**The molecular mechanism behind protein kinase B natural mutant E17K affecting the allosteric inhibitor sensitivity: A molecular dynamics simulation study**

**SUPPLEMENTARY DATA**

**Tunnel analysis**

The preset reaction coordinates of ASMD were determined with the aid of tunnel analysis. Tunnel analysis was carried out by using the CAVER 3.0 software package(Chovancova et al., 2012). CAVER is a software tool widely used for identification and visualization of tunnels and channels of a specified protein structure. Based on the trajectory generated by MD simulations, CAVER 3.0 was capable of finding the possible egress tunnels from the binding side to the surface of the enzyme, enabling automatic analysis of large ensembles of protein conformations. Several pathways with nearly identical axes may be classified into one cluster. The lowest cost pathway is selected and all the other pathways in the cluster are discarded. A trajectory of MD simulation serves as the input, while the detailed characteristics of individual transport pathways and their time evolution are the outputs. The mass centers of Gly311, Thr312 for IQO systems and Gly294, Leu295 for BAY systems were chosen as the starting point for tunnel searching. For each trajectory, 1000 snapshots were extracted from the last 100 ns simulation. The probe radius and the clustering threshold were set to 1.0 and 8.5, respectively. For the other parameters, their default values were taken throughout the calculations. The obtained tunnels were visualized with VMD(Humphrey, Dalke, & Schulten, 1996) (Figure S1). The direction of the pathway was chosen as the pulling direction in the ASMD simulations.



**Figure S1. The highest possible tunnels (colored in purple) for IQO and BAY egressing from Akt1. The direction of the pathway was chosen as the pulling direction in the ASMD simulations.**

**Table S1. The results of H++ online server calculations for the histidine residues in 3O96 crystal structure.**

|  |  |
| --- | --- |
| Histidine | PKa value |
| 13 | 5.2 |
| 89 | <0.0 |
| 143 | 5.6 |
| 194 | 3.0 |
| 207 | 6.5 |
| 220 | 5.7 |
| 238 | 2.7 |
| 265 | 6.1 |
| 287 | 5.9 |
| 354 | 5.9 |
| 405 | 2.2 |
| 415 | 5.7 |

**Binding free energy calculations**

Based on the last 200ns of each system, binding free energies were calculated by using MM-GBSA method. For each system, 160 frames were extracted from the last 200ns trajectory files. The binding free energies calculations for all the 160 frames for each system are all taken the entropy into consideration and the entropy was calculation by using Normal Mode Analysis (nmode). The results are shown in Figure S2. As shown in Figure S2, the binding free energies for both wild type and E17K mutant systems did not fluctuate much in the last 200ns simulation time, which implied the simulated systems were in the equilibrium state.



**Figure S2. Binding free energies (with entropy included) variations during the last 200ns for IQO and BAY systems.**



**Figure S3. The RMSF for the IQO and BAY systems. (A) and (B). The IQO system RMSF obtained by cMD and ASMD simulations. (C) and (D). The BAY system RMSF obtained by cMD and ASMD simulations. All the residues in ASMD simulations with higher RMSF values were labelled.**

**REFERENCES**

Chovancova, E., Pavelka, A., Benes, P., Strnad, O., Brezovsky, J., Kozlikova, B., . . . Medek, P. (2012). CAVER 3.0: a tool for the analysis of transport pathways in dynamic protein structures. PLoS computational biology, 8(10).

Humphrey, W., Dalke, A., & Schulten, K. (1996). VMD: visual molecular dynamics. Journal of molecular graphics, 14(1), 33-38.