

## FARADAY COMMUNICATIONS

## Long Range H-Bond Specific Interactions between Nucleosides

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Molecular recognition, of which nucleoside pairing is the best known example, usually involves hydrogen bonding. The binding energies of nucleosides have previously been measured, but the range of the specific forces is not known, and so is measured here. The range is of interest because it is presumably related to the dynamics of association. This is not only of intrinsic interest, but also potentially important for applications in biomolecular engineering.

The surface-forces apparatus (SFA)<sup>1,2</sup> allows the direct measurement of surface interactions and their range.<sup>3</sup> This technique gives the interaction energy between two surfaces as a function of their separation which, in our system,<sup>4</sup> is measured with a precision of  $\pm 0.01$  nm. Using this technique, we have studied surfaces covered with layers of nucleosides, either adenosine (A) or ribosylthymine (T), at known densities (Fig. 1). For this purpose, we used two lipids which have T or A as a headgroup, and another which has a methylated ribosylthymine (MeT) as a headgroup.

The new lipids were synthesized by coupling the unprotected nucleosides with 2-[1,3-di(oleoyloxy)propyl]hemisuccinate {2-[1,3-di(octadec-9-enyloxy)propyl]hemisuccinate} using a modified DCC/DMAP method.<sup>5</sup> They were deposited<sup>6,7</sup> by the Langmuir–Blodgett technique onto mica surfaces. Each mica surface was covered with two different monolayers. First, a monolayer of dimyristoylphosphatidylethanolamine (DMPE: purchased from Avanti Polar Lipids, USA) was deposited onto the mica (at a surface pressure of  $42 \text{ mN m}^{-1}$ ) to make it hydrophobic. Then, the lipid to be studied was deposited (at  $38.5 \text{ mN m}^{-1}$ ) so that its polar headgroup faced the solution.

The molecules were irreversibly bound to the surface. As a test, we have directly measured the desorption of the functionalised lipids from large mica sheets coated as in the experiments by immersing them in a large vessel of pure water (2 l) and, after several hours, retrieving the molecules. The desorption was smaller than 10% (the error bar) in 4 h (the force measurements are generally performed 2 h after the

lipids are deposited), which is not enough to exhibit a measurable hydrophobic effect. We have also measured the desorption of these lipids at the air/water interface.<sup>3</sup> After 8 h, the desorption was  $4.5 \pm 0.3\%$  for A. The high reproducibility in time of the force–distance profiles confirms that no significant desorption occurred.

A Langmuir trough was used to measure compression isotherms<sup>6,7</sup> for each surfactant, to perform the Langmuir–Blodgett depositions and to determine the deposition ratio of the molecules from the water to the mica surface. From the deposition ratio we calculate the deposited surface density of the lipids. The high surface pressure produces a high surface density: the molecular areas on mica are  $0.63$ ,  $0.56$  and  $0.51 \text{ nm}^2$ , for A, T and MeT, respectively. This ensures that the nucleosides on one surface are oriented towards those on the opposite surface. However, the pairing is possible only if the nucleosides can take one particular position and orientation of one relative to the other, which requires some orientational and translational freedom. The lipids were therefore synthesized with a succinyl spacer between the nucleosides and the hydrocarbon chains, and the latter were unsaturated so that the surface density corresponds to a fluid state of the layer. We therefore expect the bases to be free to rotate about the normal to the surface, and to translate within the layer.

In a recent paper,<sup>3</sup> we have measured T–T, A–A and A–T interactions in water using a similar technique. In all cases, the nucleoside interactions were attractive over the measurable range (in contact and 30 nm above). The adhesion energies and thus the binding energy per pair of molecules were obtained ( $2.5 \text{ kcal mol}^{-1}$  for A–T,  $1.2 \text{ kcal mol}^{-1}$  for A–A and  $0.9 \text{ kcal mol}^{-1}$  for T–T). These values are in good agreement with those obtained by other methods<sup>3</sup> and show that the A–T attraction is strongest at short distances. However, at longer distances (between 30 and 60 nm), the A–A attraction is the strongest. The A–A and A–T interaction curves must intersect somewhere between 0 and 30 nm, although our previous technique did not allow us to measure forces at closer separation. For the current study, we have improved the technique to allow measurements down to a separation of 15 nm.

The range over which the A–T attraction becomes dominant is especially important because it is related to the range of the A–T specific interaction. The A–T and A–A force–distance curves intersect at  $20 \pm 4 \text{ nm}$  (Fig. 2). The slope of A–A varies more smoothly than that of A–T, for which a large variation in slope occurs at  $38 \pm 4 \text{ nm}$ .

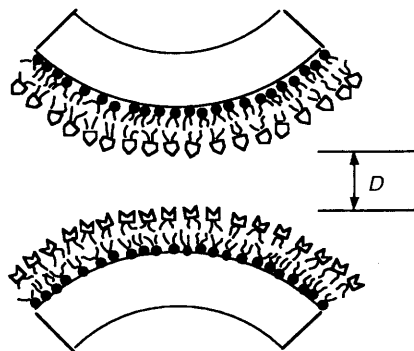
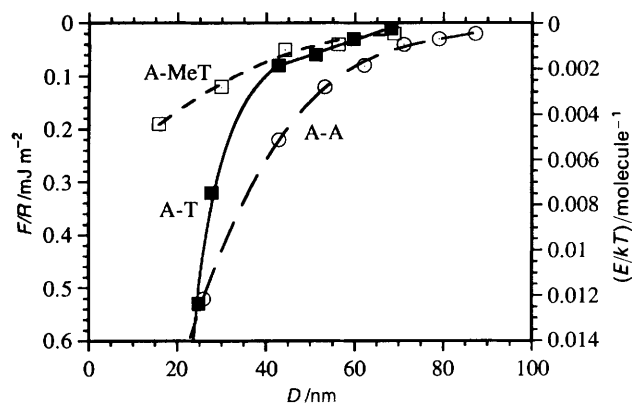


Fig. 1 Configuration of the coated surfaces: lipids whose polar heads are nucleosides were synthesized and deposited as the outer layer of a bilayer on mica using the Langmuir–Blodgett technique<sup>6,7</sup>



**Fig. 2** Variation of the internucleoside interaction vs. distance: the ordinate shows the interaction energy between two flat parallel surfaces. On the right-hand side of the graph, this energy has been divided by the density of A, and expressed in  $kT$  units where  $k$  is Boltzmann's constant and the temperature  $T$  is  $20^\circ\text{C}$ .  $D$  is the distance between the nucleosides. (■) One surface coated with A and the other with T; (○) both surfaces coated with A; (□) one surface coated with A and the other with MeT. The lines are guides for the eye.

The intersection and the shapes of the A-A and A-T curves suggest that the A-T interaction has two different regimes in contrast to A-A. For separations above 38 nm, all curves are attractive and similar in shape. This suggests a long-range non-specific attraction. The second regime occurs below *ca.* 38 nm where the A-T interaction shows a much stronger dependence on separation. This suggests that a specific A-T attraction begins to operate at a range of at least 38 nm.

According to this hypothesis, one would expect the second regime to disappear if the specific pairing were precluded by modification of either A or T. To test this prediction, MeT was synthesized. In this molecule, the hydrogen which forms an H-bond with adenosine in both Watson-Crick and Hoogsteen pairing is replaced by a methyl group.

The A-MeT and A-T interactions were similar at separations  $>38$  nm. However, below 38 nm, the A-T attraction started to increase much faster than the A-MeT one (Fig. 2). Further, the A-MeT curve did not intersect the A-A curve.

In pure water, the A-T interaction may thus be considered specific to a range of 20 nm, but the total range of the interaction giving rise to this specificity is rather greater: perhaps as great as 38 nm. The nature of this long-range specific force is unknown. Mediation by water is unlikely because the correlation length of water<sup>8</sup> does not exceed 1 nm. Local molecular geometries should also be unimportant on this scale. Electrostatic interactions are excluded because the T-T interactions (not shown) are attractive. We stress that the interaction we report is measured between surfaces and not between two individual molecules. In these macroscopic surfaces, the collective molecular behaviour<sup>3</sup> of large numbers of molecules is possible and this may contribute to the long-range interaction.

The range of this apparently specific interaction is much larger than that expected in a system whose chemical specificity is primarily due to H bonds. Among the many surface interactions investigated in colloid science,<sup>1,9</sup> no similar attraction has been reported.

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