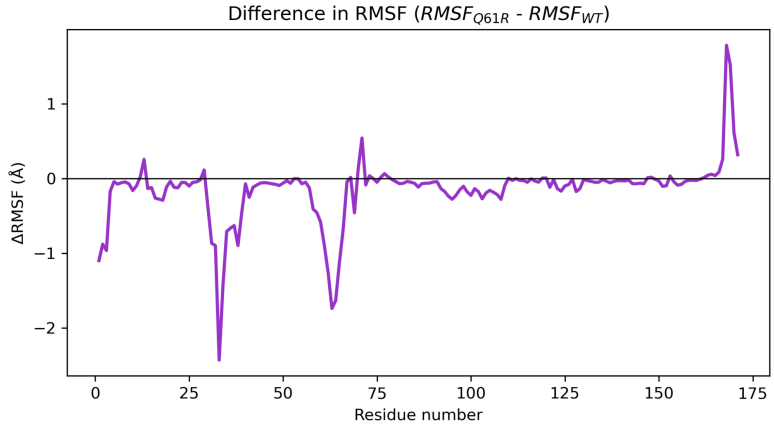
**For all figures, residue indexing ranges [1,171], such that the mutated residue 61 is plotted and labeled as residue 62. However, for consistency with relevant studies, the mutation itself will be referred to as Q61R.

1a



1b

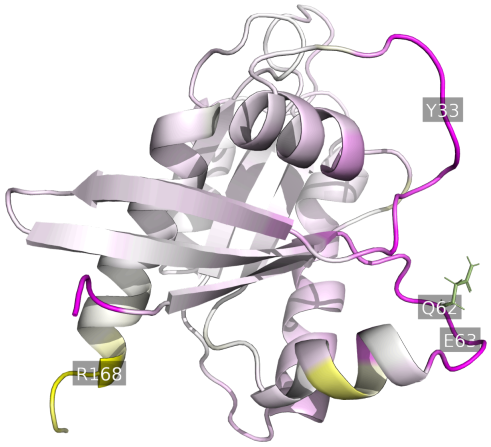


Fig. 1a: Difference in Root Mean Square Fluctuation (ΔRMSF) plot (Q61R − WT). RMSF values were computed for backbone Cα atoms from WT and mutant simulation data. RMSF differences were calculated by subtracting WT RMSF values from mutant RMSF values. Negative values correspond to regions more flexible in the WT, and positive values to those more flexible in the Q61R mutant.

Fig. 1b: ΔRMSF (Q61R − WT) values mapped onto WT structure. Regions in magenta indicate residues with greater flexibility in the WT, and regions in yellow indicate greater flexibility in the Q61R mutant. The WT residue of interest, Q62, is highlighted in green and labeled. Other labeled residues at positions 33, 63, and 168 are local minima and maxima in the corresponding plot in Fig.1a.

2

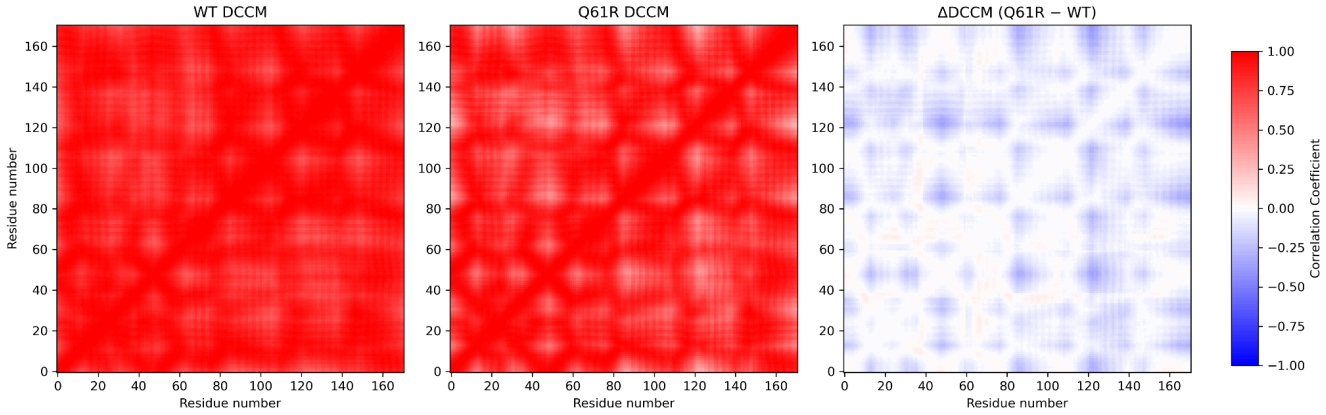


Fig. 2: Dynamic Cross-Correlation Maps (DCCM) for WT (left) and Q61R (middle), and ΔDCCM (Q61R − WT) (right). DCCM values were computed for backbone Cα atoms from WT and mutant simulation data. For the left and middle plots, the color represents the correlation coefficient of movement between each pair of residues in the protein. Red regions indicate positively correlated residues, while blue regions indicate negatively correlated residues. The ΔDCCM values were calculated by subtracting WT DCCM from mutant DCCM. In that plot, the color represents the difference in dynamic cross-correlation between WT and mutant proteins. Red regions indicate a more positive correlation between those residues in the mutant than in WT. Blue regions indicate a more negative correlation between those residues in the mutant than in WT. In this case, as there are little to no negatively correlated residues in either WT or mutant structures, blue regions indicate residues with weak or no correlation in the mutant, but strong positive correlation in the WT. This is substantiated by the observation that blue regions in the ΔDCCM plot tend to be close to white in the Q61R DCCM and red in the WT DCCM.

Discussion

Figure 1a and 1b confirm the previous claim that the Q61R mutation decreases the flexibility of the NRAS protein. This is due to the presence of prominent negative peaks in the ΔRMSF plot. Two labeled residues (Fig. 1b), Y33 and E63, are at local minima in Fig. 1a and are located in magenta regions of Fig. 1b. These residues are located within the switch I (residues 25-40) and switch II (59-75) domains of NRAS. Those switch motifs are known to be the sites of the largest conformational change between GTP-bound “on” state and GDP-bound “off” state, as they encircle the GTP/GDP binding pocket. These findings suggest that the Q61R mutation increases the rigidity of this binding pocket, locking the protein into a narrower range of conformations. This could be consistent with the fact that Q61R is an activating mutation. The Q61R mutation swaps a medium-sized, neutral residue for a large, positively-charged one, which appears to stabilize the protein due to additional charge-charge or hydrogen-bonding interactions formed by arginine, possibly with residues further away that were out of reach for glutamine. The mutation could be locking the NRAS protein into a perpetual GTP-bound conformation, promoting the downstream effects of GTP-binding even in the absence of GTP. Further studies would include a structural alignment and comparison between GTP-bound WT NRAS, GDP-bound WT NRAS, and the Q61R mutant created in this study, in order to compare the conformations of these switch domains. If the Q61R mutant’s structural conformation in these regions is found to be more similar to the GTP-bound WT, this would strongly suggest and be consistent with the claim that Q61R is an activating mutation. It can also be noted that these figures show a local maximum at R169, 2 residues from the C-terminus. This indicates that the Q61R mutation increases the flexibility of the C-terminal end of the NRAS protein. Further analysis would be required to study the effect of this specific flexibility change.

Figure 2 offers insight not shown in any previous figures. Most residues in both WT and mutant proteins appear to be strongly coupled, suggesting a relatively rigid structure. However, the figure also shows a relative decoupling of residues in the mutant trajectory. The blue regions in the ΔDCCM plot show areas where this decoupling occurs, as those regions are generally strongly red in the WT DCCM and white in the mutant DCCM. This is consistent with the notion that the mutation locks the NRAS protein’s switch domains (and the GTP/GDP binding site, as a result) into a more rigid conformation. This figure shows that this increased rigidity in certain regions is strong enough to generally decouple the protein’s motions, and thus very effectively and robustly hinder its activity. In the present molecular dynamics analysis, the Q61R mutation is seen to cause strong rigidity in the NRAS switch domains, in such a way that the protein’s overall motions are decoupled. This is consistent with the Q61R’s biological characterization as an activating mutation, as it could be locking the protein into an “on” GTP-bound conformation even in the absence of GTP. The analyses chosen for this study, ΔRMSF, DCCM, and ΔDCCM, offered insight into the extent of the relative rigidity of the mutant protein compared to the WT, and were necessary to show the decoupling effect of the mutation.