SOME ASSEMBLY REQUIRED

Probing the Physics of Order and Disorder in the Manoharan Lab

by Caroline Martin and Amelia Paine

Above: Amelia Paine displays a detailed model of the MS2 protein capsid alongside a simpler one highlighting its icosahedral symmetry. The actual virus is 3,000,000 times smaller.

The past year has fundamentally changed the way we think about the world. In the midst of all the upheavals of the ways we worked, socialized, and lived, there was a new hyper-awareness of the microscopic. Suddenly, questions that scientists had long been trying to answer felt far more urgent: How do micrometer-sized aerosols floating in the air spread through a poorly circulated room?

What are the forces acting on an infectious agent in an evaporating droplet left behind on a doorknob or a package? What drives the runaway assembly of viruses as they hijack a cell?

Professor Vinothan Manoharan's lab has been answering questions about the principles that govern the microscopic world for over a decade. We study the spontaneous emergence of complex, ordered structure from simple, disordered components. Our experiments probe the underlying physics that drives this process of self-assembly. We use this understanding to design new, useful materials, such as vibrant pigments that get their color from their microscopic structure. With our research on the emergence of order, whether in biological systems such as viruses or in model systems such as colloidal suspensions, we are gaining a deeper understanding of the physics that drives organization, assembly, and life itself.

Do viruses understand physics?

Viruses have an unmistakable elegance, despite their potentially disastrous effects on their hosts. Honed by evolution to make do with as small a genome as possible, they take advantage of symmetry to be efficient. The simplest viruses, like the bacteriophage MS2 studied in our lab, consist of a protein shell, or capsid, protecting an RNA genome. MS2 and many other viruses exhibit icosahedral symmetry, with a capsid made from 180 copies of a single protein assembled into a structure that resembles a soccer ball. We can think of viruses as one of the simplest examples of how biological systems create order out of the chaotic soup of the universe.

For many viruses, this assembly into perfect icosahedral capsids can even be reproduced in a test tube. Add some viral RNA to some coat protein and give it a good shake, and you may find yourself with functional viruses, spontaneously formed by the process of self-assembly. It's a frightening thought to some, but in our lab, it's only the viruses' bacterial hosts that are scared. Self-assembly, a term coined specifically to describe the way viral capsids form these ordered structures, is driven by the minimization of free energy—the balance between enthalpy and entropy.

But how does this ordered structure form so reliably? To create a spherical shell, the proteins that make up the capsid must form twelve perfectly placed defects, much like the twelve black pentagons on a traditional soccer ball. There are so many ways for this structure to go wrong, so how is it able to go right? Or, as Manoharan puts it, "how does the virus know where to put the defects?" For systems with a huge number of degrees of freedom, like a folding protein or an assembling capsid, there are a nearly infinite number of incorrect configurations available, but somehow, these systems find the correct one.

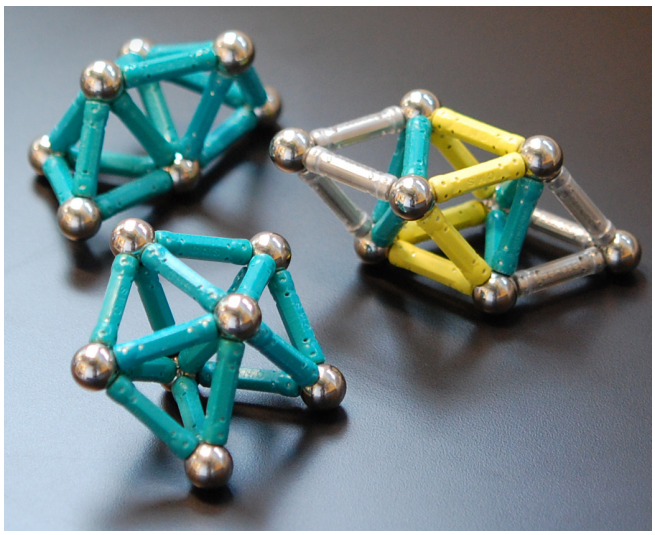
To understand how this happens, we need to observe the capsid as it assembles and rearranges itself in solution. The challenge is the length scale. Because our viruses are much smaller than the wavelength of light, observing them in action is not as simple as putting the components under a microscope and watching as they put themselves together. While it is possible to see the viruses in incredible detail with an electron microscope, we wouldn't be able to see any of their dynamics. They'd be frozen in place.

As small as they are, however, viruses can still scatter light, and as a capsid grows during assembly it will scatter more and more. Using a technique called interferometric scattering microscopy, we look at the interference between this scattered light and a weak reference beam to trace the size of individual viral particles as a function of time. With this method, we've been able to determine how MS2 assembles: very slowly at first, then rapidly after overcoming a nucleation barrier.

The results of these experiments have opened more questions than they have answered. We've found that it only takes a few properly aligned proteins to start rapid growth, and after the capsid reaches that critical size it grows to completion, without backtracking to correct for errors. We still don't know how it avoids errors, but one possibility is that the virus's own RNA guides the assembly process.

That hypothesis raises a related question: how does the virus assemble so reliably around its own RNA, when its host cell is full of other RNA that the cell needs to function? Tim Chiang, a fifth-year graduate student, is working to answer this question. "The virus's task is like finding and isolating needles in a giant haystack," says Chiang. Understanding how viruses successfully carry out this task could lead to developments in medicine and self-assembling biomaterials further down the road.

In his experiments, Chiang has been able to control the self-assembly of MS2 to selectively package its own RNA, as it does in a cell. He now wants to understand what fundamental characteristics of the coat protein and RNA allow for this selective packaging. MS2 coat protein can form ordered capsids around any RNA molecule of a reasonable size, so how can it reliably encapsulate its own RNA while avoiding cellular competitors? Chiang hypothesizes that the energetic barrier to viral self-assembly could play a role by preventing self-assembly



Attractive colloidal particles form clusters whose structures can be controlled by introducing DNA-mediated specific interactions. These structures can be modeled using the magnetic sticks and balls shown here.

from initiating on random molecules. "This is one example of an intriguing possibility—that biomolecules have evolved according to the rules of fundamental physics to perform increasingly complex tasks," he says, "to a point where their collective behaviors represent those of living things."

Colloids: atomic "spherical cows"

Stop us if you've heard this one before: When plagued by low milk production, a dairy farmer asks a local university for advice. Failing to get any help from the ecologist or the engineer, he turns to the physicist. Weeks and weeks of calculations later, the physicist returns with a solution! But it can only work for a spherical cow suspended in vacuum.

This joke highlights the tendency of physicists to abstract. When faced with a messy system, we simplify the components until we're left with a model we can work with. For questions of self-assembly, one such model system is a colloidal suspension. While not in a vacuum, the simplest colloids are indeed spheres, around a micrometer in diameter, suspended in a fluid. Milk, in fact, is a colloidal suspension of blobs of fat, and it is light scattering from these suspended particles that gives milk its white color. Colloidal suspensions are useful model systems because they're both controllable and large enough to easily image. If we can induce some kind of interaction between these small spheres, we can then study them as though they were big atoms, big proteins, or big molecules, and watch as they put themselves together in interesting ways.

Researchers have gotten creative in devising controllable interactions between colloidal spheres, taking inspiration from biology and using strands of DNA to make the spheres stick together. DNA is famous for its double-stranded helical structure that forms only between complementary sequences. By coating the spheres with complementary strands of DNA, we can make the colloids sticky—when the spheres come close enough, the DNA strands on one surface zip together with the complementary strands on the other, like selective, programmable Velcro. But because these interactions are highly dependent on temperature, the DNA acts like a special kind of Velcro that zips together when it's cold and melts away if it gets too hot.

DNA-coated particles offer an enormous range of possibilities for bespoke self-assembly. Imagine a system where each particle sticks only to certain other particles. When you mix them together, the particles arrange and rearrange themselves until they finally find the structure you want. Instead of building such a structure up block by block, you can make the particles do the work for you and self-assemble into your desired configuration.

It's important to remember that the colloidal particles aren't cleverly coordinating amongst themselves to create the final structure. Rather, they're lumbering along in random Brownian motion until they manage to find the free energy minimum that we initially design the system to have.

One way to add complexity to the system is by introducing additional strands of DNA to the solution. We can add strands that can disrupt the stickiness between particles by binding to the single strands, or strands that can strengthen the stickiness by forming an additional reinforcing beam between two strands. With control over these additional DNA strands in solution, we could take a system that is predetermined and static and make it more adaptive and more responsive to its environment.

Colloidal particles are not just controllable, though. They are also, in the grand scheme of things, large. Large enough that we can directly image the particles coming together, that is. With a light microscope, we can directly observe every step of the assembly process in three dimensions, which is not so easy to do for atomic crystallization or protein folding. For third-year graduate student Jessica Sun, this feature is crucial for answering a deceptively simple question: how does a crystal change when its substrate isn't flat, but instead has some curvature? "Almost everything in our world has curvature, either macroscopically or microscopically," says Sun, "and this curvature can have a huge impact on the formation of structure."

Consider, for example, trying to gift-wrap a ball. As Sun explains, "the gift wrap always has to crinkle." That happens because the curvature of the ball is incommensurate with the flat plane of the wrapping, and therefore the wrapping must tear or deform to conform to the surface. "The same thing happens when you have crystallization on a sphere. There are going to be defects," Sun adds.

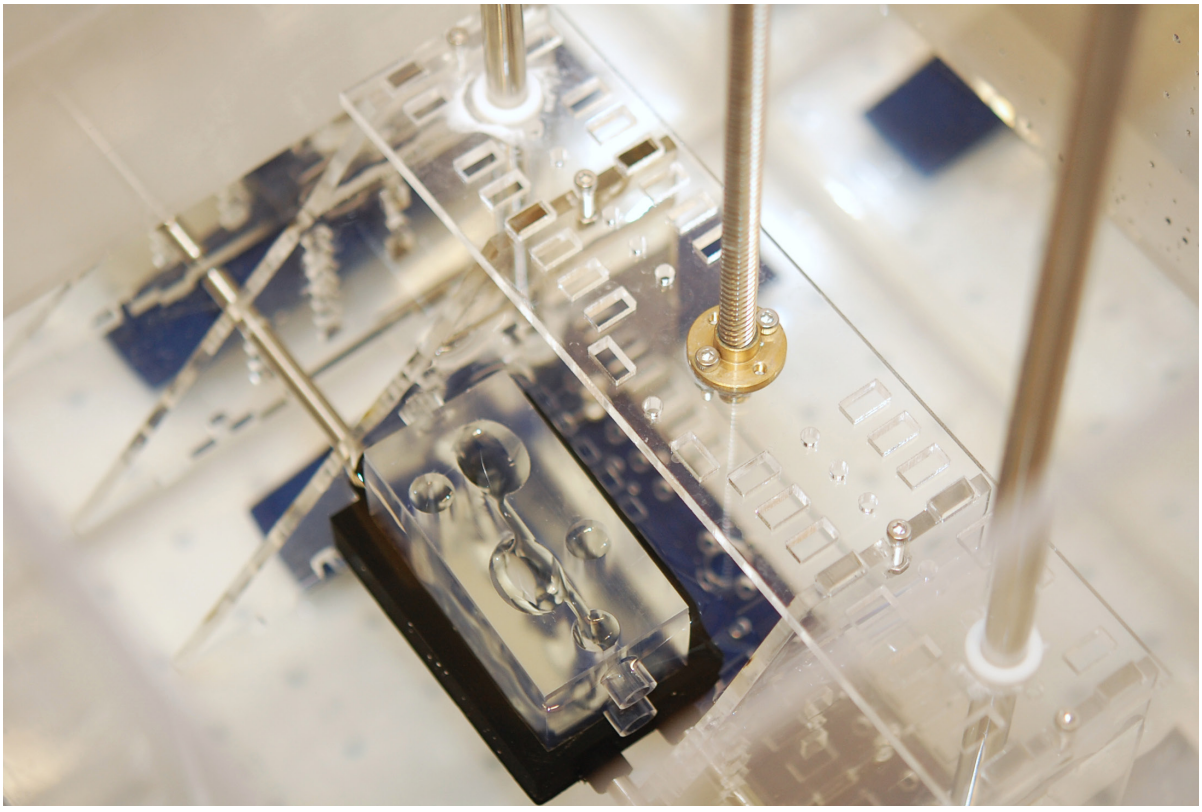
Even when we imagine a different kind of curvature, like a cone or a cylinder, the structure still determines the kinds of defects we observe. When wrapping a cone, for example, the strip of paper may lie flat, but once you bring the two ends together, you'll see that the pattern on your wrapping paper will not perfectly match up. For crystal growth, this rotation of crystal orientation means that there will be some incompatibility when the growth fronts meet, resulting in interesting regions of defects.

"When I first started working on this project, I was sure it would be a simple one," Sun remarks. "Cones are one of the basic shapes you learn in elementary school, so how hard could they be, right? But I'm continually surprised by the rich physics in this simple problem."

Assembly in action

Colloids are more than just a model system. They can also be used as building blocks for designable, self-assembled materials, like Legos that can put themselves together. One such designable feature of these materials is their color. For colloidal systems, color can arise from light scattering and interference, rather than from absorption by chemical dyes. It's like constructing a house from colorless blocks and realizing that the finished product somehow looks blue.

"Structural color exists in all kinds of biological systems," says sixth-year graduate student Annie Stephenson, "from beetles to butterflies to bird feathers." Many of the most vivid colors of nature come from some nanoscale structure rather than from traditional pigment. These nanostructures can be crystalline (which creates an angle-dependent, iridescent effect) or disordered (which creates a color that remains consistent regardless of your viewing angle). In our group, we've taken inspiration from the cotinga, a bright blue bird whose color arises from the close, random packing of air pores within the small-scale structure of the feather. The packing of these spherical bubbles can be mimicked with colloidal spheres, resulting in the same angle-independent blue shade.



An apparatus built by Ahmed Sherif to manipulate millimeter-scale objects at the interface between air and water.

Structural color has far-reaching applications, including cosmetics, paints, and displays, which all require colors that appear the same regardless of the angle you're looking from. To achieve this consistency, our lab focuses on understanding how to use disordered packings of colloids to produce colors. As postdoctoral fellow Ming Xiao explains, "we can control structural colors by tuning how the colloidal particles pack." By altering the volume fraction, we can change the spacing between the spheres, change the way the structure scatters light, and thus change the color of the sample. We can even dynamically change the color by using an external stimulus to stretch or compress the sample and thus change the particle spacing. "It's similar to the way that cephalopods can change their skin color and texture," notes Xiao.

Structural color is particularly appealing because it allows flexibility in our choice of the constituent material. Because the structure itself provides the color, we aren't confined to a particular dye or chemical to provide a pigment. This means we're free to design a material to be biodegradable, biocompatible, or more environmentally friendly. As second-year graduate student Jennifer McGuire explains, "we're inspired by nature for these systems, but we are not confined to what nature does. The goals we have for materials can be very different from how evolution drives things."

An essential tool in this design process is an accurate predictive model for the color of a particular sample. With a Monte Carlo model developed by our group, we can simulate the path of thousands and thousands of photons traveling through the material. We can predict which photons will scatter back to our eyes and determine the color of the sample for a given set of experimental parameters. We can even optimize for a particular color and determine how best to make it. "It's so exciting to be able to accurately and quickly predict the connection between color and structure. That's a really powerful design tool," says Stephenson. "Now that we have such a good model for structural color," adds McGuire, "we're exploring more and more applications, and heading beyond the visible spectrum."

We're also heading beyond the microscopic. One of the newest members of the group, second-year graduate student Ahmed Sherif, is working on ways to assemble much bigger objects. He takes advantage of capillary forces, which arise from the surface tension of a liquid interface.

You may already be familiar with one effect that arises from capillary forces. If you've ever glanced at an almost-empty bowl of Cheerios during your morning breakfast, you might have noticed that the remaining pieces of cereal floating in the milk tend to clump together. This phenomenon, called the Cheerio effect, occurs because of surface tension; the cereal deforms the surface of the milk, and that deformation is reduced when the cereal pieces are touching, driving the lone pieces together into bigger clumps.

The spectacular plumage of a male spangled cotinga. Photo by Greg Hume.

It turns out that capillary forces can be repulsive too. Sherif likes to demonstrate this by showing a video of a bowl of milk with Cheerios and a (somewhat less appetizing) pushpin floating nearby. The pushpin is repelled from the Cheerios because, as Sherif explains, "it pulls the interface down while the Cheerios bend it upwards." As a result, when the pushpin approaches a Cheerio, the interfacial area must increase, creating a restoring force.

Sherif wants to use the gentle push and pull of these forces to build structures from millimeter-scale objects. He does this by mixing floating objects of different wettabilities, shapes, and weights at a liquid interface. It's not unlike the approach the lab takes with DNA-coated colloids: in both cases, we use a diversity of interactions to drive assembly of complex structures.

However, the physics is different when menisci are involved. "That's both a challenge and an opportunity," says Sherif. Phenomena like contact-line pinning and contact-angle hysteresis complicate the task of assembling objects at a liquid interface, but they also open many possibilities for making new interactions. We're just starting to explore what those possibilities are, and how they might lead to different types of assembly from what's currently possible at the nanometer (virus) scale or micrometer (colloid) scale.

Scientific progress: a random walk?

"When I started at Harvard, I never thought my group would be working on systems whose sizes span six orders of magnitude," says Manoharan. "In those days, we did experiments on colloids. Now we work with viruses, DNA, microfabricated cones, and cereal-sized chunks of matter." The lab hosts a motley array of tools to study all these systems. A holographic microscope that can detect the nanometer-scale movements of colloidal particles sits not too far from a shaker used to grow bacteria and the viruses that kill them. In the corner sits a 3D printer used to make the aforementioned cereal-sized chunks. And that's just the hardware. "I guess I'm more surprised that simulations have become such an integrated part of our research" says Manoharan, "but we go where the science takes us."

With support from the department, collaborations with other groups, and inspiration from our fellow students, Harvard makes it easy or us to follow the science. The path is a winding one, and at times our exploration may feel like a bit of a random walk. But we aren't lumbering along in aimless Brownian motion; we're purposely chasing down the next discovery, wherever the science leads.

Annie Stephenson and Jennifer McGuire design and fabricate structurally colored materials inspired by nature. The vivid blue and green within the test tubes come from the microscopic structure of the sample.