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Peculiarities of the Interaction of Short Oligonucleotides with Supported Lipid Films and *Langmuir* Monolayers

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Summary. The method of electrostriction was applied to supported bilayer lipid membranes (sBLM) and *Langmuir* monolayers with the aim to study the peculiarities of the interaction of short oligonucleotides with lipid films and of the duplex formation between complementary oligonucleotides. The bilayer lipid membranes (sBLM) were formed on an agar support, whereas *Langmuir* monolayers were generated on the air-water interface. As an oligonucleotide, the 15-mer 5′-cholesterolphosphoryl-*dT15* (*CHpdT15*) was synthesized. We could show that the interaction of *CHpdT15* with sBLM resulted in a considerable increase of the elasticity modulus perpendicular to the membrane plane ($E_\perp$) and an increase of the surface potential. Interaction of complementary oligodeoxyadenylate (*dA15*) with sBLM modified by *CHpdT15* resulted in a slight increase of the surface potential whereas $E_\perp$ slightly decreased. *CHpdT15* forms monomolecular layers on the air/water interface. Interaction of *dA15* with such monolayers resulted in an increase of the surface pressure, probably due to an increase of the surface charge of the monolayer; similar effects were observed for lipid monolayers modified by *CHpdT15*. Prospects of using such interactions for detecting *DNA* hybridization are discussed.

Keywords. Bilayer lipid membranes; Monolayers; Electrostriction; Surface potential; Oligonucleotides; Hybridization.

Introduction

The diagnostics of infectious diseases and gene detection require fast and easy-to-use methods for the determination of *DNA* hybridization [1, 2]. A considerable effort in this direction has been made by means of electrochemical [3, 4] and

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acoustic [5–10] methods. The latter commonly involve the quartz crystal microbalance (QCM) technique. Optical methods [11, 12] and the surface plasmon resonance technique [13] have also been used for the detection of *DNA* hybridization. A new and attractive approach for fabricating *DNA* biosensors consists of the application of free suspended bilayer lipid membranes (BLM) [14] and supported bilayer lipid membranes (sBLM) [15, 16]. In this case, the oligonucleotide is chemically modified by a hydrophobic anchor that immobilizes the *DNA* on the surface of the lipid film. It has been shown that various membrane properties, such as conductance [15, 16] or capacitance relaxation [16], influence hybridization.

The interpretation of the results of hybridization detection using lipid films requires knowledge of the mechanisms of interaction of oligonucleotides with sBLM or monolayers. In this work we therefore studied the peculiarities of interaction of short oligonucleotides with supported lipid films and lipid monolayers. For this purpose we first chemically modified the oligodeoxythiolate *dT15* by cholesterol. The obtained 5′-cholesterolthiophosphoryl-*dT15* (*CHpdT15*) was then used as a probe for anchoring single-stranded *DNA* to sBLM *via* the cholesterol residue. It was shown that *CHpdT15* interacts with sBLM and lipid monolayers and is even able to form monomolecular layers at the air-water interface. The interaction of *CHpdT15* with sBLM on agar support resulted in a considerable increase of the elasticity modulus perpendicular to the membrane plane ($E_\perp$); an increase of the surface potential of sBLM was also observed. The interaction of the complementary oligodeoxyadenylate (*dA15*) with sBLM modified by *CHpdT15* resulted in a further increase of the surface potential, whereas $E_\perp$ slightly decreased. Interaction of *dA15* with lipid monolayers modified by *CHpdT15* or with monolayers formed by *CHpdT15* resulted in an increase of the surface pressure of the monolayer. The implications of these observations on the biosensing of *DNA* hybridization are discussed.

Results and Discussion

The unmodified sBLM of *DOPC* + *HDA* formed in 1 *M* KCl were characterized by $E_\perp = 4.0 \pm 0.2 \times 10^7$ Pa, $C_s = 4 \times 10^{-3}\,\mathrm{F/m^2}$ (thickness: ~ 4.6 nm), and $\Delta\Phi_m = 32.2 \pm 5.0$ mV. The potential difference can be explained by poorly matched Ag/AgCl electrodes used as reference. A typical plot of the kinetic changes of elasticity modulus, membrane capacitance, and surface potential ($E_\perp$, C_s, and $\Delta\Phi_m$) are presented in Fig. 1. Addition of *CHpdT15* to the electrolyte resulted in a sharp increase of $E_\perp$ by a factor of 1.6, whereas C_s started to increase after an initial decrease (Fig. 1a). Incorporation of oligonucleotide was accompanied by sharp changes of the surface potential (~ 21 mV, Fig. 1b). The addition of the complementary chain (*dA15*) had no substantial effect on the measured values except a slight increase of surface potential and membrane capacitance and a decrease of the elasticity modulus. The characteristic time required for the changes was approximately 30 s.

The increase of $E_\perp$ might be due to at least two effects: (*i*) incorporation of the cholesterol residue into the membrane and condensation effect of cholesterol on the lipid bilayer, (*ii*) and increase of ordering of the polar part of the membrane due

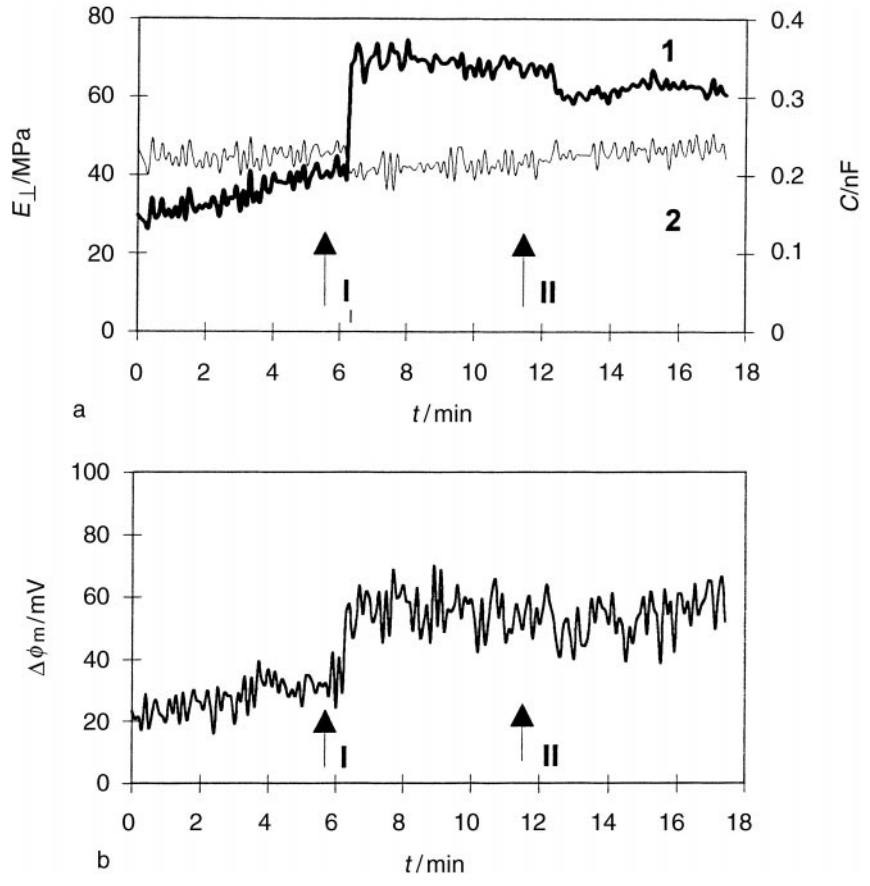

Fig. 1. Kinetics of changes of a) elasticity modulus ($E_\perp$, 1) and capacitance (C, 2) and b) surface potential ($\Delta\Phi_m$) of sBLM following addition of *CHpdT15* (I) and *dA15* (II) at a final concentration of 100 n*M*; electrolyte: 1 *M* KCl+10 m*M* HEPES, $pH = 7$

to electrostatic interaction of opposite charges (positive surface charge of the membrane surface and negative charge of the oligonucleotide). The condensing effect of cholesterol on membranes [26], resulting in an increase of elasticity modulus [27], is well known. Another effect contributing to the increase of $E_\perp$, *i.e.* an increase of the compressibility of sBLM perpendicular to the membrane plane, might be a decrease of repulsive forces between positively charged *HDA* molecules at the polar part of the membrane due to incorporation of negatively charged oligonucleotides.

In order to obtain more information about the adsorption of oligonucleotides on sBLM, we determined surface charge densities of sBLM in shielding experiments. For this purpose the sBLM were formed at low electrolyte concentrations (10 m*M* KCl + 10 m*M* HEPES, $pH = 7$). The electrolyte concentration in the teflon tube was 0.11 *M* KCl. The increase of ionic strength of the electrolyte in which the membrane was formed allowed us to determine the surface charge density of sBLM. An increase of ionic strength should result in a decrease of the *Gouy-Chapman* potential due to shielding of surface charges by ions [22]. Therefore, for sBLM formed initially at asymmetrical electrolyte conditions (*i.e.* 0.01 *M*/0.11 *M*) we

should expect a decrease of the overall surface potential following the achievement of symmetrical condition (*i.e.* 0.11/0.11 *M*). The degree of decrease of this potential will depend on the surface charge density. Therefore, we can determine the surface charge density (see Ref. [22] for detailed procedure). We showed that unmodified sBLM of *DOPC+HDA* was characterized by surface a charge density of 0.096± 0.01 e/nm^2, *i.e.* one elementary charge per 10.4 nm^2. Addition of *CHpdT15* in its final concentration of 100 n*M* resulted in an increase of the surface potential by 20.9 mV corresponding to the appearance of a negative surface charge on the membrane with a density of 0.83 ± 0.06 e/nm^2. The charge Q carried by one single stranded *DNA* molecule with n anionic phosphate groups is given by $Q = 2ne_0 f$, where $e_0 = 1.6 \times 10^{-19}$ C is the elementary charge, the factor 2 refers to the contribution of both cation and polyanion, and $f \approx 0.4$ is the reduction factor due to counterion screening in the relative motion of the cations and the polyanion [28]. Hence, for a 15-mer $Q = 12e_0$. Considering the area of sBLM of approximately 2×10^{-3} cm^2, there are *ca.* 1.4×10^{10} molecules of *CHpdT15* on the membrane surface. This is about 40 times lower than in the case of chemisorption of thiol-modified *DNA* onto gold support [29]. Addition of *dA15* only slightly increases the surface potential (by ∼4 mV), slightly decreases $E_\perp$, and increases C_s (Fig. 1). A possible reason for the weak effect of hybridization of *DNA* on the membrane potential could be connected with changes of charge distribution at the BLM surface. Certainly, for single stranded *DNA* we could expect random conformations of oligonucleotide chains with a preferred orientation parallel to the membrane surface (due to the positive surface charge of BLM containing hexadecylamine there should exist an electrostatic attraction between oligonucleotides and the sBLM surface). Double-stranded *DNA* formed due to hybridization is, however, characterized by a large persistent length (∼50 nm) [30]. Therefore, the *DNA* molecules should change conformation and orientation from random to extended conformation. Very probably, the double-stranded *DNA* should be oriented perpendicular to the membrane plane. Changes of orientation will result in a decrease of attractive forces between negative charges of *DNA* and positive ones at the sBLM surface. Due to this process, the increased negative charge of *DNA* will be compensated by not shielded positive charges of the sBLM.

The *P-A* isotherm of monolayers composed of an oligonucleotide modified chemically with cholesterol (*CHpdT15*) represents a monotonic function (Fig. 2, curve 1). The surface pressure increases with a decrease of area per molecule. Addition of complementary oligonucleotide resulted in a shift of the isotherm towards higher area per molecule, *i.e.* the surface pressure increased. This points to an increase of repulsive forces at the monolayer due to increasing surface charge density. Addition of non-complementary oligonucleotides containing only one adenine (*A1T8C6G7*) had a weaker effect on the *P-A* isotherm. However, the tendency to increase surface pressure is similar to that of fully complementary chains. Evidently, the non-specific interaction of *DNA* is strong enough to induce significant changes in surface pressure.

The *P-A* isotherm of lipid monolayers composed of a mixture of egg*PC*, *HDA*, and cholesterol has a typical shape (Fig. 3, curve 1) characteristic for egg*PC* monolayers containing cholesterol [31]. Up to a surface pressure of 35 mN/m the typical condensation isotherm was observed; above this value, fracture of the

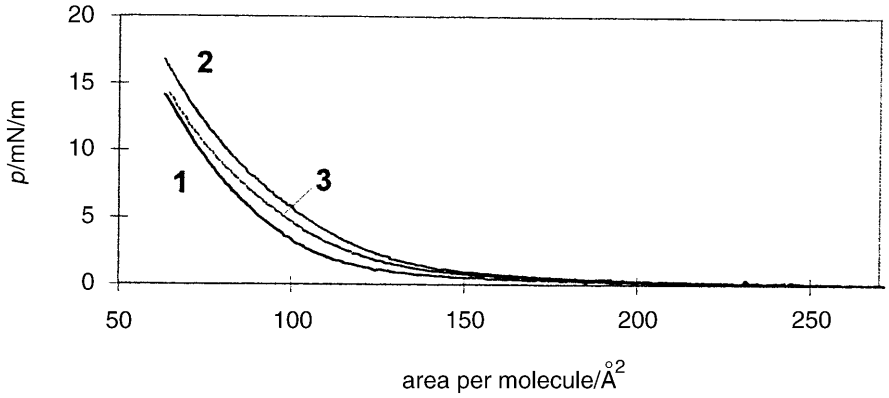

Fig. 2. *P-A* isotherm of molecular films composed of oligonucleotide *CHpdT15* (1) and in the presence of 0.17 n*M* complementary oligonucleotide *dA15* (2) or 0.17 n*M* non-complementary oligomer d(*A1T8C6G7*) (3)

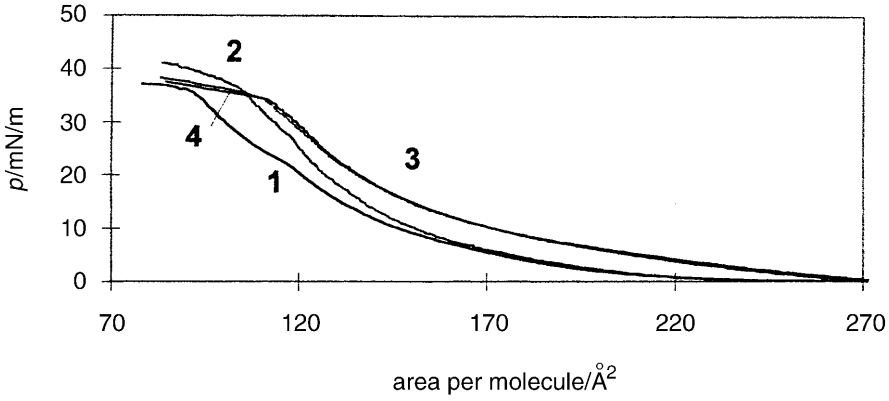

Fig. 3. *P-A* isotherm of (1) an unmodified monolayer composed of phospholipid mixture (egg*PC*+cholesterol+*HDA*); (2) lipid monolayer as in (1) modified by *CHpdT15*; (3) in the presence of 0.17 n*M* complementary oligomer *dA15*; (4) in the presence of 0.17 n*M* non-complementary oligomer d(*A1T8C6G7*)

monolayer took place. Addition of *CHpdT15* had no substantial effect on the shape of the *P-A* isotherm up to a surface pressure 10 mN/m; however, a significant increase of the pressure was observed at further compression of the monolayer (curve 2). Also, the fracture of the monolayer started at a higher area per molecule (90 Å^2) and at slightly higher pressure than that for non-modified lipid monolayers ($\sim$75 Å^2). A further increase of surface pressure took place following the addition of *dA15* to the subphase (curve 3). The *P-A* isotherm corresponding to the addition of a non-complementary chain (*A1T8C6G7*, curve 4), however, did not differ significantly from that affected by the complementary oligonucleotide. These isotherms were also characterized by a further shift of the fracture of monolayer towards a higher area per molecule. We should also note that a variation in the fracture pressure observed in experiments with lipid monolayers (Fig. 3) is characteristic for miscible components [32]; the miscibility of egg*PC* and cholesterol as well as various phospholipids with not extremely long chains in

monolayers has been proved earlier [31]. Differences in fracture pressure due to interaction of oligonucleotides with monolayers therefore cannot be explained by lipid or cholesterol phase separation in the lipid monolayer. The increase of the surface pressure following the modification of the lipid monolayer by oligonucleotides could be attributed to electrostatic repulsion between negatively charged oligonucleotides. The increase of surface pressure of the monolayer composed of neutral phospholipid diphytanoylphosphatidylcholine following addition of plasmid *DNA* (5.6 kbp) has been reported also by *Spassova et al*. [33]. This effect has been less expressed than in our case (probably due to a lack of positive charges at monolayer that facilitate the adsorption of negatively charged *DNA*) and was explained by interaction of *DNA* with lipid head groups, leading to a less dense lipid packing. The authors of Ref. [33] stated that in the *DNA*/lipid complex there is a large distance between the lipids, thus causing an increase in the surface pressure. This effect was much stronger than the condensation effect of cholesterol that would be expected when *CHpdT15* is incorporated into the monolayer. In this respect, much of the effect of *CHpdT15* on sBLM can be explained by the considerably higher oligonucleotide concentration (more then 10^4 times) mode possible by the considerably smaller volume of the measuring chamber ($\sim 0.5\,cm^3$ in case of sBLM experiments). We can, however, expect a different behavior of the *P-A* isotherm by variation of *HDA* and oligonucleotide concentration in the lipid monolayer. Accordingly, the application of sBLM and monolayers for detecting hybridization would require optimization of the film composition and oligonucleotide concentrations.

Conclusions

The approach based on supported lipid films (sBLM) and *Langmuir* monolayers allows to study some peculiarities of the interaction of oligonucleotides with lipid bilayers and monolayers. Application of sBLM could be useful for detecting the interaction of chemically modified oligonucleotides with lipid films. However, the *DNA* hybridization event only slightly influences the physical properties of sBLM and monolayers under the used experimental conditions. Hence, the use of sBLM for transducing the hybridization of oligonucleotides requires further effort in order to optimize chemical composition of sBLM as well as the chemical modification of oligonucleotides.

Materials and Methods

Modification of oligonucleotides; chemicals

5′-Cholesterolthiophosphoryl (*CHpdT15*) and its complementary oligodeoxyadenylic acid (*dA15*) were synthesized in *Rosenberg*'s laboratory according to a methodology described earlier [17, 18]. *dT15* and *dA15* were prepared by the phosphoramidite method. A cholesteryl residue was introduced at the 5′-end of oligothymidylate by the *H*-phosphonate methodology using cholesterol-(*H*)-phosphonate and adamantanecarbonyl chloride as condensing agent. Purification of *CHpdT15* after deblocking with aqueous ammonia was achieved by reversed phase chromatography on a Dynamax 300 C18 column (10×250 mm, $3\,cm^3$/min, 0–40% CH_3CN in 0.1 *M* triethylammonium acetate,

$pH = 7.1$, 40 min). All oligonucleotides were finally desalted on a Sephadex G25 column (5 cm^3), evaporated, and stored at −20°C. For details, see Refs. [17, 18].

sBLM were prepared from a mixture of dioleoylphosphatidylcholine (*DOPC*) or egg phosphatidylcholine (egg*PC*) and hexadecylamine (*HDA*) (*PC*:*HDA* = 2:1, Sigma, St. Louis, MO, USA), and cholesterol (4:1) dissolved in *n*-heptane (concentration: 20 mg/cm^3). As an electrolyte, 1 *M* KCl (Merck, Darmstadt, Germany) in deionized water buffered by 10 m*M* Hepes (Sigma, St. Louis, MO, USA, $pH = 7$) was used. Water was purified by passage through a Milli-Q cartridge filtering system (Milli-Q, Millipore, El Paso, TX, USA). All chemicals were of analytical grade.

Formation of sBLM

sBLM were formed on the tip of a teflon capillary (inner diameter 0.5 mm) filled by agar containing 1 *M* KCl according to a method described earlier [19, 20] by spreading a small amount of lipid solution from a *Pasteur* pipette on the freshly cut tip of the teflon tube. The formation of sBLM was controlled by measuring the electrical capacitance.

Measurement of elasticity modulus, electrical capacitance, and surface potential

The electrostriction method allows the simultaneous measurement of the *Young* modulus of elasticity perpendicular to the membrane plane ($E_\perp$) the membrane capacitance (C), and the surface potential ($\Delta\Phi_m$) by applying an AC voltage (amplitude: $U_0 = 50$ mV; and frequency: $f = 1$ kHz) to the lipid bilayer. The membrane capacitance is given by Eq. (1), where I_1 is the amplitude of the 90° component of the first current harmonic. The modulus of elasticity perpendicular to the membrane surface is given by Eq. (2), where $\Delta d/d$ is the relative change in membrane thickness resulting from the application of pressure p.

$$C = I_1/2\pi f U_0 \tag{1}$$

$$E_\perp = -p/(\Delta d/d) \tag{2}$$

In our setup, this pressure results from the applied AC voltage (electrostriction) and induces a time-dependent change of the bilayer thickness reflected in a third harmonic component of the current (amplitude I_3, see Refs. [20, 21]). Using $p = C_s U_0^2/2d$ for the electrostrictive pressure, where C_s is the specific BLM capacitance per unit area, the *Young* modulus can be calculated from Eq. (3).

$$E_\perp = C_s U_0^2 I_1/4dI_3 \tag{3}$$

For this calculation we used C_s determined from the electrical capacitance of sBLM ($C_s = C/A$ where A is the area of lipid film calculated from the microscopically determined film diameter). The thickness was determined from $d = \varepsilon\varepsilon_0/C_s$ ($\varepsilon = 2.1$ (relative dielectric permittivity of the hydrophobic part of the lipid film), $\varepsilon_0 = 8.85 \times 10^{-12}$ F/m (dielectric permittivity of vacuum [20])). The value of C_s was determined from the final equilibrated state of the membrane.

Two different sources of potentials are present in the system: electrode potentials (ΔU_{el}) and bilayer surface potentials ($\Delta\Phi_m$). The surface potential consists of the *Gouy-Chapman* surface charge potential (U_{GC}) and the surface dipole potential (U_D):

$$\Delta\Phi_m = U_{GC} + U_D \tag{4}$$

Of these two components, only the *Gouy-Chapman* potential at the aqueous boundary can be determined independently by means of shielding experiments. This determination is based on the dependence of U_{GC} on the concentration of the electrolyte, whereas U_D does not depend on electrolyte concentration (see Ref. [22] for more details). The relation between surface charge density (σ) and U_{GC} is given by Eq. (5) (c_i: bulk concentration of species i with charge z_i).

$$\sigma = (8\varepsilon_0\varepsilon RTc_i)^{1/2}\sinh(z_i F U_{GC}/2RT) \tag{5}$$

In contrast to the *Gouy-Chapman* potential, the dipole potential does not depend on ionic strength and can be expressed by Eq. (6), where μ is the average component of the lipid molecular dipole moment perpendicular to the membrane plane, ε_0 is the permittivity of free space, ε is the local dielectric permittivity, and A is the area per lipid molecule [23]. The changes in intrinsic potential can be determined using Eq. (7), where I_2 is the amplitude of the second harmonic of the membrane current with frequency $2f$ which is generated if the membrane is compressed simultaneously both by AC and DC voltage; U_1 is the DC voltage externally applied to the BLM. The method of measurement of $\Delta\Phi_m$ is based on simultaneous determination of the amplitudes of the current harmonics I_2 and I_3 [20].

$$U_D = \mu/(A\varepsilon_0\varepsilon) \tag{6}$$

$$\Delta\Phi_m = -U_1 + U_0 I_2/4I_3 \tag{7}$$

For a classical BLM separating two aqueous phases the electrode potentials can generally be closely matched, making their net contribution in the total circuit very small. With metal supported membranes there is an inherent asymmetry in the electrode materials (stainless steel or silver wire *vs.* calomel electrode) and environments (lipid *vs.* aqueous phase). Thus, the electrode potentials on the two sides of the bilayer may be quite different and cannot be determined easily. Similarly, the different environments at the two faces of the BLM will introduce further asymmetry into the system. Both the configurations of the head group layer in contact with the metal support and the electrical interaction with the support (*e.g.* mirror charges) will differ from the situation at the aqueous boundary [24]. For agar-supported membranes, however, this asymmetry is rather small and is mostly determined by the salt concentration in the agar bridge and in aqueous solution.

Measurements of the electromechanical and electrical parameters were carried out under the control of an IBM PC/AT 286 computer 20°C.

P-A isotherms

Surface pressure-monolayer area (*P-A*) isotherm measurements were performed with a computer-controlled LB trough 611 (NIMA Technology Ltd., Coventry, UK) as described elsewhere [25]. *Langmuir* monolayers of phospholipids were formed by spreading a small amount (200 mm^3) of $CHCl_3$ solution of the above mentioned lipid mixture (*PC* + *HDA* + cholesterol; stock solution: 0.1 μM) on the surface of the electrolyte. Modification of the lipid monolayer by *CHpdT15* was performed by addition of 50 mm^3 of a 0.1 μM aqueous solution of *CHpdT15* into the water subphase (300 cm^3). After complete solvent evaporation, floating monolayers were compressed by a mobile teflon barrier at a speed of ~11.7 $Å^2$/molecule per minute. Each *P-A* isotherm was measured with the newly formed lipid monolayer after carefully cleaning the LB trough. The films composed by *CHpdT15* were formed in a similar way. In this case, 50 mm^3 of a stock solution of the oligonucleotide in purified water (concentration: 0.1 μM) was added onto the electrolyte-air interface. After formation of lipid or oligonucleotide monolayer, about 50 mm^3 of complementary or non complementary oligonucleotide was added into the bulk electrolyte solution to a final concentration of 0.17 n*M*. *P-A* isotherm measurements were started 30 min after oligonucleotide addition; this time was more than sufficient for hybridization to take place (the hybridization of a 15-mer under the used experimental conditions usually does not take more than 5 min). As an electrolyte, 1 *M* NaCl in doubly distilled water was used. All experiments were performed at room temperature (18–20°C).

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