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Long-lived ionic nano-domains can modulate the stiffness of soft interfaces†

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Metal ions underpin countless processes at bio-interfaces, including maintaining electroneutrality, modifying mechanical properties and driving bioenergetic activity. These processes are typically described by ions behaving as independently diffusing point charges. Here we show that Na⁺ and K⁺ ions instead spontaneously form correlated nanoscale networks that evolve over seconds at the interface with an anionic bilayer in solution. Combining single-ion level atomic force microscopy and molecular dynamic simulations we investigate the configuration and dynamics of Na⁺, K⁺, and Rb⁺ at the lipid surface. We identify two distinct ionic states: the well-known direct electrostatic interaction with lipid headgroups and a water-mediated interaction that can drive the formation of remarkably long-lived ionic networks which evolve over many seconds. We show that this second state induces ionic network formation via correlative ion–ion interactions that generate an effective energy well of ~0.4k_BT/ion. These networks locally reduce the stiffness of the membrane, providing a spontaneous mechanism for tuning its mechanical properties with nanoscale precision. The ubiquity of water-mediated interactions suggest that our results have far-reaching implications for controlling the properties of soft interfaces.

Introduction

In soft and biological systems, electrostatic interactions frequently rely on alkali cations dissolved in aqueous solutions. Ions can have a profound impact on the structure, molecular organisation and dynamics of these soft systems, with key examples found in protein folding and lipid organisation within membranes. Lipids – key structural components of biological membranes – play as active a role in vivo as the proteins that were once thought to dominate function and as such the specific interactions of ions with lipid bilayers are of great general interest. The mobility, curvature and robustness of lipid membranes are all affected by ions in solution, which in turn has important implications for cellular-scale processes, including budding, endocytosis and protein-mediated channel regulation. The molecular interactions which underpin these processes are however far from trivial: the lipid head-group chemistry, ionic composition of the solution and the precise adsorption location and hydration of the ions all influence the macroscopic properties of the membrane. This has occasionally resulted in conflicting pictures of the nature and strength of ions’ binding to lipid bilayers.

These intricate and diverse interactions between ions and lipids are partly driven by the latter’s highly hydrated headgroups, which allows indirect complexes, mediated by water molecules, to form. Complexes such as these can significantly affect the properties of the interface itself, altering the molecular dynamics and mechanical properties on the nanoscale. Indeed, recent studies on mineral interfaces have shown that interfacial water can mediate attractive ion–ion correlations, and reduce the mobility of ions at the interface by orders of magnitude. Despite the quantity and breadth of studies probing lipid–ion interactions, there remains a significant gap in our understanding of how these molecular-level phenomena influence membranes. This is particularly true at the mesoscale, where spatial correlations can give rise to emergent properties. Part of the problem comes from our traditional view of ions at interfaces, which is shaped by continuum theories that reduce ions to point charges in a continuous, homogenous medium and considers the interface only as a structureless boundary. While successful at the macroscale, this view can fail markedly where molecular details are important. Indeed, it has long been known that the species-dependent hydration properties of an ion can determine its specific interactions with proteins (often referred to as Hofmeister effects) and membranes. Ion-specific effects such as these are the norm in biology and can often induce spatial correlations by coupling molecules together. Our general understanding of ionic effects at biointerfaces is thus hampered by the lack of a

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comprehensive theoretical framework to describe these nanoscale correlations and to what extent they influence the structure and mechanical properties of soft biological material. To fully capture this complexity, investigations need to be able to access molecular details of the interface while simultaneously retaining a mesoscale view of the system, over hundreds of square nanometres and up to minutes.

Here, we address this challenge using high-resolution atomic force microscopy (AFM) and all-atom molecular dynamics (MD) simulations to probe the organisation and time evolution of alkali cations at the surface of a negatively charged lipid bilayer in solution. AFM can detect and track the dynamics of singly-adsorbed ions at the electrolyte interface over long timescales, and can directly probe mechanical properties with Ångström-scale resolution. Molecular dynamics simulations complement this by revealing binding and conformational modes of the ions’ interactions with both water and the lipids. Thus, the combination of the techniques allows atomistic insight into the interfacial structure as well as the tracking of the ions’ evolution over many seconds.

Our lipid bilayer is composed of 1,2-dipalmitoyl-sn-glycero-3-phosphate (DPPA), an anionic lipid with a phosphate headgroup. Despite being relatively underrepresented in the liposome, DPPA’s headgroup plays a key role in signal transduction and pH sensing, and the lipid shows potential for application in liposomal drug delivery. Additionally, anionic lipids such as these are crucial for mediating protein interactions in the cytosol and signalling cell oncogenesis among others, and their strong electrostatic profile is likely to affect their mechanical response to ion adsorption.

We show that the alkali cations, sodium and potassium, form correlated nano-domains at the lipid-electrolyte interface, with binding modes distinct to each ion. Rubidium, on the other hand, binds too weakly to observe these domains but still forms indirect complexes via hydration water. The domains of Na⁺ and K⁺ ions evolve remarkably slowly over many seconds, mediated by attractive interactions of order ~0.4$k_B$T/ion and reduce the effective stiffness of the lipids by up to 12%. We show that this mechanical perturbation is driven by the water-mediated domains competing with directly bound ions for access to the lipid headgroups and allowing the membrane to become more flexible.

Results and discussion

Ionic structure and hydration at the bilayer interface

Fig. 1a–c illustrates the single-ion level resolution images that were obtained using amplitude-modulation AFM (AM-AFM) of the supported DPPA bilayer in aqueous solutions of NaCl, KCl or RbCl at biological concentrations (150 mM). When using gentle imaging conditions (high setpoint) and cantilever amplitudes comparable to the size of a hydrated ion (~2.0 nm), the tip probes the interfacial region where the lipid headgroups meet the electrolyte. Thus, the changes in height (purple/yellow) reflect the adsorbed ions’ modification of the lipids’ hydration landscape, as well as the underlying hexagonal symmetry of the gel-phase membrane (see also the Fourier transforms in ESI Fig. S3†). The interface’s appearance clearly depends characteristically on the species of ion present in the solution. In NaCl (Fig. 1a, topography), amorphous clusters of different heights form.

The different heights reflect the multiplicity of sodium’s binding states as it interacts with the headgroups: upper topographic levels reflect ions adsorbed in outer-sphere coordination with an intervening water molecule – an interpretation further verified in the context of the MD results below. These outer-sphere domains induce a relatively high phase difference.
(Fig. 1a, right, dashed circle) that indicates a more viscous interaction between the tip and bilayer. In contrast, lower levels are characterised by regions of reduced phase, reflecting a more elastic response (solid circle). In KCl (Fig. 1b), two adsorption states are still visible, but the interface's topographical features are markedly more regular: the roughness drops from $R_{q}^{Na} = 0.037 \pm 0.003$ nm to $R_{q}^{K} = 0.027 \pm 0.002$ nm. This reflects the fact that potassium ions adsorb more frequently in an inner-sphere configuration – that is, directly to the lipid headgroups – due to their lower charge density. Images obtained in the RbCl solution (Fig. 1c) revealed a lipid headgroup symmetry particularly marked in one direction, giving the appearance of molecular rows, with large (~200 pm), isolated protuberances that straddle these rows (white arrow) and dominate other features. The size and location of these on the bilayer is consistent with single rubidium ions adsorbed as inner-sphere complexes, implying that Rb$^+$ adsorb too rarely in this system to show significant domain formation.

We quantified the ability of Na$^+$ and K$^+$ to form these ionic domains by using a semi-automated algorithm based on height-thresholding to identify the locations of adsorbed ions (details in ESI section 4.2†). The results confirm that the mean number of nearest neighbors for bound potassium, $NN_{K}$ = 2.9 ± 0.1 is substantially larger than for a random distribution of equal coverage, $NN_{Na} = 1.3 ± 0.1$. Sodium displays an even larger number of nearest neighbors, with $NN_{Na} = 3.2 ± 0.2$, presumably due to its more varied binding state. This quantitatively confirms the cations' predilection to form non-random networks through the hydration water-mediated interactions which dominate at this scale.

The interpretation of the topography and phase images of Fig. 1a–c can be directly confirmed using phase-distance spectroscopy (Fig. 1d), in which variations in the oscillation phase, $\Delta \theta$, are monitored as a function of the average separation between the AFM tip and the lipids, $d_{li}$ (see ESI Fig. S6† for details). The phase is particularly sensitive to local perturbations of the interfacial hydration landscape and highlights differences in how each ion interacts with the lipids. Sodium presents a complex hydration structure with two peaks in $\Delta \theta$ at $d_{li} < 3$ Å. However, the perturbation is relatively small (its trace in Fig. 1d is multiplied by four), likely related to the sodium ions' non-trivial binding structure, which induces the heterogeneous topography of Fig. 1a. Potassium, in contrast, produces two well-defined peaks separated by 3 Å (about the diameter of a water molecule), with the second at a larger $d_{li}$ than for Na$^+$. This agrees with the more regular features observed via AM-AFM imaging (Fig. 1b), as well as the interpretation of the binding modes being mediated by water rather than by direct electrostatics. Rubidium, as the largest cation, adsorbs furthest away from bilayer interface and predominantly in single, broad configuration at $d_{li} \sim 3$ Å, although with a small shoulder at ~5.5 Å. This reflects rubidium's preference for forming inner-sphere complexes due to its relatively low charge density, as well as the observation of mostly single ions while imaging (Fig. 1c).

The distinctive organisation of the different cations at the interface with the bilayer has obvious consequences for their affinity to the membrane. Independent electrophoretic measurements of the ions' binding constants to DPPA reveal a direct Hofmeister series with $K_{Na} > K_{K} > K_{Rb}$ (Fig. 1e). These agree with the hydration landscape measured by AFM; Na$^+$, which has a complex, multilevel hydration structure binds most strongly and resists being displaced by the probe. The weaker attraction of K$^+$ to the bilayer allows for a better-defined hydration structure (Fig. 1d), leading to higher imaging resolution. In RbCl, the AFM tip displaces most of the adsorbed ions, leaving water dipoles and hydronium ions to screen the DPPA surface charge (see ESI Fig. S4† for a detailed discussion about ion removal by the tip).

While the AFM and electrophoretic results clearly demonstrate ion-specific coordination and ordered ionic networks at the surface of the lipid membrane, the results do not permit true atomic exploration of the underlying mechanism responsible for the formation of the networks. To address this issue, we performed all-atom MD simulations using NAMD 2.9. The goal is to replicate as closely as possible the AFM results so as to gain atomistic insights into the interactions at play and ensure that the AFM observations are not due to tip effects, but still stand in the absence of an AFM tip. Our system consisted of 136 DPPA molecules in a free-standing, tensionless bilayer at 298 K and 1 atm pressure, and in contact with aqueous 150 mM NaCl, KCl or RbCl, hence directly mirroring our experimental set-up but without the influence of the AFM tip.

In the simulations, the interactions were modelled using the CHARMM36 force field with improved parameters for the ions. All technical details of the simulations are described in the ESI. A typical snapshot is shown in Fig. 2a for KCl. The analysis of the radial correlation functions between the different (non-equivalent) oxygen atoms of the lipids, the cations and water molecules (see ESI for details) allows us to identify binding of cations to oxygen atoms of the lipids and the sharing of water molecules in the first coordination shell of ions and lipids. We observe two distinct binding modes, illustrated in Fig. 2b and c for the case of KCl. Cations in the first binding mode (Fig. 2b, green, inner-sphere) have at least one lipid oxygen atom in the first coordination shell of the cation (with average values of 2.0, 2.5 and 2.8 lipid oxygens in the coordination shells of adsorbed Na$^+$, K$^+$ and Rb$^+$ respectively). Cations are typically shared between two lipids and adsorb deep into the membrane region occupied by the headgroups, as shown in Fig. 2b. In the second mode (Fig. 2c, yellow, outer-sphere), adsorbed cations retain their full hydration shell but at least one water molecule is shared between the first hydration shell of the cations and the lipid atoms. In this case, oxygen atoms from lipid headgroups are found in the second hydration shell of cations and ions are adsorbed in more external locations of the membrane (see Fig. 2c). The fraction of ions bound in this second mode decreases following the sequence Na$^+ > K^+ > Rb^+$, mirroring the affinities shown in Fig. 1e, with the inverse true for the
and 4.55 Å for Rb⁺, corresponding in each case to cation–oxygen–cation contacts (see ESI Fig. S14–17). These correlations are mediated by the sharing of water molecules and/or lipid molecules in the first coordination shell of the ions (Fig. 2e).

Most outer-sphere ions share their solvation water with ions adsorbed in the inner mode, as illustrated in Fig. 2f. These correlated chains suggest that the domains observed in Fig. 1 are indeed induced by ionic networks at the surface of the membrane. By using similar colours for ions that share water or lipid oxygens with each other, Fig. 2g shows that in fact the majority of ions belong to such nano-networks, with many forming larger-scale structures of four ions or more (dashed regions). Surprisingly, the Cl⁻ counterions in solution do not play any role in these networks. Therefore, the simulations indicate that the mechanism behind the formation of ionic networks is the sharing of lipid oxygen atoms and/or the sharing of water molecules in the first coordination shell of cations.

**Dynamics of ionic domains**

Fig. 1 and 2 highlight the complex networks formed between cations and mediated by water molecules. These ionic networks cannot be explained with traditional continuum theories and are believed to dramatically affect the charging⁷,⁴¹ and dynamics¹⁵ at aqueous interfaces. Here, although the MD results show ions to be essentially static at the bilayer surface once they bind, the simulations’ ~100 ns length do not allow us to explore the evolution of the cationic networks over biologically relevant timescales (ms–s). However, the stability of the images such as those presented in Fig. 1 suggests that the ionic structures are remarkably long-lived, with dynamics that can be interrogated by AFM. Indeed, observations of ions at the mica–electrolyte interface have shown that the energetics associated with ion adsorption generated relaxation times ranging from seconds³⁸ to minutes,⁴² although neither of those techniques allowed for direct identification of ionic networks, as is the case here. Fig. 3 shows the evolution of the networks observed by AFM with single-ion resolution over consecutive images. We objectively tracked the ions’ locations using the same algorithm as above, but in successive images, thus allowing time-resolved information to be obtained. The ions are highlighted in Fig. 3a by white circles, and those participating in nearest-neighbour interactions are joined by red bars. Significantly, the image demonstrates that the ionic nano-networks persist long enough to be identified with AFM, and that those techniques allowed for direct identification of ionic networks, as is the case here. Fig. 3 shows the evolution of the networks observed by AFM with single-ion resolution over consecutive images. We objectively tracked the ions’ locations using the same algorithm as above, but in successive images, thus allowing time-resolved information to be obtained. The ions are highlighted in Fig. 3a by white circles, and those participating in nearest-neighbour interactions are joined by red bars. Significantly, the image demonstrates that the ionic nano-networks persist long enough to be identified with AFM, and that those techniques allowed for direct identification of ionic networks, as is the case here. Fig. 3 shows the evolution of the networks observed by AFM with single-ion resolution over consecutive images. We objectively tracked the ions’ locations using the same algorithm as above, but in successive images, thus allowing time-resolved information to be obtained.
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in amplitude modulation mode for imaging, exactly as in Fig. 1 & 3, while the frequency and phase of the second is controlled (i.e. in frequency modulation) in order to extract quantitative information from the ion–lipid interface (see ESI Fig. S7† and discussion for details).

Fig. 4a shows a bimodal AFM scan acquired on the DPPA bilayer in 150 mM KCl. The topographic and stiffness maps clearly display equivalent periodicity and symmetry, emphasising the global impact of ions on the membrane biophysics. However, a line profile comparing topographic and stiffness variations reveals the two quantities to be exactly out of phase; the presence of outer-sphere adsorbed ions decreases the interfacial stiffness, here by ~12% (Fig. 4b). This is in agreement with the phase data exhibited in Fig. 1; networks of ions adsorbed via their hydration shells make the lipid–electrolyte interface more “fluid-like” (large $\Delta t$; light blue in Fig. 1a-c and peaks in Fig. 1d), thus reflecting the induced reduction in $E_{\text{eff}}$. From the characteristic variation in lipid hydration landscape (Fig. 1a–d), binding affinity (Fig. 1e) and adsorption mode (Fig. 2d) with each cation species, we similarly expect the Young’s modulus to be ion-dependent. Specifically, rubidium, for which a larger fraction of ions directly links lipids together in inner-sphere configuration, is expected to stiffen the lipids more than sodium or potassium, which rely on indirect, water-mediated binding. Averaging of $E_{\text{eff}}$ over the entirety of >10 images for each electrolyte confirms this hypothesis (Fig. 4c), with the highest stiffness given by Rb$^+$ ($E_{\text{eff}}^\text{Rb} = 10.0 \pm 0.6$ GPa), followed by K$^+$ ($E_{\text{eff}}^\text{K} = 4.7 \pm 0.7$ GPa) and Na$^+$ ($E_{\text{eff}}^\text{Na} = 6 \pm 1$ GPa). We note that the uncertainty in stiffness for sodium is much greater than that of either rubidium or potassium, likely related to sodium’s less well-defined binding state (Fig. 1a and d). The overall trend of $E_{\text{eff}}$ versus ionic species, as well as its order of magnitude, was confirmed with conventional ‘static’ force spectroscopy (ESI Fig. S9†), thereby ruling out possible viscoelastic effects. We note that there is a systematic offset between the static and bimodal values of $E_{\text{eff}}$, and as such the absolute values must be taken with caution, especially due to the use of a continuum indentation model to extract molecular-level stiffness variations over a 5 nm thick membrane. However, the trend shown in Fig. 4c should remain valid regardless.

The present findings appear to contrast with the traditional view that dissolved salts always have a stiffening effect on biomembranes by reducing electrostatic repulsion between lipids and forming hydrogen bonds with the headgroups.\textsuperscript{7,37,48,49} Crucially however, and in contrast to many previous studies, the DPPA membrane is charged, inducing severe electrostatic repulsion at the headgroup level. Thus, the precise adsorption location of the ions will have a large impact on the membrane stiffness, an effect that is rarely taken into account. Here, we have shown that ions with greater inner-sphere populations (rubidium) indeed stiffen the membrane in a traditional manner. As the propensity of an ion to adsorb indirectly via water molecules increases however (the case for sodium and potassium), the relative rigidity of the lipids is decreased, presumably by locally disturbing inner-sphere ions and competitive adsorption. To confirm this mechanism, we quantified the effect on the membrane stiffness of altering the ratio between Na$^+$ and hydronium ions (H$_3$O$^+$) in solution – that is, reducing the pH. H$_3$O$^+$, being composed of protonated water, cannot bind in an outer-sphere configuration, and a decrease in pH for a given NaCl concentration should allow more direct binding and hence stiffen the membrane. The experiment was conducted within a pH range where no (de)protonation of the lipid headgroups or the silicon oxide AFM tip are expected,\textsuperscript{50} hence allowing a straightforward analysis. Fig. 4d shows a clear stiffening of the membrane with decreasing pH, confirming our hypothesised mechanism. The high-resolution topography images associated with this experiment confirm that hydronium ions compete with Na$^+$ for access to the DPPA headgroups, and that the number of adsorbed Na$^+$ increases with pH (ESI Fig. S5†).

Overall, this validates the mechanism by which ions in solution are able to locally reduce the membrane stiffness, by
forming remarkably slowly evolving nano-networks on the membrane surface mediated by water and competing with directly-adsorbed charges.

Conclusions

Our combined experimental and computational study evidences long-lived ionic nano-networks at the surface of a model lipid bilayer in solution. The formation of these water-mediated networks depends on the charge density of the ions through their binding strengths and hydration properties at the membrane. Here K⁺ and Na⁺ ions form nano-networks that evolve over tens of seconds, driven by correlation energies of the order of −0.4k_BT/ion. These networks induce softening of the bilayer through local replacement of directly bound ions. Our model system is in gel-phase, unlike most biomembranes, and thus its lipids are broadly static, with small areas-per-molecule. This undoubtedly will play a role in structuring the interfacial fluid, but the networks’ origin in short-ranged hydration interactions (Fig. 2) and the elongated timescales of order of seconds (Fig. 3) imply that lipid mobility would not dramatically alter such phenomena. Indeed, similar ion-dependent effects have been observed on fluid bilayers and in principle, these effects could occur at any soft interface, as they rely only on water molecules mediating these common ions’ interaction with themselves and the lipids. At biological interfaces, we expect this mechanism to modulate the local viscoelastic properties on a timescale relevant for influencing processes such as molecular adsorption or the gating of mechanosensitive protein channels.

Experimental

A more in-depth description of all procedures and materials used may be found in the ESI.†

Small unilamellar vesicles (SUVs) and supported lipid bilayers (SLBs)

1,2-Dipalmitoyl-sn-glycero-3-phosphate (DPPA) was purchased in powder form from Avanti Polar Lipids (AL, USA) and used without further purification. For electrophoretic measurements, small unilamellar vesicles of diameter ~100 nm were produced by rehydrating the lipids to a concentration of 1 mg ml⁻¹ with ultrapure water buffered to pH 7.45 with 5 mM sodium phosphate dibasic. They were then bath-sonicated at 55 °C for 30 minutes, chilled to −18 °C for 30 minutes and bath-sonicated for a final 30 minutes (the so-called freeze–thaw method) to produce a uniformly clear solution. The vesicles were then extruded through a 100 nm filter (WhatMan, Sigma Aldrich) at least 15 times, diluted with the appropriate mix of ultrapure water and electrolyte solution to a final lipid concentration of 0.1 mg ml⁻¹ and then used immediately.

The procedure for SLBs differed slightly; it used unbuffered solutions, to avoid perturbing the hydration landscape when imaging with AFM. Further, after the freeze–thaw procedure, the vesicles were diluted with 150 mM NaCl to a lipid concentration of 0.1 mg ml⁻¹ and extruded in the same way as above. The solution was then pipetted onto a freshly-cleaved mica substrate, and the vesicle fusion method was used to produce at least one uniform bilayer on the surface (ESI Fig. S1†).

Electrophoresis measurements

SUV suspensions (see above) at varying concentrations of NaCl, KCl and RbCl were pipetted into a folded cuvette and their drift velocity as a function of applied potential, V, was measured using laser-Doppler interferometry with a Zetasizer NS (Malvern Instruments, UK). This allows the calculation of the vesicles’ potential at the hydrodynamic slip plane, or ζ-potential of the lipids. Knowing ζ, the bulk ion concentration, I, and the areal lipid density, ρ, allows the calculation of the fractional adsorbed surface charge, Γ, as a function of ionic concentration. Combining Γ with an appropriate adsorption model (here Frumin–Fowler–Guggenheim) allows the extraction of the binding constant, K, for each ion as well as energy of correlation for the ions, E_c.

Atomic force microscopy (AFM)

The images shown in Fig. 1a–c and Fig. 3a were all produced by a commercial Cypher ES AFM (Asylum Research, CA, USA) operated in amplitude-modulation mode (AM-AFM). Briefly, this entails exciting a cantilever with an attached nanometre-sharp tip near its fundamental resonance frequency so that it oscillates sinusoidally within the imaging fluid. The amplitude of oscillation is kept constant by a feedback loop, while the phase difference between the excitation signal and the cantilever is allowed to vary. This allows the interfacial topography to be tracked with Ångström-resolution, while information relating to the energy dissipated by the tip is carried in the phase signal.

The small amplitudes used here ensure that the tip interacts mostly with interfacial fluid, retaining its approximately simple harmonic motion and allowing for single-ion resolution to be achieved. Arrow UHF AuD cantilevers (Nanoworld, Neuchâtel, Switzerland) were used with flexural stiffnesses k₁ = 1.71 N m⁻¹ (Fig. 1 and 3), k₂ = 0.956 N m⁻¹ (Fig. 4a and c) and k₃ = 6.58 N m⁻¹ (Fig. 4d).

For the bimodal operation of Fig. 4, the cantilever was simultaneously excited at its fundamental and second resonance frequency, the latter of which is controlled via frequency modulation, which keeps the second-mode amplitude, A₂, constant and its phase, θ₂, locked to 90°. The first mode amplitude, A₁, and second mode frequency shift, Δν₂, are used to calculate E_eff with errors of <5% using the equations of Amo et al. Fully atomistic MD simulations were carried out using CHARMM36 force fields with TIP3P water. The bilayers had 68 DPPA molecules in each leaflet and covered an area of 64.45 Å × 64.45 Å. The simulation box contained 6463 waters,
156 cations (either Na\(^+\), K\(^+\) or Rb\(^+\)) and 20 Cl\(^-\) anions (equivalent to 150 mM in each case). The temperature was kept constant at 298 K and the pressure was 1 atm. For each simulation, there were ~20 ns of equilibration followed by 100 ns of production run. All snapshots shown in Fig. 2 were produced using Visual Molecular Dynamics (VMD).\(^{54}\)

**Conflicts of interest**

There are no conflicts to declare.

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**References**

1. E. Lacroix, A. R. Viguera and L. Serrano, *J. Mol. Biol.*, 1998, **284**, 173–191.
2. D. R. Slochower, Y.-H. Wang, R. W. Tourdot, R. Radhakrishnan and P. a. Janmey, *Adv. Colloid Interface Sci.*, 2014, **208**, 177–188.
3. P. A. Janmey and P. K. J. Kinnunen, *Trends Cell Biol.*, 2006, **16**, 538–546.
4. R. A. Böckmann, A. Hac, T. Heimburg and H. Grubmüller, *Biophys. J.*, 2003, **85**, 1647–1655.
5. L. Piantanida, H. L. Bolt, N. Rozatian, S. L. Cobb and K. Voitchovsky, *Biophys. J.*, 2017, **113**, 426–439.
6. B. Ali Doosti, W. Pezeshkian, D. S. Bruhn, J. H. Ipsen, H. Khandelia, G. D. M. Jeffries and T. Lobovkina, *Langmuir*, 2017, **33**, 11010–11017.
7. S. García-Manyes, G. Oncins and F. Sanz, *Biophys. J.*, 2005, **89**, 1812–1826.
8. W. B. Huttnner and J. Zimmerberg, *Curr. Opin. Cell Biol.*, 2001, **13**, 478–484.
9. J. Dai, H. P. Ting-Beall and M. P. Sheetz, *J. Gen. Physiol.*, 1997, **110**, 1–10.
10. A. a. Gunterenko and I. Vattulainen, *J. Phys. Chem. B*, 2008, **112**, 1953–1962.
11. J. Faraudo and A. Travesset, *Biophys. J.*, 2007, **92**, 2806–2818.
12. Y.-C. Wen, S. Zha, X. Liu, S. Yang, P. Guo, G. Shi, H. Fang, Y. R. Shen and C. Tian, *Phys. Rev. Lett.*, 2016, **116**, 016101.
13. A. Martin-Molina, C. Rodriguez-Beas and J. Faraudo, *Biophys. J.*, 2012, **102**, 2095–2103.
14. A. Catte, M. Girych, M. Javanainen, C. Loison, J. Melcr, M. S. Miettinen, L. Monticelli, J. Määttä, V. S. Oganesan, O. H. S. Ollila, J. Tynkkynen and S. Vilov, *Phys. Chem. Chem. Phys.*, 2016, **18**, 32560–32569.
15. M. Ricci, W. Trewby, C. Cafolla and K. Voitchovsky, *Sci. Rep.*, 2017, **7**, 43234.
16. J. Song, J. Franck, P. Pincus, M. W. Kim and S. Han, *J. Am. Chem. Soc.*, 2014, **136**, 2642–2649.
17. M. Ricci, P. Spijkers and K. Voitchovsky, *Nat. Commun.*, 2014, **5**, 4400.
18. J. N. Israelachvili, *Intermolecular and Surface Forces*, Elsevier, 3rd edn, 2011.
19. N. Schwierz, D. Horinek and R. R. Netz, *Langmuir*, 2015, **31**, 215–225.
20. D. Bastos-González, L. Pérez-Fuentes, C. Drummond and J. Faraudo, *Curr. Opin. Colloid Interface Sci.*, 2016, **23**, 19–28.
21. W. Kunz, *Curr. Opin. Colloid Interface Sci.*, 2010, **15**, 34–39.
22. H. I. Petracek, T. Zemb, L. Belloni and V. A. Parsegian, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 7982–7987.
23. A. Martin-Molina, C. Rodriguez-Beas and J. Faraudo, *Phys. Rev. Lett.*, 2010, **104**, 168103.
24. C. A. Amo, A. P. Perrino, A. F. Payam and R. Garcia, *ACS Nano*, 2017, **11**, 8650–8659.
25. J. J. Shin and C. J. Loewen, *BMC Biol.*, 2011, **9**, 85.
26. B. P. Young, J. J. H. Shín, R. Orij, J. T. Chao, S. C. Li, X. L. Guan, A. Khong, E. Jan, M. R. Wenk, W. A. Prinz, G. J. Smits and C. J. R. Loewen, *Science*, 2010, **329**, 1085–1088.
27. M. M. A. E. Claessens, B. F. Van Oort, F. A. M. Leermakers, F. A. Hoekstra and M. A. C. Stuart, *Biophys. J.*, 2004, **87**, 3882–3893.
28. R. García and R. Pérez, *Surf. Sci. Rep.*, 2002, **47**, 197–301.
29. K. Voitchovsky, J. J. Kuna, S. A. Contera, E. Tosatti and F. Stellacci, *Nat. Nanotechnol.*, 2010, **5**, 401–405.
30. T. Fukuma, M. Higgins and S. Jarvis, *Phys. Rev. Lett.*, 2007, **98**, 106101.
31. K. Voitchovsky, *Phys. Rev. E: Stat., Nonlinear, Soft Matter Phys.*, 2013, **88**, 022407.
32. E. J. Miller, W. Trewby, A. Farokh Payam, L. Piantanida, C. Cafolla and K. Voitchovsky, *J. Visualized Exp.*, 2016, 1–10.
33. W. Trewby, D. Livesey and K. Voitchovsky, *Soft Matter*, 2016, **12**, 2642–2651.
34. S. S. Lee, P. Fenter, K. L. Nagy and N. C. Sturchio, *Langmuir*, 2012, **28**, 8637–8650.
35. B. Klaszczyk, V. Knecht, R. Lipowsky and R. Dimova, *Langmuir*, 2010, **26**, 18951–18958.
36. K. Kobayashi, Y. Liang, S. Murata, T. Matsuoka, S. Takahashi, N. Nishi and T. Sakka, *Langmuir*, 2017, **33**, 3892–3899.
37. Y. A. Ermakov, K. Kamaraju, S. Sukharev, Y. R. Shen and C. Tian, *Phys. Chem. Phys.*, 2015, **17**, 3899–3906.
38. S. S. Lee, P. Fenter, K. L. Nagy and N. C. Sturchio, *Nat. Commun.*, 2017, **8**, 15826.
39. J. C. Phillips, R. Braun, W. Wang, J. Gumbart, E. Tajkhorshid, E. Villa, C. Chipot, R. D. Skeel, L. Kalé and K. Schulten, *J. Comput. Chem.*, 2005, **26**, 1781–1802.
40 J. Yoo and A. Aksimentiev, *Phys. Chem. Chem. Phys.*, 2018, 20, 8432–8449.
41 J. Wang and A. J. Bard, *J. Phys. Chem. B*, 2001, 105, 5217–5222.
42 U. Raviv, P. Laurat and J. Klein, *J. Chem. Phys.*, 2002, 116, 5167.
43 H. Yamashita, K. Voïtchovsky, T. Uchihashi, S. A. Contera, J. F. Ryan and T. Ando, *J. Struct. Biol.*, 2009, 167, 153–158.
44 V. A. Parsegian and T. Zemb, *Curr. Opin. Colloid Interface Sci.*, 2011, 16, 618–624.
45 I. C. Bourg and G. Sposito, *J. Colloid Interface Sci.*, 2011, 360, 701–715.
46 N. Shimokawa, H. Himeno, T. Hamada, M. Takagi, S. Komura and D. Andelman, *J. Phys. Chem. B*, 2016, 120, 6358–6367.
47 M. M. A. E. Claessens, F. A. M. Leermakers, F. A. Hoekstra and M. A. Cohen Stuart, *J. Phys. Chem. B*, 2007, 111, 7127–7132.
48 K. Voïtchovsky, S. Antoranz Contera, M. Kamihira, A. Watts and J. F. Ryan, *Biophys. J.*, 2006, 90, 2075–2085.
49 G. Pabst, A. Hodzic, J. Štrancar, S. Danner, M. Rappolt and P. Laggner, *Biophys. J.*, 2007, 93, 2688–2696.
50 D. Marsh, *CRC Handbook of Lipid Bilayers*, CRC Press, 2nd edn, 2013.
51 M.-P. Mingeot-Leclercq, M. Deleu, R. Brasseur and Y. F. Dufrêne, *Nat. Protoc.*, 2008, 3, 1654–1659.
52 R. Richter, A. Mukhopadhyay and A. Brisson, *Biophys. J.*, 2003, 85, 3035–3047.
53 I. Reviakine and A. Brisson, *Langmuir*, 2000, 16, 1806–1815.
54 W. Humphrey, A. Dalke and K. Schulten, *J. Mol. Graphics*, 1996, 14, 33–38.