Hydration forces at solid and fluid biointerfaces

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Abstract

We review the different molecular mechanisms giving rise to the repulsive hydration force between biologically relevant surfaces such as lipid bilayers and bio-ceramics. As we will show, the hydration force manifests itself in very different and subtle ways depending on the substrates.

Soft, mobile surfaces such as lipid bilayers tend to exhibit monotonic, decaying hydration force, originated from the entropic constriction of the lipid head groups. Solid surfaces on the other hand, tend to exhibit a periodic oscillatory hydration force, originated from the surface induced polarization of water molecules. In this review we will describe both subtle faces of this important interaction by first describing the early experiments performed on solid surfaces and their interpretation by recent simulation studies. Then we will describe the hydration force between fluid interfaces such as bilayers and explain how experimentally researchers have unraveled the dominant role of the lipid head groups conformation.

INTRODUCTION

For decades, it was perceived that the Derjaguin Landau Verwey and Overbeek (DLVO) theory[1, 2], a combination of repulsive electrical double layer forces and attractive van der Waals forces, is able to describe the properties of wide range of colloids and bio-colloids. According to this theory, at separations below 2-4 nm, attractive van der Waals forces always dominates over the repulsive double layer forces leading to an adhesive contact. However, several phenomena (re-peptization, hydrophobic colloids, and silica particles) were found not to obey this theory as these materials neither coagulated nor swelled as predicted by DLVO theory. Thus, the idea of a repulsive force, the hydration force, acting at a few nanometer separation distance, overcoming the van der Waals primary adhesive minimum arose. These observations, yet, were not sufficient to prove the existence of an additional force simply because other repulsive contributions such as the Stern layers compression or the possible presence of steric repulsive interactions had to be ruled out [3]. Frens and Overbeek [4, 5] proposed a model which explained the removal of the primary adhesive minimum by shifting the hard wall cut off to a finite separation of \( D = D_0 + 2d \), where \( D_0 \) was the zero distance and \( d \) the diameter of ions. This theory showed that the net repulsion could continue to rise steeply as the inter-particle distance separation decreased to contact even when the surface charge density or potential was low. After this first intent, the necessity of directly measuring the interaction forces vs. separation distance was the subject of intense research in order to experimentally verify if the force laws measured can be fitted using DLVO-Stern model or if it requires some extra force to be fitted. In what
follows, we will describe the experimental characterization of the hydration forces, in two main systems, namely lipid membranes and solid substrates, the apparent discrepancies in the experimental observations that have emerged and the common ground between different researches. We describe the various experimental methods that are still used to determine such forces and continue with a detailed description of the results obtained from Surface Force Apparatus (SFA).

**FORCE MONITORING TECHNIQUES**

Several direct force measuring techniques have been developed till date. Some were devised for specific purposes while others have been implemented for a broad range of applications. The first technique to provide the quantitative data into the short range hydration forces is the Osmotic Pressure (OP) technique. OP is able to measure the repulsive forces in aqueous solutions between surfactants or lipid bilayers [6-8], aligned clay sheets [9, 10] and aligned macromolecules [11]. In this method, osmotic pressure is applied to the aqueous sample and the equilibrium spacing between surfaces is monitored by X-rays. This technique, however, is unable to measure interaction forces at longer range and is thus limited to only the repulsive parts of the force laws. OP is not able to detect any adhesive forces. Later, SFA was developed by Tabor, Winterton and Israelachvili [12-14] to measure the van der Waals forces between molecularly smooth mica surfaces in air or vacuum. Israelachvili further modified the apparatus and enabled it to monitor forces in liquid medium as well. This opened the gateway to the broad field of modern surface science for instance rheology [15], tribology [16], sensing [17], electrochemistry [18], corrosion [19].

Figure 1A shows the schematic representation of the SFA apparatus. A white light beam is directed through two curved discs covered with back-silvered mica sheets of thickness 2 to 5 μm. The silver thickness on mica is about 45 nm to 50 nm to allow at least 95% reflection. The discs are placed in cross cylinder geometry creating a three layer interferometer. The interference fringes emerging from the apparatus are called fringes of equal chromatic order (FECO). The transmitted light is directed to a spectrometer for further analysis of FECO fringes. The interaction force, \( F \), acting between the surfaces is measured by the deflection of a spring on which one of the surfaces is mounted [20]. This technique has distance and force resolutions of 1 Å and 1 μN respectively [21]. The detailed description of the instrument is given in ref. [22]. Using white light interferometry, the separation distance, the radius of curvature and the interacting force can be in-situ monitored in SFA experiments.[23] Hence, absolute distance referencing and normalization of the measured forces by radius of curvature remain unambiguous. Yet, in SFA experiments, the contact area is macroscopic and typically in the range of hundreds or thousands of μm² which is much larger than that the typical contact area measured in single molecule force spectroscopy [24].

The Atomic Force Microscope (AFM) technique is widely used to measure interaction forces between surfaces at the molecular scale[24]. The schematic of AFM is shown in Figure 1B. In an AFM experiment, a small tip attached to a lever is moved toward a surface. Meanwhile the deflection of the lever and the displacement of the base to which the lever is mounted are measured as shown in Figure 1B. The deflection of the lever is measured using a laser beam reflected into a four quadrant diode and converted into interaction force using Hooke's law. The zero distance (the situation at which the tip is in contact with the sample) in an AFM experiment, however, is assumed by calibration with a hard wall of unknown origin. The exact shape and the
roughness of an AFM tip in contact with the surface is usually unknown which often complicates the direct comparison even between different AFM experiments.

Fig. 1: Schematic representation of A) Surface Force Apparatus system and B) Atomic Force Microscope
In this report, we review the origin and evolution of the repulsive hydration force in various electrolyte solutions taking into account especially the SFA results, and corroborate the results by simulation and other experimental techniques wherever applicable.

**FORCES BETWEEN SOLID SURFACES IN ELECTROLYTE SOLUTION**

The very first, accurate and direct measurement of forces between solid mica surfaces immersed in aqueous electrolyte using SFA by Israelachvili and Adams [25] is shown in Figure 2 (the results obtained by Pashley el al. is also combined in Figure 2A). It shows the forces between two charged hydrophilic mica surfaces in K⁺ solutions in the concentration range of 1 to $10^{-5}$ M at 20°C at pH 6. Figure 2A shows pure DLVO interaction below $10^{-3}$ M and an additional monotonic short range repulsion at or above this concentration for K⁺ ions. Pashley boldly reported the rise of hydration forces only above *a certain critical bulk concentration which depends on the electrolyte* [26]. Hydration forces become apparent only when hydrated cations adsorbed on mica are prevented from desorbing the surfaces upon approach. Pashley showed that this force is *completely absent at ~5 × 10^{-6} M for Na⁺ and in hydrochloric acid solutions up to 1.2 × 10^{-3} M* [27]. Pashley and Israelachvili [28] further reconfirmed the rise of an additional short-range repulsive hydration force only above a certain electrolyte concentration by careful examination of the interaction forces below 2 nm of separation distance in aqueous KCl solutions. The authors showed that the increase of the hydration force occurred both in magnitude and range (attaining 4–5 nm) with the increase of adsorbed ions on the surface. Pashley calculated the magnitude of the hydration force which followed the series Na⁺ > Li⁺ > K⁺ > Cs⁺ using a simple site-binding model to describe the charging behavior of interacting mica surface [29]. The DLVO-regulation theory was subtracted from the total measured force on the assumption that the mica surfaces apparently were fully covered with adsorbed cations. Qualitatively, with the exception of sodium, it seemed that the smaller the ions the stronger is its

![Fig. 2 A](image-url) Fig. 2 A: Semi-logarithmic plot of force vs. distance measured between curved mica surfaces in aqueous K⁺ solution ranging from $10^{-5}$ M to 1 M. B: Comparison of the hydration force of monovalent K⁺ of $10^{-1}$ M with divalent ions Mg²⁺ and Ca²⁺ of 3 M and 5 M respectively . Adapted from ref [25-29] and [32].
binding capacity to mica. Similar results were observed by Hribar et al. and Goldberg et al. [30, 31].

Pashley and Israelachvili [32] also measured the interaction forces between mica surfaces in divalent metal (Mg$^{2+}$, Ca$^{2+}$, Sr$^{2+}$, and Ba$^{2+}$) chloride solutions. They found that at concentrations $\geq 1.0$ M, the divalent cations firmly bind to the interacting mica surfaces giving rise to a repulsive short-range force (see Figure 2B) seemingly due to the residual hydration shells of the bound cations. The authors inferred that the divalent cations were more strongly hydrated than monovalent cations and therefore did not easily shed their hydration layers in order to bind to the mica surface and give rise to hydration force at higher concentrations than monovalent cations do. The magnitude of the hydration force followed the series Mg$^{2+}$ > Ca$^{2+}$ > Li$^+$~Na$^+$~K$^+$~Cs$^+$. Israelachvili and Adams also observed that the double layer forces were much reduced in Ca(NO$_3$)$_2$ and BaCl$_2$ solutions compared to those in KNO$_3$ solutions and were not accurately described by DLVO theory [33]. Using a modified set up of SFA, Rabinovich et al. [34] carried out direct measurements of the interaction forces between gold spheres and crossed quartz filaments in air within the region of distances from 10 to 100 nm. The authors observed deviations from DLVO theory below 5 nm which they attributed to the influence of structural forces. Ruckenstein et al. [35] suggested that bilayers of charges formed by the adsorption of hydrated ions and that charged bilayer generated a polarization in the neighboring water molecules which propagates into the liquid. The repulsive hydration forces were generated by the overlap of the polarization layers of the two plates.

All the mentioned studies confirmed independently the existence of an interaction force, different from the normal van der Waals and double-layer forces. This force was found to be independent to the type of electrolyte. Therefore it was classified as an additional force and not a modification of the double-layer force. Hence, for the first time, the repulsive forces which later will be defined as hydration forces was introduced. The general inference that is drawn by these early studies is that the force was intrinsically repulsive and decayed monotonically with

Fig. 3 Force measured between two curved mica surfaces as a function of distance in $10^{-3}$ M KCl solution. Adapted from ref [38].
distance out to a separation distance of around 5 nm.

On one hand, these results were considered as a breakthrough since the full force law was possible to be determined/verified experimentally but on the other hand, they were contradicting the results of computer simulations [36, 37] which were predicting an oscillatory density profile extending several molecular diameters into the liquid. Abraham [36] suspected that the hydration force would probably arise between hydrophilic surfaces, such as silica and mica, since strongly H-bonding surface groups modify the H-bonding network of nearby liquid water molecules.

In 1983 Israelachvili and Pashley [38] meticulously measured the hydration force in $10^{-3}$ M KCl solution of pH 5.5 between molecularly smooth mica surfaces. The authors reported that the hydration force was not monotonic once the separation distance was smaller or equal to 20 Å. The hydration force was oscillatory having a minima and maxima of periodicity $2.5 \pm 0.3$ Å, roughly the diameter of a water molecule (Fig. 3). The schematic in Figure 3 shows that the adsorption of ions on the mica surface orders water molecule at the surface. Using atomic force microscopy (AFM) and computer simulations, Ricci et al. [39] recently showed that cations adsorbed on the surface of mica could induce ordered water layers at the surface of homogeneous solids in aqueous solutions as shown in Figure 4. Similar results were reported in a recent study using molecular dynamics simulations between two mica surfaces in an aqueous KCl electrolyte solution by Leng et al. [40]. Urbic et al. [41] also came to the same conclusion in a simulation study. The authors used the Mercedes-Benz (MB) model of water, in NVT and µVT Monte Carlo computer simulations and observed oscillations in the forces between inert plates, due to water structure, even at plates separation of 5-10 water diameters. Cherepanov et al. [42] showed the increase of the hydration repulsion with ionic strength by molecular dynamics simulation. Henderson et al. [43] reported 7 force oscillations below 2 nm between spheres brought in contact using a non-continuum molecular theory. Using grand canonical molecular-dynamics simulations. Li et al. [44] reported oscillatory solvation forces between hydrophilic mica and glass surfaces. Fenter et al. [45, 46] studied the mica (001)-water interface under ambient conditions using High-resolution x-ray reflectivity. The results revealed density
oscillations of the water oxygen atoms in the normal direction to the surface, providing evidence of interfacial water ordering. The spacing between neighboring water layers in the near-surface region, were approximately 2.5(2) – 2.7(2) Å, close to the size of a water molecule. The density oscillations extended to about 10 Å above the surface and did not strictly maintain a constant periodicity. The authors came to the conclusion that the primary hydration layer was followed by a weakly modulated hydration structure that extended more than 1 nm above the surface.

The described experimental and simulation results show that for monovalent ions the hydration force between hard surfaces appears only above a critical ion concentration and is not only monotonically repulsive but has an oscillatory component superimposed to it.

Attempts have been made to measure the forces between surfaces other than mica. Horn et al. [47] measured the monotonic short-range repulsive force devoid of oscillatory component in NaCl solutions between silica surfaces. The measured force was found insensitive to the ionic conditions making it apparently intrinsic to the surfaces. Similar forces have been measured between silica surfaces by Vigil et al. [48], Ducker et al. [49], between mica surfaces by Shubin et al. [50], between (0001) sapphire surfaces by Drucker et al. [51], and between glass fibers by Rabinovich et al. [34] using different techniques. According to Iler [52, 53], silica is amorphous and its negative surface charges are located at the ends of short silica hairs protruding a few angstroms from the surfaces which shifts the Outer Helmholtz Plane farther out than the physical solid-liquid interface (the van der Waals plane) causing the DLVO interaction to be more repulsive. A similar effect is observed for finite sized counterions adsorbed on mica surfaces [54, 55]. Chapel [56] measured forces between two pyrogenic silica sheets immersed in monovalent electrolytes (CsCl, KC1, NaC1, LiC1). Contrary to the previous results, his results showed that the strength and the range of the hydration force decrease with increasing the degree of hydration of the counter ion. This is opposite to the behavior of mica for which adsorbed counter ions have been reported to generate a hydration repulsion. The effects of counter ions on hydration forces, weakening for silica and enhancing for mica, show that the origin of the short-range interaction is not unique.

The existence of hydration force was evidenced by several researchers using various modes of AFM [49, 57-67]. As postulated by Israelachvili et al. [21], experimentally shown by Atkins et al. and Zeng et al. [68, 69], and theoretically shown by Yang et al. [70]; a tip roughness greater than the molecular diameter of the water molecule plays an important role in smearing out the oscillatory component of the hydration force. Adsorbed chemical compounds can also alter significantly the stability of the hydration layer as recently shown by Akrami et al. [71]. Direct in situ characterization of the AFM tip or of any interacting surface cannot be performed till date which limits our understanding of the role of surface roughness or chemistry in the appearance or disappearance of hydration forces. For more details, readers are suggested to consider refs [68, 72-77].

As discussed, the simulation studies have always been leading showing the layered density profile [36, 78-80] analogous to the hydration shell and/or evidences of interfacial layering near interfaces [81-93] for various types of substrates. Layered density profile were also experimentally shown under potential control [94-97]. The recent advancement of X-ray techniques [98-103] have enabled atomic-scale studies of interfaces. Liquid density oscillations near solid surfaces have been observed in many systems [104-108]. Neutron scattering...
measurements have also demonstrated the existence of structured fluid layers under confinement [88, 89], [109-112]. Mineral-water interface using X ray has been studied by Fenter et al. [46, 113-118]. For details mineral–water interfacial structures revealed by synchrotron X-ray scattering refer to references [45, 119]

Thus from above close examination of the studies, both experimental and theoretical, we can draw the following conclusions:

1. Above the critical concentration, the adsorption of cation on the mica surface is the cause of the repulsive hydration force and the hydration force is not only monotonically repulsive but an additional oscillatory component is superimposed to it below 2 nm and its periodicity is equal to the mean molecular diameter of water.

2. The critical concentration depends on the type of the electrolyte. The critical concentration for divalent metal ion is always higher than that for the monovalent metal ion solution.

3. The magnitude of the oscillatory force component depends on the extent of adsorption of cation on the surface which in turn depends on the concentration of the solution. The more the cations adsorbed the strong is the hydration force.

4. Surface roughness plays an important role in smearing out the oscillatory component of the hydration forces.
Lipid bilayers have been extensively studied in the past 50 years. Lipid bilayer are formed by the self assembly, in water, of lipid molecules into a bilayer structure through hydrophobic interactions between hydrocarbon tails of the molecules. As a consequence, the interfacial properties of a bilayer differs significantly from those of solid surfaces mostly due to the inherent mobility of the lipid molecules forming the bilayers. As we will see, such mobility dramatically modulates the interaction of the bilayer with water molecules, solvated ions and with other bilayers as well. In what follows, we describe the recent efforts that have been made towards a better understanding of such interactions and the future directions of research that these recent results are offering.

The water-bilayer interface

The structure of the water bilayer interface has been studied extensively using spectroscopic techniques such as Nuclear Magnetic Resonance (NMR) and Infrared (IR spectroscopy).[120] Early studies performed using NMR and molecular dynamic simulation (MDS) focused on identifying the number of molecules necessary to form the hydration shell of lipids. It is commonly accepted that this number varies between 17-22 water molecules depending on the lipid head group, lipid area [121] and availability of ions or solutes and temperature (see Figure 5).[122] More recent simulations have suggested that the interfacial water can mostly be classified into three categories which are [123, 124] (i) buried water located close to the carbonyl groups, (ii) first external hydration shell near the phosphorylcholine groups and (iii)

**Figure 5:** molecular representation of the water-lipid interface in a model bilayer made of DPPC. Also shown is the water density profile across the bilayer-water interface.
experimentally, there are clear experimental evidences of strongly bound water molecules to lipid head groups.

Water distribution around lipid head group can be easily disturbed by external factor. In particular, presence of ions, especially cations have been shown experimentally to alter lipid molecular area and diffusivity. Since Na$^+$ is the most physiologically abundant, its interaction with lipid bilayers has been extensively studied, theoretically and experimentally. For example Fluorescence Correlation Spectroscopy (FCS) has been used to characterize POPC self-diffusion coefficient in presence of varying concentration of NaCl at different temperatures. The study shows that independent to the fluorescent marker used, the augment in concentration of salt tend to decrease the diffusion of POPC molecules.[125] Simulation studies confirmed this observation and revealed that tight coordination complexes involving one Na$^+$, three lipid molecules and 1-2 water molecules could be formed. Similar results were reported with mixed lipid bilayers.[126]

The binding of other types of species such as organic molecules have also been reported to strongly affect the behavior of water molecules near the bilayer interface. For example, dimethyl sulfoxide (DMSO) has been widely studied for its ability to preserve cellular membrane during cryo-preservation. Such important property was recently explained using PFG NMR and the SFA.[127]

The interaction forces acting between apposing lipid bilayers include the following: [52]

(i) van der Waals interactions forces between the bilayer hydrocarbons layer across water,
(ii) hydration forces due to the expulsion of water molecules upon compression of the bilayers,
(iii) the hydrophobic interaction between lipid tails across water,
(iv) the protrusion interaction forces due to the restriction of lipids position fluctuations along the direction of compression,
(v) the lipid head group overlap involving conformational change of the head groups upon compression,
(vi) the undulation forces due to bending fluctuations of the bilayers and
(vii) the electrostatic interaction forces due to bilayer surface charging.

Each of these contributions have been studied both experimentally and theoretically. Mathematical expressions of these interactions are provided in Table 1 and the reader is referred to the original references for further details of their derivation.

Interaction forces between lipid bilayers or lipid membranes have been measured using a variety of techniques. The two main techniques have been the osmotic pressure technique (OP) and the SFA. As can be seen in Figure 6A and B, both measurement techniques give similar results on single component lipid bilayers and multi-component lipid bilayers/membranes. Important differences should also be noticed: when using the OP technique, the thickness of the lipid bilayers increases (the lipid molecular area decreases) as the applied pressure increases; while in
the SFA measurement, the lipid bilayer becomes thinner (the molecular area increases) at the contact area due to lateral diffusion of lipids during the compression. These important differences explain why hemifusion/fusion of bilayers is rarely reported using the OP technique and can be easily observed using the SFA.

**Figure 6:** Short range forces measured between single component lipid bilayers (A) and multi-component lipid bilayers and lipid membranes (B). (A) Interaction forces between lipid bilayers of egg PC measured independently with the SFA and by the osmotic pressure technique (OP). Both measurements show the presence of a net repulsive force exponentially decreasing with the bilayer separation distance. The characteristic decay length of this interaction is 2.5 Å. Note that in the SFA experiment only, membrane hemifusion is observed at a pressure of 10 MPa [128]. (B) Interaction forces between multi-component membranes. Measurement performed between myelin lipid bilayers using the SFA in buffer saline show that addition of calcium in the medium promotes hemifusion of the membranes. Experiment performed with the osmotic pressure method show the interaction forces between integral myelin membranes from [129]. The results illustrate that the long range portion of the interaction forces measured by SFA and OP, mainly caused by the electrostatic interaction, are in very good agreement besides the presence of transmembrane proteins in the myelin membranes which are absent in myelin lipid bilayers. Interestingly, myelin membranes do not present any signs of hemifusion during the experiment while hemifusion was clearly observed with the SFA at a surface pressure of approximately 7-10 MPa. Adapted from Refs [129], [130], [139]
Table 1: Interaction forces commonly encountered at lipid bilayers and at solid surfaces

<table>
<thead>
<tr>
<th>Contribution</th>
<th>Equation</th>
<th>Reference</th>
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<tr>
<td>Head group overlap</td>
<td>$P(D) = \frac{kT}{s^3} \left[ \left( \frac{2L}{D} \right)^{3/4} - \left( \frac{D}{2L} \right)^{3/4} \right]$ for $D &lt; 2L$</td>
<td>(Eq. 1) [130, 131]</td>
</tr>
<tr>
<td></td>
<td>$P(D) = \frac{100}{s^3} kT e^{-\pi D/L}$ for $0.4L &lt; D &lt; 1.8L$</td>
<td>(Eq. 2)</td>
</tr>
<tr>
<td>Undulation</td>
<td>$P(D) = \frac{3\pi^2 (kT)^2}{64k_bD^3}$</td>
<td>(Eq. 3) [132]</td>
</tr>
<tr>
<td>Hydrophobic interaction</td>
<td>$P(D) = \frac{e^{-D/D_H}}{(1 - (1 - e^{-D/D_H})^{1/2}) + \frac{1}{2}(e^{-D/D_H}(1 - e^{-D/D_H})^{-1/2})}$</td>
<td>(Eq. 4) [133-135]</td>
</tr>
<tr>
<td>Protrusion interaction</td>
<td>$P(D) = 2.7(\Gamma kT/\lambda)e^{-D/\lambda}$, $\lambda = kT/\pi \delta y_{l}$</td>
<td>(Eq. 5) [136, 137]</td>
</tr>
<tr>
<td>van der Waals</td>
<td>$P(D) = -\frac{A}{6\pi D^3}$</td>
<td>(Eq. 6) [52]</td>
</tr>
<tr>
<td>Electrostatic</td>
<td>$P(D) = 64kT \rho \tan h^2(ze\psi_0/4kT)e^{-D/\rho D}$</td>
<td>(Eq. 7) [52]</td>
</tr>
<tr>
<td>Hydration</td>
<td>$P(D) = W_0 e^{-D/D_H}$</td>
<td>(Eq. 8) [52]</td>
</tr>
<tr>
<td>Oscillatory (structural)</td>
<td>$P(D) \approx W_0/\sigma e^{-D/\sigma} \left[ 2\pi \sin \frac{2\pi D}{\sigma} - 1 \right]$</td>
<td>(Eq. 9) [52]</td>
</tr>
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</table>

It is important to note that all these interaction forces originate from very distinct causes and therefore exhibit very different magnitudes and act over very different length scales. As a consequence, the separation distance $D$ between the bilayer does not have the same origin point or (operational plane) in each contribution.[52] For example, the plane of origin of the electrostatic interaction should be located at the position where surface charges are located which might be quite different from the origin plane of the van der Waals interaction. Shifting of the plane of origin of an interaction potential is usually done by replacing the separation distance $D$ by $D-D_0$ where $D_0$ is the shifting distance.

All the interactions presented in Table 1 do not have to be necessarily considered in every situation. For example in a physiological fluid or in a buffer saline solution containing salt concentrations of typically 150 mM (1:1 electrolyte), the van der Waals interaction is usually found to be negligible compared to other contributions. The presence of free ions in the medium
screens the zero-frequency term of the van der Waals interaction over a distance equals to the Debye screening length (roughly 1 nm at physiological conditions).

The hydrophobic interaction is usually considered to play a minor role in the total interaction between bilayers mainly because it is generally counterbalanced by other contributions such as the electrostatic or the head group overlap force. Hydrophobic forces should only be considered when hemifusion/fusion of the bilayers occurs. As the bilayers are compressed, thinning will start to occur and will favor the exposure of the hidden hydrophobic core of the bilayer to the aqueous medium. It is clear then that appearance of the hydrophobic interaction between facing bilayers depends on lipids molecular coverage and its variation with confinement. Donaldson et al. [133] provided a simple expression for the molecular area of elastic lipid bilayers in presence of the hydrophobic interaction:

\[ a(D) = a_0 \left(1 - e^{-D/D_H}\right)^{-1/2} \]  
(Eq. 8)

Using the expression of the hydrophobic interaction together with the electrostatic and steric contributions, Banquy et al. described the interaction forces between two model myelin lipid bilayers immersed in buffered saline solution.[138] The authors showed that consideration of the hydrophobic interaction allowed to predict the onset of hemifusion of the bilayers which occurred when the lipid molecular area increased by almost 30% (see Figure 7A). The critical molecular area at which hemifusion appears, i.e. the point where the hydrophobic interaction counterbalances all other repulsive interactions, is strongly dependent on the cohesive strength of the bilayers and the lipid molecular area.

Figure 7B presents a situation where a photosensitive bilayer was deposited on mica by the vesicle deposition method. When compressed, the bilayers hemifused at a critical molecular area which was 16% higher than the equilibrium value. When illuminated under UV light, the surfactant molecules changed conformation and became more hydrophilic which dramatically expanded both the interfacial energy and the molecular area of the molecules at rest. Thus, the critical molecular area at hemifusion was found to be 25% higher than the value at equilibrium.
Figure 7: (A) Interaction energy between two myelin lipid bilayers in presence of 2 mM Ca\(^{2+}\) ions. Calcium ions facilitate the hemifusion of the bilayers as denoted by a breakthrough instability in the interaction energy profile. Such instability is governed by the hydrophobic interaction between the hydrocarbon tails of the lipids that are being exposed during compression and thinning of the bilayers. Adapted from [139] (B) Interaction force measured between photosensitive surfactant bilayers. In this system the hydrophobic force was modulated by the exposure of the light sensitive hydrocarbon tails to UV light leading to different energetic barrier to hemifusion. Adapted from [139]

The undulation interaction has by far the longest range and is comparable in magnitude to the van der Waals interaction. The magnitude of this interaction can be strongly by different external parameters. For example, lipid bilayer supported on a solid substrate will almost no undulations. As shown in Eq. 3, undulation forces are also extremely sensitive to temperature which can be useful in experimental settings where fine tuning of this interaction is required. Ions have also an important impact on undulation forces. Free ions in solution can either adsorb on lipid
membranes, alter head group dissociation degree or their hydration level. All these factors will impact directly or indirectly the bending modulus of the bilayer and therefore the undulation forces. For low salt concentration (or large surface charges), [140] derived the following expression for the bending modulus of symmetrical bilayers:

\[
   k_b = \frac{\varepsilon\lambda_0}{\pi} \left(\frac{kT}{e}\right)^2
\]

(Eq. 9)

Considering that \(\lambda_0 = 0.304/\sqrt{\varepsilon}\) for a 1:1 electrolyte at room temperature, simplification of Eq. 9 leads to \(P \propto \sqrt{\varepsilon}\) which results in a weak dependence on salt concentration. In the high salt regime, the expression of the bending modulus becomes:

\[
   k_b = \frac{3\pi(\lambda_0)^3\sigma^2}{\varepsilon}
\]

(Eq. 10)

where \(\sigma\) is the surface charge and \(\varepsilon\) the dielectric constant of the medium. Using the Grahame equation for a 1:1 electrolyte, the expression for \(\sigma\) becomes \(\sigma = \sqrt{8\varepsilon\varepsilon_0 kT \sinh(e\psi_0/kT)\sqrt{\varepsilon}}\) which leads to the same weak dependence for the interaction pressure \(P \propto \sqrt{\varepsilon}\).

The head group overlap and protrusion forces are usually difficult to distinguish from each other and are often termed "steric hydration" forces. The protrusion force is related to the hydrophilicity of the bilayer while the head group overlap force is more related to the hydration of the head groups. In both cases, it is important to remember that these forces do not originate from water structuring at the bilayer-water interface. Since both interactions exhibit an exponential decay (see Eq. 2 and 5), comparison of their characteristic decay length should in principles provide useful insights into the contribution that dominates the total interaction. A back of the envelope calculation already provides good estimates of the expected values of these two parameters. Considering the interfacial tension between water and hydrocarbon to be 50 mJ/m\(^2\) and the cross sectional radius of the hydrocarbon chain to be close to 0.2 nm, we found using Eq. 5, \(\lambda = 0.13\) nm. On the other hand, the size of a phosphatidyl head group is expected to be close to 0.8 nm giving (Eq. 2) \(\lambda = 0.25\) nm. Since these two decay lengths are comparable, it is a priori difficult to identify the dominant one.

In a recent study by Schrader et al. [141], the authors characterized the effect of DMSO, in water, on the interactions between bilayers. The authors elegantly demonstrated that by increasing the concentration of DMSO in water from 0% to 7.5%, the decay length of the interaction force markedly decreased from 0.2 to 0.15 nm (see Fig. 9). Since the interfacial tension between water/DMSO and hydrocarbon decreases with increasing the DMSO content, it is clear that the measured change in decay length could not be accounted by the presence of protruding forces. However, using NMR diffusion measurements, the authors showed that the change in decay length observed in presence of higher concentrations of DMSO was correlated with a change in hydrodynamic radius of the head group ionic moieties. The authors concluded that the interaction forces between DPPC bilayers at separation distance lower than 2 nm were dominated by the head group overlap force which is tuned by the competition between DMSO and water molecules to interact with the lipid head group.
Fig. 8 Short range interaction forces measured between DPPC lipid layers in presence of a water-DMSO solution. Measurements show a decrease of the range and the decay of the interaction forces which is a characteristic signature of a conformational change of the lipid head group mediated by DMSO. Adapted from [142].

These recent and previous studies [142-144] underscore the important fact that the so called "steric hydration" is actually an steric-entropic force similar to the polymer-brush interaction described by Alexander and deGennes. The role of water is solely to modulate the hydration, and therefore the conformation, of the lipid head groups. The effect of water on the degree of hydration of a lipid head group is a solvation effect which in term of interaction forces will manifests itself as a shift in the interaction, while the effect on the lipid head group will change the decay length and the range of the interaction.

The understanding of these subtle effects provides a powerful framework to study the interaction of a large number of solute molecules such as ions or small molecules such as peptides interacting directly or indirectly with the surface of lipid bilayers.

In light of this review of the literature on the measurements of the hydration interaction we can highlight a few similarities and differences when comparing the force laws obtained with solid and soft (fluid) surfaces. In terms of similarities, for both systems, the hydration force exhibits a monotonically decaying component whose decay length varies between 0.1 and 1 nm. The origin of this component is quite different in both systems: for solid surfaces it involves water molecules bound to the surfaces either directly or via strongly adsorbed ions, while for soft lipid bilayers it is related to the lipid head-group conformation and level of hydration. A clear evidence that origin of the hydration force in both systems is different can be appreciated in the fact that the hydration force between solid surfaces appears only above a critical salt concentration which is not the case for soft bilayers.

Hard atomically flat surfaces are the only system exhibiting an oscillatory component of the hydration superimposed to the monotonically decaying component. The appearance of this component is in part related to water conformational polarization induced by the presence of ions on the surface.
Finally, it is important to remind that the appearance of the hydration force has been quite often associated mainly to the ordering water molecules on the surfaces. Hydration forces rise from the overlapping and therefore rearrangement of this ordering of water molecules. There is obviously a connection between ordering/structuring of water and the hydration force, but both phenomena should be always carefully distinguished.
References

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