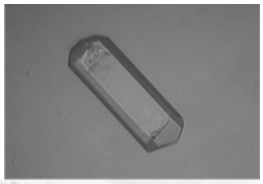
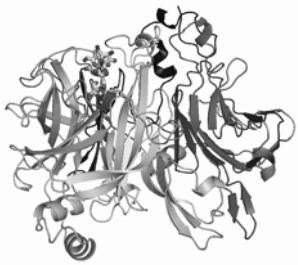
國家同步輻射研究中心
National Synchrotron Radiation Research Center
cjchen@nsrrc.org.tw

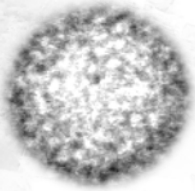


X光在生物分子結構的應用

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

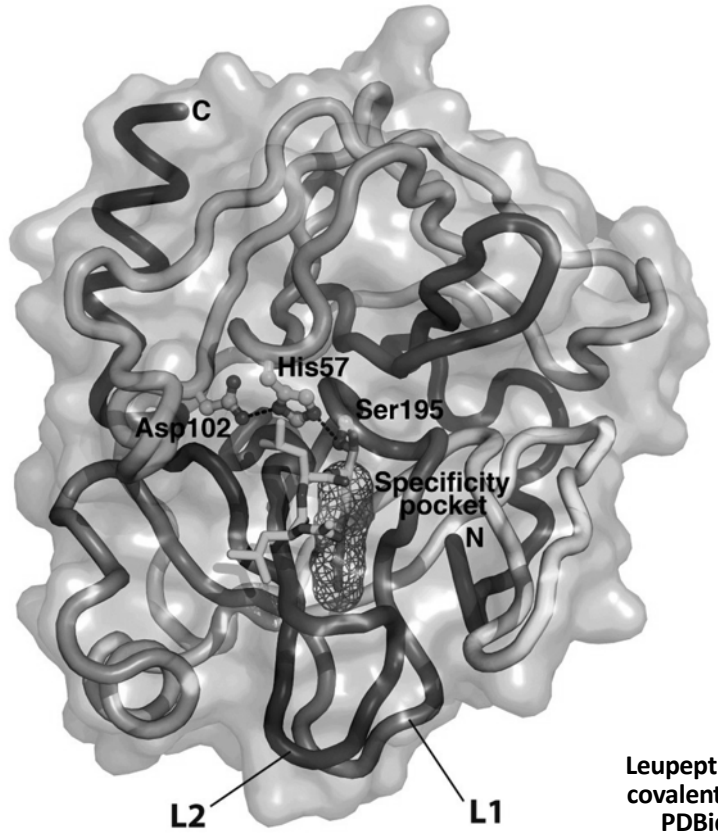
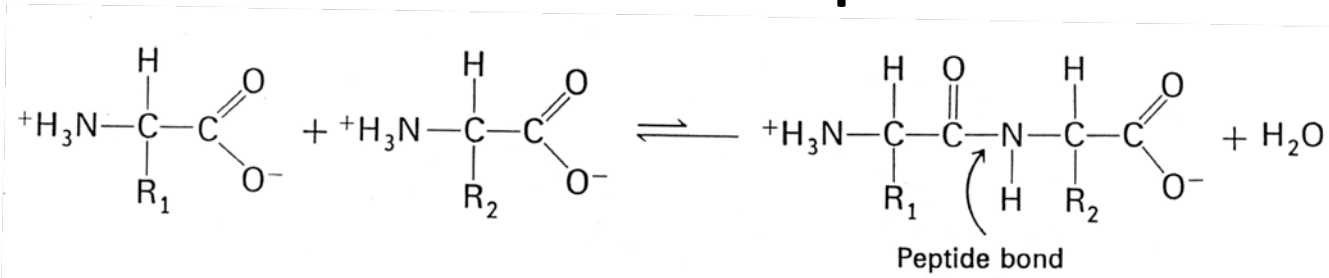
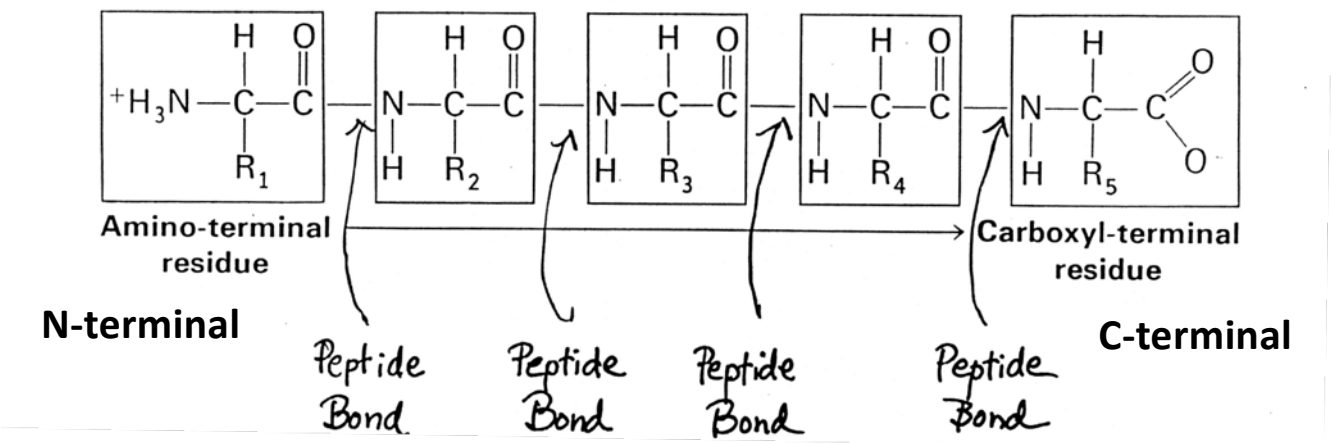


Figure 11-25

Leupeptin-trypsin covalent complex
PDBid 2AGI

Amino Acids >> Peptides

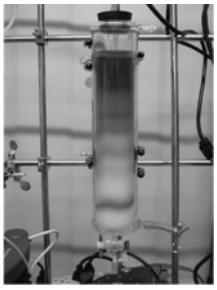




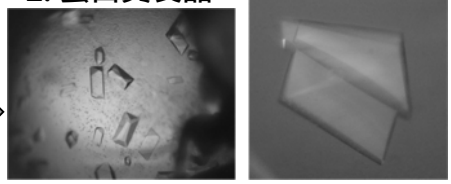
蛋白質結構與結晶學

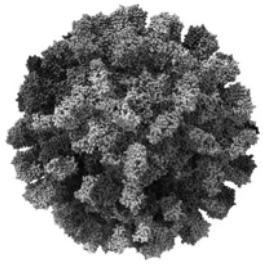
- 各生物體中蛋白質是實際執行功能的機器，人體中有超過25,000種蛋白質在作用。
- 蛋白質功能與結構息息相關，唯有了解每個蛋白質的獨特結構，才能徹底了解蛋白質的作用與反應機制。

1. 蛋白質純化生產

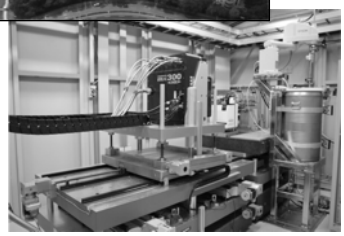


2. 蛋白質長晶

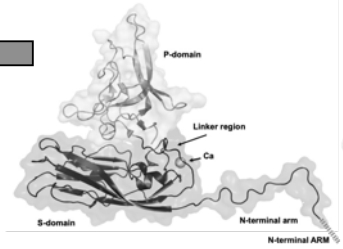


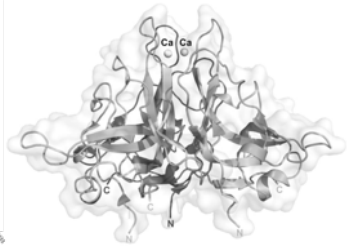


3. 同步輻射X光繞射

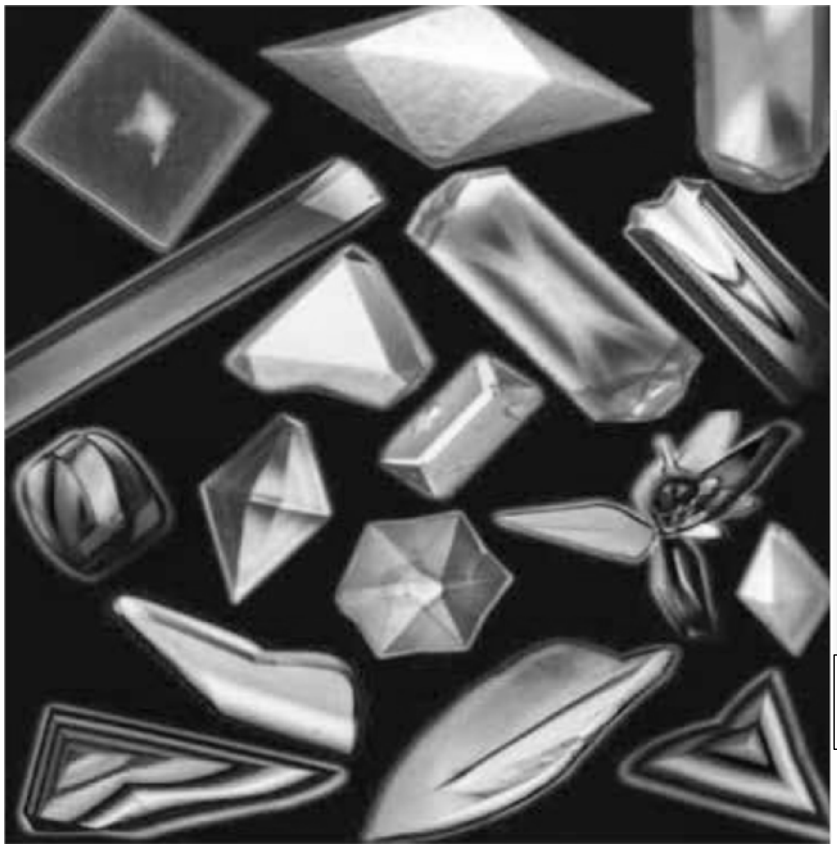


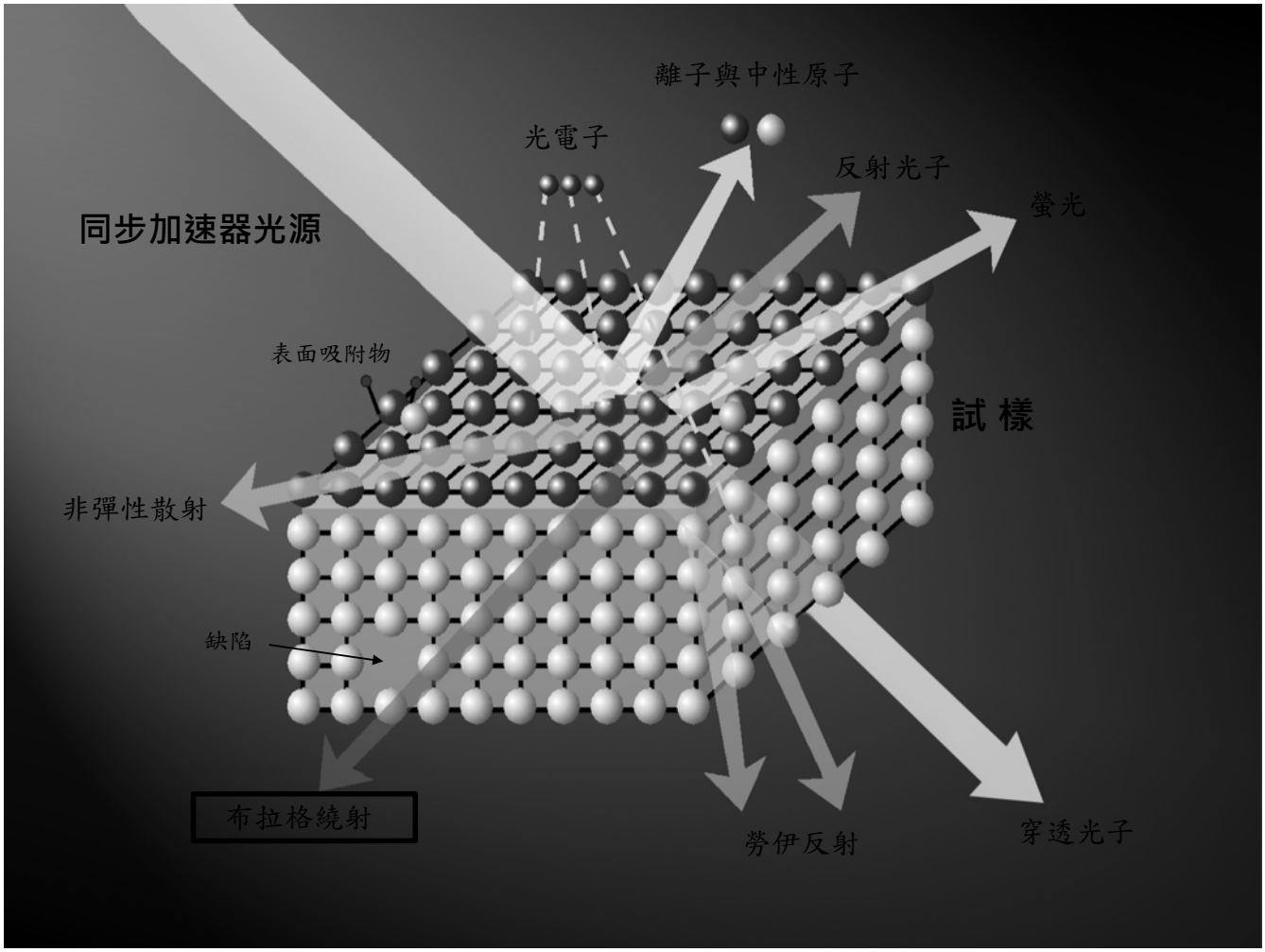
4. 蛋白質3D結構解析





5. 生技醫藥應用





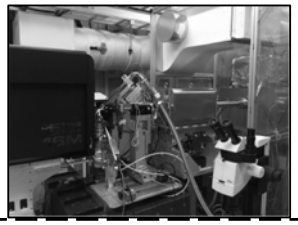
同步輻射蛋白質結晶學核心設施

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

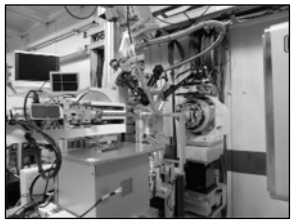
3.0 GeV
台灣光子源

- TPS 05A 微米蛋白質結晶學
- 2016.9 開放用戶使用



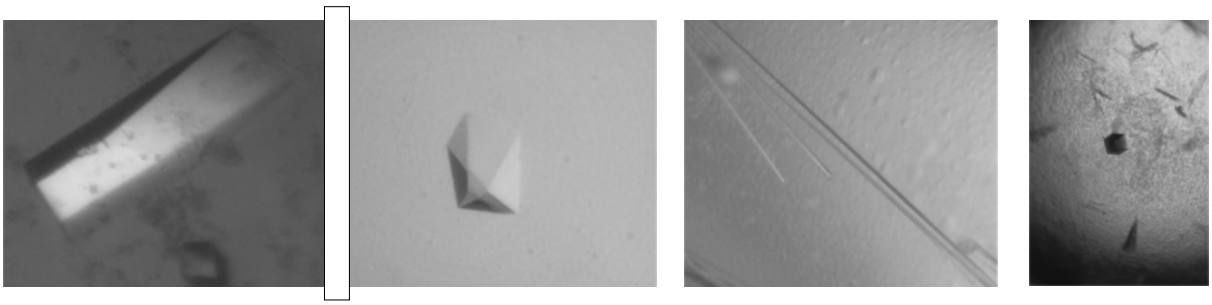
- TPS 07A 微米聚焦蛋白質結晶學
- 2021.11 開放用戶使用

8 GeV 日本春川



- 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

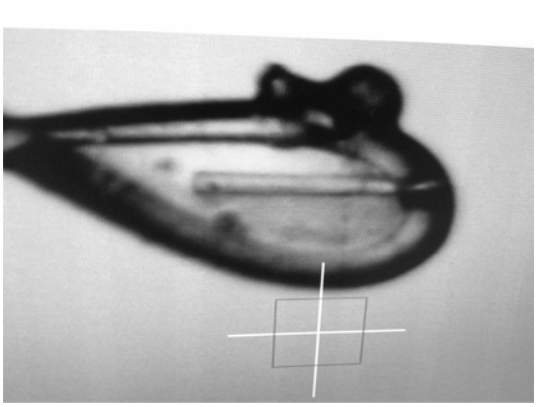


100 µm 50 µm 5 µm 1 µm

Crystal size

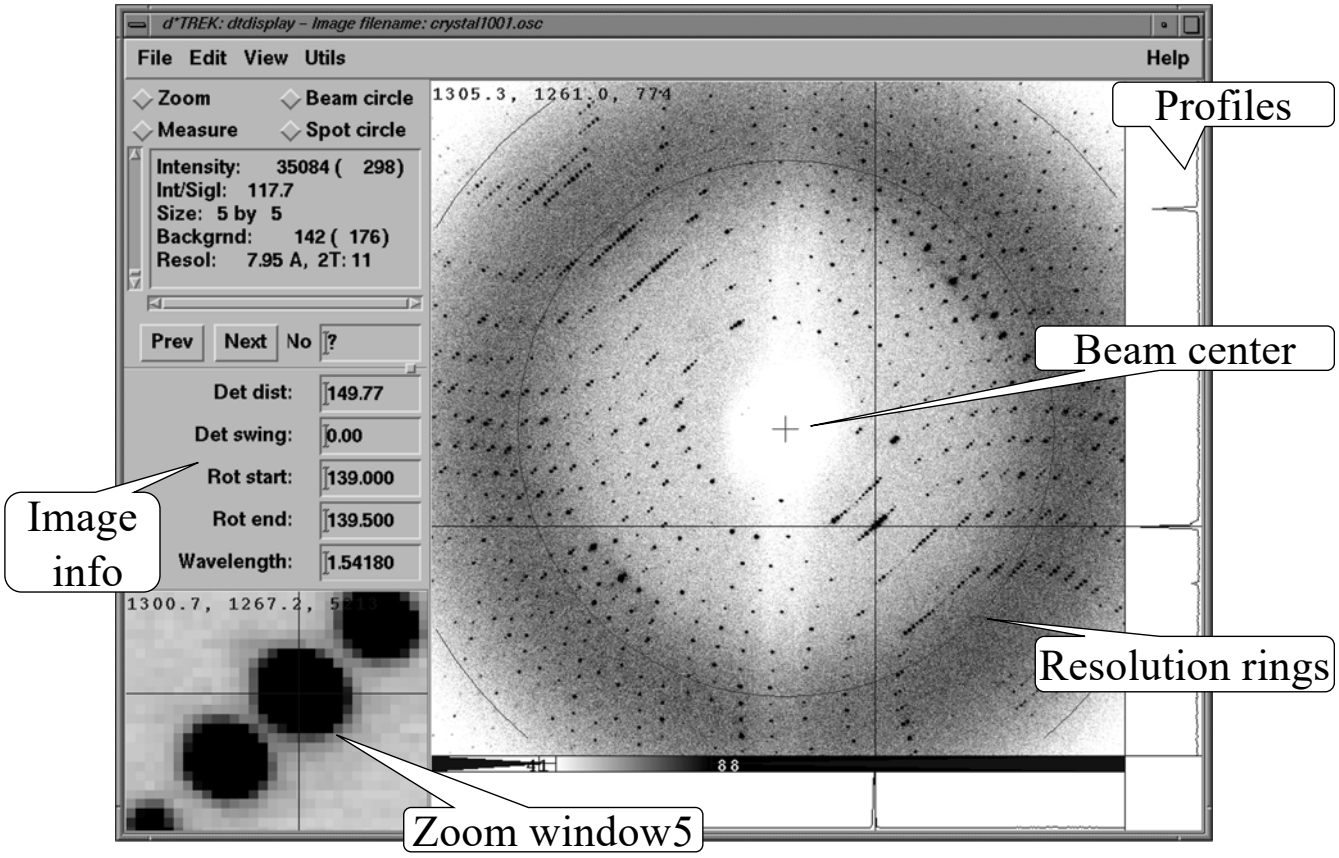
Micro-focus PX Beamline TPS 07A

Open a door for structure determination from micro-size crystals

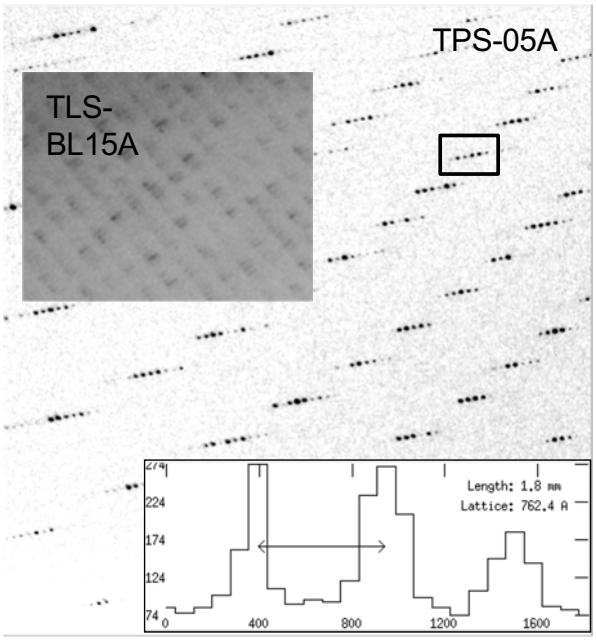


2 : 30 am

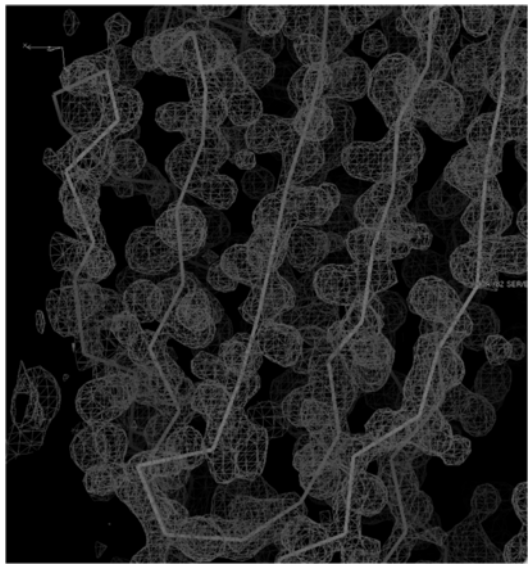
Diffraction Pattern



繞射點蘊藏著原子中電子的訊息



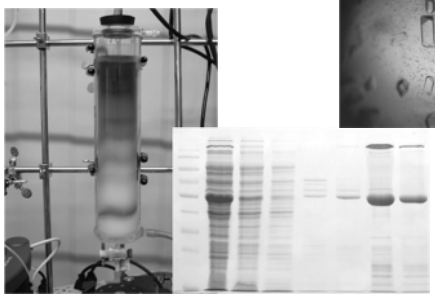
Large unit cell > 750 Å



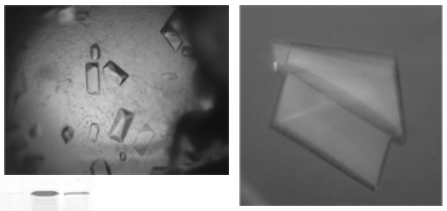
- Resolution: 1.6 Å (0.1 s exposure)
- Data set was completed in 18 sec
- 1 Se atom of 119 residues

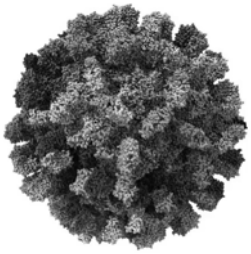
台灣光子源X-光蛋白質結晶學

1. 蛋白質純化製備

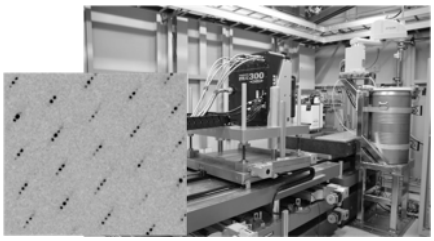


2. 蛋白質晶體培養

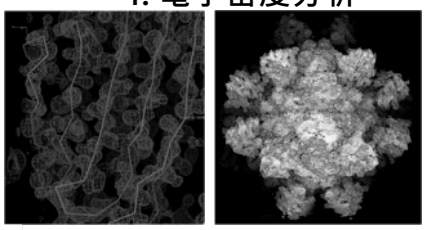




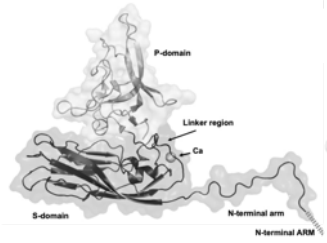
3. 同步輻射X光繞射

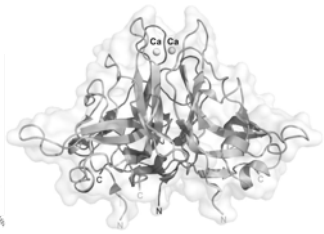


4. 電子密度分析



5. 結構解析





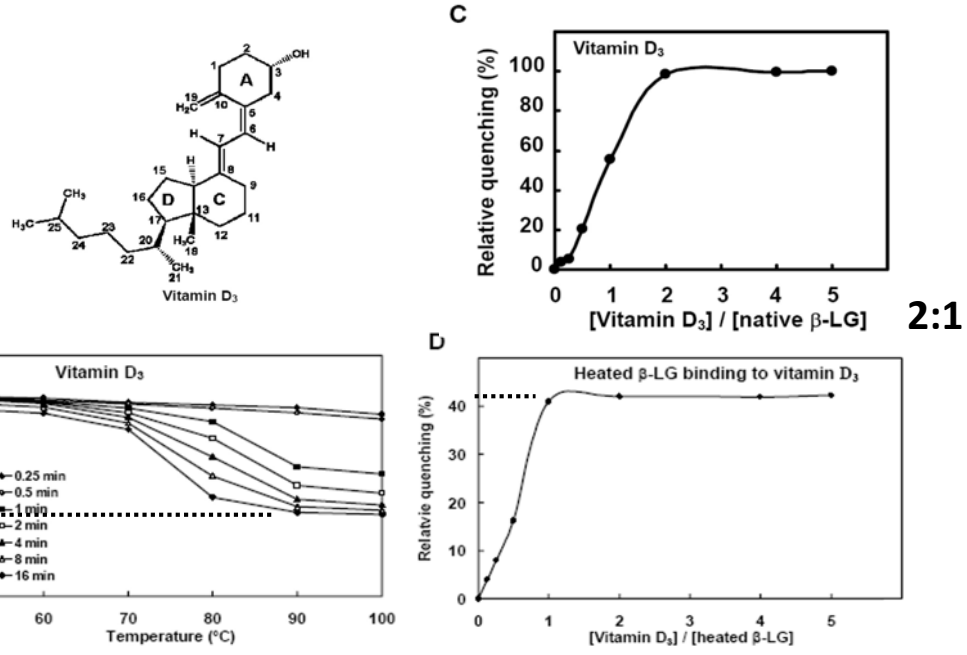
6. 生技醫藥應用

β-lactoglobulin

β-乳球蛋白：牛奶及羊奶中的主要乳清蛋白

- β-lactoglobulin (β-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.

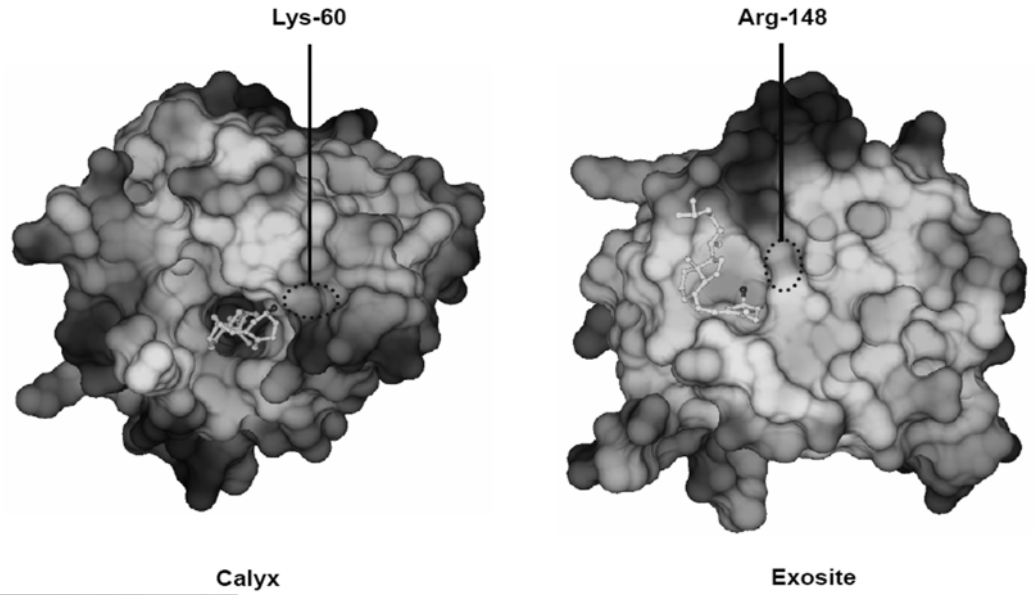
Effect of heating on β-LG binding to Vitamin D3

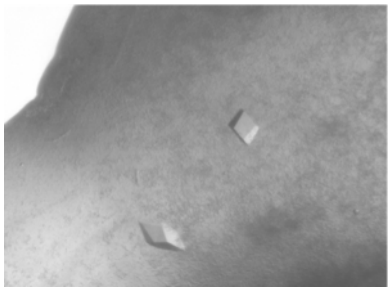


β-LG was heated between 50 and 100℃ for 15s to 16min before the addition of these ligands.

This data suggest that there is another thermally independent vitamin D_3 binding site, which is remote from the calyx.

Molecular interaction of β-LG with vitamin D_3



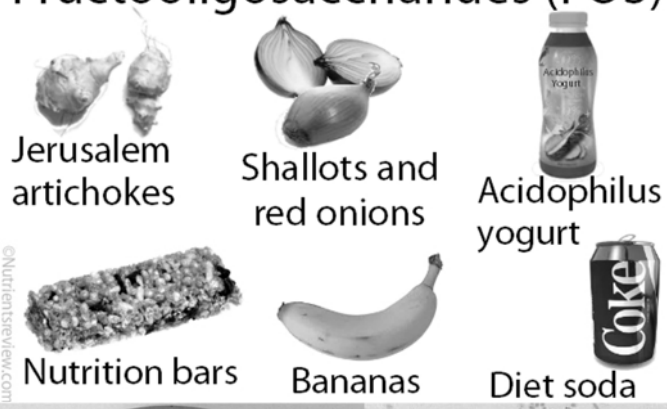


Hydrophobic residues

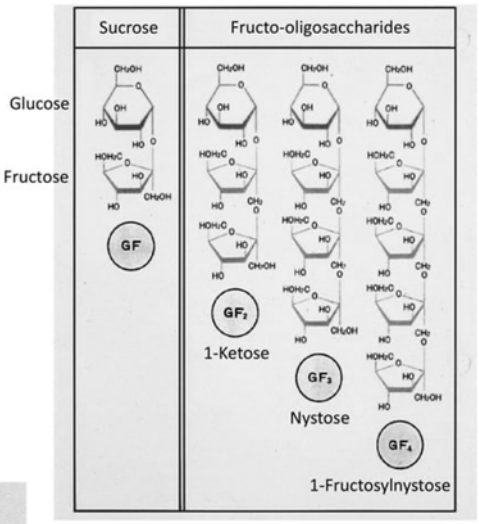
Hydrophilic residues

富含果寡糖的食物

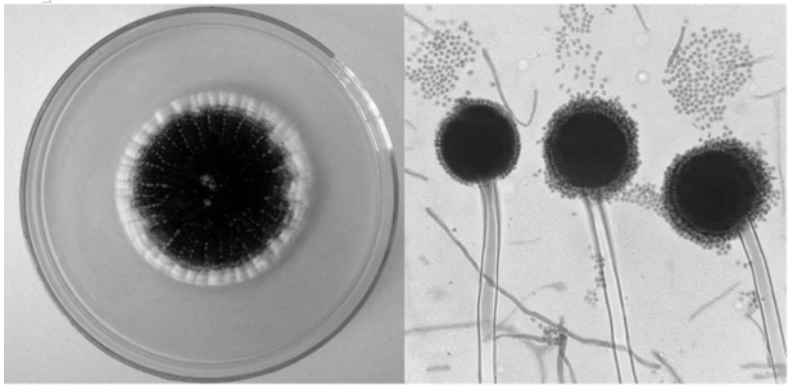
Fructooligosaccharides (FOS)



Major component molecules included in FOS



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

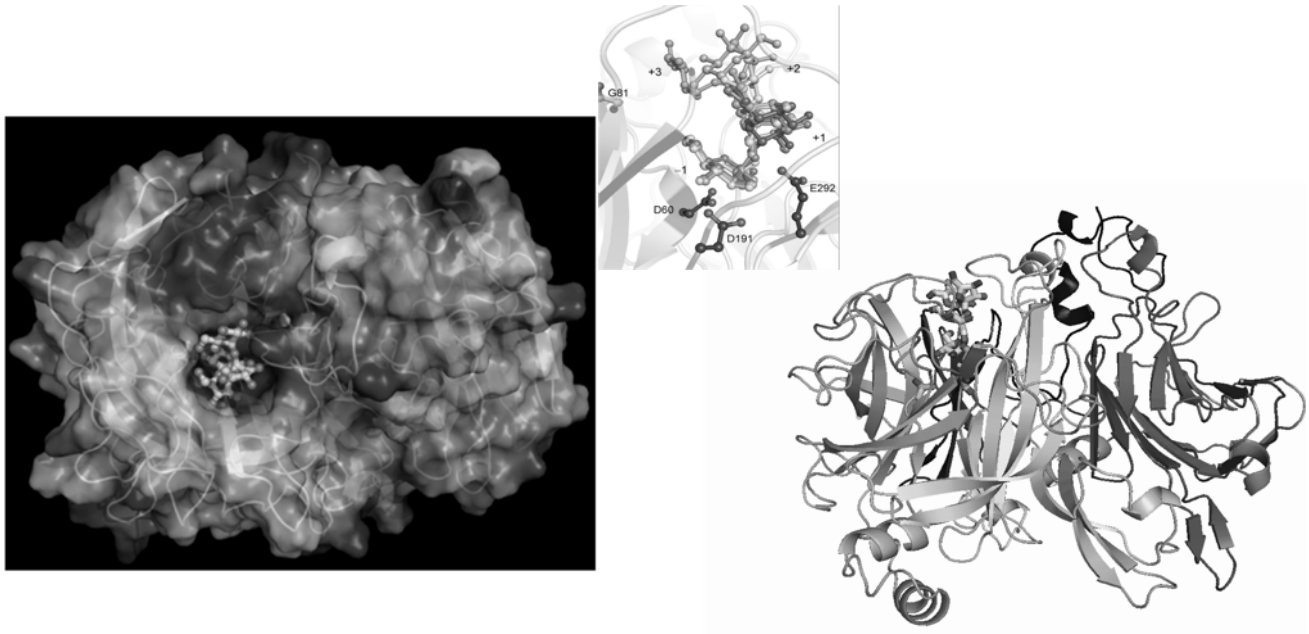


Aspergillus japonicus
日本麴黴

Fermentation (2022) 8, 437

FOS production:
Sucrose + fructosyltransferase by *Aspergillus japonicus*

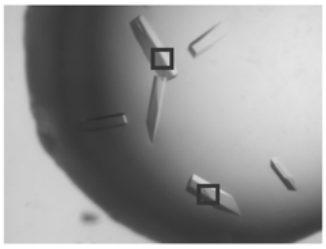
The complex structure of *Aj*FT with substrates

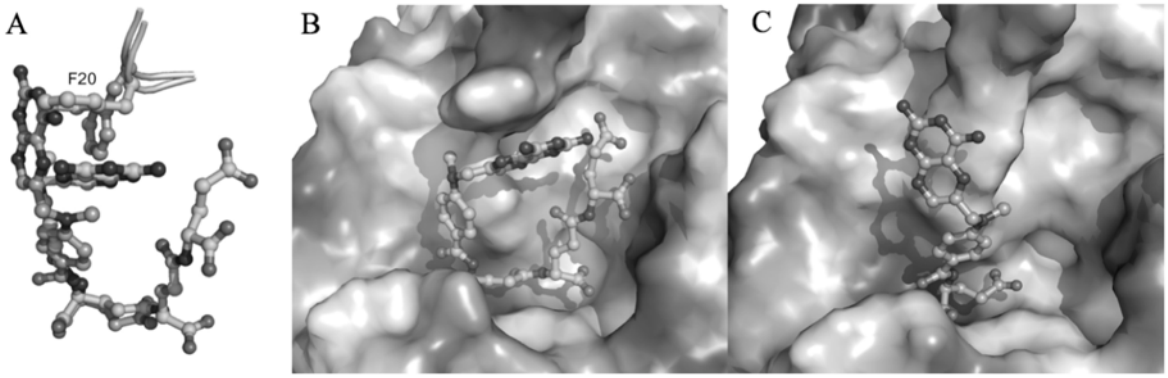


Crystal structure of *Aspergillus japonicus* fructosyltransferase (*Aj*FT) complex with nystose. The structure of *Aj*FT composed of an N-terminal catalytic domain comprising a five-blade β-propeller linked to a C-terminal β-sandwich domain is presented as ribbons and electrostatic surface (positive 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 γ-glutamyl hydrolase

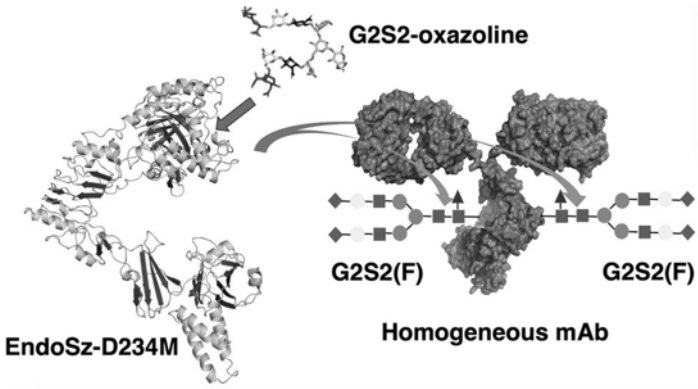
***J. Med. Chem.* (2013) 56, 7625**

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

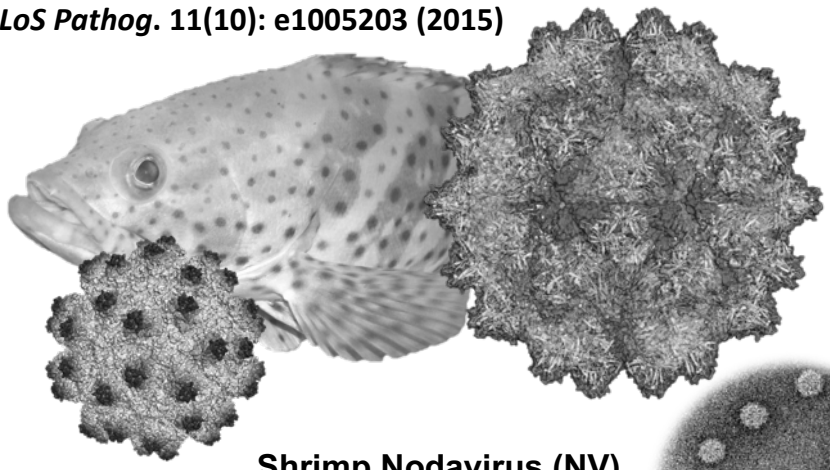
利用蛋白質結晶學光束線解析酵素及聚醣複合物在不同狀態下的晶體結構



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。

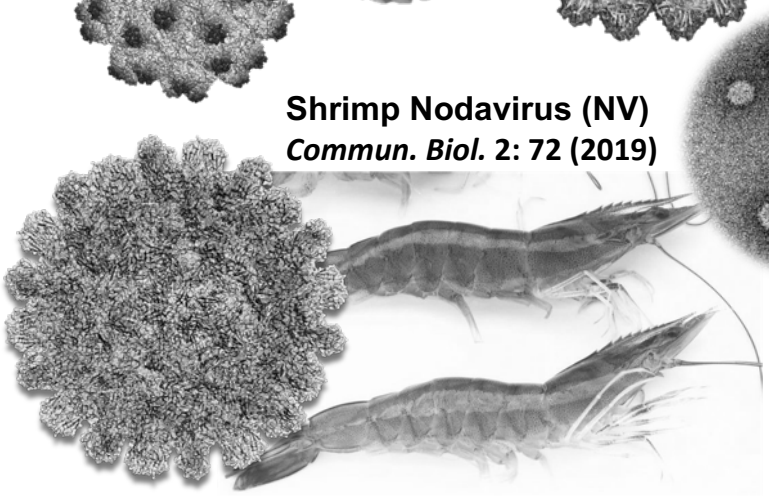
Virus Studies

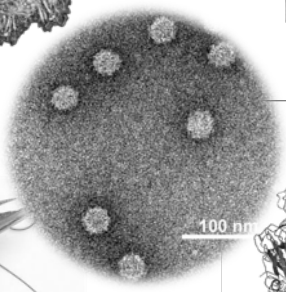
Grouper Nervous Necrosis Virus (GNNV)
***PLoS Pathog*. 11(10): e1005203 (2015)**

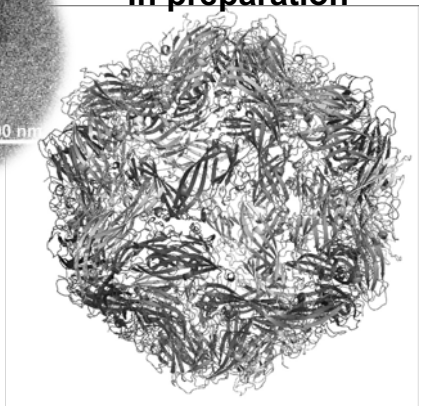


Porcine Virus
In preparation

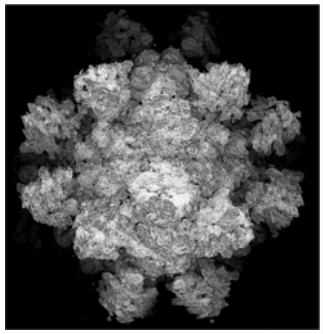
Shrimp Nodavirus (NV)
***Commun. Biol.* 2: 72 (2019)**

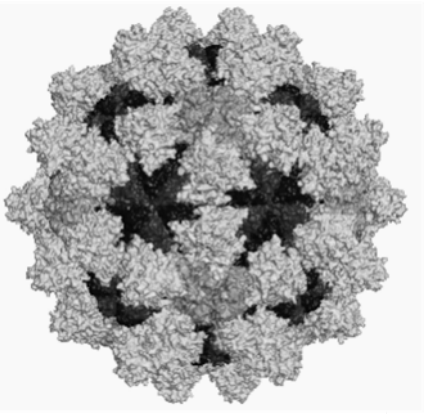


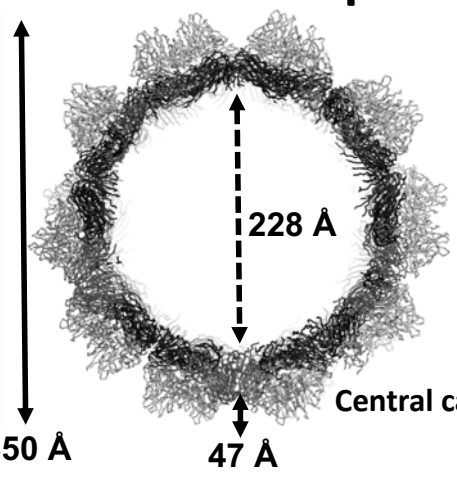


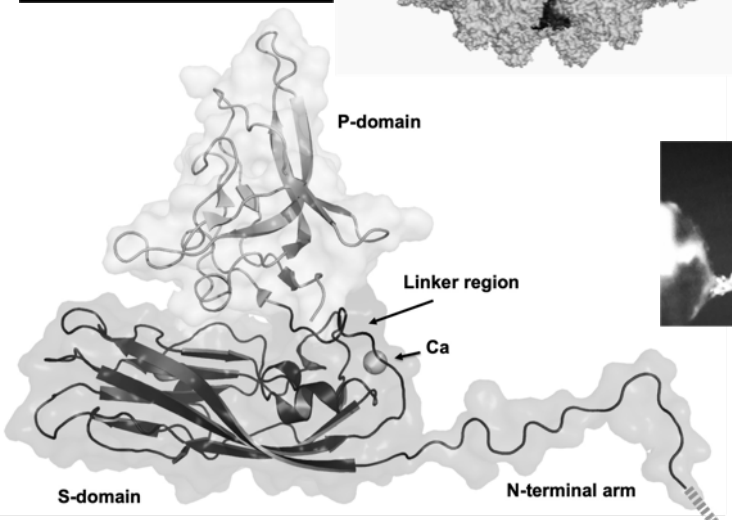


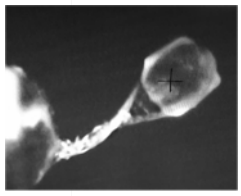
T=3 GNNV-LPs and Structure of Capsid

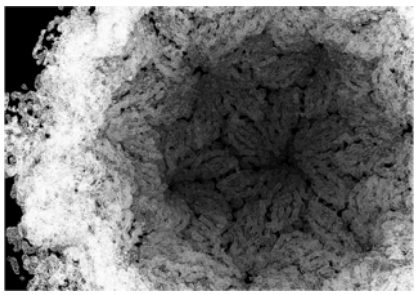












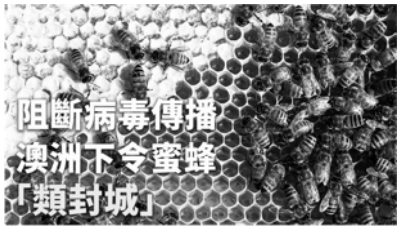
PLoS Pathog. 11(10): e1005203 (2015)

N-terminal ARM

MVRKGEKKLAKP....ATTKAANPQPRRRANNRRRSNRTDAPVSKASTVTGFGR

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**



BBC 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**.

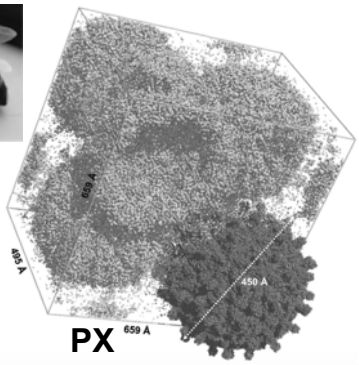
nature communications (2023) 14:545

Chen, *et al.*

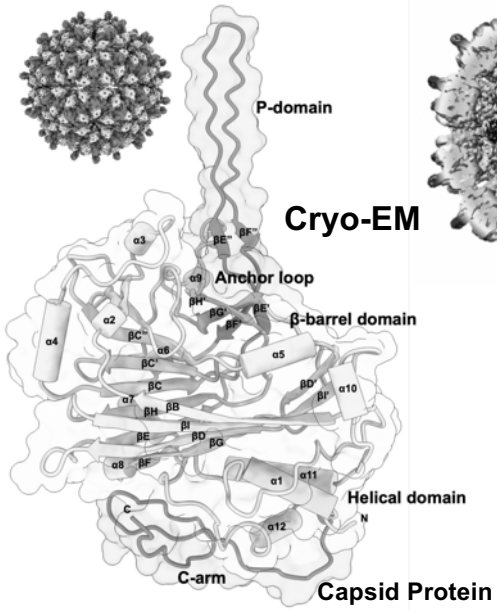
Article

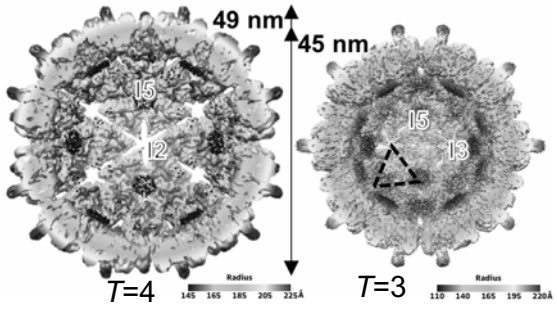
Structures of honeybee-infecting Lake Sinai virus reveal domain functions and capsid assembly with dynamic motions

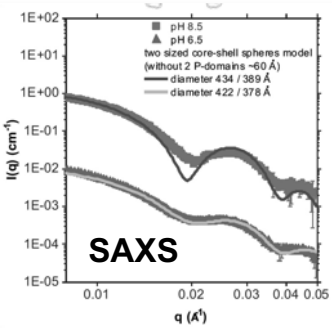
Combination of PX, Cryo-EM, SAXS, MALDI-TOF and other biophysical techniques for structural biology

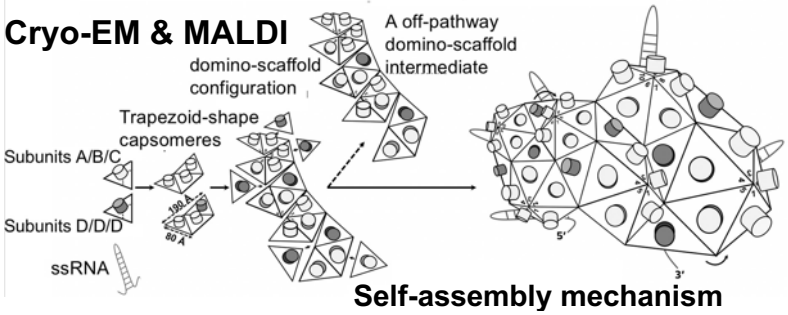


PX

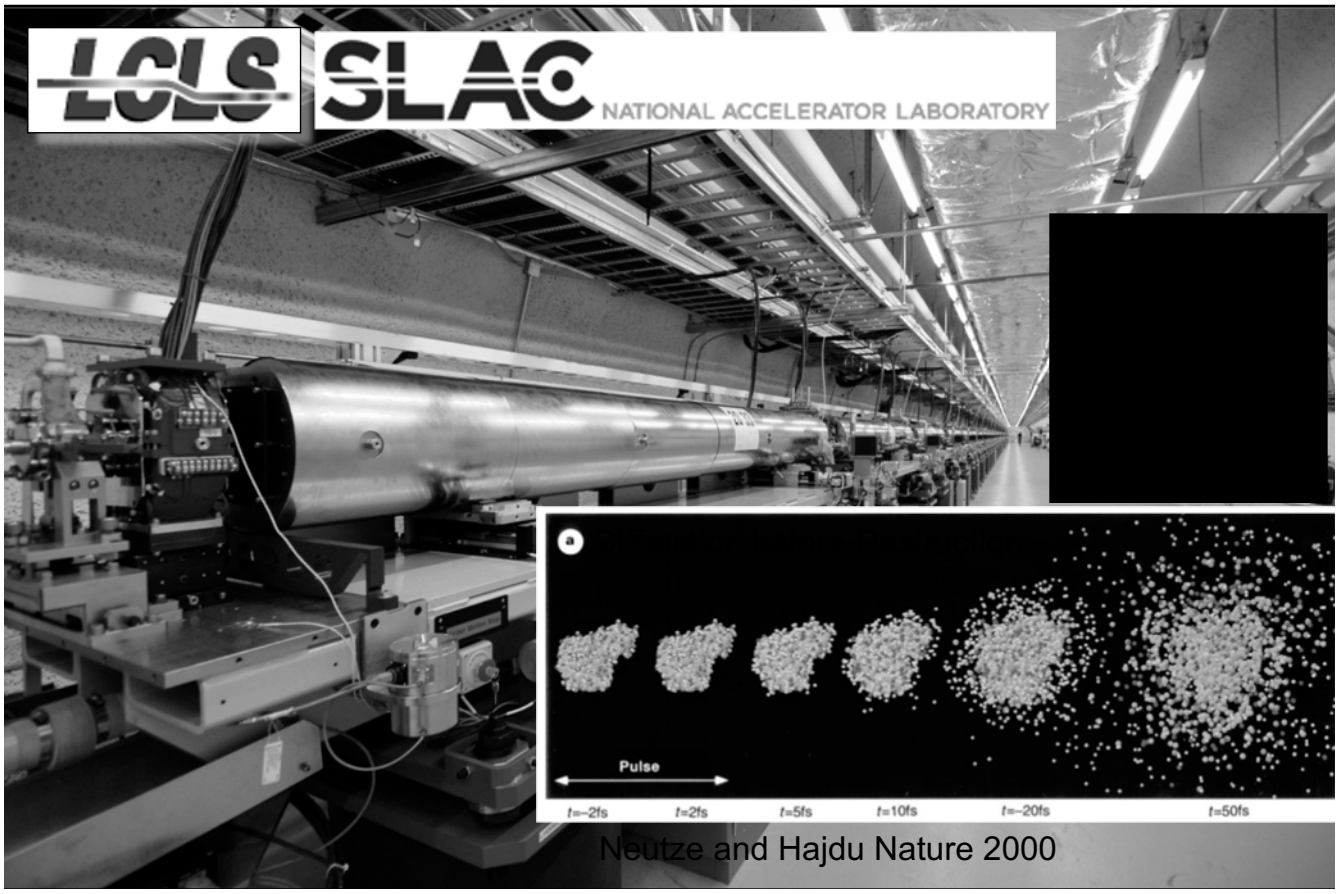








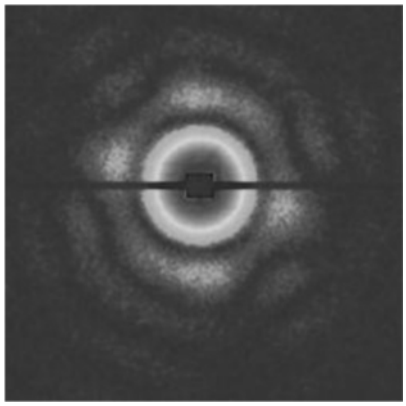
The Linac Coherent Light Source Free electron: the first hard X-ray XFEL in the world

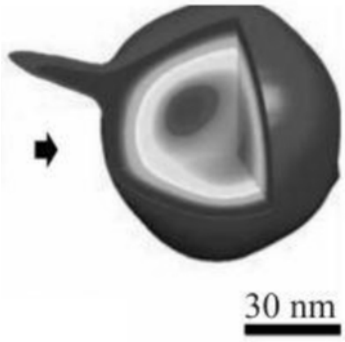


Neutze and Hajdu Nature 2000

Applications of XFEL

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

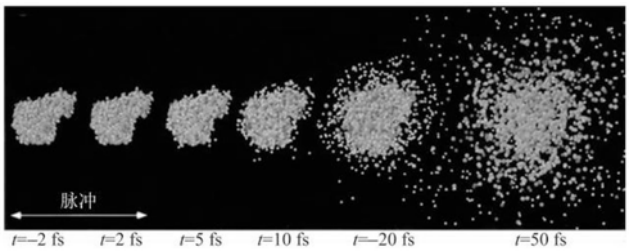


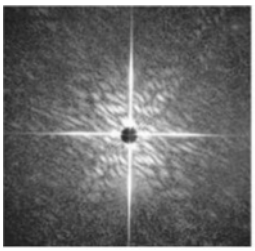


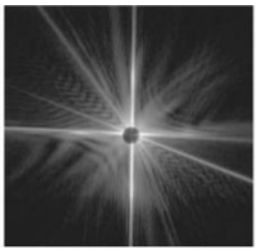
- pump-probe technology and crystallography for time-resolved dynamic study on structures.

Applications of XFEL

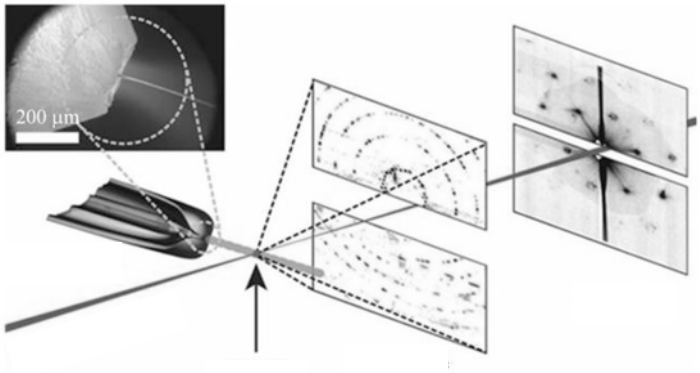
- Radiation-damage-free structures of macromolecules



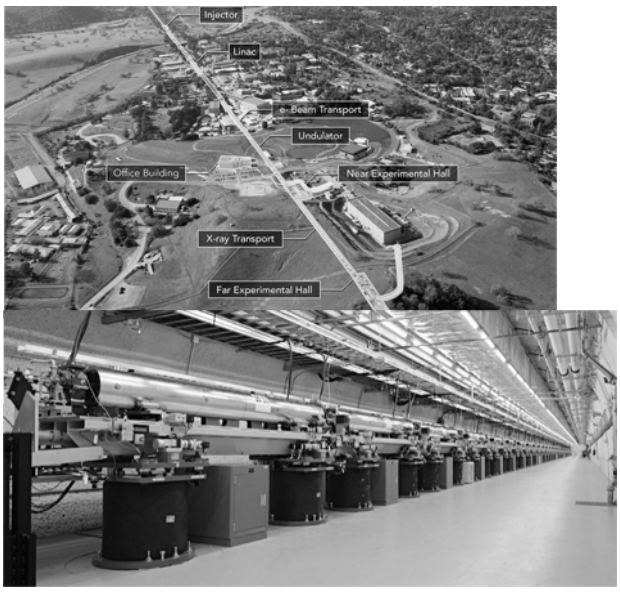




- Serial femtosecond crystallography, SFX



Strategy for Serial Femtosecond Crystallography



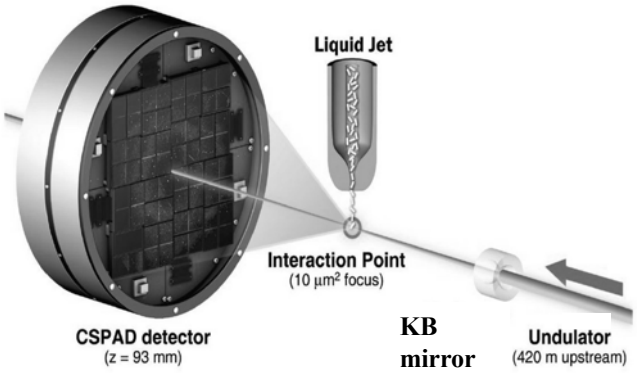
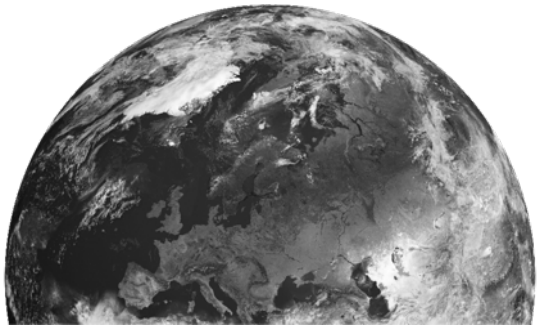


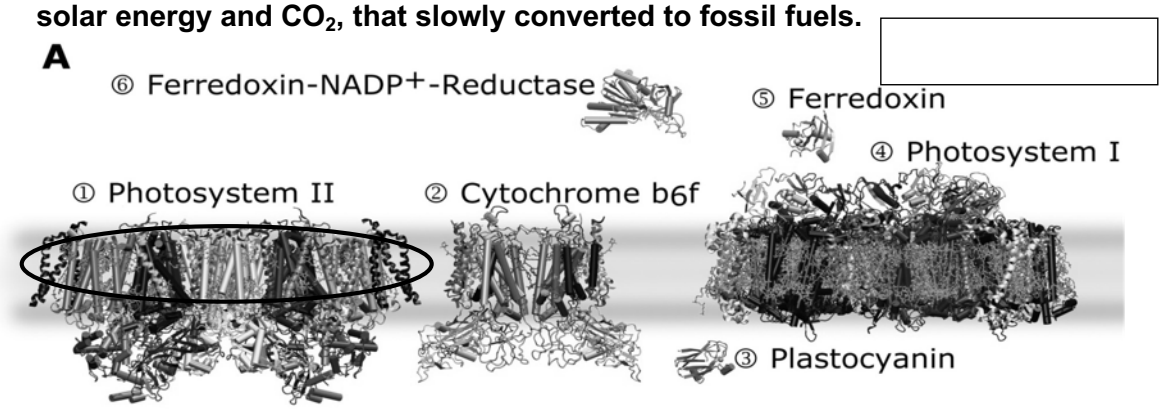
Image from Boutet et.al (2012)

- Size of crystal: small (typically 0.1-10 μm)
- Crystals in mother liquor, 'RT'
- Liquid injection
- Light source (xFEL): 'coherent', frequency = 120 Hz, higher flux (~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

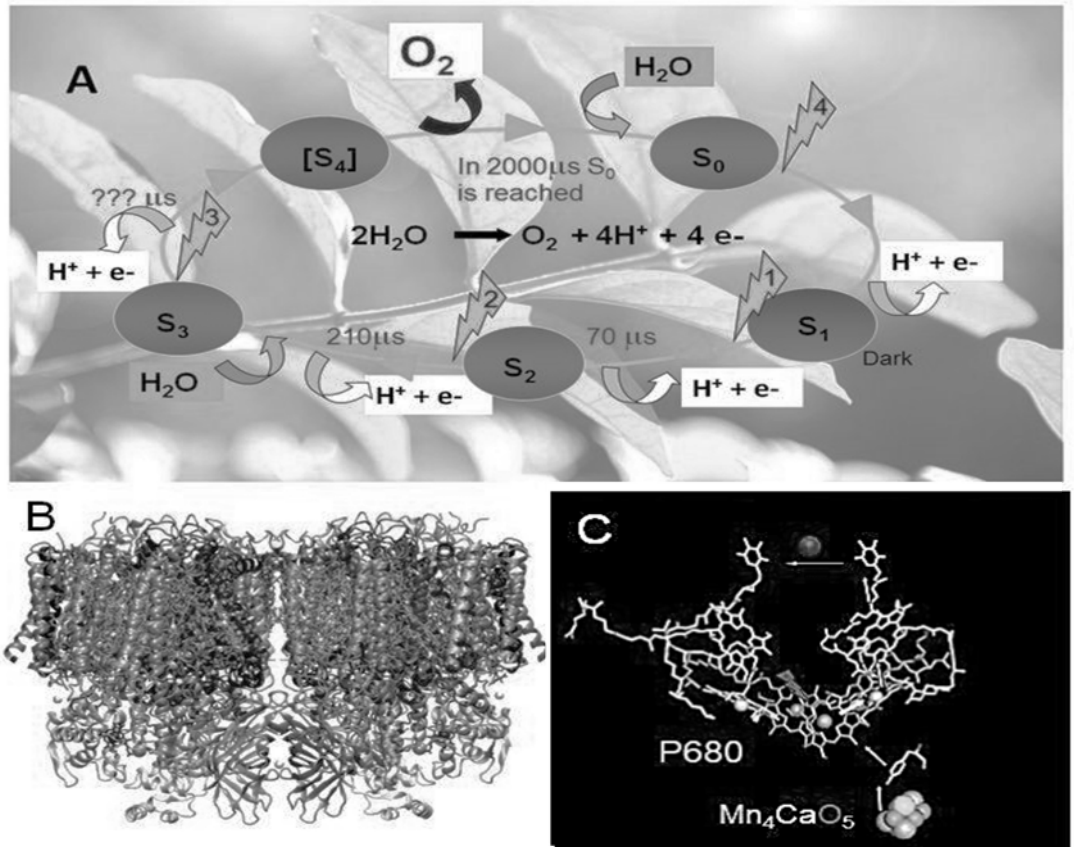


Photosynthesis transformed our planet 2.5B years ago producing oxygen, capturing solar energy and CO_2, that slowly converted to fossil fuels.

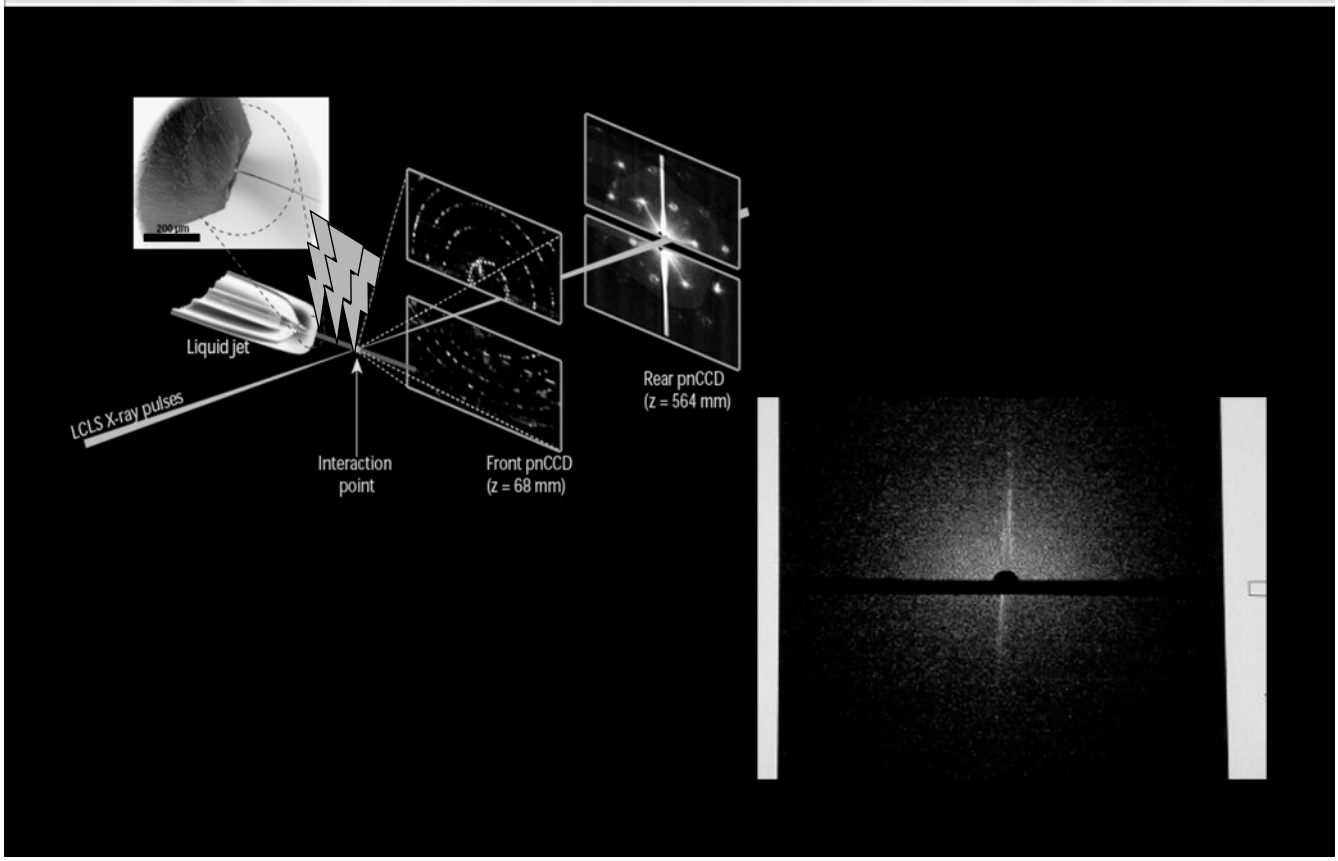


$2\ H_2O \rightarrow O_2 + 4\ H^+ + 4\ e^-$

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



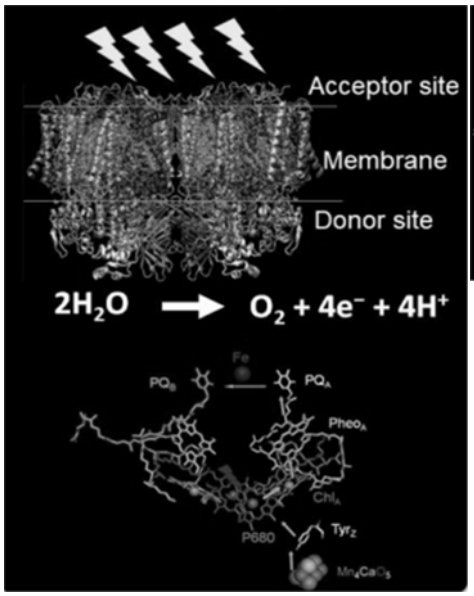
Jet setup for initial TR-SFX experiments on PSII



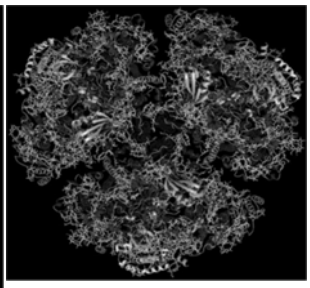
Using Protein crystallography & X-ray free electron lasers (XFELS) to Study Photosystems I & II project

Project Goals of Photosystem I & Photosystem II protein complexes

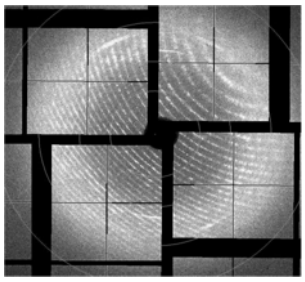
- Obtain a molecular movie of electron transfer in Photosystem I
- Resolve the structural changes in Photosystem II and the oxidation states of the Mn atoms in the transient S4 state of the water splitting process



Structure of Photosystem II, responsible for water splitting during photosynthesis



Structure of Photosystem I

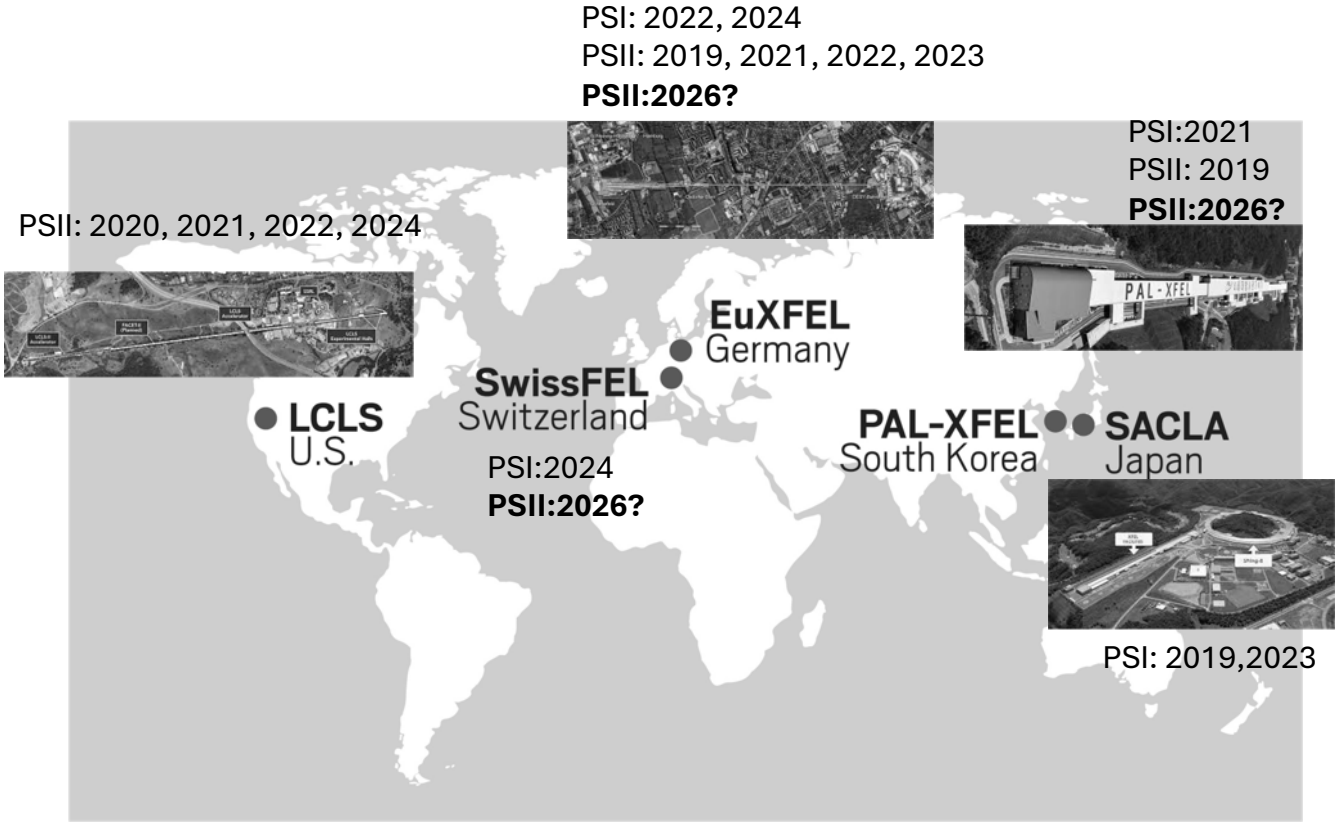


Diffraction pattern of shrunk PSII crystals

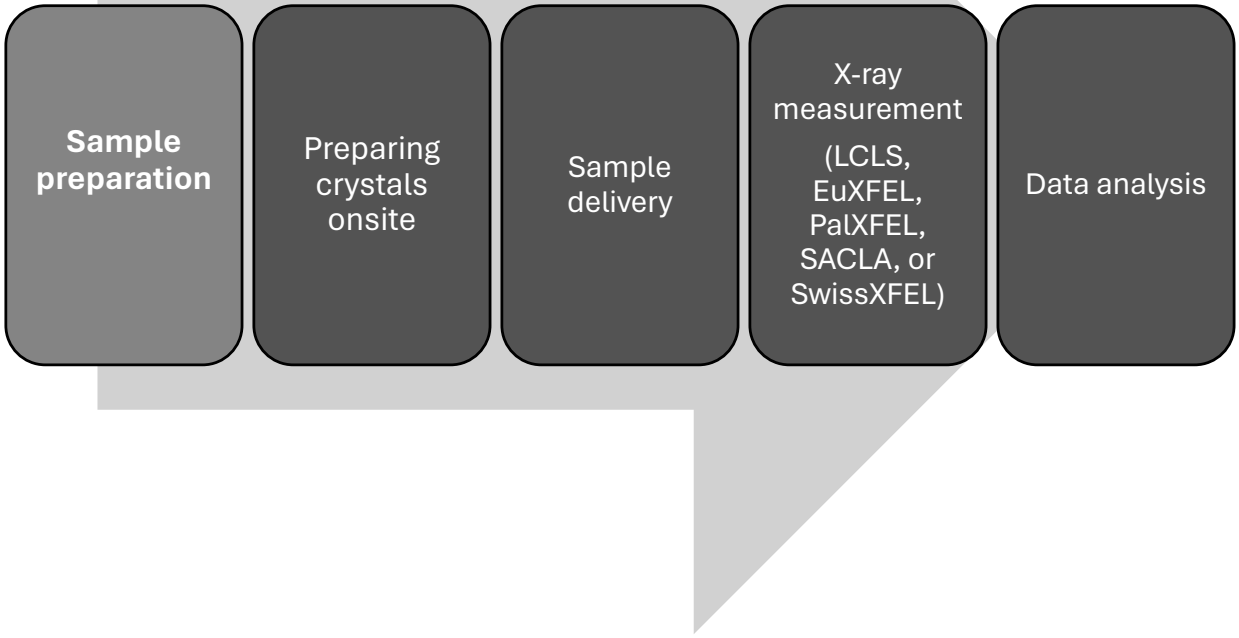
MFX beamline used for PSII water splitting experiments

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

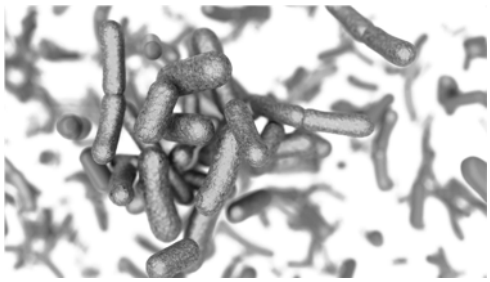
Use of XFEL for PSI and PSII



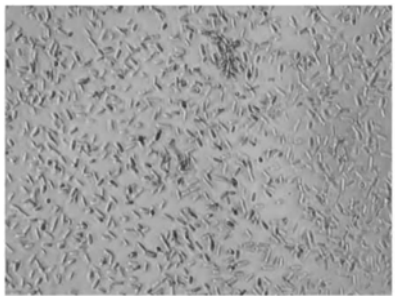
Beamtime season

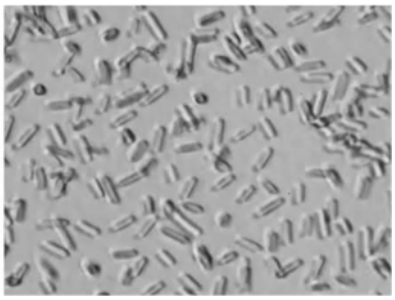


Bacteria grow and PSII purification & crystallization

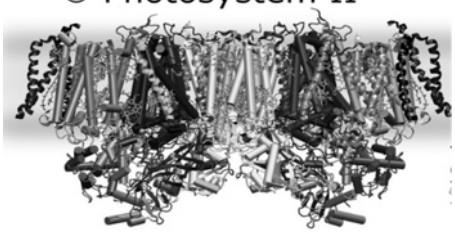


單細胞嗜熱藍綠菌，生長於約 50-60°C 的鹼性溫泉中等高溫環境。廣泛分佈於亞洲（如日本、台灣）的非酸性溫泉中。

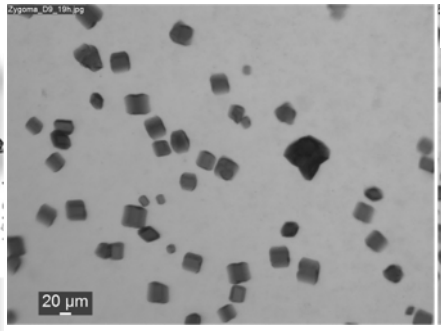


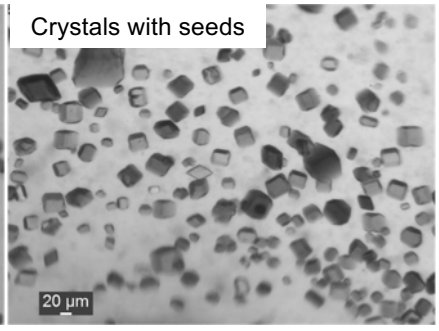


① Photosystem II

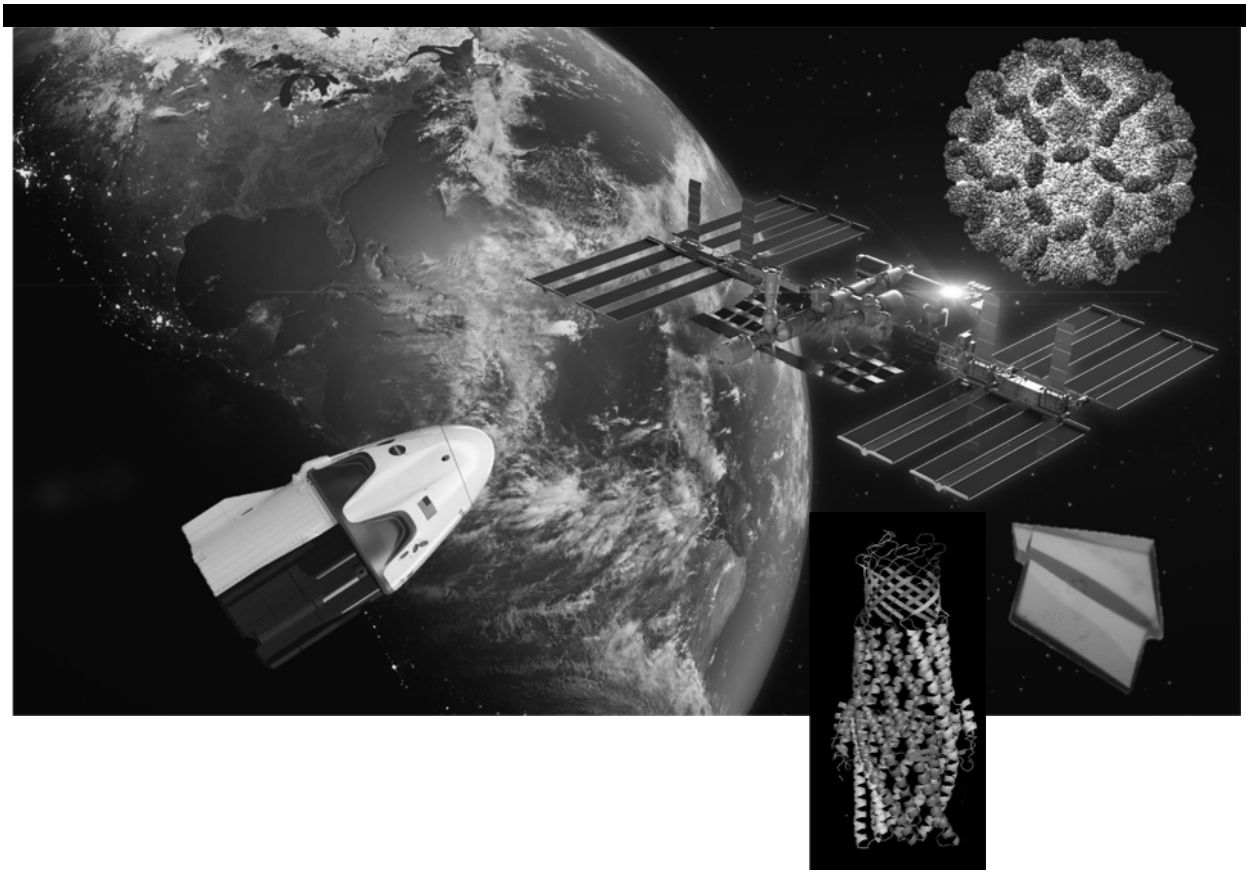


$2\,H_2O \rightarrow O_2 + 4\,H^+ + 4\,e^-$



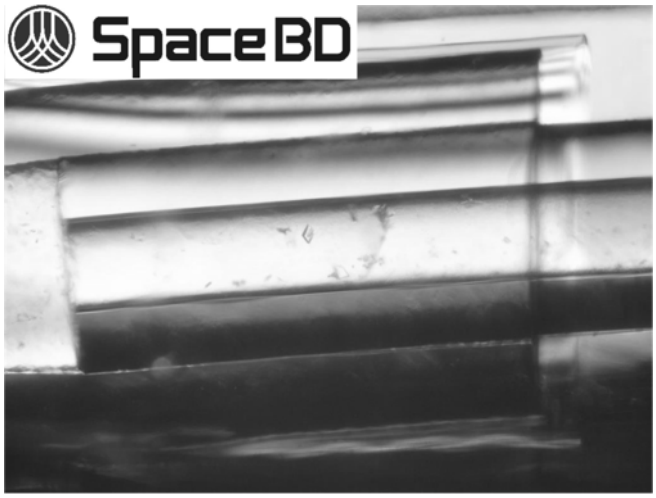


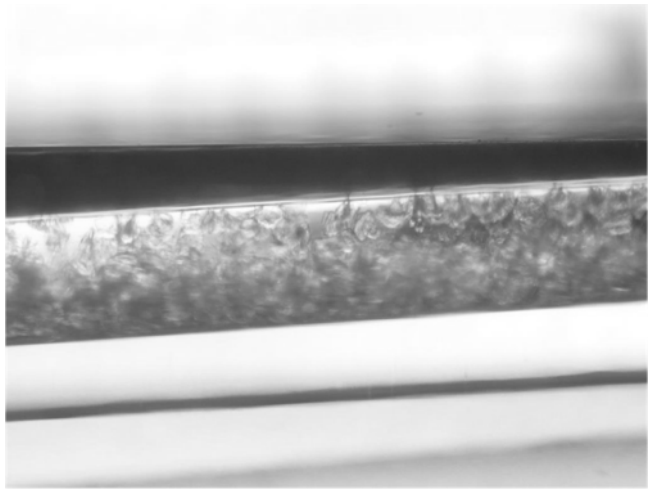
Crystallization under Microgravity in Space

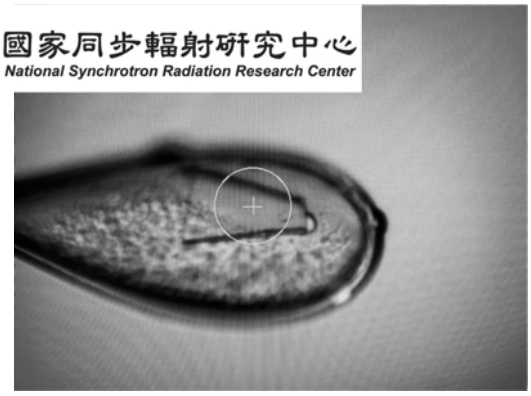


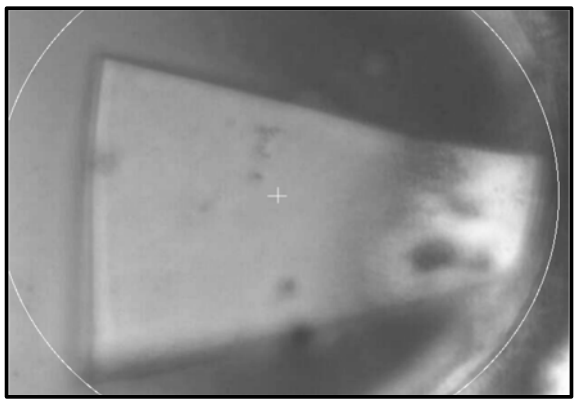
CRS-24 Ready for Launch 2021.12.21

Space Crystals Returned to NSRRC Lab (2022.02.07)





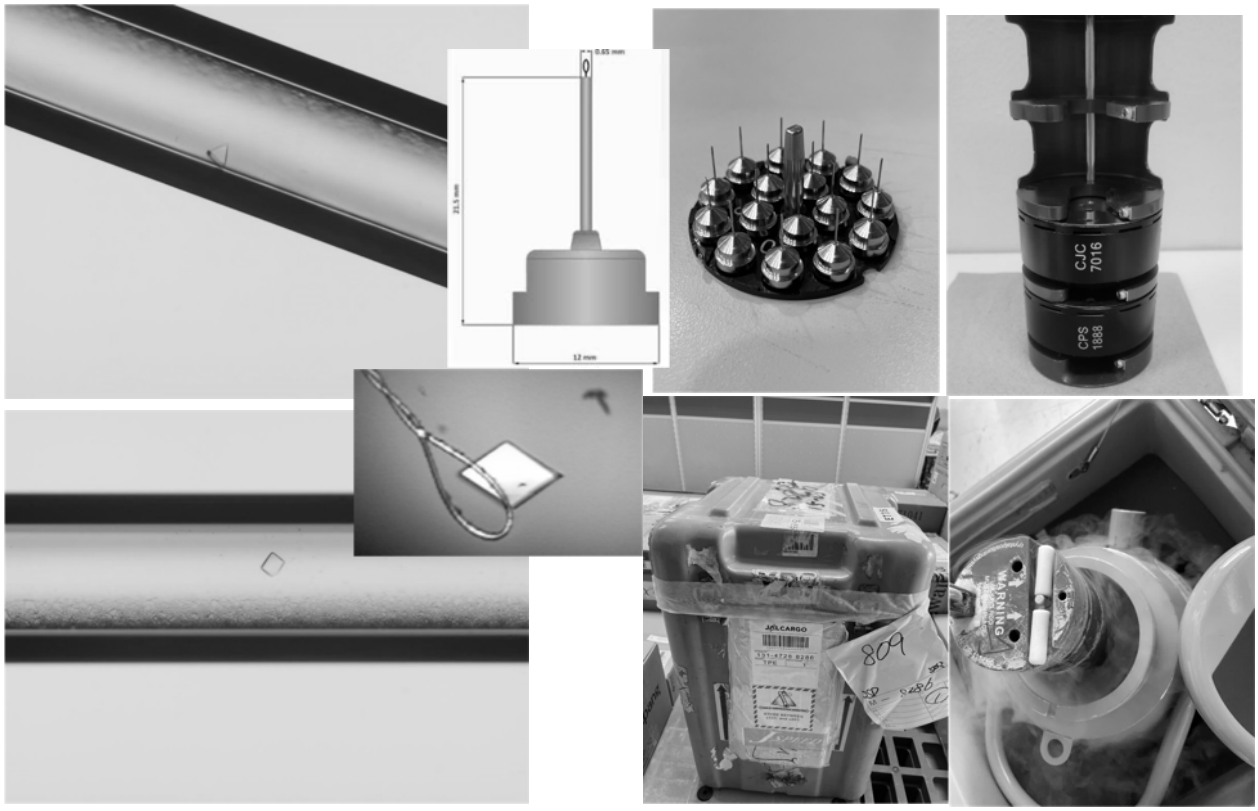




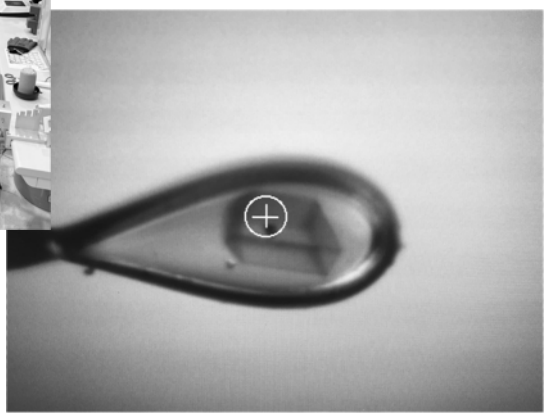
CRS-26 Launch on 2022.11.27 (3:20am TW)

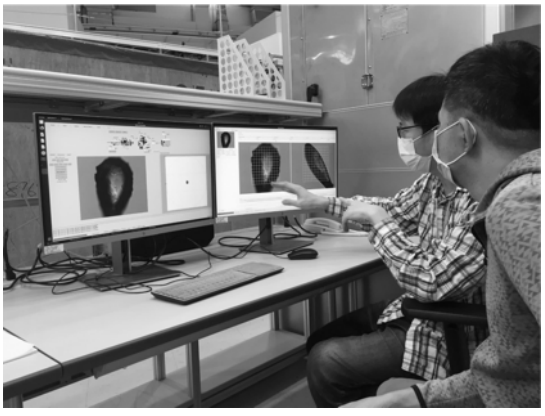
Returning Space Crystal Samples

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