

# Site-Directed Mutagenesis of Catalytic Residues in $N^5$ -Carboxyaminoimidazole Ribonucleotide Synthetase

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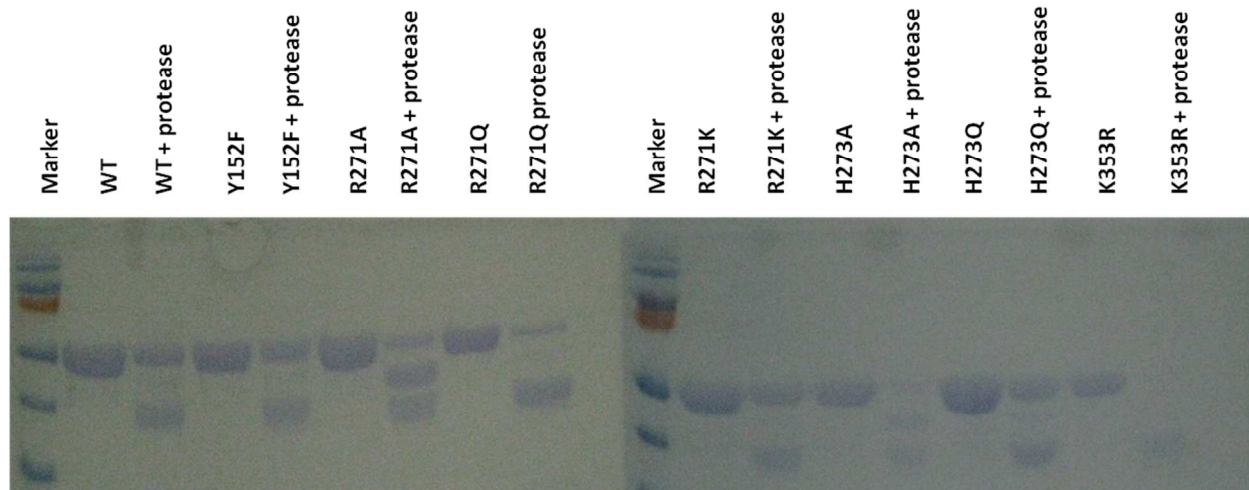
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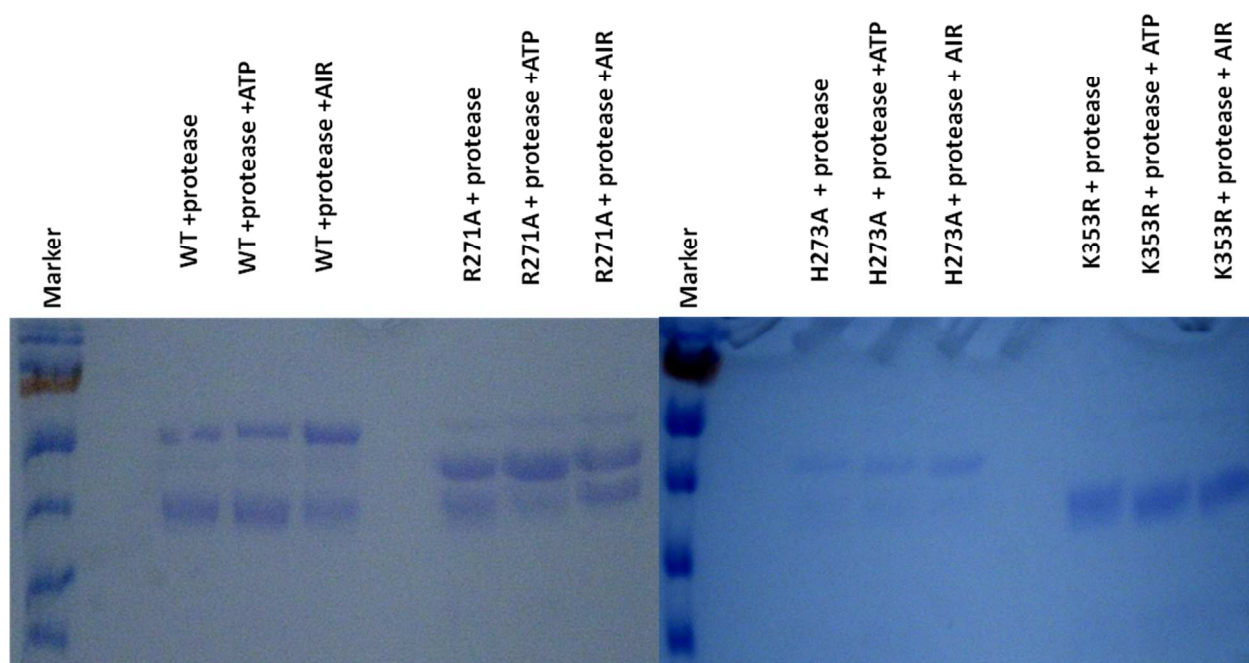
## Primers used for the creation of mutant N<sup>5</sup>-CAIR Synthetase

Mutant	Primers
R271A	5' tgcgagatcgccagcgcgatccacaactcgggc 3' 5' gcccgagttgtggatcgcgctggcgatctcgca 3'
R271Q	5' tgcgagatcgccagc <del>ca</del> aatccacaactcgggc 3' 5' gcccgagttgtggat <del>tt</del> gctggcgatctcgca 3'
R271K	5' tgcgagatcgccagc <del>ca</del> agatccacaactcgggc 3' 5' gcccgagttgtggat <del>ct</del> tgtggcgatctcgca 3'
H273A	5' atgccagccgcatcgcgaactcgggccactac 3' 5' gtagtgcccaggtcgcgatgcggtggcgat 3'
H273Q	5' atgccagccgcatc <del>ca</del> aaaactcgggccactac 3' 5' gtagtgcccaggt <del>tt</del> tgatgcggtggcgat 3'
Y152F	5'tcgaagaccatggcgttcgatggacgtggaac 3' 5'gtttcacgtccatcgaacgccatggtcttca 3'
K353R	5'gccaagcccggccgcgaatggggccacatcacc 3' 5'ggtgatgtggccattcggcggccgggcttggc 3'

Primers for the mutations E73A, Y152A, D153A, R155A, and K353A were used from reference 7.



**Supplementary Figure.** SDS-polyacrylamide gel (12%) analysis of subtilisin treatment of wild-type (WT) N<sup>5</sup>-CAIR synthetase and mutants described in the paper. Protocol is described in the experimental section of the paper.



**Supplementary Figure.** SDS-polyacrylamide gel (12%) analysis of subtilisin treatment of wild-type (WT) N<sup>5</sup>-CAIR synthetase and R271A, H273A and K353R mutants in the presence of different substrates. Enzyme samples were incubated with protease, 1 mM of ATP or saturated levels of AIR as determined experimentally from the enzyme kinetics.