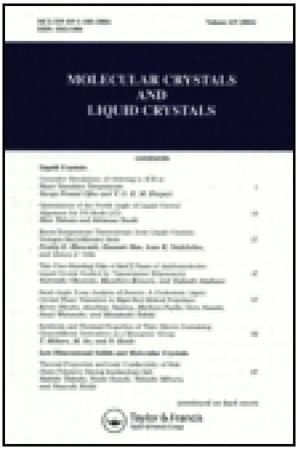

Molecular Crystals and Liquid Crystals

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl16

Lipid Bilayers in the “Fluid State: Computer Simulation and Comparison with Model Compounds

Vincenzo Busico [a] & Michele Vacatello [a]
[a] Istituto Chimico dell' Università, Via Mezzocannone, 4-80134, Naples, Italy

Version of record first published: 17 Oct 2011.

To cite this article: Vincenzo Busico & Michele Vacatello (1983): Lipid Bilayers in the “Fluid State: Computer Simulation and Comparison with Model Compounds, Molecular Crystals and Liquid Crystals, 97:1, 195-207

To link to this article: http://dx.doi.org/10.1080/00268948308073150

Mol. Cryst. Liq. Cryst., 1983, vol. 97, pp. 195–207
0026-8941/83/9704–0195/$18.50/0

Printed in the United States of America

Lipid Bilayers in the "Fluid" State: Computer Simulation and Comparison with Model Compounds†

VINCENZO BUSICO and MICHELE VACATELLO
Istituto Chimico dell' Università, Via Mezzocannone, 4-80134 Naples, Italy

(Received January 27, 1983)

Monte Carlo simulations of mono- and bilayers of alkyl chains at variable surface density have been carried out, whose results are in good agreement with experimental data on model compounds. In particular, the perturbation to the conformation of the alkyl chains in the liquid crystalline environment derived from excluded volume effects is described in detail.

INTRODUCTION

Due to segregation of the ionic parts from the aliphatic residues, hydrocarbon chain molecules with ionic end groups give microphase separation both in bulk and in aqueous dispersion above a critical concentration.[1-3]

Of the possible resulting structural arrangements, bilayers are of overwhelming importance. Stackings of bilayers indeed are found in the crystalline state, and in many plastic and liquid crystalline phases of alkali metal n-alkanoates, n-alkylammonium halides and natural and synthetic phospholipids of different classes.[1,2,4] Single bilayers of lipid molecules, on the other hand, are considered to constitute the "matrix" of cell membranes.[5]

Thermotropic polymorphism is usually observed in these systems. Below a given temperature T_c, the polymethylenic chains are crystallized

---

†Presented at the Ninth International Liquid Crystal Conference, Bangalore, December 6–10, 1982.

mainly in the planar zig-zag conformation. Above T_c, they change to a state variously defined as "fluid," "disordered" or "liquid-like."[5]

It is generally accepted that the fluid state originates from the onset of rotational isomerism of the chains around their skeletal bonds.

Nevertheless, in spite of the abundant literature covering many experimental and theoretical aspects of the problem, the current picture of the conformational disorder of the alkyl chains in the fluid state of lipid bilayers is still poorly defined. This seems to be only one side of a more general lack of comprehension of partially disordered hydrocarbon systems in which the conformational freedom is limited to some extent by directional constraints.

"The detailed organization of the hydrocarbon chains" in the fluid state indeed "has been variously described as (...) chaotic, extended and ordered, significantly ordered, and partly ordered with some rotational isomerism."[5]

The reason for such often contrasting opinions is mainly the apparent contradiction between the disorder of the polymethylenic moieties—as evidenced by X-ray diffraction and spectroscopic techniques[6,7]—and the high degree of overall structural organization of the fluid state. In particular, the presence of close neighboring chains with one extremity bound to a plane is considered to reduce drastically the degrees of conformational freedom available to the system.[8]

In other words, out of the set of conformations accessible to a chain in the hypothetical unperturbed "gaseous" state, only a limited subset should not be forbidden by excluded volume interactions with neighboring chains in this layered fluid state.

It seems likely that the conformations of such a subset be preferentially extended in a direction perpendicular to the layers. This is confirmed by the high values of the measured interlayer distances and by ^{13}C- and D-Magnetic Resonance studies.[9,10]

Apart from FTIR and Raman spectroscopic experiments, for which interpretations are often rather ambiguous, a technique presently available in order to obtain direct information on the conformational statistics of hydrocarbon chain molecules in a layer environment appears to be computer simulation, provided that all the interactions be taken into account with reasonable accuracy and the model be large enough to give statistically significant results.

A number of attempts to model the local situation in layers of alkyl chains have been made in recent times using Monte Carlo and molecular dynamics methods.[11-14]

Up to now, however, only the calculation of Ref. 14 could be assumed to approximate to the equilibrium distribution of chain conformations in real layer systems because both the size of the model and the torsional and nonbonded potentials used appear to be adequate. On the other hand, the n-decane molecules simulated are, in our opinion, too short to provide conformational data free from the influence of the chain termini.

We have performed Monte Carlo computer simulations of mono- and bilayers of n-hexadecane molecules at different surface densities, and the average conformation of the alkyl chains in the equilibrium state of our models has been examined in some detail. The outcome of these simulations will be presented and discussed in the following.

Preliminarily, model compounds synthesized in our laboratories will be described, for which a number of physical parameters were measured as a guide to or a check for the computer simulations.

STUDIES ON MODEL COMPOUNDS

In any system in which long hydrocarbon groups are organized in layers, the area A available to each group on the layer surface is fundamental to the resulting structure. In fact, A is the average cross-section of the hydrocarbon residues in planes parallel to the layers, and $1/A$ is the so-called "surface density."[15]

For a linear chain close-packed in the polyethylenic lattice,[16] A is 18 Å^2. In crystalline phospholipids,[17] A is of the order of 25 Å^2; for phospholipids in the fluid state and some cell membranes,[18,19] A is in the range 30–35 Å^2.

We have synthesized model compounds of the general formula (n-$C_nH_{2n+1}NH_3)_2MX_4$ (henceforth denoted CnMX) with M a divalent metal, X a halogen and n varying between 8 and 18. In these compounds, the alkyl residues are linked, through ammonium groups, to a high-melting framework of composition MX_4^{2-}. The crystal structure of such systems results from the stacking of bilayers constituted by a thin inorganic region sandwiched between two thick hydrocarbon regions.[20] The details of the structure have been described elsewhere.[21-23]

With an appropriate choice of M and X, we have been able to obtain[20-25] structures with A values ranging from 26 to 40 Å^2.

At low temperatures, the alkylammonium groups are crystallized mainly in the planar zig-zag conformation (modification II).

For lower A values, the chains, in order to achieve a close packing, must incline with respect to the perpendicular to the layers, at an angle α so that their cross-section may increase from 18 to A Å^2. The resulting structure is shown in Figure 1 for a representative case. In practice, the value of α is determined univocally by the value of A. Typical figures are: A = 26 Å^2, α = 45°;[20] A = 30 Å^2, α = 53°.[26]

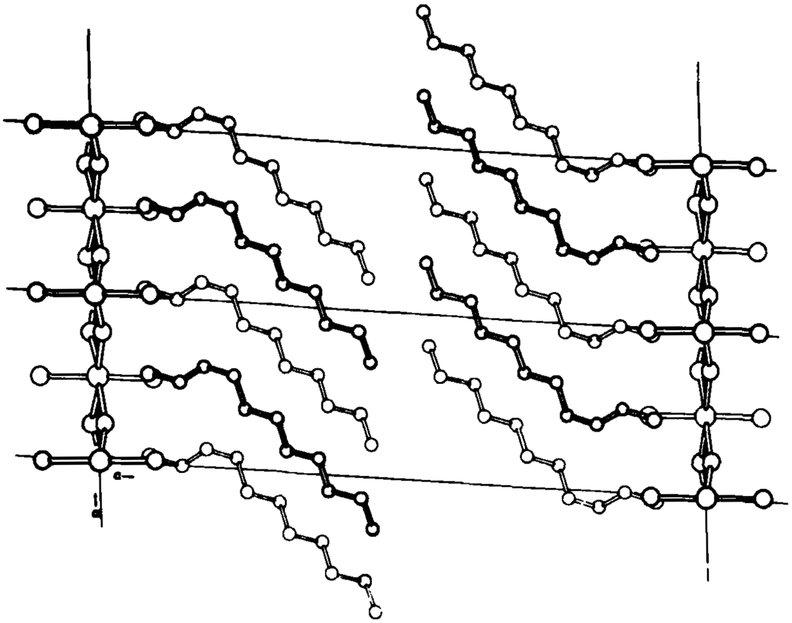

FIGURE 1 Crystal structure of C10MnCl in modification II.[21]

When A is about 36 Å^2 or greater, another packing mode becomes possible: the chains are roughly perperpendicular to the layers and there is intercalation of chains from adjacent layers (Figure 2). Actually, the latter mode is always observed in our model compounds$^{20,22-24}$ for $A \geqslant 36$ Å.^{2}

At a temperature T_c dependent on M, X, n and generally lying between 270 and 400 K, the compounds CnMX change reversibly from modification II to another crystalline form (modification I) through one or more high entropy solid-solid phase transitions. Modification I differs from modi-

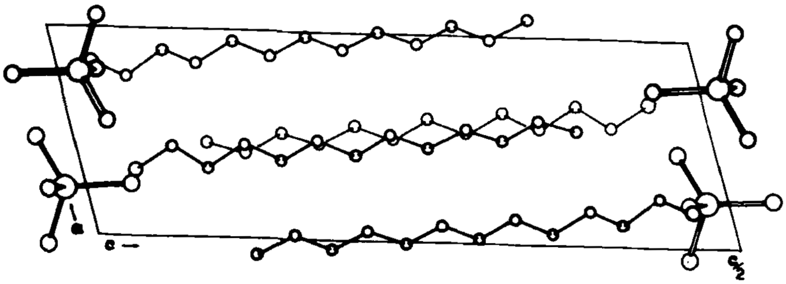

FIGURE 2 Crystal structure of C12ZnCl in modification II.[22]

fication II in that the aliphatic chains of the alkylammonium groups are in a liquid-like state (Figure 3); the inorganic regions—hence the value of A—remain practically unchanged at the transition(s).[27]

The liquid character of the chains in modification I has been shown with a number of independent techniques. In particular:

i) the short spacing region in the X-ray powder diffraction patterns in transmission changes at the transition(s) as shown in Figure 4. The spectrum of modification I looks like the diffraction pattern of a hydrocarbon melt;[28]

ii) the IR spectrum of modification I is, for the hydrocarbon part, similar to that of a liquid, and coincides with the spectrum of the melt for those systems in which melting takes place before thermal decomposition (Figure 5);[28]

iii) the solid-solid transition entropy per mole of hydrocarbon chains is close to the molar melting entropy for the n-alkanes of comparable chain length;[27]

iv) when melting is prior to decomposition, the entropy of fusion is observed to be independent of the length of the alkylammonium groups, and compares with the melting entropy of compounds of stoichiometry A_2B without conformationally flexible bonds.[27]

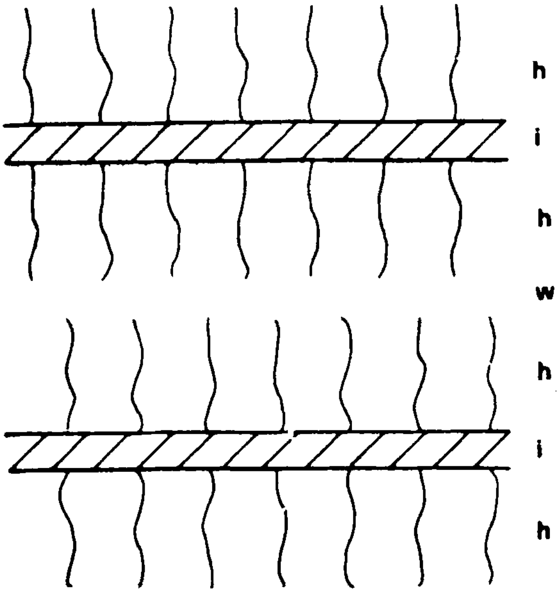

FIGURE 3 Schematic structure of the compounds CnMX in modification I.

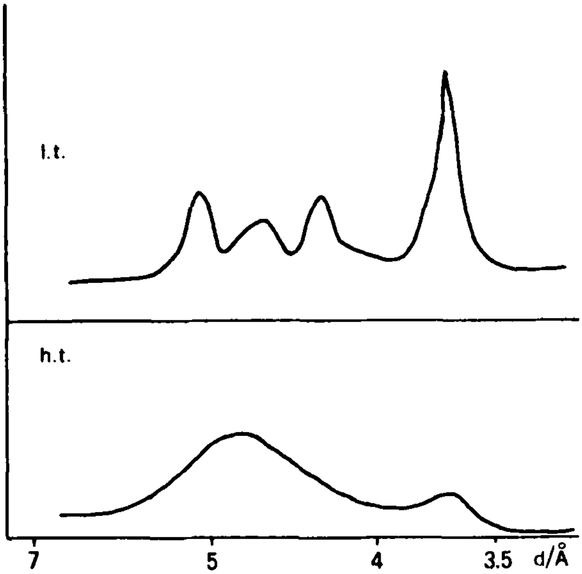

FIGURE 4 X-ray powder transmission spectrum of C14MnCl. l.t.: modification II; h.t.: modification I.

In our opinion, the compounds CnMX offer several advantages as model systems of lipid bilayers, as far as the conformation of the alkyl chains in the fluid state is concerned. In particular, single crystals of well-defined stoichiometry suitable for complete X-ray characterization are easily available. In addition, within each series CnMX, the structure of modifications I, II is substantially the same for homologues with different values of n; derivation of average structural and physicochemical parameters is then straightforward and significant.

A number of such parameters is listed in Table I. For each pair M, X are indicated:

i) the value of A;

ii) the value of $\overline{\Delta S_t}$, the mean increment of the entropy of transition (modification II → modification I) per mole of alkyl chains following the addition of one carbon atom (1C) to the chains;

iii) the value of $\overline{\Delta z}$, the mean increment of interlayer half-distance following the addition of one carbon (1C) atom to the chains in modification I;

iv) the value of $v(CH_2)$, the average volume per methylene in modification I, calculated as $A \cdot \overline{\Delta z}$;

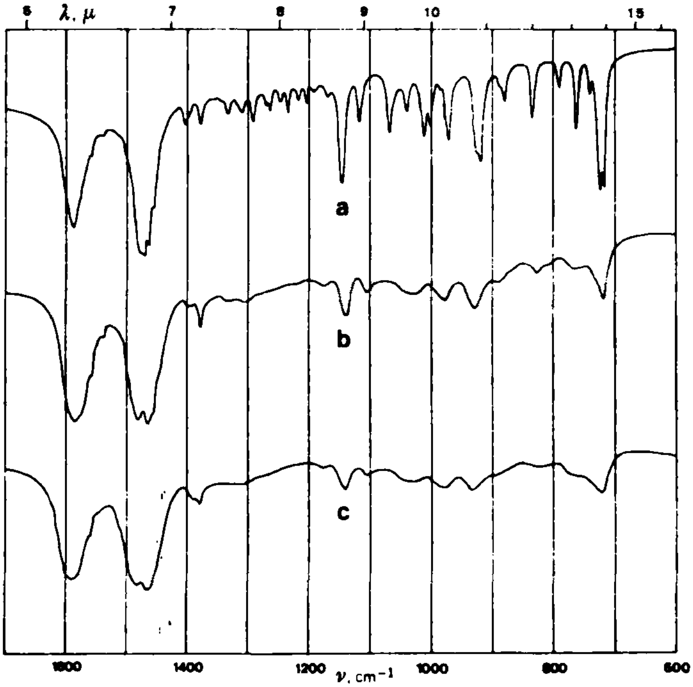

FIGURE 5 1700–600 cm^{-1} region of the IR spectrum of Cl2CoCl. (a) Modification II; (b) modification I; (c) melt.

TABLE I

Some average structural and physicochemical parameters for compounds CnMX[†]

M	X	A (Å^2)	$\overline{\Delta S_t}$ (R)	$\overline{\Delta z}$ (Å)	$\nu(CH_2)$ (Å^3)	ρ
Mn	Cl	26	0.9	1.05	27	n.d.
Cu	Cl	26	0.9	1.05	27	0.7*
Hg	Cl	30	0.8	0.9	27	n.d.
Zn	Cl	36	1.0	0.8	29	n.d.

[†] For explanation see text.

*From: M. Kozelj *et al.*, *J. Chem. Phys.*, **74**, 4123 (1981).

v) the value of ρ, the average order parameter for the chain interior (for definition read further) when known.

Table I shows for $\overline{\Delta z}$, (which can be viewed as the average projection of each C—C bond in the direction perpendicular to the layers in modification I), values in the range 0.8–1.05 Å. This means that the alkyl chains are strongly extended along the layer normal. On the other hand, $\overline{\Delta z}$ is seen to change with A so as to give a substantially constant value of $v(CH_2)$, of the order of 27–29 Å^3. This value coincides with the volume per methylene found in liquid n-alkanes and in amorphous polyethylene at comparable temperatures.[29] Accordingly, the values of $\overline{\Delta S_t}$ (0.8–1.0 R) are only slightly lower than the melting entropy per mole of methylene for n-hydrocarbons (1.1–1.2 R).[30]

Reference to the parameters of Table I will be made in the following section.

STUDIES ON COMPUTER MODELED SYSTEMS

Monolayers and bilayers of n-hexadecane molecules at variable surface density were modeled *via* computer simulation, using Monte Carlo techniques.[31]

n-Hexadecane molecules were represented in our calculations as sets of 16 point units with 1.54 Å bond lengths and tetrahedral valence angles. The distribution of conformations available to each molecule was reduced, according to the rotational isomeric approximation, to a discrete set of conformers in which only *trans*, *gauche* plus and *gauche* minus rotations around internal bonds were allowed (t, g_+ and g_-, with torsion angles 180°, 60° and −60°, respectively). The energy difference between *gauche* and *trans* states was taken as $E_g = 2.1$ kJ mol^{-1}. Repulsive interactions between units belonging to different chains or to the same chain but separated by more than three bonds were simply modeled with a hard sphere potential with a sphere diameter of 3.4 Å. This causes $g_\pm g_\mp$ two bond sequences to be forbidden, in agreement with the well-known "pentane effect."[31]

The choice of a hard sphere potential allowed us to reduce calculation times drastically. On the other hand, it is consistent with the widely accepted opinion that the organization of densely packed liquid (or liquid-like) phases is mainly determined by the shape and dimensions of the constituent molecules.[32]

Arrays of molecules (36 for monolayers, 72 for bilayers) packed at a given surface density with the first units placed in the basal plane(s) at the nodes of a hexagonal array were equilibrated, starting from an arbitrary configuration, with complete translational, orientational and conformational freedom subject to the following constraints:

i) the first unit of each molecule was free to move in the basal plane only;

ii) intermolecular and long range intramolecular contacts shorter than 3.4 Å were forbidden;

iii) distances between methylene units and the basal plane shorter than 1.7 Å were forbidden, but for the first and the second units of each molecule.

We report in the following preliminary results relative to a monolayer at a surface density corresponding to $A = 27$ Å^2, and a bilayer at a surface density corresponding to $A = 32$ Å^2 and thickness 28.71 Å (average volume per methylene *ca.* 28 Å^3, Table II).

Figure 6 shows the average height, z of each chain unit over the basal layer, as obtained in our computer models, vs the unit index, i. In close agreement with the experimental data on the model compounds CnMX (Table I), the average projection, $\overline{\Delta z}$ of a C—C bond along the layer normal in the chain interior is 1.07 Å for the monolayer, 0.9 Å for the bilayer. The close linearity of the plot shows that the density of the simulated systems is constant along the chains, with only minor deviations at the free ends.

The calculated values of the order parameter ρ_i, defined as $\rho_i = \frac{3}{2}\langle\cos^2 \vartheta_i\rangle - \frac{1}{2}$ (ϑ_i being the angle between C_{i-1}/C_{i+1} vectors and the normal to the basal plane) are plotted against the unit index, i in Figure 7.

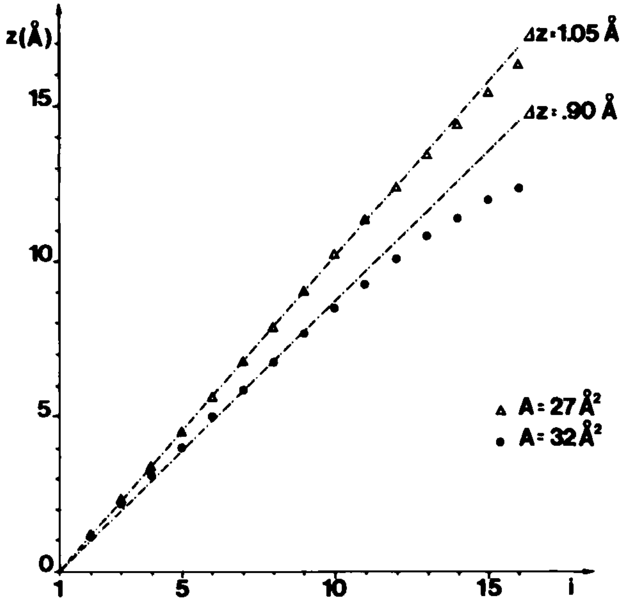

FIGURE 6 Average height, z of each chain unit over the basal layer vs unit index, i. (△) monolayer at $A = 27$ Å^2; (●) bilayer at $A = 32$ Å^2. The dotted lines reproduce the experimental $\overline{\Delta z}$ for the model compounds CnMX at corresponding surface densities (Table I).

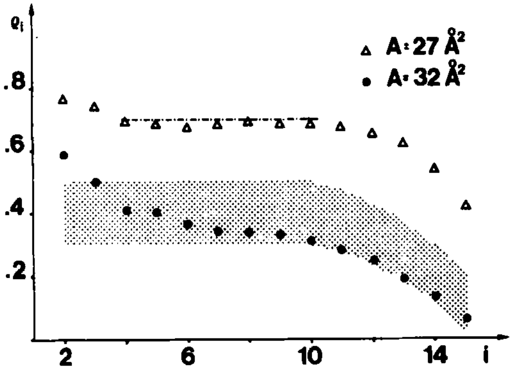

FIGURE 7 Calculated values of the order parameter ρ_i (for definition see text) vs unit index, i. (Δ) monolayer at $A = 27$ Å²; (●) bilayer at $A = 32$ Å². The dotted line gives the plateau value of ρ_i experimentally observed for ClOCuCl ($A = 26$ Å², Table I) and predicted by the lattice model of Dill and Flory[33] at $A = 25$ Å². The shaded area includes[5] experimental ρ_i values for bilayers with A in the range 30–35 Å².

The trends and the plateau values experimentally observed for all the investigated lipid bilayers and predicted by the lattice model of Dill and Flory[33] at comparable surface densities are correctly reproduced (see also Table I).

The close agreement between the experimental and calculated $\overline{\Delta z}$ and ρ_i values in the interior of our models makes us confident that the equilibrium distribution of conformations of the alkyl chains in the simulated systems must be representative of that in real bilayers at the same surface density. Such conformations have been examined in some detail.

In Table II, we report the average fraction, f_p of sequences in several given conformations as obtained from the model systems, compared with the corresponding values, f_u evaluated according to known methods[34] for an unperturbed n-hexadecane molecule ($g_\pm g_\mp$ two bond sequences disallowed) at 300 K.

From the table, it appears that, in agreement with current views,[8,35,36] the alkyl chains in the layer environment reach the extended conformation evidenced by the high values of $\overline{\Delta z}$ through both an increased fraction of all-*trans* (t_n) sequences and a clear tendency toward the occurrence of "kink-like" sequences.

That *gauche* bonds in extended chains tend to be located between t_n sequences with odd n is an obvious fact which was pointed out in previous papers.[8,35] Nevertheless, a model in which *gauche* bonds are comprised of $g_\pm t g_\mp$ sequences (kinks) only[18] appears from Table II to be by far too restrictive.

Figure 8 shows the fraction, f_g of C_i — C_{i+1} bonds in a g state vs the unit index, i. It is seen that, apart from marked odd-even effects — likely to be a consequence of the influence of the basal layer upon the conformation of the first few bonds, tending to be perpetuated along the chains by the cooperative character of the allowed sequences — the local conformation of the chain interior is quite independent of the distance from the basal layer.

CONCLUSIONS

The overall picture emerging from the characterization of the model compounds CnMX as well as of the computer modeled systems is that disordered polymethylenic chains in a layer environment are perturbed in such a way as to assume preferably conformations extended along the perpendicular to the layer.

Relative to the unperturbed "gaseous" state, these extended conformations are obtained through both an increased number of *trans* bonds and the occurrence of suitable sequences of torsional states (of which "kink-like" sequences are a considerable fraction) allowing such an extension (*e.g.* $g_{\pm}tg_{\mp}$, $g_{\pm}tttg_{\mp}$, etc.).

According to our results anyway, the perturbing effects are not so conditioning as often claimed in the literature.[5,8] For instance, more than half

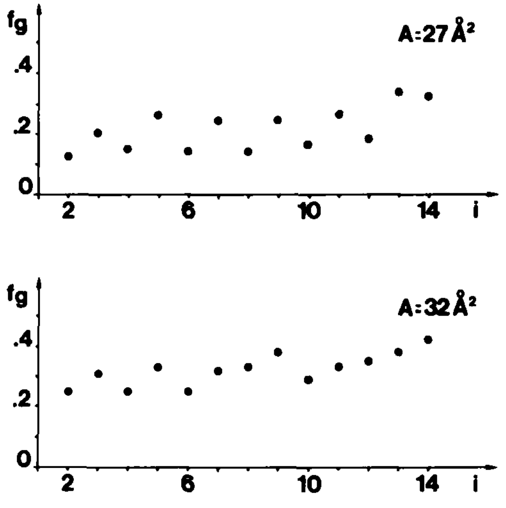

FIGURE 8 Fraction f_g of C_i—C_{i+1} bonds in a g state vs unit index, i for the model monolayer (A = 27 Å^2) and bilayer (A = 32 Å^2).

TABLE II

Fraction of sequences in various conformations[†]

Sequence	f_p (A = 27 Å^2)	f_p (A = 32 Å^2)	f_u
t	.79	.69	.64
tt	.60	.45	.38
$tg_\pm$	.098	.12	.13
$g_\pm g_\pm$	.013	.034	.047
ttt	.52	.32	.22
$g_\pm tg_\pm$	.014	.021	.028
$g_\pm tg_\mp$	.033	.031	.028
$ttttt$	.39	.17	.076
$g_\pm tg_\pm tt$	.005	.007	.012
$g_\pm tg_\mp tt$	.020	.016	.012
$ttg_\mp tg_\pm$	.020	.016	.012
$g_\pm tg_\mp tg_\pm$	.006	.005	.004
$g_\pm tttg_\pm$	.004	.007	.010
$g_\pm tttg_\mp$	.008	.011	.010

[†] As obtained from the model monolayer (A = 27 Å^2) and bilayer (A = 32 Å^2); f_p and calculated for an unperturbed n-hexadecane molecule at 300 K, f_u ($g_\pm g_\mp$ two bond sequences dissallowed).

the chains in our models have at least one of the first five internal bonds (starting from the basal plane) in a *g* state. Hence, our results do not support the recent suggestion that very long all-*trans* sequences (about eight bonds) are largely present in this region of the layers, the conformational disorder being concentrated in the remaining part of the molecules.[37] Further, *g* bonds can occur also out of kinks (Table II), though to a lesser extent than in the unperturbed state.

Lastly, the strength of the perturbation is seen to decrease rapidly with decreasing surface density. The trend of the results in Table II indeed suggests, for systems with surface densities corresponding to *A* values higher than 32 Å^2, conformational statistics with only minor differences relative to the unperturbed state.

Acknowledgment

The authors are heavily indebted to Prof. Paolo Corradini for valued suggestions and enlightening discussions. Thanks are also due to the National Research Council (CNR) and to the Ministry of Public Education of Italy for financial support.

References

1. A. E. Skoulios and V. Luzzati, *Acta Cryst.*, **14**, 278 (1961).
2. V. Busico, P. Cernicchiaro, P. Corradini and M. Vacatello, *J. Phys. Chem.*, in press.

3. See for example: J. N. Israelachvili, P. J. Mitchell and B. W. Ninham, *Biochim. Biophys. Acta*, **470**, 185 (1977).
4. *Biological membranes* (Ed., D. Chapman), Academic Press, New York, NY, 1968.
5. P. J. Quinn and D. Chapman, *Critical Rev. Biochem.*, **8**, 1 (1980).
6. D. Chapman, *J. Chem. Soc.*, **152**, 784 (1958).
7. D. Chapman, R. M. Williams and B. D. Ladbrooke, *Chem. Phys. Lipids*, **1**, 445 (1967).
8. J. Seelig, *Quarterly Rev. Biophys.*, **10**, 353 (1977).
9. M. Kozelj, V. Rutar, I. Zupancic, R. Blinc, H. Arend, R. Kind and G. Chapuis, *J. Chem. Phys.*, **74**, 4123 (1981).
10. J. Seelig and A. Seelig, *Quarterly Rev. Biophys.*, **13**, 19 (1980).
11. P. Bothorel, J. Belle and B. Lemaire, *Chem. Phys. Lipids*, **12**, 96 (1974).
12. H. L. Scott, Jr., *Biochim. Biophys. Acta*, **469**, 264 (1977).
13. A. J. Kox, J. P. Michels and F. W. Wiegel, *Nature*, **287**, 318 (1980).
14. P. van der Ploeg and H. J. C. Berendsen, *J. Chem. Phys.*, **76**, 3271 (1982).
15. A. Tardieau, V. Luzzati and F. C. Reman, *J. Mol. Biol.*, **75**, 711 (1973).
16. G. Avitabile, R. Napolitano, B. Pirozzi, K. D. Rouse, M. W. Thomas and B. T. M. Willis, *J. Polym. Sci. Polym. Lett. Ed.*, **13**, 351 (1975).
17. D. Chapman, R. M. Williams and B. D. Ladbrooke, *Chem. Phys. Lipids*, **3**, 234 (1969).
18. H. Träuble and D. H. Haynes, *Chem. Phys. Lipids*, **7**, 324 (1971).
19. D. Engelman, *J. Mol. Biol.*, **58**, 153 (1971).
20. E. Landi, V. Salerno and M. Vacatello, *Gazz. Chim. Ital.*, **107**, 27 (1977).
21. M. R. Ciajolo, P. Corrandini and V. Pavone, *Gazz. Chim. Ital.*, **106**, 807 (1976).
22. M. R. Ciajolo, P. Corradini and V. Pavone. *Acta Cryst.*, **B33**, 553 (1977).
23. G. Avitabile, M. R. Ciajolo, R. Napolitano and A. Tuzi, *Gazz. Chim. Ital.*, in press.
24. V. Busico, D. Castaldo and M. Vacatello, *Mol. Cryst. Liq. Cryst.*, **78**, 221 (1981).
25. M. Vacatello, M. De Girolamo and V. Busico, *J. Chem. Soc. Faraday Trans. I*, **77**, 2367 (1981).
26. V. Busico, V. Salerno and M. Vacatello, *Gazz. Chim. Ital.*, **109**, 581 (1979).
27. E. Landi and M. Vacatello, *Thermoc. Acta*, **13**, 441 (1975).
28. C. Carfagna, M. Vacatello and P. Corradini, *Gazz. Chim. Ital.*, **107**, 131 (1977).
29. W. Dollhopf, H. P. Grossmann and U. Leute, *Coll. Polym. Sci.*, **259**, 267 (1981).
30. A. Bondi, *Chem. Rev.*, **67**, 565 (1967).
31. M. Vacatello, V. Busico and P. Corradini, *J. Chem. Phys.* **78**, 590 (1983).
32. P. J. Flory and G. Ronca, *Mol. Cryst. Liq. Cryst.*, **54**, 289 (1979).
33. K. A. Dill and P. J. Flory, *Proc. Natl. Acad. Sci. USA*, **77**, 3115 (1980).
34. P. J. Flory, *Statistical Mechanics of Chain Molecules*, Wiley Interscience, New York, NY, 1969.
35. M. Vacatello and P. Corradini, *Atti Accad. Sci. Fis. Mat. (Naples) IV*, **XLIV**, 505 (1977).
36. D. W. R. Gruen, *Chem. Phys. Lipids*, **30**, 105 (1982).
37. H. Vogel and F. Jähnig, *Chem. Phys. Lipids*, **29**, 83 (1981).