Cheiron School 2007 by AOFSRR SPRing-8

13 September 2007

Protein Crystallography

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

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Outline

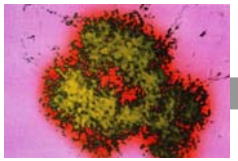
1. **Introduction to protein crystallography**

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

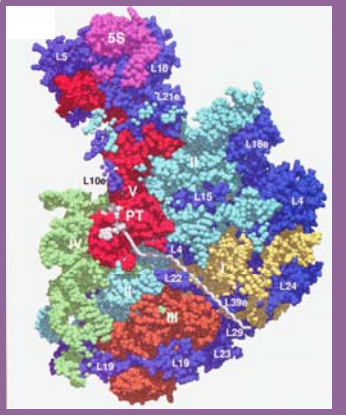
3. **Structural proteomics on post-translational modification and transport of proteins: protein-protein interactions in membrane traffic**

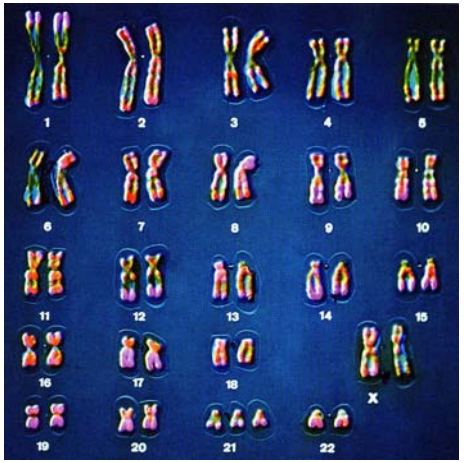
Role of Structural Biology

Genome



DNA→RNA→ **Proteins**



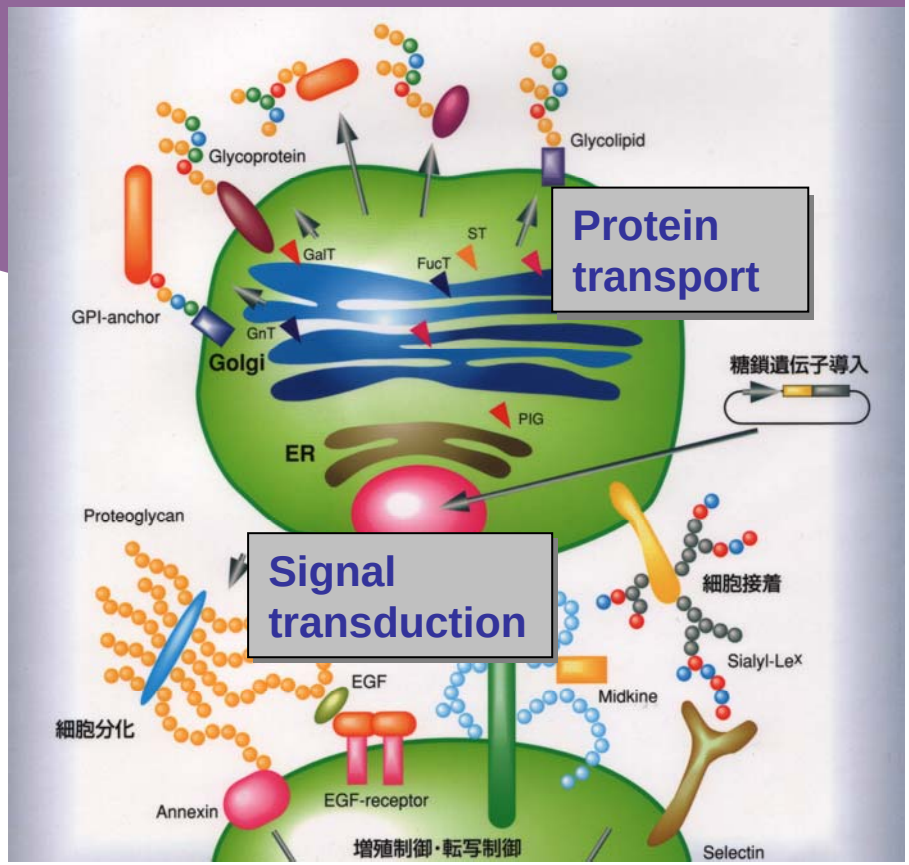


Molecular machines
（ribosomes, enzymes）
Information network

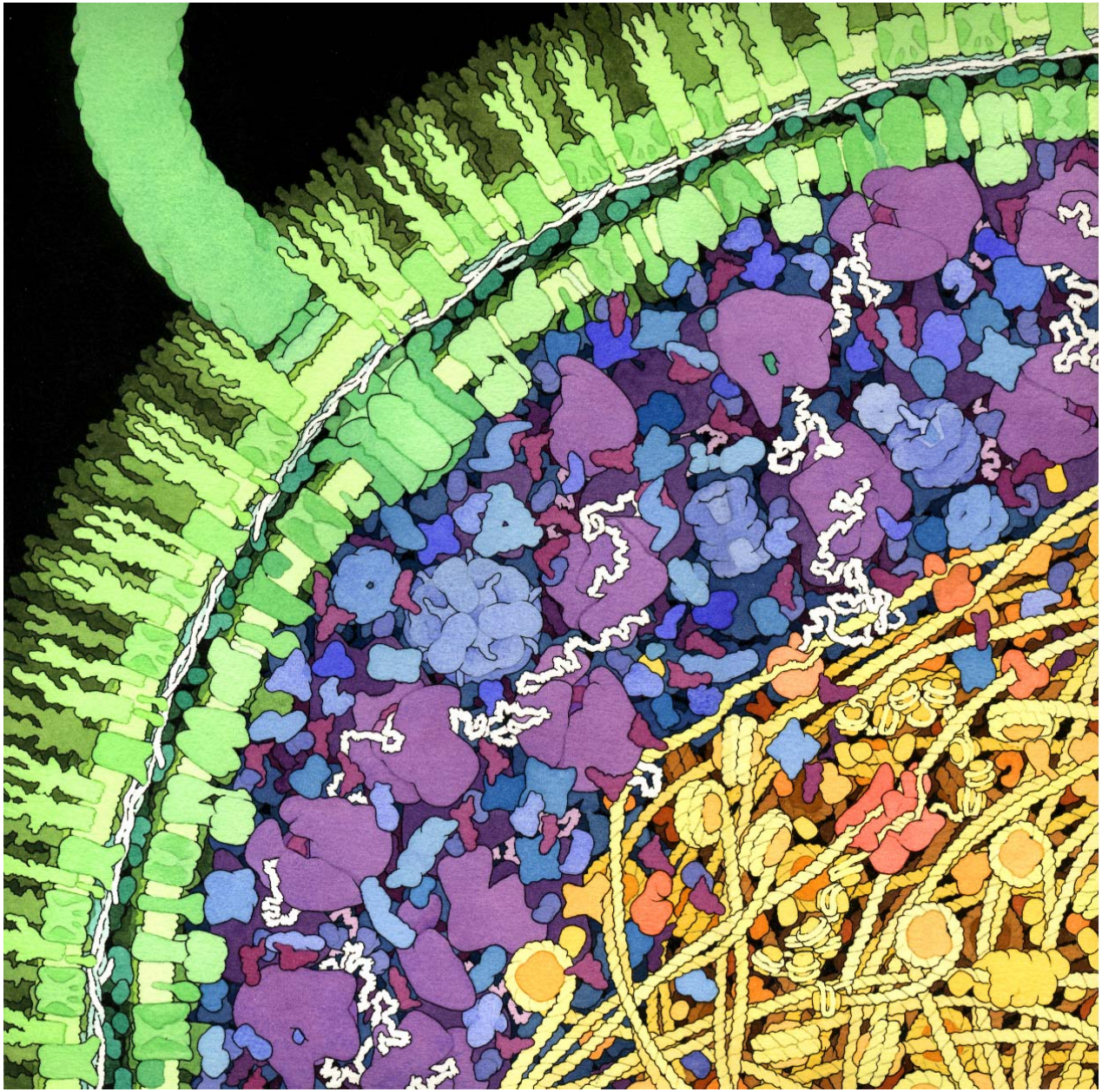
Atomic resolution analysis
○Protein structure and dynamics
○Interactions between molecular machines

Biology
(Basic research)

Medicine, drug design
(industrial applications)

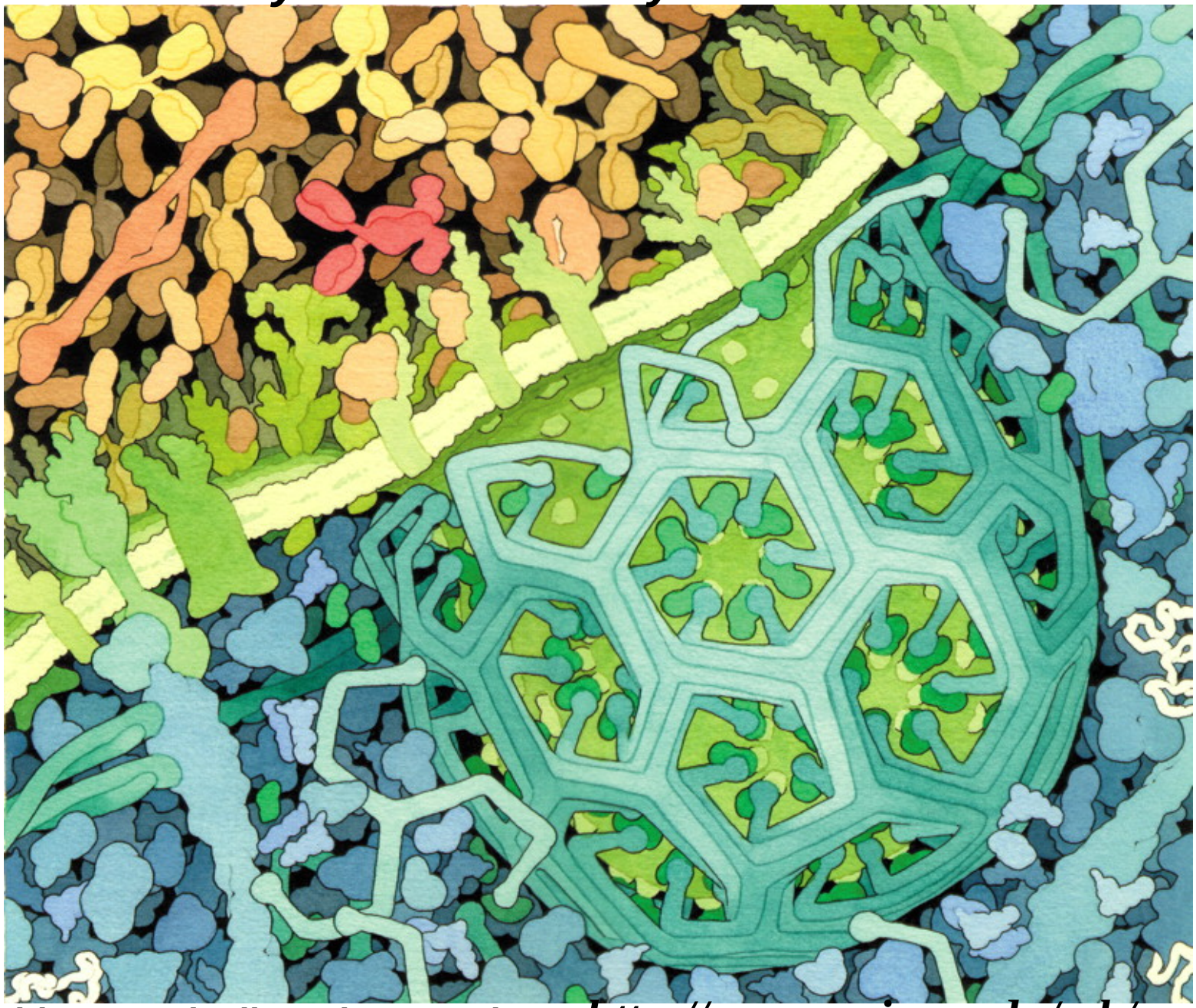


E. Coli cell

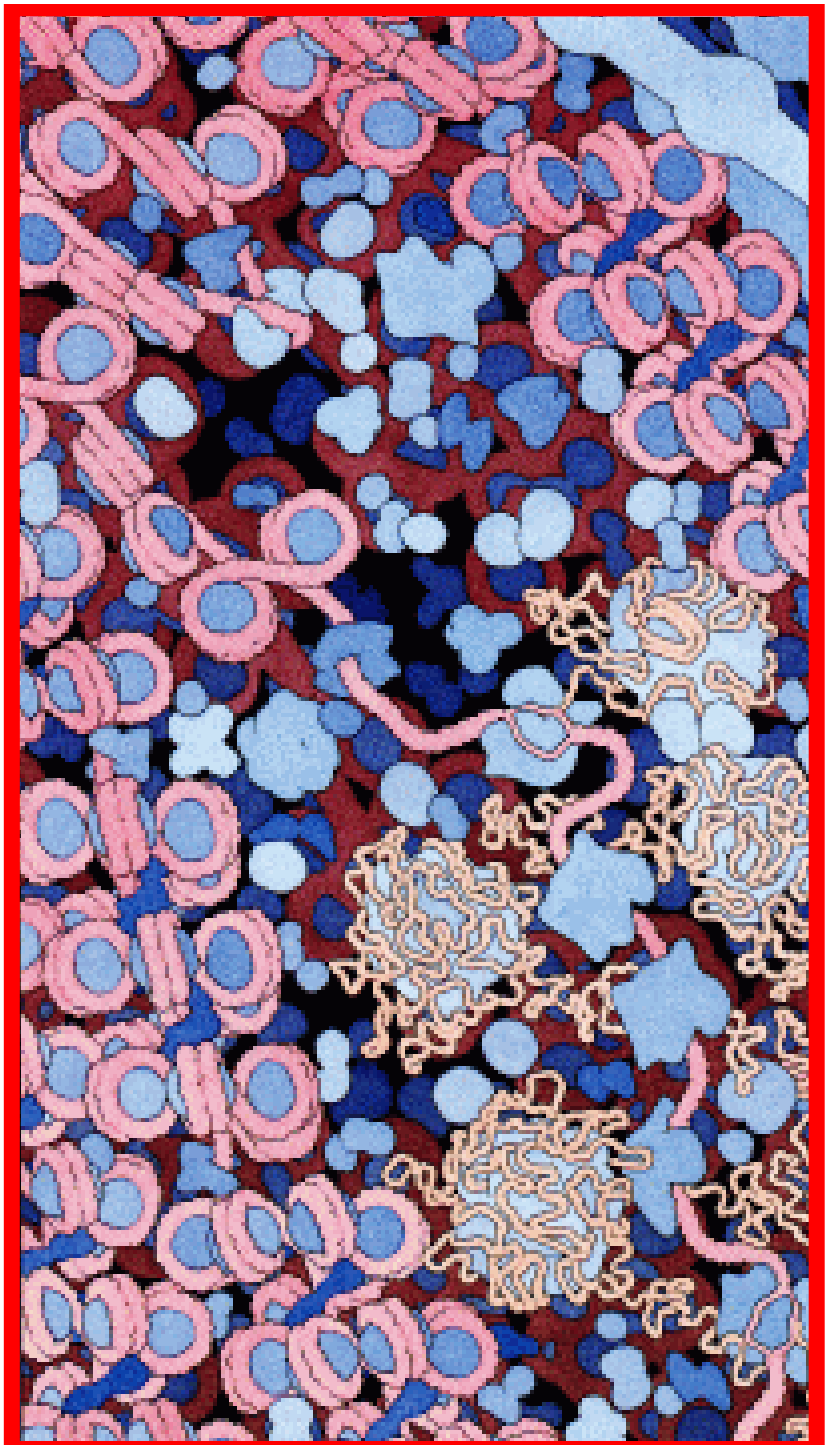


David S. Goodsell, Scripps Institute, http://www.scripps.edu/mb/goodsell/

Endocytosis of toxin by a clathrin vesicle

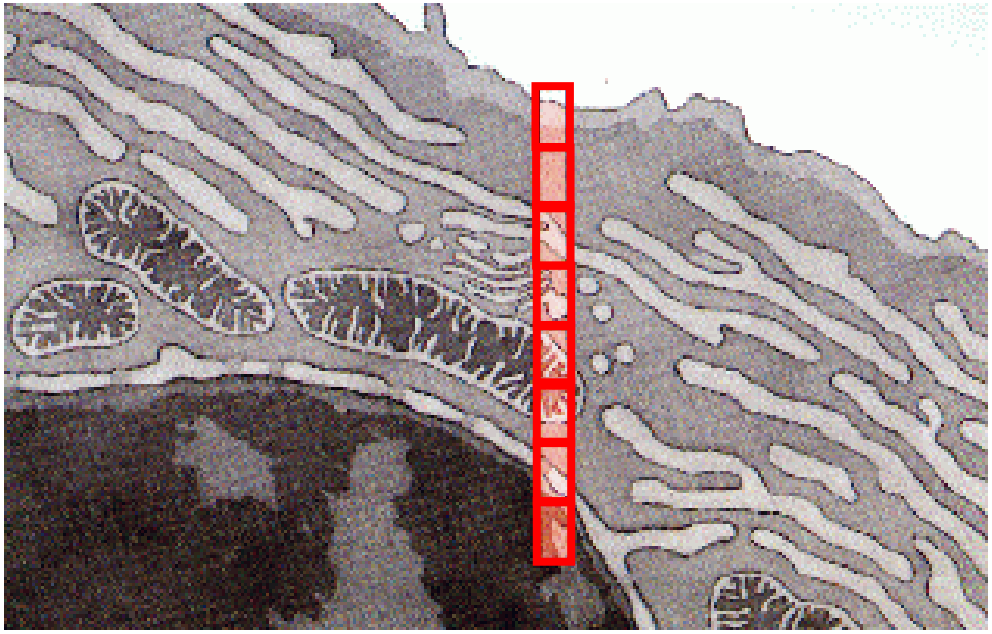


David S. Goodsell, Scripps Institute, http://www.scripps.edu/mb/goodsell/



Panoramic view of a eukaryotic cell

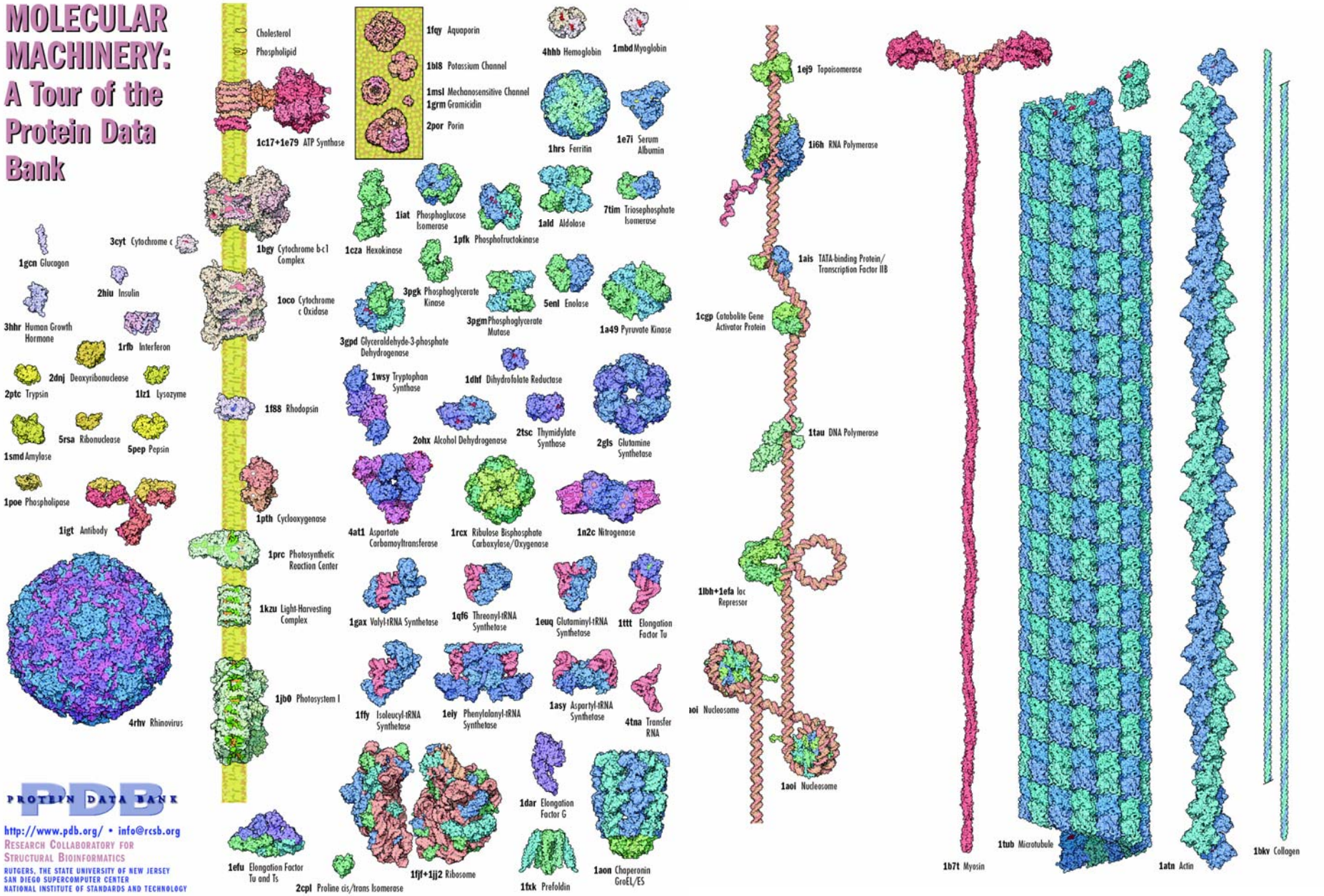
http://www.scripps.edu/mb/goodsell/



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



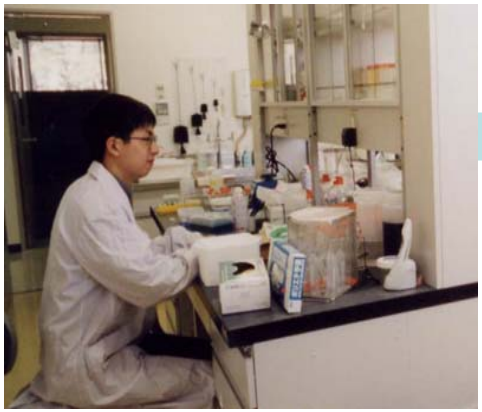
David S. Goodsell, Scripps Institute

Acetylcholine esterase

Prof. Joel Sussmann
Weizmann Institute, Israel

- ..¥Joel Sussman¥richardnew.mpg

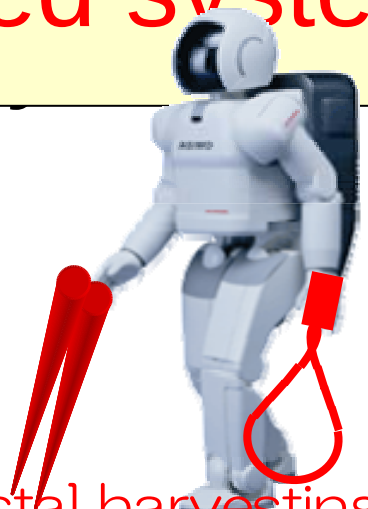
Future: automated/integrated system



Expression and purification

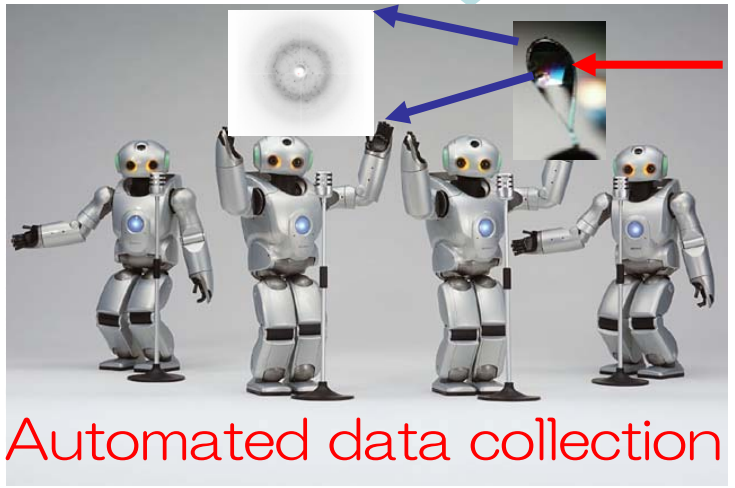
Crystallization robot

Crystallization

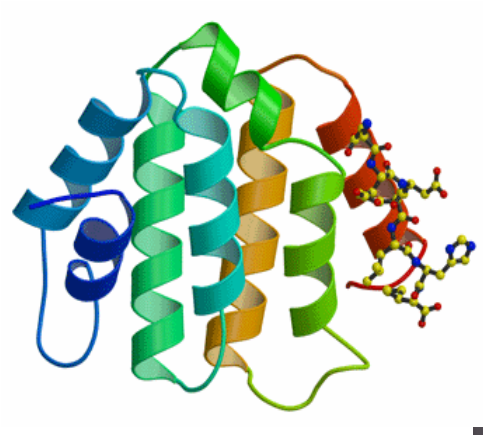


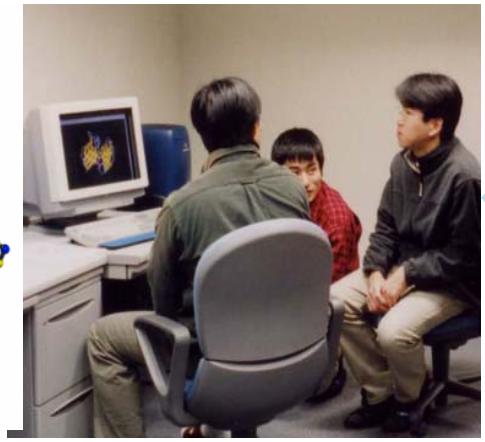
Crystal harvesting robot

Crystal harvesting



Mouting & data collection





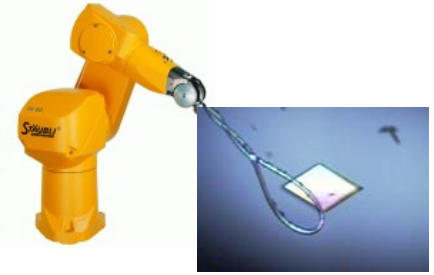
Data analysis

Flow of protein structure analysis

（1）Large scale protein expression and purification system

Crystalli-zation robot

（2）Crystal evaluation/harvesting system



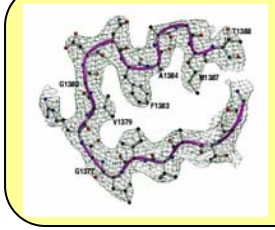
（3）High brilliance microfocus MAD beam line

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

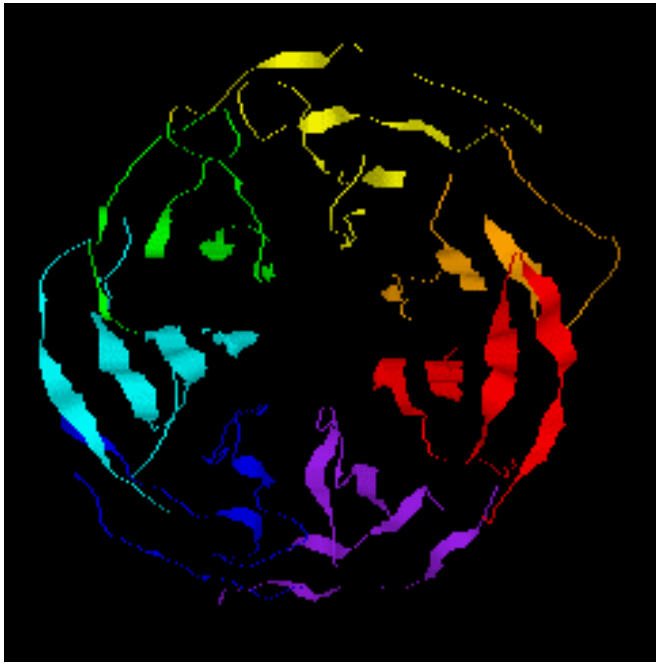
Guanine nucleotide exchange factor Sec12p

```
   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

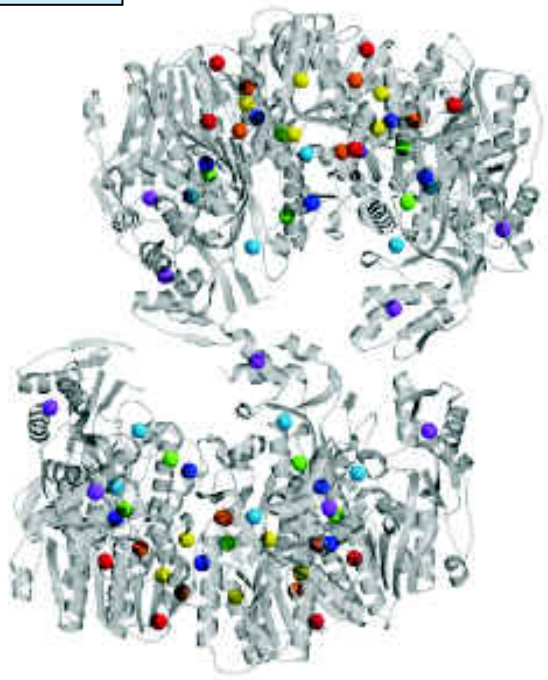
Phase Determination using Multiple Anomalous Dispersion

Methionine

Sulfur ⟶ Selenium (SeMet)

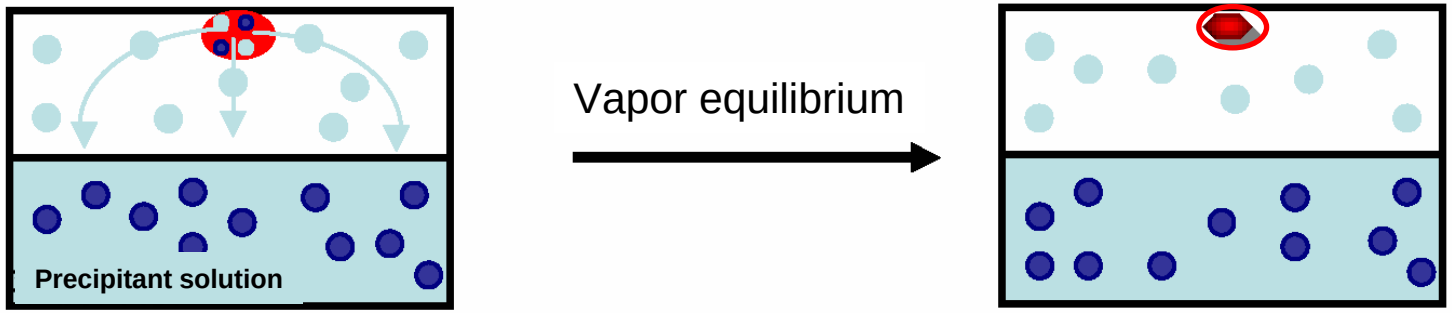
FVTASYNVGYPAYGAKFLNNDTLLVAGGGGEGNNGIPNKLTV
LRVDPTKDTEKEQFHILSEFALEDNDDSPTAIDASKGIILVGCNENSTKITQGKGNKH
LRKFKYDKVNDQLEFLTSVDFDASTNADDYTKLVYISREGTVAAIASSKVPAI**M**RIID
PSDLTEKFEIETRGEVKDLHFSTDGKVVAYITGSSLEVISTVTGSCIARKTDFDKNWS
LSKINFIADDTVLIAASLKKGKGIVLTKISIKSGNTSVLRSKQVTNRFKGITS**M**DVD**M**
KGELAVLASNDNSIALVKLKDLS**M**SKIFKQAHSFAITEVTISPDSTYVASVSAANTIH
IIKLPLNYANYTS**M**KQKISKFFTNFILIVLLSYILQFSYKHNLHS**M**LFNYAKDNFLTK
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

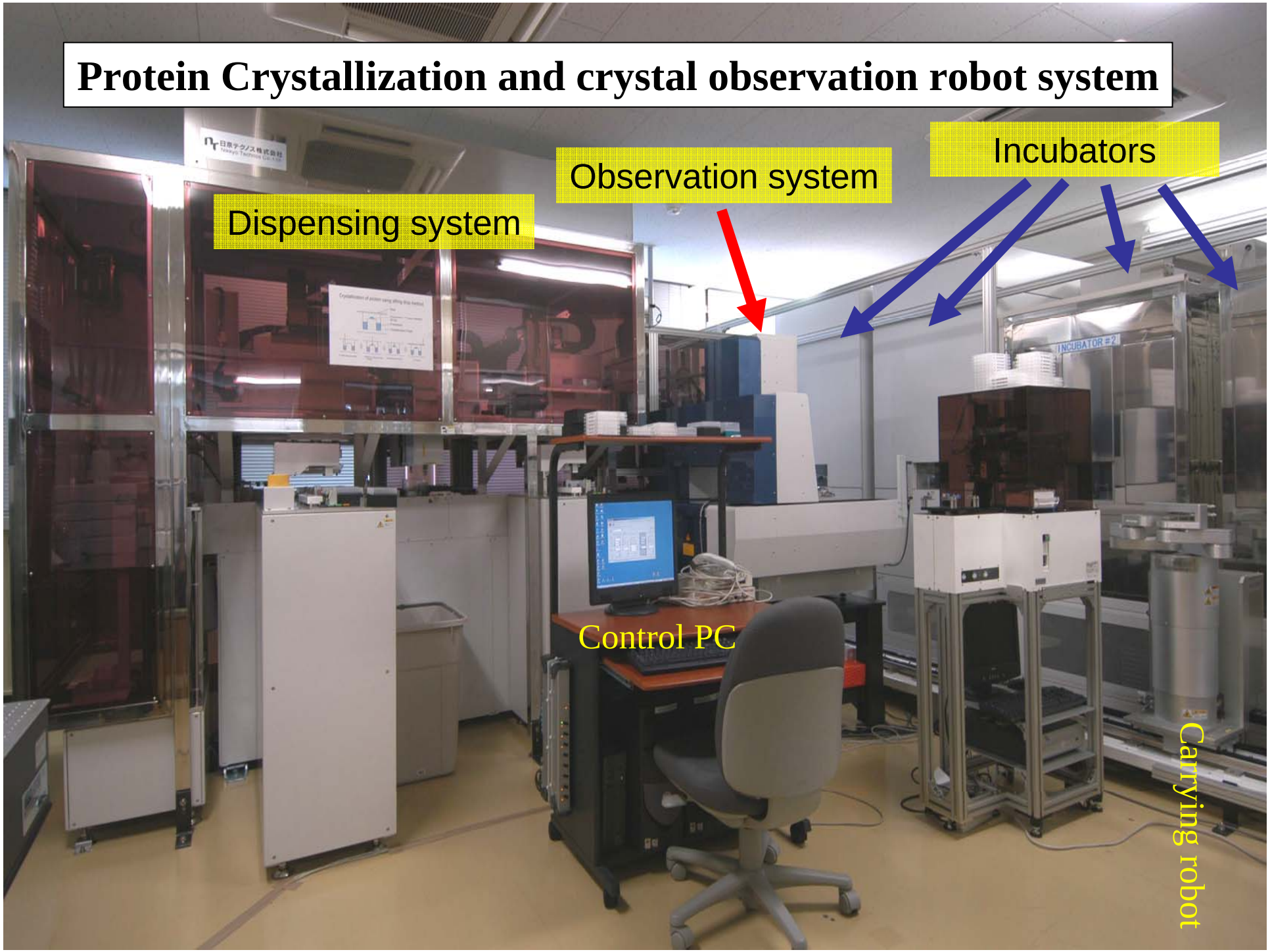
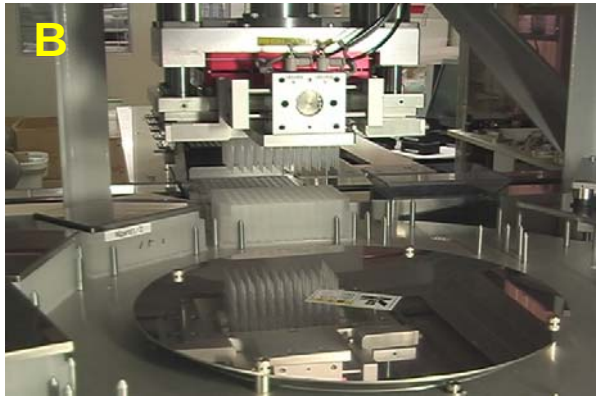
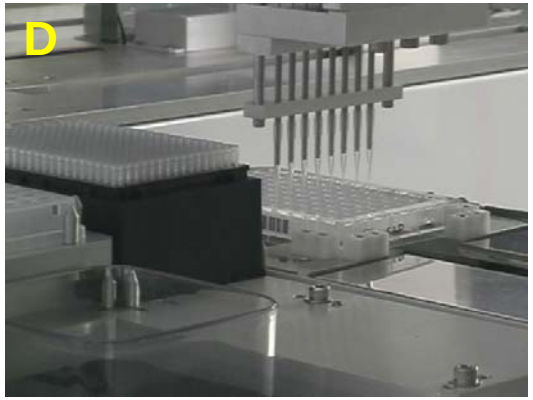
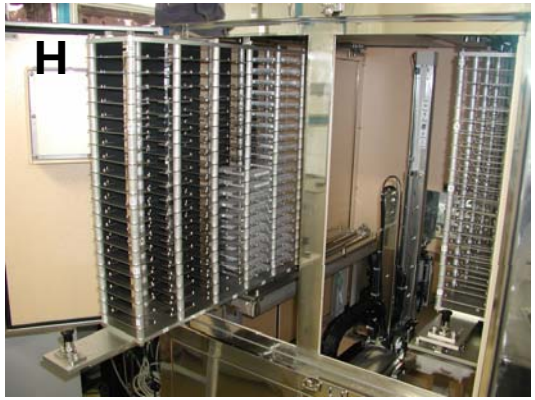

Protein Crystallization and crystal observation robot system
Dispensing system
Observation system
Incubators
Control PC
Carrying robot

Fig. 3



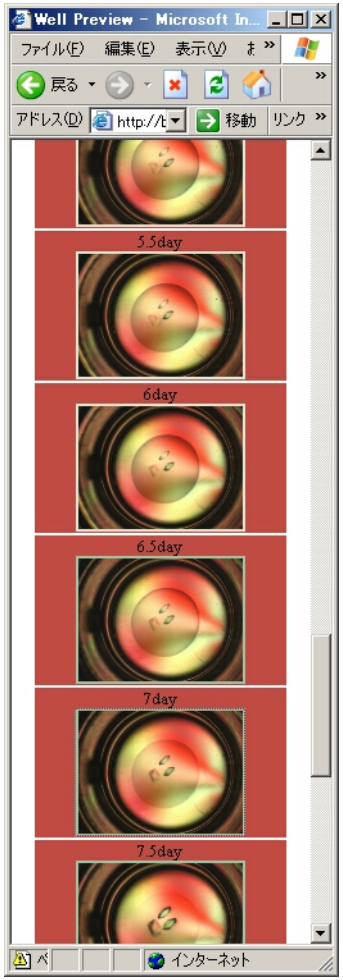


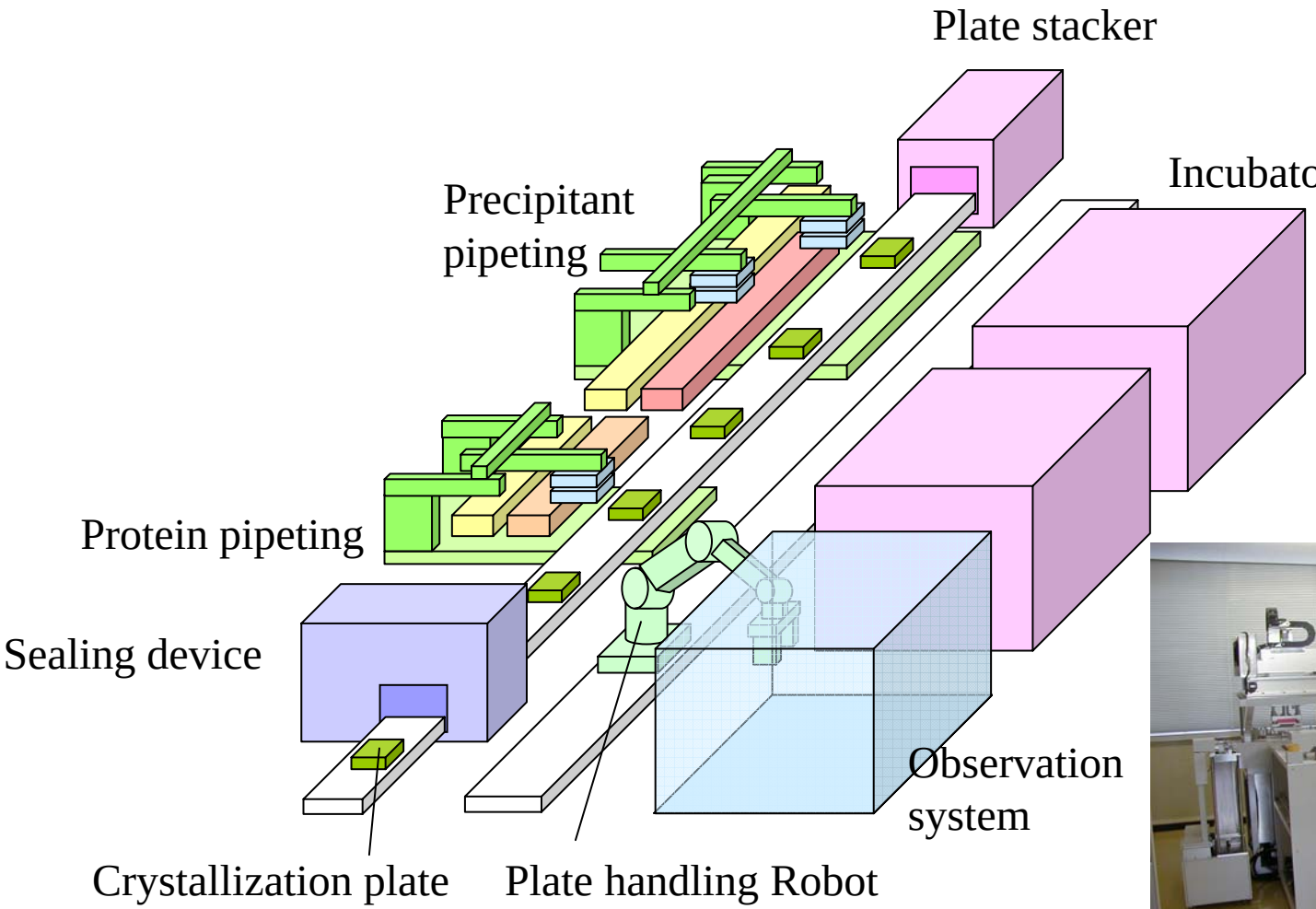


Protein Crystallization and crystal observation robot system

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

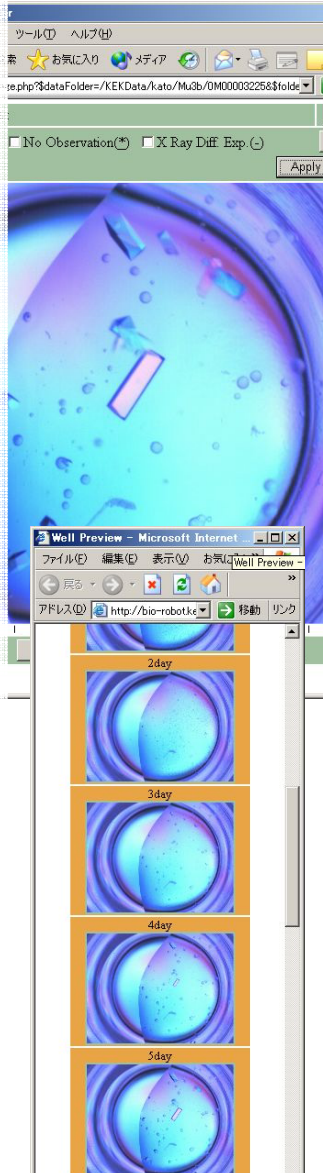
Sitting drop volume: 500 nL later down to 50 nL



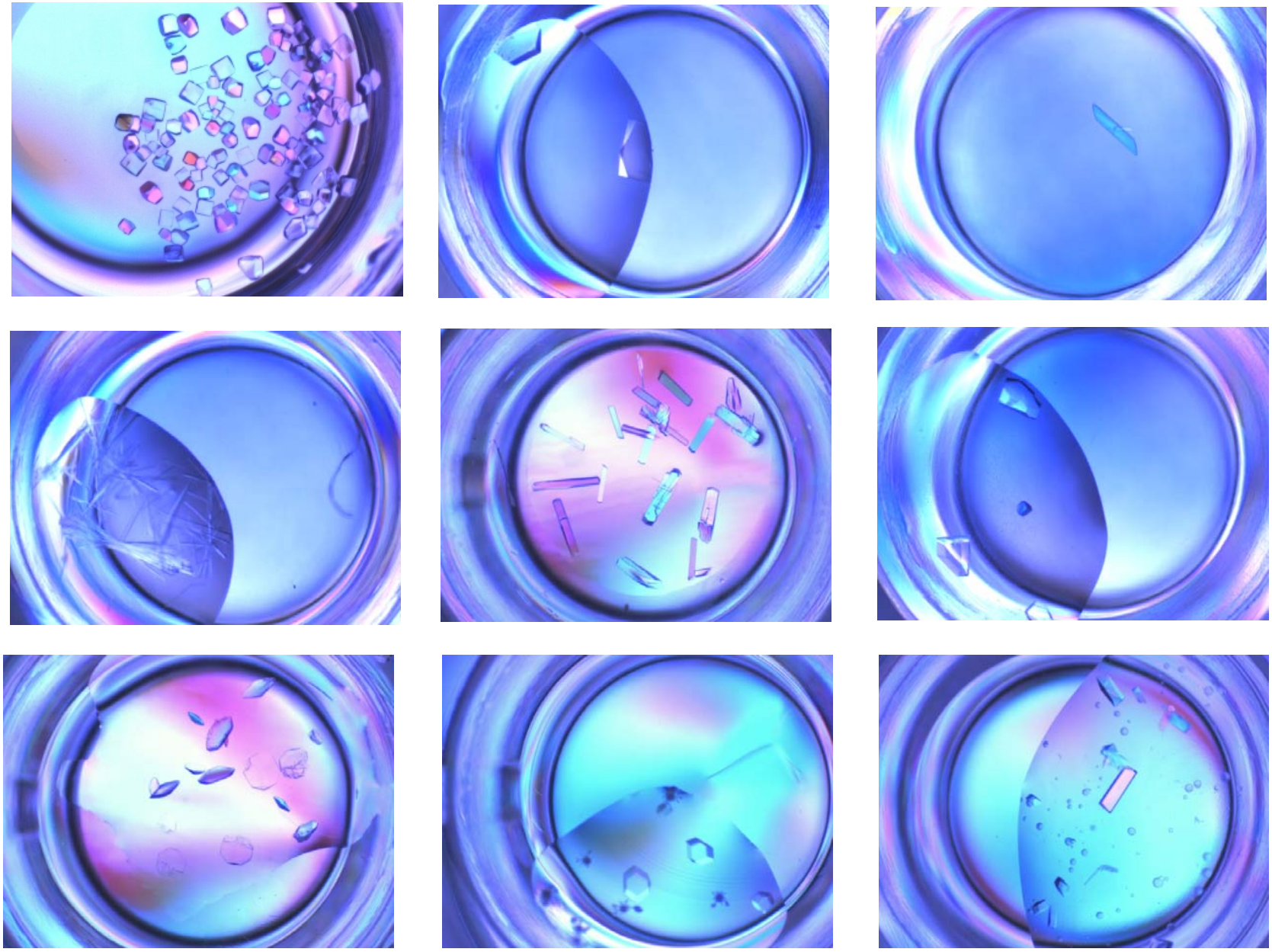


Protein Crystallization and crystal observation robot system

- **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**

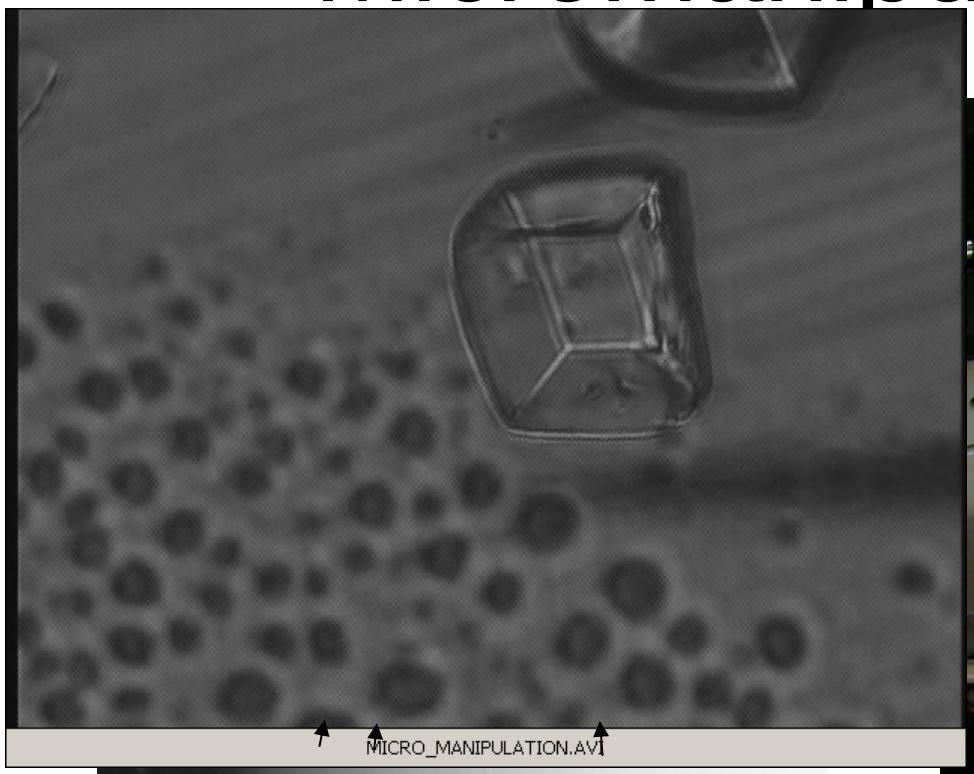


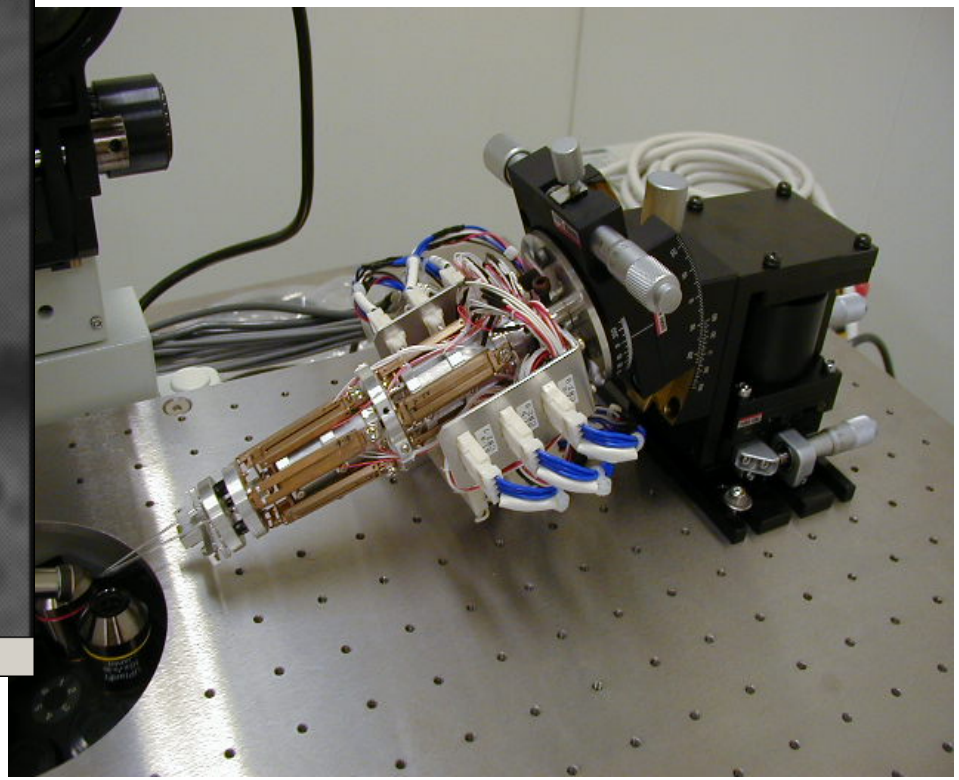
Protein Crystals

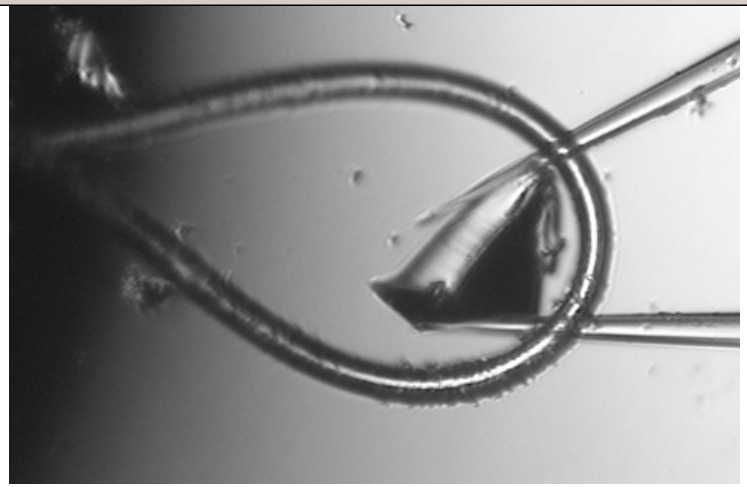


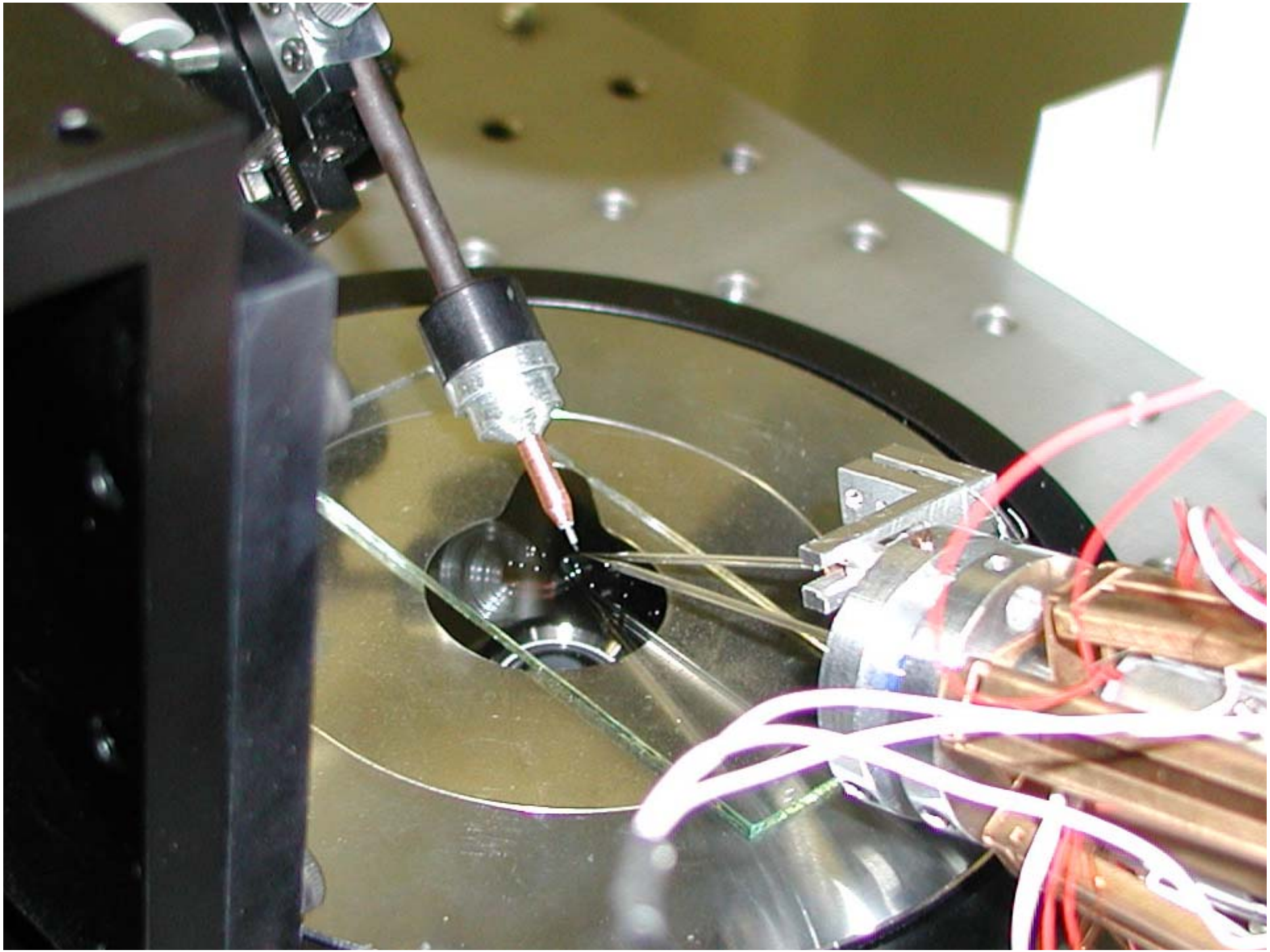
Micromanipulator system

In collaboration with Dr.Tanikawa (AIST)



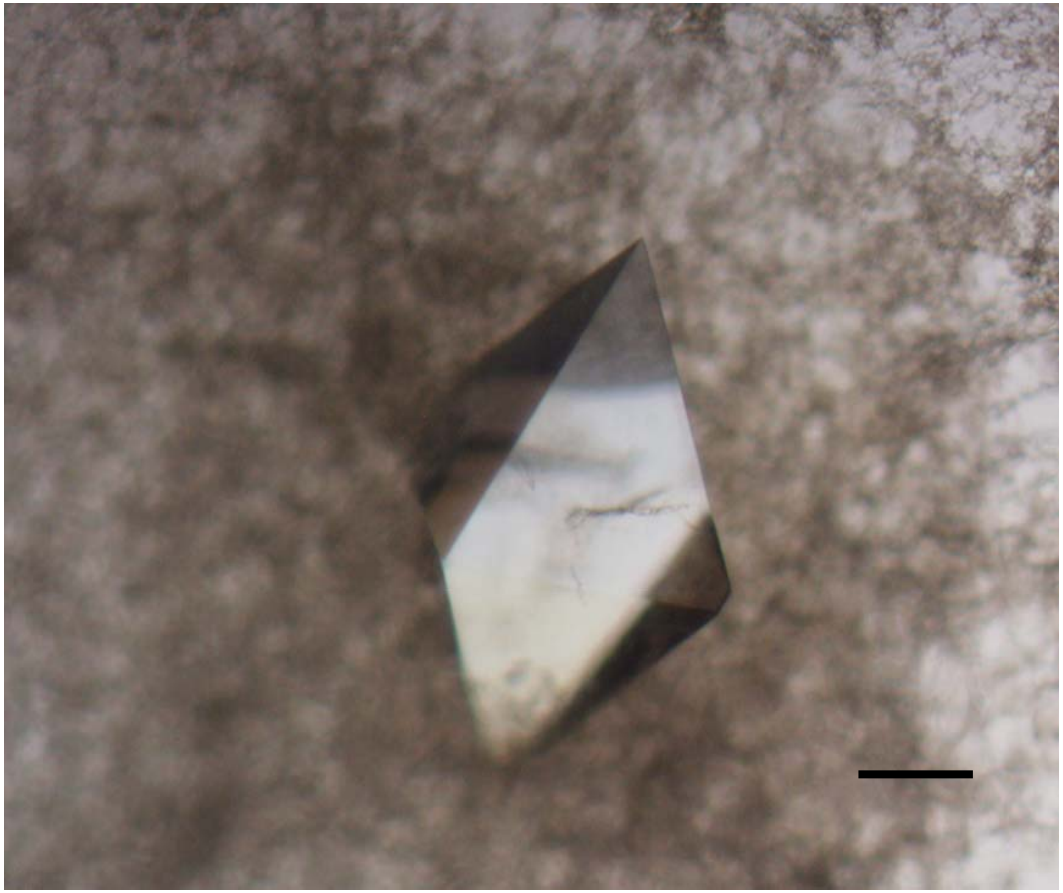






~10^{12} proteins in a typical (good size) crystal

Crystal of Human GGA1 VHS domain



bar = 0.1 mm

Crystallization method: hanging drop vapor diffusion

Protein conc.: 13 mg / ml

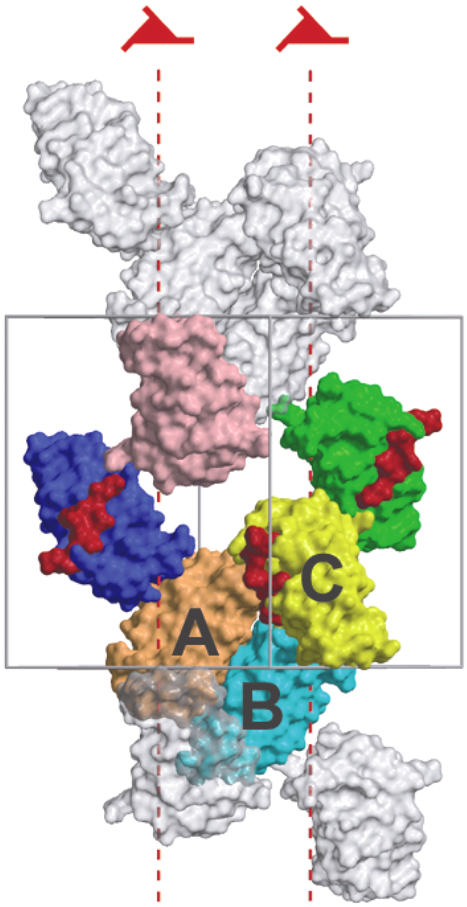
Precipitant: 17 % (w/v) PEG3350, 0.2 M KH_2PO_4

Buffer: 0.1 M Tris-HCl (pH 7.5)

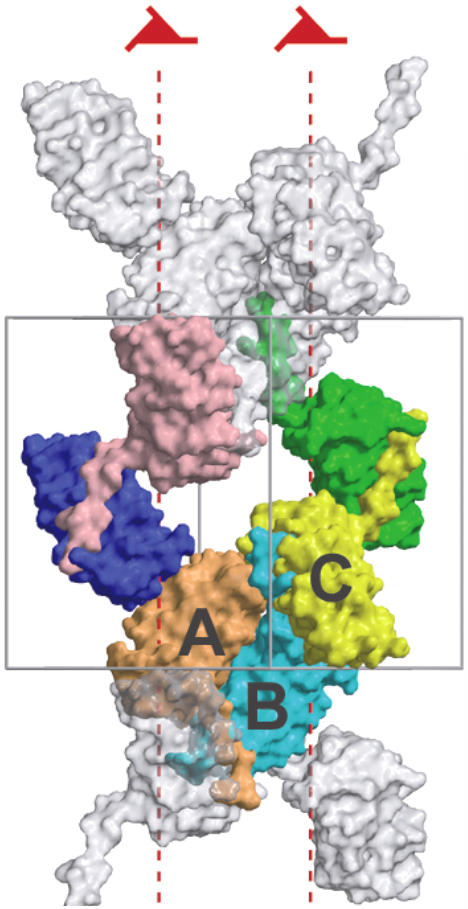
Temperature: 20 °C

Packing of proteins in a crystal

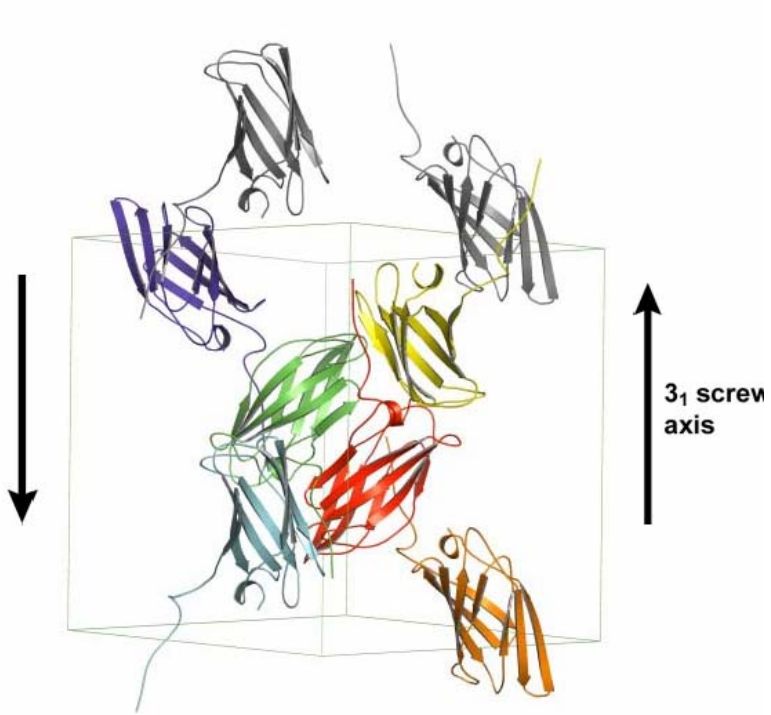
30~70 % volume of protein crystals is solvent!

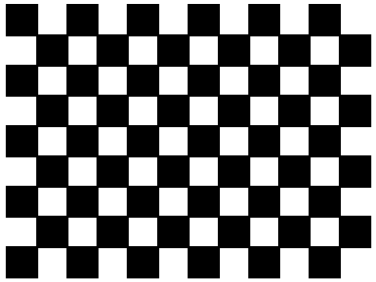


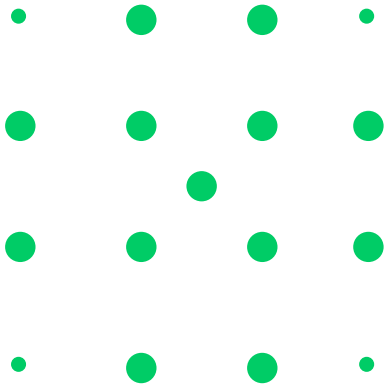
Cocrystal

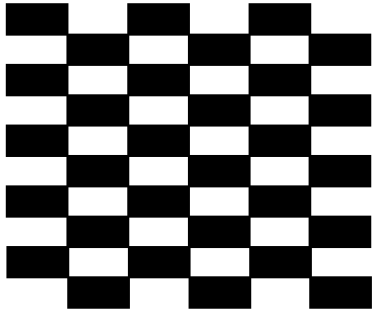


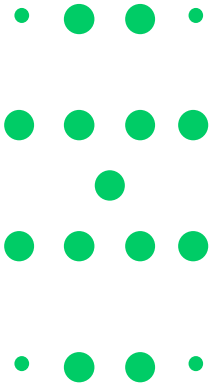
Peptide-tagged

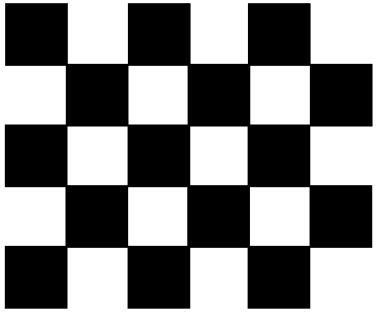


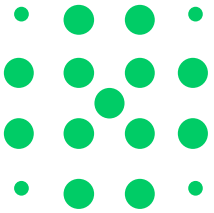


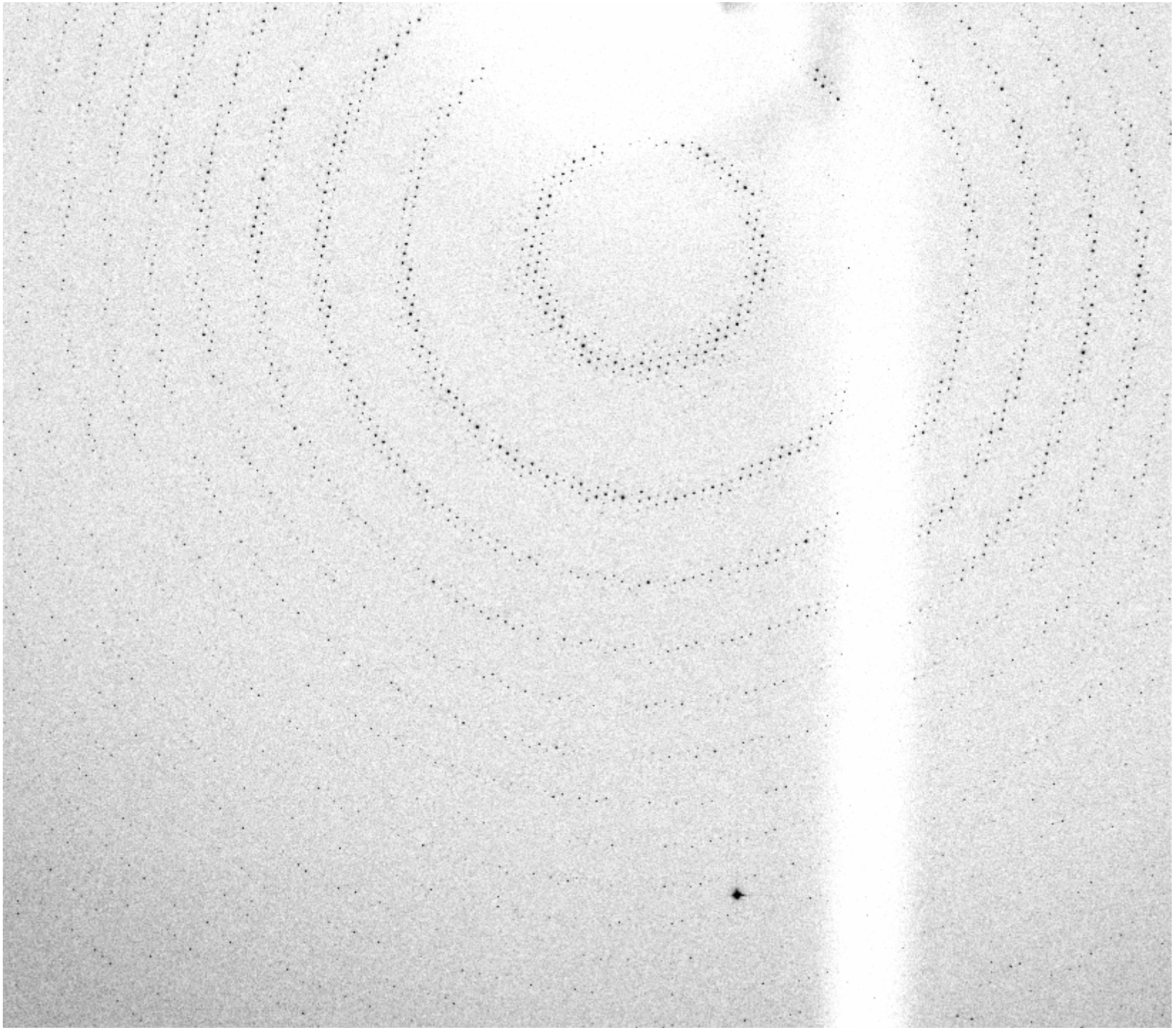


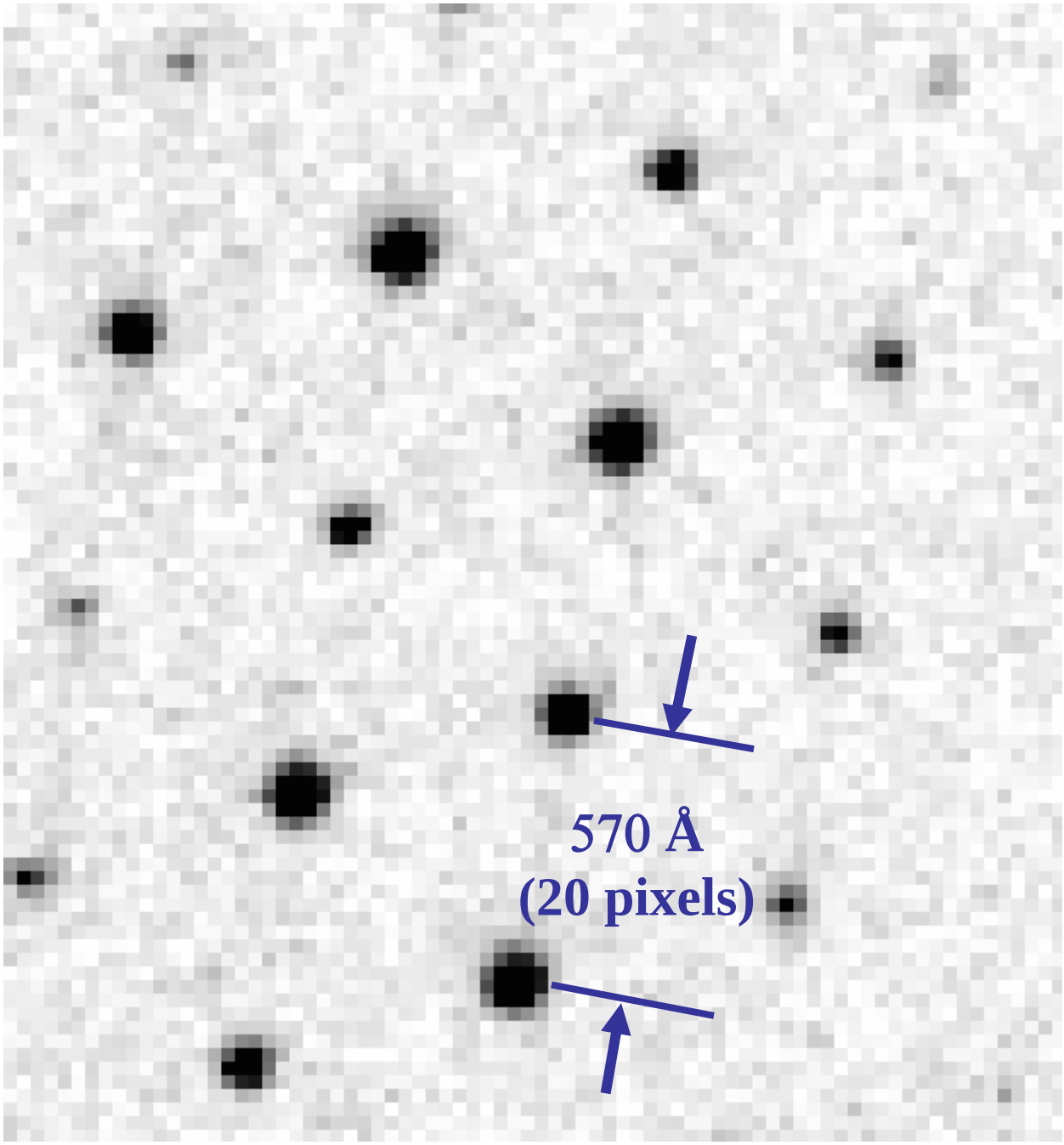












Blue Tongue Virus

Core Particle 1

$P2_12_12$

755 X 796 X 825 $Å^3$

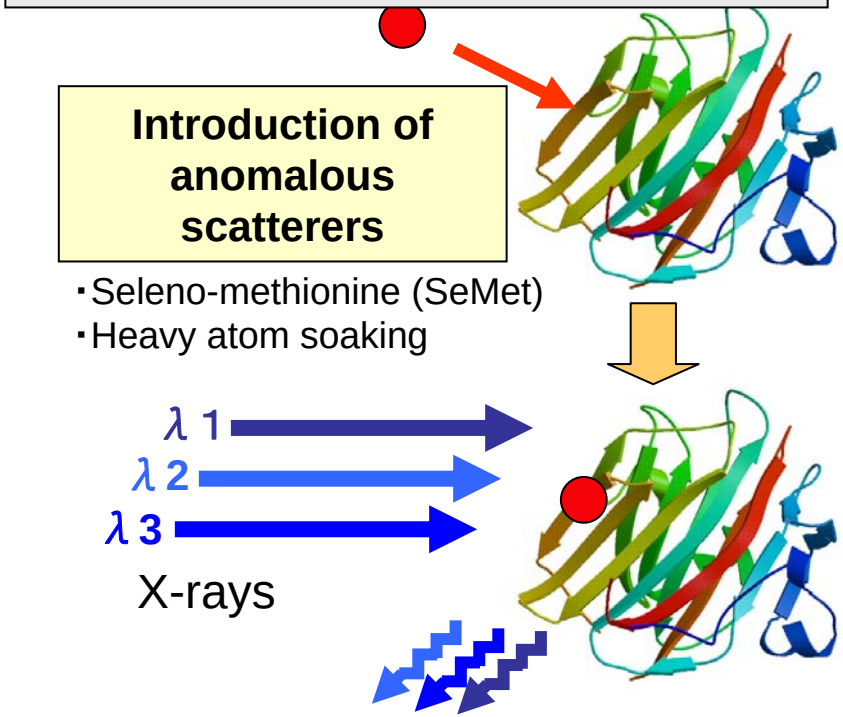
ESRF ID14/EH3

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)

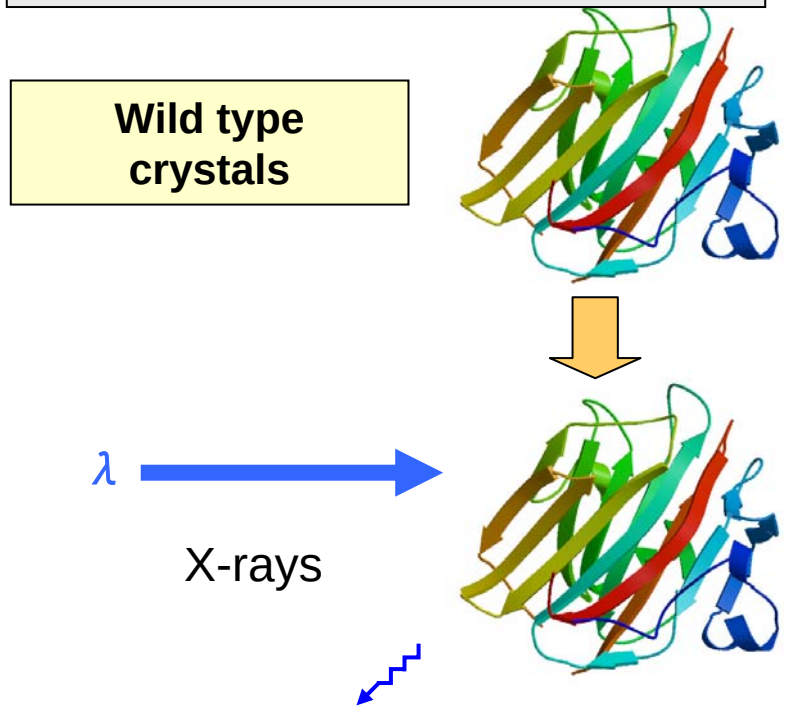


Multi wavelength anomalous dispersion

・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)

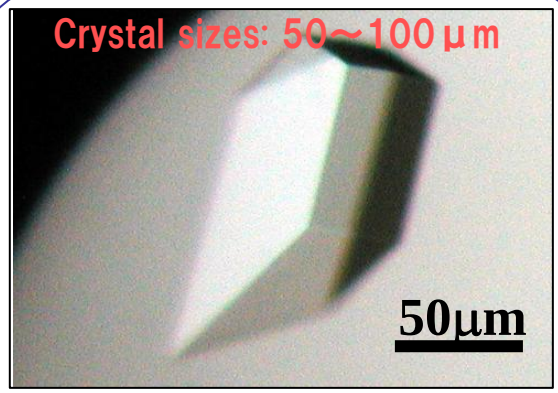


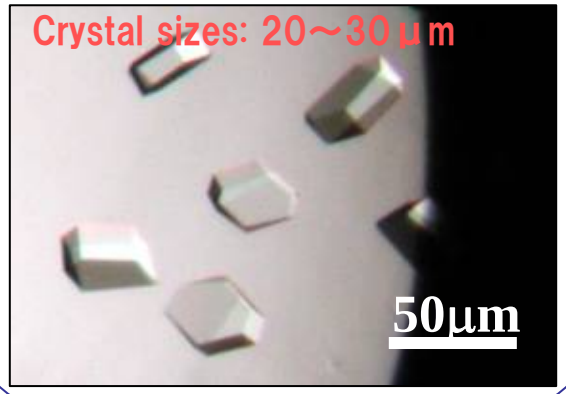
Anomalous signal from light atoms (eg: S)

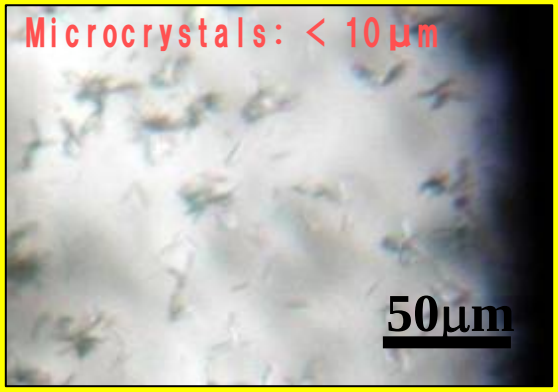
・No need for preparing heavy atoms: highly applicable for very difficult cases for which heavy atom derivatives cannot be prepared
・Light elements need low X-ray energy for higher anomalous signal
・Weak anomalous signal necessitates extremely highly accurate data, thus high redundancy

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

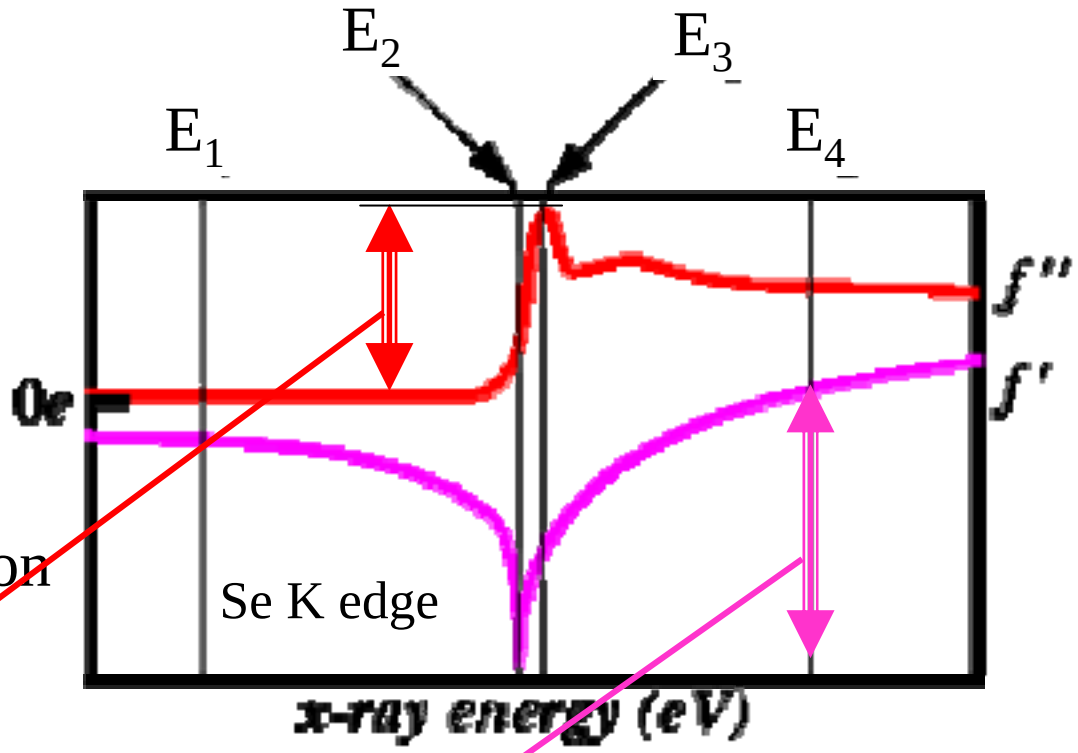
(Multiple Anomalous Dispersion)

Bijvoet pairs

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

Bijvoet Differences

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



MAD experimental maps:

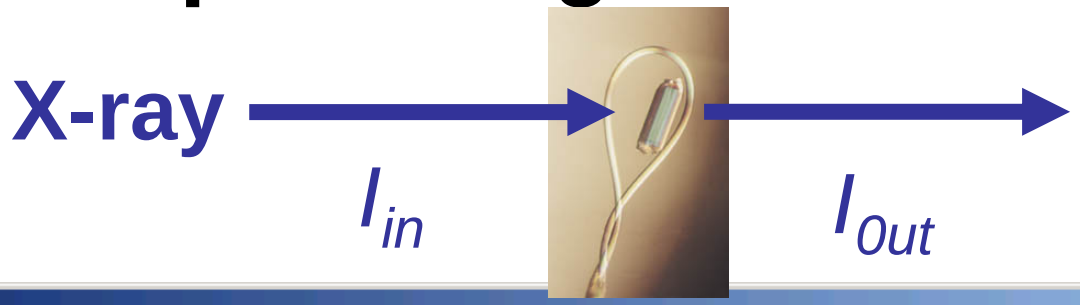
Anomalous difference Patterson

ΔF_{E3}^2 (at the peak)

Dispersive Patterson

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

Absorption edge measurement



XAFS Measurement

Status: Idle
Master: NW12adsc
FancyBox: dcmode
STOP

Select Unit: eV Å
Select Atom: Se

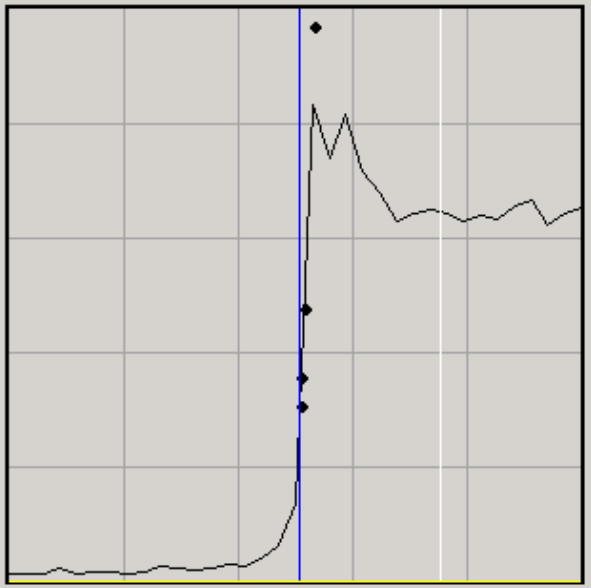
Select Wavelength: 0.90000 0.97682 Å Drive
Beam Alignment: Start Undo Slit Size mm

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
I_{in}
I_{Out}
0.000

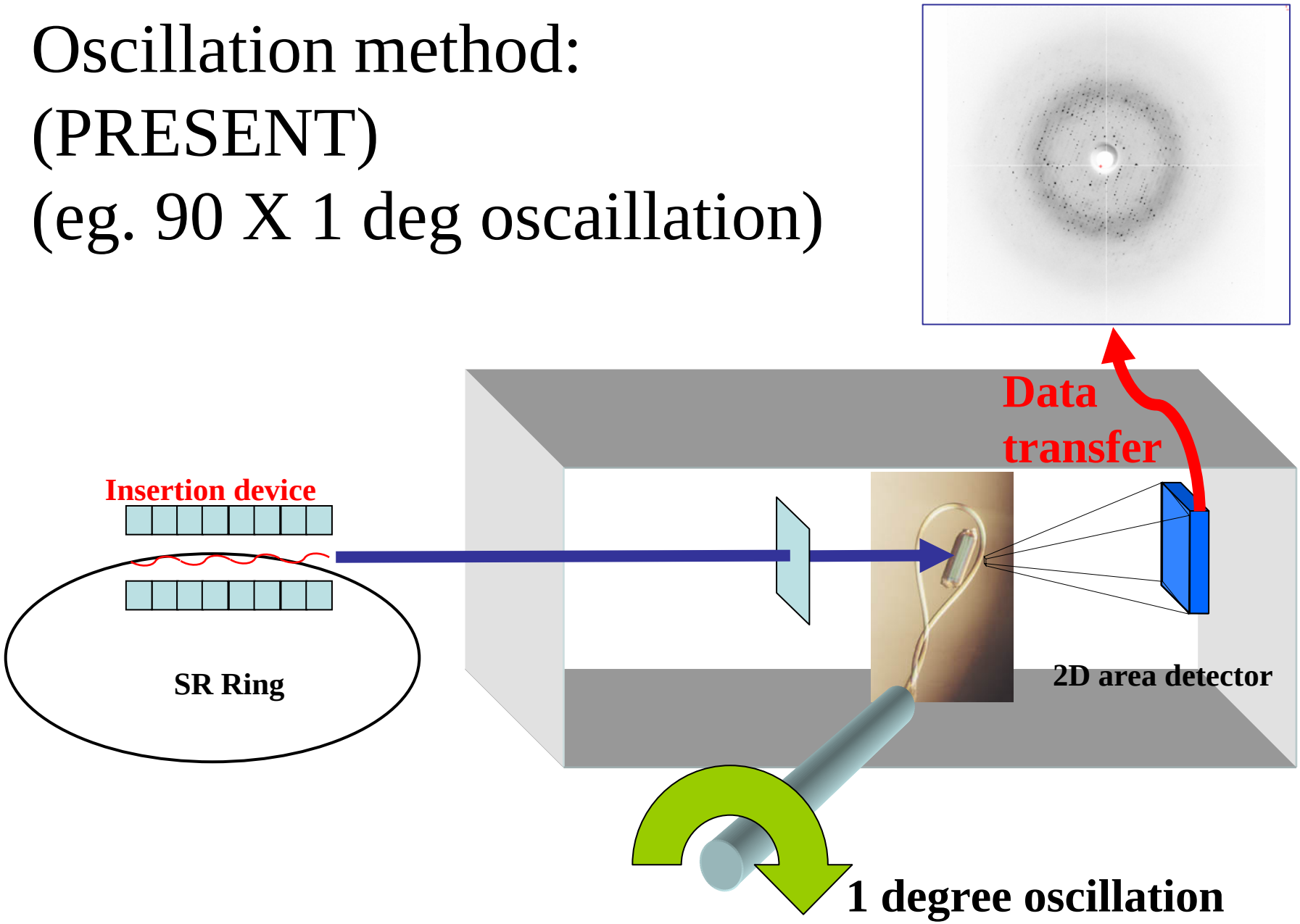


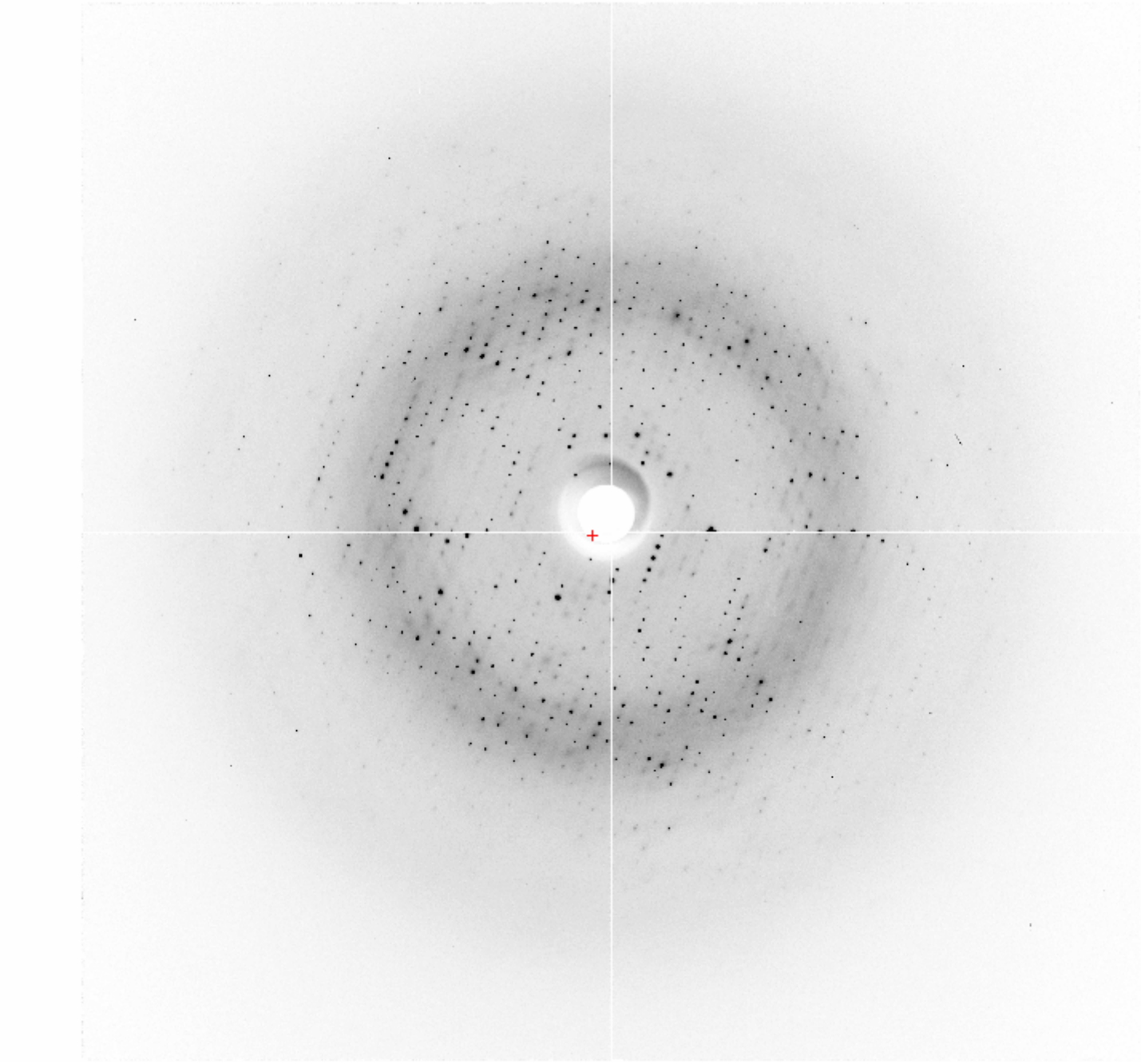
12590.04 Energy (eV) 12726.293
0.98478 Wavelength(Å) 0.97424
Lower < > Upper
Target

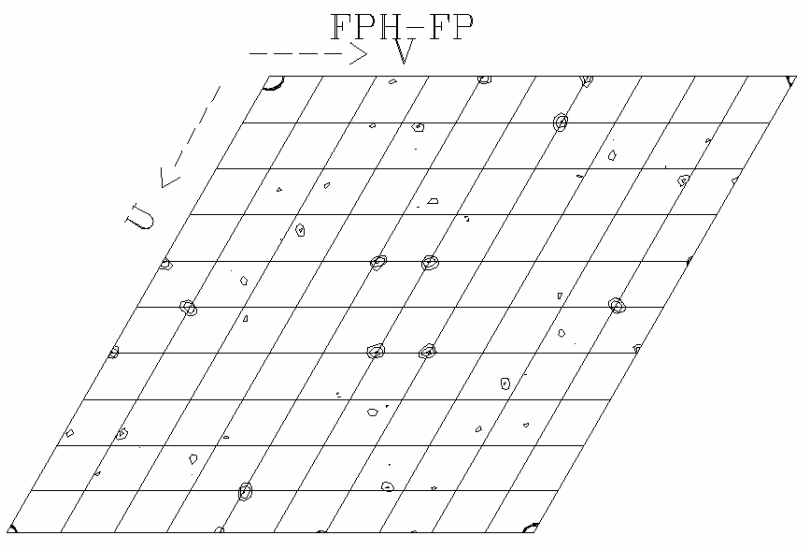
Energy

Close

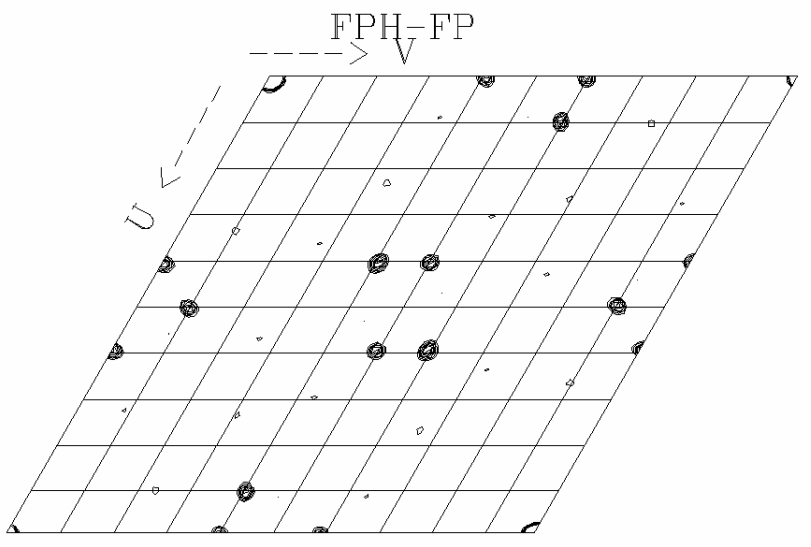
Oscillation method:
(PRESENT)
(eg. 90 X 1 deg oscaillation)



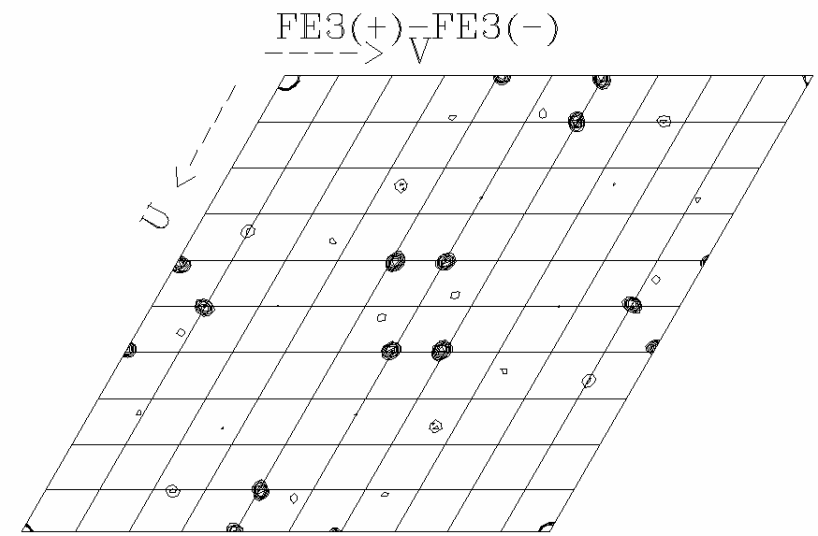




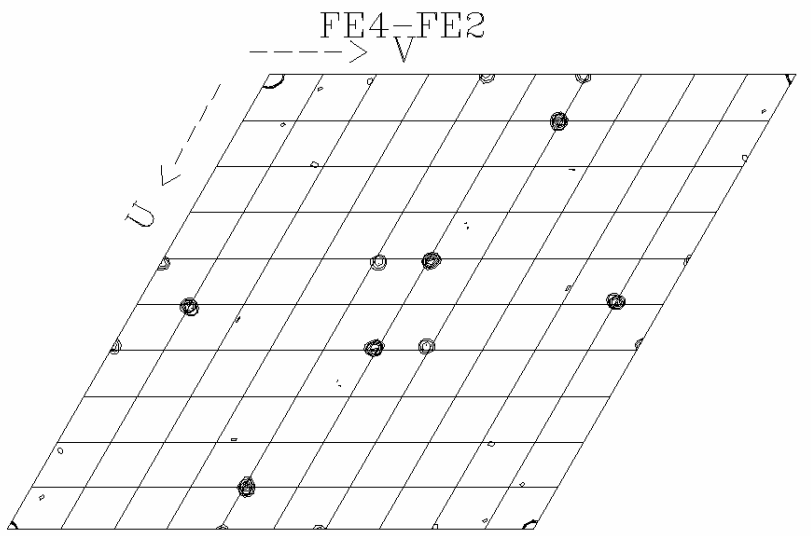
FPH(SeMet)-FP(native) difference Patterson Map



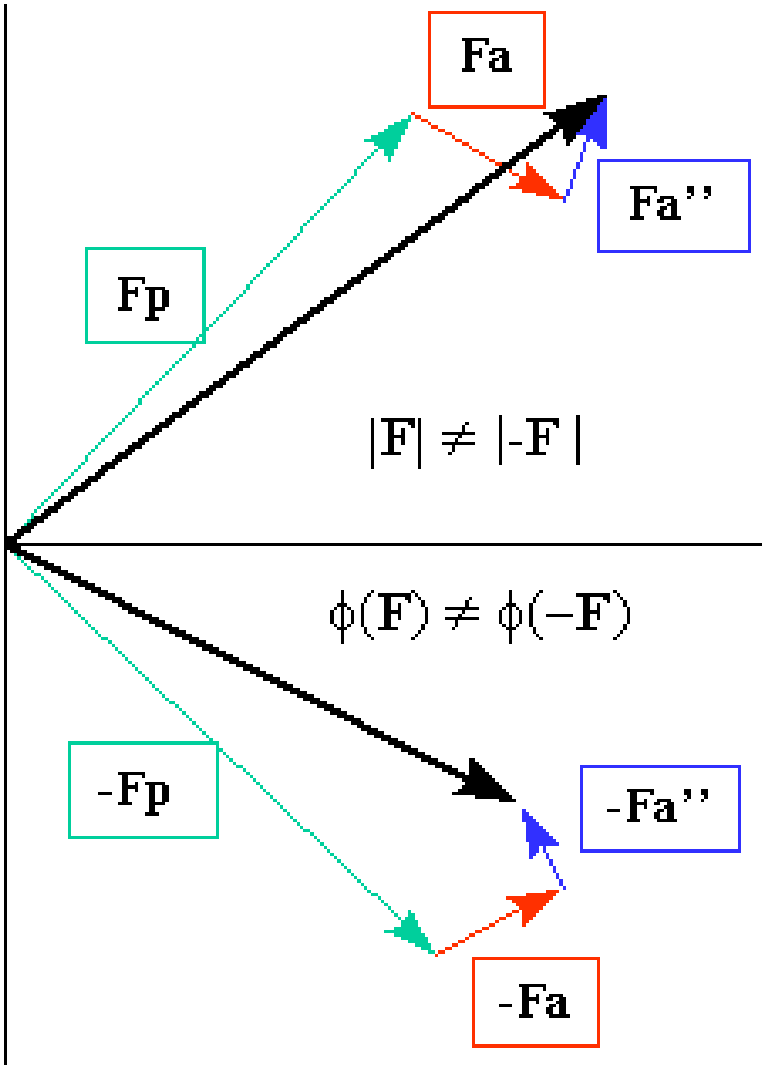
FPH(SeMet)-FP(native) anomalous difference Patterson Map

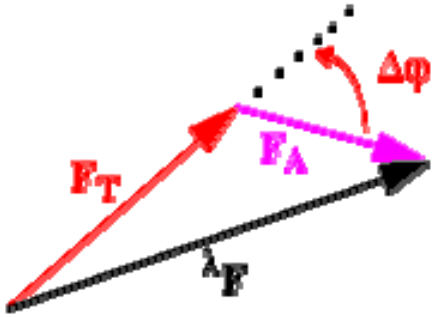


FE3(+)-FE3(-) anomalous difference Patterson Map



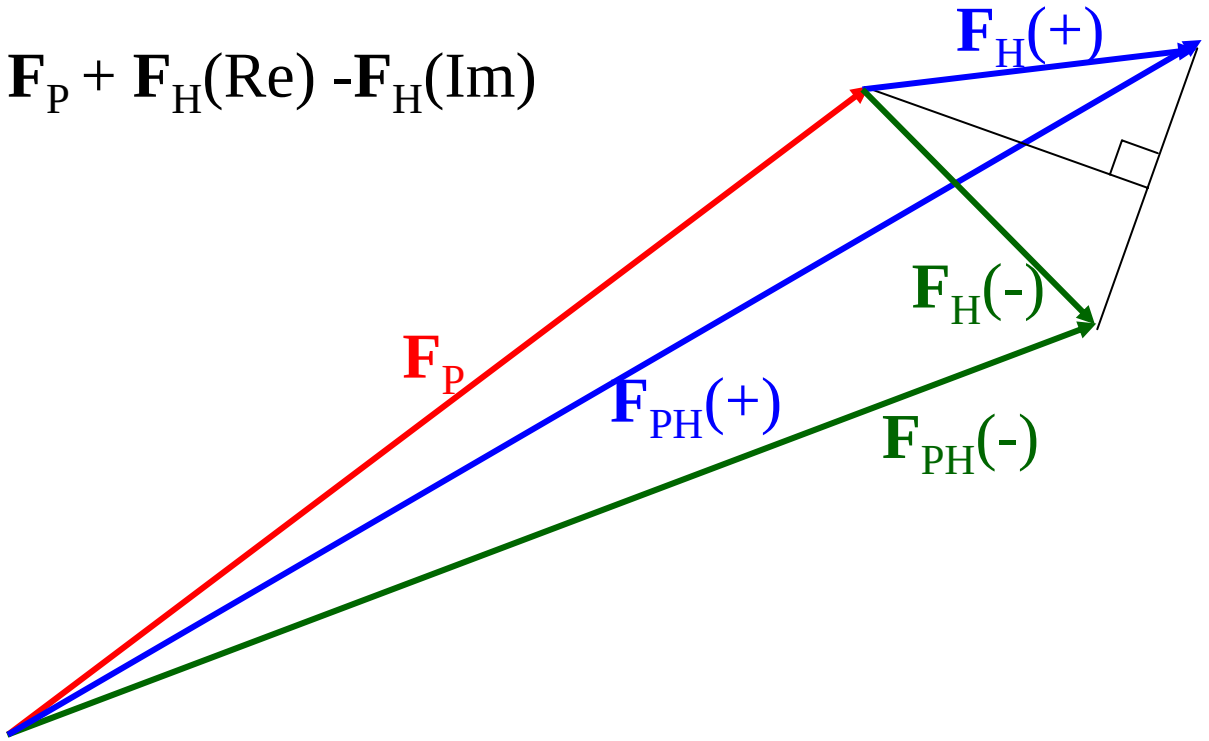
FE4(remote1)-FE2(edge) dispersive difference Patterson Map


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


Δφ
F_T
F_A
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

$|F_P|$

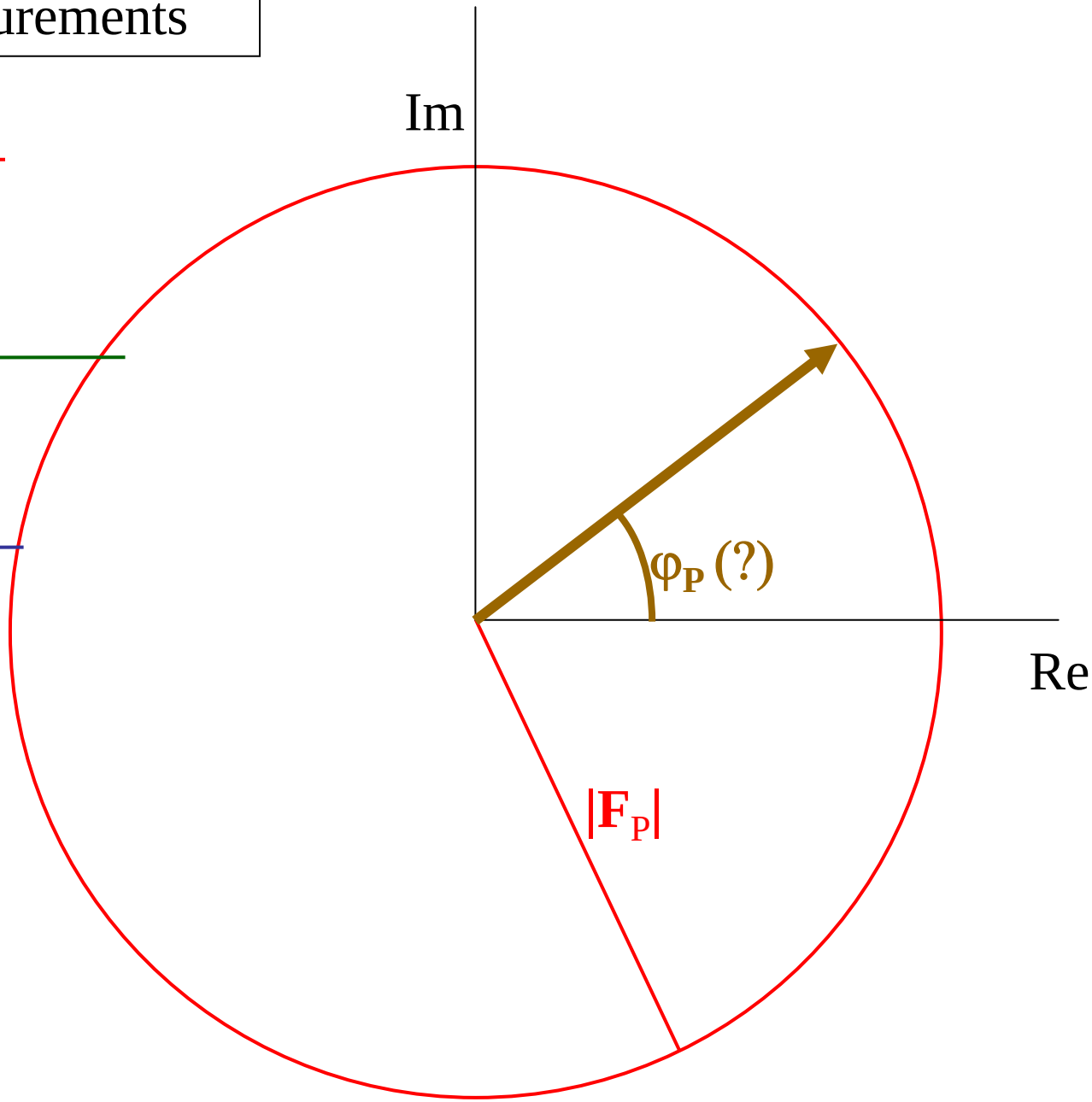
$|F_{PH}(-)|$

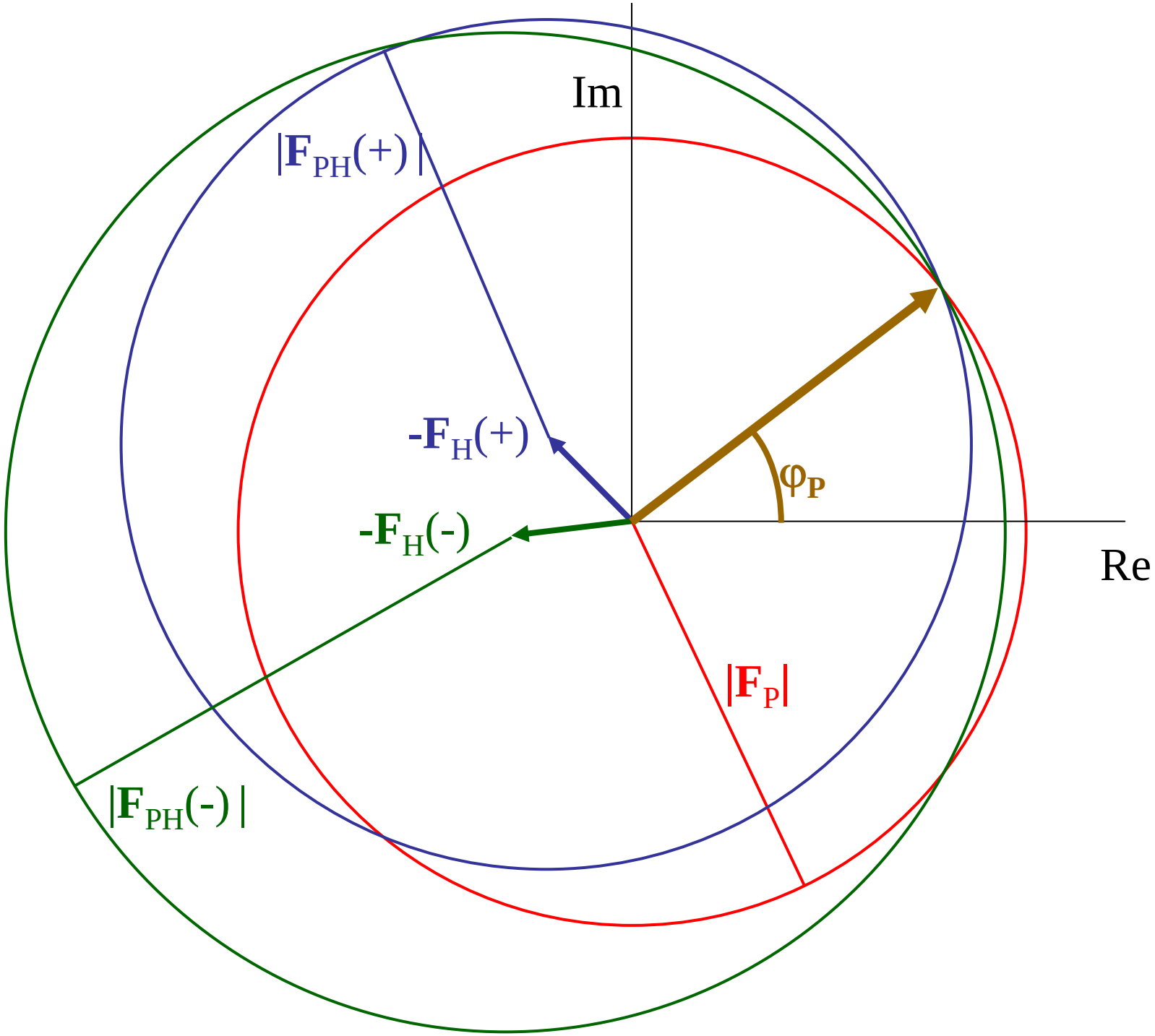
$|F_{PH}(+)|$

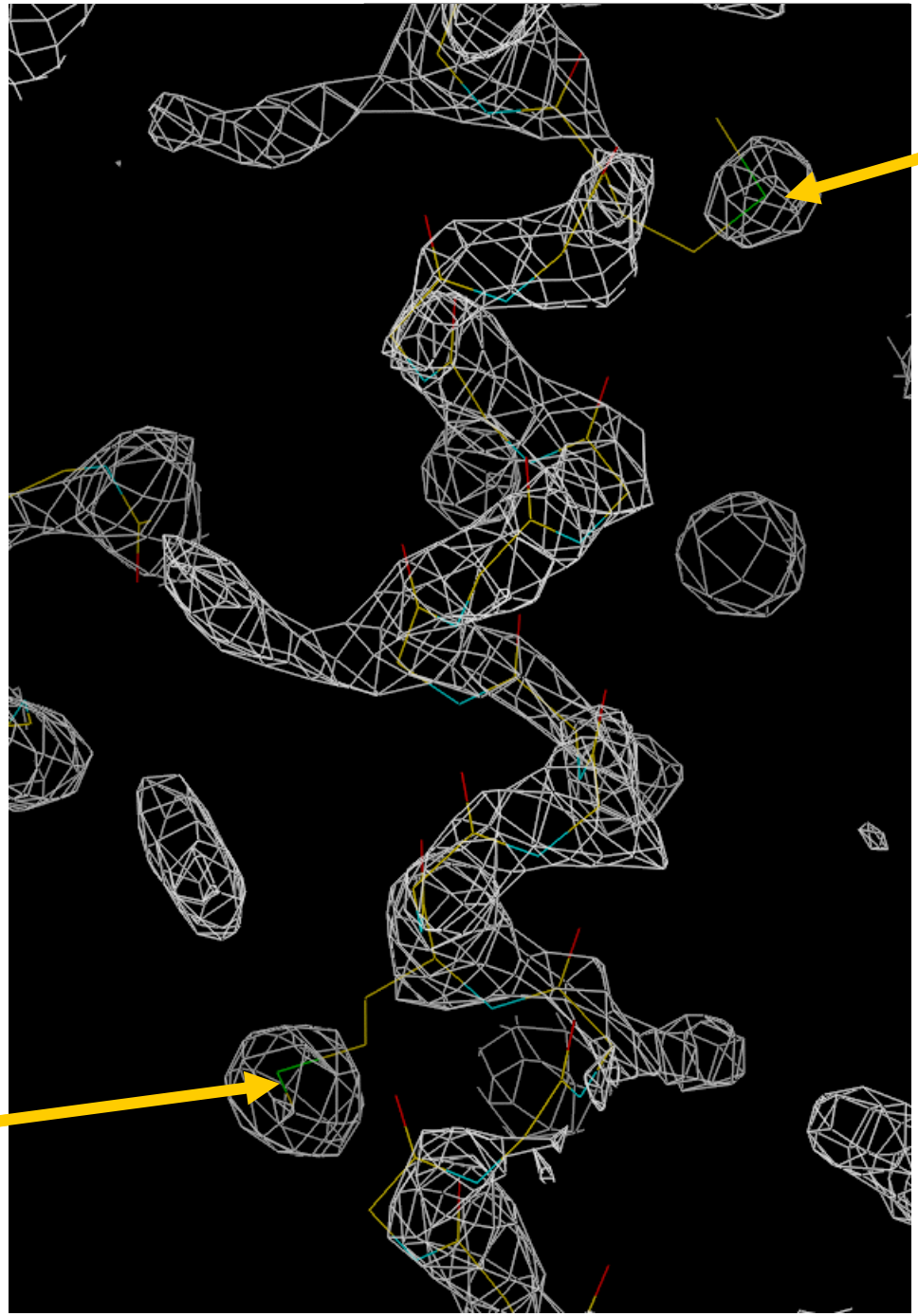
From Se positions

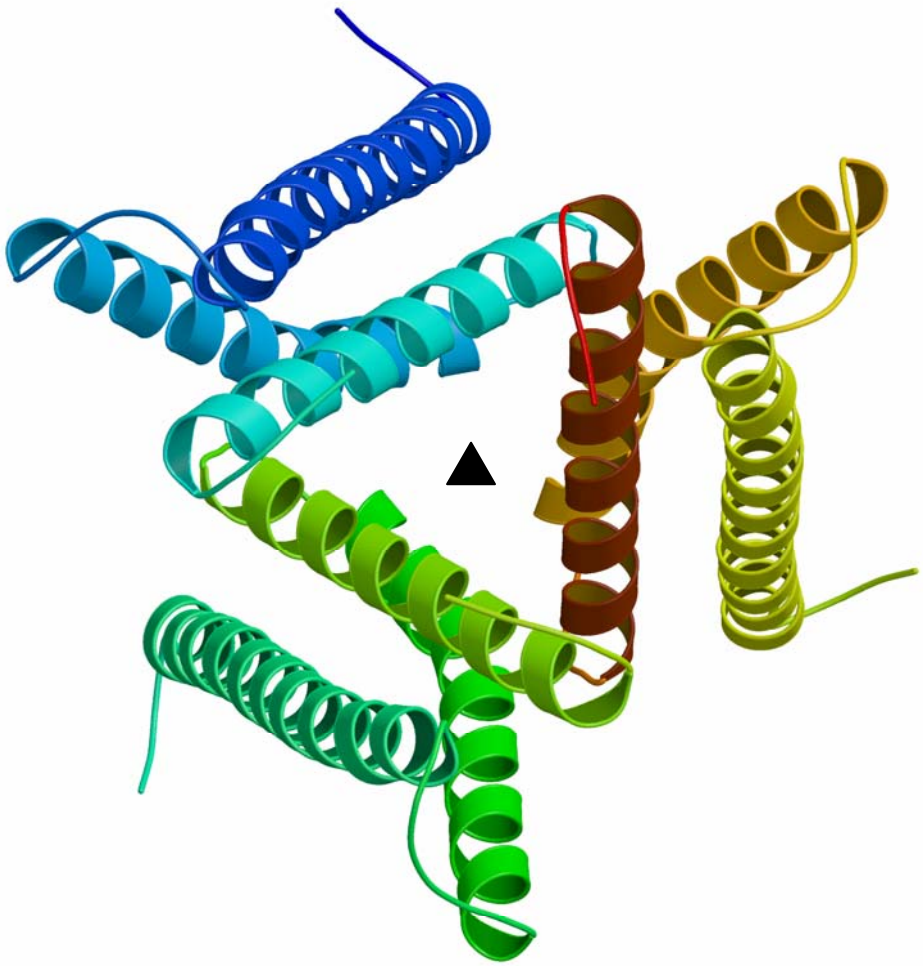
$-F_H(+)$

$-F_H(-)$




Im
Re
|F_PH(+)|
-F_H(+)
φ_P
-F_H(-)
|F_P|
|F_PH(-)|


Met94
Met84



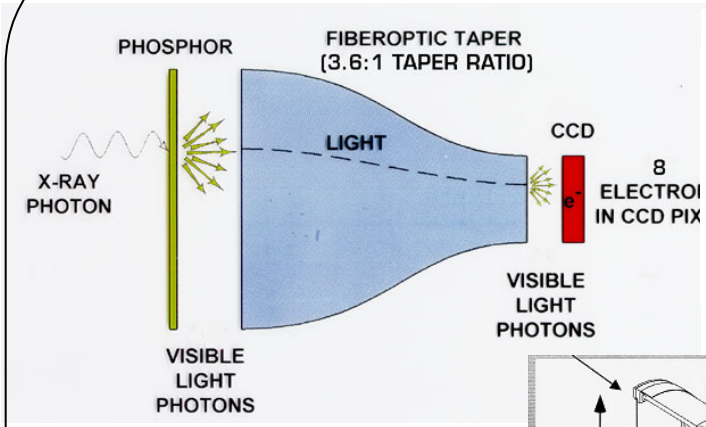
Ribbon diagram of trimer of GGA1 GAT domain

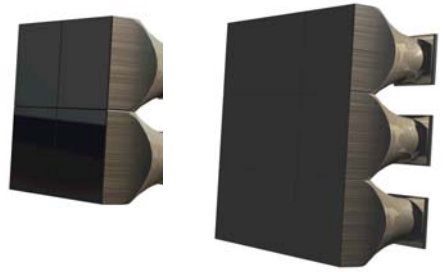
Triangle is threefold axis.

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

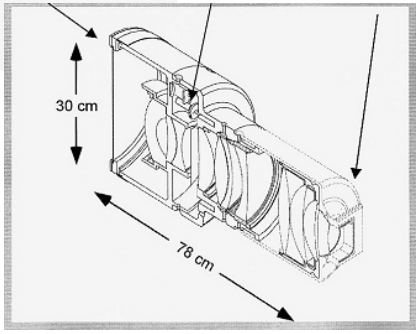
Imaging Plates

Tapered fiber optics CCD



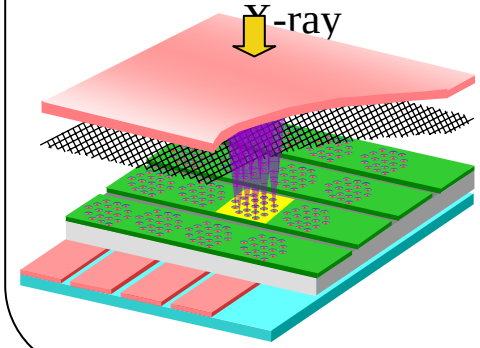


Lens-coupled CCD

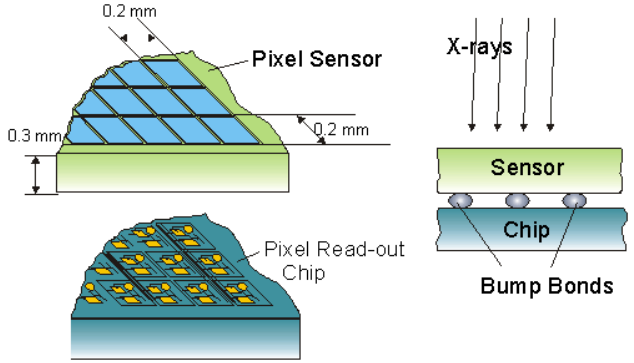


Flat Panel Detectors

HARP based Field Emitter Array (FEA)



Pixel Array Detectors (PAD)



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

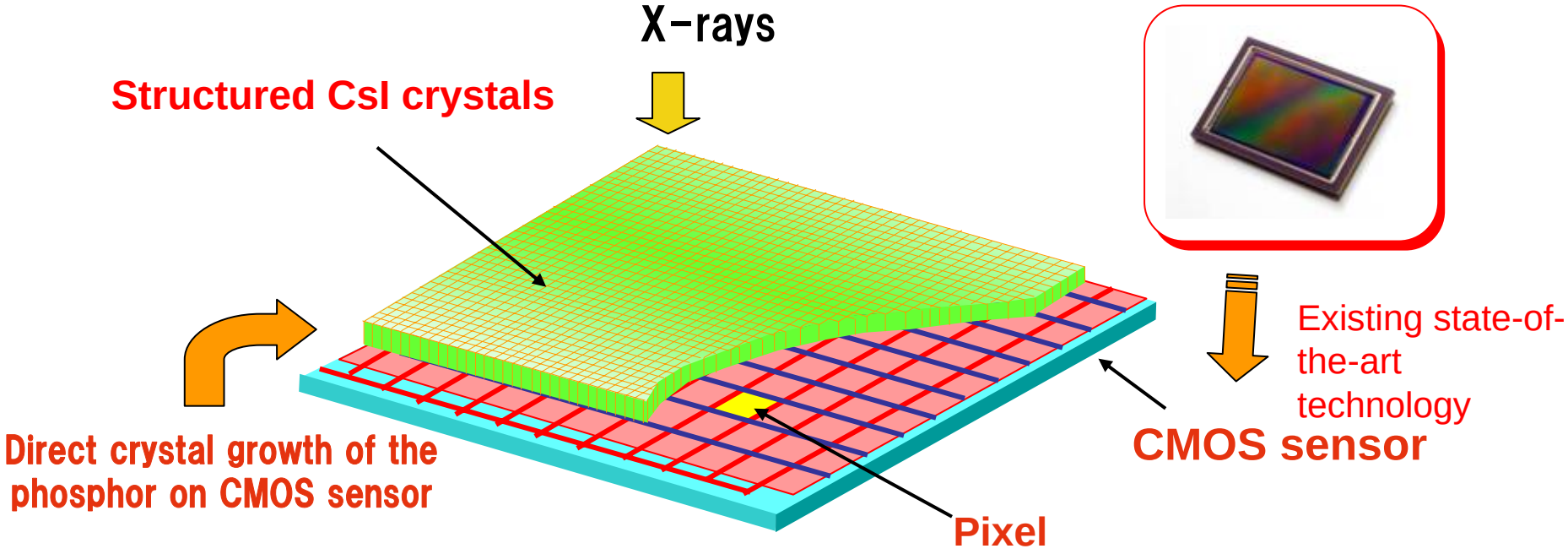
Detectors	Pros	Cons
On-line imaging plates	Large dynamic range, large area	Slow readout (20 to 200 sec per image) -> poor duty cycle Relatively broad PSF Relatively inexpensive
Off-line imaging plates	Large dynamic range, large area	Slow read-out (20 to 200 sec per image) -> poor duty cycle Relatively 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

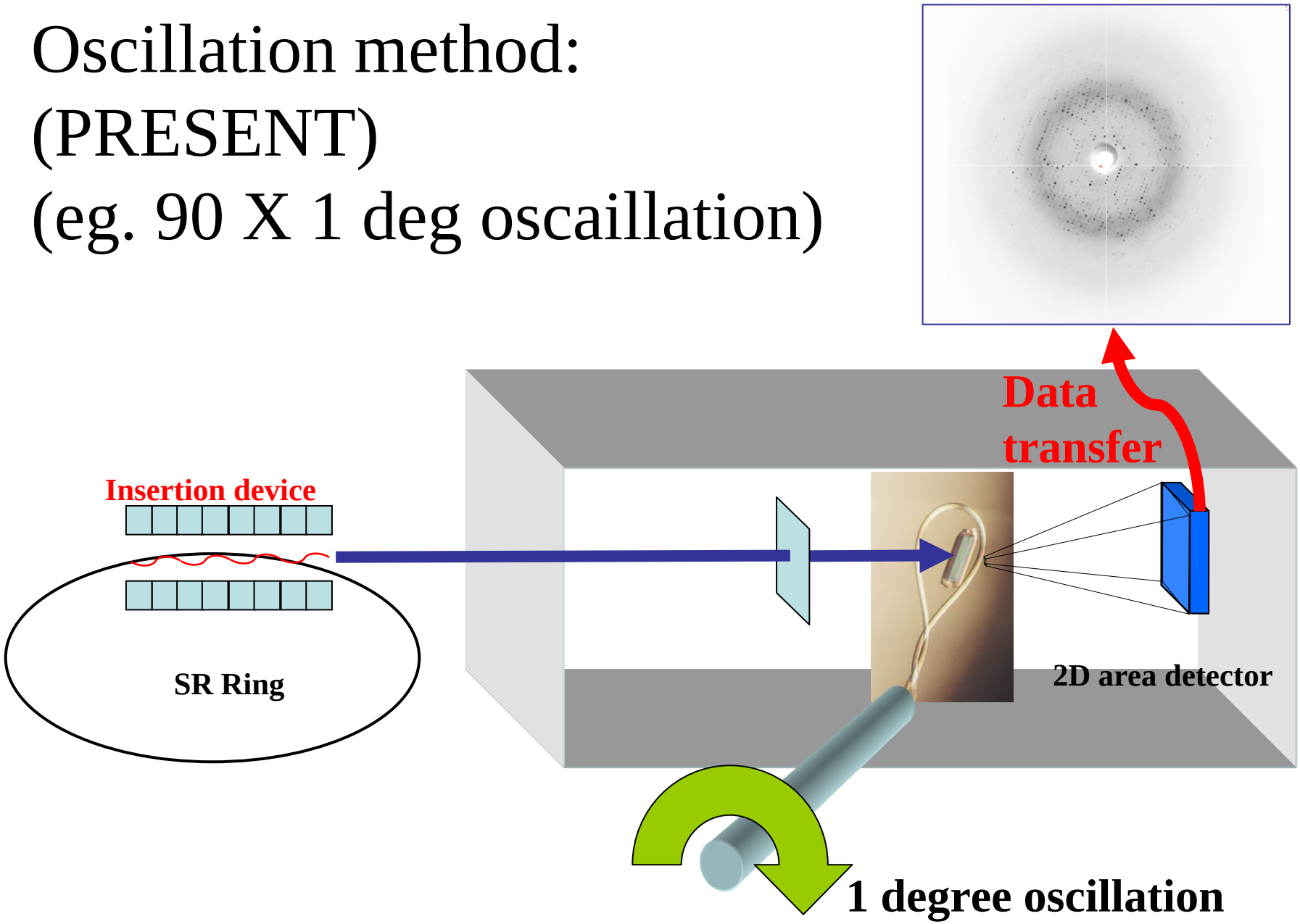


（1） Improvement of sensitivity and spaitial resolution using structured CsI crystals

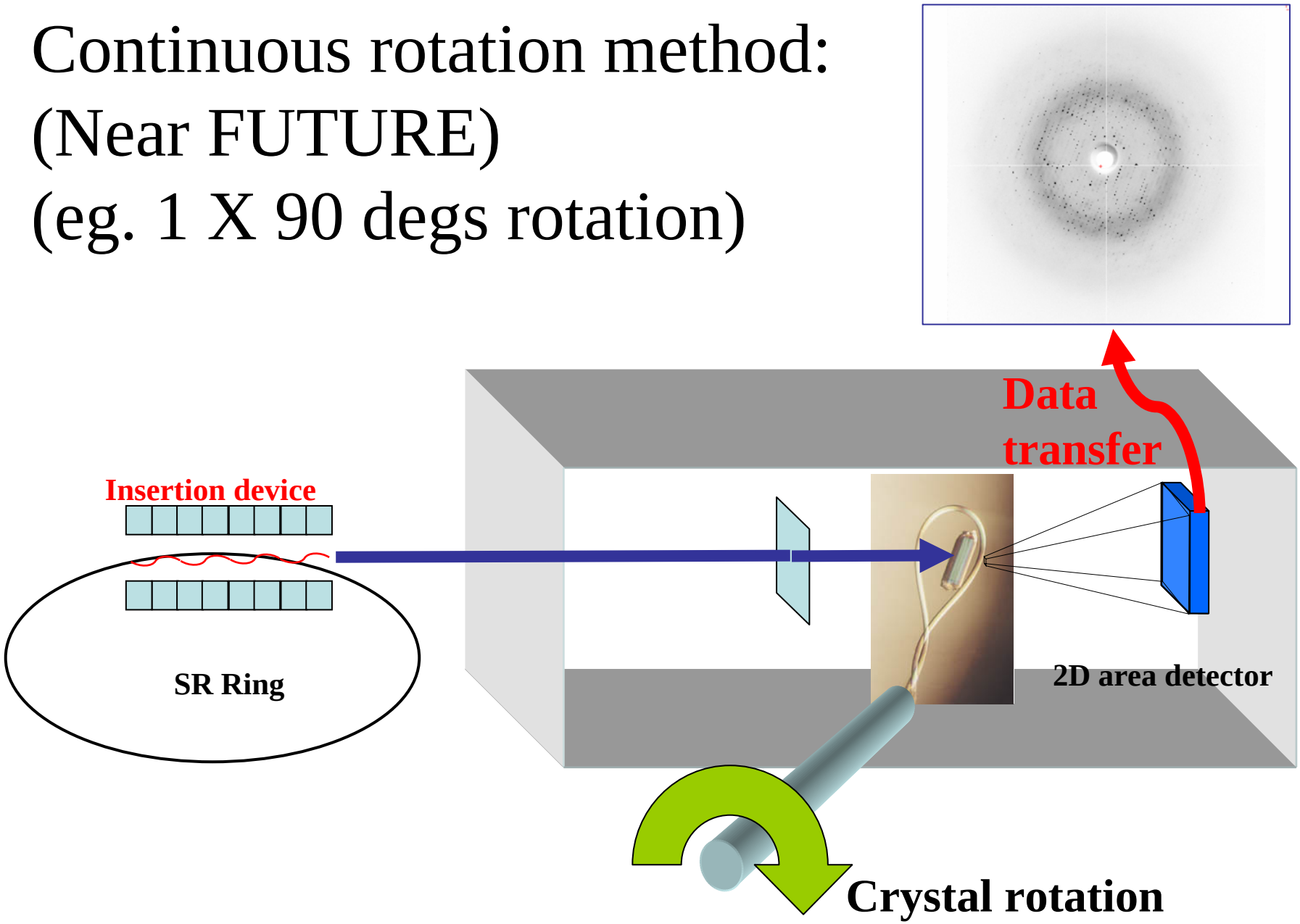
（2） **Cost down by using existing state-of-the-art technologies**

Courtesy of Dr. Masaki Yamamoto

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

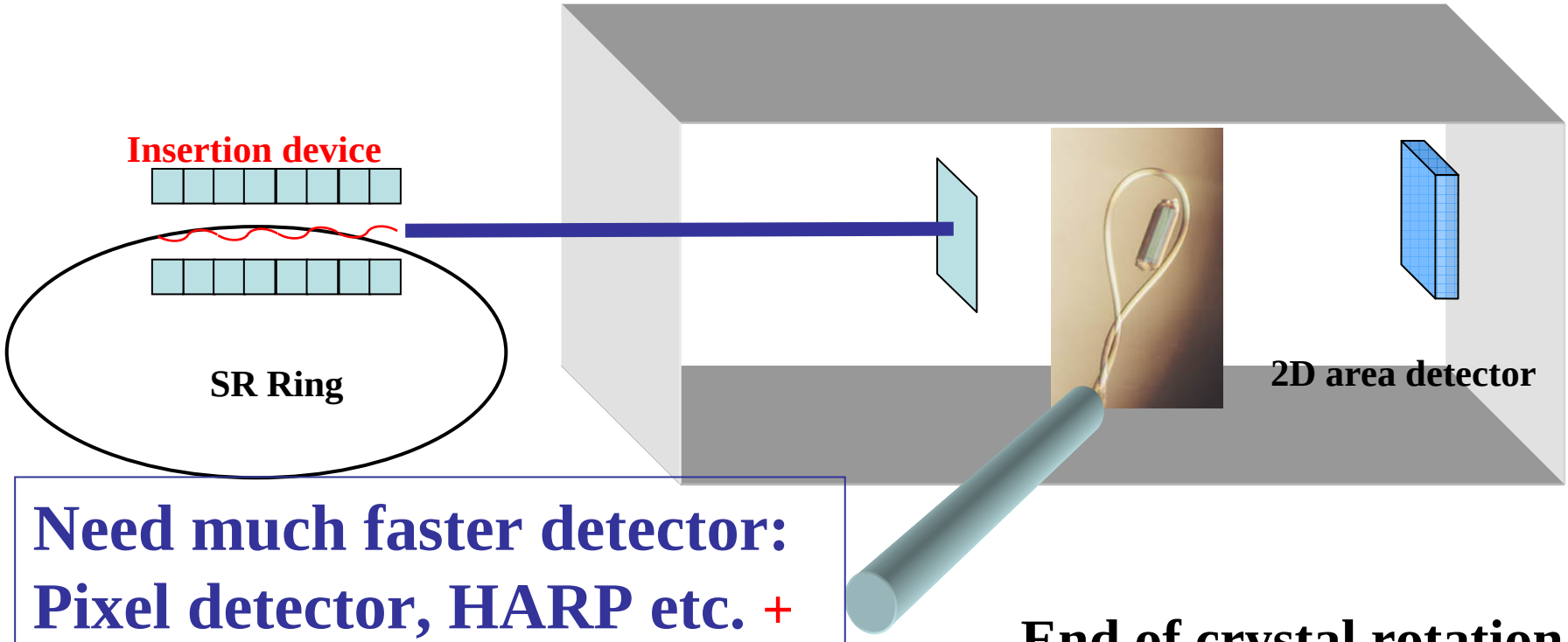


Continuous rotation method:
(Near FUTURE)
(eg. 1 X 90 degs rotation)



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

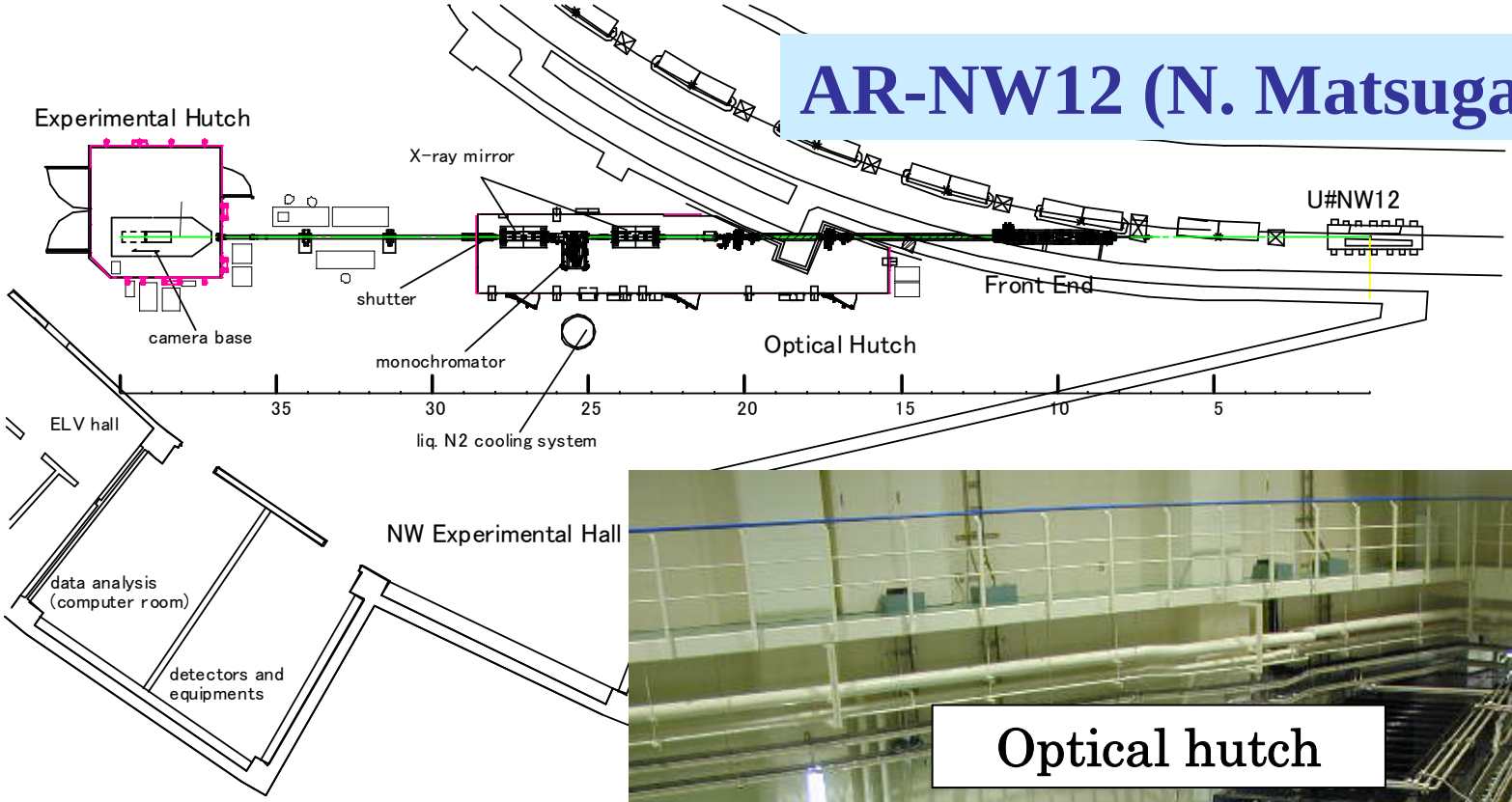
Complete Dataset!

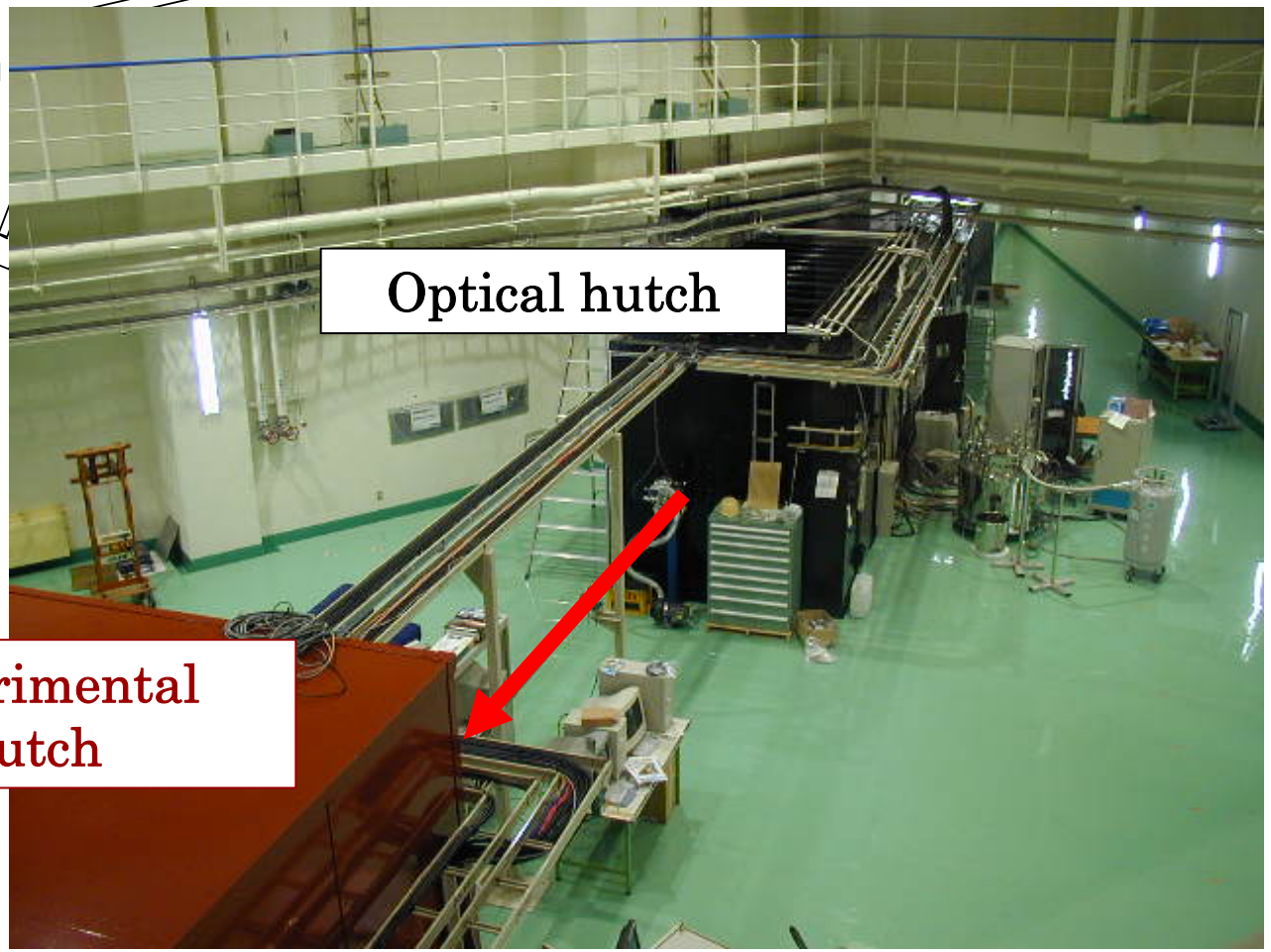


Need much faster detector:
Pixel detector, HARP etc. +
3D profile fitting

End of crystal rotation

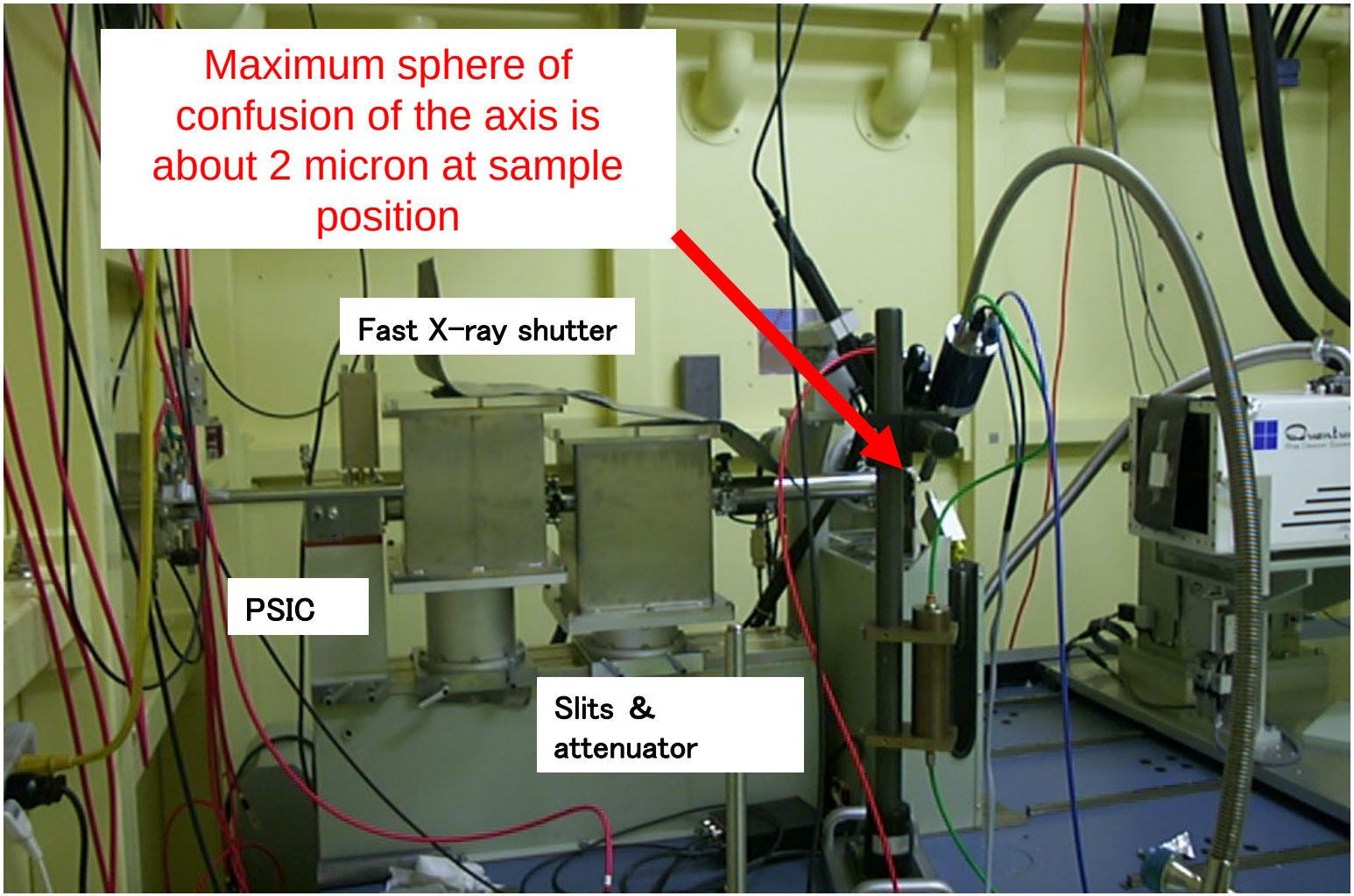
AR-NW12 (N. Matsugaki et al.)





PF-AR NW12: Highthroughput MAD beam line

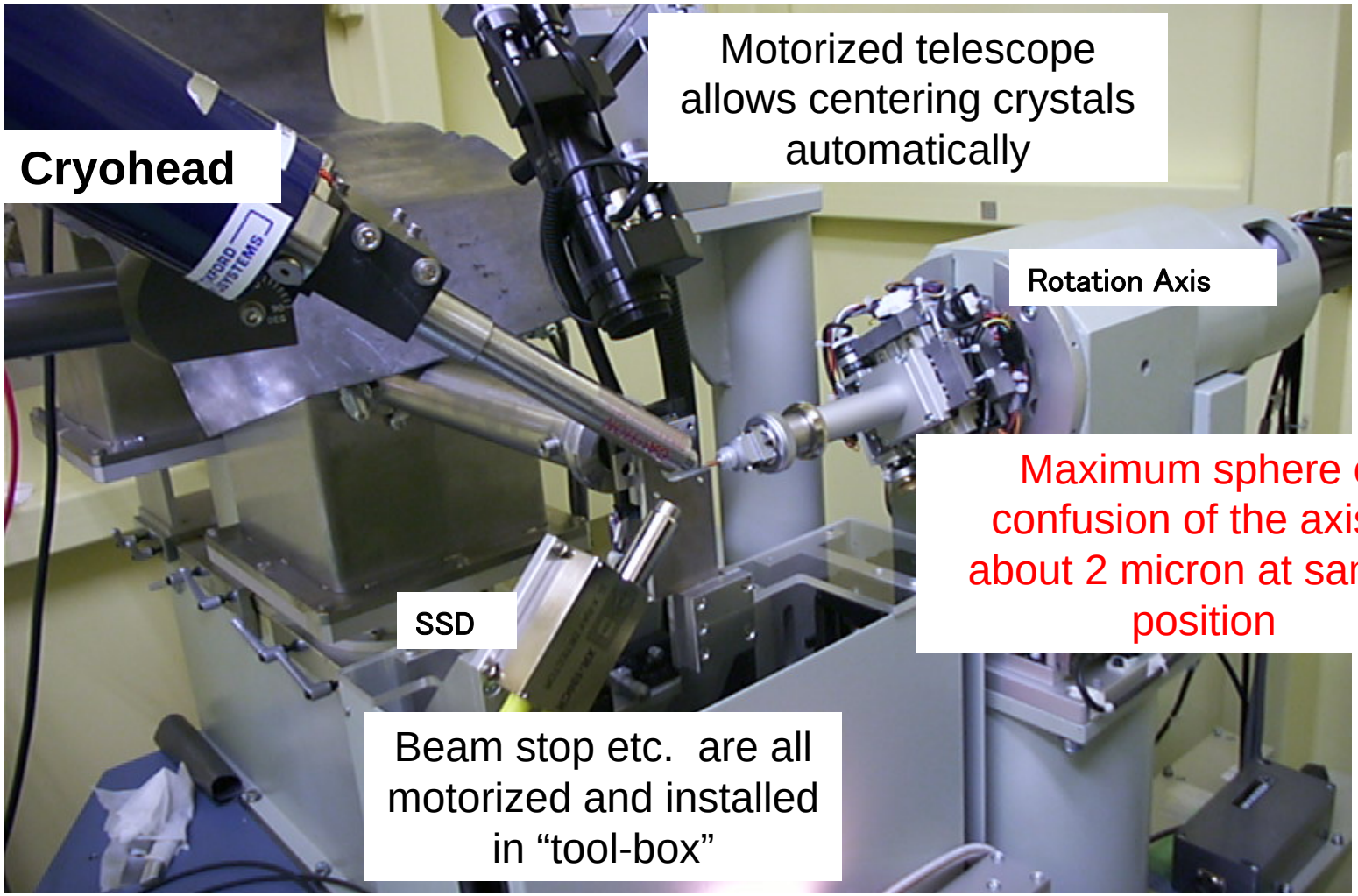
Total data collection time for 180 frames: 10 to 30 min



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.

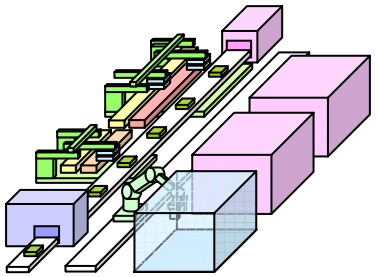
Tool box makes manual crystal mounting easy.



KEK Photon Factory

Crystallization

200,000 trials/day



Structural Biology Research Center

6.5GeV PF-AR

2.5GeV PF ring

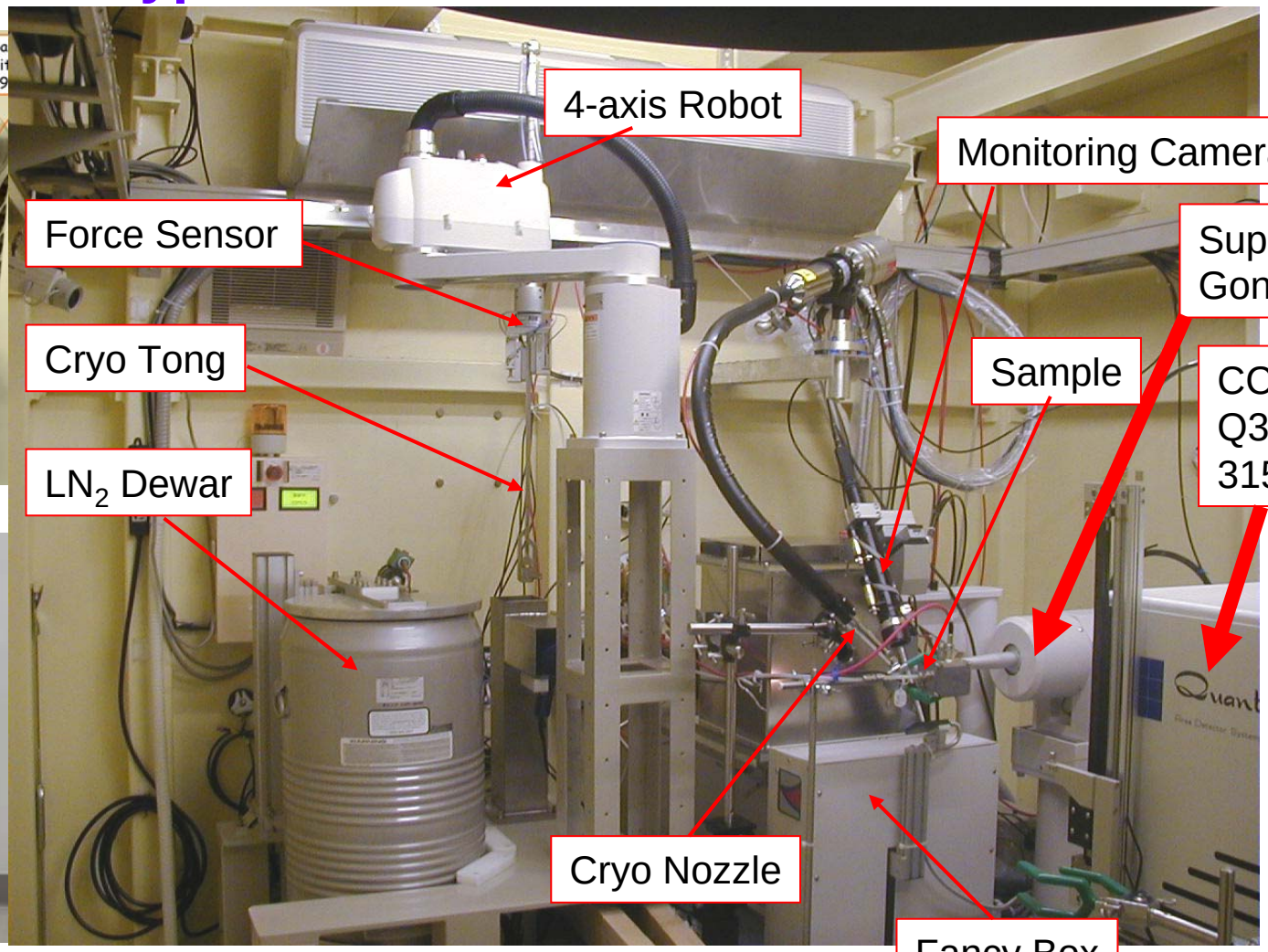
NW12A (2003)

BL-6A

BL-5A (2004)

BL-17A (2006)

SSRL-type robot installed on MAD Beamline BL-5



KEK modification: double tongue

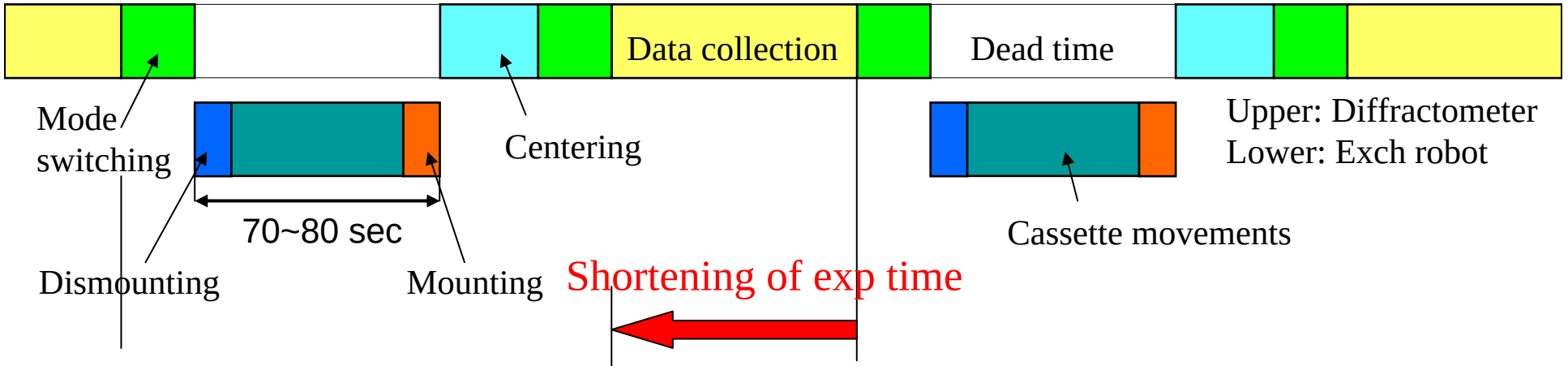
20 datasets/day ⇒ 100s datasets/day

Also in Taiwan and Australia under commissioing. Canada?

Compatibility with SPring8 pins and other standards

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 mechanisms

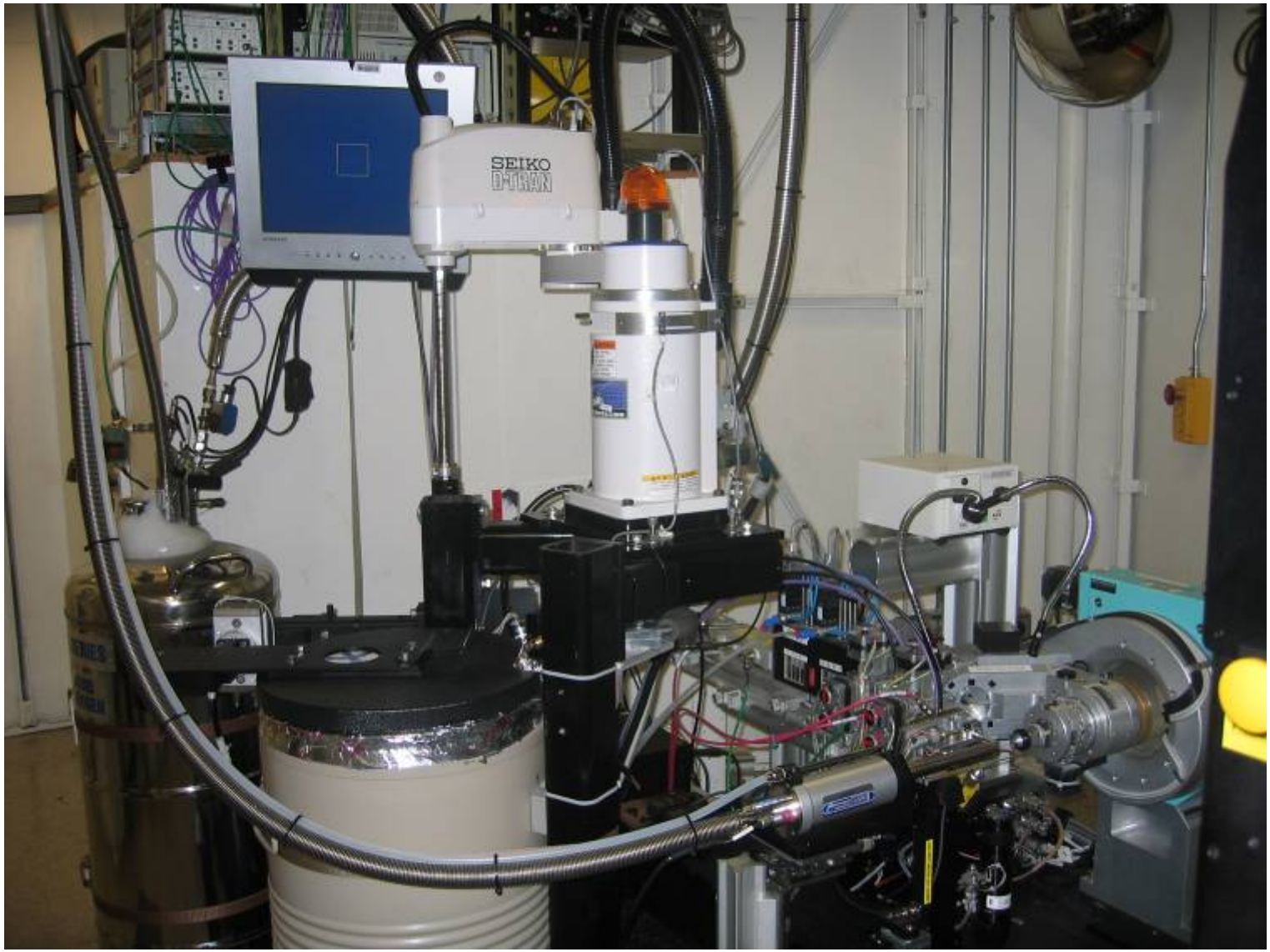
Double tongs crystal exchange on PF BL-17A

Trip to the SSRL (44 hours on 25, 26 July, 2005)

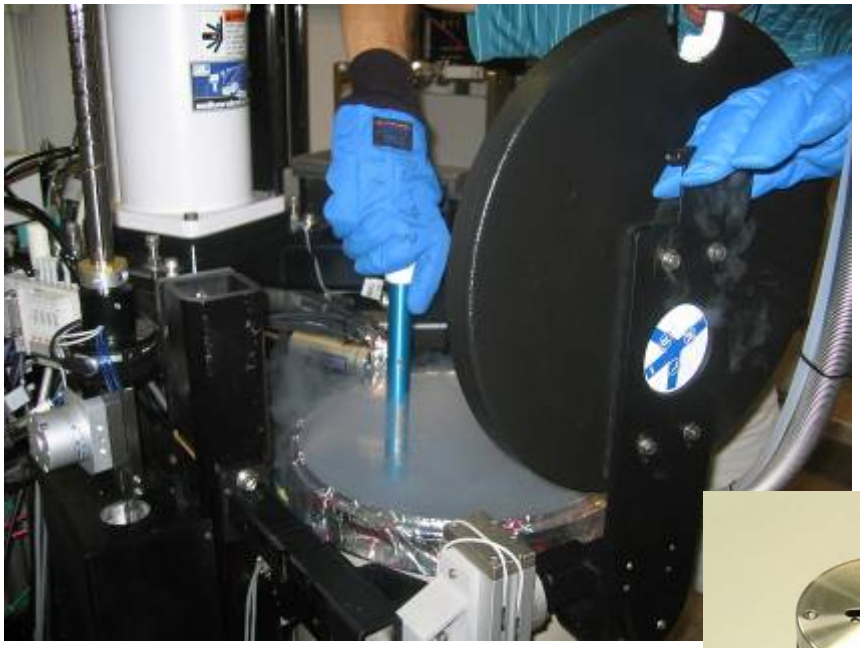
- 59 crystals frozen into a cassette, FedExed to the SSRL in a dry shipper prior to the beamtime on BL9-2.
- 9 data sets including 2 MAD data sets. One structure solved during the experiment.

- Fucosidase project in collaboration with Prof. Kenji Yamamoto, Kyoto University
- Fucosidase: 1959 amino acids, molecular weight 200,000
  ⇒ Catalytic core: 899 residues.
- A first-year master course student came to PF at the end of May, had obtained 1.2 Å resolution native data at PF.
- At the SSRL, we obtained 3.0 Å MAD and 1.6 Å SAD data sets. The MAD data was used to solved the structure while we were still at the beamline.
- Out of 1798 a.a. in a dimer, 1730 residues are assigned in 18 peptides, out of which 1721 a.a. match the sequence.

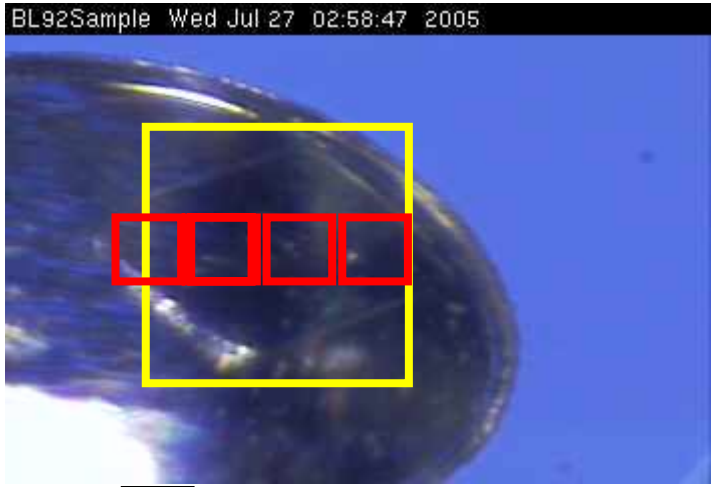
Multipole Wiggler Beam Line BL9-2 of SSRL, Stanford, USA



Crystal cassette: MPW MAD beamline BL9-2 of SSRL

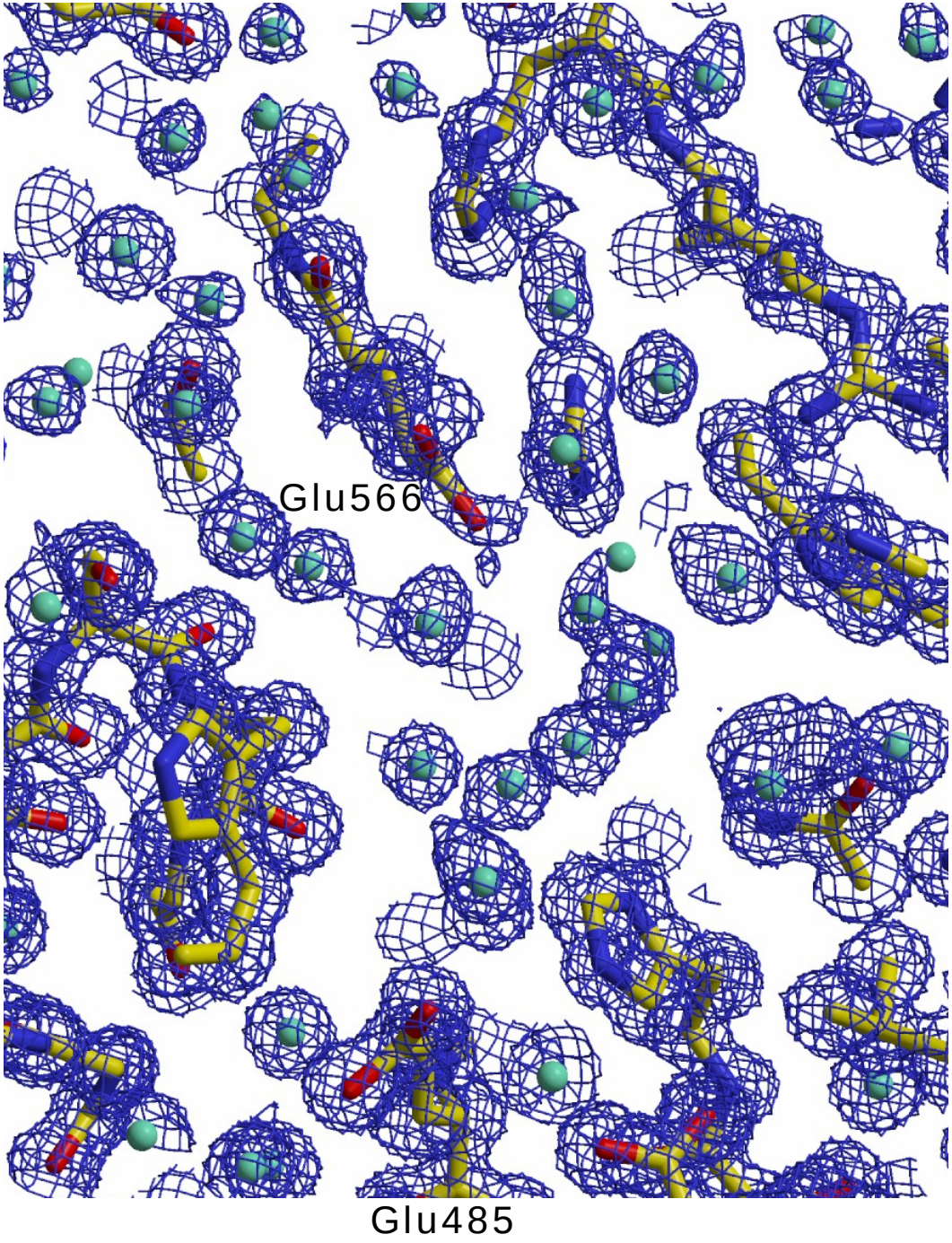


Searching the best spot on multi-crystals of fucosidase



50 μm square


MAD Phasing of Mb and SP18 with Kr

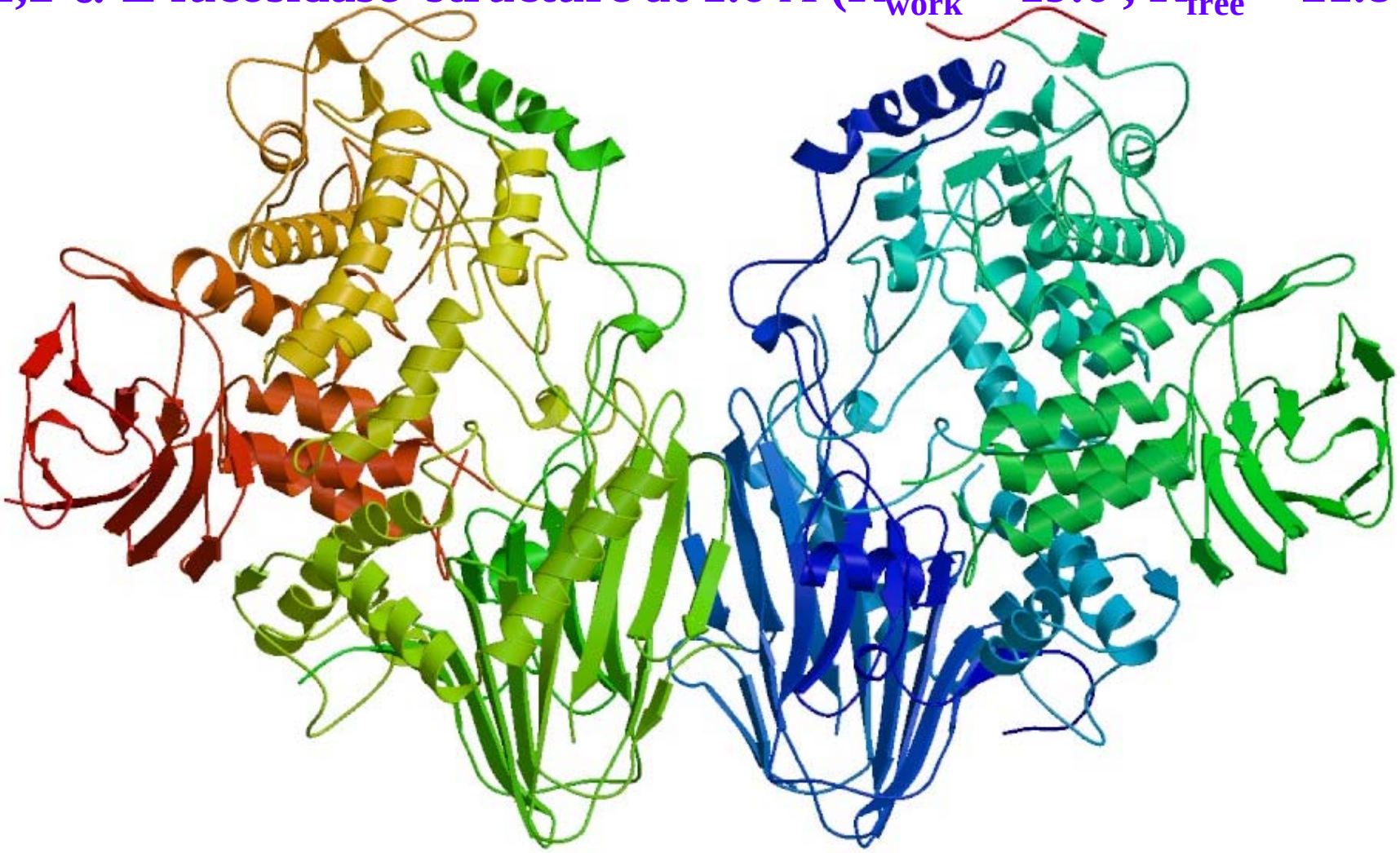


1.12Å electron density map

Active site of fucosidase

Collaboration with Prof. Kenji Yamamoto, Kyoto University

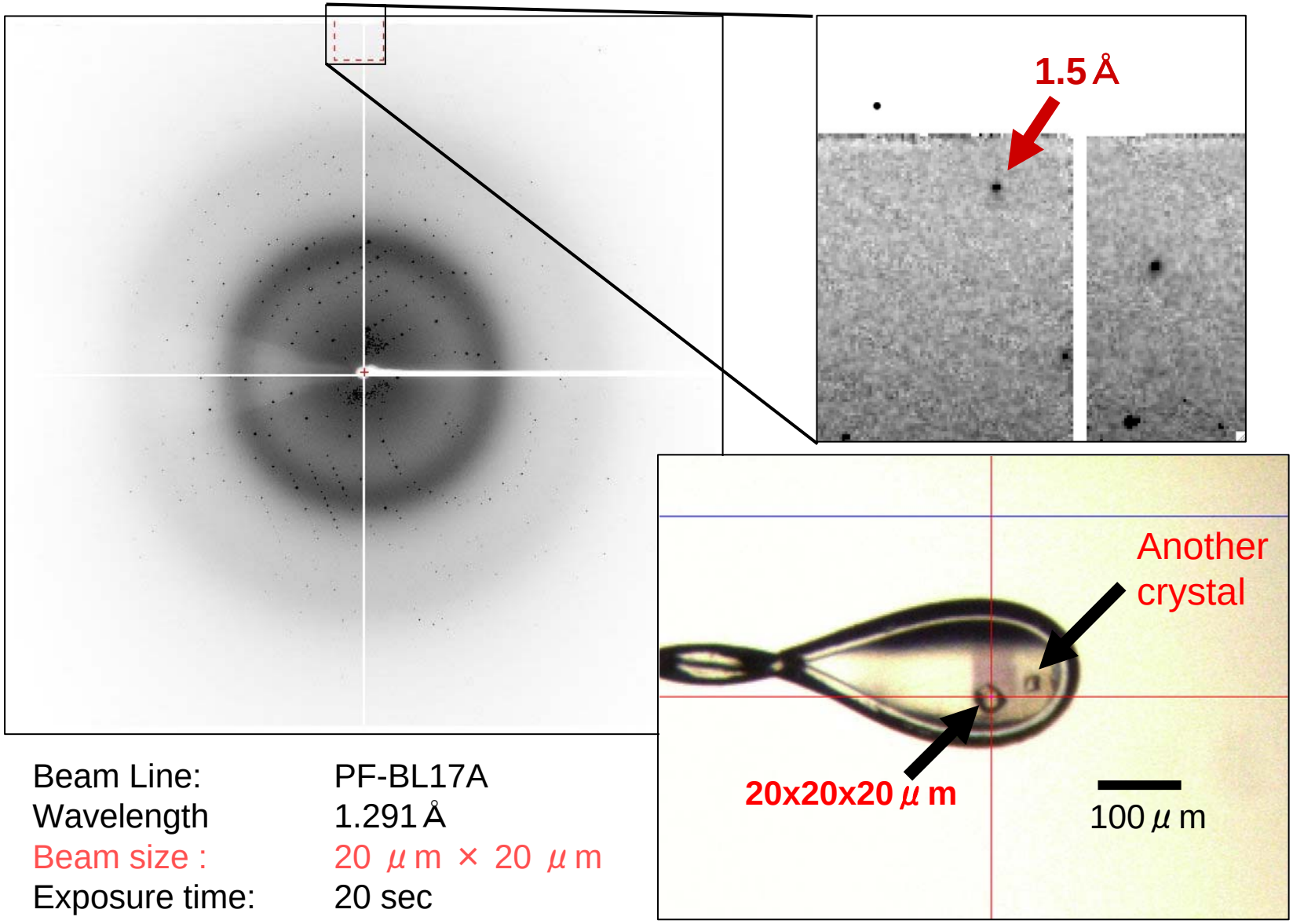
1,2-α-L fucosidase structure at 1.6 Å (R_{work} = 19.6 , R_{free} = 21.8)



Crystallographic project started in May 2005, and the 899 a.a. dimer structure determined in August the same year.

NEW BL17A short gap undulator beam line: Example of small crystals

(funded by JST Frontier Technology Development Project)

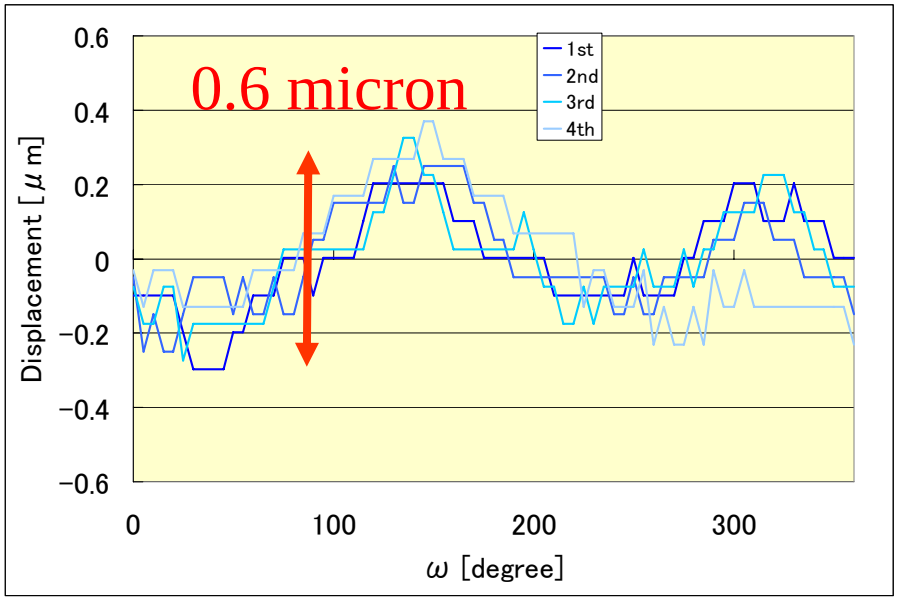


Beam Line:	PF-BL17A
Wavelength	1.291 Å
Beam size :	20 μm × 20 μm
Exposure time:	20 sec

Provided by Dr. Tadanobu Tanaka of Showa University

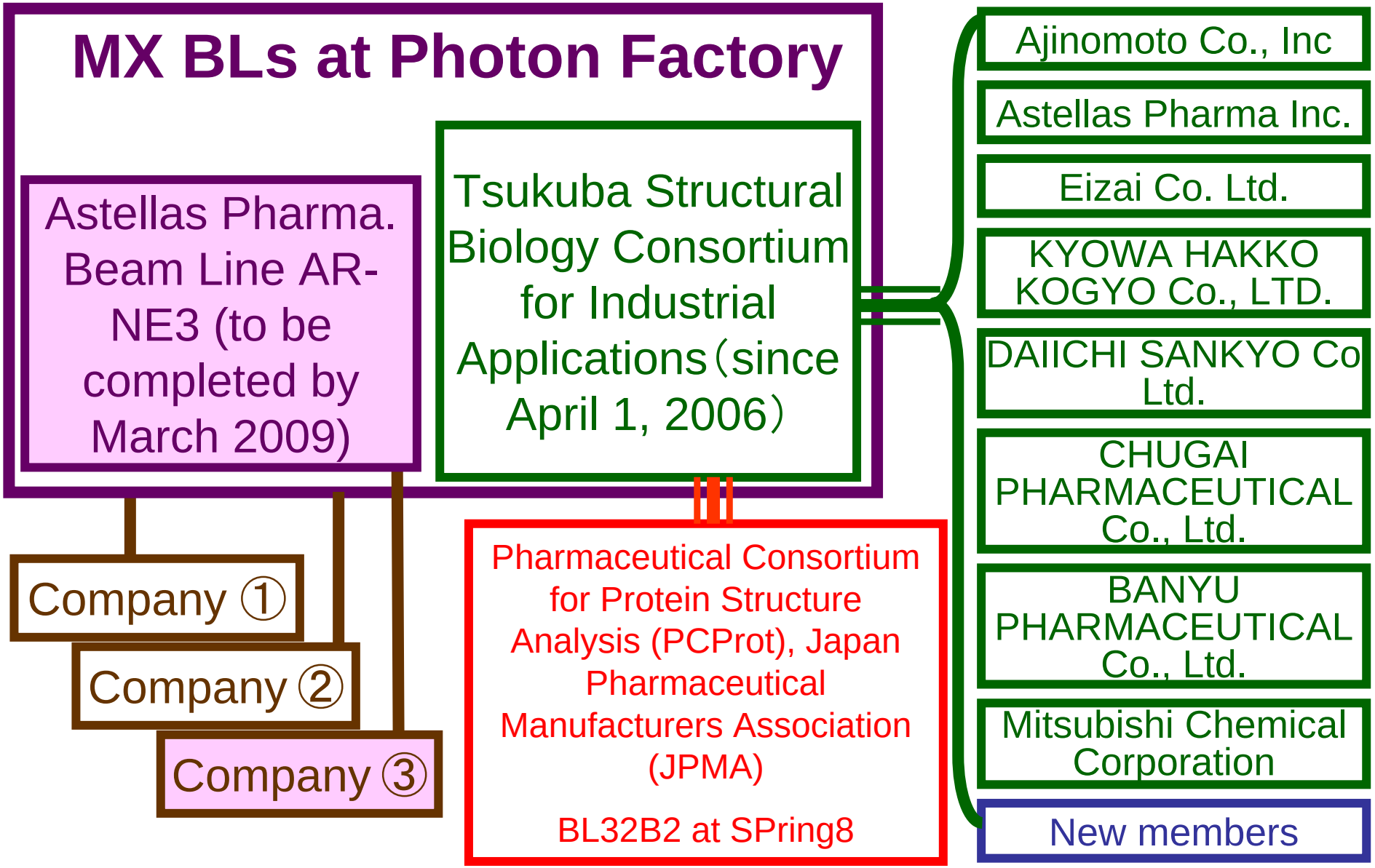
High precision one axis diffractometers with XYZ stages

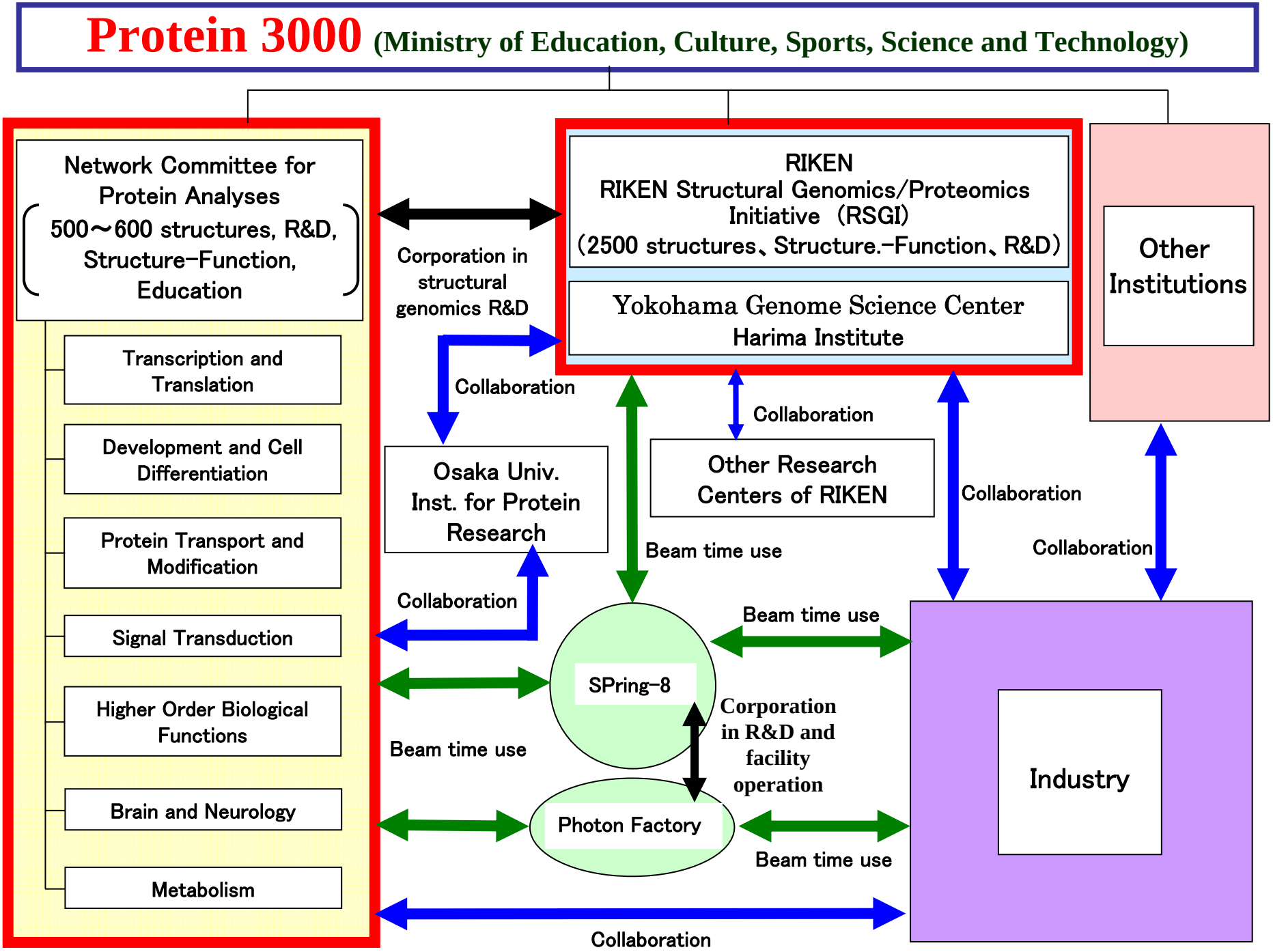
BL	BL-6A	NW12	BL-5	BL-17
Year started	2000*	2003	2004	2006 ⇒ 2009
Max deviation（μm）	10	2.2	1.0	0.6⇒ 0.1
Xtal size（μm）	100	22	10	6 ⇒ 1



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

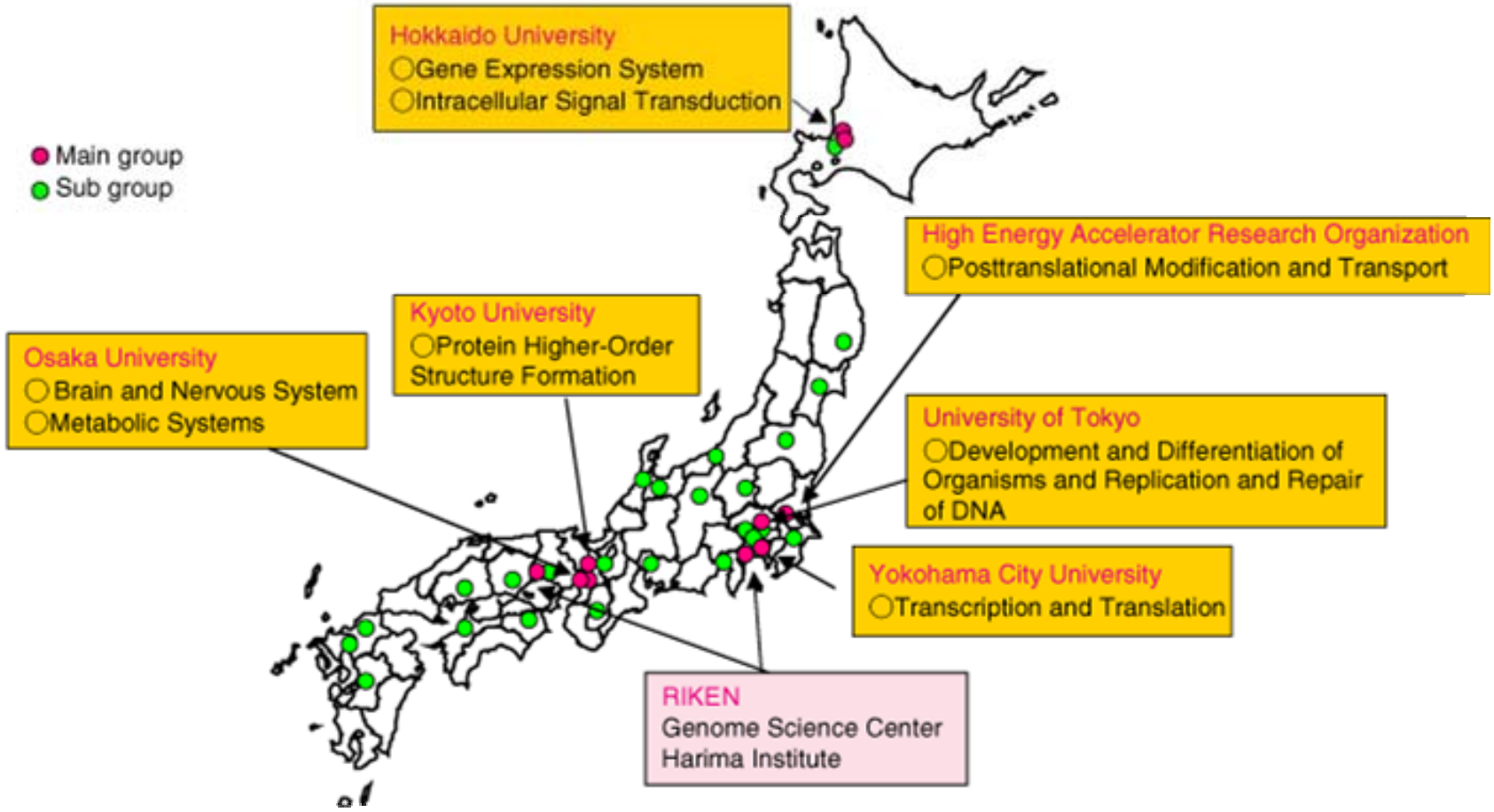
Industrial Use and Collaborations between KEK and Industry (~8% of beamtime)




Protein 3000 (Ministry of Education, Culture, Sports, Science and Technology)
Network Committee for Protein Analyses
500～600 structures, R&D, Structure-Function, Education
Transcription and Translation
Development and Cell Differentiation
Protein Transport and Modification
Signal Transduction
Higher Order Biological Functions
Brain and Neurology
Metabolism
Corporation in structural genomics R&D
RIKEN
RIKEN Structural Genomics/Proteomics Initiative (RSGI)
(2500 structures、Structure.-Function、R&D)
Yokohama Genome Science Center
Harima Institute
Other Institutions
Collaboration
Collaboration
Osaka Univ. Inst. for Protein Research
Other Research Centers of RIKEN
Collaboration
Collaboration
Beam time use
Collaboration
Beam time use
SPring-8
Beam time use
Corporation in R&D and facility operation
Photon Factory
Beam time use
Industry
Collaboration

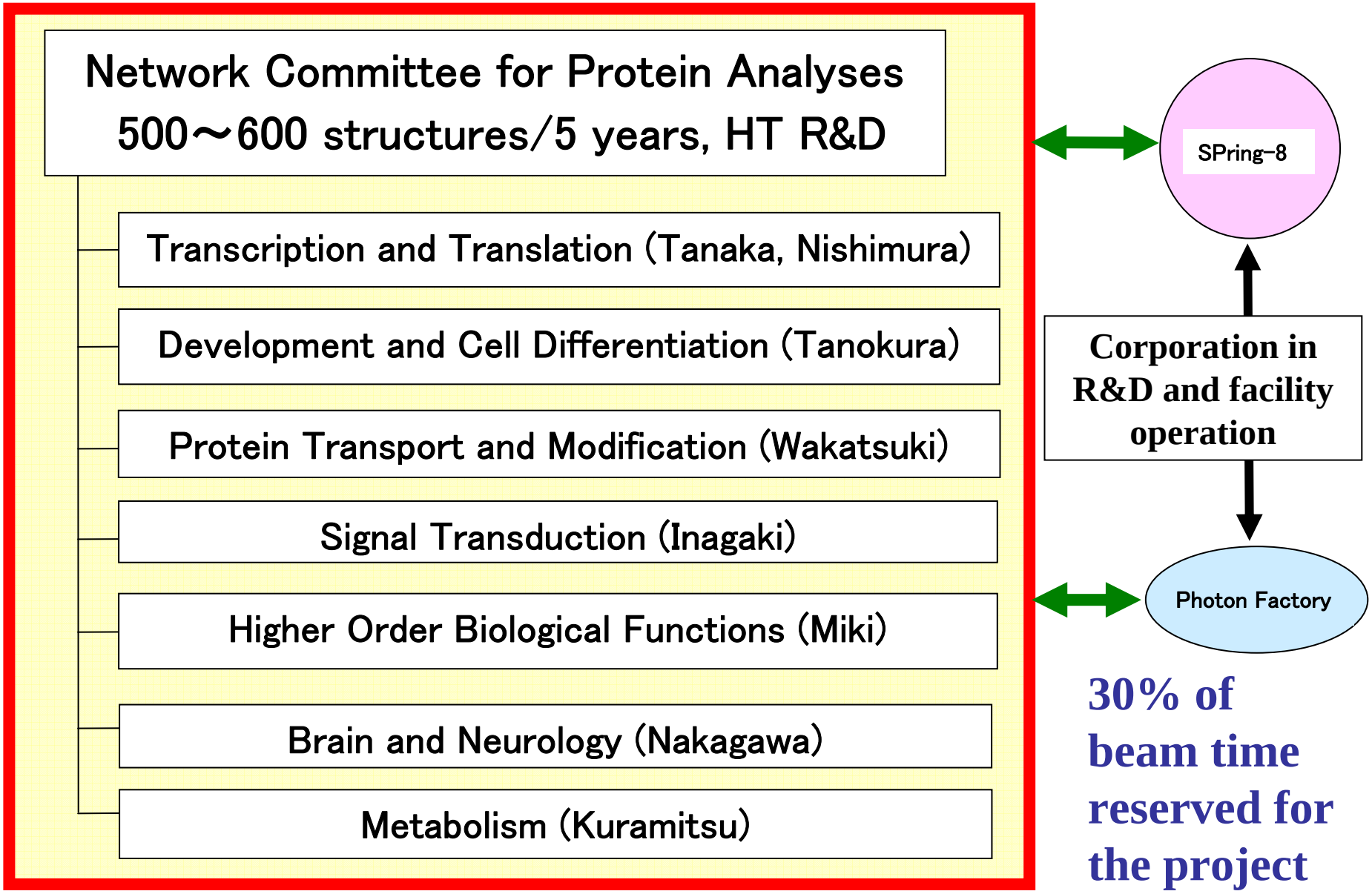
Protein 3000 (FY2002-2006)

National Project on Protein Structural and Functional Analyses

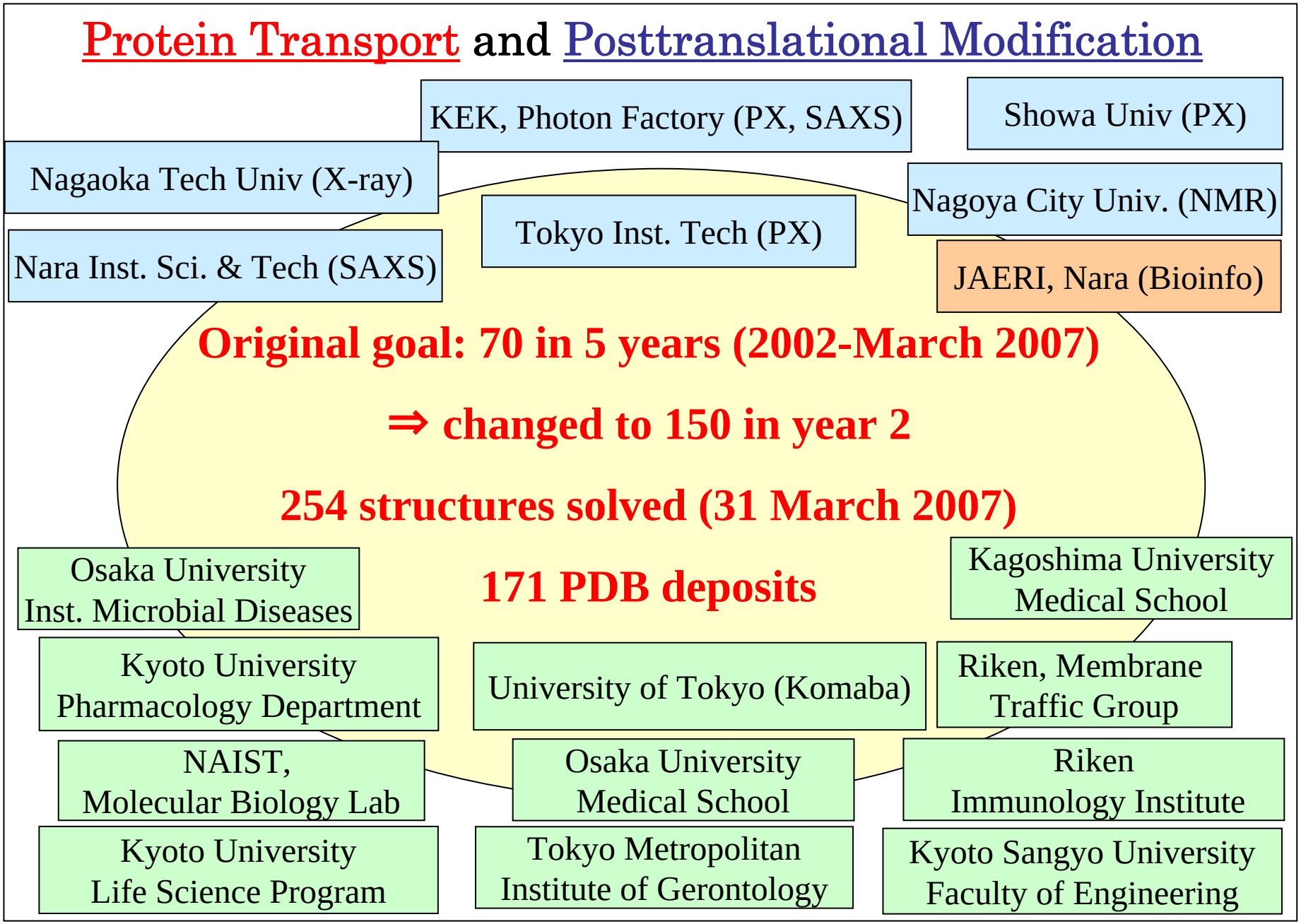


> 150 groups involved

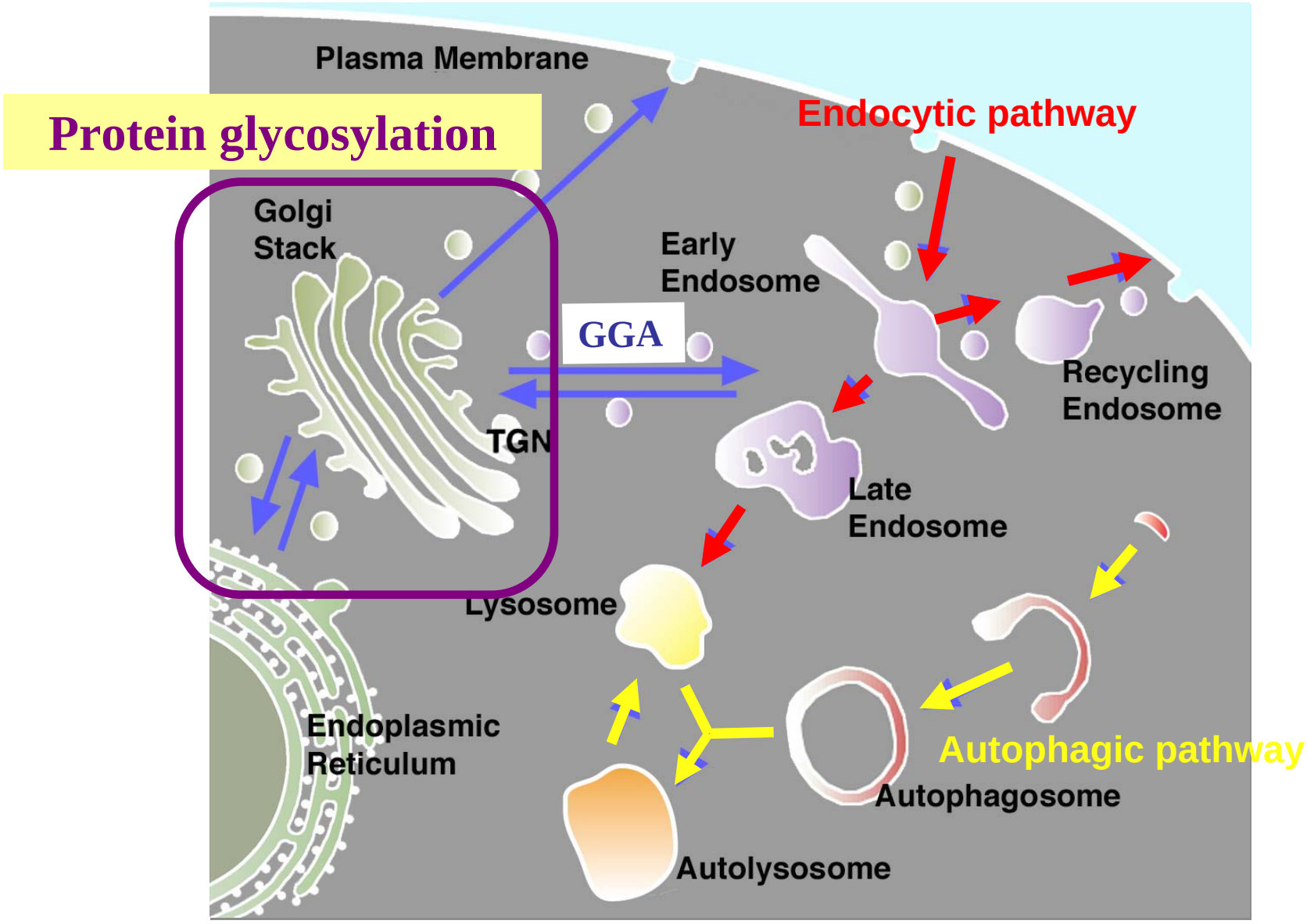
8 Consotia: Target oriented structural genomics of Protein 3000 (FY2002-2006)



Protein 3000 Tsukuba Structural Biology Consortium (21 groups)

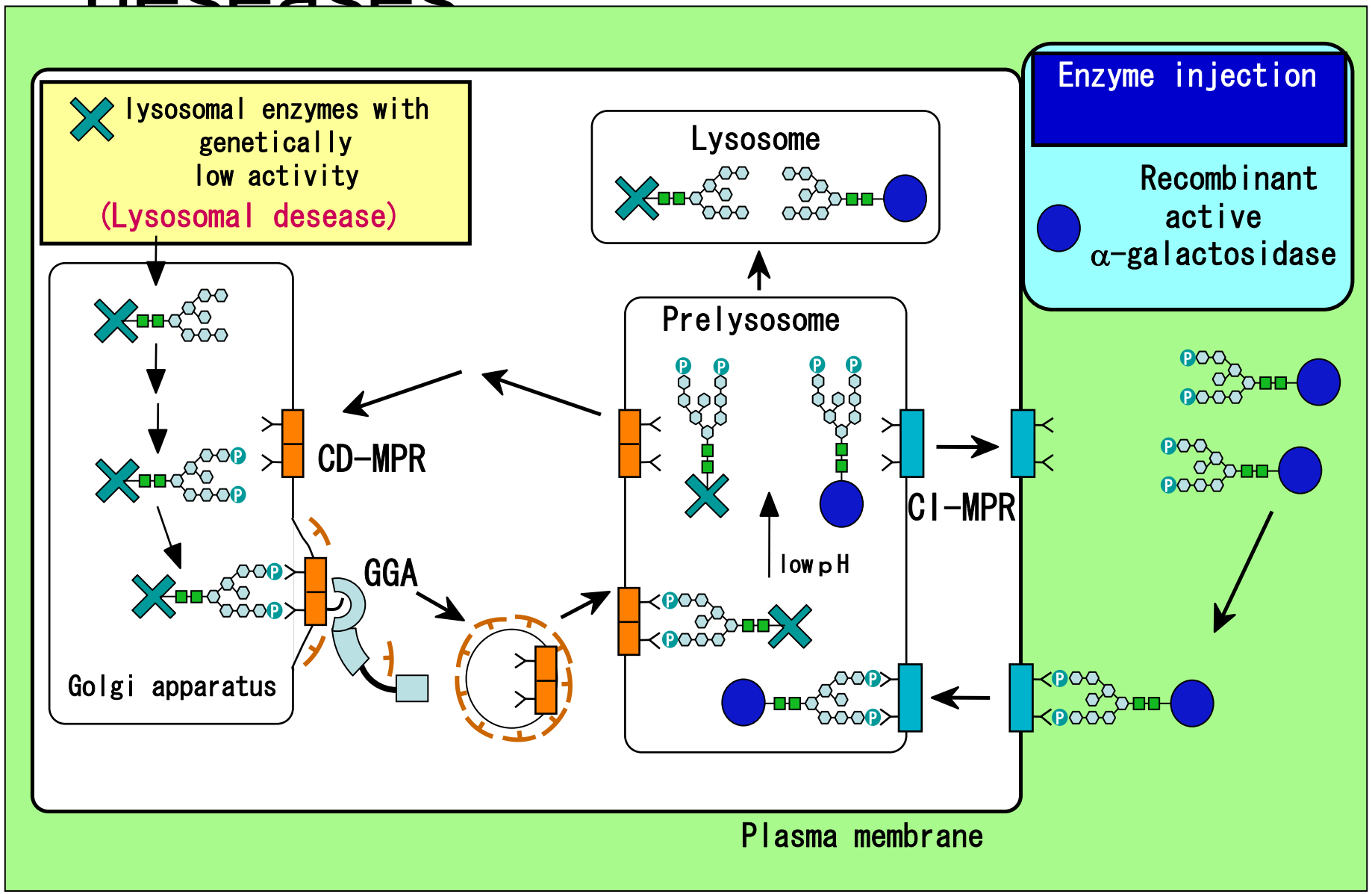


Protein glycosylation and transport



T. Yoshimori

Treatment of lysosomal deseases

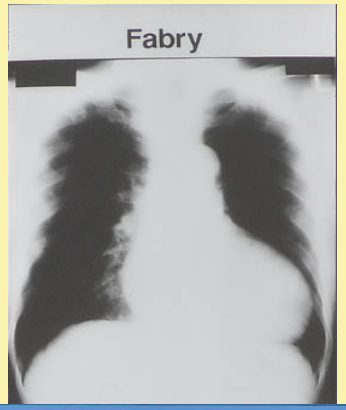


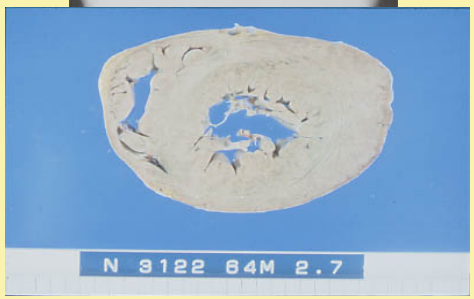
Collaboration outside of Japan

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. **ESCRT proteins: 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)

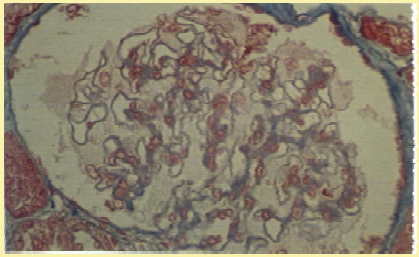
Fabry Disease and Enzyme Replacement Therapy

Fabry disease：A disease caused by mutation of α-galactosidase gene, which degrades enzymatic activity of the hydrolase in lysosome leading to accumulation of glycolipids

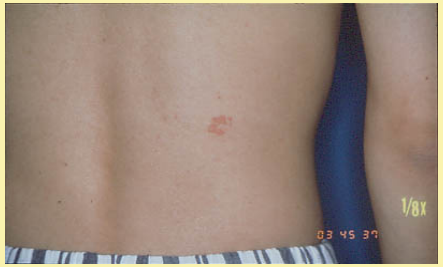




Enlargement of the heart

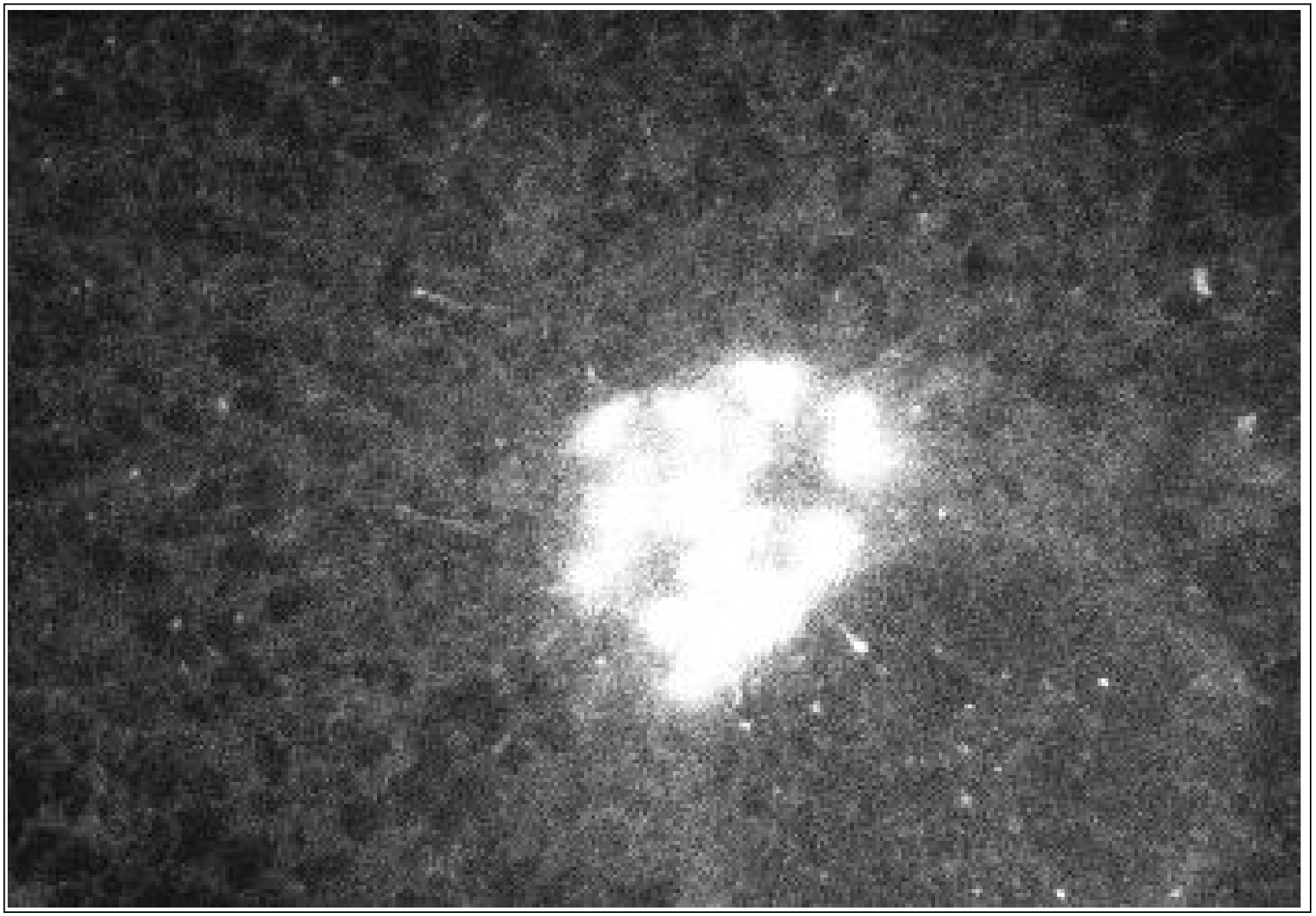


Accumulation of glycolipids in glomerulus

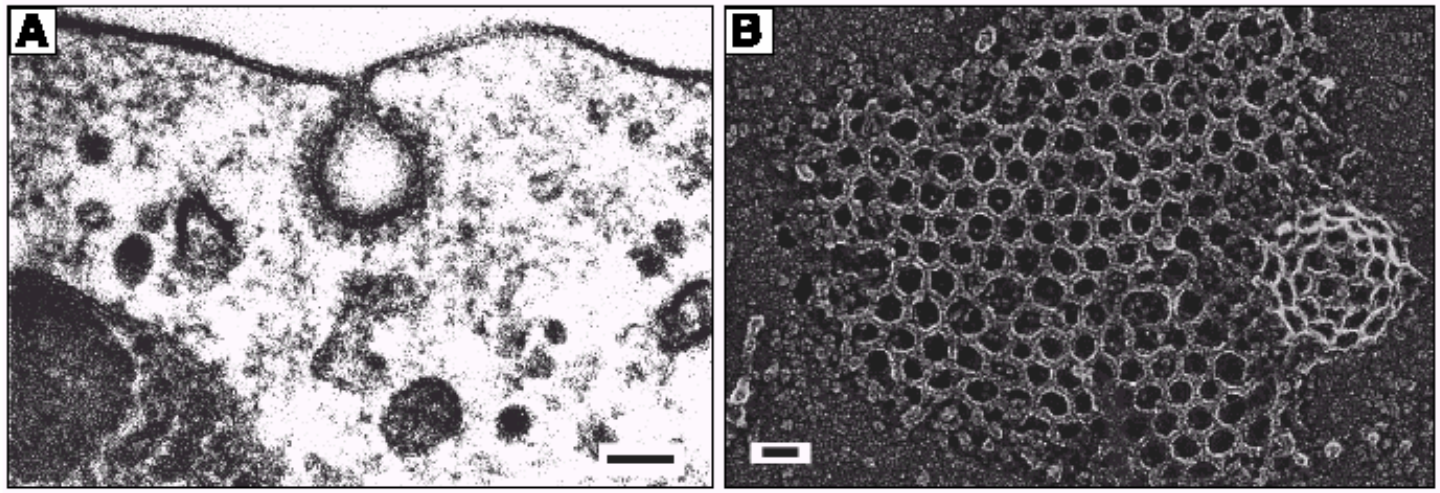


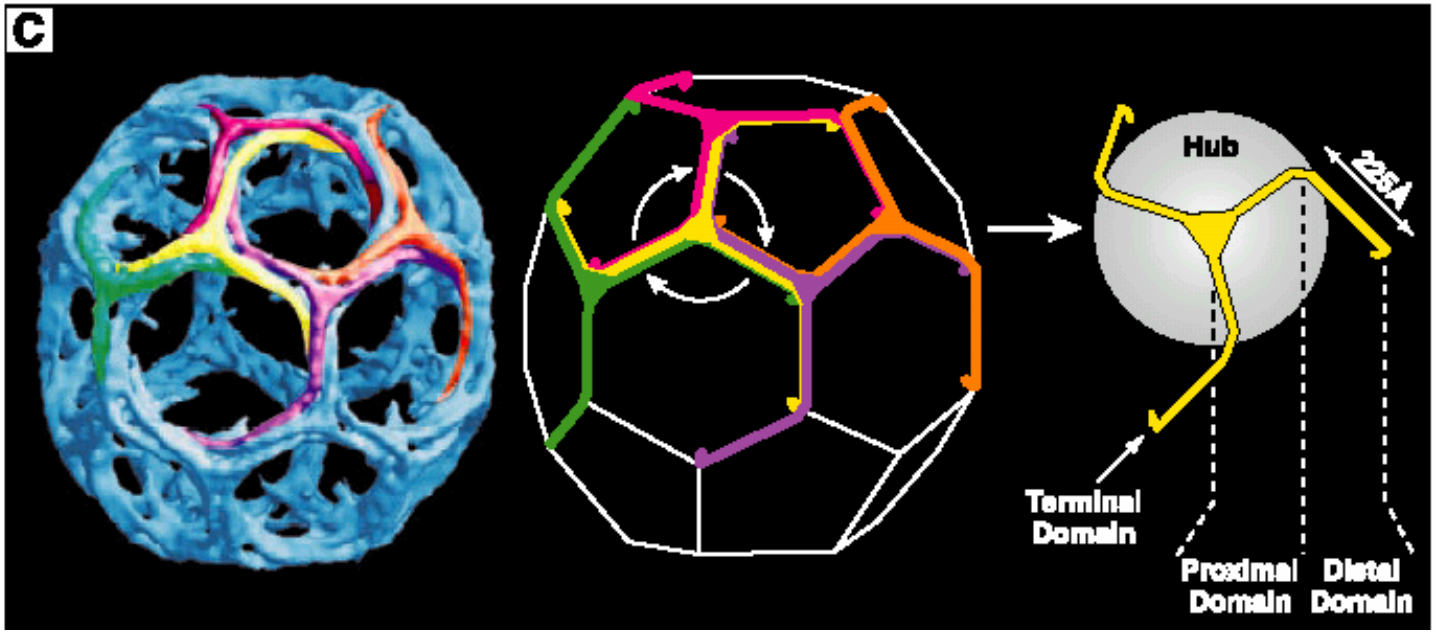
Angiokeratoma

Vesicle transport from the ER to the Golgi apparatus



Lippincott-Schwartz, J. (1998) MBC 9, 1617



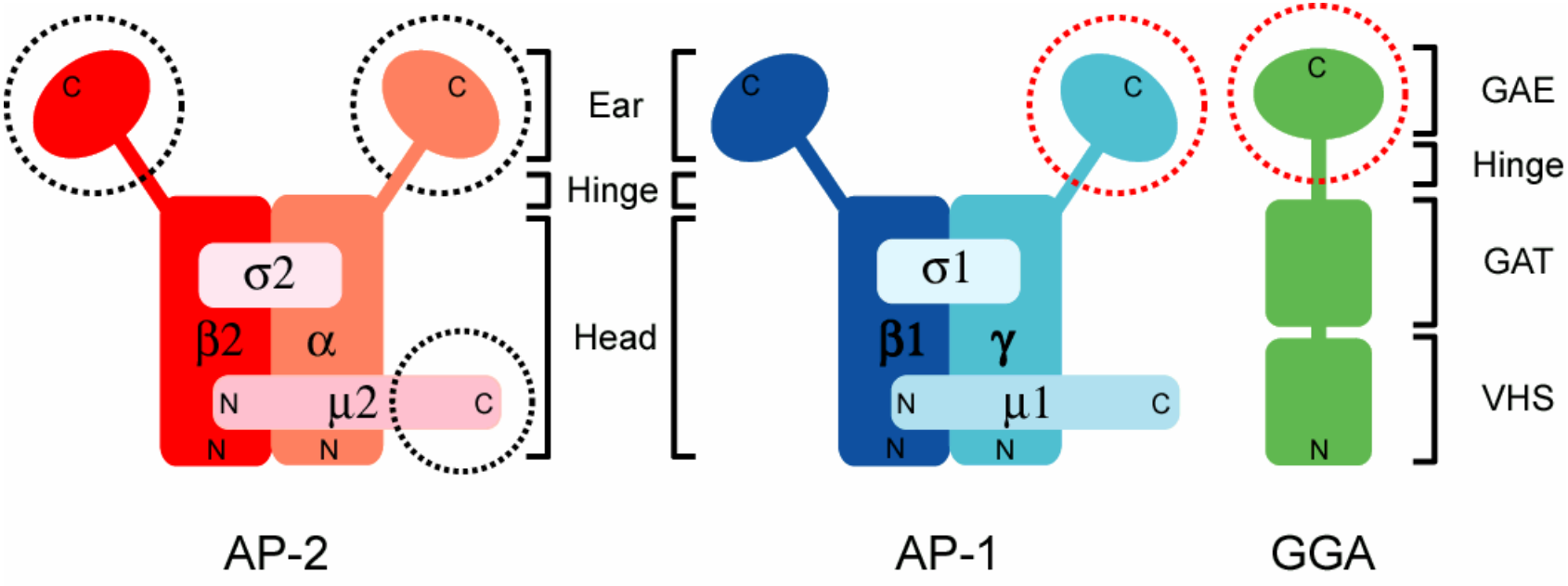


Clathrin movie by Allison , Harvard University

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

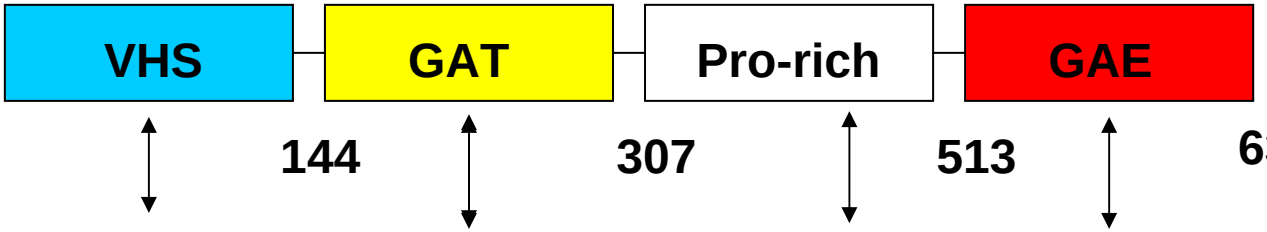
http://www.hms.harvard.edu/news/clathrin/

Two families of adaptor proteins: AP and GGA



Partners of human GGA1

The list is still growing!



Packaging of cargo by recognizing acidic di-leucine motifs:

- Mannose 6P receptors
- β-secratase
- Sortilin
- LRP

ARF-GTP (membrane docking)

- Rabaptin-5
- Ubiquitin

Clathrin cage formation

- GGA-VHS (auto-inhibithion)
- GGA-GAE
- γ-ear

Accessory/ regulatory proteins

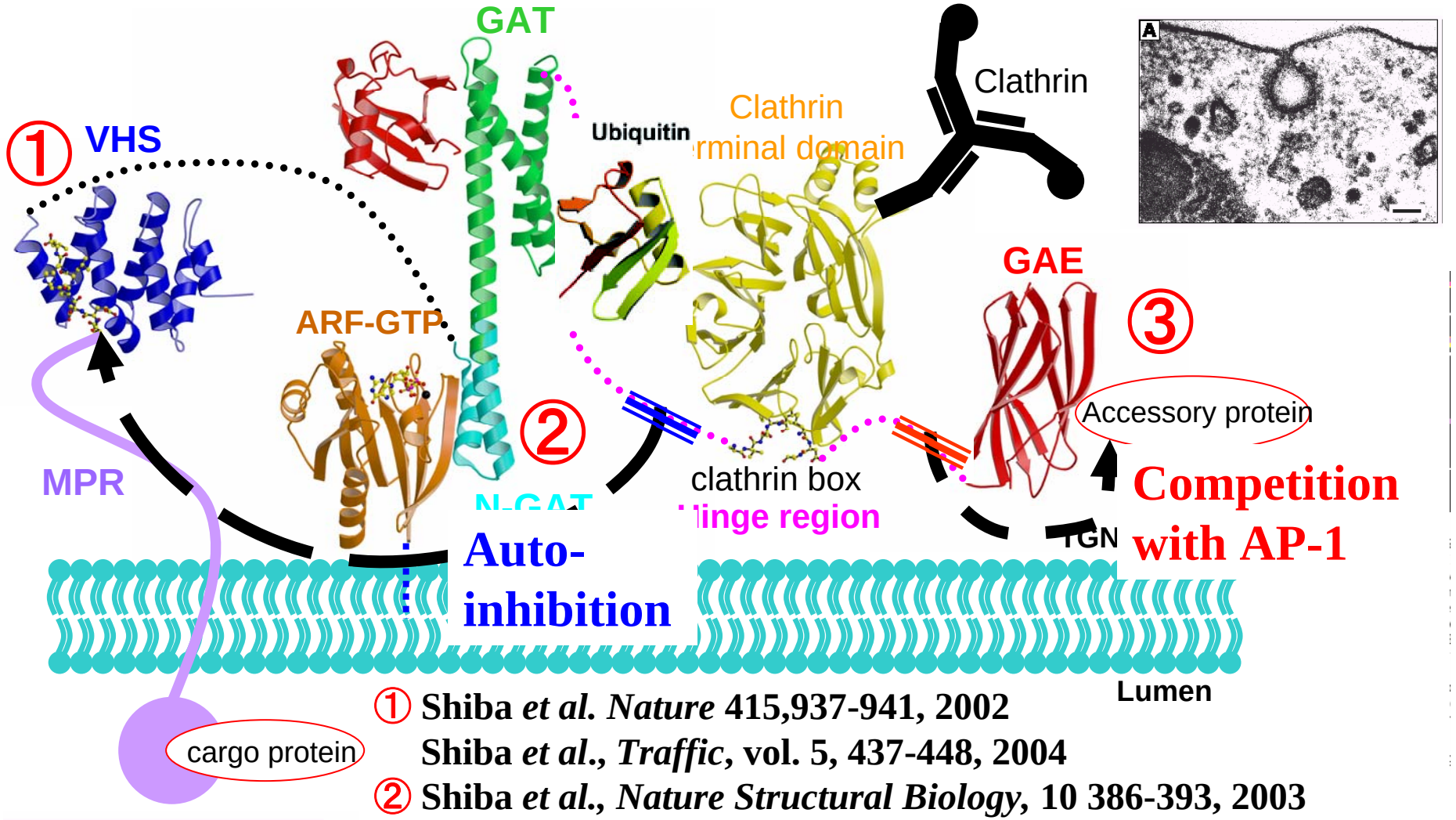
- Rabaptin-5
- γ-synergine
- Eps15
- EpsinR
- p56
- GGA-hinge

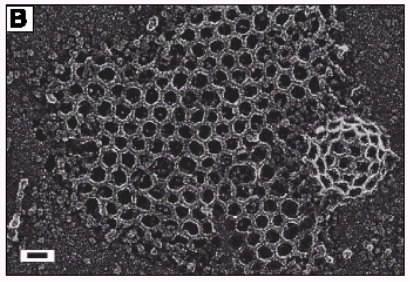
VHS: Vps27p/Hrs/STAM Domain

GAT=GGAH: GGA Homology Domain

GAE=AGEH: Adaptor γ Ear Homology Domain

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

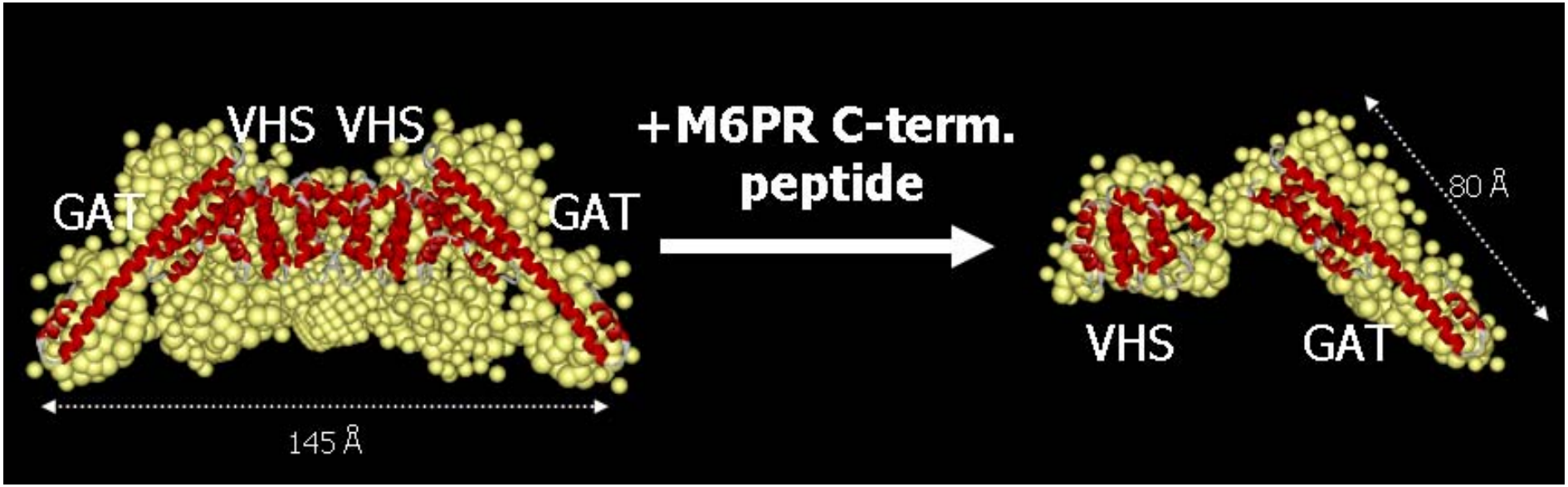
③ Nogi *et al. Nature Structural Biology*, 9, 527-531, 2002

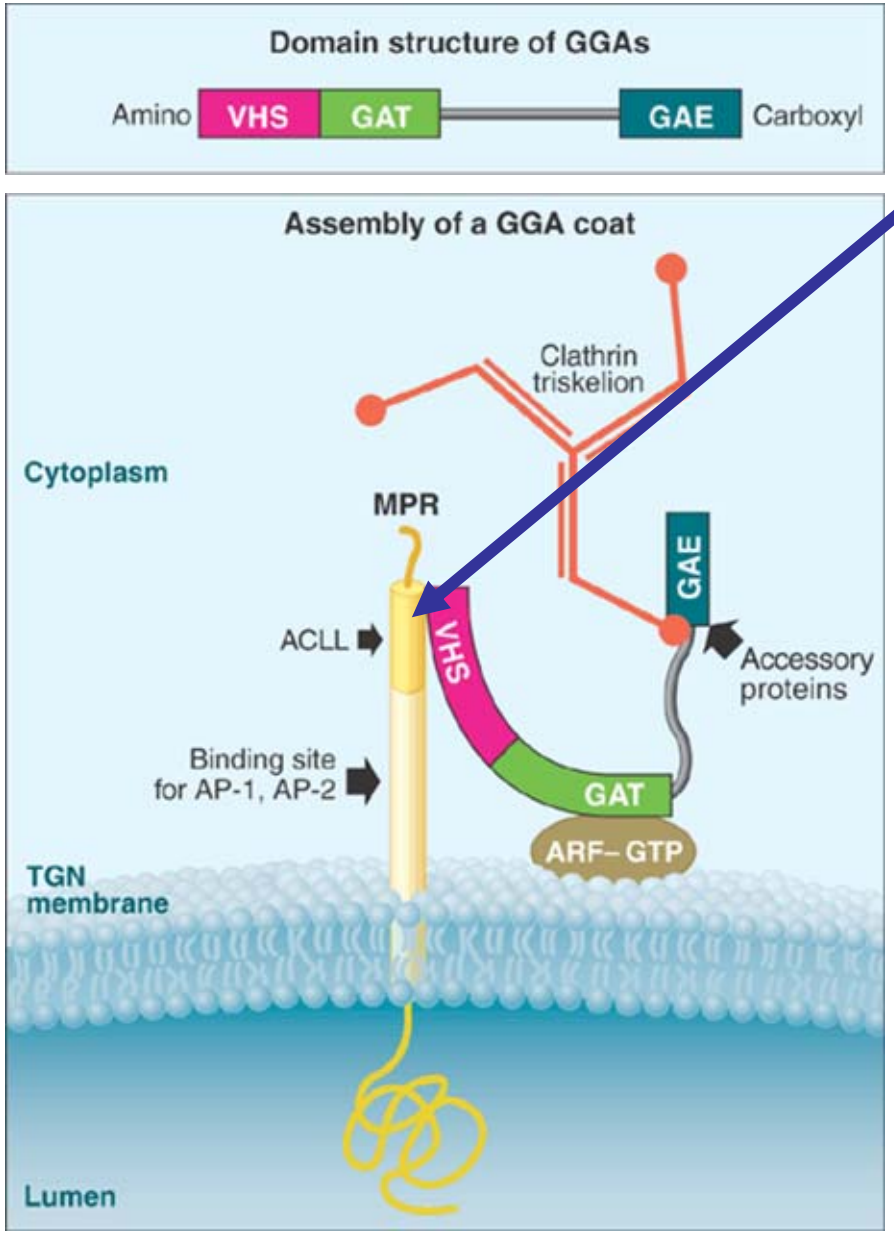
Inoue *et al. Traffic*, 8, 904-913, 2007

Structural changes of the domain arrangement of VHS－GAT domain upopn mannose 6-phosphate receptor C-term peptide binding using **small angle X-ray scattering**

(H. Kamikubo, M. Kataoka et al., in preparation)

Data collected on PF-BL10C





ACLL (acidic dileucin) motif

ACLL Peptides recognized by GGA1-VHS domain

LRP3	-MLEASDDEALLVC
CD-MPR	-EESEERDDHLLPM
CI-MPR	-SFHDDSDEDLLHI
Sort(WT)	-GYHDDSDEDLLE
Sort(DD/NN)	-GYHNNSDEDLLE
Sort(S/A)	-GYHDDADEDLLE
Sort(S/D)	-GYHDDDDEDLLE
Sort(DED/NQN)	-GYHDDSNQNLLE
Sort(LL/AA)	-GYHDDSDEDAAE
β-secretase	-QHDDFADDISLLK

Red: acidic residues

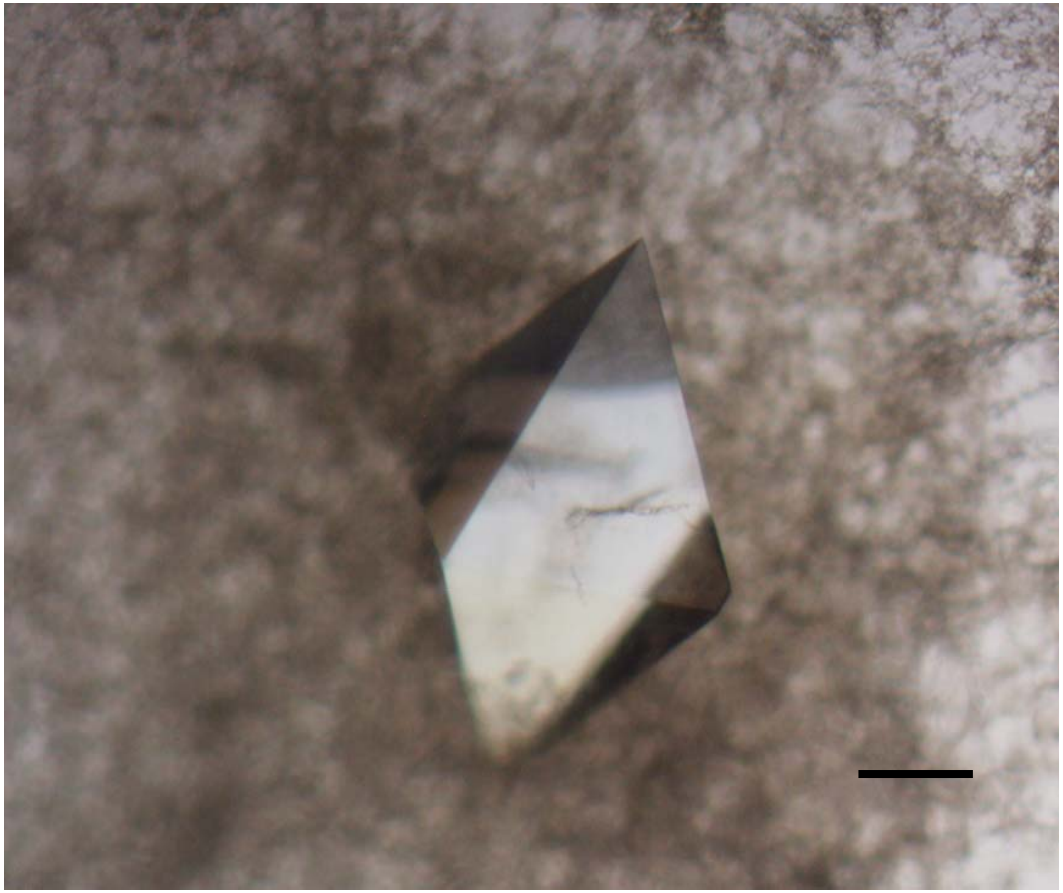
Blue: leucine pairs

Purple: serine residues that can be phosphorylated by CK-II

Takatsu et al, J. Biol. Chem. 276, 28541-28545

From S. A. Tooze, Science, vol. 292, 1 June, 2001

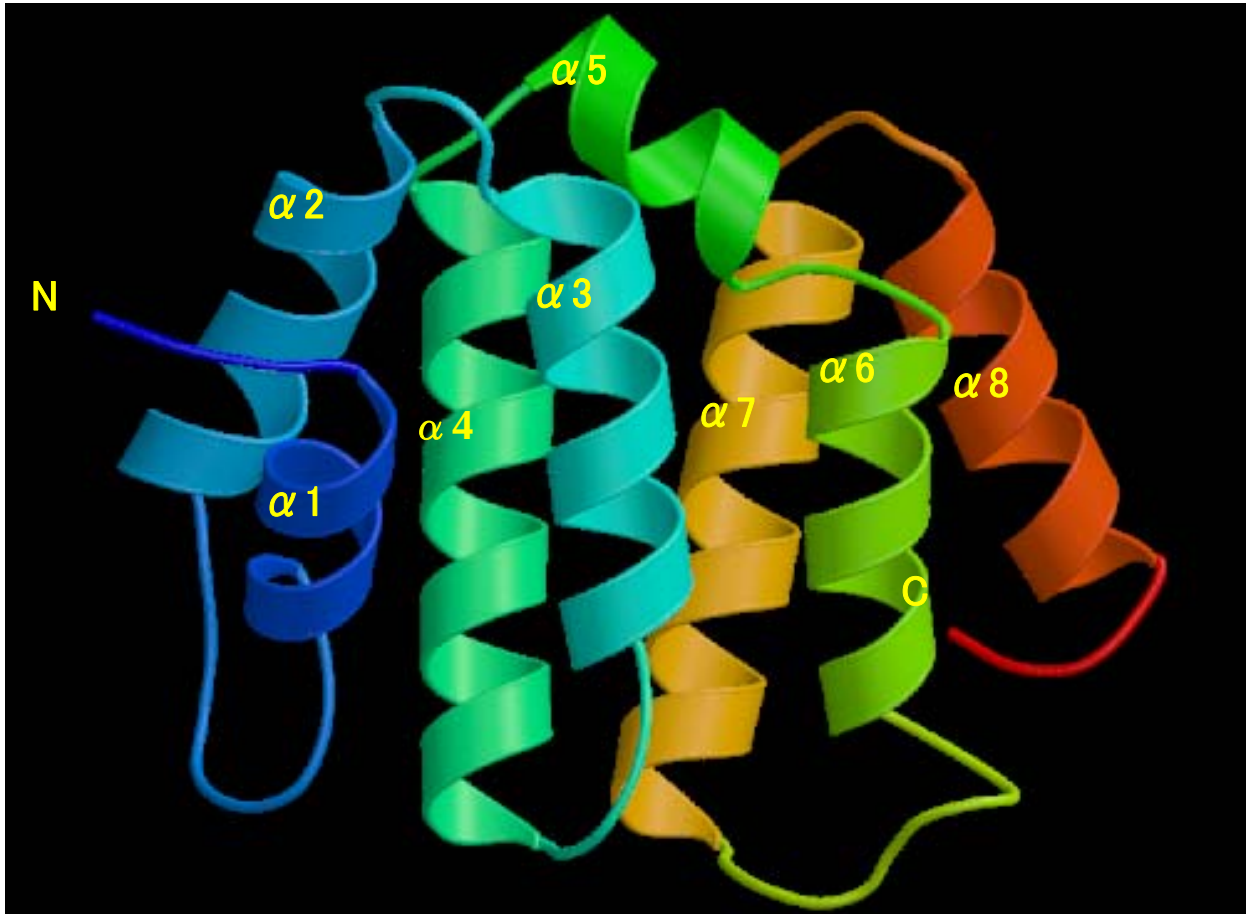
Crystal of Human GGA1 VHS domain



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 ºC**

2.1A structure of the VHS domain of a human GGA protein in the apoform



Protein preparation started on 23 April, 2001

Monday 5 PM, 13 August, complex crystals FedExed to ALS

Wednesday 1 PM, 15 August, 1.8A data set collected at ALS!

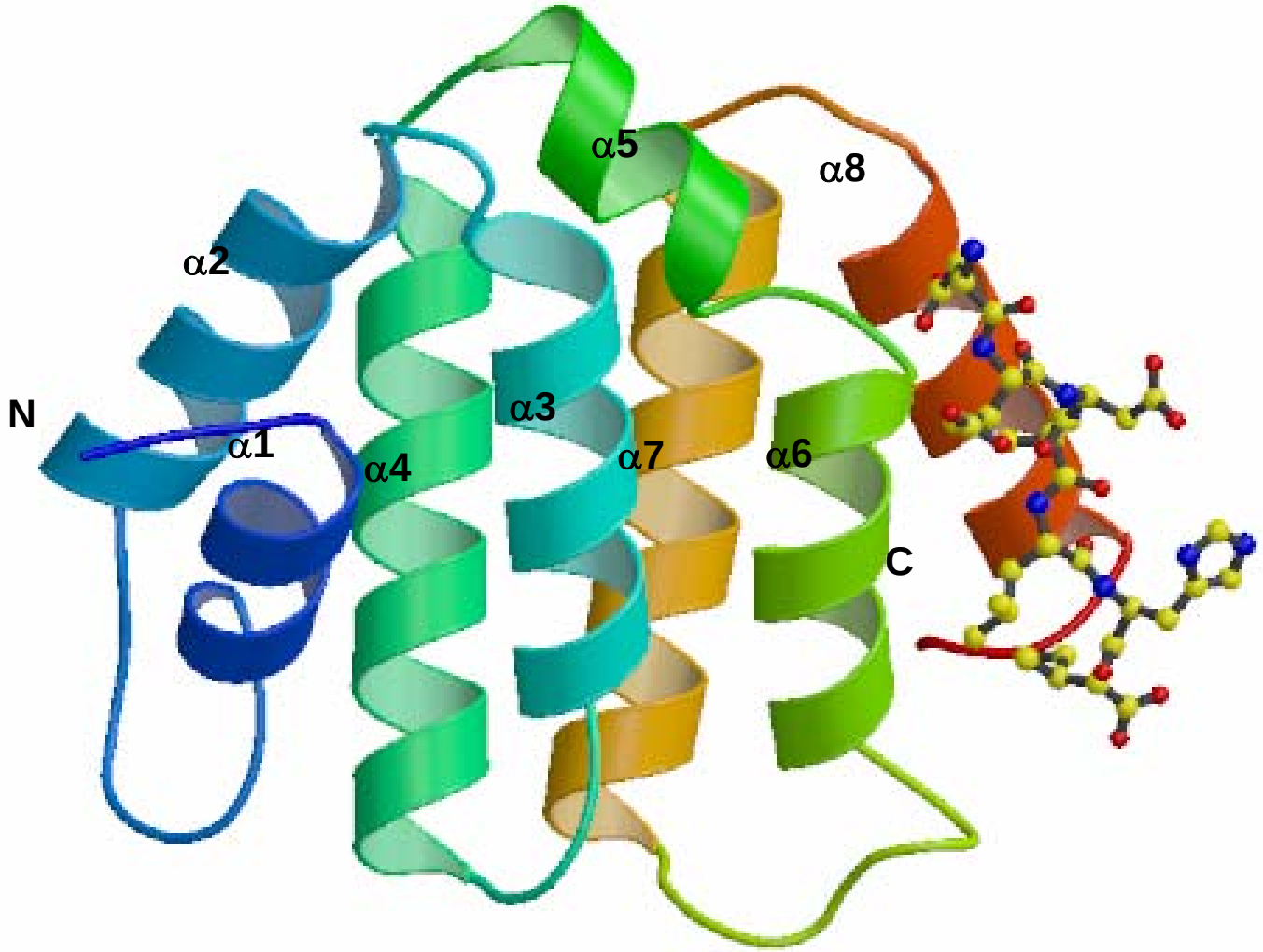
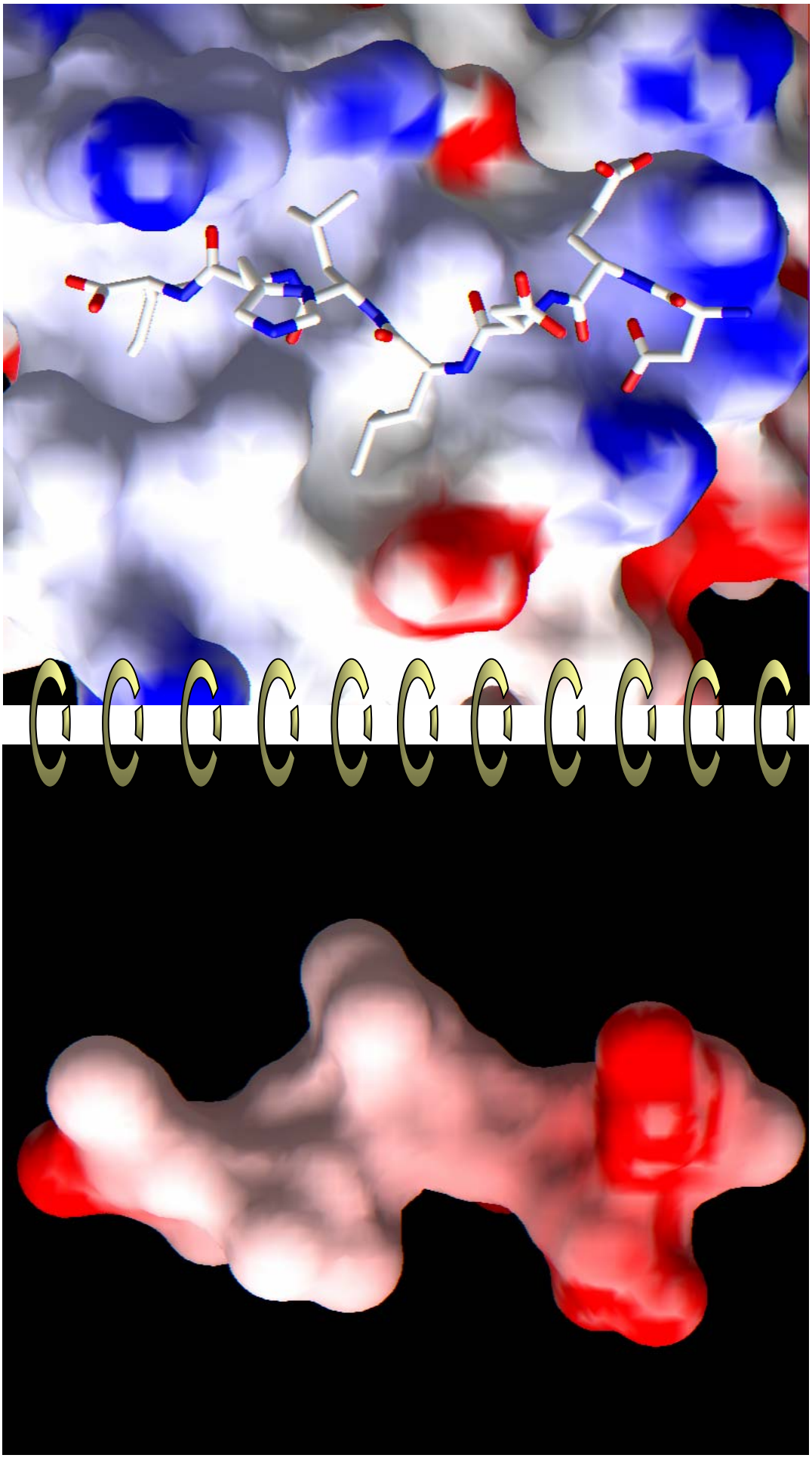
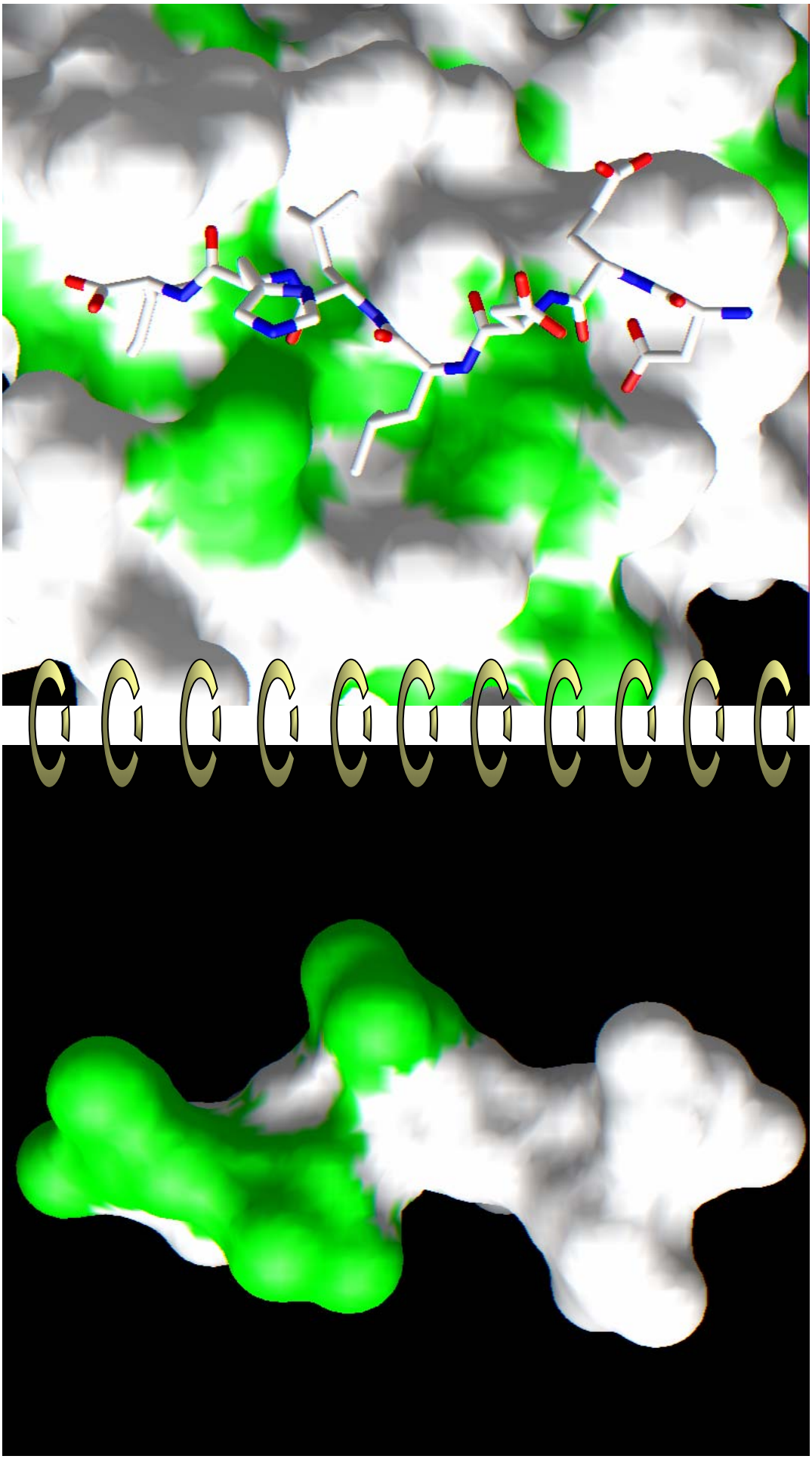


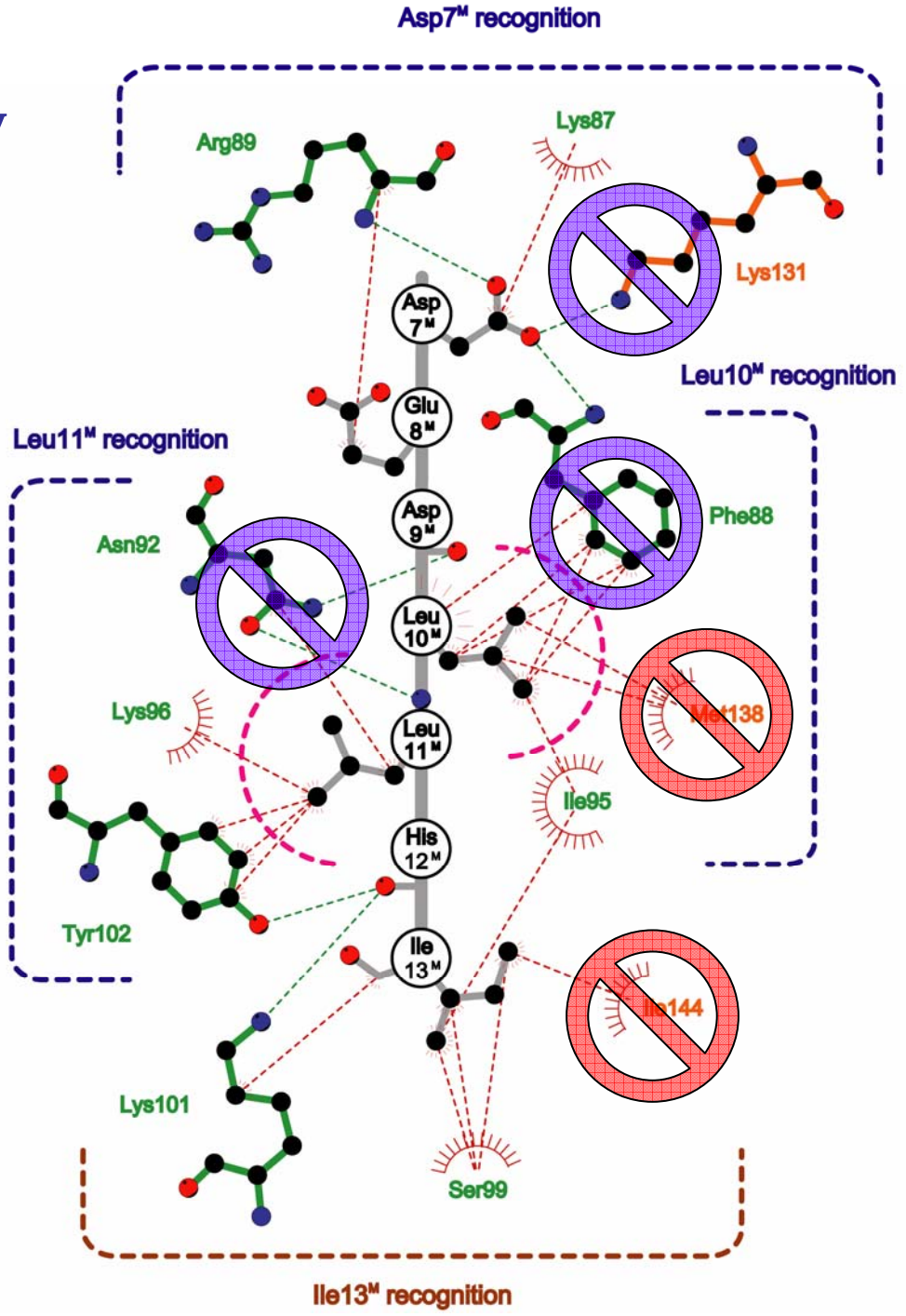
Fig.1
Ribbon diagram of VHS domain of human GGA1 complex with M6PR peptide. The peptide molecule is shown as a ball-and-stick model colored according to atom type (nitrogen, blue; carbon, yellow; oxygen, red).




GCCCCCCCCCCC

M6PR recognition by GGA1-VHS

The two-hybrid experiments of the mutants confirm the importance of specific interactions between the peptide and the protein.



NATURE |VOL 415 | 21 FEBRUARY 2002

Structural basis for acidic-cluster-dileucine sorting-signal recognition by VHS domains

Saurav Misra*, Rosa Puertollano†, Yukio Kato†, Juan S. Bonifacino† & James H. Hurley*

** Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892, USA*
† Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892, USA

pp 933-937

Structural basis for recognition of acidic-cluster dileucine sequence by GGA1

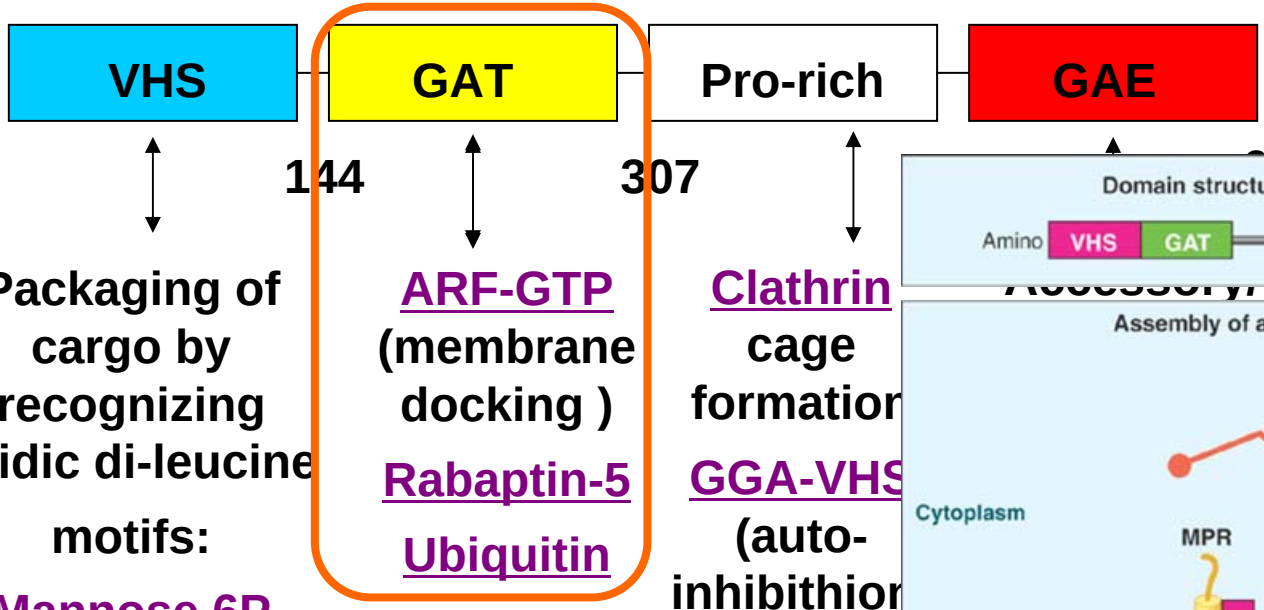
Tomoo Shiba*†‡, Hiroyuki Takatsu‡§, Terukazu Nogi*, Naohiro Matsugaki*, Masato Kawasaki*, Noriyuki Igarashi*, Mamoru Suzuki*, Ryuichi Kato*, Thomas Earnest‖, Kazuhisa Nakayama§ & Soichi Wakatsuki*

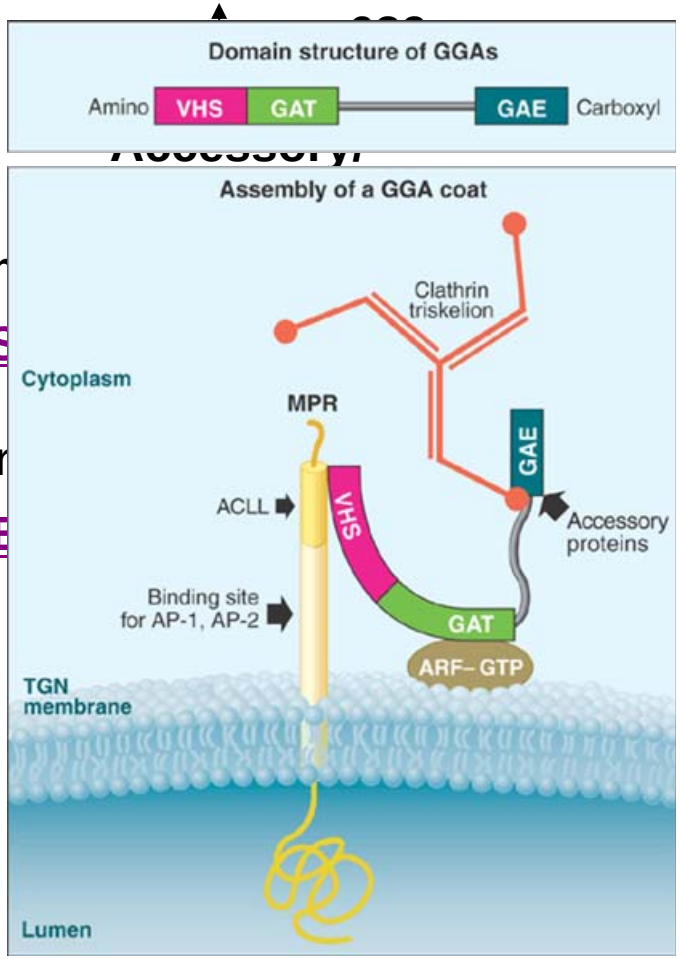
** Photon Factory, Institute of Materials Structure Science, High Energy Accelerator Research Organization (KEK), Tsukuba, Ibaraki 305-0801, Japan*
† Foundation for Advancement of International Science (FAIS), Tsukuba, Ibaraki 305-0062, Japan
§ Institute of Biological Sciences and Gene Research Center, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan
‖ Advanced Light Source, Berkeley, Berkeley Center for Structural Biology, Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, California 94720, USA
‡ These authors contributed equally to this work

pp 937-941

PART 3: Membrane anchoring:

Collection of adapter proteins to the TGN membrane surface

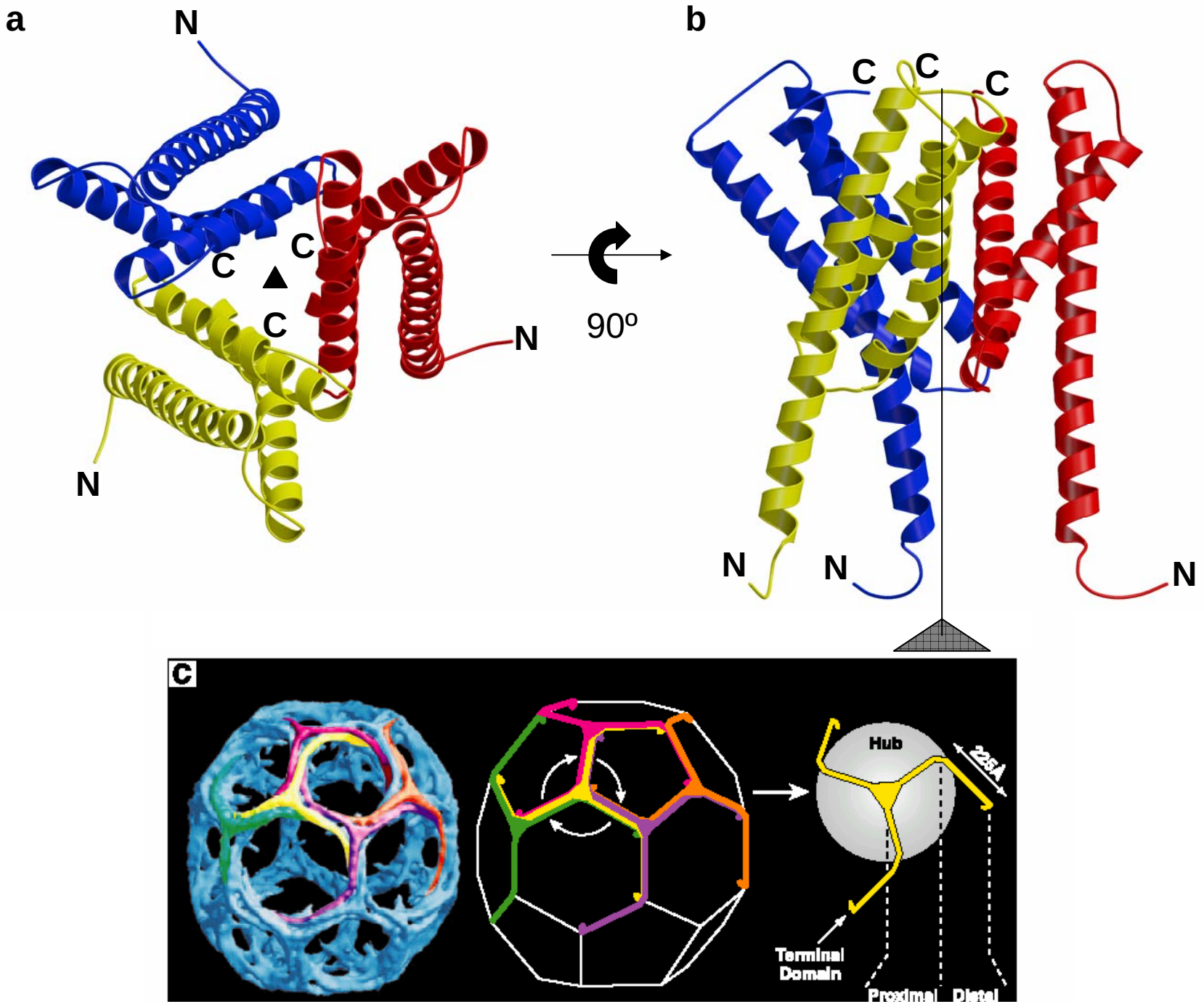




VHS: Vps27p/Hrs/STAM Domain

GAT=GGAH: GGA Homology Domain

GAE=AGEH: Adaptor γ Ear Homology Domain


a
N
C
C
C
N
N
90º
b
C
C
C
N
N
N
C
Hub
22Å
Terminal
Domain
Proximal
Distal

Four groups independently solved the structure of GGA1 GAT domain almost at the same time

Group	PDB	Resolution (Å)
KEK[1]	1J2H	2.1
Univ. of Cambridge[2]	1NAF	2.8
NIH[3]	1NWM	2.4
OMRF, Oklahoma[4]	1OXZ	2.8

2003, May [1]Shiba, T. *et al*. *Nature Struct. Biol*. **10**, 386-393

2003, March [2]Collins, B. M. *et al*. *Dev. Cell*, **4,** 321-332

2003, April [3]Suer, S. *et al*. *Proc. Natl. Acad. Sci*. **100,** 4451-4456

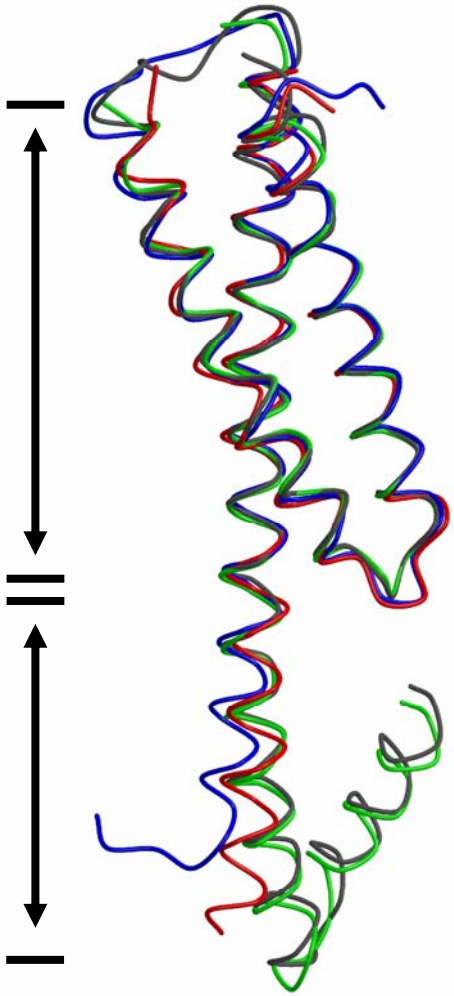
2003, June [4]Zhu, G. *et al*. *Biochemistry* **42**, 6392-6399

Three helices or four helices?

R.m.s.d. of C-terminal region (199-299)

KEK / Cambridge	**1.19** Å
KEK / NIH	**1.13** Å
KEK / Oklahoma	**1.29** Å

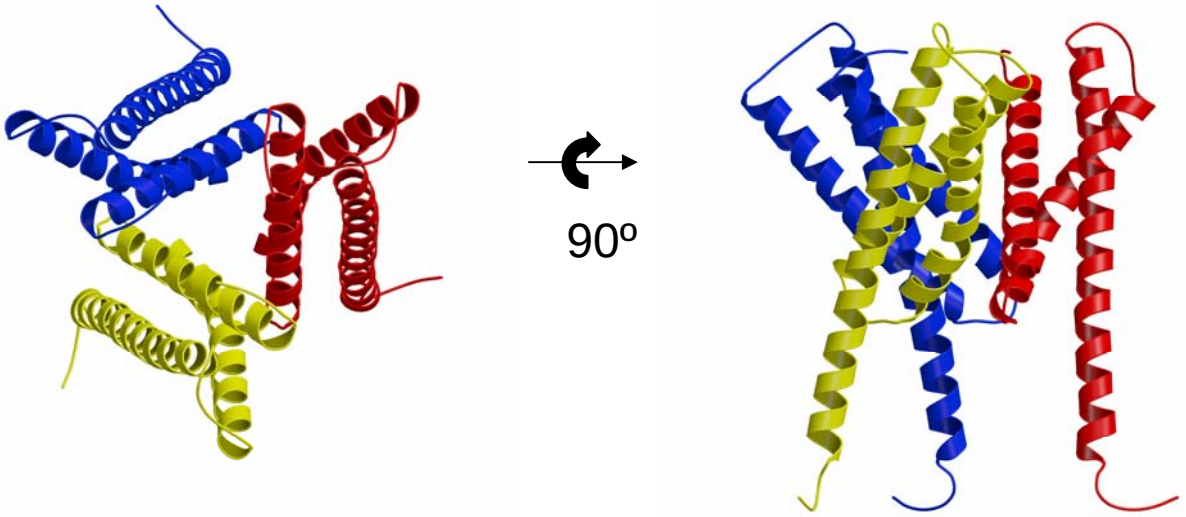
N-terminal ARF-binding region is in various conformations



KEK: **blue**
Cambridge: **green**
NIH: **red**
Oklahoma : **gray**

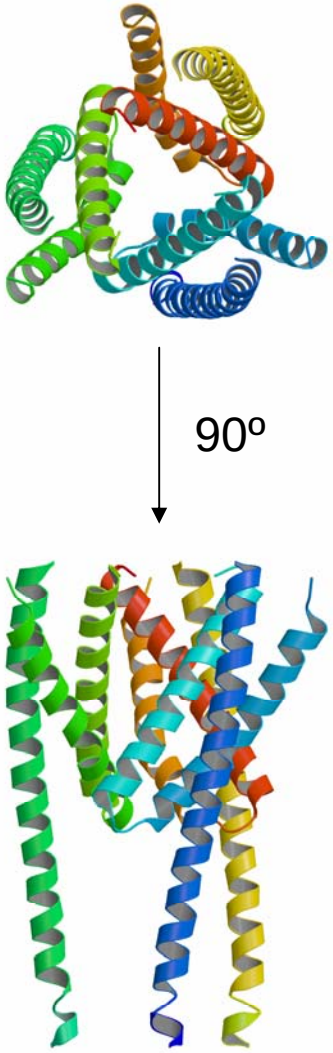
Trimerization of GGA1 GAT domain

Our group *R*3 *NSB*, 7 April 2003

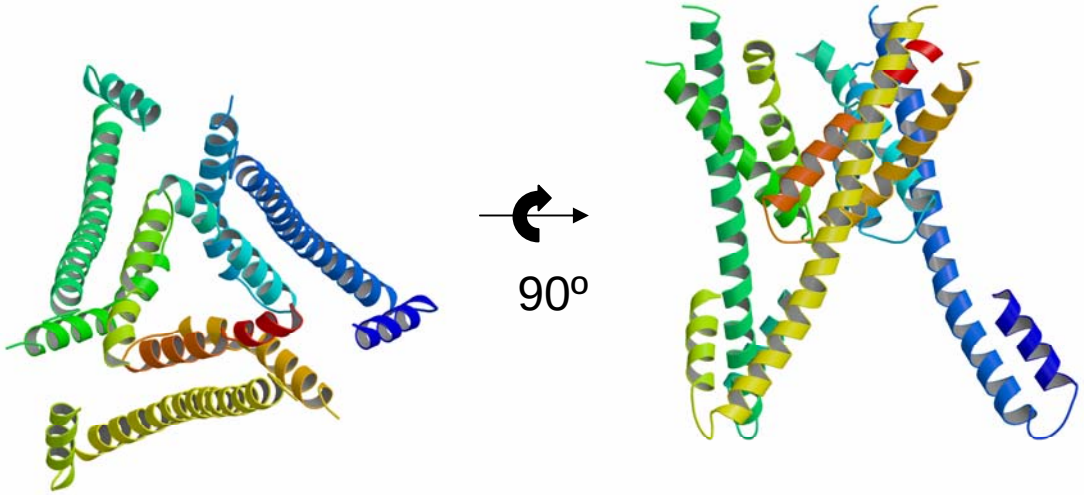


J. H. Hurley (*R*3)

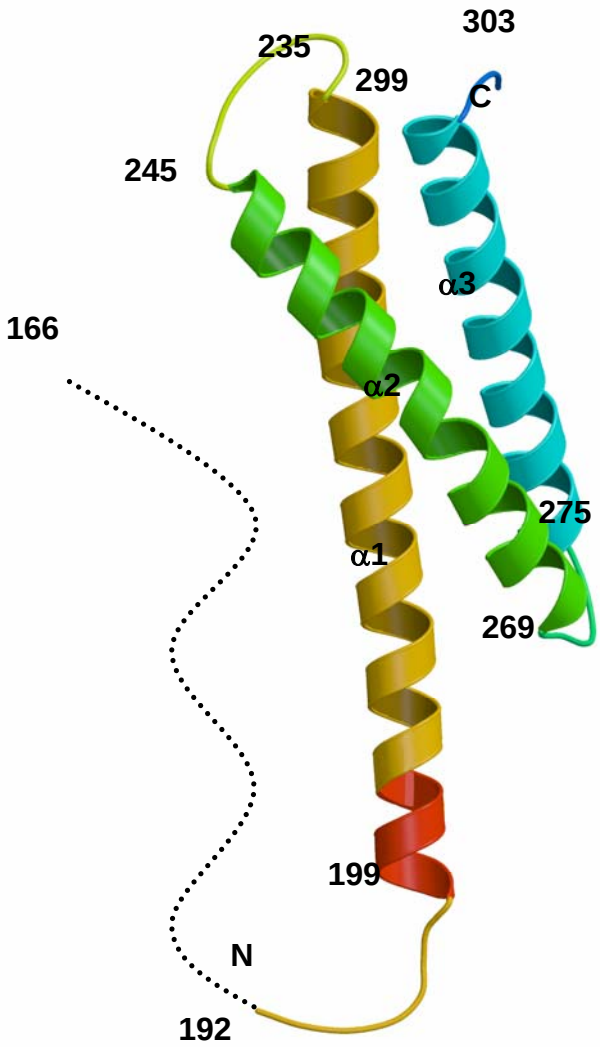
***PNAS*, 31 Mar 2003**



D. J. Owen $P6_3$ *Develop. Cell, 11 Mar 2003*

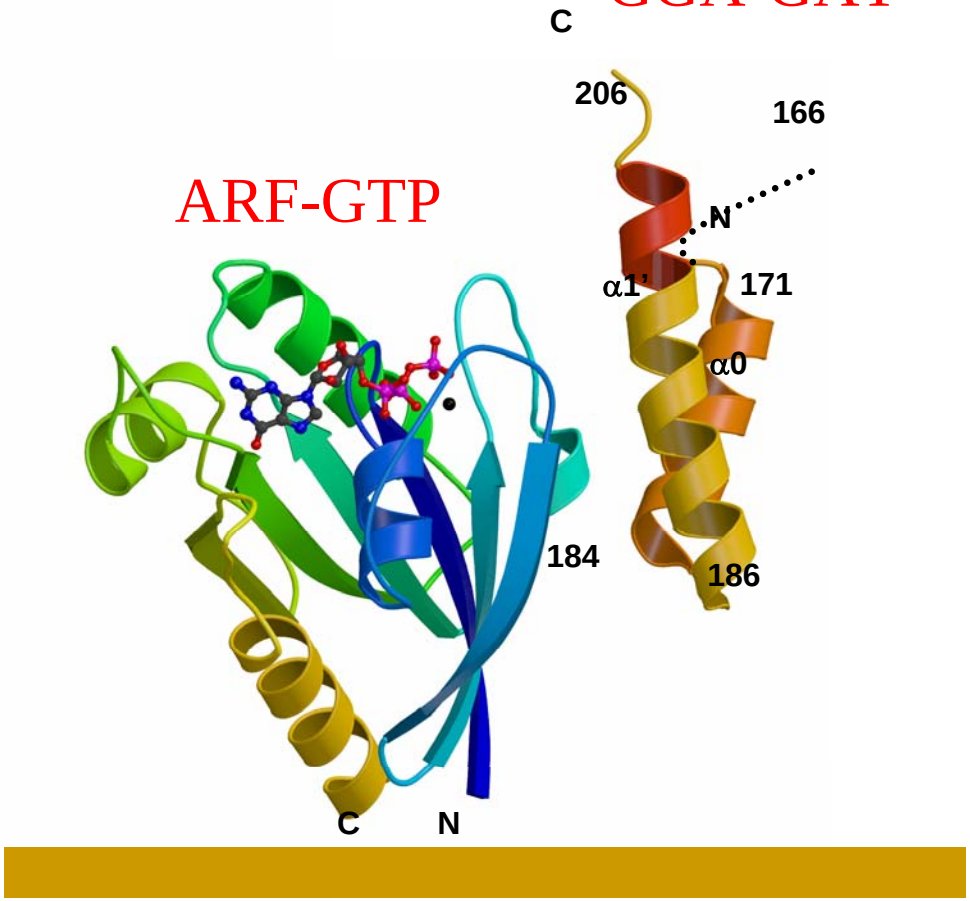


4 helices without ARF-GTP!!!



GAT domain

GGA-GAT

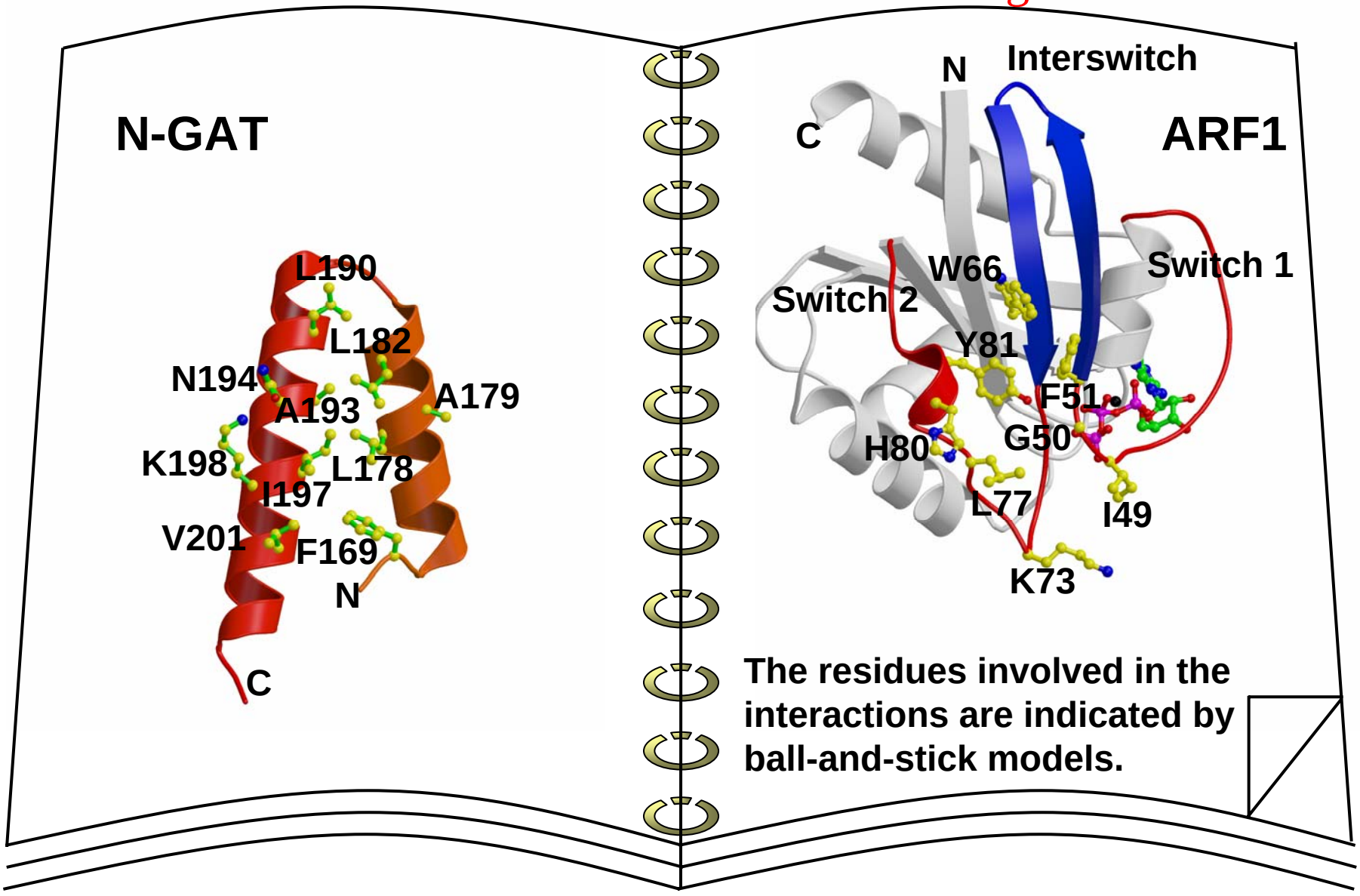


Trans Golgi Network membrane

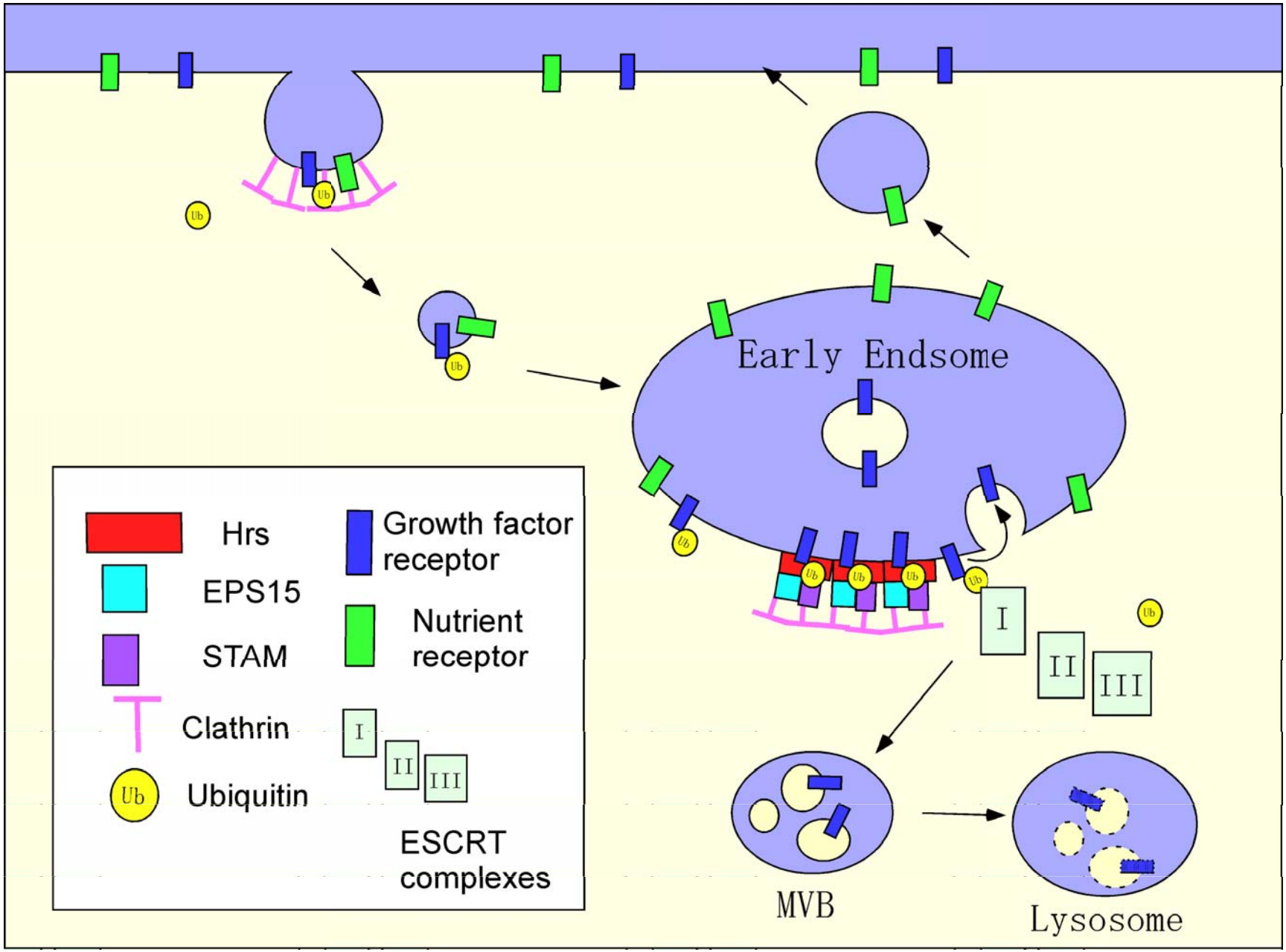
N-GAT & ARF1-GTP complex

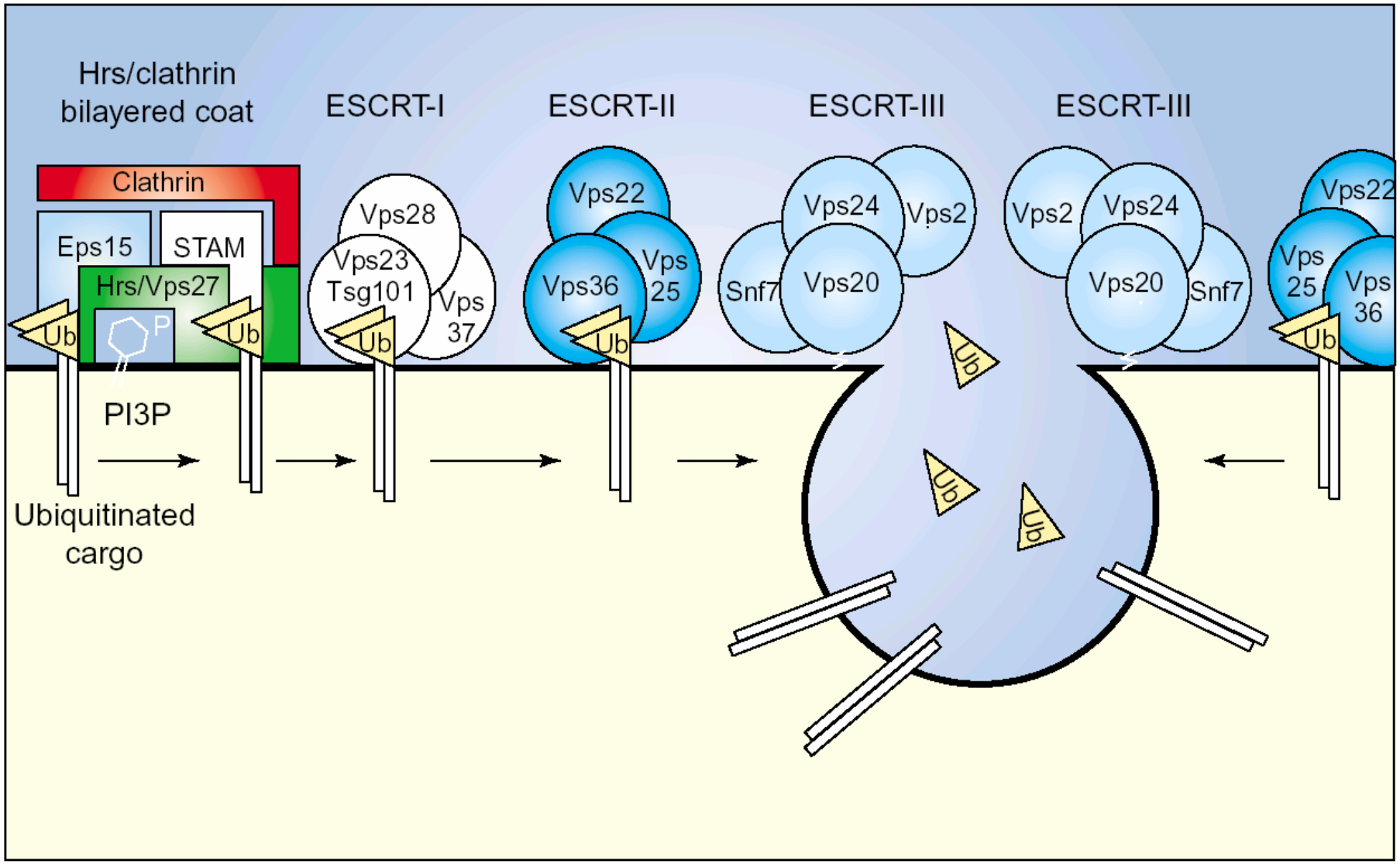
Interaction surfaces of N-GAT and ARF1-GTP

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



Downregulation of growth factor receptors

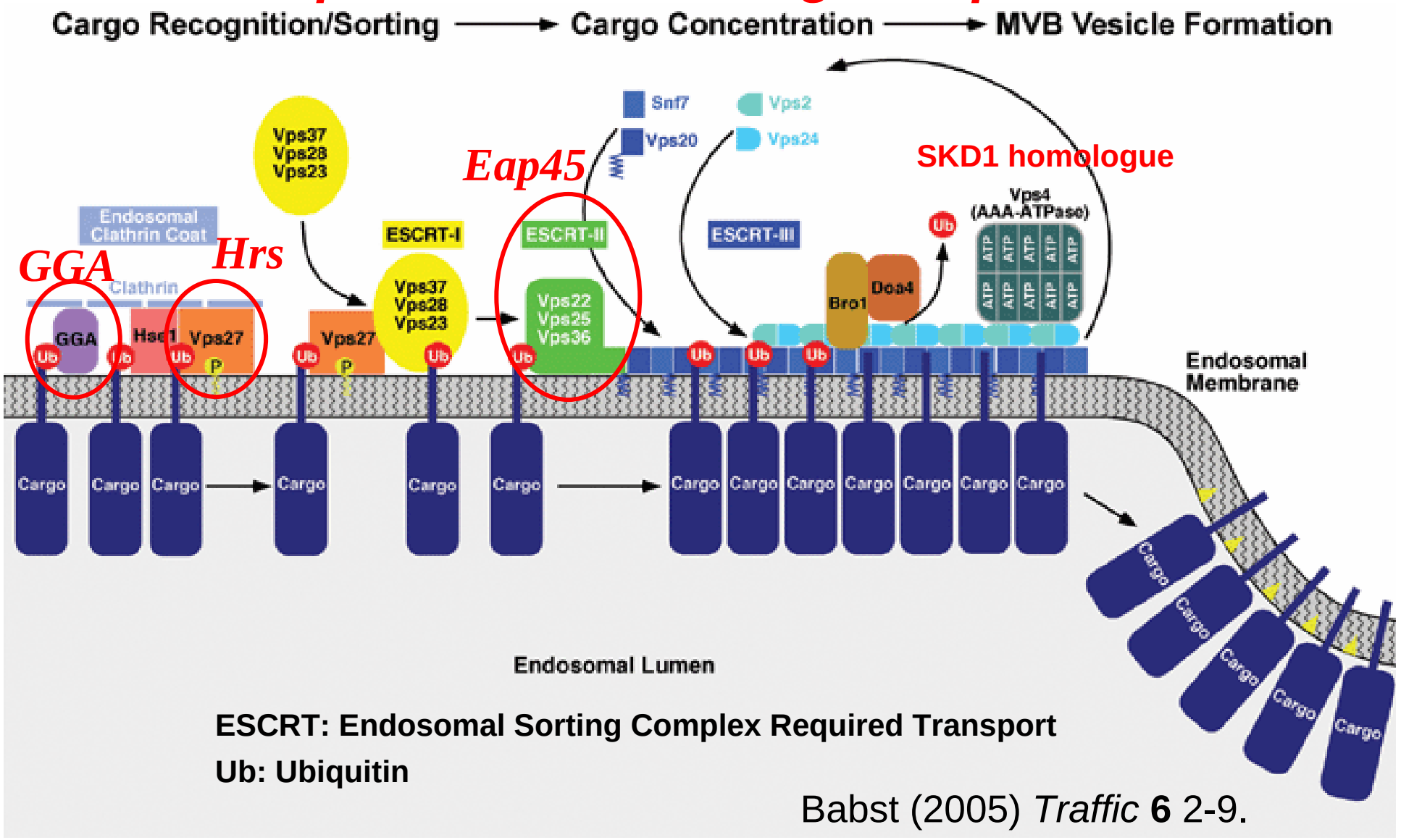




Raiborg, Rusten and Stenmark 2003 Current Opinion in Cell Biology , vol. 15 pp. 446–455

Model for the Multivesicular Body, MVB, Sorting (ESCRT) of cell surface receptors such as EGFR

Ubiquitin and PI binding is important!

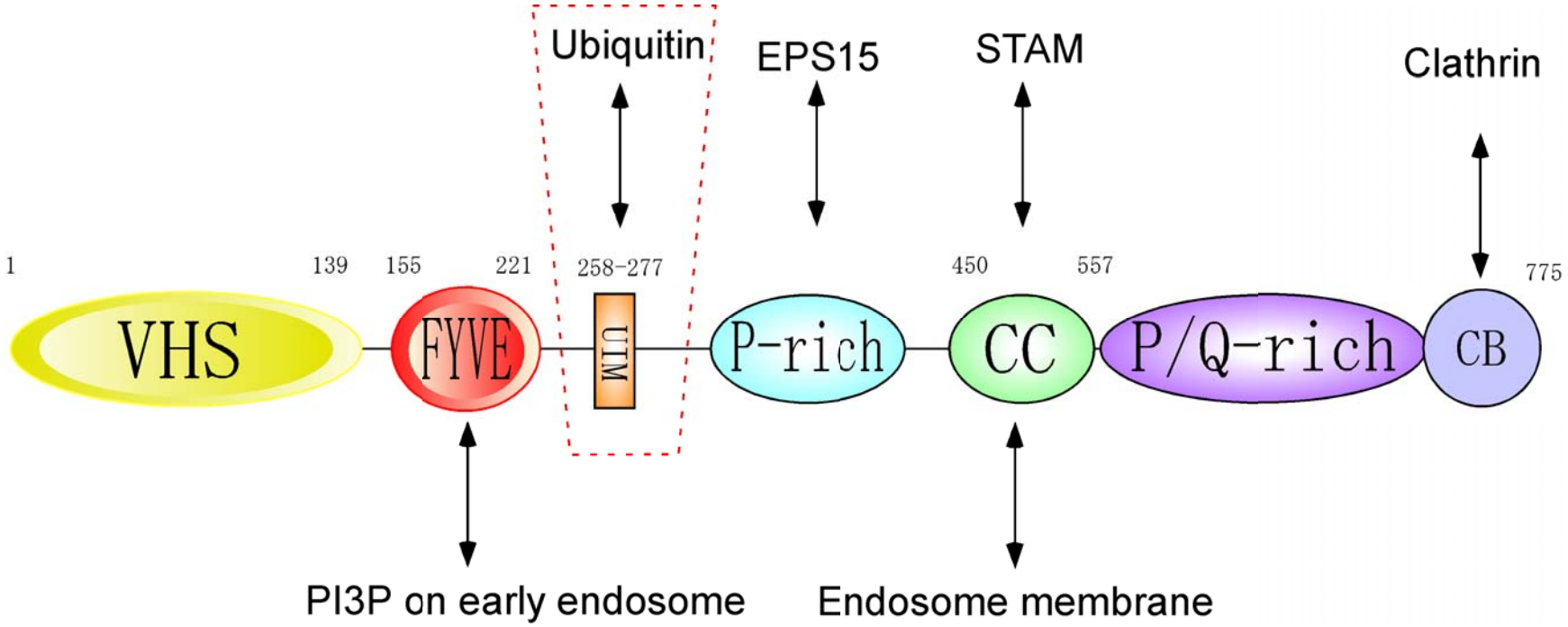


ESCRT: Endosomal Sorting Complex Required Transport

Ub: Ubiquitin

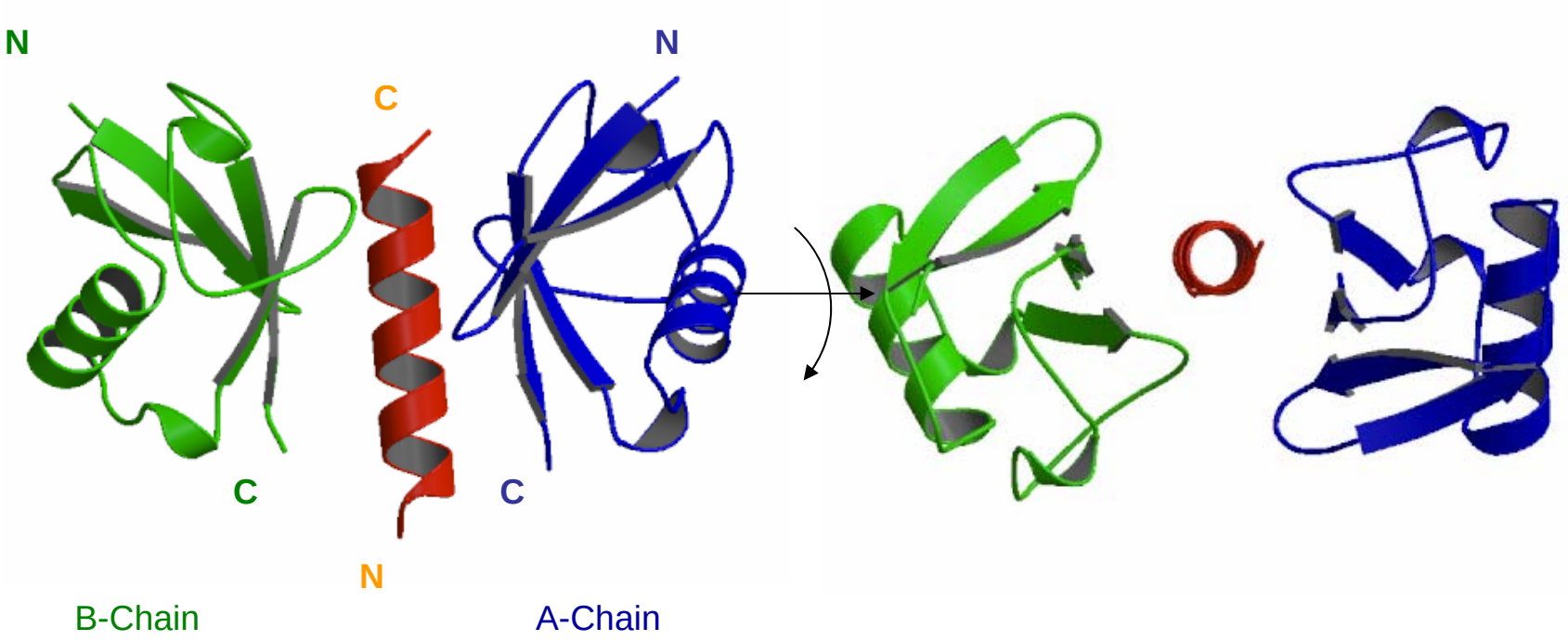
Babst (2005) *Traffic* **6** 2-9.

Domain structure of Hrs

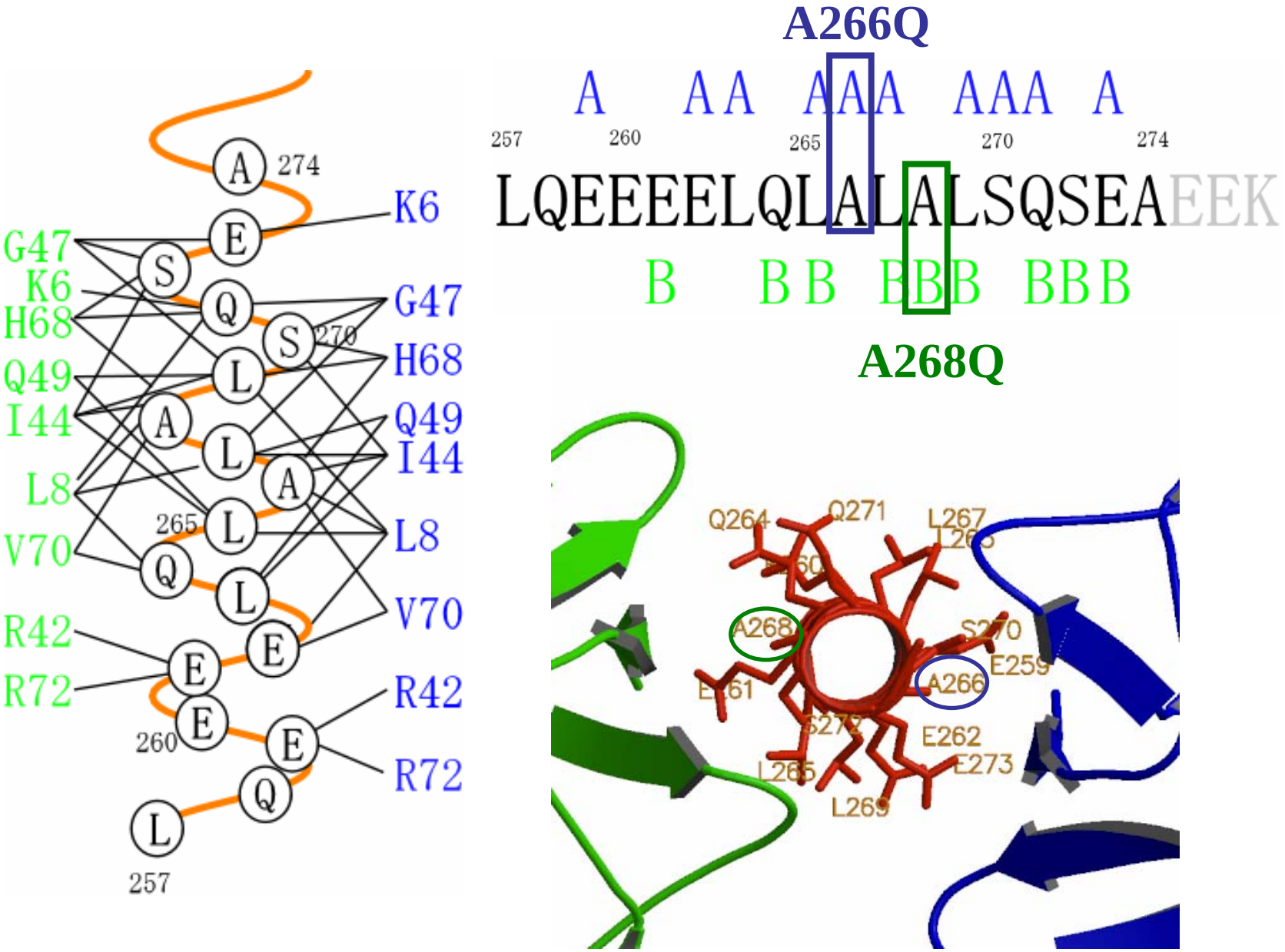


S. Hirano et al., in collaboration with Harald Stenmark, Norway

Complex structure of Hrs-UIM:Ubiquitin ①



One Hrs-UIM interacts with TWO ubiquitin molecules.



S. Hirano et al., *Nature Struct. & Mol. Biol.*, 13, 272-7, March 2006

Canon on a theme of ubiquitin

E__EL_LAL_LSQ_E

LQEEEELQLALALSQSEAEEK

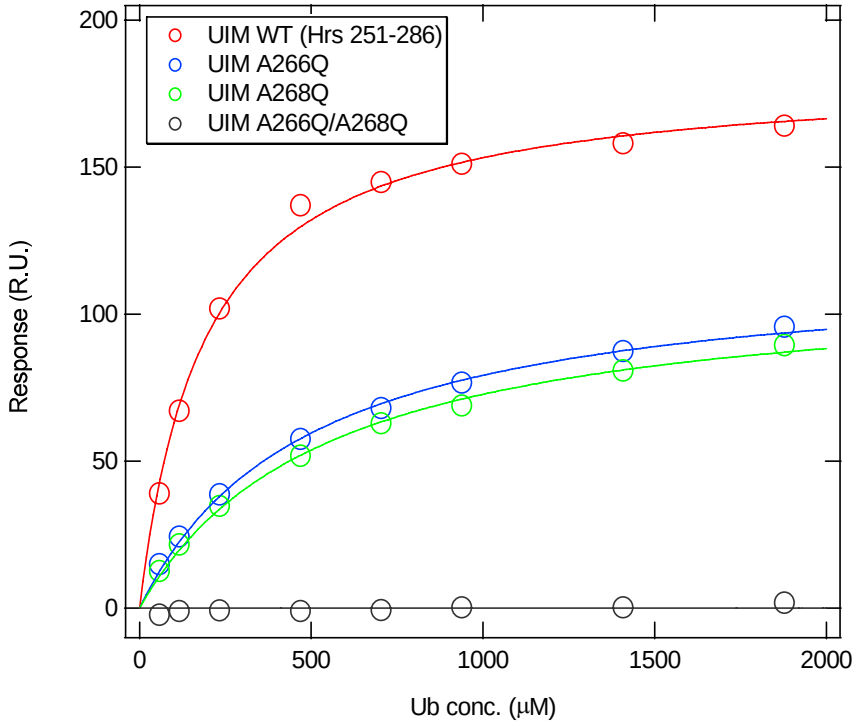
E__QL_LAL_QSE_E

Double sided ubiquitin binding

S. Hirano et al., *Nature Struct. & Mol. Biol.*, 13, 272-7, March 2006

Binding assay of Hrs-UIM

20 mM sodium phosphate pH 6.0, 10 mM NaCl, 0.005% P20, 25℃



	Kd(μM)	RUmax
Hrs-UIM	190 ±14	182 ± 3
A266Q	491 ±30	118 ± 3
A268Q	543 ± 38	112 ± 3
A266Q/A268Q	N.D.	N.D.

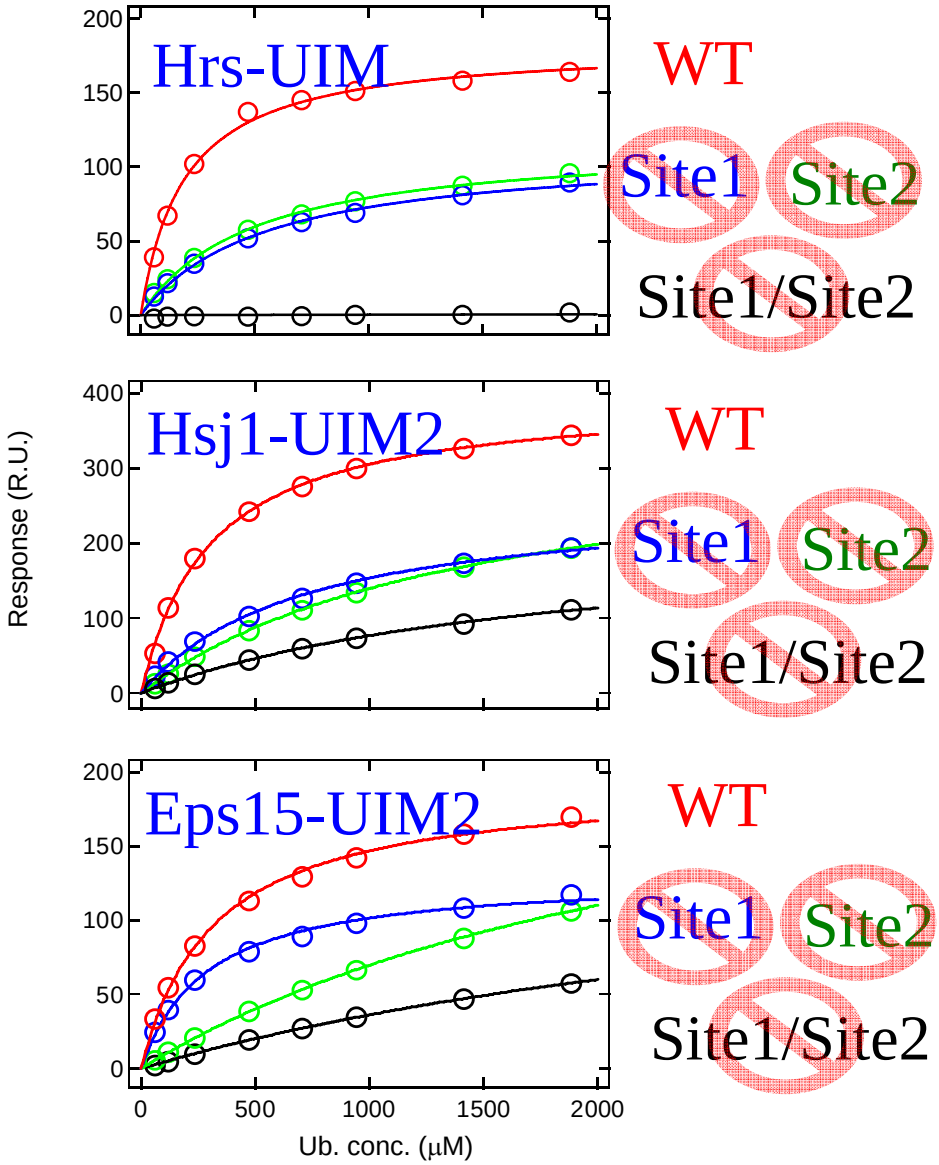
RUmax of wild type is about double of single mutants.
→Hrs-UIM has two binding sites.

Double sided Ub binding is ubiquitous!

Double-sided UIMs/Single UIMs																							
Hs Hrs UIM		257	L	Q	E	E	E	E	L	Q	L	A	L	A	L	S	Q	S	E	A	E	E	K	O14964
Hs USP25 UIM		96	G	D	D	K	D	D	L	Q	R	A	I	A	L	S	L	A	E	S	N	R	A	Q9UHP3
Hs MEKK1 UIM	*	1166	A	E	E	E	E	A	L	A	I	A	M	A	M	S	A	S	Q	V	A	L	P	Q13233
Sp STAM-like UIM	*	163	K	R	E	E	E	E	L	Q	Y	A	L	A	L	S	L	S	E	S	T	A	Q	O74749
Double-sided UIMs/Tandem UIMs																							
Mm Eps15 UIM-2		877	Q	Q	E	Q	E	D	L	E	L	A	I	A	L	S	K	S	E	I	S	E	A	P42567
Mm Eps15R UIM-2		888	R	Q	E	Q	E	D	L	E	L	A	I	A	L	S	K	A	D	M	P	A		Q60902
Hs HSJ1 UIM-2		249	L	S	E	D	E	D	L	Q	L	A	M	A	Y	S	L	S	E	M	E	A	A	P25686
Sc Ufo1 UIM-3		650	N	N	V	D	E	D	L	Q	L	A	I	A	L	S	L	S	E	I	N			Q04511
At C7A10.500 UIM-1		64	D	F	D	K	E	E	I	E	C	A	I	A	L	S	L	S	E	Q	E	H	V	O23197
Hs LOC130617 UIM-2	*	220	C	Q	E	E	E	D	L	A	L	A	Q	A	L	S	A	S	E	A	E	Y	Q	Q8WV99
Double-sided motif					e	x	e	x	ϕ	x	ϕ	A	ϕ	A	z	S	z	S/A	e					
Single-sided UIMs/Single UIMs																							
Hs STAM UIM		170	K	K	E	E	E	D	L	A	K	A	I	E	L	S	L	K	E	Q	R	Q	Q	Q92783
Mm HBP UIM		164	N	K	E	D	E	D	I	A	K	A	I	E	L	S	L	Q	E	Q	K	Q	Q	O88811
Single-sided UIMs/Tandem UIMs																							
Sc Vps27p UIM -1		284	E	D	E	E	E	L	I	R	K	A	I	E	L	S	L	K	E	S	R	N	S	P40343
Sc Vps27p UIM-2		300	E	E	E	D	P	D	L	K	A	A	I	Q	E	S	L	R	E	A	E	E	A	
Mm Eps15 UIM-1		851	P	S	E	E	D	M	I	E	W	A	K	R	E	S	E	R	E	E	E	Q	R	P42567
Mm Eps15R UIM-1		862	G	N	E	E	Q	Q	L	A	W	A	K	R	E	S	E	K	A	E	Q	E	R	Q60902
Hs Epsin UIM-1		182	G	E	E	E	L	Q	L	Q	L	A	L	A	M	S	K	E	E	A	D	Q	E	BAB14041
Hs Epsin UIM-2		207	R	G	D	D	L	R	L	Q	M	A	I	E	E	S	K	R	E	T	G	G	K	
Hs Epsin2 UIM-1		275	G	E	E	E	L	Q	L	Q	L	A	L	A	M	S	R	E	V	A	E	Q	E	O95208
Hs Epsin2 UIM-2		300	R	G	D	D	L	R	L	Q	M	A	L	E	E	S	R	R	D	T	V	K	I	
Hs S5a UIM-1		210	P	S	A	D	P	E	L	A	L	A	L	R	V	S	M	E	E	Q	R	Q	R	P55036
Hs S5a UIM-2		281	M	T	E	E	E	Q	I	A	Y	A	M	Q	M	S	L	Q	G	A	E	F	G	
Hs HSJ1 UIM-1		206	N	G	V	P	D	D	L	A	L	G	L	E	L	S	R	R	E	Q	Q	P	S	P25686
Sc Ufo1 UIM-1		546	D	D	E	D	E	Q	L	R	R	A	L	E	E	S	Q	L	I	Y	E	T	Q	Q04511
Sc Ufo1 UIM-2		582	D	E	D	D	E	E	F	L	R	A	I	R	Q	S	R	V	E	D	E	R	R	
At C7A10.500 UIM-2		109	E	D	E	D	E	E	Y	M	R	A	Q	L	E	A	A	E	E	E	E	R	R	O23197
At C7A10.500 UIM-3		171	L	E	E	D	E	L	L	A	K	A	L	Q	E	S	M	N	V	G	S	P	P	
Hs LOC130617 UIM-1	*	206	L	S	E	D	E	A	L	Q	R	A	L	E	M	S	L	A	E	T	K	P	Q	Q8WV99
Single-sided motif					e	e	x	x	ϕ	x	x	A	ϕ	x	e/ϕ	S	z	x	e					

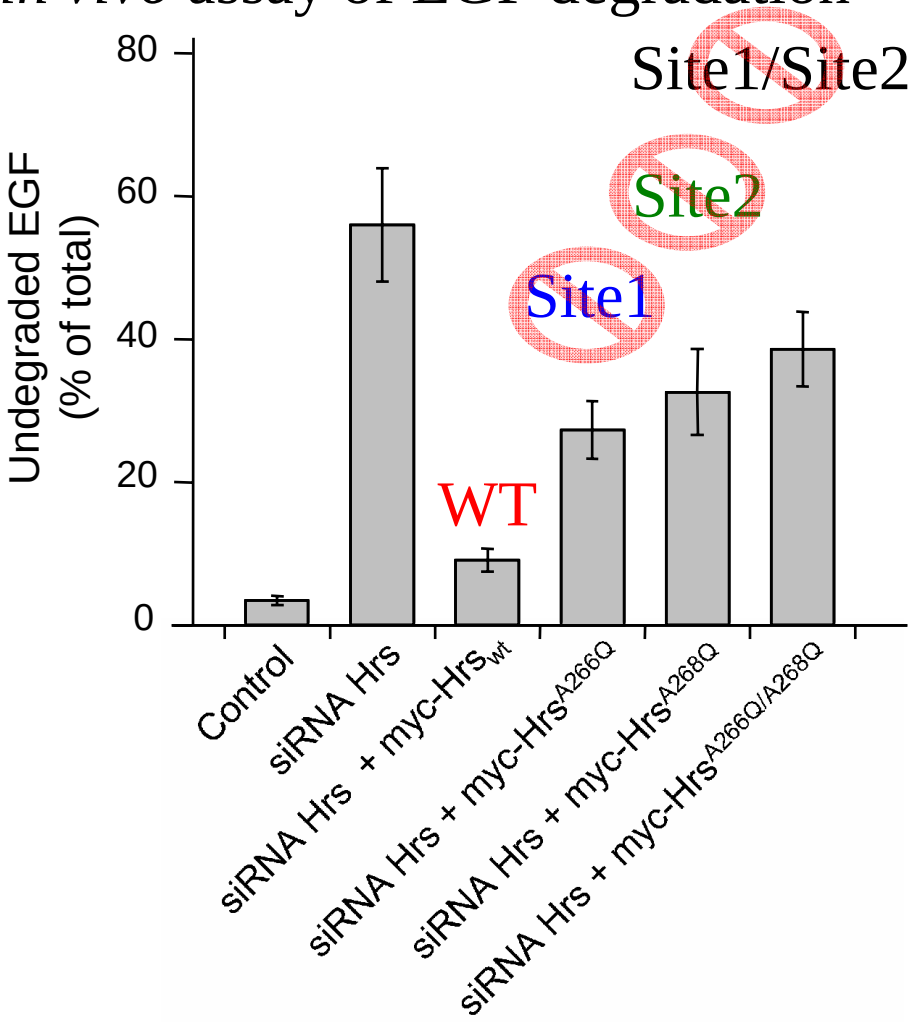
Hsj1 & Eps15: double sided UIMs!

in vivo assay using BiaCORE



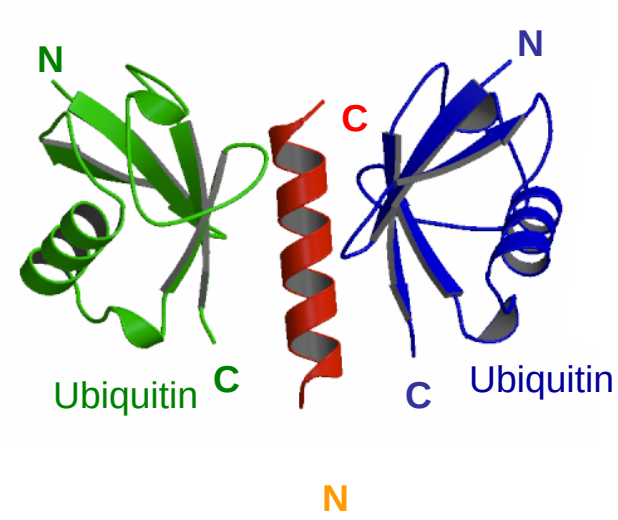
Both ubiquitin sites are needed for EGF receptor transport

in vivo assay of EGF degradation



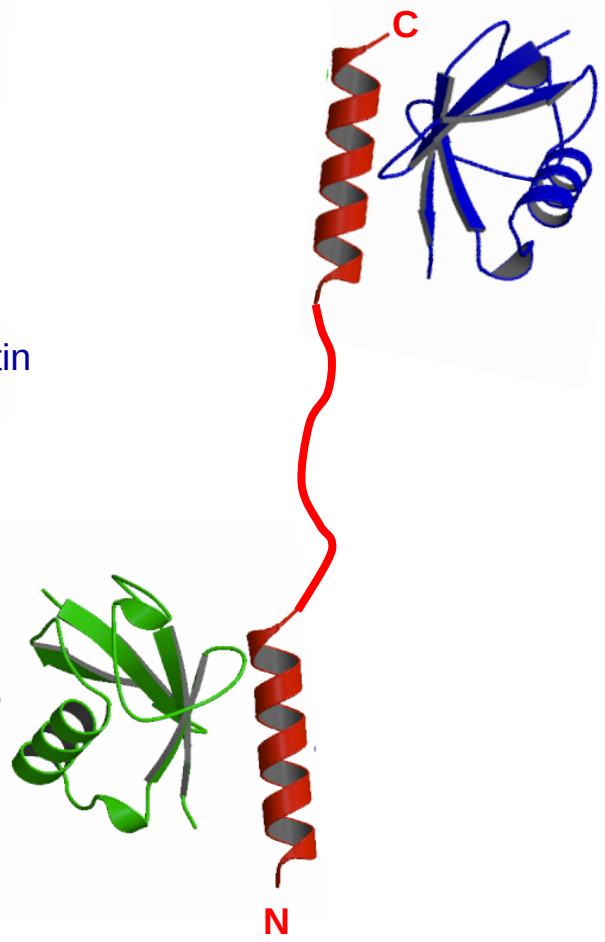
Double sided Hrs-UIM (human) vs. single sided tandem Vps27-UIM (yeast)

Double sided only:
Hrs-UIM (human)

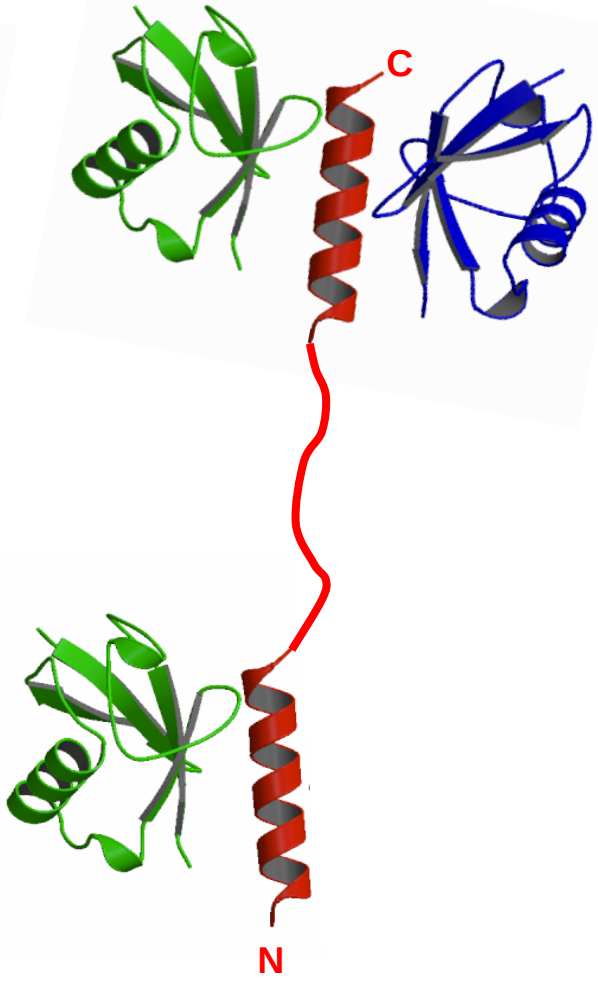


S. Hirano et al.,
Nature Struct. & Mol. Biol., 13, 272-7,
March 2006

Tandem single sided:
Vps27p-UIM (yeast)

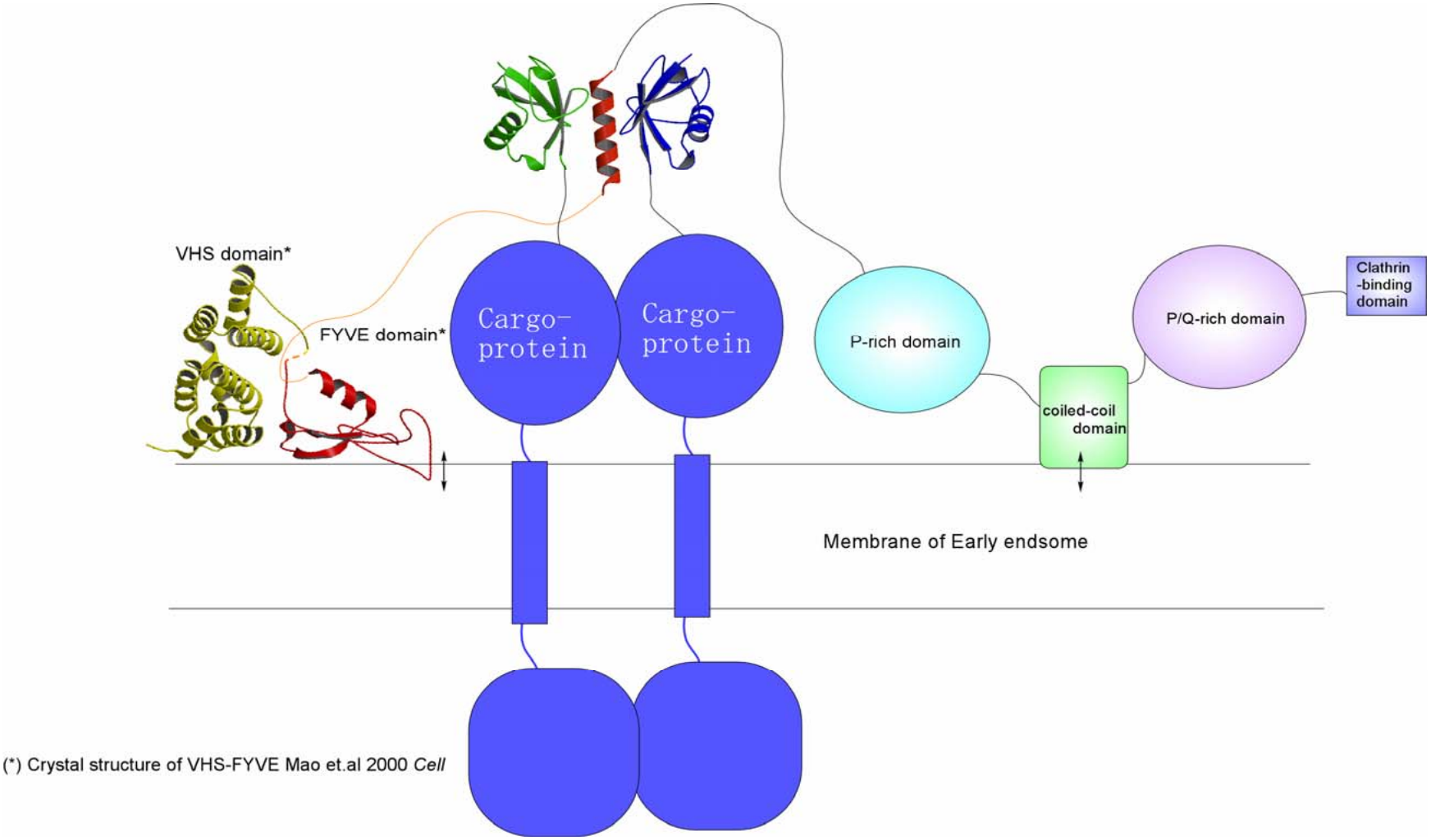


Tandem single sided:
Eps15-UIM (mouse)



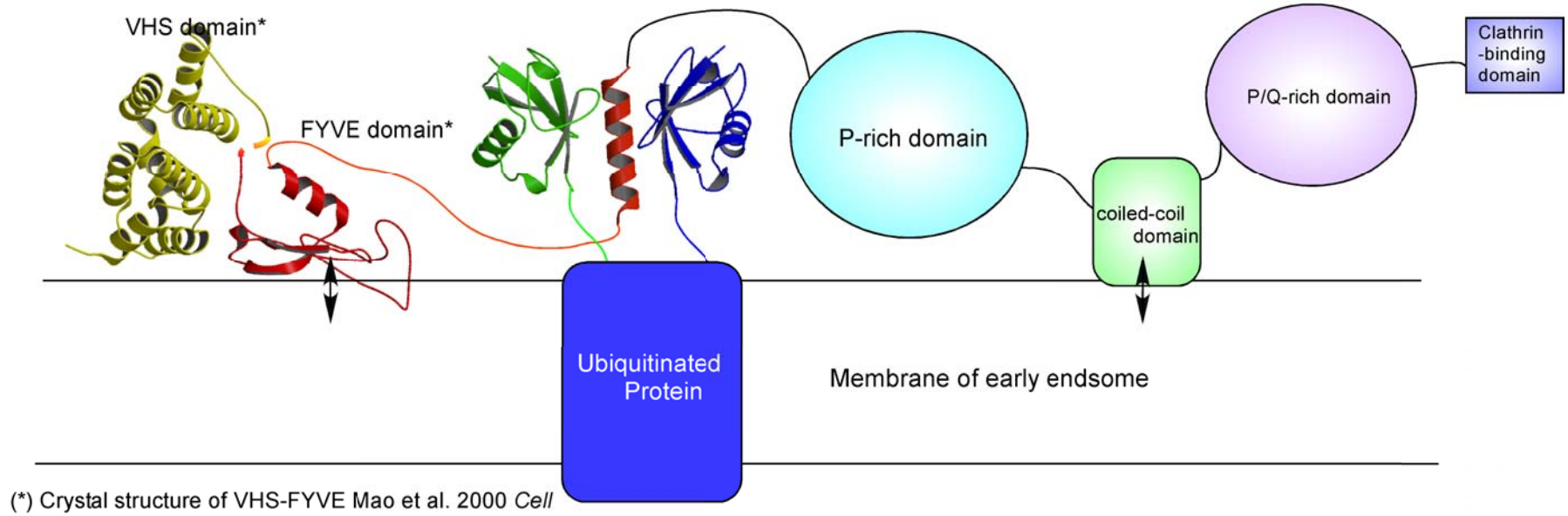
Double sided Ub binding is ubiquitous!

Model I: Hrs-UIM can bring receptors together.



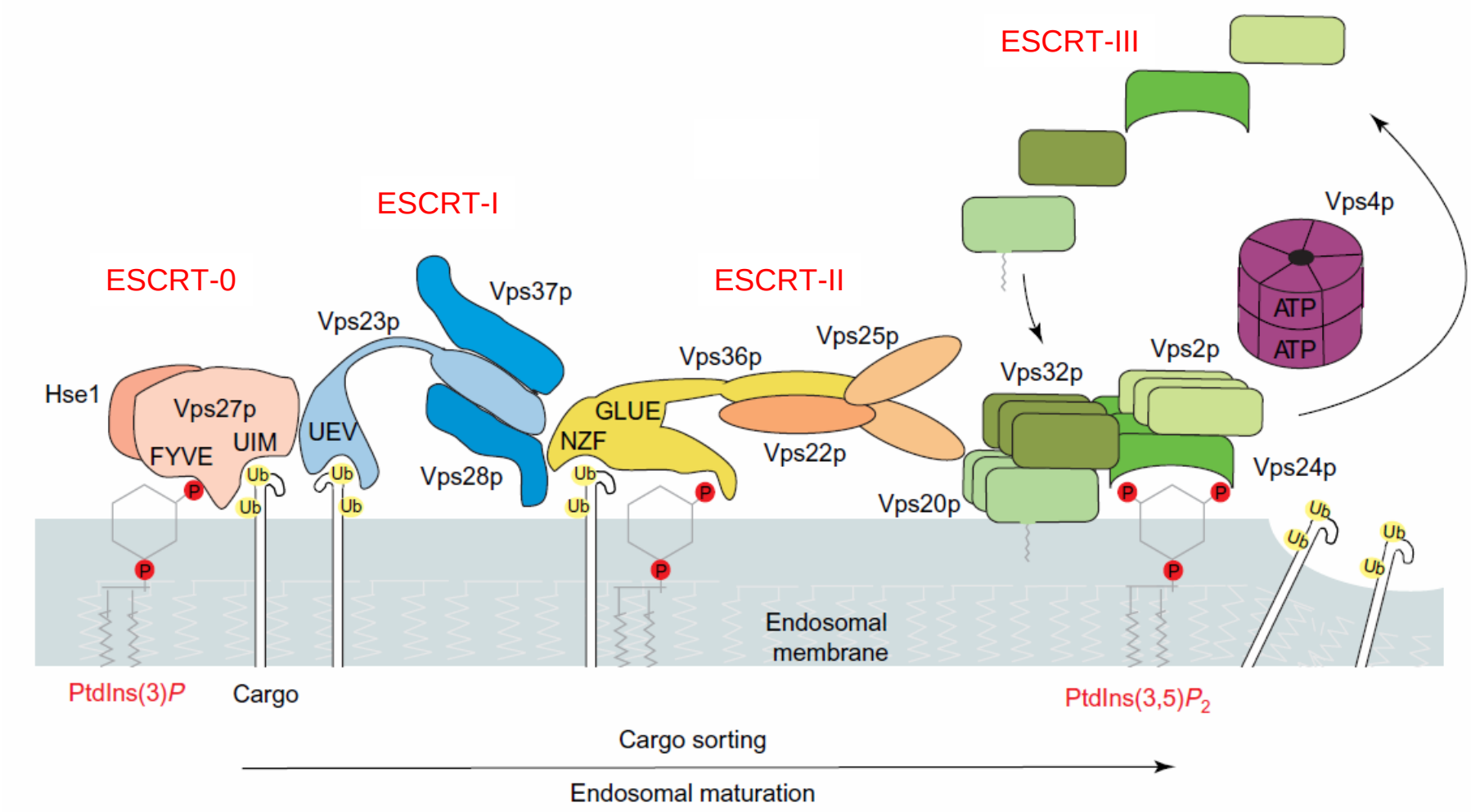
Double sided Ub binding is ubiquitous!

Model II: Hrs-UIM has higher affinity for multiply monoubiquitinated receptors.



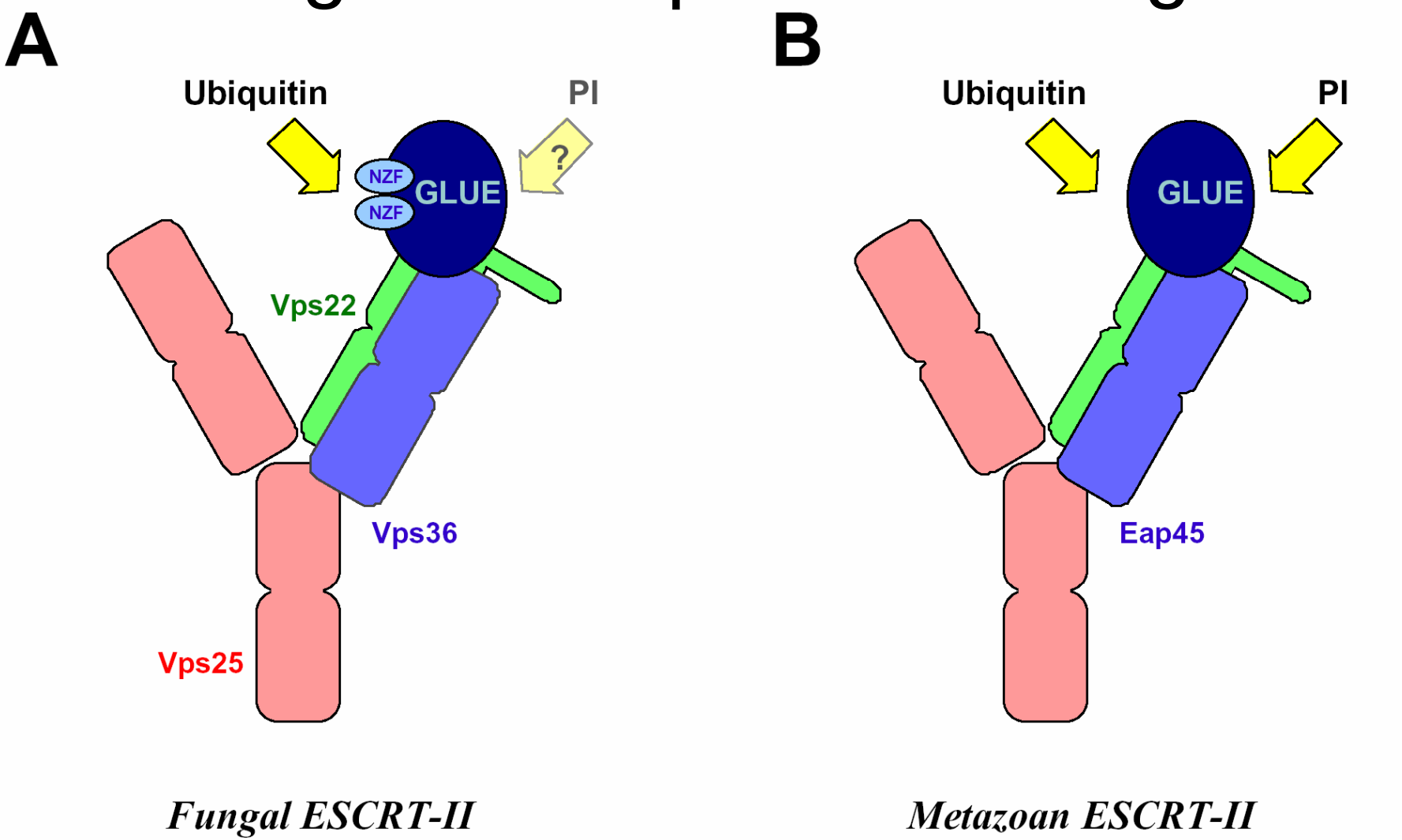
(*) Crystal structure of VHS-FYVE Mao et al. 2000 *Cell*

Function of ESCRTs in endosomal sorting of ubiquitinated membrane proteins



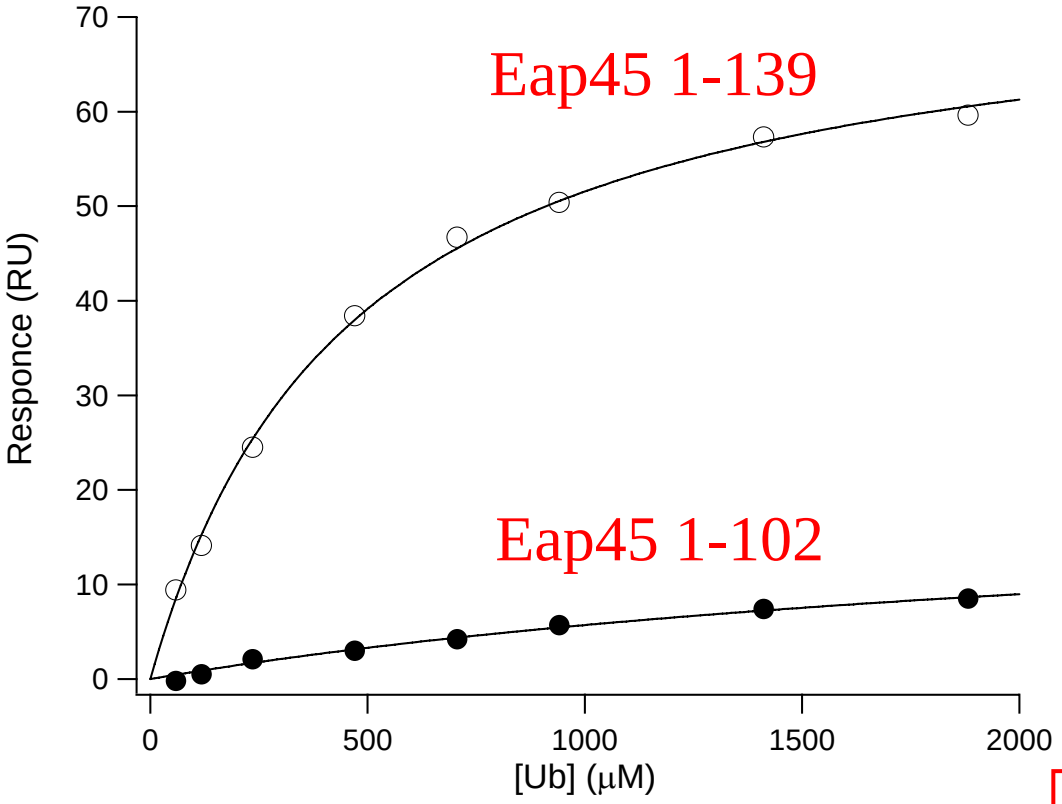
From Slagsvold, T. *et al*. *Trends. Cell Biol*. (2006) 16:317-326

How does mammalian ESCRT II recognize ubiquitinated cargos?

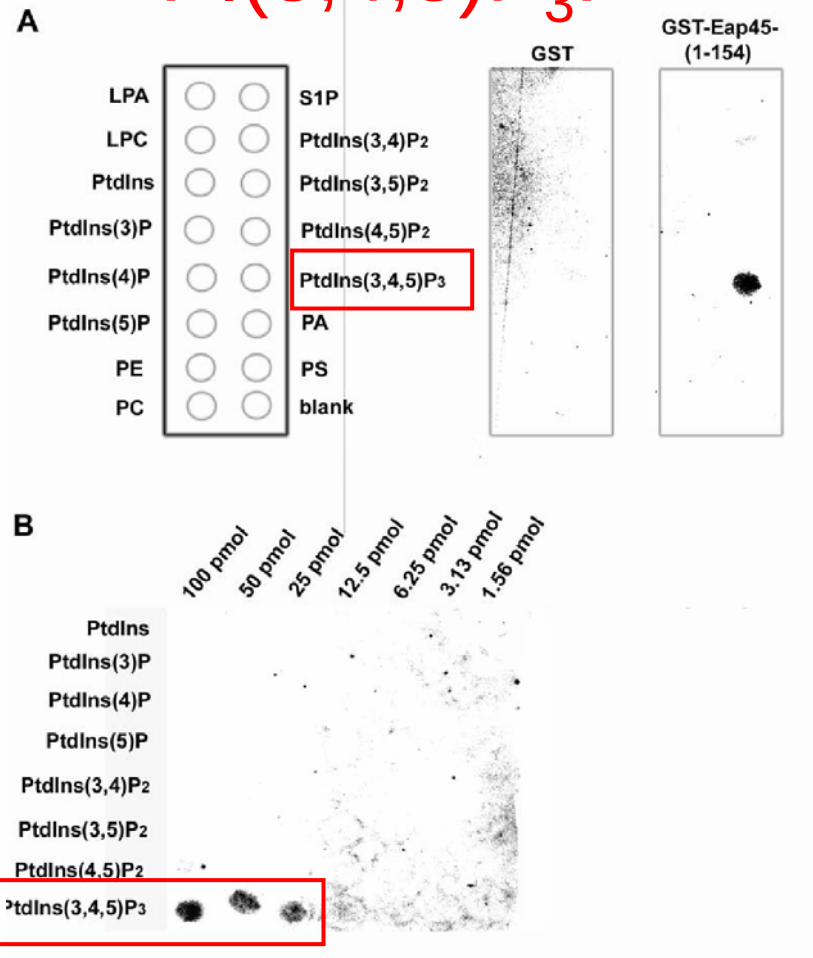


Slagsvold, et al., J. Biol. Chem.Vol. 280, May 20, 19600–19606, 2005

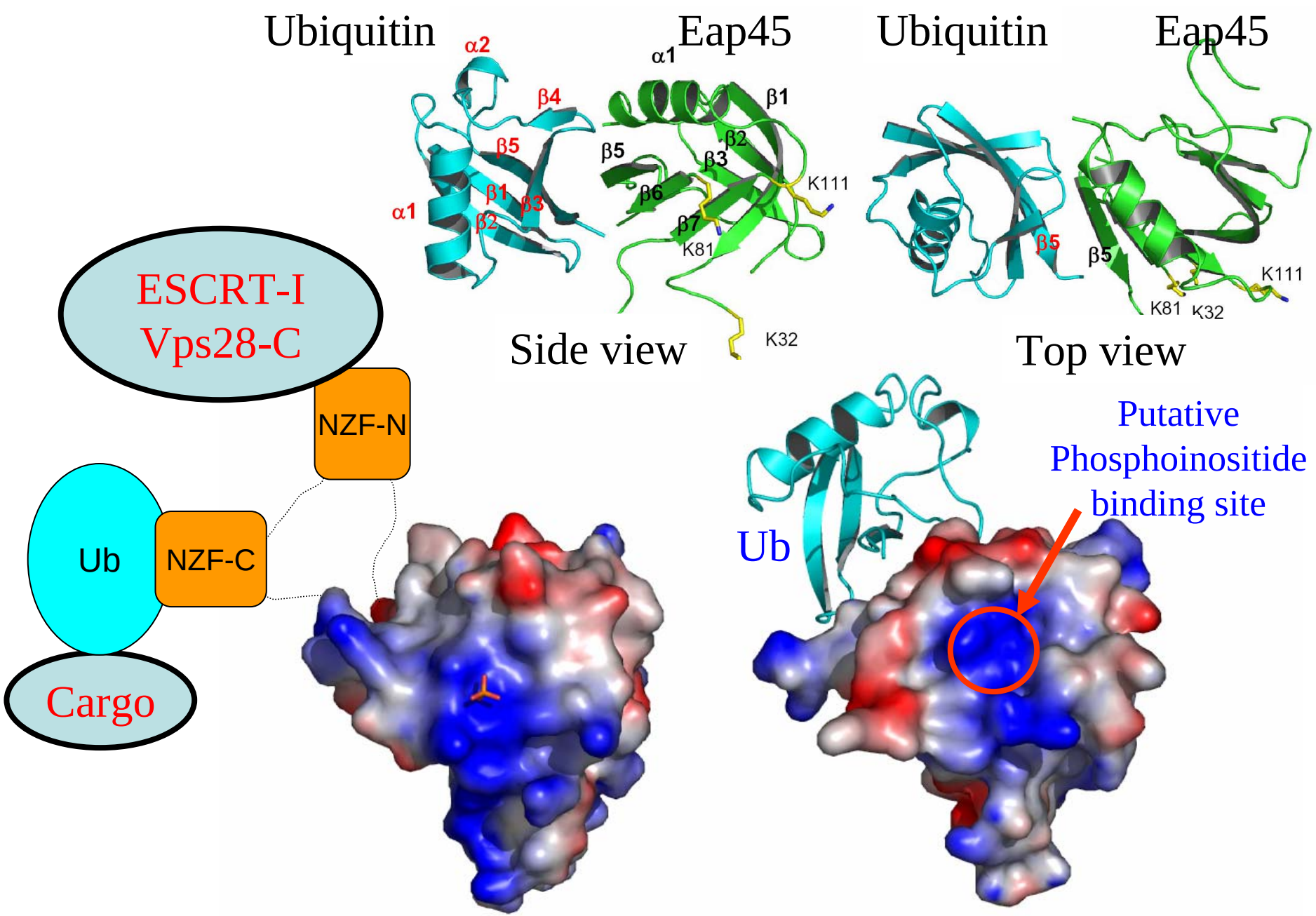
Eap45recognizes ubiquitinated cargos



It also recognized PI(3,4,5)P_3!



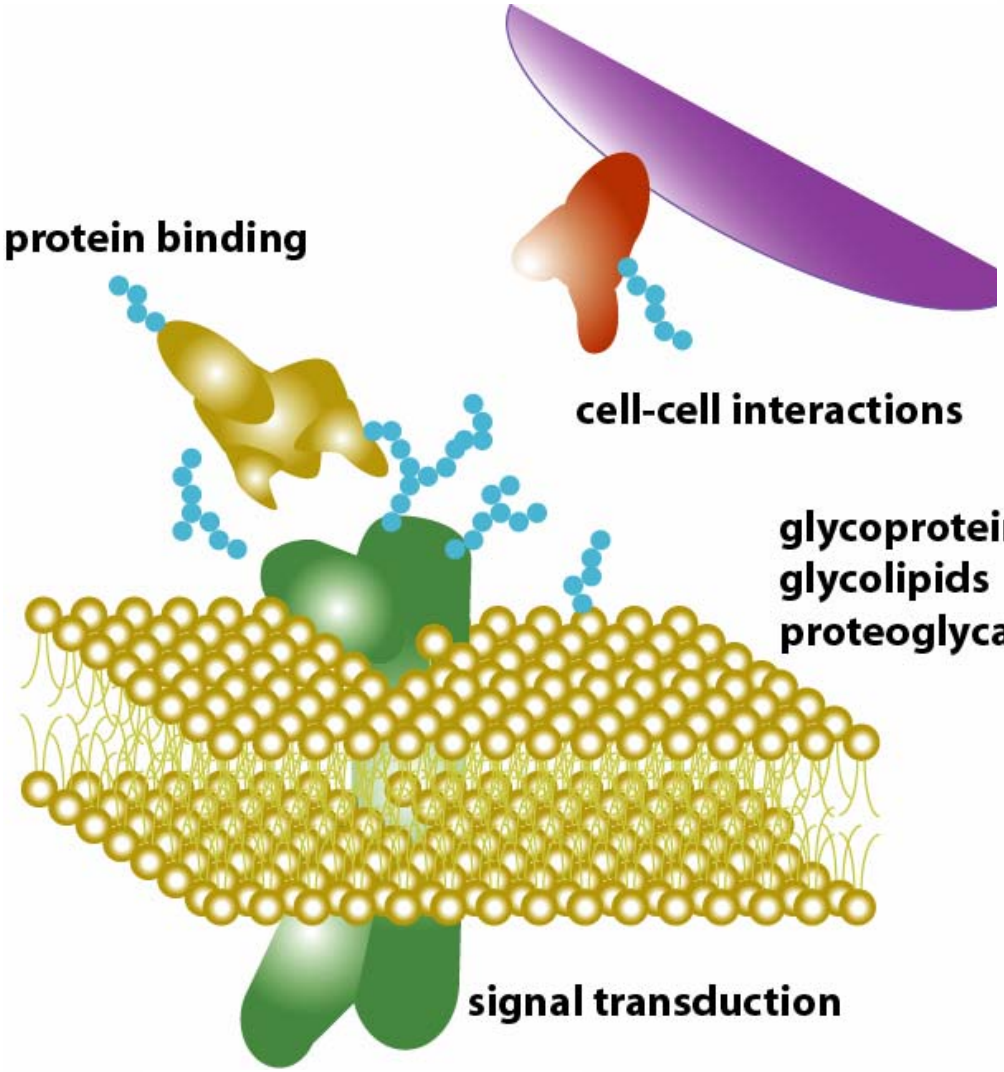
Slagsvold, et al., J. Biol. Chem.Vol. 280, May 20, 19600–19606, 2005

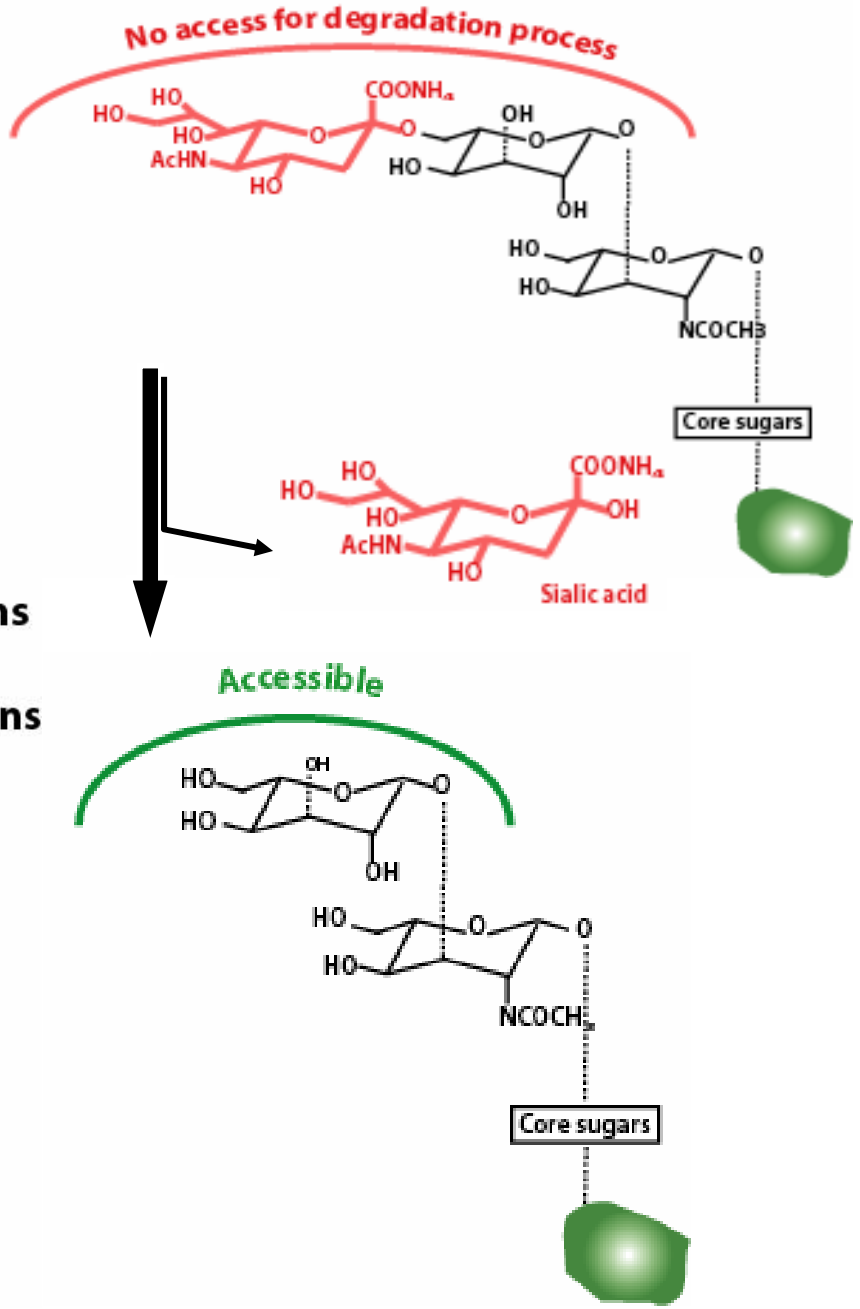


Yeast Vps36p GLUE (Theo,,,,
R. Williams, *Cell*, 2006)

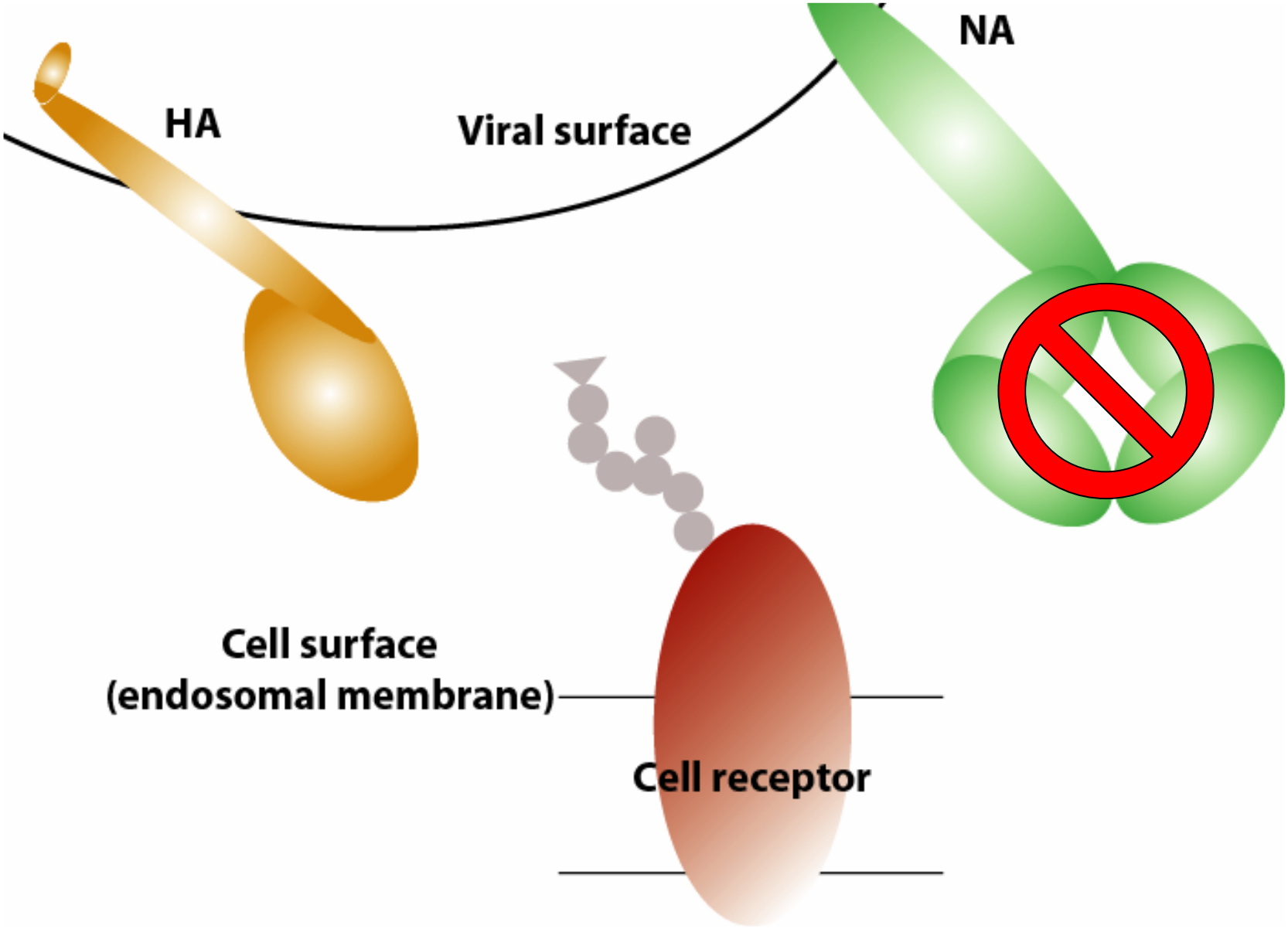
Eap45-GLUE (*NSMB*, Nov, 2006)

Functions of sialidases





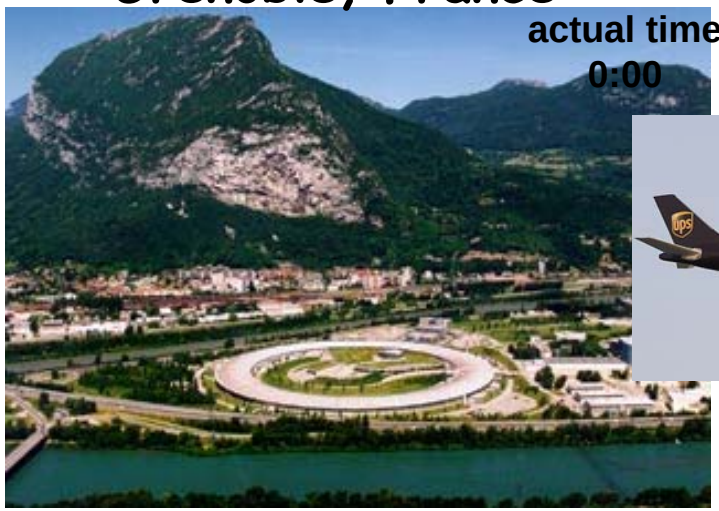
Structural studies towards new Influenza virus inhibitors



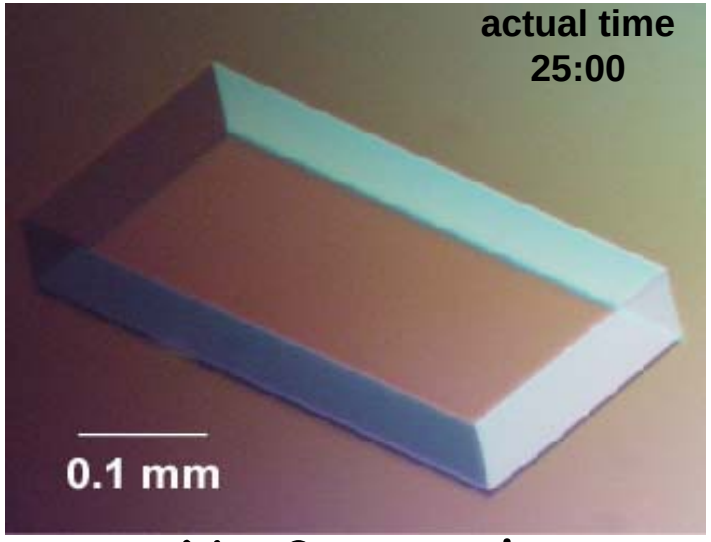
International travel of Neu2

Milano, Italy

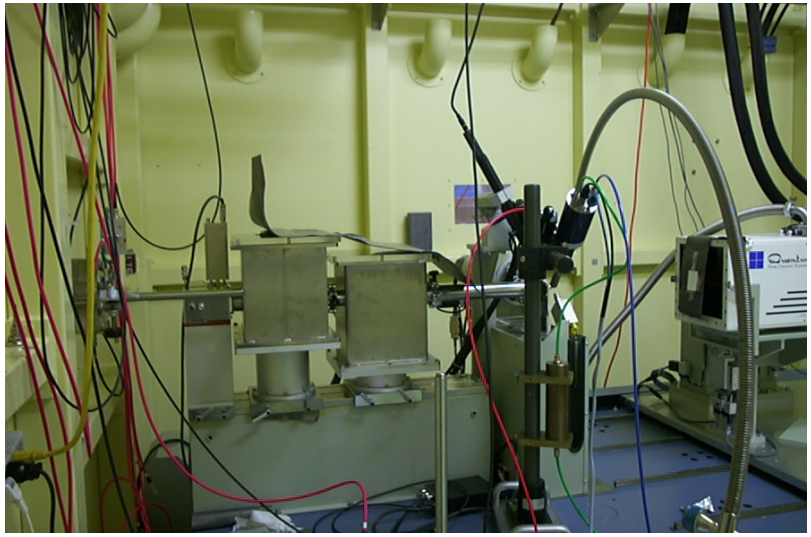
Grenoble, France



Photon Factory, Tsukuba



Neu2 crystal



PF-AR NW12

The first mammalian sialidase structure: human Neu2

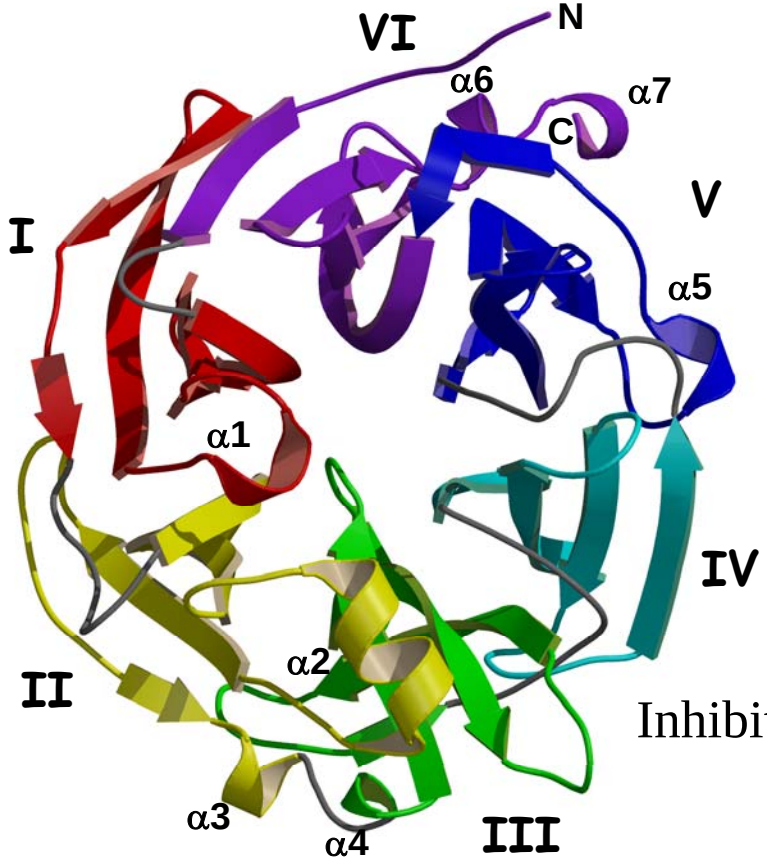
Leo Chavas et al. in preparation

Collaboration with Eugenio Monti, University of Brescia, Italy

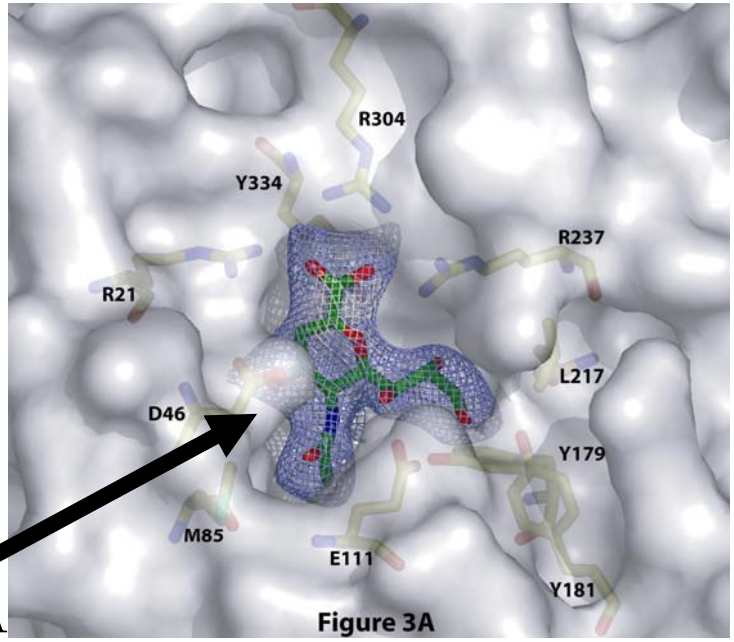
380 a.a. expressed in E. coli.

Inhibitor development for influenza viruses through comparative studies. Collaboration with Prof. M.v. Itzstein, Institute for Glycomics, Griffith University, Australia

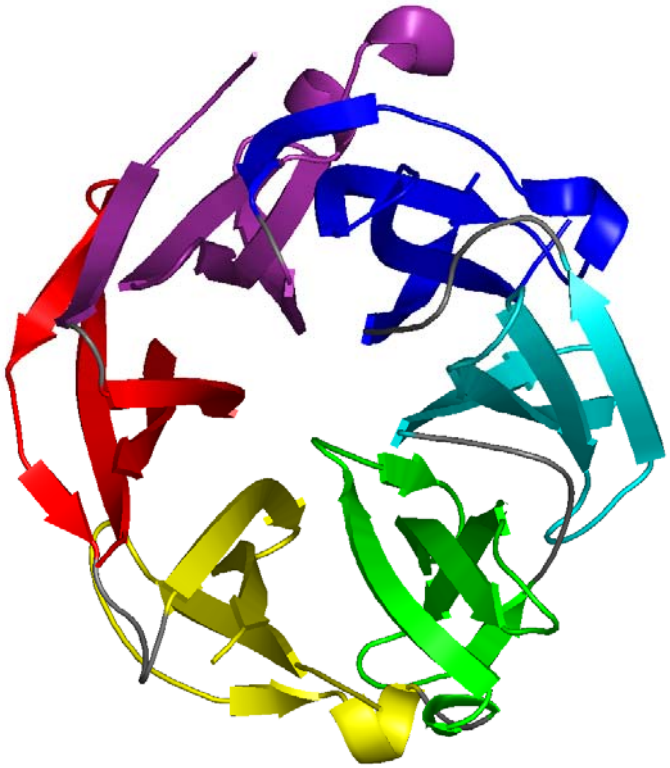
Chavas et al., J. Biol. Chem. 2005 vol 280, 469-75



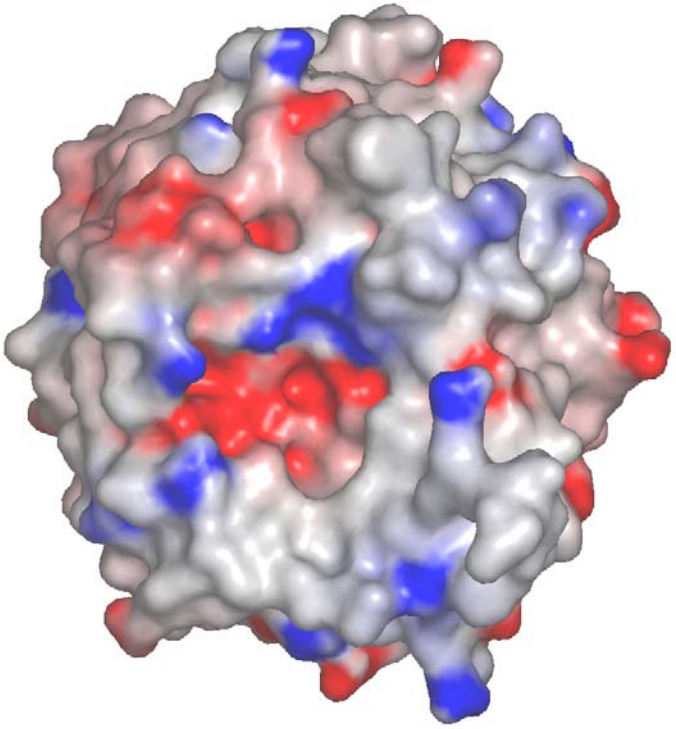
Inhibitor:DANA



Neu2 folding and electrostatic surfaces



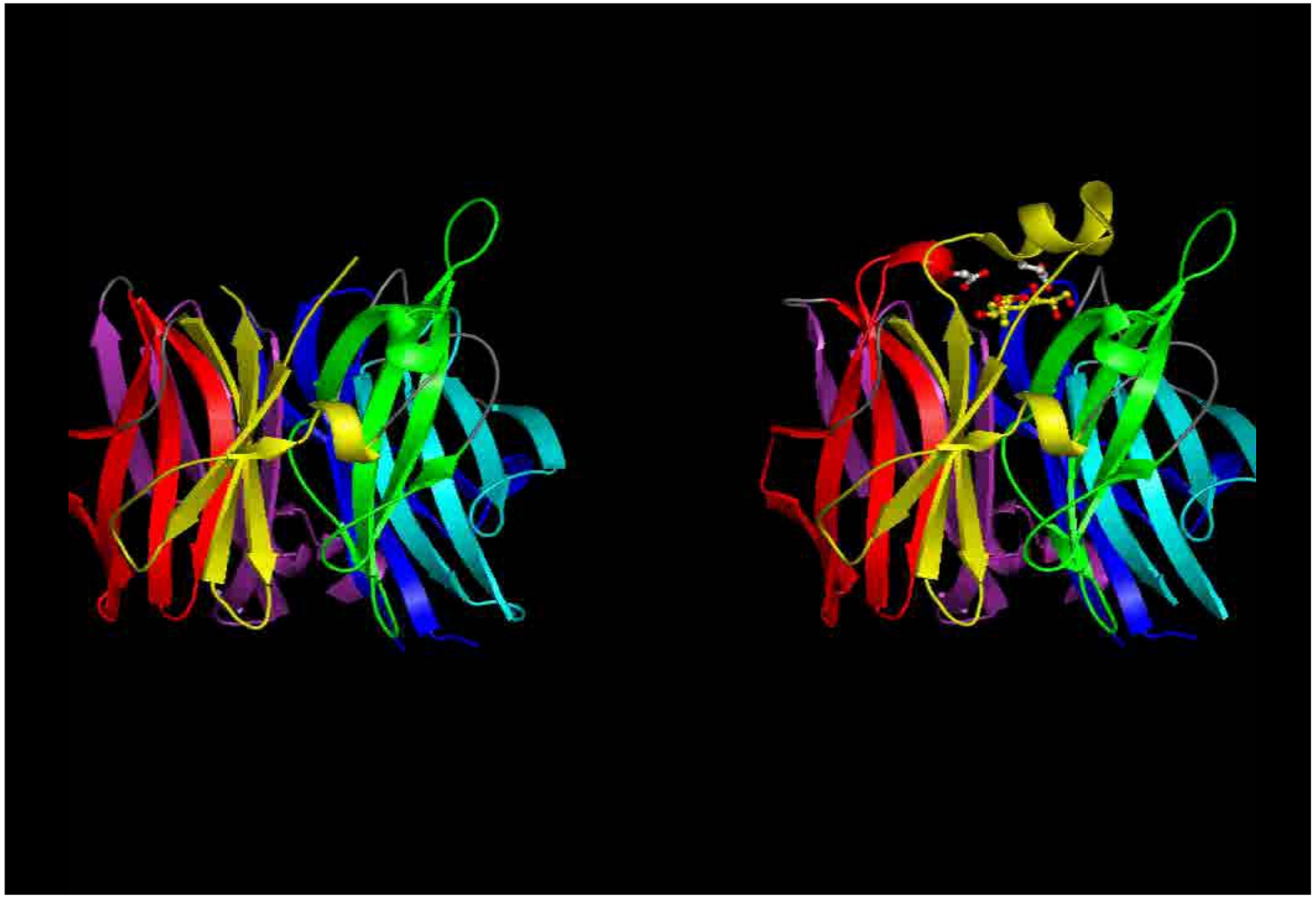
Ribbon diagram representation (view from the active site)



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

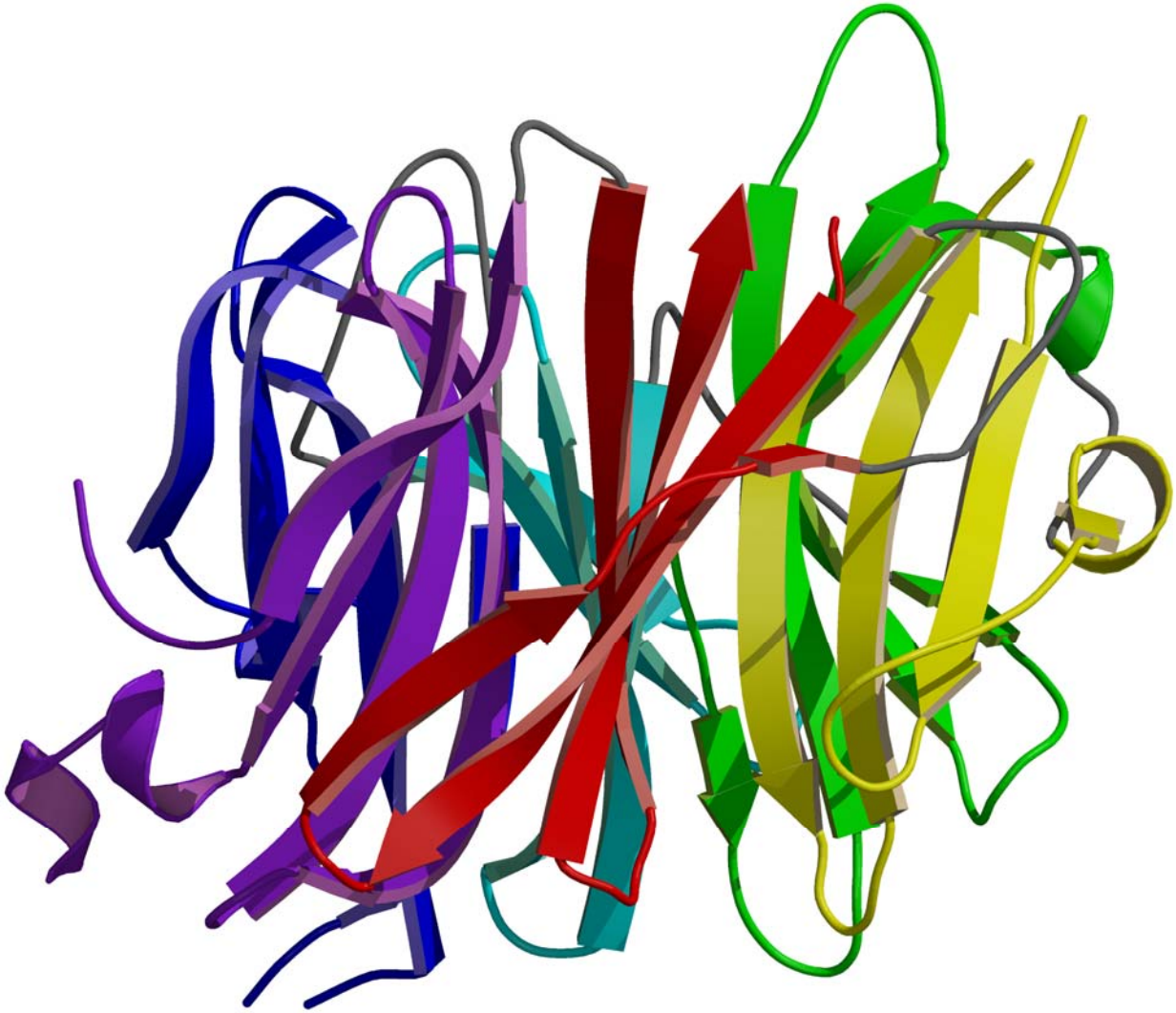
Chavas et al., J. Biol. Chem. 2005 vol 280, 469-75

Human sialidase Neu2 structure and its complex with DANA



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

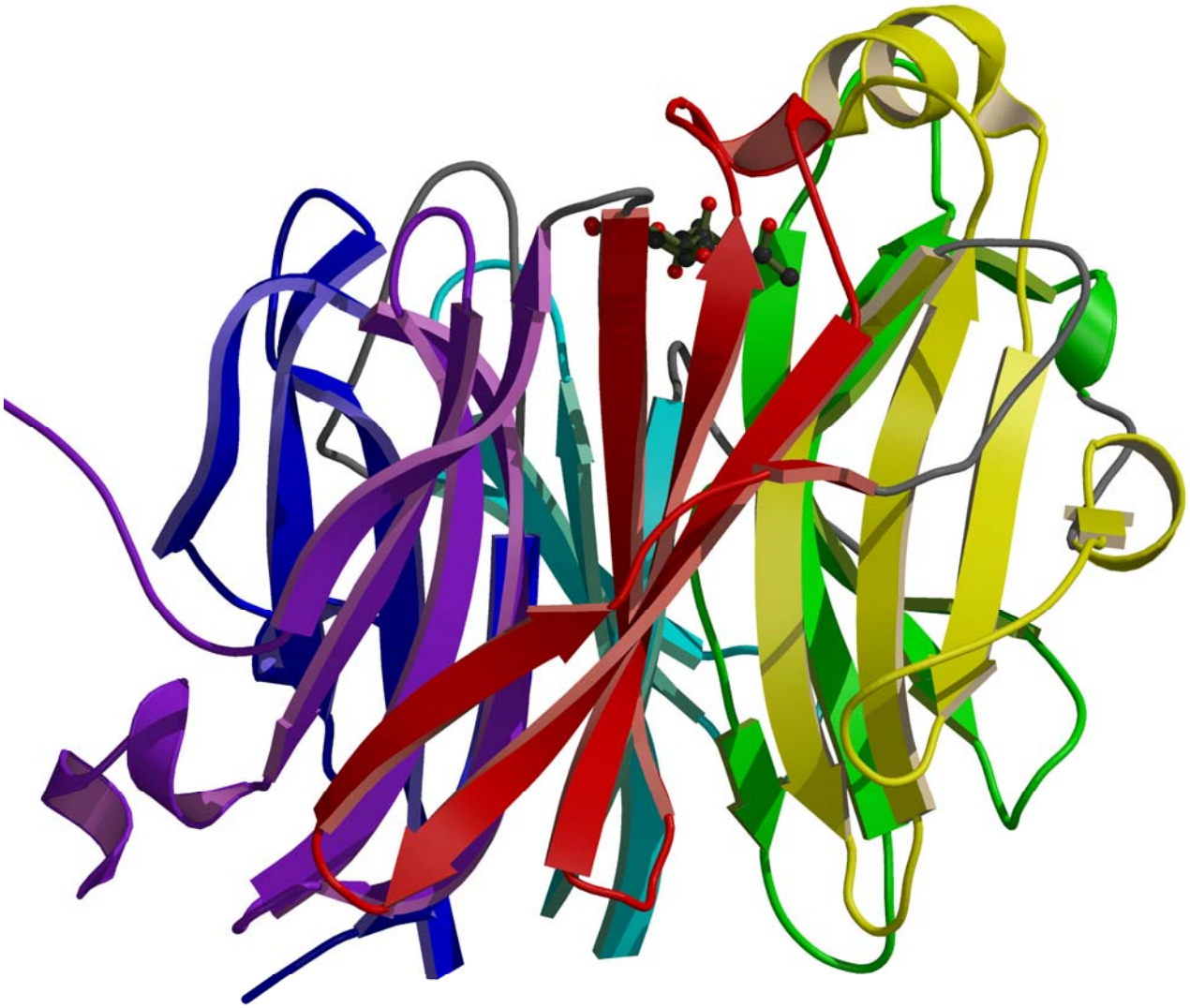
Neu2 apo form



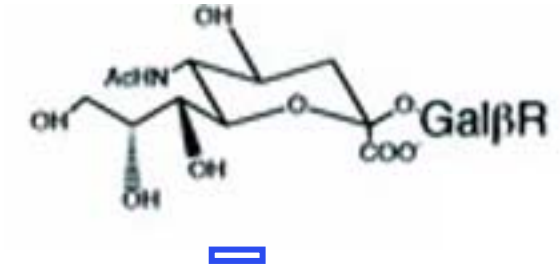
Neu2 monosaccharide induced form

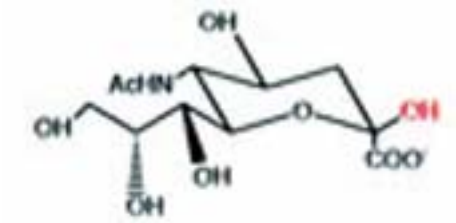
Soaking the crystal in 50 mM of one of the following mono-saccharides, galactose, glucose or maltose, for 4 hours

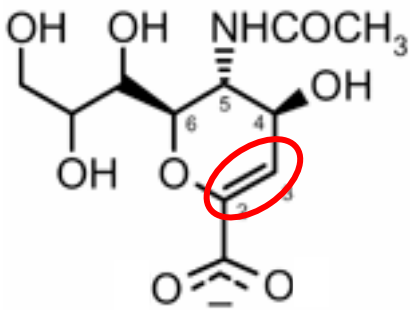
Neu2 in complex with DANA



Inhibitors







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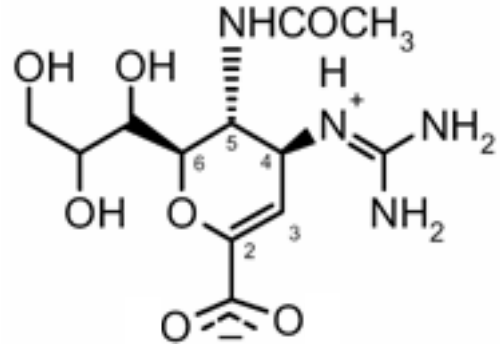
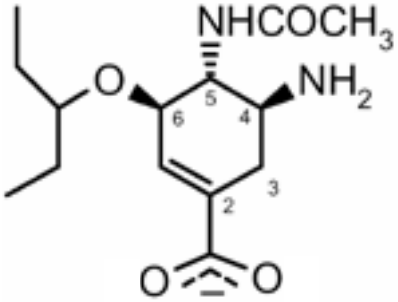
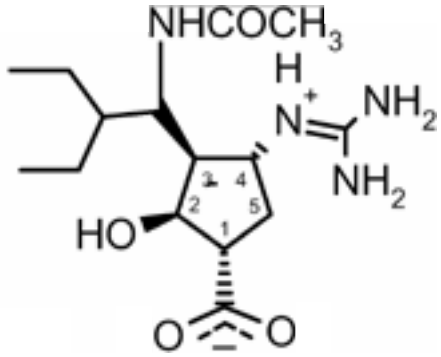


Zanamivir

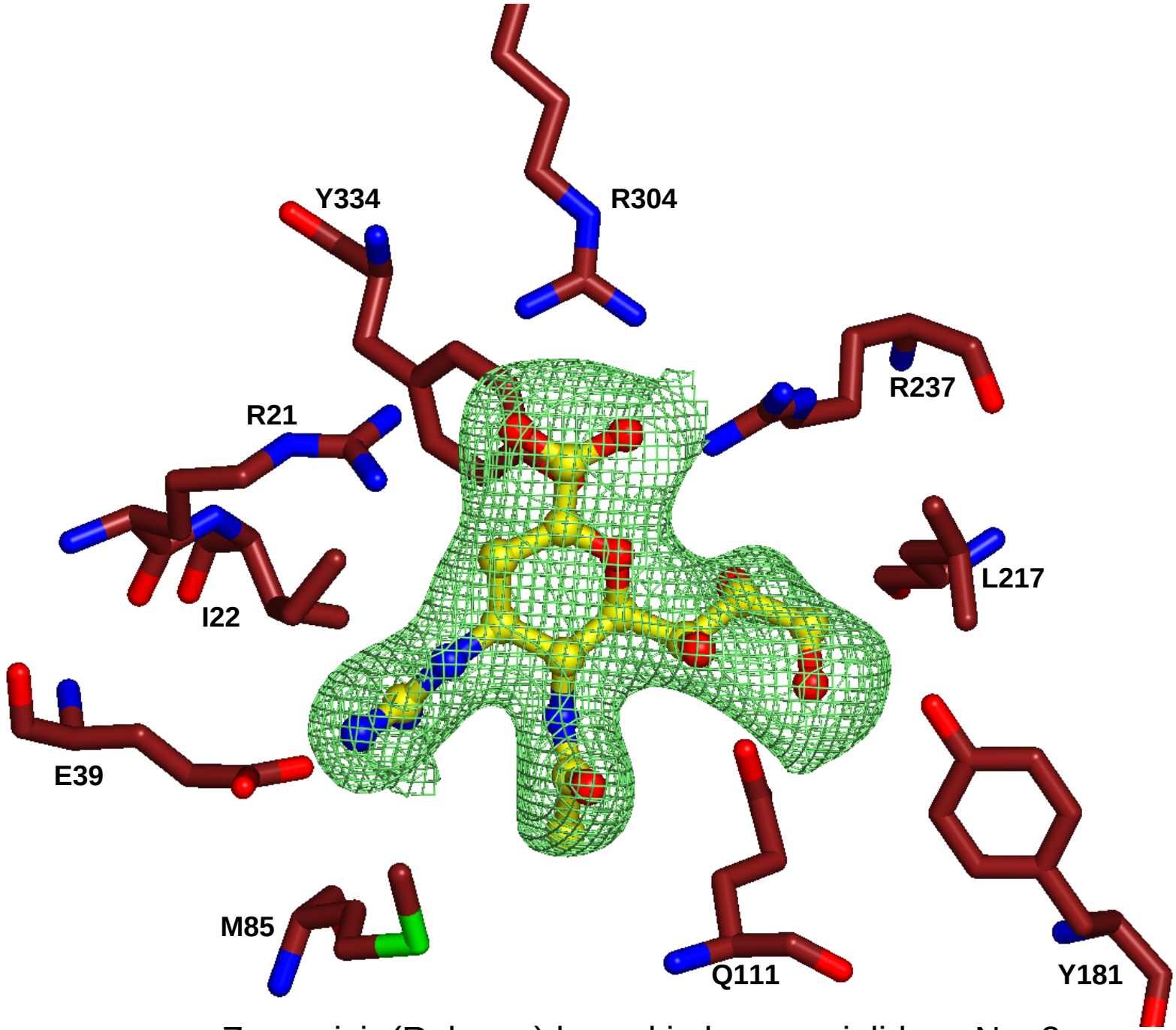


Oseltamivir Carboxylate (derived from Tamiflu)



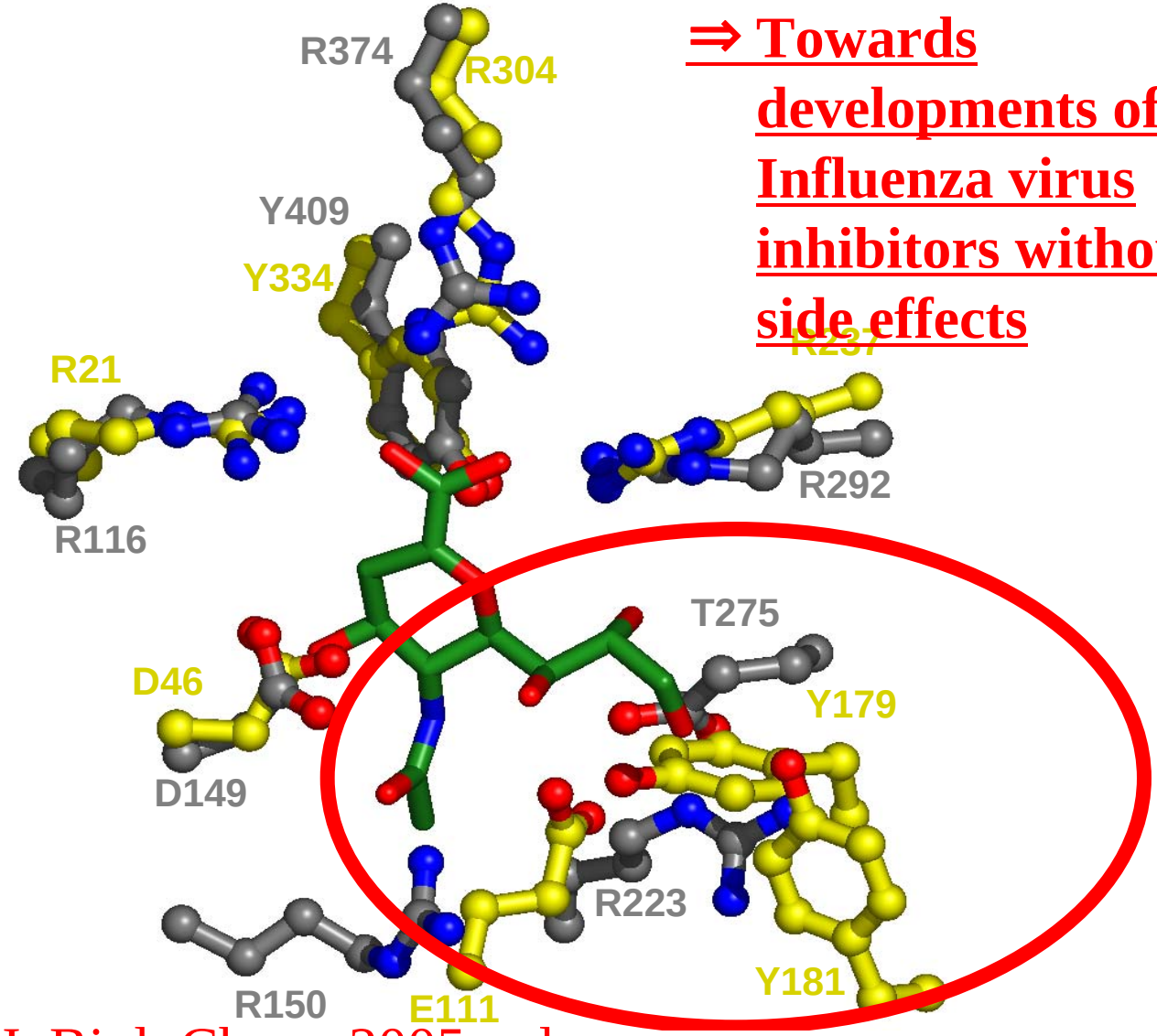
BCX – 1812
RWJ 270201

Structural studies towards development of new inhibitors: collaboration with M.v. Itzstein, Institute for Glycomics, Griffith University, and Peter Colman, Jennifer Mckimm-Breschkin, Australia



Zanamivir (Relenza) bound in human sialidase Neu2

Structural Differences between Human Neu2 and Influenza Virus Neuraminidase



⇒ Towards developments of new Influenza virus inhibitors without side effects

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

Human Neu2

Influenza virus neuraminidase (1VCJ)

Summary and future outlook

- Target oriented structural genomics on protein transport and posttranslational modification: over 254 structures solved for mostly eukaryotic proteins.

  → **More difficult targets (membrane and large protein complexes)**

- → Target Protein Research Project

Target Protein Project (5 years: 2007-2011)

- Proposal deadline: 20 April 2007
- Selection results published: 15 June 2007
- 43 teams selected
- No. of PIs and CoPIs: ~150
- Inauguration meeting: 3 & 4 September 2007, Kyoto!

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

	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 (2006-2011) The Analysis Core

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

PF

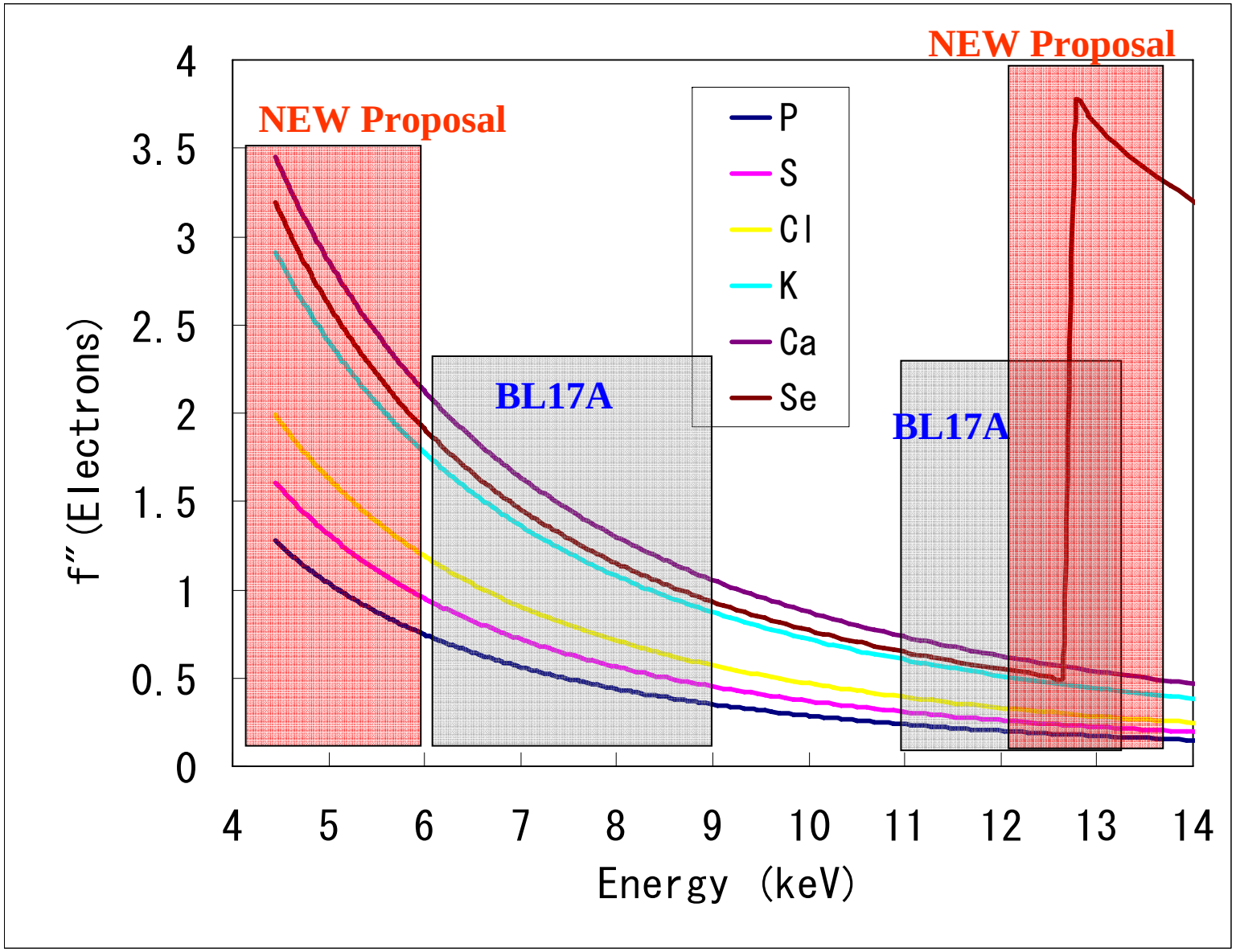
② Microfocus Beam Line Low to medium energy (Invacuum short gap undulator)

① Microfocus Beam Line Medium to high energy

SPring-8

1. To be completed by end of FY 2009
2. Compatibility: SPring-8 and PF robots

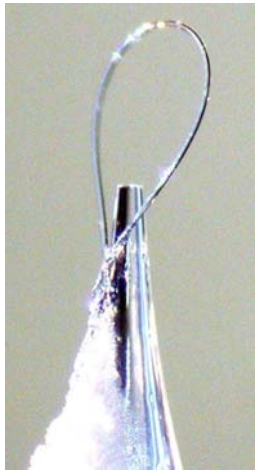
In-vacuum Short Gap Undulator Microfocus Beam Lines: softer X-rays

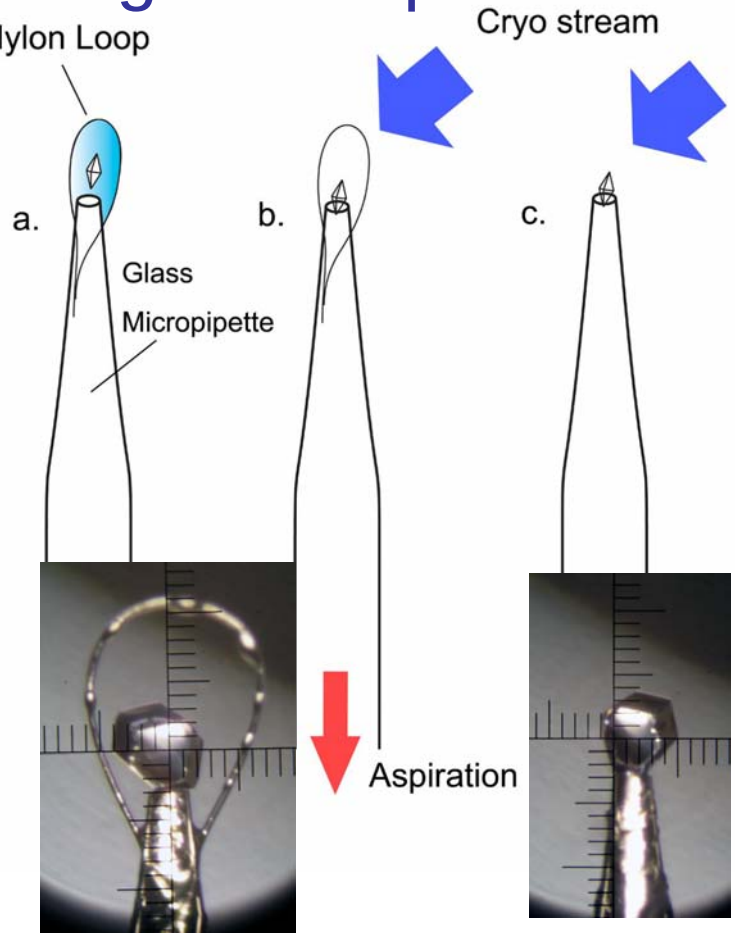


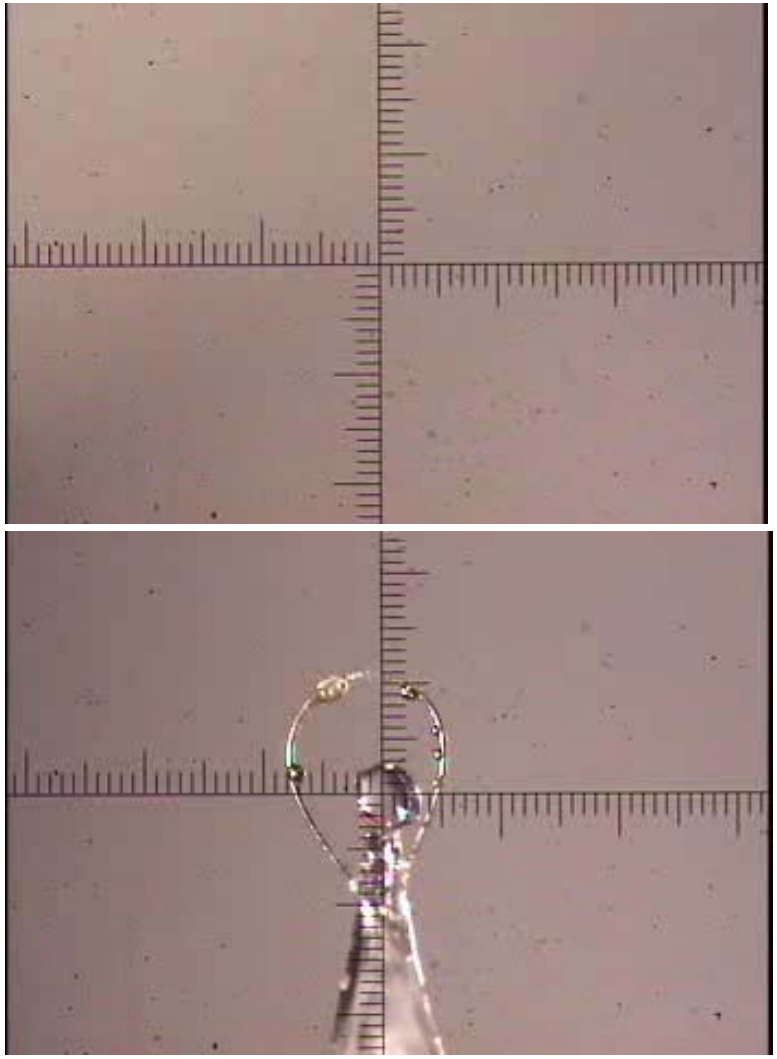
Loopless crystal mounting method

Towards lower background:

Promising techniques developed by other groups.







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

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

Acknolwledgements

Beam Line developments & robotics

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Neu2

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