Equal-Time Cross-correlation Map

Equal-Time Cross-correlation is an effective method to probe the correlated dynamics over the simulation. Here the cross-correlation of the atomic fluctuations between two residues [1] is calculated using the following equation:

$$C(i,j) = \frac{\left\langle \Delta_i(t_k) \cdot \Delta_j(t_k) \right\rangle}{\sqrt{\left\langle \Delta_i^2(t_k) \right\rangle \cdot \left\langle \Delta_j^2(t_k) \right\rangle}}$$

where $\langle \cdots \rangle$ represents the average of an ensemble, $\langle \Delta_i(t_k) \rangle$ denotes the displacement of heavy atom i from the average position over the trajectory. This calculation is carried out using the *ptraj* module in AMBER[2] on a residue basis. The correlation between two residues in the Cartesian coordinate space is measured by $C(i,j)$, which is equal to 1 if the motions of two residues during the simulation timescale are always in the same direction. $C(i,j) =$ - indicates motions are in opposite directions.

Principle Component Analysis (PCA)

A covariance matrix was constructed using the coordinates of all heavy atoms (2025 atoms) of the SAM-I riboswitch from each of the two 200 ns trajectories. The diagonalization of the covariance matrix generates a diagonal matrix of eigenvalues and a transformation matrix comprising eigenmodes. The modules *g_covar* and *g_anaeig* in GROMACS package [3] are used for the PCA calculation.

Clustering Analysis

In order to classify sub-states of conformations generated from the simulation, WoSAM_TRAJ was submitted to clustering analysis. Snapshots are extracted from WoSAM_TRAJ for every 50 ps. *ptraj*[4] is employed for the clustering analysis using a *k*-means algorithm with $k = 3$. Here RMSD is chosen as the criterion to carry out the clustering. Representative snapshots from each cluster shown in Figure 3 are chosen from the "centroid" (refs) identified for each cluster.

Electrostatic Potential (ESP) Maps

The electrostatic potentials of crystal structure and the representative snapshots (only RNA is included) from the clustering are calculated using APBS[5]. Two orthogonal planes slice through the magnesium binding site that is close to SAM binding pocket. The contour map of the electrostatic potential is shown on the slice plane. These figures are generated using Amira 5.1

MD simulations in the absence of Mg

SI Movie 3 and SI Movie 4 are generated using VMD[6] from the trajectories calculated without Mg present.

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SI Figure 1. RMSF of each simulation and the B-factors from the crystallographic structure.

SI Figure 2. Time evolution of RMSD of the whole SAM-I riboswitch with reference to the crystallographic structure and the average structure from the simulations.

SI Figure 3. (a) RMSF plots of SAM_TRAJ and WoSAM_TRAJ along their first 5 eigenmodes (color label is the same as Figure 1b). b) Eigenvalues and cumulative percentage of the eigenmodes for SAM_TRAJ and WoSAM_TRAJ.

SI Figure 4. (a) Cross-correlations maps for SAM_TRAJ and WOSAM_TRAJ by residue. (b)The difference cross-correlation map between SAM_TRAJ and WoSAM_TRAJ. The regions that reflect the anti-correlation between the P1 helix and the P3 helix in WOSAM_TRAJ are highlighted in rectangular frames. The color scheme used here is 1 (red), 0.5 (orange), 0 (white), -0.5 (cyan) and -1 (blue).

SI Figure 5. Radius of gyration of the set of phosphates defining a Magnesium binding pocket between J1/2 and J3/4. Like the SAM binding pocket, this Mg binding pocket is much more compact and stable in SAM_TRAJ than in WOSAM_TRAJ.

SI Figure 6. Torsion angle monitor of A9 during the simulations.

SI Figure 7. Inner product of the first 10 eigenmodes from the 1st 100 ns and the 2nd 100 ns of SAM_TRAJ and WoSAM_TRAJ.

SI Figure 8. A second 200 ns trajectory run under similar conditions to SAM_TRAJ yields similar conclusions. Top panel shows the RMSD with reference to the starting structure. The A9/U63 non-adjacent dinucleotide stack is observed transiently for periods in the tens of nanosecond range, as monitored by the Lennard Jones potential energy of interaction. Strong coordination is observed between a bound Mg and phosphate atoms on J1/2 and J3/4. In contrast to the original SAM_TRAJ, there is not a clear correlation between the dinucleotide stack and Mg-A9 phosphate distances.

SI Movie 1. The essential dynamics of WOSAM_TRAJ along the 1st eigenmode. The arrows are drawn in the same way as described in Materials and Methods. Here five “non-physical” snapshots displayed in tube representation have been chosen to sample five evenly distributed values of the first principal component, for individual strands within P1 and P3. The crystallographic structure is present in the background (the color scheme of sub-regions is the same as Figure 1b).

SI Movie 2. Motions of SAM_TRAJ along the 1st eigenmode with the view to show the transient nonadjacent dinucleotide stack between A9 and U63. The RNA is visualized with the New Cartoon representation in VMD with the same color scheme as Figure 1b. SAM is displayed with surface representation in yellow color. SAM is fixed in the movie. Five “non-physical” snapshots used in the movie are generated as described in SI Movie 1.

SI Movie 3 Trajectory resulting from MD simulations in the absence of Mg and the presence of SAM. The color scheme of the RNA is the same as Figure 1. Sodium ions that are within 5 Å of the phosphate atoms on residue 9 to 11 and residue 62 to 64 are traced during the simulation. The ions are color coded by their residue ID. The magnesium from the crystal structure is shown in transparent in the movie as a position reference.

SI Movie 4 Trajectory generated as for SI Movie 3 but with SAM removed**.**

SI pdb files Coordinate sets for representative snapshots for each of three clusters identified from SAM _TRAJ and as WOSAM_TRAJ, combined as a "zip file".