Bio-Science and Imaging Beamlines

ID21 BioSAXS: <u>Hyeong Jin Kim¹</u>, Ji-hun Kim², and Kyeong-Sik Jin²

ID22 BioNX: Mi-Jeong Kwak² and Yeon-Gil Kim² BM10 HEM: Yong Sung Park² and Jae-Hong Lim²

¹Research Center for Beamline, Korea Basic Science Institute ²4GSR Accelerator Research Division, Pohang Accelerator Laboratory

Nov. 5-6, 2025



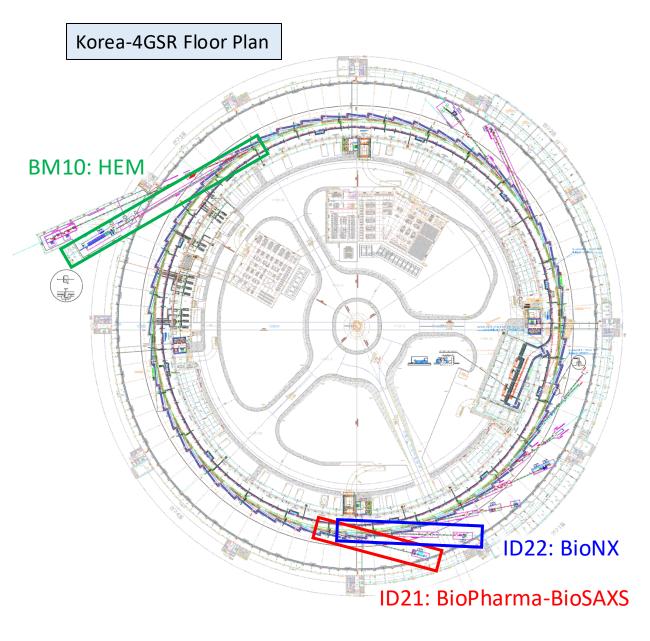


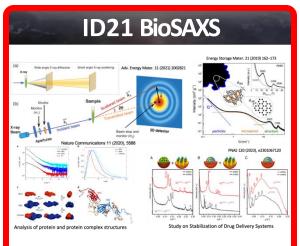


Outline

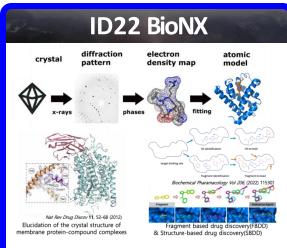
- 1. Overview
- 2. ID21 BioPharma-BioSAXS (Small angle X-ray Scattering)
- 3. ID22 BioNX (Bio Nano crystallography)
- 4. BM10 HEM (High Energy Microscopy)
- 5. Summary

1. Overview

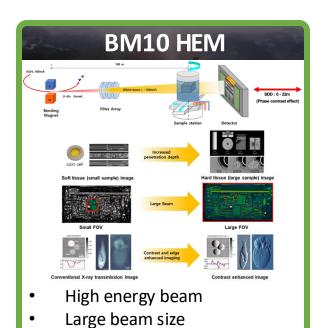




- High-throughput experiment
- Variable q-range vacuum chamber
- Efficiency / Convenience



- High-throughput experiment
- Micro-focusing beam
- High flux, rapid beam resizing, stability



X-ray phase contrast imaging

	BioSAXS	BioNX	HEM
Photon Source	IVU24	IVU20	BM (2T)
Energy Range (mainly)	8 ~ 23 keV (12)	8 ~ 25 keV (12.4 and 20)	20 ~ 150 keV
Beam Flux (ph/s)	> 5 × 10 ¹²	> 1 × 10 ¹⁴	~ 1 × 10 ¹³
Beam Size (μm²) (H × V, FWHM)	 Partially Focused: ~ 200 × 200 Focused: ~ 40 × 10 	 12.4 keV: 1 × 1 ~ 50 × 50 20 keV: 1 × 1 ~ 5 × 5 	• 100m: 200 × 25 mm ²
Spatial Resolution	8 ~ 3800 Å	0.5 ~ 1.55 Å	> 1.0 μm
Technique	SAXS/WAXS, SEC-SAXS	RSX, SSX, ISX, HTS* MX	Projection Imaging





1. Overview

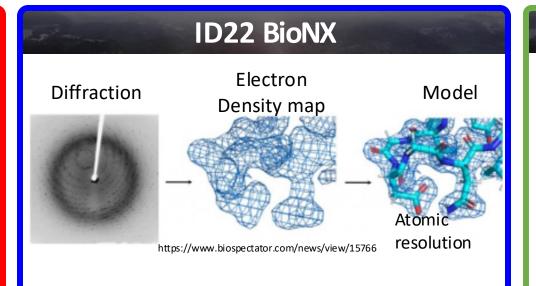
Priority support for industries

ID21 BioSAXS SAXS profile Model Molecular resolution s (1/nm Validation Parameters Current Research in Struc. Biol. (2020) Sample: Biomacromolecules in solution

Design Keyword

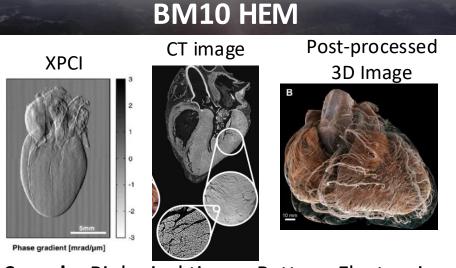
- q-vector optimizing layout
- High-throughput (automatic sample exchange robot)
- Convenience

Support for Academic R&D



Sample: Biomacromolecules as crystallized **Design Keyword**

- Micro-focusing and pulsed beam
- High flux, rapid beam resizing, stability
- High-throughput (sample exchange robot)



Sample: Biological tissue, Battery, Electronic components, Developmental prototype, etc.

Design Keyword

- High energy beam (increased penetration depth)
- Large beam (fast scan time)
- Phase contrast effect (edge enhanced imaging)



Hyeong Jin Kim KBSI



Ji-hun Kim PAL



Kyeong-Sik Jin PAL, BioSAXS Advisor



Mi-Jeong Kwak PAL



Yeon-Gil Kim PAL, BioNX Advisor



Yong Sung Park PAL

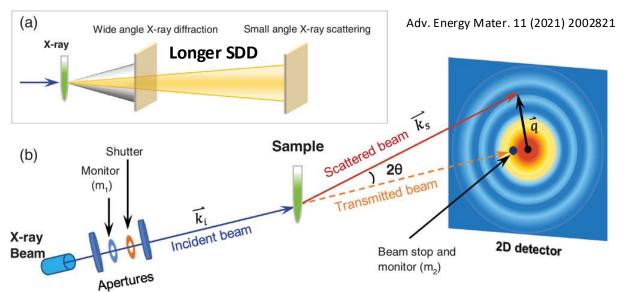


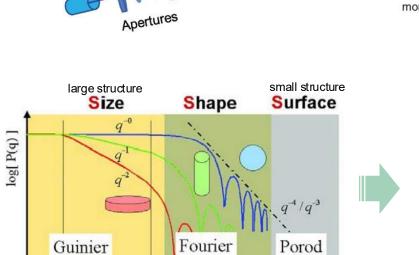
Jae-Hong Lim PAL, HEM Advisor





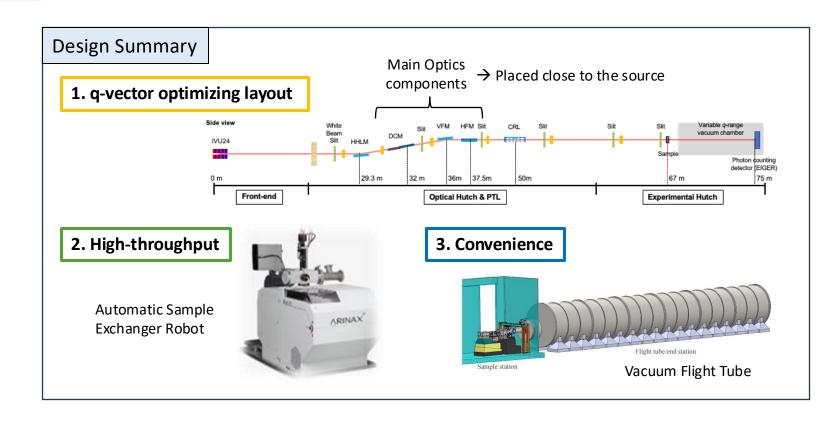
Solution Small Angle X-ray Scattering (SAXS)



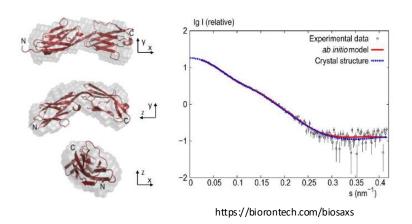


Radius of Gyration / Cross-section Structure / Surface per Volume

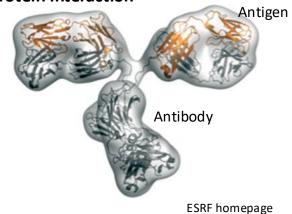
- 1. Size of particle $I(q) \sim \exp(-q^2 R_q^2/3)$
- 2. Form of particle $I(q) \sim q^{-1^{-2}}$
- 3. Surface structure $I(q) \sim q^{-4}$



Protein structure characterization in solution



Protein-Protein interaction

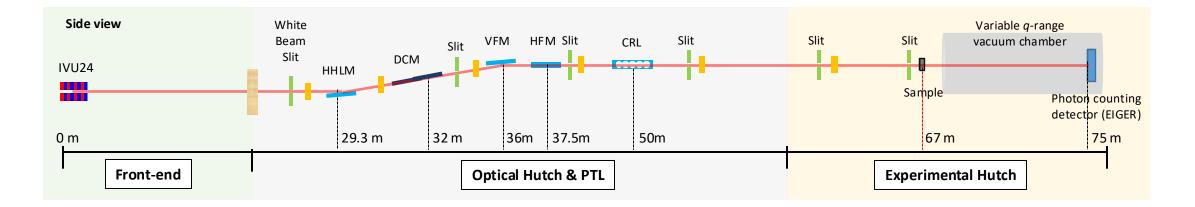








Beamline Layout



Specification

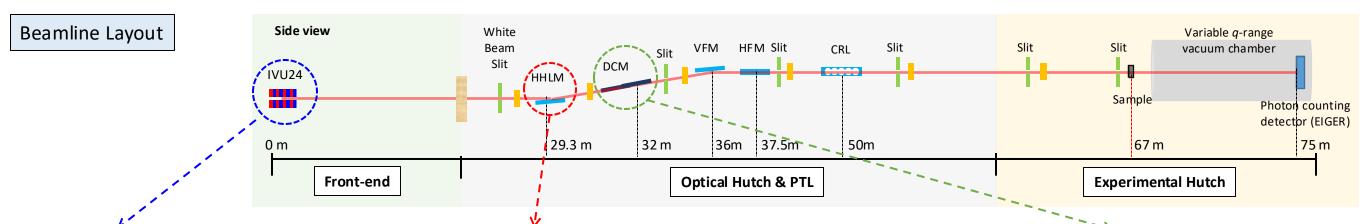
Beamline	ID21 BioPharma-BioSAXS
Light source	In Vacuum Undulator 24 (3m)
Energy range [keV] (mainly)	8 ~ 23 (12)
Energy resolution (ΔΕ/Ε)	< 2 x 10 ⁻⁴
Techniques	Solution SAXS
Beam size at sample (μm²) Η × V, FWHM	~ 200μm × 200μm ~ 40μm × 10μm
Beam flux (ph/s)	~ 5 × 10 ¹²

Beamline Features

- As an industry-prioritized beamline, an <u>automated sample exchange robot</u> will be implemented to enable high-throughput experiments.
- A large vacuum chamber including the detector allows rapid adjustment of the sample-to-detector distance from 0.5 to 8 meters, enabling efficient analysis of samples with various sizes.
- The system is designed to <u>perform experiments under a wide range of</u>
 <u>conditions</u>—such as pH, temperature, and denaturant concentration for studies
 on the 3D structure and stability of biomolecular proteins.

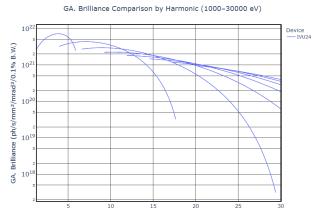




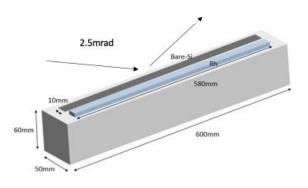


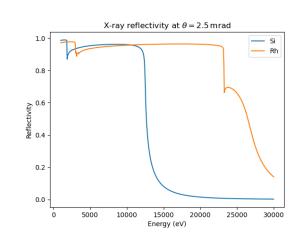
1. In-Vacuum Undulator (IVU24)

Total Length	3 m
Period Length	24 mm
Period Number	125
Deflection Constant	2.745 @ 5 mm of gap
Max. Magnetic Field	1.225T @ 5 mm of gap
Total power	17.9 kW @ 5 mm of gap
Max. power density	165.4 kW/mrad2 @ 5 mm of gap



2. High Heat Load Mirror (HHLM)





Specification	HHLM
Distance from Source	29.3 m
Incident Angle	2.5 mrad
Shape	Plane
Beam Size (H x V)	1.353 x 1.287 mm ²
Footprint (H x V, 4σ)	1.35 x 515 mm ²
Substrate Size (L x W x H)	600 x 50 x 60 mm ³
Coating material	Rh(50nm), Bare-Si
Roughness(R.M.S.)	< 0.5 nm
Slope error(R.M.S.)	< 0.2 μrad (tangential) / < 0.5 μrad (sagittal)
Absorbed Power (W)	53.7(Rh), 156.7(Bare-Si)
Max. Power Density (W/mm²)	0.185(Rh), 0.338(Bare-Si)

→ Reducing the heat of the white beam

3. Horizontal-Double Crystals Monochromator (H-DCM)

Specification	1 st Crystal	2 nd Crystal
Shape	Pla	ine
Footprint (4 σ) (maximum)	1.42 x 7.77 mm ²	1.43 x 7.79 mm ²
Size	60(L) x 25(W) x 50(H) mm ³	150(L) x 25(W) x 30(H) mm ³
Substrate	Si<111>	
Energy resolution	$< 2 \times 10^{-4}$	
Bragg angle	4.9° (23 keV) ~14.3° (8 keV)	
Slope error (R.M.S.)	< 1 µrad	
Roughness (R.M.S.)	< 0.1 ~ 0.2nm	

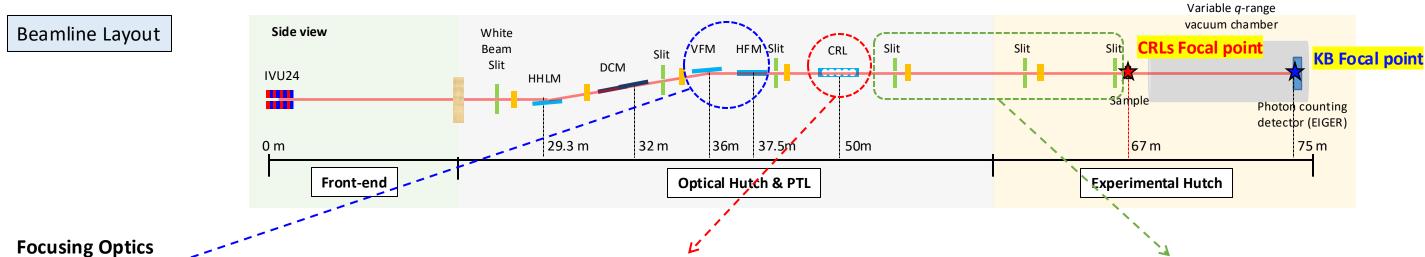
→ Monochromatize the beam with high stability





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2. BioPharma-BioSAXS

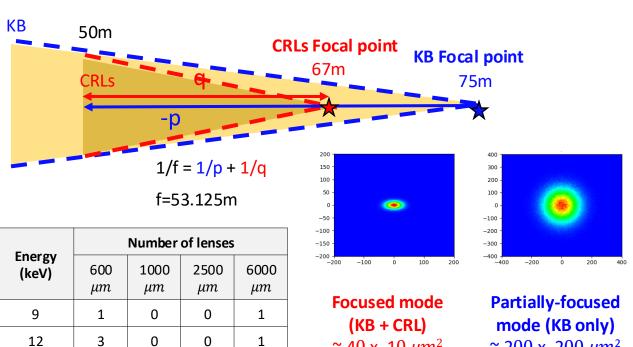


3-1. KB mirror

Specification	Vertical focusing	Horizontal focusing
Distance from source	36 m	37.5 m
Shape	Elliptical cylinder	Elliptical cylinder
Beam size @ ~12keV	1.59 x 1.66 mm ²	1.60 x 1.69 mm ²
Footprint (4σ) @ ~12keV	1.59 x 665 mm ²	1.60 x 675 mm ²
Substrate Size (L x W x H)	700 x 50 x 60 mm ³	700 x 50 x 60 mm ³
Substrate material	Si	
Coating material	Rh(50nm)	
Incident angle	2.5mrad	
p, q parameter	p: 36m q: 39m p: 37.5m q: 37.5m	
Slope error (R.M.S.)	< 0.2μrad (tangential), < 0.5μrad (sagittal)	
Roughness (R.M.S.)	0.5nm	

3-2. CRL (Compound Refractive Lens)

3



 $\sim 40 \times 10 \ \mu m^2$

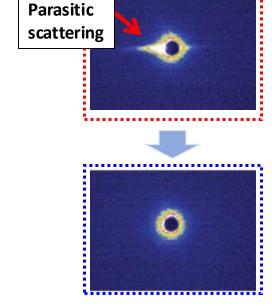
1

3

Removing the parasitic scattering

4. 3-slit system

 $\sim 200 \times 200 \ \mu m^2$



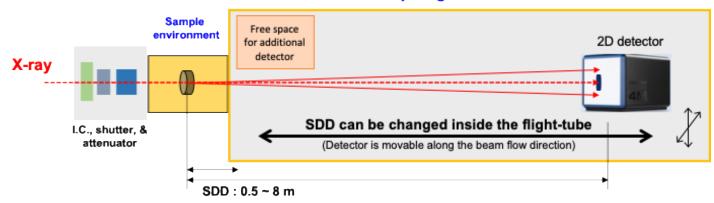




End-Station

- **♦** Rapid sample to detector distance (SDD) changing system
- Sample fixed, Detector move

Variable *q*-range vacuum chamber



- **Time reduction** for sample to detector distance (SDD) change
- By fixing the sample environment position, it is easy to install robot system and build applied experiment tools
- **Improvement of data quality** owing to minimizing air scattering (background)
 - ~ 0.33 electrons /ų (water)
 - ~ 0.43 electrons /Å3 (biomolecules)

Vacuum chamber examples







13A BioSAXS, TPS

12ID-B. SAXS, NSLS-II

♦ Automated Sample Exchanger Robot

ARINAX BioSAXS

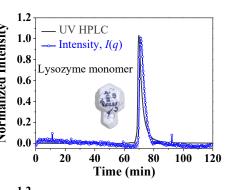


High-throughput experiments: ~ 50 samples / 1 hour

Performances		
Solution transfer volume	5 to 200 uL	
Typical cycle time - loading/unloading - cleaning (wash, rinse, dry)	50 s 15 s / 15 s 20 s	
Controls		
Sample exposure modes	Static Flow (0.05 to 20 uL/s)	
Temperature	Exposure (2 ~ 60 °C) Storage (4 ~ 40 °C)	
Dimension (W x H x D)		
Main unit (robot)	550 x 800 x 1100 mm ³	
	•	

♦ Size-Exclusion Chromatography (SEC)-SAXS





Continuous-flow in-line sample purification

In-line purification of solution

— UV HPLC BSA monomer Normalized Intensity ightharpoonup Intensity, I(q)BSA dimer

Time (min)

- Agilent infinity 1260 system, RI, UV, MALS, & DLS
- Separation and Analysis of polydisperse species or mixture

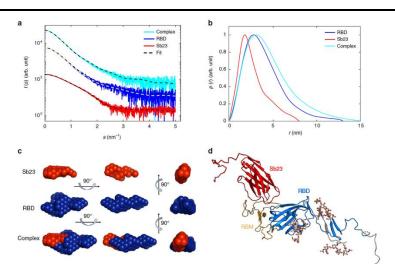




POHANG ACCELERATOR LABORATORY

Beamline Applications

Conformational Ensembles of Flexible Protein



Nature Communications 11, 5588 (2020)

Data collection parameters	Sb23	RBD	Sb23+RBD
	3023	KBU	3023+RBD
Data collection parameters Instrument	EA ADI	DIO (DETDA)	II DECV
Instrument	EMBL	P12 (PETRA I Hamburg)	II, DESY,
Beam geometry (mm ²)		0.2 × 0.12	
Wavelength (nm)		0.124	
s range (nm ⁻¹)		0.03-5.0	
Exposure time (s)		4 (20 × 0.2 s)
Temperature (K)		293	
Concentration range (mg ml-1)		0.37-4.0	
Structural parameters			
R_o (nm) (from $P(r)$)	2.2 ± 0.1	3.2 ± 0.2	3.7 ± 0.2
R _e (nm) (from Guinier plot)	2.1 ± 0.1	3.1 ± 0.2	3.5 ± 0.2
D _{max} (nm)	8.0 ± 0.5	13 ± 1	15 ± 1
Porod volume estimate, V_p (nm ³)	20 ± 2	66 ± 2	86 ± 5
Molecular weight determination	(kDa)		
From Porod volume (Vp/-1.6)	13 ± 1	37 ± 5	54 ± 3
From consensus Bayesian assessment	15±3	41 ± 1	53 ± 6
From I(0)	21 ± 2	33 ± 9	62±9
Calculated monomeric MW from sequence	15.7	32.2	47.9
Software employed			
Primary data reduction	SASFLOW	SASFLOW	SASFLOW
	PRIMUS		
	CORAL		
Computation of model intensities	CRYSOL	CRYSOL	CRYSOL
3D graphics representations	PYMOL	PYMOL	PYMOL

•••••• Industrial application

Therapeutic Antibody

mRNA-LNP

(b) go

SEC-SAXS AF4-SAXS

fit (4F5S.pdb)

Sci Rep 13 15764 (Sep 2023)

ex) Tracking structure of antibody molecules in solution

Company: Boehringer

Facility: ESRF

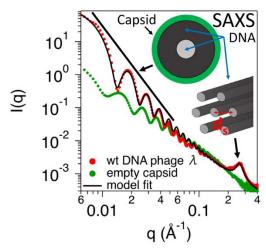
Sample: Antibody molecule in solution

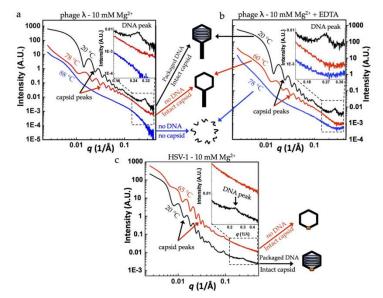
Other cases

Antibody type (Company) - Beamline, year

- 1. lgG1 mAb (Genetech) SSRL BL4-2, 2024
- 2. Pembrolizumab (Merch & Co.) Diamond B21, 2024
- 3. TrYbe (UCB Pharma) ESRF BM29, 2023
- 4. \lg G1 mAb2 (Eli Lilly x UT Austin) CHESS ID7A, 2023

DNA & Capsid structure





Journal of Virology 89, 9288-9298 (2015)

Quantitative size-resolved characterization of mRNA nanoparticles

Company: BioNTech SE Facility: PETRA III - P12

Sample: mRNA-LPX (clinical cancer vaccine candidate)

Other cases

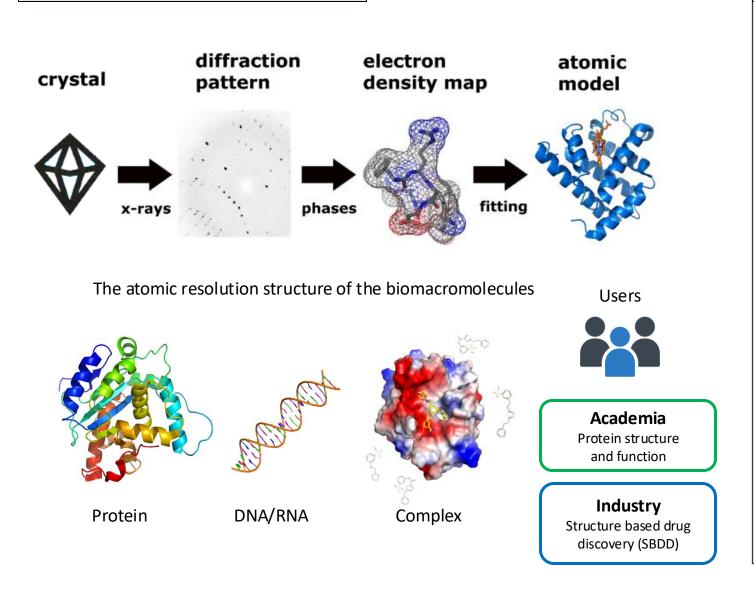
Sample type (Company) – Beamline, year

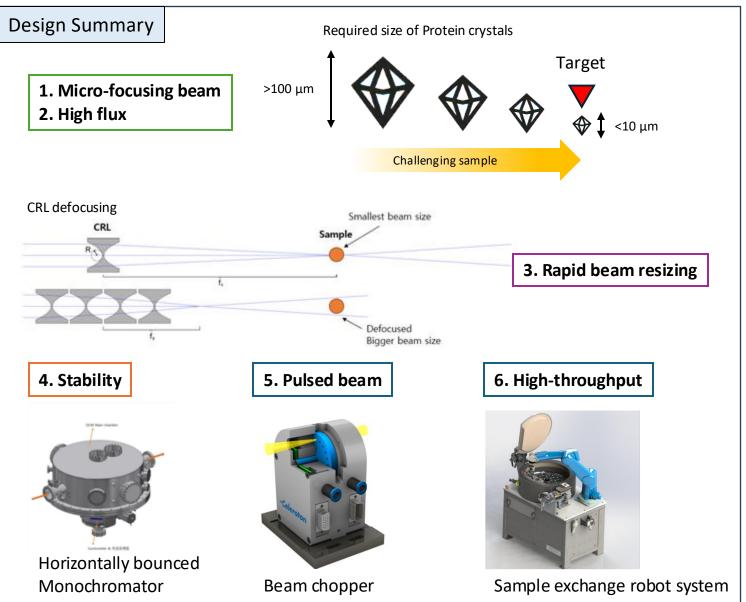
- 1. IMP-1 saRNA vaccine (CPI) Diamond B21, 2023
- 2. ASO loaded LNP library (Genetech/Roche)— ALS 12.3.1, 2023
- 3. mRNA-LNP (AstraZeneca) MAX IV SAXS, 2024
- 4. Moderna bivalent vaccine (Moderna) NSLS-II I22, 2024





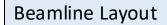
Macromolecular crystallography (MX)

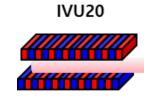


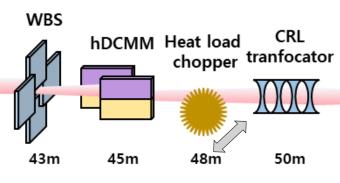


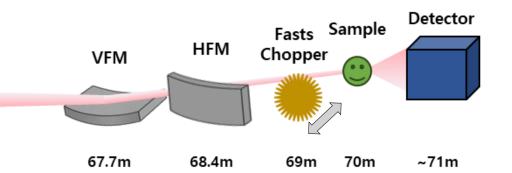












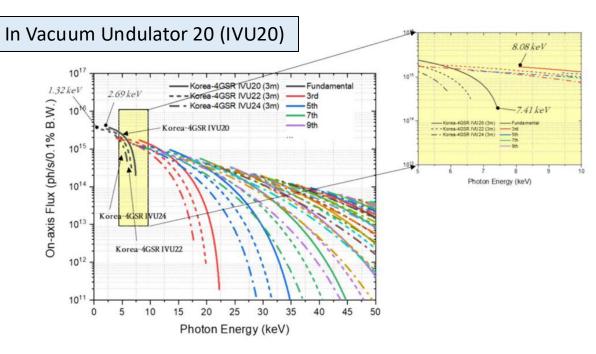
Specification

Beamline	ID22 BioNX
Light source	IVU20 (3 m)
Photon energy (keV)	8 – 25 (12.4, 20 keV mainly)
Wavelength (Å)	0.5~1.55
Energy resolution ($\Delta E/E$)	$< 2 \times 10^{-4}$ (DCM), $\sim 1\%$ (DMM)
Poam sizo at samplo (um²)	1x1 ~ 50x50 @ 12.4 keV
Beam size at sample (μm²)	1x1 ~ 5x5 @ 20 keV
Photon flux (ph/s)	>10 ¹⁴
Techniques	RSX, SSX, ISX, HTS* MX
Measurement speed	>100 Hz
Processing capacity	600 crystals/day
Auxiliary Facilities	On-site sample preparation laboratory

Beamline Science

- 1. Micro-crystallography
- 2. Room-temperature crystallography
- 3. Automated high-throughput screening for drug discovery





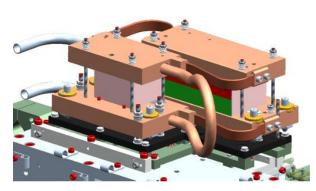
IVU Type	IVU20	IVU22	IVU24
On-Axis Flux	Best	90% of IVU20's	80% of IVU20's
On-Axis Flux	best	between 10-30 keV	between 10-25 keV
Coherent Flux	Best	80% of IVU20's	60% of IVU20's
Conerent Flux	Best	between 10-30 keV	beween 10-30 keV
Spectral Continuity	Missing/compromised between 7.41 keV and 8.08 keV	Perfect at gap size <5.58 mm	Perfect at gap size <6.46 mm
Heat Load on			
Beamline by	Best: 12 kW at the	15 kW at the minimum	17 kW at the
Off-Axis	minimum gap size	gap size	minimum gap size
Radiation			

✓ High flux

Ha, et al., JKPS (2024)

- ✓ Low heat load
- ✓ Missing 7.41 keV and 8.08 keV (limited the usage of this range) (Cobalt (7.7 keV) energy scan range)

Optics Features



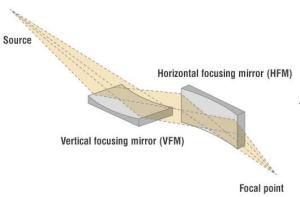
Combined horizontal DCMM Si(111) crystal Ru/B4C multilayer



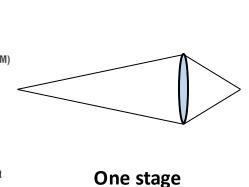
Compound refractive lens (CRL)
Diamond lens (large aperture)



Beam chopper
Titanium disk
~230 Hz, ~90 us pulsed beam



Elliptical mirrors
Si/Rh/Pt coating

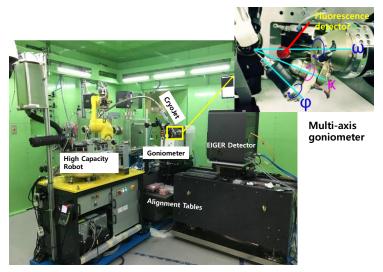


full beam
Simplifying optics
Improved manageability





End-Station



Layout

High precision diffractometer



- Sphere of confusion: 100 nm
- Raster scan at 15 mm/s
- Rotation speed: 720 deg/s
- Easy to change various goniometer heads

Silicon drift X-ray detector



- X-ray fluorescence
- Element scan



EIGER2 XE 16M

- Frame rate: 560 Hz (16 bit), 700 Hz (8 bit)
- Active area: 311 x 328 mm2
- Energy range: 6-40 keV

High-Capacity Sample Exchange Robot



592 sample/storage chamber ~600 sample/day

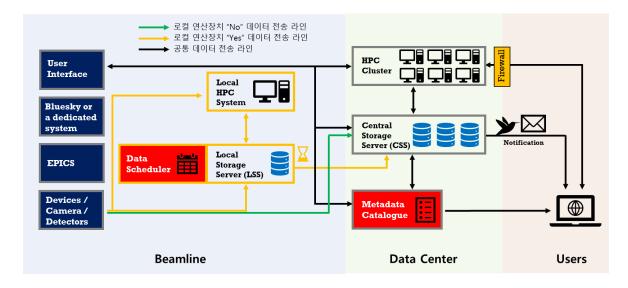
DAQ

Data acquisition/management

Web-based User Interface (WUI)
Remote access



Computing infrastructure



Local HPC system and data center

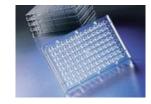




Sample Preparation Laboratory



~ fragment library on ECHO plates (ex. 1200 fragments)



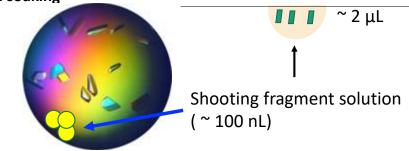
~ Crystallization Plates (ex. 1200 crystal drops)



Precise, Accurate, and Efficient Liquid transfer using sound

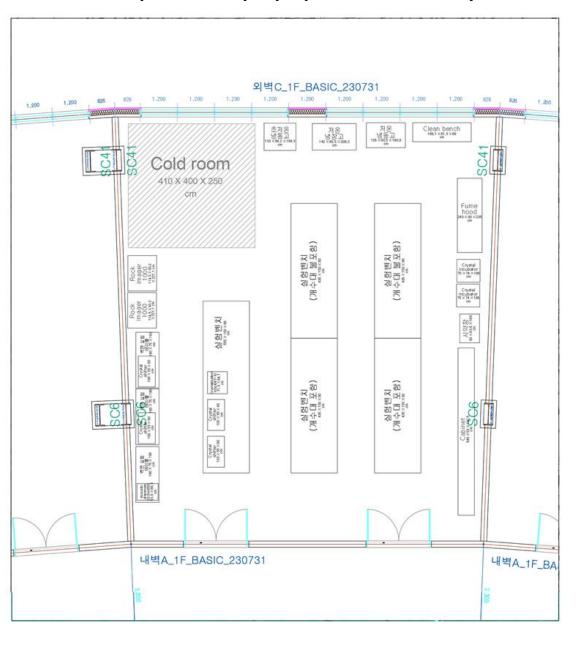


- ~ Soaking fragments into crystal drops on plate
- ~ 30 sec for 100 fragments (one crystallization plate)
- ~ singleton soaking



- Facility for sample preparation of FBDD experiment
- Support drug discovery experiment

Floor plan of sample preparation laboratory





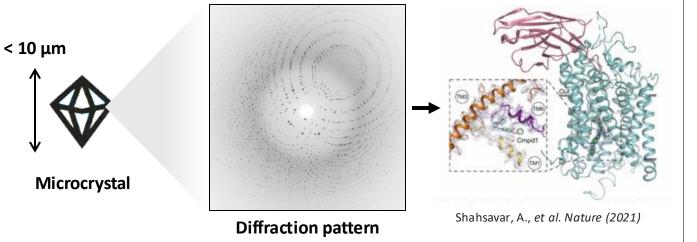


Zhao, Y., et al. ACS Chem. Neuro. (2022)

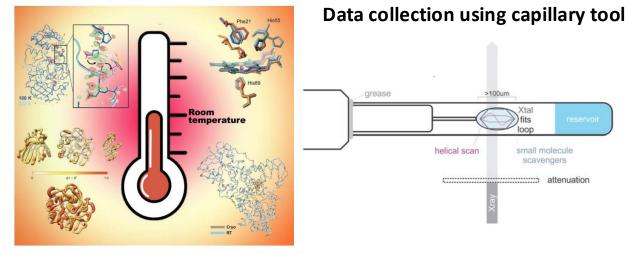
3. Bio Nano crystallography

Beamline Applications

High-quality data from microcrystal



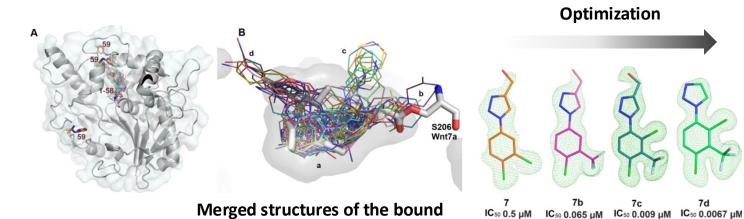
Room-temperature crystallography



Steiner, R. A., Struct. Biol. (2023)

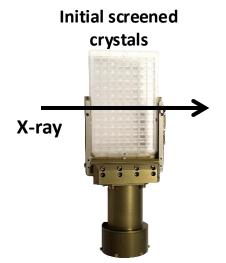
Fischer, M., Quarterly Reviews of Biophysics (2021)

Drug discovery



fragments in the enzymatic pocket

Quick screening of initial crystal



Diffraction or not?



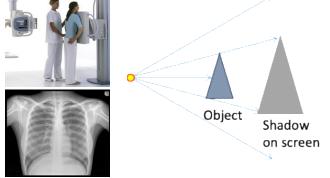


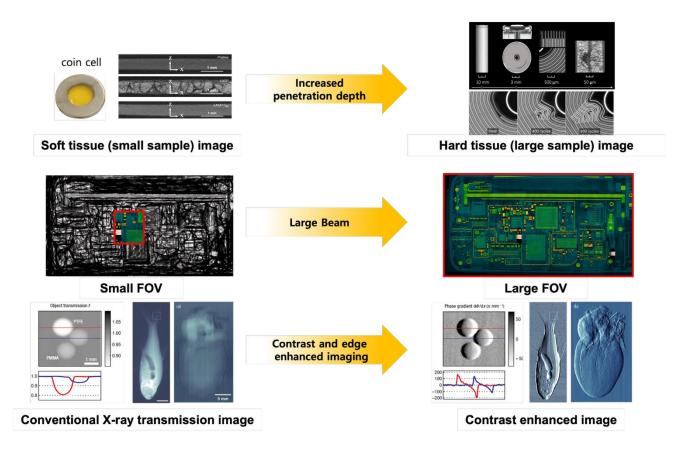
Projection imaging

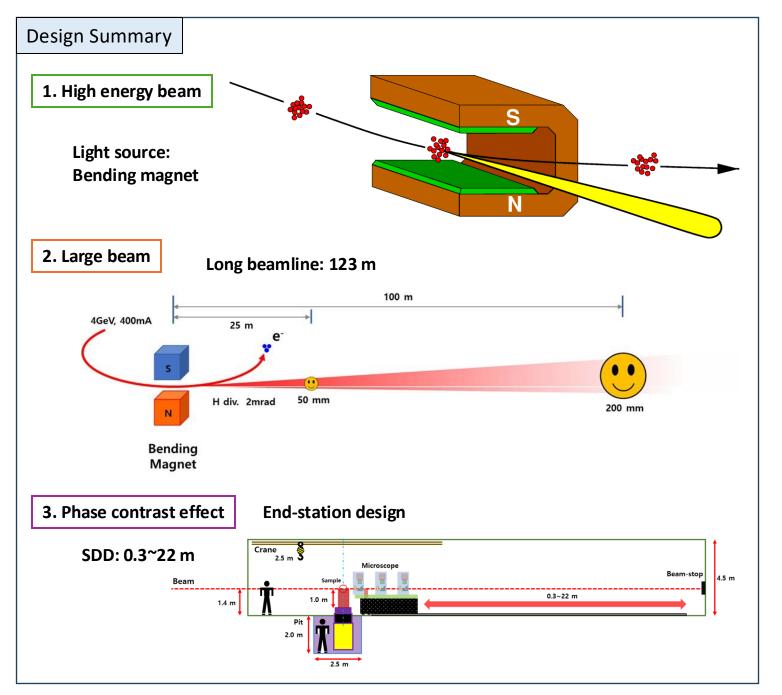
Synchrotron source

- High photon flux
 - > Fast scan
 - ➤ In situ/operando imaging studies
- High resolution imaging

Conventional projection image

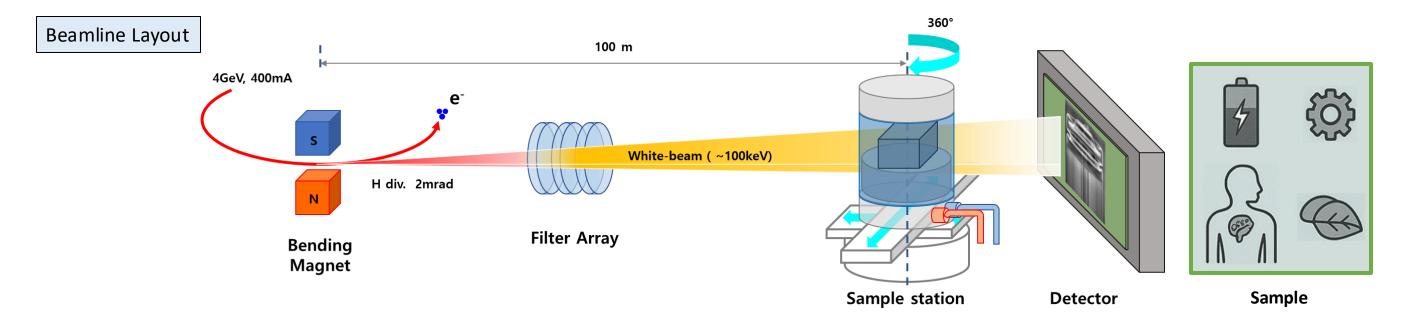












Specification

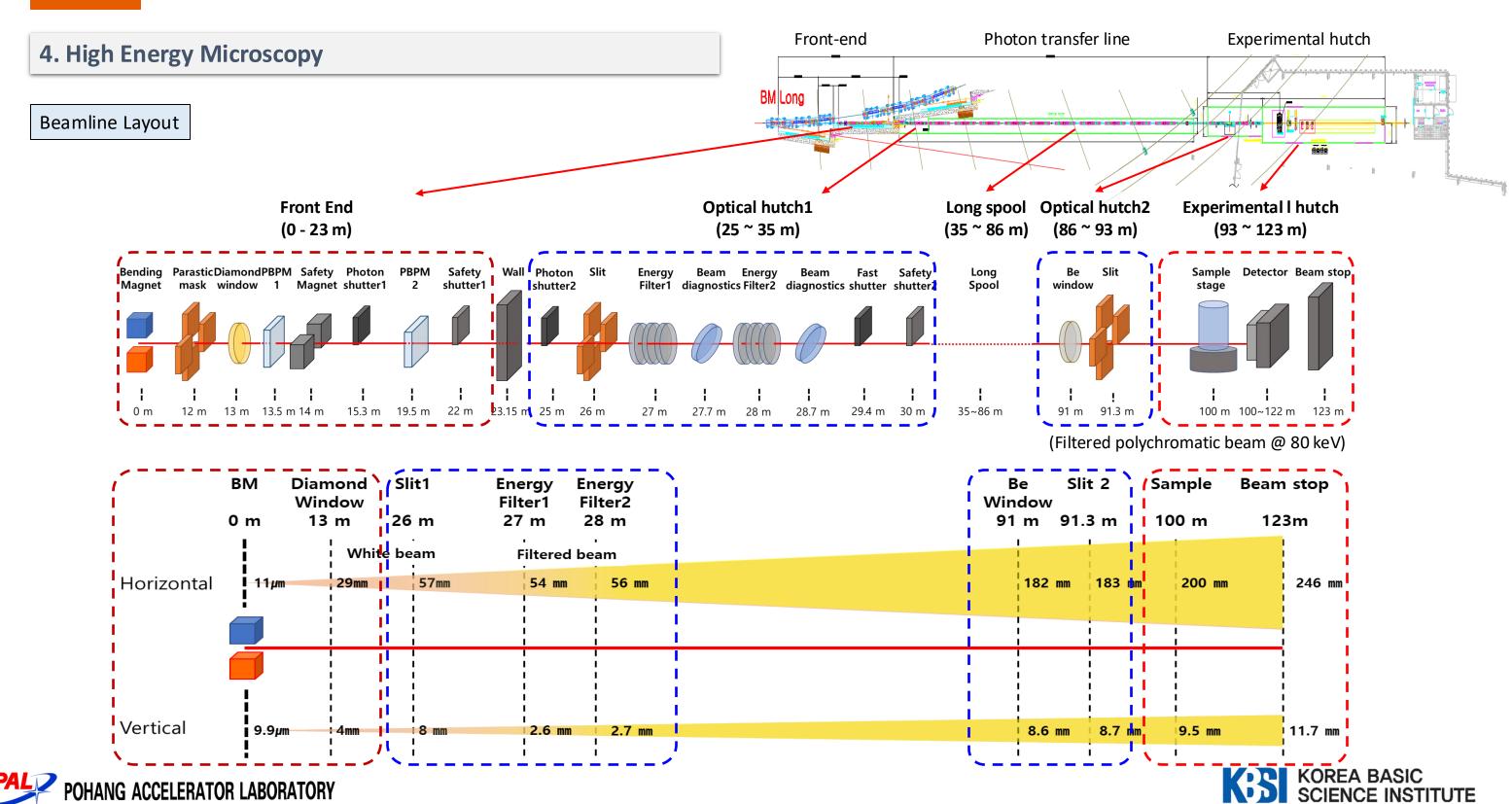
Beamline	BM10 HEM
Light Source	Bending Magnet (2T)
Photon energy	$20 \sim 150 \text{ keV} (E_c: 21.2 \text{ keV})$
Beam size (FWHM)	200 mm x 25 mm @ 100 m
SDD	< 22 m
Spatial resolution	> 1.0 um
Technique	Projection imaging
Sample space	a few m (100 kg)

Beamline Science

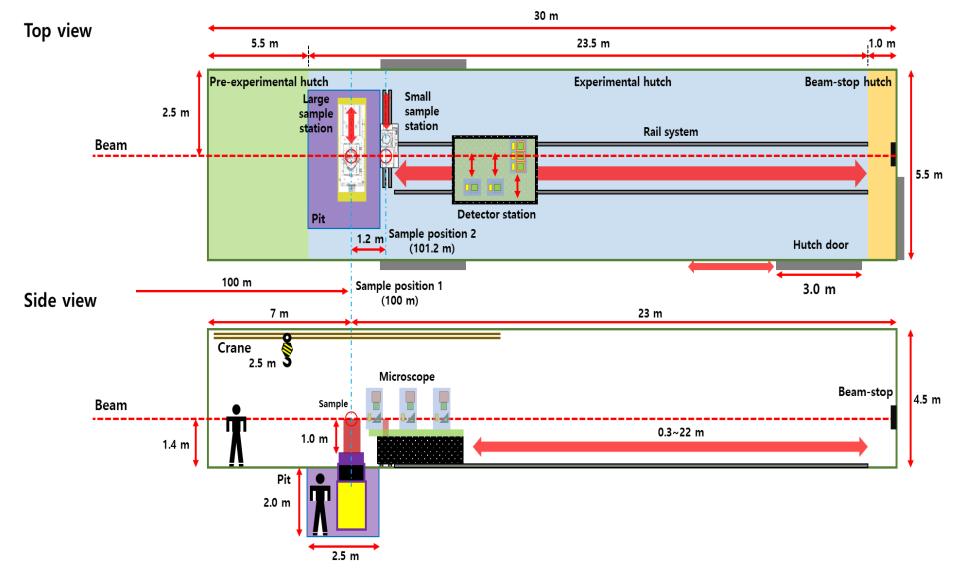
- High-energy beam: Energy & Materials applications
- Large beam size: Industrial applications
- Phase contrast effect: Bio-medical imaging



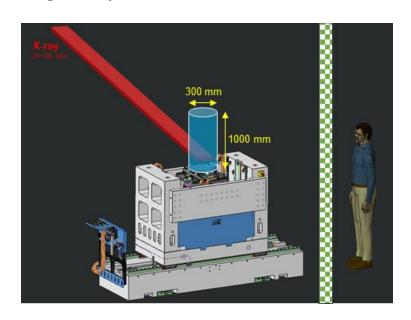




End-Station



> Large sample station



> Large sample stage Specifications

Data	Dimension
Sample weight	100 kg
Sample dimensions	H=1.0 m, D=0.3 m
Rotation stage	RT500S
Angular accuracy	< 2 arcsec
Max. speed	60 rpm

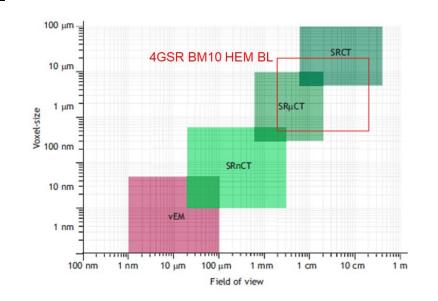




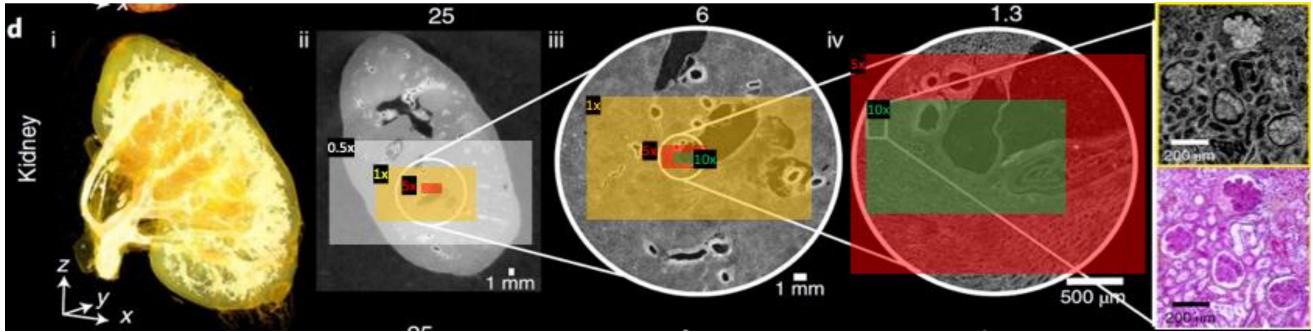
Multiple scale CT scan (Establishing a strategy of the collection of Large FOV image and Mass Data Process)

> Image specification (4GSR BM10)

X-ray microscope	Array detector	Single detector			
Field of View	200 x 25 mm ²	38 x 21 mm ²	19 x 11 mm ²	3.8 x 2.1 mm ²	1.9 x 1.1 mm ²
Magnification	0.25x	0.5x	1x	5x	10x
Pixel size	20 μm	9.2 μm	4.6 μm	0.92 μm	0.46 μm
Pixel number	26 million	9 million			
Data size	52 MB/image	18 MB/image			
3D data size	205 GB/set	25 GB/set			



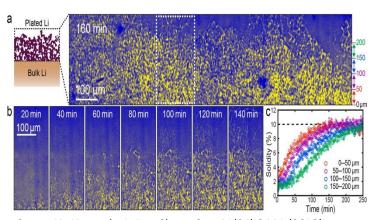
Multiscale Phase-Contrast Tomography (ESRF-EBS BM18)





Beamline Applications

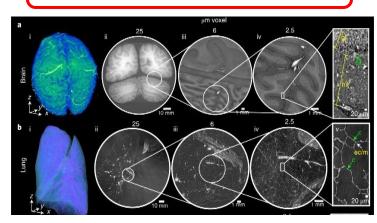
Energy Material Science



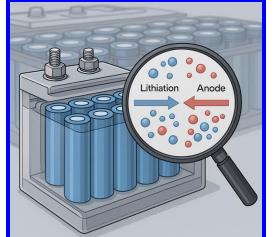
Seung-Ho Yu et al., J. Am. Chem. Soc. 41(21) 8441 (2019)

real-time observation of internal structural changes of batteries

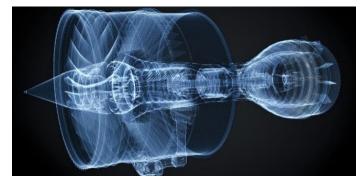
Life Science



Walsh, C. L., et al. (2021). Nature methods, 18(12), 1532-1541.



Multi scale imaging In-situ X-ray imaging Phase contrast imaging



Industrial Applications

Dragoljub Vujić, Scientific-Technical Review, vol. LIII, no.2, (2003)

non-destructive inspection of internal defects in metal and composite components.

Cultural Heritage



불상 속 비밀, 관음보살좌상 (2019) 국립중앙 박물관





Summary

	BioSAXS	BioNX	HEM
Photon Source	IVU24	IVU20	BM (2T)
Energy Range (mainly)	8 ~ 23 keV (12)	8 ~ 25 keV (12.4 and 20)	20 ~ 150 keV
Beam Flux (ph/s)	> 5 × 10 ¹²	> 1 × 10 ¹⁴	~ 1 × 10 ¹³
Beam Size (μm²) (H × V, FWHM)	 Partially Focused: ~ 200 × 200 Focused: ~ 40 × 10 	 12.4 keV: 1 × 1 ~ 50 × 50 20 keV: 1 × 1 ~ 5 × 5 	• 100m: 200 × 25 mm ²
Spatial Resolution	8 ~ 3800 Å	0.5 ~ 1.55 Å	> 1.0 µm
Sample	Biomacromolecules in solution	Biomacromolecules as crystallized	Biological tissue, Battery, Electronic c omponents, Developmental prototyp e, etc.
Technique	SAXS/WAXS, SEC-SAXS	RSX, SSX, ISX, HTS* MX	Projection Imaging

Thank You For Your Attention



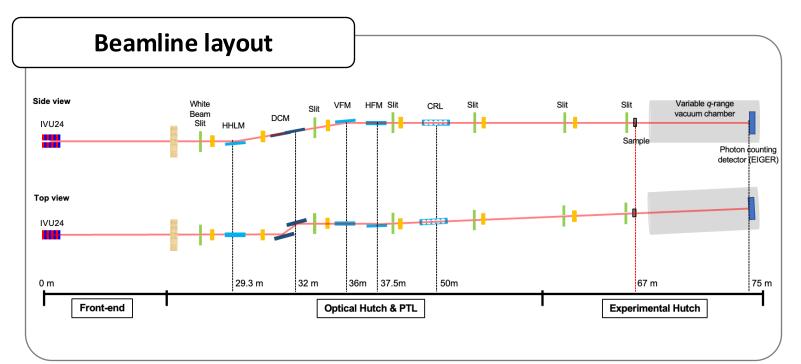
ID21 BioPharma - Bio Small-Angle X-ray Scattering (SAXS)

Study on 3D structural characteristics of biological molecule systems

Principle and application Energy Storage Mater. 21 (2019) 162-173 Adv. Energy Mater. 11 (2021) 2002821 Nature Communications 11 (2020), 5588 0.05 0.10 0.15 0.20 0.25 0.30 0.35 0.40 Study on Stabilization of Drug Delivery Systems Analysis of protein and protein complex structures

Feature of Beamline

- Introduction of an automatic sample exchange robot to facilitate high-throughput experiments.
- Variable q-range vacuum chamber allows rapid adjustment of sample-to-detector distance within a range of 0.5 m to 8 m, enabling efficient analysis of various systems.
- Supporting various sample environments for the structural analysis of biological molecules.

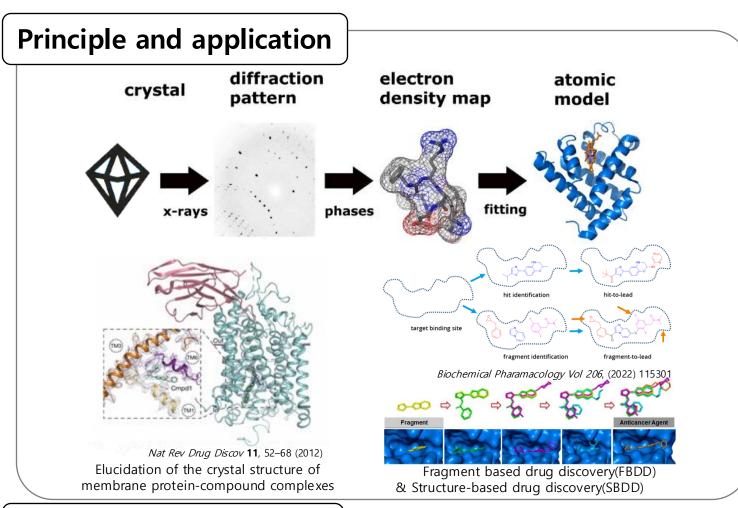


Specification

Photon Source	In Vacuum Undulator 24
Energy Range	8 ~ 23 keV
Energy resolution	< 2 x 10 ⁻⁴
Beam flux (ph/s)	~ 5 × 10 ¹²
Beam size (µm²) H × V, FWHM	< 200 x 200 (partially focused mode) ~ 40 × 10 (focused mode)
Spatial resolution	8 ~ 3800 Å

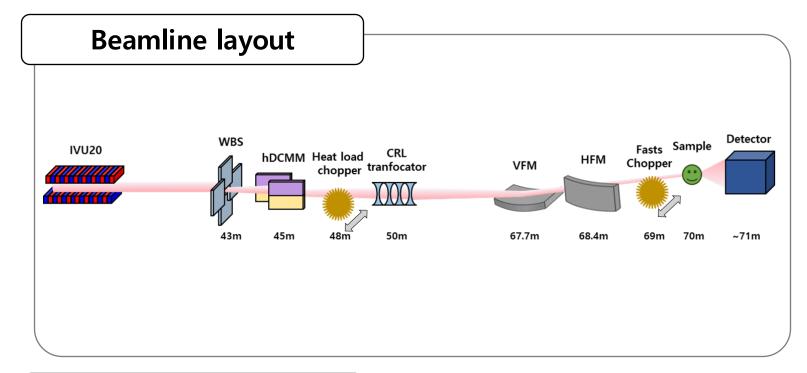
ID22 Bio Nano crystallography (BioNX)

High-throughput screening for rapid determination of protein-compound structures in drug discovery



Feature of Beamline

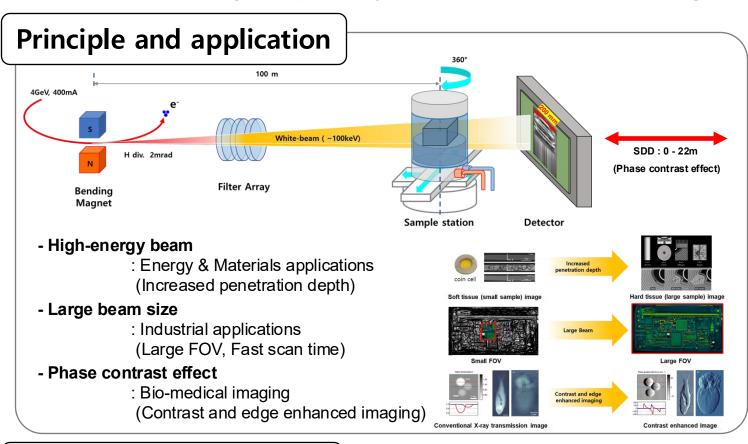
- Provide a minimum beam size of 1 micron and high beam flux, supporting serial and in-situ crystallography experiments. This enhances accessibility to challenging protein crystal samples.
- Supports high-throughput experiments with the introduction of the an automated sample exchange robot.
- Operates a sample preparation laboratory to support drug discovery research, such as fragment library screening



Specification

Photon Source	In Vacuum Undulator 20	
Energy Range (mainly)	8 ~ 25 keV (12.4 and 20)	
Energy resolution	< 2 x 10 ⁻⁴ (DCM), ~1% (DMM)	
Beam flux (ph/s)	> 1 × 10 ¹⁴	
Beam size (µm²)	1x1 ~ 50x50 @ 12.4 keV	
H × V, FWHM	1x1 ~ 5x5 @ 20 keV	
Spatial resolution	0.5 ~ 1.55 Å	
Measurement frequency	> 100Hz	

High-quality multiscale 3D image analysis using phase contrast effects



Beamline layout Bending Magnet(0m) Shielding wall(24m) Shielding

Feature of Beamline

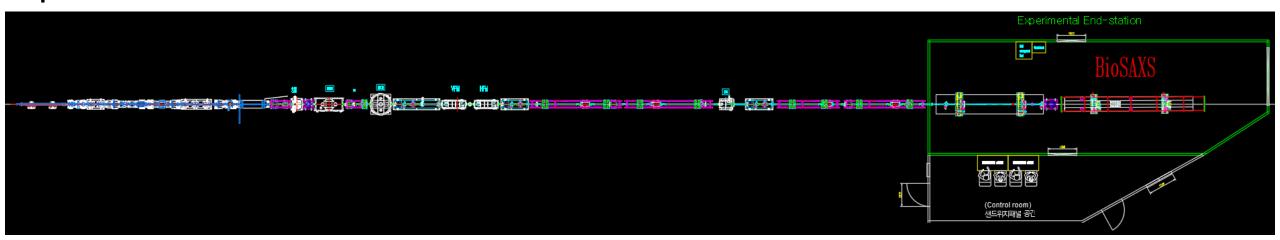
- The high energy microscope beamline is based on projection image utilizing high-energy X-ray beams above 100keV and a long beamline of over 100 meters.
- The bending magnet illuminates the sample at a distance of 100 m with a beam of 200 mm width and 25 mm height, and acquires a high quality projection image by phase contrast effect.
- Phase contrast imaging improves spatial resolution and contrast, allowing researchers to resolve finer structural details in three dimensional imaging and expand the range of observations in materials and biological specimens.

Specification

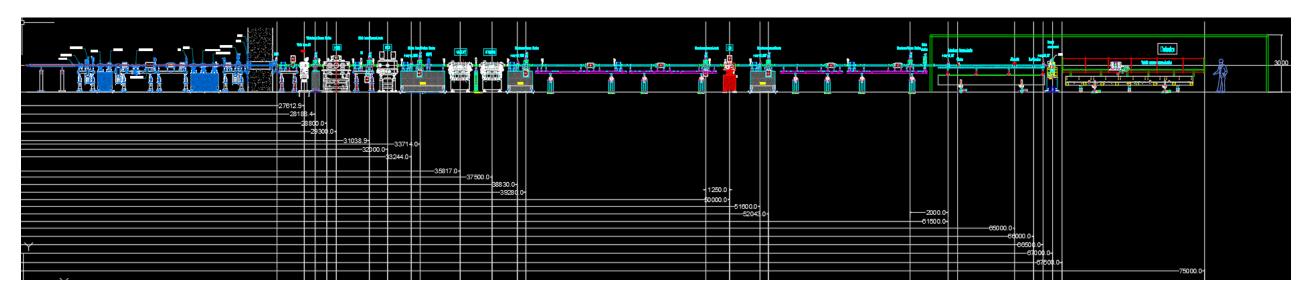
Photon Source	Bending Magnet (2 T)	
Energy Range	20 ~ 150keV (E _c :21.2 keV)	
Beam size (FWHM)	200mm x 25mm @ 100 m	
Spatial resolution	> 1.0 um	
Technique	Projection imaging	
Sample dimensions	H=1.0 m, D=0.3 m (100 kg)	

Design Drawing: BioSAXS

Top view



Side view

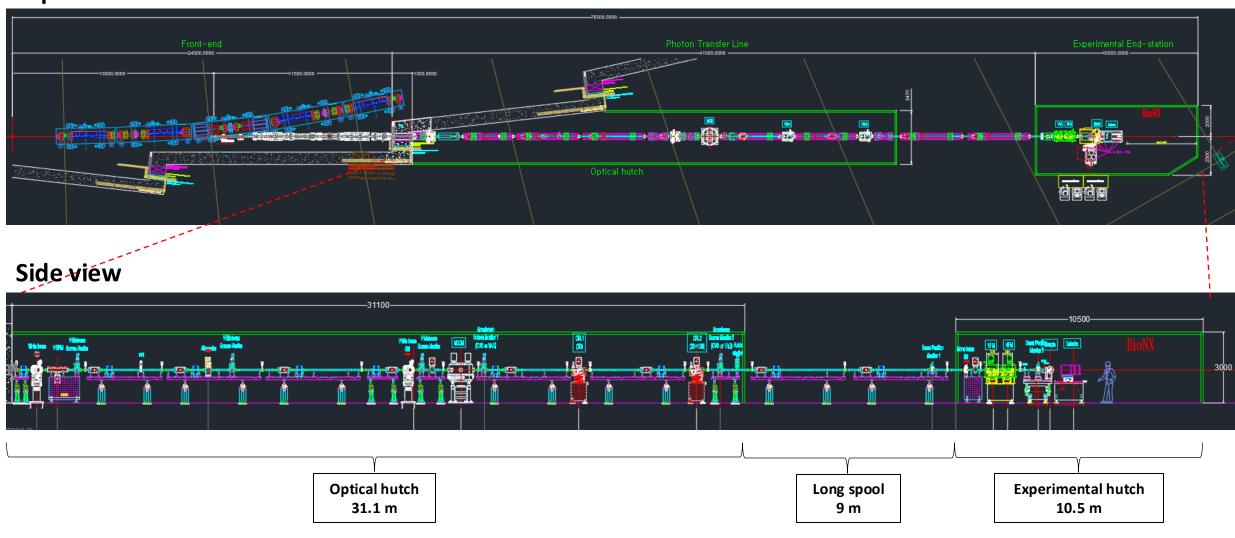






Design Drawing: BioNX

Top view







Design Drawing: HEM

