Supplemental Figure 1. Alignment of trCUG-3 with two different GAAA tetraloop/receptor structures. (A) Structure of trCUG-3 and a symmetry mate used for comparison to other tetraloop/receptor structures. Magnesium ions are numbered. (B) Alignment of the tetraloop/receptor of trCUG-3 (blue) with that of 1GID (orange). The first three magnesium ions are near the cobalt hexamine ion found in 1GID. The fourth magnesium ion near the receptor is near a corresponding magnesium ion in 1GID. (C) Alignment of the tetraloop/receptor of trCUG-3 (blue) with that of 1HR2 (orange). Again, the first three magnesium ions in trCUG-3 are near a magnesium ion found in 1HR2. The second, third and fourth magnesium ions also have counterparts near the receptor.

Supplemental Figure 2. Graphical representation of the average overlap of the bases in the 5’CUG/3’GUC motif. An illustration of the base stacking of all the available CU/UG base steps can be found in Figure 3. Average overlap was calculated using 3DNA from all currently available 5’CUG/3’GUC motifs (*17-19*, *37*). Any base step involving a non-canonical U-U pair (CU/UG and UG/CU) shows very little overlap while the GC/GC base steps stack have significant overlap. Error bars are standard deviation.