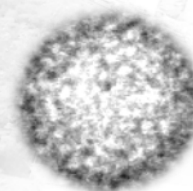
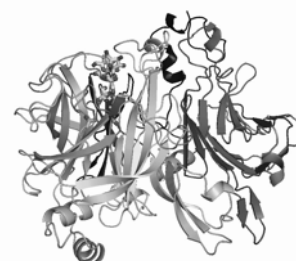


# X光在生物分子結構的應用 Structures of Biological Macromolecules with X-ray

陳俊榮  
Chun-Jung Chen



## Scales of Human Structures



1 (m)  $10^{-1}$   $10^{-2}$   $10^{-3}$   $10^{-4}$   $10^{-5}$   $10^{-6}$   $10^{-7}$   $10^{-8}$   $10^{-9}$   $10^{-10}$

奈米 埃 Å

# Bovine Trypsin & Its Inhibitor Leupeptin

胰蛋白酶  
在小腸工作  
分解蛋白質為肽  
再分解為胺基酸  
人體吸收

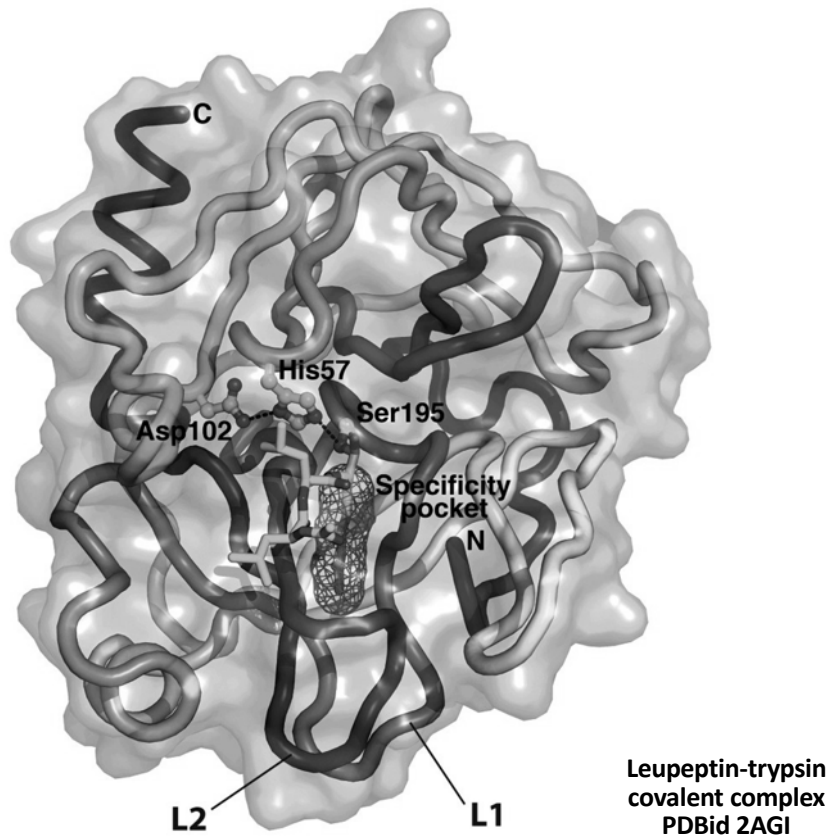
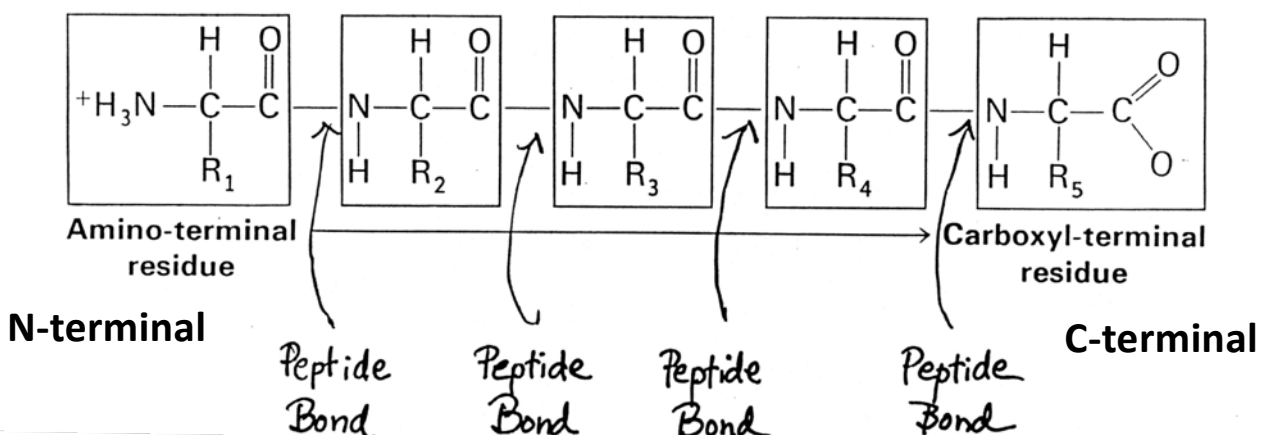
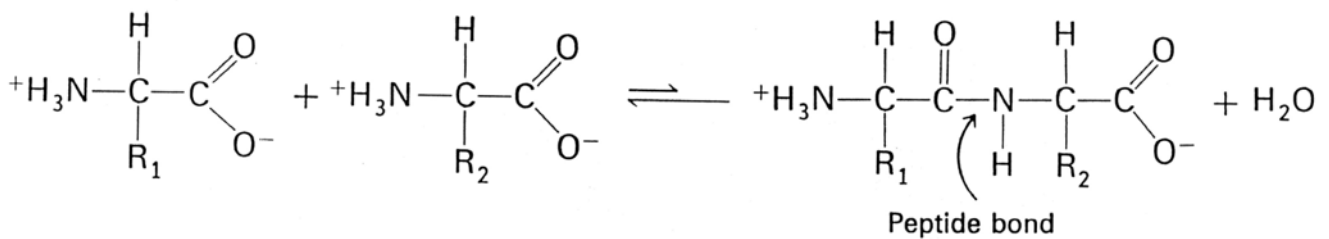


Figure 11-25  
© 2013 John Wiley & Sons, Inc. All rights reserved.

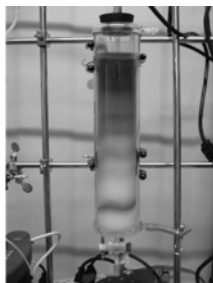
## Amino Acids >> Peptides



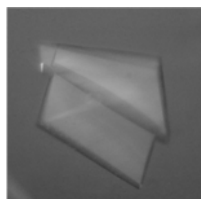
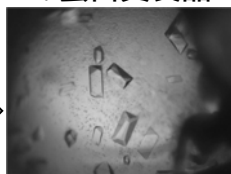
# 蛋白質結構與結晶學

- 各生物體中蛋白質是實際執行功能的機器，人體中有超過25,000種蛋白質在作用。
- 蛋白質功能與結構息息相關，唯有了解每個蛋白質的獨特結構，才能徹底了解蛋白質的作用與反應機制。

## 1. 蛋白質純化生產



## 2. 蛋白質長晶



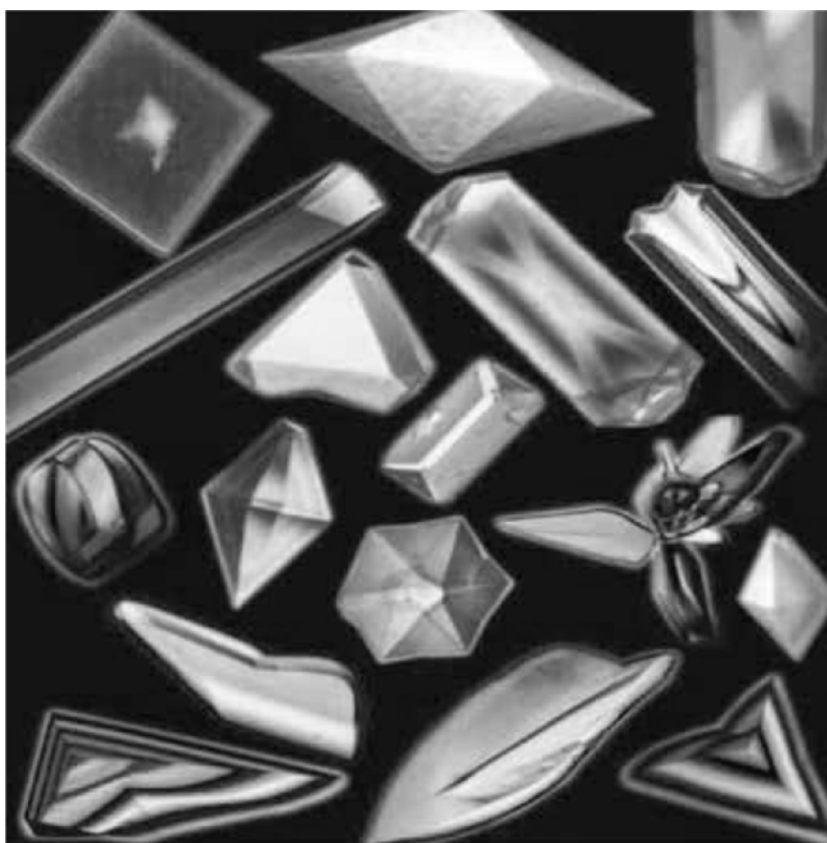
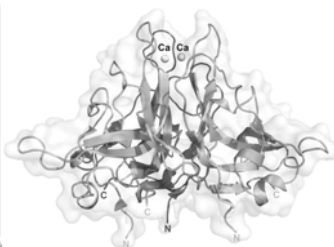
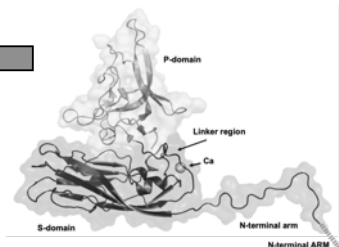
## 3. 同步輻射X光繞射

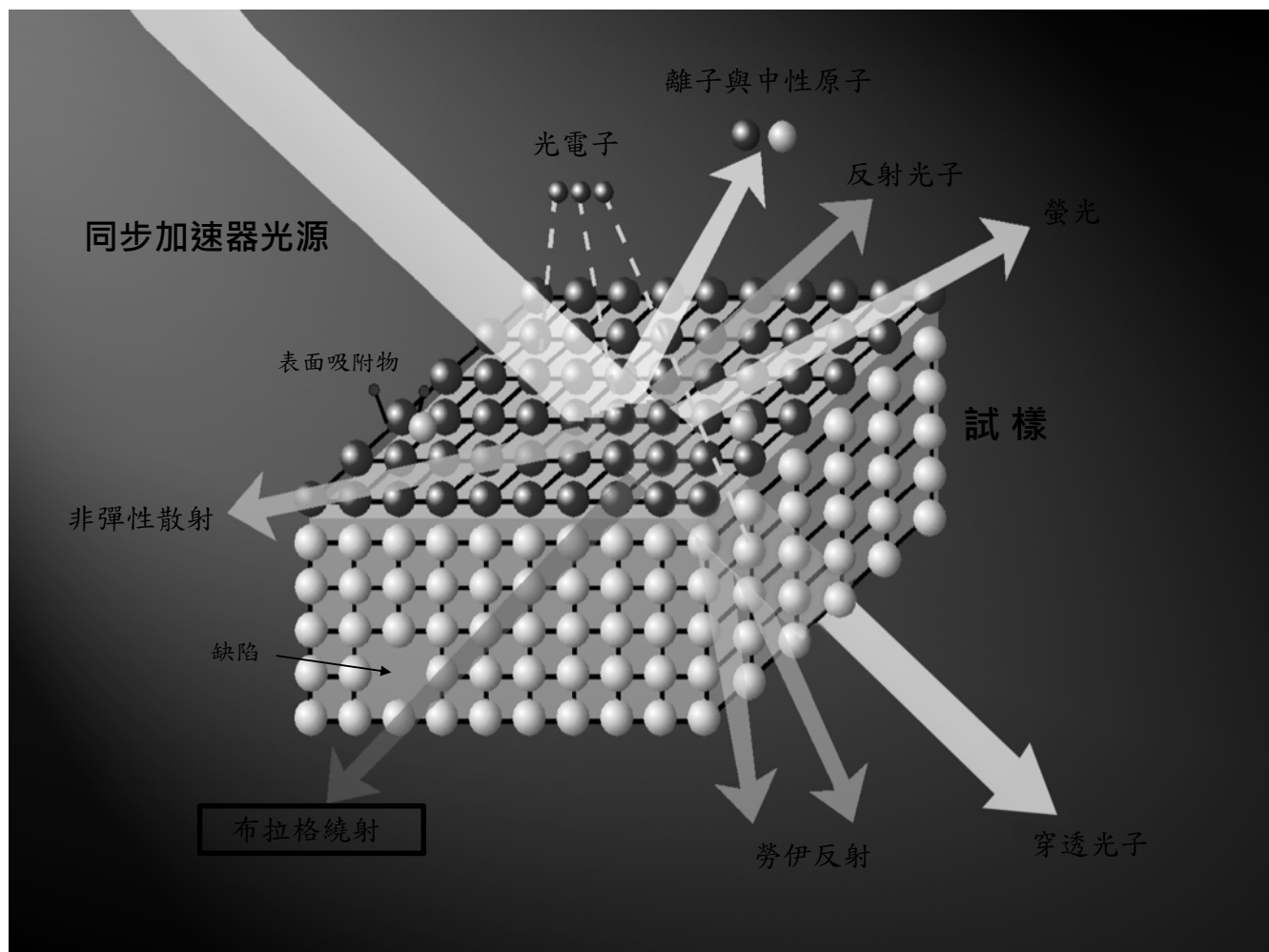


## 5. 生技醫藥應用



## 4. 蛋白質3D結構解析





## 同步輻射蛋白質結晶學核心設施



1.5 GeV  
台灣光源



- TLS 15A1 高效能蛋白質結晶學
- 2013.01 開放用戶使用



3.0 GeV  
台灣光子源



- TPS 05A 微米蛋白質結晶學
- 2016.9 開放用戶使用



- TPS 07A 微米聚焦蛋白質結晶學
- 2021.11 開放用戶使用



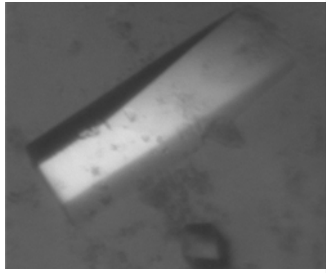
8 GeV 日本春



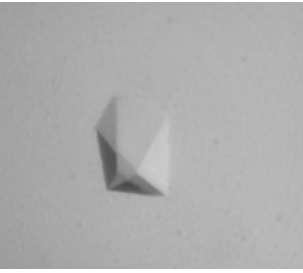
- SP44XU 大阪大學白質結晶學  
@日本 SP-8
- 2010.01 開放台灣用戶使用



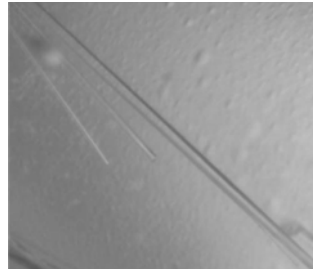
# Crystallization leads to crystals of various sizes



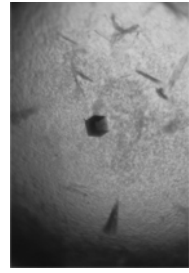
100  $\mu\text{m}$



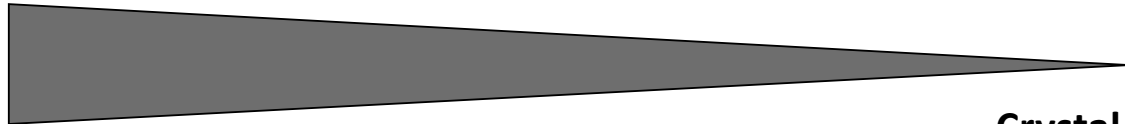
50  $\mu\text{m}$



5  $\mu\text{m}$



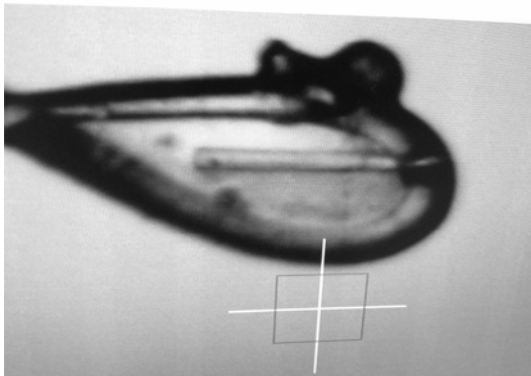
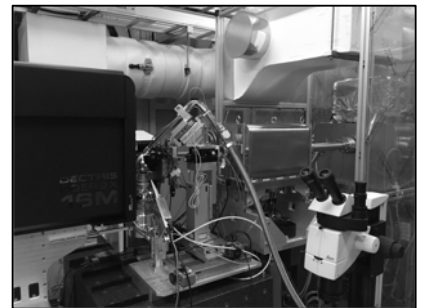
1  $\mu\text{m}$



Crystal size

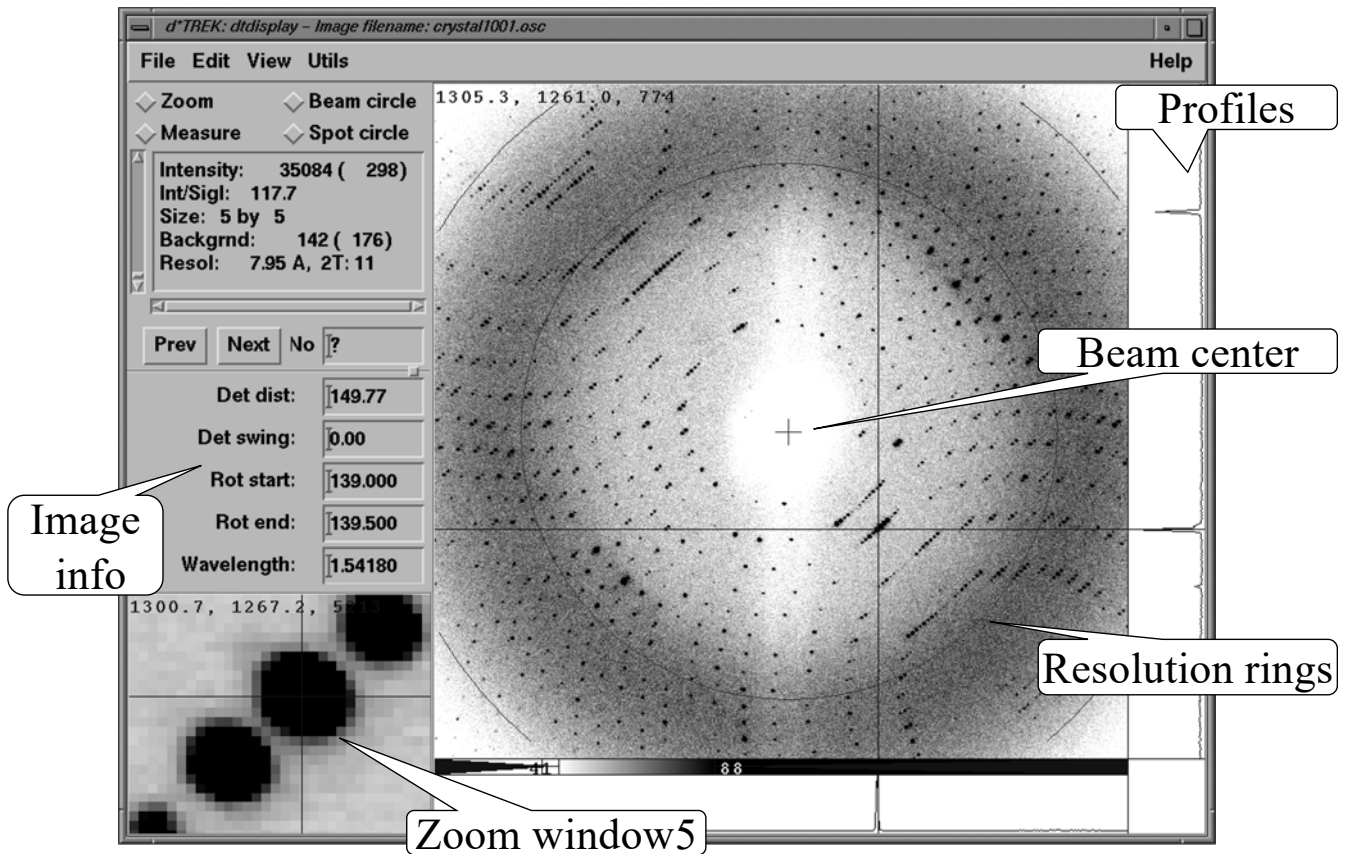
Micro-focus PX Beamline TPS 07A

Open a door for structure determination  
from micro-size crystals

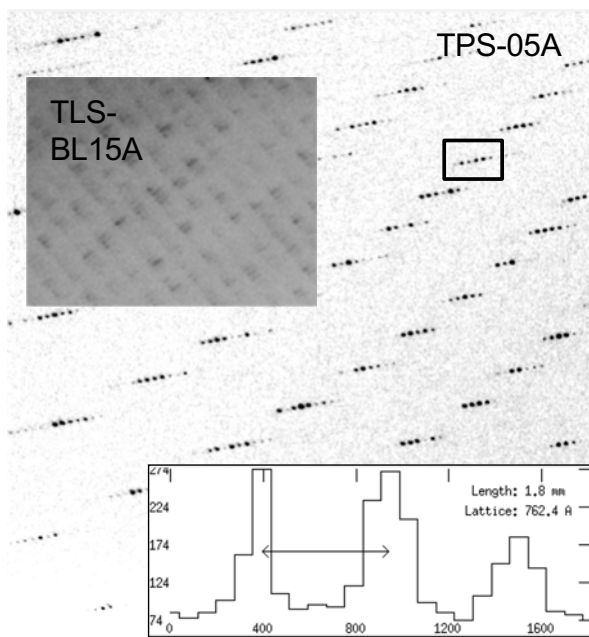


2 : 30 am

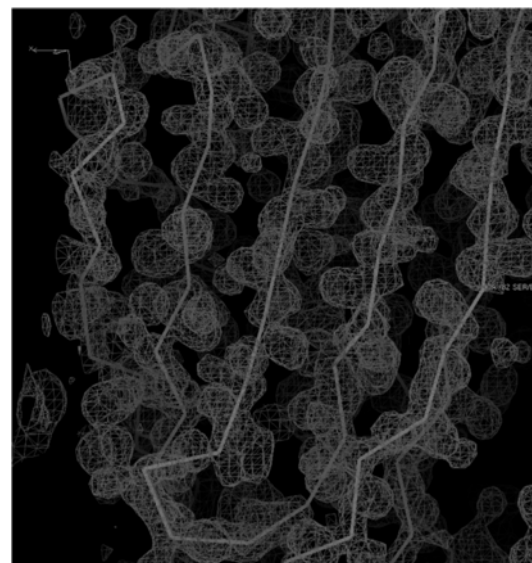
# Diffraction Pattern



繞射點蘊藏著原子中電子的訊息



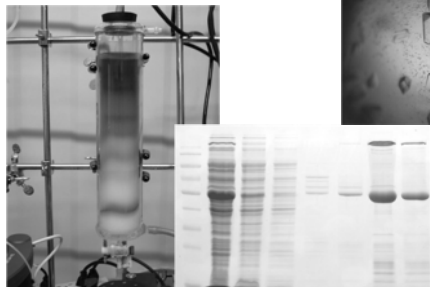
Large unit cell > 750 Å



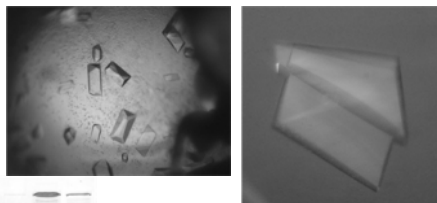
- Resolution: 1.6 Å (0.1 s exposure)
- Data set was completed in 18 sec
- 1 Se atom of 119 residues

# 台灣光子源X-光蛋白質結晶學

## 1. 蛋白質純化製備



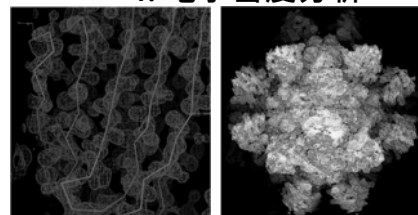
## 2. 蛋白質晶體培養



## 3. 同步輻射X光繞射



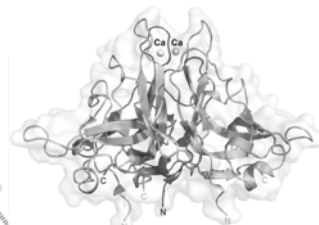
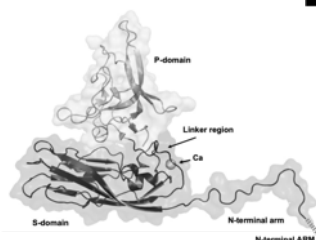
## 4. 電子密度分析



## 6. 生技醫藥應用



## 5. 結構解析



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RCSB PDB PROTEIN DATA BANK 244,290 Structures from the PDB archive 1,066,577 Computed Structure Models (CSM) Enter search term(s), Ligand ID or sequence Include CSM Help

PDB-101 PDB EMDatResource NAKB wwPDB Foundation PDB-IHM f x v i n

Redesigned PDB Statistics Support Enhanced Functionality Explore Statistics

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RCSB Protein Data Bank (RCSB PDB) enables breakthroughs in science and education by providing access and tools for exploration, visualization, and analysis of:

- Experimentally-determined 3D structures from the Protein Data Bank (PDB) archive
- Integrative 3D Structures from the PDB Archive
- Computed Structure Models (CSM) from AlphaFold DB and ModelArchive

NEW Explore Integrative Structures

PDB-101 Training Resources

November Molecule of the Month

GLP-1 Receptor Agonists

Latest Entries As of Tue Oct 28 2025

9K8F Cryo-EM structure of monomer of designed zinc-induced tetrahedron Cage-132-Zn1-HEHE-35

Features & Highlights

Register Now for Webinar: Exploring the Workhorses of Biotechnology

On November 6, learn how to use RCSB.org to analyze plastic-degrading enzymes

Register for the Nov 24 Virtual Office Hour on Exploring CSMs at RCSB.org

Learn how to use RCSB.org features to navigate 3D Computed Structure Models from AlphaFold DB and ModelArchive

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Register Now for Webinar: Exploring the Workhorses of Biotechnology

On November 6, learn how to use RCSB.org to

News

Publications

Coming Soon: Remediation of Metalloprotein-containing PDB Entries and CCDs

Existing polyatomic metal containing PDB entries and CCDs will be updated to make metalloprotein structures findable and reusable with more complete chemical description and metal coordination annotation.

~ 10/30/2025

Watch Molecules in Motion

Subscribe to the RCSB PDB YouTube channel for webinars, molecular animations, and more

~ 10/28/2025

Explore Green Fluorescent Protein

In a new tutorial, use RCSB PDB resources to find and explore the 3D structure of GFP

~ 10/21/2025

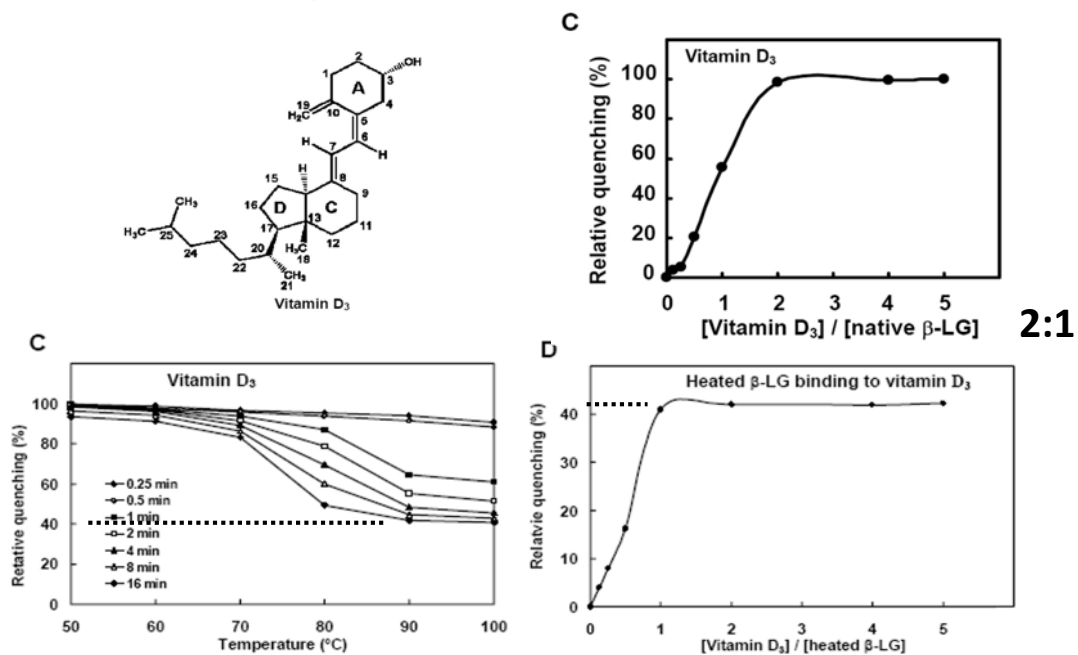
# $\beta$ -lactoglobulin

## $\beta$ -乳球蛋白：牛奶及羊奶中的主要乳清蛋白

- $\beta$ -lactoglobulin ( $\beta$ -LG), one of the most investigated proteins, is a major whey protein in bovine milk to an extent about 50%, and sensitive to thermal denaturation.
- The essential biological functions of the protein include cholesterol lowering, modulation of the immune system, transport of retinol, fatty acid, and vitamin D, and prevention of oxidative stress.
- Structurally, it belongs to the lipocalin superfamily which can carry some hydrophobic ligands.



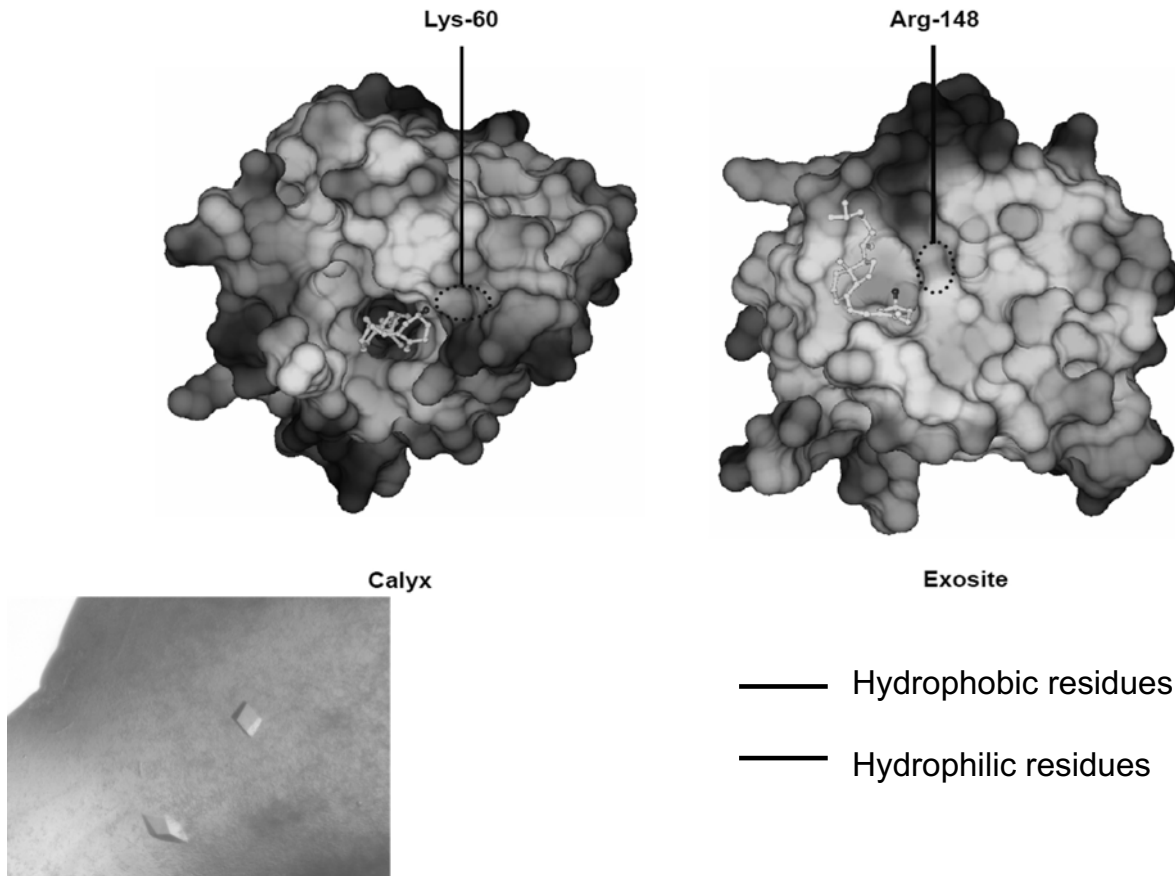
## Effect of heating on $\beta$ -LG binding to Vitamin D<sub>3</sub>



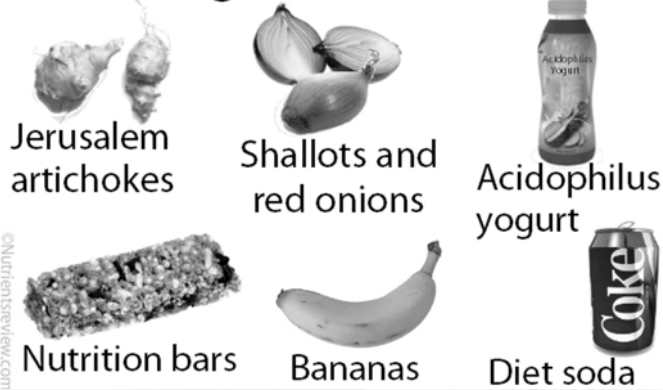
$\beta$ -LG was heated between 50 and 100°C for 15s to 16min before the addition of these ligands.

This data suggest that there is another thermally independent vitamin D<sub>3</sub> binding site, which is remote from the calyx.

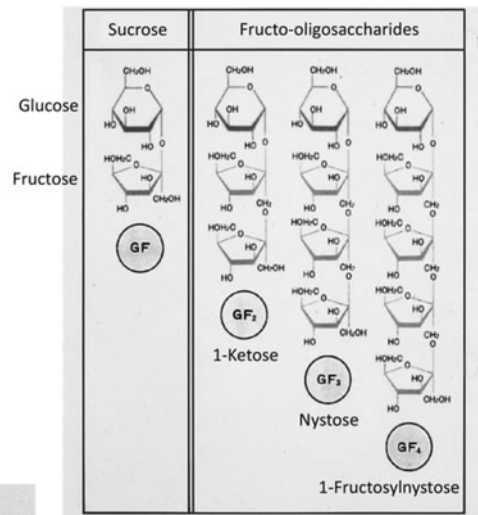
# Molecular interaction of $\beta$ -LG with vitamin D<sub>3</sub>



## 富含果寡糖的食物 Fructooligosaccharides (FOS)



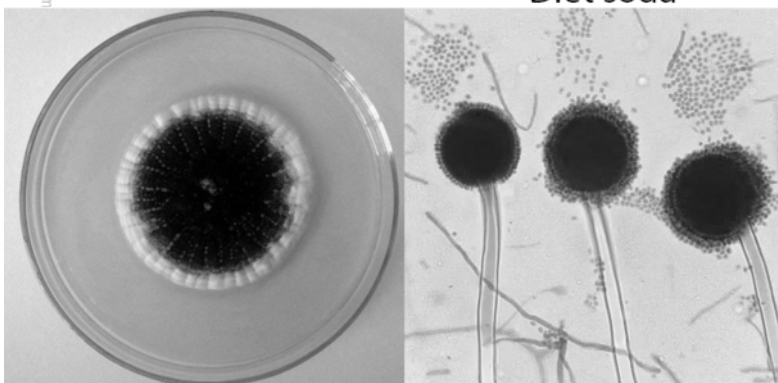
## Major component molecules included in FOS



Improve digestion, reduce inflammation, enhance immune function, and prevent diseases.



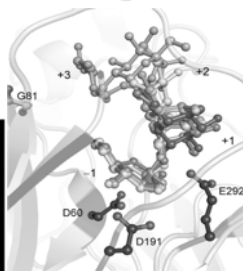
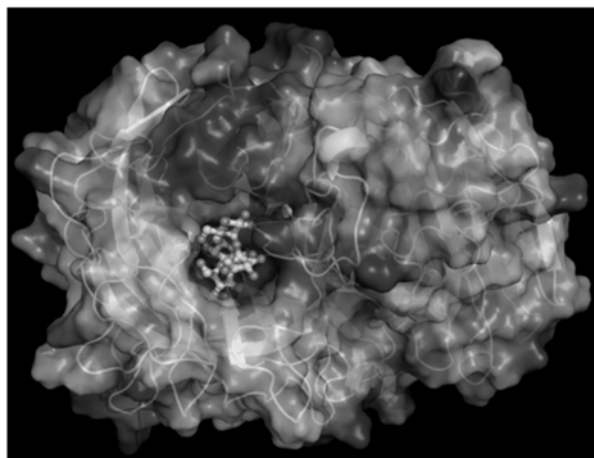
FOS production:  
Sucrose + fructosyltransferase  
by *Aspergillus japonicus*



*Aspergillus japonicus*  
日本麹黴

Fermentation (2022) 8, 437

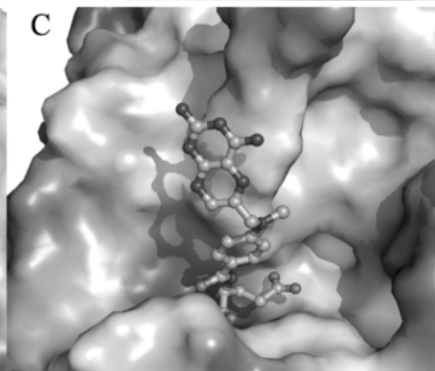
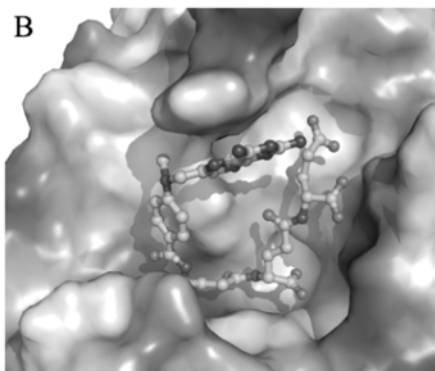
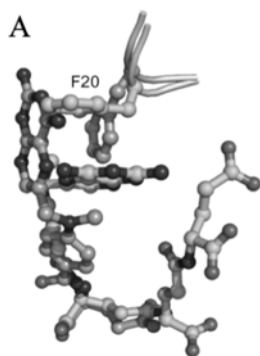
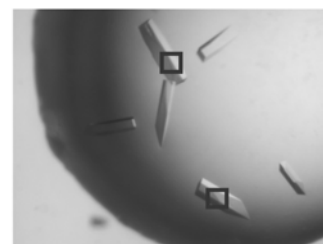
# The complex structure of *Aj*FT with substrates



Crystal structure of *Aspergillus japonicus* fructosyltransferase (*Aj*FT) complex with nystose. The structure of *Aj*FT composed of an N-terminal catalytic domain comprising a five-blade  $\beta$ -propeller linked to a C-terminal  $\beta$ -sandwich domain is presented as ribbons and electrostatic surface (positive charge: blue; negatively charge: red). A nystose substrate (in ball and stick) is bound at the active-site pocket.

## Scientific Opportunities

### Structure-based Drug Design



Polymorphism of methotrexate (cancer drug) by  $\gamma$ -glutamyl hydrolase

*J. Med. Chem.* (2013) 56, 7625

To elaborate the details of interactions between the drug target and drug hits/leads, the access of the stable micro-focus X-ray beam to obtain high-resolution data from screened micro-crystals is crucial for drug discovery.



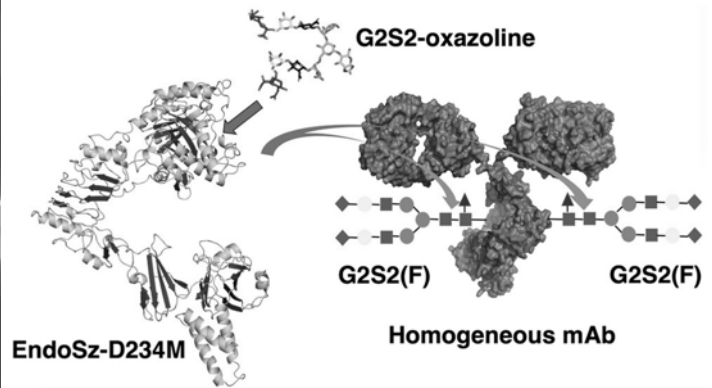
## 產學合作計畫

探討生技醫療應用的新酵素結構與功能，大幅提高抗體醫療活性



ACS Au 2024, 4, 6, 2130–2150

利用蛋白質結晶學光束線解析酵素及聚醣複合物在不同狀態下的晶體結構

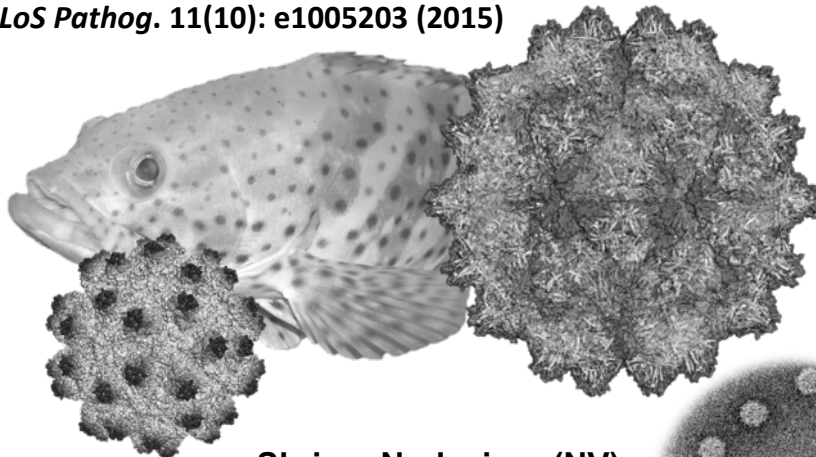


利用此新酵素可建立一個高效能的均質化抗體平台，有效提升抗體醫療活性，比目前國際市場上的醫療抗體製程更佳且自主掌握性高。對精準醫療發展有很大的貢獻。

## Virus Studies

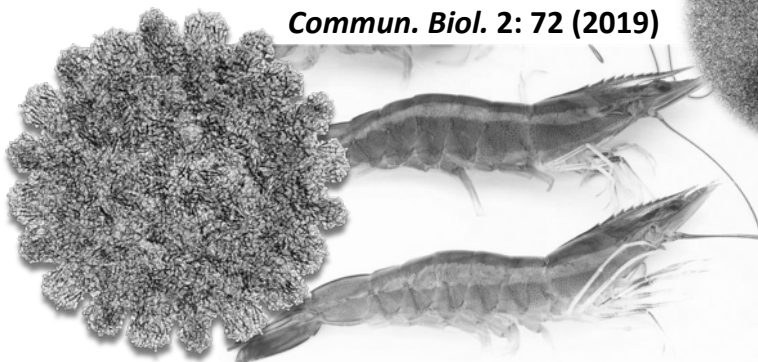
**Grouper Nervous Necrosis Virus (GNNV)**

*PLoS Pathog.* 11(10): e1005203 (2015)

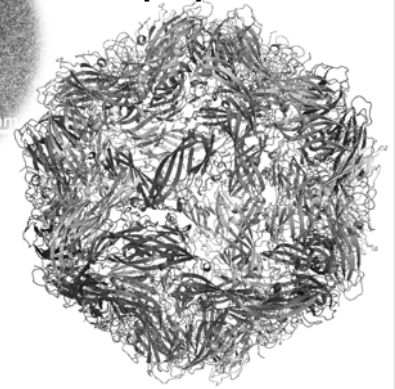


**Shrimp Nodavirus (NV)**

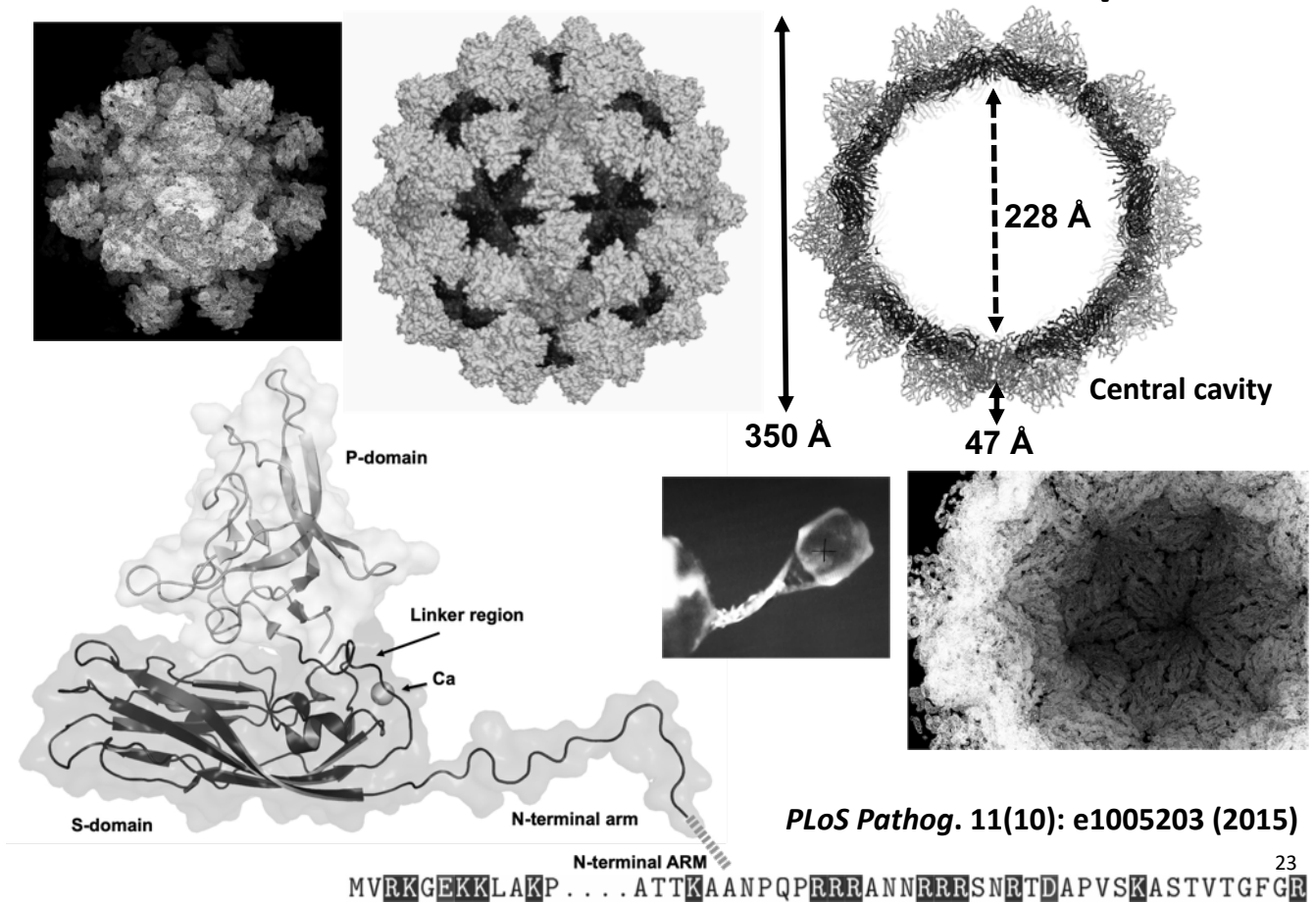
*Commun. Biol.* 2: 72 (2019)



**Porcine Virus**  
In preparation



## T=3 GNNV-LPs and Structure of Capsid



23

## Honeybee Infected by viruses

- Infection alone or through mite complex
- The central nervous system
- **Abnormal behavior** (hair loss, black tail, body shrinking, inapparent pattern, deformed wing, abnormal posture, muscle tremors...)
- High mortality



**B B C**  
**NEWS**

29 June 2022

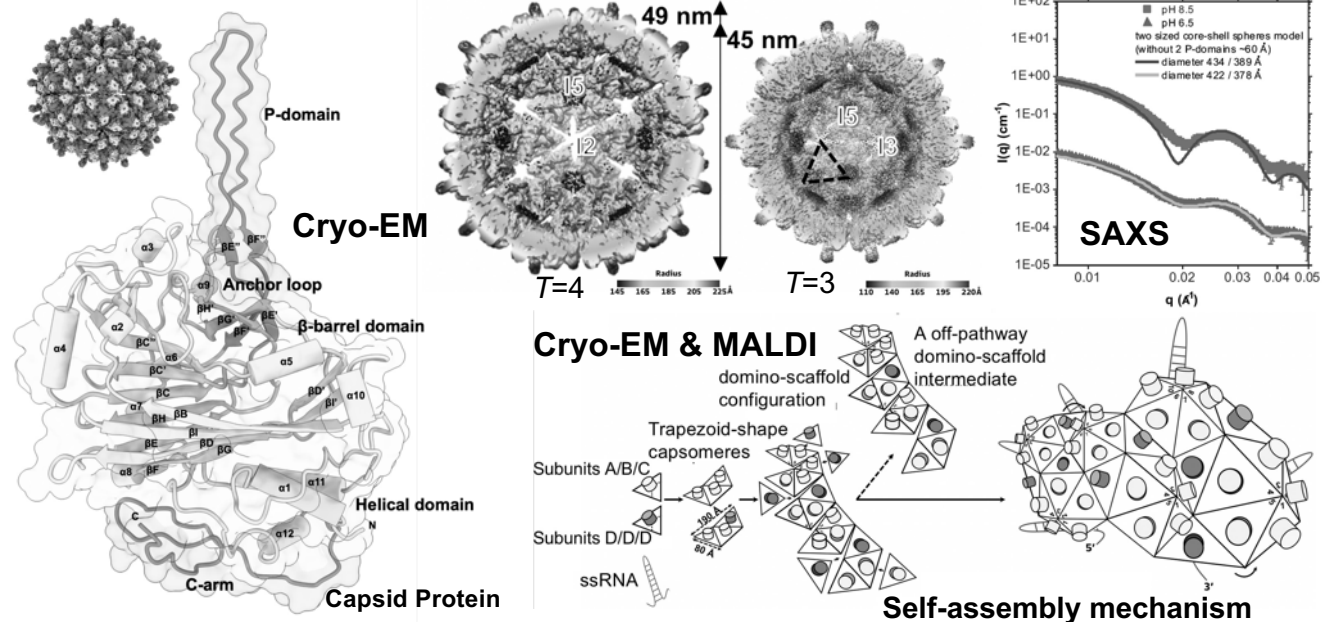
Australia honey bees put in lockdown due to deadly varroa parasite

The tiny varroa mite can kill bee colonies by feasting on them and transmitting **viruses**.

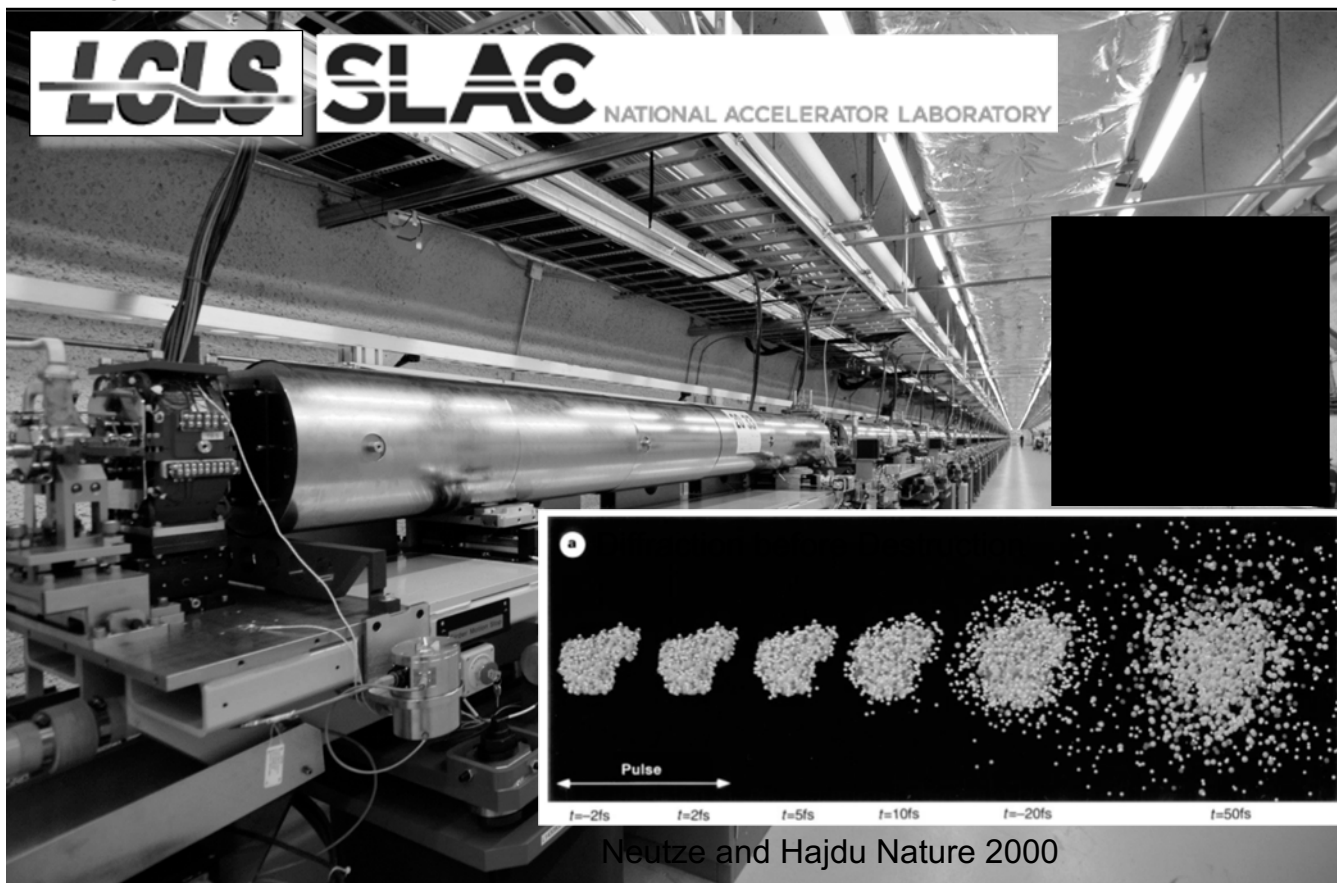
阻斷病毒傳播  
澳洲下令蜜蜂  
「類封城」

# Structures of honeybee-infecting Lake Sinai virus reveal domain functions and capsid assembly with dynamic motions

Combination of PX, Cryo-EM, SAXS, MALDI-TOF and other biophysical techniques for structural biology

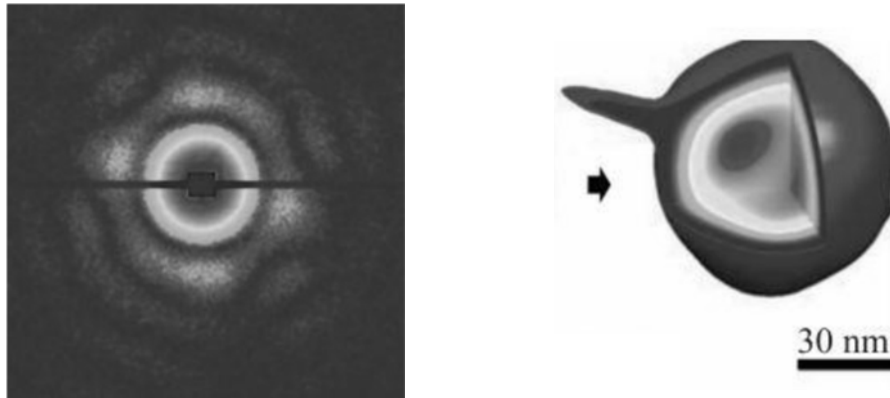


## The Linac Coherent Light Source Free electron: the first hard X-ray XFEL in the world



# Applications of XFEL

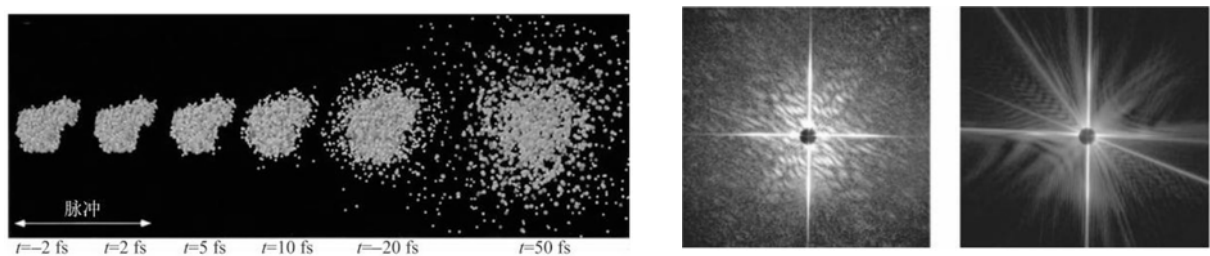
- Single Particle Imaging (SPI), such as virus particles



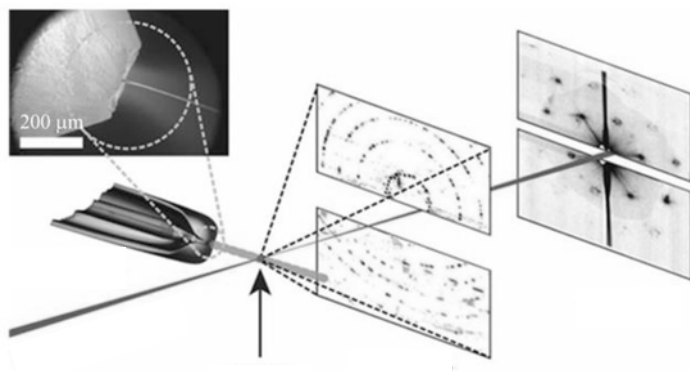
- pump-probe technology and crystallography for time-resolved dynamic study on structures.

# Applications of XFEL

- Radiation-damage-free structures of macromolecules



- Serial femtosecond crystallography, SFX



# Strategy for Serial Femtosecond Crystallography

**SLAC** NATIONAL ACCELERATOR LABORATORY

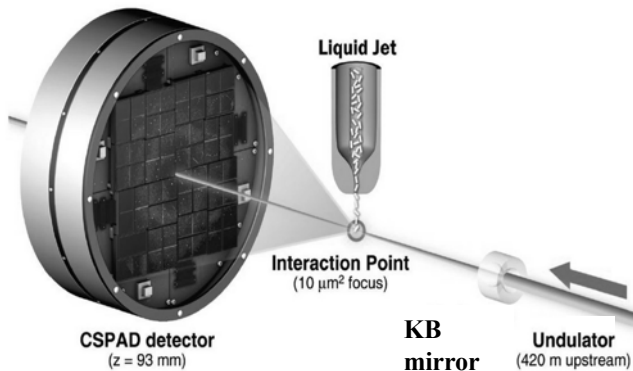
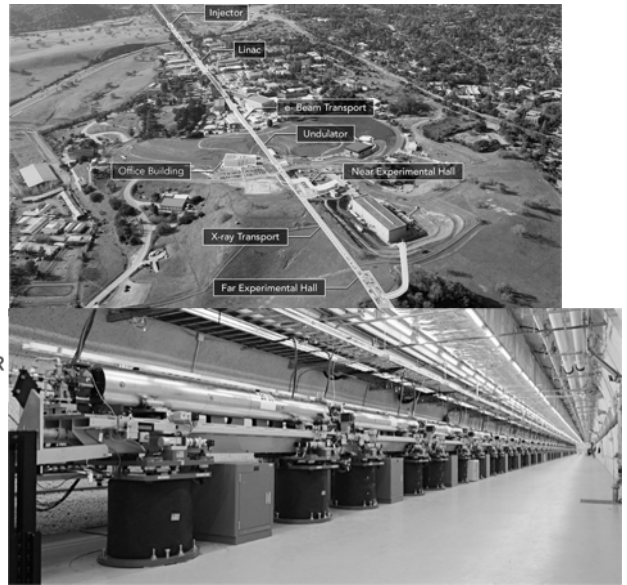


Image from Boutet et.al (2012)



- Size of crystal: small (typically 0.1-10  $\mu\text{m}$ )
- Crystals in mother liquor, 'RT'
- Liquid injection
- Light source (xFEL): 'coherent', frequency = 120 Hz, higher flux ( $\sim 10^{12}$  photons/pulse) and high peak brilliance
- Femtosecond exposure outruns radiation damage
- Time-resolved study for both reversible and irreversible processes possible

## Photosynthesis changed planet Earth



Photosynthesis transformed our planet 2.5B years ago producing oxygen, capturing solar energy and  $\text{CO}_2$ , that slowly converted to fossil fuels.

**A**

⑥ Ferredoxin-NADP<sup>+</sup>-Reductase

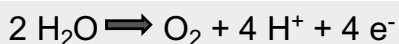
⑤ Ferredoxin

④ Photosystem I

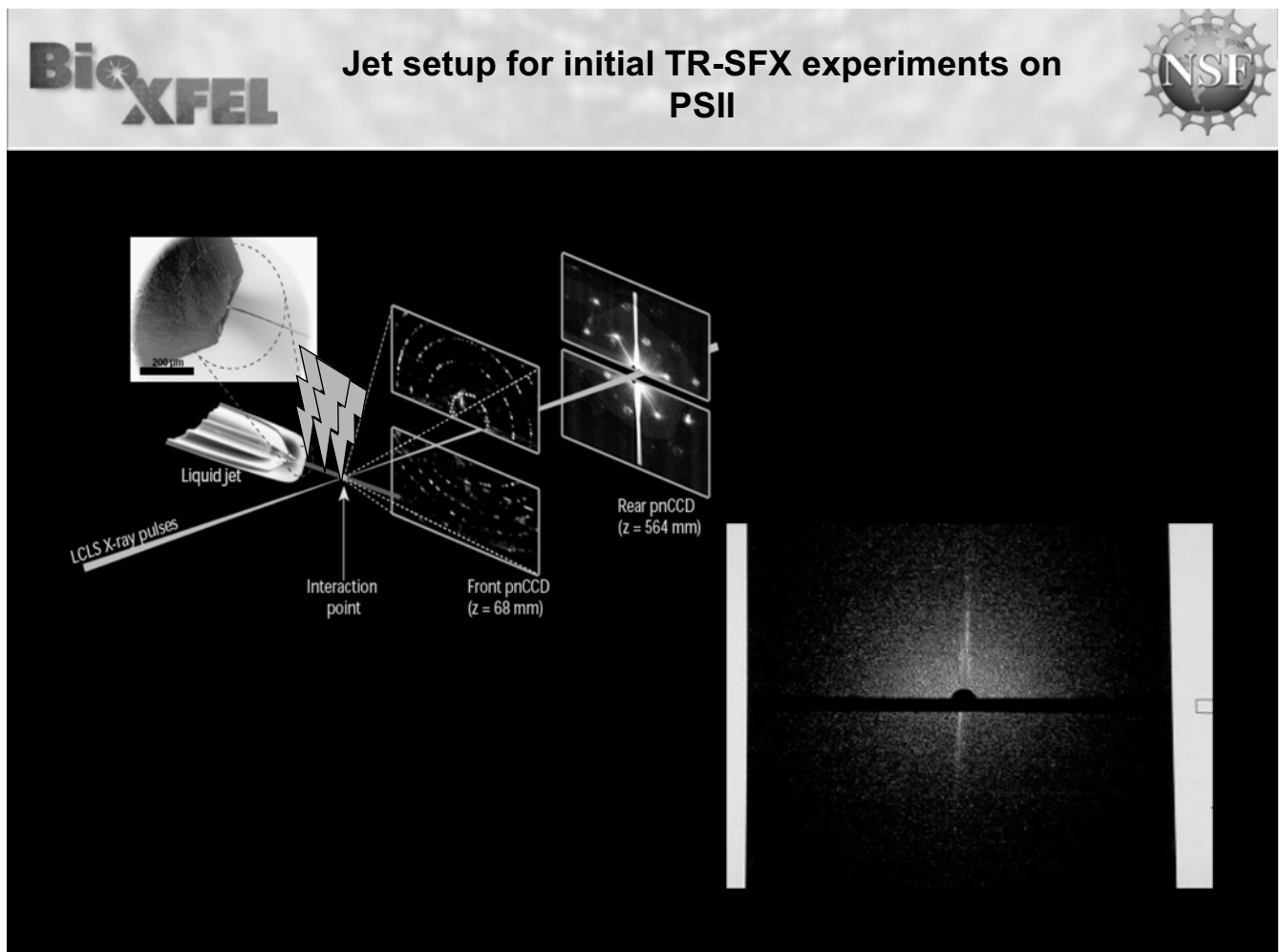
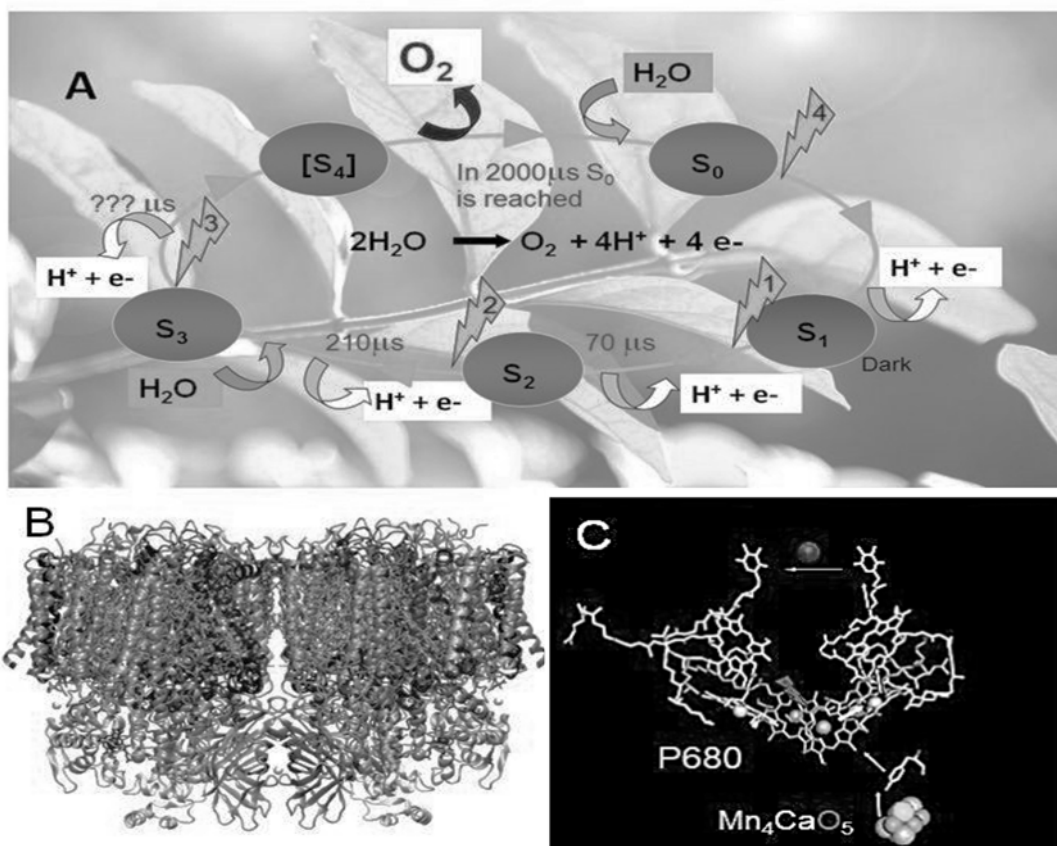
① Photosystem II

② Cytochrome b6f

③ Plastocyanin



## Use short pulse durations to resolve the structural changes in Photosystem II in transient states of water splitting process

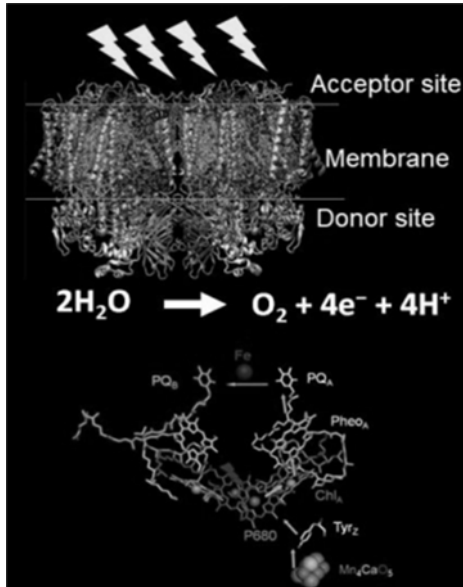




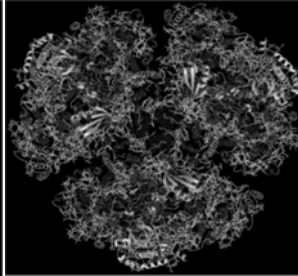
# Using Protein crystallography & X-ray free electron lasers (XFELs) to Study Photosystems I & II project

## Project Goals of Photosystem I & Photosystem II protein complexes

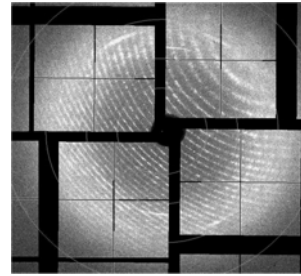
- Obtain a molecular movie of electron transfer in Photosystem I
- Resolve the structural changes in Photosystem II and the oxidation states of the Mn atoms in the transient S4 state of the water splitting process



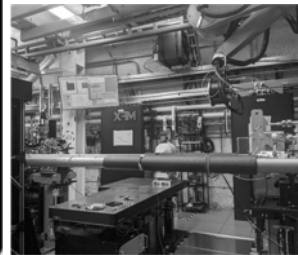
Structure of Photosystem II, responsible for water splitting during photosynthesis



Structure of Photosystem I



Diffraction pattern of shrunk PSII crystals



MFX beamline used for PSII water splitting experiments



The Fromme lab scientists observing real-time PSII diffraction at LCLS

## Use of XFEL for PSI and PSII

PSI: 2022, 2024

PSII: 2019, 2021, 2022, 2023

**PSII:2026?**

PSII: 2020, 2021, 2022, 2024



● **LCLS**  
U.S.

● **SwissFEL**  
Switzerland

PSI:2024  
PSII:2026?

● **EuXFEL**  
Germany



PSI:2021

PSII: 2019

**PSII:2026?**



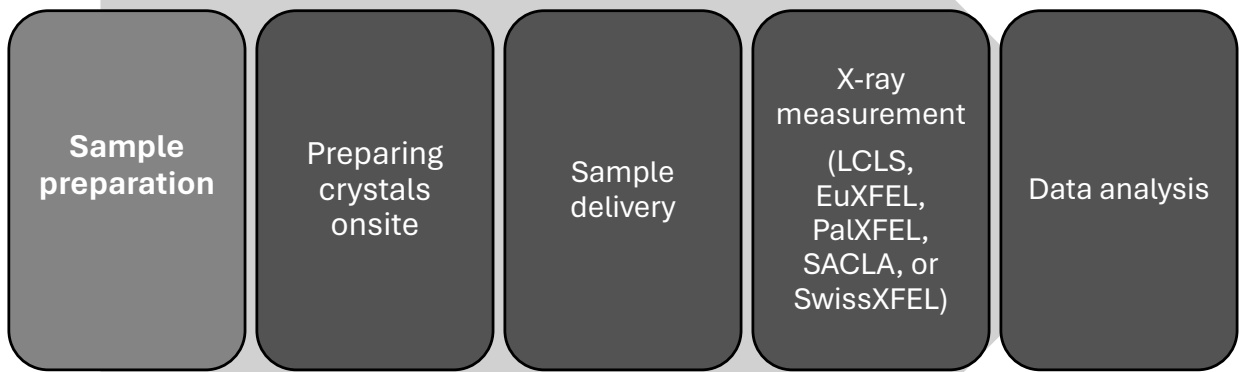
● **PAL-XFEL**  
South Korea

● **SACLA**  
Japan

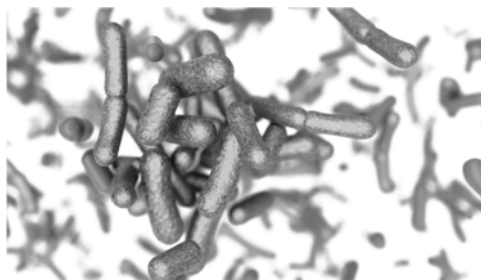


PSI: 2019,2023

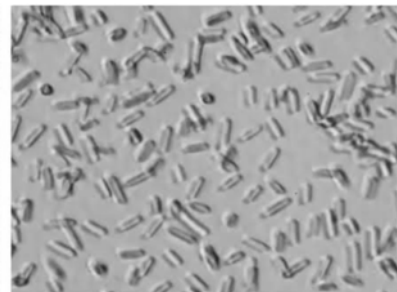
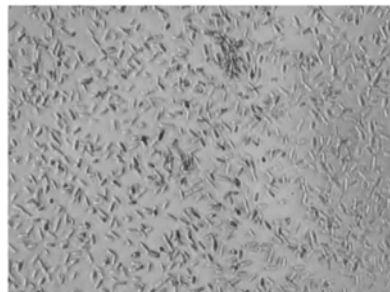
# Beamtime season



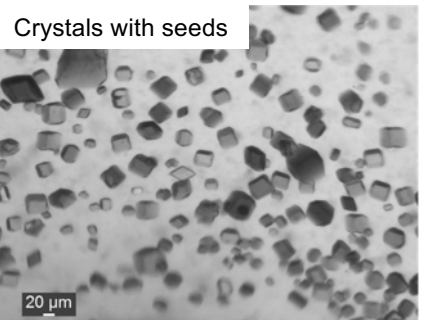
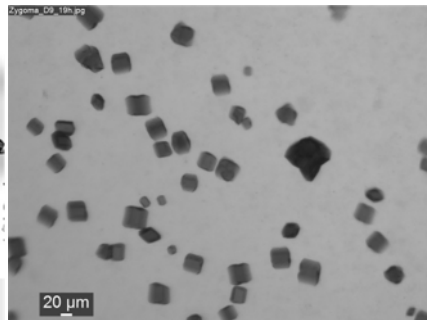
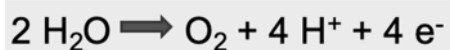
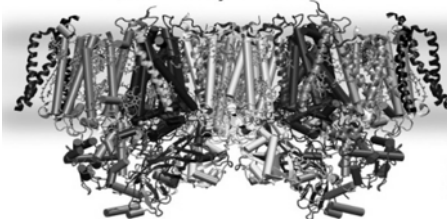
## Bacteria grow and PSII purification & crystallization



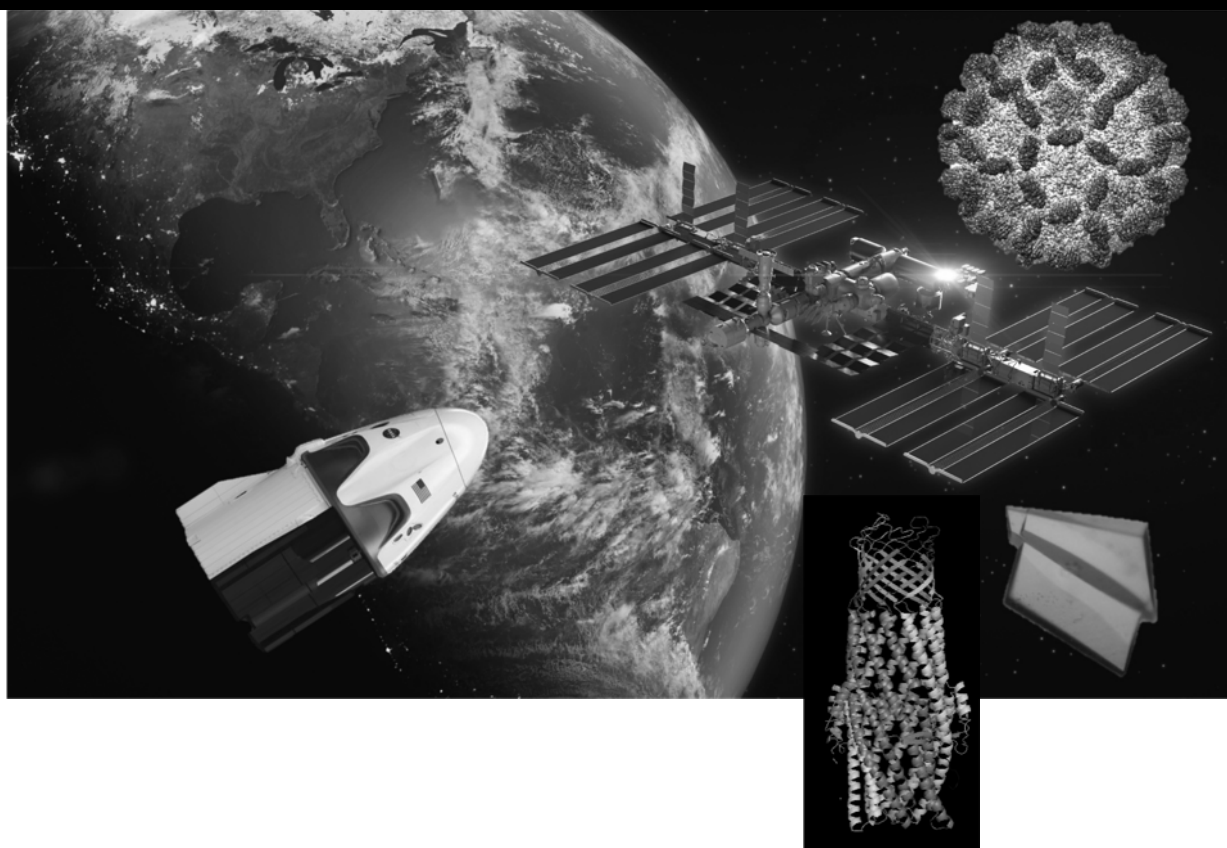
單細胞嗜熱藍綠菌，生長於約 50-60°C 的鹼性溫泉中等高溫環境。廣泛分佈於亞洲（如日本、台灣）的非酸性溫泉中。



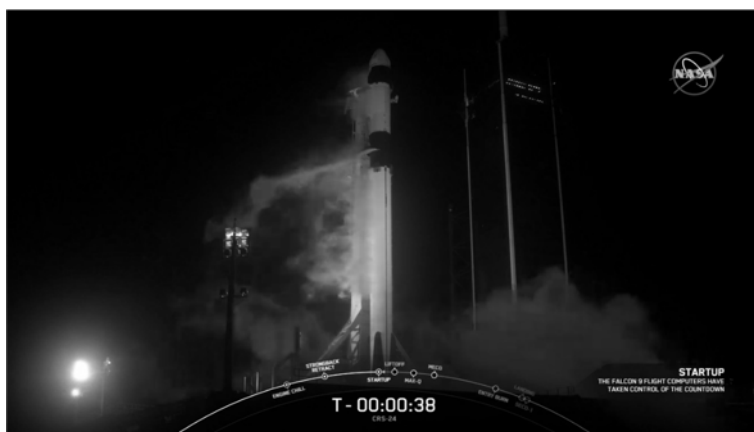
① Photosystem II



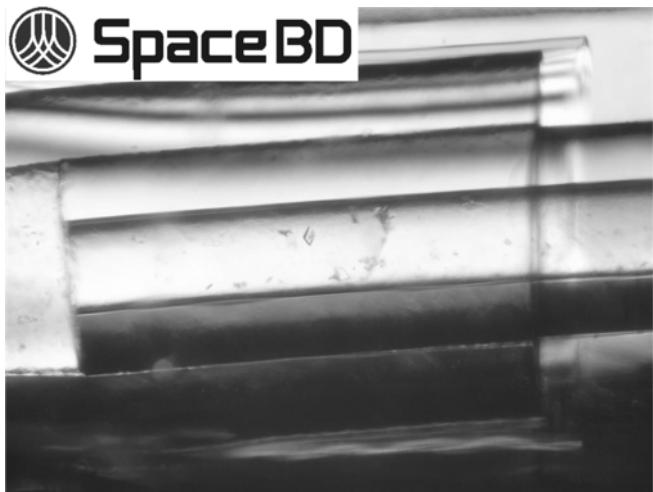
# Crystallization under Microgravity in Space



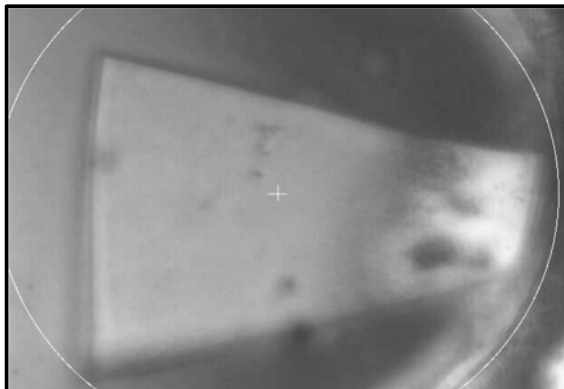
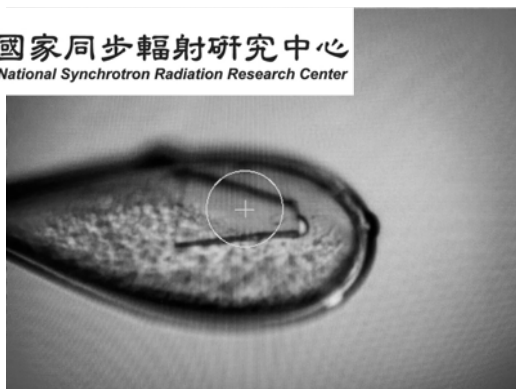
## CRS-24 Ready for Launch 2021.12.21



## Space Crystals Returned to NSRRC Lab (2022.02.07)



國家同步輻射研究中心  
National Synchrotron Radiation Research Center



## CRS-26 Launch on 2022.11.27 (3:20am TW)

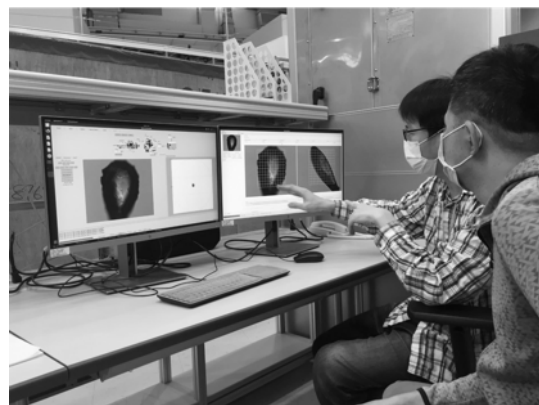
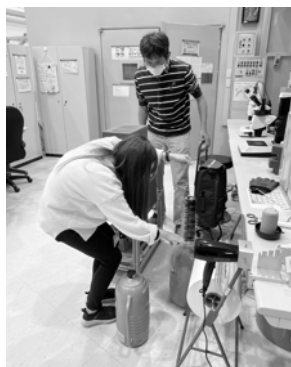


# Returning Space Crystal Samples

Crystals were shipped back to Japan from the launch site under controlling the same temperature as it was in space. Crystals were removed from capillaries, frozen, and shipped back to NSRRC, Taiwan.



## Crystal Analysis at beamline



***Thank you for your attention***

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**0922270612**

