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Bio-inspired Assembly at Two Length Scales: Bridging Intermolecular Peptide Self-Assembly and Particle Phase Behavior in Two-Dimensions

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14. ABSTRACT
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1. Summary of accomplishments:

The over arching goal of this grant was to couple the molecular and colloidal length scales by co-assembling periodically sequenced polypeptides and anisotropic Janus particles at interfaces. The work was divided into four tasks First, we synthesized several high molecular weight polypeptides with an alternating periodicity resulting in amphiphilic β-strands when confined to interfaces. A total of 14 dipeptides were synthesized, and four were successfully polymerized. Second, we synthesized several anisotropic Janus particles, varying the properties of the Janus particles along two distinct axes, (1) the size of the particles and (2) the degree of anisotropy. Third, we used a Langmuir trough duplexed with a micro-Brewster angle microscope to image the interfacial assembly and the phase behavior of the polypeptides and the particles under confinement. Fourth, we examined both the single-component and two-component phase behavior of the periodically sequenced polypeptides and the Janus particles confined at an air-water interface. Section 3 includes several details regarding these accomplishments.

2. Objectives

The grant “Bio-inspired Assembly at Two Length Scales: Peptide and Particles” (FA9550-14-1-0263) aims to couple the self-assembly of amphiphilic peptides and Janus particles confined at fluid-fluid interfaces to create biomimetic periodic structures, connecting the length scales of molecular self-assembly and colloidal phase behavior. To this end, we developed two components, a molecular scale surface active polypeptide and a colloidal scale amphiphilic anisotropic particle. The two-component interfacial system was studied to determine the phase behavior and interfacial self-assembly. Individually, a Janus particle system was studied to determine the ability of anisotropy to lead to laterally assembled structures and jammed phases

at interfaces, and we found that surface contact angle and particle anisotropy define the assembly and wrinkling behavior for particles confined in two-dimensions. A monodisperse high molecular weight polypeptide system was also studied at the air-water interface, where the self-assembly and jamming occurred at a length- and time-scale distinct from the particle assemblies.

The original proposed objectives did not change during the grant period. There were several hurdles that were overcome as we studied this system that resulted in publications highlighting non-intuitive behavior. That work and the relevant manuscripts are described below.

3. Status of effort

The grant period has concluded, and our team has completed the stated objectives of the work. Figure 1 includes the four objectives and the proposed timeline. The grant period was extended several months to complete the proposed work on the assembly and characterization of particles and peptides at interfaces.

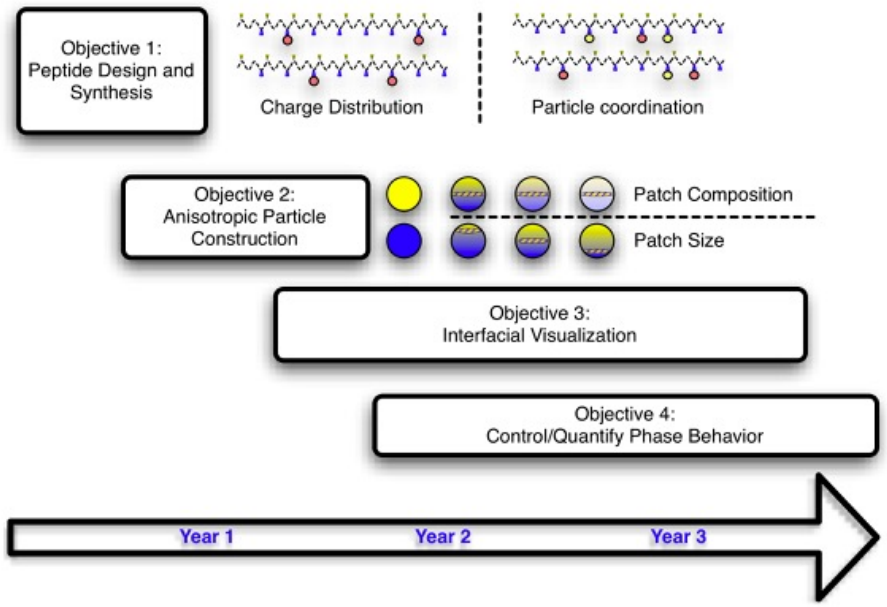


Figure 1. Original objectives and timeline included in the grant.

We have published eight papers on the particles and polypeptide systems. Those publications and the findings are described below. Also, we have three publications pending. First, we have a manuscript describing the centrifugal purification of nanoscale Janus particles. Prior to this study, it was only possible to synthesize micron sized anisotropic particles, but this paper

highlights a new method to construct nanometer sized Janus particles. Second, we have a manuscript describing the analysis of interfacial forces using colloidal probe microscopy, focusing on the contact angles, attachment energies and pinning at the air-water interface. Third, we have a manuscript describing the collective behavior of anisotropic particles at the surface of the Langmuir trough.

We demonstrate the use of biomacromolecules and anisotropic colloids to rapidly create a 2D quasi-periodic structure, mimicking the hierarchical assemblies of biology and connecting the molecular and colloidal length scales. The project is divided into four tasks. Details on those tasks and our findings are included below.

3.1. Design and synthesize high molecular weight β-strand polypeptides

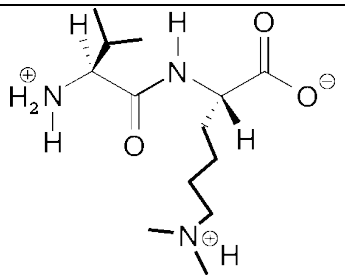
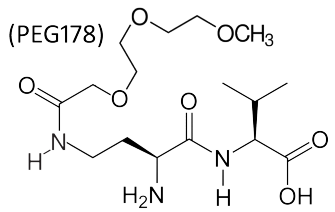
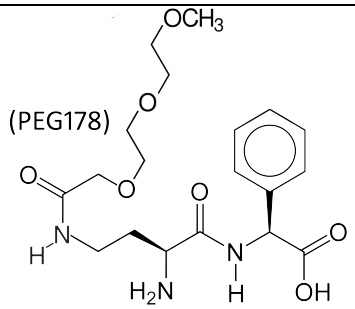
The goal of the first task of the proposed work was to synthesize a set of high molecular weight rationally designed periodically sequenced polypeptides. Because of the periodicity, the resulting polymers are both surface active and capable of lateral self-assembly at the air-water interface. Therefore, these polymers ca be employed to control 2D phase behavior at the molecular length scale (and coupled with the 2D assembly of particles at the colloidal length scale).

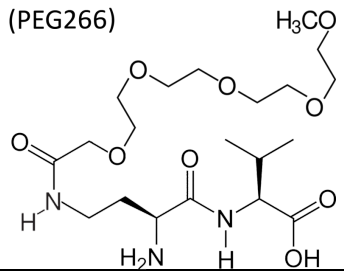
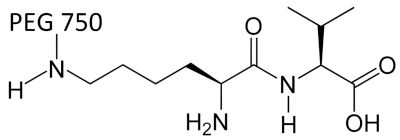
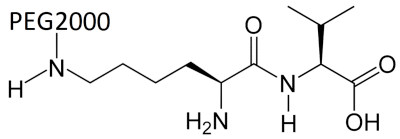
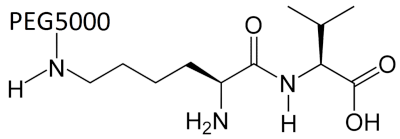
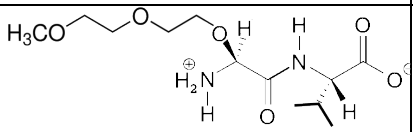
Findings.

Starting from the first year of this work, we worked on developing a synthetic protocol involving the polycondensation reaction of a dipeptide, where one of the amino acids is hydrophobic (i.e. valine) and one of the amino acids is hydrophilic (i.e. PEG-lysine). Constructing a periodically sequenced amphipathic polypeptide is typically a challenge because the polydispersity index increases with increasing molecular weight (at high degrees of polymerization, PDI -> 2.0). To circumvent this issue, we are designing synthetic amino acid dimers that are both water-soluble and amphipathic. The resulting peptide backbone will have alternating hydrophilic/hydrophobic side groups, which is the typical periodicity for beta-sheet forming polypeptides. Using this approach, we can influence the polydispersity of the growing polypeptide chains, controlling the kinetics of growth through transport-limited chain elongation. Our experiments show that in the absence of an interface, standard bulk-phase condensation polymerization occurs. Conducting the polymerization in the presence of micelles, we can control the molecular weight in a transport-limited manner. The amphipathic character of the peptide chain increases with

increasing molecular weight, resulting in a polypeptide that partitions to interface as a function of the degree of polymerization.

This type of kinetically limited growth serves to narrow the polydispersity of our periodically sequenced polypeptide. We quantify the dynamics of chain elongation and interfacial assembly using multi-angle light scattering, and we define the evolving sheet-like secondary structure using circular dichroism. A list of our 14 dipeptides that were developed for the transport limited polymerization are included in table 1:

#	Dimer Name	Molecular Structure	Dimer description/polymerization
1	Dimethyl Lysine-Valine		The hydrophilic amino acid is a partially methyated lysine that holds a charge (proton) on the ε-amino group with a pK_a similar to that of unmodified lysine. The two methyl groups transform the highly-reactive primary amine into a tertiary amine that is nearly inert under polymerization conditions. The hydrophobic amino acid is valine, which has a high sheet forming propensity. Paired together, the dimer is water soluble and amphipathic.
2	Lysine(PEG178)-Valine		The hydrophobic valine is coupled to a hydrophilic short-chain PEG group. This linkage significantly impacts the solubility of the dimer in water, where the dimer is sparingly soluble in water, but it does readily dissolve in alcohols. As a result, this dimer offers the potential to study the same transport-limited polymerization mechanisms in non-aqueous, two phase systems.
3	Lysine(PEG178)-Phenylalanine		Similar to lysine(PEG178)-valine, this dimer is not suited for use with aqueous systems. The PEG side chain retains the affinity for alcohols and other mid-range polar solvents while the phenylalanine presents a larger side group that prefers a non-polar environment. This dimer is slated for use in the same systems as the lysine(PEG178)-valine, but is expected to behave with an appreciably stronger affinity for the interface.

4	Lysine(PEG750)-Valine		This dimer is structurally identical to the lysine(PEG178)-valine system, but increases the hydrophilic character of the system with two additional ethylene oxide repeats to the point that the molecule dissolves in water.
5	Lysine(PEG750)-Valine		This dimer is structurally identical to the lysine(PEG178)-valine system, but increases the hydrophilic character of the system via a much longer PEG sidechain (more ethelene oxide units per attachment). The resulting system is then suitable for aqueous polymerization, but the polypeptide length will be attenuated due to the steric effects of the PEG side chains.
6	Lysine (PEG 2000)-Valine		This dimer is structurally identical to the lysine(PEG178)-valine system, but has a still-longer PEG group. Again, this dimer's steric resistance to polymerization is expected to greatly decrease the accessible molecular weight. In this case, a small amounts of this dimer could be pre-activated and added to systems of other dimers, greatly impacting the solubility of those peptides.
7	Lysine (PEG 5000)-Valine		This dimer is identical to the lysine(PEG2000)-valine, and is imagined for similar use. With a PEG chain over twice as long, however, the solubility impacts are found to be significantly stronger. This dimer is imagined for use as a form of solubility anchor, in which the addition of a single dimer molecule to a growning polypeptide chain will solubilize the polymer and cap the polymerization process.
8	Synthetic PEG Amino Acid-Valine		A synthesis method for creating this dimer has been developed, but remains untested at this time. This molecule is again similar to the lysine(PEG178)-valine dimer, but is not formed from lysine. It removes the associated hydrocarbon stretch and the secondary amide bond from the hydrophilic side. This change is expected to give rise to a dimer that is again water-soluble.

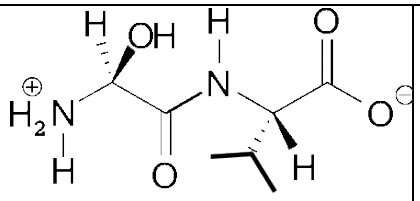
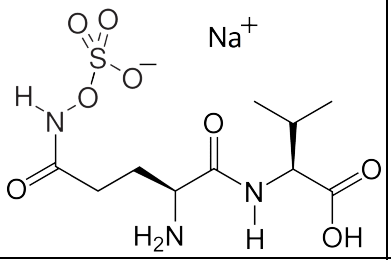
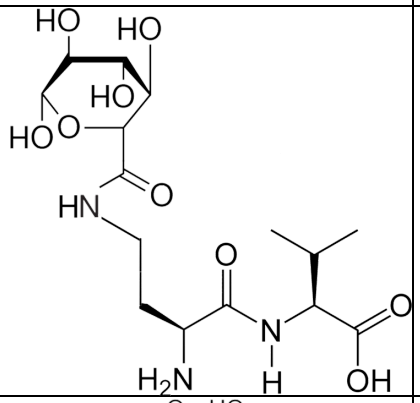
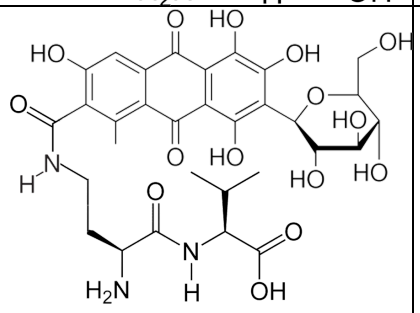
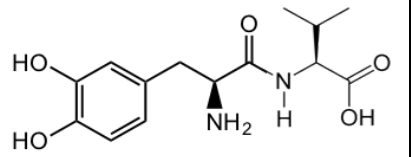
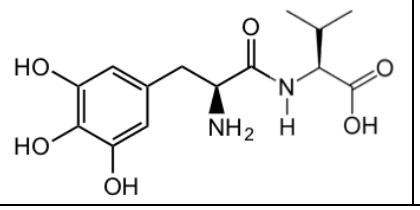
9	Serine-Valine		This dimer was created primarily as an intermediate reagent in the synthesis of the synthetic PEG amino acid-valine molecule discussed previously (8). With one side hydrophilic and the other hydrophobic, this dimer could be used to test the onset of appreciable polydispersity effects arising from the polymerization of an amphipathic peptide.
10	Glutamate(O-Sulfonic Acid)-Valine		This dimer is expected to polymerize with an end result similar to that seen for dimethyl lysine-valine. Here, a charged hydrophilic sulfonic acid sidegroup is paired to a hydrophobic valine to build the amphipathic character. The charge in this case is not pH dependent.
11	Lysine (Glucuronic Acid)-Valine		This dimer modifies the hydrophilicity of the lysine group with the attachment of a sugar group. The hydrophobic amino acid is valine. The molecule is completely water soluble.
12	Lys(Carminic Acid)-Valine		This dimer alternates the hydrophilicity of carminic acid with the hydrophobicity of valine. The molecule is completely water soluble. Additionally, carminic acid is a dye molecule that expresses two colors (red and purple), which is switchable via changes in pH. As a result, the molecule is easy to quantify and visualize, as well as serve as a simple pH indicator.
13	3,4-Dihydroxy-L-phenylalanine)-Valine		This dimer alternates between the catechol 3,4-Dihydroxy-L-phenylalanine and valine. The molecule was designed to have a structure defining valine amino acid and an adhesive DOPA derived from tyrosine. This was designed to control light adsorption properties as well as the mechanics of adhesion.
14	3,4,5-Trihydroxy-L-phenylalanine-Valione		This dimer is similar to 13 and alternates between the catechol 3,4,5-trihydroxy-L-phenylalanine (gallol analogue) and valine. The molecule was designed with a similar goal of controlling coloration and adhesive materials properties.

Table 1. Fourteen dipeptides that we have synthesized for polycondensation reactions. Four were effectively used for polymerization, namely 1, 2, 3 and 10.

Four of those polymers were successfully polymerized: Dimethyl Lysine-Valine (**1**), Lysine(PEG178)-Valine (**2**), Lysine(PEG178)-Phenylalanine (**3**) and Glutamate(O-Sulfonic Acid)-Valine (**10**). The polymerization was characterized using Multi-angle light scattering and MALDI-TOF Mass Spectroscopy.

Multi-angle light scattering. We use multiangle light scattering to ascertain both the molecular weight and the polydispersity index. A typical polymerization is described here for the dimethyl lysine-valine dipeptide (dipeptide #1 in table 1). The dipeptide was polymerized in the presence of varied surfactant (Triton X-100) concentrations. Triton-X 100 has a critical micelle concentration of 0.6 g/liter and, as such, we examined surfactant concentrations from zero added Triton-X to 50 g/L. Polymerization took place with strong mixing, minimizing mass transfer resistance to peptide partitioning onto those interfaces.

The varied availability of this micellar surface area during polypeptide growth was examined via SEC-MALS (Figure 2). Due to the size exclusion effects of the column, the largest polypeptides in each sample were separated from the smaller as they flowed through the instrument, allowing them to self-assemble in the instrument stream. This phenomena gives rise to the two peaks in the data shown. The left peak of each run, corresponding to a fast elution time, represents large, self-assembled polypeptide aggregates. The right peak shows low MW, non-assembled, individual molecule species. Given that the goal of this task is to synthesize polypeptides with a high affinity for self-assembly, ideal results would show a large high MW (left) peak paired with a small low MW (right peak). We observe this with increasing concentrations of Triton-X.

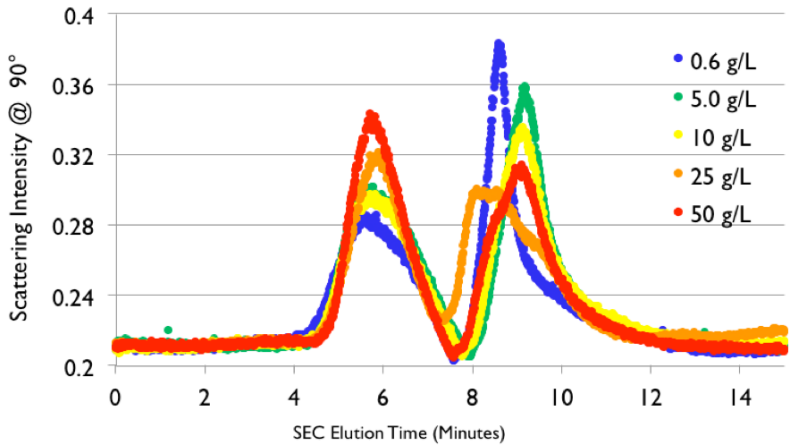


Figure 2. Multi-Angle Light Scattering data for polypeptides isolated after polymerization while exposed to varying Triton X-100 concentrations. A trend towards self-assembled polypeptides (large left peak) develops with increasing surfactant concentration present during polymerization.

To more clearly quantify these two distinct peaks in water, calculations to determine the polydispersity index (PDI) for each polypeptide were performed (Table 2). The PDIs of the small molecule peaks center around 2.0, typical of condensation polymerizations. The large molecule aggregates have low PDI's, sometimes approximately 1.0. Given that this would signify monodispersity, this number is unlikely to refer to individual polypeptide strands. Rather, it's likely a measure of the self-assembled state.

Concentration of Triton X-100	PDI of Aggregates	PDI of Small Species
0.6 g/L	1.033	1.832
5.0 g/L	1.294	1.855
10 g/L	1.203	1.721
25 g/L	1.057	2.221
50 g/L	1.084	2.256

Table 2. Multi-Angle Light Scattering data for peptides isolated after being polymerized in varied, aqueous Triton X-100 concentrations. Small species show PDI's reminiscent of condensation polymerization. Aggregates show near-monodisperse PDI's, likely in reference to a particular self-assembly, not individual polymer strands.

MALDI-TOF Mass Spectrometry. The large MW, high charge density and periodicity of our polypeptide samples prevented clear TOF interpretation and determining an ideal matrix has not always clear for MALDI samples. Still, TOF data is included below for monomeric DOPA-Val dipeptides, figure 3 and Poly-Glutamate(O-Sulfonic Acid)-Valine, figure 4.

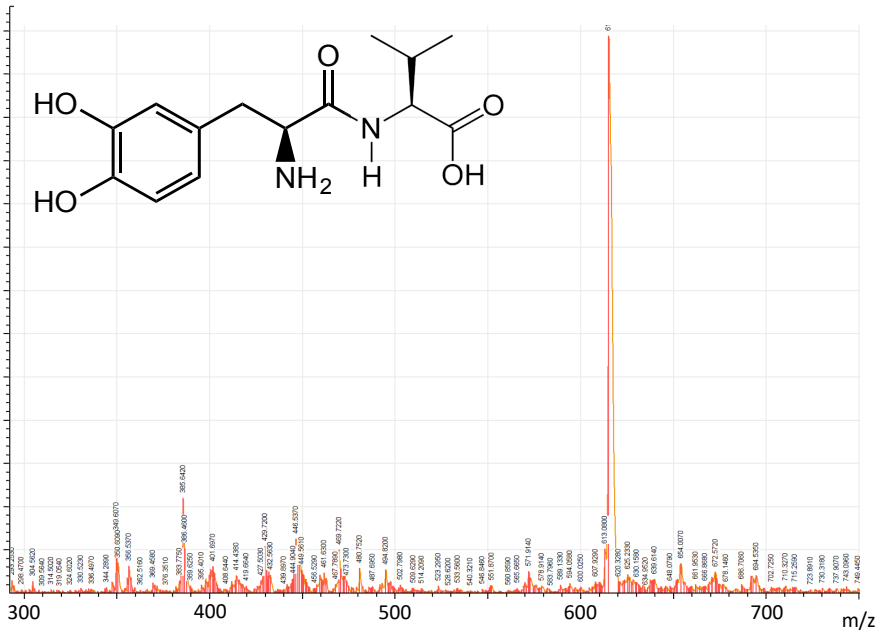


Figure 3. MALDI-TOF Mass Spec DOPA-Valine. The single peak represents the

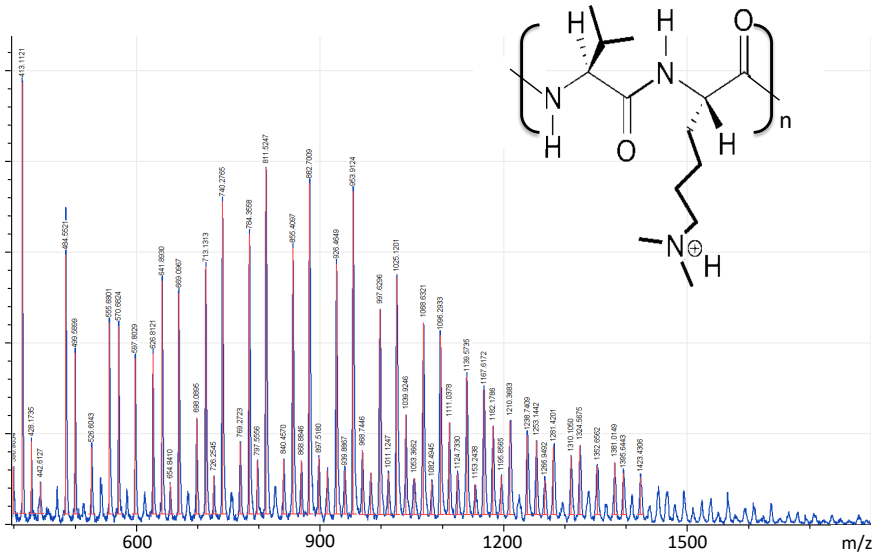


Figure 4. Mass Spec data for Poly-Glutamate(O-Sulfonic Acid)-Valine showing polymerization has occurred.

A key issue in the polymerization of these dipeptides was solubility. Several efforts was made to bio-conjugate periodically sequenced polypeptides with PEG to enhance solubility. Manipulation of the polypeptide architecture and resulting formation of large scale aggregates is highlighted in two publications, Soft Matter and Physical Chemistry Chemical Physics.

3.2. Anisotropic (Janus) particles construction

The goal of this task was to construct homogenous and anisotropic particles of various degrees of hydrophobicity. In the case of the anisotropic particles, we were aiming to form Janus particles, where one hemisphere was silica or polystyrene with varying amounts of dichloro-dimethylsilane and the opposite hemisphere was covered in a thin (~10 nm) film of gold with varying amounts of alkane thiols. Similar to the work with the periodically sequenced polypeptide, our goal was to build a colloidal scale structure that would be confined at fluid-fluid interfaces and self-assemble laterally as a function of the concentration and surface anisotropy.

Synthesis of particles with varying degrees of hydrophobicity. Initially, our experiments were completed on micron sized homogenous silica particles that were 1 μm in size. These particles are hydrophilic with a contact angle of 30° prior to modification. We used the surface silanol groups as a target for conjugation chemistry. In order to promote surface confinement, we functionalized the silanol groups with a dichloro-dimethylsilane group. The two-step reaction includes a hydrolysis step of dichlorodimethylsilane, followed by a condensation step to the surface silanols:

Hydrolysis: $2H_2O + Cl_2Si(CH_3)_2 \rightarrow (OH)_2Si(CH_3)_2 + 2\ HCl$

Condensation: $SiOH + (OH)_2Si(CH_3)_2 \rightarrow SiOSi(OH)(CH_3)_2 + H_2O$

We can control the hydrophobicity of the particle surface by either controlling the concentration of dichloro-dimethylsilane or by controlling the amount of time that we allow the reaction to proceed. In our case, we control the degree of hydrophobicities by varying the concentration of dichloro-dimethylsilane in cyclohexane. We use concentrations of 10^{-x} M dichloro-dimethylsilane. The remainder of this document will describe these homogenous particles as S_x, *where* x = 1, 2, 3, 4 or ∞. Therefore, x = 1 is the most hydrophobic and x = ∞ is a particle without modification.

Characterization of hydrophobicity. Measuring the contact angle on a micron-sized curved surface is challenging. We quantified the degree of silanization on these homogenous particles using three different methods. In the first method, we used a clean silicon wafer that was functionalized with precisely the same protocol, and we measured the wafer's contact angle as a proxy for the degree of surface modification on the curved surface. In this case, we imaged the droplet shape using the edge detection feature of a tensiometer (Attention Theta tensiometer), figure 5a.

In the second method, we attempt to more directly evaluate the degree of silanization using close-packed monolayers of surface-modified silica particles. We use a well-packed film of particles on a Langmuir trough and transfer those particles directly to a silicon wafer using the Langmuir–Schaefer deposition method. Subsequently, we imaged the droplet shape using the edge detection feature of a tensiometer as we did on the modified silicon wafers, figure 5b. The figure below shows the characterization of contact angle using both techniques below. Finally, in the third method, we use colloidal probe microscopy quantify the advancing and receding contact angles. That work is described below.

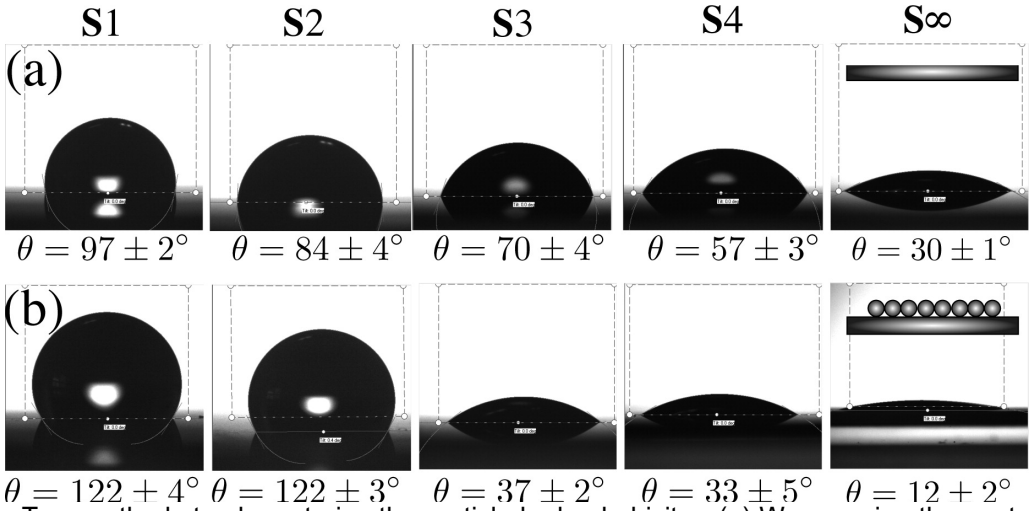


Figure 5. Two methods to characterize the particle hydrophobicity. (a) We examine the contact angles of the flat silica surface that have been treated with dichloro-dimethylsilanes. (b) We directly examine the contact angles' of particles deposited as a monolayer on the surface.

Synthesis of Janus particles with varying degrees of hydrophobicity. Anisotropic particles with one hydrophobic face and one hydrophilic face are constructed via an evaporative technique. Either silica or sulfated polystyrene particles are initially arranged in a monolayer on pre-cleaned microscope slides. Subsequently, the slides are placed in a metal evaporator and a 5 nm titanium adhesive layer is deposited on the exposed face followed by a 20 nm gold cap. Janus particle are sonicated off the glass slides into water contained in a petri dish. The petri dish with the particle suspension is then dried in an oven at ~70°C until all water has evaporated. The dried particle residue is transferred into a glass vial and weighted yielding Janus particle powder of 20-50 mg.

We can remove the particles from the interface using the Langmuir−Schaefer deposition method. Figure 6 displays the SEM image of a Janus particle layer formed at the interface and

deposited on a silicon wafer. The bright side of each particle represents the gold cap, whereas the darker region indicates the PS base particle. By investigating the orientation of each particle in this image, the frequency of different cap orientation, including cap-up, cap-down and cap-sideways, is evaluated as shown in the histogram. Janus particles are randomly oriented at the interface with the majority of the particles exhibiting sideways configuration (55%). Since particle-particle interactions depend on the specific sides of each particle pair that are facing each other, the type and strength of these interactions varies at different locations in the Janus monolayer.

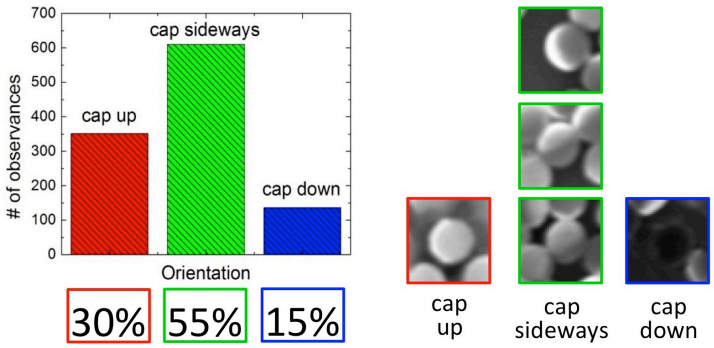


Figure 6. (right) SEM images of Janus particles constructed from polystyrene and gold caps. The particles were confined on LB interfaces and transferred onto a silicon wafer for imaging. Statistics of the various particles orientations are included (left).

Synthesis and centrifugal separation of nanoscale Janus particles. Initial experiments with the co-assembly of particles and polypeptides at the liquid-air interface showed that the phase separation was dominated by the particle interactions, which is likely an outcome of the overwhelming strength of the capillary interactions between particles. Therefore, we begun a process of synthesizing nano-scale Janus particles. Typically, the process of constructing Janus particles uses physical vapor deposition (PVD) to deposit a metal cap on colloids. This technique involves first making monolayers of particles and then placing them in a chamber underneath a coiled filament containing the metal to be deposited. The chamber containing the monolayers is pumped down to vacuum and, when a sufficiently low pressure is reached, a current is run through the filament. The metal vaporizes and radiates downward to coat the top, exposed half of the particles, as shown in Figure 7. For surface coverage less than 50%, one can use Glancing Angle Deposition (G.L.A.D.). This involves changing the angle of incidence at which the metal contacts the particles and shadowing effects allow for different surface coverage and patch geometries (Figure 7, D)

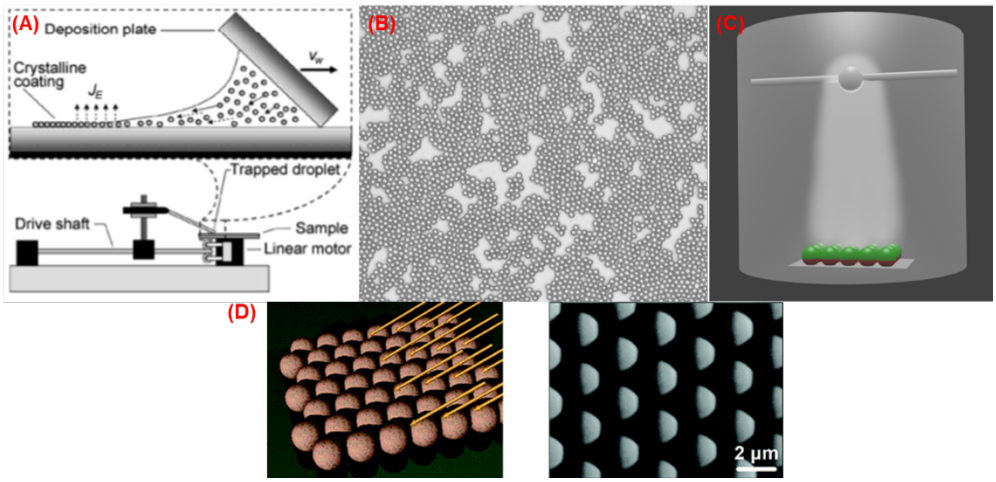


Figure 7. Janus particle and patchy particle synthesis using Physical Vapor Deposition. (A) Monolayer formation via convective assembly(2) (B) Image of monolayer to be placed in the PVD. (C) Schematic of the inside of the PVD, where the filament containing metal is represented by the grey sphere and rays of metal vapor can be seen radiating downward to coat the top half of the particles (shown in green). (D) Left – Schematic of G.L.A.D. where the incident angle of the metal hitting the particles (shown in yellow) has been change. Right – the resulting patches caused by the shadowing effects of other particles (right) SEM images of Janus particles constructed from polystyrene and gold caps. The particles were confined on LB interfaces and transferred onto a silicon wafer for imaging. Statistics of the various particles orientations are included (left).

The benefits of using physical vapor deposition for Janus and patchy particles synthesis are (1) it is a well-established, and metal capped anisotropic particles can be applied for controlling optical properties (metals have higher refractive indices than typical polymers), catalysis, and colloid swimmers. Unfortunately, one of the challenges of PVD is monolayer synthesis, particularly for patchy particle synthesis and for making Janus particles less than 500 nm in diameter. Patchy particle synthesis via G.L.A.D. requires a close packed monolayer in order to ensure uniform shadowing effects across the particle layer. However, close packed monolayers are challenging to make on a large scale. Additionally, for applications in optical properties, the size of the particles needs to be on the order of the wavelength of light. While monolayers can be made for particles smaller than 500 nm, the rate of evaporative assembly decreases tremendously (approximately 2 hours to make a monolayer on one 2”x3” glass slide). This rate is not feasible for interfacial assembly where milligrams of particles are needed for a single experiment.

To address this issue, we have developed a separation protocol using density gradient centrifugation (DGC) to separate out Janus from non-Janus particles. If multilayers are present, only the top layer of particles will be capped with metal in the PVD and the bottom layers will be unmodified. The addition of the cap increases the density of the particle as described by the equation below:

$$\rho_{JP} = \rho_{PS} * Vol\ Frac\ PS + \rho_{Au} * Vol\ Frac\ Au + \rho_{Ti} * Vol\ Frac\ Ti$$

where: ρ_{JP} = density of the Janus or patchy particle, ρ_{PS}, ρ_{Au}, ρ_{Ti} = density of polystyrene, gold and titanium, respectively, $Vol\ Frac\ PS$, $Vol\ Frac\ Au$, $Vol\ Frac\ Ti$ = volume fraction of polystyrene, gold and titanium, respectively.

In density gradient centrifugation (DGC), one makes layers in a centrifuge tube of sucrose solutions with different concentrations, as shown in Figure 8. The sample to be separated is placed at the top of the tube and the tube is centrifuged. If the density of the sucrose layer is greater than that of the material, then the material will not able to settle within that layer and it stops at that point in the tube. After centrifugation, the different layers are separated and rinsed to give the purified sample.

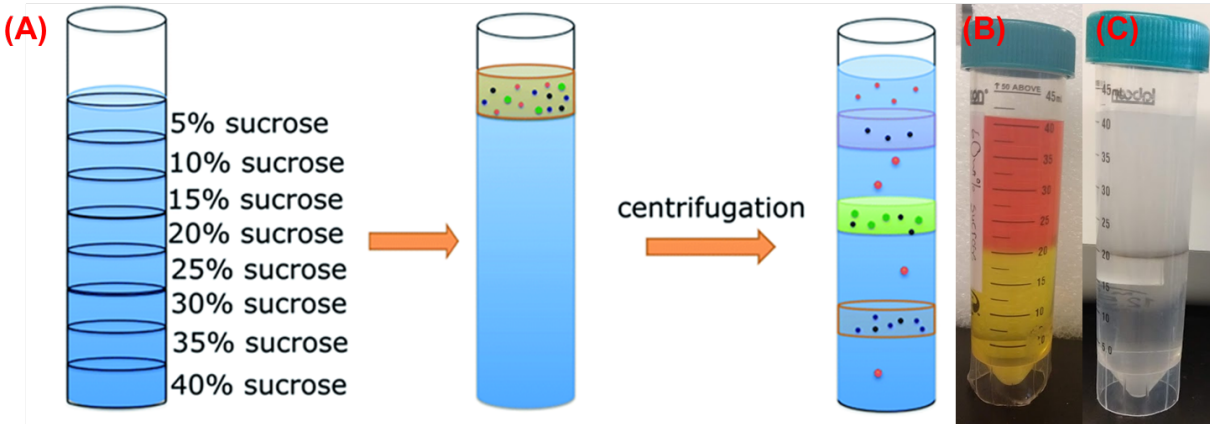


Figure 8. (A) Schematic of density gradient centrifugation showing the layering of sucrose solutions of varying concentrations (left), the placement of the sample on top of the tube (middle), and finally the separation of the sample into different layers based on the density of the material (right). (4) (B) 25% sucrose solution (red) layered on top of 60% sucrose (yellow) that have been dyed using food coloring and centrifuged to show that the layers do not mix during the separation. (C) Image of a tube containing sucrose solution and separated patchy particles. The opaque solution at the top contains a mix of unmodified polystyrene particles and 10% covered patchy particles, the black band in the middle ranges from 20-50% surface coverage, and at the bottom of the tube are any gold flakes that came off of the slides during sonication.

Thus far, DGC has been applied to make Janus particles using 4000 nm polystyrene particles capped with 10 nm of titanium and 50 nm gold and on 100 nm polystyrene particles capped with 5 nm of titanium and 10 nm gold. 4000 nm polystyrene particles have also been used to make patchy particles using a 10° angle of incidence and a nonuniform particle layer (Figure 9, C).

These samples have been characterized using scanning electron microscopy (SEM) and initial results indicate that the separation was successful in all cases. Figure 3 shows images of the separated samples. One challenge that has been encountered is charging of the polystyrene

during imaging, resulting in lower resolution images. Currently, we are still working to optimize parameters within the SEM to improve the quality of the images and enable more robust image analysis.

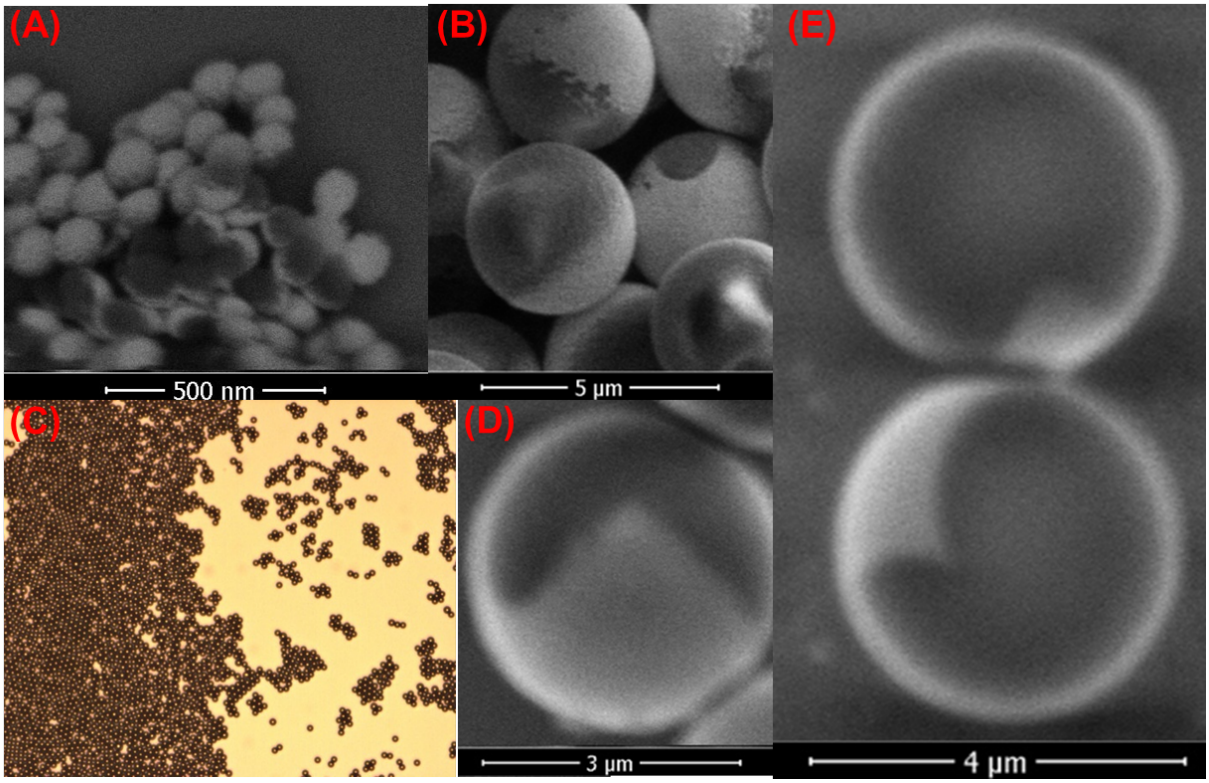


Figure 9. (A) Janus particles made from 100 nm polystyrene Janus particles capped with 5 nm Ti and 10 nm Au, ρ = 3.18 g/cm3, separated from plain polystyrene using 40 % sucrose solution. (B) Janus particles made from 4000 nm polystyrene Janus particles capped with 10 nm Ti and 50 nm Au, ρ = 1.15 g/cm3, separated from plain polystyrene using 30 % sucrose solution. (C) Image of one of the slides used for synthesizing patchy particles taken using optical microscopy. On the left you see multilayers and on the right is a diffuse monolayer. (D) Patchy particles made from 4000 nm polystyrene Janus particles capped with 10 nm Ti and 50 nm Au, taken from the lower layer of the DGC separation corresponding to 20-50% surface coverage, ρ = 1.08-1.15 g/cm3, (E) Patchy particles made from 4000 nm polystyrene Janus particles capped with 10 nm Ti and 50 nm Au, taken from the upper layer of the DGC separation corresponding to 10% surface coverage, ρ = 1.06 g/cm3

This methodology is the subject of a forthcoming manuscript. After the construction of these nano-sized Janus particles, we began the process of characterizing the self-assembly and co-assembly of particles and polypeptides at the air-water interface using the Langmuir trough. We aimed to quantify the interfacial stability and phase behavior in one- and two-component systems.

3.3. Interfacial visualization

The goal of this task of the proposed work was to develop new tools for the measurement of interfacial phase behavior. Additionally, we aimed to measure the stability and visualize the phase behavior of our periodically sequenced polypeptides and anisotropic particles developed in tasks 1 and 2.

Findings

In order to characterize phase behavior at the fluid-fluid interface, we have developed a set of tools for the visualization of particles and peptide self- and co-assemblies. Two techniques were used to define molecular phase transitions, stability, and interparticle aggregation in two dimensions: (1) Langmuir Blodgett techniques can be used to confine particles and peptides to the fluid-fluid interface and characterize phase transitions via isothermal compression and expansion and (2) A duplexed instrument combines fluorescence microscopy and Brewster angle microscopy for the visualization of particle translational dynamics.

Brewster Angle Microscopy (BAM) and Fluorescense microscopy. Using the AFOSR funding, we purchased a new liquid-liquid Langmuir Blodgett trough and a KSV micro-BAM. In addition to the BAM set-up, the trough also includes a transparent window allowing for fluorescent visualization from the aqueous-side. Fluorescence microscopy is applied to visualize the formation of ordered structures through the aqueous phase, where structure development can be examined as a function of surface concentration.

Polypeptide isotherms. An example of the phase behavior of one of our polymerized dipeptides Poly(lysine(PEG)-valine) (Table 1, Dipeptide #3) is included here. In this case, the polypeptide is soluble in most organic solvents, but it is insoluble in water. On the air-water interface, the material is highly amphipathic. To form a surface film, the grown polymer was dissolved in ethyl acetate and deposited onto the water surface of a Langmuir-Blodgett trough. Surface forces were then measured during compression and subsequent expansion, Figure 10. The film shows phase transitions while undergoing compression, and there is hysteresis that occurs on expansion. Moreover, due to the high molecular weights of the polypeptides confined in 2D, we observe that the isotherms change as a consequence of the compression rate. At slow compressions, the polypeptides are able to slowly reptate, organize and hydrogen bond, leading to a higher surface pressure. At faster compressions, the polypeptide jams into a structure that cannot reach the same surface pressures.

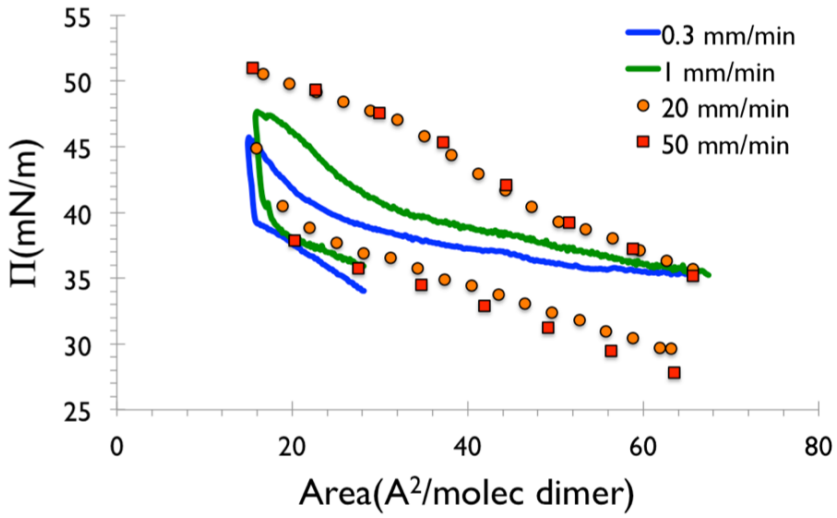


Figure 10. ε-amino PEGylated Poly(lysine-valine) surface pressure data during compression and expansion. Hysteresis is observed on expansion due to the hydrogen bonding network. Additionally, there is a dependence of the isotherm on the compression rate.

Visualization of phase behavior. Visualization of the macroscopic β-sheets was accomplished with Brewster angle microscopy. Images were captured of the developing assemblies at different pressures, Figure 11. Below surface pressures of 8 mN/m, no clear images are seen. At Π = 12 mN/m, faint fibrillar assemblies appear. At Π = 25 mN/m, fibrillar assemblies appear in high contrast, alongside islands of amorphous structures. By Π = 40 mN/m, solid bands of material stand in contrast to patches of bare air-water interface, with fibrillar assemblies between them. By Π = 44 mN/m, the patches of clean interface have collapsed, with the interface now completely coated by a contiguous-film. On expansion to surface pressures of 35 mN/m, the self-assembled structures persist but openings can be observed between dense aggregates.

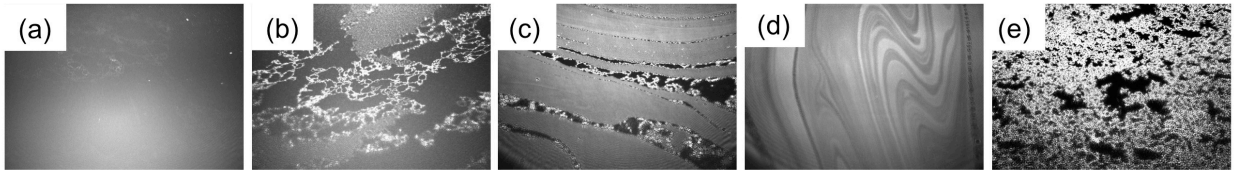


Figure 11. εBrewster angle microscopy images of β-sheet assemblies of PEGylated Poly(lysine-valine) on an air-water interface. a.) Π = 12 mN/m, b.) Π = 25 mN/m, c.) Π = 40 mN/m, d.) Π = 44 mN/m, and d.) Π = 35 mN/m, post-reexpansion

Additional efforts to visualize polypeptides at a liquid crystal interface resulted in a Soft Matter publication, highlighting our effort to understand the sheet forming activity and self-assembly at low concentrations. In this case, we discovered that a periodically sequenced polypeptide was able to co-assemble with surfactants, and the liquid crystal surface reflected the phase behavior of the polypeptide adsorbed at the surface. Figure 12 shows the three states of the liquid crystal as polypeptides adsorbed to the surface.

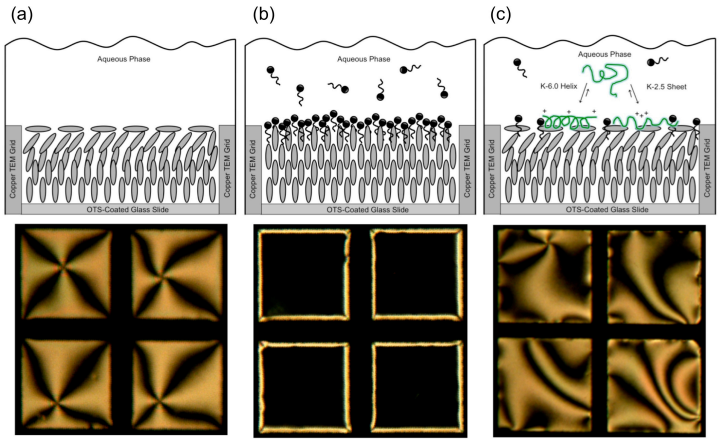


Figure 12. (top) 5CB liquid crystal changes orientation at the fluid-fluid interface from tilted to homeotropic back to tilted when interacting with water, surfactant and polypeptide. (bottom) The resulting polarized microscopy images show the phase behavior can be monitored even at low (femto-mole) surface adsorbed amounts.

3.4 Interfacial stability and phase behavior

The goal of the final task of the proposed work was to characterize the self- and co-assembly of the anisotropic particles and periodically sequenced surface active polypeptides confined at the air-water interface. Our results, described above, highlighted that the co-assembled case showed phase behavior dominated by capillary interactions of the micron sized particles. Therefore, we developed a new protocol to synthesize nanometer sized Janus particles. This section will highlight the self-assembly cases, where the colloidal and polypeptide systems demonstrate various degrees of interfacial stability.

Langmuir trough. Langmuir trough experiments were conducted by coupling our typical interfacial experiment with optical microscopy (in contrast to the BAM microscopy described above). Our goal in this effort is to visualize the micron-sized particles of varying degrees of hydrophobicity and the Janus particles that assemble at an interface. 7.5 mg of hydrophobically modified silica or Janus particles are dispersed in 200 µL of a 70:30 wt % isopropyl alcohol and water mixture. These mixtures are sonicated for 2 min to remove any aggregated particles. We verified that there is no role of dynamics in these samples by compressing at a lower

compression speed (0.05 cm/min), and no difference in the behavior of the particle layers observed.

Isotherms for particles in 2D. The method described above to determine the surface pressure(π)–area isotherms. Figure 13 shows the outcome of particles compressed in 2D using this tool. Thermodynamically, these π-A phase diagrams are a consequence of the configurational entropy of the particles confined in 2D as well as the inter-particle interactions, both factors are influenced by the degree of hydrophobicity. Figure 13 (left) shows a Janus particle laden film that is compressed and expanded multiple times. The surrounding figures show the film packing into a hexagonal monolayer, and, subsequently, the particle instability leads to a wrinkling behavior. This result was published as a Lanmguir paper in 2015. For hydrophobic particles, folding occurs, but for Janus particles, we observed a subduction phenomenon that is a consequence of particle assembly and film stiffening at the air-water interface. This is a result was published as a JACS article in 2015.

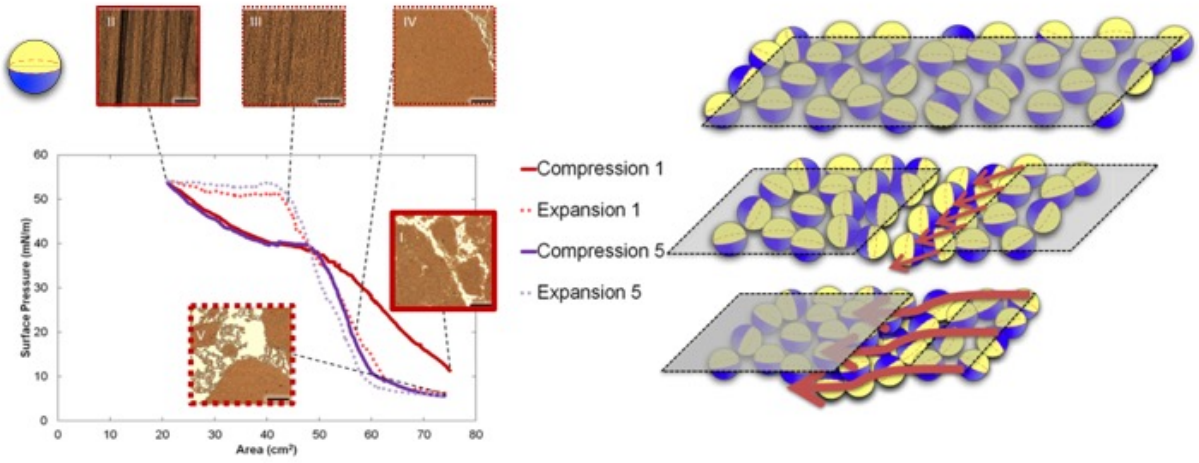


Figure 13. (left) Multiple compression and expansion isotherms of a surface active Janus particle. (right) the particle assembly and organization leading to a 'subduction' instability at the air-water interface.

Interfacial forces with colloidal probe microscopy. In the final year of the grant cycle, we aimed to quantify the capillary forces as a function of contact angle and particle size. Theoretical force curves were generated to determine meniscus shape at the perimeter of the Janus particles pinned in the air-water interface. Experimentally, colloidal probe microscopy was used to quantify the free energy landscape for Janus particles at an air-water interface. Taken together, connecting the theoretical force curves to the experimentally measured curves, will allow us to understand the meniscus shape around the particles as well as the role of this shape in the

pinning force. Figure 14 shows, a “jump” to the interface that indicates the formation of a capillary bridge that pulls the particle downwards into the bubble. Capillary bridge formation has been also observed in molecular dynamics simulation from our group. Understanding the shape of the meniscus formed is significant to understanding the free energy landscape of the particle at the interface and gives insight into capillary forces which may arise between Janus particles.

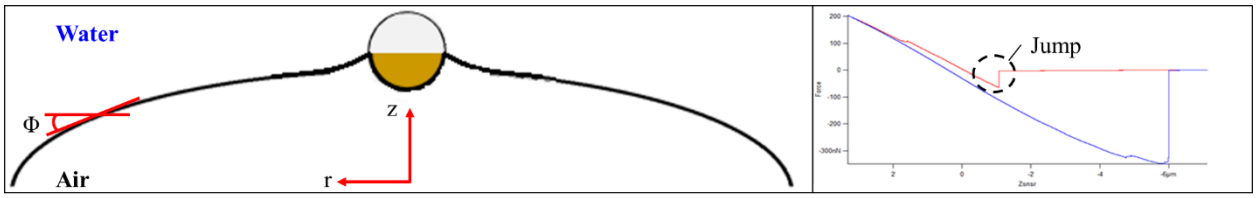


Figure 14. Left – schematic of force curve modelling showing Janus particle probing bubble, guessed meniscus shape, and fundamental equations. Right – an example force curve from colloidal AFM experiments showing jump to the air-water interface.

In order to model force curves and determine the shape of the interface, a method similar to that used in the pendant drop technique is used. The shape of the interface is solved for using the Young Laplace equation in cylindrical coordinates, as shown in Figure 14. Here s is the arclength measured from the edge of the particle.

$$\Delta P = \gamma[\frac{1}{R_1}+\frac{1}{R_2}]; \frac{dz}{ds} = sin\phi; \ \frac{dr}{ds} = cos\phi; \ \frac{1}{R_1} = \frac{d\phi}{ds}; \ \frac{1}{R_2} = \frac{sin\phi}{r}$$

First, a meniscus shape must be assumed; for example, we can assume that the interface pins at the Janus boundary. In this case, for the initial conditions at the edge of the particle (s=0), z and r are fixed and ϕ (tangent angle) and ΔP (pressure differences) are guessed. The Young Laplace equation is integrated from s=0 to the stage’s surface. At this point, the value of r is evaluated to see if the calculated radius matches the measured radius of the bubble. Subsequently, the volume of the bubble can be calculated. The radius of the bubble is known from microscopy images during the experiments. From this, bubble volume is also known, assuming that the contact angle of the bubble on the hydrophobic slide is consistent with measurements of the contact angle of water measured on the same surface. In order to match the correct values for bubble radius and volume, ϕ and ΔP are adjusted using the Newton-Raphson method. Once the correct values for ϕ and ΔP have been determined for a particle at various heights at the interface, force curves can be generated for that meniscus shape. These theoretical force curves are then compared to the force curves from the AFM experiments to determine which meniscus shape best matches the measured values.

The resulting understanding of the contact angles and pressure differences can be compared to the adhesions forces measured by the colloidal probe microscopy experiments. Figure 15 shows the normalized adhesion forces measured as a function of the angle of the particle as it approaches the air-water interface.

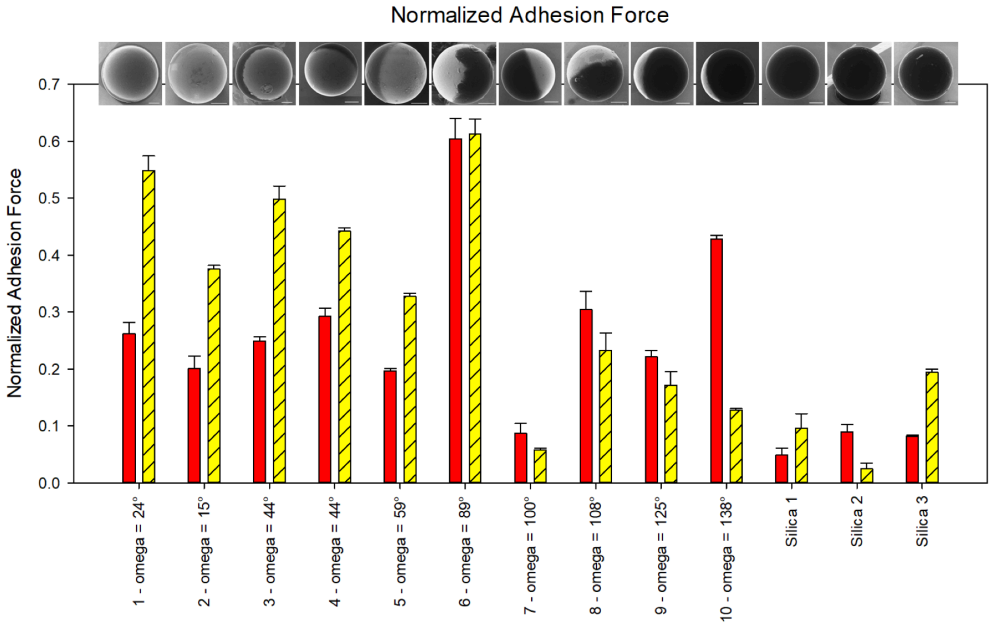


Figure 15. Normalized adhesion force for cantilevers of various orientations. Top view helium ion microscopy images of the Janus particles glued to AFM cantilevers are shown above the graph. Scale bars are 2 μm.

These findings are currently being composed in a manuscript that will be submitted in May 2019. Subsequent experiments will examine the final components of this proposed work, where nanometer sized particles will be examined in a similar fashion and probed on a bubble that has been functionalized with either surfactants, block co-polymers or high molecular weight periodically sequenced polypeptides.

4. Personnel Supported

In addition to the co-PIs (Raymond Tu - Faculty, CCNY - CUNY, Department of Chemical Engineering and Ilona Kretzschmar - Faculty, CCNY - CUNY, Department of Chemical Engineering), seven researchers were supported over the course of this grant:

Bin Ren - Post-Doc, CCNY - CUNY, Department of Chemical Engineering.

Ellen Knapp - Graduate Student, CCNY - CUNY, Department of Chemical Engineering.

Lake Kubilius - Graduate Student, CCNY - CUNY, Department of Chemical Engineering.

Joseph Badami - Graduate Student, CCNY - CUNY, Department of Chemical Engineering.

Sean O'Neill - Graduate Student, CCNY - CUNY, Department of Chemical Engineering.

Thomas Long - Graduate Student, CCNY - CUNY, Department of Chemical Engineering.

Zohreh Jalilvand - Graduate Student, CCNY - CUNY, Department of Chemical Engineering.

5. Publications

During the grant period the following manuscripts have been published acknowledging AFOSR funding:

'Reverse' Hofmeister effects on the sol-gel transition rates for an α-helical peptide-PEG bioconjugate. O'Neill, S; Weber, J; Tu, R. S. Phys Chem Chem Phys. 2018, 20, 20287-20295. DOI: 10.1039/C8CP03316A.

Evolution of mechanics in α-helical peptide conjugated linear-and star-block PEG. O'Neill, S.; Bhuiyan, Z.; Tu, R. Soft matter 2017, 13, 7521. DOI:10.1039/c7sm00968b

Nguyen, Hung and Tu, Raymond "Comprehensive Biomaterials: Chapter - Surface Engineering Using Amphiphilic Peptides" ISBN: 9780081006917, Elsevier, June 2017

Circular Dichroistic Impacts of 1-(3-Dimethylaminopropyl)-3-ethylurea: Secondary Structure Artifacts Arising from Bioconjugation Using 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide. Kubilius, M. B.; Tu, R. S. ACS Omega 2017, 2 (11), 8308–8312. DOI: 10.1021/acsomega.7b01288

Mechanical Stability of Polystyrene and Janus Particle Monolayers at the Air/Water Interface. Lenis J, Razavi S, Cao KD, Lin B, Lee KYC, Tu RS, Kretzschmar K. J. Am. Chem. Soc., 2015, 137 (49), 15370–15373. DOI: 10.1021/jacs.5b10183

Adsorption of rationally designed ''surf-tides'' to a liquid-crystal interface. Badami JV, Bernstein C, Maldarelli C and Tu, RS. Soft Matter, 2015, 11, 6604-6612. DOI: 10.1039/c5sm01431j

Collapse of Particle-Laden Interfaces under Compression: Buckling vs Particle Expulsion. Razavi S, Cao KD, Lin B, Lee KY, Tu RS, and Kretzschmar I. Langmuir, 2015, 31 (28), pp 7764–7775. DOI: 10.1021/acs.langmuir.5b01652

7. Interactions/Transitions

a. Participation/presentations at meetings, conferences, seminars:

Invited:

1. Tu RS, Nanostructured films confined at interfaces constructed from periodically sequenced polypeptides, 25 June 2018, Argonne National Labs **Advanced Photon Source- ANL**

2. Tu RS, Nanostructured films with periodically sequenced polypeptides, **ACS** – National Meeting – PMSE session, 5 April 2017, San Francisco, CA

3. Tu RS, The quantification of biomolecular adsorption dynamics at soft interfaces: Implications for drug product development of biologics, Lecture for **American Association of Pharmaceutical Scientists** (AAPS) 23 October 2017, New York, NY

4. Tu RS, Periodically sequenced peptides: a new(ish) tool for nanoscale materials 8 December 2017, Blacksburg Virginia, **Virginia Tech**

5. Tu RS, Dynamics of Protein and Peptide Interactions at the Aqueous/Liquid Crystal Interface. AIChE-SCEJ Session on Nano/Bio/Interfacial Phenomena. 2016. **AIChE**, San Francisco, CA.

6. 7. Tu RS, Periodically sequenced peptides: a new(ish) tool for nanoscale materials 11 October 2016 – UCSB Chemical Engineering Seminar, Santa Barbara, CA.

7. Tu RS, Pattern formation of surface confined periodically sequenced peptides. 2016 **ACS** – Meeting. Designing functional biomaterials: connecting experiment with theory and simulation. Fall 2016, Philadelphia, PA.

8. Tu RS, Periodically sequenced peptides: A new-ish tool for nanoscale materials synthesis. 2016. Chemical Engineering Department Seminar. **Drexel University.**

9. Tu RS, Dynamics of periodically sequenced polypeptides at the aqueous/liquid interface. 2016 **ACS** Colloid and Surface Science Symposium. Harvard University.

10. Tu RS, Periodically sequenced peptides: A new-ish tool for nanoscale materials synthesis. 2016. Chemistry Department Seminar **Stony Brook University.**

11. Tu RS, Dynamics of periodically sequenced polypeptides at the aqueous/liquid crystal interface. 2016 **ACS – Mid Atlantic Regional Meeting**. Spring 2016, Riverside, NY.

12. Tu RS, Dynamics of periodically sequenced polypeptides at the aqueous/liquid crystal interface. 2016 **ACS – Meeting**. Proteins and polymers under confinement. Spring 2016, San Diego, CA.

13. Tu RS, Measuring the dynamics of protein and polypeptide surface adsorption. Seminar. **Bristol-Myers Squibb**. 2015

14. Tu RS, High Molecular weight periodically sequenced polypeptides. 2015. **ACS** National Meeting. Denver, Colorado.

15. Tu RS, Periodically sequenced peptides: A new-ish tool for nanoscale materials synthesis. 2015. Department of Chemical Engineering Seminar **University of California – Irvine**.

16. Tu RS, Periodically sequenced peptides: A new-ish tool for nanoscale materials synthesis. 2014. Chemistry Department Seminar **Lehman College**

Conferences:

1. Effect of Amphiphilicity and Janus Cap Orientation on Janus Particles at an Air-Water Interface. EM. Knapp, RR. Dagastine, I Kretzschmar and RS Tu, October 2018. AIChE Annual Meeting. Pittsburgh, PA

2. “Examining the Stability of Amphiphilic Janus Particle-Laden Interfaces” Ellen Knapp – AIChE, October 2017, Minneapolis, MN

3. “Effect of Degree of Amphiphilicity on Janus Particle-laden Interfaces.” Ellen Knapp – ACS – Colloids and Surface Science Symposium, July 2017, New York, NY

4. “Evolution of Mechanics in α-Helix Peptide Bioconjugated Linear- and Star-Block Peg” Sean O’Neill – AIChE, October 2017, Minneapolis, MN

5. “Mechanical Evolution in alpha helical peptide tethered linear- and star-block PEG.” Sean O’Neill ACS – Colloids and Surface Science Sypmposium, July 2017, New York, NY

6. “Enhanced Liquid-phase synthesis and interfacial self-assembly of amphipathic polypeptides”, MB. Kubilius, RS Tu, Conference Presentation AIChE 2015, Nanoscience and Engineering Forum.

7. “Dynamics of Periodically Sequenced Polypeptides at the Aqueous/Liquid Crystal Interface” JV. Badami, RS Tu, ACS 2015 Colloid & Surface Science Symposium. Proteins and Polymers under Confinement or at Interfaces

8. “Surfactant-mediated polydispersities in the synthesis of self-assembling, amphipathic polypeptides” MB. Kubilius, RS Tu, ACS 2015 Colloid & Surface Science Symposium. Proteins and Polymers under Confinement or at Interfaces

9. "Surfactant-Mediated Polydispersities in the Synthesis of Self-Assembling, Amphipathic Polypeptides" MB. Kubilius, RS Tu, Conference Proceedings AIChE 2014, Food, Pharmaceutical & Bioengineering Division.

10. "Integration of Periodically Sequenced "Surftides" into Acoustically Active, Lipid--Shelled Microbubbles" JV Badami, RS Tu, AIChE presentation 2014, Biomolecules at Interfaces.

11. "Dynamics of Protein and Peptide Interactions at the Aqueous/Liquid Crystal Interface"JV Badami, RS Tu, AIChE presentation 2014, Interfacial Dynamics.

12. “Surfactant-Mediated polydispersity in the synthesis of self-assembly, amphipathic polypeptides" MB Kubilius, RS Tu, AIChE presentation 2014, Biomolecular Engineering.

13. "Incorporation of Periodically Sequenced 'Surftides' into Lipid-Based Microbubbles." JV. Badami, RS. Tu; ACS 2014 Colloid & Surface Science Symposium. Biocolloids: Contrast Agents.

14. "Surfactant micelle-mediated polydispersity in the synthesis of amphipathic, self-assembling polypeptides.” MB. Kubilius, RS. Tu; ACS 2014 Colloid & Surface Science Symposium. Molecular Self-Assembly.

b. Consultative and advisory functions to other laboratories and agencies, especially Air Force and other DoD laboratories: none.

c. Technology Assists, Transitions, and Transfers:

Rein Ulijn (ASRC - CUNY) – bubble stability

Joel Koplik (CCNY) – particle field MD Simulations

Ka Yee Lee (U Chicago) – wrinkling @nm length scale

BinHua Lin (Argonne Nat’l Labs) – Interfacial characterization of polypeptide assembly

James Gilchrist (Lehigh) – continuous particle fabrication

Aaron Lau (Strathclyde) – adhesion gallol

Herb Waite (UCSB) – adhesion catechol

Ray Dagastine (U Melbourne) – Colloidal probe microscopy

Nathan Gianneschi (Northwestern) – Supraball assembly dynamics

8. New discoveries, inventions, or patent disclosures: none at this time.

9. Honors/Awards:

FaSTRAC Research Award, ChemMatCARS – Argonne National Labs - 2018

Provost's Prize for Pedagogical and Curricular Innovation - 2016

Changes in research objectives, if any: None

Change in AFOSR program manager, if any: Aura Gimm, PhD
AFOSR/RTB-2
afosr.nature@us.af.mil

Extensions granted or milestones slipped, if any:

An 8-month no cost extension was requested in July 2017 resulting in an ending date of April 14th 2018