

# Competitive Binding of $Mg^{2+}$ and $Na^+$ Ions to Nucleic Acids: From Helices to Tertiary Structures

Kun Xi,<sup>1</sup> Feng-Hua Wang,<sup>2</sup> Gui Xiong,<sup>1</sup> Zhong-Liang Zhang,<sup>1</sup> and Zhi-Jie Tan<sup>1,\*</sup>

<sup>1</sup>Center for Theoretical Physics and Key Laboratory of Artificial Micro- and Nano-structures of Ministry of Education, School of Physics and Technology, Wuhan University, Wuhan, China and <sup>2</sup>Engineering Training Center, Jiangnan University, Wuhan, China

**ABSTRACT** Nucleic acids generally reside in cellular aqueous solutions with mixed divalent/monovalent ions, and the competitive binding of divalent and monovalent ions is critical to the structures of nucleic acids because of their polyanionic nature. In this work, we first proposed a general and effective method for simulating a nucleic acid in mixed divalent/monovalent ion solutions with desired bulk ion concentrations via molecular dynamics (MD) simulations and investigated the competitive binding of  $Mg^{2+}/Na^+$  ions to various nucleic acids by all-atom MD simulations. The extensive MD-based examinations show that single MD simulations conducted using the proposed method can yield desired bulk divalent/monovalent ion concentrations for various nucleic acids, including RNA tertiary structures. Our comprehensive analyses show that the global binding of  $Mg^{2+}/Na^+$  to a nucleic acid is mainly dependent on its structure compactness, as well as  $Mg^{2+}/Na^+$  concentrations, rather than the specific structure of the nucleic acid. Specifically, the relative global binding of  $Mg^{2+}$  over  $Na^+$  is stronger for a nucleic acid with higher effective surface charge density and higher relative  $Mg^{2+}/Na^+$  concentrations. Furthermore, the local binding of  $Mg^{2+}/Na^+$  to a phosphate of a nucleic acid mainly depends on the local phosphate density in addition to  $Mg^{2+}/Na^+$  concentrations.

## INTRODUCTION

Nucleic acids have important biological functions for genetic storage, expression, and regulation, and their functions are strongly coupled to their structures and structural changes (1,2). Since nucleic acids are highly charged polyanions, their native structure formation undergoes strong intramolecular Coulombic repulsions (1–26). However, metal ions in the solution can bind to nucleic acids and significantly reduce the Coulombic repulsion, thus favoring the structure folding of nucleic acids (1–11,13,23,25). Compared with monovalent ions, multivalent ions play a more important and efficient role in structure folding (e.g.,  $Mg^{2+}$  of millimolar concentration can generally induce RNA folding), whereas  $Na^+$  can only cause RNA folding at approximately molar concentrations (8,9,12–36). More importantly, a nucleic acid generally resides in mixed divalent/monovalent ion solutions (1–7). Therefore, the competitive binding of divalent and monovalent ions is critical to the structure folding and stability of nucleic acids (8,9,12,32–45).

To understand ion-nucleic acid interactions, several classic polyelectrolyte theories have been developed and

employed (15–22,35,46–58). The counterion condensation theory and the Poisson-Boltzmann (PB) theory have been successful in predicting various electrostatic properties of nucleic acids (20–22,46–58). However, the counterion condensation theory was developed for line-charge structure models at very dilute ion concentrations. Thus, it is inapplicable for nucleic acids with complex structures at finite salt concentrations (46,47). The PB theory ignores the ion-ion correlations and underestimates the role of multivalent ions in stabilizing nucleic acid structures (49–63) and helix assembly (53,64). The tightly bound ion (TBI) model has recently been developed to account for ion-ion correlation in binding to nucleic acids (52,53), and the model has been shown to improve predictions regarding the effect of  $Mg^{2+}$  in stabilizing RNA/DNA helices/hairpins and tertiary structures (52–54,61,62,64). However, the TBI model in its present version assumes all the atom charges are distributed in phosphate groups and thus would not result in reliable prediction of detailed ion binding properties in the vicinity of nucleic acids (54,62,64).

As an important complementary method to theories and experiments, all-atom molecular dynamics (MD) simulations can offer reliable predictions of the detailed ion-binding properties and dynamics of biomolecules (10,65–87). The binding of divalent or monovalent ions to double-stranded (ds) DNA or dsRNA in pure divalent or monovalent

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\*Correspondence: zjtan@whu.edu.cn

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ion solutions has been investigated extensively by all-atom MD simulations (27,28,66,72,74,75,82,85). Recently, all-atom MD simulations have been employed for dsDNA and dsRNA in mixed  $Mg^{2+}/Na^+$  solutions to investigate the competitive binding of  $Mg^{2+}/Na^+$  to dsDNA and dsRNA, and it has been shown that the competitive binding of  $Mg^{2+}$  and  $Na^+$  is strongly dependent on the relative divalent/monovalent ion concentration (27,28,66,82). However, these existing MD-based studies on the binding of divalent/monovalent ions generally involved dsDNA or dsRNA with a given length and seldom covered the competitive binding of divalent/monovalent ions for nucleic acid structures beyond the standard B-form and A-form helical structures (42,43,73,86,87), although the existing experiments indicate that the global binding of  $Mg^{2+}/Na^+$  (or  $K^+$ ) can be strongly dependent on nucleic acid structures (1–6,10,63). Therefore, there is still a lack of comprehensive understanding on the competitive binding of divalent/monovalent ions, especially to various nucleic acid structures.

Furthermore, an important issue in simulating nucleic acids with arbitrary structures in mixed divalent/monovalent ion solutions via MD simulations is to efficiently simulate a nucleic acid solution with desired bulk divalent/monovalent ion concentrations so as to conveniently model experimental systems and analyze ion concentration effects (63,73,82,87). This is challenging, because the competitive binding of divalent/monovalent ions may be sensitive to the structures of nucleic acids as well as the relative divalent/monovalent ion concentrations, and an MD cell generally cannot become very large because of the extremely high computation cost. To simulate a 25-base-pair (bp) dsRNA in various mixed  $Mg^{2+}/Na^+$  solutions, Kirmizialtin and Elber employed a neutralization technique to partition  $Mg^{2+}$  and  $Na^+$  in their MD simulations, although the obtained bulk  $Mg^{2+}/Na^+$  concentrations deviated apparently from the desired concentrations (27,66). To simulate a 24-bp dsDNA in mixed  $Mg^{2+}/Na^+$  solutions via MD simulations, Yoo and Aksimetiev attempted to obtain the desired bulk  $Mg^{2+}/Na^+$  concentrations through adjusting ion numbers in their MD simulations and repeating the procedure, but the resultant bulk  $Mg^{2+}/Na^+$  concentrations still deviated visibly from the desired concentrations, and such treatment was obviously not efficient (82). To model S-adenosylmethionine (SAM-I) riboswitch in mixed  $Mg^{2+}/K^+$  solution via MD simulations, Hayes et al. also chose the numbers of ions in the MD cell through repeated MD trials to obtain the desired bulk  $Mg^{2+}/K^+$  concentrations (73). Therefore, it is still necessary to obtain a general and efficient method for simulating a nucleic acid with arbitrary structure in a mixed  $Mg^{2+}/Na^+$  solution with desired bulk ion concentrations.

In this work, we first proposed a general and efficient method for simulating a nucleic acid with arbitrary structure in a mixed  $Mg^{2+}/Na^+$  solution with desired bulk ion concentrations and examined the method through extensive

MD simulations. Afterward, we investigated the competitive binding of  $Mg^{2+}$  and  $Na^+$  to extensive nucleic acids, including short dsDNA, dsRNAs, and several RNA tertiary structures. Our calculations were compared with available experimental data, and the global and local binding of  $Mg^{2+}$  and  $Na^+$  were analyzed in detail for various nucleic acids. The global binding of ions is characterized as the binding ions in excess of bulk ion concentration (27,28,66,82), and the local binding of ions is characterized by the closely binding of ions near phosphates. Our comprehensive analyses suggest the global binding of  $Mg^{2+}$  over  $Na^+$  to a nucleic acid is mainly dependent on the surface charge density and  $Mg^{2+}/Na^+$  concentrations, and the local binding of  $Mg^{2+}$  over  $Na^+$  to a phosphate of a nucleic acid depends mainly on the local phosphate density (i.e., the number of phosphates around a phosphate) in addition to  $Mg^{2+}/Na^+$  concentrations.

## MATERIALS AND METHODS

### Method for simulating a nucleic acid solution with desired bulk divalent/monovalent ion concentrations via MD simulations

Because the competitive binding of divalent/monovalent ions may be strongly dependent on many factors, such as divalent/monovalent ion concentrations and the length and structure of nucleic acids, it is challenging to propose a general and efficient method for simulating a nucleic acid in a mixed divalent/monovalent ion solution with desired bulk ion concentrations via MD simulations (27,66,73,82). The TBI model has been shown to successfully predict the global competitive binding between divalent/monovalent ions to various DNA and RNA structures (54). Through extensive TBI-based calculations for various DNA and RNA structures in mixed  $Mg^{2+}/Na^+$  solutions with wide range of  $Mg^{2+}/Na^+$  concentrations (54), an empirical equivalent formula between divalent and monovalent ions in neutralizing various nucleic acids has been derived (54):

$$\log [Na^+]_{Mg} = A \log [Mg^{2+}] + B, \quad (1)$$

where  $[Na^+]_{Mg}$  and  $[Mg^{2+}]$  are in millimolar and can achieve the equivalent ionic neutralization for a nucleic acid (54). The two parameters  $A$  and  $B$  in Eq. 1 are dependent on the length and compactness of a nucleic acid (54):

$$A = 0.65 + \frac{4.2}{N} \left( \frac{R_g}{R_g^0} \right)^2; \quad B = 1.80 - \frac{9.8}{N} \left( \frac{R_g}{R_g^0} \right)^2, \quad (2)$$

where  $N$  and  $R_g$  represent the number of phosphates and radius of gyration for a nucleic acid.  $R_g^0$  is the radius of gyration for the A-form RNA helix with the same length  $N$  (–nt), which has been fitted to an empirical formula  $R_g^0 = 0.406N + 130/(N + 11)$  (54). Based on Eq. 1, for a nucleic acid in a mixed  $Mg^{2+}/Na^+$  solution with desired bulk concentrations  $[Mg^{2+}]$  and  $[Na^+]$ , the numbers  $N_{Mg}^c$  and  $N_{Na}^c$  of divalent/monovalent counterions for neutralizing the nucleic acid can be given by

$$N_{Mg}^c = N \frac{0.5[Na^+]_{Mg}}{[Na^+]_{Mg} + [Na^+]}; \quad N_{Na}^c = N \frac{[Na^+]}{[Na^+]_{Mg} + [Na^+]}, \quad (3)$$

respectively. Therefore, the total numbers for divalent/monovalent ions in the simulation cell are given by  $N_{Mg} = N_{Mg}^c + N_{Mg}^s$  and  $N_{Na} = N_{Na}^c + N_{Na}^s$ , respectively. Here,  $N_{Mg}^c$  and  $N_{Na}^c$  are the numbers of divalent/monovalent ions dissociated from the added divalent/monovalent salts and can be calculated conveniently with the MD cell size and bulk salt concentrations ( $[Mg^{2+}]$  and  $[Na^+]$ ). Our simulation systems were kept neutral with addition of coions  $Cl^-$ .

## All-atom MD simulations

In this work, we performed all-atom MD simulations in mixed  $Mg^{2+}/Na^+$  solutions to 1) examine the method proposed above for simulating nucleic acids in mixed divalent/monovalent ion solutions with desired bulk divalent/monovalent ion concentrations; and 2) perform comprehensive analyses on the competitive binding of divalent/monovalent ions to diverse and representative constructs of nucleic acids (63,82). The nucleic acids used in our MD simulations include a 24-bp B-form dsDNA, an A-form dsRNA with different lengths (8-, 12-, and 24-bp), and four RNA tertiary structures: 28-nucleotide (nt) beet western yellow virus (BWYV) pseudoknot (Protein Data Bank (PDB): 437D) (88), 58-nt ribosomal RNA (rRNA) fragment (PDB: 1HC8) (89), 76-nt Yeast tRNA<sup>Phe</sup> (PDB: 1TRA) (90), and 94-nt SAM-I riboswitch (PDB: 2GIS) (91). The sequences of the dsDNA and dsRNAs are in Table S1. The initial structures of the dsDNA and dsRNA structures were built using the Nucleic Acid Builder of AMBER (AMBER Software, San Francisco, CA) (92), and the initial structures of RNA tertiary structures were taken directly from the PDB (93). The structures of the nucleic acids used in the work are shown in Fig. 1, and the nucleic acids, ion conditions, and the respective simulation cells are listed in Table 1. For the dsDNA and dsRNA structures, the simulation cells were chosen according to the previous simula-

tional work (82) for the convenience of direct comparison, and for the RNA tertiary structures, the simulation cells were selected to ensure the distance between the edge of the cells and the surface of the RNA tertiary structures was larger than two times the Debye-Hückel length. All the details for the numbers of ions ( $Mg^{2+}$ ,  $Na^+$ , and  $Cl^-$ ) added in the simulation cells were listed in Table S2. In this work,  $Na^+$  was chosen rather than  $K^+$  according to the related experimental (63) and simulation works (27,28,66,73,82), although  $K^+$  is more prevalent than  $Na^+$  in intracellular environment.

In our MD simulations, the Amber parmbsc0 force field (92) was employed for DNA and RNA molecules in mixed  $Mg^{2+}/Na^+$  ion solutions, combined with the TIP3P water model and Joung/Cheatham ion model for  $Na^+$  and  $Cl^-$  (94) and Åqvist ion model for  $Mg^{2+}$  (95), in which ions were added by replacing the water molecules at least 20 Å away from the surface atom of nucleic acids, i.e., the atoms with the closest distance from the added ions. All the simulation systems were optimized, thermalized (298 K), and equilibrated with the program Gromacs 4.5 (96), with the periodic boundary conditions and particle mesh Ewald method for long-range interaction (97). All atoms in the nucleic acids were restrained by a harmonic potential with force constant 1000 kJ/mol·nm<sup>-2</sup> in *x*-, *y*- and *z*-directions; hence, the effect of nucleic acid flexibility on ion binding was ignored. Each MD simulation was continued for 200 ns in the isothermic-isobaric ensemble (time step = 2 fs,  $P = 1$  atm, and  $T = 298$  K). Generally, our MD simulations for ion binding to various nucleic acids qualitatively nearly reach equilibrium after ~20 ns, as shown in Figs. S1 and S2. During all our simulations, the change on the box sizes is always less than 0.5 Å in each dimension, and it leads to a change of ~1.3% on the box volumes; thus, it would only have a slight effect on the calculated bulk ion concentrations. Additionally, we have examined the  $Mg^{2+}$  models of Allnér and Villa (98) and Li and Merz (99) for a 12-bp dsDNA (72) and did not find a significant

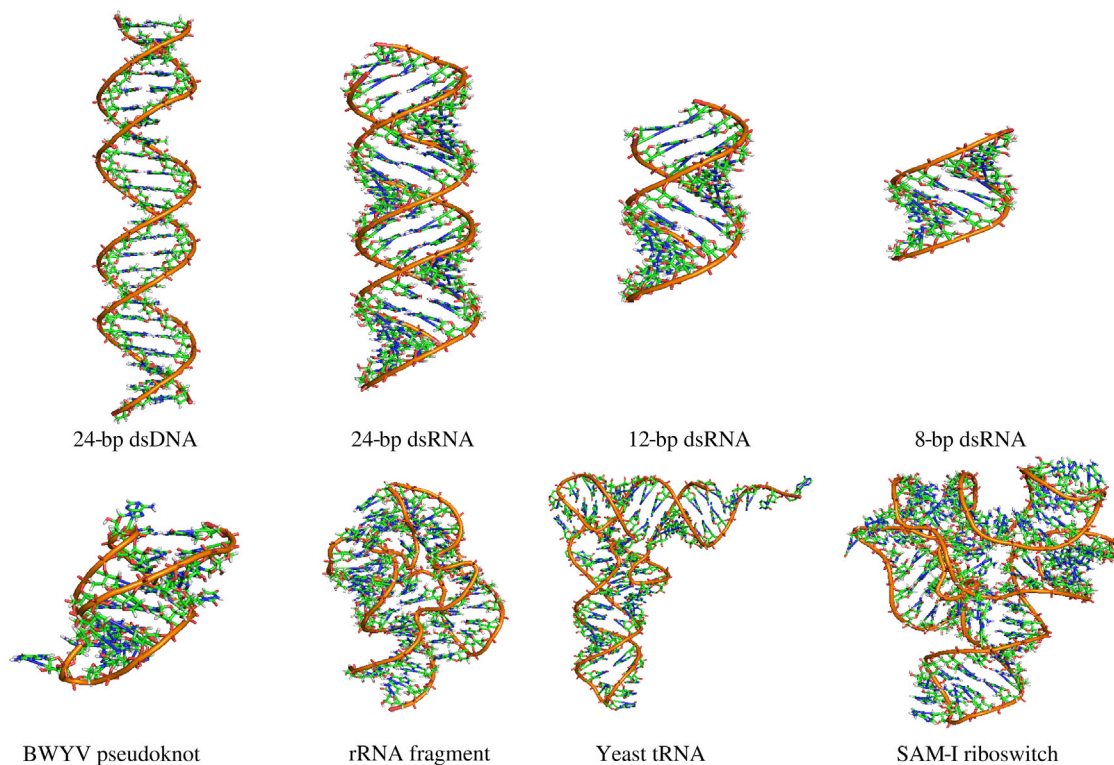


FIGURE 1 The DNA and RNAs used in our simulations, including 24-bp dsDNA; 24-bp, 12-bp, and 8-bp dsRNAs (63); BWYV pseudoknot (88); 58-nt rRNA fragment (89); yeast tRNA<sup>Phe</sup> (90); and SAM-I riboswitch (91). The structures for the nucleic acids are displayed with PyMol (<http://www.pymol.org>). To see this figure in color, go online.

**TABLE 1** The DNA/RNA Molecules and the Respective MD Simulation Details in the Work

DNA or RNA	Desired Bulk [Mg <sup>2+</sup> ]/[Na <sup>+</sup> ] (mM)	Cell Size (Å <sup>3</sup> )	Simulation Time (ns)	Bulk [Mg <sup>2+</sup> ]/[Na <sup>+</sup> ] in Reference <sup>a</sup> (mM)	Our Bulk [Mg <sup>2+</sup> ]/[Na <sup>+</sup> ] <sup>b</sup> (mM)
24-bp dsDNA	1/20	150 × 150 × 90	200	–	1.1 ± 0.1/20.2 ± 0.3
	5/20	150 × 150 × 90	200	7/18	5.4 ± 0.1/20.0 ± 0.2
	5/40	150 × 150 × 90	200	8/36	5.3 ± 0.1/40.4 ± 0.3
	5/70	150 × 150 × 90	200	8/72	5.2 ± 0.1/70.3 ± 0.2
	5/240	150 × 150 × 90	200	6/237	5.2 ± 0.2/241.6 ± 0.4
	5/420	150 × 150 × 90	200	7/418	5.3 ± 0.1/421.1 ± 0.3
	10/20	150 × 150 × 90	200	–	10.1 ± 0.1/20.8 ± 0.3
	20/20	150 × 150 × 90	200	–	20.9 ± 0.2/19.7 ± 0.2
8-bp dsRNA	50/20	150 × 150 × 90	200	–	50.8 ± 0.7/20.7 ± 0.5
	1/20	150 × 150 × 48	200	–	1.0 ± 0.1/21.5 ± 0.1
12-bp dsRNA	10/20	150 × 150 × 48	200	–	10.9 ± 0.2/20.4 ± 0.2
	1/20	150 × 150 × 58	200	–	1.0 ± 0.1/21.6 ± 0.8
24-bp dsRNA	10/20	150 × 150 × 58	200	–	10.5 ± 0.3/19.7 ± 0.2
	1/20	150 × 150 × 90	200	–	1.1 ± 0.1/20.2 ± 0.3
28-nt BWYV Pseudoknot	5/20	150 × 150 × 90	200	–	5.1 ± 0.2/21.6 ± 0.3
	10/20	150 × 150 × 90	200	–	10.1 ± 0.1/21.6 ± 0.1
	50/20	150 × 150 × 90	200	–	50.8 ± 0.3/21.7 ± 0.1
	1/20	120 × 110 × 110	200	–	1.0 ± 0.1/20.8 ± 0.2
58-nt rRNA Fragment	10/20	120 × 110 × 110	200	–	10.9 ± 0.2/20.5 ± 0.1
	1/20	130 × 130 × 120	200	–	1.1 ± 0.2/20.5 ± 0.1
76-nt Yeast tRNA <sup>Phe</sup>	10/20	130 × 130 × 120	200	–	10.2 ± 0.2/19.5 ± 0.1
	1/20	130 × 145 × 160	200	–	1.1 ± 0.1/19.4 ± 0.3
94-nt SAM-I Riboswitch	10/20	130 × 145 × 160	200	–	10.3 ± 0.1/19.1 ± 0.1
	1/20	120 × 145 × 140	200	–	1.0 ± 0.1/21.0 ± 0.2
	10/20	120 × 145 × 140	200	–	10.1 ± 0.1/20.5 ± 0.1

In the table, the sequences of dsDNA and dsRNAs are listed in Table S1, and BWYV pseudoknot, rRNA fragment, Yeast tRNA<sup>Phe</sup>, and SAM-I riboswitch represent a 28-nt beet western yellow virus (BWYV) pseudoknot fragment (PDB: 437D) (88), a 58-nt ribosomal RNA fragment (PDB: 1HC8) (89), 76-nt Yeast tRNA<sup>Phe</sup> (PDB: 1TRA) (90), and 94-nt S-adenosylmethionine riboswitch mRNA (PDB: 2GIS) (91), respectively.

<sup>a</sup>The bulk [Mg<sup>2+</sup>]/[Na<sup>+</sup>] values are obtained from Yoo and Aksimietiev (82).

<sup>b</sup>The bulk [Mg<sup>2+</sup>]/[Na<sup>+</sup>] values are obtained from our MD simulations.

effect of Mg<sup>2+</sup> models on the Mg<sup>2+</sup> distribution around the 12-bp dsDNA; see Fig. S3. We have also performed additional simulations for 24-bp dsDNA and 58-nt rRNA fragment in 1 mM Mg<sup>2+</sup>/20 mM Na<sup>+</sup> solution with different initial positions of ions.

### The Poisson-Boltzmann calculations

In addition to the all-atom simulations described above, we also calculated the binding ions in excess of bulk ions and the surface electrostatic potentials for various nucleic acids with the PB theory (52–54,59,60). For calculating the binding ions in excess of bulk ions to nucleic acids, we used the three-dimensional finite-difference algorithm developed in the TBI model for our convenience (52). A three-step focusing process was employed, and the resolutions for the three steps were kept as 2.0, 1.0, and 0.5 Å per grid respectively. The grid size ( $L_x, L_y, L_z$ ) of the first, second, and third runs were kept larger than 10, 6, and 2 times the Debye-Hückel length from the surface of a nucleic acid and varied with different DNA and RNA molecules. Other details about the solvent have been described in (52,54). For calculating the surface electrostatic potential for nucleic acids, we used the APBS software (49), since the obtained surface potential can be conveniently displayed with VMD (100). In all the PB calculations, the dielectric constant inside nucleic acids was set to be two, and that of solvent was taken as 78.5. The radii of Mg<sup>2+</sup> and Na<sup>+</sup> were taken as 3.0 Å (101) and 2.0 Å (102), respectively.

## RESULTS AND DISCUSSION

First, we have examined the proposed method for efficiently simulating a nucleic acid in mixed divalent/monovalent so-

lutions with desired bulk ion concentrations via MD simulations. Second, we have performed a detailed comparative analysis on the binding of Mg<sup>2+</sup> and Na<sup>+</sup> to dsDNA and dsRNA. Third, we have focused on the binding of Mg<sup>2+</sup> and Na<sup>+</sup> to various RNA tertiary structures with different lengths and compactness. Finally, we have summarized what governs the global and local binding of Mg<sup>2+</sup> and Na<sup>+</sup> to various nucleic acid structures. In addition, our calculations are compared with the available experimental data and cover the wide ranges of ion concentrations and nucleic acid structures. In our analyses, we will use the binding ions in excess of bulk ions to characterize the global binding of ions to nucleic acids (37,54) and use detailed ion distributions and closely binding ions near phosphates to characterize the local binding of ions to various nucleic acids.

### Simulating a nucleic acid in mixed Mg<sup>2+</sup>/Na<sup>+</sup> solutions with desired bulk ion concentrations

As described in the Materials and Methods, we proposed a method aiming to efficiently model the system of a nucleic acid in mixed Mg<sup>2+</sup>/Na<sup>+</sup> solutions with desired bulk ion concentrations via MD simulations. In the following, we have examined whether a one-time MD run with the use

of the proposed method can yield the desired bulk ion concentrations for various RNA/DNA molecules in extensively mixed  $\text{Mg}^{2+}/\text{Na}^+$  conditions. The ion concentration  $c_\alpha(r)$  around a nucleic acid can be calculated by

$$c_\alpha(r) = \frac{N_\alpha(r+dr) - N_\alpha(r)}{V(r+dr) - V(r)}, \quad (4)$$

where  $V(r)$  and  $N_\alpha(r)$  denote the volume and the number of ions within a distance  $r$  from the nucleic acid, respectively, and  $\alpha$  stands for ion species. For dsDNA and dsRNA helices,  $r$  is the radial distance from the helical axis that was obtained with the program of Curves+ (103),  $V(r)$  stands for the cylinder volume within  $r$  without excluding DNA or RNA volume, and  $c_\alpha(r)$  was previously used to analyze ion distribution around dsDNA/dsRNA (19,20,26,74,95). For RNA tertiary structures in which an axis cannot be well defined,  $r$  stands for the distance from the surface atoms (the atoms with closest distance from the examined ions), and  $V(r)$  excludes the volume of the RNAs. According to Eq. 4, the bulk ion concentrations can be obtained at large  $r$ , as shown in Figs. S4–S6. In the following, we have examined the obtained bulk divalent/monovalent ion concentrations for various nucleic acids in comparison with the desired concentrations.

For dsDNA and dsRNA of different lengths, we made extensive examinations on bulk ion concentrations over the wide range of  $[\text{Mg}^{2+}]$  and  $[\text{Na}^+]$ . As shown in Table 1, our proposed method can yield the bulk  $[\text{Mg}^{2+}]$  and  $[\text{Na}^+]$ , which are very close to the desired values. The mean relative deviations of  $[\text{Mg}^{2+}]$  and  $[\text{Na}^+]$  from the desired ones are  $\sim 5$  and  $\sim 4\%$ , respectively. Here, the mean relative deviation of  $[\text{Mg}^{2+}]$  is slightly larger than that of  $[\text{Na}^+]$  because the desired bulk  $[\text{Mg}^{2+}]$  is generally lower ( $\sim \text{mM}$ ) than the desired bulk  $[\text{Na}^+]$ . Although the largest relative deviation of  $[\text{Mg}^{2+}]$  comes from a mixed solution of 1 mM  $\text{Mg}^{2+}$  and 20 mM  $\text{Na}^+$ , the absolute deviation of  $[\text{Mg}^{2+}]$  is only  $\sim 0.1$  mM. Compared with the previous work (e.g., (82)), our obtained bulk  $[\text{Mg}^{2+}]$  and  $[\text{Na}^+]$  are much closer to the desired values (e.g., the mean relative deviations of  $[\text{Mg}^{2+}]$  from our MD and (82) for the total cases are  $\sim 5$  and  $\sim 44\%$ , respectively), given the same simulation cell (82) (see Table 1). Additionally, our method is much more efficient because only one MD run is required for each case with the proposed method described in the Materials and Methods.

For various RNA tertiary structures ranging from 28-nt BWYV pseudoknot to 94-nt SAM-I riboswitch, the obtained bulk  $[\text{Mg}^{2+}]/[\text{Na}^+]$  in our MD simulations are all close to the desired values, as shown in Table 1. The mean relative deviations of  $[\text{Mg}^{2+}]$  and  $[\text{Na}^+]$  from the desired values are rather small ( $\sim 5\%$  for  $[\text{Mg}^{2+}]$  and  $\sim 4\%$  for  $[\text{Na}^+]$ ). In a recent MD simulation of SAM-I riboswitch in mixed  $\text{Mg}^{2+}/\text{K}^+$  solution, Hayes et al. obtained the desired bulk ion concentrations with the slightly larger mean relative de-

viations of  $\text{Mg}^{2+}$  ( $\sim 5.1\%$  for  $[\text{Mg}^{2+}]$ ) by repeating several MD trials (73), in which the slightly smaller mean relative deviation of  $[\text{K}^+]$  ( $\sim 2\%$ ) may be caused by the high desired bulk concentration ( $\sim 100$  mM) (73). It should be noted that our proposed method only requires one MD run even for various RNA tertiary structures (see the Materials and Methods) to yield desired bulk ion concentrations for simulating various RNA tertiary structures in mixed  $\text{Mg}^{2+}/\text{Na}^+$  solutions, although larger RNA tertiary structures ( $>94$  nt) have not been examined here.

Therefore, the above extensive examinations show that our proposed method is effective and efficient for simulating a nucleic acid with arbitrary structure in mixed divalent/monovalent ion solutions with wide ranges of desired bulk ion concentrations. It is encouraging that only a single MD simulation is required for a nucleic acid in a mixed  $\text{Mg}^{2+}/\text{Na}^+$  solution to yield the desired bulk  $\text{Mg}^{2+}/\text{Na}^+$  concentrations, and the method is expected to work for other mixed divalent/monovalent ion solutions such as mixed  $\text{Mg}^{2+}/\text{K}^+$  solutions.

### Competitive binding of $\text{Mg}^{2+}/\text{Na}^+$ to duplexes: dsDNA versus dsRNA

#### Global binding of $\text{Mg}^{2+}/\text{Na}^+$ and comparison with experiments

Since the global binding of ions is directly coupled to the global stability of nucleic acids (1–9,36–38), we calculated the number of global binding ions for both dsDNA and dsRNA (27,28,66). According to (27,28,66,72,82), the numbers  $N_\alpha^b$  of global binding ions of species  $\alpha$  in excess of bulk ion concentrations can be calculated as those ions:

$$N_\alpha^b = \int_0^\infty (c_\alpha(r) - c_\alpha^0) d^3r. \quad (5)$$

Based on the ion concentration distributions and the bulk ion concentrations  $c_\alpha^0$  shown in Table 1 and Figs. S5 and S6, the numbers  $N_{\text{Mg}}^b$ ,  $N_{\text{Na}}^b$ , and  $N_{\text{Cl}}^b$  of global binding  $\text{Mg}^{2+}/\text{Na}^+/\text{Cl}^-$  around the 24-bp dsDNA or dsRNA were calculated through Eq. 5.

As shown in Fig. 2, A and B and in Table S2, for both dsDNA and dsRNA, with the increase of  $[\text{Mg}^{2+}]$  from 1 to 50 mM,  $N_{\text{Mg}}^b$  increases apparently and  $N_{\text{Na}}^b$  decreases simultaneously, suggesting the anticooperative binding between  $\text{Mg}^{2+}$  and  $\text{Na}^+$ . Since the number of negative charges on a nucleic acid is unchanged, the increase of  $N_{\text{Mg}}^b$  (or  $N_{\text{Na}}^b$ ) causes the decrease of  $N_{\text{Na}}^b$  (or  $N_{\text{Mg}}^b$ ). Fig. 2, A and B also show that  $N_{\text{Mg}}^b$  for dsRNA is always slightly larger than that for dsDNA over the range of  $[\text{Mg}^{2+}]$  from 1 to 50 mM, and correspondingly,  $N_{\text{Na}}^b$  for dsRNA is slightly smaller than that for dsDNA. The larger number  $N_{\text{Mg}}^b$  for dsRNA than for dsDNA can be attributed to the more

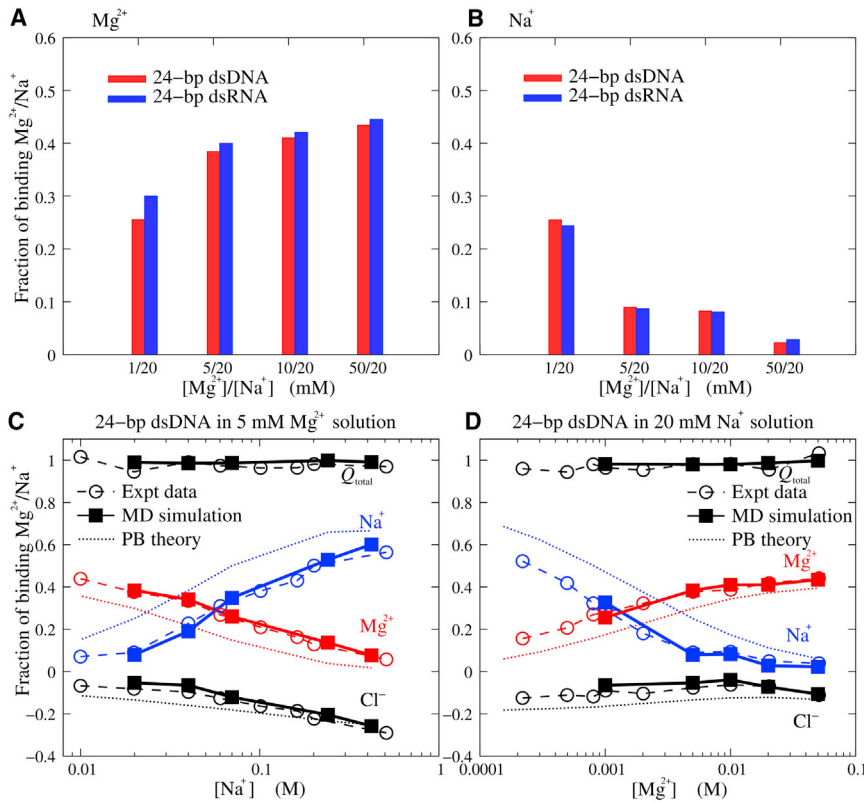


FIGURE 2 (A and B) The fractions of binding  $Mg^{2+}$  (A) and  $Na^+$  (B) per nucleotide around the 24-bp dsDNA and dsRNA. (C and D) These panels show the fractions of binding  $Mg^{2+}$ ,  $Na^+$ , and  $Cl^-$  per nucleotide and the total charge fraction  $Q_{total}$  of the ion atmosphere ( $Mg^{2+}$ ,  $Na^+$ , and  $Cl^-$ ) for the 24-bp dsDNA as functions of  $[Na^+]$  (C) and  $[Mg^{2+}]$  (D). Dashed lines with circles represent experimental data (63); solid lines with squares represent MD simulations; and dotted lines represent PB theory. To see this figure in color, go online.

compact structure of dsRNA: A-RNA is slightly shorter than B-DNA, which results in higher charge density and stronger  $Mg^{2+}$  binding for dsRNA.

Fig. 2, C and D show the experimental data (63), our MD calculations, and the PB calculations for  $N_{Mg}^b$  and  $N_{Na}^b$  around the 24-bp dsDNA. First, the total charge fraction  $Q_{total}$  of binding ions ( $Mg^{2+}$ ,  $Na^+$ , and  $Cl^-$ ) almost keeps unity, suggesting that the systems are neutral. Second,  $N_{Mg}^b$  (or  $N_{Na}^b$ ) increases with the increase of  $[Mg^{2+}]$  (or  $[Na^+]$ ), and the increase (decrease) of  $N_{Mg}^b$  is generally accompanied with the decrease (increase) of  $N_{Na}^b$ . As discussed above, increased ion ( $Mg^{2+}$  or  $Na^+$ ) concentration reduces the entropic penalty for ion binding and consequently favors the ion ( $Mg^{2+}$  or  $Na^+$ ) binding, and the interplay between  $N_{Mg}^b$  and  $N_{Na}^b$  is attributed to the unchanged number of negative charges on a nucleic acid. Third, our MD calculations are in accordance with the experimental data for  $N_{Mg}^b$ ,  $N_{Na}^b$ , and  $N_{Cl}^b$  (55), suggesting that all-atom MD simulations can capture the ion-binding properties despite the computation cost. Fourth, the PB theory apparently underestimates  $N_{Mg}^b$  and correspondingly overestimates  $N_{Na}^b$ , although it predicts the trend of ion binding with ion concentration. Physically, the PB theory assumes the mean fluid-like ion distribution and consequently ignores the discrete properties of ions such as ion correlations, which would allow ions to self-organize to low-energy states and favor the binding of multivalent ions (52–54,61,62). Thus, the PB theory under-

estimates  $Mg^{2+}$  binding and overestimates  $Na^+$  binding. The systematic deviations of the PB theory were also observed previously in the Monte Carlo simulations and PB calculations for polymeric DNA in mixed salt solutions (20). Our additional calculations show the influence of the choice of dielectric constant of nucleic acids and radii of  $Mg^{2+}$  and  $Na^+$  for the PB results on the numbers of binding ions; see Fig. S7.

#### *Mg<sup>2+</sup>/Na<sup>+</sup> distributions around duplexes: B-DNA versus A-RNA*

Beyond the above-described global binding, we performed analyses on  $Mg^{2+}/Na^+$  distributions around dsDNA and dsRNA. As shown in Fig. 3, the radial concentration distributions of  $Mg^{2+}$  and  $Na^+$  around dsDNA and dsRNA share the following similar features: 1) there are two major peaks in the radial distributions of  $Mg^{2+}$  at two radial locations, and the height of the peaks increases with the increase of bulk  $[Mg^{2+}]$ ; and 2) the radial distribution of  $Na^+$  appears much lower than that of  $Mg^{2+}$  and decreases for higher bulk  $[Mg^{2+}]$ . The higher peaks of  $Mg^{2+}$  and lower peaks of  $Na^+$  for higher  $[Mg^{2+}]$  are due to the lowered binding entropy penalty of  $Mg^{2+}$ , and the overall lower radial concentration of  $Na^+$  than  $Mg^{2+}$  is attributed to the higher charge of  $Mg^{2+}$  and the corresponding stronger binding of  $Mg^{2+}$ . The two major peak locations of  $Mg^{2+}$  suggest the two different  $Mg^{2+}$  binding modes for dsDNA and dsRNA.

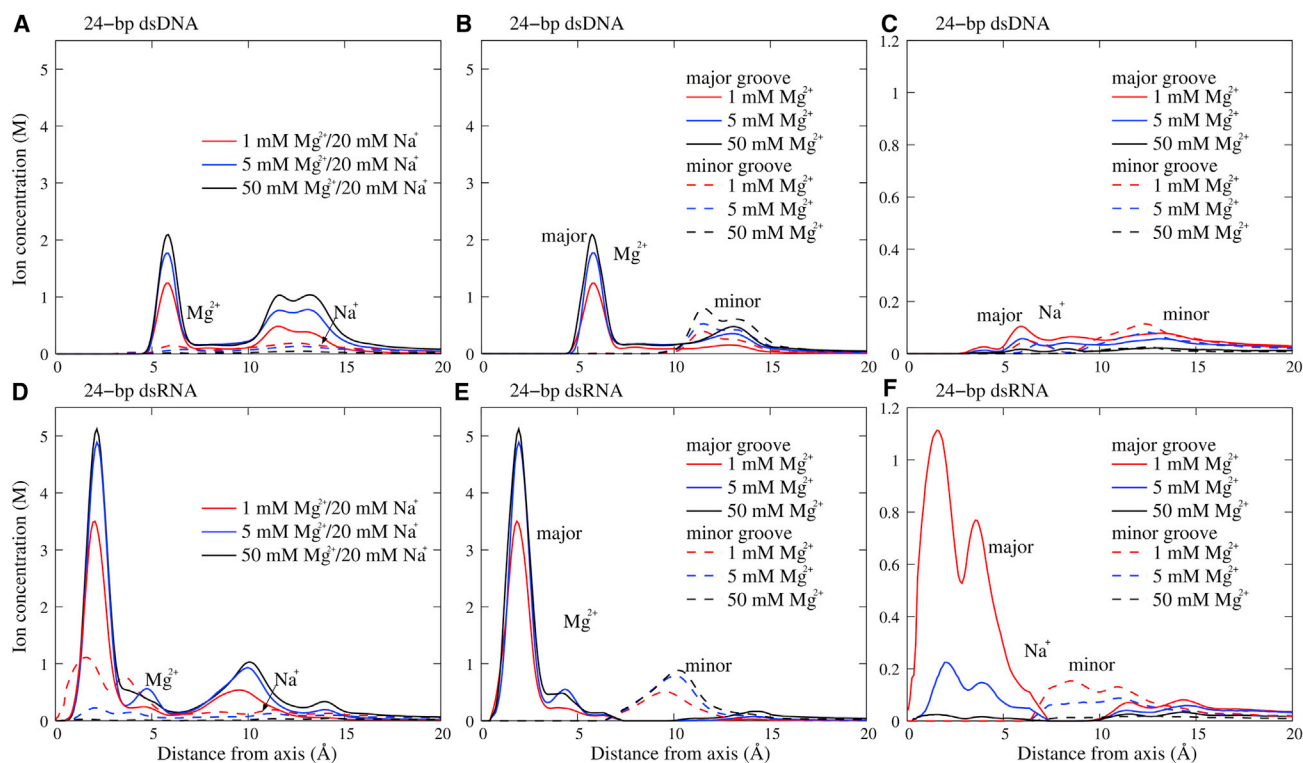


FIGURE 3 (A and D) The radial concentration distributions of  $\text{Mg}^{2+}$  and  $\text{Na}^+$  around 24-bp dsDNA (A) and dsRNA (D) in 20 mM  $\text{Na}^+$  solutions with different  $[\text{Mg}^{2+}]$  values. (B and E) These panels show the  $\text{Mg}^{2+}$  concentration distributions in/above the minor groove (dashed lines) and the major groove (solid lines) for the 24-bp dsDNA (B) and dsRNA (E). (C and F) These panels show the  $\text{Na}^+$  concentration distributions in/above the minor groove (dashed lines) and the major groove (solid lines) for the 24-bp dsDNA (C) and dsRNA (F). To see this figure in color, go online.

The detailed comparison between dsDNA and dsRNA also shows a distinctive difference in ion binding (e.g.,  $\text{Mg}^{2+}$ ): the radial locations for two major peaks are apparently different ( $\sim 6$  and  $\sim 12.5$  Å for dsDNA, and  $\sim 2$  and  $\sim 10$  Å for dsRNA), and the heights of the first peaks of  $\text{Mg}^{2+}$  and  $\text{Na}^+$  (near-radial distance of  $\sim 2$  Å) for dsRNA are much higher than those for dsDNA (near-radial distance of  $\sim 6$  Å). To understand this difference, we divided the radial concentration distributions of  $\text{Mg}^{2+}$  and  $\text{Na}^+$  into those in (or over) major and minor grooves for dsDNA and dsRNA. As shown in Fig. 3, B and C, for dsDNA,  $\text{Mg}^{2+}$  can bind into the major groove at a radial distance of  $\sim 6$  Å, whereas  $\text{Mg}^{2+}$  can bind more deeply and strongly into the major groove of dsRNA at a radial distance of  $\sim 2$  Å. The next preferred binding of  $\text{Mg}^{2+}$  is above the minor groove (at a radial distance of  $\sim 12.5$  Å) for dsDNA, and between phosphate strands in the minor groove (at a radial distance of  $\sim 10$  Å) for dsRNA.  $\text{Na}^+$  can also bind more deeply and strongly into the major groove of dsRNA than to dsDNA, although the radial concentration of  $\text{Na}^+$  is much lower than that of  $\text{Mg}^{2+}$  because of the lower ionic charge, as shown in Fig. 3, E and F. The apparent difference in  $\text{Mg}^{2+}$  binding between dsDNA and dsRNA is attributed to their different helical structures: A-form dsRNA has a much deeper/narrower major groove and a wider minor groove than B-form dsDNA, which causes very different surface

electrostatic potentials for dsDNA and dsRNA (see Figs. 1 and 4). Accordingly, we illustrated the spatial concentration distributions of  $\text{Mg}^{2+}$  and  $\text{Na}^+$  around dsDNA and dsRNA at different bulk  $\text{Mg}^{2+}/\text{Na}^+$  concentrations. As shown in Fig. 4, for dsRNA, the deep and narrow major groove is the most preferred binding domain for both  $\text{Mg}^{2+}$  and  $\text{Na}^+$ , and  $\text{Mg}^{2+}$  binding to the minor groove can become visible at high bulk  $[\text{Mg}^{2+}]$  because the narrow major groove can be almost fulfilled by  $\text{Mg}^{2+}$ . However, for dsDNA, the binding to the major groove and minor groove are both visible, whereas this appears weaker than the binding to the major groove of dsRNA. As  $[\text{Mg}^{2+}]$  increases,  $\text{Mg}^{2+}$  binding to major and minor grooves becomes stronger for both dsDNA and dsRNA, and simultaneously,  $\text{Na}^+$  binding becomes weaker. Our additional simulations with different initial positions of ions and with longer cell size in the  $z$  axis for 24-bp dsDNA yielded similar bulk ion concentrations and ion distributions; see Fig. S8. Additionally, we observed more binding  $\text{Mg}^{2+}$  ions in the GGG region of the major groove of the 24-bp dsDNA because the guanine bases can hold  $\text{Mg}^{2+}$  ions more strongly (74); see Fig. S9.

The different ion binding modes for dsDNA and dsRNA have been suggested to be responsible for the different multivalent ion-dependent condensation behaviors (27,28,34,66,73,82) and flexibilities for dsDNA and dsRNA (64,65).

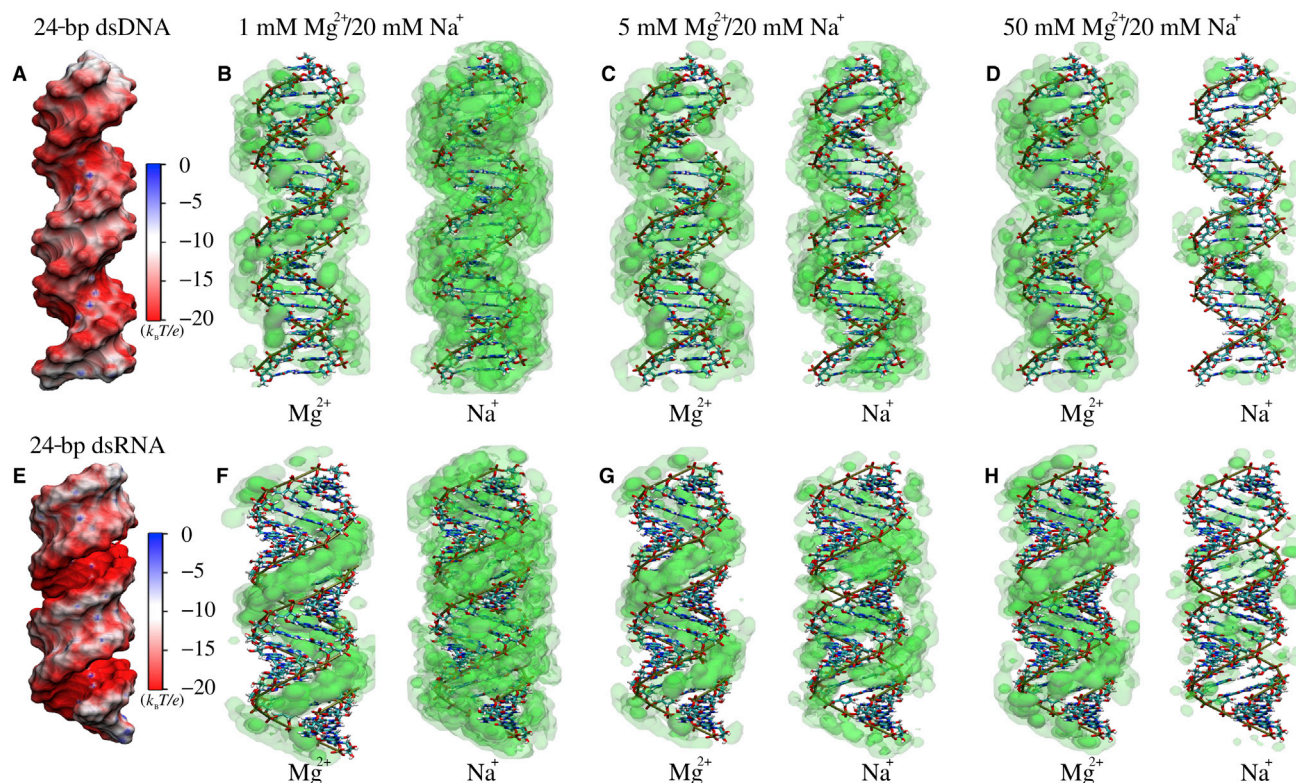


FIGURE 4 (A and E) The electrostatic potentials at the  $\text{Mg}^{2+}$  accessible surface of the 24-bp dsDNA (A) and dsRNA (E) were calculated by APBS (49). Shown is the electrostatic potential computed 3 Å away (the radius of hydrated  $\text{Mg}^{2+}$  is  $\sim 3$  Å (80)) from the surface by VMD (100). (B–D and F–H) The spatial ion concentration distributions of  $\text{Mg}^{2+}$  and  $\text{Na}^+$  around the surface of 24-bp dsDNA and dsRNA are displayed in green of three-level opacities (0.5 M–5 M, 5 M–10 M, and  $>10$  M for  $\text{Mg}^{2+}$  and 0.25 M–0.5 M, 0.5 M–2.0 M, and  $>2.0$  M for  $\text{Na}^+$ , respectively) by VMD (100). To see this figure in color, go online.

### Competitive binding of $\text{Mg}^{2+}/\text{Na}^+$ to RNA tertiary structures

Beyond dsDNA and dsRNA helices, we analyzed the competitive binding of  $\text{Mg}^{2+}$  and  $\text{Na}^+$  to various RNA tertiary structures, including 28-nt BWYV pseudoknot, 58-nt rRNA fragment, 76-nt Yeast tRNA<sup>Phe</sup>, and 94-nt SAM-I riboswitch.

#### Global binding of $\text{Mg}^{2+}$ and $\text{Na}^+$ to RNA tertiary structures

Since numbers of binding  $\text{Mg}^{2+}/\text{Na}^+$  are associated with the global stability of RNA tertiary structures, we calculated the numbers ( $N_{\text{Mg}}^b$  and  $N_{\text{Na}}^b$ ) of binding  $\text{Mg}^{2+}/\text{Na}^+$  to various RNA tertiary structures as those in excess of bulk ion concentrations according to Eq. 5. As shown in Fig. 5, A and B and in Table S2,  $N_{\text{Mg}}^b$  and  $N_{\text{Na}}^b$  from the MD simulations are in accordance with those from the empirical formula from the TBI model, which has been quantitatively tested by the extensive experimental data for various RNA tertiary structures (54). Fig. 5, A and B also show that for higher bulk  $[\text{Mg}^{2+}]$ ,  $N_{\text{Mg}}^b$  increases and  $N_{\text{Na}}^b$  simultaneously decreases because of the lower entropy penalty of  $\text{Mg}^{2+}$  at higher  $[\text{Mg}^{2+}]$ . Furthermore, compared with the predictions from the MD simulations and the TBI model, the PB theory

significantly underestimates  $N_{\text{Mg}}^b$  and simultaneously overestimates  $N_{\text{Na}}^b$ . As described above, this is because the PB theory ignores the ion correlation and fluctuation that allow ions to self-organize to low-energy states and favor multivalent ion binding (52–54,61,62).

For different RNA tertiary structures,  $N_{\text{Mg}}^b$  exhibits the following order: 94-nt SAM-I riboswitch  $>$  58-nt rRNA fragment  $>$  76-nt Yeast tRNA<sup>Phe</sup>  $>$  28-nt BWYV pseudoknot. If we include the DNA/RNA helices, the order of  $N_{\text{Mg}}^b$  is as follows: 94-nt SAM-I riboswitch  $>$  58-nt rRNA fragment  $>$  76-nt Yeast tRNA<sup>Phe</sup>  $>$  24-bp dsRNA  $>$  28-nt BWYV pseudoknot  $>$  24-bp dsDNA  $>$  12-bp dsRNA  $>$  8-bp dsRNA, and  $N_{\text{Na}}^b$  follows the reversal order. This indicates that the global competitive binding of  $\text{Mg}^{2+}/\text{Na}^+$  to RNAs may depend not only on RNA length but also structure compactness, and global  $\text{Mg}^{2+}$  binding appears stronger for RNAs with longer length and more compact structures.

#### $\text{Mg}^{2+}/\text{Na}^+$ distributions around RNA tertiary structures

Beyond the analyses described above on the global binding of  $\text{Mg}^{2+}$  and  $\text{Na}^+$ , we have performed analyses on the detailed binding of  $\text{Mg}^{2+}$  and  $\text{Na}^+$  to various RNA tertiary



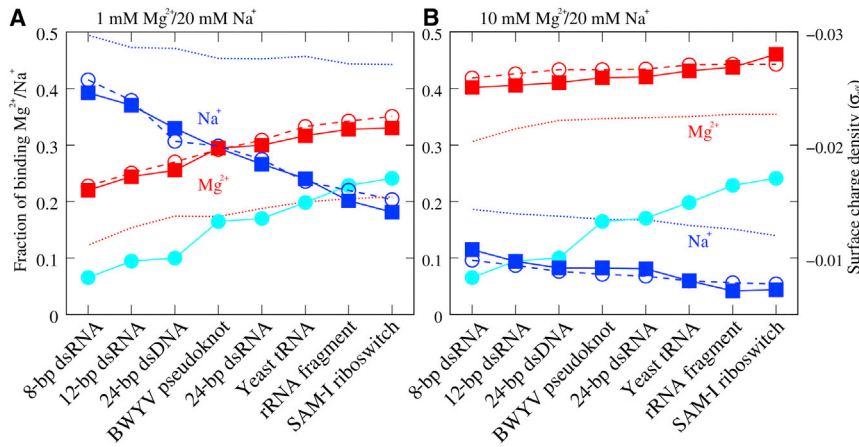


FIGURE 5 The number fractions of binding  $Mg^{2+}$  and  $Na^+$  per nucleotide for various RNA/DNA molecules in mixed 1 mM  $Mg^{2+}/20$  mM  $Na^+$  (A) and 10 mM  $Mg^{2+}/20$  mM  $Na^+$  (B) ion solutions. Solid lines with squares represent MD simulations; dotted lines represent PB theory; dashed lines with circles represent TBI predictions (Eq. 5 in (54)); and solid lines with spheres represent the effective surface charge density  $\sigma_{eff}$ . Here, RNA/DNA molecules were listed according to the order of fraction of binding  $Mg^{2+}/Na^+$ . The effective surface charge density (in units of  $e/\text{\AA}^2$ ) was calculated through considering an RNA/DNA as an ellipsoid; see the Supporting Material. To see this figure in color, go online.

structures, including the BWYV pseudoknot, rRNA fragment, yeast tRNA<sup>Phe</sup>, and SAM-I riboswitch.

Since RNA tertiary structures are generally more complex than DNA/RNA duplexes and there is no well-defined axis, we calculated the ion concentrations  $c(r)$  from the surface atoms of the RNA tertiary structures according to Eq. 4. As shown in Fig. 6, the concentration distributions of  $Mg^{2+}$  and  $Na^+$  share a similar trend for various RNA tertiary structures: 1) the location of the peaks of  $Mg^{2+}$  is  $\sim 4$  Å, and the peak heights of  $Mg^{2+}$  are apparently higher than those of  $Na^+$ ; and 2) with the increase of  $[Mg^{2+}]$ , the peak height of  $Mg^{2+}$  increases, and correspondingly, that of  $Na^+$  decreases. The location of  $\sim 4$  Å from surface atoms suggests that  $Mg^{2+}$  keeps its hydrated shell in binding to various RNAs because of the very high dehydration penalty for  $Mg^{2+}$  (33–35,66), and it is hard for  $Mg^{2+}$  to become dehydrated within finite MD simulation time (72,81). However, monovalent ions can become partially dehydrated in binding to RNAs because of the much lower dehydration energy (71–73). The stronger  $Mg^{2+}$  binding and lower  $Na^+$  binding at higher bulk  $[Mg^{2+}]$  is attributed to the lowered penalty for  $Mg^{2+}$  binding and the anticooperative binding between  $Mg^{2+}$  and  $Na^+$ . Despite the above described simi-

larity in ion binding, the peak heights of  $Mg^{2+}$  distributions appear different for various RNA tertiary structures and follow the same order as that of  $N_{Mg}^b$ : 94-nt SAM-I riboswitch > 58-nt rRNA fragment > 76-nt Yeast tRNA<sup>Phe</sup> > 28-nt BWYV pseudoknot; see Fig. 5, A and B. This order nearly follows the same order of RNA length except for the 58-nt rRNA fragment, which appears relatively more compact than tRNA<sup>Phe</sup> and BWYV pseudoknot (54,62). This also suggests that  $Mg^{2+}$  binding to RNA tertiary structures may be dependent on not only RNA length but also its structural compactness. Compared to  $Mg^{2+}$  binding, the difference in  $Na^+$  binding between different RNA tertiary structures appears relatively slight because of the strong  $Mg^{2+}$  binding.

To further reveal the detailed binding of  $Mg^{2+}/Na^+$  to RNA tertiary structures, we have illustrated the surface electrostatic potentials and spatial concentration distributions in Fig. 7. Although the detailed binding of  $Mg^{2+}/Na^+$  exhibits specific patterns for different RNA tertiary structures, the detailed binding of  $Mg^{2+}$  and  $Na^+$  to various RNA tertiary structures share the following similar features: 1)  $Mg^{2+}$  prefers to bind deeply into the major groove of A-form helical stems and the junction pocket between helical stems even at

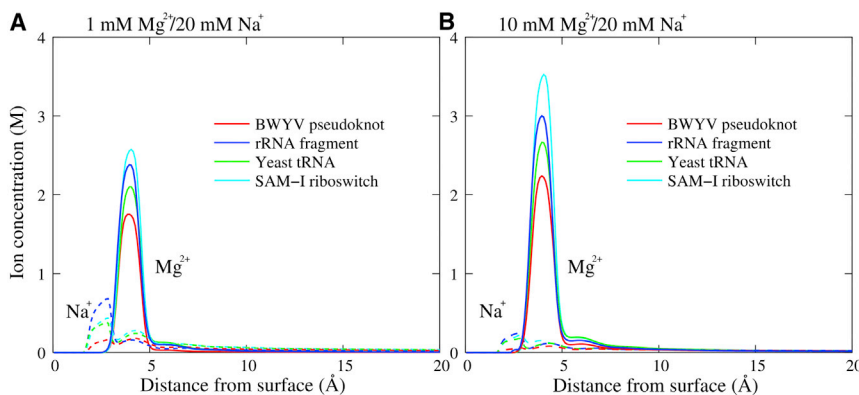


FIGURE 6 The ion concentration distributions of  $Mg^{2+}$  and  $Na^+$  around various RNA tertiary structures in mixed 1 mM  $Mg^{2+}/20$  mM  $Na^+$  (A) and 10 mM  $Mg^{2+}/20$  mM  $Na^+$  (B) solutions. The RNA tertiary structures include BWYV pseudoknot (red), 58-nt rRNA fragment (blue), yeast tRNA<sup>Phe</sup> (green), and SAM-I riboswitch (cyan), and distance from surface denotes that from surface atoms. To see this figure in color, go online.

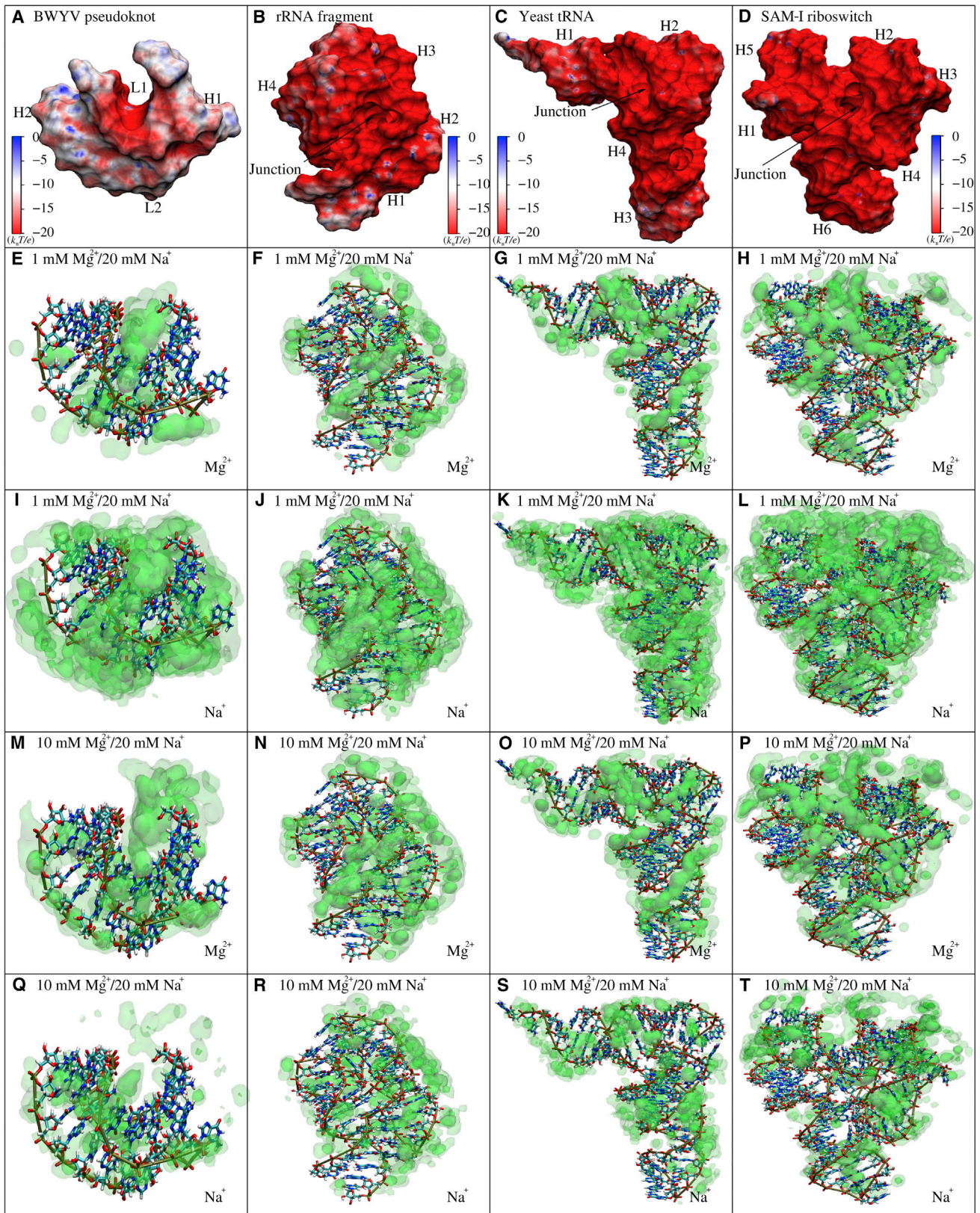


FIGURE 7 (A–D) The electrostatic potentials at the Mg<sup>2+</sup> accessible surface of BWYV pseudoknot (A), 58-nt rRNA fragment (B), yeast tRNA<sup>Phe</sup> (C), and SAM-I riboswitch (D) were calculated by APBS (49). Shown is the electrostatic potential computed 3 Å away (the radius of hydrated Mg<sup>2+</sup> is ~3 Å (80)) from the surface by VMD (100). (E–T) The spatial ion concentration of Mg<sup>2+</sup> and Na<sup>+</sup> around BWYV pseudoknot (E, I, M, and Q), 58-nt rRNA fragment

(legend continued on next page)

low  $[\text{Mg}^{2+}]$ ; 2) the binding of  $\text{Na}^+$  into major grooves of helical elements/junction pocket is overwhelmed by  $\text{Mg}^{2+}$  binding, and  $\text{Na}^+$  prefers to bind relatively loosely around RNA tertiary structures; and 3) the increase of  $[\text{Mg}^{2+}]$  causes more pronounced  $\text{Mg}^{2+}$  binding in both of the binding locations (to major groove/junction). These features for different RNA tertiary structures are understandable. As shown in Fig. 7 and Fig. S11, there is visible coupling between the local concentration distribution of  $\text{Mg}^{2+}$  and the local surface electrostatic potential. The surface potential is very negative in major grooves of helical stems and the junction pocket, and these sites are favorable for  $\text{Mg}^{2+}$  binding rather than  $\text{Na}^+$  binding because of the higher charge of  $\text{Mg}^{2+}$ .  $\text{Na}^+$  can only bind loosely to RNAs because of low ionic charge. The increase of  $[\text{Mg}^{2+}]$  decreases the entropy penalty of  $\text{Mg}^{2+}$  binding, which would enhance  $\text{Mg}^{2+}$  binding and more strongly suppress  $\text{Na}^+$  binding. It is noted that the binding of  $\text{Mg}^{2+}$  to a multibranch junction may be essential in stabilizing specific RNA tertiary structures (1,5–10,12,37–39,42–44,73,86,87).

### What governs the binding of $\text{Mg}^{2+}$ and $\text{Na}^+$ to a nucleic acid?

The global and local binding of  $\text{Mg}^{2+}$  and  $\text{Na}^+$  to various nucleic acids has been analyzed and discussed extensively. However, the question remains as to whether there is a simple rule that governs the competitive binding of  $\text{Mg}^{2+}$  and  $\text{Na}^+$  to different nucleic acid structures in addition to  $\text{Mg}^{2+}/\text{Na}^+$  concentrations. Therefore, in this study, we have attempted to explore such a rule for global binding of  $\text{Mg}^{2+}/\text{Na}^+$  to various nucleic acid structures. We also attempted to explore the rule for the local binding of  $\text{Mg}^{2+}/\text{Na}^+$  to various RNA tertiary structures. Here, our analyses cover both ds nucleic acids and RNA tertiary structures to understand the general rule for competitive binding of  $\text{Mg}^{2+}/\text{Na}^+$ , although the binding of  $\text{Mg}^{2+}$  and a variety of other cationic ligands binding to single-stranded and ds nucleic acids has been reported and discussed previously (6,15–23).

#### Global binding of $\text{Mg}^{2+}/\text{Na}^+$ to nucleic acid structures

According to the above analyses, the global binding of  $\text{Mg}^{2+}/\text{Na}^+$  mainly depends on the length and the compactness of RNA/DNA structures. We investigated whether there is a simple parameter related to RNA/DNA structure for quantifying the global binding of  $\text{Mg}^{2+}/\text{Na}^+$ . Since surface charge density has been previously shown to be a determining parameter for ion binding to spherical and cylindrical polyelectrolytes (47,48), we calculated the surface

charge densities for various RNA/DNA structures (47,48). Since the surface charge density cannot be clearly defined for a nucleic acid with complex structure, we can approximately model a nucleic acid as an ellipsoid, and the surface charge density  $\sigma_{\text{eff}}$  of the nucleic acid can be estimated as that of the ellipsoid (see the Supporting Material for details).

As shown in Fig. 5, A and B, with the increase of the effective surface charge density  $\sigma_{\text{eff}}$  for various RNA/DNA molecules, the number  $N_{\text{Mg}}^b$  of binding  $\text{Mg}^{2+}$  increases monotonously and that of  $\text{Na}^+$  ( $N_{\text{Na}}^b$ ) decreases monotonously in both 1 mM  $\text{Mg}^{2+}/20$  mM  $\text{Na}^+$  and 10 mM  $\text{Mg}^{2+}/20$  mM  $\text{Na}^+$  solutions. Such correlations between  $N_{\text{Mg}}^b$  (and  $N_{\text{Na}}^b$ ) and  $\sigma_{\text{eff}}$  are interesting and reasonable. Physically,  $\text{Mg}^{2+}$  ( $\text{Na}^+$ ) binding would become more pronounced (weaker) to a nucleic acid with higher surface charge density because  $\text{Mg}^{2+}$  with higher ionic charge interacts more strongly with a nucleic acid. Therefore, we propose that the effective surface charge density  $\sigma_{\text{eff}}$  mainly governs the global binding of  $\text{Mg}^{2+}/\text{Na}^+$  to various nucleic acids, including RNA tertiary structures, in addition to  $\text{Mg}^{2+}/\text{Na}^+$  concentrations. It is noted that our results in Figs. 5 and S12 are also consistent with the experimental analyses of the interactions of  $\text{Mg}^{2+}$  and  $\text{Na}^+$  with nucleic acids and the effects of mixtures of these ions on protein-DNA binding (15,16). Our further analyses suggest that length  $N$  may also affect the global binding of  $\text{Mg}^{2+}/\text{Na}^+$  when nucleic acids are not large, and an empirical linear coupling between the fraction of binding  $\text{Mg}^{2+}/\text{Na}^+$  and  $(1 - 3/N)\sigma_{\text{eff}}$  has been found for all the nucleic acids we used, as shown in Fig. S12. This Coulombic effect and  $1/N$  dependence of ion accumulation/binding have been previously observed for nucleic acid oligomers in a series of PB- and Monte Carlo-based theoretical studies (17–19,21–23).

#### Local binding of $\text{Mg}^{2+}/\text{Na}^+$ to RNA tertiary structures

Beyond the global binding to a nucleic acid, what governs the local binding of  $\text{Mg}^{2+}/\text{Na}^+$  to the nucleic acid? Here, we will use the closely binding of ions near phosphates to characterize the local binding of  $\text{Mg}^{2+}/\text{Na}^+$ .

Because the negative charges of a nucleic acid converge on phosphate groups and the local binding of  $\text{Mg}^{2+}/\text{Na}^+$  is generally around phosphates, we will examine the local competitive binding of  $\text{Mg}^{2+}/\text{Na}^+$  to phosphates for various RNA tertiary structures. First, we calculated the local phosphate density (i.e., local number  $N_p$  of phosphates around a phosphate) within a distance of  $\sim 9$  Å, which was taken according to the phosphate-phosphate radial distribution function for various RNA tertiary structures in Fig. S13 A. According to the different number of  $N_p$  centered at all phosphates, the phosphates of RNA tertiary structures can

(F, J, N, and R), yeast tRNA<sup>Phe</sup> (G, K, O, and S), and SAM-I riboswitch (H, L, P, and T) are displayed in green of three-level opacities (0.5 M–5 M, 5 M–10 M, and >10 M for  $\text{Mg}^{2+}$  and 0.25 M–0.5 M, 0.5 M–2.0 M, and >2.0 M for  $\text{Na}^+$ , respectively) by the VMD (100). In panels (A–D), the names of helical stems, loops, and junctions correspond to those in the secondary structures in Fig. S10. The view of the panels in the opposite direction can be found in Fig. S11. To see this figure in color, go online.

be classified into several groups, and the larger  $N_p$  represents the more compact domain with higher local charge density as shown in Fig. 8 A. Simultaneously, the fractions  $f_{Mg}/f_{Na}$  of closely binding  $Mg^{2+}/Na^+$  to phosphates were obtained by calculating the numbers of  $Mg^{2+}/Na^+$  within a small distance around the phosphate atoms, and the small distance was chosen according to the phosphate- $Mg^{2+}$  radial distribution function in Fig. S13 B. The distributions of  $N_p$  and  $f_{Mg}$  along phosphate strands for various RNA tertiary structures are shown in Fig. S14, and there is visible correlation between  $N_p$  and  $f_{Mg}$  for respective phosphates. Furthermore, we calculated the average number fractions of closely binding  $Mg^{2+}/Na^+$  per phosphate for different  $N_p$ . As shown in Fig. 8, B and C and in Table S3, we found that the average number fraction of closely binding  $Mg^{2+}$  ( $\bar{f}_{Mg}$ ) increases apparently with the increase of the local phosphate density  $N_p$  and becomes saturated at high  $N_p$ . Such an increment is more pronounced, and the saturated value of  $\bar{f}_{Mg}$  is larger for higher  $[Mg^{2+}]$ . In contrast, the increase of the fraction of closely binding  $Na^+$  with  $N_p$  is weak and appears visible at low ( $\sim 1$  mM)  $[Mg^{2+}]$ . This is because the higher charge of  $Mg^{2+}$  causes more sensitive binding of  $Mg^{2+}$  to the increased local charge density ( $N_p$ ) of RNAs than that of  $Na^+$ . The increase of  $[Mg^{2+}]$  enhances the close binding of  $Mg^{2+}$  and weakens the close binding of  $Na^+$  because of the lowered binding entropy penalty and the anticooperative binding between  $Mg^{2+}$  and  $Na^+$ . The saturation of  $\bar{f}_{Mg}$  at large  $N_p$  may be attributed to the reduced  $Mg^{2+}$ -accessible space around a phosphate with the increase of  $N_p$  for the phosphate. Our additional simulation with different initial ion positions for 58-nt rRNA fragment shows a similar ion concentration distributions and local binding of ions; see Fig. S16. Additionally, our simulation for 28-nt BWYV pseudoknot without constraint does not show a significant effect of RNA flexibility on bulk ion concentrations

and local binding of ions; see Fig. S17. It is interesting that the average number fractions of closely binding  $Mg^{2+}$  as functions of  $N_p$  converge for different RNA tertiary structures. This is understandable. The local close binding of  $Mg^{2+}/Na^+$  near a site may be mainly dependent on the local charge density around the site rather than the whole RNA structure because ion binding/screening would diminish the electrostatic potential from other phosphates of RNAs beyond the vicinity of the binding site.

## CONCLUSIONS

In this study, we performed all-atom MD simulations for various nucleic acids including dsDNA, dsRNA, and several RNA tertiary structures. A simple and efficient method for partitioning divalent/monovalent ions to simulate a nucleic acid system with desired divalent/monovalent bulk ion concentrations was proposed and validated for extensive nucleic acids in mixed  $Mg^{2+}/Na^+$  solutions. The global binding and local binding of  $Mg^{2+}$  and  $Na^+$  were analyzed for various nucleic acids including several RNA tertiary structures, and our calculations are in accordance with the available experimental data. Through extensive calculations and analyses, we have obtained the following major conclusions:

- 1) We have proposed a simple and efficient method for partitioning divalent/monovalent ions to simulate a nucleic acid in mixed divalent/monovalent ion solutions, and only one MD simulation is required for a nucleic acid with arbitrary structure in a mixed  $Mg^{2+}/Na^+$  solution to obtain desired bulk divalent/monovalent ion concentrations. The method has been tested and validated for extensive nucleic acids and a wide range of mixed divalent/monovalent ion conditions.

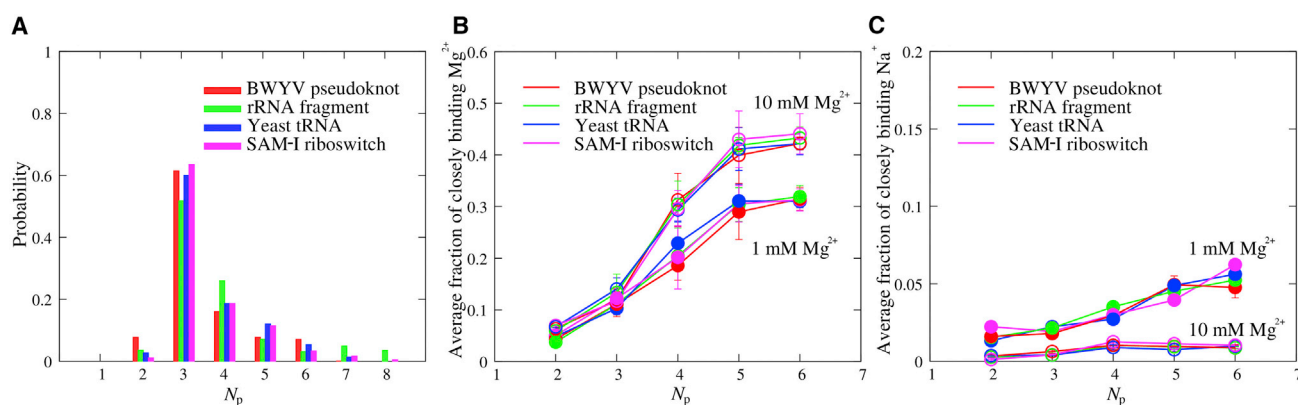


FIGURE 8 (A) The normalized distribution probability of the local phosphate density  $N_p$  for various RNA tertiary structures;  $N_p$  for a phosphate denotes the number of phosphates within a distance of 9 Å around the phosphate. (B and C) These show the average number of closely binding  $Mg^{2+}$  (B) and  $Na^+$  (C) around phosphates within a distance of 5 Å for RNA tertiary structures in 1 mM  $Mg^{2+}/20$  mM  $Na^+$  and 10 mM  $Mg^{2+}/20$  mM  $Na^+$  solutions, respectively. The cutoff distances for  $N_p$  and for closely binding  $Mg^{2+}/Na^+$  were selected according to the radial distributions of phosphates and  $Mg^{2+}$  around phosphates (see Fig. S13). The change of cutoff distance from 5 to 6 Å for phosphate- $Mg^{2+}$  does not significantly change the curves of average fractions of closely binding  $Mg^{2+}$  and  $N_p$  (see Fig. S15). To see this figure in color, go online.

- 2) The global binding of  $Mg^{2+}$  ( $Na^+$ ) to dsRNA is slightly stronger (weaker) than that to dsDNA, and  $Mg^{2+}$  binding is much more pronounced than  $Na^+$  binding for both dsRNA and dsDNA. Furthermore,  $Mg^{2+}$  binding to dsRNA is more likely to occur in the deep groove than that to dsDNA. Such difference is attributed to the different helical structures: A-form dsRNA is shorter and has deeper/narrower major grooves and wider/shallower minor grooves in comparison with B-form dsDNA.
- 3) The global binding of  $Mg^{2+}/Na^+$  to RNA tertiary structures depends on the structure compactness and chain length in addition to  $Mg^{2+}/Na^+$  concentrations. Through comprehensive analyses, we found the apparent correlation between global binding of  $Mg^{2+}/Na^+$  and the effective surface charge density for various nucleic acid molecules.
- 4) Local binding of  $Mg^{2+}/Na^+$  near the RNA surface is dependent on the local structures around binding sites, and favorable binding sites are generally in the major grooves of stems and junctions. The local binding of  $Mg^{2+}/Na^+$  near a phosphate is found to be visibly correlated to the local phosphate density for various RNA tertiary structures.

Although our proposed partition formula works very well for extensive nucleic acids over the wide range of mixed divalent/monovalent ion conditions, and our predictions agree well with the available experimental data, there are still some simplifications and limitations in our work. First, we employed the Åqvist force field for  $Mg^{2+}$  (95) rather than other  $Mg^{2+}$  models (81,98,99), such as the NBFIX hydrated  $Mg^{2+}$  model (81). However, this treatment should not significantly affect our major analyses and conclusions because previous simulations have shown that  $Mg^{2+}$  ions generally become hydrated before accumulating near nucleic acids, and the majority of  $Mg^{2+}$  ions would generally keep their hydrated shell (72,86). The minority of possible chelated  $Mg^{2+}$  ions would be somewhat related to the adopted force fields for  $Mg^{2+}$  (86). Second, our MD simulations did not cover the salt concentrations below 1 mM [ $Mg^{2+}$ ], since too-low salt concentrations would make the simulation cell too large and bring a huge computation cost. Finally, in our MD simulations, all the nucleic acids are restrained with a strong harmonic potential, and we consequently ignored the effect of flexibility of nucleic acids on ion binding. The interplay of flexibility and ion binding for RNA tertiary structures is an important issue and deserves to be discussed elsewhere. Nevertheless, this work proposed a simple and useful method for simulating a nucleic acid solution with desired mixed divalent/monovalent ion concentrations, and our analyses and results will be helpful for understanding the binding of divalent/monovalent ion to extensive DNAs and RNAs.

## SUPPORTING MATERIAL

Supporting Materials and Methods, seventeen figures, and three tables are available at [http://www.biophysj.org/biophysj/supplemental/S0006-3495\(18\)30297-2](http://www.biophysj.org/biophysj/supplemental/S0006-3495(18)30297-2).

## AUTHOR CONTRIBUTIONS

Z.-J.T., K.X., and F.-H.W. designed the research. K.X., F.-H.W., and Z.-L.Z. performed the calculations. Z.-J.T., K.X., F.-H.W., G.X., and Z.-L.Z. analyzed the data. K.X., F.-H.W., and Z.J.T. wrote the manuscript. All authors discussed the results and reviewed the manuscript.

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