Supplementary Information

A critical switch in the enzymatic properties of the Cid1 protein deciphered from its product-bound crystal structure

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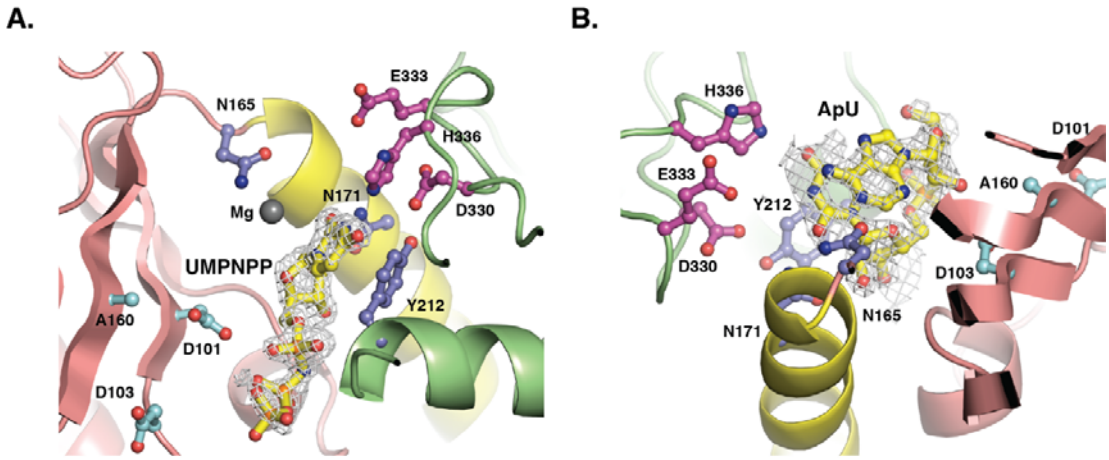
Supplementary Tables

Table SI. Primers for mutagenesis

Mutant	Primer forward (5'→3')	Primer reverse (5'→3')
R139A	TTTTACAAAGGGCAGCAATTCCCATTATC	GATAATGGGAATTGCTGCCCTTTGTAAAA
K144A	CAAGAATTCCCATTATCGCATTAACATCTGATACG	CGTATCAGATGTTAATGCGATAATGGGAATTCTTG
D160A	CGTTTCAATGTGCTATTGGATTTAAC	GTTAAATCCAATAGCACATTGAAACG
N165A	GATATTGGATTTAACACGCGTCTAGCTATTC	GAATAGCTAGACGCGTGTTAAATCCAATATC
F332D	GCGATTGAAGATCCTGACGAGATTTCACATAATG	CATTATGTGAAATCTCGTCAGGATCTTCAATCGC
H336A	CTTTCGAGATTTCAGCTAATGTGGGTAGG	CCTACCCACATTAGCTGAAATCTCGAAAG
Δ310-322	GGATGGACTTCAGCTGACAGGTATATTCTTGCG	CGCAAGAATATACCTGTCAGCTGAAGTCCATCC

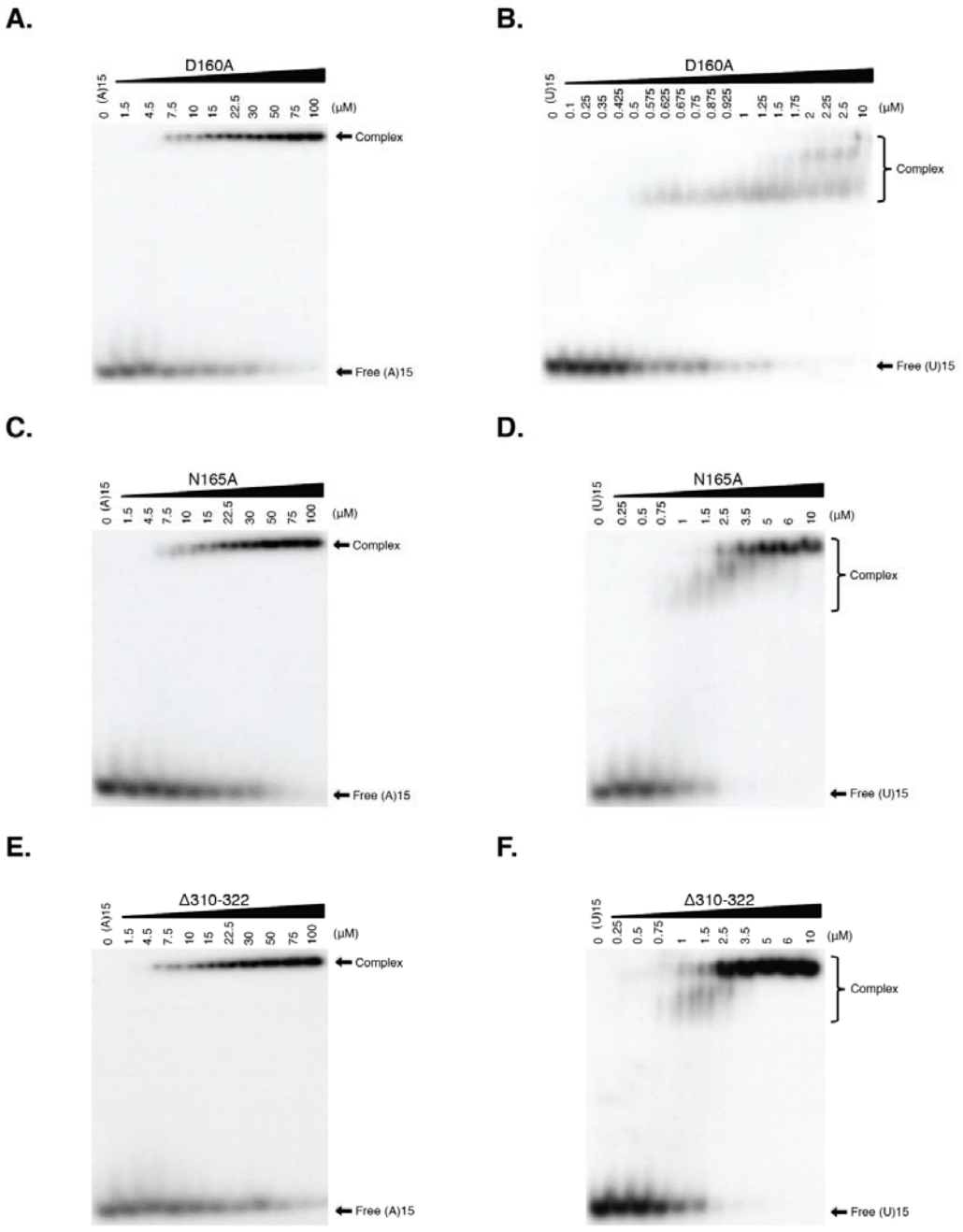
Supplementary Figures and Legends

Figure S1: View of the Fo – Fc difference density maps of the UMPNPP and ApU compounds bound to Cid1.



(**A**) Fo – Fc difference density map of the UMPNPP nucleotide bound to Cid1. The Cid1 protein is shown in lime (CD domain) and salmon pink (CAT domain) with helix 4 in yellow. The NRM loop residues are colored in light magenta, the aspartate triad residues are colored in cyan and the major residues interacting with the ligands are shown in slate. Side chains and ligands are shown using a ball and stick representation (carbon as indicated before, nitrogen in blue, oxygen in red). The difference map was calculated using the refined protein as a model and is shown as a gray mesh (contoured at 2.5σ). (**B**) Fo – Fc difference density map of the minimal product ApU bound to Cid1. The difference map was calculated using the refined protein as a model and is shown as a gray mesh (contoured at 2.5σ). Side chains and ligand are shown as in panel A.

Figure S2: Characterization of the RNA binding properties of the WT and mutant Cid1 proteins by electrophoretic mobility shift assay (EMSA).



(A) EMSA showing the association between D160A mutant and an A_{15} probe. (**B**) EMSA showing the interaction between D160A mutant and U_{15} RNA. (**C**) EMSA showing the binding between N165A mutant and A_{15} RNA. (**D**) EMSA illustrating the binding between N165A mutant and a U_{15} probe. (**E**) EMSA displaying the interaction of Δ310-322 mutant and A_{15} RNA. (**F**) EMSA showing the association of Δ310-322 mutant and a U_{15} probe. Individual reactions in all the panels contained no protein or the indicated amounts.