Task 1:

1. Answer

From the results of molecular docking, the compound 22 binds to ERK3 with similar binding mode to that of compound 7, 18, and 21 (Figure 1). This binding mode allows the DFG loops to have space to move from the active (in) conformation to the inactive (intermediate) conformation.

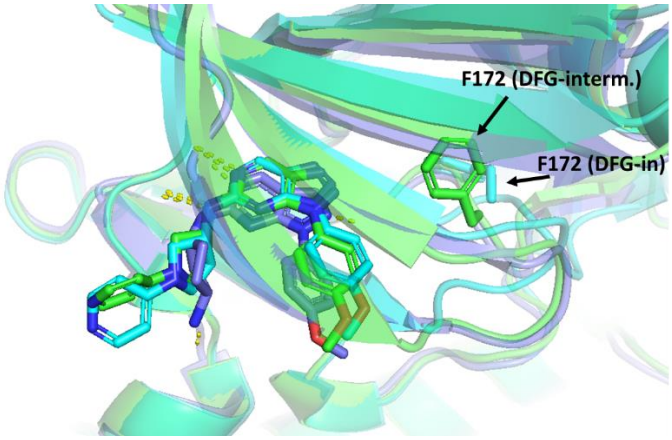

Figure 1. Predicted binding mode of compound 22(blue) compared to compound 18 binding to activate ERK3 (cyan) and inactive ERK3 (green). The compounds would not clash with residue F172 of the DFG-intermediate conformation.

In another hand, the docking results suggested that compound 13 could potentially adopts 2 different binding modes. Both binding modes of compound 13 would clash with residue F171 in the DFG-inter conformation (Figure 2). This prevents the DFG loop to adopt the intermediate conformation and prevent ERF3 to transition from active to inactive state. In fact, when docking the compound 13 to the inactive/DFG-intermediate conformation ERK3 template, the DFG loop was forced to adopt the out conformation to form pi-pi stacking interaction with the benzene ring of compound C13.

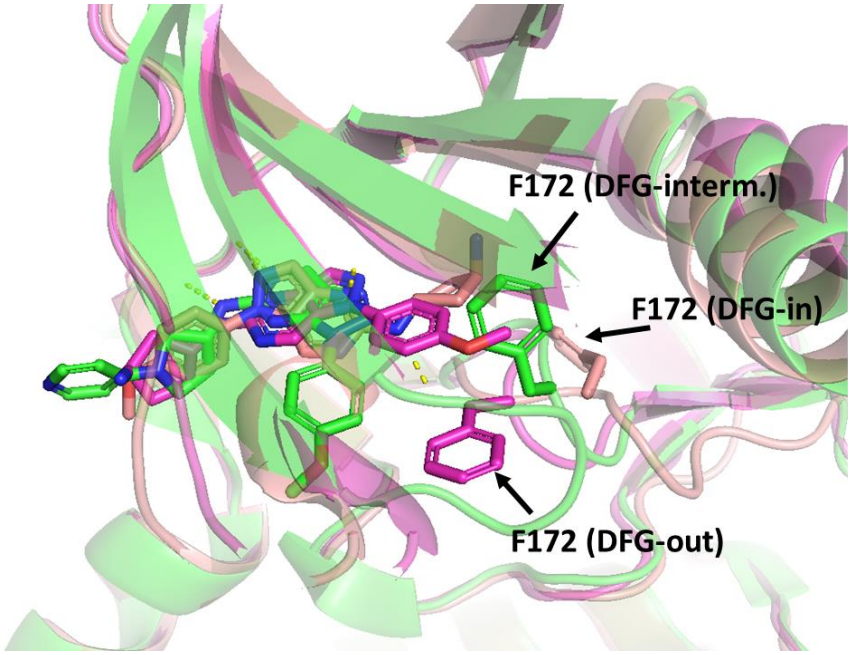

Figure 2.Predicted two binding modes of compound 13 (pink and magenta) compared to compound 18 binding to inactive ERK3 (green). In both predicted binding poses, the compound 13 clashes with residue F172 of the DFG-intermediate conformation (geen).

Hence, compound 22 showed activity in the potency assays while compound 13 did not.

2. Docking compound C13, C18, and C22 to ERK3:

a. Docking protocol:

A conformer library of 300 conformations of each compound was built with BCL:Conf application (doi: 10.1021/acs.jcim.0c01140).

All four-conformation variation (Chain A-D) of the crystal structure of ERK3-C18 complex were used as templates for docking. I used RosettaRemodel application (doi: 10.1371/journal.pone.0024109) to add missing loops to the four crystal structure variants, and then used RosettaRelax application (https://doi.org/10.1371/journal.pone.0059004) to minimize and optimize the sidechain conformations of the template structures before docking.

I generated 10,000 docking models for each compound using RosettaLigand application (doi: 10.1016/j.jmb.2008).

b. Validation of RosettaLigand docking by redock compound 18

The compound 18 was redocked to ERK3 with RosettaLigand to test the docking application's performance on predicting the native binding pose. The Rosetta predicted binding energy vs RMSD plot showed that the output docking models converse at the binding poses that is ~1A from the native binding pose from the crystal structure (PDB-ID: 6YKY, 2.5 Å resolution). This signified that the software could predict the native-like binding poses for similar inhibitors of ERK3. The best model of compound 18 has the interface energy of -23.6 REU

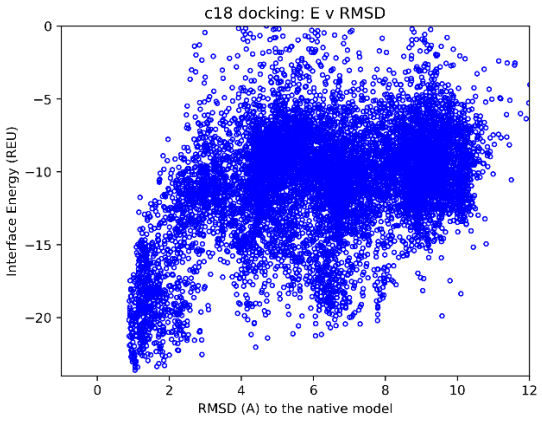

Figure 3. Rosetta predicted binding energy vs RMSD to the native binding pose of compound 18.

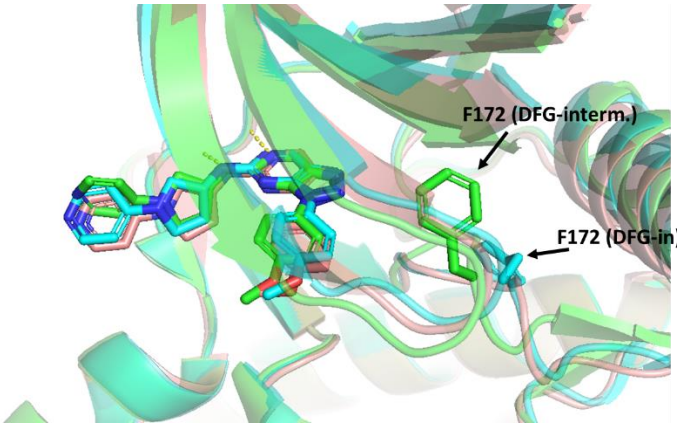

Figure 4. Predicted binding mode of compound 18 (pink) compared to crytal structures of compound 18 binding to activate ERK3 (cyan) and inactive ERK3 (green).

c. Docking results of compound 22

Similarly, compound 22 was docked to the same binding pocket of compound 18. Overall, the output models conversed well to a docking pose that is very similar to that of compound 18. The best model of compound 22 has the interface energy of -22.8 REU, which is slightly higher than that of compound 18.

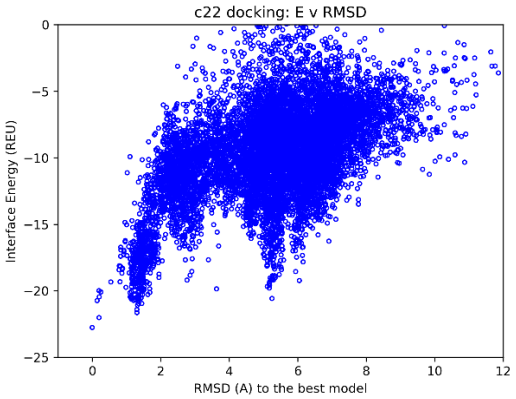

Figure 5. Rosetta predicted binding energy vs RMSD to the native binding pose of compound 22.

d. Docking results of compound 13

Similarly, compound 13 was docked to the same binding pocket of compound 18. There are two prominent potential binding posed that at the RMSD of 0A and 8A from the best score model. The best interface scores for two potential binding modes are -20.8 and -19.9 (REU), which is higher than those of compound 22 and 18.

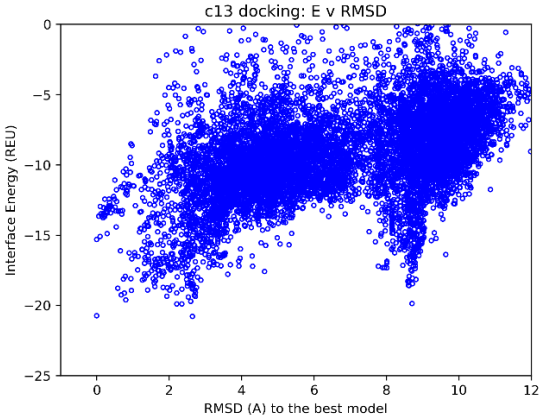

Figure 6. Rosetta predicted binding energy vs RMSD to the native binding pose of compound 13.