ONLINE METHODS

Sample preparation. The unlabeled, site-specific 2% ^{15}N-labeled deoxyribose-substituted and deazaguanine-substituted RNA oligonucleotides were synthesized at the Yale oligonucleotide synthesis facility and de-protected according to the manufacturer's instructions. Unlabeled, uniformly ^{13}C,^{15}N-labeled, and residue-type-specific ^{13}C,^{15}N-labeled *sc1* RNAs were transcribed *in vitro* using T7 RNA polymerase. The ^{13}C,^{15}N-labeled nucleotide tri-phosphates were prepared according to procedures reported previously[53]. All RNA and modified oligonucleotides were purified on the 20% polyacrylamide/8 M urea gels followed by electroelution, anion-exchange chromatography and ethanol precipitation as described previously. Unlabeled and uniformly ^{13}C,^{15}N-labeled FMRP RGG peptides were expressed in *E.coli*[54] as GST-fusions and purified by affinity chromatography. GST was cut off with PreScission protease and removed by affinity chromatography. The peptide was lyophilized, dissolved in water and purified by the reverse-phase HPLC chromatography on Deltapac C18 column (Waters). Unlabeled peptides were also purchased from Rockefeller University peptide facility.

NMR spectroscopy. NMR experiments were performed on 600 MHz Varian and 600, 700, and 800 MHz Bruker NMR spectrometers in a buffer containing 50 mM deuterated K-acetate, pH 6.8 at 25 ºC, unless otherwise specified.

Imino protons of the *sc1* RNA were assigned unambiguously using site-specific low-enrichment labeling approach[18]. Since ^{15}N-labeled deoxyguanine rather than unavailable ^{15}N-labeled guanine was used in this labeling strategy, we recorded NOESY spectra of the complex after each site-specific incorporation to take into account and correct for any chemical shift changes that could perturb the assignment analysis. Site-specific deoxyribose-for-ribose substitutions also allowed facile identification of aromatic protons including guanine and adenine H8 protons and uracil and cytosine H6 protons[20].

A series of through-bond correlation experiments were performed using complexes containing uniformly ^{13}C,^{15}N-labeled and residue-type-specific ^{13}C,^{15}N-

labeled *sc1* RNA. Guanine H8 protons were assigned by through-bond connectivities to the imino protons via ^{13}C5 by HMBC experiment[18]. Through-bond connectivities between the aromatic protons and sugar H1′ protons were established through their correlations to ^{15}N9 in G and A residues, and ^{15}N1 in U and C residues[21]. Ribose sugar protons were connected using COSY, TOCSY and 3D HCCH-COSY and HCCH-TOCSY experiments[22].

FMRP RGG peptide resonances were assigned using standard triple resonance experiments on uniformly ^{13}C,^{15}N-labeled peptide[27,28]. Backbone resonances were correlated using a combination of ^{15}N-^{1}H HSQC, ^{13}C-^{1}H HSQC, HNCO and HNCOCA. Side-chain resonances were correlated using HNCACB, HBHACONH, HCCH-TOCSY, HCCH-COSY, ^{13}C-^{1}H HSQC, COSY and TOCSY experiments.

RNA base-pair and G-tetrad alignments, as well as peptide-RNA intermolecular hydrogen-bonds were identified by HNN-COSY experiments[23,24]. Interproton distances were measured by using NOESY-type experiments at various mixing times, including 2D NOESY and 3D ^{13}C-edited or ^{15}N-edited NOESY spectra. *sc1* RNA sugar puckers were restrained according to values of the $^{3}J_{H1'-H2'}$ coupling constants qualitatively estimated from the ^{1}H-^{1}H COSY spectrum. Nucleotides G9, U10, G12, A13, G15, G16, A17, G18, U19, G20, G21, C22, U23, G24, G26, U27 and U28 were restrained to C2′-*endo* sugar pucker based on their strong H1′-H2′ COSY peaks.

Structure calculations. Calculations on the FMRP RGG peptide - *sc1* RNA complex were initially done in vacuum using XPLOR-NIH, followed by computations in explicit water with K^+ cations using INSIGHTII with AMBER force field.

Initially, only the G-quadruplex part of the RNA was folded (step 1, with only experimental restraints for quadruplex portion active during computations), followed by folding of the full RNA molecule (step 2), and finally the peptide-RNA complex was generated by imposing the full set of restraints onto the pre-folded RNA and unstructured peptide (step 3). Convergence of the molecules was further improved by filtering through negative restraints (step 4), with subsequent computations in explicit water and in the presence of K^+ cations (step 5).

Steps 1 and 2 were essentially performed as published in ref. 55. with details listed under computational protocols in Supplementary Methods.

Step 3: The single best RNA structure obtained from preceding steps was combined in a file with extended randomized peptide chain positioned along the deep groove of RNA duplex with the closest distance of 8 Å between the neighbor atoms of peptide and RNA. This molecular complex was subjected to torsion dynamics protocol with details listed in Supplementary Methods.

Step 4: Based on the geometry-refined averaged structure calculated from the ensemble of molecules in the preceding step (for RGG peptide residues 3-17), negative restraints were introduced, where pairs of atoms exhibiting no experimental NOE cross-peaks were restricted to a distance larger than 6 Å. Hydrogen-bonding and soft planarity restraints (7 kcal $mole^{-1}$ $Å^{-2}$) were also imposed on R15/G7 and R10/G31 side chains (conformational analysis for R10 and R15 is described in Supplementary Methods). The full set of restraints (with negative restraints excluded) was incorporated during the final molecular dynamics and minimization stage, which resulted in 10 best structures, which were subjected to refinement with explicit water and cations.

Step 5: Two K^+ cations were placed interactively between the G-tetrad layers, coordinating eight O6 atoms between two adjacent G tetrads. The RNA-peptide complex was solvated with a 10 Å thick layer of TIP3P water, which resulted in about 1800 water molecules added. The system was neutralized with 27 K^+ cations. Detailed protocol of molecular dynamics and molecular mechanics computations are described in Supplementary Methods.

The resulting 10 best structures (peptide spanning residues G1 to Q17) are shown on Fig. 4a, with structural statistics presented in Table 1. The Ramachandran plot statistics of peptide residues were as follows: 91.4% and 8.6% in most favored and additional allowed regions, respectively. We used PROCHECK to assess the quality of our structures, including side chain distribution of peptide residues, and hydrogen bond lengths.

Unexpectedly, we identified a K+ cation-binding site lined by phosphate oxygens at a junction point between the duplex and the quadruplex (Fig. 4c), for all complexes refined by dynamics in water shell with K^+ cations.

Filter binding assays. Filter binding assays were performed and analyzed as previously described[12,57], using GST-RGG box fusion proteins (described in detail in the Supplementary Methods section). Briefly, *sc1* RNA was heated to 75^{o} for 10 minutes in 1X SBB (Selex Binding Buffer: 10 mM Tris-acetate, pH 7.7, 200 mM K-acetate, 5 mM Mg-acetate) and allowed to renature at room temperature for 5 minutes. Proteins were diluted to the indicated concentrations in 1X SBB by serial dilution including a blank with no protein and a "total" which was spotted directly onto a filter with no washing. After incubation of protein with RNA in a total volume of 50 microliters for 10 minutes at room temperature, samples were filtered under vacuum over pre-wet 0.45 um HA membrane filters (Millipore HAWP02500) and washed with 4 ml of 1 X SBB buffer, dried, and counted in 5 ml of ReadiSafe scintillant. The data was analyzed using Kaleidagraph software (Synergy software version 4.02) using a binding algorithm to calculate Kds.