Assignment 7

Laura McDonnell

Approach

Goal: Identify a small molecule that binds to NRAS Q61R and blocks its signaling.

Target Structure Selection: After considering the structures from Assignment 6, I selected both the GDP-bound and GTP-bound Q61R NRAS structure for downstream analysis of possible binding sites in FTMap. This would allow me to determine similar sites between both conformations and identify unique sites that could be used for more selective targeting.

Binding Site Identification: FTMap was used to identify potential binding hotspots on both GTP-bound and GDP-bound structures of NRAS Q61R, as shown in figure 1. This revealed several binding sites, with a particularly interesting hotspot located near the Switch II region (residues 67-76) on the GDP-bound structure. This binding site only appeared on the GDP-bound structure, which was perfect for selective targeting of this conformation. Additionally, this region undergoes significant conformational changes between active and inactive states. It is also critical for some of the protein-protein interactions we looked at before, including BRAF binding, and shows structural differences between WT and Q61R mutant forms. Importantly, this site is in proximity to R61, providing an opportunity for selective targeting. So, this Switch II region seemed particularly attractive as a binding site because stabilizing it in the GDP-bound conformation would prevent the structural rearrangements necessary for effector binding and downstream signaling.

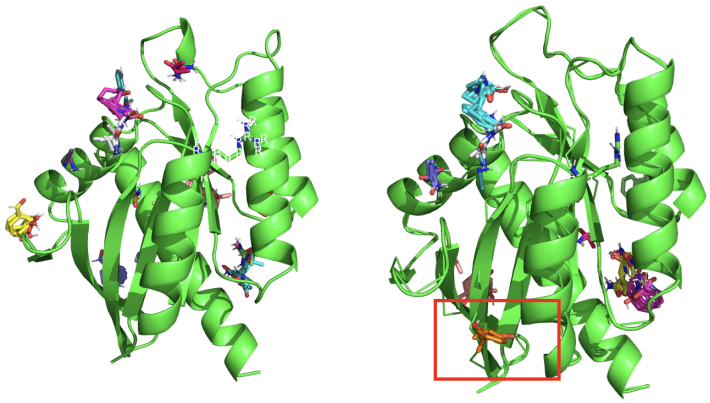

Figure 1: FTMap results showing binding hotspots on NRAS Q61R GTP-bound (left) and GDP-bound structure (right). The Switch II region indicated in red (residues 67-76) shows selective probe clustering, indicating a druggable pocket.

Pharmacophore Development Based on the FTMap results and analysis of the binding site composition, a pharmacophore model targeting the Switch II region was developed. The binding site featured key residues including tyrosine, glutamine, and lysine, which guided the pharmacophore design. The final pharmacophore included hydrogen

bond acceptor features to interact with lysine and the hydroxyl group of tyrosine, hydrogen bond donor features to form interactions with glutamine, and an aromatic feature to engage in π-stacking with tyrosine. Hydrophobic features were ultimately removed for the final pharmacophore. This pharmacophore was designed to achieve both potent binding and selectivity for the Q61R mutant over wild-type NRAS.

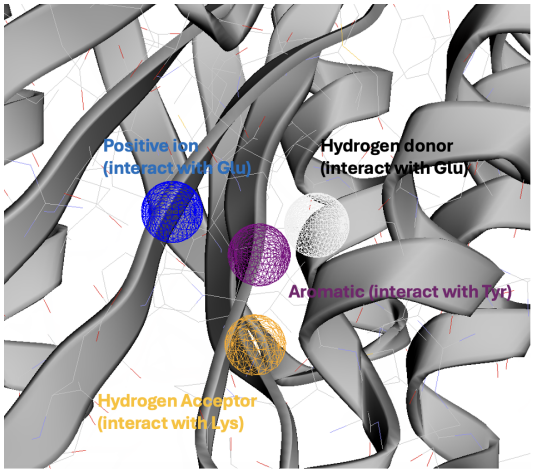

Figure 2: Pharmacophore model targeting the Switch II region of NRAS Q61R. Color coding: hydrogen bond acceptors (yellow), hydrogen bond donors (white), aromatic features (purple), positive ionizable feature (blue).

Virtual Screening and Compound Selection The pharmacophore model was implemented in Pharmit to screen several compound libraries, including ChemDiv and ChemBL34. The top hit from each of these screenings were then minimized using Smina to refine binding poses and improve affinity predictions. Selection of the final compound was based on multiple criteria including binding affinity, interactions with key residues in the Switch II region, and ability to stabilize the inactive conformation.

Results

Selected Compound Properties The screening and minimization protocol yielded a promising lead compound with favorable properties for targeting NRAS Q61R. The selected compound demonstrates strong binding affinity with a Vina score of -7.60115 kcal/mol, favorable positioning in the Switch II binding pocket, multiple key interactions with residues in the binding site, and drug-like physicochemical properties. This compound was chosen from among the top hits because it best satisfied all selection criteria while maintaining a favorable binding pose in the target site.

Mechanism of Action The selected compound achieves efficacy through an allosteric mechanism rather than direct competition with GTP binding. By binding to the Switch II region in the GDP-bound conformation, it stabilizes the inactive state of NRAS, making the transition to the active conformation energetically unfavorable. The compound creates a steric hindrance that prevents the proper reorientation of Switch II required for effector binding and disrupts the network of interactions necessary for GTP signaling. This mechanism is particularly advantageous as it allows normal GDP binding and release while specifically blocking the oncogenic signaling caused by the Q61R mutation. The compound essentially acts as a blocker, preventing the structural transitions that would normally occur during activation.

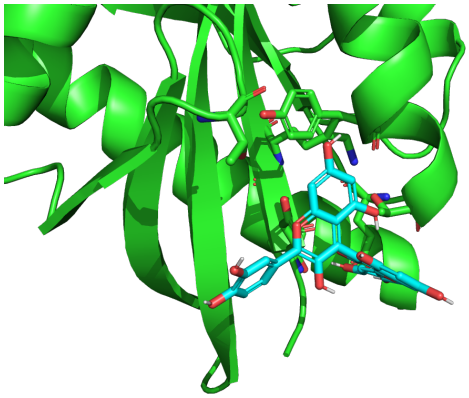

Figure 3: Binding pose of the selected compound in the Switch II region of NRAS Q61R. The compound forms key interactions with critical residues in this region.