Slow formation of stable complexes during coincubation of a minimal rRNA and

ribosomal protein S4

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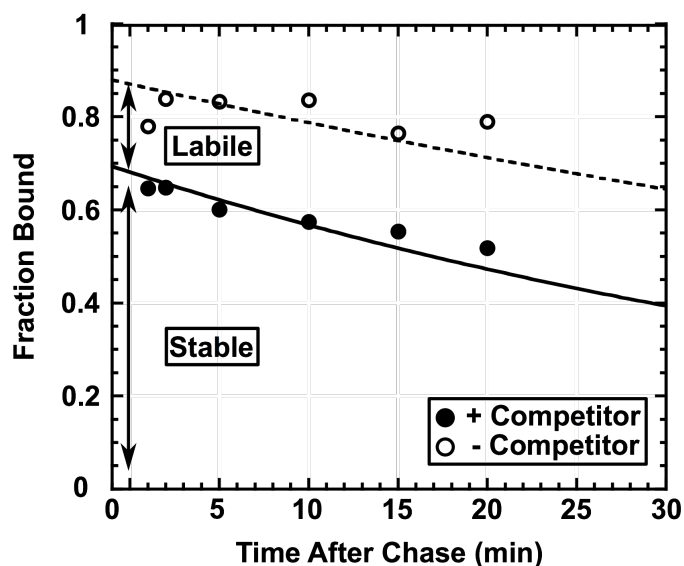

Figure S1: 5WJ kinetic competition assay analysis. Plot of fraction bound versus time after chase for the 5WJ (compare to Figure 3b). The stated amplitude of the stable phase is the difference between the initial value of the first point of the control reaction (open circles, dotted line) and the value at the first point of the experimental reaction (filled circles, solid line). Data were fit to $f_B = A \exp(-k_{obs} t)$.
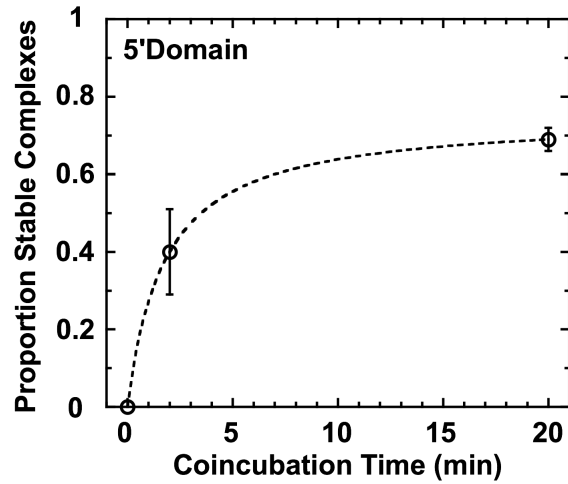
Figure S2: Effect of S4 coincubation time on S4 – 5'domain complexes. The average amplitude of the stable phase is plotted as a function of 42°C S4 coincubation time for complexes in which the 5'domain rRNA was prefolded for 20 min. Compare to Figure 3c, showing the 5WJ.
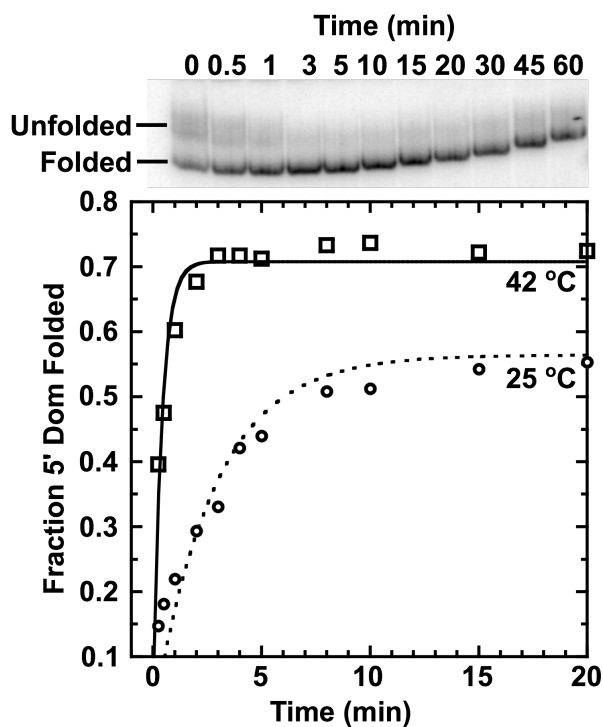
Figure S3: Temperature dependence of 5'domain folding kinetics. Representative EMSA and corresponding plot from a 5'domain kinetic folding assay. 0.5 nM of ^{32}P-labeled 5' domain was incubated with HKM4 buffer at 25 °C (circles, dotted line) or 42°C (squares, solid line) and loaded onto native polyacrylamide gels. The observed folding rates were obtained by fitting data to $f_N = f_N(0) + A(1 - \exp(-k_{obs}t))$. $f_N(0)$ is the fraction folded in the first 15 s, A is the amplitude, and k_{obs} is the observed rate constant.
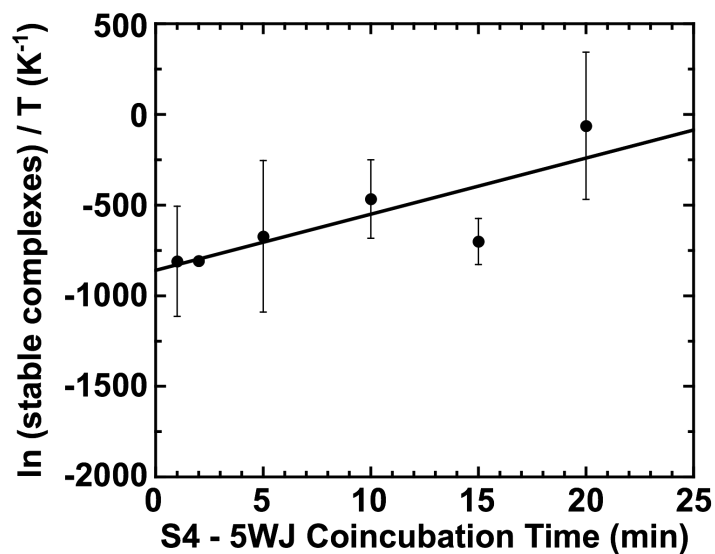
Figure S4: Temperature dependence of stable S4 – 5WJ complexes decreases linearly with increasing S4 coincubation. A modified Arrhenius plot (as in Figure 3d) was generated for 5WJ-S4 complexes coincubated for 1, 2, 5, 10, 15, and 20 min (1 and 20 min data shown in Figure 3d). The modified Arrhenius data was fit to a line for each coincubation time, and the slope of this line is plotted above as a function of coincubation time. The error bars represent the error to the linear fit on the modified Arrhenius plot.
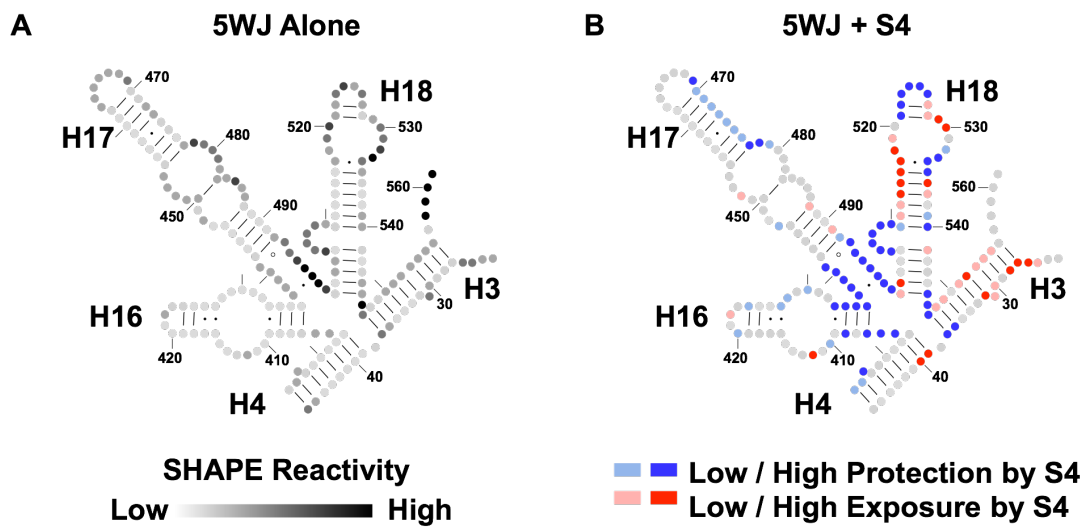
Figure S5: S4 reduces the flexibility of the 5WJ regardless of 5WJ prefolding time. (a) Schematic showing reactivity of 5WJ nucleotides in the absence of S4 after 2 min of 5WJ prefolding. Coloring as in Figure 5b. (b) Effect of S4 on 5WJ reactivity for 5WJ prefolded 2 min before S4 addition. Coloring as in Figure 5c.

Supplementary Table 1: Nucleotide SHAPE Reactivities providing the value of ρ (see Methods) with standard deviations for all nucleotides analyzed in this study. The column labels are formatted as follows: rRNA prefolding temperature & prefolding time; S4 coincubation time. This table is available as a separate Excel file.