

The specific amine-mica interaction investigated with the Surface Forces Apparatus

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received 24 January 2021

Summary. — The amine functional group is fundamental for bio adhesion and in this work we employ the Surface Forces Apparatus to explore the specific interaction between an amine-terminated polymer and a negatively charged surface. In addition, we present the first results about the role of ions at the biological solid-liquid interface, that open new possibilities of research.

1. – Introduction

Amines are a central functionality in biologic systems, *e.g.*, they play an important role in biologic adhesives [1]. Also, they are the basis used to build the amino-acids, which are bond together into proteins and other molecular elements that are crucial for life. Naturally, understanding amine-based functionality brings together physics, chemistry and biology, in the effort of comprehend its effect and structure at the biological solid-liquid interface.

For instance, polymers and lipids have been utilized to mimic real proteins [2], and to improve specific adhesion on metal surfaces by grafting an amine termination on them [3]. The amine group is also crucial in more complex real molecules, such as catecholamine or dopamine. The latter has been extensively studied in the last decade by scientists who aimed to prove its predominant role in the mechanism of bio-adhesion of surfaces in salt environment [4, 5]. In addition to specific molecular bindings of functionalities such as amines, the environment plays an important role. To date there is no conclusive understanding of competitive ion/functionality interaction reported.

The Surface Forces Apparatus (SFA) has been used to investigate salt effects, electric double layer forces, as well specific interactions, like for polymers terminated with functional groups [6] or ligand-receptor configurations [7]. The main point of this work is to report on the specific interaction between an amine group and a negatively charged surface, and testing how ions in solution may influence interaction forces, to lay the basis for a predictive approach to adhesion in complex salt environments.

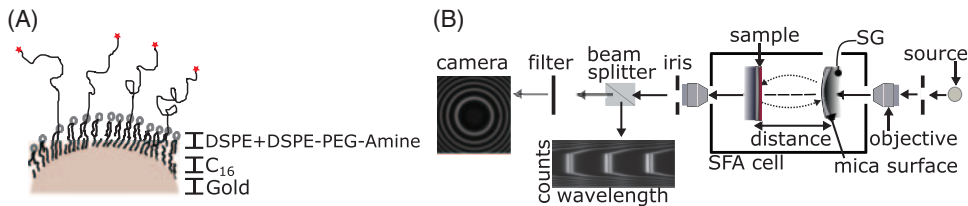


Fig. 1. – (A) Sketch of LMS. The red little stars indicate the amine functional termination. (B) SFA set-up. The sample is deposited on the left surface and indicated in red. The dashed arrows represent the multiple beam interferometry. The full arrows indicate the optical path. Below the beam splitter the FECO are displayed.

2. – Methods

2.1. Bottom-to-top approaches. – In this work we adopt the bottom-to-top approach as strategy to define and build our lipid model system (LMS). It consists in starting from a simple model that is understandable in all of its parts, followed by increasing the system complexity step-by-step, until one can observe a behaviour similar to what is found in real samples. The advantage of this method consists in the stepwise approach, which makes it easier to decipher what could be the molecular level mechanism of a new effect observed.

In our recent publication, we showed that a LMS adsorbed on gold by thiolate molecules as inner layer, is stable even in conditions of high adhesion. In addition, we proved that modifying the inner layer with molecules that are constituted of a polymeric part, it is possible to observe undulation of the interface and adhesion mechanisms that are typical of real membranes [8].

2.2. The lipid model system (LMS). – We extend the principle of the bottom-to-top approach in the direction of studying the specific amine-mica interaction (AMI). The new LMS is presented in panel (A) of fig. 1.

The substrate is gold, because we assure a good stability through the chemical adsorption of the thiol group. Hence, the inner layer is C_{16} 1-hexadecanethiol 99%, and as outer layer we have 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine (DSPE) mixed with *n*1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (ammonium salt) (DSPE-PEG-Amine), indicated by a star in the sketch. The lipid molecules are provided by Avanti Lipids, while the C_{16} is purchased by Sigma Aldrich.

The presence of lipids linked with the amine-terminated polymers, allow us the investigation of the AMI: since we use a Langmuir Blodgett Trough to deposit the outer layer, we can exactly calculate how many polymers, and therefore how many amines, we do have per unit area. This enables us to measure the interaction of a well-controlled number densities, even providing the possibility to extract single molecular information from the data.

This LMS is tested in asymmetric configuration against a back silvered negatively charged mica surface, in sodium chloride solution (min 99% from Carl Roth). The percentage of PEG-Amine on the outer layer is 10% of all the DSPE molecules.

2.3. The surface forces apparatus (SFA). – In panel (B) of fig. 1 the SFA set-up is presented. In brief, the SFA is an optical technique based on the multiple beam interferometry taking place in the optical cavity defined by two semi-reflecting mirrors [9].

The distance D that separate the tested surfaces is controlled by a piezo electric crystal and it induces an interference condition that allows only certain wavelengths to leave the cavity. The light beam goes through several optical elements and is divided in two by a beam splitter. The less intense path goes to a camera where one can observe the typical interference pattern, called Newton's ring. The other path goes to a spectrometer where a grating induces the diffraction. The figure obtained as output are fringes of equal chromatic order (FECO). These are visualized as a spectrum in wavelength which is a function of D and of the refractive index of the materials involved. Inverting the wavelengths one can obtain a direct measurement of the distance D between the mirrors [9].

The distance measurement requires a dry contact experiment between the bare back silvered mica and the gold surface to define the thickness of the first material layer, namely the mica. In this way, whatever LMS is adsorbed on the gold side, it is possible to reconstruct its thickness by further reference contact experiments [9]. The data acquired by the SFA are processed by the SFA Explorer software developed in our laboratories [10].

Typically, the force was calculated using Hook's law, from the measured distance and the elastic constant of a double cantilever system at which one of the surfaces was attached [9]. Recently, we have developed a novel strategy to measure simultaneously and independently the distance and the interaction force. The optical measurement is accompanied by a strain gauge sensor (SG) measurement which provides an electronic signal showing the force [8].

3. – Results and discussion

In fig. 2(A) we present the interaction force profile F/R normalized over the radius of curvature R , plotted against D . The sample is LMS with a 10% presence of DSPE-PEG-Amine. The experiment is performed at 10 mM sodium chloride. We indicate with $D_{\text{jumps-in}}$ the points at which we expect to have the mechanical instability that can push one surface in contact with the other. The aspect of this jumps depends on the charge distribution of the surfaces in play. However, the instability observed here, does not depend on inter-molecular forces, but by the specific AMI. Thus, around $D_{\text{jumps-in}}$ the amine termination of the polymers start to feel the presence of the binding sites distributed on the mica, producing this long-range attraction. The force presents a minimum and then increases exponentially in the contact repulsion, where we measure the thickness of the established layers indicated in the figure ($\simeq 4$ nm in high compression).

During separation (see the triangles in fig. 2(B)) the system experiences another mechanical instability, the so-called adhesion hysteresis, that produces a jumps apart of the two surfaces to a region of no interaction at $D > 30$ nm. We observe that the distance in contact is not constant during the process of retraction. This indicates that the material is soft and experiences an elongation along the direction of the applied load. This dynamics is in agreement with our previous result on model systems having polymers in their structure [8].

Figure 2(B) shows the effect of adding ions up to 1 M. The mechanical instabilities of jumps-in discussed above does not show up here, and we observe that the surfaces easily go into and out of contact, without significant adhesion.

This behaviour confirms the hypothesis that the core of the interaction force is the specific AMI. An increase of the electrolyte concentration increases the ions adsorbed on the mica side likewise. Therefore, the amines do not experience a long-range interaction at $D_{\text{jumps-in}}$, because of the lack of binding sites. Once in contact, a steric repulsion is observed, which is not related to the formation of amine/mica bonds due to the high

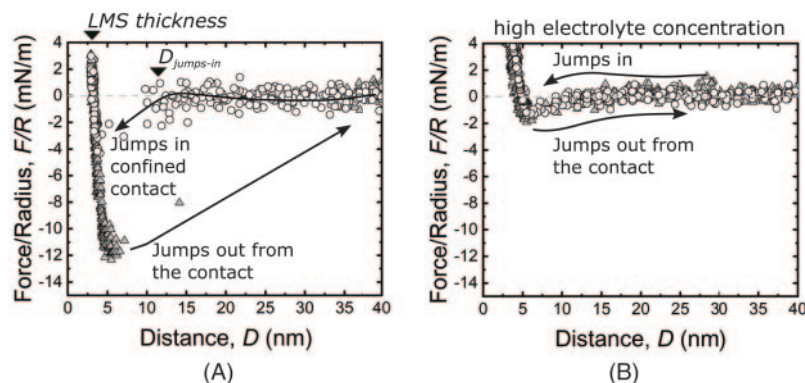


Fig. 2. – Interaction forces plotted as a function of the distance between the surfaces at (A) low and (B) high electrolyte concentration. The circles indicate the points recorded while one surface is moving toward the other, and the triangles indicate the opposite direction.

ion adsorption. Rather, a steric repulsion of adsorbed ions is observed, and consequently during retraction no adhesion is measured. Ion adsorption effectively competes with amine bonds.

4. – Conclusion

Using the SFA we tested a model system developed for measuring the specific AMI. We observed the interaction force profile at 10 mM describing the instability points. Increasing the presence of sodium chloride in solution, we observed a damping down of the adhesion. This confirmed that the instabilities recorded at 10 mM are related to the specific amine mica interaction. These results are an initial step containing the information about the molecular exchange and competition between specific interactions and ions with respect to a given surface. In the following projects of the group, we aim to display a scale of concentration and fully explore all the possibilities of investigation that the SFA can provide on such systems.

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The authors thank the European Research Council for the support given through the ERC Grant No. 677663 (CSI.interface).

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