Modeling and docking

8.6 Peng Jiang

Modeling and docking

Submit the Sequence

Using protein simulation tool RoseTTAFold web (https://robetta.bakerlab.org/) to submit PobR wild-type animo acid sequence, at the same time try to install and run in the laptop RoseTTAFold, database download in mobile hard disk. RoseTTAFold link (https://github.com/RosettaCommons/RoseTTAFold).

8.13 Shuhan Liu

Gradient HMA concentration induction assay & fluorescence re-screening

We used gradient HMA concentration induction assay to further study the operational range of P5-B7.

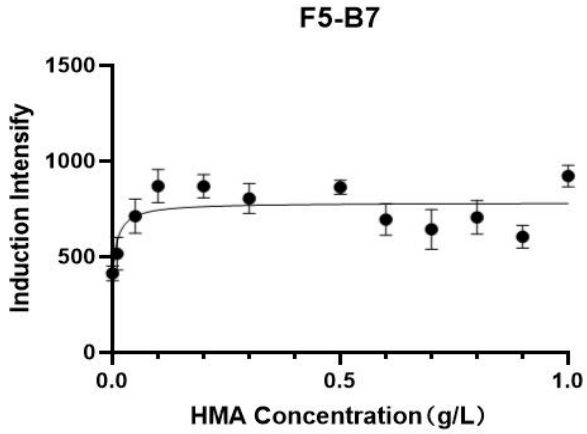

Figure 1 Gradient HMA concentration induction assay of P5-B7

Figure 2 Sequencing of the subclone of P5-B7. Above, subclone C9; below, subclone C7.

Pollution were found in the culture of P5-B7. Therefore, we used streak plate method to isolated monoclones of 10 clones with the highest ratio of IA/I0 in re-screening last time. Picked 3 monoclones of each clone in 10 clones on the plate to have

fluorescence re-screening.

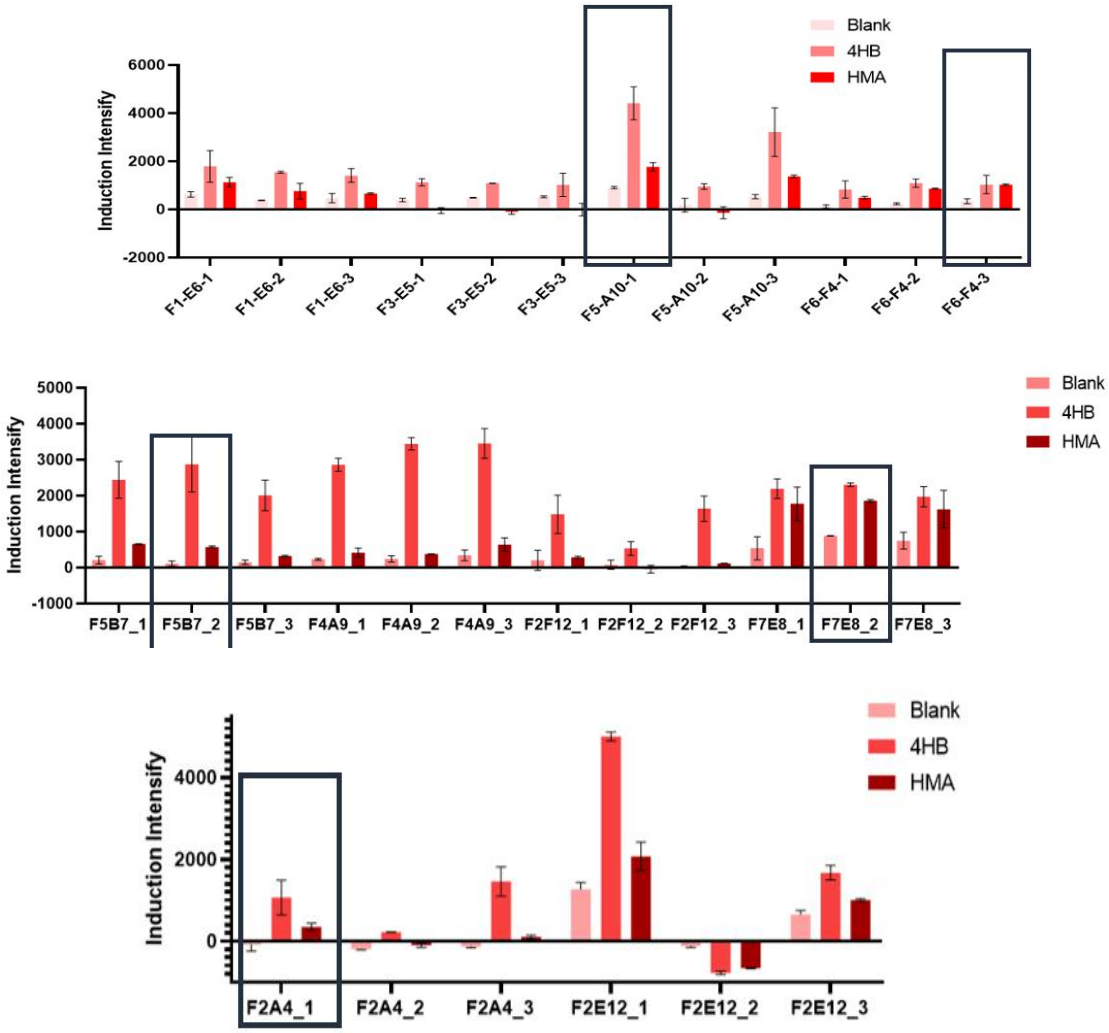

Figure 3 The induction intensity of monoclones of each clone in 10 clones

From the result we found that the HMA induction of F7E8_3, F5B7_2, F6F4_3, F5A10_1, F2A4_1 are better. Therefore, these 5 clones were used in the further study. We used gradient HMA concentration induction assay to further study the operational Range of P7-E8-3, P5-A10-1, P6-F4-3, P5-B7-2.

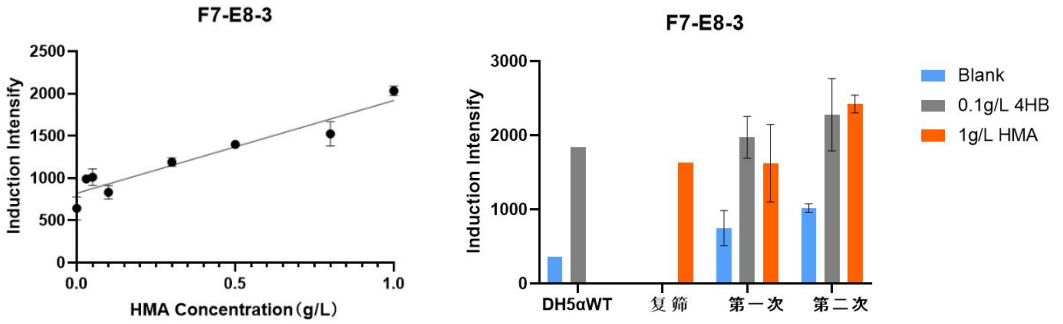

Figure 4 Left, gradient HMA concentration induction assay of P7-E8-3. Right, Data collection of P7-E8-3. The average value of IA/I0 = 2.3.

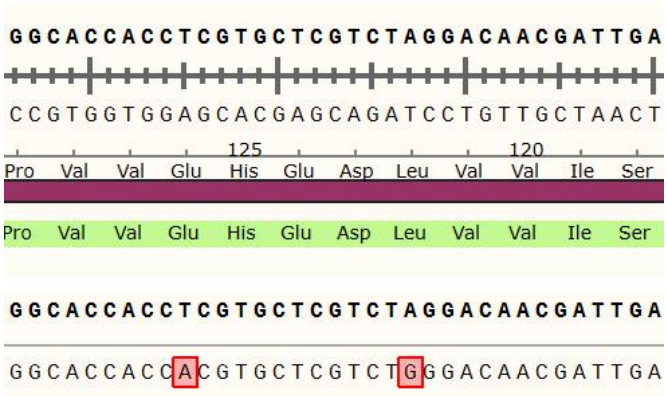

Figure 5 Sequencing of F7-E8-3

The IA of F7-E8-3 does not saturate induced by 1.0g/L HMA. DNA sequencing showed that F7-E8-3 has 2 base substitution mutation, L123P, E126V.

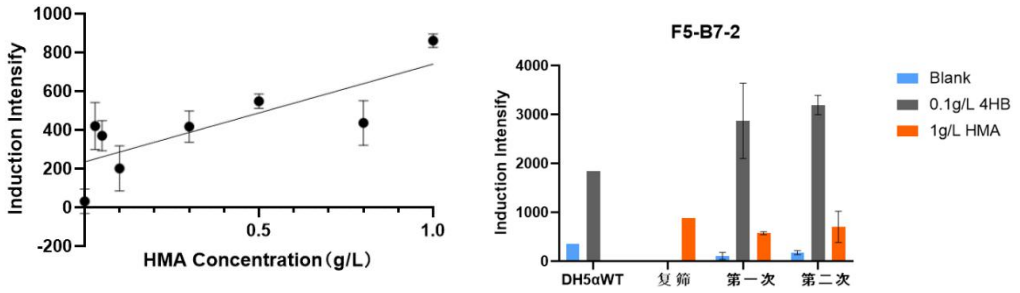

Figure 6 Left, gradient HMA concentration induction assay of P5-B7-2. Right, Data collection of P5-B7-2. The average value of IA/I0 = 3.8.

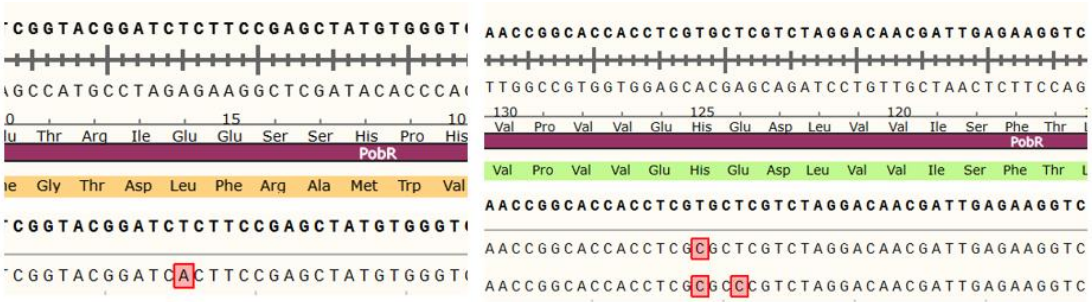

Figure 7 Sequencing of P5-B7-2

From figure 16 we found that the IA of P5-B7-2 does not saturate induced by 1.0g/L HMA. DNA sequencing showed that P5-B7-2 has 3 base substitution mutation, E16V, E124G, H125R. The amino acid mutation sites are different from the result of docking. Therefore, we tried to construct single amino acid mutants E16V, E124G, H125R to speculate important sites for the improved induction of HMA to PobR.

8.15 Peng Jiang

Modeling and docking

Docking between structure by RoseTTAFold and 4HB

The PobR protein structure was simulated by RoseTTAFold.

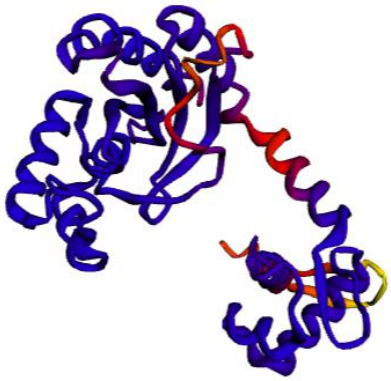

Figure 8 The protein structure simulated using RoseTTAFold

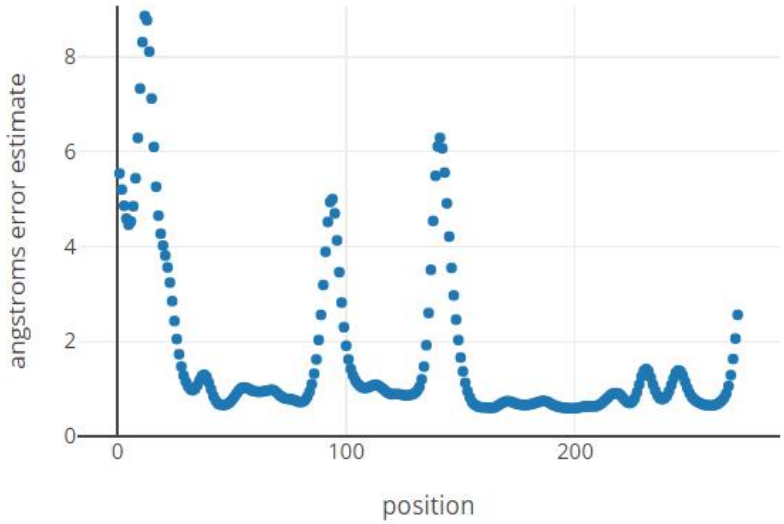

Figure 9 The predicted error of the protein structure, except for three regions

From small organic molecules Pubchem database (https://pubchem.ncbi.nlm.nih.gov/) for the chemical structure of the 4 hb file, use Autodock 4.2 for semi flexible docking RoseTTAFold and 4 hb. There are 70 conformations out there, The binding energy of the optimal conformation is -5.52 kcal/mol.

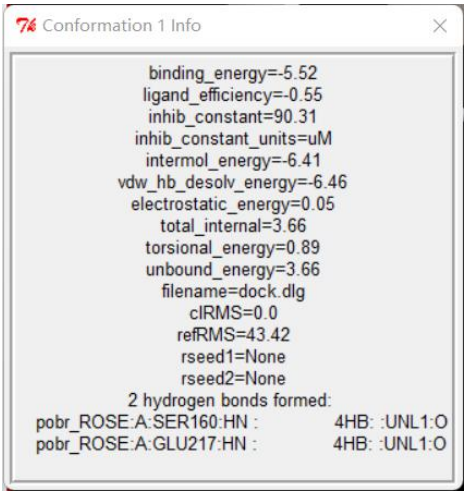

Figure 10 The parameters of this configuration by Autodock 4.2

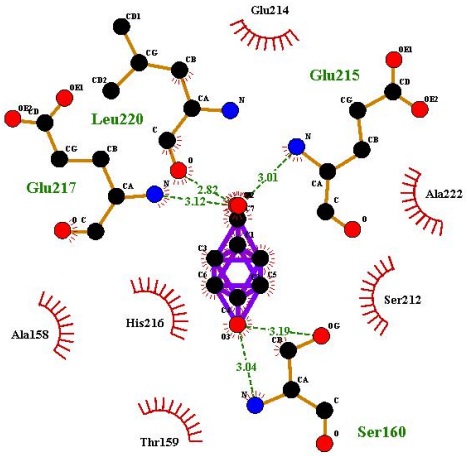

Figure 11 The interaction between the small molecule and the protein receptor in this conformation analyzed by Ligplus

As can be seen from the figure, the carboxyl side of 4HB forms hydrogen bonds with E215, E217 and L220, the hydroxyl side forms hydrogen bonds with S160, and the aromatic ring forms hydrophobic interactions with A158, T159, S212, E214, H216 and A222.

8.16 Yangjinghui Zhang

Modeling and docking

Docking between structure by AlphaFold 2 and 4HB

The protein structure of PobR was simulated using AlphaFold2, and AlphaFold2 was run through cloud computing platform (https://www.cloudam.cn/).

AlphaFold 2 generated five model structures, and we selected the top-ranked model for subsequent docking, pLDDT=91.36 (pLDDT (predicted LDDT-Cα), which is a measure of local confidence per residue in the 0-100 range. PLDDT can vary significantly along a chain, allowing the model to express high confidence in the domain, but low confidence in the linker between domains. The researchers present some evidence that regions with low pLDDT may be isolated non-structures. Regions with pLDDT<50 should not be interpreted, or interpreted as "possible disordered predictions".)

Autodock Vina was used to dock the model predicted by Alphafold 2 with 4HB, and the docking parameters, Vina search space coordinates were set as center_x = -0.83, center_y = -1.292, Center_z = 7.229. Dimensions of search space were set as size_x = 33.75, size_y = 37.5, Dimensions of search space were set as size_x = 33.75, size_y = 37.5, Size_z = 37.5. Exhaustiveness was set at 10.

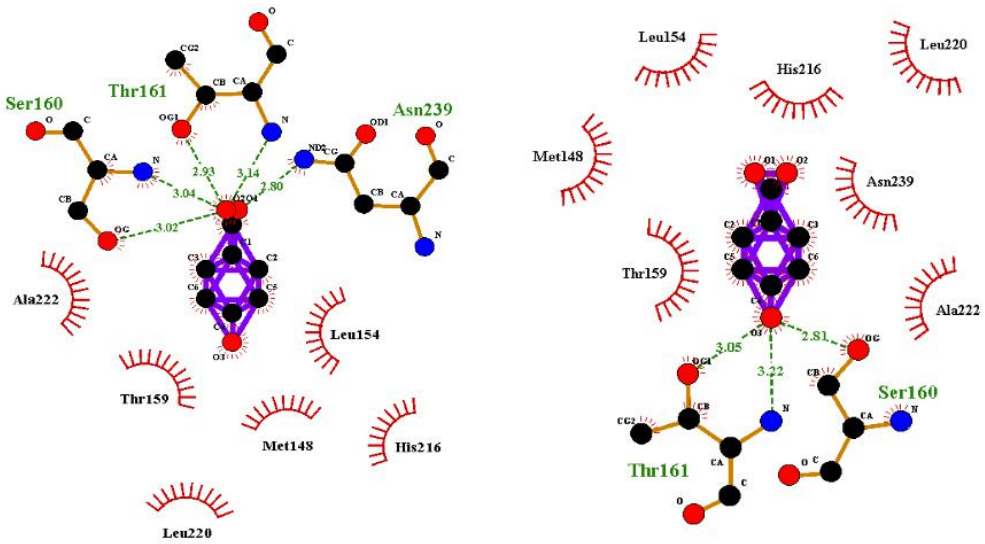

Figure 12 The top two conformations and binding energies of -6.0 Kcal/mol and -6.2Kcal/mol

In Figure 12 left, the carboxyl side of benzene ring forms hydrogen bonds with S160, T161, and N239, and forms hydrophobic interactions with aromatic rings M148, T159, L154, H216, L220, and A222. In Figure 12 right, the hydroxyl side of the benzene ring forms hydrogen bonds with T161 and S160, and forms hydrophobic interactions with aromatic rings M148, L154, T159, H216, L220, A222, and N239.

It is worth noting that the two conformations are very similar, but the docking position of the small molecule ligand has changed by 180°. Therefore, it is necessary to further confirm the accuracy of the docking results.

8.17 Chenhao Yang

Modeling and docking

Comparison of RoseTTAFold and AlphaFold 2

We also used protein structure simulated by RoseTTAFold to dock with the natural inducer 4HB. Coordinates Vina search space coordinates were set as center_x = -19.611, center_y =-15.137, Center_z = 26.083. Dimensions of search space were set as size_x = 33.75, size_y = 37.5, Size_z = 37.5. Exhaustiveness was set at 10.

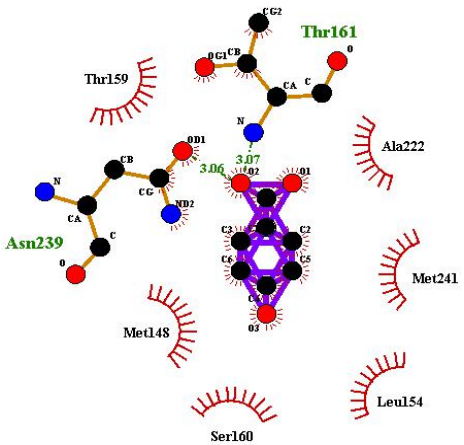

Figure 13 Schematic diagram of model by RoseTTAFold and 4HB

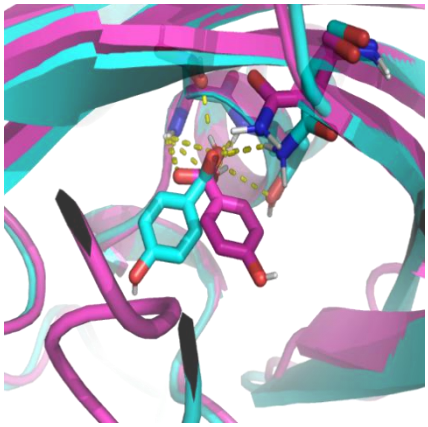

Figure 14 The cyan conformation from AlphaFold 2 and the magenta conformation from RoseTTAFold

It can be seen that T161, A239 form hydrogen bonds with the carboxyl side of 4HB, and the aromatic ring forms hydrophobic interactions with M148, L154, T159, S160, A222, and M241. The comparison shows that this conformation is very similar to the first conformation of 8.16. We observe the differences between the two conformations in three dimensions. Given the similarity of ideas and the important sites mentioned in the literature, the conformation of hydrogen bonding on the carboxyl side is more plausible.