Boundary mode lubrication of articular cartilage with a biomimetic diblock copolymer

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Abstract

We report the design of a diblock copolymer with architecture and function inspired by the lubricating glycoprotein lubricin. This diblock copolymer, synthesized by sequential reversible addition–fragmentation chain-transfer polymerization, consists of a cationic cartilage-binding domain and a brush-lubricating domain. It reduces the coefficient of friction of articular cartilage under boundary mode conditions (0.088 ± 0.039) to a level equivalent to that provided by lubricin (0.093 ± 0.011). Additionally, both the EC50 (0.404 mg/mL) and cartilage-binding time constant (7.19 min) of the polymer are comparable to purified human and recombinant lubricin. This diblock copolymer is an example of a synthetic polymer with lubrication properties equal to lubricin under boundary mode conditions, suggesting its potential utility as a therapy for joint pathologies like osteoarthritis.

Significance

Lubricin is a glycoprotein that protects cartilage in weight-bearing joints under boundary mode conditions, which defines the conditions under which physical cartilage damage takes place. The natural expression of lubricin decreases with age, and when the joint is damaged. Replacement of lubricin by intraarticular injection reestablishes boundary mode lubrication and can protect against osteoarthritis. However, the manufacture of lubricin is difficult. We set out to create a synthetic mimetic of lubricin by mimicking its structure and functional composition. The result is a diblock copolymer that reduces the coefficient of friction on cartilage, under boundary mode conditions, to a level equivalent to native lubricin. This paper reports coefficients of friction in a boundary mode system that are equivalent to lubricin.


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We report the design of a diblock copolymer with architecture and function inspired by the lubricating glycoprotein lubricin. This diblock copolymer, synthesized by sequential reversible addition–fragmentation chain-transfer polymerization, consists of a cationic cartilage-binding domain and a brush-lubricating domain. It reduces the coefficient of friction of articular cartilage under boundary mode conditions (0.088 ± 0.039) to a level equivalent to that provided by lubricin (0.093 ± 0.011). Additionally, both the EC50 (0.404 mg/mL) and cartilage-binding time constant (7.19 min) of the polymer are comparable to purified human and recombinant lubricin. Like lubricin, the tribological properties of this polymer are dependent on molecular architecture. When the same monomer composition was evaluated either as an AB diblock copolymer or as a random copolymer, the diblock effectively lubricated cartilage under boundary mode conditions whereas the random copolymer did not. Additionally, the individual polymer blocks did not lubricate independently, and lubrication could be competitively inhibited with an excess of binding domain. This diblock copolymer is an example of a synthetic polymer with lubrication properties equal to lubricin under boundary mode conditions, suggesting its potential utility as a therapy for joint pathologies like osteoarthritis.

As the primary bearing surface, articular interfaces exhibit remarkable tribological function over decades of use (1). The impressive lubricity and durability of articular cartilage has inspired the design of synthetic macromolecules that mimic the architecture of lubricating biomolecules (2–9). However, very few synthetic polymers achieve lubrication comparable to endogenous articular surfaces, strongly supporting the need for new synthetic materials that rival the efficacy of natural materials. Here, we report the design and synthesis of a diblock copolymer with substantial lubrication capacity whose architecture is inspired by the structure of lubricin, a natural glycoprotein that lubricates joints under boundary mode conditions (i.e., high normal load and slow speed).

Lubricin is a glycosylated protein found in synovial fluid (10) which plays a pivotal role in joint boundary mode lubrication (11, 12) and the prevention of osteoarthritis (11, 13, 14). Lubricin reduces the coefficient of friction (COF) of articular cartilage under boundary mode conditions by as much as 70% (12). The potent lubrication arises from its structure: a central mucin-like domain to attract and retain water and a cartilage-binding domain at the C terminus to affix the molecule to the cartilage surface (15). This architecture of lubricin is crucial to boundary mode lubrication of articular cartilage, as denaturation in either domain of lubricin causes partial or complete loss of lubrication capability (15, 16). We defined the design criteria for a synthetic boundary mode lubricant inspired by lubricin’s architecture: a diblock copolymer consisting of a large lubrication block (Mn∼200 kDa) to mimic the mucin-like domain of lubricin and a small cartilage-binding block (Mn∼3 kDa) to mimic the C-terminus domain (Fig. 1). The lubrication domain of the diblock copolymer is made of a polyacrylic acid (pAA) backbone with polyethylene glycol (PEG) brushes, which enables the polymer to retain water and to resist compression. The binding domain is made of a pAA backbone decorated with quaternary ammonia groups to nonspecifically interact with negatively charged cartilage surface components such as aggrecan. Applying this polymer to lubricin-deficient bovine articular cartilage in PBS resulted in a significant reduction in COF under boundary mode conditions.

Results and Discussion

The diblock copolymer was synthesized in three steps, as shown in Fig. 2. Starting with RAFT (reversible addition–fragmentation chain-transfer) polymerization of 2-(dimethylamino)ethyl acrylate, a precursor “prebinding” domain was produced (17). Subsequent RAFT polymerization of poly(ethylene glycol) methyl ether acrylate (Mn 480), using the “prebinding” domain as the macrorinitiator, added the lubrication domain to the copolymer. Finally, the tertiary amines in the “prebinding” cartilage-binding domain precursor were converted to quaternary by treatment with an excess of ethyl bromide to give the final product [Mn∼200 kDa, polydispersity index (PDI) = 1.6].

To evaluate the polymer as a synthetic boundary mode lubricant, we assessed its tribological characteristics using a custom-built tribometer (18). Cartilage samples were obtained from the patellofemoral groove of neonatal bovine stifles and incubated in PBS.
cartilage tribology, the measured COFs were 0.493
oscillation speed of 30
under boundary conditions (6-MPa compression load and linear
Mica surfaces that were preincubated with the poly-
types (20). Mica surfaces that were preincubated with the poly-
lubricity characteristics were consistent between different surface
and lubrication was completed using negatively charged mica
portance of the diblock copolymer architecture. An accompa-
The failure of this polymer to lubricate articular cartilage under
monomer composition as the diblock copolymer was synthesized.

The importance of the binding block to lubrication and its
behavior (Fig. 4), wherein higher concentrations of the binding
were measured after exposure to solutions composed of combi-
COFs of samples incubated with different molar ratios of
were not able to efficiently bind to either cartilage or mica sur-
interfaces, which again demonstrates the importance of the diblock
architecture for lubrication.

To better understand the effectiveness of the diblock co-
other lubrication characteristics were measured and compared with those of natural
Specifically, a dosing study was performed using carti-
lage samples that were treated with polymer solutions ranging
from 0.01 to 10 mg/mL. The COFs (Fig. 6A) exhibited a dose-
response behavior ($R^2 = 0.89$), which at the higher concentra-
tions ($EC_{50} = 0.404$ mg/mL) were effectively reduced to a level
comparable to that of naturally lubricated cartilage. Also, the
binding kinetics of the diblock copolymer to the cartilage surface
was measured in which cartilage samples were incubated at a
saturation concentration (1 mg/mL) of polymer over time. When
fit to a one-phase decay model ($R^2 = 0.95$), the binding kinetics
curve (Fig. 6B) revealed a binding time constant ($\tau$) of 7.19 min,
which is comparable to that of natural lubricin (~9 min; ref. 12).

Synthetic polymers with structures that mimic natural bio-
lubricants have been extensively studied over the past few de-
cades. Inspired by natural bottle-brush polyelectrolytes, Spencer
and coworkers explored a range of mucin analogs featured with a
polylysine backbone and grafted PEG (2, 3, 22) or dextran (23)
side chains to reduce the COFs on mica surfaces. Israelachvili
and coworkers (5) also reported a bioinspired bottle brush
polymer that exhibited extremely low friction and Amontons-like
behavior characterized by SFA. Both works report effective

1.5 M NaCl to remove surface-associated synovial fluid contents
Samples were incubated in PBS and then in polymer (3 mg/mL
in PBS) for 120 min to saturate the cartilage surface. The cartilage
samples were loaded onto the tribometer in a PBS bath and
evaluated under boundary mode conditions (30% compressive
strain and linear oscillation speeds of 0.3 mm/s). Incubating the
stripped cartilage with the diblock copolymer solution resulted in
a decrease in COF from 0.391 ± 0.020 to 0.088 ± 0.039 ($n = 4–11,$
$P < 0.0001$), which is equivalent to lubricin-treated groups
(COF = 0.093 ± 0.01112, shown as the dashed line in Fig. 3). To
establish the importance of the diblock architecture on lubrica-
tion, the individual cartilage-binding and cartilage-lubricating
domains were also evaluated under the same conditions. Nei-
eral individual domain decreased COF, supporting the premise
that both the binding and lubricating blocks of the copolymer are
necessary to lubricate cartilage under boundary mode conditions.

The importance of the binding block to lubrication and its
interaction with the cartilage surface was further demonstrated by
a competitive binding analysis. The COFs of cartilage samples
were measured after exposure to solutions composed of combi-
nations of the binding block and the diblock copolymer in molar
ratios ranging from 100:1 to 1:1 (binding block:diblock copolymer).
The COFs of samples incubated with different molar ratios of
[binding block: diblock copolymer] exhibited a dose–response behavior (Fig. 4), wherein higher concentrations of the binding
domain inhibited lubrication by the diblock copolymer, sug-
gest that intimate interaction of the polymers with the carti-
gle surface is crucial for effective cartilage lubrication.

To further establish the importance of the diblock copolymer
architecture on lubrication, a random copolymer with the same
monomer composition as the diblock copolymer was synthesized.
The failure of this polymer to lubricate articular cartilage under
the same tribological conditions (Fig. 5A) emphasized the impor-
tance of the diblock copolymer architecture. An accompa-
nying study on the effect of molecular architecture on binding
and lubrication was completed using negatively charged mica
surfaces in a surface force apparatus (SFA) to show that the
lubricity characteristics were consistent between different surface
types (20). Mica surfaces that were preincubated with the poly-
mers in solution at 3 mg/mL for 120 min were sheared in PBS
under boundary conditions (6-MPa compression load and linear
oscillation speed of 30 μm/s). Similar to the results obtained for
cartilage tribology, the measured COFs were 0.493 ± 0.082 for

Fig. 1. (A) Schematic representation of lubricin, the native boundary mode
lubricant in synovial fluid showing the PEX-like cartilage-binding domain
and the mucin-like lubricating domain. (B) The synthetic diblock copolymer
mimetic of lubricin showing the molecular compositions that mimic the
functional domains of lubricin.

Fig. 2. Synthesis of a lubricin–mimetic diblock copolymer. (i) ACPA, CPADB,
anisole, 70 °C. (ii) ACPA, DMAEA, anisole, 65 °C. (iii) EtBr, acetone, room
temperature.
The lubricin–mimetic diblock copolymer significantly decreases COF of articular cartilage compared with samples treated with PBS, binding block only, or lubrication block only (*P < 0.0001). Dashed line represents COF of samples tested in recombinant human lubricin solution at 50 μg/mL (12). One-way ANOVA was used to determine the statistical difference among the tribological results associated with the polymers in solution and the controls on articular cartilage.

Fig. 3. In this work, where both key lubrication characteristics are engineered into the lubricant by adopting a diblock architecture that facilitates binding and localization of the polymer to the cartilage surface, we show the importance of polymer architecture to lubrication. The diblock copolymer with its lubricin-mimetic structure successfully lubricates articular cartilage surfaces in the boundary mode as effectively and efficiently as natural lubricin. This work demonstrates the importance of the polymer architecture to its tribological properties and the necessity for the coexistence of binding and lubrication blocks for effective lubrication under these conditions. This approach toward the molecular design of boundary mode lubricants could serve as a guide for future synthetic lubricants with even greater efficacy.

Materials and Methods

All chemicals were purchased from Aldrich or Fisher at the highest purity grade. All monomers were purified by a column filled with aluminum oxide (base or neutral) before use. The 1H NMR spectra were performed on an Inova 400-MHz spectrometer with deuterated chloroform or deuterium oxide as the solvent. Broad or overlapping peaks, noted in spectra of polymers, are denoted “br.” Degree of polymerization was determined by initial monomer to CTA ratio and monomer conversion. Gel permeation chromatography (GPC) was performed with phosphate buffer saline (pH 7.4) at a flow rate of 0.8 mL/min. The eluent flowed through a Waters gel permeation chromatography system equipped with three Waters Ultrahydrogel columns in series (2,000 Å, 50 Å, and, 250 Å) at 30 °C. The molecular weights were measured relative to poly(methacrylic acid), sodium salt standards (1,670–110,000 g/mol). DMAEA = 2-(dimethylamino)ethyl acrylate, ACPA = 4,4‘-azobis(4-cyanopentanoic acid), CPADB = 4-cyan-4-(phenylcarbonylthio)pentanoic acid, and PEGMEA = poly(ethylene glycol) methyl ether acrylate, Mn = 480. R = -(CH2)3(CHN(CH3)2CH2CHOH and R′ = -(SC(SPh)2).

Synthesis of PDMAEA42. DMAEA (4.30 g, 30 mmol) was added to a solution containing ACPA (14.0 mg, 0.05 mmol) and CPADB (135.5 mg, 0.5 mmol) in 5 mL of anisole. The mixture was deoxygenated by five freeze–vacuum–thaw cycles before it was heated to 70 °C with stirring for 48 h. The reaction was then quenched by liquid nitrogen freezing, and the polymer gel was precipitated by the addition of 20 mL of hexane and vigorously stirring for 30 min. After decanting the solvent, the residue was redissolved in 5 mL of dichloromethane and precipitated after addition of 20 mL of hexane and vigorously stirring for 30 min (process was repeated four times) to yield a gel (1.68 g, 39% yield); 1H NMR (400 MHz, CDCl3): δ 4.13 [br, 2H, -Cδ(CH3)-CH2-], 2.55 [br, 2H, -O-CH2-CH2-N(CH3)2-], 2.31 [br, 7H, -N(CH3)2-], 1.92–1.36 [m, 2H, -CH2CH(C=O)-], 5.30 (s, residue dichloromethane).

Synthesis of PDMAEA42–PEGMEA480 (Diblock Copolymer). PEGMEA (3.46 g, 7.2 mmol) was added to a solution containing PDMAEA42 (30.9 mg, 0.009 mmol) and ACPC (0.5 mg, 0.0018 mmol) in 6 mL of anisole. The mixture was deoxygenated by five freeze–vacuum–thaw cycles before it was heated to 65 °C with stirring for 8 h. The reaction was then quenched by liquid nitrogen freezing, and the residue polymer gel was precipitated by addition of 20 mL of hexane and vigorously stirring for 30 min. After decanting the solvent, the residue was redissolved in 5 mL of dichloromethane and precipitated by addition of 20 mL of hexane and vigorously stirring for 30 min (process repeated four times) to yield a gel (1.68 g, 48% yield); 1H NMR (400 MHz, CDCl3): δ 4.13 [br, 2H, -Cδ(CH3)-CH2-], 2.37–3.44 (m, 34H, -O-CH2-CH2-O), 3.35 (s, 3H, -O-CH3), 2.24 [br, -1H, -CH2CH(C=O)-, -N(CH3)]1.93–1.26 [m, 2H, -CH2CH(C=O)-], 5.30 (s, residue dichloromethane). The resulting polymer was characterized by GPC using the method described above: Mn = 44,900; PDI = 1.58.

Synthesis of qPDMAEA42–PEGMEA480 (Diblock Copolymer). Ethyl bromide (0.3 mL, 4.0 mmol) was added dropwise into a solution containing PDMAEA42–PEGMEA480 (863.9 mg) in 3 mL of acetone at 0 °C. The mixture was stirred for
Fig. 5. (A) Random copolymer with the same composition but different architecture failed to lubricate cartilage and mica in comparison with the diblock copolymer architecture (n = 4–6, *P < 0.0001). (B) Mica samples treated with random copolymer had smaller film thickness under both uncompressed and compressed conditions in comparison with diblock copolymer using SFA measurement (n = 3–5, **P < 0.001, ***P < 0.01), indicating a less efficient binding of random copolymer to the mica surface. Two-way ANOVA was used to determine the statistical difference among the tribological results of the diblock copolymer and random copolymer under uncompressed and compressed conditions individually. Data are presented as mean ± SD.

48 h at room temperature and was then concentrated by evaporating the solvent with a dry nitrogen flow. The residue was dissolved in 3 mL of dichloromethane and precipitated with addition of 15 mL of hexane and vigorously stirring for 30 min (process repeated five times). The product was then dissolved in deionized (DI) water and further purified by dialysis using Spectra/Por regenerated cellulose dialysis tubing (2 kDa MWCO) against DI water for an additional 48 h before lyophilization. The product (851 mg) was collected as viscous gel in 98% yield; $^1$H NMR (400 MHz, D$_2$O): δ 4.08 [br, 2H, -O-CH$_2$-CH$_2$-], 3.75–3.46 (m, 34H, -O-CH$_2$-CH$_2$-O), 3.21 (s, 3H, -O-CH$_3$), 3.02 [s, -N-(CH$_2$)$_6$CH$_2$CH$_3$], 2.21 [br, 1H, -CH$_2$CH(C = O)], 2.01–1.36 [m, 2H, -CH$_2$CH(C = O)-], 1.25 [br, ~0.1 H, -N-(CH$_2$)$_6$CH$_2$CH$_3$]. The resulting polymer was characterized with GPC using the method described above: Mn = 46,100; PDI = 1.58.

Synthesis of qPDMAEA$_{24}$-PEGMEA$_{400}$ (Random Copolymer). Ethyl bromide (0.5 mL, 6.7 mmol) was added dropwise into a solution containing PDMAEA$_{24}$ (1.47 g) in 5 mL of acetone at 0 °C. The freeze–vacuum–thaw cycles before it was heated to 65 °C with stirring for 8 h. The reaction was then quenched by liquid nitrogen freezing, and the polymer gel was precipitated by addition of 20 mL of hexane and vigorously stirring for 30 min. After decanting the solvent, the residue was redissolved in 5 mL of dichloromethane and precipitated by the addition of 20 mL of hexane and vigorously stirred for 30 min (process repeated four times) to yield a gel (1.70 g, 40% yield). The structure of the purified product was characterized by $^1$H NMR (400 MHz, CDCl$_3$): δ 4.12 [br, 2H, -(C = O)O-CH$_2$CH$_3$], 3.81–3.39 [m, 34H, -O-CH$_2$-CH$_2$-O], 3.34 (s, 3H, -O-CH$_3$), 2.10 [br, 1H, -CH$_2$CH(C = O)], 2.02–1.36 [m, 2H, -CH$_2$CH(C = O)], the resulting polymer was characterized by GPC using the method as described above: Mn = 33,800; PDI = 1.52.

Synthesis of PDMAEA$_{24}$-PEGMEA$_{400}$ (Random Copolymer). PEGMEA (3.46 g, 7.2 mmol) was added to a solution containing DMAEA (61.8 mg, 0.43 mmol), ACPA (0.5 mg, 0.0018 mmol), and CPADB (1.3 mg, 0.0045 mmol) in 2 mL of anisole. The mixture was deoxygenated by five freeze–vacuum–thaw cycles before it was heated up to 70 °C with stirring for 48 h. The reaction was then quenched by liquid nitrogen freezing, and the polymer gel was precipitated by addition of 20 mL of hexane and vigorously stirred for 30 min. After decanting the solvent, the residue was redissolved in 5 mL of dichloromethane and precipitated by the addition of 20 mL of hexane and vigorously stirred for 30 min (process repeated four times) to yield a gel (865 mg, 25% yield); $^1$H NMR (400 MHz, CDCl$_3$): δ 4.12 [br, 2H, -(C = O)-O-CH$_2$CH$_3$], 3.75–3.44 (m, 34H, -O-CH$_2$-CH$_2$-O), 3.35 (s, 3H, -O-CH$_3$), 2.26 [br, ~1H, -CH$_2$CH(C = O)-, -N(CH$_2$)$_6$], 1.93–1.36 [m, 2H, -CH$_2$CH(C = O)-], 5.30 (s, residue dichloromethane). The resulting polymer was characterized with GPC using the method described above: Mn = 32,700; PDI = 1.94.

Synthesis of PEGMEA$_{400}$ (Lubrication Block). PEGMEA (3.46 g, 7.2 mmol) was added dropwise into a solution containing CPADB (2.51 mg, 0.0036 mmol) and ACPA (0.5 mg, 0.0018 mmol) in 6 mL of anisole. The mixture was deoxygenated by five freeze–vacuum–thaw cycles before it was heated to 65 °C with stirring for 8 h. The reaction was then quenched by liquid nitrogen freezing, and the polymer gel was precipitated by addition of 20 mL of hexane and vigorously stirring for 30 min. After decanting the solvent, the residue was redissolved in 5 mL of dichloromethane and precipitated by the addition of 20 mL of hexane and vigorously stirring for 30 min (process repeated four times) to yield a gel (1.70 g, 40% yield). The structure of the purified product was characterized by $^1$H NMR (400 MHz, CDCl$_3$): δ 4.12 [br, 2H, -(C = O)O-CH$_2$CH$_3$], 3.81–3.39 [m, 34H, -O-CH$_2$-CH$_2$-O], 3.34 (s, 3H, -O-CH$_3$), 2.10 [br, 1H, -CH$_2$CH(C = O)], 2.02–1.36 [m, 2H, -CH$_2$CH(C = O)], the resulting polymer was characterized by GPC using the method as described above: Mn = 33,800; PDI = 1.52.
mixture was stirred for 48 h at room temperature and was then concentrated by evaporating most of the solvent with a dry nitrogen flow. The residue was dissolved in 3 mL of dichloromethane and precipitated with addition of 15 mL of hexane and vigorously stirred for 30 min (process repeated five times). The purified product was then dissolved in DI water and further purified by dialysis using SpectraPor regenerated cellulose dialysis tubing (2 Kd MWCO) against DI water for an additional 48 h before lyophilization to yield a gel (1.36 g, 93%). 1H NMR (400 MHz, D2O) δ 4.08 [br, –2H, -(C=O)-CH2-], 3.75–3.44 (m, 34H, -(O-CH2-CH2-O)2), 3.21 (s, 3H, -(O-CH2-), 3.02 [br, –0.2H, -N(CH2)3CH2CH3], 2.21 [br, 1H, -(CH2=CHC=O)], 2.02–1.44 [m, 2H, -CH2CH(C(O)=O)-], 1.25 [br, –0.1H, -(CH2)3,CH2CH3]. The resulting polymer was characterized with GPC using the method described above: Mn ≈ 33,400; PDI = 1.71.

General Procedure for Tribological Testing. Friction coefficients were measured on our custom-built tribometer (18). Cartilage samples were obtained from the patellofemoral groove of neonatal (1 to 3 d old) bovine stifles. Shaped into a cartilage disk (6 mm in diameter by 2 mm high) by biopsy punch, samples were incubated in 1.5 M NaCl solution for 30 min, in PBS for an additional 60 min, and then in polymer solution (in PBS) for 0 to 120 min. The cartilage samples were loaded onto the tribometer against a polished glass flat counterface in a PBS bath with a tilt pad configuration (18). Before a friction test, samples were compressed to 30% strain and depressurized to an average normal load of 3.4 N within 60 min. After the fluid pressure reached equilibrium, the glass counterface was reciprocated at a linear oscillation speed of 0.3 mm/s. Both the normal load and friction force were measured by a biaxial load cell. Coefficients were calculated as an average ratio of friction force to normal load during the sliding and averaged for both the forward and reverse sliding directions. One-way ANOVA and Student’s t test were used to determine the statistical difference.

SFA Experiment, Materials. Mica was purchased from S&J Trading Inc. as optical grade. PBS was purchased from Sigma-Aldrich.

SFA Experiment, Sample Preparation. Thin, homogeneous pieces of freshly cleaved, silvered mica were glued silver-side-down onto semicircularly fused silica discs (R = 1 cm and R = 2 cm) with UV curing glue (Norland 61). The surfaces were mounted opposing each other in a cross-cylindrical configuration in the SFA. After mounting, 50 μL of 3 mg/mL polymer solution in PBS was injected between the surfaces and incubated for 1 h. The surfaces were then rinsed in PBS, and a droplet of PBS was injected, leaving only a surface-bound layer of polymer for friction and film thickness measurements.

SFA Experiment, Friction Force Measurement. Once samples were prepared in the SFA, both normal and friction force measurements were performed. For normal force measurements, the lower surface was mounted on a double-cantilever spring (k = 1,650 N/m and k = 185 N/m) for normal force detection. For friction force measurements, the lower spring was mounted on a double-cantilever spring (k = 1,650 N/m) attached to a piezoelectric bimorph slider. The bimorph slider was sheared across the upper surface, which was mounted on a semiconductor strain gauge at 30 μm/s to detect friction. Friction measurements were measured under incrementally increasing loads and friction coefficients were calculated using the slope of the friction vs. load data, \( \Delta F_{\text{friction}}/\Delta F_{\text{load}} \).

SFA Experiment, Film Thickness Measurement. Once samples were prepared in the SFA, the surfaces were brought together using a stepper motor at speeds <10 nm/s. The interference pattern, known as fringes of equal chromatic order, was recorded and analyzed to determine both the uncompressed film thickness (the film thickness where surface interaction is initially detected) and the compressed film thickness.

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