The goal of running this trajectory was to evaluate the potential benefits of adding an adamantyl group to the thiazole scaffold to facilitate CYP3A4-selective inhibition and determine whether the compound is worth pursuing synthetically. Just because there is convergence, or there are contacts predicted, does not mean this molecule will be a potent inhibitor.
Results at a Glance: Gross Problems with Trajectory Data?

The first places to look to determine whether there are significant setup or output errors with the trajectory are the:

**Protein-Ligand RMSD Plot**
- Does the ligand stabilize or find multiple discrete binding poses?
- Does the protein stabilize?

**Protein-Ligand Contacts Histogram**
- Are stable, high percentage contacts identified?
- Are conserved/expected contacts present?
Let’s determine whether this ligand is “good” by taking a closer look at its behavior over the course of the full trajectory, and the quality of the contacts predicted.

Looking at this output, one can identify three time periods of interest that will help to understand what is happening with this ligand. An initial binding pose, a transitional period, and a second binding pose.
Initial Binding Pose (~0 – 5 ns)

Here residue contacts are identified that have sustained interactions with the inhibitor for the majority of the time interval. Contacts that are blinking in and out or are only briefly stable are omitted to distill out the most crucial contacts for this portion of the trajectory.

Two water bridges formed with Arg106 and Glu374 (yellow), and an aromatic H-bond (grey) with Phe213 are identified. These interactions provide little stabilization.
Transition to New Binding Pose (~5 – 11 ns)

This transition period shows residue contacts that are by nature less stable. As such, characterizing this timeframe in the trajectory is more about novel interactions that exist in this regime rather than extraordinarily stable contacts. Contacts from the first timeframe either die off or be maintained. Contacts from the second binding pose will begin to manifest.

Water bridge(s) between Gly481 and the inhibitor can be visualized (yellow). Further analysis (Protein-Ligand Contacts Histogram) shows that the interactions between Arg375 and the inhibitor are general hydrophobic interactions.
Second Binding Pose (~11 – 20 ns)

This second binding pose allows for water bridges with Ala370 and Ser119 (yellow), π-stacking interactions with Phe57, Phe213, and Phe108 (blue), and aromatic H-bonds (grey). Again, these interactions are generally not as stabilizing as other polar interactions.

This, along with the fact that the adamantyl group offers no unique interactions, suggests that this inhibitor may not be a potent inhibitor. However, the steric size of the inhibitor may afford selectivity over other smaller active sites.
Ligand has at least two discrete binding poses. Can one identify what changes between these poses with respect to the ligand rather than with respect to the protein sidechain interactions?

Yes.

The ligand atoms with the highest RMSF are likely the atoms in the ligand that have changed their position, and thus side chain interactions over the course of the trajectory/exploration of binding poses.

The trajectory structures/video can be used to correlate what groups of side chains previously identified are crucial to each unique phase of the trajectory, as well as to what part of the ligand they interact with to facilitate these ligand conformational changes.

*There is also a protein RMSF plot output that is more usefully considered dynamically, and as such is not shown here.*
Ligand Torsion Profile

If one is interested in determining what parts of a molecule exhibit greater flexibility in the active site, rather than a gross change in position, the ligand torsion profile can be referenced. A narrow distribution on this continuous graph indicates little/no rotation about the labeled bond, whereas multiple distributions or a broad distribution indicates greater flexibility about that bond.

It is known from the ligand RMSF that the central thiazole and adamantyl group move relative to the active site. However, is this motion a result of flexibility in the ligand, or a repositioning of the ligand in the active site? One can see that the bonds adjacent to the thiazole show single, relatively narrow distributions. This indicates that the high RMSF associated with this portion of the molecule is due to repositioning rather than flexibility. However, bonds that attach the adamantyl group show two subpopulations indicating that there are torsional conformers explored by this part of the ligand that led to the higher RMSF. This indicates that this area of the active site may be less sterically challenged, or perhaps the interactions with this group are not strongly stabilizing. Lastly, one can identify that one of the OMe groups (dark green) is in an area of greater flexibility in the active site due to its broader distribution.
This diagram will report (above a user-defined threshold) ligand-protein contacts and the percentage of frames in the trajectory that they are observed. These diagrams typically report fewer contacts than will be seen in the histogram or in the residue contacts time course map, acting as a distilled version of these pieces of information. Further, the ribbon surrounding the ligand gives some perspective on the steric of the environment, solvent-exposed portions of the ligand, and the polarity of active site microenvironments.

In this case one can see that there are only a few interactions that are present for greater than 30% of the trajectory. Further, these interactions are weakly stabilizing water bridges present for less than half of the trajectory, with one pi-stacking interaction present for only 38% of the trajectory. These interactions do not suggest that this inhibitor would be potent. Additionally, the adamantyl group of the inhibitor is located in a polar part of the active site, suggesting that this functional group may not be optimized for the properties of the active site. One positive takeaway, however, is that the active site can accommodate this size of ligand without issue.
Ligand Properties

These reports show general physical properties of the ligand over the course of the trajectory, if applicable.

Of interest for this particular system are the solvent accessible surface area (SASA) and polar surface area (PSA). One can see that as the trajectory converges on the second binding pose, the ligand’s new pose reduces the solvent accessible polar surface area of the molecule as it engages in additional contacts that obscure polar oxygen and nitrogen atoms. However, as this repositioning occurs and oxygen and nitrogen atoms are less accessible, the total SASA of the molecule increases. This suggests that as the ligand converges on its second binding pose, it does so to bury or engage polar groups at the cost of exposing other areas of the molecule.
Protein Secondary Structure

Here one can see how the secondary structure of the protein changes over the course of the trajectory, reported as an overall percentage of residues that are a part of secondary structure, as well as a per-residue map showing what and when secondary structure is formed.

In this case, one can identify residues in the upper panel that are not in stable secondary structure by looking for residues with lower, but nonzero percent secondary structure (%SSE). Identifying these residues could potentially inform about the destabilization of secondary structure by the presence of a ligand. In this case, however, if these residues are compared with the time course of their engagement in secondary structure in the bottom panel, one can see that they are immediately adjacent to secondary structural elements, and blink in and out of participation randomly over the course of the entirety of the trajectory. This fluctuation is not unexpected, and likely not a consequence of the ligand's presence. The purple arrow highlights how engagement of this part of the sequence in an alpha helix is temporally constrained, suggesting some event precludes engagement of these residues in secondary structure following its occurrence. This may not be a direct consequence of the ligand's presence, but it is worth considering.