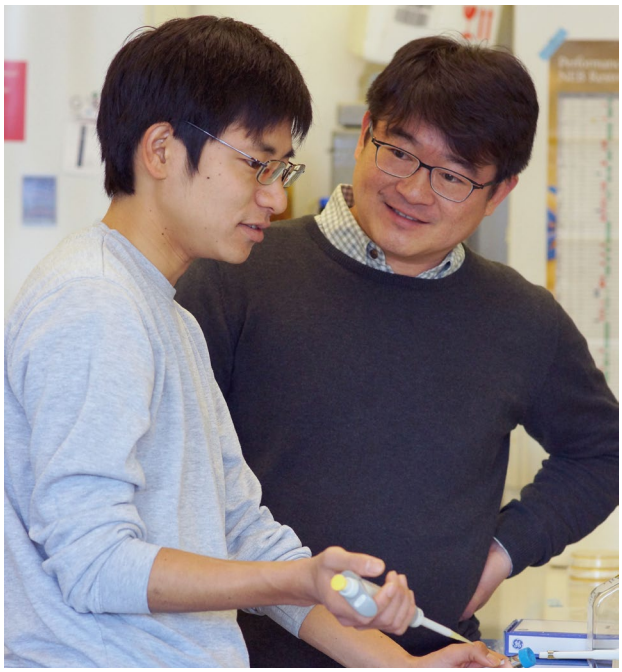
Fathoming natural nanomachine

SNI team looks inside nuclear pore complexes for the first time

The 2016 Nobel Prize in chemistry was awarded for the design and synthesis of a nanomachine. Argovia Professor Dr. Roderick Lim has also been investigating a nanomachine – the nuclear pore complex – for several years, and hopes to use his findings to build artificial nanomachines. In May 2016, Lim published the first ever video of the dynamic processes occurring in a natural nuclear pore complex in the journal "Nature Nanotechnology"*. Using a high-speed atomic force microscope, Yusuke Sakiyama – an SNI PhD student on Lim's team – was the first person on Earth to watch individual nuclear pore complexes at work, thereby confirming the hypothesis proposed by Lim on how nuclear pore complexes function.



Natural nanomachines as molecular filters

Inside the cells of higher organisms, numerous perfectly functioning nanomachines are hard at work. There are small factories providing energy or forming a wide range of compounds. Alongside them are minute motors and complete transport systems which ensure that the right substances reach the various areas of the cell. One of these ingenious transport systems is the nuclear pore complex. Vast numbers of these remarkable pores, which act as highly effective molecular filters, regulate the exchange of compounds between the cell nucleus and the surrounding cytoplasm. Water and smaller molecules are able to pass through these barriers along a concentration gradient by diffusion. However, most larger molecules are prevented from entering the cell nucleus. Only those which perform certain functions in the cell nucleus are able to enter it by binding to transport proteins.

Transport and selection are regulated by specific proteins in the pore known as phenylalanine-glycine nucleo-

* Yusuke Sakiyama, Adam Mazur, Larisa E. Kapinos and Roderick Y.H. Lim
Spatiotemporal dynamics of the nuclear pore complex transport barrier resolved by high-speed atomic force microscopy
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porins, or FG Nups. These proteins form the molecular barrier that only allows specific molecules to pass. The overall structure of nuclear pores has been known for a long time. However, it has so far remained unclear just how FG Nups operate, and how they prevent larger molecules from passing through the pore.

New findings obtained thanks to high-speed AFM

This year, Roderick Lim and his team gained new insights into how nuclear pore complexes work. Using a high-speed atomic force microscope (HS-AFM), Yusuke Sakiyama, a PhD student in Roderick Lim's laboratory, and Adam Mazur of the Biozentrum's Research IT group were able to observe the passage of molecules through the nuclear pores for the first time, and even capture the phenomenon in a short film.

To this end, the researchers examined the comparatively large nuclear pores of a frog. Even here, the diameter of the central pore channel measures just 40 nanometers. "It is simply fantastic that we are now in a position to observe natural vital processes on the nanometer scale in real time, and finally gain insights we have been working toward for years," said Roderick Lim of the innovation.

A barrier of undulating tentacles

Watching the first recordings, the researchers soon noticed that some pores became "blocked" by molecules passing through them. In order to conduct a more detailed examination, Lim's team then observed "open" pores with no molecules lodged in the central pore channel. To obtain the film sequences, the researchers recorded still images of the pore under examination at intervals of around 100 milliseconds, which they then edited together. The resulting films revealed that the arrangement and structure of the FG Nups changed constantly. The FG Nups behaved like tentacles, extending into the pore, elongating and momentarily interlinking to form short-lived "condensates" inside the central pore channel. At no point in the observations did the arrangement of the FG Nups stay the same, even though their individual tethering points on the pore walls remained constant. The FG Nups also occasionally formed sieve-like structures, although these were not static states as sometimes described in the literature.

"The speed of these dynamic movements determines which molecules are allowed to pass through the pore," explains Lim. "The FG Nups move at a faster rate than large proteins, denying them access to the nuclear pore complex. Small molecules, meanwhile, are more agile, enabling them to pass through the FG Nups barrier."

Further questions remain

The footage supports the hypothesis proposed by Roderick Lim that transport is not determined by the static arrangement of FG Nups, but rather by their dynamic shape-changing behavior. While a number of questions have been answered by these first striking recordings of the nuclear pore complex, it still remains unclear how large molecules are able to overcome the FG Nups barrier. "We are nevertheless confident that further investigation will resolve this issue, too," claims Lim.

Understanding how these selective transport systems work would pave the way for synthetic creation of similar nanomachines, for instance as components in tiny molecular factories.