[SQUEAKING] [RUSTLING] [CLICKING]

JUEJUN HU:

OK. So as I said, this is not the topic I proposed. You proposed it. So this is my 3071 final project. Now you get a chance to hear the presentation and grade it.

All right. I also just also want to mention that this is a topic that's not in my area of expertise, obviously. It's actually, in fact, pretty far. But it's good. I actually get to learn a lot of new stuff. So that's part of the good thing I sometimes say about academia. People actually pay you to learn new things.

All right. So we are talking about biomineralization. Specifically, I'm going to talk a lot about what the role amorphous material has played in that kind of process here. Some references. Obviously, you can always find these books. Read a bit more. These are some of the ones I referenced in the process here.

This is the outline of the lecture today. I will start out by giving you a brief introduction of biomineralization. I will then delve into a few specific examples about biomineralization and talk about some of the processes there.

Then I'll move on to talk about some common features that you will see in biomineralization. In particular, if you look from a structural perspective, a lot of these materials form in this process. They all have a hierarchical microstructure. So what I specifically mean by that is that there are different length scales. And at different length scales, from macroscopic to mesoscopic, microscopic to nanoscale, you have different kind of structure entities that forms this kind of hierarchical microstructure.

We'll go into that. That's a very common feature in this kind of biomineralized materials. And in order to form this kind of hierarchical microstructure, turns out the common way of doing deposition and crystallization, the common material growth techniques, they do not really apply. And that's actually where amorphous material comes in.

What you will see is that in a lot of these materials, instead of directly forming the final microstructure, they always go through an intermediate step by having this kind of transient amorphous phase or transient amorphous precursors and then crystallize it into the final crystalline form. So amorphous material does play a very big role in biomineralization.

So you picked a good topic that's very relevant to this class. And in the end, I'm going to just talk about some applications. Basically, some bioinspired different kind of applications leveraging the principles of biomineralization.

So what is biomineralization? Now, specifically, what we are going to focus on in this lecture is what we call the biologically controlled mineralization. If you look at literature, people like to categorize biomineralization into two main classes. You have so-called biologically induced mineralization. You also have biologically controlled mineralization.

So for biologically induced mineralization, the organism does not play an active role in trying to control essentially growth steps of the materials. What they did is they essentially facilitate or create the microenvironment that then triggers mineralization. So the example will be some bacteria. For example, their metabolic products becomes essentially the essential ingredient for subsequent mineral precipitation.

But in that case, the bacteria has no active role in controlling the microstructure of those kind of precipitation. So that's quite different in the case of biologically controlled mineralization, which is the focus of today, where the organism actually play a very active role in shaping the final product. We'll go through that in the next few slides.

So definition here. As I said, it's basically the formation of inorganic solids by living organisms. But when we say inorganic solids here, it turns out usually it's a composite. It's a composite that contains usually organic matrix and then and inorganic phase, which comes from this precipitation process.

So I'm giving you a few examples here. This is where your first proposal, talking about glass sponge. I'm going to discuss about glass sponge a little bit. But you also see other examples, even in us. We have teeth. We have bones. That's very typical products of biomineralization.

So now, let me go through a few examples talking about biomineralization. The first one here, seashells. I actually have a little YouTube video here, which I believe talk about the material structure very well.

[VIDEO PLAYBACK]

- But durability goes right along with this. You see, we have-

-

JUEJUN HU:

There we go.

- Thanks to Curiosity Stream for supporting PBS Digital Studios.

Seashells and chalk are both made of calcium carbonate. By itself, the mineral is brittle, crumbly, and soft enough to write out a homework assignment. Seashells, on the other hand, are wonderfully resilient. They're tough, they resist breaking, and they definitely can't be used to doodle on the sidewalk, but they're still primarily $CaCO_3$. So what makes seashells so different?

How can this and this be made of the same stuff? We're talking about the mineral chalk, by the way. Sidewalk and classroom chalk are usually made from a different mineral, known as gypsum, but we digress. Both seashells and mineral chalk are the product of living things. Those chalk deposits were generally formed from the gradual buildup and compression of single-celled ocean organisms with calcified shells.

But bivalve mollusks, like oysters and mussels, are much more sophisticated shell builders. When we use that mineral chalk, it's long dead, and all that's left is the crumbly stuff. But these mollusks are pulling dissolved carbonate and calcium from the ocean for some pretty fancy biological architecture.

Seashells contain 95% or more calcium carbonate. The rest is mostly protein and a bit of sugar. But a little biochemistry goes a long way. Calcium carbonate can take on a variety of forms. Even though its chemical formula never changes, its crystal structure, which is the 3D arrangement of its atoms in space, does change.

The two forms we care about right now are calcite and aragonite. Calcite tends to be more stable than aragonite, but many bivalve shells contain a layer of each-- calcite on the outside of the shell and aragonite on the inside. One benefit to layering is that the crystal structures are oriented differently in space. If the calcite layer fractures, that fracture will probably stop when it hits the aragonite layer because aragonite doesn't break the same way.

But that alone isn't nearly enough to make seashells so much tougher than chalk. We told you mollusks were amazing builders. The aragonite layer in a shell like an oyster's is called nacre, or mother of pearl. Here's how scientists have speculated mollusks actually make the stuff. Nacre is made up of layers of aragonite crystals separated by very thin layers of protein. Those layers are anchored by a chain of sugars called chitin.

Mono and disaccharides, the kind of sugars we eat, are water soluble and not good building materials by themselves. Try to build a castle out of sugar cubes and see how long it lasts. But chain them together in long strings called polysaccharides, and they can become tough and fibrous. Chitin is one such polysaccharide.

Proteins attached to the chitin layer give the calcium carbonate crystals a place to start growing. Those proteins have charged regions that attract the minerals. Because of how those regions are spaced, the crystals can grow in regular chunks until they get the next layer and meet each other at the edges.

That's not everything the proteins and polysaccharides do. Shell formation requires a whole army of complex biological molecules, and scientists are still trying to understand how it all goes down. Nacre is literally thousands of times tougher than chalk, thanks to the layers of protein and chitin and the precise structural control. And then the oysters go on to make pearls. They're so talented.

What all that means is that mollusks don't just wear these minerals as a hat. They control how the shell is made and what kind of crystal forms, whether calcite or aragonite. They can make all sorts of neat shell shapes that don't look anything like a stick of chalk. And they can even remodel their shells throughout their lifetime. And that's why you can't make sidewalk doodles with a seashell, which does work out in the animal's favor after all.

Thank you to Curiosity Stream for supporting PBS Digital Studios.

[END PLAYBACK]

JUEJUN HU:

You get to see a little bit about what seashells are composed of. So as the video says, basically, it's similarly a composite material. You have a mineral phase, which is either the calcite or aragonite. These are two polymorphs of calcium carbonate. And there's also some organic materials like chitin, the polysaccharide, as well as some proteins.

So the matrix proteins, they actually play a very important role because scientists have performed studies looking into essentially how these proteins can actually suppress or promote the formation of one polymorph over the other. Some proteins will actually induce precipitation, for example, of only aragonite, but not the other one.

And the reason they need these two kind of polymorphs, if you look at what I'm showing you here, this is basically what the two polymorphs look like. This is basically abalone shell. So if you look at the inside of abalone shell, this is basically nacre. It has this kind of prismatic color with a smooth surface. This is actually a nacre phase.

Outside is what they call the prismatic phase. But this is basically the calcite. So inside is aragonite, outside is calcite. They have different complementary mechanical properties. Calcite tends to be very resistant to puncture, to puncture damage. And they also help to suppress any kind of crack propagation. Whereas nacre, because of the interlayer organic phase, is actually a lot more ductile and compliant compared to calcite. So this combination of materials will give a very excellent mechanical toughness of the seashells.

OK. So one more thing I also want to mention here is that these mollusks, they do not directly form this crystalline phase. What they will do, before they form the crystalline phase, is they always go through a Amorphous Calcium Carbonate or ACC. This is one of the transient amorphous precursors we're going to be talking about.

Now, this is a very common strategy for this kind of organisms. In biomineralization, they almost always go through amorphous phase before they go to crystalline phase. There are a few reasons for that. From a genetic perspective, the amorphous structure may look a lot similar to the liquid phase that come from solution.

So this structural similarity, what it also means is that the interface energy between the amorphous phase and the solution phase tends to be a lot smaller compared to the crystalline phase and the solution phase. So lower interface energy essentially means there's a lower energy barrier to nucleation. So kinetically, it can be more favorable to form the ACC first, and then go to crystalline phase.

Also, we'll be talking about this process a little bit more later. But typically, when they actually precipitate out this kind of amorphous phase, it comes out in the form of very small clusters, nanoscale clusters. So these nanoscale amorphous clusters, they reside in the solution phase. So it's basically like a liquid with these amorphous clusters.

It's very easy for this phase to essentially infiltrate into usually the porous structure of organic scaffold. And then these little part of clusters then attach to the scaffold. And this will be a pretty quick process. This kind of infiltration and precipitation, it can take place within a few seconds to minutes. And this is actually advantageous because, in lots of cases, the organisms, or the animal, basically, want to have essentially the biomineralized products quickly to adapt to different kind of environments.

We are going to talk about crustaceans. For example, crustaceans, when they molt, they essentially shed their old shell, put on a new one. They need to have that new shell quickly, within a matter of days, a couple of days, or even shorter, just to protect themselves. So having this amorphous phase is actually very convenient. This allows this very fast deposition of the amorphous materials to form essentially the prototype of the shell.

And then after that, the actual phase transformation, going from amorphous crystalline, this tend to be a slower process. This can take hours or days. And in the process, the kinetics is basically controlled by a lot of the organic molecules. They're called inhibitory organic molecules. They essentially suppress the crystallization so that the animal can actually control what is the kinetics of the phase transformation.

And that's actually useful because, in lots of cases, in order to form this kind of hierarchical microstructure, the organic template will then dictate what is the orientation and alignment of the resulting crystals. If you do that very quickly-- from kinetics, you know that it becomes very difficult to actually control the orientation of those crystals-- essentially, you get a lot of random atomic attachment. You get highly polycrystalline solids with a random orientation of grains.

It's sort of like growing microepitaxy. In order to get to this kind of microepitaxy process, you need to control the atomic detachment, the phase transformation, so that it doesn't go too fast and screw up everything. So that's why having this kind of amorphous phase is actually quite important. And controlling the kinetics of its precipitation and growth is also quite important.

So this is basically one example. We're going to see that in other cases as well. So the second example I'm going to talk about, these are the exoskeleton of crustaceans. So I'm talking about basically crabs, shrimps, lobsters, crayfish. In terms of composition-- oh, by the way, I forgot one thing about the chemical reaction here. I should mention this.

This is a chemical reaction for the seashells' formation. Basically, the mollusks, they were taking calcium from the ocean. There's actually lots of calcium in the dissolved solution form in the seawater. They're also taking this ion here, HCO_3. It basically come from dissolved carbon dioxide in seawater. Then they can react, form the ACC, precipitate out, and that's how you actually form the seashell materials.

It's actually a very similar chemical reaction when it also comes to the crustaceans that actually live in the ocean. They basically use the same kind of chemical reaction. So in terms of composition of the exoskeleton, it's also quite similar to seashells. You similarly have chitin, the polysaccharides. You get some proteins. You get minerals.

The minerals are mostly ACC and calcite. Depending on different species, it's a mix of the two. Now let's take a look at actual structure of the exoskeleton. It's one typical example of this kind of hierarchical architecture. So if you look at the shell-- let's say a lobster shell-- there are actually multiple layers of the shell.

You have so-called exocuticle. You get the smaller ones. The outer layer is called epicuticle. It's basically a layer that has essentially a wax-like feeling on the surface. And then you get exocuticle. And then you get endocuticle. So different layers there.

Now if you look closely into the microstructure there, they all consist of essentially these kind of basically little fibers. The fibers are essentially made of chitin, but it's a chitin that's heavily mineralized. So chitin decorated with calcite crystals. So these fibers, if you look at how they're actually arranged, they form this kind of a multilayer structure between the layers.

The orientation of fibers actually rotated with respect to each other. People call this helicoidal Bouligand stacking. I don't know where the name comes from, but basically, this actually has a pretty important implication of mechanical properties. The reason you have different orientations here, one is that suppose there's some cracks developed in one of the layers. Then, because of the rotation of the orientation of the fibers between different layers, it means that when the crack tries to propagate between layers, it also has to rotate, change orientation, and then essentially also have to branch out in the process there.

So because of this kind of configuration, it means that as the crack propagates, the area of the cracked surface has to expand as it goes through multiple layers. Now that means you have to overcome more surface energy barrier. So it effectively helps you to absorb a lot of the energy. And absorbing energy corresponds to an enhanced toughness of the material.

Also, if you just look at the chitin fiber by itself, it obviously has this kind of anisotropic mechanical properties-- stronger along the direction and then weaker in the parallel direction. But by having this kind of stacking with a rotating orientation, it also eventually creates isotropic material that has uniform properties along different directions, which will be useful.

And finally, the mixture of organic phase in the organic phase is also what made the shells actually highly flexible, despite the fact that it's made of calcite, which is a pretty hard mineral. Now, one thing I have also found out while I was looking for the information about crustacean exoskeleton formation is the process called molting.

So molting just means that, for example, crabs, as they grow bigger, they need to essentially shed away the old shell and actually put on a new one so that they can accommodate their growing size. In this process here, as the crab, for example, dumps its old shell, the old shell contains lots of calcium. So one priority for the animal is that it needs to be able to essentially accumulate lots of calcium and then deposit them to form new shells, as I said, within a short period of time, just to protect itself.

So this comes with the question-- how do they actually get this large amount of calcium within a short period of time? For animals living in the ocean, like lobsters, this is much less of an issue because, as I said, ocean water, seawater, actually has a high concentration of calcium in itself. So it's easier for them to get it right.

But for terrestrial and freshwater crustaceans, for example, like crayfish, it does not have access to this huge reservoir of solution-phase calcium ion. So what they would do is, in fact, before they actually molt, they will start to reabsorb calcium in the exoskeleton before they actually molt. And in that process, they have to store the calcium somewhere. So they store it in the form of a so-called gastrolith.

Have you heard of the term? It's basically like little stones, technically. They form little stones in their stomach. I don't know if you have eaten crayfish.

AUDIENCE: Yeah.

JUEJUN HU: OK.

AUDIENCE: Do I eat the stones?

JUEJUN HU: Sometimes. Only sometimes you see it. It's right before they molt you get those kind of stones. So the stomach of crayfish is actually very close to their head. It's right behind their head, which you don't eat the head. But you may find stones in there if you look carefully.

The stones can get actually pretty big. You see the size here. This is actually-- sorry, they're scaled a bit small. This is 5 millimeters. So they can be this big. Yeah. And they primarily consist of ACC, Amorphous Calcium Carbonate. Now the reason they put in ACC is because, as I said, ACC is easy to deposit from the solution phase.

And when they need to return it into the solution phase, to return it to a body fluid to build the exoskeleton, the dissolution process can also be very efficient and very fast. So they essentially use this amorphous phase sort of as a reservoir that's easily accessible and can be expediently transformed into the calcium in the body fluid. Then they can transport it to actually build the exoskeleton.

So essentially, this is a diagram that actually shows the microstructure of this exoskeleton. This is what it looks like in terms of cross-section. And this last one here, this panel, shows the microstructure after you etch away the ACC phase. So what's left behind here is basically the organic scaffold. So it's basically an organic scaffold with lots of ACC essentially infiltrated in there. This is how these animals actually were able to very quickly build the exoskeleton, with this kind of storage.

AUDIENCE: And they're taking it from their environment or they're already--

JUEJUN HU: Well, both. They essentially accumulate it essentially from the environment. That's kind of the primary source. They have to do it. But then, as I said, before they molt, they also reabsorb the calcium from their own exoskeleton.

AUDIENCE: Kind of like-- OK.

JUEJUN HU: Yeah. That's actually what they do. And so that essentially minimizes the calcium loss when they actually molt. So they recycle it. But you only see the gastrolith, as I said, in terrestrial or freshwater crustaceans. You don't see that in lobsters. You never see in lobsters because they have lots of calcium at their disposal. So next time, if you try crayfish, look out for this kind of a gastrolith.

All right. Now let's come back and talk about ourselves. So vertebrates like us also always go through a biomineralization process in order to form bone and teeth. Now, for bone and teeth, one thing that's quite different from seashells is their composition is different. We do not rely on calcium carbonate. We rely on a different kind of mineral, essentially a calcium phosphate. That's the main difference.

So if you look closely into either the bone or teeth, they have similar compositions. They always consist of three different phases. Here you have a mineral phase, which is basically taking the form of hydroxyapatite. Essentially, it's a hydrolyzed form of calcium phosphate. You then also have an organic matrix. Now, mainly, it's actually collagen. And some proteins, of course.

And then finally, some water phase. The water phase is basically just to provide the hydration. So the specific composition really depends on the specific kind of tissue there. If you're talking about bone, you typically have anywhere from 50% to 60% of the mineral phase and then maybe 20% to 30% organic phase, the rest being the solution phase.

If it's dentin, which is basically the core of the teeth, dentin has about 70% mineral, maybe 20% organic phase. So it's more like bone. But for the surface layer of teeth, which is the white outside, the white shiny piece, that's called the enamel. The enamel is pretty much just the mineral phase. It's a very dense mineral phase. It's about 95% calcium phosphate. And then just a little bit of organic proteins. So the composition also depends on what tissue you're looking for.

Now enamel is actually quite an interesting part of the tissue that I actually want to talk a bit more about in subsequent slides. If you look at enamel here, it also has this kind of hierarchical architecture here. It actually consists of tiny little rods called enamel rods. And enamel rods basically consists of mineralized collagen fibers. They stack together in a regular array to form the enamel. If you look at it under a microscope, you see these tiny little rods arranged in more or less a hexagonal lattice.

Now, how are those formed? How do we humans, for example, form these kind of minerals? Again, amorphous phase. We still use the precursor. In this case, it's Amorphous Calcium Phosphate, or ACP. Now, the reason ACP is used is very similar to why other animals use ACC. It reduces also interfacial energy. It also provides the shape flexibility because, again, these kind of little clusters in the solution phase can very easily infiltrate into the tissues, bone tissues, precipitate on them, and eventually transform into the crystalline form of hydroxyapatite.

So one example of a mineralization process that's actually actively ongoing, even after you form the bone and teeth, is the enamel. Because enamel is actually a surface layer of teeth, it's always in interaction with the microenvironment in the mouth. So for example, if you eat some sugary food which feed bacteria, the bacteria's metabolic products tend to be acidic, or you drink lemon juice or whatever, that's also acidic, then it can actually push the chemical equilibrium goes the other way around. You start to cause dissolution of the hydroxyapatite or the enamel layer. Go back into the saliva.

So essentially you lose the mineralization. That's how you actually damage the teeth. You can actually also reverse the process by going to a more basic solution. And you supply it with sufficient amount of calcium ion as well as the phosphate ion. If you do that, they can actually go through a so-called remineralization process and repair any damage to the enamel layer. I will go through that later when we talk about the applications of this kind of process there. But that's a biomineralization process that's actually reversible and is actually ongoing always.

All right. Now, next example, we are talking about different class of minerals now, silica or glass. So there are quite a few different kinds of microorganisms that can actually mineralize SiO_2, silica. The two most notable examples is diatoms and glass sponge. So you obviously know glass sponge. That's how you propose the lecture topic.

Diatoms, these are essentially single-cell plankton that live in the ocean. And they are able to essentially convert the silica dissolved in seawater into a glass. And for diatoms, in fact, what they will do is, essentially, similar to glass sponge, they basically build the skeleton, or basically their houses they live in, out of glass, which is quite remarkable. So I have a little YouTube video here that essentially discusses about this process. So let me see.

[VIDEO PLAYBACK]

- The odds are high that you'll run into a diatom. They're both abundant and easy to spot because of the shells they encase themselves in. The results are beautiful, exacting geometries that create a living kaleidoscope in the microcosmos. And even if you lived your entire life without ever seeing a diatom, without ever hearing the word diatom, you would still be living a life that's shaped by them, all the way down to the oxygen you breathe, thanks in no small part to their outsized contribution to the world's photosynthesis.

But what remains captivating about diatoms is, well, the thing that remains even when the diatom itself is gone-- the hard silica shell they once lived in, also known as the frustule. We've talked about frustules a bit before and how they've given diatoms the nickname jewels of the sea. And today, we're going to dive a bit more into how those frustules are made and the role they play in our world.

Diatoms are the architects of their own glass houses, and the scale of this project is immense. The silica shells aren't just a smooth, uniform exterior. There are pores and elongated channels, creating ornate microscopic patterns that the organism depends on to survive.

[MUSIC PLAYING]

The source of diatoms' building blocks is geology. As rocks and land get weathered and eroded, silica enters the water, where it dissolves into silicic acid, which can then diffuse into the diatom and get converted into silica. But if diffusion isn't bringing in enough material, the diatoms can also rely on silicon transporter proteins in their membranes to gather more.

Inside the diatom is a special area called the silica deposition vesicle, and it's there that the silica bits get linked together to form bigger and bigger pieces. But while we know that this is where the silica walls get put together, the chemistry and biology of how they get put together is still unclear.

[MUSIC PLAYING]

We know that the silica can itself form a gel-like network, so that could make up a key assembly step. But there may also be other organic molecules, like proteins, that bind to the silica and help create the final structure. While scientists are still untangling those mechanisms, they have observed the way that the silica deposition vesicle and other structures in the cell, including the mitochondria, can help to mold the shape of the final silica structure.

For example, elongated symmetric diatoms, also called pennate diatoms, often have a slit called the raphe. The slit helps the diatom move around, serving as an opening through which they can secrete a viscous fluid to glide on. And one of the ways that diatoms make the raphe is by physically molding it through the arrangement of their microtubules.

[MUSIC PLAYING]

Many diatoms are non-motile, meaning they don't move around, and the lack of visible movement from the outside is striking when you consider all of the activity that has gone on inside of them to make those frustules happen. Like we said earlier, they're doing more than creating a smooth, transparent surface. They're creating a molded, ornate structure personalized to their own shape, like a house couture.

And not only is this process a lot of work, it renders diatoms dependent on silica, a chemical they can't make for themselves. So why do it? Well, for one, it's cheaper than both making and assembling your own materials. Silica is already out there, so why not take advantage of it? One study found that the materials in diatoms' frustules might help the organism take in carbon dioxide, improving their photosynthetic capabilities.

[MUSIC PLAYING]

These advantages seem to translate over into the immense capacity of diatoms to grow and dominate over other algae that share their waters. But taking over a body of water is all fun and games until the silica runs out. Diatoms are known for their ability to form large, visible blooms, whether in your local lake or the Arctic Ocean. But scientists studying those Arctic blooms found that as the diatom population expanded, they also depleted their waters of silicic acid, and that in turn halted their growth and began to increase the number of dead diatoms.

And both of these things, the lack of growth and the dying, have another impact on diatom populations-- sinking. Healthy growing diatoms can up their buoyancy to keep them afloat, while dying cells cannot. It might be sad to imagine those blooms dying and descending to lower depths, the ornate jewels collapsing like a microbial Empire. But nature doesn't trade in inconsequential tragedies.

The death of diatoms leaves behind frustules like this one, which means it leaves behind silica. Some of that silica can be recycled by other diatoms. But over time, it will eventually descend further and further down until it gathers on sediments. When a diatom takes in silicic acid, it's taking in the remnants of a geological process-- the weathering and erosion that brought the silicon to water in the first place.

And then, through the construction of its shell, it recycles and transforms the dissolved silicon into a form that can become geological once again. And how that silica gets used again can vary, but as always with the diatom, your chances of running into it again are high. Scientists found that dust from a diatom shell-rich sediment in Chad might travel as far as the Amazon. The diatom itself may not be making the trip, but the microcosmos and the things built in it have a way of sticking around.

[END PLAYBACK]

JUEJUN HU:

So you see, these amazing creatures that can make these highly delicate silica structures. In fact, in the first slide, I showed you some photos of this kind of structures that were made by diatoms. You see the geometric variety of the structure they were able to make, this kind of highly irregular structure there.

And also, I want to mention that the silica shell they made is actually not just a solid piece. It's actually a highly porous structure with very large surface-to-volume ratio. So the porous structure is actually useful. It helps them to essentially-- they need to exchange change with the surrounding environment. So that's why they actually make it into the kind of porous structure. Again, the hierarchical structure.

So we'll see later on that how this kind of structure can be useful for practical applications, leveraging the fact that they have this kind of nanoporous architecture there. Now, in terms of how the silica shell is formed, similarly, it's also a composite with organic phase and the mineral amorphous silica. And the organic molecules also play similar roles. It serves as a scaffold that catalyzes the precipitation of silica and also guides its microstructure.

So in the case of diatoms, they actually use a protein called silaffins. So silaffins basically can actually trigger precipitation of silica from surrounding silicic solutions and then they can actually interact with other molecules to actually form organized nanostructures that form the intricate structure of the glass house for diatoms. For glass sponges, they develop their own chemistry, interestingly. All the silica-grown animals, they all have different chemistry to actually do it.

For glass sponge, they use silicatein, which is actually an enzyme that catalyzes the precipitation of silica. So similar biochemistry process, but with very different specific molecules involved. Now, because you talk about glass sponge, I'm going to discuss a little bit more about glass sponges. So glass sponge is actually quite interesting because they have these highly hierarchical structures in their glass skeleton there.

So I quote this figure from the science paper here, which gives a very nice description about how the structural hierarchy are distributed in silica structure there. So macroscopically this is sort of like a cage-like structure that was precipitated by one of these glass sponges. Now if you look closely, you see that you have this kind of square lattices that's actually reinforced by these diagonal struts or diagonal rods.

So later on, we'll see that scientists actually study this kind of structure. They figure that this is actually an optimized configuration that give it the maximum resistance against buckling deformation with the minimum amount of material, which is quite remarkable. Again, we're going to talk about that in a few slides.

But if you look closely, basically, this silica fibers actually has core-shell structure. So you can see these are actually silica fibers. It's a bundle. And then, within bundle, you have this essentially fibrils. Each fibril is actually smaller. And then they have a core-shell structure. With the core, it basically contains more protein or the organic phase, while the outer layers, these are primarily essentially amorphous silica.

In between them, they are bound together by these very thin, organic interlayers. So there are some interesting implications for this kind of structure. One is that this actually helps to improve the mechanical robustness. Same thing. Because you are combining essentially flexible-- it's sort of like a laminated glass. It's one good example of naturally occurring laminated glass. You have organic phase binding different kinds of silica plates together.

Also, it also means that there's actually a refractive index gradient from the core to go to the outside there. It turns out, the core tends to have a higher refractive index. The cladding tends to have low refractive index. So effectively, you have a fiber-optic waveguide.

People actually look at it. This is actually a little photo. These glass sponges can grow these kind of fibers to centimeters in length. And people have actually measured optical loss by sending light from one end of this fiber and then measure how much light actually escapes from the other end of fiber. You can actually see this picture here.

This is actually a fiber. It's glowing because there's actually light scattered from the surface as they propagate inside. And they were able to measure loss that's actually less than 1 dB per centimeter, which is better than some of the waveguides people actually made, for example, on chip. That's quite remarkable.

Now if you look even more closely, look at one of these silica layers, it's, again, not a solid piece of silica glass. Instead, you see this kind of granular structure, indicating that when this layer is actually formed, it's also formed through attachment of silica nanoparticles, which is a very typical process in biomineralization.

So this serves as a good introduction into some of the interesting kinetic processes that help to shape the unique microstructure of the biominerals. So what I'm going to do in this next section here, I will essentially be giving you a few-- introducing a few general features about the kinetic processes in microstructure evolution of these kind of biominerals.

So first thing is that, as we have mentioned, the formation of this kind of biominerals almost always involves a transient amorphous phase. So this can be summarized with this quick phrase here. It's always amorphous first, crystalline later. The reason this amorphous phase is desirable primarily is it helps to lower the nucleation barrier. So it's a lot easier to form from solution phase.

This actually is dictated by the so-called Ostwald's step rule. I don't know if you talk about it in your other classes, but essentially what it says is that in a non-equilibrium process, when you're trying to precipitate some solid phase from a solution, what usually precipitates out tends to be thermodynamically least stable phase. It's not the most stable phase. Because part of the argument there is that the thermodynamically most stable phase tends to be structurally most dissimilar compared to the solution phase.

So the kinetic barrier is actually high. And that's why there's always an intermediate phase that's thermodynamically unstable, but kinetically expedient to form, guide the process. So in this case, it's actually usually the amorphous phase that actually forms first, consistent with the Ostwald's step rule. So it helps to reduce interfacial energy. I mentioned that.

And also, these organic matrices, they also provide preferential nucleation sites. That's how they actually guide the microstructural assembly in this kind of precipitation process. And then subsequently-- So when you actually grow these minerals, you always start out with a solution-phase infiltration deposition. As I said, this is a kinetically fast process. Takes seconds, maybe two minutes.

The subsequent crystallization is a lot slower. It's kinetic. It's basically controlled by these organic molecules that serve as inhibitory agents that control the kinetics of the crystal growth process. And that's why you can actually vary-- these organisms can control the orientation and spatial arrangement of those crystals when they actually form.

So this is one example of the so-called non-classical crystallization process. I have this schematic here that's trying to illustrate the difference of this non-classical crystallization with classical crystallization. So for classical crystallization process, this is what you have learned in your kinetics class. You have a supersaturation of ions in the solution. Then, once you have a little nuclei or you have little nucleus, then these ions can attach to the surface essentially ion by ion. And then you build up the crystal there. That's the classical nucleation process.

Now, in bioorganisms, usually this kind of process is not what actually happens. What happens is that these ions will start out by forming these little amorphous clusters. Instead of attaching to a solid ion by ion, its actually cluster by cluster. And then that's eventually how you actually form the bulk crystal. So this is actually one good example here in this figure. This schematically shows how the ACC actually attaches to the surface in the form of clusters.

And that's why if, you look at the microstructure for a lot of the biominerals, you tend to see this essentially nanoparticle formation in the solid phase there because of the kinetic pathway. Now, one example to actually illustrate this kind of kinetic pathway here, I'm showing you, in this case, this is actually a sea urchin. Basically the spikes of the sea urchin.

The spikes are basically calcites, calcium carbonate. Now, calcium carbonate is actually in the solution phase. If it's supersaturated, it can crystallize within a matter of seconds. So in order to control the kinetics of the amorphous phase so that you can actually control the microstructure, different organisms, like sea urchin, they have developed their own strategies how to regulate the kinetics of crystallization.

There can be different chemical processes that's involved. For example, you can have organic macromolecules, whether it's protein or polysaccharides. And the way these organic molecules essentially slows down the kinetics is by essentially they attach to the surface and block nucleation sites. So that way, it can slow down the crystallization.

You can also use small ions, like magnesium, in ACC. Magnesium works by essentially distorting the local coordination environment and making nucleation more difficult. Water and hydration shells. This is also another commonly used strategy here. By essentially forming this kind of liquid-like hydration shell, it basically creates a kinetic barrier to prevent the growth of the crystals, which also helps to control the kinetics.

And then finally, geometric confinement also plays a role. Lots of the organic scaffolds, they have this kind of nanoporous structures. So it means that the actual space that allows for crystal growth is highly constrained. So this prevents the formation of large randomly oriented crystals and is essentially used as a template to guide the crystal assembly in the formation process.

So one example, as I said, is this kind of sea urchins here. So let me explain what they actually did. So basically, essentially, the scientists collected these urchins. They intentionally cut the little spines or the sharp ends of these little spines. So then the sea urchin actually goes ahead and repairs it. And they look at what happens during this kind of repairing process.

So what I have noticed here is that this is actually an example of one of these spicules that was actually cut. And after a few days, you notice that this is what the surface actually looks like. This liquid-like structure, this is actually amorphous calcium carbonate. This is basically ACC formation. And they use this kind of liquid phase to first infiltrate the structure and then transform it into crystalline phase.

Now, to stabilize this ACC, they actually combine all the three strategies I'm talking about here. It's actually a magnesium-rich hydrated ACC, also stabilized with a specific kind of protein. So that's how they actually control the kinetics there. And as this kind of molecules, they dehydrate, this usually causes the precipitation of the crystalline phase and transformation.

What's also remarkable is that by controlling the kinetics, the spicules that were formed, each of the spicules is actually a single crystal calcium carbonate, which is quite remarkable because naturally occurring process usually don't lead to this kind of single crystal minerals. In most cases, it's polycrystalline.

If you want to get single crystalline, you need to introduce in epitaxial process. You basically have to do this transformation very, very slowly. That's what you learn from kinetics. If you have lots of molecules, you dump all the molecules into the surface, they are just going to randomly form essentially randomly oriented polycrystalline material.

If you want to get the crystalline phase, you need to ensure that all the atoms essentially slowly move, find the lowest energy position, which corresponds to atomic lattice sites, and then they can form actually the single crystal. So the whole idea here is that you want to be able to suppress the crystallization kinetics, to the extent that it's slow enough so that you can have this templated epitaxial process.

So there's something that people actually have verified using diffraction and TEM to find that this indeed is actually a single crystal calcium carbonate. OK. All right. So that's about the kinetics and the involvement of amorphous transient phases in the formation of the biominerals.

So another structural feature I just want to highlight here is that this kind of process also enables the formation of hierarchical microstructures. The typical kinetic process, as I mentioned here, is that they involve the formation of amorphous precursor phase. You also have a mesoscale scaffold that mostly consists of organic materials.

The organic materials, they get mineralized because of infiltration of the amorphous precursors. And then over time, they then slowly transform into the final crystalline structures with the nanocrystals essentially being-- their arrangement, their alignment, and orientation being controlled by the local organic matrix.

So one example for this kind of hierarchical structure is basically bone, like human bone. So this diagram shows the hierarchical structure of the human cortical bone. So cortical bone is basically the surface layer of bone, which is the most dense. And that's why it's also called the compact bone.

So if you look at the structure here, the basic structural unit is this kind of collagen molecules. The collagen molecules have this kind of long-chain form. And then these long-chain organic molecules, they basically get mineralized. A lot of the hydroxyapatite crystals attach to this kind of long fibril to form, essentially, a mineralized composite.

And then these kind of fibrils then stack together to form the collagen fibers. These collagen fibers then subsequently assemble into this kind of structure here. Essentially it's sort of like core-shell filaments where, within each core-shell filaments, this is called the osteons. The osteons, they typically have a size of maybe a couple hundred microns in terms of diameter.

And in the osteon, the inside basically is a hollow core. That's where the blood vessels and nerves run through. The outside layers is this collagen decorated with hydroxyapatite that give you the mechanical strength. So that's the basic structure. You need to know both. But you can see that you have this kind of hierarchy here, structural hierarchy here, from microscale, which is the basic osteon, then go to essentially the submicron scale, where you have the collagen fibers, and eventually you go to even smaller, go to essentially the individual mineralized strands of collagen fibrils.

So this is a very typical characteristic of a lot of biominerals having this kind of structural hierarchy there. And amorphous materials, again, plays a very important role in forming this kind of hierarchical microstructure here. I probably don't have to talk too much about this. I have already mentioned about that, that it helps to essentially create this kind of a structural flexibility by this infiltration deposition process.

Also, by slowing down the crystallization kinetics, it avoids the formation of large, randomly oriented crystals, which may actually cause a lot of internal stress and cracking. So it helps to decrease the brittleness of the composite in the formation process and ensure its structural integrity. And then kinetics control. The organisms can also essentially control the amorphous formation by essentially using these inhibitory molecules to control kinetics and integrate them into hierarchical microstructure.

All right. So that's basically about the kinetics of this kind of biomineralized process. Any questions about that before we move on to talk about how people learn from the processes?

AUDIENCE: Well, maybe this will be related. For the sponges, the amorphous silica, is that the same-- I guess you said it was used for waveguides. Is it the same structure as amorphous silica that we--

JUEJUN HU: Not exactly.

AUDIENCE: --hydrated? I tried to look at it.

JUEJUN HU: It contains protein. That's the main difference. It's a composite material. Yeah. It's not pure silica. Yeah. It always contains a little bit of protein.

AUDIENCE: It still worked well enough?

JUEJUN HU: Yeah. I mean, protein itself is pretty transparent to certain wavelengths of light. So that's why it can work as basically a fiber-optic cable, basically. Yeah. But it's essentially silica. But when they die, essentially, the organic molecules, they decompose. So what's really left behind becomes pure silica, essentially, in a more porous form, in nanoporous form. Yeah.

AUDIENCE: So that maybe would be better? Well, I don't know. And then how are they turning things in this kinetic control so that you don't get a polycrystalline thing?

JUEJUN HU: For silica, it's easy because silica practically almost never crystallizes. So that's quite straightforward. You need to inhibit the crystallization mostly for calcium carbonate and calcium phosphate.

AUDIENCE: So they're purposefully slowing everything down? Because every process-- it felt like every process we talked about this semester takes place at some sort of annealing temperature or something like that. And high temperatures.

JUEJUN HU: In fact, bio organisms, as I said, purposefully, they want to slow it down.

AUDIENCE: Slow it down. OK. So the issue is that it's--

JUEJUN HU: The issue is not that they cannot crystallize it. It's usually they want to do it slowly. Yeah. So that's a strategy I talk about, for example, for the crustacean, when they're molting. The idea always is they want amorphous phase to form essentially a raw shape quickly, but then they slowly try to essentially mold the microstructure into the desired geometry.

AUDIENCE: OK.

JUEJUN HU: Yeah. And that always takes time. Whether it's the spicules for sea urchin or it's the exoskeleton for crustaceans, they all have this specific microstructure. If you allow the crystallization to happen normally, on pace, then you always get randomly oriented crystals that does not follow this hierarchical microstructure. They need a templated process, such that the crystals only attached to the side they want. And to do that, you need to do it slowly. Yeah. To ensure the--

AUDIENCE: I guess I was surprised that it's kinetically favorable to crystallize even--

JUEJUN HU: It's kinetically-- I mean, it's thermodynamically always favorable to crystallize. But it won't kinetically slow it down, that process. And kinetically, by forming this amorphous phase first, that gives them a lot of control in terms of geometry and the controlled crystallization pathways.

AUDIENCE: OK. OK. I'm back on track.

JUEJUN HU: Yeah. So that's why. OK?

AUDIENCE: All good.

JUEJUN HU:

All right. Now just a few examples. I mean, this slide I actually quoted from one of the previous lectures. This is essentially a bioinspired nacre-like composite. You have essentially these kind of glass platelets. In between, you put the polymer layers. So when you actually try to deform it, essentially the glass will slide with respect to each other because of the interpolymeric layers. And that actually helps to improve the toughness of these kind of glass plates. So that's just one example how these kind of hierarchical structures can help boost the strength of manmade structures.

Now, as I promised, I'm going to talk about glass sponge. So this is another example where we humans can learn from essentially nature to create a mechanically robust design. So this is basically the silica skeleton, glass skeleton, of a specific kind of a sponge. I will not pretend that I know Latin. I have no idea how to pronounce it, but it's a kind of sponge, basically.

So as I said, it has this kind of very unique arrangement of the silica skeleton here. You have essentially a square lattice that's essentially reinforced by these kind of diagonal rods. So there are actually two sets of diagonal rods if you look at it. So what the scientists did, they try to model this structure here with a set of geometric parameters.

So you have, for example, the lattice period. You have the width of the square lattice. You have the width of the struct. All those kind of things. What they do is then they put it into a model. They try to optimize all those parameters to maximize the resistance of the structure against buckling deformation. They also look into several reference structures. These are alternative designs.

For all the alternative designs, you also have a few set of geometric parameters that characterize essentially the arrangement. So similarly, they optimize all those parameters to maximize its buckling resistance. And what ends up happening here, when you look at experiment, this structure that's bioinspired by sponges, exhibits the highest buckling resistance compared to all the other optimized structures.

What they also found out is that the geometric parameters coming from the natural sponge is actually very close to what people optimized using finite-element simulation. So in other words, the constraint they are putting about here, the figure of merit, is the maximum buckling resistance with the same amount of total material, total silica.

So it essentially means that these glass sponges, they are really ingenious architects. They were able to find out this design, use the minimum amount of silica, it gives the maximum mechanical strength. They're very close to optimal, within a few percent. So to me that's quite remarkable, how nature were able to get to this kind of optimized design.

So that's one example here. We're talking about mechanical properties. Now let's take a look at another example. In this case, let's look at diatoms. So the silica shell of diatoms, as I mentioned, they have this kind of nanoporous structure here. So this nanoporous structure and also the fact that it's basically silica-- it's very abundant, it's biocompatible-- has attracted lots of attention for applications like sensing or drug delivery.

So this is actually one example of a single-molecule sensor people were able to develop using essentially this diatom. So the technique they're using here is called SERS, Surface Enhanced Raman Spectroscopy. It's basically using Raman scattering. We talk about Raman scattering. Different molecules, they essentially have different kind of phonon energy, different vibrational energy. And that means that if you essentially program, excite them using a laser, they're going to emit different frequencies of Raman-scattered signals. From that, they serve as a molecular signature to identify different species.

Downside, which I did not talk about previously, is that Raman is actually a very weak process. Compared to Rayleigh scattering, Raman-scattering cross-section is billions of times weaker compared to Rayleigh scattering. So normally, Raman tends to be quite an insensitive technique. And that's why, in fact, Raman spectroscopy was not even used when Raman discovered Raman scattering. Nobody cared about it because it's so weak, until '60s, '70s, when people actually develop lasers.

They now have a very powerful light source that can actually excite enough Raman photons. That's why it actually became useful. Now, that said, if you want to detect a single molecule using Raman, you can imagine it can be quite difficult. So the way it works is to essentially create these kind of microstructures, where you can concentrate the optical field into a very small regime to get into high enough photon density so that you can excite enough number of molecules. That's the basic idea of SERS.

So in this case, this kind of porous structure of the silica, biosilica, produced by diatom, actually is very useful here. It sort of acts optically as a photonic crystal. So when it's actually decorated with these metallic nanoparticles, they can actually interact with the metallic nanoparticles to cause essentially a plasmonic enhancement also with the photonic crystal effect. And that produces a high density of what are called hotspots.

So hotspots is the terminology used in SERS to essentially describe regions where you have very high local electromagnetic field. In those regions, if you put a molecule there, it's going to generate very strong Raman scattering. That's how you can actually detect it. So this kind of porous structure helps you to amplify the density of these hotspots through basically a photonic crystal effect.

It also helps to have large surface area. You are able to absorb lots of these metallic nanoparticles to have plasmonic enhancement. So optically, the nanoporous structure is quite useful. So that's one part.

What's really ingenious about this study is that they also use the fact that silica is silica. So it has hydrophilic surface. It also has this kind of nanoporous geometry. So it means that it actually wets solution really well. So imagine you put a droplet onto one of these substrates that contain this kind of diatoms, biosilica. Then as the solution dries, this diatom, the silica, biosilica, essentially pulls the drying liquid droplets towards itself.

In the process, anything that's originally in the solution phase, all the molecules, essentially get concentrated down into the surface of the silica shell here. So this also creates very good spatial overlap between the molecules originally in the solution phase to the hotspots, the SERS hotspots on the surface there. So that's why they were able to actually detect single molecules by using this kind of architecture here. It's like a self-concentrating mechanism in the solution and also provides optical enhancement.

AUDIENCE: Can you repeat how the optical enhancement--

JUEJUN HU: Yeah, the optical enhancement happens basically through-- so what they did is they have to essentially decorate the surface with silver nanoparticles. So silver nanoparticles provide plasmonic enhancement. Essentially, because of oscillations of free electrons, it creates a very high electromagnetic field at the surface. So by having this kind of porous nanostructure, you can have essentially many attachment sites for the silver nanoparticles, which helps in that regard.

Itself is also photonic crystal. So that helps essentially also locally concentrate electromagnetic field. And the whole game about SERS is just try to concentrate electromagnetic field to get enhanced scattering. So basically, that's why they call it double enhancement. You have essentially the fluid enhancement, essentially pulling the molecule towards it. Also, optically, it helps to boost the signal. That's why I think it's quite an interesting study there. Yeah.

All right. Next example for this kind of nanoporous structure. Because it has very large surface-to-volume ratio, you can imagine that they can also be used for drug delivery, to transport drug molecules to biological tissues. So in this particular study I'm quoting here, you can see this is actually a diatom, biosilica, they're using. You can see it's a highly porous structure.

So what they did is they actually genetically engineered the diatom. So when they build the silica shell, the silica shell, the surface is actually decorated with a specific kind of protein. And this protein actually binds to antibody that's present on the surface of the targeted cells, targeted biological cells. They want to essentially deliver the drugs specifically to only one kind of biological cells.

So that's why they biological engineer, genetically engineer the biosilica, the diatom. So in this plot here, this basically shows essentially the surface-binding capabilities. This one, WT, this is basically a wild type. This is ungenetically modified diatom. It doesn't really bind. It only has some physical adsorption to the kind of proteins.

But if it's genetically modified, these four strands, you can see that they exhibit much stronger surface affinity to the antibodies. So they can selectively bind to the cell. Also, this porous surface also means that they can actually be essentially-- they can electrostatically attract to the hydrophobic drug molecules with a larger surface-to-volume ratio. They can also carry lots of drug molecules.

So then they can then use this kind of little biosilica as a little vehicle here to transport the drug molecules in a site-specific manner to target the cells. So this is actually the image here. This is a fluorescent image of the cells. So the blue part is basically the nuclei. The red part is the surrounding region there. And on the surface of the cells, you actually see this little silica, little vehicles. They serve as vehicles that eventually attach to the surface and deliver the drug molecules specifically to that kind of cell there. So another example of using these kind of diatom structures.

Now, last part I want to quickly mention here is kind of a material synthetic strategy that people have developed by copying what the nature is doing in biomineralization. It's often called a Polymer-Induced Liquid Precursor, PILP. The PILP process is really a direct replica of the natural biomineralization process.

You start out by adding these polymers that contains ionic species in a solution that's already supersaturated with minerals. In this case, because the solution is supersaturated with mineral ions, the ions then electrostatically attract to the polymer chain. And then they can then assemble into essentially these nanoclusters that contains amorphous mineral with the polymer matrix.

So these are the nanoclusters. These clusters, people use them to infiltrate whatever template they have made. And in the end, this template, you then essentially trigger the crystallization of those nanoclusters. So then you form the material that follows whatever template you have set for the crystallization process. So as you can tell, this is very, very similar to the biomineralization process.

Some examples about the application of this kind of PILP process. This is an example where people are trying to develop essentially a bone-like microstructure for a composite that contains essentially collagen with essentially mineralized hydroxyapatite that's decorated. So create artificial bone. That's what people are trying to do.

So what's shown here in this plot here, this is basically they call the collagen sponge. It's basically a porous structure containing many collagen fibers. And if you look at the SEM, this is what the sponge looks like. You can see this kind of fibrous structures. Now, if you directly use a conventional crystallization deposition process, this is what you end up getting.

You see these little spheres? These are essentially a calcium carbonate that crystallizes and form little crystals that directly then attach to the surface there. So this is not a desirable microstructure. If you use the polymer-induced liquid precursor approach, then you get these kind of nanoclusters that then attach to the surface of the collagen fibers here. And you get this kind of microstructure that's actually very similar to what you get in the bone.

What's also interesting is that they actually found out that the nanocrystals attached to fiber, they are all aligned crystallographically. The long axis of the fibers, they all align with 011 direction of the calcite crystals that was formed, which is quite remarkable. This is actually the same as what people observe actually in human bone. Same idea. The crystals crystallographically are aligned along the fibers.

So one example about how we can learn from nature to create microstructures that resembles natural biominerals. Now, another example, this is about enamels, the teeth. So as I mentioned, if you eat sugary foods, if you drink acidic liquids, you can push the chemical equilibrium to the other direction. You start to get what's called demineralization of the enamel.

And what shows up is this kind of a so-called white spot lesion. This is actually one example. You see these little white spots here, like right here? So this is actually indication of the initial phase of demineralization. You are essentially extracting the hydroxyapatite from the surface, leaving behind the porous enamel, which then, of course that's bad. Later on, you're going to expose essentially dentin. And then you have basically a cavity.

So as I mentioned, good thing is that this process is actually reversible. So if you supply it more basic solution, you supply enough amount of essentially calcium phosphate, you can reverse the process. The problem though, however, is that in order to form the microstructure that's similar to what you have in enamel, you need to have a transient amorphous phase, ACP.

So ACP itself is also not stable. It will crystallize quickly, within a matter of minutes. So that's why people have developed different kinds of strategies, even different kind of molecules, to essentially try to stabilize the ACP molecules in the solution phase. I'm not going to go through the details here. There are many options people have tried.

One, probably the most commonly used option, is to use a kind of molecule called a Casein Phosphopeptide, CPP. CPP is basically a polypeptide where it has a certain arrangement in its polypeptide chain that can selectively attract the ions and then prevent the crystallization. So that's why this kind of complex, called CPP, ACP, if you do a Google search, you'll find lots of dental products that contain this kind of complex there. CPP. ACP.

You find toothpaste, like dental or tooth mousse that you can put on your teeth that contain these kind of molecules. And there are also lots of papers that study that, indeed, clinically, they have been verified to be useful in catalyzing so-called the remineralization process. So remineralization essentially means that they can essentially repair damages, something like white spot lesions, by essentially redepositing this calcium phosphate onto the teeth.

So this is actually the one example I quoted from this paper that shows one example of this kind of remineralization process. They do not use CPP. They use another molecule called PCBAA. I'm not a biochemist. I'm not going to tell you what exactly a molecule is. But what they claim is that this PCBAA serves two functions. On one hand, it helps to slow down the crystallization kinetics of the calcium phosphate, and they help with the remineralization process.

They also have this added function that they can prevent the formation of biofilms. So the idea is that if you think about the surface of tooth, there are lots of bacteria. These bacteria, their metabolic activities form this kind of biofilm that covers the surface of the tooth, which prevents attachment of the calcium phosphate clusters. So if you want to have a sustainable, robust remineralization process, you need to inhibit the formation of biofilms.

So that's what they found out, that this molecule also helps to inhibit the formation of the biofilm to facilitate the remineralization process. And yeah. So that's why. This could be useful here. All right.

So OK. I think we are running out of time towards the end of class here. This is the last slide I quoted from a review paper that tells you that there are lots of exciting applications of biomimetic mineralization process that have yet to be explored, with a lot of exciting prospects. So I'm going to stop here. Any questions? How do you grade my final presentation?

AUDIENCE: Great. Yeah. Thank you. This is cool.

JUEJUN HU: OK, great. Yeah. So you should get to the same level for final presentation.

AUDIENCE: Oh, yeah.

JUEJUN HU: No. It's fine. But anyway, if you have any questions, let me know. I think this is a quite exciting process. Well, I got to learn a lot myself.