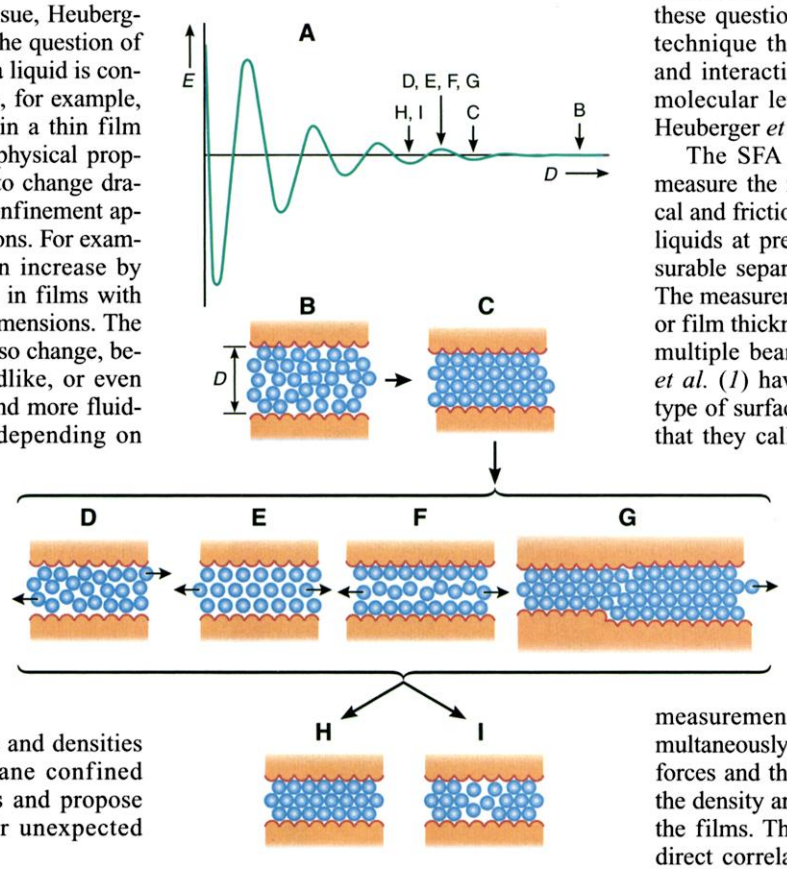
PERSPECTIVES: LIQUIDS

Putting Liquids Under Molecular-Scale Confinement

Jacob Israelachvili and Delphine Gourdon

On page 905 of this issue, Heuberger *et al.* (*1*) address the question of what happens when a liquid is confined within a small volume, for example, in an ultrathin capillary or in a thin film between two surfaces. The physical properties of liquids are known to change dramatically as the degree of confinement approaches molecular dimensions. For example, a liquid's viscosity can increase by several orders of magnitude in films with molecular or "nanoscale" dimensions. The "structure" of a liquid can also change, becoming more ordered, solidlike, or even crystalline or less ordered and more fluidlike than the bulk liquid, depending on how the microscopic shape and atomic structure of the confining walls match that of the liquid molecules (*2*). Many aspects of these changes remain poorly characterized and understood. Heuberger *et al.* (*1*) report unprecedentedly detailed measurements of the forces and densities of thin films of cyclohexane confined between two mica surfaces and propose new explanations for their unexpected observations.



How does the confined liquid respond? The short-range oscillatory "solvation" force (also known as the potential of mean force) between two surfaces in a liquid varies between attraction and repulsion with a periodicity equal to some dimension of the confined liquid molecules (**A**). This force is intimately related to the "structure" that the confined liquid is forced to adopt under confinement (also known as the density distribution function), as illustrated in (**B**) to (**I**).

The properties of confined liquids (and solids) are of great interest and importance in areas as diverse as materials science, microfabrication, adhesion and lubrication, biology, geology, and the budding new fields of nanoscience and nanoengineering. These disciplines all deal with increasingly complex, multicomponent systems that are often structured at the microscopic and nanoscopic scales, with a network of internal surfaces, interfaces, and "interphases" (thin films trapped between two surfaces) that ultimately determine the overall properties of the whole system.

Van Megen and Snook (*3*) were the first to predict large periodic density variations and an oscillatory force between two smooth (unstructured) hard planar walls approaching each other in a simple liquid (see panel A in the figure). These predictions were later confirmed experimentally (*4*, *5*) in surface forces apparatus (SFA) measurements of the oscillatory forces between surfaces across various liquids. But despite much theoretical and experimental work since then, the details of these phenomena are still unclear because most confining surfaces are not planar and are themselves structured at the atomic scale.

Some of the outstanding questions are illustrated in the figure. As two surfaces approach each other, does the confined liquid film undergo a succession of liquid-to-solid-to-liquid phase transitions (B → C → D → H), does the film collapse in an ordered fashion (C → E → H), or do individual layers get forced out through dislocations (C → G → H)? And are there both out-of-plane and in-plane (lateral) heterogeneities, such as two-dimensional domains, in the films (F or I)? To answer these questions experimentally requires a technique that can probe both structure and interactions in real time at the submolecular level (<0.1 nm). This is what Heuberger *et al.* (*1*) have achieved.

The SFA (*6*) is traditionally used to measure the normal and lateral (rheological and friction) forces between surfaces in liquids at precisely controllable and measurable separations at the angstrom level. The measurement of the surface separation or film thickness is achieved optically with multiple beam interferometry. Heuberger *et al.* (*1*) have designed and built a new type of surface force–measuring apparatus that they call an extended surface forces apparatus (eSFA). Using fast spectral correlation spectroscopy to record the interference fringes, they were able to measure surface separation D and film refractive index n at least 10 times more accurately than in conventional SFA measurements. This enabled them to simultaneously measure both the interaction forces and the refractive index (and hence the density and, indirectly, the structure) of the films. This allows for the first time a direct correlation between these two intimately related factors.

Using the eSFA, Heuberger *et al.* measured the oscillatory force profile between two molecularly smooth surfaces of mica across liquid cyclohexane, C_6H_6, a roughly spherical molecule with a mean diameter of 0.53 nm, at separations below 5 nm. They also measured the variation of the film refractive index n with D, from which they could determine how the fluid density varies with D. They conclude that the adhesive minima in the oscillations lie close to the continuum van der Waals force curve—a very interesting and potentially important result because it suggests that van der Waals adhesion cannot be enhanced by the deep energy minima of an oscillatory solvation force. They also measured the fluctuations about the equilibrium state at different film thicknesses and found (not surprisingly) that these are greatest when the mean density is lowest. Less expectedly, they observed a reduction of the film density to half the bulk liquid value even for films as thick as

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three to four molecular layers and report that at certain film thicknesses the density distribution is bimodal.

The authors argue that their results indicate the existence of unexpectedly large density variations, manifested by long-lived solid-liquid-gas phase transitions within the confined film and a highly statistical force distance profile. The study highlights the difficulty of distinguishing between a thermodynamic phase and fluctuations about the equilibrium state in small systems. Indeed, the concept of a thermodynamic phase or state, where the time-averaged properties of all the molecules should be the same everywhere, does not apply to molecularly thin films, whose molecules interact with the confining surfaces in an anisotropic manner, and, consequently, neither does the concept of a phase transition.

Heuberger *et al.* do not compare their results with any quantitative theory or model. It would be interesting to compare the results for the forces and structure with theoretical predictions based on grand canonical Monte Carlo or molecular dynamics simulations of cyclohexane between two molecularly smooth mica surfaces. Experimental results at different lateral locations of the film would provide information about in-plane correlations and fluctuations. Meanwhile, the results support some current thinking on the effects of confinement on the properties of matter (whether liquid or solid) but also raise new and unexpected questions.

References

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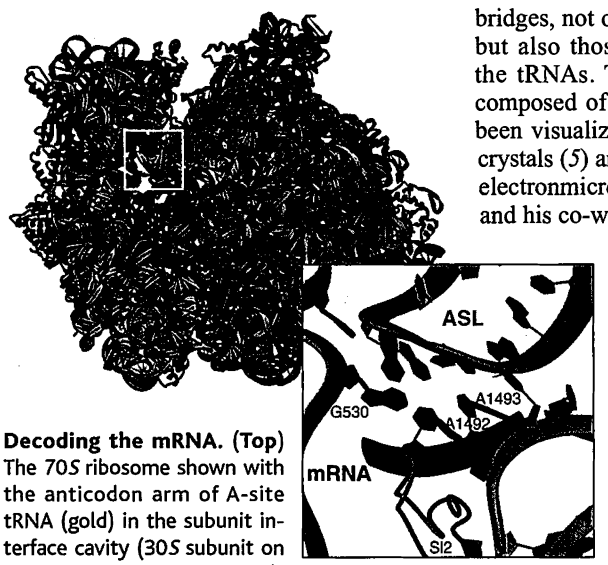
PERSPECTIVES: RIBOSOME STRUCTURE

The Ribosome in Action

Albert E. Dahlberg

Proteins, one of the basic building blocks of cells, are synthesized by linking amino acids together in macromolecular factories called ribosomes. The 20,000 ribosomes in a bacterial cell translate messenger RNA (mRNA) with remarkable speed and accuracy, and two articles in this issue provide fascinating new insights into how this is achieved. These publications come at a time of rapid progress in structural studies of the ribosome. Within the past year both the 30*S* and 50*S* subunit structures have been determined at atomic resolution (*1*–*3*); the large subunit (50*S*) and the small subunit (30*S*) comprise the whole ribosome (70*S*). Unlike previous reports, however, Noller, Ramakrishnan, and their co-workers have now more closely approximated the functional state of the ribosome by including transfer RNA (tRNA) and mRNA in their crystals, thus providing views of the ribosome in action.

The first article (on page 883 of this issue) represents an enormous achievement both in effort and content as Noller and his colleagues report on the complete structure of the *Thermus thermophilus* 70*S* ribosome at 5.5 Å resolution in the presence of mRNA and cognate tRNAs bound in the A (aminoacyl), P (peptidyl), and E (exit) sites (*4*). The 70*S* structure represents more than the sum of its parts. The careful analysis and interpretation of the numerous intersubunit bridges that mediate the functional interactions between the two subunits and the three tRNAs—built upon a wealth of biochemical, genetic, and structural data (much of it from Noller's own lab) accumulated over many decades—is intensely illuminating.



Decoding the mRNA. (Top) The 70*S* ribosome shown with the anticodon arm of A-site tRNA (gold) in the subunit interface cavity (30*S* subunit on left, 50*S* subunit on right). **(Bottom)** The mRNA codon (purple) and cognate tRNA (gold) shown in the A site of the 30*S* subunit with A1492 and A1493 (red) sensing Watson-Crick pairing in the first two base pairs of the codon-anticodon double helix. G530 (red) and S12 (brown) both contact A1492.

All three ribosome binding sites for tRNA (A, P, and E) are in universally conserved regions of the ribosome structure. The significant distance the tRNA must move (about 20 Å) during translocation from the A to the P site underscores the active nature of translation. Additionally, we can see precisely how these highly conserved nucleotides in ribosomal RNA (rRNA) and tRNA fit together in the peptidyl transferase and decoding sites.

Arguably, the observation with the greatest implication for understanding how the amino acid carriers (the tRNAs) are ratcheted through the center of the ribosome is the delineation of the numerous bridges, not only between the two subunits but also those between the subunits and the tRNAs. These bridging interactions, composed of both RNA and protein, have been visualized at lower resolution in 70*S* crystals (*5*) and, originally, by elegant cryo-electronmicroscopy studies (*6*), but Noller and his co-workers now define them at the molecular scale. That these bridges involve the translocating tRNAs marks these regions as dynamic, and thus it is no surprise that three bridges are located in regions of the large subunit that were disordered in the 50*S* crystal structure (*1*). In addition to orienting the subunits and facilitating movement, the bridges also may function in signaling between the subunits, coordinating the multiple steps in the cycle of peptide elongation. No doubt, many groups will now seize on this information to test their models.

An equally exciting report comes from the Ramakrishnan lab (on page 897 of this issue), describing in atomic detail the crystal structure of the *T. thermophilus* 30*S* subunit complexed with fragments of the cognate A-site tRNA and mRNA (*7*). Their structure shows how the ribosome checks for proper codon-anticodon interaction in order to achieve fidelity of translation. Viewing figure 4 of their manuscript (see the figure, lower panel) is like uncovering an ancient drawing, depicting in exquisite detail a universal mechanism

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