Physics for the 21st Century

Unit 9: Biophysics

Vinothan Manoharan and Harald Paganetti

Biophysics is a branch of science that applies ideas from physics to problems in biology.

Vinothan Manoharan, of Harvard University, is studying the phenomenon of self-assembly – when particles interact with one another, spontaneously arranging themselves into organized structures.

Researchers have seen self-assembly as the future of manufacturing, as well as a hope for curing diseases.

> VINOTHAN MANOHARAN: *The dream of self-assembly from the nanotechnology perspective was that you'd take a bunch of components, throw them into a pot, wait for a long time, and have it come out as your latest generation microchip, microprocessor for your computer. Biological systems, ourselves included do much more complex things all the time.*

Harald Paganetti, of Massachusetts General Hospital and Harvard Medical School, is looking for more effective ways to use particle accelerators to improve cancer treatment.

Proton therapy offers the hope of more effective treatment with fewer side effects. But because patients' lives are at stake, the inevitable uncertainties of science take on even greater significance.

> HARALD PAGANETTI: *If you simulate a detector, for example at CERN, at your experiment you most likely know exactly the geometry of your detector. You know the materials. And let's assume the physics is accurately known. You can simulate very accurately what's happening in your detector of your experiment. But here, your detector is the patient.*

Manoharan and Paganetti are using the tools and methods of physics to find better ways of treating disease.

Part I: Particle Self-Assembly Vinothan Manoharan

Imagine if doctors could stop disease by manipulating the structure of a virus. This is the dream of biophysicists investigating self-assembly, a phenomenon in which particles interact, without external direction, to organize themselves into ordered structures.

Picture taking a box of Legos, gently shaking the box for a few minutes, to find that the pieces assembled themselves into perfect cubes. It sounds outlandish, but it's a good analogy for what happens in some biological systems.

Vinothan Manoharan, Associate Professor of Chemical Engineering and Physics at Harvard University, looks to both physics and biology in his investigations of the principles of self-assembly.

> VINOTHAN MANOHARAN: *There are things that each discipline can learn from the other. And the boundaries have become sort of blurred between them. And I am sitting at that blurry boundary between condensed matter and biophysics.*
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> *My particular research and interest now has to do with systems of many particles that are interacting at once. I'm interested in this very general problem. Which is, how do many particles come together? How do they self-assemble to form some organized whole?*
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> *Analogies in biology might be how do proteins come together to form a virus.*

Viruses have a protein shell called a capsid that protects the genetic material that viruses use to reproduce, and to infect host cells. In some viruses the capsid appears to be completely self-assembled. Some researchers believe that understanding capsid self-assembly could present new ways to fight disease by disrupting virus reproduction.

> VM: *I think that coming from the perspective of -as I said – nanotechnology where we spent a lot of time trying to build things through self-assembly, that that should be relatively straightforward, because we find that it's actually very easy to disrupt the self-assembly process. But you have to know the right way to do it. We have to understand something about that process in order to disrupt it.*

The first problem Manoharan and his researchers want to solve is, what are the physical rules behind self-assembly?

> VM: *To do that, to actually understand -how these fantastically organized virus states form - we either need new tools, or we need to look at some slightly different systems. My research tends to say we can understand some of the physics of how these viruses form, not by looking at the viruses themselves, but by looking at model systems consisting of colloidal particles.*

To model self-assembly in nature, Manoharan's research team creates colloids. A colloid is a type of mixture in which one substance is dispersed evenly throughout another. Manoharan's colloids are solid particles suspended in liquid.

The particles in Manoharan's colloids are polystyrene spheres between 10 nanometers and several microns in size. For comparison, the average human hair is about 100 microns thick.

Manoharan's researchers observe various colloidal systems under a microscope, to watch how the particles interact and self-assemble into structures.

> VM: *Most proteins we can't see using visible light. We work with larger-scale systems, these colloidal systems because they behave in some ways like a very, very simplified protein, but they're large enough that we can actually see directly, with our own eyes, without disturbing the system, what's going on.*

In the most basic form of self-assembly a large number of colloidal particles will settle into a crystal formation. Crystals are solids with regular geometric shapes formed from regular arrangements of particles.

> VM: *So this is what we consider to be one simple example of self-assembly where you have particles that are sitting in a fluid and eventually once they come together at a high enough density they'll spontaneously form a crystal. And those colors are light that's being diffracted off the crystals.*

The simplest self-assembled structures Manoharan studies consist of six identical spherical particles.

> VM: *We take these simple spherical particles and we put them in different geometries and we watch how they self-assemble.*

Manoharan's team places drops of the colloids on a microscope slide containing thousands of micro-wells separating the particles into distinct groups. Researchers observe the particles using an ordinary optical microscope.

Guangnan Meng is a postdoctoral researcher studying the self-assembly of small numbers of colloidal particles.

> GUANGNAN MENG: *We use these spheres to model the self-assembly of the other materials at equilibrium. So here we control the interactions between the individual spheres very carefully, so they have a little attraction between them, so they make structures.*

The particles exhibit a weak attraction so that they only stick to each other when they happen to touch.

> GM: *Actually you can also change the interaction by changing the temperature or solvent conditions.*

Some of the simpler systems are yielding data contributing to the very beginnings of a set of rules for self-assembly.

In the six-particle samples two distinct structures have self-assembled.

> GM: *These* are *the two structures made by six particles. One is called octahedron. The other is called poly-tetrahedron.*
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> VINOTHAN MANOHARAN: *When we had six particles we found that we get two different structures. And these are the two different structures. So this is an octahedron. It contains six spheres, there are twelve rods that are connecting the spheres, and it's a very symmetric shape.*
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> *The one I have in my left hand consists of three tetrahedra that are base to base. We call it a poly-tetrahedron. And it also consists of six spheres; it also has twelve bonds or twelve rods connecting those spheres. These two structures are the only two structures that occur for six particles.*

Manoharan expected to see the particles predominantly assemble into symmetrical structures. Instead he found an overwhelming predominance of asymmetrical structures.

> VM: *It was very surprising to us that this highly symmetric structure would occur so much less frequently than this asymmetric structure. The question is: why? And the only answer that seems to work is that it depends on entropy.*

Entropy is a measure of disorder, and is determined by the number of different possible states a system can be in. According to the second law of thermodynamics, isolated systems tend to become more disordered over time. States with higher entropy are favored in nature.

There are different forms of entropy. Vibrational entropy describes the number of ways that a structure can flex or vibrate without breaking. The poly-tetrahedrons have greater vibrational entropy than the octahedrons – there are more distinct ways they can flex.

If vibrational entropy was the only factor contributing to the entropy, poly-tetrahedrons would be twice as likely to form as octahedrons. But Manoharan found a far greater predominance of poly-tetrahedrons.

> VM: *This one, the less symmetric one, occurs a factor of 24 times as often as this one -much more often.*

Another form of entropy, rotational entropy, makes a much larger contribution to the total entropy of the structures forming in the colloids. Rotational entropy is related to the number of distinct ways the particles can be arranged in the same structure. The greater the number of arrangements, the greater the entropy.

There are exactly 720 possible ways six particles can be arranged into the highly symmetrical octahedron, and also 720 for the asymmetrical poly-tetrahedron.

But there is redundancy within these numbers. In some cases different arrangements are revealed to be the same when the structure is rotated about an axis of symmetry. To get a true number of possible permutations to find the true rotational entropy, all arrangements that are the same when rotated should be counted as a single arrangement.

The octahedron is much more symmetrical than the poly-tetrahedron, so many more of its arrangements are the same when rotated.

> VM: If I look at it from different angles I can see that it has many *different rotational symmetry axes. I can rotate it in many different configurations and get exactly the same thing.*

Taking this into account, the octahedron has only 30 possible permutations. The poly-tetrahedron has 360. The rotational symmetry accounts for the predominance of the poly-tetrahedron by a factor of 12.

Since the poly-tetrahedron has more possible arrangements than the octahedron, and it can flex in more ways, the poly-tetrahedron's entropy is much greater. Therefore, poly-tetrahedrons will form more often than the simpler, more symmetrical octahedrons.

Rotational entropy accounts for the predominance of poly-tetrahedrons by a factor of 12. Vibrational entropy accounts for a far smaller factor of two. Together, they explain why poly-tetrahedrons form approximately 96% of the time.

> VM: *This is an example of one of the physical rules that we hope to discover. That in this system, when we have short-range attraction, asymmetry is strongly favored.*

To observe simpler systems Manoharan's team can use traditional tools, like optical microscopes. More sophisticated observations require more advanced tools.

> VM: *If we want to study for example the dynamics of how the particles come together then it's not such a useful tool because we can only see one 2-D slice at a time. What we'd really like to be able to do is to see the entire volume, see all the particles at once, which may be on different focal planes on our microscope.*

To observe more complex systems --for example, those with more particles – 3-D imaging is a better tool.

Samples are imaged by taking a series of computerized holographic snapshots. Data from each hologram is used to reconstruct a three dimensional image. When put in sequence, these images form a 3-D movie of the particles in the sample.

> VM: *The technique is fast! So I can take a series of 2-D images, and -very short time scales, say a millisecond or so, and then from that be able to tell what each particle on this entire volume is doing on millisecond time scales.*

The 3-D movie allows the researchers to track self-assembly in more complex systems.

Manoharan's experiments are providing new ways of looking at how these model systems self-organize. But they are only the first steps toward answering some of the most basic questions about how particles ultimately form complex structures. He believes the answers will open exciting opportunities in the fields of medicine and nanophysics.

VM: *Self-assembly, this phenomenon of self-assembly*

understanding it, harnessing it, for nanotechnology, and at the same time understanding it in order to do something useful in medicine relies on understanding complexity.

I think what really excites me is when we make one of these crazy systems that we do, and then just watch what happens. And we find something that's entirely counterintuitive. And this happens quite frequently, so maybe our intuitions are bad, or maybe we're discovering some new physics along the way.

Part II: Proton Therapy

Harald Paganetti

Research into the physics of how microscopic structures self-assemble might someday be used to interrupt virus replication, providing new treatments for disease. Another active area of medical research is in cancer treatment.

Right now, cancer is the second leading cause of death in the United States, with over 1.5 million new cases diagnosed every year.

As Associate Professor of Radiation Oncology at Harvard Medical School and Director of Physics Research at Massachusetts General Hospital, it's Harald Paganetti's job to look for new and better ways to deliver cancer therapy.

HARALD PAGANETTI: *A tumor is a part of uncontrolled growing tissue. What you want to do, you want to destroy the tumor cells. And that's the purpose of the treatment.*

Conventional photon radiation therapy kills tumors using high-energy photons, such as X-rays. Photon radiation therapy is very effective, but has an important limitation: while it does destroy cancerous tissue it also damages a large volume of healthy tissue.

As X-rays enter the patient's body, they deposit much of their energy in healthy tissue, before reaching the tumor. Higher doses must be used to penetrate deeply enough to reach most tumors.

Compared to photon therapy, including X-ray and gamma ray treatments, proton treatments can be much more precise, causing less damage to healthy tissue.

HP: A big advantage of using protons for cancer treatment is that

with protons, you can deliver the dose more accurately.

When energetic protons enter tissue, they release most of their energy as they come to rest at the site of the tumor.

Immediately after the peak of energy release, or the Bragg Peak, the radiation dose falls near zero. Less radiation affects healthy tissue in front of the tumor, and virtually none of it affects healthy tissue behind the tumor. This results in far less damage to nearby organs and structures. Because the healthy tissue receives less radiation, higher doses may be delivered to the tumor more accurately, leading to more effective treatment.

HP: *Proton therapy was invented, or the idea came out of the Harvard Cyclotron Lab by Robert Wilson in the late '40s, where he had this idea that particle beams could be used for cancer treatment.*

The Harvard Cyclotron Lab was no longer interested in doing basic physics experiments because the machine was getting too old - the machine was built in 1948. So, basically the energy region that this machine could cover was no longer of any interest for basic nuclear or atomic physics. So they had this idea, together with Mass General Hospital, to build this program to use protons for cancer therapy.

The first patient was treated with protons in 1954 at the Lawrence Berkeley Laboratory in California. In 1961 collaboration began between the Harvard Cyclotron Laboratory and the Massachusetts General Hospital to pursue proton therapy.

At the center of a cyclotron, a source emits a charged particle, such as a proton, which is immediately accelerated by a voltage. The proton travels through a magnetic field that curves its path into a spiral as it continues to accelerate. Eventually, it spirals out of the cyclotron at a high speed.

The protons are directed down a beam line to collide with a target. In proton therapy that target is a tumor.

HP: *Protons stop in tissue. So, they're stopped somewhere. So, you can make them, by adjusting the energy, you can make them stop in the target. So, you will have dose deposited in the path going through the target, but most of the dose is really going to be in the target. And that's a big advantage.*

Patients treated at Massachusetts General Hospital's Francis H. Burr Proton Therapy Center receive multiple doses over a number of visits.

DANIEL COPPINGER: *I'm here to be treated for spinal and brain tumors. I found out about this type of treatment through my doctors because they said that you would have had to go through back surgeries all the time like I did and I'm on my fourth week right now. I've never seen anything like this before. When I first saw it, I didn't know what it did or what I was supposed to do. But now I'm getting really used to it.*

Proton beams are modulated so that the area of maximum energy deposition coincides with the tumor volume.

Brass apertures that modulate the proton beam and plastic compensators that control the depth of the beam's penetration are milled in the hospital's machine shop, according to the medical team's precise specifications for each patient.

JOHN MACDONALD: *On the brass pieces the shape that we cut in there is to shape the proton beam to the shape of the tumor. And the plastic piece that's on the back side of it controls the depth of the beam, wherever the tumor is inside the body.*

HARALD PAGANETTI: *These holes that are drilled by the milling machine resemble basically the outer shape of the tumor. So before we treat the patient we have to mount the aperture and the compensator on the treatment head.*

While proton therapy is complex and difficult it may be one of the most effective kinds of radiation treatment available. Although some side effects result, they are fewer and less severe than in other radiation treatments.

DANIEL COPPINGER: *I have had other treatments but this treatment is a lot better. When I go in there I'm fine but when I leave I'm just like really really tired. Nothing too bad at all.*

While current proton beam therapy treatment is highly effective Paganetti believes it can be improved.

HARALD PAGANETTI: *What makes our research unique is that we're really trying to make it more precise or to understand more precisely what is happening in the human body.*

Treatment plans calculate dosage up to a centimeter beyond the tumor to ensure that the entire tumor is destroyed. The treated area outside the tumor is known as the safety margin.

Although the collateral dosage outside the tumor ensures that the entire volume is treated, it results in some exposure of healthy tissue.

An aim of Paganetti's research is to reduce this collateral dosage.

> HP: *If you simulate a detector - for example, at CERN -at your experiment, you most likely know exactly the geometry of your detector. You know the materials. And let's assume the physics is accurately known. You can simulate very accurately what's happening in your detector of your experiment. But here, your detector is the patient.*
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> *And there's always this uncertainty about how your detector meaning the patient really reacts. And that's where a lot of research is currently being done to understand that. And to make sure that we're delivering enough dose to the tumor at the same time to deliver not too much dose to the organs at risk so that the side effects that cannot be avoided, but that the side effects are acceptable.*

Reducing uncertainty is the goal of treatment planning -allowing radiologists to target the entire tumor with less risk to healthy tissue.

> HP: *So, these predictions are basically based on analytical dose calculation methods because they're very fast, and they're reasonably accurate.*

Analytical dose calculations require information about tissue density to determine the optimum proton dosage. This information comes from Computed Tomography, or CT scans, that combine a series of X-ray images to produce cross-sectional images of structures inside the body.

To reduce collateral dosage in more complex geometries, such as a human head, calculations must incorporate data about the biological makeup of the bones and tissue.

> HP: *So, if you want to reduce that safety margin, which is desirable, because then you treat less healthy tissue - we need a more accurate dose calculation. So, Monte Carlo is that tool.*

Monte Carlo simulation is a mathematical modeling technique originally developed for nuclear weapons research in the 1940's. It is widely used in systems in which probability determines the outcome of an experiment.

The technique can model proton therapy by simulating a beam of protons with a particular energy distribution as it enters the body. Researchers know the probability that each proton will deposit its energy at a certain point, but do not know what each individual proton will do.

A computer randomly assigns the behavior of each proton in a manner consistent with underlying rules of physics. The result is a precise and thorough simulation of the deposited dose.

Paganetti predicts that the Monte Carlo method will lead to far more accurate treatment dosage plans than the analytical calculations. To test his theory, he compared the two calculations.

> HP: *This shows nicely how Monte Carlo dose calculations are giving us more accurate results than analytical dose calculations. On the right side you see what our planning system predicts as the dose being delivered to this patient. On the left side is what the Monte Carlo simulation predicts. We see quite a remarkable difference here, which has to do with soft tissue and bone interfaces that are difficult to handle in this analytical dose calculation, because particles are scattered in and out at these interface, whereas in the Monte Carlo this is handled correctly because the physics is implemented in a more accurate way.*

There is a drawback to using Monte Carlo in treatment planning. The technique relies on simulating many individual protons interacting in the patient, each using different sets of randomly generated values. Millions of individual proton tracks have to be simulated to achieve a result with high statistical accuracy. Currently the process takes too long to be usable for treatment planning.

> HP: *So, our research focuses, for example, in making Monte Carlo very, very fast. In nuclear physics experiments people are used to running Monte Carlo simulations over days and it's not really a problem for them usually. But when we do a dose calculation for a patient, our clinicians are going to have the result within let's say five to ten minutes, or maybe a half an hour -but certainly not longer than that. And analytical methods are able to achieve that.*
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> *Based on our research -on making Monte Carlo faster - we're getting into the timeframe that we can really use Monte Carlo routinely in the clinic.*

By developing innovative ways to combine new and existing technology and physics, enhanced proton therapy treatments are resulting in fewer side effects, improving patient outcomes.

> HP: *So, the conclusion that we're getting from the research right*

now is that we're treating the patients correctly. In other words, we're really administering the dose to the tumor that we plan for. But what it also shows is that we can probably reduce some of these safety margins if we understand the physics and the biology in the patient better.

Physicists like Vinothan Manoharan and Harald Paganetti are making progress toward the development of better treatments for serious diseases by applying the principles of physics to biological systems.