Negatively Charged Lipids Exhibit Negligible Effects on the Water Repellency of Montmorillonite Films

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ABSTRACT: Amphiphilic molecules can alter the wettability of soil minerals. To determine how the headgroup chemistry of amphiphiles determines these effects, we investigate a system of the clay montmorillonite with long-chain phospholipids. We use phosphatidylglycerol (PG) phospholipids to contrast with our previous work using phosphatidylethanolamine (PE) lipids. Zwitterionic PE lipids can sorb to the negatively charged montmorillonite surface, whereas negatively charged PG lipids cannot. Employing a suite of techniques from molecular dynamics, atomic force microscopy, fluorescence microscopy, and contact angle measurements, we define sample characteristics from molecular-scale structure to the macroscopic wettability. We find that PG lipids do not significantly alter montmorillonite wetting characteristics, such as the contact angle, flow viscosity, and the characteristic time scale for droplet imbibition. On comparing PE and PG lipid/clay films, we find that, among the phospholipids compared, they must have three characteristics to change clay/lipid film wettability: they must bind to the mineral surface, be solid at room temperature, and have a relatively continuous distribution throughout the film.

1. INTRODUCTION

Soil water repellency (SWR) is a phenomenon in which soils do not readily absorb and retain water.1 While SWR negatively affects a number of environmental phenomena, such as causing nonuniform water infiltration2 and increasing surface runoff and erosion,3 the underlying mechanisms that cause it are not well understood.

Organic matter in soil is generally considered a main source of SWR.4 Numerous prior studies suggested that amphiphilic molecules have a particularly pronounced impact on SWR.5 Most of the previous work characterizing SWR has been done with either natural organic matter6 or bulk soils.7 While such studies show that different soils have different degrees of water repellency and that organic matter and soil water content are important factors controlling water repellency, only a few studies determine the underlying mechanisms by performing both imaging and wettability measurements on a particulate surface.8 Therefore, to determine the mechanism by which lipids cause SWR, we investigated model systems consisting of one lipid and one particulate mineral.

Previously, we analyzed a system of montmorillonite and zwitterionic phosphatidylethanolamine (PE) lipids.9 One saturated PE lipid, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE), and one unsaturated PE lipid, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), were studied to determine if the state of the lipid—liquid or solid—altered wettability. We found that the liquid, unsaturated lipid and the solid, saturated lipid had very different distributions in the film at the nanoscopic and microscopic levels. The solid, saturated DSPE was present as isolated islands in the film, while the liquid, unsaturated DOPE was distributed throughout the sample film. The most significant alteration of wettability (as measured by contact angle and imbibition kinetics) occurred when DSPE was melted and then mixed with the montmorillonite particles before cooling, creating a situation where a solid lipid was distributed throughout the film. Previous literature reports have hypothesized that water flow in clay is non-Newtonian,10 that is, the liquid viscosity changes under stress. Our results agree with these prior reports, as we found that the spreading characteristics of water droplets on the films implied that the water exhibited non-Newtonian behavior in the form of shear thinning (reduction of viscosity) during imbibition into the film.10

Received: February 4, 2020
Accepted: May 4, 2020
Published: May 15, 2020
Relative to clay alone, melted DSPE caused water to have a higher, more viscous fluid index and, therefore, more Newtonian behavior than was present in the clay alone. This suggested that a solid lipid distributed through the film could change the wettability more than a solid, nondistributed lipid or a liquid, distributed lipid. In all cases, the PE lipids were able to sorb to the montmorillonite surface.

For this work, we selected lipids that do not readily bind with mineral surfaces and thus should have a less pronounced effect on wettability. To this end, we investigated the mechanisms by which water repellency is altered by negatively charged phosphatidylglycerol (PG) lipids on montmorillonite. Due to the PG headgroup’s negative charge, PG lipids are not expected to sorb to the montmorillonite planar surface. One saturated, solid lipid (DSPG) and one unsaturated, liquid lipid (DOPG) were investigated for direct comparison with DSPE and DOPE. All four lipids (DOPE, DSPE, DOPG, and DSPG) have two 18-carbon tails. Our results indicate that both the headgroup and tailgroup chemistry influence lipid-induced changes in SWR.

2. EXPERIMENTAL SECTION

Techniques were selected to encompass scales from molecular to macroscopic. At the smallest scale, molecular dynamics (MD) simulations provide information on the type of aggregates formed by the lipids and the binding strength of these aggregates to the mineral surface. Atomic force microscopy (AFM) and fluorescence microscopy were used to characterize the film surface and distribution of lipid through the films. Wettability was measured with the water contact angle, flow viscosity, and kinetics of infiltration. A complete description of the methods and materials used is provided in Kessenchik et al., and the associated supplement.9

2.1. Materials and Sample Preparation. The lipids 1,2-dioleoyl-sn-glycero-3-phospho-1’-rac-glycerol (DOPEG) and 1,2-distearoyl-sn-glycero-3-phospho-1’-rac-glycerol (DSPG) were acquired from Avanti Polar Lipids. Montmorillonite was purchased from the Clay Minerals Society and further ion-exchanged to fully saturate the sorption sites with sodium cations. Lipid/montmorillonite exchanged to fully saturate the sorption sites with sodium cations. Lipid/montmorillonite films were prepared as follows. Lipids were suspended in water via the gentle hydration method for making small unilamellar vesicles; in the case of DSPG, the lipids were heated over the melting point for several minutes to facilitate suspension.

The lipids were then mixed with montmorillonite suspensions, at ratios where the surface area of the lipid added was known, assuming a monolayer lipid coverage on the mineral surface

\[
\text{% coverage} = 100 \times \frac{\text{total lipid surface area}}{\text{total montmorillonite surface area}}
\]

Equation 1 does not represent the true coverage, but only a theoretical maximum coverage as a lipid monolayer (reported as the percent coverage as monolayer, %CAM). An area per molecule11,12 of 60 Å² and a montmorillonite surface area13 of 22.7 m²/g were used.

The aqueous mixtures of lipid and clay were then pipetted onto freshly cleaved mica surfaces and allowed to air-dry overnight. For wettability measurements, fluorescence microscopy, and large-scale AFM, 16 mg/mL montmorillonite suspensions were used; 1 mg/mL Montmorillonite was used for small-scale AFM.

2.2. Atomic Force Microscopy (AFM). To identify lipid aggregates on the surface of the films, an Asylum Cypher ES was utilized to image at scales under 5 μm. To image the film topography, a Bruker ICON was used to image at scales of 50 μm. In all cases, imaging was performed with Asylum AC240TS cantilevers, which have a spring constant of 2 N/m and a frequency of 70 kHz.

2.3. Molecular Dynamics (MD) Simulation. The system was modeled the same way as in our previous paper using a CLAYFF force field for the montmorillonite, CHARMM36 force field for the lipids, and the TIP3P force field for the water.9 The shapes of the DSPG lipid aggregates were also inferred in a similar way; we computed the density of tail carbon atoms and head phosphorous atoms, as DSPG does not have a nitrogen atom in the headgroup. We calculated the binding energy of a single DSPG lipid onto the surface of the clay using replica exchange umbrella sampling where we bias the distance between the phosphorous atom in the lipid head and the surface of the clay. We simulated 35 windows, each 0.1 nm apart with a constraint of 500 kJ/(mol nm²). All other simulation protocols remain the same as in the previous paper.

2.4. Fluorescence Microscopy. To map the distribution of the lipid within the film across hundreds of microns, 1 mol % of rhodamine–DMPE dye was mixed into the lipids prior to the film preparation. The resulting distribution of fluorescence intensity was imaged with a Nikon Y-FL epifluorescence microscope. Brightness in the fluorescence images is proportional to the amount of lipid present in a given location.

2.5. Wettability. Initial contact angle and droplet shape over time were monitored with a Kruss DSA100. Five or more droplets were observed for each sample. In addition to the initial contact angle, the droplets were video-recorded for 70 s as they spread out and imbibed into the sample films. The radius was extracted using a custom MATLAB script and fit with eq 2 using a Python’s Scipy optimize.curve_fit function.

We determined the wettability of our systems with a method adapted from Chao et al.14,15 Briefly, a water droplet is placed on the sample surface, and the change in the droplet radius is monitored over time. The radial expansion of a droplet spreading across a porous surface where the fluid can also infiltrate into the sample is described as follows

\[
\frac{R}{R_0} = [1 + K t]^n
\]

(2)

where \( R \) is the droplet radius, \( R_0 \) is the droplet radius at \( t = 0 \), \( K \) is an inverse time constant (\( TC = 1/K \)), and \( n \) is a proxy for the fluid index. \( K \) depends on a number of material properties of the film and fluid. For our system, \( n \) is related to the fluid index \( n \) by

\[
a = \frac{n}{1 + n}
\]

(3)

The fluid index \( n \) indicates the deviation of the fluid behavior away from that of a Newtonian fluid. In a Newtonian fluid, the fluid viscosity is independent of strain, the fluid index \( n = 1 \), and \( a = 0.5 \). Shear-thinning fluids, which have reduced viscosity under strain, have \( n < 1 \) and \( a < 0.5 \), while shear-thickening fluids have \( n > 1 \) and \( a > 0.5 \).

The wettability variables of the initial contact angle, TC, and \( a \) will be used below to characterize the wettability of the films.

2.6. Image Processing and Quantification. Fluorescence and large-area (50 × 50 μm²) AFM images of sample topography were analyzed using the same procedure as in our previous publication. Briefly, each image was turned into a
binary image at three different thresholds: 25, 50, and 75% (Figure 1). The threshold percentage (e.g., 25%) renders everything below the percentile of the gray value as white and everything above it as black. Using multiple thresholds enables the capture of morphological detail that would be lost if only one threshold was used.

After binarization, each image was skeletonized with ImageJ, which reduces the dark portions of the binarized images to a line 1 pixel wide down the center of the prior black region. The skeletonized images were then analyzed with the ImageJ macro Analyze Skeleton to obtain the number of pixels where the lines meet, which gives an indication of the interconnectedness of the AFM topography. Junctions are reported as the number of junction pixels per square micron.

The ratio of dark-to-light pixels in the skeletonized images was also recorded, as this gives an indication of ridge spacing in the case of AFM topography images and of lipid density in the case of fluorescence images. The ratio will henceforth be abbreviated as the skeleton ratio. For AFM topography images such as Figure 1A, the skeleton ratio is inversely proportional to the separation of the ridges in the topography. For fluorescence images such as in Figure 4, the skeleton ratio reflects the degree to which the lipid spreads through the film (a film with a very patchy lipid distribution will have a small skeleton ratio, and a film with a more continuous lipid distribution will have a larger skeleton ratio).

3. RESULTS AND DISCUSSION

3.1. Lipid Morphology. AFM imaging showed two dominant lipid morphologies on the film surface: small, roughly circular lipid aggregates and bilayer sheets (Figures 2 and S5). Relative to PE lipids, PG lipid aggregates strongly preferred edge sites on the montmorillonite flakes (Table 1 and Figures 2 and S2). DSPG formed spherical aggregates under a 30% coverage and formed patchy bilayer sheets above 30% coverage (Figure 2B,C). This behavior is similar to that of the saturated lipid DSPE, which switched from forming small aggregates to forming bilayers at a 40% coverage. We conclude that this change in morphology is linked to the state of the lipid at the temperature...
at which the film is prepared, as both DSPE and DSPG are solid at room temperature, while DOPG and DOPE, which continue forming spherical aggregates at higher coverage, are liquid at room temperature. (DSPE can be forced to form spherical aggregates at higher coverage by preparing the films above its melting temperature; this case will be referred to as “melted DSPE” or “heated DSPE”.)

Aggregate heights were measured for DSPG at a 25% coverage. Aggregates ranged in height from 0.3 to 6.4 nm, with the average height at 2.8 ± 1.7 nm. As the average aggregate was around 13 nm in diameter, the taller heights (>2 nm) are consistent with a hemimicellar surface structure. Shorter heights may represent collapsed micelles or hemimicelles.

3.2. Simulation Results. Molecular dynamics suggests that DSPG lipids, which have negatively charged headgroups, do not bind to the surface (Figure 3). The montmorillonite surface is also negatively charged, so the lipids are repelled from it and freely float in water instead (note that water molecules are not shown in Figure 3 for clarity). A single DSPG lipid molecule prefers bulk water by 2 kJ/mol over the montmorillonite surface. The maximum in the free-energy profile lies at the surface of the clay. This also means that the aggregates seen on the montmorillonite with AFM (Section 3.1) were most likely forced onto the surface by the drying process. As is the case with single lipids, the aggregates also prefer the bulk water. The headgroups orient toward the water, forming perfect spherical micelles at a 25% coverage or cylindrical micelles at a 50% coverage. We infer this from the symmetric nature of all four number density profiles in Figure 3D. The tail density profiles for both coverages are unimodal. The phosphate density for 25% does not have any peak, and that of 50% is bimodal. Based on these MD simulations, we hypothesize that DSPG and DOPG form micelles in solution, rather than vesicles or bilayers. This supports the data from Blosser et al. who found that DPPG lipids do not readily form vesicles in water. (Note that in Blosser et al., the ternary phase diagrams are marked “inaccessible” for the 100% DPPG composition, indicating that vesicles larger than the optical limit did not form. We expect DSPG to behave similarly to DPPG given the similarity in structure.)

3.3. Lipid Distribution in the Film. Both DOPG and DSPG are relatively continuously distributed through the film (Figures 4 and S7). As seen before, the weblike pattern in the fluorescence is linked to the film’s texture at the scale of tens of microns. However, DSPG is solid at room temperature and, as such, we expected its distribution to be discontinuous, consisting of isolated aggregates that tend to lie away from the ridges, as was the case with DSPE (Figure 4C). The reason for the difference between the distribution of DSPG and DSPE is unclear. DSPG’s melting temperature, 55 °C, is lower than DSPE’s of 74 °C, so we hypothesize that in between these temperatures, there is a melting point that divides continuous from discrete distributions or a temperature range over which

| lipid | % coverage | fraction of aggregates on clay edges | aggregate diameter (standard deviation) |
|-------|------------|-------------------------------------|----------------------------------------|
| DOPG  | 25         | 0.80                                | 7.9 ± 1.8                              |
|       | 50         | 0.11                                | 6.6 ± 1.2                              |
| DSPG  | 10         | 1.00                                | 11.8 ± 2.1                             |
|       | 25         | 0.97                                | 13.3 ± 2.6                             |
|       | 30         | 0.92                                | 5.4 ± 1.3                              |
|       | 40         | NA; forms bilayers                  | NA; forms bilayers                     |
|       | 50         | NA; forms bilayers                  | NA; forms bilayers                     |

“Typically, over 30 aggregates were counted for each sample, except for DOPG at a 10% coverage, which was difficult to image. The number of aggregates on the edge was then divided by the total number of aggregates to obtain the fraction of aggregates on the edge.

Figure 3. Results from MD simulations. Representative simulation snapshots are shown in panels A (25% CAM) and B (50% CAM), where lipid phosphorus is represented in orange and oxygen in red. The clay oxygen, silicon, aluminum, and magnesium are represented in red, yellow, pink, and cyan, respectively. The ions and water molecules in the simulation are not shown for clarity. The free energy of single lipid aggregate binding to the surface of the montmorillonite is not favorable for single DSPG molecules (C). Density plots for the freely floating aggregates are presented in (D).
the distribution transitions from continuous to discrete. We hypothesize that such a temperature could be the critical temperature separating the formation of micelles and the formation of bilayers. As shown in the MD simulations, DSPG forms spherical micelles in solution (Figure 3), whereas DSPE aggregates were in the form of bilayers.

3.4. Wettability. DSPG does not significantly change the contact angle or the value of \(a\). DOPG reduces the contact angle slightly but does not alter either the fluid index proxy \(a\) or the time constant of imbibition TC (Figures 5A–C and S4). Humidity likely changes \(a\), as the \(a\) measured for montmorillonite in these experiments was lower than that reported in our previous paper, and the only known factor that was different was the laboratory’s relative humidity. For the previous paper, the wettability was measured at between 30 and 40% relative humidities, whereas this data was collected between 40 and 50% humidities. Both sets were collected at 29 °C.

That PG lipids do not significantly change \(a\) or TC is unsurprising, given that the non-Newtonian flow—i.e., the degree of shear thinning (see eq 2)—in clay is due to water–clay interactions. Therefore, if the PG lipids do not stick to the clay

Figure 4. Film topography and lipid distribution. (A–C) Fluorescence images of the film. Scale bars are 50 \(\mu m\). Brighter regions contain more lipid and darker areas contain less lipid. (A) DSPG at a 26% coverage. (B) DOPG at a 26% coverage. (C) DSPE at a 26% coverage; reprinted with permission from Kessenich et al., J. Colloid Interface Sci. 2019, 555, 498–508, copyright 2019 Elsevier. (D) AFM image of the film topography. For additional fluorescence images, please refer to the Supporting Information. The contrast in the fluorescence images has been increased for feature visibility.

Figure 5. Comparison of PE and PG lipid wetting characteristics. Error bars indicate standard deviations. (A) Contact angle. (B) Exponent \(a\). Note that the value of \(a\) is likely lowered by increased humidity. (C) Time constant TC. (D) Comparison of the time constant TC and flow viscosity (a) for PE and PG lipids. (E) Comparison of the contact angle and \(a\). (F) Comparison of the contact angle and TC. PE data reprinted with permission from Kessenich et al., J. Colloid Interface Sci. 2019, 555, 498–508, copyright 2019 Elsevier. See Figures S4 and S5 to view PG data on expanded axes.
and thus alter the interfacial water–clay interactions, no change in fluid behavior is expected.

Among all lipids tested, melted DSPE caused the largest changes in contact angle, $a$, and TC (Figure 5A–C and Table 2). The PG lipids caused the least change, with DOPE and DSPE having intermediate effects. The primary difference between the PG and PE lipids is that the PG lipids do not bind to the clay surface, while PE lipids do bind. Therefore, we conclude that, to alter wettability, the lipids must have a headgroup that interacts with the montmorillonite surface.

The degree of shear thinning is not, in the case of the PG lipids, linked to the time constant of imbibition (Figures 5D and S5), but as there is essentially no variation in either $a$ or TC, this finding is also reasonable given that the time constant must be affected by the fluid viscosity. In contrast, for the PE lipids where both parameters vary over a substantial range, there is a strong correlation between water that exhibits less shear thinning (larger, more viscous $a$) and slower flow (larger TC) (Figure 5D). There is also a strong relationship between a higher contact angle and lower degree of shear thinning (larger $a$) for heated/melted DSPE (Figure 5E, eq 3). This suggests that, for some lipids, more surface hydrophobicity and less shear thinning are linked. This result is consistent with the conclusion in our previous study $^9$ that heated DSPE leads to less shear thinning, because the molten lipid, which spreads throughout the film, solidifies upon cooling and thus creates larger separations.

Table 2. Percent Difference between the Measurement on Plain Montmorillonite and the Measurement with Lipid That Most Deviates from the Montmorillonite Value $^a$

| Largest % difference for each lipid and each wettability variable |
|---------------------------------------------------------------|
| Binds to surface | Does not bind to surface |
|-------------------|-------------------------|
| DOPG | DSPG |
| DOPE | DSPE | DSPE-melted | DOPG | DSPG |
| Contact Angle | -16 | -9 | +54 | -19 | -11 |
| TC (1/K) | -49 | -97 | +174 | +17 | +19 |
| $a$ | +54 | -56 | +140 | +17 | +11 |

$^a$Darker colors indicate a larger difference.

Table 3. Overview of Correlation Strength for Linear Comparisons of PG Wetting Variables to Physical Variables $^a$

| | DOPG | DSPG |
|-------------------|-------|------|
| Contact angle | $X$ | $X$ |
| TC | $X$ | $X$ |
| $a$ | $X$ | $X$ |

$^a$Wetting variables are listed across the top and physical variables down the side. Refer to Figures 1 and 2 for definitions of the physical variables. The cell shading indicates the value of the $r^2$. Darker colors indicate a higher $r^2$, and lighter colors indicate a lower $r^2$. Pairings and $r^2$ value $>$0.80 are also marked with an $X$. See Table S2 for a complete listing of $r^2$ values.

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between the individual montmorillonite flakes as the film dries. These larger spaces mitigate the shear-thinning effect of smaller interflake separation in the lipid-free film. However, while there is also a positive correlation between contact angle and time constant (Figure 5F), that correlation is weaker. Thus, the other material factors that impact TC appear to weaken the correlation between surface hydrophobicity and the speed of imbibition even when surface hydrophobicity and degree of shear thinning are strongly correlated.

3.5. Connections between Wettability (a, TC, and Contact Angle) and Physical Parameters (Lipid Aggregate Density and Location, Lipid Distribution, and Film Topography). Relative to PE lipids, PG lipids exhibited a few strong correlations between wettability and physical variables (Tables 3 and S2 and Figures 6, S8, and S9). As the PG lipids have limited impact on a and TC, even strong linear correlations have little causative meaning.

For DSPG, the contact angle showed correlations with the size, density, and edge preference of the lipid aggregates (Figure 6B), but the error bars preclude strong conclusions about the mechanistic implications, if they exist at all. TC correlated with the aggregate diameter and fluorescence measure of lipid distribution, but these lines were essentially flat given that TC does not vary much over the concentrations investigated (Figure S8). However, the sudden increase in TC at a 40% coverage, which is the same concentration that DSPG switches from forming spherical aggregates to forming bilayer patches, is potentially of note. The value of a for DSPG is also correlated with the aggregate edge preference, though the error bars on a indicate that this correlation is unlikely to represent a true causative relationship (Figure 6A).

DOPG exhibited even fewer correlations than did DSPG. A tentative connection exists between contact angle and aggregate edge preference (Figure 6B). Since aggregates on the edge of clay flakes could alter pore space or how the surface traps air, this correlation may actually represent causation. TC is correlated with measures of the topography, but these points were present in a tight cluster, so a causative relationship between these factors is unlikely (Figure S9).

The limited changes in PG montmorillonite film wettability that are observed are mostly correlated to measures of the aggregate distribution on the surface (see Section 3.1). By contrast, measures of lipid distribution across hundreds of microns had the largest impact on PE montmorillonite film wettability. Therefore, we conclude that headgroup chemistry not only determines trends in wettability, but the mechanism by which the wettability is altered may also change with headgroup chemistry.

4. SUMMARY AND CONCLUSIONS

Whether and to what degree phospholipids affect montmorillonite wettability depends on both the lipid head and tail chemistry. Overall, melted DSPE caused the largest changes in the measured wettability variables, raising the contact angle, fluid index, and time constant for imbibition. Thus, we hypothesize that to change the contact angle, imbibition time constant, and fluid index, a lipid must (1) interact strongly with the clay surface, (2) be solid at room temperature, and (3) be relatively continuously distributed through the film. DSPG meets criteria (2) and (3) but does not bind to the surface and thus does not alter wettability as drastically as melted DSPE. As soil wettability also depends on whether the soil is wet or dry, our results suggest that even in a dry state, PG lipids do not alter wettability.

In the case of PG lipids, which do not bind to the clay surface, there is additionally no relationship between the degree of shear thinning in clays and the time constant of imbibition. For PE lipids, which do bind, there is a positive linear trend for longer time constants and lower shear thinning. PE lipids overall caused greater magnitude changes in a, TC, and the contact angle. Therefore, we conclude that phospholipids that bind to the clay surface have the most influence on wettability. Also, that influence is maximized when the lipids can be distributed throughout the clay film in a liquid state, but exist in a solid state at the temperature of exposure to water. This latter conclusion highlights the potential role that temperature may play in the development of high SWR due to lipid–soil interactions during wetting and drying cycles. Our results support the use of clay as a method of SWR remediation, given its intrinsic hydrophilicity and resistance to organic-induced hydrophobicity among the molecules studied here.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c00499.

Table of wettability data, plots of all contact angle data points, aggregate height and diameter data, PG wettability data on expanded axes, comparison of PG wettability variables, cartoons of lipid surface morphology, additional fluorescence micrographs, table of all r² values, and linear regression plots for all variables with r² > 0.8 (PDF)

ASSOCIATED CONTENT

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https://dx.doi.org/10.1021/acsomega.0c00499

ACS Omega 2020, 5, 12154–12161
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Notes
The authors declare no competing financial interest.

Acknowledgments
The authors thank Odeta Qafoku and John Loring (PNNL) for providing processed montmorillonite and Elias Nakouzi (PNNL) for the development of image analysis routines for contact angle measurements. B.L.K. is grateful for lipid expertise, wet lab space, and microscope use through Prof. Sarah Keller and her group at UW. B.L.K. is supported by the National Science Foundation Graduate Research Fellowship Program under Grant No. DGE-1762114. J.K.K. acknowledges support for data analysis and AFM imaging from The National Science Foundation through the UW Molecular Engineering Materials Center, a Materials Research Science and Engineering Center (DMR-1719797), including the Alliances for Learning and Vision for Underrepresented Americans (ALVA) summer research program. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation. Sample synthesis, image analysis algorithms, and model development were supported by the U.S. Department of Energy (DOE), Office of Basic Energy Sciences (BES) Chemical Sciences, Geosciences, and Biosciences Division at the Pacific Northwest National Laboratory (PNNL). PNNL is a multiprogram national laboratory operated for DOE by Battelle under Contract No. DE-AC05-76RL01830. This work was facilitated through the use of advanced computational, storage, and networking infrastructure provided by the Hyak supercomputer system at the University of Washington. Atomic force microscopy was conducted at the Molecular Analysis Facility, a National Nanotechnology Coordinated Infrastructure site at the University of Washington, which is supported in part by the National Science Foundation (grant NNCI-1542101), the University of Washington, the Molecular Engineering & Sciences Institute, the Clean Energy Institute, and the National Institutes of Health.

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