when a Ca$^{2+}$-free buffer was subsequently flowed through the device. Control experiments carried out with unmodified SiNWs (Fig. 4B) did not exhibit a conductance change when Ca$^{2+}$ is added and thus demonstrate that the calmodulin receptor is essential for detection. In addition, the observed conductance decrease in modified SiNWs is consistent with expected chemical gating by positive Ca$^{2+}$, and the estimated dissociation constant, 10$^{-5}$ to 10$^{-6}$ M, is consistent with the reported $K_d$ for calmodulin (27).

References and Notes

15. SiNWs with diameters of 10 to 50 nm were suspended in ethanol and flow aligned on oxidized Si substrates (1 to 100 nm-thick, 600-nm thick; Silicon Sense), and contact leads (50 nm Al or Ti) were defined with electron-beam lithography. The separation between contacts was typically 2 to 4 μm. The conductance of SiNW devices as a function of time was determined directly with a computerized apparatus with lock-in-amplifier (Stanford Research, SR 830); a 317-Hz sine wave with 30-mV amplitude at zero Δc bias was used in most measurements. The conductances of the SiNW devices were between 500 and 2000 nS (resistance, 2 mohms to 500 kilohms). This relatively small range testifies to the good control of doping in our NWs.
16. Surface-functionalized SiNW devices were prepared by cleaning in an oxygen plasma (0.35 torr, 25 W power for 20 s) to remove contaminants, immersion in 1% ethanol solution of APTES (Aldrich) for 20 min, rinsing with ethanol for three times, followed by heating at 120°C for 5 min. The different pH solutions were made from 10 mM phosphate buffers with 100 mM NaCl. Solutions were flowed through PDMs microchannels (10, 17) (100- to 200-μm width and height) at a flow rate of 0.5 μl/min. The parasitic conductance through the solution was the constant (about 10 nS) and less than the signal from the SiNW (about 100 nS).
20. Biotin-modified SiNWs were prepared by depositing a drop (~20 μl) of phosphate-buffered solution (PBS) (250 μg/ml; pH 5.6) solution of biotinamide-coupled bovine serum albumin (Sigma) on SiNWs for 2 hours, followed by a five times rinse with buffer solution. The solutions used to probe biotin-streptavidin binding were 1 mM phosphate buffer (pH 9) with 10 mM NaCl. The biotin—saturated streptavidin solution was prepared by adding four equivalents of biotin (Sigma) to one equivalent of streptavidin. All the solutions used in biotin and m-antibiotin (Sigma) binding studies were 1 mM phosphate buffer (pH 7) with 5 mM NaCl.
22. In addition, the detection sensitivity can be changed by the doping concentration and should enable single-molecule detection at sufficiently low concentration. As an example, a single charge on the NW surface will be detected if it generates a sufficiently large local potential barrier (>100 meV at room temperature) for electronic motion. Assuming that a single charge is ~1 nm away from a 20-nm-diameter NW, the carrier concentration will most likely be lower than the order of ~1000 electrons/μm (or a few electrons/nm) for detection, which translates into 3 × 10$^{-5}$ to 10$^{-6}$ cm$^{-2}$.
26. Sequence analysis shows that the binding region of antibiotin (IgG1) is positively charged at pH 7 (24). The remaining domains of this large protein are relatively distant from the SiNW and thus should have little effect on SiNW conductance.
28. We thank L. Lauhon, L. Chen, and Q. Cui for helpful discussion and T. Deng for technical assistance. C.M.L. acknowledges support of this work by the Office of Naval Research and the Defense Advanced Projects Research Agency.
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**Stable Ordering in Langmuir-Blodgett Films**

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Defects in the layering of Langmuir-Blodgett (LB) films can be eliminated by depositing from the appropriate monolayer phase at the air-water interface. LB films deposited from the hexagonal phase of cadmium arachidate (CdA$_2$) at pH 7 spontaneously transform into the bulk soap structure, a centrosymmetric bilayer with an orthorhombic herringbone packing. A large wavelength folding mechanism accelerates the conversion between the two structures, leading to a disruption of the desired layering. At pH > 8.5, though it is more difficult to draw LB films, almost perfect layering is obtained due to the inability to convert from the as-deposited structure to the equilibrium one.

Langmuir-Blodgett films are made by pulling a substrate through a monolayer of amphiphilic molecules at the air-water interface. Under the appropriate conditions, the monolayer is transferred to the substrate (1, 2). Although the LB technique has been used for decades, application of the method has been frustrated by defects ranging from pinholes to larger scale reorganization of the layers (Fig. 1A) (3, 4). We show here that this reorganization is the progression from the as-deposited structure to the thermodynamic equilibrium structure. However, as Fig. 1B shows, the reorganization can be slowed to the point that nearly perfect LB multilayer films can be made by depositing from a different monolayer phase that exists at the air-water interface at pH > 8.5. The high-pH monolayer phase has a more condensed and lower energy lattice structure than the monolayer at pH
force microscopy (AFM) have revealed a remarkably rich polymorphism for fatty acids and other amphiphilic molecules at the air-water interface (5) and in LB films (2, 8). On a subphase containing millimolar cadmium at pH 7, arachidic acid [CH₃(CH₂)₁₈COOH] monolayers have short-ranged hexagonal order. LB monolayers deposited from these conditions are made of the fatty acid salt, two monoalvalent anionic fatty acids complexed with a single divalent cationic cadmium, as expected by charge neutralization (9, 10). Monolayer LB films deposited from pH 7 have a short-range hexagonal packing similar to that found at the air-water interface (Fig. 2A) (11, 12). However, LB films with three or more layers condense into an orthorhombic, herringbone packing with long-range, crystalline order (11, 13) (Fig. 2A). The reduction in area per molecule that accompanies this transition to the orthorhombic packing likely leads to the proliferation of pinholes in the deposited films. Corkery pointed out that the lattice parameters and symmetry measured for fatty acid salt LB multilayers were identical with the equilibrium structure of the corresponding bulk metal soaps (14). Hence, even three-layer LB films have evolved to the equilibrium structure under these conditions.

For cadmium and other fatty acid salts, the equilibrium structure is a centrosymmetric bilayer, with one fatty acid molecule on either side of a central metal ion (14). Before deposition, however, the air-water interface constrains the fatty acid salt to be asymmetric—the cadmium ion in the aqueous phase, with both alkane chains on the air side of the interface. The lack of long-range order and the larger area per molecule in the asymmetric monolayer reinforce the idea that the centrosymmetric packing is energetically favored; the asymmetric structure is likely strained and the alkane chains cannot pack efficiently (6, 7). A LB monolayer on a hydrophilic substrate, however, must retain the asymmetry of the air-water interface, which explains the differences observed between LB monolayers and multilayers (15). The transition between these two configurations—the asymmetric structure enforced by LB deposition and the centrosymmetric structure favored by equilibrium—is the driving force behind LB film reorganization. As soon as three asymmetric monolayers have been deposited on the substrate, a headgroup-to-headgroup interface is formed that facilitates the exchange of ions between fatty acids as illustrated schematically in Fig. 2B. Equally important to the kinetics of the reorganization, the hexagonal packing at the air-water interface can condense into the bulk soap structure by shrinking in the next-nearest-neighbor (NNN) direction with only a negligible expansion in the nearest-neighbor (NN) direction (Fig. 2A). The cylindrical symmetry of the alkane chains in the hexagonal phase is broken on this transformation; there is a regular orientational ordering of the chains in the herringbone packing.

The conversion from the hexagonal structure present at the air-water interface at pH 7 to the equilibrium structure also leads to the large-scale disruption of the layering (Fig. 1A). To show the consequences of the reorganization, we deposited alternating layer LB films of cadmium stearate (CdSt₂, C₁₈ carbon chain) and cadmium lignocerate (CdL₂, C₂₄ carbon chain, deposited at 32°C (16)) at pH 7. The difference in layer thickness between these two fatty acids is easily resolved in AFM images. These films formed multilayer islands and holes with the equilibrium lattice structure (15), as did CdA₂ (Fig. 1A). The roughness of the terraces did not increase during the reorganization, showing that the fatty acids from different layers did not mix at the molecular level. The area of the holes was roughly equal to the area of the multilayer islands, so there was negligible loss of material by solubilization (15).

During every “downstroke” in which the LB films are in the subphase, two asymmetric monolayers are present tail-to-tail (Fig. 3).

R E P O R T S
One way to proceed to the equilibrium structure is via a large-scale bilayer folding process that leads to a headgroup-headgroup interface. Two possible mechanisms are shown in Fig. 3, “overturning” and “creeping.” In the overturning mechanism, the bilayer folds back on itself and the bottom monolayer is now on top; the bilayer orientation is lost (15, 17). In the creeping mechanism, the bilayer detaches and slides over the remaining bilayer; the orientation of the bilayer is preserved. In both mechanisms, a new headgroup-headgroup interface is formed. This allows the transition between asymmetric and centrosymmetric structures, and a condensation of the hexagonal lattice into the lower energy equilibrium structure.

Figure 3 shows the two possible outcomes of these mechanisms on a reorganized film consisting of alternate layers of CdSt2 and CdL2. Table 1 shows the predicted bilayer step heights for the two mechanisms, along with the average value of the step heights measured on LB films with CdSt2 deposited first, followed by CdL2; or CdL2 deposited first, followed by CdSt2. The measured step heights show that reorganization proceeds by the overturning mechanism. Overturning reorients the deposited layers, which is consistent with x-ray diffraction measurements of alternating layer films that show a partial intermixing of the layers (17, 18).

However, Fig. 1B shows that this reorganization does not occur if the LB films were deposited between a phase of pH $\geq 8.5$. Whereas the film deposited at pH 7 (Fig. 1A) had an overall height variation in excess of 12 nm (about two bilayers), the pH 8.8 film was flat to <1 nm (Fig. 1B). X-ray photoelectron spectroscopy (XPS) of the low-pH and high-pH films showed that the stoichiometry was about 40:1 of carbon to cadmium, confirming that the stoichiometry of both films was CdA2 (9).

One reason that reorganization is inhibited in monolayers deposited from the higher pH is that the molecules are better ordered. X-ray diffraction by Leveiller et al. (19, 20) showed CdA2 adopts a pseudo-herringbone orthorhombic lattice (Fig. 2A) with longer ranged order at the higher pH. Lattice energy calculations showed that the pseudo-herringbone packing is not as favorable as the herringbone packing; however, the difference in energy is less than that between the hexagonal packing and the equilibrium structure (5, 6). More important, as shown in Fig. 2B, the unit cell of the pseudo-herringbone packing is significantly smaller in the NN direction than the hexagonal or herringbone packing. In order for the high-pH pseudo-herringbone lattice to convert to the equilibrium structure, the lattice must expand in the NN direction to allow for rotation of the chain axes, which effectively moves the molecules through the hexagonal packing on the way to condensing into the equilibrium structure (Fig. 2A). This expansion likely requires an activation energy; hence, the kinetics of the transition should be significantly slower, as is observed.

The consequences of this inhibited transition are shown in the molecular-resolution lattice images of the LB films shown in Fig. 4. An AFM image (Fig. 4A) of a five-layer film deposited at pH 7 shows that the as-deposited hexagonal structure has completely converted into the equilibrium herringbone structure. AFM and electron diffraction show that the typical grain size in CdA2 films deposited at pH 7 is 10 to 100 μm (8, 12, 21). Figure 4B shows a five-layer film of CdA2 deposited at pH 8.8. These films consist of many 5- to 10-nm-sized crystallites. Fourier transforms from various crystallites reveal lattice spacings corresponding to all three packings shown in Fig. 2A: (i) the equilibrium herringbone structure, (ii) the hexagonal packing, and (iii) the as-deposited pseudo-herringbone packing. The conversion from as-deposited and equilibrium lattice structure is inhibited by deposition from the high-pH subphase. Attenuated total reflection–Fourier transform infrared (ATR-FTIR) spectra of five-layer CdA2 films deposited onto germanium crystals at high and low pH

Table 1. Comparisons of predicted and actual island heights for two alternating layer films allowed to reorganize. In (1), a monolayer of CdSt2 was deposited as described previously onto a silicon wafer at 25°C and a surface pressure of 30 mN/m, followed by a monolayer of CdL2 deposited at 32°C and 30 mN/m. In (2) a monolayer of CdL2 was deposited at 32°C and 30 mN/m, followed by a monolayer of CdSt2 at 25°C and a surface pressure of 30 mN/m. Both (1) and (2) were allowed to reorganize at 25°C under water for 15 minutes. The resulting films were then removed through a clean air-water interface to strip off the top monolayer. This results in a film with monolayer and three-layer regions. All step heights measured were consistent with the overturning mechanism.

<table>
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<tr>
<th>Film</th>
<th>Predicted</th>
<th>Measured</th>
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<tr>
<td></td>
<td>Overturning</td>
<td>Creeping</td>
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<td>(1) CdSt2/Li2</td>
<td>6.8</td>
<td>5.9</td>
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<tr>
<td>(2) CdL2/ST2</td>
<td>5.1</td>
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depositing the LB films. The reorganization is also likely caused by a dramatic increase in the monolayer viscosity at high pH as shown by canal viscometry. [Details of experiments are available; see (22, 23).] For the pH 7 monolayer, the monolayer viscosity was 0.98 ± 0.04 dyne-s/cm, similar to that measured earlier for fatty acid films on cadmium subphases (24). However, at pH 8.8 the surface viscosity was 41.8 ± 0.93 dyne-s/cm, a 40-fold increase, consistent with the increased ordering of the pseudo-herringbone structure (Fig. 2A). This higher viscosity of the monolayer requires a slower dipping speed for deposition. Although the increased viscosity is likely due to the increased ordering of the alkane chains of the monolayer at pH >8.5, sum-frequency generation (SFG) spectroscopy (Fig. 5) shows that the water adjacent to the headgroup region is also better ordered. The SFG signal occurs only if there is a lack of inversion symmetry at the interface; any ordering of the water adjacent to the interface breaks the inversion symmetry and leads to the increased signal seen at the higher pH (25, 26). The more-ordered water layer could also allow the rearrangement of the counterions necessary for the transformation from the as-deposited to the bulk soap structure, as well as contribute to the higher monolayer viscosity.

Figure 6A shows an AFM image of two monolayers of CdA deposited at pH 7, transferred to a pH 8.8 subphase to soak for 1 hour, with a final monolayer deposited at the higher pH. In Fig. 6B, two monolayers were deposited at pH 8.8, allowed to soak for 1 hour at pH 7, with a final monolayer deposited at pH 7. The films initially deposited at pH 7 reorganized, whereas the films deposited at pH 8.8 did not, even when exposed to the lower pH. Reorganization is inhibited by the lattice structure of the deposited film rather than the local conditions during the reorganization. Thus, it should be possible to prepare near-perfect LB films for specialized applications (such as sensors, waveguides, etc.) in nanotechnology using a two-stage deposition process.

References and Notes
9. XPS measurements were done by T. Mates using a Kratos Analytical XPS spectrometer (Chesnut Ridge, NY) on monolayers of cadmium arachidate deposited from subphases at either pH 7 or 8.8. The theoretical ratio of carbon to cadmium in CdA is 40:1; the measured ratios were 34:1 for the pH 7 films and 39:1 for the pH 8.8 films.
16. The hexagonal to pseudo-herringbone transition that occurs by increasing pH in CdA can also be induced by lowering the temperature to 7°C. For the longer chain cadmium behenate [CdL2], this transition occurs at about 20°C. In general, phase transitions of molecules with similar headgroups increase by 5° to 10° for each additional two carbons in the chain. CdL2 is in the low-viscosity, hexagonal phase at this deposition temperature, which is why it reorganizes.
22. Web figure 1 is available at Science Online at www.sciencemag.org/cgi/content/full/293/5533/1292/DC1.
28. Preliminary viscosity measurements were done by J. Ding. Financial support for this project was provided by the National Institutes of Health (GM47334 and HL51177), the Tobacco Related Disease Research Program (BRT-0077), and the Materials Science and Engineering Research Center program of the NSF (DMR-9632716).

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