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Structural Contribution to Solute-Solute Interaction*

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The response of a strongly correlated solvent, *e. g.* water, to a perturbation caused by a solute molecule is not local, *i. e.* the solvent structure is modified some distance away from the solute. This modification inevitably leads to an indirect solute-solute interaction. We examine here the indirect interaction of proteins in bilayer membranes and different types of solute interaction in aqueous solutions.

INTRODUCTION

Most interesting biological processes involve solvents with strong intermolecular interactions. For example, the positions and orientations of neighbouring water molecules, or conformations of neighbouring lipid chains in the interior of membranes are all strongly correlated. The introduction of a foreign solute into such strongly correlated systems results in a significant perturbation of solvent structure. The nature of the perturbation will depend on the range of the solute-solvent interaction. Direct solute-solvent interaction always affects the state of solvent molecules in the immediate neighbourhood of the solute. However, the direct solute-solvent interaction will reach beyond the nearest neighbours of the solute only if the solute molecule is charged.

Although in many cases the direct solute-solvent interaction is short--ranged, the perturbation of the solvent structure will spread beyond the first neighbours. The change in the position and/or orientations of the first neighbours of the solute induces further changes in the subsequent layers, and the perturbation is propagated some distance away from the solute. The form and the extent of this propagation depends on the nature of correlations in solvent structure. However, if long-range Coulomb forces are not involved, the asymptotic form of the decay of the perturbation is normally exponential. The characteristic decay length of the exponential, *i. e.* the length ξ in the form $\exp(-r/\xi)$, describes the range of correlations in solvent structure. In other words, the response of the solvent to the perturbation is not local on the spatial scale of the characteristic length ξ, usually referred to as the *correlation length.*

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Perturbation of solvent structure introduced by solute molecules affects many properties of the solution. At low solute concentrations, some properties, *e. g.* the entropy or the free energy of solvation, depend on the interaction of a single solute molecule with the solvent. For example, negative solvation entropies typical of hydrophobic solutes in aqueous solutions indicate an increase in solvent order induced by the solute molecule[1,2].

When the distance between two solute molecules is comparable to the solvent structure correlation length ξ, the regions of perturbed solvent structure will overlap, resulting in a modification of the solvent perturbation. The free energy of the system is a function of the total perturbation of solvent structure, which in turn depends on the solute-solute separation (and relative orientation). The result is an indirect solute-solute interaction mediated by the perturbation of solvent structure. This interaction may be observed by following, *e. g.*, the behaviour of the osmotic pressure of the solution, or the tendency of solute molecules to form aggregates.

To obtain a quantitative measure of the indirect solute-solute interaction, it is first necessary to select a suitable way of describing the perturbation of solvent structure. A very simple way of describing such a perturbation is through the concept of a spatially-dependent *order parameter* $\eta(\mathbf{r})$, familiar from the theory of phase transitions. The choice of a relevant order parameter for a given system will depend on the physical situation, and particularly on the nature of the introduced perturbation. In the case of water, the appropriate order parameter may be, *e. g.*, the degree of tetrahedral coordination between water molecules, or the induced dielectric polarization if electric fields are involved. A structural perturbation can then be described as a deviation of the order parameter from its bulk value. The free energy is a function of the order parameter, and if the functional form is known, the resulting solute-solute interaction can be calculated.

In the subsequent sections, we shall review recent work on several very different examples of indirect solute-solute interaction. The systems under consideration are quite complex, and the analysis is necessarily rather simplified. For example, for aqueous solutions considered in the last two sections, the changes in spatial and orientational correlation functions of water near the solute molecule are much too complex to be described by a single order parameter. Nevertheless, the present analysis improves the conceptual understanding of the processes involved and may serve as a guide in future work which will necessarily have to involve extensive use of numerical simulation methods.

INTERACTION OF MEMBRANE-BOUND PROTEINS

At physiological temperatures, lipid chains in the interior of bilayer membranes are partially ordered. The order parameter is best measured by deuteron quadrupole resonance[3,4]: it describes the tendency of the chains to be orientated along the normal to the plane of the bilayer. Let this direction coincide with the z axis. If, for a carbon atom C_i, the angle between the normal to the plane spanned by C_i—H bonds and the z axis is called Θ_i, the parameter η_i is defined as the thermal average

$$\eta_i = \langle 3/2 \cos^2 \Theta_i - 1/2 \rangle \qquad (1)$$

The value of the order parameter will vary between the usual limits of 1 (fully ordered system, *i. e.* chains orientated along the z direction) and zero (random orientation of chain segments).

The effect of protein molecules on the order of the surrounding lipid chains has been described in several recent reports[5–7]. The proteins studied were cytochrome *P450*,[6] cytochrome oxidase[5] and ATPase[7]. The general conclusion reached is that lipid molecules in the closest proximity to a protein form a relatively rigid ring, termed the »annulus«, around a protein molecule. In other words, the order of the first neighbour lipid chains is increased.

The perturbation introduced by the protein molecule cannot abruptly vanish beyond the first neighbour annulus. We have therefore examined the full spatial dependence of the change in lipid order in the neighbourhood of a protein[8]. The calculation was performed with a version of the most realistic model[4,9] of the lipid chain structure currently available. It is a molecular field model describing the ordering of lipid chains, with the required statistical mechanics sums running over all conformations of a single chain. Lipid chains were assumed to occupy the sites of a two-dimensional hexagonal lattice (the same as in a crystalline state). Assuming nearest-neighbour interaction, the order at each lattice site depends on the order of the six nearest neighbours which determine the strength of the molecular field at that site. For first neighbours of the protein, molecular field is modified depending on the strength of the lipid-protein interaction, which is a single parameter in the calculation.

A typical order parameter profile is shown in Figure 1. While lipid order is increased in the neighbourhood of protein molecules, it returns to the equilibrium value 3 or 4 layers away from the protein. In the configuration shown in the Figure, there are only 4 lipid layers between the proteins. Within

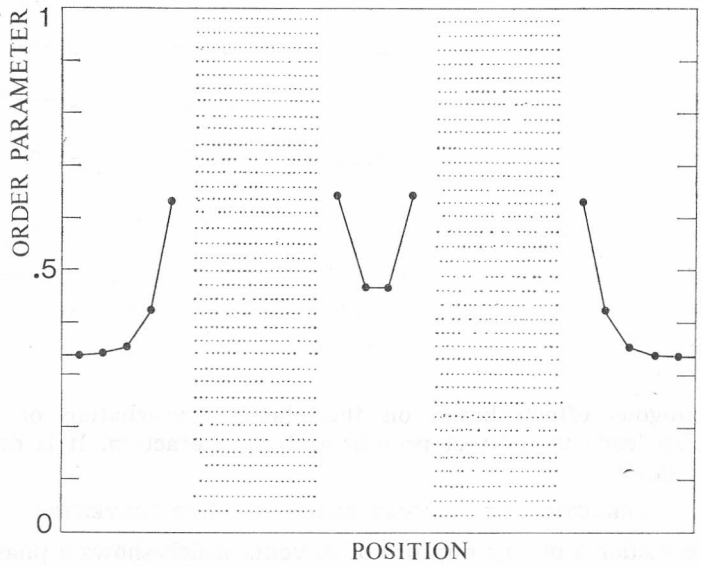

Figure 1. Calculated order parameter profile around two protein molecules in a bilayer membrane. The shaded area is occupied by protein molecules. Each protein molecule occupies 19 lipid lattice sites, temperature is 35 °C (10 °C above the phase transition), and lipid-protein interaction strength is 0.9 of the lipid-lipid interaction strength in the frozen membrane.

that distance, the order parameter cannot reach the equilibrium value and this is the source of the interaction between the two protein molecules.

The perturbation of lipid structure increases the free energy of the system. In this example, the total perturbation is clearly diminished when protein molecules are in the same vicinity, and the indirect interaction is therefore attractive.

Some typical values of the resulting protein-protein potential are given in Table I. It is seen that the interaction strength is comparable to the thermal energy kT and therefore represents a significant effect. Another important point is that closer to the phase transition temperature of bulk lipids, the lipid-mediated interaction has a longer range (due to an increase in lipid-lipid correlation length) and increased strength. As the temperature is decreased, the increase in indirect interaction may cause protein aggregation.

TABLE I

*Change in membrane free energy as a function of protein separation**

Protein separation (lipid layers)	$\Delta G/kT$ $V_{lp} = .9\ V_0$	$.7\ V_0$	$.5\ V_0$
1	-2.02	-.830	-.221
2	-1.71	-.625	-.140
3	-1.36	-.397	-.070
4	-1.07	-.200	-.034
5	-.75	-.071	-.015
6	-.26	-.034	-.007
7	-.08	-.005	-.003

* T = 26 °C (one degree above the phase transition in this calculation), and each protein is assumed to occupy 19 lipid sites. The parameter V_{lp} describes the strength of lipid-protein interaction relative to the maximum value (frozen state) lipid-lipid interaction V_0.

An analogous effect, based on the elastic perturbation of membrane structure, also leads to indirect protein-protein interaction. It is described in detail by Gruler[10].

INTERACTION OF POLYMER BEADS IN MIXED SOLVENTS[11]

Let us consider a binary mixture of solvents which shows a phase separation at lower temperatures. Near the critical point, the mixture exhibits large fluctuations in relative concentration c. The fluctuations are characterized by the correlation length $\xi(c, T)$, which becomes very large near the critical point.

A perturbation in solvent composition is carried over a characteristic distance $\xi(c, T)$, and near the critical point even a weak perturbation may have a significant effect.

Investigations on polystyrene chains[12] in mixtures of good and bad solvents have shown that solvent composition near the chain is modified. Close to the critical point of solvent binary mixture, such a perturbation of solvent composition will lead to a long-range monomer-monomer interaction, which will affect polymer conformation.

A single monomer situated at $\mathbf{r} = \mathbf{r}_i$ will induce a local change in the relative concentration of the solvent, described by the function $\delta c(\mathbf{r})$. The chemical potential of the monomer is of the form

$$\mu = \mu_0 + \mu_1 \delta c(\mathbf{r}_i). \tag{2}$$

As a good approximation, the Ornstein-Zernike form of the response function is used, and the change in relative concentration is given as

$$\delta c(\mathbf{r}) = \chi(\mathbf{r} - \mathbf{r}_i)\mu_1 = \frac{\mu_1 \bar{\chi}}{4\pi\xi^2} \frac{\exp(|\mathbf{r} - \mathbf{r}_i|/\xi)}{|\mathbf{r} - \mathbf{r}_i|} \tag{3}$$

where $\bar{\chi}$ is the integral of $\delta c(\mathbf{r})$ over all space

$$\bar{\chi} = 4\pi \int_0^\infty \delta c(r)\, r^2\, dr.$$

The second monomer is now introduced at the position $\mathbf{r}_j$. It feels the change in relative concentration $\delta c(\mathbf{r}_j)$ and the interaction energy

$$\Delta G(r_{ij}) = \mu_1 \delta c(\mathbf{r}_j) = -\frac{e^2}{r_{ij}} e^{-r_{ij}/\xi} \tag{5}$$

where $e^2 = \mu_1^2 \bar{\chi}/(4\pi\xi^2)$, and $r_{ij} = |\mathbf{r}_i - \mathbf{r}_j|$.

The interaction has the form of a screened Coulomb force and is always attractive. It should be noted that the derivation neglects the size of the monomer, and consequently the form (5) is valid when r_{ij} is larger than the monomer size.

The indirect interaction described here has to be considered alongside with the excluded volume interaction (and, in the case of polyelectrolytes, Coulomb repulsion) in calculations of the size of the solute chain. Several interesting physical situations are described by deGennes in his original letter[11].

REPULSION OF LECITHIN BILAYERS

A recent important experiment[13] has provided precise data on the repulsion of egg lecithin bilayers in aqueous dispersions. The repulsion was determined by measuring osmotic pressure, volume fractions and X-ray repeat distance for multilayer dispersions. The result is an exponential law of the form $P = P_0 \exp[-d/\xi]$, where d is the distance between lecithin-water interfaces, $P_0 = 1.0 \times 10^{11}$ dyne/cm and $\xi = 1.93$ Å.

On the basis of NMR[14–17] and differential scanning calorimetry[18] experiments, it is known that water structure is modified near the interface with lecithin. The most detailed NMR experiment[16] indicated several types of »bound«

water and detected up to 21 oriented water molecules per molecule of lecithin. At the same time, the rate of molecular motion near the interface was found to be restricted.

Lecithin zwitterions produce a strong local electric field which acts to orientate nearby water molecules. The resulting perturbation of bulk water order is propagated away from the interface and results in an indirect interaction of lecithin bilayers[19].

Let us assume that the lecithin/water interfaces are positioned at $x = d/2$ and $x = -d/2$, and describe the perturbation of water structure with the order parameter $P(x)$. $P(x)$ is tentatively identified with the dielectric polarization induced on the interfaces by zwitterion dipoles. For small perturbation from bulk order, the Landau expansion of the free energy density has the form

$$g = g_0 + \frac{\varepsilon}{2\chi}\left\{P^2(x) + \xi^2\left(\frac{\partial P(x)}{\partial x}\right)^2\right\} - PE_0 + \ldots, \tag{6}$$

where E_0 is the applied electric field, $P = \chi E$, $E = E_0 - 4\pi P$ and ε is the dielectric constant. The coefficient $\varepsilon/2\chi$ is obtained by comparing the free energy change for uniform polarization with that given by macroscopic electrostatics, and ξ is the corresponding correlation length. The electric field due to zwitterions is localized, and we assume that its only effect is to impose the polarization $\pm P_0$ at the respective lecithin-water interfaces. The minimization of the total free energy leads to the differential equation

$$\frac{d^2 P(x)}{dx^2} - \frac{1}{\xi^2} P(x) = 0. \tag{7}$$

The solution satisfying the boundary conditions $P(d/2) = -P(-d/2) = -P_0$ is

$$P(x) = -\frac{P_0 \sinh(x/\xi)}{\sinh(d/2\,\xi)}, \tag{8}$$

The form of the solution is shown in Figure 2. The free energy per unit area is

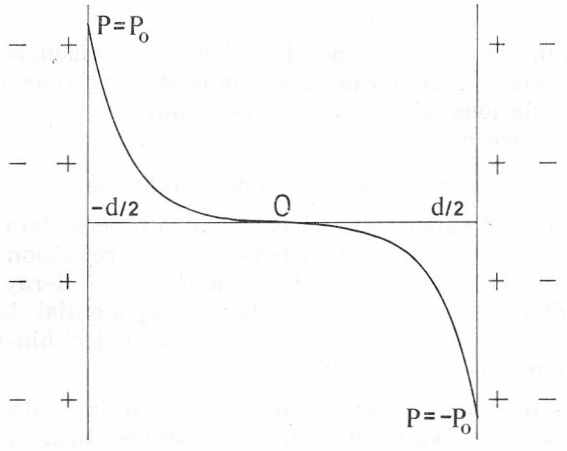

Figure 2. Order parameter configuration between two lecithin bilayers. Different sign of polarization at lecithin-water interfaces facing each other leads to the repulsive interaction.

$$\Delta G = \int_{-d/2}^{d/2} (g - g_0)\, dx = (\varepsilon\, \xi/\chi)\, P_0^2 \coth (d/2\, \xi), \tag{9}$$

while the pressure on the interface is

$$\pi = \frac{\partial \Delta G}{\partial d} = -\frac{(\varepsilon/2\,\chi)\, P_0^2}{\sinh^2 (d/2\, \xi_0)}. \tag{10}$$

For $d > \xi$, the repulsion follows an exponential law

$$\pi = -(2\, \varepsilon/\chi)\, P_0^2\, e^{-d/\xi}. \tag{11}$$

Using the value $P_0 = 2.5$ Debye/molecule (full orientation of water dipoles) leads to $2\, \varepsilon\, P_0^2/\chi = 4.4 \times 10^{12}$ erg/molecule (1.5×10^{11} dynes/cm^2), while the value determined experimentally by LeNeveu, Rand and Parsegian[13] is 10^{11} dynes/cm^2. In systems with short-range interaction, the correlation length is normally of the order of intermolecular separation. The value determined in the experiment[13] is $\varepsilon = 1.9$ Å.

At this point, it is appropriate to examine the basic difference between Figures 1 and 2. Symmetric boundary conditions (cf. Figure 1) lead to solute--solute attraction. The opposite sign of polarization at the adjacent lecithin interfaces, *i. e.* antisymmetric boundary conditions, results in repulsive interaction (Figure 2).

SOLUTE-SOLUTE INTERACTION IN AQUEOUS SOLUTIONS

In this last section we shall consider aqueous solutions of nonelectrolytes. The interaction of hydrophobic solutes in aqueous solutions, or shorter, the hydrophobic interaction, has received ample attention since its importance in stabilizing native conformations of protein molecules has been recognized by Kauzmann[21]. The first detailed study was presented by Nemethy and Scheraga[22], who estimated the maximum hydrophobic interaction for a number of amino acid side group pairs *in contact*. Subsequently, hydrophobic interaction for simple alkanes was discussed by Ben-Naim[23]. In a recent Monte Carlo study[24] on a sample of 62 water molecules and 2 methane molecules, Dashevsky and Sarkisov have obtained the first information concerning the spatial dependence of the hydrophobic interaction.

Despite the extensive work on hydrophobic interaction, both conceptual and quantitative, understanding of the underlying molecular mechanism is not satisfactory. To quote from a recent review by Franks[25]: »Although hydrophobic hydration and solute association are now accepted phenomena, it must be emphasized that, as yet, we have no knowledge of the origin of such behaviour.«

In 1945, Frank and Evans presented the interpretation of thermodynamic data on solvation entropies and free energies of hydrophobic solutes. The large negative solvation entropies indicate an increase in solvent order induced by solute molecules. Water structure near small hydrophobic solutes must to some degree resemble the clathrate structure known from the study of crystalline clathrate hydrates. The resulting dynamic structure increases the tetrahedral coordination (indicated by negative solvation energies) and restricts the orientational freedom and the rate of motion of neighbouring water molecules. Correlation of water structure will propagate the perturbation to more distant neighbours of the solute.

A formalism similar to that employed in previous sections will allow a simple description of the corresponding solute-solute interaction. Let us describe the solute induced perturbation of water structure by the order parameter field $\eta(\mathbf{x})$. The associated free energy density change is

$$\Delta g(\mathbf{x}) = a\{\eta^2(\mathbf{x}) + \xi^2[\nabla \eta(\mathbf{x})]^2\} + \ldots, \tag{12}$$

This form of the free energy density corresponds to the Ornstein-Zernike form of the order parameter correlation function. The minimization problem leads to the differential equation:

$$\xi^2 \nabla^2 \eta(\mathbf{r}) - \eta(\mathbf{r}) = 0. \tag{13}$$

This equation is solved subject to the boundary condition $\eta = \eta_0$ on the surface of two solute molecules. The solutions depend on two parameters: $a\eta_0^2$ (free energy density associated with the perturbation at the solute-solvent contact) and ξ (order parameter correlation length). The value of $a\eta_0^2 = 1.2 \times 10^9$ erg/cm^3 is determined[20] by fitting the solvation free energies for simple n-alkanes (corresponding to the solutions of eqn. (13) for single sphere, single cylinder and intermediate shapes). The correlation length $\xi = 1.9$ Å is taken from the experiment on lecithin bilayer repulsion[13]. Although the perturbation induced by hydrophobic solutes is not equivalent to the perturbation at the lecithin-water interface, we expect that the correlation length which is basically determined by the properties of the hydrogen bond network in water will be similar in both cases. The method of solution of eqn. (13) is described in detail in ref. 20.

The form of the solute-solute attraction is shown in Figure 3. The solutions for different solute radii were obtained by using $\xi = 1.9$ Å. For example, for methane-methane interaction $R = 2$ Å and $v_0 a\eta_0^2 = .77\ kT$ ($v_0 = 27$ Å^3 is the volume of the water molecule). The resulting interaction energy is similar to the Monte Carlo results of ref. 24. The depth of the well is 1 kcal/mole, compared to 1.4 kcal/mole obtained in the numerical simulation[24]. While the Monte Carlo results should be better than the present values for molecules in close proximity, the exponential decay of the interaction at larger distances could not be obtained in the Monte Carlo study due to the small size of the sample.

The solute-solute interaction in aqueous solutions of polar or partly polar molecules is easily understood using the same concepts. In this case, the osmotic pressure and the activity coefficient data provide useful information[26] on solute-solute interaction. Using the McMillan-Meyer picture, the osmotic virial coefficient is expressed in terms of the solute-solute interaction potential as

$$B_2^* = -(2V)^{-1} \int \{\exp[-U(2)/kT] - 1\}\, d\{2\}, \tag{14}$$

where integration is carried over all molecular pair distances and orientations. The minimum repulsive contribution to B_2^* is the hard core repulsion R_2, which may be estimated on the basis of molecular geometry. The minimum attractive contribution is therefore[26]

$$A_2 = R_2 - N_A B_2^*, \tag{15}$$

where N_A is the Avogadro number.

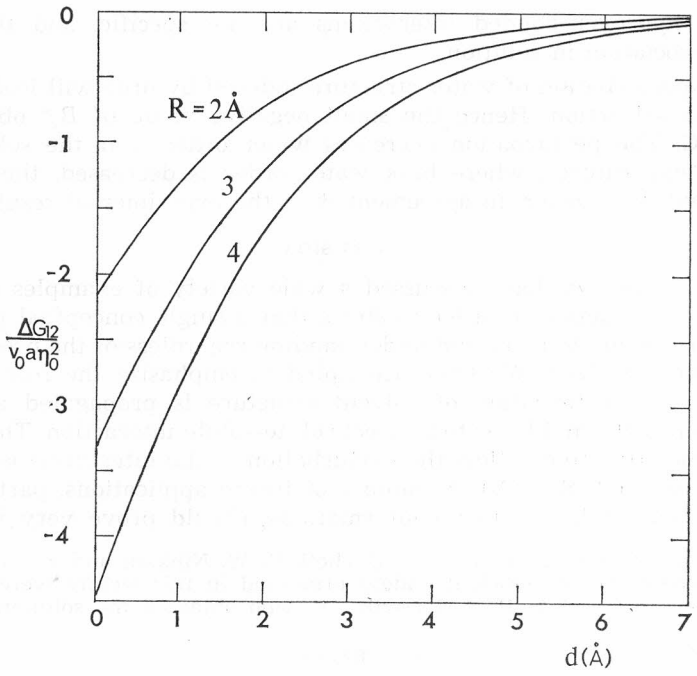

Figure 3. Solute-solute interaction potential in aqueous solutions as a function of solute separation. For methane solutes, $R = 2$ Å and $v_0 a \eta_0^2 = 0.77\ kT$.

The attractive contribution to the integral in eqn. (14) is easily computed for a binary solution of hard spheres with different radii[20]. In that case, a contribution arising from density perturbation induced by a hard sphere solute in hard sphere solvent is responsible for the attractive solute-solute interaction. However, when the solute radius is larger than or equal to the solvent radius, the attractive contribution is smaller than the hard-core repulsion, leading to positive values of B_2^*.

The magnitude of B_2^* for a number of aqueous solutions considered in ref. 26 is comparable to that expected on the basis of hard sphere analogy. However, very small or even negative values of B_2^* observed for aqueous solutions of alcohols or urea cannot be explained without taking into account the specific structure of water as a solvent.

The hydrophobic interaction strength found for simple methane solutes could readily explain very small (positive or negative) values of B_2^* measured for aqueous solutions of alcohols. The increase of the hydrophobic interaction strength with temperature is understood within the same picture[20]: increased solvent order induced in the neighbourhood of the solute causes a larger increase in free energy at higher temperatures, where bulk water structure becomes increasingly disordered. As a result, the hydrophobic interaction is stronger at higher temperatures.

An example of a solute, which strongly disorders water structure, is provided by urea. Recent NMR data[27] on aqueous solutions of urea show that the long-range order characteristic of pure water is destroyed. Short-lived

urea-water hydrogen-bonded interactions are not specific, and there is no urea self-association in solution.

Strong perturbation of water structure induced by urea will lead to strong solute-solute attraction. Hence the small negative value of B_2^* observed for urea at 0 °C. The perturbation decreases water order near the solute: hence at higher temperatures, where bulk water order is decreased, the urea-urea attraction will be weaker, in agreement with the experimental results.

CONCLUSION

In this lecture we have discussed a wide variety of examples of indirect solute-solute interaction in order to stress that a single conceptual picture can provide a degree of guidance and understanding regardless of the precise nature of the system involved. We have attempted to emphasize the role of *solvent correlation:* the perturbation of solvent structure is propagated away from the solute molecule, and leads to indirect solute-solute interaction. The resulting forces will be attractive, unless the perturbation at the interacting surfaces has an opposite sign (cf. Sec. IV). A number of future applications, particularly to the calculations of biopolymer conformations, should prove very interesting.

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DISCUSSION

G. Zerbi:

You tickled my curiosity on chain interactions. What kind of potential are you using in your theory of lipid interactions? In the field of lattice dynamics of ordered systems (take even the simplest polyethylene) we do not know as yet the origin, magnitude and distance of the interactions between chains which keep the crystal together and make them vibrate. This is a general still unsolved problem of molecule--molecule interactions in organic systems.

S. Marčelja:

We are far behind the precision of Professor Zerbi's calculations. All membrane theories, except two-dimensional models by Nagle, are mean-field calculations. In considering intrachain interaction only one *trans* and two *gauche* states are considered (without allowing for vibrations). The interchain interaction is included through the molecular field potential of the form $V_0 (3/2 \cos^2 \Theta - 1/2)$, usually used in describing the ordering of liquid crystals.

D. Bäckström:

I think the vectorial orientation of membrane proteins is to a great extent determined by the local hydrophobic and electrostatic regions of the membrane proteins. A general property of membrane proteins is a low isoelectric point ≈ 4. Does this influence the orientation to the hydrophilic part of the phospholipid bilayer?

S. Marčelja:

It does. At present time conformations of the polar region of phospholipid bilayers are being investigated, and we have no realistic idea of the corresponding interactions. The question leads back to the unsolved problem of interaction of ions **in aqueous solutions.** These questions are much more difficult than the description of interactions within the hydrophobic region of the bilayer.

S. Maričić:

Were you puzzled by the finding of Dr. J. W. DePierre that there was no conformation change of cytochrome *P450* (constant spectra, constant activity) on removing the polar heads from microsomal phospholipids by phospholipase C?

S. Marčelja:

I was surprised. This may indicate that the lipid-mediated protein interaction, which I discussed previously, may be the dominant contribution to the interaction between cytochrome *P450* molecules.

S. Maričić:

Is the 20 °C-phase transition in your graph directly related to an actual transition in real membranes?

S. Marčelja:

It is — the model calculation leads to realistic transition temperatures without adjustable parameters.

SAŽETAK

Strukturni doprinos interakciji otopljenih čestica

S. Marčelja

Reakcija jako koreliranog otapala poput vode na perturbaciju izazvanu otopljenom molekulom nije lokalna, tj. struktura se otapala modificara do neke udaljenosti od otopljene molekule. Takva modifikacija strukture otapala neminovno vodi do indirektne interakcije otopljenih molekula. U radu se ispituju indirektne interakcije proteina u dvosloju membrana i različitih molekula u vodenim otopinama.

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