

Solvation Behavior of Elastin-like Polypeptides in Divalent Metal Salt Solutions

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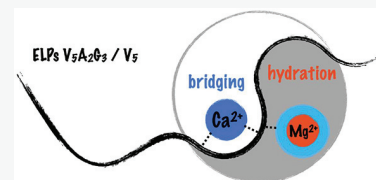


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Supporting Information

ABSTRACT: The effects of CaCl_2 and MgCl_2 on the cloud point temperature of two different elastin-like polypeptides (ELPs) were studied using a combination of cloud point measurements, molecular dynamics simulations, and infrared spectroscopy. Changes in the cloud point for the ELPs in aqueous divalent metal cation solutions were primarily governed by two competing interactions: the cation–amide oxygen electrostatic interaction and the hydration of the cation. In particular, Ca^{2+} cations can more readily shed their hydration shells and directly contact two amide oxygens by the formation of ion bridges. By contrast, Mg^{2+} cations were more strongly hydrated and preferred to partition toward the amide oxygens along with their hydration shells. In fact, although hydrophilic ELP $\text{V}_5\text{A}_2\text{G}_3$ was salted-out at low concentrations of MgCl_2 , it was salted-in at higher salt concentrations. By contrast, CaCl_2 salted the ELP sharply out of solution at higher salt concentrations because of the bridging effect.



The phase behavior of macromolecules in aqueous solutions can be modulated by salts through a combination of ion–ion, ion–water, and macromolecule–water (hydration shell) interactions.^{1–6} Although the dominant effect of salts has been attributed to anion effects, a direct cationic Hofmeister series has been proposed for the salting-out of proteins from aqueous solutions:^{7–10} $\text{N}(\text{CH}_3)_4^+ > \text{NH}_4^+ > \text{Cs}^+ > \text{Rb}^+ > \text{K}^+ > \text{Na}^+ > \text{Li}^+ > \text{Ca}^{2+} > \text{Mg}^{2+}$. However, the mechanism driving the salting-in–salting-out behavior in this series is not fully understood. Studies based on small solutes such as butyramide and macromolecules like poly(*N*-isopropylacrylamide) (PNIPAM) as well as proteins have indicated that weakly hydrated monovalent cations tend to be depleted from the solute whereas strongly hydrated divalent cations interact weakly with the amide moiety.^{2,5,11} Bruce et al.⁵ proposed a salting-in contribution due to the interaction of Ca^{2+} and CaCl^+ solvent-shared ion pairs with the amide oxygen based on a combination of cloud point experiments and MD simulations. However, the cumulative effect of all the interactions still led to net salting-out behavior for PNIPAM. Understanding these ion effects on the conformations of macromolecules is imperative, particularly in the case of intrinsically disordered proteins (IDPs) whose aggregation is believed to play an important role in neurodegenerative diseases.^{12–15} The binding of ions, especially multivalent cations, with IDPs may promote/inhibit chain conformations which may accelerate the formation of neurotoxic aggregates,^{13,15} which motivates us to provide atomistic insight into cation–IDP interactions. To that end, we consider a typical IDP-like model system, the elastin-like polypeptides (ELPs).¹⁶ The ELPs are pentameric repeats of VPGXG, whereby X represents different combinations of G, A, and V in the fourth position. We study the phase behavior of two ELP sequences,

namely, V_5 -120 and $\text{V}_5\text{A}_2\text{G}_3$ -120 (where 120 represents the total number of pentamers per ELP chain) in chloride salts of divalent cations, Ca^{2+} and Mg^{2+} . ELPs exhibit a lower critical solution temperature (LCST) transition in aqueous solutions with an expanded–collapsed conformational change.^{17–19} The cloud point of ELPs depends on the guest residue, the molecular weight, salts, and other cosolvents.^{17,20–28} Significantly, we observe that the Mg^{2+} –amide oxygen interaction can lead to salting-out (V_5) or salting-in ($\text{V}_5\text{A}_2\text{G}_3$) behavior at a high salt concentration. By contrast, Ca^{2+} –amide oxygen bridging interactions lead to salting-out of both V_5 and $\text{V}_5\text{A}_2\text{G}_3$. Molecular dynamics simulations show that the effect of divalent salts on the phase behavior of ELPs is mainly determined by a competition between the cation–amide oxygen and the cation–hydration water interactions. Additionally, the ionic interactions are strongly influenced by the presence of triglycine motifs (pentamers with G as guest residues).

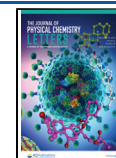
Figure 1 depicts the molecular mechanism for the observed salt effects on the phase behavior of ELPs derived from atomistic molecular dynamics simulations, thermodynamic measurements, and infrared spectroscopy. The divalent cations, Ca^{2+} and Mg^{2+} , interact weakly with the amide oxygens (it should be noted that most of the cations are depleted from the ELP chain). Ca^{2+} cations are able to shed

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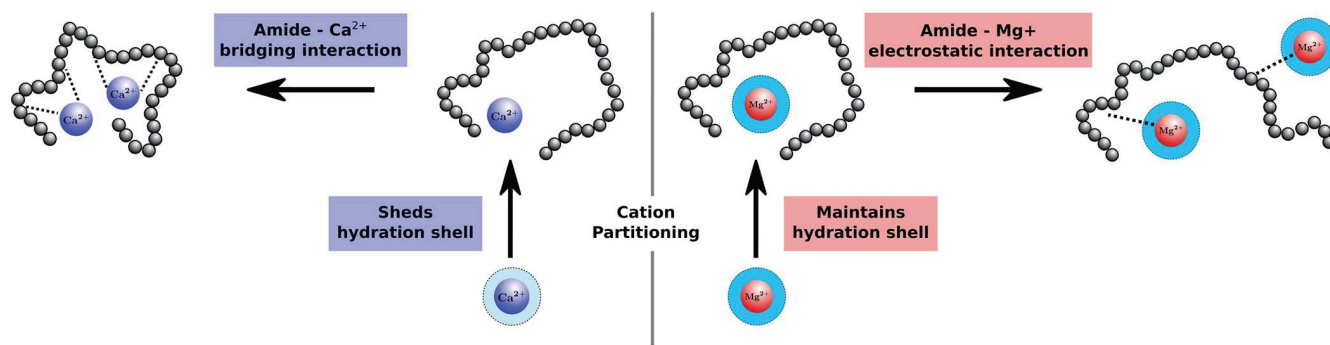


Figure 1. Schematic illustration of the interaction between two divalent cations, Ca^{2+} and Mg^{2+} , and ELPs, as derived from molecular dynamics simulations, thermodynamic measurements, and infrared spectroscopy.

their hydration shell water molecules and engage in the formation of ion bridges among two amide oxygen atoms. This mechanism leads to a rapidly decreasing cloud point temperature (salting-out). On the other hand, Mg^{2+} cations bind their hydration water tightly as compared to Ca^{2+} . As a result, the Mg^{2+} ions partition to the ELP chain along with their hydration waters and are therefore not able to form ion bridges. The partitioning of the hydrated Mg^{2+} cations to the ELP chain increases with increasing salt concentration, which leads to the salting-in of the ELP at higher salt concentrations. Moreover, the triglycine motifs in $\text{V}_5\text{A}_2\text{G}_3$ (which makes the polymer chain more flexible and less hydrophobic compared with V_5) are revealed to play a significant role in regulating the phase behavior of ELPs in salt solutions.

Phase Behavior of ELPs in Aqueous Salt Solutions. The top panel of Figure 2 shows the cloud point temperature of two 120 pentamer repeat ELP sequences, $\text{V}_5\text{A}_2\text{G}_3$ -120 and V_5 -120 (5 mg/mL of each ELPs), in CaCl_2 and MgCl_2 solutions. Readers are referred to Section S1 in the Supporting

Information for details pertaining to the materials and experiments. For $c < 1.5$ M, the cloud point temperature decreases with increasing salt concentration, indicating the salting-out effect of CaCl_2 with both ELPs. At CaCl_2 concentrations above $c > 1.5$ M, the cloud point of both ELPs decreases sharply with increasing salt concentration. Interestingly, the two ELPs have different phase behaviors in the MgCl_2 solution. $\text{V}_5\text{A}_2\text{G}_3$ showed salting-out behavior (decreasing cloud point) at low MgCl_2 concentration ($c < 1.5$ M), but salting-in behavior (increasing cloud point) was observed at high MgCl_2 concentration ($c > 1.5$ M); see Figure 2a. By contrast, the cloud point of V_5 monotonically decreased with increasing salt concentration at both low and high MgCl_2 concentration; see Figure 2b. Therefore, MgCl_2 had a similar effect as CaCl_2 on the phase behavior of V_5 , albeit the cloud point decreased to a smaller extent in the solution with MgCl_2 . Two key observations from Figure 2 are the effect of divalent metal salts on the phase behavior of ELPs is (a) cation-specific and salt concentration dependent and (b) sequence dependent. The difference between the cloud point behavior of the two ELPs in divalent metal salt solutions is likely caused by the presence of flexible triglycine motifs in $\text{V}_5\text{A}_2\text{G}_3$.

ELP–Cation Interactions. To understand the mechanisms governing the observations in Figure 2a, we studied the interactions of Ca^{2+} and Mg^{2+} with two 20 pentamer repeat ELP sequences, $\text{G}_6\text{A}_4\text{V}_{10}$ (seq1) and $\text{G}_3\text{A}_2\text{V}_{10}\text{A}_2\text{G}_3$ (seq2), using atomistic molecular dynamics (MD) simulations at two salt concentrations, 1.0 and 2.5 M. In seq1, the flexible triglycine motifs (pentamers with G as the guest residue, VPGGG) are placed in one block of the ELP chain (G_6 on one terminus). By contrast, in seq2, the triglycine motifs are placed on either end of the ELP chain (G_3 on both terminals). For each salt concentration, the averaged thermodynamic properties were computed from 3 independent 1 μs simulation trajectories, which sufficiently sampled the conformational ensemble of the ELP chain. Details pertaining to the force fields and simulations can be found in Section S2, Figures S1 and S2, and Table S1 in the Supporting Information. The two sequences were studied to investigate the role of triglycine motifs. The change in the gyration radius of the ELP chain with increasing salt concentration from the MD simulations (Figure S3 for more details) agreed with the cloud point trends observed in Figure 2. Previous simulation studies involving PNIPAM in aqueous CaCl_2 solutions have shown that the divalent cations locally accumulate around the amide oxygen.⁵ Given this observation, we examined the distribution of divalent cations around the amide oxygen atoms of the ELPs.

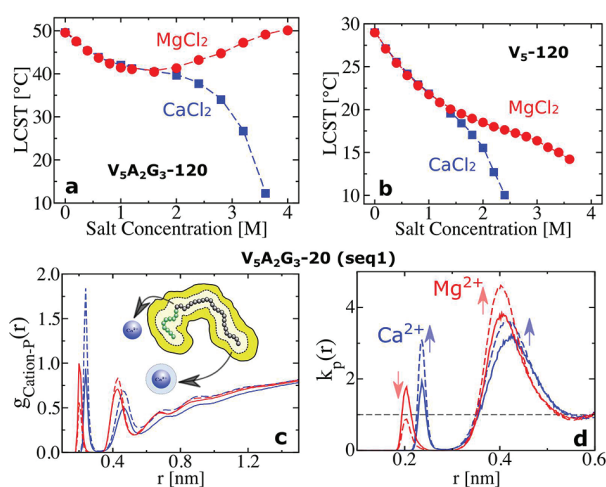


Figure 2. Top Panel: The cloud point temperature of (a) $\text{V}_5\text{A}_2\text{G}_3$ -120 and (b) V_5 -120 in MgCl_2 and CaCl_2 solutions as a function of salt concentration. Note, the unit M represents the molar concentration of salt. Bottom Panel: The variation of the (c) cation and amide oxygen radial distribution function, $g(r)$, and (d) the partition coefficient, $k_p(r)$, with r , which corresponds to the proximal cation/water–amide oxygen distance for seq1 from molecular dynamics simulations. For the bottom panel, the solid and dashed blue lines show the data in CaCl_2 at 1.0 and 2.5 M, respectively, and the solid and dashed red lines are the results in MgCl_2 at 1.0 and 2.5 M, respectively.

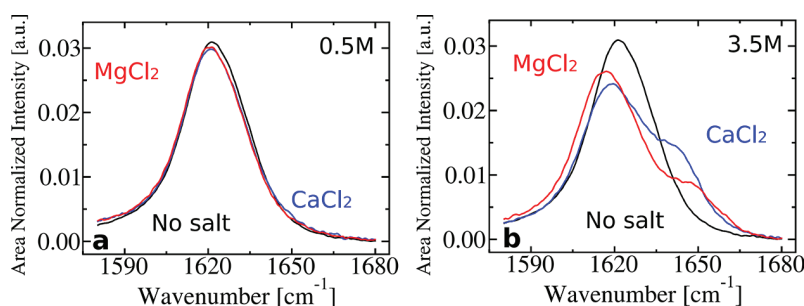


Figure 3. Infrared spectra of NMA at 0.5 M (a) and 3.5 M (b) salt concentration. The black line shows the data in pure D₂O water, and the red and blue lines are the results for MgCl₂ and CaCl₂, respectively.

Figure 2c shows the radial distribution function (rdf) between the amide oxygen (OA) of seq1 and the divalent cations (M^{2+}). The first and second peaks of rdf can be attributed to contact OA– M^{2+} and solvent-separated OA– M^{2+} interactions, respectively. The data indicate that Ca²⁺ ions, shown in blue, shed their hydration waters and interacted more strongly with the amide oxygen atoms via contact interactions than via solvent-separated interactions. Moreover, the Ca²⁺ ions were engaged in bridging interactions involving two amide oxygen atoms (a snapshot of the formed Ca²⁺ bridge can be seen in Figure S4 in the Supporting Information). The formation of these Ca²⁺-ion bridges provides an explanation for why the LCST data shown in Figure 2a fell rapidly as a function of the CaCl₂ concentration, especially at high salt concentrations. Quantitative analysis indicated that the Ca²⁺ bridging interactions involved a combination of local (bridged oxygen atoms (i, j) less than three amino acid residues away, $|i - j| \leq 2$) and global ($|i - j| > 2$) bridging contacts, but most bridging interactions were local. The fraction of the global Ca²⁺ bridges grew slightly at higher salt concentration but a more pronounced increase was observed for the maximum bridging range ($|i - j|_{\max} = 99$ at 2.5 M; see Section S3 and Tables S2 and S3 for more details).

In contrast to Ca²⁺ ions, Mg²⁺ cations preferred to engage in solvent-separated interactions with the amide oxygen atoms (see red curves in Figure 2c), and thus no Mg²⁺ bridges were formed. This observation can be attributed to the fact that Mg²⁺ cations are strongly hydrated and bind their hydration waters tighter than Ca²⁺ cations.^{29,30} With increasing MgCl₂ concentration, the number of contact OA–Mg²⁺ interactions decreased, whereas the number of solvent separated interactions increased. This occurs due to the tendency of Mg²⁺ ions to maximize their favorable electrostatic interactions with amide oxygen atoms without shedding their hydration shell water molecules. This enhanced partitioning of Mg²⁺ along with their hydration waters explains the salting-in of the ELP at high MgCl₂ concentrations (Figure 2a).

The different effects of the two divalent cations were also observed in the local bulk partition coefficient $k_p(r)$ values as a function of position (see eq S6 in the Supporting Information). This quantity has the property $k_p(r) > 1$ when cations partition more favorably than water to the ELP chain, while $k_p(r) < 1$ (cations are depleted) in the opposite scenario. As such, $k_p(r)$ provides a measure of the ion-to-water affinity. Figure 2d, shows that with increasing salt concentration, Ca²⁺ preferably accumulated in the contact OA–Ca²⁺ shell (first peak) whereas Mg²⁺ ions tended to preferably accumulate in the solvent shared OA–Ca²⁺ shell (second peak), leading to the contrasting behavior observed in Figure 2c. For seq2, identical

trends were observed, as shown in Figure S5 in the Supporting Information. Therefore, the effect of divalent cations on the phase behavior of ELPs can be explained as a competition between the nature of the cation–amide oxygen electrostatic interactions (contact interactions and bridges) and the strength of cation hydration (solvent-shared interactions). This mechanism explains the different cloud point temperature trends for ELP V₅A₂G₃ shown in Figure 2a.

We have also calculated the number of cation–chloride contact ion pairs (CIPs) and solvent-shared ion pairs (SIPs) in salt solutions without the ELP. The results are shown in Figure S6 in the Supporting Information. As the salt concentration increased, the absence of Mg²⁺–Cl[−] CIPs and the increasing number of Mg²⁺–Cl[−] SIPs further verified our observation that Mg²⁺ ions bound their hydration waters tightly. By contrast, the progressively growing number of Ca²⁺–Cl[−] CIPs confirmed that Ca²⁺ cations shed their hydration shells. Bruce et al. have shown that the partitioning of the Ca²⁺–Cl[−] SIPs to PNIPAM provided a salting-in contribution to the polymer chain.⁵ Our analysis of cation–chloride SIPs in ELP solutions (Figure S7 in the Supporting Information) suggested that a similar contribution may be present in the case of ELPs in MgCl₂ solution: the increased number of Mg²⁺–Cl[−] SIPs in the solvation shell of the ELP correlated with the salting-in of the ELP at high MgCl₂ concentrations. In contrast to Mg²⁺, the increased number of Ca²⁺–Cl[−] CIPs correlated with the salting-out of the ELP at high CaCl₂ concentrations. This suggests that the salting-out contribution due to bridging provided by Ca²⁺–Cl[−] CIPs overcompensated for the salting-in contribution provided by the Ca²⁺–Cl[−] SIPs (see the Supporting Information for more details).

Amide Oxygen–Cation Pairing and Infrared Spectroscopy. The inferences obtained from the MD simulations can be experimentally confirmed by infrared spectroscopy, which can be used to detect the presence of ELP amide oxygen–cation contact and solvent-shared shells by monitoring the amide I band. However, the amide I band for ELPs is highly dependent on the hydrogen bonding network of the amide oxygen and susceptible to the changes in secondary structures of the ELPs (see Figure S8 in the Supporting Information). To avoid this problem, we used *N*-methylacetamide (NMA) as a model system instead. It has no secondary structures and thus changes to its amide I band in salt solutions were caused by perturbation of the hydration shell via interactions with the ions. The infrared spectra of NMA with CaCl₂ and MgCl₂ are shown in Figure 3. One can be seen that the spectra of NMA in 0.5 M CaCl₂ or MgCl₂ solutions are very similar to pure D₂O water (Figure 3a). This changes at 3.5 M salt. The main resonance at 1622 cm^{−1} in neat D₂O red shifts to 1617.4 and

1619.5 cm^{-1} for MgCl_2 and CaCl_2 , respectively. This red shift has previously been assigned³¹ to a local electric field along the dipole of the amide (Figure 3b). Herein, this peak is assigned to the cation–amide oxygen solvent-separated interactions and is larger for Mg^{2+} . The second peak centered at 1649.0 cm^{-1} for Mg^{2+} and 1647.1 cm^{-1} for Ca^{2+} grows with higher salt concentrations and is assigned to the cation–amide oxygen contact interactions. A smaller quantity of contact interactions is observed for Mg^{2+} , which is consistent with the all-atom simulation results.

Role of Triglycine Motifs. The top and middle panels of Figure 4 compare the rdf of M^{2+} with the whole ELP chain to the rdf

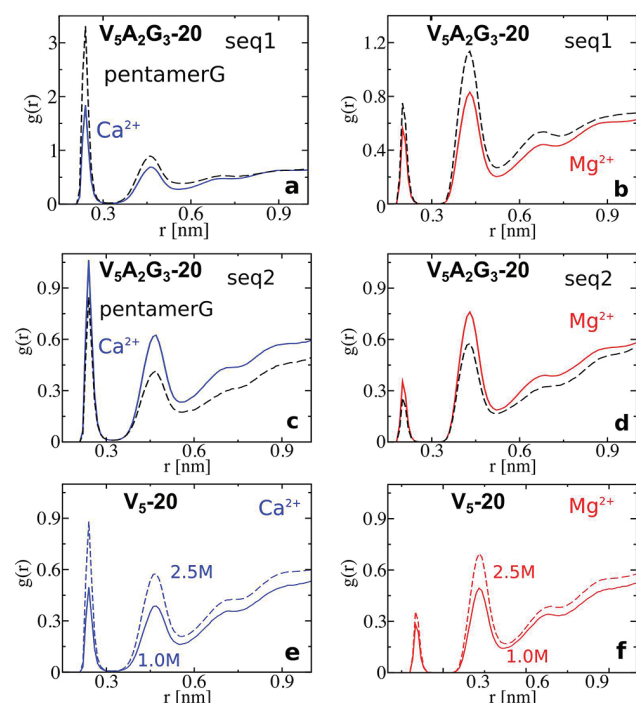


Figure 4. Top and Middle Panels: Comparison of the radial distribution function $g(r)$ between the whole chain (solid lines) and pentamerG (dashed lines) for the ELP $\text{V}_5\text{A}_2\text{G}_3$ 20-mer at a 2.5 M salt concentration. Plots (a) and (b) are for seq1 in CaCl_2 and MgCl_2 solutions, respectively. Plots (c) and (d) are the same as plots (a) and (b) but for seq2. Data at 1.0 M salt concentration can be found in Figure S9 in the Supporting Information. Bottom Panel: M^{2+} –ELP rdf of the V_5 20-mer. Plots (e) and (f) are the results for Ca^{2+} and Mg^{2+} , respectively. The solid and dashed lines are for 1.0 and 2.5 M, respectively.

of M^{2+} with only the pentamerG (–(VPGGG)– tryglycine motifs) units of the ELP chain for seq1 and seq2. For seq1 (see Figure 4a,b), both Ca^{2+} and Mg^{2+} preferentially partitioned to the segments with triglycine motifs as the first and second peak intensities of the rdf for M^{2+} –pentamerG are larger than the M^{2+} –ELP rdf. On the other hand, the partitioning of the M^{2+} ions to the triglycine motifs of seq2 was lower than the rest of the chain (see Figure 4c,d). Additionally, the overall partitioning of the divalent cations to seq2 was lower than that of seq1 at both considered salt concentrations. Seq1 has a long flexible terminus, in comparison to seq2, as all segments with triglycine motifs (which are very flexible) have been placed in one block. The presence of this single long flexible terminus in seq1 allows for conformations that may lead to an efficient interaction between the divalent cations and the amide

oxygen atoms as compared to seq2. This is supported by the observation that more Ca^{2+} bridges were found within pentamerG segments in seq1 in comparison to seq2 (see Table S3 in the Supporting Information). This shows that both the location and the length of the triglycine motifs play important roles in regulating ELP–cation interactions for 20-mer ELP chains.

The bottom panel of Figure 4 shows the M^{2+} –ELP rdf for the more hydrophobic and less flexible V_5 20-mer in CaCl_2 and MgCl_2 solutions. The peak intensities of the M^{2+} –ELP rdfs for the V_5 20-mer were smaller than those for seq2 at both salt concentrations that were considered (note that the M^{2+} –ELP rdfs for seq2 are much smaller than seq1). This observation further supports our conclusion that triglycine motifs enhanced ELP–cation interactions.

We have shown that the phase behavior of two ELPs, $\text{V}_5\text{A}_2\text{G}_3$ and V_5 , in solution with divalent metal cations is regulated by a competition between amide oxygen–cation electrostatic interactions and the strength of cation hydration. The divalent cation, Ca^{2+} , was able to shed its hydration shell and bridge two amide–oxygen atoms together, leading to the salting-out of ELPs, which was far more enhanced at higher salt concentrations. By contrast, Mg^{2+} interacted predominantly with the amide oxygen atoms via solvent-separated electrostatic interactions that led to a salting-in effect for ELP $\text{V}_5\text{A}_2\text{G}_3$ -120 at high salt concentrations. Moreover, the presence of triglycine motifs was found to regulate the phase behavior of ELPs as evidenced by the different LCST behaviors of $\text{V}_5\text{A}_2\text{G}_3$ and V_5 (with and without triglycine motifs) in MgCl_2 and CaCl_2 solutions.

Although the contrasting effects of Ca^{2+} and Mg^{2+} on the phase behavior of these two ELPs are present only at molar salt concentrations, analogous Ca^{2+} bridging behavior occurs at physiological salt concentrations with charged carboxylate residues. An in vivo example of this concerns the EF Hand motif in Ca^{2+} binding proteins.³² Also, Bilkov et al. have shown that the preferential bridging of phosphate moieties by Ca^{2+} over Mg^{2+} can inhibit the binding of PH Domains to PI(4,5)P2 lipids in biomembranes.³³ It would therefore seem that the bridging of protein sites in water is a common characteristic of Ca^{2+} over Mg^{2+} with a number of different chemistries. Additionally, it is possible that direct physiological implications of the Ca^{2+} bridging of amide oxygens might occur under exceptionally high salt concentrations such as in the Dead Sea in Israel (1.65 M Ca^{2+} and 0.42 M Mg^{2+}) or in the Don Juan Pond in Antarctica (4.1 M Ca^{2+} and 0.1 M Mg^{2+}).^{34–37}

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jpclett.3c02476>.

Experimental details, simulation details (force field parameters (Table S1), derivative of activity coefficients vs concentration and PMF (Figures S1 and S2)), tables of backbone separation $|i - j|$ of Ca^{2+} ion bridges (Table S2), fraction of Ca^{2+} ion bridges in pentamerG (Table S3), Gyration radius as a function of salt concentration (Figure S3), calcium ion bridges (Figure S4), radial distribution function and k_p for seq2 (Figure S5), radial distribution function for cation–chloride pairing (Figure S6), cumulative number cation–chloride pairing (Figure

S7), infrared spectra of $V_5A_2G_3$ (Figure S8), triglycine motif effects (Figure S9) (PDF)

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Notes

The authors declare no competing financial interest.

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