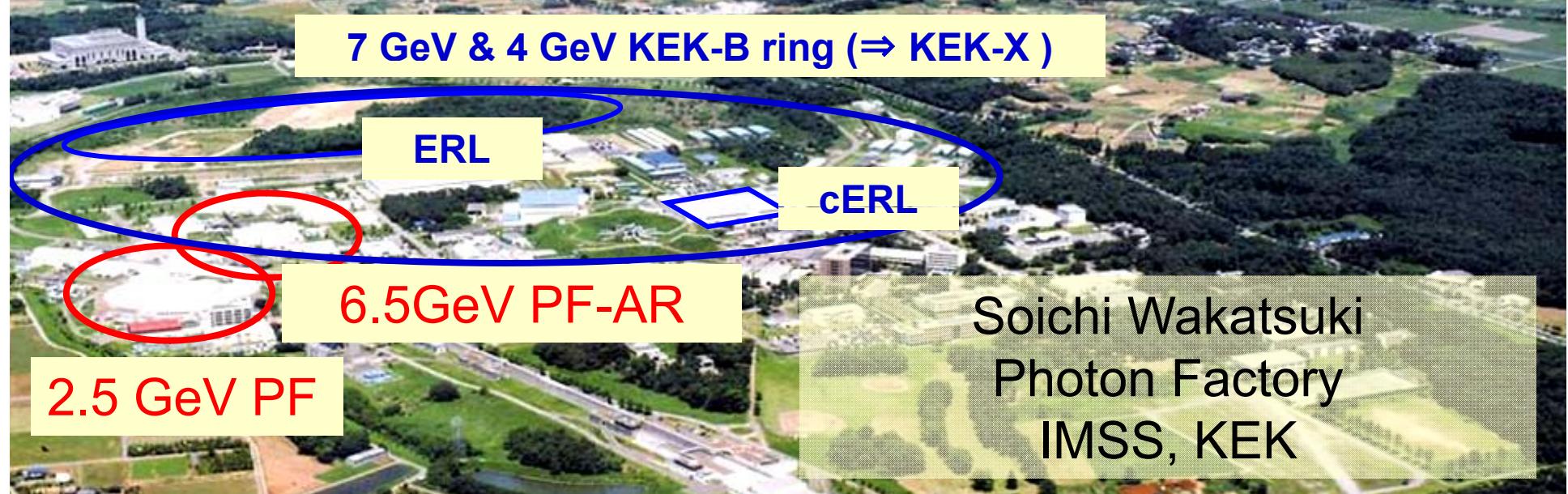


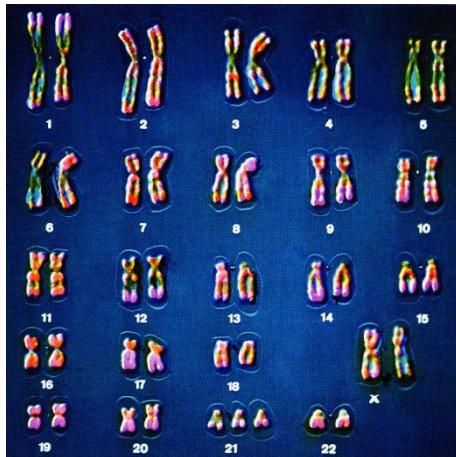
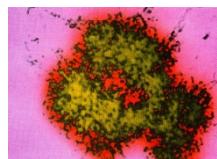


# Protein Crystallography: Synchrotron X-rays in quest of new horizons in the protein universe



# Role of Structural Biology

# Genome



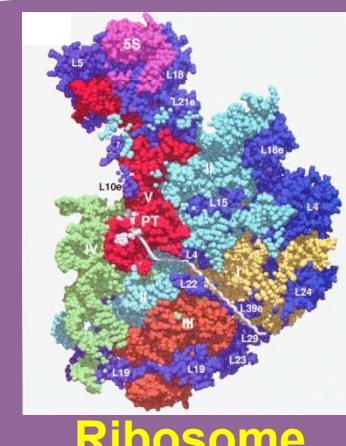
# *Transcription*

## DNA → mRNA →

# Proteins

# *Translation* Molecular machines (ribosomes, enzymes)

# Information network



# Ribosome

## Atomic resolution analysis

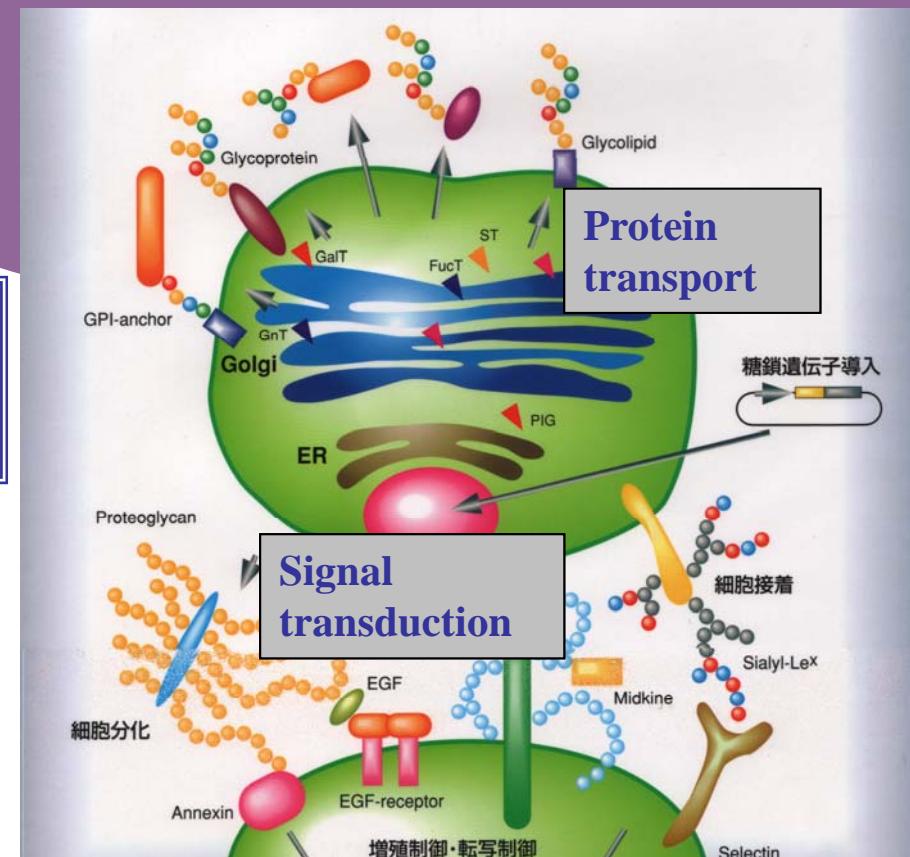
O Protein structure and dynamics

## ○Interactions between molecular machines

# Biology

## (Basic research)

## Medicine, drug design (industrial applications)

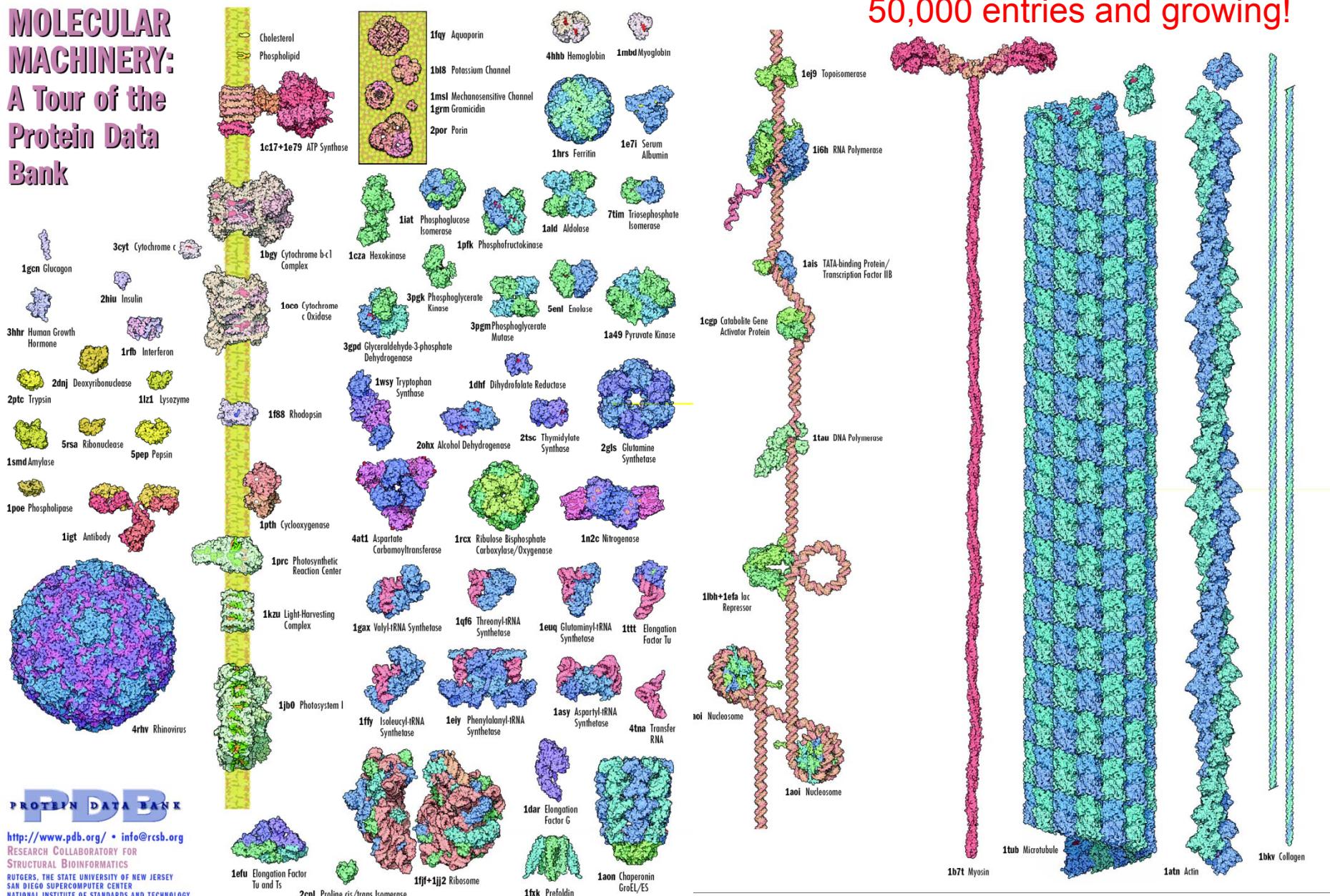


# The Worldwide Protein Data Bank (wwPDB)

<http://www.wwpdb.org/index.html>

50,000 entries and growing!

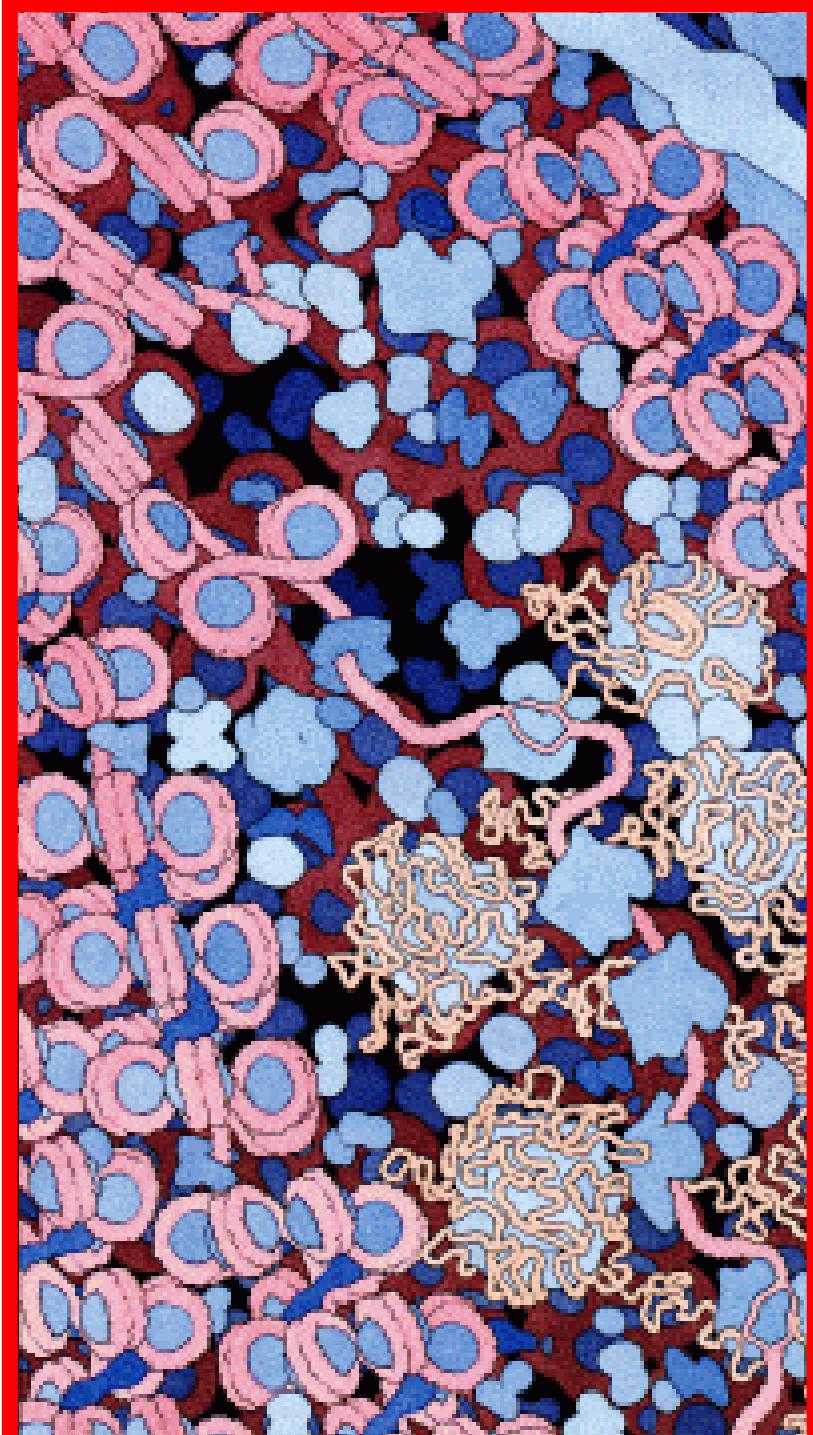
## MOLECULAR MACHINERY: A Tour of the Protein Data Bank



**David S. Goodsell, Scripps Institute**

# How large is the protein universe?

- How many genes?
  - Meta genomes --- J. Craig Venter Institute's Global Ocean Sampling Expedition
  - Human microbiomes (Gill, et.al. David Relman, Stanford, Science 2006)
  - Emerging infectious diseases
- Splicing variants – exon: cut and paste
- Non-coding RNA: largely uncharted
- Protein-protein, protein-carbohydrate, protein-lipid, protein-nucleic acid interactions
- Posttranslational modifications



## ***Panoramic view of a eukaryotic cell***

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

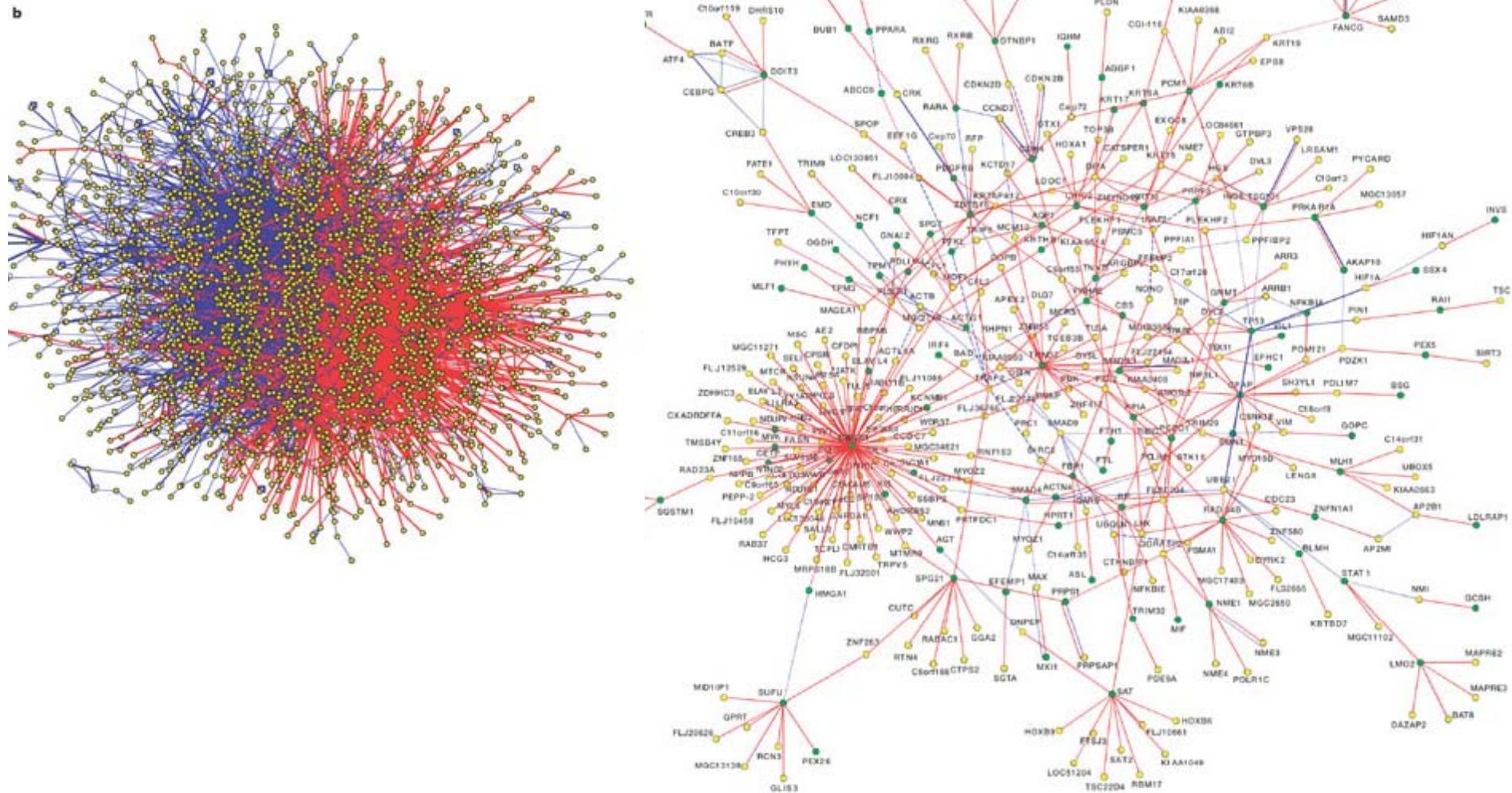
Movements



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

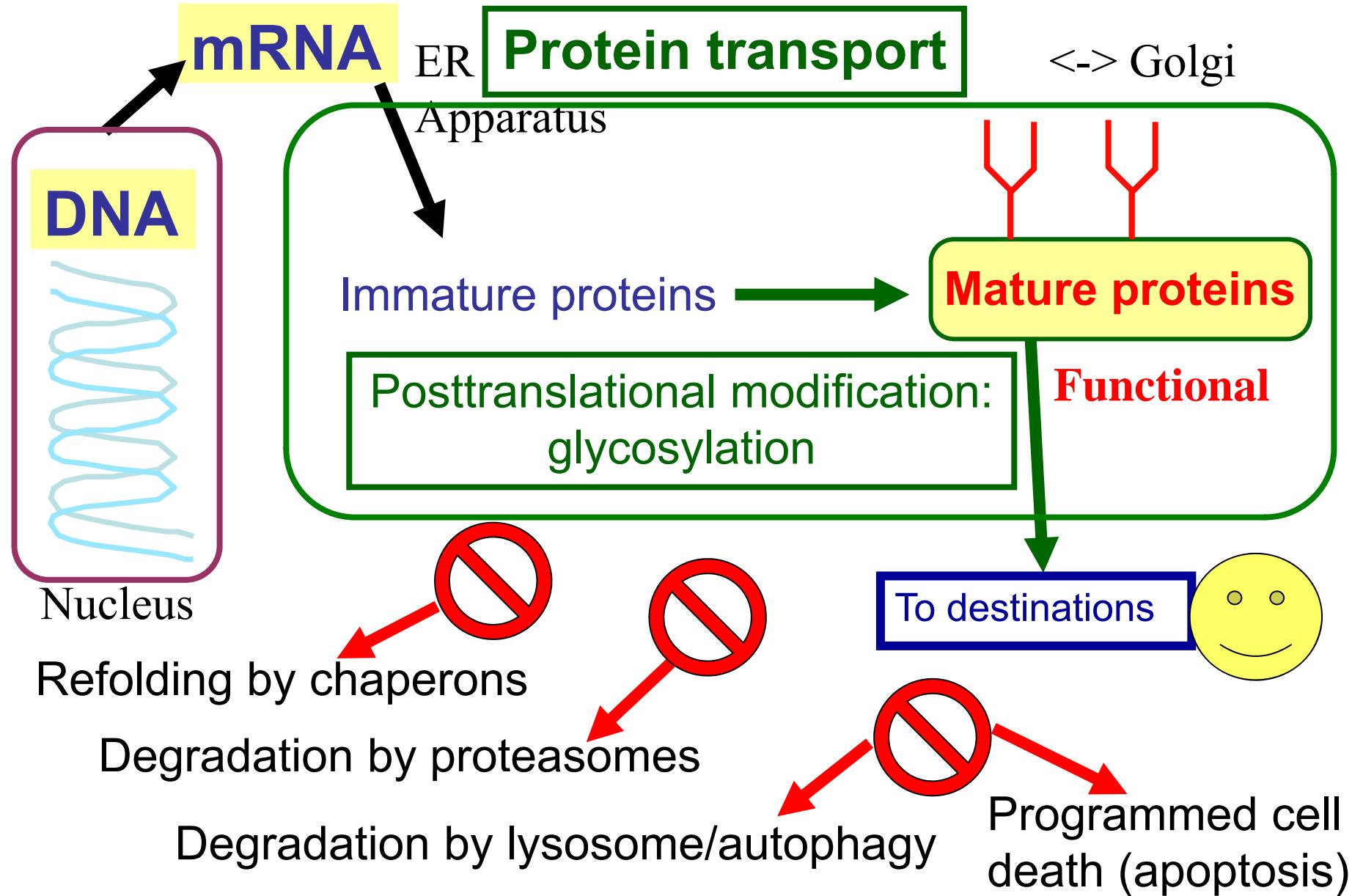
***David S. Goodsell, Scripps Institute***

# Protein-protein interaction network



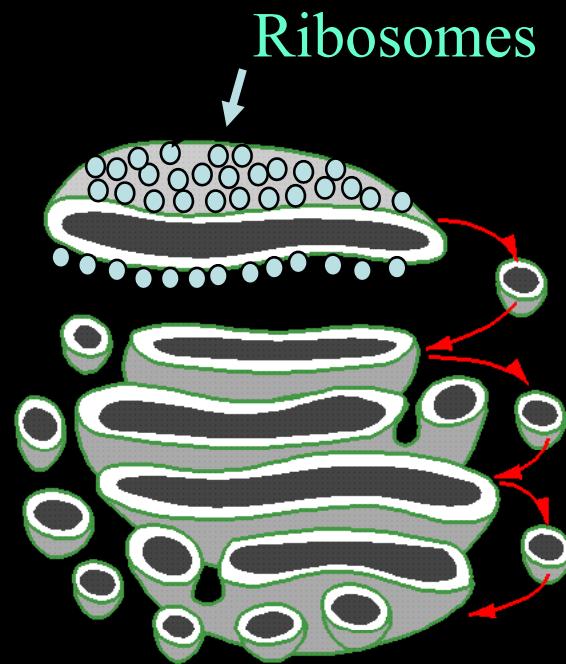
Taken from Rua et al., Nature 2005

# Protein life cycle and posttranslational modification



# What are posttranslational modifications of proteins?

- Modification of proteins after biosynthesis
- Not directly coded in the DNA sequences
- Add functionality, modify surface properties, transport signals
  - Glycosylation
  - Ubiquitylation (SUMO, NEDD etc.)
  - Lipidation
  - Phosphorylation
  - Acetylation
  - And more



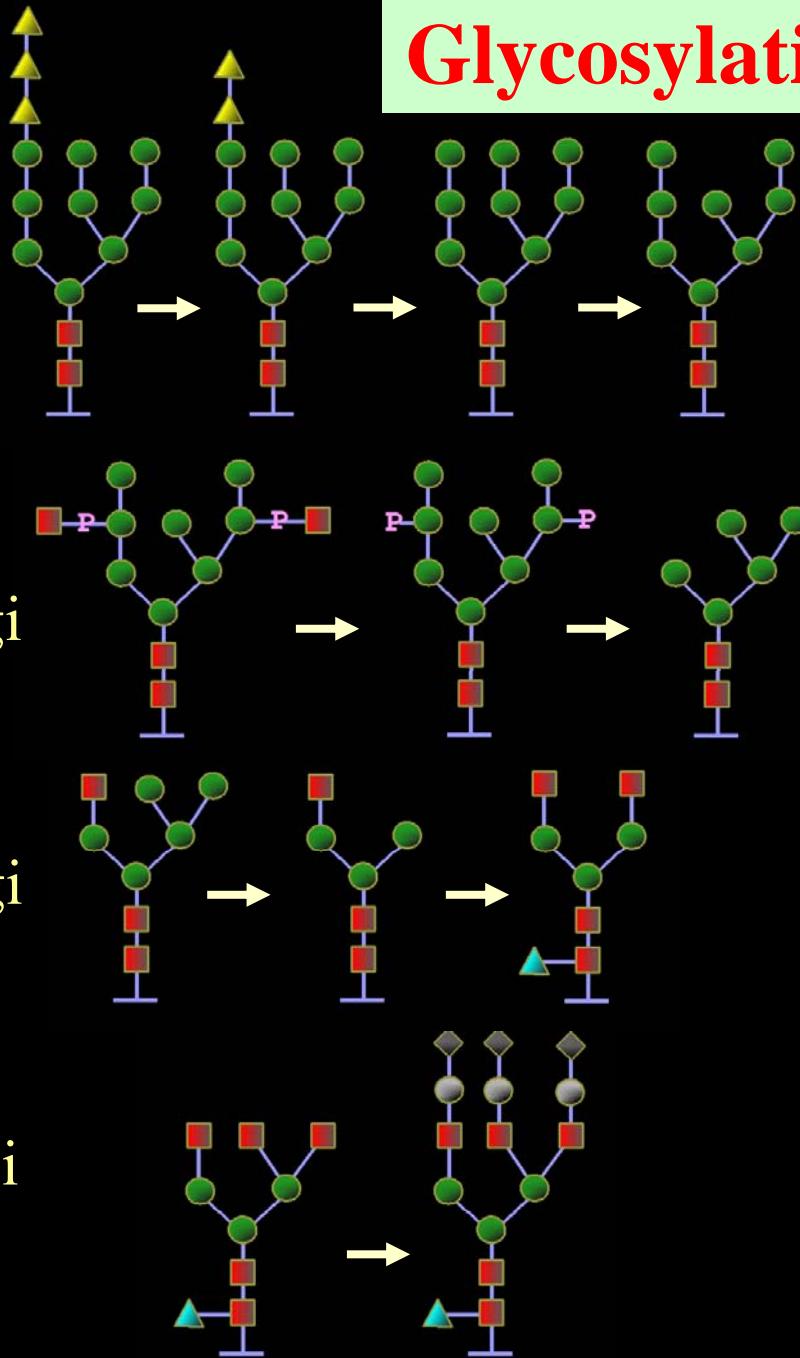
Cis-Golgi

Medial-Golgi

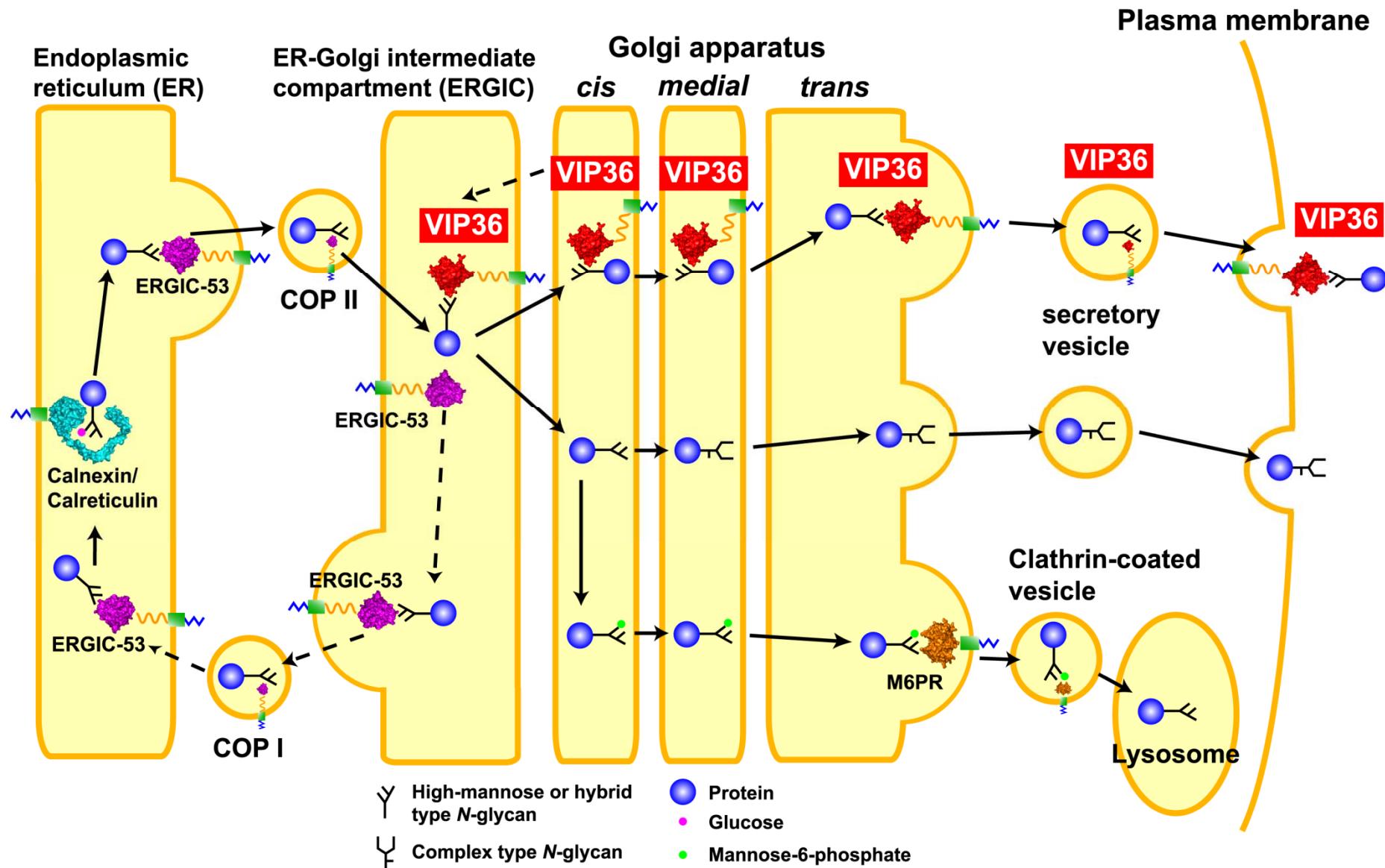
Trans-Golgi

- GlcNAc
- Mannose
- ▲ Glucose
- Phosphate
- ▲ Fucose
- Galactose
- ◆ Sialic acid

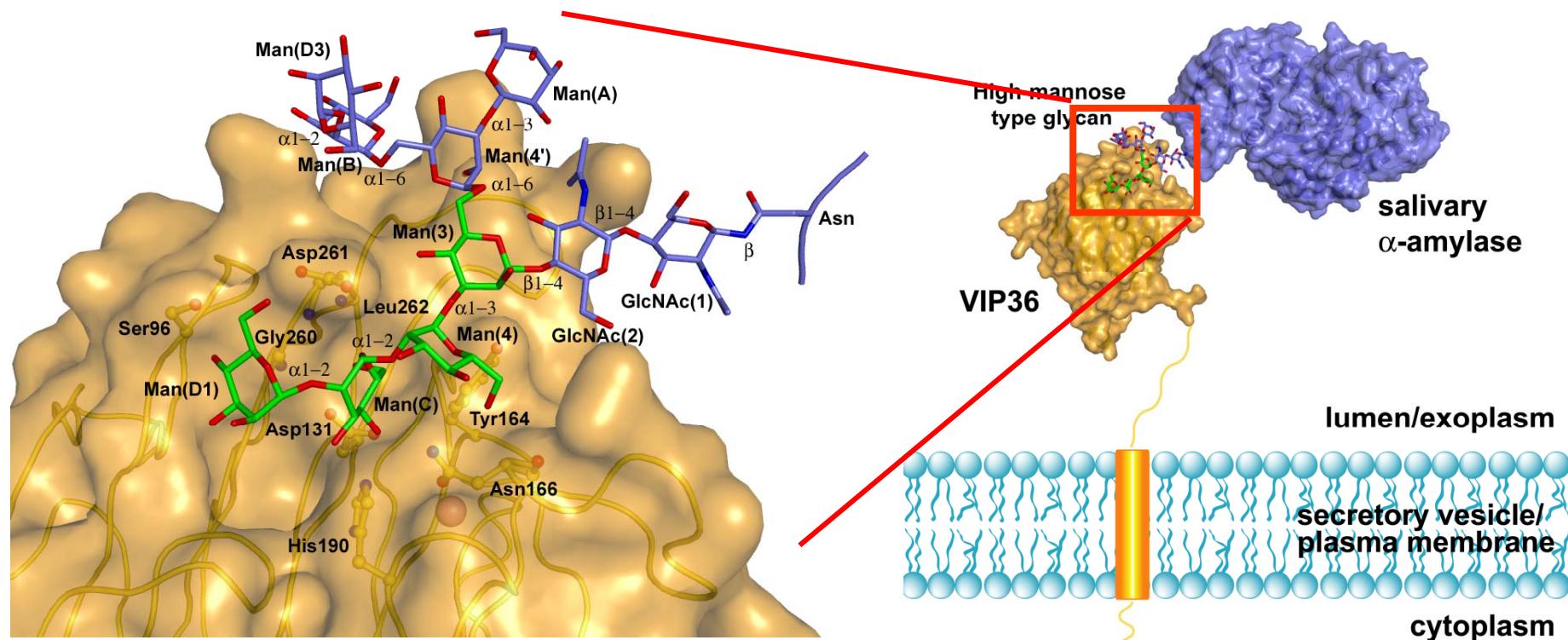
## Glycosylation



# Model for intracellular transport of N-linked glycoproteins

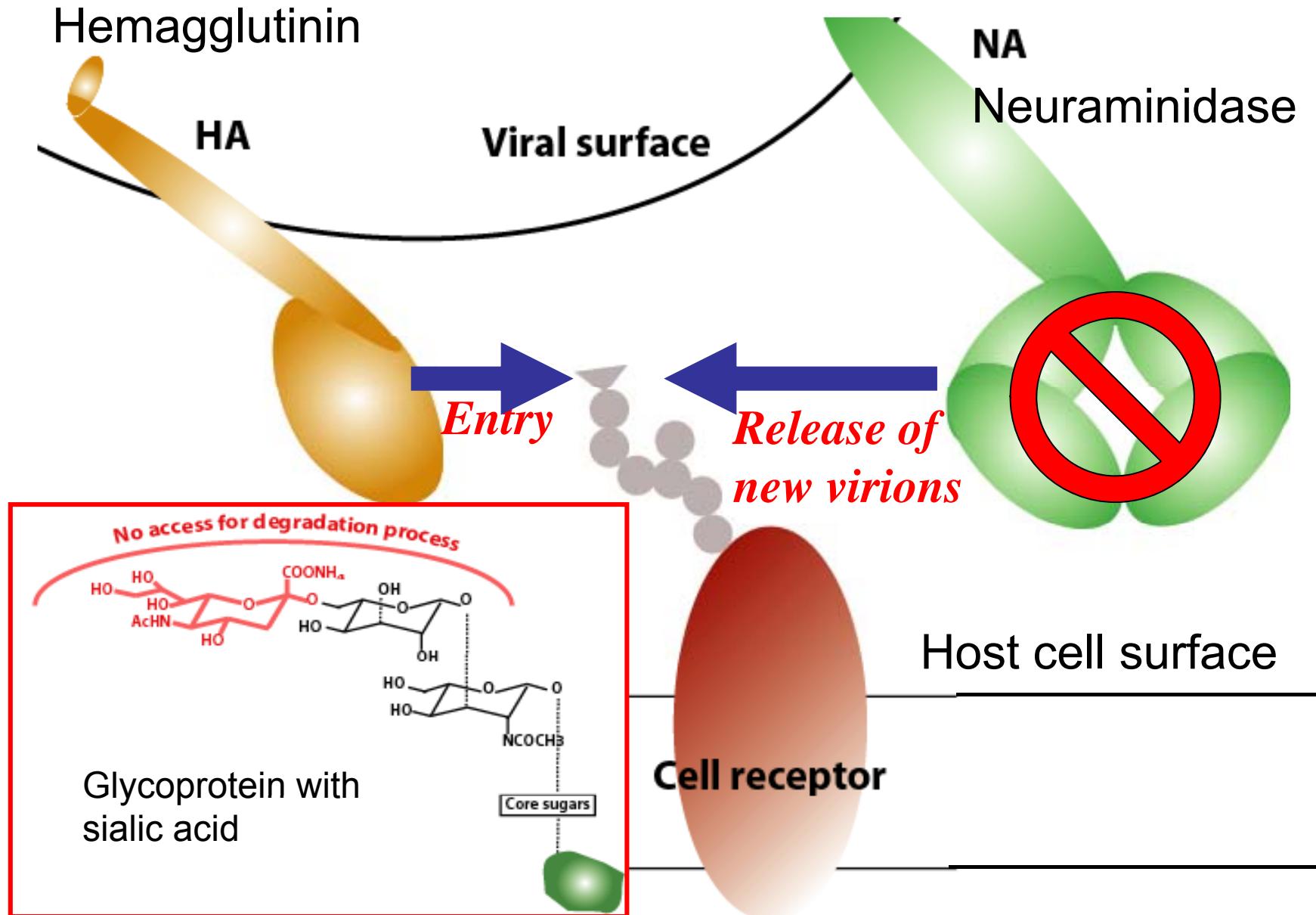


# Model for binding between VIP36 and high-mannose type glycoprotein, salivary $\alpha$ -amylase

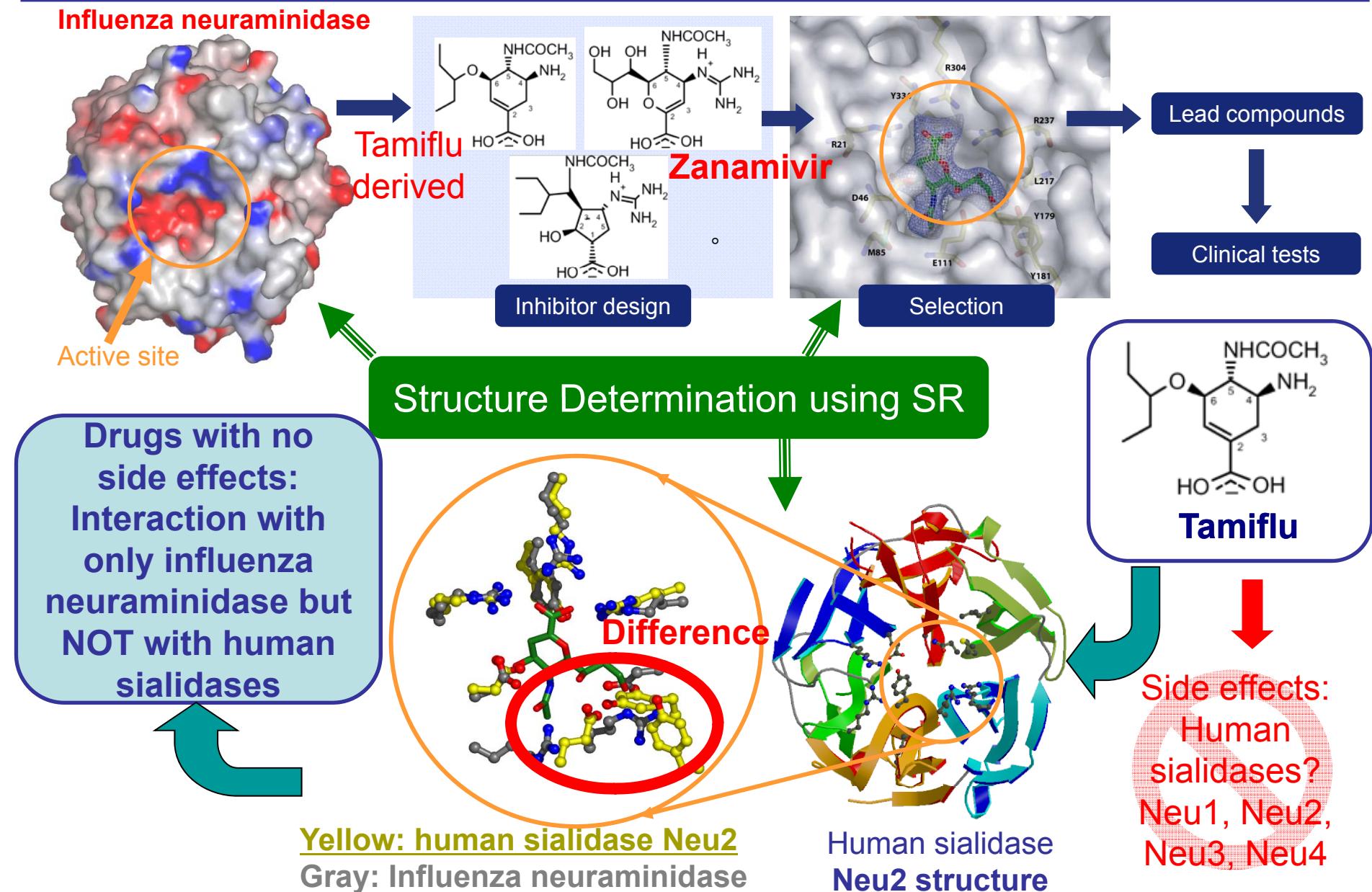


Tadashi Satoh, Nathan Cowieson, et al., J. Biol. Chem. (2007) 282, 28246

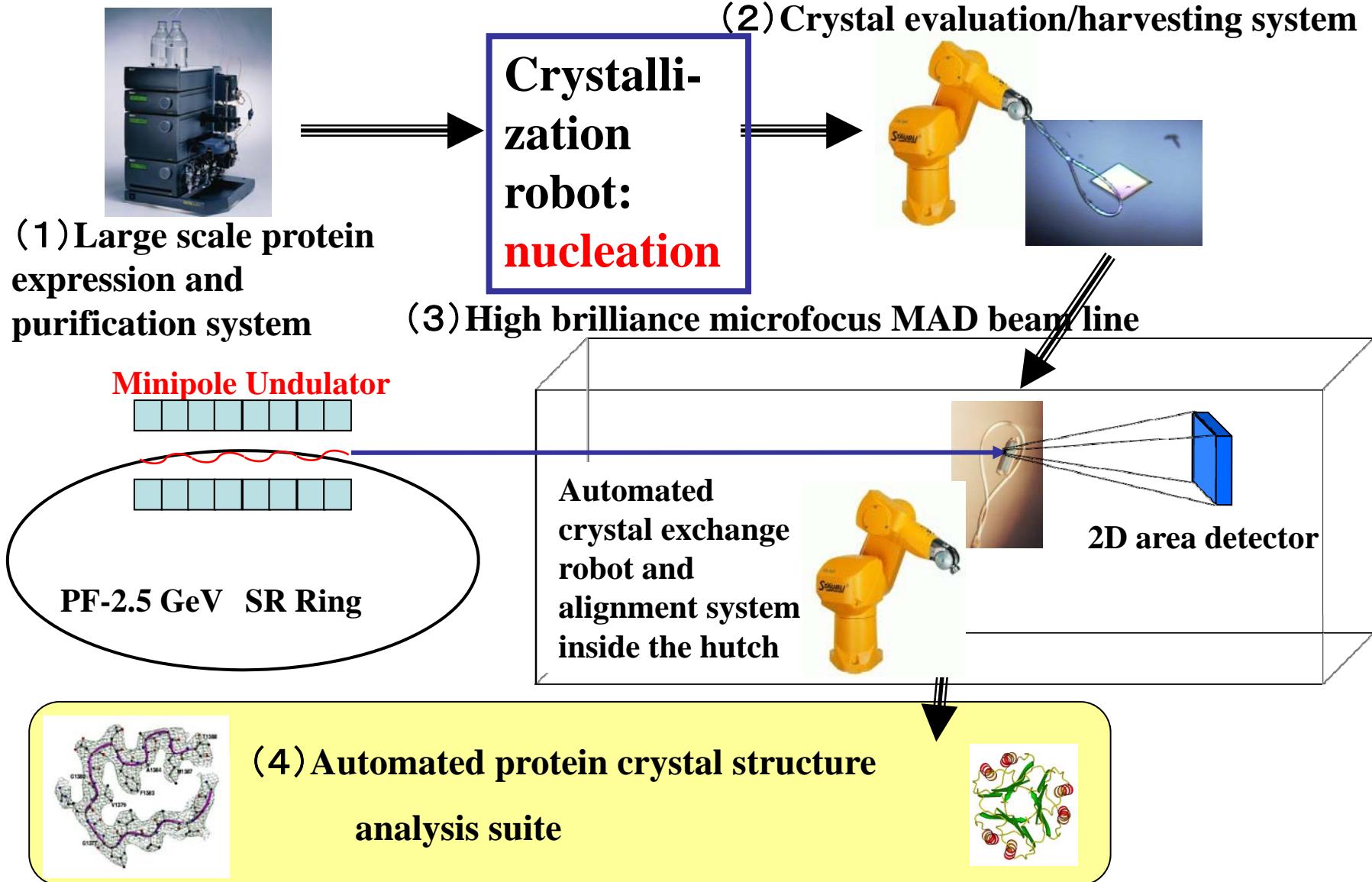
# Structural studies towards new Influenza virus inhibitors



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



# Flow of protein structure analysis



# Guanine nucleotide exchange factor Sec12p

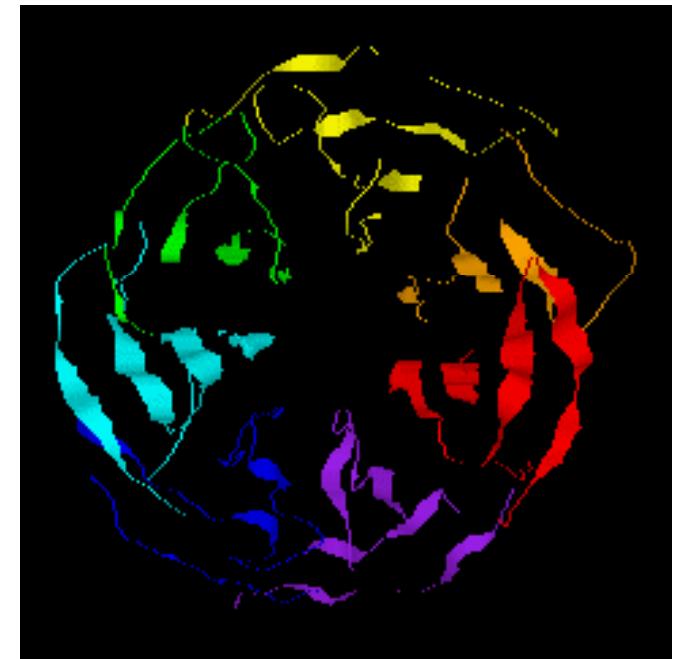
1 ttgc tgacagctag ttataacgtc gggatccctg cgtacggcgc aaaatttttg  
61 aataacgaca cattacttgt ggcaggcggt ggaggagaag gaaacaatgg cataccaaac  
121 aagctgacgg tcttgcgcgt ggatcctacc aaagatactg agaaggaaca gttcatata  
181 ttgagcggat ttgcatttggaa agacaacgac gactctccta ctgcaattga cgcttccaag  
241 ggtatcattt tggttggctg caatgaaaat agcactaaga ttacccaagg taaaggtaat  
301 aagcacttga gaaaatttaa atacgataaa gtgaatgatc aattggagtt cctcactagt  
361 gtagactttg acgcatctac aaatgcggat gactacacga agctggttt tatttcacga  
421 gaaggtaccc ttgcagctat cgcatcatct aaagtacctg ctataatgag aatcattgac  
481 ccgagcact tgacagagaa gtttgagatc gagactaggg gtgaagtaaa ggatttacac  
541 ttttccactg atggtaaggt tgttgcttat atcaccgggtt cttagtttggaa agtgatttca  
601 acagtgactg gaagttgcat tgcttagaaa acagattttg ataagaatttggagtttatct  
661 aaaataaaact tcatacgccga tgacacagta ttgatagcag cctttttaaa aaaaggaaaa  
721 ggtattgtgc tgacaaaaat aagcatcaaa tcaggaaaca cttccgtatt aagatccaaa  
781 caagtaccaa acagattcaa agggattact tctatggatg tcgacatgaa gggtaatttgg  
841 gcggtactgg caagtaatga caattccata gctcttgcga aactaaaaga cctgtcaatg  
901 tctaaaatat tcaaacaagc tcatagtttt gccattacag aggtcaactat ctctccggac  
961 tctacatatg tggcgagtgt ttccggcagcc aacactatcc acataataaa attaccgtt  
1021 aactacgcca actacacccctc aatgaaacaa aaaatctcta aattttcac caacttcata  
1081 cttattgtgc tgctttctta cattttacag ttctccctata agcacaattt gcattccatg  
1141 cttttcaatt acgcaagga caattttctta acgaaaagag acaccatctc ttccggctac  
1201 gtagttgatg aagacttaca tcaaacaact ttgttggca accacggatc aaaaacatct  
1261 gtaccttagcg tagattccat aaaagtgcattt ggcgtgcattt agacgagttc tgtgaatggaa  
1321 actgaagtct tatgtactga aagtaacatt attaataactg gagggggcaga gtttgagatc  
1381 accaacgcaa ctttcgaga aatagatgtat gct**tga**

No. of bases: 1416

$$(1416 - 3)/3 = 471$$

FVTASYNVGYPAYGAKFLNNDTLLVAGGGEGNNNGIPNKLTV  
LRVDPTKDEKEQFHILSEFALEDNDDSPATAIDASKGIILVGCNENSTKITQGKGNKH  
LRKFKYDKVNDQLEFLTSVDFDASTNADDYTKLVYISREGTVAAIASVKPAIMRIID  
PSDLTEKFEIETRGEVKDLHFSTDGKVAYITGSSLEVISTVTGSCIARKTDFDKNWS  
LSKINFIADDTVLIAASLKKKGKIGIVLTKISIKSGNTSVLRSKQVTNRFKGITSMDVDM  
KGELAVLASNDNSIALVKLKDLSMSKIFKQAHSAITEVTISPDTYVASVSAANTIH  
IIKLPLNYANYTSMKQKISKFFTNFILIVLLSILQFSYKHNHLHSMLFNYAKDNFLTK  
RDTISSLSPYVVDEDLHQTTLFGNHTKTSVPVSDSIKVHGVHETSSVNGTEVLCTESNI  
INTGGAEFEITNATFREIDDA

No of amino acid residues: 471



Model structure of Sec12

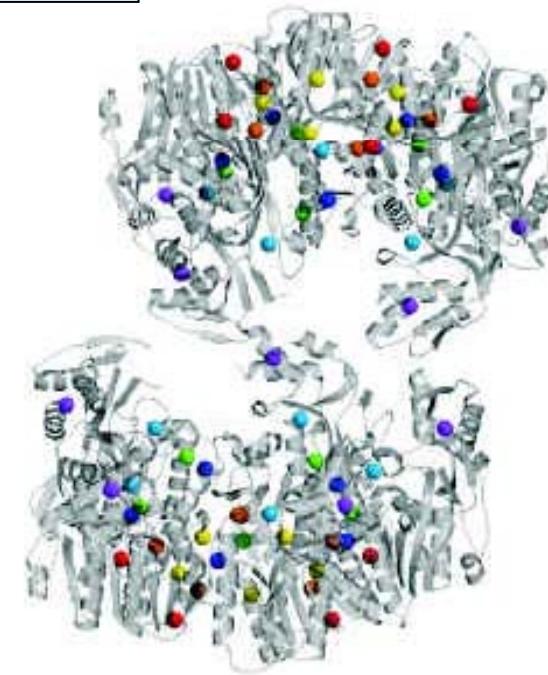
# Phase Determination using Multiple Anomalous Dispersion

Methionine

Sulfur —→ Selenium (SeMet)

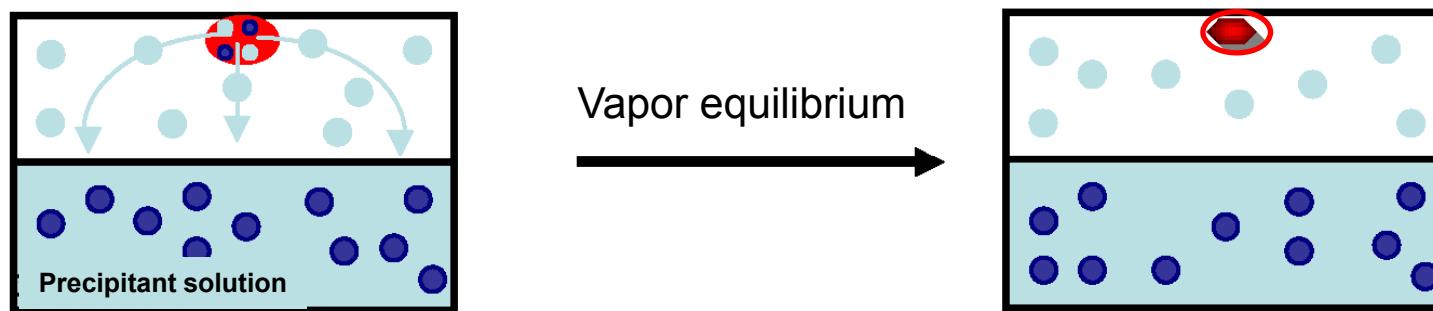
FVTASYNVGYPAYGAKFLNNDTLLVAGGGGEGNNGIPNKLTV  
LRVDPTKDTKEQFHILSEFALEDNDDSPATAIDASKGIILVGCNENSTKITQGKGNKH  
LRKFKYDKVNDQLEFLTSVDFDASTNADDYTKLVYISREGTVAAIASSKVPAIMRIID  
PSDLTEKFEIETRGEVKDLHFSTDGKVAYITGSSLEVISTVTGSCIARKTDFDKNWS  
LSKINFIADDTVLIAASLKKKGKIVLTKISIKSGNTSVLRSKQVTNRFKGITSMDVDM  
KGELAVLASNDNSIALVKLKDLSMSKIFKQAHSAITEVTISPDSITYVASVSAANTIH  
IIKLPLNYANYTSMKQKISKFFTNFILIVLLSYILQFSYKHNLSHMLFNYAKDNFLTK  
RTIISSPYVVDEDLHQTTLFGNHGTTSVPSVDSIKVHGVHETSSVNGTEVLCTESNI  
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

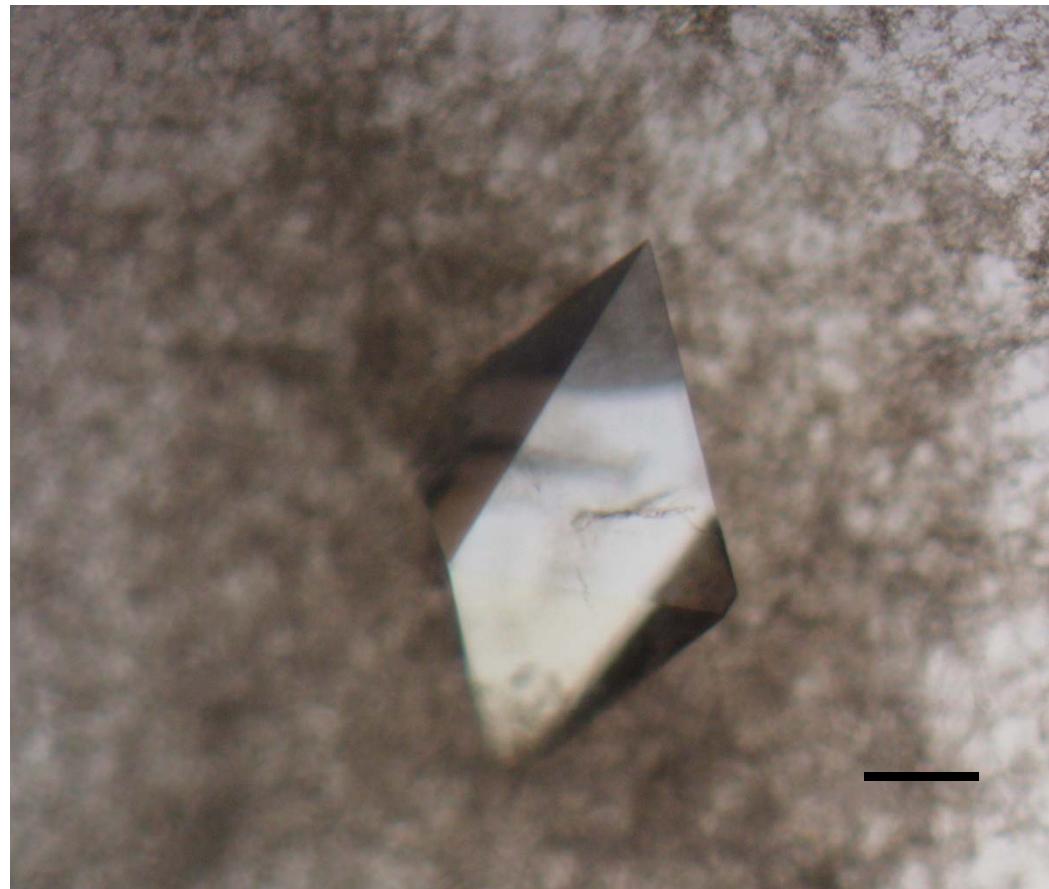


- Protein solution drop (containing water, precipitant and protein molecules)
- Precipitant molecule
- Water molecule
- ◆ Protein crystal

Drawing by Prof. Yoshiki Higuchi

**~ $10^{12}$  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**

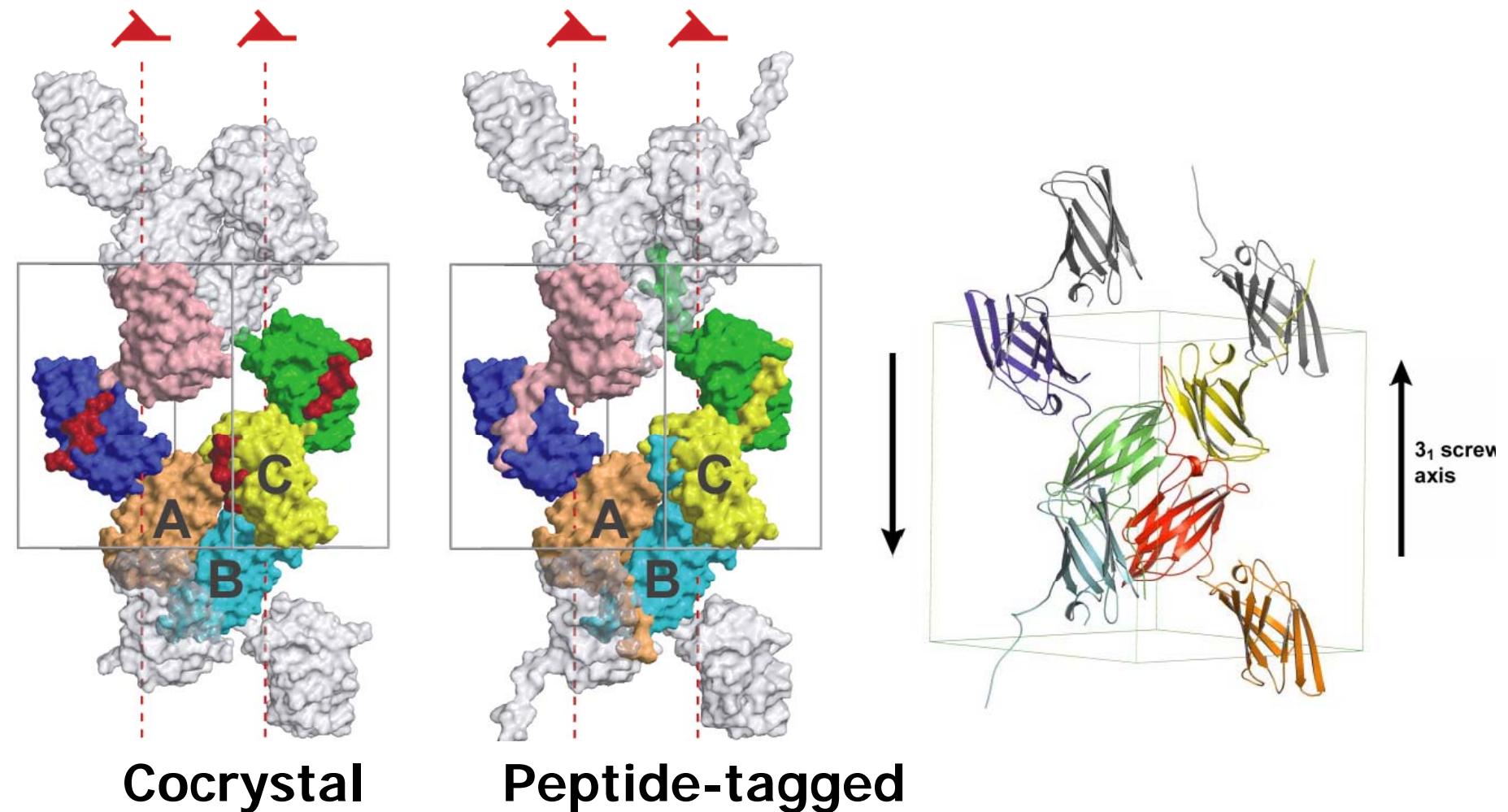
**Precipitant: 17 % (w/v) PEG3350, 0.2 M  $\text{KH}_2\text{PO}_4$**

**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!



# Why synchrotron?

- X-ray intensity      ⇒ measurements of weak reflections  
                            ⇒ high-throughput measurements
- Parallel beam        ⇒ large unit cells
- Micro beam           ⇒ micro crystals  
                            ⇒ use of best part of crystals
- $\lambda$  tunability        ⇒ phase determination

# Intensity of a reflection

$$E(hkl) = \frac{e^4}{m^2 c^4 \omega} I_0 \lambda^3 LPA \frac{\frac{V_x}{V_0^2}}{|F(h)|^2}$$

$F(h)$ : structure factor

$\omega$ : speed of crystal rotation

$I_0$ : Intensity of incoming X-ray

$\lambda$ : wavelength

$P$ : polarization factor

$L$ : Lorentz correction

$A$ : absorption correction

$V_x$ : volume of the crystal in the beam

$V_0$ : unit cell volume

# Methods to Determine Phases

## Heavy atom MAD

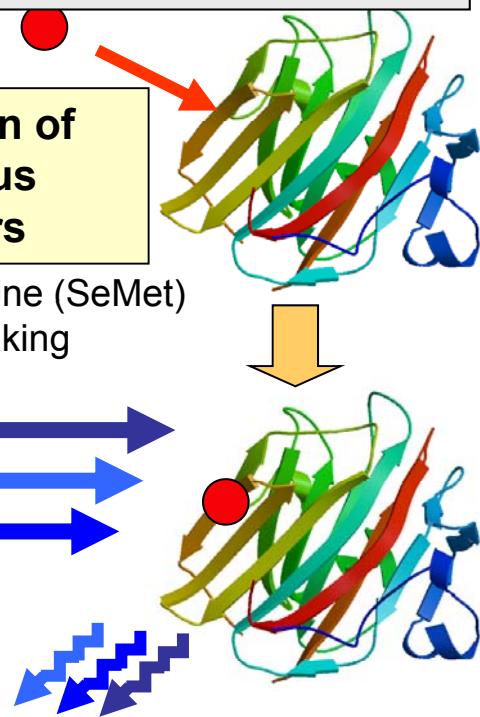
(Multiple Anomalous Dispersion)

### Introduction of anomalous scatterers

- Seleno-methionine (SeMet)
- Heavy atom soaking

$\lambda_1$   
 $\lambda_2$   
 $\lambda_3$

X-rays



### 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

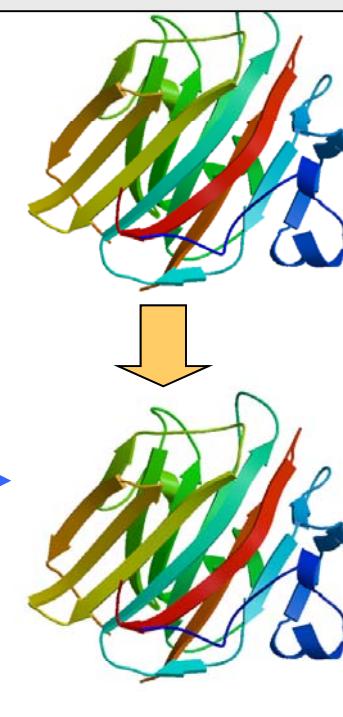
## Light atom SAD

(Single Anomalous Dispersion)

### Wild type crystals

$\lambda$

X-rays



### 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

## Phase determination using MAD

Bijvoet pairs

### (Multiple Anomalous Dispersion)

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

Bijvoet Differences

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

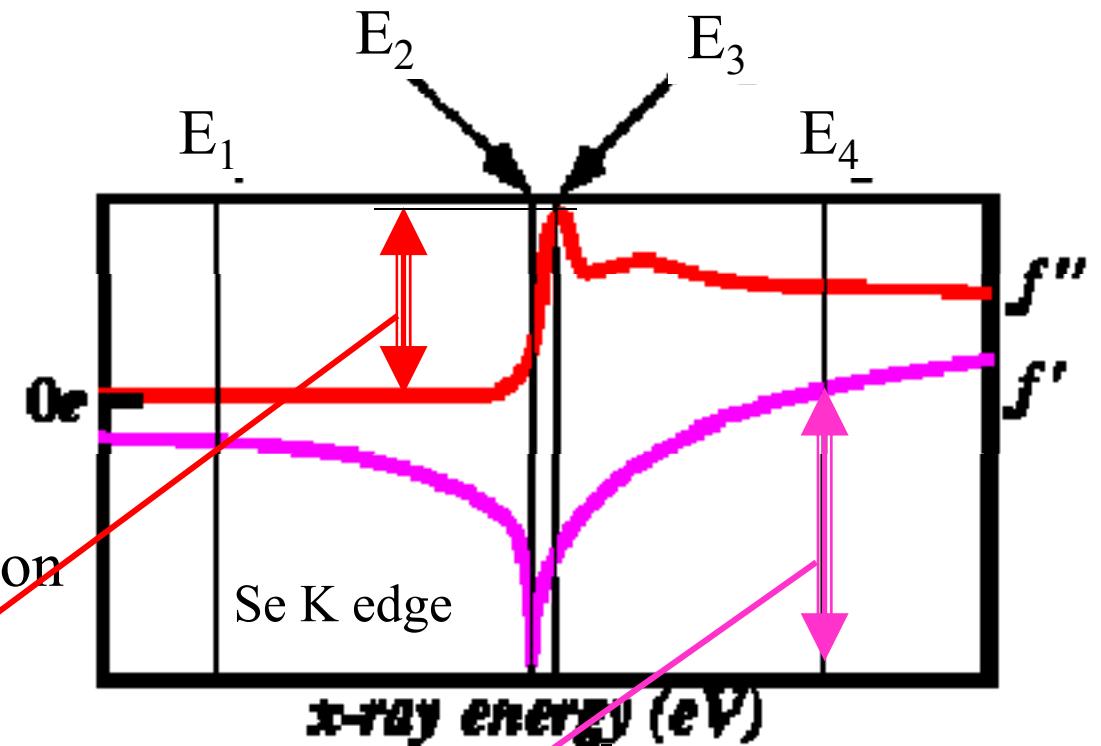
MAD experimental maps:

Anomalous difference Patterson

$$\Delta F_{E_3}^2 \text{ (at the peak)}$$

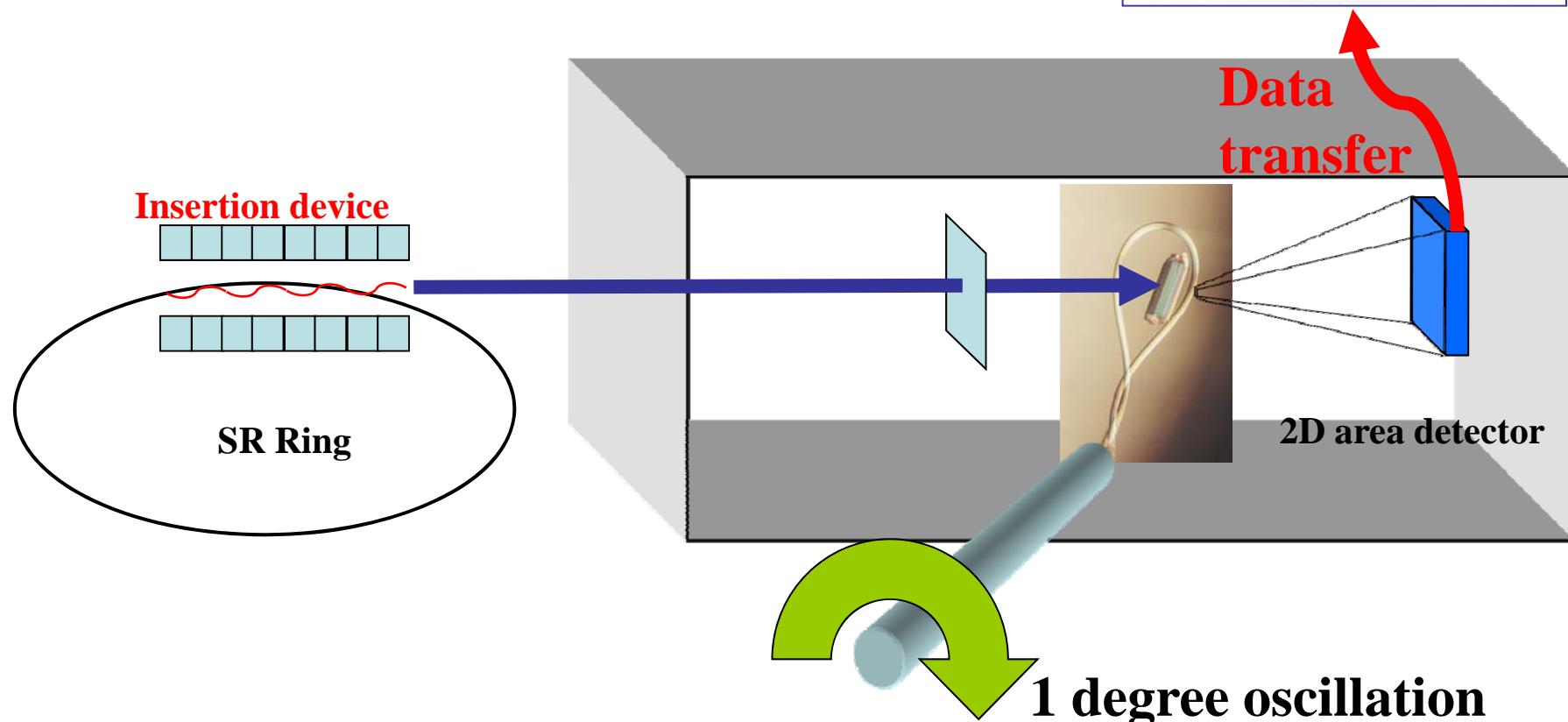
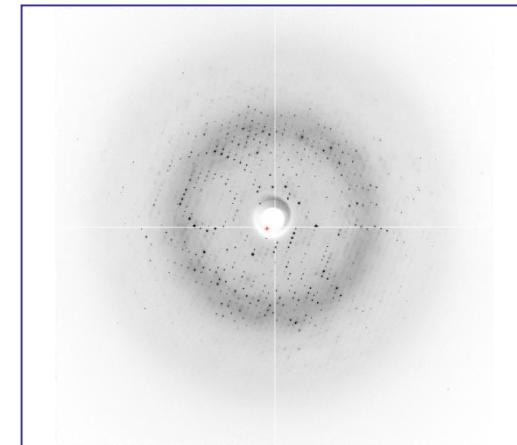
Dispersive Patterson

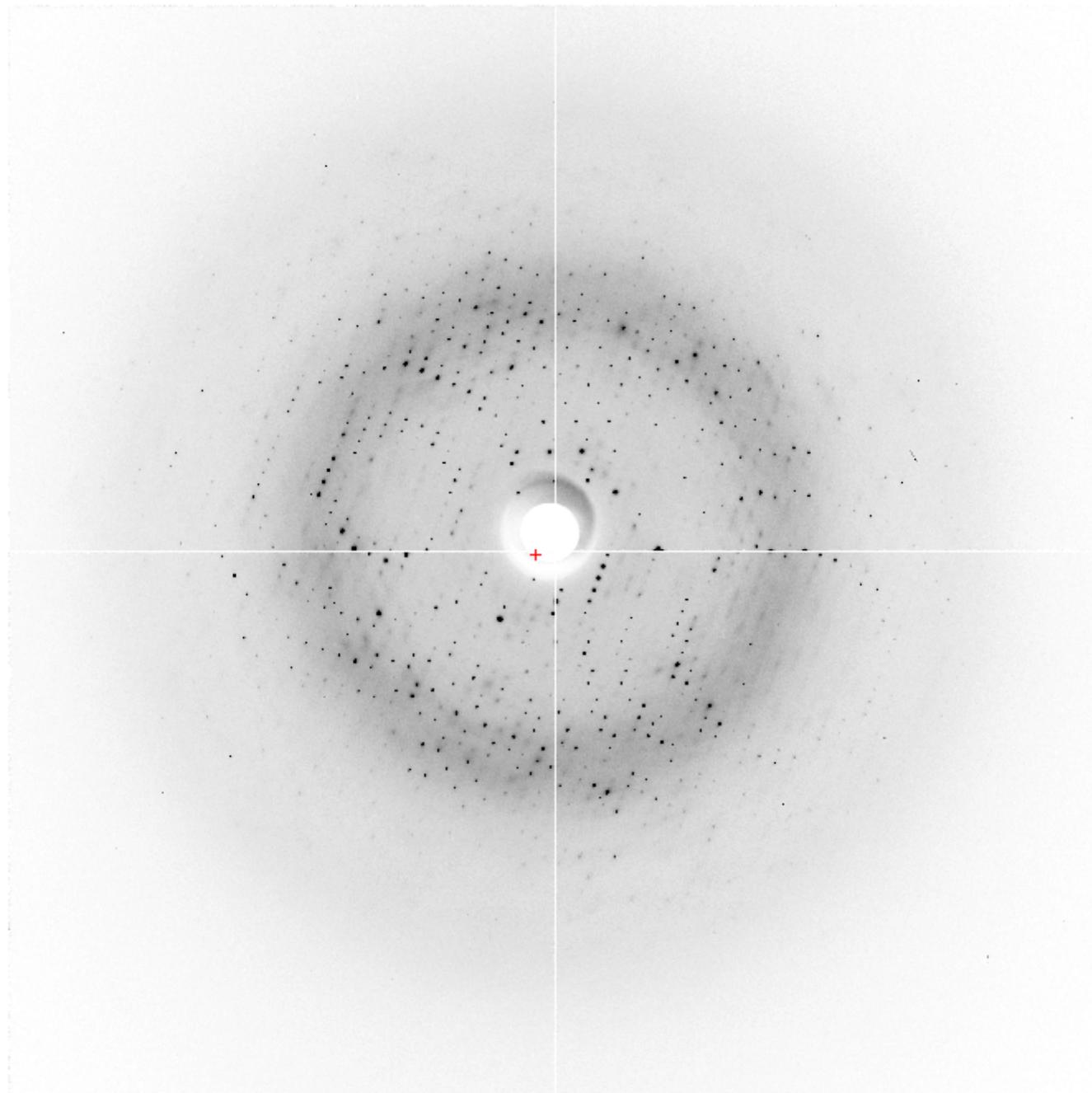
$$F_{E_4}^2 - F_{E_2}^2 \text{ (remote - inflection point)}$$



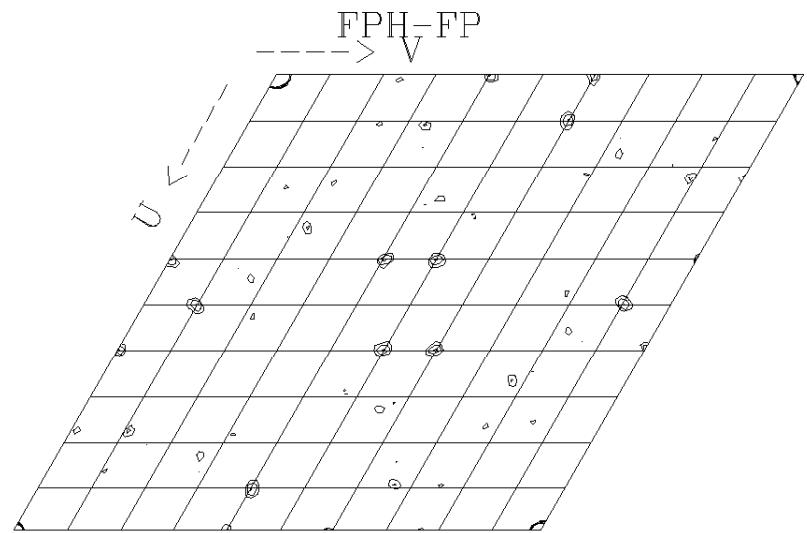
$$f(\lambda) = {}^0f + f'(\lambda) + if''(\lambda)$$

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

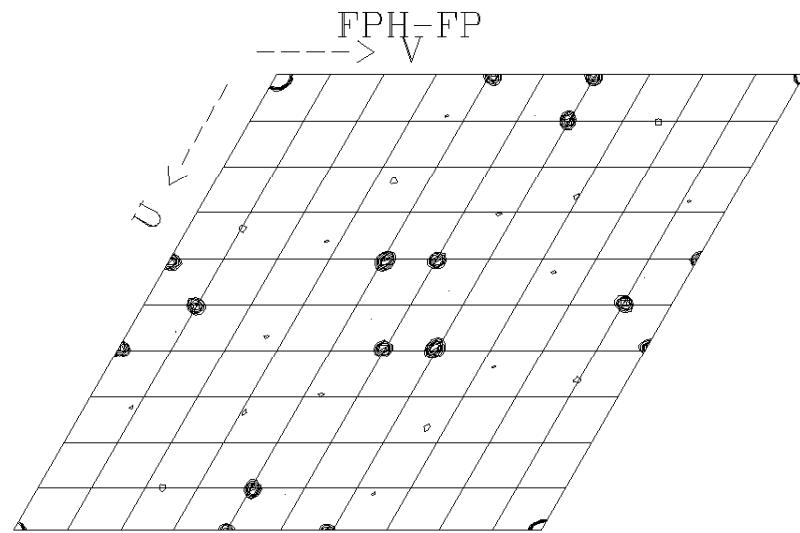




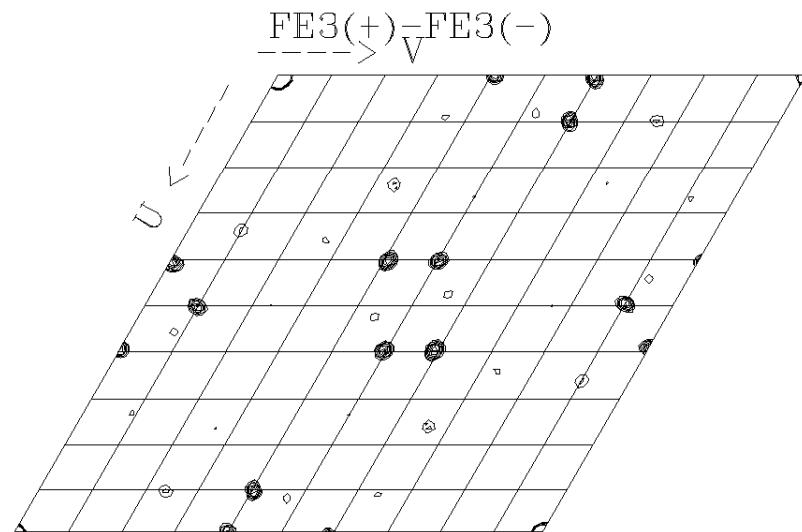
L



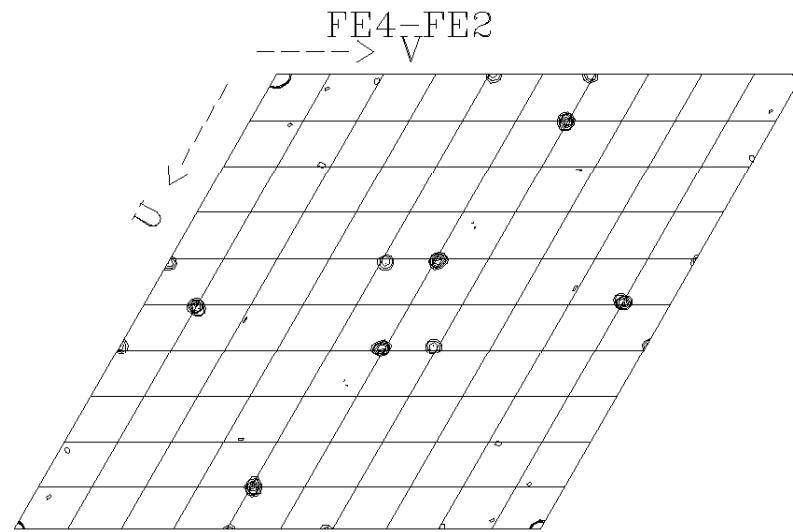
**FPH(SeMet)-FP(native) difference  
Patterson Map**



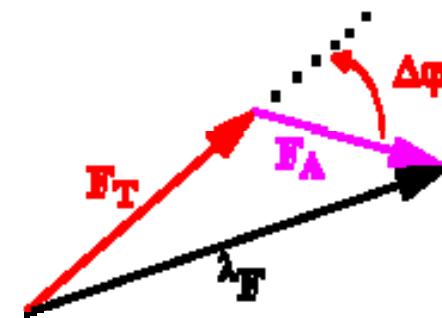
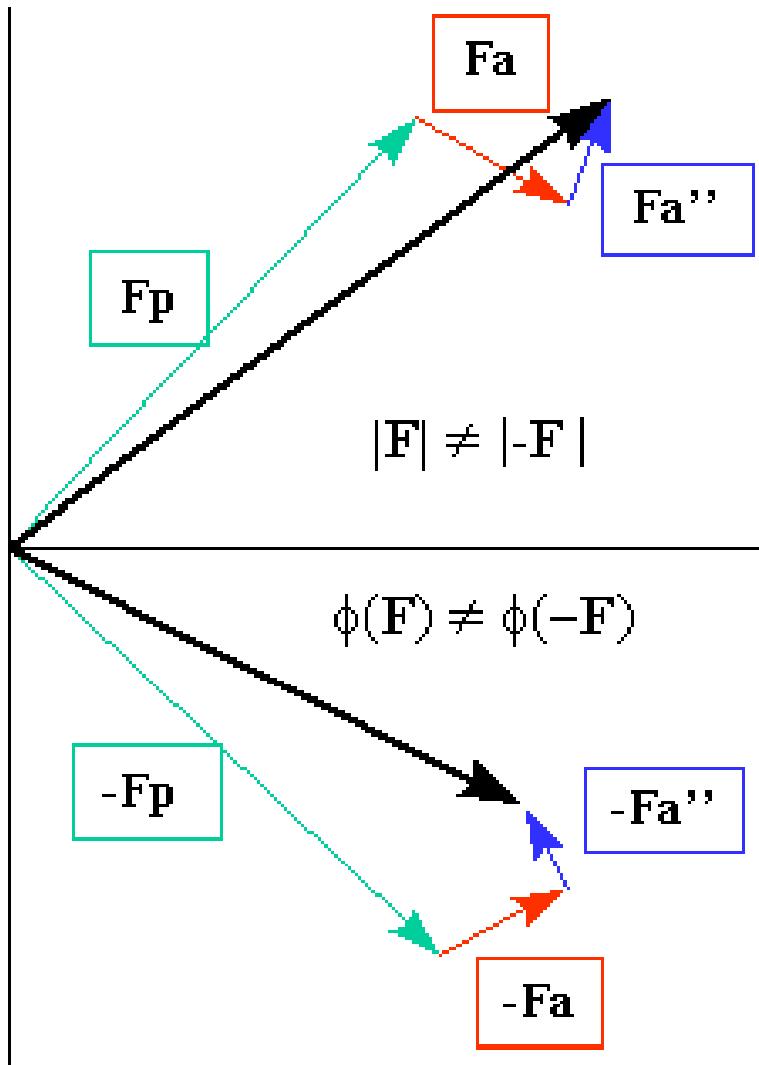
**FPH(SeMet)-FP(native) anomalous  
difference Patterson Map**



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

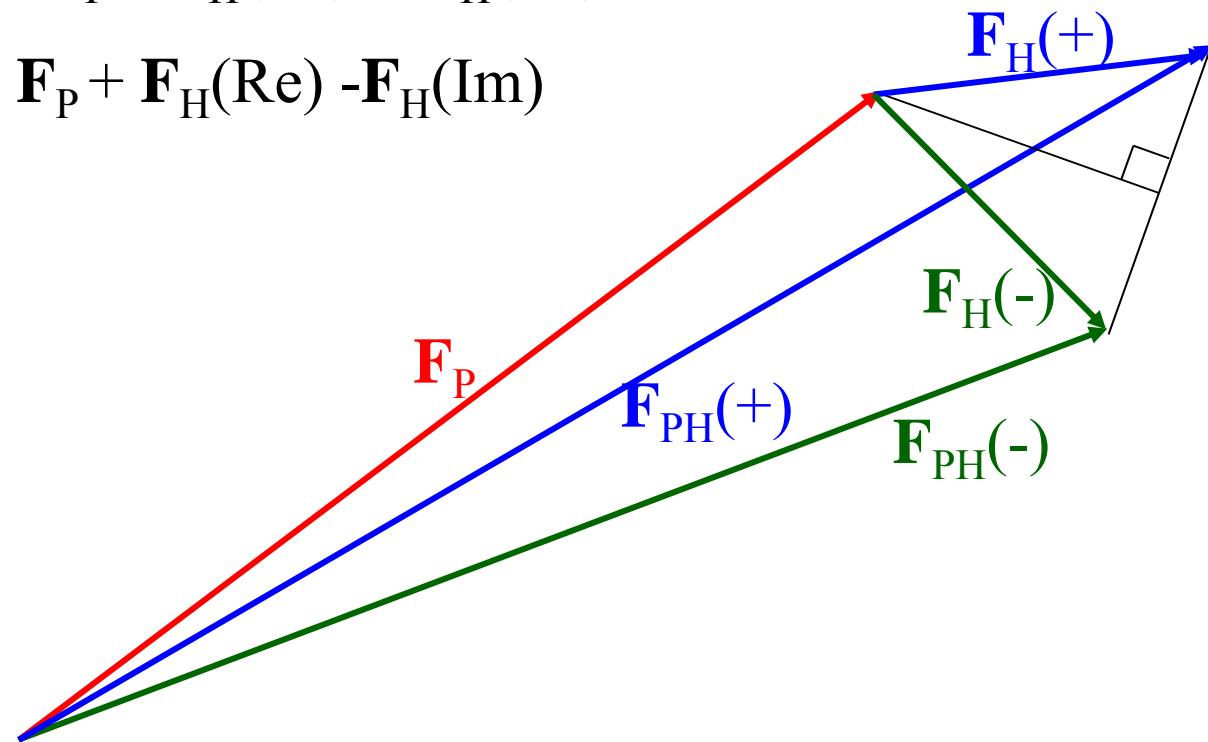


**FE4(remote1)-FE2(edge) dispersive  
difference Patterson Map**



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

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



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

From the heavy atom positions:

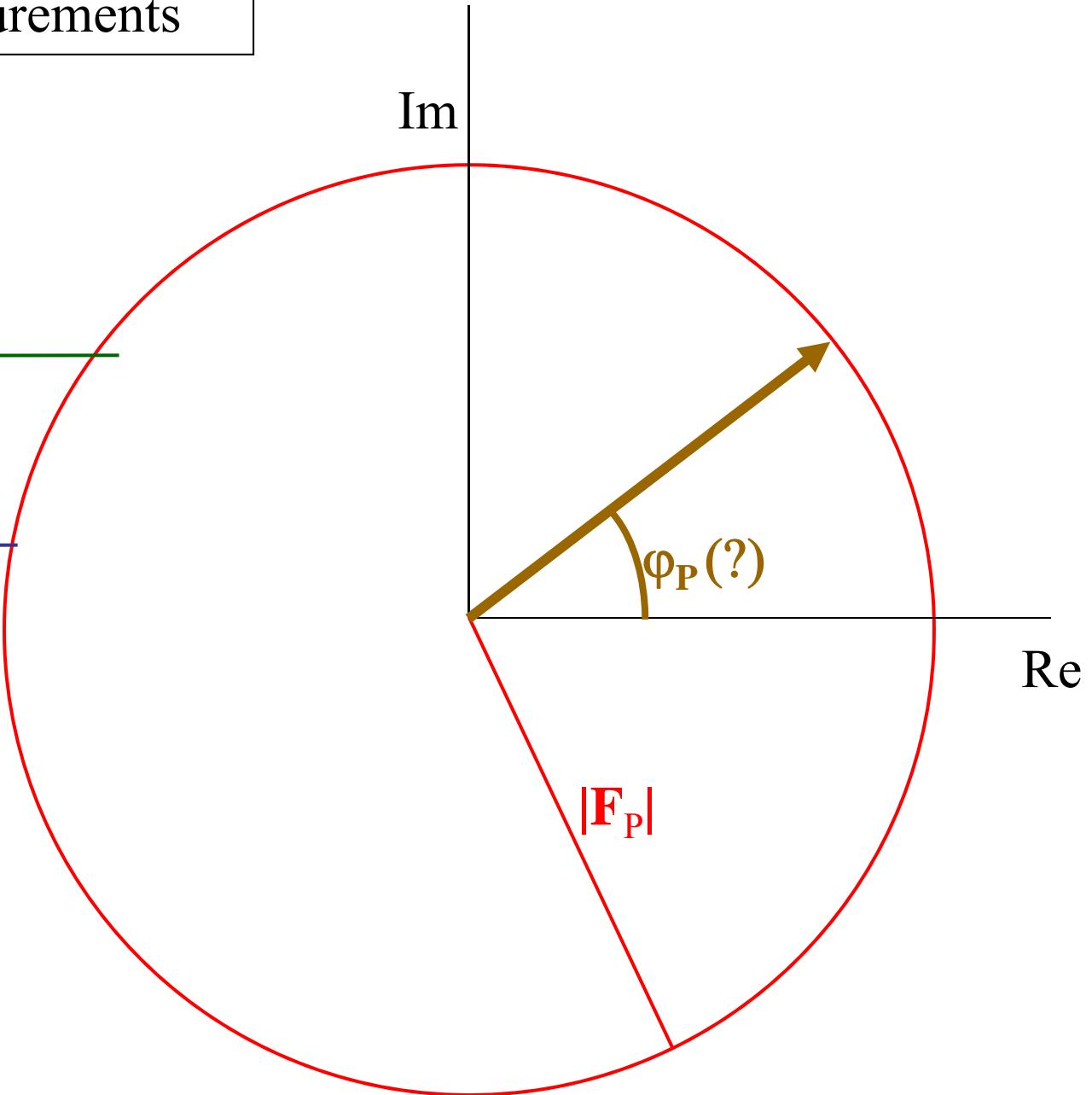
$$\mathbf{F}_H(+) \& \mathbf{F}_H(-)$$

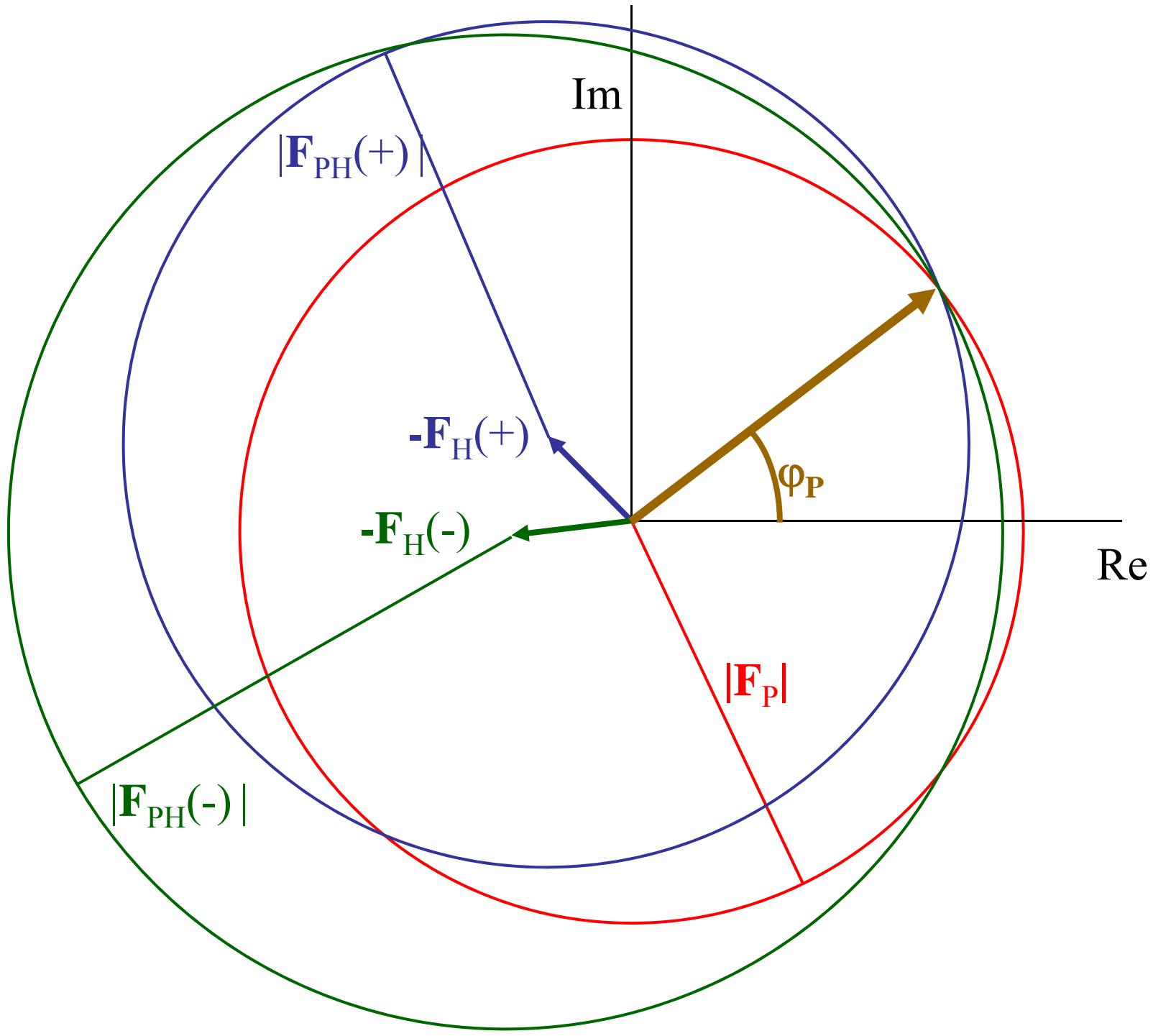
From intensity measurements

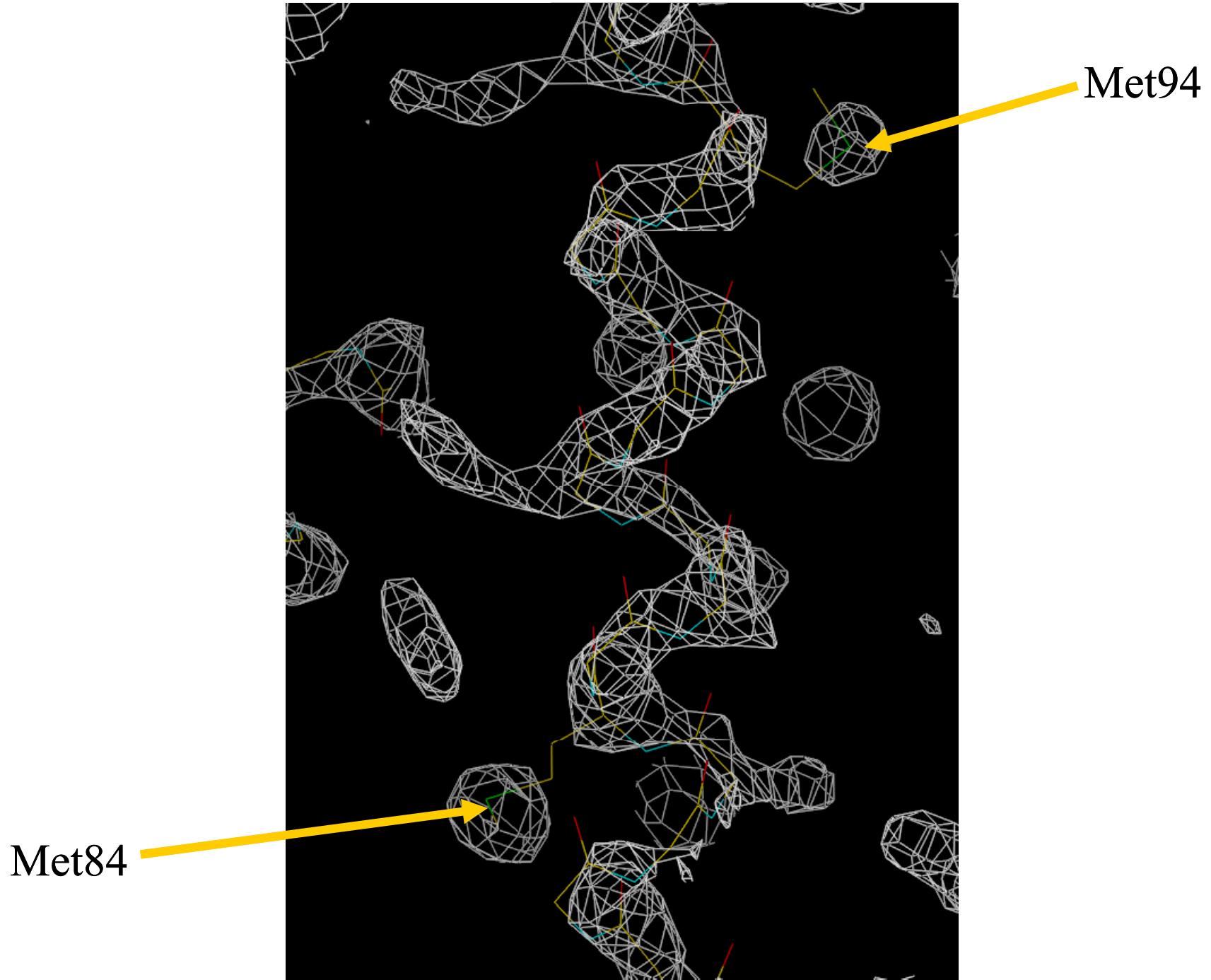
$$\left\{ \begin{array}{l} |\mathbf{F}_P| \\ |F_{PH(-)}| \\ |F_{PH(+)}| \end{array} \right.$$

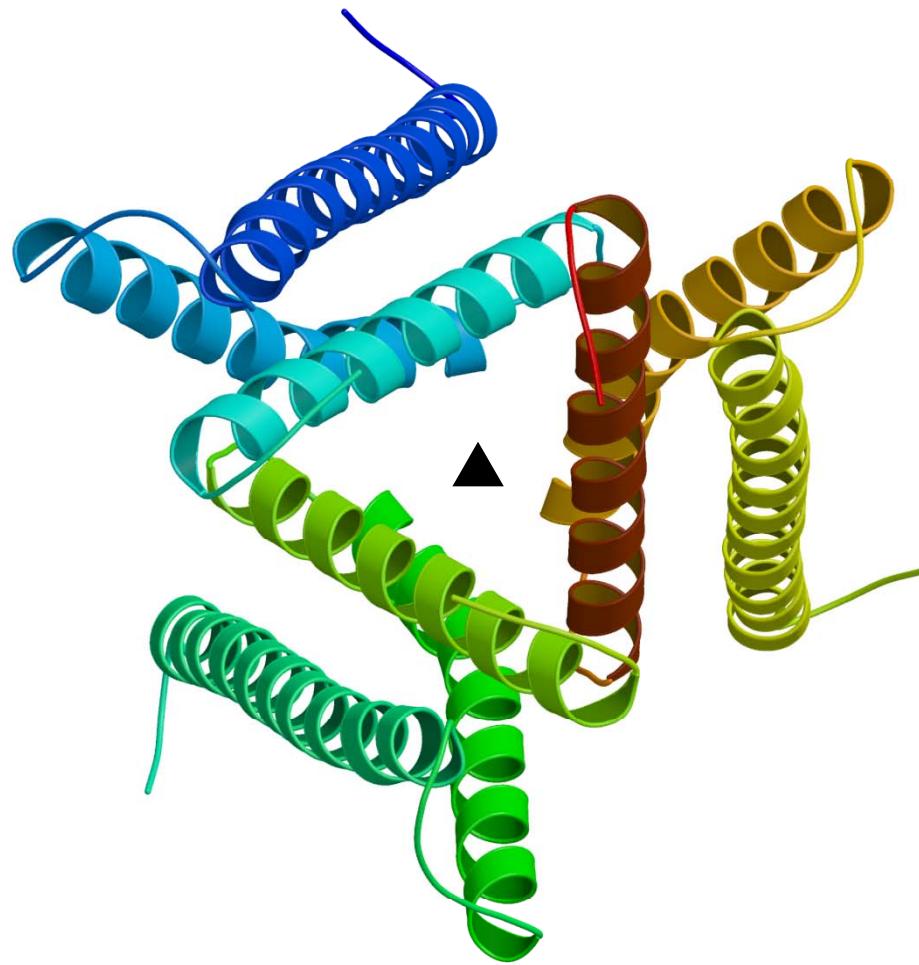
From Se positions

$$\left\{ \begin{array}{l} -F_{H(+)} \\ -F_{H(-)} \end{array} \right.$$





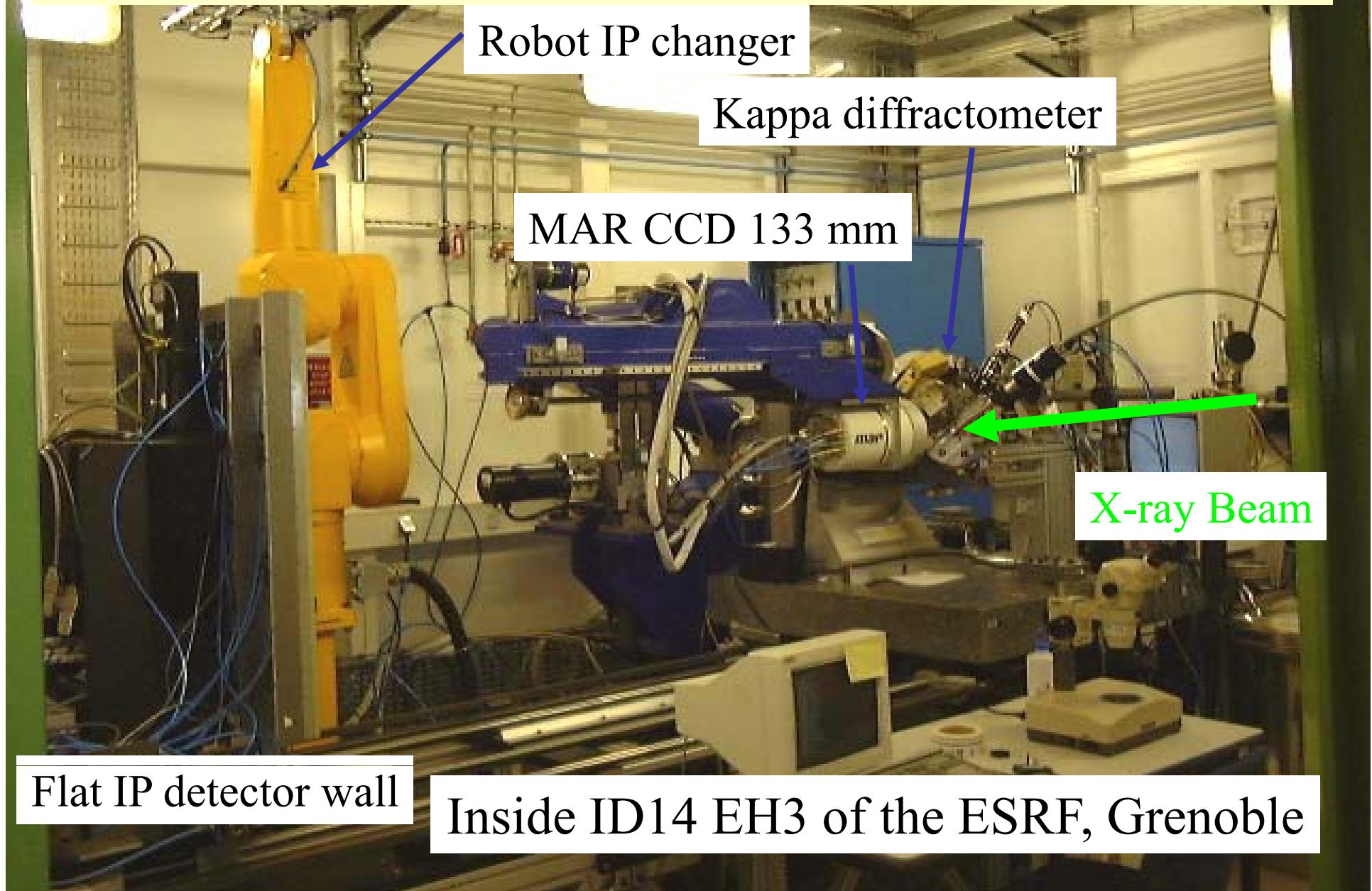


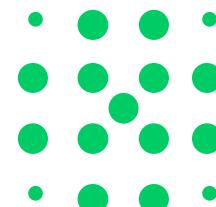
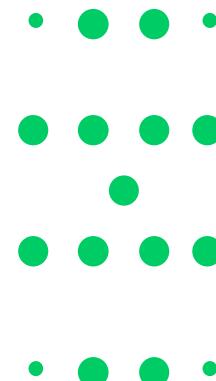
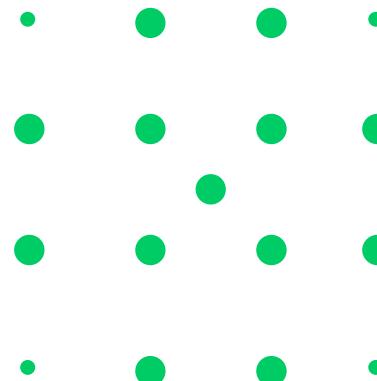
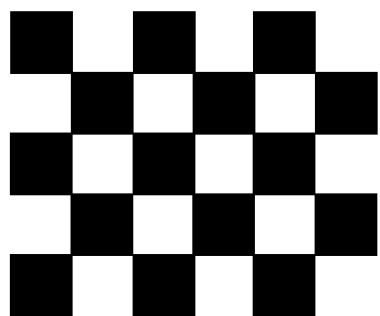
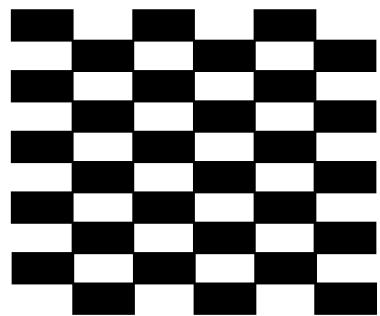
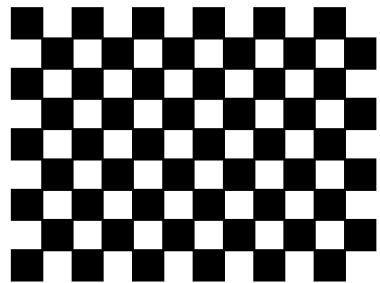


Ribbon diagram of trimer of GGA1 GAT domain

Triangle is threefold axis.

Large complexes requires high brilliance (high intensity & parallel beam) and large-area detectors.





Bluetongue virus structure analysis by D.Stuart, Univ of Oxford

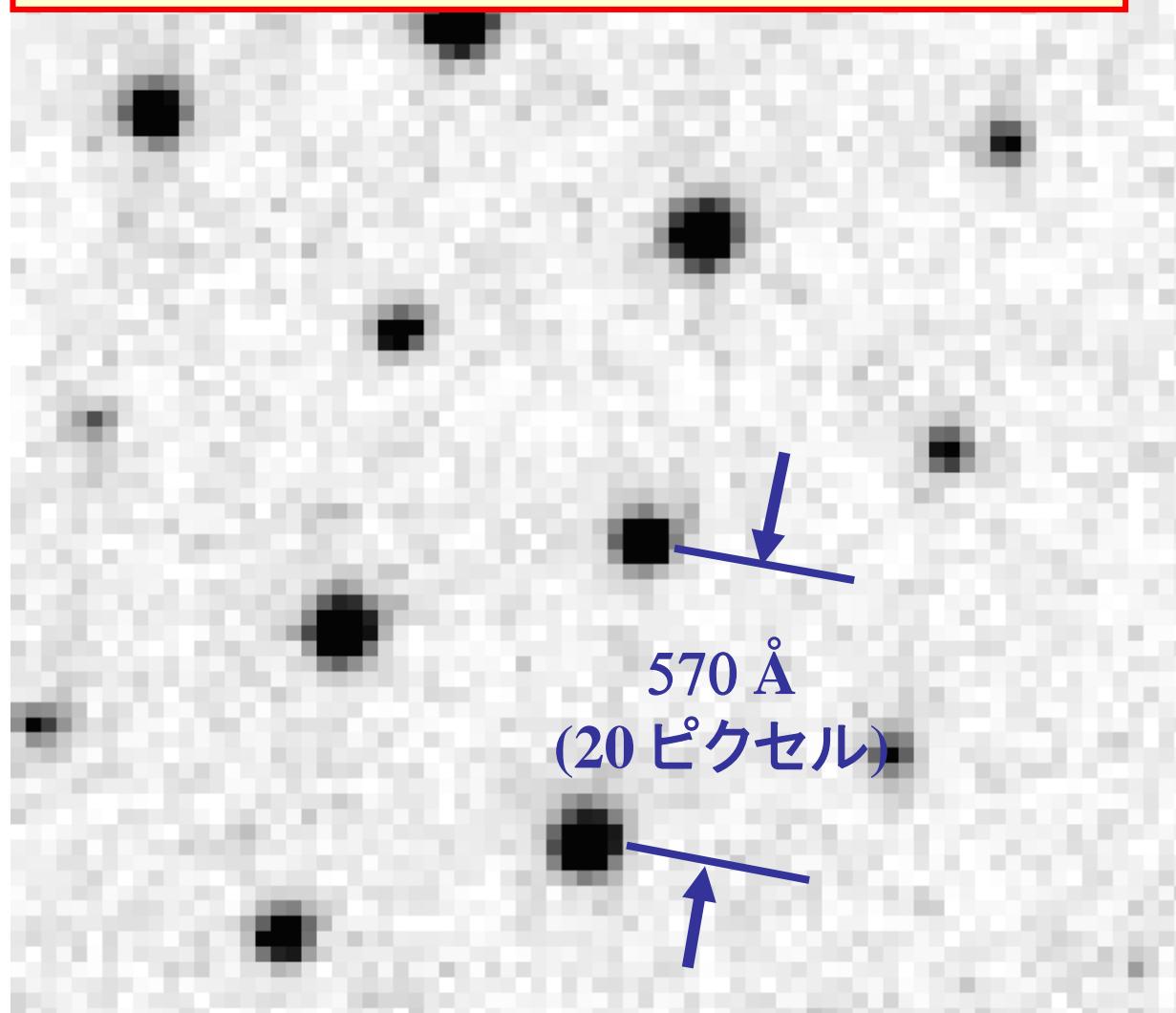


ID2PX, ESRF

Diffraction data on large IP  
detector on EH3/ID14, ESRF

Show the possibility of 2000 unit cell dimensions

Even larger with ultra low emittance?



Blue Tongue Virus

Core Particle 1

P<sub>2</sub>12<sub>1</sub>2

755 X 796 X 825 Å<sup>3</sup>

ID14/EH3

Crystal to detector distance 1250 mm

Wavelength 0.918 Å

Oscillation 0.1 deg

Exposure time 100 sec

Pixel size 100 mm

Beam size 100 mm

FWHM 181 mm

<http://www.kek.jp/ja/news/topics/2009/NobelYonath.html>

# Nobel Award in Chemistry, 2009



- Ribosome structures
- Prof. Ada Yonath was a user of the Photon Factory for 10 years from 1987
- Her plenary lectures
  - Biology and Synchrotron Radiation/Medical Applications in Synchrotron Radiation in Melbourne between February 15-19, 2010
  - Symposium of Target Protein Research Program March in Tokyo, on 5<sup>th</sup>, 2010
  - Photon Factory Symposium in Tsukuba on March 9, 2010



Prof. Ada Yonath( 2<sup>nd</sup> from right) & Prof Noriyoshi Sakabe (3<sup>rd</sup> from left)  
In ICCBM conference in Beijing, June 2004

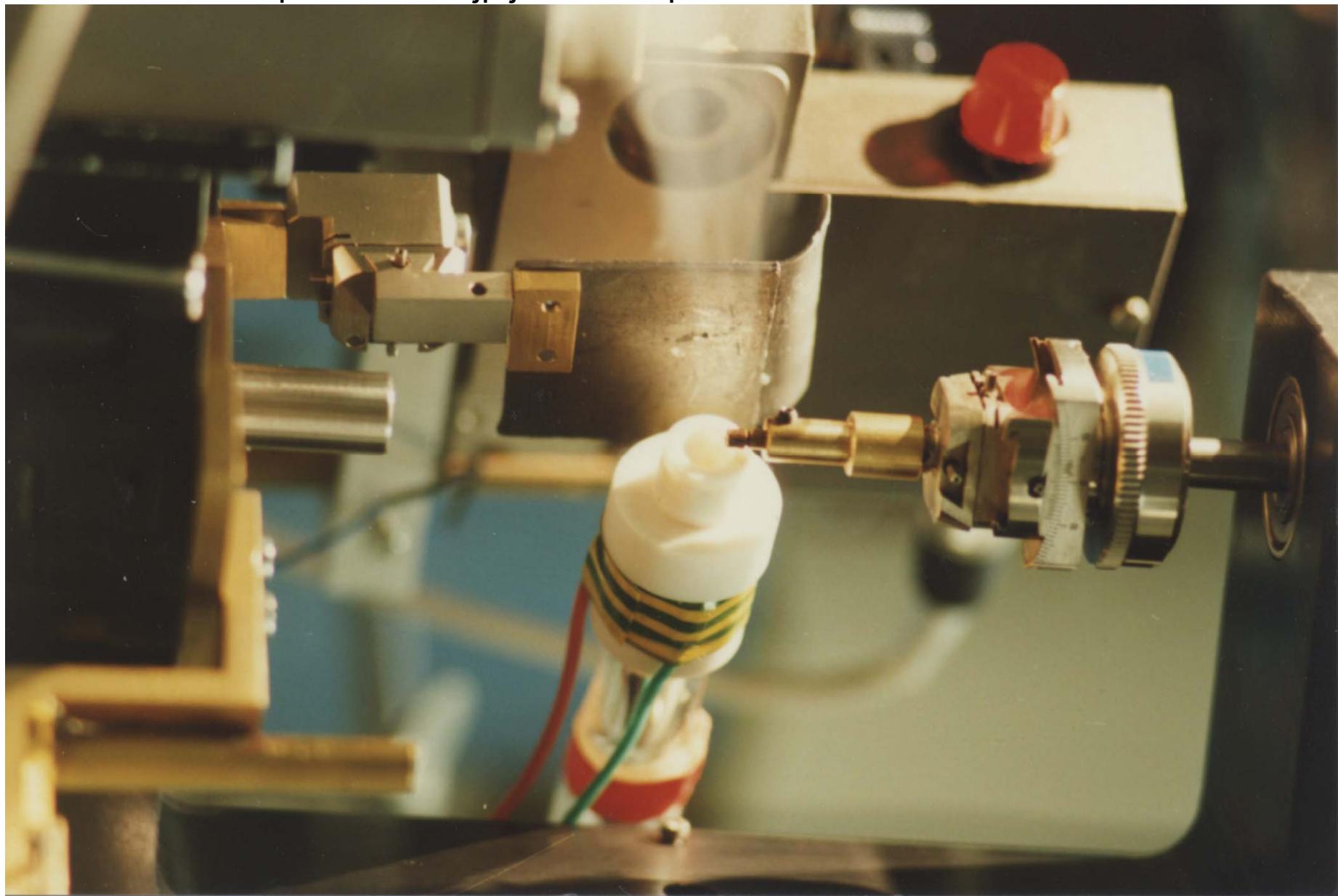
<http://www.kek.jp/ja/news/topics/2009/NobelYonath.html>



'92 3 2

Prof. Ada Yonath (left). At a BSR92 (4<sup>th</sup> international conference on Synchrotron and Biology) with Prof. Wayne Hendrickson of Columbia University, USA (center) , Dr. Joseph Zaccai of Laue-Langevin Institute, Grenoble, France (right) .

<http://www.kek.jp/ja/news/topics/2009/NobelYonath.html>



Cryo cooling apparatus developed by Prof. Ada Yonath (courtesy of Prof. Sakabe)

# Number of unit cells in crystals

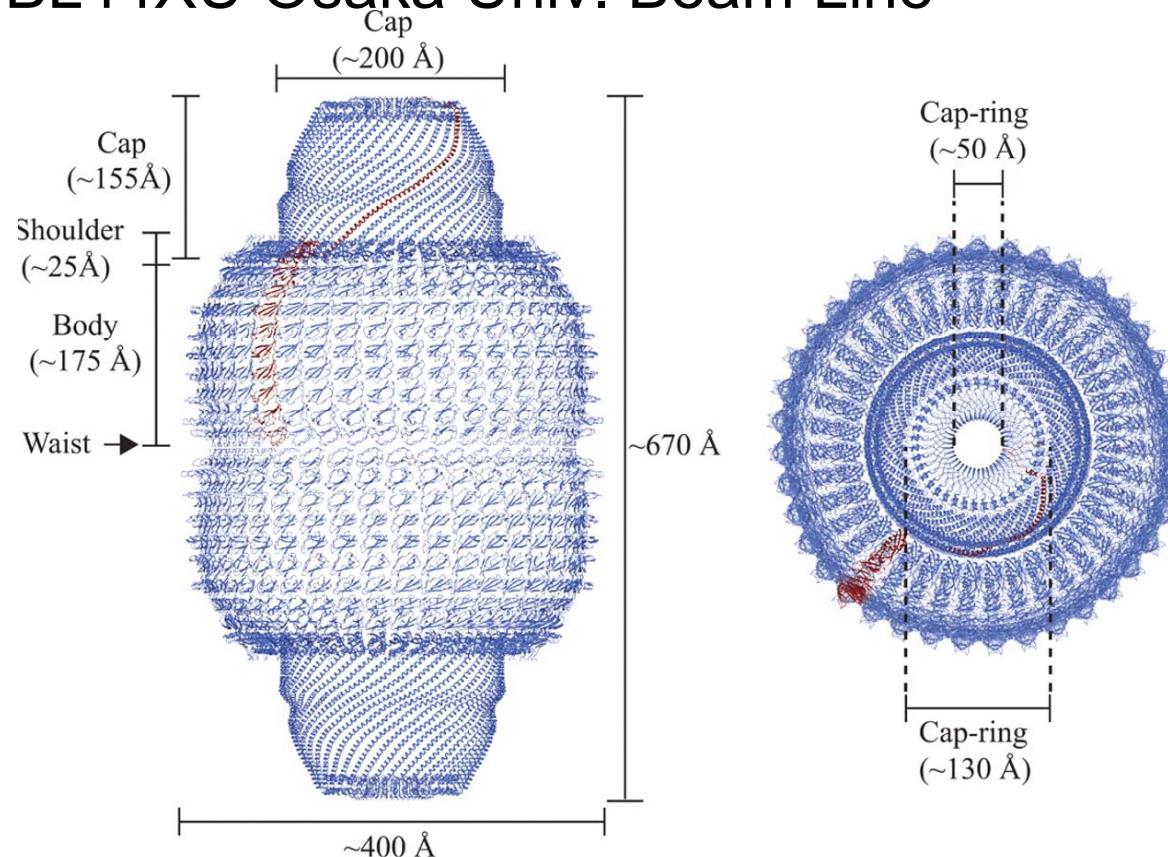
Unit cell dimension

Crystal size (micro cube)	30 Å	40 Å	50 Å	100 Å	200 Å	300 Å	500 Å	800 Å
300	1.0E+15	4.0E+14	2.0E+14	3.0E+13	3.0E+12	1.0E+12	2.0E+11	5.0E+10
200	3.0E+14	1.0E+14	6.0E+13	8.0E+12	1.0E+12	3.0E+11	6.0E+10	2.0E+10
100	4.0E+13	2.0E+13	8.0E+12	1.0E+12	1.0E+11	4.0E+10	8.0E+09	2.0E+09
50	5.0E+12	2.0E+12	1.0E+12	1.0E+11	2.0E+10	5.0E+09	1.0E+09	2.0E+08
30	1.0E+12	4.0E+11	2.0E+11	3.0E+10	3.0E+09	1.0E+09	2.0E+08	5.0E+07
20	3.0E+11	1.0E+11	6.0E+10	8.0E+09	1.0E+09	3.0E+08	6.0E+07	2.0E+07
10	4.0E+10	2.0E+10	8.0E+09	1.0E+09	1.0E+08	4.0E+07	8.0E+06	2.0E+06
5	5.0E+09	2.0E+09	1.0E+09	1.0E+08	2.0E+07	5.0E+06	1.0E+06	2.0E+05
4	2.0E+09	1.0E+09	5.0E+08	6.0E+07	8.0E+06	2.0E+06	5.0E+05	1.0E+05
3	1.0E+09	4.0E+08	2.0E+08	3.0E+07	3.0E+06	1.0E+06	2.0E+05	5.0E+04
2	3.0E+08	1.0E+08	6.0E+07	8.0E+06	1.0E+06	3.0E+05	6.0E+04	2.0E+04
1	4.0E+07	2.0E+07	8.0E+06	1.0E+06	1.0E+05	4.0E+04	8.0E+03	2.0E+03

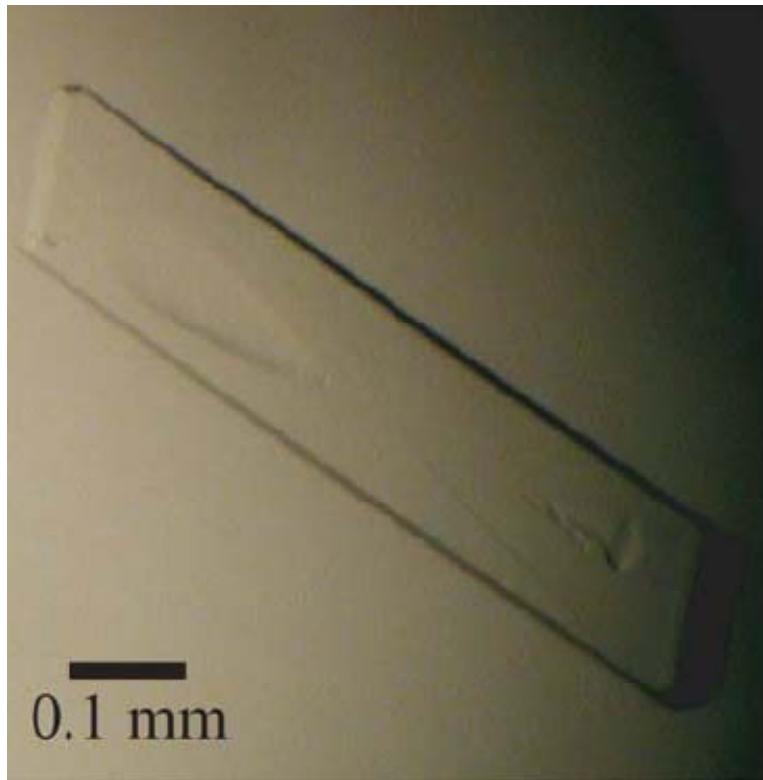
Numbers of “cubic shape” unit cells in cubic shape crystals. Colored according to the number of unit cells contains in crystals: more than  $10^8$  copies in green,  $10^7$  pink, &  $10^6$  copies yellow. (by Masaki Yamamoto, Harima RIKEN/SPring-8)

# Extremely large complex, Vault (Tsukihara Group, Science 2009)

- Structure determined at 3.5 Å resolution with 39-fold symmetry
- MVP(major vault protein) alone is  $99\text{kDa} \times 78 \text{ copies} = 7.7 \text{ MDa}$
- SPring-8 BL44XU Osaka Univ. Beam Line



# Vault crystal (Tsukihara et al., Science 2009, SPring-8 BL44XU)

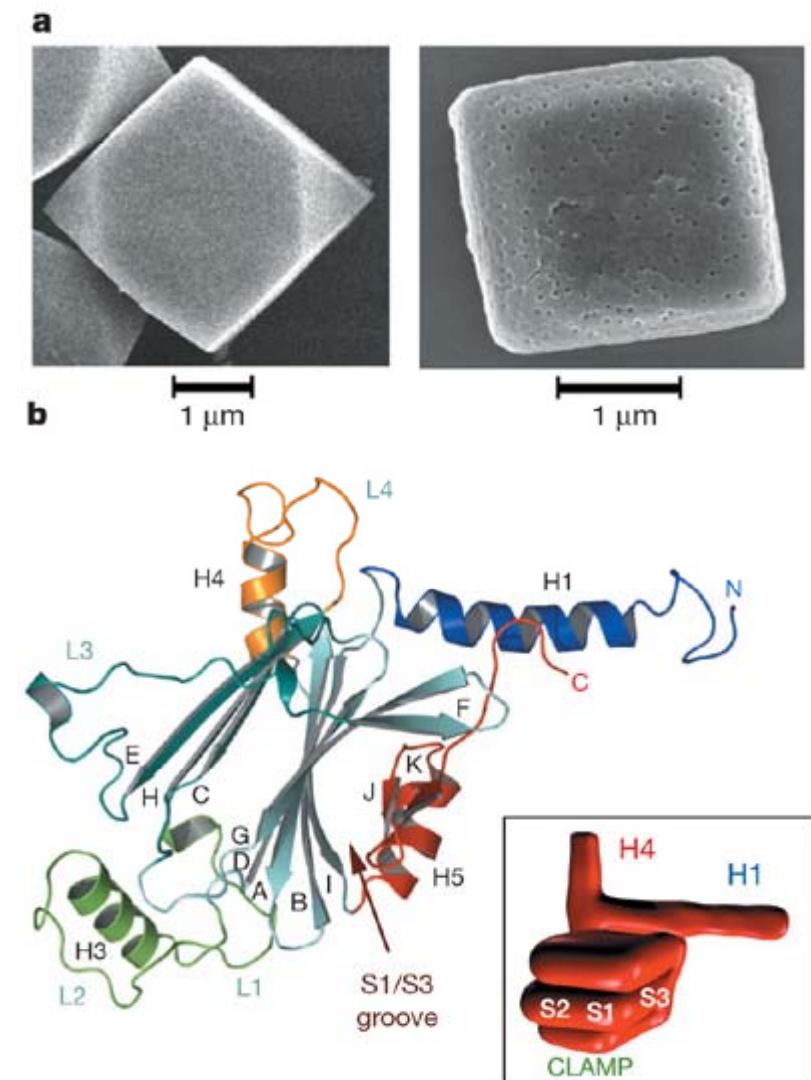


Photographs of vault crystal. The crystal was about 0.70 mm x 0.15 mm x 0.03 mm, and belongs to the space group C2, with cell dimensions of  $a = 702.2 \text{ \AA}$ ,  $b = 383.8 \text{ \AA}$ ,  $c = 598.5 \text{ \AA}$ , and  $\gamma = 124.7^\circ$ .

$$10,000 \times 4000 \times 500 = 2 \times 10^{10} \text{ unit cells}$$

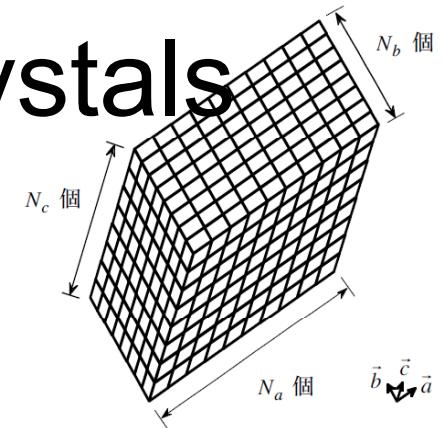
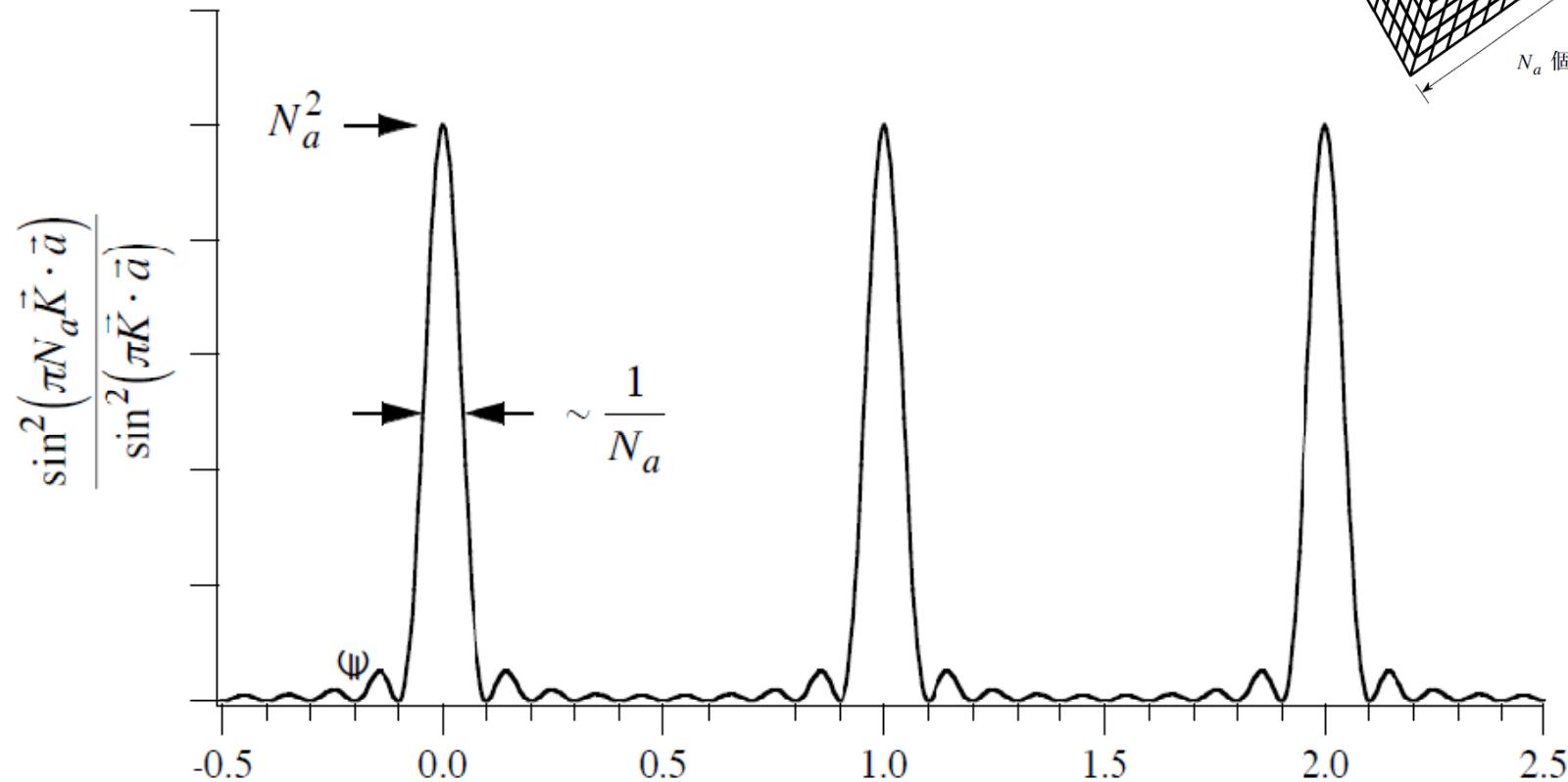
# Micro crystals of cypovirus polyhedra

- Structure at 2 Å resolution (Peter Metcalf group, Nature 2007, data collected at Swiss Light Source, presented in AsCA 2009, Beijing)
- Very robust crystals: dissolve only above pH10
- $a=b=c= 103 \text{ \AA}$ , 1 micron cube crystal  $\Rightarrow$  contains  $10^6$  unit cells  $\Rightarrow$  100 repeats along each axis



# Laue function of micro crystals

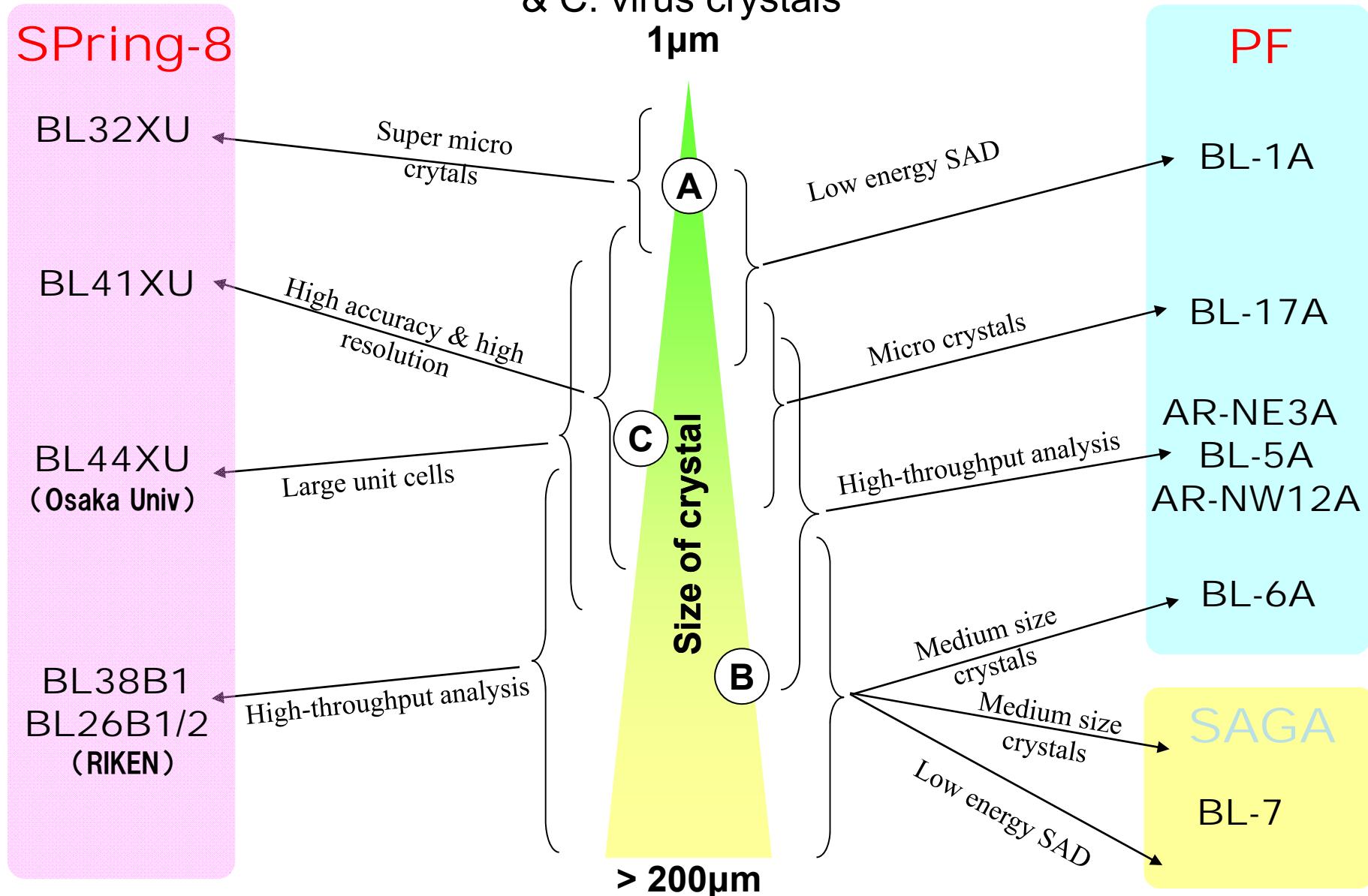
Limit  $10 \times 10 \times 10 = 1000 ?$



Laue function  $\frac{\sin^2(\pi N_a \vec{K} \cdot \vec{a})}{\sin^2(\pi \vec{K} \cdot \vec{a})}$ , For  $N_a = 10$

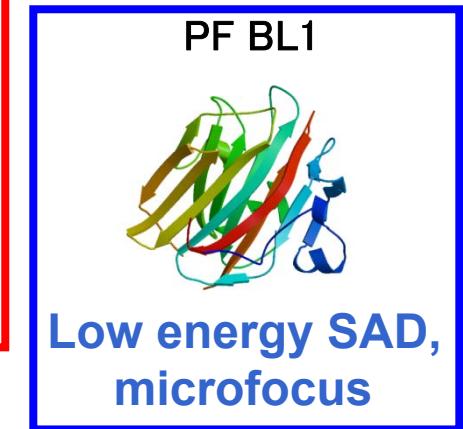
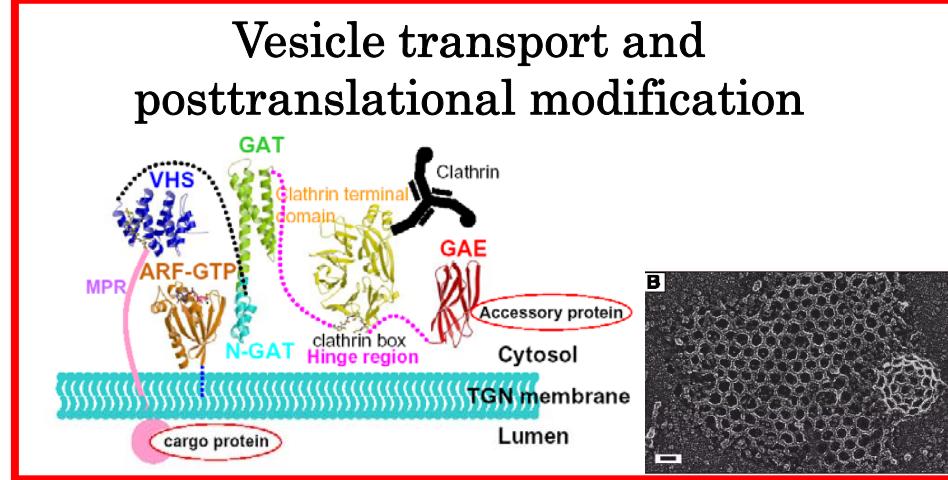
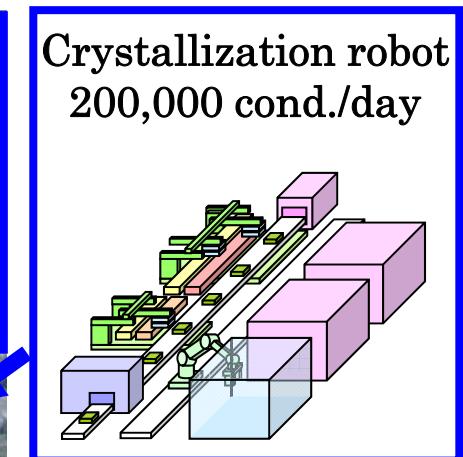
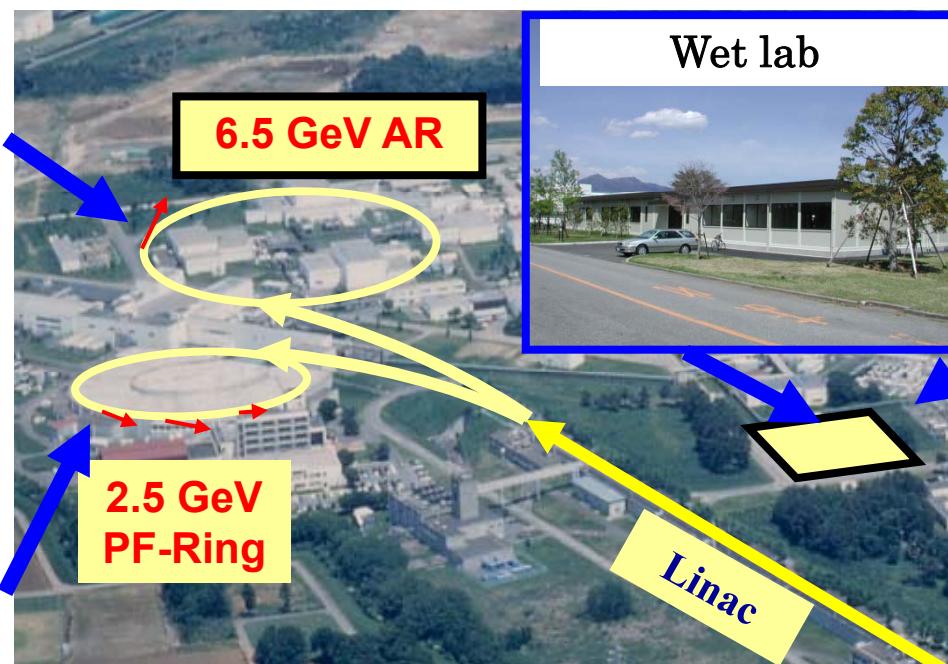
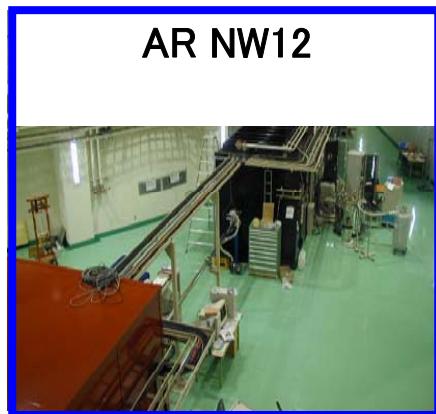
# Roadmap of protein crystallography beamlines in Japan

Examples A: membrane proteins, B: enzymes without heavy atom labels & C: virus crystals





# KEK-Photon Factory (Tsukuba) Structural Biology Research Center (since 2003)

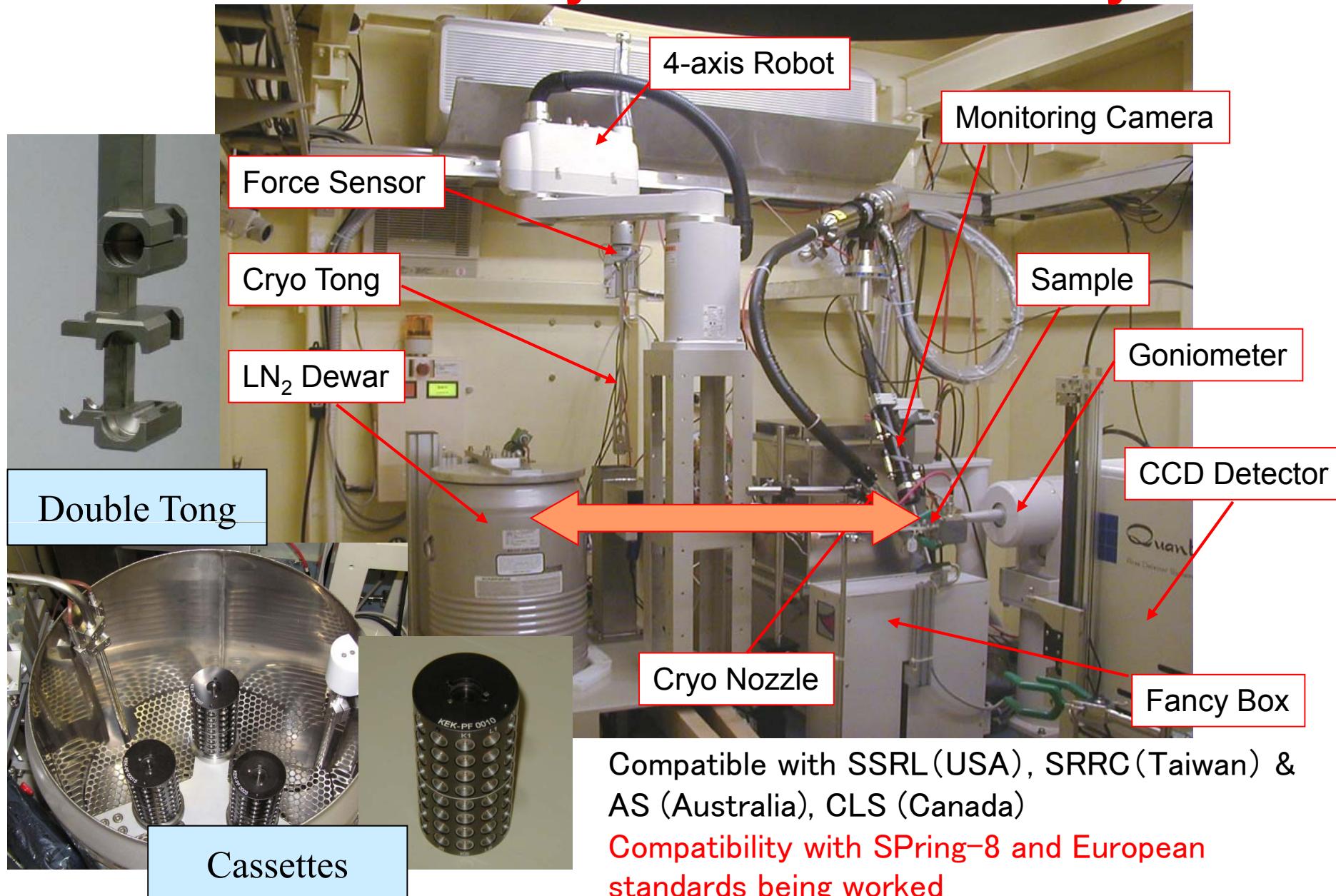


5 large grants (01~09: Total US\$ 69M, US\$40M for KEK)

Collaboration with domestic and overseas groups from 8 countries

# SSRL-type robot installed on MAD Beamline BL-5

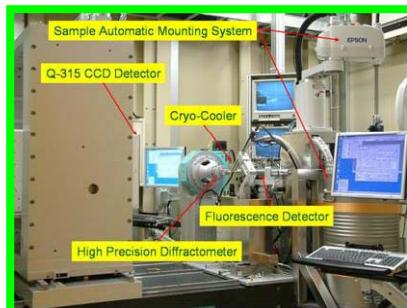
## 20 datasets/day $\Rightarrow$ 100s datasets/day



# SAM System Family



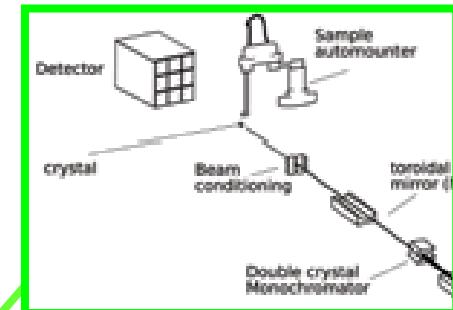
KEK-PF  
BL-1A, BL-5A, BL-17A,  
AR-NW12A, AR-NE3A



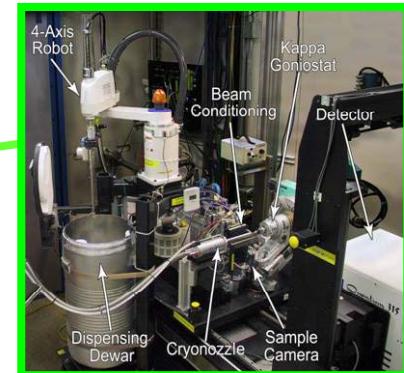
NSSRC  
BL13B1, BL13C1



ALS  
12.3.1



CLS 08B1-1(CMCF2)



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



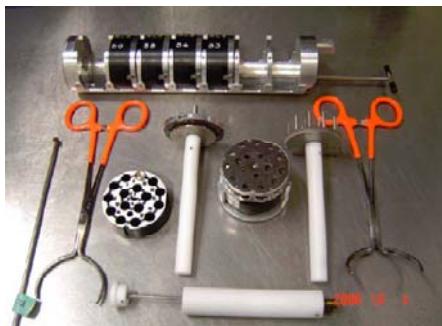
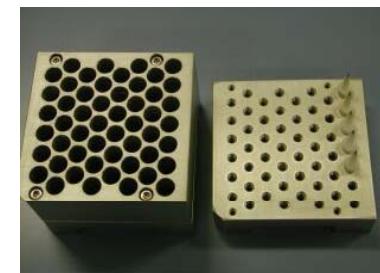
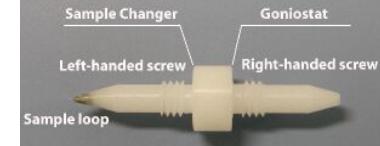
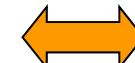
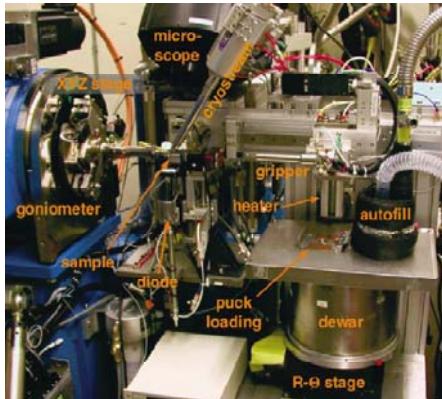
AS  
3-BM1, 3-ID

**SSRL**  
**Automated**  
**Mounting system**

# But so many different systems...



# Compatibility with other systems



BAM(ALS)



Universal Puck



SAM System

PF will accommodate to Universal Pucks

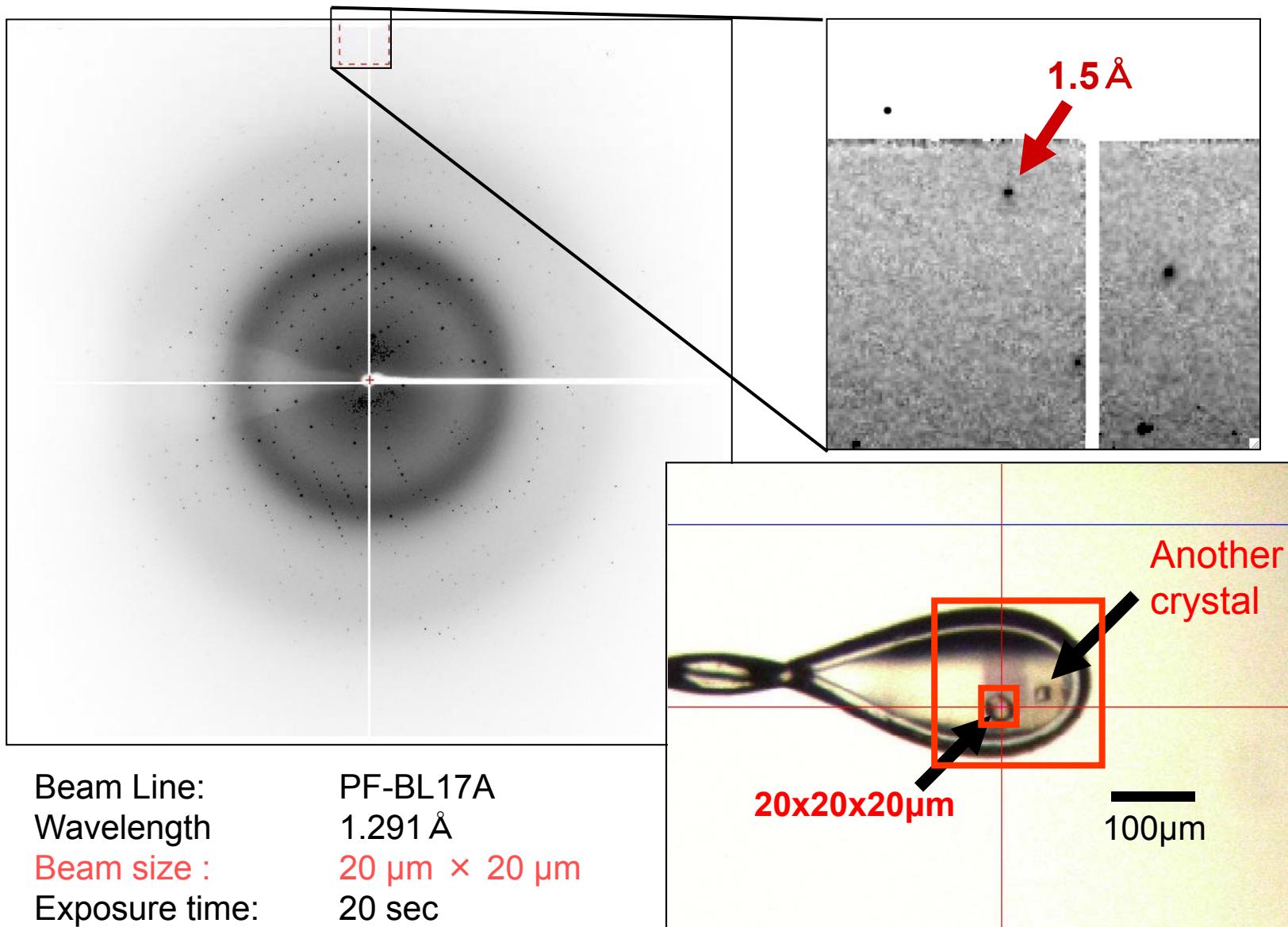
Under development within the Target Protein Research Project



Rigaku ACTOR  
DIAMOND, SOLEIL, SLS, APS,...

SPACE(SPring-8)

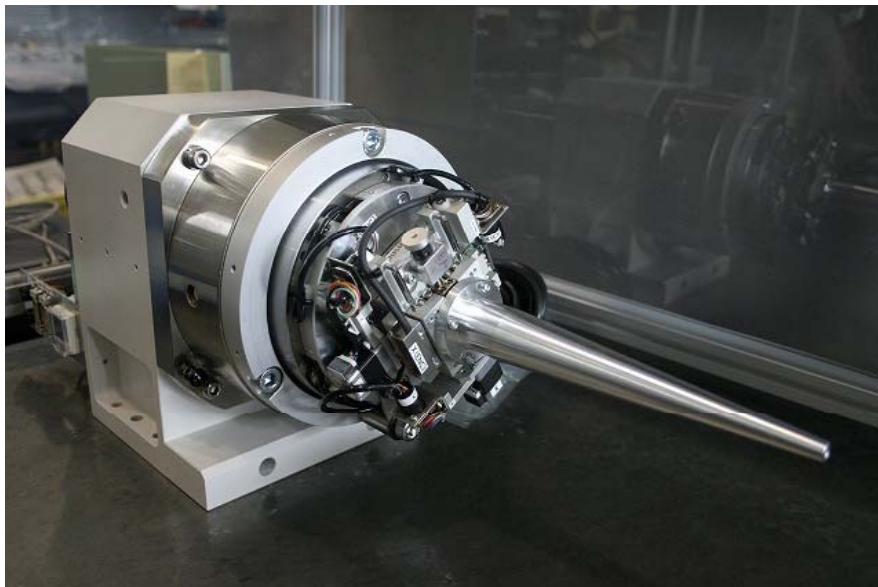
# NEW BL17A short gap undulator beam line: Example of small crystals (funded by JST Frontier Technology Development Project)



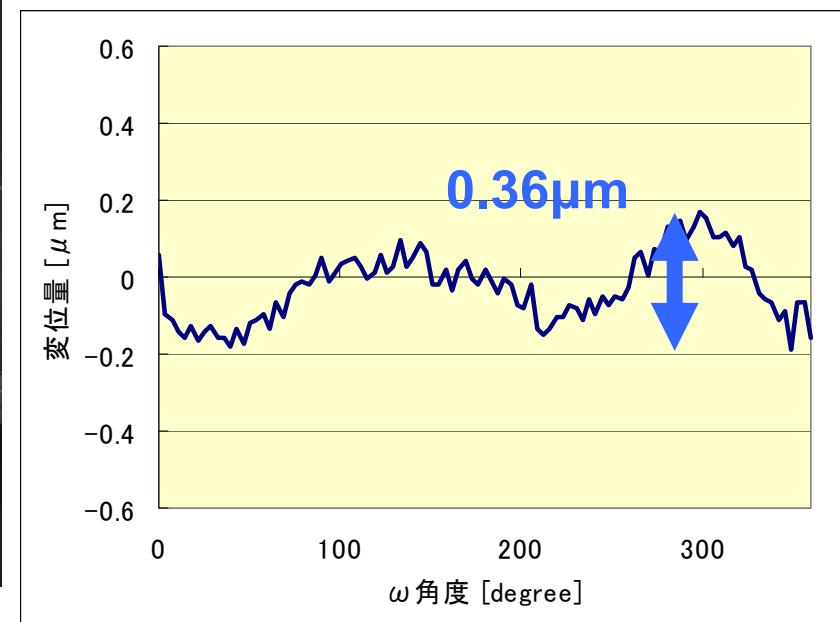
Provided by Dr. Tadanobu Tanaka of Showa University

## High precision one axis diffractometers with XYZ stages

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

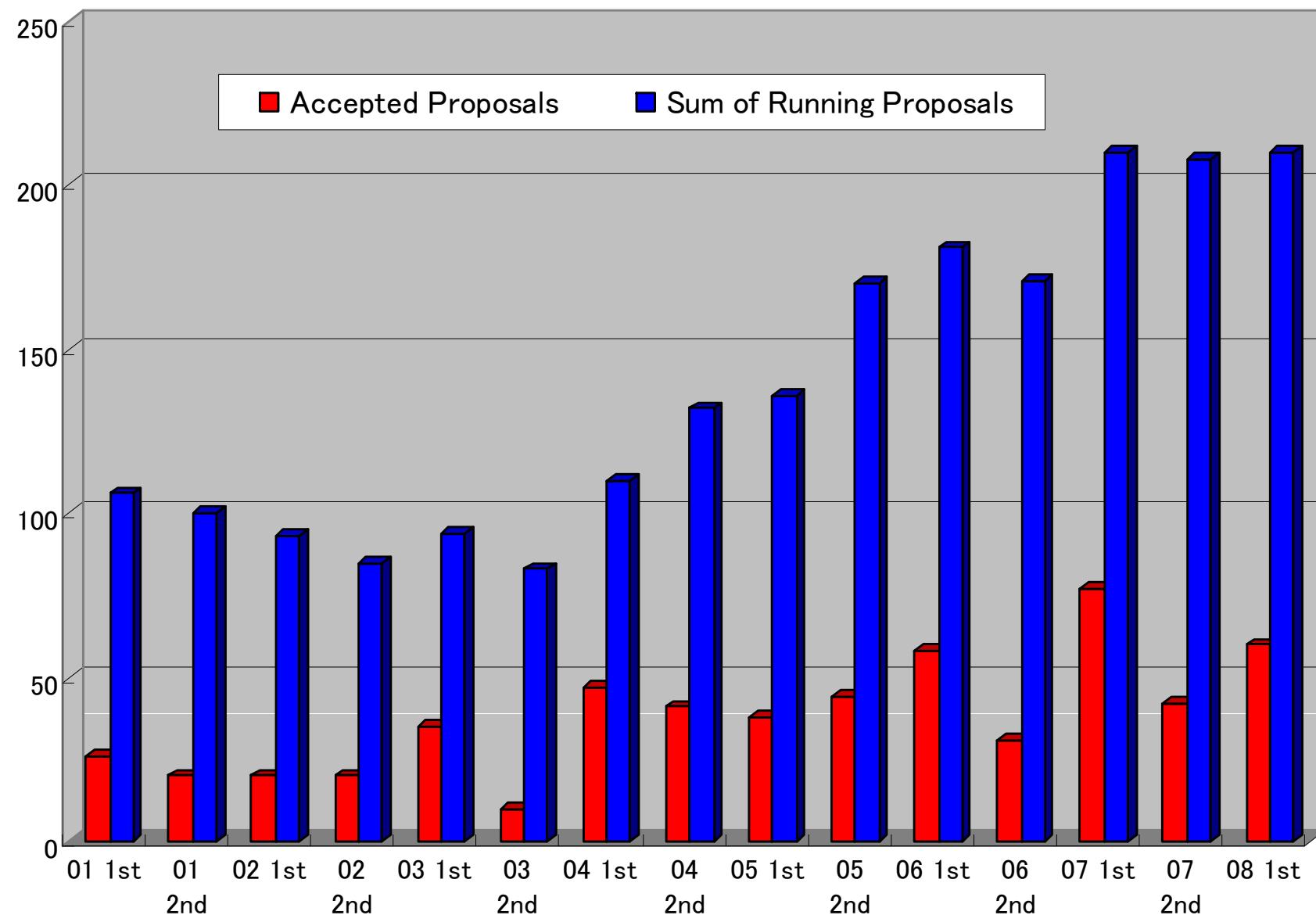


Air bearing & magnetic x-y stages



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

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



# Industrial Use and Collaborations between KEK and Industry (~16% of beamtime)

## MX BLs at Photon Factory

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

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

Company ①

Company ②

Astellas ③

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

BL32B2 at SPring8

Ajinomoto Co., Inc

Astellas Pharma Inc.

Eizai Co. Ltd.

KYOWA HAKKO  
KOGYO Co., LTD.

DAIICHI SANKYO Co.  
Ltd.

CHUGAI  
PHARMACEUTICAL  
Co., Ltd.

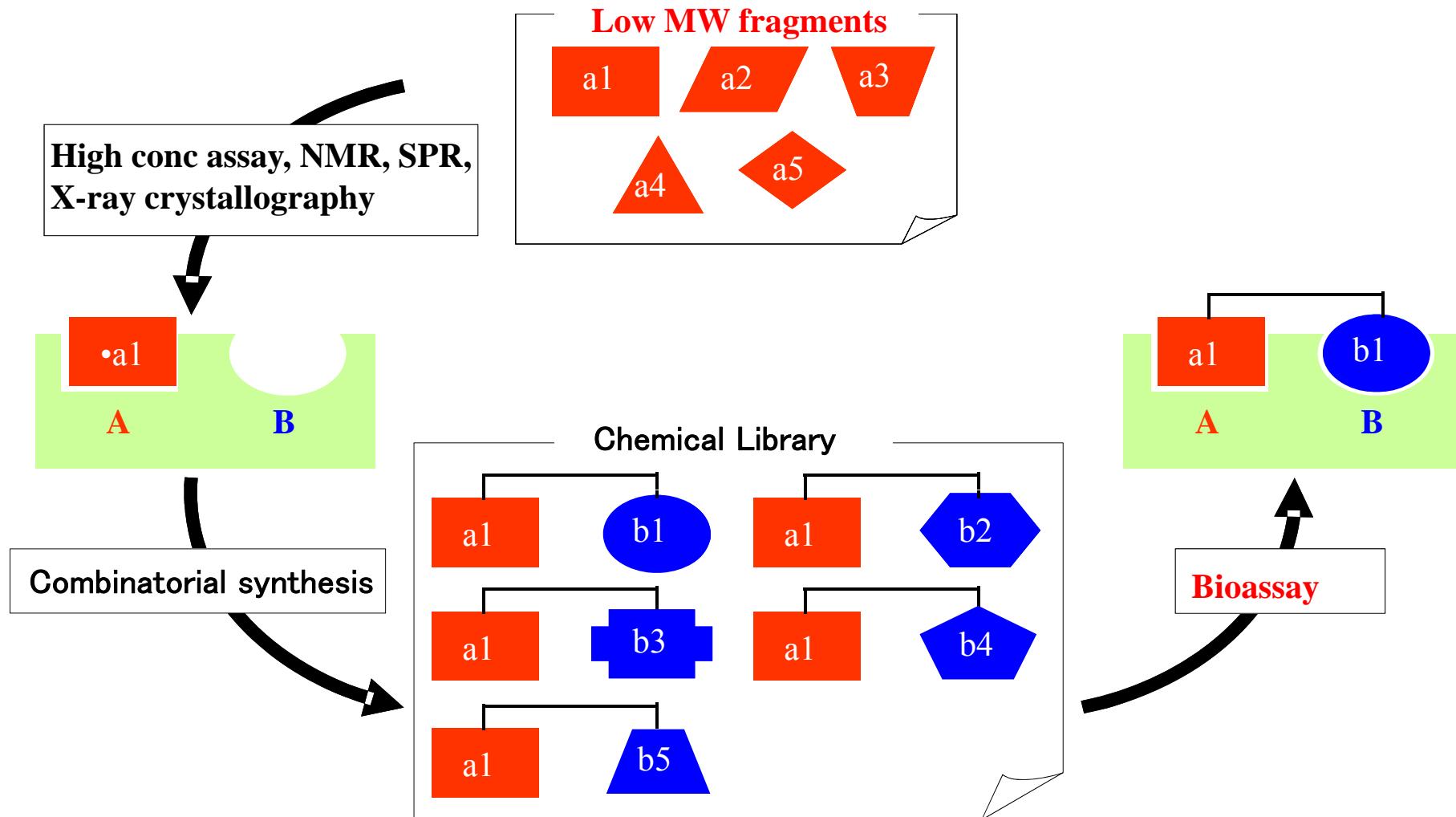
BANYU  
PHARMACEUTICAL  
Co., Ltd.

Mitsubishi Chemical  
Corporation

New members

# Fragment Based Lead Discovery

## “Fragment Evolution”

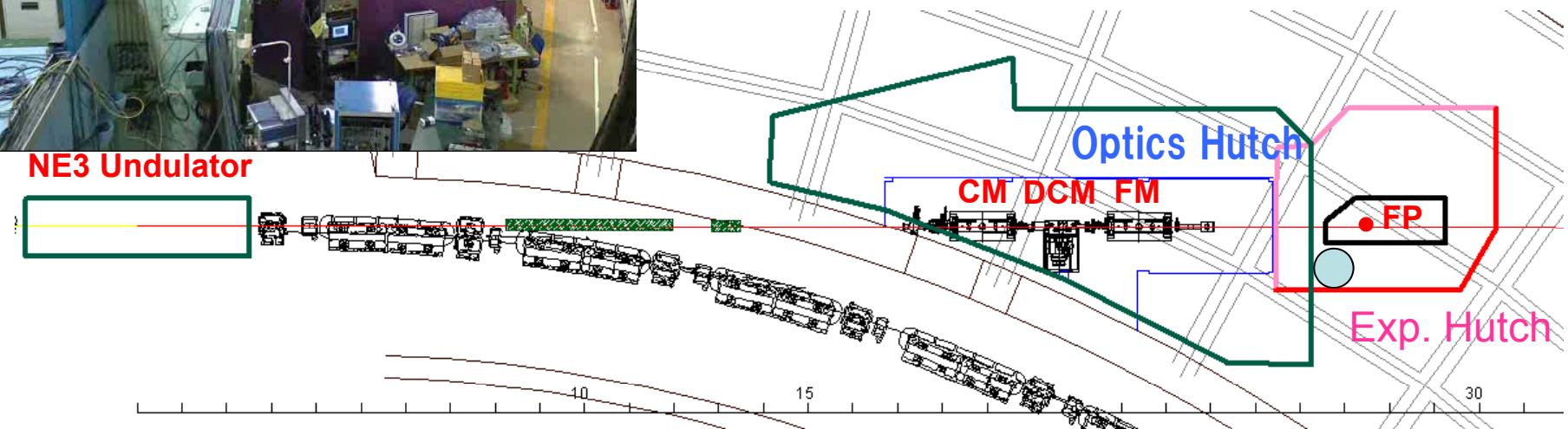




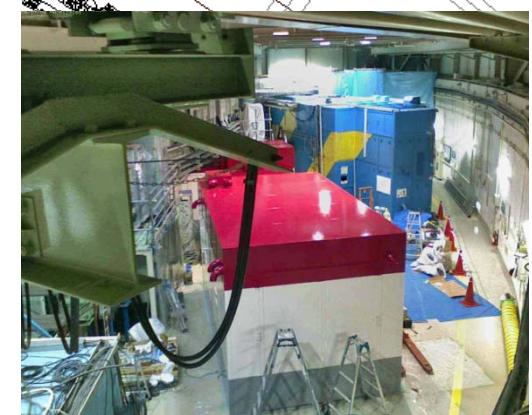
NE3 Undulator

# Astellas Pharma Beam Line: PF-AR NE3

Available from Apr. 2009



- Stronger than AR-NW12A by a factor of 3
- Astellas Pharma will have priority access for certain amount of beam time during 10 years from April 2009.
- The rest of the beam time can be used for general user operation including use by other pharmaceutical companies.
- High throughput: 450 datasets in 3 days



# International Collaborations

Australian BL20B  
since 1992, one of the  
most productive BLs at  
PF

⇒ Also industrial use (2009~)



ANBF-Photon Factory

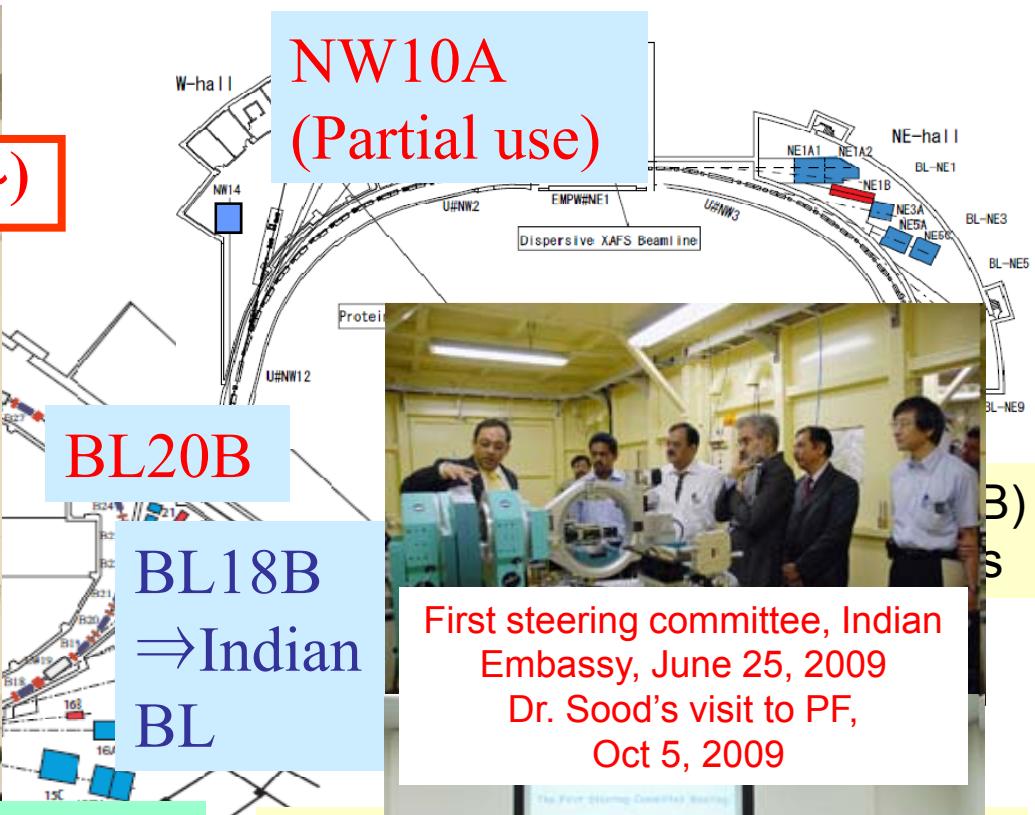
698 Experiments

~1,500 scientist visits to Japan

>> 2000 Days of beamtime

Many hundreds of publications

3000 registered users  
500~600 papers published each year



NW10A  
(Partial use)

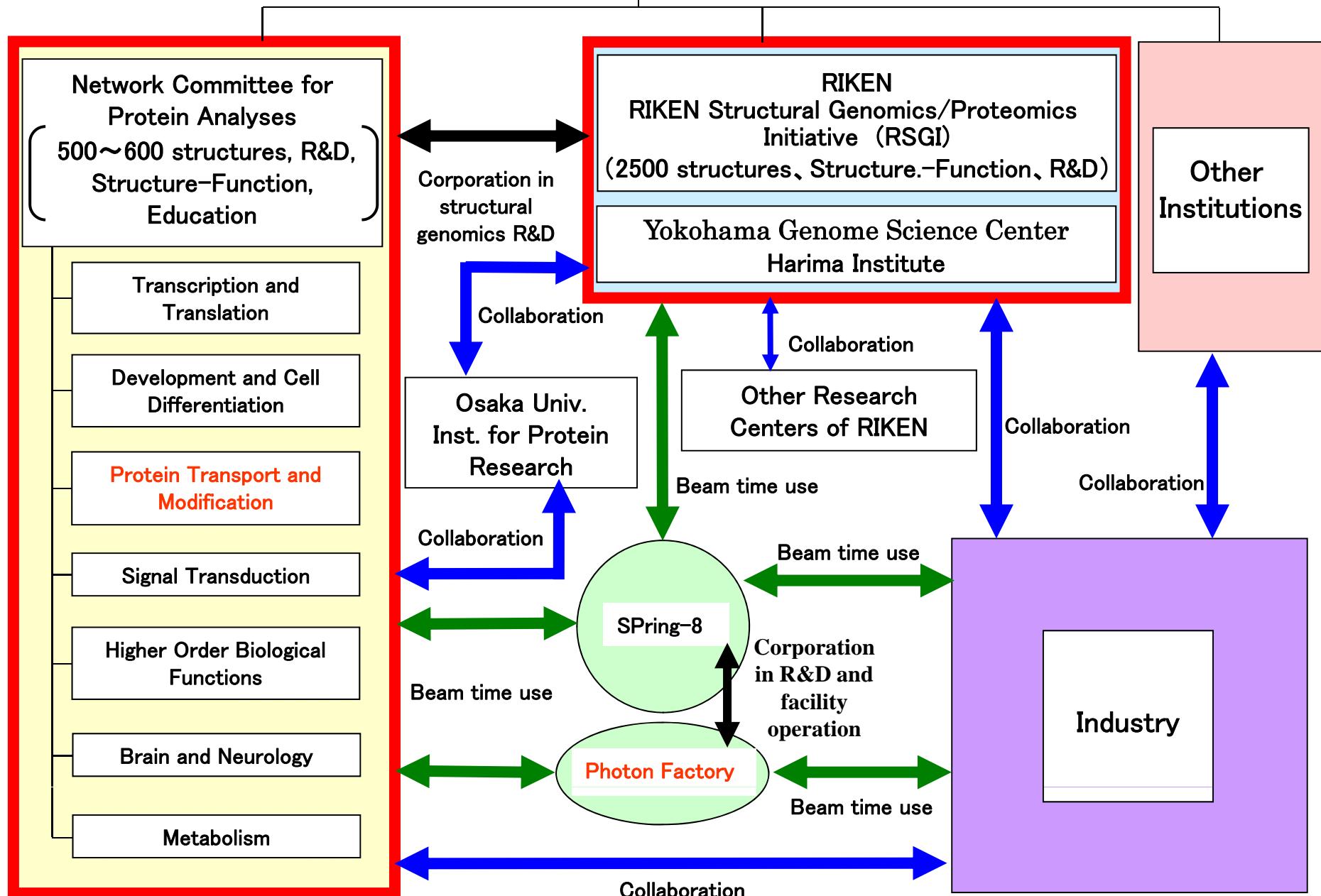
BL20B  
BL18B  
⇒ Indian  
BL

PF (2)  
56 (4)

First steering committee, Indian  
Embassy, June 25, 2009  
Dr. Sood's visit to PF,  
Oct 5, 2009

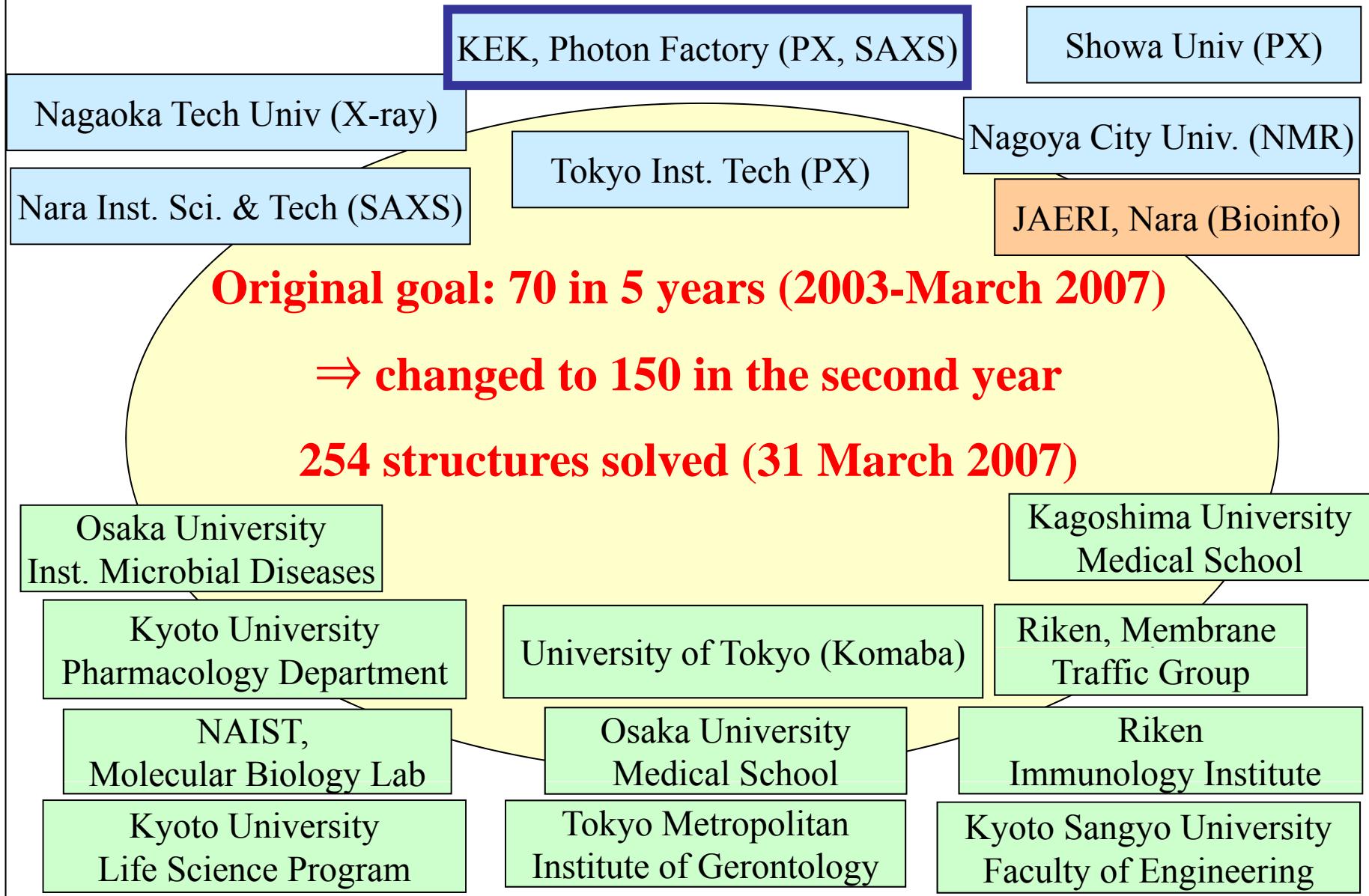


# Protein 3000 Project (the MEXT) 2002~2007



# Protein 3000 Tsukuba Structural Biology Consortium (21 groups)

## Protein Transport and Posttranslational Modification



# Target Protein Project (5 years: 2007-2011)

- Yearly budget: 5.5 billion yen, US\$ 44.5M, 33.3 MEuro,  
(includes 30% overhead)
- 43 teams selected on 15 June 2007
- KEK: Vesicle transport & Structural Analysis Core

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

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

Photon Factory

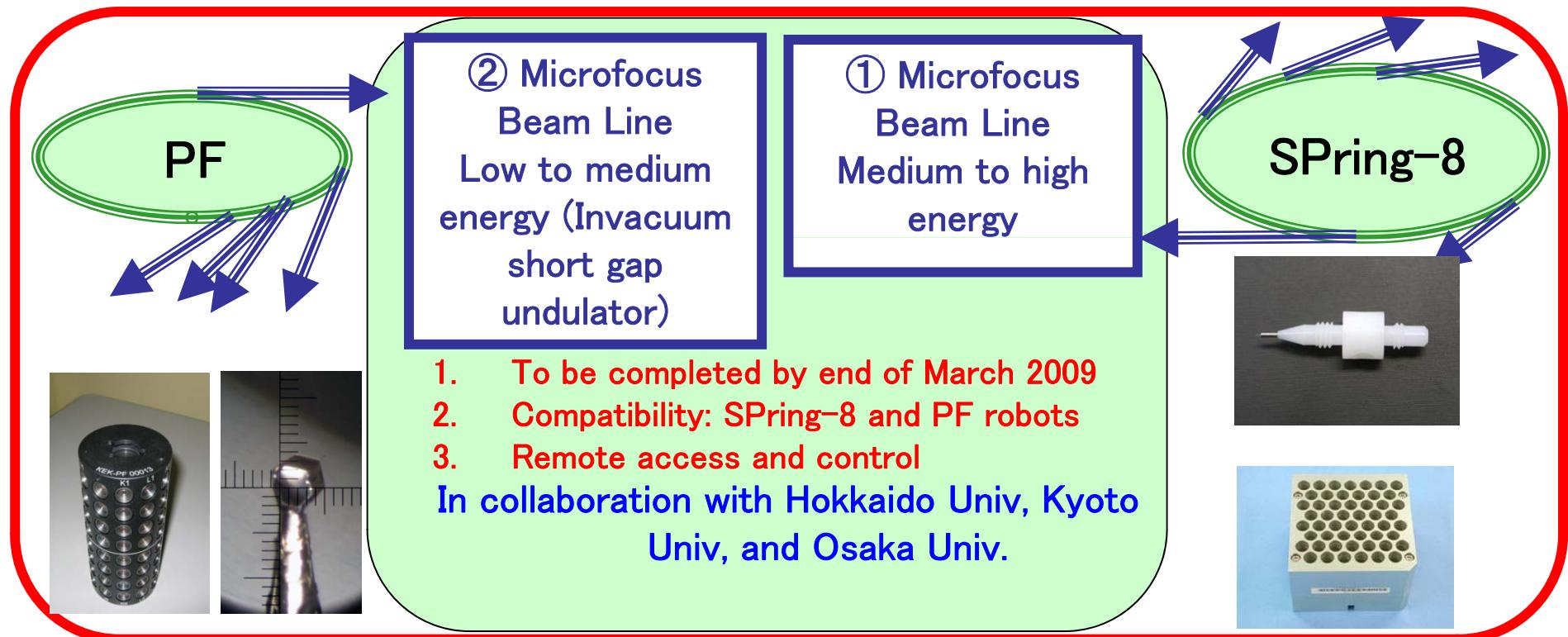
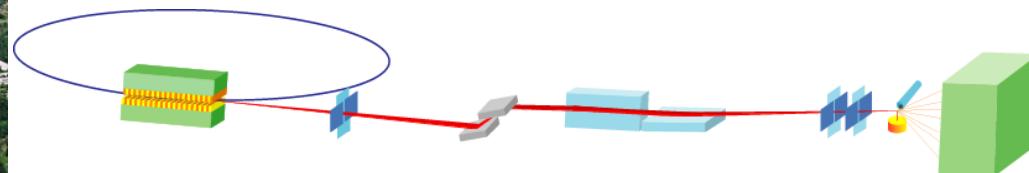


# MEXT: Target Protein Project (2007-2011)

SPring-8



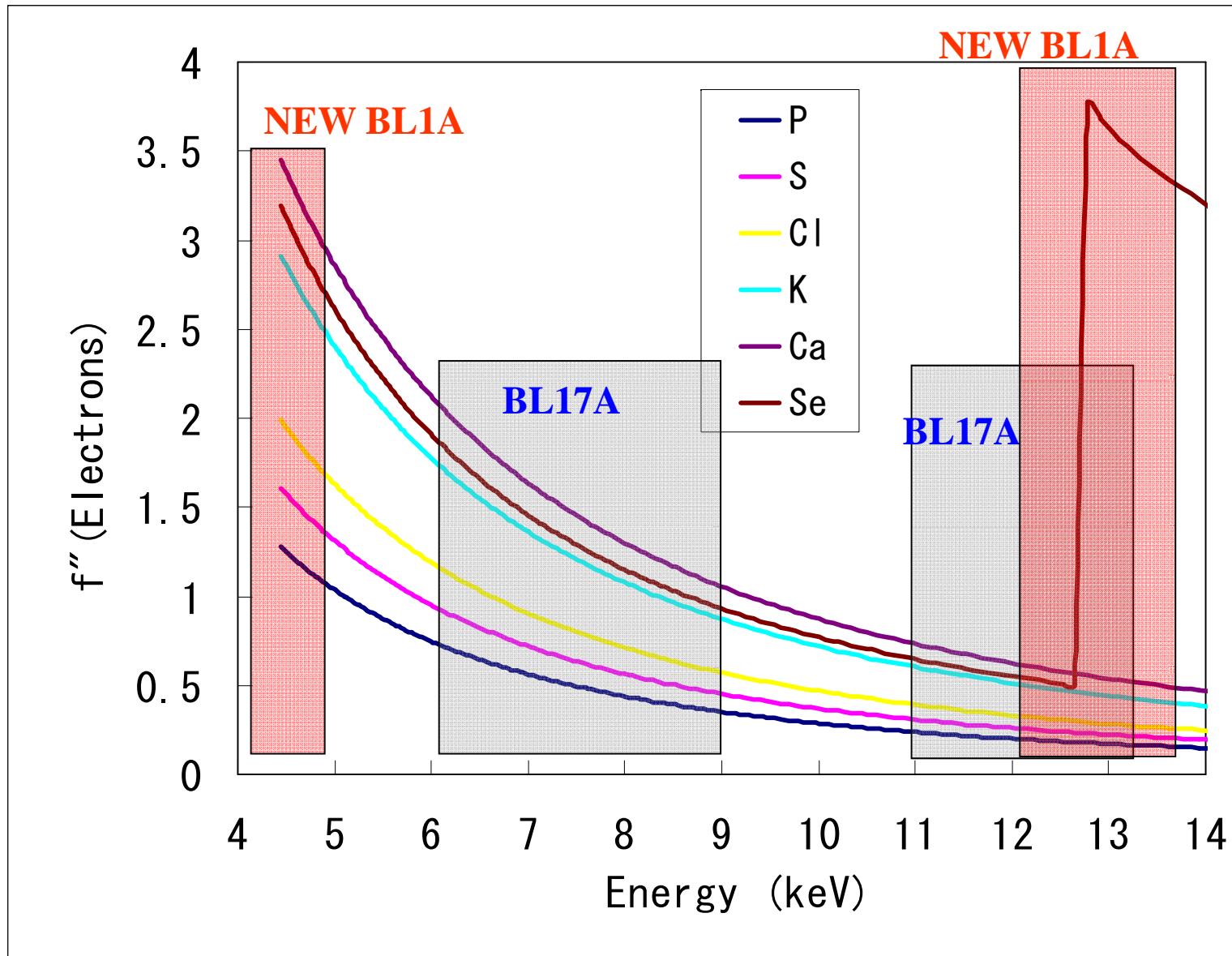
Joint Proposal by SPring-8 and PF:  
Two **Complementary** New Beam Lines



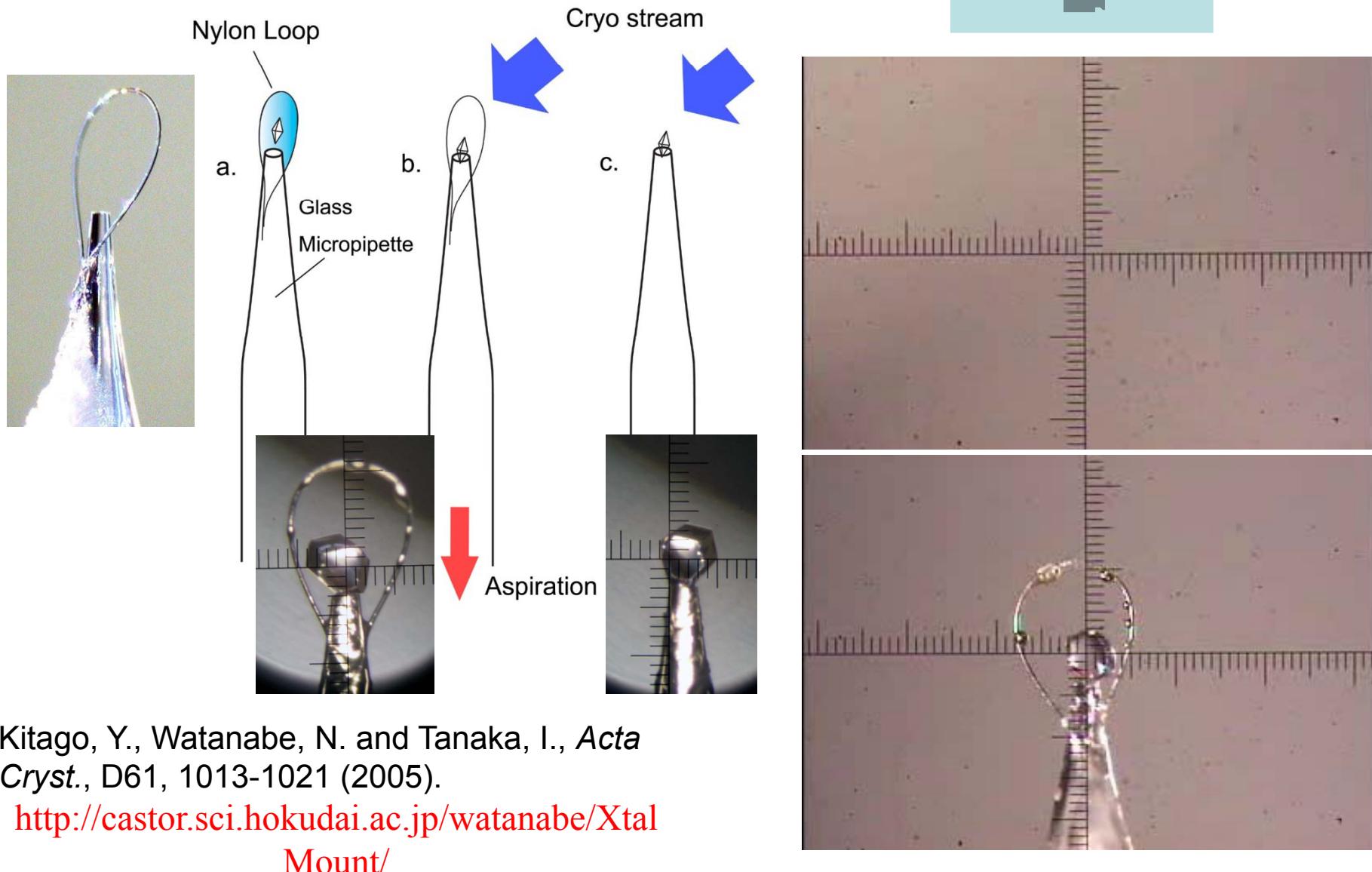
1. To be completed by end of March 2009
2. Compatibility: SPring-8 and PF robots
3. Remote access and control

In collaboration with Hokkaido Univ, Kyoto Univ, and Osaka Univ.

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



# Towards lower background: Promising techniques developed by other groups. Loopless crystal mounting method



Towards lower background:  
Promising techniques developed by other groups.

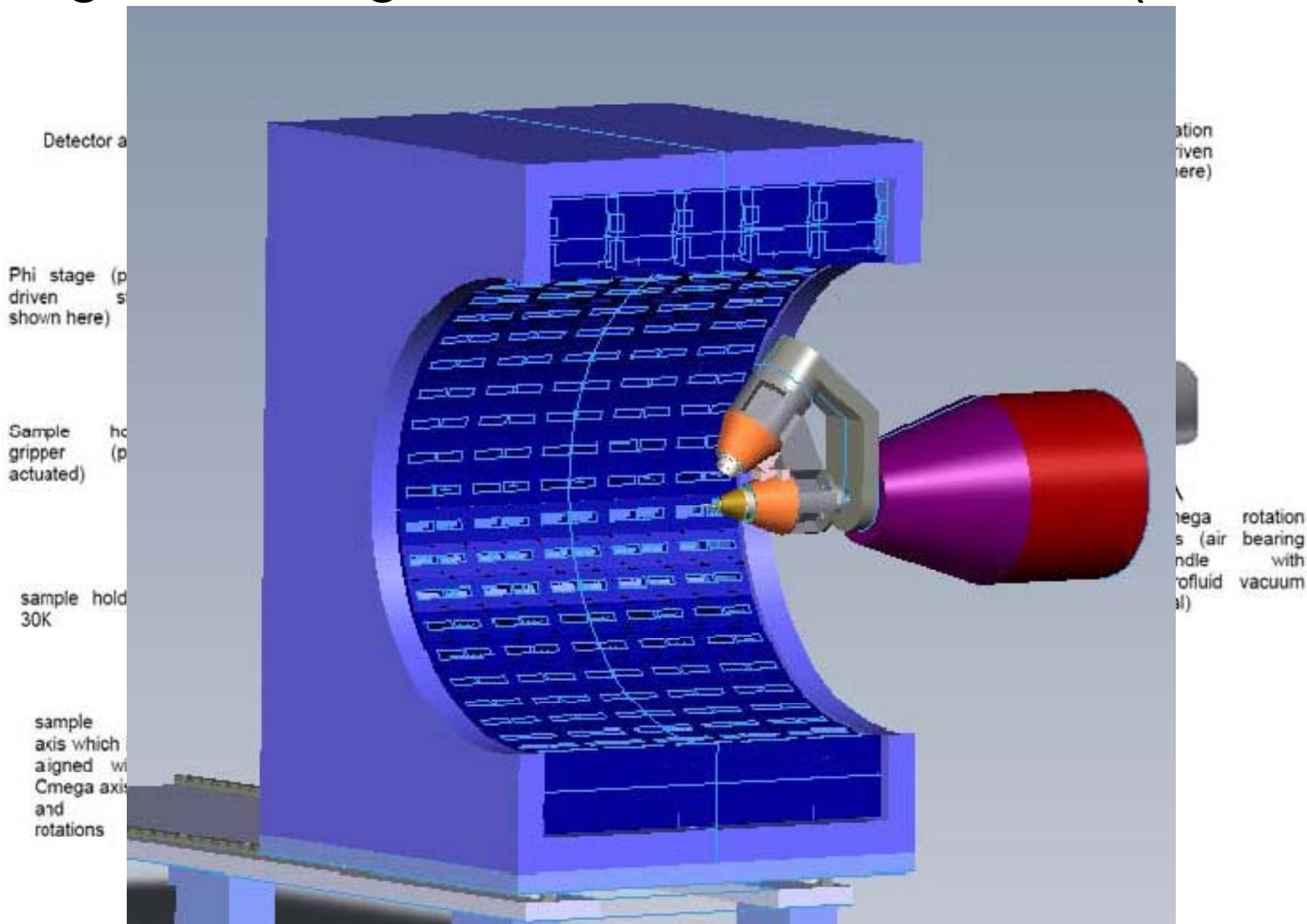
OR CUT AWAY the rest with a 193 nm UV laser



Courtesy of Nikon Ltd. & Sosho Ltd.

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

# Long Wavelength MX BL at Diamond:I23 (2~4 Å)

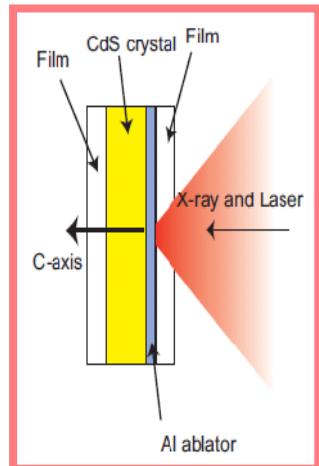


Courtesy of Armin Wagner, Talk on Friday 11:45

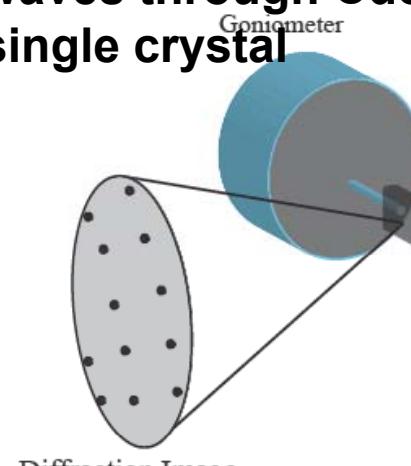
# PF-AR NW14A: ERATO Project (~2009)

Dynamics studies with 100 ps time resolution for innovation in materials and biological sciences

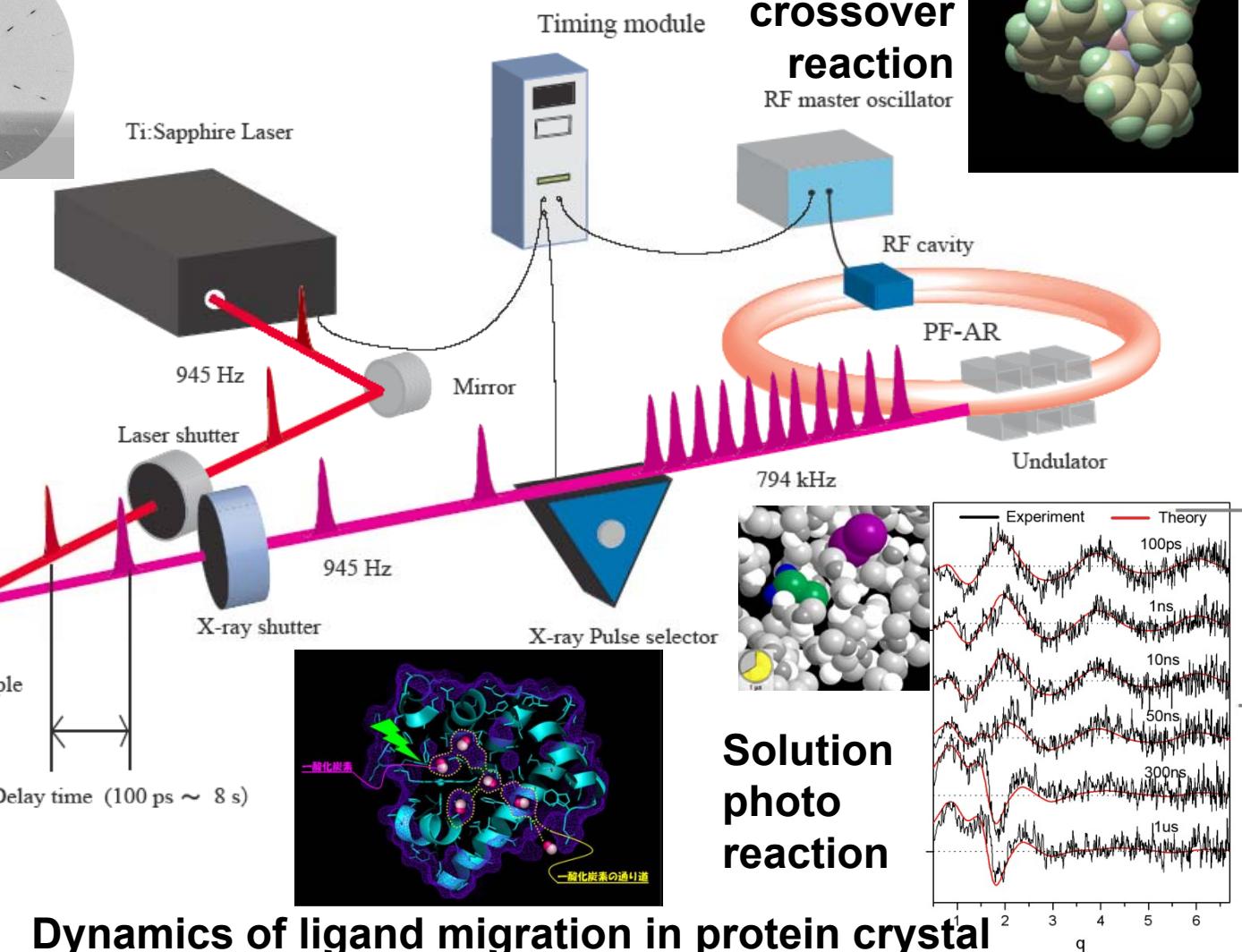
Tokyo Institute of Technology, ERATO (JST) S. Koshihara, KEK-PF Shin-ichi Adachi



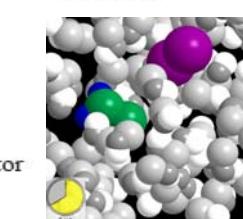
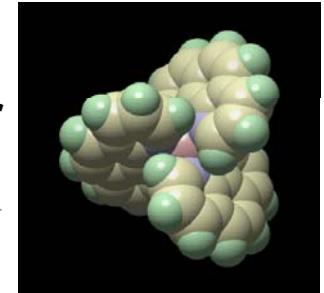
**Laser driven shock waves through CdS single crystal**



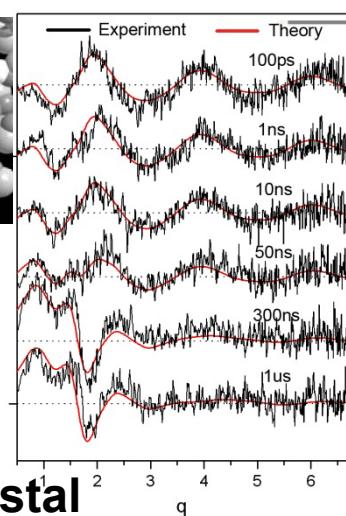
**Dynamics of ligand migration in protein crystal**



**Laser induced spin crossover reaction**

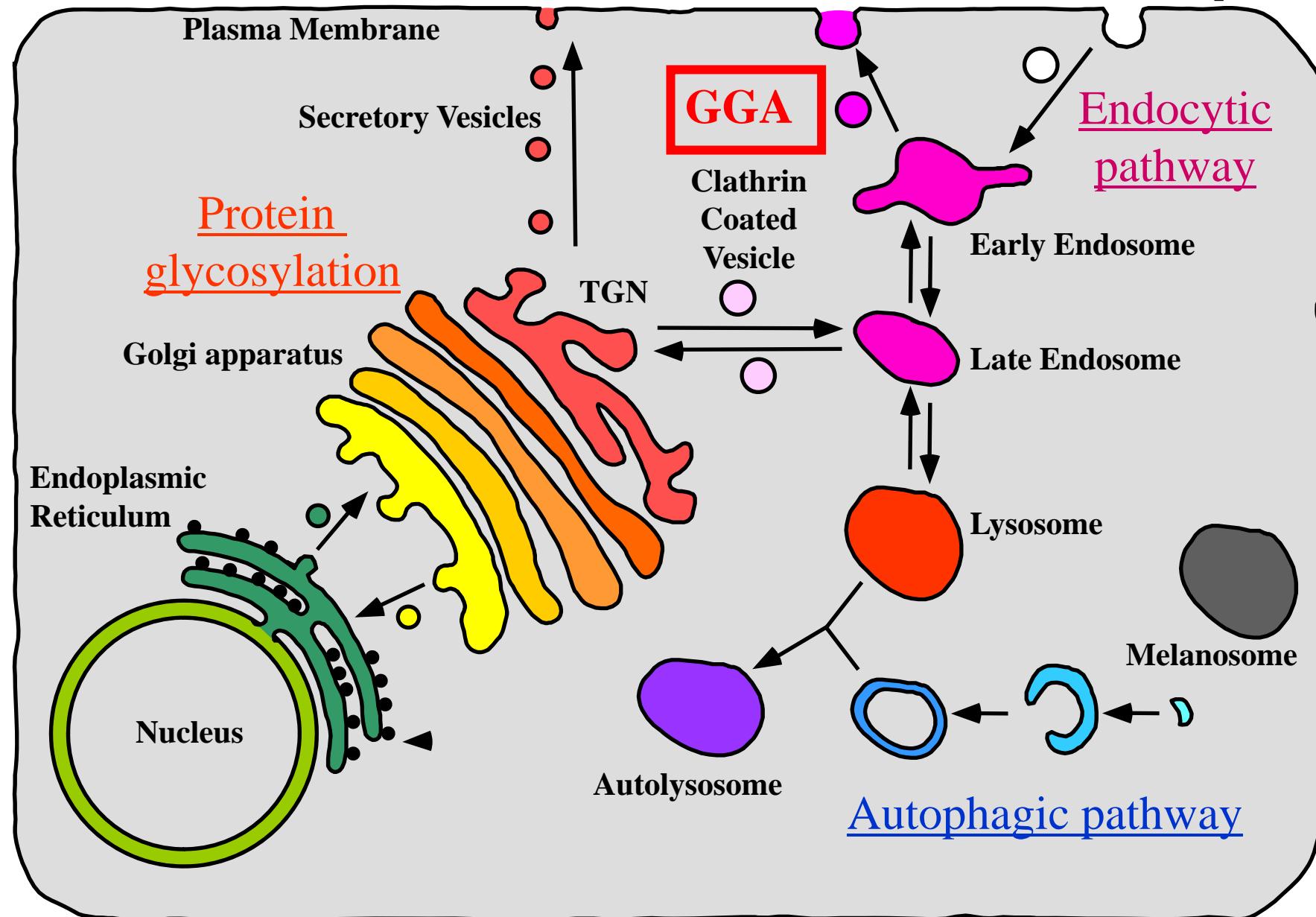


**Solution photo reaction**



# Systems Structural Biology

## Posttranslational modification and transport

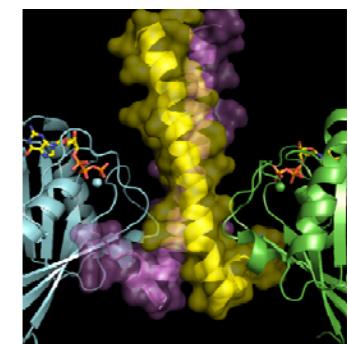
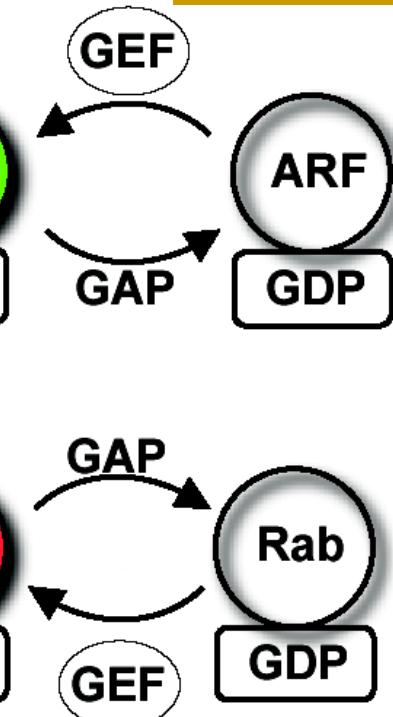
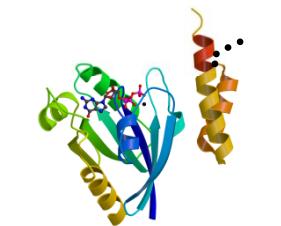
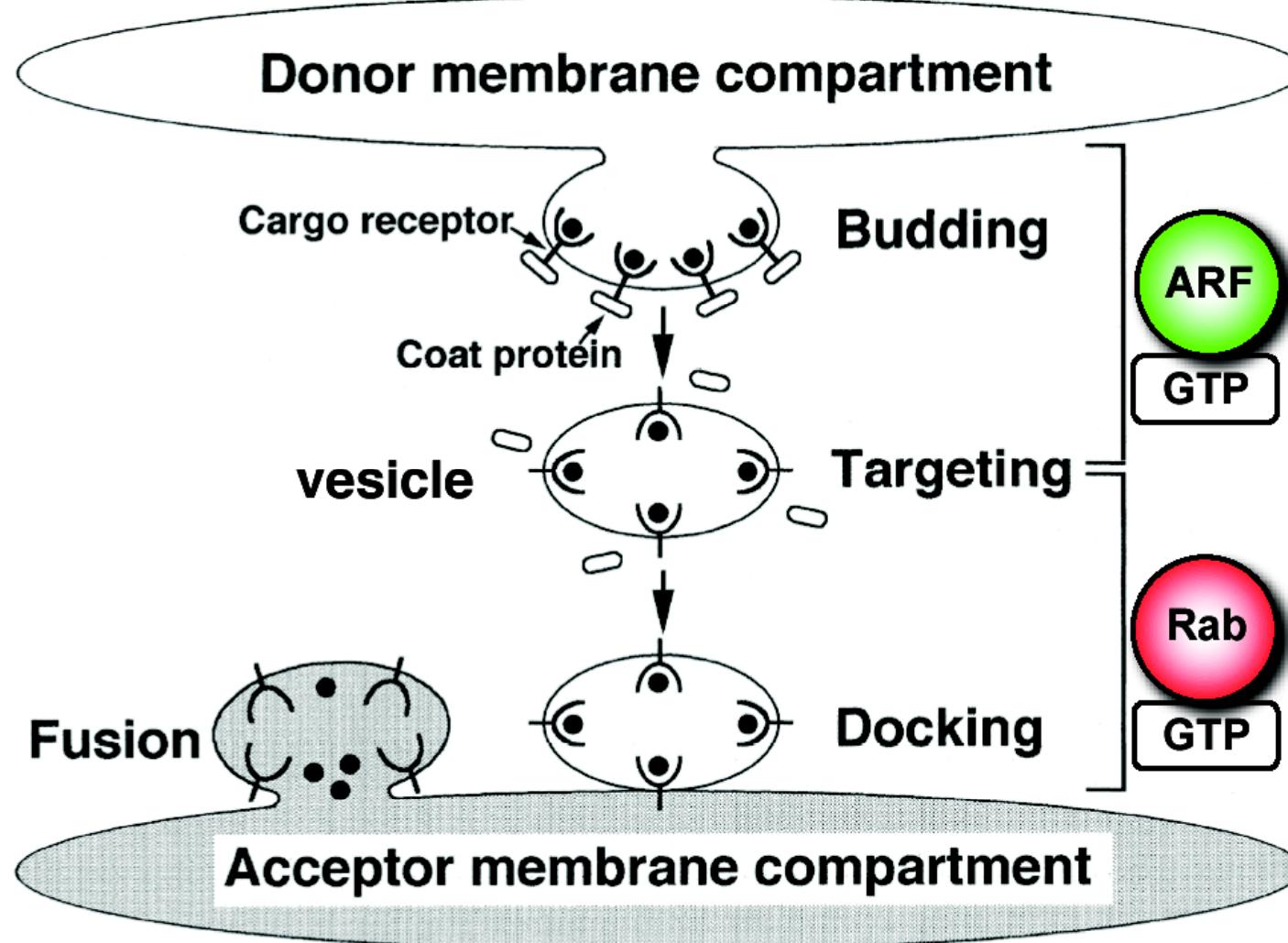


**Small GTPases, motor proteins  
involved in vesicle transport:**

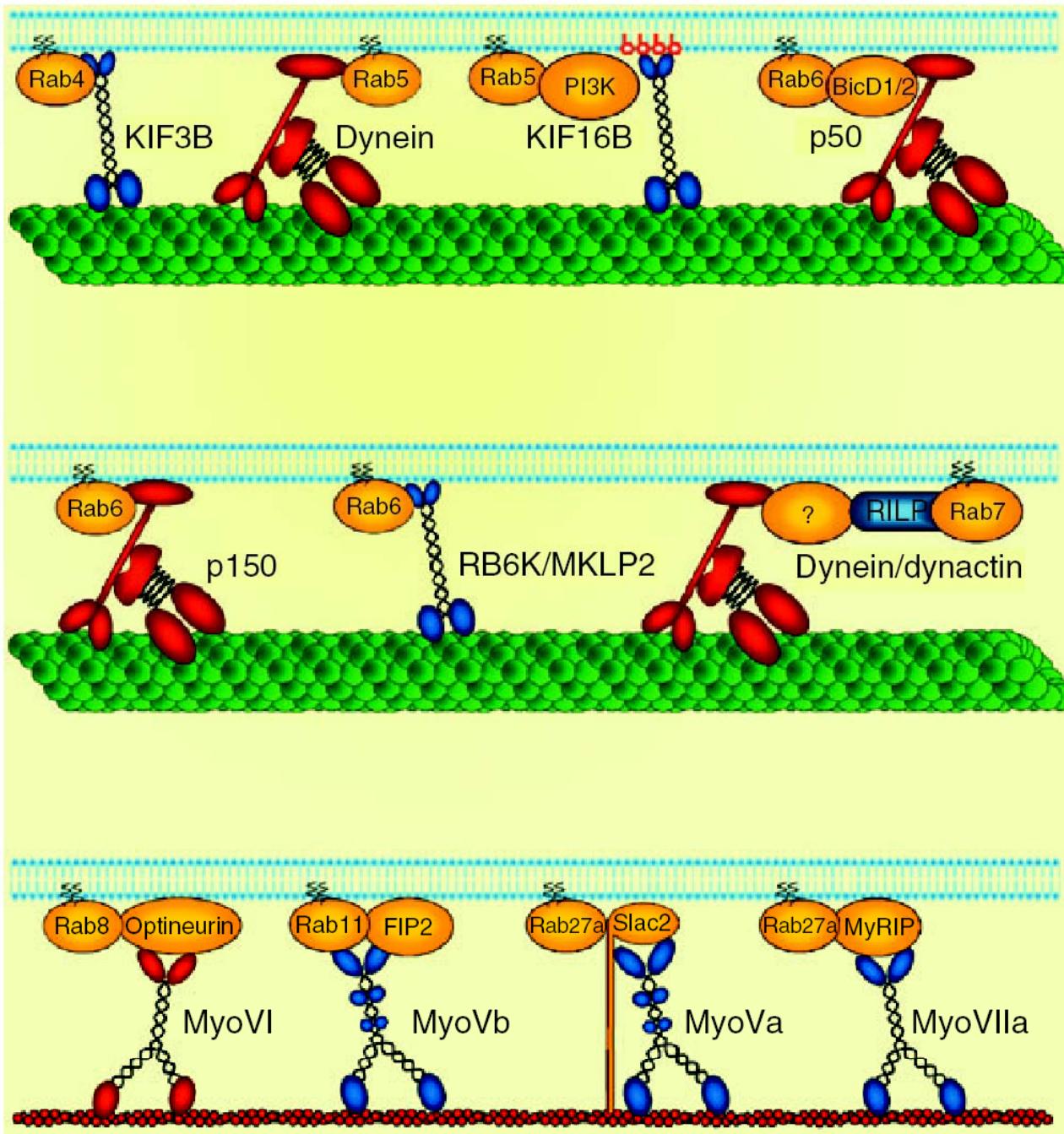
**Rab and Arf, kinesin, and  
clathrin**

# Small GTPases, ARF and Rab, in vesicular trafficking

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



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

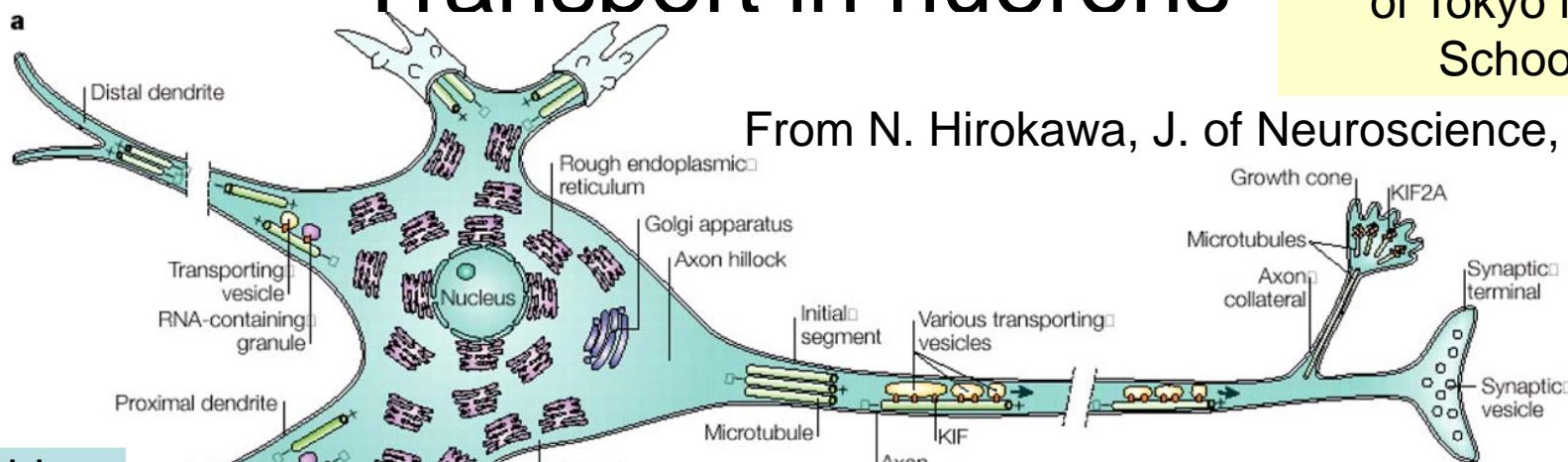


Taken from I. Jordens, et al., J.Neefjes, Traffic 2005; 6: 1070–1077

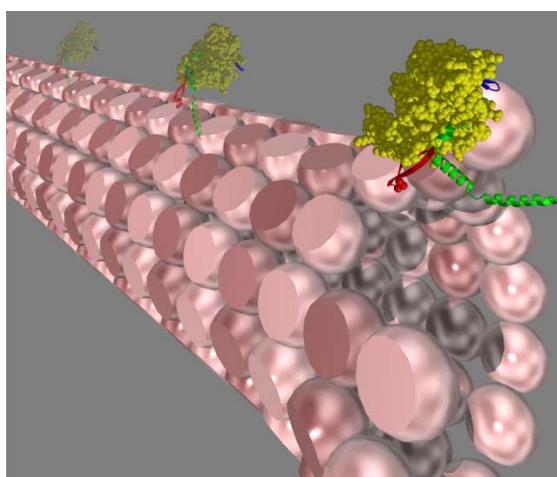
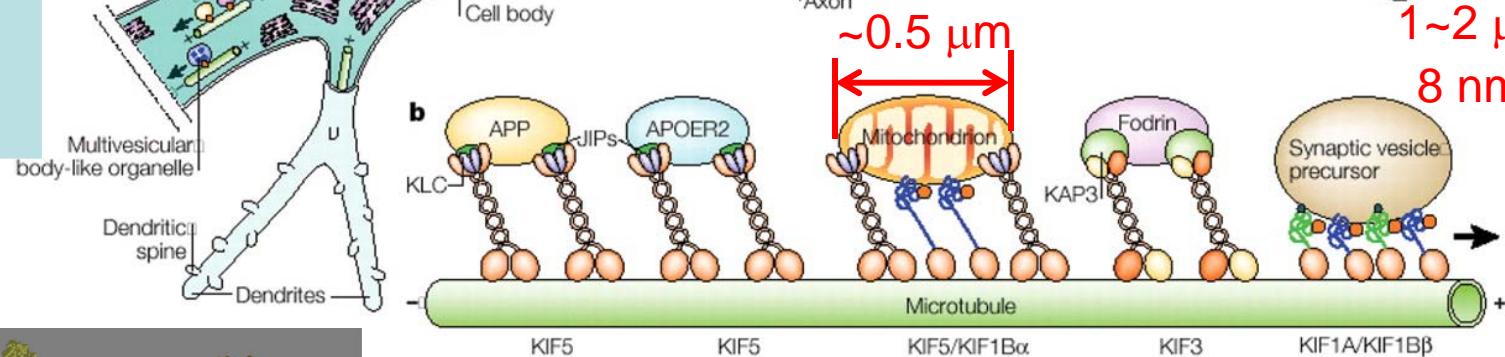
# Transport in neurons

N. Hirokawa, Univ  
of Tokyo Med  
School

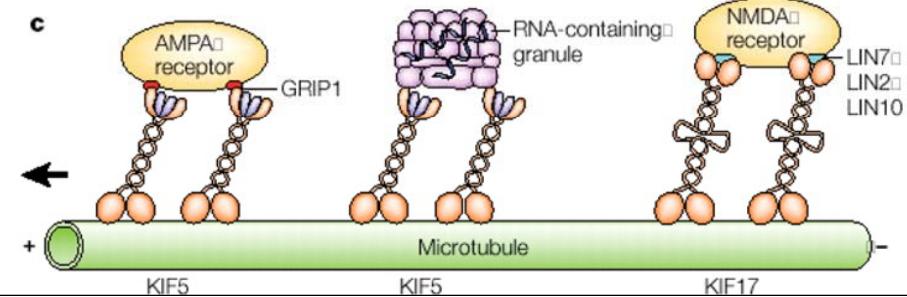
From N. Hirokawa, J. of Neuroscience, 2006



Double headed kinesin



Ogawa, et al., Cell, 2004

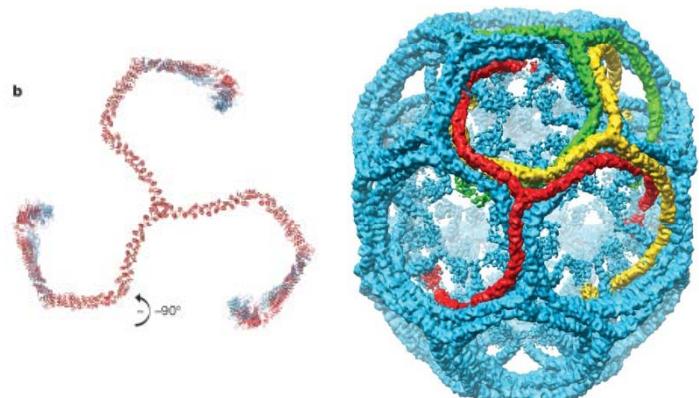


KIF1A: single headed motor, Nitta, Hirokawa et al., *Science*  
2004, 305, 678 – 683, Data collected on PF-BL6A & 19ID APS

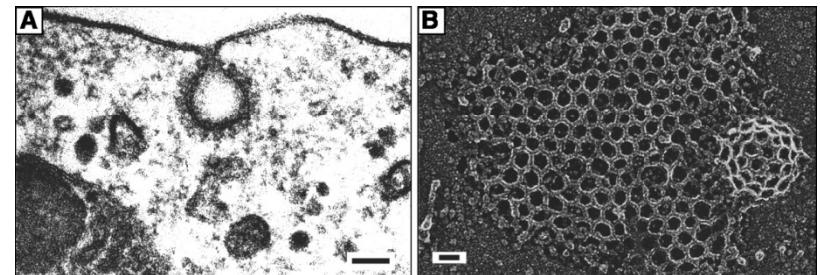
# Higher order protein complexes localized on organelle membranes

EM

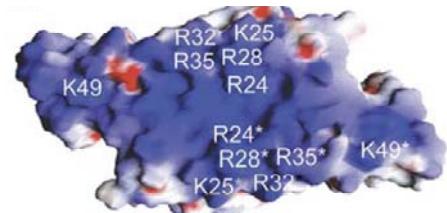
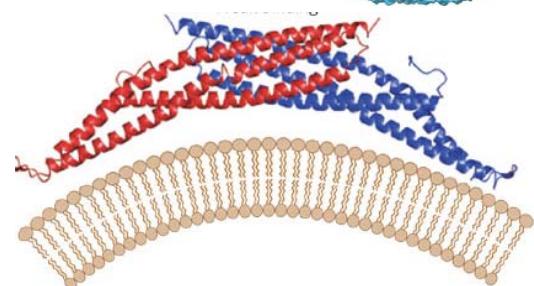
Confocal microscopy (live cell imaging)  
*in vitro* reconstruction system  
Synchrotron X-ray diffraction imaging



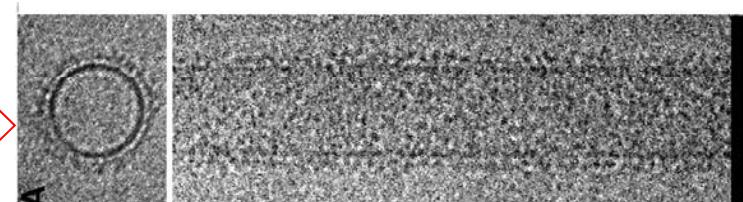
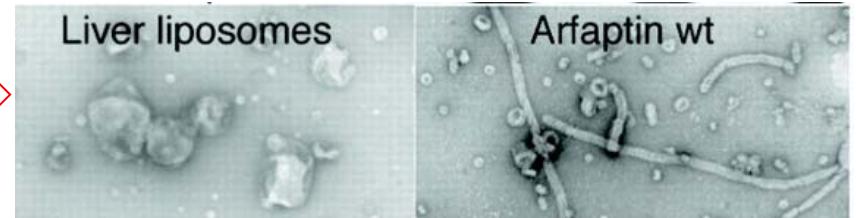
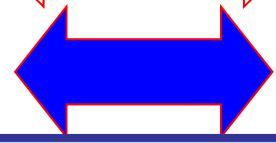
AP, & GGA



BAR domain



ESCRT-III



Organelle morphology

High resolution protein structures

Multiple Arf/Rab interactions with their effectors

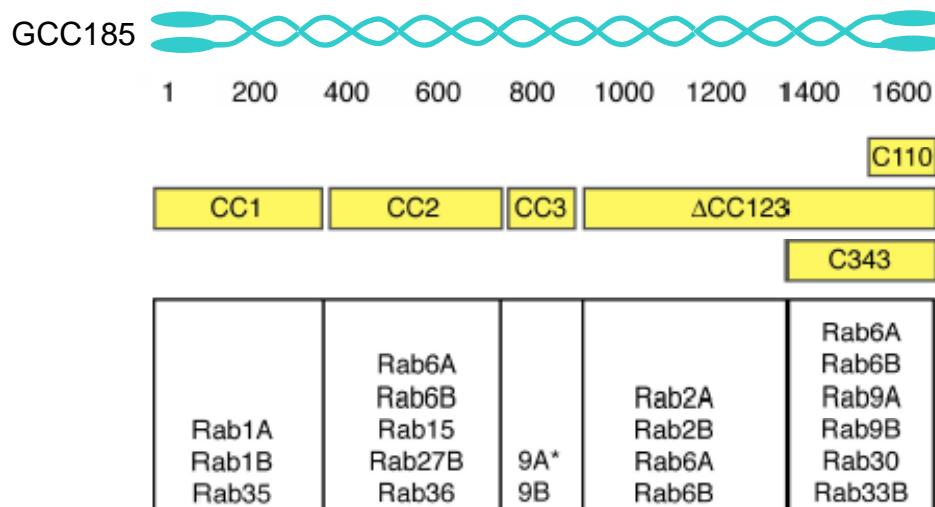
Organelle structure and function

# Golgin: multi effector protein for Arf/Rab GTPases

Golgin proteins (GCC185)

Very long coiled-coil structure

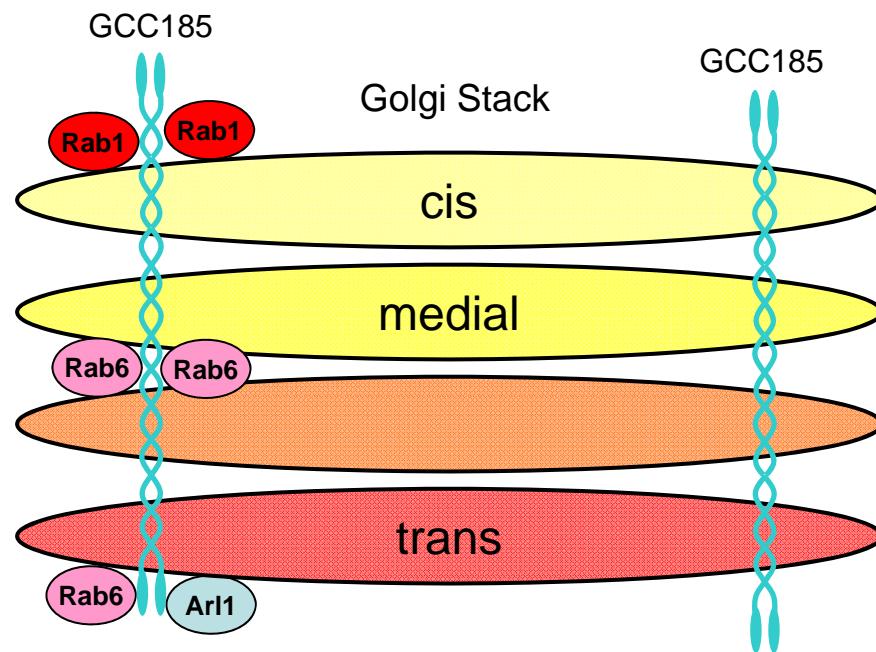
Scaffold for maintaining Golgi stacks



Sinka et al., 2008 *J. Cell. Biol.* 183, 607.

Hayes et al., 2009 *Mol. Biol. Cell* 20, 209.

Rab small GTPases (> 60) are localized on specific organelles



Molecular level

Golgin structure



Specific interactions between  
Golgin and Rab/Arf

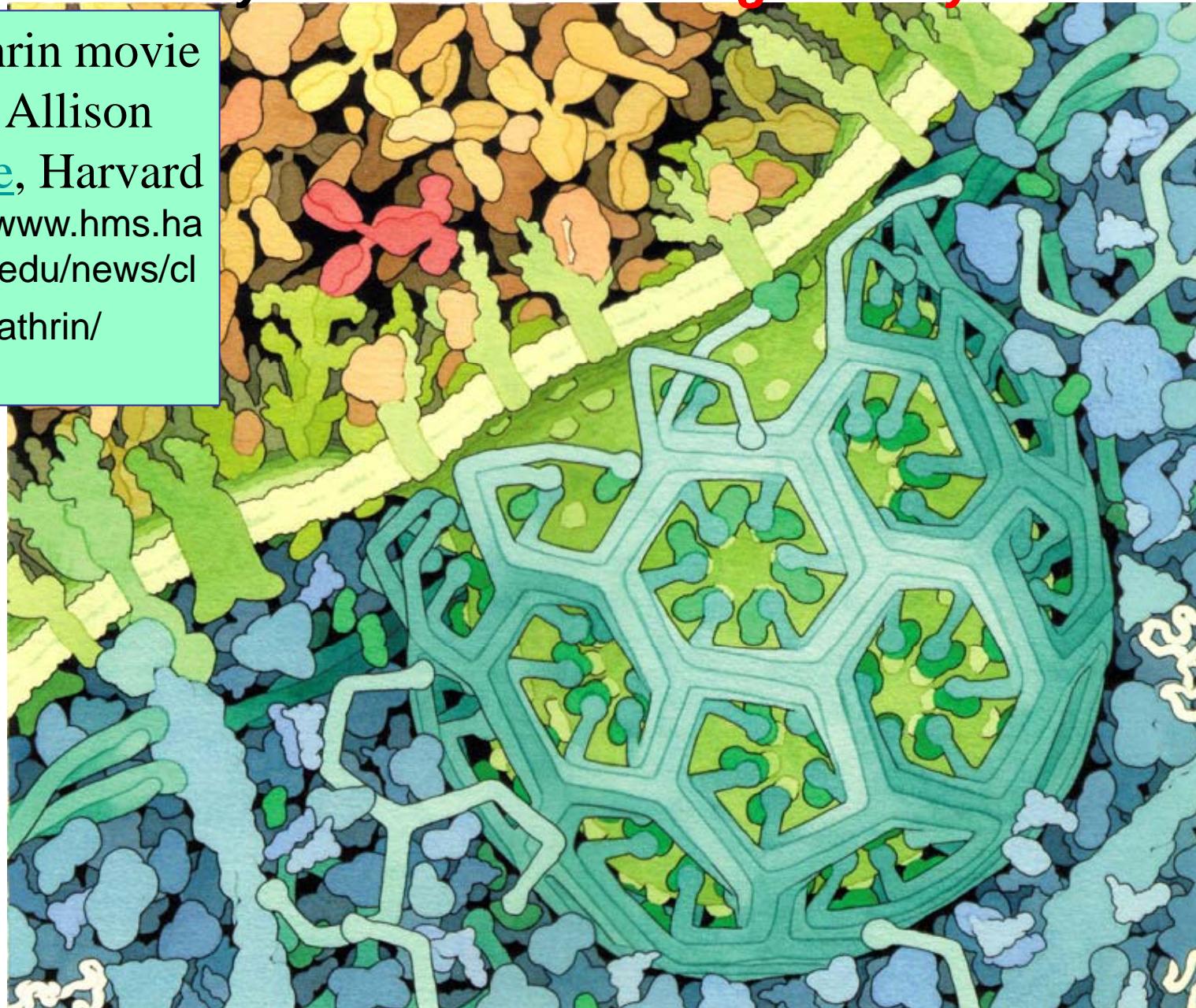
Cell level

Maintenance of Golgi stack structure

Localization of Rab/Arf on  
different Golgi compartments

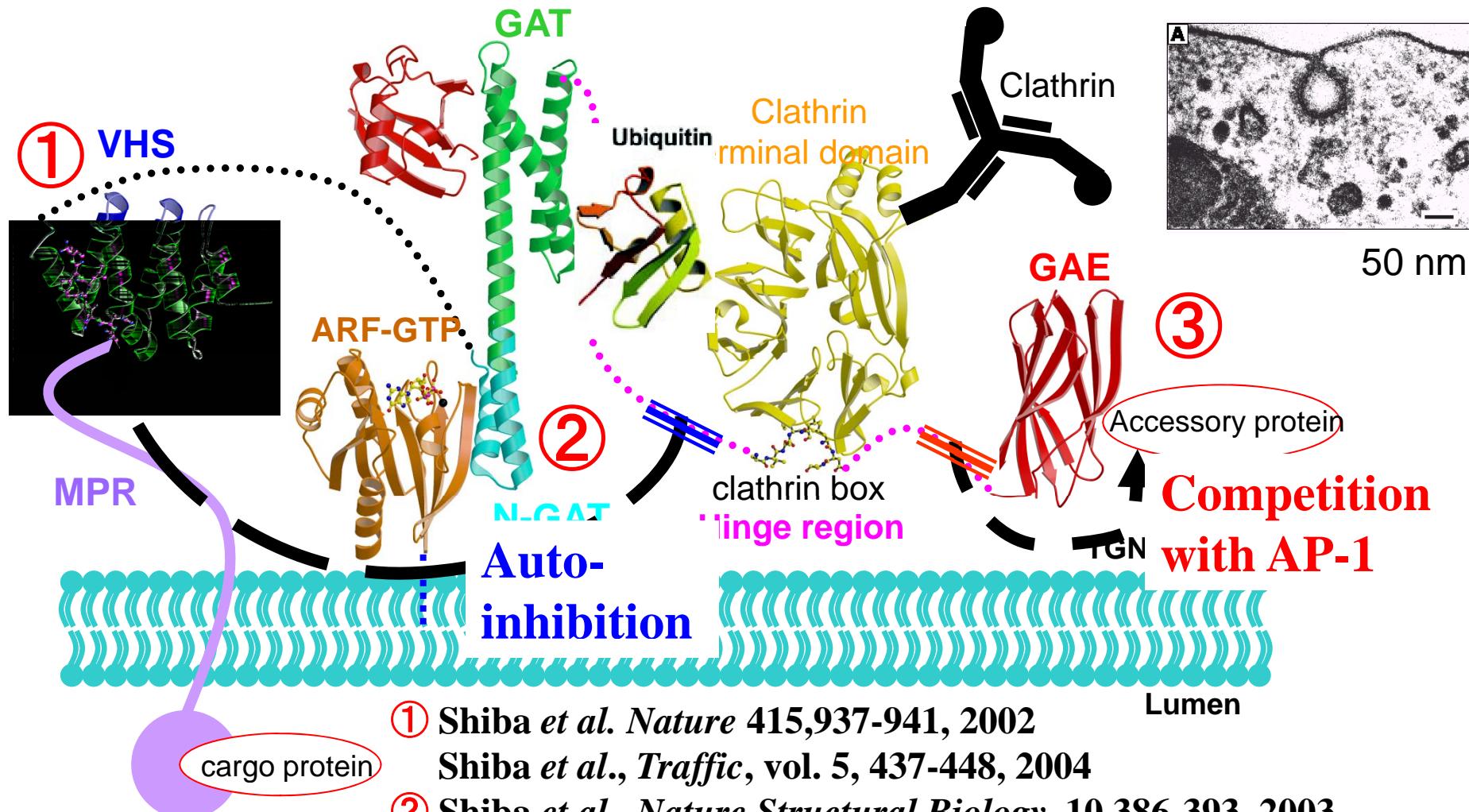
## ***Endocytosis of toxin -> Drug delivery***

Clathrin movie  
by Allison  
[Bruce](#), Harvard  
<http://www.hms.harvard.edu/news/clathrin/>



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

# Human GGA: a new class of adaptor proteins

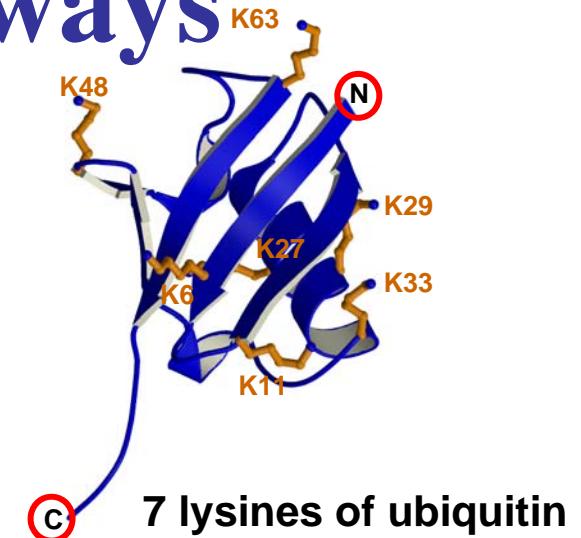


- ① Shiba *et al.* *Nature* 415, 937-941, 2002  
Shiba *et al.*, *Traffic*, vol. 5, 437-448, 2004
- ② Shiba *et al.*, *Nature Structural Biology*, 10, 386-393, 2003  
Shiba *et al.*, *J. Biol. Chem.* 279, 7105-11279, 2004  
Kawasaki *et al.*, *Genes to Cells*, 10, 639–654, 2005  
Yogosawa *et al.*, *BBRC*, 350, 82-90, 2006
- ③ Nogi *et al.* *Nature Structural Biology*, 9, 527-531, 2002  
Inoue *et al.* *Traffic*, 8, 904-913, 2007

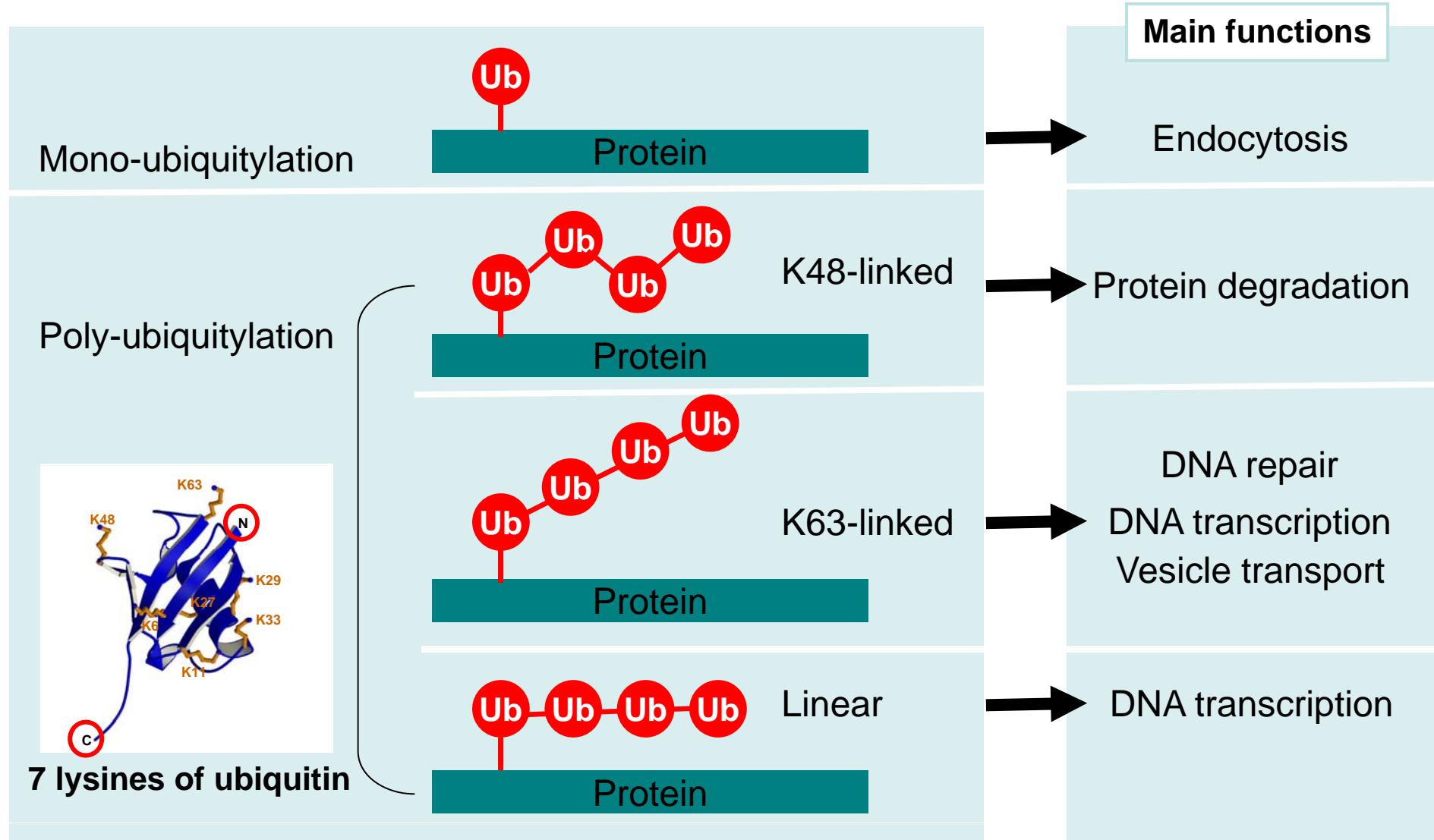
**Ubiquitin (Ub): ubiquitous protein modifier with only 76 amino acid residues, 27000 papers published to date**

**Can connect Ub to another Ub using 7 (now 8) different ways**

**How are specific linkages recognized for signaling?**

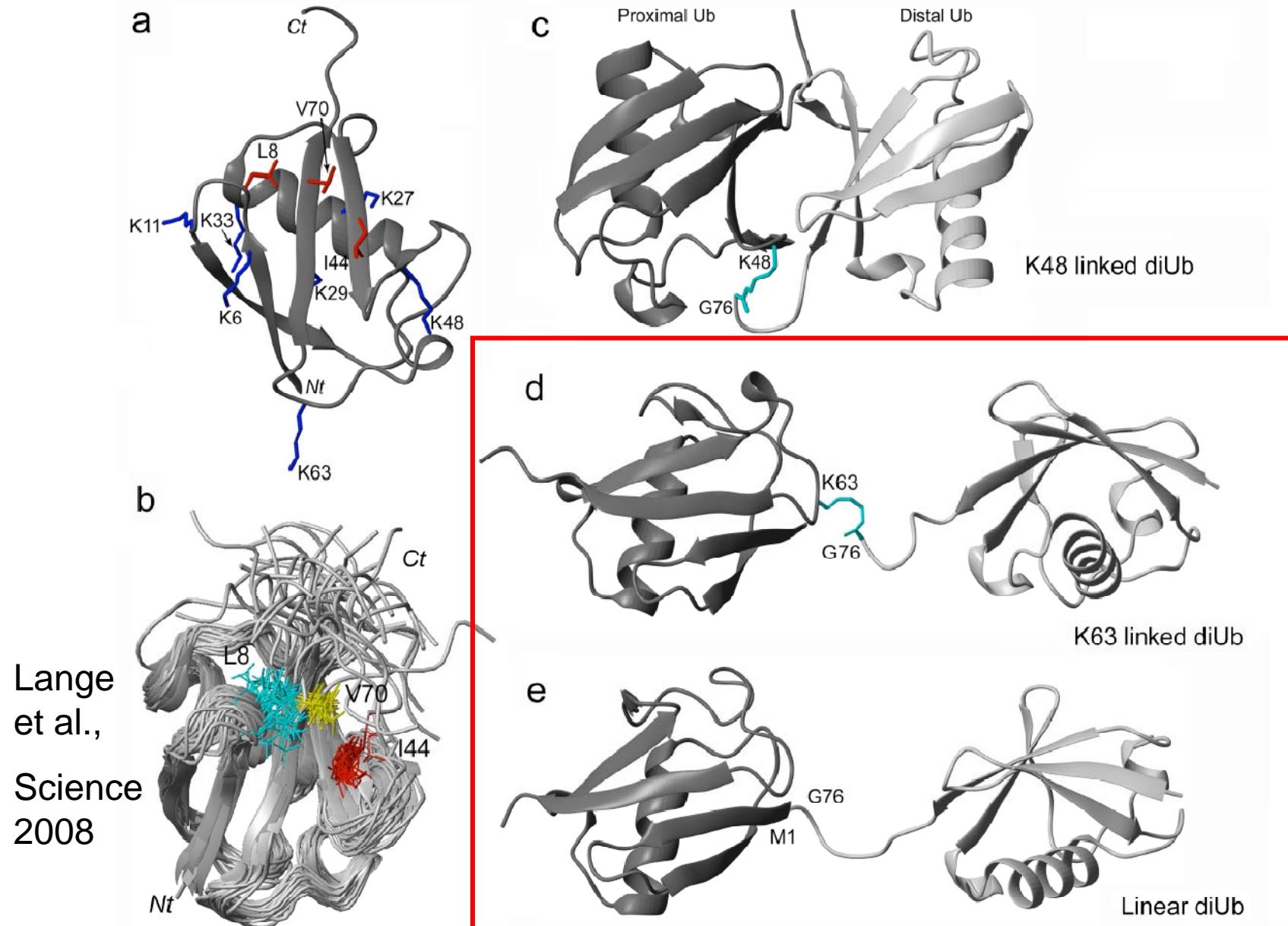


# Polyubiquitin: linkage specificity vs. function



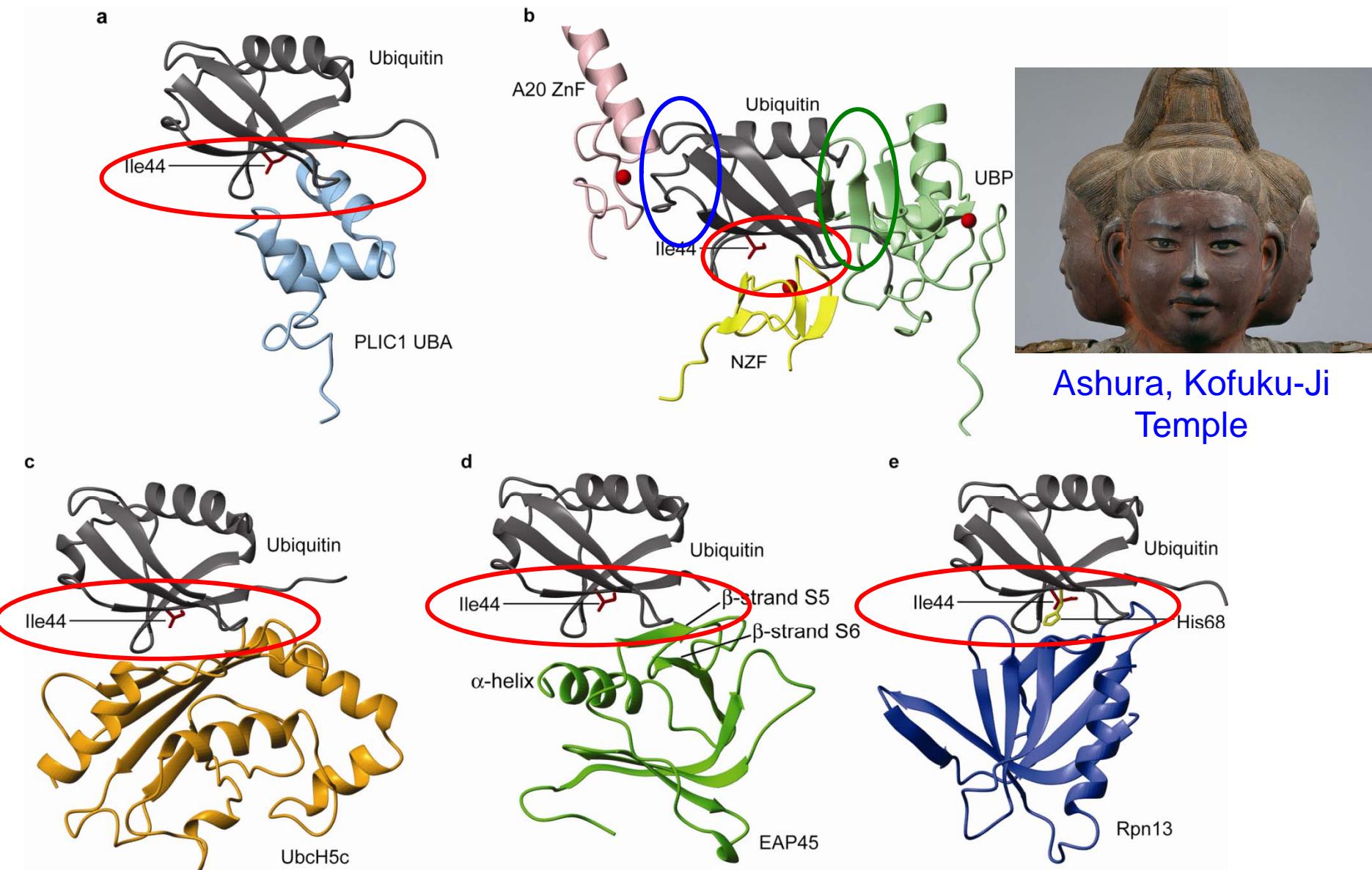
Linear-ubiquitylation and NEMO (Rahighi, et al., Wakatsuki, Dikic, Cell 2009; Tokunaga et al., Iwai, Nature Cell Biol., 2009)

# 7 lysines, K48, K63 and Linear



Taken from Dikic, Wakatsuki, Walter, *Nat. Rev. Mol. Cell Biol.*, in press

# Ubiquitin is a multifaceted protein

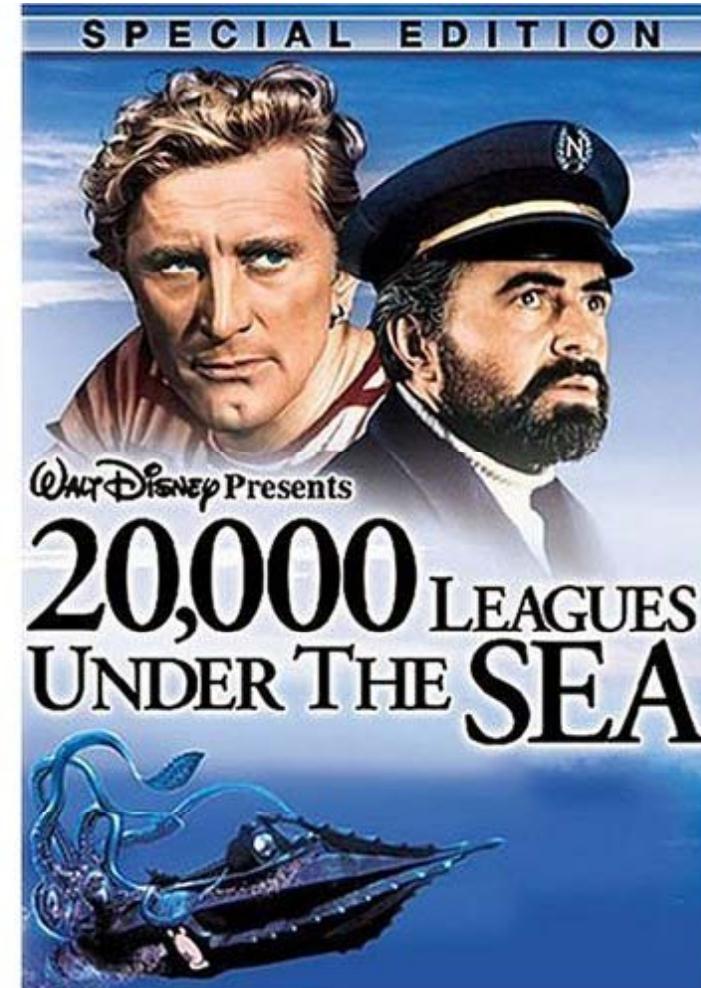




# Finding NEMO



Structural Biology Research Center  
Photon Factory, IMSS, KEK, Tsukuba,  
Japan



Goethe University, Frankfurt, Germany  
MRC, Cambridge, UK

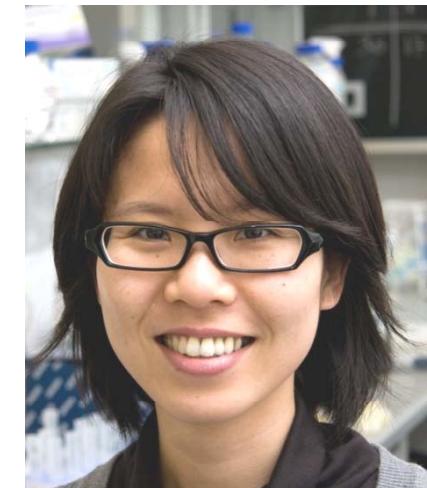
# Teams behind the Ubiquitin/NEMO project



Simin Rahighi



Ivan Dikic



Fumiyo Ikeda

**Ivan Dikic & Fumiyo Ikeda, Inst. of Biochemistry, Goethe Univ Med School, Frankfurt, Germany**

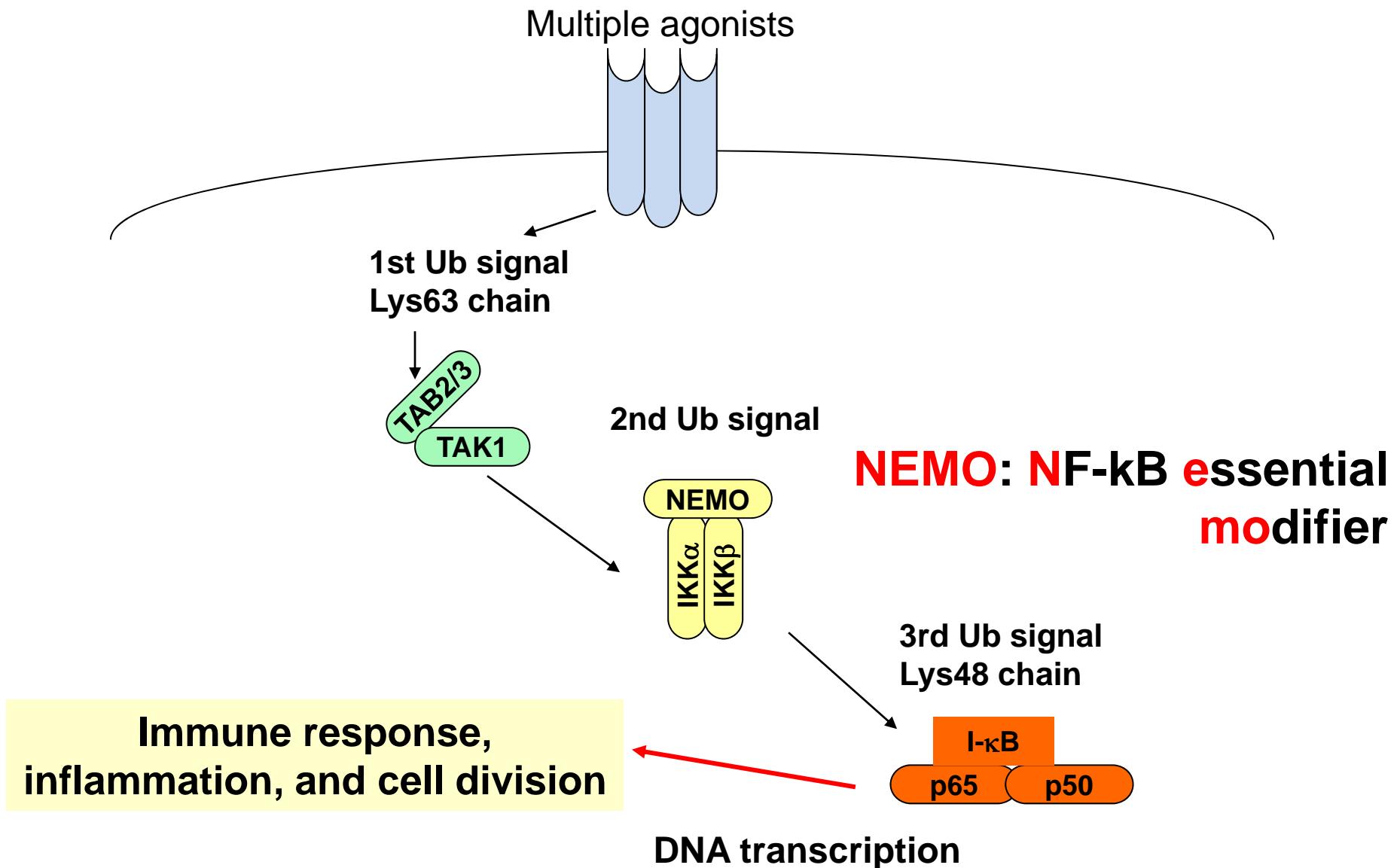
**Mark Schmidt Supprian, Germany**

**Felix Randow, MRC, Cambridge, UK**

**David Komander, MRC, Cambridge, UK**

**Simin Rahighi, Masato Kawasaki, Nobuhiro Suzuki, Tamami Uejima, Ryuichi Kato, SBRC, KEK-PF, Tsukuba, Japan**

# Ubiquitin signals in NF-κB activation

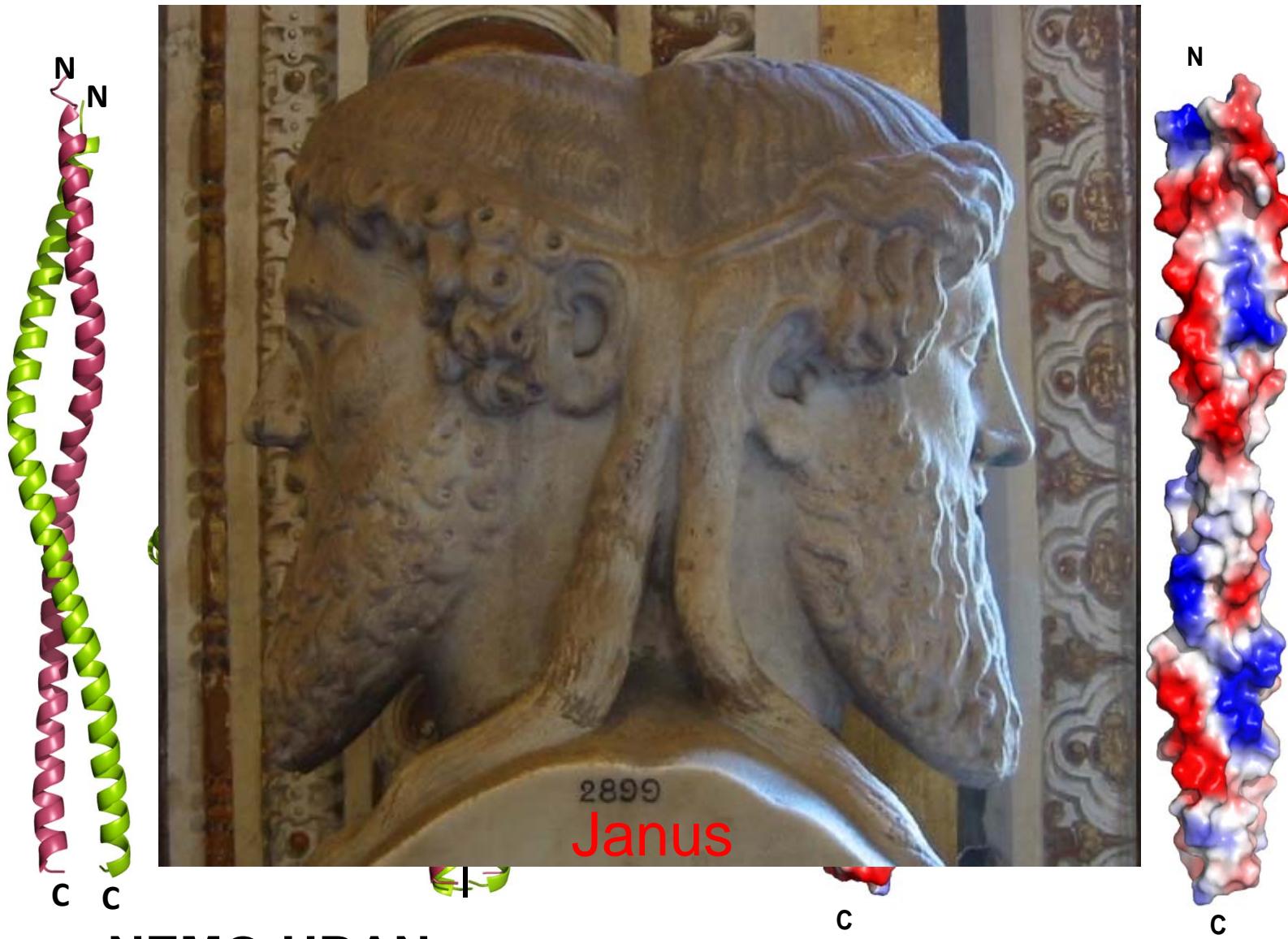


# UBAN - Ub binding domains present in ABIN and NEMO proteins



NEMO	Mm	(286)	IVMETVPVLKAQADIYKADFAQERHAREKLVEKKEYLQEQLQLQR
	Hs	(293)	IVMETVPVLKAQADIYKADFAQERQAREKLAEKKEFLQEQLQLQR
	Bt	(293)	IVMETVPVLKAQADIYKADFAQERQAREKLAEKKEFLQEQLQLQR
	Dm	(263)	-KQEVIKGLQIQNDIYRRDFEMERADREKNAGEKDQYLMDLRSILQR
Optineurin	Mm	(459)	EDLETMAVLRAQMEVYCSDFHAERAAREKIHEEKEQLALQLAILLK
	Hs	(456)	EDLETMTILRAQMEVYCSDFHAERAAREKIHEEKEQLALQLAVLLK
ABIN-1/Naf1	Mm	(467)	ELVTQNELLKQQVKIFEEDFQRERSDRERMNEEKEELKKQVEKLQA
	Hs	(454)	ELVTQNELLKQQVKIFEEDFQRERSDRERMNEEKEELKKQVEKLQA
ABIN-2	Mm	(292)	TALERVQMLEQQILAYKDDFKSERADRERAHSRIQELEEKIMSLMY
	Hs	(291)	AALERVQMLEQQILAYKDDFMSERADRERAQSRIQELEEKVASLLH
ABIN-3/LIND	Hs	(192)	EMRTEMEVLKQQVQIYEEDFKKERSDRERLNQEKEELQQINETSQS

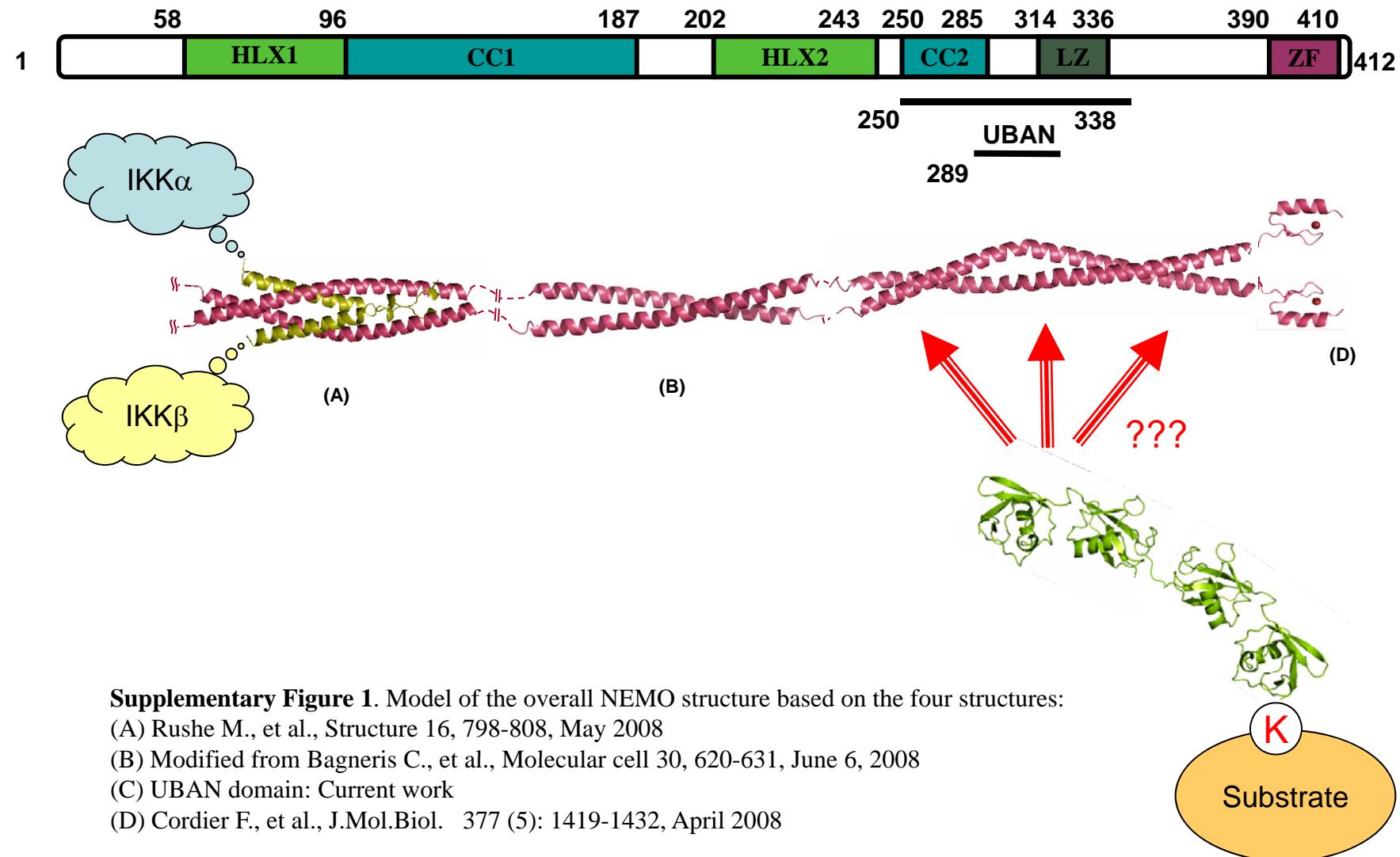
# Structure of ubiquitin binding domain of NEMO



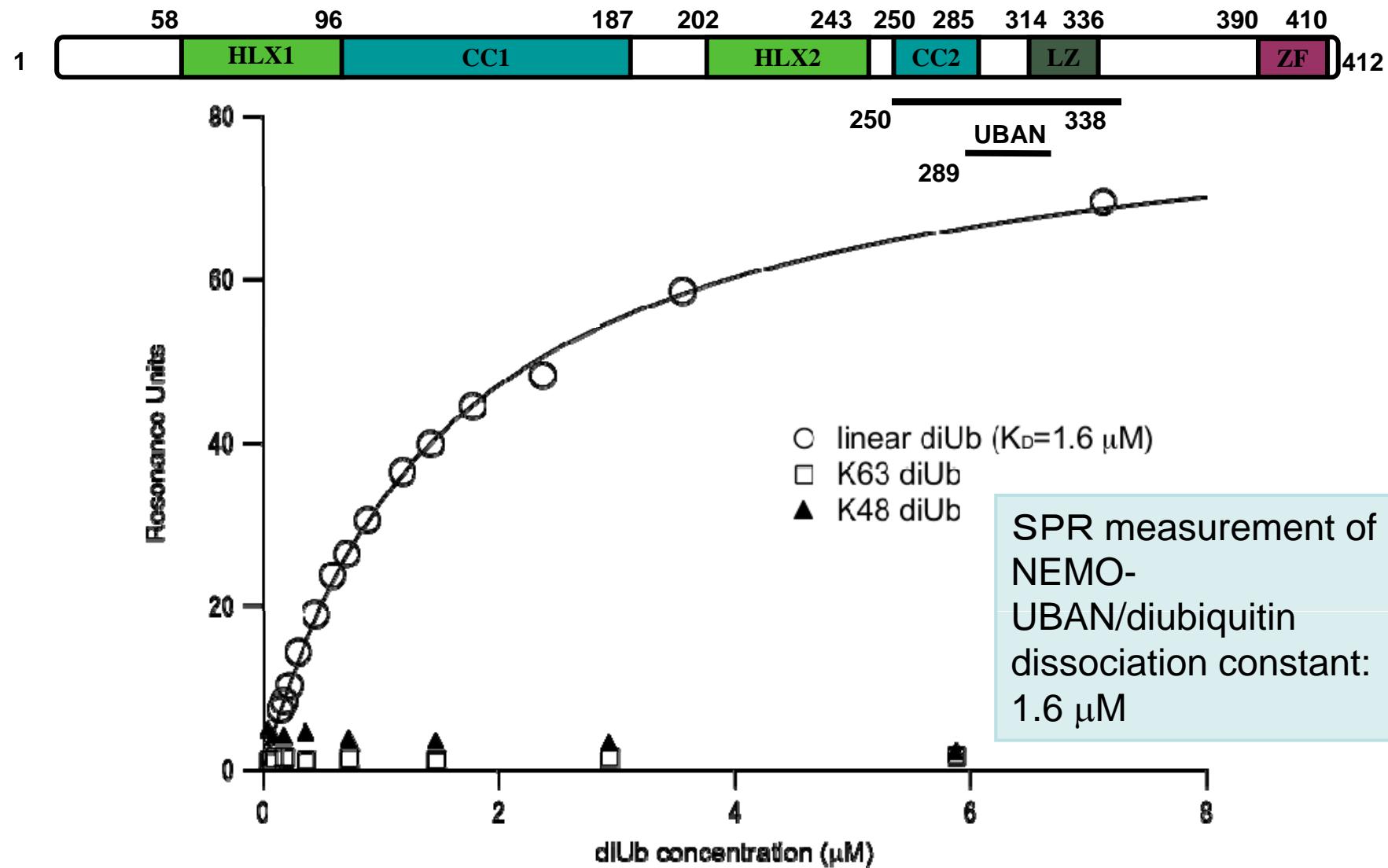
NEMO-UBAN

# How is linear ubiquitin chain recognized by NEMO?

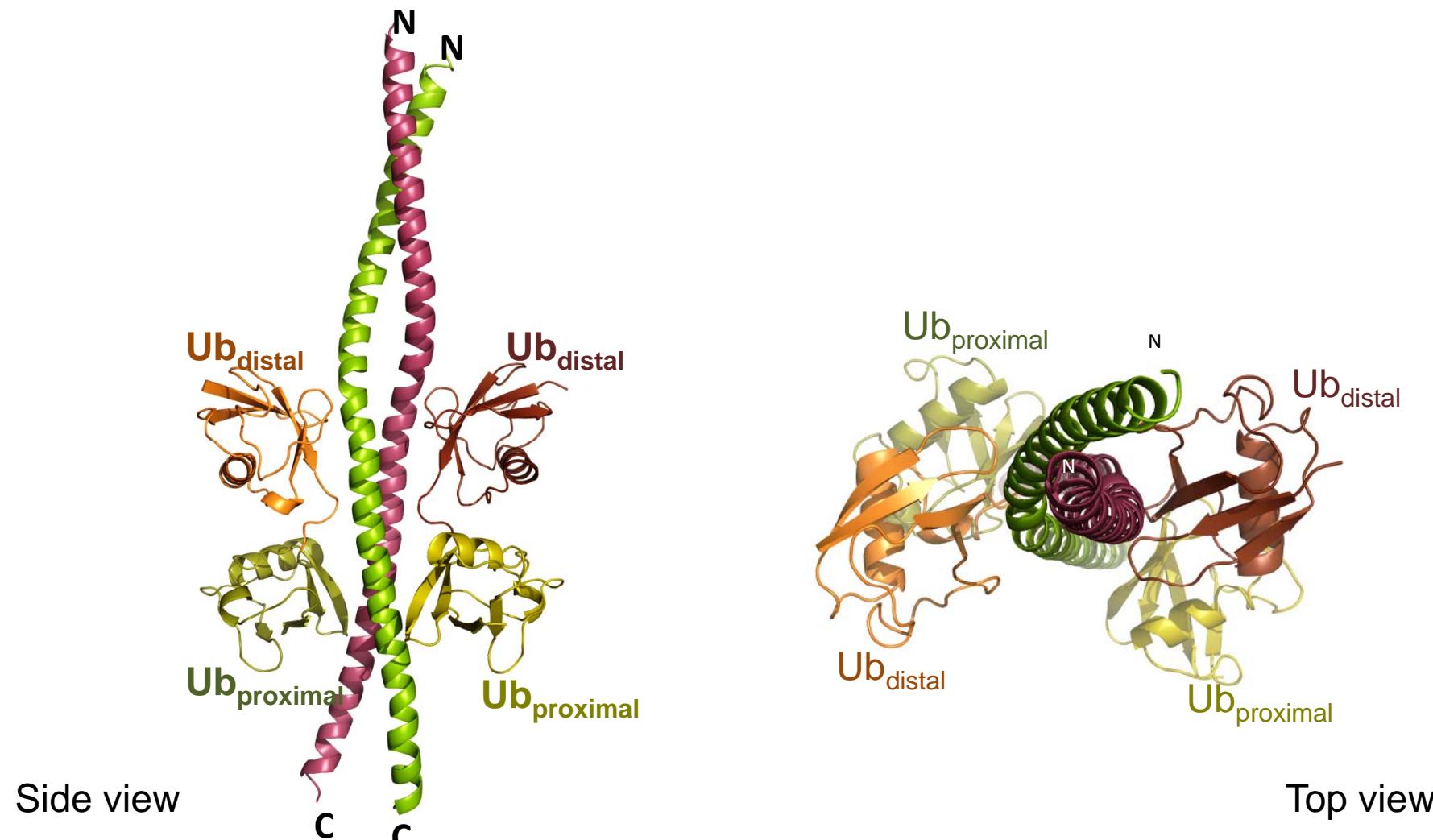
- How does NEMO differentiate linear vs. K63 linkage —
- How does Ub binding activates IKK? —



# Ubiquitin binding domain of NEMO (UBAN) specifically recognizes linear-diubiquitin

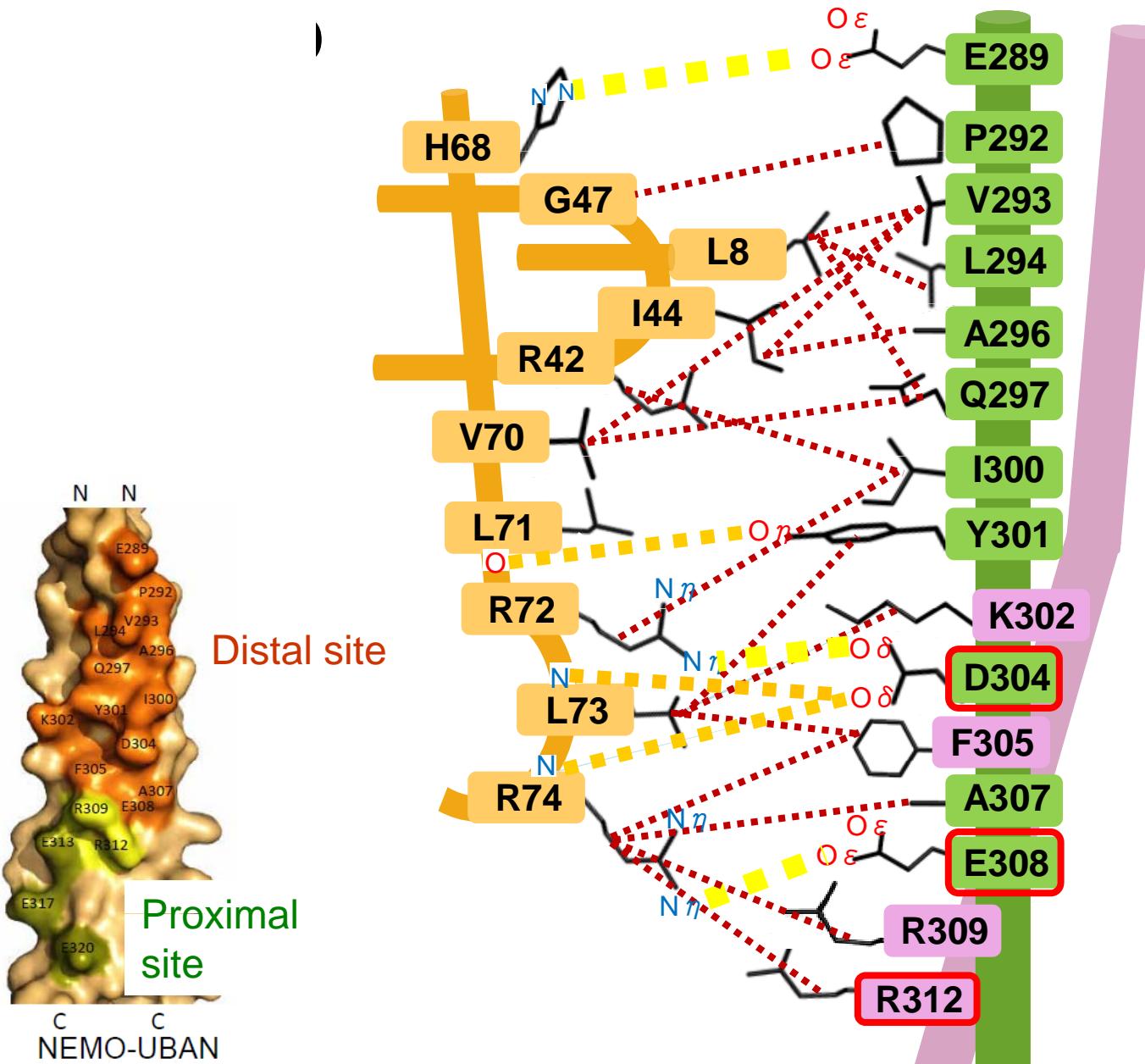


# Structure of ubiquitin binding domain of NEMO

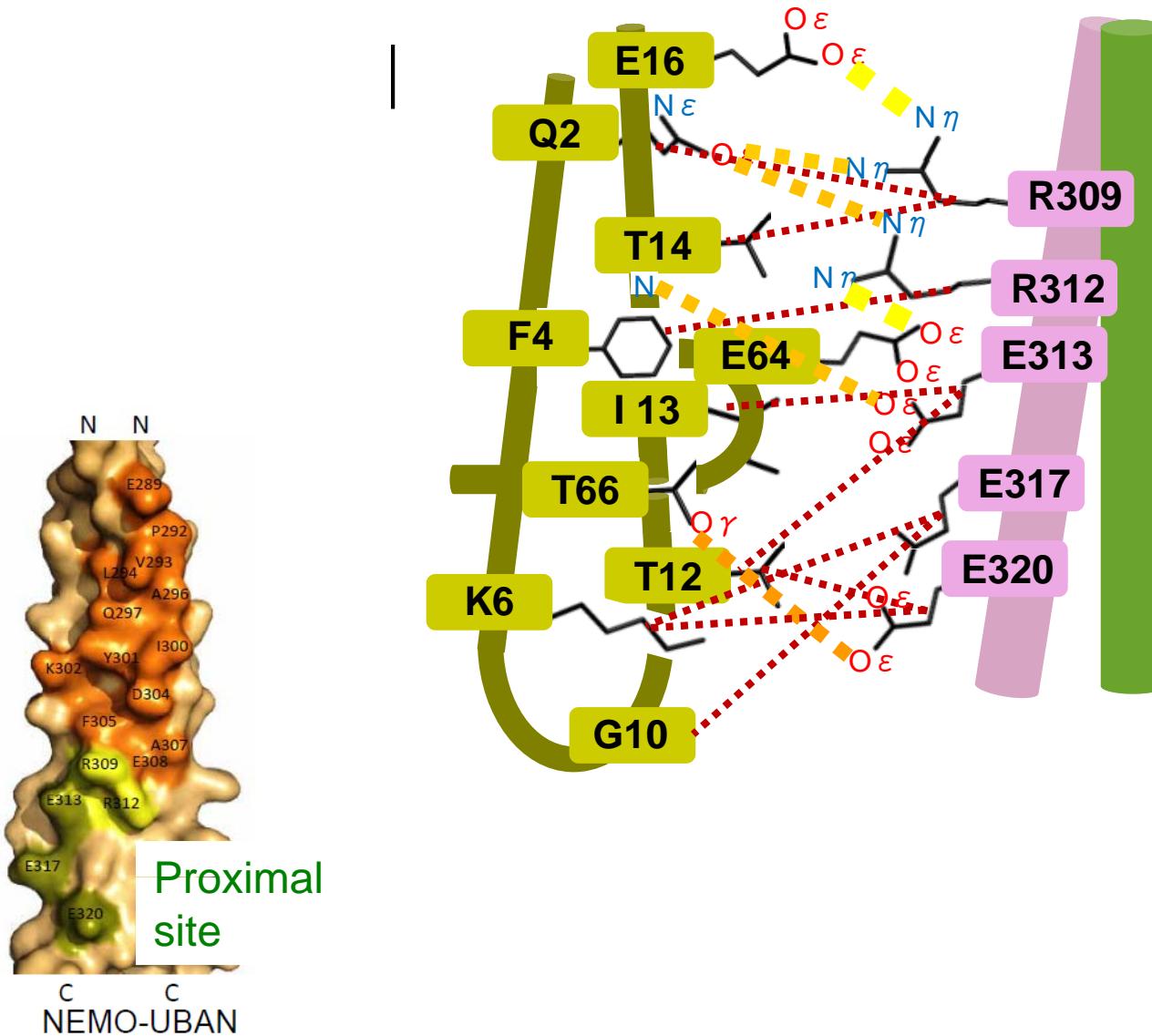


UBAN dimer in complex with two linear diubiquitin molecules

# Distal ubiquitin interaction



# Proximal ubiquitin interaction



# Structural Basis for Recognition of Diubiquitins by NEMO

Cornell Group's models based on NMR titration, ITC, mutagenesis

Molecular Cell  
Article

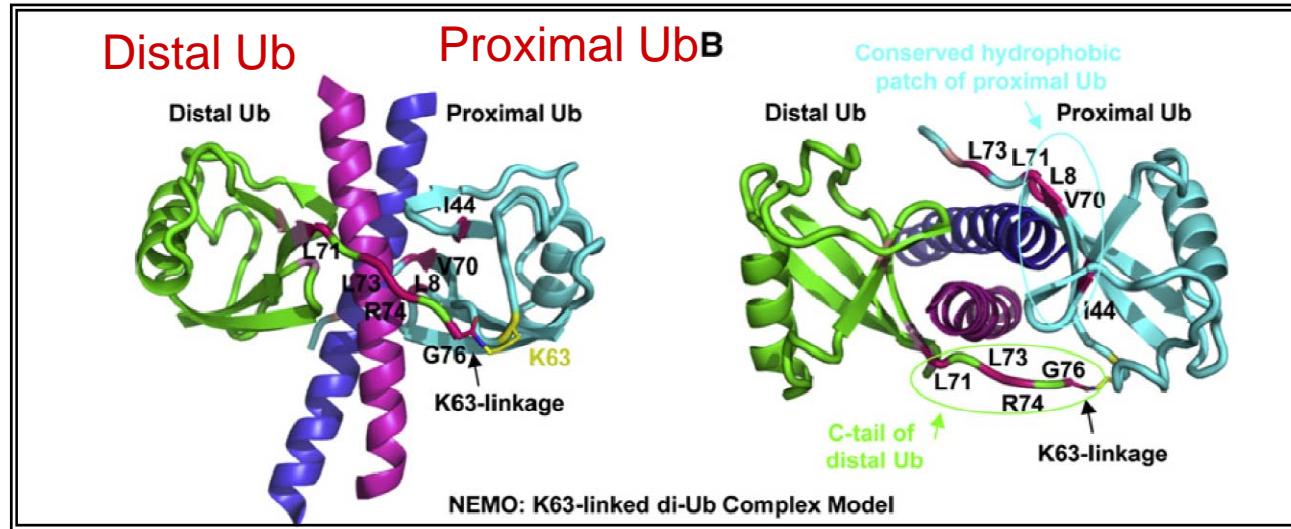
Yu-Chih Lo,<sup>1</sup> Su-Chang Lin,<sup>1</sup> Carla C. Rospigliosi,<sup>1</sup> Dietrich B. Conze,<sup>2</sup> Chuan-Jin Wu,<sup>2</sup> Jonathan D. Ashwell,<sup>2</sup> David Eliezer,<sup>1</sup> and Hao Wu<sup>1,\*</sup>

<sup>1</sup>Department of Biochemistry, Weill Cornell Medical College, New York, NY 10021, USA

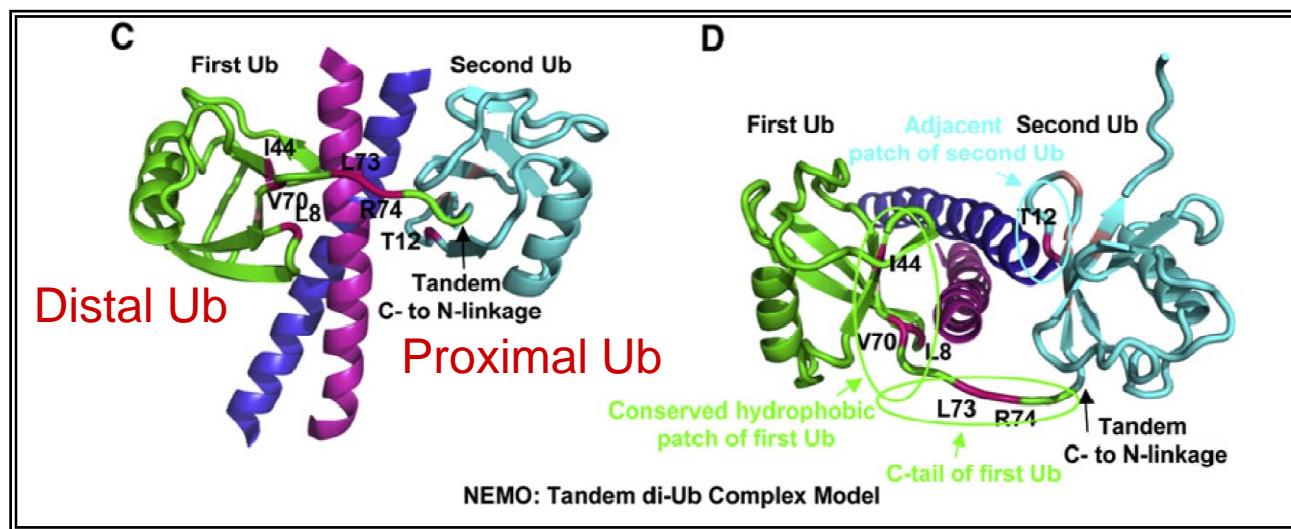
<sup>2</sup>Laboratory of Immune Cell Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA

\*Correspondence: haowu@med.cornell.edu

K63-linked diubiquitin

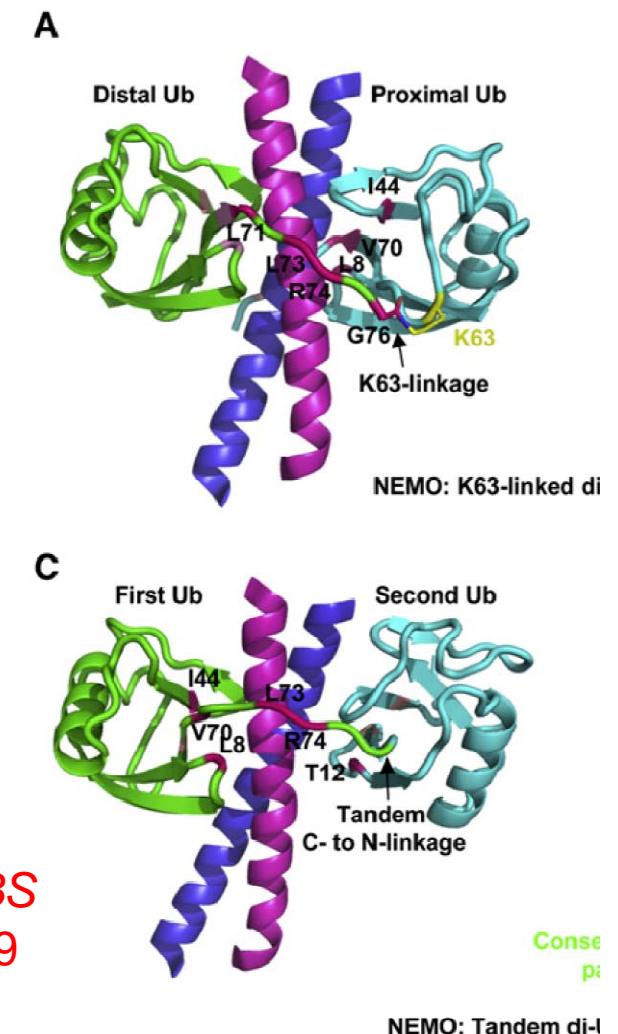
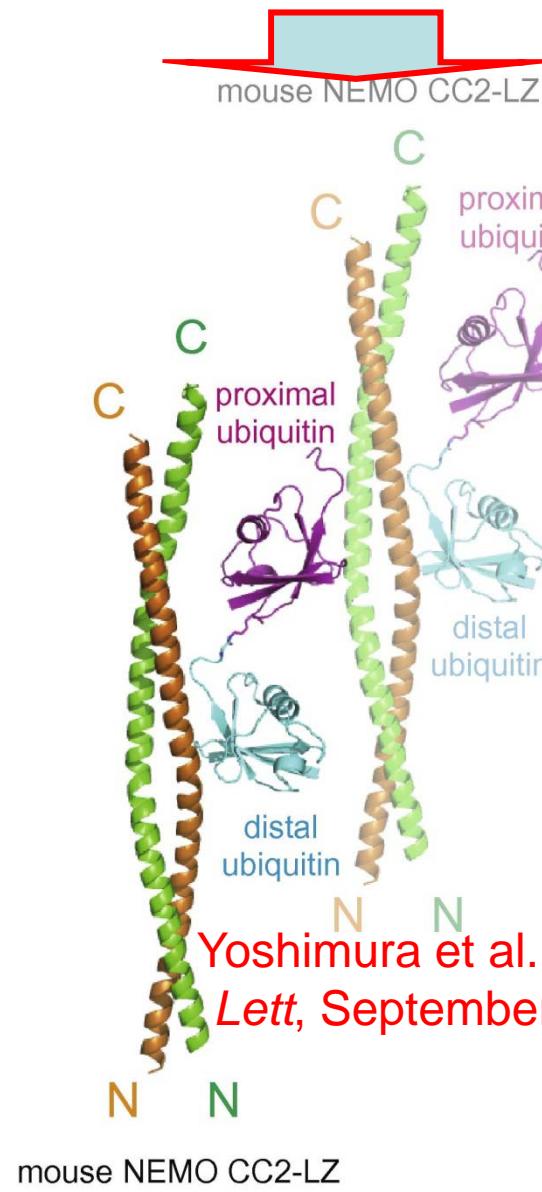
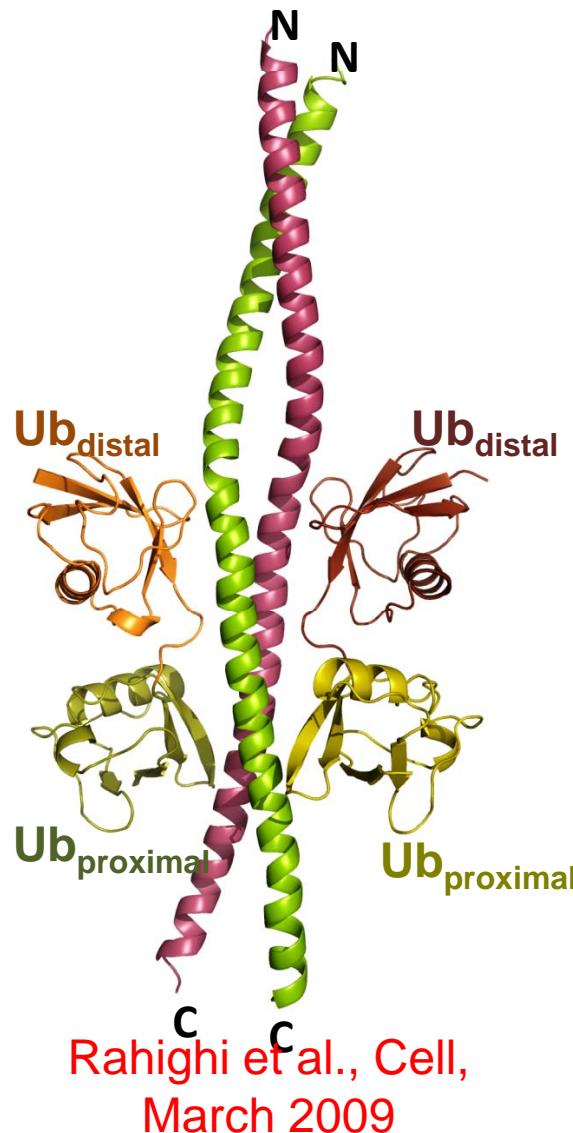


Linear diubiquitin



Lo et al., *Mol Cell*, EPUB 29 Jan, 2009

# K63 and NEMO: 2:1 structure by Fukai's group, FEBS Letters, Sep, 2009



Lo et al., Molecular Cell, January 2009

# Structural Basis for Recognition of Diubiquitins by NEMO

Yu-Chih Lo,<sup>1</sup> Su-Chang Lin,<sup>1</sup> Carla C. Rospigliosi,<sup>1</sup> Dietrich B. Conze,<sup>2</sup> Chuan-Jin David Eliezer,<sup>1</sup> and Hao Wu<sup>1,\*</sup>

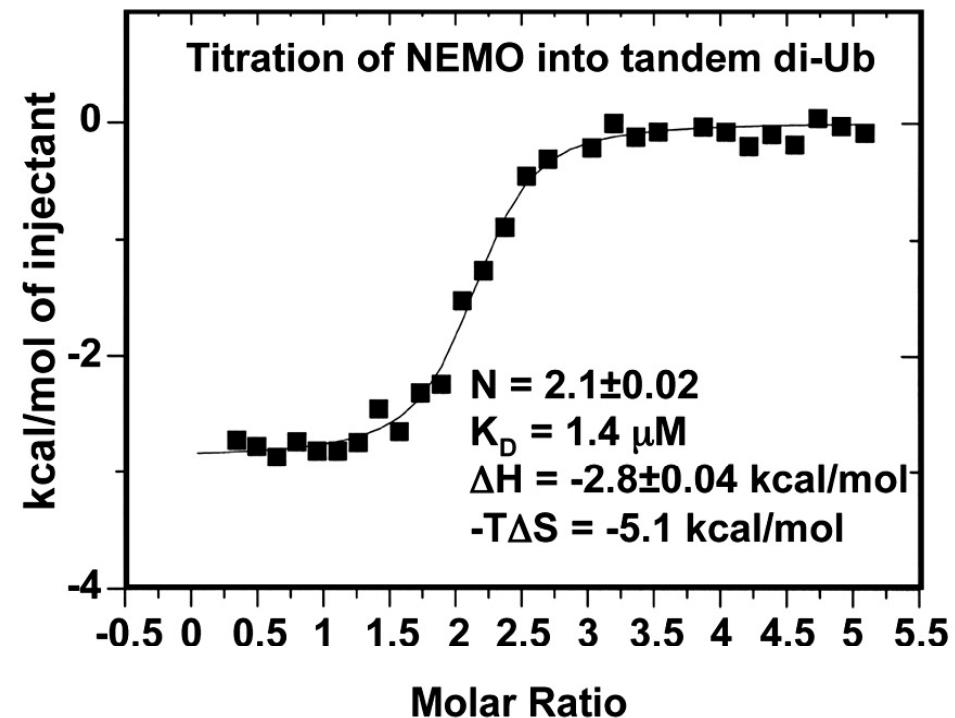
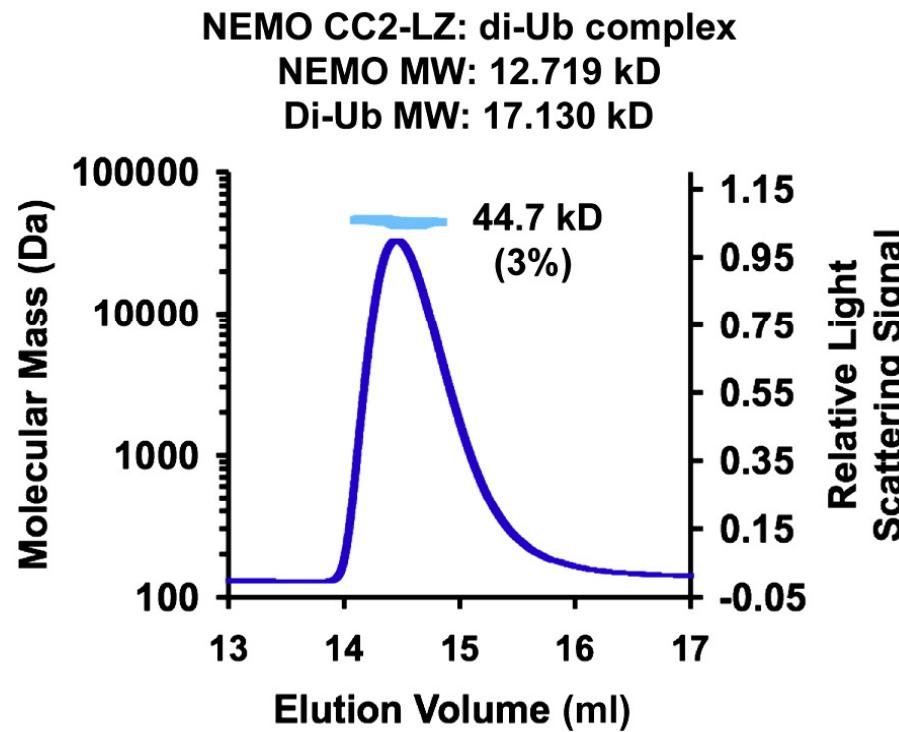
<sup>1</sup>Department of Biochemistry, Weill Cornell Medical College, New York, NY 10021, USA

<sup>2</sup>Laboratory of Immune Cell Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA

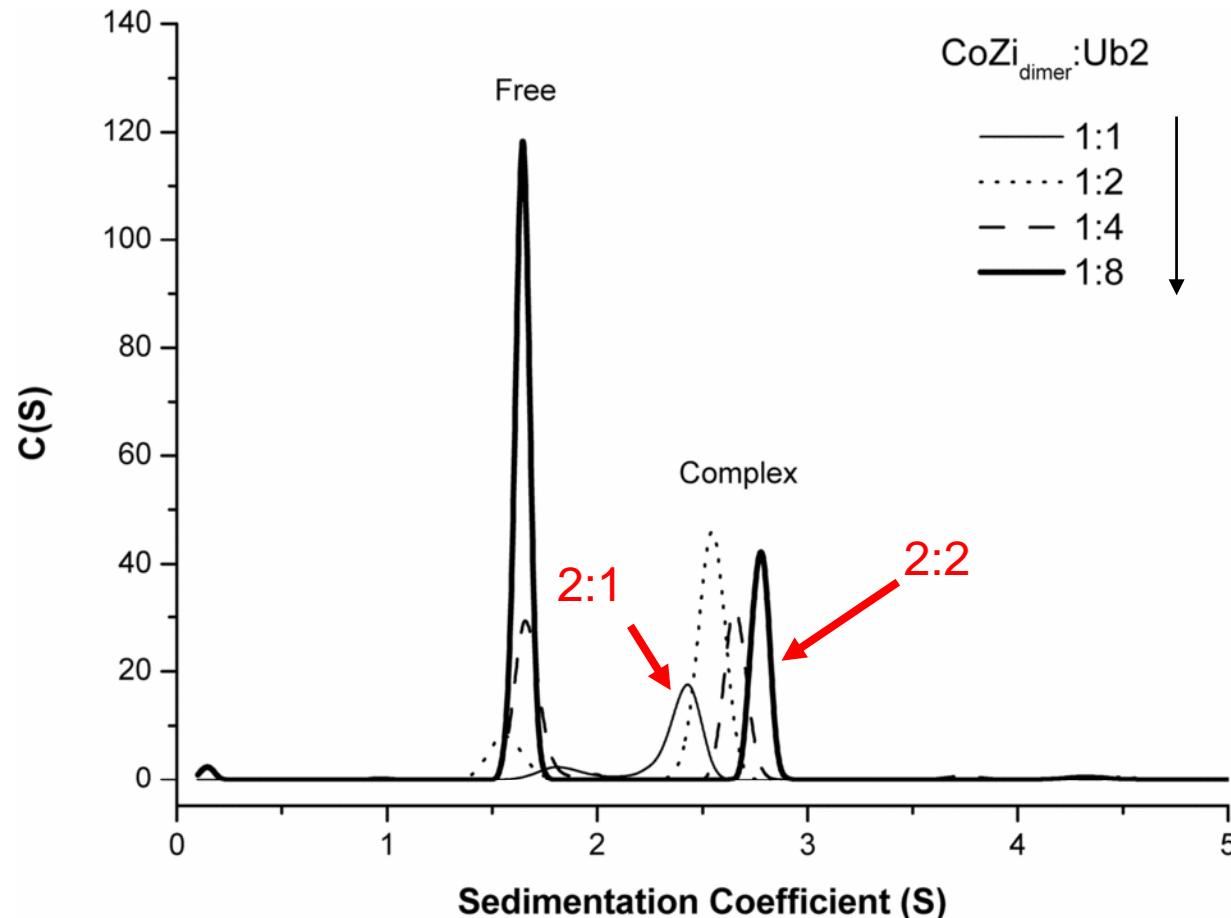
\*Correspondence: haowu@med.cornell.edu

Wu's group  
UBAN:diUb = 2:1

Multi Angle Light Scattering (MALS) and ITC,  
ITC: later by Ritter and KEK

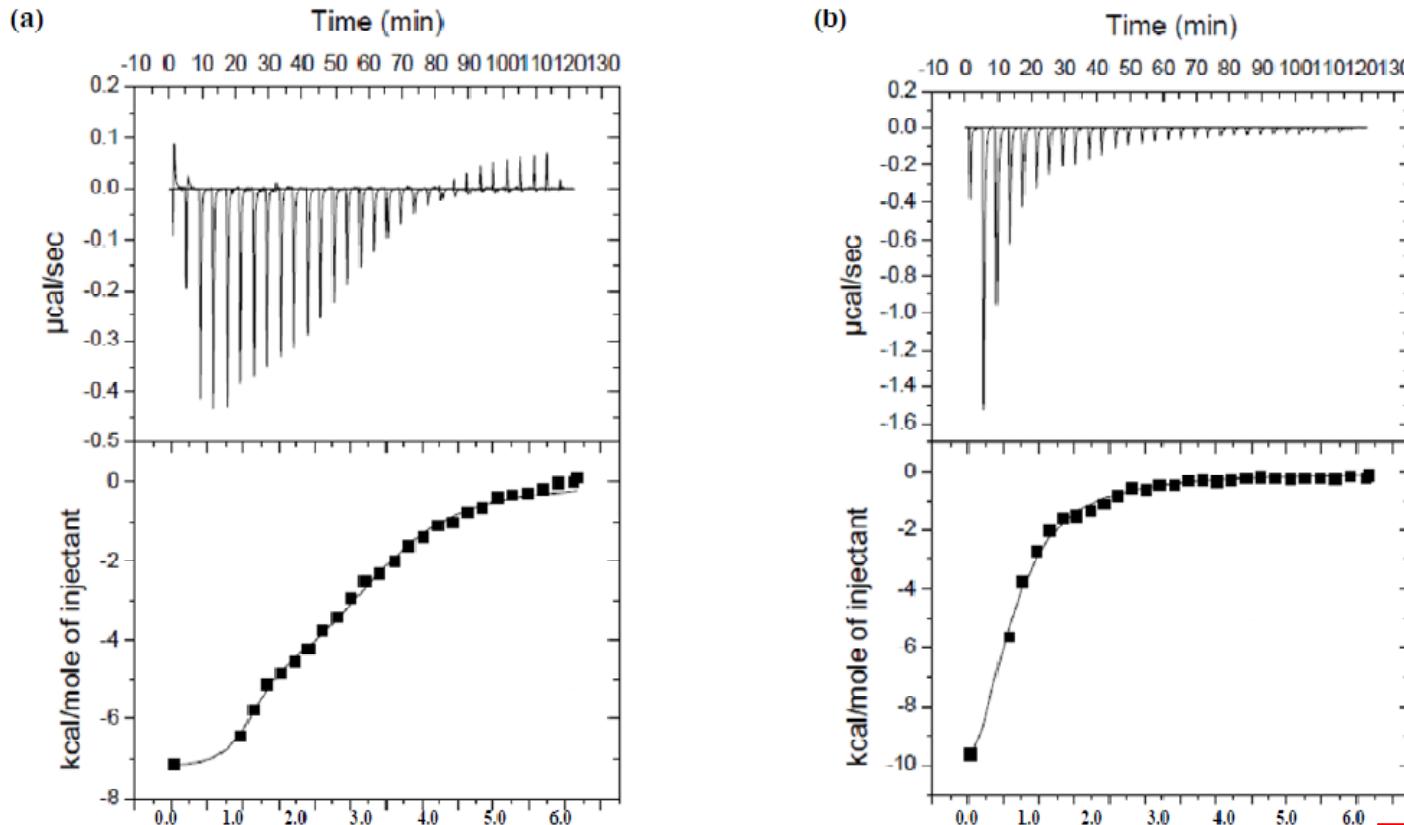


Stoichiometry controversy, continued: sedimentation velocity analysis by Rittinger's group, Ivins et al,  
*Biochemistry J*, 421 243–251, 2009



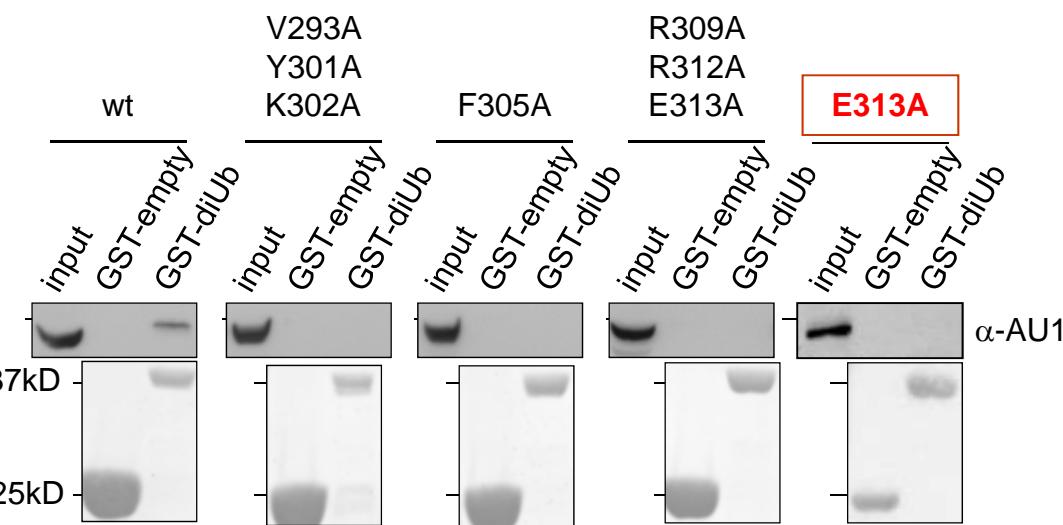
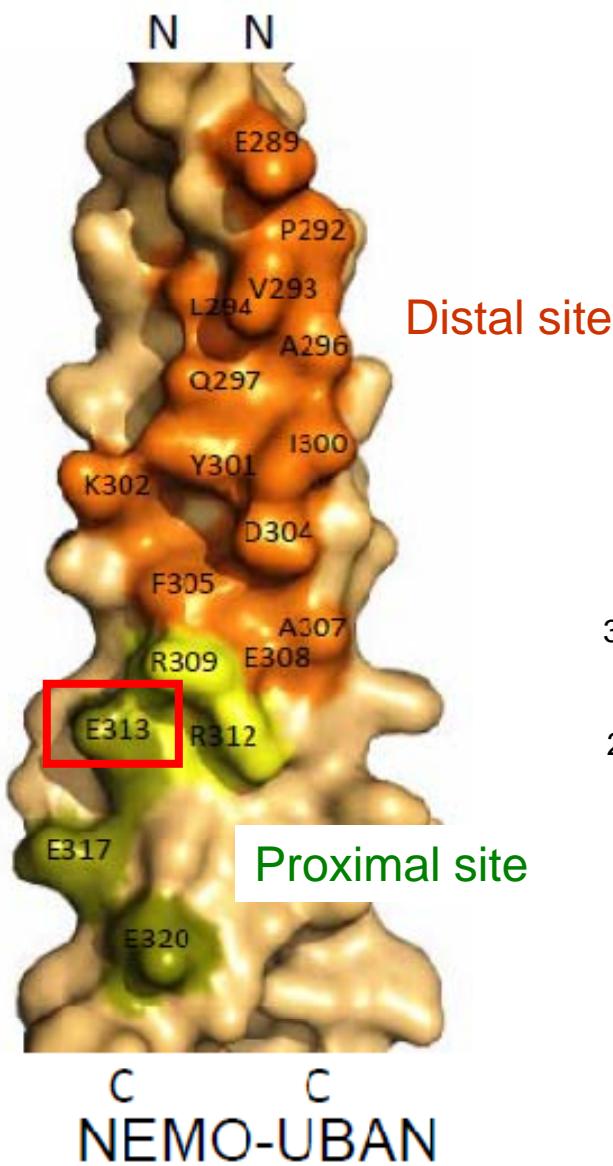
CoZi and di-Ub interact to form **CoZi dimer/diubiquitin 2**. Best fit C(s) functions for sedimentation velocity analysis of CoZi/Ub2 interaction(s).

# Isothermal calorimetry shows both 2:1 & 2:2

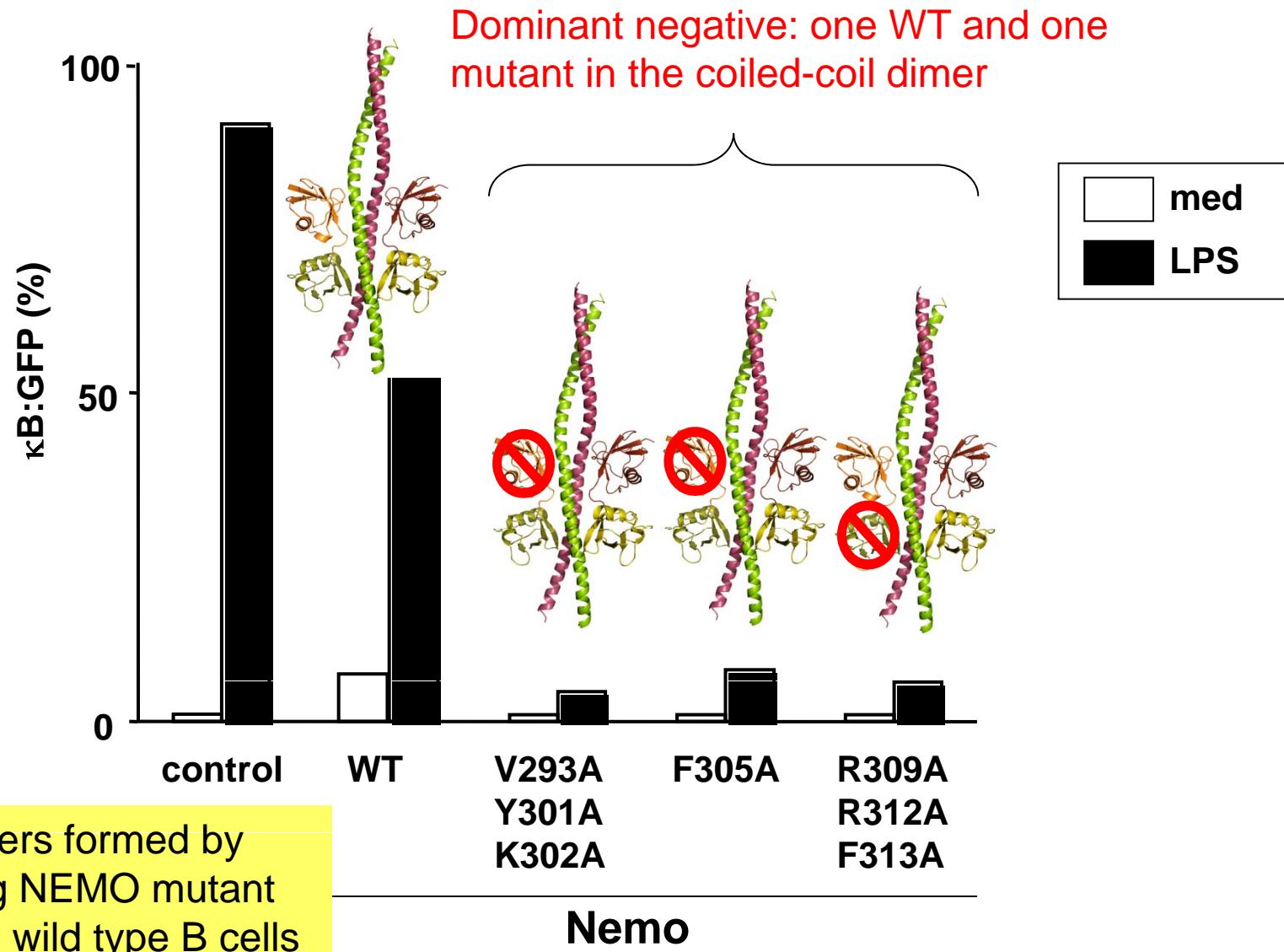


Injecting Component (mM)	Component in Cell (mM)	T	Fitting model	N	$K_a$ ( $\mu\text{M}$ )	$\Delta H$ (kcal/mol)	Stoichiometry NEMO CoZi/diUb
NEMO CoZi (0.4)	LineardiUb (0.015)	30°C	Two set of sites	$N_1: 1.05 \pm 0.06$ $N_2: 1.83 \pm 0.05$	$K_{a1}: 16.30 \pm 9.24$ $K_{a2}: 0.30 \pm 0.05$	$\Delta H_1: -7.24 \pm 0.17$ $\Delta H_2: -5.26 \pm 0.42$	2:2 2:1
LineardiUb (0.4)	NEMO CoZi (0.015)	30°C	One set of sites	$N: 0.52 \pm 0.03$	$K_a: 0.19 \pm 0.02$	$\Delta H: -16.38 \pm 1.73$	2:1

# Purely proximal E313A NEMO mutation

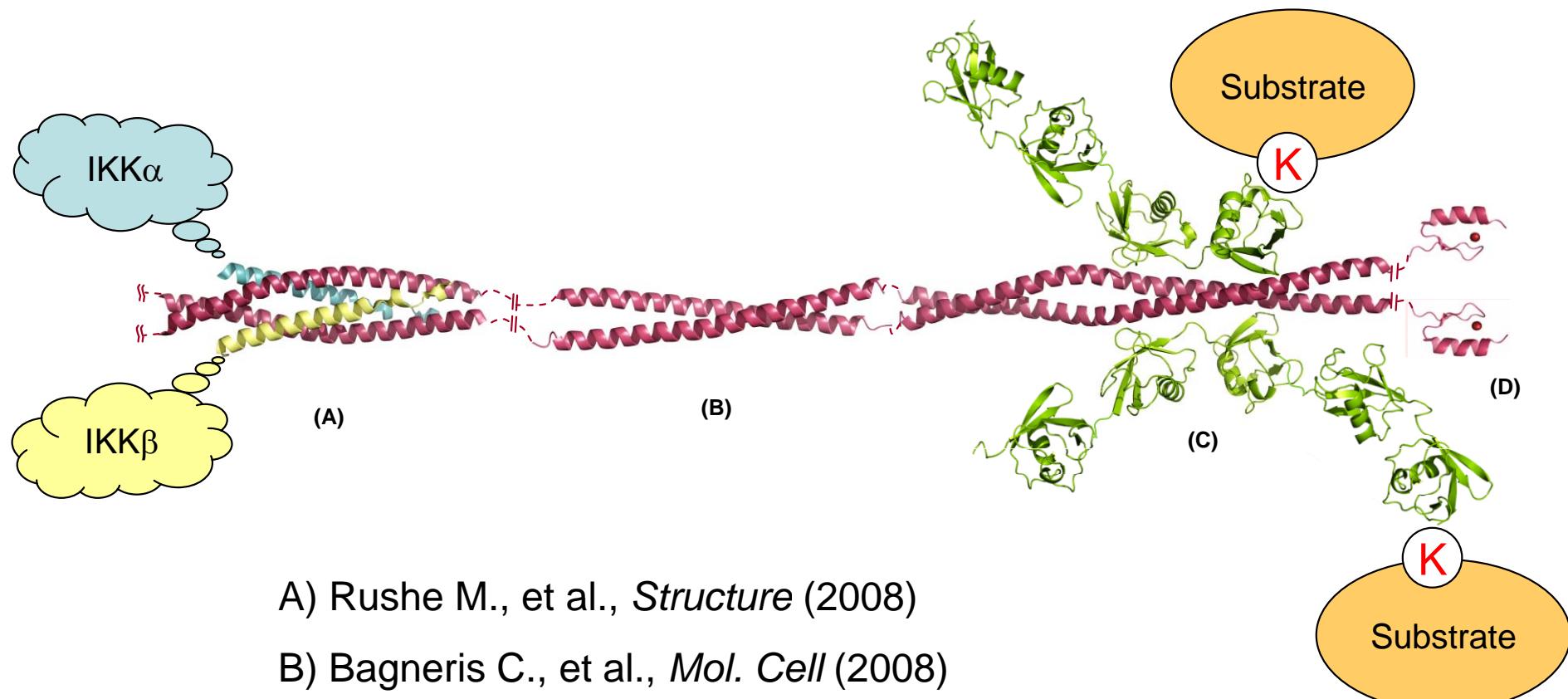


# Linear-diUb recognition of NEMO is critical for LPS-induced NF- $\kappa$ B activation in vivo



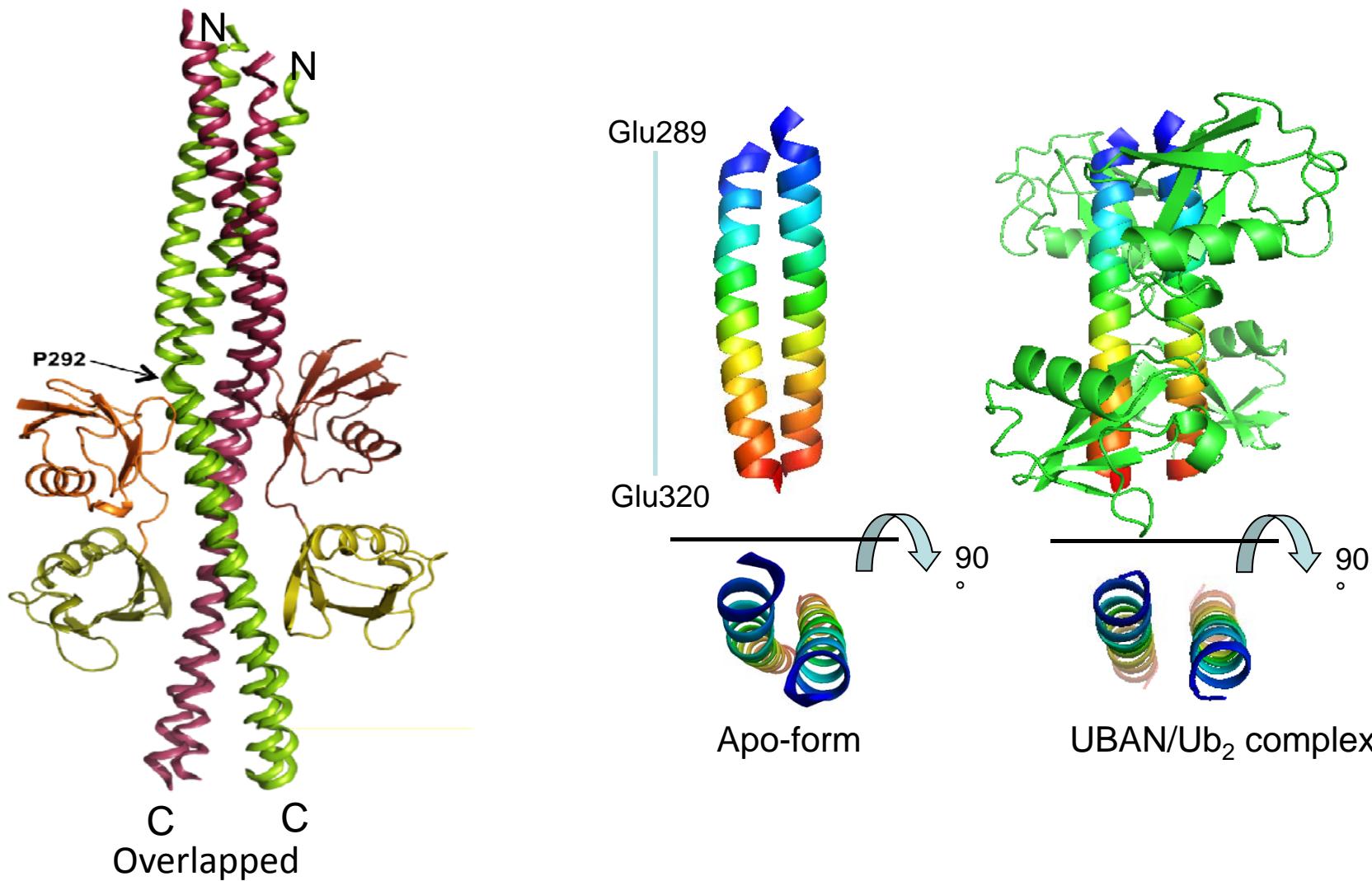
# NEMO is a long alpha helical dimer

## How does Ub binding activate IKK $\alpha$ and IKK $\beta$ ?

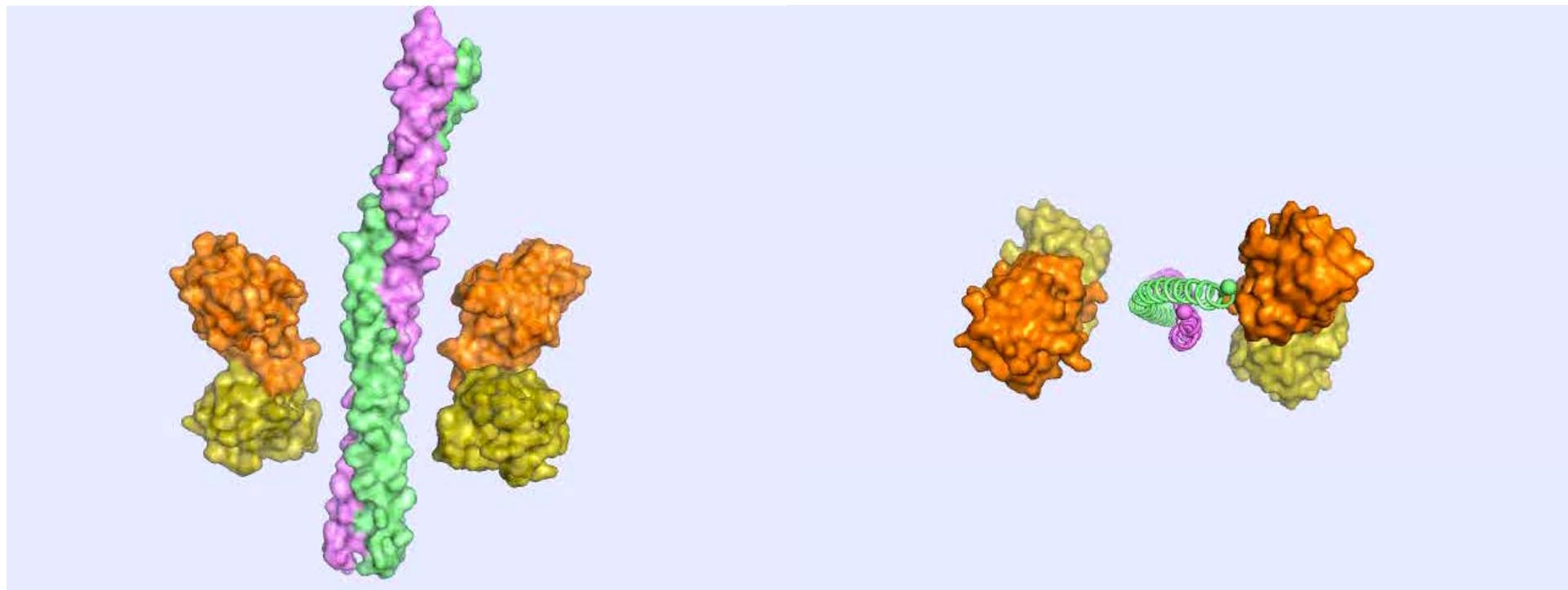


- A) Rushe M., et al., *Structure* (2008)
- B) Bagneris C., et al., *Mol. Cell* (2008)
- C) Current work
- D) Cordier F., et al., *J. Mol. Biol.* (2008)

# Conformational change in NEMO upon binding to linear diubiquitin chains

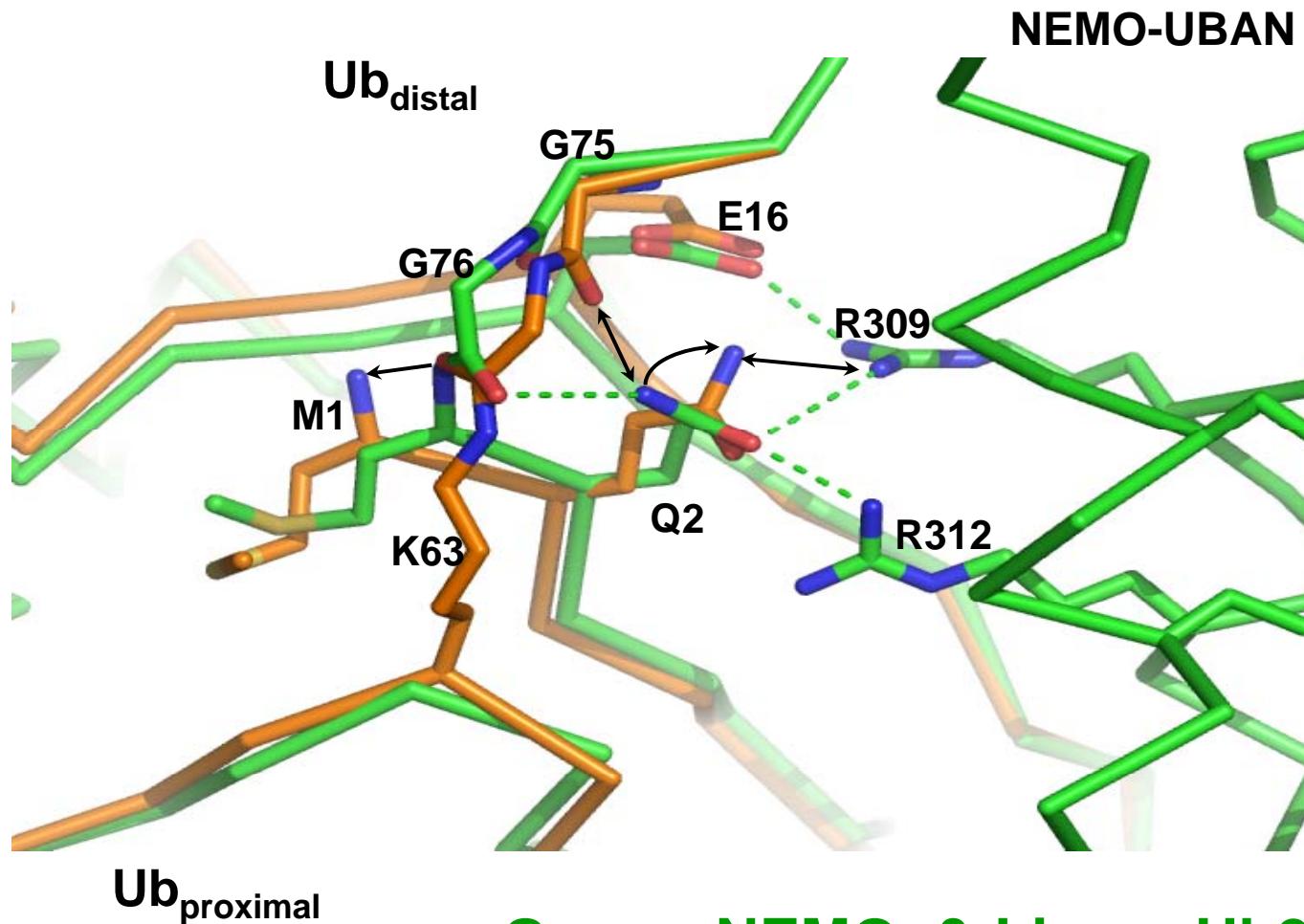


Binding of two linear ubiquitin chains unwinds,  
slightly, the NEMO-UBAN domain  
⇒ may lead to structural change of the entire NEMO—

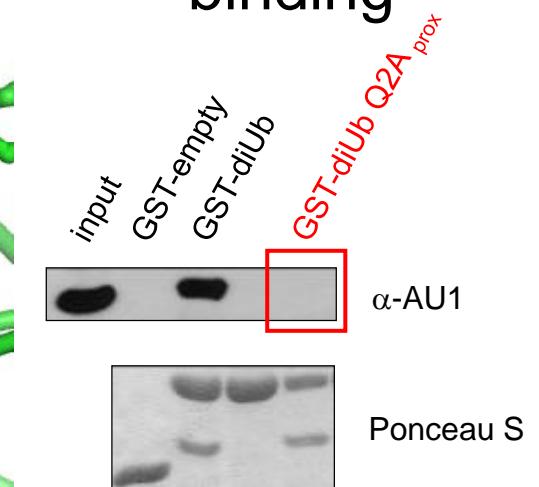


Rahigi & Ikeda et al., Cell, online publication: March 20, 2009  
In collaboration with Ivan Dikic, Goethe Univ. Frankfurt,  
David Komander & Felix Rando, MRC Cambridge

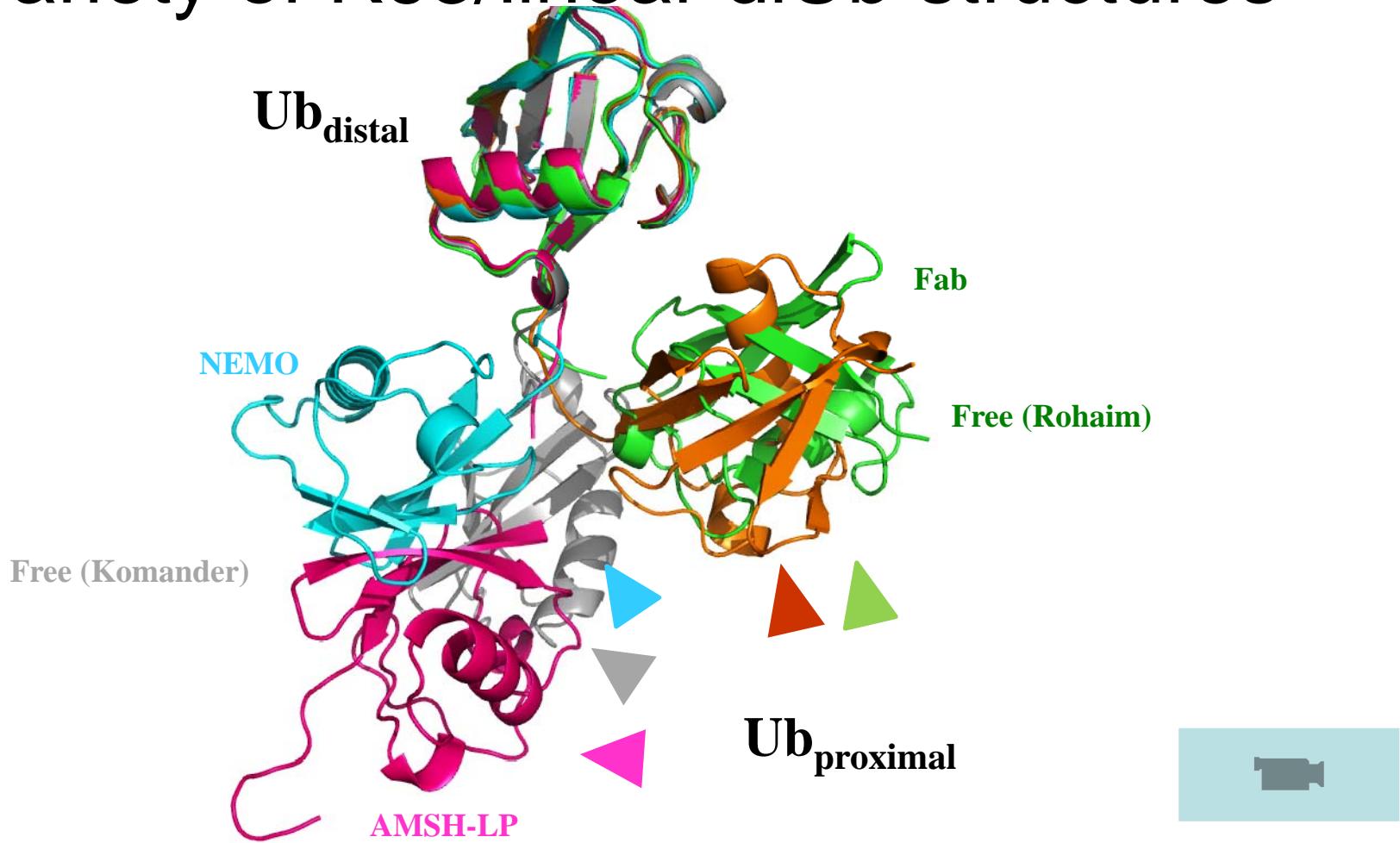
# Specific interaction of NEMO-UBAN to linear vs. K63-linked diubiquitin linkages



Q2A mutation in proximal Ub disrupts NEMO binding



# Variety of K63/linear diUb structures



K63 diUb (Free K63): Komander et al., *EMBO Reports*, 2009

K63 diUb (Fab): Newton et al., *Cell*, 2008

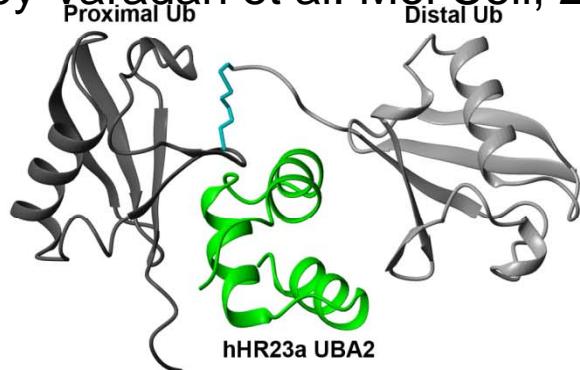
K63 diUb (AMSH-LP): Sato et al., *Nature*, 2008

Linear diUb (NEMO): Rahighi et al., *Cell*, 2009

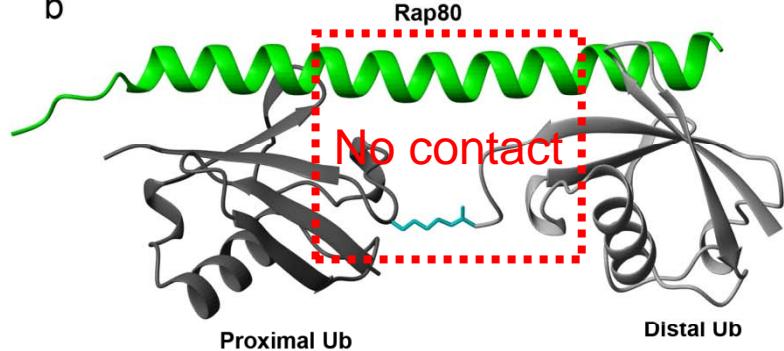
Linear diUb: Rohaim et al. (unpublished)

## K48 diubiquitin

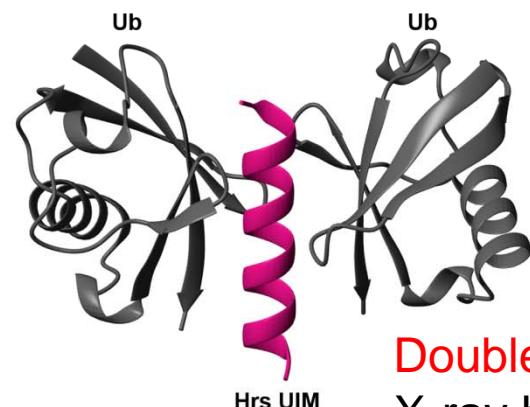
NMR by Varadhan et al. Mol Cell, 2005  
a



b



d

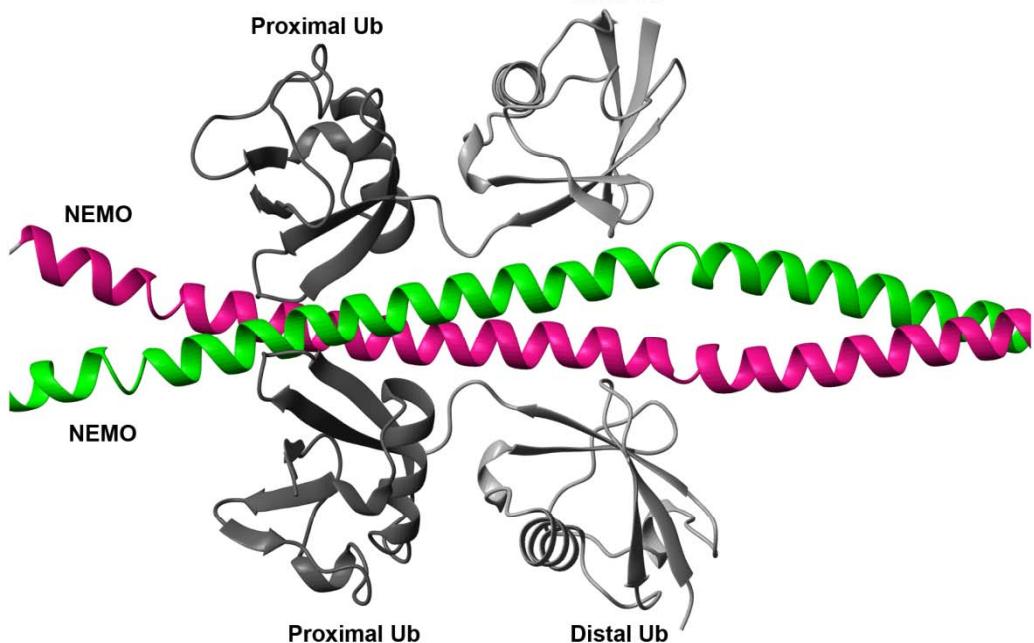


## Double sided UIM

X-ray by Hirano et al., NSMB, 2005

## Linear diubiquitin

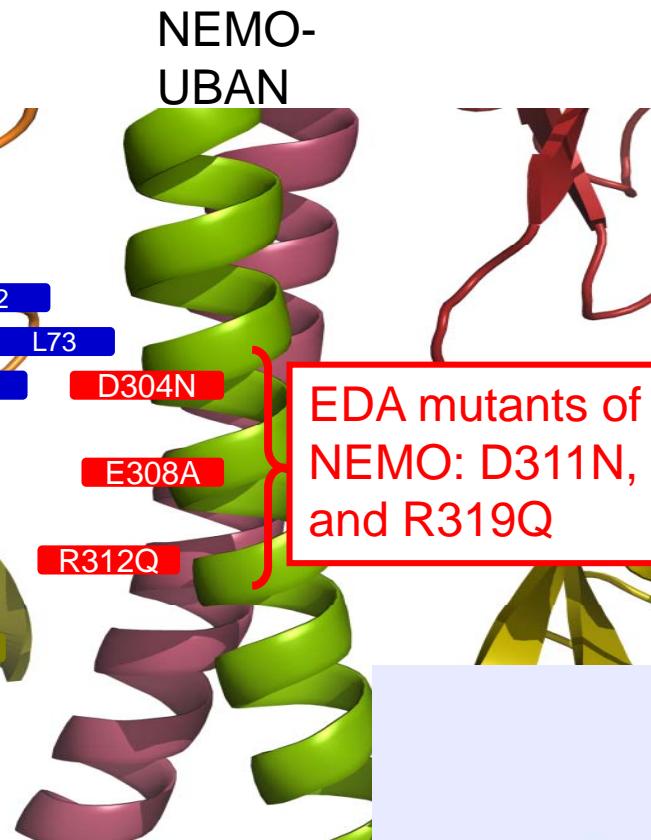
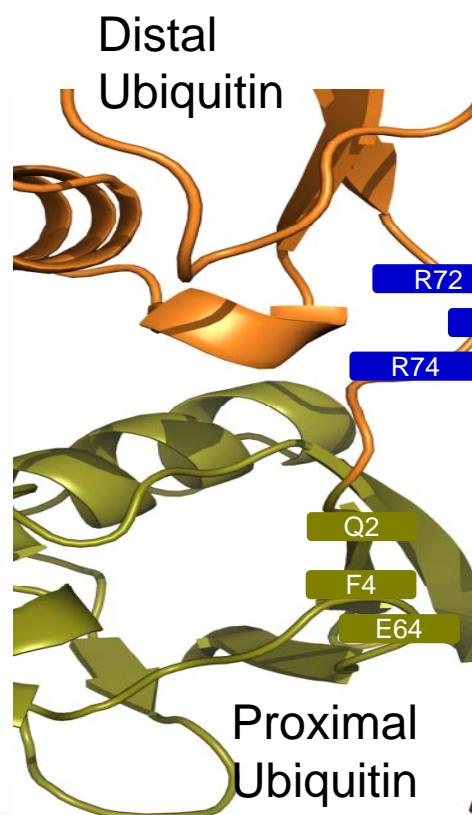
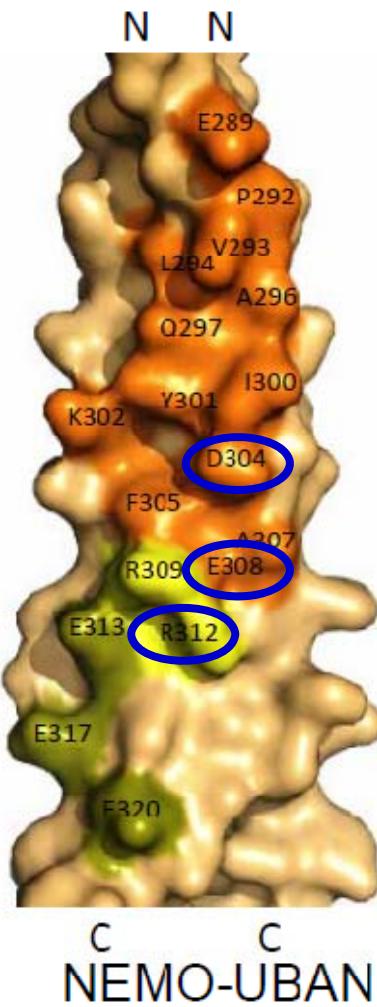
X-ray by Rahighi et al., Cell, 2009



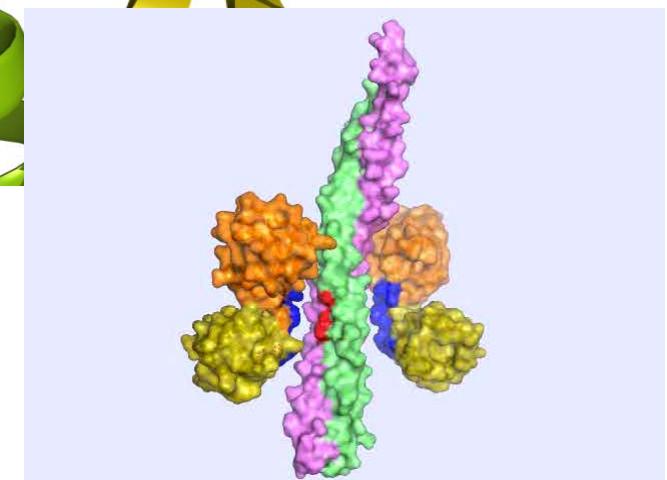
## K63 diubiquitin

Model by Sims & Cohen, Mol Cell 2009  
X-ray by Sato et al., EMBO J., advance online publication 18 June 2009

# Mutations in NEMO in patients suffering from ectodermal dysplasia with immunodeficiency

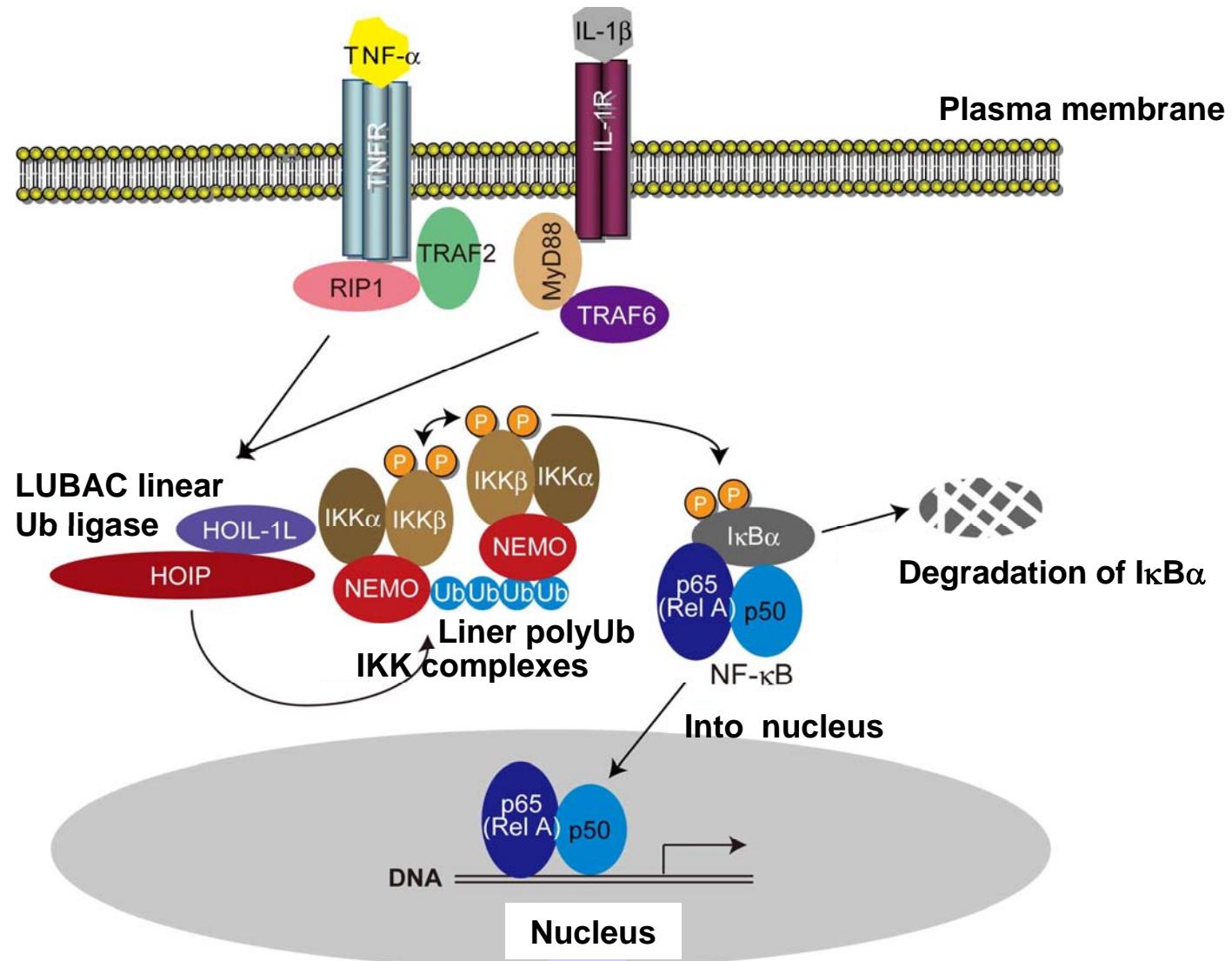


EDA mutants of human NEMO: D311N, E315A and R319Q



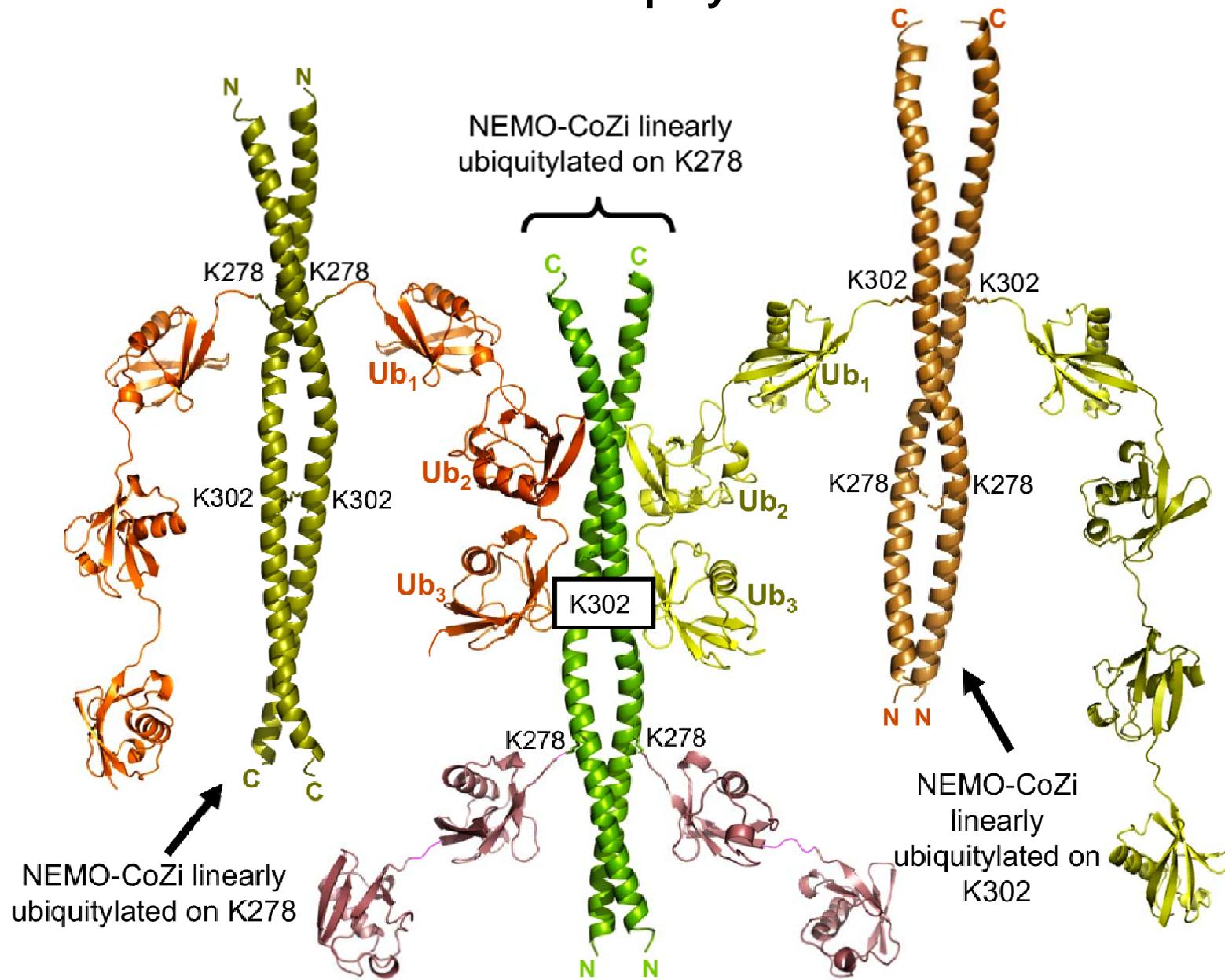
# Model of Ub signaling in the NF-κB pathway

## New paradigm in ubiquitin signaling in the NF-κB pathway: linear ubiquitin recognition

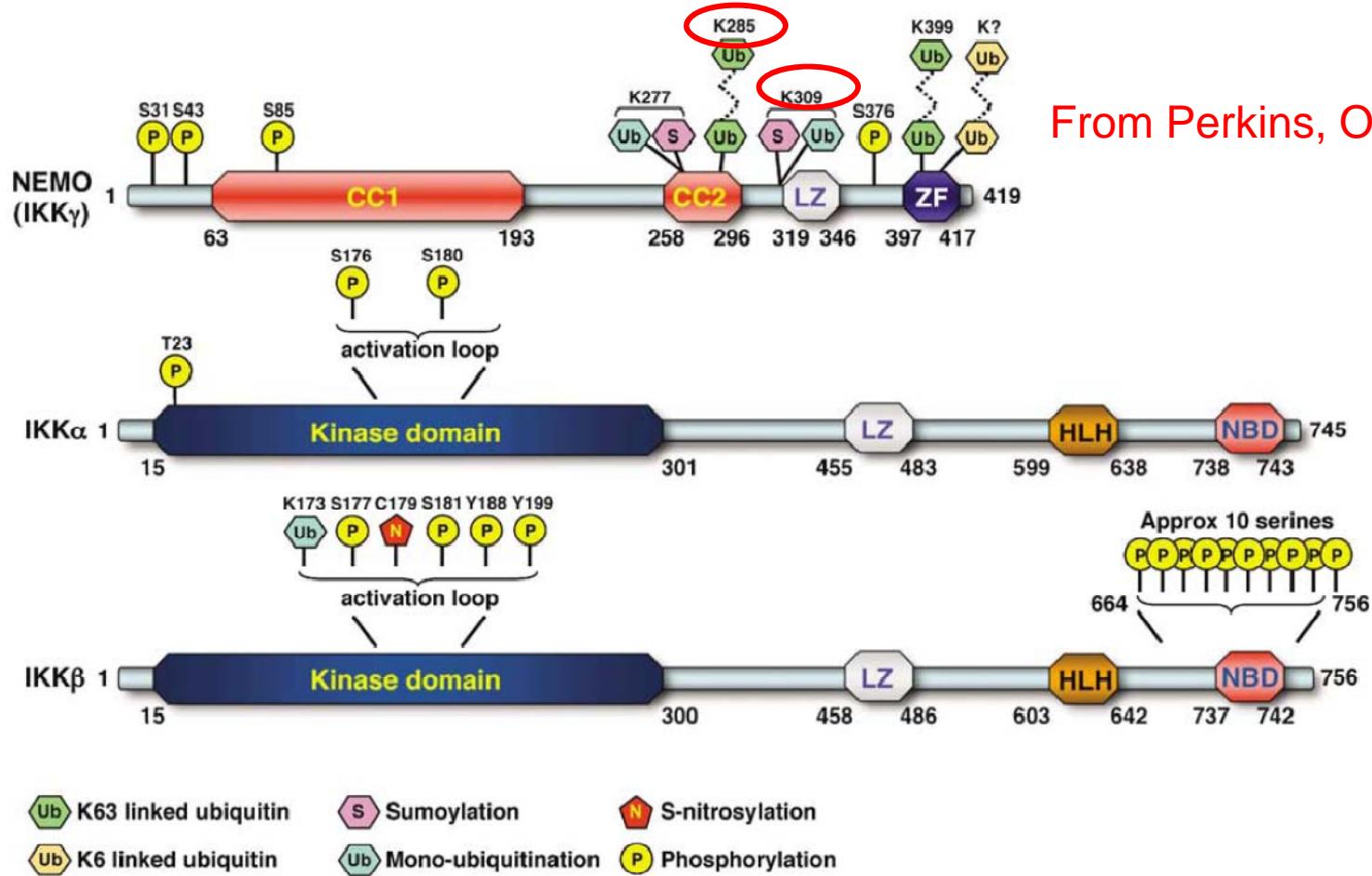




# A model for the NEMO linear Ubiquitin binding and ubiquitylation



# Complex patterns of posttranslational modifications of IKK complex

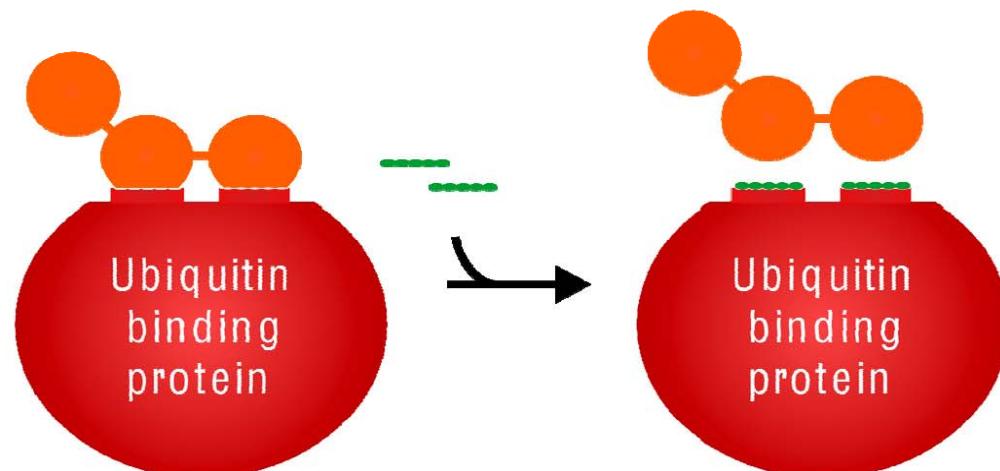


**Figure 1** Post-translational modifications and structure of the IKK subunits. Shown are schematic diagrams of the principal structural motifs of NEMO, IKK $\alpha$  and IKK $\beta$  in relation to the known post-translational modifications that regulate their activity. It is possible that IKK $\alpha$  may undergo similar modifications to those seen in IKK $\beta$ , but these have not yet been described in the literature. Phosphorylations at the C-terminus of IKK $\beta$  are indicated as being approximately 10, since the exact number were not definitively established by the original mutagenesis studies (Delhase *et al.*, 1999). Numbering is from the human proteins, although some definitions of where a domain begins and ends might differ between publications. Abbreviations: CC1 and CC2, coiled coil regions 1 and 2; LZ, leucine zipper motif; ZF, zinc-finger domain; HLH, helix-loop-helix domain; NBD, NEMO-binding domain. The code for the different modifications is shown in the figure.

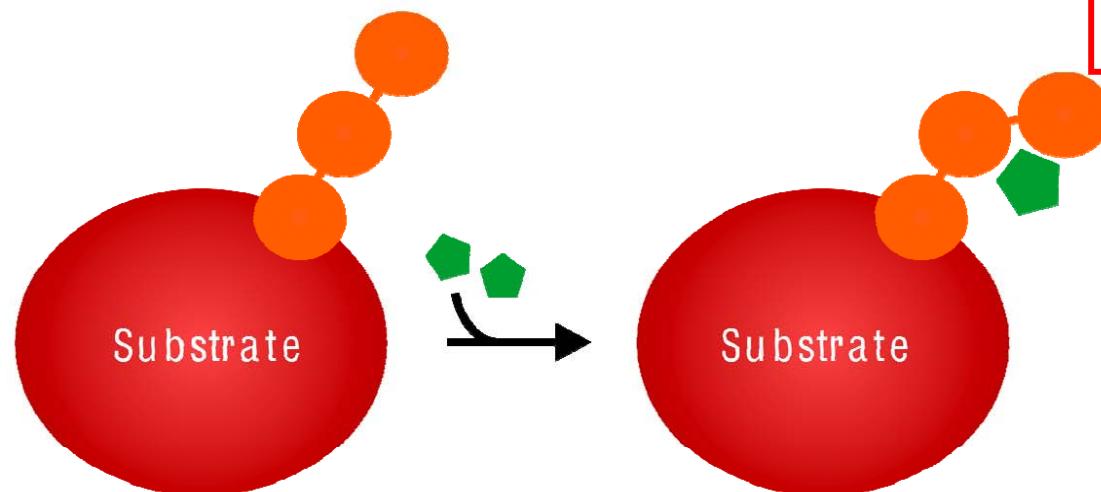
From Perkins, Oncogene, 2006

# Drug design targets

A

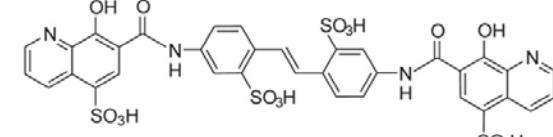


B

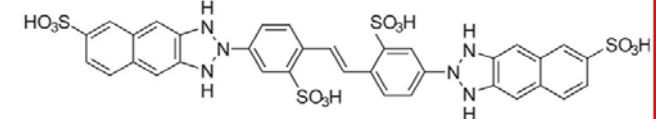


Targeting ubiquitin itself with **ubistatins**: Verma et al, R.W. King, Science, 2004

C92 (Ubistatin A)

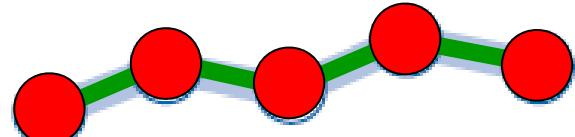


C59 (Ubistatin B)



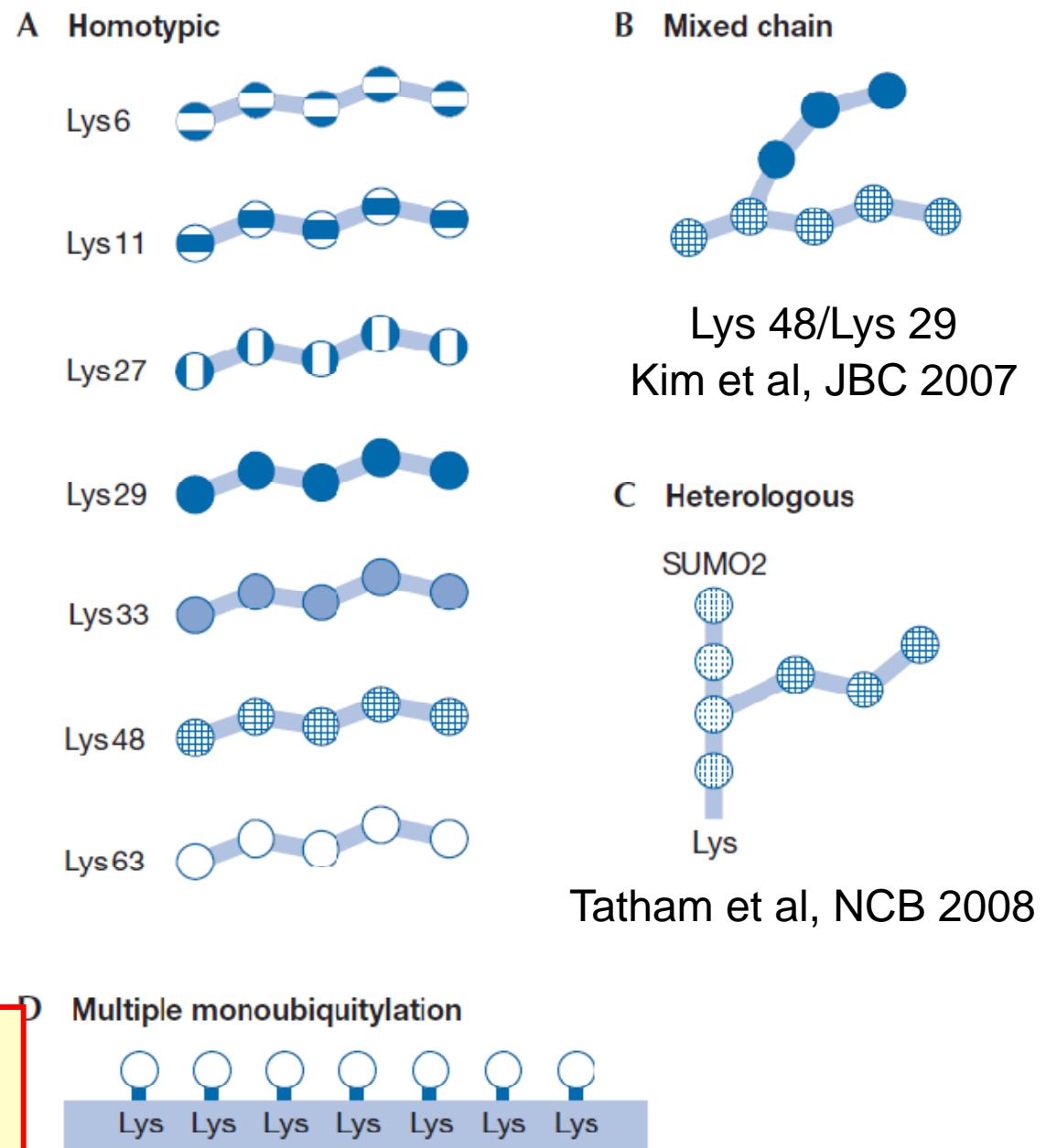
Drug  
 Ubiquitin

Plethora of  
ubiquitin chains  
may lead to  
numerous  
biological signals  
⇒ New ubiquitin  
world  
Linear!



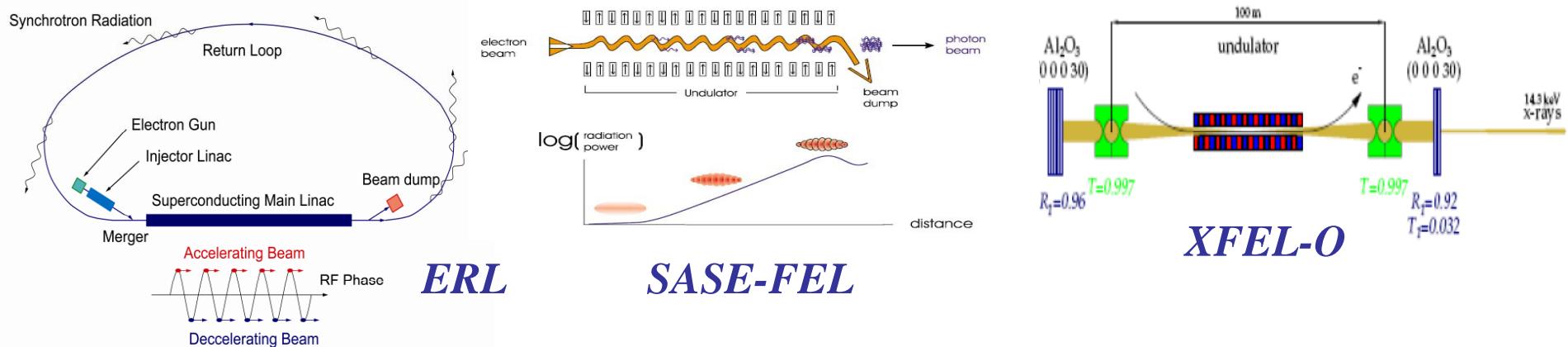
Tokunaga, Iwai et al.,  
NCB, 2009

Previously:  $7 \times 7 \times 7 \times 7 \times 7 = 16807$   
With linear:  $8 \times 8 \times 8 \times 8 \times 8 = 32768$



Taken from Ikeda & Dikic, EMBO Reports, 9, 536-542 (2008)

# Comparison of ERL, SASE-FEL and XFEL-O

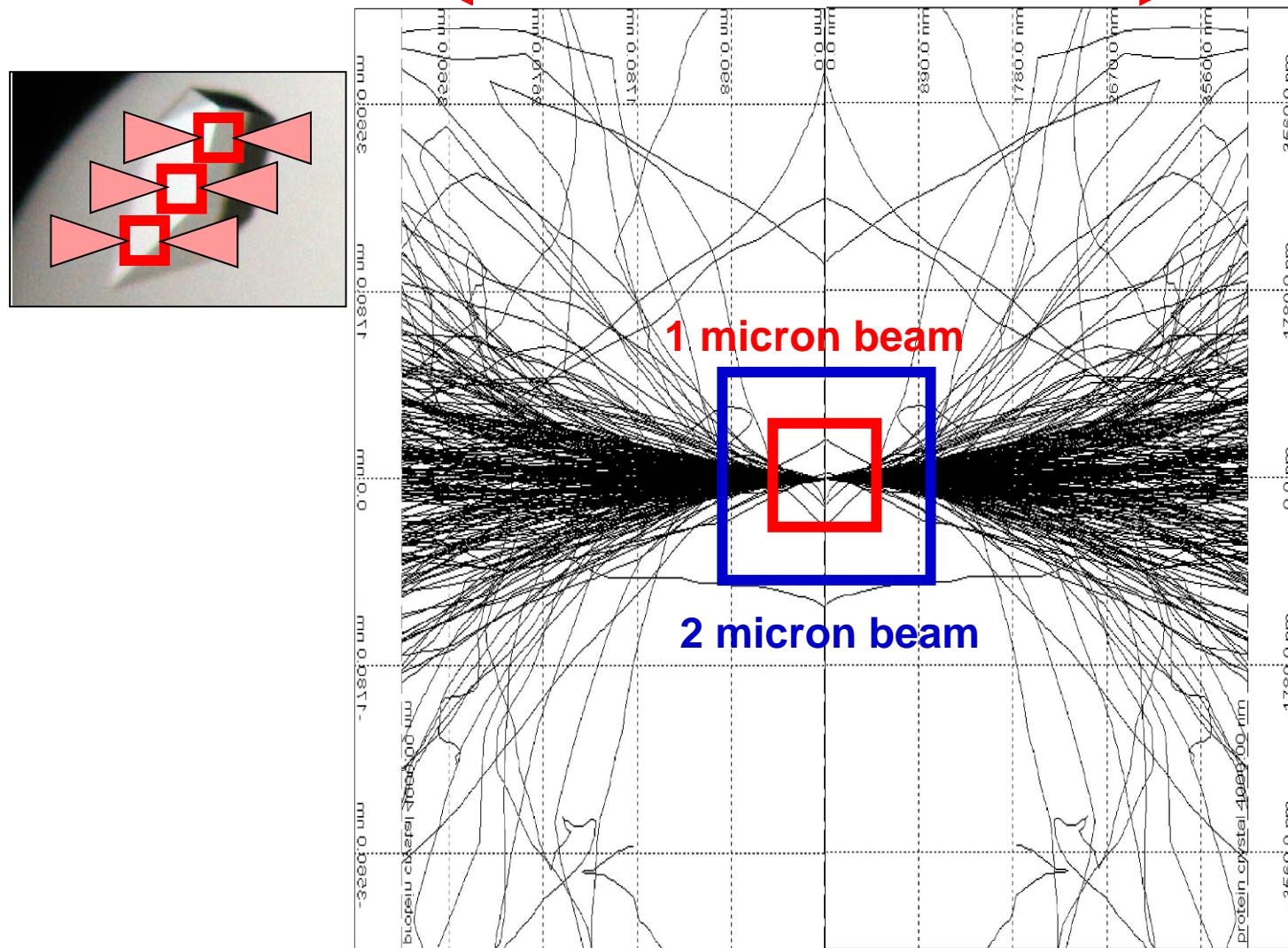


	average brilliance	peak brilliance	repetition rate (Hz)	coherent fraction	bunch width(ps)	# of BLs	Remark
<b>ERL</b>	$\sim 10^{23}$	$\sim 10^{26}$	<b>1.3G</b>	$\sim 20\%$	<b>0.1~1</b>	$\sim 30$	Non-perturbed measurement
<b>XFEL-O (Option)</b>	$\sim 10^{27}$	$\sim 10^{33}$	$\sim 1M$	<b>100%</b>	<b>1</b>	<b>few</b>	Single mode FEL
<b>SASE-FEL</b>	$\sim 10^{22\sim 24}$	$\sim 10^{33}$	<b>100~10K</b>	<b>100%</b>	<b>0.1</b>	<b>~1</b>	One-shot measurement
<b>3<sup>rd</sup>-SR</b>	$\sim 10^{20\sim 21}$	$\sim 10^{22}$	$\sim 500M$	<b>0.1%</b>	<b>10~100</b>	$\sim 30$	Non-perturbed measurement

(brilliance : photons/mm<sup>2</sup>/mrad<sup>2</sup>/0.1%/s @ 10 keV)

# Radiation damage: photoelectrons escape sideways.

Polarization direction of the SR



Will reduced radiation damage occur with very small crystals?  
Colin Nave and Mark A. Hill, J. Synch. Rad. (2005). 12, 299–303

# New Ubiquitin World with Accelerator Technologies

Analysis of receptors upon agonist binding

Neutron reflectivity  
GI-SANS & GI-SAXS

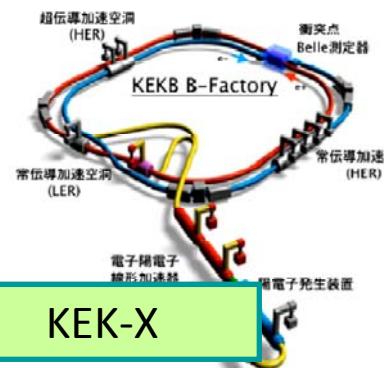
NF- $\kappa$ B pathway

Macroscopic structural changes  $\Leftrightarrow$  function

Hierarchical structure of ubiquitin recognition



Protein Crystallography



SAXS

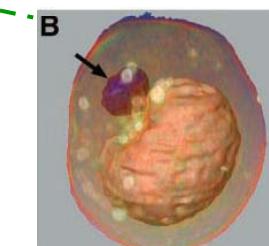
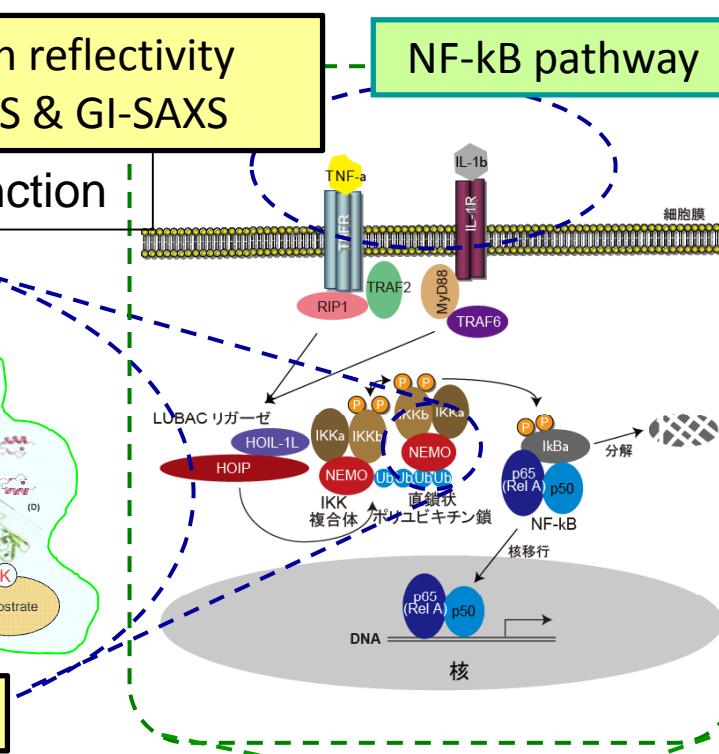
Time resolved solution structural analyses  
of posttranslational modifications & complex formation

Accelerate developments of drugs

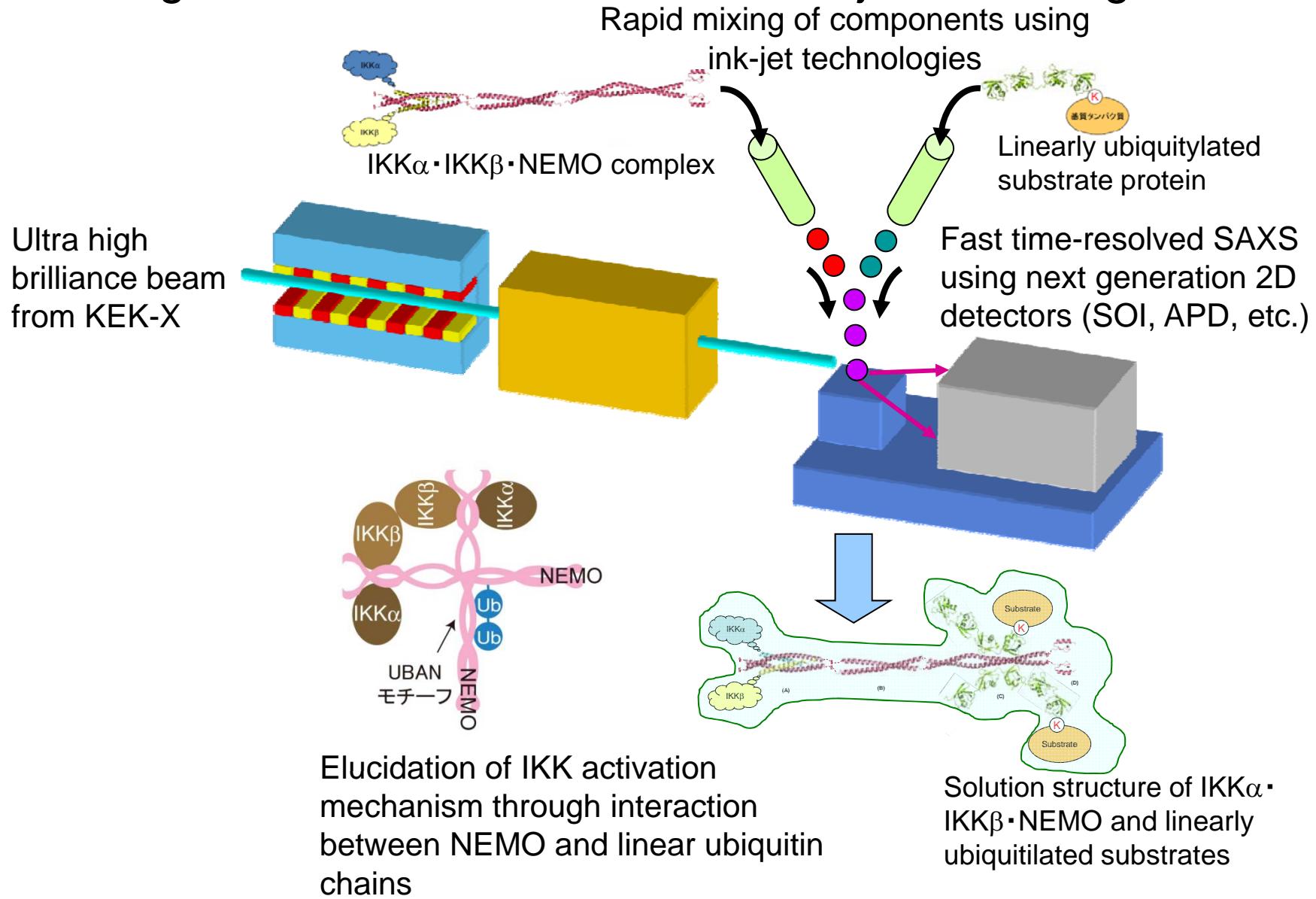
Imaging localization of protein  
complexes with high resolution and  
contrast

SR X-ray tomography

Structural studies of polyubiquitin synthesis & recognition  
In vivo analysis of drug delivery and signal transduction



# Transient structural dynamics of signal transduction complexes: Small Angle X-ray Scattering (SAXS) with next generation 2D detectors and ink-jet technologies



# How large is the protein universe?

- How many genes?
  - Meta genomes --- J. Craig Venter Institute's Global Ocean Sampling Expedition
  - Human microbiomes (Gill, et.al. David Relman, Stanford, Science 2006)
  - Emerging infectious diseases
- Splicing variants – exon: cut and paste
- Non-coding RNA: largely uncharted
- Protein-protein, protein-carbohydrate, protein-lipid, protein-nucleic acid interactions
- Posttranslational modifications

# PSI:Biology of NIGMS/NIH

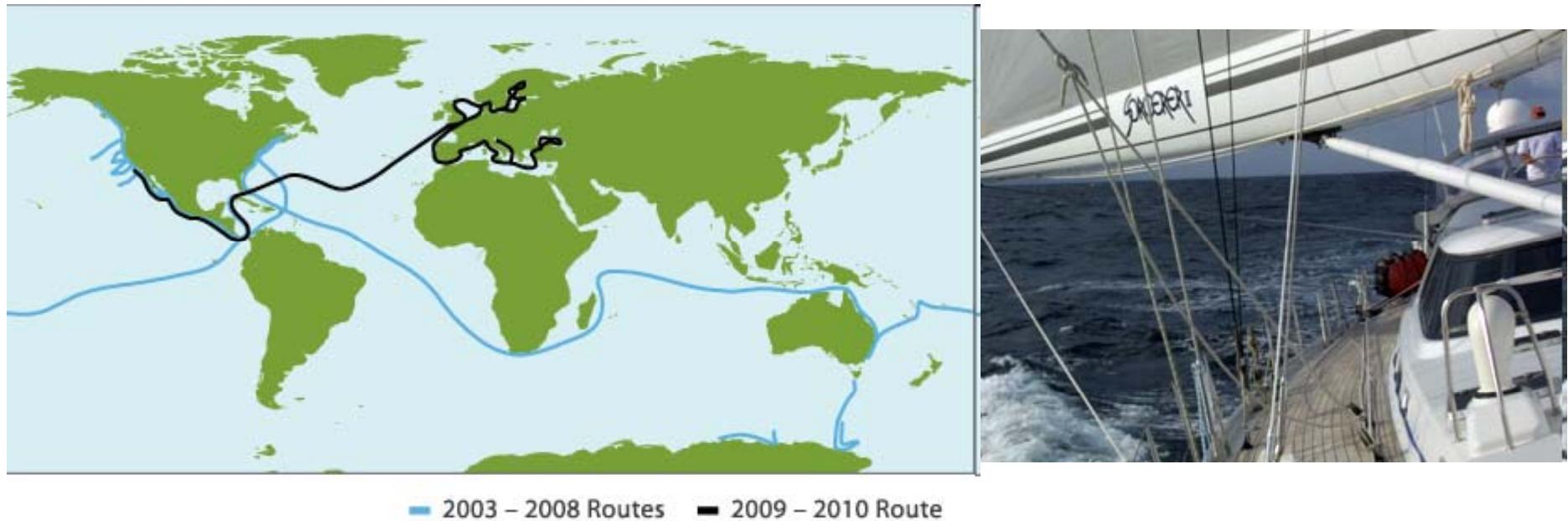
PSI Biology large centers: Applications by Oct 28th

<http://www.nigms.nih.gov/Initiatives/PSI>

1. **Concept Clearance:** High-Throughput Structural Biology  
[http://www.nigms.nih.gov/News/Reports/council\\_concept\\_clearance\\_2009.htm](http://www.nigms.nih.gov/News/Reports/council_concept_clearance_2009.htm)
2. Recommendations for the Future of the PSI  
[<http://www.nigms.nih.gov/News/Reports/PSIAC\\_Future\\_2009.htm>](http://www.nigms.nih.gov/News/Reports/PSIAC_Future_2009.htm) (PSI Advisory Committee)
3. Report of the Future Structural Genomics Initiatives Meeting  
[<http://www.nigms.nih.gov/News/Reports/FutureSGMeeting102008.htm>](http://www.nigms.nih.gov/News/Reports/FutureSGMeeting102008.htm)
4. Annual PSI Advisory Committee Meeting Report  
[<http://www.nigms.nih.gov/News/Reports/PSIAC2008.htm>](http://www.nigms.nih.gov/News/Reports/PSIAC2008.htm)
5. PSI Steering Committee Meeting Report  
[<http://www.nigms.nih.gov/News/Reports/psi\\_steering\\_2008.htm>](http://www.nigms.nih.gov/News/Reports/psi_steering_2008.htm)

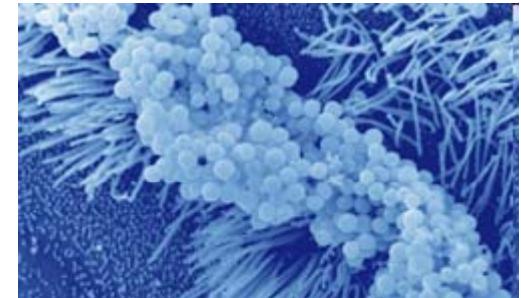
# Global Ocean Sampling Expedition

- Sorcerer II of Craig Venter Institute
- GOS analysis identified some **6.12 million proteins** from 7.7 million newly discovered sequences



# Human microbiomes

- Within the body of a healthy adult, microbial cells are estimated to outnumber human cells by a factor of ten to one.
- Human gut: 1000 microbiomes,  $1 \times 10^{14}$  cells, 1 kg per person, but so far only 40 species have been characterized.
- The NIH Roadmap Initiative now includes a Human Microbiome Project (HMP, <http://nihroadmap.nih.gov/hmp>)
- JCSG (NIGMS PSI) collaboration on structures



# Structural Biology Research Center, Photon Factory, KEK

**Soichi Wakatsuki** Professor, Director of Structural Biology Research Center

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