Supplementary Material For:
Coupling RNA annealing and strand displacement: a FRET-based microplate reader assay for RNA chaperone activity

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Supplementary Figure S1. Fluorescence emission spectra of single- and double-stranded CyDye-labeled RNAs. Fifty nanomoles of Cy3™-21R- and Cy5-21R+ each were either kept on ice or heated to 95°C and then slowly cooled to room temperature. Measurement was in 200 μL annealing buffer at room temperature. In a Perkin-Elmer LS50B spectrometer, the sample was excited at 540/10 nm and fluorescence emission was scanned from 550–680 nm. Upon hybridization, fluorescence resonance energy transfer (FRET) occurs and results in an emission peak at 665 nm in addition to the Cy3 emission peak at 565 nm. IU, international units.
Supplementary Figure S2. Data transformation of fluorescence resonance energy transfer (FRET) kinetics. (A) RNA annealing causes a decrease in Cy3 (thin lines) and a concomitant increase in Cy5 fluorescence emission (thick lines). Measurements in the absence or presence of 1 μM StpA were performed in quadruplets each. The microplate reader used here (Tecan GENios Pro™) and the others tested (see text) start each kinetic measurement with different initial relative fluorescence units (RFU) indicated by the varying starting points. (B) The FRET index is derived by calculating the ratio of F_{Cy5}/F_{Cy3}. (C) Since the annealing reaction starts with the injection of one reactant onto the other, the FRET index values are set to 0 at t0. (D) To visualize the overlapping individual measurements of each set and to better indicate whether phase II after injection of the competitor RNA at t_{200} describes continuous RNA annealing or strand displacement, the data were normalized to 1 at t_{180}. No information is lost since the amplitudes in panels B and C are not absolute but only indicative. Thin black lines in panels B and C indicate the curve fit with the respective functions, and the calculated k_{obs} is not affected by the amplitude transformations.
Supplementary Figure S3. Binding of StpA to CyDye-labeled RNA. Five nanomoles of RNA oligonucleotides were incubated in 100 μL annealing buffer at 37°C in microplate wells with the indicated concentrations of StpA protein. Anisotropy readings were taken in a Tecan GENios Pro reader and fitted with $y = y_0 + A/(1 + (K_{1/2}/c)^n_H)$ where $A$ is the amplitude, $c$ is the protein concentration, and $n_H$ is Hill coefficient. The calculated $K_{1/2}$ is 1053.2 nM for Cy3-21R- and 118.3 nM for Cy5-21R+. 