## Supporting Information

# Analysis of GTP addition in the reverse (3′-5′) direction by human tRNA$^{His}$ guanylyltransferase

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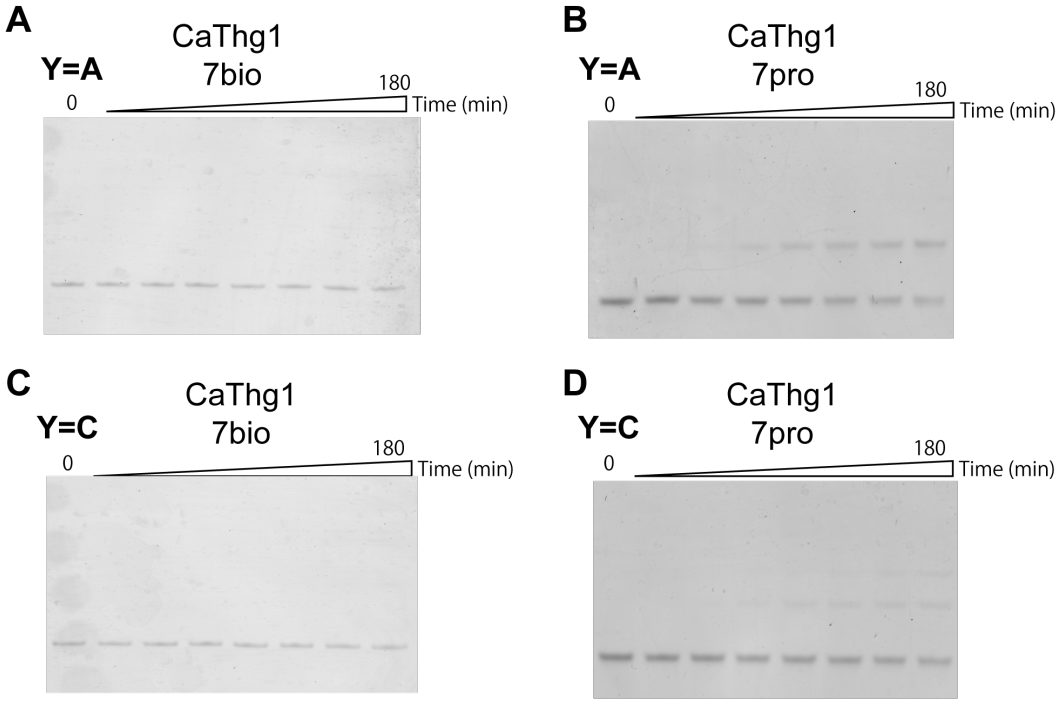


**Figure S1**: Nucleotide addition reaction of 7bio and 7pro by CaThg1 with two-piece tRNAs. CaThg1 has almost no activity for single RNA labeling using 3′RF-A (**A** and **B**) and for multiple RNA labeling using 3′RF-C (**C** and **D**)

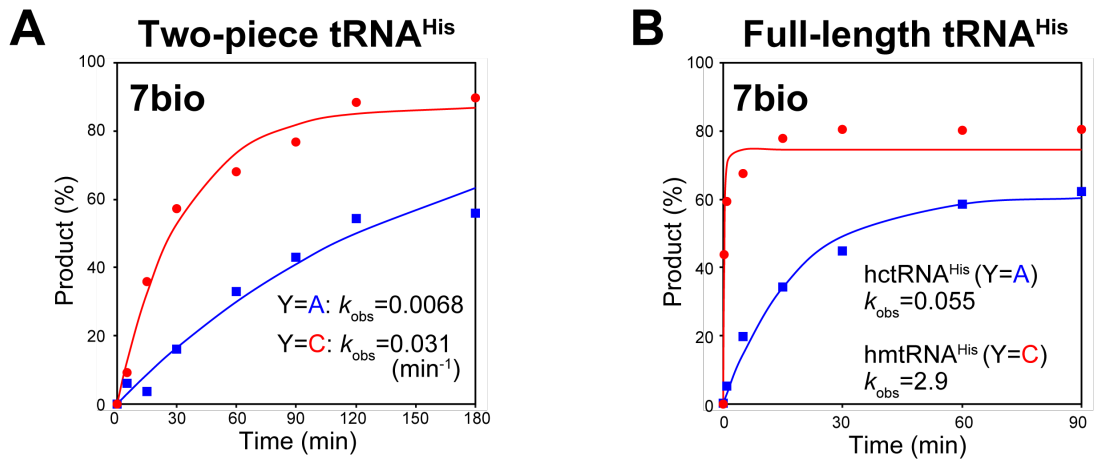


**Figure S2**: Time course experiments of 7bio addition reactions into two-piece tRNA$^{His}$s **(A)** or full-length tRNA$^{His}$s **(B)**. Lines represent each time course fitted to a single-exponential equation (eq. 1) to yield $k_{obs}$.

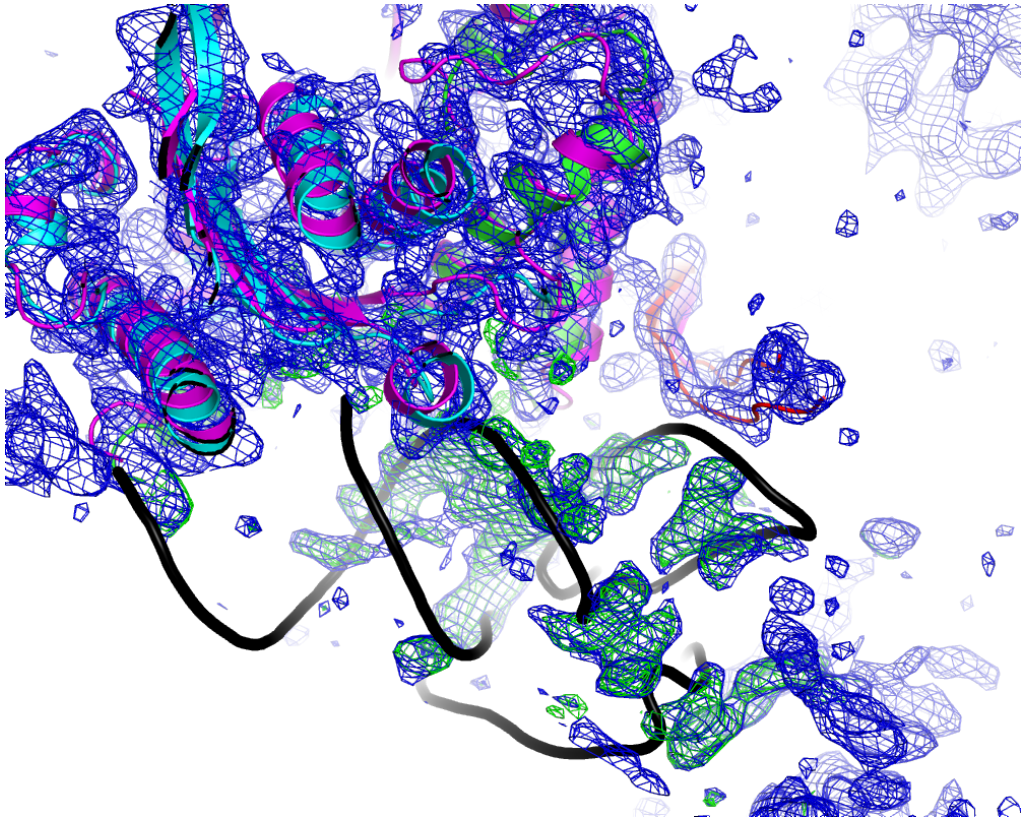

**Figure S3**: Superposing the CaThg1-tRNA structure (magenta and black) onto the HsThg1-tetramer (green and cyan). The interface region between the D-loop and the T-loop (elbow region) of the $tRNA^{His}$ superposes onto the unknown electron density map (2*Fo-Fc* map (1.5 σ); blue, and the *Fo-Fc* map (2.5 σ);green).