Title	Self-assembling soccer ball-shaped protein nanoparticles : towards next generation’s nanomaterials
Sub Title	
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Publisher	Faculty of Science and Technology, Keio University
Publication year	2023
Jtitle	New Kyurizukai No.37 (2023. 8) ,p.2- 3
JaLC DOI	
Abstract	
Notes	Man-made proteins from Keio's Faculty of Science and Technology : invention and creation through researching self-assembling proteins Norifumi Kawakami : senior assistant professor, Department of Biosciences and Informatics The research
Genre	Article
URL	https://koara.lib.keio.ac.jp/xoonips/modules/xoonips/detail.php?koara_id=KO50001003-00000037-0002

The Research This issue features Senior Assistant Professor Norifumi Kawakami and his ongoing research about creating soccer ball-shaped protein nanoparticles.

Self-Assembling Soccer Ball-Shaped Protein Nanoparticles

Towards next generation's nanomaterials

Proteins: a fundamental building block of all living organisms. Norifumi Kawakami has harnessed the properties of proteins in his creation of soccer ball-shaped nanoparticles. These nanoparticles can be broken down and still return to their original shape, meaning that they are expected to have incredible utility as nanocapsules for delivering medications inside the human body.

Creating a name and legacy through a molecule

When Professor Kawakami was hired to his current position in April 2014, he set to work on a research project that would "create a molecule that's never existed before in our world." In explaining his motivation for this project, Kawakami says, "I've done research in so many different areas that you could say that there's been no real consistency to my work. I realized that in order to survive as a researcher, I would need to make a name for myself, some achievement that would automatically be associated me, almost like a research 'calling card.'"

Because of Kawakami's frequent encounters working with proteins in the past, he decided to use them as his focus when creating the new type of molecule. For his research "motif" he landed on the idea of a soccer ball. "When I was a student, I was fascinated by the beauty of fullerenes. A fullerene is a form of sixty carbon atoms arranged in a pattern like a soccer ball. (Fig. 1) I later learned that this soccer-ball shape occurs naturally in everything from plants to outer space, so I thought there might be something special about it and some reason why it forms so readily. This was my inspiration in wanting to design a soccer ball-shaped molecule."

Fig.1 "Soccer ball" patterns appearing in various settings
A fullerene composed of 60 carbon atoms (left), newly budding pincushion flowers (center), and Kronberger 61, a planetary nebula shaped like a soccer ball (right). Rightmost photo courtesy of the International Gemini Observatory/AURA

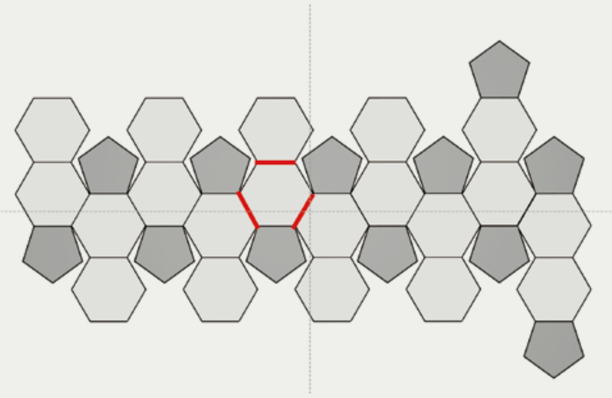

Fig.2 The geometric net of a soccer ball
Soccer balls are made up of 12 pentagons and 20 hexagons. The vertices of connected pentagons (red lines) naturally form hexagons.

Making a soccer ball-shaped molecule using fusion proteins

So how exactly is it possible to take proteins and make them into a soccer ball-esque molecule? Kawakami's chosen molecular design method was to use fusion proteins.

Inside our bodies, proteins are at constant work, spontaneously and automatically collecting into complex structures. Around the year 2000, researchers began to investigate ways that they could use this property to manipulate proteins into man-made shapes.

Then, in 2014, an American team of researchers successfully constructed a polyhedral molecule by using fusion proteins which combined two types of proteins together. However, a problem emerged; the proteins could take multiple different polyhedral shapes. Mixed types in a product create a major roadblock to industrial applications. Kawakami saw this issue and began to explore whether

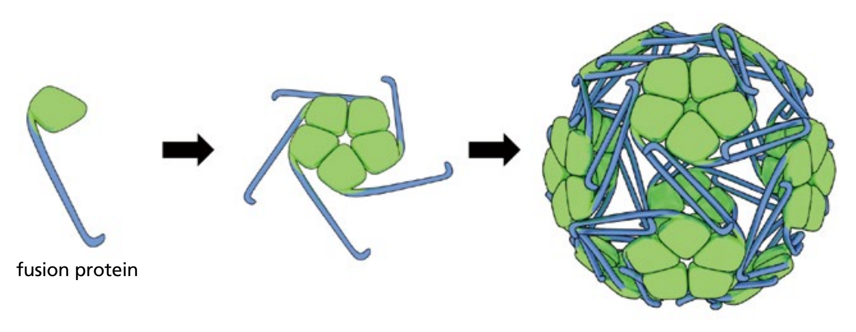

Fig.3 Design concept for creating the soccer ball-shaped molecules.
The scientists fused two types of natural proteins so that when 60 of them were collected, they would form the shape of a soccer ball. The green and blue proteins have properties that are attracted to each other. Furthermore, the blue proteins are hooked, meaning that when they latch to each other, they stabilize the molecule's structure. In practice, the fusion proteins are created when their genes are introduced to bacteria from the large intestine (E. coli). The bacteria synthesize the proteins, which then spontaneously gather and form into the shape of soccer balls.

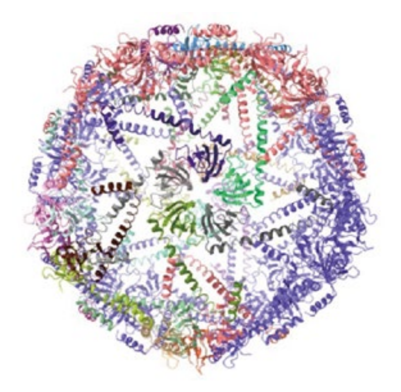

Fig.4 The model (photo) and actual structure of the molecule as revealed through cryo-EM (above)
Just as intended, the 60 individual proteins ended up forming into a soccer ball-shape. The photographed piece of the model shows how five proteins are amalgamated into a pentagonal piece. The final 60-molecule nanoparticle is a truncated icosahedral protein, which led to the name "TIP60."

there was a way of only producing soccer ball-shaped molecules.

"Soccer balls are essentially just polyhedrons made up of 12 pentagons and 20 hexagons, so my first thought was to figure out some sort of pattern that combined these shapes. The problem was that I had no idea how to arrange them together in a way that made sense and no clue how to make their edges line up." However, as Kawakami stared down the blueprints for his molecule model, he realized something. "I was stuck on the idea that hexagons were essential to building a soccer ball, but then I realized that if you simply use lines to connect pentagons to each other at their vertices, inevitably you will end up with hexagons." (Fig. 2)

Using Euler's theorem on polyhedrons—(number of vertices) - (number of edges) + (number of faces) = 2—it follows that a polyhedron consisting of hexagons and pentagons must include 12 pentagons. In other words, it is impossible to create any shape other than that of a soccer ball if relying on a mechanism which connects the lines extending from pentagons' vertices. "This is it!" Kawakami was confident he had found the solution.

The project researchers then designed a fusion protein (Fig. 3) and were able to confirm that the only types of molecules that should result from their experiments would be shaped like soccer balls. However, these particles were microscopic—only 22 nanometers in diameter (a nanometer is one billionth of a meter)—meaning that any tests to categorically determine whether the molecules were structured like soccer balls would require advanced techniques and equipment. Thanks to the help of the joint researchers on his team, Kawakami was able to conduct an analysis using cryo-electron microscopy (cryo-EM), a technique for which the inventors were awarded the 2017 Nobel Prize in Chemistry. Five years after the particles were first constructed, Kawakami was finally able to demonstrate conclusively that the molecules were, in fact, shaped like soccer balls(Fig.4)

Potential applications for nanocapsules and nanomaterials

While Kawakami originally started this research so that he could have an identifiable accomplishment associated with his name, the response to his soccer ball-nanoparticles in academic papers and at conferences was enormous. Because the centers of the nanoparticles were hollow, it was possible to fill them with medicine, allowing them to be used as "nanocapsules" or for other futuristic applications.

It was at this point that Kawakami then invented a way for the nanoparticles to disassemble and reassemble themselves into their soccer ball-form. If a manufacturer adds a substance when the particles are reassembling into their original shape, this substance will be trapped inside the ball.

In a more recent development, one of the students working on the research project figured out how to produce large quantities of the soccer ball nanoparticles at a low cost. The team is also working to produce a soft, gel-like material where the nanoparticles form large structures that store water around them. This mechanism would make it so that when the gel is activated, the soccer-ball particles will "collapse" allowing whatever they carry inside to be released.

When asked about his thoughts and aspirations for the future of nanoparticles, Kawakami says, "My goal is to have people all over the world use these soccer ball nanoparticles. That way, when other researchers see how we were able to package other substances inside them, turn them into a gel, and find different uses for them, they might look back on our work making the soccer balls when exploring materials and chemicals of their own. That would feel incredible."

(Interview and text writer: Chisato Hata)