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Organizational consequences of the hydrophobic interaction

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Abstract. If otherwise hydrophobic molecules also contain hydrophilic groups, they become known as "amphiphilic" and they are then subject to a potent organizational effect, which orients each molecule so as to satisfy as far as possible the thermodynamic requirements of both parts of the molecule. One consequence is the formation of monolayers of oil on water, which has been of great historical importance in physics and chemistry, leading to the first determinations of molecular size, symmetry and flexibility. The most important role of this kind of organizational force, however, is in biology. Here amphiphilic phospholipid molecules are organized into bilayers that are essential to the very existence of a living cell, defining the boundary between the inside of a cell and its environment. The formation of specific structures of proteins, too, is dominated by the hydrophobic interaction: in this case the need to fold the protein in such a way (intricately, with many twists and turns) as to minimize contact between hydrophobic groups and the surrounding aqueous medium. It is not an exaggeration to say that hydrophobic interactions are essential for all aspects of the chemistry of life as we know it.

Keywords. Monolayers; bilayers; lipids; molecular dimension; living cells; proteins.

1. Introduction

The "hydrophobic effect" as a topic in physical chemistry – how to account for the unusual thermodynamic attributes of aqueous solutions of hydrocarbons and certain other poorly soluble solutes – would by itselt be a rather academic subject, of interest to relatively few scientists. The effect happens however to be a major factor in the creation of a potent force for structural organization. The existence of this force has played an important role in the history of physics and chemistry, in the development of clear ideas about the nature of molecules. Its ability to generate spatial organization, with molecules all oriented the same way, made it possible to "see" molecular properties with a clarity that is not possible for unoriented molecules.

Structural organization based on the hydrophobic effect has proved more recently to be even more important in biology, where it is used by nature itself to create structural organization. The very existence of a living cell, the definition of a boundary between the inside of a cell and its environment (irrespective of what the contents of the cell may be) is absolutely dependent on the hydrophobic effect. And within the cell, the existence of enzymes and transport proteins with highly specific functions is likewise absolutely dependent on the influence of hydrophobic interactions. The phenomenon that forms the subject of this volume is essential for virtually every aspect of life.

I shall focus in this paper on this organizational effect, partly in historical fashion, beginning with the first recorded observation of it by Benjamin Franklin more than 200 years ago.

2. Amphiphilic solutes. The size of water molecules

Structural organization implies molecular asymmetry. The effects which will be described in this paper are in fact unique to "amphiphilic" organic molecules, molecules that contain hydrophilic domains as well as hydrophobic domains. Long chain fatty acids or their anions are typical: palmitic or stearic acid, for example, with saturated alkyl chains of 16 and 18 C atoms, respectively, or oleic acid, which also has 18 carbon atoms but contains a double bond, that prevents internal rotation and confers a mandatory kink on the hydrocarbon chain at that point. Each molecule terminates in a hydrophilic COOH or COO^- group.

The biological molecules of greatest interest are described schematically in figure 1. The hydrocarbon chains in this figure represent the fully extended states, with a bend in the middle for the oleyl chain at the position of its double bond. Normally, in the liquid state, the chains have considerable flexibility, with a shorter end-to-end distance, but the difference between saturated and unsaturated chains persists. Unsaturated hydrocarbons have a larger cross-sectional area (perpendicular to the direction of the chain backbone) than saturated ones.

The molecules in figure 1 are all derivatives of fatty acids, containing 2 or 3 hydrocarbon chains per molecule, attached to a common hydrophilic base, namely glycerol. The glycerol molecule has three points of attachment for side chains. Three oleic acid chains are attached in triolein. Three stearic acid chains are attached in tristearin. Only two fatty acid chains are used in phospholipid molecules, the third position on the hydrophilic base being occupied by another strong hydrophilic group. Many different kinds of fatty acids may be used in phospholipid molecules. Typically one of them has a double bond and the other does not, as in the illustration in figure 1.

All molecules of this kind, hydrophobic at one end because of the uninterrupted hydrocarbon chains, hydrophilic at the other end, are torn between the opposing thermodynamic demands of these entities. A specific molecular orientation is the only possible way to try to meet these demands. If an amphiphilic molecule in an aqueous environment has a *random* orientation, then the molecule is bound to move and pivot to try to find its state of minimal free energy.

If an interface is available, on one side of which there is no water, then that is

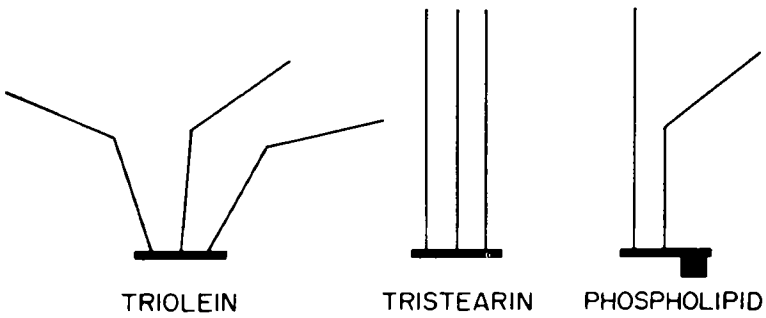

Figure 1. Fats, oils and phospholipids. Schematic representations of triolein (principal component of olive oil), tristearin (typical "saturated" animal fat) and a phospholipid molecule. The straight and kinked lines represent the hydrocarbon chains of stearic and oleic acids, respectively.

where the molecule must move, with its hydrophilic part in the water and its hydrophobic part out of the water. If no interface is available, then the molecule must create its own interface – concerted action among an assembly of amphiphilic molecules becomes necessary to create a volume within the system, within which the hydrophobic parts of the molecule can be in contact with each other and shielded from contact with water.

It is worth noting that these simple consequences of the structure of an amphiphilic molecule or ion depend on an aspect of the hydrophobic interaction that is often not greatly stressed – the fact that water molecules are very small. It is an excellent approximation, not only conceptually but almost quantitatively, to look upon the domains of amphiphilic molecules as nearly independent of each other in their interactions with water. This would not be possible if a water molecule were not small compared to the size of the domains.

3. Benjamin Franklin: Oil on troubled waters

The first scientific report of this powerful organizing effect came from Benjamin Franklin in 1774 (Franklin 1774). Franklin was in London on a diplomatic mission, trying to negotiate an agreement between the King's government and a few of the American colonies to redress grievances that had to do with fiscal matters – taxes and import duties. However, he had no legal standing as a diplomat because only foreign countries could have "ambassadors" with access to the King and Privy Council. The American colonies were part of England, ruled by Governors appointed by the Crown, one for each colony, their positions not unlike the position of the Viceroy in India a century later. Mere citizens were not supposed to go to London to bargain directly with the authorities, even when they were sent by local governing bodies at home, but were supposed to approach the government through the proper channels. Needless to say, progress in processing Franklin's petitions was slow, and he had plenty of time for travel and other activities.

Though diplomatically without status, Franklin did have an honorable standing in England as a scientist. In this respect the perception of America as English "property" helped him. Franklin was a Fellow of the Royal Society, an organization open only to British citizens, having been elected while he was still in Philadelphia, on the basis of his brilliant work on electricity. He had also been awarded the Royal Society's prestigious Copley Medal for this work. He was therefore welcomed by the scientific community when he came to England, as someone already well known to them from his publications. Most of his friends and associates during his many years in England were in fact scientists or philosophers. He was a regular attendee at Royal Society meetings, and was often consulted and befriended by younger scientists, such as Joseph Priestley.

There were still people in England at that time who did not believe in molecules, but Benjamin Franklin certainly did. He was a Newtonian and, like most English scientists, believed in "ultimate particles" of matter, which is what molecules are. But when he did the experiment that led to his 1774 paper he wasn't (at first) thinking about molecules at all, but about the age-old practice of mariners to pour oil on troubled waters – vegetable or fish oil was supposed to calm the waves in a storm. Franklin was skeptical, but he wanted to try the experiment, and one day on

the pond at Clapham Common outside London he did so, using olive oil that you could buy at any grocery store.

And it worked. Just a teaspoonful was enough. It was a windy day and the surface of the pond was rough, but the oil produced an instant calm over a space of several square yards, which extended itself gradually until an area that Franklin estimated to be about half an acre became, in his own words, "as smooth as a looking glass".

He repeated the experiment many times, often for the edification of friends or fellow-scientists, and it always worked. What is remarkable, however, is that Franklin had (in spite of being relatively untutored) an excellent scientific insight and realized right from the start that the calming of the water was not the most interesting part of the phenomenon he was observing. The intriguing part was the *spreading* of the teaspoonful of oil to such a large area. In his own words (Franklin 1774).

"In these experiments, one circumstance struck me with particular surprise. This was the sudden, wide and forcible spreading of a drop of oil on the face of the water, which I do not know that anybody has hitherto considered. If a drop of oil is put on a polished marble table, or on a looking glass that lies horizontally; the drop remains in place, spreading very little. But when put on water it spreads instantly many feet round, becoming so thin as to produce the prismatic colors, for a considerable space, and beyond them so much thinner as to be invisible, except in its effect of smoothing the waves at a much greater distance."

And he puzzled over it. There are oil particles and water particles. You put some oil particles on a smooth surface like a looking glass placed horizontally, and the oil doesn't spread. And you put water on the same surface, it doesn't spread either But you put the oil particles on top of the water particles, and you get this extraordinary spreading to a huge area. (If you calculate it, the spreading factor here is 10^7!). He puzzled about it, but could not come up with an answer.

And we cannot blame Franklin for this because the time was not ripe for a correct molecular interpretation. As I said earlier, the concept of a "molecule" as the smallest particle with the chemical attributes of a substance was accepted. But it was natural to assume that these defining attributes would be distributed uniformly throughout the mass of the molecule, that every part of each molecule would have the same properties as the bulk substance from which it was derived. The idea that an olive oil molecule could be composed of distinct domains – attracted to water at one end and repelled by it at the other end – could not have been conceived.

But that is precisely what is *essential* for an explanation of the spreading phenomenon. It requires the simultaneous presence of both attractive and repulsive forces, the attractive force to make sure that each oil molecule is at least in part immersed in the water, the repulsive force to make sure that at least a part of each molecule remains outside the water. Only in this way can a monomolecular film be formed.

And, it might be observed, it is difficult to imagine how the enormously spread-out film can be anything other than a monolayer. It is clear that there is cohesiveness between oil molecules, for otherwise there would not be a compact "drop" of oil to start the experiment. And the spreading on water does not continue indefinitely, as both Franklin and subsequent observers reported. There comes a point when the spreading stops, when the film has reached some kind of limit.

What other interpretation can there be than that the limit represent a layer of the ultimate oil particles – when no further expansion could occur without disrupting the last bit of the force of attraction between these particles?

4. Molecular dimensions

There is one puzzle, which has not been satisfactorily answered. Ben Franklin knew that a drop of oil, when spread out over an area of half an acre, becomes astonishingly thin, thin enough for us to observe the prismatic colors, and even thinner than that, where the presence of the oil would have been undetectable but for the smoothing of the waves. But he did not calculate precisely how thin, and nobody really understands why. He was a practical man, accustomed to weights and measures, and certainly knew how to make the calculation: given that he had a teaspoonful (2 cc) and that it spread to an area of half an acre (2000 m^2), he would have arrived at the incredibly low value of 10^{-7} cm for the film thickness – less than one ten millionth's of an inch is what Franklin would have said.

Anyway, he didn't make the calculation. If he had done so, he would surely also have realized that 10^{-7} cm must be about the value of the dimensions of the olive oil molecule – or at least that the molecule could not possibly be larger than that. Had Franklin done so, his paper would have come down in history as one of the great classics – the first measurement of molecular size! Although the *existence* of "ultimate particles" was fairly generally accepted, nobody had the slightest idea what size they would turn out to be.

As it happened, Franklin's method – reduced to laboratory scale – was in fact the method by which molecular dimensions were first measured directly. But it didn't happen till 100 years later, when Lord Rayleigh repeated the experiment, which he did deliberately for the purpose of determining molecular dimensions. "In view of the great interest which attaches to the determination of molecular magnitudes," he said, "the matter seemed well worthy of investigation." And investigate it he did (Rayleigh 1890).

For quantitation, in his own laboratory, Rayleigh at first used a circular bath with a diameter of about 1 meter, and he used the motion of camphor chips as a measuring device. The chips move about spontaneously on an uncontaminated water surface, but motion ceases where the surface is covered by oil. The thickness measurement that Rayleigh reported required successive measurements with increasing amounts of oil until he reached a volume of oil that was "about enough" to "very nearly stop" all movement of the camphor.

About the same time Agnes Pockels made the same measurement, but she did it better (Pockels 1891). She invented the prototype of what is now known as a "Langmuir trough", a shallow *rectangular* container with movable strips that can be used both to sweep surfaces clean of impurities and also to compress or expand the film at will while keeping the amount of adsorbed material constant. Pockels used surface tension as a measure of the state of the surface, while Langmuir later used lateral surface pressure, but the two quantities are related by an equation: the important aspect was that measurements could now be made reversibly and continuously over a wide range of areas for a single application of oil.

Agnes Pockels was one of the most remarkable woman scientists of all time

(Giles and Forrester 1971). She was a young lady without formal education, who did all of her work in her own kitchen, in Braunschweig, Germany, with home-made equipment. The idea for sweeping an aqueous surface to keep it clean apparently came to her from the kitchen technique of skimming fat off the top of soup or stew. She had no idea whether or not her amateur work on surface physics had any value, and didn't receive much encouragement from colleagues of her brother's, who was a professor of physics at the University of Göttingen. Then her brother saw Lord Rayleigh's paper on oil films in the Proceedings of the Royal Society, and showed it to his sister. She then had the courage to write to Rayleigh about her own work, and he immediately recognized its value. He helped her to get her technique and results published in *Nature*, and himself copied her apparatus and used it for his own later measurements.

As table 1 shows, all the data for the thickness of an olive oil monolayer are in good agreement, including that derived from the later work of Irving Langmuir, who of course measured more than just film thickness. Note that Avogadro's Number was not yet known at the time of Rayleigh and Pockels, so that they both determined film thickness from the ratio of total volume to total surface area. Avogadro's number was established by the time of Langmuir, so that he knew how many molecules he had in the amount of oil he placed on the surface, and could report his results directly in molecular dimensions.

5. Molecular shape and orientation

It is rather curious that Rayleigh himself was blind to aspects of his experiment other than the one dimension he reported. In spite of his stated great interest in the "determination of molecular magnitudes", he appears not to have speculated at all about molecular *shape*. The structural formulas for organic molecules are only representations of how atoms are linked to each other, and cannot tell us anything about the disposition of the atoms in space. The molecules of oil on water might be long and thin, imitating in nature the way we write the formulas on paper. But it makes equally good sense to think that the molecules might be coiled up into compact balls to occupy minimal space. (Protein molecules with incredibly long chains – thousands of atoms – in fact do that, as we shall mention below.) Rayleigh did not discuss this problem in his papers. It was Irving Langmuir who addressed himself to the problem and thereby in effect introduced the modern concept of molecular "conformation" (Langmuir 1917).

Table 1. Thickness of a fully extended film of olive oil on water.

	Amount	Area	Film thickness (Å)
B Franklin (1774)	1 teaspoon (2 cc)	½ acre	10
Lord Rayleigh (1890)	0·8 mg (0·0009 cc)	5500 cm^2	16
Agnes Pockels (1892)	1·0 mg (0·0011 cc)	8460 cm^2	13
I Langmuir (1917)	—	—	13

Table 2. Molecular dimensions from surface area measurements.

	Cross-sectional area ($Å^2$)	Square root of same (Å)	Length (Å)
Palmitic acid (C16)	21	4·6	24
Stearic acid (C18)	22	4·7	25
Tristearin	66	8·1	25
Oleic acid (C18)	46	6·8	11·2
Triolein	126	11·2	13

From Langmuir 1917.

Some of Langmuir's results are shown in table 2. They give for the first time real information about molecular asymmetry – information that can be obtained only because the molecules are all identically oriented at the air/water interface. They confirm the conceptual thinking that explains the orientation, that it arises from the opposing needs of the hydrophobic and hydrophilic ends of the molecules. For example, tristearin has the same molecular length as stearic acid, but three times the cross-sectional area, as expected on this basis. The absolute numbers provided for the first time an experimental measure of hydrocarbon chain flexibility: molecular lengths are less than expected for fully extended chains. The difference between oleic acid and stearic acid illustrates the influence of the double bond.

Today we are accustomed to seeing molecular "pictures", based on model building or x-ray diffraction studies of crystals. In 1917, such sophisticated tools were not available. Langmuir's measurements on hydrophobically oriented films thus had a truly revolutionary impact at a time when understanding of molecular structure was virtually non-existent.

6. Modern use of monolayers

The ability to produce so easily layers of molecules that have a unique orientation in space continues to be exploited by chemists to the present day. And, by some strange quirk, monolayer experimentation has involved at least one other prominent person like Benjamin Franklin, whose first claim to fame is in geopolitics rather than in science. In the modern era this is Margaret Thatcher, the Prime Minister of Britain. She started her professional life as a chemist, and wrote a respectable paper (under her maiden name of Margaret Roberts) on a comparison between the kinetics of ester hydrolysis in a monolayer (similarly oriented molecules side-by-side) and in bulk solution. She found no significant difference in activation energy, though the absolute rates, of course, were different (Jellinek and Roberts 1951).

More recent monolayer work includes spectral studies, where the defined orientation of adjacent chromophores helps to define the interaction between them more precisely (Möbius 1981), and experiments to discriminate directly between stereo-isomers in intermolecular forces (Arnett *et al* 1982).

7. Importance for Biology

By the year 1900 it was known that living organisms are composed of "cells". Many microscopic organisms – bacteria, yeasts, amoeba – are unicellular, all their life functions contained within a single cell. The visible plants and animals around us, however, contain millions or billions of cells, functionally differentiated, so that the organism is capable of a much more complex life.

Life originated in the ocean, and water remains to this day the universal medium for living things. Thus all the cells of multicellular organisms are bathed in an aqueous extracellular fluid, usually a rather salty fluid, with a very high content of Na^+, and millimolar levels of Ca^{2+} and Mg^{2+}. The internal volume of cells is likewise aqueous, but does not usually contain the same inorganic salts as the extracellular medium: K^+ is the most abundant cation instead of Na^+, and levels of Ca^{2+} are extremely low, usually below 1 μM. This difference in salt composition is one of the manifestations of "life", but it is of course only a small part of the difference between the inside and outside of cells. All the machinery for life, all the metabolites for energy production, amino acids for protein synthesis and the proteins they build, and the nucleic acids that carry the genetic information – all these substances are inside the cell and must be kept there, must not be allowed to escape.

It is clear – and was clear to most people by the year 1900 – that cells must be defined by *membranes*, by thin films that enclose the cell contents and prevent mixing with the external solutions. But the chemical nature of membranes was not yet known.

In the year 1899 Charles Ernest Overton investigated the question (Overton 1899). He investigated the permeability characteristics of muscle cell membranes by studying substances to which the membranes were in fact quite permeable (alcohols, for example). His most important discovery was a striking correlation between membrane permeation rates and solubilities in – surprisingly, olive oil! This discovery led to several more or less obvious conclusions:

1. There must be some resemblance between a cell membrane and olive oil. The membrane must be impregnated with molecules of the same class as the olive oil molecule – either fats or lipids.
2. The correlation with a "solubility" measurement suggests (perfectly reasonably) that the way molecules get across the membrane is by first dissolving in the membrane interior, then diffusing across the membrane thickness, and, finally entering the aqueous medium on the other side. And that the ability to dissolve in the lipid-like interior is the critical factor, limiting how fast the overall process can occur.
3. This mechanism provides, for the first time, a simple explanation for the vital function of the membrane – enclosure and confinement of the cell contents. The molecules (and the inorganic cations) that need to be confined are mostly water-soluble, strongly hydrophilic. Their solubility in olive oil is slight. By inference their solubility in the membrane interior is also low, and this explains the low rate of escape from the cell.

Overton (1899) was astute enough to realize that "containment" could not by itself suffice to define a viable membrane. The substances to be contained must be brought in from the outside, and the already mentioned differences in salt

concentrations must be continuously maintained. Inward transport often needs to be "uphill", from low concentration to high concentration, contrary to the direction of spontaneous diffusion, which requires expenditure of metabolic energy, specially designed engines to use energy for this unique purpose. Overton realized that that these processes represent a problem quite separate from the problem of containment. "This must be a phenomenon quite different from the simple diffusion of substances through the protoplasts", he said.

And it was soon realized that a real living membrane had to be a mosaic surface, containing proteins for communication across the membrane, as well as lipid for containment. About the same time, lipid chemists discovered that the particular lipids involved in membrane formation were principally *phospholipids* (figure 1), or similar lipid types with two fatty acid chains per glycerol moiety. The structural advantage of having the third position occupied by a strong polar group is to magnify the need that one side of the molecule remains in contact with water. The hydrophilic groups of oils and fats are only weakly hydrophilic, and cells that store oils and fats as food reserves commonly contain globules of lipid in which the many lipid molecules have no contact with water. For phospholipids the thermodynamic cost of incorporation into such globules would be prohibitively high.

8. Gorter and Grendel. Recognition of the lipid bilayer

Evart Gorter, a Dutch pediatrician, accepted the fact that cell membranes were composed of lipids, and, when he read Irving Langmuir's paper after World War I, immediately saw what it implied for cell membranes (figure 2). The membrane's function is to divide one aqueous compartment from another aqueous compartment, a physical situation quite different from that at Langmuir's air/water interface. Here the lipid hydrocarbon tails must avoid the water on *both sides* of the membrane. The only way to accomplish that is to have the ionic lipid head groups (which of course need to be hydrated) *facing the water on both sides*. The membrane must be a *bimolecular* layer. There is no rational way to accommodate "extra" lipid molecules (more than needed for the bilayer) within the bilayer's hydrocarbon interior.

Gorter had to wait several years(until his appointment as Professor of Pediatrics) before he was allowed to have a laboratory in which this conclusion could be tested. Then, in 1925, with the help of a student, F Grendel, he carried out one of the great experiments in the history of biology (Gorter and Grendel 1925). Gorter and Grendel extracted all the lipids from the membranes of red blood cells, and measured their surface area when spread as a monolayer on water. They then compared this with the surface areas of the cells themselves, based on microscope pictures. Some of their results are shown in table 3. The conclusion is clear. There is a difference of exactly a factor of two, independent of whether one is using animals with large or small red blood cells. The cell membrane must be a *bilayer*, exactly two lipid molecules thick.

It is remarkable how much time elapsed before this now seemingly obvious principle was generally accepted. Experimentalists looking at membranes through early versions of the electron microscope had insufficient resolution to be very exact about membrane thickness, and, as late as the mid-1950's, a theoretically

Figure 2. Schematic diagrams of a lipid monolayer and bilayer. Black circles represent hydrophilic ends of the lipid molecules, wavy lines the hydrocarbon chains. The pictures are cross-sections, lacking a third dimension. Both monolayers and bilayers normally occur as extended surfaces. Bilayers tend to fold upon themselves to eliminate the peripheral edge, creating closed vesicles with an internal water-filled space.

The thermodynamic driving force that dictates the orientation of lipid molecules at an interface is the need for the hydrophilic end of each molecule to be dissolved in the water, and the simultaneous need for the hydrocarbon chains to be excluded from water. At the interface between two aqueous solutions, hydrophilic groups must face *both sides* of the layer – a bimolecular film is a necessity. The pressure exerted by the water on the two sides holds the two halves of the bilayer tightly together.

Table 3. Typical results of Gorter and Grendel (1925).

	Amount of blood used for analysis (gm)	No. of cells per mm^3	Surface of one cell (μ^2)	(*A*) Surface of total cells used in expt. (m^2)	(*B*) Monolayer surface of lipids (m^2)	Ratio *B*/*A*
Dog A	40	8,000,000	98	31·3	62	2
	10	6,890,000	90	6·2	12·2	2
Sheep 1	10	9,900,000	29·8	2·95	6·2	2·1
	9	9,900,000	29·8	2·65	5·8	2·2
Rabbit A	10	5,900,000	92·5	5·46	9·9	1·8
	10	5,900,000	92·5	5·46	8·8	1·6
	0·5	5,900,000	92·5	0·27	0·54	2·
Rabbit B	1	6,600,000	74·4	0·49	0·96	2
	10	6,600,000	74·4	4·9	9·8	2
	10	6,600,000	74·4	4·9	9·8	2

questionable model by Danielli and Davson (1935), dominated the thinking of biologists about the arrangement of the constituents of cell membranes. The Danielli–Davson model's thickness was left *unspecified*: layers of three lipid molecules were considered as likely as bilayers. Moreover, the Danielli–Davson model had protein coating the *outside* of the lipid layers, where it could not have catalyzed the transport function required for physiological viability.

9. Proteins

Proteins are the catalysts that create and maintain the chemistry of life. Every biological reaction, some of them exquisitely specific, some of them impossible to duplicate in the laboratory, is made possible by specific group configurations on the

surface of a protein molecule. There are thousands of different proteins, each precisely tailored to its task.

The basic structure of proteins is quite simple. Each protein is a "chain polymer", made from hundreds of amino acid units, as shown in figure 3. Twenty different kinds of amino acids (different "R" groups in the diagram) are used in nature, arranged for each protein in a strictly defined sequence. Variations in the length of the chain, variations in composition (constituent amino acids), and, especially, variations in sequence, clearly permit the generation of virtually an infinite number of distinct molecules. The protein molecules that *actually* exist are genetically determined and synthesized error-free – there is nothing random or statistical in the synthetic process.

The twenty amino acids that enter into protein formation include some that donate ionic and highly polar side chains, and also some that provide hydrophobic side chains. Table 4 lists some of the more important of the latter. These hydrophobic entities of the molecule, though quite small relative to the long hydrocarbon chains of the molecules pictured in figure 1, make an extended structure of the protein chain, everywhere in contact with solvent, thermodynamically unstable. Because hydrophobic side chains are distributed all along the chain they cannot be removed from contact with water by any simple universal structural organization. What must happen instead is that the chain must coil into a compact "globular" structure with a complex pattern of twists and turns, so as to shield as many hydrophobic entities as possible from the solvent, while at the same time keeping hydrophilic side chains on the outside in contact with water. Exactly how this is done – the ultimate three-dimensional folding – is unique to each individual protein. For the majority of arbitrarily chosen sequences (by a computer, for example) there is probably no way to achieve the desired result at all, but, as I have said, there is nothing arbitrary about the protein sequences that exist. Ones that cannot fold to a compact structure have not survived the evolutionary process.

It should be noted that the backbone of the chain structure, the continually repeated peptide group, is obviously hydrophilic. However, it is capable of internal hydrogen-bonding that can satisfy the requirements of polarity without absolute necessity for contact with water. The well-known "α-helix" is the most frequent example. It is probable that the transition between an open chain, with each peptide group bonded to water molecules, and a cooperative water-free internally hydrogen-bonded structure, makes almost no contribution to thermodynamic balance between an overall unfolded structure and a specifically folded three-

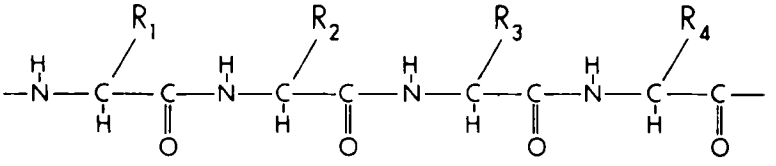

Figure 3. Chemical formulas for protein molecules. Each molecule is a "chain polymer", made from hundreds of amino acid units. Twenty different amino acids (different "R" groups in the diagram) are used in nature, arranged for each protein in a strictly defined sequence. Some of the amino acids provide ionic or highly polar side chains, some are hydrophobic.

Table 4. Typical hydrophobic side chains (R Groups of figure 3)

$-CH_3$	Alanine
$-CH(CH_3)(CH_3)$	Valine
$-CH(CH_3)(CH_2-CH_3)$	Isoleucine
$-CH_2-CH_2-S-CH_3$	Methionine
$-CH_2-C_6H_5$	Phenylalanine

dimensional organization. The internalization of hydrophobic groups is probably the major thermodynamic factor.

It might also be mentioned that the small size of the hydrophobic side chains permits the existence of imperfections, i.e., the thermodynamic cost of leaving an occasional hydrophobic group in contact with water is dependent on the area of contact with water, and in the case of protein side chains is therefore relatively small. If 100% sequestration of hydrophobic groups were required, then it might not be possible to find any structure that would satisfy the thermodynamic requirements within the constraints of allowed bond distances, bond angles and hydrogen-bond orientations along the macromolecular backbone.

The beautiful patterns of folding that result from the need to satisfy the hydrophobic and hydrophilic thermodynamic driving forces have been described in many places: a good recent summary is given by Richardson (1981). What is important here is not the beauty of the ultimate structures, but the catalytic functional specificity that results from it. Because the overall manner of folding is unique, it rigidly locks the hydrophilic groups at the surface into unique arrangements that can serve as sites for recognizing and binding specific ligands, or as catalytic centers for making and breaking organic chemical bonds.

10. Proteins in membranes

The typical enzyme or binding protein envisaged in the preceding paragraph is water-soluble by virtue of the hydrophilic amino acid side chains that cover its surface – the specific binding or catalytic site *per se* involves only a small part of the surface. But proteins must also be part of functioning biological membranes, and some of them must traverse the membrane from one side to the other in order to provide for regulated communication between the internal aqueous medium of a cell (the so-called "cytoplasm") and the extracellular medium, or between the cytoplasm and otherwise sealed-off intracellular compartments, such as mitochondria and the endoplasmic reticulum. The specific proteins involved here must have part of their surface, after folding, in contact with the hydrocarbon chains of the

interior of a membrane bilayer. Indeed, this turns out to be the case. Membrane-incorporated proteins, just beginning to be studied in detail, do not have wholly hydrophilic surfaces, but contain segments that reside preferentially in a non-aqueous medium. Figure 4 provides a schematic illustration.

All proteins, including membrane proteins, are synthesized within the cell, i.e., on the cytoplasmic sides of membranes. How membrane proteins are actually incorporated into phospholipid bilayers, with some hydrophilic part of each molecule on both sides of the membrane, remains an unsolved problem. The important thermodynamic feature is that the proteins we actually find in membranes are thermodynamically stable once located there, by virtue of appropriately designed segments of hydrophobic surface. A protein with a wholly hydrophilic surface would be unstable in the membrane, no matter what the mechanism whereby it arrived there, and would spontaneously seek an entirely aqueous environment. Thermodynamics has priority over other aspects of biochemical mechanism.

11. Micelles and emulsions

There is still another kind of molecular organization that amphiphilic molecules can undergo, namely the formation of micelles or emulsions, aggregates of many small molecules, in principle like phospholipid bilayers with a hydrophobic interior and a hydrophilic surface, differing from bilayers in that the surfaces are curved, generating aggregates of limited size instead of extended sheet-like structure. The basis for this difference rests on molecular geometry: the relation between the size of the hydrophilic end of an amphiphile molecule and the molecular volume of the hydrocarbon portion (Tanford 1978).

Micelles and emulsions are of great importance industrially and socially (soaps and detergents), but they are also used in biological systems, in the handling of facts by living organisms, e.g., in the absorption of fats by the gut in mammalian digestion. Micelle-forming detergents are also used as reagents by biochemists who want to break up biological membranes so as to be able to study individual

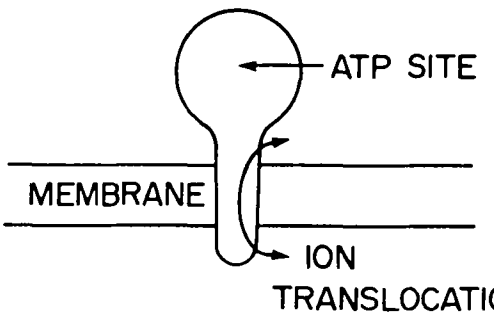

Figure 4. Schematic representation of a protein molecule incorporated into a biological membrane. The segment of the protein in contact with the bilayer interior has a surface made up of hydrophobic amino acid side chains (table 4) and firmly anchored in the membrane by the hydrophobic exclusion from the bulk media on either side. Functionally, the protein is intended to represent an "ion pump", a catalyst that energetically couples ATP hydrolysis to uphill transport of cations (Ca^{2+} or Na^{+}).

membrane proteins or other components, but want to maintain the existence of a hydrocarbon/water interface similar to that of the native membrane (Tanford and Reynolds 1976).

Because of their practical importance, the substances that form micelles and emulsions (loosely termed "surfactants") are much better known to chemists than are lipids and proteins (see, for example, Tanford 1980), and for that reason I have not discussed them in this paper. It should be noted that the most recent international symposium on surfactants in fact took place in India, and the proceedings are in press.

12. Discussion

One might ask the question, why has Nature chosen the hydrophobic effect as the mechanism whereby it achieves the super-molecular organization without which Life could not exist at all? The probable answer lies in the fact that life originated in water and has continued to evolve in an aqueous environment. It is economical to employ the means already at hand and that's what has happened here. A similar phenomenon is apparent with respect to signalling across membranes (by means of proteins that span the membrane). Na^+, K^+ and Ca^{2+} ions are used for most such trans-membrane signalling, the most readily available constituents of the oceans, rather than more esoteric messenger molecules that might be capable of more refined function but would need to be specifically synthesized.

For cell membranes, there is another advantage in organization that is based on the hydrophobic effect. The "seal" that holds the membrane together – hydrocarbon chains of the lipids, hydrophobic side chains of proteins – is a *liquid* seal. It allows the cell to be deformed, squeezed by contact with other cells, etc., without disruption of the containment wall that holds the cell contents together. The normal presence on membrane lipids of a saturated and unsaturated hydrocarbon chain on the same lipid molecule (figure 1) has undoubtedly developed for the same reason – to prevent crystallization of the membrane interior at cold temperatures.

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