NMR Spectroscopy and Molecular Dynamics Simulation of r(CCGCUGCGG)$_2$ Reveal a Dynamic UU Internal Loop Found in Myotonic Dystrophy Type 1

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Materials and Methods

Preparation and Purification of RNA Samples

RNA was purchased from Dharmacon. The ACE protecting groups were cleaved using Dharmacon's deprotection buffer. The sample was lyophilized and resuspended in water and then passed through a PD-10 gel filtration column (GE Healthcare) and eluted with DEPC-treated nanopure water. The RNA was then purified using thin layer chromatography as previously described (*1*). Sample purity was assessed using reverse phase HPLC using a C-18 column and was greater than 95%. For NMR experiments, the lyophilized RNA was dissolved in 0.01 M phosphate buffer, pH 7.2, 0.1 M NaCl, and 0.05 mM EDTA in 100% D_2O. For water experiments, the sample was dissolved in 10% D_2O/90% H_2O.

NMR Spectroscopy

All spectra were acquired on a Varian NMR spectrometer operating at either 400 or 500 MHz on proton.

NOESY spectra were acquired at 40°C with 100, 200, and 400 ms mixing times; 256 FIDs per experiment were collected. For each FID, 64 transients with 4,096 data points were collected. For two-dimensional spectra, the second dimension was zero filled to 4k data points, and a shifted sine bell curve apodization was used in both dimensions.

A two-dimensional water NOESY at 10°C with a 100 ms mixing time and the same number of FIDs per experiment, transients and data points per FID described as above was collected. Water suppression was achieved with a 1-1 Jump Return pulse sequence.

DQF-COSY spectra were recorded with 512 FIDs (32 transients with 4,096 data points per FID). The spectra were processed with a shifted sine bell curve in both dimensions.

Assignments were made by analysis of the NOESY and DQF-COSY spectra. Table 1 lists the chemical shift assignments for non-exchangable protons.

Structure Calculations

All calculations were carried out on Dell GX270 Linux workstation using CYANA 2.1.(*2*) The following distance restraints were used for 1H-1H distances: 2.0 to 2.6 Å for peaks equal to or stronger that the CH-H5/H6 crosspeak; 2.6 to 3.75 Å for peaks with similar intensities as the CH-H5/H6 crosspeak; and 3.75 to 5.5 Å for peaks with less intensity than the CH-H5/H6 crosspeak. For overlapped peaks, the upper limit was extended 5 or 5.5 Å. Base pair hydrogen bond restraints and base pair planarity restraints (C1' to C1' distances) were also used for all base pairs (excludes the UU mismatch). The global structure of the CUG loop was generated using torsion angle dynamics (TAD) (*3*) in CYANA 2.1. The TAD calculation was repeated 100 times with random initial structures. These structures were evaluated and the top 20 structures imported into MOLMOL (*4*) molecular visualization software and superimposed. These structures were then further refined using Amber 11 (*5*) with the NOE distance and Watson-Crick base pair restraints listed above.

NMR Refinement of Cyana Structure

The three-dimensional structure from Cyana was further refined in Amber 11 using NOE distance and Watson-Crick base pair restraints. A total of 223 NOE distance restraints were applied for structural calculations. Out of 223 restraints, 180 were NOE distance restraints (112 intra- and 68 inter-residue) and the rest (43) as Watson-Crick base pair restraints (Tables 2 and 3). The structure was then subjected

to 100 cycles of minimization using the steepest descent algorithm to remove molecular strain and relax the system. In order to sample conformational space, the structure was subjected to 100 ps of simulated annealing refinement, which involved heating the system from 0 K to 600 K, recooling from 600 K to 100 K, and finally cooling to 0 K. All Amber calculations were conducted with the ff99sb force field using a generalized Born (GB) solvent continuum model (*6*) (Amber igb parameter = 1). A series of 25 structures were generated in Amber (Amber ig parameter = -1). All structures agreed with the experimental NOE distance restraints within 0.2 Å. All molecular graphics analysis and visualization was performed using Pymol (*7*) and VMD (*8*). Helical parameters were calculated using X3DNA (*9*).

Molecular Dynamics Calculations

Amber calculations were performed with a 16-processor Linux cluster. The NMR refined Cyana structure was subjected to energy minimization with ff99sb force field followed by simulated MD. The MD simulations were conducted using Amber 11 with the amber 99 force field (ff99sb). PDB files generated in Cyana structure calculations were modified using SED editor so that atom names could be read into Amber 11. The LEAP Amber module was used for generating parameter and topology files for MD. Further using LEAP, Na^+ ions were added to neutralize the negatively charged system. The whole system was then solvated with octahedral solvent box with thickness of 8 Å using TIP3P model. This resulted in solvated structure consisting of 578 RNA molecule atoms, 16 Na^+ ions and 3141 water molecules.

Energy minimization was performed in two stages using the Sander module of Amber 11. In the first stage of energy minimization, the RNA molecule was subjected to 500 steps of steepest descent and conjugate gradient energy minimization. The RNA was held fixed with a force constant of 500 kcal mol^{-1} $Å^{-2}$ while allowing the movement of ions and solvent during the course of the minimization. This energy minimization was performed to relax the system and remove kinks and other unnecessary strains left form the NMR structure. In the second stage of energy minimzation, the entire system comprised of

RNA, water, and ions were minimized with 2500 steps of steepest descent followed by 1000 steps of conjugate gradient energy minimization protocol.

Once the structure was successfully minimized and optimized, MD simulating was performed. MD studies were performed using the Sander module of Amber. The system was subjected to 500 ps of warm-up dynamics to allow water, ions, and the RNA molecule to equilibrate. This involved heating the system gradually from 0 to 300 K using a force constant of 10 kcal mol^{-1} $Å^{-2}$ to keep RNA molecule atoms restrained. This equilibration process prevented wide fluctuations. The SHAKE algorithm was used to contrain bond lengths involving hydrogen. SHAKE removes the fastest bond stretching motion, thus allowing larger time steps (10). Once the RNA system was successfully equilibrated at constant volume, production dynamics was performed for 1 ns with time steps of 2 fs under constant pressure (1 atm) and temperature (300 K) conditions without any restraint on the RNA. The output files generated after the completion of production dynamics were "mdcrd" and "restart", which contain the coordinates from a particular MD simulation run. The mdcrd file is the coordinate file with periodic boundary coordinate box (crdbox) and gives information about the coordinates of the atoms and their motion throughout the simulation. Atomic coordinates were saved every 0.1 ns during the production dynamics run. RMSD analysis indicates that after 20 ps, the system shows a fairly stable RMSD, thus confirming the stablilty of the dynamics (Figure S1). These files are used as the input for the next stage of the simulation.

The Amber Carnal and Ptraj utilities were then used to analyze the properties of the system such as the presence of hydrogen bonds, the root mean square deviation (RMSD), *etc*. The distance and angle cutoff for hydrogen bonds was a maximum donor-acceptor distance of 3.5 Å and minimum donor-acceptor-acceptor angle of 120^{o}.

Helical Parameters and Electrostatic Calculations

Helical parameters were calculated using X3DNA. Table 4 lists the helical parameterfor the 0, 1 and 2 hydrogen bonded structures. Electrostatic surface potential was calculated by solving the non-linear

Poisson–Boltzmann equation (*10*). The partial charges were assigned to the system based on the Amber force field. The surface electrostatic potential for RNA molecule was calculated with APBS (*11*). The electrostatic maps were visualized. Figure S2 shows the stacking pattern and electrostatic interactions as viewed from the minor grove.

Table 1. Chemical shift assignments for the non-exchangable protons in r(CCGCUGCGG)$_2$ at 40˚C

	H1'	H2'	H3'	H4'	H5	H5"/H5'	H6/H8
C1	5.438	4.461	4.493	4.338	6.009	3.929/4.05	8.048
C2	5.642	4.597	4.694	-	5.636	-	7.983
G3	5.774	4.557	4.69	-	-	4.185	7.658
C4	5.568	4.385	4.455	-	5.29	-	7.622
U5	5.539	4.324	4.464	-	5.532	4.134	7.651
G6	5.808	4.555	4.678	-	-	4.165	7.838
C7	5.586	4.618	4.521	-	5.27	-	7.676
G8	5.763	4.588	4.53	-	-	4.141	7.499
G9	5.862	4.086	4.255	-	-	-	7.364

Table 2. 1H-1H NOE distance restraints and C1'-C1' planarity restraints

Residue Number	Residue	Atom	Residue Number	Residue	Atom	Lower bound	Upper bound
1	RC5	H6	1	RC5	H5	2.40	2.60
1	RC5	H5	1	RC5	H6	2.40	2.60
1	RC5	H2'1	1	RC5	H1'	2.60	3.75
1	RC5	H2'1	1	RC5	H6	2.60	3.75
1	RC5	H3'	1	RC5	H6	2.60	3.75
1	RC5	H4'	1	RC5	H6	4.50	5.50
1	RC5	H3'	1	RC5	H1'	3.75	5.00
1	RC5	H1'	1	RC5	H6	3.75	5.00
1	RC5	H5'1	1	RC5	H6	3.75	5.00
1	RC5	H5'2	1	RC5	H6	3.75	5.00
1	RC5	H2'1	2	RC	H6	2.00	2.60
1	RC5	H2'1	2	RC	H5	2.60	3.75
1	RC5	H6	2	RC	H6	3.75	5.00
1	RC5	H5	2	RC	H5	2.60	3.75
1	RC5	H1'	2	RC	H6	3.75	5.00
1	RC5	H3'	2	RC	H6	3.75	5.00
1	RC5	H1'	2	RC	H5	3.75	5.00
2	RC	H6	2	RC	H5	2.40	2.60
2	RC	H5	2	RC	H6	2.40	2.60
2	RC	H2'1	2	RC	H1'	2.60	3.75
2	RC	H2'1	2	RC	H6	2.60	3.75
2	RC	H3'	2	RC	H6	2.60	3.75

2	RC	H1'	2	RC	H6	3.75	5.00
2	RC	H1'	3	RG	H8	2.60	4.50
2	RC	H2'1	3	RG	H8	2.00	2.60
2	RC	H6	3	RG	H8	3.75	5.00
3	RG	H2'1	3	RG	H1'	2.60	5.00
3	RG	H2'1	3	RG	H8	2.60	4.50
3	RG	H3'	3	RG	H8	2.60	3.75
3	RG	H1'	3	RG	H8	3.75	5.00
3	RG	H5'1	3	RG	H8	3.75	5.00
3	RG	H1'	4	RC	H6	3.75	5.50
3	RG	H2'1	4	RC	H6	2.00	2.60
4	RC	H3'	4	RC	H6	2.60	5.00
4	RC	H6	4	RC	H5	2.40	2.60
4	RC	H5	4	RC	H6	2.40	2.60
4	RC	H2'1	4	RC	H1'	2.60	3.75
4	RC	H2'1	4	RC	H6	2.60	3.75
4	RC	H1'	4	RC	H6	3.75	5.00
4	RC	H2'1	4	RC	H5	3.75	5.00
4	RC	H2'1	5	RU	H6	2.60	5.00
4	RC	H2'1	5	RU	H5	2.60	5.00
4	RC	H5	5	RU	H5	2.60	3.75
4	RC	H1'	5	RU	H6	3.75	5.00
4	RC	H3'	5	RU	H6	2.60	5.00
5	RU	H1'	5	RU	H6	2.60	4.50

5	RU	H6	5	RU	H5	2.40	2.60
5	RU	H6	5	RU	H6	2.60	4.50
5	RU	H2'1	5	RU	H1'	2.60	3.75
5	RU	H2'1	5	RU	H6	2.60	3.75
5	RU	H3'	5	RU	H2'1	2.00	2.60
5	RU	H5'1	5	RU	H6	3.75	5.00
5	RU	H3'	5	RU	H6	3.75	5.00
5	RU	H3'	6	RG	H8	2.60	5.00
5	RU	H2'1	6	RG	H8	2.00	2.60
5	RU	H1'	6	RG	H8	3.75	5.00
5	RU	H6	6	RG	H8	3.75	5.00
5	RU	H2'1	6	RG	H1'	3.75	5.00
6	RG	H2'1	6	RG	H1'	2.60	3.75
6	RG	H2'1	6	RG	H8	2.60	3.75
6	RG	H3'	6	RG	H8	2.60	3.75
6	RG	H1'	6	RG	H8	3.75	5.00
6	RG	H5'1	6	RG	H8	3.75	5.00
6	RG	H1'	7	RC	H6	3.75	5.00
6	RG	H2'1	7	RC	H5	2.60	4.50
6	RG	H1'	7	RC	H5	2.60	4.50
6	RG	H8	7	RC	H5	3.75	5.50
6	RG	H2'1	7	RC	H6	2.00	2.60
7	RC	H3'	7	RC	H6	3.75	5.00
7	RC	H6	7	RC	H5	2.40	2.60

7	RC	H2'1	7	RC	H1'	2.60	3.75
7	RC	H2'1	7	RC	H6	3.75	5.00
7	RC	H1'	7	RC	H6	3.50	5.00
7	RC	H2'1	8	RG	H8	2.00	2.60
7	RC	H1'	8	RG	H8	3.75	5.00
7	RC	H5	8	RG	H8	3.75	5.00
7	RC	H6	8	RG	H8	3.75	5.50
7	RC	H3'	8	RG	H8	2.60	3.75
7	RC	H6	8	RG	H8	3.75	5.00
8	RG	H2'1	8	RG	H1'	2.60	3.75
8	RG	H1'	8	RG	H8	2.60	3.75
8	RG	H3'	8	RG	H8	2.60	3.75
8	RG	H5'1	8	RG	H8	3.75	5.00
8	RG	H2'1	8	RG	H8	3.75	5.00
8	RG	H3'	9	RG3	H8	2.60	3.75
8	RG	H2'1	9	RG3	H8	2.00	2.60
8	RG	H1'	9	RG3	H8	3.75	5.00
8	RG	H8	9	RG3	H8	3.75	5.50
9	RG3	H2'1	9	RG3	H8	2.60	3.75
9	RG3	H3'	9	RG3	H8	2.60	3.75
9	RG3	H3'	9	RG3	H2'1	2.00	2.60
9	RG3	H1'	9	RG3	H8	3.75	5.00
1*	RC5	H6	1*	RC5	H5	2.40	2.60
1*	RC5	H5	1*	RC5	H6	2.40	2.60

1*	RC5	H2'1	1*	RC5	H1'	2.60	3.75
1*	RC5	H5'1	1*	RC5	H6	2.60	3.75
1*	RC5	H2'1	1*	RC5	H6	2.60	3.75
1*	RC5	H3'	1*	RC5	H6	2.60	3.75
1*	RC5	H1'	1*	RC5	H6	3.75	5.00
1*	RC5	H5'2	1*	RC5	H6	3.75	5.00
1*	RC5	H2'1	2*	RC	H6	2.00	2.60
1*	RC5	H2'1	2*	RC	H5	2.60	3.75
1*	RC5	H6	2*	RC	H6	3.75	5.00
1*	RC5	H1'	2*	RC	H5	3.75	5.00
1*	RC5	H1'	2*	RC	H6	3.75	5.00
1*	RC5	H3'	2*	RC	H6	3.75	5.00
1*	RC5	H5	2*	RC	H5	2.60	3.75
2*	RC	H6	2*	RC	H5	2.40	2.60
2*	RC	H5	2*	RC	H6	2.40	2.60
2*	RC	H2'1	2*	RC	H1'	2.60	3.75
2*	RC	H2'1	2*	RC	H6	2.60	3.75
2*	RC	H3'	2*	RC	H6	2.60	3.75
2*	RC	H1'	2*	RC	H6	3.75	5.00
2*	RC	H2'1	3*	RG	H8	2.00	2.60
2*	RC	H6	3*	RG	H8	3.75	5.00
2*	RC	H1'	3*	RG	H8	2.60	4.50
3*	RG	H2'1	3*	RG	H1'	2.60	5.00
3*	RG	H2'1	3*	RG	H8	2.60	4.50

3*	RG	H3'	3*	RG	H8	2.60	3.75
3*	RG	H1'	3*	RG	H8	3.75	5.00
3*	RG	H5'1	3*	RG	H8	3.75	5.00
3*	RG	H2'1	4*	RC	H6	2.00	2.60
3*	RG	H1'	4*	RC	H6	3.75	5.00
4*	RC	H3'	4*	RC	H6	2.60	5.50
4*	RC	H6	4*	RC	H5	2.40	2.60
4*	RC	H5	4*	RC	H6	2.40	2.60
4*	RC	H2'1	4*	RC	H1'	2.60	3.75
4*	RC	H2'1	4*	RC	H6	2.60	3.75
4*	RC	H1'	4*	RC	H6	3.75	5.00
4*	RC	H2'1	4*	RC	H5	3.75	5.00
4*	RC	H2'1	5*	RU	H6	2.60	5.00
4*	RC	H2'1	5*	RU	H5	2.60	5.00
4*	RC	H5	5*	RU	H5	2.60	3.75
4*	RC	H1'	5*	RU	H6	3.75	5.00
4*	RC	H3'	5*	RU	H6	2.60	5.00
5*	RU	H1'	5*	RU	H6	2.60	4.50
5*	RU	H6	5*	RU	H5	2.40	2.60
5*	RU	H2'1	5*	RU	H1'	2.60	3.75
5*	RU	H2'1	5*	RU	H6	2.60	3.75
5*	RU	H3'	5*	RU	H2'1	2.00	2.60
5*	RU	H5'1	5*	RU	H6	3.75	5.00
5*	RU	H3'	5*	RU	H6	3.75	5.00

5*	RU	H2'1	6*	RG	H8	2.00	2.60
5*	RU	H1'	6*	RG	H8	3.75	5.00
5*	RU	H6	6*	RG	H8	3.75	5.00
5*	RU	H2'1	6*	RG	H1'	3.75	5.00
5*	RU	H3'	6*	RG	H8	2.60	5.00
6*	RG	H2'1	6*	RG	H1'	2.60	3.75
6*	RG	H2'1	6*	RG	H8	2.60	3.75
6*	RG	H3'	6*	RG	H8	2.60	3.75
6*	RG	H1'	6*	RG	H8	3.75	5.00
6*	RG	H5'1	6*	RG	H8	3.75	5.00
6*	RG	H1'	7*	RC	H6	3.75	5.00
6*	RG	H1'	7*	RC	H5	2.60	4.50
6*	RG	H2'1	7*	RC	H5	2.00	3.75
6*	RG	H2'1	7*	RC	H6	2.00	2.60
6*	RG	H8	7*	RC	H5	3.75	5.50
7*	RC	H2'1	7*	RC	H6	3.75	5.00
7*	RC	H3'	7*	RC	H6	3.75	5.00
7*	RC	H6	7*	RC	H5	2.40	2.60
7*	RC	H2'1	7*	RC	H1'	2.60	3.75
7*	RC	H1'	7*	RC	H6	3.50	5.00
7*	RC	H2'1	8*	RG	H8	2.00	2.60
7*	RC	H1'	8*	RG	H8	3.75	5.00
7*	RC	H5	8*	RG	H8	3.75	5.50
7*	RC	H3'	8*	RG	H8	2.60	3.75

7*	RC	H6	8*	RG	H8	3.75	5.50
8*	RG	H2'1	8*	RG	H1'	2.60	3.75
8*	RG	H1'	8*	RG	H8	2.60	3.75
8*	RG	H3'	8*	RG	H8	2.60	3.75
8*	RG	H5'1	8*	RG	H8	3.75	5.00
8*	RG	H2'1	8*	RG	H8	3.75	5.00
8*	RG	H3'	9*	RG3	H8	2.60	3.75
8*	RG	H2'1	9*	RG3	H8	2.00	2.60
8*	RG	H1'	9*	RG3	H8	3.75	5.00
8*	RG	H8	9*	RG3	H8	3.75	5.50
9*	RG3	H2'1	9*	RG3	H8	2.60	3.75
9*	RG3	H3'	9*	RG3	H8	2.60	3.75
9*	RG3	H3'	9*	RG3	H2'1	2.00	2.60
9*	RG3	H1'	9*	RG3	H8	3.75	5.00
1	RC5	C1'	9*	RG3	C1'	1*.60	1*.90
2	RC	C1'	8*	RG	C1'	1*.60	1*.90
3	RG	C1'	7*	RC	C1'	1*.60	1*.90
4	RC	C1'	6*	RG	C1'	1*.60	1*.90
6	RG	C1'	4*	RC	C1'	1*.60	1*.90
7	RC	C1'	3*	RG	C1'	1*.60	1*.90
8	RG	C1'	2*	RC	C1'	1*.60	1*.90
9	RG3	C1'	1*	RC5	C1'	1*.60	1*.90

Table 3. Watson-Crick base pair restraints

Residue Number	Residue	Atom	Residue Number	Residue	Atom	Lower Bound	Upper Bound
1	RC5	O2	9*	RG3	H21	1.80	2.00
1	RC5	O2	9*	RG3	N2	2.70	3.00
1	RC5	N3	9*	RG3	H1	1.80	2.00
1	RC5	N3	9*	RG3	N1	2.70	3.00
1	RC5	H41	9*	RG3	O6	1.80	2.00
1	RC5	N4	9*	RG3	O6	2.70	3.00
2	RC	O2	8*	RG	N2	2.70	3.00
2	RC	N3	8*	RG	H1	1.80	2.00
2	RC	N3	8*	RG	N1	2.70	3.00
2	RC	H41	8*	RG	O6	1.80	2.00
2	RC	N4	8*	RG	O6	2.70	3.00
3	RG	N2	7*	RC	O2	2.70	3.00
3	RG	H1	7*	RC	N3	1.80	2.00
3	RG	N1	7*	RC	N3	2.70	3.00
3	RG	O6	7*	RC	H41	1.80	2.00
3	RG	O6	7*	RC	N4	2.70	3.00
4	RC	O2	6*	RG	H21	1.80	2.00
4	RC	O2	6*	RG	N2	2.70	3.00
4	RC	N3	6*	RG	H1	1.80	2.00
4	RC	N3	6*	RG	N1	2.70	3.00
4	RC	H41	6*	RG	O6	1.80	2.00
4	RC	N4	6*	RG	O6	2.70	3.00

6	RG	N2	4*	RC	O2	2.70	3.00
6	RG	H1	4*	RC	N3	1.80	2.00
6	RG	N1	4*	RC	N3	2.70	3.00
6	RG	O6	4*	RC	H41	1.80	2.00
6	RG	O6	4*	RC	N4	2.70	3.00
7	RC	O2	3*	RG	H21	1.80	2.00
7	RC	O2	3*	RG	N2	2.70	3.00
7	RC	N3	3*	RG	H1	1.80	2.00
7	RC	N3	3*	RG	N1	2.70	3.00
7	RC	H41	3*	RG	O6	1.80	2.00
7	RC	N4	3*	RG	O6	2.70	3.00
8	RG	N2	2*	RC	O2	2.70	3.00
8	RG	H1	2*	RC	N3	1.80	2.00
8	RG	N1	2*	RC	N3	2.70	3.00
8	RG	O6	2*	RC	H41	1.80	2.00
8	RG	O6	2*	RC	N4	2.70	3.00
9	RG3	N2	1*	RC5	O2	2.70	3.00
9	RG3	H1	1*	RC5	N3	1.80	2.00
9	RG3	N1	1*	RC5	N3	2.70	3.00
9	RG3	O6	1*	RC5	H41	1.80	2.00
9	RG3	O6	1*	RC5	N4	2.70	3.00

Table 4. Helical parameters for 0(a), 1(b) and 2(c) hydrogen-bonded structures. Displacement is of middle C1'-C1' point from the helix; Angle is the inclination between C1'-C1' vectors; Twist is the angle between successive C1'-C1' vectors and Rise is the helical projection of the vectors connecting consecutive C1'-C1' points onto the helical axis.

a)

Base Pair	Displacement [Å]	Angle [^{0}]	Twist [^{0}]	Rise [Å]
C1-G9*	6.71	20.32	25.07	2.58
C2-G8*	7.66	12.95	36.2	3.17
G3-C7*	8.39	9.24	25.56	3.06
C4-G6*	8.64	1.14	30.48	2.64
U5-U5*	7.78	8.98	36.99	2.91
G6-C4*	6.38	12.71	19.13	2.81
C7-G3*	5.92	9.13	28.49	2.42
G8-C2*	8.87	2.14	32.29	3.55
G9-C1*	9.69	1.12	---	---

b)

Base Pair	Displacement [Å]	Angle [^{0}]	Twist [^{0}]	Rise [Å]
C1-G9*	8.66	18.92	35.73	2.11
C2-G8*	8.72	21.58	30.39	1.95
G3-C7*	8.94	19.68	37.86	1.8
C4-G6*	7.79	17.79	28.34	2.05
U5-U5*	7.54	22.17	24.64	2.9
G6-C4*	7.81	24.29	38.01	1.95
C7-G3*	8.74	25.08	34.87	2.02
G8-C2*	8.17	23.6	35.39	2.09
G9-C1*	7.53	20.37	---	---

c)

Base Pair	Displacement [Å]	Angle [^{0}]	Twist [^{0}]	Rise [Å]
C1-G9*	7.89	6.28	31.66	2.46
C2-G8*	7.81	9.37	21.4	2.77
G3-C7*	8.54	8.3	28.57	3.66
C4-G6*	8.62	9.36	31.1	3.07
U5-U5*	6.76	8.39	34.66	2.57
G6-C4*	7.15	9.37	24.76	2.73
C7-G3*	6.94	12.02	27.19	3.09
G8-C2*	8.17	8.33	36.94	2.78
G9-C1*	8.2	7.14	---	---

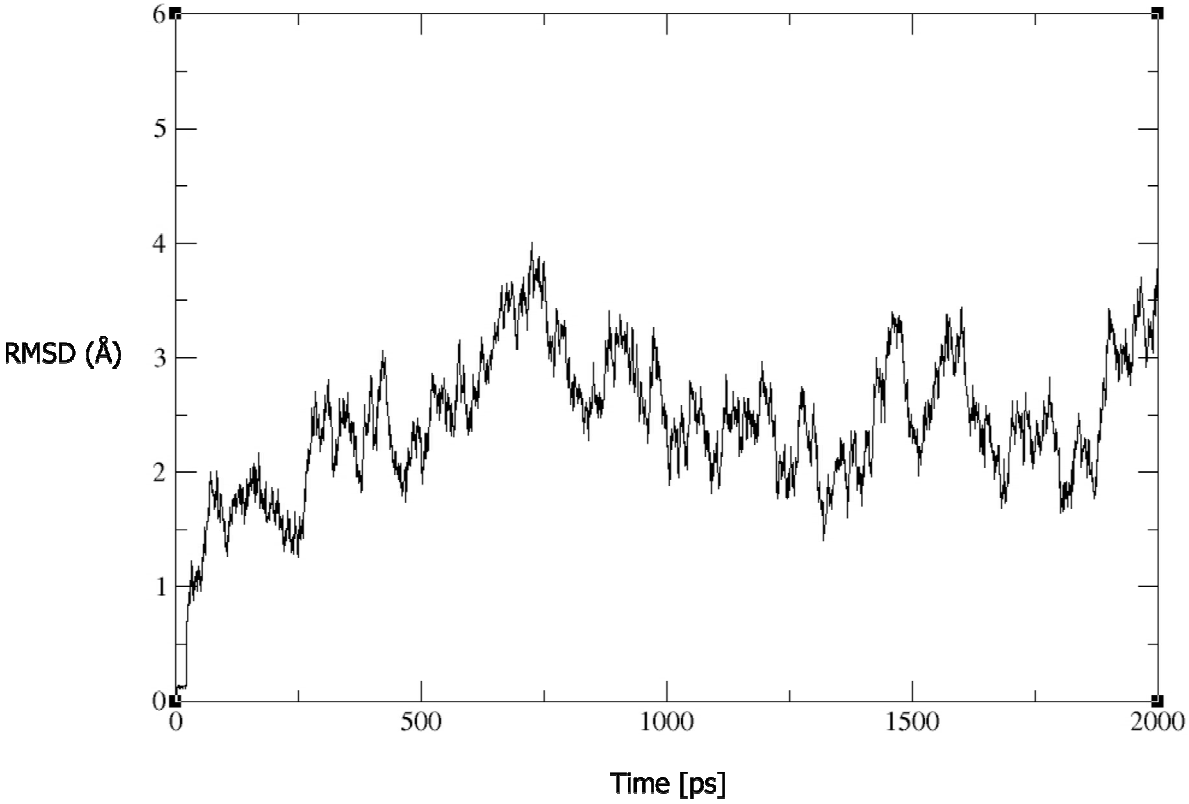

Figure S1. Time course plot of the RMSD value of $r(CCGCUGCGG)_2$ over a period of 1 ns with respect to the starting NMR refined structure. Note that RMSD of backbone atoms remained low for the first 20 ps, which is due to the restraints applied during the simulation. After this time period, the RMSD is fairly stable indicating equlibration.

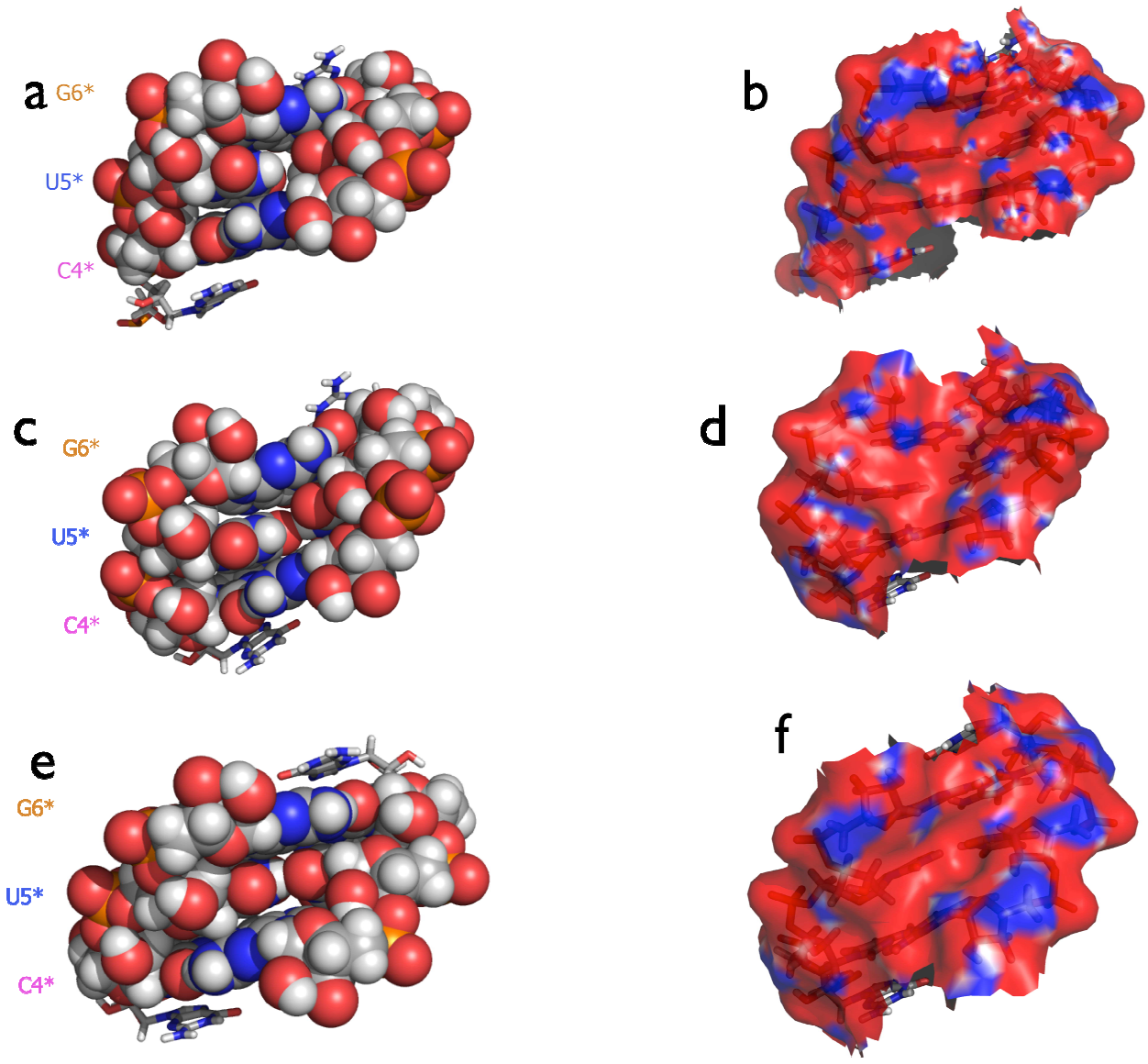

Figure S2. *Left,* space filling model depicting the minor grove view of r(CCGCUGCGG)$_2$ for the 0. 1, and 2 hydrogen bonded structures (a, c, and e, respectively). *Right,* electrostatic surface view of the minor grove for the 0, 1, and 2 hydrogen bonded structures (b, d, and f, respectively).

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