



Figure Legends

Figure S1: Structural heterogeneity in the GAAA tetraloop-receptor RNAs assayed by native gel electrophoresis. The relative FRET efficiencies for each band are indicated by the false color image, with donor (Cy3) and acceptor (Cy5) fluorescence emission shown in green and red, respectively. (a) Native gel on the A₇, U₇, and A₁₄ constructs. Multiple bands per lane are resolved with differing relative FRET efficiencies. (b) Control gel with various combinations of the individual oligonucleotide strands for the A₇ construct (see Figure 1). Arrows indicate the assignment of the strand(s) corresponding to each band. Lane 1 contains all three strands for the A₇ construct. Lane 2 lacks the biotin-strand, but the GAAA tetraloop-receptor interaction is still formed. Lanes 4 and 6 are only the Cy3 and Cy5 strands, respectively. Lane 3 is the Cy3 strand and the (non-fluorescent) biotin strand; these two strands do not have complementary sequences and thus do not form a complex. The slower migrating band in lane 5 is the Cy5 strand-biotin strand complex, and the faster migrating band is the free Cy5 strand. Lanes 5 and 6 show direct excitation and detection of the Cy5 acceptor rather than FRET. This control gel shows that none of the bands in (a) correspond to single strands, demonstrating that this GAAA tetraloop-receptor system exists in multiple conformations under the conditions of the gel. The bands of differing relative FRET efficiencies observed for each construct in (a) is consistent with these RNAs having at least two sub-populations with different docking abilities. Given the slow timescale for

separating species by gel electrophoresis, these bands most likely correspond to an actively docking/undocking species and a much less active species.

Native polyacrylamide gel electrophoresis protocol. Electrophoresis was performed in 12% polyacrylamide gels under native conditions favoring docking (10 mM Mg^{2+}) with 1X THE running buffer (34 mM Tris, 66 mM HEPES, 0.1 mM EDTA) at 4 °C. Bands were imaged via fluorescence using a Typhoon 9400 Variable Mode Imager. Fluorescence was excited at a wavelength absorbed primarily by the donor (532 nm). The relative FRET efficiency for each band was determined by separating donor and acceptor emission with a dichroic mirror and defining Cy3 and Cy5 emission with 565-595 nm and 655-685 nm by band pass filters, respectively. To qualitatively show the relative FRET efficiency of each band, the gels are displayed in false color with donor and acceptor emission in green and red, respectively. For lanes lacking a donor-labeled strand, the bands are imaged by directly exciting the Cy5 acceptor at 655 nm rather than via FRET excitation, leading to only red color in these bands.