Structural Biology with X-FEL and ERL: expectations and limitations

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Outline

I. Structural genomics and structural biology

- **New national project of Protein 3000**
- **Target oriented structural genomics on protein modification and transport of proteins**

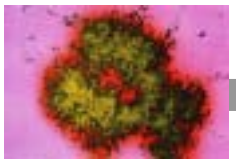
II. Limitations of the current methods

- **Beam line development and high-throughput R&D**

III. Key issues in realizing single particle and nano-crystal structural biology

Part I Role of Structural Biology & Structural Genomics

Genome



DNA→RNA→ **Proteins**

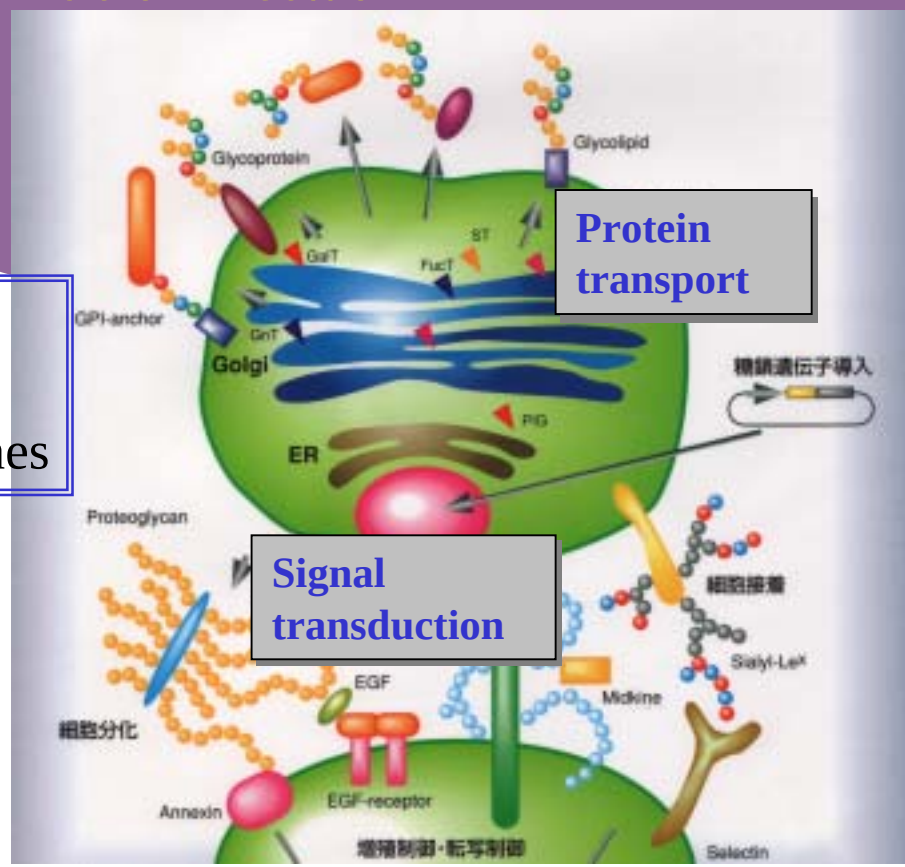
Molecular machines
(ribosomes, enzymes)

Information network

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

Biology
(Basic research)

Medicine, drug design
(industrial applications)



Currently 35 Structural Genomics Projects Worldwide

Internationl Structural Genomics Organization

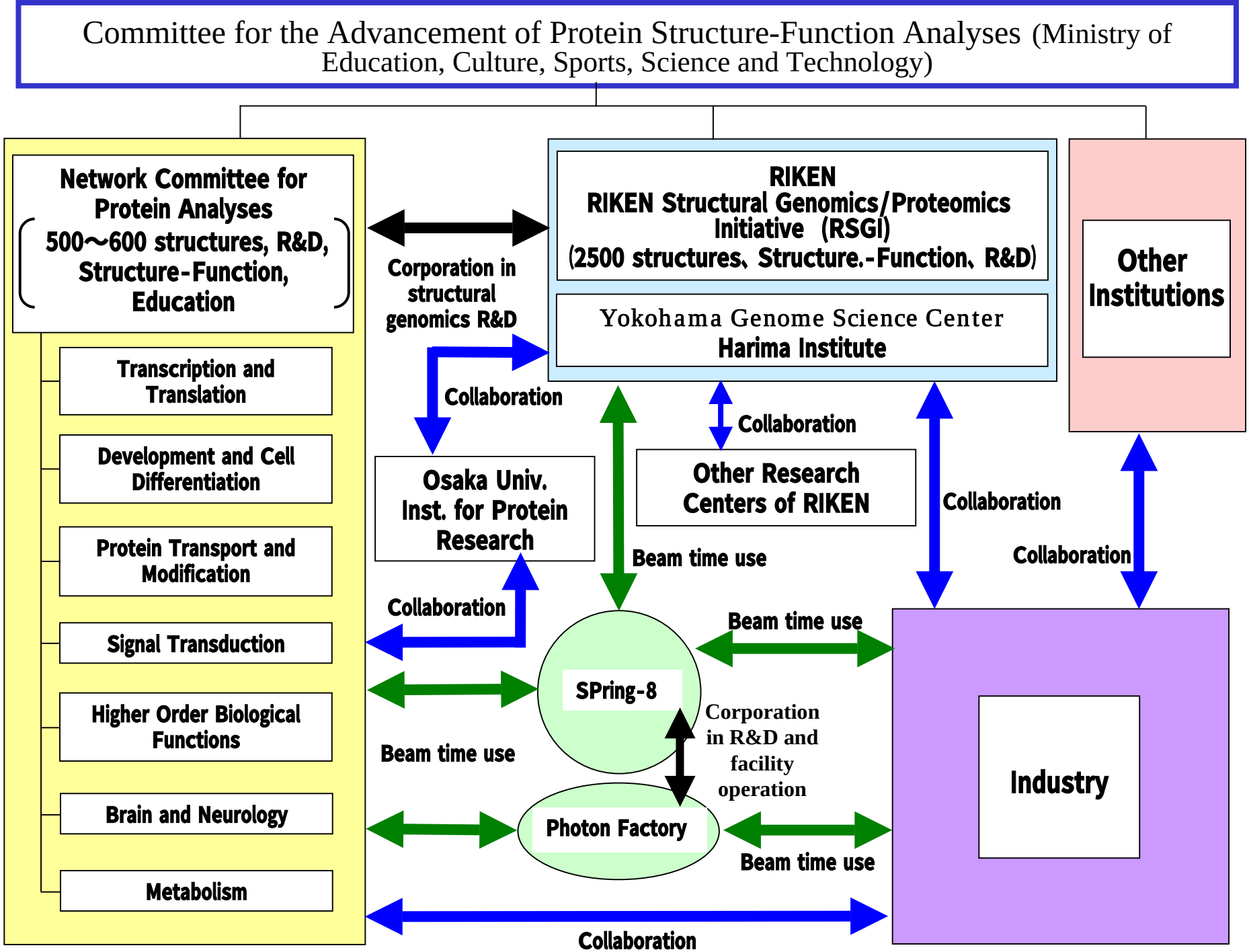
http://www.isgo.org

Structural Genomics and Proteomics Project list

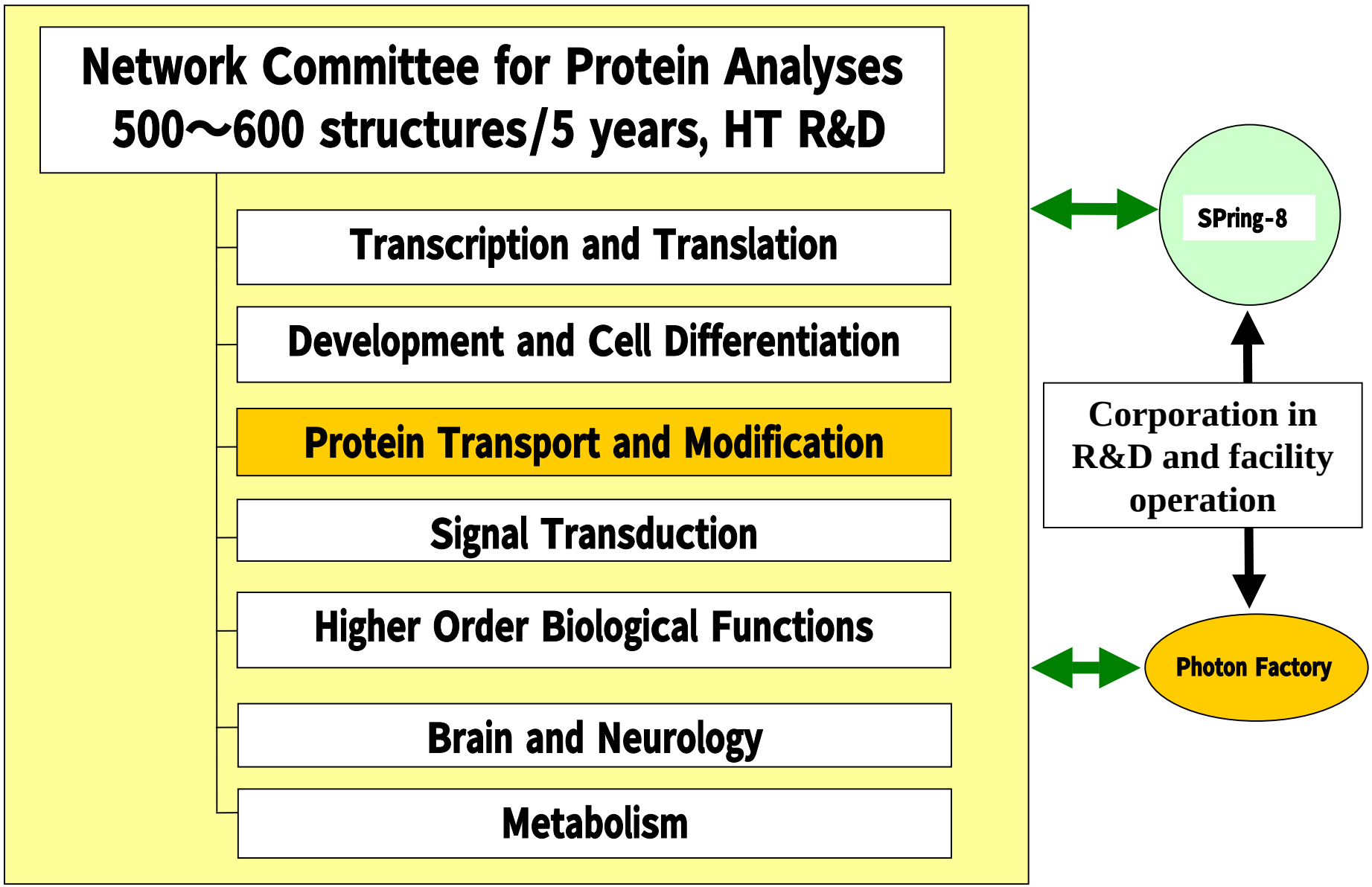
-Worldwide Initiatives -

Australia / Canada / EU / France / Germany / Japan / Korea / Switzerland / UK / USA

Australia:	**3 planned**	**Canada**	**4**
EU	**1**	**France**	**4**
Germany	**1 & 1 planned**	**Japan**	**10**
Korea	**1**	**Switzerland**	**1**
UK	**3**	**USA**	**10**


Committee for the Advancement of Protein Structure-Function Analyses (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
Collaboration
Collaboration
Osaka Univ. Inst. for Protein Research
Other Research Centers of RIKEN
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

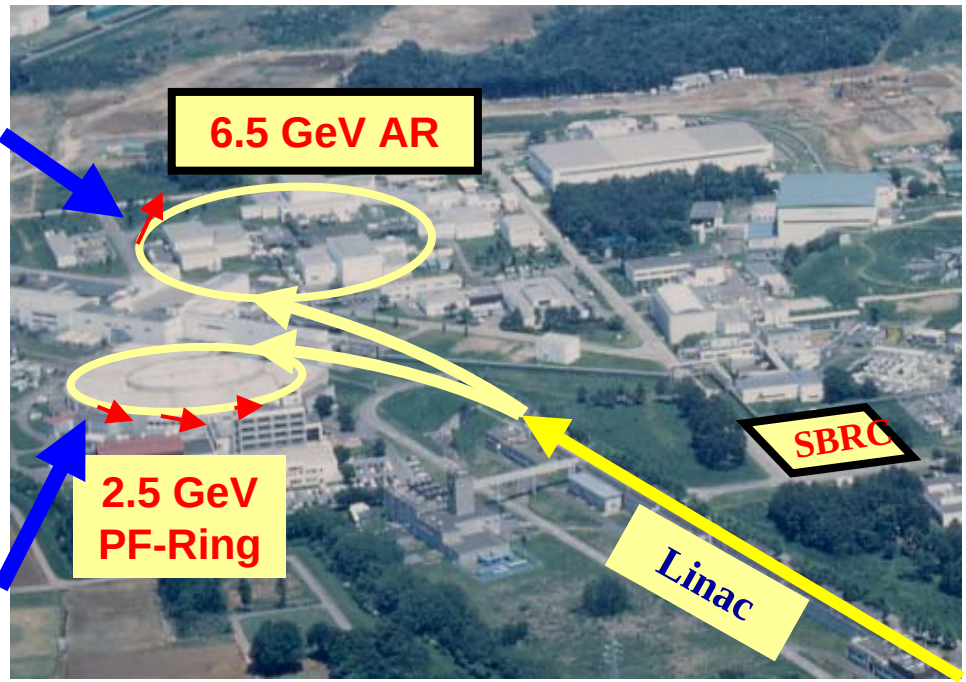
Target oriented structural genomics consortia of universities and national institutes



KEK-PF Strcutural Biology Reseach Center

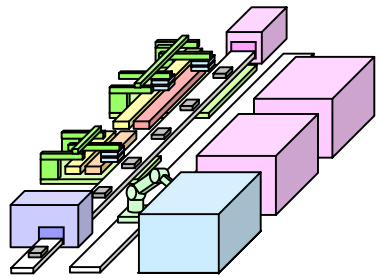
AR NW12

PF BL5

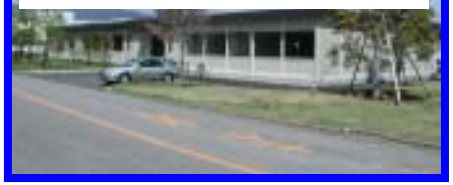


Crystallization

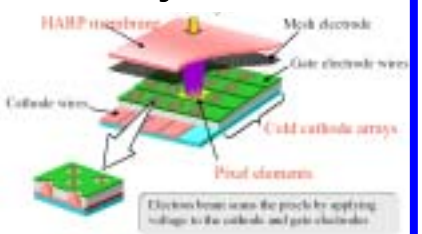
200,000 trials/day



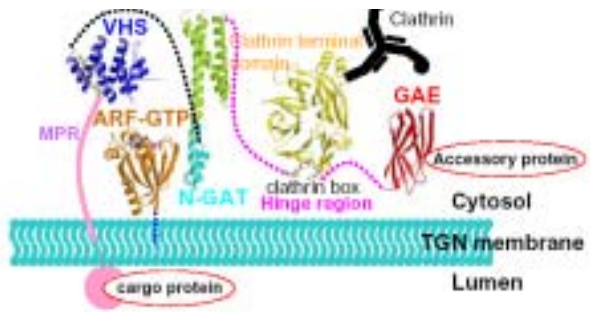
Newly extended building



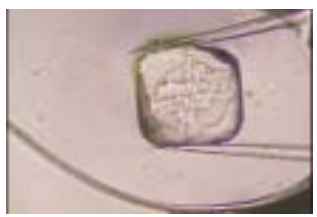
Next generation detector: X-ray-HARP



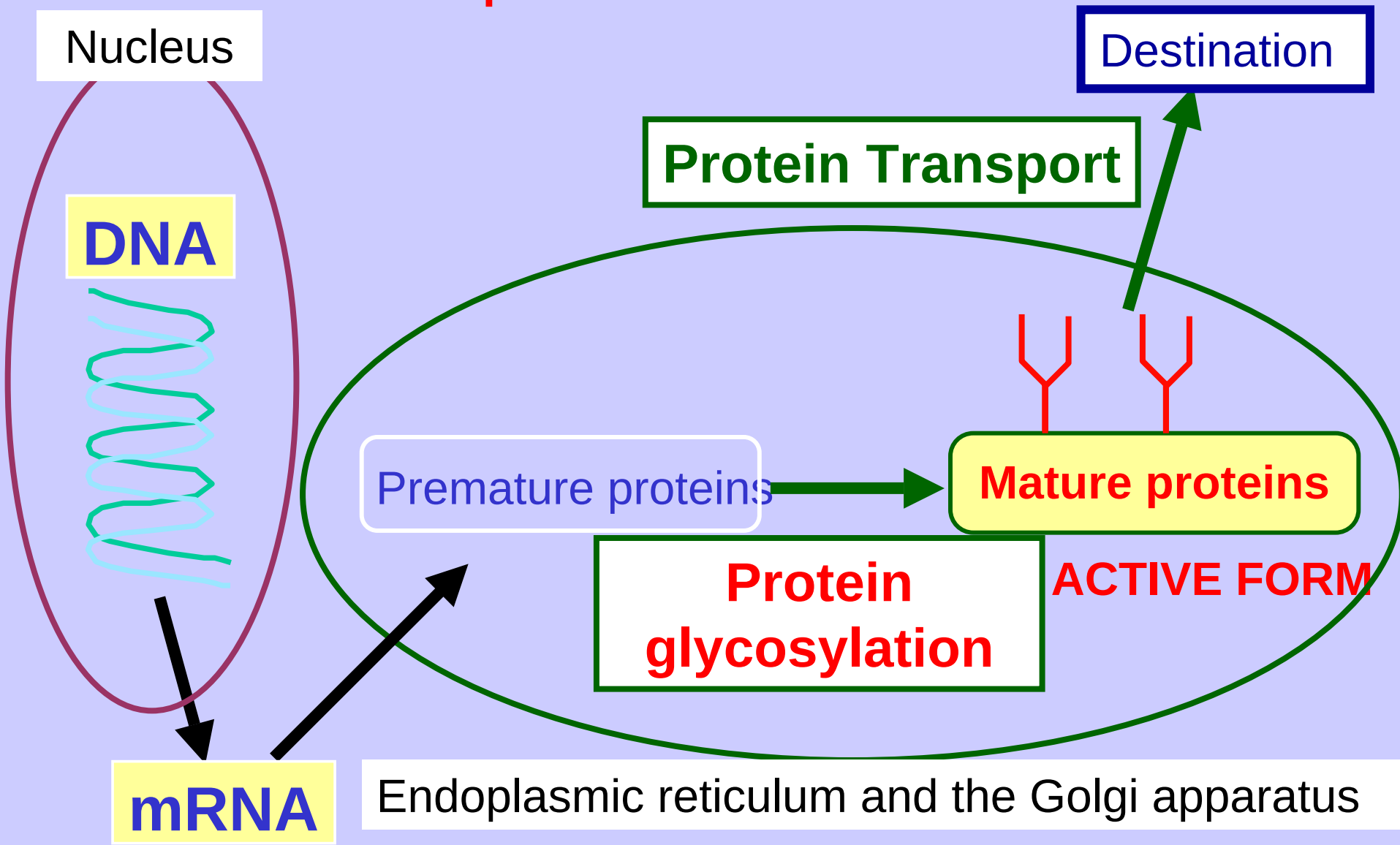
Post-translational modification and transport of proteins

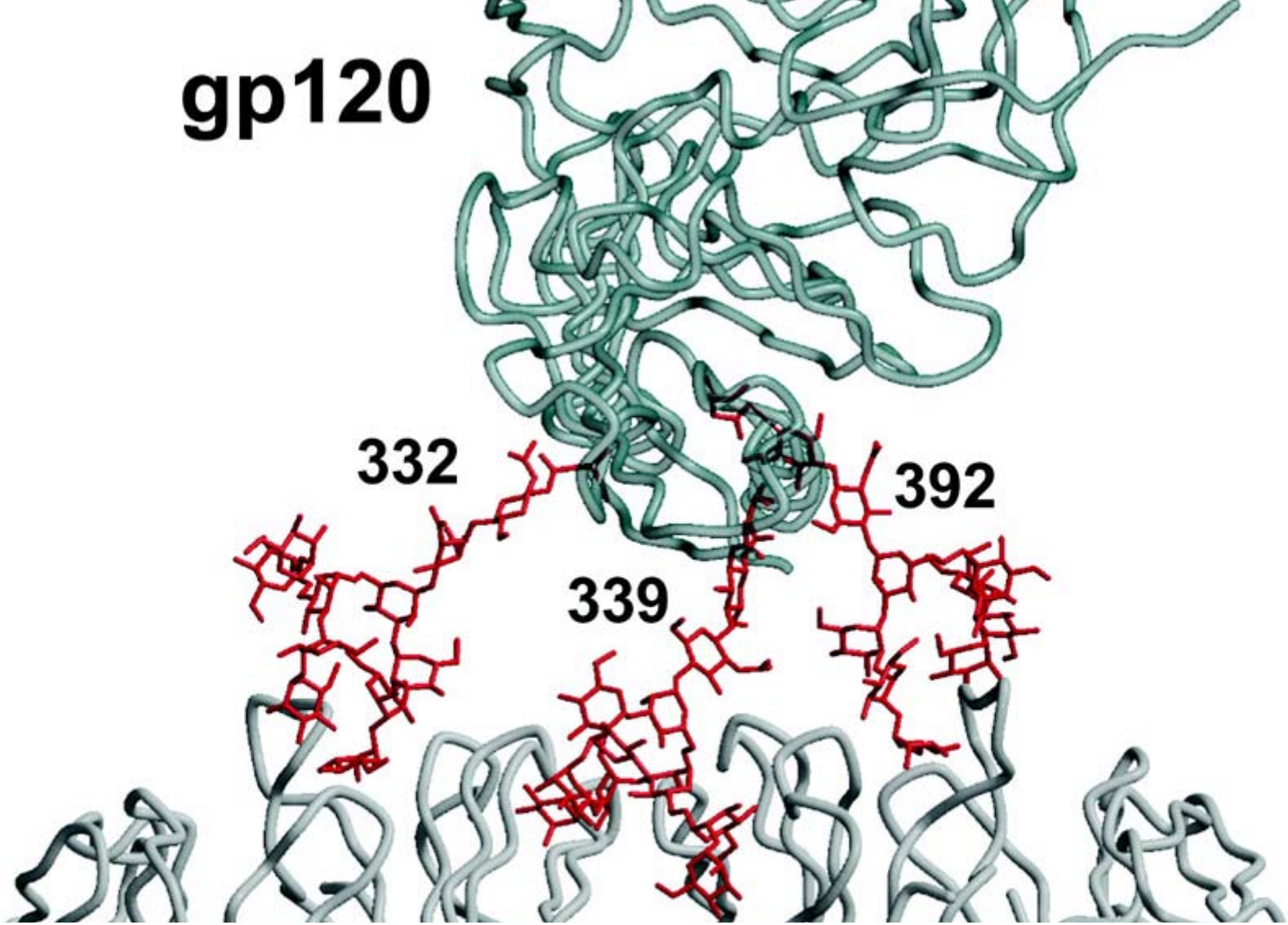


Micro-manipulator



More than half of our proteins are modified with oligosaccharides (glycosylated) to become mature proteins and transported to correct destinations





Model of 2G12 glycan recognition of gp120. On the basis of our model, three separate $Man_9GlcNAc_2$ moieties, shown in red (two in the primary combining sites and one in the V_H/V_H' interface), potentially mediate the binding of 2G12 to gp120. D.A. Calarese et al., *Science* 2003 June 27; 300: 2065-2071.

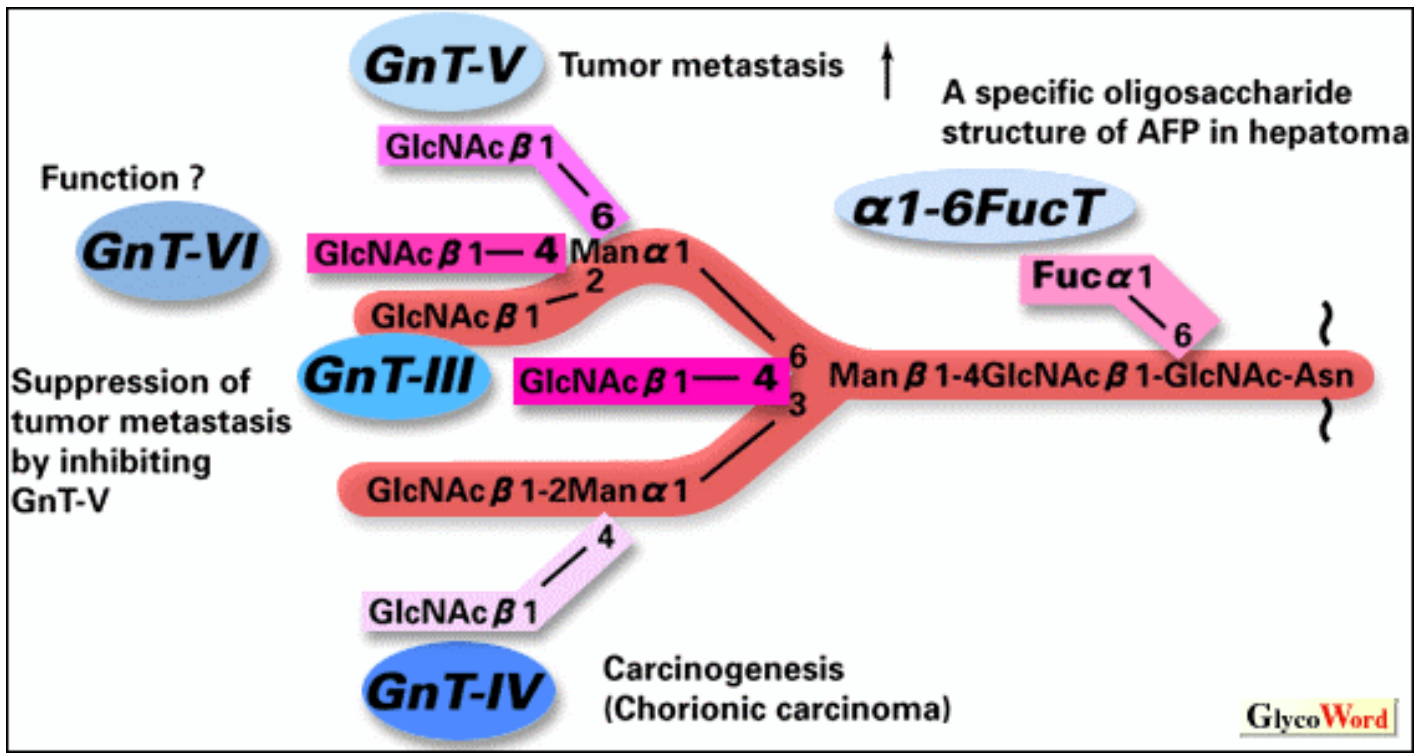
Target oriented structural genomics!

(first proposed in Aug. 2000)

Protein modification is closely regulated by the cell's transport mechanism.

We chose protein glycosylation and protein transport as the target of the structural genomics project.

Development of technologies for production of glycosylated human proteins in their active forms.

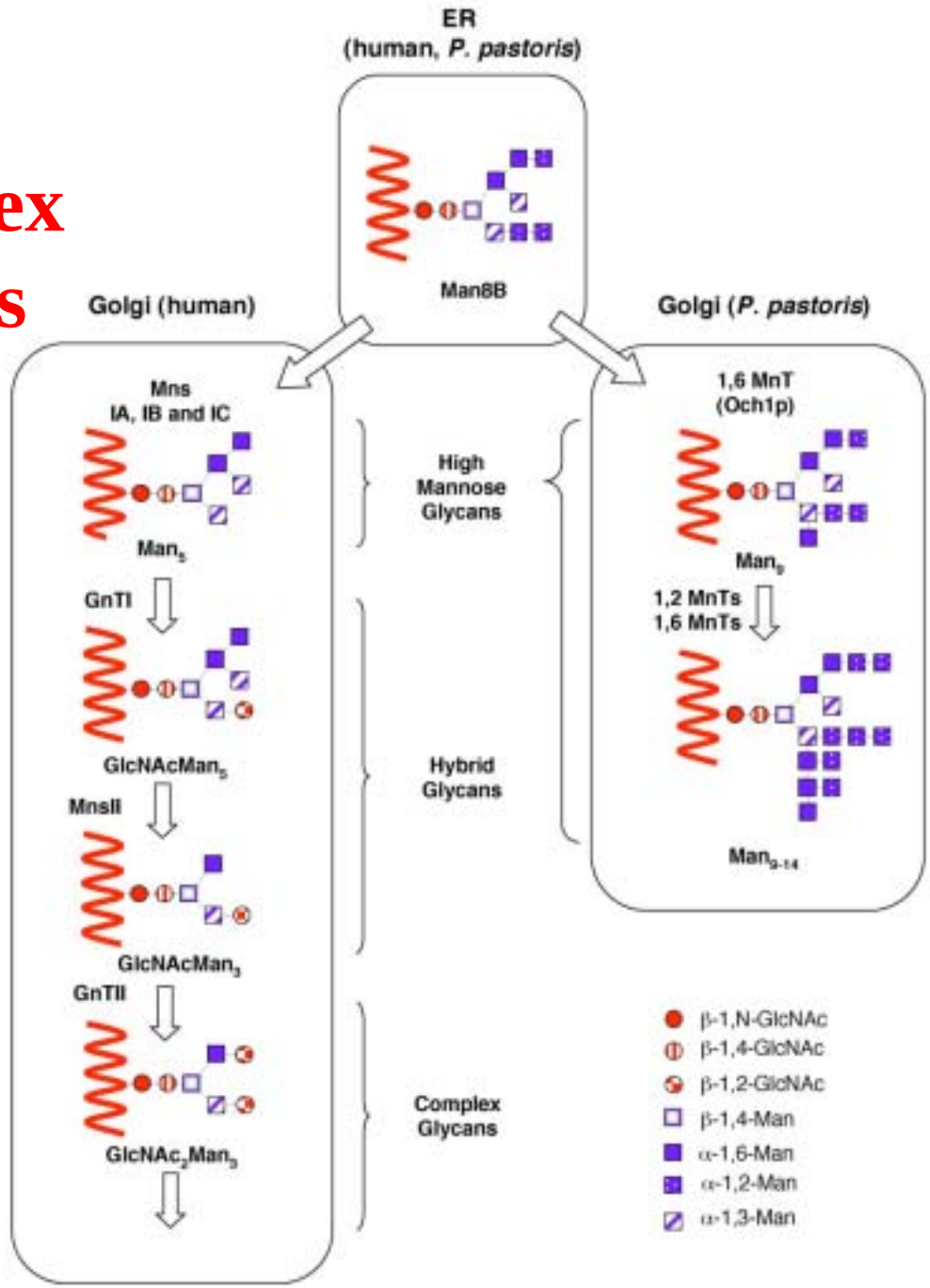


Production of Complex Human Glycoproteins in Yeast

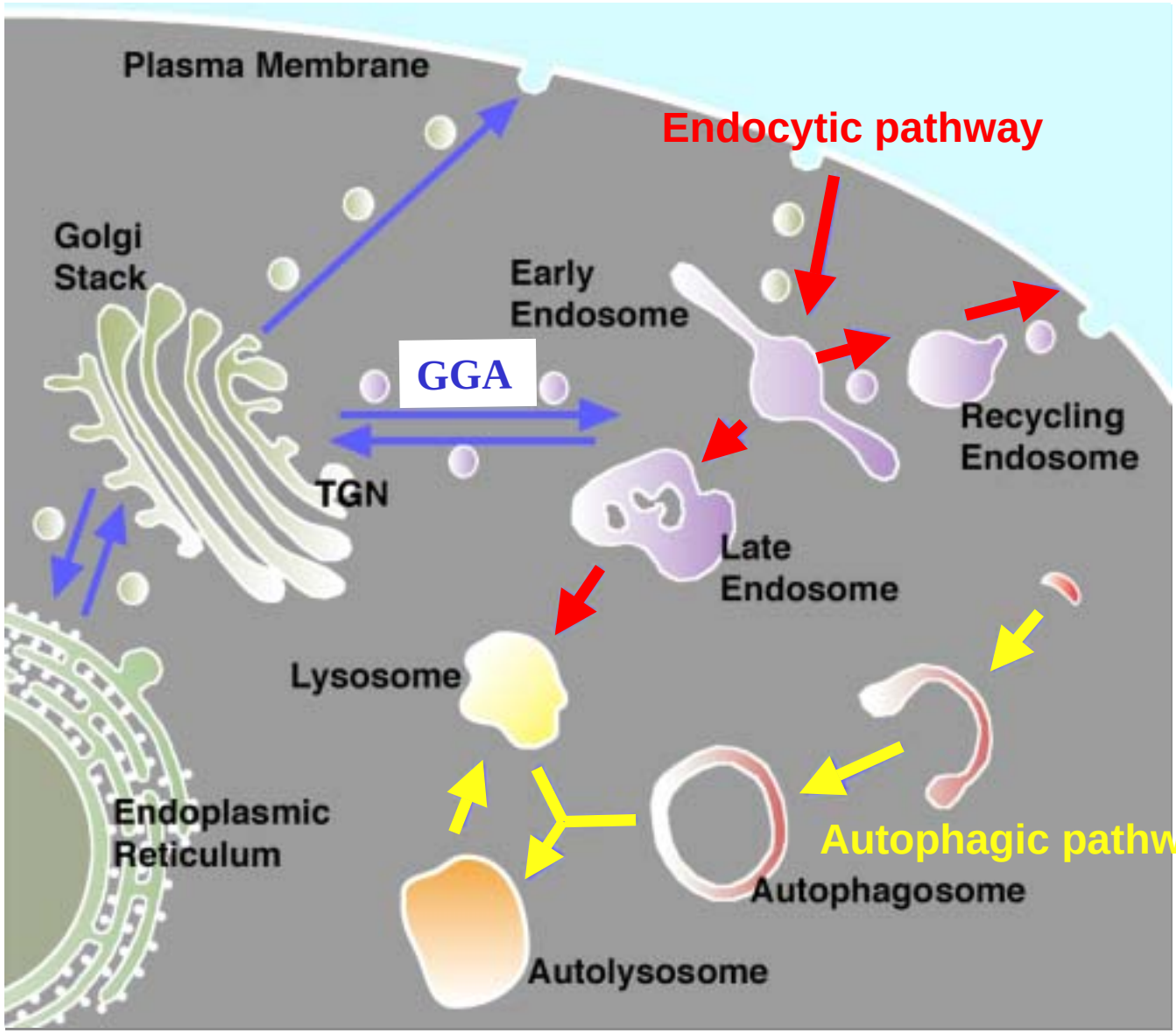
Stephen R. Hamilton et al.

Science 2003 301: 1244-1246.
29 Aug 2003

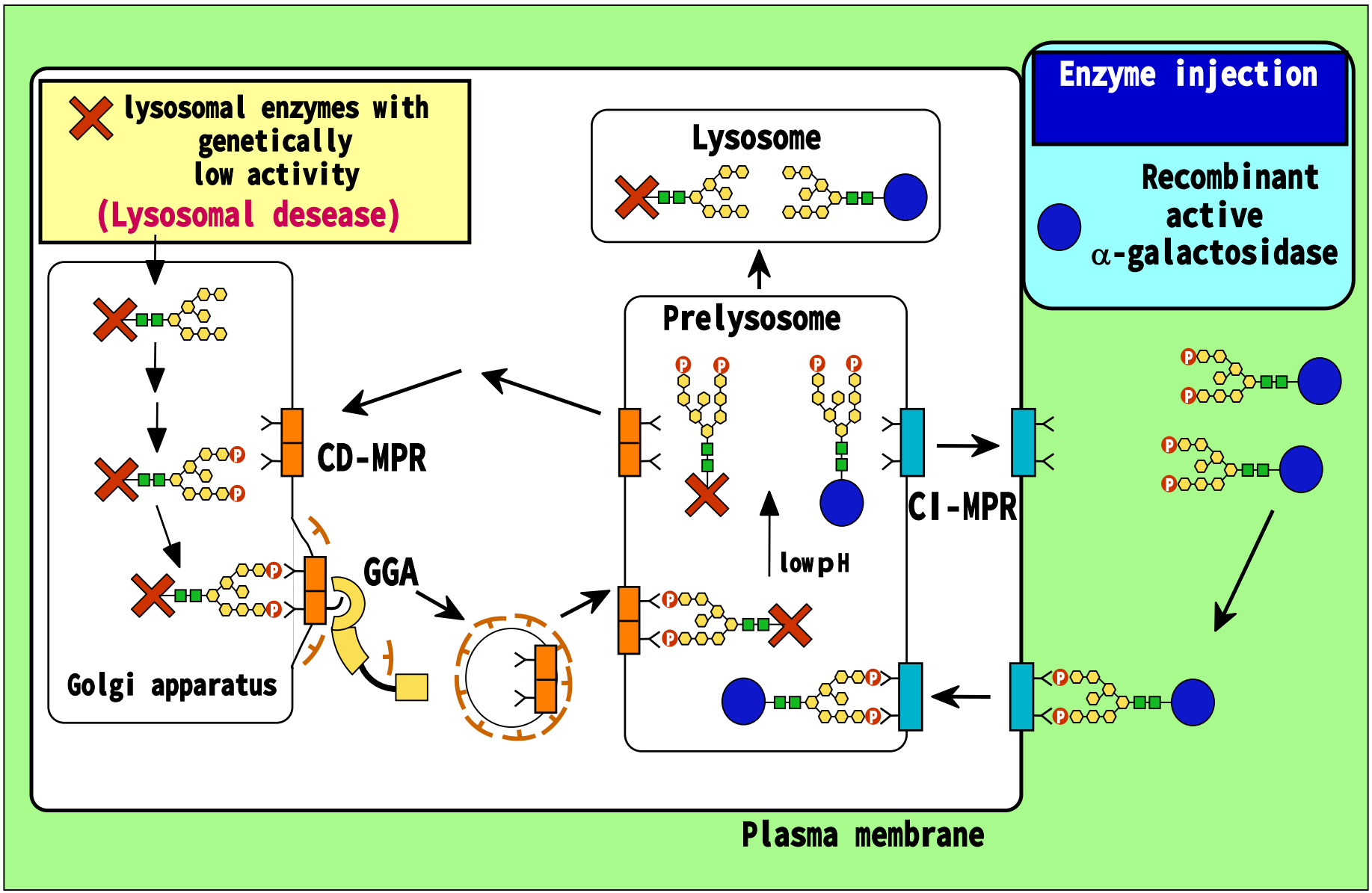
New frontiers of highly effective drugs



Lysosomal Function Depends on Membrane Traffic

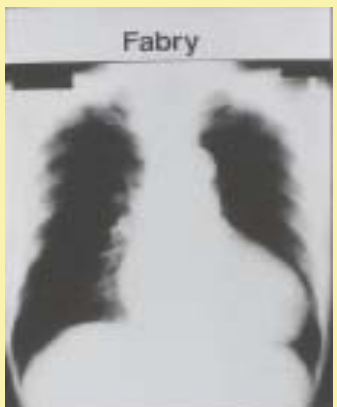


Treatment of lysosomal deseases

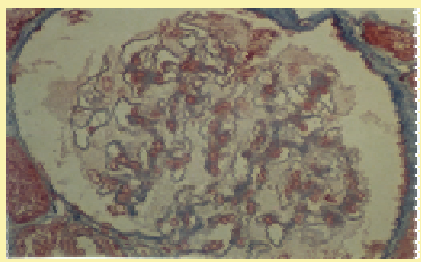


Fabry Disease and Enzyme Replacement Therapy

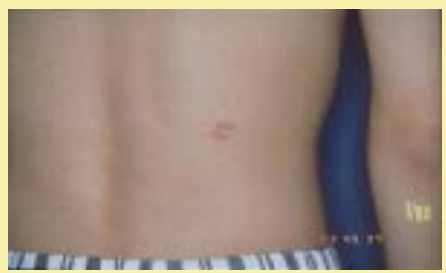
Fabry disease：A disease caused by mutation of α-galactosidase gene, which degrades enzymatic activity of the hydrolase in lysosome leading to accumulation of glycolipids



Enlargement of the heart

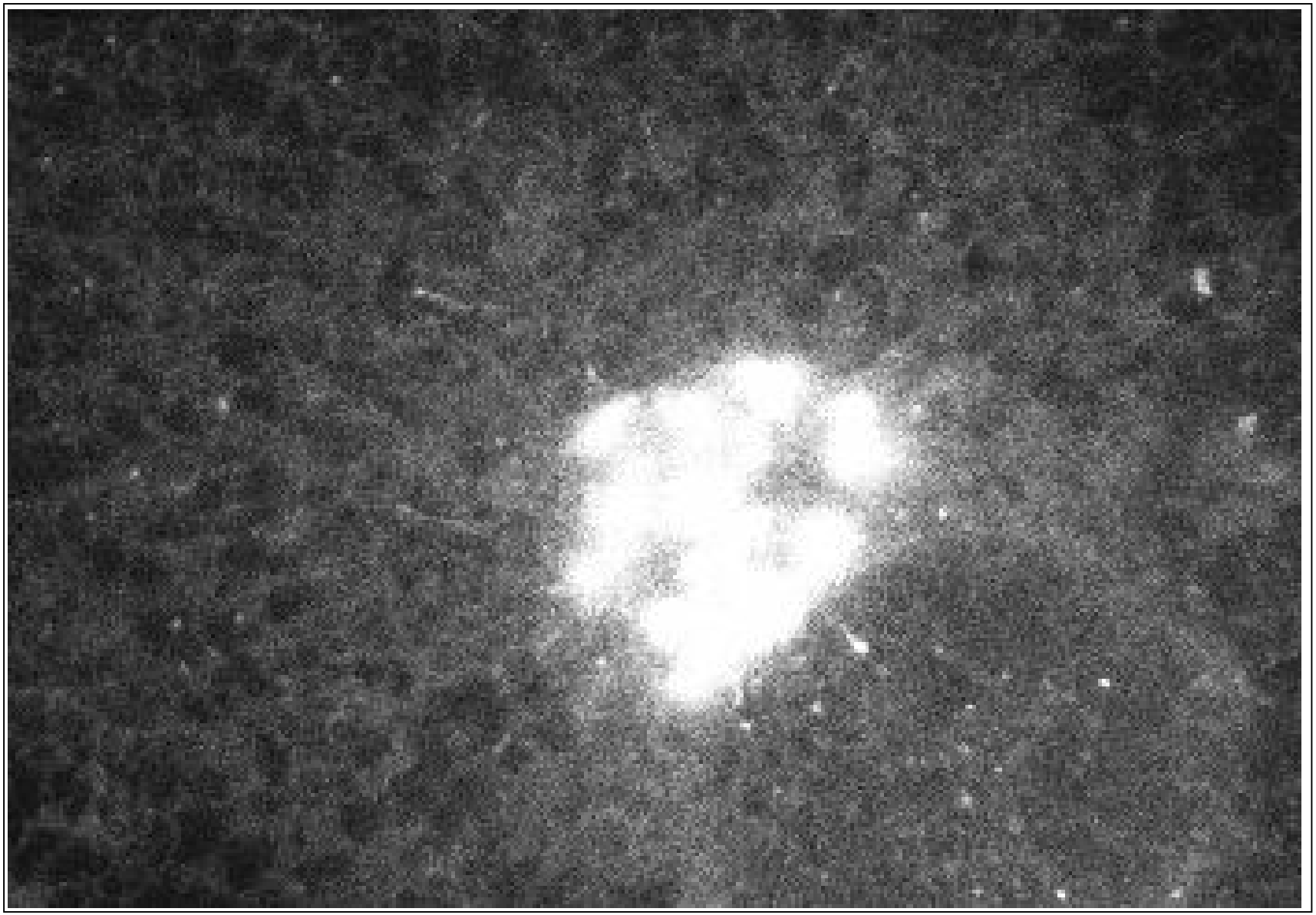


Accumulation of glycolipids in glomerulus

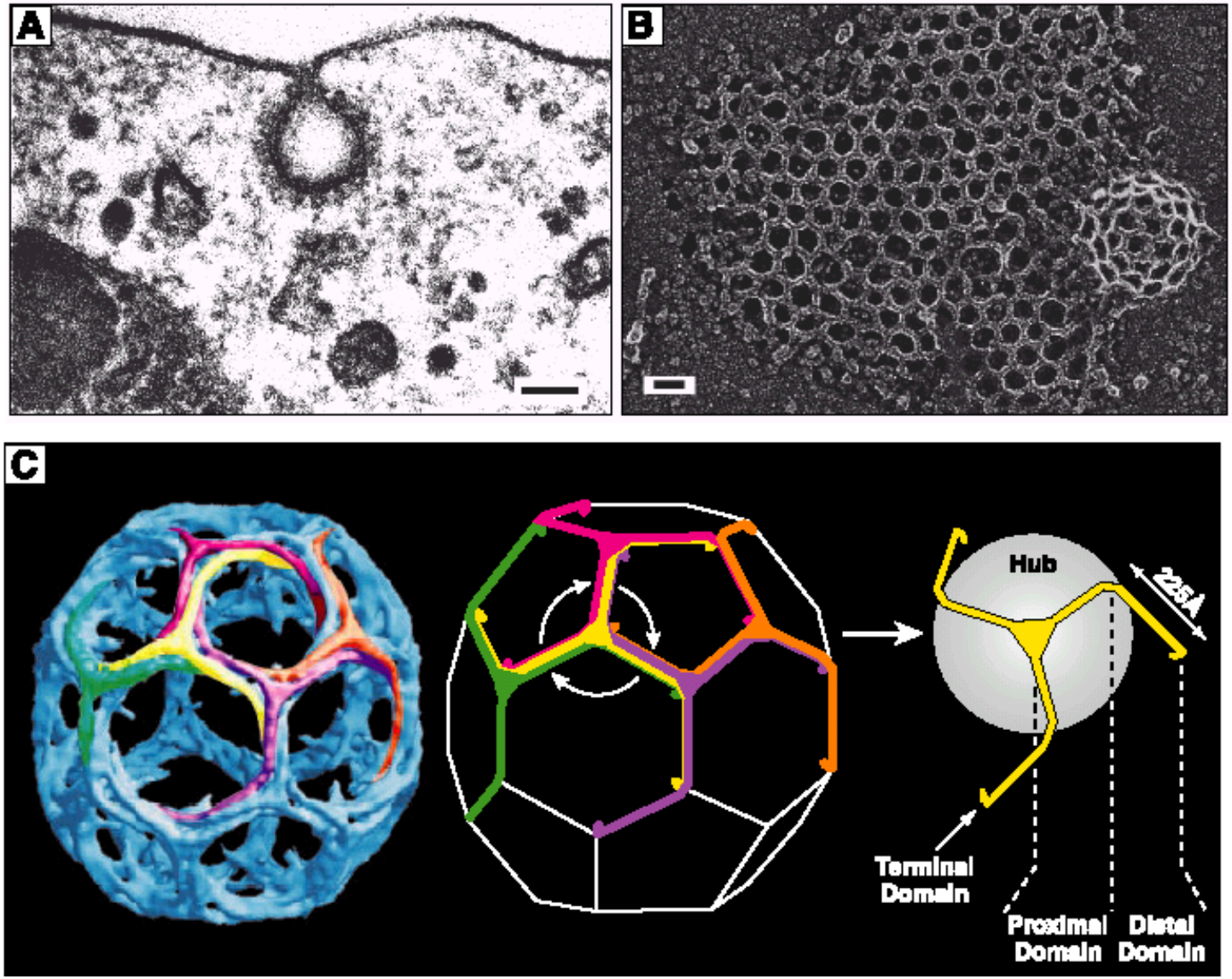


Angiokeratoma

Vesicle transport from the ER to the Golgi apparatus



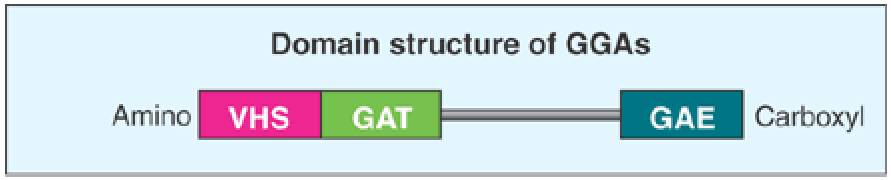
Lippincott-Schwartz, J. (1998) MBC 9, 1617

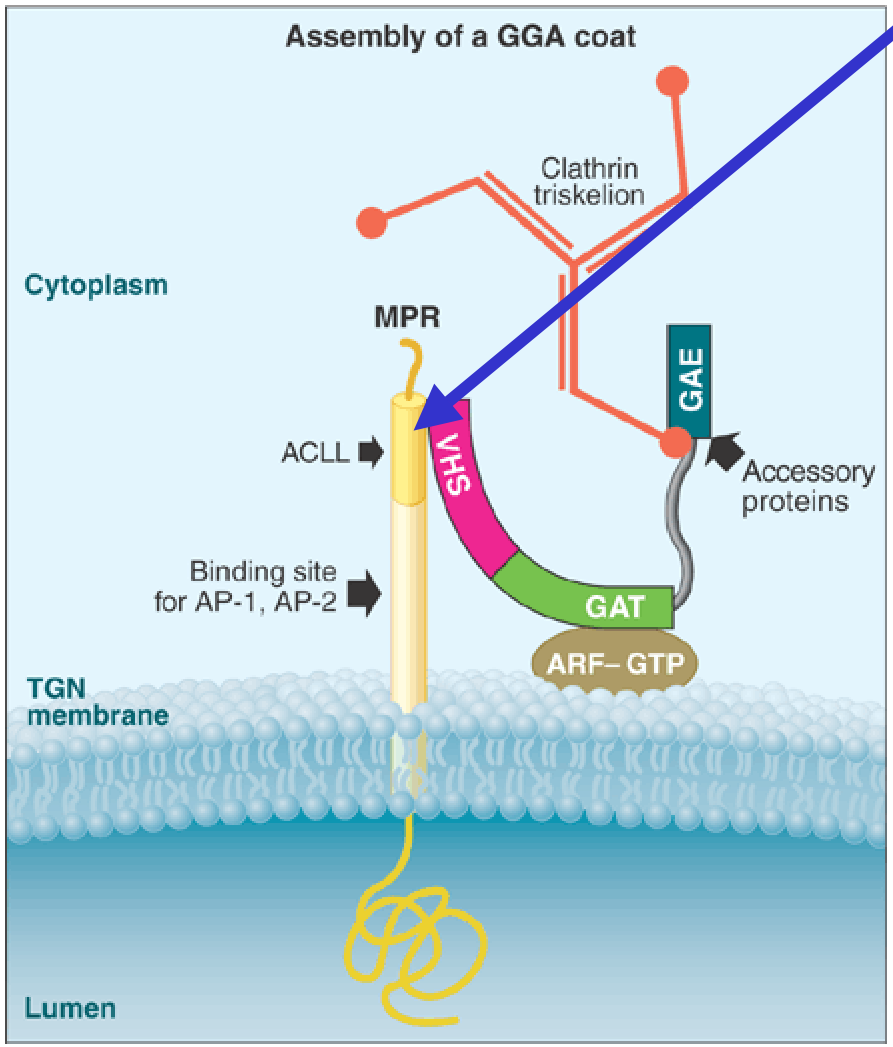


M. Marsh and H. T. McMahon, Science, 1999, Vol. 285, 215

Clathrin movie by Allison Bruce, Harvard University

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





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

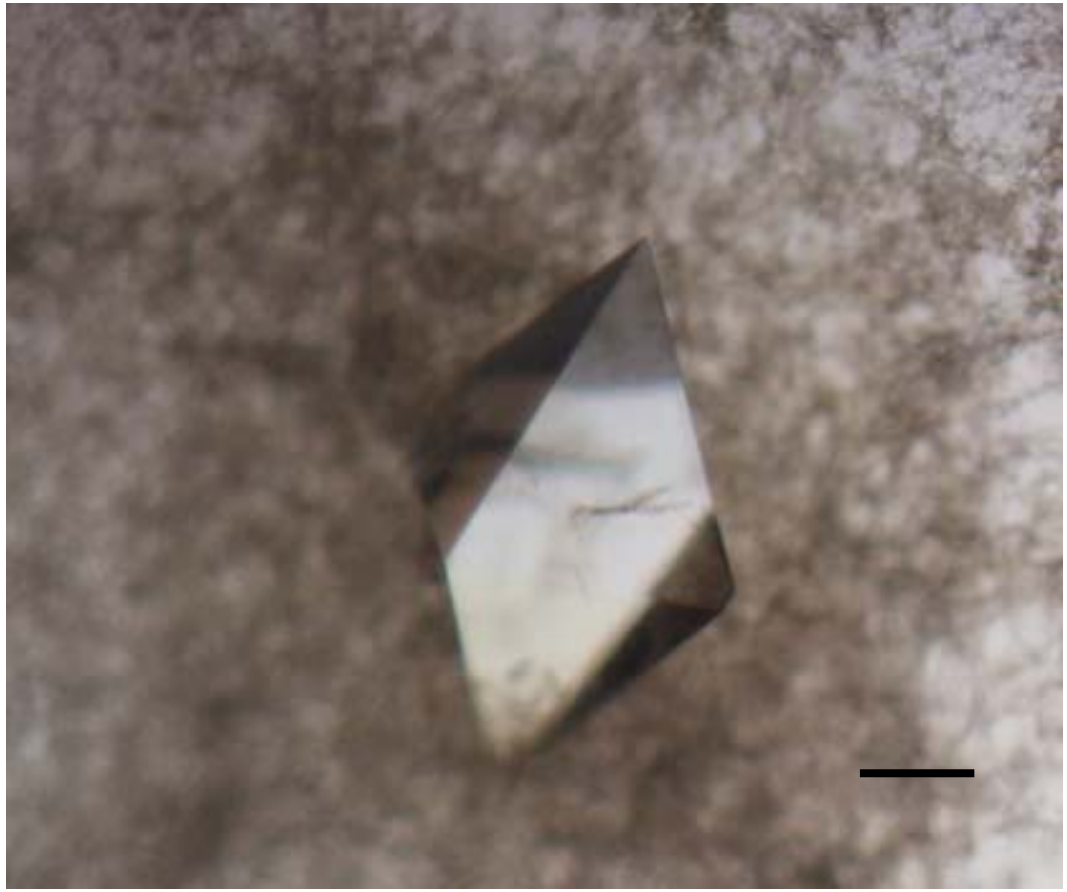
Blue: leucine pairs

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 °C

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

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

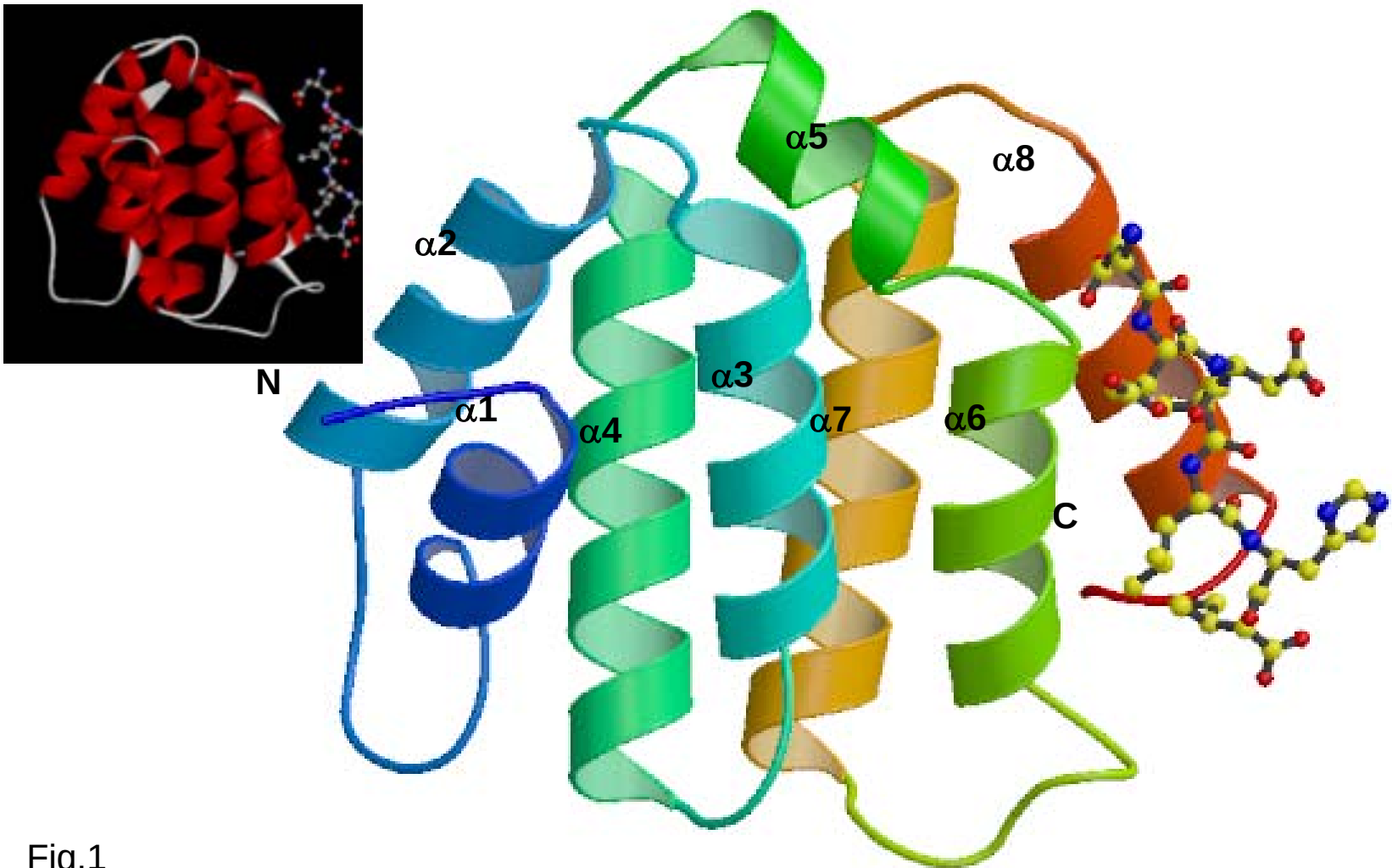


Fig.1
Ribbon diagram of VHS domain of human GGA1 complex with M6PR peptide. The peptide molecule is shown as a ball-and-stick model colored according to atom type (nitrogen, blue; carbon, yellow; oxygen, red).

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*
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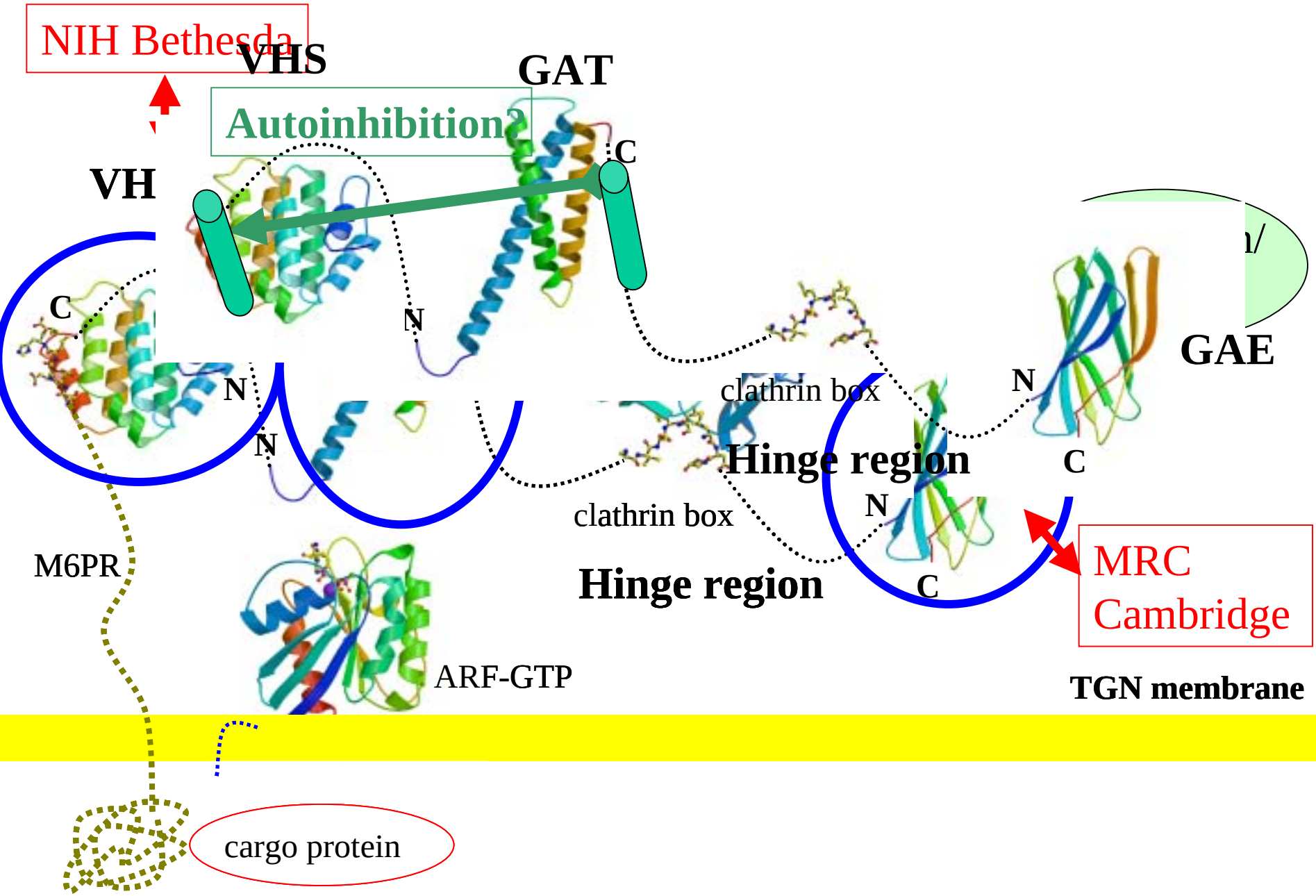
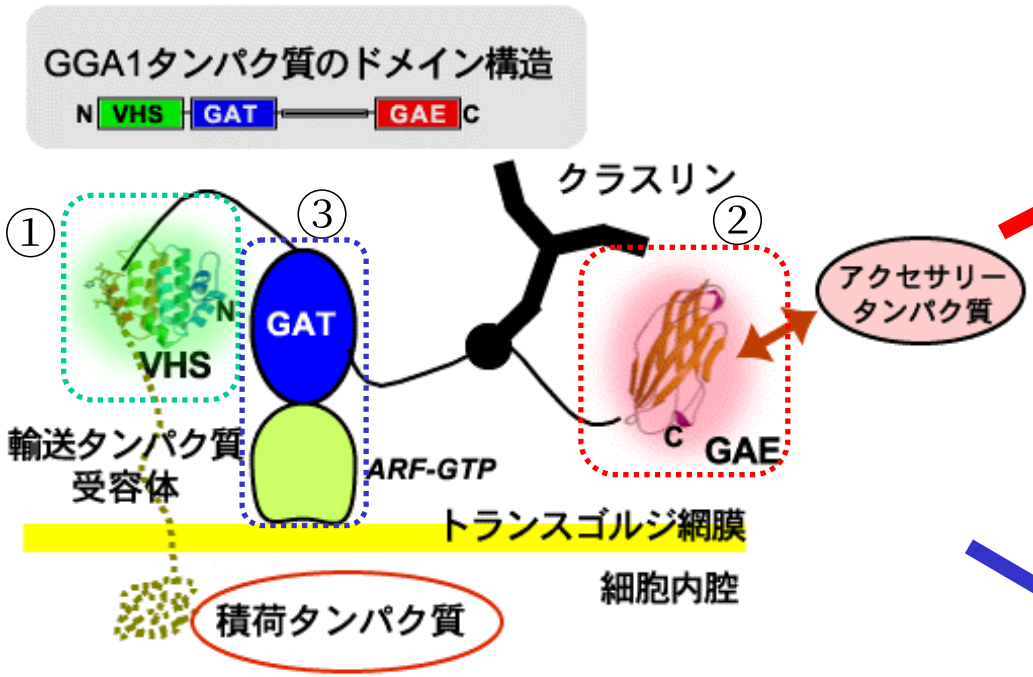
‖ *Advanced Light Source, Berkeley, Berkeley Center for Structural Biology, Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, California 94720, USA*
‡ *These authors contributed equally to this work*

pp 937-941

Model for the assembly of GC



Future Directions of structural and functional analyses of posttranslational modifiation and intracellular transport



X-ray Small Angle Scattering

M. Kataoka (Nara)

Oligosaccharide modification and recognition

↓

Combination of X-ray, NMR and Mass Spec

High resolution structural analysis of single molecules

↓

ERL/X-FEL

GGA domains are far apart! May be impossible to crystallize the entire protein? What else can we do?

Small Angle X-ray scattering?

Energy Recovery Linac (Next generation SR)?

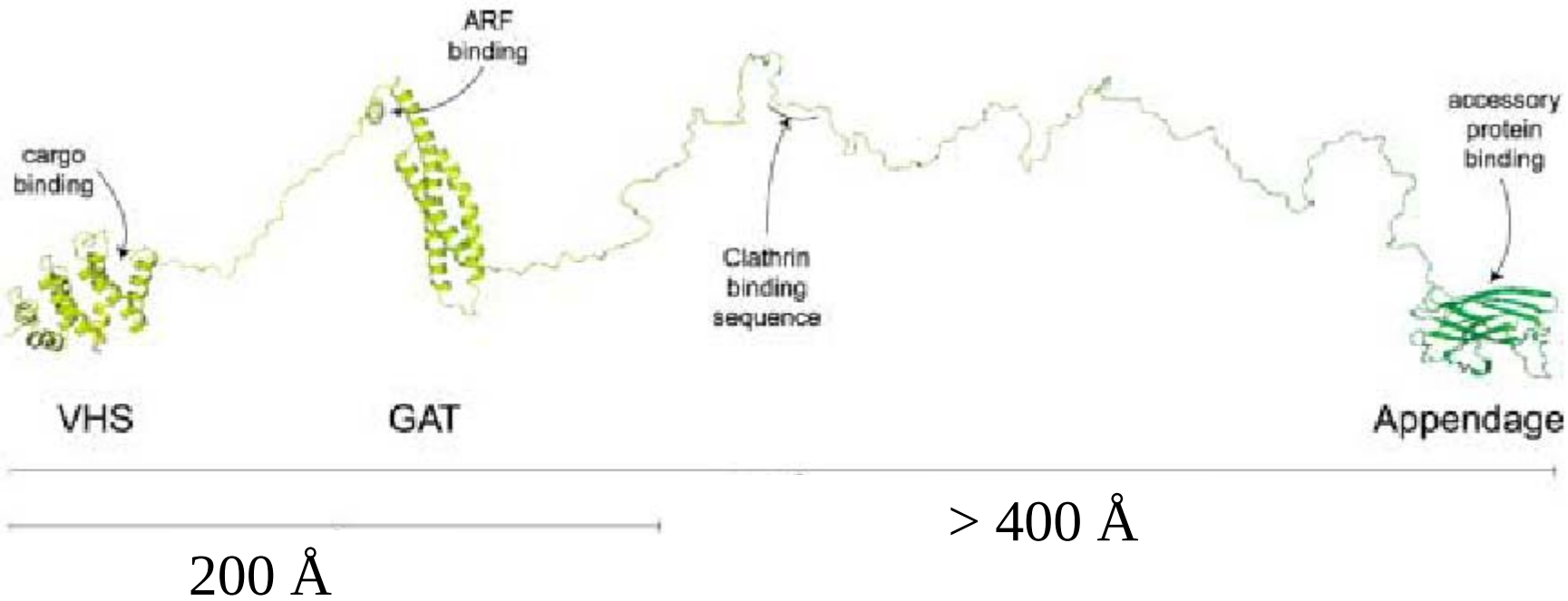
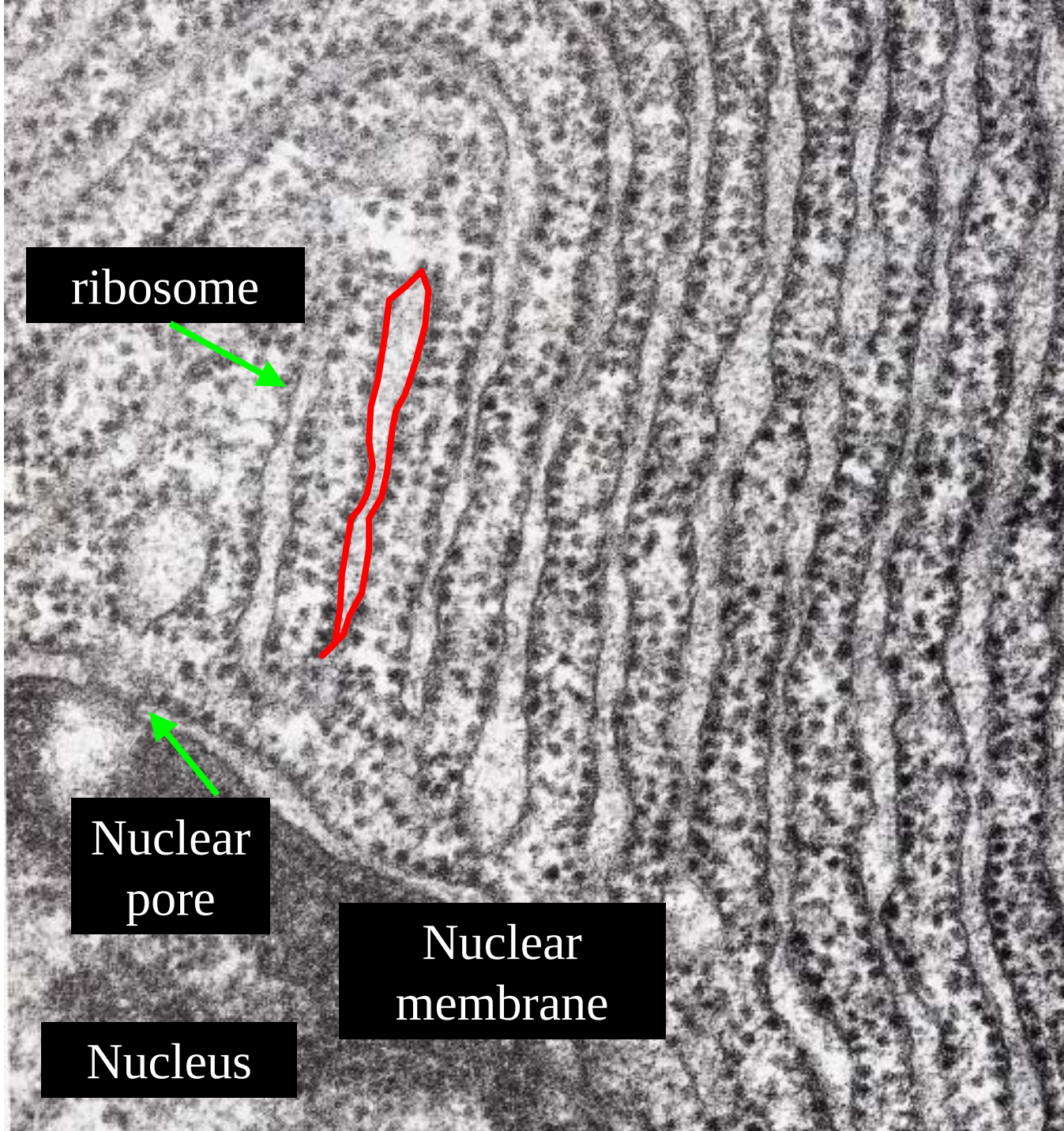


Figure 6. Model Showing the Overall Structure and Dimensions of the Full-Length GGA1 Protein

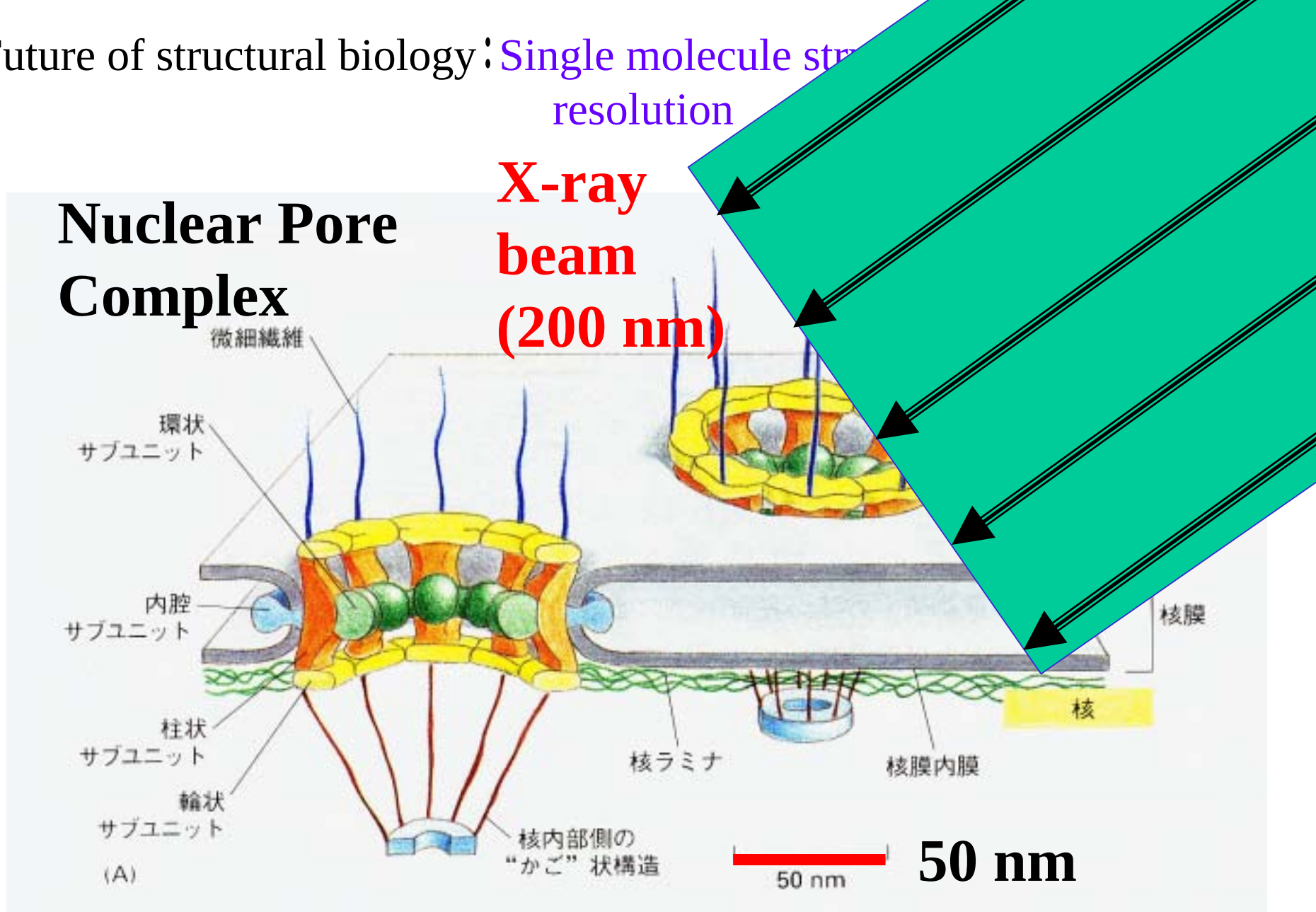
•**From B.M. Collins et al., *Developmental Cell,* Vol 4, 321-332, March 2003**

イヌすい臓の外分泌細胞

B. Alberts et al.「Molecular Biology of the Cell」



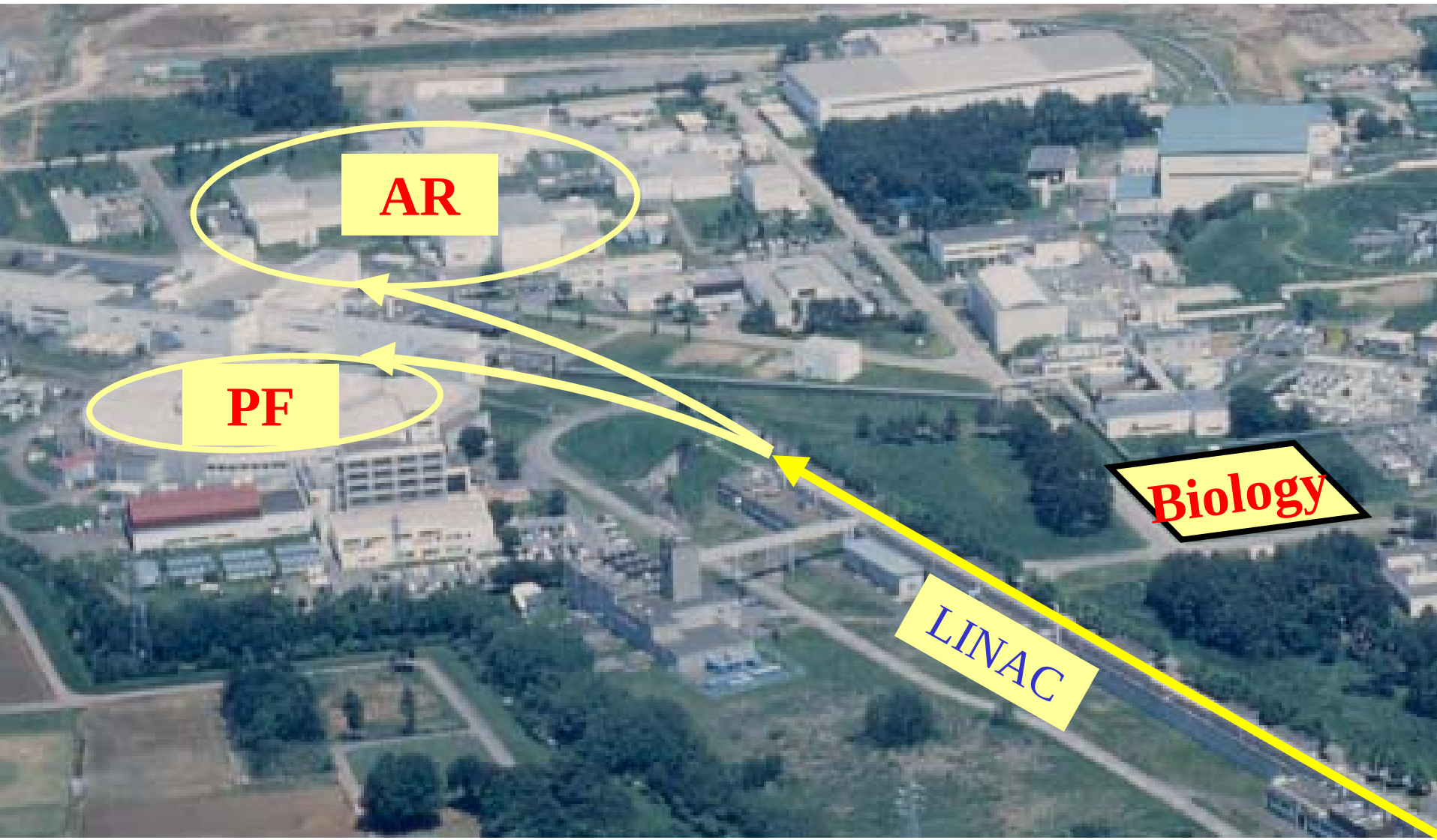
Future of structural biology：Single molecule str
resolution



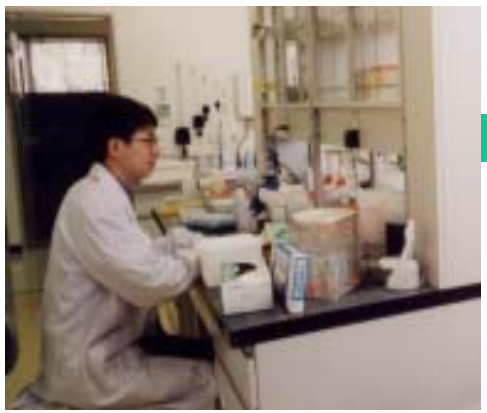
B. Alberts et al. “Molecular Cell Biology”

PART II. Limitations of the current methods

- **Beam line development and high-throughput R&D**



Future: automated/integrated system

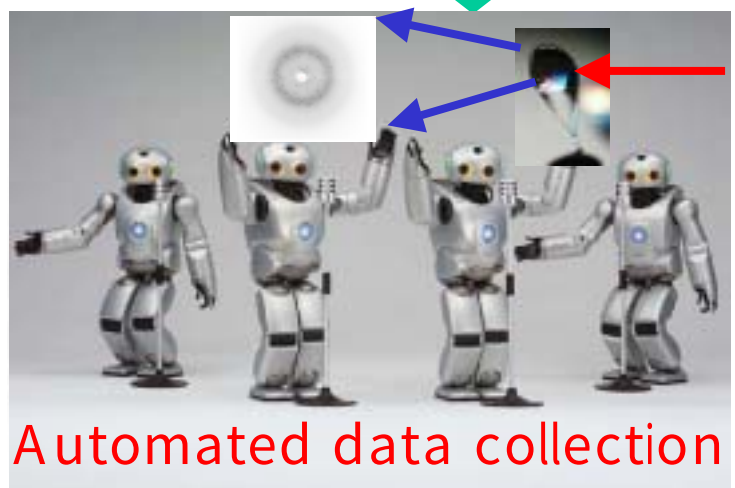


Expression and purification

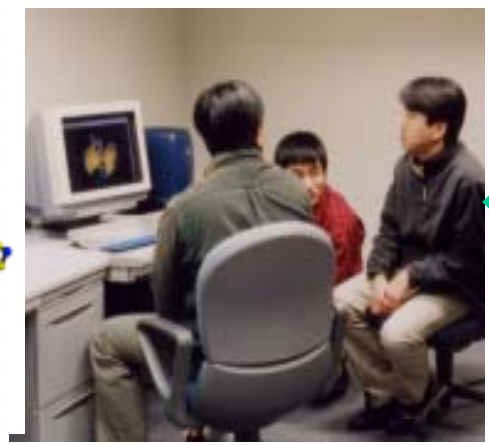
Crystallization robot

Crystallization

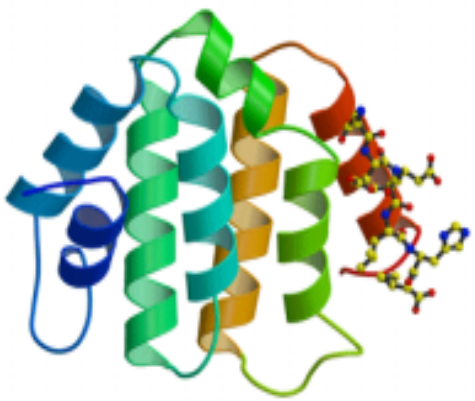
Crystal harvesting



Mouting & data collection

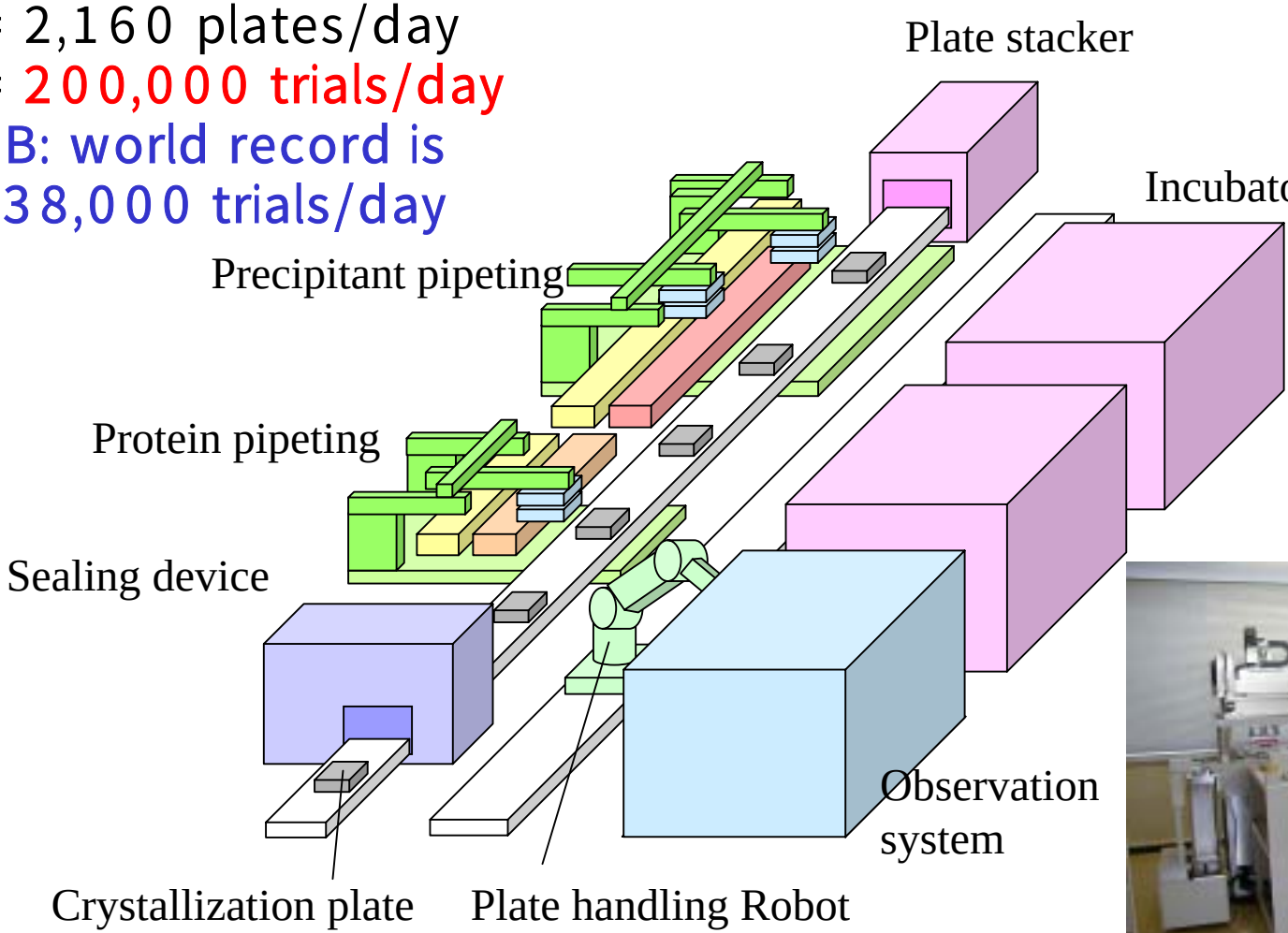


Data analysis

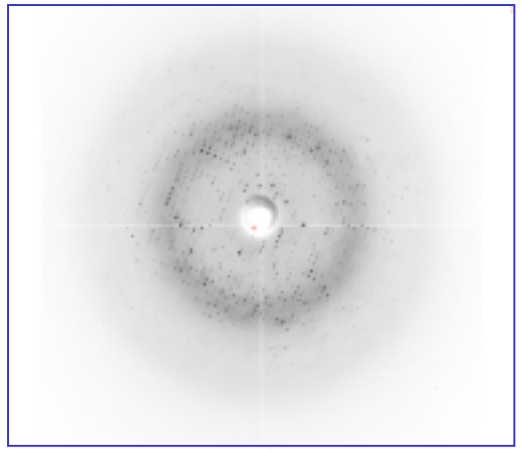


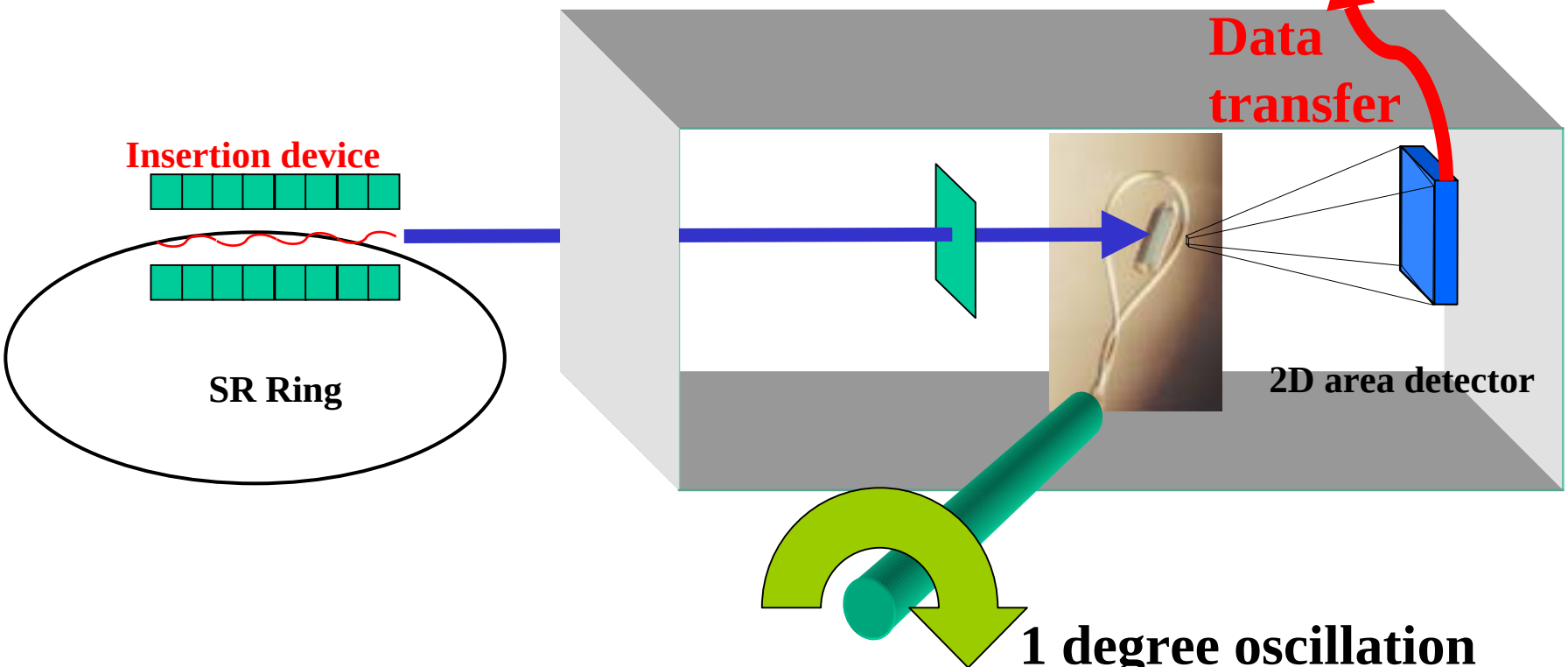
Protein Crystallization and crystal observation robot system to be completed in Autumn 2003

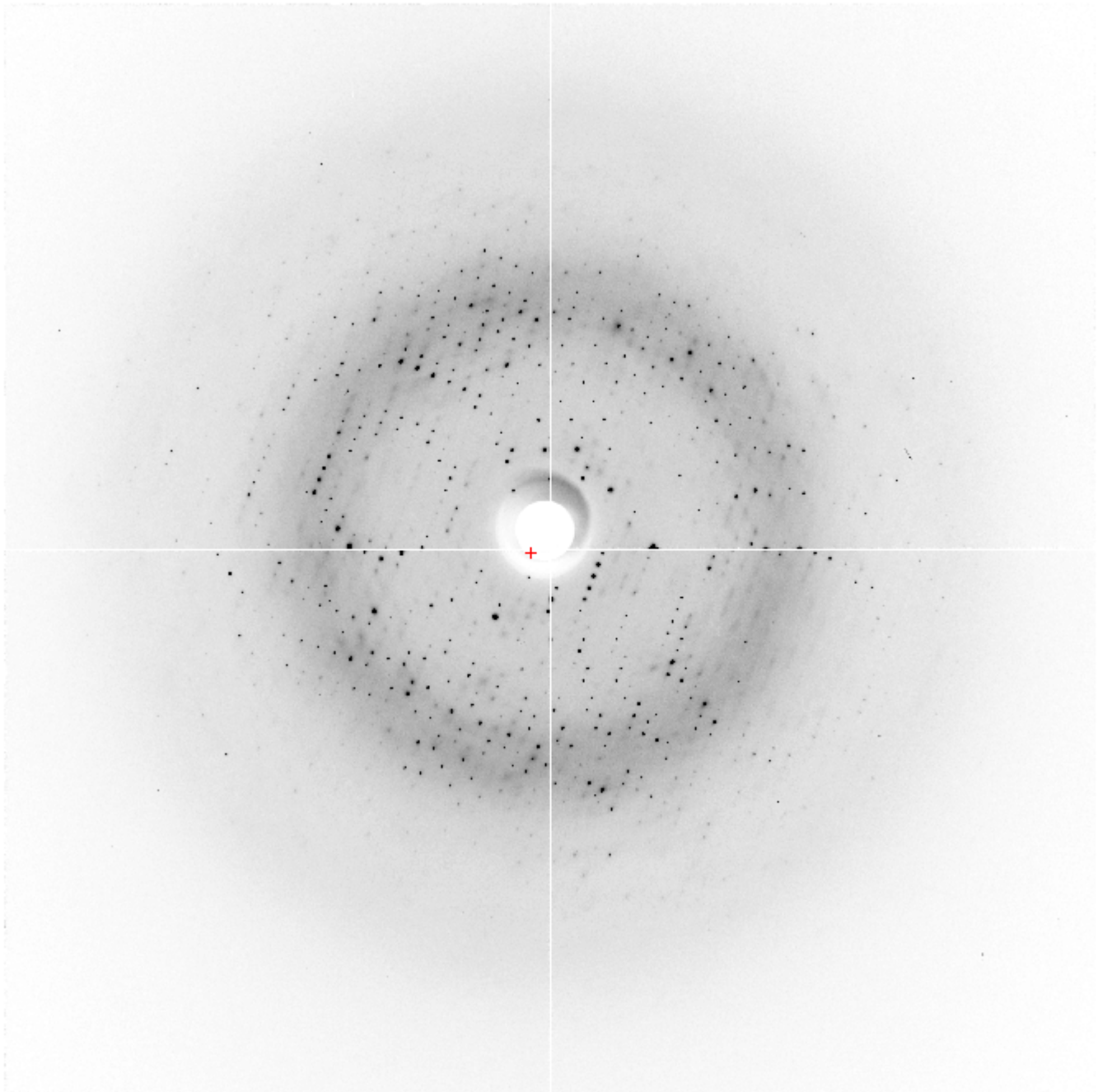
Target: 1 plate/40 sec
= 2,160 plates/day
= 200,000 trials/day
NB: world record is
138,000 trials/day



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







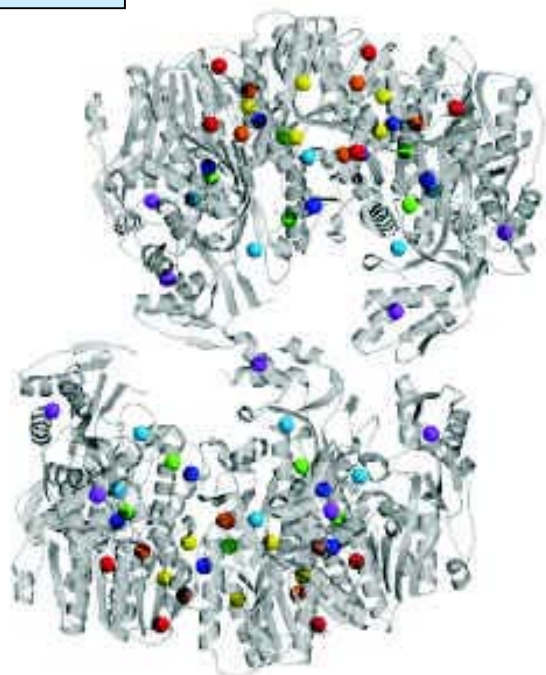
Phase Determination using Multiple Anomalous Dispersion

Methionine

Sulfur ⟶ Selenium (SeMet)

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

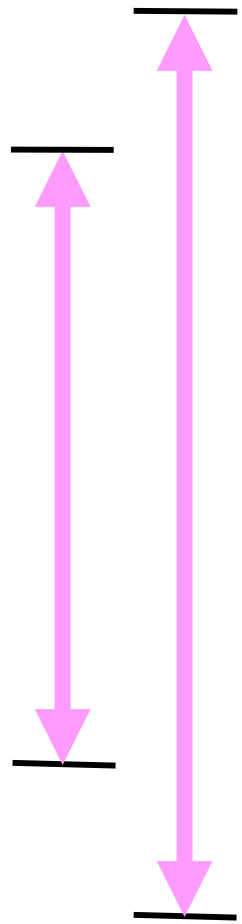
6 methionines out of 471 residues



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

The absorption edges of the elements frequently used in protein crystallography

Xe	0.3587Å	34.57keV
U	0.7223Å	17.16keV
Br	0.9202Å	13.48keV
Pb	0.9511Å	13.08keV
Se	0.9795Å	12.66keV
Hg	1.0093Å	12.29keV
Au	1.0402Å	11.92keV
Pt	1.0722Å	11.57keV
Zn	1.2837Å	9.66keV
Cu	1.3808Å	8.98keV
Sm	1.6625Å	7.46keV
Fe	1.7433Å	7.11keV



Phase determination using MAD

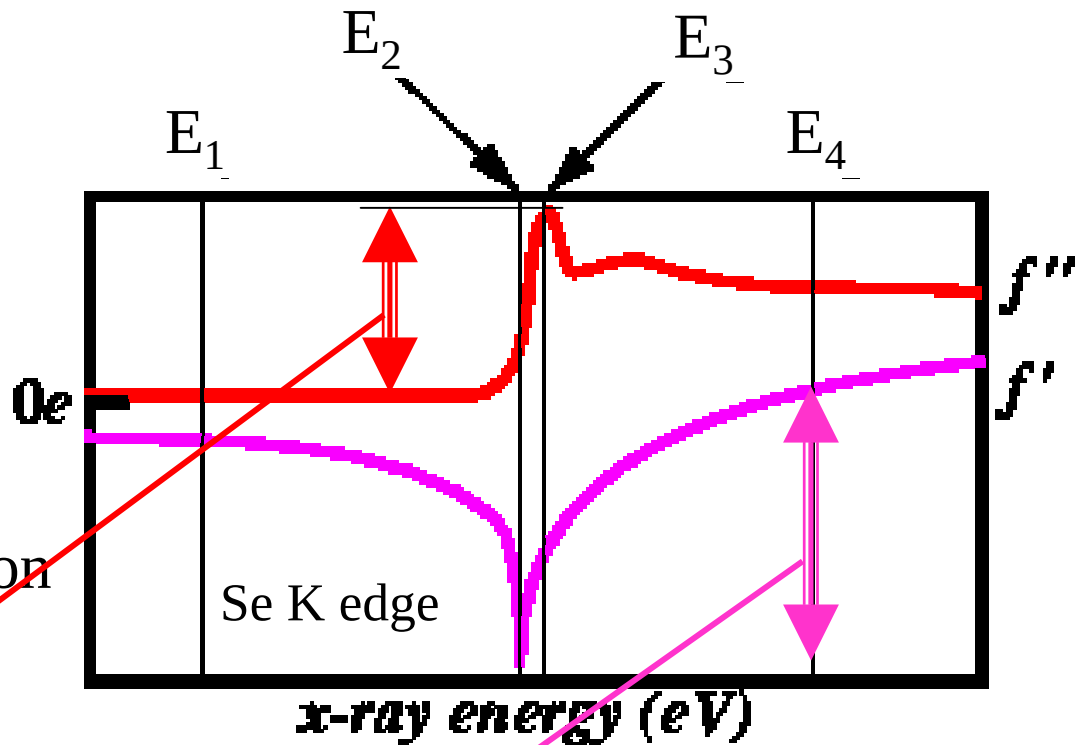
(Multiple Anomalous Dispersion)

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

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



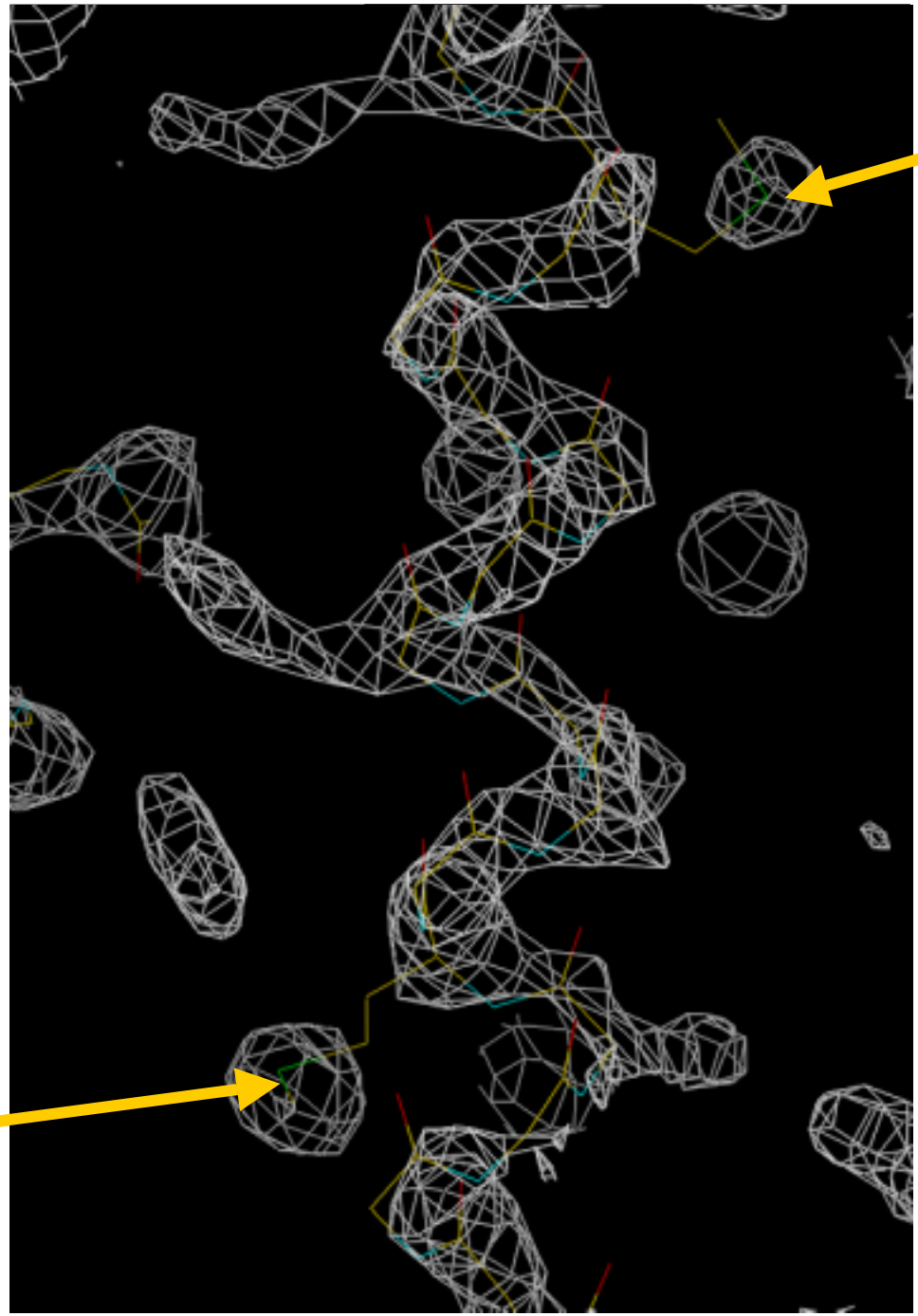
MAD experimental maps:

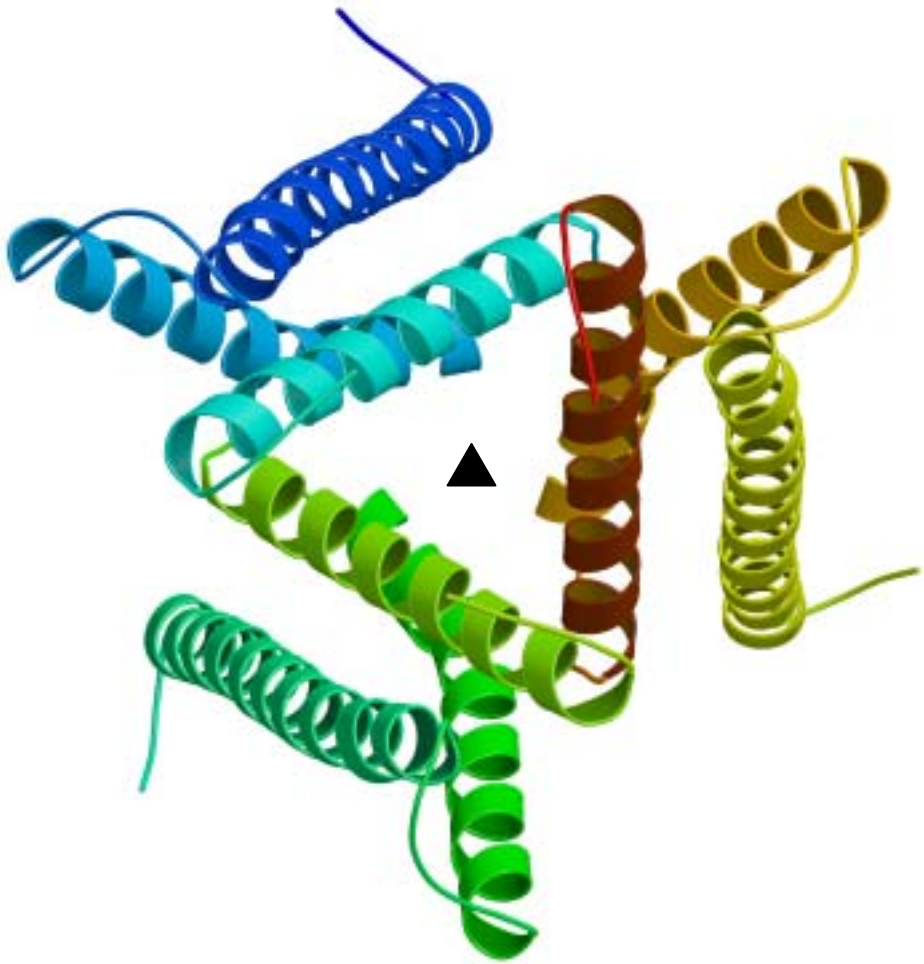
Anomalous difference Patterson

ΔF_{E3}^{2} (at the peak)

Dispersive Patterson

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


Met94
Met84

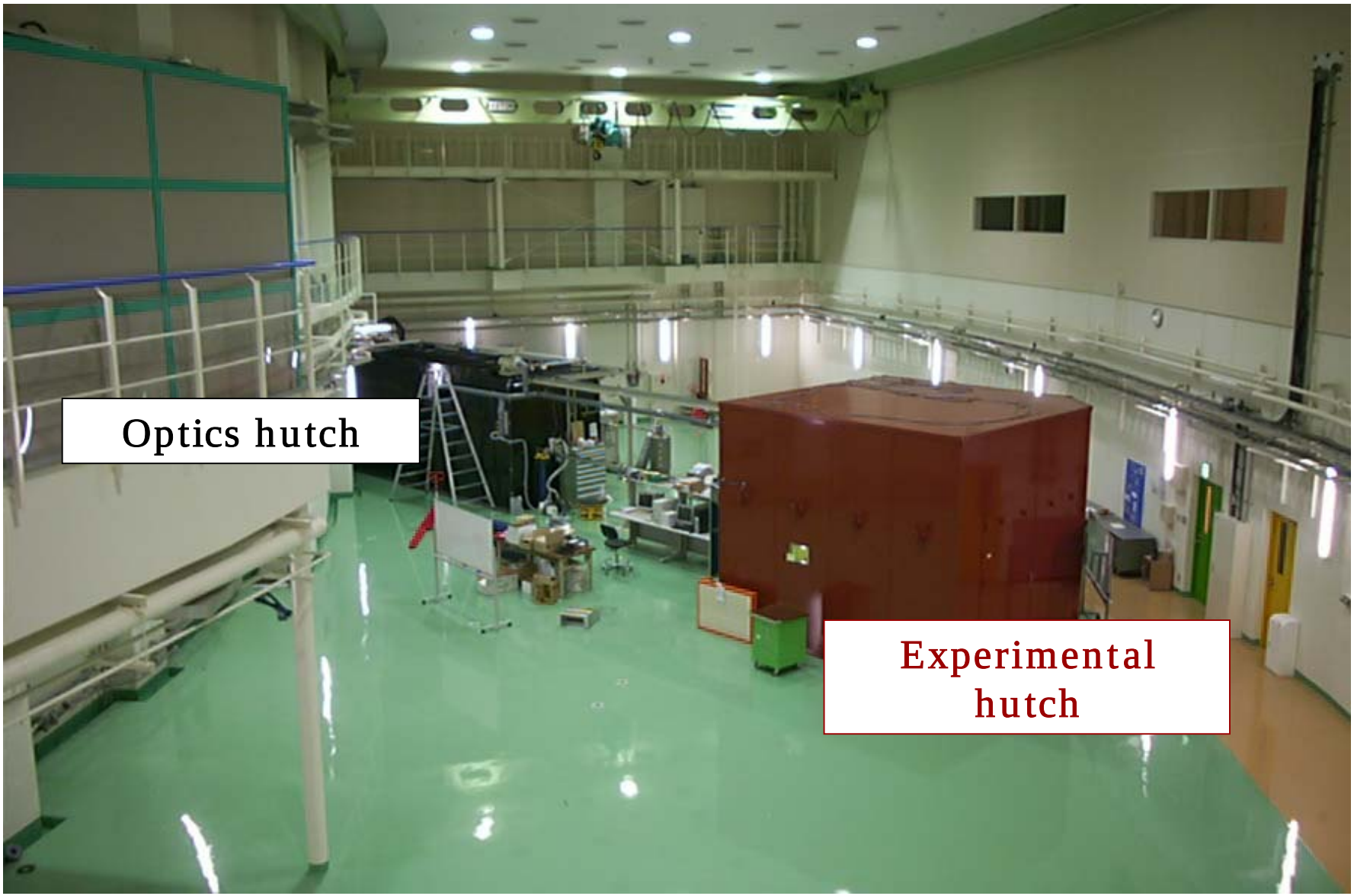


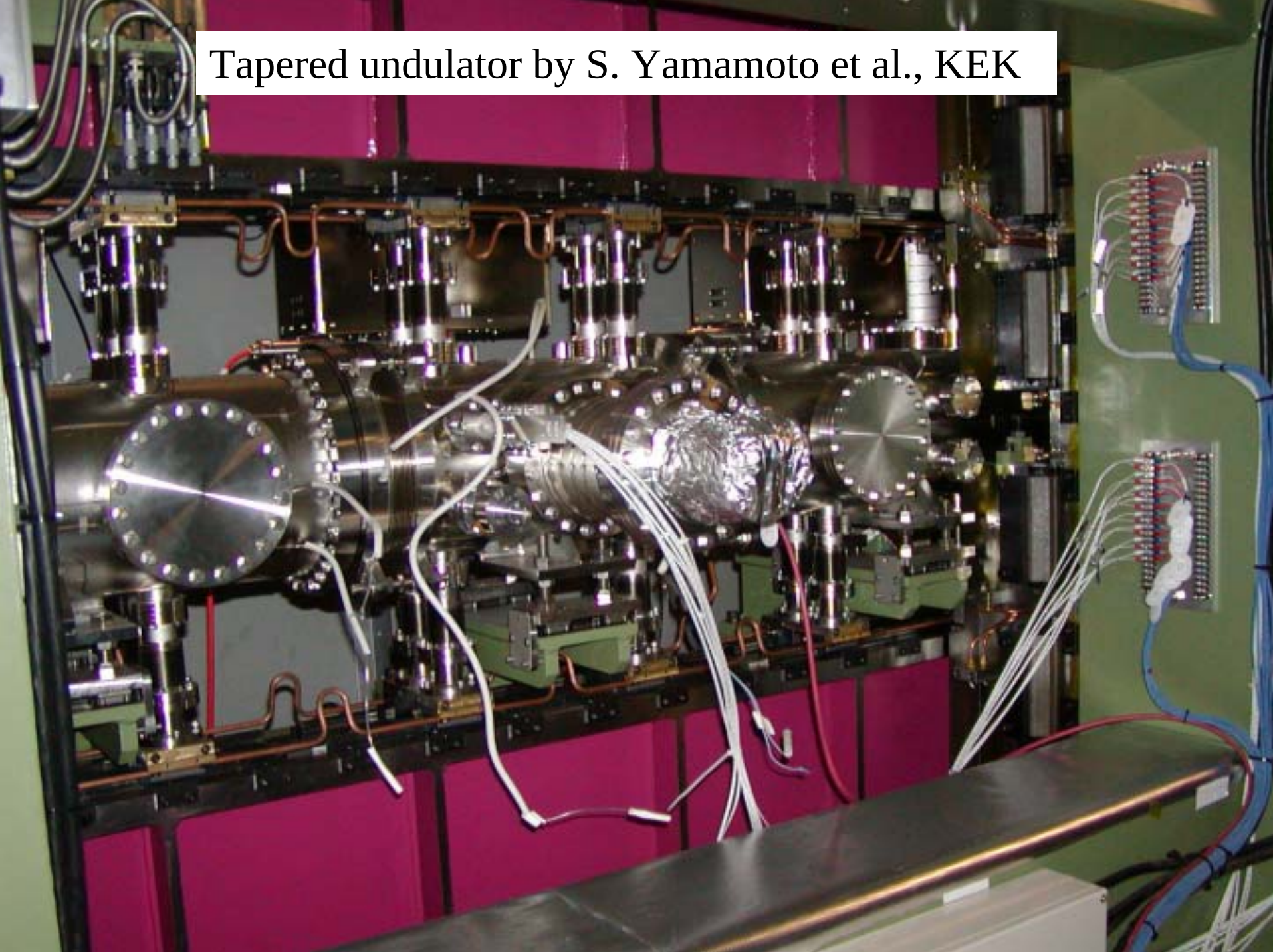
Ribbon diagram of trimer of GGA1 GAT domain

Triangle is threefold axis.

One of the fastest MAD beam lines in the world

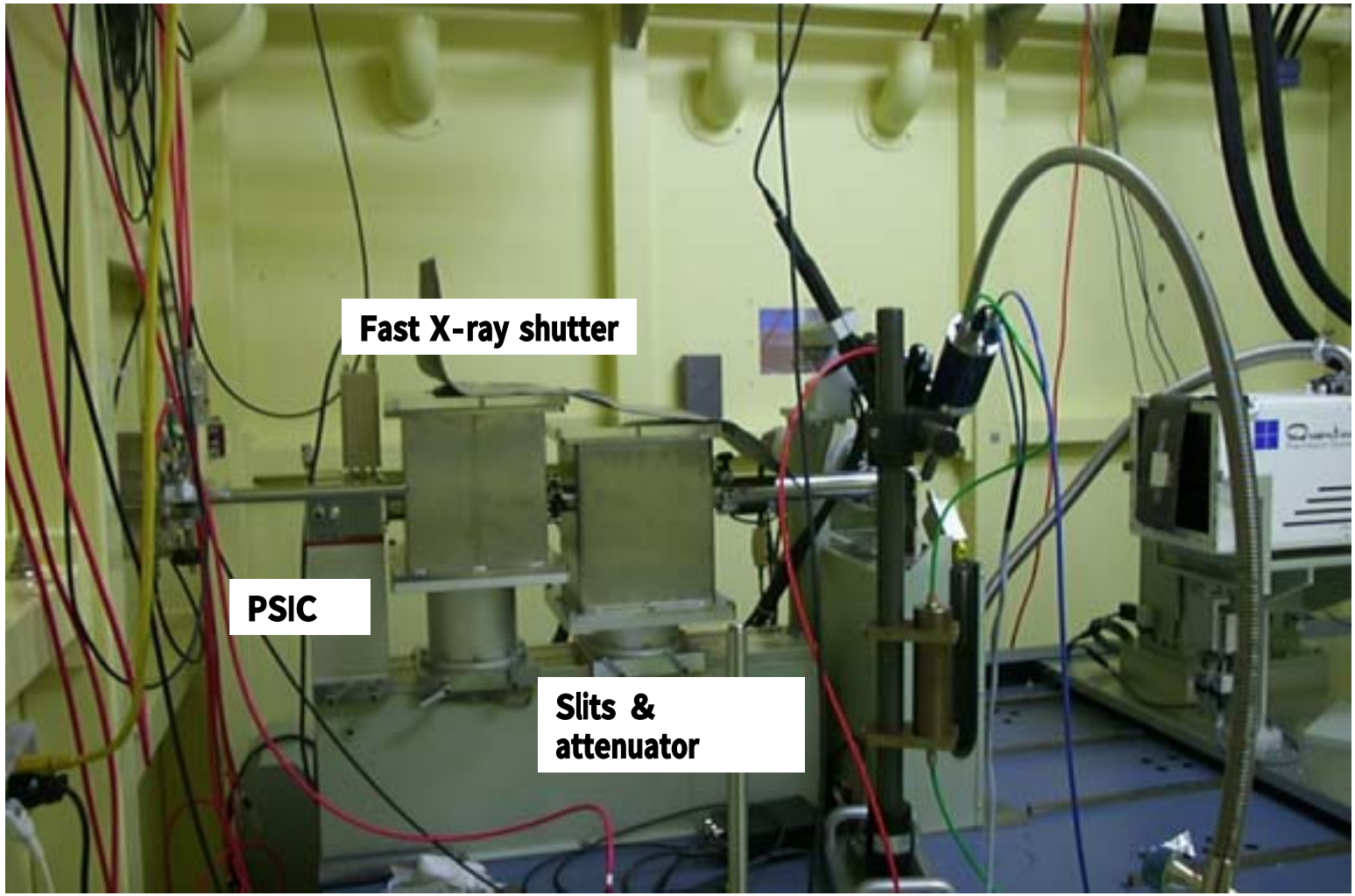
PF-AR NW12




Tapered undulator by S. Yamamoto et al., KEK

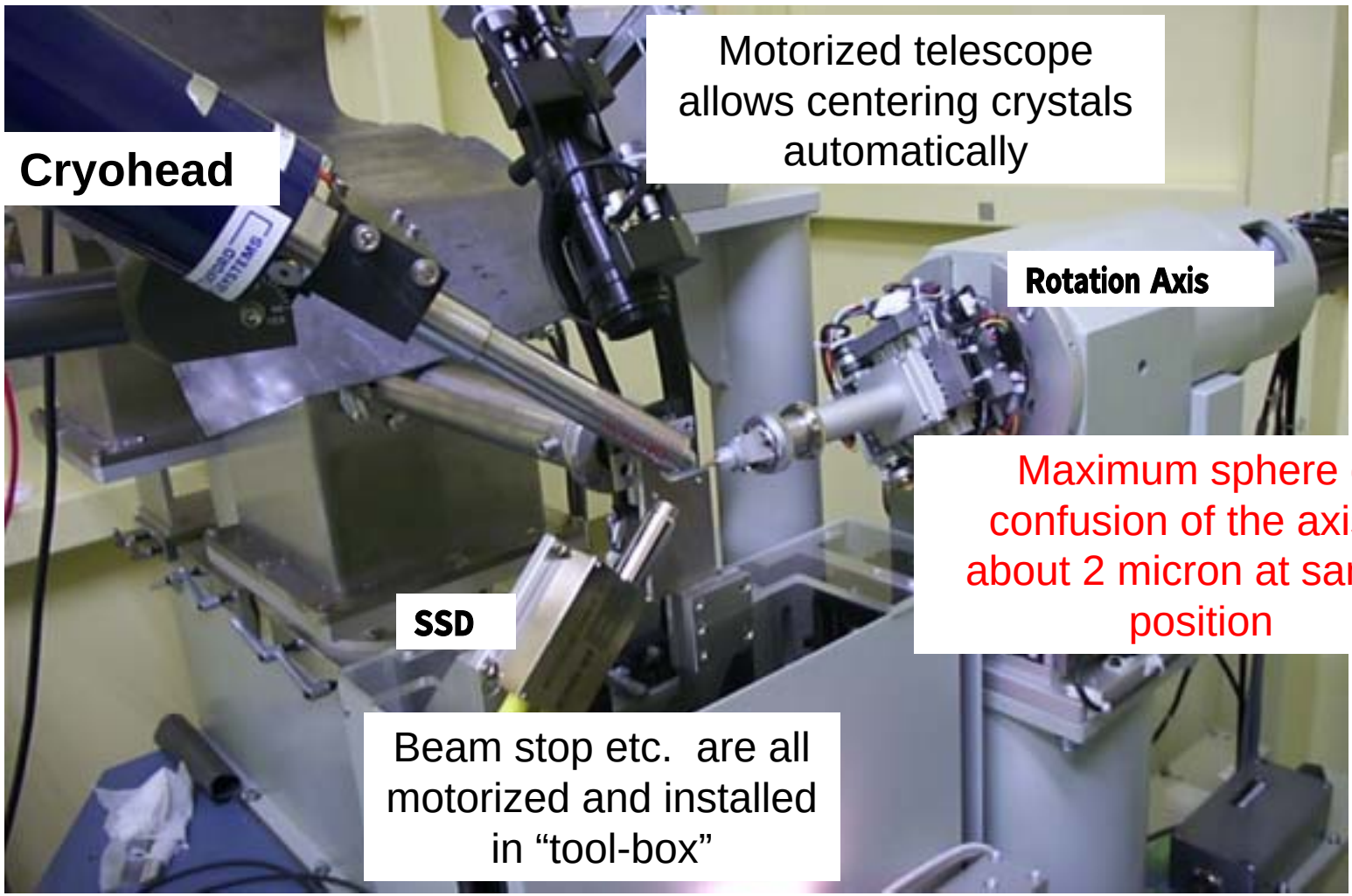
NW12 is one of the fastest MAD beam lines in the world.

Total data collection time (min): 10 to 30 min



NW12 allows for data collection from very small crystals.

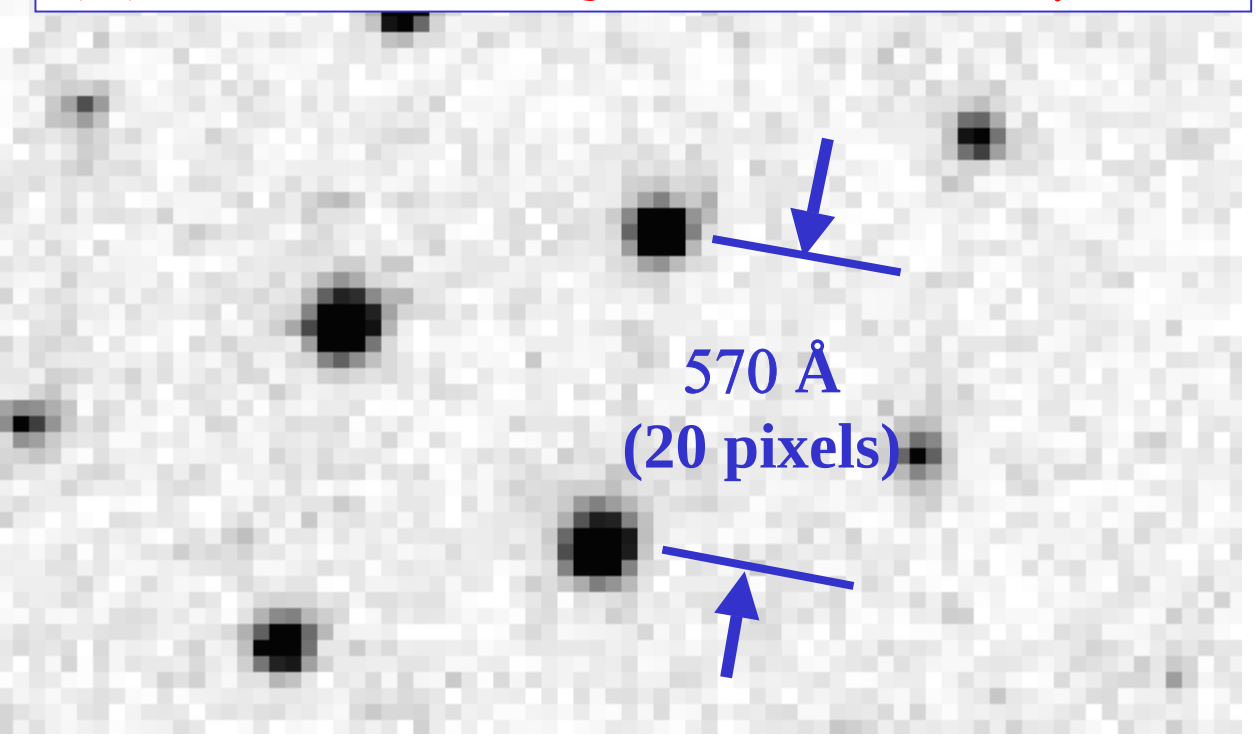
Tool box makes manual crystal mounting easy.



Limits of third generation synchrotron sources

(1) The largest unit cell dimension : 2000 Å

(2) Smallest crystals: a few μm³



Blue Tongue Virus

Core Particle 1

$P2_12_12$

755 X 796 X 825 Å³

ID14/EH3

Crystal to IP: 1250 mm

Wavelength	0.918Å
Oscillation	0.1 deg
Exposure	100 sec
Pixel size	100 μm
Beam size	100 μm
FWHM	181 μm

Part III

Key issues in realizing single particle/nano-crystal structural biology

Averaging

in crystal **conventional crytallography**

in computer **single molecule analysis**

Expectation from the 4th generation synchrotron

Future of structural biology： **Single molecule** or **nanocrystals**
structural analysis at atomic resolution

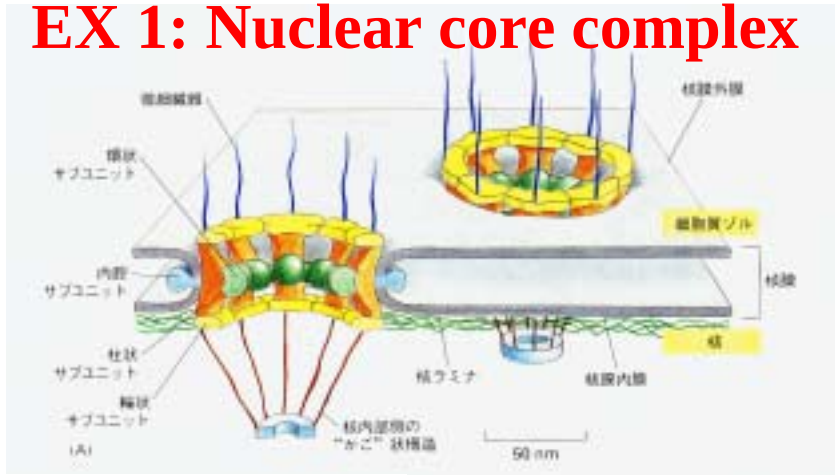
Properties

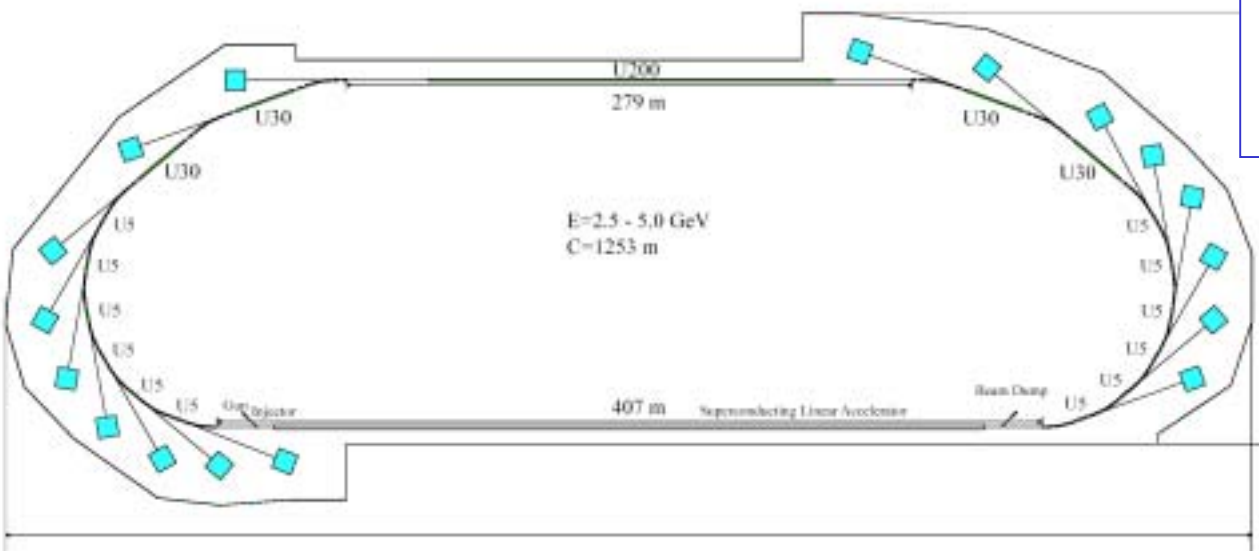
Low cost operation of multiple beam lines owing to the energy recovery

Brilliance: 1000 to 10000 times of the 3rd generation synchrotrons (ESRF, APS, Spring-8)

Very short pulse length：100 femto seconds（1/100）

EX 1: Nuclear core complex





EX 2: Nano crystals 10 to 100 nm size

Weak signal and many more images to collect

Critical conditions for successful single molecule structural analysese

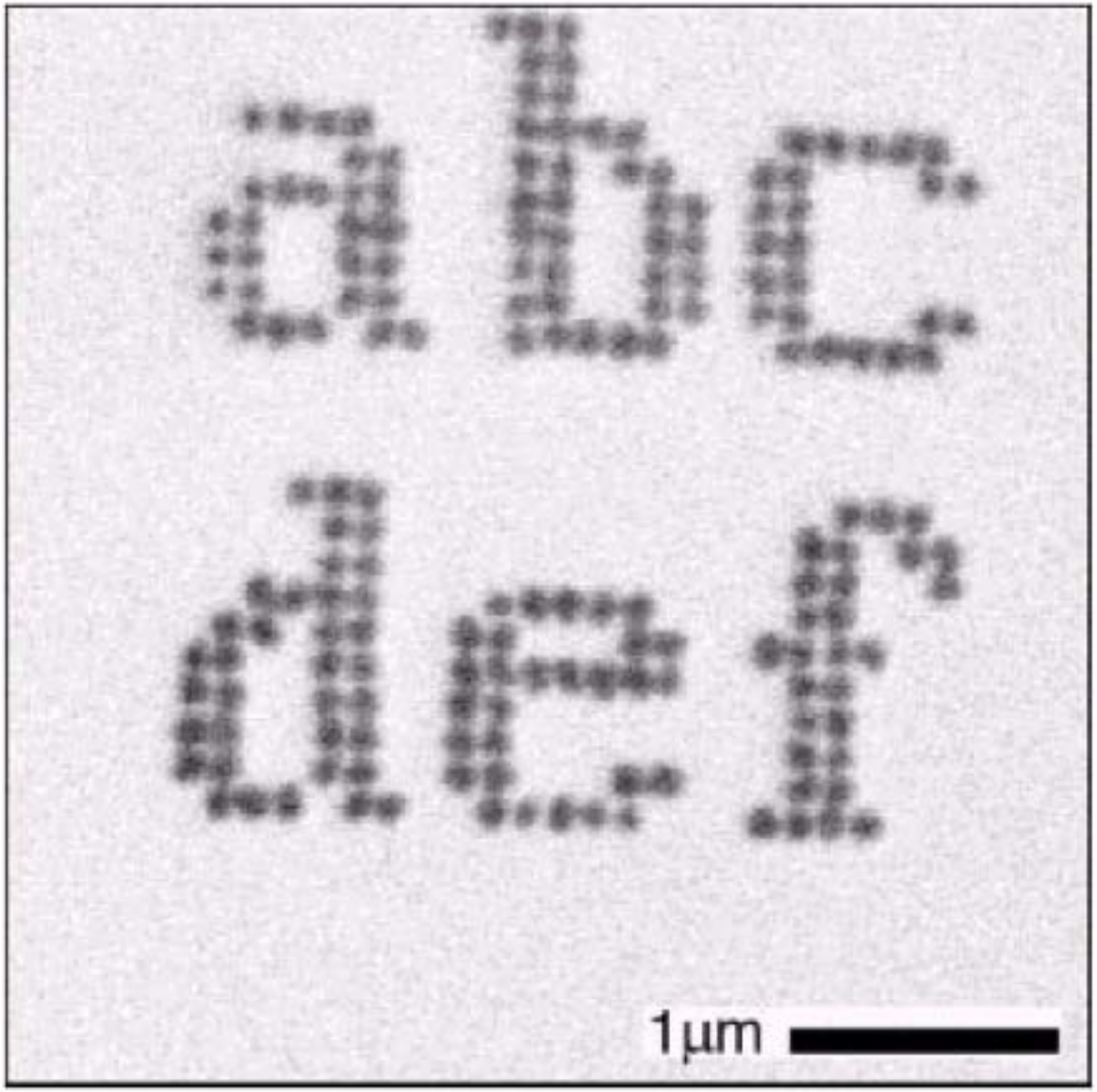
- Determination of orientation of single molecules
- Phase determination using the oversampling method
- Radiation damage
- Sample manipulation
- Sufficient S/N ratio from averaging single molecules

Extending the methodology of X-ray crystallography to allow imaging of micrometre-sized non-crystalline specimens

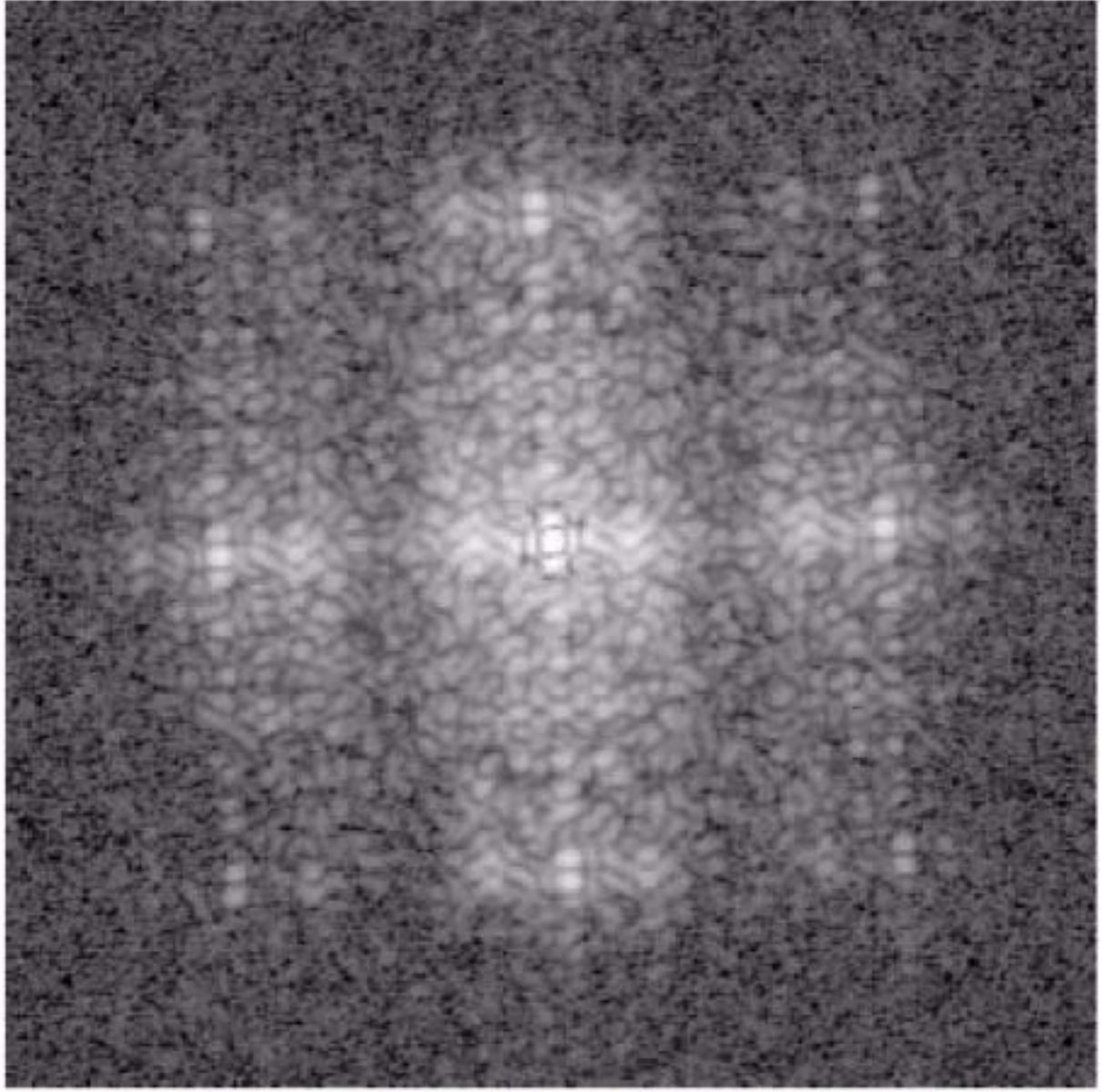
Jianwei Miao*, Pambos Charalambous†, Janos Kirz* & David Sayre*‡

** Department of Physics and Astronomy, State University of New York, Stony Brook, New York 11794-3800, USA*

† Kings College, Strand, London WC2R 2LS, UK



A scanning electron microscope image of the specimen. The specimen was fabricated by depositing gold dots, each ~100 nm in diameter and 80 nm thick, on a silicon nitride membrane.

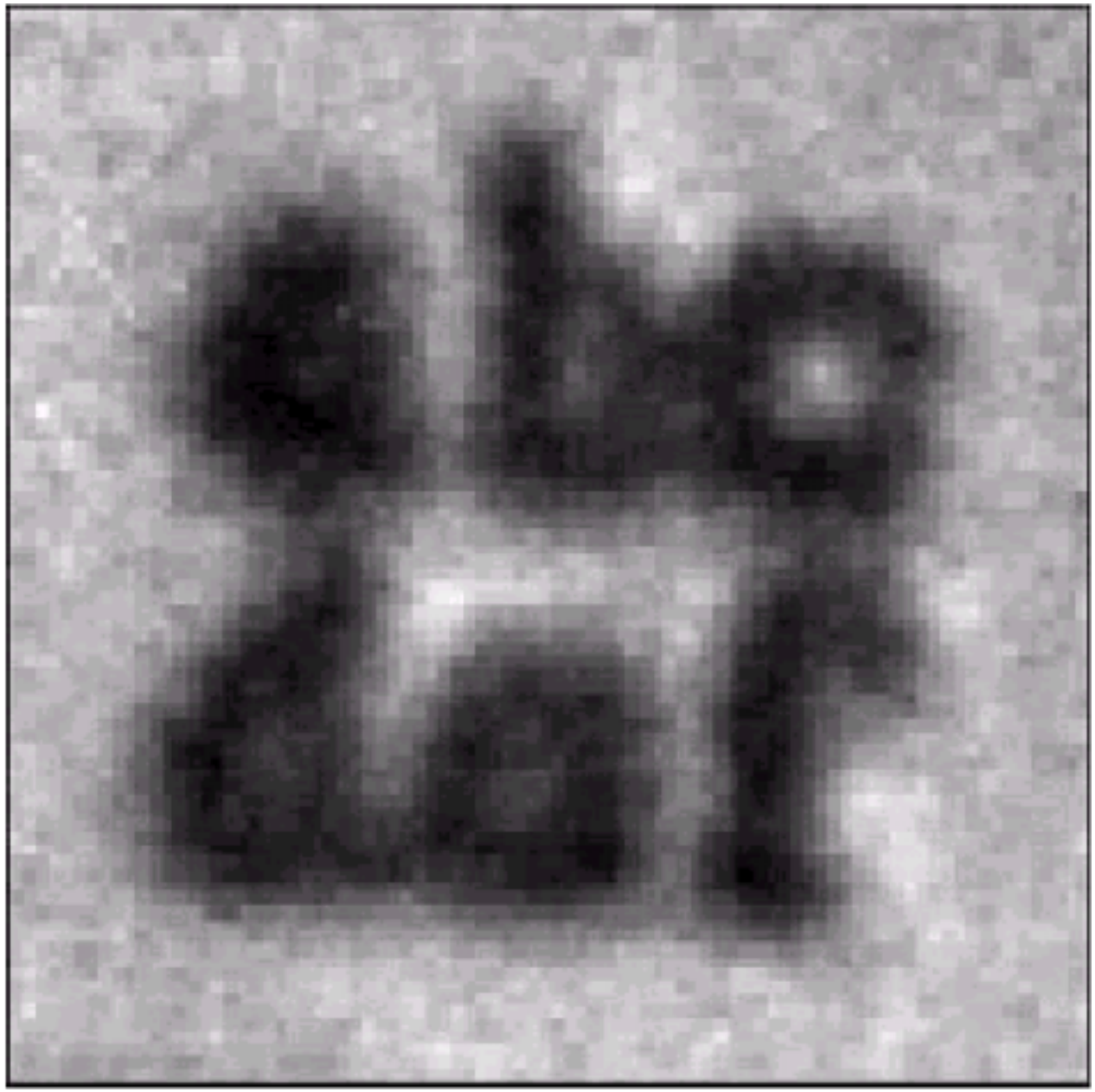


λ=1.7 nm, X1A NSLS

CCD: 512 x 512

(24μm pixel)

A diffraction pattern of the specimen (logarithmic intensity scale). The central 15-pixel-radius circular area is supplied by the squared magnitude of the Fourier transform of the optical microscope image.



An optical microscope image of the specimen.

Strucure amplitudes

Electron density

$$F(\mathbf{k}) = \sum_{\mathbf{x}=0}^{N-1} f(\mathbf{x}) \exp(2\pi i \mathbf{k} \cdot \mathbf{x} / N) \quad (1)$$

$$\left|F(\mathbf{k})\right| = \left|\sum_{\mathbf{x}=0}^{N-1} f(\mathbf{x}) \exp(2\pi i \mathbf{k} \cdot \mathbf{x} / N)\right| \quad (2)$$

Eq. (2)	No of equations (A)	No of unknown variables (B)	(B) / (A) underdetermined
f(x): real	$N^3/2$	N^3	2

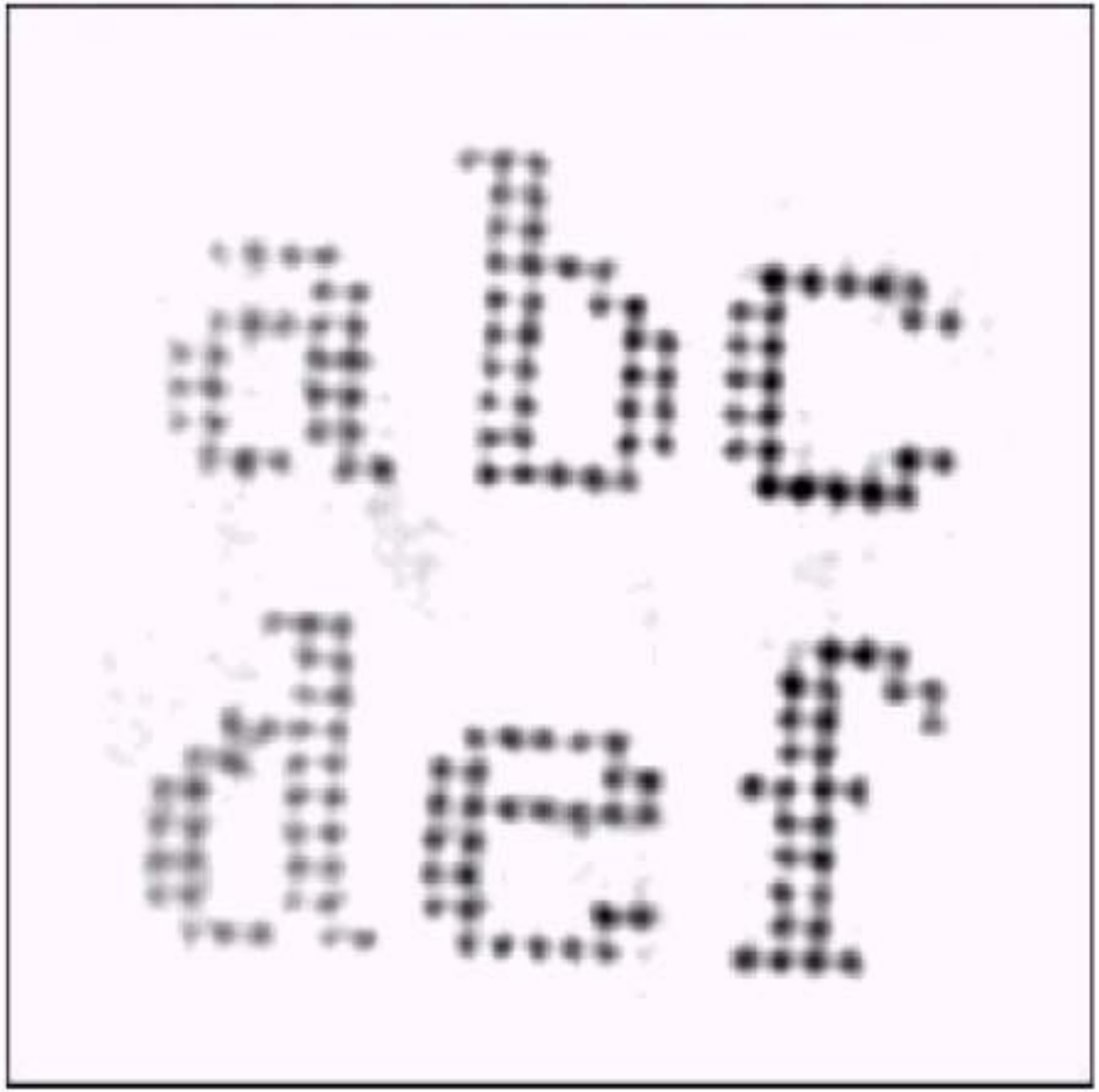
Determination of Phases

- Given the magnitute of a Fourier tranform sampled at the Bragg density, the phase problem is underdetermined by a factor 2.
- Thus at least in principle, oversampling the magnitute of a Fourier transform by factor of $2^{1/3}$ (=1.26) in each dimension is necessary to retrieve the phase of a 3D object

Phase Problem

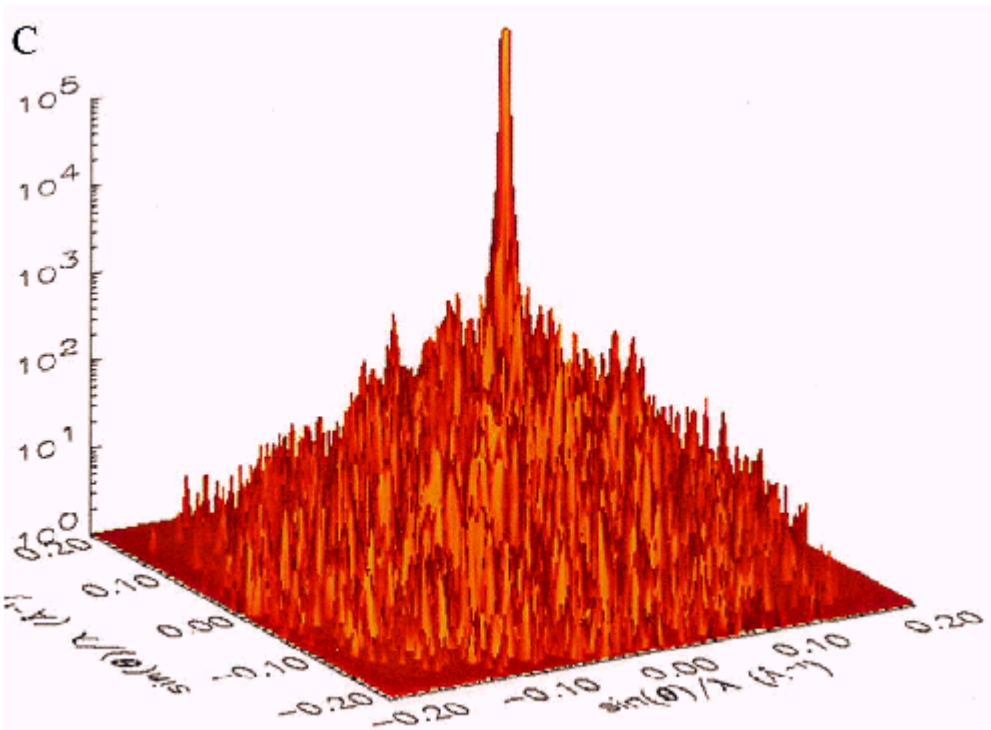
1) Decrease the number of unknown-valued pixels based on the knowledge of the object – finite support; density outside the protein is zero (Protein Crystallography→Density Modification)

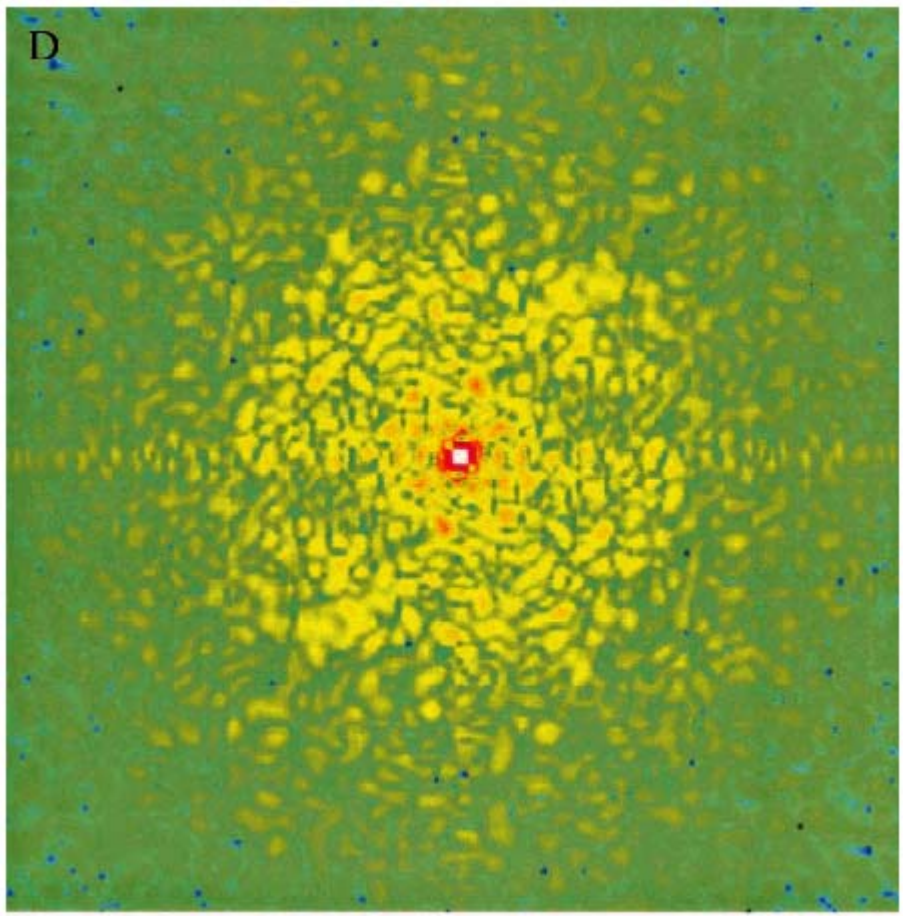
2) Oversampling by factor 2



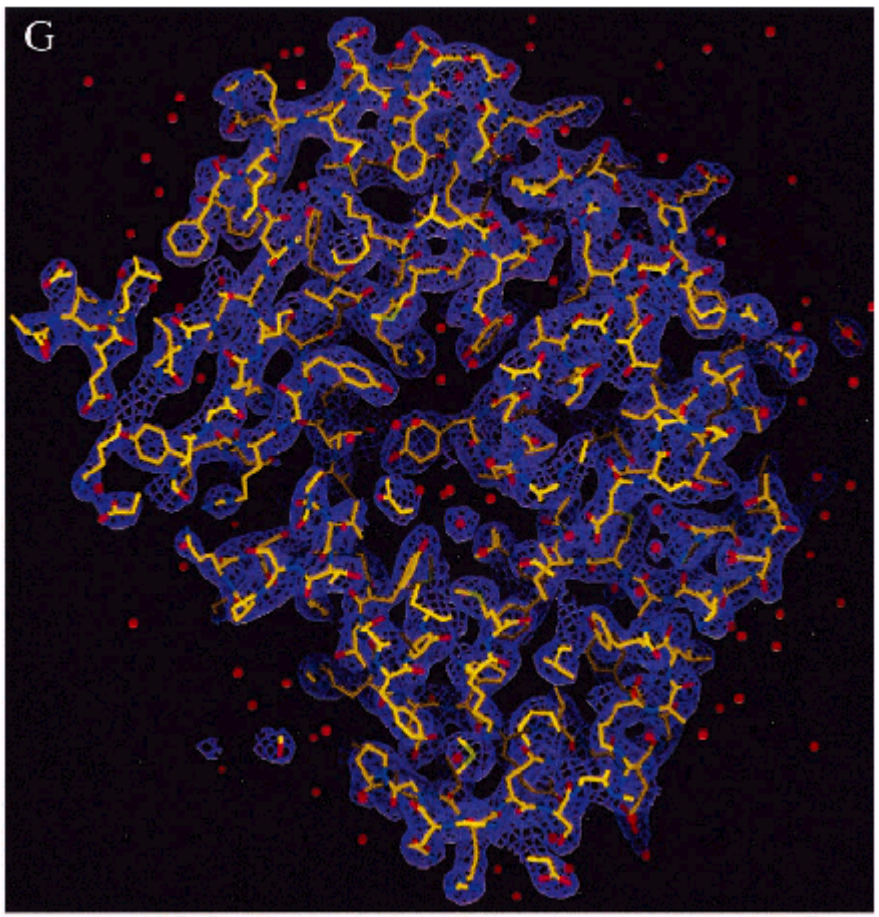
The specimen image as reconstructed from the diffraction pattern

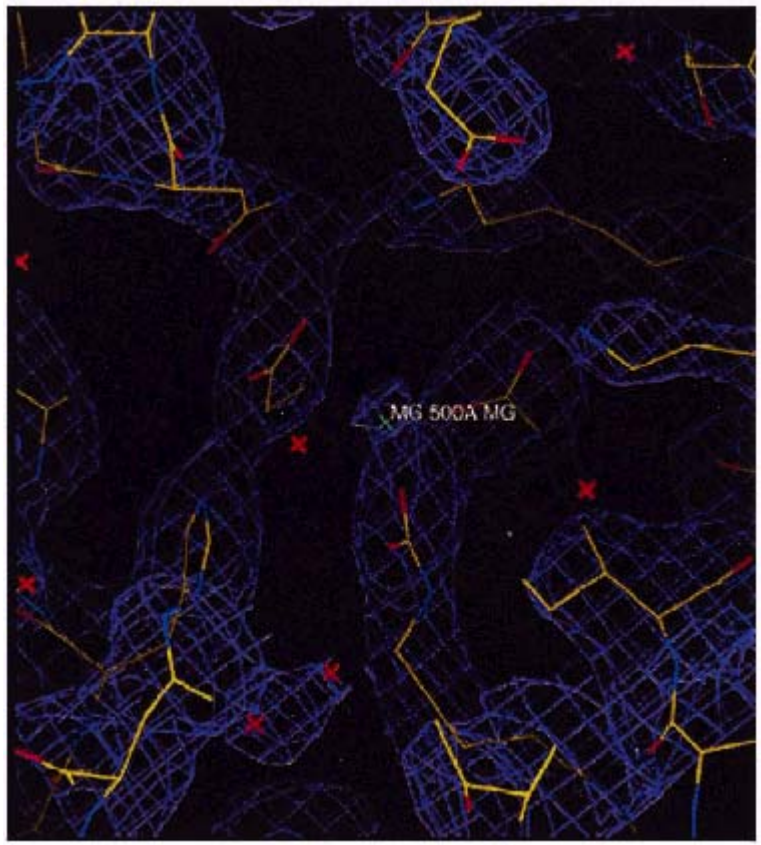
3D structural determination of single rubisco molecules utilizing a simulated X-FEL and direct phase retrieval by the oversampling technique. Miao，Hodgson，Sayer，PNAS，June 5, 2001, vol. 98, 6641‘645





3D structural determination of single rubisco molecules utilizing a simulated X-FEL and direct phase retrieval by the oversampling technique. Miao，Hodgson，Sayer，PNAS，June 5, 2001, vol. 98, 6641‘645





Simulated diffraction images

X-ray energy:	12 keV
Integrated X-ray intensity	3×10^{12}
	(3.8×10^{6} per A^{2})
Detector	100 mm by 100mm (128 by 128 pixel)
Sample to detector distance:	100 mm
Resolution limit at the rim of the detector:	2.2 Angstroms

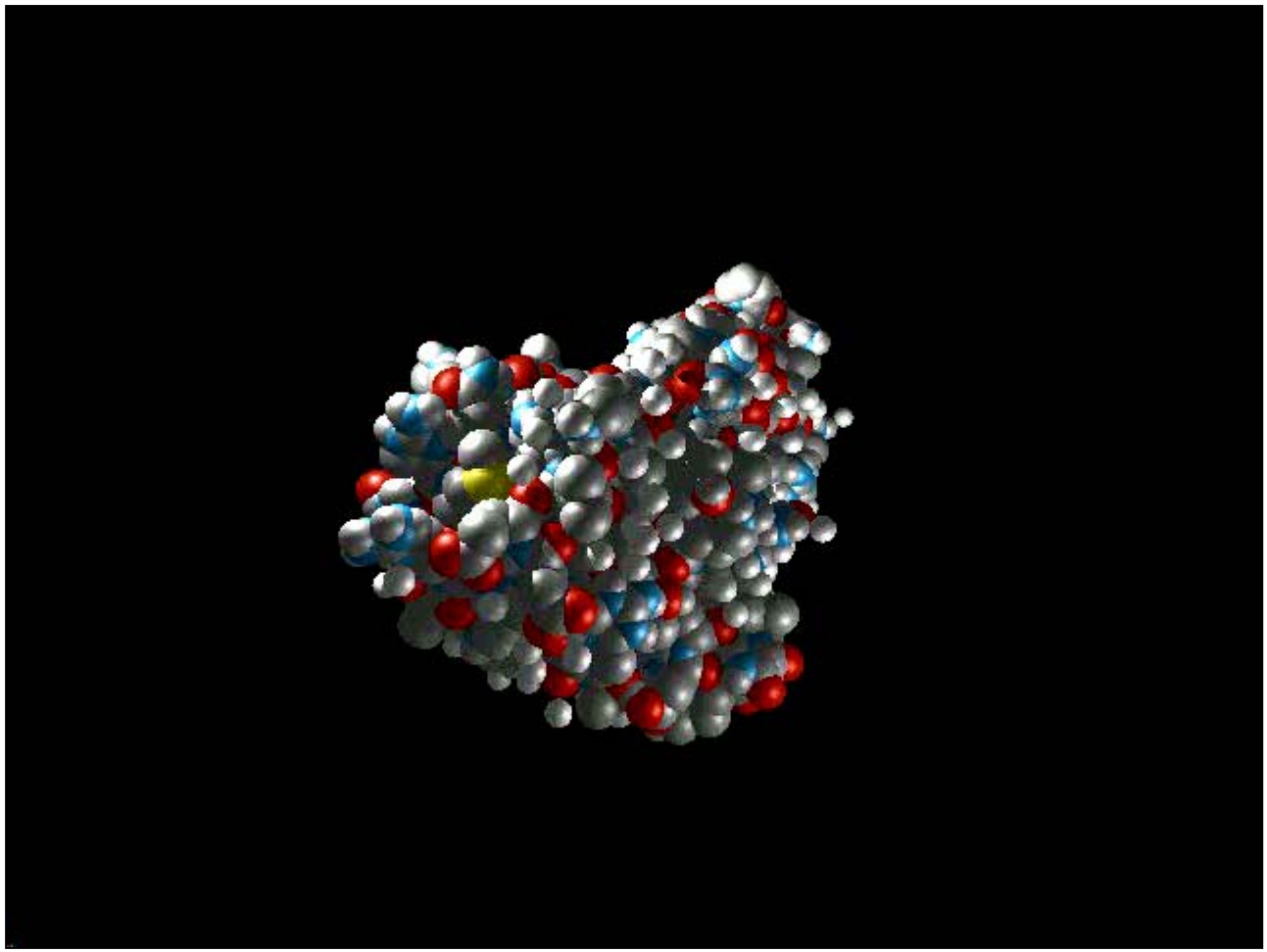
NO BACKGROUND WAS TAKEN INTO ACCOUNT although they are aware of sources of various errors.

Potential for biomolecular imaging with femtosecond X-ray pulses

Richard Neutze*, Remco Wouts*, David van der Spoel*, Edgar Weckert†‡ & Janos Hajdu*

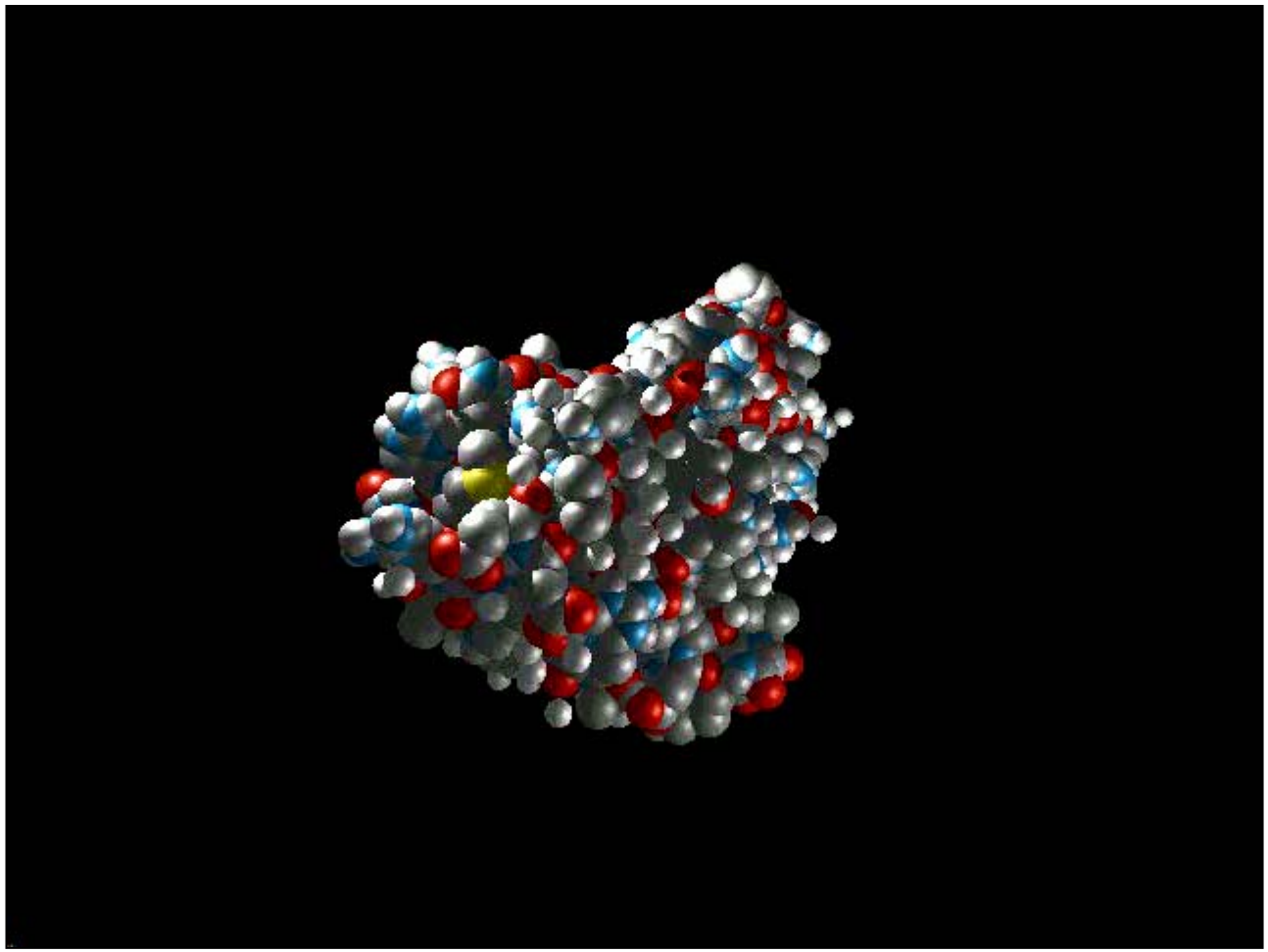
** Department of Biochemistry, Biomedical Centre, Box 576, Uppsala University, S-75123 Uppsala, Sweden*
† Institut für Kristallographie, Universität Karlsruhe, Kaiserstrasse 12, D-76128 , Germany

200 fsec X-ray pulse onto lysozyme single molecule



200 fsec pulse, 3 x 10^{11} photons in 100 nm spot. Radiation damage interferes with atomic positions and the atomic scattering factors

100 fsec X-ray pulse onto lysozyme single molecule



100 fsec pulse, 3 x 10^{11} photons in 100 nm spot. One could record data prior to onset of significant radiation damage

Manipulation of nano-scale samples

Sphere of frustration (Paul Sigler, Yale)

•Visualization of samples smaller than 100 nm

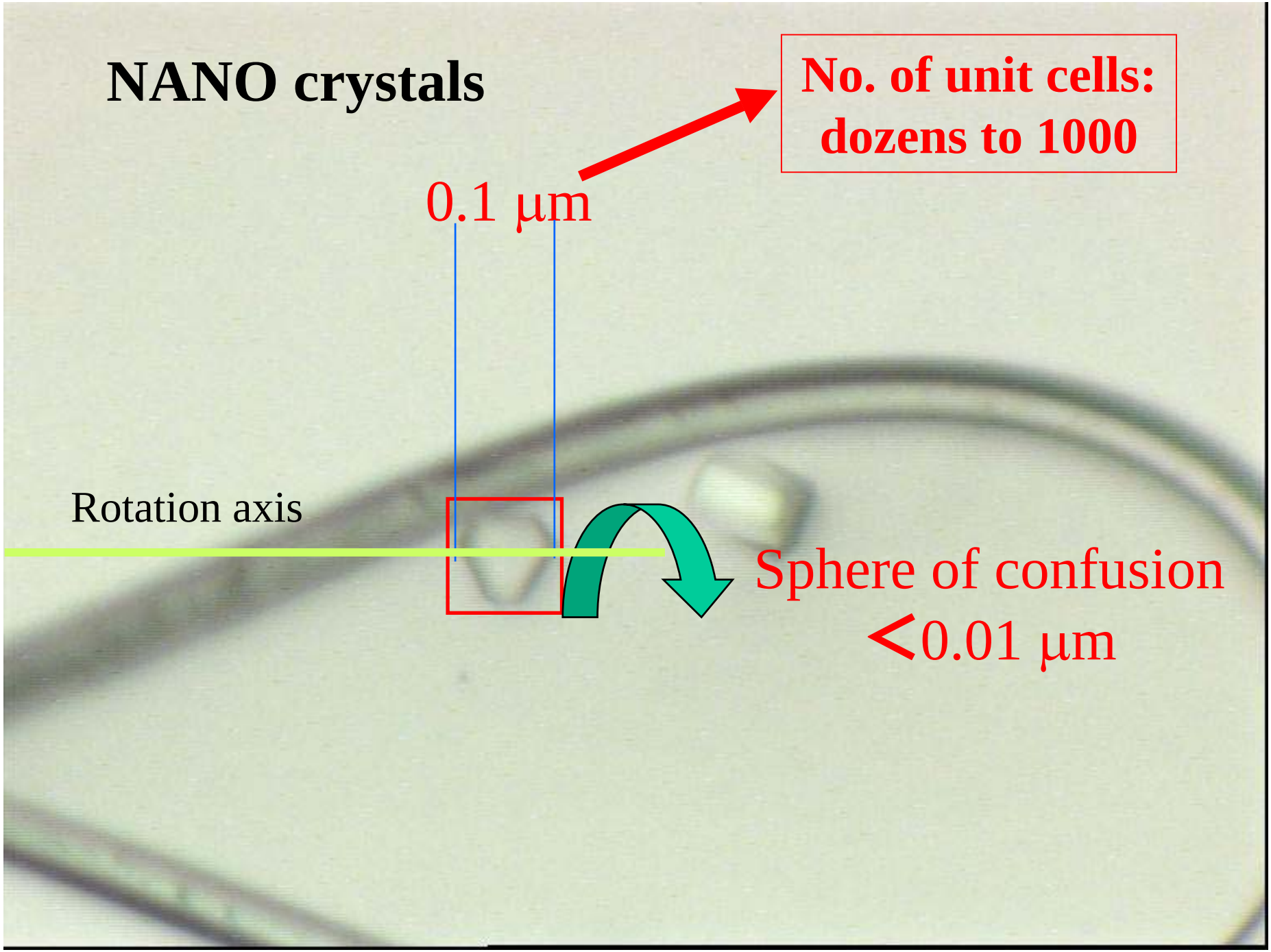
•Goniometer with sphere of confusion better than 10 nm

- Electrospary techniques no control of sample rotation
- Vitrous ice-EM techniques tilt angle limits

- Immobilization of protein molecule(s) onto a substrateu using multiple chemical bonds.
- Laser tweezers

NANO crystals

No. of unit cells: dozens to 1000

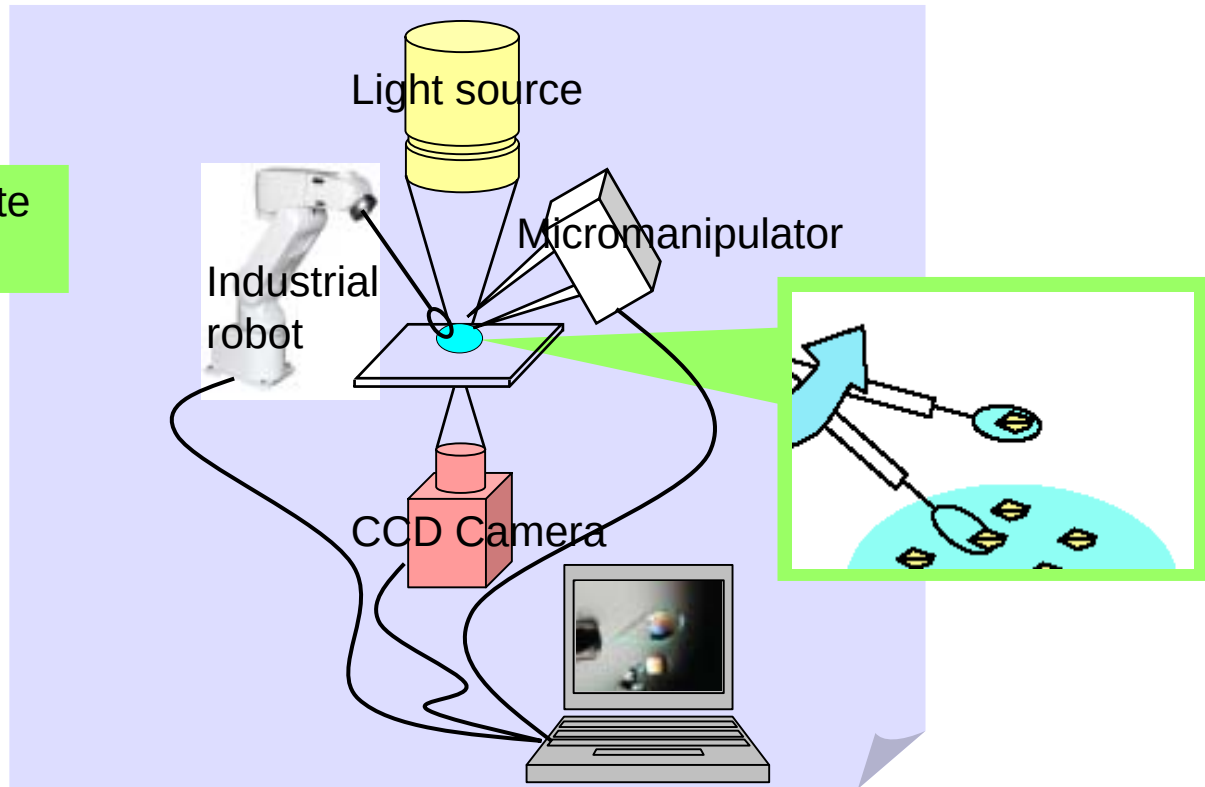


Carrying Robot

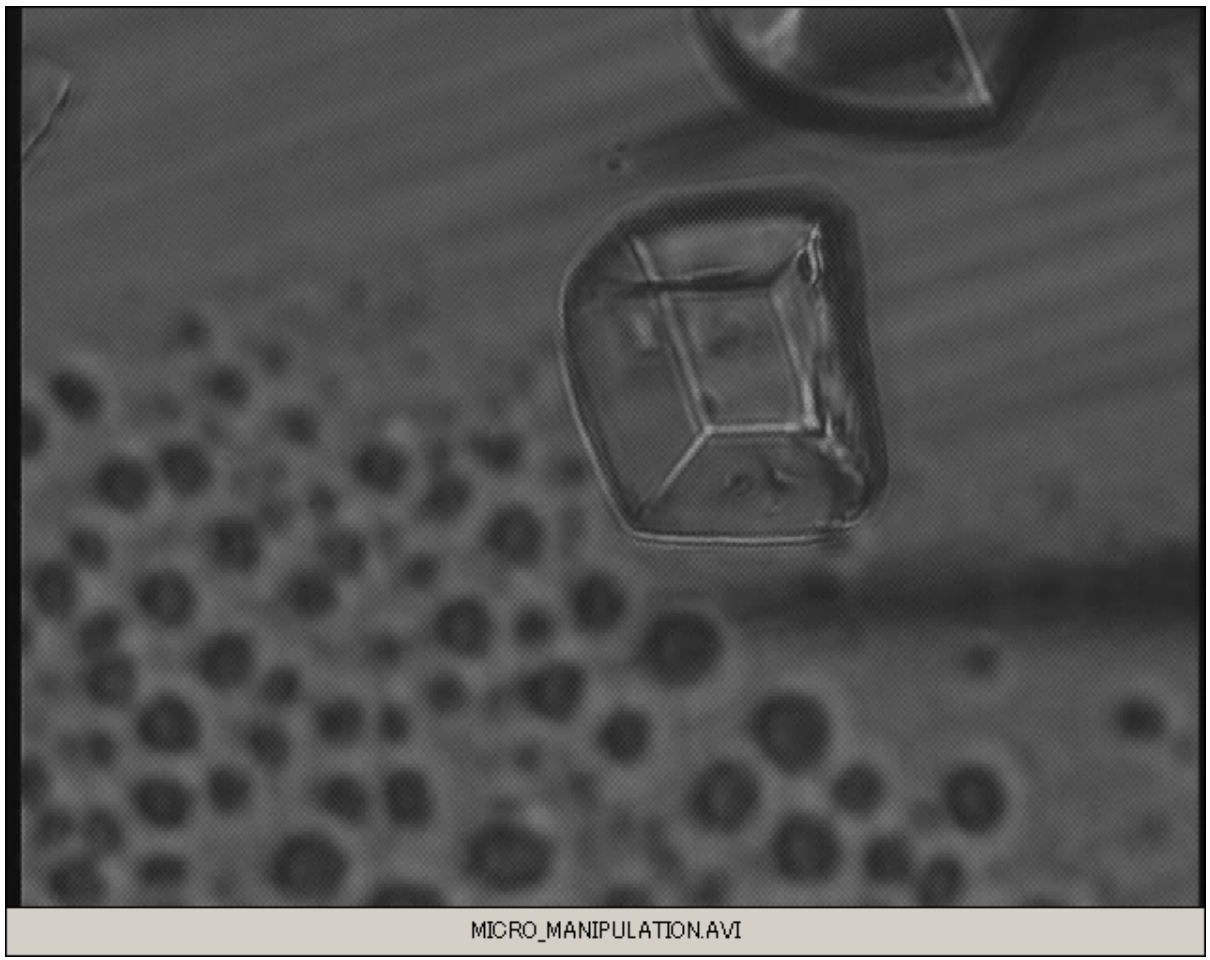
Loops

Crystal Harvesting System

Decides an appropriate size of a loop

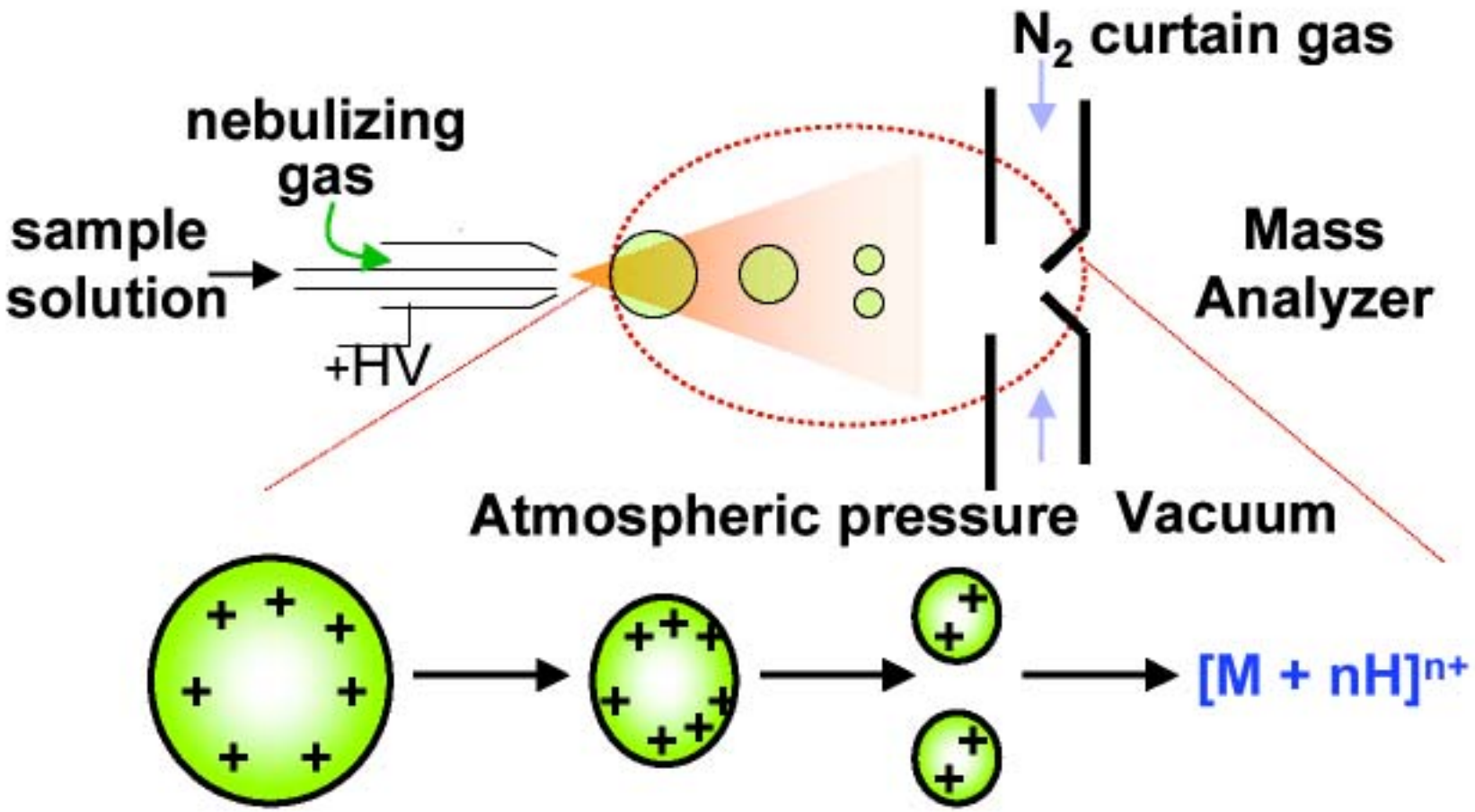


Mounting/Data Acquisition System



Micromanipulation (international patent to be submitted, Wakatsuki & Tanikawa)

Electrospray Ionization (ESI)



1. Solvent evaporation
2. Coulombic repulsion

Averaging is necessary anyway for atomic structure determination!

1. **in crystal** **conventional crytallography**
2. **in computer** **single molecule analysis**

Simulation: effect of the water molecules

Parameters in for the structure amplitudes calculation

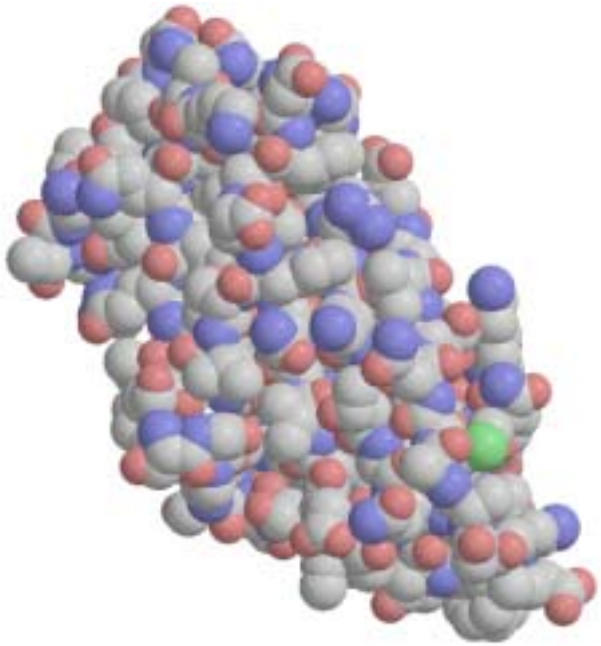
Resolution rage:	30.0 – 2.5 Å
Spacegroup:	P1
Unit cell:	$a = b = c = 700$ Å, $\alpha = \beta = \gamma = 90$ °

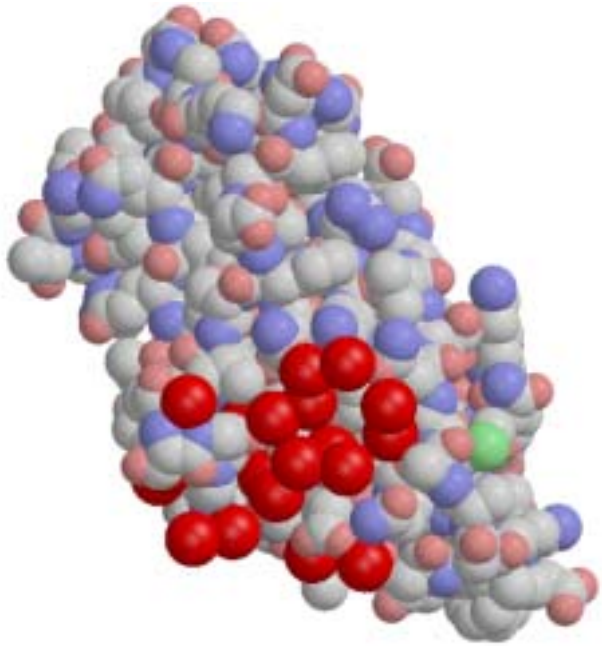
Coordinate: γ1-ear domain complex with γ-synergin peptide (PDB ID:1UI4)
(from the crystal of the peptide tagged protein)

Resolution:	1.85 Å
Number of residues:	124
Number of water molecules:	140

Protein only

Protein and 17 water molecules

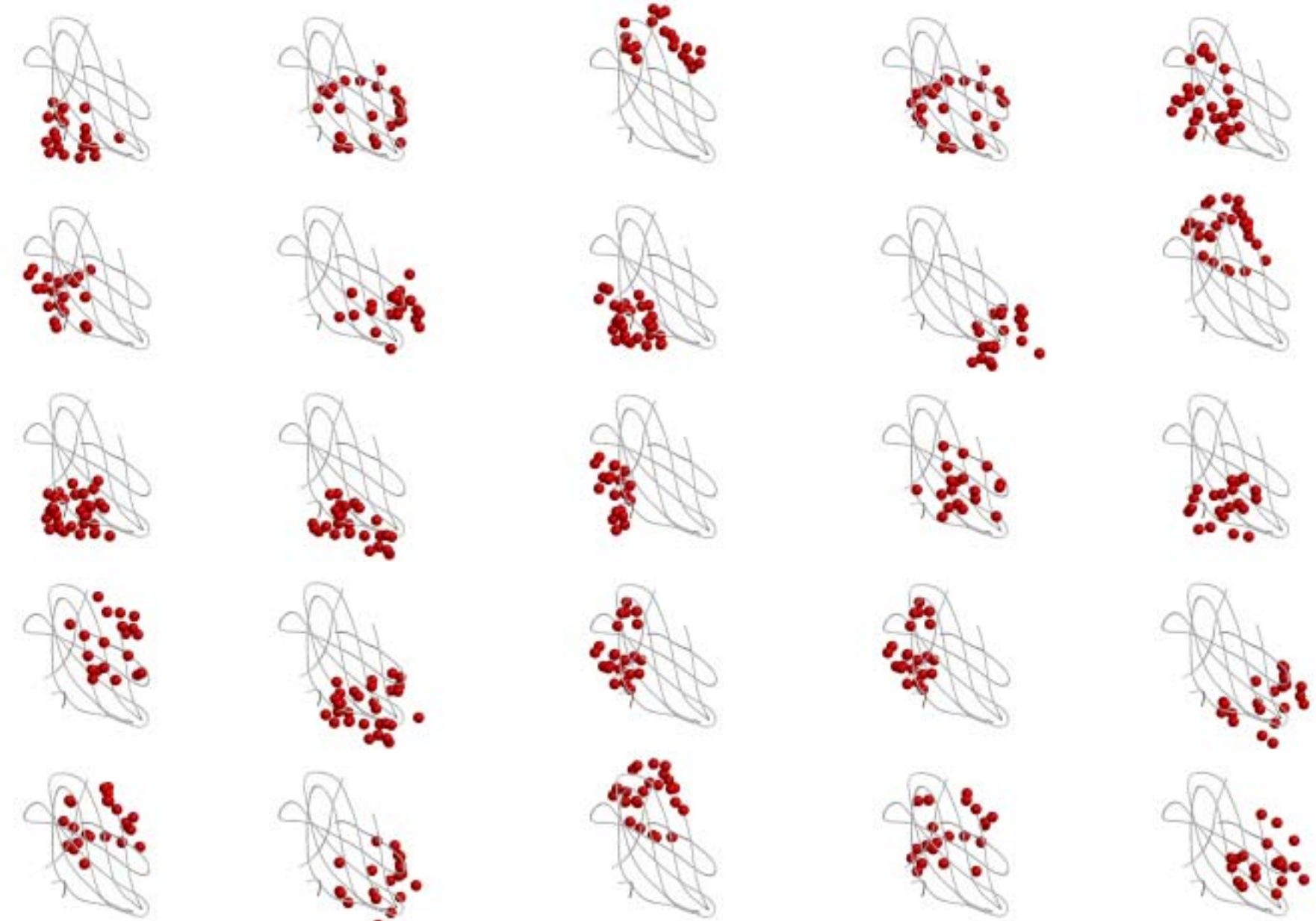




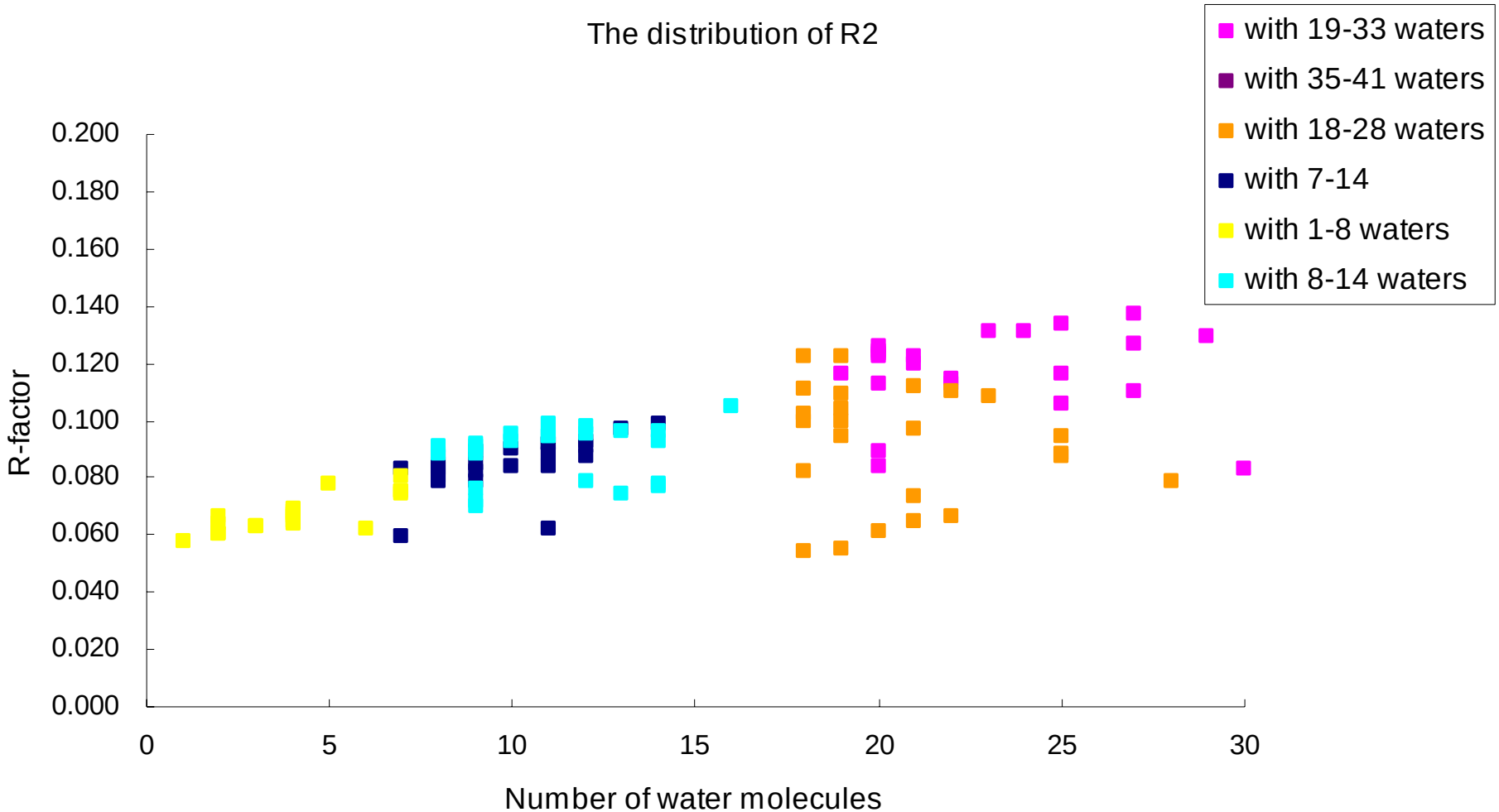
$F_{\text{protein}} \longleftrightarrow F_{\text{protein-water}}$

$$R1 = \frac{\sum_h \| F\text{protein} - \text{water} | - | F\text{protein} \|}{\sum_h | F\text{protein} |}$$

Twenty-five coordinates were generated so that each coordinate contains the protein molecule and 19 ~ 33 water molecules which are within a sphere of 12 Å radius. The sphere was created randomly.

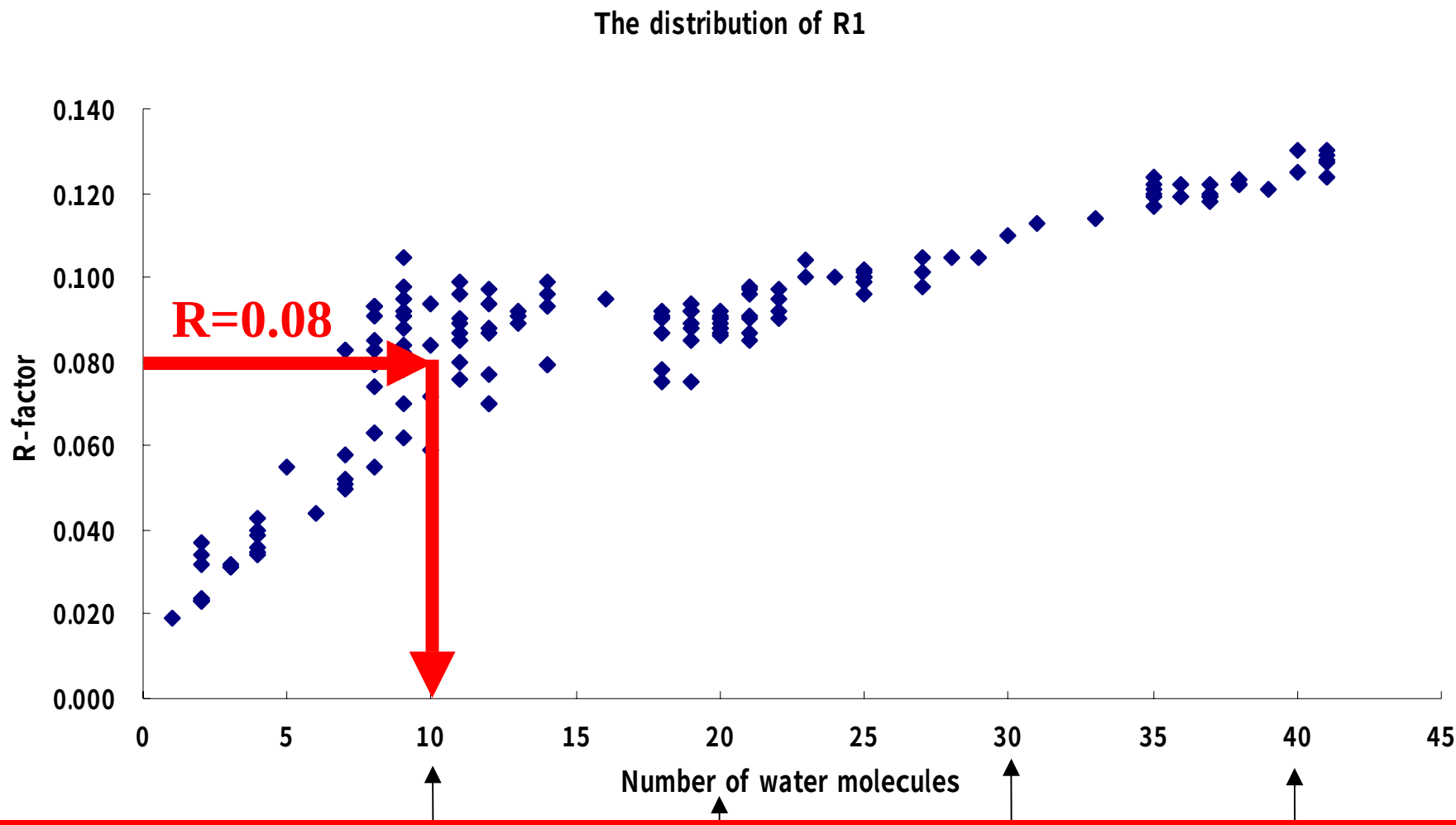


Difference between structures with different number of water molecules



Deviation from the protein structure without water →

Water content should be less than 1 %.



XFEL REQUIREMENTS FOR THE PLANNED BIOLOGY EXPERIMENTS

Studies on large structures

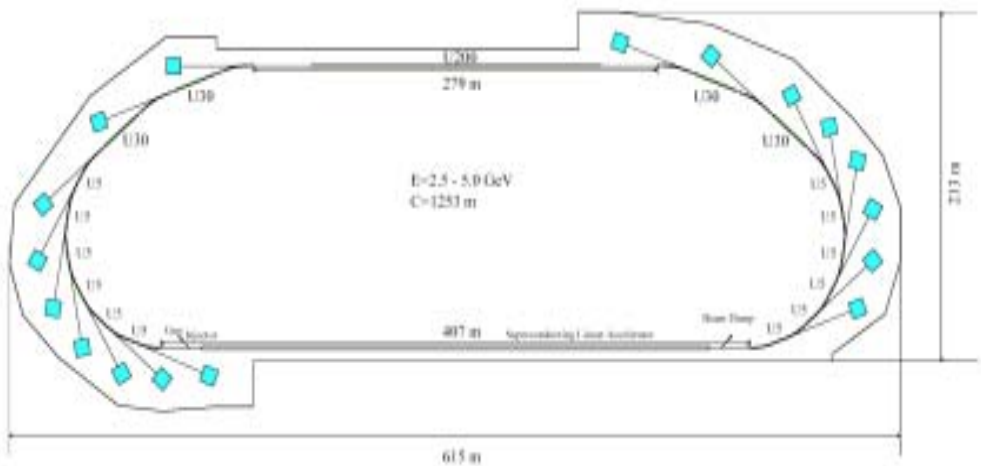
	J. Hajdu et al.	S.W.'s comments
Bandwidth	**0.2%**	**Could be much broader for experiments without anomalous signals.**
Pulse length	**230 fs and then shorter (while maintaining high dose/pulse)**	**Much longer for non-dynamic experiments**
Pump-probe?	**May be possible (delay pump-probe)** **Synchronization requirement: around 200 fs**	**Then short pulse**
Polarization (hor/vert)	**Not relevant**	**Must be known**
Pulse-to-pulse fluctuations	**Not a factor (normalization)**	**BIG problem since it will be almost impossible to normalize.**

Comparison of various types of the coherent X-ray sources (by N. Kulipanov)

	ESRF storage ring	LCLS linac	MARS
Wavelength, nm	.1	.15	.1
Electron energy, GeV	6	14	5.4
Average current, A	.2	3×10^{-8}	10^{-3}
Peak current, A		3.4×10^{3}	1
Relative energy spread		2×10^{-4}	1×10^{-5}
Emittance, nm εx εz	4 2.5×10^{-2}	3×10^{-2}	3×10^{-3}
Undulator period, cm	4.2	3	1.5
Undulator length, m	5	100	150
Coherent flux, photon/s	6×10^{12}	6×10^{14}	7×10^{13}
Bandwidth	10^{-2}	10^{-3}	10^{-4}
Average brightness, ph/s/mm^2/mrad^2/0.1%BW	10^{20}	6×10^{22}	3×10^{23}
Peak brightness, --//--		5×10^{33}	3×10^{26}
Transverse size of source (standard deviation), μm	σ_x 350 σ_y 8	9	10
Radiation transverse divergence (standard deviation), μrad	$\sigma_{x'}$ 13 $\sigma_{y'}$ 3	2	1

PF-ERL Main Parameters

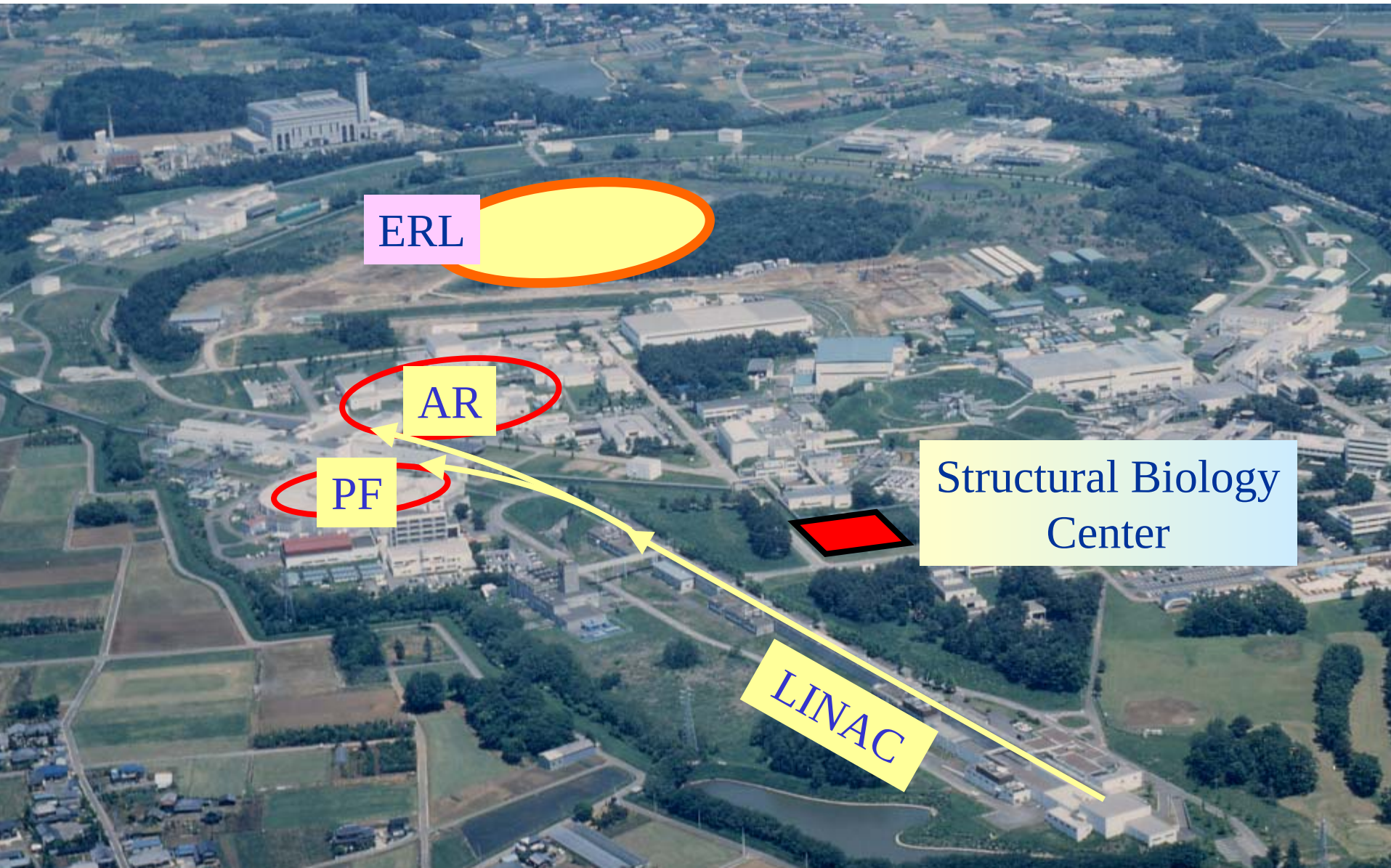
Beam Energy	2.5 ~ 5.0 (GeV)
Injection Energy	10 (MeV)
Circumference	1253 (m)
Beam Current	~100 (mA)
Normalized Emittance	~0.1 (μmrad)
Horizontal Emittance	~10.0 (pmrad) at 5.0 GeV
Vertical Emittance	~10.0 (pmrad) at 5.0 GeV
Energy Spread	~5×10^{-5}
Bunch Length	1 (ps) ~ 100 (fs)
RF Frequency	1.3 (GHz)
ACC. Gradient	~20 (MV/m)
Long Undulator	200 (m) x 1
Middle Undulator	30 (m) x 4
Short Undulator	5 (m) x12



SUMMARY

1. There will a very large number of biological problems that will require single molecule or nanocrystal structural studies
2. Radiation damage due to X-FEL pulse(s) →still a problem
3. Nano crystals (smaller than 1 μm cube)
   - Very promising as a way to overcome the limitations of the 3rd generation synchrotron sources
4. Single molecule analysis
   - Phase determination using oversampling →seems possible
   - Sample manipulation and determination of sample orientation →requires a lot of work
5. Storage ring type SR facilities will not be replaced by XFEL -- cohabilitation

Future Plan for Synchrotron Sources at KEK



PF Structural Biology Research Center

- Ryuichi Kato (Assoc. Prof.)
- Mamoru Suzuki (Research Assoc.)
- Noriyuki Igarashi (Research Assoc.)
- Naohiro Matsugaki (Research Assoc.)
- Masato Kawasaki (Research Assoc.)
- Masahiko Hiraki (Research Assoc.)
- Minora Nagai (Robotics technician)
- Tomoo Shiba (Post-doc)
- Shinsuke Hiramoto (Post-doc)
- Tamami Uejima (Staff scientist)
- Tadashi Sato (Post-doc)
- Satoshi Hirano (Post-doc)
- Michio Inoue (Ph.D. student)
- Yusuke Yamada (Ph.D. student)
- Yurii Gaponov (Scientific programmer)
- Leo Chavas (Ph.D. student ~Oct 2002, EMBL, Grenoble, France)

Vacancies

- Staff scientists (a few)
- Post-docs (4~6)
- Ph.D students (1~2)
- Engineers
- Technicians