SUPPLEMENTAL INFORMATION

Structural dynamics in Ras and related proteins upon nucleotide switching

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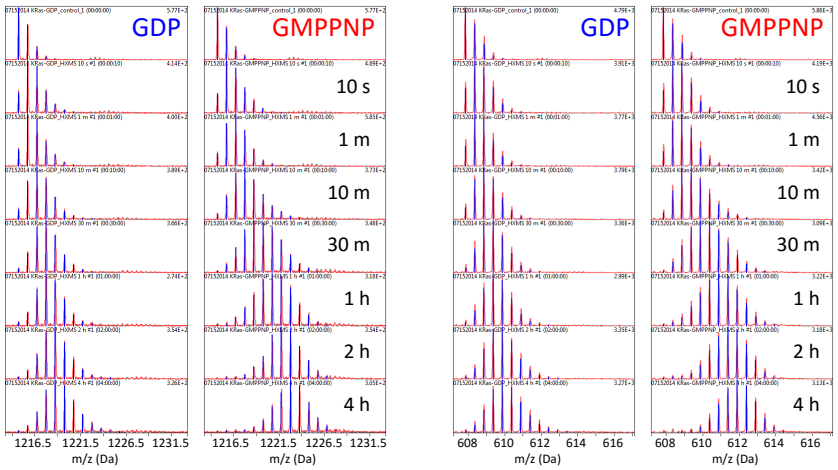
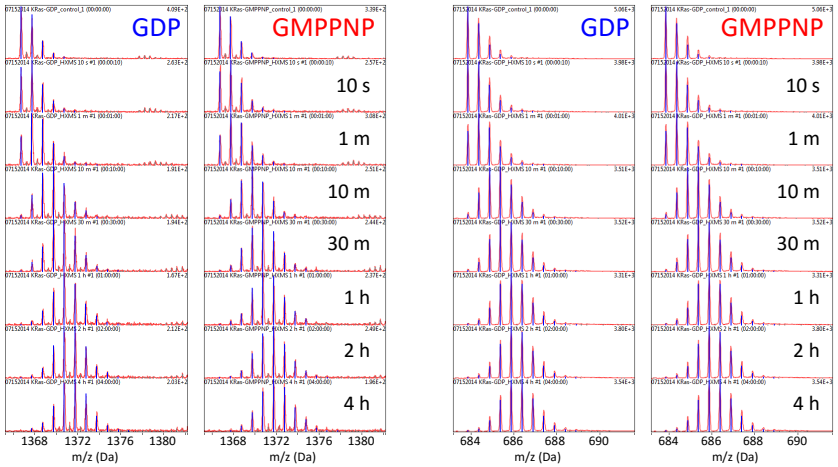
Supplemental information contains Figures S1 – S6

(a) VVVGAGGVGKSALT (+1, +2)



(b) LARSYGIPFIET (+1, +2)



(c) YTLVREIRKHKEK (+2, +3, +4)

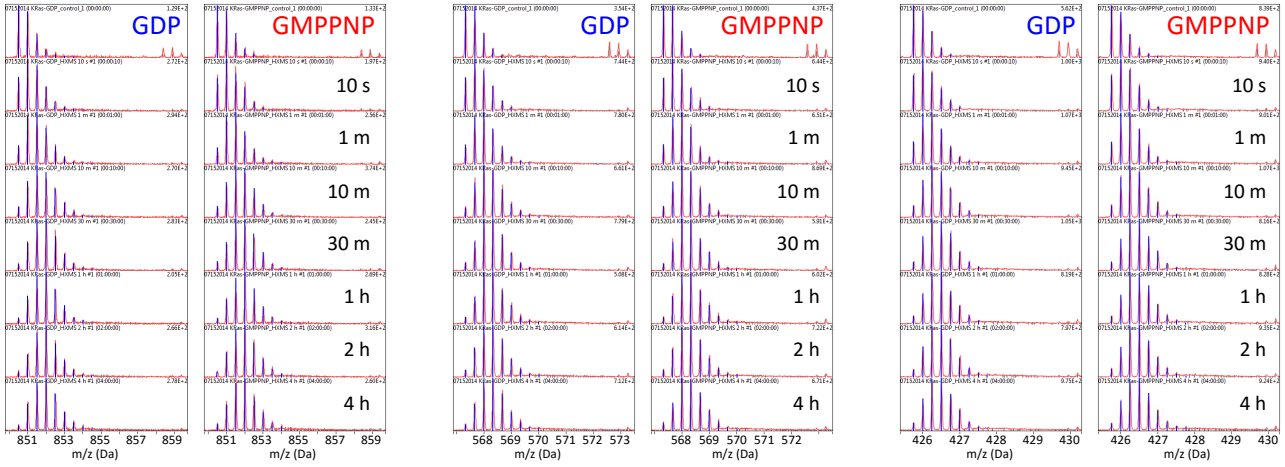


Figure S1. Mass spectra of representative peptides from K-Ras, each spanning multiple charge states. For each peptide, spectra associated with the lowest charge state is on the far left, and charge state increases moving from left to right. Undeuterated controls are shown at the top, labeled either GDP or GMPPNP depending on bound nucleotide, and labeling time increases from top to bottom. (a) peptide VVVGAGGVGKSALT, 7-20 (b) peptide LARSYGIPFIET, 133-144 (c) peptide YTLVREIRKHKEK, 157-169.

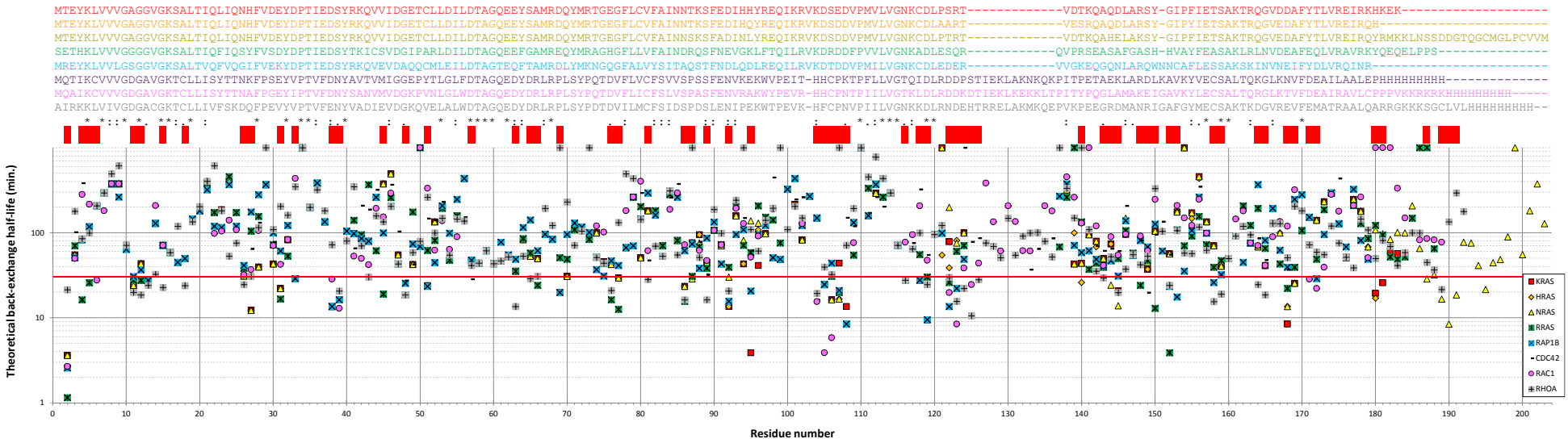


Figure S2. Theoretical back-exchange half-life [Bai et al. (1993). Proteins 17,75-86] of every backbone amide hydrogen for all proteins studied, as calculated using HXPep (created by Dr. Zhongqi Zhang, Amgen, Thousand Oaks, CA). Prolines do not have amide hydrogens and are represented by placing a symbol at the top of the Y-axis. Aligned sequences of each protein are shown at the top of the figure, including symbols denoting sequence conservation. Residue numbering consistent with Figure 1a is on the X-axis. Red flags above the graph indicate amino acid positions for which the back-exchange half-life varies significantly between proteins. In order for a position to be flagged, the minimum theoretical back-exchange must be less than 30 minutes, and the relative standard deviation of all theoretical back-exchange values at that position must exceed 10%. There is a red line drawn at the back-exchange half-life of 30 minutes, above which differences in theortical back-exchange half-life are likely irrelevant due to the timing of HDX MS experiments. A value of 30 is conservative given the vast majority of peptides experience less than 10 minutes total between sample thawing and analysis by the mass spectrometer.

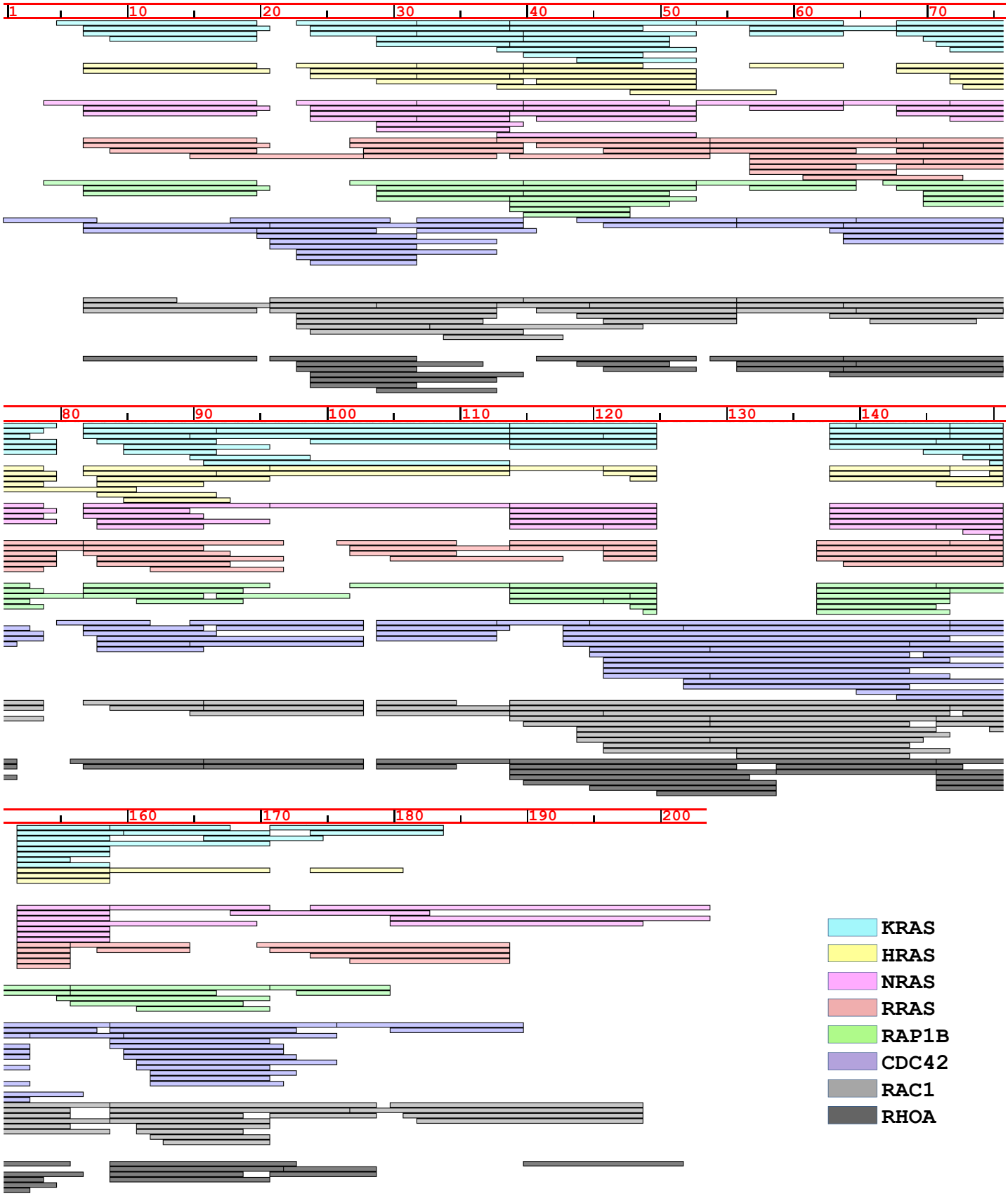


Figure S3. Peptic peptide coverage maps for each Ras protein analyzed in this work. Peptides that include any portion of N-terminal purification tags are not shown. Residue numbering is consistent with Figure 1a.

(a)

Protein	GDP (%) in Inactive Samples	GMPPNP (%) in Active Samples
K-Ras	100	71
H-Ras	100	65
N-Ras	100	73
R-Ras	88	87
Rap1B	74	61
CDC42	100	66
Rac1	100	64
RhoA	100	80

(b)

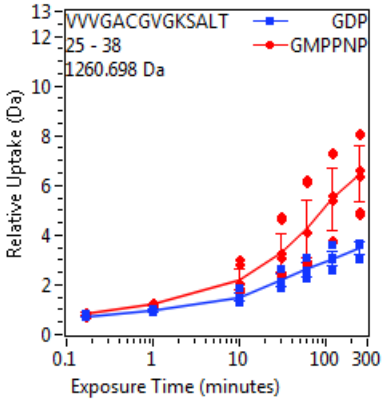


Figure S4. (a) Results of nucleotide loading as measured by HPLC-UV. Values shown correspond to the biological replicates used to generate HX MS data used in Figures 2, 3, and 4. (b) The effects of nucleotide loading on HX MS data, shown on the deuterium uptake plot of a critical peptide from a K-Ras mutant. At each time point and for each state, each cluster of data points represents a distinct biological replicate analyzed in duplicate. The majority of observed variation in deuteration is due to variation in nucleotide loading, especially for GMPPNP (red). Variation in the deuteration of the GDP variant (blue) is due to the fact that each biological replicate was prepared using different solutions and analyzed on a different day. This variation is most likely the result of slight differences in the pH of solutions and slight variations in the LC-MS.

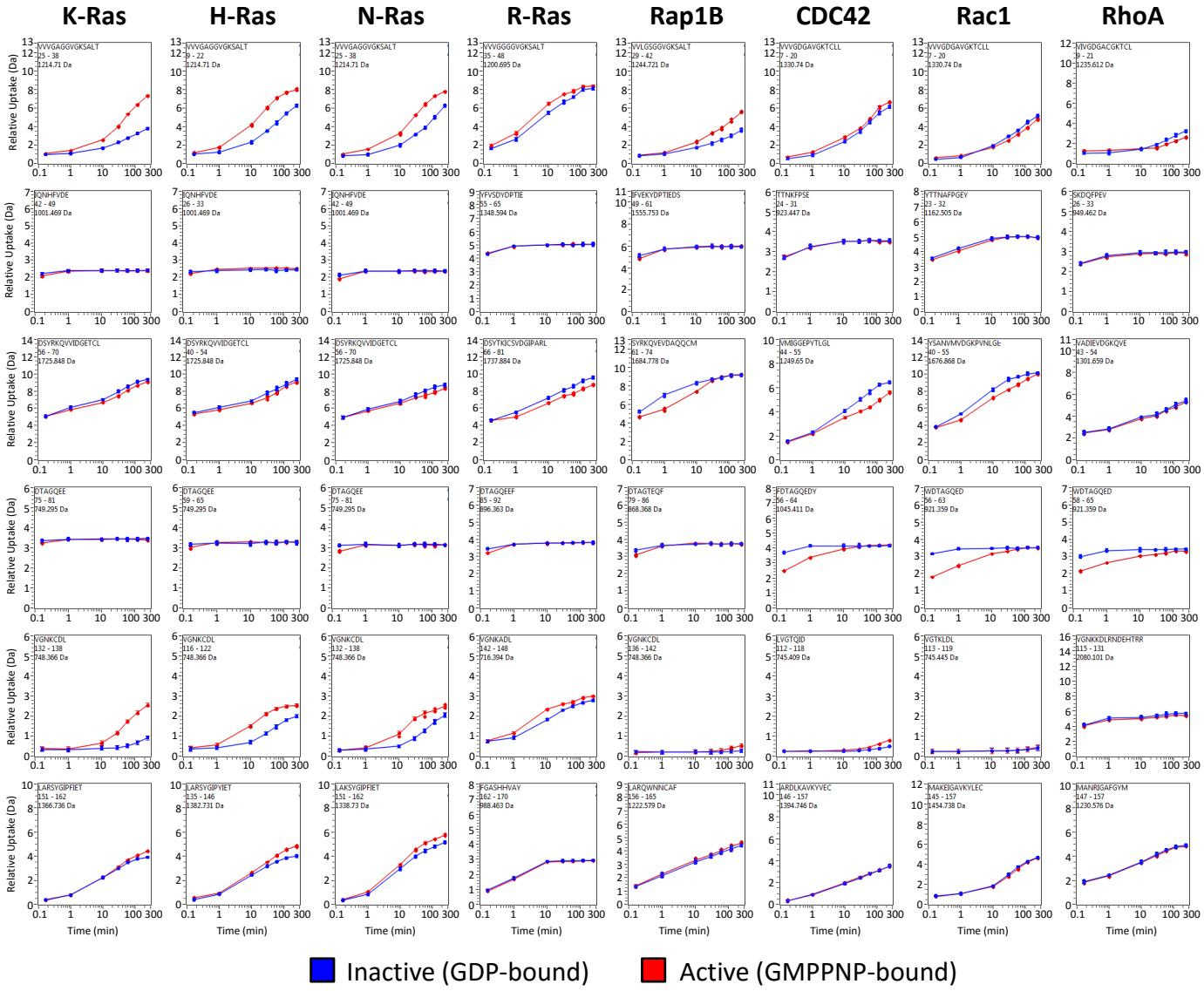


Figure S5. Deuterium uptake plots for key peptides showing characteristic differences in deuterium incorporation between the active (red) and inactive (blue) states.

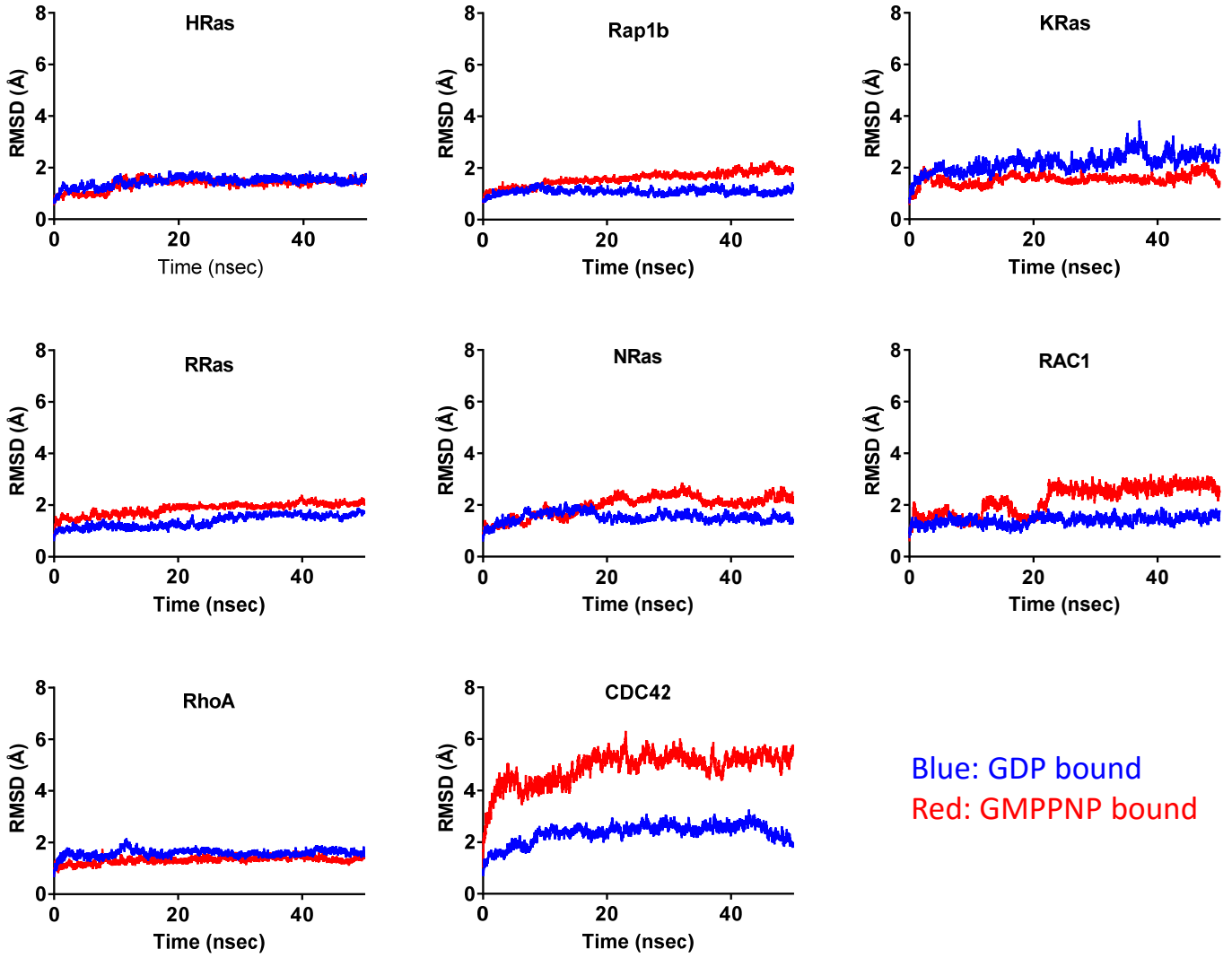


Figure S6. Molecular dynamics trajectories. Protein backbone (Cα) root mean square deviation (RMSD) was used to measure the average change in displacement of Cα in Å relative to a reference frame. RMSD over the span of the entire simulation time are shown for each protein model at a resolution of 10 picoseconds. Data were truncated from the beginning and end of simulations to exclude regions of non-equilibrium.