Supplemental Information

Mutual effect between single-stranded DNA conformation and Na⁺-Mg²⁺ ion competition in mixed salt solution

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S1. The MCTBI model

Ions are considered to be fully hydrated in the MCTBI model. Specifically, the mono- and divalent ion radii correspond to one hydrated shell and two hydrated shells, respectively,^{1,2} giving rise to $r_+ = 3.5$ Å, $r_{2+} = 4.5$ Å, and $r_- = 4.0$ Å for the Na⁺, Mg²⁺, and Cl⁻ ions, respectively. For a ssDNA-mixed ion system, the MCTBI calculation follows three steps: (1) establishing the demarcation between tightly bound (TB) and diffusely bound (DB) regions; (2) sampling the discrete distributions of the TB ions (in the TB region) using the Monte Carlo "insertion-deletion" (MCID) algorithm; and (3) calculating the free energy of the DB ions (in the DB region) based on the TB ion distributions.

(a) Determination of the TB region

For the ssDNA with a fixed conformation, we first run the nonlinear Poisson-Boltzmann (NLPB) calculation with three-step focusing process³ (with the grid sizes of 1.7, 0.85, and 0.425Å) to roughly estimate the local ion concentration c(r). Based on c(r) around the ssDNA, the spatial distribution of the ion strength S(r) can be calculated:

$$S_{\alpha}(r) = \frac{(Z_{\alpha}e)^2}{k_{\rm B}T\varepsilon_{\rm w}a_{\alpha}(r)}$$
(S1)

Here the subscript α denotes the ion species. Z_{α} , $k_{\rm B}$, T, and $\varepsilon_{\rm W}$ are the valency of ion α , Boltzmann constant, temperature, and solvent dielectric constant (= 78.5 at room temperature), respectively. $a_{\alpha}(r)$ represents the Wigner-Seitz radius of the ion species α at position r, which is determined by the local ion concentration:⁴

$$\frac{4\pi}{3}[a_{\alpha}(r)]^{3}[c_{\alpha}(r)-c_{\alpha}]=1$$
(S2)

Here c_{α} is the bulk concentration of ion α . The region where the ion strength $S_{\alpha}(r)$ is larger than the critical strength $S_c = 2.6$ (the value for gaseous to liquid phase transition in the ion system⁵) is defined as the TB region. In general, the width of the TB region usually from zero to several angstroms. For the monovalent ions, the width of the TB region is less than 0.2Å even at a high ion bulk concentration. Therefore, the TB region of the monovalent ions can be ignored and the monovalent ions are considered as the DB ions. For the divalent ions, a higher bulk concentration can lead to a larger TB region.

(b) The discrete distributions of the TB Mg²⁺ ions

In the TB region, we use simple cubic lattice with lattice size l_b to sample the discrete distribution of the TB Mg²⁺ ions. Each lattice site can be occupied by at most a Mg²⁺ ion. The distributions of the TB ions are generated by adding ions one by one in the TB region. The sum over all possible distributions of N_b ions in TB region gives the total statistical weight $W(N_b)$:

$$W(N_{\rm b}) = \prod_{i=1}^{N_{\rm b}} w(i) \tag{S3}$$

Here the product " Π " represents the process of inserting the first, second,, N_b -th ion into the TB region. The individual statistical weight w(i) for the *i*th added ion is given by:

$$w(i) = l_{\rm b}^3 \sum_{k=1}^{m_i} e^{-U_i(k)/k_{\rm B}T}$$
(S4)

Here m_i represents the number of available sites in the TB region for the *i*th inserted ion.

 $U_i(k)$ in Eq. (S4) is the interaction energy between the inserted ion at site k and other pre-existing particles:

$$U_{i}(k) = \sum_{j} \frac{Z_{i}Z_{j}e^{2}}{\varepsilon_{R}r_{ij}} + \sum_{j} \varepsilon_{ij} [(\frac{\sigma_{ij}}{r_{ij}})^{12} - (\frac{\sigma_{ij}}{r_{ij}})^{6}] + (\frac{1}{\varepsilon_{W}} - \frac{1}{\varepsilon_{R}}) \sum_{j} \frac{Z_{i}Z_{j}e^{2}}{\sqrt{r_{ij}^{2} - B_{i}B_{j}} \exp(-r_{ij}^{2}/4B_{i}B_{j})} + (\frac{1}{\varepsilon_{W}} - \frac{1}{\varepsilon_{R}})(\frac{1}{B_{i}} - \frac{1}{B_{i}^{0}})Z_{i}^{2}e^{2}$$
(S5)

Here the subscript *j* represents the pre-existing particle *j* in the TB region (including the beads of the ssDNA and other TB ions already therein). The first term in the above equation is the Coulombic energy between charges *i* and *j*. $\varepsilon_{\rm R}$ is the relative dielectric constant (=20 for the ssDNA), and r_{ij} is the distance between the two particles. The second term is the Lennard-Jones (LJ) potential with $\varepsilon_{\rm lj}$ (=0.35) as the LJ strength and $\sigma_{\rm ij}$ as the equilibrium distance between particles *i* and *j*. As only the TB Mg²⁺ ions are treated as discrete, the LJ potential describes the volume exclusions between Mg²⁺ ions and between Mg²⁺ ions and ssDNA beads. The third and forth terms denote the mutual polarization energy induced by other particles and self-polarization of the *i*th inserted TB ion, respectively. $B_{i(\text{or }j)}$ is the Born radius of particle *i* (or *j*) in the TB region and B_i^0 is the Born radius of an isolated TB ion.

To calculate the total and individual statistical weights $W(N_b)$ and w(i), we configure the TB ions in the TB region by adding the ions one by one. However, the newly added ion will perturb the distribution of the pre-existing TB ions. To take the above feedback effect into account, we will use the MCID algorithm⁶ to sample the TB ion distributions. The MCID algorithm involves the insertion step and deletion step.

The insertion step. In this step, we insert the TB ions one by one into the TB region until there are N TB ions (here N is equal to the nucleotide number of the ssDNA). After (i-1)ions have been inserted the TB region, to insert the newly *i*th ion, we enumerate all available sites $k=1, 2, ..., m_i$ and calculate the interaction energy $U_i(k)$ corresponding to each site. The newly added *i*th ion has the probability $p_i(i,k)$ to be placed at site k:

$$p_{\rm f}(i,k) = \frac{e^{-U_i(k)/k_{\rm B}T}}{\sum_{k=1}^{m_i} e^{-U_i(k)/k_{\rm B}T}}$$
(S6)

Here $\Sigma_k p_f(i,k) = 1$. The site with low interaction energy has high probability to be visited by the *i*th ion. At the end of this step, a *N*-TB ion distribution is generated.

The deletion step. In this step, the ions in the TB region will be removed one by one until there is no ion in the TB region. A TB ion *j* has the probability $p_b(j,k)$ to be removed from the site *k*:

$$p_{b}(j,k) = \frac{e^{U_{j}(k)/k_{\rm B}T}}{\sum_{j} e^{U_{j}(k)/k_{\rm B}T}}$$
(S7)

Here $U_j(k)$ represents the interaction energy between the ion *j* at site *k* and other charged particles (including the phosphates of the ssDNA and other TB ions). With removing the first, second,...*i*th ion from the TB region, there are N-1, N-2,, N-i ions remaining in the TB region. According to the removing probability in the above equation, the remained ions tend to a low-energy distribution. For each (i-1)-ion distribution, we then can calculate the individual statistical weight w(i) using Eq. (S4).

For each N-ion distribution generated in the insertion step, we repeat the deletion step M_b times

to calculate the averaged w(i). The total statistical weight $W(N_b)$ for N_b -ion distribution then can be calculated by Eq. (S3). In our calculations, the *N*-ion distribution will be generated M_f independent samples to compute the averaged $\overline{W(N_b)}$. Our previous test indicated that $M_b = N$ (the number of ssDNA nucleotides) and $M_f = N_s$ (the number of lattice sites) yield the optimal balance between the sampling efficiency and accuracy.

(c) The free energy of the DB ions

We classify the TB-ion distributions according to the number N_b (=0, 1,, N) of the TB ions. For each N_b , we can compute a Boltzmann-averaged mean TB ion distribution from the discrete N_b -ion distributions obtained by the MCID algorithm. According to the mean TB ion distribution, we implement the NLPB calculation to compute the corresponding free energy ΔG_d of the DB ions in the DB regions:^{7,8}

$$\Delta G_{d} = \frac{1}{2} \int \sum_{\alpha} c_{\alpha}(r) Z_{\alpha} e[\varphi(r) + \varphi'(r)] d^{3}r + \int \sum_{\alpha} [c_{\alpha}(r) \ln(\frac{c_{\alpha}(r)}{c_{\alpha}}) - c_{\alpha}(r) + c_{\alpha}] d^{3}r$$
(S8)

Here $\varphi(r)$ and $\varphi'(r)$ denote the electric potentials at position *r* with and without the DB ions in the solution. The first integral includes the free energy for the interaction between the DB ions and the charged particles (charged phosphates and TB ions) in the TB region and the enthalpic part of the free energy for the DB ions. The second integral provides the entropic part of the free energy for the DB ions.

S2. The charge ratio of depleted Cl⁻

In order to calculate the binding fractions f_+ of the excess Na⁺ ions and f_{2+} of excess Mg²⁺ ions, in addition to the Eqs (12) and (13) in the main paper, we need one more function that involves the binding fraction f_- of the depleted Cl⁻ ions per nucleotide. To this end, we build a function similar with the excess Na⁺ ion contribution R_+ (see Eq. (6) in main paper), as the ratio of depleted anion Cl⁻ charges against the sum of itself and the excess Mg²⁺ charges:

$$R_{-} = \frac{-f_{-}}{2f_{2+} - f_{-}} \tag{S9}$$

In general, the smaller value of R_- with a fixed f_{2^+} indicates that less anions are excluded from the ion atmosphere by the negatively charged phosphates of the ssDNA. The ion binding fraction f_{2^+} (or f_-) of the excess Mg²⁺ (or depleted Cl⁻) ions can be calculated by the MCTBI model.

For the initial conformation of CG ssDNA poly(dT)₄₈ generated from the software X3DNA, the MCTBI-calculated R_- under various ion concentrations are shown by the scatters in the Fig. S2(a). At a fixed c_+ , a high c_{2+} favors the Mg²⁺ ions to compete with the Na⁺ ions, increasing the number of excess Mg²⁺ ions in the ion atmosphere. These excess Mg²⁺ ions can prevent Cl⁻ ions from leaving the ion atmosphere due to the strong electrostatic Mg²⁺-Cl⁻ attractions. As a result, we can find that R_- decreases with the increase in c_{2+} . On the contrary, if the excess Na⁺ ions are dominant in the atmosphere, as Na⁺-induced screening/neutralizing effects are relatively weak, more Cl⁻ ions will be excluded away from the ion atmosphere. Therefore, a high c_+ results in a low R_- at a fixed c_{2+} . In addition, we also show the MCTBI-based calculation results of the conformation-dependent R_- in Fig. S2(b). The accumulation of the phosphates favors Mg²⁺ in the Na⁺-Mg²⁺ competition, which increases the number of excess Mg²⁺ ions and consequently decreases the depletion of the anions. Therefore, R_- decreases with the increase of the linear density of the phosphates ρ_P .

According to the MCTBI calculations, we find that the the ratio of Cl⁻ charge depletion R_{-} is highly dependent on the Na⁺-Mg²⁺ competition,⁹ which is governed by the ion concentrations c_{+} and c_{2+} and the ssDNA conformations. Therefore, similar with R_{2+} (see Eq. (12) in main paper), R_{-} is also can be phenomenologically described as:

$$R_{-} = \frac{(c_{+})^{A_{+}} \rho_{P}^{-1}}{(c_{+})^{A_{+}} \rho_{P}^{-1} + (\frac{c_{+}}{2c_{2+}})^{A_{-}} (2c_{2+})^{A_{2+}} \rho_{P}}$$
(S10)

Here the best-fit values of the two parameters $A_{+} = 1.37$ and $A_{2+} = 0.965$ come from the Eq. (12) in the main paper. We obtain the value of parameter $A_{-} = 0.27$ by fitting the Eq (S10) with the MCTBI-based calculation results shown in Fig. S2.

S3. The radius of gyration for ssDNA in single salt solution

We can use LD simulation to study the conformations of ssDNA poly(dT)₁₂₀ in the single NaCl or MgCl₂ solution. In the simulations, the effective charge $Q = Q_+$ (= C_s/l_B) in NaCl solution and $Q = Q_{2+}$ (= $C_s/2l_B$) in MgCl₂ solution, respectively. Here the charge space $C_s = 4.4$ Å¹⁰ on the ssDNA backbone and Bjerrum length $l_b = 7.15$ Å at room temperature. Fig. S4 shows the simulation results of the Mg²⁺ ion concentration c_{2+} -dependent R_g (red squares) for the ssDNA in pure MgCl₂ solution and the Na⁺ concentration c_+ -dependent R_g (black cycles) for the ssDNA

The radius of gyration R_g for the ssDNA can be considered as the sum of two components, which can be expressed as:

$$R_{\rm g} = R_{\rm g}^{\infty} + R_{\rm g}^{\prime} \tag{S11}$$

Here R_{g}^{∞} represents the intrinsic radius of gyration for the ssDNA without the electrostatic repulsion between the charged phosphates, which can be regarded as the ssDNA at the solution with infinitely high ion concentration. R_{g}' is the contribution of the ion-involved bead P-bead P electrostatic repulsion to R_{g} . Inspired by the treatments of persistence length in reference,¹¹ the radius of gyration can be expressed by:

$$R_{g} = R_{g}^{\infty} + \frac{R_{g}^{0} - R_{g}^{\infty}}{(a_{\alpha}c_{\alpha})^{n_{\alpha}/2} + 1}$$
(S12)

Here R_g^0 denotes the radius of gyration for ssDNA at pure water (without the salt). The subscript α represents the ion species (Na⁺ or Mg²⁺). In addition, the radii of gyration R_g^0 and R_g^{∞} for the ssDNA at the extreme ion conditions $c_{\alpha} = 0$ M and $c_{\alpha} = \infty$ M, respectively, can be calculated form the Flory scaling relationship:¹²

$$R_{g}^{\circ} = \mathbf{B}_{\infty} N^{\nu_{\infty}}$$

$$R_{g}^{0} = \mathbf{B}_{0} N^{\nu_{0}}$$
(S13)

Here both the prefactor B and Flory exponent v are sensitive to the ion condition. Based on our previous studies,¹³ at infinitely high ion concentration $B_{\infty} = 4.37$ and $v_{\infty} = 0.51$, while at pure water $B_0 = 2.43$ and $v_{\infty} = 0.73$. As a result, for the ssDNA poly(dT)₁₂₀, $R_g^0 = 80.1$ Å and $R_g^{\infty} = 50.2$ Å by Eq. (S13). Using the values of R_g^0 and R_g^{∞} in Eq. (S12), we can obtain the parameters

 $a_{+} = 20.7$ and $n_{+} = 2.3$ for the ssDNA-NaCl system (see black line in Fig. (S3)) and $a_{2+} = 384$ and $n_{2+} = 1.36$ for the ssDNA-MgCl₂ system (see red line) by fitting Eq. (S12) to the simulation results.

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magg (Da)		mp		ms	m _B	
mass (Da)		95		76	120	
mating(8)		ґр		ŕs	ľВ	
radius(A)		1.53	1	.49	1.96	
		P-S	S	S-P	S-B	
bond potential ^a	$k_{ m b}$	4.5	1	9.5	18.0	
	b_0	4.11		3.8	4.3	
		P-S-P	S-P-S	P-S-B	B-S-P	
angle potential ^b	ka	4.0	5.8	10.6	11.8	
	$ heta_0$	1.91	1.57	1.66	2.23	
			P-S-P-	S		
	k_1		k_2	k_3	k_4	
	1.60		0.85	0.33	0.23	
	ϕ_1		ϕ_2	ϕ_3	ϕ_4	
dihedral angle	0.08	-	0.87	-0.52	-0.66	
potential ^b		S-P-S-P				
	k_1		k_2	k_3	k_4	
	1.37	-	0.70	0.38	-0.28	
	ϕ_1		ϕ_2	ϕ_3	ϕ_4	
	0.04		0.11	-1.28	0.35	

Table S1. Parameters in the PACG model

^a the units of the parameters k_b and b_0 are $k_BT/Å^2$ and Å.

^b the units of k_a and k_n are k_BT/rad^2 , and the units of θ_0 and ϕ_n are radian.

$c_{+} = 0.005 \mathrm{M}$		$c_{+} = 0.02 \mathrm{M}$		$c_{+} = 0.1 M$		$c_{+} = 0.5 M$						
$C_{2+}/1$ VI	f_{2^+}	f_+	f-	f_{2^+}	f_+	f-	f_{2^+}	f_+	f-	f_{2^+}	f_+	f-
0.00001	0.020	0.702	-0.234	0.002	0.733	-0.261	0.000	0.688	-0.305	0.000	0.608	-0.348
0.00003	0.049	0.648	-0.228	0.007	0.725	-0.260	0.001	0.687	-0.305	0.000	0.608	-0.348
0.00006	0.081	0.591	-0.221	0.014	0.712	-0.258	0.001	0.686	-0.305	0.000	0.608	-0.348
0.0001	0.112	0.537	-0.213	0.022	0.697	-0.256	0.002	0.684	-0.305	0.000	0.608	-0.348
0.0003	0.200	0.387	-0.188	0.056	0.635	-0.248	0.007	0.677	-0.303	0.001	0.607	-0.347
0.0006	0.260	0.290	-0.169	0.094	0.569	-0.238	0.013	0.666	-0.302	0.001	0.606	-0.347
0.001	0.303	0.222	-0.154	0.132	0.502	-0.228	0.021	0.652	-0.299	0.002	0.604	-0.347
0.003	0.375	0.110	-0.130	0.241	0.319	-0.195	0.055	0.592	-0.290	0.007	0.596	-0.346
0.006	0.407	0.063	-0.122	0.308	0.210	-0.173	0.095	0.524	-0.279	0.014	0.585	-0.344
0.01	0.423	0.040	-0.118	0.352	0.141	-0.156	0.135	0.454	-0.267	0.023	0.571	-0.342

Table S2. The MCTBI model-calculated binding fractions of excess Na⁺ and Mg²⁺ (f_+ and f_{2+} , respectively), and depleted Cl⁻ (f_-) for poly(dT)₄₈ with the initial structure at various ion concentrations.

c_{2+}/M	$ ho_{ ext{P}^{ ext{a}}}$ /Å-1	$ ho_{ ext{P}}^{ ext{b}}$ /Å-1
0.0001	0.962 ± 0.001	0.964 ± 0.001
0.0003	$0.985{\pm}0.001$	0.996 ± 0.001
0.0006	0.993±0.001	1.022 ± 0.001
0.001	1.022 ± 0.001	1.035 ± 0.001
0.003	1.060 ± 0.001	1.076 ± 0.001
0.006	1.083 ± 0.001	1.104 ± 0.001
0.01	1.110 ± 0.001	1.118 ± 0.001
0.03	1.142 ± 0.001	1.138 ± 0.001
0.06	1.165 ± 0.001	1.185 ± 0.004

Table S3. The simulation data of c_{2+} -dependent ρ_P for poly(dT)₃₀ at $c_{+} = 0.02M$

^a the simulations use the effective charge of bead P obtained from Eq. 15 in the main paper.

^b the simulations use the effective charge of bead P obtained from Eq. 16 in the main paper with the parameter $A_B = 0.5$.

c_{2+}/M	$c_{+} = 0.02 \mathrm{M}$	$c_{+} = 0.05 M$	$c_{+} = 0.2 M$
0	72.4±0.1	64.3±0.1	55.3±0.1
0.00001	73.0±0.1	65.8±0.1	56.0±0.1
0.00003	70.8±0.1	64.5±0.1	54.6±0.1
0.00006	71.6±0.1	64.3±0.1	56.1±0.1
0.0001	68.7±0.1	63.2±0.1	55.2±0.1
0.0003	65.4±0.1	62.0±0.1	55.2±0.1
0.0006	63.3±0.1	59.1±0.1	54.6±0.1
0.001	62.0±0.1	58.4±0.1	54.2±0.1
0.003	60.0±0.1	56.5±0.1	53.1±0.1
0.006	57.4±0.1	56.1±0.1	53.5±0.1
0.01	55.8±0.1	55.5±0.1	51.8±0.1
0.03	52.9±0.1	52.6±0.1	51.2±0.1
0.06	53.1±0.1	50.3±0.1	50.4±0.1
0.1	51.1±0.1	51.7±0.1	50.4±0.1

Table S4. The simulation data of the c_{2+} -dependent R_g for the ssDNA poly(dT)₁₂₀ at various Na⁺ concentrations c_+ .

c_+/M	$c_{2+} = 0.0001 \mathrm{M}$	$c_{2+} = 0.001 \mathrm{M}$	$c_{2^+} = 0.01 \mathrm{M}$			
0	77.9±0.1	69.6±0.1	58.2±0.1			
0.0001	77.2±0.1	71.3±0.1	58.5±0.1			
0.0003	76.2±0.1	70.9±0.1	58.9±0.1			
0.0006	75.9±0.1	69.8±0.1	58.2±0.1			
0.001	75.2±0.1	70.0±0.1	59.5±0.1			
0.003	74.0±0.1	69.0±0.1	57.4±0.1			
0.006	72.9±0.1	65.9±0.1	58.3±0.1			
0.01	71.7±0.1	64.2±0.1	56.7±0.1			
0.03	67.0±0.1	60.9±0.1	55.9±0.1			
0.06	61.9±0.1	58.3±0.1	54.6±0.1			
0.1	57.3±0.1	55.2±0.1	53.0±0.1			
0.3	52.6±0.1	52.7±0.1	52.5±0.1			
0.6	52.3±0.1	50.8±0.1	50.8±0.1			
1	52.7±0.1	50.3±0.1	52.2±0.1			

Table S5. The simulation data of the c_+ -dependent R_g for the ssDNA poly(dT)₁₂₀ at various Mg²⁺ concentrations c_{2+} .



Fig. S1. (a) Ten distinct conformations of the ssDNA $poly(dT)_{48}$ selected from the ensemble of 300 conformations. The numbers are the conformation indices in the conformation pool. (b and c) The distributions of the radius of gyration (b) and end-to-end distance (c) for the 300 conformations.



Fig. S2. (a) The c_{2+} -dependent ratio of Cl⁻ depleted charges R_{-} at various mixed ion conditions for the initial conformation of the CG ssDNA poly(dT)₄₈. (b) The ssDNA conformation-dependent R_{-} at various mixed ion conditions. The ssDNA conformation is characterized by the linear density $\rho_{\rm P}$ of the phosphate beads. The scatters and lines are the calculation results from the MCTBI model and Eq. (S10), respectively.



Fig. S3. The training of the unpaired base-Mg²⁺ ion interaction term $\chi_B = 1 + A_B$ (for the totally non-base-paired ssDNA) in Eq. (16) of the main paper by comparing with the results from ref. 14. Scatters are the experimental data and the dashed line are the results from the hill equation $f_{2+} = F_{2+}(c_{2+}/M_{2+})^n [1+(c_{2+}/M_{2+})^n]$ in ref. 14. Here $F_{2+} = 0.49$, $M_{2+} = 1.09$ mM and n = 0.88. The plots of Mg²⁺ binding fraction f_{2+} versus Mg²⁺ concentration c_{2+} show that $A_B = 0.5$ leads to the best agreement between the calculation results of our simulation model and the hill equation in ref. 14.



Fig. S4. The ion concentration c_{α} -dependent radius of gyration R_g for the ssDNA poly(dT)120 in single NaCl (red) or MgCl₂ (black) solution. Here α represents the cation species Na⁺ or Mg²⁺. The scatters are the simulation results with the effective change constant $Q = Q_+$ in NaCl solution and $Q = Q_{2+}$ in MgCl₂ solution. The lines are the analytical results calculated from Eqs. (12) and (13). Here $a_+ = 20.7$ and $n_+ = 2.3$ for the ssDNA-NaCl system and $a_{2+} = 384$ and $n_{2+} = 1.36$ for the ssDNA-MgCl₂ system.