ABSTRACT: The ubiquitous molecule spermidine is known for its pivotal roles in the contact mediation, fusion, and reorganization of biological membranes and DNA. In our model system, borosilicate beads were attached to atomic force microscopy cantilevers and used to probe mica surfaces to study the details of the spermidine-induced attractions. The negative surface charges of both materials were largely constant over the measured pH range of pH 7.8 to 12. The repulsion observed between the surfaces turned into attraction after the addition of spermidine. The attractive force was correlated with the degree of spermidine protonation, which changed from +3 to +1 over the measured pH range. The force was maximal at pH 7.8. To explain the observed pH and spermidine concentration dependence, two different theoretical approaches were used: a chemical model of the charge equilibrium of spermidine and Monte-Carlo simulations of the orientation of the rodlike spermidine molecules in the gap between the borosilicate and mica surfaces. Monte-Carlo simulations of the orientational ordering of the rodlike spermidine molecules suggested the induction of attractive interactions between the surfaces if the gap was bridged by the molecules. For larger gaps, the orientational distribution function of the spermidine molecules predicted a considerable degree of parallel attachment of the molecules to the surfaces, resulting in reduced effective surface charge densities of both surfaces, which reduced their electrostatic repulsion.

INTRODUCTION

As early as 1677, van Leeuwenhoek described crystals in human spermatozoa. These crystals contain an amazingly high concentration of 3.3 mg/mL of spermine, which is synthesized from spermidine. Spermidine is a rodlike polycation with three nitrogen atoms in a carbon chain (Figure 1). Depending on the pH, it may carry a maximum of three positive charges located at the two terminal amino groups and the central imine group. The investigation of their pH-dependent protonation by 13C NMR spectroscopy revealed \( pK_1 = 8.25 \pm 0.09, pK_2 = 9.71 \pm 0.12, \) and \( pK_3 = 10.90 \pm 0.21. \) Spermidine has a wide variety of physiological functions, for example, in inflammation reduction, lipid metabolism, regulation of cell growth, proliferation, apoptosis, and so forth. The presence of spermidine or spermine is required for the stabilization of double-stranded RNA during replication. Spermidine has been shown to induce autophagy in a wide variety of higher organisms and even in worms or yeast. Positive effects on the aging process have also been discussed. Spermidine, which is triprotonated under physiological conditions, plays a role in specific receptor–ligand binding processes and may mediate the unspecific interaction of negatively like-charged surfaces, for example, of cells, vesicles, and macromolecules in many biological processes. The agglutination of giant unilamellar vesicles (GUVs) has been used as a model system of mutually repulsive negatively like-
charged surfaces.10–12 Spermidine is also important for contact control in semibiological systems, for example, in the process of implant integration.11–13

The DLVO theory provides a straightforward approach to explaining these effects by considering the superposition of electrostatic and van der Waals forces.14,15 In the classical approach, electrostatic double-layer repulsion, which dominates at long distances may be overcome by van der Waals attraction at short distances. This approach correctly describes many phenomena in biological systems, for example, unspecific interactions, which are important in the initial processes of aggregation and adhesion.16

The DLVO theory has been refined by ion-correlation and ion-condensation effects.17 Ion correlation has experimentally been observed with polyvalent macroions by Kirkwood and Shumaker before it was studied with Monte-Carlo simulations.18,19 Oosawa predicted the condensation of large ions after he investigated charge fluctuations in the counterion cloud of polyions.20 These fluctuations generate attractive forces between the induced dipole of the ion clouds of adjacent electric double layers.

To investigate how the degree of spermidine protonation modulates the attractive forces between negatively like-charged surfaces, we used freshly cleaved sheets of muscovite mica and borosilicate beads as a model system. The charge of these surfaces is known to be largely pH-independent above pH 7.0.3–5 We attached commercial borosilicate beads to atomic force microscopy (AFM) cantilevers to investigate the spermidine concentration and pH dependence of the interaction forces of the negatively like-charged surfaces.

Mica (KAl₃[Si₃AlO₁₀]₉(OH)₂) is a 2:1 clay mineral, that is, one elementary sheet contains two layers of [Si₃AlO₁₀]₉⁻ that are zipped together by a layer of partly hydroxylated Al³⁺ ions. Potassium ions compensate for the resulting net charge and form linkages between the single sheets. The chemical weakness of this coupling results in the good cleavability of mica, yielding nearly ideal, atomically flat thin sheets.24,25

Borosilicate beads are known for their pH-independent negative zeta potential.25,26 This potential results from the isoelectric point of borosilicate being below pH 3.27 Chemically, the pH-independent zeta potential is based on the hydroxyl groups of silicon and boron,28 which provide the major charge contributions.29

## EXPERIMENTAL SECTION

### AFM Setup

The force measurements were carried out with a NanoWizard II AFM (JPK Instruments, Berlin, Germany) mounted on an inverted fluorescence microscope (Axio Observer A1, Carl Zeiss, Jena, Germany). The AFM head was equipped with a linearized piezoelectric scanner with a 15 μm z-range and an infrared laser-elevator system. The measurements were conducted in Petri dishes (diameter 40 mm; TPP, Trasadingen, Switzerland), which were placed in a thermostated Petri dish heater (JPK Instruments, Berlin, Germany). The measurement parameters were determined using JPK’s SPM Control Software v. 3.

### Solutions

Crystalline spermidine trihydrochloride (Sigma-Aldrich, Taufkirchen, Germany) was dissolved in deionized water to a concentration of 1 mg/mL. This solution had a pH of 5.90 ± 0.05. The desired pH values of 7.0, 7.8, 9.0, 10.5, or 12 were achieved by the addition of sodium hydroxide (Carl Roth, Karlsruhe, Germany) with stirring and pH monitoring. To prepare the spermidine dilution series, the pH-adjusted spermidine stock solution was diluted with titrated deionized water. At each pH value, the AFM measurements were started in deionized water without spermidine and then continued in solutions of spermidine at different concentrations in an ascending order over a range of 0.01 to 0.1 mg/mL. At each pH value, 1000 force-distance curves were recorded before the Petri dish was rinsed with 10 mL of the measuring solution of the next spermidine concentration to be measured. Measuring volumes of 3 mL ensured concentration to be measured. Measuring volumes of 3 mL ensured pH stability during the measurement time. Before each measurement, the system was allowed to equilibrate for 30 min.

### Borosilicate Beads

The borosilicate beads (Cat. no. 07666, Polysciences Inc., Eppelheim, Germany) were checked for clean surfaces by scanning electron microscopy (Figure 2). Energy-dispersive X-ray spectroscopy tests revealed no relevant contaminations of the bead surfaces except for traces of calcium and of the omnipresent carbon atoms (data not shown). Because exact surface charge data were not available for the borosilicate beads used, we conducted measurements using single particle electrophoresis (Supporting Information). The obtained charge densities between −0.0115 and −0.0294 As/cm² suggest zeta potentials in the range of −80 to −150 mV for the measurement conditions used in the force experiments.

### Bead-Functionalized Cantilever

For measurements, tipped (tip length: 3.5 μm), 100 μm-long, arrow-shaped, silicon-nitride cantilevers (Pyrex nitride probes, PNP-DB, NanoWorld, Neuchâtel, Switzerland) with a nominal spring constant of 0.48 N/m and a nominal resonance frequency of 67 kHz were used. The cantilevers were functionalized with the borosilicate beads, which were attached to the cantilever tips with resin glue (Endfest 300, UHU GmbH & Co KG, Bühl, Germany). To circumvent normalization of the maximum detachment forces (MDFs) and the maximum snap-to-contact force (MSFs) to the bead diameter using criteria that would depend on the mechanism of bead–surface interactions, beads with similar diameters (pH 7: 11.12 μm; pH 7.9: 10 μm; pH 9: 10.2 μm; pH 10.5: 9.9 μm; and pH 12: 8.71 μm) were microscopically selected. The functionalized cantilevers were allowed to settle for at least one day before the bonding seams between the cantilevers and beads were checked by light microscopy (Figure 2). Cantilevers with excess glue were not used. The spring constant of each cantilever was determined with the thermal noise (Figure 2). Cantilevers with excess glue were not used. The spring constant of each cantilever was determined with the thermal noise (Figure 2).
which prevented more elaborate measurements in the lower pH range (compare to ref 23).

**Mica Surfaces.** Muscovite mica sheets (PLANO, Wetzlar, Germany) were cut with a sharp blade to fit into the Petri dishes before being cleaved to a thickness of approx. 0.28 mm and attached to the bottom of the dishes by moderate pressure. Mica is well-known to have a negative surface charge with typical surface potentials in the range of −60 to −130 mV depending on the exact chemical composition of the mica type.31 The negative zeta potential of mica is strongly pH-dependent in the range of pH 5 to 7 but largely constant over the pH range used in this study.23

**Registration of Force–Distance Curves.** The AFM measurements started from a force-free position. During the approach of the cantilever at a velocity of 20 μm/s, it experienced a hydrodynamic force until bead–mica contact. Further extension led to increasing cantilever deflection, represented by a steep slope in the force–distance curve. After the setpoint force of 1.5 nN was reached, the cantilever was retracted at 20 μm/s. Attractive interactions during retraction led to a downward deflection of the cantilever tip. The MDF was determined before bead detachment. The hydrodynamic force during retraction induced a deflection opposite to that during the approach. No differences in the force behavior were registered when approach and retraction velocities of 5 μm/s were used. For further details, see refs 32 and 33.

Under certain conditions, electrostatic attraction did overcome the spring constant of the cantilever in the approach mode, resulting in a snap to contact. Snap-to-contact events are characterized by their head-to-surface distance and the MSF. In rare cases, attraction was observed in the retraction curves up to 800 nm. This was attributed to debris in the measurement medium. Such exceptionally long distances were not observed at the highest spermidine concentrations used.

**Preparation of Giant Unilamellar Vesicles.** GUVs were prepared by electroformation at room temperature according to Angelova et al. with the modifications described by Tomšíč et al.34,35 The synthetic lipids cardiolipin (1,1′,2,2′-tetraoleoylcardiolipin), POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine), and cholesterol were purchased from Avanti Polar Lipids, Inc. (Alabaster, USA). Weighted amounts of POPC, cardiolipin, and cholesterol were dissolved in 2:1 chloroform/methanol (v/v) before thorough mixing. To obtain charged GUVs, cardiolipin, POPC, and cholesterol were mixed in a 4:4:2 ratio. For microscopic observations, the GUVs were transferred to the measurement chamber before agglutination was induced by spermidine addition.

**Data Interpretation.** The Young’s modulus of borosilicate glass ranges from 65 to 70 GPa, whereas muscovite mica is a highly anisotropic crystal with orientation-dependent elastic constants. Its effective elastic modulus is between 20 and 60 GPa.36 These values suggest that the Hertz deformation can be neglected in the investigated force range up to 1.5 nN. Because the surface of mica is molecularly smooth,32 it must be expected that the surface roughness of the bead will determine the true contact area, which differs from the apparent contact area. For rough elastic surfaces, the true contact surface is generally proportional to the normal force, whereas the size of the microcontacts is known to only weakly depend on the load.32,36

For the beads’ surface charges, Gaussian distributions were obtained (Supporting Information). This permitted ANOVA testing of the pH dependence of the surface charge. The force spectroscopy data were statistically evaluated with JMP 10 data analysis software (JMP, Böblingen, Germany) and tested for their Gaussian distribution using the Shapiro–Wilk test. Accordingly, the mean values of the force distributions were used in the following considerations.

### RESULTS AND DISCUSSION

**Force Spectra: pH and Spermidine Effects.** Figure 3 shows three different force–distance curves illustrating qualitative pH-related spermidine effects. Without spermidine, snap-to-contact and attractive forces were observed only at pH 7.0 (Figure 3A). The MSF and MDF were 11 ± 41 and 1078 ± 951 pN, respectively. At a slower retraction velocity (5 μm/s), the MDF and its standard deviation were lower than those at 20 μm/s. An influence of the approach velocities on the MSF was not detected. In the pH ranging from 7.8 to 12, only repulsive forces were observed in both the approach and retraction profiles (Figure 3B). The detectable MDF values were below the noise level (1.8 pN RMS). The addition of spermidine induced attractive interactions over the pH range of 7.8 to 10.5 but not at pH 7.0 (Figure 5). In the presence of spermidine (0.02 mg/mL), the MDF decreased for increasing pH, with 1815 ± 275, 801 ± 99, and 25 ± 3 pN at pH 7.8, 9.0, and 10.5, respectively. Above pH 10.5, all attractive interactions disappeared in both the presence and absence of spermidine.

At 20 μm/s, the largest interaction distances at which deflections of the cantilever were detectable were approx. 100 nm in the approach and retraction force curves in the absence of spermidine and in the presence of low and intermediate spermidine concentrations. For spermidine concentrations of 0.1 mg/mL and above (data not shown), snap to contact was never observed in the approach curves.

For interaction distances above 100 nm, electrostatic explanations can be excluded because the Debye–Hückel length was below 100 nm even at the very low ionic strengths used in the experiments. A possible explanation involves the soft elastic soak layers on the surfaces. Such soak layers of 80–90 nm thickness have been described to develop on borosilicate glass but not on mica surfaces.24 The layers generate complex, pH-dependent force effects, which lead to deformations in the approach curves. Here, we attribute the distortions in the

![Figure 3. Force–distance curves measured with borosilicate beads over a mica surface.](Image)
force—distance curves, which were observed especially at longer measurement times, to the indentation and elastic relaxation effects of the soak layers.

The pH dependence of the attractive interactions was most pronounced at a spermidine concentration of 0.02 mg/mL. The attraction was maximal at pH 7.8. In the presence of spermidine, the snap-to-contact distances in the approach profiles decreased with increasing pH.

Snap to contact was never observed at pH 10.5. At pH 7.8 and 9.0, the detected MSFs were $201 \pm 32$ and $56 \pm 5$ pN, respectively. At pH 9, the snap-to-contact disappeared at spermidine concentrations higher than 0.02 mg/mL, and repulsive interactions were detected in the approach profiles (Figure 3D). At the highest spermidine concentration of 0.1 mg/mL, repulsion was detected in all approach profiles above pH 7.8. Interestingly, attraction was detected in the retraction profiles at pH 7.8. Figure 4 summarizes the measured MDF data at different pH values and spermidine concentrations.

**Figure 5.** GUVs with a negative surface charge in the absence (A) and in the presence (B) of spermidine.

---

**Spermidine-Induced Attractive Interaction Between GUVs.** GUVs are spherical membrane bodies. Their physical properties, such as surface charge and membrane fluidity, are determined by their lipid composition. We produced GUVs composed of cardiolipin, POPC, and cholesterol with a negative surface charge density similar to that of biological cells, as described by Perutkova et al.,41 to demonstrate the biological relevance of the spermidine-induced force effects. Under physiological conditions, the GUVs were microscopically observed as well-separated spherical objects (Figure 5A). Spontaneous agglutination was observed after the addition of spermidine (Figure 5B).

**Basic Assumptions.** It is known that the surface potentials of mica and borosilicate surfaces may approach 0 mV under strongly acidic conditions.32,36 Nevertheless, at pH 7.8 and above constant negative potentials with similar surface charge densities can be assumed for both like-charged surfaces, whereas the spermidine charge decreases within the measured pH range. The spermidine molecule is triprotonated at pH 7 and monoprotonated at pH 10.5.2

For simplicity, ionic strength effects were not taken into account. Because the presence of counterions other than spermidine was neglected, the spermidine concentration had to be assumed according to the electroneutrality condition, that is, higher spermidine concentrations were assumed for smaller gaps. In the simulations, a room temperature of 300 K was assumed.

**Attractive Interactions without Spermidine.** For spermidine-free solutions, snap to contact at pH 7.0 can be explained by a positively charged mica surface (Figure 3A). Electrostatic repulsion would be the straightforward explanation for the observed forces at pH 7.8 (Figure 3B). Nevertheless, the correct explanation must include osmotic effects. Without ions, the aqueous gap between the like-charged surfaces would be field free. For simplicity, let us assume that only counterions are introduced into the gap to ensure electroneutrality of the whole system. The ions would repel one another and would be collected at the surface of the aqueous medium, similar to the electronic charges at the surface of a charged metallic body. The approach of the two surfaces would increase the counterion concentration inside the gap. The resulting increase in osmotic pressure would drive water into the gap, generating repulsion.31 This repulsion will be higher for narrower gaps and higher counterion concentrations.32 These relationships are described by the contact value theorem,17 which allows the determination of the osmotic pressure from the electric properties of the system. The theorem can be applied as long as no counterions are adsorbed on the surfaces, which would change the effective surfaces charge densities.

**Monte-Carlo Simulations of the Orientational Ordering of Spermidine.** Monte-Carlo simulations were conducted to consider the role of the orientational ordering of the positively charged, rod-like spermidine molecules in attracting the negatively like-charged borosilicate and mica surfaces. The standard Monte Carlo Metropolis algorithm43 was applied to an electroneutral, periodic system with 2D symmetry using an implementation of the Lekner—Sperb method.44,45 The algorithm used was similar to that of Moreira and Netz.46 The canonical system of 100 diprotonated molecules considered was constrained by two impenetrable charged surfaces, which were treated as planar with respect to the size of the molecules. For simplicity, the simulations were only performed for diprotonated molecules. In each simulation step, a single molecule was randomly chosen and randomly translated or rotated around its center, assuming the same probability for the two types of movement. The run time of a simulation was approx. $5 \times 10^7$ steps. For the protonation sites, pointlike charges with two different separation distances of $L = 1$ nm (rodlike model) and $L = 0.01$ nm (pointlike model) were considered (see Figures 6 and 7). The assumption of pointlike diprotonated molecules with $L = 0.01$ nm permitted the comparison of the Monte-Carlo results with the predictions of the Poisson–Boltzmann standard model.
corresponds to the length midplane between the two surfaces (Figure 6A). This gap width densities of 0.023 A s/m² and gap widths of 1 (A), 1.8 (B), 3 (C), and 10 nm (D). The distributions were obtained by Monte-Carlo simulations with intramolecular charge separation distances of L = 1 nm (filled circles) and L = 0.01 nm (filled triangles). The results of the mean-field Poisson–Boltzmann theory for monovalent (solid line) and diprotonated (dashed line) molecules are given for comparison.

For a gap width of d = 1 nm, the number density of the centers of mass shows a maximum at x = d/2, that is, at the midplane between the two surfaces (Figure 6A). This gap width corresponds to the length L = 1 nm of the rodlike model. The roof shape of the density distribution results from the increasing steric restrictions in the spherical gyration of molecules, which approach the hard wall surfaces. For d = 1 nm, free rotation is ensured only for positions of the molecular centers at x = d/2. For larger gap widths, two peaks are obtained in the number density of the centers of mass, appearing approximately 0.5 L away from the surfaces (Figure 6B–D). For gap widths of d = 10 nm ≫ L (Figure 6D), a relatively high number of molecules are attached in parallel to the surfaces, resulting in increased number densities of the centers of mass and of the charges near the surfaces. At this gap width, the charge distribution profiles of the Monte-Carlo simulations and of the Poisson–Boltzmann mean-field approach almost overlap for diprotonated pointlike molecules. This hints at the convergence of the two approaches for larger gap widths and small L values. At a very short distance from the surfaces, the Monte-Carlo profiles are decreased because of entropic effects driven by the thermal motion of the molecules.

For the rodlike model, the degree of rotational freedom is reflected in the contribution of the rotational entropy to the total free energy of the system. Accordingly, the predicted peak positions in the number density distributions of the centers of mass correspond to optimal positions for a minimal free energy of the system. For a gap width of d ≈ L, the highest number density of the centers of mass was predicted at the midplane, whereas the number density of elementary charges was lowest at this position (Figure 6A). Molecules located with their centers of mass at the midplane, may electrostatically interact with both charged surfaces. For larger gap widths, electrostatic interaction must occur predominantly with either one of the charged surfaces (Figures 6B–D and 7).

For d = 1.8 nm, the mass number density profile shows minute peaks at surface distances of approximately L/2. The corresponding charge number density profile exhibits two weak maxima around a distance L from both charged surfaces, suggesting that a considerable number of molecules is attached in a perpendicular orientation. The free ends of these molecules overlap in the midplane (Figure 7B), generating a charge number density that is larger than those near the surfaces (Figure 6B). When the two surfaces are approaching in the presence of diprotonated rodlike molecules, the local increase in the charge number density at the midplane creates a local electrostatic energy barrier, which must be overcome during the approach or retraction of the surfaces. It can be assumed that the electrostatic repulsion of the charged molecular tips generates forces, which induce correlated charge patterns when the tip layers penetrate one another.

These properties result in strong deviations of the charge number densities predicted by the Poisson–Boltzmann theory for pointlike charges, especially for gap widths of 1.8 and 3.0 nm (Figure 6B,C).

At a gap width of 3.0 nm, the charge number density profile shows small peaks at a distance of 1 nm from the surfaces. This is the distance bridged by molecules that are perpendicularly attached to the surfaces (Figure 6C). Because of the increasing number of molecules with nonperpendicular orientations, the charge number density at the surfaces slightly exceeds the 1 nm peaks.

Figure 7 presents the positions and orientations of rodlike molecules within the gap. The figure illustrates the special features found in the Monte-Carlo simulation, such as intermolecular charge correlation and steric restrictions in the spatial distributions of mass centers and charges. For clarity in the schematic presentation, the majority of molecules are neglected. The total number of molecules in the simulations was determined from the electroneutrality condition in the gap, which is schematically fulfilled in Figure 7. Molecules attached...
to the surfaces in parallel orientation reduce the effective surface charge densities at larger gap widths. The order parameters $S$ given in Table 1 were averaged over the degree of orientational ordering of the diprotonated spermidine molecules for the gap widths in Figure 7. As described therein, the random orientation of the rodlike molecules corresponds to $S = 0$, whereas the average orientations in parallel and perpendicular to the charged surfaces correspond to $S = -0.5$ and $S = 1$, respectively. The order parameter for $d = 1$ nm indicates that only a minority of molecules are oriented perpendicular to the surfaces. At first glance, this is due to steric effects because only molecules with a center of mass located at the midplane may orient perpendicular to the surfaces (see also Figure 6A). The closer the center of mass is to one of the surfaces, the stronger the steric restrictions are, resulting in a more parallel orientation to the surface. Actually, the orientation is predominantly because of electrostatic effects. Namely, for a gap width of 1 nm and large surface charge densities, a considerable number of molecules are located with their center of mass in the vicinity of the midplane, generating strong attractive bridging forces between the like-charged surfaces (Figure 6A). For larger gap widths, the order parameter approaches zero because the two charges of the rodlike molecules cannot interact with both planar surfaces at the same time.

**Table 1. Averaged Order Parameters $S$ of Diprotonated Rodlike Molecules between Two Like-Charged Surfaces for Different Gap Widths ($d$)**

<table>
<thead>
<tr>
<th>$d$ [nm]</th>
<th>$S$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>-0.235</td>
</tr>
<tr>
<td>1.8</td>
<td>-0.091</td>
</tr>
<tr>
<td>3.0</td>
<td>-0.073</td>
</tr>
<tr>
<td>10.0</td>
<td>-0.067</td>
</tr>
</tbody>
</table>

**pH-Dependent Spermidine Charge and MDF: a Phenomenological Model.** The decrease in MDF with increasing pH (Figure 4) suggests that the triprotonated spermidine species make the highest contributions to the MDF. The contribution decreases for diprotonated, monoprotonated, and nonprotonated spermidine.

The pH-dependent probability of the protonation of the three N groups depends on their position within the spermidine molecule. This probability is described by the $pK_a$ values of 8.25, 9.71, and 10.90, permitting the titration to determine the molecule’s overall charge number. For our experimental pH range, the protonation is changed from 1+ to 2+ at approximately pH 10.5 and from 2+ to 3+ at approximately pH 8.5. The protonation of spermidine is described by three equilibrium reactions with the equilibrium constants $K_1$, $K_2$, and $K_3$:

$$\text{H}_3\text{SP}^{3+} \rightleftharpoons K_1 \text{H}_2\text{SP}^{2+} + H^+$$

$$\text{H}_2\text{SP}^{2+} \rightleftharpoons K_2 \text{HSP}^+ + H^+$$

$$\text{HSP}^+ \rightleftharpoons K_3 \text{SP}^0 + H^+$$

The equilibrium constants $K_1$, $K_2$, and $K_3$ were derived by Kimberly and Goldstein from the chemical shifts observed in $^{13}$C NMR experiments. The overall spermidine concentration $[\text{SP}]$ is the sum of the non- [SP$^0$], mono- [HSP$^+$], di- [H$_2$SP$^{2+}$], and triprotonated [H$_3$SP$^{3+}$] spermidine species

$$[\text{SP}] = [\text{SP}^0] + [\text{H}_2\text{SP}^{2+}] + [\text{H}_3\text{SP}^{3+}]$$

Correlating the abundance of the different spermidine species to the generated MDF, the MDF is

$$\text{MDF} = f_0 [\text{SP}^0] + f_1 [\text{HSP}^+] + f_2 [\text{H}_2\text{SP}^{2+}] + f_3 [\text{H}_3\text{SP}^{3+}]$$

with the constants $f_0$, $f_1$, $f_2$, and $f_3$ weighting the specific MDF contributions of each species. To reduce the number of free parameters in the pH-dependent MDF equation, the weak force contributions of the nonprotonated and monoprotonated spermidine species were neglected ($f_0 = f_1 = 0$). The concentrations of the triprotonated [H$_3$SP$^{3+}$] and diprotonated species [H$_2$SP$^{2+}$] are

$$[\text{H}_3\text{SP}^{3+}] = \frac{[\text{H}^+]^3 [\text{SP}]}{[\text{H}^+]^3 + [\text{H}^+]^2 K_1 + [\text{H}^+] K_2 + K_3}$$

$$[\text{H}_2\text{SP}^{2+}] = \frac{K_1 [\text{H}^+]^2 [\text{SP}]}{[\text{H}^+]^2 + [\text{H}^+] K_1 + K_2 + K_3}$$

The pH-dependent MDF is described by the sigmoidal decay function

$$\text{MDF} = \frac{[\text{H}^+]^3 [\text{SP}](f_2 K_1 + f_3 K_1)}{[\text{H}^+]^3 + [\text{H}^+]^2 K_1 + [\text{H}^+] K_2 + K_3}$$

The force constants $f_2$ and $f_3$ were obtained by fitting the MDF to the pH values and spermidine concentrations.

In contrast to Figure 4, Figure 8 shows individual fits to the MDF data at certain spermidine concentrations. The plateau of these pH-dependent curves is defined by $f_3$, that is, the maximum contribution of the triprotonated spermidine. At the first glance, the most striking difference from that in Figure 4 is the inconstant plateau height at different spermidine concentrations. However, as one can see from Figure 8, there is no obvious trend in differing plateau heights for increasing spermidine concentrations. In addition to the strong variation in the plateau height, a change in the position of the decay flank near $pK_1$ is also observed.

**Figure 8. MDF dependence on pH.** The curves are fits of eq 8 ($R^2 = 0.95$) to the data in Figure 4 (0.01 mg/mL (filled circles/solid line), 0.02 mg/mL (open circles/medium-dashed line), 0.03 mg/mL (filled triangles pointed down/short-dashed line), 0.04 mg/mL (open triangles/dash-dotted line) and 0.05 mg/mL (filled squares/long-dashed line)). The measurement points at pH 7 were excluded from the fit.
In Figure 9, the measured points of each spermidine concentration were fitted using eq 8. Systematic features are difficult to comprehend from the plot. Nevertheless, Figure 10 reveals a characteristic spermidine concentration dependence of $f_2$ and $f_3$.

![Figure 9. Sigmoidal fit of the MDFs (eq 7) for the dependence on the spermidine concentration.](image)

The decreasing force contributions toward higher spermidine concentrations suggest that the surfaces are saturated above 0.05 mg/mL. This is in line with an MSF that disappeared above 0.02 mg/mL and turned into repulsion at pH 9.0 (Figure 3D).

**CONCLUSIONS**

Our experiments showed that spermidine may induce electrostatic attraction between the negatively like-charged surfaces of borosilicate and mica. One possibility to explain this finding is to employ the DLVO theory. It describes the total interaction energy between objects or surfaces by superimposing electrostatic and van der Waals forces. At short distances, also small counterions may induce charged surfaces may be overcome by attractive van der Waals interactions induced by bridging effects between similarly charged objects, which can be predicted using a refined mean-field approach. Short-range attractions induced by bridging effects have been confirmed by Monte-Carlo simulations.

In the experiments, repulsion (no MSF) was detected above 0.02 mg/mL spermidine at pH 9.0, where the molecules were mostly diprotonated (Figure 3D). A possible explanation involves the reduced effective surface charge by the molecules attached to the surface in parallel orientation (Figure 7D) and an energy barrier generated by the repulsion of the positively charged headgroups of the perpendicularly oriented spermidine molecules approaching the midplane (cf. to discussion on ion correlation in the text). When the setpoint force overcomes this barrier, the “bridging force mechanism” may come into effect, inducing the detected MDF. In contrast, no repulsion was observed at pH 7.8, independent of spermidine concentration. We suggest that the higher effective charge of the di- and triprotonated spermidine species at this pH reduces the effective surface charge, leading to reduced free energy of the surfaces and decreased electrostatic repulsion (Figure 3C, compare to $f_2$ $f_3$ in Figure 10). Large polyions may even overcompensate for the surface charge, inducing a charge reversal of one or both surfaces, which may explain the lack of repulsion or a more complex behavior. We believe that our model system provides a platform for studying the mechanism behind the force interactions between like-charged surfaces. AFM measurement with GUVs will be complicated. For liposomes with diameters larger than 150 nm, there is a delicate balance between the adhesion of stable spherical structures and spreading.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.7b04199.

Surface charge of the borosilicate beads determined by particle electrophoresis (PDF)

**AUTHOR INFORMATION**

*E-mail: jan.gimsa@uni-rostock.de. Phone: +49 381 498 6020. Fax: +49 381 498 6022 (J.G.).

*E-mail: ales.iglic@fe.uni-lj.si (A.I.).
ACKNOWLEDGMENTS

The authors are grateful to the DFG (German Research Council) graduate school GRK1505/2 "Welisa" for funding the PhD positions of P.W. and T.W. and consumables for the experiments. We are grateful to Mojca Frank-Bertoncelj, University Hospital Zurich, Switzerland, for providing the liposome pictures. Dr. Andreas Körtge, University of Rostock, Germany, is acknowledged for his help in the AFM experiments.

ABBREVIATIONS

AFM, atomic-force microscopy; GUV, giant unilamellar vesicle; MDF, maximum detachment force; MSF, maximum snap-to-contact force; POPC, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine

REFERENCES


