Thermal-mechanical fluctuations enhance repulsion between bimolecular layers

(steric repulsion/lamellar undulations/hydration forces/electrostatic double layers/bilayer assembly)

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ABSTRACT Beginning with the free energy potential for long-range interactions between parallel flat sheets and the mechanical energy of bending those sheets, we have derived an upper bound for repulsion enhanced by thermal undulations of sheets in a multilamellar array. Through a self-consistent (mean-field) potential, we cover the full range of layer separations from close-in to far-apart, where the enhanced repulsion approaches the weak steric interaction derived previously by Helfrich [Helfrich, W. (1978) *Z. Naturforsch.* 33a, 305–315]. We have examined the effect of the fluctuation-enhanced repulsion in experimental studies of osmotic (and mechanical) compression of multilamellar lipid arrays, mechanical compression of bilayers immobilized on mica substrates, and controlled adhesion of giant bilayer membrane vesicles.

The prescient work of Helfrich (1) has made us aware of the possibility that mechanical fluctuations contribute to the repulsive forces observed between bilayers formed by hydration of surfactant molecules. These fluctuations are expressed as undulative displacements of the bilayers from planar configurations. This phenomenon has been embraced in particular by Sornette and Ostrowsky (2), who have argued (3) that “fluidization” of phospholipid bilayers coincident with acyl chain melting might be responsible for the large differences observed in the hydration repulsion of such bilayers in the gel versus liquid crystal states (4). We have undertaken this analysis of mechanical (bending) excitations to estimate an upper bound for augmentation of repulsion between free bilayers at separations greater than the polar head group dimensions. Other fluctuations—e.g., head group motions or surface density variations—have not been considered because they are expected to act primarily at close range near molecular contact.

In his original formulation, Helfrich (1) computed the magnitude of fluctuations and the consequent steric repulsion in the absence of long-range interbilayer forces. Here we modify Helfrich’s approach to estimate fluctuations (and their associated energies) as variations from a planar elastic state governed by a general set of long-range forces between layers. We have used a self-consistent (mean-field) elastic potential to account for undulations in a strongly nonlinear potential (i.e., bilayer displacements from a region of weak or negligible interaction to a region of strong repulsion). From comparison with the Helfrich model of a bilayer between rigid walls, we conclude that confinement of membrane fluctuations by the generalized elastic potential greatly enhances the free energy of interaction for separations over a considerable range of separations. In the limit of very large separations, the models converge.

Bending excitations can significantly expand the stress-free equilibrium separation of fully hydrated, electrically

neutral bilayers. On the basis of our upper bound calculation for repulsion, earlier estimates for the Hamaker/van der Waals attraction must be increased to match separation distances published for fully hydrated systems. The ratio of fluctuation stress to total repulsion is found to diminish as the bilayers are pushed closer together from the fully hydrated state. Similarly, for charged bilayers, the fluctuation stress contribution becomes appreciable at large separations and may even overwhelm the van der Waals attraction to prevent stable adhesion (i.e., eliminate the secondary minimum). Fluctuations appear to explain the “stretching” of electrostatic double layer forces between charged bilayers.

ANALYSIS

We consider the free energy of a one-dimensional N -layered crystal to be the sum of a pure elastic energy E_0 for assembly of uniformly spaced plane layers plus a contribution $F' = E' - TS'$ from thermal excitation of the crystal planes. Variation of the total free energy ($F_T = E_0 + F'$) with separation determines the total mechanical stress σ_t ,

$$\sigma_t = \frac{1}{(N \cdot A)} \cdot \frac{\partial F_T}{\partial z}. \quad [1]$$

The total stress σ_t is the sum of an elastic stress σ_e and a “fluctuation stress” σ_f , A is the area of a single bilayer, and z is the separation distance between layers. The elastic stress is derived from the energy E_0 for assembly of the bilayers considered as rigid plane surfaces,

$$\sigma_e = \frac{1}{(N \cdot A)} \cdot \frac{\partial E_0}{\partial z} \quad \text{or} \quad E_0 = (N \cdot A) \int_{-\infty}^z \sigma_e \cdot dz'. \quad [2]$$

By definition, σ_e would be the stress in the absence of thermal fluctuations. The fluctuation stress is given by

$$\sigma_f = \frac{1}{(N \cdot A)} \cdot \frac{\partial}{\partial z} (E' - T \cdot S'). \quad [3]$$

For small strains and small curvatures, we approximate the elastic energy of deformation due to fluctuations in bilayer spacing as

$$E' = \frac{1}{2} \int_{-A}^A \int \left[K_3 \left(\frac{\partial u}{\partial n} \right)^2 + K_c \left\{ \left(\frac{\partial^2 u}{\partial x_1^2} \right) + \left(\frac{\partial^2 u}{\partial x_2^2} \right) \right\}^2 \right] dx_1 \cdot dx_2 \cdot dn, \quad [4]$$

where u is the local displacement in the z direction and n refers to the n th layer of the array (illustrated schematically in Fig. 1). K_3 is the elastic stiffness (or derivative of the elas-

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Abbreviation: Ole₂-PtdCho, dioleoyl phosphatidylcholine.

tic stress in the z direction); K_c is the bending or curvature elastic modulus of a single bilayer (5, 6). We recognize that the elastic potential E_0 and the stiffness coefficient K_3 are strong functions of the separation distance. As such, the stiffness depends on higher derivatives of the elastic potential, which gives rise to nonlinear terms in the displacement energy function, Eq. 4. These nonlinear terms become extremely important close to and beyond the stress-free state ($\sigma_e = 0$). Unfortunately, inclusion of these terms in Eq. 4 creates an intractable analytical problem. Consequently, we will employ a self-consistent or mean-field estimate for K_3 that is defined by

$$\frac{1}{2} \bar{K}_3 \left\langle \left(\frac{\partial u}{\partial n} \right)^2 \right\rangle = \int_{-\infty}^{\infty} P(\zeta) \cdot E_0(z + \zeta) \cdot d\zeta - E_0(z), \quad [5]$$

where $P(\zeta)$ is the fraction of bilayer states with relative displacement magnitude $|\partial u / \partial n|$ that lie between ζ and $\zeta + d\zeta$.

We express the displacement function as a sum of periodic modes (q) of thermal excitation,

$$u(x_1, x_2, n) = \sum_q \text{Re}\{u_q \cdot e^{i(q_1 x_1 + q_2 x_2 + q_3 n)}\}, \quad [6]$$

which depends on three positional coordinates (x_1, x_2, n). Introducing Eq. 6 into Eq. 4 and accepting the mean-field approximation for the elastic stiffness, Eq. 5, we find that the average elastic energy per fluctuation mode is given by

$$E'_q \approx \left(\frac{N \cdot A}{2} \right) \{ \bar{K}_3 \cdot q_3^2 + K_c \cdot q_1^4 \} \langle u_q^2 \rangle, \quad [7]$$

where $q_1^2 \equiv q_1^2 + q_2^2$. From the equipartition of energy theorem, we know that the energy for each mode q is constant and equal to $\frac{1}{2} kT$. This leads to spectral function that specifies the mean-square amplitude for each fluctuation mode,

$$\langle u_q^2 \rangle = \left(\frac{k \cdot T}{N \cdot A} \right) / \{ \bar{K}_3 \cdot q_3^2 + K_c \cdot q_1^4 \}. \quad [8]$$

There can be no variation in internal energy; hence, the free energy variation due to fluctuations is exclusively entropic,

$$\delta F'_T = -T \cdot \delta \left(\sum_q S'_q \right). \quad [9]$$

It is obvious that resonant (coherent) excitations as in a regular crystal lattice contribute negligibly to the heat or entropy content of the system. However, because of the presence of an interlamellar liquid (water), the layers uncouple so that displacements of layer position may become uncorrelated. Since our purpose here is to derive an upper bound or conservative estimate of the excess free energy and stress caused by thermal-mechanical excitations, we assume (as did Helfrich) that the modes are randomly distributed and completed uncorrelated. As such, the mean-square amplitude measures the number of complexions for each mode so that the average entropy per mode relative to the noninteracting system is given by

$$S'_q = \frac{k}{2} \cdot \ln \left\{ \frac{\langle u_q^2 \rangle_z}{\langle u_q^2 \rangle_\infty} \right\} = \frac{k}{2} \ln \left\{ \frac{q_1^4}{(q_3^2 / \lambda^2) + q_1^4} \right\}, \quad [10]$$

where

$$\lambda^2 \equiv K_c / \bar{K}_3.$$

In our expression for the entropy per fluctuation mode, the elastic potential acts to confine bilayer displacement. Steric

FIG. 1. Random displacements from the planar geometry of an n -layered crystal array.

interactions as originally considered by Helfrich were based on a similar equation for the entropy per mode where an "apparent force constant" was introduced for the compressional stiffness. This constant was determined by the requirement that the root-mean-square displacement of a single layer be less than the interlayer spacing—i.e.,

$$\left\langle \left(\frac{\partial u}{\partial n} \right)^2 \right\rangle \leq \mu \cdot z^2, \quad [11]$$

where μ is an arbitrary factor between 0 and 1. With the use of the self-consistent elastic stiffness, $\bar{K}_3$, it is not necessary to specify the parameter μ ; Eq. 11 is always satisfied provided that the elastic potential is repulsive at close range.

Based on the expression for the average entropy per mode, Eq. 10, the free energy excess per mode is expressed by

$$F'_q = -k \cdot T \cdot \ln \left\{ \frac{q_1^4}{(q_3^2 / \lambda^2) + q_1^4} \right\} \quad [12]$$

To evaluate the free energy due to fluctuations, we convert the sum over modes to an integral over q -space,

$$\sum_q (\dots) \rightarrow \frac{N \cdot A}{(2\pi)^2} \int_{\pi/N}^{\pi} dq_3 \int_0^{\infty} dq_1^2 (\dots), \quad [13]$$

where we have assumed that the area of individual layers is sufficiently large that q can be arbitrarily small[‡] and that each layer is sufficiently continuous so that q can be arbitrarily large. The spatial frequency q_3 is taken from a lower limit that reflects the total number of layers N and goes to the so-called "infra-red" cutoff or the edge of the first Brillouin zone determined by the interlayer spacing. Therefore, the free energy due to fluctuations can be derived as a free energy density,

$$F' / N \cdot A = (\pi \cdot k \cdot T / 16) (\bar{K}_3 / K_c)^{1/2} \cdot (1 - 1/N^2). \quad [14]$$

Clearly, even for a small number of layers, the $1/N^2$ term can be neglected.

To examine steric interaction and the self-consistent stiffness parameter, $\bar{K}_3$, we use the integral over modes with Eqs. 6 and 8 to derive an expression for the variance in separation,

$$\left\langle \left(\frac{\partial u}{\partial n} \right)^2 \right\rangle / z^2 = \pi \cdot k \cdot T / [16 \cdot z^2 \cdot (K_c \cdot \bar{K}_3)^{1/2}]. \quad [15]$$

[‡]Sornette and Ostrowsky (2) have considered the effect of finite extent of the layers. However, it is obvious that this factor can be neglected in any practical situation where simple continuum properties are expected to hold.

Here, the self-consistent character of $\overline{K}_3$ becomes apparent—i.e., the stiffness determines the mean-square relative displacement consistent with the definition for the mean-field energy, Eq. 5. Ideally, for random uncorrelated modes, we would use a Gaussian distribution. However, because the interaction energy is not defined for z less than or equal to zero, we must use a truncated distribution function. One simple approach is to use a triangular distribution with the proper variance. (This is a good approximation to a normal distribution and has the practical advantage that Eq. 5 can be easily integrated in closed form.)

Finally, the added stress due to fluctuations is derived from Eq. 14 as specified by Eq. 3,

$$\sigma_f = [\pi \cdot k \cdot T / 32 (K_c \cdot \overline{K}_3)^{1/2}] \cdot \left(\frac{\partial \overline{K}_3}{\partial z} \right). \quad [16]$$

A stable stress-free equilibrium state can exist when the sum of elastic and fluctuation stresses is zero and the derivative of the total stress with respect to separation distance is positive.

It is generally agreed that the nonspecific or colloidal interactions between bilayers include hydration repulsion, van der Waals attraction, and electrostatic repulsion. Based on established theories (7–10), the forms of the van der Waals attraction and electrostatic repulsion between bilayers are given and approximated, respectively, by the following stress relations:

$$\sigma_{vdw} = (A_H / 6\pi) \{1/z^3 - 2/(z+t)^3 + 1/(z+2t)^3\} \quad [17]$$

and

$$\sigma_{es} \approx -\sigma_{es}^0 \cdot e^{-z/\lambda_D} \quad (z \gg \lambda_D), \quad [18]$$

where A_H is the Hamaker coefficient, t is the bilayer thickness, and λ_D is the Debye length. [Note: The planes that define the origin may differ between these interactions. In the absence of clear definitions for these planes, we have assumed that all forces are measured relative to the same zero separation and that the separation distances are defined by the “mass-average” water gap between layers—i.e., the Luzzati approach to x-ray diffraction analysis (11).]

The hydration repulsion is more empirical in character, but theoretical models (12–15) have been introduced to support the observed form for this interaction,

$$\sigma_{hyd} \approx -P_{hyd} \cdot e^{-z/\lambda_{hyd}} \quad (z \gg \lambda_{hyd}). \quad [19]$$

It is apparent that even for simple representations of these interactions, there are a minimum of three physical coefficients (A_H , σ_{es}^0 , and P_{hyd}) and two decay distances (λ_{hyd} and λ_D) that must be evaluated in any experimental correlation. To examine the effect of fluctuation-enhanced repulsion on the total interaction between layers, we have chosen the following parsimonious approach to analysis of experimental data: (i) We correlate the expression for total stress (including fluctuation-enhanced repulsion) with stresses derived from compression of neutral lipid multibilayers. In addition, we require that the free energy potential for assembly of bilayers to the stress-free separation distance correlate with values for bilayer adhesion energy measured by vesicle-vesicle adhesion. The results yield values for the physical coefficients (P_{hyd} and A_H) and the hydration decay distance (λ_{hyd}). (ii) We assume that the parameters for interactions between neutral bilayers are applicable to charged bilayers (an assumption of little consequence where electrostatic forces dominate). A further restriction is that the Debye length for decay of the electrostatic repulsion be specified by

FIG. 2. Optimum fit of the theoretical total stress (—) to data derived from electrically neutral egg phosphatidylcholine experiments (○) (22, 28); the van der Waals attraction coefficient A_H was found to be 1.1×10^{-13} erg (1 erg = 10^{-7} J) to agree with the observed separation distance at full hydration and with adhesion energy values derived from experiments on giant bilayer vesicle adhesion [$\approx 10^{-2}$ erg/cm² (30, 33)]. For comparison, a van der Waals attraction coefficient comparable to earlier estimates (7×10^{-14} erg) was used to calculate the total stress shown as — — —. Plotted as — — — is the elastic planar stress associated with the optimum fit (minus the contribution of fluctuation stress); this is the level of stress expected to act between immobilized bilayers. The hydration repulsion is characterized by a magnitude $P_{hyd} = 9 \times 10^9$ dynes/cm² (1 dyne = 10^{-5} N) and a decay length of 2.2 Å.

classical theory. (iii) We evaluate the total stress, including electrostatic and fluctuation-enhanced repulsion.

RESULTS

Neutral Bilayers. The stresses between bilayers as a function of separation distance have been derived from dehydration (by osmotic stress, vapor-phase equilibria, or mechanical compression) of multilamellar lipid phases in water (4, 22–29). Interlayer distances and layer thicknesses are derived from the lamellar repeat spacing seen by x-ray diffraction combined with data for known lipid-to-water weight ratios. The stresses are determined by either the chemical potential of water in equilibrium with the multilamellar phase or the mechanical stress of compression. Data typical of phosphatidylcholines (zwitterionic lipid bilayers) in the liquid (L_α) state are shown in Fig. 2. Shown is the optimum fit (solid line) of the theoretical total stress between layers, including “fluctuation-enhanced” repulsion.[§] For comparison the sum of elastic stress without the fluctuation contribution (broken line) is also plotted in Fig. 2.

The results given in Fig. 2 demonstrate that a significant outward shift in the stress-free separation (i.e., from 22 to 27 Å) could be caused by bending fluctuations. The total stress near the stress-free separation is greatly augmented by the fluctuation contribution. The parameters calculated for the strong hydration repulsion, both magnitude and decay length, differ slightly from the values determined previously where fluctuations were neglected (4, 28, 29). However, the value of the van der Waals (Hamaker) coefficient deduced from the correlation is 1.5- to 1.6-fold greater than previous estimates. The strong effect of the van der Waals coefficient on the location of the stress-free state is shown in Fig. 2. With the parameters derived from the stress-separation correlation, the predicted value for the total free energy per unit area at the secondary minimum compares very well with values of adhesion energy measured directly by micropipet-controlled adhesion of giant lipid bilayer vesicles (30, 33).

[§]In these calculations, we have taken $K_c = 10^{-12}$ erg for the elastic bending modulus, on the basis of experimental results of Servuss *et al.* (16) and theoretical considerations of Evans and Skalak (17). Also see Schneider *et al.* (18).

FIG. 3. Based on the stress correlations for the multilamellar system (Fig. 2), integrations of the total stress with fluctuation-enhanced repulsion (—) and the planar elastic stresses (---) provide bounds on the free energy of interaction between bilayers. Data from mechanical compression of two phosphatidylcholine bilayers immobilized on mica substrates (stippled area) were derived with the Derjaguin approximation applied to the forces measured between the curved surfaces to give the interaction energy per unit area (31). Data are for temperatures above those of chain melting. The form of the elastic energy without fluctuation-enhanced repulsion more closely resembles that of the shaded region but is displaced inward by 2–3 Å, a systematic difference well within the limits of resolution for the experimental technique.

Suppression of fluctuations, however, does not appear to account for the weaker repulsion seen between bilayers in the solid crystalline (L_β') phase compared to that between bilayers of the same lipids in the liquid chain (L_α) phase—e.g., dipalmitoyl phosphatidylcholine data at 25°C and 50°C (4). Thus there seems to be a reduced coefficient P_{hyd} for the solid phase; this reduction was noted in earlier studies and appeared to correlate with lipid molecular areas (3, 29) as confirmed by later studies (31).

Another type of experiment to examine interactions between surfaces, and recently interactions between bilayer surfaces, involves measurement of the total force $\bar{f}$ between orthogonal (crossed) cylinders of radius R . Using the "Derjaguin approximation" $|\bar{f}| = 2\pi \cdot E \cdot R$, one relates the force $\bar{f}$ to the free energy density E (i.e., work per unit area) needed to bring planar surfaces together. This work per unit area is the integral of the total stress from large separation to any position, z . We have calculated the dehydration free energy density associated with assembly of two bilayers to specific separation distances. These results are shown in Fig. 3 along with the data of Marra and Israelachvili (31) for neutral lipids [earlier data have been reported by Horn (32)]. As before, the total energies, both with and without contribution from fluctuations, are shown. Here in excellent correlation, the two relations bracket the data for interaction energies between two immobilized bilayers adsorbed onto rigid mica substrata.

Charged Bilayers. Mechanical fluctuations *do* seem to explain why electrostatic double layer repulsion between charged bilayer membranes is observed to decay more slowly than expected from classical theory, a vexing anomaly that evaded explanation (23–25). Here, we mention two examples: charged lipids (phosphatidylserine and phosphatidylglycerol) with fixed charges in 0.1 M NaCl (25) and a neu-

FIG. 4. Optimum fit of the theoretical total stress with fluctuation-enhanced repulsion (—) to data derived from dehydration tests on charged [phosphatidylserine (○) and phosphatidylglycerol (●)] arrays in 0.1 M NaCl (25); the parameters for hydration repulsion ($\sigma_{es} = 7 \times 10^7$ dynes/cm²) and van der Waals attraction were those derived previously from data for the electrically neutral lipid arrays (Fig. 2). The characteristic decay length of the electrostatic repulsion was prescribed by the classical Debye length ($\lambda_D = 9.7$ Å). Also shown (---) is the elastic planar stress without enhanced repulsion. The two-headed arrow parallel to the abscissa marks the range of separations observed in the low stress state near full hydration.

tral lipid [dioleoyl phosphatidylcholine (Ole₂-PtdCho)] with charges induced by divalent ion absorption from 30 mM CaCl₂ (19, 20, 23, 24). As demonstrated in Fig. 4, we find that there is a marked enhancement of the repulsive stress with fluctuations (relative to the anticipated elastic stress). We predict that the stress-free state (secondary minimum) can be eliminated by the fluctuation-enhanced repulsion. When fluctuation stresses were not recognized, strangely low values for the van der Waals attraction coefficient were deduced (25) from the separation distances near full hydration. The repulsion between phosphatidylserine and phosphatidylglycerol bilayers in 0.1 M NaCl now follows a constant potential curve with proper exponential decay rate and a van der Waals attraction consistent with that of neutral lipids.

Earlier attempts (23–25) to explain the anomalous decay invoked variable surface potentials of significantly greater magnitude than the 16 mV measured by electrophoresis of Ole₂-PtdCho vesicles in 30 mM CaCl₂ at 25°C (S. McLaughlin, personal communication; also, see data for egg phosphatidylcholine in 100 mM NaCl and CaCl₂ in ref. 21). Now, the force data for Ole₂-PtdCho in 30 mM CaCl₂ are described by $\sigma_{es} = 1.06 \times 10^7$ dynes/cm². This corresponds to a constant potential of about 19.5 mV computed with a linear equation or about 22 mV with the full nonlinear electrostatic equation (23). The difference from the electrophoretic measurement is small enough to be reconciled by a combined shift of only 2–3 Å in assumed plane of ion binding or plane of electrophoretic slip, which has already been proposed (21).

DISCUSSION

As we have shown, fluctuation-enhanced repulsion caused by confinement of bilayer undulations in an elastic potential (without postulation of "hard-wall" or "excluded volume" restrictions) correlates well with available experimental data. In Fig. 5, comparison of the calculated variances in separation distance for the self-consistent versus linearized elastic potentials shows that the restriction embodied in Eq.

FIG. 5. Variance in interlamellar separation predicted for neutral (egg phosphatidylcholine) and charged (phosphatidylserine or phosphatidylglycerol) multilamellar arrays based on either the linearized elastic potential (---) or the self-consistent (mean-field) approximation (—). Note that the variance diverges for the calculations based on the linearized elastic potential where the stiffness K_3 goes to zero. This demonstrates that nonlinear terms in the elastic potential must be taken into account.

11 cannot be satisfied at—or beyond—the stress-free state unless the nonlinear terms in the local expansion of the elastic potential are taken into account. With a mean-field approach, the variance in separation approaches a constant value of 0.17 as the separation becomes very large (based on the use of a triangular probability distribution function). The limiting value establishes the empirical constant for Helfrich's steric restriction, Eq. 11. Thus, the excess free energy due to fluctuations approaches the inverse-square law derived by Helfrich. This result leads to a simple metastable relation for repulsion versus attraction,

$$2(\pi \cdot k \cdot T / 16)^2 / (\mu \cdot K_c) \sim A_H / 6\pi.$$

Hence, stable adhesion is predicted to be promoted by membrane rigidification (increased K_c), a feature recognized by Helfrich and currently under investigation in his laboratory (34). [A caveat is that membrane rigidification acts to oppose adhesion if the surfaces must deform to adhere (33).] Bilayers (with or without charge) might be prevented from adhesion by steric repulsion at very large separations. On the other hand, a small effort (force) to push the layers together might overcome the steric stresses (tiny fractions of an atmosphere in magnitude) at long range that could lead to adhesion.

As a final statement, we emphasize that our analysis is a conservative estimate, an upper bound, for thermal-mechanical fluctuation effects at long range. Coherent modes of excitation, fluid damping (which acts to couple the layers), etc. reduce the effects. Further diminution of fluctuations will occur if the layers are under tension. In-plane tension effectively limits the maximum surface wavelength for undulations—i.e., places a lower bound on spatial frequencies given approximately by $q_1^2 > T_m / K_c$, where T_m is the bilayer tension. Tensions in unpressurized vesicles (and within the contact zone of adherent vesicles) can be very low (less than 10^{-4} dyne/cm), so this threshold would not be important. At higher tensions (i.e., pressurized vesicles), the expectation is that fluctuation effects would be diminished and could facili-

tate adhesion. (But note that tension increases resistance to deformation of the vesicle and this opposes contact formation.) Given all these reservations, one significant observation supports the use of fluctuation models to derive estimates of enhanced repulsion—i.e., large progressive increases in broadening of x-ray patterns are seen as multilamellar arrays are hydrated towards low stress separation distances. Undulative displacements from the planar configuration occur and increase significantly with mean separation.

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