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The conformational landscape of the ribosomal protein S15 and its influence on the protein interaction with 16S RNA.

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Abstract

The interaction between the ribosomal protein S15 and its binding sites in the 16S RNA, was examined from two points of view. First, the isolated protein S15 was studied by comparing NMR conformer sets, available in the PDB and recalculated using the CNS-ARIA protocol. Molecular dynamics trajectories were then recorded starting from a conformer of each set. The recalculation of the S15 NMR structure, as well as the recording of MD trajectories, reveals that several orientations of the N terminal α helix $\alpha 1$ with respect to the structure core are populated. Molecular dynamics trajectories of the complex between the ribosomal protein S15 and RNA were also recorded in presence and in absence of ions Mg^{2+} . The ions Mg^{2+} are hexa-coordinated by water and RNA oxygens. The coordination spheres mainly interact with the RNA phosphodiester backbone, reducing the RNA mobility and inducing electrostatic screening. When the ions Mg^{2+} are removed, the internal mobility of the RNA and of the protein increases at the interaction interface close to the RNA G-U/G-C motif, due to a gap between the phosphate groups in the UUCG capping tetraloop and to the disruption of S15-RNA hydrogen bonds in that region. On the other hand, several S15-RNA hydrogen bonds are reinforced and water bridges appear between the three way junction region and S15. The network of hydrogen bonds observed in the loop between $\alpha 1$ and $\alpha 2$ is consequently reorganized. In absence of Mg^{2+} , this network has the same pattern as the network observed in the isolated protein, where the helix $\alpha 1$ is mobile with respect to the protein core. The presence of Magnesium ions may thus play a role in stabilizing the orientation of the helix $\alpha 1$ of S15.

1 Introduction

During the formation of the ribosome 30s subunit, the protein S15 is among the first proteins, along with S18, S6 and S11, interacting with the 30s central domain [1]. S15 binds to a phylogenetically conserved three-way junction formed by the intersection of helices H20, H21 and H22 of eubacterial 16S RNA (Fig. 1). The free 16S RNA undergoes an equilibrium [2] between an unfolded conformation, in which the junction is planar with approximately 120 degrees inter-helical angles,

and a folded conformation, in which two helices, H21 and H22, become collinear and the third, helix H20, forms a 60 degrees angle with respect to H22. From the observations made in the literature, it was concluded [2] that the Magnesium ions as well as the protein stabilize the RNA folded conformation.

The ribosomal protein S15 from *Thermus thermophilus* was co-crystallized with a 57 nucleotide 16S rRNA fragment containing shortened helices H20, H21 and H22 [3]. The two helices H21 and H22 are capped by UUCG tetraloops, which will be called TL1 and TL2 in the following, according to Ref. 4. The 2.8 Å resolution of the crystal structure of the complex (PDB id: 1dk1, Fig. 2a) shows [3] that S15 interacts in the minor groove with the three-way junction (first binding site) and a G-U/G-C motif (second binding site). The protein structure consists of four α helices, the helices α 1 (residues 3-15), α 2 (residues 24-45), α 3 (residues 49-73), and α 4 (residues 73-81). The S15 structure in the complex is similar to the structure of the free protein previously determined by NMR (PDB id: 1ab3, Fig. 2b) [5]. Nevertheless, in the crystallographic structure of the free S15 protein from *Bacillus stearothermophilus* (PDB id: 1a32, Fig. 2c) [6], the N-terminal helix α 1 protrudes out from the body of the molecule to make contacts with a neighboring protein in the crystal lattice.

Nine ions Mg^{2+} were positioned in the S15-RNA complex structure according to the observation of strong peaks of electronic density [3], and three of them are supposed to contribute to the junction stability. The ions Mg^{2+} were previously shown in the literature to play an important role in the mechanism of ribozyme catalysis [7, 8], in the folding of RNA [9–13], in the stability of the ribosome [14], and in the interaction between RNA and proteins [15, 16] and ligands [17]. Molecular dynamics simulations were performed to study the influence of Mg^{2+} ions on the conformation of an RNA kissing loop [18], on the conformation of the 5S rRNA loop E motif [19], and on the conformation of the RNA-protein complex between *E. Coli* loop E/helix IV rRNA and L25 protein [20].

The ions Mg^{2+} are supposed to stabilize the folded form of the RNA three-way junction, and thus to enhance the S15 binding [2], by increasing the bimolecular association rate. However, the effect of the ions Mg^{2+} on the protein-RNA interaction at the atomic level, has not yet been investigated. We analyze here molecular dynamics simulations of the S15-RNA complex in absence and in presence of the ions Mg^{2+} , in order to study the influence of Mg^{2+} on the protein-RNA interaction. Molecular dynamics trajectories of the protein S15 are also analyzed in order to explore the conformational landscape of the free protein, and to relate it to the protein behavior in the complex. As said previously, the interaction of S15 with 16S RNA is one of the first steps in the 30s subunit formation, and a detailed

inspection of the S15-RNA interaction features may thus be important for targeting the ribosome formation [21]. Indeed, S15 was detected in a crystal structure of the 70s ribosome [22] in a bridge spanning the ribosomal subunit interface and seems to be essential for an efficient formation of the ribosome from the subunits 30s and 50s [23].

The use of molecular dynamics simulations has proved to be a valuable tool for analyzing the behavior of systems including RNA (see Ref. 24 and references cited therein). Nevertheless, the electrostatics of the ions Mg^{2+} is modeled here using a single point charge without including the polarisability. This approximation is certainly crude, but cannot be avoided, as the introduction of the polarisability [25] would result into an excessive computational load for a several nanosecond trajectory. Another limitation of the MD simulations of nucleic acids is the difficulty to model the conformation of the phosphodiester backbone [26]. But, there is not much drift of the phosphodiester backbone angles from the crystallographic structure, during the time interval studied in the present work.

2 Materials and Methods

The starting point of the MD trajectories recorded on the protein S15 was the first conformer of the PDB NMR structure [5] of S15 (PDB id: 1ab3), and the smallest-energy conformer of the NMR structure recalculated in the present work using the CNS-ARIA protocol. The starting conformation for the simulations of the S15-RNA complex was the X-ray crystallographic structure [3] of the S15-RNA complex (PDB id: 1dk1). Two molecular conformations are present for the residues 27-29 of RNA in the 1dk1 file: the conformation A corresponding to the maximum number of formed basepairs was selected.

The parm98 parameter set [27] and the TIP3P model for water [28] were used in simulations. The motion equations were integrated with the versions 6 and 7 of the program AMBER [29] respectively for the isolated protein, and for the complex. The cutoff of the Lennard-Jones interaction was 9 Å, and the long-range electrostatics interactions were calculated using the Particle Mesh Ewald protocol [30]. The Lennard-Jones parameters of the ions Mg^{2+} were taken from Ref. 31, and correspond to an ϵ radius of 0.7926 Å and a well depth of 0.8947 kcal/mol. Trajectories of 15 ns were recorded for each studied system: the 1ab3 NMR conformer of S15, the 2fmx conformer of S15 calculated in the present work, and the Xray structure of the S15-RNA complex in presence and in absence of Mg^{2+} ions, with a temperature of 298K and a pressure of 1 atm. The atom coordinates were

saved each ps.

In case of the simulation of S15-RNA in presence of ions Mg^{2+} , the solute includes the 9 crystallographic ions Mg^{2+} , but not the 3 ions Na^+ and K^+ observed in the crystal. The counter-ions were added in the vicinity of the charged groups of the solute using a Coulombic potential on a grid, using the command `AddIons` of `leap`. The charge of the isolated protein was neutralized with chloride ions, whereas sodium ions were added to neutralize the complex S15-RNA. For the simulation in absence of ions Mg^{2+} , the ions Na^+ were not placed at the positions of removed Mg^{2+} . The resulting modeling is thus related to the presence or the absence of a counter ion, more than the electronic properties (polarisability) of the ions. This counterbalances the limitations of the treatment of electrostatics in the system, and the uncertainty of the determination of the ions type in crystallographic structures.

The concentration of ions Mg^{2+} is around 40 mM, which is a value of the same order as the experimental concentration used to study the S15-RNA interaction [2]. On the other hand, the concentrations of ions Na^+ in the S15-RNA simulations are around 140 mM in presence of ions Mg^{2+} and of 220 mM in absence of these ions. The removal of the ions Mg^{2+} thus introduces only a small perturbation of the electrostatics field around the complex, and the effect produced by their disappearance is not produced by a non-specific electrostatic effect.

The solute conformations were hydrated by a box of water molecules in a pseudo-equilibrated configuration. The water molecules farther than 9 Å from any solute molecule were discarded. The simulation parameters (box dimensions, numbers of water molecules and atoms, type and number of counter-ions) are given in Table 1, along with a name for each trajectory.

Heating and system equilibration were performed as follows: 10 ps of heating up to 298 K at constant volume were done while restraining solute atom locations with an harmonic restraint of 25 kcal/mol Å², followed by 5 ps of MD at constant volume and four MD rounds of 2.5 ps at constant pressure. The restraints on the solute atoms were progressively reduced to zero during these six rounds. No restraint was applied on the positions of ions Mg^{2+} , Na^+ or Cl^- . The isothermal-isobaric unrestrained simulations were performed using the Berendsen algorithm [32] for temperature bath coupling. The equations of motion were integrated using a time-step of 2 fs. All covalent bonds involving hydrogen atoms were kept rigid using SHAKE [33].

The analysis was mainly performed with the program `ptraj` [29]. The analysis of the conformational drift during the trajectories of the S15-RNA complex, and the monitoring of the S15, RNA and complex electrostatic energies (data not

shown) show that the S15-RNA trajectories are stabilized in the 11-15 ns interval. The comparison of the s15-rna-mg and s15-rna trajectories was then based on mean and standard deviation values calculated on this interval. On the other hand, the trajectories s15-pdb and s15-aria were included integrally into the analysis, as the purpose of their analysis was to compare the conformational drift and the conformational landscapes of the structures 1ab3 and 2fkx.

During the simulation of the complex, the protein residues are numbered from 1 to 86 (corresponding to the 101-186 residues of the 1dk1 file), the RNA residues are numbered from 87 to 143 (corresponding to the 1-57 residues of the 1dk1 file), and the ions Mg^{2+} are numbered from 144 up to 152 (corresponding to residues 200-206 and 208-209 of the 1dk1 file). The residue numbers given in the analysis are those used in the MD simulation, the corresponding numbers of the 16S RNA, if they exist, are underlined in parentheses.

The calculation of S15 conformers under NMR restraints was performed using the simulated annealing protocol available in the CNS-ARIA protocol [34], which was recently used for the recalculation of NMR structures in the RECOORD database [35]. The scripts were downloaded from <http://www.ebi.ac.uk/msd-srv/docs/NMR/recoord/scripts.html>. The non-bonded force field was PROLSQ and the covalent geometry was parall-hdg5.3.pro [36]. The conformers of the NMR structure and the conformations of S15 observed in the trajectories were thus obtained in presence of two different force fields, which alleviates the possible bias induced by the use of a unique force field.

The NMR restraints were: 965 NOE distance restraints and 38 dihedral restraints provided by H. Berglund and already applied in Ref. 5. These restraints were supplemented by adding in α helices 76 hydrogen bond restraints ($i, i + 4$) between amide hydrogens and carboxyl oxygens. The definition of the α helices was taken from the header of the 1ab3 file. The 25 best-energy conformers were selected from the 100 conformers calculated using the CNS-ARIA protocol. Among them, conformers displaying outliers orientations of the helices $\alpha 1$ and $\alpha 4$, were removed. The set of S15 conformers and the restraints used are deposited at the Protein Data Bank (id: 2fkx).

The NMR structures were analyzed using PROCHECK v.3.5.4 [37] and WHATIF 8.1 [38]. The root-mean square deviation (RMSD) values between the conformers coordinates were calculated on the backbone atoms. The nuclear Overhauser effect (NOE) distance restraints were examined, in the NMR structures and in the trajectories, by calculating, for each restraint, all distances r_i separating the hydrogen pairs involved in the NOE. The mean distance value R was then determined

as:

$$R^{-6} = \langle r_i^{-6} \rangle_i \quad (1)$$

the mean value $\langle \rangle_i$ being calculated on the hydrogen pairs, and on the trajectory snapshots or on the NMR conformers. The NOE restraint is violated for values of R larger than $L + 1$ Å, where L is the restraint upper-bound. Each dihedral restraint was evaluated by calculating the mean dihedral angles along the trajectory, and is violated if the mean value is located outside the restraint interval.

The variation of S15 tertiary structure was monitored by calculating the angles and the distances between the helix axes. The points belonging to each helix axis were determined as the middles of the backbone atom segments (N(i), N(i+2)), (C α (i), C α (i+2)) and (C'(i), C'(i+2)), where the residue numbers i vary along the secondary structure element. The angle between two axes was defined as the angle between the vectors linking the first and the last point of each axis. The distance between two helix axes was calculated as the minimum distance between the points defining the axes.

3 Results and discussion

3.1 Analysis of the S15 NMR structures

The comparison of 2fkx and 1ab3 conformers (Fig. 3) shows a large increase in 2fkx of the mean percentage of residues located in the core Ramachandran regions determined by PROCHECK (Table 2a). Similarly, the percentages of residues in the allowed and in the generously allowed regions decrease. Thus, the 2fkx conformers display better Ramachandran parameters than the 1ab3 conformers. The mean number of bad contacts observed by PROCHECK is also considerably reduced if the conformers are calculated with the CNS-ARIA scripts.

The WHATIF Z-scores (Table 2b) show a picture similar to the PROCHECK analysis. The mean Z-score values are improved, and all Z-scores are in the $-4/4$ range for the 2fkx conformers. The number of bumps detected by WHATIF is also much reduced in the 2fkx conformers. The improved quality of 2fkx comes from the optimization of the conformers in the torsion angle space [39, 40] as well as from the use of a final refinement step in water [41].

The number of violated nuclear Overhauser effect (NOE) restraints is divided by two in the 2fkx conformers with respect to the 1ab3 conformers (Table 2c): this decrease comes from a much smaller number of long-range violations, as well as

a smaller number of $(i, i + 1)$ violations (Table 3). Nevertheless, the number of violated dihedral restraints slightly increases in 2fkx (Table 2c).

The relative orientations of the α helices show different patterns in 1ab3 and 2fkx (Table 4). The mean values of the angles between $\alpha 1$ and $\alpha 2$ and between $\alpha 3$ and $\alpha 4$ are smaller in 2fkx than in 1ab3: the helices $\alpha 1$ and $\alpha 4$ are thus less parallel to the protein core formed by $\alpha 2$ and $\alpha 3$. The standard deviations of angles between the axes of α helices in 1ab3 are much smaller than those observed in 2fkx. A similar trend is observed for the distances between the helix axes and particularly between the axes of $\alpha 1$ and $\alpha 2$. Thus, the helix $\alpha 1$ and to a lesser extent, the helix $\alpha 4$, display a much wider range of orientations in 2fkx than in 1ab3. Because of the variability of the $\alpha 1$ and $\alpha 4$ orientations, larger RMSD values between the conformers of the complete protein are observed in the 2fkx than in the 1ab3 structure. But, backbone RMSD values calculated by superimposing only the helices $\alpha 2$ and $\alpha 3$ of the protein (residues 24-70), are smaller for 2fkx than for 1ab3 (Table 2c).

The large variability observed for the $\alpha 1$ and $\alpha 4$ orientations arises from the sparsity of the NOE restraints network. Indeed, among the 91 long-range NOE restraints, 8 are applied between $\alpha 1$ and $\alpha 2$, 6 between $\alpha 1$ and $\alpha 4$ and 4 between $\alpha 2$ and $\alpha 4$. In $\alpha 1$, only one residue, Phe-14, located at the C-terminal end of the helix, is involved in inter-helix interactions, whereas in $\alpha 4$, five residues, Arg-76, Tyr-77, Leu-80, Lys-83 and Leu-84 are involved in restraints with other helices. It is thus not surprising that the orientation of $\alpha 4$ with respect to the protein core is better defined than the orientation of $\alpha 1$. Furthermore, between $\alpha 2$ and $\alpha 3$, which have the best-defined relative orientation, 21 restraints exist, and they involve 5 residues in each helix.

The mean orientations of the α helices were also compared (Table 4), in the NMR (1ab3 and 2fkx) and in the X-ray (1a32 and 1dk1) structures of S15, as well as in the S15 structures present in the 3.5 Å resolution X-ray structures (2avy and 2aw7) of the ribosome 70s of *E. Coli* [42]. The $\alpha 1, \alpha 2$ and $\alpha 2, \alpha 3$ angles of the 2fkx conformers are between the values observed in the structures 1a32 and 1ab3. The angles and the distances between the α helices in 1dk1, 2avy and 2aw7 are close to those observed in 1ab3.

The backbone angles ϕ and ψ of the residues 15-23, which form the loop connecting the helices $\alpha 1$ and $\alpha 2$, exhibit quite different mean values in the NMR structures (1ab3, 2fkx), as well as in the X-ray structures (1a32, 1dk1, 2avy, 2aw7) (Figs. 4a and b). Various conformations of the loop are thus observed in the different S15 structures.

The 2fkx conformers display a better structure quality and a lighter internal

strain than those of the 1ab3 structure. Nevertheless, the convergence of the structure is worse: the orientations of the helices $\alpha 1$ and $\alpha 4$ with respect to the protein core are more variable in the 2fkx conformers. This feature is in agreement with the sparse network of long-range NOE restraints and with the X-ray structure of S15 (PDB id: 1a32) where the first helix is extended far apart from the other protein helices [6].

3.2 Molecular dynamics simulations of the isolated protein S15

A name for each MD trajectory is given in Table 1, along with the main simulation parameters. In the two MD simulations recorded on the protein S15, the variation of the coordinate RMSD with respect to the starting point, shows different trends (Fig. 5a). RMSD values larger than 7.0 Å are observed for s15-pdb, whereas values of about 4.5 Å are observed in the case of s15-aria. The protein conformation in s15-aria thus displays a better stability than in s15-pdb, which is probably due to the lighter internal strain of the structure, observed in the previous section.

The large RMSD values observed in the trajectory s15-pdb mainly arise from a conformational drift observed for the loop connecting the helices $\alpha 2$ and $\alpha 3$ (Fig. 5b). RMSD values larger than 2.0 Å are also observed on the loop connecting $\alpha 1$ and $\alpha 2$, in the 2-4 ns interval (data not shown). The whole protein, except few residues (residues 3-15 ($\alpha 1$) and residues 35-40 ($\alpha 2$)) displays consequently larger atomic fluctuations in s15-pdb than in s15-aria (Fig. 5c). The differences in fluctuations are particularly large in the 15-25 and in the 42-52 regions, which correspond to the loops between helices $\alpha 1, \alpha 2$ and between helices $\alpha 2, \alpha 3$.

The angles between the helices $\alpha 1, \alpha 2$ (Fig. 5d) and $\alpha 3, \alpha 4$ (Fig. 5f) vary in the same range (90-140°) during both trajectories. This variability of the orientations of $\alpha 1$ and $\alpha 4$ with respect to the protein core is in agreement with the observations made above on 2fkx. On the other hand, the angle between $\alpha 2$ and $\alpha 3$ (Fig. 5e) undergoes a transition in the non-stabilized part of the trajectory s15-pdb and is stable in the trajectory s15-aria.

The secondary structure elements have been monitored along the trajectories (data not shown) by calculating the mean and the standard deviation values of the backbone NH...O hydrogen bond distances in the α helices. In both trajectories, the helix $\alpha 1$ (residues 3-15) is not well-defined, as well as the C-terminal part of $\alpha 2$ and the N-terminal part of $\alpha 3$. The C-terminal regions of $\alpha 3$ and $\alpha 4$ are less stable in s15-pdb than in s15-aria.

The numbers of violations for the $(i, i+1)$, $(i, i+2)$, $(i, i+3)$ NOE restraints are similar (Table 3) for both trajectories. On the other hand, s15-aria displays 7

more violations for the intra-residue (i, i) NOEs, whereas 10 more violations are observed in s15-pdb for the long-range NOEs. The visualization of the violated NOEs on a contact map (Fig. 6) demonstrates that very long-range NOE restraints (i, j) such that $|i - j| > 30$ are observed in s15-pdb and not in s15-aria. On the other hand, most of the (i, j) NOE violations observed in s15-aria are such that $|i - j| < 10$. This distribution of the violations is in agreement with the larger conformational drift observed in s15-pdb than in s15-aria.

The observations quoted above show that the trajectory starting from a 2fmx conformer is more stable than the one recorded from a 1ab3 conformer. This is probably due to the calculation of the 2fmx structure in the torsion angle space as well as to the use of a final refinement step in water, as it was shown [35, 39, 41] that these two ingredients improve the quality of the structure, and thus the stability in MD simulation. On the other hand, the better convergence of 1ab3, which is the sign of a larger internal strain of the structure, is correlated to a larger conformational drift in the s15-pdb simulation [43].

The variable orientations observed for the helices in S15 may arise from the bundle helix structure of the protein S15. Indeed, in p8^{MTPC1} [44], a protein consisting of three α helices, and having a similar tertiary structure, the third helix displays internal motion and large displacements in MD simulations [45], and this behavior is in agreement with dipolar coupling measurements [46].

3.3 Internal mobility in the S15-RNA complex

The mobility of the partners of S15-RNA interaction was analyzed during the trajectories s15-rna-mg and s15-rna, by calculating separately on S15 and on RNA the atomic fluctuations by residues from the 11-15 ns interval of the trajectories. In the protein, a slight increase of fluctuations is observed in absence of Mg^{2+} (Fig. 7a) for the residues 37-50, in the helices $\alpha 2$ and $\alpha 3$ and in the loop connecting them.

In absence of Mg^{2+} , an increase of flexibility of RNA is observed in four regions (Fig. 7b): the residues 92-100 (588-650) in the TL1 tetraloop, the residues 102-104 (652-654) and 133-134 (748-749) near the three-way junction, the residue 115 (665) close to the G-U/G-C motif, the residues 121-123 in the TL2 tetraloop. Among these residues, A103 (653), A115 (665) and C133 (748) are not involved in canonical base-pairs in the structure 1dk1. The increase of RNA fluctuations is in agreement with the positions of the ions Mg^{2+} in the crystal, close to the G-U/G-C motif and to the three-way junction motif. The increase of flexibility observed in the protein helices $\alpha 2$ and $\alpha 3$, in the loop $\alpha 2$ $\alpha 3$ (Fig. 7a) and in the

TL2 tetraloop (Fig. 7b), are consistent, because these regions interact.

3.4 Conformational drift of S15

In presence of ions Mg^{2+} , the RMSD of S15 atomic coordinates from the initial conformation, converges to a plateau of 3 Å after 11 ns (Fig. 7c). The trajectory recorded in absence of ions Magnesium reaches the same value after 3 ns (Fig. 7c). The variation of the coordinate RMSD of the protein S15 in presence of Mg^{2+} displays a jump in the 7.8-11.5 ns range (Fig. 7a) before attaining the plateau value. The conformations corresponding to the minimum RMSD values in this interval (7881 ps: 2.31 Å, 9895 ps: 2.41 Å) were compared (Fig. 8a) with the conformations corresponding to the maximum RMSD values (8837 ps: 3.11 Å, 11347 ps: 3.29 Å). The conformation of the loop $\alpha1,\alpha2$ changes and the residues 15-19 undergo the largest displacement (Fig. 8b). The residues 3-7 move simultaneously (Fig. 8c), and the backbone hydrogen bond between Lys-4 and Gln-8 is disrupted. The simultaneous variation of conformation for the residues 3-7 and 15-19 is consistent with a mechanical link between the loop connecting the helices $\alpha1$ and $\alpha2$ and the helix $\alpha1$.

As the $\alpha1\alpha2$ loop is located at the intermolecular interface of the S15-RNA complexes in the crystal, the loop conformation is probably disturbed by crystal packing effects. The loop drifts during all MD trajectories, recorded on the S15-RNA complex and on S15, as similar mean ϕ and ψ values are calculated from the whole MD trajectories in the loop connecting $\alpha1$ and $\alpha2$ (Figs. 4c and d), whereas different values are observed in the different NMR and Xray structures (Figs. 4a and b). Transitions of the ϕ and ψ values are thus observed (Fig. 9) in residues located in the $\alpha1\alpha2$ loop, in presence or in absence of ions Mg^{2+} .

The angles between the S15 helices and the distances separating them vary less than 10° and than 1 Å respectively (data not shown) during the trajectories s15-rna-mg and s15-rna: the presence of RNA freezes the variability of the helices orientations, observed for the isolated protein. Nevertheless, the angle between $\alpha2$ and $\alpha3$ is increased by 6° if the ions Mg^{2+} are removed, and because the helices are anti-parallel, they are thus more apart in presence of ions Mg^{2+} . Also, when the ions Magnesium are removed, the distance between $\alpha1$ and $\alpha2$ is reduced by 1 Å after 10 ns.

3.5 RNA Conformation

The variation of the coordinate RMSD of the RNA in presence of Mg^{2+} displays two jumps at around 4 and 6 ns (Fig. 7b). The conformations exhibiting the maximum RMSD values (3812 ps: 3.82 Å, 6212 ps: 4.22 Å) were compared to conformations exhibiting the minimum RMSD values (3261 ps: 2.20 Å, 4782 ps: 2.01 Å, 6347 ps: 2.28 Å). The 3' and 5' extremities spanning the 87-90 (584-586) and 140-143 (755-757) residues exhibits different conformations.

The inter-phosphate distances between phosphate groups facing each other in the RNA backbone, were calculated on the 11-15 ns interval of the trajectories *s15-rna-mg* and *s15-rna*. All distance differences are smaller than 1.5 Å, except the four following ones. The pairs of residues A113(663)-G127(742), G114(664)-G126(741), G116(666)-U125(740) and U119-G122 show variations of distance of 2.0 Å, 2.1 Å, 2.0 Å and 4.6 Å. These residues are located in the G-U/G-C region and in the tetraloop TL2. The phosphate groups thus move apart in absence of Mg^{2+} ions, which is in agreement with the increase of internal flexibility in this RNA region (Fig. 7b).

The values of the RNA phosphodiester angles were compared in presence and in absence of Mg^{2+} ions, by calculating their mean and standard deviation values in the 11-15 ns interval (Fig.10). Few angles display a difference of mean values significant with respect to the standard deviation values. These differences concern, for the angle α , 11 residues, for the angle β 1 residue, for the angle γ , 7 residues, for the angle δ , 6 residues, for the angle ϵ , 6 residues and for the angle ζ , 4 residues. These residues cluster in the UUCG TL2 tetraloop, in the three-way junction, and in the helix H22, close to the positions of the ions Mg^{2+} . These phosphodiester angle variations are thus in agreement with the stabilization of the RNA structure by the ions Mg^{2+} [2]. But, the hydrogen bond parameters (data not shown) involved in base triple between G104(654), G137(752) and C139(754) are not modified between *s15-rna* and *s15-rna-mg*.

The comparison of the mean angle values from the trajectories with the corresponding angles measured in 1dk1 shows (Fig.10) that, for the majority of the residues, the trajectories angles are in a similar angular region than the values observed in the structure. The X-ray angles were compared to the interval of variation of the trajectory angles, and the residues for which the X-ray angle is more than 90° outside of the interval are the following ones: G92 (588), U102 (652), G107 (657), A113(663), G114(664), A115 (665), U120, G122, G123, U125(740), G127(742), C133(748), C134(749), G135 (750), A138 (753) and C143.

The angle values calculated on the trajectories and measured on the 1dk1 struc-

ture were also compared to the rotameric classification introduced recently [47] to describe the RNA backbone. The mean absolute difference value between the RNA angles and the rotameric angles was calculated over the 53 sugar-to-sugar angles suites present in the RNA. For each pair of angle values p and q , the difference was the smallest absolute value of the three numbers: $p - q$, $p - q + 360^\circ$ and $p - q - 360^\circ$. The RX structure of S15-RNA display 18 suites with means difference larger than 20° and the maximum mean difference observed is 79.9° . On the other hand, the *s15-rna-mg* and *s15-rna* trajectories have 10 and 13 such suites and their maximum mean differences are 46.4° and 73.8° . The conformations explored during the 11-15 ns trajectory intervals thus seem to be in reasonable agreement with the analysis of rotamers performed on 132 RNA crystallographic structures [47].

The simulation of UUCG loops was used as a test case in molecular dynamics simulations [48, 49], as the first NMR structure of the UUCG loop [50] was incorrect, and was corrected [51] by discovering a misassignment. The use of combined locally enhanced sampling and Particle Mesh Ewald was shown [49] to allow the simulation to find the correct structure starting from the incorrect one. On the other hand, several simulations [48] of the two UUCG structures concluded to the formation of the hydrogen bond U1(O2')-U2(O5') and not U1(O2')-G4(O6).

The conformation of the two UUCG tetraloops was compared (Fig. 11) to experimental structures of UUCG (1dk1, 1f7y, 1hlx, 1i6u, 1kuq). The structure 1hlx is the NMR structure of the P1 helix from group I self-splicing introns [51], in which only one UUCG loop is present. The others are the S15-RNA [3, 4] and S18-RNA [52] X-ray structures, in which a three-way-junction RNA is present, with both tetraloops TL1 and TL2. The values of the phosphodiester backbone angles are similar in structures and in MD trajectories. The angles displaying the largest variations between *s15-rna-mg* and *s15-rna* vary also among the experimental structures.

A set of hydrogen bonds already analyzed in a molecular dynamics simulation of UUCG [48], was compared in the MD trajectories and in X-ray and NMR UUCG structures (Table 5). The comparison between TL1 and TL2 inside the MD trajectories shows that, in *s15-rna-mg*, over 9 distances, 6 show a difference larger than 1 \AA , whereas, in *s15-rna*, only 2 distances show a difference larger than 1 \AA . This reduction in the number of differences is in agreement with the homogenization of the environment of the UUCG loops obtained by removing the ions Mg^{2+} . The loop TL1 shows the same pattern of formed hydrogen bonds in both MD trajectories, but the percentage of hydrogen bond formation generally decreases in absence of Mg^{2+} .

The comparison between the MD trajectories and the structures, gives the following results. The pattern of formed hydrogen bonds in TL1 is qualitatively similar to those obtained for TL1 in X-ray and NMR structures. In TL2, the distances vary much between the X-ray structures, and consequently, the formation of hydrogen bond observed in the MD trajectories is always in agreement with a least one X-ray structure. The hydrogen bond between U1(O2') and G4(O6) is partially formed in TL1, but not in TL2 for s15-rna-mg. In all X-ray structures, this hydrogen bond is formed in TL1, but in the NMR structure, it is partially formed.

The hydrogen bond between U1(O2') and U2(O5') is stronger than the one between U1(O2') and G4(O6) for TL1 and TL2 in both S15-RNA trajectories. This observation is in agreement with [48], but contradicts the observations made on the X-ray structures, as it was already pointed out by [4]. Nevertheless, the analysis of the NMR structure 1hlx shows that the hydrogen bonds between U1(O2') and G4(O6) and U1(O2') and U2(O5') are both partially formed in the NMR structure, with formation percentages of the same order of value. In all X-ray structures, the UUCG loop is included in a larger RNA sequence. On the other hand, the only structure of an isolated UUCG loop is the NMR structure 1hlx. The non-availability of a crystallized structure for this molecule may be the sign of a large internal flexibility, which is also observed in MD trajectories.

The previous analysis of the hydrogen bonds inside loops UUCG showed that the removal of ions Mg^{2+} induce a reorganization of the hydrogen bond network, but according to the limitation of the modeling of ions electrostatics, it is not possible to state that this behavior is specific of ions Mg^{2+} . One should also notice that: (i) the ions Mg^{2+} observed in the S15-RNA crystal may have not be all present, (ii) in the NMR sample of the P1 helix from group I self-splicing introns, no ions Mg^{2+} were present, but other ions may be present (G Varani, personal communication). The comparison between the MD trajectories, the X-ray and the NMR structures reveals that floppier hydrogen bonds are generally observed in the NMR structure, which agrees with the observations made during the MD trajectories. Furthermore, the large heterogeneity of distances in TL2, agrees with a the larger number of disrupted hydrogen bonds in TL2 during the MD trajectories. The overall analysis performed on the phosphodiester backbone angles and on the hydrogen bonds in TL1 and TL2 shows that the overall topology of the simulated TLs did not change much with respect to the crystallographic structures.

The relative position of the three RNA helices forming the three-way-junction, was monitored (Fig. 12) by calculating the two angles formed by the atom triplets

(107-C6, 114-N2, 142-O2) and (107-C6, 114-N2, 98-N1). The first angle describes the relative orientation of the helices H22 and H20, and the second angle describes the relative orientation of the helices H22 and H21. Both angles are slightly larger in absence of ions Mg^{2+} , which means that the helices have a tendency to go apart, in agreement with the observations of the Ref. 2.

3.6 Positions of the counter-ions and of the water bridges

The positions of the nine crystallographic ions Mg^{2+} (Fig. 13) were analyzed along the full trajectory *s15-rna-mg* by determining, for each snapshot, the atoms forming the coordination cage of the ions Mg^{2+} . The six oxygens closest to each Mg^{2+} were determined for each snapshot. The Table 6 describes the coordination partners of each ion Mg^{2+} , along with their residence times.

The partners of ions Magnesium do not vary during the simulation. The only exception is the ion Mg-151: it interacts in the initial conformation with the atoms O1P and O2P of G107 (657), but the hydrogen bond with O2P is broken after 60 ps. This stability of the partners of ions Mg^{2+} during the simulation shows that the simple model used to model the Mg^{2+} electrostatics can nevertheless give a description of the system in agreement with the X-ray crystallographic measurements.

The ion 144 is coordinated by five water oxygens with mean values of coordination distances around 2.0 Å. The sixth coordination partner of Mg-144 is always at a distance of around 3.7 Å, and is quite variable. The two most frequent coordination partners are O6-99 (7.5 ns) and O2P-99 (1.1 ns). The ions Mg-145 and Mg-151 are hexa-coordinated with five oxygens of water molecules and a phosphate oxygen (for Mg-145: O2P-G112 (662), for Mg-151: O1P-G107 (657)). The ion Mg-146 is hexa-coordinated with four oxygens of water molecules, and two atoms O6 of bases G107 (657) and G135 (750). The ions Mg-147, Mg-148, Mg-149 and Mg-150 are hexa-coordinated with oxygens of water molecules. The ion Mg-152 is hexa-coordinated with five water oxygens and the ribose oxygen O2' in residue A138 (753). The direct interactions of the Mg^{2+} ions are thus established mainly with water, phosphate or O6 oxygens, which corresponds to the observations previously made in MD simulations [18, 53]. The distances between the atoms involved in the coordination cage of the magnesium ions have a mean standard deviation of 0.1 Å: the geometry of the coordination complex does not vary and the oxygens form a rigid cage around the ion Mg^{2+} , as it was observed in the Ref. 19. The few direct interactions of the Mg^{2+} ions with RNA residues involve G135 (750), A138 (753) and G107 (657), which are located in the vicinity

of the hinge between the helices H20, H21 and H22.

The water molecules forming the coordination cage of the ions Mg^{2+} were then analyzed to detect their hydrogen bond partners. The residues Gln-61 and Asp-73 of S15 and the residues G91 (587), G92 (588), C93 (589), C94 (590), U95, G99 (649), A103 (653), G108 (658), G116 (666), G117 (667), C118, U119, G122, U125 (740), G127 (742), U128 (743), C133 (748), G137 (752) of RNA are bound to a Magnesium cluster more than 1ns. These RNA residues are located in the H22 helix on the side non-interacting with the protein and close to the G-U/G-C motif. The Magnesium clusters are mainly bound to phosphate groups, but also to atoms O6 of G99 (649) G116 (666), G117 (667), G127 (742), to atom O4 of U125 (740) and U128 (743), and to atoms O2 and N3 of C133 (748). As the weight of a Magnesium cluster coordinated to six water molecules is 120 g/mol, it counterbalances the weight of a phosphate group (PO_4 : 95 g/mol), and thus reduces the flexibility of RNA phosphodiester backbone, while screening the phosphate charges. The ions Mg^{2+} are also bound to the sidechain carboxyl groups of Gln-61 and Asp-73. Over the 48 water molecules bound to Mg^{2+} , 23 are bound to less than five RNA or protein groups. The ions Mg-147, Mg-148 and Mg-149, which are coordinated only to water molecules, visit the largest amount of groups.

The closest neighbors of each ion Na^+ were determined for the 11-15 ns interval in s15-rna-mg and s15-rna. As the coordination partners of the ions Na^+ do exchange much faster than for the ions Mg^{2+} , only coordination partners present for more than 0.5 ns are displayed in a Table 7. In s15-rna-mg, only 26 such coordination partners are detected for 10 ions Na^+ over the 31 ions Na^+ present in the simulation. From these partners, 19 are water oxygens, 4 are base oxygens and 3 are phosphate oxygens. In s15-rna, 200 coordination partners are observed for 20 ions Na^+ over the 49 ions Na^+ present in the simulation. The coordination partners are thus more tightly bound to ions Na^+ in absence of ions Mg^{2+} .

The water bridges between solute chemical groups, were analyzed in the 11-15 ns intervals of s15-rna-mg and s15-rna, by detecting the water molecules in which the oxygen is an hydrogen bond donor, and at least one hydrogen is an hydrogen bond acceptor. The selected hydrogen bonds correspond to distances smaller than 2.0 Å. For each water molecule, the total lifetime of its involvement in bridges was calculated. In s15-rna-mg, 75 water molecules are involved in bridges more than 100 ps, 4 of them form bridges for more than 1 ns, with lifetimes in the 1.0-2.7 ns range. In s15-rna, 110 water molecules form bridges for more than 100 ps, 9 bridges have lifetimes larger than 1 ns in the 1.0-4.0 ns range. The more rigid water bridges observed in s15-rna may be a compensating effect for the increased

flexibility induced by the magnesium ions removal.

On the other side, the formation time of each observed bridge, as well as the number of water molecules involved in the bridge, are displayed (Table 1: SI) for the formation times larger than 100 ps. Thirty three such bridges are observed in s15-rna-mg, whereas 39 are observed in s15-rna. The removal of ions Mg^{2+} increase much the number of bridges observed inside S15 in the $\alpha1/\alpha2$ loop. The number of bridges observed inside S15 in the $\alpha2/\alpha3$ loop decreases, and the bridges observed between His-41, Asp-48 and Ser-51, disappear. The number of bridges between S15 and RNA increase from 9 to 13 when the ions Mg^{2+} are removed. In particular, bridges involving triplets of residues (Gln-61,G140(755),C-141(756)), (Asp-48,G-116(666),U-125(740)) and (Arg-37,His-41,G-126(741)) appear between loop $\alpha2/\alpha3$, helix $\alpha3$ and RNA. Four water bridges between S15 and RNA and close to the three way junction: (Thr-21,C-134(749)), (Thr-24,U-136(751)), (Tyr-68,A-138(753)) appear in absence of ions Mg^{2+} . In s15-rna-mg, inside RNA, 2 bridges are observed in TL1, and 5 in the TL2 region, whereas in s15-rna, 5 bridges are observed in TL1.

3.7 S15-RNA interaction interface

The S15-RNA interaction was analyzed by monitoring on the 11-15 ns interval, distances chosen in the following way. A set of hydrogen bonds between protein and RNA was first selected by running the program HBPLUS [54] on the initial and final conformations of trajectories s15-rna-mg and s15-rna. Another set of distances was the set of distances quoted in Table 2 in Ref. 55. Finally, the distances between solute donor and acceptor groups were calculated for each snapshot of s15-rna-mg and s15-rna, and the pairs corresponding to distances smaller than 2.0 Å for more than 100 ps in one trajectory gave the third set of distances analyzed. The total set of atom pairs was analyzed along the trajectories s15-rna-mg and s15-rna, by calculating the mean and standard deviation values for the distance hydrogen-acceptor, and the angle donor-hydrogen-acceptor. Only the distances displaying a mean value smaller than 3 Å in at least one trajectory, are shown in Tables 8 and 9.

Four groups of distances are detected. The first group includes distances connecting the helix $\alpha2$ and the G-U/G-C motif, and distances connecting RNA residues inside the tetraloop TL2. The second group contains distances connecting the helix $\alpha3$ and the three-way junction. The third group is a set of distances between the $\alpha1,\alpha2$ loop and the three-way junction. The fourth group is formed by intra-protein distances in the loop $\alpha1,\alpha2$. As the last group of distance is ana-

lyzed in the S15-RNA complex as well as in the isolated protein and in structures and MD trajectories, the results are displayed in Table 9.

In the group 1 (G-U/G-C motif and TL2), three hydrogen bonds between the residues Arg-34, His-41, Asp-48 of the protein, and G126(741), C124(739) and G117(667) of RNA, are disrupted when the ions Mg^{2+} are removed. These observations are in agreement with the increase of mobility of the complex partners, observed near the G-U/G-C motif. On the other hand, Arg-34 forms a transient hydrogen bond with the phosphate group of G107(657), which is located in the helix H22 toward the three-way junction. The variations of distances involving residues from the helix $\alpha 2$, are in agreement with the decrease of distances between $\alpha 1$ and $\alpha 2$ described at the end of the section 3.4. Indeed, if the helix $\alpha 2$ reduces its interaction with the G-U/G-C motif, located at one edge of the RNA structure, this helix has more possibility to come closer to $\alpha 1$. In the capping tetraloop TL2, the formation and disruption of hydrogen bonds between U119, U120, C121, G122 and G123, agree with the gap between the phosphate groups of TL2 described in section 3.3.

Concerning the interaction of the RNA three way junction with the helix $\alpha 3$ of S15 (group 2), Gln-61, Arg-64 and Arg-71 reinforce hydrogen bonds with G140(755), A138(753) and C139(754) in s15-rna. This observation is consistent with the observation made previously that the helix $\alpha 2$ is reducing its interaction with the G-U/G-C motif (group 1 of distances).

In the interaction between the $\alpha 1, \alpha 2$ loop and the RNA three way junction (group 3), the hydrogen bonds between the NH_3^+ sidechain group of Lys-7 and the phosphate groups of G108(658) and U109(659) are reinforced in s15-rna. Asp-20 establishes an hydrogen bond with to G135(750), as well as Gln-27 with to C106(656) and U136(751). On the other hand, the hydrogen bond between Thr-21 and G107(657), is broken in s15-rna. Inside the junction, the residues A138(753) and C139(754) form an hydrogen bond when the ions Mg^{2+} are removed: the formation of this hydrogen bond is facilitated by the absence of Mg^{2+} , as A138 is otherwise bound to a magnesium.

From the previous description, one sees that, in the vicinity of the three way junction, the intermolecular hydrogen bonds between S15 and RNA are mainly strengthened when the ions Mg^{2+} are removed, which is in agreement with the appearance of water bridges between S15 and RNA in that region (section 3.6). Another effect of the magnesium ions removal is the reorganization of the network of hydrogen bonds involving inside S15, residues located in the loop connecting helices $\alpha 1$ and $\alpha 2$ (Table 9a). Hydrogen bonds are disrupted between the residues Arg-16, Asp-20, Thr-21, Gly-22, Thr-24, Glu-25 and Gln-27 and are formed be-

tween residues Ser-23, Val-26 and Gln-27. These residues, except Arg-16 and Asp-20, are conserved [56] in S15 proteins from several organisms. This behavior agrees with the water bridges observed between the same group of residues in s15-rna. The N-terminal part of the loop connecting the helices $\alpha 1$ and $\alpha 2$ is less stable in absence of ions Mg^{2+} . The MD trajectories s15-rna (Table 9a) as well as s15-pdb and s15-aria (Table 9b) and the 1ab3 and 2fkx NMR structures of S15 (Table 9c) display the same pattern of formed and non-formed hydrogen bonds, and the isolated S15 proteins (s15-pdb, s15-aria, 1ab3, 2fkx) exhibit a variability of the orientation of $\alpha 1$ with respect to the protein core. A similar pattern of hydrogen bonds is observed in the X-ray structure 1a32 (Table 9d), where $\alpha 1$ protrudes away from the protein core, and in the S15 structures from 2avy and 2aw7 (Table 9d), where no high occupancy site for Mg^{2+} was observed in crystallographic difference density map $F_o - F - c$ (J. Cate, personal communication). On the other hand, the hydrogen bond pattern observed in s15-rna-mg is also observed in 1dk1, where the S15-RNA complex is in presence of Mg^{2+} .

4 Conclusion

The recalculation of the NMR structure of S15, as well as the MD trajectories recorded on the isolated protein, show that the N terminal helix $\alpha 1$ displays probably different orientations in solution with respect to the protein core. This observation is in agreement with the X-ray structure of the isolated S15 [6], where $\alpha 1$ protrudes away from the protein core, due to intermolecular interactions in the crystal. In the S15-RNA complex, it was proposed [2] that the protein stabilizes the RNA, which is otherwise unfolded. The observations made here on S15, indicate that the protein displays an internal mobility and probably undergoes a conformational drift as well as the RNA during the establishment of the interaction. Measurements of residual dipolar couplings [57] could be used to obtain more precise information of the relative orientations of the α helices inside the protein.

The Mg^{2+} ions form hexa-coordinated clusters in the MD trajectory of the S15-RNA complex. Most of these clusters, which interact mainly with the RNA phosphodiester backbone, behave as rigid bodies, which is not very surprising, but makes the work presented here consistent with previous observations from molecular dynamics simulations [19]. In the region of the G-U/G-C motif, the removal of ions Mg^{2+} induce an increased mobility of the RNA and of the protein in agreement with the crystal structure in which an ion Mg^{2+} (Mg-150) stabilizes

the UUCG tetraloop. On the other hand, in the vicinity of the three-way junction, the removal of the ions Magnesium induces the appearance of protein-RNA water bridges and induces a reinforcement of several hydrogen bonds between S15 and RNA. The absence of ions Mg^{2+} modifies the network of hydrogen bonds inside the protein loop connecting $\alpha 1$ and $\alpha 2$. This network then becomes similar to those observed in isolated S15 and in S15-RNA complexes observed in absence of or in low occupancy of ions Mg^{2+} . As the $\alpha 1$ $\alpha 2$ loop is the hinge permitting the mobility of $\alpha 1$ with respect to the protein core, the variation of hydrogens bond network in this loop may play a role in stabilizing the orientation of $\alpha 1$ into the S15-RNA complex. The importance of this hydrogen bond network in the S15-RNA complex could be experimentally investigated by mutagenesis. Such an approach was already used [58] to analyze the importance of residues located in the N-terminal part of the helix $\alpha 1$, and the helix was concluded to play no significant role in the specific binding of S15 to the rRNA. These results do not contradict the present work, as we focus here on residues located in the $\alpha 1\alpha 2$ loop.

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6 Tables

Simulation name	Solute	number of water molecules	number of atoms	Box size ($\text{\AA}, \text{\AA}, \text{\AA}$)	Duration (ns)	Ions
s15-pdb	S15 (1ab3)	6130	19905	73,66, 55	15	8 Cl^-
s15-aria	S15 (2fkx)	6405	20730	75,67, 53	15	8 Cl^-
s15-rna-mg	S15-RNA (1dk1)	11219	37018	79,67, 69	15	9 Mg^{2+} , 31 Na^+
s15-rna	S15-RNA (1dk1)	11018	36424	79,66, 69	15	49 Na^+

Table 1: Molecular dynamics simulations run on the S15 isolated molecule, and on the S15-RNA complex. The PDB ids of the structures providing the starting points, are given in parentheses after the solute name.

(a)	PROCHECK core (%)	PROCHECK allowed (%)	PROCHECK gener. allowed (%)	
1ab3	59.4 ± 2.9	32.6 ± 3.2	7.5 ± 1.9	
2fkx	82.8 ± 3.6	14.3 ± 2.9	1.5 ± 1.5	
	PROCHECK non-allowed (%)	PROCHECK Mean numbers of bad contacts		
1ab3	0.5 ± 0.6	18.1 ± 3.0		
2fkx	1.4 ± 1.1	0.1 ± 0.3		
(b)	WHATIF Z score 2nd generation packing quality	WHATIF Z score Ramachandran plot appearance	WHATIF Z score chi-1/chi-2 rotamer normality	
1ab3	-4.7 ± 0.3	-6.4 ± 0.4	-4.6 ± 0.3	
2fkx	-0.6 ± 0.7	-3.6 ± 0.7	-0.1 ± 0.6	
	WHATIF Z score Backbone conformation	Mean numbers of bumps		
1ab3	-7.6 ± 1.0	104.1		
2fkx	-3.4 ± 1.4	12.0		
(c)	Number of conformers	Backbone RMSD (Å) (residues 24-70)	NOE violations	dihed violations
1ab3	26	3.1	48	13
2fkx	18	2.1	26	18

Table 2: Structural quality of the NMR conformers of S15 from Ref. 5 (PDB id: 1ab3) and recalculated using the CNS-ARIA scripts (PDB id: 2fkx). The analysis was performed using PROCHECK v.3.5.4 [37] and WHATIF [38]. The violations are consistent violations, i.e. observed in more than 50 % of the conformers.

NOE restraints	1ab3	2fkx	s15-pdb	s15-aria
i,i	9	10	15	21
i,i+1	15	8	8	10
i,i+2	0	0	1	1
i,i+3	5	3	9	9
$ i-j > 3$	19	6	37	27
total	48	26	70	68

Table 3: Numbers of NOE restraint violations in the NMR structures (1ab3 and 2fkx) and in the MD trajectories (s15-pdb and s15-aria) recorded on the isolated protein. The violations are consistent violations, i.e. observed in more than 50 % of the conformers. The trajectory names are those given in Table 1.

	Angle $\alpha 1\alpha 2$ (degrees)	Angle $\alpha 2\alpha 3$ (degrees)	Angle $\alpha 3\alpha 4$ (degrees)
1ab3	140.9 ± 6.6	159.3 ± 6.0	147.8 ± 5.3
2fkx	104.8 ± 39.2	154.9 ± 14.5	139.7 ± 17.0
1a32	62.5	150.5	145.2
1dk1	140.0	153.0	149.1
2avy	140.8	150.4	145.1
2aw7	141.8	150.1	143.1
	Distance $\alpha 1\alpha 2$ (Å)	Distance $\alpha 2\alpha 3$ (Å)	Distance $\alpha 3\alpha 4$ (Å)
1ab3	9.2 ± 0.2	8.2 ± 0.4	7.3 ± 0.1
2fkx	8.5 ± 1.2	9.7 ± 0.8	7.1 ± 0.3
1a32	21.1	9.3	7.4
1dk1	8.6	9.3	7.6
2avy	8.2	9.0	7.3
2aw7	8.2	9.0	7.2

Table 4: Geometry of the S15 tertiary structure, in the NMR structures (1ab3 and 2fkx), in the X-ray structures (1a32 and 1dk1) and in the structures of S15 in two conformations of the ribosome of *E. Coli* (2avy and 2aw7).

Hydrogen bond	Distance (Å)	Angle (degrees)	Formation (%)	
s15-ma-mg				
TL1 U1(O2) G4(N2/H21)	2.4 ± 0.4	139.5 ± 12.8	45.9	
TL1 U2(O2P) C3(N4/H41)	3.8 ± 0.4	37.8 ± 6.7	0	
TL1 U1(O2) G4(N2/H22)	4.0 ± 0.4	25.6 ± 7.7	0	
TL1 U2(O2P) C3(N4/H42)	2.1 ± 0.4	155.3 ± 13.2	77.3	
TL1 U1(HO'2) G4(O6)	3.9 ± 0.9	57.3 ± 42.4	12.9	
TL1 U1(HO'2) U2(O5')	2.5 ± 0.72	40.1 ± 37.5	46.4	
TL1 U1(H3) G4(O6)	6.3 ± 0.4	74.1 ± 6.1	0	
TL1 U1(O2) G4(N1)	1.9 ± 0.2	153.3 ± 12.1	90.1	
TL1 U1(O4) G4(N1)	6.3 ± 0.3	147.4 ± 10.3	0	
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TL2 U1(O2) G4(N2/H21)	6.6 ± 0.7	72.4 ± 6.8	0	
TL2 U2(O2P) C3(N4/H41)	3.7 ± 0.2	37.8 ± 5.2	0	
TL2 U1(O2) G4(N2/H22)	7.1 ± 0.7	41.6 ± 7.3	0	
TL2 U2(O2P) C3(N4/H42)	2.0 ± 0.2	162.2 ± 8.7	88.5	
TL2 U1(HO'2) G4(O6)	6.1 ± 0.9	53.3 ± 41.7	0	
TL2 U1(HO'2) U2(O5')	3.1 ± 0.6	59.7 ± 28.9	1.2	
TL2 U1(H3) G4(O6)	6.3 ± 0.4	74.1 ± 6.1	0	
TL2 U1(O2) G4(N1)	4.9 ± 0.6	62.1 ± 7.1	0	
TL2 U1(O4) G4(N1)	8.0 ± 0.4	45.4 ± 5.8	0	see the
<hr/>				
s15-ma				
TL1 U1(O2) G4(N2/H21)	2.1 ± 0.3	148.9 ± 13.7	75.4	
TL1 U2(O2P) C3(N4/H41)	3.7 ± 0.4	38.2 ± 6.6	0	
TL1 U1(O2) G4(N2/H22)	3.8 ± 0.3	31.0 ± 9.3	0	
TL1 U2(O2P) C3(N4/H42)	2.0 ± 0.4	159.6 ± 11.1	86.8	
TL1 U1(HO'2) G4(O6)	4.5 ± 0.6	49.7 ± 19.1	2.0	
TL1 U1(HO'2) U2(O5')	2.4 ± 0.4	34.4 ± 17.2	26.9	
TL1 U1(H3) G4(O6)	6.6 ± 0.5	79.1 ± 6.5	0	
TL1 U1(O2) G4(N1)	2.2 ± 0.4	144.7 ± 12.8	62.5	
TL1 U1(O4) G4(N1)	6.6 ± 0.4	136.8 ± 11.3	0	
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TL2 U1(O2) G4(N2/H21)	2.6 ± 0.5	131.6 ± 11.8	23.7	
TL2 U2(O2P) C3(N4/H41)	4.0 ± 0.8	39.7 ± 6.9	0	
TL2 U1(O2) G4(N2/H22)	4.3 ± 0.4	20.0 ± 7.2	0	
TL2 U2(O2P) C3(N4/H42)	2.3 ± 0.8	162.4 ± 9.8	67.2	
TL2 U1(HO'2) G4(O6)	7.5 ± 0.5	31.5 ± 14.4	0	
TL2 U1(HO'2) U2(O5')	2.4 ± 0.3	30.8 ± 16.5	28.1	
TL2 U1(H3) G4(O6)	3.8 ± 0.3	132.7 ± 9.3	0	
TL2 U1(O2) G4(N1)	1.9 ± 0.1	158.6 ± 10.6	96.8	
TL2 U1(O4) G4(N1)	5.8 ± 0.3	149.5 ± 8.5	0	

caption of Table 5 on the next page

Hydrogen bond	1dk1 (Å)	1f7y (Å)	1i6u (Å)	1kuq (Å)
TL1 U1(O2) G4(N2)	3.3	3.3	3.7	2.8
TL1 U2(O2P) C3(N4)	2.9	3.0	2.9	3.4
TL1 U1(O2') G4(O6)	2.5	2.4	2.8	2.5
TL1 U1(O2') U2(O5')	3.7	3.7	3.6	4.4
TL1 U1(N3) G4(O6)	5.9	5.9	5.8	6.3
TL1 U1(O2) G4(N1)	2.8	2.9	2.9	2.8
TL1 U1(O4) G4(N1)	7.0	7.0	6.9	7.1
TL2 U1(O2) G4(N2)	4.8	3.2	3.5	7.5
TL2 U2(O2P) C3(N4)	2.6	2.7	2.9	7.6
TL2 U1(O2') G4(O6)	4.6	3.0	2.6	3.7
TL2 U1(O2') U2(O5')	3.8	3.9	5.0	4.0
TL2 U1(N3) G4(O6)	3.2	6.5	5.9	5.5
TL2 U1(O2) G4(N1)	3.0	3.2	3.0	5.3
TL2 U1(O4) G4(N1)	7.0	7.3	7.1	9.7
1hlx				
Hydrogen bond	Distance (Å)	Angle (degrees)	Formation (%)	
TL1 U1(O2) G4(N2/H21)	3.9 ± 0.	27.6 ± 3.2	0	
TL1 U2(O2P) C3(N4/H41)	3.8 ± 1.1	131.5 ± 5.5	0	
TL1 U1(O2) G4(N2/H22)	2.1 ± 0.2	145.5 ± 5.8	65	
TL1 U2(O2P)C3(N4/H42)	5.2 ± 1.1	40.5 ± 2.9	0	
TL1 U1(HO'2) G4(O6)	2.7 ± 0.3	72.5 ± 18.9	15	
TL1 U1(HO'2) U2(O5')	3.0 ± 0.4	84.0 ± 26.8	10	
TL1 U1(N3) G4(O6)	6.6 ± 0.2	61.3 ± 2.1	0	
TL1 U1(O2) G4(N1)	2.0 ± 0.1	153.7 ± 6.8	90	
TL1 U1(O4) G4(N1)	6.5 ± 0.1	158.8 ± 7.0	0	

Table 5: Parameters of hydrogen bonds in UUCG loops calculated on the 11-15 ns time interval of the trajectories s15-rna-mg and s15-rna, and on PDB structures (1dk1: [3], 1f7y: [4], 1hlx: [51], 1i6u: [52], 1kuq) . The hydrogen bond mean distance is given as well as the donor-hydrogen-acceptor mean angle, when available. The formation is the percentage of trajectory time or of NMR conformers in which the mean distance is smaller than 2.2 Å.

Mg ²⁺ ion	Coordination number	Coordination partners
144	5	OW-8570(15.0) OW-8572(15.0) OW-8577 (15.0) OW-8584 (15.0) OW-8596 (15.0)
145	6	O2P-112(13.3) OW-4913(15) OW-5012 (15.0) OW-5076 (15.0) OW-5151 (15.0) OW-5551 (15.0)
146	6	O6-107(13.3) O6-135(13.3) OW-8017 (15.0) OW-8035 (15.0) OW-7978 (15.0) OW-8032 (15.0)
147	6	OW-6061(14.9) OW-6063(14.9) OW-6064 (14.9) OW-6171 (14.9) OW-6174 (14.9) OW-8595 (14.9)
148	5	OW-5524(14.4) OW-5568(14.5) OW-5986 (14.6) OW-5992 (14.4) OW-6020 (14.5)
149	6	OW-3038(14.9) OW-3048(14.8) OW-5541 (14.9) OW-5545 (14.8) OW-5564 (14.8) OW-5565 (14.8)
150	6	OW-2368(15.0) OW-3033(15.0) OW-3036 (15.0) OW-3039 (15.0) OW-3045 (15.0) OW-3046 (15.0)
151	6	O1P-107(13.3) OW-5532 (15.0) OW-5543 (15.0) OW-5585 (15.0) OW-5586 (15.0) OW-3101 (14.9)
152	6	O2'-138(13.3) OW-8578 (15.0) OW-8583 (15.0) OW-8585 (15.0) OW-8602 (15.0) OW-9046 (15.0)

Table 6: Coordination numbers of the ions Mg²⁺, partner names and residence times (ns) in parentheses.

Na ⁺ ion	Coordination partners
<hr/>	
s15-rna-mg	
153	06-137 (1.4), OW-2191 (0.6), OW-8836 (0.6), OW-9739 (1.5), OW-11692 (0.6)
156	OW-3089 (0.7), OW-5937 (0.8), OW-9739 (1.5), OW-10949 (0.5) OW-11171 (0.9)
157	O2P-104 (0.5), O2P-105 (0.8), OW-3924 (0.7), OW-4219 (0.5) OW-9507 (0.7)
162	O1P-103 (0.8)
163	O4-95 (0.7)
165	O6-137 (1.4), OW-3165 (0.7), OW-3883 (0.7), OW-11171 (0.9)
171	O6-122 (0.8), OW-8870 (0.5)
175	OW-3883 (0.7)
178	OW-10949 (0.5)
179	OW-8621 (0.5)
<hr/>	
s15-rna	
153	OW-1046 (1.0), OW-1579 (0.5), OW-2042 (1.2), OW-2958 (1.5), OW-3304 (1.0), OW-5978 (0.9), OW-6046 (0.6), OW-8032 (1.2), OW-8925 (0.6), OW-9450 (0.8), OW-10607 (0.5), OW-10823 (0.7), OW-11171 (0.5)
155	OW-884 (0.5), OW-889 (0.7), OW-910 (0.5), OW-1186 (1.2), OW-2958 (1.5), OW-3060 (0.6), OW-4398 (0.8), OW-4419 (0.6), OW-4476 (0.8), OW-5978 (0.9), OW-7116 (1.3), OW-7246 (0.5), OW-7992 (0.6), OW-8344 (0.8), OW-8925 (0.6), OW-9195 (0.6), OW-9427 (0.8), OW-10462 (0.5), OW-10607 (0.5), OW-11171 (0.5), OW-11300 (1.5)
156	OW-1815 (0.8), OW-1864 (0.7), OW-2000 (1.0), OW-2592 (0.7), OW-2648 (0.6), OW-4526 (0.6), OW-4962 (0.7), OW-5556 (0.8), OW-6984 (0.6), OW-7803 (1.2), OW-8028 (0.6), OW-8032 (1.2), OW-8163 (0.7), OW-9510 (0.7), OW-11031 (0.8), OW-11118 (0.7), OW-11267 (0.6), OW-11374 (0.6)
157	OW-2000 (1.0), OW-2918 (0.5), OW-3215 (0.9), OW-4715 (0.9) OW-5128 (0.5), OW-7803 (1.2), OW-10025 (0.7)
159	OW-796 (1.2), OW-1652 (0.6), OW-2452 (0.7), OW-2786 (0.8) OW-5515 (0.8), OW-7327 (1.2), OW-7877 (0.7), OW-11323 (0.6)
163	OW-1360 (0.8), OW-7862 (1.0), OW-8032 (1.2), OW-8791 (0.6) OW-8913 (1.8), OW-8959 (0.7), OW-9450 (0.8), OW-9479 (0.7)
166	OW-3234 (0.7), OW-10314 (0.7)
167	O6-88 (0.7), O6-89 (0.8), OW-1309 (0.6), OW-1369 (0.5), OW-2323 (1.2), OW-5412 (0.6), OW-5589 (1.0), OW-5684 (0.5), OW-6101 (0.8), OW-7821 (0.6), OW-8468 (0.6), OW-8573 (1.9), OW-10307 (0.5), OW-11336 (1.0)

see the caption of Table 7 on the next page

Na ⁺ ion	Coordination partners
s15-rna	
169	OW-3119 (0.5), OW-8912 (2.5)
170	O1P-106 (0.5), OW-4913 (0.7), OW-5267 (0.7), OW-5705 (0.9), OW-5786 (3.3), OW-5791 (1.3), OW-7545 (1.1), OW-8785 (0.9), OW-8791 (0.6), OW-8959 (0.7), OW-9365 (1.1), OW-9646 (0.8), OW-9749 (0.7), OW-11340 (0.5)
171	OW-2323 (1.2), OW-8468 (0.6)
173	OW-1186 (1.2), OW-1360 (0.8), OW-2042 (1.2), OW-2373 (0.7), OW-3234 (0.7), OW-3667 (1.0), OW-3851 (0.6), OW-4398 (0.8), OW-4476 (0.8), OW-5211 (0.6), OW-5276 (0.7), OW-5978 (0.9), OW-5997 (1.1), OW-7057 (0.5), OW-7125 (0.7), OW-7297 (0.5), OW-7862 (1.0), OW-8032 (1.2), OW-8163 (0.7), OW-8344 (0.8), OW-8913 (1.8), OW-9217 (0.7), OW-9421 (0.5), OW-9479 (0.7), OW-9646 (0.8), OW-9649 (0.5), OW-10530 (1.1), OW-11300 (1.5)
175	OW-5997 (1.1)
176	OW-3215 (0.9)
177	OW-1046 (1.0), OW-1186 (1.2), OW-1865 (1.0), OW-4011 (0.5), OW-8344 (0.8), OW-8913 (1.8), OW-9541 (1.0), OW-10607 (0.5)
179	OW-7803 (1.2), OW-9559 (1.0), OW-11064 (0.7), OW-11238 (0.6)
180	OW-1124 (0.7), OW-1624 (0.8), OW-2452 (0.7), OW-3119 (0.5), OW-4108 (1.5), OW-4598 (2.0), OW-6288 (0.6), OW-6858 (0.7), OW-7877 (0.7), OW-8323 (0.6), OW-9291 (0.8), OW-9687 (0.5), OW-10405 (0.5), OW-11323 (0.6)
181	OW-2373 (0.7), OW-7297 (0.5)
182	OW-947 (0.6), OW-1186 (1.2), OW-1360 (0.8), OW-1855 (0.9), OW-2544 (0.7), OW-2592 (0.7), OW-2648 (0.6), OW-3215 (1.0), OW-3234 (0.7), OW-3667 (1.0), OW-3851 (0.6), OW-4398 (0.8), OW-5978 (0.9), OW-6046 (0.6), OW-6998 (0.6), OW-7444 (0.7), OW-8028 (0.6), OW-8032 (1.2), OW-8147 (1.2), OW-8785 (0.9), OW-8913 (1.8), OW-9217 (0.7), OW-9510 (0.7), OW-9559 (1.0), OW-5997 (1.1), OW-9649 (0.5), OW-9842 (0.5), OW-10768 (0.6), OW-11267 (0.6), OW-11300 (1.5), OW-11374 (0.6)
183	OW-6984 (0.6), OW-7821 (0.6)

Table 7: Coordination partners of the ions Na⁺ detected more than 500 ps during the 11-15 ns interval of s15-mg-rna and s15-rna. The detection times (ns) are in parentheses.

Atom pair				s15-rna-mg		
atom1	res1	atom2	res2	Distance (Å)	Angle (deg)	Formation (%)
group 1						
HH12	Arg-34	O1P	G107(<u>657</u>)	6.0 ± 0.5	108.4 ± 9.7	0.0
HH22	Arg-34	O1P	G107(<u>657</u>)	7.9 ± 0.5	107.8 ± 7.5	0.0
HH21	Arg-34	O1P	G126(<u>741</u>)	1.9 ± 0.1	156.5 ± 10.2	96.6
Hδ1	His-41	O2'	C124(<u>739</u>)	2.0 ± 0.2	153.3 ± 12.6	85.3
Oδ2	Asp-48	H22	G117(<u>667</u>)	1.8 ± 0.1	166.7 ± 7.2	99.2
HO	U119	O2	U120	3.6 ± 1.9	119.3 ± 31.8	39.7
O2	U119	HO	U120	2.6 ± 1.2	127.1 ± 54.4	67.5
O2P	U119	H41	C121	2.3 ± 0.5	148.3 ± 16.7	59.0
O4	U120	H22	G123	10.3 ± 1.0	144.1 ± 13.0	0.0
HO	G122	O1P	G123	2.8 ± 1.1	144.2 ± 26.3	51.9
group 2						
He21	Gln-61	O1P	G140(<u>755</u>)	7.6 ± 0.7	39.7 ± 8.5	0.0
HH11	Arg-64	O2P	G140(<u>755</u>)	3.6 ± 1.5	109.9 ± 49.8	36.4
HH	Tyr-68	O1P	A138(<u>753</u>)	1.9 ± 0.4	156.8 ± 15.5	92.5
HH22	Arg-71	O3	A138(<u>753</u>)	3.9 ± 0.2	162.5 ± 10.5	0.0
HH12	Arg-71	O1P	C139(<u>754</u>)	2.2 ± 0.5	140.9 ± 15.4	61.7
HH22	Arg-71	O1P	C139(<u>754</u>)	2.0 ± 0.3	149.1 ± 13.5	87.2
HH22	Arg-71	O5'	C139(<u>754</u>)	2.9 ± 0.4	130.0 ± 13.0	4.7
group 3						
Hζ1	Lys-7	O1P	G108(<u>658</u>)	2.9 ± 0.7	77.9 ± 47.1	25.4
Hζ2	Lys-7	O1P	G108(<u>658</u>)	2.7 ± 0.7	90.8 ± 51.2	37.6
Hζ3	Lys-7	O1P	G108(<u>658</u>)	2.8 ± 0.7	83.3 ± 46.7	29.6
Hζ3	Lys-7	O2P	U109(<u>659</u>)	2.9 ± 0.7	79.3 ± 45.8	0.0
Oδ2	Asp-20	HO2'	G135(<u>750</u>)	5.2 ± 0.6	105.7 ± 13.3	0.2
O	Thr-21	HO2'	G107(<u>657</u>)	1.8 ± 0.1	165.1 ± 8.2	97.2
Oγ	Thr-21	H22	G107(<u>657</u>)	2.3 ± 0.4	141.4 ± 11.5	97.2
H	Gly-22	O2'	G135(<u>750</u>)	2.7 ± 0.5	138.7 ± 12.0	16.8
O	Gly-22	H22	G135(<u>750</u>)	2.2 ± 0.4	163.9 ± 8.6	70.5
He1	Gln-27	O2	C106(<u>656</u>)	4.0 ± 0.7	77.1 ± 11.4	0.2
HO	A138(<u>753</u>)	O2P	C139(<u>754</u>)	3.8 ± 0.3	143.5 ± 18.1	0.0

see the caption of Table 8 on the next page

Atom pair		s15-rna				
atom1	res1	atom2	res2	Distance (Å)	Angle (deg)	Formation (%)
group 1						
HH12	Arg-34	O1P	G107(<u>657</u>)	2.1 ± 0.7	150.2 ± 19.1	83.4
HH22	Arg-34	O1P	G107(<u>657</u>)	2.6 ± 1.2	137.6 ± 17.6	52.8
HH21	Arg-34	O1P	G126(<u>741</u>)	9.7 ± 0.8	87.3 ± 14.1	0.0
Hδ1	His-41	O2'	C124(<u>739</u>)	5.3 ± 0.5	89.2 ± 14.1	0.0
Oδ2	Asp-48	H22	G117(<u>667</u>)	3.3 ± 0.2	141.7 ± 6.3	0.0
HO	U119	O2	U120	2.6 ± 0.5	120.8 ± 19.9	24.5
O2	U119	HO	U120	7.1 ± 0.5	35.4 ± 22.3	0.0
O2P	U119	H41	C121	8.8 ± 0.6	87.6 ± 10.9	0.0
O4	U120	H22	G123	2.0 ± 0.3	156.9 ± 13.7	83.6
HO	G122	O1P	G123	3.9 ± 0.3	129.5 ± 19.2	0.0
group 2						
Hε21	Gln-61	O1P	G140(<u>755</u>)	2.0 ± 0.3	155.4 ± 13.1	87.5
HH11	Arg-64	O2P	G140(<u>755</u>)	1.8 ± 0.1	161.5 ± 8.5	99.5
HH	Tyr-68	O1P	A138(<u>753</u>)	1.9 ± 0.4	161.7 ± 11.3	92.3
HH22	Arg-71	O3	A138(<u>753</u>)	2.0 ± 0.3	160.2 ± 12.4	85.0
HH12	Arg-71	O1P	C139(<u>754</u>)	1.9 ± 0.3	152.8 ± 16.2	93.0
HH22	Arg-71	O1P	C139(<u>754</u>)	2.9 ± 0.4	123.6 ± 10.8	3.2
HH22	Arg-71	O5'	C139(<u>754</u>)	3.7 ± 0.4	135.2 ± 13.9	0.0
group 3						
Hζ1	Lys-7	O1P	G108(<u>658</u>)	1.8 ± 0.1	158.7 ± 11.3	99.2
Hζ2	Lys-7	O1P	G108(<u>658</u>)	3.1 ± 0.2	62.5 ± 9.2	0.0
Hζ3	Lys-7	O1P	G108(<u>658</u>)	3.3 ± 0.2	48.3 ± 7.2	0.0
Hζ3	Lys-7	O2P	U109(<u>659</u>)	1.8 ± 0.1	159.2 ± 10.3	98.7
Oδ2	Asp-20	HO2'	G135(<u>750</u>)	2.0 ± 0.2	145.1 ± 10.4	85.5
O	Thr-21	HO2'	G107(<u>657</u>)	2.0 ± 0.2	133.8 ± 9.4	88.4
Oγ	Thr-21	H22	G107(<u>657</u>)	2.2 ± 0.2	143.8 ± 10.6	62.2
H	Gly-22	O2'	G135(<u>750</u>)	2.4 ± 0.3	134.2 ± 13.2	23.6
O	Gly-22	H22	G135(<u>750</u>)	2.1 ± 0.2	116.4 ± 8.0	78.9
Hε1	Gln-27	O2	C106(<u>656</u>)	2.6 ± 0.5	123.5 ± 17.1	23.0
HO	A138(<u>753</u>)	O2P	C139(<u>754</u>)	1.7 ± 0.1	158.1 ± 8.8	99.3

Table 8: Interactions at the atomic level in the S15-RNA complex. The mean hydrogen-acceptor distances (Å) and the mean donor-hydrogen-acceptor angles (deg) were calculated on the 11-15 ns interval. The formation percentage is the percentage of trajectory time during which the distance is smaller than 2.2 Å. The results are compared in s15-rna-mg (previous page) and in s15-rna (this page).

(a) Atom1	res1	Atom2	res2	s15-rna-mg distance (Å)	Angle (deg)	Formation (%)
H	Thr-24	O	Gly-22	2.2 ± 0.2	126.4 ± 13.0	48.3
H	Thr-24	Oε1	Gln-27	2.2 ± 0.2	132.7 ± 11.1	57.2
H	Gly-25	O	Ser-23	2.1 ± 0.3	147.2 ± 8.1	66.9
H	Arg-16	Oδ1	Asp-20	1.9 ± 0.2	156.6 ± 11.4	92.7
H	Gln-27	O	Ser-23	5.5 ± 0.2	68.3 ± 3.2	0.0
H	Val-26	O	Ser-23	4.3 ± 0.3	140.1 ± 7.8	0.0
Atom1	res1	Atom2	res2	s15-rna distance (Å)	Angle (deg)	Formation (%)
H	Thr-24	O	Gly-22	4.0 ± 0.2	100.2 ± 4.2	0.0
H	Thr-24	Oε1	Gln-27	6.0 ± 0.3	42.2 ± 6.3	0.0
H	Gly-25	O	Ser-23	3.2 ± 0.2	75.6 ± 6.7	0.0
H	Arg-16	Oδ1	Asp-20	4.1 ± 0.3	100.8 ± 8.5	0.0
H	Gln-27	O	Ser-23	2.3 ± 0.2	155.8 ± 11.3	40.4
H	Val-26	O	Ser-23	2.4 ± 0.3	130.1 ± 14.2	31.9
(b) Atom1	res1	Atom2	res2	s15-pdb distance (Å)	Angle (deg)	Formation (%)
H	Thr-24	O	Gly-22	4.0 ± 0.4	99.8 ± 6.7	0.0
H	Thr-24	Oε1	Gln-27	9.0 ± 1.0	32.0 ± 27.3	0.0
H	Gly-25	O	Ser-23	3.2 ± 0.4	82.6 ± 10.1	0.2
H	Arg-16	Oδ1	Asp-20	5.7 ± 1.2	27.7 ± 23.9	0.0
H	Gln-27	O	Ser-23	4.0 ± 0.5	140.0 ± 8.7	0.1
H	Val-26	O	Ser-23	2.5 ± 0.4	158.0 ± 13.0	27.1
Atom1	res1	Atom2	res2	s15-aria distance (Å)	Angle (deg)	Formation (%)
H	Thr-24	O	Gly-22	4.1 ± 0.3	99.1 ± 4.9	0.0
H	Thr-24	Oε1	Gln-27	6.2 ± 1.1	54.2 ± 31.1	0.0
H	Gly-25	O	Ser-23	3.3 ± 0.2	78.7 ± 8.6	0.0
H	Arg-16	Oδ1	Asp-20	4.1 ± 0.4	24.1 ± 5.9	0.0
H	Gln-27	O	Ser-23	2.3 ± 0.4	150.6 ± 13.4	49.4
H	Val-26	O	Ser-23	2.4 ± 0.3	123.7 ± 13.2	27.8

see the caption of Table 9 on the next page

(c) Atom1							
res1	Atom2	res2	1ab3				
			distance (Å)	Angle (deg)	Formation (%)		
H	Thr-24	O	Gly-22	3.9 ± 0.8	93.3 ± 15.5	0.0	
H	Thr-24	Oε1	Gln-27	7.0 ± 1.5	70.6 ± 41.0	0.0	
H	Gly-25	O	Ser-23	3.6 ± 0.4	78.7 ± 6.7	0.0	
H	Arg-16	Oδ1	Asp-20	5.1 ± 1.4	59.8 ± 27.4	5.5	
H	Gln-27	O	Ser-23	3.0 ± 0.9	142.6 ± 11.2	27.8	
H	Val-26	O	Ser-23	3.2 ± 0.8	116.6 ± 20.5	0.0	
Atom1							
res1	Atom2	res2	2fxx				
			distance (Å)	Angle (deg)	Formation (%)		
H	Thr-24	O	Gly-22	3.9 ± 0.8	93.4 ± 15.2	4.0	
H	Thr-24	Oε1	Gln-27	6.9 ± 1.6	73.5 ± 42.3	0.0	
H	Gly-25	O	Ser-23	3.6 ± 0.4	78.7 ± 8.1	0.0	
H	Arg-16	Oδ1	Asp-20	5.3 ± 1.4	52.7 ± 28.0	4.0	
H	Gln-27	O	Ser-23	2.9 ± 1.0	147.3 ± 13.3	28.0	
H	Val-26	O	Ser-23	3.2 ± 0.9	117.1 ± 17.9	0.0	
(d) Atom1							
res1	Atom2	res2	1a32	1dk1	2avy	2aw7	
N	Thr-24	O	Gly-22	4.1	3.3	3.3	3.5
N	Thr-24	Oε1	Gln-27	5.9	4.0	6.7	6.7
N	Gly-25	O	Ser-23	3.4	2.6	3.3	3.6
N	Arg-16	Oδ	Asp-20	12.3	2.8	3.1	3.9
N	Gln-27	O	Ser-23	2.9	5.4	2.8	2.9
N	Val-26	O	Ser-23	3.2	4.4	2.8	3.2

Table 9: Mean values of intra-protein distances (Å) located in the $\alpha 1\alpha 2$ loop. The mean hydrogen-acceptor distances (Å) and the mean donor-hydrogen-acceptor angles (deg) were calculated on the 11-15 ns interval. The formation percentage is the percentage of trajectory time or of NMR conformers in which the distance is smaller than 2.2 Å. The MD trajectories (previous page: (a): s15-rna-mg, s15-rna, (b): s15-pdb, s15-aria), the NMR structures (this page: (c): 1ab3 and 2fxx), the X-ray structures (d: this page) of isolated S15 (1a32) and of S15-RNA complex (1dk1), and the structures of S15 in two conformations of the ribosome of *E. Coli* (2avy and 2aw7) are analyzed. In 1a32 and 1dk1, the atom Oδ1 of Asp-20 is used, whereas in 2avy and 2aw7, the atom Oδ2 of Asp-20 is used.

7 Figure Captions

Figure 1

Secondary structure of the three-way junction RNA involved in the interaction with S15. The base-pairing is displayed according to the ontology developed by 59. The base-pair between G51 and C53 was not displayed to improve the figure readability. This figure was realized with S2S [60].

Figure 2

The structures of the S15-RNA complex (X-ray (1dk1): a), and of S15 (NMR (1ab3): b, X-ray (1a32): c). The structures are shown in cartoon, the RNA is brown, and S15 is green and red in the α helices. The N and C terminal parts of the protein are shown as well as the 5' and 3' ends of RNA. The α helices S15 and the helices H20, H21, H22 of RNA are also shown in the figure. This figure was realized with pymol 0.98 [61].

Figure 3

Conformers of the NMR structures 1ab3 (a) and 2fkx (b) shown in cartoons. The conformers are superposed to minimize the RMSD on the complete structure (a) or on the residues 24-70 (b). The Figure was realized with pymol 0.98 [61].

Figure 4

Mean values of the ϕ (a, c) and ψ (b, d) angles in the loop connecting the $\alpha 1$ and $\alpha 2$ helices of S15. The angle values measured in the NMR and X-ray structures (a,b) and calculated during the 11-15 ns interval of the MD trajectories (c,d). These angle values are plotted along the residue numbers. The color code in (a) and (b) is: 1ab3 (red), 2fkx (blue), 1dk1 (pink), 1a32 (green), 2avy (cyan), 2a7w (orange). The color code in (c) and (d) is: s15-pdb (red), s15-aria (blue), s15-rna-mg (pink), s15-rna (green). The x-axis describes the residue numbers, and the y-axis the angle values in degrees.

Figure 5

Comparison of trajectories s15-pdb and s15-aria. Coordinate RMSD from the starting point calculated on: (a) all atoms (b) the loop connecting helices $\alpha 2$ and $\alpha 3$ (residues 41-57). (c) Atomic fluctuations by residues. Angles between helices $\alpha 1, \alpha 2$ (d), $\alpha 2, \alpha 3$ (e), $\alpha 3, \alpha 4$ (f). The full line is for the trajectory s15-aria, and the dotted/dashed lines for the trajectory s15-pdb. The x-axis describes the trajectory time in ns and the residue numbers, and the y-axis describes the angle values in degrees or the RMSD value and atomic fluctuations in Å. The secondary structures of S15 are shown under (c).

Figure 6

Contact plot of the violated NOEs during the trajectories of the isolated protein S15 (empty circles: s15-pdb, filled circles: s15-aria). The x- and y-axes describe the residue numbers.

Figure 7

Comparison of the trajectories s15-rna-mg and s15-rna. Atomic fluctuations by residues in S15 (a) and in RNA (b), calculated on the time interval 11-15 ns.

The RMSD values from the starting point are calculated on: (c) all atoms in S15, (d) all atoms in RNA. The full line is for the trajectory s15-rna, and the dotted/dashed lines for the trajectory s15-rna-mg. The x-axis describes the trajectory time in ns or the residue numbers, and the y-axis describes the atomic fluctuations (Å) or the RMSD (Å). The secondary structures of S15 and RNA are shown under (a) and (b).

Figure 8

Superposition of the S15-RNA conformations recorded at 7881, 8837, 9895 and 11347 ps in presence of ions Mg^{2+} . The RNA is in brown, and the S15 conformers are in cyan (7881 ps), green (8837 ps), magenta (9895 ps) and red (11347 ps). (a) The full complex structure shown in cartoon. A blue arrow indicates the $\alpha 1\alpha 2$ loop, and a red arrow the N-terminal region. The residues Arg-16 and Lys-4 are drawn in sticks. (b) Zoom on the loop linking $\alpha 1$ and $\alpha 2$, with Arg 16 drawn in sticks. (c) Zoom on the N-terminal part of $\alpha 1$ with Lys 4 and Gln 8 drawn in sticks. This figure was realized with pymol 0.98 [61]

Figure 9

Variation of the angles (a) ϕ of Ala-15, (b) ϕ of Thr-21, and (c) ψ of Asp-20 in the loop connecting the helices $\alpha 1$ and $\alpha 2$ in S15. The full line is for the trajectory s15-rna, and the dotted line for the trajectory s15-rna-mg. The x-axis describes the trajectory time in ns, and the y-axis describes the angle values in degrees.

Figure 10

Angles of the RNA phosphodiester backbone ((a) α , (b) β , (c) γ , (d) δ , (e) ϵ , (f) ζ). The mean values and the standard deviations, calculated on the 11-15 ns range, are given along the residue number (dashed line: s15-rna-mg, full line: s15-rna). The angle values measured on the 1dk1 structure are shown by diamonds. The secondary structures of RNA are shown at the bottom.

Figure 11

Values of the phosphodiester backbone angles for the U1 (a), U2 (b), C3 (c) and G4 (d) residues in UUCG tetraloops. The values obtained for the PDB files (1dk1: 3, 1f7y: 4, 1hlx: [51], 1i6u: [52], 1kuq) are shown with empty circles. The values calculated from the 11-15 ns intervals in s15-rna-mg and s15-rna are shown with dots and crosses respectively. For the PDB file 1hlx and the MD trajectories, the mean values of the angles are shown, along with the standard deviations in dotted line.

Figure 12

Variation of the angles between (a) H22 and H20 and between (b) H22 and H21 in the RNA during the MD trajectories of the complex. The full line is for the trajectory s15-rna, and the dotted line for the trajectory s15-rna-mg. The two angles formed by the atom triplets (107-C6, 114-N2, 142-O2) and (107-C6, 114-N2, 98-N1) are plotted in degrees, according to the trajectory time. The first angle describes the relative orientation of the helices H22 and H20, and the second angle describes the relative orientation of the helices H22 and H21. The x-axis describes the trajectory time in ns, and the y-axis describes the angle values in degrees.

Figure 13

The structures of the S15-RNA complex (X-ray (1dk1) with the ions Mg^{2+} drawn in cpk. The structures are shown in cartoon, the RNA backbone is brown, the RNA bases are cyan and S15 is green. This figure was realized with pymol 0.99 [61].

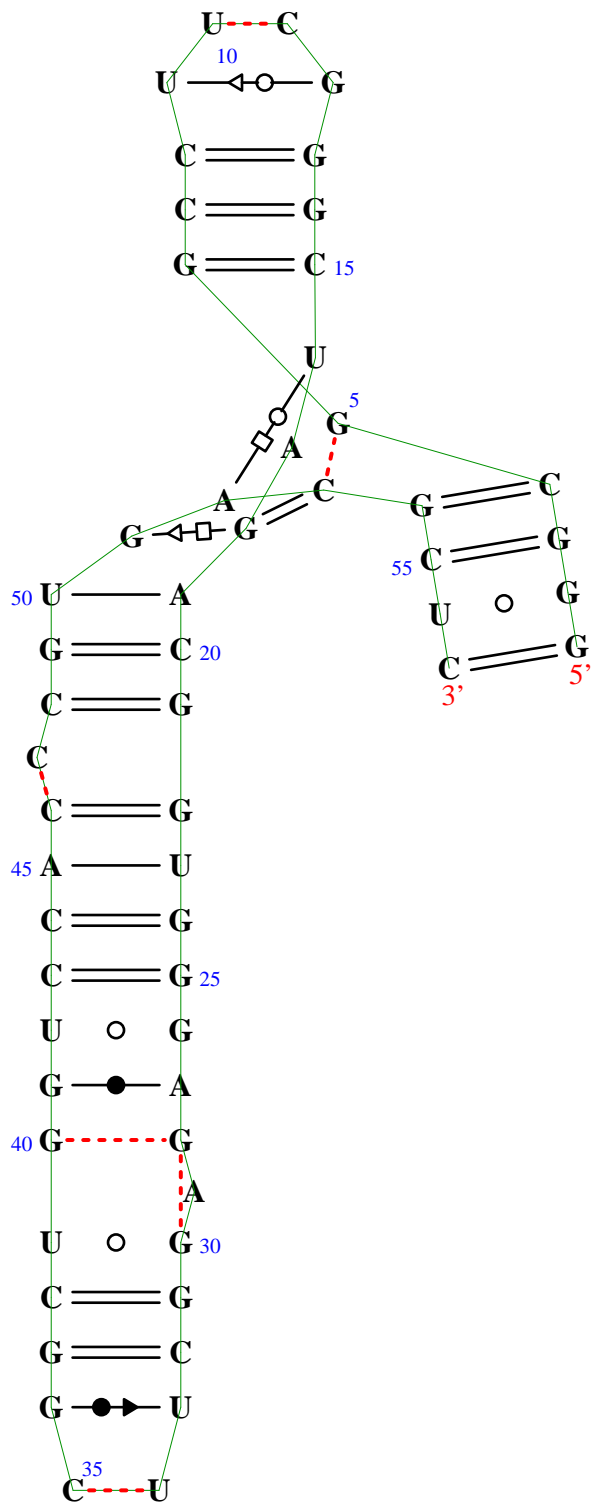


Figure 1:

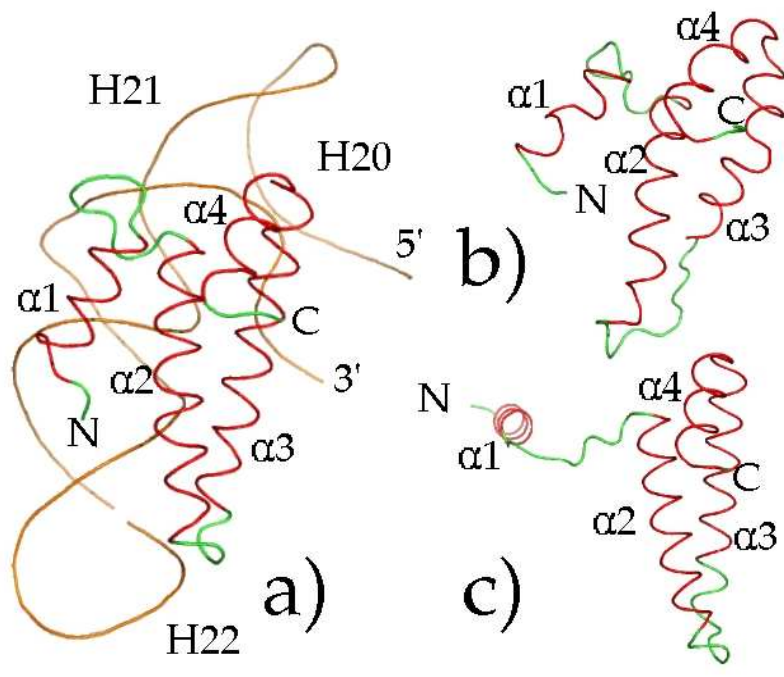


Figure 2:

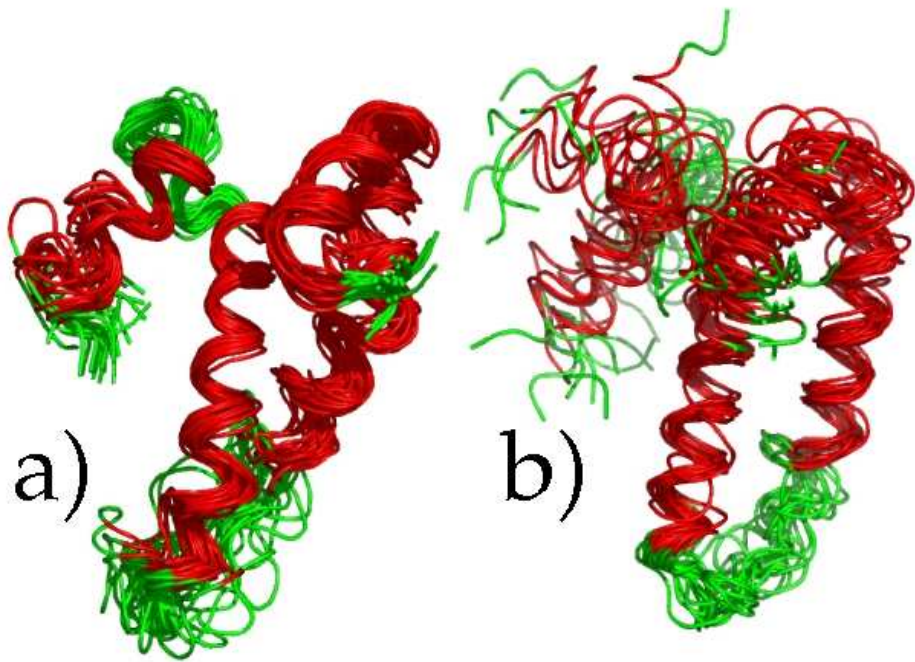


Figure 3:

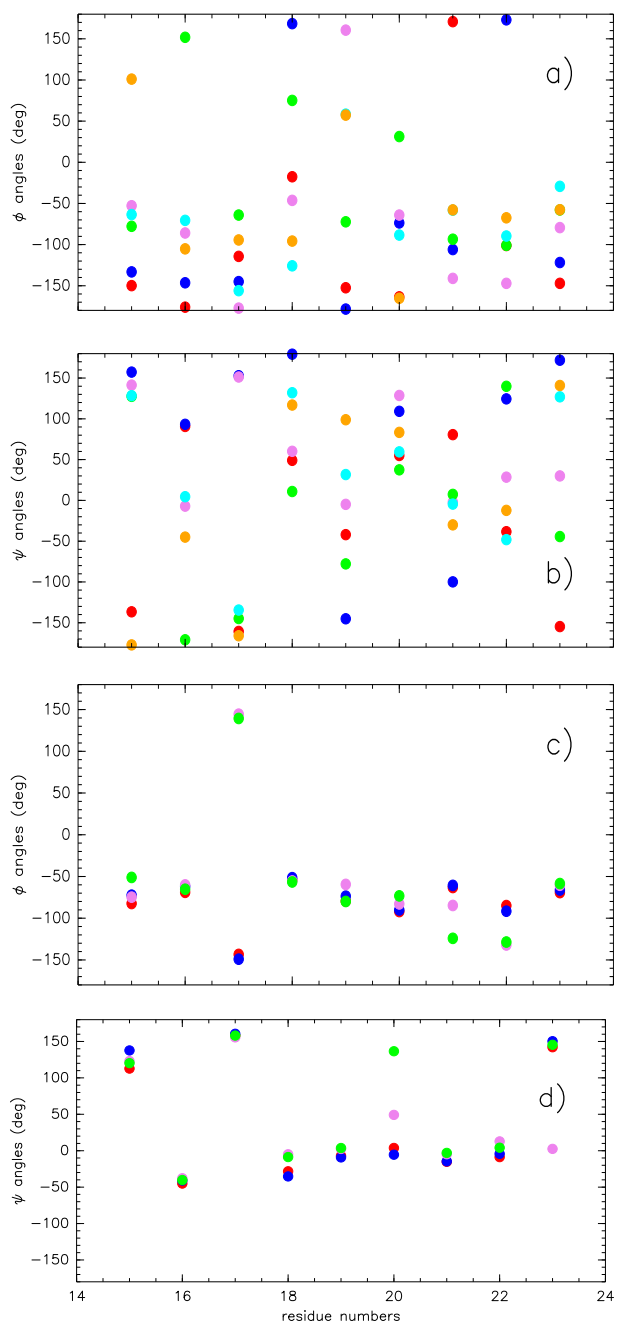


Figure 4:

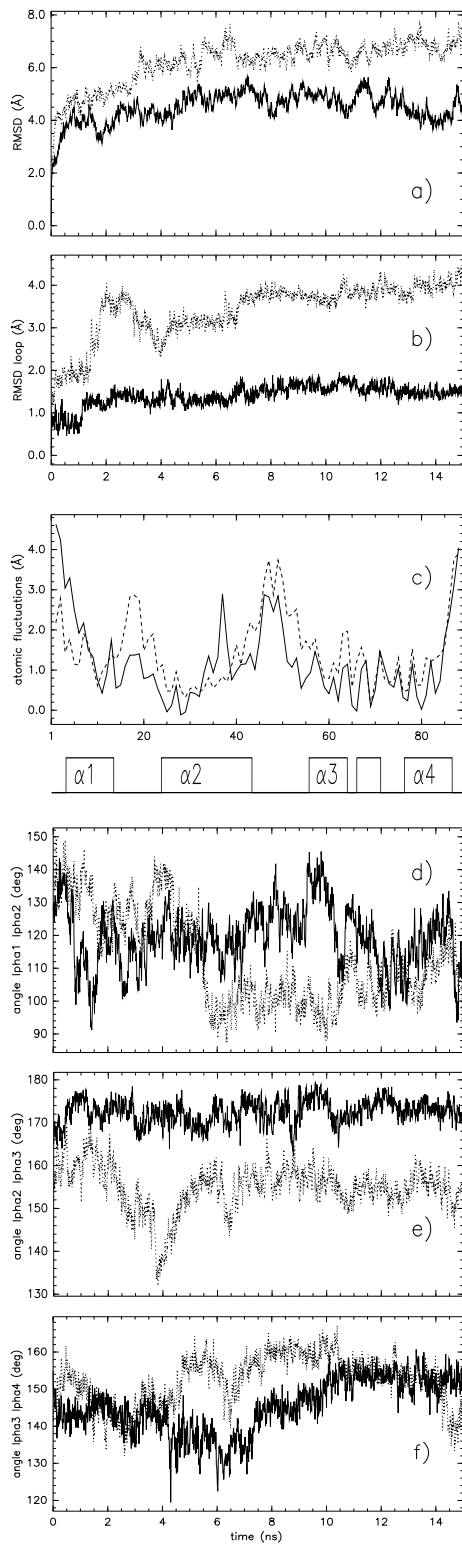


Figure 5:

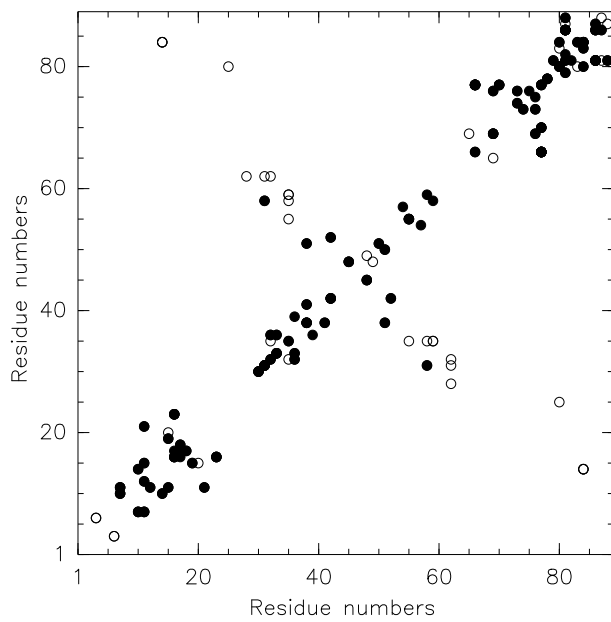


Figure 6:

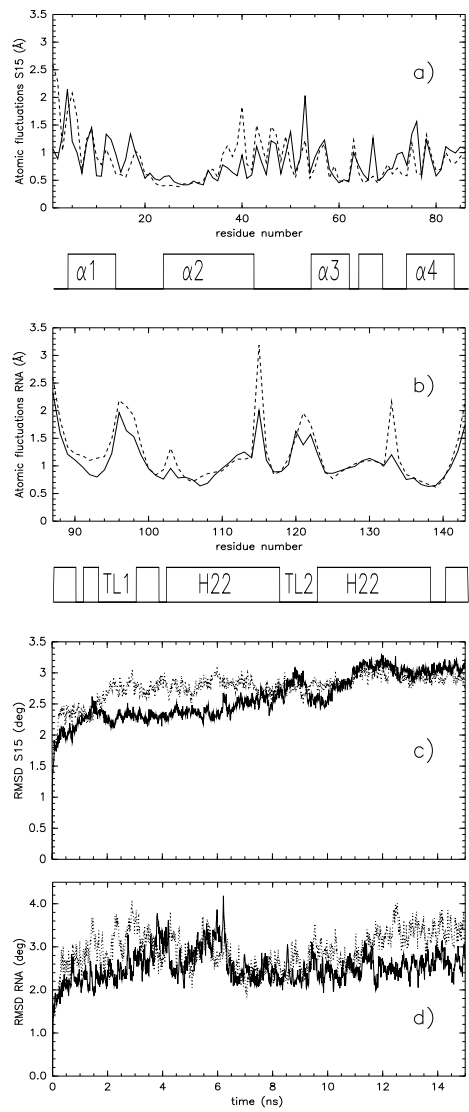


Figure 7:

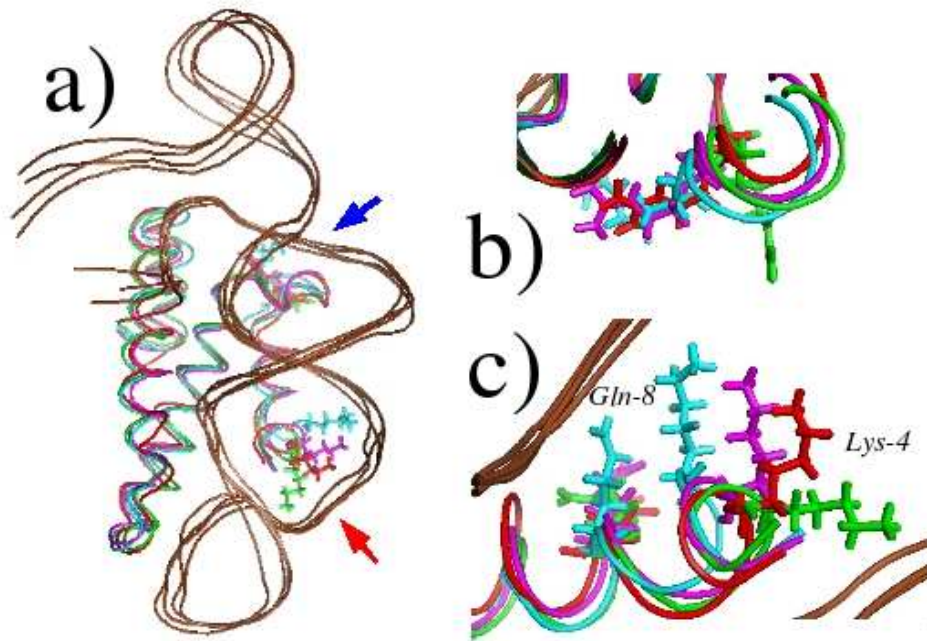


Figure 8:

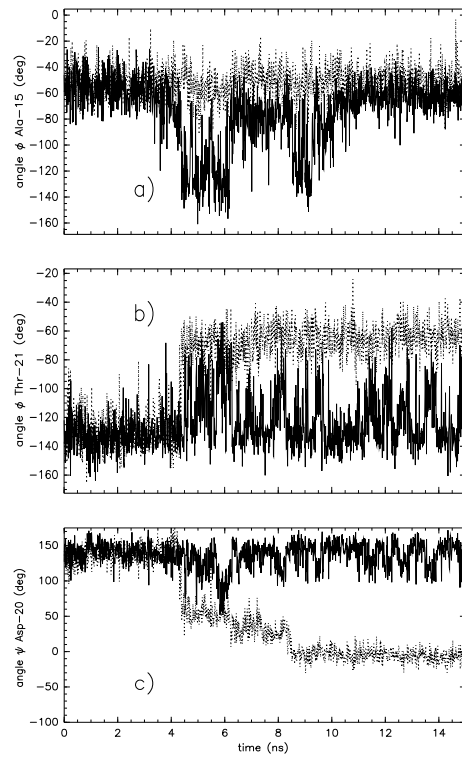


Figure 9:

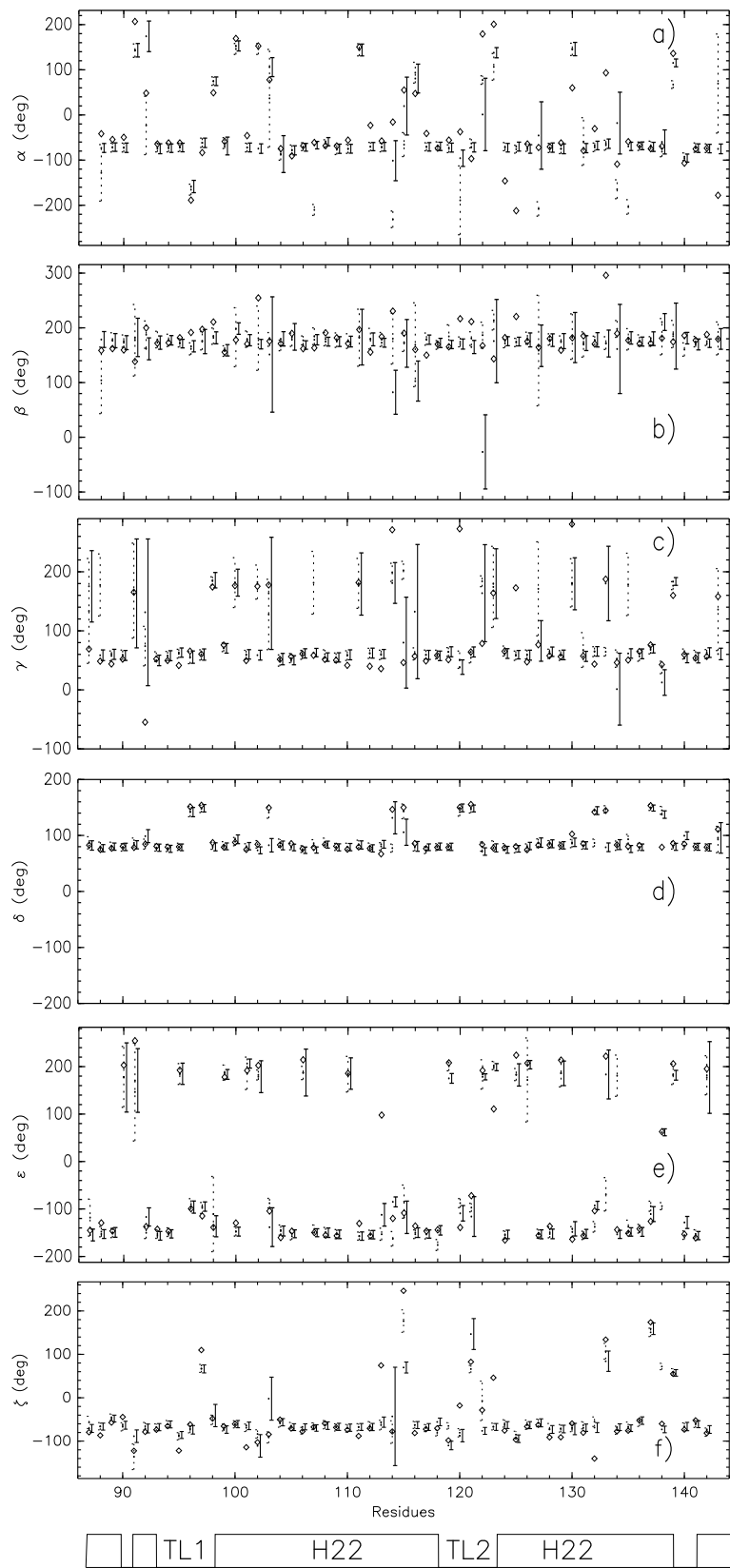


Figure 10:

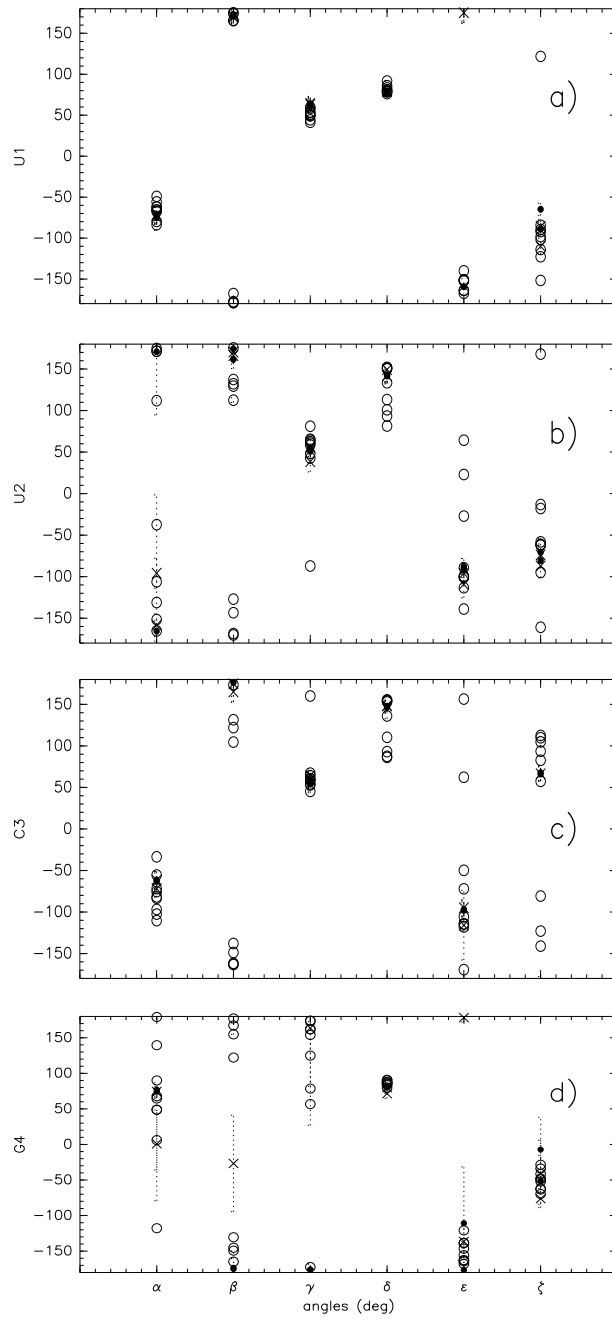


Figure 11:

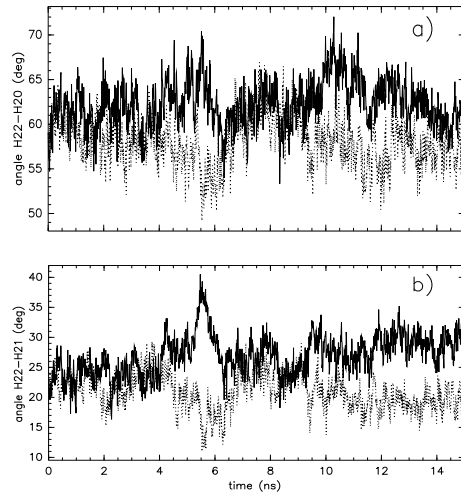


Figure 12:

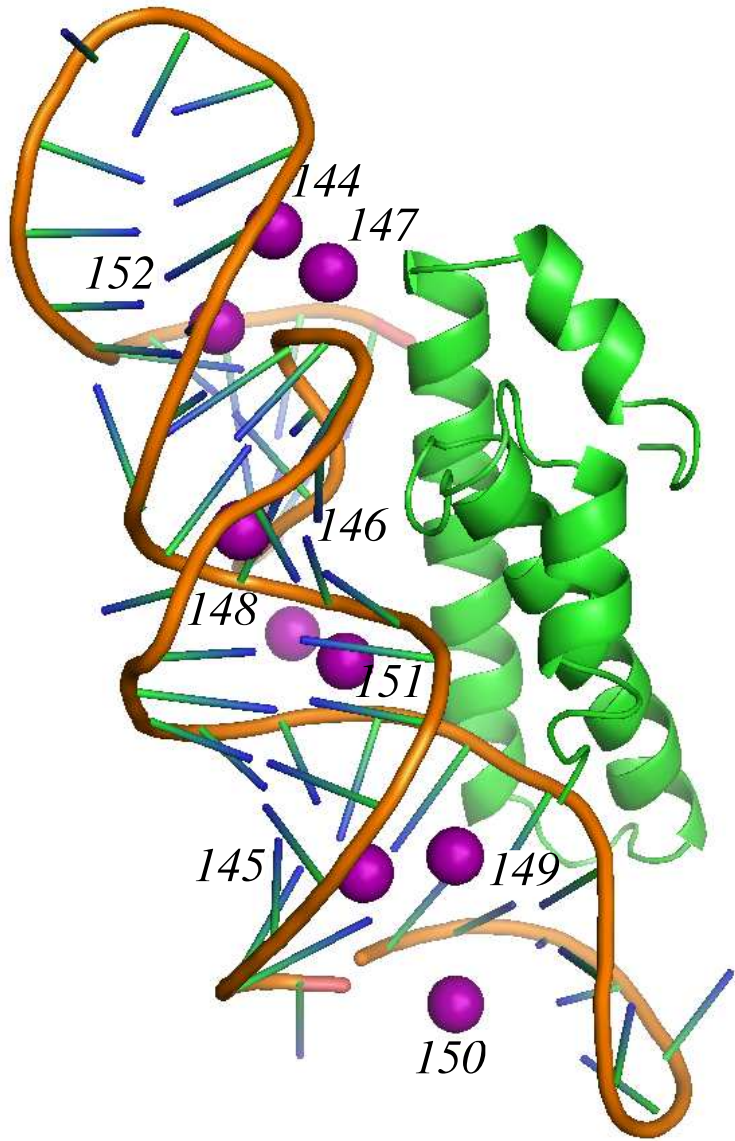


Figure 13: