Supporting Information

Structural dynamics govern substrate recruitment and catalytic turnover in H/ACA RNP pseudouridylation

Andreas Schmidt[#], Gerd Hanspach[#], Martin Hengesbach

Figure S1:

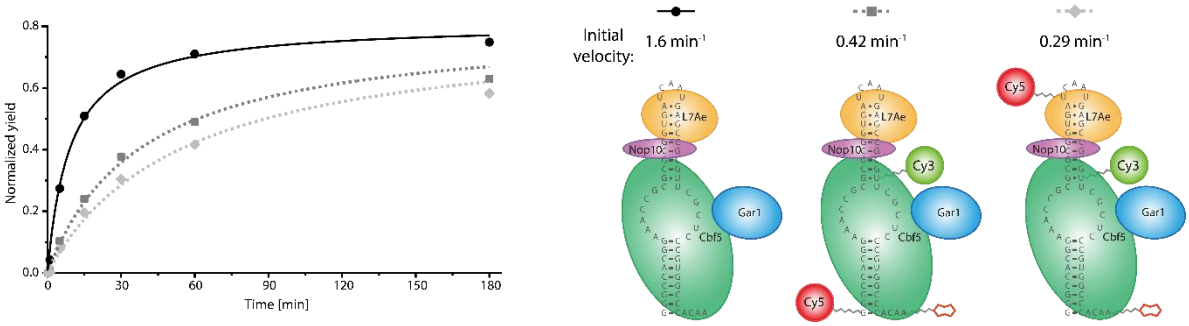

Figure S1: Comparison of different unlabeled and labeled H/ACA complexes. Black: unlabeled complex. Grey: Different FRET label positions as depicted on the right. Both fluorophore-labeled RNAs reconstitute significant levels of catalytic activity in H/ACA RNP (c = 50 nM) complexes with a 20-fold excess of target RNA at 70 °C.

Figure S2

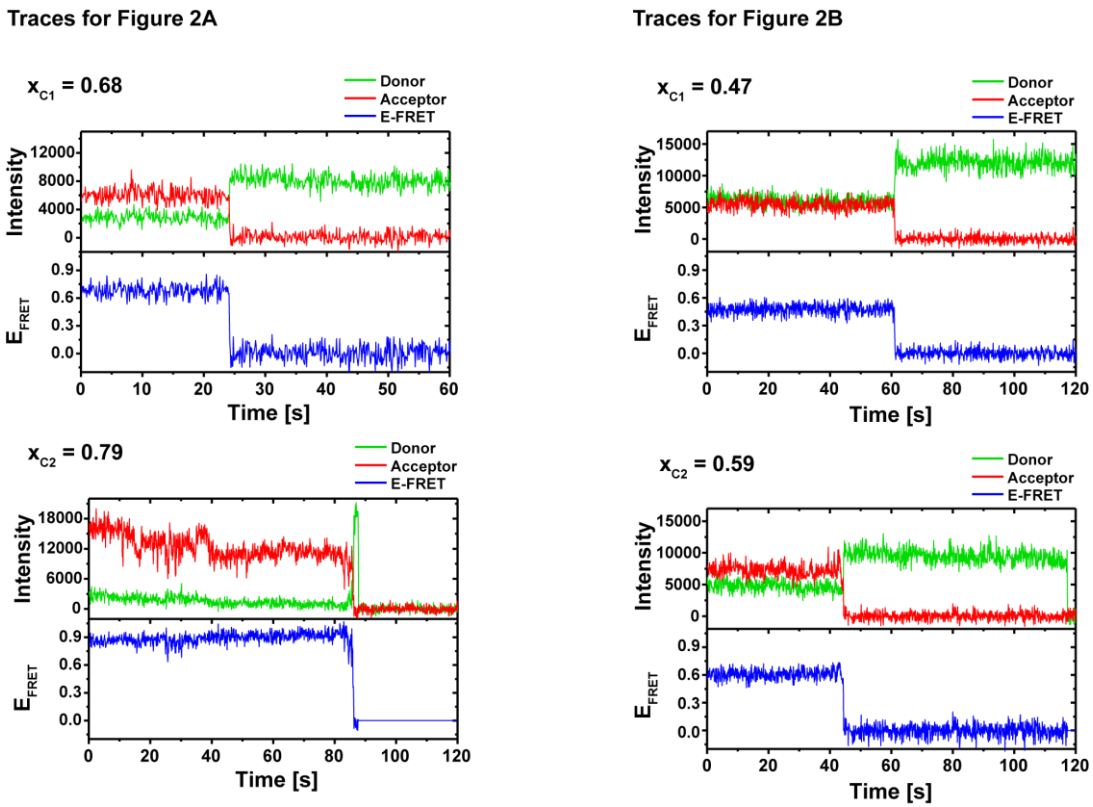

Figure S3: FRET time traces representing different E_{FRET} states as described in Figure 2 of the main manuscript.

Figure S3:

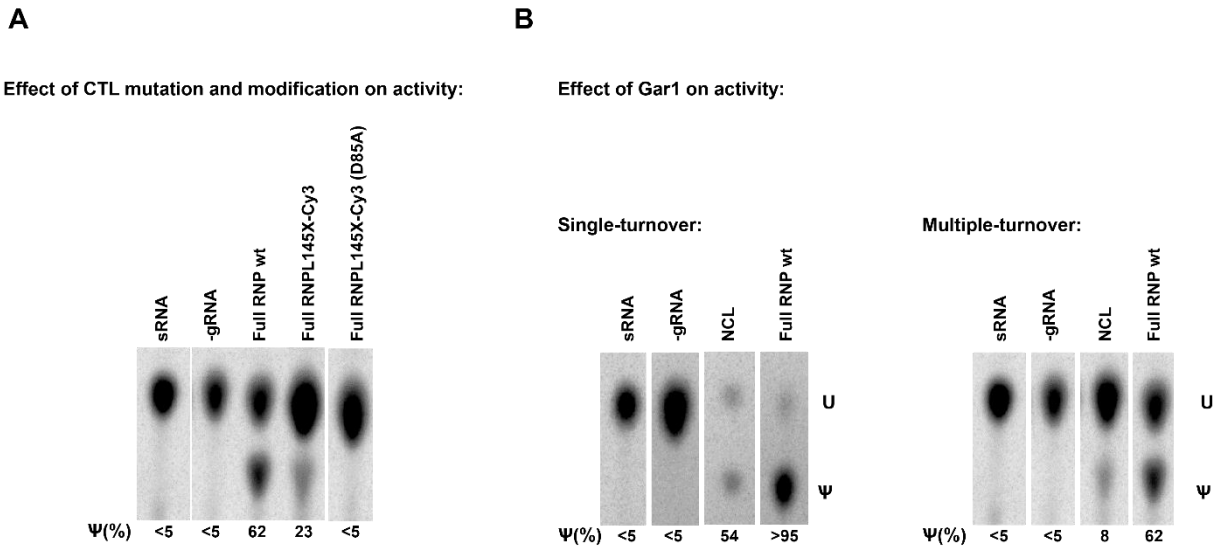

Figure S3: A) Effect of Cbf5 CTL mutation and modification on pseudouridylation. B) Effect of Gar1 presence on activity under single and multiple turnover conditions.

sRNA, Gar1 and L7Ae are required to convey noticeable levels of activity to the complex.

Reconstituted complexes contained the following components:

NC: Nop10, Cbf5, sRNA

NCG: Nop10, Cbf5, Gar1, sRNA

-sRNA: only proteins Cbf5, Nop10, Gar1 and L7Ae;

NCGL: sRNA, Cbf5, Nop10, Gar1 and L7Ae (fully reconstituted H/ACA complex).

Figure S4:

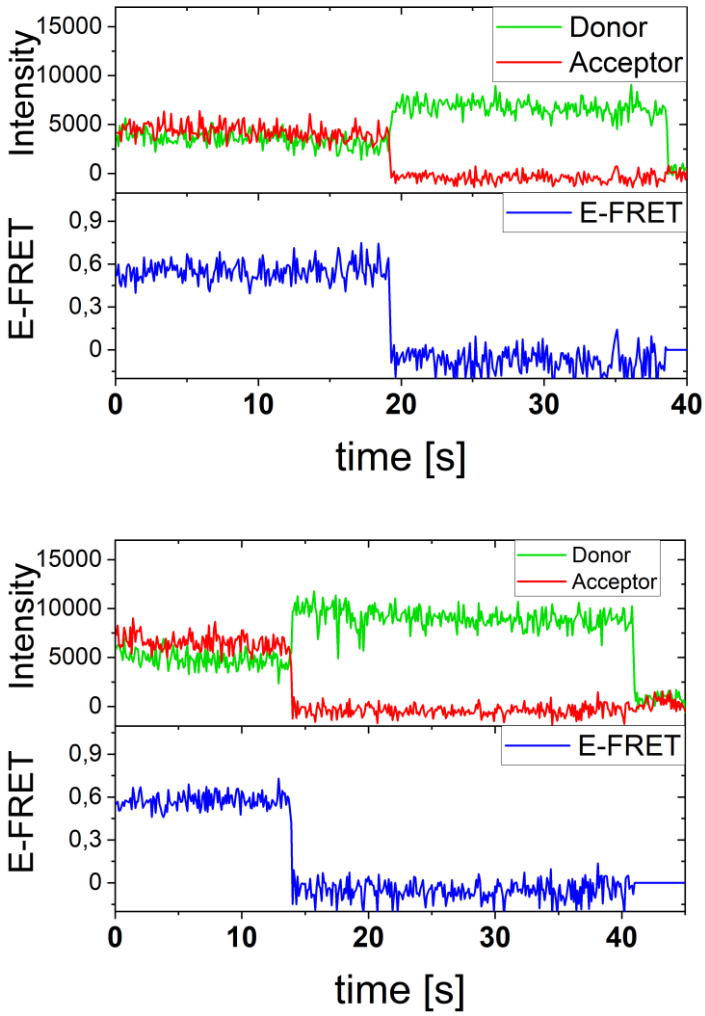

Figure S4: Exemplary time-resolved FRET traces representing the state in Figure 3 of the main text. The traces show no apparent dynamics of interconversion between distinct FRET states.

Figure S5:

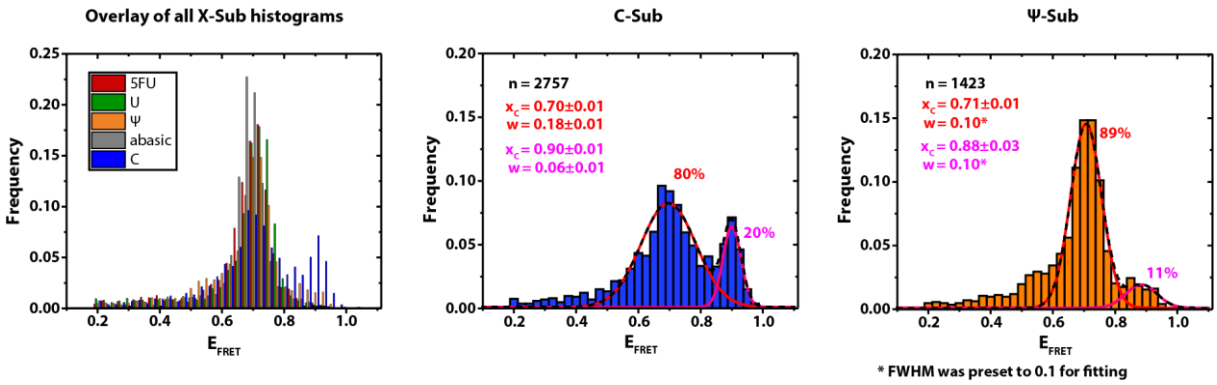

Figure S5: FRET histograms from constructs immobilized via a biotinylated target RNA. Left: overlay of histograms for all target nucleotides. Center: Target cytosine. Right: Target pseudouridine, representing the reaction product.

Figure S6:

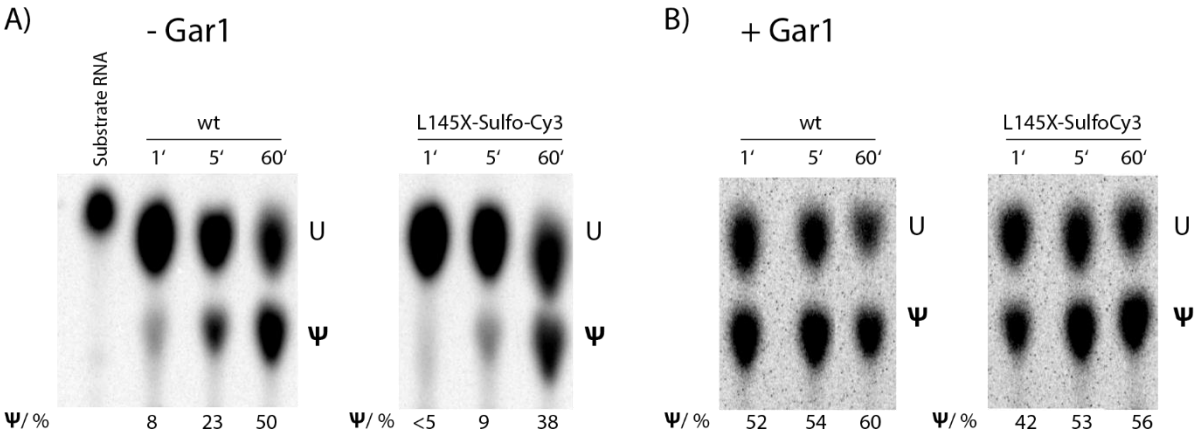

Figure S6: Time-course of pseudouridylation activity of H/ACA complexes without ("-Gar1") and with ("+Gar1") Gar1. Wt: unmodified protein constructs. L145X-SulfoCy3: Cbf5 containing propargyllysine at position 145, with Sulfo-Cy3-azide coupled to the protein. Coupling and purification were performed as described [17].

Figure S7:

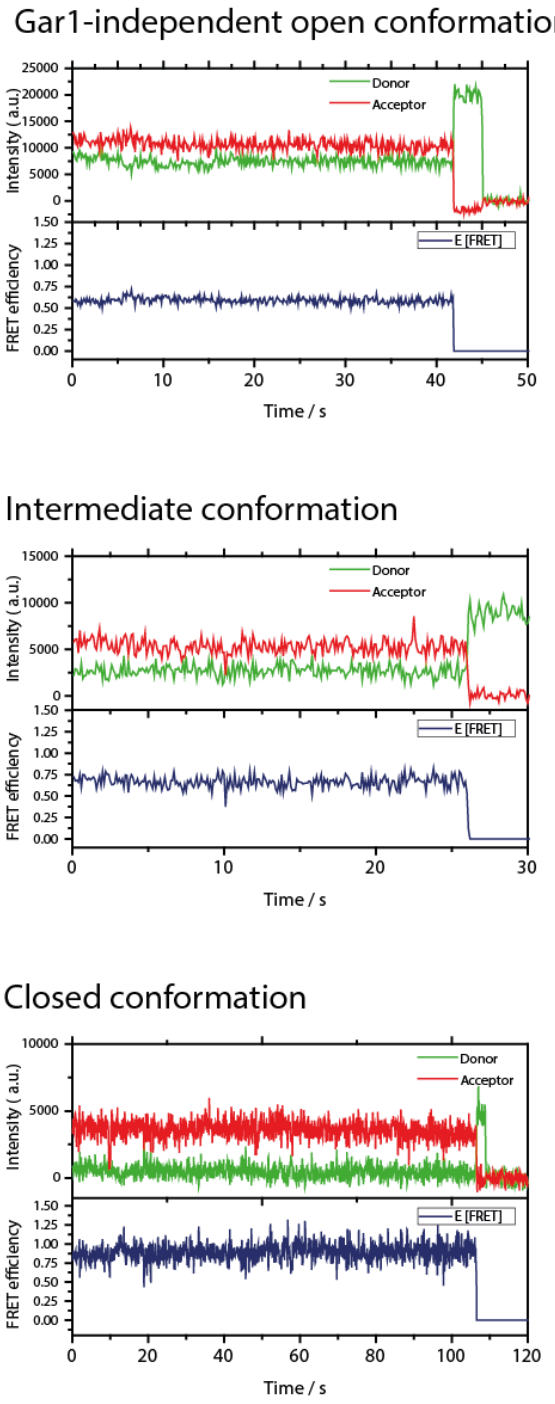

Figure S7:

Exemplary time-resolved FRET traces representing each of the states in Figure 5 of the main text. The traces show no apparent dynamics of interconversion between distinct FRET states.