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SPECIFIC FORCES BETWEEN DNA BASES

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Molecular recognition occurs at all levels of living matter but the mechanisms are not understood in physical terms. One striking example is that of DNA whose properties are intimately related to the specific molecular interactions of four nucleosides, based on hydrogen-bonds and size complementarities. We have directly measured the interaction between two of them, adenosine and thymidine, using a surface force apparatus. In these experiments, lipids functionalised with nucleosides were synthesised, and used to coat the surfaces between which forces were measured. The interactions of complementary molecules were compared to those, markedly different, for which the complementarity was hindered by a small modification of one of the molecules. The distance range of the specific forces, deduced from this comparison, was surprisingly long. The adhesion energy of the surfaces covered by these nucleosides were highly specific. Binding energies obtained from these measurements were in good agreement with values from the literature. The results also show that without the size effect existing in DNA, H-bonds alone can generate the specificity. An unusual behaviour, attributed to the sticky and fluid character of the layers, was pointed out. A long-range non specific interaction, also unexpected, was found. These features observed on surfaces coated with chemical functional groups may partly result from a collective behaviour. They illustrate the variety of physical effects one can obtain by playing on the chemistry of a surface.

1. Biological Recognition, Cell Adhesion and Intermolecular Forces

Biological recognition, resulting from specific molecular interactions that are sometimes called key-lock interactions, occurs at all levels in living matter, in such processes as cell adhesion, DNA replication, movement of the cytoskeleton[1] etc. The cell adhesion plays a dominant role in numerous biological processes such as infections, migration of cells in organisms, cell differentiation, and in the formation of tumoral metastasis.[2] In biological transport, a cell or a cell component will selectively reach its target generally by the means of a ligand and its receptor. Many bioengineering applications, such as drug delivery systems, biosensors and smart

materials involve the recognition of functional molecules.[3] Their efficiency is based on the dynamics of association which not only depends on the binding energy of the molecules involved, but also on the distance range at which the specific forces exist. The equilibrium constant of association of soluble species are often known, but the interaction however is not understood in physical terms. Owing to their complexity, we are hardly capable of predicting whether two biomolecules will recognise each other, and we do not know the distance range of the specific interactions. It is generally admitted that the latter are a combination of the geometry of the molecules with known physicochemical forces. Quite a variety of these forces has been studied in the framework of colloid and surface science[4,5] because they make up the interactions between colloidal particles. They include the van der Waals and electrostatic double-layer forces, the forces due to the structure of the liquid and the entropic forces to name only a few. Many of them have been thoroughly studied by direct forces measurements.[5] Some of them (van der Waals and double layer electrostatic forces for instance) have been given a clear explanation and an accurate theoretical description, allowing to predict the force/distance profile of the interaction. Other ones still remain unexplained. For instance, the attractions between hydrophobic surfaces sometimes display a van der Waals profile but other times are longer ranged.[6] The case of surfaces bearing hydrogen bonds (H-bonds) has, so far, received much less attention.

The surface force technique[7] (SFA technique) allows to measure directly the forces between two surfaces in liquid media, separated by a distance monitored at the angström scale. Its advent has brought immense progress in the knowledge of physicochemical interactions and colloid stability. Most of the non-specific interactions have been thoroughly studied in this way which allowed an extensive comparison between theory and experiment. This technique is now applied to surfaces coated with the molecules to be studied rather than to bare surfaces as in the early days and it is possible to measure the forces resulting from specific intermolecular interactions. It has been shown[8,9] that the range of the streptavidin–biotin interaction is in the nanometer range. This ligand-receptor couple has one of the strongest specific interactions known in biology and is often used as a biological "glue" to anchor other biomolecules on substrates. The development of the atomic force microscope (AFM) has also allowed to obtain new informations on the forces related to molecular recognition. Florin *et al.*[10] have measured the force necessary to break up the bond between streptavidin and biotin molecules and have managed to evaluate the force necessary to break one bond. Hoh *et al.*[11] have analyzed the force between an AFM tip and a surface in terms of the breakage of single H-bonds.

Many specific molecular interactions involve H-bonds. From the purely physicochemical point of view, the interaction of surfaces bearing H-bonds is not precisely known. The simplest, and though most important specific interactions occurring in biology, those between the complementary bases of DNA, also involve H-bonds.[1] DNA is a linear polymer of nucleosides monophosphates (nucleotides). It has a helical structure and can replicate (Fig. 1). Its extraordinary properties are due to four

complementary bases, adenine, thymine, cytosine, and guanine which specifically bind to each other in pairs: adenine binds only to thymine and cytosine only to guanine. Their interaction is due to H-bonds and to a size complementary relative to the distance between the two DNA strands.[12] In addition to these preferential interactions, proof-reading mechanisms ensure an error free replication. This works so well that a fragment of DNA can be indefinitely copied with a process almost as simple as photocopy, called polymerase chain reaction (PCR). The pairing of the bases in DNA is referred to as the Watson–Crick configuration, but they can associate in other modes, called reversed Watson–Crick, Hoogsteen and reversed Hoogsteen configurations in which different H-bonds sets between the bases are involved.[12] The bases are also known to have a tendency to associate by setting their planes parallel to each other, a phenomenon called "stacking" which also contributes to the DNA structure. Although the binding energy of the nucleotides in DNA has already been determined from thermodynamic measurements[13] and quantum mechanical computation for the interaction in vacuo,[14,15] the underlying forces are not known.

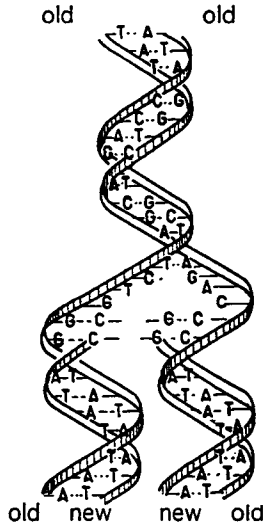

Fig. 1. Replication of DNA: The two strands separate and replicate by the means of the specific interaction between adenine and thymine, and cytosine and guanine.

We have chosen to study these interactions by measuring them between adenine and thymine with the SFA technique. For this purpose, the two surfaces in the SFA were coated by Langmuir–Blodgett deposition[16] of lipids functionalised with nucleosides.[17,18] This procedure offers a comprehensive control of the physicochemical parameters. We have thus designed and synthesised the appropriate functionalised lipids.

2. Methods

2.1. Lipids functionalised with nucleosides

To measure specific intermolecular forces, it is not enough to attach functional groups on the surfaces of an SFA; certain requirements have to be met:

(i) the functional groups must be accessible to the ones of the other surfaces; this means that each functional group needs some rotational and translational clearance to adopt a position allowing a specific interaction to occur with its opponent;
(ii) the surfaces must be stable, i.e. desorption of the groups and chemical damage must be negligible.
(iii) the forces that bind the groups to the surfaces must be larger than the interaction to be measured;
(iv) the surface density of groups must be above some minimum value, the force sensitivity of the SFA being 100 nN.

In view of these requirements, the Langmuir–Blodgett deposition of functionalised lipid monolayers gathers many advantages. It allows to control the density of the deposited molecules, and to fix their orientation[15] while letting the molecules freely diffuse inside the layer. In order for the lipids to have translational freedom, the monolayer has to be in a fluid state. One way to obtain this is to have lipids with unsaturated alkyl chains: while DOPC (dioleoyl phosphatidylcholine), for instance, which has two unsaturated C_{18} alkyl chains with one double bond, forms multibilayer phases that have a gel/fluid transition at $-22°C$, the corresponding saturated compound DSPC (distearoyl phosphatidylcholine) has a gel/fluid transition at $55°C$.[19] To provide the functional groups with some rotational freedom, they are anchored to the lipid moiety by the means of a flexible spacer which will allow the functional headgroup to position itself in the most favourable configuration.

The functionalised lipids were therefore designed with two unsaturated C_{18} alkyl chains, and a flexible spacer between the functional groups and the chains. We have synthesised two functionalised lipids which have one nucleoside, thymidine (T) or adenosine (A), as a headgroup (see Fig. 2). The only difference between a nucleoside and a nucleotide is the presence of a phosphate group at the 5'-position on the sugar backbone that does not play any rôle in the specific interactions. Another lipid which has a methylated thymidine (MeT) as a headgroup (see Fig. 2) was also synthesised. In MeT, the original proton that in thymidine is responsible for one of the two hydrogen bonds involved in the A–T pairing is replaced with a methyl group. Consequently the modified base is not able any more to form a highly stable pair with A. This group was synthesised in order to measure the effect of hindering the pairing on the forces. These lipids were obtained by coupling the unprotected nucleosides with 2-(1,3-dioleyloxy) propyl hemisuccinate using a modified DCC/DMAP method.[20] In principle, these dioleoyl-succinyl lipids

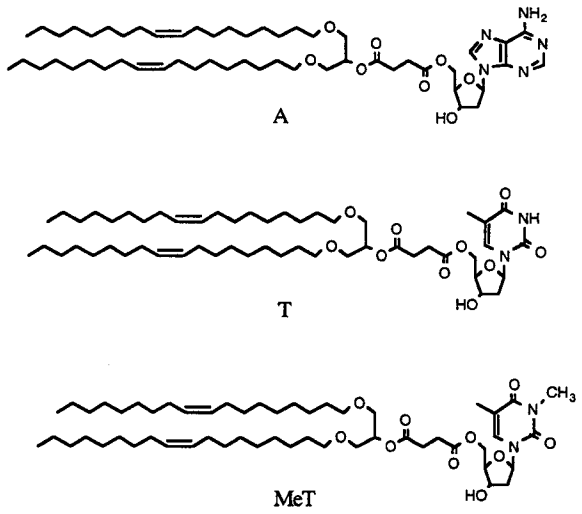

Fig. 2. Chemical structures of the functionalised lipids: A, T and MeT.

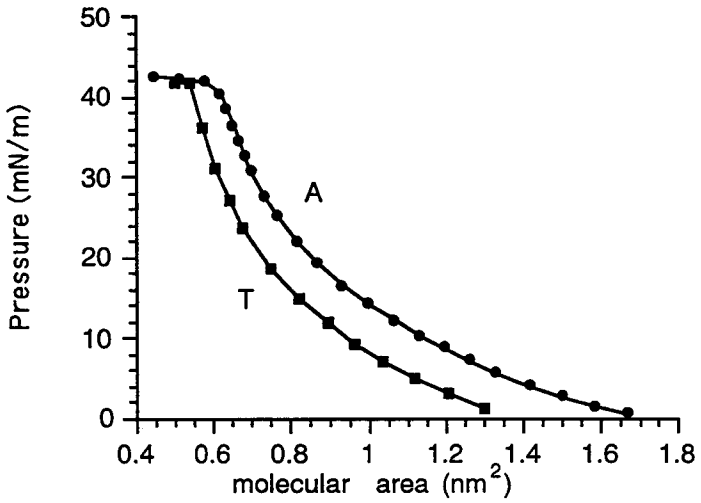

Fig. 3. Compression isotherms of lipids A (circles) and T (squares).

functionalised with nucleosides should be called DOST, DOSA, and DOSMeT. However, for clarity and conciseness, they will be called A, T and MeT.

Such synthetic lipids could be spread as insoluble monolayers at the air/water interface in a Langmuir trough[21] and bidimensionally compressed. The compression isotherms of these monolayers were highly reproducible, and are those of a purely fluid monolayer, showing no phase transition plateau[16] (see Fig. 3). We have also verified the stability of the monolayer by keeping it under a constant surface pressure

of 37.5 mN/m for several hours and measuring its total area as a function of time. This way any desorbed or chemically damaged molecule produces a reduction of the total monolayer area directly measurable on the Langmuir trough with a ruler. The monolayers were very stable: 5% of desorption for A after 6 hours, while, for DOPC which is considered as an insoluble lipid, the desorption was almost 10% over 6 hours (see Fig. 4) under the same surface pressure.

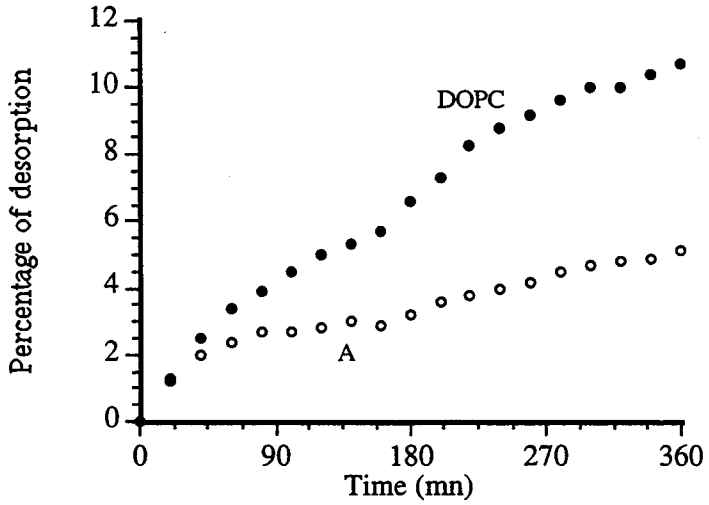

Fig. 4. Desorption of DOPC (closed circles) and A (open circles) at the air/water interface.

2.2. *Langmuir–Blodgett deposition of functionalised lipids*

The Langmuir–Blodgett deposition technique[16] consists in spreading a monolayer at an air/water interface, and vertically moving a solid substrate across the interface (here, at a speed of 14 mm/min). In the course of this movement, a monolayer is transferred to the solid. As some molecules leave the air/water interface and go to the mica surface, the surface density and therefore the surface pressure decrease. To compensate for this and to keep constant the surface density and pressure, the area of the monolayer is decreased. Each mica surface was covered with two different monolayers. First, a monolayer of DMPE (dimyristoylphosphatidylethanolamine) in a solid state was deposited onto the mica (at a surface pressure of 43 mNm^{-1}) to make it hydrophobic. This permitted the deposition of a second layer with the headgroups oriented towards the aqueous medium. This second layer was made of the lipid to be studied (see Fig. 5) and was deposited in a fluid state (at 39.5 mNm^{-1} for A, T and DOPC, 37.5 mN/m for MeT. As the area per molecule changes upon deposition on the mica, the deposition ratios (i.e. the ratio between the area of the monolayer at the air/water interface that was deposited and the area of the mica) were measured and found to be equal to: 0.95 ± 0.10 for all molecules. These

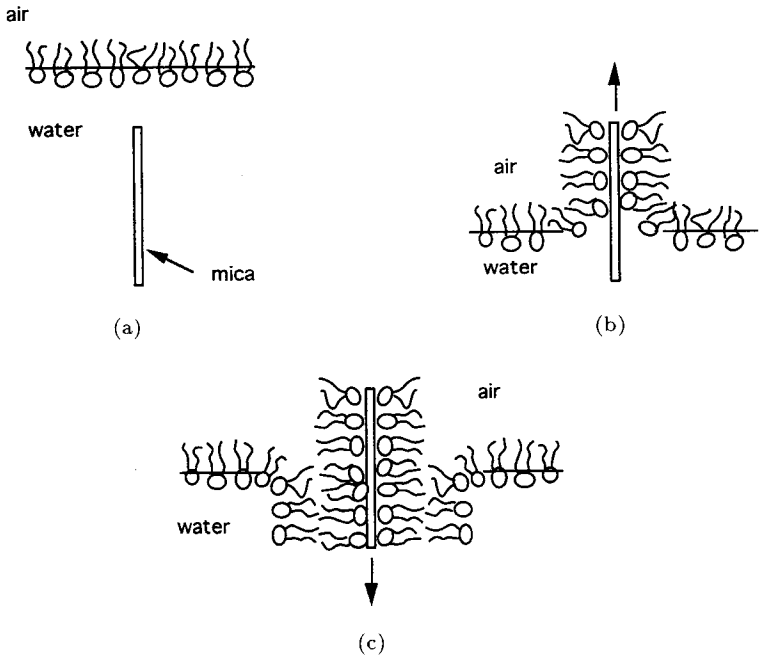

Fig. 5. Langmuir–Blodgett deposition of two monolayers on mica. (a) Substrate ready for monolayer deposition; (b) Uptake of the first monolayer upon the first crossing of the interface; (c) deposition of the second monolayer.

ratios are necessary to determine the number of deposited molecules on mica. The molecular areas of the deposited lipids are 0.56 nm^2 for T, 0.63 nm^2 for A and 0.51 nm^2 for MeT.

2.3. *Stability of the coated surfaces*

It is essential to be sure that the molecules were irreversibly bound to the surface to avoid any time dependent effect. The stability of a deposited monolayer should correlate with the above tested stability at the air–water interface. So we can expect our molecules to be irreversibly bound to the surface once deposited. To cross-check this point, the desorption of the functionalised lipids from the DMPE coated surfaces was directly measured: a first layer of DMPE and a second layer of functionalised lipid were deposited on a large mica sheet (several cm^2); this sample was then transferred under water in a large lipid free vessel (2 litres); after 4 hours (time scale of a force experiment), the sample was re-transferred in a Langmuir trough, and the lipids re-deposited to the air/water interface by emerging the mica sheet; the re-deposited lipid molecules could then be counted by surface pressure vs. area measurements; less than 10% of the lipids were lost which is within the experimental error for a such experiment. These results ensure that no significant desorption occurred during a force measurement.

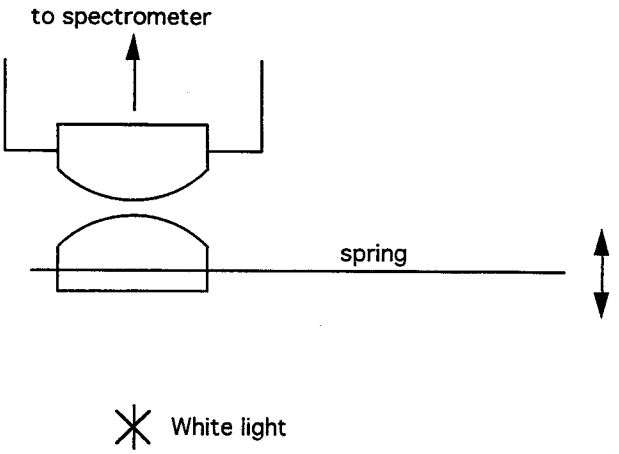

Fig. 6. Schematic representation of the SFA technique.

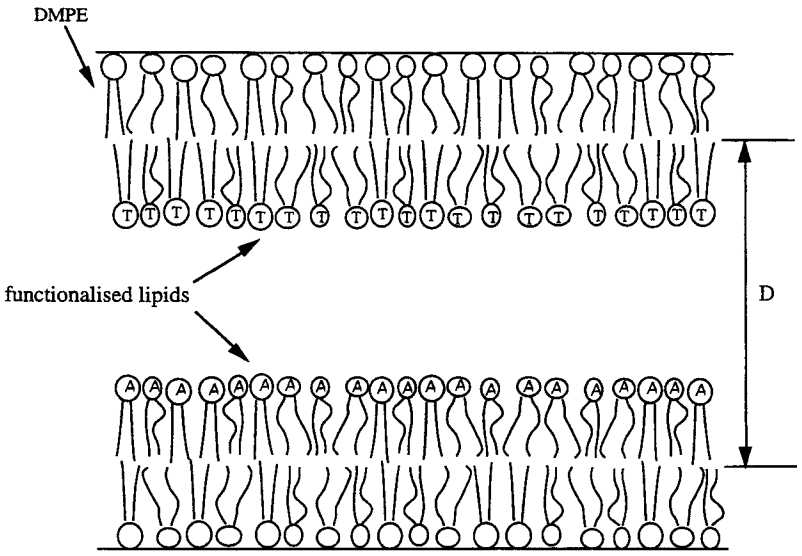

Fig. 7. Two complementary surfaces facing each other.

2.4. *Surface force apparatus*

To measure the free energy of interaction of two surfaces coated with nucleosides, we have used the surface force technique developed by Israelachvili.[7] This technique gives the force F between two mica surfaces in a crossed cylinders geometry (see Fig. 6), immersed in a liquid, as a function of the separation distance D. The distance D is that between the DMPE layers (see Fig. 7). The reference distance $D = 0$ is obtained by removing the outer layers and bringing into contact the two DMPE layers.

The distance between the surfaces is measured by multiple beam interferometry in white light. This also enables to obtain independently the refractive index of the intervening medium and the shape of the surfaces, including their radius of curvature R. In our set-up, the distance is measured with an accuracy of 10 pm by coupling the interferometric system with a home-made CCD camera. To keep the advantages of such an accuracy, molecularly smooth mica surfaces are used.

The force is determined by measuring the deflection of the spring on which one of the surfaces is attached. The accuracy is about 100 nN which becomes 0.01 kT in terms of free energy per molecule. The experiments are entirely computer-controlled. The crossed cylinders geometry is equivalent to a sphere/plane one if $D \ll R$ which is the case in the experiments. The free energy E of interaction per unit area, between plane parallel surfaces, is obtained from the measurements through the Derjaguine approximation: $F(D) = 2\pi RE(D)$.

This technique allows any repulsive force to be measured. In contrast, there are some limitations for the measurement of attractive forces. If the force gradient is larger than the spring constant, an instability appears leading to a jump (analogous to those occurring when two magnets are brought towards each other, one of which being suspended from a spring).

3. Nucleoside–Nucleoside Interactions

To evaluate the specific part of the A/T interactions, it is necessary to compare it with non preferential cases. Different situations were examined:

— two complementary surfaces: A/T.
— two "pseudo complementary" surfaces, i.e. A/MeT.
— two identical surfaces facing each other: A/A, T/T, MeT/MeT (symmetrical cases).

3.1. *General features*

The interactions were always attractive at all distances and in every case. The absence of double-layer repulsion in the symmetrical cases shows that all the surfaces are electrostatically neutral. Three features common to all the experiments were found in the energy-distance profile. Firstly, an attraction sets in from 60 nm and increases on approaching the surfaces until the force gradient becomes higher than the spring constant. At this point, the surfaces jump into adhesive contact of the two outer monolayers and flatten. Secondly, when the surfaces remain in contact, the distance decreases down to a value that corresponds to some loss of the lipids from the outer layer. This unusual behaviour will be discussed below as the "sticky fluid monolayer" behaviour. Thirdly, when the surfaces were pulled apart, the distance increased up to the size of fully-packed layers and the surfaces separated. The measurement of this "pull off force" gives access to the energy of adhesion.

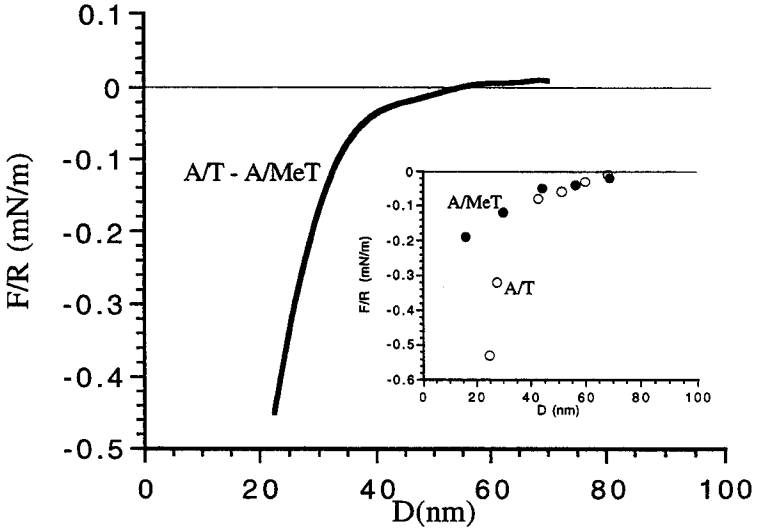

Fig. 8. Specific attraction between A and T (line) obtained by substracting the A/MeT interaction (closed circles) from the A/T interaction (open circles).

All these experiments were reproducible for several approaches of the same surfaces and also from one experiment to another.

3.2. *Range of the specific attraction*

MeT was designed in order to make a minimal change in T compatible with a hindering of the A/T pairing. It can be assumed that all non-specific (such as van der Waals) interactions remain unchanged by this modification. Therefore, the difference between the A/T and the A/MeT curves is a good estimation of the specific interaction (Fig. 8). The specific effects are noticeable below 38 nm. At this distance, an abrupt change in the slope can be noticed in the A/T curve (Fig. 8). The origin of this unexpected long range is not easy to determine. It is approximately two orders of magnitude greater than the size of the molecules. This implies that the range of the specific attraction cannot be due to the local geometry of the molecules. As the linkage between A and T is achieved through two H-bonds, the hypothesis of a water mediated specific interaction may be considered. However, as the correlation length in water is about 1 nm,[22] a given configuration of H-bonds cannot propagate in water at distances as large as 38 nm. This rules out the possibility for a mediation through water. Long-range attractions have also been measured between two mica surfaces made hydrophobic with a surfactant monolayer. Then it may seem reasonable to attribute such long-range forces to hydrophobic effects. MeT was obtained from T by removing one H-bond and replacing it by a more hydrophobic group, a CH_3 group. It is therefore more hydrophobic and would, if the hypothesis of a hydrophobic origin were valid, generate a stronger attraction. As this is not the case, this hypothesis is also to be rejected. Finally, it does not seem

possible for one single uncharged molecule to interact with another one at 38 nm distance. The origin is likely to result from the use of densely coated surfaces rather than single molecule.

3.8. *Binding energies and "pull off" forces*

The measurement of specific forces at a distance does not prove so far that these forces are the ones that drive DNA bases to establish hydrogen bonds and to form pairs. One way to ensure it is to check that the adhesion energies obtained from these measurements are compatible with the already known binding energies of nucleosides for A/T, T/T and A/A. This can be achieved in a straightforward manner by dividing the adhesion free energy by the number of bases involved.

The Johnson, Kendall and Roberts theory[23] (known as JKR theory) predicts that the force F necessary to separate a sphere of radius R in contact with a plane (i.e. the pull off force, see Sec. 3.1) is related to the adhesion free energy E per unit area of their surfaces by $F = 3\pi Re/2$. It also predicts that the surfaces are flattened just before separation. Another theory proposed by Derjaguine, Muller and Toporov[24] (known as DMT theory) predicts that the area of contact before separation falls down to zero, and that the relation between F and E is: $F = 2\pi RE$. Maugis[25] has reconciled these two theories by describing the JKR to DMT transition, ascribing the JKR behaviour to deformable surfaces with a strong adhesion, and the DMT behavior to the opposite case. Tangential and non-tangential contact profiles refer respectively to the DMT and JKR regimes.

Table 1. Values of F/R at separation, of the adhesion energy, of the density of molecules on the surfaces and of the binding energy. F/R at separation is the "pull off forces" divided by the radius of curvature of the surfaces. The adhesion energy is deduced from F/R at separation, and the binding energy is deduced from the adhesion energy and the density of molecules.

	A/T	T/T	A/A
F/R at separation ($mN.m^{-1}$)	110	43	50
Adhesion energy ($mJ.m^{-2}$)	23.3	9.1	10.6
Density of molecules (nm^{-2})	1.58	1.78	1.58
Binding energy (kcal/mole)	2.1	0.7	1.0

As the nucleoside coated surfaces always flatten in contact with a non-tangential profile, the relevant theory to quantify the adhesion energy is the JKR one. The pull off forces divided by the radius of curvature of the surfaces are reported in Table 1 as well as the adhesion energies deduced from JKR theory. Assuming a one to one pairing, the binding energies can be deduced from the adhesion energy and

the density of molecules. These values are reported in Table 1. In the A/T case, the relevant density is that of A because it is the lowest (see Table 1).

In the symmetrical cases the surfaces could be indefinitely approached, contacted and separated with no measurable change in the forces. The A/T adhesion energy is the same within 10% as that of hydrophobic DMPE coated mica surfaces inducing a tearing of the layers after 70% of the established contacts.

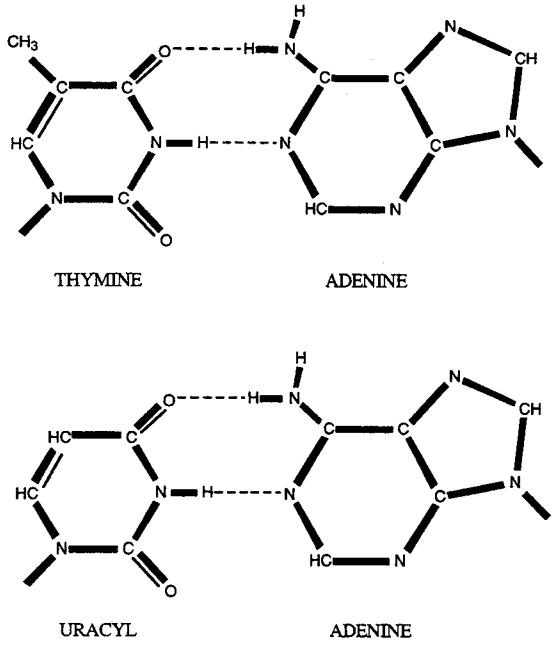

Fig. 9. Watson–Crick pairs of DNA bases: T–A and U–A.

Table 2. Comparison of the binding energies obtained by SFA and by thermodynamical methods. Comparison of the values obtained by SFA and by quantum mechanical calculations for the differences between the A/T, A/A and T/T values.

	SFA values (kcal/mole)	Other methods (kcal/mole)
		Thermodynamical:
A/T	2.1	2.2 (A/U)
		Quantum mechanical:
A/T–A/A	1.2	1.4
A/T–T/T	1.4	1.8

The binding energies given by this direct method can be compared to values obtained by thermodynamical methods and by quantum mechanical calculations in vacuo (see Table 2). The adenine-uracyl binding energy was experimentally determined by Tinoco *et al.*[13] who obtained 2.2 kcal/mole. Uracyl has the same H-bonds

configuration as T, and binds to A in the same manner (Fig. 9). Quantum mechanical calculations were performed for the interaction in vacuo, and gave different binding energies from those obtained in water as water molecules can establish hydrogen bonds with the bases too. Comparing the differences between each case in water, to those in vacuo is nevertheless possible. These differences are in good mutual agreement (see Table 2).

As the area of the flattened contact zone can be measured, it is possible to estimate the number of pairs that bind the surfaces in contact. The force necessary to break one bond can then be directly deduced from the knowledge of the "pull off" force. The contact zone is a circle of radius ≈ 3 μm. With a "pull off" force of 2.2 mN for A/T and an area per molecule of 0.63 nm^2, the force to rupture a single A/T bond is 49 pN. The interaction of an adenine coated AFM tip with a thymine coated surface has recently been performed.[26] These results give 54 pN for the rupture of one A/T bond, a value very close to our estimation.

One may wonder which of the Watson–Crick and the Hoogsteen configuration is favoured by the geometry of the SFA experiments. As the binding energies are not very different, one may not obtain the answer from the measurements. Figure 10 shows that the Hoogsteen configuration is less likely to occur than the Watson–Crick one because of the geometry of the molecules.

3.4. *Sticky fluid monolayers*

The contact distance D_c corresponds to two fully packed functionalised lipid layers ($D_c = 4.5$ nm). The surfaces are flattened (see Fig. 11). With time and compression the distance decreases down to about 2 nm (see Fig. 12). However, under compression, fusion is never observed on the time scale of one hour. When a force to separate the surfaces is applied and increased, the distance remains constant within 0.3 nm. Above some pulling force threshold, it increases, with a sharp discontinuity in the $F(D)$ curve, to reach D_c just before the surfaces jump apart. Surface forces between lipid monolayers which have both a fluid state and a strong adhesion have never been investigated before. This behaviour, hereinafter called "sticky fluid behaviour", is never observed with usual lipids such as lecithin[4,27–29] for which either fusion occurs after its nucleation and rapid spreading over the contact zone or no distance decrease is observed. The MeT monolayers are much less stable than the other functionalised lipid monolayers since they fuse in the experiments in which they are involved. A naive explanation involves both the fluid character of the monolayer and the strong affinity between the nucleoside molecules. When in contact, the lipids form complexes in which many H-bonding sites are occupied (see Fig. 12), in a similar manner as in Ref. 30. These complexes are more hydrophobic than the individual species; instead of behaving like the supramolecular aggregates that they formed as individual monolayers, they may collectively behave more like paraffin: they flow under pressure and gather into a micromeniscus at the edge of the contact zone. As the binding energy of nucleosides is higher in organic media

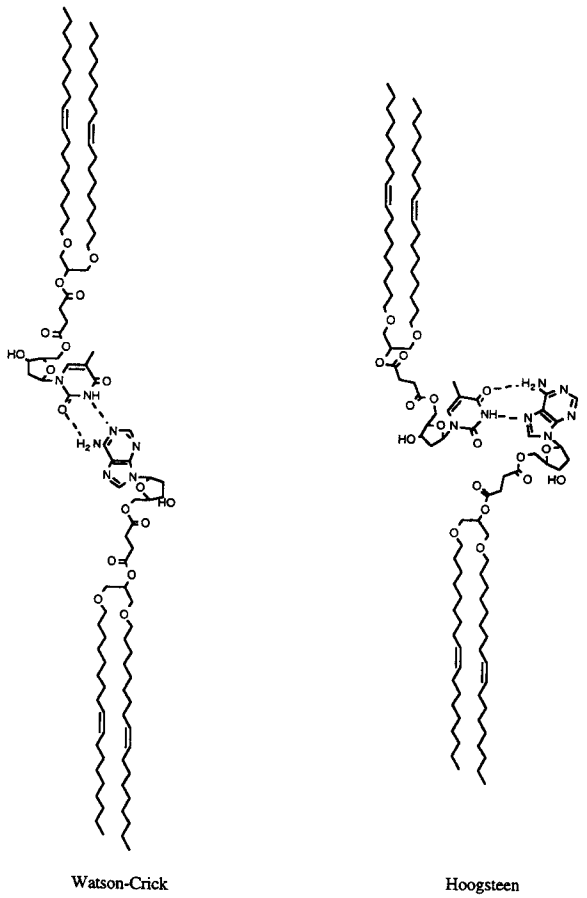

Fig. 10. The functionalised lipids in the Watson–Crick and Hoogsteen configurations.

than in water,[12] the stability of the complexes is enhanced by the paraffinic character of their environment. Upon applying a force of separation, the lipids return to the contact zone under the field of force generated by the two hydrophobic DMPE surfaces and the two outer monolayers retrieve their initial structure. Adenine and thymine functionalised lipid layers in a gel phase do not produce this behaviour while they also have a strong adhesion.[31] This result, in addition to the fact that DOPC (which has the same unsaturated hydrocarbon chains) does not produce the sticky fluid behaviour, supports this hypothesis.

3.5. *Long range attractions*

The above mentioned features result in a large part from the specific A/T interaction. However, a surprising long-distance attraction observed in all the cases sets in at a separation of 60 nm. A/A shows the largest effect (see Fig. 13).

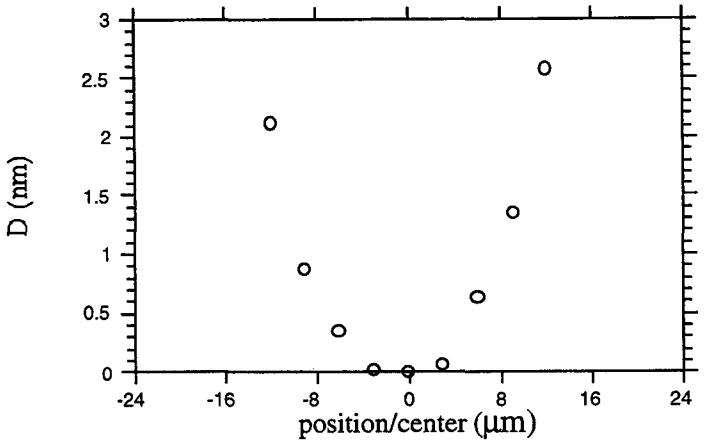

Fig. 11. Shape of the surfaces in contact.

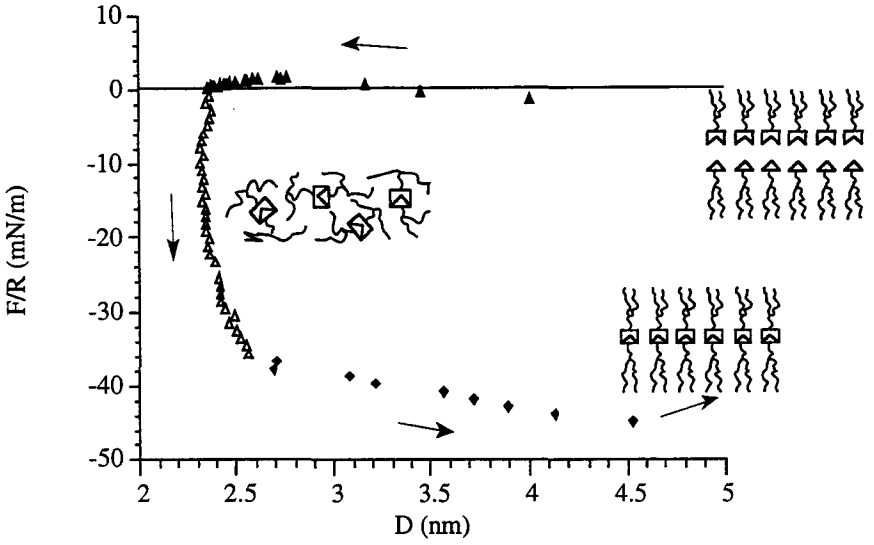

Fig. 12. Behaviour of two contacting surfaces in sticky fluid behaviour.

With the polar heads of the functionalised lipids facing the solution, the surfaces are hydrophilic. They are not charged since no repulsion occurs in the A/A and T/T cases. Such long range attractions have never been observed between two hydrophilic neutral surfaces: usual uncharged lipids in the fluid or gel state have an attraction at less than 10 nm distance (see Fig. 13).

The attraction between nucleoside lipids is very similar in range and magnitude to that between two surfaces made hydrophobic by a monolayer of lipids deposited

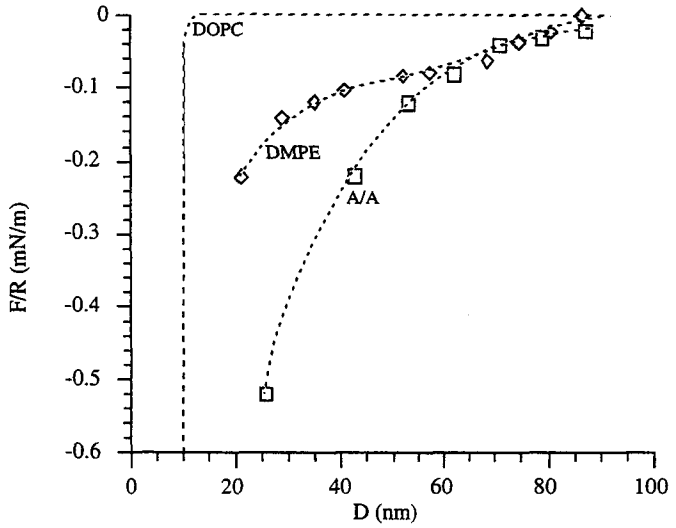

Fig. 13. Comparison of DOPC/DOPC van der Waals attraction with the long range A/A or DMPE/DMPE attractions.

by the Langmuir–Blodgett technique (see Fig. 13). This attraction that has often been referred to as "hydrophobic attraction" was not observed between other hydrophobic systems, such as plasma polymerised mica surfaces,[32] Its controversial origin has recently been discussed[6] and it occurs only when the mica is coated with amphiphilic monolayers in a crystalline state. This led the authors to the conclusion that this ordering is responsible for the long-range attraction which results from polarised domains of the layers which correlate their orientation from one surface to another. According to this explanation, long-range attractions could also exist between two hydrophilic surfaces. The similarities suggest that both "hydrophobic attractions" and nucleoside ones may have the same origin, i.e. a two-dimensional ordering of the monolayers. The functionalised lipids have been designed to give fluid state monolayers which should hinder any such arrangement. However, the stacking of DNA bases may induce a bidimensional arrangement. This was observed with soluble adenine and thymine adsorbed on epitaxed gold.[33] With its two cycles, adenosine has a higher stacking free energy ($\Delta G = -1.00$ kcal/mole) than thymidine ($\Delta G = 0.06$ kcal/mole). This correlates with a larger attraction for A/A than for T/T. Stacked structures are known to be most favoured in aqueous solvents.[34] Long-range attractive forces between gel state monolayers of thymine lipids were also observed[31]; in this reference, the adenine lipid is charged and the long-range attraction is masked. All these facts support the description of these forces in terms of bidimensional ordering of the layers.

The A/T curve shows a sudden change in the gradient at 38 nm, absent in the A/A curve, which indicates the transition between the long-range attraction and the

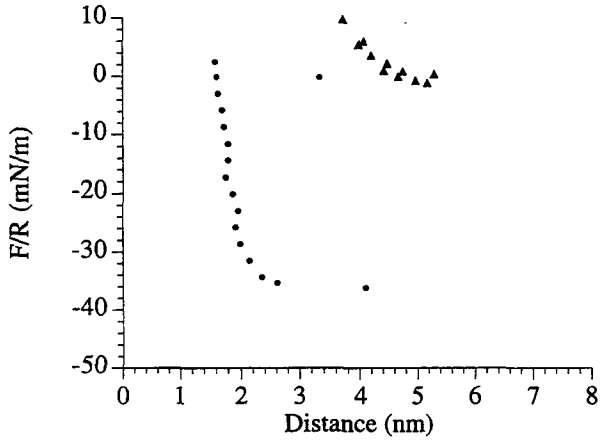

Fig. 14. Interactions between mixed T/DOPC layers (50/50 mol/mol).

specific regime. This discontinuity indicates that both attractions obey to different mechanisms.

3.6. *Phase separation in mixed monolayers*

A dilution of T with a non functionalised lipid, DOPC, was investigated. Monolayers containing a 1:1 mixture of T and DOPC were deposited and the forces measured between two such identical surfaces. Two distinct types of behaviour (see Fig. 14) were obtained: T/T interaction (25% of the cases) or DOPC/DOPC interaction (75% of the cases). This suggests an unmixing of the lipids in the monolayer. The mixed monolayers were spread on water in two different ways: either from a mixture in chloroform solution or by spreading the T first and the DOPC subsequently. In both cases, the same behaviour was observed.

4. New Specific and Non-Specific Effects

DNA bases can associate by forming H-bonds and by stacking. Used as polar heads of lipids they also can self assemble. All of this can give rise to a rich variety of molecular assemblies and their related interactions. Using a surface forces apparatus on this system has not only provided the values of already known parameters, but also measurements of new phenomena. To our knowledge the binding energies of A/A and T/T, although already calculated by quantum mechanical methods, have never been measured. The range of the specific A/T forces in water is as large as 38 nm and it does not resemble any known surface force. The results also show that without the size effect existing in DNA, H-bonds alone can generate the specificity. The non-specific long range attraction occurring between the nucleoside covered surfaces was also unexpected and its origin remains unclear. It resembles the unexplained attraction between some hydrophobic surfaces and may help to elucidate it. Contacting two fluid surfaces bearing groups that can strongly bind to

each other provided the opportunity to observe the formation of an isotropic liquid made of the molecular complexes in a confined medium.

Performed with the simplest molecular recognition system, this approach has given a wide range of physical phenomena. It has also shown that a small chemical change can noticeably modulate the interactions. This approach can be extended to more complicated functionalised molecules such as those involved in cell adhesion and in cytoskeleton. The understanding of key-lock interactions will contribute to the development of applications ranging from targeted drug delivery systems and anti-adhesive therapies to biosensors.

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