Supporting Information

Do Collective Atomic Fluctuations Account for Cooperative Effects? Molecular Dynamics Studies of the U1A-RNA Complex

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Simulation Details

The molecular dynamics (MD) simulation of the U1A-RNA system was based on the x-ray cocrystal structure of the N-terminal RRM of U1A bound to stem loop 2 (SL2) of U1 snRNA solved at 1.92 Å resolution,[1] PDB[2] ID: 1URN. Biological unit 2 was chosen for the initial structure as it contains structural information for the entire SL2 RNA. As described in Pitici et al.,[3] the U1A protein was extended to obtain a construct containing residues 2 – 102, and two point mutations (H31Y and R36Q) were introduced to revert the protein to the wild type sequence.

MD simulation of the U1A-RNA complex was carried out using the AMBER[4,5] suite of programs with the parm96[6] force field. The system was solvated in a periodic box of explicit TIP3P[7] water molecules extending a minimum distance of 12 Å from the solute atoms. Na^+ and Cl^- ions were added to the system to reach a salt concentration of 250 mM. Long-range interactions were treated using the particle mesh Ewald method[8] with a 9 Å cutoff for direct space non-bonded calculations and a 0.00001 Ewald convergence tolerance. The simulation protocol involved energy minimization of the initial structure for 2000 steps, heating to 298 K for 10 ps, equilibration at 298 K and 1 atm for 50 ps and a production run of 10 ns. Additional details may be found in the protocol described by Pitici et al.[3] The results presented here are based on a production run of 10 ns.

The all-atom RMSD from the equilibrated structure over the 10 ns trajectory on the U1A-RNA system stabilized after 0.6 ns and remained stable throughout the remainder of the simulation. Comparison to previous MD studies on the U1A-RNA system [9-13] was used to verify the trajectory for this analysis. The average RMSD of 1.4 Å (computed for the protein backbone and over all RNA atoms) is consistent with the prior studies.

Cross-Correlation Calculations

The extent to which the fluctuations of a system are correlated is dependent on the magnitude of the cross-correlation coefficient, C_{ij}, given by the following equation: [14, 15]

$$C_{ij} = \frac{\left\langle \Delta \mathbf{r}_i \cdot \Delta \mathbf{r}_j \right\rangle}{\left(\left\langle \Delta \mathbf{r}_i \right\rangle^2 \left\langle \Delta \mathbf{r}_j \right\rangle^2 \right)^{1/2}}$$

where i and j may be any two atoms, residues or domains, $\Delta \mathbf{r}_i$ and $\Delta \mathbf{r}_j$ are displacement vectors of i and j, and the angle brackets denote an ensemble average. This involves calculation of all inter-atomic cross-correlations of atomic fluctuations, forming a matrix whose elements C_{ij} may be displayed in a graphical representation as a positional cross-correlation map. If $C_{ij} = 1$ the fluctuations of i and j are completely correlated (same period and same phase), if $C_{ij} = -1$ the fluctuations of i and j are completely anticorrelated (same period and opposite phase), and if $C_{ij} = 0$ the fluctuations of i and j are not correlated.

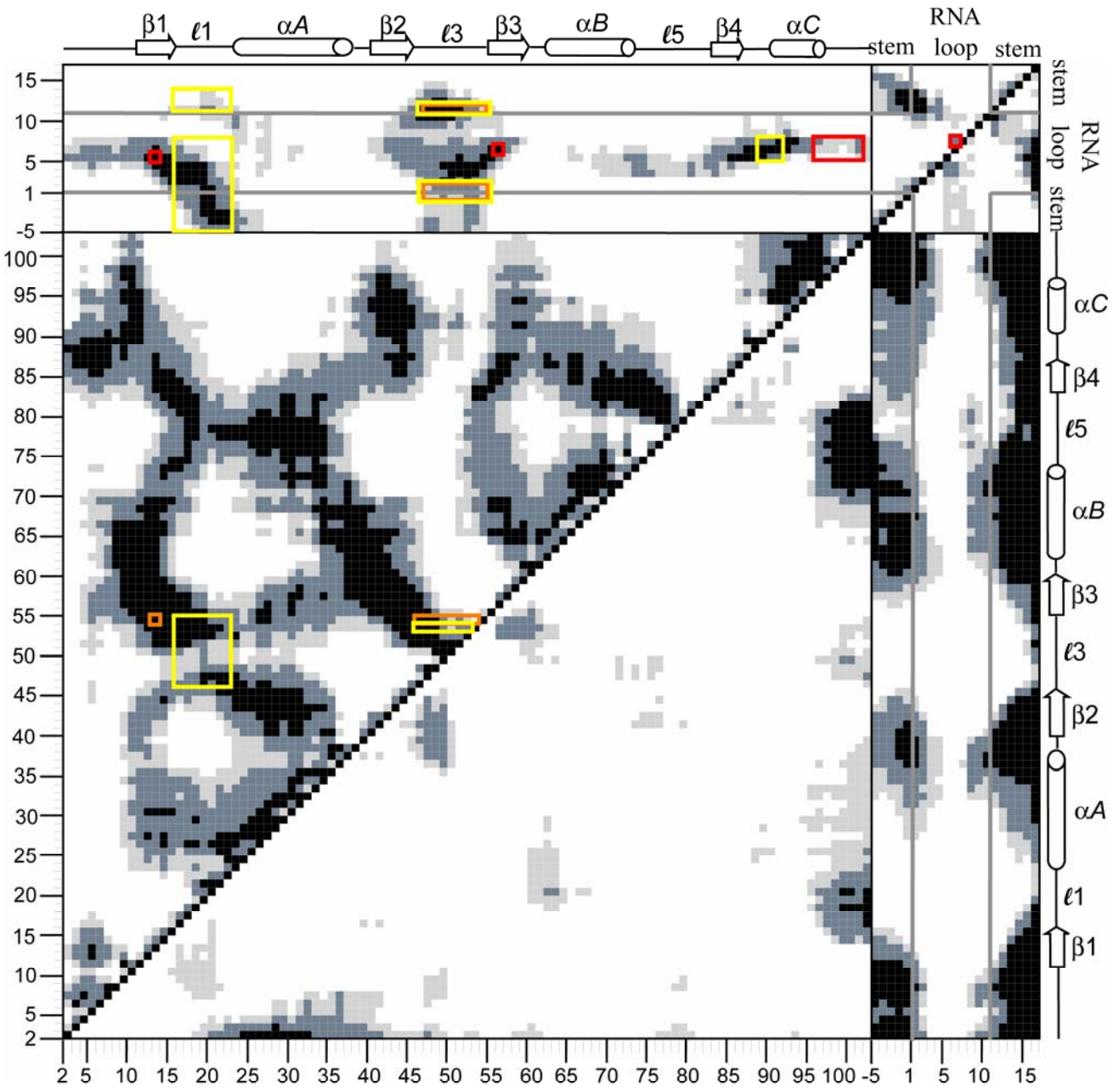
For the results presented herein, structures for positional cross-correlation analysis were superimposed by minimizing the RMSD over all C^a to the equilibrated structure. C_{ij} elements have been computed by residue over the center of mass of N, C^a and C backbone atoms in U1A and by nucleotide over the center of mass of all atoms in SL2 RNA. The analysis is based on an ensemble average of one structure per picosecond over the 10 ns trajectory.

Fluctuational Cross-Correlation Map Details

The results presented in Figure 1 represent a two dimensional graphical presentation of the covariance matrix of all residue fluctuations in U1A-RNA. Positive correlations are collected in the upper triangle and negative correlations are collected in the lower triangle. Only correlations stronger than ±0.25 are shown and darker colors indicate stronger correlated fluctuations: black indicates $C_{ij} = \pm\ 0.75 - 1.00$,

slate gray indicates C_{ij} = ± 0.50 – 0.75, and light gray indicates C_{ij} = ± 0.25 – 0.50. Note that not only are protein-protein correlated fluctuations obtained, but protein-RNA and RNA-RNA correlations are obtained, as well. The U1A protein is numbered 2 – 102 and SL2 RNA is numbered -5 – 16. Gray lines have been drawn on the plot to distinguish between SL2 stem (-5 through -1, 11 through 16) and loop (1 through 10) nucleotides.

A large-scale view of the fluctuational cross-correlation map for U1A-RNA is shown in Figure S1. Included in this figure is a detailed description of the three cooperative networks of amino acid residues for which evidence has been shown by Hall and coworkers,[12,16-18] Baranger and coworkers[19-21] and Laird-Offringa and coworkers.[22, 23] This encompasses the residues shown (to date) to be involved in cooperative networks in the U1A-RNA system using biophysical methods. These networks are color-coded and have been mapped in Figure S1.



Network 1: Tyr13 through C5 and Phe56 through A6 and C7, RNA to C-terminal residues, Lys96 - Phe101
Network 2: Tyr13 to Gln54 through loop 3 to the RNA closing C-G base pair and A1
Network 3: Gly53 through loop 3 through loop 1, loops 1 and 3 through RNA to Thr89, Asp90 and Ser91

Figure S1. Calculated fluctuational cross-correlation map for the U1A-RNA complex. Cooperative networks of residues are mapped on the DCCM and are distinguished according to color. The specific residues involved in each of the networks are indicated below the picture and color coded to match those mapped in the DCCM. The residues that reveal cross-correlation coefficients between -0.25 – 0.25, indicated by white space, are important for further clarification of the specific residues involved in the cooperative networks.

Complete Reference 25: See reference 5 below.

References

(1) Oubridge, C.; Ito, N.; Evans, P.R.; Teo, C.H.; Nagai, K. *Nature.* **1994,** *372*, 432-8.

(2) Berman, H.M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T.N.; Weissig, H.; Shindyalov, I.N.; Bourne, P.E. *Nuc. Acids Res.* **2000,** *28*, 235-42.

(3) Pitici, F.; Beveridge, D.L.; Baranger, A.M. *Biopolymers.* **2002,** *65*, 424-35.

(4) Pearlman, D.A.; Case, D.A.; Caldwell, J.W.; Ross, W.S.; Cheatham, T.E.; DeBolt, S.; Ferguson, D.; Seibel, G.; Kollman, P. *Comp. Phys. Com.* **1995,** *91*, 1-41.

(5) Case, D.A.; Darden, T.A.; Cheatham III, T.E.; Simmerling, C.L.; Wang, J.; Duke, R.E.; Luo, R.; Merz, K.M.; Wang, B.; Pearlman, D.A.; Crowley, M.; Brozell, S.; Tsui, V.; Gohlke, H.; Mongan, J.; Hornak, V.; Cui, G.; Beroza, P.; Schafmeister, C.; Caldwell, J.W.; Ross, W.S.; Kollman, P. *AMBER 8*, University of California: San Francisco, 2004.

(6) Kollman, P.A.; Dixon, R.; Cornell, W.; Fox, T.; Chipot, C.; Pohorille, A. In *Computer Simulation of Biomolecular Systems*. Wilkinson, A.; Weiner, P.; van Gunsteren, W. F., Eds. Kluwer: London, 1997; Vol. 3, pp 83-96.

(7) Jorgensen, W.L.; Chandrasekhar, J.; Madura, J.D.; Impey, R.W.; Klein, M.L. *J. Chem. Phys.* **1983,** *79*, 926-36.

(8) Darden, T.A.; York, D.M.; Pedersen, L.G. *J. Chem. Phys.* **1993,** *98*, 10089-92.

(9) Blakaj, D.M.; McConnell, K.J.; Beveridge, D.L.; Baranger, A.M. *J. Am. Chem. Soc.* **2001,** *123*, 2548-51.

(10) Hermann, T.; Westhof, E. *Nat. Struct. Biol.* **1999,** *6*, 540-4.

(11) Reyes, C.M.; Kollman, P.A. *RNA.* **1999,** *5*, 235-44.

(12) Showalter, S.A.; Hall, K.B. *Biophys. J.* **2005,** *89*, 2046-58.

(13) Tang, Y.; Nilsson, L. *Biophys. J.* **1999,** *77*, 1284-305.

(14) McCammon, A.J.; Harvey, S.C. *Dynamics of Proteins and Nucleic Acids*. Cambridge University Press: Cambridge, 1986.

(15) Brooks III, C.L.; Karplus, M.; Pettitt, B.M. *Proteins: A Theoretical Perspective of Dynamics, Structure and Thermodynamics*. John Wiley and Sons: New York, 1988.

(16) Kranz, J.K.; Hall, K.B. *J. Mol. Biol.* **1998,** *275*, 465-81.

(17) Kranz, J.K.; Hall, K.B. *J. Mol. Biol.* **1999,** *285*, 215-31.

(18) Showalter, S.A.; Hall, K.B. *J. Mol. Biol.* **2002,** *322*, 533-42.

(19) Nolan, S.J.; Shiels, J.C.; Tuite, J.B.; Cecere, K.L.; Baranger, A.M. *J. Am. Chem. Soc.* **1999,** *121*, 8951-2.

(20) Shiels, J.C.; Tuite, J.B.; Nolan, S.J.; Baranger, A.M. *Nuc. Acids Res.* **2002,** *30*, 550-8.

(21) Tuite, J.B.; Shiels, J.C.; Baranger, A.M. *Nuc. Acids Res.* **2002,** *30*, 5269-75.

(22) Katsamba, P.S.; Bayramyan, M.; Haworth, I.S.; Myszka, D.G.; Laird-Offringa, I.A. *J. Biol. Chem.* **2002,** *36*, 33267-74.

(23) Law, M.J.; Chambers, E.J.; Katsamba, P.S.; Haworth, I.S.; Laird-Offringa, I.A. *Nuc. Acids Res.* **2005,** *33*, 2917-28.