UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY

The Role of Mg^{+2} Ion-interactions in Folding of Twister Ribozyme Revealed Through Umbrella Sampling Combined With Oscillating Chemical Potential Grand Canonical Monte Carlo - Molecular Dynamics Simulations

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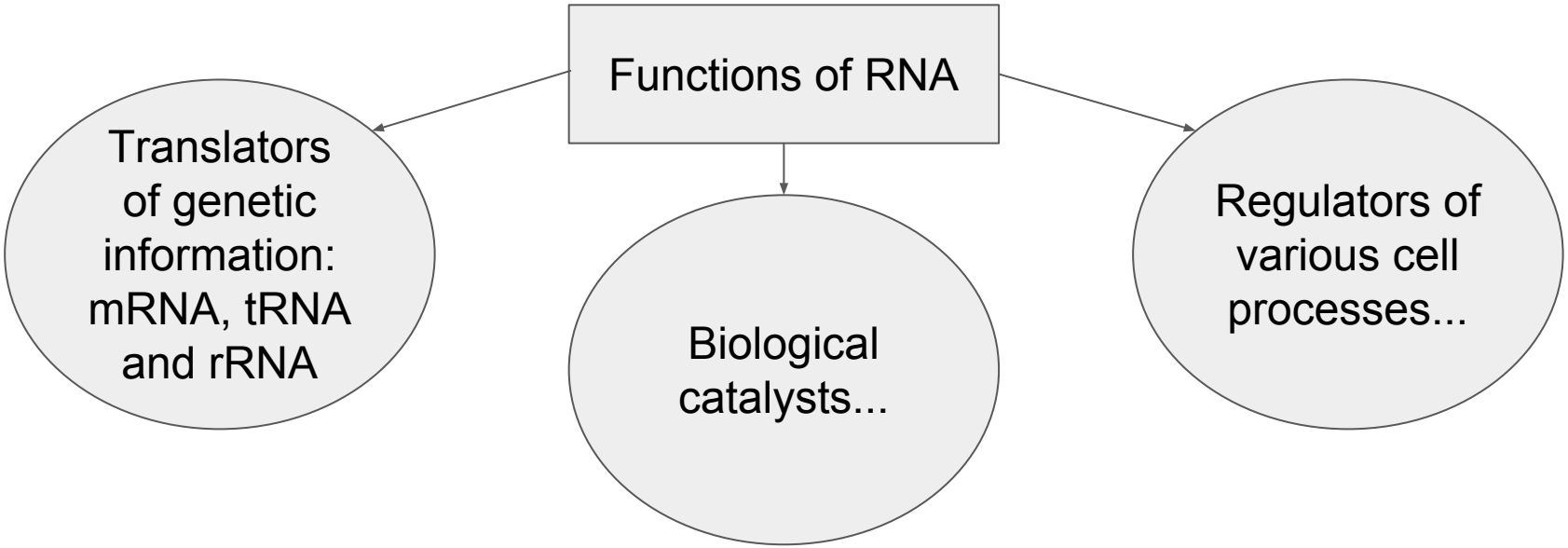
Abstract

RNA molecules perform a variety of biological functions, including as ribozymes where they catalyze chemical reactions, for which the correct three-dimensional structure is essential. However it is challenging to resolve atomic-details of the folding landscape of ribozymes as they assume the tertiary structures required for catalytic activity. Metal ions, especially Mg^{+2} ions, are known to neutralize these negatively charged nucleic acids and specifically stabilize RNA tertiary structures. Studies have determined specific binding sites of Mg^{+2} ions for the folded conformations, but their role in RNA folding processes is still unclear.

Here, we use a computational approach that combines umbrella sampling with oscillating chemical potential Grand Canonical Monte Carlo/Molecular Dynamics (GCMC/MD) simulations to capture the atomic-level details of the intermediate states of RNA folding and identify the ion-RNA interactions that drive the folding pathway. The oscillating excess chemical potential allows for the sampling of Mg^{+2} ion distributions of the partially folded states of RNA without any bias from initial Mg^{+2}-RNA configurations. We investigate the unfolding of the twister ribozyme, along reaction coordinates similar to that used in experimental studies. We also simulate the ribozyme at different ion concentrations and capture the effect of ions on the folding potentials of mean force. Results reveal the stability of intermediate states and track the ion-mediated conformational changes. Overall, the present study establishes a better understanding of how Mg^{+2} ion-interactions contribute to RNA folding.

Background



Why do we care about how RNA folds?

- Huge diversity with combinations of secondary and tertiary interactions
- Misfolding of RNA impacts many diseases like cystic fibrosis etc
- Knowledge of RNA folding process is essential for development of novel drugs.

Metal ions in RNA folding

Metal ions Screen negatively charged phosphates of backbone

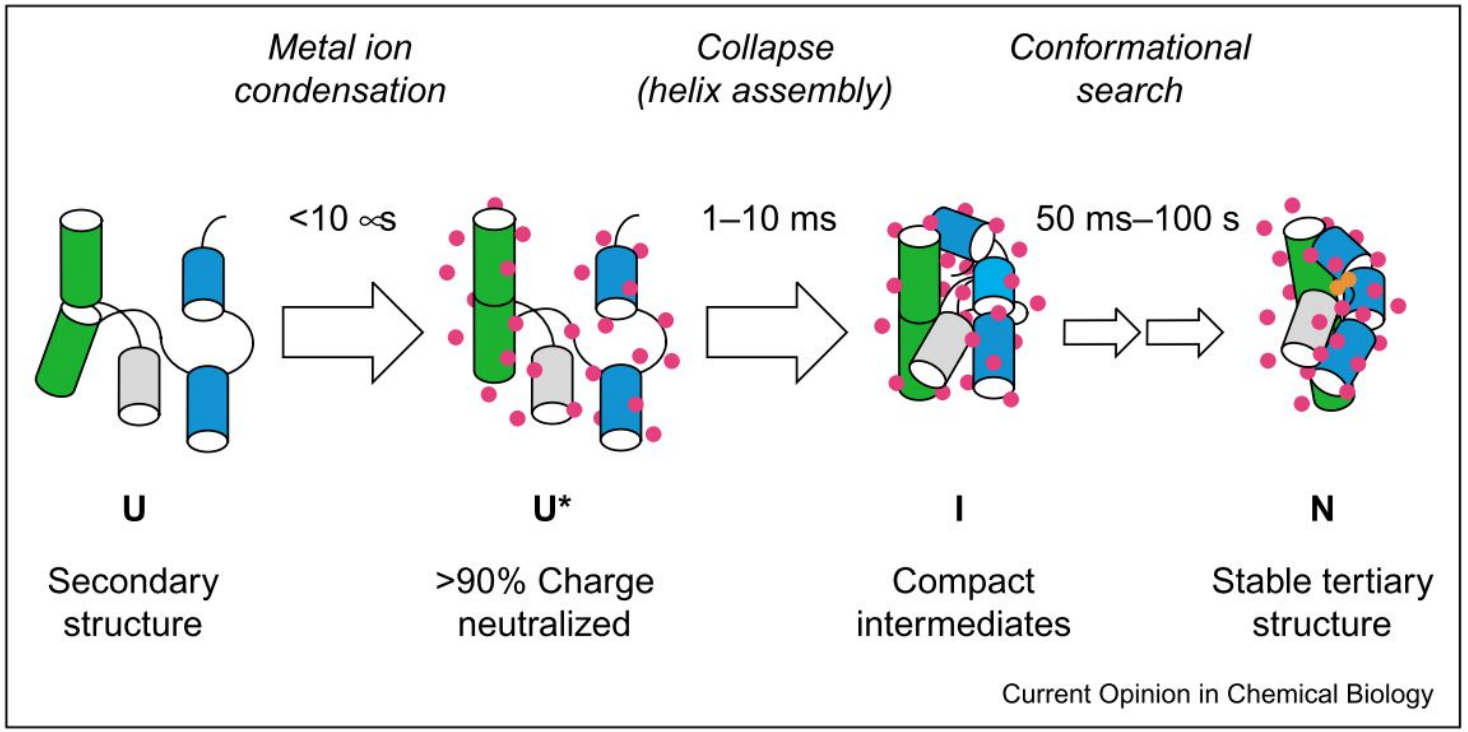


Image: Woodson, S. A. (2005). Current Opinion in Chemical Biology, 9(2), 104–109.

PROBLEM

Exchange rates

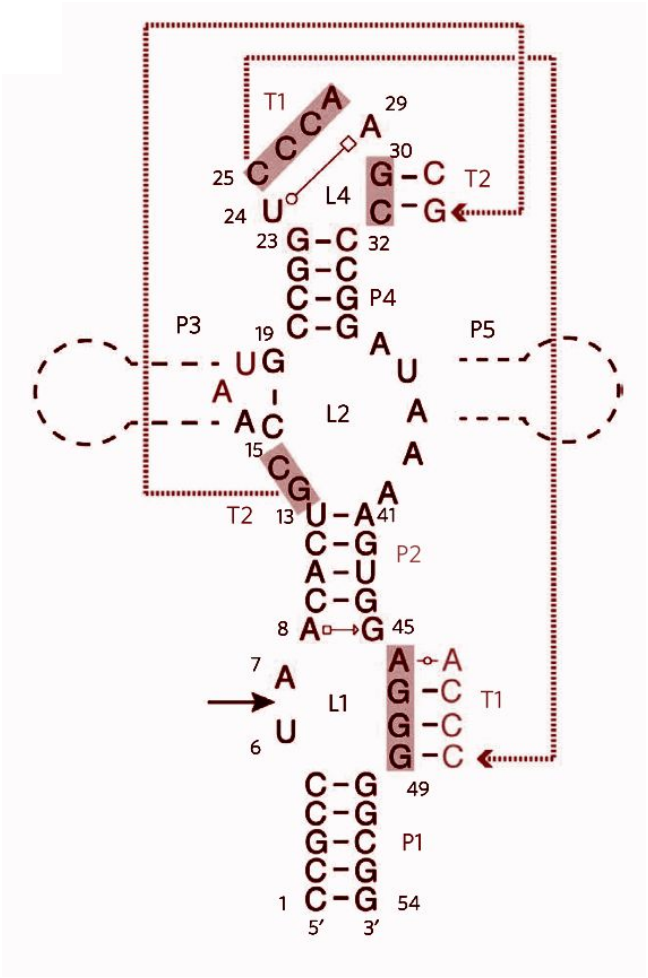
Water complexed with Mg^{+2} ions → μs time scale (6.7×10^5 s-1)

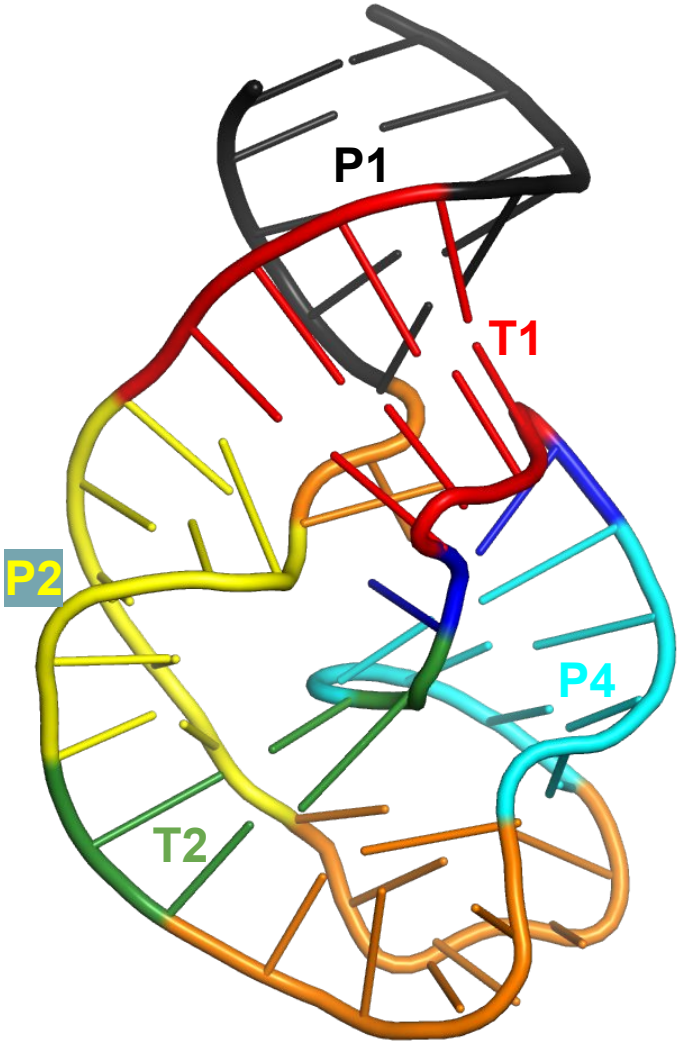
Mg^{+2} complexed with Phosphate → ms time scale (0.5 to 2.5×10^3 s-1)

Allnér et. al., *JCTC* 2012. **8**: p. 1493-1502.

Twister Ribozyme

PDB - 4OJI, 54 Nucleotides
5 Mg^{+2} ions : 2 direct contact + 3 diffuse ions





Liu et. al. (2014) *Nature Chemical Biology*, *10*(9), 739–744

Twister Ribozyme

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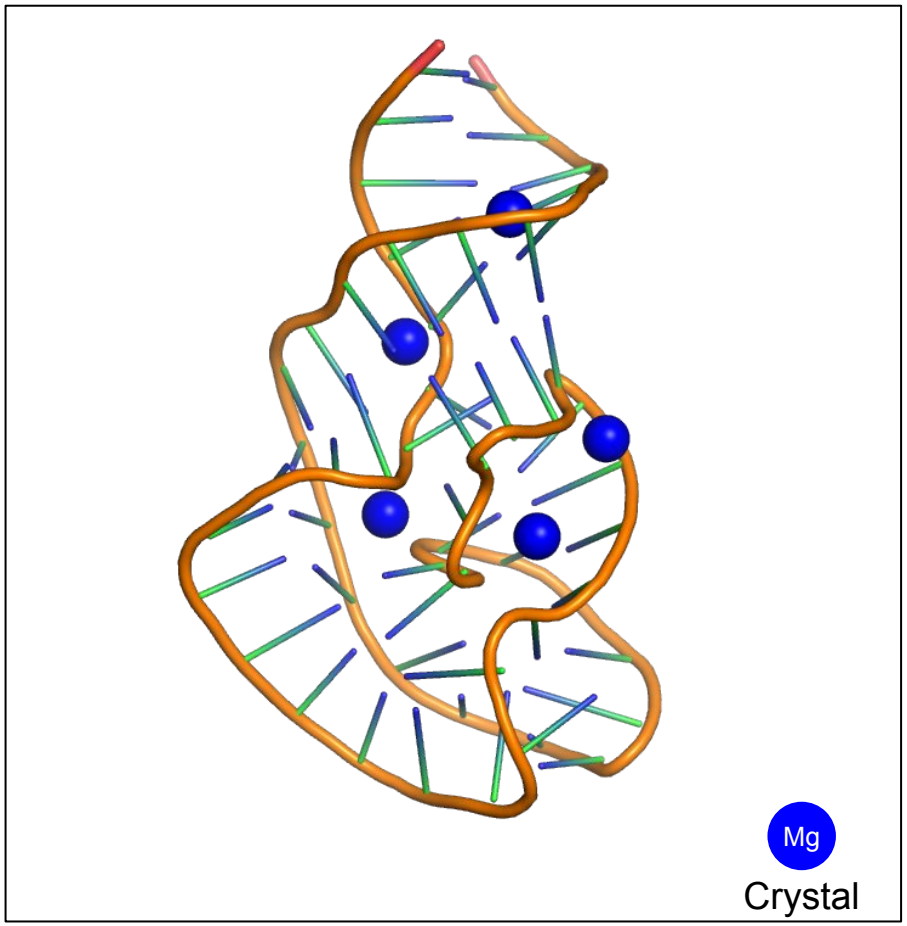


Systems for simulation:

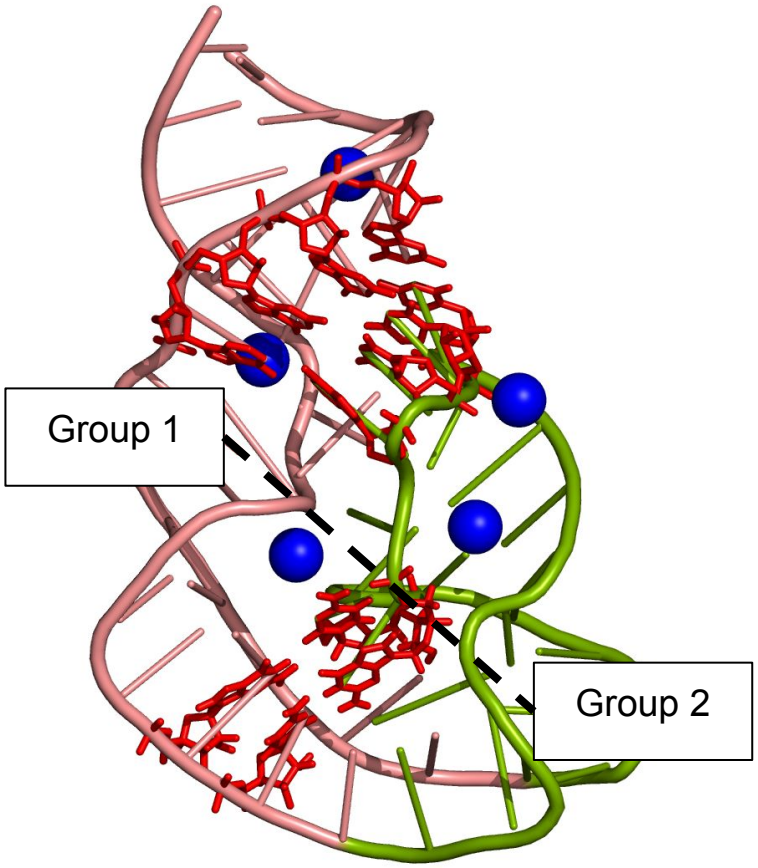
1. 0 mM $MgCl_2$ + 100 mM KCl
2. 10 mM $MgCl_2$ + 100 mM KCl
3. 20 mM $MgCl_2$ + 100 mM KCl
4. 100 mM $MgCl_2$ + 100 mM KCl

FF : CHARMM36 + modified-TIP3P
Boxsize : 90×90×90 Å (~ 68000 atoms)

100 ps NPT equilibration in CHARMM
3 replicates of 200-ns NPT in OpenMM

10 mM ~ 5 Mg^{+2} ions ~ crystal positions
20 mM ~ 8 Mg^{+2} ions ~ random positions
100 mM ~ 40 Mg^{+2} ions ~ random position

Umbrella sampling of twister ribozyme unfolding



Reaction Coordinate (RC)

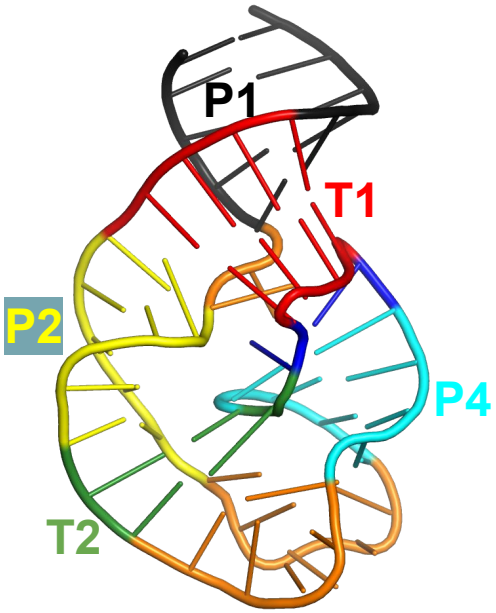
distance { com(group1), com(group2) }

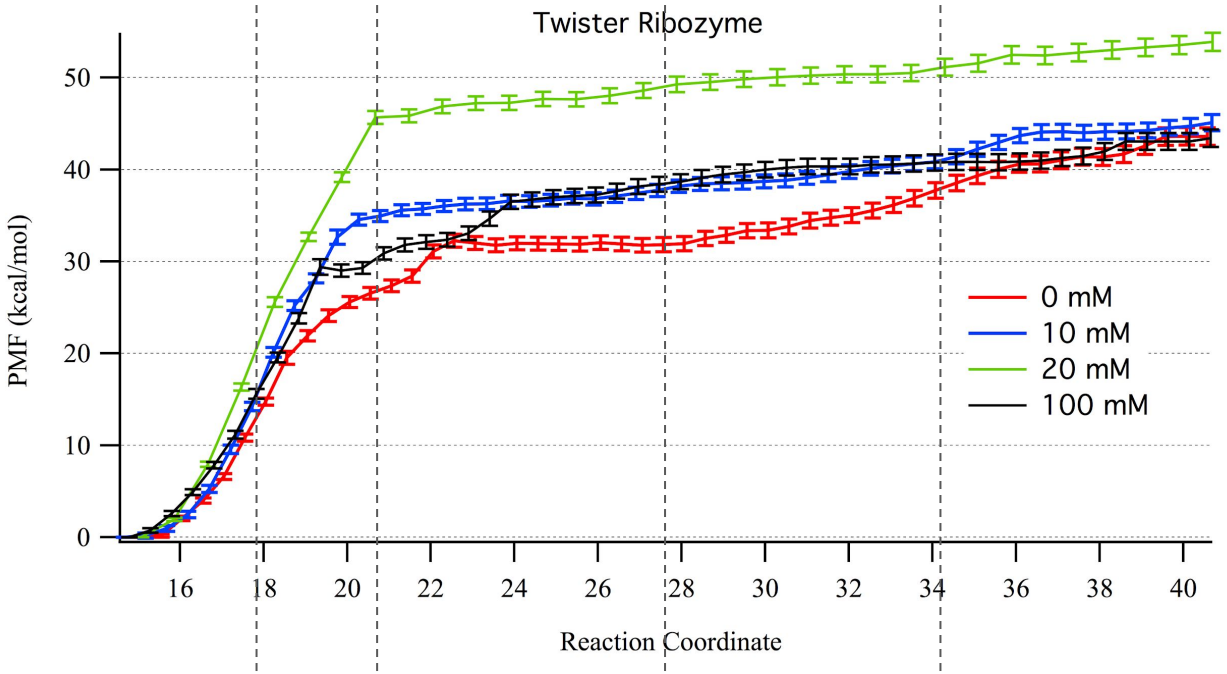
Umbrella sampling along COM-COM distance

- Windowmaking in CHARMM

  10 ps per window with restraints on secondary base pairs
- Production run for 10 ns per window in OpenMM with PLUMED

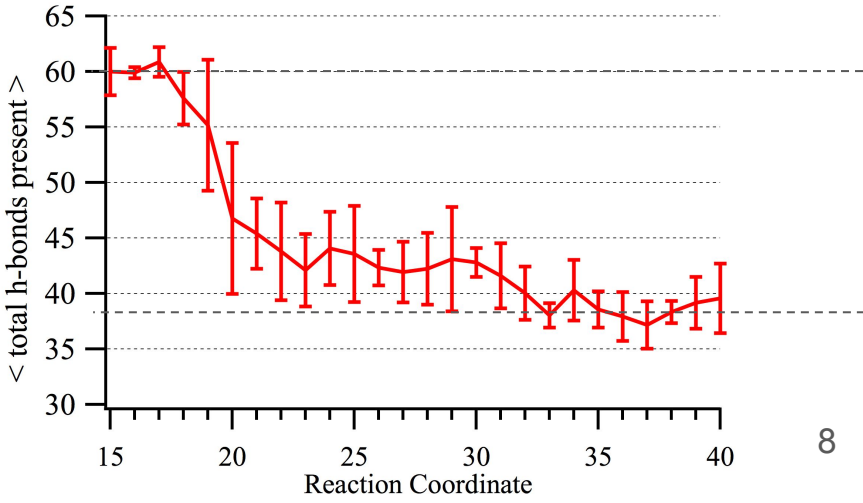
Mg^{+2} ion-interaction adds to the PMF for unfolding



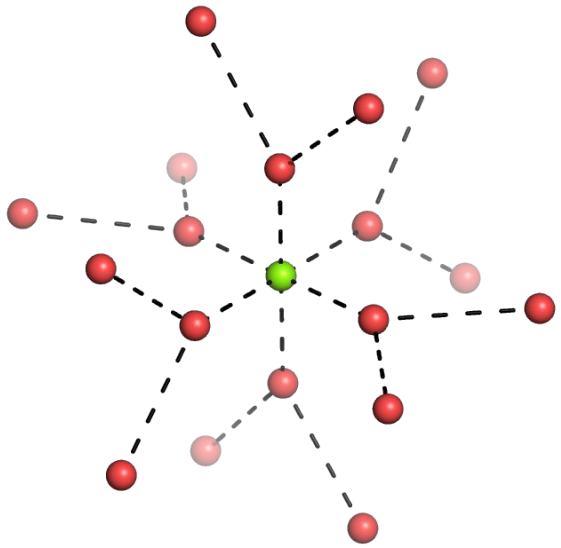


Free energy per hydrogen-bond ≈ -2 kcal/mol

Stofer et. al. 1999 *JACS* vol: 121 (41) pp: 9503-9508



Hydration of Mg^{+2} ion and coordination interactions



Mg^{+2} ion hydration scheme from DFT optimized geometry

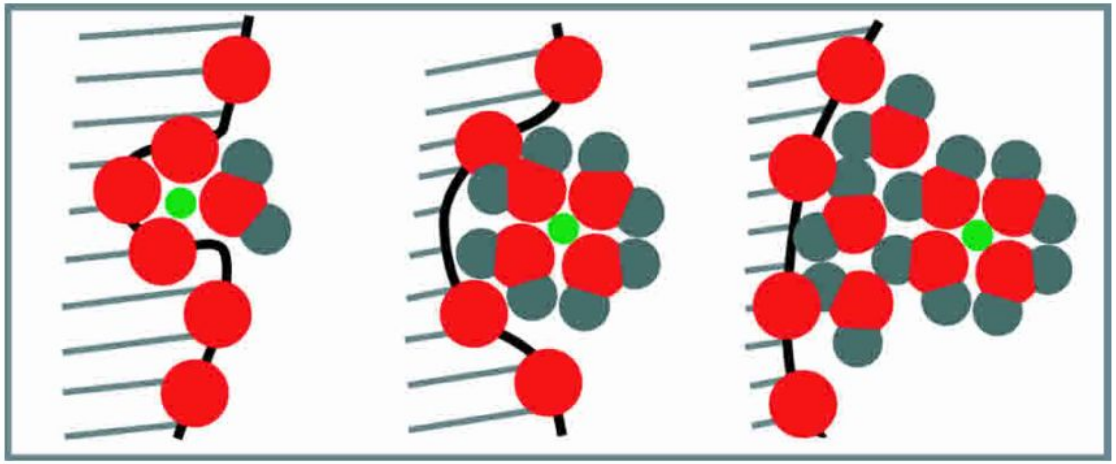


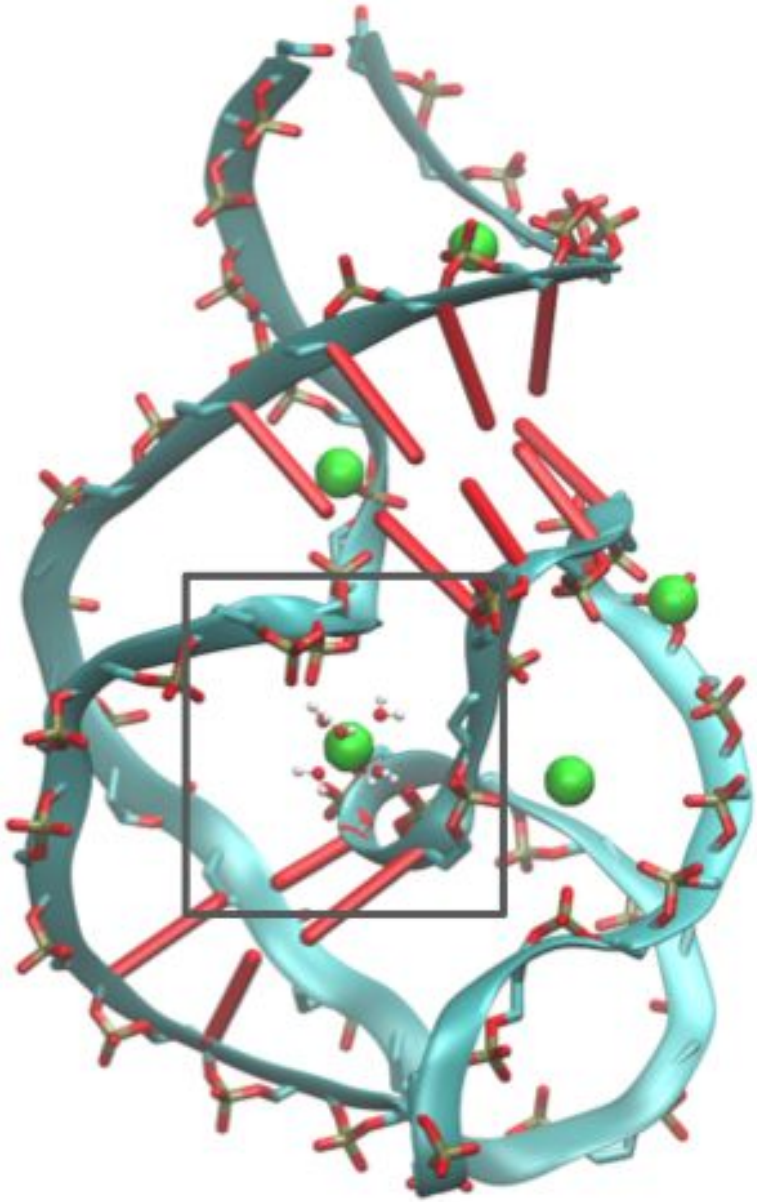
Image: Draper, D. E. (2004). A guide to ions and RNA structure. Rna, 10(3), 335–343.

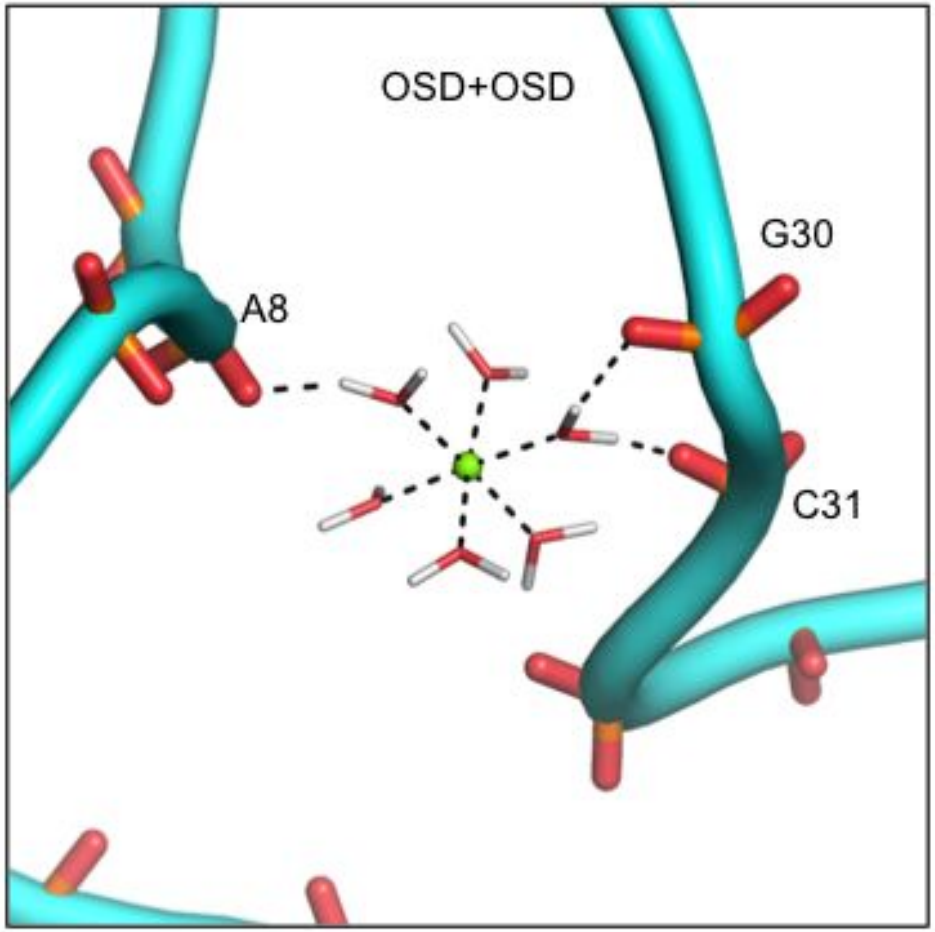
Inner-shell dehydration (ISD)

Outer-shell dehydration (OSD)

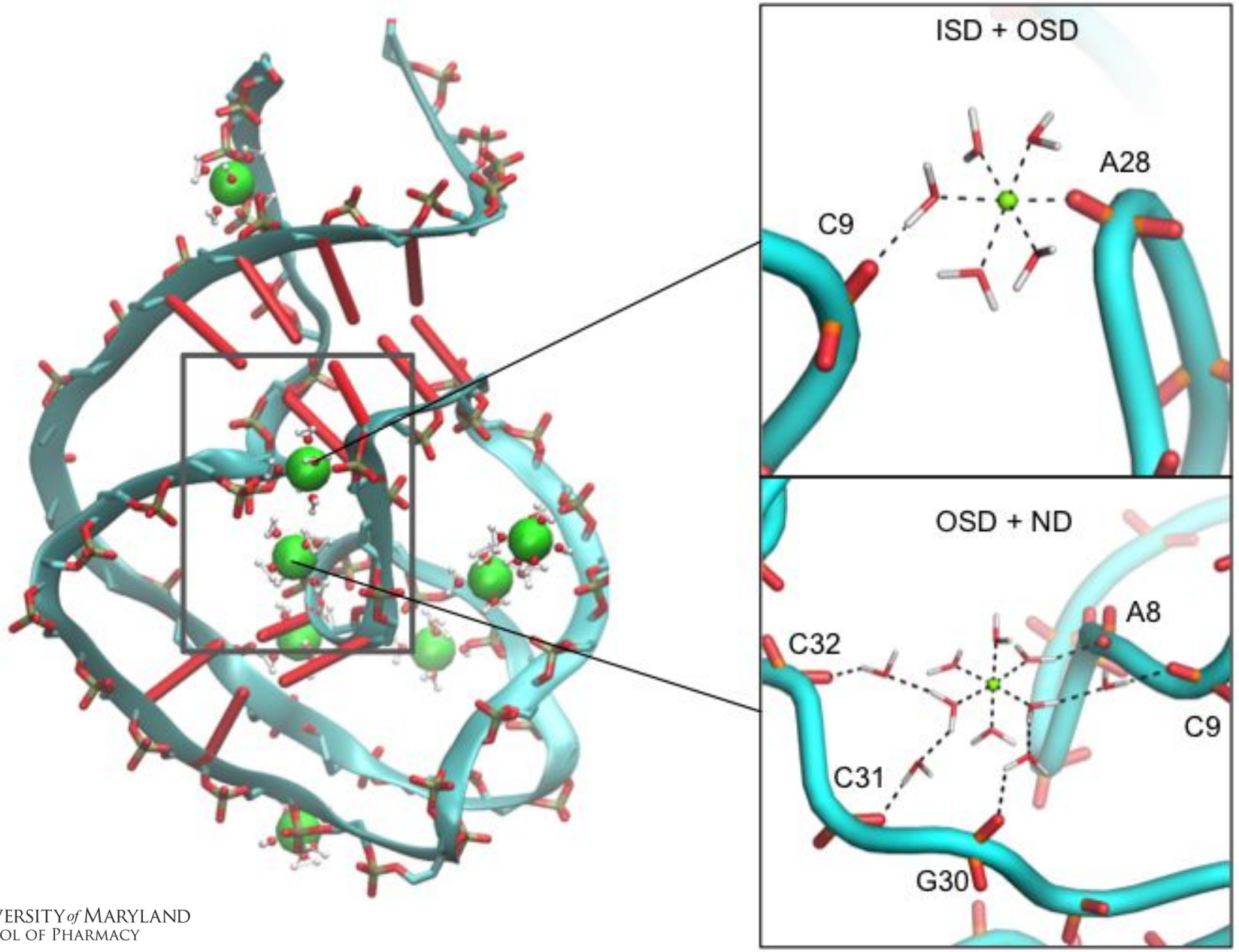
No dehydration (ND)

At 10 mM $MgCl_2$





At 20 mM $MgCl_2$



Validation of new GCMC-MD protocol

Harmonic restraints on the positions of RNA backbone atoms

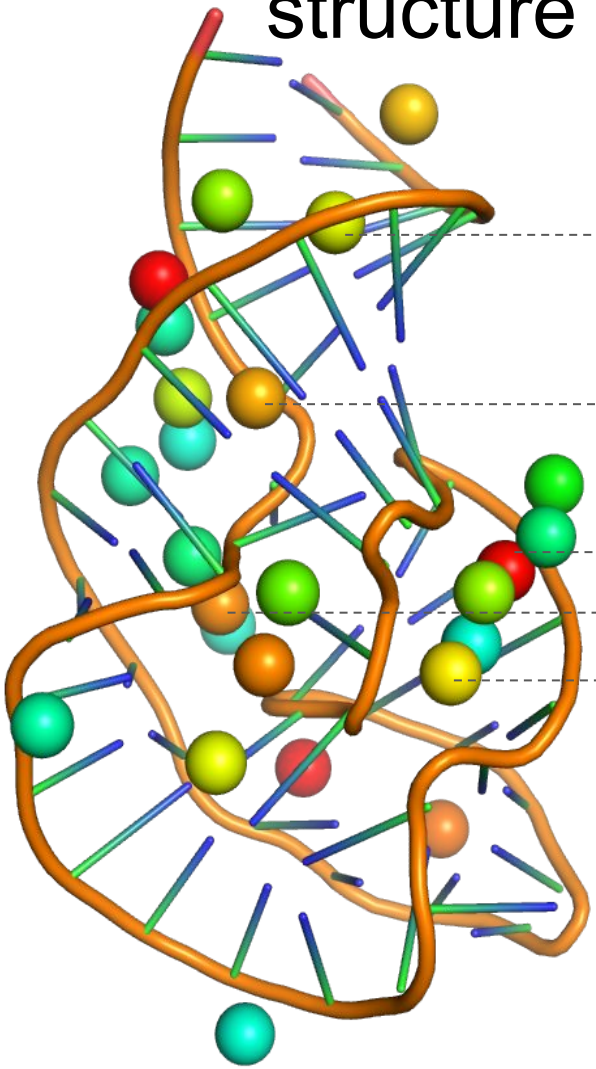
4 RNA structures with 50 mM $MgCl_2$ and 100 mM KCl, Box Size: cubic 65 Å

4 sets per RNA - 50 cycles per set

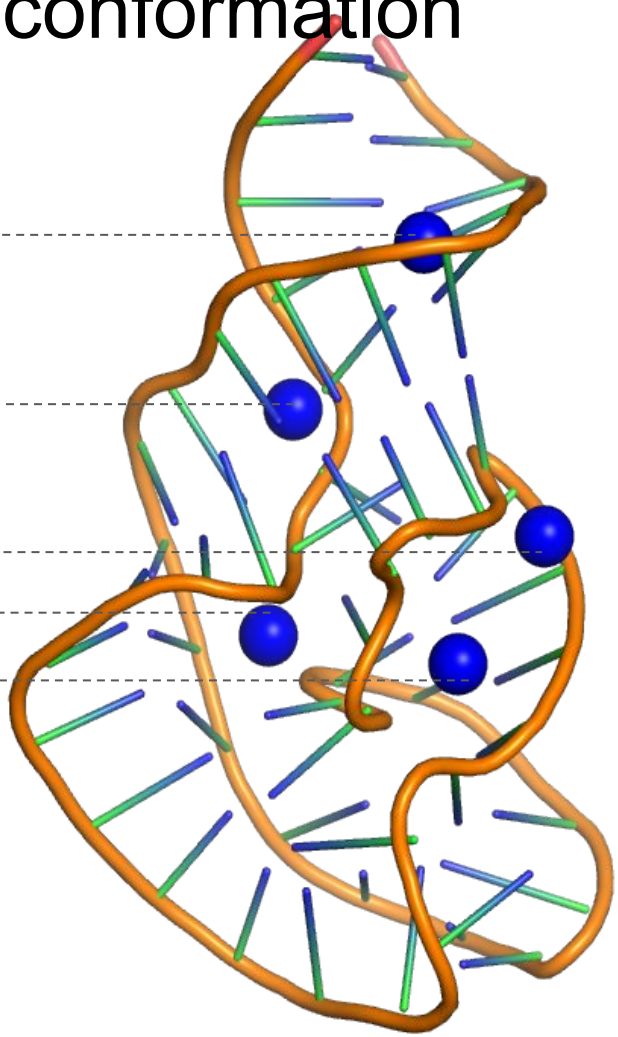
<u>One cycle includes:</u>

1. Delete Mg^{+2} and insert K^+ (Upto 200000 steps)
2. Rotate and translate all fragments (80000 steps)
3. Repeat 1 and 2 until the system in neutral
4. Insert Mg^{+2} and delete K^+ (Upto 200000 steps)
5. Rotate and translate all fragments (80000 steps)
6. Repeat 4 and 5 until the system is neutral
7. Minimize (5000 SD Steps), equilibrate (500-ps) and run production MD (5-ns) in OpenMM.

GCMC-MD identified MG^{+2} binding sites from crystal structure in native conformation



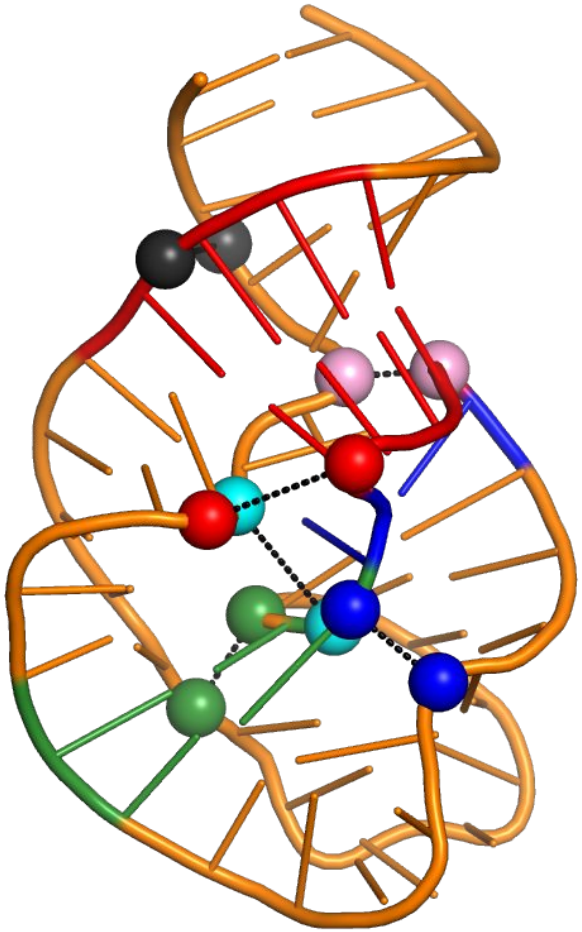
Cluster centers colored based on rank
Red being the most populated

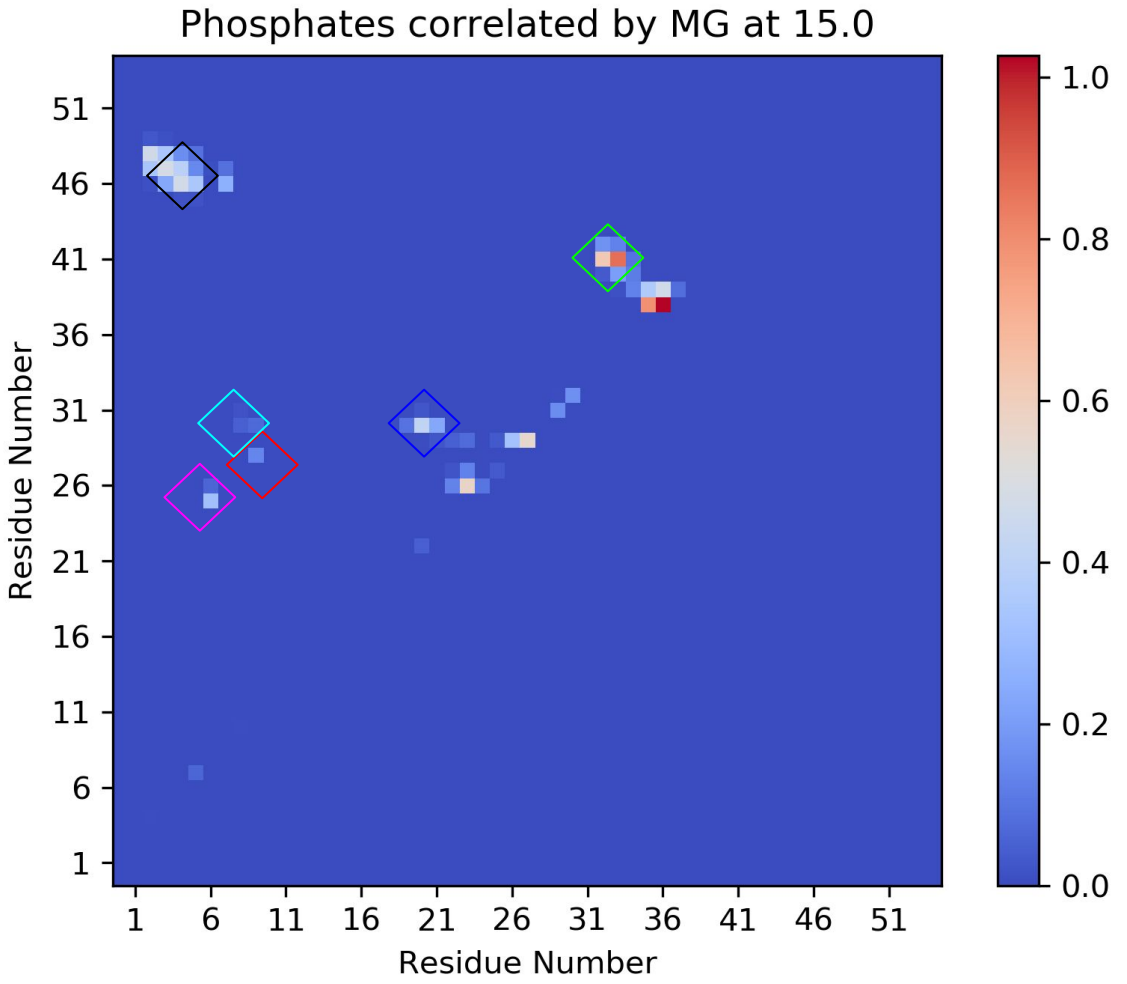


Crystal Structure

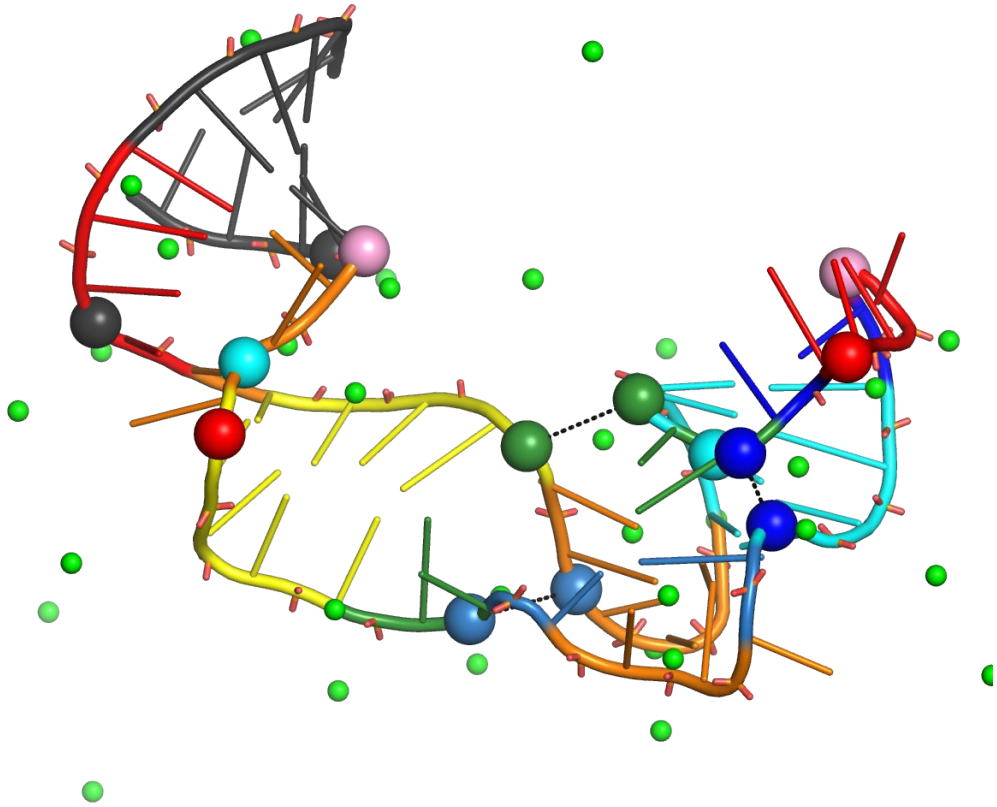
Mg
Crystal

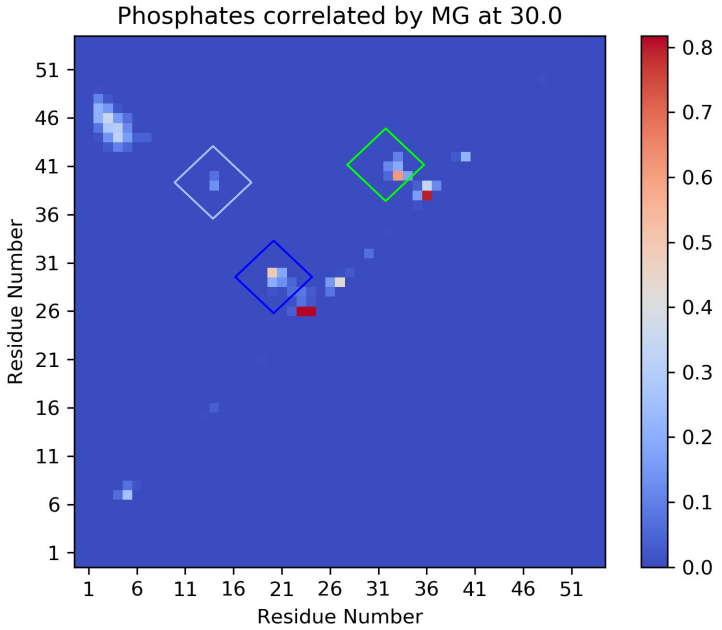
Pairs of Phosphates frequently coordinated by Mg^{+2} at native state

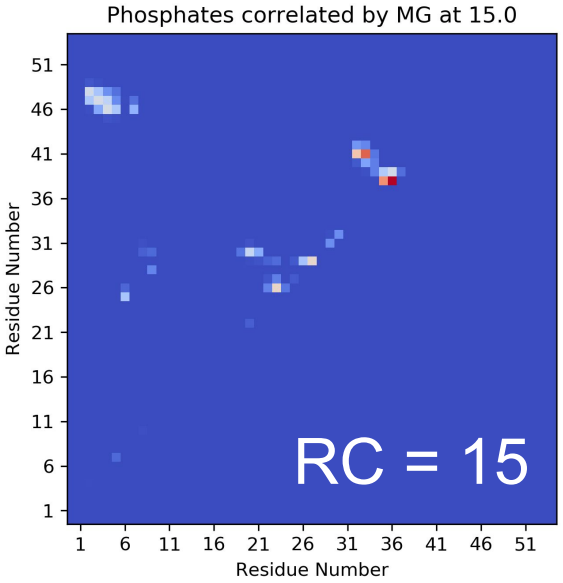


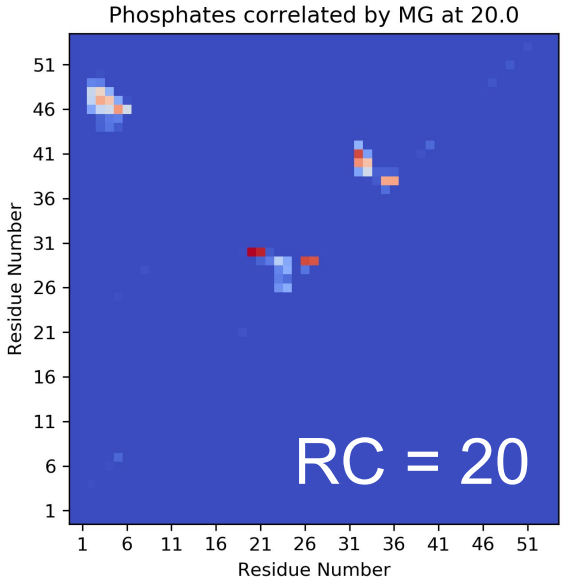


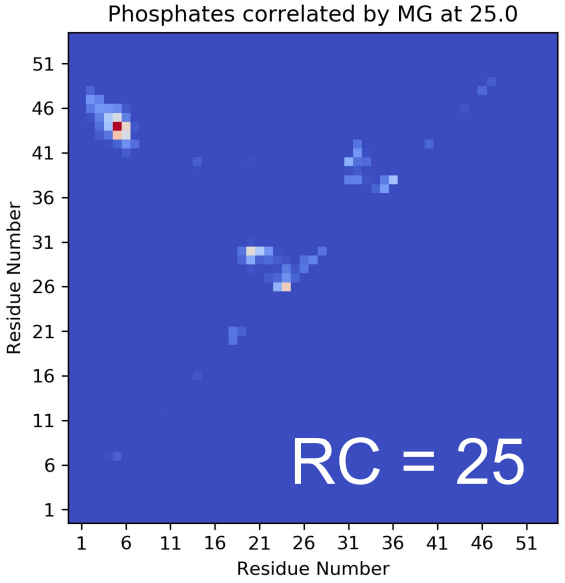
Pairs of Phosphates frequently coordinated by Mg^{+2} at intermediate stage

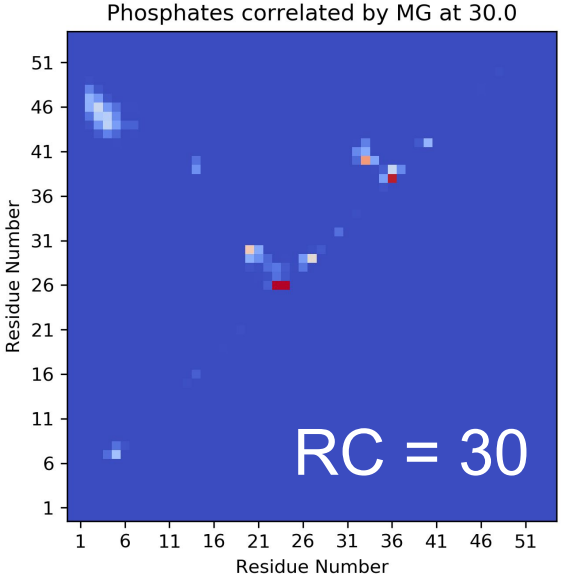


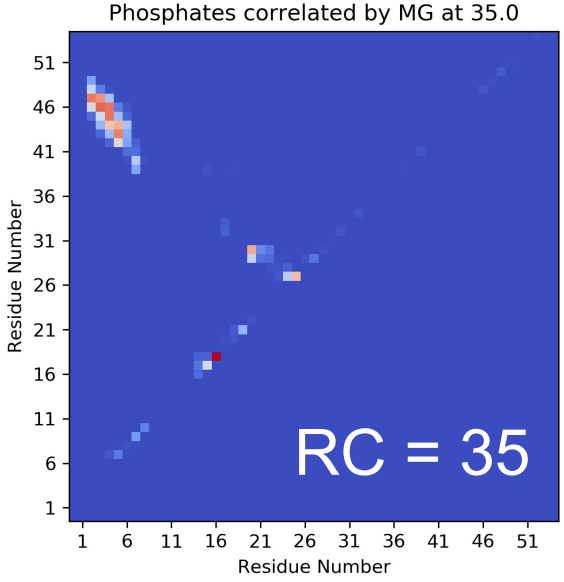


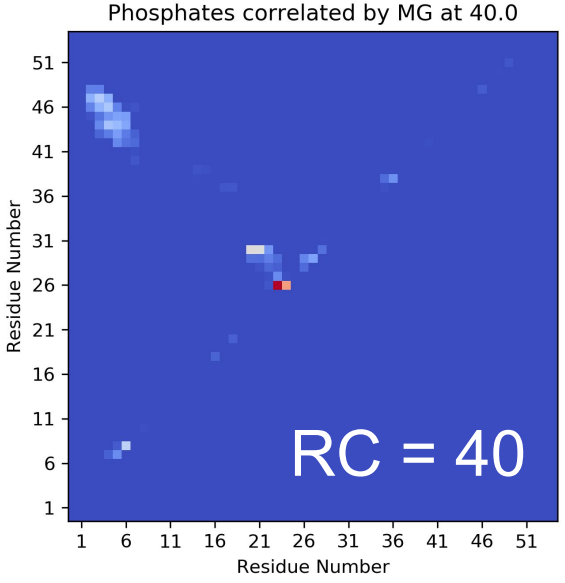












Frequency of two residues being coordinated by Mg^{+2} ions

Conclusions

- Umbrella sampling provided a thermodynamic estimate of how the various levels of Mg^{+2} ion-interactions contribute to potential of mean force to unfold the ribozyme.
- GCMC-MD allowed us to identify the pairs of phosphates being connected at various intermediate stages. Coordination properties of Mg^{+2} ions nucleate the folding of RNA by bringing two or more phosphate groups together.
- Overall, enhanced sampling with GCMC-MD provides better understanding of Mg^{+2} ion-interactions and their role by overcoming the time-scales of exchanging Mg^{+2} ions.