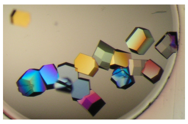
Synchrotron Radiation and its application to the Structural Biology



Synchrotron Radiation Research Center
Nobuhisa Watanabe

Welcome to my lecture today.

My name is NW, and I am officially belonging to the SR Center.

Topics

- What is Synchrotron Radiation?
- Protein crystallography & SR

  (high-pressure protein crystallography using SR)

This is the outline of today's talk.

OK. Shall we start.

What is Synchrotron Radiation?

electromagnetic radiation emitted when charged particles are accelerated radially

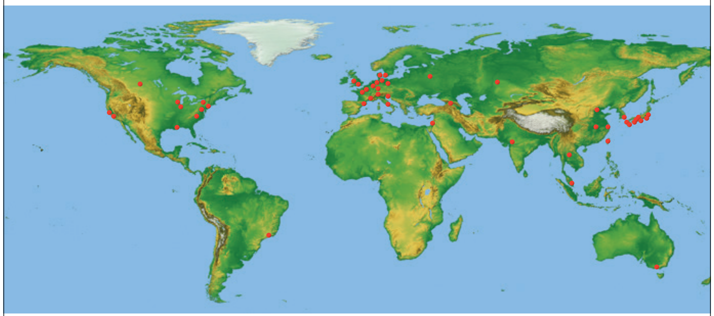
Benefit us?

Have you ever heard of Synchrotron?

World's synchrotron facilities

@2015

47 facilities / 23 countries



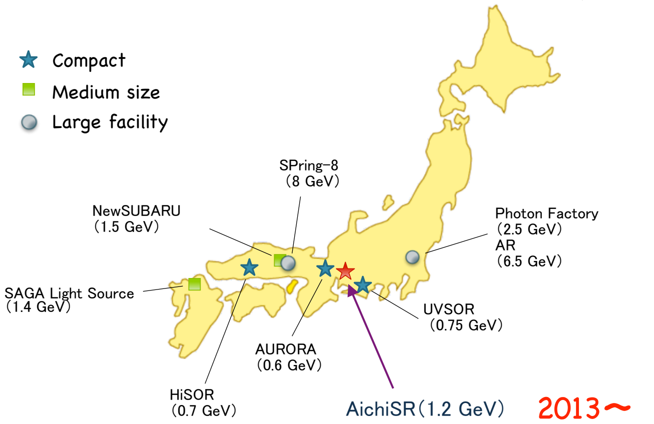
Nature Photonics, 9, 281 (2015)

In fact, there are 47 SR facilities in the word.
23 countries have some facilities.

How about your home country?

And we have …

Synchrotron facilities in Japan



We have 8 SR facilities now.

And we have a plan to construct new one in Tohoku.
You can see there is a blank area, here at northern part of Japan.

A familiar synchrotron radiation

Crab Nebula (Taurus)

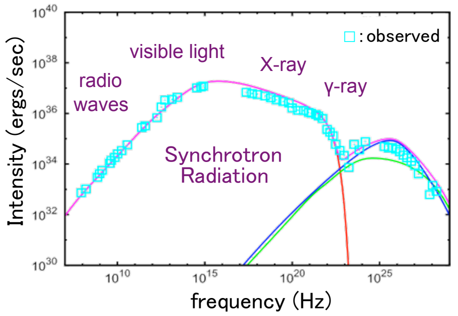
http://www.nasa.gov/multimedia/imagegallery/

OK. Come back to the synchrotron…

This is a image of the crab nebula at NASA web page.

I don't know what do you get from "familiar", but we can observe synchrotron radiation from there.

The Crab spectrum



Aharonian, F.A. & Atoyan, A.M., 1998, nspt.conf, 439A

This is a spectrum from the crab nebula.

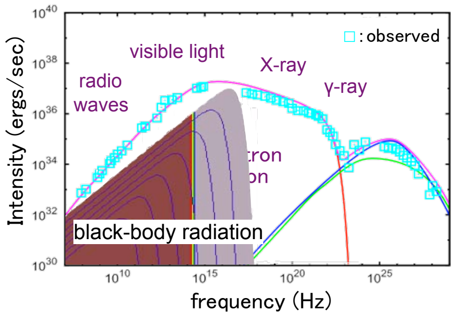
The horizontal axis of this graph is in frequency, I think you may not be familiar with. And vertical axis is intensity of the light.

Ignore the higher frequency domain, this large area from radio waves to gamma-ray is the synchrotron radiation.

As a light source in the space, I think you may think about the "sun".

But…

The Crab spectrum



Aharonian, F.A. & Atoyan, A.M., 1998, nspt.conf, 439A

The "sun" emits as a black-body, and the shape of the spectrum from it is like this,

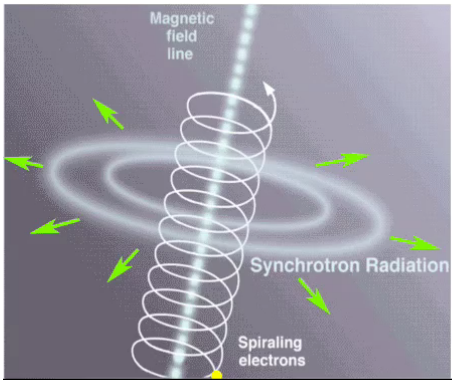
even if the "sun" has not realistic very very high temperature.

Do you know the surface temperature of the sun?
6,000 K

Can you guess what is the temperature of this spectrum? It's Millions degrees!

Important difference for us is that SR has a strong X-ray component.

Mechanism of the synchrotron radiation

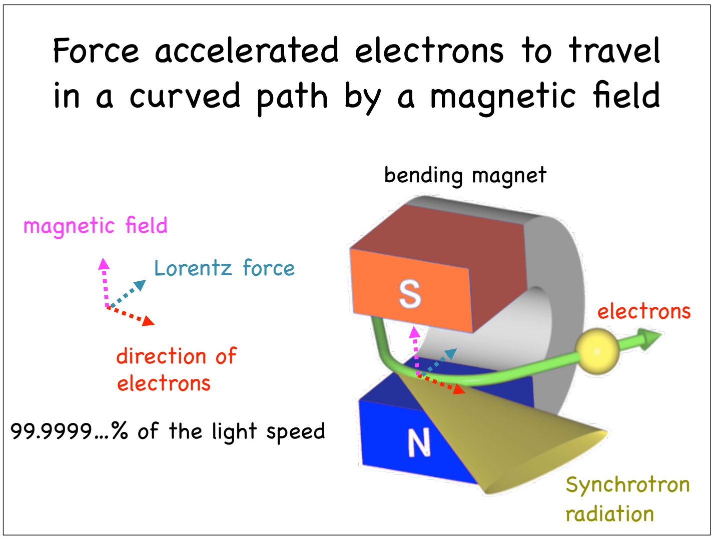


(movie)

This is the mechanism of how the SR is generated.

If very high-speed electrons run under the magnetic field, the pathway of the electron will bend and SR emits from there.

This means, we can make this kind of "galaxy" on the earth, if we can accelerate electrons enough and introduce them into a magnetic field.

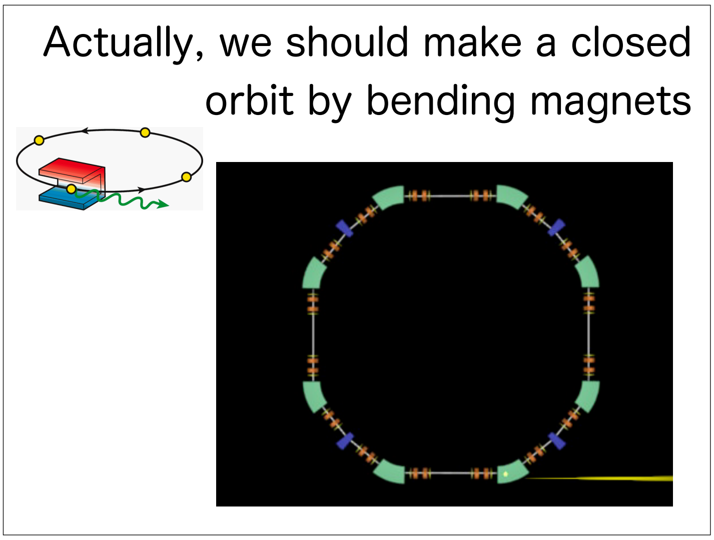


… Like this conceptual figure.

The speed of the electrons should be almost the light speed.

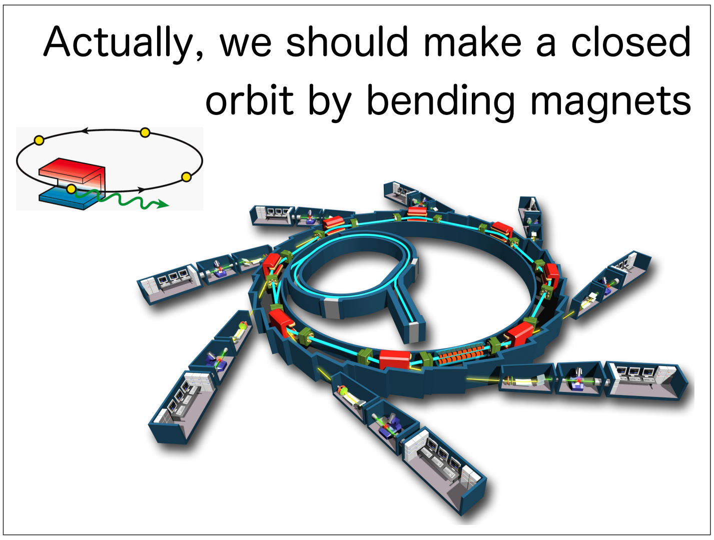
Under magnetic field, the direction of the electron is bent by the Lorentz force, and the SR is emitted tangentially.

I think you might learn about the Lorentz force somewhere.



(movie)
The high-speed electrons should be kept in the “ring” shape orbit, like this.

So, we call the facility as a “storage ring”.



With this ring structure, we can make several experimental “stations” around the ring.

Properties of synchrotron radiation

1. High Flux high intensity photon beam
2. High Brilliance highly collimated photon beam
3. Broad Spectrum
   which covers from microwaves to hard X-rays

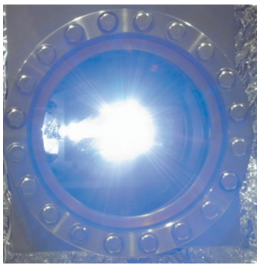
Now, let's move on to the properties of SR.

SR has several beneficial features.

The 1st one is…

Properties of synchrotron radiation

1. High Flux high intensity photon beam



UV-SOR

1st.

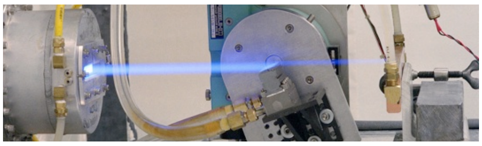
SR is a high flux, or a high intensity photon beam.

This photo was taken at the UV-SOR in Okazaki.

Have you ever been Okazaki?

Properties of synchrotron radiation

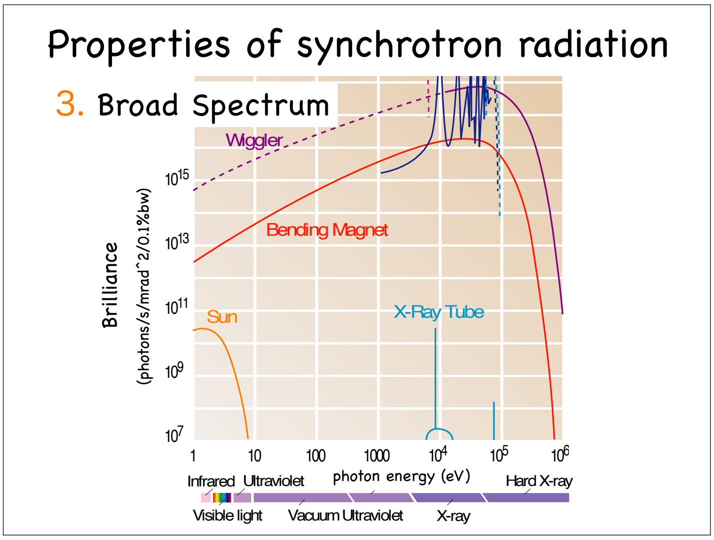
2. High Brilliance highly collimated photon beam



National Synchrotron Light Source, USA

2nd.

SR has high brilliance.
Like a laser, SR is a directional beam.



3rd.

As we have already seen at the Crab Nebula in space, SR has a broad spectrum form infrared to X-ray region.

This is a spectrum from SPring-8, Hyogo.

You can also compare the brilliance (intensity) with the sun and X-ray generators in laboratories.

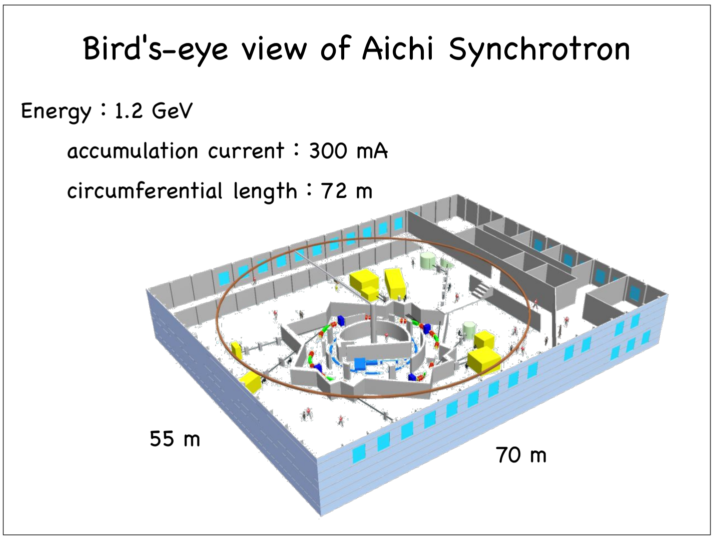
Then, I will show you our Synchrotron.

Unfortunately, not in this campus, but we have one, the Aichi SR, at Toujishiryokan Minami Sta of the Linimo.

Have you ride on the Linimo?

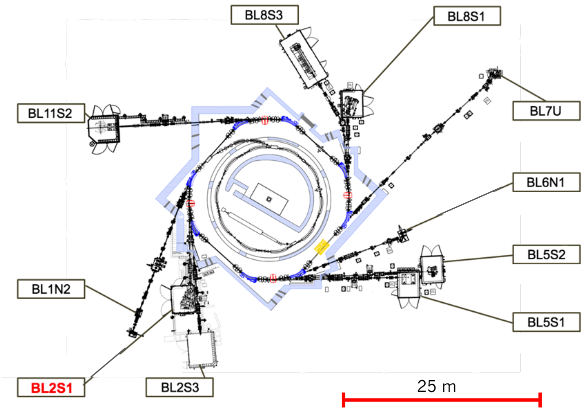
It takes about 40 min from here, but from the station, you can go there only 2 min on foot.

In the respect of accessibility, I think this is the most convenient SR facility in Japan.



This figure shows the size of the facility.

Beamlines of Aichi SR



And there are several Beamlines. (We call these apparatus as "beamline".)

By the way, for the radiation safety, as you can see, the "ring" is installed inside the 1m thick heavy concrete wall, which I have designed.

And, Nagoya U has BL for protein crystallography.

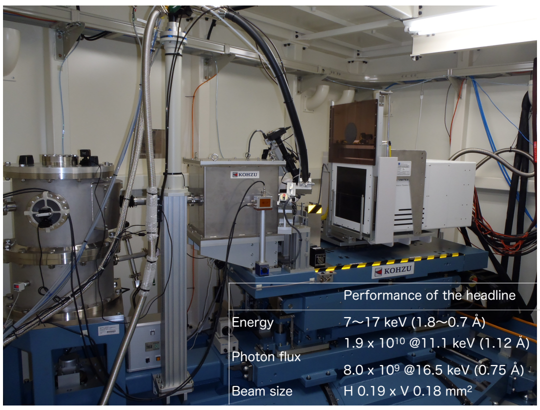
Beamline for Protein Crystallography

BL2S1（Nagoya University BL）

The BL commences the service at May 2015

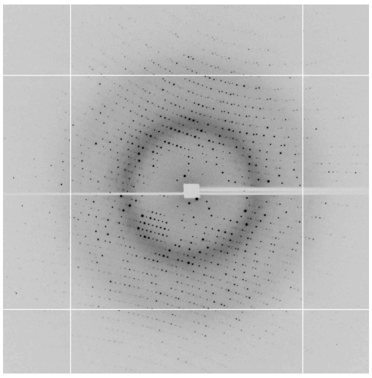
This is the Nagoya U BL, BL2S1 for protein crystallography.

Diffractometer of BL2S1



This is an inside view of the beamline.
And here you can see some parameters of the beamline.

Diffraction photos at BL2S1



in total 110 images

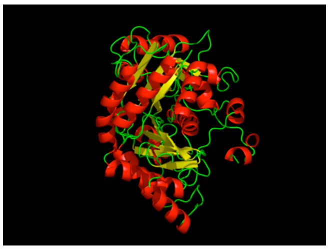
ADSC Q315r detector

At BL2S1, you can get this kind of X-ray diffraction photograph from protein crystals.

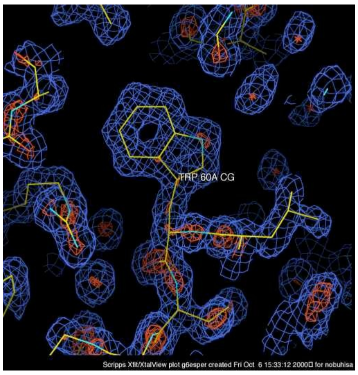
And from these photos, you may solve the protein structure.
That is the “X-ray Protein crystallography”.

What we can see by X-ray

X-rays interact with electrons



“structure”



X-ray interacts with electrons in the crystal.
So, we can observe where and how electrons are distributed.
Electron rich means that there are atoms, and we can build molecules, like this.

Protein crystallography & Synchrotron radiation

OK

Now let’s move on the next part, how the protein crystallography use SR.

Properties of synchrotron radiation

1. High Flux — high intensity photon beam

2. High Brilliance — highly collimated photon beam

3. Broad Spectrum
   which covers from microwaves to hard X-rays

As we have already seen, SR has such properties.
And …

Properties of synchrotron radiation

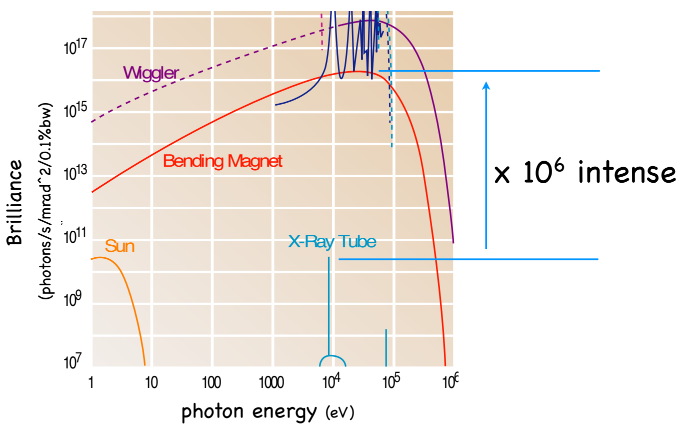
1. High Flux — high intensity photon beam

2. High Brilliance — highly collimated photon beam

make data collection faster & easier

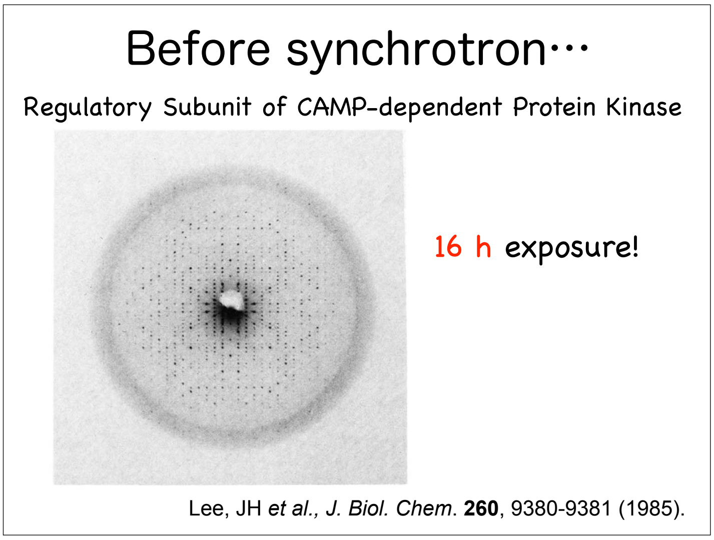
These 2 properties make data collection of protein crystallography faster & easier.

High flux & high brilliance



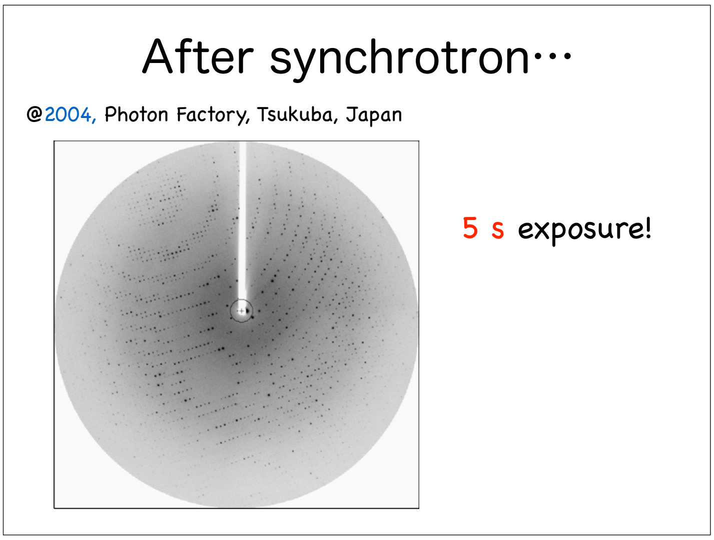
Comparing with the traditional X-ray source, SR has an intensity million times brighter.

This means, or this makes the time for experiments very short.



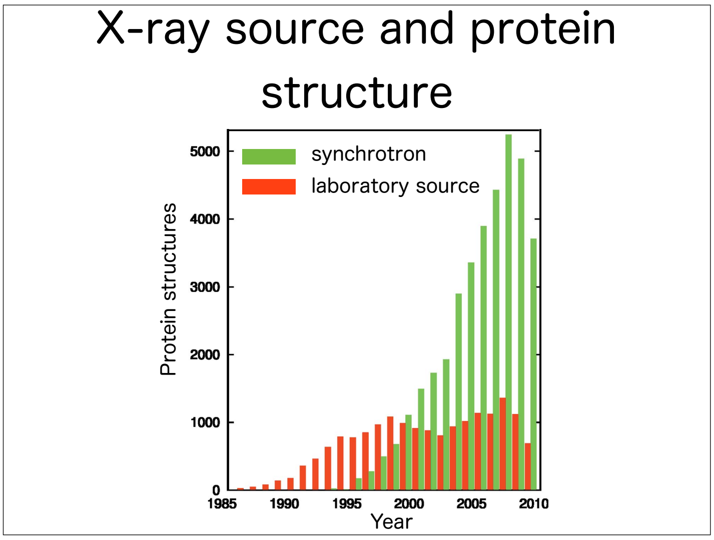
Before SR, it took more than 10 h exposure to get this kind of photo.

However,



After SR, the exposure time is dramatically reduced. For example, this image could be taken in only seconds.

Can you guess what this means?



SR bring in a “speed” into the protein crystallography.

I think you were born at around 2000, when SR surpassed lab X-ray.
After that, the structure solution of proteins is highly accelerated.

Properties of synchrotron radiation

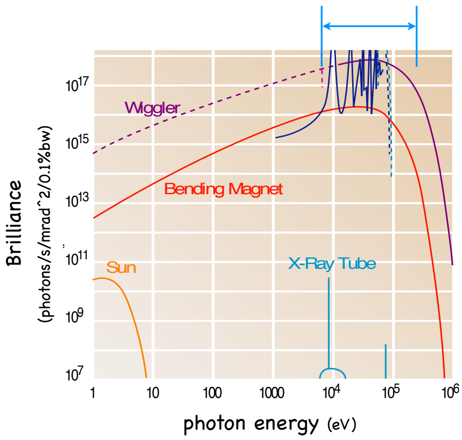
1. High Flux — high intensity photon beam

2. High Brilliance — highly collimated photon beam

3. Broad Spectrum
   which covers from microwaves to hard X-rays

And the 3rd property of SR is…

Broad Spectrum



Before SR, we can only use this sharp X-ray wavelength depending to the metal target.
However, with SR, we can choose any X-ray wavelength we want.

Utilization of broad spectrum of SR

longer wavelength (low energy) X-ray

structure solution method using sulfur atoms

shorter wavelength (high energy) X-ray

high-pressure structural study
using a diamond anvil cell (DAC)

As my subject of research, longer wavelength X-ray is used for a structure solution method, and shorter wavelength X-ray can be used for high-pressure study.

Utilization of broad spectrum of SR

longer wavelength (low energy) X-ray

structure solution method using sulfur atoms

shorter wavelength (high energy) X-ray

high-pressure structural study
using a diamond anvil cell (DAC)

Since time is limited today, we will see the utility of shorter wavelength X-ray here.

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using shorter wavelength X-ray

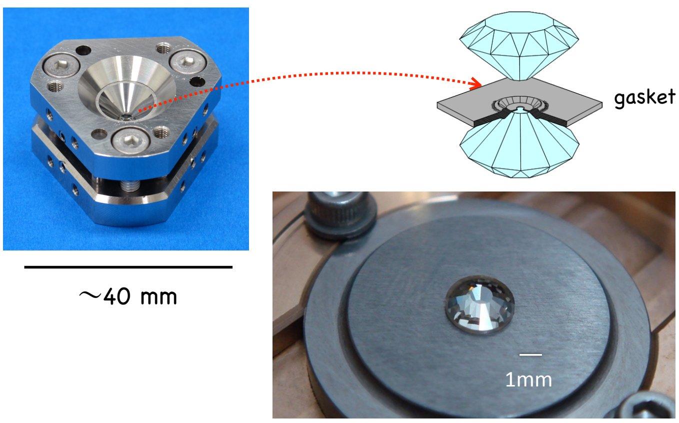
~0.7Å (7 nm)

high-pressure structural study
using a diamond anvil cell (DAC)

Shorter wavelength means here is X-ray which wavelength is shorter than 0.7 Å, or 7 nm.

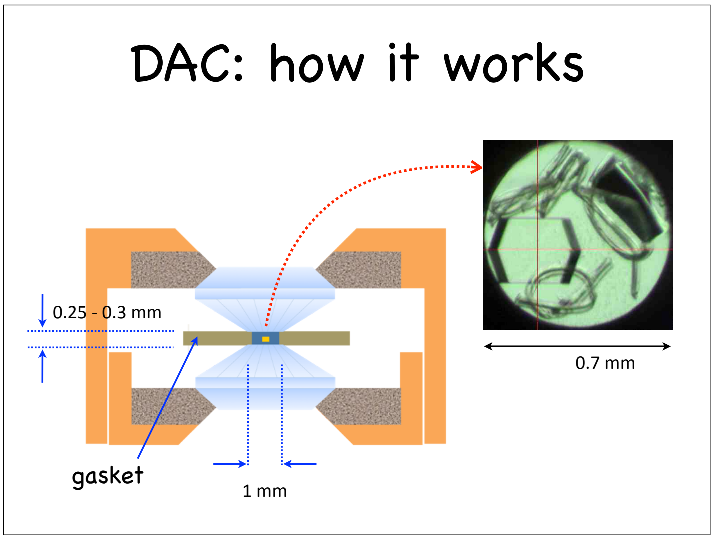
The reason of using shorter wavelength is…
we use diamonds for the sample cell.

Diamond anvil cell (DAC)

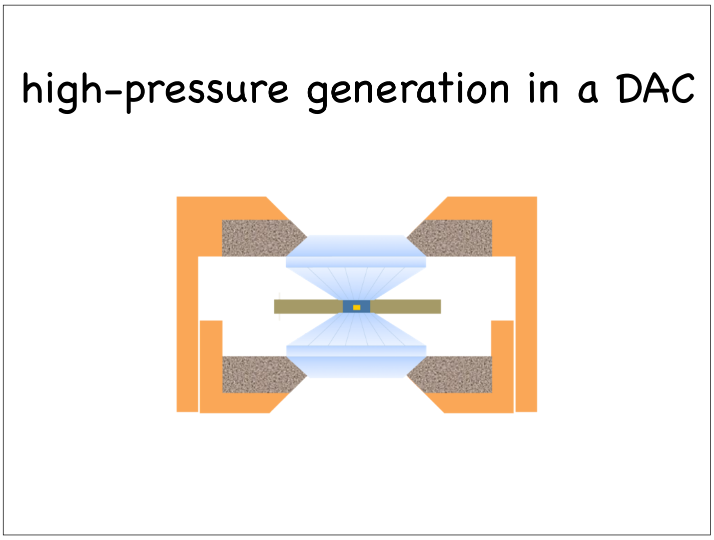


One of the key tools of HPPX is a pressure-generating device, DAC.

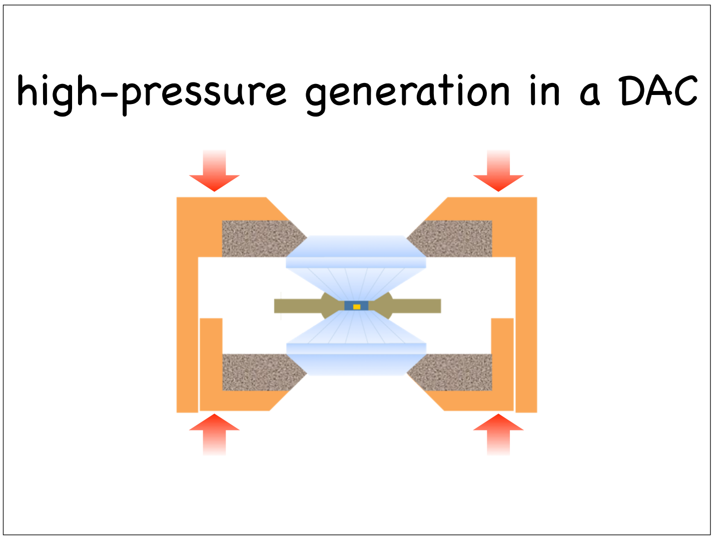
The DAC consists of a pair of diamonds and a metal gasket.
The flat faces at the tip of the diamond, called culet, and a small hole of the gasket make a sample chamber.



And we will mount protein crystals in there.
These are fiber knots to fix crystals in the chamber.

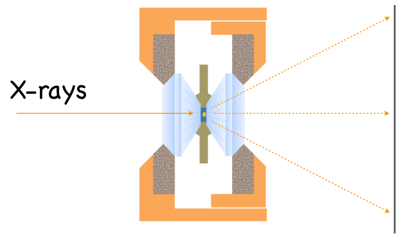


The protein crystals in the sample chamber will be compressed with the uniform hydrostatic pressure generated by the squeezing of the camber volume between the two diamond's culets.



like this.

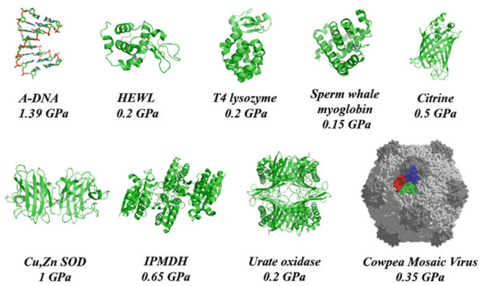
X-ray diffraction measurement using DAC



using shorter wavelength X-ray is necessary to reduce the absorption by the diamond

Then the X-ray diffraction measurements can be done as usual, through the two diamonds.

Examples of macromolecular structures investigated by HPPX



Dhaussy, AC & Girard, E, Fig 11.4 in "High Pressure Bioscience" Akasaka & Matsuki eds (2015) Springer

HPPX does not so popular yet, but it has been applied to the studies from nucleic acid to large virus capsid.

And our HPPX studies have been made for several proteins, including this IPMDH...

Two aims of our HPPX study

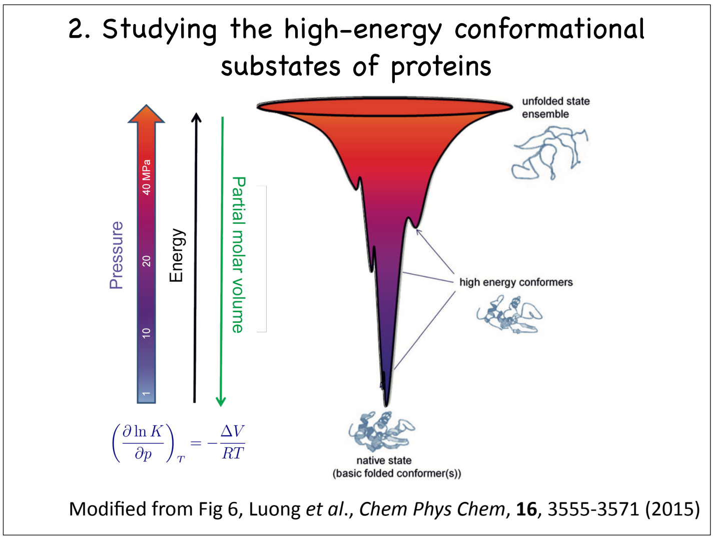
High-pressure protein xtallography

1. Understanding of life under high-pressure conditions

2. Studying the high-energy conformational substates of proteins

I think there are two aims for our HP study on protein crystals.

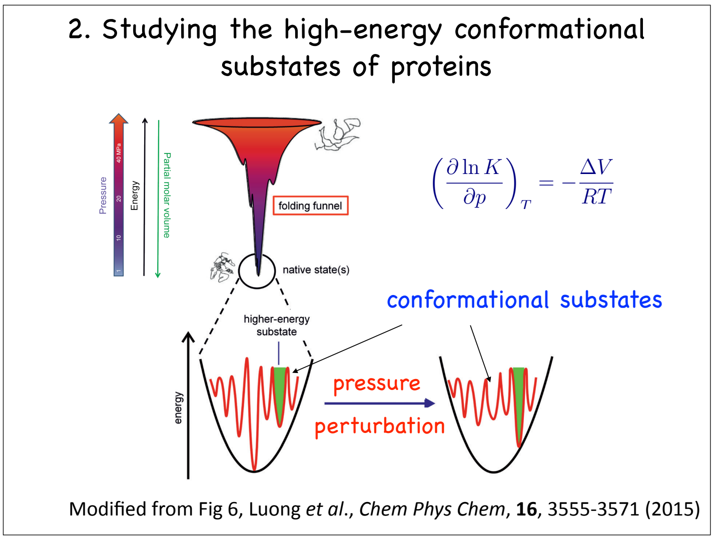
One is understanding the mechanism of pressure response of proteins itself, and the other is studying the higher energy conformational states of proteins to make structural excursions along the energy landscape of proteins.



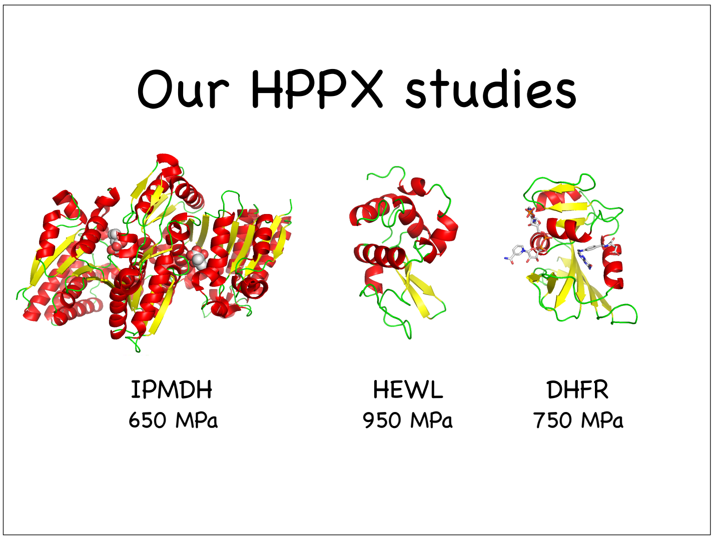
The possibility of HPPX is studying the energy landscape of proteins.

Under pressure, proteins behave after the “Le Chatelier's Principle”.
It means, if we pressurize the system, the partial molar volume of the system decreases.

Generally, proteins at the higher energy substates have smaller partial molar volume.



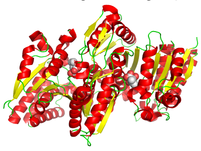
This means that we can use pressure as a perturbation to induce higher energy conformational substates.



We have already some results on these proteins.

Two aims of our HPPX study

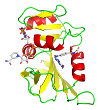
1. Understanding of life under high-pressure conditions



IPMDH
650 MPa

2. Studying the high-energy conformational substates of proteins

HEWL
950 MPa



DHFR
750 MPa

The aims of the studies may be compiled like this.

IPMDH is studied for the 1st subject, and these two are for the 2nd subject.

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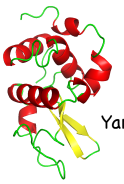
2. Studying the high-energy conformational substates of proteins

Reaction mechanism of hen egg white lysozyme



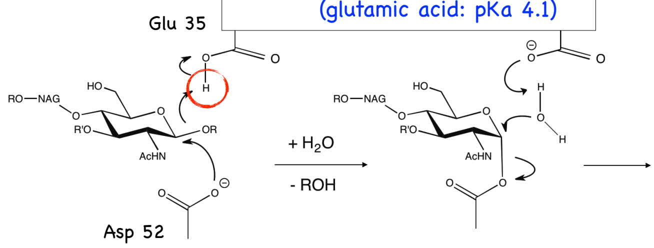
Yamada, Nagae & Watanabe, Acta Cryst., **D71**, 742-753 (2015)

Today, I will show you the results on lysozyme as an example of the 2nd aim of our HPPX.

Reaction mechanism of hen egg white lysozyme

pKa 6.0 ~ 6.8

(glutamic acid: pKa 4.1)



As you may know, lysozyme is an enzyme that hydrolyzes a glycosidic bond.

And two residues, Asp52 and Glu35, are the catalytic residue.

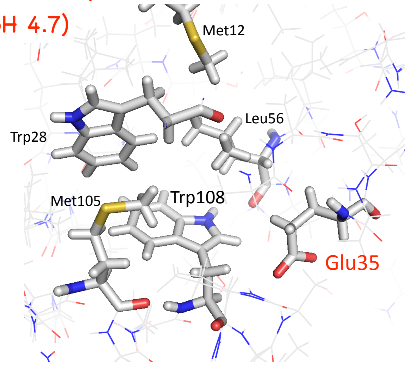
Before the first step of the reaction, carboxyl group of Glu35 should be protonated.

And many biochemical studies showed that the pKa value of Glu35 is quite-high as 6.0 to 6.8, but the mechanism of high pKa is not clear.

High-resolution crystal structure of HEWL

0.65 Å resolution, P1
(pH 4.7)

PDB: 2vb1



Wang, *et al.*, *Acta Cryst.*, **D63**, 1254-1268 (2007)

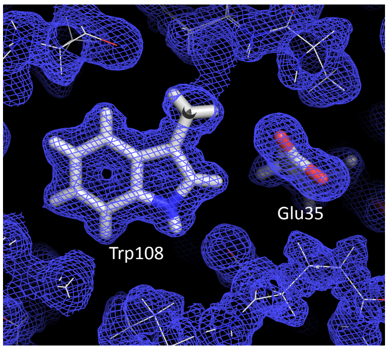
This is a crystal structure around the Glu.
Structure of lysozyme was already solved at ultra-high-resolution of 0.65 Å and this pH.

On the other hand, many biochemical studies have been performed, and they thought that the hydrophobicity of this Trp is believed to the key to keep the high pKa value of Glu.
However, as you can see, the side chain of this Trp does not directory interact with the side chain of Glu.

High-resolution crystal structure of HEWL

0.65 Å resolution, P1
(pH 4.7)

PDB: 2vb1



Wang, *et al.*, *Acta Cryst.*, **D63**, 1254-1268 (2007)

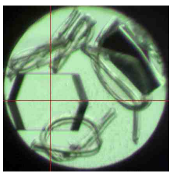
And even in this ultra-high-resolution structure, the Glu seems not to be protonated.

The mechanism of high pKa is not clear from crystal structures.

Therefore, we made a HPPX study on HEWL.

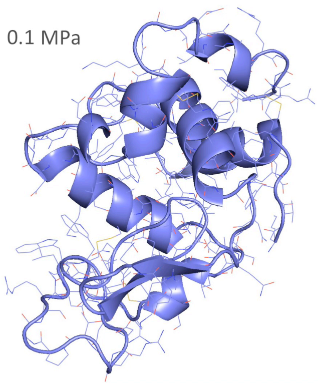
HEWL structure change by pressure

0.1 → 950 MPa



tetragonal
$P4_32_12$

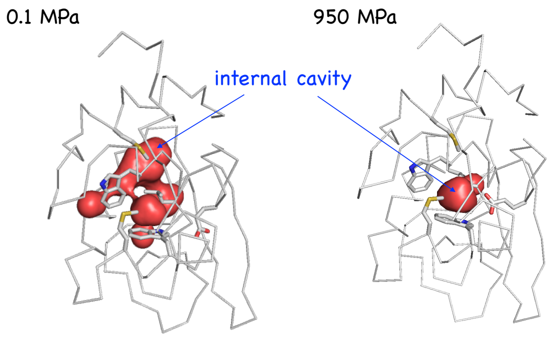
pH 4.5



(movie)
This is a morph movie shows the structures change between ambient pressure and 950 MPa.

At 950 MPa, the lysozyme molecule is compressed or become smaller after the “Le Chatelier's Principle”.

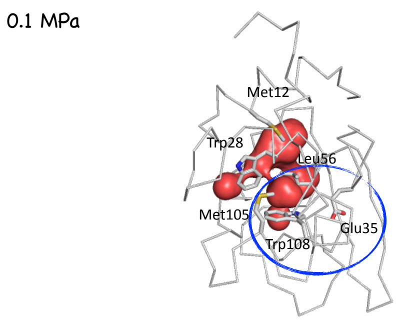
Compression of internal cavities



The structure change was caused mainly by compression of its internal cavities, like this figure.

Large internal cavities at ambient pressure became smaller at 950 MPa.

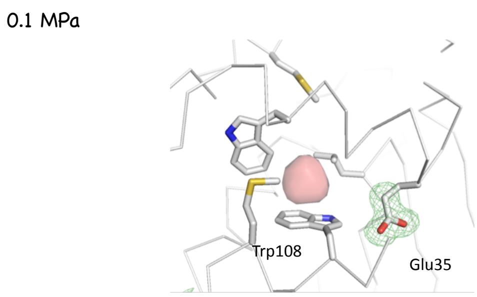
Active residue Glu35, and Trp108



The location of the active residue Glu35 is here.
And at the upper side of Trp, there is a hydrophobic cavity.

In our HP study, we had observed structural changes around those residues with pressure.

HEWL structure near Glu35

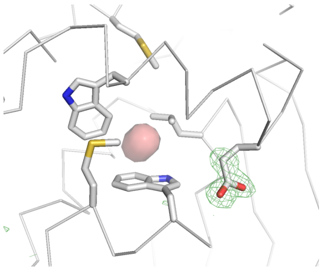


This is a magnified view around the hydrophobic cavity at the ambient pressure.

The omit electron density of the side chain of Glu35 is also shown at 3 sigma level.

HEWL structure near Glu35

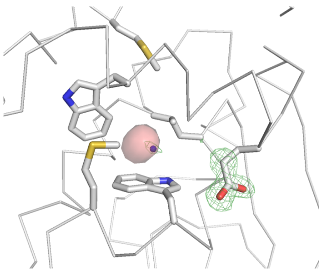
600 MPa



Then, if the crystal is pressurized to 600 MPa, the hydrophobic cavity is compressed as expected.

HEWL structure near Glu35

700 MPa



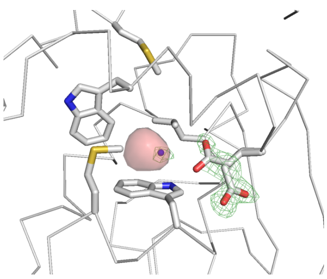
But, at 700 MPa, a new electron density peak emerged over the side chain of Trp.

Can you guess what is this density?
It seems a water molecule.

Since the environment of the cavity is hydrophobic, this water molecule seems to be stabilized by lone pair-pi interaction with Trp side chain.

HEWL structure near Glu35

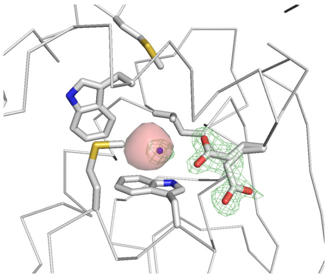
800 MPa



And at 800 MPa, with growing the water density, the side chain flipping of Glu seems to be initiated, and some of the side chain has inward conformation.

HEWL structure near Glu35

890 MPa

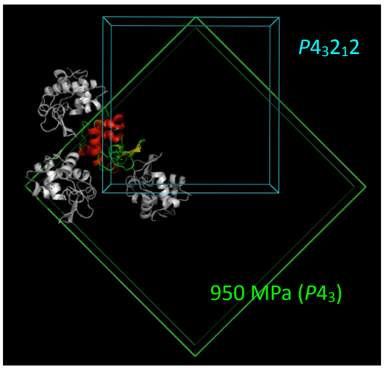


At 890 MPa, the water peak grows up, and the two electron densities of the side chain of Glu become comparable, or inward conformation seems to be more than outward conformation.

And at little above this pressure, unexpected happens.

Crystallographic phase transition happens

between 890 MPa → 950 MPa



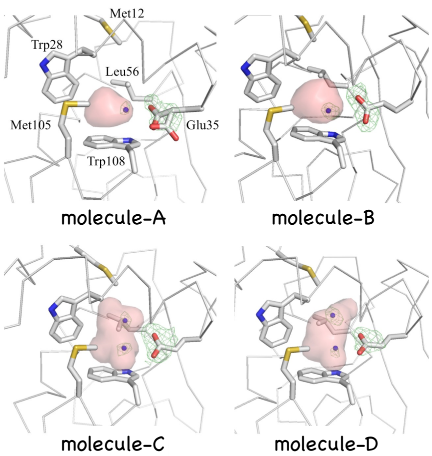
Interestingly, crystallographic phase transition was happen between 890 to 950 MPa.

The new cell contains 4 molecules in the asymmetric unit.

If we observe these four molecules carefully, …

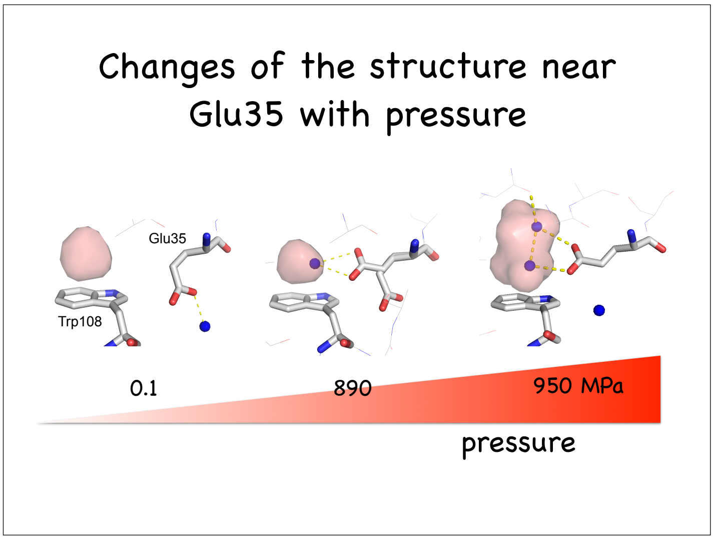
HEWL structure near Glu35

950 MPa



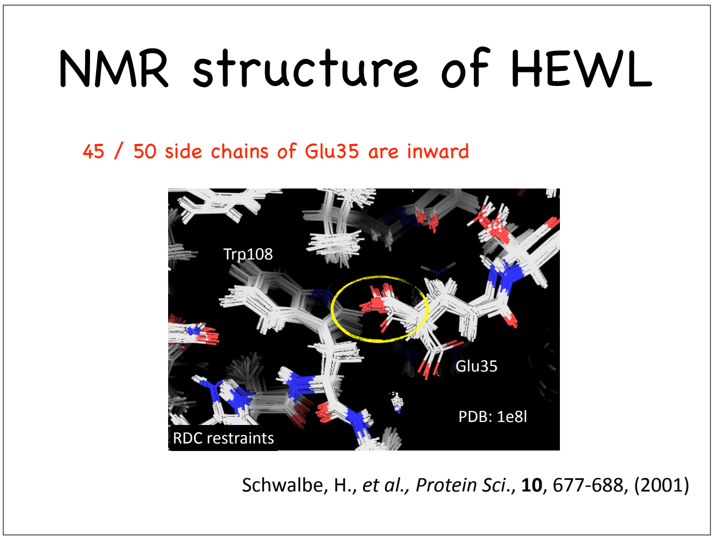
Interestingly, three of four structures of Glu have only inward conformation.

And in these two molecules, a second water molecule has penetrated into the hydrophobic cavity at Trp.



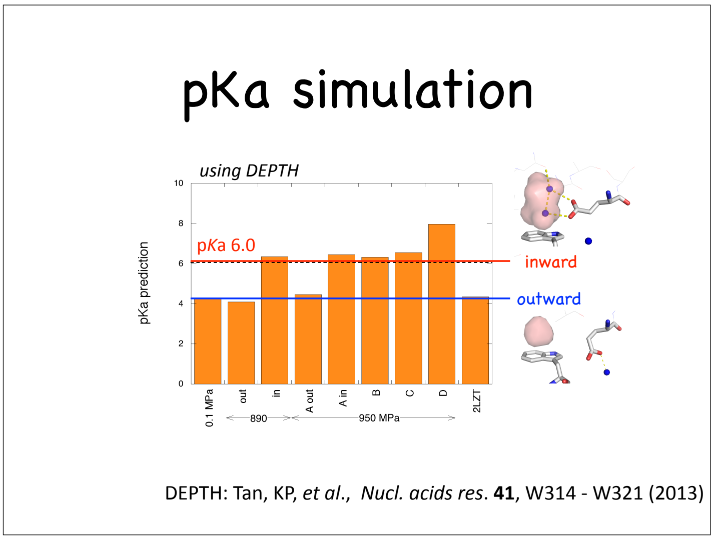
If we summarize the results, the side chain of Glu35 and water molecules above Trp108 change with pressure like this figure.

Now the question is the meaning of this inward conformation.



In the PDB structure database, we found that the refined NMR structure of lysozyme shows that the inward conformation of Glu35 is popular in solution. Surprisingly, 45 of the 50 low-energy structures have the inward conformer of Glu35.
In this paper, the authors did not discuss anything about this, but the inward conformation of Glu35 appears to be common in the solution state.

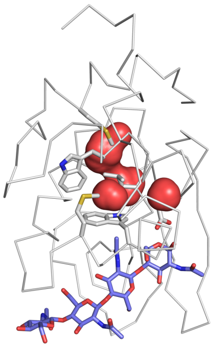
Why conventional crystallography does not detect the inward conformation at ambient pressure is not clear.



However, if we estimate the pKa of Glu35 with these structures, the inward conformations of Glu35 give high pKa value fitted well with that of biochemical studies.

(GlcNAc)$_4$ complex

920 MPa



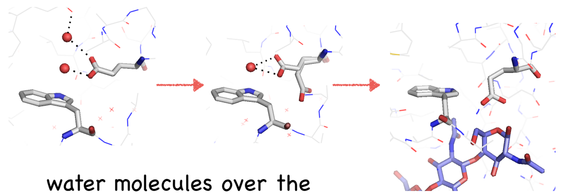
the side chain of Glu35 maintains outward structure

no phase transition was observed

And if we pressurize a complex crystal of lysozyme and its product, the side chain of Glu remains outward from ambient pressure to high pressure of 920 MPa, and no crystallographic phase transition was observed.

Summary

Glu35 reaction mechanism



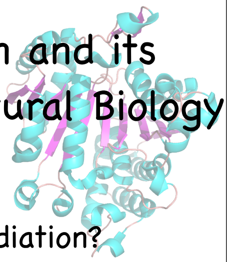
water molecules over the "hydrophobic" Trp108 keeps the high pKa value of Glu35

Yamada, Nagae and Watanabe, *Acta Cryst*. D71, 742-753 (2015).

From these results, we think the mechanism of the high pKa value of Glu35 of lysozyme comes from the water molecules at the hydrophobic cavity above Trp108.

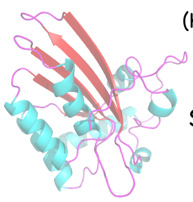
This means that, not hydrophobicity but hydrophilicity of Trp, which is provided by the lone pair–pi interaction, seems important to the high pKa of Glu.

Synchrotron Radiation and its application to the Structural Biology



- What is Synchrotron Radiation?
- Protein crystallography & SR

(high-pressure protein crystallography using SR)



Synchrotron Radiation Research Center
Nobuhisa Watanabe

Any Q?