made. When multiple forces were combined on the same mutant, most of them had no effect on each other, but some combinations showed strong synergistic effect; 2. Statistical summary of all the point mutations that significantly improved the structure of the team and significantly reduced the free energy of folding under the FOLDX prediction, and identified more than 20 point mutations that were finally used for combined mutations: T29D, A40P, S54W, S58P, A80V , S92P, T113P, T116R, Q119D, S121E/P, Q127L, S141P, Q182I, D186F, S193R, A202C, S214F, E231C, K252L, R260F, T266P, N275P/F. The basic principle of our combined mutation strategy is to average the single mutation in the spatial structure distribution and force type, and take into account the disulfide bond constructed by S121E / D186F, A202C/ E231C, S58P / S141P and other pairs of point mutations, or the synergistic effect of DDG value on improving thermal stability, a total of 543 twelve combination mutations were obtained, and energy prediction was started.  Figure 12 S121E/D186F Synergy

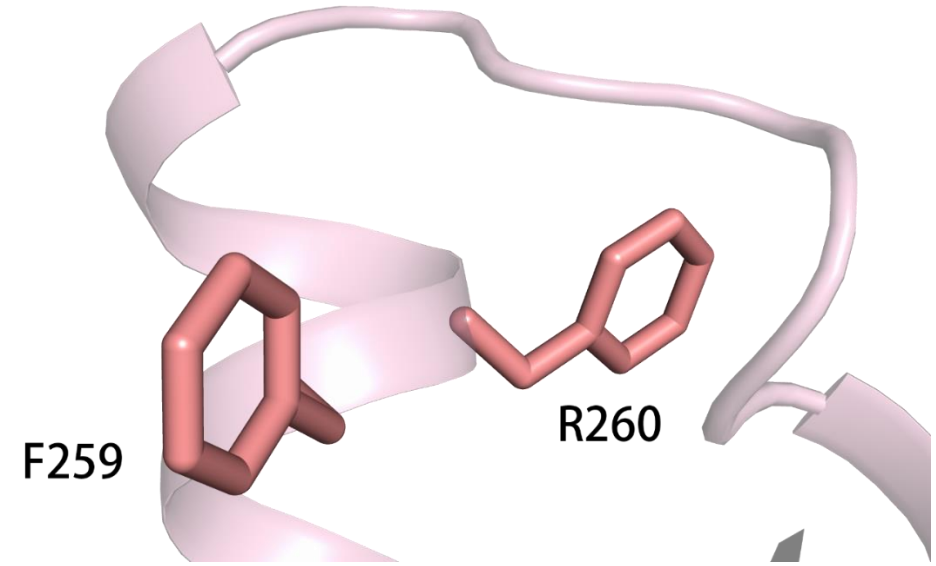
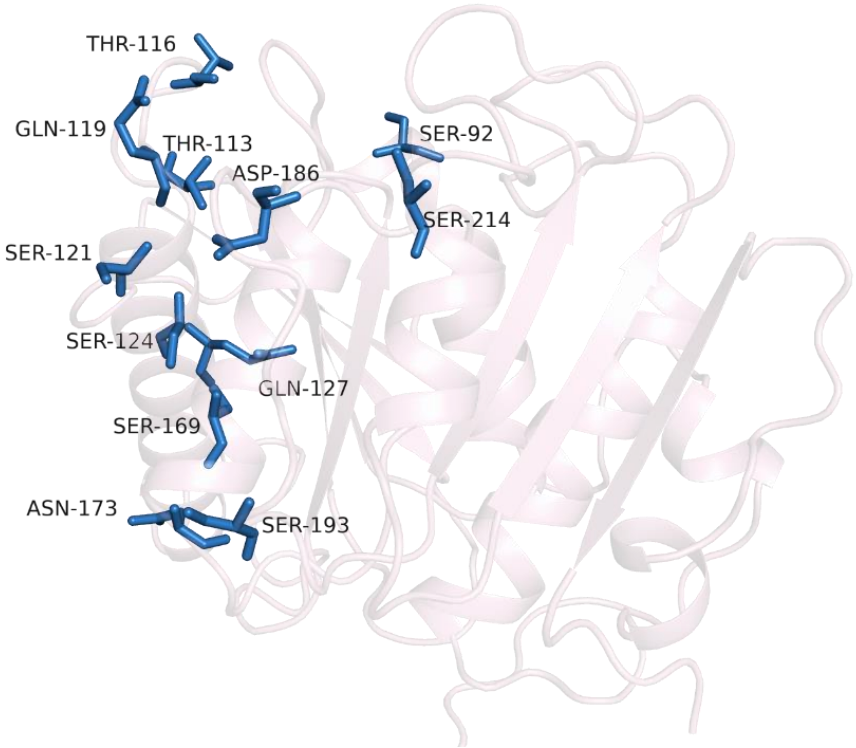
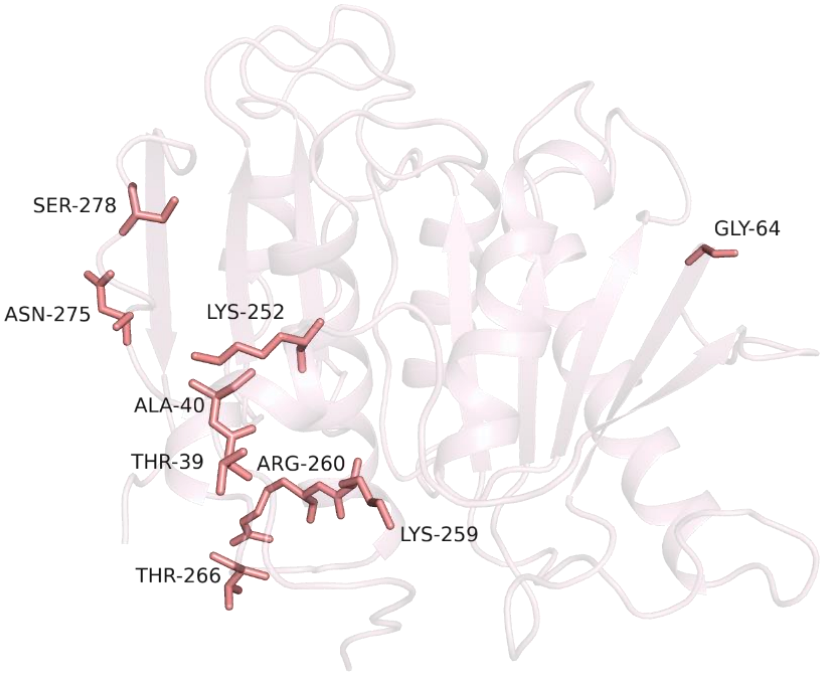
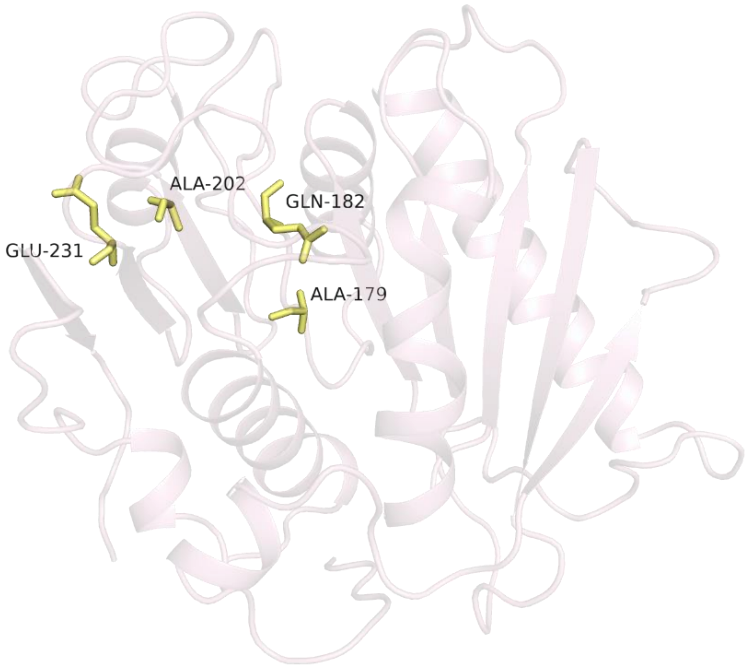
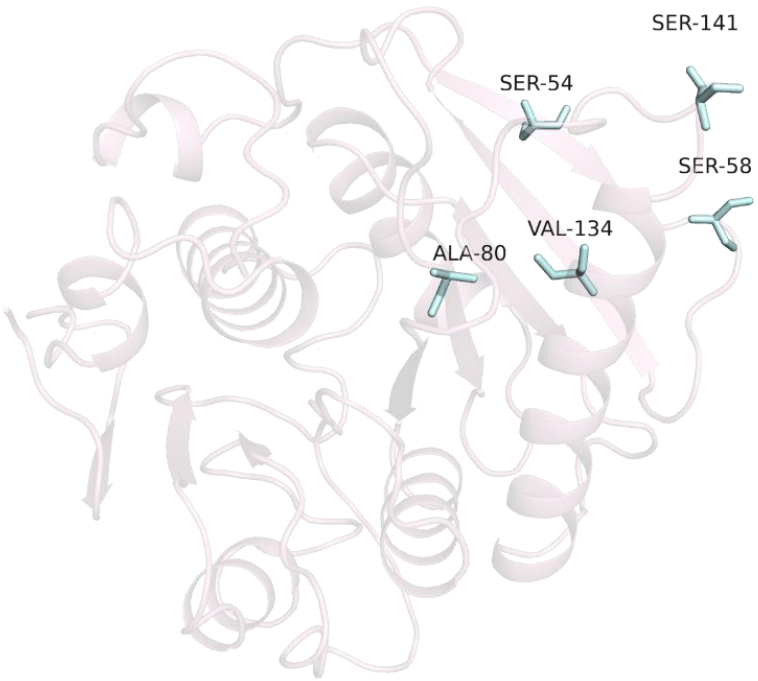


Figure 13 K259F/R260F Synergy




SER-278
GLY-64
ASN-275
LYS-252
ALA-40
THR-39
ARG-260
LYS-259
THR-266


ALA-202
GLN-182
GLU-231
ALA-179

	 Figure Group 14 Spatial distribution of selected mutation points. According to the different spatial positions, the selected sites are divided into 4 groups.

SEPTEMBER

WEEK1-2

Date	09/01/2020-09/12/2020
Aim	1. Calculate 543 combination mutations of twelve-point mutations and screen the results; 2. Analysis of combined mutant structure
Participant	Yuan Wang, Yuhan Li, Xujia Liu, Yufei Zhou, Dewu Liu, Haoyang Shi, He Zhang
Content	Calculate the calculation of 543 combined mutations. For the obtained 543 combination mutants with superior DDG values, the two mutant combinations satisfying DDG<-28, -28<DDG<-27 were selected respectively, and these mutants were integrated and analyzed, and then assigned to different fields. The person in charge performs structural analysis, using JMOL to analyze the number of salt bridges, viewing the structure of proline in the mutant, visually analyzing hydrogen bonds with PyMOL, visually analyzing disulfide bonds, and changes in protein hydrophobicity.
Result	Completed the calculation of 543 combined mutations. 40 mutants satisfying DDG<-28 and nearly a hundred mutants satisfying -28<DDG<-27 were screened out. Through the visual screening, 12 best combination mutants were initially selected: N37R/T39D/S54W/Q119D/Q127L/A202C/S214F/E231C//R260F/T266P/T270R/N275F S54W/T72E/N73R/T77E/N114R/Q119D/Q127L/A202C/S214F/E231C/R260F/N275

F
S54W/T72E/N73R/T77E/S115R/Q119D/Q127L/A202C/S214F/E231C/R260F/N275F
S54W/T72E/N73R/T77E/Q119D/Q127L/A202C/S214F/E231C/R260F/T266P/N275F
S54W/T72E/N73R/T77E/N114R/Q119D/Q127L/A202C/S214F/E231C/T266P/N275F
S54W/T72E/N73R/T77E/S115R/Q119D/Q127L/A202C/S214F/E231C/T266P/N275F
S54W/T72E/N73R/T77E/S115R/Q119D/Q127L/A202C/E231C/R260F/T266P/N275F
S54W/T72E/N73R/T77E/N114R/Q119D/Q127L/A202C/E231C/R260F/T266P/N275F
S54W/T72E/N73R/T77E/Q119D/Q127L/A202C/S214F/E231C/R260F/T266P/N275A
S54W/T72E/N73R/T77E/N114R/Q119D/Q127L/A202C/S214F/E231C/R260F/N275A
S54W/T72E/N73R/T77E/S115R/Q119D/Q127L/A202C/S214F/E231C/R260F/N275A
for subsequent MD screening.

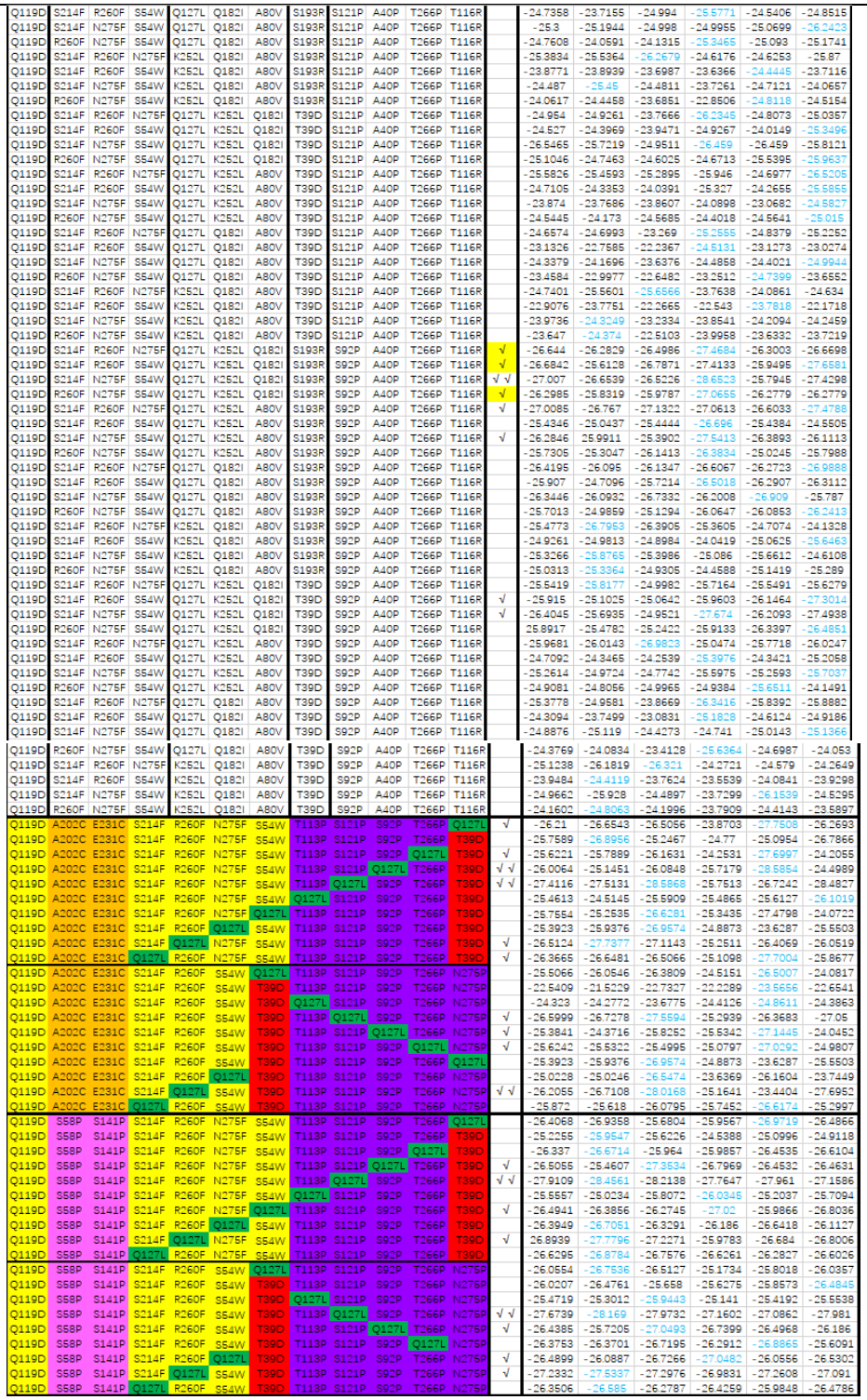
sites											level	DDG					
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	S193R	T113P	S121P	A40P	N275P	√	-25.8813	-25.1201	-27.2543	-25.9904	-25.8334	-25.2082
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	S193R	T113P	S121P	A40P	N275P		-25.1601	-25.4956	-24.9309	-25.2296	-25.294	-24.8507
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	S193R	T113P	S121P	A40P	N275P		-25.0489	-24.3799	-25.9627	-25.2596	-24.778	-24.8641
Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	S193R	T113P	S121P	A40P	N275P		-24.0234	-24.8079	-24.2374	-23.4448	-24.1712	-23.4555
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	T39D	T113P	S121P	A40P	N275P		-24.7403	-24.441	-26.4011	-24.5946	-24.8319	-23.4328
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	T39D	T113P	S121P	A40P	N275P		-24.5554	-25.2388	-24.2201	-24.032	-24.8601	-24.4259
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	T39D	T113P	S121P	A40P	N275P		-23.9455	-23.3649	-24.7618	-23.8484	-24.3646	-23.3877
Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	T39D	T113P	S121P	A40P	N275P		-23.4067	-24.9129	-23.0283	-22.9728	-22.9338	-23.1858
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	S193R	T113P	S92P	A40P	N275P	√	-26.71	-26.7454	-26.4129	-27.8413	-26.1774	-26.3732
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	S193R	T113P	S92P	A40P	N275P		-26.3083	-26.6816	-26.2022	-26.1424	-26.8204	-25.695
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	S193R	T113P	S92P	A40P	N275P	√	-26.6124	-26.6526	-25.8236	-27.1974	-26.5052	-26.8834
Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	S193R	T113P	S92P	A40P	N275P		-25.6252	-25.5761	-24.892	-25.7265	-25.5883	-26.3429
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	T39D	T113P	S92P	A40P	N275P		-25.6535	-25.9391	-26.6364	-25.5518	-25.0113	-25.1289
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	T39D	T113P	S92P	A40P	N275P		-24.9322	-25.3154	-24.8	-24.7079	-24.7223	-25.1155
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	T39D	T113P	S92P	A40P	N275P		-25.2605	-25.5735	-26.0698	-25.0055	-24.8839	-24.7701
Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	T39D	T113P	S92P	A40P	N275P		-24.275	-25.5639	-24.582	-24.3422	-23.0738	-23.8132
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	S193R	S121P	S92P	A40P	N275P		-24.7727	-24.4485	-25.6552	-24.8257	-24.577	-24.6573
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	S193R	S121P	S92P	A40P	N275P		-24.1275	-23.8991	-23.7813	-23.9398	-24.4149	-24.6026
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	S193R	S121P	S92P	A40P	N275P		-23.9905	-23.3667	-24.7587	-23.5556	-24.1064	-24.165
Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	S193R	S121P	S92P	A40P	N275P		-23.2302	-23.8681	-24.0485	-22.1016	-23.292	-22.8408
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	T39D	S121P	S92P	A40P	N275P		-23.9709	-23.9871	-23.9659	-24.1689	-24.6307	-23.1019
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	T39D	S121P	S92P	A40P	N275P		-23.3769	-23.3801	-22.9029	-23.4524	-23.6165	-23.5328
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	T39D	S121P	S92P	A40P	N275P		-23.0284	-22.3697	-22.894	-23.7357	-23.3378	-22.8046
Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	T39D	S121P	S92P	A40P	N275P		-22.33	-23.6339	-22.5307	-21.0749	-22.6117	-21.7988
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	S193R	T113P	S121P	T266P	N275P	√	-26.6462	-25.9106	-26.4966	-27.767	-26.752	-26.305
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	S193R	T113P	S121P	T266P	N275P		-25.8169	-25.6419	-24.9663	-26.0904	-26.0066	-26.3793
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	S193R	T113P	S121P	T266P	N275P	√	-26.207	-25.7673	-26.7605	-25.9896	-25.4016	-27.1162
Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	S193R	T113P	S121P	T266P	N275P		-24.8518	-25.3687	-25.4043	-24.8029	-24.7807	-23.9024
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	T39D	T113P	S121P	T266P	N275P	√	-26.7745	-25.9763	-27.6106	-26.844	-27.1101	-26.3316
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	T39D	T113P	S121P	T266P	N275P		-26.1996	-25.8003	-26.227	-26.3282	-26.6196	-26.0227
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	T39D	T113P	S121P	T266P	N275P		-26.3902	-25.9985	-26.9677	-26.1703	-26.8492	-25.9655
Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	T39D	T113P	S121P	T266P	N275P		-24.9094	-26.0559	-25.3898	-23.8986	-24.7948	-24.408
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	S193R	T113P	S92P	T266P	N275P	√ √	-27.6781	-26.8903	-28.344	-27.2594	-28.3813	-27.5156
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	S193R	T113P	S92P	T266P	N275P	√	-26.9268	-26.8797	-26.7306	-27.3391	-26.7928	-26.8919
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	S193R	T113P	S92P	T266P	N275P	√ √	-27.1197	-26.4994	-26.4792	-28.242	-27.7278	-26.6503
Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	S193R	T113P	S92P	T266P	N275P	√	-25.9952	-25.439	-25.4088	-26.1434	-25.8411	-27.1439
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	T39D	T113P	S92P	T266P	N275P	√ √	-27.3229	-27.0693	-28.1339	-27.2373	-27.0369	-27.1372
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	T39D	T113P	S92P	T266P	N275P	√	-27.1701	-27.1484	-27.0523	-26.9624	-27.0184	-27.669
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	T39D	T113P	S92P	T266P	N275P	√ √	-27.2979	-26.7223	-26.6709	-27.93	-27.1074	-28.0588
Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	T39D	T113P	S92P	T266P	N275P		-26.3857	-26.3924	-26.6421	-26.5862	-26.3704	-25.9375
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	S193R	S121P	S92P	T266P	N275P		-25.2245	-24.5555	-24.3998	-26.3128	-25.521	-25.3336
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	S193R	S121P	S92P	T266P	N275P		-25.328	-24.3866	-25.1584	-25.7548	-25.8067	-25.5335
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	S193R	S121P	S92P	T266P	N275P		-25.4051	-24.7236	-25.8006	-25.6879	-25.8737	-24.9398
Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	S193R	S121P	S92P	T266P	N275P		-24.3895	-23.9186	-25.0425	-25.0432	-24.2737	-23.6693
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	T39D	S121P	S92P	T266P	N275P		-25.4731	-24.6747	-25.4837	-26.1666	-25.5061	-25.5346
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	T39D	S121P	S92P	T266P	N275P		-25.2255	-24.5366	-25.1201	-25.587	-25.6115	-25.2724
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	T39D	S121P	S92P	T266P	N275P		-25.551	-25.0421	-25.8589	-26.003	-25.8994	-24.9516
Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	T39D	S121P	S92P	T266P	N275P		-24.2613	-24.4309	-25.6017	-23.8722	-23.7194	-23.6825
Q119D	S214F	R260F	N275F	Q127L	K252L	Q182I	S193R	T113P	S121P	A40P	T266P	√	-26.674	-25.909	-27.4508	-27.3717	-26.5991	-26.0392

	Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	S193R	T113P	S121P	A40P	T266P		-26.161	-25.333	-26.1632	-26.1303	-26.3459	-26.8327	
	Q119D	S214F	N275F	S54W	Q127L	K252L	Q182I	S193R	T113P	S121P	A40P	T266P	√ √	-27.356	-26.4986	-28.4694	-28.2269	-27.3394	-26.2454	
	Q119D	R260F	N275F	S54W	Q127L	K252L	Q182I	S193R	T113P	S121P	A40P	T266P		-26.0493	-25.5241	-26.8503	-26.0334	-26.5888	-25.2496	
	Q119D	S214F	R260F	N275F	Q127L	K252L	A80V	S193R	T113P	S121P	A40P	T266P		-26.0529	-25.9543	-25.6477	-26.5815	-26.2753	-25.8058	
	Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	S193R	T113P	S121P	A40P	T266P		-25.5736	-25.6056	-25.441	-25.1423	-25.6876	-25.9916	
	Q119D	S214F	N275F	S54W	Q127L	K252L	A80V	S193R	T113P	S121P	A40P	T266P	√	-26.404	-25.6554	-26.2998	-27.0162	-26.6107	-26.4376	
	Q119D	R260F	N275F	S54W	Q127L	K252L	A80V	S193R	T113P	S121P	A40P	T266P		-25.5773	-25.2127	-24.8185	-25.7835	-25.4926	-26.5793	
	Q119D	S214F	R260F	N275F	Q127L	Q182I	A80V	S193R	T113P	S121P	A40P	T266P		-25.5414	-24.7939	-26.4392	-25.374	-25.8175	-25.2824	
	Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	S193R	T113P	S121P	A40P	T266P		-25.0204	-24.2282	-26.0821	-24.9068	-24.9968	-24.8882	
	Q119D	S214F	N275F	S54W	Q127L	Q182I	A80V	S193R	T113P	S121P	A40P	T266P	√	-26.5734	-25.4278	-26.9394	-27.3916	-26.7656	-26.3425	
	Q119D	R260F	N275F	S54W	Q127L	Q182I	A80V	S193R	T113P	S121P	A40P	T266P		-25.4041	-24.5446	-25.5035	-25.4852	-26.1475	-25.3397	
	Q119D	S214F	R260F	N275F	K252L	Q182I	A80V	S193R	T113P	S121P	A40P	T266P		-24.9167	-24.9916	-25.8212	-24.8371	-24.9825	-23.951	
	Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	S193R	T113P	S121P	A40P	T266P		-24.7939	-24.6615	-25.4086	-24.5635	-24.0322	-25.3037	
	Q119D	S214F	N275F	S54W	K252L	Q182I	A80V	S193R	T113P	S121P	A40P	T266P		-25.3231	-25.6167	-25.6679	-25.2949	-25.4814	-24.5548	
	Q119D	R260F	N275F	S54W	K252L	Q182I	A80V	S193R	T113P	S121P	A40P	T266P		-25.1413	-25.1118	-25.8182	-24.6919	-24.8954	-25.189	
	Q119D	S214F	R260F	N275F	Q127L	K252L	Q182I	S193R	T113P	S121P	A40P	T266P	√	-26.674	-25.909	-27.4508	-27.3717	-26.5991	-26.0392	
	Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	S193R	T113P	S121P	A40P	T266P		-26.161	-25.333	-26.1632	-26.1303	-26.3459	-26.8327	
	Q119D	S214F	N275F	S54W	Q127L	K252L	Q182I	S193R	T113P	S121P	A40P	T266P	√ √	-27.356	-26.4986	-28.4694	-28.2269	-27.3394	-26.2454	
	Q119D	R260F	N275F	S54W	Q127L	K252L	Q182I	S193R	T113P	S121P	A40P	T266P		-26.0493	-25.5241	-26.8503	-26.0334	-26.5888	-25.2496	
	Q119D	S214F	R260F	N275F	Q127L	K252L	A80V	S193R	T113P	S121P	A40P	T266P		-26.0529	-25.9543	-25.6477	-26.5815	-26.2753	-25.8058	
	Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	S193R	T113P	S121P	A40P	T266P		-25.5736	-25.6056	-25.441	-25.1423	-25.6876	-25.9916	
	Q119D	S214F	N275F	S54W	Q127L	K252L	A80V	S193R	T113P	S121P	A40P	T266P	√	-26.404	-25.6554	-26.2998	-27.0162	-26.6107	-26.4376	
	Q119D	R260F	N275F	S54W	Q127L	K252L	A80V	S193R	T113P	S121P	A40P	T266P		-25.5773	-25.2127	-24.8185	-25.7835	-25.4926	-26.5793	
	Q119D	S214F	R260F	N275F	Q127L	Q182I	A80V	S193R	T113P	S121P	A40P	T266P		-25.5414	-24.7939	-26.4392	-25.374	-25.8175	-25.2824	
	Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	S193R	T113P	S121P	A40P	T266P		-25.0204	-24.2282	-26.0821	-24.9068	-24.9968	-24.8882	
	Q119D	S214F	N275F	S54W	Q127L	Q182I	A80V	S193R	T113P	S121P	A40P	T266P	√	-26.5734	-25.4278	-26.9394	-27.3916	-26.7656	-26.3425	
	Q119D	R260F	N275F	S54W	Q127L	Q182I	A80V	S193R	T113P	S121P	A40P	T266P		-25.4041	-24.5446	-25.5035	-25.4852	-26.1475	-25.3397	
	Q119D	S214F	R260F	N275F	K252L	Q182I	A80V	S193R	T113P	S121P	A40P	T266P		-24.9167	-24.9916	-25.8212	-24.8371	-24.9825	-23.951	
	Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	S193R	T113P	S121P	A40P	T266P		-24.7939	-24.6615	-25.4086	-24.5635	-24.0322	-25.3037	
	Q119D	S214F	N275F	S54W	K252L	Q182I	A80V	S193R	T113P	S121P	A40P	T266P		-25.3231	-25.6167	-25.6679	-25.2949	-25.4814	-24.5548	
	Q119D	R260F	N275F	S54W	K252L	Q182I	A80V	S193R	T113P	S121P	A40P	T266P		-25.1413	-25.1118	-25.8182	-24.6919	-24.8954	-25.189	
	Q119D	S214F	R260F	N275F	Q127L	K252L	Q182I	S193R	T113P	S92P	A40P	T266P	√ √	-27.2522	-26.8567	-28.2362	-28.0493	-27.1926	-25.9271	
	Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	S193R	T113P	S92P	A40P	T266P	√ √	-26.9177	-26.1641	-28.2111	-26.7269	-27.0444	-26.4419	
	Q119D	S214F	N275F	S54W	Q127L	K252L	Q182I	S193R	T113P	S92P	A40P	T266P	√ √	-28.2678	-27.2917	-28.2081	-28.21	-29.0928	-28.5363	
	Q119D	R260F	N275F	S54W	Q127L	K252L	Q182I	S193R	T113P	S92P	A40P	T266P	√ √	-27.4007	-26.8755	-28.0379	-28.2084	-27.1787	-26.703	
	Q119D	S214F	R260F	N275F	Q127L	K252L	A80V	S193R	T113P	S92P	A40P	T266P		-26.3686	-26.4455	-25.5707	-26.2207	-26.9014	-26.7048	
	Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	S193R	T113P	S92P	A40P	T266P		-26.2708	-26.8344	-25.4511	-26.9429	-25.9118	-26.2138	
	Q119D	S214F	N275F	S54W	Q127L	K252L	A80V	S193R	T113P	S92P	A40P	T266P	√ √	-27.3812	-26.4679	-26.8321	-28.217	-27.8763	-27.5124	
	Q119D	R260F	N275F	S54W	Q127L	K252L	A80V	S193R	T113P	S92P	A40P	T266P	√	-26.5034	-27.1276	-24.999	-27.0215	-26.7019	-26.6669	
	Q119D	S214F	R260F	N275F	Q127L	Q182I	A80V	S193R	T113P	S92P	A40P	T266P	√	-26.6928	-26.3696	-26.3432	-27.7338	-26.4536	-26.5637	
	Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	S193R	T113P	S92P	A40P	T266P	√	-26.6339	-26.2806	-26.7558	-27.8411	-26.1409	-26.1512	
	Q119D	S214F	N275F	S54W	Q127L	Q182I	A80V	S193R	T113P	S92P	A40P	T266P	√ √	-27.6374	-27.3819	-27.459	-27.6713	-28.1166	-27.5584	
	Q119D	R260F	N275F	S54W	Q127L	Q182I	A80V	S193R	T113P	S92P	A40P	T266P	√	-26.7693	-26.5132	-25.8668	-27.4895	-27.5686	-26.4085	
	Q119D	S214F	R260F	N275F	K252L	Q182I	A80V	S193R	T113P	S92P	A40P	T266P		-24.4319	-24.1716	-25.0983	-23.762	-24.7	-24.4278	
	Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	S193R	T113P	S92P	A40P	T266P		-24.8184	-25.2943	-25.1286	-24.5168	-24.9489	-24.2035	
	Q119D	S214F	N275F	S54W	K252L	Q182I	A80V	S193R	T113P	S92P	A40P	T266P	√	-26.9357	-27.1085	-27.0622	-27.0741	-27.2382	-26.1956	
	Q119D	R260F	N275F	S54W	K252L	Q182I	A80V	S193R	T113P	S92P	A40P	T266P		-25.4339	-26.2752	-25.892	-25.1542	-25.533	-24.315	
	Q119D	S214F	R260F	N275F	Q127L	K252L	Q182I	S193R	T113P	S92P	A40P	T266P	√ √	-27.2522	-26.8567	-28.2362	-28.0493	-27.1926	-25.9271	
	Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	S193R	T113P	S92P	A40P	T266P	√ √	-26.9177	-26.1641	-28.2111	-26.7269	-27.0444	-26.4419	
	Q119D	S214F	N275F	S54W	Q127L	K252L	Q182I	S193R	T113P	S92P	A40P	T266P	√ √	-28.2678	-27.2917	-28.2081	-28.21	-29.0928	-28.5363	
	Q119D	R260F	N275F	S54W	Q127L	K252L	Q182I	S193R	T113P	S92P	A40P	T266P	√ √	-27.4007	-26.8755	-28.0379	-28.2084	-27.1787	-26.703	
	Q119D	S214F	R260F	N275F	Q127L	K252L	A80V	S193R	T113P	S92P	A40P	T266P		-26.3686	-26.4455	-25.5707	-26.2207	-26.9014	-26.7048	
	Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	S193R	T113P	S92P	A40P	T266P		-26.2708	-26.8344	-25.4511	-26.9429	-25.9118	-26.2138	
	Q119D	S214F	N275F	S54W	Q127L	K252L	A80V	S193R	T113P	S92P	A40P	T266P	√ √	-27.3812	-26.4679	-26.8321	-28.217	-27.8763	-27.5124	
	Q119D	R260F	N275F	S54W	Q127L	K252L	A80V	S193R	T113P	S92P	A40P	T266P	√	-26.5034	-27.1276	-24.999	-27.0215	-26.7019	-26.6669	
	Q119D	S214F	R260F	N275F	Q127L	Q182I	A80V	S193R	T113P	S92P	A40P	T266P	√	-26.6928	-26.3696	-26.3432	-27.7338	-26.4536	-26.5637	
	Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	S193R	T113P	S92P	A40P	T266P	√	-26.6339	-26.2806	-26.7558	-27.8411	-26.1409	-26.1512	
	Q119D	S214F	N275F	S54W	Q127L	Q182I	A80V	S193R	T113P	S92P	A40P	T266P	√ √	-27.6374	-27.3819	-27.459	-27.6713	-28.1166	-27.5584	
	Q119D	R260F	N275F	S54W	Q127L	Q182I	A80V	S193R	T113P	S92P	A40P	T266P	√	-26.7693	-26.5132	-25.8668	-27.4895	-27.5686	-26.4085	
	Q119D	S214F	R260F	N275F	K252L	Q182I	A80V	S193R	T113P	S92P	A40P	T266P		-25.973	-25.7548	-26.4054	-25.3694	-25.9741	-26.3615	
	Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	S193R	T113P	S92P	A40P	T266P		-24.8184	-25.2943	-25.1286	-24.5168	-24.9489	-24.2035	
	Q119D	S214F	N275F	S54W	K252L	Q182I	A80V	S193R	T113P	S92P	A40P	T266P	√	-26.9357	-27.1085	-27.0622	-27.0741	-27.2382	-26.1956	
	Q119D	R260F	N275F	S54W	K252L	Q182I	A80V	S193R	T113P	S92P	A40P	T266P		-25.4339	-26.2752	-25.892	-25.1542	-25.533	-24.315	
	Q119D	S214F	R260F	N275F	Q127L	K252L	Q182I	S193R	S121P	S92P	A40P	T266P		-25.3026	-25.9424	-25.7656	-24.0887	-25.2217	-25.4947	
	Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	S193R	S121P	S92P	A40P	T266P		-25.2476	-24.4847	-25.5092	-25.3657	-25.7479	-25.1305	
	Q119D	S214F	N275F	S54W	Q127L	K252L	Q182I	S193R	S121P	S92P	A40P	T266P	√	-26.505	-25.5844	-26.6187	-26.7619	-25.9659	-27.5941	
	Q119D	R260F	N275F	S54W	Q127L	K252L	Q182I	S193R	S121P	S92P	A40P	T266P		-25.1764	-24.9776	-26.5728	-25.1932	-24.1925	-24.9459	
	Q119D	S214F	R260F	N275F	Q127L	K252L	A80V	S193R	S121P	S92P	A40P	T266P		-26.0809	-26.6545	-25.6384	-25.8376	-26.1116	-26.1624	
	Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	S193R	S121P	S92P	A40P	T266P		-24.3621	-23.9515	-24.1318	-24.4813	-24.6934	-24.5526	
	Q119D	S214F	N275F	S54W	Q127L	K252L	A80V	S193R	S121P	S92P	A40P	T266P		-25.6902	-25.1589	-24.8877	-26.4578	-26.0392	-25.9076	
	Q119D	R260F	N275F	S54W	Q127L	K252L	A80V	S193R	S121P	S92P	A40P	T266P		-24.9258	-24.3518	-25.0824	-25.3063	-25.0032	-24.8856	
	Q119D	S214F	R260F	N275F	Q127L	Q182I	A80V	S193R	S121P	S92P	A40P	T266P		-25.5036	-24.8011	-25.3374	-26.5302	-26.0488	-24.8002	
	Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	S193R	S121P	S92P	A40P	T266P		-24.1376	-24.4705	-23.833	-24.2544	-24.5668	-23.5634	
	Q119D	S214F	N275F	S54W	Q127L	Q182I	A80V	S193R	S121P	S92P	A40P	T266P		-25.5497	-26.024	-25.5931	-25.5131	-25.5911	-25.0272	
	Q119D	R260F	N275F	S54W	Q127L	Q182I	A80V	S193R	S121P	S92P	A40P	T266P		-24.4789	-23.8037	-25.8175	-23.5039	-25.3009	-23.9687	
	Q119D	S214F	R260F	N275F	K252L	Q182I	A80V	S193R	S121P	S92P	A40P	T266P		-24.4899	-23.8938	-24.1563	-23.3007	-24.8607	-26.2382	
	Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	S193R	S121P	S92P	A40P	T266P		-24.1408	-24.711	-24.0684	-23.1177	-24.5362	-24.2708	
	Q119D	S214F	N275F	S54W	K252L	Q182I	A80V	S193R	S121P	S92P	A40P	T266P		-24.7174	-24.3679	-24.9806	-24.2632	-25.1833	-24.7919	
	Q119D	R260F	N275F	S54W	K252L	Q182I	A80V	S193R	S121P	S92P	A40P	T266P		-24.3051	-25.0924	-23.2548	-24.1862	-24.5742	-24.4177	
	Q119D	S214F	R260F	N275F	Q127L	K252L	Q182I	S193R	S121P	S92P	A40P	T266P		-25.3026	-25.4947	-25.2217	-24.0887	-25.7656	-25.9424	
	Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	S193R	S121P	S92P	A40P	T266P		-25.2476	-25.1305	-25.7479	-25.3657	-25.5092	-24.4847	
	Q119D	S214F	N275F	S54W	Q127L	K252L	Q182I	S193R	S121P	S92P	A40P	T266P	√	-26.505	-25.5844	-26.6187	-26.7619	-25.9659	-27.5941	
	Q119D	R260F	N275F	S54W	Q127L	K252L	Q182I	S193R	S121P	S92P	A40P	T266P		-25.1764	-24.9776	-26.5728	-25.1932	-24.1925	-24.9459	
	Q119D	S214F	R260F	N275F	Q127L	K252L	A80V	S193R	S121P	S92P	A40P	T266P		-26.0809	-26.6545	-25.6384	-25.8376	-26.1116	-26.1624	
	Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	S193R	S121P	S92P	A40P	T266P		-24.3621	-23.9515	-24.1318	-24.4813	-24.6934	-24.5526	
	Q119D	S214F	N275F	S54W	Q127L	K252L	A80V	S193R	S121P	S92P	A40P	T266P		-25.6902	-25.1589	-24.8877	-26.4578	-26.0392	-25.9076	
	Q119D	R260F	N275F	S54W	Q127L	K252L	A80V	S193R	S121P	S92P	A40P	T266P		-24.9258	-24.3518	-25.0824	-25.3063	-25.0032	-24.8856	
	Q119D	S214F	R260F	N275F	Q127L	Q182I	A80V	S193R	S121P	S92P	A40P	T266P		-25.5036	-24.8011	-25.3374	-26.5302	-26.0488	-24.8002	
	Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	S193R	S121P	S92P	A40P	T266P		-24.1376	-23.5634	-24.5668	-24.2544	-23.833	-24.4705	
	Q119D	S214F	N275F	S54W	Q127L	Q182I	A80V	S193R	S121P	S92P	A40P	T266P		-25.5497	-25.0272	-25.5911	-25.5131	-25.5931	-26.024	
	Q119D	R260F	N275F	S54W	Q127L	Q182I	A80V	S193R	S121P	S92P	A40P	T266P		-24.4789	-23.9687	-25.3009	-23.5039	-25.8175	-23.8037	
	Q119D	S214F	R260F	N275F	K252L	Q182I	A80V	S193R	S121P	S92P	A40P	T266P		-24.4899	-26.2382	-24.8607	-23.3007	-24.1563	-23.8938	
	Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	S193R	S121P	S92P	A40P	T266P		-24.1408	-24.2708	-24.5362	-23.1177	-24.0684	-24.711	
	Q119D	S214F	N275F	S54W	K252L	Q182I	A80V	S193R	S121P	S92P	A40P	T266P		-24.7174	-24.7919	-25.1833	-24.2632	-24.9806	-24.3679	
	Q119D	R260F	N275F	S54W	K252L	Q182I	A80V	S193R	S121P	S92P	A40P	T266P		-24.3051	-24.4177	-24.5742	-24.1862	-23.2548	-25.0924	
	Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	S193R	T113P	S121P	N275P	T116R		-25.654	-25.2891	-26.2103	-26.8319	-25.0584	-24.8804	
	Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	S193R	T113P	S121P	N275P	T116R		-25.6419	-25.3049	-26.1008	-25.3912	-26.3832	-25.0293	
	Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	S193R	T113P	S121P	N275P	T116R		-25.3406	-25.2478	-25.7306	-24.6425	-24.9826	-26.0993	
	Q119D	S214F	R260F	S54W	Q182I	K252L	A80V	S193R	T113P	S121P	N275P	T116R		-25.1967	-26.1959	-25.5482	-23.9886	-24.8565	-25.3945	
	Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	T39D	T113P	S121P	N275P	T116R		-26.1757	-25.5335	-26.7587	-26.7265	-25.4731	-26.3867	

Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	T39D	T113P	S121P	N275P	T116R		-25.8938	-25.7434	-25.8448	-25.5908	-25.8286	-26.4613	
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	T39D	T113P	S121P	N275P	T116R		-25.6838	-25.1699	-26.0729	-25.298	-26.5782	-25.3	
Q119D	S214F	R260F	S54W	Q182I	K252L	A80V	T39D	T113P	S121P	N275P	T116R		-25.4362	-24.9218	-25.8812	-25.3338	-26.1311	-24.9132	
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	S193R	T113P	S92P	N275P	T116R	√	-27.0682	-26.1135	-27.099	-27.5565	-27.2723	-27.2998	
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	S193R	T113P	S92P	N275P	T116R		-25.8085	-25.8383	-25.8068	-25.3051	-26.4266	-25.6656	
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	S193R	T113P	S92P	N275P	T116R	√	-26.0483	-25.8866	-26.2559	-27.2368	-25.8519	-25.0102	
Q119D	S214F	R260F	S54W	Q182I	K252L	A80V	S193R	T113P	S92P	N275P	T116R		-25.3054	-25.9222	-25.2792	-24.7835	-24.9591	-25.5831	
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	T39D	T113P	S92P	N275P	T116R	√ √	-27.0556	-26.6613	-28.6798	-26.7676	-26.5122	-26.6571	
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	T39D	T113P	S92P	N275P	T116R	√	-26.9407	-26.4679	-26.8964	-27.8536	-26.5864	-26.8994	
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	T39D	T113P	S92P	N275P	T116R	√	-26.4667	-26.1396	-27.4489	-26.2706	-26.6171	-25.8572	
Q119D	S214F	R260F	S54W	Q182I	K252L	A80V	T39D	T113P	S92P	N275P	T116R	√	-26.0662	-27.2262	-26.5744	-26.1576	-25.4068	-24.966	
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	S193R	S121P	S92P	N275P	T116R		-24.7685	-24.709	-24.8798	-24.5392	-25.5598	-24.1549	
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	S193R	S121P	S92P	N275P	T116R		-24.7186	-25.2963	-25.2315	-24.7329	-24.5208	-23.8114	
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	S193R	S121P	S92P	N275P	T116R		-24.7862	-24.1983	-24.4411	-25.7267	-25.5436	-24.0213	
Q119D	S214F	R260F	S54W	Q182I	K252L	A80V	S193R	S121P	S92P	N275P	T116R		-23.3538	-22.3332	-23.823	-23.232	-23.9372	-23.4435	
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	T39D	S121P	S92P	N275P	T116R		-25.1994	-25.3581	-25.8951	-24.8468	-25.4586	-24.4386	
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	T39D	S121P	S92P	N275P	T116R		-24.5893	-25.1096	-24.3567	-24.7167	-24.7078	-24.0556	
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	T39D	S121P	S92P	N275P	T116R		-24.4065	-24.2907	-24.2352	-24.0408	-25.2782	-24.1877	
Q119D	S214F	R260F	S54W	Q182I	K252L	A80V	T39D	S121P	S92P	N275P	T116R		-23.6428	-23.1681	-23.0629	-23.4339	-24.5413	-24.008	
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	S193R	T113P	A40P	N275P	T116R	√	-26.3104	-26.01	-27.278	-26.4059	-26.1498	-25.7083	
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	S193R	T113P	A40P	N275P	T116R		-25.6837	-25.6617	-26.0115	-25.6636	-25.3068	-25.7747	
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	S193R	T113P	A40P	N275P	T116R		-26.1364	-25.9085	-26.0543	-26.9029	-26.4365	-25.3801	
Q119D	S214F	R260F	S54W	Q182I	K252L	A80V	S193R	T113P	A40P	N275P	T116R		-24.7929	-24.1346	-24.7691	-24.1124	-25.1441	-25.8041	
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	T39D	T113P	A40P	N275P	T116R		-25.8194	-26.078	-24.4871	-26.1122	-26.6483	-25.7713	
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	T39D	T113P	A40P	N275P	T116R		-25.0038	-24.3835	-24.7652	-25.5782	-24.6388	-25.6636	
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	T39D	T113P	A40P	N275P	T116R		-25.0909	-25.7024	-24.0502	-25.9822	-25.088	-24.6319	
Q119D	S214F	R260F	S54W	Q182I	K252L	A80V	T39D	T113P	A40P	N275P	T116R		-24.3135	-23.8929	-24.0765	-23.7526	-23.947	-25.8983	
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	S193R	T113P	T266P	N275P	T116R	√ √	-27.0048	-26.5368	-26.1136	-27.0348	-28.5615	-26.7774	
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	S193R	T113P	T266P	N275P	T116R	√	-26.4418	-26.6974	-27.0528	-26.7541	-25.3889	-26.3157	
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	S193R	T113P	T266P	N275P	T116R	√	-26.4398	-26.5837	-27.2706	-26.3397	-25.9624	-26.0428	
Q119D	S214F	R260F	S54W	Q182I	K252L	A80V	S193R	T113P	T266P	N275P	T116R		-25.8863	-26.4121	-26.3069	-25.5212	-25.9046	-25.2867	
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	T39D	T113P	T266P	N275P	T116R	√	-27.3528	-27.2208	-27.089	-27.7903	-27.6867	-26.9772	
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	T39D	T113P	T266P	N275P	T116R	√	-27.1337	-27.5073	-27.5903	-27.1926	-26.5231	-26.855	
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	T39D	T113P	T266P	N275P	T116R	√	-27.063	-27.2117	-26.8189	-27.2861	-27.4137	-26.5846	
Q119D	S214F	R260F	S54W	Q182I	K252L	A80V	T39D	T113P	T266P	N275P	T116R		-26.0504	-25.6584	-25.8367	-25.3886	-26.5773	-26.7913	
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	S193R	S121P	A40P	N275P	T116R		-25.3265	-26.4013	-24.7384	-25.5625	-25.6179	-24.3126	
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	S193R	S121P	A40P	N275P	T116R		-24.2424	-25.1646	-24.2262	-23.6619	-24.2422	-23.9174	
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	S193R	S121P	A40P	N275P	T116R		-24.2457	-23.9071	-24.7052	-24.1149	-24.9306	-23.5706	
Q119D	S214F	R260F	S54W	Q182I	K252L	A80V	S193R	S121P	A40P	N275P	T116R		-23.7839	-23.7473	-24.5027	-23.4068	-23.5286	-23.7342	
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	T39D	S121P	A40P	N275P	T116R		-24.3081	-25.2265	-23.4992	-25.2191	-23.465	-24.1308	
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	T39D	S121P	A40P	N275P	T116R		-23.5639	-24.1682	-23.5427	-23.311	-23.2039	-23.5935	
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	T39D	S121P	A40P	N275P	T116R		-23.4814	-23.6476	-24.338	-23.9701	-22.774	-22.6774	
Q119D	S214F	R260F	S54W	Q182I	K252L	A80V	T39D	S121P	A40P	N275P	T116R		-23.1616	-23.6062	-23.9201	-21.9779	-22.578	-23.7259	
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	S193R	S121P	T266P	N275P	T116R		-25.8865	-25.2731	-25.9749	-25.2303	-26.7769	-26.1776	
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	S193R	S121P	T266P	N275P	T116R		-25.1584	-25.2443	-25.4282	-25.3429	-24.9727	-24.8041	
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	S193R	S121P	T266P	N275P	T116R		-25.1047	-25.7418	-24.9224	-24.9574	-25.6487	-24.2533	
Q119D	S214F	R260F	S54W	Q182I	K252L	A80V	S193R	S121P	T266P	N275P	T116R		-23.9073	-23.4427	-23.8917	-24.411	-23.6563	-24.1348	
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	T39D	S121P	T266P	N275P	T116R		-26.4391	-26.6606	-26.0517	-26.4924	-26.6272	-26.3637	
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	T39D	S121P	T266P	N275P	T116R		-25.4064	-24.9942	-25.3627	-26.0642	-24.9597	-25.6514	
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	T39D	S121P	T266P	N275P	T116R	?	-25.5278	-24.4313	-24.9180	-26.0173	-26.2667	-26.0056	
Q119D	S214F	R260F	S54W	Q182I	K252L	A80V	T39D	S121P	T266P	N275P	T116R		-24.3149	-24.6405	-24.8826	-23.8063	-23.6604	-24.5845	
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	S193R	S92P	A40P	N275P	T116R	?	-25.6352	-25.2770	-25.8155	-25.7408	-26.542	-24.8008	
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	S193R	S92P	A40P	N275P	T116R		-25.0051	-24.7186	-25.1623	-25.0575	-25.0552	-25.0320	
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	S193R	S92P	A40P	N275P	T116R		-25.2638	-24.2609	-25.1667	-25.987	-25.8427	-25.0617	
Q119D	S214F	R260F	S54W	Q182I	K252L	A80V	S193R	S92P	A40P	N275P	T116R		-24.1579	-24.7702	-24.6082	-23.9171	-23.1729	-24.3213	
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	T39D	S92P	A40P	N275P	T116R		-24.7395	-24.6892	-24.9304	-24.6838	-24.0973	-25.2966	
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	T39D	S92P	A40P	N275P	T116R		-24.2381	-24.2381	-23.3706	-24.4206	-23.7969	-25.2641	
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	T39D	S92P	A40P	N275P	T116R		-24.3714	-23.5196	-24.6564	-24.3032	-24.3103	-25.0674	
Q119D	S214F	R260F	S54W	Q182I	K252L	A80V	T39D	S92P	A40P	N275P	T116R		-23.2755	-24.3057	-22.8980	-22.6042	-23.4962	-23.0732	
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	S193R	S92P	T266P	N275P	T116R	√	-26.5387	-25.8514	-27.4214	-26.3436	-26.1928	-26.8842	
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	S193R	S92P	T266P	N275P	T116R	?	-26.0459	-25.4206	-25.8915	-26.3237	-25.6768	-26.9169	
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	S193R	S92P	T266P	N275P	T116R	√	-26.0909	-24.8256	-25.9450	-27.4193	-26.1528	-26.1117	
Q119D	S214F	R260F	S54W	Q182I	K252L	A80V	S193R	S92P	T266P	N275P	T116R		-24.8115	-25.2160	-25.9844	-24.5060	-24.5951	-23.7559	
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	T39D	S92P	T266P	N275P	T116R	√	-26.7846	-26.2494	-27.9070	-26.0998	-25.6913	-27.9756	
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	T39D	S92P	T266P	N275P	T116R		-24.9898	-25.2475	-24.0214	-24.9604	-24.7965	-25.9231	
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	T39D	S92P	T266P	N275P	T116R		-24.9098	-24.4856	-23.4400	-26.5502	-24.9290	-25.1440	
Q119D	S214F	R260F	S54W	Q182I	K252L	A80V	T39D	S92P	T266P	N275P	T116R		-24.5772	-24.9904	-25.1675	-23.6161	-24.3042	-24.8076	
Q119D	S214F	R260F	N275F	Q127L	K252L	Q182I	S193R	T113P	S121P	A40P	T116R	√	-26.2207	-25.1688	-26.0525	-27.5163	-27.4355	-24.9305	
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	S193R	T113P	S121P	A40P	T116R		-25.364	-24.5744	-26.0237	-25.4477	-25.2449	-25.5291	
Q119D	S214F	N275F	S54W	Q127L	K252L	Q182I	S193R	T113P	S121P	A40P	T116R		-26.1474	-26.1327	-26.2344	-25.7496	-26.0565	-26.5597	
Q119D	R260F	N275F	S54W	Q127L	K252L	Q182I	S193R	T113P	S121P	A40P	T116R		-25.7148	-25.7072	-26.8096	-26.0387	-24.6276	-25.3083	
Q119D	S214F	R260F	N275F	Q127L	K252L	A80V	S193R	T113P	S121P	A40P	T116R		-25.2472	-25.0247	-25.3356	-25.1225	-25.1382	-25.5944	
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	S193R	T113P	S121P	A40P	T116R		-24.4274	-24.7011	-24.233	-24.3345	-24.5438	-24.3482	
Q119D	S214F	N275F	S54W	Q127L	K252L	A80V	S193R	T113P	S121P	A40P	T116R		-25.516	-25.288	-26.0487	-25.3353	-25.2112	-25.7658	
Q119D	R260F	N275F	S54W	Q127L	K252L	A80V	S193R	T113P	S121P	A40P	T116R		-25.1441	-25.0345	-25.6495	-25.2765	-24.8484	-24.9342	
Q119D	S214F	R260F	N275F	Q127L	Q182I	A80V	S193R	T113P	S121P	A40P	T116R		-24.8892	-24.5599	-25.9365	-24.1712	-25.0076	-24.7233	
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	S193R	T113P	S121P	A40P	T116R		-24.7269	-23.8314	-24.3452	-25.2633	-25.0978	-25.086	
Q119D	S214F	N275F	S54W	Q127L	Q182I	A80V	S193R	T113P	S121P	A40P	T116R		-25.8744	-25.4335	-25.0156	-25.8083	-26.2433	-26.7993	
Q119D	R260F	N275F	S54W	Q127L	Q182I	A80V	S193R	T113P	S121P	A40P	T116R		-24.62	-24.4335	-24.7998	-24.2247	-24.2432	-25.3923	
Q119D	S214F	R260F	N275F	K252L	Q182I	A80V	S193R	T113P	S121P	A40P	T116R		-24.6728	-23.9314	-25.0356	-25.0618	-25.0251	-24.2371	
Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	S193R	T113P	S121P	A40P	T116R		-23.89	-24.6313	-23.1035	-23.6775	-24.0971	-23.9351	
Q119D	S214F	N275F	S54W	K252L	Q182I	A80V	S193R	T113P	S121P	A40P	T116R		-24.8573	-24.6534	-24.739	-25.0631	-24.2954	-25.5374	
Q119D	R260F	N275F	S54W	K252L	Q182I	A80V	S193R	T113P	S121P	A40P	T116R		-24.5462	-24.8768	-24.7613	-24.9256	-23.8363	-24.3231	
Q119D	S214F	R260F	N275F	Q127L	K252L	Q182I	T39D	T113P	S121P	A40P	T116R		-25.8285	-25.5602	-26.6907	-26.0139	-26.1324	-24.7808	
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	T39D	T113P	S121P	A40P	T116R		-25.0305	-24.7993	-24.0695	-25.4736	-25.3429	-25.5484	
Q119D	S214F	N275F	S54W	Q127L	K252L	Q182I	T39D	T113P	S121P	A40P	T116R		-25.832	-25.6881	-25.6108	-25.9776	-25.8086	-26.0764	
Q119D	R260F	N275F	S54W	Q127L	K252L	Q182I	T39D	T113P	S121P	A40P	T116R		-24.5336	-24.524	-23.9668	-24.0998	-25.11	-24.9912	
Q119D	S214F	R260F	N275F	Q127L	K252L	A80V	T39D	T113P	S121P	A40P	T116R		-24.5292	-24.7911	-24.3398	-24.8123	-23.4914	-25.3052	
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	T39D	T113P	S121P	A40P	T116R		-23.9246	-24.6623	-23.2113	-24.655	-23.7586	-23.3355	
Q119D	S214F	N275F	S54W	Q127L	K252L	A80V	T39D	T113P	S121P	A40P	T116R		-25.0527	-24.7906	-25.2908	-24.138	-25.157	-25.8873	
Q119D	R260F	N275F	S54W	Q127L	K252L	A80V	T39D	T113P	S121P	A40P	T116R		-24.1797	-23.1867	-23.735	-24.8388	-24.4448	-24.6932	
Q119D	S214F	R260F	N275F	Q127L	Q182I	A80V	T39D	T113P	S121P	A40P	T116R		-24.0765	-24.0255	-24.4892	-23.7817	-24.1955	-23.8908	
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	T39D	T113P	S121P	A40P	T116R		-23.5257	-24.0927	-23.5834	-24.5466	-22.8195	-22.5862	
Q119D	S214F	N275F	S54W	Q127L	Q182I	A80V	T39D	T113P	S121P	A40P	T116R		-24.1462	-24.2062	-23.34	-24.1061	-25.1977	-23.881	
Q119D	R260F	N275F	S54W	Q127L	Q182I	A80V	T39D	T113P	S121P	A40P	T116R		-24.3325	-23.903	-24.4917	-24.9175	-23.3754	-24.9748	
Q119D	S214F	R260F	N275F	K252L	Q182I	A80V	T39D	T113P	S121P	A40P	T116R		-23.565	-24.9403	-23.3369	-22.982	-22.5406	-24.0251	
Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	T39D	T113P	S121P	A40P	T116R		-23.4994	-23.0434	-23.4996	-22.5854	-24.5346	-23.8338	
Q119D	S214F	N275F	S54W	K252L	Q182I	A80V	T39D	T113P	S121P	A40P	T116R		-24.2237	-25.4361	-24.1543	-23.0294	-24.6644	-23.8342	
Q119D	R260F	N275F	S54W	K252L	Q182I	A80V	T39D	T113P	S121P	A40P	T116R		-23.6389	-24.882	-24.1197	-22.7284	-23.0733	-23.3913	
Q119D	S214F	R260F	N275F	Q127L	K252L	Q182I	S193R	T113P	S92P	A40P	T116R	√	-26.4044	-26.0312	-27.8646	-27.5494	-25.5364	-25.0406	

Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	S193R	T113P	S92P	A40P	T116R	√	-26.4387	-26.4008	-27.5252	-26.2137	-26.5387	-25.5149
Q119D	S214F	N275F	S54W	Q127L	K252L	Q182I	S193R	T113P	S92P	A40P	T116R	√	-27.0365	-26.5654	-27.5509	-27.3166	-27.3825	-26.3668
Q119D	R260F	N275F	S54W	Q127L	K252L	Q182I	S193R	T113P	S92P	A40P	T116R	√	-26.739	-26.7127	-27.7653	-27.1123	-26.7902	-25.3145
Q119D	S214F	R260F	N275F	Q127L	K252L	A80V	S193R	T113P	S92P	A40P	T116R		-25.0446	-25.0285	-25.0302	-24.655	-25.3678	-24.1418
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	S193R	T113P	S92P	A40P	T116R		-25.4388	-25.8524	-25.3249	-25.7952	-24.9273	-25.294
Q119D	S214F	N275F	S54W	Q127L	K252L	A80V	S193R	T113P	S92P	A40P	T116R	√	-26.4329	-25.9752	-26.34	-26.4952	-26.2252	-27.1291
Q119D	R260F	N275F	S54W	Q127L	K252L	A80V	S193R	T113P	S92P	A40P	T116R		-26.0281	-26.0588	-25.8202	-26.888	-25.4741	-25.8995
Q119D	S214F	R260F	N275F	Q127L	Q182I	A80V	S193R	T113P	S92P	A40P	T116R		-25.2945	-26.2246	-25.0841	-25.2274	-24.6559	-25.2806
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	S193R	T113P	S92P	A40P	T116R		-25.7093	-26.2907	-26.0217	-25.4186	-24.7357	-26.1799
Q119D	S214F	N275F	S54W	Q127L	Q182I	A80V	S193R	T113P	S92P	A40P	T116R	√	-26.4164	-27.1212	-25.7984	-26.221	-25.9292	-27.0121
Q119D	R260F	N275F	S54W	Q127L	Q182I	A80V	S193R	T113P	S92P	A40P	T116R		-25.6833	-26.491	-25.7968	-25.104	-25.305	-25.7195
Q119D	S214F	R260F	N275F	K252L	Q182I	A80V	S193R	T113P	S92P	A40P	T116R		-25.0658	-25.3255	-25.9842	-23.947	-24.7828	-25.2894
Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	S193R	T113P	S92P	A40P	T116R		-24.7502	-25.8418	-24.3913	-24.0421	-24.9116	-24.5644
Q119D	S214F	N275F	S54W	K252L	Q182I	A80V	S193R	T113P	S92P	A40P	T116R	√	-25.7605	-27.0678	-25.9997	-26.2195	-24.422	-25.0935
Q119D	R260F	N275F	S54W	K252L	Q182I	A80V	S193R	T113P	S92P	A40P	T116R		-25.0255	-26.3087	-25.1205	-23.4394	-25.2455	-25.0133
Q119D	S214F	R260F	N275F	Q127L	K252L	Q182I	T39D	T113P	S92P	A40P	T116R		-25.9596	-26.3328	-26.6187	-26.0779	-26.5527	-24.2157
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	T39D	T113P	S92P	A40P	T116R		-25.7717	-25.6387	-26.2936	-26.2665	-25.8805	-24.7794
Q119D	S214F	N275F	S54W	Q127L	K252L	Q182I	T39D	T113P	S92P	A40P	T116R	√	-26.6322	-27.7212	-27.6559	-26.1209	-26.113	-25.5497
Q119D	R260F	N275F	S54W	Q127L	K252L	Q182I	T39D	T113P	S92P	A40P	T116R		-25.7987	-26.0107	-26.6733	-26.1849	-24.6816	-25.4431
Q119D	S214F	R260F	N275F	Q127L	K252L	A80V	T39D	T113P	S92P	A40P	T116R		-24.4058	-24.2933	-23.7833	-25.112	-24.5625	-24.2779
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	T39D	T113P	S92P	A40P	T116R		-24.5926	-24.8676	-24.3073	-25.0154	-24.1479	-24.6249
Q119D	S214F	N275F	S54W	Q127L	K252L	A80V	T39D	T113P	S92P	A40P	T116R		-25.3628	-26.2475	-24.5092	-25.1931	-25.4323	-25.4921
Q119D	R260F	N275F	S54W	Q127L	K252L	A80V	T39D	T113P	S92P	A40P	T116R		-24.9551	-25.4613	-24.8014	-24.9087	-24.5689	-25.0353
Q119D	S214F	R260F	N275F	Q127L	Q182I	A80V	T39D	T113P	S92P	A40P	T116R		-24.5397	-25.4751	-25.3345	-24.2547	-24.4924	-23.1417
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	T39D	T113P	S92P	A40P	T116R		-24.5454	-25.21	-23.8665	-25.3021	-23.9867	-24.3619
Q119D	S214F	N275F	S54W	Q127L	Q182I	A80V	T39D	T113P	S92P	A40P	T116R		-25.3151	-26.116	-25.2949	-25.111	-25.533	-24.5204
Q119D	R260F	N275F	S54W	Q127L	Q182I	A80V	T39D	T113P	S92P	A40P	T116R		-25.2347	-25.9445	-25.0614	-24.8837	-25.0607	-25.2234
Q119D	S214F	R260F	N275F	K252L	Q182I	A80V	T39D	T113P	S92P	A40P	T116R		-23.6563	-24.4074	-24.5538	-24.1579	-23.1519	-22.0107
Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	T39D	T113P	S92P	A40P	T116R		-23.8509	-25.1386	-23.9906	-23.731	-23.4553	-22.9391
Q119D	S214F	N275F	S54W	K252L	Q182I	A80V	T39D	T113P	S92P	A40P	T116R	√	-24.8101	-27.2609	-25.1018	-23.4636	-23.9385	-24.2855
Q119D	R260F	N275F	S54W	K252L	Q182I	A80V	T39D	T113P	S92P	A40P	T116R		-24.4959	-25.4295	-24.5347	-24.169	-23.7767	-24.5697
Q119D	S214F	R260F	N275F	Q127L	K252L	Q182I	S193R	S121P	S92P	A40P	T116R		-24.8006	-24.7469	-25.0723	-24.7919	-24.7575	-24.6345
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	S193R	S121P	S92P	A40P	T116R		-23.5794	-23.865	-24.8465	-23.0075	-23.3274	-22.8505
Q119D	S214F	N275F	S54W	Q127L	K252L	Q182I	S193R	S121P	S92P	A40P	T116R		-25.4319	-24.9006	-25.902	-25.4671	-25.6372	-25.2526
Q119D	R260F	N275F	S54W	Q127L	K252L	Q182I	S193R	S121P	S92P	A40P	T116R		-24.8819	-24.2996	-25.4331	-24.8818	-24.8341	-24.961
Q119D	S214F	R260F	N275F	Q127L	K252L	A80V	S193R	S121P	S92P	A40P	T116R		-25.4904	-25.2892	-25.6222	-25.5913	-25.1554	-25.7936
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	S193R	S121P	S92P	A40P	T116R		-23.8164	-23.6028	-23.6572	-24.237	-24.0801	-23.5049
Q119D	S214F	N275F	S54W	Q127L	K252L	A80V	S193R	S121P	S92P	A40P	T116R		-24.7799	-24.3038	-24.1408	-25.0266	-25.3119	-25.1165
Q119D	R260F	N275F	S54W	Q127L	K252L	A80V	S193R	S121P	S92P	A40P	T116R		-24.3445	-23.8367	-24.5427	-24.3705	-24.4052	-24.5676
Q119D	S214F	R260F	N275F	Q127L	Q182I	A80V	S193R	S121P	S92P	A40P	T116R		-24.7782	-24.3179	-24.8296	-24.5311	-25.6442	-24.5682
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	S193R	S121P	S92P	A40P	T116R		-23.4424	-22.7664	-23.726	-23.3108	-24.0857	-23.3231
Q119D	S214F	N275F	S54W	Q127L	Q182I	A80V	S193R	S121P	S92P	A40P	T116R		-24.4432	-24.3286	-25.1373	-23.7721	-23.9333	-25.0446
Q119D	R260F	N275F	S54W	Q127L	Q182I	A80V	S193R	S121P	S92P	A40P	T116R		-24.0988	-23.2416	-24.3548	-23.9281	-25.0156	-23.9539
Q119D	S214F	R260F	N275F	K252L	Q182I	A80V	S193R	S121P	S92P	A40P	T116R		-24.2676	-24.8349	-25.1822	-24.2664	-23.2624	-23.7921
Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	S193R	S121P	S92P	A40P	T116R		-23.0009	-23.4561	-23.7286	-22.2533	-23.0215	-22.545
Q119D	S214F	N275F	S54W	K252L	Q182I	A80V	S193R	S121P	S92P	A40P	T116R		-24.0588	-25.2421	-23.7173	-22.765	-23.69	-24.8795
Q119D	R260F	N275F	S54W	K252L	Q182I	A80V	S193R	S121P	S92P	A40P	T116R		-23.3847	-23.8279	-24.5341	-23.2997	-22.6828	-22.5792
Q119D	S214F	R260F	N275F	Q127L	K252L	Q182I	T39D	S121P	S92P	A40P	T116R		-23.5752	-23.5946	-23.5311	-22.8595	-24.5469	-23.3438
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	T39D	S121P	S92P	A40P	T116R		-24.1442	-23.5436	-24.0621	-24.1763	-23.9695	-24.9694
Q119D	S214F	N275F	S54W	Q127L	K252L	Q182I	T39D	S121P	S92P	A40P	T116R		-25.1396	-24.8642	-24.8	-25.0261	-25.2916	-25.7158
Q119D	R260F	N275F	S54W	Q127L	K252L	Q182I	T39D	S121P	S92P	A40P	T116R		-24.5176	-23.7836	-25.0481	-23.8616	-24.4967	-25.3978
Q119D	S214F	R260F	N275F	Q127L	K252L	A80V	T39D	S121P	S92P	A40P	T116R		-24.4278	-24.7695	-23.5916	-24.8305	-25.555	-23.3922
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	T39D	S121P	S92P	A40P	T116R		-23.11	-22.8475	-22.9805	-23.3492	-23.0301	-23.3447
Q119D	S214F	N275F	S54W	Q127L	K252L	A80V	T39D	S121P	S92P	A40P	T116R		-23.9924	-24.1737	-22.9238	-23.6229	-24.7397	-24.5021
Q119D	R260F	N275F	S54W	Q127L	K252L	A80V	T39D	S121P	S92P	A40P	T116R		-23.4831	-23.1391	-23.5009	-24.7553	-23.2034	-22.8168
Q119D	S214F	R260F	N275F	Q127L	Q182I	A80V	T39D	S121P	S92P	A40P	T116R		-23.8026	-23.3242	-23.9325	-23.2842	-25.2608	-23.2111
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	T39D	S121P	S92P	A40P	T116R		-22.7046	-21.9929	-23.4208	-22.062	-23.5818	-22.4654
Q119D	S214F	N275F	S54W	Q127L	Q182I	A80V	T39D	S121P	S92P	A40P	T116R		-23.8619	-23.278	-24.1086	-23.2927	-24.8813	-23.749
Q119D	R260F	N275F	S54W	Q127L	Q182I	A80V	T39D	S121P	S92P	A40P	T116R		-23.0732	-22.2145	-23.775	-22.9667	-23.6304	-22.7795
Q119D	S214F	R260F	N275F	K252L	Q182I	A80V	T39D	S121P	S92P	A40P	T116R		-22.8683	-23.8512	-22.6108	-23.3631	-22.2066	-22.3098
Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	T39D	S121P	S92P	A40P	T116R		-22.4603	-23.0878	-22.7522	-21.4746	-22.1123	-22.8746
Q119D	S214F	N275F	S54W	K252L	Q182I	A80V	T39D	S121P	S92P	A40P	T116R		-23.2643	-25.25	-22.7396	-21.5099	-22.9735	-23.8487
Q119D	R260F	N275F	S54W	K252L	Q182I	A80V	T39D	S121P	S92P	A40P	T116R		-22.8598	-23.6236	-23.2659	-21.8455	-22.8388	-22.7251
Q119D	S214F	R260F	N275F	Q127L	K252L	Q182I	S193R	T113P	S121P	T266P	T116R	√	-26.6071	-26.6761	-27.162	-27.2489	-26.0644	-25.8844
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	S193R	T113P	S121P	T266P	T116R	√	-25.911	-25.4302	-25.5005	-27.4403	-25.2214	-25.9627
Q119D	S214F	N275F	S54W	Q127L	K252L	Q182I	S193R	T113P	S121P	T266P	T116R	√	-27.1605	-26.7349	-27.3414	-27.4991	-26.5676	-27.6596
Q119D	R260F	N275F	S54W	Q127L	K252L	Q182I	S193R	T113P	S121P	T266P	T116R	√	-26.5623	-26.2717	-27.1551	-27.1	-26.4978	-25.7869
Q119D	S214F	R260F	N275F	Q127L	K252L	A80V	S193R	T113P	S121P	T266P	T116R	√	-26.207	-26.4579	-26.1527	-27.2122	-25.7309	-25.4813
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	S193R	T113P	S121P	T266P	T116R		-25.2313	-25.0763	-24.6045	-25.4358	-25.8271	-25.213
Q119D	S214F	N275F	S54W	Q127L	K252L	A80V	S193R	T113P	S121P	T266P	T116R	√	-26.7093	-26.3624	-26.2181	-26.8713	-27.2069	-26.8876
Q119D	R260F	N275F	S54W	Q127L	K252L	A80V	S193R	T113P	S121P	T266P	T116R		-25.7396	-25.5355	-25.8247	-25.9753	-26.0141	-25.3487
Q119D	S214F	R260F	N275F	Q127L	Q182I	A80V	S193R	T113P	S121P	T266P	T116R		-25.9702	-25.8055	-26.6734	-25.9479	-25.3468	-26.0775
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	S193R	T113P	S121P	T266P	T116R		-25.0218	-25.0686	-25.9838	-23.5686	-24.436	-26.0519
Q119D	S214F	N275F	S54W	Q127L	Q182I	A80V	S193R	T113P	S121P	T266P	T116R		-26.0642	-25.9753	-25.8716	-26.1961	-25.7803	-26.4979
Q119D	R260F	N275F	S54W	Q127L	Q182I	A80V	S193R	T113P	S121P	T266P	T116R		-25.7231	-25.1661	-25.8417	-25.6159	-26.5358	-25.456
Q119D	S214F	R260F	N275F	K252L	Q182I	A80V	S193R	T113P	S121P	T266P	T116R		-25.0776	-26.0205	-25.6539	-24.7429	-25.1831	-23.7878
Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	S193R	T113P	S121P	T266P	T116R		-24.6176	-25.516	-25.3958	-23.4264	-24.7229	-24.027
Q119D	S214F	N275F	S54W	K252L	Q182I	A80V	S193R	T113P	S121P	T266P	T116R		-25.7913	-26.4433	-26.3173	-26.073	-25.3838	-24.7392
Q119D	R260F	N275F	S54W	K252L	Q182I	A80V	S193R	T113P	S121P	T266P	T116R		-25.0477	-26.1859	-25.0336	-24.8022	-25.1542	-24.0624
Q119D	S214F	R260F	N275F	Q127L	K252L	Q182I	T39D	T113P	S121P	T266P	T116R	√	-26.7649	-26.9326	-27.9532	-26.6038	-27.1807	-25.1544
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	T39D	T113P	S121P	T266P	T116R	√	-25.8733	-25.4784	-27.0934	-25.8458	-25.5303	-25.4189
Q119D	S214F	N275F	S54W	Q127L	K252L	Q182I	T39D	T113P	S121P	T266P	T116R	√ √	-27.4379	-26.8868	-28.4484	-27.2319	-27.3893	-27.2327
Q119D	R260F	N275F	S54W	Q127L	K252L	Q182I	T39D	T113P	S121P	T266P	T116R		-26.4012	-26.4127	-27.2371	-26.4103	-26.1976	-25.7485
Q119D	S214F	R260F	N275F	Q127L	K252L	A80V	T39D	T113P	S121P	T266P	T116R		-26.485	-26.6068	-26.3922	-26.8471	-26.2127	-26.3663
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	T39D	T113P	S121P	T266P	T116R		-25.7236	-25.383	-25.4405	-26.5576	-25.9535	-25.2834
Q119D	S214F	N275F	S54W	Q127L	K252L	A80V	T39D	T113P	S121P	T266P	T116R	√	-26.8807	-26.5732	-26.3325	-27.216	-27.2371	-27.0447
Q119D	R260F	N275F	S54W	Q127L	K252L	A80V	T39D	T113P	S121P	T266P	T116R		-26.2863	-25.7133	-26.8344	-25.7941	-26.5014	-26.5886
Q119D	S214F	R260F	N275F	Q127L	Q182I	A80V	T39D	T113P	S121P	T266P	T116R		-25.8563	-25.9533	-26.54	-25.5507	-25.4634	-25.774
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	T39D	T113P	S121P	T266P	T116R		-25.4907	-25.0722	-26.2434	-24.9324	-25.8732	-25.3322
Q119D	S214F	N275F	S54W	Q127L	Q182I	A80V	T39D	T113P	S121P	T266P	T116R	√	-26.5745	-25.9884	-26.9456	-25.7699	-27.4843	-26.6844
Q119D	R260F	N275F	S54W	Q127L	Q182I	A80V	T39D	T113P	S121P	T266P	T116R		-26.3279	-25.6725	-26.3384	-26.8085	-25.8671	-26.9532
Q119D	S214F	R260F	N275F	K252L	Q182I	A80V	T39D	T113P	S121P	T266P	T116R		-25.3962	-26.2791	-26.4452	-24.5556	-25.3813	-24.3197
Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	T39D	T113P	S121P	T266P	T116R		-25.0552	-25.6811	-24.669	-24.2417	-24.9407	-25.7435
Q119D	S214F	N275F	S54W	K252L	Q182I	A80V	T39D	T113P	S121P	T266P	T116R	?	-25.8569	-26.804	-26.1427	-25.055	-26.3026	-24.9805
Q119D	R260F	N275F	S54W	K252L	Q182I	A80V	T39D	T113P	S121P	T266P	T116R		-25.366	-26.3426	-25.2271	-24.59	-25.608	-25.0622
Q119D	S214F	R260F	N275F	Q127L	K252L	Q182I	S193R	T113P	S92P	T266P	T116R	√ √	-26.8455	-26.4415	-28.49	-26.2487	-27.3073	-25.7399
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	S193R	T113P	S92P	T266P	T116R	√ √	-26.8195	-26.392	-28.3575	-26.8407	-25.4471	-27.0601
Q119D	S214F	N275F	S54W	Q127L	K252L	Q182I	S193R	T113P	S92P	T266P	T116R	√ √	-28.0753	-27.9109	-28.6	-28.903	-27.4238	-27.5388
Q119D	R260F	N275F	S54W	Q127L	K252L	Q182I	S193R	T113P	S92P	T266P	T116R	√ √	-27.2368	-26.3968	-28.3462	-27.0784	-27.5368	-26.8256
Q119D	S214F	R260F	N275F	Q127L	K252L	A80V	S193R	T113P	S92P	T266P	T116R	√	-26.5472	-26.0798	-26.0463	-26.6972	-27.3437	-26.5688

	Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	S193R	T113P	S92P	T266P	T116R	√	-26.5261	-26.2842	-26.0959	-27.1922	-26.7378	-26.3201	
	Q119D	S214F	N275F	S54W	Q127L	K252L	A80V	S193R	T113P	S92P	T266P	T116R	√	-27.6349	-25.455	-27.0236	-27.8567	-28.0344	-27.8048	
	Q119D	R260F	N275F	S54W	Q127L	K252L	A80V	S193R	T113P	S92P	T266P	T116R	√	-26.5302	-25.9875	-26.6522	-27.6636	-26.2854	-26.0625	
	Q119D	S214F	R260F	N275F	Q127L	Q182I	A80V	S193R	T113P	S92P	T266P	T116R	√	-26.1425	-27.0689	-24.6075	-24.008	-24.6431	-27.3851	
	Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	S193R	T113P	S92P	T266P	T116R		-26.212	-26.0777	-26.9599	-25.3915	-25.9098	-26.7211	
	Q119D	S214F	N275F	S54W	Q127L	Q182I	A80V	S193R	T113P	S92P	T266P	T116R	√	-27.3837	-26.7695	-27.8263	-27.0365	-27.7146	-27.5713	
	Q119D	R260F	N275F	S54W	Q127L	Q182I	A80V	S193R	T113P	S92P	T266P	T116R	√	-26.8811	-26.2489	-27.1046	-27.0311	-27.7627	-26.2581	
	Q119D	S214F	R260F	N275F	K252L	Q182I	A80V	S193R	T113P	S92P	T266P	T116R		-26.0667	-26.7918	-26.0365	-26.9077	-25.1866	-25.411	
	Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	S193R	T113P	S92P	T266P	T116R		-25.6582	-26.2625	-25.1174	-25.7108	-25.7667	-25.4335	
	Q119D	S214F	N275F	S54W	K252L	Q182I	A80V	S193R	T113P	S92P	T266P	T116R	√ √	-27.1627	-27.2859	-26.9659	-28.0296	-26.3827	-27.1494	
	Q119D	R260F	N275F	S54W	K252L	Q182I	A80V	S193R	T113P	S92P	T266P	T116R		-25.8839	-26.9567	-26.2557	-24.9439	-26.4529	-24.8106	
	Q119D	S214F	R260F	N275F	Q127L	K252L	Q182I	T39D	T113P	S92P	T266P	T116R		-26.0163	-25.7686	-26.7736	-26.5413	-25.5241	-25.4738	
	Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	T39D	T113P	S92P	T266P	T116R	√ √	-27.2276	-26.7549	-28.5185	-28.1502	-27.0757	-25.6386	
	Q119D	S214F	N275F	S54W	Q127L	K252L	Q182I	T39D	T113P	S92P	T266P	T116R	√ √	-28.2017	-28.1346	-28.8366	-27.8974	-27.824	-28.3158	
	Q119D	R260F	N275F	S54W	Q127L	K252L	Q182I	T39D	T113P	S92P	T266P	T116R	√ √	-27.4863	-26.8726	-28.4968	-27.0803	-28.4377	-26.5439	
	Q119D	S214F	R260F	N275F	Q127L	K252L	A80V	T39D	T113P	S92P	T266P	T116R	√	-27.0994	-26.2687	-27.8028	-26.8163	-27.537	-27.0724	
	Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	T39D	T113P	S92P	T266P	T116R	√	-26.947	-26.6706	-27.0743	-27.0599	-26.1121	-27.3683	
	Q119D	S214F	N275F	S54W	Q127L	K252L	A80V	T39D	T113P	S92P	T266P	T116R	√ √	-27.7115	-27.7112	-27.2054	-27.7626	-27.2934	-28.5851	
	Q119D	R260F	N275F	S54W	Q127L	K252L	A80V	T39D	T113P	S92P	T266P	T116R	√	-27.0421	-26.5296	-27.4846	-27.8448	-26.449	-26.9023	
	Q119D	S214F	R260F	N275F	Q127L	Q182I	A80V	T39D	T113P	S92P	T266P	T116R	√	-26.841	-27.0025	-27.3042	-26.2091	-26.7956	-26.8938	
	Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	T39D	T113P	S92P	T266P	T116R	√	-26.3006	-26.0128	-27.1601	-25.4652	-26.8209	-26.044	
	Q119D	S214F	N275F	S54W	Q127L	Q182I	A80V	T39D	T113P	S92P	T266P	T116R	√ √	-27.4201	-27.017	-27.955	-26.4587	-28.2703	-27.3998	
	Q119D	R260F	N275F	S54W	Q127L	Q182I	A80V	T39D	T113P	S92P	T266P	T116R	√	-26.583	-26.2101	-27.0914	-25.9764	-26.3056	-27.3314	
	Q119D	S214F	R260F	N275F	K252L	Q182I	A80V	T39D	T113P	S92P	T266P	T116R		-26.4418	-26.9649	-26.4378	-26.6769	-26.9061	-25.2234	
	Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	T39D	T113P	S92P	T266P	T116R		-25.6289	-26.4524	-26.5979	-24.6289	-26.2352	-24.2299	
	Q119D	S214F	N275F	S54W	K252L	Q182I	A80V	T39D	T113P	S92P	T266P	T116R	√	-26.702	-27.4766	-26.6718	-27.0424	-26.7097	-25.6092	
	Q119D	R260F	N275F	S54W	K252L	Q182I	A80V	T39D	T113P	S92P	T266P	T116R	√	-26.0551	-27.0938	-26.2646	-25.0747	-27.1142	-24.7285	
	Q119D	S214F	R260F	N275F	Q127L	K252L	Q182I	S193R	S121P	S92P	T266P	T116R	√	-25.5226	-25.294	-27.013	-24.9203	-26.0771	-24.3088	
	Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	S193R	S121P	S92P	T266P	T116R		-24.5884	-24.2402	-25.0373	-24.137	-24.5887	-24.9389	
	Q119D	S214F	N275F	S54W	Q127L	K252L	Q182I	S193R	S121P	S92P	T266P	T116R	√	-26.4653	-25.9895	-26.6469	-25.6976	-26.6895	-27.333	
	Q119D	R260F	N275F	S54W	Q127L	K252L	Q182I	S193R	S121P	S92P	T266P	T116R		-25.2828	-24.604	-26.9923	-25.0537	-24.7889	-24.9749	
	Q119D	S214F	R260F	N275F	Q127L	K252L	A80V	S193R	S121P	S92P	T266P	T116R		-25.9379	-25.9828	-26.8105	-25.438	-25.9277	-26.1607	
	Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	S193R	S121P	S92P	T266P	T116R		-24.5794	-24.1542	-24.2471	-24.5348	-24.4981	-25.463	
	Q119D	S214F	N275F	S54W	Q127L	K252L	A80V	S193R	S121P	S92P	T266P	T116R		-25.7824	-25.5299	-25.5354	-25.8394	-25.8245	-26.1826	
	Q119D	R260F	N275F	S54W	Q127L	K252L	A80V	S193R	S121P	S92P	T266P	T116R		-25.1941	-24.185	-25.3206	-25.8337	-24.6159	-26.015	
	Q119D	S214F	R260F	N275F	Q127L	Q182I	A80V	S193R	S121P	S92P	T266P	T116R		-25.5451	-25.2107	-25.6375	-26.2406	-25.6403	-24.9967	
	Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	S193R	S121P	S92P	T266P	T116R		-24.424	-24.4573	-25.047	-23.1201	-24.3758	-25.1197	
	Q119D	S214F	N275F	S54W	Q127L	Q182I	A80V	S193R	S121P	S92P	T266P	T116R		-25.4761	-25.8335	-25.4054	-24.81	-25.7027	-25.6289	
	Q119D	R260F	N275F	S54W	Q127L	Q182I	A80V	S193R	S121P	S92P	T266P	T116R		-24.6441	-24.7002	-24.8116	-23.2707	-25.6613	-24.7769	
	Q119D	S214F	R260F	N275F	K252L	Q182I	A80V	S193R	S121P	S92P	T266P	T116R		-24.4719	-25.9775	-24.6907	-23.916	-23.3536	-24.4219	
	Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	S193R	S121P	S92P	T266P	T116R		-23.6472	-24.4809	-23.9905	-22.3807	-23.0599	-24.3239	
	Q119D	S214F	N275F	S54W	K252L	Q182I	A80V	S193R	S121P	S92P	T266P	T116R		-25.0731	-25.0077	-25.9878	-25.0109	-25.0358	-24.3234	
	Q119D	R260F	N275F	S54W	K252L	Q182I	A80V	S193R	S121P	S92P	T266P	T116R		-24.1478	-24.9341	-24.7774	-23.6148	-24.4948	-22.918	
	Q119D	S214F	R260F	N275F	Q127L	K252L	Q182I	T39D	S121P	S92P	T266P	T116R	√	-25.626	-25.5645	-27.145	-25.067	-25.1316	-25.2221	
	Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	T39D	S121P	S92P	T266P	T116R		-25.0307	-24.4861	-25.0732	-25.4188	-24.8945	-25.2808	
	Q119D	S214F	N275F	S54W	Q127L	K252L	Q182I	T39D	S121P	S92P	T266P	T116R	√	-26.7972	-26.0744	-27.4093	-27.1673	-27.4242	-25.9107	
	Q119D	R260F	N275F	S54W	Q127L	K252L	Q182I	T39D	S121P	S92P	T266P	T116R	√	-25.5283	-24.6909	-25.7408	-24.5341	-25.1582	-27.5176	
	Q119D	S214F	R260F	N275F	Q127L	K252L	A80V	T39D	S121P	S92P	T266P	T116R		-26.284	-25.9847	-26.4209	-26.3952	-26.5679	-26.0513	
	Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	T39D	S121P	S92P	T266P	T116R		-24.987	-24.3789	-24.8814	-25.4718	-24.7666	-25.4364	
	Q119D	S214F	N275F	S54W	Q127L	K252L	A80V	T39D	S121P	S92P	T266P	T116R		-25.8883	-25.6718	-25.7066	-26.1058	-25.3903	-26.5672	
	Q119D	R260F	N275F	S54W	Q127L	K252L	A80V	T39D	S121P	S92P	T266P	T116R		-25.1296	-24.2283	-25.2088	-26.1266	-24.9568	-25.1274	
	Q119D	S214F	R260F	N275F	Q127L	Q182I	A80V	T39D	S121P	S92P	T266P	T116R		-26.0052	-25.624	-26.2521	-25.1608	-26.9462	-26.0431	
	Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	T39D	S121P	S92P	T266P	T116R		-24.9972	-24.8332	-24.9782	-25.0421	-25.3172	-24.8151	
	Q119D	S214F	N275F	S54W	Q127L	Q182I	A80V	T39D	S121P	S92P	T266P	T116R		-25.8542	-26.0977	-26.6936	-24.9949	-25.876	-25.7086	
	Q119D	R260F	N275F	S54W	Q127L	Q182I	A80V	T39D	S121P	S92P	T266P	T116R		-25.4173	-24.97	-25.8433	-24.3607	-25.9887	-25.9239	
	Q119D	S214F	R260F	N275F	K252L	Q182I	A80V	T39D	S121P	S92P	T266P	T116R		-25.1877	-26.1812	-25.9402	-25.2621	-24.0476	-24.5075	
	Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	T39D	S121P	S92P	T266P	T116R		-24.175	-24.7718	-24.4636	-23.5705	-24.2067	-23.8622	
	Q119D	S214F	N275F	S54W	K252L	Q182I	A80V	T39D	S121P	S92P	T266P	T116R		-24.9801	-26.326	-25.3204	-24.5787	-24.2731	-24.4023	
	Q119D	R260F	N275F	S54W	K252L	Q182I	A80V	T39D	S121P	S92P	T266P	T116R		-24.4201	-25.2081	-24.9186	-24.4153	-23.6709	-23.8878	
	Q119D	S214F	R260F	N275F	Q127L	K252L	Q182I	S193R	T113P	A40P	T266P	T116R	√ √	-27.6765	-26.3585	-28.5612	-28.243	-27.8291	-27.3905	
	Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	S193R	T113P	A40P	T266P	T116R	√	-26.4758	-25.9682	-27.5672	-26.3418	-25.8655	-26.6366	
	Q119D	S214F	N275F	S54W	Q127L	K252L	Q182I	S193R	T113P	A40P	T266P	T116R	√ √	-27.7777	-27.1076	-28.9569	-27.1387	-28.5727	-27.1125	
	Q119D	R260F	N275F	S54W	Q127L	K252L	Q182I	S193R	T113P	A40P	T266P	T116R	√	-27.0634	-26.3319	-26.5795	-27.3312	-27.3496	-27.7251	
	Q119D	S214F	R260F	N275F	Q127L	K252L	A80V	S193R	T113P	A40P	T266P	T116R	√	-26.7818	-27.0974	-26.985	-27.165	-26.9418	-25.7199	
	Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	S193R	T113P	A40P	T266P	T116R		-25.9533	-25.4712	-25.4434	-26.9673	-25.8834	-26.0013	
	Q119D	S214F	N275F	S54W	Q127L	K252L	A80V	S193R	T113P	A40P	T266P	T116R	√	-26.7684	-26.5298	-26.6389	-27.2035	-27.2131	-26.2568	
	Q119D	R260F	N275F	S54W	Q127L	K252L	A80V	S193R	T113P	A40P	T266P	T116R		-26.596	-26.8334	-26.3256	-26.6339	-26.5864	-26.6009	
	Q119D	S214F	R260F	N275F	Q127L	Q182I	A80V	S193R	T113P	A40P	T266P	T116R	√	-26.8413	-25.5604	-27.1134	-27.4791	-26.7526	-27.3012	
	Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	S193R	T113P	A40P	T266P	T116R	√	-26.1802	-25.2464	-25.7846	-27.1549	-25.6023	-27.1127	
	Q119D	S214F	N275F	S54W	Q127L	Q182I	A80V	S193R	T113P	A40P	T266P	T116R	√ √	-27.0762	-26.1573	-27.7335	-26.6311	-28.0564	-26.8026	
	Q119D	R260F	N275F	S54W	Q127L	Q182I	A80V	S193R	T113P	A40P	T266P	T116R	√	-26.2148	-25.562	-25.6214	-25.7504	-26.9014	-27.2388	
	Q119D	S214F	R260F	N275F	K252L	Q182I	A80V	S193R	T113P	A40P	T266P	T116R		-25.65	-26.2636	-26.025	-25.5982	-25.6435	-24.7199	
	Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	S193R	T113P	A40P	T266P	T116R		-25.5384	-25.8305	-25.942	-25.0113	-25.0672	-25.8408	
	Q119D	S214F	N275F	S54W	K252L	Q182I	A80V	S193R	T113P	A40P	T266P	T116R		-25.8366	-26.553	-25.8814	-25.4342	-26.0702	-25.244	
	Q119D	R260F	N275F	S54W	K252L	Q182I	A80V	S193R	T113P	A40P	T266P	T116R		-25.5983	-26.0882	-25.1254	-25.5523	-26.2941	-24.9315	
	Q119D	S214F	R260F	N275F	Q127L	K252L	Q182I	T39D	T113P	A40P	T266P	T116R	√ √	-26.9182	-26.5112	-26.4811	-28.0433	-26.3267	-27.2286	
	Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	T39D	T113P	A40P	T266P	T116R		-25.898	-25.8815	-25.5408	-26.6904	-25.6729	-25.7041	
	Q119D	S214F	N275F	S54W	Q127L	K252L	Q182I	T39D	T113P	A40P	T266P	T116R	√	-26.7551	-26.1785	-26.9291	-26.7636	-26.3249	-27.5796	
	Q119D	R260F	N275F	S54W	Q127L	K252L	Q182I	T39D	T113P	A40P	T266P	T116R		-25.9178	-26.5143	-26.3666	-26.7393	-25.1733	-24.7958	
	Q119D	S214F	R260F	N275F	Q127L	K252L	A80V	T39D	T113P	A40P	T266P	T116R		-26.4661	-26.9072	-26.9324	-26.3028	-25.5755	-26.6128	
	Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	T39D	T113P	A40P	T266P	T116R		-25.3977	-25.4117	-25.4045	-26.0807	-25.0175	-25.0741	
	Q119D	S214F	N275F	S54W	Q127L	K252L	A80V	T39D	T113P	A40P	T266P	T116R	√	-26.559	-26.1471	-26.3014	-26.8995	-25.9395	-27.5074	
	Q119D	R260F	N275F	S54W	Q127L	K252L	A80V	T39D	T113P	A40P	T266P	T116R		-25.9625	-26.6337	-25.9745	-26.0752	-25.1759	-25.9533	
	Q119D	S214F	R260F	N275F	Q127L	Q182I	A80V	T39D	T113P	A40P	T266P	T116R		-25.8812	-24.767	-24.7784	-27.146	-26.2645	-26.4501	
	Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	T39D	T113P	A40P	T266P	T116R		-25.0134	-24.3599	-24.2591	-26.195	-24.9977	-25.2552	
	Q119D	S214F	N275F	S54W	Q127L	Q182I	A80V	T39D	T113P	A40P	T266P	T116R		-26.3955	-25.779	-26.6166	-25.9615	-26.8038	-26.8164	
	Q119D	R260F	N275F	S54W	Q127L	Q182I	A80V	T39D	T113P	A40P	T266P	T116R		-25.4528	-24.6878	-24.309	-26.651	-25.8912	-25.8149	
	Q119D	S214F	R260F	N275F	K252L	Q182I	A80V	T39D	T113P	A40P	T266P	T116R		-25.2448	-26.2546	-25.4066	-24.6653	-24.5798	-25.3176	
	Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	T39D	T113P	A40P	T266P	T116R		-24.3863	-25.8025	-23.9435	-23.4627	-24.119	-24.6039	
	Q119D	S214F	N275F	S54W	K252L	Q182I	A80V	T39D	T113P	A40P	T266P	T116R		-25.6232	-26.0709	-25.4589	-25.5434	-24.9847	-26.0581	
	Q119D	R260F	N275F	S54W	K252L	Q182I	A80V	T39D	T113P	A40P	T266P	T116R		-24.9115	-26.0794	-24.4014	-25.083	-25.2179	-23.7759	
	Q119D	S214F	R260F	N275F	Q127L	K252L	Q182I	S193R	S121P	A40P	T266P	T116R		-25.9297	-25.0316	-25.7927	-26.9244	-25.9818	-25.9179	
	Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	S193R	S121P	A40P	T266P	T116R		-24.721	-24.6156	-24.3173	-25.213	-23.6289	-25.83	
	Q119D	S214F	N275F	S54W	Q127L	K252L	Q182I	S193R	S121P	A40P	T266P	T116R	√	-26.4184	-26.6872	-26.1064	-27.0005	-25.7732	-26.5248	
	Q119D	R260F	N275F	S54W	Q127L	K252L	Q182I	S193R	S121P	A40P	T266P	T116R		-25.4031	-24.7054	-25.8109	-26.4651	-24.9227	-25.1115	
	Q119D	S214F	R260F	N275F	Q127L	K252L	A80V	S193R	S121P	A40P	T266P	T116R		-26.2493	-25.8099	-26.6499	-26.5131	-26.6604	-25.6133	
	Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	S193R	S121P	A40P	T266P	T116R		-24.601	-24.0512	-24.9662	-24.8673	-24.8698	-24.2506	
	Q119D	S214F	N275F	S54W	Q127L	K252L	A80V	S193R	S121P	A40P	T266P	T116R		-25.3349	-25.3165	-24.5447	-26.288	-25.0308	-25.4945	
	Q119D	R260F	N275F	S54W	Q127L	K252L	A80V	S193R	S121P	A40P	T266P	T116R		-24.958	-24.4127	-25.4491	-25.2169	-25.0816	-24.6299	
	Q119D	S214F	R260F	N275F	Q127L	Q182I	A80V	S193R	S121P	A40P	T266P	T116R		-26.0435	-25.5421	-25.0571	-26.7376	-25.9595	-26.921	



Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	S193R	S121P	A40P	T266P	T116R		-24.7358	-23.7155	-24.994	-25.5771	-24.5406	-24.8515
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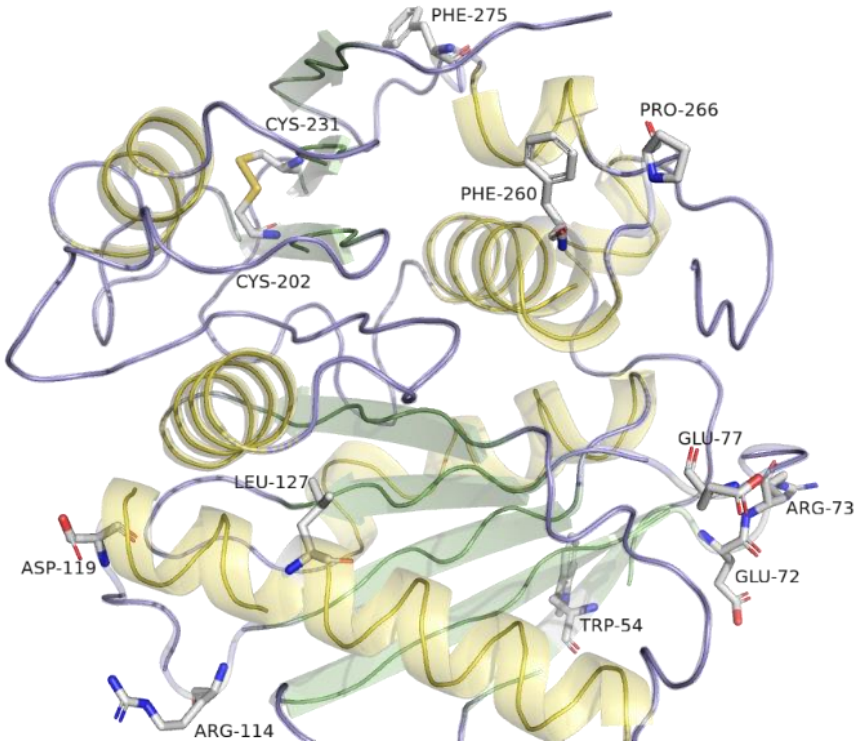
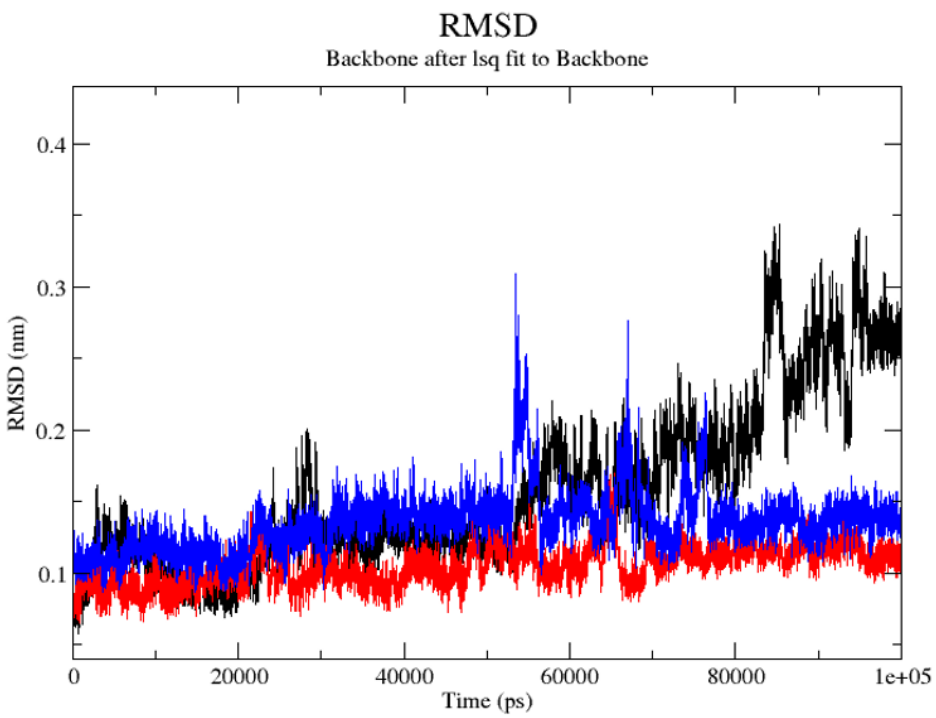
Q119D	R260F	N275F	S54W	Q127L	Q182I	A80V	S193R	S121P	A40P	T266P	T116R		-24.7608	-24.0591	-24.1315	-25.3465	-25.093	-25.1741
Q119D	S214F	R260F	N275F	K252L	Q182I	A80V	S193R	S121P	A40P	T266P	T116R		-25.3834	-25.5364	-26.2679	-24.6176	-24.6253	-25.87
Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	S193R	S121P	A40P	T266P	T116R		-23.8771	-23.8939	-23.6987	-23.6366	-24.4445	-23.7116
Q119D	S214F	N275F	S54W	K252L	Q182I	A80V	S193R	S121P	A40P	T266P	T116R		-24.487	-25.45	-24.4811	-23.7261	-24.7121	-24.0657
Q119D	R260F	N275F	S54W	K252L	Q182I	A80V	S193R	S121P	A40P	T266P	T116R		-24.0617	-24.4458	-23.6851	-22.8506	-24.8118	-24.5154
Q119D	S214F	R260F	N275F	Q127L	K252L	Q182I	T39D	S121P	A40P	T266P	T116R		-24.954	-24.9261	-23.7666	-26.2345	-24.8073	-25.0357
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	T39D	S121P	A40P	T266P	T116R		-24.527	-24.3969	-23.9471	-24.9267	-24.0149	-25.3496
Q119D	S214F	N275F	S54W	Q127L	K252L	Q182I	T39D	S121P	A40P	T266P	T116R		-26.5465	-25.7219	-24.9511	-26.459	-26.459	-25.8121
Q119D	R260F	N275F	S54W	Q127L	K252L	Q182I	T39D	S121P	A40P	T266P	T116R		-25.1046	-24.7463	-24.6025	-24.6713	-25.5395	-25.9637
Q119D	S214F	R260F	N275F	Q127L	K252L	A80V	T39D	S121P	A40P	T266P	T116R		-25.5826	-25.4593	-25.2895	-25.946	-24.6977	-26.5208
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	T39D	S121P	A40P	T266P	T116R		-24.7105	-24.3353	-24.0391	-25.327	-24.2655	-25.5855
Q119D	S214F	N275F	S54W	Q127L	K252L	A80V	T39D	S121P	A40P	T266P	T116R		-23.874	-23.7686	-23.8607	-24.0898	-23.0682	-24.5827
Q119D	R260F	N275F	S54W	Q127L	K252L	A80V	T39D	S121P	A40P	T266P	T116R		-24.5445	-24.173	-24.5685	-24.4018	-24.5641	-25.015
Q119D	S214F	R260F	N275F	Q127L	Q182I	A80V	T39D	S121P	A40P	T266P	T116R		-24.6574	-24.6993	-23.269	-25.2555	-24.8379	-25.2252
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	T39D	S121P	A40P	T266P	T116R		-23.1326	-22.7585	-22.2367	-24.5131	-23.1273	-23.0274
Q119D	S214F	N275F	S54W	Q127L	Q182I	A80V	T39D	S121P	A40P	T266P	T116R		-24.3379	-24.1696	-23.6376	-24.4858	-24.4021	-24.9944
Q119D	R260F	N275F	S54W	Q127L	Q182I	A80V	T39D	S121P	A40P	T266P	T116R		-23.4584	-22.9977	-22.6482	-23.2512	-24.7399	-23.6552
Q119D	S214F	R260F	N275F	K252L	Q182I	A80V	T39D	S121P	A40P	T266P	T116R		-24.7401	-25.5601	-25.6566	-23.7638	-24.0861	-24.634
Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	T39D	S121P	A40P	T266P	T116R		-22.9076	-23.7751	-22.2665	-22.543	-23.7818	-22.1718
Q119D	S214F	N275F	S54W	K252L	Q182I	A80V	T39D	S121P	A40P	T266P	T116R		-23.9736	-24.3249	-23.2334	-23.8541	-24.2094	-24.2459
Q119D	R260F	N275F	S54W	K252L	Q182I	A80V	T39D	S121P	A40P	T266P	T116R		-23.647	-24.374	-22.5103	-23.9958	-23.6332	-23.7219
Q119D	S214F	R260F	N275F	Q127L	K252L	Q182I	S193R	S92P	A40P	T266P	T116R	√	-26.644	-26.2829	-26.4986	-27.4684	-26.3003	-26.6698
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	S193R	S92P	A40P	T266P	T116R	√	-26.6842	-25.6128	-26.7871	-27.4133	-25.9495	-27.6581
Q119D	S214F	N275F	S54W	Q127L	K252L	Q182I	S193R	S92P	A40P	T266P	T116R	√ √	-27.007	-26.6539	-26.5226	-28.6523	-25.7945	-27.4298
Q119D	R260F	N275F	S54W	Q127L	K252L	Q182I	S193R	S92P	A40P	T266P	T116R	√	-26.2985	-25.8319	-25.9787	-27.0655	-26.2779	-26.2779
Q119D	S214F	R260F	N275F	Q127L	K252L	A80V	S193R	S92P	A40P	T266P	T116R	√	-27.0085	-26.767	-27.1322	-27.0613	-26.6033	-27.4788
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	S193R	S92P	A40P	T266P	T116R		-25.4346	-25.0437	-25.4444	-26.696	-26.4384	-24.5505
Q119D	S214F	N275F	S54W	Q127L	K252L	A80V	S193R	S92P	A40P	T266P	T116R	√	-26.2846	-25.9911	-25.3902	-27.5413	-26.3893	-26.1113
Q119D	R260F	N275F	S54W	Q127L	K252L	A80V	S193R	S92P	A40P	T266P	T116R		-25.7305	-25.3047	-26.1413	-26.3834	-25.0245	-25.7988
Q119D	S214F	R260F	N275F	Q127L	Q182I	A80V	S193R	S92P	A40P	T266P	T116R		-26.4195	-26.095	-26.1347	-26.6067	-26.2723	-26.9888
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	S193R	S92P	A40P	T266P	T116R		-25.907	-24.7096	-25.7214	-26.5018	-26.2907	-26.3112
Q119D	S214F	N275F	S54W	Q127L	Q182I	A80V	S193R	S92P	A40P	T266P	T116R		-26.3446	-26.0932	-26.7332	-26.2008	-26.909	-25.787
Q119D	R260F	N275F	S54W	Q127L	Q182I	A80V	S193R	S92P	A40P	T266P	T116R		-25.7013	-24.9859	-25.1294	-26.0647	-26.0853	-26.2413
Q119D	S214F	R260F	N275F	K252L	Q182I	A80V	S193R	S92P	A40P	T266P	T116R		-25.4773	-26.7953	-26.3905	-25.3605	-24.7074	-24.1328
Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	S193R	S92P	A40P	T266P	T116R		-24.9261	-24.9813	-24.8984	-24.0419	-25.0625	-25.6463
Q119D	S214F	N275F	S54W	K252L	Q182I	A80V	S193R	S92P	A40P	T266P	T116R		-25.3266	-25.8765	-25.3986	-25.086	-25.6612	-24.6108
Q119D	R260F	N275F	S54W	K252L	Q182I	A80V	S193R	S92P	A40P	T266P	T116R		-25.0313	-25.3364	-24.9305	-24.4588	-25.1419	-25.289
Q119D	S214F	R260F	N275F	Q127L	K252L	Q182I	T39D	S92P	A40P	T266P	T116R		-25.5419	-25.8177	-24.9982	-25.7164	-25.5491	-25.6279
Q119D	S214F	R260F	S54W	Q127L	K252L	Q182I	T39D	S92P	A40P	T266P	T116R	√	-25.915	-25.1025	-25.0642	-25.9603	-26.1464	-27.3014
Q119D	S214F	N275F	S54W	Q127L	K252L	Q182I	T39D	S92P	A40P	T266P	T116R	√	-26.4045	-25.6935	-24.9521	-27.674	-26.2093	-27.4938
Q119D	R260F	N275F	S54W	Q127L	K252L	Q182I	T39D	S92P	A40P	T266P	T116R		25.8917	-25.4782	-25.2422	-25.9133	-26.3397	-26.4851
Q119D	S214F	R260F	N275F	Q127L	K252L	A80V	T39D	S92P	A40P	T266P	T116R		-25.9681	-26.0143	-26.9823	-25.0474	-25.7718	-26.0247
Q119D	S214F	R260F	S54W	Q127L	K252L	A80V	T39D	S92P	A40P	T266P	T116R		-24.7092	-24.3465	-24.2539	-25.3976	-24.3421	-25.2058
Q119D	S214F	N275F	S54W	Q127L	K252L	A80V	T39D	S92P	A40P	T266P	T116R		-25.2614	-24.9724	-24.7742	-25.5975	-25.2593	-25.7037
Q119D	R260F	N275F	S54W	Q127L	K252L	A80V	T39D	S92P	A40P	T266P	T116R		-24.9081	-24.8056	-24.9965	-24.9384	-25.6511	-24.1491
Q119D	S214F	R260F	N275F	Q127L	Q182I	A80V	T39D	S92P	A40P	T266P	T116R		-25.3778	-24.9581	-23.8669	-26.3416	-25.8392	-25.8882
Q119D	S214F	R260F	S54W	Q127L	Q182I	A80V	T39D	S92P	A40P	T266P	T116R		-24.3094	-23.7499	-23.0831	-25.1828	-24.6124	-24.9186
Q119D	S214F	N275F	S54W	Q127L	Q182I	A80V	T39D	S92P	A40P	T266P	T116R		-24.8876	-25.119	-24.4273	-24.741	-25.0143	-25.1366
Q119D	R260F	N275F	S54W	Q127L	Q182I	A80V	T39D	S92P	A40P	T266P	T116R		-24.3769	-24.0834	-23.4128	-25.6364	-24.6987	-24.053
Q119D	S214F	R260F	N275F	K252L	Q182I	A80V	T39D	S92P	A40P	T266P	T116R		-25.1238	-26.1819	-26.321	-24.2721	-24.579	-24.2649
Q119D	S214F	R260F	S54W	K252L	Q182I	A80V	T39D	S92P	A40P	T266P	T116R		-23.9484	-24.4119	-23.7624	-23.5539	-24.0841	-23.9298
Q119D	S214F	N275F	S54W	K252L	Q182I	A80V	T39D	S92P	A40P	T266P	T116R		-24.9662	-25.928	-24.4897	-23.7299	-26.1539	-24.5295
Q119D	R260F	N275F	S54W	K252L	Q182I	A80V	T39D	S92P	A40P	T266P	T116R		-24.1602	-24.8063	-24.1996	-23.7909	-24.4143	-23.5897
Q119D	A202C	E231C	S214F	R260F	N275F	S54W	T113P	S121P	S92P	T266P	Q127L	√	-26.21	-26.6543	-26.5056	-23.8703	-27.7508	-26.2693
Q119D	A202C	E231C	S214F	R260F	N275F	S54W	T113P	S121P	S92P	T266P	T39D		-25.7589	-26.8956	-25.2467	-24.77	-25.0954	-26.7866
Q119D	A202C	E231C	S214F	R260F	N275F	S54W	T113P	S121P	S92P	Q127L	T39D	√	-25.6221	-25.7889	-26.1631	-24.2531	-27.6997	-24.2055
Q119D	A202C	E231C	S214F	R260F	N275F	S54W	T113P	S121P	Q127L	T266P	T39D	√ √	-26.0064	-25.1451	-26.0848	-25.7179	-28.5854	-24.4989
Q119D	A202C	E231C	S214F	R260F	N275F	S54W	T113P	Q127L	S92P	T266P	T39D	√ √	-27.4116	-27.5131	-28.5868	-25.7513	-26.7242	-28.4827
Q119D	A202C	E231C	S214F	R260F	N275F	S54W	Q127L	S121P	S92P	T266P	T39D		-25.4613	-24.5145	-25.5909	-25.4865	-25.6127	-26.1019
Q119D	A202C	E231C	S214F	R260F	N275F	Q127L	T113P	S121P	S92P	T266P	T39D		-25.7554	-25.2535	-26.6281	-25.3435	-27.4798	-24.0722
Q119D	A202C	E231C	S214F	R260F	Q127L	S54W	T113P	S121P	S92P	T266P	T39D		-25.3923	-25.9376	-26.9574	-24.8873	-23.6287	-25.5503
Q119D	A202C	E231C	S214F	Q127L	N275F	S54W	T113P	S121P	S92P	T266P	T39D	√	-26.5124	-27.7377	-27.1143	-25.2511	-26.4069	-26.0519
Q119D	A202C	E231C	Q127L	R260F	N275F	S54W	T113P	S121P	S92P	T266P	T39D	√	-26.3665	-26.6481	-26.5066	-25.1098	-27.7004	-25.8677
Q119D	A202C	E231C	S214F	R260F	S54W	Q127L	T113P	S121P	S92P	T266P	N275P		-25.5066	-26.0546	-26.3809	-24.5151	-26.5007	-24.0817
Q119D	A202C	E231C	S214F	R260F	S54W	T39D	T113P	S121P	S92P	T266P	N275P		-22.5409	-21.5229	-22.7327	-22.2289	-23.5656	-22.6541
Q119D	A202C	E231C	S214F	R260F	S54W	T39D	Q127L	S121P	S92P	T266P	N275P		-24.323	-24.2772	-23.6775	-24.4126	-24.8611	-24.3863
Q119D	A202C	E231C	S214F	R260F	S54W	T39D	T113P	Q127L	S92P	T266P	N275P	√	-26.5999	-26.7278	-27.5594	-25.2939	-26.3683	-27.05
Q119D	A202C	E231C	S214F	R260F	S54W	T39D	T113P	S121P	Q127L	T266P	N275P	√	-25.3841	-24.3716	-25.8252	-25.5342	-27.1445	-24.0452
Q119D	A202C	E231C	S214F	R260F	S54W	T39D	T113P	S121P	S92P	Q127L	N275P	√	-25.6242	-25.5322	-25.4995	-25.0797	-27.0292	-24.9807
Q119D	A202C	E231C	S214F	R260F	S54W	T39D	T113P	S121P	S92P	T266P	Q127L		-25.3923	-25.9376	-26.9574	-24.8873	-23.6287	-25.5503
Q119D	A202C	E231C	S214F	R260F	Q127L	T39D	T113P	S121P	S92P	T266P	N275P		-25.0228	-25.0246	-26.5474	-23.6369	-26.1604	-23.7449
Q119D	A202C	E231C	S214F	Q127L	S54W	T39D	T113P	S121P	S92P	T266P	N275P	√ √	-26.2055	-26.7108	-28.0168	-25.1641	-23.4404	-27.6952
Q119D	A202C	E231C	Q127L	R260F	S54W	T39D	T113P	S121P	S92P	T266P	N275P		-25.872	-25.618	-26.0795	-25.7452	-26.6174	-25.2997
Q119D	S58P	S141P	S214F	R260F	N275F	S54W	T113P	S121P	S92P	T266P	Q127L		-26.4068	-26.9358	-25.6804	-25.9567	-26.9719	-26.4866
Q119D	S58P	S141P	S214F	R260F	N275F	S54W	T113P	S121P	S92P	T266P	T39D		-25.2255	-25.9547	-25.6226	-24.5388	-25.0996	-24.9118
Q119D	S58P	S141P	S214F	R260F	N275F	S54W	T113P	S121P	S92P	Q127L	T39D		-26.337	-26.6714	-25.964	-25.9857	-26.4535	-26.6104
Q119D	S58P	S141P	S214F	R260F	N275F	S54W	T113P	S121P	Q127L	T266P	T39D	√	-26.5055	-25.4607	-27.3534	-26.7969	-26.4532	-26.4631
Q119D	S58P	S141P	S214F	R260F	N275F	S54W	T113P	Q127L	S92P	T266P	T39D	√ √	-27.9109	-28.4561	-28.2138	-27.7647	-27.961	-27.1586
Q119D	S58P	S141P	S214F	R260F	N275F	S54W	Q127L	S121P	S92P	T266P	T39D		-25.5557	-25.0234	-25.8072	-26.0345	-25.2037	-25.7094
Q119D	S58P	S141P	S214F	R260F	N275F	Q127L	T113P	S121P	S92P	T266P	T39D	√	-26.4941	-26.3856	-26.2745	-27.02	-25.9866	-26.8036
Q119D	S58P	S141P	S214F	R260F	Q127L	S54W	T113P	S121P	S92P	T266P	T39D		-26.3949	-26.7051	-26.3291	-26.186	-26.6418	-26.1127
Q119D	S58P	S141P	S214F	Q127L	N275F	S54W	T113P	S121P	S92P	T266P	T39D	√	26.8939	-27.7796	-27.2271	-25.9783	-26.684	-26.8006
Q119D	S58P	S141P	Q127L	R260F	N275F	S54W	T113P	S121P	S92P	T266P	T39D		-26.6295	-26.8784	-26.7576	-26.6261	-26.2827	-26.6026
Q119D	S58P	S141P	S214F	R260F	S54W	Q127L	T113P	S121P	S92P	T266P	N275P		-26.0554	-26.7536	-26.5127	-25.1734	-25.8018	-26.0357
Q119D	S58P	S141P	S214F	R260F	S54W	T39D	T113P	S121P	S92P	T266P	N275P		-26.0207	-26.4761	-25.658	-25.6275	-25.8573	-26.4845
Q119D	S58P	S141P	S214F	R260F	S54W	T39D	Q127L	S121P	S92P	T266P	N275P		-25.4719	-25.3012	-25.9443	-25.141	-25.4192	-25.5538
Q119D	S58P	S141P	S214F	R260F	S54W	T39D	T113P	Q127L	S92P	T266P	N275P	√ √	-27.6739	-28.169	-27.9732	-27.1602	-27.0862	-27.981
Q119D	S58P	S141P	S214F	R260F	S54W	T39D	T113P	S121P	Q127L	T266P	N275P	√	-26.4385	-25.7205	-27.0493	-26.7399	-26.4968	-26.186
Q119D	S58P	S141P	S214F	R260F	S54W	T39D	T113P	S121P	S92P	Q127L	N275P		-26.3753	-26.3701	-26.7195	-26.2912	-26.8865	-25.6091
Q119D	S58P	S141P	S214F	R260F	Q127L	T39D	T113P	S121P	S92P	T266P	N275P	√	-26.4899	-26.0887	-26.7266	-27.0482	-26.0556	-26.5302
Q119D	S58P	S141P	S214F	Q127L	S54W	T39D	T113P	S121P	S92P	T266P	N275P	√	-27.2332	-27.5337	-27.2976	-26.9831	-27.2608	-27.091
Q119D	S58P	S141P	Q127L	R260F	S54W	T39D	T113P	S121P	S92P	T266P	N275P		-26.3506	-26.685	-26.2787	-26.4259	-25.9848	-26.4786

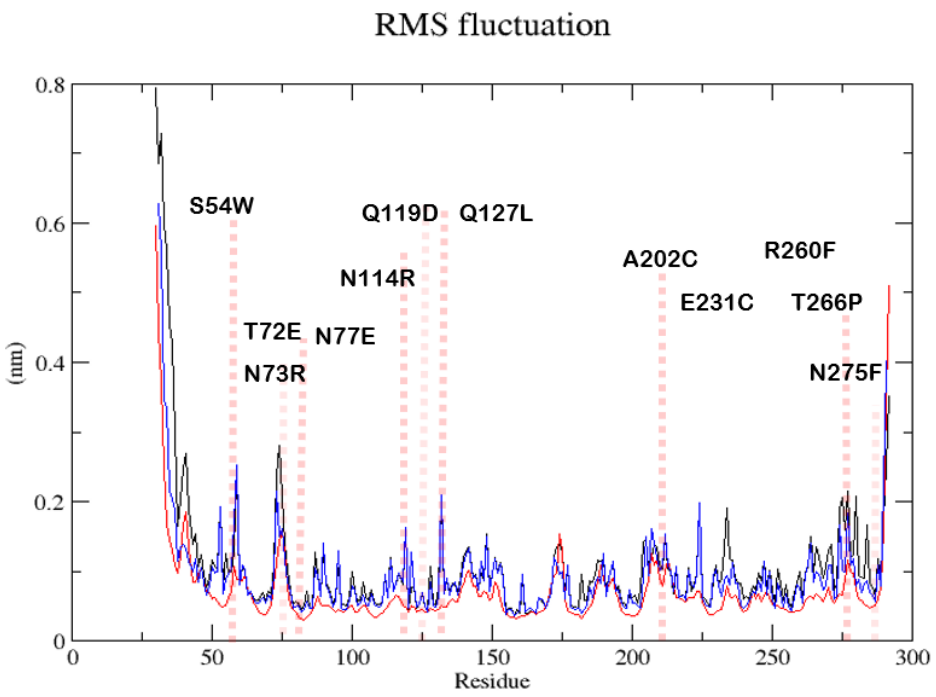
Figure Group 15 All of the DDG value of each Combinatorial mutant calculated. The first few columns are the mutated sites combined in each mutant, which are followed by the stability level that more check marks mean higher stability. The DDG values are showed at the last few columns. The first column is the average of the five values, and the lowest value among the five is showed in blue.

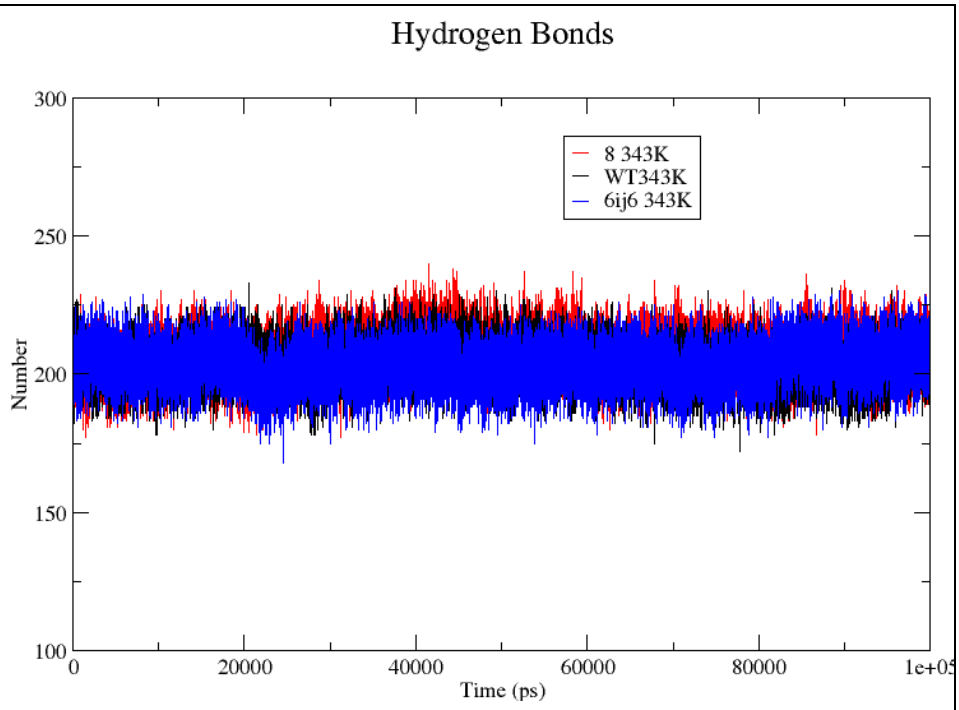
WEEK3

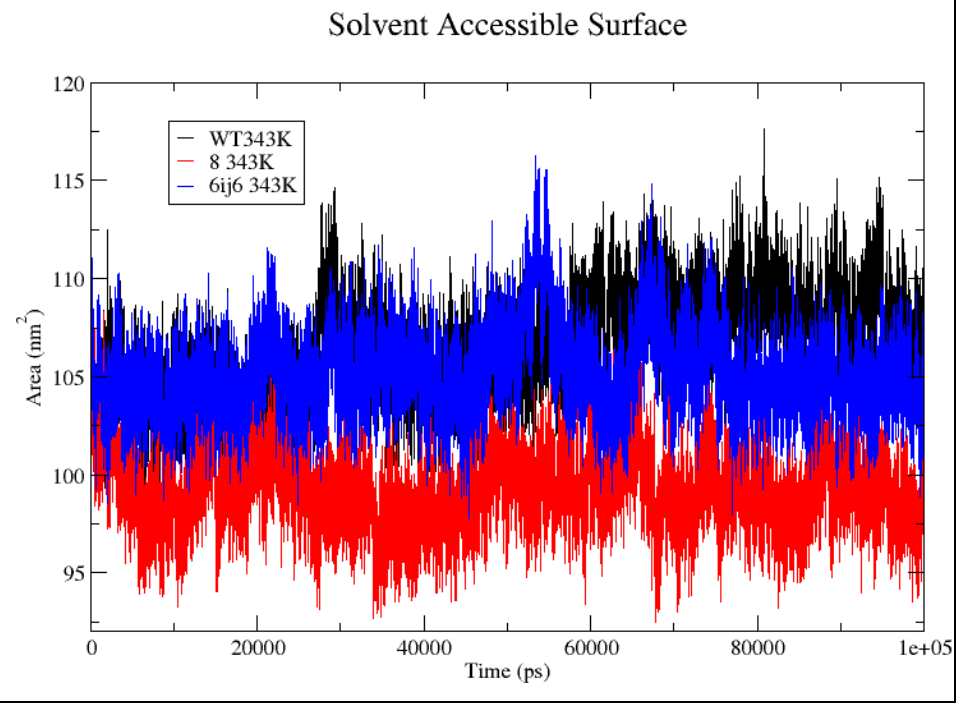
Date	09/13/2020-09/19/2020

Aim	1. Try to use ROSETTA for stability analysis similar to FoldX; 2. Use MD to screen mutants with the best thermal stability.
Participant	Shuying Wu, Dewu Liu, Haoyang Shi, Kegen Chen, Yansong Bai
Content	1. Learn to install and use ROSETTA to predict mutant thermal stability; 2. MD and molecular docking analysis were performed on the combined mutation screening results.
Result	1. There were many problems with ROSETTA compilation, such as virtual machine downtime and installation failure, so we decided to use PYROSETTA with similar functions to work. 2. Determine the best mutant: S54W/T72E/N73R/T77E/N114R/Q119D/Q127L/A202C/E231C/R260F/T266P/N275F  Figure 16 The best combinatorial mutant. It contain the mutated sites S54W/T72E/N73R/T77E/N114R/Q119D/Q127L/A202C/E231C/R260F/T266P/N275F.


RMSD
Backbone after lsq fit to Backbone
RMSD (nm)
0.4
0.3
0.2
0.1
0
20000
40000
60000
80000
1e+05
Time (ps)


RMS fluctuation
(nm)
0.8
0.6
0.4
0.2
0
S54W
Q119D
Q127L
A202C
R260F
N114R
E231C
T266P
T72E
N77E
N73R
N275F
0
50
100
150
200
250
300
Residue


Hydrogen Bonds
8 343K
WT343K
6ij6 343K
Number
Time (ps)
300
250
200
150
100
0
20000
40000
60000
80000
1e+05


Solvent Accessible Surface
WT343K
8 343K
6ij6 343K
Area (nm^2)
Time (ps)
120
115
110
105
100
95
0
20000
40000
60000
80000
1e+05

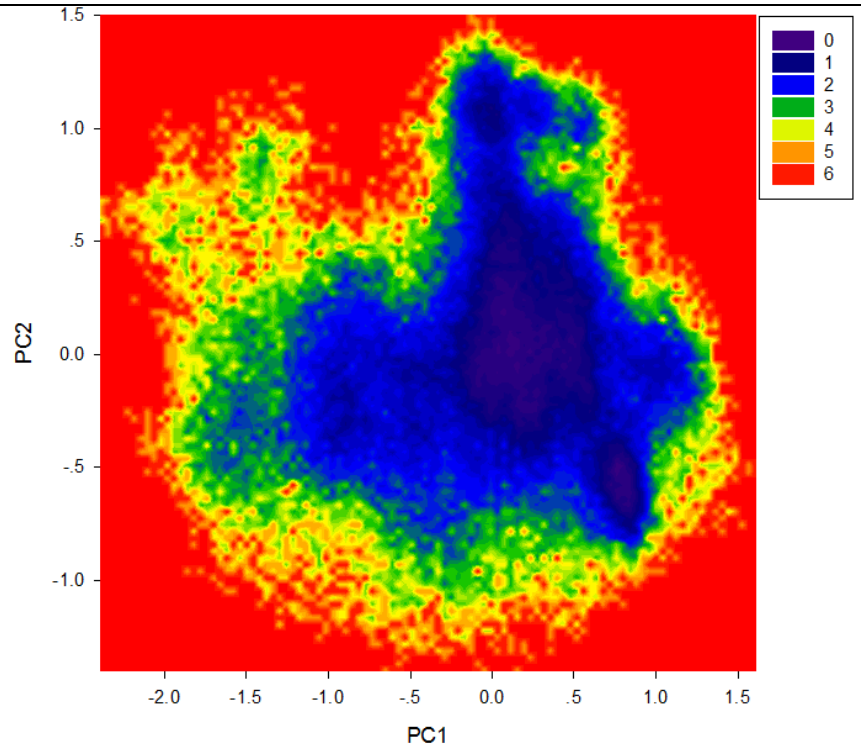


Figure Group 17 The consequence of MD analysis on the best combinatorial mutant.

WEEK4

Date	09/20/2020-09/30/2020
Aim	Try to replace Rosetta with pyrosetta
Participant	Dewu Liu, Kegen Chen
Content	Learn to install and use PyROSETTA to predict the thermal stability of mutants.
Result	The virtual machine configuration cannot meet the requirements of compiling pyrosetta, and finally the attempt to use ROSETTA and pyrosetta can only be abandoned.