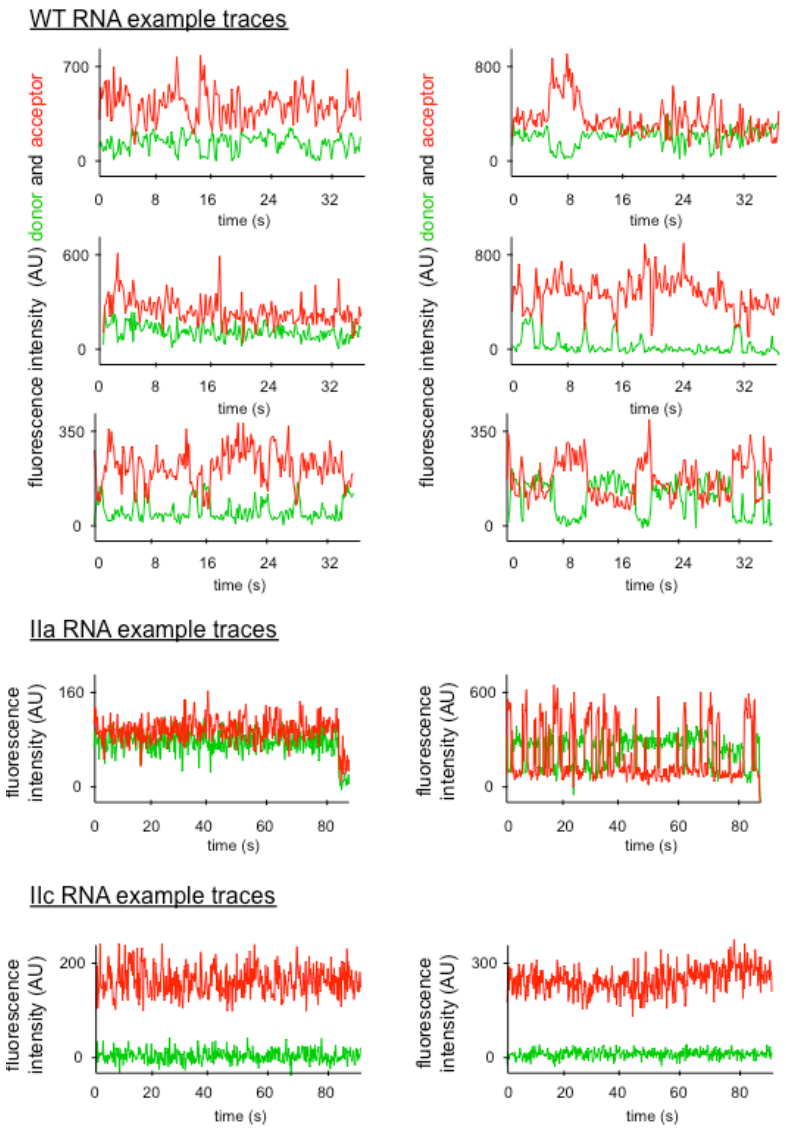
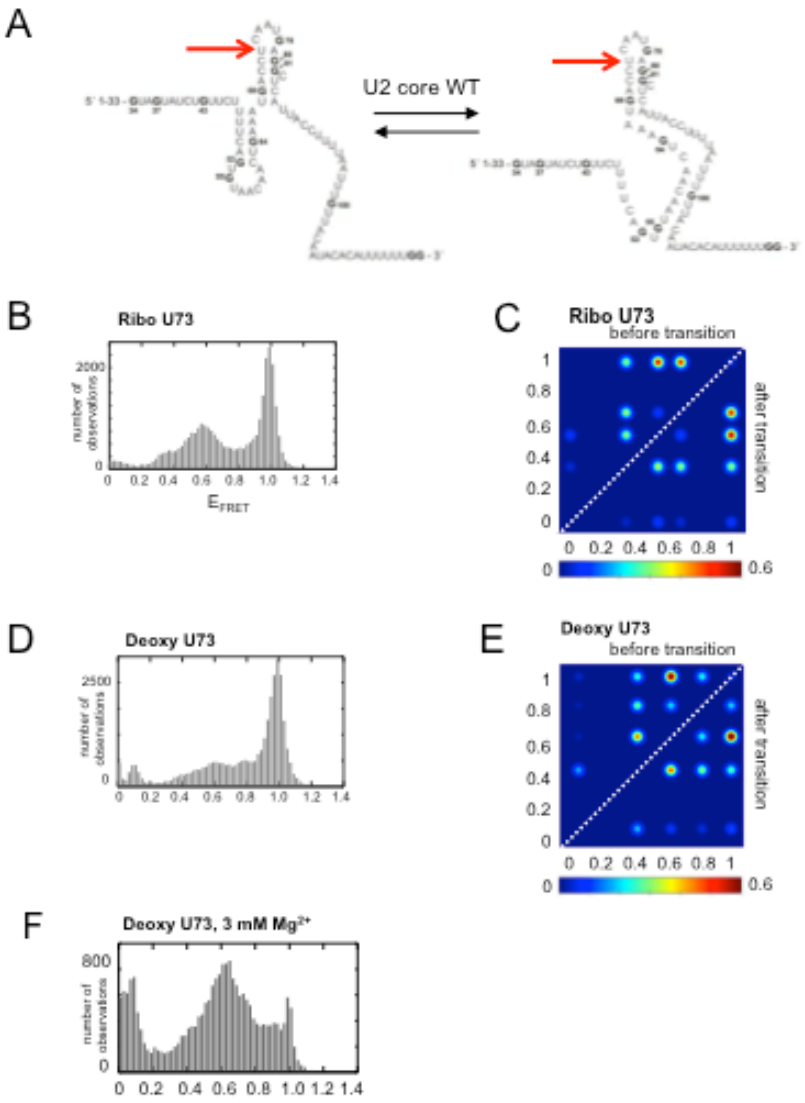
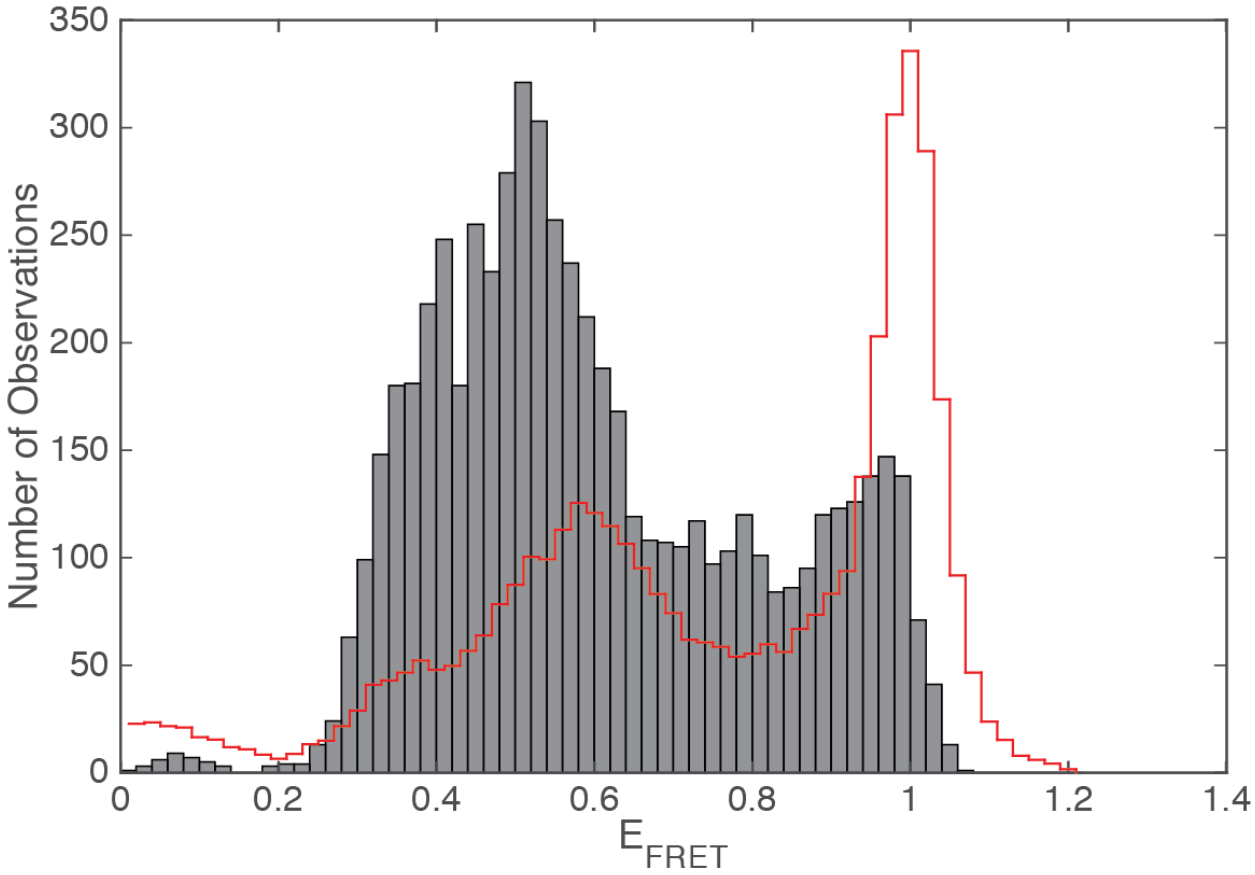
Supplemental Figures and Table



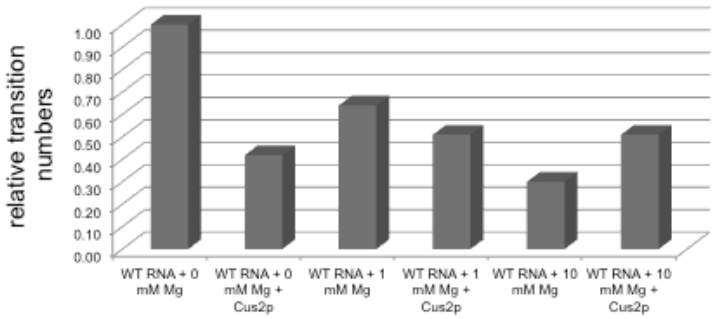
Supplemental Figure S1. Example smFRET data from individual molecules of the WT U2 core RNA and mutants showing anticorrelated changes in Cy3 donor (green) and Cy5 acceptor (red) fluorescence indicative of FRET dynamics. While many molecules of the WT and stem IIa-stabilized RNAs fluctuate between different conformations, very few transitions are observed with the stem IIc RNA.



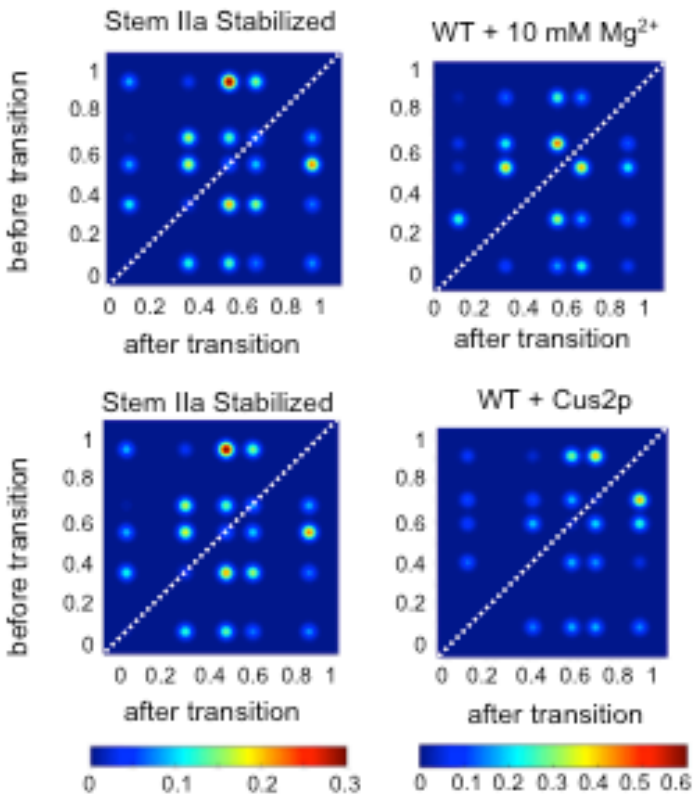
Supplemental Figure S2. **(A)** Position of the deoxy substitution at U73 in stem IIb. **(B)** Histogram of E_{FRET} values obtained from the WT construct, data reproduced from **Figure 1D**. **(C)** TODP from the WT construct, data reproduced from **Figure 2B**. **(D)** Histogram of E_{FRET} values obtained from the deoxy U73 RNA. The deoxy substitution in stem IIb causes an increase in the relative number of stem IIc E_{FRET} values observed compared with stem IIa. **(E)** TODP from the deoxy U73 RNA. The TODP shows a change in the patterns of transitions when compared with the WT RNA in **(C)**. **(F)** Histogram of E_{FRET} values of the deoxy U73 RNA obtained in 3 mM Mg^{2+}. A deoxy substitution in stem IIb allows for persistence of some stem IIc E_{FRET} states even in the presence of magnesium. No IIc E_{FRET} states were observed with the deoxy U73 RNA in the presence of Cus2p (not shown).



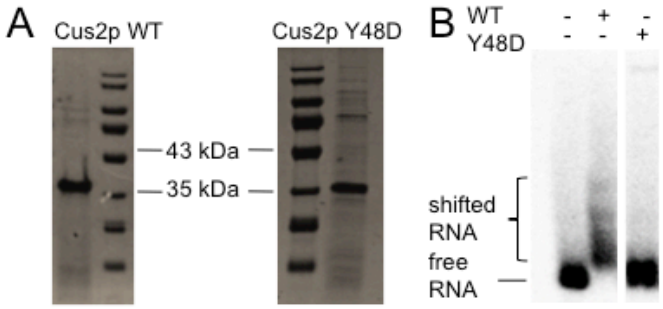
Supplemental Figure S3. Histogram analysis of E_{FRET} values obtained for a stem II FRET reporter RNA containing the entire 5' region of U2 including stem I and the intact BSL (grey). The histogram has been superimposed with that obtained using the smaller U2 stem II core fragment (red). Use of a larger RNA also decreases the relative abundance of the highest E_{FRET} values corresponding to stem IIc formation.



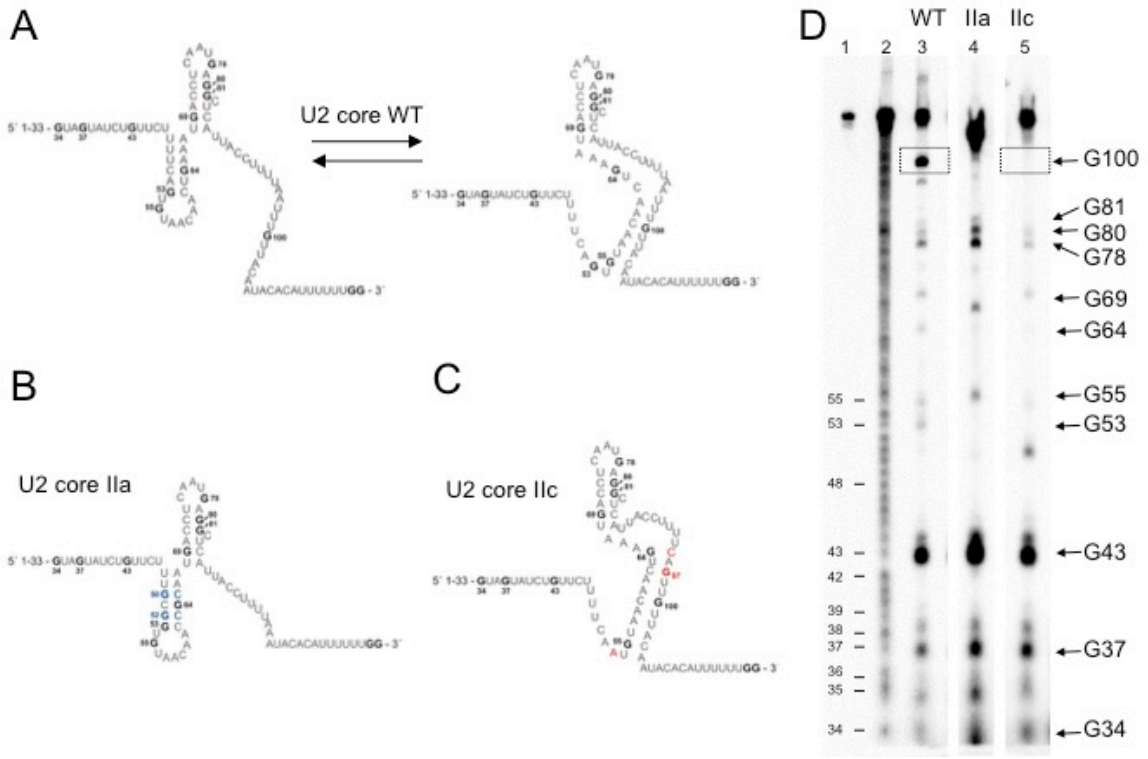
Supplemental Figure S4. Relative number of changes in E_{FRET} value (transitions) observed with WT core RNAs in the presence or absence of cofactors. The presence of magnesium or Cus2p reduce the number of dynamics observed in smFRET experiments. Equivalent-sized data sets were used in this comparison and the number of transitions were normalized to those observed with the WT RNA.



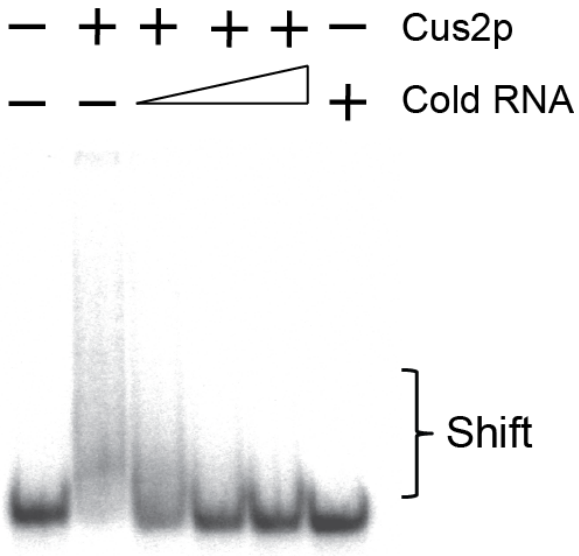
Supplemental Figure S5. Side-by-side comparison of TODPs from Figures 2 and 3 to facilitate comparison between transitions observed with the stem IIa RNA and the WT RNA in the presence of Cus2p or magnesium.



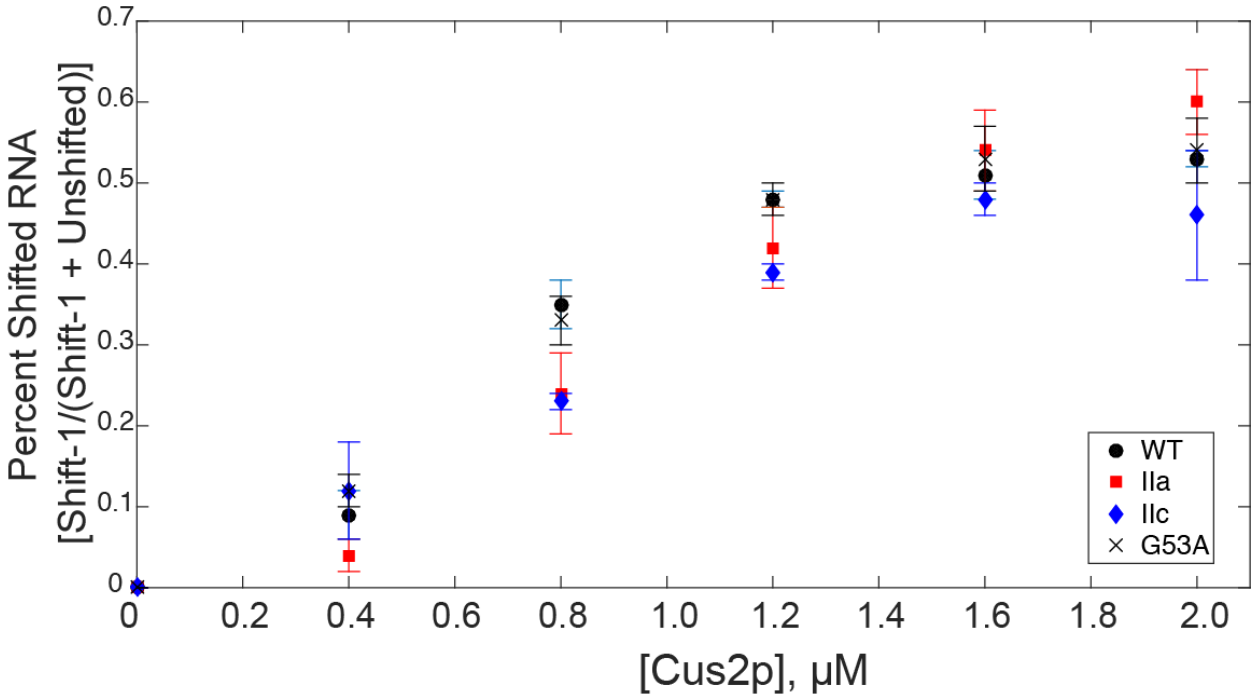
Supplemental Figure S6. (A) SDS-PAGE gel of purified WT and Y48D histag-Cus2p. Cus2p migrates just above the 35 kDa molecular weight marker, in agreement with a predicted molecular weight of 34.977 kD. **(B)** WT Cus2p (2 μM) shifts WT U2 core RNA but no shifts were observed with the Y48D mutant (2 μM). Shifts were observed by incubation of the protein with the RNA followed by native PAGE and phosphorimaging.



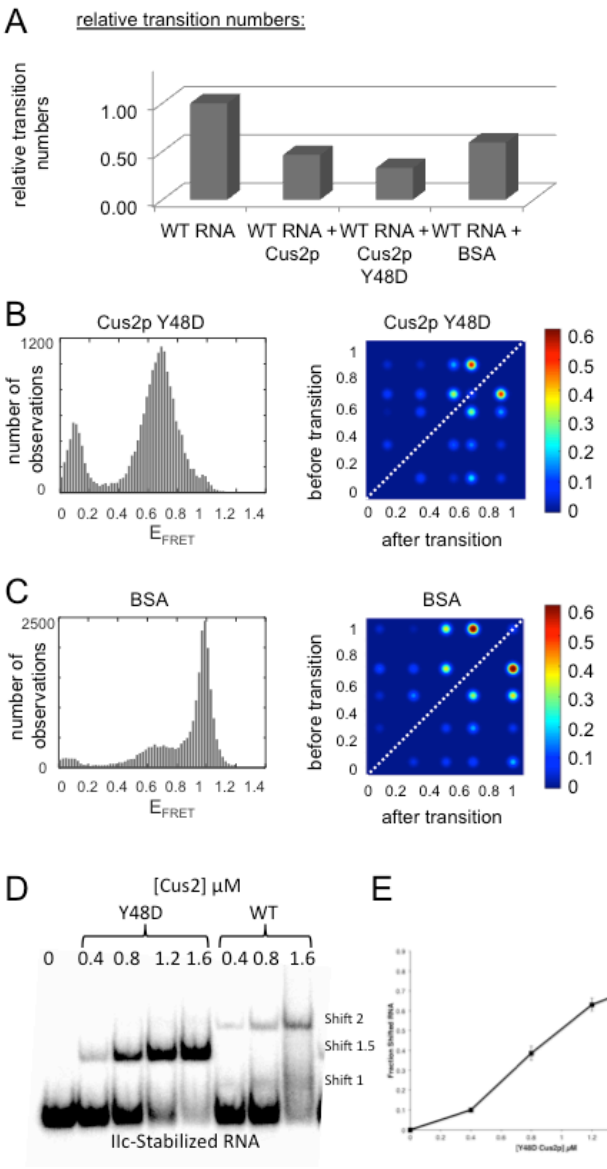
Supplemental Figure S7. (A) Schematic of the WT U2 core RNA used in EMSAs. Guanine nucleotides are shown in bold and numbered. The RNA is shown in the stem IIa (left) and IIc (right) conformations. **(B)** Schematic of the stem IIa stabilized U2 core RNA used in EMSAs. Stem IIa stabilizing mutations are shown in blue. **(C)** Schematic of the stem IIc stabilized U2 core RNA used in EMSAs. Stem IIc stabilizing mutations are shown in red. **(D)** PAGE analysis of U2 core RNAs after RNase T1 digestion. Lane 1: undigested WT RNA; Lane 2: RNA hydrolysis ladder; Lane 3: T1 digested WT RNA; Lane 4: T1 digested stem IIa stabilized RNA; Lane 5: T1 digested stem IIc stabilized RNA. The G100 nucleotide is predicted to be basepaired in the stem IIc conformation and not the stem IIa conformation; T1 cleavage is detected with the WT RNA and not the IIc stabilized RNA in agreement with this prediction. G100 is absent in the stem IIa RNA due to use of the ΔCC sequence.



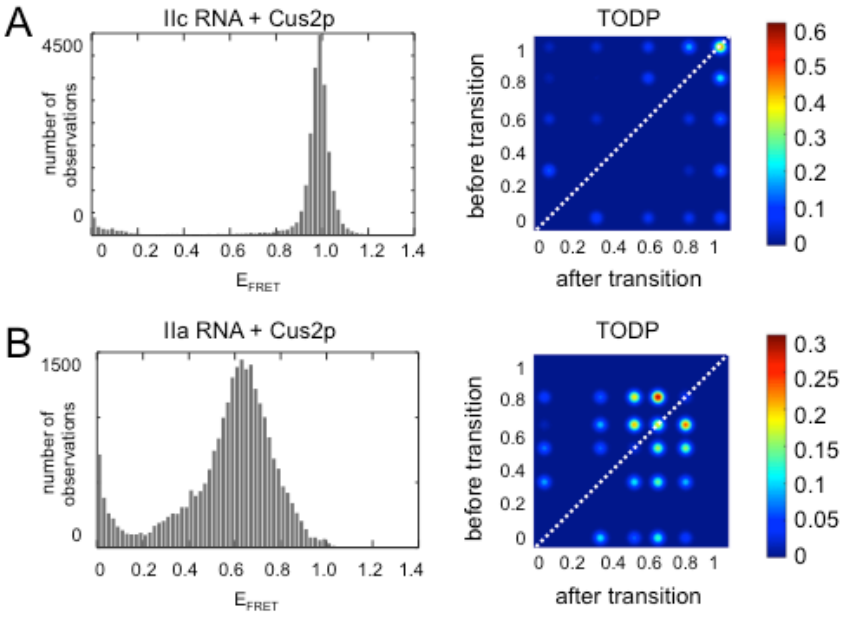
Supplemental Figure S8. Competition assay of [^{32}P]-labeled WT stem II RNA (2 nM) with excess unlabeled RNA for Cus2p binding. Unlabeled RNA was added to final concentrations of 0.5, 1.0, 2.0, and 2.0 μM in lanes 3-5, respectively. Cus2p was at a concentration of 1.2 μM when present.



Supplemental Figure S9. Results from EMSA analysis of Cus2p binding to stem II core RNAs (**Fig. 4**). No significant difference could be detected between the fraction of RNA appearing in Shift-1 relative to the sum of Shift-1 and unshifted RNA. Each data point represents the average of three separate experiments ±S.D.



Supplemental Figure S10 (A) Relative number of changes in E_{FRET} value (transitions) observed with WT core RNAs in the presence or absence of proteins. **(B)** Histogram of E_{FRET} values observed for the WT U2 core RNA in the presence of Y48D Cus2p (2µM) and TODP analysis. The Y48D mutant protein diminishes the abundance of the stem IIc E_{FRET} state near 1 relative to mid- E_{FRET} states, as does the WT protein. TODP analysis shows that the RNA dynamics observed in the presence of the Y48D mutant protein are different than those observed with the WT protein—the mutant protein shows a prevelant transition to a ~0.3 E_{FRET} state. **(C)** Histogram of E_{FRET} values observed for the WT U2 core RNA in the presence of BSA and TODP analysis. BSA does not eliminate the predominance of the high E_{FRET} state associated with stem IIc formation. **(D)** EMSA analysis of interactions between the Y48D mutant of Cus2p with the stem IIc-stabilized RNA. A single shift is observed (Shift 1.5) intermediate between Shifts 1 and 2 that are observed with WT Cus2p interacting with the same RNA. **(E)** Quantification of the EMSA results shown in **D** for Y48D Cus2p. Data points represent the average of triplicate experimental results ± S.D.



Supplemental Figure S11. (A) Histogram of E_{FRET} values observed for the stem IIc U2 core RNA in the presence of WT Cus2p and TODP analysis. Stem IIc stabilizing mutations prevent Cus2p from facilitating accumulation of mid E_{FRET} states. **(B)** Histogram of E_{FRET} values observed for the stem IIa U2 core RNA in the presence of WT Cus2p and TODP analysis. Stem IIa stabilizing mutations do not prevent Cus2p from suppressing E_{FRET} transitions to the 0.3 E_{FRET} state (although this state is slightly more prominent than in the WT RNA).

Supplemental Table S1.

Ranges of E_{FRET} Values Observed with U2 Core RNAs Under Various Conditions

E_{FRET}	0.01-0.07	0.28-0.35	0.51-0.57	0.65-0.70	0.85-0.91	0.94-0.98
IIc	X					X
WT	X	X	X	X		X
WT + 1 mM Mg	X	X	X	X		X
WT + 10 mM Mg	X	X	X	X	X	
WT + Cus2	X	X	X	X	X	
WT+Cus2+1 mM Mg	X	X	X	X	X	
WT+Cus2+10 mM Mg	X	X	X	X	X	
IIa	X	X	X	X	X	
Extended RNA	X					