

# Chapter 4

## Hydration Forces Between Lipid Bilayers: A Theoretical Overview and a Look on Methods Exploring Dehydration

Helge Pfeiffer

**Abstract** Although, many biological systems fulfil their functions under the condition of excess hydration, the behaviour of bound water as well as the processes accompanying dehydration are nevertheless important to investigate. Dehydration can be a result of applied mechanical pressure, lowered humidity or cryogenic conditions. The effort required to dehydrate a lipid membrane at relatively low degree of hydration can be described by a disjoining pressure which is called hydration pressure or hydration force. This force is short-ranging (a few nm) and is usually considered to be independent of other surface forces, such as ionic or undulation forces. Different theories were developed to explain hydration forces that are usually not consistent with each other and which are also partially in conflict with experimental or numerical data.

Over the last decades it has been more and more realised that one experimental method alone is not capable of providing much new insight into the world of such hydration forces. Therefore, research requires the comparison of results obtained from the different methods. This chapter thus deals with an overview on the theory of hydration forces, ranging from polarisation theory to protrusion forces, and presents a selection of experimental techniques appropriate for their characterisation, such as X-ray diffraction, atomic force microscopy and even calorimetry.

**Keywords** Hydration forces • Controlled hydration • Osmotic stress • Polarization • Protrusion • Sorption • Percolation • Plasticizer

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## 4.1 Introduction

### 4.1.1 Fundamentals

During the last decades, it has been more and more accepted that hydration plays a complex, functional role in living cells, and in this context it becomes clear that phospholipid membranes are much more than semi-permeable barriers (Disalvo et al. 2008; Scharnagl et al. 2005).

Native membranes usually exist at excess water conditions. However, investigations on phenomena arising from partial or complete dehydration in membranes and other biological surfaces are important as well, such as for the case of cell fusion, stress on cartilage or especially for anhydrobiosis, i.e. the ability of different organisms to survive even complete dehydration. There are different potential applications for anhydrobiosis, such as tailored cryosurgery in medicine (Balasubramanian et al. 2009), biopreservation, i.e. the storage of biological material (Aksan et al. 2009; Franca et al. 2007), and also applications in space projects (Ricci et al. 2005; Rothschild and Mancinelli 2001).

The degree of hydration can on the one hand be described by the integral water concentration, and on the other hand by the fraction of water that is chemically or physically attached on their respective host molecules under excess water conditions. The quantification of such bound water mostly depends on methods and models (Jayne 1982) and one should not be confused if there appear deviating or even contradicting results in the literature; bound water can e.g. be defined by a decay constant, or by the amount of non-freezable water (Kodama et al. 2004). Furthermore, one should emphasise that in some cases, the integral water concentration or the hydration number do not necessarily give information on the actual position of the water molecules within the membrane. Some water can e.g. also be trapped in diffusionally restricted or sealed micro-volumes such as water-filled pockets or pores (see e.g. Binder and Gawrisch 2001).

The global hydration between lipid surfaces is frequently expressed by the molar hydration number, i.e. the molar ratio of water and lipid  $R_w$  (Eq. 4.1), where  $n_w$  and  $n_L$  are the molar number of water and lipid resp.

$$R_w = \frac{n_w}{n_L} \quad (4.1)$$

or by the water layer thickness  $d_w$  (corresponds to the broad arrows in Figs. 4.9 and 4.10). The water layer thickness was frequently calculated according the model of Luzatti (Marsh 1990) but also other approaches are used that e.g. make use of the electron density profile of lipid headgroups (Schmiedel et al. 2001).

The free enthalpy of water transferred from a reference phase into the lipid bilayer is expressed by the chemical potential of water,  $\mu_w$ . It has close relationships with the water activity,  $a_w$  or the water potential,  $\Psi_w$ , and the corresponding relationship is given by Eq. 4.2 (Moore 1972):

$$\mu_W = \mu_{W,0} + RT \ln a_w \quad (4.2)$$

where  $\mu_{w,0}$  is the chemical potential of water at reference conditions,  $R$  is the gas constant and  $T$  the absolute temperature. The water potential  $\Psi_w$  is defined by Eq. 4.3:

$$\Psi_W = \frac{\mu_W - \mu_{W,0}}{V_W} \quad (4.3)$$

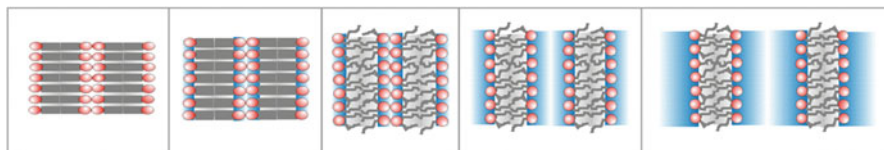
where  $V_W$  the molar volume of water. The water potential is in the respective literature usually composed of different terms (Adam et al. 1995) representing the osmotic potential (entropic origin), “turgid potential” (hydrostatic pressure) and matrix potential (contains contributions from the interactions with colloids, as e.g. in capillary and surface interactions).

### 4.1.2 Phase Behaviour at Low Hydration

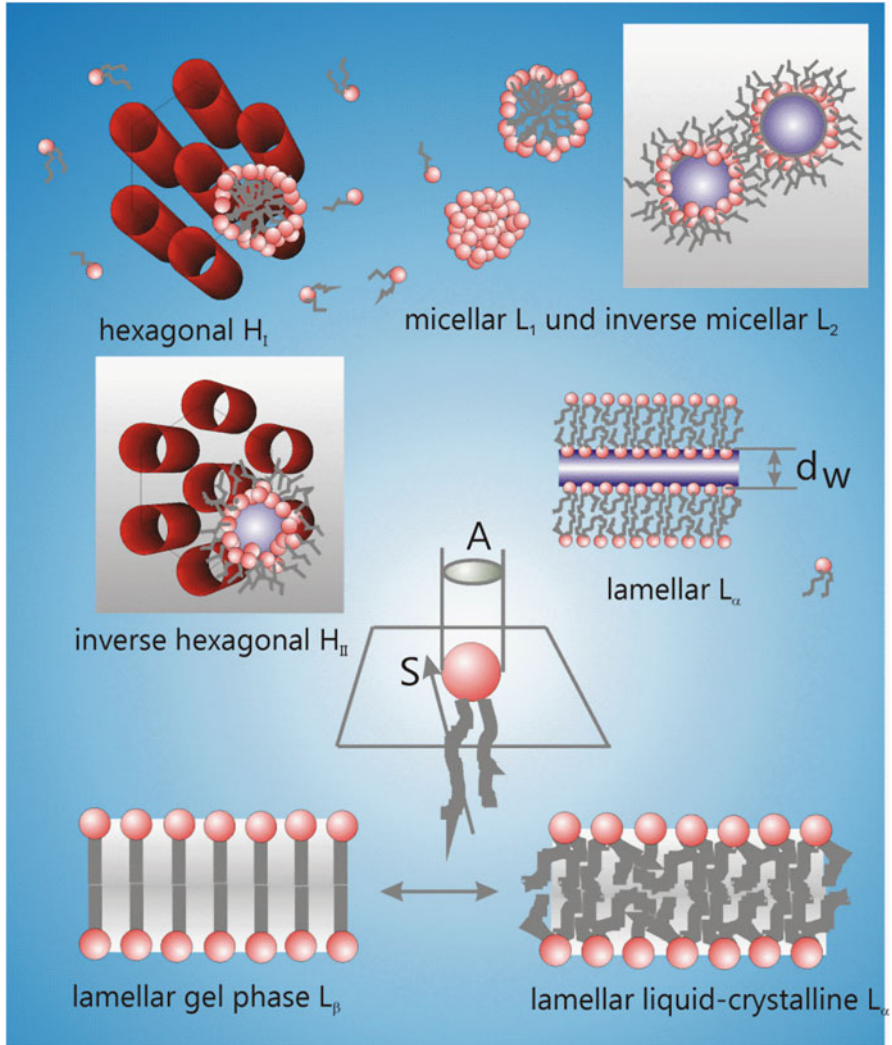
During dehydration, intermolecular interactions and motional degrees of freedom within the membrane are affected and the loss of mobility leads in general to solidification. For almost all pure, dehydrated lipids with saturated hydrocarbon chains, a solid lamellar phase can be found at ambient temperatures, in most cases the  $L_\beta$ ,  $L_{\beta'}$  or the  $L_c$  phase (Kranenburg and Smit 2005). For lipids with unsaturated chains, such as DOPC, at room temperature a liquid-like phase can exist. For higher temperatures, also non-lamellar phases are reported for the case of low water content (see e.g. Jürgens et al. 1983) (Fig. 4.1).

Even in lipid-water systems containing only one lipid species, up to three coexisting lipid phases can be observed, at least in the range of broad phase transitions and at low hydration degrees as e.g. revealed by calorimetry, X-ray diffraction and  $^2\text{H}$  and  $^{31}\text{P}$ -NMR spectroscopy for the case of POPC (Pfeiffer et al. 2013a). This can be rationalised by the Gibbs phase rule and experimental data showing that these phases are all lamellar phases with different degrees of hydration, i.e. there is obviously an equilibrium between lipid layers with different, but distinct hydration degrees. Outside phase transitions, these separate phases cannot be observed, but this might also mean that our methods are not yet able to detect them.

The question of non-lamellar phases typically applies to mixtures, especially if lipids are mixed with other kinds of amphiphiles, such as surfactants. Here, diverse



**Fig. 4.1** Hydration of solid lipids, frequently inducing the liquid-crystalline phase and swelling

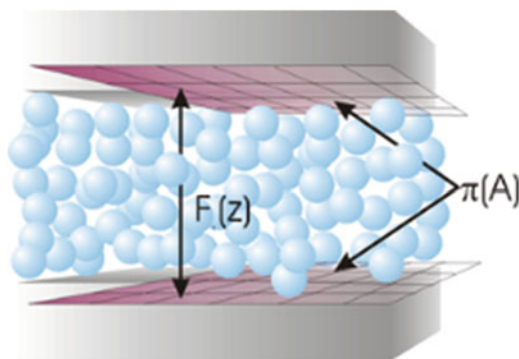


**Fig. 4.2** Different hydration states of lipids induce different lyotropic phases

non-lamellar phases, such as hexagonal, inverse-hexagonal or inverse micellar phases can be observed at ambient temperatures when hydration is sufficiently reduced (Koynova and Tenchov 2001; Klose et al. 1995a; Funari et al. 1996; Pfeiffer et al. 2012). In native systems, the transition into non-lamellar phases denatures membranes as such which is considered to be a major factor of concern in cryobiology.

**Table 4.1** Overview of surface forces with their principal dependence upon water layer distance,  $d_w$ 

Interaction	Pressure $P(d_w)$
Electrostatic	$P_{el} \propto \exp(-d_w/C_{el})$
Van der Waals	$P_{vdw} \propto 1/d_w^3$
Undulation	$P_{und} \propto 1/d_w^3$ (Helfrich 1978)
Undulation	$P_{und} \propto 1/d_w$ (Freund 2013)
Peristaltic	$P_{per} \propto 1/d_w^5$ (Helfrich 1978)
Steric	$P_{ster} \propto 1/d_w^{(9/4)} - d_w^{(3/4)}$
Protrusion	$P_{pro} \propto \exp(-d_w/C_{prot})$ (Israelachvili and Wennerström 1992)
Polarisation	$P_{pol} \propto \exp(-d_w/C_{pol})$ (Cevc 1991)

**Fig. 4.3** Highly simplified representation of hydration force  $F(z)$  between polar surfaces,  $\pi(A)$  denotes the surface pressure

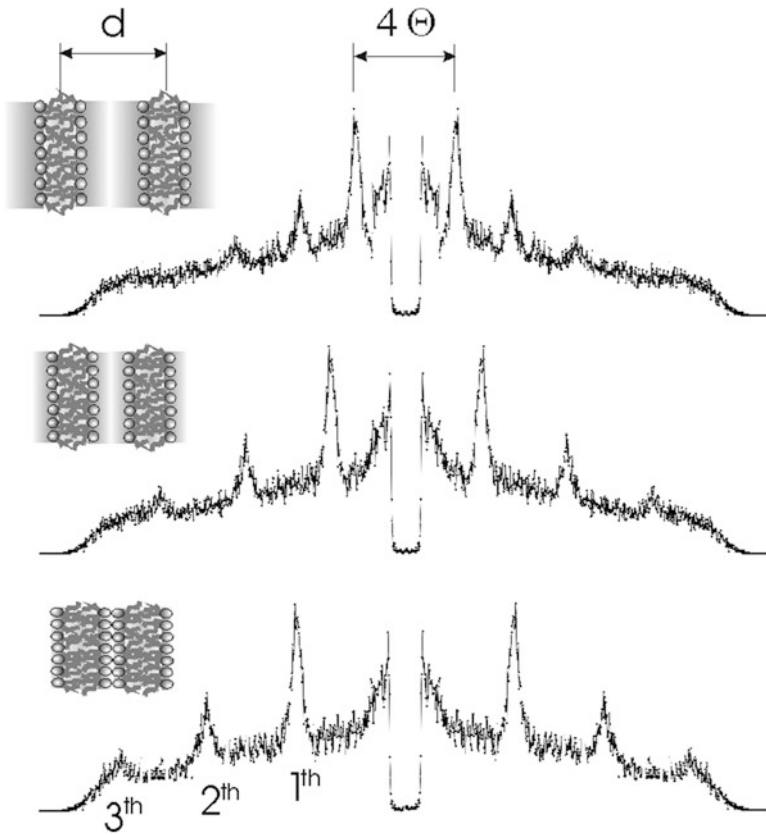
## 4.2 Hydration Force

### 4.2.1 Motivation and Possible Definitions

In physical-chemical terms, the force between hydrated surfaces is mostly defined by the hydrostatic pressure maintaining the chemical equilibrium between the hydration water and free water at reference conditions.

It is generally accepted that various interactions apply when hydrated phospholipid membranes approach, such as the Van der Waals, electrostatic (not applicable for zwitterionic lipids in pure solvents), undulatory, peristaltic and steric (=hard core) interactions (Nagle and Tristram-Nagle 2000) (Table 4.1).

There is however one interaction concerning magnitude and distance between steric repulsion and Van der Waals attraction, that is not predicted by established interaction models (Israelachvili and Wennerström 1996). It is mostly called hydration pressure or hydration force (Fig. 4.3) and it can empirically be described by an exponential function versus the water layer distance (Eq. 4.4) or the molar ratio of water and lipid,  $R_w$  (see Eq. 4.1):



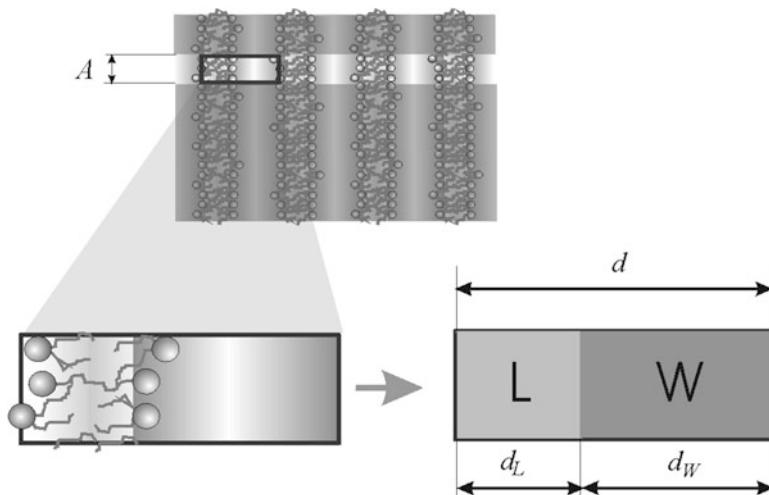
**Fig. 4.4** Idealised representation of the wide-angle diffraction patterns at varying repeat distances of the corresponding lipid layers. The numbers give the diffraction orders

$$P_h = P_{h,0} \exp\left(-\frac{R_w}{R_{w0}}\right), P_h = P_{h,0} \exp\left(-\frac{d_w}{d_{w0}}\right) \quad (4.4)$$

The characteristic constants  $P_{h,0}$  are the hydration pressure or hydration force at hypothetical zero-hydration and  $R_{w,0}$  and  $d_{w,0}$  are the corresponding decay constants (Eq. 4.4). The repeat distance is frequently determined by X-ray diffraction (Fig. 4.4).

In the range from 0.2 to 2 nm, the hydration force is considered to be the dominating interaction, therefore, in many cases the force curves obtained for that hydration range are considered as the hydration forces curves as such.

One should not be confused that there are different representations of hydration force used in the literature. The hydration force,  $F_h$ , for instance is for convenience frequently expressed in pressure units. When measuring with the surface force apparatus (SFA) however, the force is usually given as the force normalized with respect of the “effective radius” of approaching cylinders, typically with the



**Fig. 4.5** Composition of the repeat distance according to the LUZZATTI model

dimensions  $[F/R] = \mu\text{N/m}$  (see also Sect. 4.2.3.2). In the case of atomic force microscopy however, a force as such is provided, mostly given in “nanoscopic” dimensions, i.e.  $[F] = \text{nN}$  or  $\text{pN}$  (see also Sect. 4.2.3.3) and derived from the frequencies of an oscillating scanning tip.

For comparing hydration forces in lipids with different surface cross sections, proteins and other materials, it makes sense to use the water layer thickness or a surface related hydration number instead of a molar hydration number, otherwise it would be difficult to compare hydration force parameters. One should however keep in mind that geometrical quantities at this molecular level strongly depend on models, such as the model of Luzzati (Luzzati and Chapman 1968) (Fig. 4.5) which is presented in short below.

Thus, the repeat distance,  $d$ , is considered as the sum of the water layer thickness,  $d_W$ , and the thickness of the bilayer,  $d_L$ :

$$d = d_W + d_L \quad (4.5)$$

The volume fractions,  $\Phi_L$  and  $\Phi_W$ , can be expressed by the corresponding apparent specific volumes  $V$  weighted by their concentrations. Using Eq. 4.5 one can write:

$$\frac{d - d_W}{d_W} = \frac{\bar{V}_L c_L}{\bar{V}_W (1 - c_L)} \quad (4.6)$$

The weight concentration,  $c_L$ , is known from sample preparation and the apparent specific volumes are obtained from densitometry (Wilkinson and Nagle 1981). Usually, one assumes that they are independent of hydration. However, this is an assumption that is not always reasonable, especially at very low hydration. A

detailed investigation even shows contradicting results, to be attributable to different methods, assumptions and used instrumentation (White et al. 1987; Scherer 1987).

The final expression for the water layer thickness is:

$$d_w = d \frac{1}{\left( \frac{\bar{V}_L}{\bar{V}_W} \frac{c_L}{1-c_L} - 1 \right)} \quad (4.7)$$

Hydration pressure, introduced by Langmuir for explaining disjoining pressure in colloids, is thus a phenomenological expression and it was introduced to explain the colloidal stability for many hydrophilic biological surfaces such as for stress on cartilage, cell surfaces, osmotic dehydration or freezing induced dehydration (Wolfe et al. 1994); it is found for DNA, proteins (Valle-Delgado et al. 2011), polyelectrolytes and polysaccharides (Rand and Parsegian 1989; Parsegian et al. 1995). Interesting options of using hydration pressure related phenomena in gels are even given by recent applications in liquid-detecting sensors using hydration triggered thresholds for percolation conductivity (Pfeiffer et al. 2011, 2014).

As mentioned above, the first concept of hydration pressure was introduced by Langmuir in 1938 (Evans and Wennerström 1994) and Le Neveu et al. (1976) applied the concept to lipid bilayers. In 1985, Marra and Israelachvili (1985) have been the first who directly measured the force curves with a surface force apparatus (SFA). Nowadays, two basic concepts try to explain the origin of this force. But these concepts are sometimes contradictory (Israelachvili and Wennerström 1996; Parsegian and Rand 1991) and the efforts to make a definitive decision for one of these theories have not been wholly successful (see also Sect. 4.3).

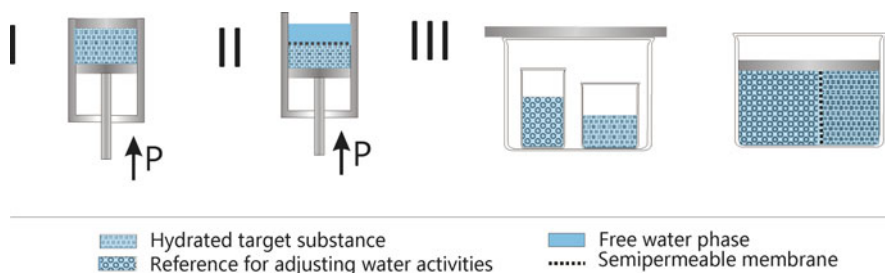
## 4.2.2 Controlling Hydration and Pressure

The exploring of hydration-dependent pressure effects requires defined thermodynamic boundary conditions. Therefore, this section will present a small overview on principal configurations to adjust hydrostatic pressure, chemical potential and/or water concentration (Fig. 4.6), see also Table 4.2.

Configuration I – Iso-compositional: This is the standard configuration to investigate high-pressure effects on closed lipid dispersions (Winter and Pilgrim 1989) which are situated in a sealed vessel preventing a change of the global solvent concentration. Changes in the hydration state of the lipid by hydrostatic pressure means amplification or weakening of solvent-solute interactions probably leading to a change of hydration numbers. Furthermore, the thermodynamic activity of all components is enhanced with respect to a reference phase because the hydrostatic pressure is increased (Moore 1972).

Configuration II – Iso-potential: This configuration represents the original hydration pressure experiment (Leneveu et al. 1976, 1977), see Sect. 4.2.3.1. The chemical potential of water is kept constant (= iso-potential) at every step of





**Fig. 4.6** Different configurations to adjust hydration and/or hydrostatic pressure, *left* iso-compositional, *middle*: iso-potential, *right*: isopiestic

**Table 4.2** Relationship between hydration  $n_w$ , hydrostatic pressure  $P_{hst}$  and chemical potential of water  $\mu_w$  at different experimental configurations

Configuration	Constant	Variable
I	$n_w$	$P, \mu_w$
II	$\mu_w$	$n_w, P$
III	$P_{hst}$	$\mu_w, n_w$

hydration enabled by a semi-permeable membrane that mediates the chemical equilibrium of the membrane water with a water phase at standard conditions (free water). The amount of water released into the free water phase is according to the hydration force function correlated to the hydrostatic pressure.

Configuration III – Isopiestic: This configuration represents hydration under constant, ambient pressure and the chemical potential is varied by humidities or by appropriate osmotic solutions (see e.g. vapour pressure and osmotic stress method) (Parsegian et al. 1979). One obtains functional pairs of chemical potential hydration which are also known as sorption isotherms. The water activity can be adjusted by the water vapour via saturated salt solutions, by inert co-solutes, such as dextran or polyethylene-glycols (PEG) or by devices that use a calibrated gas stream composed of water vapour and other gasses, such as nitrogen (Binder et al. 1999a; Baumgart and Offenhäusser 2002).

## 4.2.3 Measuring Hydration Force: A Selection of Piezotropic Methods

### 4.2.3.1 The Original Method by LeNeveu and Rand

The original experiment on hydration force, published in 1976, is performed according to the iso-potential configuration (Configuration II). For achieving the osmotic pressure, the lecithin bilayers were deposited in a dextran solution which was in contact with a reference water phase via a semi-permeable membrane.

Dextran is chemically inert with respect to the lipids and the macromolecules do not penetrate into the bilayer phase (Leneveu et al. 1976, 1977). The osmosis-driven hydrostatic pressure in the dextran/lipid solution was directly determined by conventional pressure gauges. Furthermore, the repeat distances were determined by small angle X-ray diffraction (SAXD) (Fig. 4.3) and for calculating the water layer thickness, the Luzzati model was applied (Luzzati and Chapman 1968).

The Luzzati model (see above) considers the explicit assumption that water and lipid layers do not penetrate. Although, this assumption is relatively unrealistic, that model was also frequently applied in the literature, and even if the weaknesses are taken into account, valuable results were obtained. The determination of the zero approach is however also here a big challenge, such as in many other methods for determining hydration force (Butt et al. 2005). Newer models make use of the electron density profile obtained from X-ray scattering (see e.g. (Schmiedel et al. 2001)).

#### 4.2.3.2 Surface Force Apparatus (SFA)

The surface force apparatus (Israelachvili and Adams 1978) also operates under the iso-potential configuration (Configuration II) because the pressurised water layer is also (should be) in equilibrium with an outer water phase at reference conditions. Practically, two crossed cylinders with a diameter of approximately 1 cm are moved against each other along a line perpendicular to their axes. When measuring the force between the cylinders by a deflected spring and the distance by optical interferometry using fringes, accurate surface force curves are obtained. For the cylinder, pure or coated mica surfaces are used, (Marra and Israelachvili 1985) but also silica surfaces are possible as substrate (Orozco-Alcaraz and Kuhl 2013). In contrast to the AFM method described below, relatively flat surfaces are applied with a radius that is large with respect to the thickness of the water layer. In this sense, the experiments are very close to the original experiment on hydration force (Sect. 4.2.3.1), except of the fact that with the SFA, the lipid layers are supported leading to reduced undulatory motions.

Furthermore, the force curves of several systems show oscillatory behaviour (Christenson and Horn 1983) attributed to the layer-wise removal of hydration water, also observed for other solvents. This is an interesting phenomenon because oscillatory means that even the sign of the force changes, i.e. hydration repulsion changes into hydration attraction and so forth.

When the cylinders are coated with lipids, no oscillatory but smooth curves are measured (Trokhymchuk et al. 1999). These smooth curves can be interpreted as Hilbert transforms because that mathematical operation provides envelopes connecting the maxima of an oscillation curve. In the literature, the lack of oscillatory behaviour was in physical terms explained by a dynamically “smearing out” of those oscillations (Leckband and Israelachvili 2001) (see also Sect. 4.2.4.1) which is in fact nevertheless a remarkable phenomenon; one could alternatively also assume that the positive and negative oscillatory “peaks” arithmetically average.

### 4.2.3.3 Atomic Force Microscopy (AFM)

Another direct method for measuring hydration forces is atomic force microscopy (AFM) (Fukuma et al. 2007; Higgins et al. 2006). Here, a ultrathin tip scans the sample perpendicular and planar along different hydration layers, comparable to the operation mode of a record player. Usually, a frequency modulation technique is applied (FM-AFM) and variations of the resonance frequency of the oscillating tip are directly correlated to local forces.

It is more difficult to categorise that experiment under the configurations mentioned under Sect. 4.2.2. This is due to the very local nature of the measurements being at molecular scale. But the set-up strongly resembles the iso-potential configuration (Configuration II) because pressurised water molecules under the tip can dynamically exchange with free water in the neighbourhood of the tip.

Already in 1995, Cleveland et al. reported “oscillatory” forces acting between water-filled mineral surfaces measured by AFM (Cleveland et al. 1995), comparable to the results obtained with surface force apparatus (see Sect. 4.2.3.2). But later on, the “oscillatory” hydration forces were also observed in the presence of lipids (Fukuma et al. 2007), in contrast to the SFA measurements where these oscillations are usually “smeared out” (Leckband and Israelachvili 2001).

Unfortunately, phospholipids obviously need to be in the solid state for in-depth analysis of force curves, otherwise the ultrathin tip breaks through the soft lipid-crystalline layer at a certain distance. The “breakthrough distance”, i.e. the distance between the tip and zero-distance at membrane disruption is a well-investigated parameter seriously limiting further exploration of hydration pressure under liquid-crystalline conditions (Butt et al. 2005). Finally, it was also reported that the chemical nature of the tip itself has a strong influence on the parameters of the obtained curves (Butt et al. 2005). Accordingly, it was also tried to coat the tip with a lipid bilayer which however seems to be a quite demanding procedure.

### 4.2.3.4 The Piezotropic Phase Transitions Method (PPM)

Another method for obtaining hydration pressure in bulk phases was proposed a couple of years ago (Pfeiffer et al. 2003a). It uses configuration I, i.e. a hydrostatic pressure is applied on a closed lipid dispersion. In contrast to AFM or the SFA method, the pressure does not act on supported lipid bilayers, but on non-oriented bulk layers.

Using the PPM, the shift of the main phase transition pressure of lipids is measured at different hydration degrees. For that purpose, the diamond anvil cell (DAC) can be used that is filled with lipids at known water concentration and spectroscopic pressure gauges, such as quartz (Wong et al. 1985) or BaSO<sub>4</sub>. The phase transition can be determined by FTIR spectroscopy making use of the pressure-tuned gauche – all-trans transition, detectable by the  $\nu$ -CH<sub>2</sub> stretching vibrations (Dunstan and Spain 1989; Spain and Dunstan 1989). The hydration-dependent shift of the hydrostatic phase transition pressure can be considered as

numerically equal to the hydration pressure (see derivation below). The approach requires almost no theoretical assumptions, the only problem is that the method requires a relatively sharp phase transition in a measurable range. In this context, it is quite difficult to determine the magnitude of the hydrostatic pressure sufficiently accurate because of spectroscopic parameters are used taken from the internal pressure gauges. The thermotropic method (Sect. 4.2.4.3) which is based on the same formalism partially establishes a solution to this problem because a scanning calorimeter can be used.

The presented piezotropic approach thus gives a simple relationship between hydration pressure and the shift of the main phase transition pressure. Let us consider a phospholipid/water dispersion existing in a two-state phase equilibrium, liquid crystalline phase,  $L_\alpha$  – gel phase,  $L_\beta$ . Water and lipid are considered as the two components of the dispersion. According to the equilibrium condition, the chemical potential of the lipid in the liquid crystalline phase must be equal to the chemical potential of the gel phase (Eq. 4.8).

$$\mu_{L,liq} = \mu_{L,gel} \quad (4.8)$$

From this follows (Eq. 4.9):

$$d\mu_{L,liq} = d\mu_{L,gel} \quad (4.9)$$

The relationship between the change of the chemical potentials of lipid and water,  $d\mu_L$  and  $d\mu_W$  is given by the Gibbs-Duhem relation (Eq. 4.10).

$$0 = n_L d\mu_L + n_W d\mu_W \quad (4.10)$$

which is applied at both the left and the right side of Eq. 4.9 (Eq. 4.11):

$$R_{w,liq} d\mu_{L,liq} = R_{w,gel} d\mu_{L,gel} \quad (4.11)$$

The change of the chemical potential can be expressed by the second terms of the corresponding Taylor series (Eq. 4.12).

$$d\mu = \left( \frac{\partial \mu}{\partial p} \right)_{a_w} dp + \left( \frac{\partial \mu}{\partial a} \right)_p da \quad (4.12)$$

The derivative of the chemical potential with respect to pressure is the molar volume,  $V_W$ , and the derivative with respect to the activity can be obtained from the well-known relationship,  $\mu_w = \mu_{w,0} + RT \ln a_w$ , which links the activity to the chemical potential (Eq. 4.13).

$$d\mu = V_W dp + RT d \ln a_w \quad (4.13)$$

A combination with Eq. 4.11 provides Eq. 4.14:

$$R_{W,liq} (V_{W,liq} dp + V_{W,liq} d\Psi_W) = R_{W,gel} (V_{W,gel} dp + V_{W,gel} d\Psi_W) \quad (4.14)$$

where  $\Psi_W$  is the isopiestic water potential. Rearrangement leads to Eq. 4.15:

$$(R_{W,gel} V_{W,gel} - R_{W,liq} V_{W,liq}) dp = - (R_{W,gel} V_{W,gel} - R_{W,liq} V_{W,liq}) d\Psi_W \quad (4.15)$$

From this follows Eq. 4.16:

$$dp = -d\Psi_W \quad (4.16)$$

The absolute values of the differential shift of the transition pressure and the isopiestic water potential are thus numerically equal (Eq. 4.17):

$$\int_{P_{re} (a_w=1)}^{P_{tr}} dp = - \int_0^{\Psi_W} d\Psi_W \quad (4.17)$$

Integration of Eq. 4.17 gives the relationship between the isopiestic water potential and the shift of the piezotropic phase transition pressure (Eq. 4.18):

$$P_{tr} - P_{tr,0} = \Delta P_{tr} = -\Psi_W \quad (4.18)$$

where  $P_{tr,0}$  is the transition pressure at full hydration (Eq. 4.19).

$$P_h = \Delta P_{tr} \quad (4.19)$$

Given the relationship between hydration pressure and isopiestic water potential, one can conclude that the hydration pressure is equal to the dehydration-induced shift of the main phase transition pressure,  $P_h = \Delta P_{tr}$  (Eq. 4.19).

## 4.2.4 Measuring Hydration Force: A Selection of Isopiestic Methods

### 4.2.4.1 Osmotic Stress Method (OSM)

One of the most frequently applied methods for adjusting hydration force is the so-called “osmotic stress method” (OSM). It replaces the technically more demanding piezotropic experiments (configuration II) by an isopiestic set-up (configuration III), enabled by the assumed equivalence of the configurations II and III, i.e. the isopiestic water potential is considered to be numerically equal to the piezotropic hydration pressure. This is a reasonable assumption proven by consistent results

(see also Sects. 4.2.3.4 and 4.2.4.3), but there also are some warning remarks on the methodological side mentioned later on.

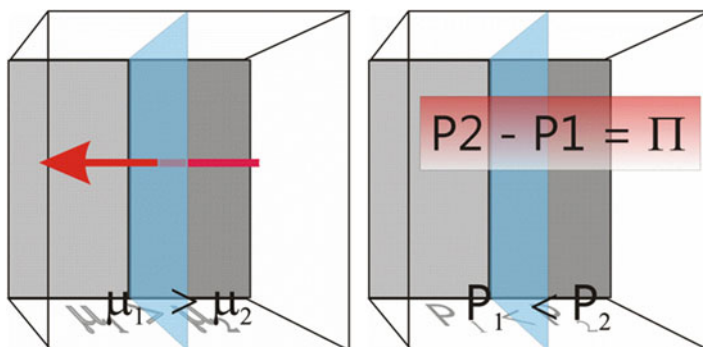
According to the OSM, the lipids are deposited in an isopiestic arrangement according to the configuration III. The water activity,  $a_w$ , is adjusted by a water vapour or by an osmotic solution containing inert solutes. The hydration pressure can be calculated using the well-known formula (Eq. 4.20):

$$P_h = -\frac{RT}{V_w} \ln a_w \quad (4.20)$$

where  $R$  is the gas constant,  $T$  the absolute temperature and  $V_w$  is the molar volume of water (Leneveu et al. 1977). If one determines a characteristic hydration quantity, such as the water content per lipid,  $R_w$ , or/and the water layer thickness,  $d_w$ , one obtains functional pairs of pressure and hydration. It is important to mention that the curves do not show oscillatory behaviour (see Sects. 4.2.3.2 and 4.2.3.3). This is explained by an averaging, or “smearing out” of these oscillations arising from lipid domains with different hydration degrees (Leckband and Israelachvili 2001).

It was implicitly suggested (Parsegian et al. 1986) that the experiments according to the configurations II and III are equivalent in the sense that the isopiestic water potential (Eq. 4.3) is numerically equal to the hydration pressure. However, the chemical potential of water in configuration II is constant at all hydration steps, in configuration III it is not. All properties that are correlated to the chemical potential are different as well. Thus, this approach is finally based upon the silent assumption that the underlying hydrophilicity of the hydrated substances is independent of the hydrostatic pressure. However, this needs to be checked in every case. One example is the wavenumber of the  $\nu$ -CH<sub>2</sub> stretching vibrations of POPC seen by FTIR spectroscopy (Pfeiffer et al. 2013b). With decreasing hydration, i.e. with increasing isopiestic “hydration pressure”, a reduction of the wavenumber, also called red-shift, is observed, but when applying hydrostatic pressure, blue shift is measured. Another example is reported by Di Primo et al. (DiPrimo et al. 1995) who have investigated the influence of “osmotic pressure” and hydrostatic pressure on the low-spin-high-spin transition for cytochrome 450. The authors found that both “pressures” induce antagonistic effects, i.e. hydrostatic pressure promotes the high spin to low spin transition and “osmotic pressure” promotes the low spin to high spin transition. But if one correlates that spin-transition with the water activity, there is indeed no antagonistic effect, because the water activity is increased at hydrostatic pressure and decreased by “osmotic pressure”. The “antagonistic effect” in this view appears less mysterious. Indeed, it has been correctly suggested by Di Primo et al. that hydrostatic pressure and “osmotic pressure” probe different properties of the transition. Therefore, care has to be taken, at least when using spectroscopic quantities to derive hydration force parameters, see also Sect. 4.2.4.2.

The quantity “hydration pressure” in the configuration II (Rand and Parsegian 1989) is thus used as a synonym for the isopiestic water potential,  $\Psi_w$ . To show the difference, let us consider the basics of osmosis.



**Fig. 4.7** Osmosis (*left*), a diffusion driven flux due to the gradient of the chemical potential. Osmotic pressure (*right*), the hydrostatic pressure to stop or prevent osmosis

The net flux of solvent molecules from one solution into another caused by different concentrations of solutes is called osmosis (Fig. 4.7). This flux in the direction of the solution with the higher concentration is driven by diffusion. The concentration difference is maintained by a selective diffusion barrier (semi-permeable membrane) that avoids the exchange of solute molecules. Osmotic pressure,  $\Pi$ , is defined as the smallest hydrostatic pressure that is required to stop or prevent osmosis (i.e. a kind of compensation pressure), i.e. the net flux of solvent. In other words, the hydrostatic excess pressure ( $\Pi = P_2 - P_1$ ) which must act on the higher concentrated solution (phase 2) to enable the chemical equilibrium with the less concentrated solution (phase 1 = reference phase) is called osmotic pressure. If the molar volume,  $V_w$ , of water can be considered to be constant, one can write Eq. 4.21:

$$\mu_1 = \mu_2 + V_w (P_2 - P_1) \quad (4.21)$$

The osmotic pressure is then defined by Eq. 4.22:

$$\Pi \stackrel{def}{=} P_2 - P_1 \quad (4.22)$$

The most typical feature of real osmotic pressure experiments is that these are piezotropic experiments, there is a physical hydrostatic pressure present and that the chemical potential of water is kept constant by a membrane and a reference solution.

The concept of “osmotic stress” have not only been applied to lipid hydration, but also to biochemical reactions to determine the amount of water that is exchanged at the reaction (Rand et al. 1993). This is important if the biomolecules (enzymes etc.) are situated in a solution which contains inert co-solutes.

A sometimes overlooked limitation of Eq. 4.20 regards the value of the molar volume of water. Due to change of the water structure it is unlikely that the density

is a constant, i.e. the molar water volume starts deviating substantially from the bulk property (Scherer 1987). From this follows that absolute data on the hydration pressure in the range of  $R_w = 1$  are most probably overestimated (Pfeiffer et al. 2003b).

#### 4.2.4.2 Nuclear Magnetic Resonance (NMR): Quadrupolar Splitting and Relaxation Time

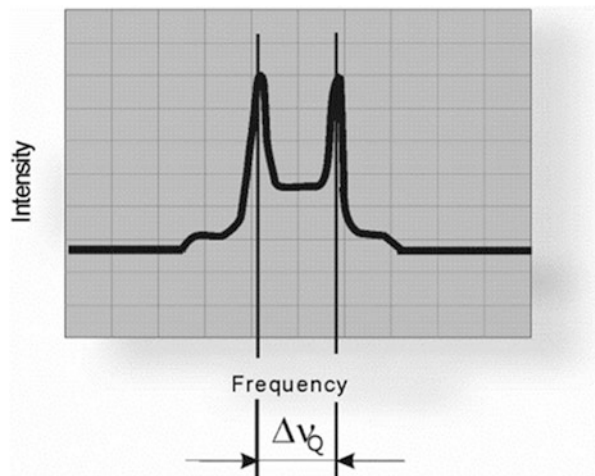
Also here, the isopiestic configuration was used (configuration III), but the method is probably not limited to this boundary condition.

A typical feature of the hydration force function is that it is exponentially decaying and such behaviour is also seen for other physical quantities at hydration. In this sense, one can argue that all hydration-tuned exponentially decaying parameters have a more or less direct correlation with the hydration force, or at least a correlation with its common root cause. An interesting example is the logarithm of the hydration-dependent shift of the lateral diffusion in phospholipid monolayers (Baumgart and Offenhäusser 2002) that has a linear correlation with the water activity. It was accordingly shown that a measurement of the lateral diffusion would provide at least an estimate of hydration force parameters.

An exponential behaviour is also seen for various NMR parameters, it has e.g. be shown that the quadrupolar splitting of  $D_2O$ ,  $\Delta\nu_Q$  that is correlated to the average orientation of water molecules (Fig. 4.8), is linearly correlated with the water activity, such as reported by Volke et al. (1994a). The corresponding equation is Eq. 4.23:

$$a_w(n_w) = \frac{\Delta\nu_Q - A}{B} \quad (4.23)$$

**Fig. 4.8** Highly idealised representation of quadrupolar splitting spectra





The coefficients  $A$  and  $B$  are free variables which depend on the nature of the lipid. From this observation, the hydration pressure can easily be derived by Eq. 4.24 making use of Eq. 4.20.

$$P_h(n_w) = \left(-\frac{RT}{V_w}\right) \ln\left(\frac{\Delta v_0 - A_0}{B}\right) \quad (4.24)$$

The method of Volke was also applied to mixtures composed of lipids and non-ionic surfactants. Although, the linearity stated by Eq. 4.23, is slightly disturbed, most probably due to admixture effects (Pfeiffer et al. 2010), the parameters  $A$  and  $B$  are very similar to the original values published before.

An important limitation of this method should be mentioned; the quadrupolar splitting is reduced at and in the range of the main phase transition, a phenomenon which is called motional narrowing (Bryant et al. 1992). The motional narrowing proceeds over a relatively wide temperature ranges (10–20 K) and its temperature dependency can mathematically be described by Eq. 4.25 (Hawton and Doane 1987),

$$\Delta v_0 \propto \sqrt{|T - T_0|} \quad (4.25)$$

where  $T_0$  is the main phase temperature. Motional narrowing is not yet completely explored, but one assumes that during domain formation at phase transitions, the average preferential water orientation within the lipid layer is partially lost. According to Eq. 4.25, the quadrupolar splitting can even disappear at the phase transition temperature  $T_0$  and the water behaves apparently as free water! in the time window of the NMR experiment. However, also in the case of phase transitions, hydration forces are measured and almost no corresponding abnormal loss or uptake of water can be detected (Pfeiffer et al. 2013a). This points to the fact that this NMR parameter only correctly reflect hydration force parameters when no phase transitions are “in the neighbourhood”.

An analogous formulae (Eq. 4.26) for the water activity (see Eq. 4.23) was proposed by Ulrich et al., but most interestingly, here applied to the CD<sub>2</sub>-groups of the headgroup of lipids (Ulrich and Watts 1994a),

$$a_w(n_w) = \left(\frac{f(n_w) - f_0}{f_s - f_0}\right) \quad (4.26)$$

where  $f$  denotes the generalised NMR parameters investigated, the  $T_1$  relaxation times of the CD<sub>2</sub> segments and the respective quadrupolar splitting,  $\Delta v_0$ . In this sense, it was consequently argued that NMR directly reflects the sorption isotherms of lipids. The equivalence of Eqs. 4.24 and 4.26 is of high importance because they show how order parameters of lipid and water are coupled, see also Ge and Freed 2003.

Also the  $T_1$  spin-lattice relaxation time of the hydration water D<sub>2</sub>O can directly be related to hydration phenomena (Eisenblätter et al. 1994). It was reported that

the mean spin-lattice relaxation time of water in the neighbourhood of phospholipid membranes is an averaged value which is composed of the different relaxation times at different positions  $x$ , it is in the range of milliseconds. The average value can be described by the following equation, Eq. 4.27:

$$\frac{1}{T_{1,N}} = \frac{1}{N} \sum_{x=1}^N \frac{1}{T_1(x)} \quad (4.27)$$

The approach is based a simple two-state model for the spin lattice relaxation time  $T_1$  assuming fast exchange between water binding sites. This model assumes two states of water, the bound state (b) and the unbound state (u). This approach resembles the assumption of a Langmuir VI isotherm where a first hydration layer is considered to be bound and the remaining one are considered to be free solvent molecules. For the spin lattice relaxation times, one finds that the relaxation of the bound water molecules are smaller than that of the unbound molecules ( $1/T_{1,b} > 1/T_{1,u}$ ). The reason is the fixation of the water molecules at the surface of the amphiphiles which enables a faster spin lattice relaxation.

The Hamiltonian for a water molecule in the state  $i$  is given by Eq. 4.28:

$$H_i(N) = \left( \Delta E - \frac{E_S}{N} \right) \sigma_i + const. \quad (4.28)$$

where  $\Delta E$  is the difference for the energy between the bound and the unbound state, and  $E_S$  the free enthalpy of hydration. The ISING-variable,  $\sigma_i$ , is set to be 1 for the bound state and 0 for the unbound state. From Eq. 4.28 one obtains Eq. 4.29,

$$\frac{1}{T_{1,N}} = \frac{1}{Z} \sum_{i=b,u} \frac{1}{T_{1,i}} \exp \left( -\frac{H_i(N)}{k_B T} \right) \quad (4.29)$$

where  $Z$  is the canonical partition function of the molecule. The final expression as published by the authors (Eisenblätter et al. 1994) is (Eq. 4.30):

$$\left( \frac{T_{1,N} - T_{1,b}}{T_{1,b}} \right) \approx \left( \frac{T_{1,F} - T_{1,b}}{T_{1,b}} \right)^{1 - \frac{n_c}{N}} \quad (4.30)$$

The relaxation time of free water is given by  $T_{1,F}$  and the constant  $n_c$  is defined by  $n_c = E_S/\Delta E$ . However, the final expression obtained from the above mentioned paper can be rewritten by using the simple relationship, Eq. 4.31:

$$a^x = e^{x \ln a} \quad (4.31)$$

In this picture, the reciprocal mean spin-lattice relaxation time  $T_{1,N}$  is given by Eq. 4.32:

$$\frac{1}{T_{1,N}} = \frac{1}{T_{1,b}} \frac{1}{\exp\left(\frac{\Delta E - E_S/N}{k_B T}\right) + 1} \quad (4.32)$$

The previous equation (Eq. 4.32) shows that the relaxation time is described by an expression containing the difference of the energy states between the bound and unbound states,  $\Delta E$ , as well as the averaged hydration Energy,  $E_S$ . Interestingly, the previous equation (Eq. 4.32) is formally analogous to the FERMI-DIRAC distribution. It represents the energy distribution of the fermions depending on the FERMI-energy,  $E_F$ . The original FERMI-DIRAC distribution is given by Eq. 4.33:

$$f(E) = \frac{1}{\exp\left(\frac{E - E_F}{k_B T}\right) + 1} \quad (4.33)$$

The original FERMI-energy,  $E_F$ , corresponds thus to the energy level between bound and unbound states and the energy,  $E$ , corresponds formally to the expression  $E_S/N$  in Eq. 4.32.

The analysis of hydration dependent spin-lattice relaxation times have thus been proposed as a tool for the characterisation of surface energy conditions of amphiphile/water systems (Eisenblätter et al. 1994). The approach established in Eq. 4.32 was in this way applied as a tool for the determination of the binding energy of water in phospholipids and non-ionic surfactants (Eisenblätter et al. 1994; Klose et al. 1995b). The value for POPC and C<sub>12</sub>E<sub>4</sub> have been given as  $E_S = 28 \pm 4$  kJ/mol and  $E_S = 24 \pm 6$  kJ/mol. However, the sorption isotherm of POPC and C<sub>12</sub>E<sub>4</sub> shows that the hydration behaviour is strongly different. The free enthalpy of hydration is only about  $\Delta G = 4$  kJ/mol instead of 24 kJ/mol. The fitting according to Eq. 4.32 thus furnishes strongly overestimated values in the case of non-ionic surfactants. There are at least two probable reasons. The quantity  $E_S$  does not correspond to the free enthalpy of hydration, or the assumptions for Eq. 4.32 are too simplified. However, the qualitative behaviour according to the FERMI-DIRAC distribution remains an interesting fact and should be further investigated.

#### 4.2.4.3 The Thermotropic Phase Transition Method (TPM)

This relatively direct method for obtaining hydration pressure curves was proposed in parallel with the piezotropic phase transition method, PPM, (Sect. 4.2.3.4) and it is based on the pressure-tuned variation of the main phase transition temperature in phospholipids (Pfeiffer et al. 2003b). Its advantage is that it can be performed under isopiestic conditions, such as the OSM method (Sect. 4.2.4.1).

Different approaches has been proposed in the past for using thermotropic phase transitions in lipid/water dispersions to obtain hydration pressure parameters. The approach of Ulrich et al. (Disalvo et al. 2008), demonstrated on DOPC, is based on the freezing point depression of the hydration water. Interestingly, the same approach was introduced by Bach et al. (1982) already in 1982, but in their case

referred to “swelling pressure”. The hydration pressure as a function of water content is given by Eq. 4.34 (Ulrich et al. 1994):

$$P_h = \frac{\Delta H_{tr,W}}{V_W} \left( 1 - \frac{T(R_W)}{T_0} \right) \quad (4.34)$$

where  $\Delta H_{tr,W}$  is the molar enthalpy change of melting ice,  $V_W$  the molar volume of liquid water,  $T(R_W)$  the hydration dependent melting temperature and  $T_0$  the melting temperature of pure water. The application of that approach provided a sound result for DOPC. However, for the hydration pressure at zero hydration one obtains  $\Delta H_{tr,W}/V_W$  implying that the freezing temperature would approach zero Kelvin at dehydration, and that the pressure at dehydration is independent of the lipid species, the last outcome clearly contradicts other results from the literature.

Cevc and Marsh (Cevc and Marsh 1985) proposed an equation relating the shift of the main phase transition temperature of lipids to the degree of hydration,

$$\Delta T_m = \Delta T_m(0) \tanh \left( \frac{n_W V_W}{\lambda A} \right) \quad (4.35)$$

but experiments by Simon & McIntosh (Simon et al. 1991) could not confirm that approach experimentally, it was concluded that calorimetric investigations of the thermotropic phase transition of the lipid might only give qualitative information on hydration processes. Another equation (Eq. 4.36) based on the same formalism relates the shift in phase transition temperature to the water potential,  $\Psi_w$  (Cevc 1987),

$$\Delta T_m = \frac{A\xi}{100\Delta S_{anh,t}} \Psi_w \quad (4.36)$$

where,  $A\xi$  is a length characteristic of the water structure and  $\Delta S_{anh,t}$  the transition entropy of the lipid in the dehydrated state. But, because the derivation follows the same formalism as for Eq. 4.35 the conclusions in Simon et al. 1991 also apply here.

However, when skipping intrinsic hydration force theories and applying fundamental thermodynamics, it is nevertheless possible (Pfeiffer et al. 2003b) to obtain quantitative information on hydration force from the shift of the main phase transition temperature. The underlying physics is based on the Gibbs-Duhem equation for linking hydration pressure and water potential, as well as on the Clausius-Clapeyron equation relating the pressure variations to the variations of the main phase transition temperature.

The hydration pressure parameters were determined for various lipids by differential scanning calorimetry, and the only assumption is the approximate constancy of the pressure-induced shift of the phase transition temperature ( $\alpha \approx 0.2$  K/MPa), which is valid for almost all compounds containing long hydrocarbon chains (Pfeiffer et al. 2003b, c).

The derivation of the relationship between the main phase transition temperature and hydration pressure thus essentially continues the formalisms for piezotropic transitions (Sect. 4.2.3.4). The relationship between the differential shift of the piezotropic phase transitions,  $dP_{tr}$ , and the water potential,  $d\psi_w$ , was given by Eq. 4.37:

$$dP_{tr} = -d\Psi_W \quad (4.37)$$

After an infinitesimal shift of temperature,  $dT$ , a new equilibrium is established. The dependence of the transition pressure,  $P_{tr}$ , on the temperature is given by the equation of Clapeyron-Clausius (Eq. 4.38), which is also valid for main phase transitions of phospholipids (Winter and Pilgrim 1989):

$$\frac{dT}{dP} = \frac{\Delta V_{L,tr}}{\Delta S_{L,tr}} \quad (4.38)$$

$\Delta V_{L,tr}$  and  $\Delta S_{L,tr}$  are the volume change and the entropy change of the lipid. A comparison of Eqs. 4.37 and 4.38 shows that the dependence of water potential on the temperature is simply given by Eq. 4.39:

$$\frac{dT}{d\Psi_W} = -\frac{\Delta V_{L,tr}}{\Delta S_{L,tr}} \quad (4.39)$$

From Eq. 4.39 thus follows a simple method for the determination of hydration pressure by using calorimetry. Taking the definition of the pressure-induced temperature increase,  $\alpha$ , given in Eq. 4.40:

$$\alpha = \frac{\Delta V_{L,tr}}{\Delta S_{L,tr}} \quad (4.40)$$

one can rewrite Eq. 4.39. The crucial point is thus the knowledge of the pressure-induced temperature increase  $\alpha$  as a function of temperature and hydration. One obtains Eq. 4.41 which gives the relationship between phase transition temperature and water potential:

$$dT_{tr} = -\alpha d\Psi_W \quad (4.41)$$

Thus, using the relationship between water potential and hydration pressure ( $P_h = -\Psi_w$ , see Sect. 3.3.3) one obtains Eq. 4.42:

$$dP_h = \frac{1}{\alpha} dT_{tr} \quad (4.42)$$

From Eq. 4.42 follows finally Eq. 4.43:

$$P_h = \frac{1}{\alpha} \Delta T_{tr} \quad (4.43)$$

where  $\Delta T_{tr}$  is the shift of the transition temperature with respect to the fully hydrated state. Furthermore, it is important to realise that  $\alpha$  is nearly a constant ( $\alpha \approx 0.2$  K/MPa (Winter and Pilgrim 1989; Pfeiffer et al. 2003c)) for about all phospholipids, because it is mainly determined by the ratio of the volume and entropy change per  $\text{CH}_2$ -segment in the hydrocarbon chain, in this way it is also not a function of hydration itself because it is even invariant concerning the use of other solvents (Pfeiffer et al. 2003d). This finally enables an estimation of hydration pressure in phospholipids when the dehydration-induced temperature shift of the main phase transition is known, i.e.  $P_h \approx 5 \Delta T_{tr}$  with  $[P_h] = \text{MPa}$  and  $[\Delta T_{tr}] = \text{K}$ .

Under Sect. 4.2.4.1 there were some critical remarks concerning the statement that “osmotic pressure”, i.e. isopiestic water potential and the hydration pressure are numerically equal. But the consistent results offered by the piezotropic and thermotropic methods, Sects. 4.2.3.4 and 4.2.4.3 give essentially a broad confirmation that this is more or less granted.

The phase transition methods (see also Sect. 4.2.3.4) should be applied to pure lipid species. In the case of mixtures, the phase transitions becomes broad and non-ideal interactions, demixing processes occur, this is even partially a problem in one-component lipid systems, i.e. transitions become broader also in this case (Pfeiffer et al. 2013a). However, this is in fact a general problem for all methods as such. When dehydration is investigated in arbitrary lipid systems by any of these methods, admixture effects will always influence the observed force curves because also the lipid water dispersion is a highly non-ideal binary mixture, see also Sect. 4.1.2.

### 4.3 Hydration Force Theories

A number of theories for exploring the nature of hydration force have been proposed within the last three decades. They can roughly be systematised into two categories, i.e. some theories focus on the influence of the surface, and others see a dominant influence coming from the solvents. The final truth will be that both parts will play a role because a replacement of the solvent on the one hand, but also the change of the lipid moieties on the other hand has remarkable influence on hydration force parameters.

#### 4.3.1 *The Langmuir VI Sorption Isotherm*

Usually, theories on absorptions isotherms, such as the Langmuir VI isotherm are mostly not considered as a hydration force theories. On the other hand, due to the dominating opinion that the osmotic stress method is a representation of hydration force, one must conclude that any analytical theory on absorptions is also automatically a “candidate” for a hydration force theory (Marsh 2011). In turn, also hydration force theories were applied for theories on absorption isotherms (see also (Marsh 2011; Klose et al. 1992)).

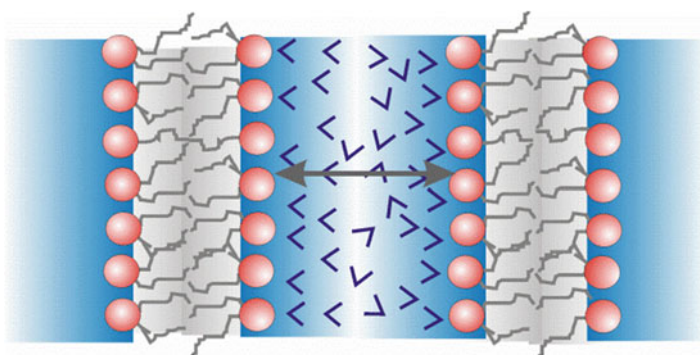
The Langmuir theory assumes a multilayer arrangement of water molecules above the absorbent and the first layer is per definition considered as bound water. Depending on the boundary conditions, the subsequent water layers belong to a free water phase, or they occupy binding sites with energies following a Boltzmann-type distribution. Many lipid isotherms fit with the Langmuir VI model, but the physical meaning of the free parameters is not always clear (König 1993).

### 4.3.2 The Polarisation Theory

The polarisation theory was originally introduced by Marčelja and Radic in 1976 (Marcelja and Radic 1976) and improved by Gruen and Marčelja in 1983 (Gruen and Marcelja 1983). The theory, based on a Landau expansion of the free energy, regards water dipoles as oriented in a confined space, showing in this way a preferential average dipole orientation (Fig. 4.9) leading to a net polarisation. With progressing surfaces approach, it is required to gradual rearrange these orientations and the entropy-based resistance against those re-orientations is supposed to be the origin of the hydration force. Refinements of that theory tried e.g. to include the explicit influence of the polarity of the surface, to modify the boundary conditions concerning the width of the polar surface and to consider non-local polarisations.

A concluding analytical expression, additionally including ideas from the Gouy-Chapman theory, was given by Cevc (1987). For the hydration pressure  $P_h$  as a function of the water layer thickness one obtains equation Eq. 4.44 (König 1993):

$$P_{pol}(x) = \frac{\chi \Pi_{h0}}{\xi} \exp\left(-\frac{x}{\xi}\right); P_h(d_W) = \frac{2\chi \Pi_{h0}^2}{\xi^2} \exp\left(-\frac{d_W}{\xi}\right) \quad (4.44)$$



**Fig. 4.9** A polar bilayer surface is the reason for a preferential dipole orientation (presented as hooks) which is supposed to be the reason of a repulsive force

The variable  $x$  is the distance between the surface and the middle of the water layer ( $2x = d_w$ ), the water structure susceptibility is given by  $\chi$  and the parameter  $\xi$  and  $\Pi_{h0}$  represent a length characteristics of the solvent structure and the bilayer hydration potential at surface contact.

A direct experimental support of the polarisation theory, probably overlooked so far, is the preferential orientation of water molecules with respect to the lipid layer measured by nuclear magnetic resonance (see also Sect. 4.2.4.2). The quadrupole splitting  $\Delta\nu_0$  of  $D_2O$  is related to the order parameter,  $S$  by the well-known equation,  $\Delta\nu_0 = \frac{3}{4} \kappa S$ , where  $\kappa$  is the quadrupole coupling constant for deuterated water (Volke et al. 1994b; Gawrisch et al. 1978). The order parameter  $S$  is directly related to the angle between O-2H bond and the membrane,  $S = 1/2 (3 \cos^2 \Theta - 1)$  and with it to the net polarization. One can see that there is a simple relationship between the experimentally determined water orientation and the reduced water activity, resp. enhanced hydration pressure (Eq. 4.23).

$$a_w = \frac{\frac{3}{8} \kappa (3 \cos^2 \Theta - 1) - A}{B} \quad (4.45)$$

Interestingly, exponentially decaying orientation angles with respect to the bilayer distance were obtained from computer simulations (Marrinck and Berkowitz 1995) and so it appears that the NMR results finally experimentally confirm polarisation effects. A final question is whether a measurement in the NMR time domain can represent a thermodynamic equilibrium or is it just a snapshot in the time window of the experiment.

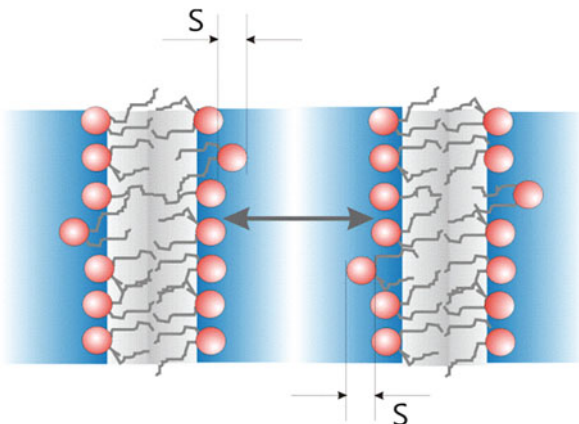
A further interesting and puzzling aspect related to the polarisation theory was found by Binder et al. (1999b). When adding water to lipids at room temperature, an endothermic reaction is observed when carefully applying titration calorimetry. The endothermic nature of the hydration process shows that it is under these conditions essentially an entropy-driven process. Obviously, hydration enables an enhancement of the configurational space for additional degrees of freedom in water populations and most probably also in lipids (Ge and Freed 2003). The entropic nature of the interactions was also pointed out by other authors before (Marrinck and Berkowitz 1995) and does also apply to the protrusion theory, see below.

### 4.3.3 *The Protrusion Theory*

There is sufficient experimental evidence that there are a number of interactions arising from thermally excited motions of membrane components (Gordeliy et al. 1996), usually referred to as entropic forces. Wavelike membrane fluctuations are known as undulations, thickness variations of the bilayers are called peristaltic movements (Helfrich 1978) and thermally excited out-of-plane movements of isolated membrane components are the so-called protrusions (Table 4.1).



**Fig. 4.10** Single-molecule displacements of membrane components are supposed to be the origin of hydration force according to the protrusion theory. The structure of the water phase is neglected



The concept presented by Israelachvili and Wennerström states that protrusions are the dominant origin of hydration pressure in phospholipids. Protrusions are indicated in Fig. 4.10 by a displacement of single lipid out of a membrane by the distance  $S$  ( $S = \Delta x$ ). It was stated that the protrusion energy  $V(x)$  of one amphiphile molecule is a linear function of the displacement (Aniansson 1978) (Eq. 4.46).

$$V(x) = \alpha x \quad (4.46)$$

Here, the coefficient  $\alpha$  is assumed to be the product of the average lateral dimension of an average protrusion movement and the surface energy, i.e.  $\alpha = \pi \sigma \gamma$ . The steric repulsion due to the “collisions” of protruding amphiphiles are thus considered as the origin of the hydration pressure and because protrusion is assumed to be governed by the Boltzmann distribution, its exponential term is finally responsible for the exponential decay of hydration pressure. The corresponding expression is given by Eq. 4.47,

$$F \approx 2,7 n \alpha \exp\left(-\frac{\alpha}{kT} \lambda_w\right) \quad (4.47)$$

where  $n$  denotes the binding sites per surface unit.

Critical comments (Parsegian and Rand 1991) referred e.g. to contradictions between the probability of protrusion movements and the solubility of the membrane components. If the protrusion theory were valid, the solubility should be larger by four orders of magnitude. Another point of criticism is the difference between the hydration pressure parameters found in membranes with solid and melted hydrocarbon chains. There is almost no such a difference, as one should expect on the basis of the protrusion theory. In the same sense, there should be a difference between the hydration pressure for membranes with one and two hydrocarbon chains, but there is not. Furthermore, addition of substances that changes the stiffness of the membrane (e.g. cholesterol) has almost no influence on hydration

pressure. But hydration pressure does occur in polyelectrolytes, something which should be impossible according to Israelachvili and Wennerström. A methylation of the head group also dramatically changes the hydration pressure parameters. Furthermore, it was criticised that it was also forgotten to include the free energy created by the free volume in the hydrocarbon core during the protrusion movement.

There were also attempts to provide experimental support for the protrusion theory as well. Gordeliy (1996) proposed to use neutron and X-ray diffraction to confirm the existence of protrusion motions by relating the out-of plane movements to measurable repeat distances. From the experimental proven increase of inter-bilayer distances and the observed loss of Bragg peak intensity (Gordeliy et al. 1996) one concluded enhanced entropic motions, including protrusion and undulation. The final question is however whether these findings can prove the validity of the protrusion theory as such because it was found by Simon et al. that the short-ranging hydration repulsion does barely vary with the temperature (Simon et al. 1995), a result which would be in contradiction with the findings of Gordeliy, at least for the short-ranging part of the interaction which is assumed to be uninfluenced by undulations and peristaltic movements.

The ultimate decision on the protrusion theory was most probably given by Binder et al. (1999c). In their study, the hydration pressure parameters for polymerised and non-polymerised lipid bilayers were compared. It was found that polymerised lipids in the liquid crystalline phase show about the same hydration pressure parameters than the liquid-crystalline bilayers composed of non-polymerised lipids. In the case of the protrusion model however, the hydration pressure for non-polymerised lipids should be much stronger because the lipid molecules are not bound by covalent bonds such as in the case of the polymerised lipids.

Another study reported hydration pressure parameters for non-ionic surfactants and it was shown that the hydration pressure parameters are independent of the chain length (see parameter  $\alpha$  in Eq. 4.46), and that they only vary with the polarity, i.e. in that case with the size of the head groups (Pfeiffer et al. 2004).

## 4.4 A Possible New Look on Water in Membranes

### 4.4.1 *Recent Developments in the Literature*

Finally, the debate on the validity of polarisation or protrusion models is not yet decided (Gordeliy et al. 1996; Binder et al. 1997) but it seems that the arguments for the polarisation model are at least less disputed.

It appeared that at the end of the 1990s, the big discussions on this topic were fading out, most probably because of a lack of new experimental results and inspiration. In the last years however, for instance enhanced computer power and subsequent in-silico studies enabled a renewed debate on hydration force (Zemb and Parsegian 2011). Besides support for one of the existing theories, new approaches emerge. This includes ideas that hydration repulsion only occurs in specific cases

(Schneck et al. 2012) or that hydration pressure might even be a phantom because interactions, such as Van der Waals and steric repulsion are already wrongly applied, i.e. “However if we subtract the predictions of two incorrect theories from a perfectly good measured force curve, we have in fact not a hydration force, but rather, non-sense” (Kunz et al. 2004). An argument in this direction is provided by the work of Freund who e.g. questioned the force law for undulation repulsion (see Table 4.1). Referring to this result, Sharma expressed that the debate “will hopefully encourage design of further experimental work” (Sharma 2013).

All by all, most of the knowledge on hydration force is still empirical, the satisfactory theory thus still needs to be found and there are essentially also not enough experimental results available (Parsegian and Zemb 2011).

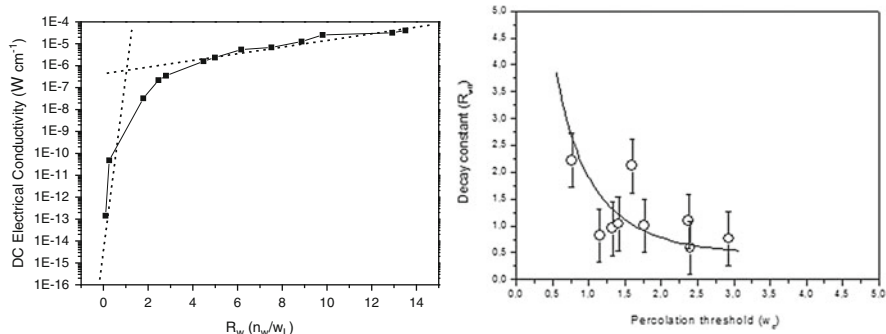
## 4.5 Percolation Theory and the Decay Constants of Hydration Pressure

Jendraziak and co-workers (Jendraziak and Smith 2004) reported studies on electrical conductivity and hydration in diverse phospholipid membranes investigated as a function of the humidity. Dehydrated lipids are essentially non-conductors, however, Jendraziak and co-workers obtained curves showing a huge increase of the electrical conductivity at relatively low humidity indicating the establishment of so-called percolation networks. This means that the first emerging water networks already enable a certain electrical conductivity that is strongly increasing with water content. Here, the continuous transfer of protons plays the major role explaining macroscopic conductance effects.

Conductance by percolation is essentially a statistical phenomenon i.e. it rises with the probability of mutual contacts between conductive domains in mixtures. For the conductive state, the percolation theory (Essam 1980) predicts a power-law behaviour for the conductance,  $\sigma$ , as a function of the fraction of the conductive component (Eq. 4.48) where  $X$  is the concentration of the statistically distributed conductive component with the percolation threshold  $X_c$ . The exponent is  $\mu$ , a semi-universal constant that also depends on the nature of the matrix;  $\sigma_{cc}$  is the conductivity of the pure conductive component.

$$\sigma = \sigma_{cc}(X - X_c)^\mu \quad (4.48)$$

Interestingly, Jendraziak et al. never explicitly referred to the percolation theory, at least not to the best of our knowledge. A simple fit also shows that the curves obtained does not support percolation models (Eq. 4.48) established for simple two- or three- dimensional systems indicating that not only statistics is determining the network establishment and that lipid hydration also might proceed in fractal dimensions. Nevertheless, this phenomenon is a percolation process by definition and one can easily define a percolation threshold for most of the experimental curves available (Fig. 4.11, left) which can be further plotted versus the exponential decay constant (Fig. 4.11, right).



**Fig. 4.11** Example of the arbitrary definition of a percolation threshold based on the data of Jendraziak et al. and relationship between decay constant as a function of the percolation threshold for a couple of different phospholipids. The *line* is just a guide to the eyes

The question is thus whether the percolation threshold has direct or indirect relationships with the hydration pressure parameters. An analysis based on the data of Jendraziak (Fig. 4.11, right) proposes that there is at least a slight trend, i.e. the decay constant of the exponential hydration force function decreases with increasing percolation threshold. This would mean that an early-established continuous water phase makes the hydration force more far-reaching. In any case, even if the trend would be neglected, reasonable regarding the error bars (all data are situated around  $R = 2 \pm 1$ ), one can safely state that the decay constant is in fact/also that hydration number marking the creation of a continuous water phase.

The fact that percolation thresholds are not a constant also points to the importance of considering fractal dimensions of lipid surfaces. This easily arises from the fact that for a perfect flat surface the percolation threshold should be a constant and it should be possible to describe it by simple percolation models, which is in fact not the case.

In this “percolation-view”, the decay constant would be rationalised concerning its relationship with the creation of a liquid phase out of single molecules, however, in most cases the hydration force is modelled starting from slight perturbations of water molecules within the liquid phase. Finally, relating the percolation threshold to the decay constant does not yet provide an exponential decay function. Here, further theoretical work would be required.

## 4.6 Water Is a Plasticizer

A plasticizer is a chemical additive that makes a material softer, more flexible and ductile. Accordingly, when water is absorbed in solid, dehydrated phospholipids, it will act as a so-called “external plasticizer”, i.e. after gradual ingress of the solvent, the mobility and deformability of the membrane strongly enhances; however, which is essential for plasticizers, without challenging the global integrity of the bilayer as such. This typical behaviour is achieved by the fact that the samples change

their mechanical properties when exposed to the solvent, but there is always a limiting factor preventing dissolution. This limiting factor arises e.g. from covalent polymerisation, such as in starch (Li et al. 1996; Heremans et al. 2000) or by the entropic effect ensuring lamellar stability of lipid bilayers in water (Ben-Shaul 1995). An interesting analogue is the gelation temperature in starch that shows a typical decay function on hydration, similar to the case of the main phase transition of phospholipids.

The fact that water is a plasticizer could in a certain sense be a source of inspiration for describing and understanding hydration force in lipids. There are essentially four plasticiser theories, the lubrication theory, the gel theory, the free volume theory and the mechanistic theory of plasticization (Daniels 2009). When compared to the hydration force theories, the mechanistic theory of plasticization is the closest one because it considers solvent molecules exchanging between different binding sites. Furthermore, also the free volume theory applies in a certain sense, especially when one considers that water acts like a spacer when entering the headgroup region of lipids forcing free volume in the hydrocarbon chains leading to liquid phases. Finally, it is very likely that hydration, resp. solvation force theories could in turn also apply in plastification theories.

## 4.7 Summary

There are arguments supporting the polarisation or protrusion theory but also reasonable arguments against them. This means that the final theory still needs to be found and/or that nature is too complex preventing a generalised solution. It might even be allowed to ask whether it is reasonable to talk about surface forces if the structural roughness of the surface itself exceeds the size of the solvent molecules and the decay length is also of the same order. Recent computer simulations seem to confirm some of these considerations leading to conclusions such as “hydration repulsion is less universal as previously assumed” (Schneck et al. 2012). It was even stated that hydration force might be a phantom because Van der Waals and steric repulsion models might be already wrongly applied, i.e. “... if we subtract the predictions of two incorrect theories from a perfectly good measured force curve, we have in fact not a hydration force, but rather, non-sense” (Kunz et al. 2004).

As a summary, one can state that what we call hydration force is observed from stiff mica surfaces up to liquid-crystalline lipid membranes indicating that the dynamics of the hydrocarbon chains has minor influence compared to water structure and surface polarity. Furthermore, the essentially endothermic nature of hydration detectable by titration calorimetry indicates an entropic origin of hydration force. Finally, the spectroscopically confirmed preferential water orientation could lead to the conclusion that hydration pressure is driven by the change of the configurational space of water molecules turning from an relatively disordered 3 dimensional bulk phase into a differently ordered intermembrane phase.

As a final summary, Table 4.3 gives a short overview to the most important facts on hydration force.

**Table 4.3** Fact sheet on hydration force in phospholipid layers

Topic	Selection
Nomenclature and/or synonyms	Hydration force Hydration pressure Hydration repulsion (Schneck et al. 2012) Swelling pressure (Bach et al. 1982) Osmotic pressure
Common characteristic	Exponentially decaying surface force with a relatively uniform decay constant (0.2–0.4 nm), the pre-exponential factor can however differ considerably Cannot be modelled by as a superposition of Van der Waals, undulations or steric interactions $\lambda_w \approx 0.2\text{--}2$ nm
Typical range in terms of water layer thickness	Pressure gauge in solution under osmotic pressure (Leneveu et al. 1976)
Methods and models for determining/demonstrating hydration force	Deflected spring within the surface force apparatus (SFA) (Marra and Israelachvili 1985) Modulated tip oscillations in atomic force microscopy (AFM) (Fukuma et al. 2007) Adjusted chemical potential in osmotic stress method (OSM) (Parsegian et al. 1979) Main phase transition pressure of lipids (PPM) (Pfeiffer et al. 2003a) Main phase transition temperature of lipids (PTM) (Pfeiffer et al. 2003b) Melting temperature of hydration water (Ulrich et al. 1994) NMR Quadrupolar splitting in lipid (-CD) and/or water (D <sub>2</sub> O) (Volke et al. 1994a) NMR relaxation times of lipids (Ulrich and Watts 1994b) Diffusion of lipids by fluorescence microscopy (Baumgart and Offenhäuser 2002)

Selection of methods for water layer thickness <sup>a</sup> determination	X-ray, using the Luzzati model (Luzzati and Chapman 1968)
	X-ray, analysing electron density profiles e.g. (Schmedel et al. 2001)
Selected theories	Optical fringes observed in surface force apparatus (SFA) (Marra and Israelachvili 1985)
	Langmuir VI isotherm (statistical mechanics) (Marsh 2011)
	Polarisation (electrodynamics, statistical mechanics and thermodynamics) (Cevc 1991)
Temperature dependence	Protrusion (statistical mechanics) (Israelachvili and Wennerström 1990)
Computer modelling	Only a few studies, obviously negligible (Simon et al. 1995)
	Atomistic simulations (Schneck et al. 2012), Molecular dynamics (Leng 2012)
	Monte Carlo (Hayashi et al. 2002)?

<sup>a</sup>Sometimes, only a molar hydration number is given. This avoids geometrical model assumptions and is acceptable for equal or similar lipid head groups

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