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# Biophysical view of the role of interfaces in biomolecular recognition

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**Abstract**

Molecular recognition plays a key role in life. Macromolecular interactions at and with interfaces are of paramount importance in this respect. It is therefore crucial to understand and quantify the forces near the surfaces of biological interest in sufficient detail. Specific binding of large molecules, such as antibodies, is affected by the proximity of polar surfaces, for example. On the one hand, the presence of the net surface charges may raise or lower the local macromolecular concentration depending on the relative sign of the charges involved. On the other hand, the ligands attached to strongly polar surfaces always attract and bind their corresponding antibodies less efficiently than the corresponding dissolved molecules. The reason for this is the non-Coulombic repulsion between the ligand-presenting polar surface and the approaching macromolecule. This force is promoted by the surface hydrophilicity and the width of the interfacial region. A simple, direct hydration force is seldom, if ever, seen in such systems. (This is owing to the very short range ($\Lambda_h \simeq 0.1$ nm) of pure hydration force.) The non-specific adsorption of proteins to the lipid bilayer is also little affected by the overall repulsion between the macromolecule and the bilayer surface; such an adsorption is governed more by the number of defects and/or by the availability of the hydrophobic binding sites in the interfacial region. Artificial lipid membranes typically offer numerous such binding sites to the surrounding macromolecules. Multiple non-specific protein adsorption, which results in partial macromolecular denaturation or complement activation, is therefore one of the main reasons for the rapid elimination of lipid vesicles from the blood stream in vivo. To promote the circulation time of an intravenously injected lipid suspension it is therefore necessary to modify the surfaces of their constituent lipid bilayers. Increasing the surface net charge density and/or increasing the bilayer surface hydrophilicity is of little use in this respect. In order to affect the non-specific bilayer–protein interactions significantly, an optimal number of water-soluble, short and sufficiently mobile polymers must be attached to the lipid head-groups. These polymers then increase the repulsive barrier of the membrane surface dramatically, due to the generation of a thick and mobile as well as strongly hydrated interface. Owing to this, the affinity for proteins of the resulting surface is lowered and the surface-induced protein denaturation or complement insertion is hampered. Polymer-coated liposomes, consequently, are not attractive for the phagocytic cells. Such liposomes, consequently, remain in the blood circulation much longer than simple lipid vesicles; the former, consequently, may spontaneously accumulate in tumors.

*Keywords:* Membrane; Lipid bilayer; Molecular adsorption; Macromolecular models; Drug delivery

## 1. Introduction

Specificity and the confinement of space, this is, the creation of cellular membranes, were the prereq-

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uisites for the development of higher life. Furthermore, molecular recognition near or by the membrane surface remains the key issue of most biological reactions to date. Many attempts have therefore been made to understand and describe the mechanisms by which such recognition works. These range from the simple picture of 'key and lock' to highly sophisticated computer simulations of protein–ligand binding with essentially atomic details.

The former approach is easy to grasp. An entirely phenomenological picture of recognition is also rather convenient for simplistic discussions. Its big disadvantage is, however, that it provides no means for quantitative evaluations or predictions. On the contrary, modern computer simulations are potentially extremely informative and precise; their handicap is, however, that they are most demanding in terms of computing time and necessary background knowledge; moreover, detailed calculations cannot be done properly for any big system as yet. (Often it is even difficult to prove that the given result of a computer simulation corresponds to the absolute — and not only to a local — energy minimum.)

The gap between these two worlds can be closed

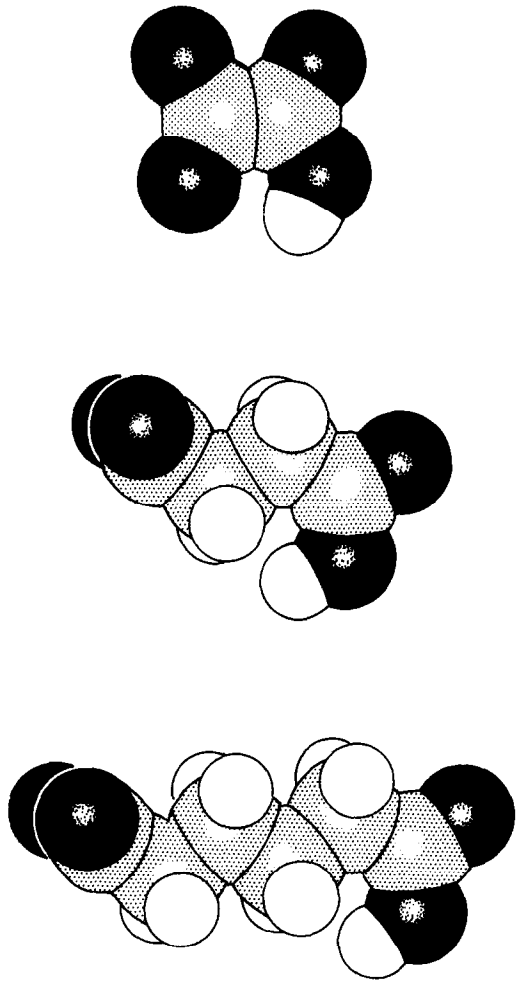

Fig. 1. Space filling model of several 'molecular rulers' of increasing length. Polar groups are shown in black; the apolar central alkane segment is grey and white. Characteristic distance, $d$, corresponds to the separation between both terminal groups.

by suitably defined semi-phenomenological models (see also the contribution by J. Olson in this issue). Such models, on the one hand, are designed with far-reaching reference to molecular details. On the other hand, they are also made to contain adjustable parameters. The latter can then be assigned values that ensure optimum agreement between the results of model calculations and experimental data. Mean-field models of surface electrostatics and hydration are good examples for this [1]. In the following, a brief survey of such currently available simple models, and a biophysical view of the role of interfaces in biomolecular recognition are given.

## 2. Simple models of hydration

Let us first consider the standard 'hydration force' model, for example. This model was introduced by S. Marčelja in the late 70's [2] to describe the results of disjoining force measurements with phospholipid multilamellae under osmotic (hydration) stress [3]. Being physically very plausible and also extremely simple to use, the same model has later been applied to the description of the repulsion between DNA strands [4], polycarbohydrate chains [5], and even individual protein segments.

The advantage of such a commonly used hydration force model is that it accounts explicitly for the phenomenon of solvation, which is generally believed to play an important role in the process of molecular stabilization and recognition [1]. The main draw-back of simplistic hydration force models is that they neglect the interfacial structure and the ambiguity about the precise range of molecular, which is typically given in terms of the decay-length of hydration phenomena, $\Lambda_h$.

It is not possible to extract the value of $\Lambda_h$ directly from the results of disjoining pressure measurements. The interdependence between such pressure and the interfacial structure precludes the success of corresponding attempts. This difficulty can only be overcome by getting rid of the interface.

[1] Hydration is not easily included into the detailed molecular simulation programs owing to the complexity of the inter-water and water–surface interactions.

## 3. Hydration range and the solvation of simple molecules

With this idea in mind we have used dissolved, bifunctional molecules with two polar termini. Such termini represent the hydrophilic segments of biologically interesting molecules or surfaces. Studies of their hydration thus provide valuable information for a basic understanding of macromolecular hydration (Fig. 1).

We have thus used selected bi-functional molecules as 'molecular rulers' [7]. This permitted us to evaluate the value of $\Lambda_h$ from the spatial dependence of the hydration-dependent contribution to the protonation energy of their polar termini. This was achieved with an analytical method proposed earlier by Kornyshev and Ulstrup [6] in combination with up-to-date molecular simulations and gave a spatial resolution better than 0.01 nm and an accuracy of at least 0.03 nm.

In a series of experiments molecular rulers with the terminal carboxy- and amino-groups were investigated. These groups were chosen as models for the hydrophilic amino-group side chains on polypeptides and proteins. The effective decay-length of hydration for such systems was then found to be $\Lambda_h \simeq 0.1 \pm 0.03$ nm. This value was essentially salt- and temperature-independent. (This suggests that the hydration of organic molecules is chiefly due to quantum-mechanical, electrochemical, and enthalpic phenomena rather than to macroscopic polarization (and entropy).)

*(Dielectric) polarity profile*: The spatial dependence of molecular hydration can be described quite accurately in terms of the effective dielectric constant profile $\epsilon(\mathbf{r})$ (cf. Fig. 2) [6]. It decays with distance approximately exponentially on the length-scale of $\Lambda_h$ between the 'proximal', high-frequency value of $\epsilon_\infty$ and the bulk, static value of $\epsilon$.

The corresponding experiment-derived dielectric function thus provides a basis for the phenomenological descriptions of the solvent effects in molecular modelling programs [7]. It also suggests the follow-

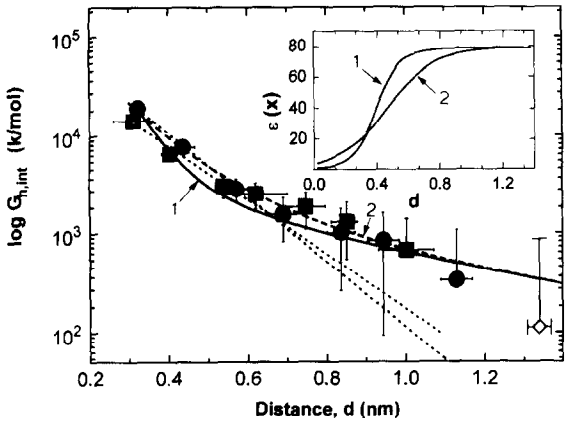


Fig. 2. Dependence of the hydration force measured with a molecular ruler as a function of the separation, $d$, between the terminal polar groups (cf. Fig. 1). Data measured with dicarboxyalkanes (● and ■, respectively) are seen to be described very accurately within the framework of a non-local electrostatic model in which the correlations between the solvent molecules are assumed to decay exponentially on the length-scale of 0.1 nm (curve 1) or 0.13 nm (curve 2). The insert shows the corresponding dielectric constant profile. Lines give the result of a quasi-exponential force decay approximation.

ing equation for the calculation of the hydration force between two polar residues at a distance, $d$:

$$F_h(d) = F_h(d=0)\exp(-d/\Lambda_h) \simeq (q_p \Lambda_h/\epsilon_0)(1/\epsilon_\infty - 1/\epsilon)\exp(-d/\Lambda_h) \quad (1)$$

where $\epsilon_0$ is the dielectric permittivity of free space.

Whilst the former expression is entirely phenomenological, and vindicated only by the experiments, the latter form of Eq. 1 can be derived within the framework of non-local electrostatics with only a few simplifying assumptions. The pre-exponential factor in Eq. 1 is proportional to the difference of the inverse dielectric constants. (For water: $1.8 \leq \epsilon_\infty \leq 4.6$ and $\epsilon \leq 82$.)

## 4. Solvation of complex molecules and surfaces

Solvation of more complex — and thus more spacious — polar termini cannot be described in terms of two hydrophilic centres and one dielectric constant profile only. In order to model the hydration of larger molecules or extended molecular aggregates one must, therefore, allow for finite size effects: every polar residue is thus taken as an independent source of solvation at $\mathbf{r}'$ and the corresponding dielectric constant profile is replaced by: $\epsilon(\mathbf{r}-\mathbf{r}')$.

The description of an extended supramolecular aggregate within the framework of a correspondingly generalized non-local electrostatic approximation numerically becomes a daunting task. The only exceptions are relatively uniform surfaces of sufficiently large, spherical or quasi-planar aggregates.

These can be described in a one-dimensional generalized 'hydration force model'. In a first approximation, such a model is obtained by using $\mathbf{r}-\mathbf{r}' \rightarrow r - r'$ or $\mathbf{r}-\mathbf{r}' \rightarrow x - x'$, where $x$ is the distance along the surface normal. The distribution of polar residues in the interfacial region, furthermore, is assumed to be given by the interfacial polarity profile, $\rho_p(r')$.

For the surfaces with an exponentially decaying polarity profile, $\rho_p(x) = (\sigma_p/d_p)\exp(-x/d_p)$, where $\sigma_p$ is the surface local excess charge density on all atoms in the interfacial region, the disjoining pressure in the linear approximation is found to contain three contributions. The first two depend on hydration decay-length, $\Lambda_h$, and on the interfacial polarity profile decay length, $d_p$, respectively. The third is a mixed term which combines these two dimension parameters.

If the interfacial thickness is much greater than the solvation decay length one gets simply:

$$p_h(d) = (1/\epsilon_\infty - 1/\epsilon)\left(\sigma_p^2/\epsilon_0\right)\left(\Lambda_h/d_p\right)^2 \times \exp(-d/d_p) \quad d_p \gg \Lambda_h \quad (2)$$

This expression is formally identical to the result of the standard hydration force model valid for infinitely narrow interfaces:

$$p_h(d) = p_h(d=0)\exp(-d/\Lambda_h) \simeq (1/\epsilon_\infty - 1/\epsilon)\left(\sigma_p^2/\epsilon_0\right)\exp(-d/\Lambda_h)$$

(Again, the latter form is only valid within the framework of a non-local electrostatic approximation.) The essential difference is that in the former equation the characteristic interfacial thickness appears in the place of solvation decay-length. For-

mally identical results are obtained when the interfacial polarity profile is terminated at half-distance, $d = d_w/2$, between the interacting surfaces. For other values of the cut-off distance, $l_c = s \cdot d$, an interfacial softness parameter, $s$, also appears in the final model result [8].

It is not very likely that the interfacial polarity profile will often decay exponentially with the distance from the polar surface. It is much more probable that such a dielectric function will resemble a Gaussian or a skewed Gaussian distribution [2]. Fortunately, this ambiguity does not affect the results of the model calculations appreciably [8]. For an interfacial polarity profile of Gaussian form it is even permissible to further use the results of an appropriate exponential approximation in a given — not too large — separation region.

When the interfacial width much exceeds the reach of segmental hydration, the spatial dependence of the hydration-dependent interfacial repulsion over significant separation decays on the length-scale of interfacial thickness (cf. Fig. 3). For the thick interfaces, the so-called 'hydration force' should hence be re-interpreted as an interfacial force that is modulated by the hydration effects. (Note that in this approximation the value of $p_h$ tends to zero if $\Lambda_h \to 0$.)

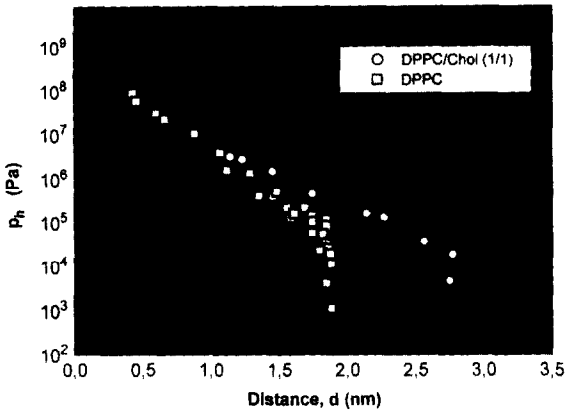


Fig. 3. Effect of the effective interfacial thickness, $d_p$, on the logarithm of hydration-dependent disjoining pressure, $p_h(x)$. Increasing the decay length of the interfacial polarity profile, $d_p$, strengthens the repulsive pressure far away from the polar–apolar interface. Experimental data are from ref. [25] and pertain to pure dipalmitoylphosphatidylcholine (DPPC) multibilayers (○) and to the corresponding DPPC/cholesterol (1/1) mixtures (□). $\xi \equiv \Lambda_h$ is solvent-order decay length (modified from ref. [9]).

## 5. Hydration-dependent interfacial repulsion

Disjoining pressure thus contains information on the molecular structure of a given interface as well as on the swelling capability of all polar residues inside the surface–water interphase. This gives rise to a 'surface softness effect'. This notwithstanding, the interfacial polarity profile is not directly mirrored in so-called 'hydration force' data [9]. Rather than this, the effective hydration-dependent interfacial repulsion integrates the hydrophilicity of the entire polar surface [10]. Fig. 4 illustrates this for a layer of glycerophosphorylethanolamine head-groups.

The measured disjoining pressure is relatively insensitive to the fine details of the interfacial structure; so is the effective force between a (supra)macromolecule approaching a polar interface. In spite of this, the equilibrium separation between two surfaces, or between a macromolecule and its adjacent polar surface, is strongly affected by the local hydrophilicity of the interfacial region (here taken to be proportional to the polarity parameter $\sigma_p$). Interfacial thickness given in terms of $d_p$ (and $s$) also plays an important role in this respect.

The surface characterizing parameters are typically closely related. In order to contain many and/or strongly hydrophilic polar residues ($\sigma_p \gg 0$) an interface must be sufficiently thick ($d_p \gg 0$). If so, it is also likely to swell extensively ($s \simeq s_{max} = 0.5$) and to respond strongly to the variations in the water content in the investigated system. Changing the water activity has the same effect.

Molecular recognition and binding are associated with the free energy minimization for a given molecule near the adsorbate surface. For a proper evaluation of this minimum all pertinent interaction potentials should be considered. In the simplest possible approximation [11] one can assume, for example, that the binding molecule will be attracted to a surface when the repulsive, disjoining interaction and

[2] If each interfacial polar segment is assumed to vibrate toward the bulk in a harmonic potential, its distribution is given by the Gaussian curve.

the attractive, van der Waals interaction sum up to a value at least slightly lower than zero. While the former force has been argued in previous paragraphs to decay approximately exponentially with the separation from the surface the latter only changes with distance with an inverse second power, $d^{-2}$, in the non-retarded case. The variability of the measured equilibrium separation data is thus chiefly caused by changes in interfacial repulsion.

This is clearly seen by correlating the calculated surface local excess charge density, $\sigma_p$, with the measured equilibrium membrane–membrane distance in excess water, $d_w$ [13]. (See Fig. 5 and the second and third column in Table 1). This distance is then found to increase with the value of $\sigma_p$, which is a simple measure of the effective surface hydrophilicity. The functional relation between these two parameter can be denoted approximately by sinh

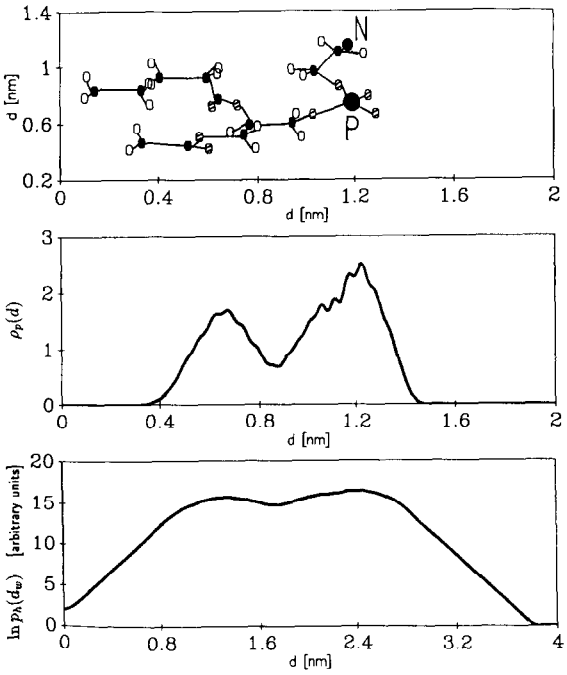


Fig. 4. Space filing representation of the diacylglycerophosphorylethanolamine (PE) building block of a polar lipid layer (top), the corresponding interfacial polarity (excess charge density) distribution function (middle) and the calculated disjoining pressure profile, $p_h(d)$ (bottom), for the planar PE layer. PE crystal structure data are from ref. [14], the local excess charge densities were calculated from the data given in ref. [26] and $\rho_p(x)$ as well as $p_h(d)$ are from ref. [15].

Table 1
Effect of the effective polarity of bilayer surface, $\sigma_p$ (As: $m^{-2}$), on the binding efficiency, $Q$ (%), of an antibody directed against ligands covalently attached to the membrane surface

| Lipid type | Temperature (°C) | Surface polarity $\sigma_p$(As $m^2$) | Binding efficiency Q(%) |
|---|---|---|---|
| DLPE/DMPE (1/1) | 35 | $0.11 \pm 0.02$ | $38.7 \pm 0.7$ |
| DMPE($CH_3$) | 37.5 | $0.14 \pm 0.03$ | $29 \pm 4$ |
| DSPC | 50 | $0.18 \pm 0.03$ | $21.2 \pm 2$ |
| DMPC | 28 | $0.26 \pm 0.02$ | $9.0 \pm 1$ |

DLPE: 1,2-dilauroyl-*sn*-glycero-3-phosphoethanolamine; DMPE: 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine; DMPE($CH_3$): 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine-N-methyl; DMPC: 1,2-dimyristoyl-*sn*-glycero-3-phosphorylcholine; DSPC: 1,2-distearoyl-*sn*-glycero-3-uphosphorylcholine.

$\sigma_p$. Such a non-linear dependence [12] may be caused by the extremely short range of the hydration phenomena in the investigated system [13] or else it may be due to the 'softening' and 'thickening' of the interfacial region with increasing surface local excess charge density.

This conclusion has a rather general validity. One can, therefore, inspect the membrane-dependent suppression of antibody binding to the corresponding haptenated membranes (see fourth column in Table 1). The effective interfacial repulsion between the studied membrane and/or a macromolecule is then always seen to get stronger with increasing surface polarity value, $\sigma_p$.

Straight-forward modeling of these experimental findings in terms of Eqs. 1 and 2 is not permissible. Non-linearity effects, which are neglected in these expressions, preclude such simple modeling. This notwithstanding the fact that it is possible to correlate the data given in the third and the fourth columns of Table 1 directly.

The results of such a correlation test are illustrated in Fig. 5. They prove that the repulsion between two polar membranes and the repulsion between a polar membrane surface and an approaching (or binding) macromolecule, such as antibody, are indeed closely related ($R = 0.98$).

## 6. Molecular basis of specific (protein) binding

The efficacy of the specific association between a protein and its ligand, discussed in the previous

paragraphs, has been argued to be dependent on the proximity of a polar surface. As a rule, the apparent binding constant is lower for a surface-attached ligand than for a freely accessible ligand. The importance of this suppression increases with increasing lipid (and thus membrane surface) polarity.

This suggests that the proximity of a highly hydrophilic surface may become quite an obstacle for the binding of proteins to their specific receptors in biological membranes. Immunological interactions at the surfaces of lipid bilayers, consequently, are promoted and enhanced by the incorporation of haptens into the relatively apolar bilayer; alternatively, and more elegantly, a similar gain can be reached by introducing a sufficiently long spacer between the presenting surface and the actual binding site. This is the reason why phosphatidylethanolamine-caproyl derivatives are much better immunogens than corresponding simple phosphatidylethanolamine derivatives [16]. This is also an explanation for the observation that phosphatidylethanolamine bilayers are better antigen presenters than phosphatidylcholine membranes, for example.

## 7. Molecular basis of non-specific (protein) adsorption

Similar phenomena are observed in studies of non-specific protein adsorption to a surface of the model membranes.

An arbitrary macromolecule approaching such a lipid bilayer surface must penetrate through the repulsive, interfacial barrier before it can adsorb non-specifically to the membrane. This normally happens only with a few peptide residues at a time which minimizes the energy cost of such a penetration. Moreover, once the repulsive barrier is overcome, the initial energy loss can be compensated for by the subsequent energy gain from the protein accommodation in the interfacial region.

While being similar in some respects, non-specific macromolecular adsorption to an interface differs in several other aspects from molecular binding to a specific receptor at the membrane surface. In the former case the participating molecules are subject to only a few structural constraints. (In fact, the denaturation of protein molecules and major changes in the lipid packing properties at the adsorption site are nearly inevitable in such a situation.) In the latter case, the bound entities are confined into an energetically unfavourable region, from the point of view of interactions with lipid molecules at least.

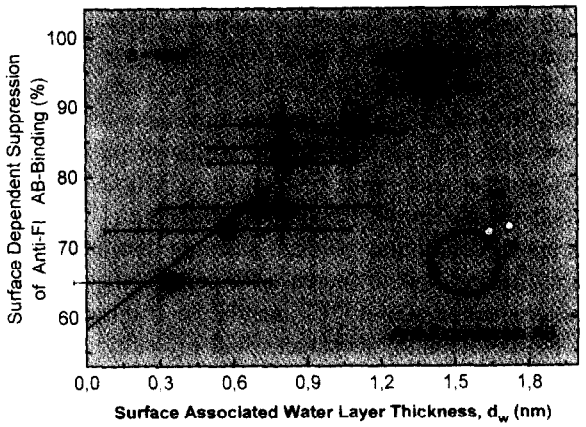


Fig. 5. The effect of surface repulsion on the efficacy of hapten–antibody binding at the lipid bilayer–solution interface. Increasing equilibrium separation between the hapten-presenting membranes, $d_w$, is a direct measure of the strength of surface repulsion and results in a progressively lower capability of macromolecules to bind to their surface-associated ligands. The line gives the results of a linear regression analysis.

The free energy gain associated with the protein adsorption to a lipid bilayer normally stems from the insertion of the protein's hydrophobic residues into the hydrocarbon membrane core [17]. Any imperfections in this core, therefore, are prone to increase the probability for and the stability of non-specific membrane–protein association.

Protein adsorption to a lipid bilayer is hence particularly strong in the phase-transition or in the phase-separation region [18]. In order to minimize the adsorption of proteins to lipid bilayers, proximity of a phase transition temperature or mixed lipid systems should thus be avoided. Ordered phases, moreover, should be given preference over fluid ones; rather than to choose lipids with a low chain-packing density (such as charged or strongly hydrophilic lipids) moderately polar and densely packed lipid bilayers should be used.

## 8. Macromolecular adsorption effects

Before any in vitro and in vivo application it is also important to assess the changes that an interfa-

cially adsorbed or bound macromolecule is going to cause in the properties of its 'accepting membrane'.

Two considerations, in this respect, are particularly important. On the one hand one should always remember that proteins adsorb to the membrane by inserting at least some of their hydrophobic peptide residues into the hydrophobic membrane core. This is apt to deteriorate the chain-packing density of the lipid bilayer and to increase its permeability to the water soluble solutes [19]; membrane affinity for the small amphiphilic substances will probably also increase and the chemical lipid stability will most likely decrease simultaneously. Secondly, the non-specifically adsorbed or specifically bound macromolecules will form a membrane 'coat'. This is going to affect all bilayer–cell interactions. Direct fusion or the transfer of material between the lipid vesicle and a non-phagocytic cell, for example, will be less probable after such an opsonization [20]. In contrast to this, the probability of the elimination of a lipid vesicle by phagocytes, most notably by liver macrophages (Kupffer cells), will increase dramatically by the presence of surface-associated, denatured protein molecules [21]. Fig. 6 illustrates this for simple and surface-modified lipid vesicles.

To avoid the accumulation of lipid vesicles in the liver, which is frequently undesired, it is thus necessary to suppress the adsorption and denaturation of proteins at the lipid bilayer surface [21–23]. To maximize the efficacy of binding of the specific vesicle binding to some selected organ or body sub-site it is important to attach the targeting molecules at some distance from the surface [24]. In both cases, chemical modification of the lipid polar head-groups is useful for this purpose.

## 9. Membrane surface modification

The simplest possibility for improving the repulsive barrier of a lipid bilayer is to make the lipid head-groups longer. The proviso for the success of

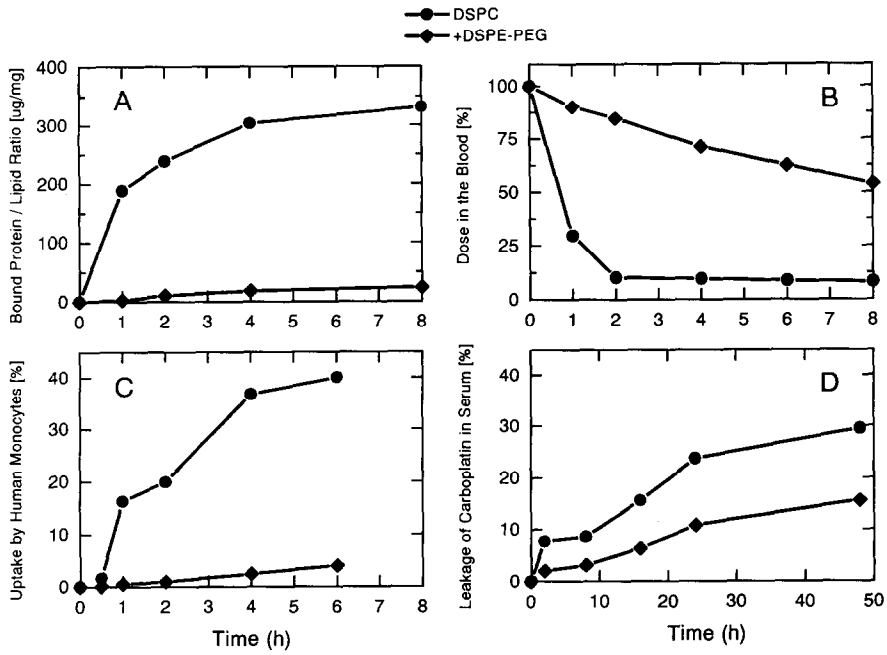


Fig. 6. Time-course of the characteristic parameters of standard phosphatidylcholine membranes (●) and of the surface-modified (PEG-ylated) liposomes (◆). Upper left (A): adsorption of serum components to the bilayer surface; upper right (B): clearance from the blood circulation, lower left (C): uptake by the THP-1 (human monocyte) cells in human plasma at 37°C; lower right (D): serum-mediated release of an antineoplastic agend, carboplatin, from the lipid vesicles at 37°C. A 10% admixture of long-headed DSPE–PEG derivative to the DSPC host membrane is seen to decrease the surface propensity for the interactions with macromolecules and cells.

such a treatment is that the overall head-group hydrophilicity and mobility are not diminished by such lipid modification.

One can understand the significance — and the origin — of such a requirement by inspecting the data given in ref. [3]. These illustrate the effects of increasing interfacial thickness ($d_p$) on the effective intermembrane repulsion [9]: interfacial thickness is seen to increase rather directly the height of the 'protective' repulsive barrier at the bilayer surface. One can, therefore, minimize the danger of an undesired protein adsorption to the bilayer surface by attaching flexible, polar molecules or molecular segments to the phospholipid head-groups [23]. Phospholipid–polyethyleneglycol (PEG) derivatives provide the best explored [22,23], but by no means the only possible example [23], for this.

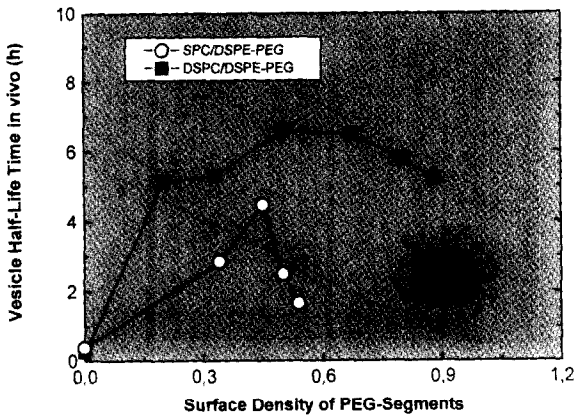


Fig. 7. Liposome elimination from the murine bloodstream as a function of time and the surface density of head-group-modified lipids (DSPE–PEG110) in the bilayer. (Host lipid matrix consisted of distearoylphosphatidylcholine (DSPC, ■) or soybean phosphatidylcholine (SPC, ○) and was labelled with $^3$H-dipalmitoylphosphatidylcholine; from ref. [21].)

## 10. Rational design of strongly repulsive membranes for applications in vivo

Fig. 6 shows the results of in vivo measurements with phosphatidylcholine liposomes mixed with phosphatidylethanolamine–PEG110. Vesicles made from such a lipid mixture circulate at least ten times longer in the blood stream than standard phosphatidylcholine liposomes when they are injected intravenously in mice. 24 hours after the application, nearly 25% of the modified liposomes are still in circulation whilst, simultaneously, less than 5% of standard phosphatidylcholine vesicles are then found in the blood.

The kinetics of protein adsorption to the surface of simple and polymer-coated phosphatidylcholine bilayers (Fig. 6A), the investigations of liposome elimination from the murine circulation in vivo (Fig. 6B), the uptake of simple and sterically stabilized liposomes by the human phagocytes in vitro (Fig. 6C), and the temporal dependence of the cytotoxic drug release from the vesicle in human serum (Fig. 6D), all convey the same message: lipid vesicles with a thick, highly polar interface, due to the presence of DSPE–PEG in the bilayer, interact with the surrounding and with the biological (macro)molecules or living cells less avidly and efficiently than simple liposomes.

In contrast with wide-spread belief, the fluidity of the hydrocarbon chains in the bilayer interior and the net negative charge density of the lipid bilayer surface only play a minor role in this; not even the net hydrophilicity of the lipid bilayer surface really matters. The predominant factor which governs the interactions between model membranes and biological molecules is the effective surface density and mobility of the polar segments in the interfacial region, this is the density of long-headed lipids in the bilayer. This factor namely enhances the repulsion between a lipid bilayer surface and the biological fluids most strongly. In order to optimize the longevity of surface-modified liposomes in vivo one thus has to get this one parameter right: lipid vesicles remain in the blood the longest when they contain some 20 or 30 mol-% of the PEG-ylated long-headed phospholipids when the host matrix is in the gel or fluid phase, respectively (cf. Fig. 7). For fluid- and gel-phase liposomes optimum surface density of such long-headed components is approximately the same.

This confirms the significance of the dynamical character of the interfacial barrier in the process of interfacial interaction. Interfacial mobility, in combination with a sufficiently, but not excessively, large thickness of the interfacial region, is crucial for preventing or slowing down the adsorption of macro-

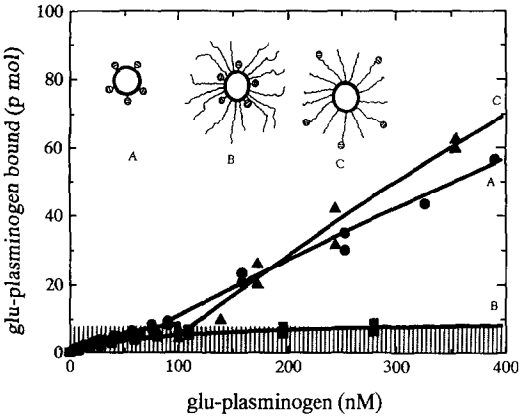


Fig. 8. Effect of the interfacial modification on the efficacy of molecular recognition at and binding to the membrane surface. Simple phosphatidylcholine–cholesterol mixed vesicles with surface attached receptors for glu–plasminogen and the corresponding polymer-coated cryptosomes with the receptors, coupled to the inner or to the outer membrane surface, respectively, bind very differently to the corresponding lipid membranes in spite of the fact that their intrinsic binding affinity is always the same, due to the extended interfacial region ('sterical' repulsion) effect. (Hatched area gives the region of experimental uncertainty. From ref. [24].)

molecules from the blood to the lipid bilayer surface. If this polymer layer at the membrane surface is too tightly packed and immobile, a new interface arises which permits a different type, but not less detrimental, macromolecular adsorption.

Based on the picture of molecular recognition given in previous sections one can conclude that all polymers with similar physico-chemical properties and low biological reactivity should be useful for the preparation of long-circulating liposomes, cryptosomes. If the resulting 'hidden-bodies' are to bind to the specific targets in the body, however, their strongly repulsive surface becomes an obstacle. In order to improve on this, the accessibility of the surface ligands for the targeting moieties must be improved by a slight modification of their surface chemistry.

Covalent receptor attachment onto the termini of lipid head-groups conveniently solves this problem, for example. This can be seen from the data given in Fig. 8. Coupling of the receptor to the outer edge of protective region also brings this receptor out of the region of strong repulsion and thus makes it more easily accessible to its ligands. This increases the efficacy of binding dramatically. Indeed, the final apparent binding efficiency for such a terminally bound receptor may be higher than that of the same receptor located at the very surface of a plain phospholipid bilayer.

Surface-modified lipid vesicles with terminally attached receptors offer all the advantages of a targetable system. Surface-modified non-targetable but long-circulating liposomes may have an unusual biodistribution in vivo per se, however. Owing to the sieving function of imperfect endothelium in the blood capillaries of inflamed or tumour tissues, for example, the effective concentration of the material that is injected in the form of cryptosomes may exceed by a factor of 10 and more the corresponding results obtained with ordinary phospholipid vesicles. This offers an interesting opportunity for drug delivery into neoplastic tissues.

## 11. Conclusion

In summary, molecular recognition at biological surfaces can be understood and described in terms of simple (bio)physical models in which all pertinent intermolecular interactions are allowed for. Solvation-dependent steric repulsion and electrodynamic (van der Waals) attraction appear to be particularly important in this respect, while direct 'hydration force' and Coulombic interactions only play an indirect role. (The former affects the interfacial swelling and the (quantum-mechanical) electrostatics at short separations and the latter chiefly modifies the molecular concentration in the vicinity of an interface.) Specific molecular binding to a surface-attached receptor, as a rule, is (partly) suppressed by the surface proximity, unless the receptor is bound at the end of a sufficiently long spacer. The latter can be very short when simple phospholipid membranes are studied but needs to be extended for systems with a thick interface. Non-specific macromolecular adsorption is less sensitive to interfacial repulsion and more to the number and type of the hydrophobic binding sites (spontaneously occurring or induced 'defects') in or at the surface. Ionic interactions may, but need not be, involved in this process.

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