Q5:
The GDP-bound WT and Q61R mutant structures are somewhat similar (Fig. 8), but complex changes in interaction of residue 61 with other residues are visible through Fig. 1a-b and 2a-b. The overall changes are seen in Fig. 3 and 4. The mutation (located on the Switch II domain, which is residues 59-75) altered interaction of its own domain with the other domains encircling the GDP/GTP binding site. The distribution of distances between residue 61 and residue 37 became noticeably more narrow, which is most apparent comparing Fig. 1b and 2b. Residues 10 and 16 are located on the P-loop (residues 10-17), while residue 37 is located in the Switch I domain (residues 25-40). The latter suggests that the Q61R mutation decreases flexibility of the Switch I domain, which aligns with previous conclusions that the mutation increases the rigidity of the protein. One explanation for this may be that the negatively charged E37 forms a strong salt bridge interaction with positively charged R61 and not with uncharged Q61, which it may have only hydrogen bonded with. This also results in a decrease of the mean minimum distance between R61 and E37 (Fig. 3). We also see a shift in the distribution of distances to residues 10 and 16, where the P-loop residues are generally farther from residue 61 in the mutant. Further analysis may be required to fully understand the functional effects of this change, as well as its exact biochemical causes. However, a possible cause of this is a repulsive force between positively charged K16 and R61. The mutant is seen to be more rigid and locked into its conformation. However, this is evidence of the generally decreased tight packing and coupling of these three binding site domains in the mutant, destabilizing the inactive GDP-bound form. This aligns with Q61R's characterization as an activating mutation.

Q6:
The most noticeable difference between the GTP-bound Q61R mutant and WT is in the distribution of minimum distances between residue 61 and residue 37 (Fig. 5a-b, 6a-b). The mutant form still existed in a conformational state where E37 was further (~7.5 Å), but primarily existed in a form where E37 was ~2.5 Å away. This maximum in the distribution is extremely narrow, indicating that this conformation is very stable and does not allow for much flexibility or relative movement between the Switch I domain (25-40) and Switch II (59-75). This supports the previous conclusion that the Q61R mutant is more rigid and is locked into its conformation. The mutation seems to stabilize an unusually tight coupling between R61 and E37, likely due to ionic interactions as well as other steric factors that allow for this interaction to be tighter here than it was in the GDP-bound mutant. In contrast, the GTP-bound WT minimum distance between Q61 and E37 has a much broader distribution where E37 is almost always further (8-14 Å) than it is in the mutant (Fig. 5a, 6a). As seen in the GDP-bound mutant as well, R61 seems to have some repulsive interaction with K16, creating a greater mean minimum distance between the two in the mutant (Fig. 7). However, this may not match the

overall interaction between Switch II and P-loop domains, as G10 is on average closer to residue 61 in the mutant than in the WT. Looking carefully at Fig. 8, it appears that the P-loop (residues 10-17) is oriented in the direction of residue 61 in the mutant. The P-loop is known to stabilize binding of the nucleotide, and otherwise move away to allow hydrolysis of GTP by water. This strongly supports the conclusion that Q61R is an activating mutation, as the P-loop appears to be drawn more into a conformation where it would lock GTP into its binding site and prevent hydrolysis to GDP. It stabilizes the active form of NRAS to prevent inactivation. Another difference seen in Fig. 7 is the increased mean minimum distance between R61 and T58. This can be explained by the increased coupling of R61 to the P-loop and large residue E37. These may result in organizational steric clashes causing T58 to move away from Q61 in order to make room for these other structures to orient more closely around R61. All these differences in interactions between GTP-bound WT and Q61R mutant NRAS support the conclusion that the mutant structure has a more stable active (GTP-bound) conformation than the WT. The mutant is less likely to hydrolyze GTP and become inactivated.