Published in final edited form as:

Methods Mol Biol. 2017 ; 1632: 1–17. doi:10.1007/978-1-4939-7138-1_1.

A new method to predict ion effects in RNA folding

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Summary

The strong interaction between metal ions in the solution and the highly charged RNA molecule is critical for RNA structure formation and stabilization. Metal ions binding to RNA can induce RNA structural changes that are important for RNA cellular functions. Therefore, quantitative modeling of the ion effects is essential for RNA structure prediction and RNA-based molecular design. Recently, inspired by the increasing experimental evidence that supports the importance of ion correlation and fluctuation in ion-RNA interactions, we developed a new computational model, Monte Carlo Tightly Bound Ion (MCTBI) model. The validity of the model is shown by the improved accuracy in the predictions for ion binding properties and ion-dependent free energies for RNA structures. In this chapter, using homodimeric tetraloop-receptor docking as an illustrative case, we show the MCTBI method for the computational prediction of the ion effects in RNA folding.

Keywords

Partition function; Ion-RNA interactions; Tightly Bound Ion model; RNA folding

1. Introduction

RNA functions are intrinsically related to RNA three-dimensional (3D) structures and their folding stabilities.^{1–5} In the folding process, counterions accumulate around RNA to neutralize and screen the strong intra-chain Coulomb repulsion between the negatively charged RNA backbone.^{6,7} Such an ion-mediated force is essential for the stabilization of RNA functional structures. Change of the ionic condition in the solution can shift the conformational equilibrium and induce RNA structural changes. In general, counterions around an RNA can be classified into three different states of RNA-binding. In one extreme, site-specific bound (or chelated) ions⁸ can be fully or partially dehydrated and trapped at specific binding sites (such as three-dimensional pockets in the RNA structure).⁹ The direct interaction between the bound ions and the RNA can often provide a stabilizing force for specific RNA tertiary contacts. In the other extreme, diffuse ions in the bulk solution away from the RNA surface usually remain hydrated and highly mobile.¹⁰ Unlike site-bound ions, diffuse ions are not in direct contact with RNA. However, the large number of diffuse ions can cause significant electrostatic effect and play a dominant role in the electrostatic contribution to RNA folding stability.

Between the site-bound ions and the mobile ions in the bulk solvent is the diffuse ions in close proximity of the RNA surface. The strong Coulomb attraction between the negatively charged polyanionic RNA and the positively charged diffuse ions can result in a high local concentration of the ions close to the RNA surface, especially around the electro-negative atoms or groups such as the phosphate groups.¹² The high ion concentration can lead to strong correlation between the ions through excluded volume and Coulombic interactions.^{11, 13, 14} The correlation effect is more pronounced for multivalent ions (e.g. Mg^{2+} ions) because they carry higher charges and hence involve stronger interactions. We call such strongly correlated diffuse ions as the Tightly Bound Ions (TBI) and the weakly correlated diffuse ions as diffusely bound ions (DBI). Physically, a TBI is less mobile than a DBI¹¹ due to inter-ion correlation.

Although the important role of ions in the RNA folding has been long recognized,^{15, 16} the problem of accurate and efficient modeling of the ion effects, especially for the multivalent ions such as Mg^{2+} ion, still remains.^{17–20} Molecular dynamics (MD) simulations can provide atomic details for the ion binding events.^{14, 21–23} However, the need to sample the huge ensemble of ion distributions renders the method to be computationally challenging.²⁴ Analytical models, such as the classical Counterion Condensation (CC) theory²⁵ and the nonlinear Poisson–Boltzmann (NLPB) theory,^{26–29} can predict the ion effects through more efficient computations. However, these methods neglect the correlation and fluctuation effects of ions, resulting in underestimation of the Mg^{2+} ion effects in RNA folding stability.³⁰

Inspired by the importance of the correlation and fluctuation effects for multivalent ions (such as Mg^{2+} ions) in RNA folding,^{14, 30, 31} several new theories have been developed.^{32–34} For example, the three-dimensional reference interaction site model (3D-RISM) uses Ornstein and Zernike integral equation theory to account for the ion-ion correlation.³³ A generalized counterion condensation theory explicitly treats Mg^{2+} ions and ion-ion correlations³⁴ with the framework of the counterion condensation theory. A hybrid model, Tightly Bound Ion (TBI) model,¹¹ was proposed to account for the discrete, correlated, many-body distributions for the (strongly correlated) TB ions. Since the model was first reported in 2005, it has been under continuous development in the past decade for better accuracy and efficiency.^{11, 30, 35–40}

Recently, we developed a new generation of the TBI-like model, Monte Carlo Tightly Bound Ion (MCTBI) model.⁴¹ A major advance in this new model is the new Monte Carlo-based sampling algorithm for the ion distribution. Specifically, we applied a two-step (“insertion–deletion”) algorithm to sample the distribution of the TB ions: We first generate the TB ion distributions by randomly inserting the ions one by one, we then remove unstable (high energy) ions randomly. We apply the Metropolis Monte Carlo algorithm for random sampling. This new enhanced sampling method enables us to treat large RNA structures (> 200 nts) that have been unattainable with the previous generations of the TBI model. Furthermore, the MCTBI model, which is based on much higher spatial resolution for the ion distributions than the previous model, offers a useful tool to predict the ion binding sites with higher accuracy.

In this article, using the tetraloop–receptor (TL–R) complex as an illustrative example, we introduce the MCTBI-based method to predict ion-mediated electrostatic effects in RNA folding. We select the TL–R complex because of its widespread structural and functional roles.^{42–47} Experimental results have shown that high Mg^{2+} concentration (several millimolar)^{46, 47} can help stabilizing the docked homodimeric TL–R complex structure. Our computational method can provide quantitative insights into the contributions of Mg^{2+} ions in the homodimeric docking process.

2. RNA structure

To compute the ion effects for a given RNA structure, we first need to have the PDB file for the RNA structure. For known (experimentally determined) structures, such as the docked structure of the homodimeric TL–R complex (see Fig. 1; PDB ID: 2I7Z⁴⁷), the structure files can be downloaded from the Protein Data Bank (PDB) website (see Note 1). The homodimeric TL–R complex is comprised of two identical chains. In this chapter, we will compute the $[\text{Mg}^{2+}]$ -dependence of the electrostatic free energy of the dimer system as a function of the inter-molecular separation as characterized by the distance R between the two (identical) monomeric TL–R structures. Here R is determined from the distance between the centers of mass of the two monomeric TL–R structures (see Note 2). We set R equal to 0 for the reference state, the native docked state of the homodimer (see Fig. 1; PDB ID: 2I7Z⁴⁷).

For system whose RNA structures are not available in the PDB database, we can build the all-atom 3D structure using structure prediction models^{49–55} such as the Vfold model⁵⁶ (see Note 3): We first input the RNA sequence into the Vfold2D module (see Fig. 2A and Note 4). For the given sequence, Vfold2D module predicts the most probable 2D structure (base pairs) of the RNA (see Fig. 2B). We then provide the sequence and the predicted 2D structure (base pair) information to the Vfold3D module (see Fig. 2C and Note 5). Finally, the Vfold3D module may output the 3D structures for the given RNA sequence (Fig. 2D). The basis of the Vfold for 3D structure construction is to use the rigid A-form helix for the helical part and PDB-derived templates for loops/junctions. Alternative, without using the loop/junction template structures, we can use the coarse-grained RNA folding model to generate the ensemble of loop/junctions conformations and then add all atoms to the coarse-grained conformations.^{57, 58}

In the current version of the MCTBI model, coarse-grained charge model is used in the assignment of RNA charges (see Note 6). Moreover, ions are assumed to remain hydrated (see Note 7).

¹URL: <http://www.rcsb.org/pdb/home/home.do>

²The two chains are restricted to keep parallel configurations without rotation. The distance R is defined as $R = R_{\text{undocked}} - R_{\text{docked}}$, where R_{undocked} and R_{docked} are the distances between the centers of mass of the two monomeric structures for the undocked and the docked states, respectively.

³URL: <http://rna.physics.missouri.edu/index.html>

⁴The sequence is 5' AUGCAUGCAUGCAAAGCAUGCAUGCAU3'.

⁵For the predicted 2D structure, the basepair information is (((((((((((.....)))))))))), where a symmetric pair of “(” and “)” indicates a base pair between the corresponding nucleotides. Unpaired nucleotides are represented by dots.

⁶As a coarse-grained RNA charge model, each phosphorus atom is assumed to carry an electronic charge ($-e$) and the rest atoms are assumed to be neutral.

3. The Monte Carlo Tightly Bound Ion (MCTBI) model

We compute the electrostatics free energy G of the whole system (RNA and the ionic solution) from the partition function Z : $G = -\ln(Z)$. In this section, we focus on the calculation of the partition function for a given RNA structure in an ionic solution.

3.1 Classification of tightly bound (TB) and diffusely bound (DB) regions

In the MCTBI model, the space around RNA is classified into two regions: a Tightly Bound (TB) region for the TB ions (strong correlation) and a Diffusely Bound (DB) region for the DB ions (weak correlations). The correlation for monovalent ions such as K^+ is usually weak because of its relatively weak Coulomb interactions. We treat all the monovalent ions as DB ions. In contrast, multivalent ions such as Mg^{2+} ions accumulated in the close vicinity of RNA surface usually involve strong Coulomb interactions and are more likely to be strongly correlated. Therefore, as shown in the steps listed below, we classify the TB and DB ions (regions) according to the multivalent ions such as Mg^{2+} ions.

1. To minimize the boundary effect, the RNA is placed at the center of a $L \times L \times L$ box whose size L is larger than 6 times of Debye length.¹¹
2. We estimate the spatial distribution of the Mg^{2+} ion concentration in the whole box by solving non-linear Poisson-Boltzmann (NLPB) equation.
3. According to the Mg^{2+} ion concentration and the corresponding spatial distribution of the ion-correlation strength, we divide the whole box into the TB region around the RNA surface for the TB ions and the DB region away from the RNA surface for the DB ions (*see* Note 8). Depending on the RNA structure and the ion concentrations, the TB region can be a thin layer (with $\sim 1-5$ Å) around the RNA.

The TB region is dependent on the RNA structure and the bulk ion concentrations, as shown in Fig. 3. Compared with an extended structure, a compact structure has a higher RNA (negative) charge density and thus a stronger tendency to induce ion accumulation around the RNA. Therefore, the TB region of a compact structure is usually larger than that of an extended structure, especially for a dilute Mg^{2+} solution (*see* Fig. 3A and C). Moreover, comparisons between Figs. 3A and C and Figs. 3B and D show that a high (bulk) ion concentration leads to a higher affinity for ion binding and thus a larger TB region.

⁷The radii of the three ion species are $r_{Mg^{2+}} = 4.5$ Å, $r_{K^+} = 4.0$ Å, and $r_{Cl^-} = 4.0$ Å, respectively.

⁸The ion-correlation strength $\Gamma(\mathbf{x})$ at position $\mathbf{x}$ is determined by the local ion concentration $c_{2+}(\mathbf{x})$:

$$\Gamma(\mathbf{x}) = \frac{(ze)^2}{\epsilon_w a_{ws}(\mathbf{x})}, \quad \frac{4\pi}{3} [a_{ws}(\mathbf{x})]^3 [c_{2+}(\mathbf{x}) - c_{2+}^0] = 1$$

Here, ze and ϵ_w ($=78$) are the charge of an ion ($z = 2$ for Mg^{2+}) and the dielectric constant of water. Position $\mathbf{x}$ belongs to the TB region if $\Gamma(\mathbf{x}) > 2.6$ and DB region otherwise.¹¹

After establishing the demarcation between the TB and DB regions, the partition function of the whole system, including the RNA and the ionic solution, can be calculated from the sum over N_b , the number of the TB ions:

$$Z = \sum_{N_b} Z_b(N_b) \times Z_d(N_d)$$

Here, Z_b and Z_d are the partition functions of the TB and the DB ions, respectively. The average interaction between the TB and DB ions are considered through an approximation in Z_d . In the above formula, N_d is the numbers of the DB ions. The charge neutrality condition gives $N_{2+} = N_b + N_d$, where N_{2+} is the total number of Mg^{2+} ions in the solution. We first compute Z_b with the different TB ion numbers N_b . We then calculate Z_d for the DB ions for each given N_b .

3.2 Partition function of tightly bound (TB) ions

The partition function is the ensemble average of the Boltzmann weight over all the possible ion distributions. To compute the TB ion partition function Z_b , we first need to sample the TB ion distributions. We use a simple cubic lattice grid to configure the ion coordinates in the TB region. For a given RNA with fixed N phosphates, the number of TB (Mg^{2+}) ions N_b is assumed to vary from 0 to N . Each grid site in the TB region can be occupied by at most one ion. Following the Rosenbluth–Rosenbluth (RR) method,⁵⁹ the partition function Z_b for an N_b -ion system can be calculated through a process with ions added one by one (see Note 9). However, the original RR method neglects the backtrack effect, namely, the redistribution of the pre-inserted ions in response to the insertion of a new ion. To account for the backtrack effect, we developed the following “insertion-deletion” algorithm for the computation of the partition function for the TB ions.⁴¹

1. For the insertion of the i th ion ($i = 1, 2, \dots, N_b$), we enumerate all the available sites $k = 1, 2, 3, \dots, m_i$ in the TB region, where m_i is the number of available sites for the ion. We calculate the total interaction energy $U_i(k)$ (see Note 10) between the i th ion (if the ion is placed at site k) and the other charged particles (phosphates and the pre-existing TB ions).
2. From the energy $U_i(k)$, we compute the probability $p_i(i, k)$ for placing the i th ion at site k (see Note 11). We then use Monte Carlo sampling method to

⁹ Assuming the TB region has totally N_s lattice grid sites, the sequentially added 1st, 2nd, ..., N_b -th TB ion would have $m_1 = N_s$, $m_2 = N_s - 1$, ..., $m_{N_b} = (N_s - N_b)$ available sites. In an ideal situation, if all the particles in the TB region are electrically neutral, the partition function of the N_b -ion system would be equal to

$$Z_b(N_b) = \frac{\prod_{i=1}^{N_b} w(i)}{N_b!} = \frac{\prod_{i=1}^{N_b} m_i}{N_b!} = 1$$

Here for non-interacting “ions”, the statistical weight for the i -th inserted ion is $w(i) = i$.

¹⁰ The total interaction energy includes Coulombic energy, Lennard–Jones (LJ) potential, and polarization energy.

¹¹ $p_i(i, k) = e^{-U_i(k)/k_B T} / \sum_k e^{-U_i(k)/k_B T}$ for the i th ion located at position k .

randomly select the a site for the i th ion according to the probability $p_i(i, k)$. The low energy sites are more likely be visited by the ions.

3. The TB ions are added to the TB region one by one until all the N ions are inserted ($N_b = N$).
4. We remove the ions one by one from the N -ion distribution generated in the above steps. The sampling is biased toward the removal of higher energy ions (see the probability function p_b in *see Note 12*).
5. With the removal of 0, 1, 2, ..., ($N - i$), ..., $N - 1$ ions (one by one), low-energy distributions are generated for N , ($N - 1$), ($N - 2$), ..., i , ..., 1-ion systems. For a distribution of i -ion system, after the ion to be removed is decided, we can calculate the statistical weight for this ion from the sum over all its m_i available sites: $w(i) = \sum_{k=1}^{m_i} e^{-\Delta U_i(k)}$
6. We repeat steps 4–5 for a large number (M_b) of times and compute the average weight $\bar{w}(i)$ ($i \in [1, N_b]$).
7. Repeat all the above steps for a large number (M_f) of times. The average gives the partition function of the N_b TB ions:

$$Z(N_b) = \frac{1}{M_f} \sum \frac{\prod_{i=1}^{N_b} \bar{w}(i)}{N_b!}$$

Through extensive tests, we find that $M_b = N$ and $M_f = N_s$ can provide the optimal balance between efficiency and accuracy.⁴¹

3.3 Partition function of diffusely bound (DB) ions

The partition function $Z_d(N_d)$ of DB ions is dependent on the distribution of the TB ions in the TB region. However, given the large number of the TB ion distributions, solving NLPB calculation for each TB ion distribution can be computationally demanding. Therefore, we treat the DB ions with an approximation.

1. From the ensemble of TB ion distributions (see steps 1–7 above), for each N_b , we calculate the Boltzmann-averaged mean distribution for the TB ions.
2. Based on the mean distribution of the N_b TB ions, we solve the NLPB equation. From the solution of the NLPB for the DB ion distribution, we calculate the free energy (*see Note 13*). G_d ¹¹
3. We compute the partition function of the DB ions: $Z_d(N_d) = e^{-G_d/k_B T}$.

¹² $p_b(j, k) = e^{-U_j(k)/k_B T} / \sum_k e^{-U_j(k)/k_B T}$ for the ion j at position k .

¹³ G_d of the DB ions is the sum of the entropic free energy of the diffuse ions (the electrostatic energy), including the (average) electrostatics interaction energy between TB ions and DB ions.

4. Results

4.1 Free energy landscape for the homodimeric TL-R complex

As a simplified approach to the conformational sampling of the system, we fix the orientations of each monomeric structure and change the separation distance between the two structures. Specifically, we increase the R value from 0 Å to 15 Å. The conformational ensemble corresponds to a specific pathway of the docking of TL-R complex. For each R , we compute the electrostatic free energy of the homodimeric system. In our calculation, we fix the K^+ concentration at 20 mM and increase the Mg^{2+} concentration from 0 mM to 20 mM. To investigate the contribution of the Mg^{2+} ions to the docking of homodimeric TL-R complex, we calculate the electrostatics free energy difference between systems with and without Mg^{2+} ions: $G = G([Mg^{2+}]) - G(0)$. The result of G for the various $[Mg^{2+}]$ and R are shown in Fig. 4. From the free energy landscape, we can also compute ion-dependence of the conformational distribution. The energy landscape shows that a higher $[Mg^{2+}]$ would results in a lower free energy G and the docked state ($R = 0$) is at the bottom of the landscape. The result suggests that Mg^{2+} ions promote the docking of the TL-R complex.

4.2 Distribution of the bound ions

From the statistical weight for each TB ion distributions, we can calculate the probability $p(k)$ for a TB ion to be placed at grid site k (see Note 14). The information about ion distribution can provide the mechanism for ion-mediated RNA stability and can further offer guidance for the computational design of RNA structure and ionic conditions for stabilizing or destabilizing specific functional motif. Based on the the experimental solution (20 mM K^+ and 16 mM Mg^{2+}),⁴⁷ we predict the distribution of the TB ions; See Fig. 5. We find that the ion binding probability decreases toward the outer boundary of the TB region (see Fig. 5A), indicating that as the ions move toward the boundary of the TB region, the ion concentration approaches that of DB ions. This result suggests the possible continuity for the transition TB→DB in the interfacial regime. Furthermore, MCTBI-predicted most probable ion binding sites are in overall agreement with the experimentally observed site-specific ion binding sites (see Fig. 5B), suggesting that the non-specific electrostatic interactions help the stabilization of site-specific bound ions.

Acknowledgments

This research was supported by NIH grant GM063732.

¹⁴The ion occupancy probability is calculated from the following formula:

$$p(k) = \sum_{N_b=0}^N \frac{n(N_b, k)}{M_f \times M_b} \times \frac{Z(N_b, N_d)}{Z}.$$

Here $n(N_b, k)$ is the number of N_b -ion distributions (out of the totally $M_f M_b$ sampled distributions) with site k occupied by a TB ion.

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Figure 1.
The secondary structure (A) and 3D structure (B) of homodimeric TL-R complex (PDB ID: 2I7Z⁴⁷). The GAAA tetraloop and receptor are highlighted in red and green, respectively.

Vfold2D (version 2.0): Predicting RNA 2D structures

(1) Temperature °C

(2) Energy parameters for base stacks, including mismatched base stacks:

* from the Turner parameters (04 version)

☐ from the MFOLD (2.3 version)

(3) Enter sequence (A,a,U,u,G,g,C,c):

(4) Job name (alphanumeric characters only):

(5) Your email address (optional, you will receive the results by email, if provided):

(A)

Vfold3D: predicting RNA 3D structure

(1) Enter sequence(A,a,U,u,G,g,C,c):

(2) Input 2D structure, without cross-linked base pairs, in dot-bracket format:

(3) Job name (alphanumeric characters only):

(4) Your email address (optional, you will receive the results by email, if provided):

(C)

Figure 2.

(A) Ascreenshot of the Vfold2D module. The sqeunce should be input in the place highlighted in red. (B) The predicted 2D structure for the sequence. (C) The screenshot of the Vfold3D module. The sqeunce and the basepair information should be input in the places highlighted in red. (D) The 3D structures predicted by the Vfold3D module.

Figure 3.

The TB regions of the homodimeric tetraloop-receptor structure in the docked state at $[Mg^{2+}] = 10 \mu M$ (A) and $[Mg^{2+}] = 10 mM$ (B), and in the undocked state ($R = 15 \text{ \AA}$) at $[Mg^{2+}] = 10 \mu M$ (C) and $[Mg^{2+}] = 10 mM$ (D)

Figure 4.

The electrostatic free energy landscape as a function of Mg^{2+} bulk concentrations and the distances between the two monomeric TL–R structures. The units for the free energy (and the color scale), Mg^{2+} ion concentration, and the inter-molecular distance R are $k_B T$ (at room temperature), M, and Å, respectively.

Figure 5. Predicted TB Mg^{2+} ion distribution in a solution with 16 mM Mg^{2+} and 20 mM K^{+} . (A) and (B) show the “far-field” and “near-field” views of the Mg^{2+} ion distribution in the TB region, respectively. The cyan balls and yellow backbone show the site-specific binding ions and docked TL–R complex structure (PDB ID: 2I7Z).