Active microrheology and simultaneous visualization of sheared phospholipid monolayers

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2011

10.1038/ncomms1321

Peer reviewed
Two-dimensional films of surface-active agents—from phospholipids and proteins to nanoparticles and colloids—stabilize fluid interfaces, which are essential to the science, technology and engineering of everyday life. The 2D nature of interfaces present unique challenges and opportunities: coupling between the 2D films and the bulk fluids complicates the measurement of surface dynamic properties, but allows the interfacial microstructure to be directly visualized during deformation. Here we present a novel technique that combines active microrheology with fluorescence microscopy to visualize fluid interfaces as they deform under applied stress, allowing structure and rheology to be correlated on the micron-scale in monolayer films. We show that even simple, single-component lipid monolayers can exhibit viscoelasticity, history dependence, a yield stress and hours-long time scales for elastic recoil and aging. Simultaneous visualization of the monolayer under stress shows that the rich dynamical response results from the cooperative dynamics and deformation of liquid-crystalline domains and their boundaries.
Fluid interfaces are ubiquitous in industry, technology and life. Interfaces separate and organize biological systems, from organelles to cells to organs, and enable gas exchange in respiration. High-interface foams and emulsions find wide use in industry, food and personal care products: emulsified oil droplets give both taste and texture to a fine espresso. Surfactants, including traditional amphiphilic fatty acids and phospholipids and non-traditional surfactants, such as colloids, block copolymers and nanoparticles, lower the free energy of interfaces and introduce a kinetic barrier to coalescence, thereby stabilizing these high-interface systems by creating novel microstructures and phases.

The static properties of interfaces have long been studied: Langmuir isotherms relate the surface tension, \( \gamma \) (or the surface pressure \( \Pi = \gamma(0) - \gamma(T) \)), to the surface concentration, \( \Gamma \), of surfactant. However, the dynamic response of interfaces—how monolayers and bilayers respond to applied forces—can be equally important, albeit less well known. The dynamic response is more challenging to study, as the bulk material generally swamps the interfacial viscoelastic response in a conventional rheometer. Probes moving within the interface feel drag from both the interface and the bulk fluid. To maximize sensitivity to the interfacial properties, the Boussinesq number,

\[
Bo = \frac{\text{drag from interface}}{\text{drag from subphase}} = \frac{\eta_P P}{\eta_A} \tag{1}
\]

must be large. Here \( \eta \) and \( \eta \) are the viscosities of the interface and the bulk, and \( P \) and \( A \) are the contact perimeter and wetted surface area of the probe. \( Bo \) establishes a practical lower limit, \( \eta > \eta A / P \), that can be measured with a given probe. High-aspect ratio probes such as knife-edges or magnetic needles maximize sensitivity, and even larger \( P / A \) ratios can be achieved with microscale colloidal spheres or ferromagnetic nanorods. Here we describe a new active microrheology technique sensitive to the surface viscoelasticity of a wide range of interfaces. Using photolithography, we make micron-scale, ferromagnetic ‘microbutton’ probes, whose surface chemistry is tuned to render them amphiphilic (Fig. 1). A known torque is applied by electromagnets to rotate a microbutton at the interface, and the ‘buttonholes’ are tracked to record the angular displacement as a function of time. The result is a monolayer, two-dimensional (2D) Couette rheometer with \( P / A \sim 1/R - 1/10(10 \mu\text{m}) \) that can measure surface viscosities of order \( \eta \sim 10^{-5} \text{N} \text{s}^{-1} \) (\( |Bo| \sim 1 \)), and can simultaneously follow the evolution of the interfacial microstructure with fluorescence microscopy. Our work builds on related, larger-scale techniques, adding not only dramatically enhanced sensitivity but also simultaneous visualization. Such direct correlation is almost impossible in traditional, three-dimensional (3D) rheology.

Results

Frequency-dependent linear viscoelasticity of liquid-condensed dipalmitoylphosphatidylcholine. Here we show that the monolayer dynamics of even a single-component monolayer of dipalmitoylphosphatidylcholine (DPPC), one of the primary lipids in lung surfactant and ubiquitous in cell membranes, can be far richer than ever expected. DPPC monolayers exhibit a disordered liquid-expanded (LE) phase at low surface pressures that transforms into a liquid-condensed (LC) phase with long-range orientational and short-range positional order (hexatic) at \( \Pi = 7 \text{mN} \text{m}^{-1} \) at 20°C (Fig. 1). All experiments are performed at \( \Pi = 9 \pm 1 \text{mN} \text{m}^{-1} \) in the LC phase, just above the coexistence plateau. A small-amplitude, oscillatory magnetic field, \( B_0 \), applied perpendicular to the magnetic moment, \( \mathbf{m} \), of a microbutton suspended at the interface (Fig. 1), exerts an oscillatory torque \( T_e \) that drives a small oscillatory rotation \( \theta = \omega t + \phi(t) \). Measuring the buttonhole orientation along with the applied torque determines the probe’s rotational resistance \( \zeta(\omega) = T_e / \omega \theta_0 \). When \( Bo \gg 1 \), the real and imaginary parts of \( \zeta(\omega) \) are proportional to the surface elastic and viscous shear moduli, \( G'(\omega) \) and \( G''(\omega) = \eta \omega \), respectively. Much to our surprise, the DPPC monolayer had a primarily elastic response \( G''(\omega) \sim 150 \text{nN} \text{m}^{-1} \) down to 0.1 Hz, indicating that the monolayer stored elastic energy, without appreciable relaxation, over 10-s time scales (Fig. 2a–c). Above 4 Hz, however, \( G'(\omega) \) and \( G''(\omega) \) crossed, and the monolayer response was primarily viscous \( (\eta \sim 70 \text{nN} \text{s} \text{m}^{-1}) \) (Supplementary Movie 1).

History-dependent rheology of DPPC. Forcing a few large-amplitude \( (180^\circ) \) rotations, however, changed the measured response dramatically (Fig. 2d–f). The DPPC monolayer exhibited a viscous (albeit frequency-thinning) response at all frequencies measured, without the elastic storage seen in a ‘freshly prepared’ monolayer. This dramatic history-dependence highlights the care required in interpreting dynamic measurements of monolayers.
Figure 2 | History-dependent linear viscoelasticity. (a) Linear viscoelasticity of as-prepared liquid-condensed dipalmitoylphosphatidylcholine (LC-DPPC) monolayers at air/water interfaces (II = 9 ± 1 mN m⁻¹). Surface shear moduli indicate a dominant low-frequency elastic response, \( G'(\omega) > G''(\omega) \) down to 0.1 Hz, crossing over to a primarily viscous response, \( G''(\omega) > G'(\omega) \) above 5 Hz. (b) Direct visualization of the DPPC monolayer reveals the elastic response to result from a network of interlocked LC domains that cannot deform without deforming neighboring domains. (c) After forcing a large-amplitude microbutton rotation, LC-DPPC appears viscous \( G'(\omega) > G''(\omega) \) at all frequencies. (d) Visualization reveals the creation of a ‘slip line’ (white arrow) within the monolayer, which enables non-cooperative deformation under shear (scale bar, 20 μm).

Our ability to visualize the monolayer while deforming it allowed us to unambiguously identify the microstructure changes responsible for this unusual behaviour. The bright lines (high fluorescent dye concentration) in Figure 2c and Supplementary Movie 1 show the boundaries of the irregularly shaped, interlocking ~10 μm LC domains, which cannot slide past each other without deforming. DPPC monolayers in the LC phase respond to a weak applied stress with small elastic deformations of the domains, rather than rearrangement of the domains or the domain boundaries. Large-amplitude deformations, however, drive the monolayer out of its equilibrium microstructure (Fig. 2f and Supplementary Movie 2). The domains deform enough that the boundary forms a continuous, almost circular slip line that effectively fractures the material. The microbutton and domains within the slip line rotate freely, with minimal deformation of the domains inside or outside of the slip line, eliminating the elastic response (Fig. 2d,e).

Ageing and healing of a single slip line. On returning the microbutton to its original orientation and removing the driving torque, the circular slip line roughens (Fig. 3), the smoothed grains heal to their interpenetrating configuration (compare Fig. 3a, e), and the original viscoelastic response (Fig. 3f) is recovered. When the slip line is formed (Fig. 3b), both \( G'_s \) and \( G''_s \) drop 20- to 100-fold (Fig. 3f), and the monolayer appears primarily viscous (\( G''_s > G'_s \)). However, after shear stops, both \( G'_s \) and \( G''_s \) increase with time: the elastic response is recovered (\( G'_s > G''_s \)) after 40 s, when the domain structures again interpenetrate (Fig. 3c). Even the detailed features of the grain boundaries and domains are largely restored after 150 s (Fig. 3e).

Surface yield stress measurements from steady rotation. Significant domain deformation should require a stress of order \( G'_s \) as well: concentrated emulsions yield at applied stresses of ~5 G/10 (ref. 5, 28, 29). Monolayers respond to a steady torque (Fig. 4 and Supplementary Movie 3) by establishing two distinct regions: an outer region in which the interface does not flow, and an inner region that appears largely structure-free and freely flowing. The boundary radius, \( R_y \), between the two is determined by the surface yield stress, \( \tau_y \); the interface flows for \( r < R_y \) where \( \tau_y > \tau_i \), but deforms elastically for \( r > R_y \) where \( \tau_y < \tau_i \). An interfacial torque balance relates applied torque to surface shear as \( T_{app} = 2\pi R_y^2 \tau_y \). Figure 4d shows that \( T_{app} \) increases linearly with \( R_y^2 \), whose slope gives a surface yield stress \( \tau_y \approx 0.01 \mu \text{N m}^{-1} \) of order \( G'_s/10 \), as expected (28, 29).

Hours-long viscoelastic recovery after steady shear. The division into flowing (yelled) and elastic regions is reminiscent of many other yield-stress materials whose yield stress results from an interlocking or system-spanning elastic network. Forcing such materials to flow destroys this structure, which gradually heals when the imposed shear is removed. The LC-DPPC monolayer, however, did not age in a straightforward manner once the driving torque was removed. Instead, to our great surprise, steadily sheared monolayers immediately begin to recoil and ‘unwind’ for minutes to even hours (Fig. 5 and Supplementary Movie 4).

Discussion
The low-frequency, linear elastic response, followed by flow at larger strains also arises in concentrated 3D emulsions, which cannot rearrange unless the applied stress is sufficiently strong to significantly deform the tightly packed droplets. Emulsions deform elastically until a critical yield stress is applied, above which they flow: mayonnaise sits like a solid under gravity, but may be easily spread with a knife. In 3D emulsions, elastic energy is stored in droplet...
Given a line density ~1, subsequent relaxation are generally quite complicated. If the topology of the domain structure were rigorously preserved (for example, domains could not slip, rearrange or break), the recovery would be complete and elastic, as indeed occurs for small applied rotations (Fig. 6). The relatively inviscid grain boundaries, however, allow stretched grains to slide along the slip lines that form within the yielding monolayer, swapping neighbours rather than deforming further. Nonetheless, the disc drives shear deformations that continually stretch and arrange domains in a ‘wound’ manner, even as they respond by slipping, rearranging and perhaps even breaking. Once shear is removed, line tension clearly drives the monolayer to recoil, whether the domains retain their topology or not. Topological changes and viscous losses thus generally give rise to a viscoelastic (partial) recovery: Figure 6 reveals the recovered (unwound) fraction to decay with applied strain by an approximate power-law – 0.6.

Our measurements and observations lead us to argue that LC-DPPC monolayers—and likely grainy LC monolayers in general—behave like high-interphase emulsions, but with novel 2D features. Line tension drives domains towards lower-energy shapes against an inhomogeneous viscous resistance: viscous grains are separated by relatively inviscid boundaries. Under small strains, the interpenetrating LC grains deform only slightly, but cooperatively, to give a viscoelastic response (Fig. 2). Large-amplitude oscillatory strains smooth the interpenetrations to form slip lines, along which localized, non-cooperative deformations occur with little resistance. If domains have not rearranged, however, ‘smoothed’ slip lines roughen to recover their initial (equilibrium) structure (Fig. 3). Monolayers fully yield under steady shear, forming extended (yet continuous) domains that continually slip past one another along interfaces, giving $G'\gammaR_0$ for droplets of size $R_0$ and surface tension $\gamma$. Extending this analogy to 2D emulsions suggests the measured surface elastic modulus $G'\sim\lambda/\gammaR_0$, is set by the line tension of the domain boundaries, $\lambda$, and the size, $R_0$, of the LC domains. The resulting line tension, $\lambda\sim G'\gammaR_0$, is in good agreement with measured values for phospholipid and fatty acid monolayers, and is consistent with a simple molecular argument: The attractive energy between molecules in a condensed liquid phase must be of order $k_B T$, so the energy cost of moving from an ordered to disordered liquid should be $~k_B T$ per molecule. Given a line density $~1$ molecule per nm, one expects a line energy $\lambda\sim k_B T$ nm$^{-1}$–pN.

Figure 5 highlights the recovery of a monolayer that had been sheared at 60 Hz for 5 min, then allowed to relax. Domains slip relative to one another (green) or neighbouring pairs can swap (adjacent light-blue domains to be neighbours) through topological transitions reminiscent of T1 events in foams and concentrated emulsions. Unlike 3D emulsions, however, highly stretched domains (red) evolve from imperceptibly thin crescents to increasingly compact structures. Such extended shapes may at first seem surprising: stretched droplets in 3D emulsions break into smaller droplets because of the Plateau–Rayleigh (capillary) instability. The absence of out-of-plane curvature in 2D, however, makes the extended domains inherently stable. 2D domains can thus stretch without breaking, maintaining their integrity while slipping past each other, until they may eventually become so thin that non-hydrodynamic forces cause their rupture. Once stress is removed, the line tension of the still-continuous boundaries acts to pull the domains back into more energetically favourable compact configurations.

The dynamics of steadily sheared LC monolayers and their subsequent relaxation are generally quite complicated. If the topology of the yielded region had appeared to be largely domain-free (Fig. 4), discrete but highly deformed LC domains have maintained their identity and liquid-crystalline orientational order. (a–d) After the stress has been relieved, line tension pulls domains (red) from imperceptibly thin, extended structures (a) to more energetically favourable compact structures (d). Domains exchange neighbours by relative sliding (green), and topological ‘T1’ transitions, wherein domains that are initially North–South neighbours (light-blue) make way for East–West neighbours (dark-blue) as the coarsening of foams and emulsions (scale bar, 20 µm).

Figure 4 | Yield stress measurement. Steady rotation at 5 Hz (a), 10 Hz (b), and 30 Hz (c) divides the monolayer into two regions: an inner region that flows with the microbutton, deforms continuously and appears domain-free; and an outer region with domains that does not deform significantly. The radius $R$ of the yielded region (white arrows) is set by the surface yield stress, $\tau_y$. A torque balance gives $\tau_y=2\pi R\lambda/\gamma$, consistent with (d); the slope gives $\tau_y\sim10^{-4}$ µN/m$^{-1}$, $G'(\omega)/10$ (Fig. 2) as expected by analogy with three-dimensional emulsions ($^5$28,29) (scale bar, 20 µm).
a power-law behaviour, decaying with initial strain to the
controlled pairs of electromagnets, and measured the frequency- and amplitude
probe to directly measure the magnetic
/f_i
e wafer is then soaked in 1H,1H,2H,2H-per
/f_t
raphy to fabricate micron-scale, ferromagnetic, amphiphilic ‘microbutton’ probes,
Fabrication of amphiphilic ferromagnetic microbuttons
Methods
fatty acids can be added to systematically construct model mon-
fluid interfaces, of scienti-
generally, our technique can interrogate the dynamical response of a
rearrange and slide relative to one another along local slip lines.
By visualizing the structure of an interface while it is being
deformed, our new technique provides an unprecedented ability to
correlate structural deformations with rheological response. More
generally, our technique can interrogate the dynamical response of a
wide variety of fluid/fluid interfaces, of scientific, biological, industrial
and technological relevance. For example, lipids, proteins and
fatty acids can be added to systematically construct model monoc-
layer of biological relevance, such as the surfactant monol-
ayer.4,17

Multiple strain lines. Line tension pulls extended strains back into
more compact shapes against the high LC viscosity, while strains
rearrange and slide relative to one another along local slip lines.

Figure 6 | Fraction of strain recovered as microbutton ‘unwinds’ after
torque is removed. Recovered strain fraction (θ — θ_i)/θ is measured as
a function of initial, applied strain θ_i. The recovered strain fraction shows
a power-law behaviour, decaying with initial strain to the ~0.6 power.
Topological rearrangements (for example, domain slipping and neighbour
swapping) and general viscoelastic deformations irreversibly lose elastic
energy that would otherwise be stored while ‘winding’.

Relating rotational drag to rheological properties. Having measured the rotational
drag coefficient of the microbutton probe, ζ(ω), we use hydrodynamic theory to
extract the viscoelastic moduli that give rise to this drag. The resistance ζ(ω) is
generally complex and frequency dependent, and depends on the visco(elastic)
modulus of the surface and bulk phases. Just as the Stokes flow around a transla-
ing sphere must be solved to determine the steady drag of a small sphere (F_D = 6πμDU),
the Stokes flow around a rotating disc in a surfactant monolayer must be solved18,24
to relate ζ(ω) to surface complex viscosity η_s*(ω) and subphase viscosity η_u.
The surface viscosity and elasticity are in turn related to the complex surface
modulus G_1(ω) = G_0(ω) + iκ_0ω according to η_s*(ω) = G_0(ω)/iω.
When η_u > 1 (which holds in the experiments reported here), the rotational resistance becomes completely dominated by the interfacial rheology, and the
subphase effectively decouples from the interface. A good approximation for
the drag coefficient can therefore be obtained by solving the 2D Stokes
equations, ∇P + η_s*∇V = 0 and ∇·V = 0. Symmetry requires the flow be
purely in the θ-direction with no pressure gradient, giving simply V/μ = 0,
with boundary conditions u_θ = QD at r = a and u_r = 0 as r → ∞. The resulting
velocity field is given by u_r = Qa^2/r, so that the torque on the disc is given by
T = 2πa^2Q(2a/(ur_i)) = 4πQa^2, revealing the rotational resistance to be
ζ = 4πQ/μ.

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Electromagnet and microbutton magnetic moment calibration. We used a Hall
probe to directly measure the magnetic field established by two independently
controlled pairs of electromagnets, and measured the frequency- and amplitude
dependence of both the current in the coils and the magnetic field. To determine
the magnetic moment of the microbuttons, we measured their dynamic response
in liquids of known viscosity. Placing the microbutton at a clean, surfactant-
free air/water interface (Bo = 1), we measured the microbutton orientation
/θ_N,θ_P^meas^ = mθ_Nθ_P^meas^ under an applied, oscillatory magnetic field. As ζ = 4πμ/μ
in the low-Bo (surfactant-free) limit, we extracted the magnetic moment from
measured rotational resistance via m = oθ_Nθ_P^meas^/θ. In this manner, we measured
the magnetic moments of 29 individual microbuttons, each with 150-nm Ni and
10-nm Au layers, from two different synthesis batches, and found m = (4.3±0.7)×
10^{-5} e.m.u.

General experimental procedure. DPPC domain structures are visualized by add-
ing 0.1 mol% of Texas red 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine
(DHPE, Invitrogen) to DPPC (Avanti Polar Lipids) in chloroform, which is
excluded from the LC domains15 so that disordered, LE phases appear bright in
the images. A Teflon Langmuir trough is cleaned with acetone, isopropanol
and deionized water at least three times. The Langmuir trough is filled with deionized
water (Milli-Q). DPPC/DHPE solution is then spread at the air/water interface
with micro-syringe. Chloroform evaporates to leave a surfactant monolayer.
A few drops of microbuttons in deionized water are added to the trough, centred
between the electromagnets. The microbuttons do not ‘see’ the interface, and sink.
The monolayer is compressed to the LC/LE coexistence phase (~6 mN/m), and
a rare earth magnet is then used to pull the microbuttons from the bottom of
the trough to the air/water interface, where their amphiphilic nature ‘pins’ them.
The monolayer is then further compressed until the desired pressure, with the rigidity
of the LC/LE interface preventing microbuttons from drifting out of the field of
view during compression.

Two pairs of independently controlled electromagnets are used to exert a speci-
ﬁed torque on the microbutton probe, and its resulting rotation is measured in real
time by accurately locating the centroids of the buttonholes.16 By exerting a known
torque ζ(ω) and measuring the resulting angular displacement δθ as ω → ∞, we extract
the rotational drag coefﬁcient ζ(ω) from the relation iδθ_q = ζ(ω)/i(ω_q).
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Acknowledgments

T.M.S. and S.Q.C. acknowledge primary support from the NSF MRSEC program (DMR-0520415), and additional support from NSF CBET-0730270, ACS PRF 47000-G7 and the Beckman Young Investigator Program. J.A.Z. and S.S. acknowledge the NIH under grants HL-51177 and GM076709. A.J. Pascal’s suggestion to evaporatively deposit the magnetic film on microbuttons is gratefully acknowledged.

Author contributions

T.M.S. and S.Q.C. conceived and S.Q.C. constructed the experimental apparatus; S.S. assisted with early and complementary measurements; all authors contributed to the material design of the experiments, S.Q.C. performed all experiments and analysed the data. S.Q.C., T.M.S. and J.A.Z. wrote the manuscript.

Additional information

Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

Competing financial interests: The authors declare no competing financial interests.

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How to cite this article: Choi, S.Q. et al. Active micro rheology and simultaneous visualization of sheared phospholipid monolayers. *Nat. Commun.* 2:312 doi: 10.1038/ncomms1321 (2011).

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