Supplemental Information

The bacterial ribonuclease P holoenzyme requires specific, conserved residues for efficient catalysis and substrate positioning

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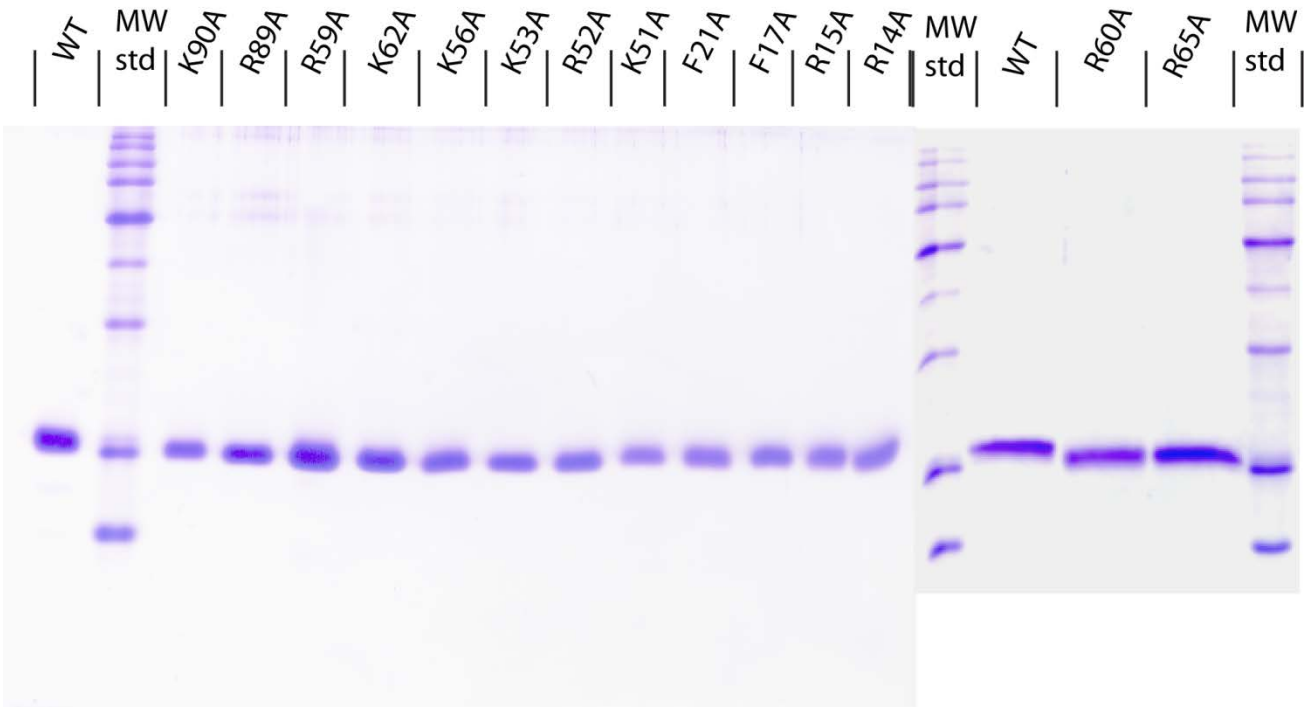
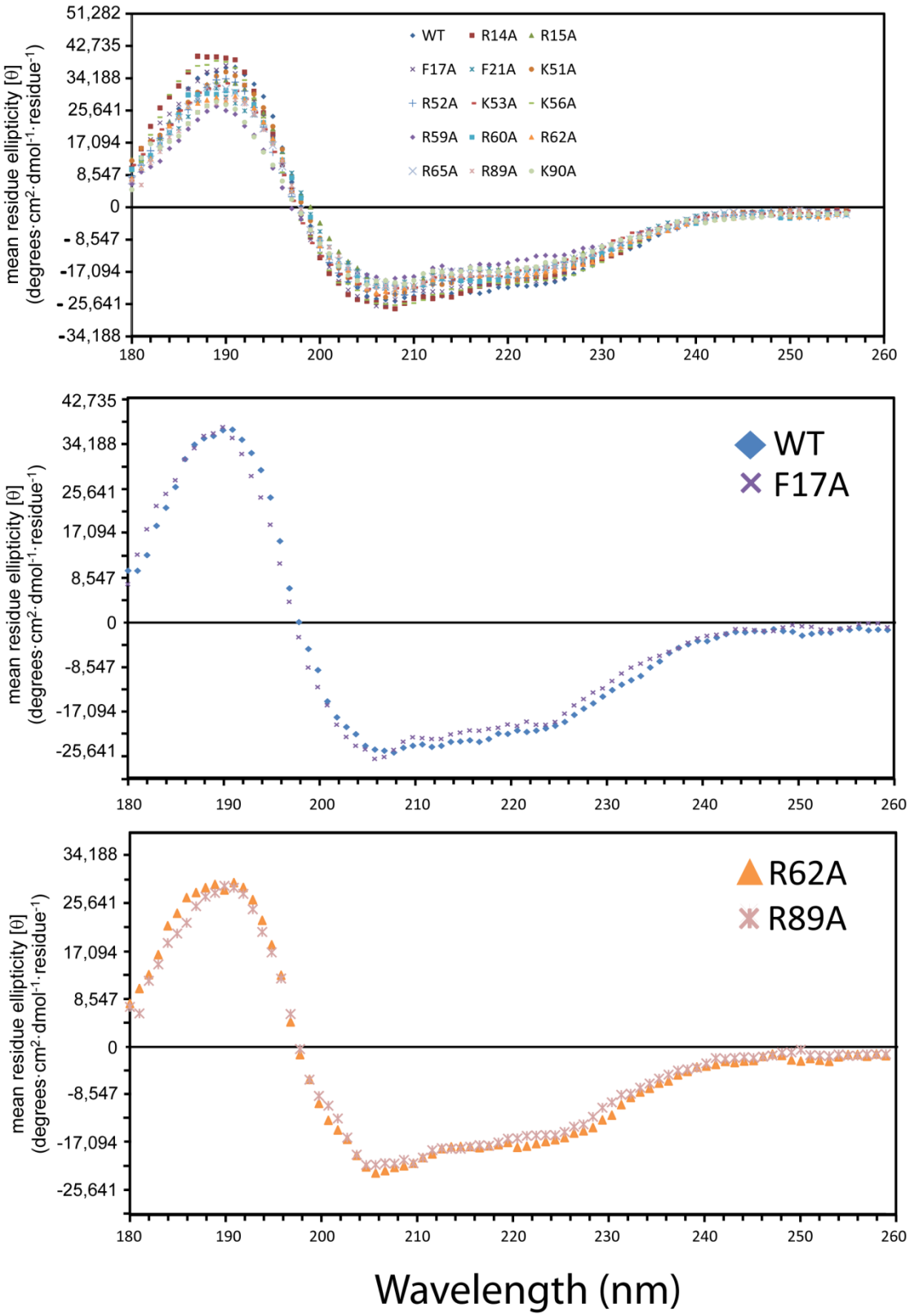


Supplemental Figure 1: SDS-PAGE gel showing the purity of all *T. maritima* RNase P protein point mutants used in the cleavage activity assays. Specific point mutants were targeted based on the structure of the complex (rcsb: 3Q1R) and/or regions of high sequence conservation across bacteria. The identity of each mutant was confirmed by DNA sequencing and mass spectrometry of the purified protein.


mean residue ellipticity [θ]
(degrees·cm²·dmol⁻¹·residue⁻¹)
WT
R14A
R15A
F17A
F21A
K51A
R52A
K53A
K56A
R59A
R60A
R62A
R65A
R89A
K90A
WT
F17A
R62A
R89A
Wavelength (nm)

Supplemental Figure 2: Overlays of the circular dichroism (CD) wavelength scans of the wild-type and RNase P protein mutants show that all mutants are well folded and with no differences in their secondary structure (top). Selective overlay of CD scans comparing the F17A and wild-type P protein (middle) and the R62A and R89A mutant proteins (bottom) reveal near identical ellipticity profiles, demonstrating that F17A and R89A are not mis-folded, but rather, adopt identical secondary structures as functionally active forms of the RNase P protein. Data were collected on a Jasco J-815 as described in the experimental methods section.

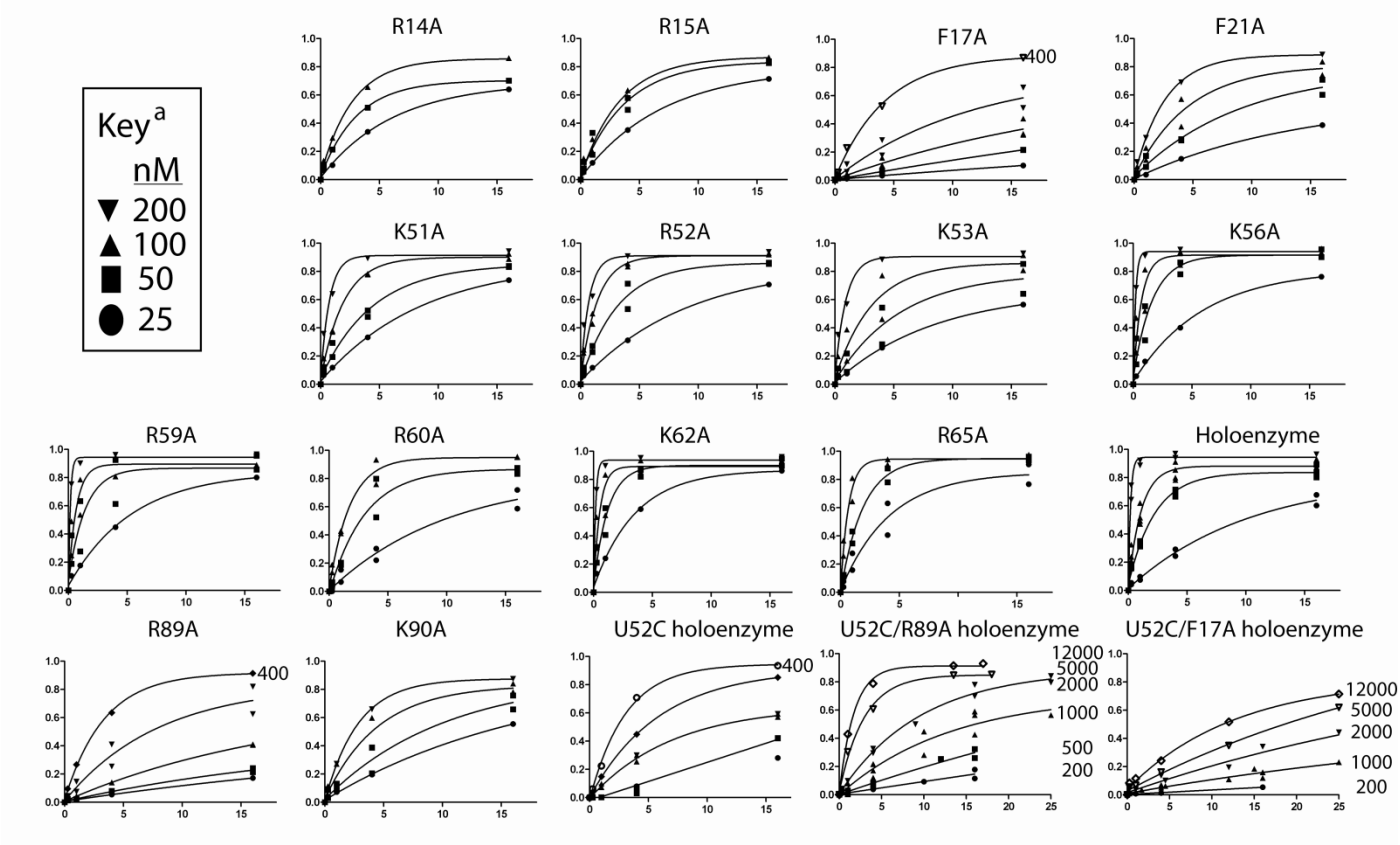


Figure 3: Representative observed kinetic profiles (k_{obs}) of the *T. maritima* RNase P protein and point mutants, showing the percentage of product (y-axis) over time (min.) (x-axis). Nearly all time course cleavage reactions were performed in duplicate and were performed at standard linear enzyme concentration ranges (25 – 200 nM), with the only exceptions being the R89A, F17A, U52C holoenzyme, and double mutants (*dm)* (U52C RNA/R89A or U52C/F17A) due to the dramatic decreases in activity. In both *dm* cases, enzyme concentrations ranged from 200 – 12000 nM.