SUPPLEMENTARY MATERIAL TO:

STRUCTURE AND DYNAMICS OF THE DEOXYGUANOSINE-SENSING RIBOSWITCH STUDIED BY NMR-SPECTROSCOPY

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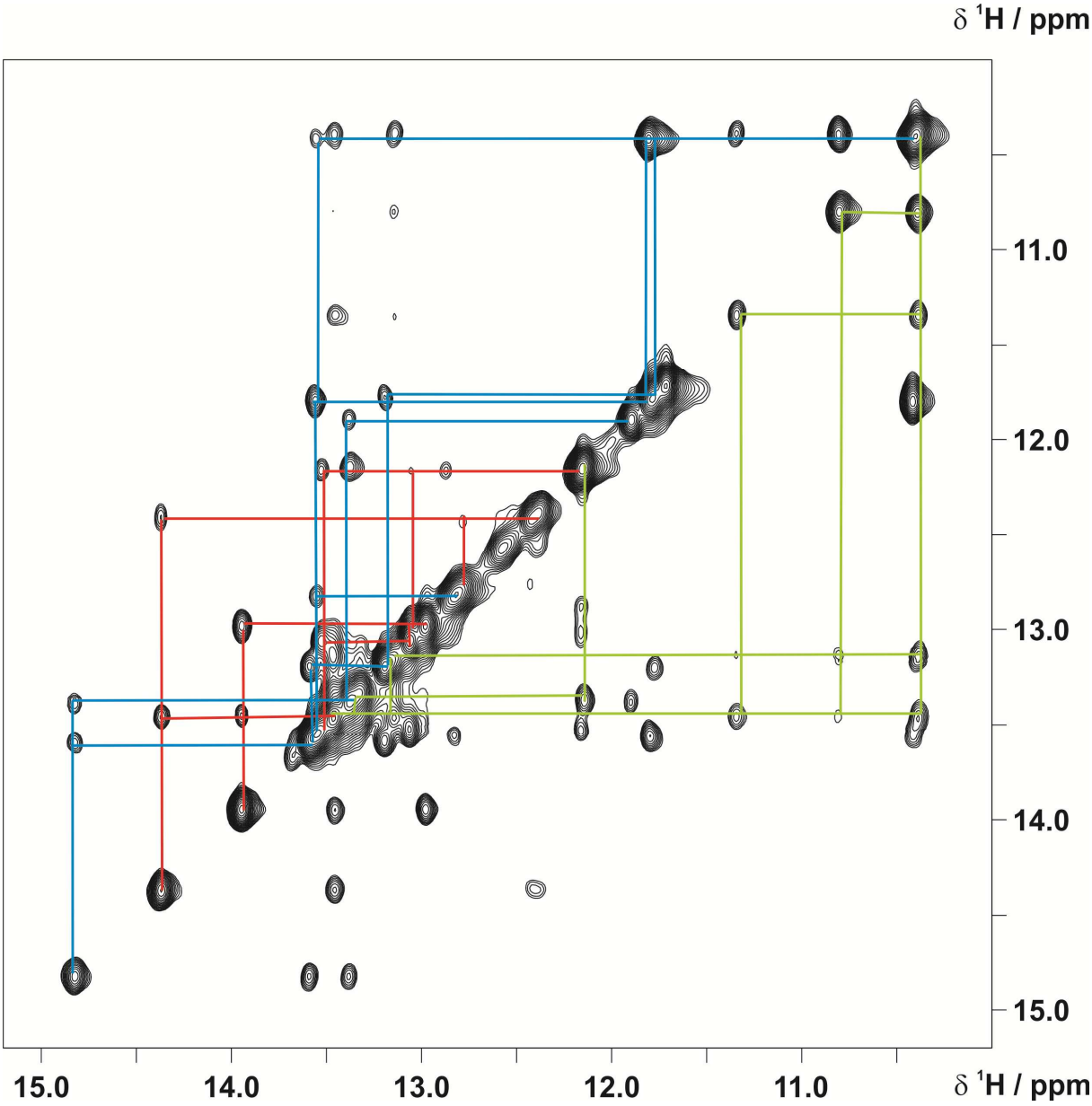
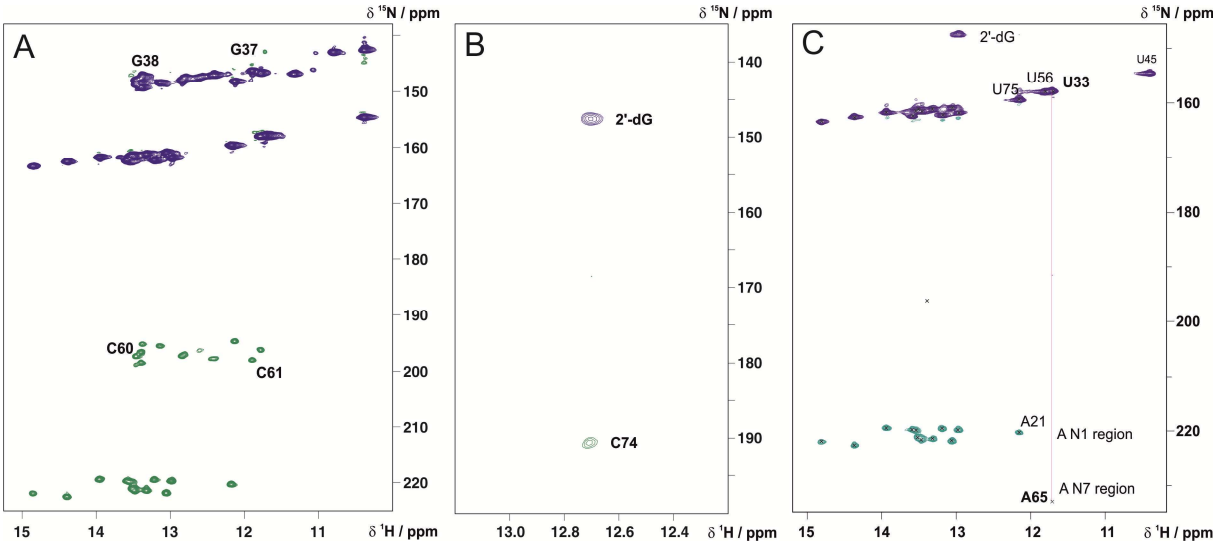
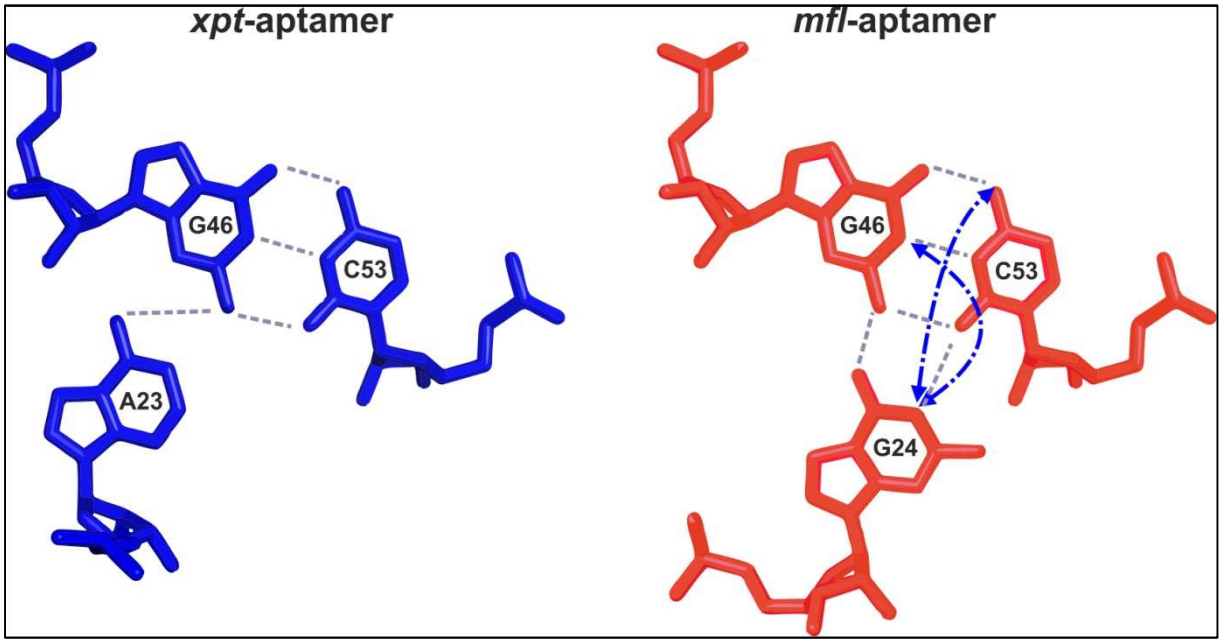


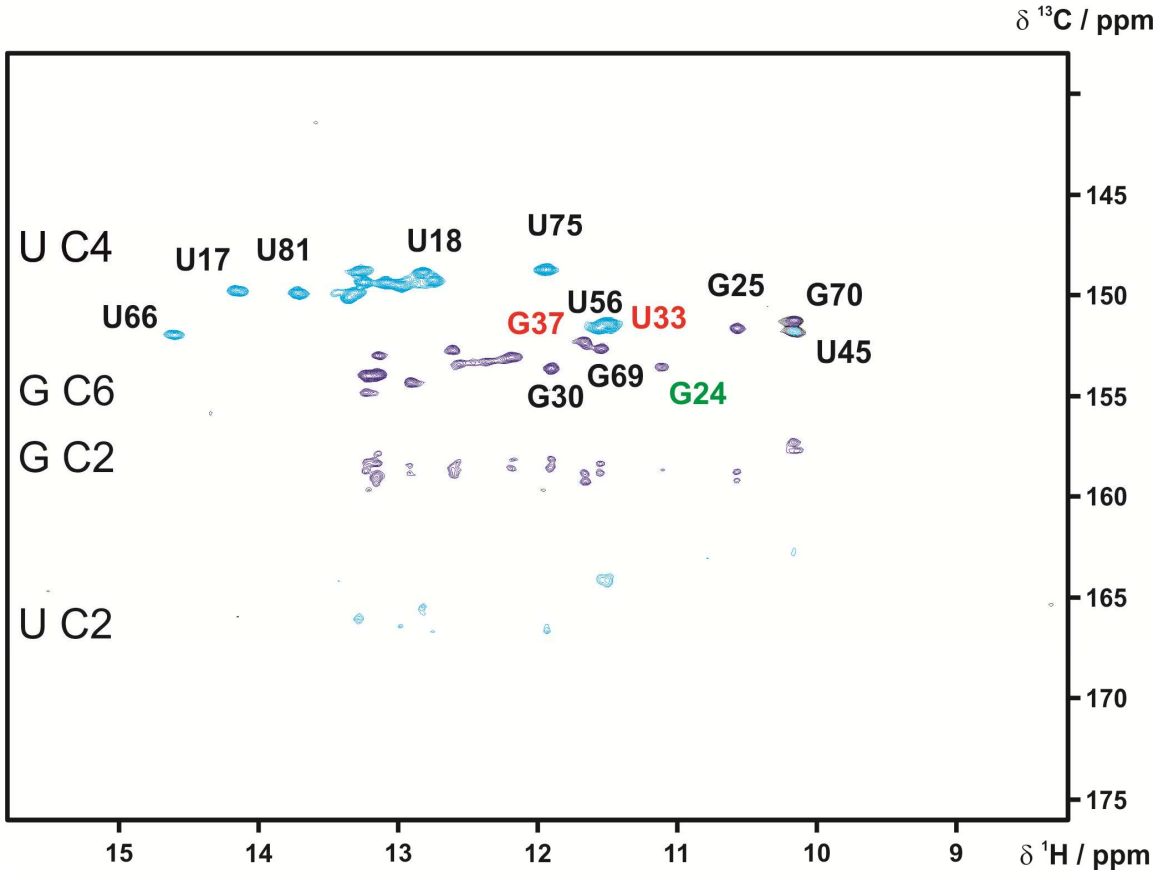
Supplementary Figure S1: Imino proton region of a ^{1}H,^{1}H-NOESY spectrum showing the imino-imino proton connectivities in the A-helical elements P1 (red), P2 (green) and P3 (blue). The NOE mixing time was 80 ms. The NOESY spectrum was recorded at 283 K on a 950 MHz spectrometer.



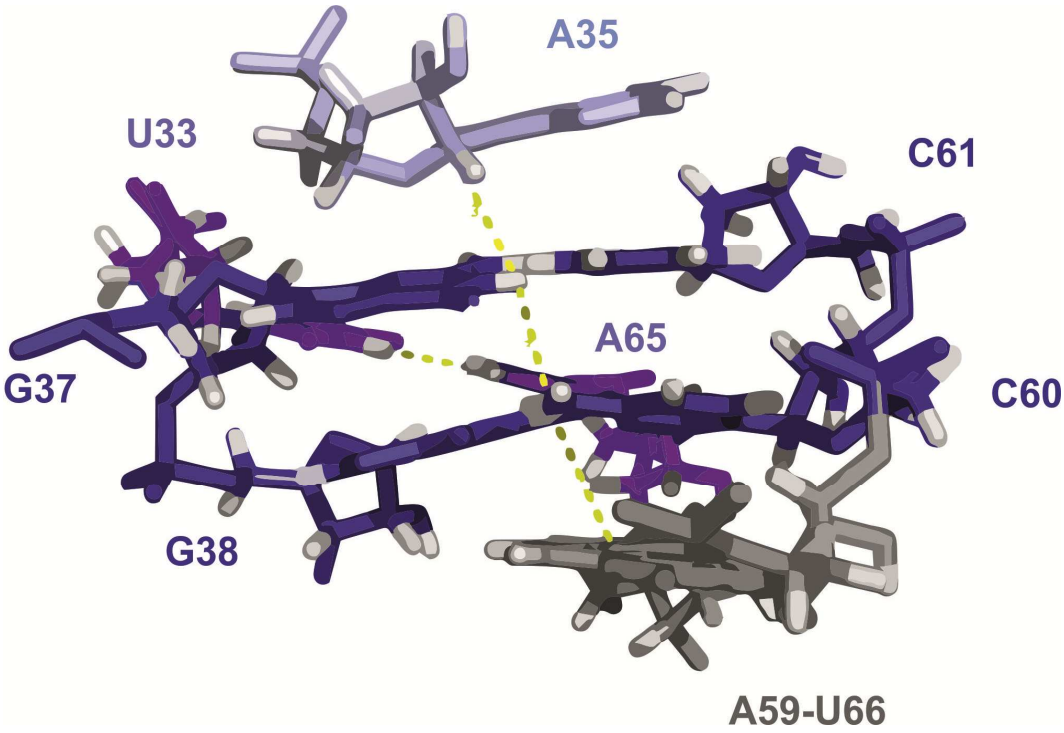
Supplementary Figure S2: HNN-COSY spectra [1] of A, uniformly ^{15}N-labelled *mfl*-aptamer RNA in complex with unlabelled 2'-dG. The loop-residues forming the WC-base pairs in the loop-loop interaction, G37-C61 and G38-C60, are assigned. B, ^{15}N-cytidine-labelled *mfl*-aptamer RNA in complex with ^{15}N-labelled 2'-dG. The hydrogen bond formed by WC base-pairing of 2'-dG to C74 is detected as a 2'-dG NH-C74 N3 cross signal. C, ^{15}N-adeine/uridine-labelled *mfl*-aptamer RNA bound to ^{15}N-labelled 2'-dG. The non-canonical H-bond formed between U33 NH and A64 N7 is indicated by a red line. The spectrum was recorded at 283 K on a 600 MHz spectrometer.



Supplementary Figure S3: left: base-triple observed for the xpt-aptamer, formed by A23, G46 and C53; right: The G24 imino proton of the mfl-aptamer shows NOE cross signals to the G46 imino proton, the C53 amino protons as well as to the U45 imino proton which, together with G25, constitutes the basal base pair of P2 (NOE contacts indicated by dashed blue arrows).



Supplementary Figure S4: HNCO-spectrum [2-4] of the *mfl*-aptamer/2'-dG complex showing the C2 carbonyl shifts if the uridine residues and the C6 carbonyl shifts of the guanosine residues. Non-Watson-Crick like carbonyl shifts are observed for U66, U56 (wobble), U33 (Hoogsteen) and U45 (wobble); and for G25 (wobble) and G70 (wobble). The spectrum was recorded at 283 K on a 600 MHz spectrometer.



Supplementary Figure S5: Model of the loop-loop interaction based on the heavy-atom coordinates of the *xpt*-aptamer-guanine complex [5] (PDB ID: 1Y27). Single-Nucleotide substitutions in loop L2 (G32A, A33U, U34A) were performed with Discovery Studio Visualizer 3.0 (Accelrys) and no further refinement was applied. Of loop L3, only C60, C61 and A66 (corresponding to A65 in the *mfl*-aptamer) are shown. The 2 conserved WC-base pairs (G37-C61, G38-C60) are shown in blue, the L3 closing base pair A59-(U67 in the *xpt*-aptamer) is shown in grey, the Hoogsteen base pair U33A65 (A33-A66 in the *xpt*-aptamer) are shown in purple and A35, which shows an NOE of its H2 proton to the G37 imino proton is shown in light blue. Distances observable as NOEs for the *mfl*-aptamer are indicated by yellow dashed lines. The three distances around the WC-base pairs are all reasonably possible in this crude structural model: U67 NH-G38 NH ~ 4.1 Å; G38 NH-G37 NH ~ 3.6 Å; G37 NH-A35 H2 ~ 3.8 Å. The distance between the U33 NH and the A65 H8 (~ 5.6 Å) is larger in this model than one would expect from the strong NOE, but this is very likely due to the fact that the atom coordinates of the *xpt*-aptamer are used for this model.

Literature for Supplementary Material:

1. Wohnert, J., et al., *Direct identification of NH...N hydrogen bonds in non-canonical base pairs of RNA by NMR spectroscopy.* Nucleic Acids Res, 1999. **27**(15): p. 3104-10.
2. Grzesiek, S. and A. Bax, *An Efficient Experiment For Sequential Backbone Assignment of Medium-Sized Isotopically Enriched Proteins.* J magn reson, 1992. **99**(1): p. 201-207.
3. Kay, L.E., G.Y. Xu, and T. Yamazaki, *Enhanced-Sensitivity Triple-Resonance Spectroscopy with Minimal H2O Saturation.* J Magn Reson, 1994. **A109**: p. 129-133.
4. Schleucher, J., M. Sattler, and C. Griesinger, *Coherence selection by gradients without signal attenuation: application to three-dimensional HNCO experiments.* Angew Chem Int Ed Engl, 1993. **32**: p. 1489-1492.
5. Serganov, A., et al., *Structural basis for discriminative regulation of gene expression by adenine- and guanine-sensing mRNAs.* Chem Biol, 2004. **11**(12): p. 1729-41.