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# FEATURE STORY



## The 2009 Nobel Prize in Chemistry and KEK's Photon Factory

**[Ribosome, Weisenberg Camera, Imaging Plate]**

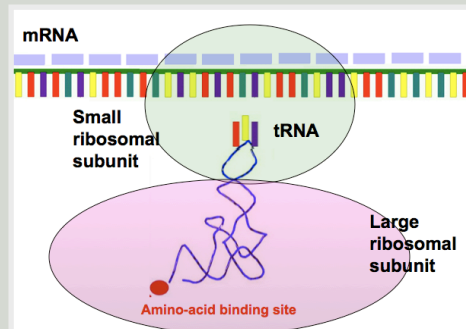
This week features the pioneering work of the 2009 Nobel Chemistry Prize winner Prof. Ada Yonath, and explains how KEK's Photon Factory played a key role in her pioneering research to determine the structure of the ribosome, the cell machinery responsible for manufacturing proteins.

The 2009 Chemistry Nobel laureate Prof. Ada Yonath, the director of the Helen and Milton A. Kimmelman Center for Biomolecular Structure and Assembly at the Weizmann Institute of Science, visited Tsukuba to attend the Photon Factory Symposium held on March 9-10, 2010. She was awarded an Honorary Supreme Professorship at KEK after giving an hour-long lecture.

**Those who pave the way to** new ideas and new technologies are those who are unafraid. They are called pioneers. Professor Ada Yonath, the 2009 Chemistry Nobel laureate, is one pioneer. Now the director of the Helen and Milton A. Kimmelman Center for Biomolecular Structure and Assembly at the Weizmann Institute of Science, she, despite widespread skepticism expressed by her colleagues, whole-heartedly pursued her dream of understanding the structure of ribosomes, the protein-manufacturing machines that reside in every organism

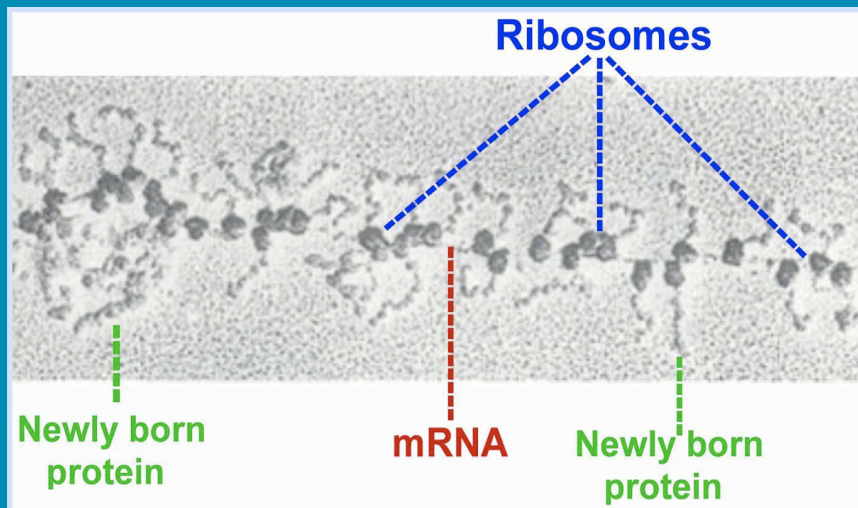
The 2009 Nobel Prize in Chemistry was shared by Venkatraman Ramakrishnan, Thomas Steitz,

and Ada Yonath. According to Prof. Noriyoshi Sakabe of KEK, "It was Ada who truly opened up the field of ribosomal crystallography for others to follow. She was the one who had the strong will and courage to make this happen." Prof. Yonath's decades-long mission was to determine the details of the complex structure of the ribosome. In her quest to develop a fully resolved image of a ribosome, Prof. Yonath traveled from continent to continent for more than two decades, looking for the cutting-edge X-ray sources to shine on her ribosome crystals. Among the facilities she used was KEK's Photon Factory—the only light source that offered Weisenberg Camera with high sensitivity imaging plates.



A ribosome has a small subunit and a large subunit. The small subunit decodes genetic information in messenger RNA, while the large subunit forms proteins according to the directions in the messenger RNA.





Prior to the success of ribosome crystallization, the above picture of ribosomes was the best available to show how proteins are manufactured by ribosomes.

## The difficulty of ribosome crystallography

Ribosomes are the cell machinery that produce proteins from amino acids using the information in DNA. This process is called translation. First, genetic information encoded in the DNA located in the nucleus of a cell is copied onto a piece of messenger RNA (mRNA). Next, the mRNA moves out of the nucleus, into the main body of the cell. Here, a ribosome binds to the mRNA, decodes the genetic code on it, and uses the information in the mRNA to create a string of amino acids. This string of amino acids is a protein.

Ribosomes consist of two subunits, unimaginatively named the small subunit and the large subunit. While there is some variation in ribosomal structure among different species, the overall structure is similar. In all cases, both subunits are complex biochemical machines. For example, one type of small subunit, one

found in bacteria and called 30S, consists of one strand of ribosomal RNA (rRNA) and up to 21 different proteins. The corresponding large subunit, called 50S, consists of two strands of rRNA and up to 34 proteins. These two subunits bind together to form a ribosome called 70S. While the small subunit decodes the genetic information in the mRNA, the large subunit creates a chain of amino acids according to the instructions in the mRNA. When finished, the chain of amino acids is a protein, an organic catalyst which ensures that chemical reactions necessary for life happen as they should.

"A ribosome is a very effective machine," explained Prof. Yonath in her invited speech at the Photon Factory Symposium held on March 9-10, 2010, in Tsukuba. "It produces 15 peptide bonds in one second, and a full protein in a few seconds."

The basic structure of DNA was determined in the 1940s. Over the next twenty years, scientists tried to do the same for the ribosome, hoping to understand how ribosomes actually produce proteins. The first electron microscopic image of ribosomes was obtained before 1970s (see image). With a bit of imagination, one could differentiate the two ribosomal subunits, the mRNA being fed into the ribosome, and

protein that came out on the other side.

However, gaining a detailed picture of entire ribosome, showing the exact locations of its constituent molecules, was much more difficult. To form an image of a ribosome, there were two crucial technological hurdles. First, scientists needed to prepare samples of whole ribosomes. These needed to be purified and crystallized as uniformly as possible. Second, scientists needed to have a high-quality X-ray source. With these two parts, a well-focused X-ray beam could be shot into the crystal. From the way the beam was scattered by the atoms and molecules within the crystal, scientists could infer the detailed structure of the crystal.

At that time, "textbooks definitively stated that ribosomes could not be crystallized," recalls Prof. Yonath. Ribosomes have "a high degree of internal mobility, flexibility, functional heterogeneity, a marked tendency to deteriorate, chemical complexity, large size, and



To gain ribosomal crystals, Prof. Yonath chose to use extremophile bacteria. The Dead Sea above is saturated with salt, where she successfully extracted ribosomes from *Haloarcula marismortui*, which could be crystallized.

asymmetric nature." Thus, crystallization seemed impossible.

## Chilling breakthrough

"Then I read in a magazine about polar bears. Before and during their hibernation, they pack their ribosomes in their cell membranes in an orderly fashion. This indicated that ribosomes could be orderly packed, in contrast to the published arguments that this was impossible. The question was why they do it," says Prof. Yonath. "I thought it was to maintain a big storage of active ribosomes to be used when they woke up. Just after awakening, they need a very intense metabolism to produce proteins."

After reading the polar bear article, Prof. Yonath immediately started experimenting. One of her colleagues, Prof. H.G. Wittmann from Max Planck Inst for Molecular Genetics in Berlin, "had experience with ribosomes but had no idea how to crystallize them, and I had an idea but I had no ribosomes. Together, we were a good team." She knew just where to look. Organisms



After the ceremony, Prof. Yonath was united with old colleagues from KEK. From left: Dr. Nobuhisa Watanabe, now professor at Nagoya University; Dr. Atsushi Nakagawa, now professor at Osaka University; Prof. Ada Yonath; Dr. Kiwako Sakabe; and Prof. Emeritus Noriyoshi Sakabe of KEK.



The first cryo-cooling of crystals was conducted at SSRL/SLAC national laboratory, with the help of Prof. Hakon Hope (left).

living in extreme conditions, known as extremophiles, usually have highly active and presumably robust ribosomes. Prof. Yonath thought their ribosomes could be made uniform more easily, making them easier to crystallize. The Dead Sea, on the eastern rim of Israel, is an extreme environment saturated by salts. The first crystals were obtained from the large ribosomal subunits from an extremophile source, *Bacillus stearothermophilus*. A few years later, crystals were obtained from the large subunits of the extreme halophilic bacterium *Haloarcula marismortui*, which lives in the Dead Sea.

Different types of crystals require different types of conditions for optimal growth. Combinations of different pH values, types of alcohols, organic materials, temperatures, and types of ribosomes gave Prof. Yonath a staggering 25,000 of different set of conditions to test. For the first four months, she and her colleague screened numbers of conditions for crystal growth for four types of bacteria ribosomes, until when they saw convergences of the trends for crystal growth into narrow ranges.

"The path to determining the structure of the ribosome was long and demanding," says Prof. Yonath in her presentation. "Frequently, we felt like we were climbing high mountains, just to discover that there is a higher mountain in front of us."

### Cryo-cooling, Eureka!

While Prof. Yonath and her team worked on the improvement of ribosome crystals, X-ray synchrotron sources around the world continued to make advancements towards higher intensity and higher resolution. For Prof. Yonath, who was simply incapable of sitting around doing nothing with ribosomal crystal in her hands, "those were very long 'short breaks'."

By the mid 1980s, high intensity, high resolution X-ray sources became readily available for imaging, but there was still one critical limitation: ribosome crystals would be destroyed after just one exposure. Because ribosomes have a complex structure with no repeating patterns or symmetry, Prof. Yonath needed to take images from every angle to discern their exact structure. "We figured out that to work under this limitation, we needed over 20,000 crystals to get a set of data," says Prof. Yonath. This was clearly impractical.

What saved the project was the polar bear again. When the high intensity X-ray beam penetrates into a ribosomal crystal, it breaks chemical bonds within the ribosomes, damaging their structure and producing free radicals—the components that lack (or have additional) charge. These progress through the crystals looking for balancing their charge and meanwhile cause more damage and more free radicals. Prof. Yonath's new idea was to minimize the damage within the crystal by cryo-cooling the crystal, so that there would be less energy and therefore the motions of the free radicals would be minimized and the damage would not progress. "Now everybody uses this technique, but at that time, no one believed in it," says Prof. Yonath. Luckily, it was not quite 'no one.' Prof. Hakon Hope at University of California at Davis was a specialist in cryo-cooling. He took the case and helped her

develop the cryo-cooling methods she needed.

This astonished KEK's Sakabe, who had tried cryo-cooling 5 years before her, and failed. "It was common knowledge before Ada that cryo-cooling of protein was not possible," says Sakabe. Frozen water diffracts X-rays, making it difficult to distinguish the effects of the ice from the effects of the sample. Ice also destroys the structure of crystals because water expands when frozen. The solution, developed by Prof. Yonath and Prof. Hope, was to immerse their sample in high duty gear oil.

"Nothing is immediate in science. We seldom felt like Archimedes discovering the principle of [buoyancy in his] bath and rushing out shouting: Eureka! Eureka! (I found it!) But finding that the cryo biocrystallography brings almost eternal life to crystals was really the moment of Eureka!" The new methods of cryo crystallography were improved over time. Consequently, the number of crystals required to obtain one data set went from a 20,000 down to less than a hundred. Prof. Yonath had now pioneered the technique of cryo biocrystallography, which later became the standard method of biocrystallography.

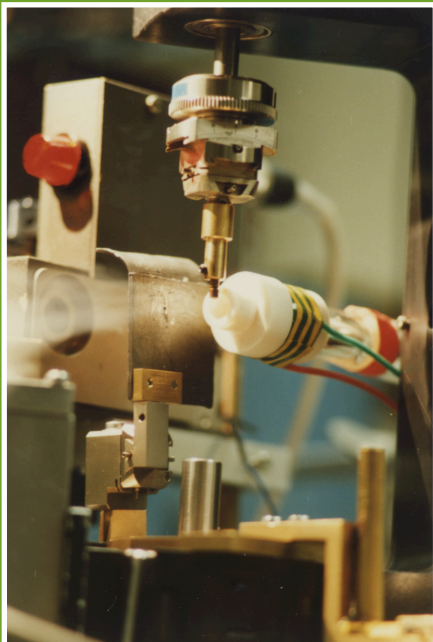
### KEK's Weissenberg camera and imaging plate

In 1986, Sakabe's beamline at KEK's Photon Factory was just about to start up. Although it was an inter-university research organization, when KEK published the user application forms, they didn't expect much from overseas institutions. This was the age of no Internet. However, as soon as the form was released, there it was, an application from Israel. "Prof. Yonath's ability to quickly seize a chance, and her persistence and determination over the decade that followed astounded us all," says Prof. Osamu Shimomura, the director of the Institute of Materials Structure Science at KEK. The next year, in 1987, Prof. Yonath arrived at KEK with a pickup truck fully loaded with her equipment.



Prof. Noriyoshi Sakabe digs out from his storage room the first generation Sakabe-Weissenberg camera used by Prof. Yonath.





Cryo-cooling apparatus developed by Prof. Yonath installed at KEK.

Prof. Yonath's objective was to use the Weisenberg camera which was newly developed by KEK's Sakabe. "Nowadays, characteristics such as coherence and intensity are the most important measures when gauging the performance of light sources. Back then, what mattered most was the performance of the detector, because a single exposure of X-ray destructed samples anyway no matter what the quality of X-ray beam was," says Sakabe.

The innovative feature of the Sakabe-Weisenberg camera was not the camera itself, but that it used an imaging plate instead of film. The imaging plate, developed by Fujifilm Co. for medical use, had many advantages over conventional camera film. First, the imaging plate reader digitalizes data automatically when reading out the imaging plate, while conventional film needed digitalization after the film development. Second, unlike film, it was easily reusable. Third, the sensitivity of the plates is linearly proportional to the intensity of beam, meaning that it requires no linearity correction. This is an important property of a detector, as linearity correction can be very complex. This property also makes the plates equally sensitive to low intensity X-rays. For lower intensity X-rays, the imaging plate was 100 times

more sensitive than conventional film. Lastly, the dynamic range—the ratio between the largest and smallest sensitivity—was 1,000 times greater than with conventional film.

"Just to take a set of data to image a ribosome was worth a Ph.D. back then. The Sakabe camera made this possible in just month's time. I could sense we were in the middle of a technology revolution," says Prof. Yonath. She maximized the use of beam time at every possible synchrotron light source available around the world—such as DESY, SSRL/SLAC, Daresbury and CHESS/Cornell. She came back to KEK frequently for the next ten years, visiting weeks at a time as a user.

"The experience at KEK was better than I had expected," smiles Prof. Yonath. "Support and assistance were excellent, and it was clear that they really wanted to understand the science." She points out that Sakabe and his wife Kiwako had a great deal of background in biology and crystallography, which was rare for synchrotron light source scientists at other overseas institutions. Two research assistants Dr. Atsushi Nakagawa, now a professor at Osaka University, and Dr. Nobuhisa Watanabe, now a professor at Nagoya University, "were the most dedicated and helpful."

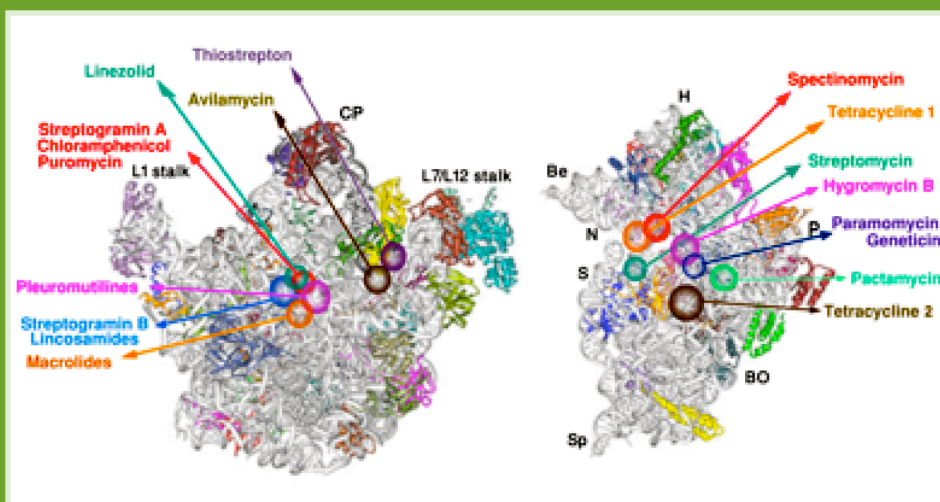
### KEK's second Honorary Supreme Professorship recipient

At the recent Photon Factory Symposium, Prof. Yonath received an Honorary Supreme Professorship from KEK's Director General, Prof. Atsuto Suzuki, for her outstanding achievements in structural and functional studies of ribosomes. She is the second recipient of this award, following KEK professor emeritus and Nobel laureate, Prof. Makoto Kobayashi. "To be recognized by people who also work [hard at] their own sciences is really wonderful," says Prof. Yonath.

Asked if she'd take a break from her job, she says "to rest is complicated for me, and to work is easy." Her plans are three-fold: to understand how antibiotics affect the ribosomes of disease-causing organisms to determine how pathogens behave, and to gain more basic scientific knowledge of how ribosome evolved.

Prof. Yonath says, "If you have curiosity, go after it. It will also be fun. Science is never sure, because you never know what will happen. Sometimes you get completely unexpected results, and sometimes you find out that you have to look for different mountains."

Prof. Yonath's driving force for the last few decades has been to understand how the ribosome works. "My question is biological, my answer is chemical, my method is physical, my calculations involve a high level of mathematics. [Knowledge and skills from all walks of science are required] to understand how nature functions." To do the best you can at whatever you do is her advice for us.



The ribosome as a target for antibacterial agents. Ribosomes are universal in organisms, and many current antibiotics target ribosomes in disease-causing organisms. Prof. Yonath's research has revealed how 20 different antibiotics functions.

#### Related Link:

[Ribosome Structure and Function, Prof. Ada E. Yonath Group](#)

[KEK Photon Factory](#)

[The 2009 Nobel Prize in Chemistry](#)

#### Paper:

[Large facilities and the evolving ribosome, the cellular machine for genetic-code translation](#)

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