SUPPORTING INFORMATION

Description and assessment of Langmuir model for combined fitting of ITC and ellipsometry data

Elucidation of molecular details of interaction in biological systems relies on the assumption that the applied model correctly describes the mechanism of the studied process. We use Langmuir model for fitting ellipsometry and isothermal titration calorimetry (ITC) data and investigate applicability of this approach for studying binding of model peptidomimetics to negatively charged lipid membranes.

Model description

The analysis of the binding/adsorption data was performed using the classic Langmuir model, described by an equilibrium reaction:

$$P + L \rightleftharpoons P_{ads} \quad [1]$$

where P and P_{ads} designate the peptidomimetic in solution and adsorbed on the surface, whereas L represents free adsorption sites on the lipid bilayer. The assumptions of this model include: a) all adsorption sites are equivalent; b) the adsorbed molecules do not interact; c) all adsorption events occur through the same mechanism; d) at maximum adsorption, only a monolayer is formed.

The equilibrium constant for this reaction is:

$$K = \frac{P_{ads}}{[P]_f \cdot L_f} \quad [2]$$

where $[P]_f$ is the molar concentration of free (non-bound) peptidomimetic, L_f is the number of free adsorption sites and P_{ads} is the amount of adsorbed molecules (all of which are unknown).

Introducing the degree of saturation, θ, defined as the ratio between the occupied (P_{ads}) and the total amount (L_t) of adsorption sites, we can write:

$$\theta = \frac{P_{ads}}{L_t} \quad [3]$$

then

$$\begin{cases} P_{ads} = \theta \cdot L_t \\ L_f = (1-\theta) \cdot L_t \end{cases} \quad [4]$$

while

$$[P]_f = C - \frac{P_{ads}}{V} = C - \frac{\theta \cdot L_t}{V} \quad [5]$$

Here C is the total concentration of the peptidomimetic and V is the volume of the reaction cell (approximately 5 mL). Substituting these into the equation for K, we obtain:

$$K = \frac{\theta}{1-\theta} \cdot \frac{V}{CV - \theta L_t} = \frac{\theta}{1-\theta} \cdot \frac{1}{C - \theta L_t / V} \quad [6]$$

Assuming that the bulk concentration of the peptidomimetic, C, is large compared to the amount adsorbed on the available surface, $\theta L_t/V$, we can write:

$$\theta L_t / V \ll C \quad [7]$$

Hence the expression for the equilibrium constant can be approximated by:

$$K \approx \frac{\theta}{1-\theta} \cdot \frac{1}{C} \quad [8]$$

and thus

$$\theta = \frac{K \cdot C}{K \cdot C + 1} \quad [9]$$

38 The above assumption is necessary for the completion of the ellipsometry data treatment. However,
39 as shown below, this correction is small (< 1%), compared to the uncertainties in determination of
40 concentrations of the reactants. The ellipsometry experiments provide values for the parameter Γ_p,
41 which is the density of the peptidomimetic adsorbed at the lipid bilayer in nmol/m^2. Using
42 parameter Γ_t, the surface density of the total number of adsorption sites, we can eliminate θ:

43 $$\theta = \frac{\Gamma_p}{\Gamma_t} \qquad [10]$$

44 Thus experimental ellipsometry data can be expressed as

45 $$\Gamma_p = \Gamma_t \frac{K \cdot C}{K \cdot C + 1} \qquad [11]$$

46 and fitted by floating the parameters Γ_t and K.

47 Each ellipsometry experiment contains 4 data points. To increase the reliability of the least-squares
48 analysis the two duplicate experiments were fitted together (increasing the number of data points to
49 8), using the same values for Γ_t and K. Two extra parameters,[i] describing the initial offset of optical
50 polarization not related to the binding process were introduced to improve the quality of the fit. The
51 standard deviations were obtained from 95 % confidence intervals of the fit, following the protocol
52 of Kemmer & Keller[1].

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[i] These two parameters do not have any thermodynamic significance and reflect the fact that even a minute concentration of peptidomimetic influences the structure of a lipid bilayer, shifting the whole data set up or down in Γ values, compared to a pure lipid. The offset parameter is essentially a transfer of the binding curve along the y-axis.

Analysis of the ITC data

The major advantage of calorimetry is the possibility to directly access the enthalpy of intermolecular interactions, ΔH. Using equation [10], we can express θ in terms of the adsorbed peptidomimetic, X (in nanomoles), which yields:

$$\theta = \frac{X}{L_t} = \frac{X}{\gamma S} \quad [12]$$

Here L_t is substituted by γS, since the total number of peptidomimetic binding sites is proportional to the total area of the membrane surface, S. The proportionality coefficient, γ, has the dimension of mol/m^2 (or nmol/m^2), which is the surface density of the adsorbed peptidomimetic at saturation. This parameter can be compared directly to the one obtained from the fitting of ellipsometry data, Γ_t

Then K becomes:

$$K = \frac{X}{(C - X/V_{cell}) \cdot (\gamma S - X)} \quad [13]$$

Rearrangement of the above leads to the quadratic equation

$$K \cdot X^2 - X \cdot \left(V_{cell} + K \cdot (CV_{cell} + \gamma S)\right) + CK\gamma S V_{cell} = 0 \quad [14]$$

which can be solved through the commonly used method for finding the discriminant, Δ, and then one of the roots, X:

$$\Delta = \left(V_{cell} + K \cdot (CV_{cell} + \gamma S)\right)^2 - 4 \cdot K^2 C \gamma S V_{cell} \quad [15]$$

$$X_{1,2} = \frac{V_{cell} + K \cdot (CV_{cell} + \gamma S) \pm \sqrt{\Delta}}{2K} \quad [16]$$

In the formulas above, the known parameters are the cell volume, V_{cell} = 1.0 mL, and the total concentration of the peptidomimetic, C. The total adsorption area, S, calculated from the molar concentration of the lipids, [l], and the surface area of a liposome, A, as:

$$S = n \cdot A \cdot V_{cell} \quad [17]$$

$$n = \frac{number\ of\ LUV's}{Liter} = \frac{[l] \cdot N_A}{number\ of\ lipids\ per\ LUV} = \frac{[l] \cdot N_A \cdot A}{2a} \quad [18]$$

$$A = \pi d^2 \quad [19]$$

Here a is the surface area of a POPC molecule in a lipid bilayer and is equal to 68 Å^2,[2] d = 100 nm is the average diameter of an LUV and N_A is the Avogadro constant.

The total heat measured by ITC, Q, is proportional to the amount (in moles) of adsorbed peptidomimetic:

$$Q = X \cdot \Delta H \quad [20]$$

and the heat produced by the i-th injection, q_i, can be expressed as:

$$q_i = Q_i - Q_{i-1} = \Delta H \cdot (X_i - X_{i-1}) \quad [21]$$

During the fitting procedure the experimental heats are compared to the calculated ones. The best fit is achieved by minimizing the variance upon changing the parameters ΔH, γ, K and q_{offset}[ii].

Validation of the Langmuir adsorption model

Two fundamental requirements for the Langmuir approach are i) the monolayer adsorption and ii) the absence of interaction between the neighboring peptidomimetics. This approximation can be evaluated by calculating the surface density of the peptidomimetics at saturation, Γ_t. For example, the estimated surface area per H-(hArg-Bn)$_6$-NH$_2$ is ~20 nm^2 (at 37 °C), obtained by dividing 85 nm/m^2 (Table 1 in the main text) by Avogadro constant. This value, which is an order of magnitude

[ii] This parameter describes the residual non-zero heat associated with the dilution of titrant after the complete saturation of the binding sites.

larger than the molecular size of the peptidomimetic can be converted into the average distance between two neighboring molecules, amounting to ~44 Å. The long average distance between two bound molecules precludes their interaction through hydrogen bonding or van der Waals forces. Furthermore, calculation of the Debye length in a 150 mM KCl solution at 37 °C according to Eq. [22].

$$\kappa^{-1} = \sqrt{\frac{\varepsilon_r \varepsilon_0 k_B T}{2 N_A e^2 I}} \qquad [22]$$

using the table values for permittivity of free space, ε_0, the dielectric constant of water, ε_r, Boltzmann constant, k_B, Avogadro number, N_A, and elementary charge, e, gives ~8 Å. The short Debye length implies that involvement of long-range electrostatic forces into peptidomimetic-peptidomimetic interaction is unlikely. Together, this demonstrates the absence of both long-range and short-range interactions between peptidomimetics and thus fulfills two requirements of the Langmuir model: 1) the absence of interaction between the adsorbed molecules and 2) monolayer adsorption.

REFERENCES

1. Kemmer, G. and Keller, S. *Nat. Protoc.* **2010**, *5*, 267.

2. Kucerka, N.; Tristram-Nagle, S.; Nagle, J.F. *J. Membr. Biol.* **2005**, *208*, 193.