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Dense Two-Dimensional Systems
of Colloidal Tri-Stars and Rhombs

A dissertation submitted in partial satisfaction of the requirements
for the degree Doctor of Philosophy in Chemistry

by

Kenny Mayoral

2013
ABSTRACT OF THE DISSERTATION

Dense Two-Dimensional Systems
of Colloidal Tri-Stars and Rhombs

by

Kenny Mayoral
Doctor of Philosophy in Chemistry
University of California, Los Angeles 2013
Professor Thomas G. Mason, Chair

In this thesis, we describe the observation of self-organized two-dimensional phases composed of simple components having non-trivial shapes which interact only by hard repulsions and are entropically excited. Through experiments and computational modeling, we investigate and explain how certain particle shapes self-organize into interesting two-dimensional phases. In order to obtain large quantities of precisely replicated particles having custom shapes for the experiments, we make a colloidal system composed of lithographically fabricated particles. As a preface to our work for dense systems, we design a novel Fourier tracking method to measure the rotational diffusion of Brownian colloidal polygons confined to two dimensions (2D) in a dilute dispersion.
Systems of convex platelet Brownian particles, capable of interdigitation, are osmotically compressed to high densities in a tilted barometric column. An equilibrated alternating stripe crystal (ASX) phase is observed at high particle density. Digital-wide-field-of-view microscopic imaging of the complete column enables us to obtain the 2D osmotic equation of state for the system and to determine the structural characteristics of the ASX phase which dominates, over a hexagonal crystal (HX) at high densities.

We introduce and apply a translational-rotational cage model (TRCM) in order to explain the experimentally observed preference for ASX over HX. The TRCM, based on a collision detection routine, determines the total number of states accessible to a mobile tri-star particle within an ASX and a HX cage. We find that the additional translational states permitted by the ASX phase exceeds the number of additional rotational states permitted by the HX phase, thus setting ASX as the entropically preferred phase. The TRCM is also used to explain the chiral symmetry breaking observed in a system of rhombs which form a rhombic lattice under high osmotic compression. In both cases, the TRCM is able to explain the phase behavior observed where models based on rotations or translations alone could not. We anticipate that future, more sophisticated programs could be designed with the TRCM which, through optimization and feed-back, would be able to make more predictive assessments on similar systems.
The dissertation of Kenny Mayoral is approved.

Peter M. Felker

Robijn F. Bruinsma

Thomas G. Mason, Committee Chair

University of California, Los Angeles

2013
To Mom, Dad, Keith, and Jenny
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PREFACE

The universe is an unimaginably complex, yet beautiful system, limitless in its potential to inspire wonder and awe in those who take a moment to contemplate it. As a child, trying to understand the little I knew of nature always brought me joy; I always gained a bit of satisfaction whenever I was able to rationalize why something is the way it is. Currently, it is unimaginable to think that we could ever explain the universe completely, yet I am comforted by the fact that we are able to simplify any small portion of the universe into a system which we can understand and explain. This thesis is about work that attempts to accomplish this, on a system which is fairly simple compared to those found in nature, yet still capable of teaching us something fundamental about our universe.

Most of matter we interact with in daily life consist of a collection of simple component particles (atoms and molecules) in a solid, liquid, or gaseous phase. How and why component particles arrange into specific phases under certain conditions are some of the most fundamental questions one could ask; the answers to which could eventually lead to innovations in how humans design and control synthetically made systems. Just as with any question, one could explore it by any number of different methods (constraining some variables, varying others), and depending on those choices, the results would tell us something particular for the given circumstances. In this thesis, we will describe the observation of self-organized two-dimensional phases composed of simple components with non-standard shapes which interact only through hard-particle repulsions. Specifically, how the component particle shape affects the self-organization of two-dimensional phases is a main focus of the work presented here.
ACKNOWLEDGEMENTS

I have been fortunate to have the support of family and friends throughout my life and I am grateful for the opportunities afforded to me. Countless others in my past are also responsible for where I am now: from my teachers who encouraged me in school, to classmates and colleagues who shared in my education, to acquaintances who's life path casually intersected mine for enough time to leave even the slightest impact. I say 'thank you' to you all.

As a graduate student, I have found no shortage of support and guidance from several people at UCLA but none more so than my advisor Tom Mason. I have become the researcher and scientist I am today because of Tom's continuous patience and encouragement. His scientific creativity and enthusiasm are infectious; I always left a meeting inspired to figure out a question or try out an idea that we had discussed. My scientific knowledge, writing, and teaching have all benefited under Tom's guidance and I appreciate how much time and effort he was willing to invest in me. His attention to detail and insightful comments have propelled my growth as a professional and I am thankful to have Tom as my advisor and as my friend.

Other faculty and staff members have been important for my scientific development at UCLA including: Benjamin Schwartz, Alex Levine, Louis Bouchard, Peter Felker, Robijn Bruinsma, Xiangfeng Duan, Arlene Russel, Johnny Pang, and Ignacio Martini. Thank you all.

Much of my time in graduate school has been spent alongside the Mason group members and I am glad to have spent it with such a diverse group of fun and intelligent people. Some gone, others new, I appreciate my interactions with all of you and wish you
the best in the future! Most of my time overlapped with Mike, Xiaoming and Terry and I'm thankful for the friendships we have developed, you guys are awesome! To all the new guys — Dimitri, Po-Yuan, Max, Sheba, and Ha-Seong — it was great to meet a young, eager group of scientists ready to explore the world of soft matter, go get 'em! The Mason group postdocs have always been exceptional and I'm glad I had the chance to learn from and hang out with Jung-Ren, Fred, Kun, KI, Clayton, Manas, and Laura. Kun and Clayton were especially helpful to me early in my graduate school career, as was Laura in the latter part of it, thanks!

During the trials and tribulations that defined the first year physical chemistry courses, I was fortunate to share the experience with a wonderful and smart group of peers. I appreciate the friendships I formed with Argyris, Soo Hong, Odo, Mauricio, Peter, Ellen, and Lu-Feng, among others. We had some really fun times! Additionally, I would be reduced to a crater if I didn't mention the greatest outcome of that first year, finding my love and girlfriend, Jenny. Thank you, Jenny, for all your support, I love you!

Additionally, my friends from outside of graduate school also encouraged me and helped make this period of my life more enjoyable. To my UCI Chemistry pals, the YMC who adopted me for a while, all my basketball friends, and friends from back home, thanks for everything! Andrew and Whitney, you guys have been always been great and thanks for all the fun memories!

Of course, I would be no where without my family and I owe everything to my mom, dad, and brother. Thanks mom and dad for all the love and support, always! Thanks brother for being my brother! Thanks to all my aunts, uncles, cousins, and
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1 Introduction and General Principles

1.1 Colloids and suspensions

Systems of dispersed colloids are ubiquitous in nature and influence many aspects of our daily lives. Different classifications of colloidal systems include: *an aerosol* - a dispersion of liquid droplets in a gas (e.g. early morning fog); *an emulsion* - a dispersion of liquid droplets in another immiscible liquid phase (e.g. milk and mayonnaise); *a colloidal suspension* - a dispersion of fine solid particles in a liquid medium (e.g. pigment-based ink)\(^1\). Herein, we are primarily concerned with colloidal dispersions.

A colloidal dispersion consists of *dispersed phase* composed of finely-sized constituents distributed in a *continuous phase*. Generally, for a dispersed constituent to be defined as a colloid, a maximal spatial dimension of a particle should lie in the range from about 1 nm to several microns, so their Brownian motion is evident\(^1\). Thus, thermally driven diffusion (i.e. entropy) is important in colloidal dispersions.

There usually exists a separation of length scales between the colloids and the continuous phase solvent molecules, which are around \(10^{-10}\) m in size. While thermal fluctuations keep both the solvent molecules and colloids in a constant state of random motion (diffusion), the larger-sized colloids are readily seen using conventional optical microscopy. Solvent molecules continuously collide with the surface of a colloid as they thermally diffuse, imparting a fluctuating instantaneous net force on the colloid\(^2-5\). These microscopic collisions result in the thermal, diffusive motion of the colloids, termed *Brownian Motion* after Robert Brown, who observed this motion in a sample of pollen grains dispersed in water\(^3\) in 1827. Curiously, Brown was not the first to report this
phenomenon, as Jan Ingenhauz studied the random motion of charcoal power on an ethanol film over 40 years earlier\textsuperscript{6}, in 1785. Nevertheless, Brownian motion causes colloids to translate and rotate through a fluid, tracing out random trajectories, in a manner similar to fluid atomic systems. If dispersed in a viscoelastic complex fluid, a colloidal particle's geometry, as well as the viscoelastic properties of the continuous phase, affect the colloid's motion in a complex fluid at a given temperature; this is the basis for passive microrheology\textsuperscript{7,8}.

In addition to the Brownian motion exhibited by colloids, it is sometimes possible to independently tune the particle shapes and their interaction potentials\textsuperscript{9}. This remains challenging in molecular systems. The thermal diffusion and tunable potential of colloids make them good analogues or mimics for molecular systems and ideal for studying the self-assembly phase behavior seen in molecular systems\textsuperscript{10}. In Chapter 2, we measure the rotational diffusion of polygons. In Chapters 3, 4, and 5, we focus on the phase behavior of two-dimensional colloidal systems as they are concentrated to high densities.

1.1.1 Interactions among colloidal particles

The behavior of colloidal particles dispersed in a fluid is governed by the colloids' interaction potentials and entropy, provided that the influence of hydrodynamic forces, gravitational forces, and external fields are negligible by comparison\textsuperscript{8}. In the simplest systems, in which both attractions and repulsions are present, short-range attractive interactions between colloids arise from van der Waals forces and repulsive interactions result from electrostatic forces. Van der Waals forces are generally short range, although
very strong compared to thermal energy. In the absence of any stabilizers, particles will often irreversibly aggregate due to this attraction. In order to prevent unwanted colloidal aggregation, particles can be stabilized in suspension through the adsorption of ionic surfactants which charge the particles' surfaces or by chemically bonding molecules onto the particles' surfaces.

Surface charges, arising from ionic surfactants adsorbed on a colloid, attract counterions (ions having opposite charge) and repel co-ions (ions having similar charge) from the solution. The collection of ions surrounding a particle's surface is called the electrostatic double layer and results in electrostatic screening of the particle surface charge. The range of a particle's electrostatic repulsion is determined by the Debye screening length, $l_D$, which is inversely proportional to the square root of the ion concentration in solution. So, as the ion concentration increases, $l_D$ decreases. If enough ions are added to solution, the repulsions will become screened enough so that only the van der Waals attractions dominate, resulting in particle aggregation. Alternatively, by adjusting the surface charge density and the solution ion concentration, one can make $l_D$ small, a few nanometers, which results in effectively hard interactions. The DLVO model potential describes the total colloidal interaction energy, which combines the van der Waals attractions with electrostatic double layer repulsions$^{1,11,13}$.

Because particles which interact through a hard-core potential do not effectively repel each other until they come in contact, the core-shape of the colloidal particles becomes an import factor for determining the types of phases observed when these particles are concentrated to high densities. The experimental studies in 2D herein involve particles that have nearly hard interactions but interesting core shapes.
1.1.2 Depletion attractions between two spheres

Depletion attractions refer to the interactions between larger-size microscale colloids in the presence of a non-zero concentration of smaller-size nanoscale depletion agents\(^{14,15}\) (e.g. polystyrene spheres, nanoemulsions, non-adsorbing polymers). Figure 1.1A shows a schematic diagram describing the how depletion agents contribute to the attractive interaction between two larger spherical colloids. Around each large colloidal particle, there is a volume that is entropically depleted of smaller particles (i.e. a volume that is not thermodynamically accessible) called the overlapping excluded volume. When two larger colloidal particles diffuse close enough to each other that their depletion zones overlap, the resulting osmotic pressure imbalance acts to force the larger colloidal particles together. Consequently, the nanoscale depletion agents have more accessible phase space to explore and the overall entropy of the system is increased\(^{14,15}\).

The depletion attraction potential \(U_d(r)\) depends on the interparticle center-to-center distance \(r\) between the microscale colloids and its magnitude can be obtained by multiplying the overlapping excluded volume by the osmotic pressure of the nanoscale depletion agents\(^{16}\). In Figure 1.1B we show \(U_d(r)\) (in units of \(\beta \phi_s k_B T\)) for two large spheres of radius \(a\) surrounded by small spheres of radius \(r_d\), where \(\beta\) is sphere diameter ratio \((a/r_d\) in Fig1.1B \(\beta = 10\)), \(\phi_{DA}\) is the nanoscale depletion agent volume fraction, \(k_B\) is Boltzmann's constant, and \(T\) is temperature. For \(r > 2a + 2r_d\), there is no overlapping excluded volume and therefore no depletion attraction \((U_d(r) = 0)\).
Figure 1.1 Schematic depiction of a depletion-induced attraction between colloidal spheres, adapted from ref. 17. (A) Two spherical colloids (gray circles) having radius, $a$, experience a net attraction when dispersed in a solution containing a substantial concentration of a depletion agent (orange circles) having radius $r_d$. The center positions of each depletion agent are depleted from the dashed volume surrounding the colloids. The overlapping excluded volume (striped area) results from the depletion zone of one colloid intersecting the depletion zone of another. (B) Plot of the dimensionless depletion potential between two macroscale spheres, including the hard-particle repulsion at $r = 2a$. Here, the ratio of diameter of large sphere to small sphere is $\beta = a/r_d = 10$. 
For particle interseparations between $2a < r < 2a + 2r_d$, the depletion zones surrounding each macroscale particle begin to overlap and they attract. When the particle distance $r = 2a$, the particles are in contact (as drawn in Fig. 1.1A) and the maximum amount of overlapping excluded volume has been achieved, but the particles cannot attract further because of the hard-core repulsions\textsuperscript{17}.

### 1.1.3 Roughness-controlled depletion attractions

A similar principle can be applied to confine a platelet colloidal particle near the flat wall of a microcapillary cell, effectively restricting it to two dimensions. Figure 1.2A shows how an idealized smooth platelet particle would be attracted to the microcapillary wall in the presence of a depletion agent. The strength of the depletion attraction can be tuned by varying the depletion agent volume fraction $\phi_{DA}$ (the ratio of the total depletion agent volume to the total system volume), while the range of interaction can be tuned by varying the depletion agent particle radius $r_d$. Additionally, if the colloidal particle can be made so that one of its surfaces is rougher than the others, one can utilize roughness-controlled depletion attractions\textsuperscript{18,19} to confine the particle to two-dimensions without strongly affecting lateral in-plane particle-particle interactions, as seen in Figure 1.2B. The magnified portion in Figure 1.2B exaggerates edge roughness and shows the depletion zone as a red or blue region for each particle.
Figure 1.2 Schematic depictions of depletion-induced attractions between platelet particles and a wall. (A) Similar to the situation between spheres, depletion agents (orange circles) can create a depletion attraction between an idealized smooth platelet particle (green rectangle - seen from side-view) and the slide wall (gray rectangle) of a microcapillary cell, effectively restricting the platelet particle to two dimensions. (B) Increased roughness on particle edges (compared to particle faces) inhibits edge-to-edge depletion attractions. A magnified view of the cartoon depiction shows the volume excluded to depletion agents by each platelet particle (red and blue regions). While there is a significant amount of excluded volume overlap (striped regions) between particles' faces and the slide wall, there is essentially negligible overlap between particles due to higher edge roughness, resulting in an anisotropic roughness-controlled depletion attraction. Thus, interactions between the particles in-plane remain nearly hard. Depletion agents are not to scale and asperities are exaggerated for clarity.
Here, asperities on the edges of the platelets have average heights greater than $2r_d$, so they keep the platelets from getting close enough to create any significant overlapping excluded volume. This inhibits edge-edge depletion attractions between neighboring platelets. On the contrary, the faces of the platelets are smoother as fabricated, and, therefore, the depletion attraction confines their diffusion to the plane just above the microcapillary's wall.

1.1.4 Creating a 2D system

The fundamental phase behavior we wish to study is more easily visualized by optical microscopy in two dimensions, because experimental methods and analysis are substantially simplified\textsuperscript{2, 20-23}. For the single-particle and multi-particle experiments described in Chapters 2 and 3, respectively, roughness-controlled depletion attractions\textsuperscript{18,19} will be used to restrict the diffusion and collisions of the platelet particles to two dimensions. The depletion agent concentration is optimized for different particle shapes individually by determining the minimum amount of depletion agent which confines all the platelet particles having a certain shape to remain near the microcapillary wall (i.e. so platelets do not exhibit Brownian tipping out of the plane). A solution having an optimized concentration of depletion agent is mixed with a dispersion of platelet particles and SDS. The volume fraction of platelet particles must also be optimized so as to minimize particle-particle dimer aggregation before particles settle along slide wall. Typically, this volume fraction is in the range: $\phi \sim 10^{-5} - 10^{-3}$. A very small amount of
dimer aggregation is seen in the 2D systems before particles settle to the lower glass wall, but their existence in these systems is not ultimately problematic. For single-particle measurements (Chapter 2), avoiding dimers is as simple as viewing a different particle. For multi-particle measurements (Chapter 3), dimers ultimately slide to the bottom of the barometric column, away from the equilibrated single-layer phases we study, so they also do not pose a problem.

1.2 Photolithography

Photolithography is one of the most important processing techniques developed in modern times, enabling the mass scale manufacture of complex integrated circuits and microchips\textsuperscript{24}. Current photolithography shares some similarities with the traditional lithographic stone-based printing developed in the late 18th century.\textsuperscript{25} These similarities include the ability to reproduce fine patterns using detailed templates. Modern photolithography has advanced dramatically since then, by offering smaller feature size, higher throughput, and sensitive resist materials. Photolithography is a fabrication process that uses light to pattern a light-sensitive chemical (photoresist) with multiple copies of a desired image. In semiconductor applications, this 'image' might be part of a transistor component or wire on an integrated circuit board or microchip\textsuperscript{26}. Exact control over image size and shape in a parallel writing process makes lithography an ideal process for microfabrication, and even nanofabrication. The same characteristics make optical projection lithography an excellent tool for mass-producing custom-shaped colloids. Adopting this process for the fabrication of custom-shaped colloids requires
some modification to the general processing steps used for traditional semiconductor microfabrication. A simple process yields stable aqueous dispersions of polymeric microplatelets\textsuperscript{27}.

We fabricate custom-shaped colloidal particles using an Ultratech XLS 2145 i-line 5:1 reduction stepper. The XLS stepper utilizes UV projection lithography, as opposed to contact or proximity printing common among mask aligners. Projection exposure provides benefits such as smaller feature sizes, greater particle fidelity, and longer mask life.

Initially, a silicon substrate is prepared prior to exposure by spin coating a sacrificial layer and a photoresist layer over the substrate, as seen in Figure 1.3A. The choice for photoresist is important because it will determine the material composition of the lithographic colloids; herein, we use SU-8 2000 series epoxy-based photoresists (MicroChem) which have excellent thermal and chemical resistance, are optically transparent, and capable of producing high aspect ratio features. Because the photoresist layer is spun on and later exposed, this process for fabricating colloids is best suited for flat, platelet-like particles, although features in the $z$-direction can be obtained by repeating the lithographic process for multiple layers\textsuperscript{27}. When spin coating an SU-8 2000 series photoresist onto a silicon wafer, the thickness of the resist layer (which will later define the particle thickness) can be adjusted to lie in the range from about 0.05 to about 5 $\mu$m.

A pattern containing multiple repeating copies of a desired particle shape (e.g. tri-star, triangle, rhomb) is designed using L-Edit and sent out to a commercial mask shop. This shop uses electron beam lithography to print a chrome-on-quartz mask. A single
Figure 1.3 Lithographic fabrication of custom-shaped colloids using an Ultratech XLS 2145 i-line reduction stepper (modified from ref. 27). (A) A silicon wafer substrate (gray disk) is prepared by spin coating on a thin sacrificial layer (orange disk). A subsequent SU-8 photoresist layer (dark green disk) is spun over the sacrificial layer and the wafer is ready for UV exposure. (B) The stepper reduces (5:1) and transfers the image containing a high density, repeating pattern of particle shapes (mask) onto the resist layer using UV light, crosslinking the resist in the exposed locations. The exposure regions are translated over the resist (stepped) to completely pattern the substrate. (C) Development dissolves away any uncrosslinked photoresist, leaving fully formed particles connected to the sacrificial layer. (D) The sacrificial layer is dissolved away during the lift-off step and particles are dispersed and stabilized in an aqueous surfactant solution.
mask can have several different designs, which can be appropriately bladed off (i.e. UV light is blocked by metal blades) controlled by the stepper's software to yield only particles of a certain shape (~10^8 particles per wafer).

Once the substrate has been prepared, it is loaded into the stepper, which automates the exposure process, controlling every aspect of the exposure for maximum particle fidelity (Figure 1.3B). Ultraviolet (UV) i-line (365 nm) light is collimated before passing through the chrome-quartz mask, that has been designed with multiple copies of the desired particle's shape. The image is reduced by 5X as it passes through the stepper's reduction lens, and the patterned light exposes the photoresist layer. A photo-initiated, acid-catalyzed epoxy ring-opening reaction leads to the eventual crosslinking of the exposed regions. The stepper translates the wafer, exposing a set of regions on the wafer as it steps, completely patterning the wafer with UV light. The wafer is then removed from the stepper, and a post-exposure bake (PEB) ensures that the thermal crosslinking reaction is complete in the exposed regions.

A development step washes away uncrosslinked SU-8 in an appropriate solvent which is insoluble to crosslinked SU-8 (Figure 1.3C). After exposure and development, the photoresist has been patterned into crosslinked regions (i.e. many discrete particles). Finally, the sacrificial layer is dissolved away using a basic aqueous solution (1.8% tetramethylammonium hydroxide aqueous solution) and the particles are dispersed in the surfactant solution (Figure 1.3D). An anionic surfactant, sodium dodecyl sulfate (SDS) is added to the aqueous solution during lift-off to inhibit particle aggregation. Subsequent centrifugations and washing steps are required to purify (i.e. remove) release material out
of the colloidal suspension. After these steps, the aqueous dispersion typically has a
dilute volume fraction of particles $\phi \approx 10^{-5}$ and SDS concentration $1 \text{ mM} \leq C_{\text{SDS}} \leq 10$
mM. Once dispersed, the particles can be used at dilute concentrations for single-particle
experiments or concentrated through mild centrifugation for dense 2D experiments.

While the stepper enables the mass production of particles at submicron
resolution, its limited feature size $^{20}$ ($\approx 300$ nm), resulting primarily from the diffraction
limit of the UV light, precludes the fabrication of sharp corners below this feature size.
This causes the corners of convex and concave particles to deviate from their ideal shapes
and become rounded. Corner-rounding can potentially affect the phase behavior of dense
systems of Brownian particles, potentially changing the equilibrated phases observed for
these particles compared to their ideally-shaped analogues. In principle, using the most
sophisticated stepper that has a much smaller minimum feature size (e.g. 20 nm) could
reduce the radius of curvature of rounded corners by as much as an order of magnitude.
However, such steppers are typically unavailable in current research settings.

### 1.3 Summary

We use and refine methods of optical lithography to create colloids that serve as
molecular analogues. This enables us to study how particle shape affects colloidal
rotational diffusion and self-assembly. As a prelude to self-assembly studies involving
concentrated multi-particle systems in 2D, we first focus on a dilute system of individual
colloids. In Chapter 2, we will examine how the rotational diffusion of polygonal
particles depends on particle shape. We also describe an image analysis technique for
rotational tracking of a single particle. In Chapter 3, the observation and characterization of an alternating-stripe crystal (ASX) phase composed of concave, tri-star particles, which have been compressed to high densities, is described. Additionally, we develop and describe a method for measuring the osmotic equation of state for two-dimensional systems. Explaining the ASX phase observed in Chapter 3 serves as a motivation for the computational modeling that follows. In Chapter 4, a translational-rotational cage model of tri-stars is used to successfully explain the entropic preference of the ASX phase over a possible hexagonal crystal (HX) phase. Finally, in Chapter 5, we extend the translational-rotational cage model to a system of rhomb particles and show that it can explain the chiral symmetry breaking observed in prior experiments.
1.4 References


2 Rotational Fourier Tracking of Diffusing Polygons

2.1 Abstract

We use optical microscopy to measure the rotational Brownian motion of polygonal platelets that are dispersed in a liquid and confined by depletion attractions near a wall. The depletion attraction inhibits out-of-plane translational and rotational Brownian fluctuations, thereby facilitating optical microscopic imaging and video analysis. By taking fast Fourier transforms (FFTs) of the images and analyzing the angular position of rays in the FFTs, we determine an isolated particle's rotational trajectory, independent of its position. The measured in-plane rotational diffusion coefficients are significantly smaller than estimates for the bulk; this difference is likely due to the close proximity of the particles to the wall, which results from the depletion attraction.
2.2 Introduction

The common availability of optical microscopes and digital video hardware in many research labs has led to the widespread use of video particle tracking microscopy (VPTM). Movies that capture many kinds of interesting and complex transport phenomena, including instabilities of driven fluids in microfluidic devices\textsuperscript{1,2}, Brownian diffusion of microscopic probe particles in viscoelastic media\textsuperscript{3}, and time-lapse dynamics of the division of biological cells\textsuperscript{4}, have been recorded. Although these movies can be readily made, performing quantitative image analysis to measure relevant transport properties can frequently be challenging. Thus, opportunities remain for improving the experimental measurement of microscopic phenomena captured by VPTM.

In particular, the rotational mobilities of non-spherical probe particles, even in a simple viscous liquid, have not been accurately measured or predicted for many different kinds of shapes having reduced symmetry. Although the translational and rotational Stokes drag, and therefore diffusion coefficients, have been calculated for circular platelets (\textit{i.e.} disks) in a bulk fluid at low Reynolds number\textsuperscript{5-7}, calculations of the Stokes drag for more complex shapes, such as polygonal platelets, have not been made. Since a variety of lithographic methods now enable production of microscale and nanoscale complex platelets\textsuperscript{8-10}, even with such intricate geometries as the alphabet\textsuperscript{9}, it is now possible to experimentally observe and record the diffusion coefficients of platelets having a wide variety of shapes in two dimensions using optical microscopy.

While significant attention has been given to determining the center positions of spherical colloidal probe particles through VPTM\textsuperscript{11-13} and more complicated laser deflection\textsuperscript{14} and interferometry methods\textsuperscript{15}, much less attention has been given to
rotational tracking. Light streak tracking, which involves detecting focused laser light scattered from a single microparticle, has achieved good accuracy in determining mean square angular displacements (MSADs) of disks\textsuperscript{16}. This method has been used to perform passive rotational microrheology of polymeric solutions\textsuperscript{17}. Although a video particle tracking routine that determines the center positions and vertices of polygons at high densities has been developed and used\textsuperscript{18,19}, this simple single vertex identification approach has somewhat limited precision. Thus, it would be desirable to develop a robust image analysis technique that more fully utilizes the angular information in digital images and could, therefore, more accurately determine the angular trajectories of a variety of custom-shaped probe particles.

Here, we introduce a versatile Fourier method for analyzing video movies that can precisely determine the angular orientation of an isolated colloidal particle that has a non-trivial cross-sectional projection. To demonstrate this method, we use optical video microscopy to measure the rotational diffusion of triangular and square shaped lithographic polygonal platelets confined in two dimensions near a solid wall by a depletion attraction. Using the Fourier approach, we can obtain angular trajectories, MSADs, and the rotational diffusion coefficients without having to locate the center positions of the particles. Because this Fourier image analysis method does not inherently depend on the type of microscopy used, in principle, it could be applied to images or movies made using other forms of microscopy, such as electron microscopy, x-ray microscopy, or even time-resolved surface probe microscopy (\textit{e.g.} atomic force microscopy).
2.3 Methods

2.3.1 Experiments

Plate-like microscale lithographic particles (e.g. equilateral triangular and square prisms) composed of an epoxy photo-resist polymer SU-8 are prepared by exposing and cross-linking select areas of SU-8 in a controlled and precise way using an Ultratech i-line XLS stepper, as described in Chapter 1. This top-down production process produces about $10^8$ microplatelets per five-inch diameter wafer. These cross-linked polymeric particles are dispersed in an aqueous surfactant solution (1 mM sodium dodecyl sulfate (SDS)) that stabilizes the released particles against aggregation.

In order to prevent thermally induced tipping and elevation far above the flat surface of the confining cuvette's glass wall, we restrict the diffusion of the platelets to two dimensions utilizing roughness-controlled depletion attractions (RCDA), generated by adding depletion agents of anionically stabilized polystyrene spheres that keep the faces of particles near the surface of a glass coverslip through attractive interactions (Chapter 1). The concentration and size of depletion agent, as well as the area contributing to the excluded volume of microscale particle, all strongly affect the strength of the depletion attraction in the system and are therefore optimized depending on the surface areas of the flat faces of the platelets. In particular, a highly dilute aqueous dispersion of regular triangular platelets (volume fraction $\phi \approx 10^{-5}$), having an edge length of 2.7 $\mu$m and a thickness of 1.0 $\mu$m, are added to an aqueous dispersion of polystyrene spheres (20 nm diameter), yielding a final volume fraction $\phi_{DA} = 0.5\%$ for the depletion agent. As fabricated, square platelets have an edge length of 4.5 $\mu$m and a thickness of 1.0 $\mu$m, so a somewhat smaller volume fraction of the same depletion agent $\phi_{DA} = 0.1\%$
can be used for squares in order to achieve a similar degree of two-dimensional confinement. Separately, these colloidal mixtures containing either triangles or squares are loaded into a rectangular glass microcuvette, sealed to inhibit convection, and imaged using optical microscopy (Nikon TE2000 inverted microscope, brightfield, 100x objective, 1.40 numerical aperture, PointGrey Flea2 CCD 1024 x 768 pixels, 30 frames per second). The particles are imaged after they have sedimented and depletion attractions restrict their diffusion in-plane just above the lower wall of the cuvette. To simplify the experimental imaging and inhibit undesired particle aggregation, we restrict our attention to a highly dilute dispersion of platelets so that only a single particle is observed in a field of view. All measurements have been made at temperature \( T = 297 \pm 1 \) K.

### 2.3.2 Image Analysis

An example of an acquired image of the triangular platelet is shown in Fig. 2.1A. Edge detection is used to create a sharp high-contrast 8-bit grayscale image of the particle, as shown in Fig. 2.1B\(^\text{12}\). The high-contrast image is fast Fourier transformed (FFT). Because the particle's edges are straight and well defined over many pixels, the resulting transform (log-scaled, amplitude-squared) exhibits a set of rays; each ray is perpendicular to a corresponding edge of the particle, as shown in Fig. 2.1C\(^\text{21}\). For each frame of the resulting pre-processed FFT video, an automated program (LabView) detects the locations of each intensity peak as a function of azimuthal angle \( \theta \) in the FFT at a particular radius around the center of the image. To obtain sharp intensity peaks
exhibiting a high signal-to-noise ratio, we set the radius in reciprocal space to be 70 pixels for triangles and 50 pixels for squares; the ring's effective width is 1 pixel [e.g. see the white ring in Fig. 2.1C]. A parabolic fitting algorithm is used to detect the centers of the peaks, six for triangles, shown as the vertical dashed lines in Fig. 2.1D. By comparing this set of peak locations with the ideal case of peaks separated by $\pi/3$ for equilateral triangles, we obtain an average orientation angle $\theta$, modulo $2\pi$. By tracking one certain peak from frame-to-frame, the routine corrects for abrupt jumps in any peak locations due to the $2\pi$ periodicity, thus providing the absolute change in angle $\theta$ over long periods of time $t$. For square particles, four rather than six rays are seen in the FFTs, corresponding to the reduced number of distinct edges, as shown in Fig. 2.2. The rotational tracking process produces absolute angular positions with a resolution of 20 milliradians (mrad) based upon the averaging of the detected peaks in each frame. In our digital videos, triangles have an approximate edge length of 29 pixels and the squares have an edge length of 37 pixels, so an edge length of about 30 pixels can provide about 20 mrad resolution. Whether for triangles or squares, after obtaining the angular trajectory, the MSAD, $<\Delta \theta^2(t)>$, is calculated and the particle's in-plane rotational diffusion coefficient $D_r$ is determined from the slope of a linear least squares fit of $<\Delta \theta^2(t)> = 2D_r t$ in the short-time limit.
Figure 2.1 Fourier transform analysis yields the angular orientation of an isolated triangular polygon without identification of its position. The real space image obtained from optical video microscopy (A) is transformed to a grayscale image using edge detection (B) and a fast Fourier transform (FFT) of the grayscale image generates (C) a spoke-like FFT having six rays with angles that reflect the triangle's orientation. The intensity of these rays is measured azimuthally along a set radius (white ring in (C) has a radius of 70 pixels), yielding $I(q)$ having six peaks, as shown in (D). Peak detection identifies the maxima in $I(q)$ (vertical dashed lines), and these angles are used to more accurately determine the absolute orientation of the triangle associated with one vertex. The real-space scale bar in (A) is 3 $\mu$m; the reciprocal space scale bar in (C) is 4 $\mu$m$^{-1}$. 
Figure 2.2 Fourier transform analysis of a square particle, paralleling the results shown in Fig. 2.1: (A) optical micrograph, (B) grayscale image after edge detection, (C) FFT showing four rays corresponding to the two sets of parallel edges of the square (white ring has a radius of 50 pixels), and (D) intensity as a function of angle $I(\theta)$ showing with peak-detected angles (vertical dashed lines). The real-space scale bar in (A) is $4 \mu m$; the reciprocal space scale bar in (C) is $1 \mu m^{-1}$. 
2.4 Results and Discussion

A representative angular trajectory $\theta(t)$ of a triangular particle is shown in Fig. 2.3A. This trajectory has the expected characteristics of a one-dimensional random walk. Figure 2.3B shows a sample probability distribution for an angular step between consecutive frames, $p(\Delta \theta)$; the distribution is Gaussian (dashed line is a fit), as expected for rotation driven by Brownian motion. Since the angular resolution is high enough, the calculated MSADs for both triangles and squares are linear down to the earliest times measured, as shown in Fig. 2.4. The angular Brownian motion of the platelet particles near the solid wall yields rotational diffusion coefficients of $D_r = 3.3 \pm 0.2 \times 10^{-2}$ rad$^2$/s for triangles and $D_r = 8.3 \pm 0.5 \times 10^{-3}$ rad$^2$/s for squares. The smaller diffusion coefficient of squares is consistent with its larger edge length (i.e. greater maximal spatial dimension) compared to triangles.

It would be interesting for us to compare our results with a relevant theory that could account for the rotational diffusion of triangular and square platelet particles in close proximity to a wall in the presence of strong depletion interactions; unfortunately, no theory that incorporates this complexity has yet been developed. Consequently, lacking such a theory, we simply compare the measured diffusion coefficients with the closest available theory, a calculation in the bulk for thin disks having similar lateral dimensions. The rotational diffusion coefficient for a thin disk having radius $a$ diffusing in a bulk fluid having a viscosity $\eta$ can be estimated in the low frequency, low Reynolds number limit as $D_r = \frac{3k_B T}{(32 \eta a^3)}$. Using the circumscribed radius of the triangle for $a$, its rotational diffusion coefficient is estimated to be $3.8 \times 10^{-1}$ rad$^2$/s. Our measured rotational diffusion coefficient for a triangle is about an order of magnitude smaller than
the estimate based on a disk in the bulk, likely due to a combination of effects that arise from additional drag from the nearby solid wall and the presence of depletion attraction, both of which can reduce the rate of rotational diffusion of the polygon. Although we have not directly measured the thickness of lubricating layer of water between the platelets and the solid wall, we estimate it to be $\approx 50$ nm, somewhat larger than the roughness on the faces of the particles, which is comparable to the size of the depletion agent.

**Figure 2.3** Example rotational trajectory data for a triangular platelet particle diffusing near the cell wall. (A) A portion of the rotational trajectory $\theta(t)$ of a triangular platelet determined using the FFT analysis technique in Fig. 2.1. (B) The measured angular step-size distribution $p(\Delta \theta)$ from a trajectory set with 1,800 frames for a diffusing triangle, corresponding to a fixed time interval between frames of 0.033 s (dashed line is a fit to a Gaussian distribution).
Figure 2.4 Calculated mean square angular displacement (MSAD) \( \langle \Delta \theta^2(t) \rangle \) versus time \( t \) for square platelets (solid squares) and triangular platelets (solid triangles). Solid lines are linear least squares fits of \( \langle \Delta \theta^2(t) \rangle = 2D_r t \), where \( D_r \) is the in-plane rotational diffusion coefficient, yielding \( D_r = 3.3 \pm 0.2 \times 10^{-2} \, \text{rad}^2/\text{s} \) (triangles) and \( D_r = 8.3 \pm 0.5 \times 10^{-3} \, \text{rad}^2/\text{s} \) (squares). The horizontal dashed line represents the lower bound of the angular resolution determined for triangles (0.020 radians).

Confining platelet particles to two dimensions using a depletion agent is one convenient way to facilitate video imaging; other realizations of experiments that provide time sequences of images of an isolated rotating particle, whether driven diffusively or convectively, could also be used. Indeed, images showing the re-orientation of complex particles that have at least one straight edge should be able to take advantage of the FFT ray-tracking algorithm, even if only 2 primary rays in the FFT are generated from one straight edge. In principle, the FFT analysis method we have demonstrated for isolated
particles can be extended to several particles in the same field of view. However, interpreting the resulting superposition of rays from different particles may become ill-posed if certain rays overlap and cannot be uniquely distinguished in the FFT intensity. Each additional particle in the field of view will add another set of rays to the FFT image. Nevertheless, by using phase information of the FFT, it may be possible to extend the technique we have presented to track the rotations of multiple particles, for which the contributions to the FFT intensity of specific rays originating from certain particles can be uniquely labeled using the FFT phase information. The FFT phase effectively encodes particle positions, and we have shown that considering the phase information is not necessary when determining the angular orientation of an isolated single particle.

While we have shown that the Fourier method explores a way to assess the orientation of a particle having a non-trivial cross-sectional projection in two dimensions, we anticipate that this method can be readily extended into three dimensions by taking the three-dimensional Fourier transforms of, for instance, individual volume frames of confocal microscopy movies of isolated facetted particles. The Fourier transform would result in a set of rays (i.e. spokes) in 3D reciprocal space emanating in directions normal to the facet facets of the particle, analogous to the rays we have shown in two-dimensions for polygons. Tracking the spokes would require adapting the current algorithm into three dimensions using an approach analogous to the method we have presented here.

Although the rotational diffusion of particles and molecules have been measured in many other cases, these results cannot be directly compared with the results of our experiments because of significant differences in the shapes and sizes of the diffusing particles and also because of the differences in boundary conditions (i.e. proximity to a
A system of rotating prolate ellipsoids, for example, has been made using a thin glass cell with thickness < 1 µm as the method of confinement\textsuperscript{22}. In this case, the ellipsoids are near two solids walls, whereas, in our experiments, the depletion attraction keeps the polygonal platelets near only one solid wall. Although measurements of diffusion coefficients for translating and rotating rods near a surface have been made\textsuperscript{23}, the friction coefficients for these objects (\textit{i.e.} shape and aspect ratio) are not similar to the polygons we have examined. More work, whether simulations or theory, on anisotropic, complex-shaped particles is needed to fully understand the complicated hydrodynamics of particles undergoing rotational and translational diffusion in the bulk and near walls. A suitable theory that could potentially address our measurements would consider the in-plane rotational Stokes drag of regular polygonal platelets confined near a solid wall by a depletion attraction, separated by only a very thin layer of lubricating viscous liquid, yielding the in-plane rotational mobility and diffusion coefficient. Such a calculation would enable a direct quantitative comparison with our experimental results and would be beneficial in advancing the understanding of the diffusion of anisotropic, complex-shaped particles.

In summary, we have shown that Fourier analysis of optical micrographs of isolated polygonal platelets can be used for rotational tracking independent of particle position. Although we have only presented detailed results for two types of regular polygons, triangles and squares, this approach could be extended to many other kinds of platelet particles. Among the easiest to track, platelets having well-defined linear edges are especially well suited, provided that the number of edges is not so large that the rays in the FFT intensity overlap and cannot therefore be distinguished by peak
detection. Furthermore, this technique can be applied in a straightforward manner for less
symmetric shapes, other than regular polygons, provided that there is at least one well-
defined straight edge on the particle that will give rise to a bright line of intensity in the
FFT. Indeed, the technique could be extended to particle shapes having complex curved
edges using pattern recognition of a complex continuous set of rays in Fourier space (i.e.
by performing correlation analysis of the Fourier pattern of an initial frame of the FFT
movie with an angularly rotated version of a Fourier pattern in a subsequent frame).
Increasing the particle's size in pixels in images and also the frame acquisition rate of the
camera could provide a means of sensitively probing rotational diffusion useful in
microrheology applications of thin layers of materials between the platelet and solid wall
over a wide dynamic range.

2.5 Conclusion

The rotational Brownian motion of complex-shaped particles can be accurately
measured by Fourier transform analysis. By tracking peaks in $I(\theta)$, one can extract
rotational diffusion coefficients for many different complex particle shapes, as we have
demonstrated with equilateral triangles and squares. Not surprisingly, the values of the
rotational diffusion coefficients of these plate-like particles confined near a solid wall by
a depletion attraction are substantially lower than an estimate of rotational diffusion
coefficients of a thin circumscribed disk diffusing in the bulk. This points out the need for
calculations of translational and rotational Stokes drag coefficients of complex shapes in
the bulk and near a wall in the presence of a depletion agent. In the future, the Fourier
analysis technique can potentially facilitate the translational tracking of the particle in a subsequent analysis, since its orientation has been pre-determined. Moreover, this analysis approach for isolated particles may also be extended to several particles in a field of view if the phase information of the FFT is appropriately considered.
2.6 References


3 Two-Dimensional Brownian System of Hard Tri-Star Colloids

3.1 Abstract

A dense system of hard Brownian tri-star particles in two dimensions exhibits a remarkable entropic disorder-order phase transition, reflected in its surface osmotic equation of state. When concentrated to high particle area fractions, $\phi_A$, while being subjected to continuous thermal excitations, lithographic microscale tri-star particles self-organize into an alternating stripe crystal (ASX), a broken-symmetry rectangular crystal phase. ASX exhibits two types of lock-and-key features: tri-stars arrange head-to-tail to form long linear stripes, and neighboring stripes interdigitate by pointing in alternating and opposite directions, creating an array of zippers. We find that entropy maximization can cause certain concave shapes, such as tri-stars, which have been concentrated to high $\phi_A$, to self-organize into extended zipper-like interdigitated structures analogous to those formed by amyloidogenic biomolecules.
3.2 Introduction

Structures of interdigitated components, such as gears\(^1\) and lock-and-key shapes\(^2,3\), are important in diverse fields ranging from mechanical engineering to molecular biology. Understanding how to direct the organized self-assembly of simple yet small components is an ultimate goal in soft nanotechnology and colloidal science\(^4,5\). In particular, advances in top-down\(^6-8\) and bottom-up\(^9-11\) fabrication methods are enabling unprecedented control over the sizes and shapes of microparticles and nanoparticles dispersed in a liquid. Entropic excitations of such designer colloidal particles lead to their continual Brownian motion, causing frequent collisions when the particles are concentrated to higher densities. Thus, entropy maximization can drive the self-organization of hard non-spherical particles into a variety of interesting and desired configurations\(^12-15\). Although some phases and structures have been observed and explained for a limited number of shapes, there are still many things to learn about how to design and control the organized self-assembly of hard colloidal components into more complicated, larger-scale structures.

Beyond directing self-assembly, roughness-controlled depletion attractions\(^16,17\) (RCDAs) have provided a key pathway for exploring and measuring the self-organized phases and jammed states of Brownian systems of hard lithographic microplatelets in two dimensions\(^18-21\). RCDAs, induced by a nanoscale depletion agent, such as nanospheres, nanodroplets, and micelles, keep the platelets confined so that they diffuse in two dimensions near a flat and smooth solid glass surface, yet in-plane interactions between the platelets are nearly hard.
Regular polygons have served as a basic starting point, and a wide range of structures have been observed. As pentagonal platelets are concentrated, the system undergoes a disorder-order transition from an isotropic liquid into a hexagonal rotator crystal\(^{20,22}\) (HRX), then rotational frustration sets in, and an order-disorder transition occurs\(^{20}\) at the highest particle area fractions \(\phi_A\). The experimentally observed system of pentagons, even when concentrated very slowly from low to high \(\phi_A\), becomes jammed into an out-of-equilibrium configuration\(^{20}\) and never reaches the conjectured highest density configuration, an alternating striped crystal (ASX) of interdigitated stripes of pentagons pointing head-to-tail in opposite and alternating directions\(^{22,23}\). For squares, the HRX phase is again observed, yet at higher densities, there is a first-order entropic crystal-crystal transition through a co-existence region into a rhombic crystal (RB), and the rhombic angle of the lattice continuously increases towards 90 degrees (\textit{i.e.} a square crystal) as the particles are further concentrated\(^{18}\). By contrast, regular triangles do not self-organize into a HRX phase, but instead undergo a transition from a liquid-like isotropic phase into a triatic liquid crystalline phase that can exhibit a form of local chiral symmetry breaking driven by entropy\(^{19}\). Achiral rhomb particles, when driven entropically in 2D, also develop non-local chiral symmetry breaking over extended single-crystalline domains and have a propensity to form twinned crystals\(^{21}\).

Although hard convex colloidal shapes, including spheres\(^{24-26}\), rods\(^{12,27-28}\), and disks\(^{13,29,30}\), have received much attention so far, hard concave shapes have received comparatively little, especially experimentally. Planar shapes are categorized as being concave if they have at least one internal angle associated with a vertex that is greater than 180\(^\circ\); convex shapes have internal angles that are all less than 180\(^\circ\). Certain
naturally occurring microscale spoke-like diatoms\textsuperscript{31} and normal human red blood cells\textsuperscript{2} are examples of biological shapes that have concave features and sizes near the upper end of the colloidal range. Alternatively, a few concave colloidal shapes, such as tetrapods\textsuperscript{9}, crosses\textsuperscript{6}, and certain letters in colloidal alphabet soup\textsuperscript{6}, have been synthetically fabricated, but their osmotic equations of state (O-EoS) and phase behavior remain unmeasured.

Here, we lithographically fabricate and study monodisperse, hard, plate-like, star-shaped particles that have 3-fold rotational symmetry and equal-length rays separated by 120° that are co-joined at a central core (\textit{i.e.} tri-stars). Tri-stars are achiral and exhibit mirror symmetry about three equivalent lines in a plane that extend through the center of each ray, so tri-stars have higher symmetry than Y-shapes that have distinguishable heads and tails. Consequently, any one of the three rays of a tri-star could equivalently be selected as the head of the particle, leaving the other two rays to form a V as the tail of the particle. Since isolated tri-star particles do not have identifiable heads and tails, some additional broken symmetry in a condensed phase of many particles would be needed for a particular ray of a given tri-star to be unambiguously identified as effectively serving as its head. It is possible that entropy maximization could lead to the formation of such broken symmetry crystal phases of hard symmetric Brownian shapes, possibly tri-star shapes in 2D. However, until now, no experimental observations of a thermodynamically stable phase of hard tri-stars have been made, nor has an equation of state been measured and correlated with the self-organized structures that form.

Herein, we report detailed experimental observations of dense 2D systems of tri-star particles having a high (\textit{i.e.} slender) aspect ratio $\zeta \approx 6.5$, given by length divided by
width of the projecting rays. We present a general method of measuring the osmotic equation of state (O-EoS) and structure of 2D Brownian systems of plate-like particles, based on real-space optical microscopy over a very large spatial scale, and perform subsequent image analysis for obtaining the O-EoS from a tilted 2D equilibrated column of tri-star platelets in a gravitational field. We show that there is a colloidal disorder-order transition\textsuperscript{32} of hard tri-star particles from a liquid-like isotropic phase at lower $\phi_t$ to a glass-like disordered state that is frustrated, due in part to significant particle interdigitation, and then, remarkably, this disordered state gives way to a spatially ordered ASX phase at even higher $\phi_t$. The stripes in ASX are highly interdigitated leading to an appearance of a repeating steric zipper motif characteristic of amyloidogenic proteins that possess concavity\textsuperscript{33,34}.

3.3 Methods

3.3.1 Fabricating and Characterizing Tri-Star Dispersions

Tri-star polymer platelet colloids are mass-produced using photolithography in a top-down process using an Ultratech i-line XLS stepper\textsuperscript{6}. A quartz-chrome mask containing many identical replicates of tri-star particles, 5x larger than actual particle dimensions, has been created using electron beam lithography based on a computer-aided design (L-Edit software by Tanner) and is robotically loaded into the stepper. A five inch silicon wafer is spin-coated with a layer ($\approx 200$ nm in thickness) of lift-off-release (LOR, supplied by MicroChem) material, and then subsequently coated with a layer of light-sensitive polymer photoresist (SU-8, supplied by MicroChem) of $t_p = 1.0$ $\mu$m thickness.
Ultraviolet exposure and a post-baking process cross-link the SU-8 to form the tri-star particles, yielding approximately 100 million monodisperse particles per wafer. After development using propylene glycol monomethyl ether acetate, which does not dissolve LOR but removes unexposed SU-8, the tri-star particles are released from the wafer by dissolving the water-soluble LOR through immersion into a 1 mM aqueous surfactant solution of sodium dodecyl sulfate (SDS) and 1.8 %w/v tetramethylammonium hydroxide. As the particles lift-off the wafer, SDS adsorbs onto their surfaces, stabilizing them against aggregation via an electrostatic repulsion that has a very short range (i.e. a Debye screening length of a few nanometers). To remove residual and unwanted LOR polymer in the continuous phase of the dispersion, the tri-star particles are allowed to settle, the majority of the continuous phase is removed, and 1 mM SDS solution is added; this process of LOR removal (i.e. washing) is repeated four times. Subsequently, again via gravitational settling and removal of the continuous phase, the tri-stars are concentrated to a particle volume fraction of $\phi \approx 2 \times 10^{-3}$.

### 3.3.2 Forming a Tilted 2D Gravitational Column

We form a 2D monolayer of tri-star particles by adapting the procedure presented in the Supplemental Material of Zhao and Mason. Roughness-controlled depletion attractions (RCDAs) are generated by adding 1.6% w/v of 20 nm diameter sulfate stabilized polystyrene spheres, which serve as the depletion agent, in a 50:50 ratio to the dilute tri-star dispersion at $\phi = 2 \times 10^{-3}$. This mixed dispersion of tri-stars and nanospheres is sealed in an optically transparent, rectangular microcuvette having internal dimensions...
of 40 mm x 2.0 mm x 0.1 mm that is placed flat on a table so the plane of the larger two dimensions is perpendicular to gravity. After the tri-stars sediment towards the lower microcuvette wall, RCDAs confine them so that their faces remain parallel with the wall. Because of the very short-range charge repulsion, a thin lubricating layer of the continuous liquid phase nevertheless remains between the particles and the wall, so the particles can still diffuse translationally and rotationally in the plane. To minimize lateral in-plane attractions between the edges of the tri-stars, the concentration of polystyrene spheres in the mixture has been optimized to be as low as possible yet still high enough that the lubricated face-wall depletion attraction keeps the tri-stars in the plane above the wall even in the presence of thermal excitations. In addition, in-plane interactions between two interacting tri-stars are effectively hard because the depletion agent is smaller than the roughness on the particles' edges\textsuperscript{16,17}. The larger roughness on the edges of the particles than on their faces is a natural consequence of the lithographic processing used to create the particles.

To examine self-organized structures of tri-stars over a wide range of particle area fractions, we create the equivalent of an equilibrated barometric column of the particles in 2D. The tri-stars have a higher mass density than water; the mass density difference is \( \Delta \rho \approx 0.2 \text{ g/cm}^3 \). By slightly tilting an optical microscope, which holds the microcuvette at an angle of \( \psi \approx 6 \text{ deg} \) with respect to the horizontal, we effectively subject the tri-stars to a slow gravitational osmotic compression in 2D, as shown schematically in Figure 3.1. The tri-stars slowly settle towards the lower end of the microcuvette, forming a monolayer in which the osmotic pressure \( \Pi_{2D} \) and the particle area fraction \( \phi_a \) both vary as a function of distance. Using optical microscopy, we scan along the length of the tilted
microcuvette each week until the area fraction of particles as a function of distance reaches a steady state and no longer evolves. Equilibration takes about two months for this tilt angle, and this slow rate of concentration provides a good approximation of a quasi-static compression. Our observations of the particles in the tilted column have been made after six months of equilibration. We have explored higher tilt angles, which do yield a faster approach to a steady-state gradient in particle area fraction, but the structures that form are not as ordered and are likely to be out-of-equilibrium jammed states rather than equilibrium phases.

Examining the monolayer of the equilibrated tilted column of particles using an inverted optical microscope enables us to observe the structure and density of tri-star particles over a wide range of $\phi_A$, as shown schematically in Figure 3.1. A Nikon TE2000 microscope is equipped with a CFI60 plan achromat 40x long-working distance objective. This objective has a correction collar that is adjusted to provide a sharp contrast between the rays of the tri-star particles and the aqueous continuous phase outside. Movies and still images are acquired using a Silicon Imaging camera (CMOS 1280 x 1024 pixels). Because the entire microscope is tilted, the plane in which the particles diffuse remains parallel with the focal plane of the microscope, enabling clear imaging of particles over the entire field of view. All optical microscopy observations are made at temperature of $T = 298$ K.
Figure 3.1 Schematic of a tilted rectangular microcuvette used to create an equilibrated 2D gravitational column of hard Brownian tri-star particles (green). An aqueous suspension, containing microscale tri-star particles and a nanoscale depletion agent, is loaded into and sealed inside a microcuvette. Tri-star particles are restricted to the 2D plane of the lower microcuvette wall (gray slab) by roughness-controlled depletion attractions. Reminiscent of the classic barometric distribution, an equilibrium steady-state gradient in the area fraction, $\phi_A$, of tri-star particles forms along $z$. At higher osmotic compressions, deeper into the column (left), the entropically excited tri-stars can self-assemble into crystals having extended domain sizes. Brightfield video imaging is performed using a Nikon TE2000 inverted microscope equipped with a CFI60 plan achromat 40x long-working distance objective (OBJ). The entire microscope, into which the microcuvette is mounted, is tilted to ensure that the tri-stars are in good focus over the entire field of view. The tilt angle with respect to the horizontal is chosen to be small, $\psi \approx 6 \text{ deg}$, to produce an effectively quasi-static 2D osmotic compression.

3.4 Results and Discussion

3.4.1 Observed Structures in an Equilibrated 2D Column

A large-area composite microscope image of an equilibrated gravitational 2D tilted column of tri-stars is shown in Figure 3.2. At the top of the column, a gas-like dilute region of tri-star particles is observed and the gradient in $\phi_A$ is substantial. Further down the column at a larger distance $d$, where $\phi_A$ and 2D osmotic pressure $\Pi_{2D}$ are
higher, the gradient in $\phi_A$ decreases. Deeper into the column, tri-stars are interdigitated, yet their structure remains disordered. A few tiny regions of HX are also observed. Even further below this, large crystalline domains of ASX are seen; these domains contain hundreds of particles. Below the largest crystallites of ASX, some smaller crystallites of HX and ASX are found; these apparently are trapped states and not able to grow into larger domains of ASX over even long observation times since $\Pi_{2D}$ is higher there and particle rearrangements occur very infrequently.

Several overlapping images, taken about 10 s apart (corresponding to the time required to move the microscope's $xy$-stage to a new viewing region), are combined (i.e. composited) digitally, thereby providing a wide-area view of the entire equilibrated column, as shown in Fig. 3.2. In regions where two overlapping images are stitched together, especially for dilute concentrations, the translational and rotational motion of individual particles is evident. When determining locally averaged properties, such as $\phi_A$, at these boundary regions in the column, we calculate the local $\phi_A$ in the overlap regions near the edges of two adjacent images prior to compositing and report the average.

Characteristic average dimensions of the tri-star particles are measured using scanning electron microscopy (SEM). A drop of a highly diluted dispersion of tri-stars is placed on a clean silicon wafer substrate, the water is evaporated, and a 5 nm layer of gold, a good electrical conductor, is sputtered over the tri-stars and wafer substrate to inhibit charge accumulation. Isolated tri-stars are imaged using SEM at an accelerating voltage of 7.0 kV and an emission current of 10 $\mu$A (see Figure 3.2(inset)). The average measured length and width of a ray of a tri-star (i.e. internal dimensions which exclude roughness) are $2.92 \pm 0.07 \, \mu m$ and $0.45 \pm 0.04 \, \mu m$, respectively, based on images of 24
different tri-star particles. The tips of the rays, which emanate from the central core of the tri-stars, are rounded as a result of the diffraction-limited lithographic fabrication process\textsuperscript{18}, and the average radius of curvature of these rounded tips is $\approx 200$ nm. From the internal dimensions of the particles, the core facial area per particle is $A_p = 4.0 \pm 0.5 \ \mu$m$^2$.

Based on atomic force microscopy (AFM) and SEM measurements, the tallest asperity heights on the faces of the tri-stars are approximately 20 nm, whereas the tallest asperity heights on the edges of the tri-stars are significantly larger, approximately 50 nm. The edge asperities of neighboring particles are sparse, so when considering the interaction between two diffusing tri-star particles in 2D, the effective thickness of the additional boundary layer due to roughness on the rays, from the perspective of collisions, is about half of the edge roughness and therefore approximately 25 nm. Including this boundary layer due to edge roughness, the effective facial area per tri-star $A_{p,r}$ that is relevant for calculating particle area fractions describing phase behavior resulting from collisions is $A_{p,r} \approx 4.5 \pm 0.5 \ \mu$m$^2$. We use $A_{p,r}$ to calculate the measured particle area fraction $\phi_t$, since it is most relevant when considering geometrical packing.
Figure 3.2
Figure 3.2 Brownian tri-star colloids self-organize into extended domains of interdigitated alternating stripe crystals (ASX) when osmotically compressed in an equilibrated, tilted, 2D gravitational column, as revealed by brightfield transmission optical microscopy. The tilt angle of the plane of particles with respect to the horizontal is $\psi \approx 6$ deg. Below a dilute disordered gas region (top) and a more concentrated disordered frustrated region (middle), three large crystallites of ASX are visible (blue regions-bottom) at higher osmotic pressures; other smaller crystallites of ASX can also be seen but are not colored. To obtain this wide-scale view, five different images in the $x$-$z$ observation plane have been digitally composited together (boundaries are marked by arrows on the right) to provide a complete picture of the density of particles as a function of distance $d$ from the top of the column, above which no particles are present. A few tiny crystallites having a hexagonal structure (HX) are also present and a few examples are shown in red. The effective direction of gravity in the plane is downward as shown. Inset: scanning electron micrograph of a lithographic tri-star particle (inset scale bar: 1 µm).

3.4.2 Two-Dimensional Osmotic Equation of State

By digitally analyzing the measured large-field-of-view image of the entire 2D equilibrated column of tri-stars, we then obtain the two-dimensional osmotic equation of state. The behavior of the 2D O-EoS is inherently linked to the shape of the particles and any underlying phase changes that Brownian systems of these particles undergo as they become more concentrated. We show that a barometric equation can be used to accurately fit the particle area fraction $\phi_A$ in the dilute gas-like regime, and that a simple semi-empirical functional form having only two parameters can be used to describe the 2D O-EoS of tri-star colloids over an extended range of $\phi_A$ while the tri-stars remain disordered even as interdigitation begins to occur. By comparison with the ideal gas law at dilute $\phi_A$, we determine an effective area per particle that is somewhat higher than the bare area, potentially reflecting the importance of the protruding arms and rotational diffusion that can occur between particle collisions. At higher $\phi_A$, the emergence of the
alternating stripe crystal phase is accompanied by an abrupt change in particle density, and the crystalline system of tri-stars is much less compressible.

### 3.4.3 Determining Particle Area Fraction in a 2D Tilted Column

Using digital video analysis of a large-scale composite image of particles in an equilibrated 2D tilted gravitational column, we have determined the center positions of all tri-star particles in Figure 3.2. The particle density clearly exhibits a changing gradient, ranging from dilute at the top to concentrated toward the bottom. From these center coordinates, we calculate the 2D osmotic equation of state (O-EoS) as follows. Here, the coordinate $z$ is along the lower surface of the microcuvette and referenced relative to an arbitrary location that is in the dense part of the column, pointing upward (i.e. opposite the direction of the component of the gravitational force that acts to concentrate the particles). The width $w$ of the image is $w \approx 210 \, \mu m$. We count the number of particles $N$ in successive vertical bins at height $z$, each having a differential thickness $dz = 15 \, \mu m$. The area fraction at height $z$ is then given by: $\phi_A(z) = N(z)[A_{p,r}/(w \, dz)]$. Except at the boundary where large crystalline domains occur, we average over nearest neighboring bins to obtain a smoother $\phi_A(z)$. Since it is conventional to report results from the dilute to concentrated particle densities, we define a distance $d$ into the column to be referenced from the top of the column in the bin having $N = 0$, just above the first bin having non-zero $N$. For the composite image shown in Fig. 3.2, given our choice of $z = 0$, the conversion between the two distances is $d = 570 \, \mu m - z$. The results of the measured $\phi_A(d)$ are shown in Fig. 3.3. We observe an initial rapid rise in $\phi_A(d)$, followed
by a change in curvature from concave up to concave down, a reduction in slope, a discontinuous increase in $\phi_A$ (marked by a vertical dashed line) where crystallization occurs, and then a trend towards zero slope at large $d$. For larger $d$ beyond the dashed line, $\phi_A$ has been determined within a single ASX domain.

We fit the dilute gas-like region of $\phi_A$ near the top of the column where $d$ approaches zero to a barometric exponential form to obtain the thermal gravitational height $h_g$ of the entropically excited particles. Strictly speaking, the classic barometric form, derived using the Boltzmann factor of statistical mechanics$^{35}$, describes a probability density of finding an isolated colloidal particle in a liquid at a height $h$ above some reference surface (i.e. opposite gravity) in the limit of zero particle density: $p(h) \sim \exp(-h/h_g)$, where $h_g = k_B T/(\Delta \rho V_p g)$, $k_B$ is Boltzmann's constant, $V_p$ is the volume of the particle, and $g$ is the gravitational acceleration. Because $h$ is referenced as positive from a bottom surface upward and $d$ is referenced as positive is from the top of the column downward, and because we choose $d = 0$ where $\phi_A = 0$, we use a growing exponential functional form that conforms to our assumptions and includes $h_g$ as a fit parameter: $\phi_A(d) \sim \exp(d/h_g) - 1$, where for a slightly tilted 2D system $h_g = k_B T/(\Delta \rho V_p g \sin \psi)$. We limit the number of data points included in the fit of $\phi_A(d)$ by progressively including more data points in the fit and identifying when the correlation coefficient of the fit begins to systematically decrease. For the measured $\phi_A(d)$ shown in Fig. 3.3, we find that the maximum value of $d$ that can be used without causing a significant reduction in the correlation coefficient of the fit corresponds to $d_{gl} \approx 100 \mu m$ (i.e. so only the first 8 data points at smallest $d$ are used for the fit). The quality of this fit is excellent for $d \leq d_{gl}$, and its correlation coefficient is 0.998, very close to unity. From this fit, we extract $h_g = 44 \pm$
2 μm. This value of $h_g$ corresponds to a very weak effective average gravitational force acting on a tri-star particle in the slightly tilted column of $k_B T/h_g \approx 9$ picodynes.

**Figure 3.3** Quasi-static gravitational osmotic compression of hard tri-stars creates a steady-state 2D area fraction profile. Shown is the dependence of the particle area fraction, $\phi_A$, on the distance $d$ into the tilted 2D column, determined from Fig. 3.2. Uncertainties in $\phi_A$ for different $d$ are shown as bars near the top. For $d \leq 100$ μm, $\phi_A(d)$ can be well fit to an exponential barometric form (solid line), yielding an effective Brownian gravitational height of $h_g = 44 \pm 2$ μm. The dashed vertical line at $d \approx 450$ μm indicates a step-change in density between the disordered region and single-domain pure ASX. Insets: Fourier transforms (FTs) of images of tri-star particles at different $\phi_A$ (0.21 - purple circles, 0.26 - green circles, and 0.30 - red circles) show disordered structures having broadened rings that become progressively more intense and larger towards larger $\phi_A$. The FT of a single crystallite of ASX (0.33 - blue circles) reveals an array of peaks and six diffuse lobes. White scale bar: 0.1 μm$^{-1}$. 
3.4.4 Obtaining the 2D Osmotic Equation of State

Using the measured \( \phi_A(d) \) and \( h_g \) determined from the fit at small \( d \), we calculate the 2D O-EoS as follows. By analogy to an approach for spherical colloids in 3D, which itself is based on long-standing barometric equations\(^{36} \), the 2D osmotic pressure at height \( z \) is given by an integral proportional to the effective gravitational force of all particles above \( z \):

\[
\Pi_{2D}(z) = \Delta \rho \, t_p \, g \sin \psi \int_z^\infty \phi_A(z') \, dz', \text{ where } z' \text{ is a variable of integration.}
\]

Substituting for \( h_g \), this becomes:

\[
\Pi_{2D}(z) = \left( k_B T / A_p \right) \int_z^\infty \phi_A(z') \left( dz' / h_g \right), \text{ where } V_p = A_p t_p. \]

This integral can be readily computed numerically. We next convert height into distance into the column, \( d = 570 \, \mu m - z \), yielding \( \Pi_{2D}(d) \). We then eliminate the common parameter \( d \) in \( \phi_A(d) \) and \( \Pi_{2D}(d) \) to obtain \( \Pi_{2D}(\phi_A) \), as shown in Figure 3.4. This approach for determining \( \Pi_{2D}(\phi_A) \) from \( \phi_A(d) \), or equivalently \( N(z) \), is not limited to tri-star shapes and is general for any system of monodisperse colloidal particles in 2D.

3.4.5 Behavior of the 2D Osmotic Equation of State

In the dilute limit, as \( \phi_A \) approaches zero, the ideal gas law in 2D should be valid for featureless disk-like hard particles. However, for hard particles such as tri-stars that have non-compact features, such as extended slender rays, the ideal gas law could potentially require modification. Because the tri-stars diffuse rotationally as they move in space, the area that each tri-star effectively occupies, for the purposes of considering collisions, is larger than its hard-core area. Lacking a theory to describe this effect in detail for a tri-star shape, we assume \( \Pi_{2D}(\phi_A) = \phi_A k_B T / A_{p,\text{eff}} \), where \( A_{p,\text{eff}} \) is an effective
facial area per particle that enforces the ideal gas law. Thus, one would not necessarily expect $A_{\text{p,eff}}$ to be exactly equal to $A_{\text{p,r}}$, given the rotational diffusion of an extended shape, yet these two areas should not be extremely different.

The units of the 2D O-EoS are energy per area or equivalently force per length; these units differ from those typical of 3D equations of state for molecules. We normalize the 2D O-EoS by a 2D thermal energy density $k_BT/A_{\text{p,eff}}$ required to give ideal gas-law behavior $\Pi_{2D}/(k_BT/A_{\text{p,eff}}) = \phi_A$ at low $\phi_A$, as shown in Figure 3.4, yielding $A_{\text{p,eff}} = 6.2 \, \mu m^2$ as the effective area of a tri-star. This $A_{\text{p,eff}}$ is near but somewhat larger than $A_{\text{p,r}} = 4.5 \, \mu m^2$. Up to $\phi_A \approx 0.27$, the entire scaled 2D O-EoS can be fit well to an empirical functional form that has a linear ideal-gas rise at low $\phi_A$ and a divergence at a critical area fraction $\phi_{A,c}$: $\Pi_{2D}(\phi_A)/(k_BT/A_{\text{p,eff}}) = \phi_{A,c}\phi_A/\left(\phi_{A,c} - \phi_A\right)$, yielding $\phi_{A,c} = 0.33 \pm 0.01$. We have chosen to display the 2D O-EoS in a dimensionless form. However, to put our measurement in perspective in absolute terms, the values of $\Pi_{2D}$ that we report for our 2D colloidal system of hard microscale tri-star particles are many orders of magnitude smaller than those that are typical of thin monolayers of molecules, which have Ångstrom-scale or nanoscale dimensions, that have been measured using other classical experimental surface methods.

The fit of the scaled 2D O-EoS for disordered tri-star particles to the semi-empirical form $\phi_{A,c}\phi_A/\left(\phi_{A,c} - \phi_A\right)$ works remarkably well up to very large $\phi_A$ close to where we begin to observe small domains of HX and larger domains of ASX. This may indicate that the average effective number of accessible states per particle in phase space would approach zero in a manner that is linearly proportional to $\phi_{A,c} - \phi_A$ for large $\phi_A$, so long as the system remains disordered. The divergence area fraction $\phi_{A,c} \approx 0.33$, potentially
analogous to a glass transition temperature for attractive molecular systems, is close to the observed phase transition area fraction for crystallization into ASX, analogous to a freezing temperature in attractive molecular systems.

Interdigitation of tri-stars occurs at relatively dilute \( \phi_A \), well below particle area fractions corresponding to the appearance of the ASX phase. A model of close packing of circles that circumscribe tri-stars in a hexagonal lattice, reminiscent of Onsager's approach for rods, would predict a \( \phi_A^* \approx 0.11 \) for the tri-star system that we have fabricated. Because each tri-star has only three slender rays, most pairs of tri-stars do not effectively collide with each other until their average \( r/D < 1 \). Interestingly, this predicted value of \( \phi_A^* \) corresponds closely to the change in curvature of \( \phi_A(d) \) in Figure 3.3 and also to the upper limit in \( \phi_A \) of the applicability of the barometric law associated with the gas-like disordered phase, beyond which interdigitation plays a major role. Thus, the simple overlap concept of \( \phi_A^* \) may serve as a rough boundary between gas-like and liquid-like disordered regions for concave shapes, such as tri-stars, that have only a few slender rays.

For \( \phi_A \geq 0.27 \), where the tri-stars are highly interdigitated and undergo structural transitions, we would not expect the semi-empirical functional form for disordered tri-stars to describe the measured 2D O-EoS well. For such large \( \phi_A \), we observe two regions of higher compressibility where the slope of the 2D O-EoS is significantly reduced; these regions could potentially mark co-existence regions of corresponding to phase transitions. The first region, corresponding to \( 0.27 \leq \phi_A \leq 0.29 \), is continuous and not abrupt, possibly reminiscent of a second-order transition or even the emergence of a liquid-like or glassy disordered state, which we call the frustrated interdigitated (FID) state. In this region of
\(\phi_A\), rotational motions of neighboring particles are strongly coupled, so rotational diffusion is highly arrested and there is no long-range spatial or rotational order. In fact, we speculate that the FID state could possibly be a thermodynamic phase of very small and intermixed transient domains of HX and ASX if these structures apparently have nearly the same entropy for the same \(\phi_A\) and therefore compete in a nucleation and growth scenario. In fact, very small isolated domains of HX are observed in this range of \(\phi_A\), as highlighted by the red tri-stars in Figure 3.2. The second region, corresponding to \(0.31 \leq \phi_A \leq 0.33\), exhibits an abrupt and discontinuous change in \(\phi_A\), indicating a first-order transition from the disordered FID state into a highly ordered and dominant ASX phase that can form large domains, as emphasized by the blue tri-stars in Figure 3.2.

**Figure 3.4**
Figure 3.4  Two-dimensional O-EoS $\Pi_{2D}(\phi_A)$ of tri-star colloids extracted by integration of $\phi_A(d)$ in Fig 3.3, scaled by the thermal energy density $k_B T/A_{p,\text{eff}}$, where $A_{p,\text{eff}} = 6.2 \, \mu m^2$ is the effective area per particle that yields ideal gas-like behavior at very small $\phi_A$. For $\phi_A < 0.27$, the O-EoS can be fit (solid line) to a form $\phi_{A,c}\phi_A/(\phi_{A,c} - \phi_A)$, where $\phi_{A,c} = 0.33 \pm 0.01$ marks the divergence in $\Pi_{2D}$ that would occur if the particles had been rapidly quenched into an out-of-equilibrium disordered glassy state using a higher rate of compression. Horizontal dashed line: transition between a disordered state of highly interdigitated tri-stars and the ASX phase. Inset: spatial pair correlation functions $g_s(r/D)$ of the center positions of all tri-stars, irrespective of pointing direction, where $D$ is the diameter of a circle that circumscribes a tri-star, at different average $\phi_A$ corresponding to the insets in part A. Primary peaks below $r/D = 1$ reflect significant interdigitation of neighboring tri-stars. Index markings for a perfectly ordered rectangular lattice of center positions are shown below $g_s$ for $\phi_A = 0.33$ in ASX.

3.4.6 Structures of Tri-Stars at High Densities

The insets in Figure 3.3 show the results of Fourier transforming real-space regions, taken from Figure 3.2, at different depths into the column. For very low $\phi_A$ in the isotropic regime, the Fourier transform (FT) is isotropic and nearly featureless, exhibiting only a slight depression in the region corresponding to non-overlap of hard particles at small wavenumbers. A ring-like isotropic FT pattern can be seen at higher $\phi_A = 0.26$; the intensity in the first, smallest ring becomes brighter as $\phi_A$ is raised. By contrast to disks and other compact convex polygons, concave tri-stars have the capacity to interdigitate significantly, so the first ring for tri-stars is broader than for compact convex shapes because there is a greater spread in center-to-center separations, $r$, between pairs of neighboring tri-star particles. As $\phi_A$ is increased into the FID regime, the first ring becomes even more prominent, and a second larger ring emerges. Beyond FID, at $\phi_A$ corresponding to ASX, we find a set of peaks and six diffuse lobes in the FT that is
characteristic of an ordered 2D crystalline striped phase of three-fold symmetric objects. We provide a detailed explanation of the origin of the features in the FT of the ASX phase in Section 3.4.9.

We have also calculated the $\phi_A$-dependent spatial pair correlation function $g_s(r/D)$, based on center positions of all tri-stars irrespective of their pointing directions (see insets in Fig. 3.4), where $D$ is the diameter of a circle that circumscribes a tri-star. For all $\phi_A$, the region of hard non-overlap of concave tri-stars, corresponding to $g_s = 0$, can be seen for $r/D < 0.5$. The existence of a peak in $g_s$ for $r/D < 1$ reflects interdigitation of neighboring tri-star particles; this is very different than the spatial correlation function for convex hard disks which has $g_s = 0$ for $r/D < 1$ at all $\phi_A$. As the particle density of tri-stars changes from gas-like to liquid-like, a small peak in $g_s$ begins to emerge near $r/D \approx 0.7$, as can be seen for $\phi_A = 0.21$. As $\phi_A$ increases, this first peak becomes higher and the value of $r/D$ associated its maximum becomes smaller, approaching $r/D \approx 0.5$. A second peak can be clearly distinguished at $r/D \approx 1.25$ for $\phi_A = 0.26$ and 0.30 in the frustrated interdigitated state. These broad and smooth peaks that damp out rapidly as $r/D$ increases are characteristic of disordered glassy structures. At yet larger $\phi_A = 0.33$, in a single ASX domain, the first peak for $r/D < 1$ splits into two closely spaced peaks, reflecting the underlying rectangular crystal structure of ASX; this rectangular structure can also been seen in the peaks at lowest wavenumbers in the corresponding FT. Moreover, $g_s(r/D)$ for ASX exhibits sharper peaks that at much larger $r/D$, indicating at least quasi-long-range spatial order, which is very different than the behavior for lower $\phi_A$ outside of the ASX phase. Although we refer to the ASX phase as a crystal for simplicity, we recognize that, strictly speaking, this ASX phase could actually be a 2D liquid crystalline phase and may
only exhibit quasi-long-range spatial order. A detailed analysis of the line widths of the FTs and the correlation functions on much larger domains than we have grown thus far would be necessary to make this determination with a high degree of confidence.

### 3.4.7 Alternating Stripe Crystals of Hard Tri-Star Colloids

Detailed characterization of the ASX phase of hard tri-stars provides further insight into its fluctuating structure. By performing detailed image analysis on a single ASX crystallite, we obtain distributions of the interparticle spacing within a stripe, the interstripe spacing, and the pointing directions of particles relative to the stripe axis. These distributions can be fit well using Gaussian functions typical of thermally driven diffusion, and the widths of these distributions reflect the underlying anisotropic elastic properties of the ASX phase. In addition, we obtain the equivalent of a light scattering experiment on a single ASX crystal by taking the 2D Fourier transform of a limited region of our real-space images. This enables us to show that the ASX phase of tri-stars can be identified through scattering experiments that reveal a rectangular structure of peaks, consistent with the rectangular real-space unit cell, in combination with a 6-fold angular intensity modulation arising from the shapes of pairs of oriented tri-stars.

### 3.4.8 Structural Characteristics of Alternating Stripe Crystals

Only after the self-organization of the ASX phase, which is accompanied by both spatial and orientational symmetry-breaking, can one unambiguously identify which ray
of a given tri-star is effectively serving as its head and which two other rays are
effectively serving as its V-like tail within a given stripe. This can be seen in the single
crystallite of ASX shown in Figure 3.5A. We use computer-aided image analysis to
determine the center locations and pointing directions of individual tri-star particles
within this crystallite. Figure 3.5B shows a selected region of an ASX crystal (taken from
Fig. 3.2 at \( z \approx 100 \mu m \) corresponding to \( \phi_A = 0.338 \) and rotated for clarity); center
locations are identified and linear least-squares fits to these center positions, which
quantitatively define the centerline of the stripes, are shown.

In Figure 3.6A, a schematic showing a portion of the idealized ASX lattice
defines the lattice parameters and pointing direction of tri-stars. Figure 3.6B displays the
normalized probability distributions of distance \( u \), corresponding to the center-to-center
separation of neighboring particles within a stripe, and also of the interstripe spacing \( s \),
obtained from the least squares fits of particle center coordinates in individual stripes.
The standard deviations of these distributions, \( \delta u \) and \( \delta s \) respectively, which are both fit
well by Gaussian functions, predominantly reflect the thermal fluctuations of the particles
within the lattice along the two different primary crystal axes. Based on our SEM
measurements, the very small contribution to these widths that arise from the inherent
polydispersity in the arm lengths resulting from the optical fabrication process, can be
neglected. Moreover, we find that \( \delta s \) is smaller than \( \delta u \). This difference reflects the
underlying anisotropy in the elasticity of the symmetry-broken ASX phase, and it
potentially implies that the crystallite possesses a higher elasticity perpendicular to the
stripe axis than along it. The measured time-averaged and number-averaged aspect ratio
is \( \langle u \rangle / \langle s \rangle = 1.15 \) for ASX at the highest \( \phi_A \) we achieve, where thermal fluctuations are
still apparent; This aspect ratio is close to $2/3^{1/2} \approx 1.1547$ that we predict geometrically for ideal slender tri-stars which are close-packed in ASX. A unit cell of ASX, shown as the blue dashed rectangle in Fig. 3.6A, has dimensions of $<u> = 3.83 \, \mu m$ by $2<s> = 6.66 \, \mu m$, an aspect ratio of $<u>/(2<s>) = 0.575$, and effectively contains two tri-stars.

We determine the equilibrium probability density of pointing directions of tri-stars within a stripe, $p_{\theta}(\theta)$, using a large single-crystalline ASX domain. The average ASX crystal axis along the stripes is obtained by fitting the center positions of tri-stars within each stripe of the ASX domain to straight lines, yielding a set of slopes (i.e. fit parameters) that are averaged. The pointing angle $\theta$ of each particle relative to this average crystal axis along the stripes is determined using video analysis, and these angles are binned to yield $p_{\theta}(\theta)$, as shown in Fig. 3.6C. We fit this distribution to a Gaussian function $p_{\theta}(\theta) \sim \exp\left[-\left(\theta-\theta_0\right)^2/(2\delta\theta^2)\right]$, yielding good agreement with the data, as shown by the line in Fig. 3.6C. The fit yields a mean of zero (i.e. $\theta_0 \approx 0$), within the uncertainty, and a standard deviation of $\delta\theta = 5.4 \pm 0.3$ deg. Thus, the average thermally-induced rotational fluctuations of tri-stars within the ASX lattice are small and bounded, since the rays of a given tri-star are strongly constrained within a cage formed by neighboring tri-stars. The degree of rotational confinement obviously depends on the specific area fraction observed; further compression of the ASX phase would reduce $\delta\theta$. 
Figure 3.5 Image analysis of a single ASX crystallite. (A) Detailed view of an ASX crystallite of 244 tri-stars from Figure 3.2 (rotated for clarity) at a particle area fraction $\phi_A = 0.338$. Scale bar: 5 $\mu$m. (B) Results of digital image analysis. The center locations of tri-stars are shown as light blue circles; linear fits to these center locations define the stripes, shown by solid red lines.
Figure 3.6 Structural characteristics of the ASX lattice of tri-star colloids, reported as spatial and angular normalized probability densities. (A) Schematic of an idealized single-domain ASX crystallite shown at $\phi_4 = 0.33$. The center-to-center spacing between two adjacent tri-stars within a stripe is $u$, and the interstripe spacing is $s$. The pointing angle $\theta$ of a tri-star particle is referenced relative to the average pointing axis of stripes in the crystallite (i.e. red lines in Fig. 4B). The blue dashed box is a rectangular unit cell of the ASX crystal. (B) Brownian fluctuations of tri-stars within the ASX crystallite shown in Fig. 3.5 lead to equilibrium normalized distributions $p_u(u)$ and $p_s(s)$. Gaussian fits to these distributions yield $<u> = 3.83 \mu$m and average relative peak width (i.e. normalized standard deviation) $<\delta u>/<u> = 0.053$, and $<s> = 3.33 \mu$m and average relative peak width $<\delta s>/<s> = 0.033$. (C) Measured normalized probability density $p_\theta(\theta)$ of pointing angles $\theta$ of tri-star particles within the observed ASX crystallite at $\phi_4 = 0.338$. A Gaussian fit, having a mean $\theta_0$ and standard deviation $\delta \theta$, is shown by the solid line. The fit yields $\theta_0 = 0.03 \pm 0.31$ deg, so the distribution is centered about zero within the uncertainty, and $\delta \theta = 5.4 \pm 0.3$ deg.
3.4.9 Reciprocal Space Characterization of a Single Crystal

To obtain the equivalent of a light scattering pattern of the ASX phase, we take FTs of an observed ASX region, shown in Fig. 3.7A, in which thermal fluctuations in the positions and orientations of tri-stars is evident, and also an idealized ASX lattice, shown in Fig. 3.7B, in which full long-range order in the positions and orientations of tri-stars has been rigidly enforced. In Fig. 3.7C, peaks are seen in the FT of the observed thermalized ASX phase towards lower wavevectors $q$, yet these peaks die off in intensity at higher $q$. This reduction in peak intensity alludes to a 2D phase that has only quasi-long-range spatial order, such as is typical of a hexatic liquid crystal. Thus, the observed thermally excited ASX phase has reciprocal space features that indicate it is not a perfect "crystal" in a formal sense; the FT indicates that the ASX phase is most likely a liquid crystal in the hexatic sense. However, the orientational order of tri-stars within a stripe appears to be long-range, at least given the largest, yet limited, domain sizes of ASX that we have observed. By contrast, in Fig. 3.7D, the FT of the idealized ASX exhibits sharp peaks out to high $q$; the locations of peaks at lower $q$ match those seen in Fig. 3.7C, although the peaks in the thermally driven ASX are not as sharp. In both Figs. 3.7C and 3.7D, the horizontal set of peaks in the middle of the FT show peaks that are more widely spaced; this larger distance in $q$-space reflects the interstripe spacing, regardless of pointing direction of the stripes. By contrast, the smallest horizontal distance between peaks in both Figs. 3.7C and 3.7D is half this distance, indicating that the unit cell (see Fig. 3.7A) has an aspect ratio that is about twice as wide as it is tall. Along the vertical direction, in the middle of the FT in Fig. 3.7C, thermal fluctuations, which create variations in the inter-particle spacing within a stripe lead to the rapid decrease in peak
intensity away from the center of the image; these peaks persist to larger $q$ in the FT of the perfectly ordered lattice of Fig. 3.7D. Moreover, a fainter, diffuse, yet noticeable six-fold lobe pattern can be seen in Fig. 3.7E, where the FT intensity has been log-scaled. This lobe pattern is indicative of the shapes and orientations of the tri-star particles. A superposition of FTs of isolated motifs of up-down and down-up pairs of tri-stars, shown in Fig. 3.7F, generate the observed six-fold lobe pattern that modulates the peaks arising from the positional structure. Thus, the FTs in Figs. 3.7C and 3.7E have features that reflect constructive and destructive interference of thermally driven tri-star shapes in a highly interdigitated ASX arrangement.
Figure 3.7 Fourier transforms (FTs) of an observed thermal ASX phase of tri-stars and a comparison with a perfect, frozen ASX lattice. (A) Observed single crystallite of ASX of tri-stars at a particle area fraction $\phi_d = 0.338$. (B) Ideally ordered ASX phase of tri-stars. (C) FT of part A, yielding $I(q)$, where $q$ is the 2D scattering wavevector, measured from the center of the FT where $|q| = 0$. Blue arrows indicate examples of peaks that appear because the spatial period between pairs of up and down pointing stripes is twice as large as the average interstripe spacing. (D) FT of part B, yielding the $I(q)$ of the idealized ASX lattice. Red arrows indicate peaks that are faint, yet visible, and seen more prominently in part C. (E) FT of part A, log-scaled and color-coded, revealing six diffuse lobes. White scale bar is 0.1 $\mu$m$^{-1}$ for parts C, D, and E. (F) Superposition of FTs of isolated up-down and down-up pairs of tri-star particles (see insets), showing six lobes that match the diffuse lobes seen in part E. Finer structures (i.e. line patterns) within the six lobes for idealized pairs would become smeared out as a result of thermal fluctuations in the relative positions and orientations of pairs of particles in part A. White scale bar for part F is 0.2 $\mu$m$^{-1}$. 
3.5 Implications and Future Directions

This work has important general implications for colloidal systems beyond tri-star particles. The large-field-of-view imaging approach and analysis routines we present can be used to determine the 2D osmotic equation of state for other shapes. The particular results we have found for tri-star shapes provide the first detailed and quantitative study of the 2D equation of state and phase behavior of hard concave shapes that have slender rays which can interdigitate. These experimental approaches, as well as specific findings for tri-stars, lead to many exciting future directions that will provide additional insight into the role of hard-core shape in dense biomolecular systems, such as amyloidogenic proteins, and other interesting forms of condensed matter.

3.5.1 General Implications for Colloidal Systems

Experimentally, this work is the first to introduce digital wide-field-of-view microscopic imaging of an equilibrated 2D gradient column of a system of colloidal particles for measuring its 2D osmotic equation of state. The 2D EoS depends upon the shapes of the constituent particles, not only on the particle area fraction. Image analysis software provides the centers of mass of the particles, enabling the connection of the particle area fraction to the 2D osmotic pressure, which is obtained through integration. This approach for measuring $\Pi_{2D}(\phi_d)$ of designer lithographic platelets is general and does not depend on the specific tri-star shape that has been the primary focus herein. We anticipate that it will be used to determine the 2D O-EoS of many other shapes. By
examining a wide range of shapes, it may be possible to determine general rules that connect features in particles shapes to observed phases and phase transitions.

3.5.2 Specific Implications Inferred from a Brownian System of Tri-Stars

Local mechanical stability is an important consideration that could frustrate the entropic selection and formation of ASX crystals when compressing a dilute system of particles. First, for comparison, we consider systems of regular pentagons. Locally, edge-edge configurations of neighboring pentagons are mechanically stable, but edge-vertex configurations of pentagons, inherent to ASX, are not locally mechanically stable under compression. Consequently, ASX for hard pentagons is not observed, and instead an order-disorder transition occurs. However, for concave tri-star particles, the insertion of an arm of a first particle into two arms that form a V of a second particle leads to a mechanically stable local configuration that promotes stripe formation; this local head-tail mechanical stability of concave tri-stars is not possible for convex pentagons. Moreover, for slender tri-stars at high osmotic pressures, ASX and not HX appears to be the entropically preferred phase. Alternating stripe phases are also observed in quasi-2D thin films of antiferromagnetic and antiferroelectric materials, yet their physical origin is different than ASX of hard Brownian tri-star particles at high $\phi_A$.

The diminished rotational diffusion measured in Chapter 2 for platelet particles confined to 2D must be considered in order to avoid out-of-equilibrium jammed states in the systems of osmotically compressed tri-star particles presented here. If the system's tilt angle, which controls the rate of concentration of particles, is too high, it is possible that
the reduced rotational and translational diffusion of the two-dimensionally confined platelet particles would inhibit the exploration of phase space necessary for particles to form one or more equilibrated phases. The tilt angle can be optimized for a balance which allows a relatively quick osmotic compression of particles into an equilibrated phase. Studying the dynamics and structure of phase formation as tilt angle is systematically varied, to distinguish jammed from equilibrium structures, remains a promising future direction.

When designing mechanical systems of interdigitating gears in a plane, such as for clocks or watches, gears within gear trains are typically carefully arranged so coupled rotations of neighboring gears do not interfere with each other and can transmit rototary motion in a desired manner\(^1\). To avoid interference of proximate gears in the plane that can lead to undesired binding yet keep the design compact, it is sometimes necessary to transmit the rotary motion out of the plane through a rigid co-axial mechanical linkage between two gears. Systems of interdigitated tri-stars at higher \(\phi_A\) bear some resemblance to many-body systems of coupled gears. Because a given particle is typically rotationally coupled to multiple nearest neighboring particles at high \(\phi_A\), the system becomes rotationally frustrated as it is compressed, typical of a spin glass\(^{38}\), while remaining spatially disordered. Thus, tri-star particles could serve as a useful model system for studying the rotational glass transition of 2D or quasi-2D systems of molecules having concave shapes.

The discovery of a dominant stable ASX phase of Brownian hard tri-stars at high \(\phi_A\) has several important implications. First, by contrast to prior work on proteins\(^{33,34}\) and shape shifting particles\(^{39}\), it demonstrates that attractive interactions are not necessary to
obtain zipper-like configurations of colloidal objects that have slender projecting structures. Instead, maximization of the combined translational and rotational entropy of a system of slender tri-stars at a high enough osmotic pressure is sufficient to generate a crystal of zipper-like alternating stripes where only hard repulsions are present. In ASX, the translational and rotational diffusion of individual interdigitated tri-stars are highly limited, and both the translational and rotational mean square displacements are bounded at long times.

### 3.5.3 Future Directions

Our observations imply that certain hard concave shapes, such as tri-stars, can self-organize to form hierarchical lock-and-key configurations that have two different types of interdigitation: head-to-tail interdigitation at the particle scale to form 1D stripes that have extended spatial range, and alternating opposite interdigitation of 1D stripes much larger than the particle scale to form zipper-like 2D ASX crystals. In the future, it would be worthwhile studying the nucleation and growth of ASX crystals during the dynamic process of applying the 2D osmotic pressure, possibly via time lapse microscopy. It is likely that tri-stars at the boundaries of a growing ASX crystallite re-orient under thermal fluctuations to become incorporated into stripes. The domains of ASX we have observed are larger along the head-tail axis than along the perpendicular interstripe axis, thus suggesting that the growth rates along different crystal directions had been anisotropic. Further control over nucleation and growth of crystals by dynamically varying the tilt angle of the sample cell may be possible and could lead to even larger crystals of ASX, or it may be possible to generate more prominent crystals of
HX at somewhat lower $\phi_A$. Likewise, the spatio-temporal kinetics of 2D melting of the ASX phase could potentially be studied by reducing the tilt angle of the sample cell to lower the 2D osmotic pressure.

It is interesting that a 3-fold rotationally symmetric particle can have a preference to self-organize into a rectangular unit cell, rather than a hexagonal one, at high $\phi_A$. Although the sizes of the observed crystalline grains of ASX are limited, Fourier transform analysis and the spatial correlation function suggest that the spatial order in ASX is only quasi-long-range, as in certain types of 2D liquid crystals$^{30,40}$.

We anticipate that future theoretical passive microrheology analysis, although complicated and tensorial$^{41}$, beyond the scope of treatment here, would provide a quantitative connection between the reported widths of the distributions in Figs. 3.6B and 3.6C and the anisotropic elastic properties of the ASX phase. In fact for the ASX phase, the full theoretical equations of microrheology will be tensorial in two aspects: one is related to the anisotropy of the probe particle shape (i.e. a tri-star) and the other is related to the anisotropy of the effective viscoelastic medium (i.e. the broken-symmetry ASX phase).

Future theoretical work could quantify the influence of rotational diffusion of slender concave hard shapes on the effective area per particle and the 2D O-EoS in the dilute limit. It is remarkable that a simple equation of state having effectively only two parameters (i.e. a divergence area fraction and an effective area per particle) describes $\Pi_{2D}(\phi_A)$ over such a large range of $\phi_A$. Our findings may be related to jamming and the glass transition of disordered particles, where the $\phi_A$ associated with the divergence might be related to a glass transition. Future experiments that extend to larger tilt angles will
explore how rapidly quenching the system into a dense state can affect the prevalence of disordered states, the defect density, and the sizes of the domains of ASX.

### 3.6 Conclusion

In the absence of attractive interactions, slender, hard tri-star colloids self-organize entropically into alternating stripe crystals when concentrated in 2D. We have reported microscopic observations and the structural and reciprocal space characterizations of a system of hard tri-star particles. We have developed a general microscopic method for measuring a fundamental property, the surface osmotic equation of state, of 2D systems of hard plate-like colloids, irrespective of the colloids 2D cross-sectional shape, and we applied this method to tri-stars. Features in the measured 2D osmotic equation of state, obtained using wide-field-of-view digital image analysis, reflect the underlying structural self-organization.
3.7 References

4 Explaining Entropic Selection of ASX using a Translational-Rotational Cage Model

4.1 Abstract

In order to explain the entropic selection of the alternating stripe crystal phase over other possible particle configurations at high 2D osmotic compression, we develop a model that considers both translational and rotational microstates of a central mobile particle in a static cage of neighboring particles. Using a collision detection routine, we calculate the total number of accessible translational-rotational microstates per particle, $\Omega$, at different $\phi_A$. By comparing $\Omega$ for both ASX and HX configurations, we show that ASX has a greater number of accessible microstates for large $\phi_A$ above the observed transition. In addition, we show that the ASX configuration enables collective translational sliding microstates involving many particles. The translational-rotational cage model that we introduce represents an improvement over purely translational and purely rotational cage models, and it can be used to predict the entropic self-organization of other hard shapes.
4.2 Introduction

Entropy maximization is an important factor in the formation of structures observed in nature, such as liquid crystals and colloidal crystals\textsuperscript{1-4}. Designing and directing the organized self-assembly of relatively simple yet small components which can interdigitate into more complex structures is at the forefront of soft nanotechnology and colloidal science\textsuperscript{5,6}. While real systems usually include both enthalpic and entropic components in energy, using particles having hard interactions can isolate entropy's contribution to the formation of exotic structures and phases. A general understanding of how monodisperse systems of hard colloidal components can entropically self-organize into more complex, larger-scale structures as a function of shape at high densities remains incomplete, whether in two or three dimensions.

Simulations have predicted interesting types of liquid crystalline phases, such as cubatic phases of crosses and tetrapods in 3D\textsuperscript{7,8}; these possess long-range orientational, but not spatial, order. In 2D, while an ASX configuration has been predicted and observed in athermal systems of macroscopic regular pentagonal particles at high densities\textsuperscript{9}, and similar ASX configurations have been predicted in simulations of strongly attractive aggregates of 3-fold symmetric Y shapes\textsuperscript{10}, no thorough quantitative treatment of the collective behavior and self-organization of entropically driven systems of hard 3-fold symmetric concave shapes yet exists, even through simulations, to the best of our knowledge.

Here, we design a translational-rotational cage model to understand how entropy can select particular broken symmetry phases at higher particle densities. The model
consists of a lattice of monodisperse, hard, 2D star-shaped particles that have 3-fold rotational symmetry and equal-length rays separated by 120° that are co-joined at a central core (i.e. tri-stars), which were the subject of experiments shown in Chapter 3. Mimicking the phases observed experimentally, these tri-stars are arranged either in a hexagonal lattice (HX), where heads and tails are indistinguishable from one another, and in an alternating stripe crystal (ASX) lattice, where a head and tail to each tri-star is unambiguously identified by the broken symmetry resulted in the ASX phase.

The computational modeling, combined with our experimental observations described in Chapter 3, provide a basis for understanding the important role that entropy plays in selecting the self-organized structure of a system of hard colloidal particles driven by Brownian forces into a symmetry-broken phase after the particles are concentrated by an osmotic pressure to a particular density. Entropy-driven selection among different possible structures of hard particles represents a new form of polymorphism\textsuperscript{11} that we call \textit{entropomorphism}. The theoretical methods that we present are general and can be extended beyond the shape of a tri-star particle in 2D that is the main subject of the work presented in Chapter 4.
4.3 Methods

In order to compare the entropy of the observed dominant ASX phase to an alternative possible crystal phase, the simple hexagonal (HX), we have developed a collision-detection routine in Mathematica for determining the different numbers of accessible microstates available to a tri-star particle in either caged ASX or HX lattices. The dimensions of the tri-star particle used for the model are the average internal dimensions determined by SEM measurements, where tips of the rays are rounded to correspond with the inset of Fig. 3.2. The routine translates a central tri-star particle (shown in red in Figs. 4.1-4.4) in 10 nm increments and rotates it in 0.5 deg increments within an idealized lattice of static neighboring tri-star particles that are evenly spaced (shown in black in Figs. 4.1-4.4). For each center position and angle, the routine checks for line-line intersections (i.e. overlap or collision) between the central particle and all neighboring particles. Positions and angles of the central particle that do not result in any overlap with a neighboring particle are treated as valid (i.e. allowed) microstates of the central mobile particle. For each $\phi_A$ and lattice type, the routine records the positions and angles of allowed microstates and increments the total number of microstates allowed. This translational-rotational approach represents an improvement over purely rotational cage models$^{12-14}$ which involve packing optimization of trioid, squaroid, and rhomboid shapes. These rotational cage models, while useful for certain shapes, implicitly assume that rotational microstates dominate over translational microstates; this assumption may not be a good approximation for all particle shapes, including tri-stars.
The results of the collision detection routine can be concisely reported as a bounding surface of accessible microstates in a special mixed three-dimensional space consisting of two orthogonal translational coordinates \((x, y)\) and one rotational angle \((\theta)\). The mixed translational-rotational free volume within this bounding surface is proportional to the average number of accessible microstates per particle, where collective fluctuations of the lattices are implicitly ignored because the cage of neighbors is fixed. Thus, neglecting collective fluctuations, the thermodynamically selected phase, corresponding to entropy maximization of the system of hard particles, at a given \(\phi_A\) is the one that has the largest translational-rotational free volume per particle in this mixed 3D translational-rotational space.

4.4 Results and Discussion

4.4.1 Single-Particle Translational-Rotational Static Cage Model of Tri-Stars

Several examples of configurations corresponding to points on the top \((i.e. \theta \geq 0)\) surface of the translational-rotational free volume corresponding to HX, calculated using the static cage model, are shown in Figs. 4.1 and 4.2. Likewise, at the same \(\phi_A\), we show several examples of the bounding surface of the translational-rotational free volume of ASX in Figs. 4.3 and 4.4. Detailed aspects of the shapes of the particles, including the rounded tips of the rays, influence the precise appearance of the surfaces shown in Figs. 4.1A and 4.3A. As expected by symmetry, for both HX and ASX, the surfaces corresponding to \(\theta \leq 0\) deg are simply the same as the surfaces for \(\theta \geq 0\) deg rotated by
180 degrees about the stripe axis (i.e. along the pointing direction of the stripes) for ASX or about one of the three equivalent pointing axes of rays of tri-stars for HX, respectively.

We show top, side, and ortho views of the surfaces defining the maximum extent of the available translational-rotational cage corresponding to HX and ASX for $\phi_A = 0.313$ in Figs. 4.2B and 4.4B, respectively, and for $\phi_A = 0.348$ in Figs. 4.2C and 4.4C, respectively. The two in-plane coordinates $(x,y)$ represent the 2D position of the center of the central particle within the cage and the out-of-plane coordinate $\theta$ represents the 1D orientation of the central particle about its center. Overall, the translational-rotational free volume corresponding to HX offers more accessible microstates at higher angles, yet fewer translational microstates at lower angles, as compared to the shape of the translational-rotational free volume for ASX at the same $\phi_A$.

### 4.4.2 Comparison of Number of Accessible Microstates: ASX versus HX

The routine is executed for several different $\phi_A$ (and therefore lattice parameters), while keeping the lattice aspect ratio $(<u>/<s>)$ of ASX fixed to 1.15 as observed experimentally and predicted for close packing of tri-stars having the slenderness aspect ratio that we have fabricated. When $\phi_A$ is increased from 0.313 to 0.369, the number of accessible microstates $\Omega$ decreases by about an order of magnitude from $4.6 \times 10^5$ to $4.6 \times 10^4$ for HX and from $4.1 \times 10^5$ to $5.0 \times 10^4$ for ASX, respectively, as shown in Fig. 4.5A. The shape of the ASX translational-rotational free volume is not as pointed along the angular axis, when compared to the volume for HX. Thus, ASX has more accessible translational states at lower angles than HX, so ASX becomes the entropically preferred phase as angular motions become more highly restricted in HX at larger $\phi_A$. 

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Figure 4.1 Calculation of the bounding surface of a volume of accessible microstates in a hybrid 3D translational-rotational space of a central tri-star particle in a static cage of neighbors in the hexagonal HX phase at a particle area fraction $\phi_a = 0.313$. (A) Top view of the surface: maximum allowed angles $\theta \geq 0$ (i.e., describing counter-clockwise particle rotations) at different center positions $(x,y)$ of the particle in the cage are color-coded. (B) Examples of allowed configurations of the central tri-star particle (red) in a cage of neighbors (black) at $\theta = 0$ deg, 5 deg, and 15 deg. Three different allowed $(x,y)$ configurations on the surface in part A are shown for each angle $\theta$ and labeled with numbers in circles.
Figure 4.2 Predicted translational-rotational free volume of a hexagonal (HX) crystal of tri-star particles, calculated using the static cage model. (A) Schematic showing a mobile central tri-star particle (red) within a fixed cage of neighboring particles (black) in the HX phase. The area fraction shown is $\phi_A = 0.313$. (B) Top, side, and ortho views (from top to bottom) of the shapes of the translational-rotational free volumes of a caged tri-star calculated using the collision detection routine at $\phi_A = 0.313$. (C) Views of the smaller translational-rotational free volume at higher $\phi_A = 0.348$. Scale bar is 0.5 µm for parts B and C. The 2D translational and 1D rotational coordinates are shown in the space between B and C; the color scale refers to the maximum allowed rotation angle $\theta$. 
Figure 4.3 Predicted bounding surface defining the volume of accessible microstates in a hybrid 3D translational-rotational space of a central tri-star particle, calculated using a static cage of neighbors in the ASX phase at a particle area fraction $\phi_t = 0.313$. (A) Top view of the surface: maximum allowed angles $\theta \geq 0$ (i.e. describing counter-clockwise particle rotations) at different center positions $(x,y)$ of the particle in the cage are color-coded. The outer light gray regions correspond to portions of the surface having angles $\theta < 0$ (i.e. clockwise rotations). (B) Examples of allowed configurations of the central tri-star particle (red) in a cage of neighbors (black) at $\theta = 0$ deg, 5 deg, and 15 deg. Three different allowed $(x,y)$ configurations on the surface in part A are shown for each angle $\theta$ and labeled with numbers in circles.
Figure 4.4 Predicted translational-rotational free volume of an alternating stripe (ASX) crystal of tri-star particles, calculated using the static cage model. (A) Schematic showing a mobile central tri-star particle (red) within a fixed cage of neighboring particles (black) in the ASX phase. The area fraction shown is $\phi_A = 0.313$. (B) Top, side, and ortho views (from top to bottom) of the shapes of the translational-rotational free volumes of a caged tri-star calculated using a collision detection routine at $\phi_A = 0.313$. (C) Translational-rotational free volume at $\phi_A = 0.348$. Scale bar is 0.5 µm for parts B and C. The 2D translational and 1D rotational coordinates are shown in the space between B and C; the color scale refers to the maximum allowed rotation angle $\theta$.

Results for $\Omega_{\text{ASX}}$ can be fit well using a cubic form $\Omega_{\text{ASX}}(\phi_A) = \Omega^*(1 - \phi_A / \phi_{A,\text{pack}})^3$, where $\phi_{A,\text{pack}} = 0.42$ is the packing limit of hard tri-stars in ASX and $\Omega^* = 2.7 \times 10^7$.

As $\phi_A$ is raised towards the packing limit, the greater accessibility to translational microstates in ASX overcomes the greater accessibility to rotational microstates of HX, and the ASX phase is entropically preferred. To show this, we plot the difference in
entropy per particle between ASX and HX, $S_{\text{ASX}} - S_{\text{HX}} = k_B \ln(\Omega_{\text{ASX}}/\Omega_{\text{HX}})$ as a function of $\phi_\lambda$ in Fig. 4.5B. When $S_{\text{ASX}} - S_{\text{HX}} > 0$, i.e. for $\phi_\lambda$ greater than the predicted transition point $\phi_{\lambda, \text{ASX}(p)} = 0.338$, then the ASX phase is the higher entropy state and is therefore spontaneously selected over HX at the highest densities. After including the effects of surface roughness, our observed ASX crystals have a measured $\phi_{\lambda, \text{ASX}(m)} = 0.334 \pm 0.004$, in good agreement with this prediction. While we do observe some domains of HX at lower $\phi_\lambda$, these are very small and infrequent, and it is likely that the spatial disorder of the FID state, as well as competition from the only slightly lower entropy ASX phase, strongly influences nucleation and growth of HX, thereby inhibiting the formation of large domains of ordered HX at lower $\phi_\lambda$, at least for this aspect ratio.

By contrast to hard tri-stars, strongly attractive tri-stars would condense into large-scale aggregates, and these aggregates would exert a much weaker to almost negligible 2D osmotic pressure that would be difficult to measure. Even adding a weak short-range attraction between particle surfaces, beyond the hard-core repulsion, would significantly alter and reduce $\Pi_{2D}$ in the measured O-EoS. As we have shown in Figures 4.1-4.5, there is a subtle change between the dominance of accessible translational and rotational microstates, so it would not be obvious a priori that ASX would be preferred over HX for all types of tri-stars that have smaller aspect ratios. In fact, this preference has never been predicted theoretically or computationally as a function of aspect ratio of hard tri-star particles. Attractive amyloidogenic proteins are known to form steric zippers\textsuperscript{15,16} and strongly attractive tri-star shapes form static aggregates that have defected ASX-like structures, in which interdigitated arms effectively touch\textsuperscript{10}, rather than centered and fluctuating, as we observe for hard tri-stars. Obviously, ASX would be
preferred over HX over a wider range of $\phi_A$ if strong short-range attractive interactions are introduced to the system of hard particles, since ASX offers the advantage of having attractive arms of neighboring particles aligned in parallel; relative positions of arms of tri-stars in attractive ASX would not be centered on average as they are for a hard system of tri-stars in ASX. The existence of such attractions could be easily detected through a structural parameter characterizing the relative translational offsets of neighboring stripes.

4.4.3 Multi-particle Collective Fluctuations: Translational Sliding Microstates

Although the single-particle static-cage model predicts the dominance of ASX over HX at higher $\phi_A$, it does not include accessible microstates arising from collective motion\textsuperscript{17,18} of two or more tri-stars. In ASX crystals, several tri-stars within one stripe can collectively slide relative to neighboring stripes that point in the opposite direction. By contrast, in HX crystals, collective sliding fluctuations are much more spatially limited by nature of the geometry. Thus, beyond the single-particle cage model, there is an additional entropy difference between the ASX phase and the HX phase primarily due to differences in the numbers of accessible collective sliding microstates.

In an idealized infinite ASX crystal of tri-star particles, if one neglects boundary conditions at infinity, a single stripe can slide relative to other stripes by a sliding length $L_{\text{ASX}}$. This sliding length is approximately equal to $<u>$, even as $\phi_A$ approaches the upper limit corresponding to close packing of tri-stars in ASX. Examples of the collective motion of particles in one stripe relative to other stripes are shown in Figs. 4.6A and 4.6B.
Figure 4.5 Alternating stripe crystal (ASX) is entropically preferred over hexagonal crystal (HX) at high area fractions $\phi_A$. (A) Number of translational-rotational accessible microstates $\Omega_{ASX}$ (solid circles) and $\Omega_{HX}$ (open circles) for a mobile central tri-star particle in a static cage of neighboring tri-star particles in the ASX and HX phases as a function of particle area fraction $\phi_A$. Solid line: least squares fit of $\Omega_{ASX}(\phi_A)$ to a cubic form, $\Omega^*(1 - \phi_A / \phi_{A,\text{pack}})^3$, where $\phi_{A,\text{pack}}$ is a packing limit of hard tri-stars, yielding $\phi_{A,\text{pack}} = 0.42$ and $\Omega^* = 2.7 \times 10^7$. Dashed line: same cubic form fit of $\Omega_{HX}(\phi_A)$, yielding $\phi_{A,\text{pack}} = 0.42$ and $\Omega^* = 3.3 \times 10^7$. Solving for $\phi_A$ when $\Omega_{ASX}(\phi_A) = \Omega_{HX}(\phi_A)$ yields a crossover area fraction predicted by the fit near $\phi_A \approx 0.338$ (vertical dashed line). (B) Entropy difference $S_{ASX} - S_{HX}$ per particle between ASX and HX determined from the calculated number of accessible single-particle translational-rotational microstates using HX and ASX static cage models as a function of $\phi_A$. For $\phi_A < 0.338$, the entropy difference is negative, so the translational-rotational cage model predicts a preference for HX; whereas for $\phi_A > 0.338$, this difference is positive, so the cage model predicts a preference for ASX.
Moving closer to the experimental system, consider a finite ASX crystallite having $N \times N$ particles, and therefore approximately $N$ stripes each containing $N$ particles, again neglecting boundary conditions at the edges of the crystallites. The total entropy difference $\Delta S^\dagger_{\text{ASX-HX}}$ between two different crystallites of ASX and HX, each containing $N^2$ particles, can be approximated by a sum of properly weighted single-particle and collective-particle terms:

$$\Delta S^\dagger_{\text{ASX-HX}} \approx N^2(S_{\text{ASX}} - S_{\text{HX}}) + N(S'_{\text{ASX}} - S'_{\text{HX}}) \approx N^2 k_B \ln(\Omega_{\text{ASX}}/\Omega_{\text{HX}}) + N k_B \ln(L_{\text{ASX}}/L_{\text{HX}}),$$

where $S'_{\text{ASX}}$ and $S'_{\text{HX}}$ represent the entropy associated with collective relative sliding motion of stripes in ASX and HX, respectively, and $L_{\text{ASX}}/L_{\text{HX}}$ represents the ratio of relative sliding lengths permitted for collective motion of stripes in ASX and HX, respectively. Regardless of the single-particle term in $\Delta S^\dagger_{\text{ASX-HX}}$, for tri-stars we consider, the collective sliding term is positive and favors the ASX configuration over the HX configuration. Since the ASX structure permits a greater number of collective sliding microstates than HX, the collective sliding contribution to the entropy could further stabilize ASX beyond the simple prediction of the single-particle translational-rotational static cage model. Thus, even beyond the single particle predictions, relative translational sliding fluctuations of tri-stars, even when they exist as quasi-one-dimensional density waves within a stripe in a finite crystallite give the lower symmetry ASX phase additional preference over the higher symmetry HX phase. For example, a collective 1D density fluctuation is enabled by the ASX structure (Fig. 4.6C), and whole groups of particles can slide relative to others (Fig. 4.6D). Thus, beyond the standard 2D density fluctuations present in the HX phase, the symmetry-broken ASX phase offers additional multi-particle microstates associated with 1D collective sliding fluctuations of tri-stars within stripes.
Figure 4.6 The ASX configuration facilitates collective sliding fluctuations that provide additional microstates to the system beyond the single-particle microstates of the translational-rotational static cage model. (A) A central stripe of tri-stars (red) can collectively shift to the right to the maximum extent possible, so the center of the leftmost red tri-star shown lies outside the region of single-particle fluctuations (blue— a monocolor projection to scale of the region shown in Fig. 4.3A). (B) A central stripe of tri-stars (red) can collectively shift to the left to the maximum extent possible, and again the center of the leftmost red tri-star lies outside the region of single-particle fluctuations (blue). (C) An example of a one-dimensional density wave of tri-stars (red) provides additional accessible translational microstates even if the wave is terminated by two tri-stars that have average equilibrium center positions (black). (D) A first 2D ASX region of tri-stars (red) can slide relative to a second 2D region of ASX tri-stars (black). Coordinated collective motions of large 2D ASX regions along a slip line are thus possible even if infrequent.
4.5 Implications and Future Directions

This work has important general implications for colloidal systems beyond tri-star particles. In particular, the translational-rotational cage model can be used to rapidly predict the entropic preference for certain particle configurations at high particle densities. The results that we have found for tri-star shapes provide a quantitative study of the unique phase behavior for shapes which have slender rays that could interdigitate. These theoretical approaches lead to many exciting future directions that will provide additional insight into the role of hard-core shape in interesting forms of condensed matter. The entropic selection of the ASX phase as the preferred polymorph at very high particle area fractions is an example of a new class of polymorphism: *entropomorphism*.

4.5.1 General Implications for Colloidal Systems

Theoretically, we have presented the first complete translational-rotational cage model for a non-trivial shape: a slender tri-star that has rounded rays. For such slender tri-stars, we have shown that the ASX phase is preferred over the HX phase at the highest particle area fractions because the self-organized ASX phase permits additional translational microstates, both single-particle and multi-particle, compared to HX. We have implemented this cage model using a computer that employs a collision detection routine because the rounded tri-star shape is complicated, but we anticipate that certain other simpler shapes would be amenable to a classical theoretical analysis based on this approach. Regardless of whether or not a computer is used to determine the average
number of accessible translational-rotational microstates per particle, maximization of the total entropy, which is proportional to the logarithm of the number of accessible states via Boltzmann's law, can provide explanations for more subtle aspects of phase behavior of certain particle shapes that cannot be captured by a purely translational cage model or by a purely rotational cage model. Beyond this, we have shown that the static cage can be deformed away from an average equilibrium configuration, and the number of microstates recomputed; so, microstates can contribute to the entropy. For tri-star shapes, collective translational sliding modes along the stripe axis in ASX appear to further stabilize ASX over HX, where such 1D sliding modes are prohibited by nature of the hexagonal geometry. Further work is needed to assess how collective microstates alter the predicted ASX transition point.

We anticipate that this translational-rotational cage model, here implemented for a 2D system, where there are two translational and one rotational degree of freedom per particle (so the boundaries of the accessible microstates can be easily visualized as a volume), can be generalized to 3D systems, where there are three translational degrees of freedom and two or three rotational degrees of freedom. The resulting effective "volume" of translational-rotational microstates would then have 4 or more dimensions, making it more difficult to visualize, yet still relevant for the entropic selection of various phases.

We also anticipate that it would be possible to create an additional computer program, which utilizes the translational-rotational cage model, that would maximize the translational-rotational volume of accessible microstates by computing this for a wide variety of different cage structures, potentially using feedback to optimize the cage structure at a given $\phi_A$. This more sophisticated computer program could rapidly provide
predictive power of the structure of the phases and osmotic equation of state for a wide range of shapes without extensive computations that are often required by Brownian dynamics or Monte Carlo simulations of many particles.

4.5.2 Entropomorphism: A New Class of Polymorphism

Polymorphism in materials science refers to the ability of a material to exhibit more than one morphology or structure. Packing polymorphism is a particular class that refers to a diversity of crystal structures of a solid material that is typically held together by attractive forces. The attractive forces in packing polymorphism are typically interatomic or intermolecular in origin. By contrast, in dispersed systems of hard particles that are compressed by an osmotic pressure to a certain area fraction, entropy maximization, not merely packing, selects the dominant morphology among possible polymorphs. This entropy maximization is related to the geometry of the hard shapes of the constituent particles in the system; it is not simply a packing problem involving attractively interacting constituents. For instance, in two dimensions, as we have shown for hard tri-star shapes, the volume defining accessible translational-rotational microstates of a central particle in the static cage model can be derived from the particle shape; the dominant observed polymorph, the alternating stripe crystal, maximizes that volume at fixed particle area fraction at high 2D osmotic pressure. We have defined entropomorphism to be selection of a particular polymorph by entropy maximization. Entropomorphism is a way of defining polymorphism that governs confined hard particle systems, and it may play an important role, but not the only role, in certain systems of
attractive particles, too. Entropomorphism is thus a form of polymorphism that is based on statistical mechanics, entropic rules of spontaneity, and the second law of thermodynamics. Entropomorphism is therefore fundamentally different than packing polymorphism, and the selection of the alternating stripe crystal over the hexagonal crystal for a system of hard tri-stars at large $\phi_A$ is a very clear example of entropomorphism. Moreover, the selection principles underlying entropomorphism can potentially be applied to liquid crystalline morphologies in addition to crystalline morphologies. In particular, entropic contributions resulting from collective motions of constituent particles, beyond the static cage model of a single particle, may be important in considering the selection of liquid crystalline entropopolymorphs.

4.5.4 Future Directions

For hard tri-stars, a high value of the aspect ratio $\zeta$ can lead to spontaneous symmetry breaking and the selection of one of three axes in the plane as being preferred in order to increase the number of translational and rotational microstates available to the particles. If one were to reduce $\zeta$, it would be possible to move from the highly concave limit towards the convex limit, and eventually hard tri-stars would resemble triangles and most likely prefer a triatic liquid crystal phase rather than an ASX phase. Using the translational-rotational cage model to explore how the system's phase behavior changes as rays of a 3-fold rotationally symmetric hard tri-star particle are systematically shortened (i.e. as $\zeta$ is reduced) is an exciting future direction.
Collective modes could play a significant role in the nucleation and growth of crystallites of tri-stars, and including these in a theory beyond the single particle cage model could potentially refine $\phi_A$ predicted for the preference for ASX over HX, as well as $\phi_A$ associated with the transition between FID and ASX.

4.6 Conclusion

Interdigitation of neighboring tri-stars, facilitated by the inherent concavity in their shape, leads to fundamentally new behavior that cannot be described by simple rotational cage models. Such models have been used to explain dense phases of convex particles in the past. Using a translational-rotational cage model of tri-stars, we have shown that, as $\phi_A$ is increased, ASX yields more accessible microstates per particle than a hexagonal crystal (HX), and, therefore, ASX is entropically preferred over HX, consistent with our experimental observations (Chapter 3). Moreover, the ASX phase better facilitates collective translational sliding motion of more than one tri-star along the axis associated with the stripes, causing further preference for ASX over HX. The entropic selection of ASX over HX is remarkable, since symmetric tri-stars do not have any inherent pointing direction; entropy maximization is enough to cause a special form of symmetry breaking that results in an identifiable pointing direction, or "head", of each particle within a given stripe. Our observations and explanations provide a physical example of entropomorphism, a form of polymorphism in which entropy maximization selects the dominant stable structure in hard-particle systems.
4.7 References


5 Long-Range Chiral Symmetry Breaking in 2D Colloidal Crystals of Achiral Rhombs

5.1 Abstract

Long-range chiral symmetry breaking (CSB) in 2D rhombic colloidal crystals of hard achiral rhomb particles has been recently observed experimentally\(^1\), but the origin of this unanticipated effect has not yet been quantitatively explained. Here, using a translational-rotational cage model (TRCM), we demonstrate the entropic selection of a chiral crystalline polymorph over an achiral crystalline polymorph in dense systems of hard (72-degree) rhombs. For several different particle area fractions, we calculate the number of accessible microstates, \(\Omega\), of a mobile central particle in a static cage of neighboring particles that have different orientational angles, \(\gamma\), relative to the bisector of the crystalline axes. We demonstrate that symmetric maxima appear in the even function \(\Omega(\gamma)\) at non-zero maximal cage orientation angles of \(\gamma_{\text{max}}\) typically less than 10 deg. Boltzmann's law and the second law of thermodynamics thus imply that the chiral polymorph is entropically preferred over an achiral polymorph. These non-zero \(\gamma_{\text{max}}\), which are in good agreement with experiment, indicate that more translational microstates become accessible in the CSB polymorph, because the pointed tips of neighboring rhombs that are slightly rotated away from the bisector reduce translational tip-tip interference. The success of this TRCM in explaining the entropic preference for this CSB morphology, which cannot be explained using purely rotational or purely translational cage models, indicates that it will have significant utility in predicting the behavior of other hard shapes in 2D.
5.2 Introduction

The existence of spontaneous symmetry breaking, where a system loses some or all of a type of symmetry, even though the laws of nature would permit that symmetry, is necessary for highly structured types of condensed matter to form and is important in colloidal phase transitions\textsuperscript{1-8}, liquid crystals\textsuperscript{9-13}, and field theory\textsuperscript{14-15}. When considering the simple crystallization of molecules from a liquid state into a solid lattice (i.e. freezing), the accessible translational microstates of individual molecules become restricted. The molecules adopt a closed packed periodic structure where continuous translational symmetries are broken in favor of discrete ones\textsuperscript{16}. Similarly, continuous rotational symmetry is broken for a transition from an isotropic liquid, which has full continuous translational and rotational symmetry, to a nematic liquid crystal, where an average preferred molecular orientation is selected and becomes long range\textsuperscript{2}. Although, these examples already present many fascinating features of condensed matter systems, it is arguably even more intriguing when a chiral structure forms as a result of symmetry breaking. Understanding how and why chirality emerges in entropically driven Brownian systems is important for the future design and control of microscale complex systems\textsuperscript{17-20} and in protein assembly\textsuperscript{21,22}.

Lithographically fabricated, colloidal dispersions of exotically-shaped Brownian hard-particles present a system that can be relatively easily observed and controlled. Only certain systems of achiral shapes lead to emergent chirality when compressed\textsuperscript{1,6,23-26}. Dispersions of stabilized, achiral, microscale, platelet particles that are concentrated to high densities in a quasi-2D system have been observed to exhibit both local short-range\textsuperscript{6} and nonlocal quasi-long range\textsuperscript{1} chiral symmetry breaking (CSB). Specifically, a local
form of CSB was observed in a system composed of a dense 2D arrangement of triangular, achiral colloids. When compressed to sufficiently high densities, the triangles spontaneously arrange themselves to form a fluctuating racemic mixture of offset dimer pairs. A trioid model, which considers only rotational contributions to the entropy, was sufficient to explain the entropic selection of these chiral pairs. In another system, involving a similar experimental setup which used achiral, 72-degree rhomb colloids instead, a robust long-range CSB was observed. At high densities, a rhombic crystal was found to entropically select one of two chiral polymorphs where the rhombic lattice was measured to have a slight deviation in "molecular" orientation from the average bisector of the lattice direction along the diagonal. This observation of long-range CSB, different than the local CSB of triangles, could not be explained with a simple rotational rhomboid model, so the authors hypothesized that to completely explain this effect would require a model that included contributions from both translational and rotational entropy. Specifically, they speculated that tip-tip interference between rhombs led to the slight tilting of the average orientation of rhombs with respect to the lattice, yielding chiral symmetry breaking. This experimental system motivates our present study as we aim to explain the entropic origin of this chiral symmetry breaking quantitatively.

Previous work on the self-organization of tri-star colloids (Chapter 4 of this thesis) serves as a starting point for the analysis of the rhombic system, using basic principles of statistical mechanics. We have adapted the translational-rotational cage model (TRCM) to study rhomb particles. This TRCM allows one to simulate a two dimensional crystal lattice using a static cage of neighboring particles and, through collision detection and discretization of space and angle, determine the number of states
available to a translating and rotating central particle. For tri-star particles, the TRCM largely explains the entropic selection of an unanticipated alternating stripe crystal phase over a simple hexagonal crystal. While other models exist for determining the role that entropy plays in colloidal systems\textsuperscript{20,28-30}, this model has the advantage of being straightforward. Including both translational and rotational contributions results in a more complete treatment of the entropic preference within a hard-particle system than either purely translational or purely rotational cage models. Although collective motions are ignored, a static cage model is a significant improvement.

Here, we show that more accessible total microstates per particle are created if the average orientation of rhombs within the lattice are slightly offset from the bisector to the lattice axes, as the area fraction, $\phi_A$, is increased. This is consistent with observations of the experimental system. We visually represent the results of the calculations as a bounding surface of accessible microstates which illustrate the interplay between both translational and rotational contributions to the entropy of the mobile particle. To better link the results with experimental observations, we convert the calculated total number of accessible states into a normalized probability distribution for the probability of finding a rhomb with a particular orientation within a rhombic lattice at a given particle density. The results explain the entropic selection of a chiral symmetry broken phase in the rhombic crystals. The increased number of available microstates per particle results when rhombs within the rhombic lattice adopt a slightly rotated configuration; this minimizes translational tip-tip interference between adjacent rhombs, thus confirming a speculation based on experiments\textsuperscript{1}. While this chapter focuses on rhombs within a rhombic lattice, the theoretical methods presented here can be extended generally to a wide variety of
shapes and lattices. The TRCM potentially offers a faster means of predicting entropically preferred polymorphs, compared to Monte Carlo or other many particle simulation methods, albeit in the limit when collective contributions to the entropy can be ignored.

### 5.3 Methods

To explain the long-range chiral symmetry breaking observed experimentally in 2D rhombic colloidal crystals, we use a collision detection routine to calculate the shapes of the accessible translational-rotational volumes and the total number of accessible translational-rotational microstates available to a central rhomb platelet particle for several static rhombic lattice cage configurations for different cage orientation angles, $\gamma$, at various $\phi_d$. A prior experiment\textsuperscript{1} defines the geometry and dimensions of the achiral rhombs used for the TRCM. The rhomb particle's edge lengths and angles are matched to experiment. Although in the experiment, the shape of rhombs exhibit corner rounding (a result of the diffraction limit of photolithographic fabrication), for these calculations we use idealized rhombs to simplify the collision detection. Additionally, while the actual experiments used three-dimensional rhomb platelet particles confined in a quasi-2D system, the translational-rotational cage model approximates the particles as fully two-dimensional, and the surrounding liquid is ignored.

The static rhombic cage of rhombs surrounding the mobile central test particle is set by two parameters; the particle area fraction, $\phi_d$, a measure of the density of rhombs within the lattice and $\gamma$, the orientational angle of the lattice. A third parameter, $\alpha$, defines
the rhombic lattice angle and its value depends on the empirically measured area fraction of the system\textsuperscript{1}. A schematic depiction of the lattice at $\phi_A = 0.75$ and $\gamma = 0$ deg used in the model is shown in Fig. 5.1A. As shown in Fig. 5.1B, both $\theta$ and $\gamma$ are defined with respect to the bisector of the angle supplementary to $\alpha$; however $\gamma$ (for the static cage particles) remains unchanged throughout any single collision detection calculation. By contrast, $\theta$ (for the central mobile particle) is systematically varied continuously throughout the calculation. Cage particles are not allowed to touch each other.

**Figure 5.1** A translational-rotational cage model is used to approximate the rhombic lattice observed experimentally by a static cage of rhombs (black) surrounding a central mobile rhomb platelet particle (red), which is translated and rotated within the static cage. (A) Eight stationary cage rhombs are arranged so that their center positions lie along the lattice points of a 2D rhombic crystal. The mobile central rhomb (center position - black dot) is translated and rotated through the available space defined by the cage. (B) The locations for the lattice points are determined by choosing an area fraction, $\phi_A$, and using the rhombic lattice angle, $\alpha$, corresponding to measurements at that $\phi_A$. The cage orientational angle, $\gamma$, which refers to the orientation of the cage particles, is the bisector to the supplementary angle of $\alpha$. The center particle is rotated by an angle $\theta$, where clockwise rotation corresponds to $\theta > 0$. The lattice shown in part A corresponds to $\phi_A = 0.75$, $\gamma = 0$ deg and $\theta = 0$ deg.
An initial configuration for the lattice is assembled at a particular $\phi_A$ and $\gamma$ and a collision detection routine translates a central rhomb by 10 nm increments in $x$ and $y$ independently and rotates it by 0.5 deg increments in $\theta$, provided there is no collision with the static cage. For each center position and angle, the routine checks for any intersections between the central particle and all neighboring particles. Any translational and rotational microstates of the central particle that do not result in a collision with rhombs in the cage are treated as valid and accumulated into the total number of microstates ($\Omega$) of the hard particle system.

The collision detection routine, written in Mathematica, systematically probes a bounded region of translational-rotational phase space using nested loops. This space is large enough to ensure complete coverage of the available states yet not so large to be computationally inefficient and records all acceptable positions and orientations. However, we do not allow $\theta$ to range to very high values corresponding to metastable configurations (e.g. orientational point defects) of the central rhomb. For configurations at higher densities, where the discretizations used for shifting position and angle are too large to provide an adequate number of states, the incremental spacing is decreased and the measured number of microstates are rescaled according to the ratio in voxel size between the different discretizations, so that all results can be presented on the same scale.

The set of all the accessible positions and angles available to the central particle within a cage at a particular $\phi_A$ and $\gamma$ represents a point-cloud microstate volume in a special combined three-dimensional space $(x, y, \theta)$. When this point-cloud volume is displayed, it reveals a bounding surface of accessible microstates. The volume of the
microstates is proportional to $\Omega$ and direct comparisons between these volumes are useful for determining entropic preferences between different states using Boltzmann's law: $S = k_B \ln \Omega$. Entropy maximization selects the preferred polymorph for a system, which can explain the long-range CSB observed experimentally in rhombic colloidal crystals.

5.4 Results

5.4.1 Bounding Surfaces for Several Cage Configurations

The relationship between translational and rotational microstates available to a central particle within a rhombic lattice is easiest to interpret when the results are reported as a bounding surface of accessible microstates in a special mixed three-dimensional space. The three-dimensional space consists of two translational coordinates $(x, y)$ centered on a lattice point and one rotational angle ($\theta$), where $\theta = 0$ deg corresponds to a pointing direction along the bisector of the lattice. The translational-rotational free volume per particle encapsulated within the bounding surface is proportional to the number of accessible microstates per particle in a rhombic lattice at a particular $\phi_A$ and $\gamma$. Any additional microstates that might be available in the experimental system due to collective sliding fluctuations are implicitly ignored in the model due to the static cage assumption. Therefore, neglecting collective fluctuations, it follows that the thermodynamically preferred lattice configuration at a given $\phi_A$ is the one that has the largest translational-rotational free volume per particle in this mixed 3D space.

Examples of translation-rotational bounding surfaces for a central particle in a lattice at $\phi_A = 0.75$ and $\gamma = 0$ deg, -8 deg, and -15 deg are shown in Figs. 5.2, 5.3, and 5.4,
respectively. Seen in part A of each figure is an ortho-angled view of the complete bounding surface, alongside a deconstructed view that highlights the bounding translational extent of a central rhomb particle rotated by a constant $\theta$ angle. The absolute value of the maximum allowed angle for the rotation of the central particle is color-coded on the surface.

To better display the information in the translational-rotational free volumes, Figs. 5.2B, 5.3B, and 5.4B show schematic depictions of the rhombic lattice configuration corresponding to select locations on the bounding surfaces. The shape of the translational extent allowed, for a central rhomb rotated by a certain $\theta$, is clearly described by example positions (1, 2, 3) in the adjacent lattice depictions. Implicit in these representations is the fact that all states within the bounding surface are also allowed microstates.

As shown in Figs. 5.2 - 5.4, the translational-rotational free volumes that result from the collision detection routine can contain multiple allowed rotational angles $\theta$ for every $(x, y)$ center position allowed, resulting in a complicated multi-valued surface that exhibits some concavity in shape. The constant-$\theta$ planes shown in Figs. 5.3A, 5.5A, and 5.5A illuminate how the geometry of lattice can restrict the total number of translational microstates accumulated for a particular configuration. Constant-$\theta$ planes for certain lattice configurations show an indentation in the translational extent available to the central rhomb; translational states are disallowed due to the geometrical constraints of
Figure 5.2 The bounding surface defining the volume of accessible microstates in a hybrid translational-rotational space of a central rhomb particle, calculated using a static cage of neighbors in a rhombic lattice at a particle area fraction $\phi_A = 0.75$ ($\alpha = 92$ deg) and having a cage orientational angle $\gamma = 0$ deg. (A) Top-front view of the surface showing the maximum allowed rotation angles ($\theta$) at different center positions ($x, y$) of the particle in the cage. The surface is color coded according to the magnitude of maximum rotation allowed. To better visualize within the volume, the surface is deconstructed, from the bottom up, into zones of similar angle (the top zone has been shifted off to the side for clarity). The bounding center-position trajectories are highlighted on each slice with blue, green, and yellow lines at $\theta = 0$ deg, 6 deg, and 12 deg, respectively. (B) Examples of allowed configurations of the central rhomb particle (red) in a cage of neighbors (black) at $\theta = 0$ deg, 6 deg, and 12 deg. Three different allowed ($x, y$) configurations on the surface of part A are shown for each angle $\theta$ and are labeled with numbers in circles. Scale bar is 0.5 $\mu$m for part A and 1 $\mu$m for part B.
the lattice. This is a principal feature of the rhombic lattice, which was not observed in previous work involving a crystal lattice of tri-star particles\textsuperscript{27}, and is primarily due to steric tip-tip interference caused by interactions between the central particle and the two static rhomb particles in the lattice which lie along the diagonal angle, supplementary to $\alpha$. The most dramatic example of tip-tip interference is seen in Fig. 5.2A for the microstate surface generated at $\phi_A = 0.75$, $\gamma = 0$ deg, and $\theta = 0$ deg. In this example, because $\theta = 0$ deg, the interference between the tips of diagonal rhombs in the lattice creates a large indentation (i.e. gouge) in the shape of this volume where translational states are not valid. In fact, the tip-tip translational interference, between the central rhomb and its neighbors, is the primary source of the experimentally observed chiral symmetry breaking. Entropy maximization favors a slight rotation in the average equilibrium pointing directions of neighboring rhombs away from the lattice bisector so as to minimize this tip-tip interference.

Top, ortho, side, and bottom views of the surfaces defining the maximum translational-rotational extent available to a central rhomb particle in a rhombic lattice at $\phi_A = 0.75$ and $\gamma = 0$ deg, -8 deg, and -15 deg are shown in Fig. 5.5. At $\phi_A = 0.75$, as $\gamma$ is decreased from 0 deg (corresponding to a counter-clockwise rotation in the lattice orientation), the total number of microstates available to the central particle increases to a maximum at $\gamma_{\text{max}} = -8.44$ deg and then decreases thereafter. Overall, the translational-rotational free volume corresponding to the rhombic lattice at $\phi_A = 0.75$ and $\gamma = -8$ deg offers more accessible microstates than the lattice at $\gamma = 0$ deg and $\gamma = -15$ deg. Here, the shapes for negative $\gamma$ were shown, but one would expect similar results had positive $\gamma$ been shown instead.
Figure 5.3 The bounding surface defining the volume of accessible microstates in a hybrid translational-rotational space of a central rhomb particle, calculated using a static cage of neighbors in a rhombic lattice at a particle area fraction $\phi_A = 0.75$ ($\alpha = 92$ deg) and having a cage orientational angle $\gamma = -8$ deg. (A) Top-front view of the surface showing the maximum allowed rotation angles ($\theta$) at different center positions ($x$, $y$) of the particle in the cage. The surface is color coded according to the magnitude of maximum rotation allowed. To better visualize within the volume, the surface is deconstructed, from the bottom up, into zones of similar angle. The bounding center-position trajectories are highlighted on each slice with yellow, green, and blue lines at $\theta = -12$ deg, $-8$ deg, and $6$ deg, respectively. (B) Examples of allowed configurations of the central rhomb particle (red) in a cage of neighbors (black) at $\theta = -12$ deg, $-8$ deg, and $6$ deg. Three different allowed ($x$, $y$) configurations on the surface of part A are shown for each angle $\theta$ and are labeled with numbers in circles. Scale bar is 0.5 $\mu$m for part A and 1 $\mu$m for part B.
Figure 5.4 The bounding surface defining the volume of accessible microstates in a hybrid translational-rotational space of a central rhomb particle, calculated using a static cage of neighbors in a rhombic lattice at a particle area fraction $\phi_A = 0.75$ ($\alpha = 92$ deg) and having a cage orientational angle $\gamma = -15$ deg. (A) Top-front view of the surface showing the maximum allowed rotation angles ($\theta$) at different center positions $(x, y)$ of the particle in the cage. The surface is color coded according to the magnitude of maximum rotation allowed. To better visualize within the volume, the surface is deconstructed, from the bottom up, into zones of similar angle. The bounding center-position trajectories are highlighted on each slice with red, orange, and blue lines at $\theta = -18$ deg, $-15$ deg, and $-3$ deg, respectively. (B) Examples of allowed configurations of the central rhomb particle (red) in a cage of neighbors (black) at $\theta = -18$ deg, $-15$ deg, and $-3$ deg. Three different allowed $(x, y)$ configurations on the surface of part A are shown for each angle $\theta$ and are labeled with numbers in circles. Scale bar is 0.5 $\mu$m for part A and 1 $\mu$m for part B.
Figure 5.5 Predicted translational-rotational free volume of a rhombic crystal of rhombs, calculated using the static cage model. (A) Top, ortho, side, and bottom views (from top to bottom) of the shapes of the translational-rotational free volumes of a caged rhomb calculated using a collision detection routine at $\phi_A = 0.75$ and $\gamma = 0$ deg. (B) Translational-rotational free volume at $\phi_A = 0.75$ and $\gamma = -8$ deg. (C) Translational-rotational free volume at $\phi_A = 0.75$ and $\gamma = -15$ deg. The 2D translational and 1D rotational coordinate systems are shown in part A. The color scale refers to the maximum allowed rotation angle $\theta$ and is shown in the space between B and C; scale bar is 0.5 $\mu$m for top and bottom views (side and ortho views are not to scale). The dashed black lines denote the plane corresponding to $\theta = 0$ deg for each volume.
For $\gamma = 0$ deg there is reflection symmetry in the shape of the bounding surface about the $\theta = 0$ deg plane, which is lost as $\gamma$ shifts to a non-zero angle. For $\gamma = 0$ deg, the greatest extent of translational microstates in $(x, y)$ is found at $\theta = 0$ deg. But as $\gamma$ decreases, the bulk number of microstates available shift from 0 deg to lower $\theta$, as seen clearly in the side views of Fig. 5.5. Additionally, the top half ($\theta > 0$ deg) and the bottom half ($\theta < 0$ deg) of the bounding surfaces loses reflection symmetry as a result of asymmetric rotational boundary conditions for the central mobile rhomb within the cage when it rotates clockwise ($\theta > 0$ deg) compared to counter-clockwise ($\theta < 0$ deg).

In Fig. 5.5, one can see the effect of the tip-tip interference in the bounding surfaces as $\gamma$ is varied. For $\gamma = 0$ deg, in Fig. 5.5A, the bulk number of microstates is centered symmetrically around $\theta = 0$ deg, although a significant portion of the potentially allowed translational states is missing due to the significant tip-tip interference found at this rotation. As the lattice is rotated to $\gamma = -8$ deg in Fig. 5.5B, near the entropically preferred $\gamma_{\text{max}} = -8.44$ deg, the bulk number of microstates available shifts to lower $\theta$, as the lattice cage shifts to accommodate the central particle by reducing tip-tip interference. Further lattice rotation to $\gamma = -15$ deg in Fig. 5.5C shows mild tip-to-tip interference at all $\theta$’s, however, at large $\gamma$, the translational extent available to the central particle has been substantially reduced at all rotation angles, thereby resulting in a significant loss in available microstates. It is worth noting that although we primarily chose to display example results for lattices having negative $\gamma$, this choice remains arbitrary. As one would expect from the symmetry of a rhombic lattice.
composed of achiral rhombic platelets, surfaces for the positive $\gamma$ lattices would result in symmetric reflected versions of the ones shown.

### 5.4.2 Determining Entropically Preferred Configurations

The TRCM routine for rhombs is executed for several $\gamma$ at several $\phi_A$ spanning from the experimentally observed onset of the rhombic crystal phase ($\phi_A = 0.62$) up to extremely high densities ($\phi_A = 0.90$) and translational-rotational free volumes are calculated throughout. The results of collision detection calculations for several rhombic lattices having different $\gamma$ at a constant $\phi_A$ yield $\Omega(\gamma)$. The results determine the entropic preference of a rhombic lattice configuration as $\gamma$ is shifted. A spline-fit interpolation of the calculation results determines the $\gamma_{\text{max}}$ at which the maxima in $\Omega(\gamma)$ are found. An example $\Omega(\gamma)$ interpolation for a rhombic lattice at $\phi_A = 0.75$ is shown in Fig. 5.6. Each blue point in the plot corresponds to a separate collision detection calculation at $\phi_A = 0.75$ for different values of $\gamma$. The same process is repeated for lattices at different $\phi_A$, from the experimentally discernible onset of the rhombic crystal phase at $\phi_A = 0.62$ to a very dense phase beyond experiments at $\phi_A = 0.90$. For the higher density lattices ($\phi_A = 0.75 - 0.90$), the density of $\gamma$ and $\theta$ angles in increased in order to ensure accurate interpolations for $\Omega(\gamma)$ at higher $\gamma$.

The total number of states within a translational-rotational free volume at a particular $\phi_A$ and $\gamma$ is collected and plotted as shown in Fig. 5.7A. As the area fraction increases, the appearance of maxima in $\Omega(\gamma)$ at non-zero gamma is observed and the
magnitude of these peaks relative to the number of states measured at zero gamma, $\Omega_0$, is observed to increase. As $\phi_A$ is increased from 0.62 to 0.90, the peak number of accessible

**Figure 5.6** Repeating the collision detection routine for several cage orientational angles, $\gamma$, yields the number of microstates for a mobile central particle within a static rhombic lattice at $\phi_A = 0.75$. Each blue point is the result of a collision detection calculation. The black line is the result of a spline-interpolation fit to the data and used to determine the orientational lattice angle corresponding to the peak number of microstates, $\gamma_{\text{max}}$ (red squares). For a central particle allowed to translate and rotate within a cage at $\phi_A = 0.75$, the maximum number of states are found at $|\gamma_{\text{max}}| = 8.5$ deg. Examples of bounding translational-rotational surfaces for $\gamma = 0$ deg, $\gamma = -8$ deg, and $\gamma = -15$ deg are shown below the curve. Dashed black lines denote the $\theta = 0$ deg plane along each surface.
Figure 5.7
Figure 5.7 The translational-rotational cage model reveals an entropic preference for an offset pointing angle of rhombs within a rhombic lattice, explaining the experimentally observed chiral symmetry breaking. (A) The number of accessible microstates (Ω) for a rhomb particle within a static cage is measured for many different cage configurations. Each point (blue) represents a different configuration where area fraction (φA) and cage orientational angle (γ) are varied. Interpolated peaks (red squares) in each plot correspond to the entropically preferred γ angle at each φA. (B) The absolute value of the cage orientational angle corresponding to the entropically preferred state (|γmax|) monotonically decreases as φA increases. A linear fit, having the constraint of γmax = 0 at φA = 1, yields a slope describing an average decrease of 1.6 deg in γmax for every 5% increase in φA. (C) The peak number of states normalized by number of states at γ= 0, \( \Omega_{\text{max}}/\Omega_0 \), gives a relative measure of the entropic preference at γmax as a function of φA. The general trend increases as area fraction increases. A linear fit to the initial data points (solid line) predicts a zero entropic cost to vary γ at φA ≤ 0.62. For φA < 0.62, Ω(γ) is almost flat in part A, corresponding to a nearly uniform distribution.

The microstates observed, \( \Omega_{\text{max}} \), decreases from \( 1.0 \times 10^4 \) to \( 5.0 \times 10^1 \). The absolute value of the orientational angle, \(|γ_{\text{max}}|\), that corresponds to the peak number of accessible microstates monotonically decreases from 11.2 deg to 2.8 deg as φA approaches 0.90, as shown in Fig 5.7B. No discernible peak is observed for φA = 0.62 when interpolating Ω(γ), so this φA is therefore not included in analysis regarding peak number of microstates. The linear fit is constrained so that γmax = 0 at φA = 1, as required by the tiling geometry of achiral rhombs. Figure 5.7C shows that as φA is increased from 0.65 to 0.90, the ratio \( \Omega_{\text{max}}/\Omega_0 \) monotonically increases from 1.05 to 1.23, although in a non-linear fashion. From the linear fit applied to the initial data points it is seen that \( \Omega_{\text{max}}/\Omega_0 = 1 \) at φA = 0.62.

To connect the results to experiment more directly, results from the collision detection routine can be converted into a normalized probability distribution representing the likelihood of observing a rhomb particle with a particular orientation within a
rhombic lattice, as illustrated in Fig. 5.8. The probability $p_\gamma(\gamma)$ is determined by solving for the Boltzmann probability, $p_\gamma(\gamma) \sim \exp\left(-\Delta E_\gamma / k_B T\right)$, where the change in free energy $\Delta E_i$ is simply related to the change in entropy ($\Delta E_\gamma = -T\Delta S_\gamma$), a result of the absence of attractions in a hard-particle system. The change in entropy of the system is defined by $\Delta S_\gamma = k_B \ln \left[\Omega(\gamma) / \Omega_{\text{ref}}\right]$, where $\Omega(\gamma)$ is the number of microstates calculated for a given rhombic lattice having $\gamma$ at $\phi_A$ and $\Omega_{\text{ref}}$ corresponding to the single state available for $\gamma = 0$ deg at $\phi_A = 1$. Including a constant, $C$, set by the normalization condition, yields a straightforward expression for the probability: $p_\gamma(\gamma) = C \Omega(\gamma)$. An example of this conversion for the rhombic lattice at $\phi_A = 0.80$, chosen because the $\gamma_{\text{max}}$ calculated is closest to the $\gamma$ observed experimentally, is shown in Figs. 5.8A and 5.8B. Remarkably, the resulting dual-peaked probability distribution can be fit surprisingly well to a simple, two parameter ($|\gamma_{\text{avg}}|, \sigma$) fit involving the sum of two Gaussian functions:

$$p_\gamma(\gamma) = \frac{1}{\sqrt{2\pi}\sigma} \left[ \exp\left[-\frac{(\gamma + |\gamma_{\text{avg}}|)^2}{2\sigma^2}\right] + \exp\left[-\frac{(\gamma - |\gamma_{\text{avg}}|)^2}{2\sigma^2}\right] \right].$$

The fit to the data results in two mean values corresponding to the peaks in the probability distributions at $|\gamma_{\text{avg}}| = 6.06$ with a standard deviation $\sigma = 4.53$. Furthermore in Fig 5.8C, the dual-peaked probability distribution is decomposed into a single Gaussian function, by plotting a normal Gaussian distribution using the $|\gamma_{\text{avg}}|$ for a single peak. This produces a single-peaked distribution which more closely resembles the experimentally measured result in which a single chiral enantiomeric crystal has been selected during the crystallization process.
Figure 5.8
**Figure 5.8** Results from the collision detection routine can be converted into a normalized probability distribution for comparison with experiment. (A) The number of microstates ($\Omega$) available to a central particle in a rhombic lattice at $\phi_A = 0.80$ is calculated for several $\gamma$ (blue circles). A spline-fit interpolation of the data (dashed line) determines the most probable value of $\gamma$ (red squares). (B) From the calculated microstates available at each $\gamma$, a normalized probability distribution is obtained (blue circles). A spline interpolation (solid black line) of the probability distribution highlights two peaks of increased probability corresponding to the two possible enantiomeric senses of the rhombic crystal expected at this density. The probability distribution is fit to the sum of two Gaussian functions (red dashed line). This two-parameter function fits reasonably well. (C) The normalized probability distribution experimentally observed for the rhombic crystal at $\phi_A = 0.63$ (orange circles) is fit to a normal Gaussian function (black line). The dual-peaked probability distribution in part B is decomposed into a single Gaussian function (blue line), which effectively selects out a single enantiomeric sense of the rhombic crystal, comparable to the experimental result. For comparison, the experimental data for a rhombic crystal having an average relative orientation $\gamma_{\text{avg}} = -5.55$ with a standard deviation $\sigma = 5.83$ at $\phi_A = 0.63$ is co-plotted with the calculated data where $\gamma_{\text{avg}} = -6.06$ with a standard deviation $\sigma = 4.53$ at $\phi_A = 0.80$.

### 5.5 Discussion

The TRCM successfully explains the entropic selection of a long-range chiral polymorph by the appearance of two maxima, at non-zero $\gamma$, in the plot of accessible microstates $\Omega(\gamma)$, as $\phi_A$ is increased. The maxima in $\Omega(\gamma)$ represent the entropically preferred CSB lattice configurations at each $\phi_A$. Each of the two peaks in the calculated distribution corresponds to one of the possible enantiomeric chiral senses of the rhombic lattice that could be observed experimentally (one peak in $p(\gamma)$ with either $\gamma_{\text{max}}> 0$ deg or $\gamma_{\text{max}} < 0$ deg). By decomposing these two peaks into individual Gaussian functions, one can recover the single distribution expected to be observed experimentally after a system of equilibrated rhombs becomes locked into a single enantiomeric sense of a rhombic crystal. Considering how the TRCM does not account for particle corner rounding and collective fluctuations, it's striking that the results succeed at qualitatively explaining the
source of the CSB observed in experiment, where other models which rely on only translations or only rotations alone, cannot.

The absolute value for the location of the peaks are found to decrease linearly until $\gamma_{\text{max}} = 0 \, \text{deg}$ at $\phi_A = 1$, at which point there would be no CSB observed because the completely tiled lattice would yield a single achiral crystalline polymorph (only a single microstate exists). Experimentally, average values for $\gamma$ were observed at $\gamma = 5.77 \, \text{deg}$ and $\gamma = -5.55 \, \text{deg}$ for a lattice measured at $\phi_A = 0.62$ and $\phi_A = 0.63$, respectively. The widths of the measured and predicted probability distributions are very similar. According to a linear fit to the data, the TRCM predicts these most probable angles to be observed around $\phi_A = 0.82 - 0.83$. Possible reasons for this difference are discussed below.

Even though the TRCM predicts the CSB observed experimentally in a rhombic crystal, it does not include accessible microstates arising from collective fluctuations\textsuperscript{31,32} of two or more rhombs. These collective fluctuations, which can become quite complicated to quantify as the configuration of the lattice is shifted, would certainly increase the number of accessible states available to a rhomb in the rhombic lattice. Most likely, the extent of these fluctuations would also be highly dependent on the amount of tip-tip interference present in a rhombic lattice having a particular configuration. Imagining the two most straightforward collective fluctuations possible in the rhombic lattice, translations along either of the two dominant crystal axes, one would see that collective translations are facilitated when the lattice is shifted to minimize tip-tip interference at $\gamma = -8 \, \text{deg}$ (Fig. 5.3B) when compared to a lattice at $\gamma = 0 \, \text{deg}$ (Fig. 5.2B). Including the number of increased microstates due to collective fluctuations could
increase the relative microstate difference at $\gamma_{\text{max}}$, by preferentially adding more states to configurations where tip-tip interference is minimized (peaks) and adding a reduced set of states for all other configurations. This would have the effect of increasing the entropic preference for the enantiomeric sense selected at a particular $\phi_A$ and would potentially decrease the predicted $\phi_A$ necessary to observe CSB, increasing the quantitative agreement with experiment. Although we haven't determined how the subtle contributions to the entropic stability arising from collective fluctuations would precisely affect the results, we would certainly except the widths of $p_{\gamma}(\gamma)$ to broaden since collective fluctuations would allow access to large rotations with the rhombic lattice. While including even two particle fluctuations in a modified TRCM capable of collective-fluctuations could be done, the extension of the model to larger fluctuations would be challenging, as keeping track of larger numbers of mobile particles translating and rotating while systematically sampling the available phase space would quickly increase the computational complexity.

Other possible factors contributing the difference in measured and predicted $\phi_A$ associated with CSB have to do with the input of experimentally measured lattice and particle parameters into the model. While the lattice angle, $\alpha$, was measured experimentally for lower $\phi_A$, an interpolation was required to estimate $\alpha$ for higher densities. The fact that peaks in Fig.5.7A are slightly asymmetric about $\gamma = 0$ deg for higher densities reflects the fact the $\alpha$ interpolation as a function of $\phi_A$ is not ideal at these densities, which could lead to small deviations in the $\phi_A$ predicted for chiral symmetry breaking.
Particle corner-rounding in the experiments could also increase the resulting total number of microstates calculated via the TRCM. Rhombs from the experiments exhibit corner rounding at particle vertices as a result of particle dimensions which lie close to the minimum feature size of the UV stepper used for rhomb fabrication. Corner rounding has been included for TRCM calculations with tri-stars previously (Chapter 4) but at the expense of computational efficiency. Where two line segments are sufficient to describe a vertex for a shape without corner rounding, many more line segments must be included to approximate a rounded vertex and this increases the number of collision detection calculations required to test if each translational-rotational is acceptable. Here, it was sufficient to use rhombs without corner-rounding to explain the CSB observed, but we expect that repeating the calculations again with corner rounding could yield even more allowed microstates near configurations with high tip-tip interference and potentially change the extent of 'gouging' seen in the shape of the translational-rotational volume (e.g. in Fig 5.2A). This could result in better agreement.

Similarly, the effective $\phi_A$ reported in the experiment could be different than the bare area fraction used as an input for the model. Because the model approximates the rhombs as perfectly formed, complete, and smooth-edged particles (a small departure from the actual characteristics of the experimental rhombs) an effective area per particle may be necessary to account for these deviations. Previously, it was necessary to account for particle edge roughness when calculating the effective area per particle relevant for describing phase behavior resulting from collisions; this could change the area per particle by up to ~10-15%, shifting the experimentally reported $\phi_A$ up closer to the calculated ideally smooth values. Additionally, it would have been helpful if
experiments had been conducted at higher osmotic pressures, too, so that we could better compare our predictions for the reduction in the entropically preferred $\gamma_{\text{max}}$ toward higher $\phi_A$.

Our calculations predict that as $\phi_A$ is decreased, the relative entropic preference at $\gamma_{\text{max}}$ is reduced and $\Omega(\gamma)$ becomes independent of $\gamma$. This is similar to the result one would expect for rhombs in a hexagonal rotator crystal phase where a single rhomb would be able to rotate completely and, therefore, show no dependence of $\Omega$ on $\gamma$. This behavior is expected for a rhomb in the experimentally observed hexagonal rotator crystal phase (RX), which is observed to occur at lower area fractions than the rhombic crystal phase\(^1\). However, the TCRM as implemented here cannot properly handle two-phase coexistence between RX and the rhombic crystal. Even so, one should be careful not to confuse the results of the collision detection routine at lower densities for a prediction of this transition; the near-independence of $\Omega$ on $\gamma$ corresponds to a uniform distribution, the central rhomb would be so far from the neighboring rhombs that it could rotate completely without a collision. Moreover, the presumed lattice of TCRM in our calculations at lower densities would still correspond to a rhombic lattice and this may not correspond to the lattice symmetry selected by entropy maximization there. To completely capture a phase transition with the translational-rotational cage model one would need to include inputs modeling how the lattice parameters shift through the transition, which may be challenging, especially if the transition passes through a coexistence region involving a mixture of two or more phases.

The translational-rotational cage model has proven itself to be a valuable tool for providing physical insight into why a hard-system of 72-degree rhombs entropically
prefers to form a single chiral enantiomeric sense of a rhombic crystal at high densities. While these results are useful for explaining experimental observations, further improvements to the routine could turn it into a better and more highly refined predictive tool in the future. Optimizing the algorithm to increase computational efficiency could maximize the capability of the routine, allowing it to calculate more states in less time, opening the opportunity to continuously vary several parameters at once, including: particle shape, lattice type, lattice parameters, lattice density, etc. This could be used, for example, to correct the empirical $\alpha$ interpolation assumed, or in a more ambitious goal, to determine the two-dimensional phase predicted for a uniquely-shaped particle within a particular lattice. An exciting future prospect remains the ability to self-optimize a particular lattice (by changing lattice angles, area fractions, lattice orientations, etc.) and search for the most entropically preferred phase out of a myriad of possibilities.
5.6 Conclusion

We have explained the entropic selection of a chiral crystalline polymorph that has been experimentally observed in a system of rhombs that self-organize into a rhombic lattice by using a translational-rotational cage model. This model, which accounts for both translational and rotational degrees of freedom in the system, determines the number of microstates available per particle. The number of microstates have been converted to a normalized probability distribution and compared to experiment. Results indicate an increase in accessible translational microstates, corresponding to a greater entropic preference for a chiral symmetry broken crystal, when the rhombic lattice orientation was shifted to a slightly offset angle, consistent with observations made experimentally. Representations of the bounding surface of a volume of accessible microstates confirm that lattice reorientation to an offset angle result in a maximization of system entropy by minimizing tip-to-tip translational interference between adjacent rhombs which would act to restrict translational microstates available to the central particle. This mechanism of CSB is different than for triangles, where a slight translational shift between two neighboring triangles increased the accessible rotational microstates. As is, the translational-rotational cage model has proven itself to be a valuable tool for explaining the thermodynamically equilibrated structures that form in hard-particle systems. We anticipate that it may provide insight into a wide variety of systems beyond tri-stars and rhombs in the future.
5.7 References


6  General Summary

The first half of this thesis has focused on experimental measurements of lithographically fabricated colloidal platelet particles having unique shapes and the observations of the two-dimensional phases which form as these particles are compressed under high osmotic pressures. The second half has focused on computational modeling, specifically the translational-rotational cage model (TRCM), which explains the different types of entropic self-organization observed in dense systems of tri-stars and rhombs.

Chapter 2 has described a novel Fourier method designed to track the rotational diffusion of two different isolated polygonal platelets, a square and a triangle, confined to two dimensions. This Fourier method can be applied readily to track any colloidal platelet particle that has at least one straight edge.

In Chapter 3 we have studied how the capability for interdigitation of tri-stars affects the equilibrated 2D phases that form at high particle densities. In a tilted barometric column, we have developed a digital wide-field-of-view microscopic imaging technique to observe a transition from a gas-like isotropic phase seen at lower densities to a frustrated interdigitated phase at higher densities. Further compression has revealed a transition to an alternating stripe crystal (ASX) at the highest osmotic pressures created. Additionally, small crystallite domains of a hexagonal crystal (HX) have been observed near the ASX transition. The structural characteristics of the rectangular crystal ASX phase have been described and we have developed a method for obtaining the 2D osmotic equation of state for particles observed in the tilted column. The results in Chapter 3 present the first quantitative study of the equation of state and phase behavior of shapes that have slender rays that can interdigitate.
Inspired by our experimental observations of the ASX phase, we have created a translational-rotational cage model (TRCM) for tri-star particles. This model has shown that the ASX phase is entropically preferred over the HX phase at conditions similar to those observed in the experiment. ASX permits additional translational microstates compared to HX. Additionally, deformations in the static cage of tri-stars reveal collective sliding modes in the ASX phase, which are prohibited in the HX phase, and these modes serve to further contribute to the entropic preference of the ASX phase.

In Chapter 5, the TRCM from Chapter 4 has been modified to rhomb-shaped particles in a 2D rhombic lattice. We have shown that the TRCM can explain the long-range chiral symmetry breaking (CSB) observed experimentally. This demonstrates that the TRCM provides explanations for observed aspects of phase behavior that cannot be captured by purely translational or purely rotational cage models. We have shown that more translational microstates become available in the CSB polymorph as a result of less tip-tip interference when the cage rhombs are slightly rotated away from the bisector, thereby explaining that the basic mechanism that causes CSB is entropy maximization. The TRCM routine is a prelude to a more sophisticated program which is capable of optimizing cage parameters to solve for the entropically preferred phase at a given $\phi_A$, incorporating a predictive element to the routine which could enable it to compete with Monte Carlo and Brownian dynamics simulations.