perhaps because of steric effects. [Ethanol forms solid complexes with cholesterol; methanol does not (5).]

For all of the cosolvents studied, the Krafft pressure was near the critical pressure of CO_2. Transitions, however, were much less sharp than those observed without cosolvent. A gradual sharpening of the peaks was seen when the pressure fell through the critical region in a solution of spin-labeled cholesterol and 3% (v/v) methanol in CO_2. The sharpening was more abrupt when *tert*-butanol was the cosolvent. When cosolvents are added to supercritical CO_2, the broadened Krafft pressure region may be due to increased polydispersity in aggregation number.

There is a good correlation between the degree of spin-spin broadening in the EPR spectrum of spin-labeled cholesterol in various CO_2-cosolvent mixtures and the observed enhancement in the rate of enzymatic cholesterol oxidation (Fig. 6). The rate of reaction increases when cholesterol is more tightly aggregated and the local concentration of polar hydroxyl groups is higher. EPR spectroscopy indicates that this aggregation is promoted by solvents isobutanol and *tert*-butanol, whereas methanol and acetone do not enhance aggregation. Cholesterol aggregation appears to be the dominant factor affecting the rate of enzymatic oxidation; increased solubility of cholesterol (due to addition of cosolvent to supercritical CO_2) does not necessarily lead to higher reaction rates.

There are several possible explanations for the enhanced enzymatic activity with the addition of aggregate-enhancing cosolvents such as isobutanol or *tert*-butanol. As with many membrane-bound proteins (*18*), cholesterol oxidase from *Nocardia rhodocrous* contains a hydrophobic anchor region that confers amphipathic properties on the enzyme, causing the enzyme to bind to hydrophobic membranes and to detergent micelles (*19*). A detergent micelle or hydrophobic surface is necessary for full enzymatic activity. Although it has not been confirmed that cholesterol oxidase from *G. chrysocreas* has a hydrophobic anchor region, such amphipathic character is likely. Increased hydrophobic surface area due to formation of larger aggregates may allow stronger binding of enzyme to cholesterol aggregates. Cosolvents such as *tert*-butanol and isobutanol may also act as stabilizing "spacers," a well-known phenomenon in liquid micellar systems (*20*). Increased enzymatic activity may then result from more favorable cholesterol spacing, as occurs in aqueous solution when cholesterol is placed in mixed micelles of dioctanoylphosphatidylcholine (*21*). Alternatively, increased activity may result from steering effects caused by the holding of cholesterol molecules in a more rigid orientation than that of monomeric cholesterol free in solution.

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14. Labeled enzyme in 50 m*M* phosphate, *p*H 7.0, was freeze-dried onto the walls of quartz high-pressure EPR cells. Cosolvents were premixed with CO_2 before addition to the enzyme.
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Cavitation and the Interaction Between Macroscopic Hydrophobic Surfaces

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The interaction in water of neutral hydrocarbon and fluorocarbon surfaces, prepared by Langmuir-Blodgett deposition of surfactant monolayers, has been investigated. The attraction between these hydrophobic surfaces can be measured at separations of 70 to 90 nanometers and thus is of considerably greater range than previously found. Spontaneous cavitation occurred as soon as the fluorocarbon surfaces were brought into contact but occurred between the hydrocarbon surfaces only after separation from contact. The very long range forces measured are a consequence of the metastability of water films between macroscopic hydrophobic surfaces. Thus the hydrophobic interaction between macroscopic surfaces may not be related to water structure in the same way that the hydrophobic effect between nonpolar molecules is related to water structure.

VERY STRONG LONG-RANGE ATTRACTIVE forces have been measured between macroscopic hydrophobic surfaces in water (*1–4*). This attraction cannot be accounted for by the classical Derjaguin-Landau-Verwey-Overbeek (DLVO) theory (*5, 6*) or Lifshitz theory (*7, 8*), and there has been no satisfactory explanation.

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Similarly, interactions between small hydrophobic moieties drive amphiphile aggregation (*9*) and help to determine the conformation of proteins in solution (*10*). The nature and origin of these hydrophobic forces present physicists, chemists, and biologists with a key problem. We report new measurements of the interaction between neutral macroscopic hydrocarbon and fluorocarbon surfaces. In comparison with our results, earlier measurements of the hydrophobic attraction do not seem very different from the continuum van der Waals force.

The hydrophobic surfaces were prepared by Langmuir-Blodgett deposition (*4*) of double-chain cationic hydrocarbon and fluorocarbon surfactants on mica. The hydrocarbon surfactant was dimethyldioctadecylammonium bromide (DDOA; deposition pressure of 25 mN/m), and the fluorocarbon surfactant was *N*-(α-trimethylammonioacetyl)-*O*,*O*′-bis-(1H,1H,2H,2H-perfluorodecyl)-L-glutamate chloride (deposition pressure of 20 mN/m). The advancing (θ_a) and receding (θ_r) contact angles of water on these surfaces were $\theta_a = 93°$, $\theta_r = 60°$ (DDOA) and $\theta_a = 113°$, $\theta_r = 60°$ (fluorocarbon surface). These surfaces were stable for days in distilled water, that is, the contact angle was not measurably affected. The surfaces were mounted in a surface force apparatus (*11*) that was subsequently filled with deaerated water; multiple-beam interferometry (*12*) was used to measure the force between the surfaces and to study the occurrence of cavitation.

The force as a function of surface separation between two DDOA-coated surfaces is shown in Fig. 1. There is a measurable attraction at 70 nm, which appears to decay exponentially with a decay length of 13 nm in the range from 30 to 70 nm. For comparison, the force measured between surfaces coated with dihexadecyldimethylammonium acetate (DHDAA) by adsorption from solution (*3*) and the continuum van der Waals force are shown.

There is a strong adhesion between DDOA-coated surfaces (typically 300 to 500 mN/m), much stronger than expected from simple extrapolation of the fit to the forces at separations beyond 30 nm. The interaction is not a simple exponential over the entire range, as was suggested in (*2*) and (*3*). On separation from contact, a vapor cavity is formed as a bridge between the surfaces (Fig. 2), as was found with DHDAA (*3*) and suggested earlier (*13*). This cavity remains stable to a separation of 1 μm but vanishes instantly if the surfaces are brought back into contact.

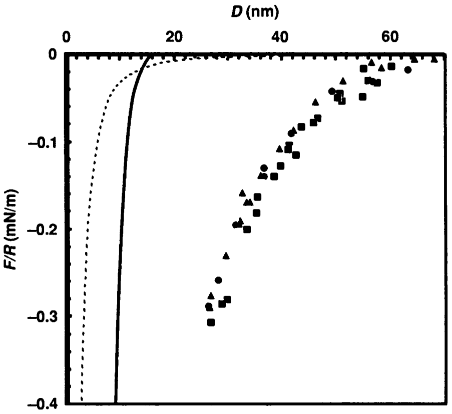


Fig. 1. The force *F* normalized by the radius of curvature *R* of the surfaces as a function of separation *D* for two mica surfaces rendered hydrophobic by deposition of dimethyldioctadecylammonium bromide (DDOA) and immersed in water. The various filled symbols are the results of separate measurements. The attractive force is well described by the equation $F/R = -2.3 \exp(-D/13)$, where F/R and D have the units shown in the figure. The solid line shows the results of (*3*) (the force measured between surfaces coated with dihexadecyldimethylammonium acetate by adsorption from solution). The dotted line is the nonretarded continuum van der Waals force for bare mica surfaces across water (Hamaker constant $A = 2 \times 10^{-20}$ J), which is an upper bound for the theoretically expected interaction in this system.

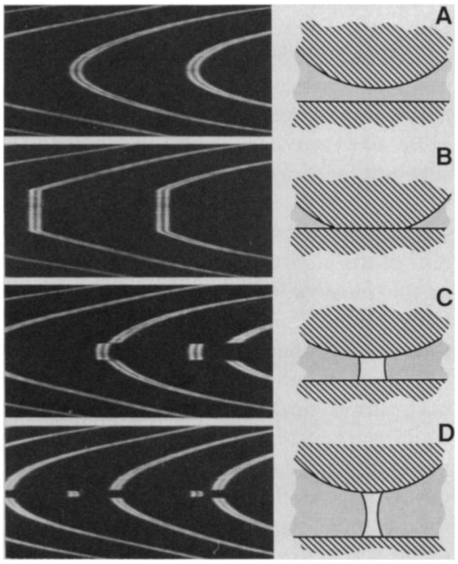


Fig. 2. Vapor cavities in water between curved ($R \sim 1$ cm) mica surfaces coated with DDOA. The mica surfaces are silvered on the back sides, and the standing wave pattern created by multiple reflections of incident white light gives an accurate reflection of the surface shape and allows calculation of the surface separation and refractive index of the intervening medium. The fringe pattern observed in a spectrometer is shown on the left, and the surface configuration, which may be deduced therefrom, is shown schematically on the right. A difference in optical path length due to a decrease in refractive index causes the interference condition to occur for shorter wavelengths (a shift to the left of the fringe). At a vapor-water interface there is thus a discontinuity in the fringes. (**A**) At a separation of 100 nm, the fringe pattern is characteristic of the curved surfaces. (**B**) As the surfaces come into molecular contact, they deform and flatten (contact diameter, 50 μm) as a result of the large adhesion. (**C**) Upon subsequent separation (150 nm), a "bridge" of refractive index 1.00 can be seen to connect the surfaces. (**D**) The diameter of this vapor cavity decreases with surface separation (1 μm).

Between fluorocarbon surfaces, the measured force is similar to that found with DDOA but of slightly longer range (measurable at 90 nm, decay length of 16 nm in the range from 30 to 90 nm). As the surfaces come into contact, however, the behavior is dramatically different. A number of small vapor cavities form spontaneously as soon as the surfaces have come into contact (Fig. 3, A and C); the surfaces often stop at a separation of 1 to 4 nm as the cavities form. Thereafter they continue into contact slowly, presumably because the cavities impede the drainage of water from between the surfaces. After separation, distinct vapor bridges connect the surfaces (Fig. 3, B and D). These bridges gradually coalesce with increasing surface separation but remain stable out to several micrometers.

The interfacial free energy of a hydrophobic surface is lower against vapor than against water (that is, the contact angle is greater than 90°), so that it is energetically favorable to replace water between the surfaces by water vapor, provided the increase in free energy resulting from the vaporization of water and the formation of a vapor-water interface do not together exceed this free energy gain. Unfortunately, a precise analysis of the conditions governing cavity formation is difficult. Spontaneous cavitation at contact seems impossible in that water would have to recede over the surface, and, since $\theta_r < 90°$, no cavitation should occur. Obviously, the macroscopic contact angle is not necessarily the correct parameter to use for a dynamic process such as cavitation (for both surfaces the measured adhesion in water is greater than in air). There is also an activation energy (*14*) that depends on the particular surface and the dynamics of cavity formation.

The pressure inside the cavity (at equilibrium) is not accurately known, but it must lie between the vapor pressure of water (with a minor correction for meniscus curvature) and atmospheric pressure (if the water is saturated with air). The pressure difference ΔP across the cavity-water interface is given in terms of the surface tension γ and the principal radii of curvature r_1 and r_2 by the Laplace equation,

$$\Delta P = \gamma(1/r_1 + 1/r_2) \qquad (1)$$

Since ΔP must be in the range 0 to −1 atm (the pressure inside the cavity is lower), the total radius of curvature is negative and must have a magnitude exceeding 700 nm. Because

Fig. 3. The appearance of (**A** and **B**) the interference fringes and (**C** and **D**) the surfaces for mica surfaces coated with a monolayer of the fluorinated surfactant *N*-(α-trimethylammonioacetyl)-*O*,*O*′-bis-(1H,1H,2H,2H-perfluorodecyl)-L-glutamate chloride and immersed in water. As the surfaces are brought into contact (A and C), many small cavities form, leading to a number of discontinuities in the fringes. After separation (B and D), several distinct cavities remain between the surfaces.

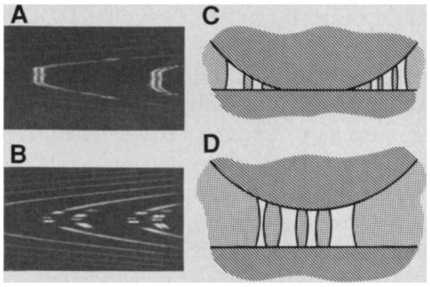
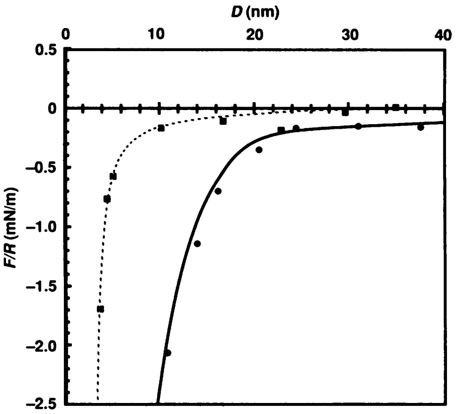


Fig. 4. A comparison of the attractive force between fluorocarbon monolayer surfaces in the absence (solid line) and in the presence (dashed line) of a vapor cavity connecting the two surfaces. Since the energy state at contact is the same in both cases, the greater attraction without the cavity (the hydrophobic force) indicates a higher energy state away from contact. The hydrophobic interaction is thus a nonequilibrium force. The magnitude of the attraction is similar to that measured by us between hydrocarbon surfaces (compare with Fig. 1).



of the flattened contact zone, an annular cavity must have one large positive radius of curvature and a negative radius of some minimum magnitude. This leads to a critical dependence of the annulus size on the contact angle. Only for contact angles approaching 90° can such a cavity become arbitrarily small. We do not know what the "microscopic" contact angle at the cavity-water interface is, but with neither surface does a single, large annulus form in contact. Such an annular cavity has been reported between contacting glass surfaces in mercury (*15*).

The many small and separate cavities formed between the fluorocarbon surfaces avoid the constraint of one large positive radius of curvature, and it becomes easier to satisfy the Laplace pressure condition. With the hydrocarbon surfaces the activation energy is higher and cavities cannot form at contact, only during or after separation. Possibly the separation from molecular contact causes a reduction in local pressure that facilitates cavitation. We cannot rule out that local defects in the deposited monolayers reduce the activation energy and are responsible, at least in part, for the difference between the hydrocarbon and fluorocarbon monolayers. After separation the cavity forms a cylindrical bridge with the two radii satisfying the curvature requirements. No cavity is observed after the surfaces are brought together again, so either it has disappeared completely or become immeasurably small.

The free energy of two hydrophobic surfaces at small separations is lowered by the presence of a cavity. At contact there is a minimum in the free energy, and it is independent of whether a cavity was present away from contact or not. To go from a finite separation to contact leads to a smaller decrease in the free energy in the presence of a cavity than in its absence. This shows up experimentally as a much reduced attractive force (the change in free energy with separation) with a cavity present (Fig. 4).

It remains to consider the reason for the much larger attraction found here as compared to earlier measurements. In the case of monolayers adsorbed from solution (*2, 3*) it is impossible to rule out the presence of a weakly adsorbed second layer [in (*3*) bilayer adsorption did eventually take place]. A weakly adsorbed bilayer would increase the stability of the interlayer of water and reduce the range of the force. Because a second layer is easily squeezed out, the short-range force, adhesion, and cavitation behavior would be similar. Earlier results with DDOA (*4*) seemed to show a shorter decay length (5.5 nm) of the attraction, but this was measured in the presence of a repulsive double-layer force. The difficulty of accurately subtracting this may account entirely for the difference.

The "hydrophobic interaction" between macroscopic surfaces in water is not a true equilibrium force, even though the measurements are reproducible. We believe that therein lies the key to the problem. We suggest that the hydrophobic attraction between macroscopic surfaces originates from a local imbalance of the kinetic and cohesive pressures (*16*) in the metastable liquid layer separating the two hydrophobic surfaces, which may lead to an attraction of considerable range and magnitude. Laplace's original formulation of the capillary pressure relation does consider the internal pressure of the liquid (*17*), and it is perhaps time to resurrect this term.

The alternative explanation of an equilibrium structural force that results in a measurable attraction at a separation of 300 molecular diameters of water appears to be less likely, particularly as the range of the repulsive hydration force between hydrophilic surfaces is 20 molecular diameters or less (*18, 19*).

An important consequence of our suggestion is that any extrapolation of the interaction measured between macroscopic surfaces down to molecular dimensions (*2*) is not justified. The hydrophobic effect between nonpolar solute molecules and the hydrophobic attraction between macroscopic surfaces are not the same thing. Nevertheless, awareness of the existence of such an extraordinarily long-range attraction between macroscopic hydrophobic surfaces must be of great importance for many technological applications such as mineral flotation, wetting of surfaces, and colloidal stability.

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