Cheiron School 2008 by AOFSRR SPring-8

5 October 2008

Protein Crystallography

High Energy Accelerator Research Organization (KEK)
Institute of Materials Science
Photon Factory
Structural Biology Research Center

Soichi Wakatsuki

soichi.wakatsuki@kek.jp

http://pfweis.kek.jp/eng/index.html

Outline

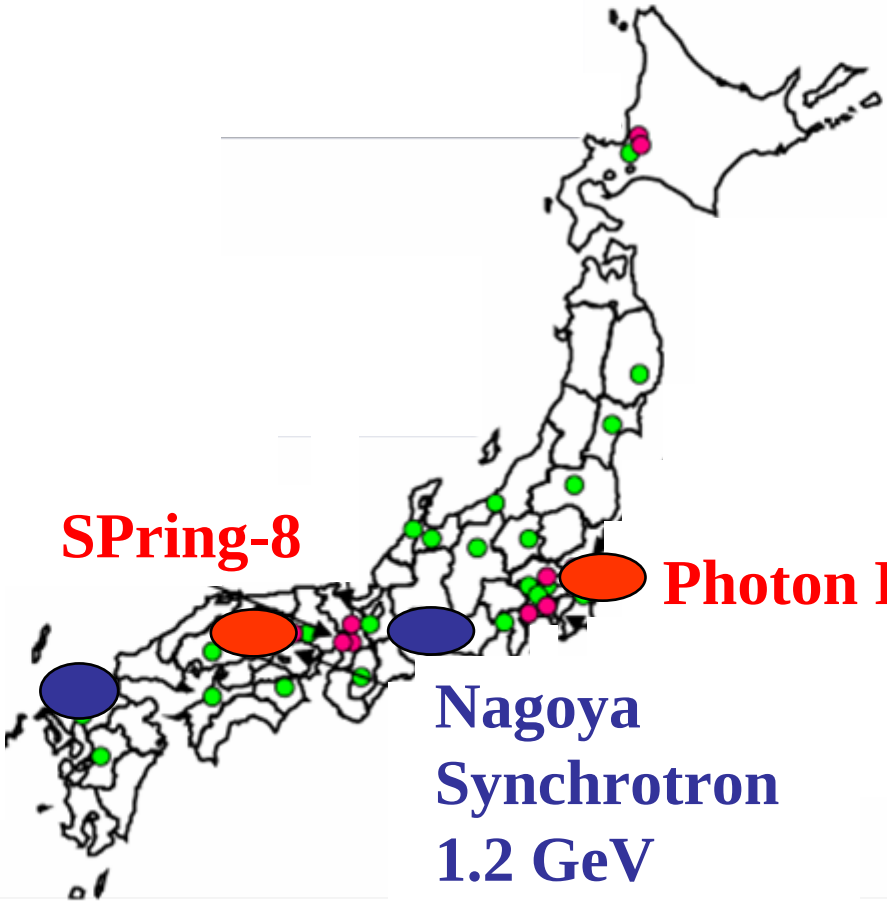
1. **Introduction to protein crystallography**

2. **High throughput technologies for synchrotron experiments**
   systems approach

3. **Structural proteomics on post-translational modification and transport of proteins: protein-protein interactions in membrane traffic**

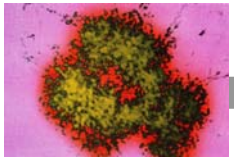
Synchrotron Facilities with PX in Japan

and new ones coming up

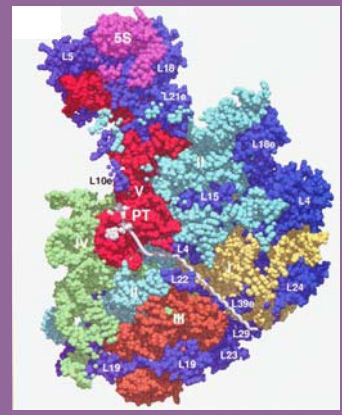


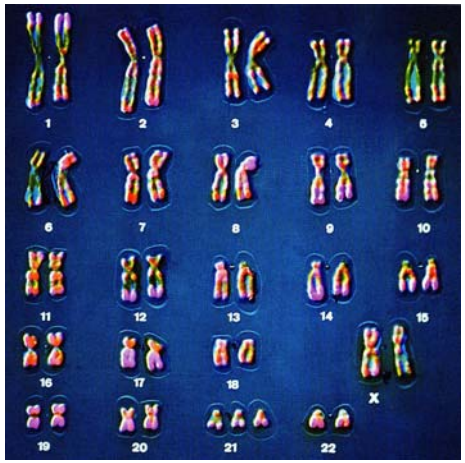
Role of Structural Biology

Genome



DNA→RNA→ **Proteins**



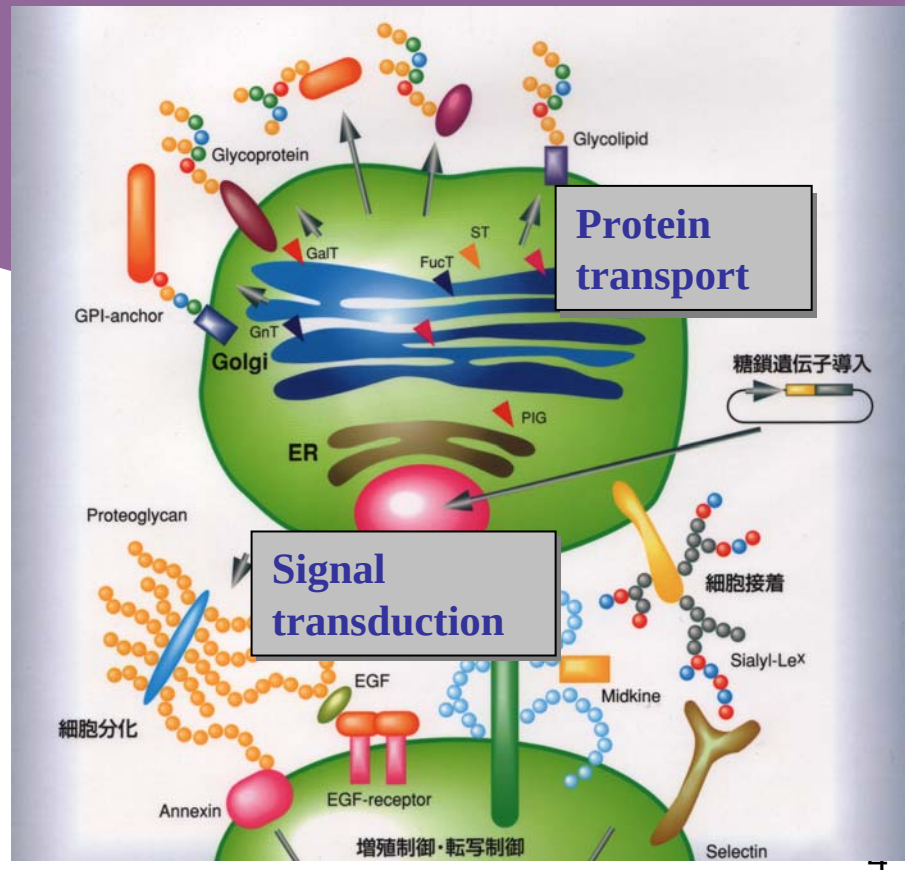


Molecular machines（ribosomes, enzymes）
Information network

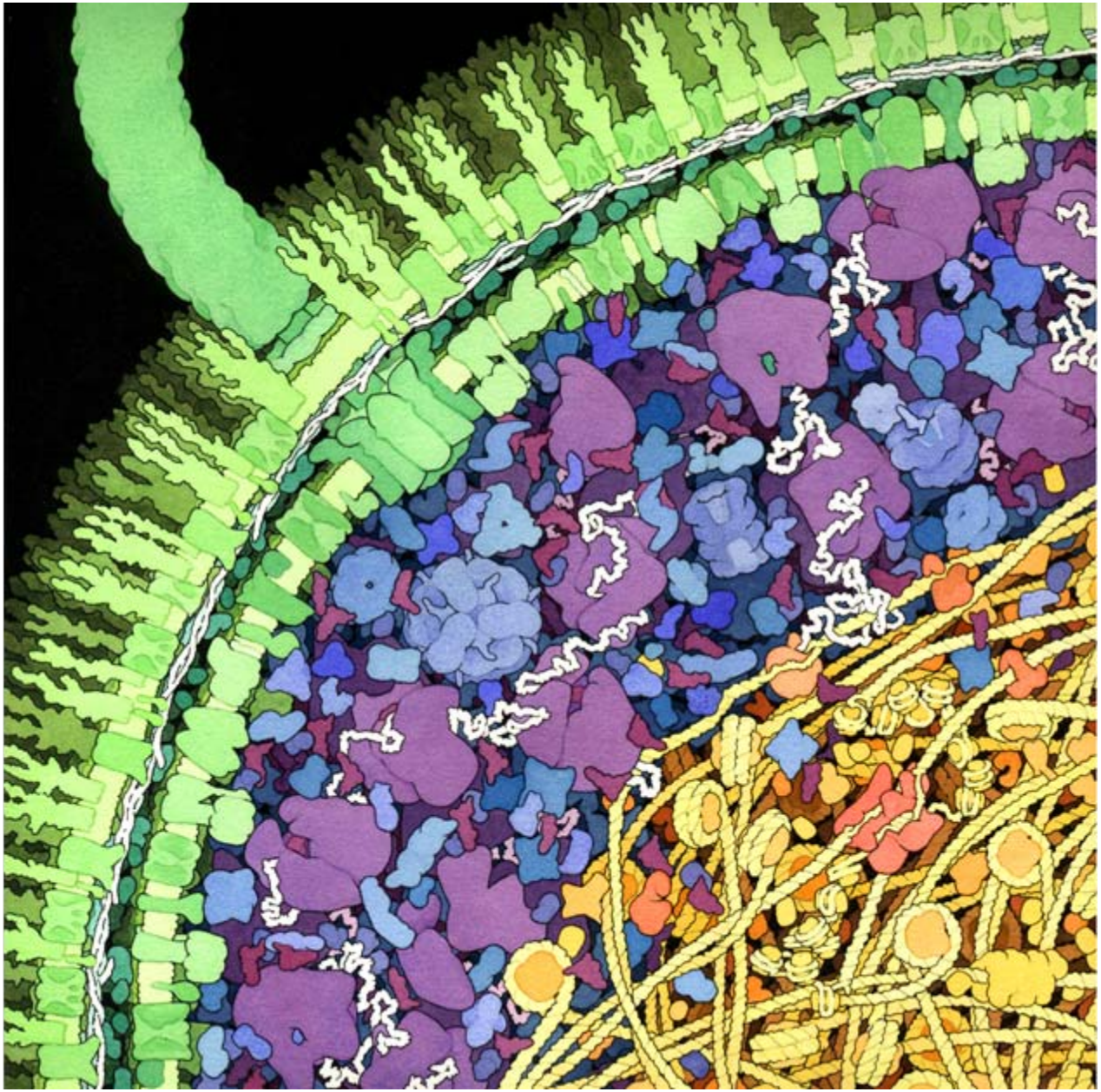
Atomic resolution analysis
- ○Protein structure and dynamics
- ○Interactions between molecular machines

Biology (Basic research)

Medicine, drug design (industrial applications)

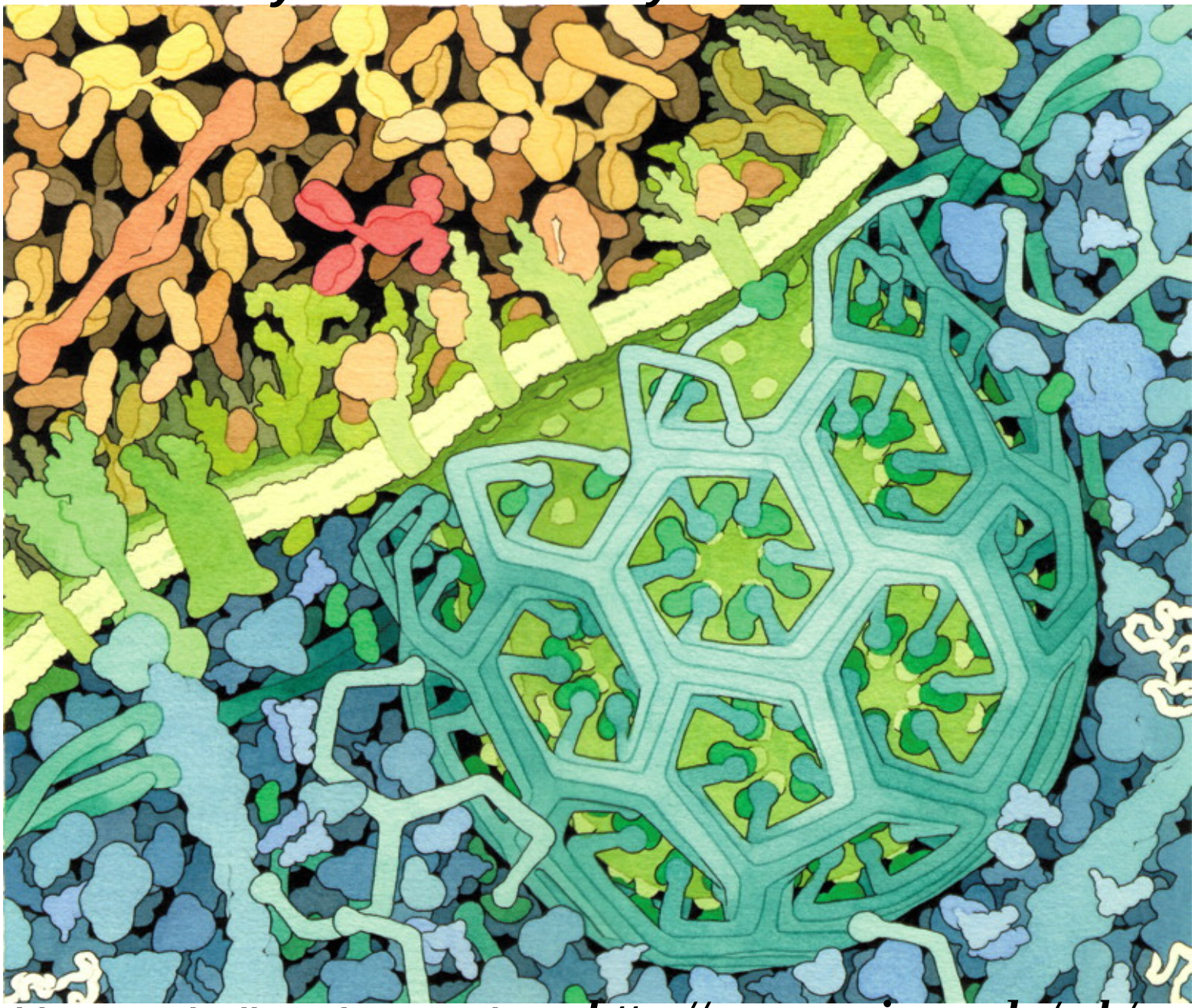


E. Coli cell

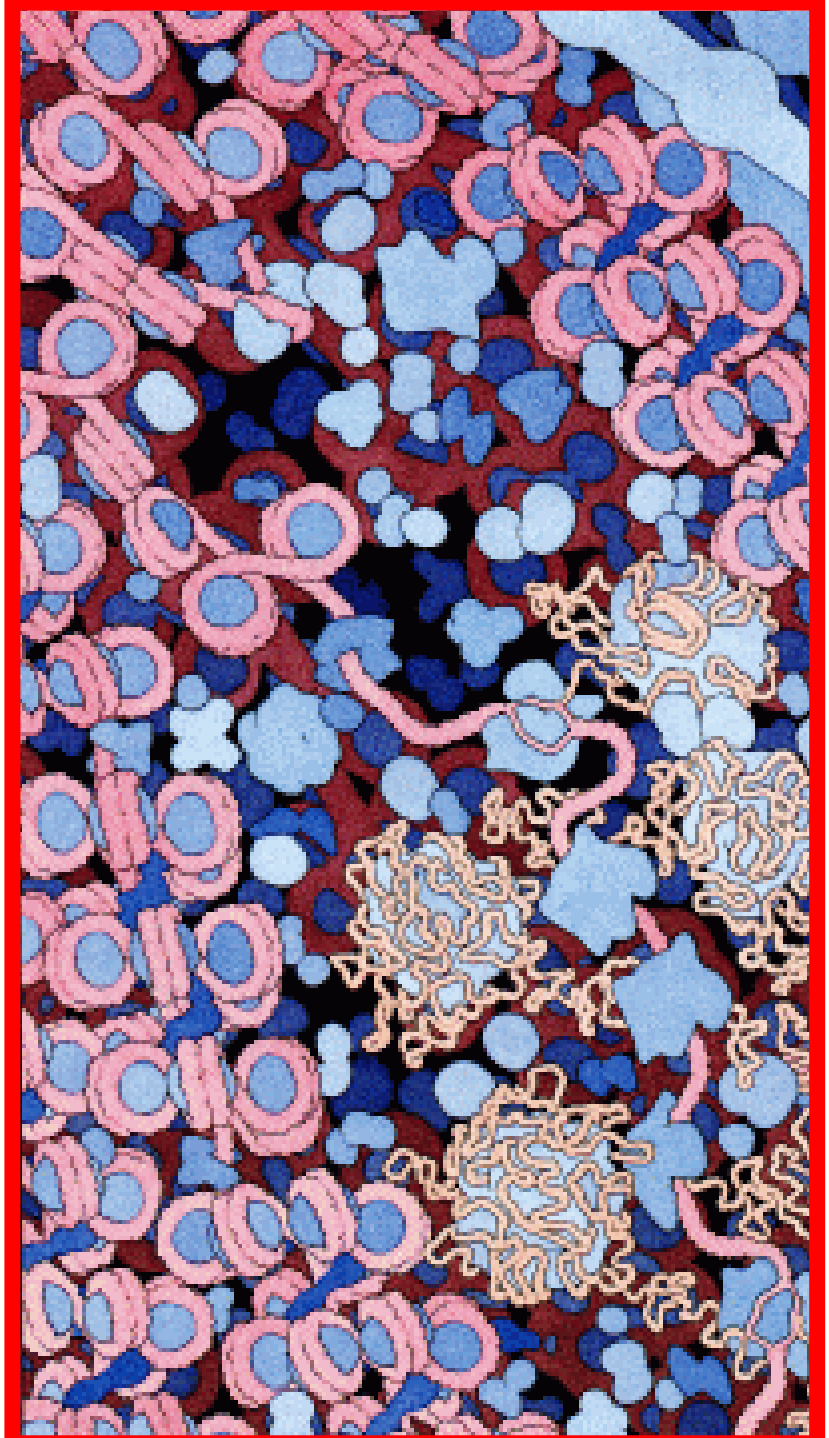


David S. Goodsell, Scripps Institute, http://www.scripps.edu/mb/goodsell/

Endocytosis of toxin by a clathrin vesicle

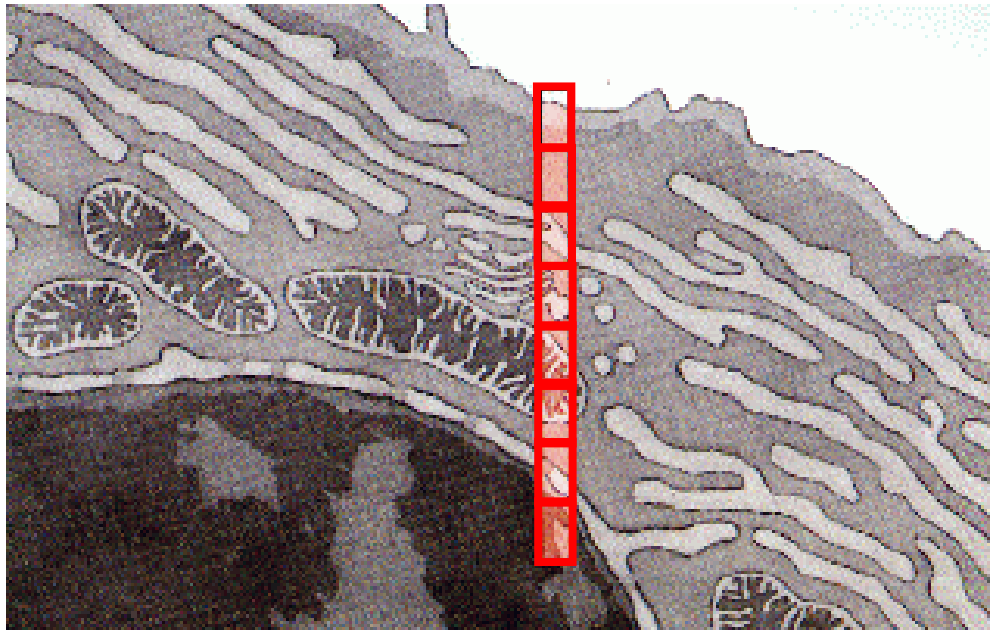


David S. Goodsell, Scripps Institute, http://www.scripps.edu/mb/goodsell/



Panoramic view of a eukaryotic cell

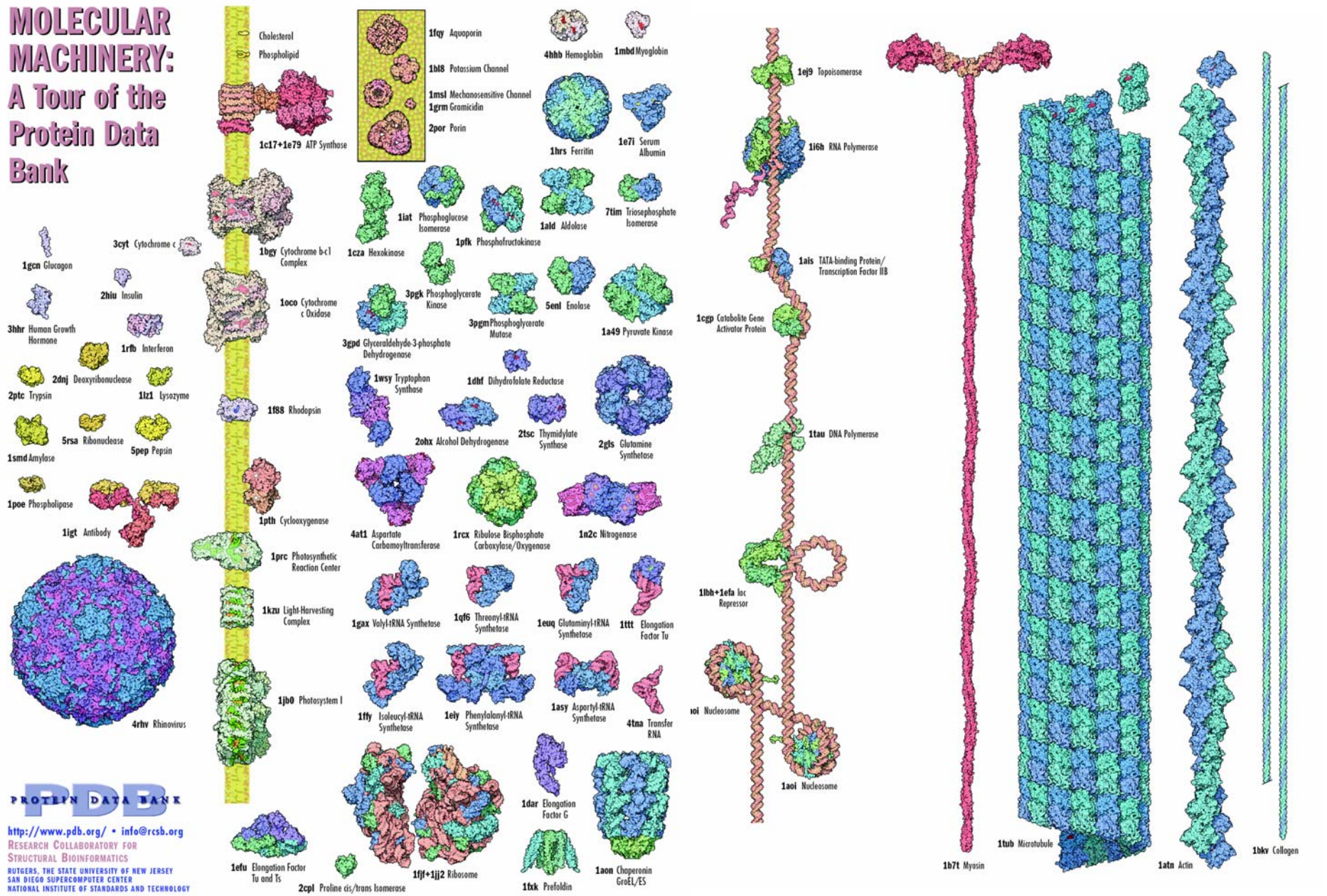
http://www.scripps.edu/mb/goodsell/



COLORS: proteins in blue, ribosomes in magenta, DNA and RNA in red and orange, lipids in yellow, and carbohydrates in green.

David S. Goodsell, Scripps Institute

The Worldwide Protein Data Bank (wwPDB) http://www.wwpdb.org/index.html



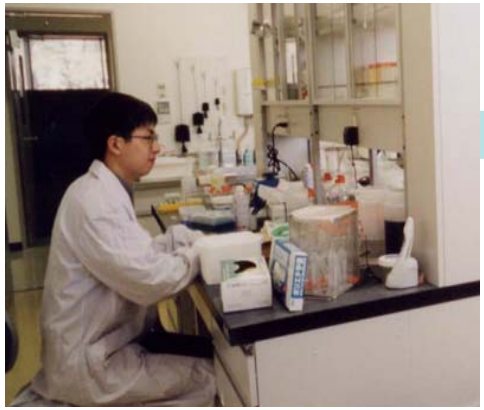
David S. Goodsell, Scripps Institute

Acetylcholine esterase

Prof. Joel Sussmann
Weizmann Institute, Israel

- ..¥Joel Sussman¥richardnew.mpg

Future: automated/integrated system

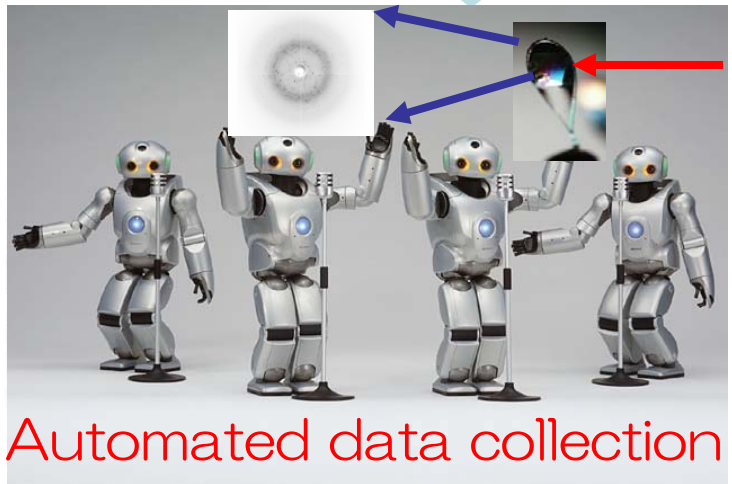


Expression and purification

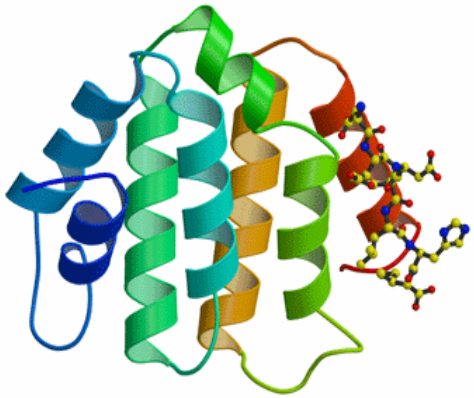
Crystallization robot

Crystallization

Crystal harvesting



Mouting & data collection



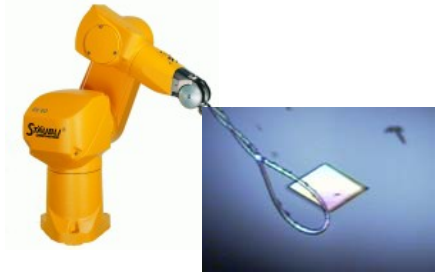
Data analysis

Flow of protein structure analysis

（1）Large scale protein expression and purification system

Crystalli-zation robot

（2）Crystal evaluation/harvesting system



（3）High brilliance microfocus MAD beam line

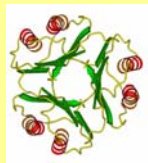
Minipole Undulator

PF-2.5 GeV SR Ring

Automated crystal exchange robot and alignment system inside the hutch

2D area detector

（4）Automated protein crystal structure analysis suite



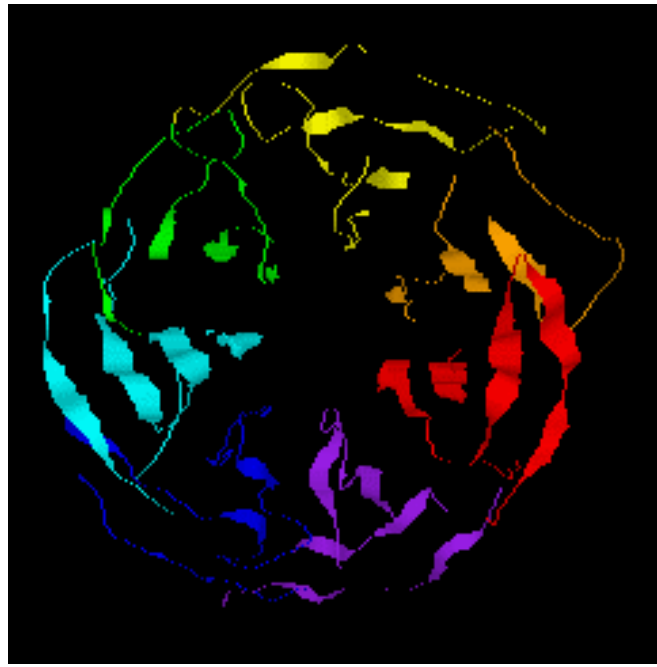
Guanine nucleotide exchange factor Sec12p

```
   1       ttcg tgacagctag ttataacgtc gggtatcctg cgtacggtgc aaaatttttg
  61 aataacgaca cattacttgt ggcaggcggt ggaggagaag gaaacaatgg cataccaaac
 121 aagctgacgg tcttgcgcgt ggatcctacc aaagatactg agaaggaaca gtttcatata
 181 ttgagcgagt ttgcattgga agacaacgac gactctccta ctgcaattga cgcttccaag
 241 ggtatcattt tggttggctg caatgaaaat agcactaaga ttacccaagg taaaggtaat
 301 aagcacttga gaaaatttaa atacgataaa gtgaatgatc aattggagtt cctcactagt
 361 gtagactttg acgcatctac aaatgcggat gactacacga agctggttta tatttcacga
 421 gaaggtaccg ttgcagctat cgcatcatct aaagtacctg ctataatgag aatcattgac
 481 ccgagcgact tgacagagaa gtttgagatc gagactaggg gtgaagtaaa ggatttacac
 541 ttttccactg atggtaaggt tgttgcttat atcaccggtt ctagcttgga agtgatttca
 601 acagtgactg gaagttgcat tgctaggaaa acagattttg ataagaattg gagtttatct
 661 aaaataaact tcatagccga tgacacagta ttgatagcag cctctttaaa aaaagggaaa
 721 ggtattgtgc tgaccaaaat aagcatcaaa tcaggaaaca cttccgtatt aagatccaaa
 781 caagtgacaa acagattcaa agggattact tctatggatg tcgacatgaa gggtgaattg
 841 gcggtactgg caagtaatga caattccata gctcttgtga aactaaaaga cctgtcaatg
 901 tctaaaatat tcaaacaagc tcatagtttt gccattacag aggtcactat ctctccggac
 961 tctacatatg tggcgagtgt ttcggcagcc aacactatcc acataataaa attaccgctt
1021 aactacgcca actacacctc aatgaaacaa aaaatctcta aatttttcac caacttcatc
1081 cttattgtgc tgctttctta cattttacag ttctcctata agcacaattt gcattccatg
1141 cttttcaatt acgcgaagga caattttcta acgaaaagag acaccatctc ttcgccctac
1201 gtagttgatg aagacttaca tcaaacaact ttgtttggca accacggtac aaaaacatct
1261 gtacctagcg tagattccat aaaagtgcat ggcgtgcatg agacgagttc tgtgaatgga
1321 actgaagtct tatgtactga aagtaacatt attaatactg gaggggcaga gtttgagatc
1381 accaacgcaa cttttcgaga aatagatgat gcttga
```

No. of bases : 1416

```
  FVTASYNVGYPAYGAKFLNNDTLLVAGGGGEGNNGIPNKLTV
LRVDPTKDTEKEQFHILSEFALEDNDDSPTAIDASKGIILVGCNENSTKITQGKGNKH
LRKFKYDKVNDQLEFLTSVDFDASTNADDYTKLVYISREGTVAAIASSKVPAIMRIID
PSDLTEKFEIETRGEVKDLHFSTDGKVVAYITGSSLEVISTVTGSCIARKTDFDKNWS
LSKINFIADDTVLIAASLKKGKGIVLTKISIKSGNTSVLRSKQVTNRFKGITSMDVDM
KGELAVLASNDNSIALVKLKDLSMSKIFKQAHSFAITEVTISPDSTYVASVSAANTIH
IIKLPLNYANYTSMKQKISKFFTNFILIVLLSYILQFSYKHNLHSMLFNYAKDNFLTK
RDTISSPYVVDEDLHQTTLFGNHGTKTSVPSVDSIKVHGVHETSSVNGTEVLCTESNI
INTGGAEFEITNATFREIDDA
```

No of amino acid residues : 471



Model structure of Sec12

(1416-3)/3＝471

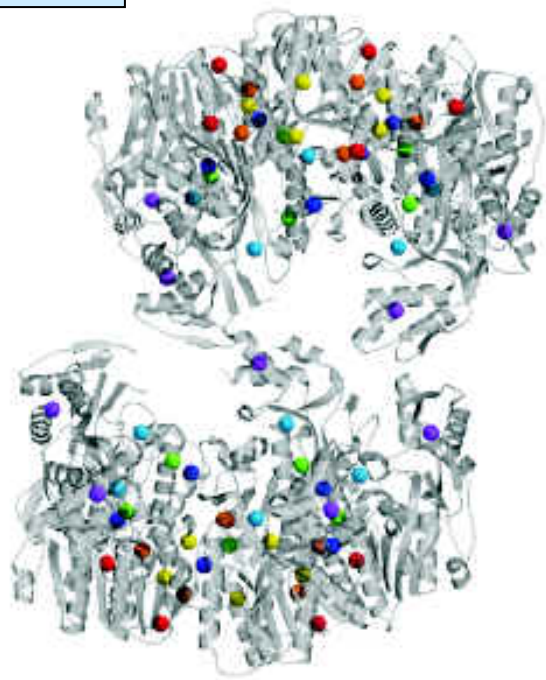
Phase Determination using Multiple Anomalous Dispersion

Methionine

Sulfur ⟶ Selenium (SeMet)

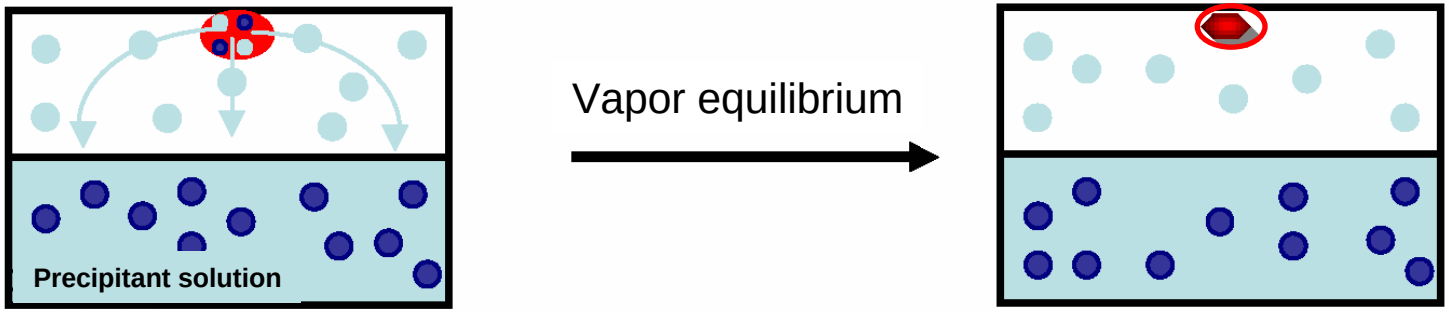
```
  FVTASYNVGYPAYGAKFLNNDTLLVAGGGGEGNNGIPNKLTV
LRVDPTKDTEKEQFHILSEFALEDNDDSPTAIDASKGIILVGCNENSTKITQGKGNKH
LRKFKYDKVNDQLEFLTSVDFDASTNADDYTKLVYISREGTVAAIASSKVPAIMRIID
PSDLTEKFEIETRGEVKDLHFSTDGKVVAYITGSSLEVISTVTGSCIARKTDFDKNWS
LSKINFIADDTVLIAASLKKGKGIVLTKISIKSGNTSVLRSKQVTNRFKGITSMDVDM
KGELAVLASNDNSIALVKLKDLSMSKIFKQAHSFAITEVTISPDSTYVASVSAANTIH
IIKLPLNYANYTSMKQKISKFFTNFILIVLLSYILQFSYKHNLHSMLFNYAKDNFLTK
RDTISSPYVVDEDLHQTTLFGNHGTKTSVPSVDSIKVHGVHETSSVNGTEVLCTESNI
INTGGAEFEITNATFREIDDA
```

No. of amino acid residues: 471
including 6 methionines



A ribbon representation of a protein with 70 selenium atoms superimposed in colour. Equivalent selenium atoms from molecule to molecule are coloured the same.

Protein crystallization by vapor diffusion method



Protein solution drop (containing water, precipitant and protein molecules)

Precipitant molecule

Water molecule

Protein crystal

Drawing by Prof. Yoshiki Higuchi

Protein Crystallization and crystal observation robot system

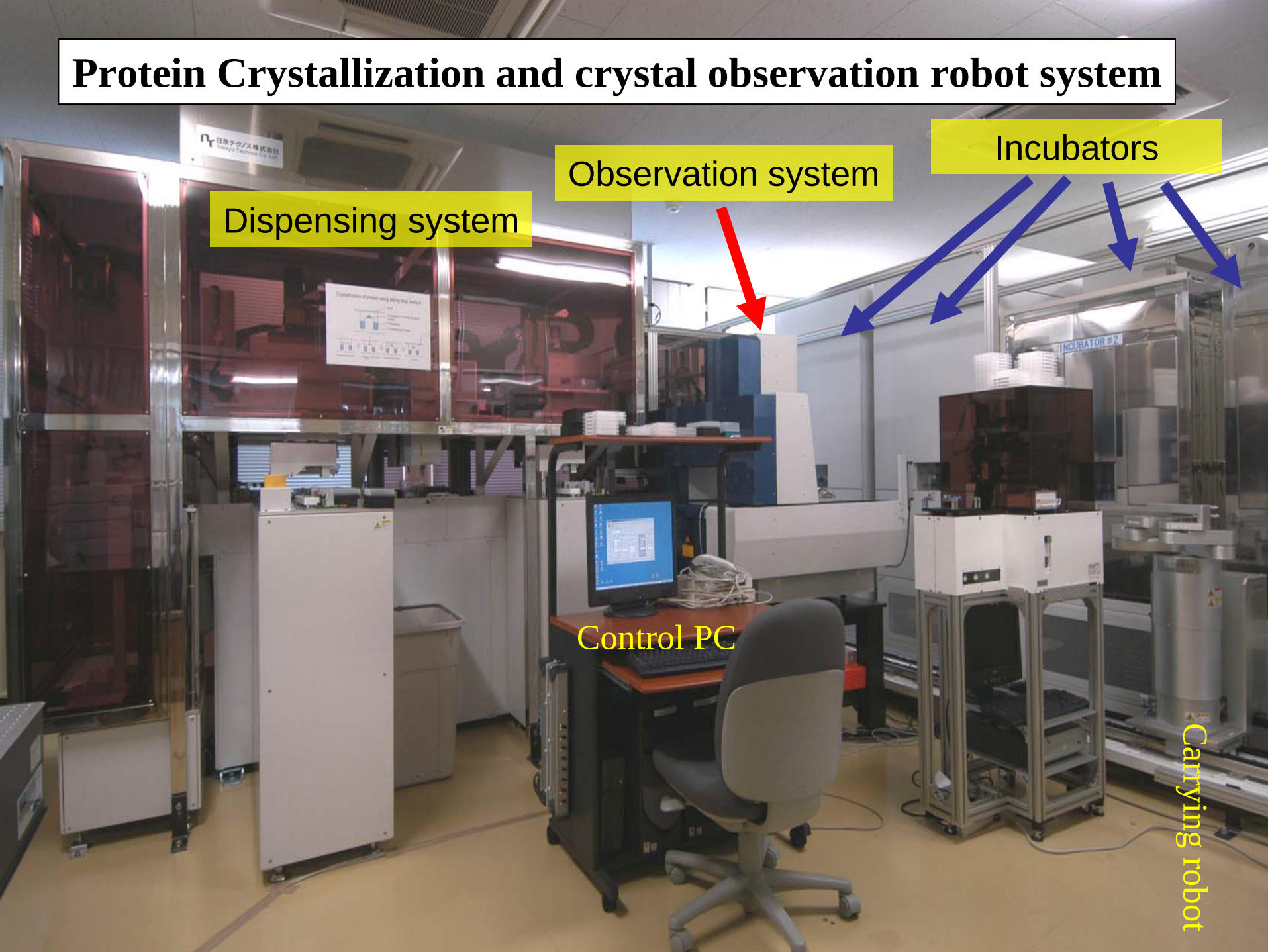
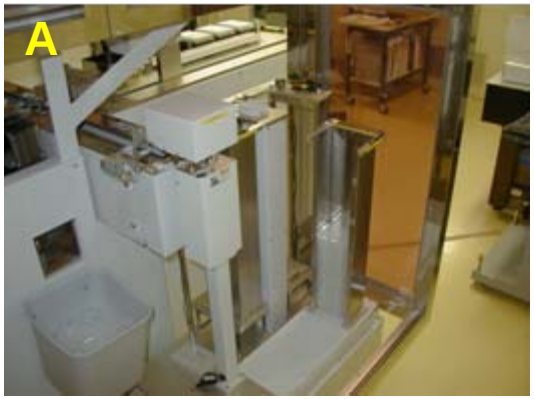
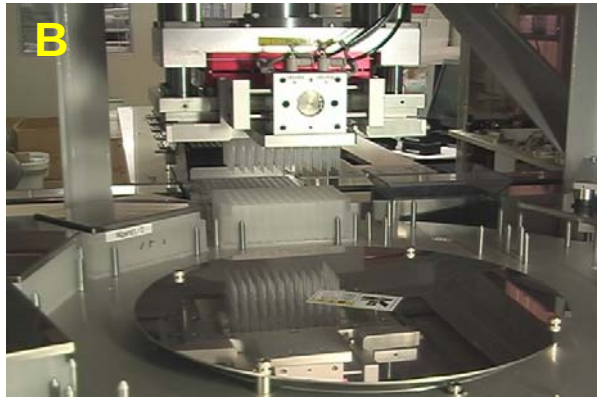
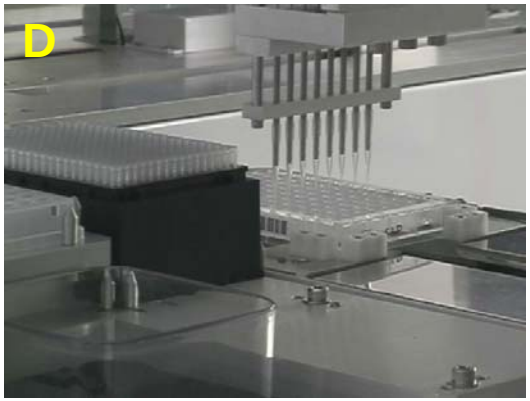
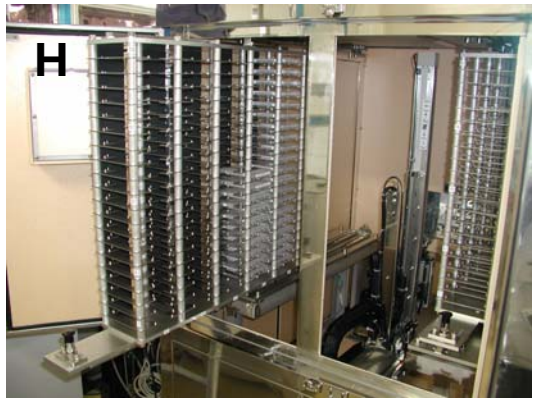


Fig. 3





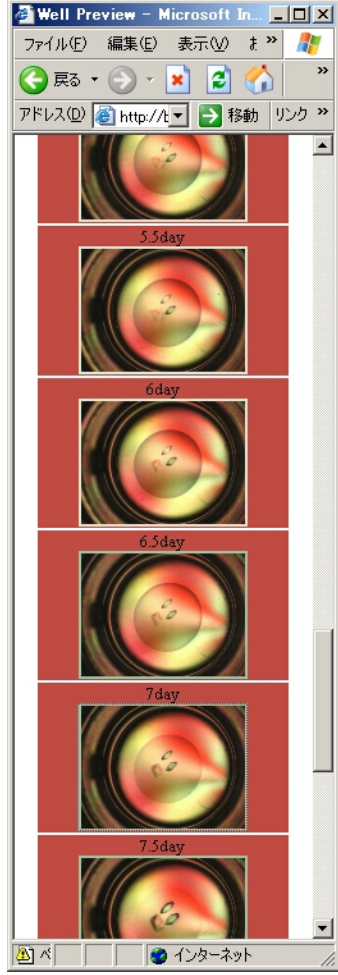


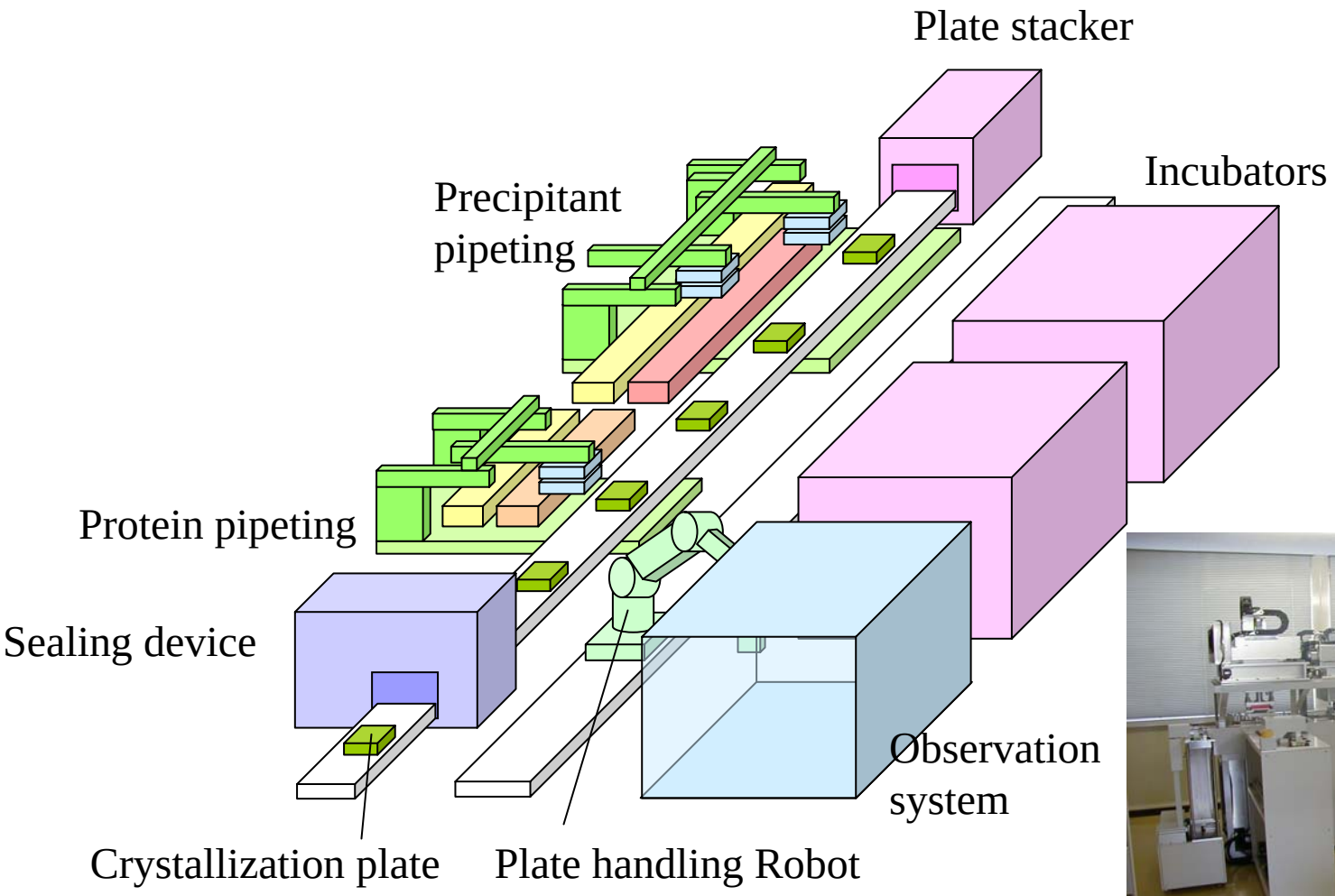


Protein Crystallization and crystal observation robot system

Speed: 1 plate/36 sec = 2,500 plates/day = 240,000 trials/day

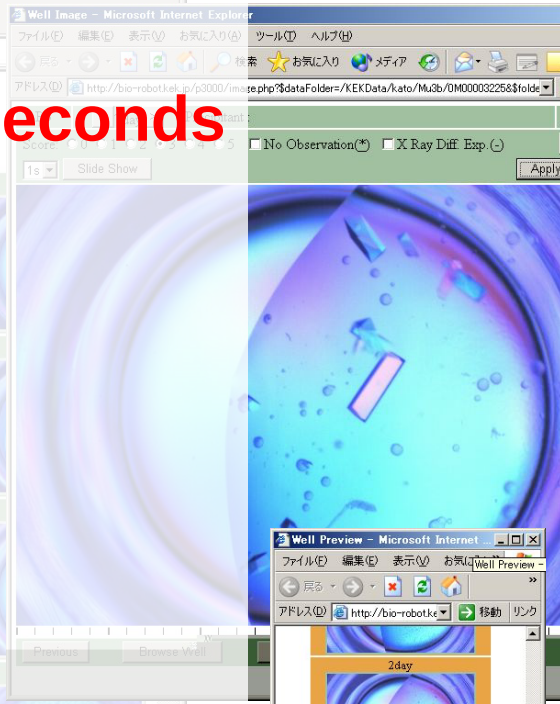
Sitting drop volume: 500 nL later down to 50 nL





Protein Crystallization and crystal observation robot system

- Fastest crystallization robot
  Dispensing : Sitting drop vapor diffusion
  : One plate (96 wells) every 36 seconds
  : 180 seconds for each plate
  (from dispensing to sealing)
  About 2,500 plates per day
  About 240,000 drops per day
  One batch capacity: 8 proteins, 960 precipitants,
  80 plates



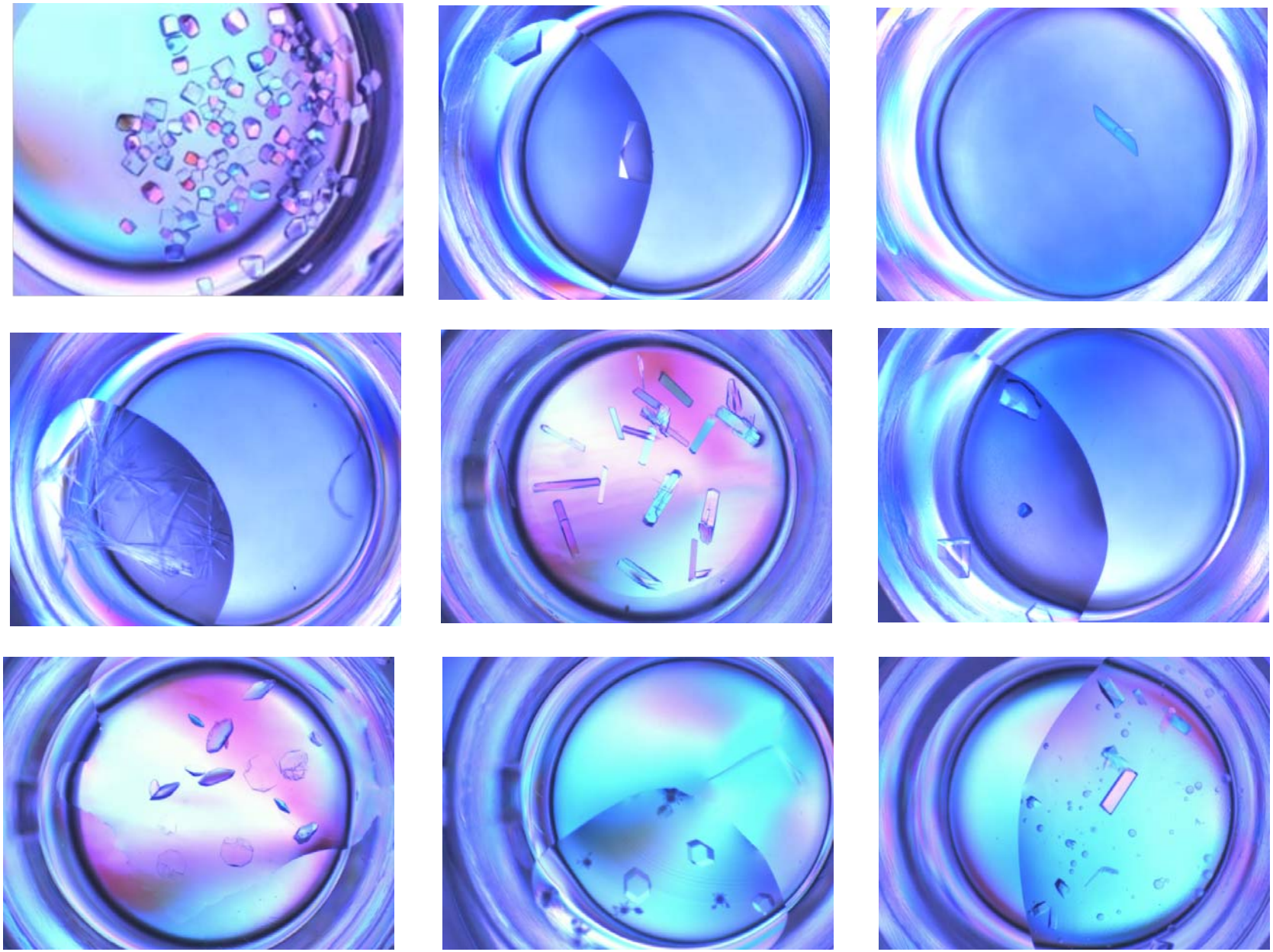
Example: 500 conditions:

2 days by manual operation

→ 10 min (everything included)

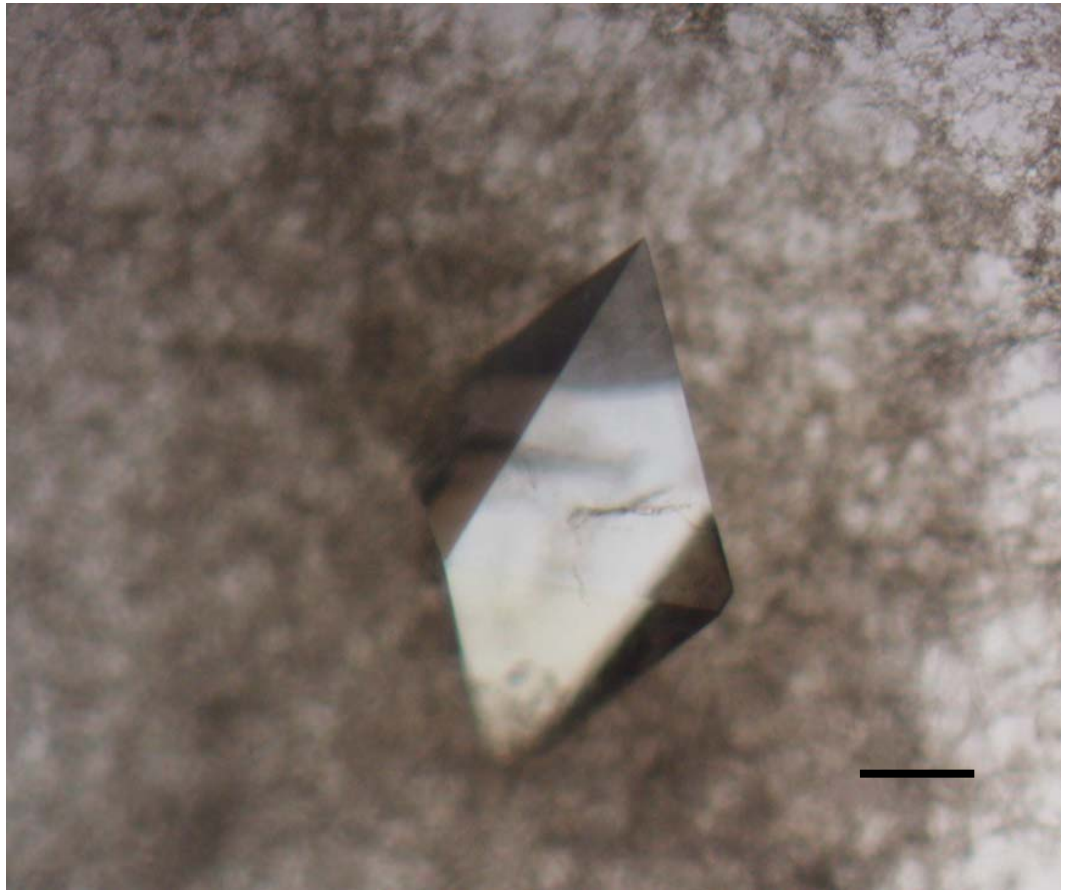
- Web browser for crystal growth observation
  - To be developed for collaboration and as a user facility

Protein Crystals



~10^{12} proteins in a typical (good size) crystal

Crystal of Human GGA1 VHS domain



bar＝0.1 mm

Crystallization method: hanging drop vapor diffusion

Protein conc.: 13 mg / ml

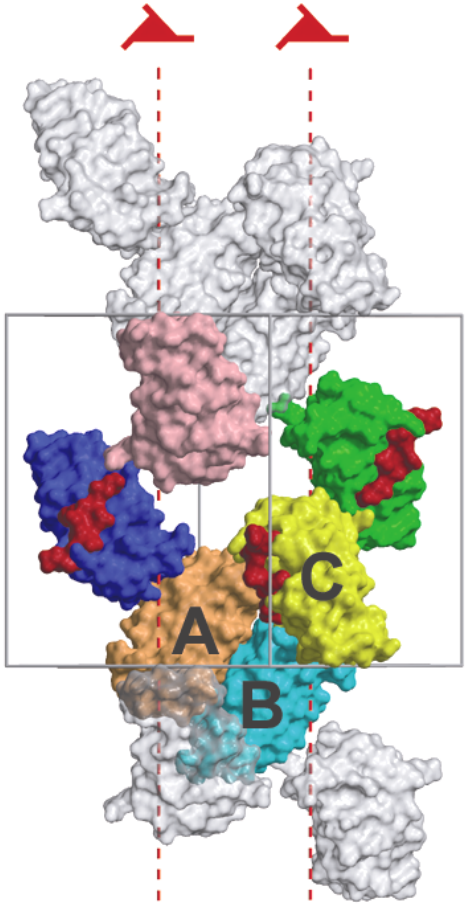
Precipitant: 17 % (w/v) PEG3350, 0.2 M KH_2PO_4

Buffer: 0.1 M Tris-HCl (pH 7.5)

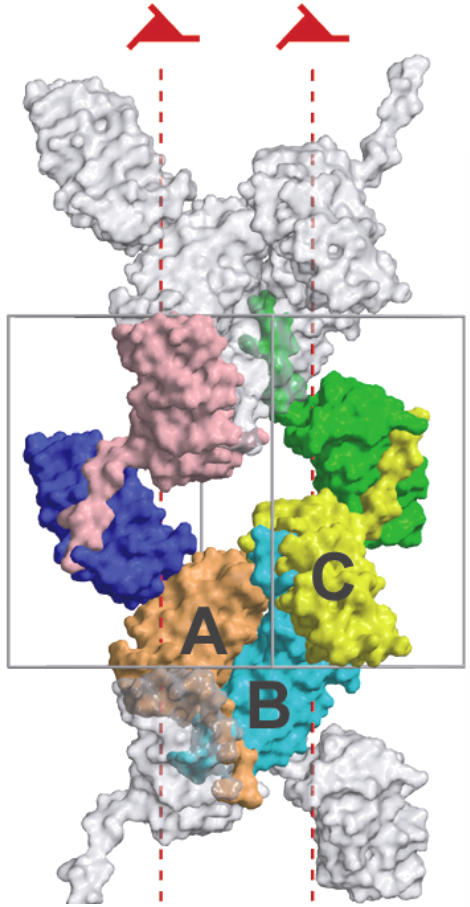
Temperature: 20 °C

Packing of proteins in a crystal

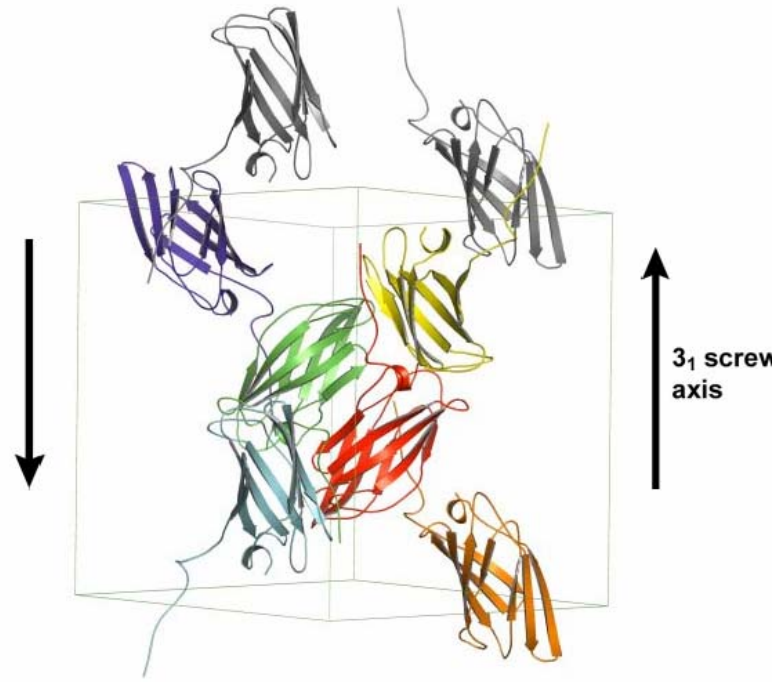
30~70 % volume of protein crystals is solvent!

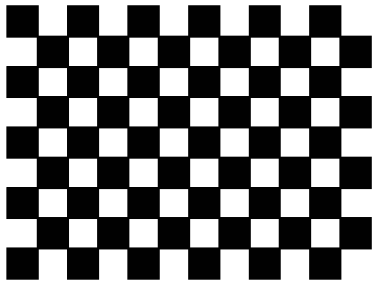


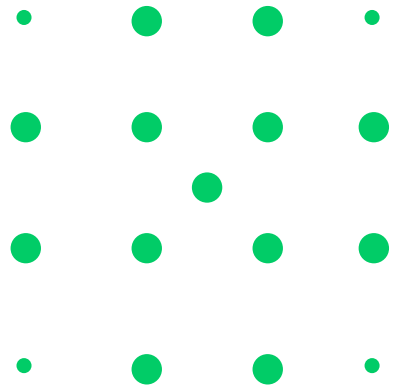
Cocrystal

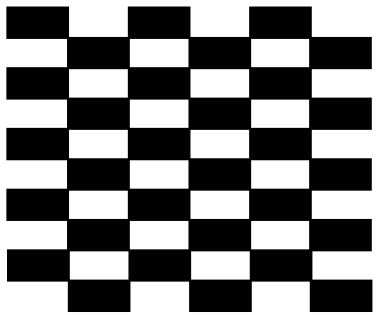


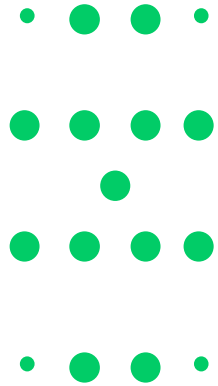
Peptide-tagged

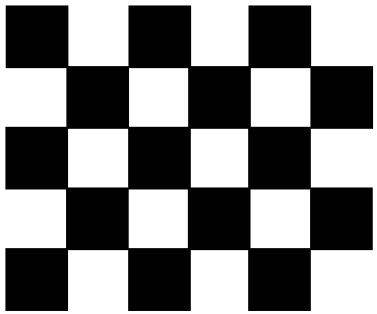


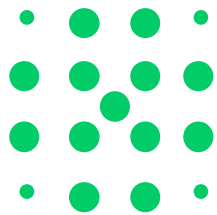


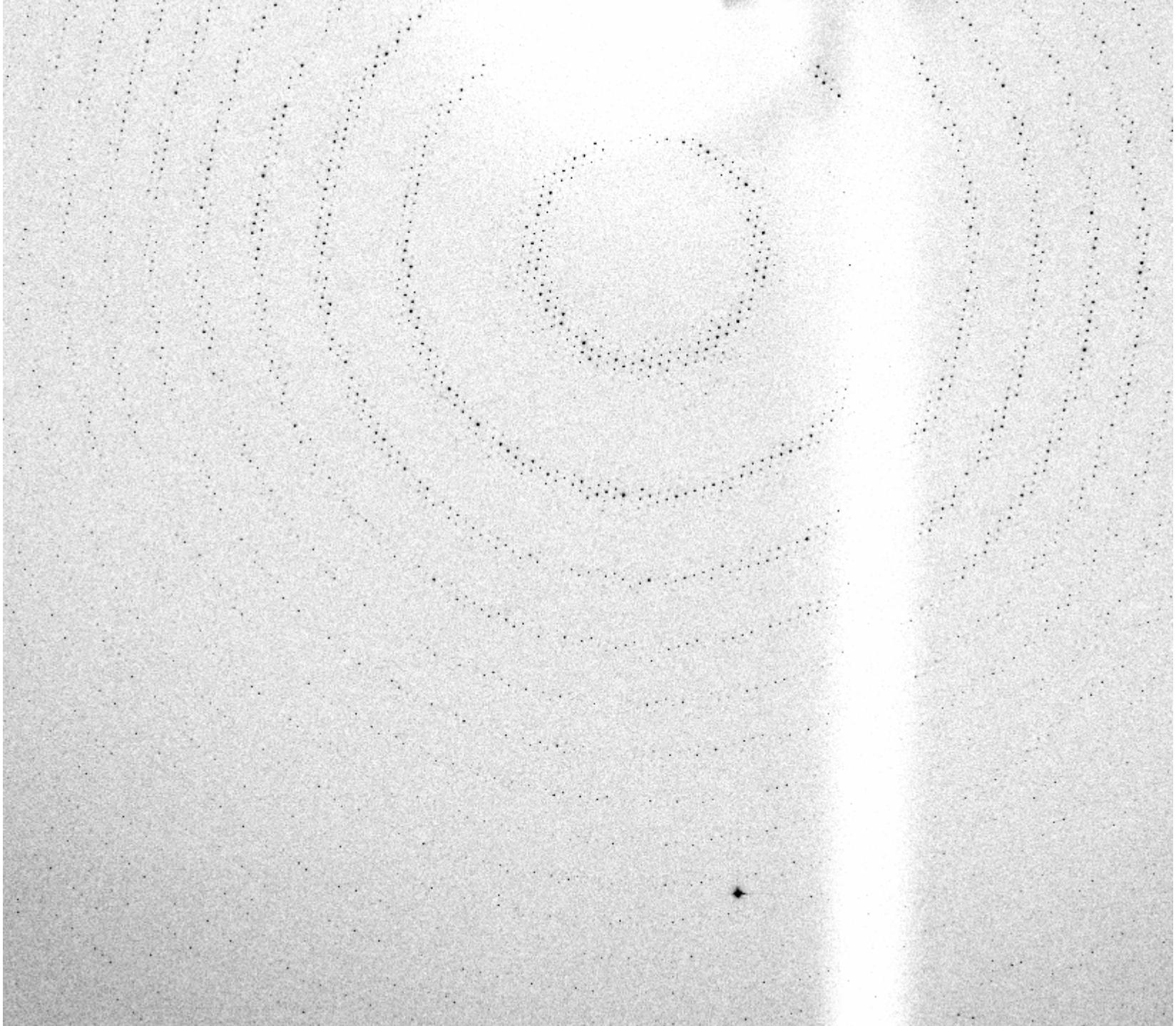


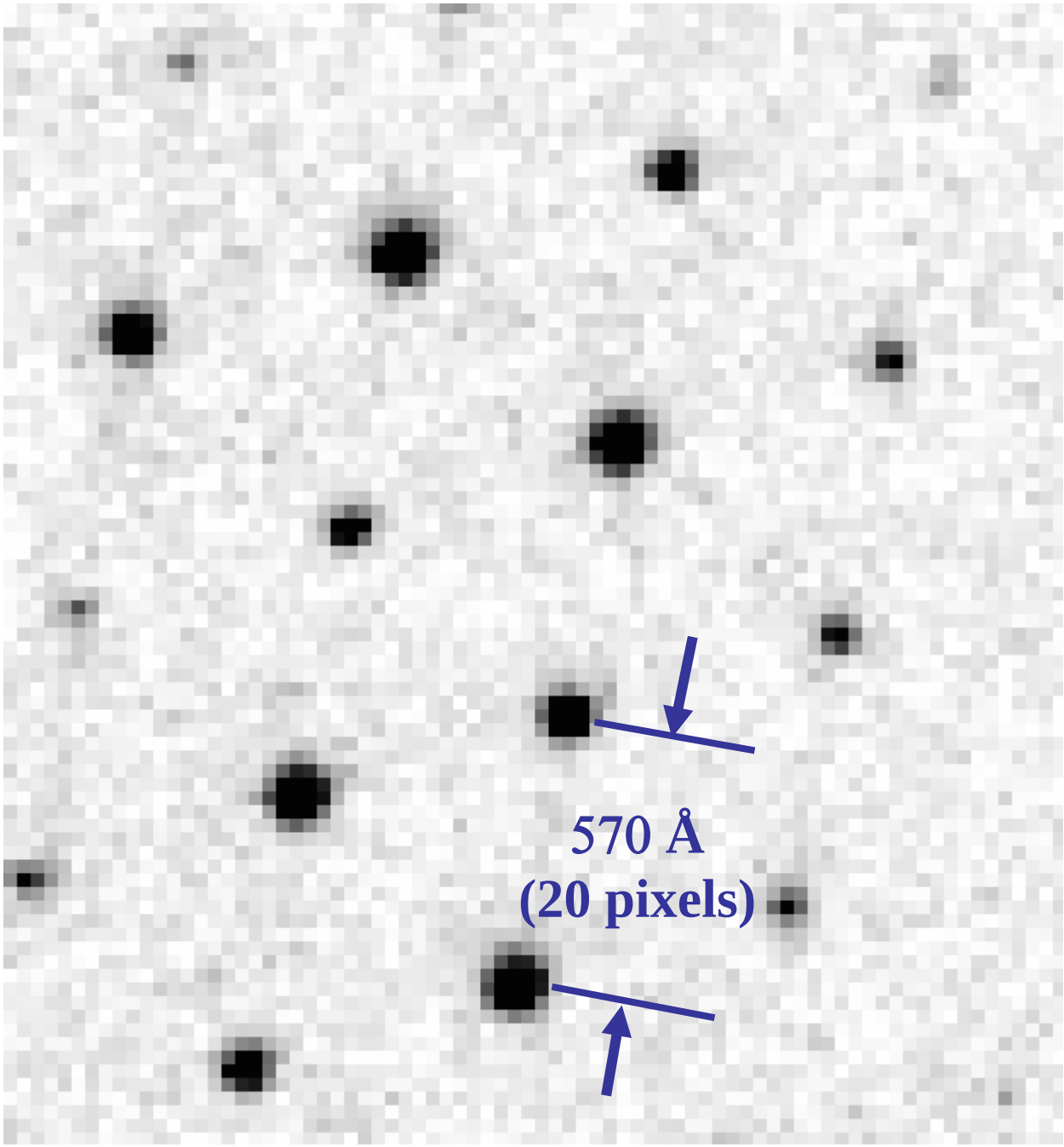












Blue Tongue Virus

Core Particle 1

$P2_12_12$

755 X 796 X 825 $Å^3$

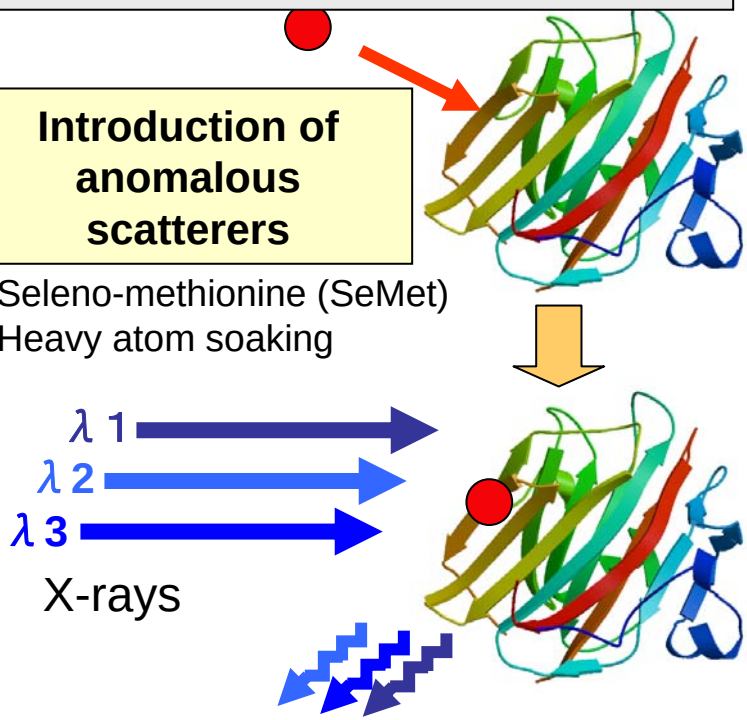
ESRF ID14/EH3

Detector: Imaging Plate

Crystal to det distance	1250 mm
Wavelength	0.918Å
Osc. Angle	0.1 deg
Exp. Time	100 sec
Pixel size	100 mm
Beam size	100 mm
FWHM	181 mm

Methods to Determine Phases

Heavy atom MAD (multiple anomalous dispersion)

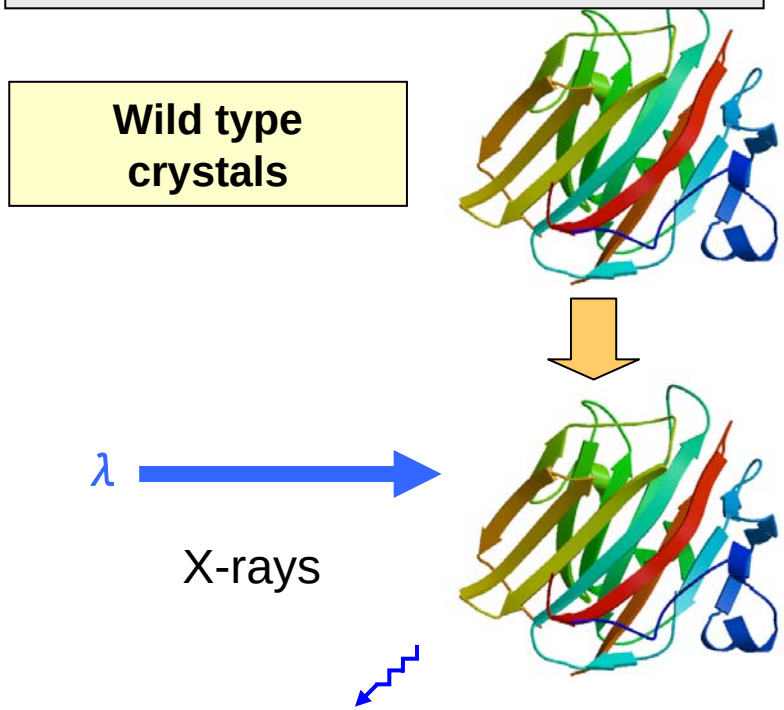


Multi wavelength anomalous dispersion

・Good method as long as heavy atom derivatives are available
・Multiple data sets need to be collected quickly
・Possible to use for extremely large complexes

Many successes

Light atom SAD (Single anomalous dispersion)

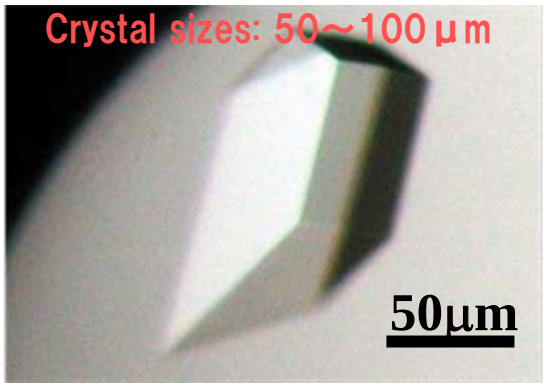


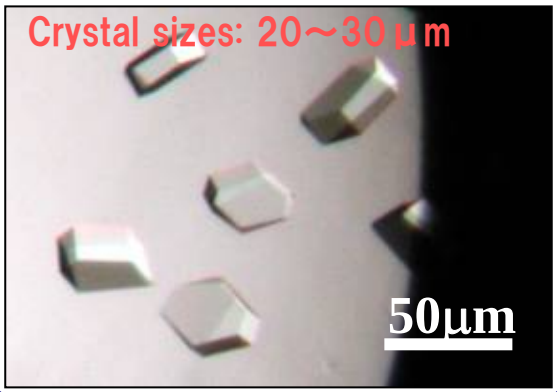
Anomalous signal from light atoms (eg: S)

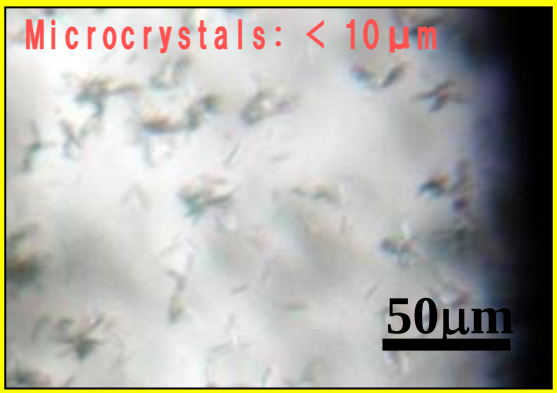
・No need for preparing heavy atoms: highly applicable for very difficult cases for which heavy atom derivatives cannot be prepared
・Light elements need low X-ray energy for higher anomalous signal
・Weak anomalous signal necessitates extremely highly accurate data, thus high redundancy

Still not plenty

Beam Lines







Needs extremely strong beams

Heavy atom labeling is difficult

SPring-8

Ultra high brilliance micro beam BL (new)

BL44XU
（SPring-8 / Osaka Univ）

BL41XU（SPring-8）

NW12A（PF-AR）
BL-5A（PF）

BL-17A（PF）

Low energy micro-focus BL

PF

Phase determination using MAD

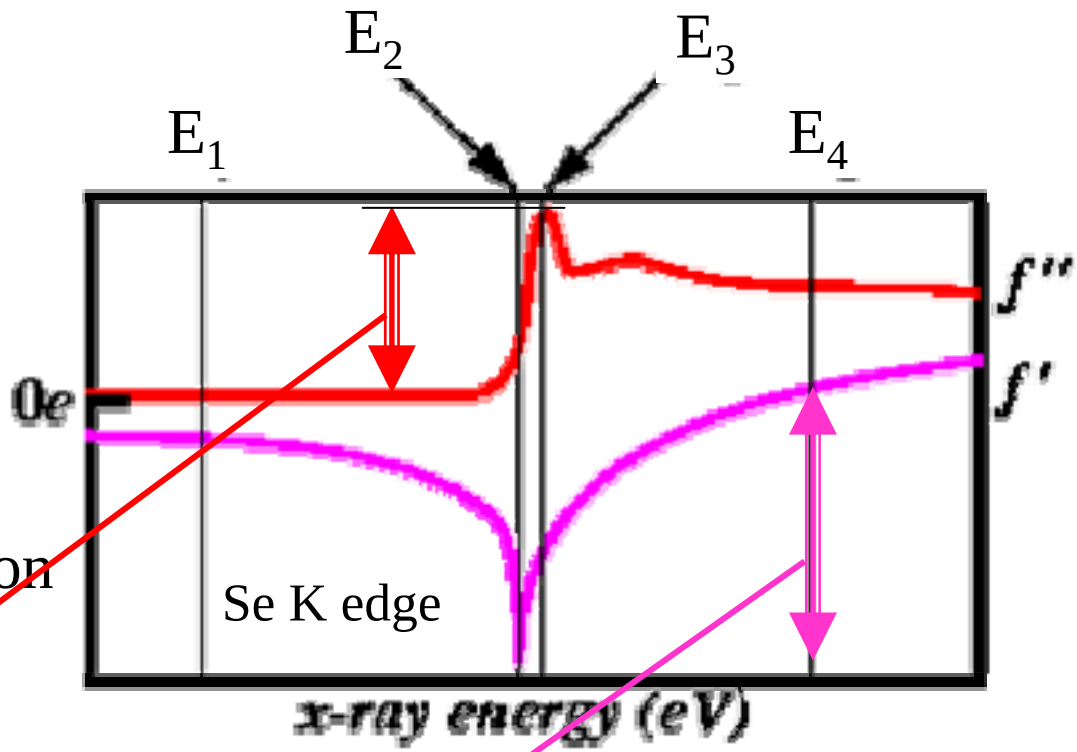
(Multiple Anomalous Dispersion)

Bijvoet pairs

$$|F^+| \equiv |F_{hkl}| = |F_{\bar{h}\bar{k}\bar{l}}| \qquad |F^-| \equiv |F_{\bar{h}kl}| = |F_{h\bar{k}l}|$$

Bijvoet Differences

$$\Delta F = |F^+| - |F^-|$$



MAD experimental maps:

Anomalous difference Patterson

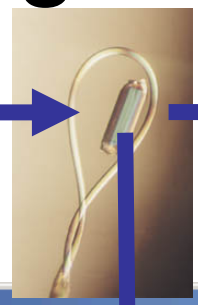
ΔF_{E3}^2 (at the peak)

Dispersive Patterson

$F_{E4}^2 - F_{E2}^2$ (remote – inflection point)

$$f(\lambda) = {}^0f + f'(\lambda) + if''(\lambda)$$

Absorption edge measurement



XAFS Measurement

Status Idle

Master NW12adsc FancyBox dcmode STOP

Select Unit eV Å

Select Atom Se

Select Wavelength 0.90000 0.97682 Å Drive

Beam Alignment Start Undo Slit Size mm

MCA Analysis Start Stop

MCA Parameter Range 1.1674 to 1.03819 Å All Channel Set

XAFS Measurement 0.98372 to 0.97395 step 0.00031 Å Start

Integration Time 1 sec No. Of Points 35

XAFS File Name forproteomix.xafs File Load Save

Select Wavelength 0.90000 0.97941 Å Drive & Measure

Attenuator 0 mm 0 mm 1 mm 2 mm 3 mm 6 mm Apply

I/Io 362325.43

Auto Scale

Y Position :

0.000

12590.04 Energy (eV) 12726.293

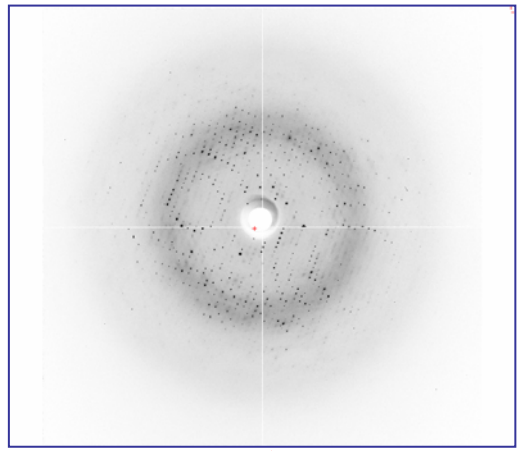
0.98478 Wavelength(Å) 0.97424

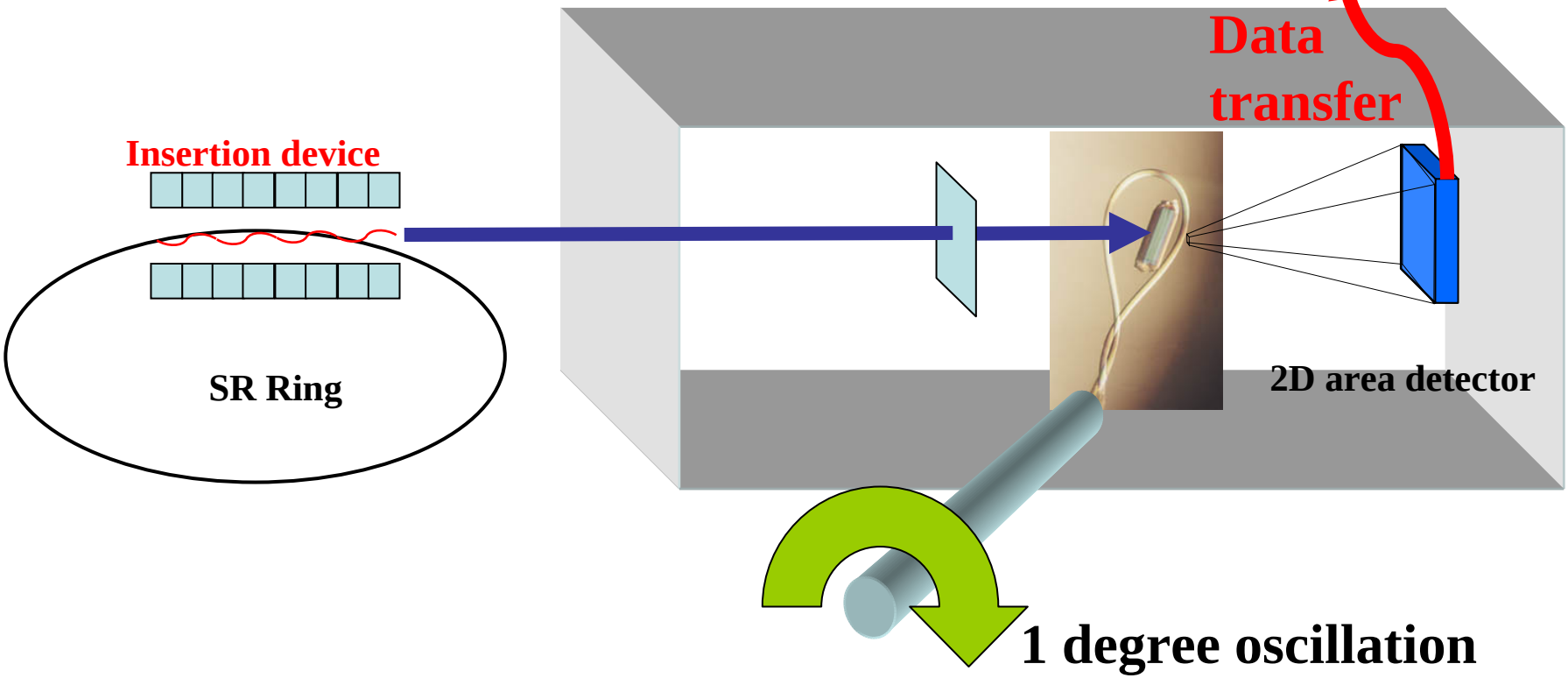
Lower < > Upper

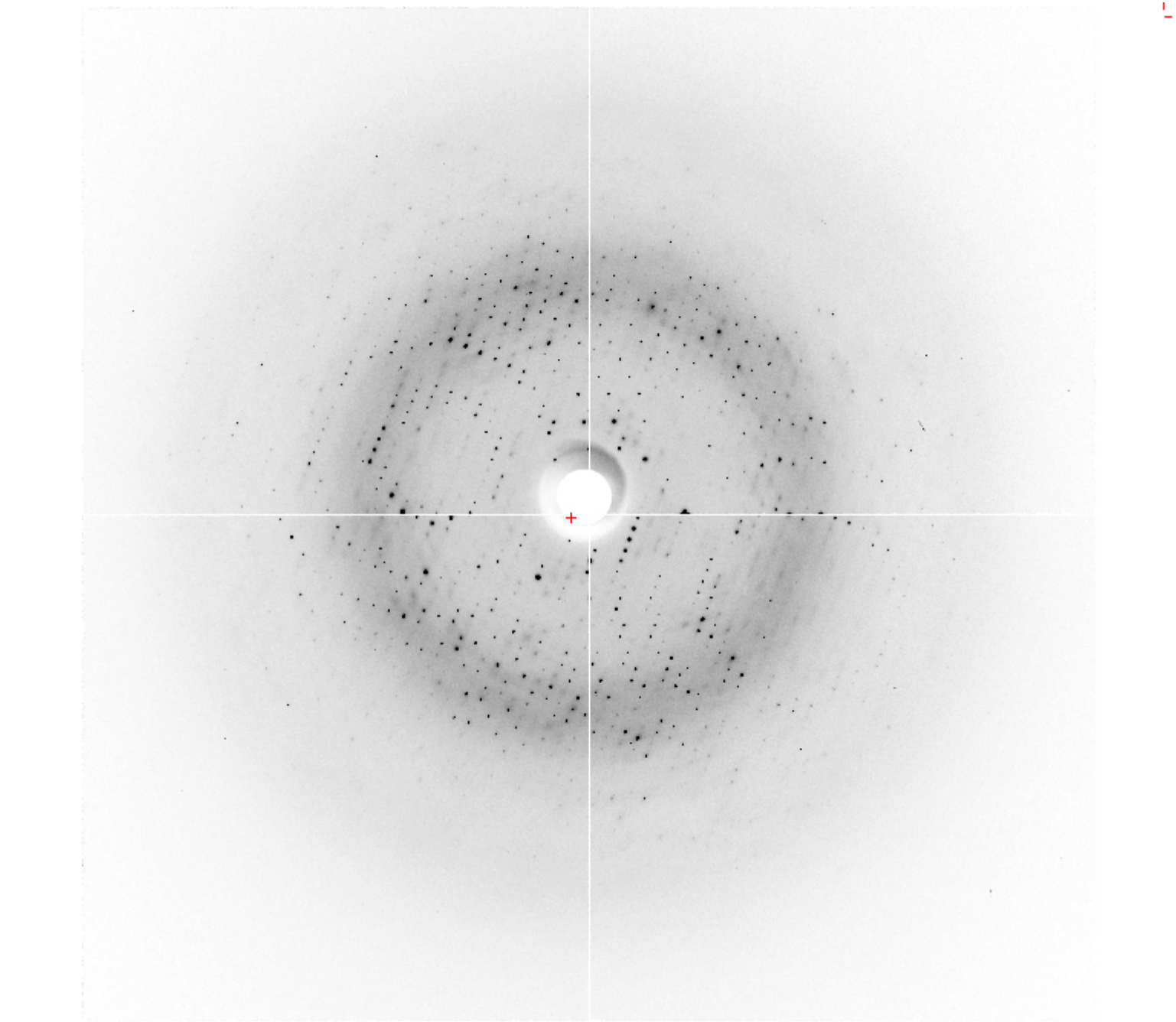
Target

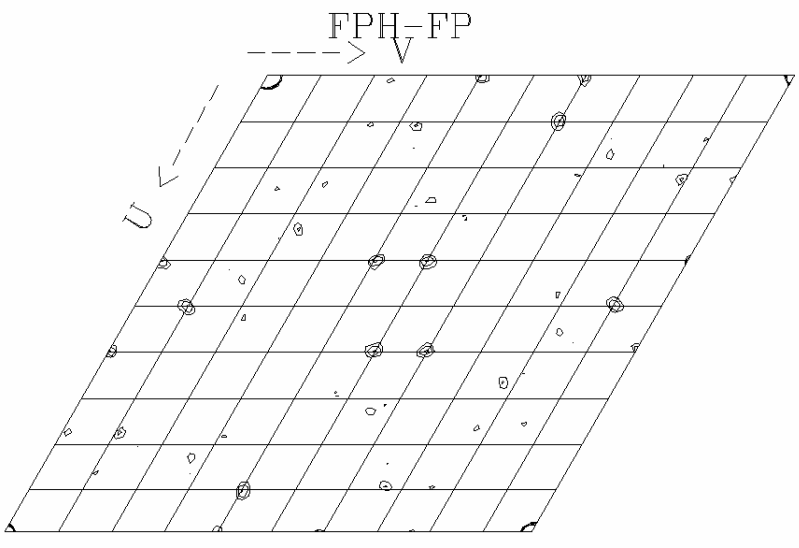
Close

Oscillation method:
(PRESENT)
(eg. 90 X 1 deg oscaillation)

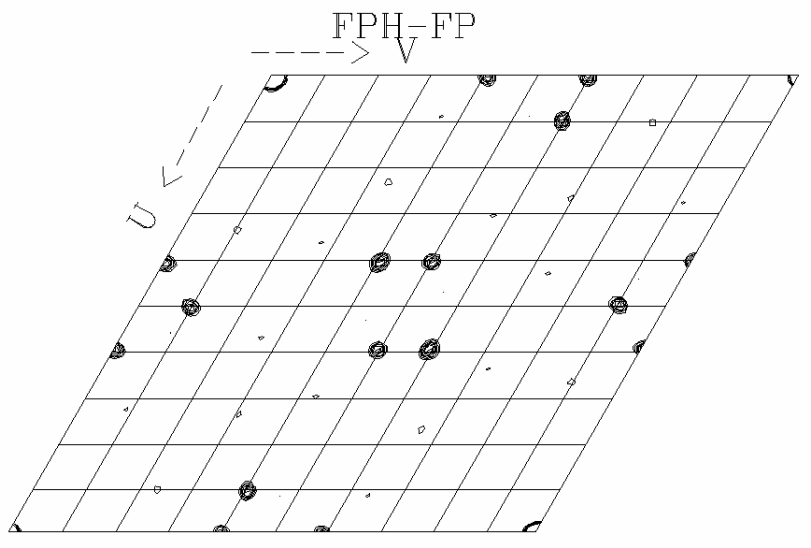




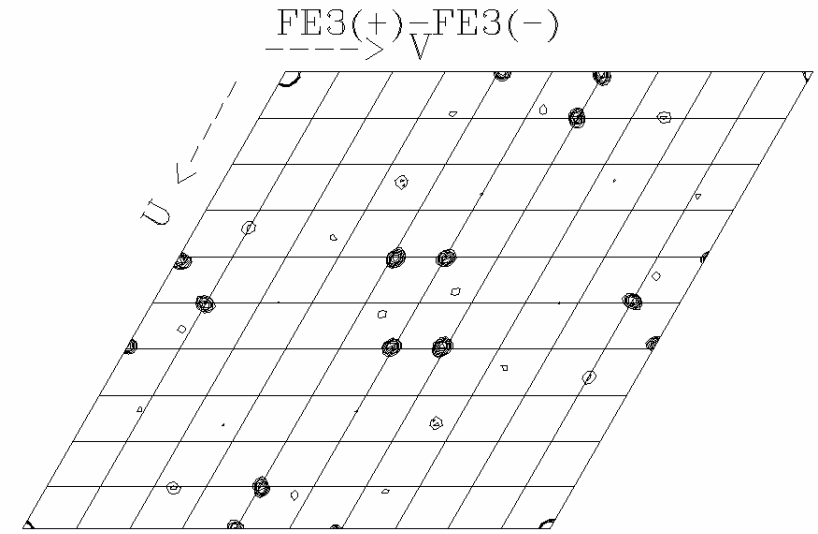




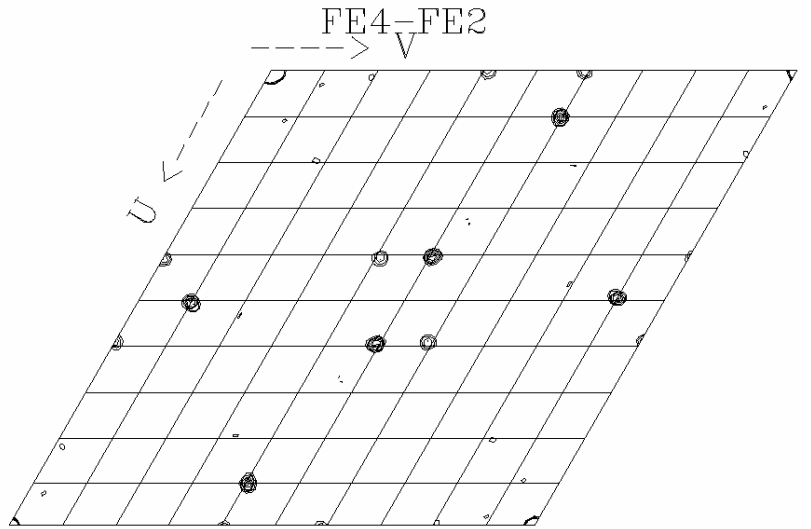
FPH(SeMet)-FP(native) difference Patterson Map



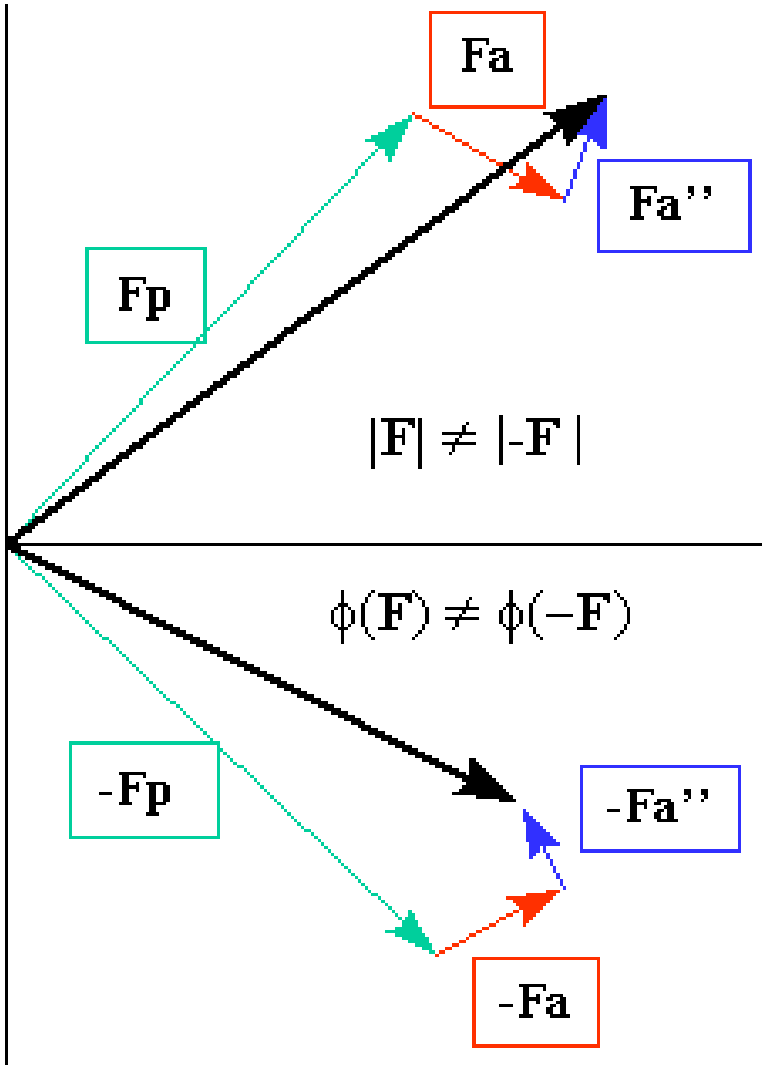
FPH(SeMet)-FP(native) anomalous difference Patterson Map

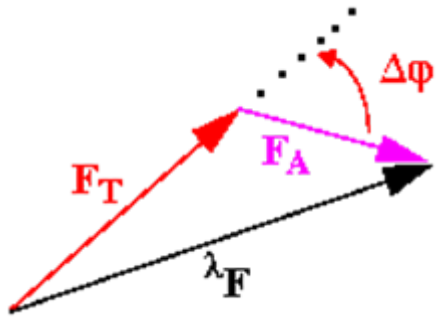


FE3(+)-FE3(-) anomalous difference Patterson Map



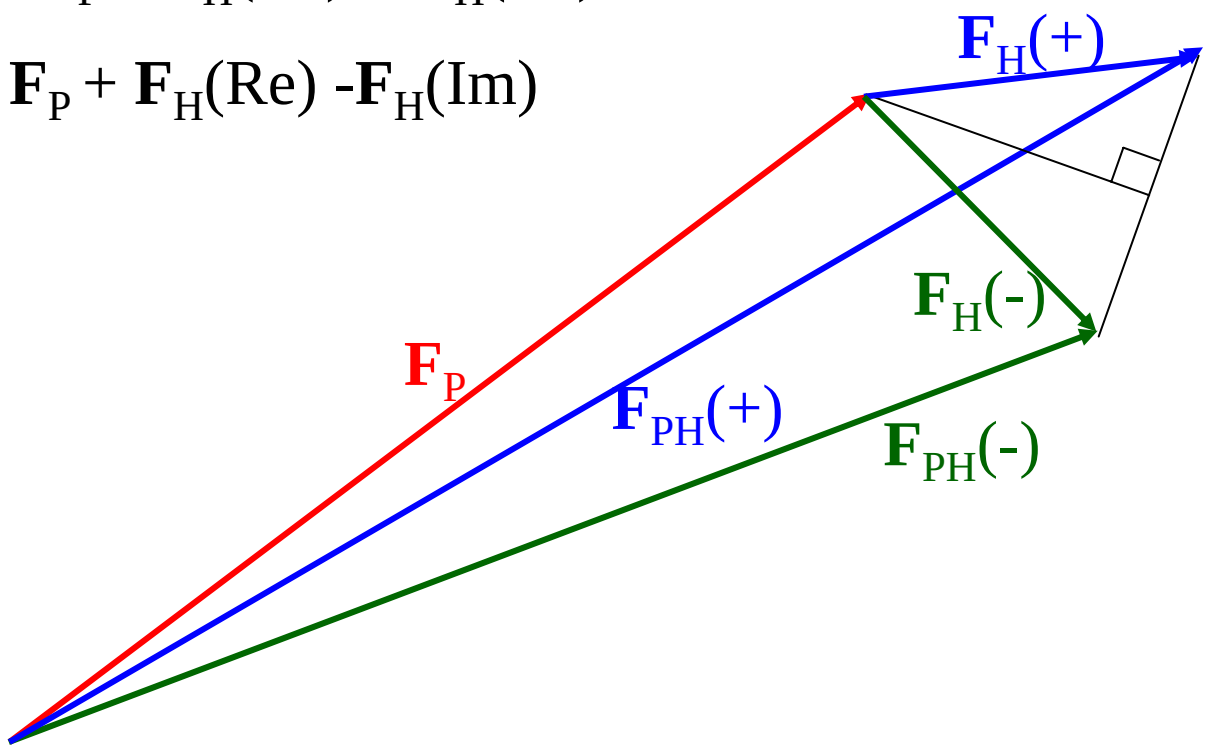
FE4(remote1)-FE2(edge) dispersive difference Patterson Map


Fa
Fa''
Fp
|F| ≠ |-F |
ϕ(F) ≠ ϕ(−F)
-Fp
-Fa''
-Fa


Δφ
F_T
F_A
λ_F

$$\mathbf{F}_{PH}(+) = \mathbf{F}_P + \mathbf{F}_H(+) = \mathbf{F}_P + \mathbf{F}_H(\mathrm{Re}) + \mathbf{F}_H(\mathrm{Im})$$

$$\mathbf{F}_{PH}(-) = \mathbf{F}_P + \mathbf{F}_H(-) = \mathbf{F}_P + \mathbf{F}_H(\mathrm{Re}) - \mathbf{F}_H(\mathrm{Im})$$



Measurements:	$\lvert\mathbf{F}_P\rvert$	at remote energy, E4
	$\lvert\mathbf{F}_{PH}(+)\rvert, \lvert\mathbf{F}_{PH}(-)\rvert$	at the peak of ***f***", E3

From the heavy atom positions:

$\mathbf{F}_H(+)$ & $\mathbf{F}_H(-)$

From intensity measurements

$|F_P|$

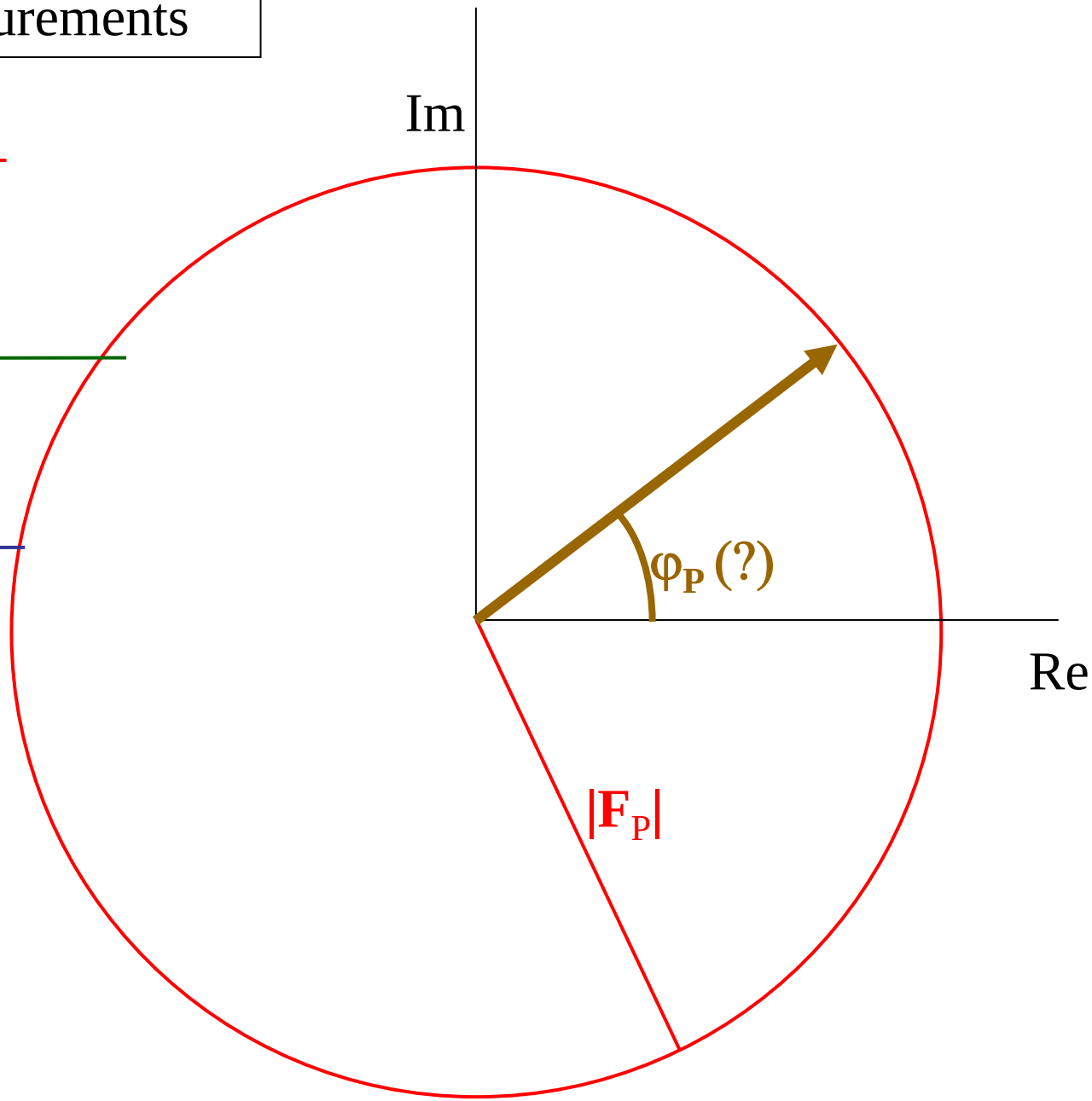
$|F_{PH}(-)|$

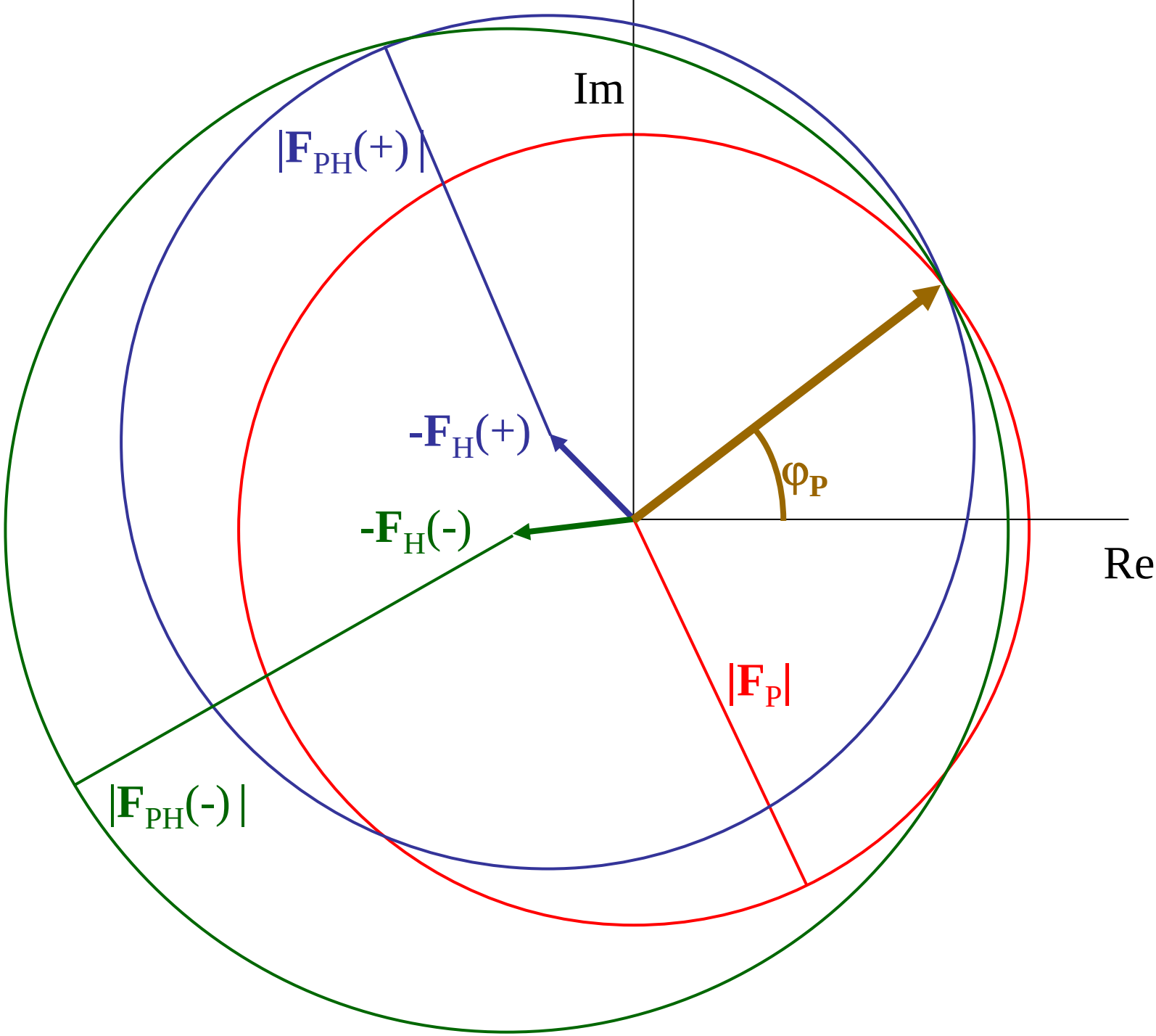
$|F_{PH}(+)|$

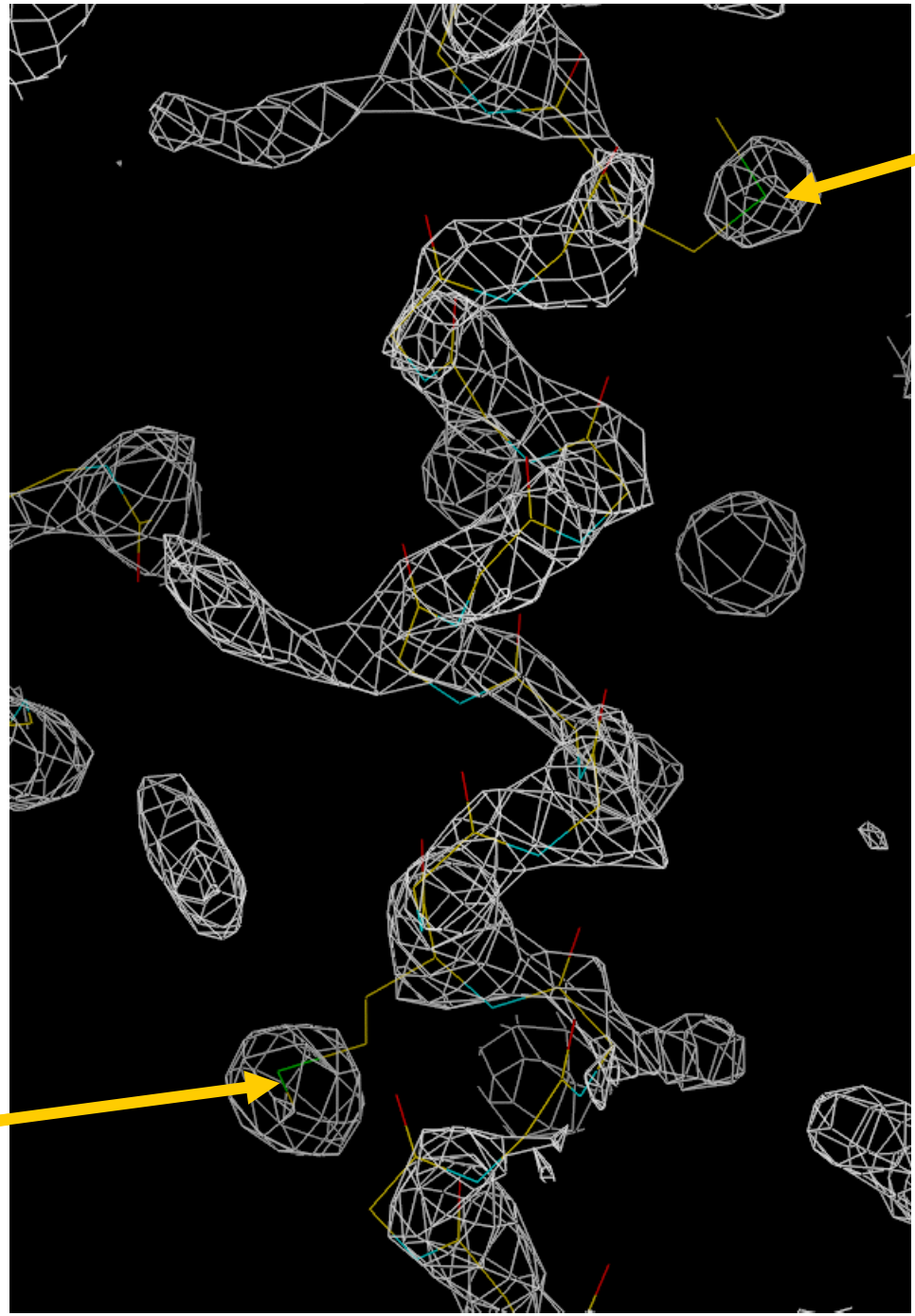
From Se positions

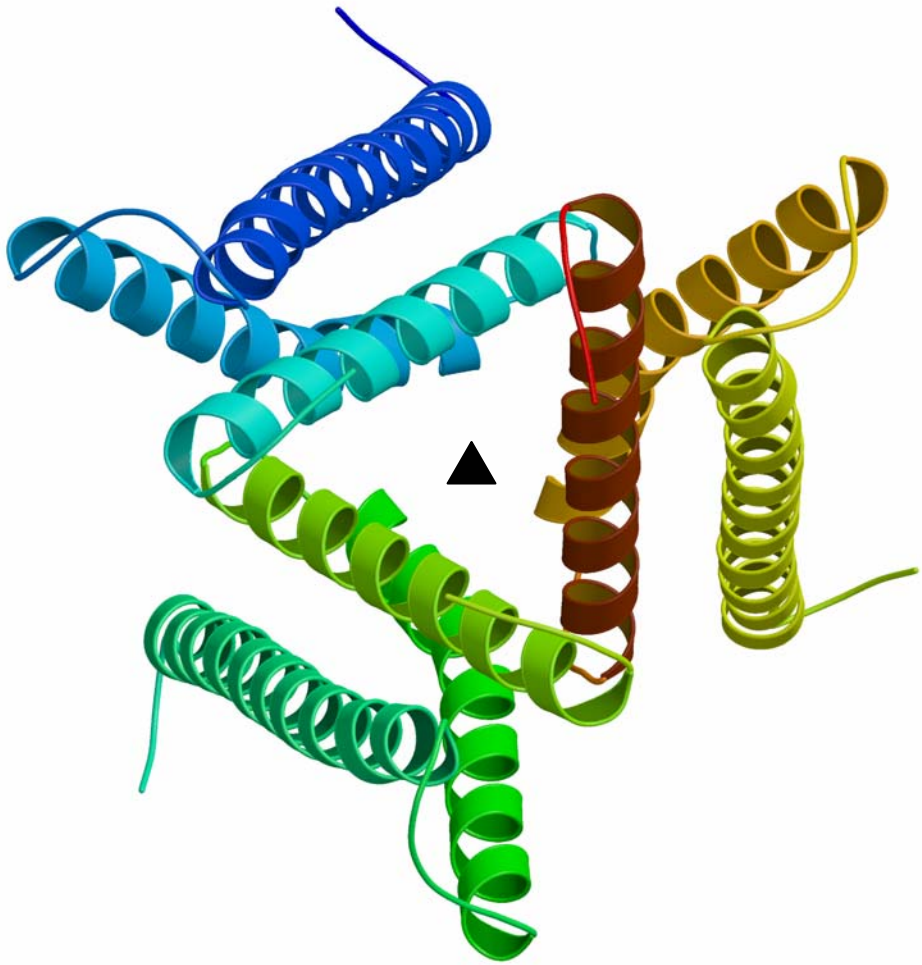
$-F_H(+)$

$-F_H(-)$




Im
Re
$|F_{PH}(+)|$
$-F_H(+)$
$-F_H(-)$
φ_P
$|F_P|$
$|F_{PH}(-)|$


Met94
Met84



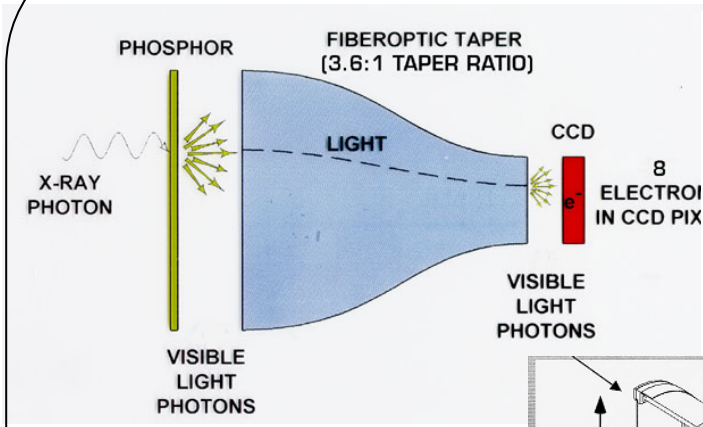
Ribbon diagram of trimer of GGA1 GAT domain

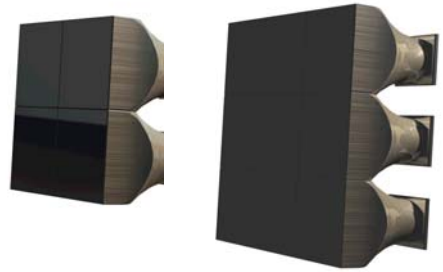
Triangle is threefold axis.

X-ray Area Detectors for Synchrotron X-ray Protein Crystallography

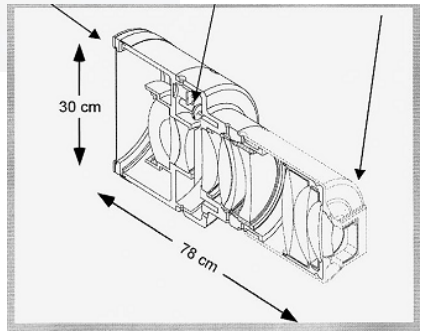
Imaging Plates

Tapered fiber optics CCD



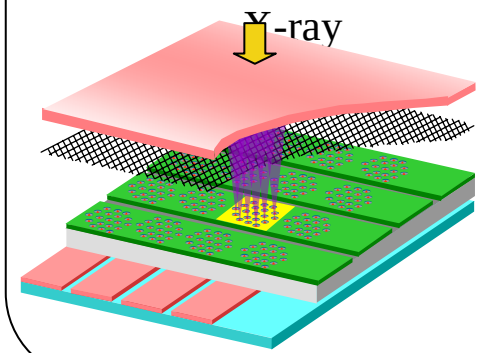


Lens-coupled CCD

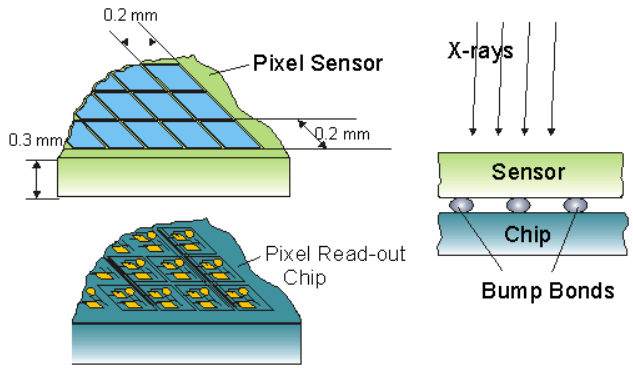


Flat Panel Detectors

HARP based Field Emitter Array (FEA)



Pixel Array Detectors (PAD)



X-ray Area Detectors for Synchrotron X-ray Protein Crystallographic Data Collection

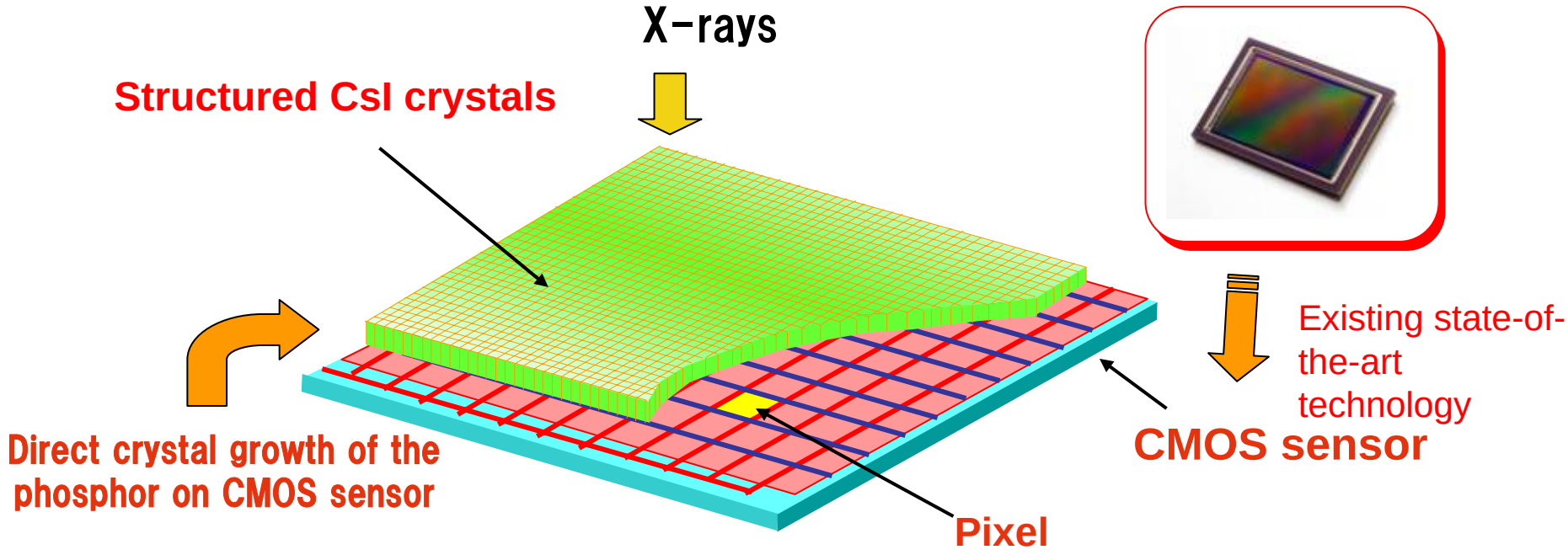
Detectors	Pros	Cons
On-line imaging plates	Large dynamic range, large area	Slow readout (20 to 200 sec per image) -> poor duty cycle Relatively broad PSF Relatively inexpensive
Off-line imaging plates	Large dynamic range, large area	Slow read-out (20 to 200 sec per image) -> poor duty cycle Relatively broad PSF Cumbersome to handle
Tiled, tapered fiber optics CCD	Fast readout (0.3 to dozens of secs)	Limited dynamic range (~<16 bits) Expensive to cover large solid angle
Lens-coupled CCD	Large active area (300 mm diameter) Inexpensive	Has gone into market very recently, and not yet established. Large (1 m long) and heavy (100 kg)
Flat Panel Detectors	Inexpensive, very light (~7 kg) Large active area (easily 400 mm square) Fast readout (a few seconds)	Inherent problem of noise
Pixel Array Detectors (PAD)	Extremely good PSF Extremely Fast readout	Still under development Difficult to tile the components to cover a large solid angle
HARP based Field Emitter Array (FEA)	Extremely sensitive (800 X CCD) Large area and very fast readout Very good PSF	At the very first stage of the development

Detector Requirements for good PX data collection

- Large, fast, reliable and inexpensive
- Must be an integrated system
  - data acquisition and storage
  - data analysis
  - archiving
- Easy to maintain

Next generation detector: large area, high sensitivity, high speed, high resolution

SPring-8 CMOS based flat panel detector development

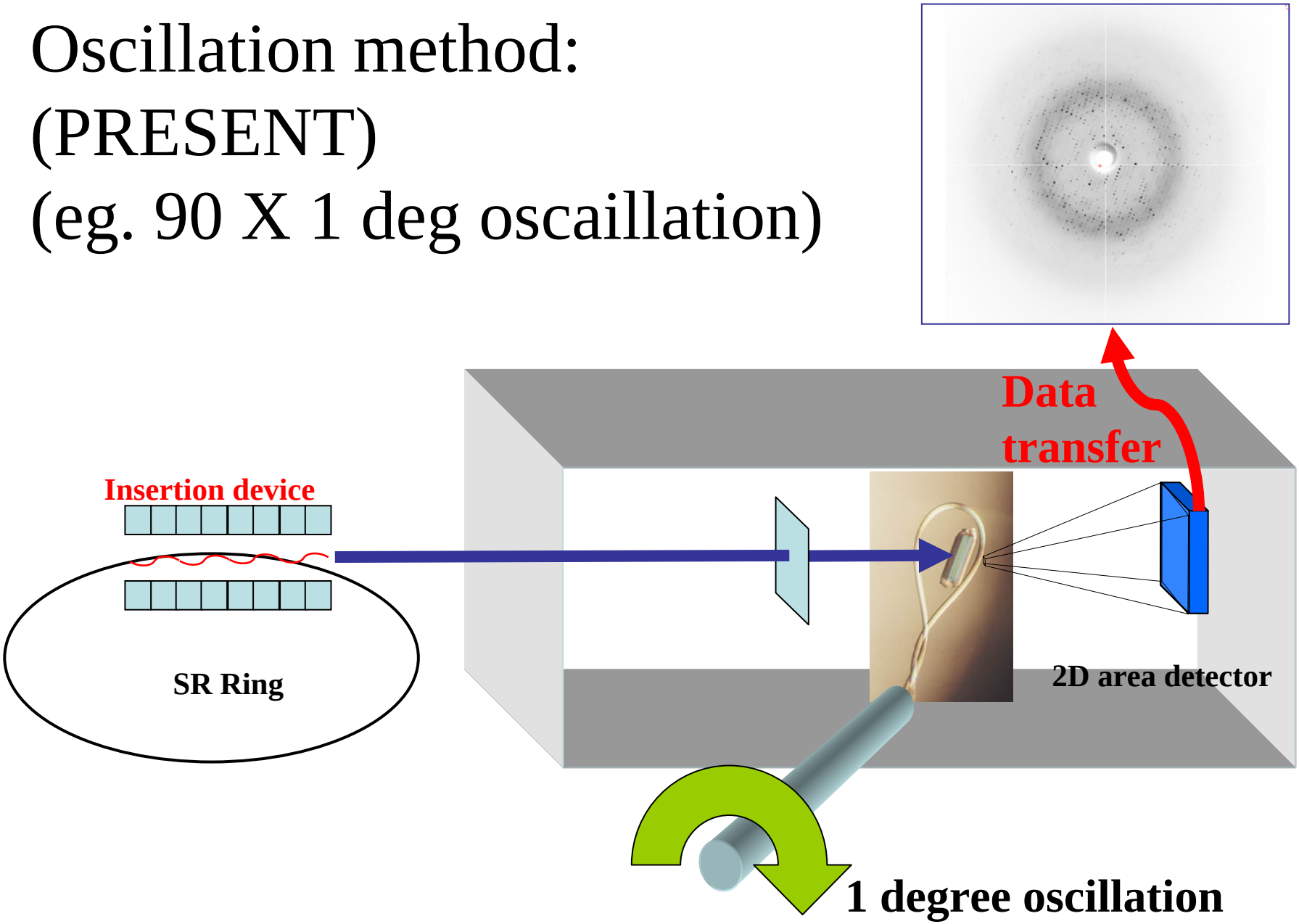


(1) Improvement of sensitivity and spaitial resolution using structured CsI crystals

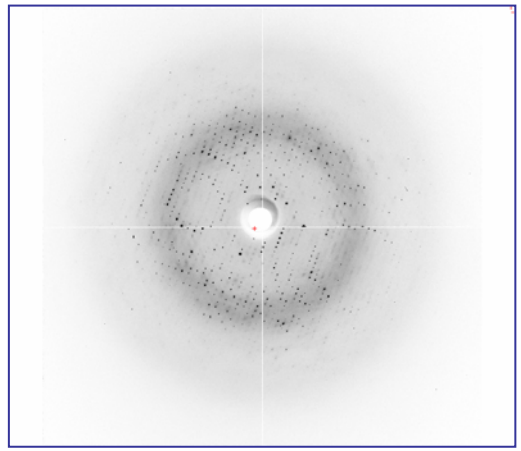
(2) Cost down by using existing state-of-the-art technologies

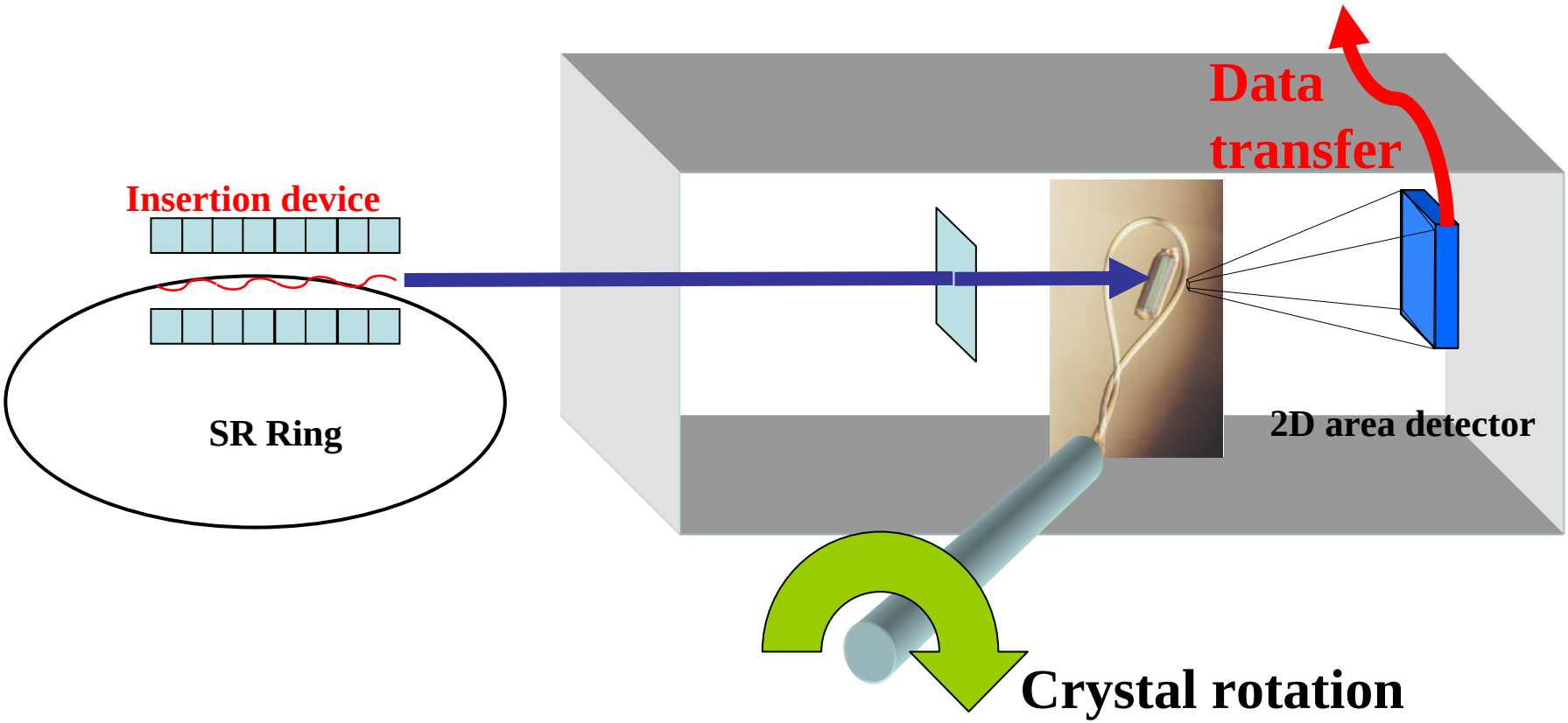
Courtesy of Dr. Masaki Yamamoto

Oscillation method:
(PRESENT)
(eg. 90 X 1 deg oscaillation)



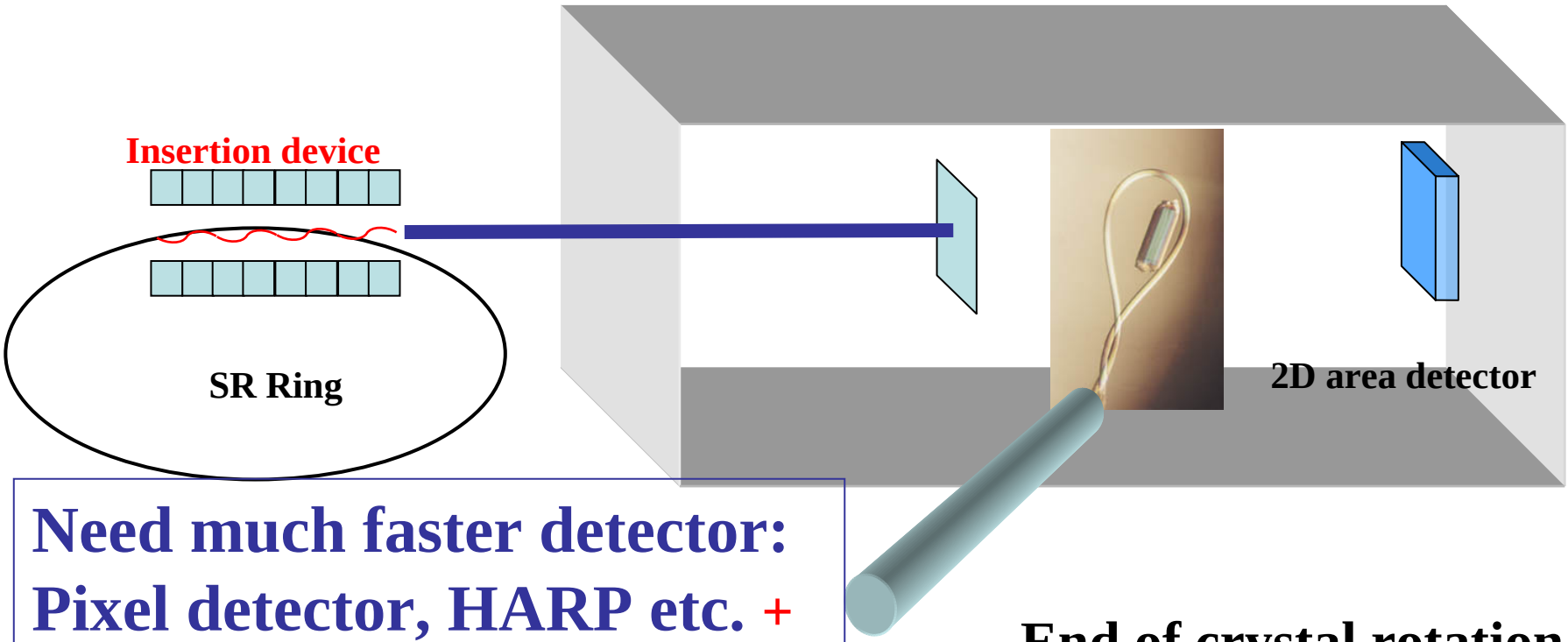
Continuous rotation method: (Near FUTURE) (eg. 1 X 90 degs rotation)





Continuous rotation method: (Near FUTURE) (eg. single sweep of 90 deg rotation)

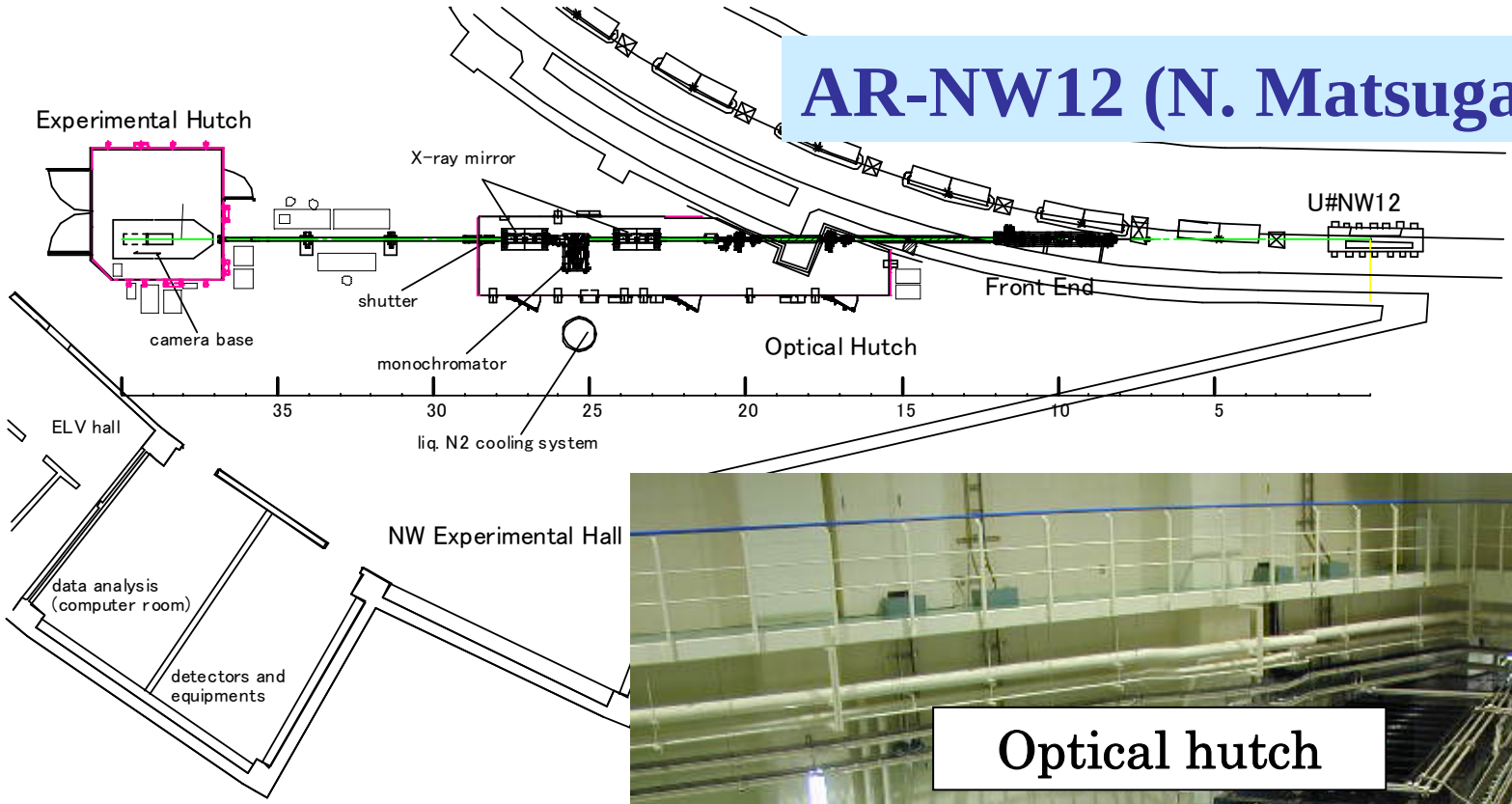
Complete Dataset!

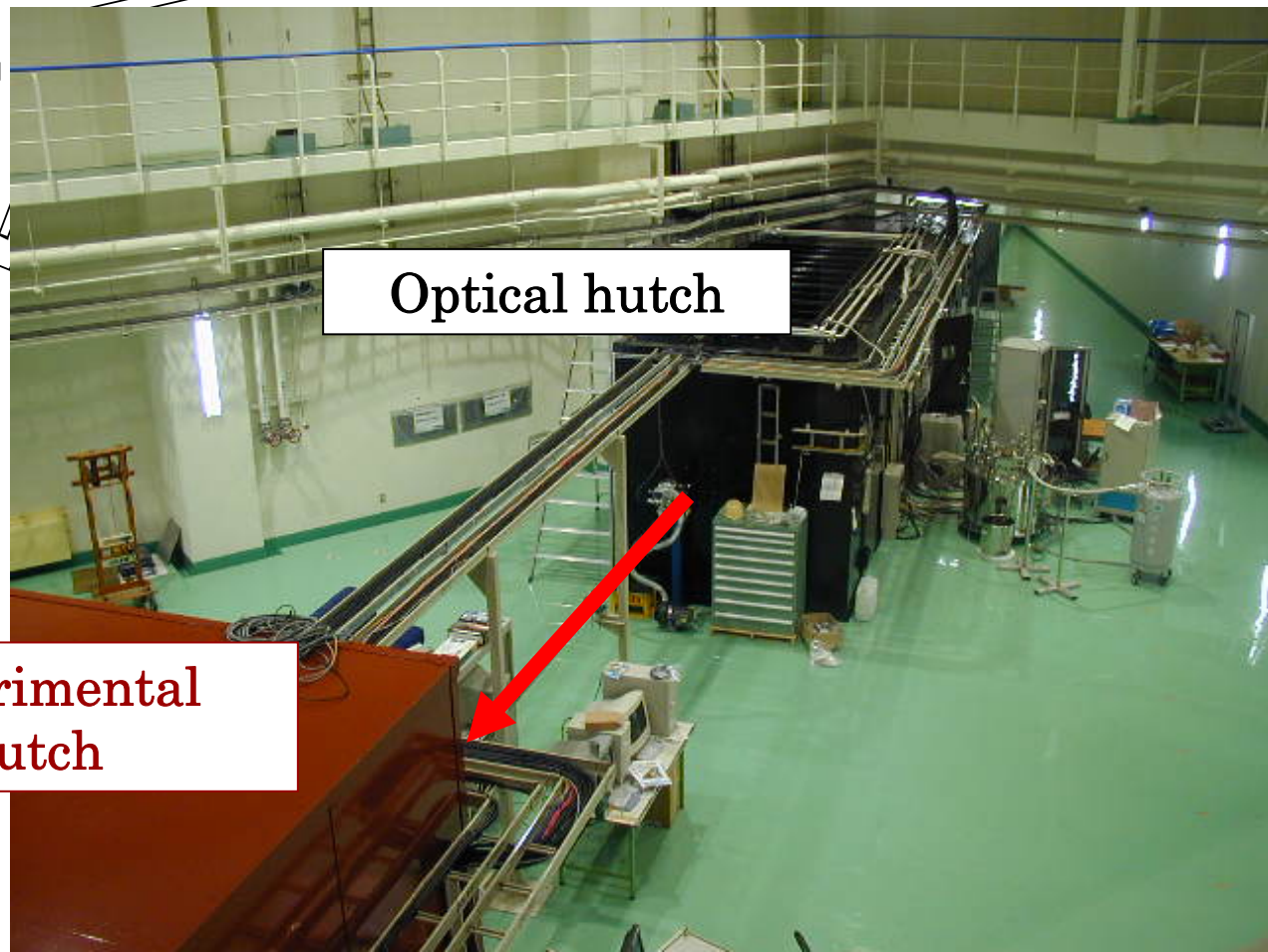


Need much faster detector: Pixel detector, HARP etc. + 3D profile fitting

End of crystal rotation

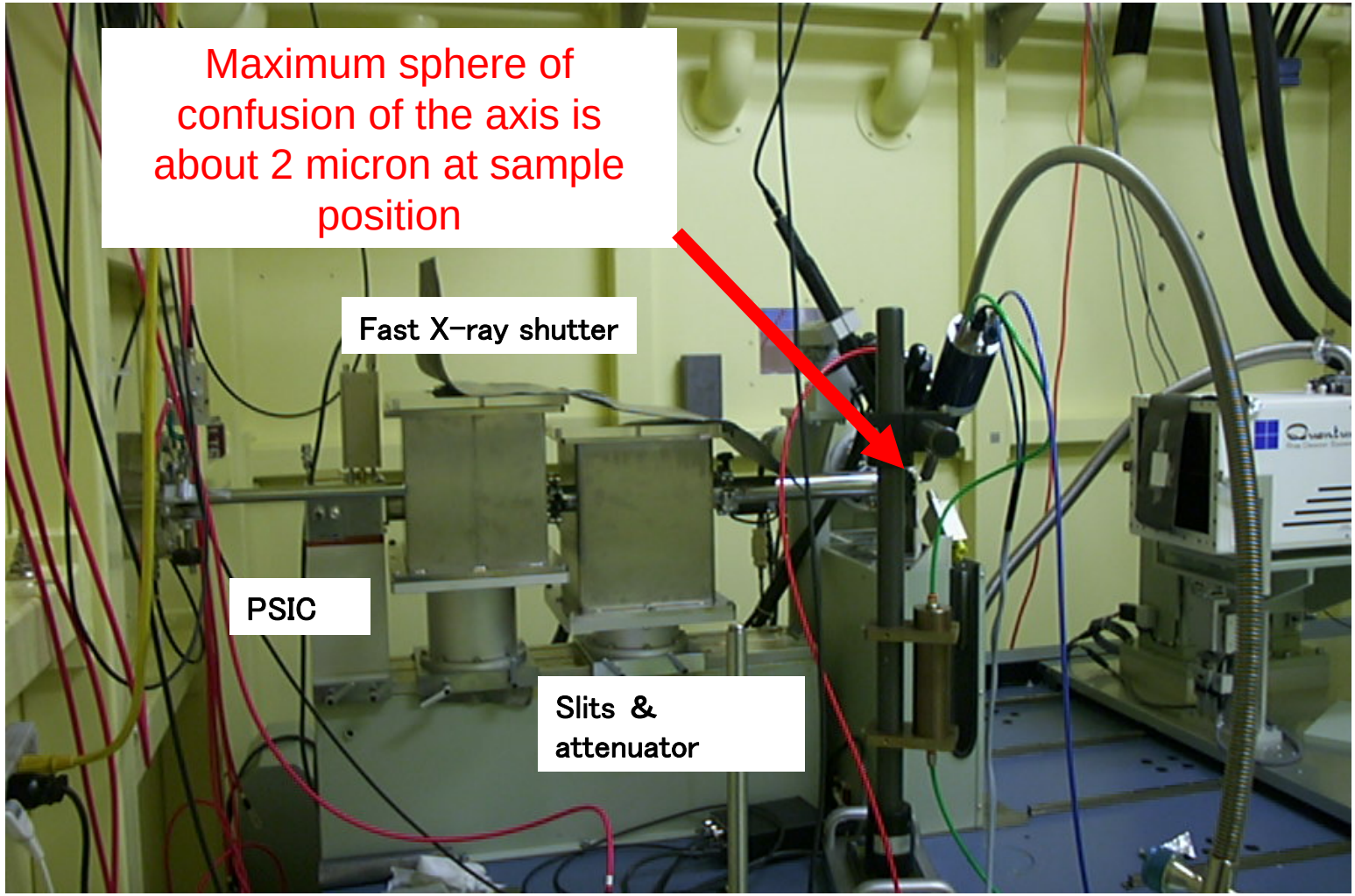
AR-NW12 (N. Matsugaki et al.)





PF-AR NW12: Highthroughput MAD beam line

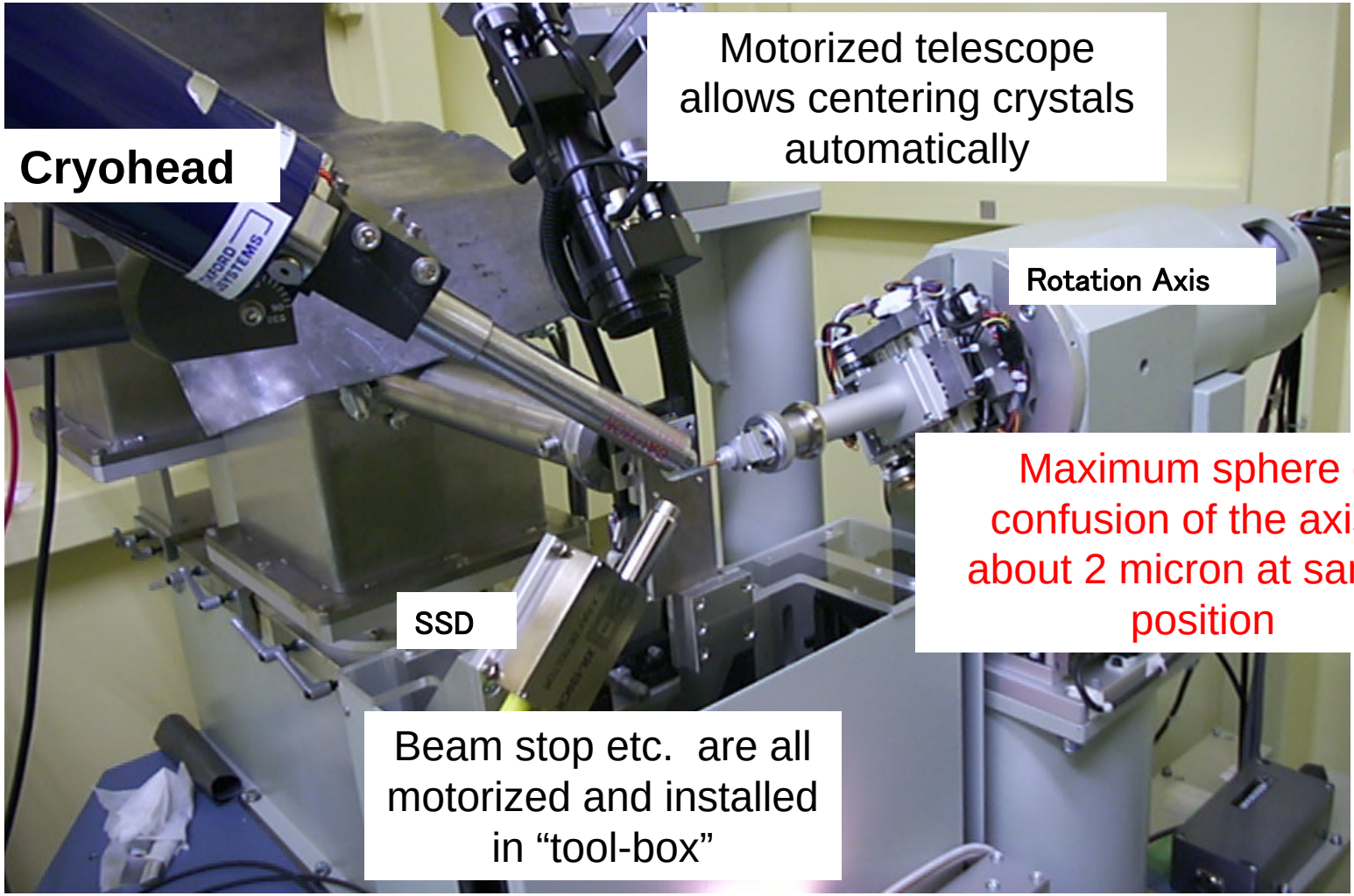
Total data collection time for 180 frames: 10 to 30 min



Long camera distance and large area size of the detector allow the data collection of crystals with large cell dimensions

NW12: data collection from very small crystals.

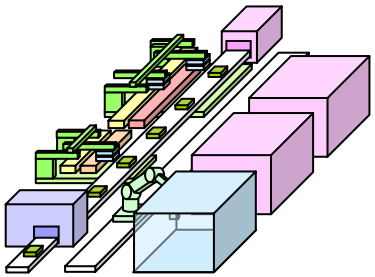
Tool box makes manual crystal mounting easy.



KEK Photon Factory

Crystallization

200,000 trials/day



Structural Biology Research Center

6.5GeV PF-AR

2.5GeV PF ring

NW12A (2003)

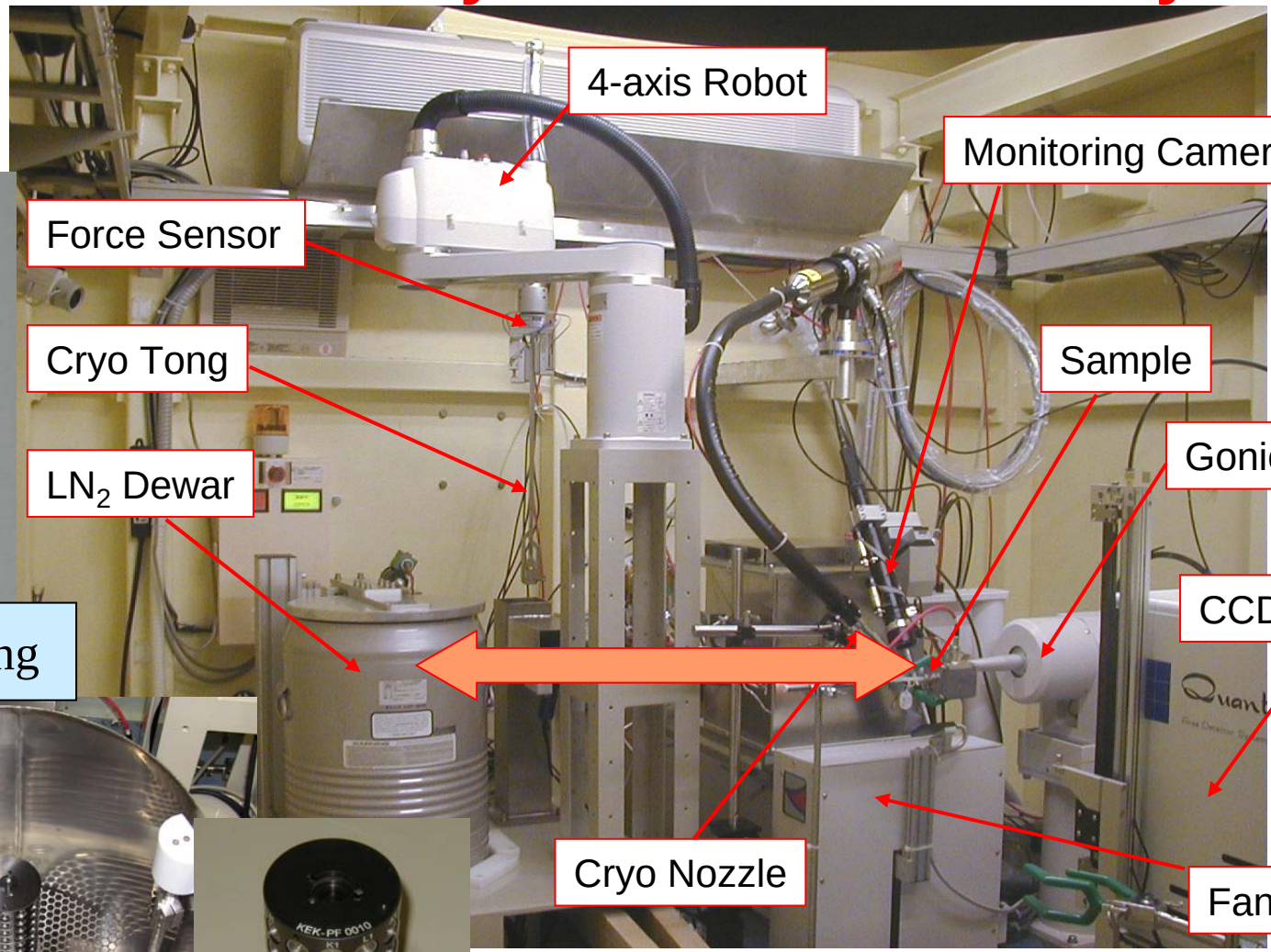
BL-6A

BL-5A (2004)

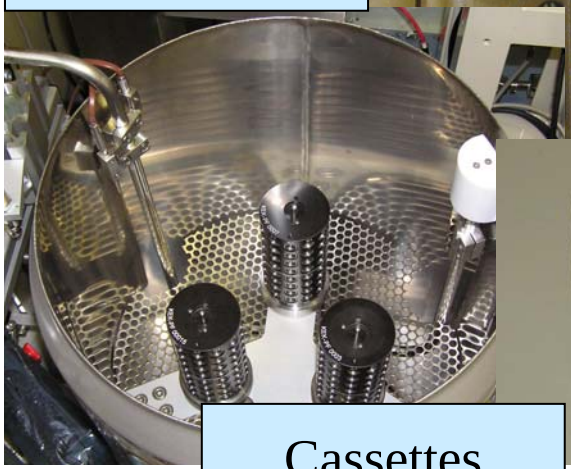
BL-17A (2006)

SSRL-type robot installed on MAD Beamline BL-5

20 datasets/day ⇒ 100s datasets/day



Double Tong



Cassettes

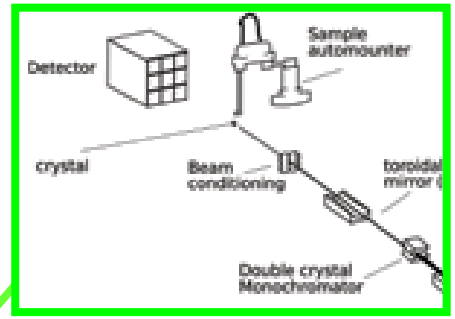
Compatible with SSRL(USA), SRRC(Taiwan) & AS (Australia), CLS (Canada)

Compatibility with SPring-8 and European standards being worked

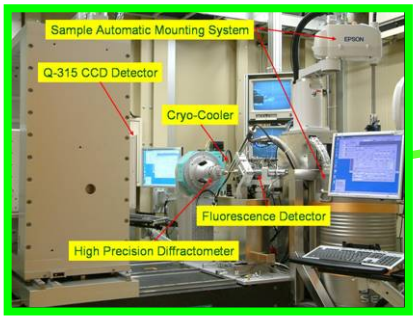
SAM System Family

KEK-PF
BL-1A, BL-5A, BL-17A,
AR-NW12A, AR-NE3A

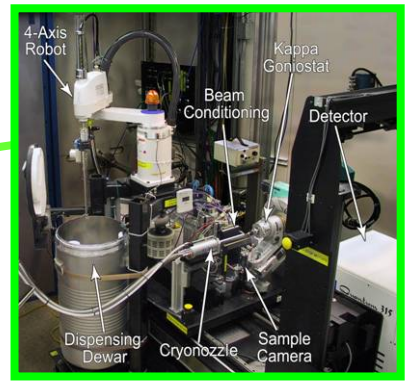
ALS
12.3.1



CLS 08B1-1(CMCF2)



NSSRC
BL13B1, BL13C1



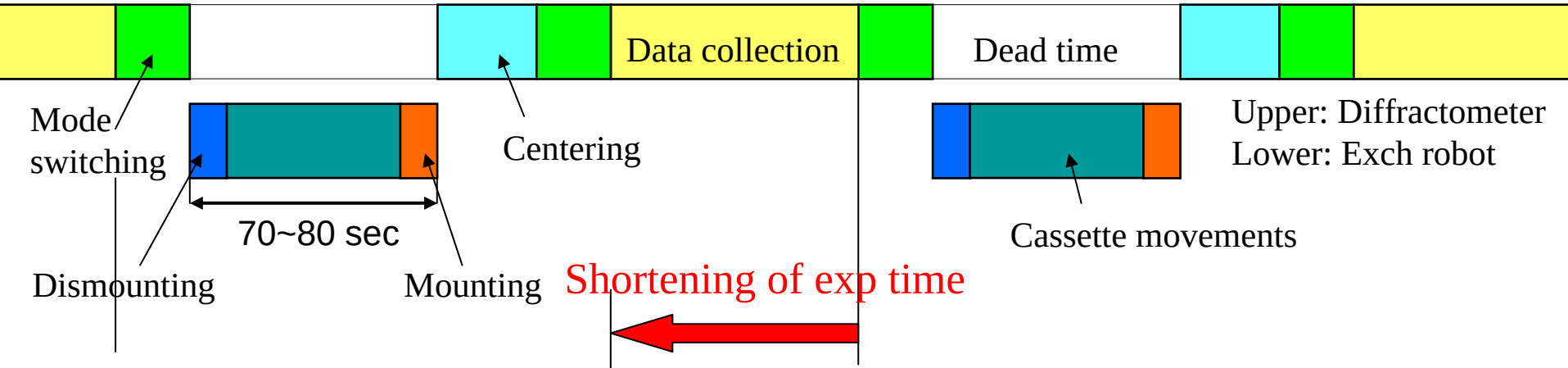
SSRL
BL1-5, BL7-1, BL9-1,
BL9-2, BL11-1, BL11-3

AS
3-BM1, 3-ID

SSRL
Automated
Mounting system

Less time for crystal exchange

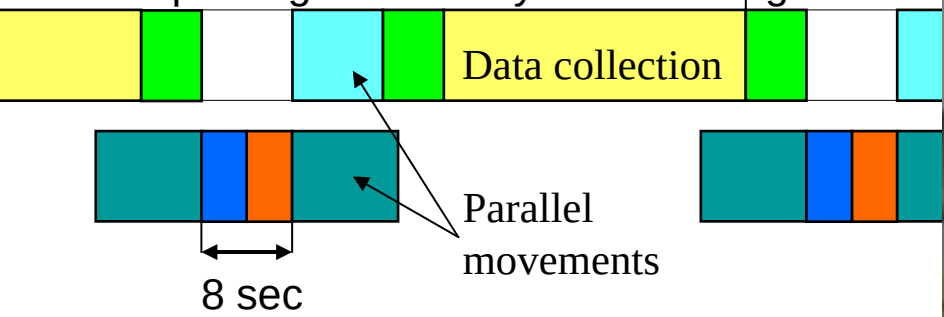
Original single tong



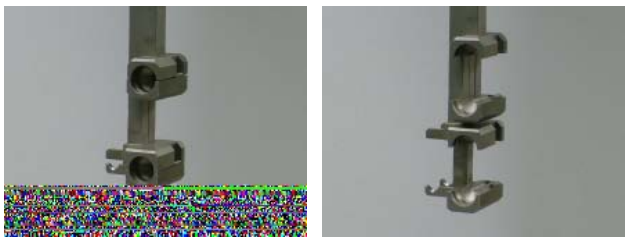
Shortening of exp time

Double tongs

+ saving time for mode change over
+ improving time for crystal centering

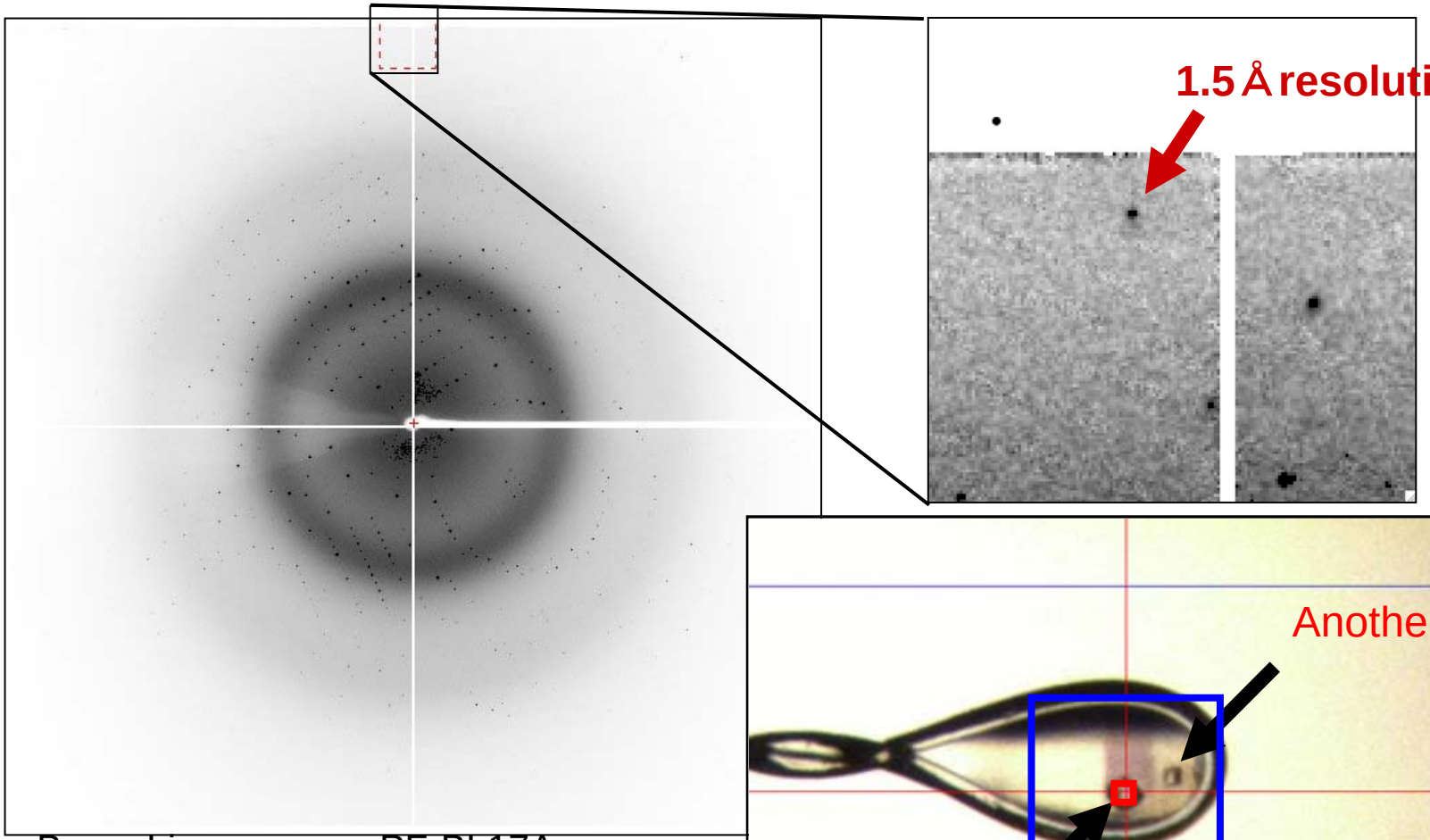


Double tongs crystal exchange on PF BL-17A



Double tongs mechanisms

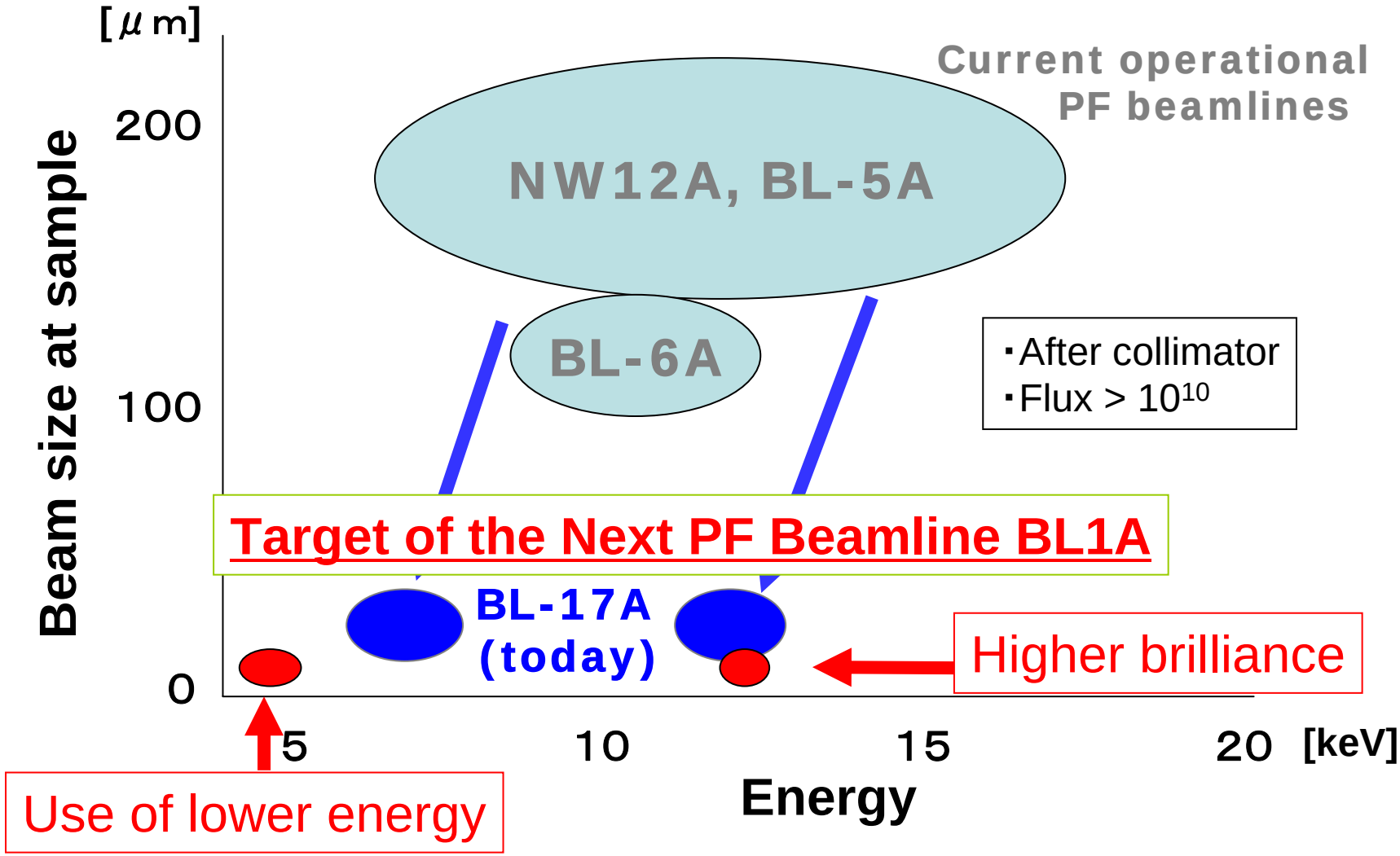
NEW BL17A short gap undulator beam line: Example of small crystals (funded by JST Frontier Technology Development Project)



Beam Line:	PF-BL17A
Wavelength	1.291 Å
Beam size :	20 μm × 20 μm
Exposure time:	20 sec

Provided by Dr. Tadanobu Tanaka of Showa University

Towards lower energy, higher brilliance



BL17: Mini-gap undulator & Experimental environment

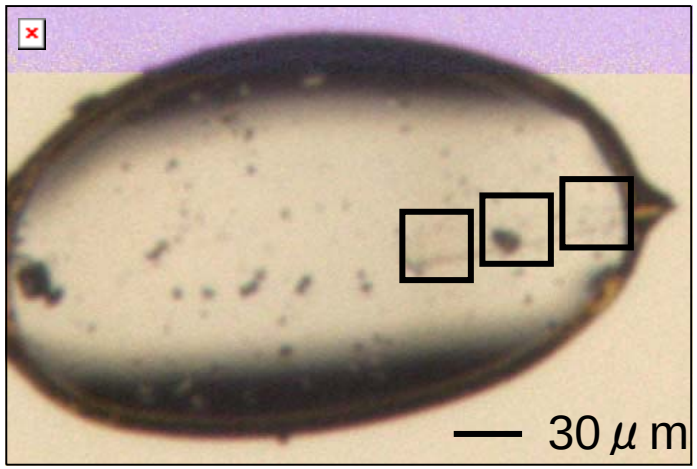
- High speed shutter
  - Precision < 1 msec.
- High precision goniometer-head
  - SOC (rotation error) < 0.7 μm
- Fast readout, large area and high gain CCD detector (ADSC Q270)
  - Readout 1.1 sec
  - Active area 270 x 270 mm^2
  - Gain 21 e-/photon at 12.4 keV
  - Minimum distance 40 mm
- Automatic sample changer
  - Exchange < 11 sec.

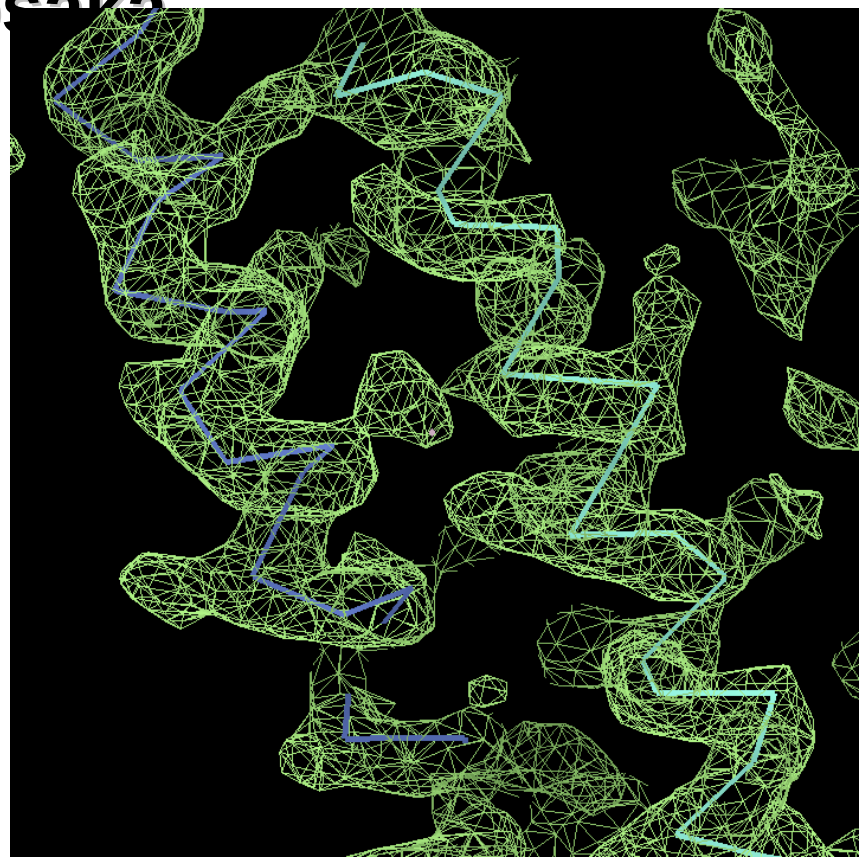
BL17 example

Se-MAD experiment: data collection at three xtal positions to avoid radiation damage

Osaka University Atsushi Nakagawa, Harumi Hosaka

- DNA binding protein
- Mol weight：37 kDa
- Xtal：2 x 10 x 150 μm^3
- 3.2 Å resolution（Se-SAD）
- Data collected at 3 positions

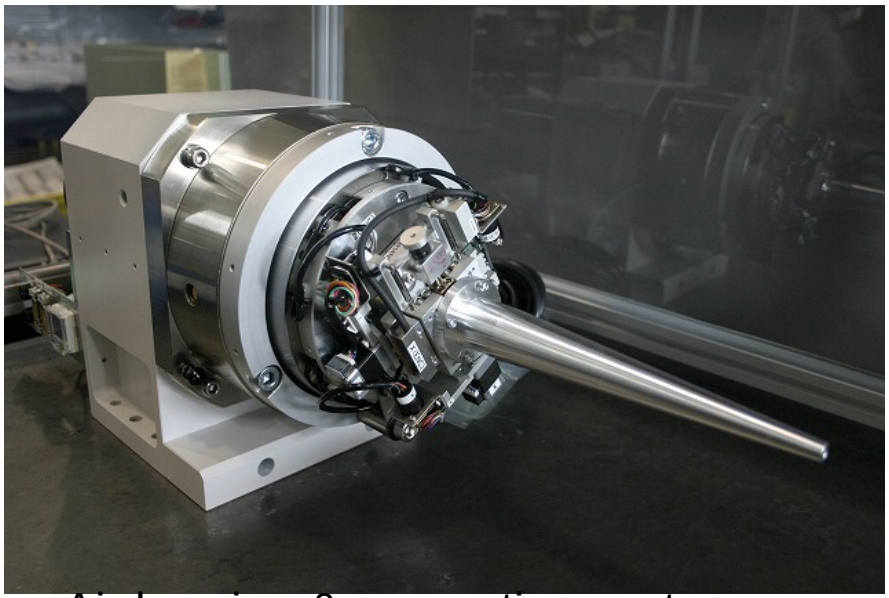




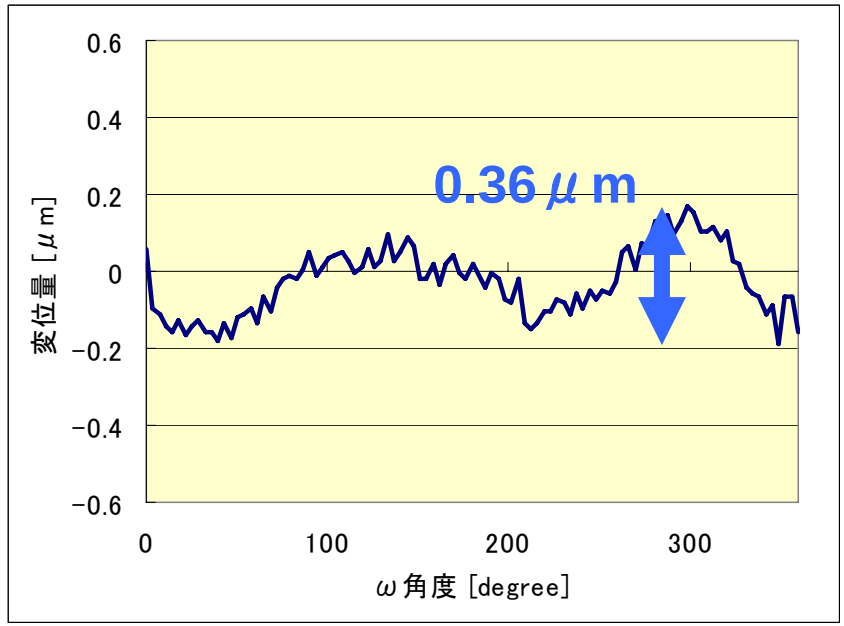
After density modification

High precision one axis diffractometers with XYZ stages

BL	BL-6A	NW12	BL-5	BL-17
Year started	2000*	2003	2004	2006
Max deviation（μm）	10	2.2	1.0	0.37(2007)⇒ 0.1(2009)
Xtal size（μm）	100	22	10	4 (2007) ⇒ 1 (2009)

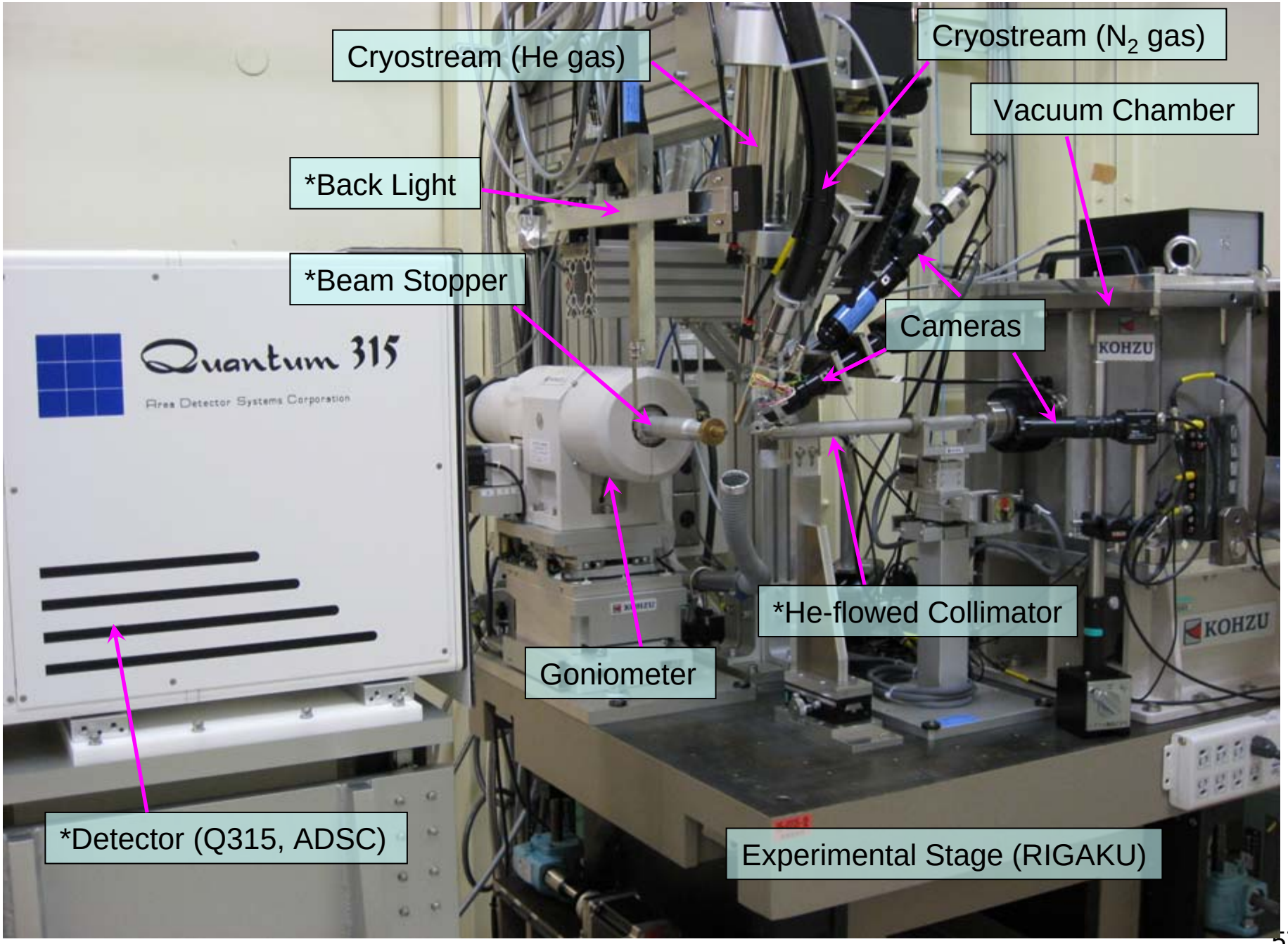


Air bearing & magnetic x-y stages



BL-5 type diffractometer -> also installed on BL41XU & BL44XU, SPring8

SPring-8 BL41XU experimental hutch



Industrial Use and Collaborations between KEK and Industry (~8% of beamtime)

MX BLs at Photon Factory

Astellas Pharma. Beam Line AR-NE3 (to be completed by March 2009)

Tsukuba Structural Biology Consortium for Industrial Applications（since April 1, 2006）

Company ①

Company ②

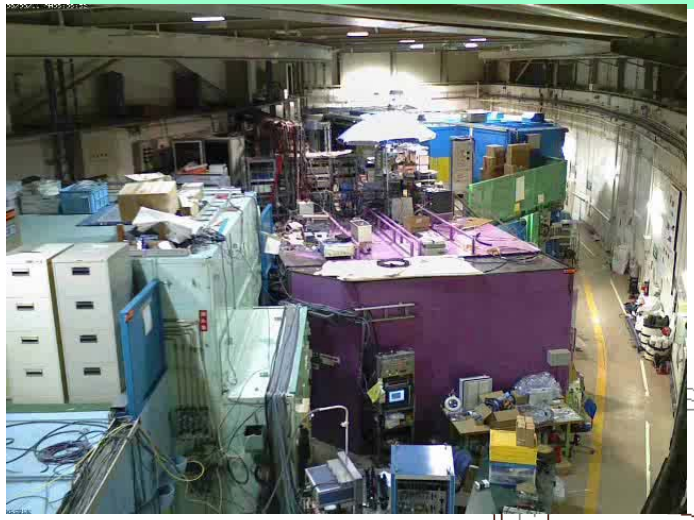
Company ③

Pharmaceutical Consortium for Protein Structure Analysis (PCProt), Japan Pharmaceutical Manufacturers Association (JPMA)

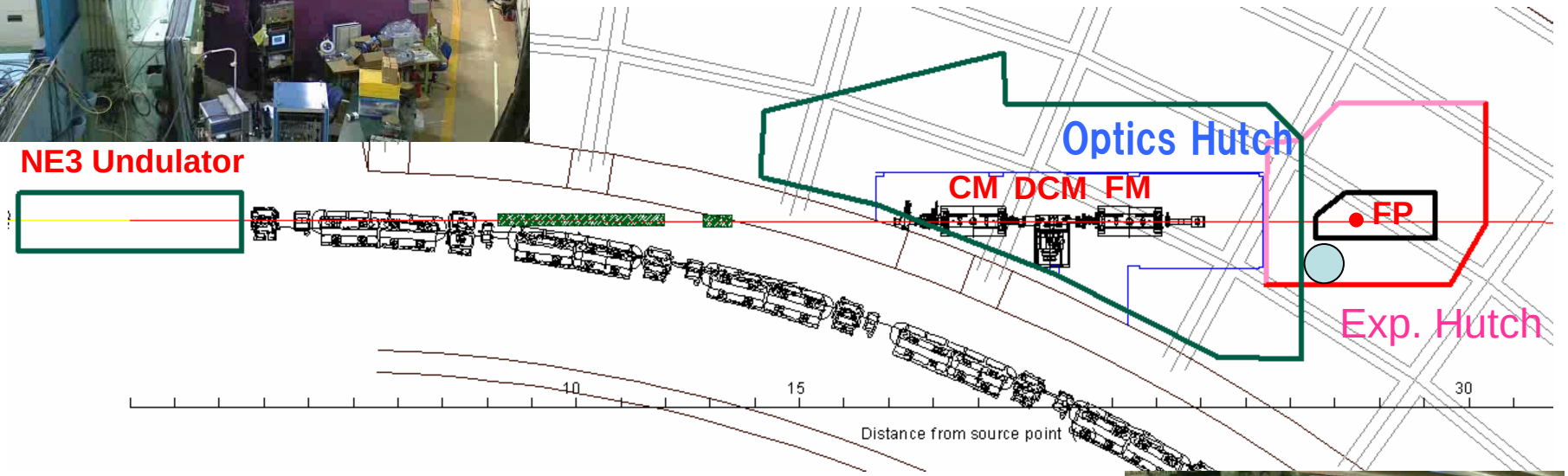
BL32B2 at SPring8

- Ajinomoto Co., Inc
- Astellas Pharma Inc.
- Eizai Co. Ltd.
- KYOWA HAKKO KOGYO Co., LTD.
- DAIICHI SANKYO Co Ltd.
- CHUGAI PHARMACEUTICAL Co., Ltd.
- BANYU PHARMACEUTICAL Co., Ltd.
- Mitsubishi Chemical Corporation
- New members

Astellas Pharma Beam Line: PF-AR NE3

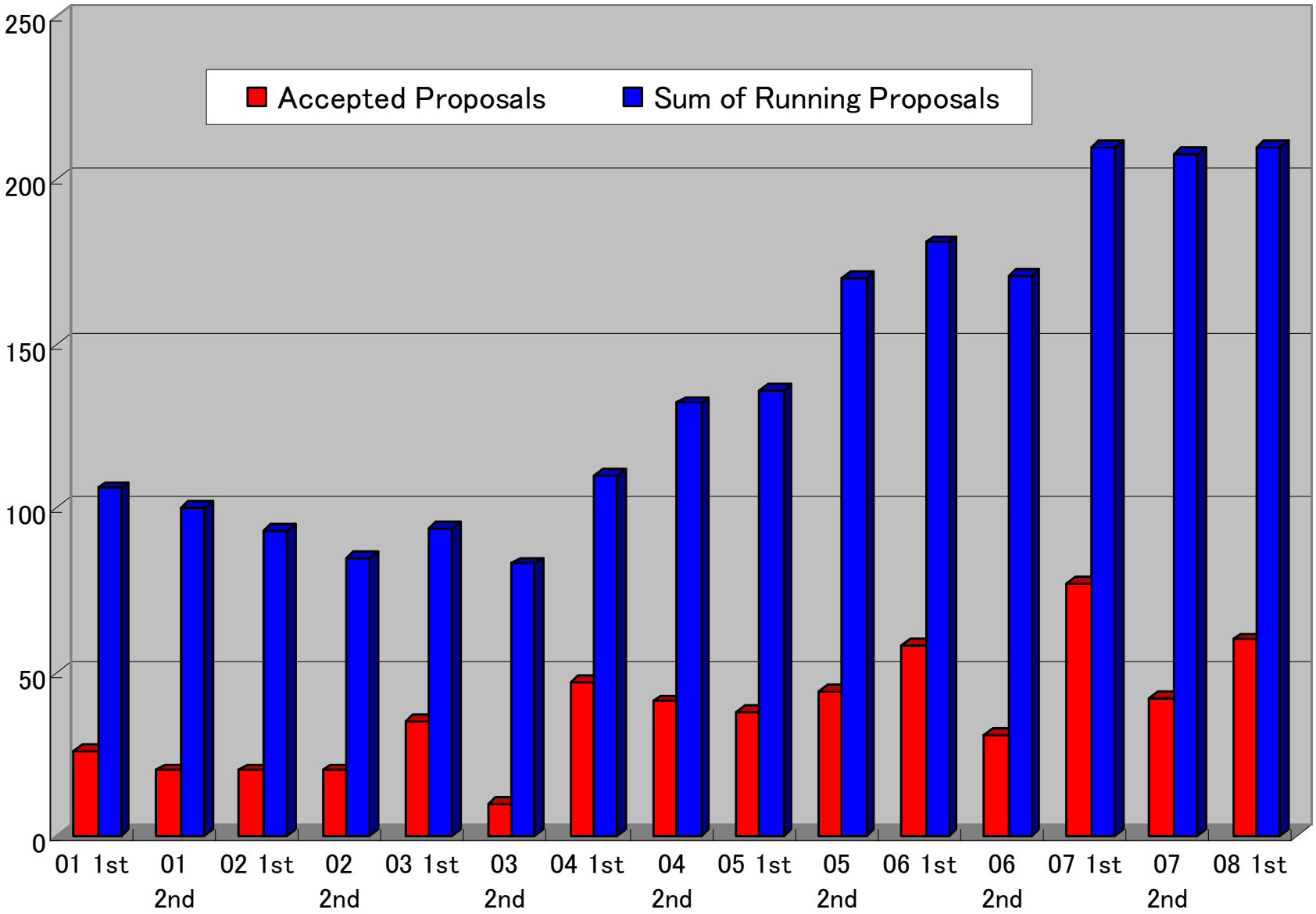


Available from Apr. 2009

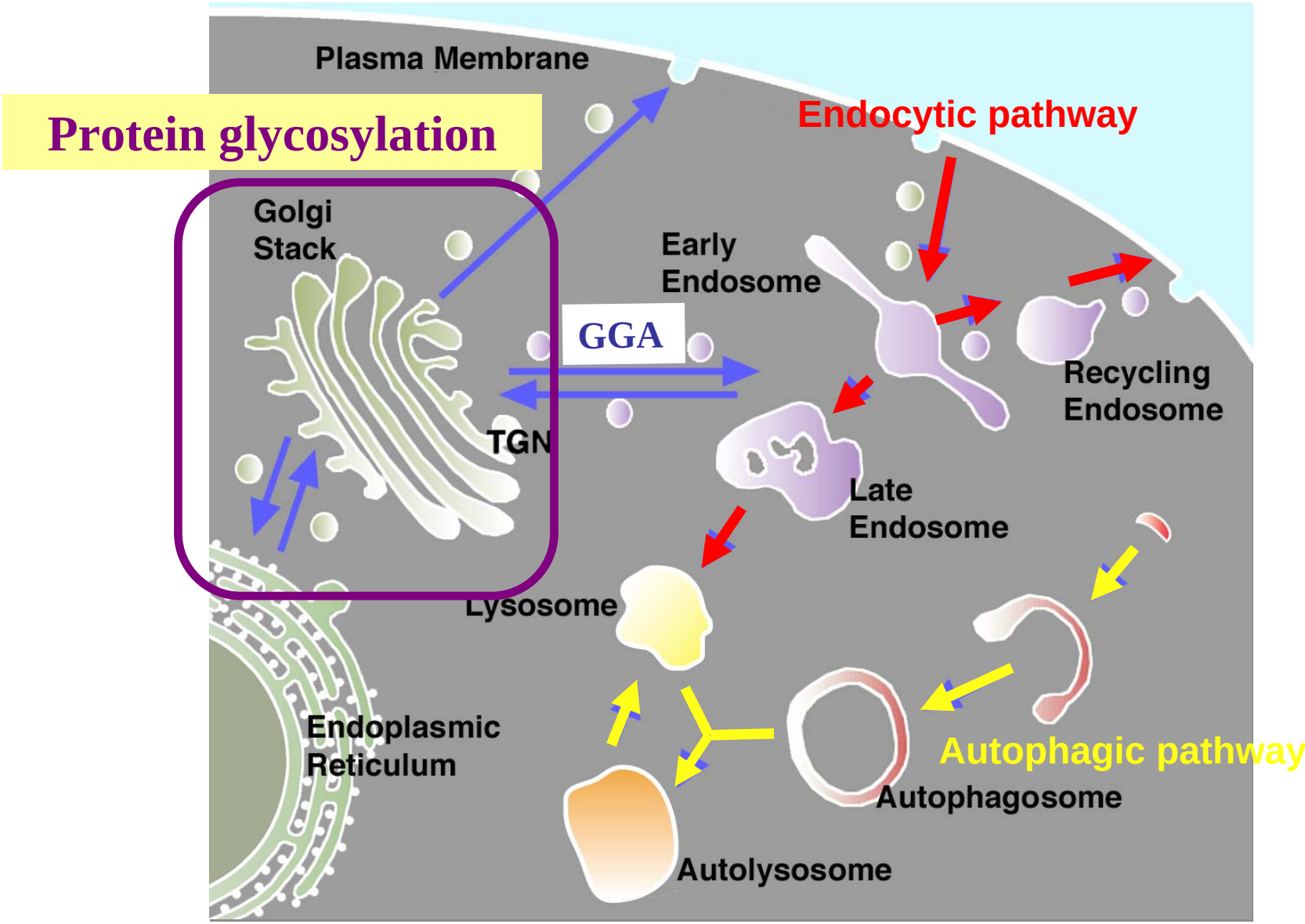


- Expected to become stronger than AR-NW12A
- Astellas Pharma will have priority access for certain amount of beam time during 10 years from April 2009.
- The rest of the beam time can be used for general user operation including use by other pharmaceutical companies.

No. of Beam Time Proposals on Protein Crystallography Beam Lines at PF
Doubled in the last 7 years.

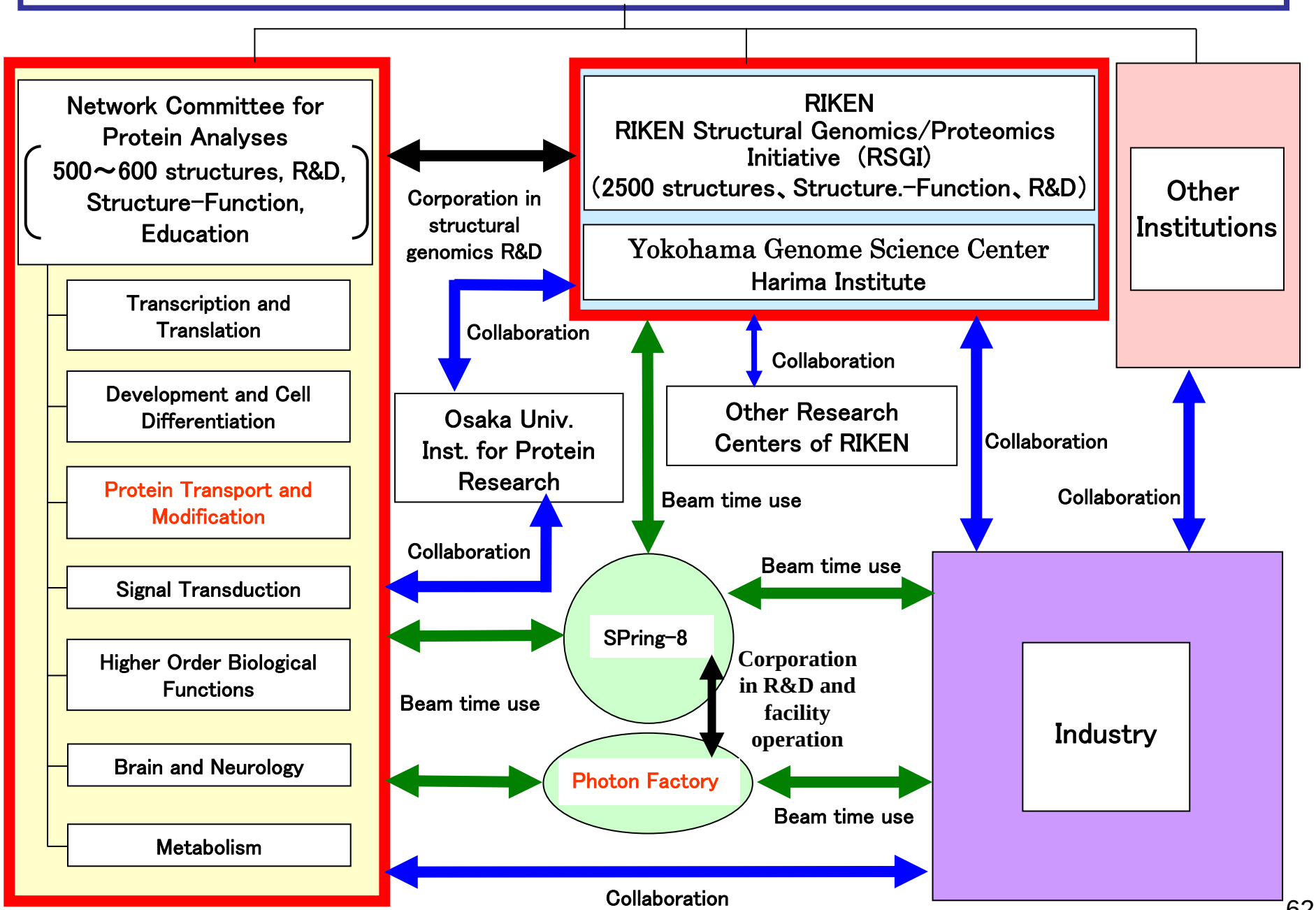


Protein glycosylation and transport



T. Yoshimori

Protein 3000 (Ministry of Education, Culture, Sports, Science and Technology)



Protein 3000 Tsukuba Structural Biology Consortium (21 groups)

Protein Transport and Posttranslational Modification

KEK, Photon Factory (PX, SAXS)

Showa Univ (PX)

Nagaoka Tech Univ (X-ray)

Nagoya City Univ. (NMR)

Tokyo Inst. Tech (PX)

Nara Inst. Sci. & Tech (SAXS)

JAERI, Nara (Bioinfo)

Original goal: 70 in 5 years (2003-March 2007)

⇒ changed to 150 last year

254 structures solved (31 March 2007)

171 PDB deposits

Osaka University
Inst. Microbial Diseases

Kagoshima University
Medical School

Kyoto University
Pharmacology Department

University of Tokyo (Komaba)

Riken, Membrane
Traffic Group

NAIST,
Molecular Biology Lab

Osaka University
Medical School

Riken
Immunology Institute

Kyoto University
Life Science Program

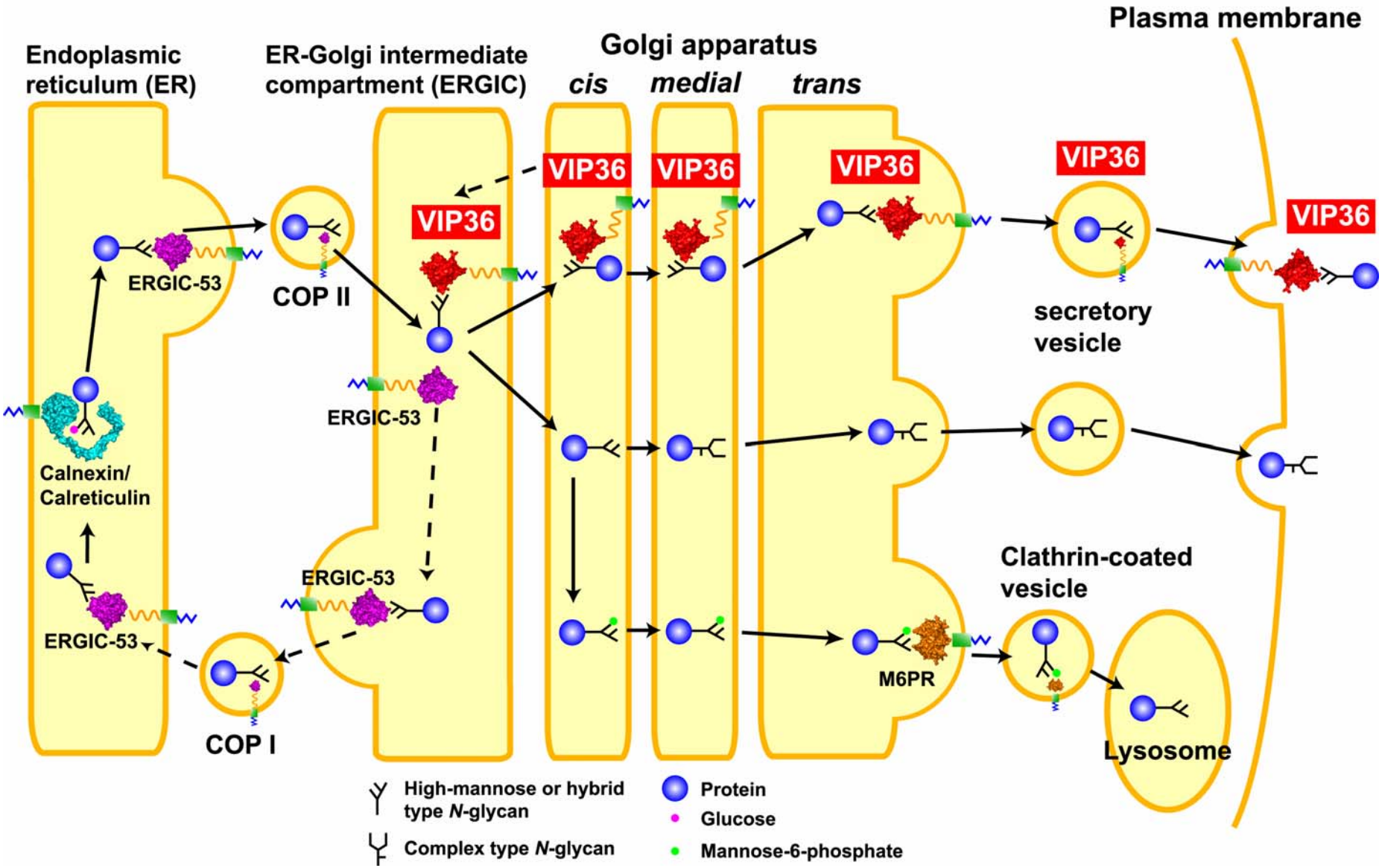
Tokyo Metropolitan
Institute of Gerontology

Kyoto Sangyo University
Faculty of Engineering

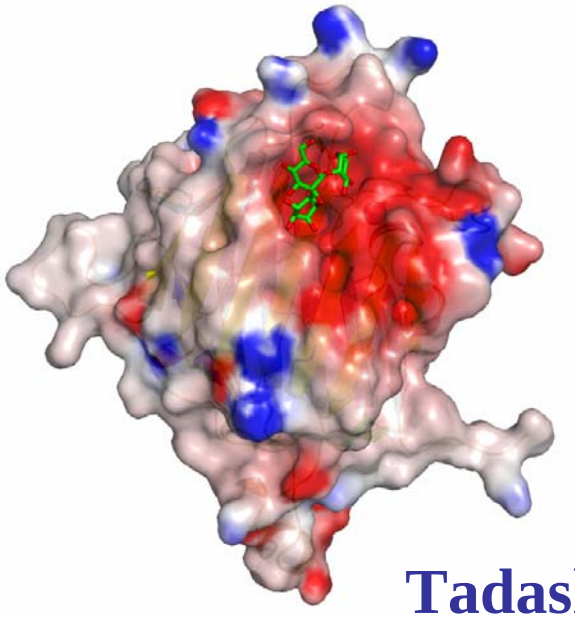
Collaboration outside of Japan

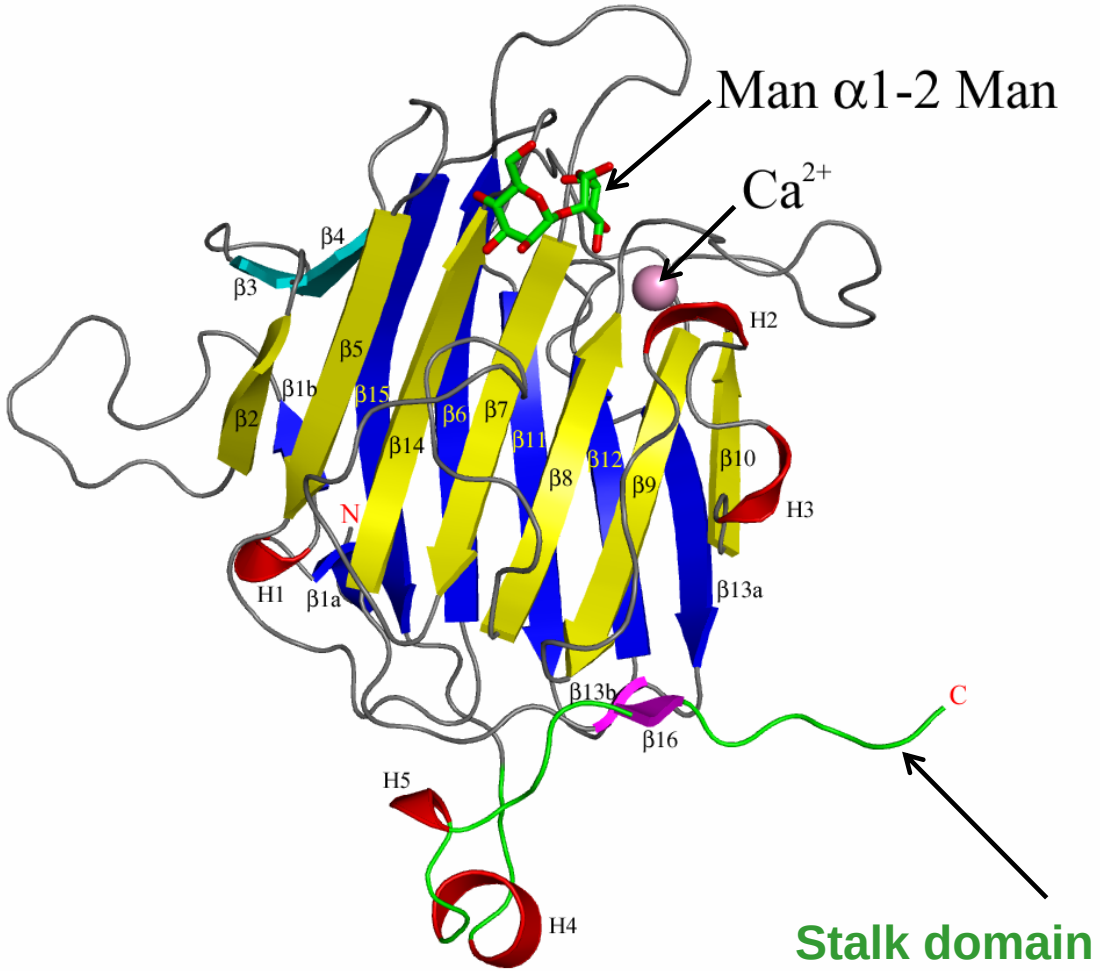
1. Human sialidase: G. Tattamanti & G. Monti, Italy,
   (Chavas et al., *JBC*, 2005)
2. Human sialidase inhibitors: M.v. Itzstein, Institute
   for Glycomics, Griffith University, Australia
3. Sialidase inhibitors: Peter Colman, Australia
   Steve Withers, Canada
4. Endocytic pathways: H. Stenmark, Oslo, Norway
   (Slagsvold et al. *JBC*, 2005, Hirano et al. *NSMB*,
   13, 272, 2006, Hirano et al. *NSMB*, 13:1031, 2006)
5. Protein carbohydrate recognition in HIV infection:
   R. Varadarajan, Bangalore, India
6. Protein carbohydrate interaction, Johan Deisenhofer,
   Univ. Texas, USA (C.-I. Chang et al. *PNAS*, June 2005)
7. Ubiquitin recognition, Ivan Dikic, Johan Wolfgan Goethe
Univ.
   Frankfurt, Germany

Model for intracellular transport of *N*-linked glycoproteins



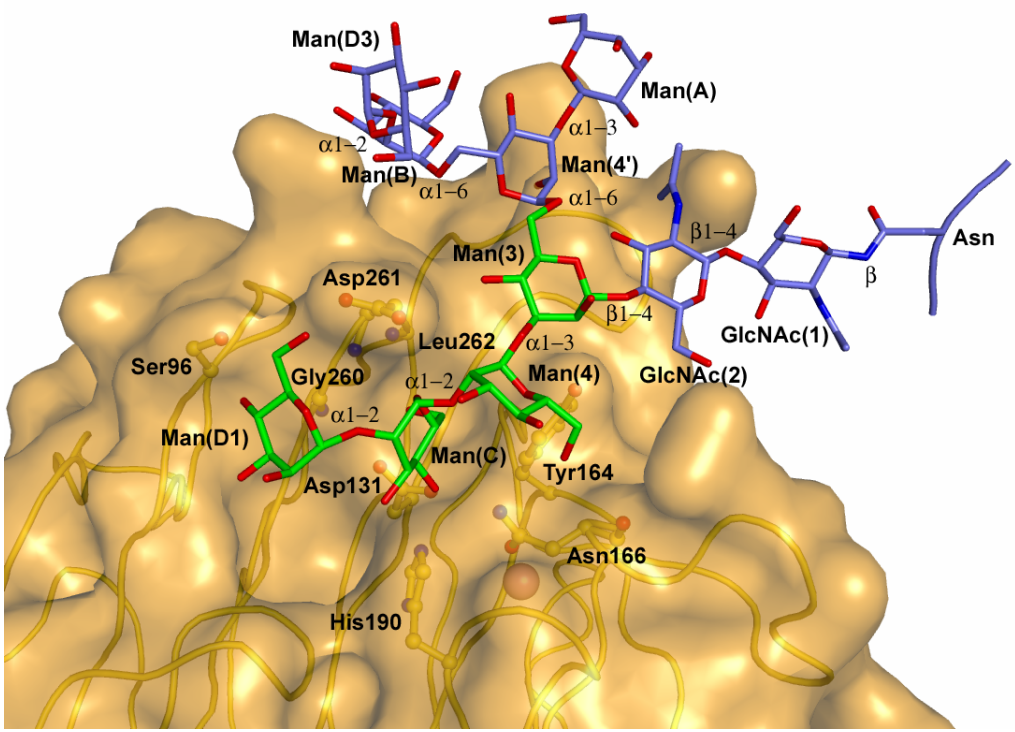
Complex structure of the Ca^{2+}/Man_2-bound VIP36 exoplasmic/lumenal domain

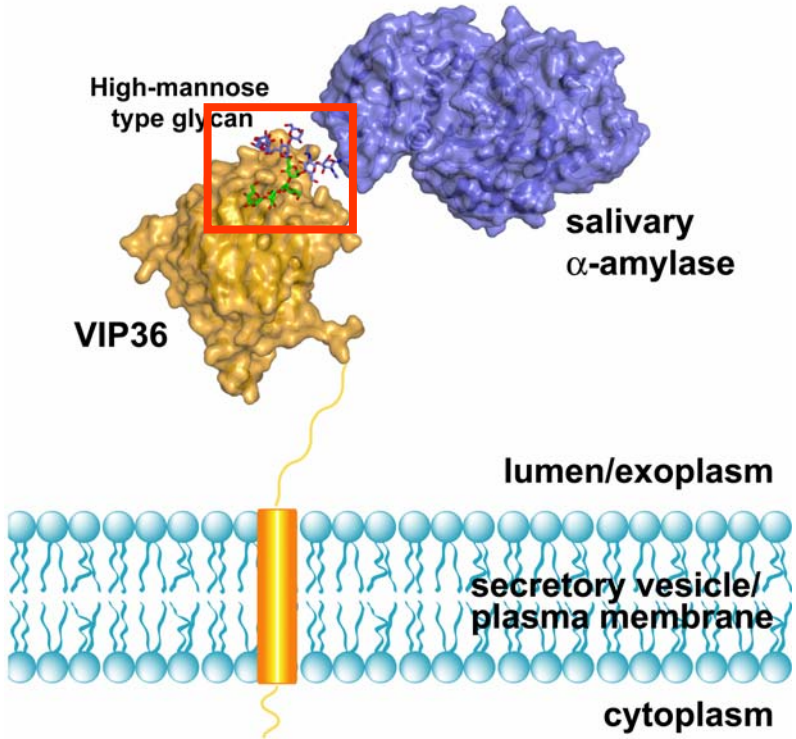




Tadashi Satoh et al., J. Biol. Chem. (2007) 282, 28246

Model for binding between VIP36 and high-mannose type glycoprotein, salivary α-amylase





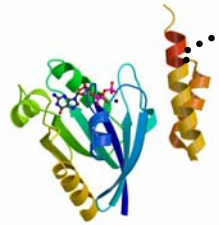
Tadashi Satoh et al., J. Biol. Chem. (2007) 282, 28246

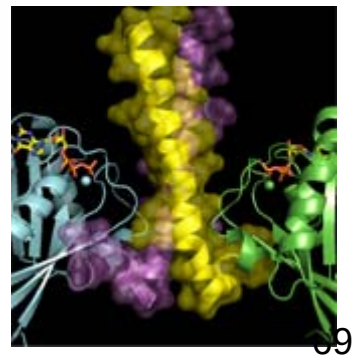
Small GTPases involved in vesicle transport:

Rab and Arf

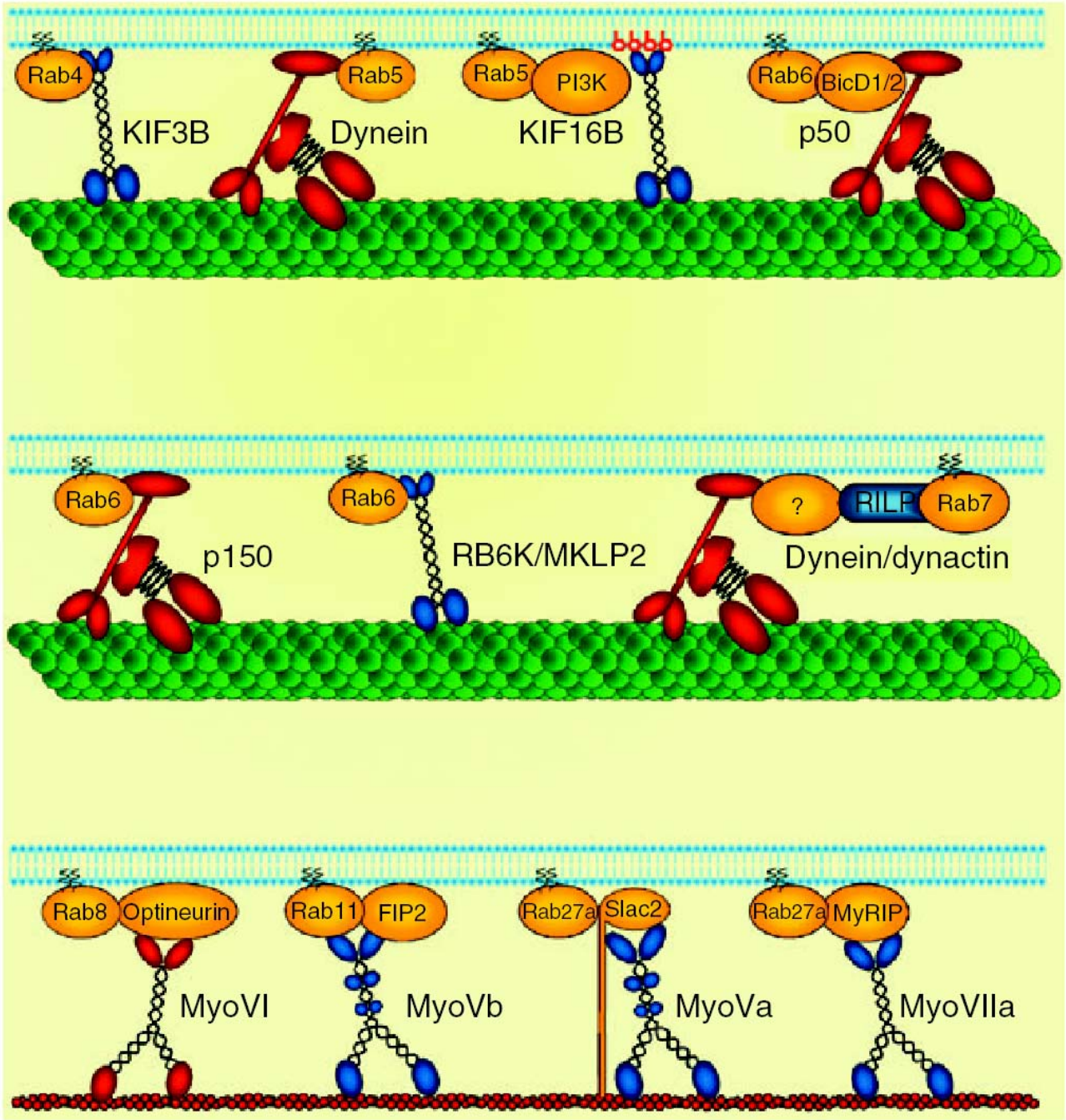
Small GTPases, ARF and Rab, in vesicular

T. Shiba, et al., *Nature Structural Biology* 10 386-393, 2003





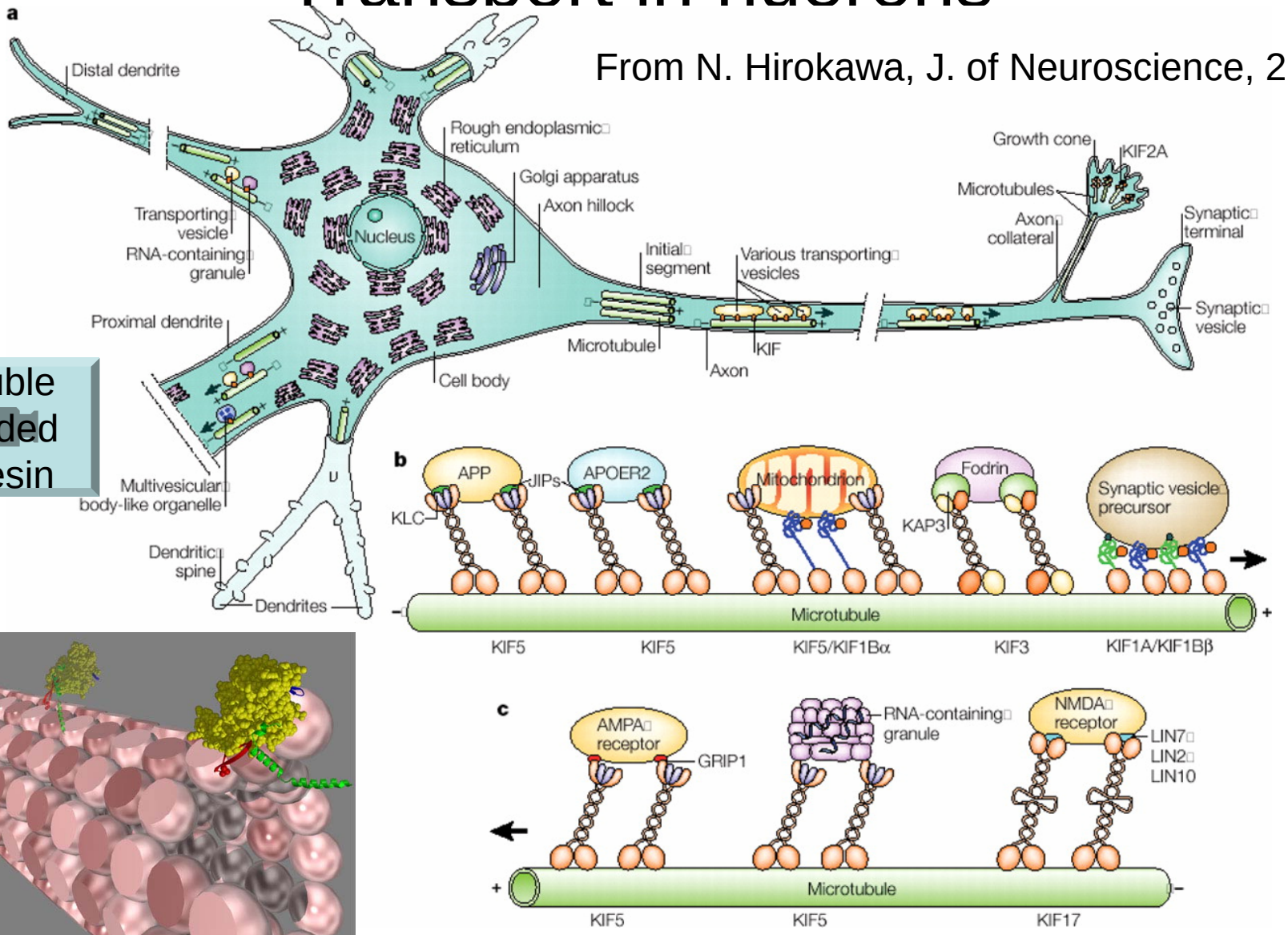
T. Shiba *et al. PNAS* 103, 15416, 2006



Taken from I. Jordens, et al., J.Neefjes, Traffic 2005; 6: 1070–1077

Transport in nuerons

From N. Hirokawa, J. of Neuroscience, 2006

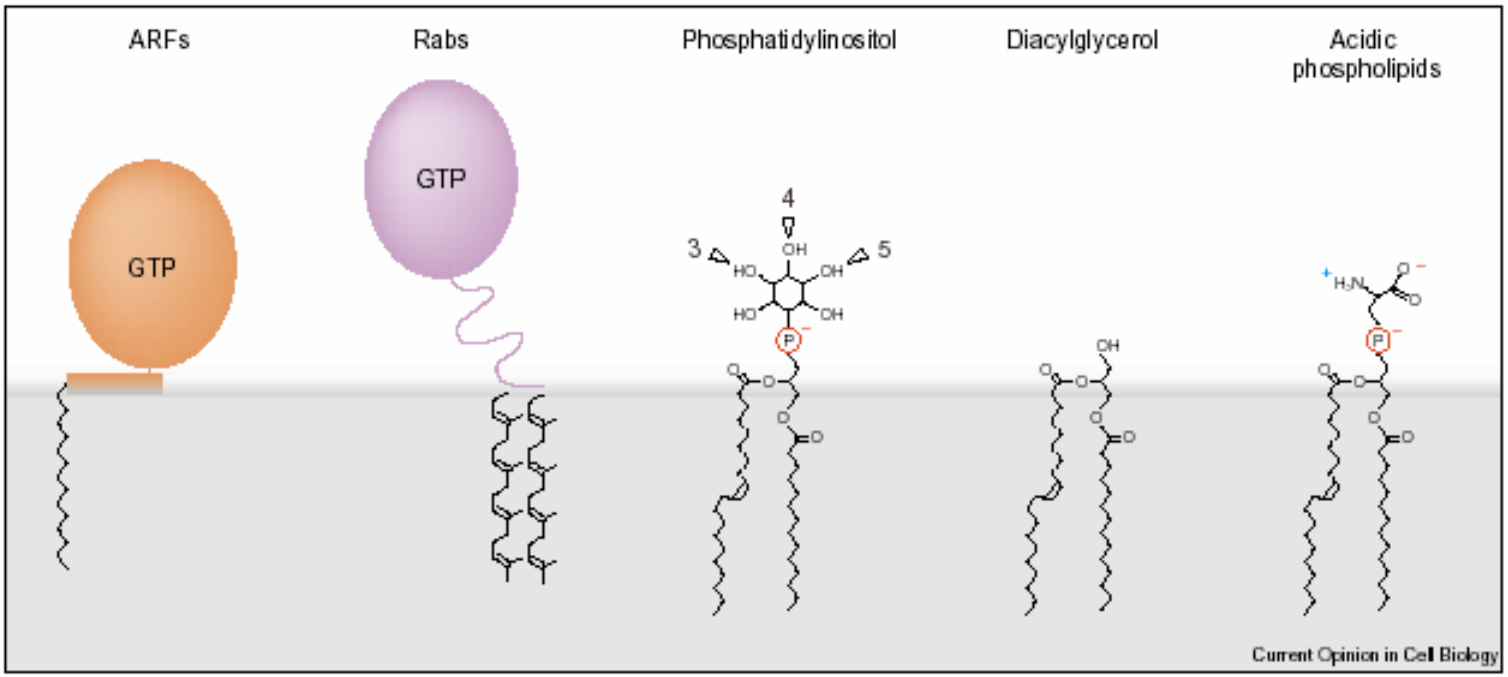


Double headed kinesin

Ogawa, et al., Cell, 2004

KIF1A: single headed motor, Nitta, Hirokawa et al., *Science* 2004, 305, 678 – 683, Data collected on PF-BL6A & 19ID APS

Membrane recruitment of effector proteins by Arf and Rab GTPases



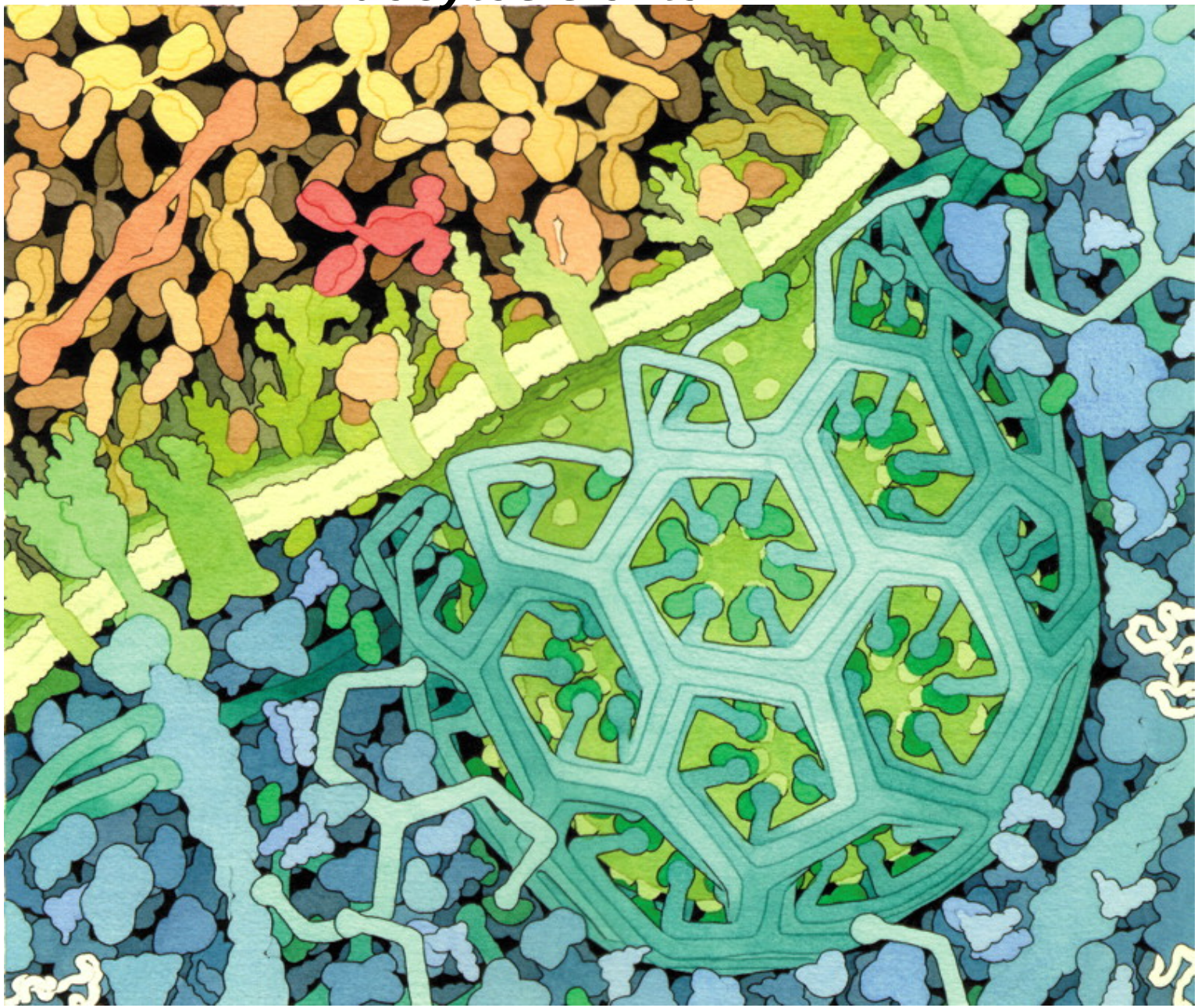
Schematic representation of the major factors known to be involved in the constitutive recruitment of peripheral membrane proteins to the cytoplasmic face of organelles. The phosphoinositides can carry their phosphates at any combination of the three positions indicated. Most of these recruitment determinants lie at or near the surface of the membrane, with the exception of the Rab proteins, which are linked to the bilayer via a poorly conserved carboxy-terminal region of 25 residues. This region appears to be unstructured, and so could extend up to 80 Å from the bilayer, about twice the diameter of the Rab itself [76].

FROM: Munro S.
Organelle identity and the targeting of peripheral membrane proteins.
Curr Opin Cell Biol. 2002, 14: 506-514.

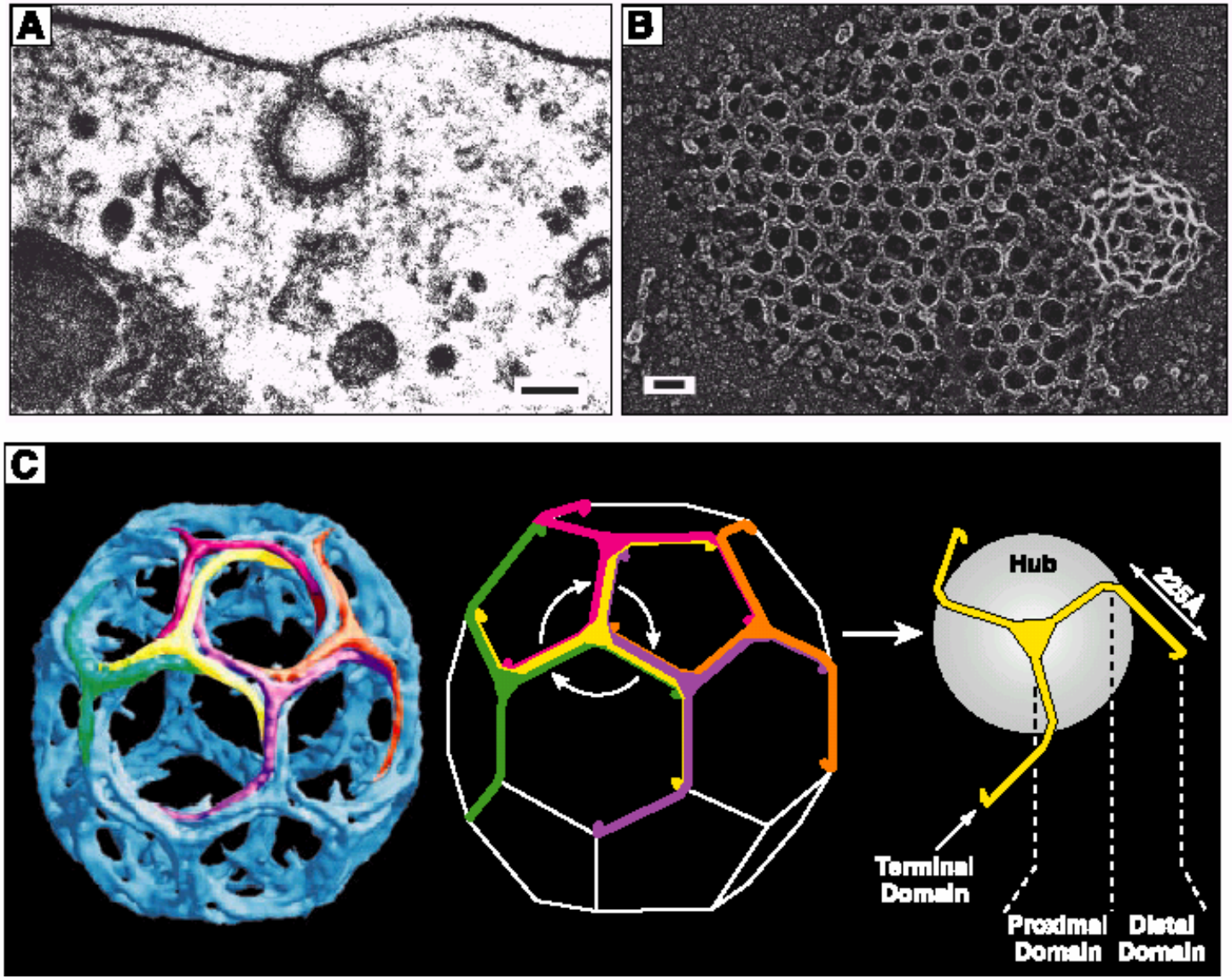
Small GTPases involved in vesicle transport:

Rab and Arf in GTP form: effector interactions

Endocytosis of toxin



David S. Goodsell, Scripps Institute, http://www.scripps.edu/mb/goodsell/

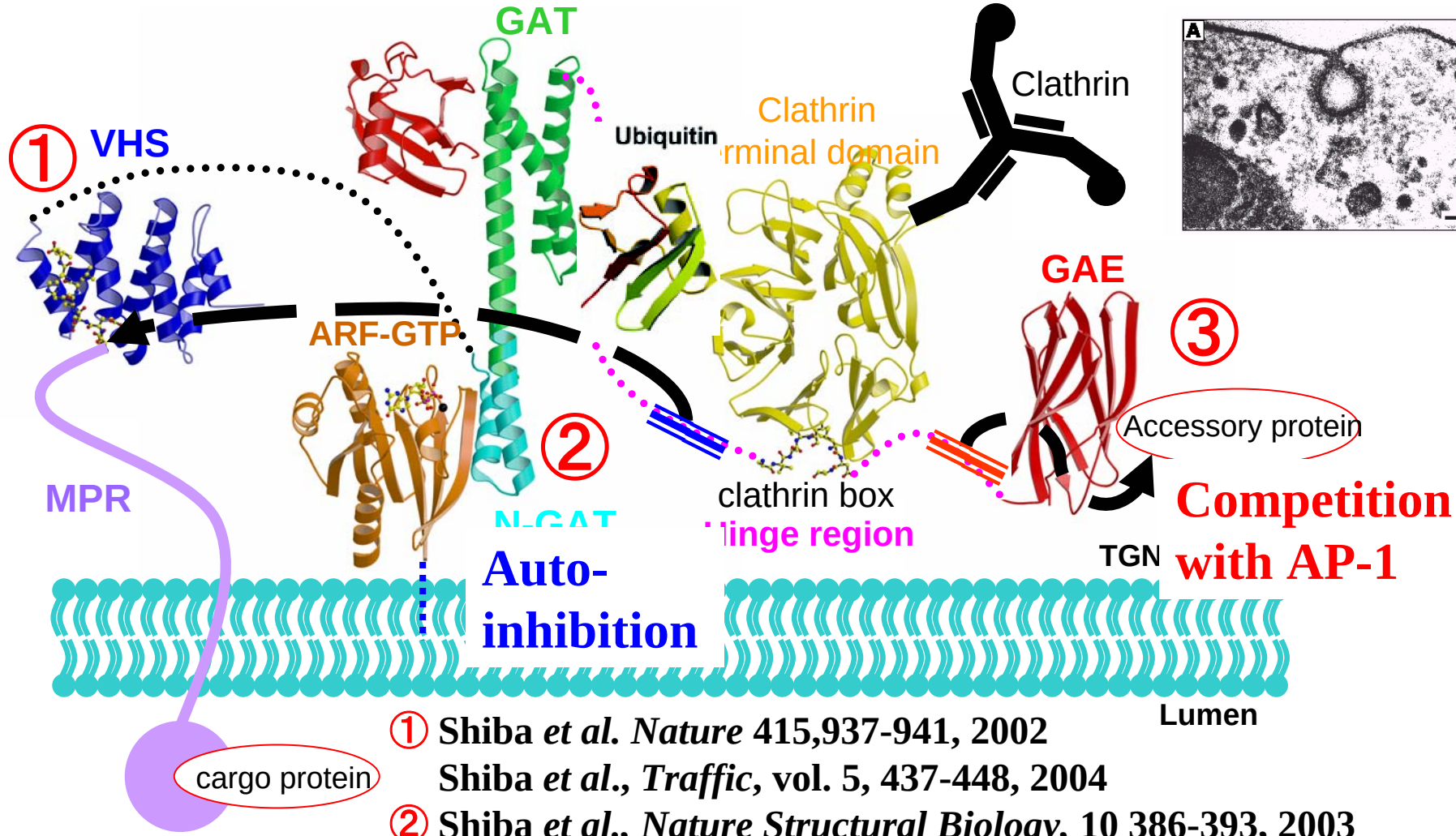


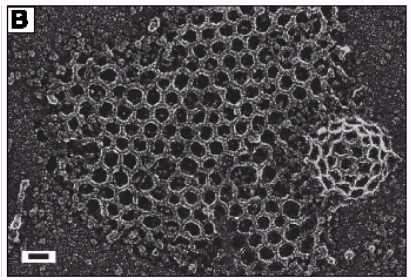
M. Marsh and H. T. McMahon, Science, 1999, Vol. 285, 215

Clathrin movie by Allison Bruce, Harvard University

http://www.hms.harvard.edu/news/clathrin/

Human GGA: a new class of adaptor proteins





① Shiba *et al. Nature* 415,937-941, 2002

Shiba *et al., Traffic*, vol. 5, 437-448, 2004

② Shiba *et al., Nature Structural Biology*, 10 386-393, 2003

Shiba *et al., J. Biol. Chem.* 279, 7105-11279, 2004

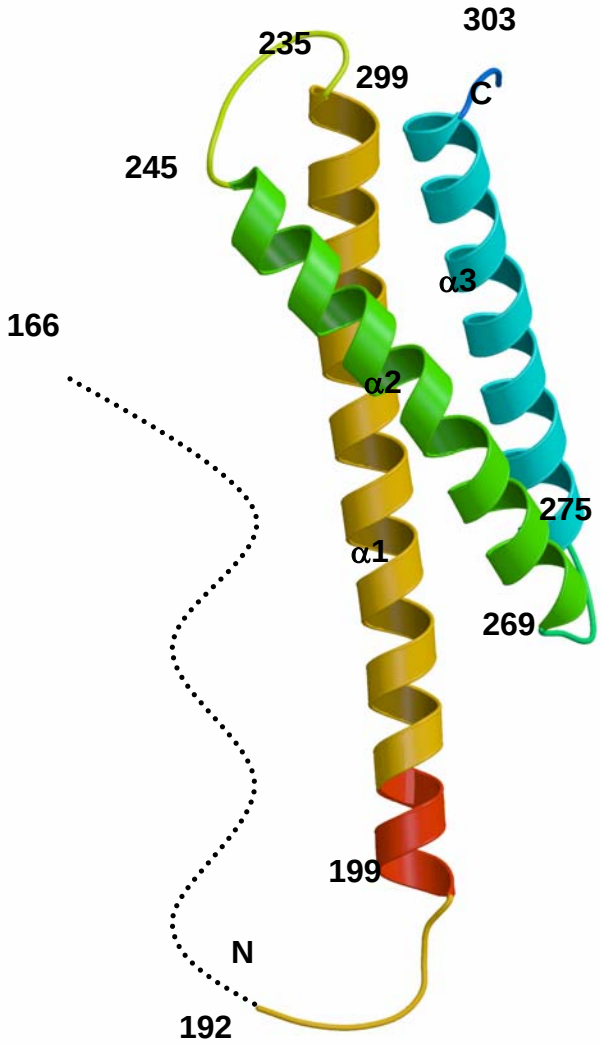
Kawasaki *et al., Genes to Cells*, 10, 639–654, 2005

Yogosawa *et al., BBRC*, 350, 82-90, 2006

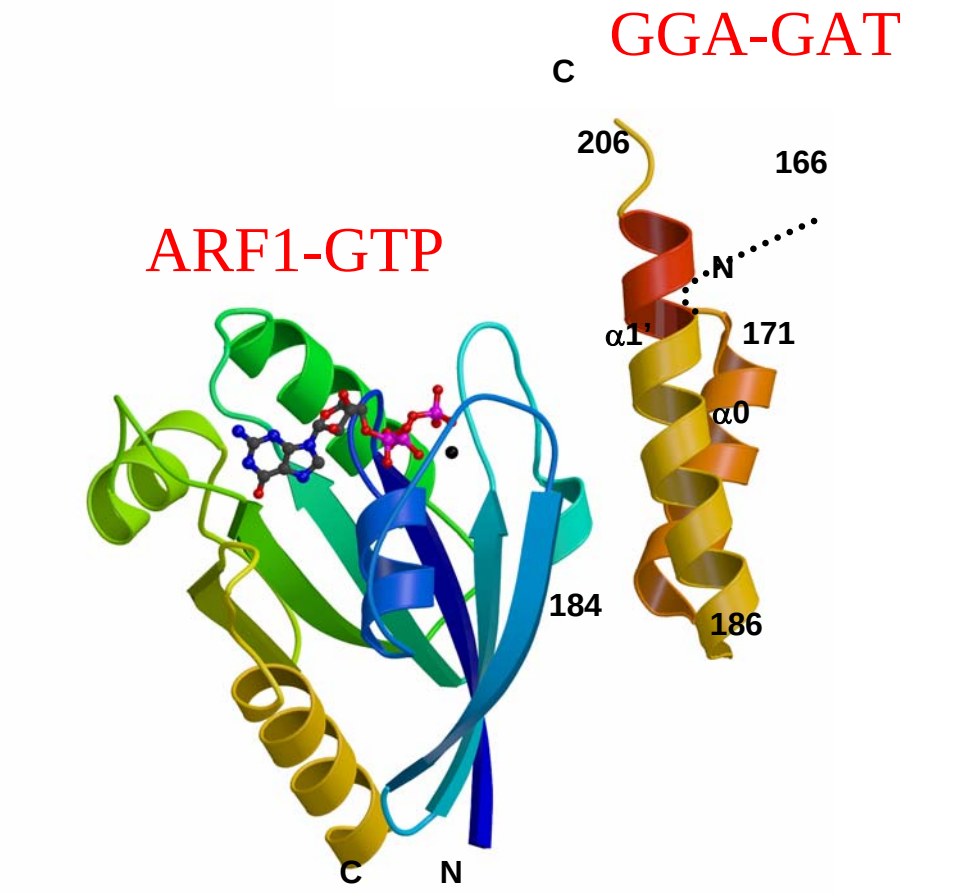
③ Nogi *et al. Nature Structural Biology*, 9, 527-531, 2002

Inoue *et al. Traffic*, 8, 904-913, 2007

GGA-GAT is indispensable for docking onto the TGN membrane



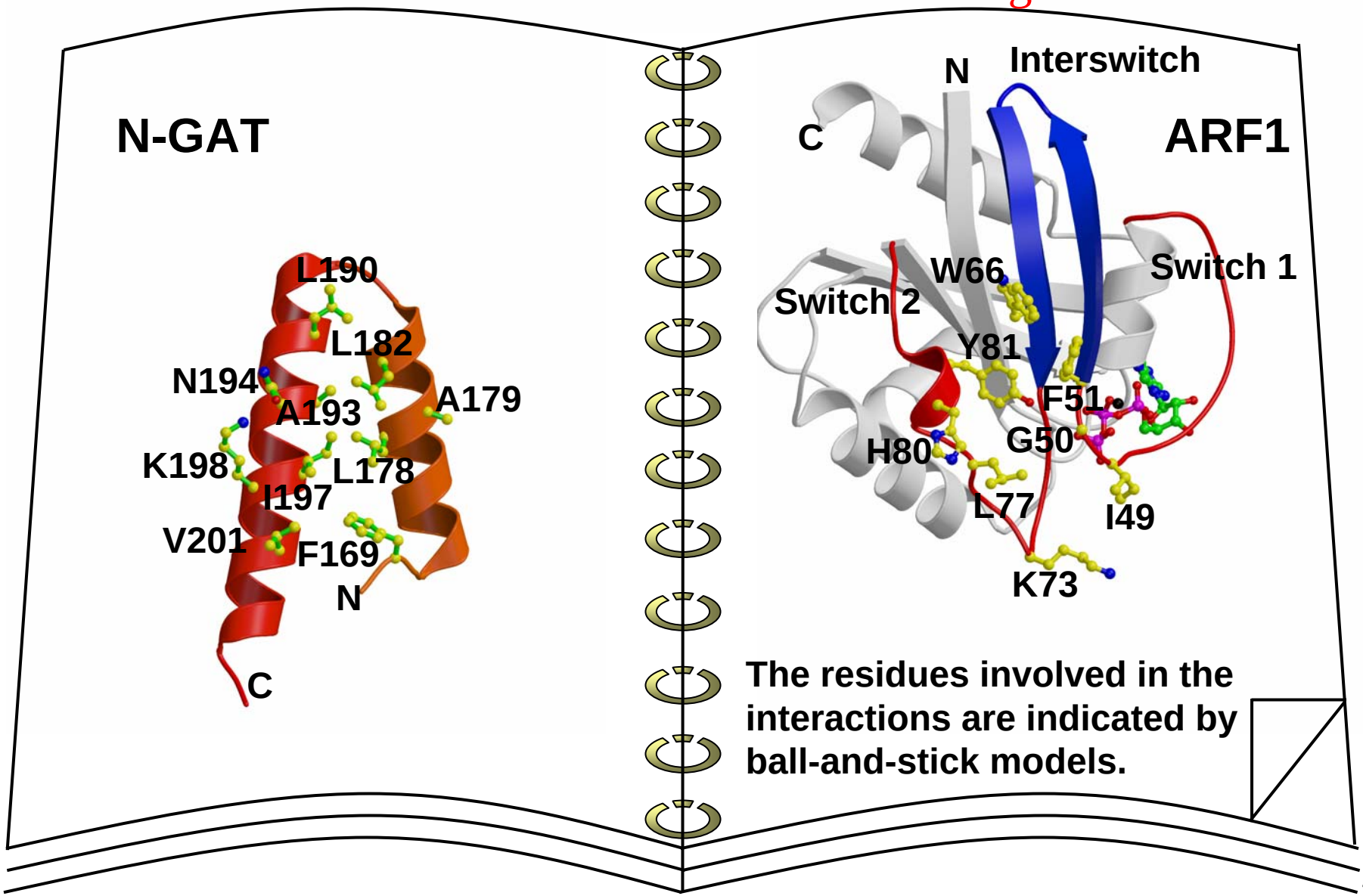
GAT domain apo-form



N-GAT & ARF1-GTP complex

Interaction surfaces of N-GAT and ARF1-GTP

GGA-GAT docks on the membrane via mostly hydrophobic interactions with Switches 1 & 2 and interswitch region of ARF1-GTP

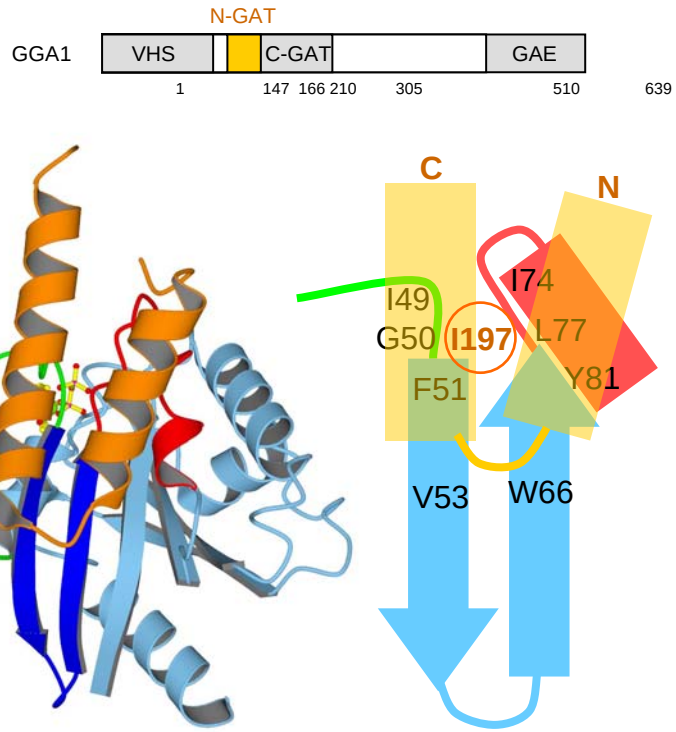


The residues involved in the interactions are indicated by ball-and-stick models.

Arf's interacation site is used by effectors and many other proteins (Kawasaki, Nakayama, &Wakatsuki, Current Opinion in Structural Biology 2005, 15, 681)

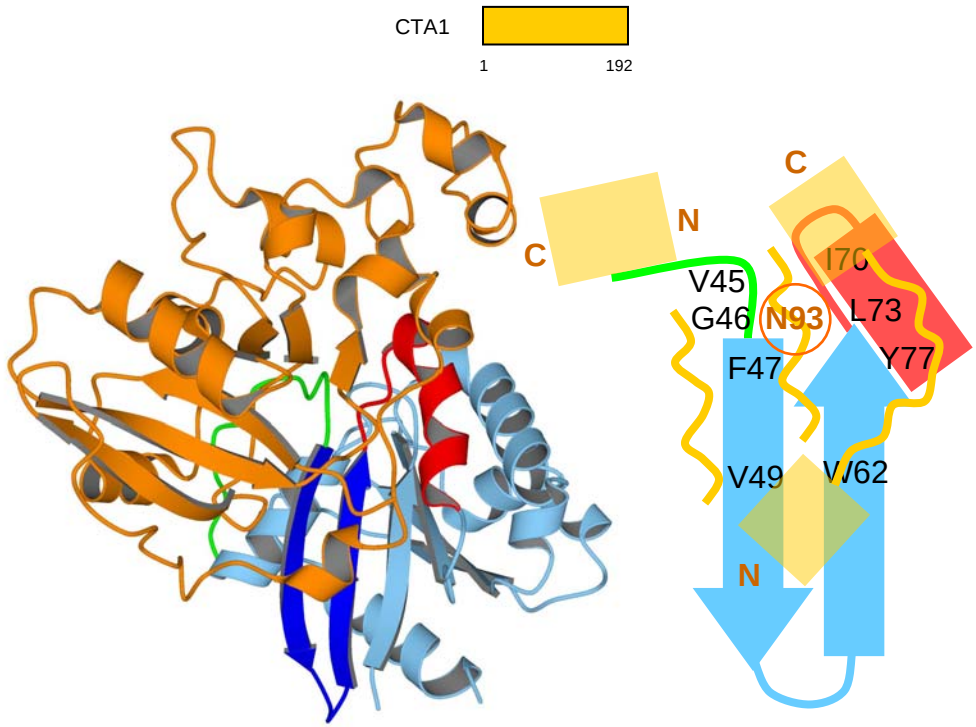
Exploitation of the human protein transport pathway by cholera toxin.

Arf1–N-GAT



T. Shiba, et al., *Nature Structural Biology* 10 386-393, 2003

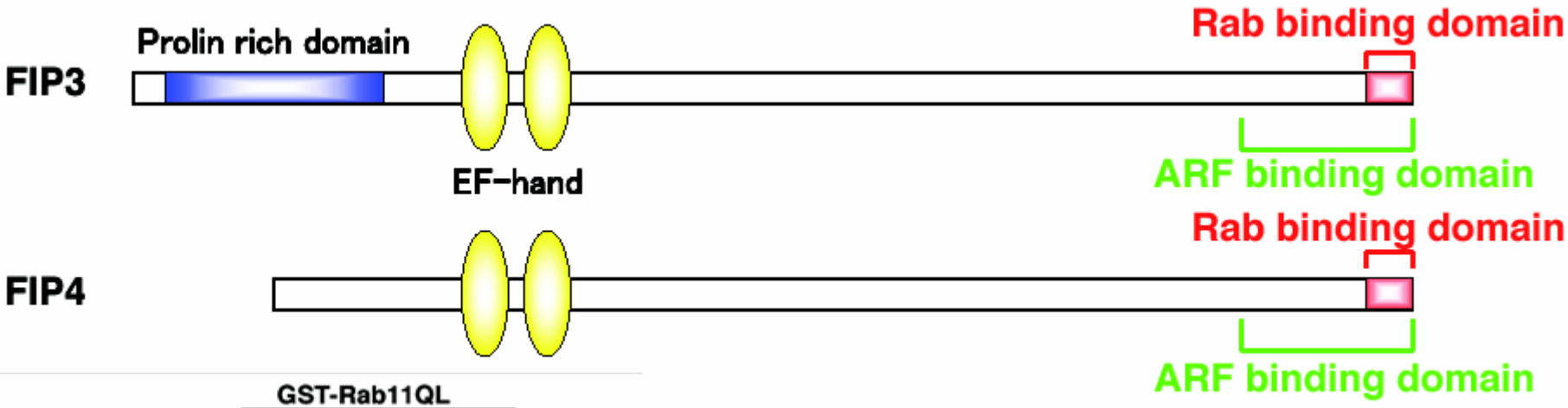
Arf6–CTA1（Cholera toxin）

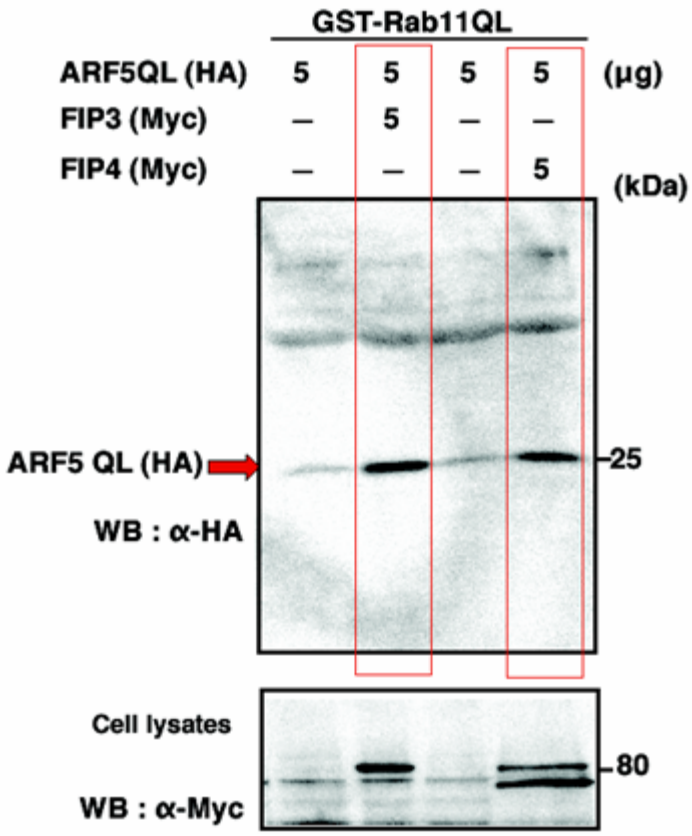


Structure by Wim Hol, Seattle, USA

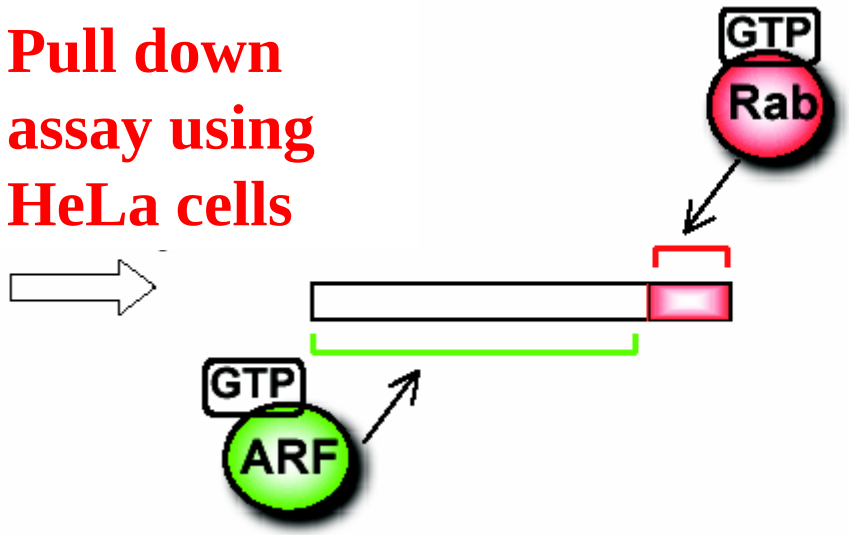
C.J. ONeal, et al. and W.G.J. Hol, *Science* 12 August 2005

FIP (Family of Rab11 interacting proteins)



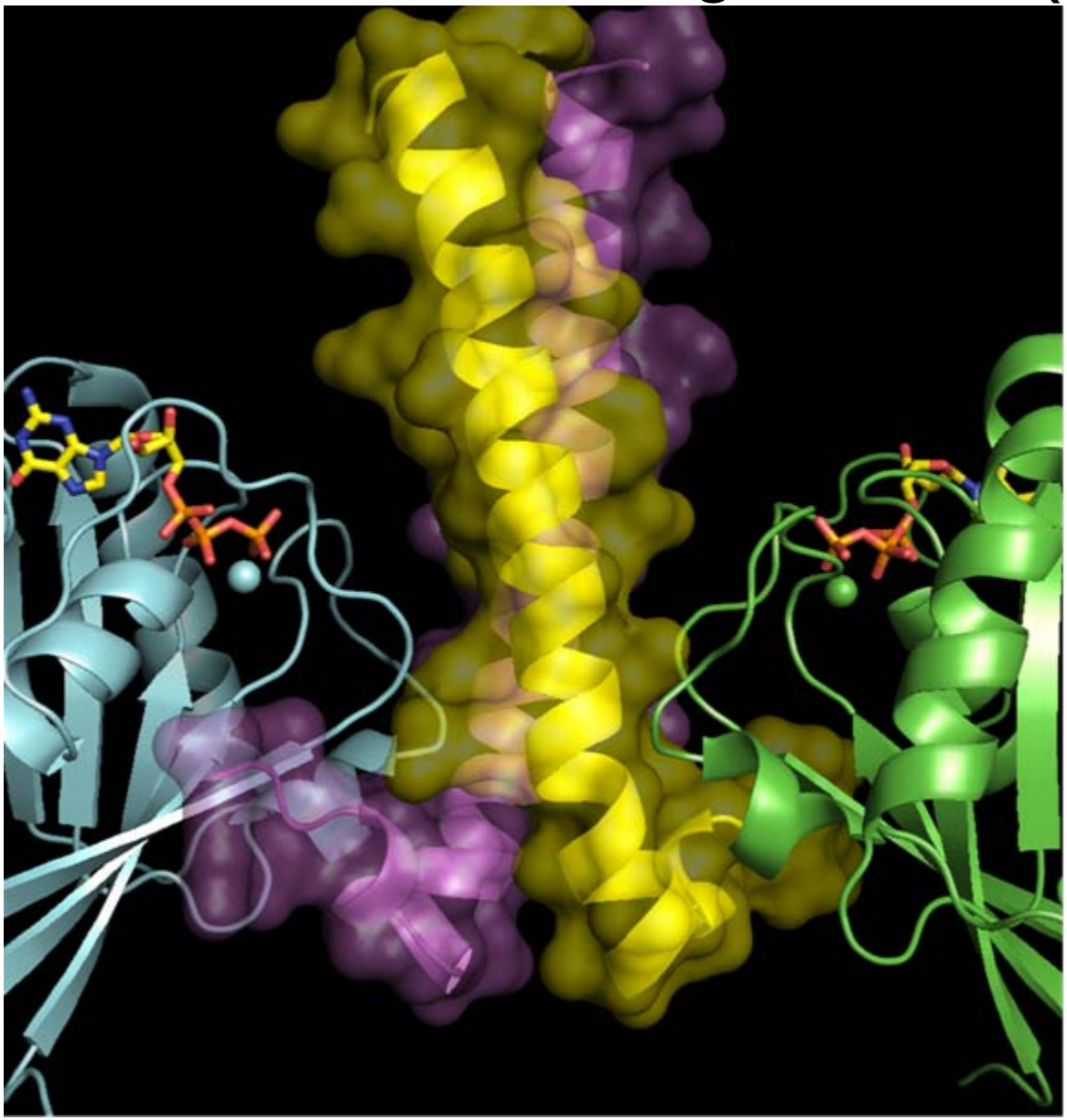


Pull down assay using HeLa cells



FIP3/4 can also interact with ARF5 and Rab11 simulateously.

Rab11/ Rab11-binding domain (RBD) of FIP3



T. Shiba *et al.*
PNAS *103: 15416, 2006.*

Also:

FIP3/Rab11 by Lambright, JMB, Aug 26, 2006

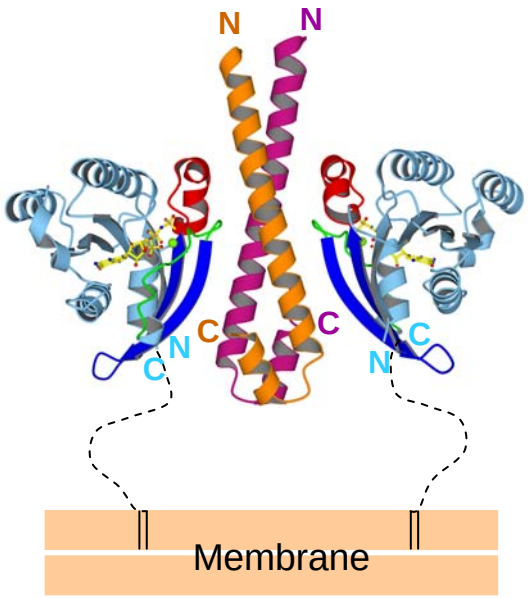
&

FIP2/Rab11 by A.R. Khan, Structure, Aug 2006

Target membrane

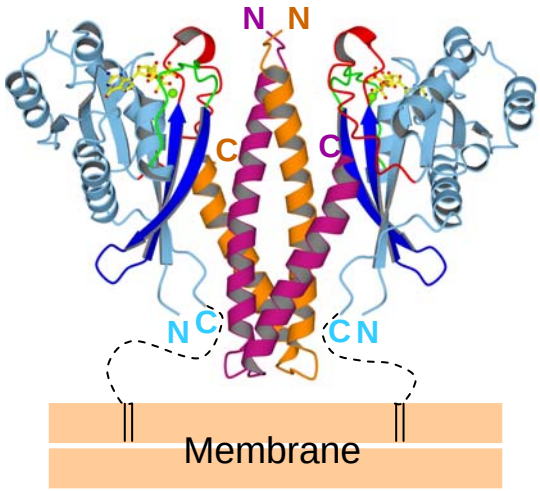
Two-fold symmetry of Rab binding domains

Rab5–Rabaptin5



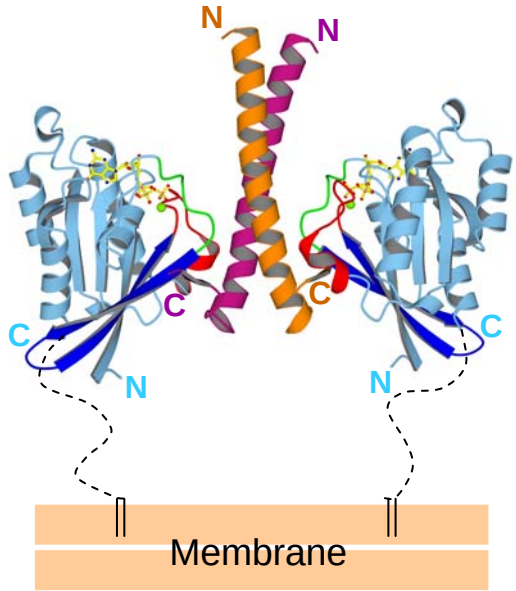
Zhu G, et al., *Nat. Struct. Mol. Biol.* 11, 975-983 (2004)

Rab7–RILP



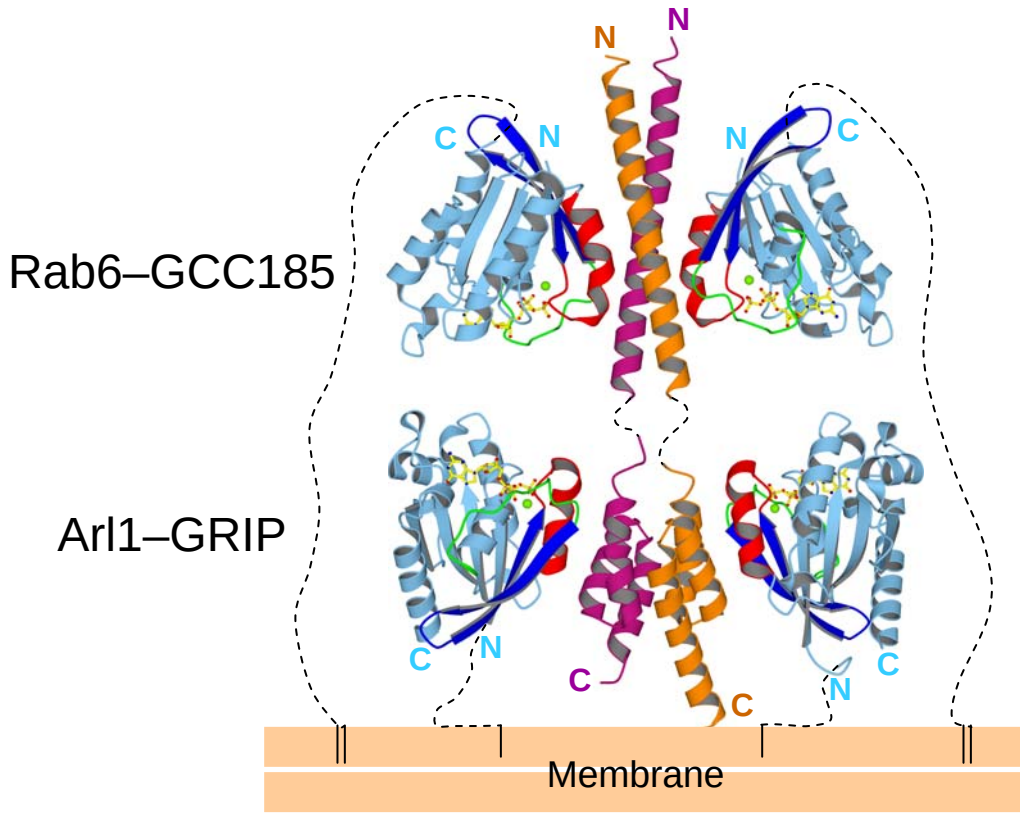
Wu M, et al., *EMBO J.* 24, 1491-1501 (2005)

Rab11–FIP3



Shiba T. *et al.* PNAS 103, 15416-15421 (2006)

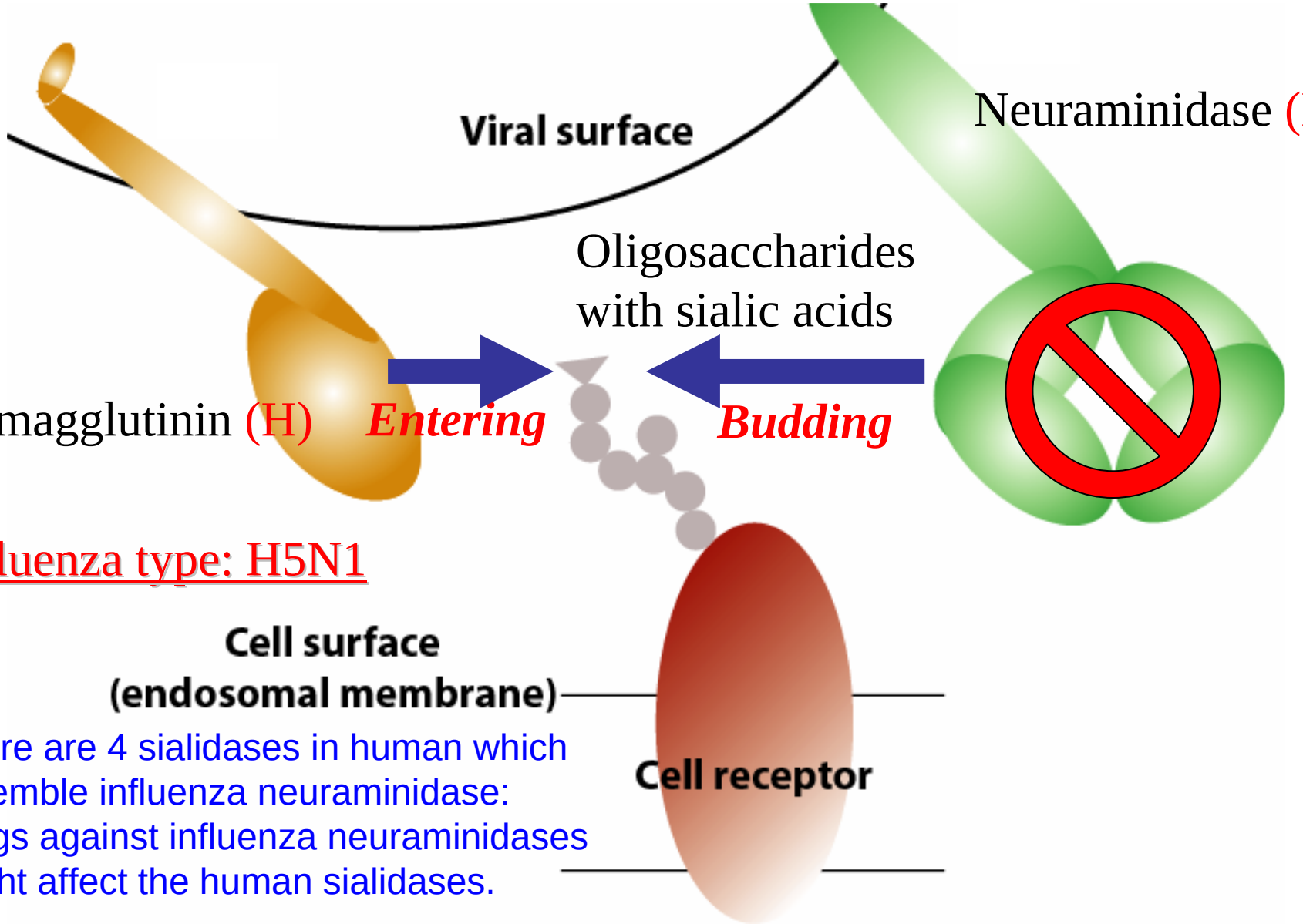
Two-fold symmetry of Arf/Rab binding domains



Burguete AS, Fenn TD, Brunger AT, Pfeffer SR, *Cell* 132, 286-298, 2008

Studies towards Structure Based Drug Design

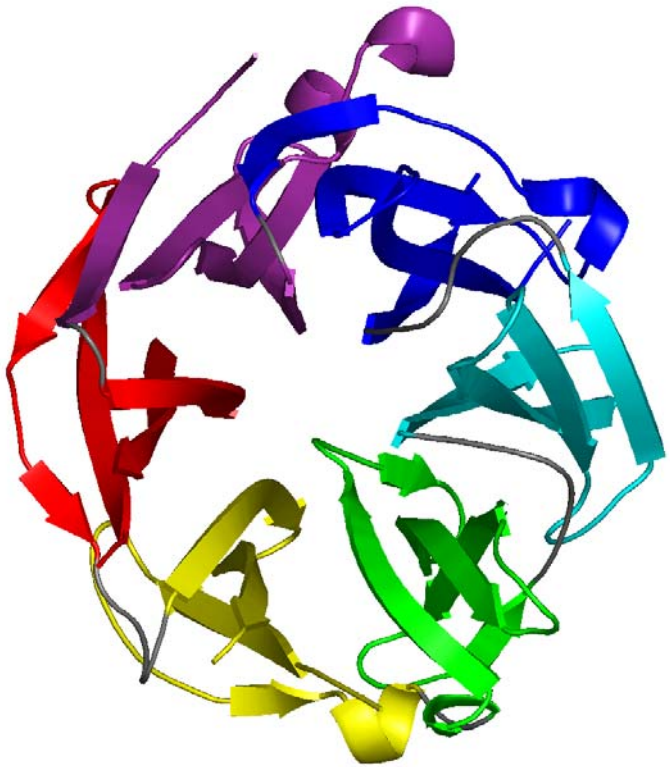
Anti-influenza drug with little or no side effects



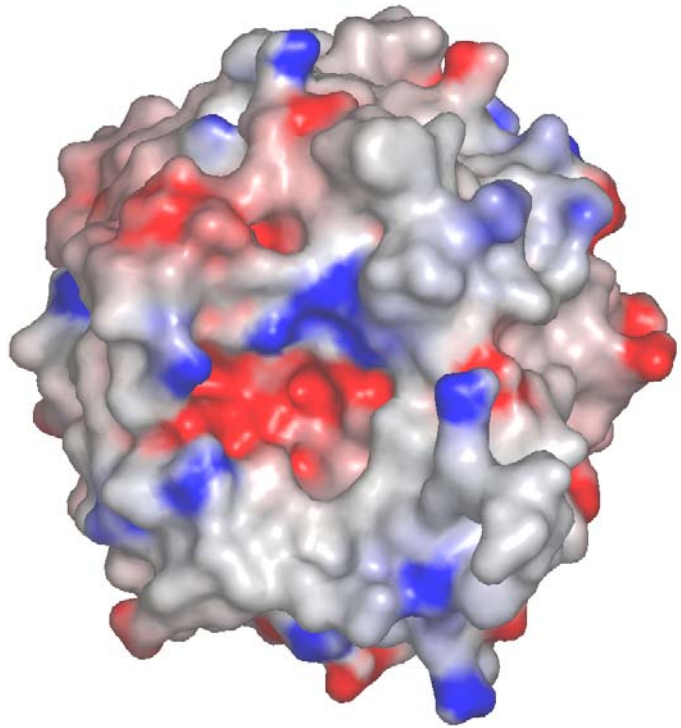
Influenza type: H5N1

There are 4 sialidases in human which resemble influenza neuraminidase: drugs against influenza neuraminidases might affect the human sialidases.

First human sialidase (~neuraminidase) structure Neu2 folding and electrostatic surfaces



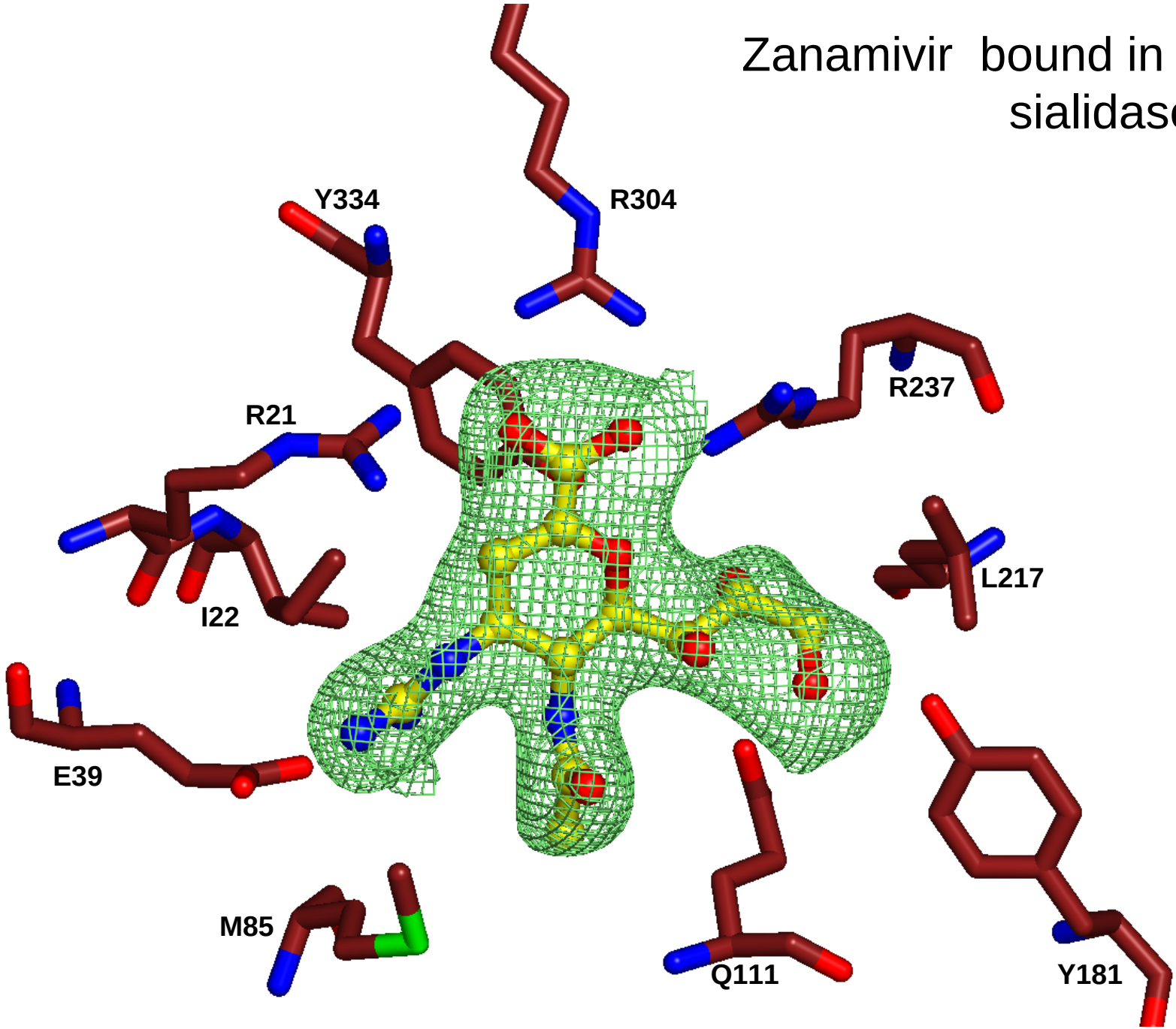
Ribbon diagram representation (view from the active site)



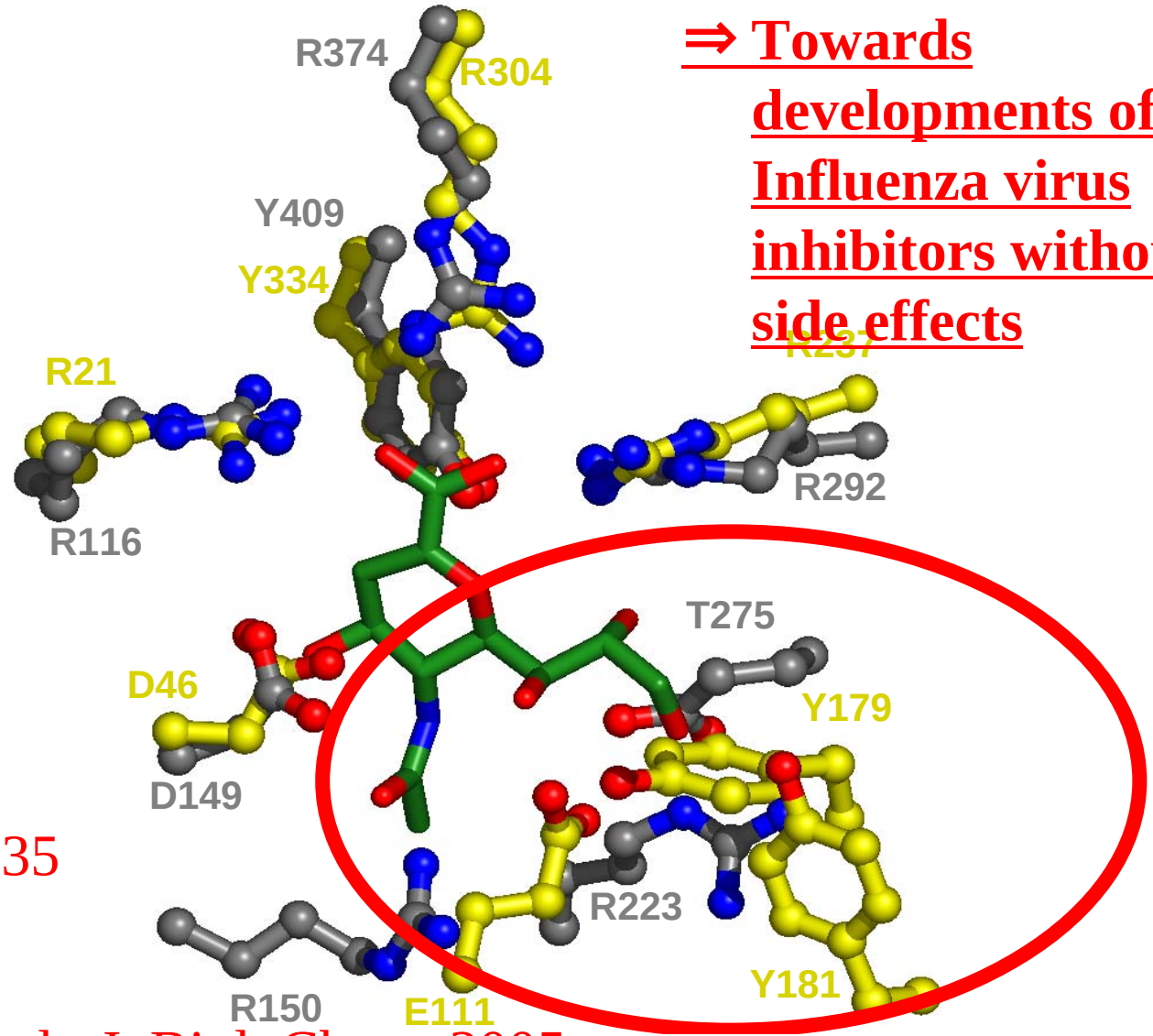
Electrostatic surface representation (blue and red regions are basic and acid surfaces respectively)

Chavas et al., J. Biol. Chem. 2005 vol 280, 469-75

Zanamivir bound in human sialidase Neu2



Structural Differences between Human Neu2 and Influenza Virus Neuraminidase



⇒ Towards developments of new Influenza virus inhibitors without side effects

Talk by Leo Chavas, 23rd Session 7, 16:35

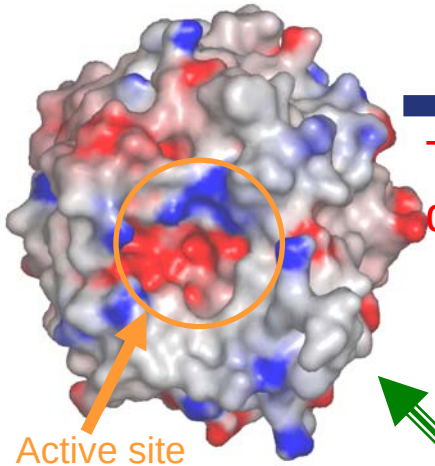
Leo Chavas et al., J. Biol. Chem. 2005 vol 280, 469-75

Human Neu2

Influenza virus neuraminidase (1VCJ)

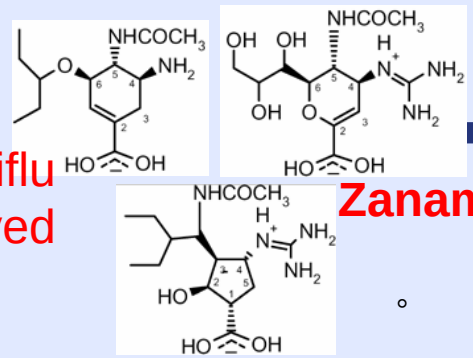
Structure Based Drug Design : more efficient and lower cost for development of
Anti influenza drug with little or no side effects (collab. with Italy, Australia, Canada)

Influenza neuraminidase



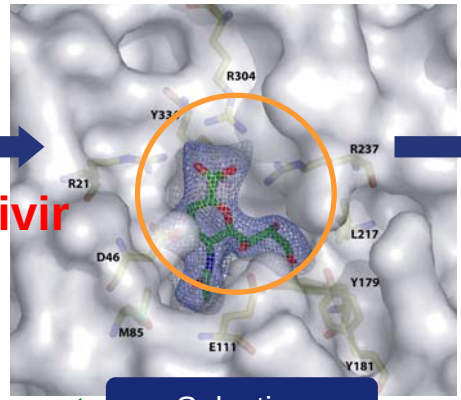
Active site

Tamiflu derived



Zanamivir

Inhibitor design

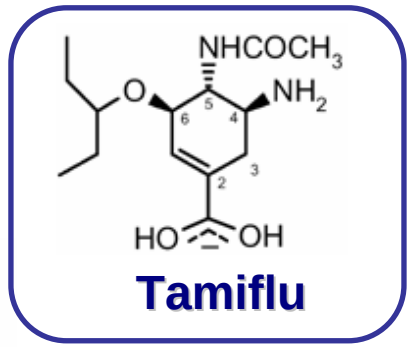


Selection

Lead compounds

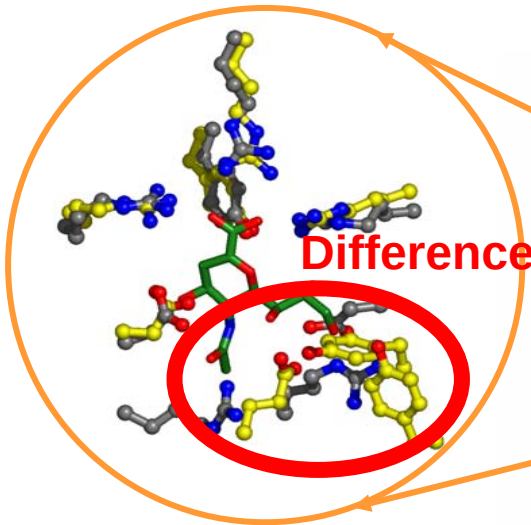
Clinical tests

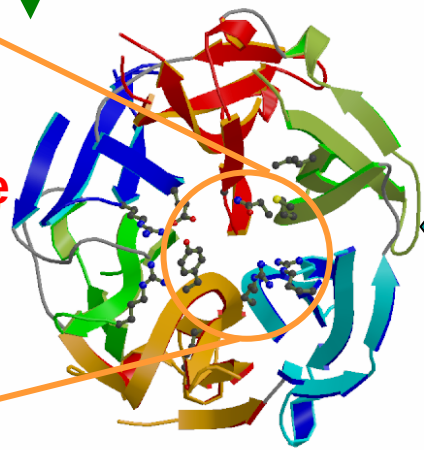
Structure Determination using SR



Tamiflu

Drugs with no side effects: Interaction with only influenza neuraminidase but NOT with human sialidases





Side effects: Human sialidases? Neu1, Neu2, Neu3, Neu4

Yellow: human sialidase Neu2

Gray: Influenza neuraminidase

Human sialidase **Neu2 structure**

Target Protein Project (5 years: 2007-2011)

- Yearly budget: 5.5 billion yen, US$ 44.5M, 33.3 MEuro, (includes 30% overhead)
- Proposal deadline: 20 April 2007
- Selection results published: 15 June 2007
- 43 teams selected

Targets:	Fundamental Biology	Medical importance/relevance	Food and environment
5 yr term	7	6	5
3 yr term	5	4	6

	Protein Production Core	Structural Analysis Core	Functional Control Core (Chem. Library)	Informatics Core
5 yr	1	1	1	1
3 yr	3	2	0	1

Target Protein Project (2007-2011)
The Analysis Core

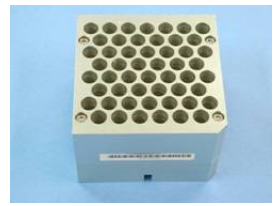
Joint Proposal by SPring-8 and PF: Two New Beam Lines

PF

② Microfocus Beam Line Low to medium energy (Invacuum short gap undulator)

① Microfocus Beam Line Medium to high energy

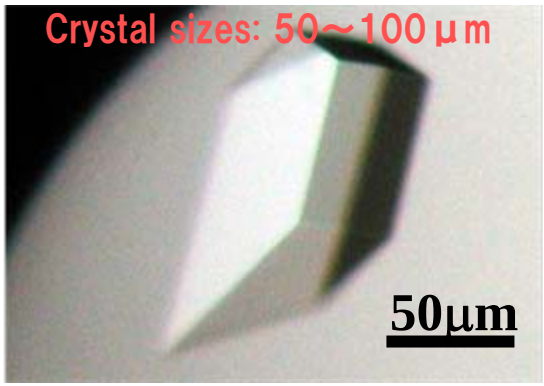
SPring-8

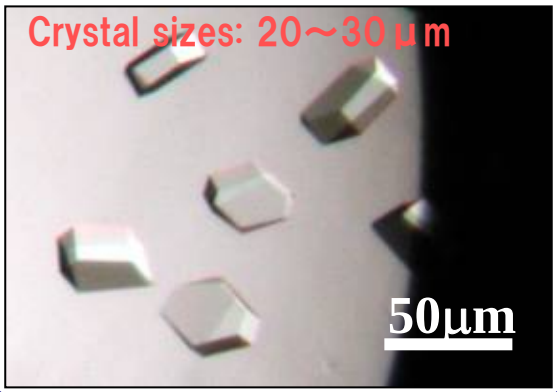


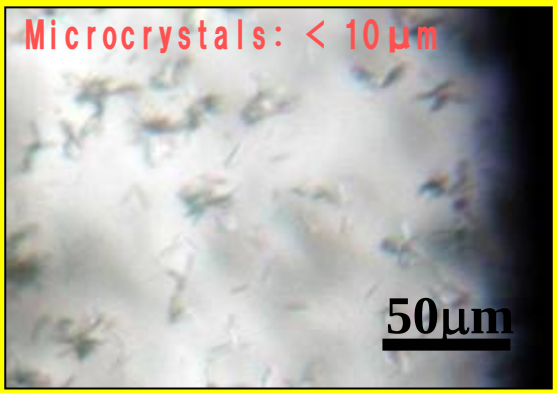
1. To be completed by 2009
2. Compatibility: SPring-8 and PF robots
3. Remote access and control

Isao Tanaka, Hokkaido Univ
Kunio Miki, Kyoto Univ
Atsushi Nakagawa, Osaka Univ.

Beam Lines







Needs extremely strong beams

Heavy atom labeling is difficult

SPring-8

Ultra high brilliance micro beam BL (new)

BL44XU
（SPring-8 / Osaka Univ）

BL41XU（SPring-8）

NW12A（PF-AR）
BL-5A（PF）

BL-17A（PF）

Low energy micro-focus BL

PF

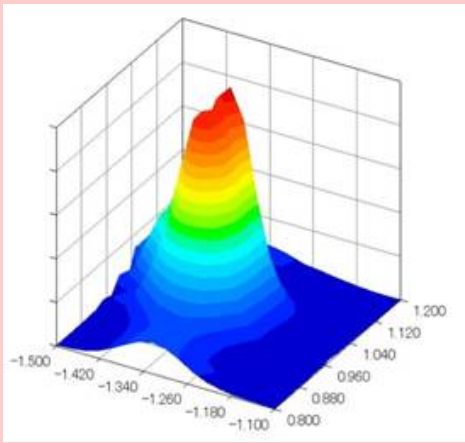
Target Protein Research Project (National Project)

SPring-8

Micro-focusing beamline for tiny crystals (<10 μm)

Goal :
10^{10} photons/sec/μm²
@ 12 keV

Current: **10^{12}** photons/sec/**100x100μm²** (BL41XU)



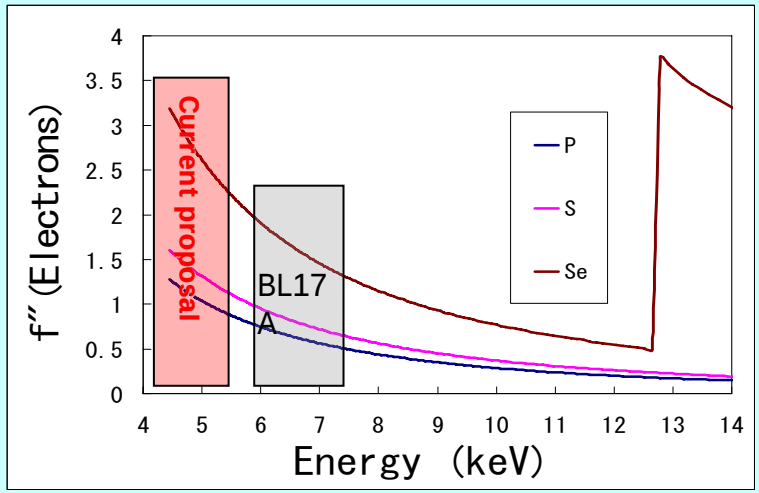
Beam profile of SPring-8 BL41XU

Further increasement of flux

PF

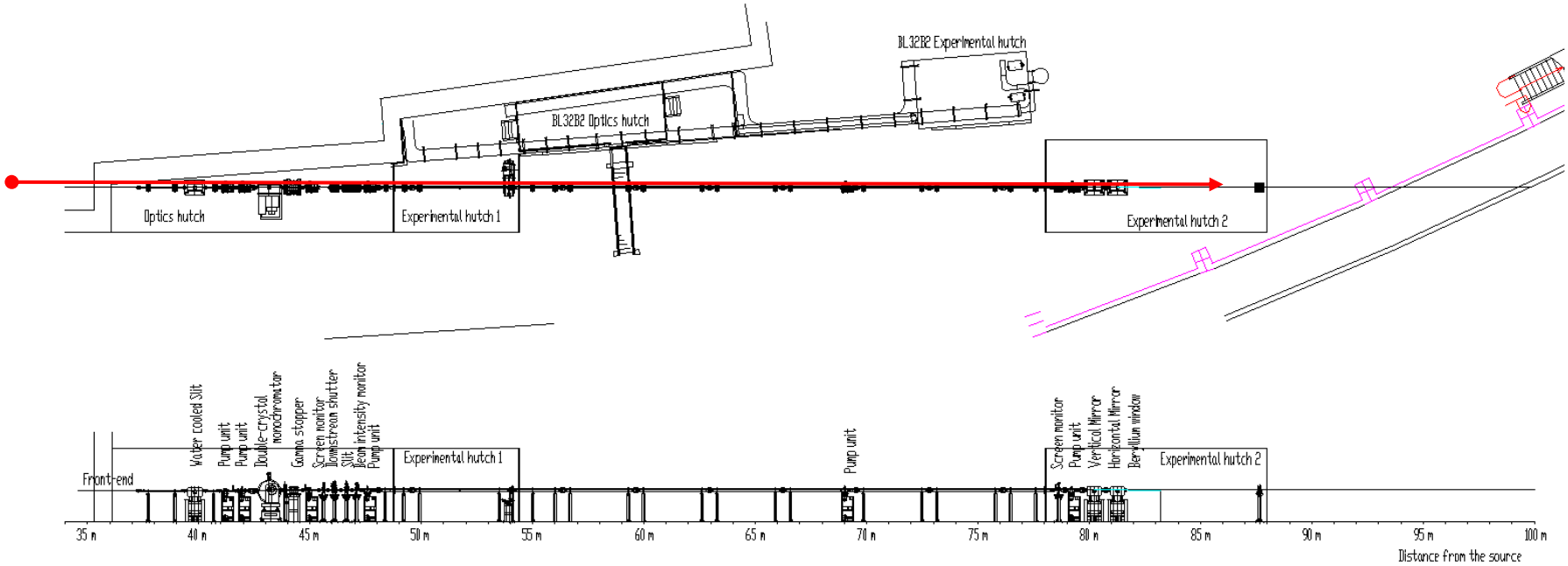
Micro-focusing beamline for sulfur-SAD phasing

Goal :
10^{10} photons/sec/10x10μm²
@ 4～5 keV



User operation from April 2010.

Beamline design of BL32XU @ SPring-8 by M. Yamamoto et al.



Concepts

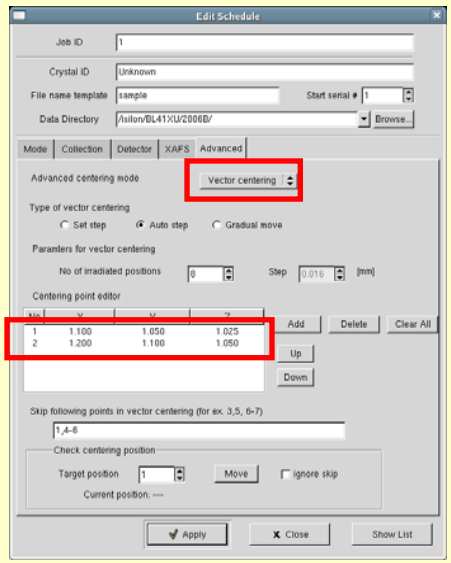
1. Making the best use of capability of SPring-8 undulator
2. Minimizing a number of optical elements to reduce mechanical errors
3. Focusing with a LARGE magnification factor

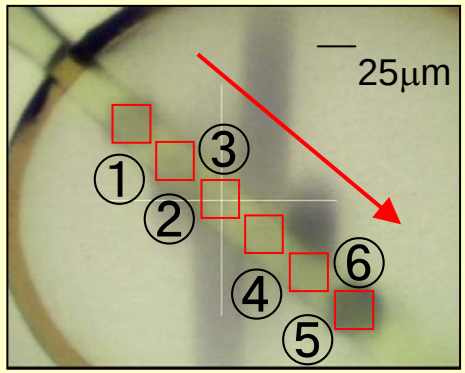
Current beamline control system @ SPring-8
by M. Yamamoto et al.

Multiple X-ray exposures on one/several crystals to avoid serious RD during data collection

・Vector Centering：

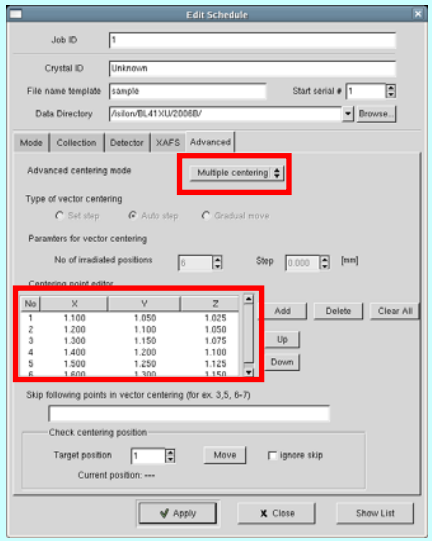
Changing exposure position linearly in every constant frames on *One crystal*

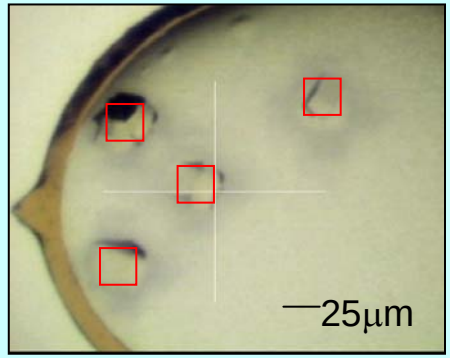




・Multiple Centering：

Multiple exposure positions using several crystals in *One cryo-loop*

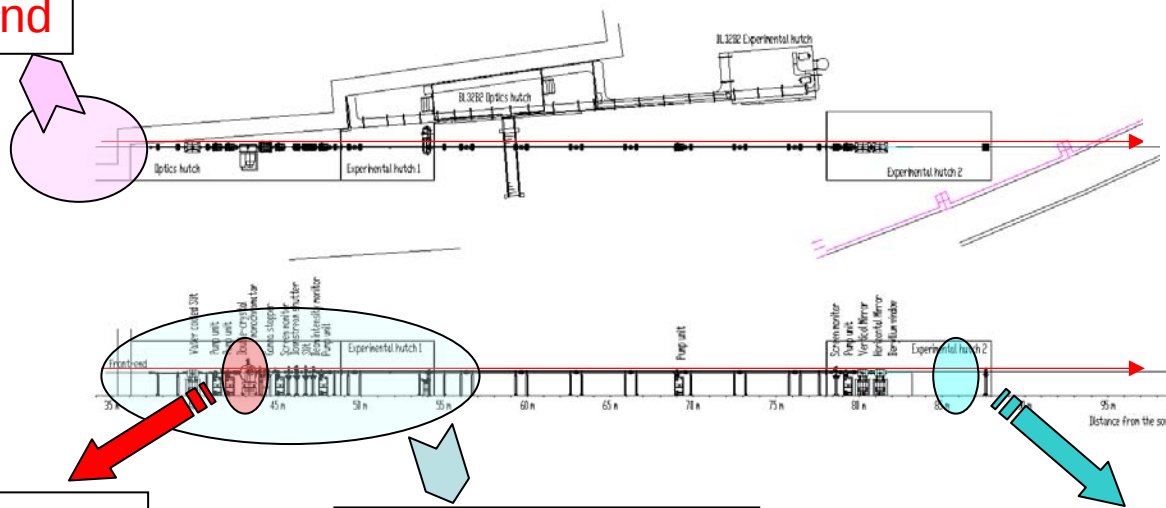


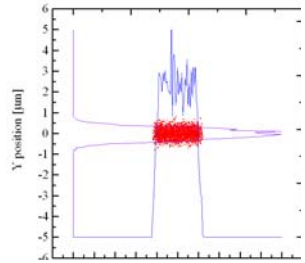


Current status of construction of BL32XU @ SPring-8

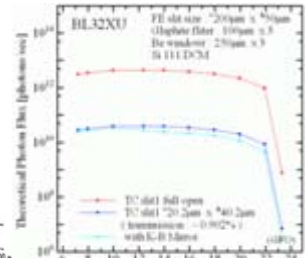
Design & ray trace

Frontend





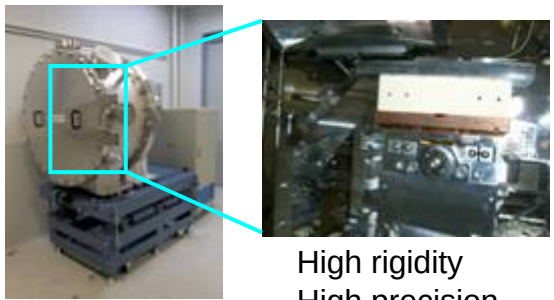
Beamsize **1 x 2 mm²**



Flux density

10^{16} phs/sec/mm²

High-precision DCM

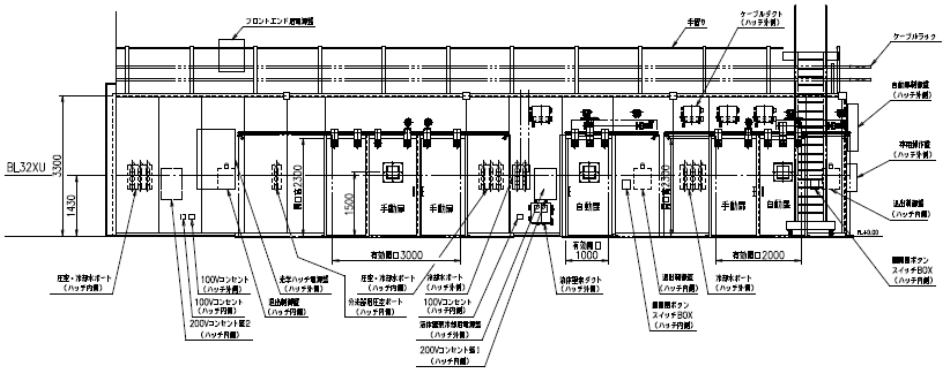


High rigidity
High precision

X-ray shield hutch

R & D of CCD detector

X-ray shield hutch of BL32XU @ SPring-8



Start
2007/12/21

Before construction

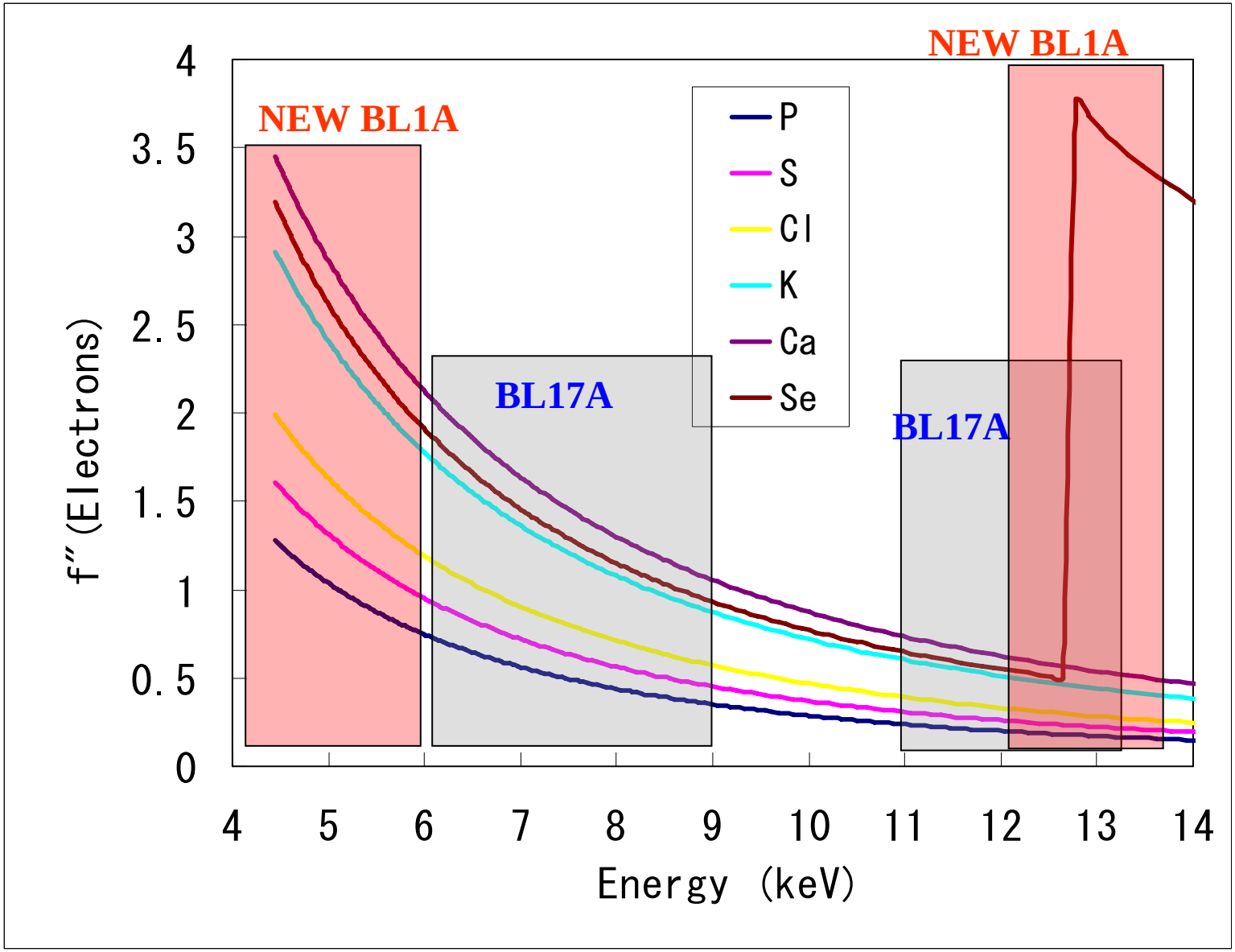
X-ray shield hutch of BL32XU @ SPring-8

Finished product...

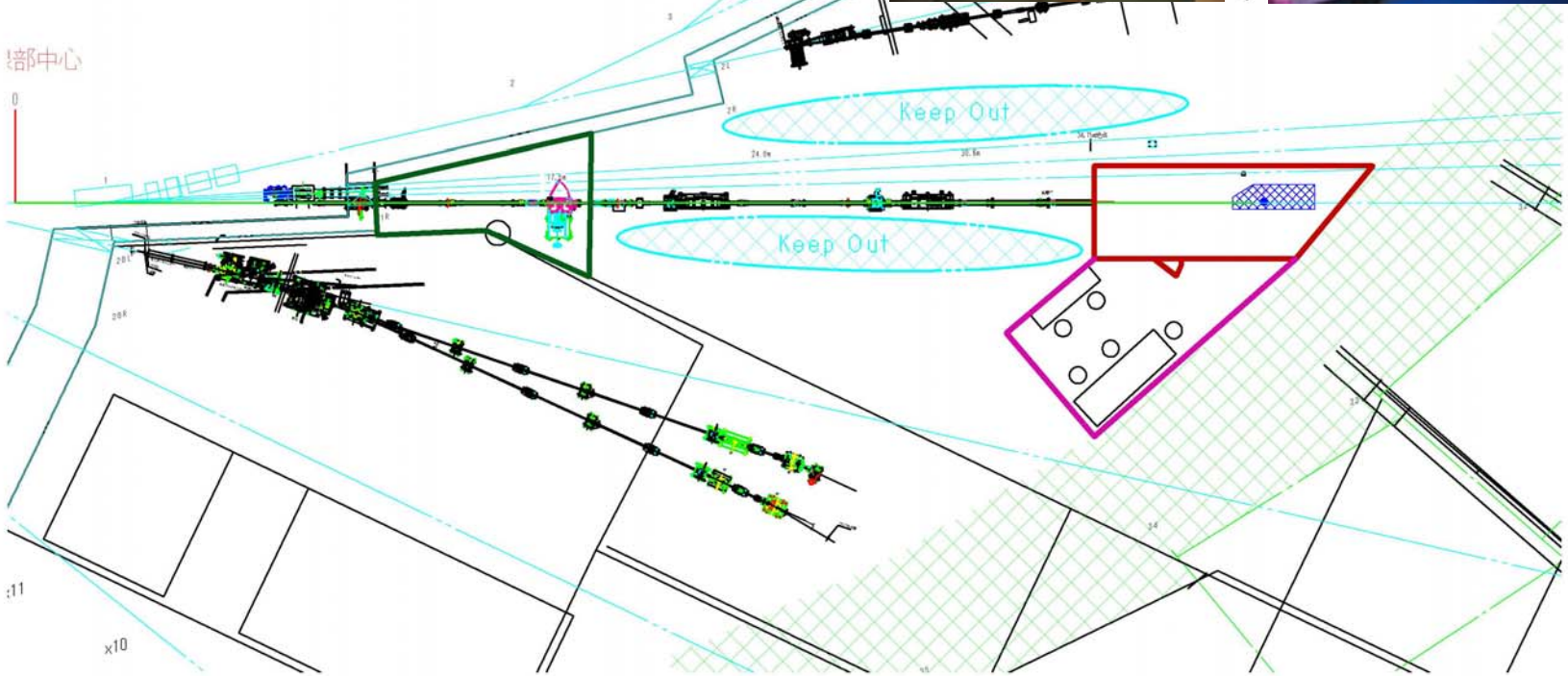
2007/12/21 ~ 2008/02/25

Sophisticated
JAPANESE workers

In-vacuum Short Gap Undulator Microfocus Beam Lines: softer X-rays @ Photon Factory



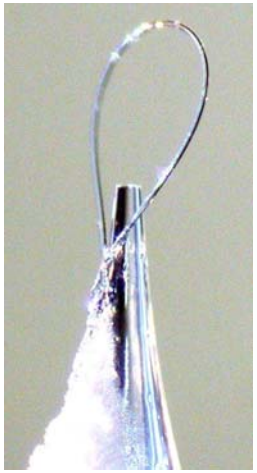
Lower Energy Microfocus undulator beam line PF-BL1A

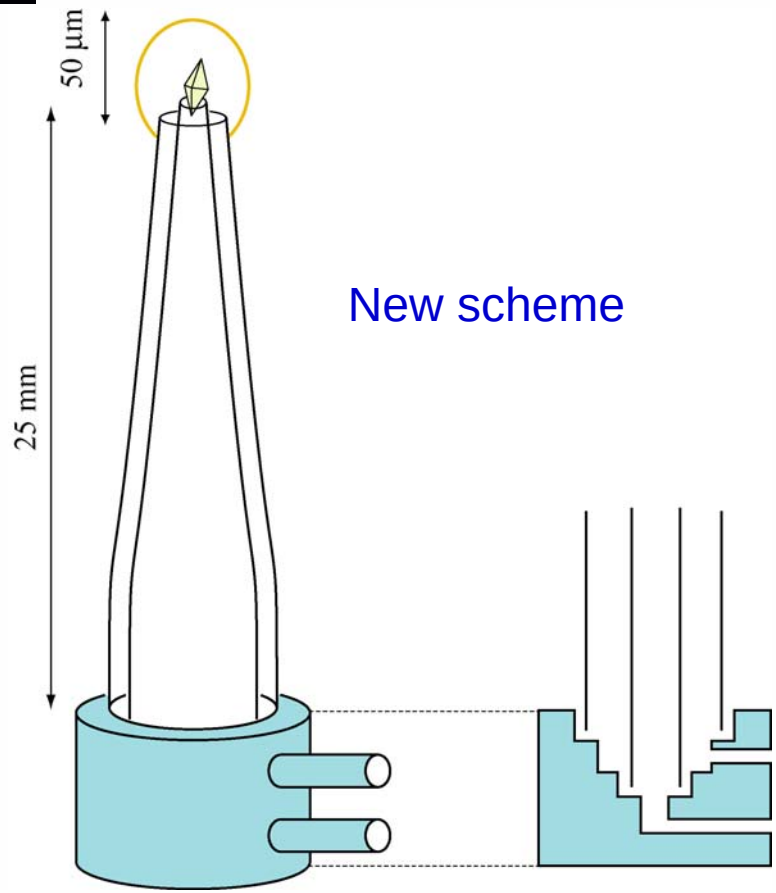


KEK-PF BL-1A: in-vacuum short gap undulator: fundamental to cover 4.1~4.5 keV

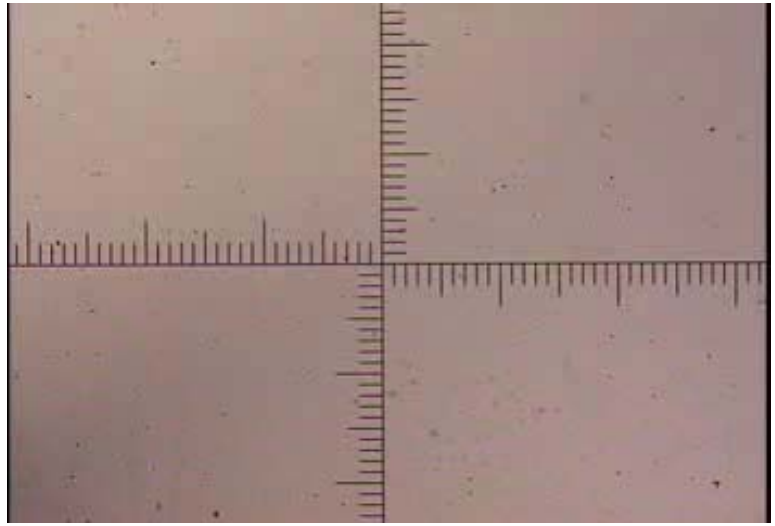
Towards lower background
by Isao Tanaka, Hokkaido University

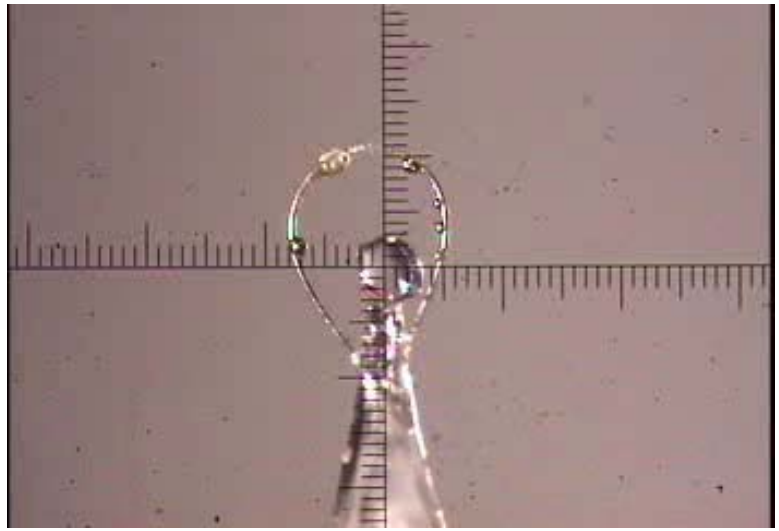
Loopless crystal mounting method





New scheme



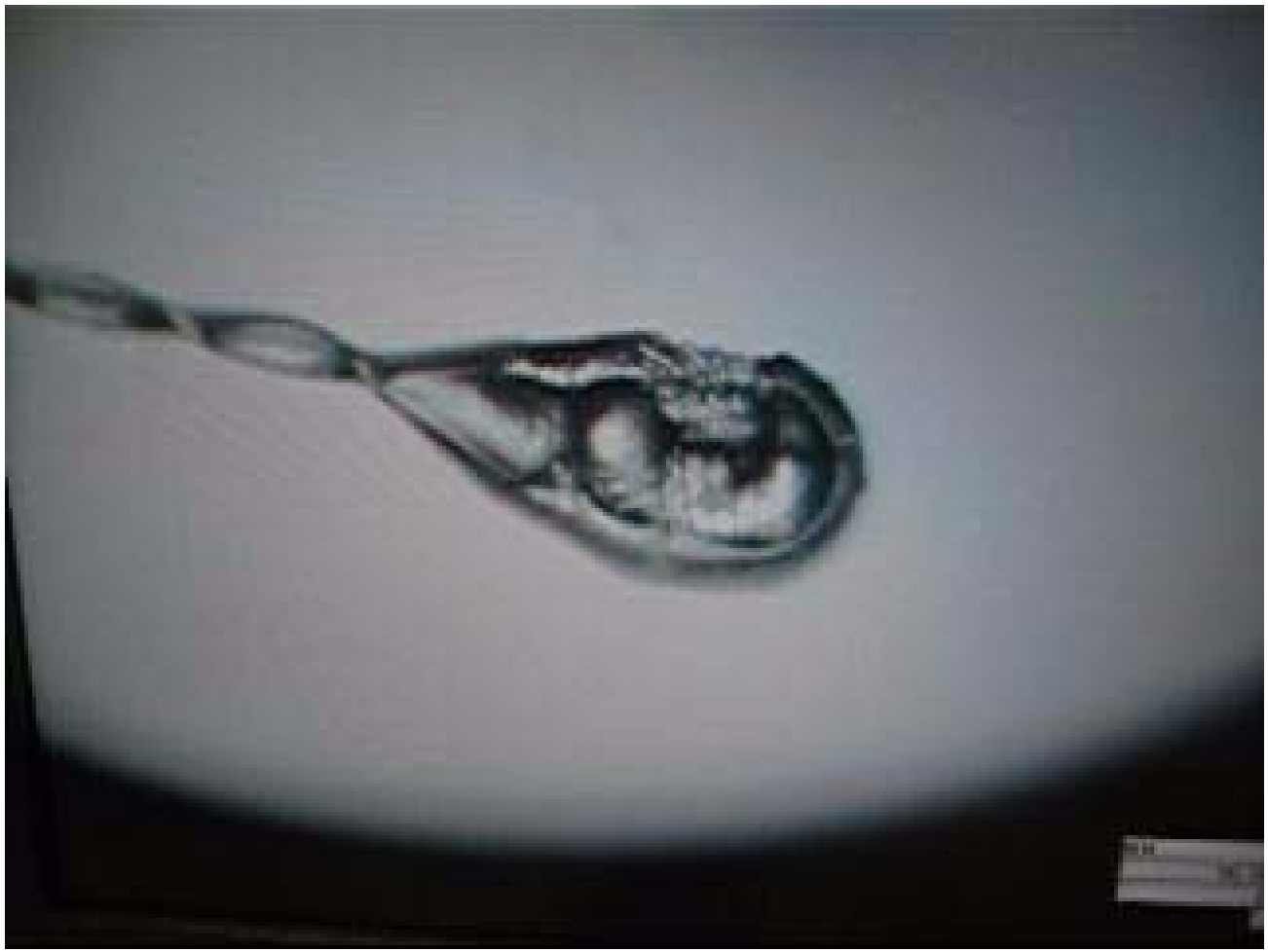


Kitago, Y., Watanabe, N. and Tanaka, I., *Acta Cryst.*, D61, 1013-1021 (2005).

http://castor.sci.hokudai.ac.jp/watanabe/XtalMount/

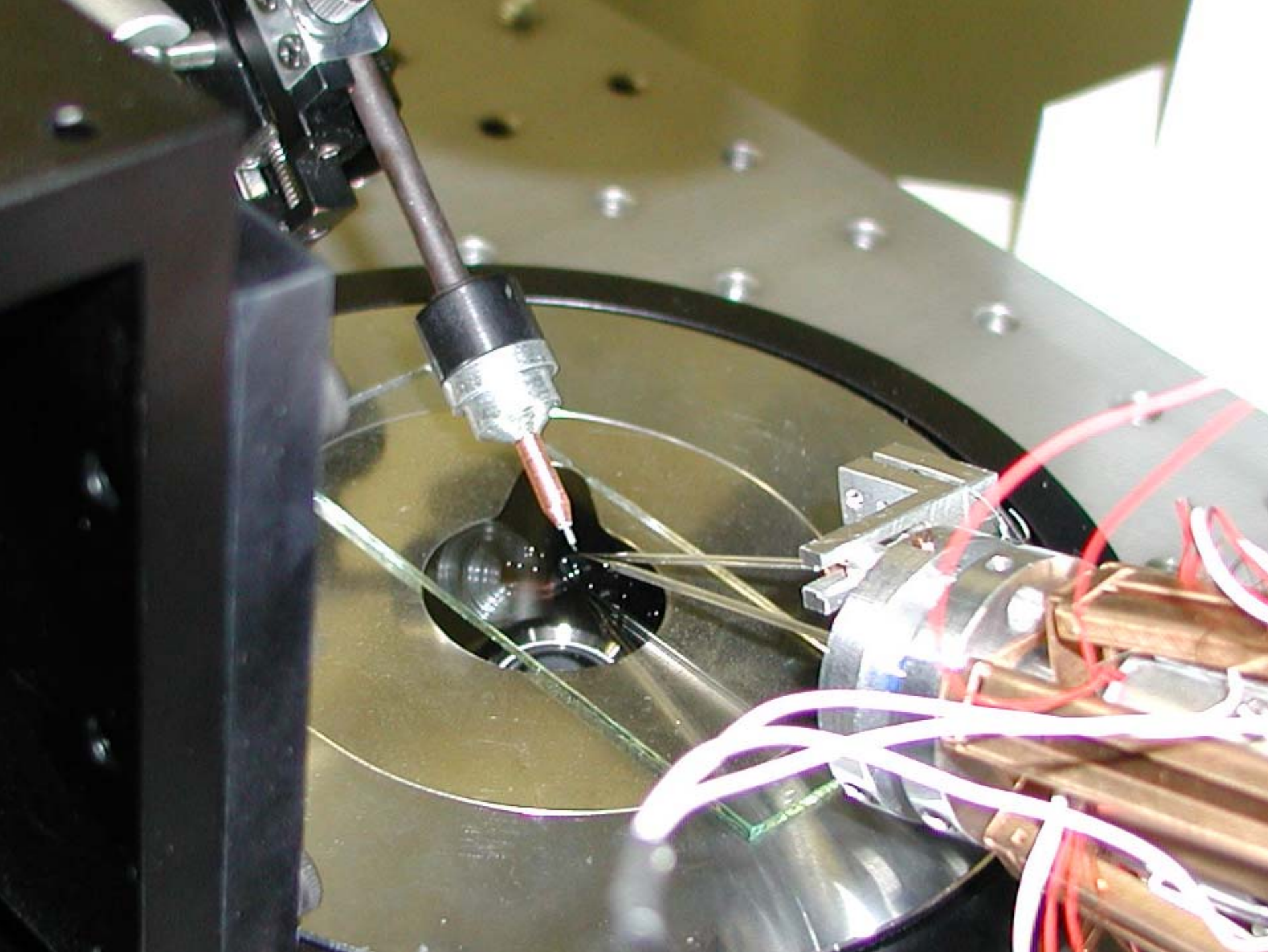
Towards lower background:
Promising techniques developed by other groups.

OR CUT AWAY the rest with a 193 nm UV laser



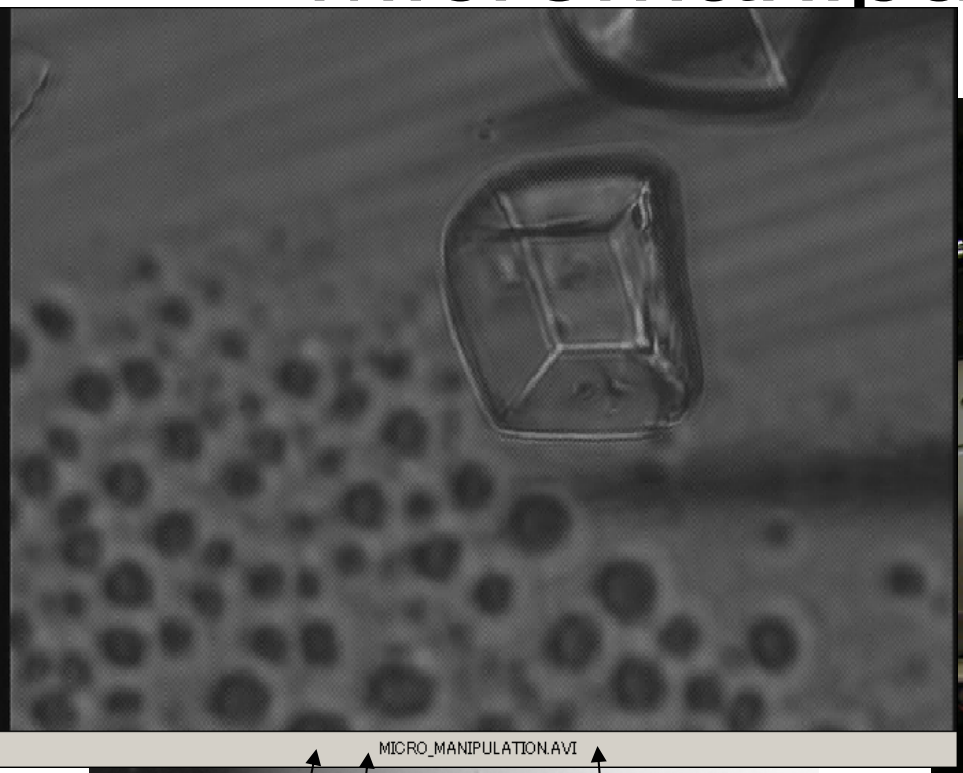
Courtesy of Nikon Ltd. & Sosho Ltd.

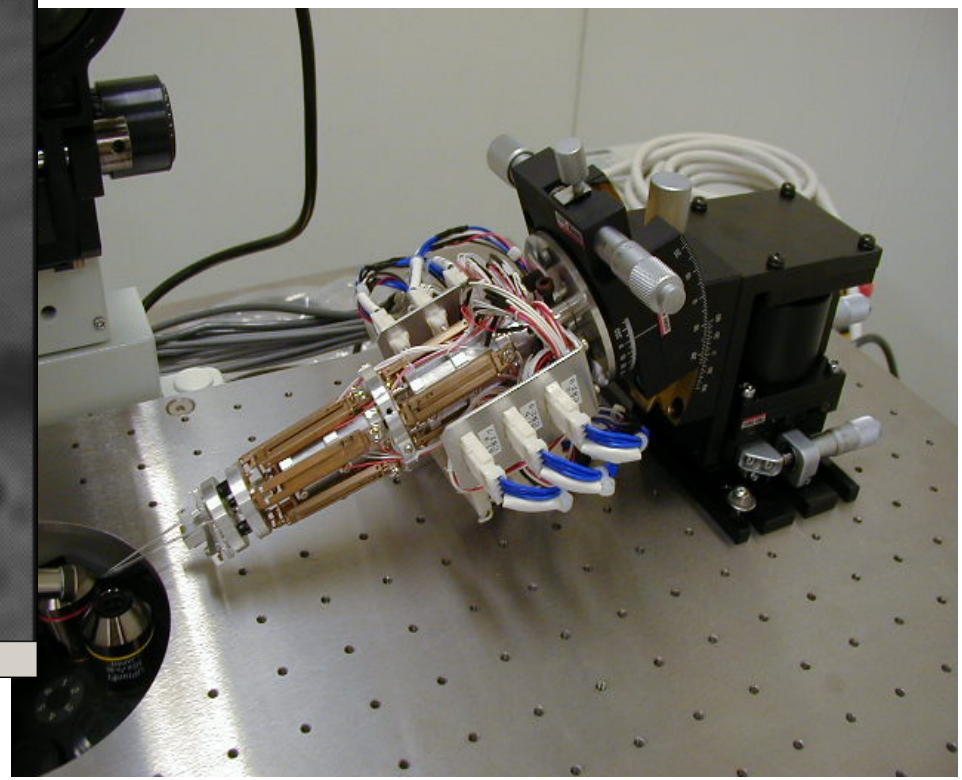
H. Adachi et al., Japanese Journal of Applied Physics Vol. 44, No. 2, 2005, pp. L54-L56 Protein

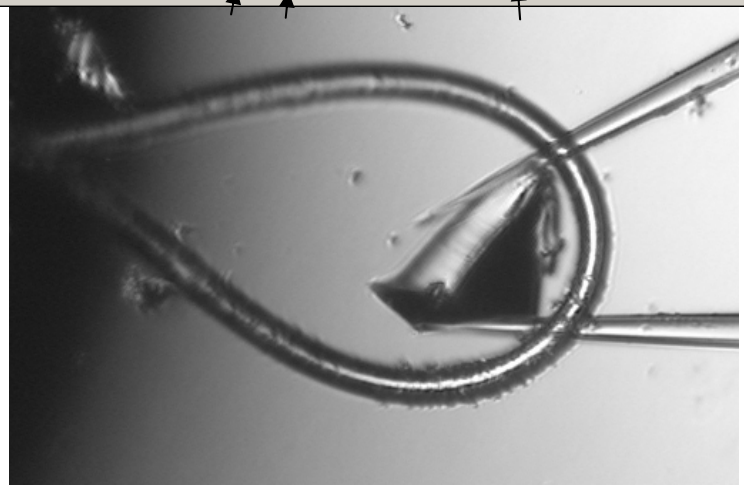


Micromanipulator system

In collaboration with Dr.Tanikawa (AIST)

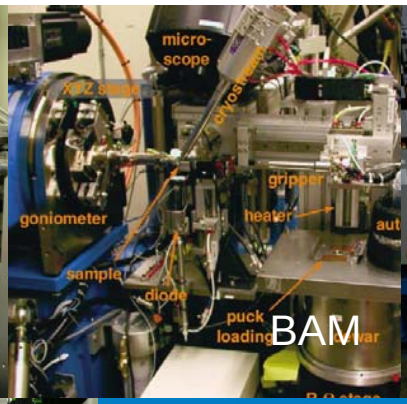




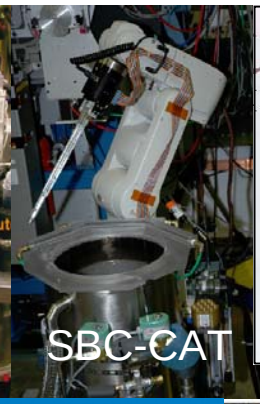


Crystal handling: so many different systems…

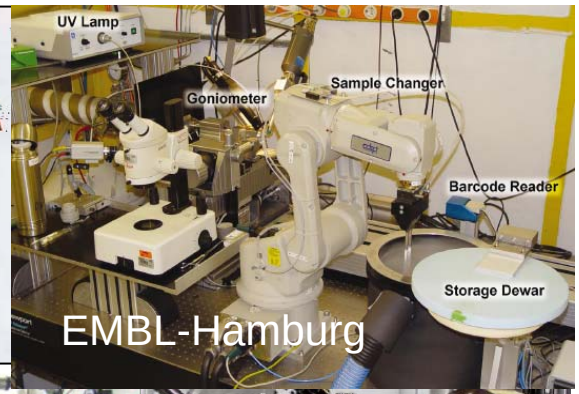

SPACE



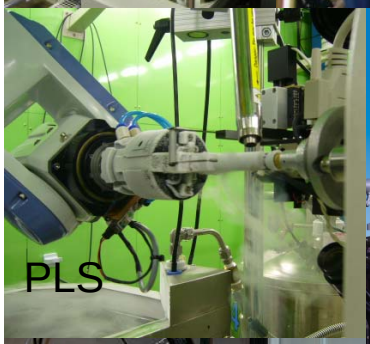
BAM

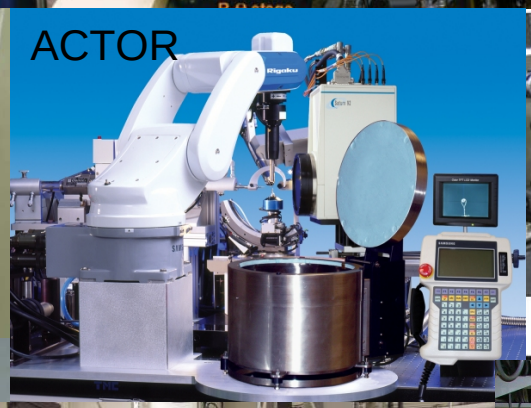

SBC-CAT


mar-CSC



EMBL-Hamburg

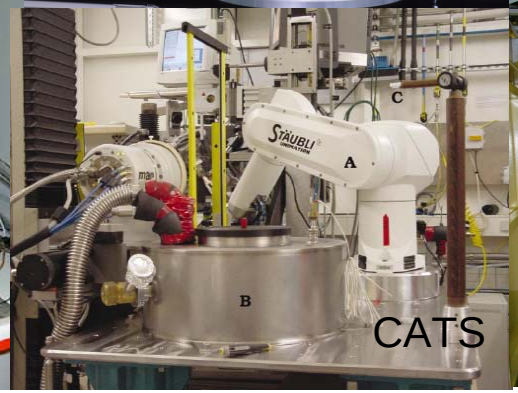

PLS


ACTOR

EMBL-SC3

DORIS


BruNo

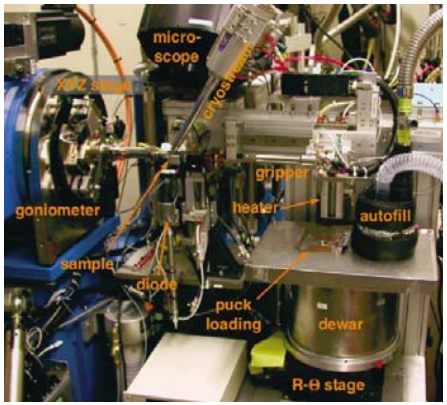


CATS


SAM


Cool-Hand-Luke

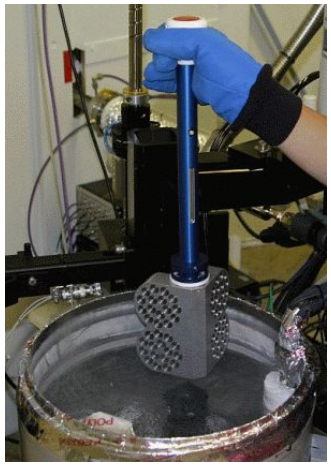
Compatibility with other systems



SAM System

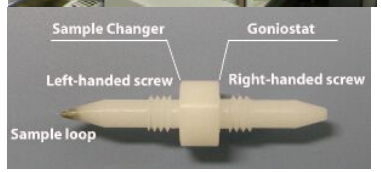
BAM(ALS)

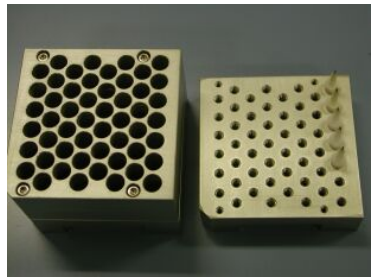
PF will accommodate to Universal Pucks



Under development within the Target Protein Research Project

Rigaku ACTOR





SPACE(SPring-8)

Universal Puck

DIAMOND, SOLEIL, SLS, APS,...

Summary

- Target Oriented Structural Proteomics of Protein Transport
- New insertion device beamline, BL-17A, has been developed at the Photon Factory 2.5 GeV ring.
- Recent progress on the stabilization of X-ray beams and the development of the extremely high precision diffractometer enable more precise data collection.
- New Target Protein Research Program: challenging protein targets (membrane proteins and complexs): two complementary micro beam BLs at SPring-8 and Photon Facotry
- New microfocus lower energy PF-BL1A will be used fo lower energy SAD phasing (and normal MAD).
- Various exchange mechanisms for cryo-pins and exchange robots

Acknowledgements

SPring-8
Masaki Yamamoto's group

Hokkaido University
Isao Tanaka

Kyoto University
Kunio Miki

Osaka University
Atsushi Nakagawa
Mamoru Suzuki

Nagoya University
Nobuhisa Watanabe

Financial Supports

Development of Systems and Technology for Advanced Measurement and Analysis (JST)

Protein 3000 Project (MEXT)

Grand-in-Aid for Young Scientists (B) 18770098 (MEXT)

Target Protein Research Program (MEXT)

Structural Biology Research Center, Photon Factory, KEK

Soichi Wakatsuki Professor, Director of Structural Biology Research Center

Structural Biology

Ryuchi Kato (Associate Prof.)
Masato Kawasaki (Research Assoc.)
Kentaro Ihara (Post Doc Fellow)
Norio Kudo (Post Doc Fellow)
Mohammad Yousef (JSPS fellow)
Tamami Uejima (Researcher)
Tamie Aoki (Lab Technician)
Honda (Lab Technician)

Ex members:
Tadashi Satoh (Post Doc Fellow)
Leonard Chavas (Post Doc Fellow)
+++

Beamline & Robotics

Noriyuki Igarashi (Research Assoc.) BL-6A, BL-17
Naohiro Matsugaki (Res. Assoc.) AR-NW12, BL1
Masashiko Hiraki (Res. Assoc.) Robotics
Yusuke Yamada (Research Assoc.) NE3
Yurii Gaponov (Researcher) Programming
Kumiko Sasajima (System Engineer)
Shokei Watanabe (Mechanical Engineer)
Nobuo Honda (System Engineer)
Kazutaka Demura (Beamline Assistant)
Kubota (Beamline Assistant)

The Graduate University for Advanced Studies

Junichi Kouyama (D4), Simin Rahigi (D2), Ahmed Rohaim (D1), Kensuke Nakamura (M1), Ken Ohkubo (M1)

Secretaries

Naomi Nagata*
Tomoko Zeniya*
Ritsuko Ebisawa*
Reiko Sukegawa

Internship Students: Atsuko Tsuchiya (Kyoto Univ. M1), Ryunosuke Nakatani (Nagaoka Univ. of Tech. B4)