*Cellular and Molecular Bioengineering*, Vol. 1, No. 4, December 2008 *(© 2008)* pp. 240–246
DOI: 10.1007/s12195-008-0025-7

*Molecular Interactions*

# The Surface Force Apparatus to Reveal the Energetics of Biomolecules Assembly. Application to DNA Bases Pairing and SNARE Fusion Proteins Folding

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*(Received 2 August 2008; accepted 19 September 2008; published online 10 October 2008)*

**Abstract**—The Surface Force Apparatus (SFA) measures directly, and with nanoscale resolution, the interaction energy vs. distance profile of planar arrays of biological molecules (e.g., lipids, polymers, or proteins). Through recent advances in the reconstitution and deposition of lipid bilayers, it is now possible to use SFA to study the interactions between membrane-incorporated biomolecules and to reveal any conformational changes and intermediate assembly states. Therein we describe two example systems. First, we show that using bilayers functionalized to carry DNA bases on their lipid headgroups, we can measure a macroscopic nucleoside–nucleoside adhesion force, from which one can obtain a molecular binding energy. Second, we describe the use of the SFA to study the interaction between SNARE proteins, which are involved in most of intracellular fusion events. Membrane fusion occurs when SNARE proteins assemble between lipid bilayers in the form of SNAREpins. SFA measurements between SNAREs embedded in lipid bilayers allowed us to elucidate the energetics and dynamics of SNAREpin folding, and to capture an intermediate binding state in SNAREpin assembly.

**Keywords**—Surface force apparatus, Interaction energy, DNA bases, SNAREs, Membrane fusion.

## INTRODUCTION

Every major process in a cell involves dynamic assemblies and/or disassemblies of biological molecules. There has always been a strong interest in quantifying these interactions but generally studies have been restricted to bulk thermodynamical measurements. The advent of nanotechniques allowed experimentalists to measure the interaction between surfaces coated with molecules whose position, density, and orientation were precisely controlled, and thus to obtain information on fundamental interactions and single bond energies.

The Surface Force Apparatus (SFA)[10] was the pioneer scientific instrument to measure nanoscale forces. It was originally designed to study colloidal interactions, including steric, electrostatic, van der Waals, and solvation forces, and today, it can also be used to monitor the assembly of biomolecules in real time. Other force measurement techniques include: (i) the atomic force microscope (AFM)[2] which measures rupture forces between the tip of a cantilever and a surface; (ii) the optical[1] or magnetic[6] tweezers that exert and measure forces on microscopic beads using a focused laser beam or a magnetic field, respectively; (iii) the Biomembrane Force Probe (BFP)[5] which gives the force between a bead and a surface using a red blood cell as a spring with tunable stiffness; and (iv) the flow chamber that provides the rate of formation and dissociation of molecular bonds submitted to a hydrodynamic flux.[18]

The SFA technique stems from right after World War II as David Tabor was studying frictional interactions between surfaces in the Cavendish laboratory. He had an industrial contract aimed at developing improved windscreen wipers, which led him to modelize the interactions between rubber and glass in water. Using hemispherical rubber samples pressed against a flat glass surface, his team could follow the interaction by interferometry. When the rubber/glass interaction experiments were performed in air, contact was immediately established and the rubber hemisphere became flattened over some area even under zero (compression) force. Under negative (pulling) force, and up to the onset of separation, the contact area

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remained nonzero, suggesting that attractive forces were operating between these two solid surfaces. This observation led his team to develop the JKR theory[13] for the adhesion between two solid bodies, which predicts that the pull off force $F$ to separate a deformable sphere of radius $R$ from a plane is equal to:

$$F = 3\pi R W_{\text{adh}}/2 \tag{1}$$

where $W_{\text{adh}}$ is the adhesion free energy per unit area of the two surfaces.

When the rubber/glass interaction experiments were performed in water, the team could not observe any adhesive contact between the rubber and the glass. Upon increasing the compression force, the rubber would deform and the contact area would flatten, but no adhesion would be produced. It soon became obvious to them that they were dealing with the so-called repulsive electrostatic double-layer forces.

At the same time, Samuel Tolansky was extensively studying the surface topography of crystals using Fringes of Equal Chromatic Order (FECO).[29] FECO are obtained by passing a white light through a Fabry–Perot interferometer, and are extremely sensitive to the surface topography down to the angstrom scale. They turned out to be also very useful for surface force measurements, since they could give the inter-surface distance and the deformation of two interacting surfaces with an unprecedented resolution.

In order to study surface forces with better-defined substrates, Tabor's team used mica which can be easily cleaved into atomically smooth thin sheets over several square centimeters. This allowed them to produce the first detailed measurements of van der Waals forces in the normal and retarded regimes.[24] One of Tabor's students, named Jacob Israelachvili, adapted the original design of the SFA to operate in liquids, and improved its mechanics. He also solved the complete set of equations of the FECO, therefore improving the accuracy of multiple-beam interferometry and making it more user-friendly.[8] Notably, this allowed him to substantially improve the measurements of van der Waals forces.[12] After this, Israelachvili and his co-workers studied various inter-surface and intermolecular forces, such as DLVO forces (repulsive electrostatic double-layer forces and attractive van der Waals forces),[10] hydration forces,[11] forces between lipid bilayers,[17] and forces between receptors and their ligands.[7]

Other groups, such as the one of Jacob Klein, focused on polymer interactions at interfaces.[16] Theory describes several cases of steric forces generated by polymer layers. Among them, the mushroom model describes the interaction of surfaces bearing end-grafted molecules which do not overlap laterally. Entropic in nature, these repulsive interactions come from the restriction of the number of conformations that the polymer can take due to the presence of the other surface[3,9]:

$$\frac{F(D)}{R} = 72\pi\Gamma k_{\text{B}} T e^{-\frac{D}{R_{\text{g}}}} \tag{2}$$

where $F$ is the force between two surfaces (radius of curvature $R$) at a distance $D$ from each other, $\Gamma$ is the surface density of the polymers, and $R_{\text{g}}$ is their radius of gyration.

As we describe below, the combination of SFA technology and polymer theory can give rise to very precise characterization of the repulsion and adhesion between two layers of proteins.

## DESCRIPTION OF THE SFA TECHNIQUE

The SFA is based on astute mechanics combined with powerful interferometry, which allow the measurement of force vs. distance profiles between molecularly smooth mica surfaces, and can resolve distances of 0.1 nm and forces of 0.1 $\mu$N.

The force is measured with a bending spring having a typical stiffness of ~100 N/m. The apparatus contains a translation device which moves the lower mica surface (connected to the force-measuring spring) toward the upper one (Fig. 1). It employs two or three systems of distance control with increasing sensitivity. A coarse level, consisting of a micrometric translation, allows for a rapid positioning of the two surfaces (accuracy ~1 $\mu$m). A finer level, using a differential spring system, has an accuracy of ~1 nm. It consists of a helical spring pressing against a cantilever spring one thousand times stiffer (Fig. 2). When the helical spring is pressed one micron further, it bends the cantilever spring by one nanometer, and thus moves the lower mica by the same distance. This already provides accuracy sufficient for most of surface force studies. Addition of a piezoelectric system can further improve the sensitivity, and allows for positioning to an accuracy of ~0.1 nm.

The distance is measured by multiple beam interferometry through a Fabry–Perot formed by the two mica sheets (of equal thickness 2–5 $\mu$m), free of crystal steps, and silvered on the backside (Fig. 3). When a white light is sent to this interferometer, the various wavelengths are reflected and transmitted across the different interfaces, and produce a transmitted spectrum made of Fringes of Equal Chromatic Order (FECO). For a planar interferometer, the fringes consist of lines in the spectrometer. For a curved interferometer, the fringes are curved, as shown in Fig. 3. These FECO give the distance between the two micas with a resolution of 0.1 nm.

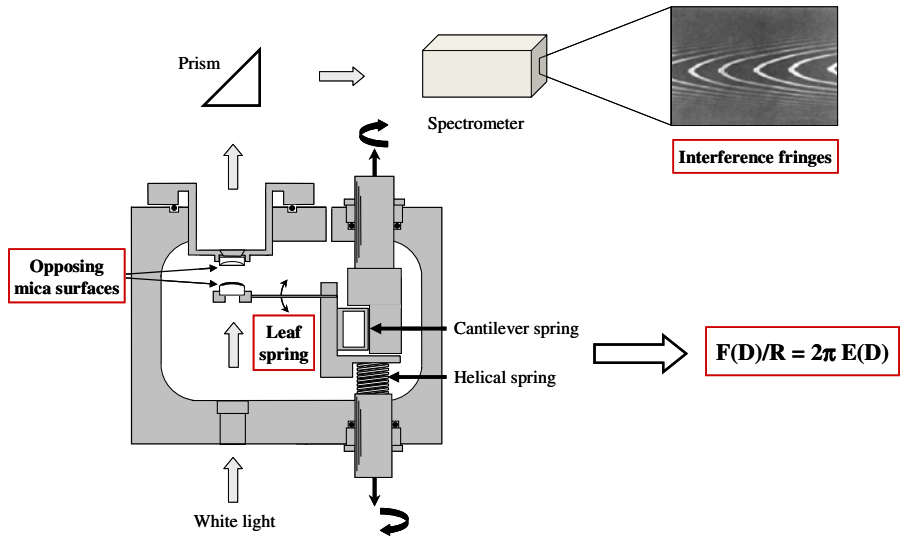


**FIGURE 1. The Surface Force Apparatus (SFA).**

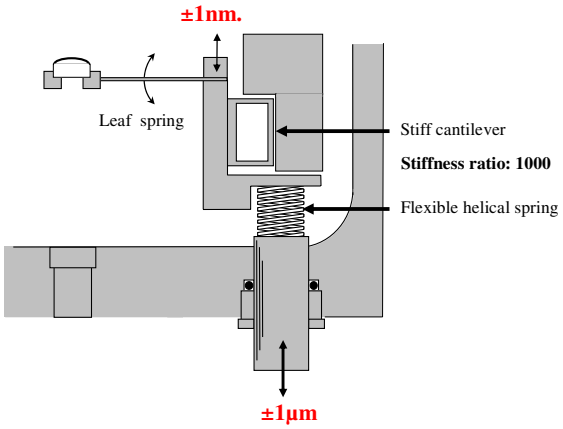


**FIGURE 2. The differential spring system.**

The mica sheets (typical area of $8 \times 8$ mm$^2$) are glued with a thermosetting epoxy onto two cylindrical lenses, which are then arranged in a crossed-cylinder geometry. This geometry (equivalent to a sphere-plane geometry) makes the alignment easier, and enables testing many different surface regions. According to the Derjaguin approximation,[4] the force, $F(D)$, between these two curved surfaces is proportional to the interaction free energy per unit area, $E(D)$, between two equivalent planar surfaces if the distance is much smaller than the radius of curvature, $R$, of the surfaces, and if the interaction energy decreases sufficiently rapidly with distance (at least in $1/D^2$):

$$F(D)/R = 2\pi E(D) \qquad (3)$$

SFA measurements thus provide directly the interaction free energy per unit area between two surfaces as a function of their separation distance.

The SFA remains a challenging technique. First of all, it is not easy to cleave mica and obtain a few square centimeters of thin sheets free of steps, of dust particles, and of mica flakes. Then, upon gluing the mica onto the cylindrical lenses and mounting the lenses into the apparatus, care has to be taken to avoid dust particles or any other contamination to reach the surfaces. The liquids and the chemicals used during the experiment have to be ultra-pure in order to reduce sources of contamination. In addition, the SFA has to be installed on a vibration-free table to avoid noise in the force measurements, and in a thermostated room to reduce thermal drifts.

Mica surfaces can be coated with various molecules of interest (lipids, polymers, proteins), which makes it possible to study the complete interaction profile of these molecules using the SFA, and to identify the nature of the forces involved. From the mid-eighties on, several SFA studies of lipid bilayer interactions have been reported which aimed at characterizing the fundamental forces between membranes[9]: van der Waals forces, double-layer forces, hydration forces, and undulation forces. Once the technique was routinely applicable to lipid bilayers, it was fairly straightforward to use functionalized lipid bilayers whose headgroups would carry the function to be studied,[19,25–27] or to follow any structure modification of two interacting lipid layers.[20] When applied to interacting proteins (whose size is typically of a few nanometers), the angstrom resolution of the technique can provide the molecular detail of their assembly, including conformational change and passage through any intermediate binding state.[15,22]

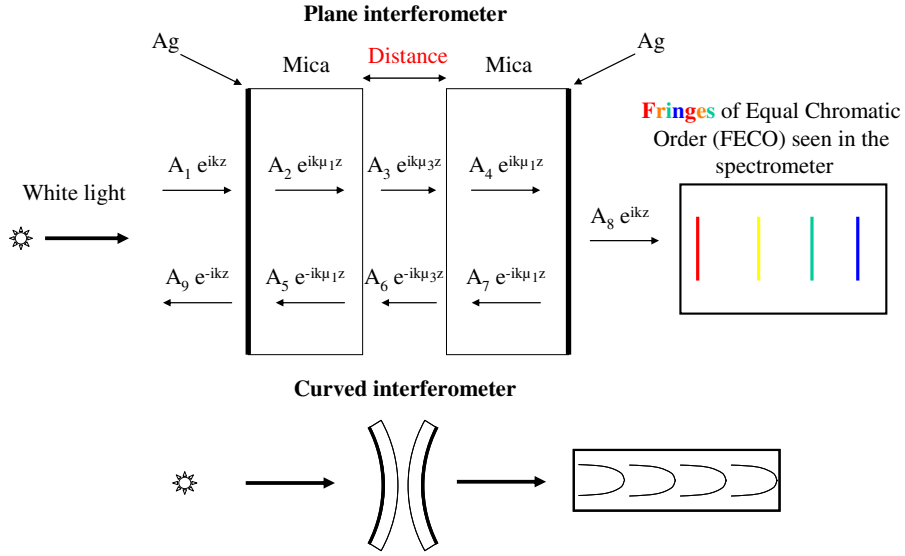


**FIGURE 3. Interferometer made of two silvered mica sheets of equal thickness with refractive index $\mu_1$, separated by another medium of refractive index $\mu_3$. A beam of incident light is transmitted to the right, after multiple reflections, as lines observable in a spectrometer. The wavelength of these lines (and the position in the spectrometer) varies when the distance between the surfaces is changed. *Bottom*: When the silvered mica sheets are curved, the fringes observed in the spectrometer are curved.**

## SFA TO MEASURE THE ENERGY OF DNA BASES PAIRING

The dynamic double helix structure of the DNA molecule is stabilized by highly specific interactions between complementary nucleotides: Adenine bonding with Thymine, and Guanine bonding with Cytosine. We have used the SFA to measure the adhesion between surfaces coated with the nucleosides (nucleotides lacking phosphate groups) Adenosine (A) or Thymidine (T). Two lipids were synthesized whose headgroups were, respectively, made of A and T nucleosides. These lipids were deposited onto monolayer-coated mica surfaces, with the Langmuir–Blodgett technique, at a film pressure sufficiently high to ensure close-packing of the lipid layers. In this configuration, the nucleosides are perpendicular to their surface and oriented toward the other surface (Fig. 4); they are thus fully accessible for binding to their complementary group residing on the opposite surface.

Experiments were performed with symmetrical (A/A and T/T) and complementary (A/T) surface pairs (Fig. 4). The adhesion measured upon separating two complementary surfaces (A/T experiments) was more than twice the adhesion of the symmetrical systems (A/A or T/T experiments). At contact, the surfaces were flattened, and therefore the JKR theory (Eq. 1) could be used to evaluate their adhesion free energy. As the number of molecules was known on each surface from the Langmuir–Blodgett deposition parameters, it was then straightforward to estimate the energy

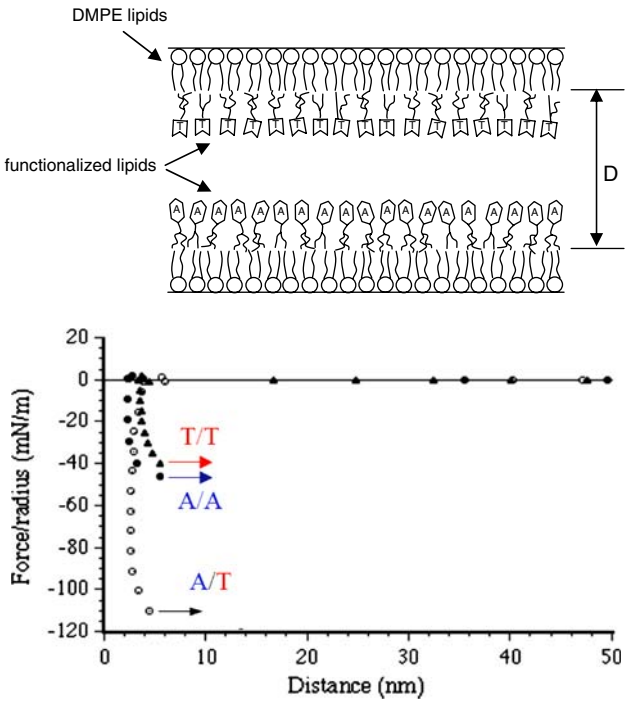


**FIGURE 4. Interaction between DNA bases measured by SFA. The mica surfaces are coated with bilayers functionalized to carry nucleosides on their lipid headgroups. The adhesion measured in the complementary system (A/T) is much larger than for the symmetrical systems (A/A and T/T).**

of the molecular bonds: the adhesion free energy per unit area $W_{adh}$ is equal to the density of bonds multiplied by the molecular binding energy $e_b$:

$$W_{adh} = \text{density of bonds} \times e_b \quad (4)$$

As the lipid layers are close-packed, every nucleoside faces a nucleoside from the other surface, and one could assume that all molecules can form a bond. However, because the binding energy of the molecules is small, two molecules which face each other at atomic distance do not necessarily form a bond. The probability that they form a bond is given by Boltzmann statistics: two interacting molecules can be either (i) unbound and in a zero energy state, or (ii) bound and in a $-e_b$ energy state. The partition function $Z$ of the system is then: $Z = 1 + \exp(e_b)$, with $e_b$ expressed in thermal energy units $k_BT$, and the probability $p_{bound}$ that a bond forms is:

$$p_{bound} = \frac{1}{1 + \exp(-e_b)} \quad (5)$$

The energy of bond formation, deduced from Eqs. 1, 4 and 5, is 4.0 $k_BT$ (2.4 kcal/mol) for A/T, 2.2 $k_BT$ (1.3 kcal/mol) for A/A, and 1.8 $k_BT$ (1.1 kcal/mol) for T/T.[19,27] These values are in very good agreement with those previously obtained from quantum mechanics calculations[21] or thermodynamic measurements.[28]

This result was followed by several similar studies using various functionalized lipid bilayers[25–27] (e.g., bilayers bearing chelating groups or groups that can interact via a combination of hydrophobic forces and hydrogen bonds). In the case of chelation bonds, three independent methods were used to estimate the binding energy (SFA, giant vesicle adhesion measurements and microcalorimetry), which gave similar results.[25] It thus confirms that from a macroscopic adhesive force measured by SFA, one can reliably deduce molecular binding energies. In addition, it shows that the SFA is a powerful tool to quantify the interactions of biomolecules in aqueous solutions, i.e. under close-to-native conditions.

## SFA TO MONITOR SNARE FUSION PROTEINS ASSOCIATION IN REAL TIME

Membrane fusion is a fundamental biological process which plays a central role in many areas of physiology, such as neuronal communication, viral infection, or mitochondrial dynamics. Because biological membranes are designed to be stable, membrane fusion does not occur spontaneously and requires the intervention of specialized proteins which provide the energy required for fusion to occur. Intracellular trafficking events are governed by the assembly of cognate vesicular (v-) and target (t-) SNARE proteins, which initially reside in apposing membranes, to form a stable bridging complex (the SNAREpin) that triggers the membranes to merge.[23,30] How much energy is made available from the SNAREpin protein-folding event, and how this energy is transmitted to the membranes destined to fuse are still very much open questions.

Through SFA interaction energy measurements between membrane-embedded cognate v- and t-SNARE proteins, we have recently provided a comprehensive description in real time of the energetics and dynamics of SNAREpin folding across membranes.[15] The cytoplasmic domains of v- and t-SNARE proteins were modified to have only a single Cysteine residue at their C-terminal end, and then chemically coupled through this Cysteine to a supported lipid bilayer consisting of 89 mol% of DOPC lipids, 10 mol% of negatively charged DOPS lipids, and 1 mol% of reacting DOPE-Maleimide lipids (Fig. 5). We tested many different pairs of SNARE bilayers, probing various regions on each of these pairs. Each SFA experiment consisted of several approaching/separation cycles during which distances and forces of interaction were measured simultaneously every 30 s.

During the approaching phase, and before they start associating (region between 20 nm and 8 nm in Fig. 5), SNARE proteins repelled each other like random polymers. Upon separating two SNARE bilayers, a strong adhesion was systematically observed. The measured adhesive forces/radius varied however substantially from one experiment to another (from 3 mN/m to 20 mN/m). Because we noticed a correlation between stronger adhesive forces and larger long-range steric repulsion, these variations were attributed to differences in local SNARE density. The surface density of SNARE proteins was estimated by modeling the long-range repulsion experienced by SNARE bilayers with the mushroom model of steric forces between polymer layers (Eq. 1). Knowing the SNARE density and the macroscopic adhesion energy of two cognate SNARE bilayers, we were thus able to deduce the SNAREpin binding energy: 35 $k_BT$ (21 kcal/mol), which corresponds closely to the energy needed to create the highly curved non-bilayer transition structures (stalks) leading to fusion (40–50 $k_BT$[14]). By these measures, the cooperative effect of a few SNAREpins at the site of fusion would therefore release enough energy to overcome the high energetic barriers of membrane merging.

The force vs. distance profiles of assembling SNAREpins also revealed that SNARE motifs begin to interact when the membranes are 8 nm apart, and that the resulting SNAREpin is partially unstructured in its C-terminal region (SNAREpin about 60–80%

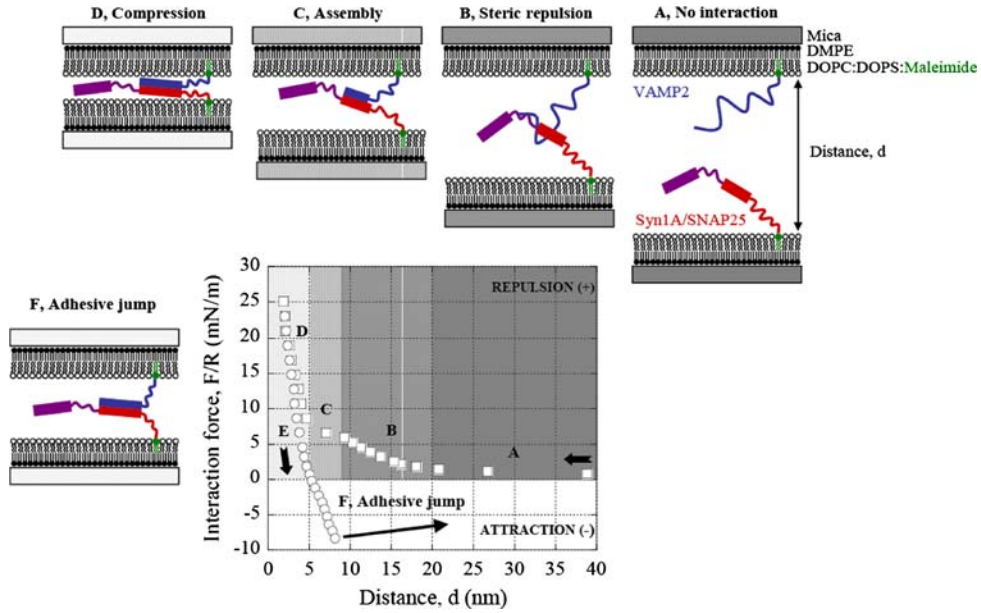


**FIGURE 5. Assembly/disassembly of lipid-anchored SNAREpins in apposing bilayers (*squares*: approach, *circles*: separation). As the bilayers are brought into close apposition, the interaction between SNAREs proceeds through a series of successive steps: no interaction, steric repulsion, initiation of complex formation, and compression of SNAREpins. The plateau observed around 8 nm indicates that SNARE proteins do not offer any resistance to compression at this distance and thus corresponds to the region where SNAREpins start to fold up between lipid bilayers. A strong adhesion is measured upon separation (the corresponding adhesive jump is symbolized by the *arrow*). Identical interaction profiles are obtained by repeated cycles of approach and separation (including the plateau at 8 nm and the adhesive jump), indicating that SNAREpins can be readily assembled and disassembled in our system.**

zippered). In addition, membrane-proximal regions of the SNAREs (rich in basic amino acids) were shown to interact with the negatively charged lipid membrane, which could contribute to partial SNARE assembly. Within a native fusion environment, such interaction could play a role in functionally coupling SNARE assembly to the bending and fusion of two apposing lipid bilayers.

## CONCLUSION

The Surface Force Apparatus is a powerful technique to study in real time and in a close-to-native environment the interactions between biological molecules. Its high resolution in distance and force allows the identification of any conformational changes and intermediate binding states that occur in the course of their assembly. In addition, as it measures the interaction produced by a large number of identical molecules, the SFA enables averaging out any effect coming from thermal fluctuations, and thus makes it possible to obtain directly the energetics of biomolecules assembly, which single molecules techniques cannot currently do in a direct manner.

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