

Modulation of density and orientation of amphiphilic DNA anchored to phospholipid membranes. Part I: Supported Lipid Bilayers

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Supporting Information

Preparation of Supported POPC layers.

The formation of POPC bilayers on a gold-coated AT-cut quartz crystal was followed in situ by means of QCM with Impedance monitoring (QCM-Z). The gold substrates were cleaned with chromic acid, rinsed thoroughly with Milli-Q and finally dried with a gentle nitrogen flow. The average contact angle of the surface was always less than 30°. Δf and ΔD values were obtained at the fundamental frequency (5 MHz) and at all the overtones, using TBS as reference baseline (baseline drift lower than 0.5 Hz for at least 1 h). After equilibrating the QCM-Z sensor with the buffer solution, the chamber was filled with

the diluted POPC liposome solution as many times as necessary to obtain surface saturation at each examined concentration. Saturation was followed by rinsing cycles with TBS until constancy of the QCM-Z signal, a detailed discussion on the step-by-step formation of POPC bilayers by means of QCM-Z, ellipsometric mapping and Dual Polarization Interferometry (DPI) will be reported in a separate paper.

The final values of frequency and dissipation changes were found to correspond to the formation of a planar bilayer with an average area per POPC molecule of $(0.58 \pm 0.05) \text{ nm}^2$ and a hydrodynamic thickness of $(5.2 \pm 0.3) \text{ nm}$. The experimental parameters obtained for the POPC phospholipid are in a good accordance with literature data: an area per lipid head group of 0.63 nm^2 was obtained both theoretically¹ and from π -A isotherms at the water-air interface²⁻⁴, whereas a bilayer thickness of 5.0 nm was measured by Coupled Plasmon-Waveguide Resonance (CPWR) spectroscopy⁵.

Analysis of the QCM-Z data with a Voigt-based model

The Voigt-based model describes the propagation and the damping of shear-bulk acoustic waves in a homogeneous viscoelastic adlayer, in contact with a semi-infinite bulk Newtonian fluid under no-slip conditions. The adsorbed layer is depicted as a homogeneous viscoelastic film with a uniform thickness, δ_f , film density, ρ_f , and a frequency dependent complex shear modulus, which is described by the following relation:

$$G = G^I + iG^{II} = \mu_f + i\omega\eta_f \quad (\text{S1})$$

where G^I describes energy storage and G^{II} describes energy dissipation, $\omega = 2\pi f_0$ is the angular frequency, μ_f is the elastic shear (storage) modulus, and η_f is the shear viscosity (loss) modulus.

For a single viscoelastic layer in a liquid medium, the change in the resonant frequency, $\Delta f_n^{\text{Voigt}}$ and in the dissipation factor, $\Delta D_n^{\text{Voigt}}$ is governed by the following expressions²:

$$\Delta f_n^{\text{Voigt}} = -\frac{f_0}{\pi\sqrt{\rho_q\mu_q}} \left\{ \delta_f\rho_f\omega - \frac{\delta_f\eta_f\omega^3}{\mu_f + \omega^2\eta_f} (\eta_l\rho_l) \right\} \quad (\text{S2})$$

$$\Delta D_n^{Voigt} = \frac{1}{n\pi\sqrt{\rho_q\mu_q}} \left\{ \frac{\delta_f\eta_f\omega^2}{\mu_f + \omega^2\eta_f} (\eta_l\rho_l) \right\} \quad (S3)$$

where ρ_l and η_l are, respectively, the viscosity and the density of the bulk liquid. In the absence of layer viscoelastic contributions, equation S2 reduces to the Sauerbrey relation (equation 1 in the main text), where $\Delta m/A = \delta_f\rho_f$.

In the present work the thickness, δ_f , the density, ρ_f , the elasticity and the viscosity of the oligonucleotide adlayer are fitted with equation S2 and S3, respectively, by using the QCMBrowse software version 2.30 (KSV Instrument Ltd., Finland). To reduce the number of fitting parameters we treat the density as fix and known, we assume that the storage modulus is much smaller than the loss modulus and we make use of multiple harmonics. The fundamental frequency data were discarded since they are influenced by energy trapping processes⁶.

Using this approach the best fit between the Voigt-based model and the measured parameters was done utilizing a Levenberg-Marquardt algorithm that search for the unknown model parameters (ρ_f , μ_f , η_f) by minimizing the χ^2 given by:

$$\chi^2 = \sum_n \left(\frac{(\Delta f_n^{Voigt} - \Delta f_n^{measured})^2}{\sigma_n^f} + \frac{(\Delta D_n^{Voigt} - \Delta D_n^{measured})^2}{\sigma_n^D} \right) \quad (S4)$$

Binding models for monolayer adsorption

The steady state analysis of the QCM-Z data has been performed in the monolayer saturation regime for both SC-ON₁ and MC-ON₁. In this part we present a brief description of the models employed to interpret the adsorption behavior together with the fitting procedure.

Briefly, different localized adsorption models adapted for neighbor-neighbor interactions were considered in the first place, since previous literature for large systems that bind to many lipids at the same time, recommend to consider models, such as the *van der Waals gas (VdW)* or Hill de Boer

equation, taking into account continuous adsorption surface where excluded volume effects generally tend to lower the number of adsorbed species.

Langmuir model

The simplest and the most used isotherm for both physical and chemical adsorption is the Langmuir isotherm⁷ which can be written as:

$$CK_d^{-1} = \frac{\theta}{1-\theta} \quad (\text{S5})$$

where $C = [\text{ON}]$ is the bulk oligonucleotide concentration, $\theta = (\Delta m/A)/(\Delta m/A)_{sat}$ is the surface coverage and K_d is the dissociation constant.

Hill model

In surface adsorption studies, where the binding of the oligonucleotides to the SLB is enhanced by the presence of more than one anchoring units on the same oligonucleotide, the **Hill** equation⁸ better explains the adsorption behavior. It describes the fraction of the SLB surface saturated by the oligonucleotide as a function of the concentration:

$$CK_d^{-1} = \left(\frac{\theta}{1-\theta} \right)^{1/n} \quad (\text{S6})$$

where n is a parameter that accounts for binding cooperativity effects. A coefficient $n = 1$ indicates completely independent binding sites and the isotherm assume a typical **Langmuir** form. Numbers greater than one indicate positive cooperativity, while numbers less than one indicate negative cooperativity.

Brag-Williams model

The Bragg-Williams (**BW**) model is derived by statistical thermodynamics and it is a simple way to study localized adsorption by introducing the interactions between adsorbed molecules⁹. The BW adsorption isotherm takes the form:

$$CK_d^{-1} = \frac{\theta}{1-\theta} e^{(h(1-2\theta))} \quad (\text{S7})$$

Here $h > 0$ denotes the effective nearest-neighbor attraction strength (mutual interactions between two molecules adsorbed on nearby sites). If $h > 0.5$ such attraction renders the adsorption process *cooperative*,¹⁰. Larger values of h lead to lateral phase separation of the adsorbed layer.

Van der Waals (VdW) or Hill de Boer model

The Hill de Boer or Van der Waals (**VdW**) model give an equation of the isotherm which takes account of the interactions and mobility of the adsorbed molecules¹¹. It takes the following form:

$$CK_d^{-1} = \frac{\theta}{1-\theta} e^{\left[\frac{\theta}{1-\theta} - 2\alpha\theta \right]} \quad (\text{S8})$$

where the first term in the exponential accounts for the excluded volume of the molecules and the constant $\alpha > 0$ denotes the attractive adsorbate-adsorbate interactions.

Modeling of the binding curve

The binding curve were fitted with all the reported equations both for the SC-ON₁ and the MC-ON₁ and the results are shown in figure S1.

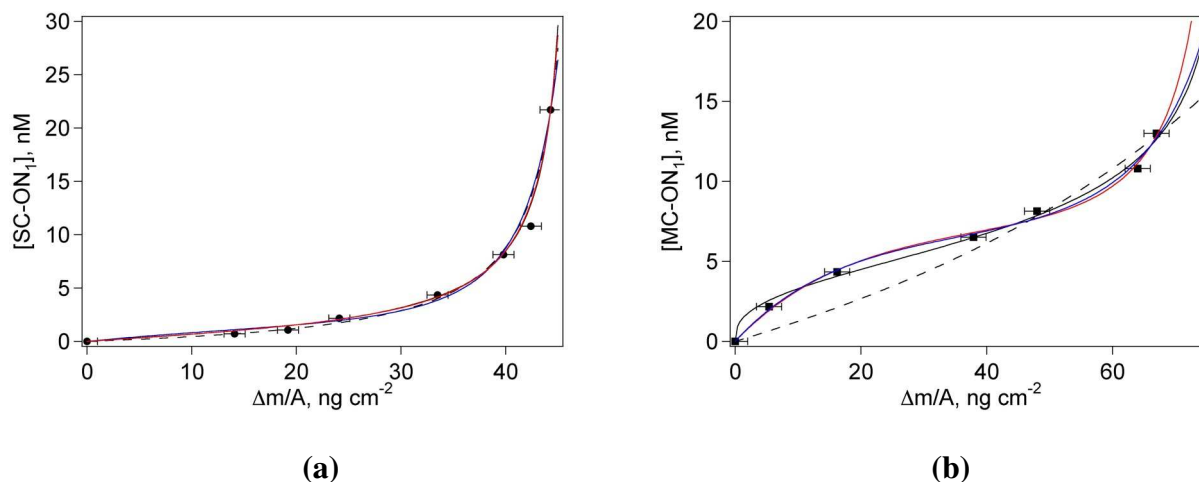


Figure S1. Bulk oligonucleotide concentration as a function of the surface density of adsorption: (a) SC-ON₁, (b) MC-ON₁. The results of the fitting are reported as solid lines: Hill (black), BW (red) VdW (blue). A comparison with a Langmuir type model is reported as a dashed line.

The dissociation constant K_d and the saturation mass density $(\Delta m/A)_{sat}$ are reported in table S1 for all the used equations together with the χ^2 values.

Table S1. Results of the fitting to equation S5-8 for the adsorption of SC-ON₁ and MC-ON₁ onto SLB surface.

	<i>SC-ON₁</i>			<i>MC-ON₁</i>		
	K_d , nM	$(\Delta m/A)_{sat}$, ng/cm ²	χ^2	K_d , nM	$(\Delta m/A)_{sat}$, ng/cm ²	χ^2
Langmuir	1.7±0.1	47.8±0.3	0.92	—	—	—
Hill	2.0±0.2	46.6±0.6	0.49	6.7±0.4	79±6	0.84
BW	2.0±0.2	47.2±0.3	0.51	6.9±0.2	80±2	0.50
VdW	6±2	60±1	0.90	48±5	113±4	0.61

The adsorption of SC-ON₁ onto SLB behaves to a Langmuir-type mechanism while the insertion of the MC-ON₁ follows a sigmoidal behavior with concentration suggesting the presence of cooperative effects. In this latter case, the cooperativity may be ascribed to the presence of strong attractive interactions between the adsorbed oligonucleotides as indicated by the values of the nearest-neighbor parameters in the Bragg-Williams ($h = 1.6$) and in the Hill-de Boer ($\alpha = 2.4$) relations.

Other models that take into accounts only the excluded volume effects such as the Volmer isotherm¹² or the adsorption equation derived from the scaled particle theory^{13,14} fails to reproduce the data. However, it is believable that the topographical constrains become even more important at the monolayer saturation, as observed for the time dependent adsorption behavior.

Double strand adsorption onto SLB surface

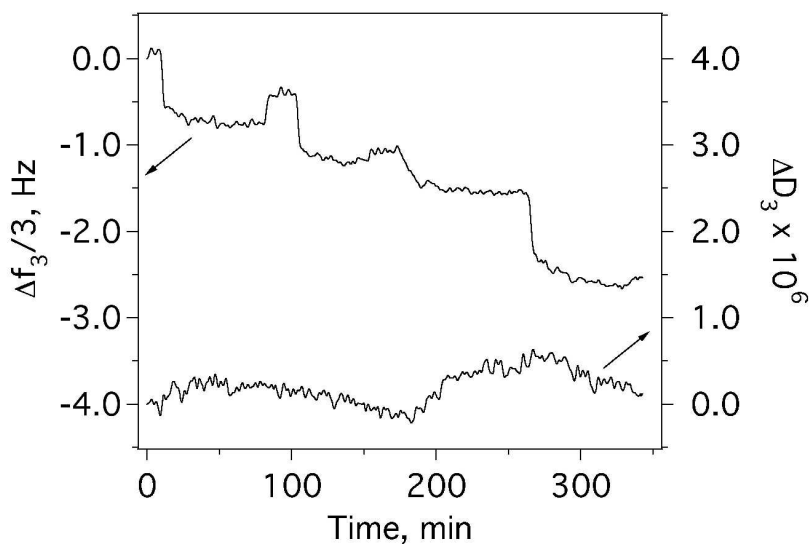


Figure S2. Normalized frequency and dissipation factor shifts for the adsorption of SC-ON₁/ON₂FAM 2.2nM onto SLB (method B).

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