Status of the Structural Biology at the Photon Factory

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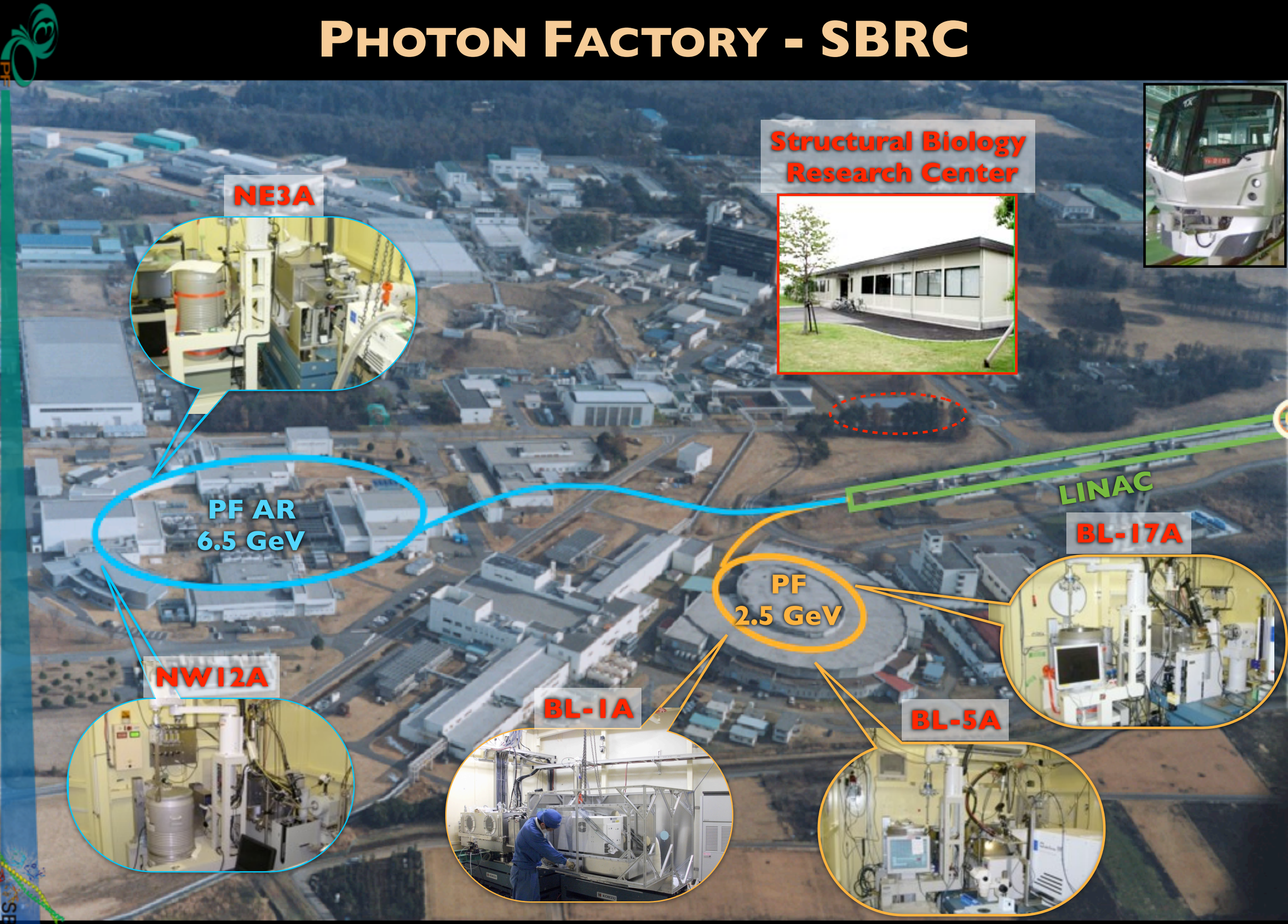
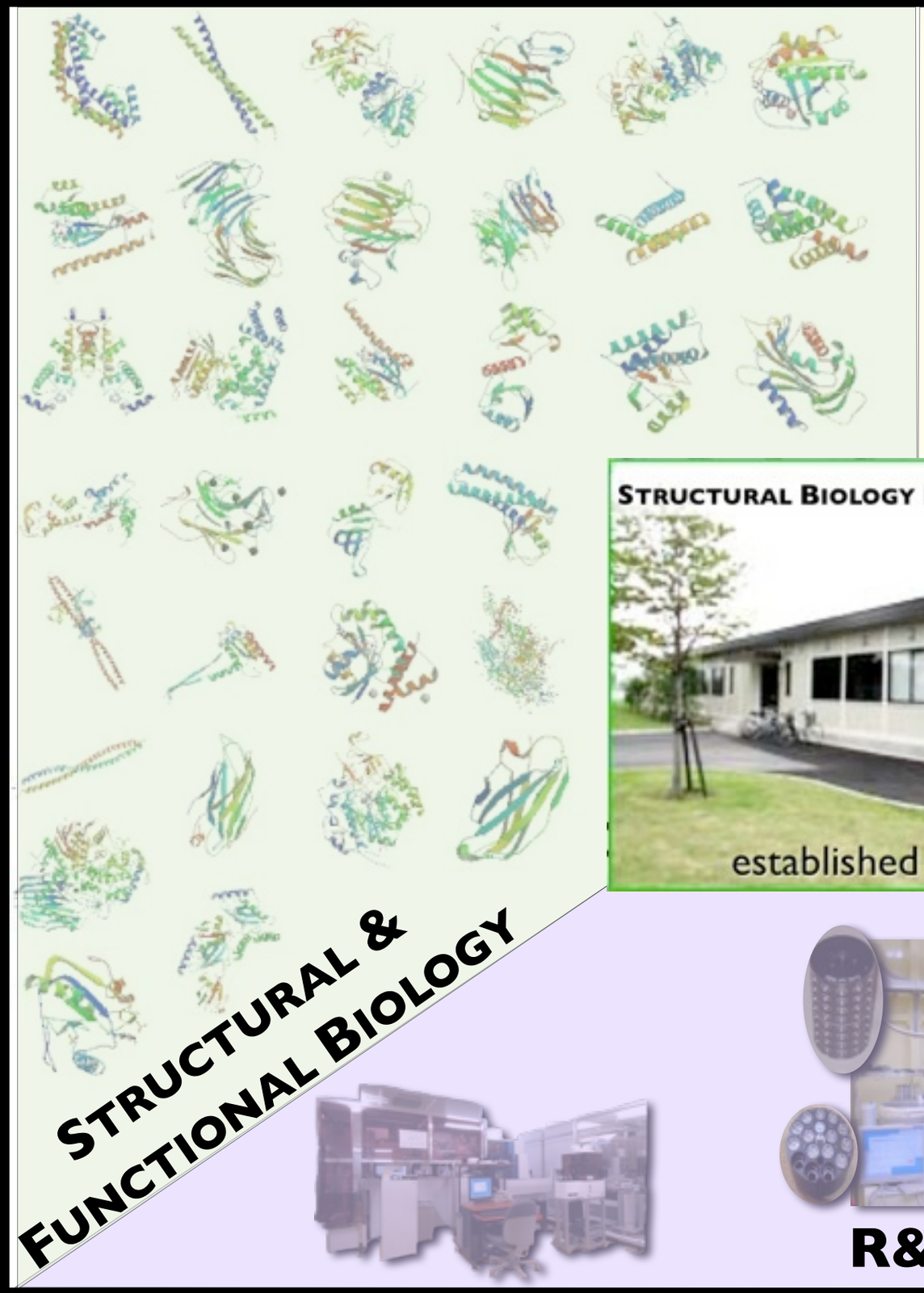
Outline

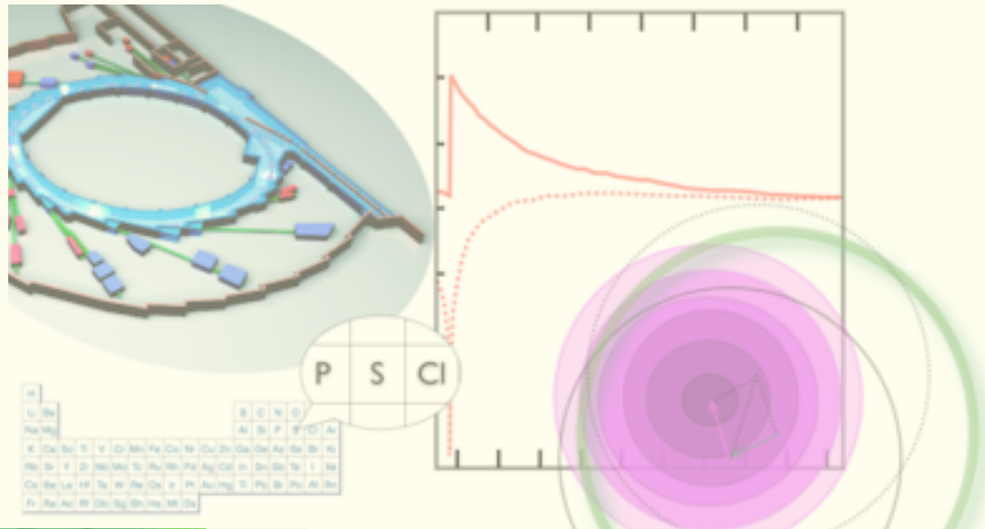
- Where are we?
- What are we doing?
  - ~ introduction on structural biology
  - ~ R&D : application to structural biology
  - ~ synchrotron science
    - ... AR-NE3A: high-throughput beamline
    - ... BL-1A: low energy beamline
- Structural biology applied to influenza virus and human neuraminidases: involvement R&D and synchrotron science
- New applications
  - ~ R & D : UV-based centering and phasing
  - ~ synchrotron science : high-pressure studies
- Conclusions
- Acknowledgments

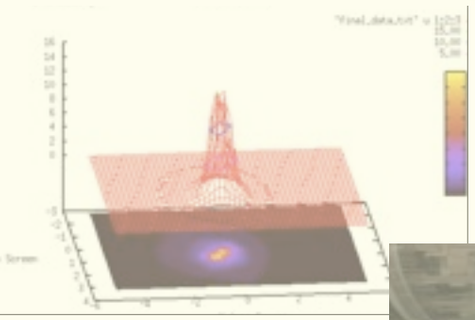
Photon Factory - SBRC



Research and Support Activities

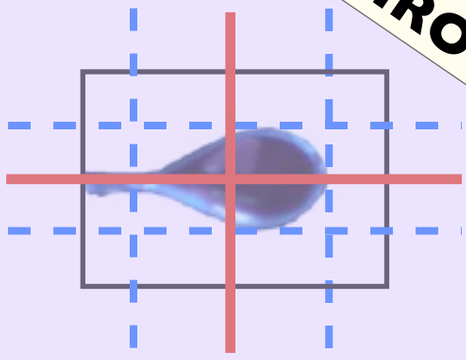






Structural & Functional Biology

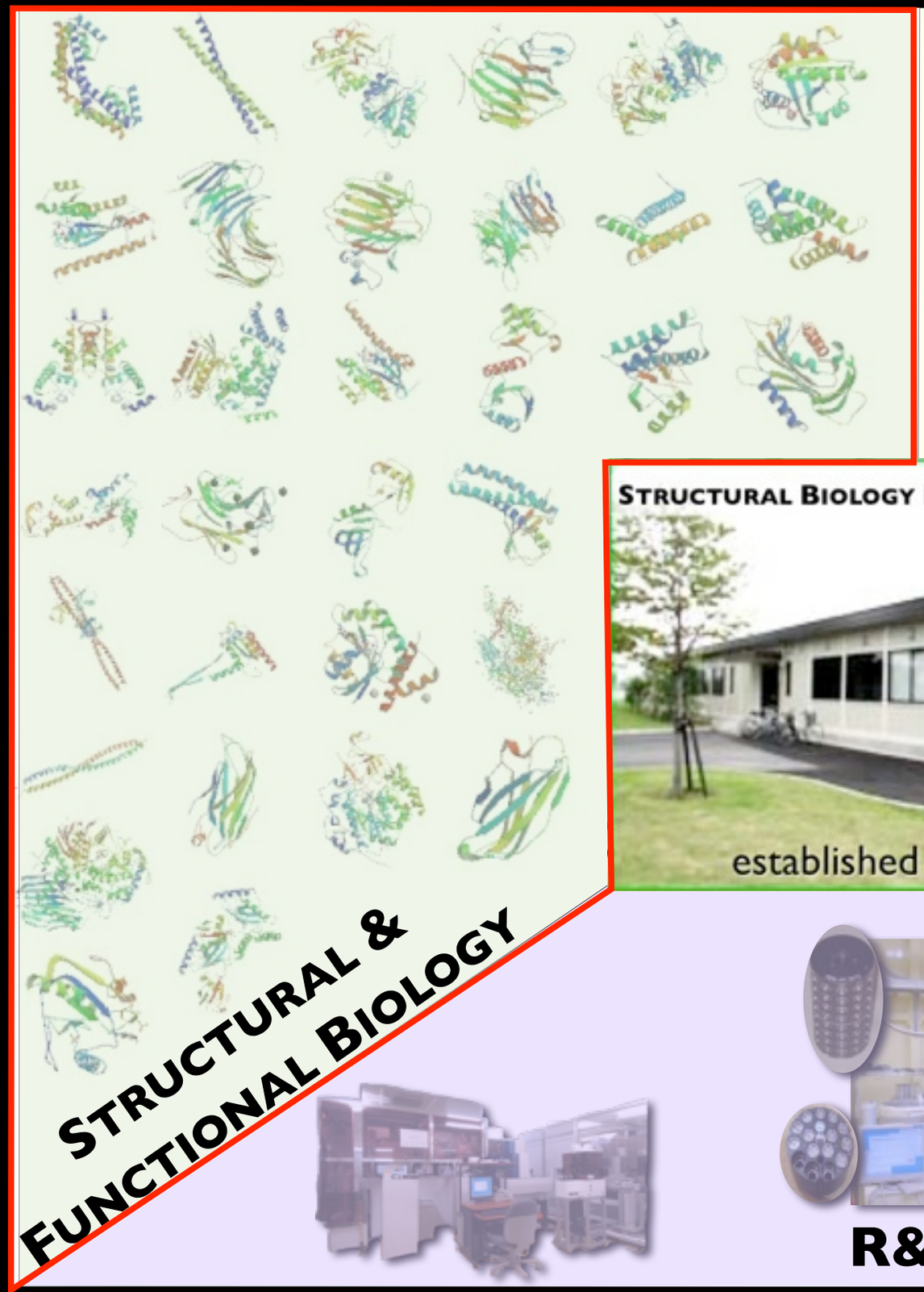
R&D



Synchrotron Science

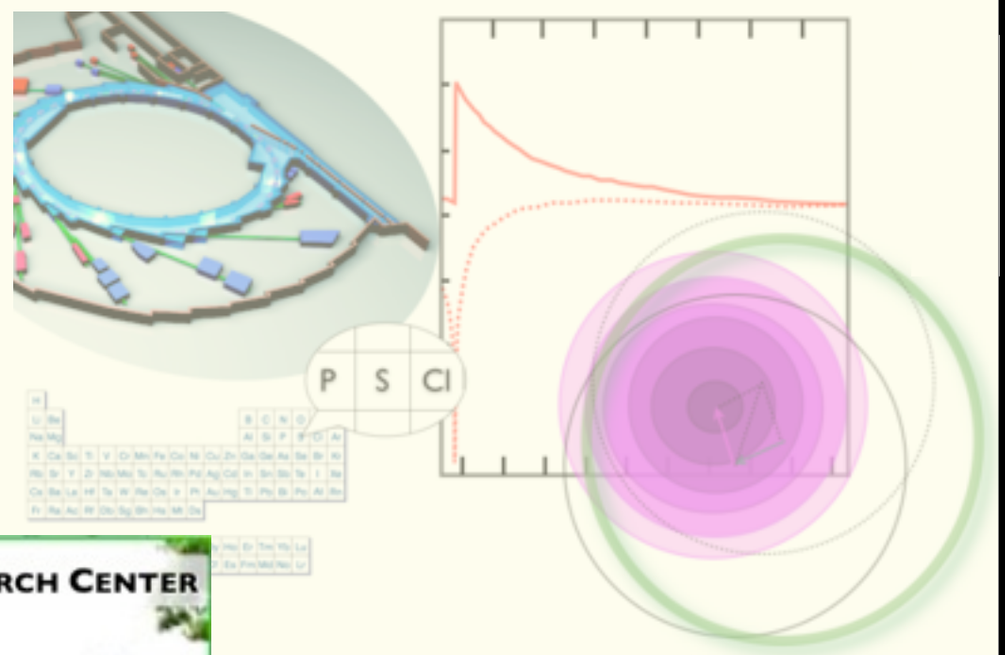
- What are we doing?

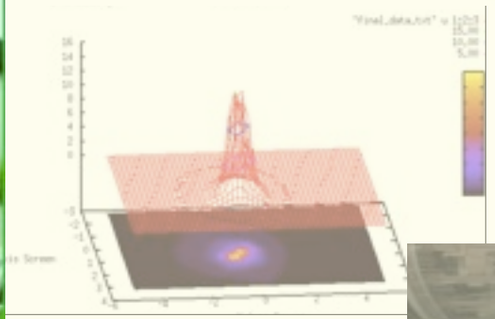
Research and Support Activities

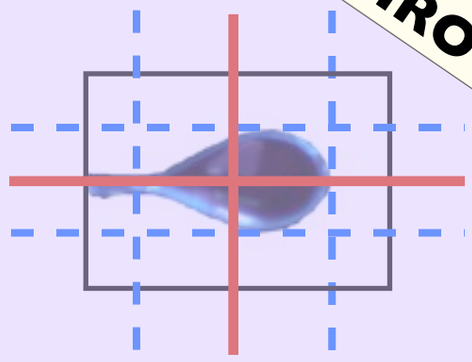


Structural & Functional Biology

R&D



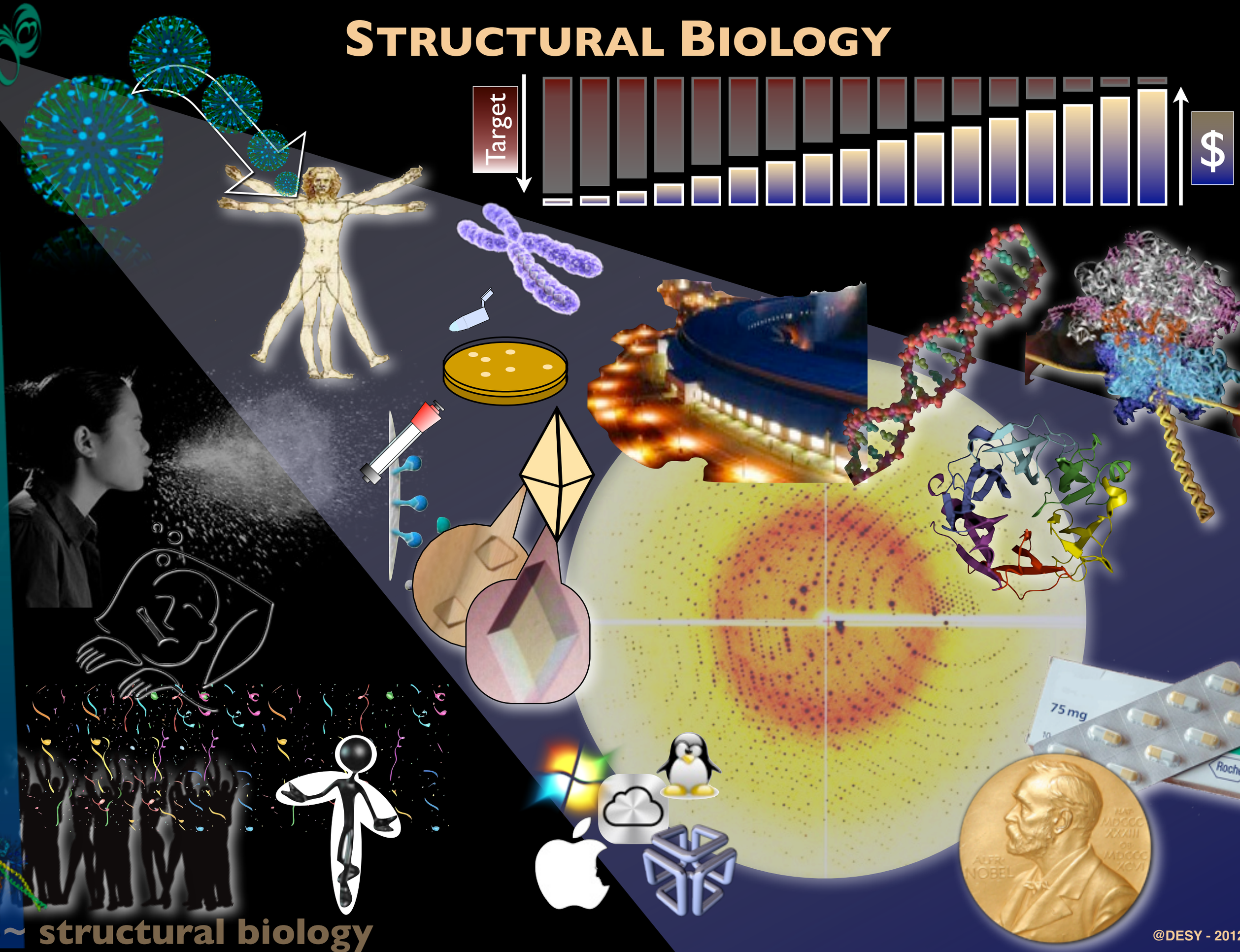




Synchrotron Science

- What are we doing?

Structural Biology



~ structural biology

Structural Biology

Identify a target model

~ structural biology

Biological Model: Influenza

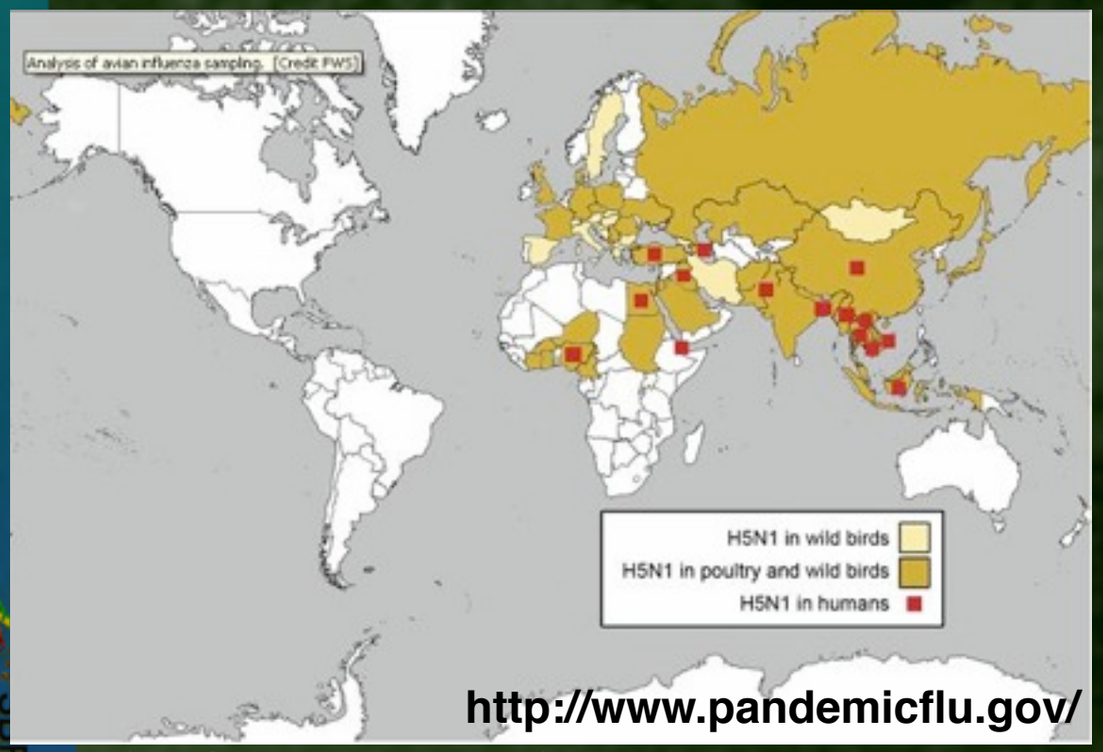
Oxford American Dictionaries:

"...a highly contagious viral infection of the respiratory passages causing fever, severe aching, and catarrh, and often occurring in epidemics..."

abnormal blue comb

swelling of the wattles

congestion of hocks and shanks



http://www.pandemicflu.gov/

"Avian (or bird) flu is caused by influenza viruses that occur naturally among wild birds. The H5N1 variant is deadly to domestic fowl and can be transmitted from birds to humans. There is no human immunity and no vaccine is available."

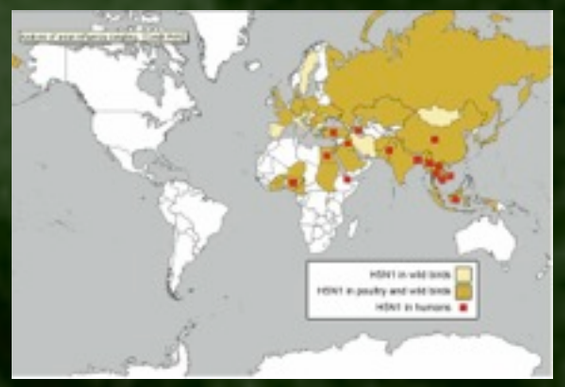
As of 2010:
- 508 human cases
- 302 deaths

... influenza virus

Biological Model: Influenza

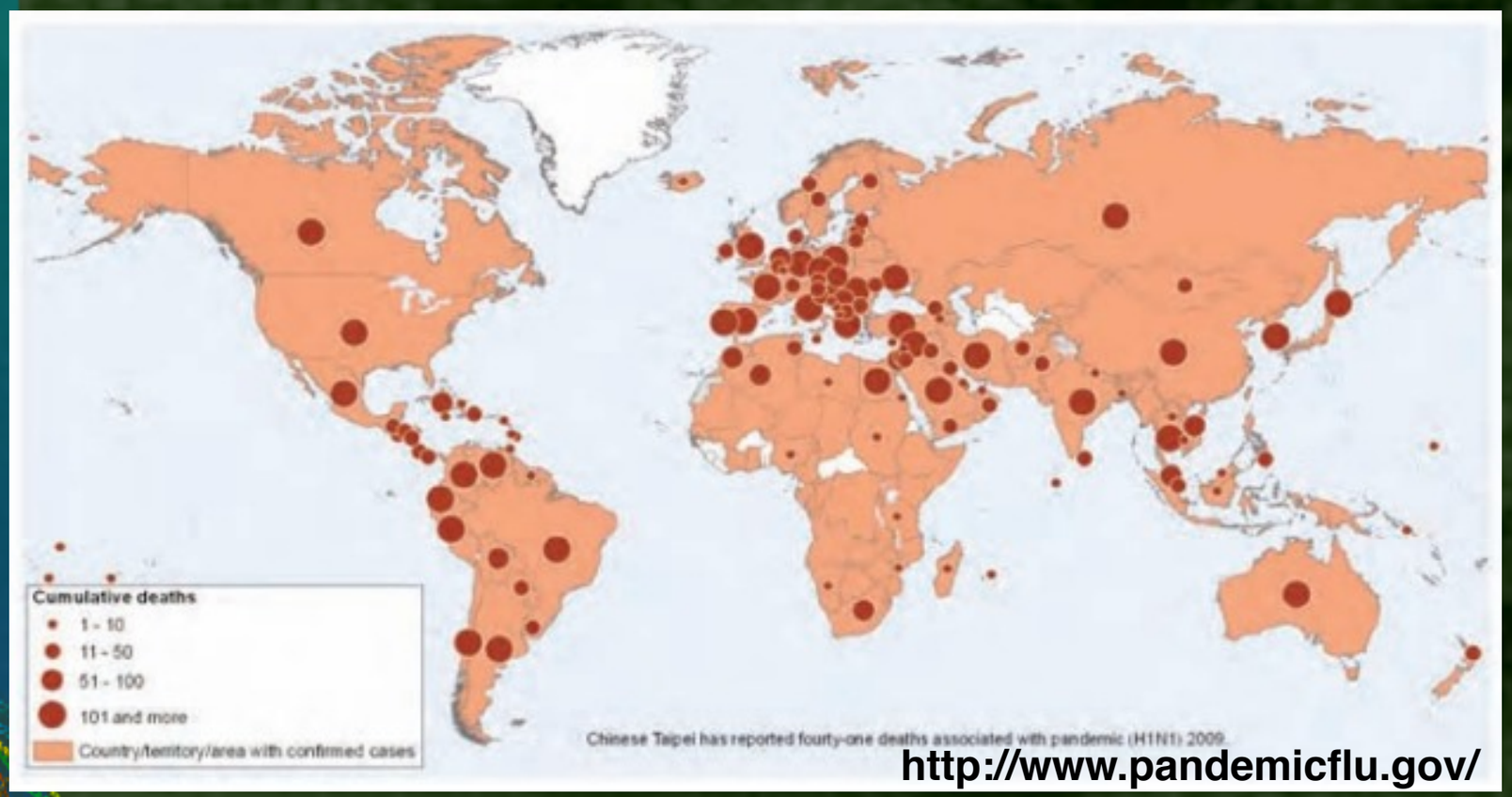
H5N1

As of 2010:
- 508 human cases
- 302 deaths



H1N1

As of 2010:
- ~ 61 million human cases
- ~ 12 470 deaths



http://www.pandemicflu.gov/

"H1N1 is a new influenza A virus that has never before circulated among humans. This virus is not related to previous or current human seasonal influenza viruses."

... influenza virus

Structural Biology

Target

$

Identify a target model

Detailed analysis

~ structural biology

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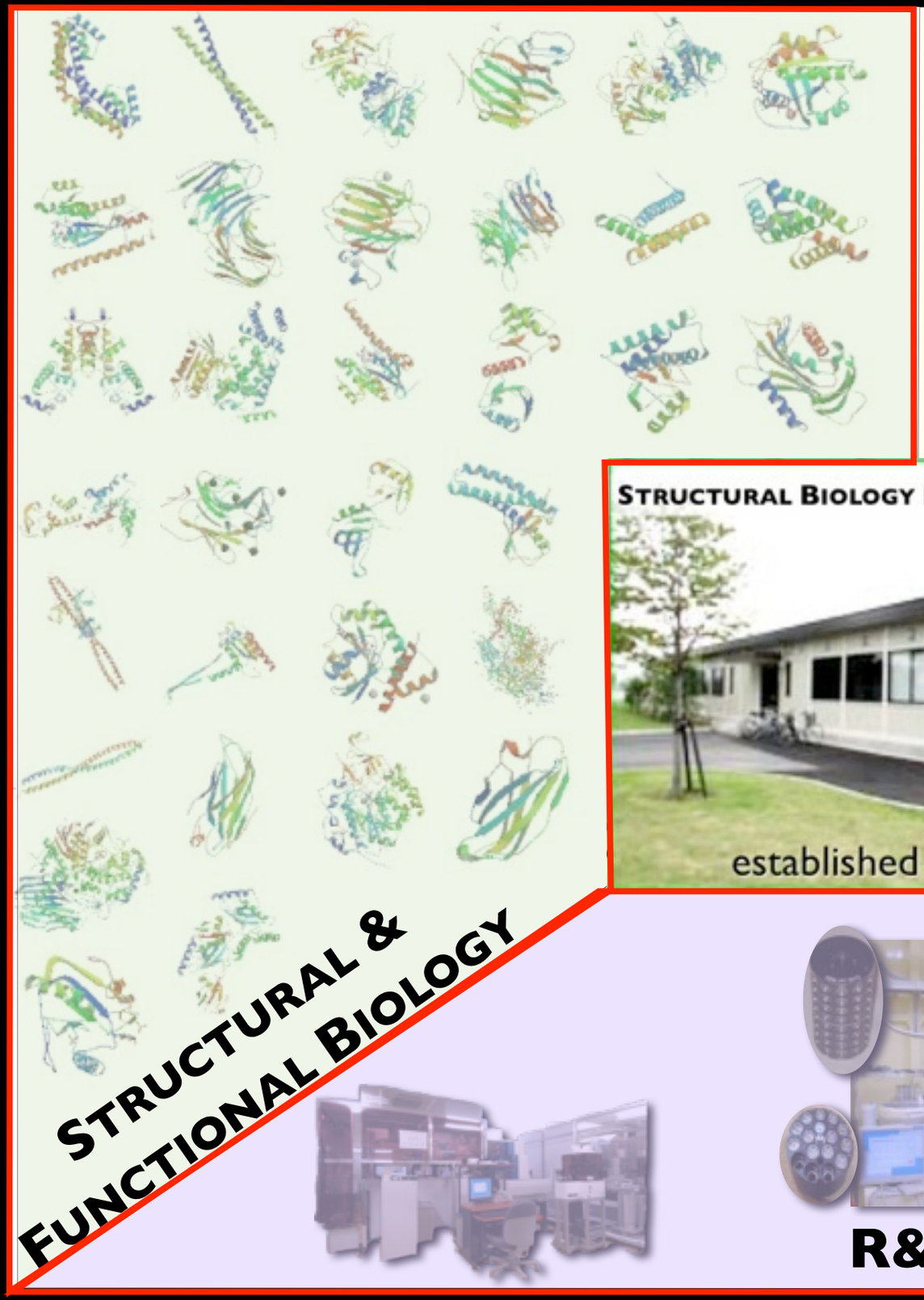
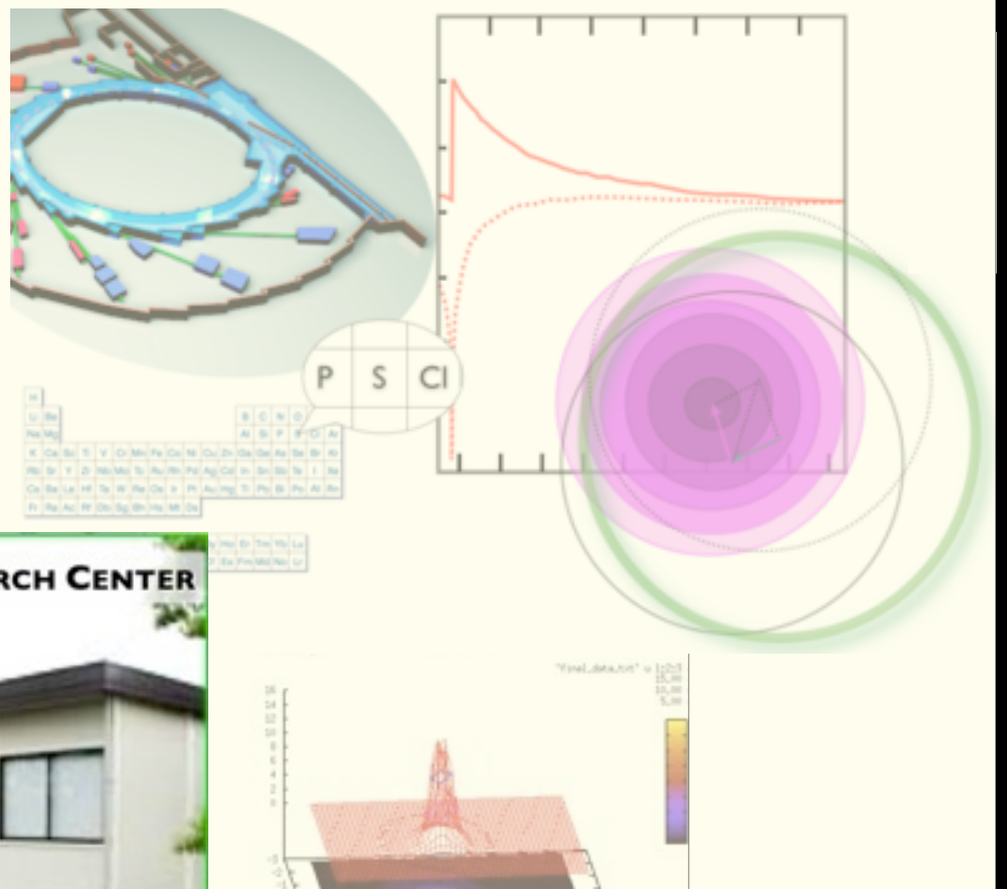
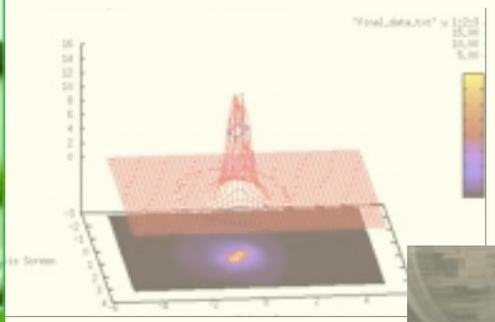
Research and Support Activities

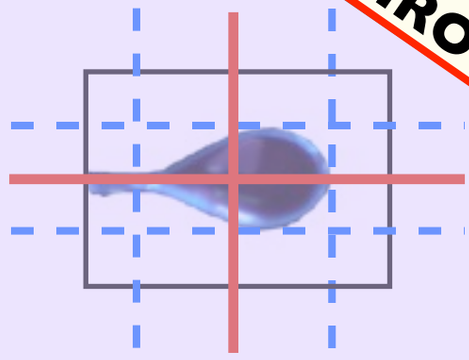






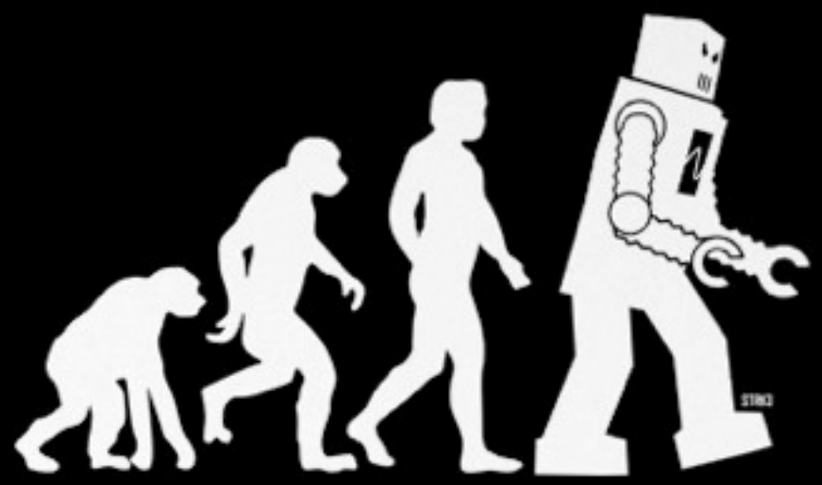
Structural & Functional Biology

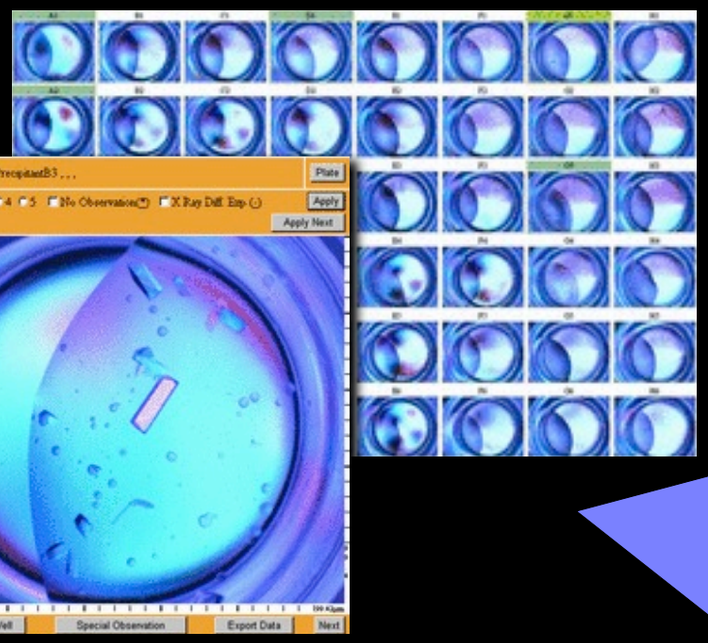
R&D



Synchrotron Science

- What are we doing?




Today
Precipitant83 , , ,
Plate
Apply
Apply Next
Slide Show
Previous
Browse Well
Special Observation
Export Data
Next

~ R & D

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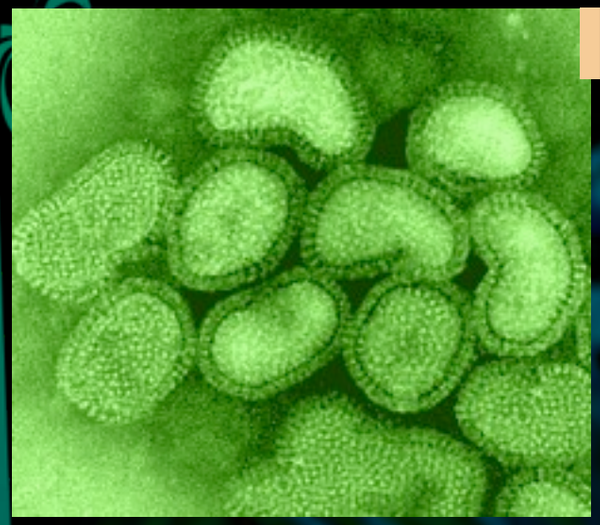
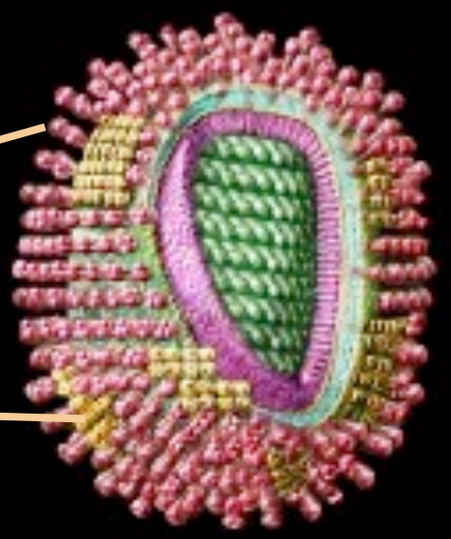
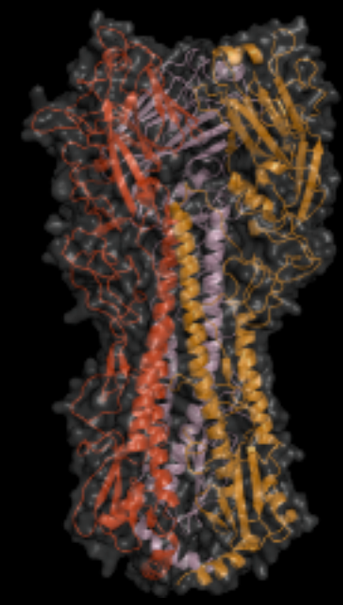
Influenza Virus Proteins





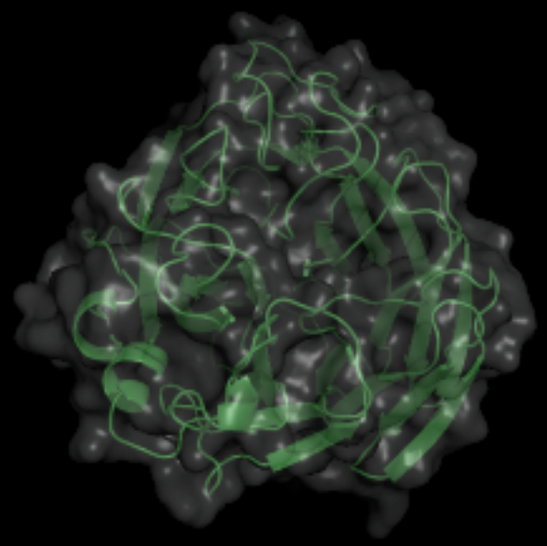
Influenza virus is an RNA type virus

Haemagglutinin (HA) & Neuraminidase (NA) are 2 surface proteins that help to parasite the host cell



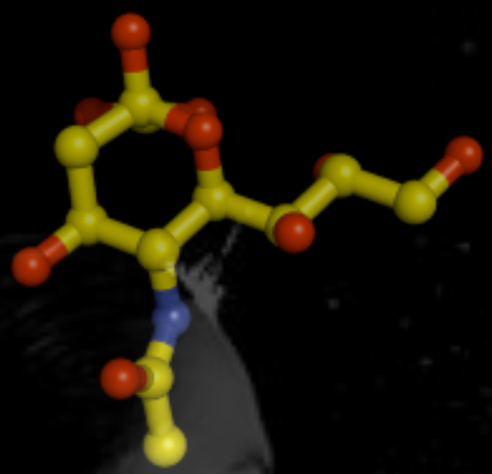
- **HA recognizes sialic acid receptors at the plasma membrane; necessary for the virus entrance**

- **NA hydrolyzes the sialic acid of the receptor; necessary for newly formed virion's release and virus propagation**

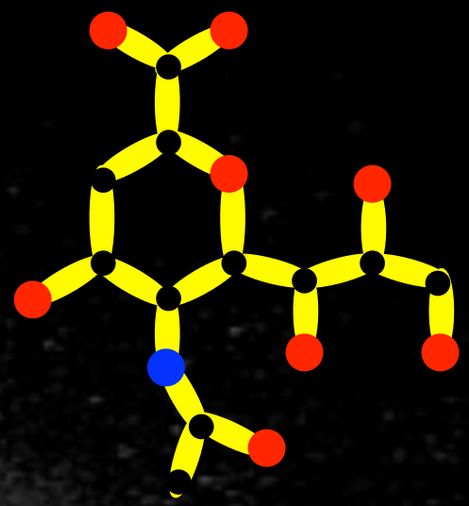


... influenza virus

Anti-Viral Drugs for Flu Treatment

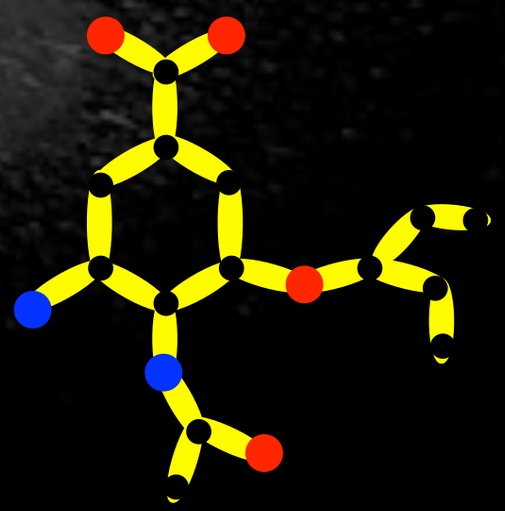


sialic acid



DANA

zanamivir
(Relenza)



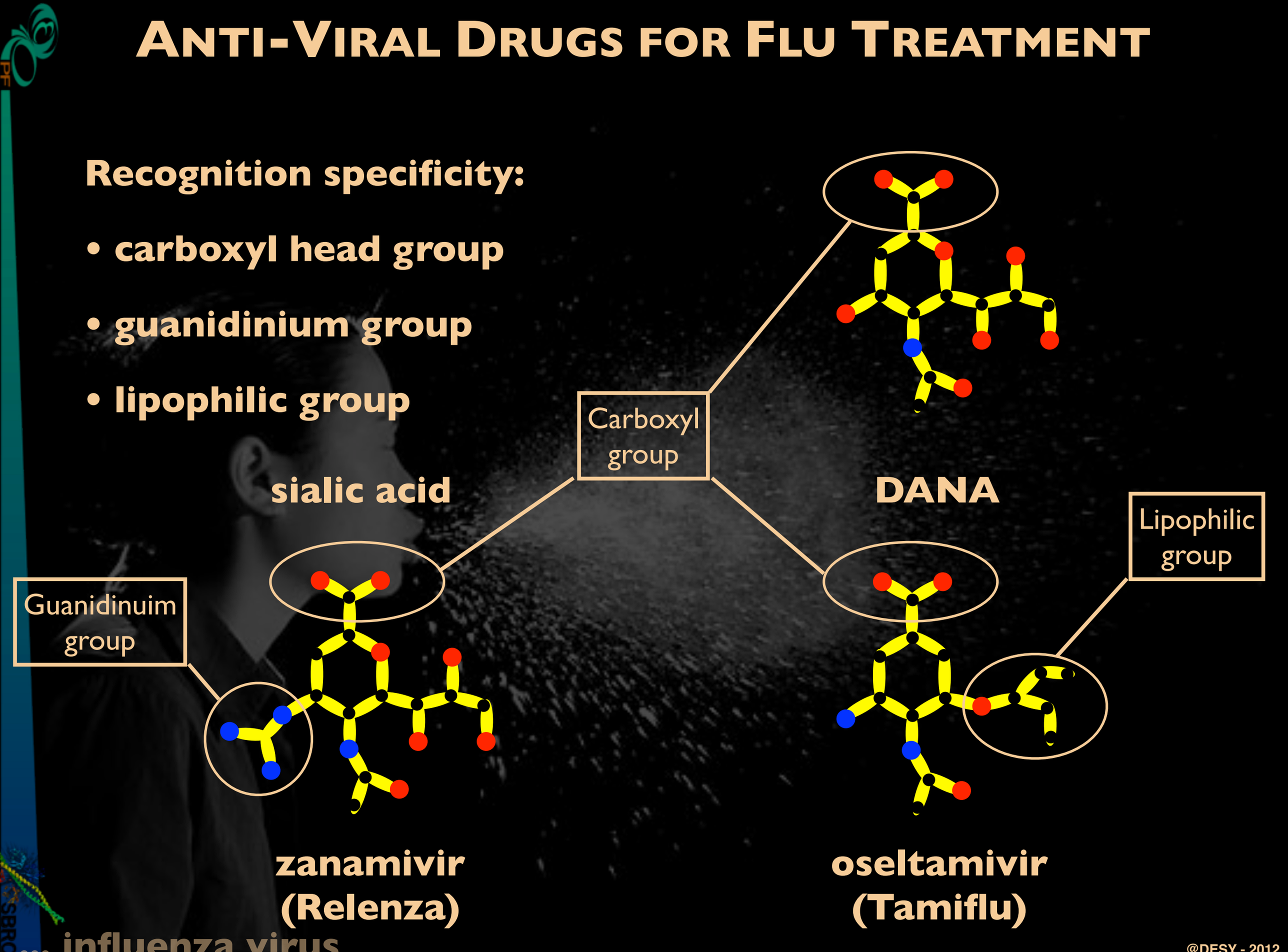
oseltamivir
(Tamiflu)

... influenza virus

Anti-Viral Drugs for Flu Treatment

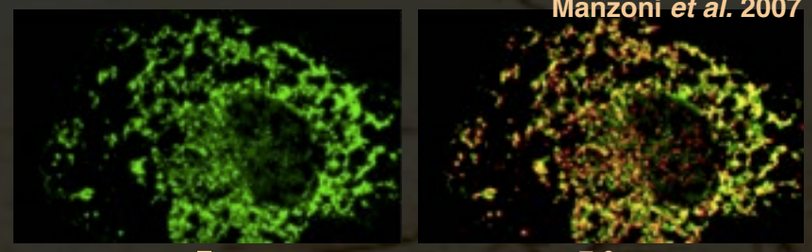
Recognition specificity:

- **carboxyl head group**
- **guanidinium group**
- **lipophilic group**

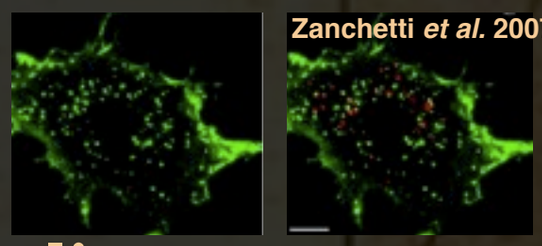


... influenza virus

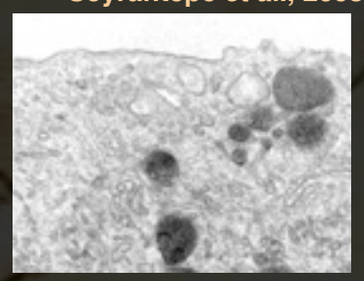
Human NAs


Manzoni *et al.* 2007

- **Neu1**
  - lysosomal membrane sialidase
  - mutations associated with lysosomal storage diseases (sialidosis; galactosialidosis)
- **Neu2**
  - cytoplasmic sialidase
  - low expression, mainly in skeletal muscle
  - hydrolysis of GM_3; weak against GM_2 or GM_1
  - induces proliferation and spontaneous differentiation of myoblast cells
  - potential role in early steps of neuronal differentiation


Zanchetti *et al.* 2007

- **Neu3**
  - plasma membrane sialidase
  - implicated in cell signaling and insulin signaling
- **Neu4**
  - mitochondrial? lysosomal sialidase?
  - implicated in apoptosis?


Seyrantepe *et al.*, 2008

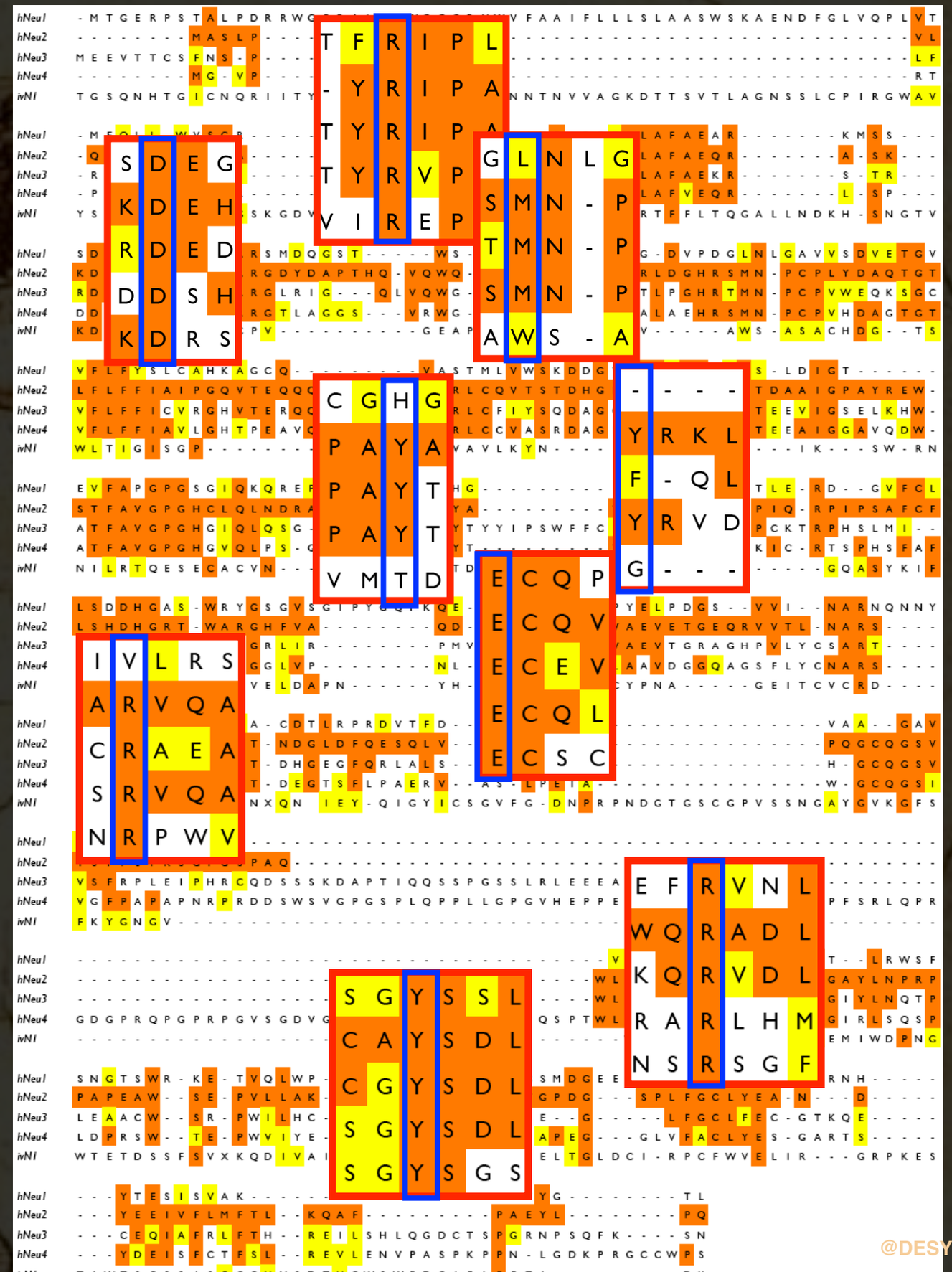
... influenza virus

Human NAs

- Human sialidases share a high sequence identity when comparing to the influenza virus neuraminidase

- Neu2 compared with:
  - Neu1.....23 %
  - Neu3.....38 %
  - Neu4.....34 %
  - influ NA...17 %

- Conserved residues for sialic acid coordination and enzymatic activity



... influenza virus

EFFECTS OF ANTI-FLU DRUGS IN HUMAN?

WED., NOV. 15TH, 2006

Tamiflu will carry caution on

Tamiflu®
Oseltamivirum

Roche Laboratories inc.:

" ...dose dependent increase in the incidence rates of a variety of minor skeletal abnormalities..."

GlaxoSmithKline:

" ...increase in the incidence rates of a variety of minor skeleton alterations..."

closely monitored for

ny Roche Holding AG

cases of delirium, hallucinations and other unusual psychiatric behavior in children treated with the drug. Most were Japanese children.

The Food and Drug Administration said a relationship between the drug and the behavior had not been established and that the

mal behavior flagged in the updated label.

The surprise change came three days before an FDA panel of outside experts was to discuss whether to recommend that the agency add the precautionary language to the Tamiflu label.

In documents

iflu and users' reported bizarre behavior. REUTERS PHOTO

of bird flu. The drug doesn't prevent flu but can reduce the length and severity of its symptoms.

Previously, Roche has cited studies from the United States and Canada

... influenza virus

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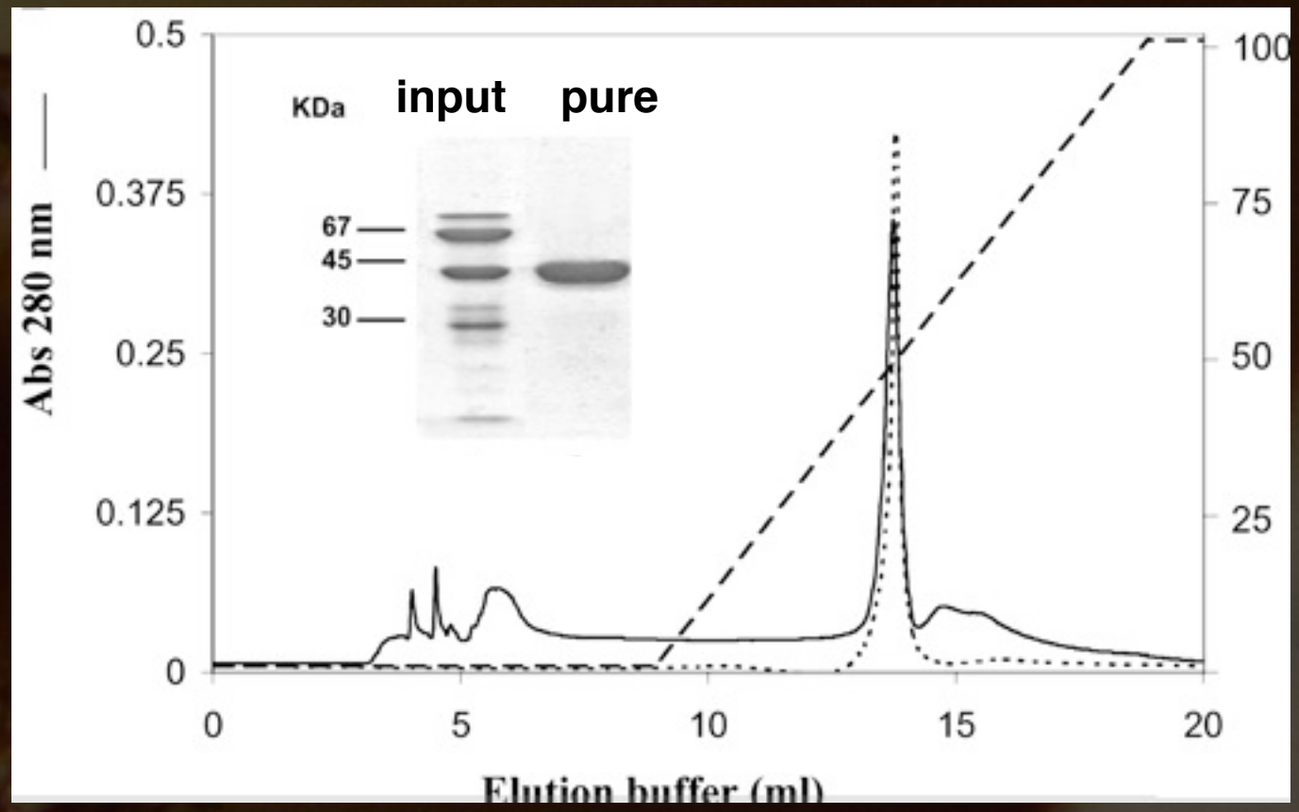
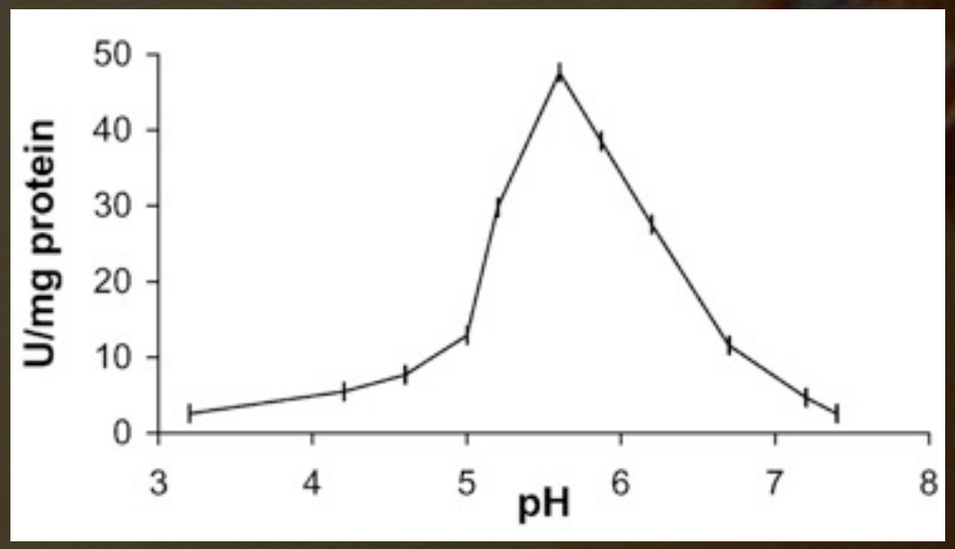
Neu2 Sample Preparation

- Recombinant expression (GST-tagged protein)
- Protein purification (affinity, ion exchange chromatography)

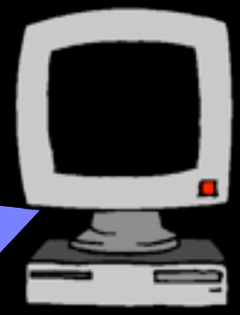
> monodisperse sample
> highly pure and intact
> ready for crystallization
> active protein

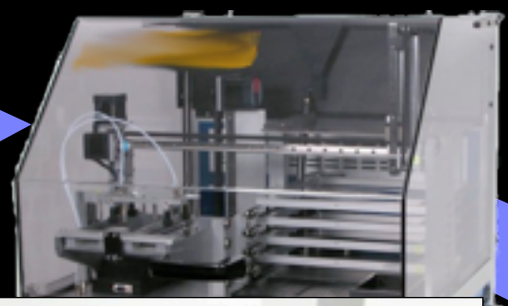


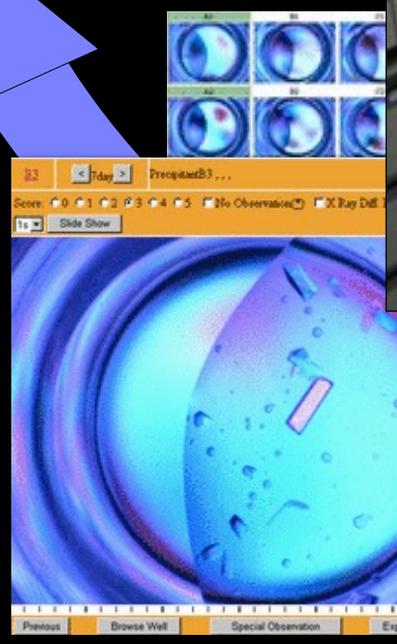


activity test at different pH

... influenza virus






Previous
Browse Well
Special Observation
Export Data
Next

~ R & D

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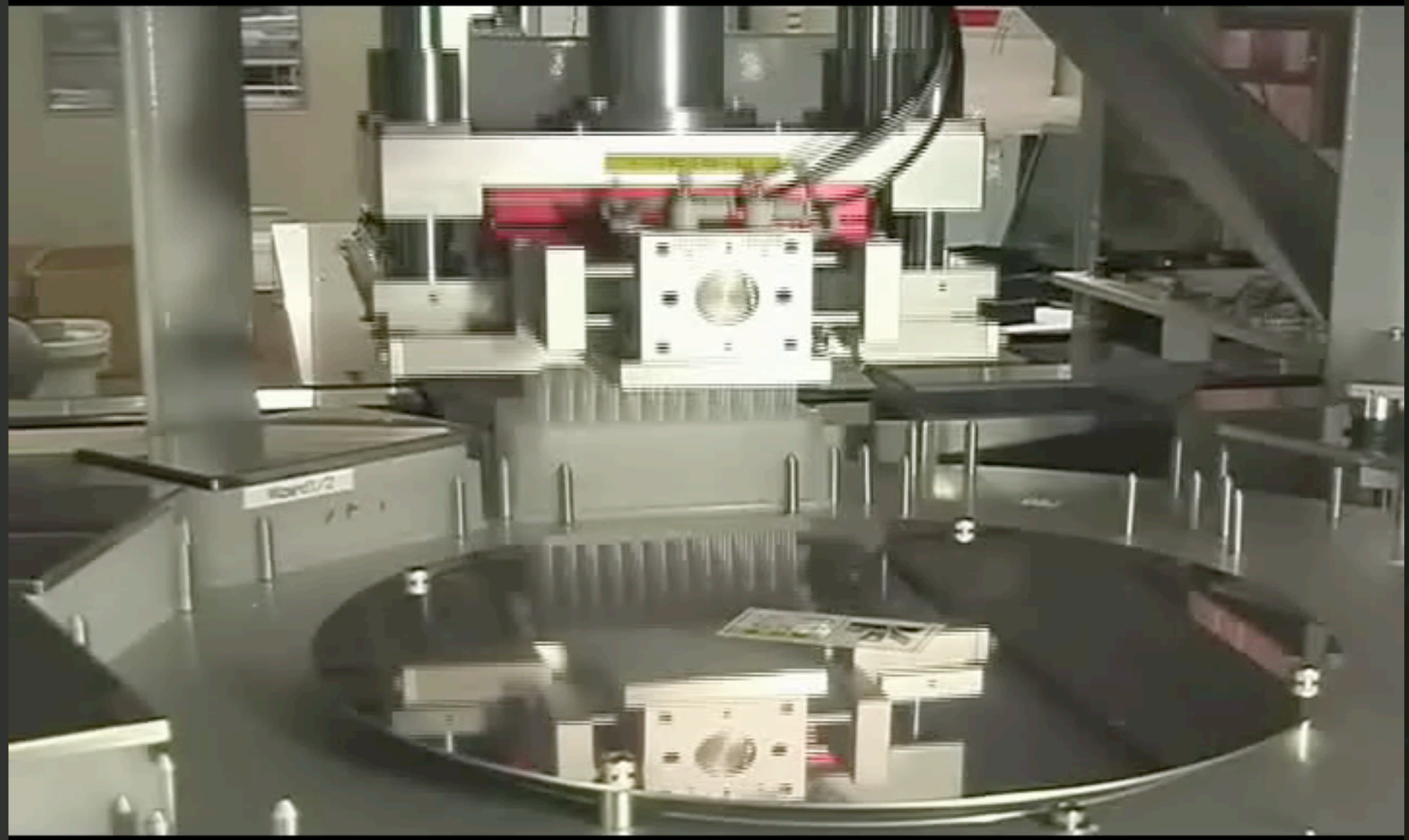
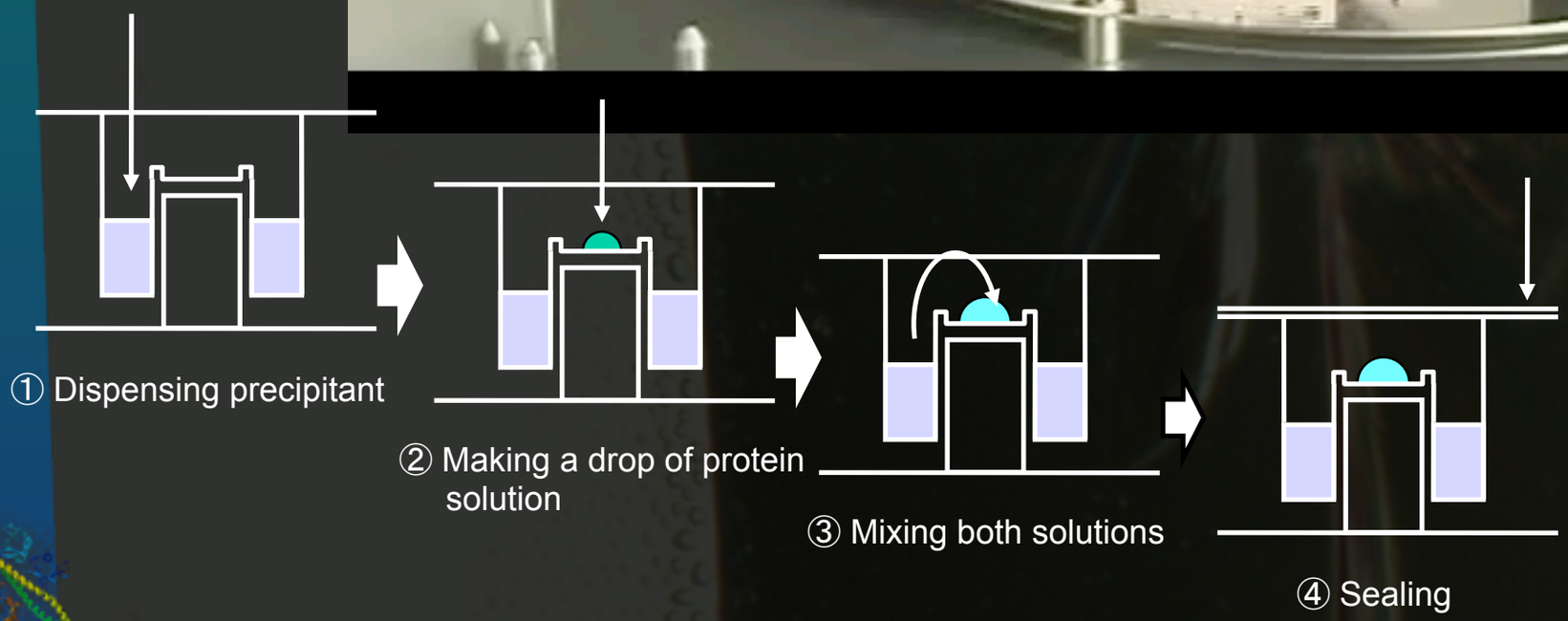
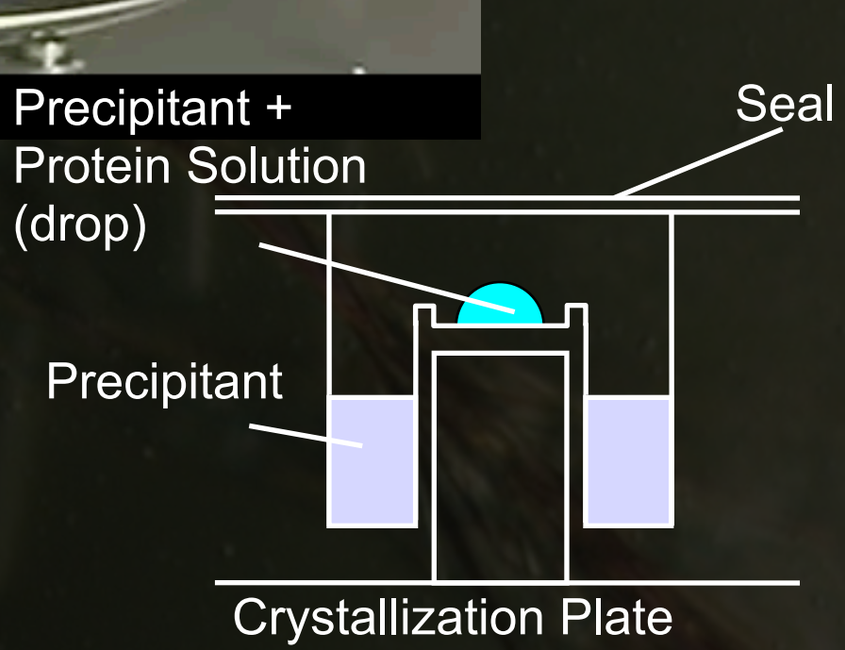
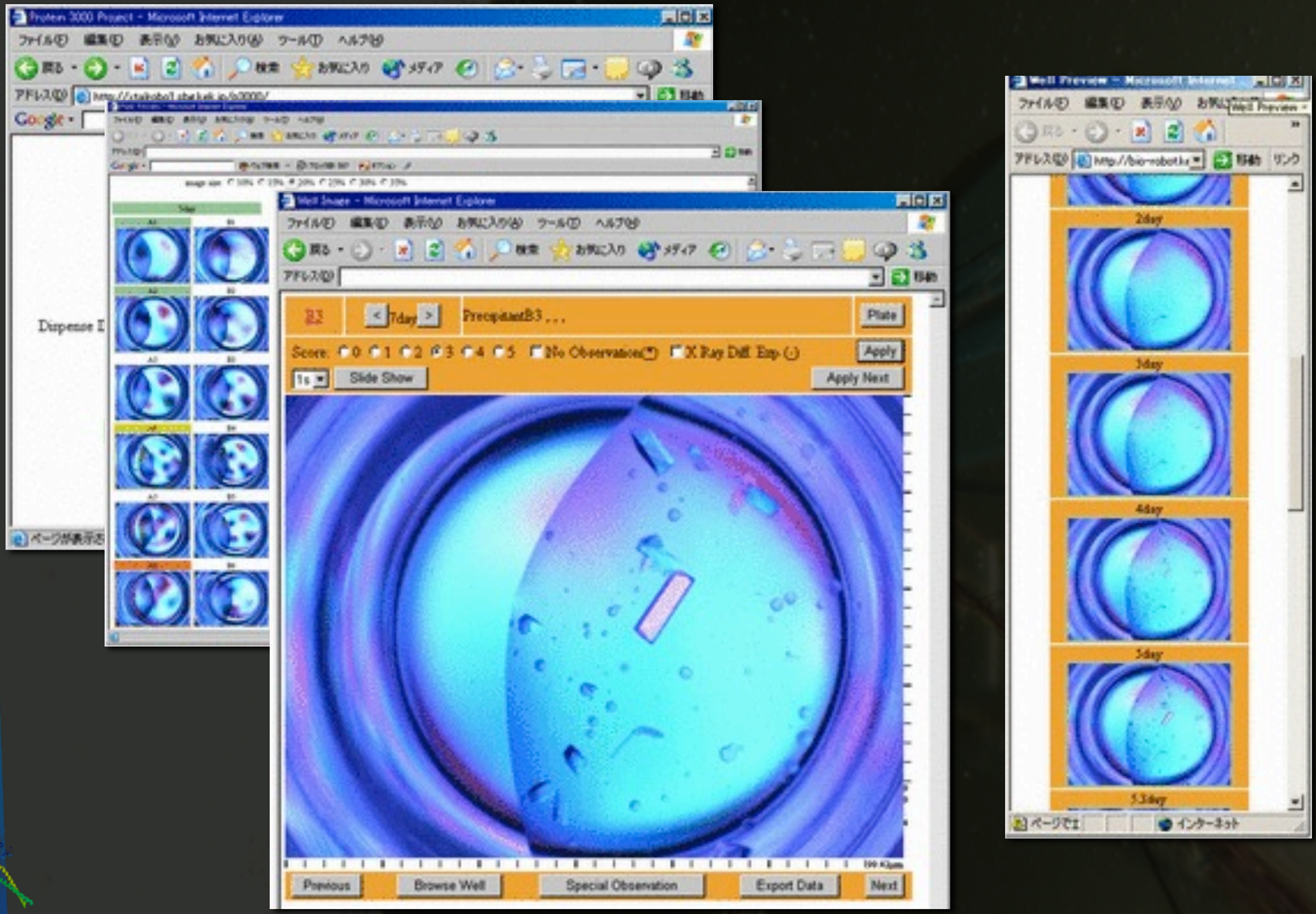
PSX Robot



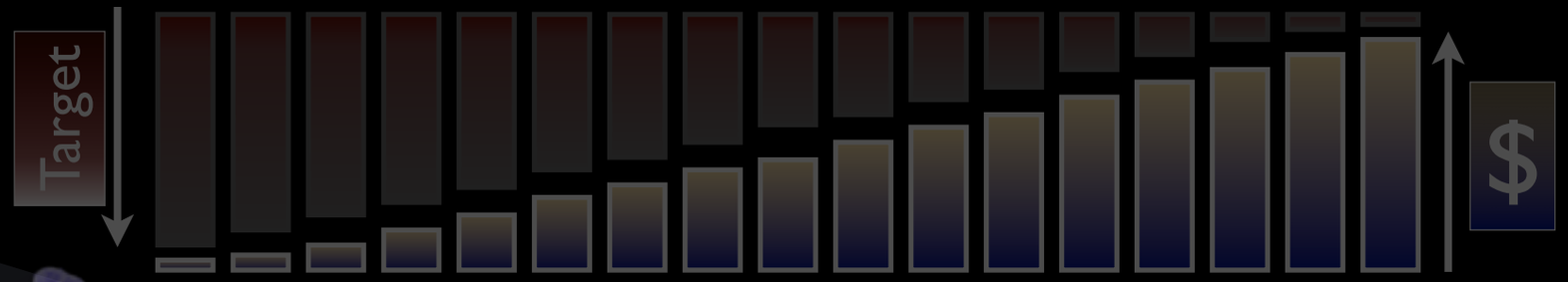

① Dispensing precipitant
② Making a drop of protein solution
③ Mixing both solutions
④ Sealing


Precipitant + Protein Solution (drop)
Seal
Precipitant
Crystallization Plate

Observation System



Structural Biology



Identify a target model

X-ray analysis

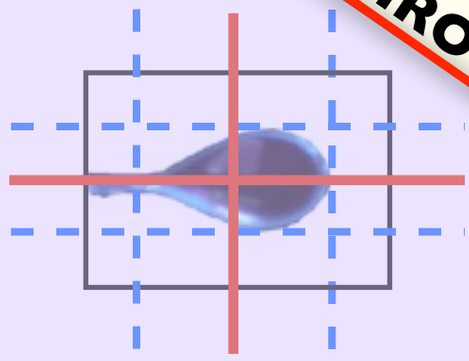
Detailed analysis

~ structural biology

Research and Support Activities

Structural & Functional Biology

R&D



Synchrotron Science

- What are we doing?

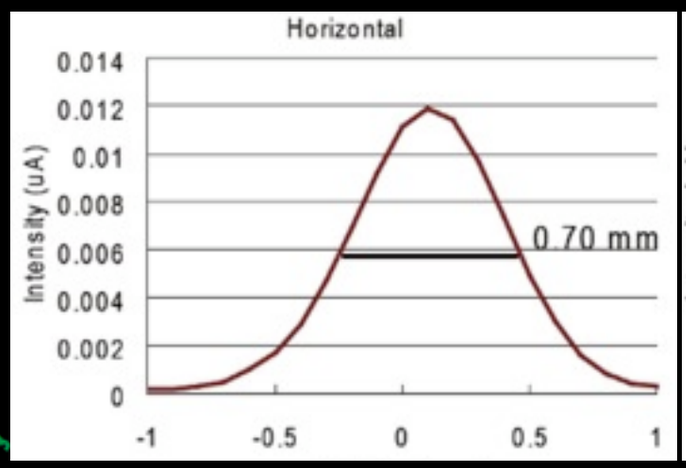
Research and Support Activities

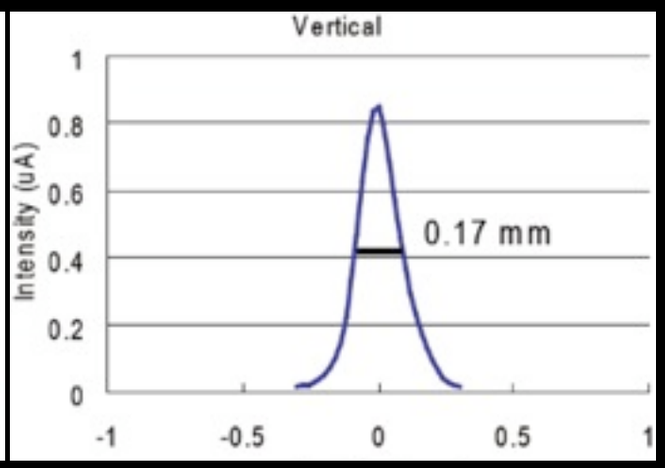
	BL-1A	BL-5A	BL-17A	AR-NW12A	AR-NE3A
Starting year	2010	2004	2006	2003	2009
Status	Operational	Operational	Operational	Operational	Operational
Synchrotron Ring		PF		PF AR	
Injection		Top-up		Twice / day (8:30. 20:30)	
X-ray source	Short-gap undulator	Multipole wiggler	Short-gap undulator	Undulator	Undulator
Wavelength (Å)	0.9 -1.1 / 2.7 - 3.0	0.7 - 1.9	0.9 - 2.1	0.7 - 1.9	0.7 - 1.9
Energy resolution (ΔE / E)	2.5×10^{-4}	2.5×10^{-4}	2.5×10^{-4}	2.5×10^{-4}	2.5×10^{-4}
Photon flux (photons / sec, @ 1.0 Å)	2×10^{10} 2×10^{9} (@2.7 Å)	2×10^{11}	6.6×10^{9} 1.3×10^{10} (@2.0 Å)	2.0×10^{11}	8.0×10^{11}
Slit size for flux measurement (μm²)	10 x 10	200 x 200	20 x 20	200 x 200	200 x 200
Detector	Quantum 270	Quantum 315r	Quantum 210r	Quantum 210r	Quantum 270
Type	CCD	CCD	CCD	CCD	CCD
Active area (mm²)	270 x 270	315 x 315	210 x 210	210 x 210	270 x 270
Pixel size (μm²)	64.8 x 64.8	102 x 102	51 x 51	102 x 102	64.8 x 64.8
Pixel number	4168 x 4168	3072 x 3072	4096 x 4096	2048 x 2048	4168 x 4168
Frame data size (MByte)	34	19	32	8.0 x 1011	34
Readout time (sec)	1.1	0.3	1	0.3	1.1
Typical exposure time (sec / deg)	Exp. time + 2.7	Exp. time + 1.6	Exp. time + 2.7	Exp. time + 1.6	Exp. time + 2.7
Data collection time per frame (sec)	11	14	24	14	11
Typical data collection time (min, 180 frames)	6	6 (24)	6	6	6
(using the IEEE1394 interface)					
Camera distance (mm)	40 - 500	60-950	40-700	60-950	60-500
Detector vector offset (mm)	0 - 100	0 - 150	0-100	0-100	0-150
Sample changer	PAM				
Software image processing	HKL2000, Mosflm, XDS…				

Research and Support Activities

Astellas Beamline : Pharmaceuticals Beamline

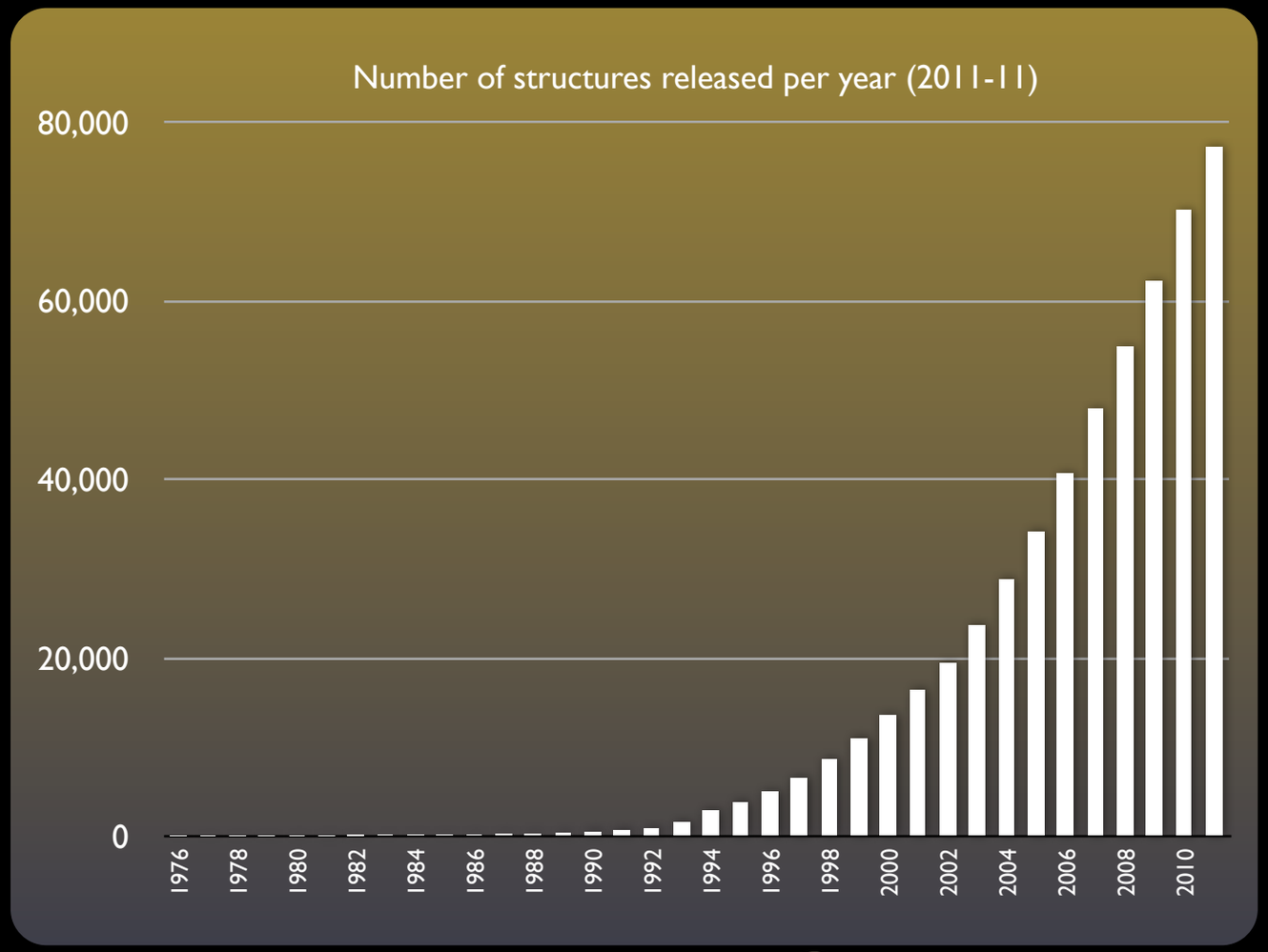
Starting year	AR-NE3A
Status	2009
Synchrotron Ring	Operational
Injection	PF AR
X-ray source	Twice / day
Wavelength (Å)	Undulator
Energy resolution (ΔE / E)	0.7 - 1.9
Photon flux (photons / sec, @ 1.0 Å)	2.5×10^{-4}
	8.0×10^{11}
Slit size for flux measurement (μm²)	200 x 200
Detector	Quantum 270
Type	CCD
Active area (mm²)	270 x 270
Pixel size (μm²)	64.8 x 64.8
Pixel number	4168 x 4168
Frame data size (MByte)	34
Readout time (sec)	1.1
Typical exposure time (sec / deg)	Exp. time + 2.7
Data collection time per frame (sec)	11
Typical data collection time (min, 180 frames)	6
(using the IEEE1394 interface)	
Camera distance (mm)	60-500
Detector vector offset (mm)	0-150
Sample changer	PAM
Software image processing	HKL2000 etc.



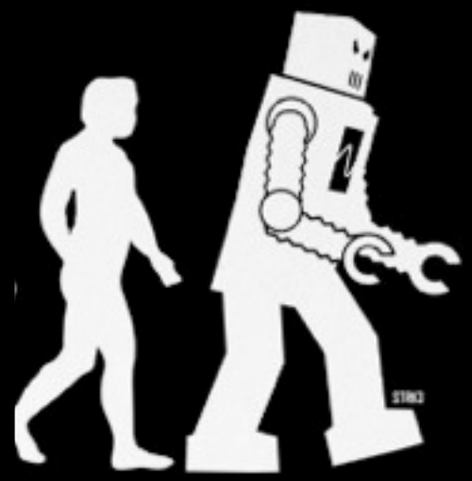


Research and Support Activities

Astellas Beamline : Pharmaceuticals Beamline



Starting year	AR-NE3A
Status	2009
Synchrotron Ring	Operational
Injection	PF AR
X-ray source	Twice / day
Wavelength (Å)	Undulator
Energy resolution (ΔE / E)	0.7 - 1.9
Photon flux (photons / sec, @ 1.0 Å)	2.5×10^{-4}
	8.0×10^{11}
Slit size for flux measurement (μm²)	200 x 200
Detector	Quantum 270
Type	CCD
Active area (mm²)	270 x 270
Pixel size (μm²)	64.8 x 64.8
Pixel number	4168 x 4168
Frame data size (MByte)	34
Readout time (sec)	1.1
Typical exposure time (sec / deg)	Exp. time + 2.7
Data collection time per frame (sec)	11
Typical data collection time (min, 180 frames) (using the IEEE1394 interface)	6
Camera distance (mm)	60-500
Detector vector offset (mm)	0-150



Sample changer PAM

Robots to the rescue !

~ synchrotron science

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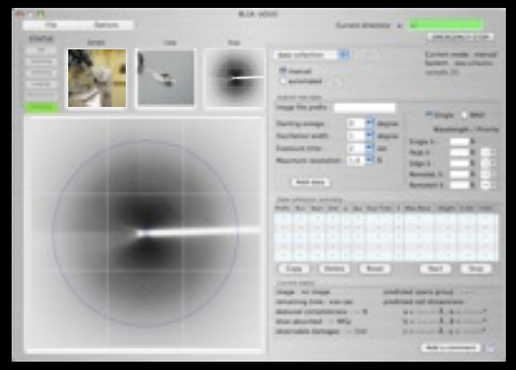
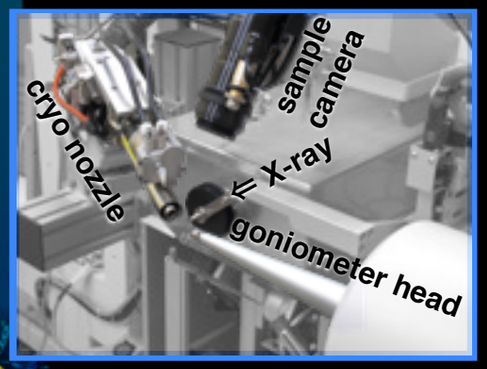
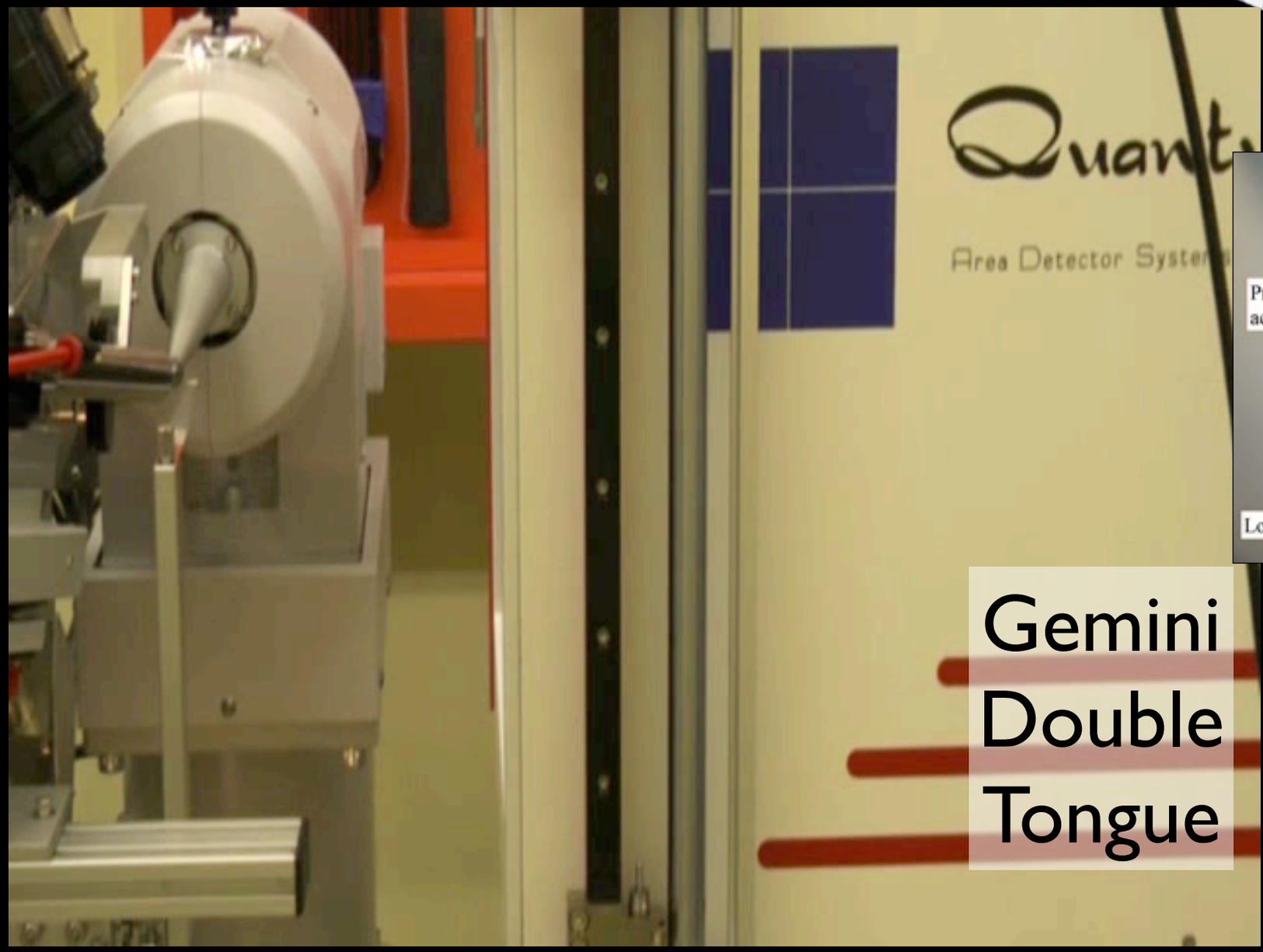
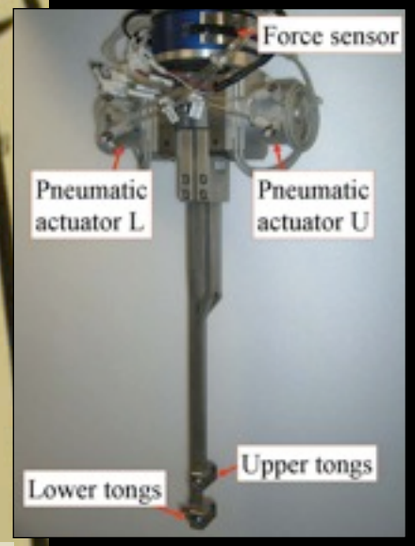
PF Automated Mounting System

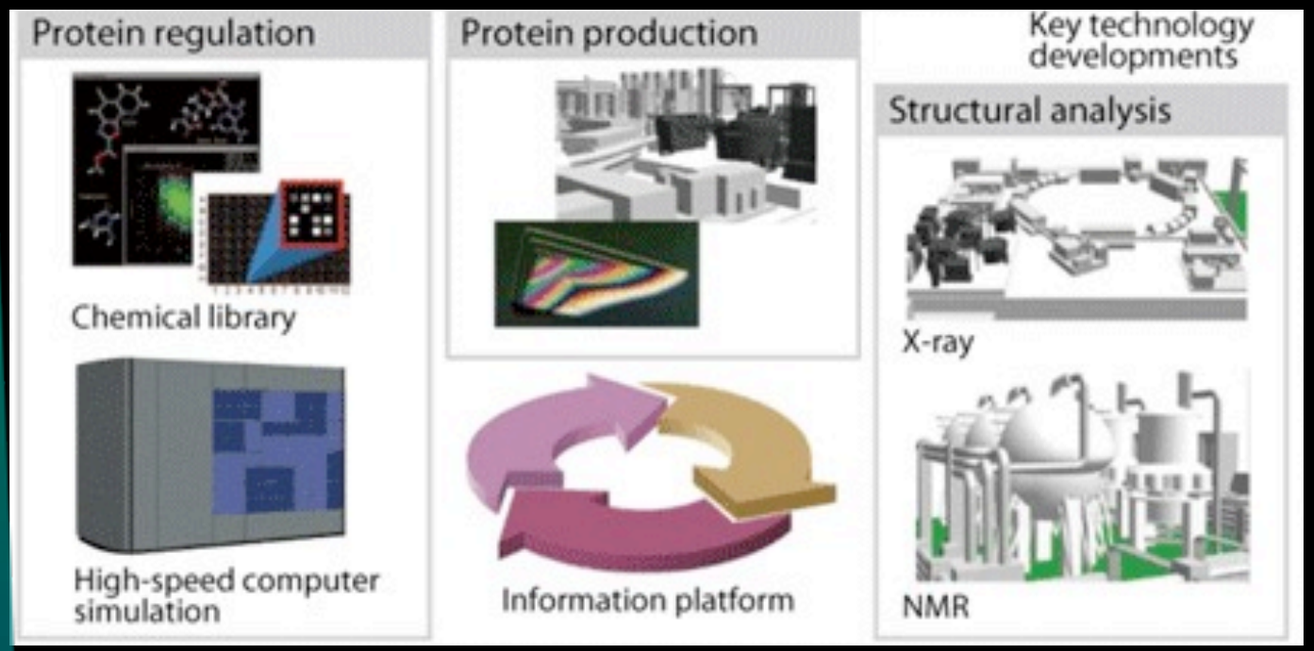








Research and Support Activities
Low Energy Beamline



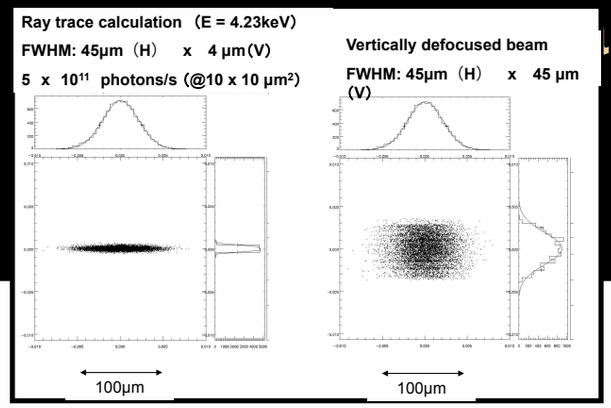
TPRP Targeted Proteins Research Program MEXT

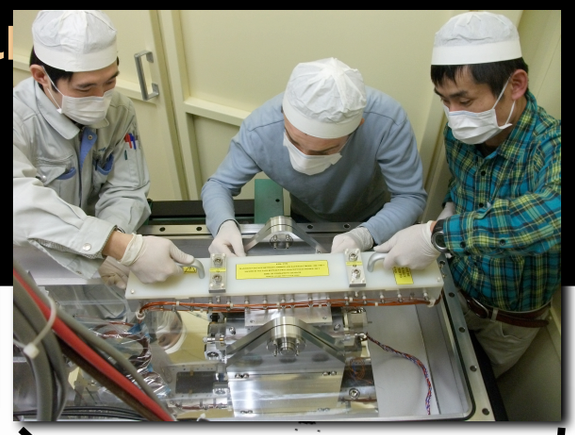
Starting year	BL-1A
Status	2010
Synchrotron Ring	Operational
Injection	PF
X-ray source	Top-up
Wavelength (Å)	Short-gap undulator
Energy resolution (ΔE / E)	0.9 -1.1 / 2.7 - 3.0
Photon flux (photons / sec, @ 1.0 Å)	2.5×10^{-4}
	2×10^{10}
Slit size for flux measurement (μm²)	2×10^{9} (@2.7 Å)
Detector	10 x 10
Type	Quantum 270
Active area (mm²)	CCD
Pixel size (μm²)	270 x 270
Pixel number	64.8 x 64.8
Frame data size (MByte)	4168 x 4168
Readout time (sec)	34
Typical exposure time (sec / deg)	1.1
Data collection time per frame (sec)	Exp. time + 2.7
Typical data collection time (min, 180 frames)	11
(using the IEEE1394 interface)	6
Camera distance (mm)	40 - 500
Detector vector offset (mm)	0 - 100
Sample changer	PAM
Software image processing	HKL2000 etc.

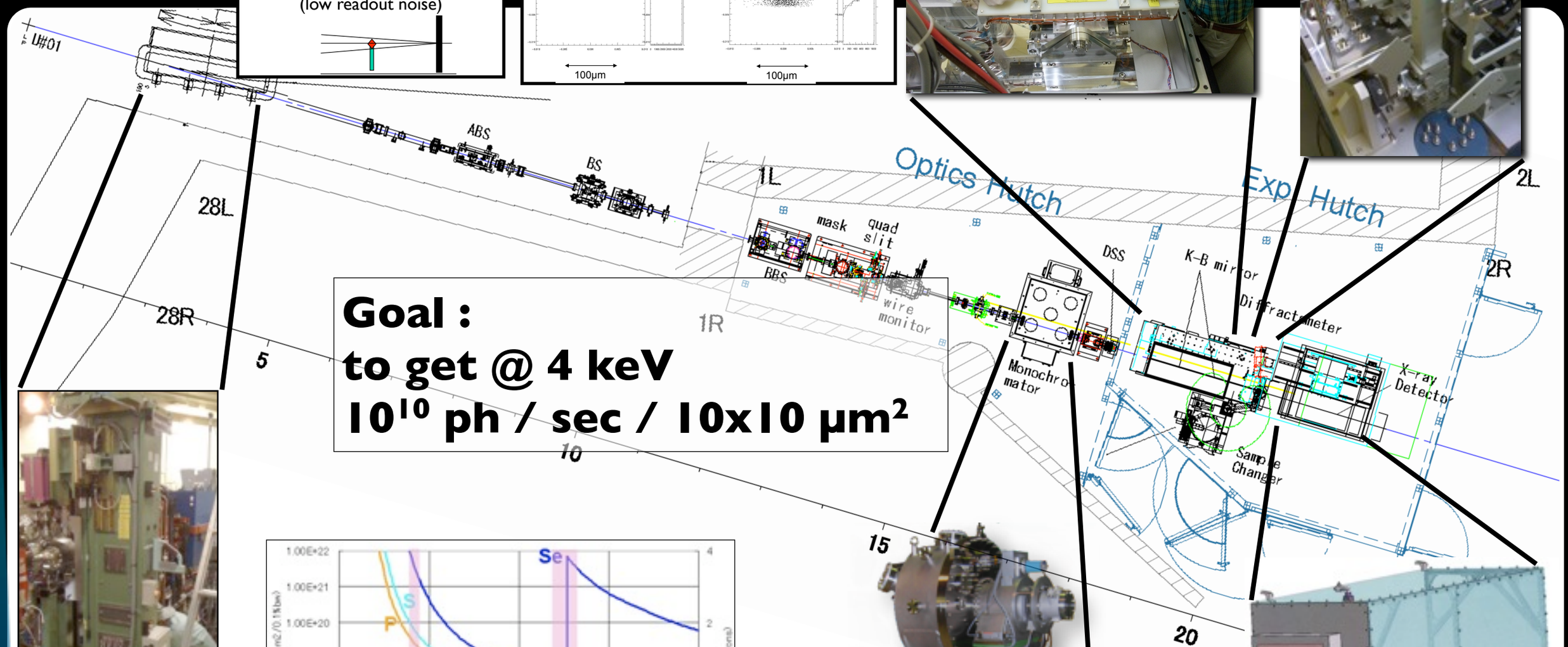
Research and Support: K/B Mirrors

Photon density focused
on the crystal
(better diffraction for small crystals)

Improved resolution
of the diffraction spots
(low readout noise)

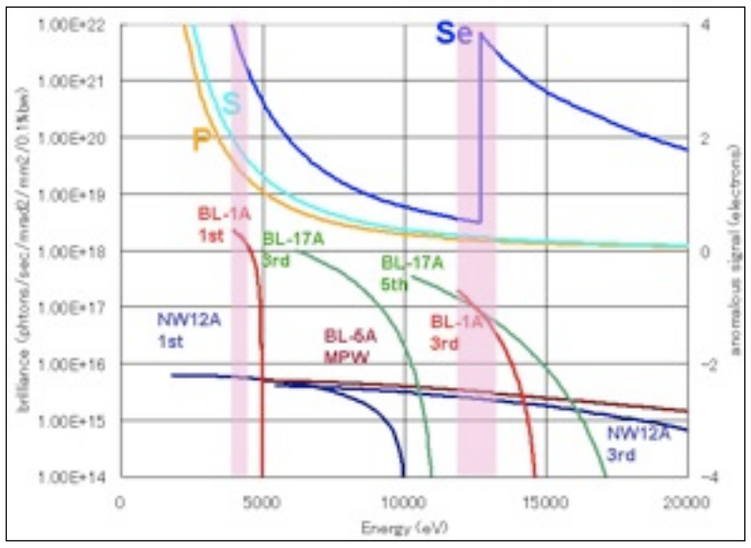


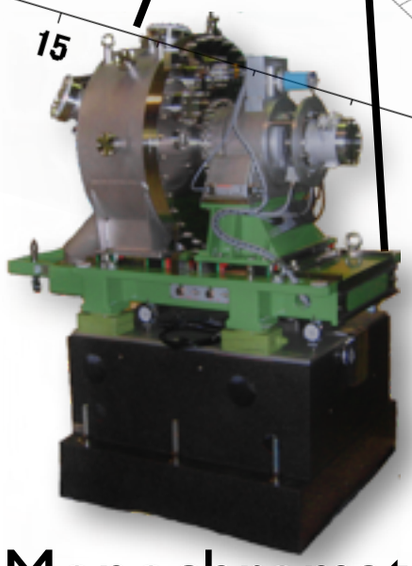




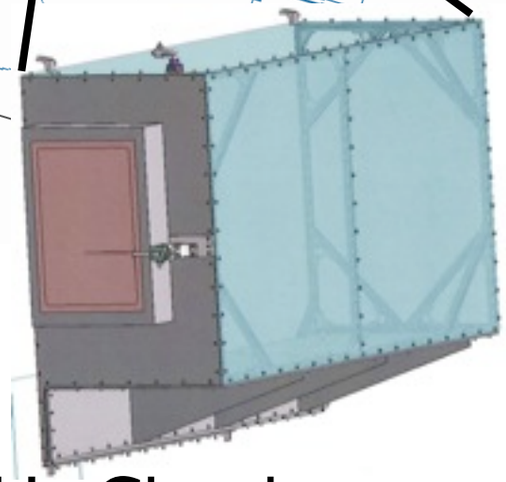
Goal :
to get @ 4 keV
10^{10} ph / sec / 10x10 μm²

Short gap
undulator





Monochromator



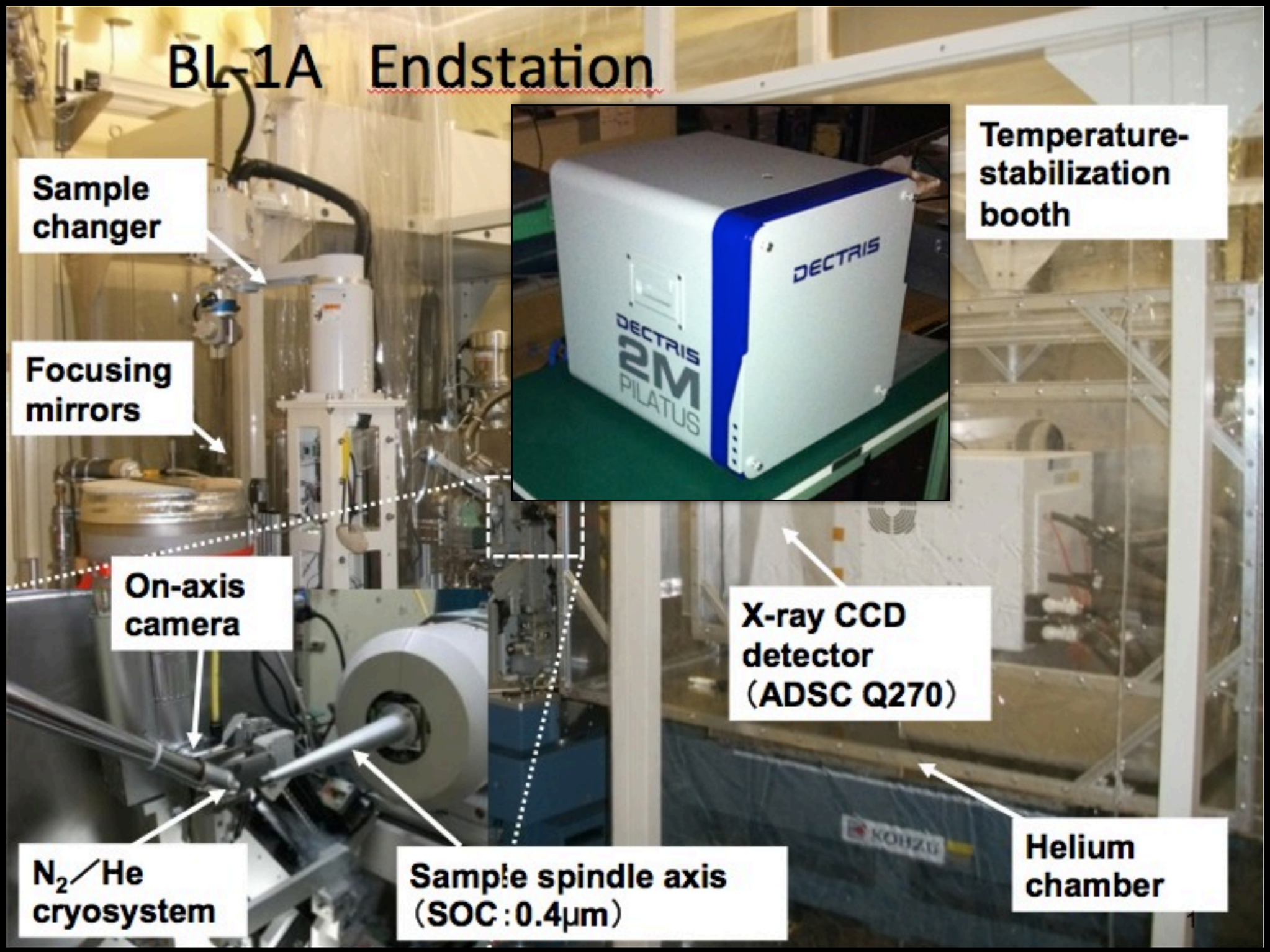
He Chamber

Monochromator

He Chamber

Research and Support Activities

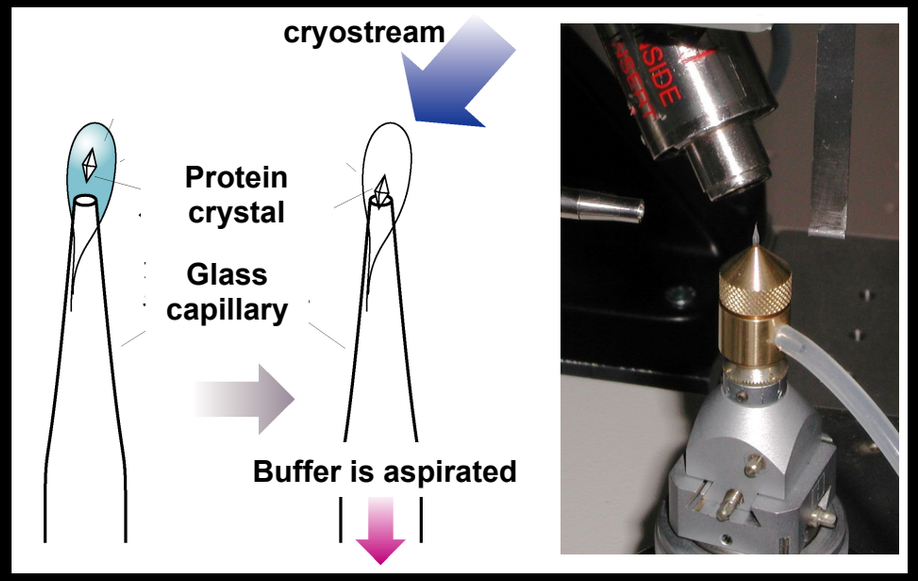
Low Energy Beamline

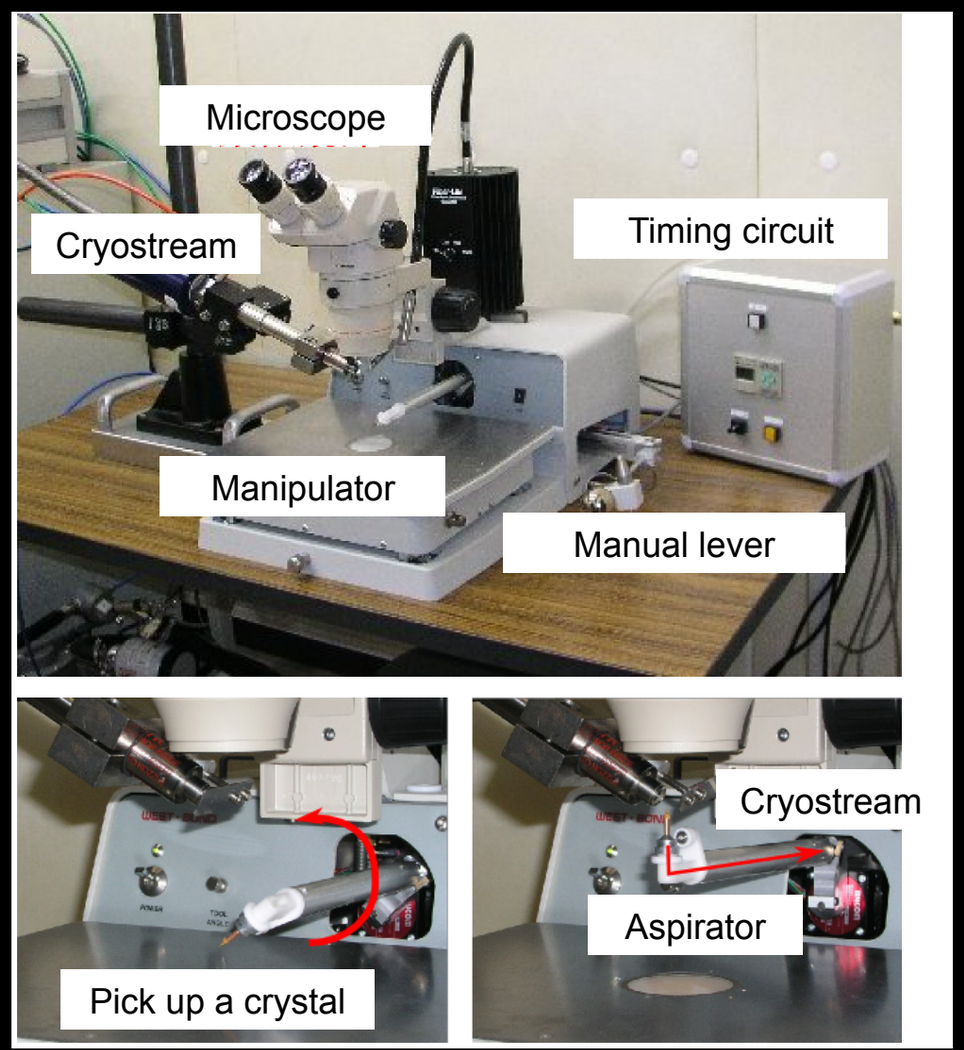


BL-1A	
2010	
Operational	
PF	
Top-up	
Short-gap undulator	
0.9 -1.1 / 2.7 - 3.0	
2.5×10^{-4}	
2×10^{10}	
2×10^{9} (@2.7 Å)	
10 x 10	
Quantum 270	
CCD	
270 x 270	
64.8 x 64.8	
4168 x 4168	
34	
1.1	
Exp. time + 2.7	
11	
6	
40 - 500	
0 - 100	
PAM	
HKL2000 etc.	

Research and Support Activities

Low Energy Beamline





	BL-1A
Starting year	2010
Status	Operational
Synchrotron Ring	PF
Injection	Top-up
X-ray source	Short-gap undulator
Wavelength (Å)	0.9 -1.1 / 2.7 - 3.0
Energy resolution (ΔE / E)	2.5×10^{-4}
Photon flux (photons / sec, @ 1.0 Å)	2×10^{10}
	2×10^{9} (@2.7 Å)
Slit size for flux measurement (μm²)	10 x 10
Detector	Quantum 270
Type	CCD
Active area (mm²)	270 x 270
Pixel size (μm²)	64.8 x 64.8
Pixel number	4168 x 4168
Frame data size (MByte)	34
Readout time (sec)	1.1
Typical exposure time (sec / deg)	Exp. time + 2.7
Data collection time per frame (sec)	11
Typical data collection time (min, 180 frames)	6
(using the IEEE1394 interface)	
Camera distance (mm)	40 - 500
Detector vector offset (mm)	0 - 100
Sample changer	PAM
Software image processing	HKL2000 etc.

Structural Biology

Target

$

Identify a target model

X-ray analysis

Detailed analysis

Data analysis

~ structural biology

@DESY - 2012

Structural Biology

Target

$

Identify a target model

Detailed analysis

X-ray analysis

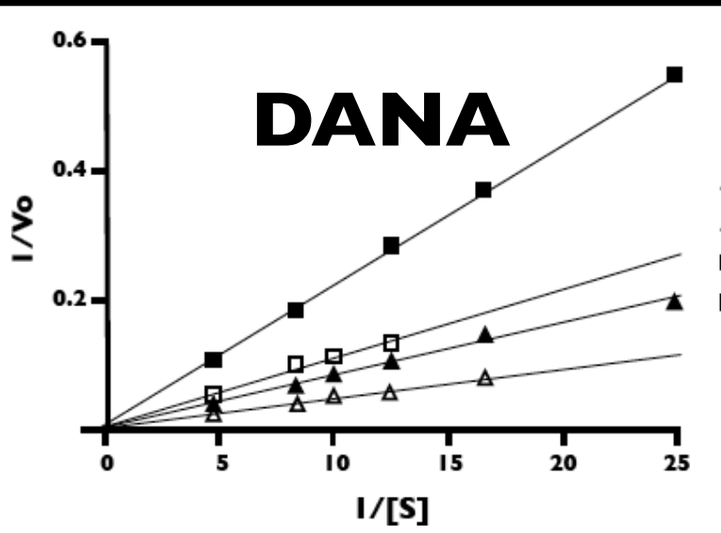
Structure determination

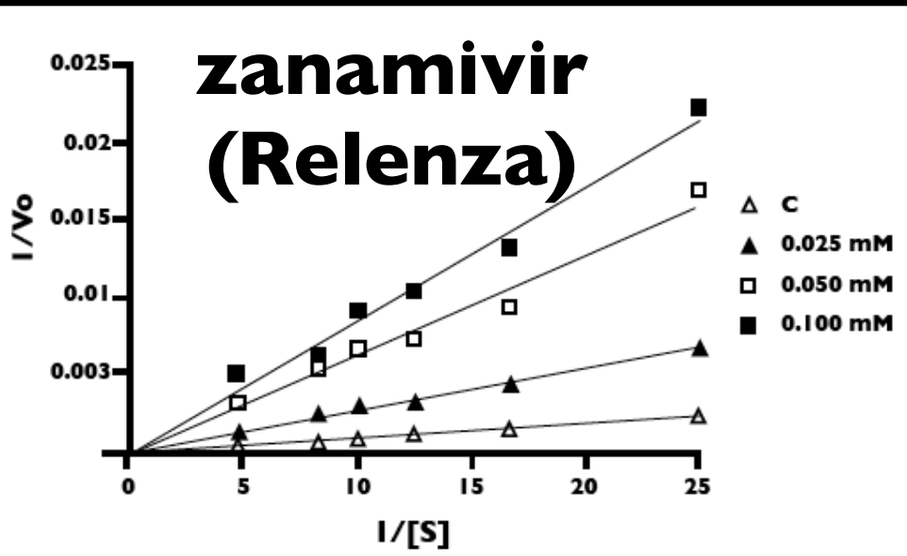
Data analysis

~ structural biology

@DESY - 2012

Neu2 Inhibition by NIs

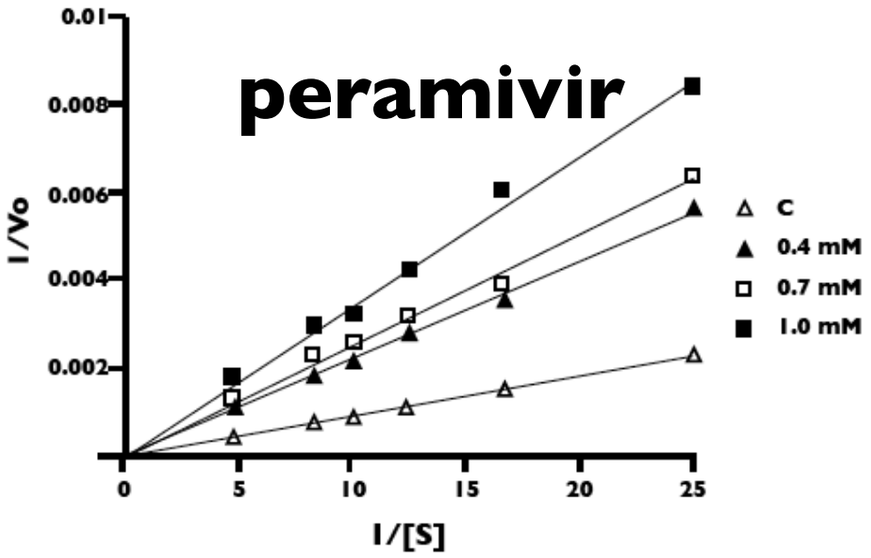


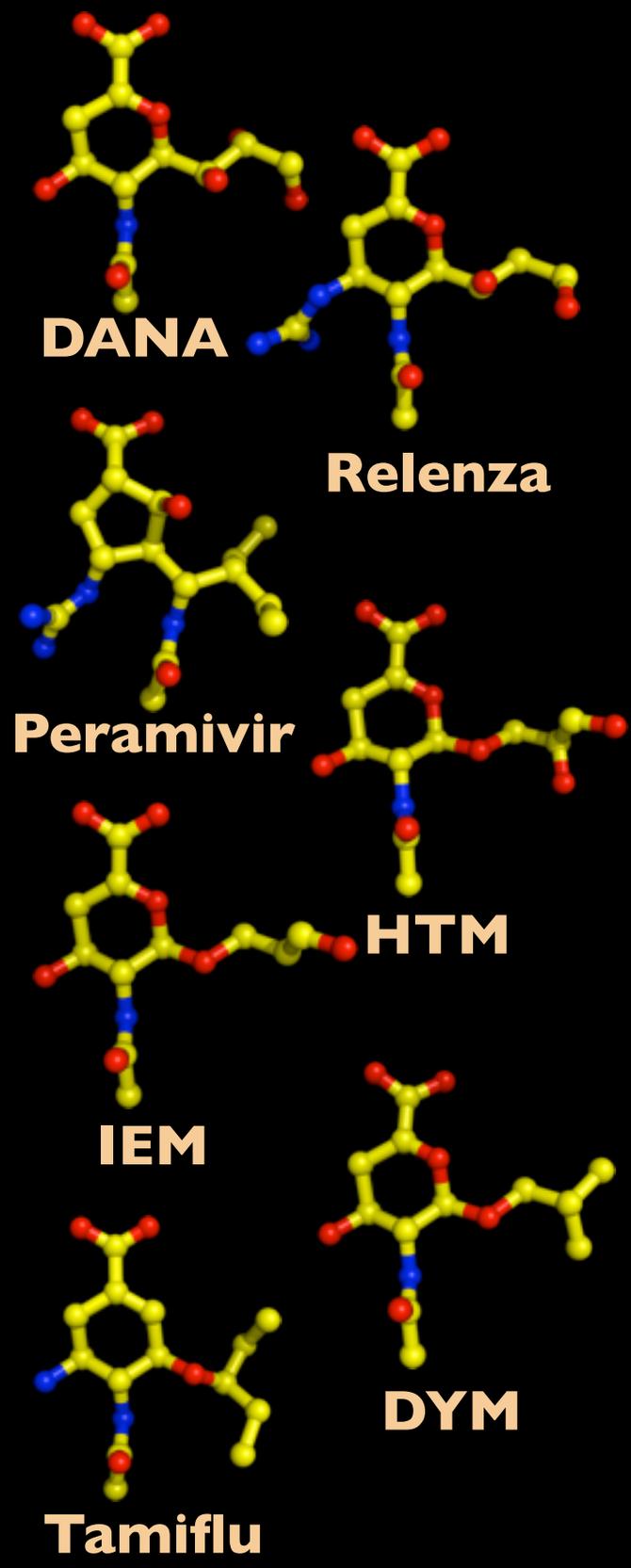


DANA: K_i=0.060 mM

zanamivir (Relenza): K_i=0.011 mM

peramivir: K_i=0.563 mM





... human Neu2

DANA

K_i=0.060 mM
H-bonds: 10

zanamivir
(Relenza)

K_i=0.011 mM
H-bonds: 13

Active site
free form

R304

Y334

R237

R21

L217

E39

Y181

N86

Y179

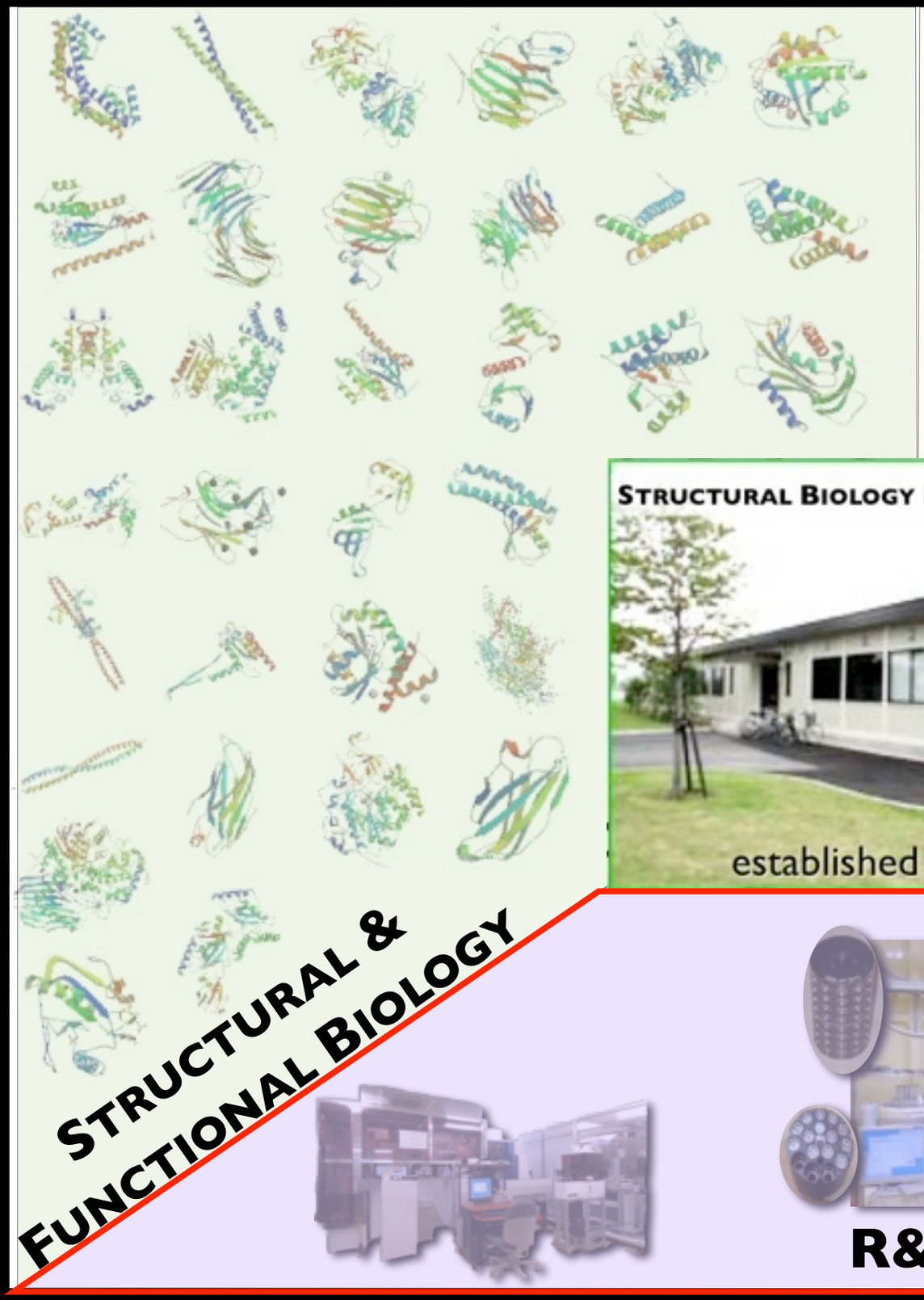
E111

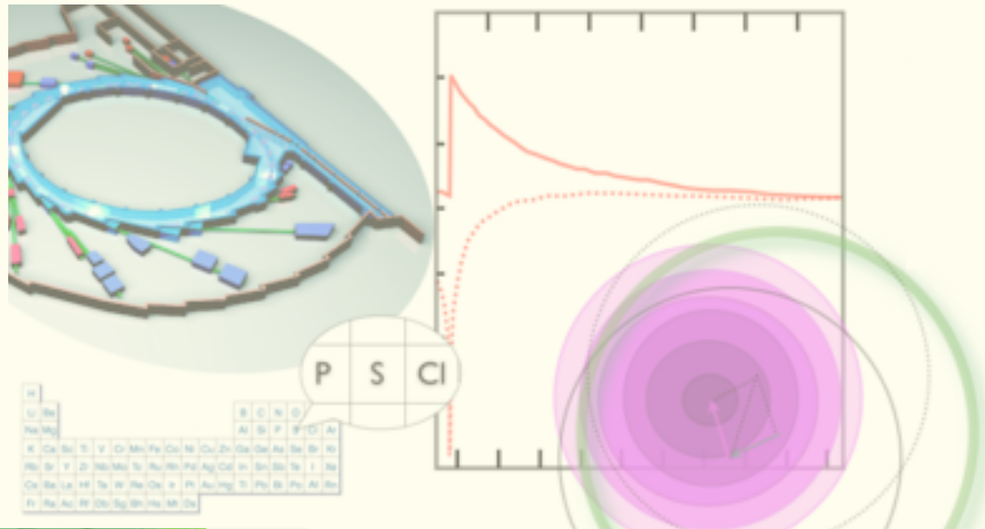
peramivir

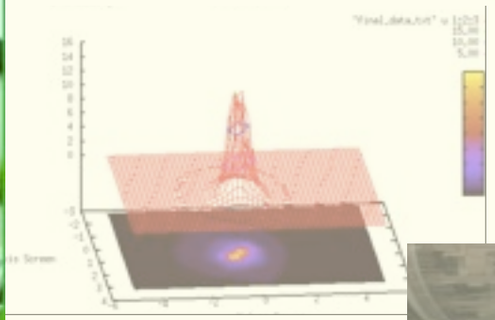
K_i=0.563 mM
H-bonds: 8

... human Neu2

Research and Support Activities

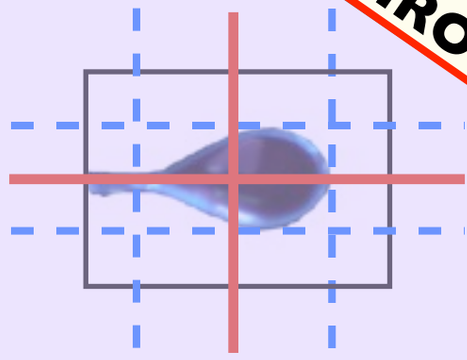






Structural & Functional Biology

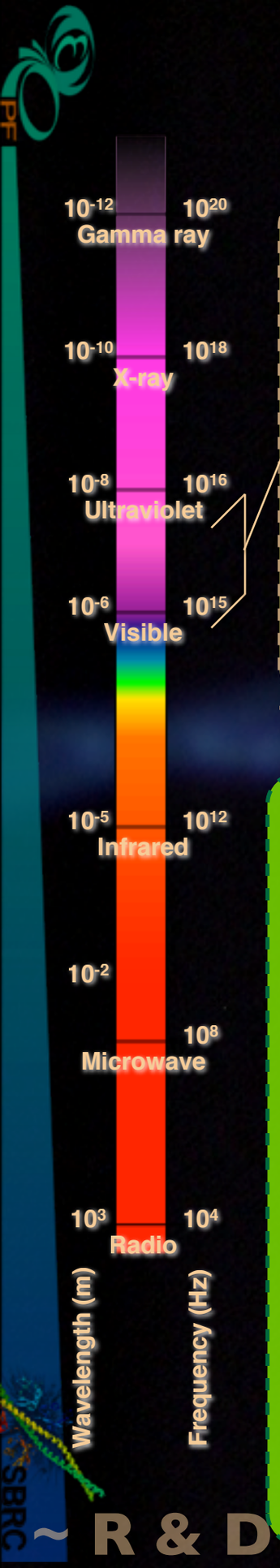
R&D



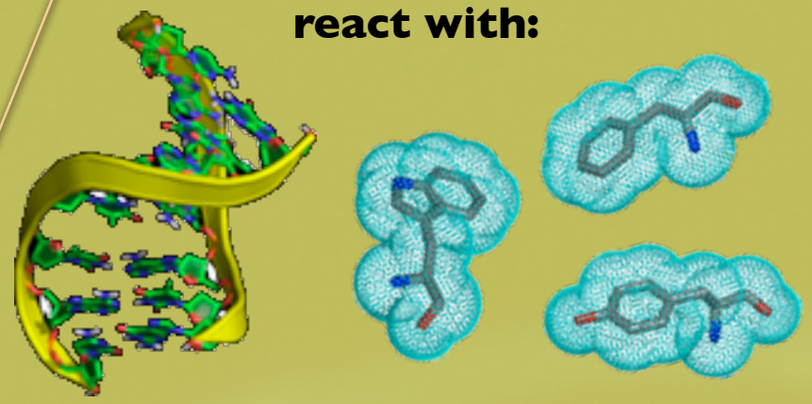
Synchrotron Science

- What are we doing?

UV for Centering & Phasing



near to middle UV (200~400 nm /3.10~6.20 eV) react with:

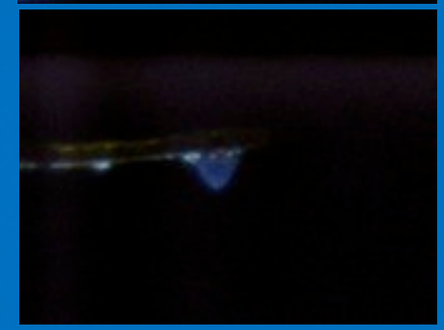


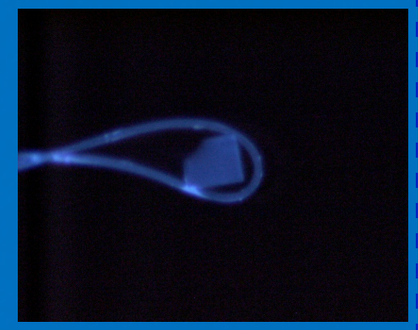
cytosines (DNA & RNA)

aromatics (proteins)

Specific Highlighting of Biological Crystals (protein / DNA / RNA)

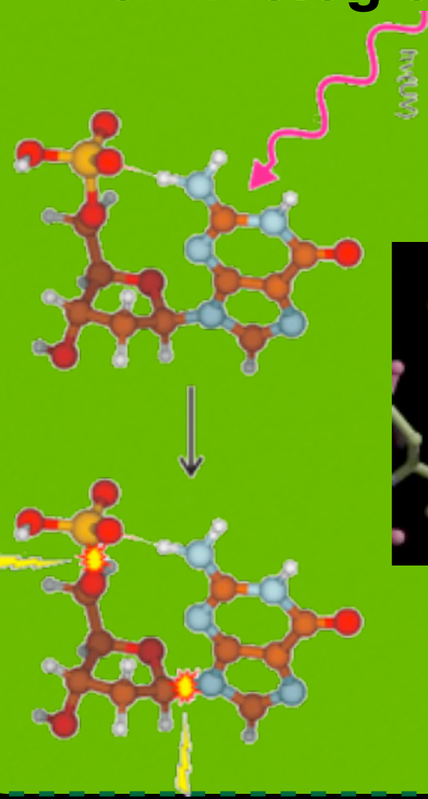
crystals in drops



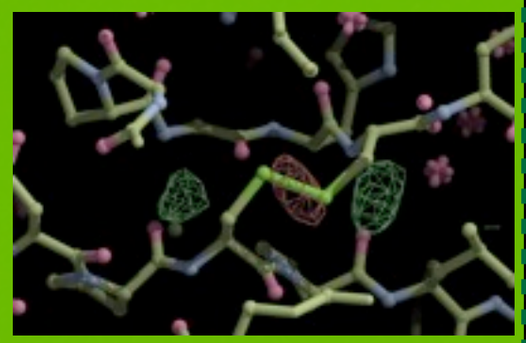


crystal identification in loops at beamlines (UV-LED based, no noticeable damage one samples)

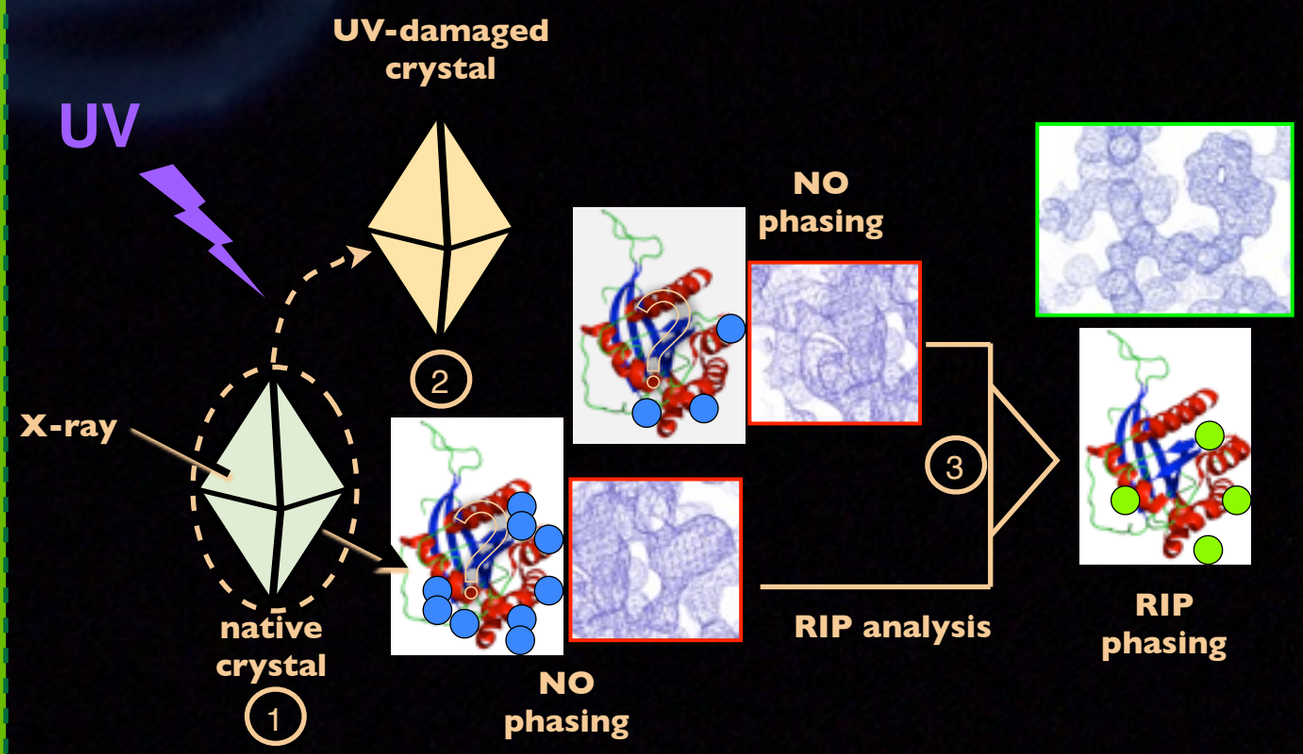
Specific Radiation Damage on Biological Samples



cytosine deamination of oligonucleotides



alteration of sulfur-carbon (Met), disulfide (Cys), and OH-carbon (Tyr) bonds in proteins



~ R & D

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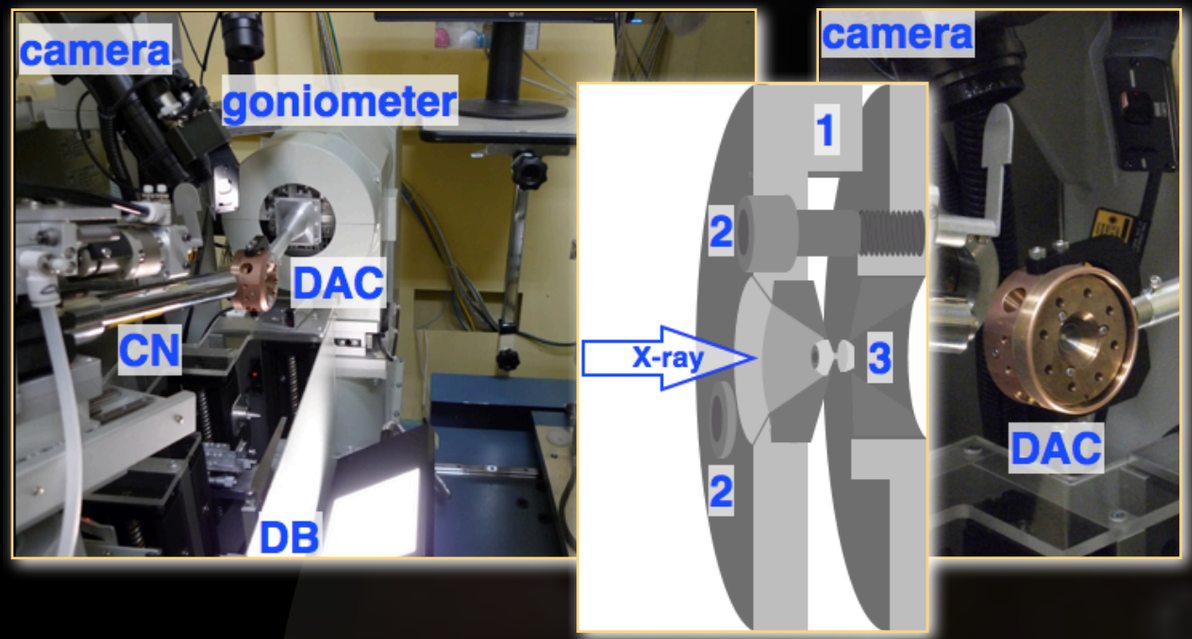
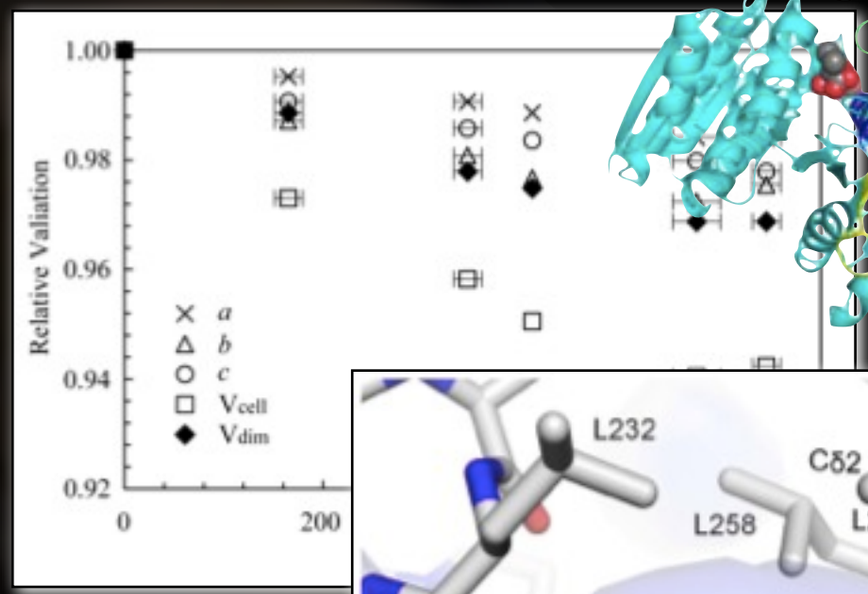
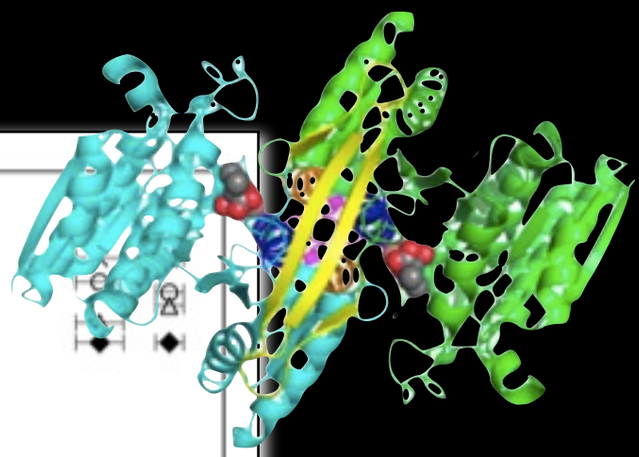
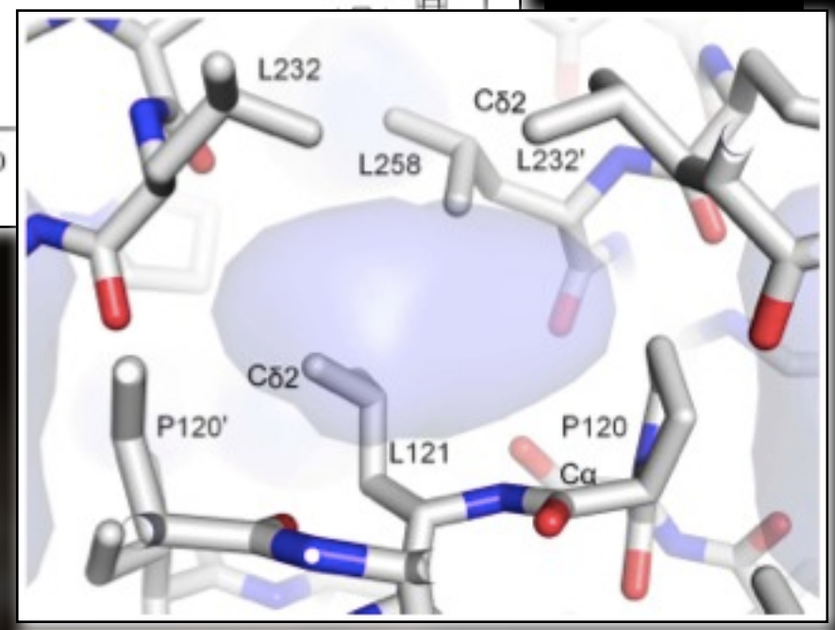
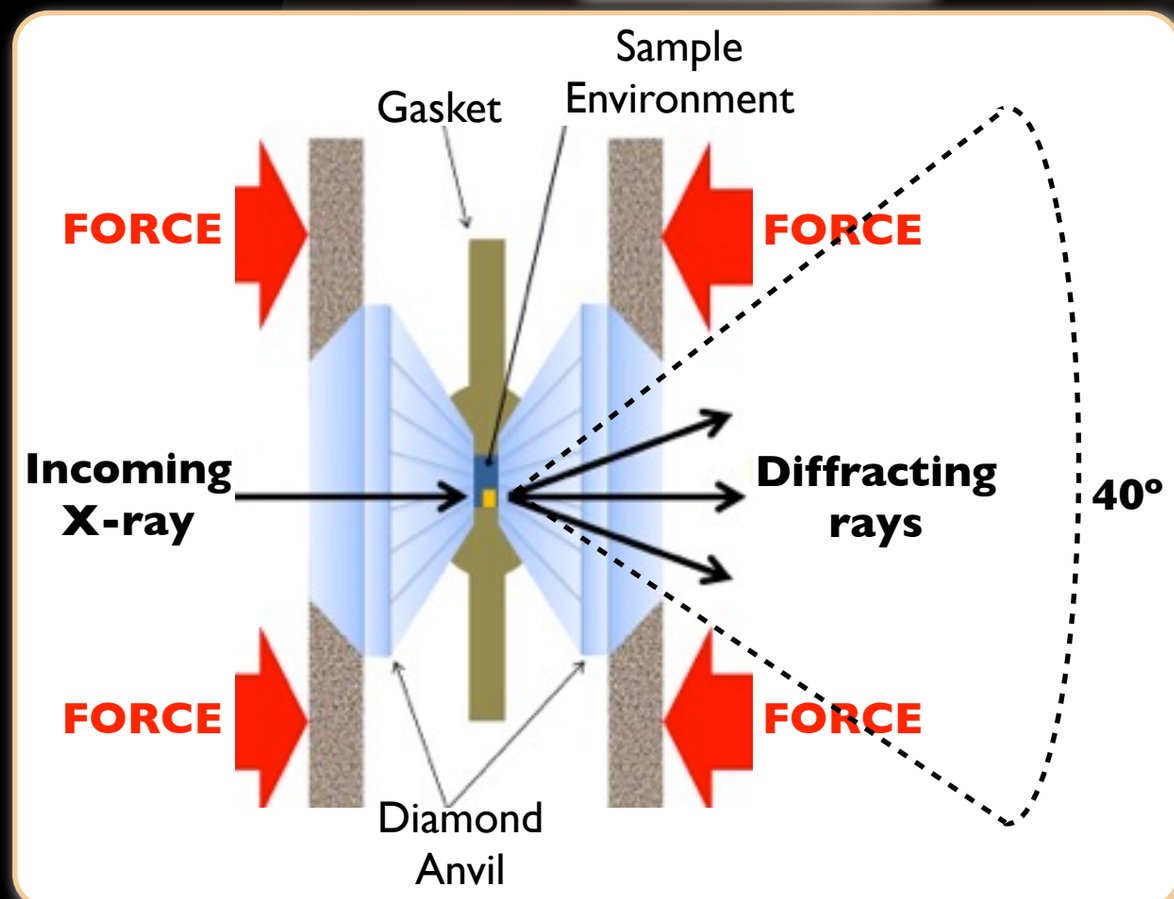
High-Pressure Set-up

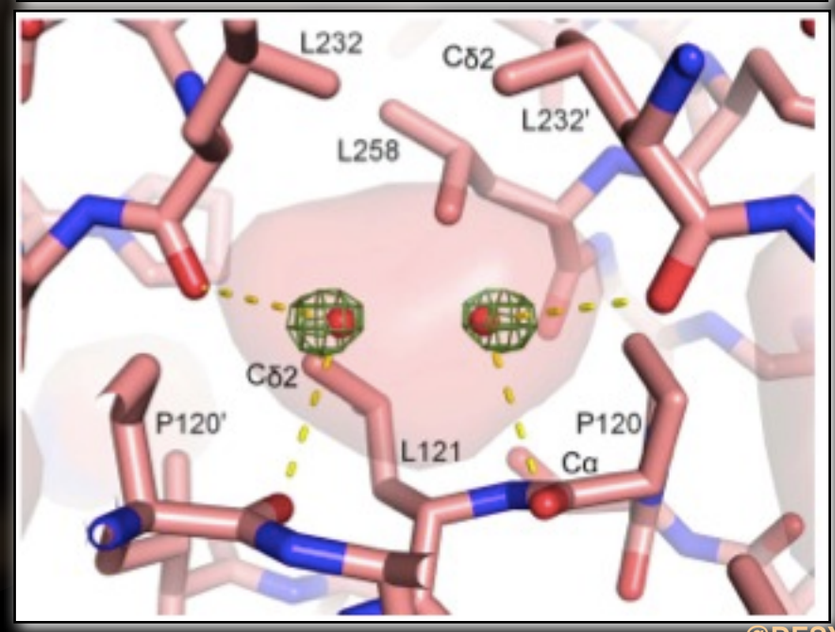




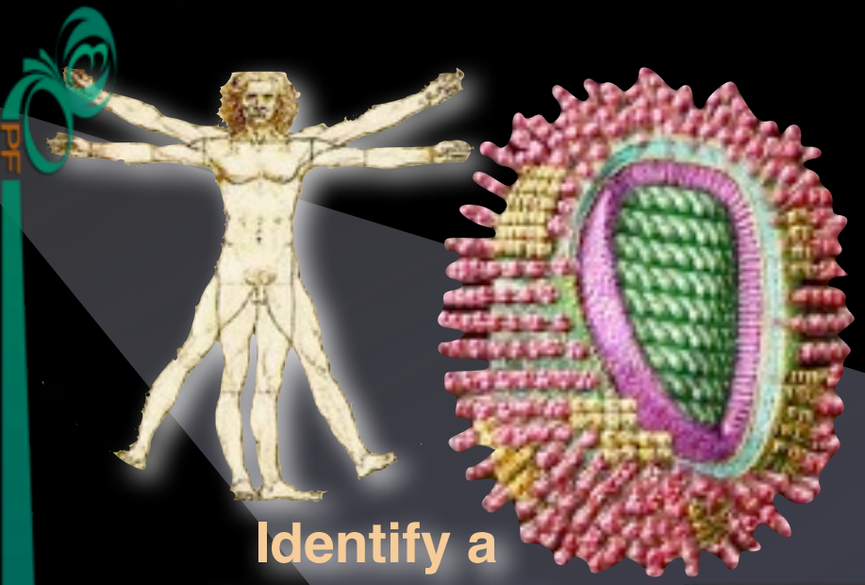






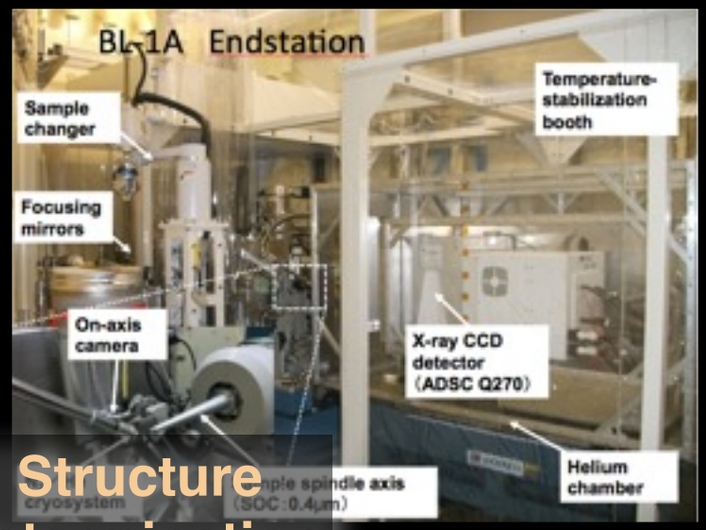


Conclusions



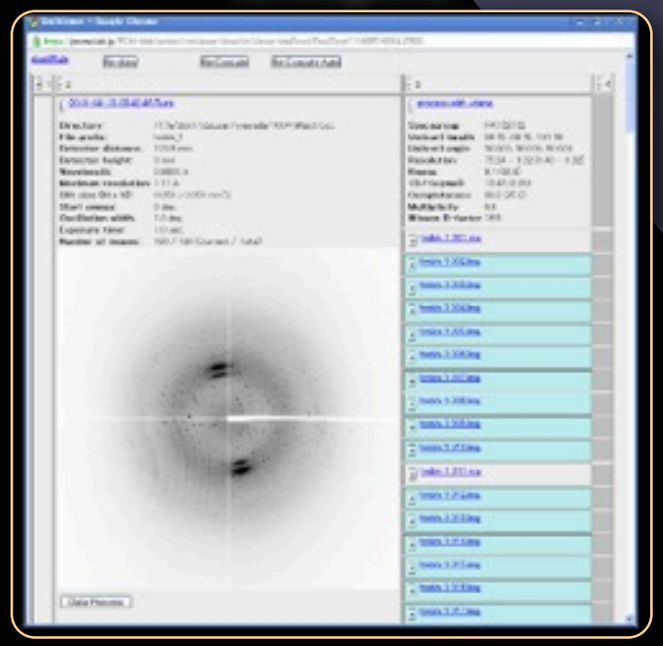
Identify a target model

X-ray analysis

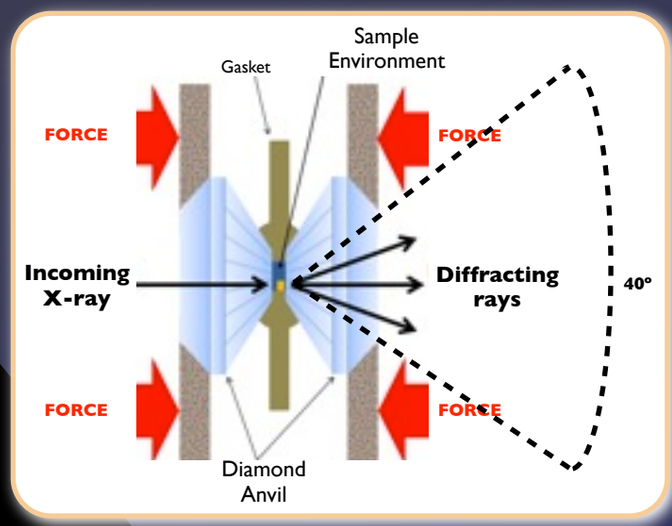


Structure determination

Detailed analysis



Data analysis



~ structural biology

Acknowledgments

KEK - PF

TPRP Targeted Proteins Research Program — MEXT

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U-VIX Corporation

astellas Astellas Pharma Inc.

… and more than
40 different collaborations worldwide
(SACLA XFEL, SPring-8, AS…)

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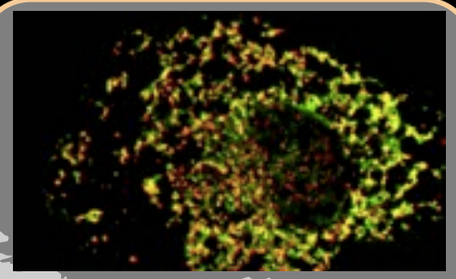
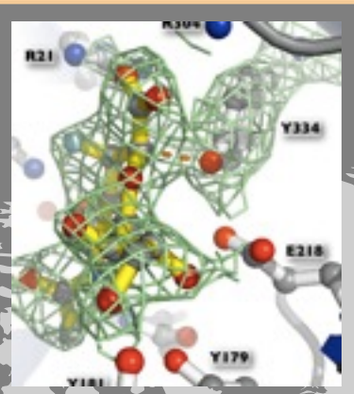
… Structural Biology Research Center

Substrate modeling
(Prof. Woods)

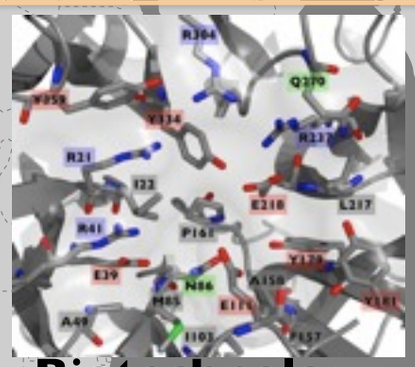


Human enzymes
(Dr. Monti)

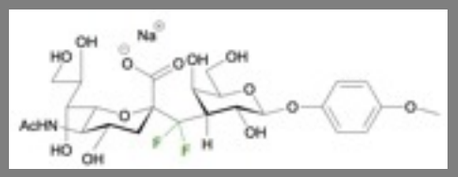


Enzymatic mechanism
(Prof. Withers)

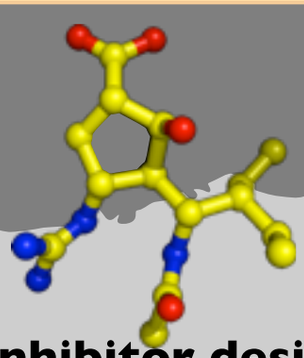
Space crystallization
(Dr. Satoh; JAXA)



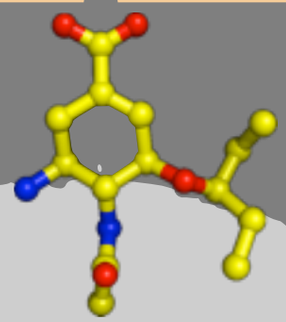
Biotechnology
(Prof. Itoh
Tokushima Univ.)



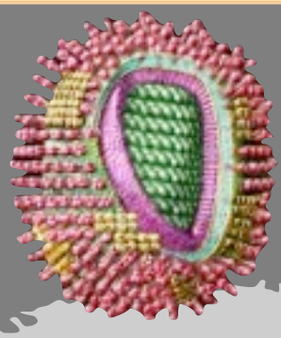
Substrate mimic
(Prof. Sodeoka
RIKEN)



Inhibitor design
(Prof. von Itzstein)



Tamiflu-related
(Prof. McKimmBreschkin)



Influenza-related
(Prof. Colman)

… Neu2 worldwide network