



16 & 17 October 2008



IMSS Symposium: Structural Biology Research Center

6.5GeV
PF-AR

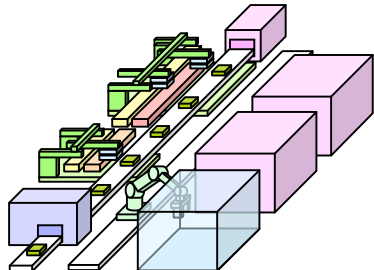
2.5 GeV PF

Soichi Wakatsuki
Photon Factory
Institute of Materials Structure
Science
High Energy Accelerator
Research Organization (KEK)

Structural Biology Research Center

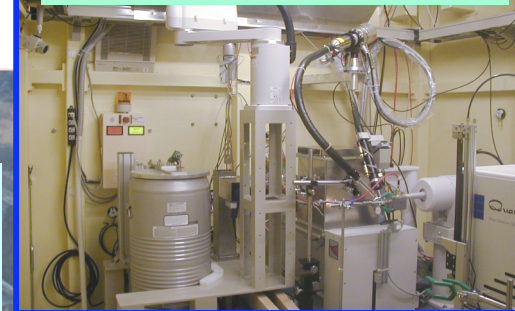
Crystallization

200,000 trials/day



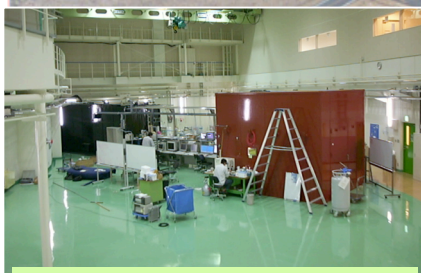
Structural Biology Research Center

Modified SSRL sample changer robot



6.5GeV PF-AR

2.5GeV PF ring



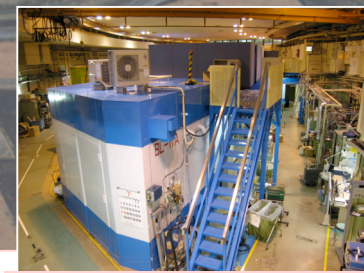
NW12A (2003)



BL-6A



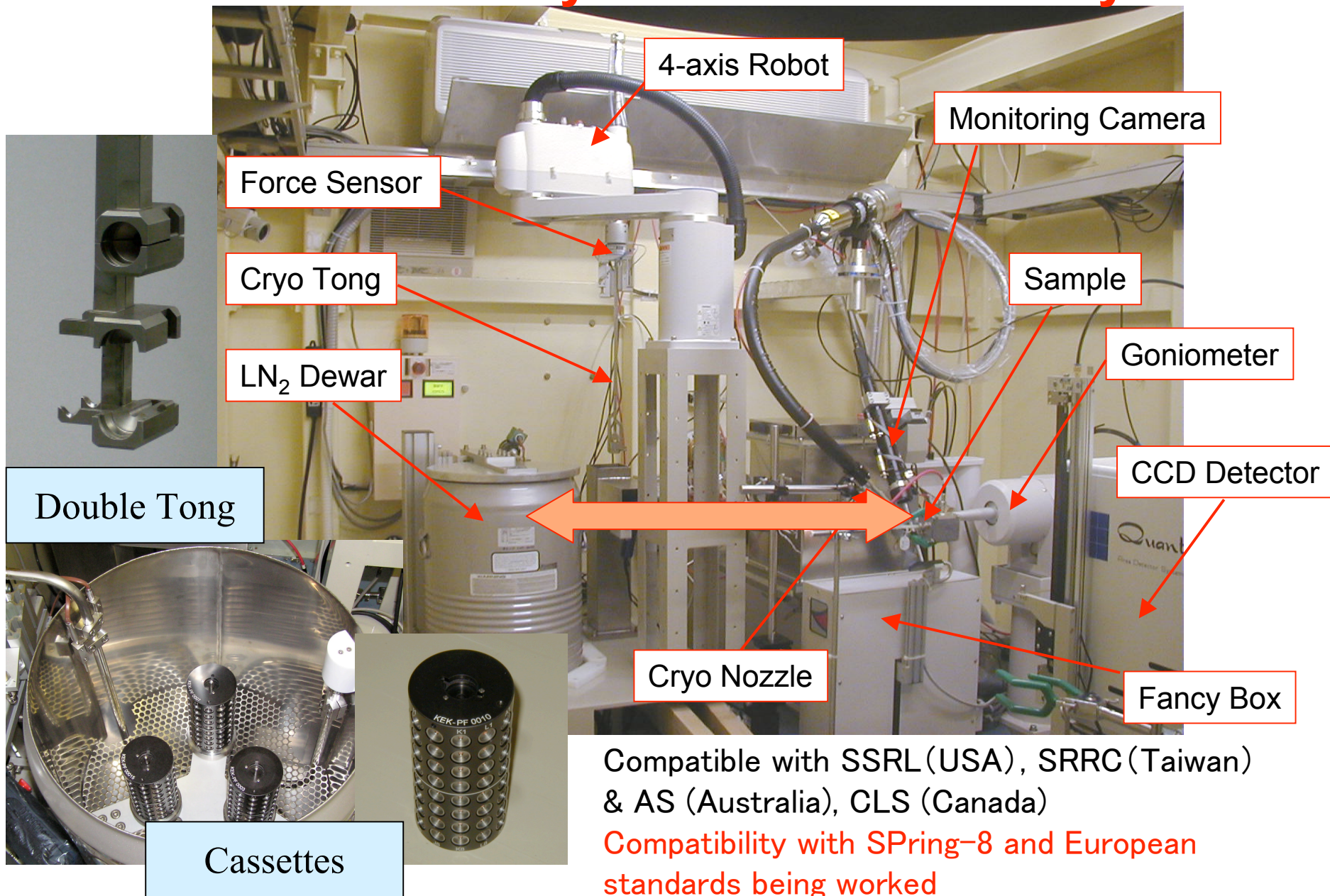
BL-5A (2004)



BL-17A (2006)

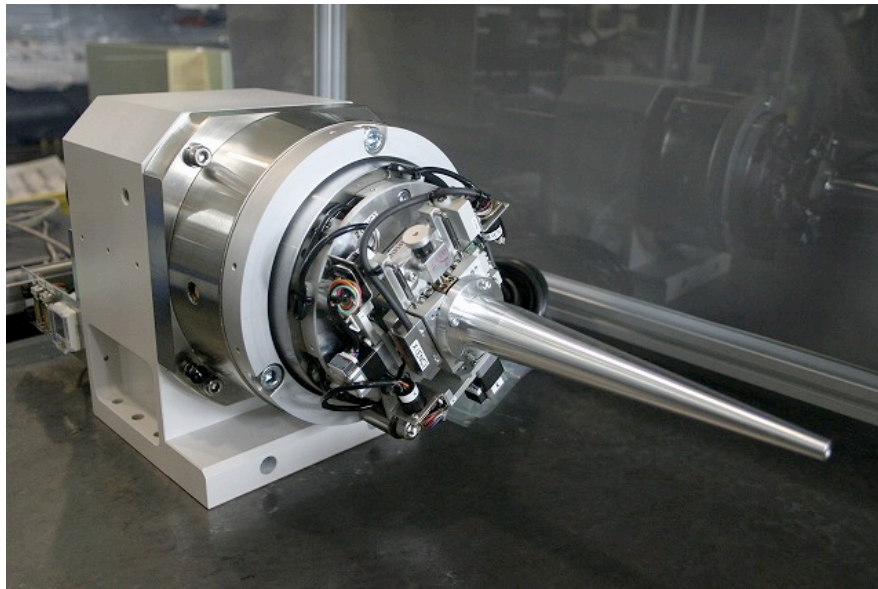
SSRL-type robot installed on MAD Beamline BL-5

20 datasets/day \Rightarrow 100s datasets/day

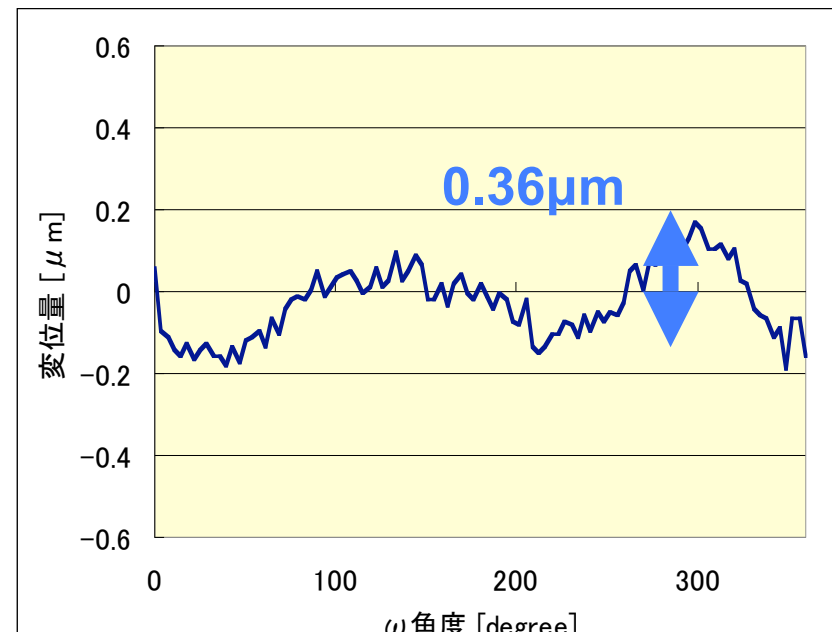


High precision one axis diffractometers with XYZ stages

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) \Rightarrow 0.1(2009)
Xtal size (μm)	100	22	10	4 (2007) \Rightarrow 1 (2009)

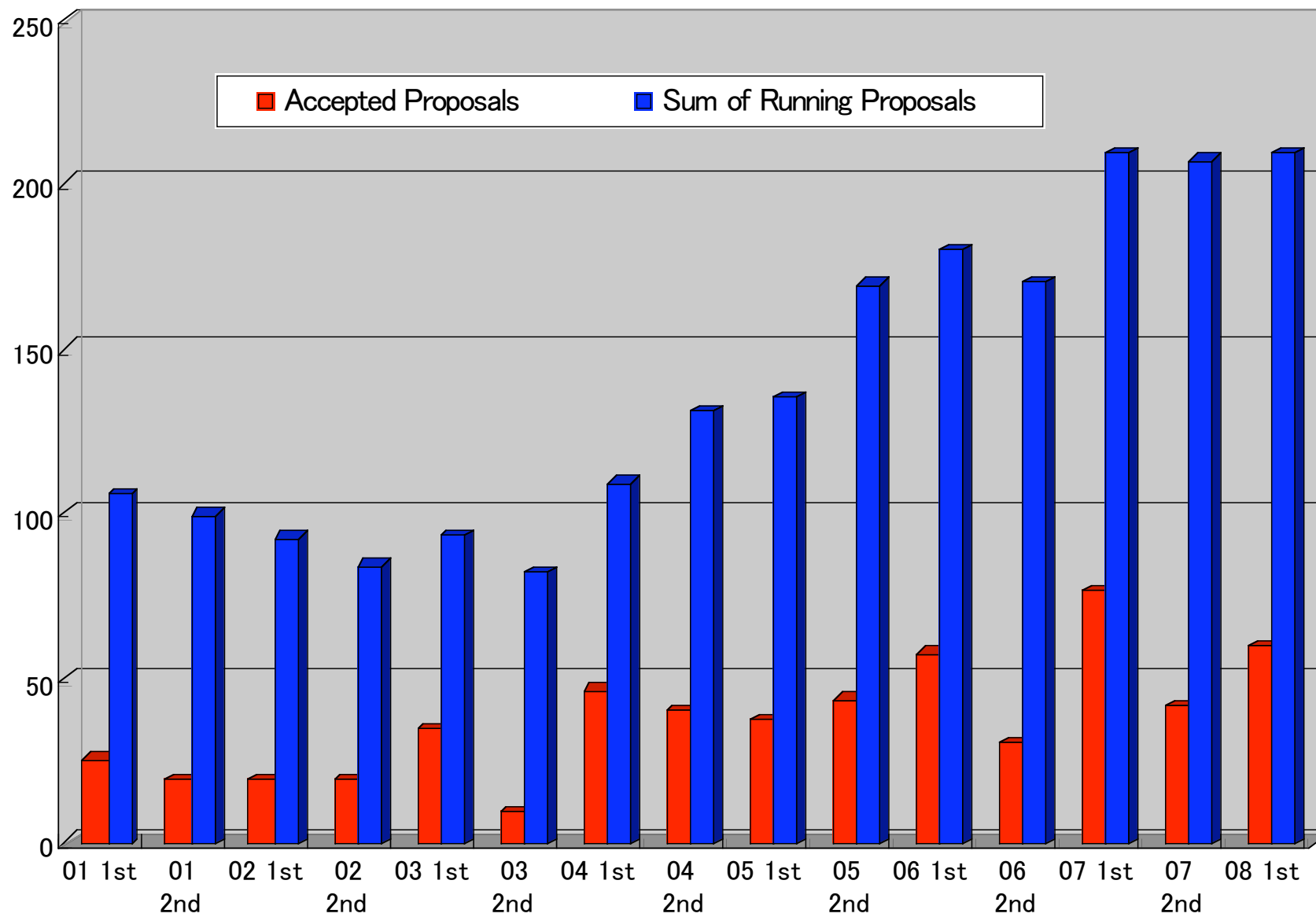


Air bearing & magnetic x-y stages



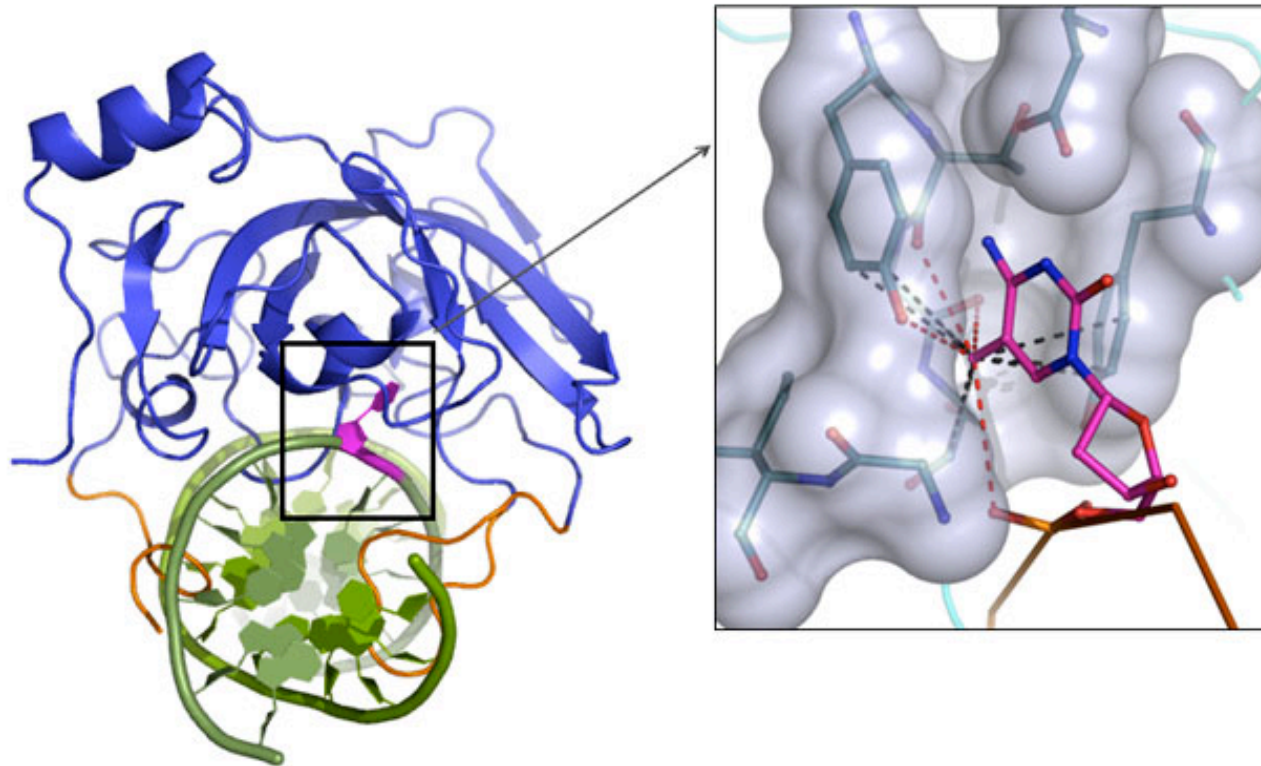
BL-5 type diffractometer -> also installed on BL41XU & BL44XU, SPring8

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



“Flipping-out” mechanism of recognition of hemi-methylated DNA by UHRF1 (BL-5A) by M. Shiraka’s group, Kyoto University

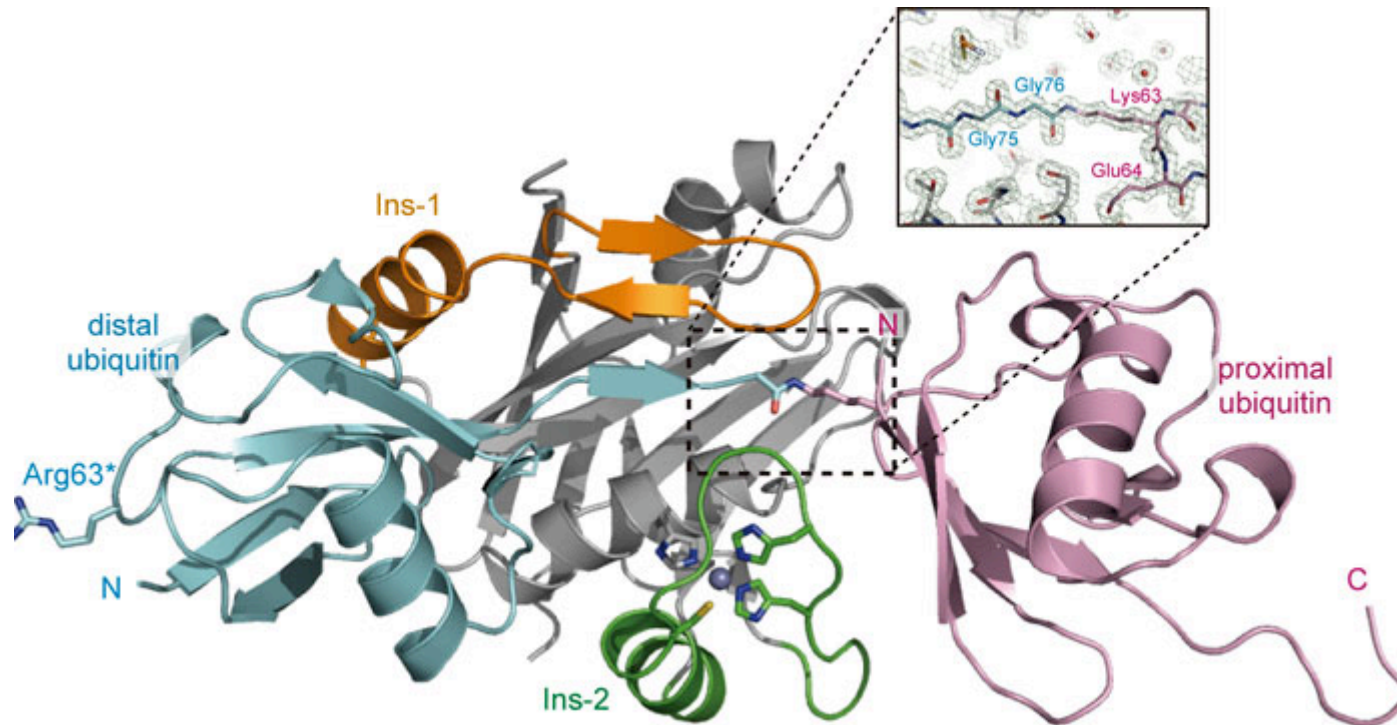
K. Arita et al., Nature, 455, 818-821 (2008).



DNA methylation is an important epigenetic modification of mammalian genomes and essential for the gene expression. Inheritance of the methylation pattern is mediated by the enzyme Dnmt1, and the protein UHRF1 recognizes hemimethylation sites and directs Dnmt1 to these sites. Crystal structure of the complex of DNA and SRA domain of UHRF1 revealed that the hemi-methylated site is flipped out of the DNA helix

Structural basis for specific cleavage of Lys 63-linked polyubiquitin (NW12A)

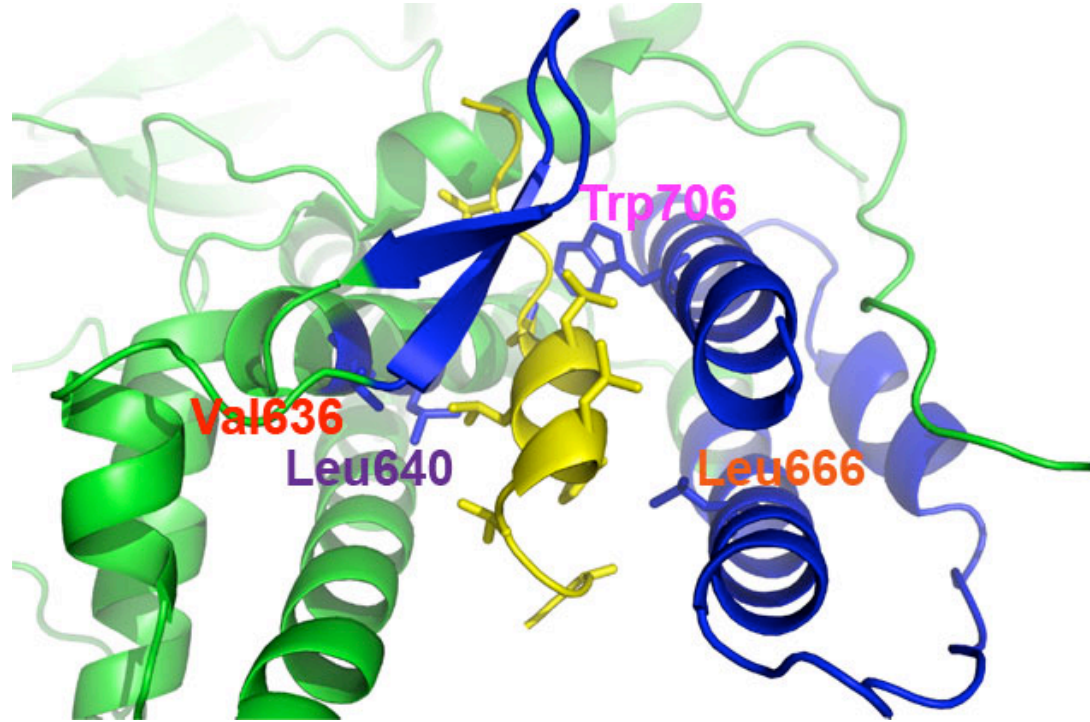
S. Fukai's group, Tokyo Univ., Res. Org. Sync. Rad.
Y. Sato et al., Nature, 455, 358-362 (2008).



Ubiquitin is a post-translational modifier that regulates a wide variety of biological processes. Lys 48-linked polyubiquitin chains are the most abundant linkage in vivo and constitute the signal for degradation by the proteasome. However, Lys 63-linked polyubiquitin chains serve proteasome-independent roles. The structure of AMSH family members which specifically cleave Lys 63-linked polyubiquitin chain was determined.

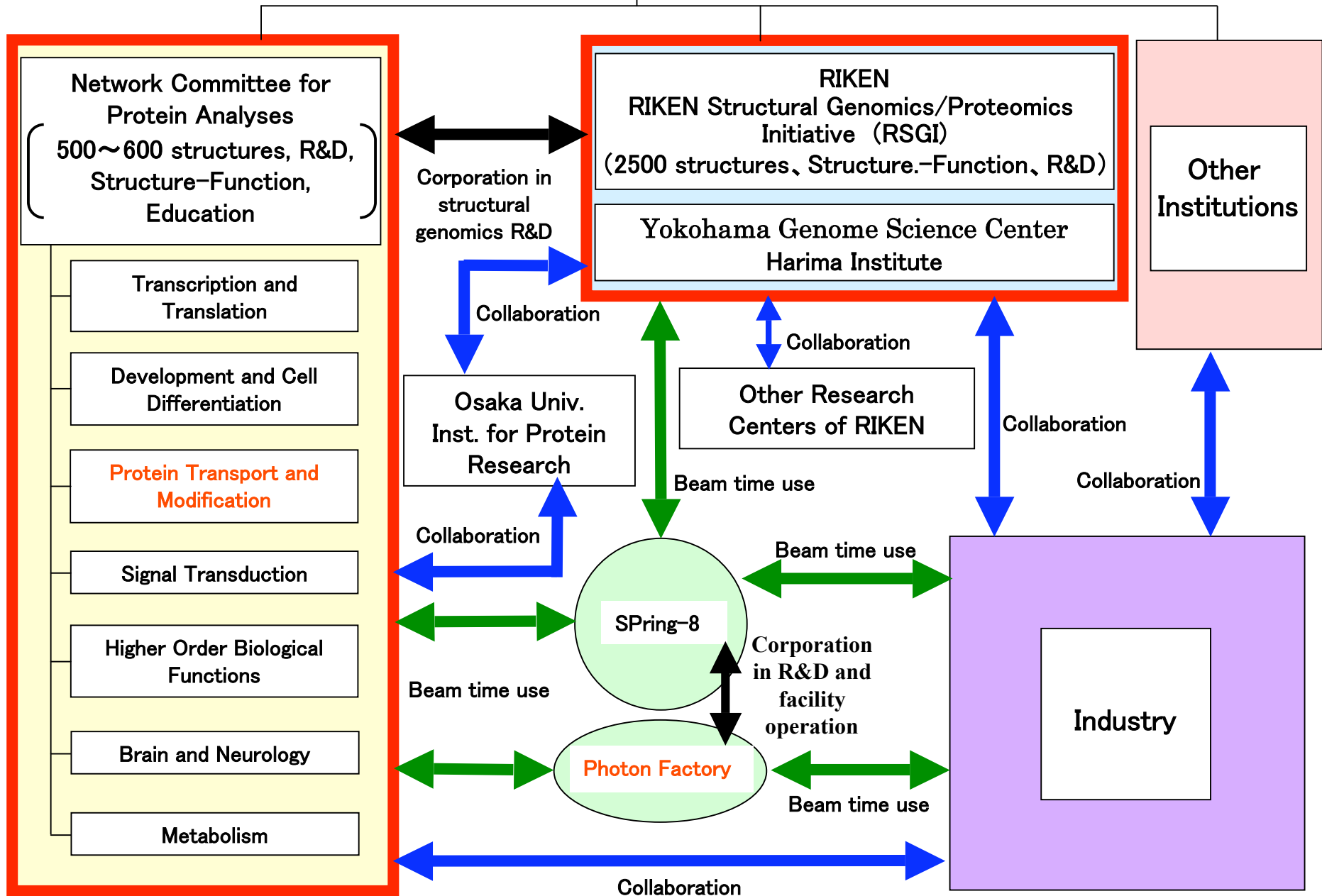
Essential subunit interaction in influenza virus RNA polymerase (BL-5A)

S. Park's group, Yokohama City University
E. Obayashi et al., Nature, 454, 1127-1131 (2008).



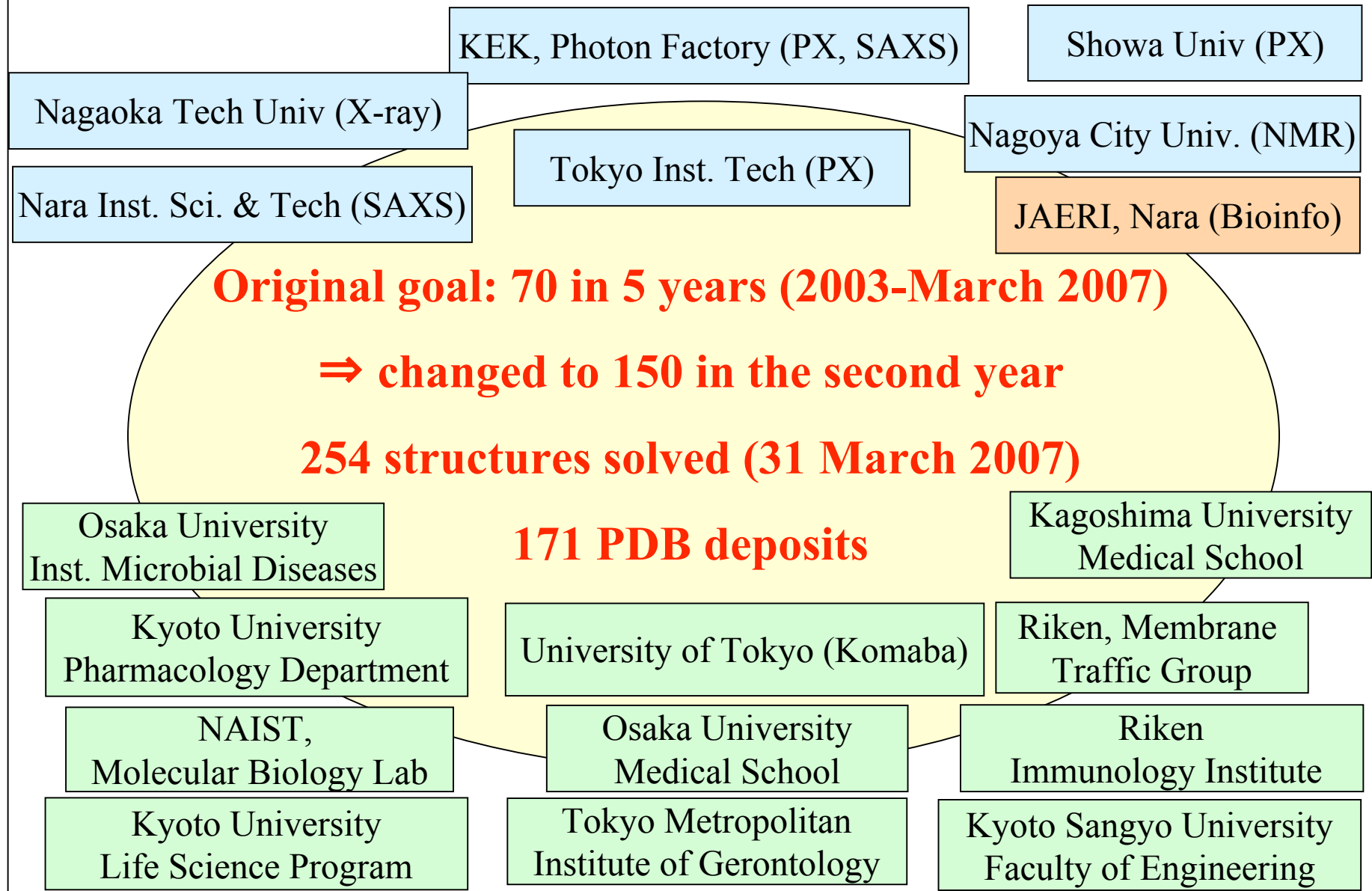
Influenza virus is a major human and animal pathogen, and reproduces rapidly, mutates frequently and occasionally crosses species. The importance to viral replication of a subunit interface in the viral RNA polymerase was demonstrated by the crystal structure. The structure presented here provide a new set of potential drug binding sites.

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

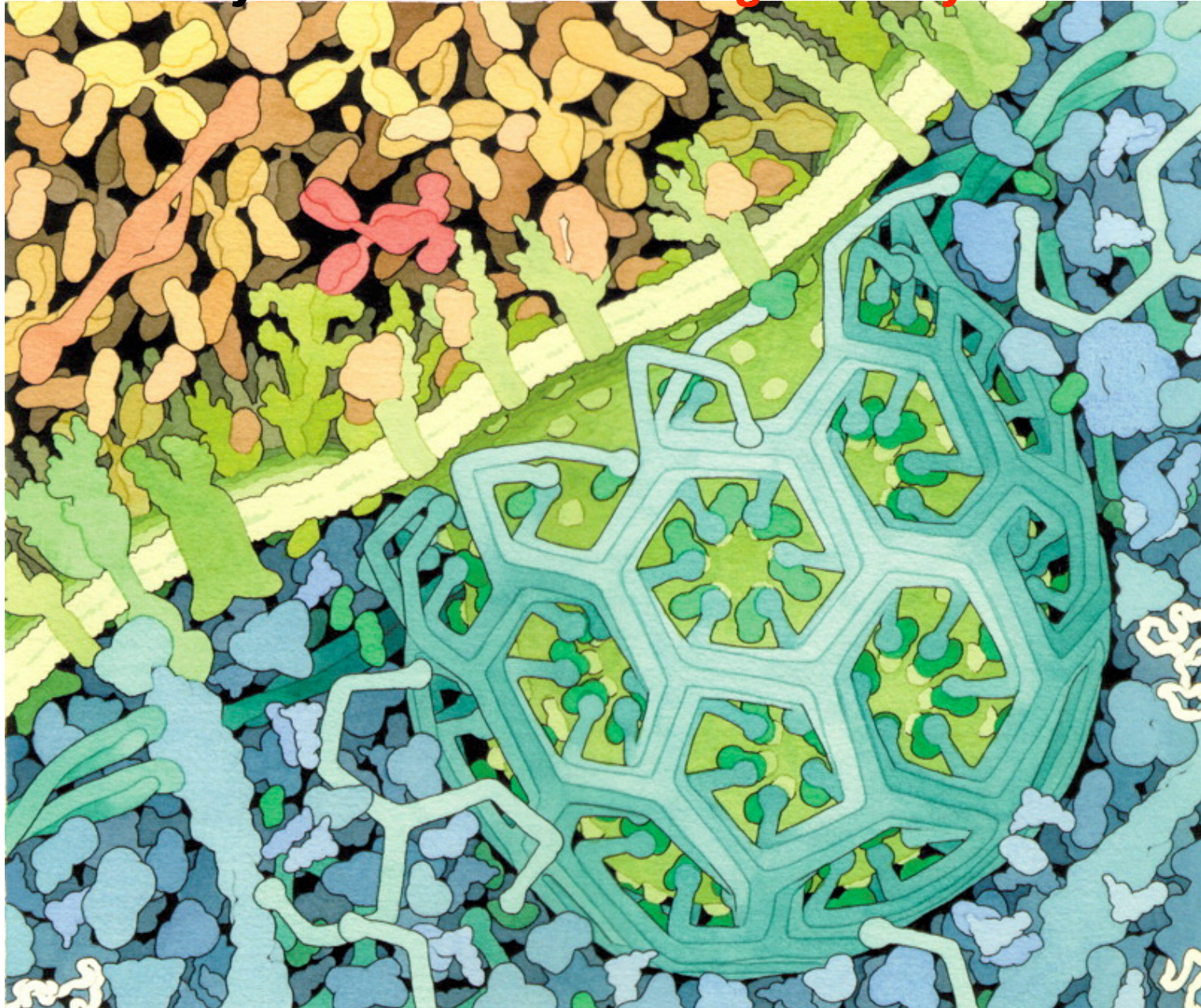


Protein 3000 Tsukuba Structural Biology Consortium (21 groups)

Protein Transport and Posttranslational Modification

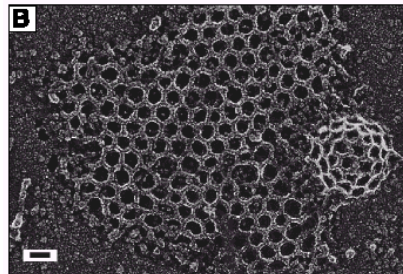
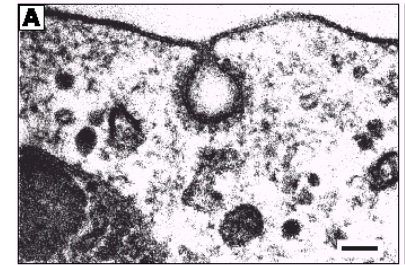
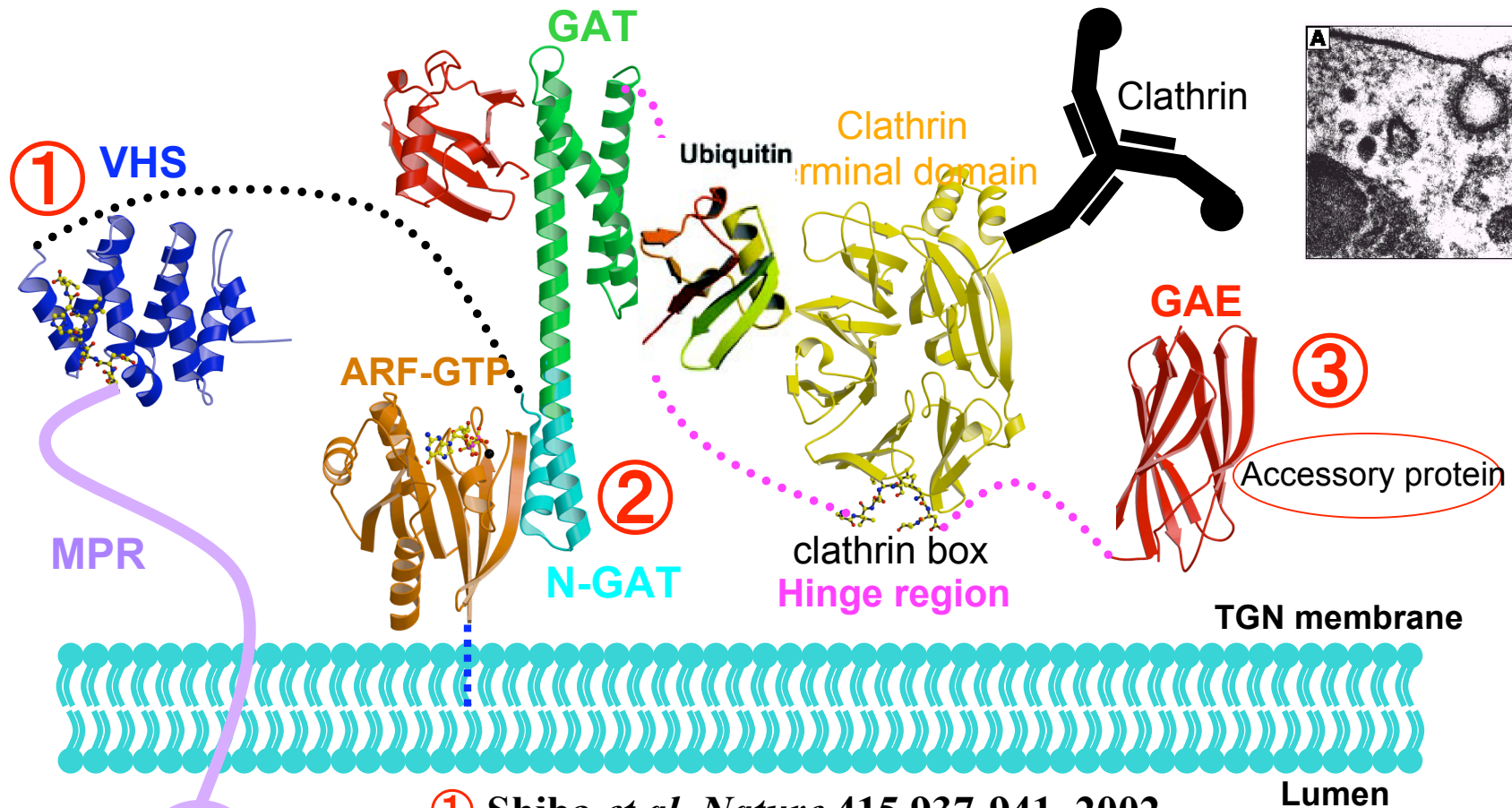


Endocytosis of toxin -> Drug delivery



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

Human GGA: a new class of adaptor proteins



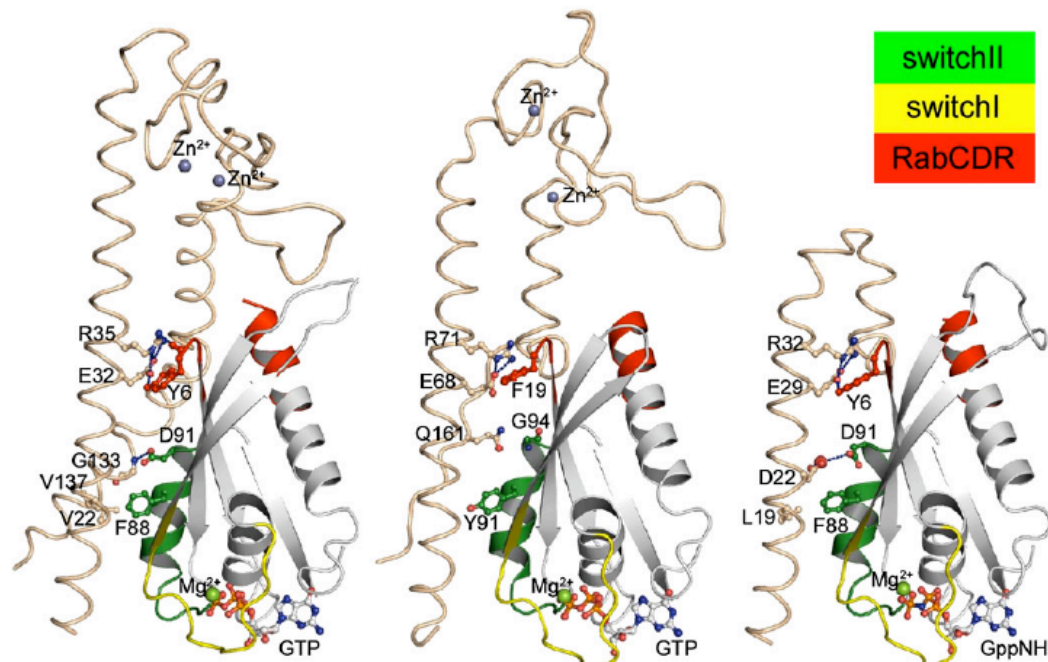
- ① Shiba *et al.* *Nature* 415,937-941, 2002
Shiba *et al.*, *Traffic*, vol. 5, 437-448, 2004
- ② Shiba *et al.*, *Nature Structural Biology*, 10 386-393, 2003
Shiba *et al.*, *J. Biol. Chem.* 279, 7105-11279, 2004
Kawasaki *et al.*, *Genes to Cells*, 10, 639-654, 2005
Yogosawa *et al.*, *BBRC*, 350,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

Protein complexes necessary for the melanosome trafficking

Structure
Previews

Review by R. Goody, *Structure*, **16**, 1437-1439 (2008).

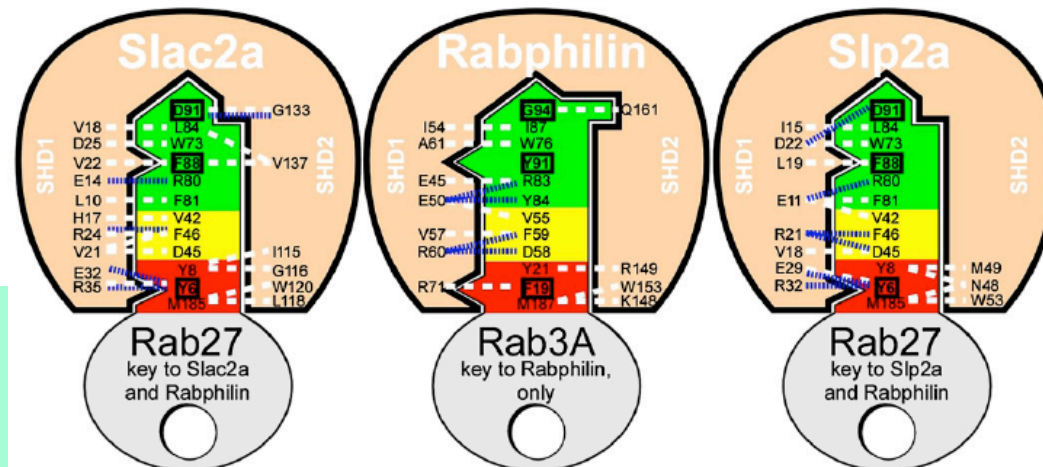
Slac2a:Rab27 Rabphilin:Rab3A Slp2a:Rab27



M. Kukimoto-Niino *et al.*, *Structure*, **16**, 1478-1490 (2008).
 SP8 BL41XU

L.M.G. Chavas *et al.*, *Structure*, **16**, 1468-1477 (2008).

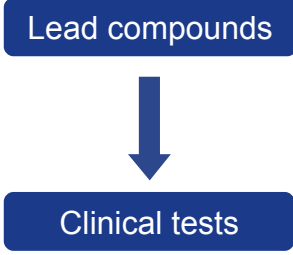
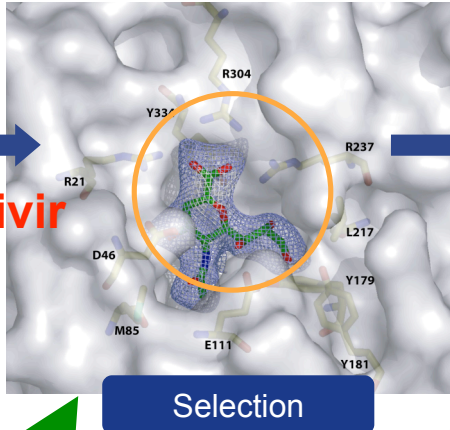
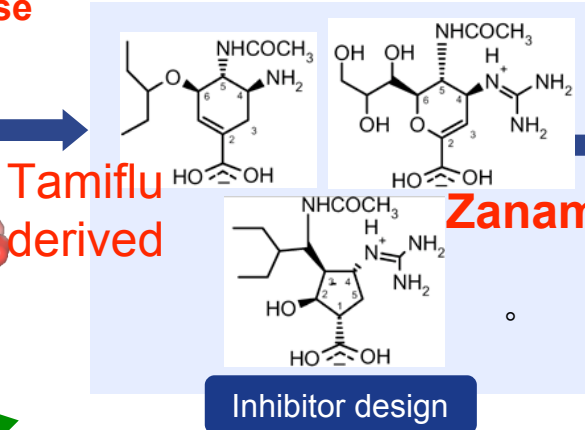
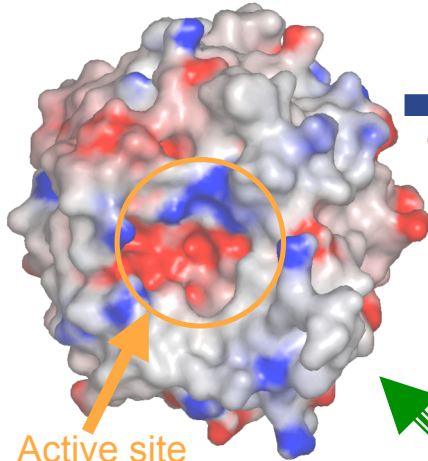
PF BL-5A



Joint Press Release
 by RIKEN and KEK
 on Oct 3rd, 2008

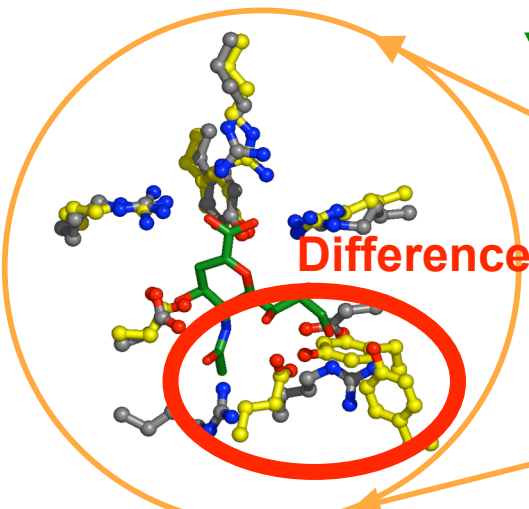
Structure Based Drug Design: more efficient and lower cost for development of Anti influenza drug with little or no side effects (collab. with Italy, Australia, Canada)

Influenza neuraminidase

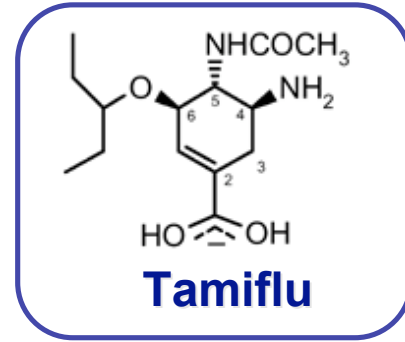
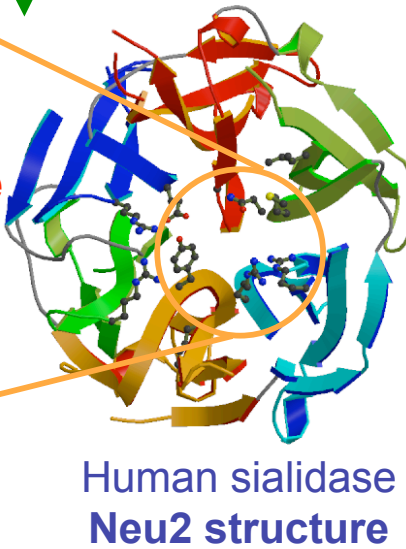


Structure Determination using SR

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

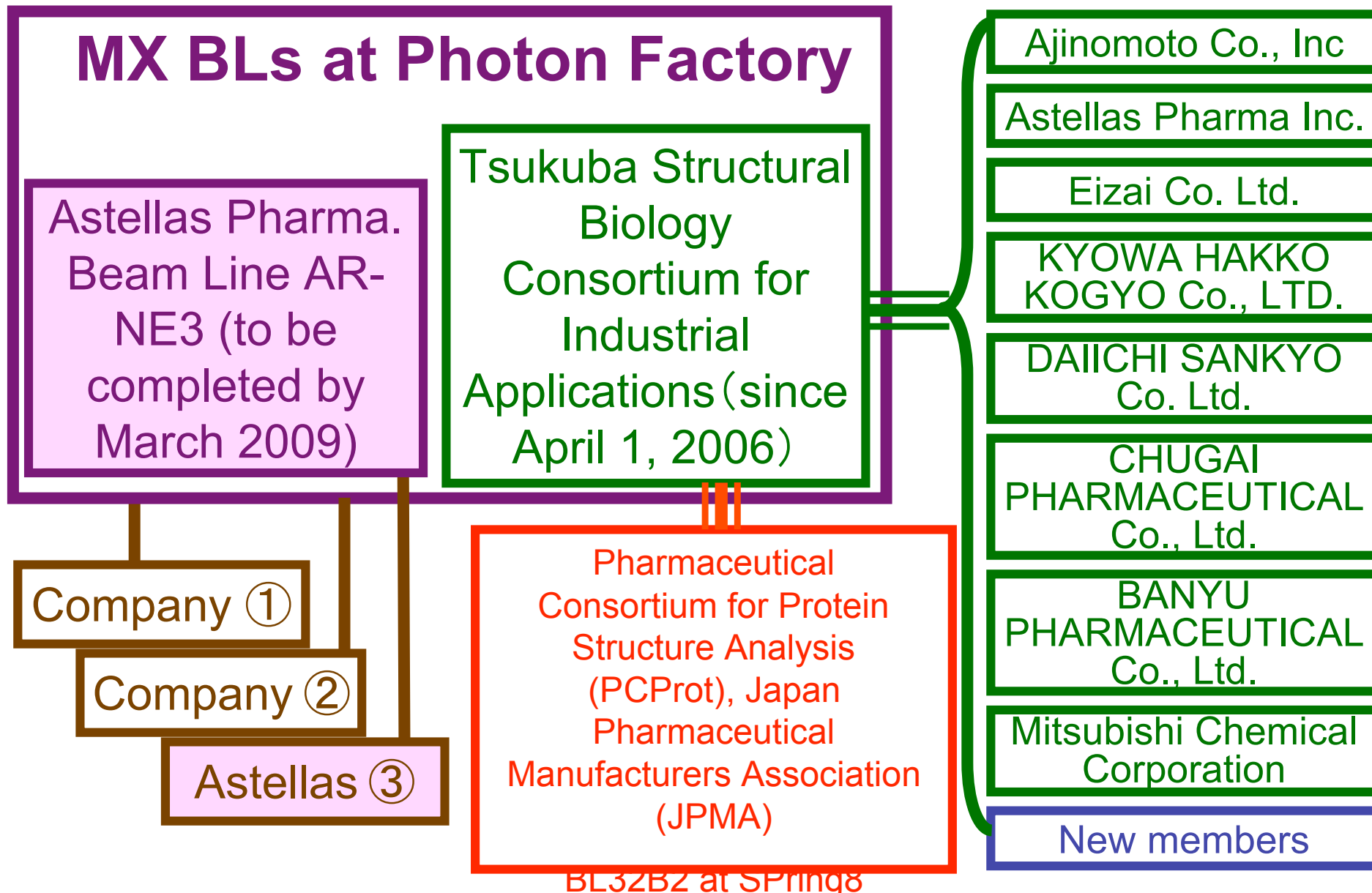


Yellow: human sialidase Neu2
Gray: Influenza neuraminidase



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

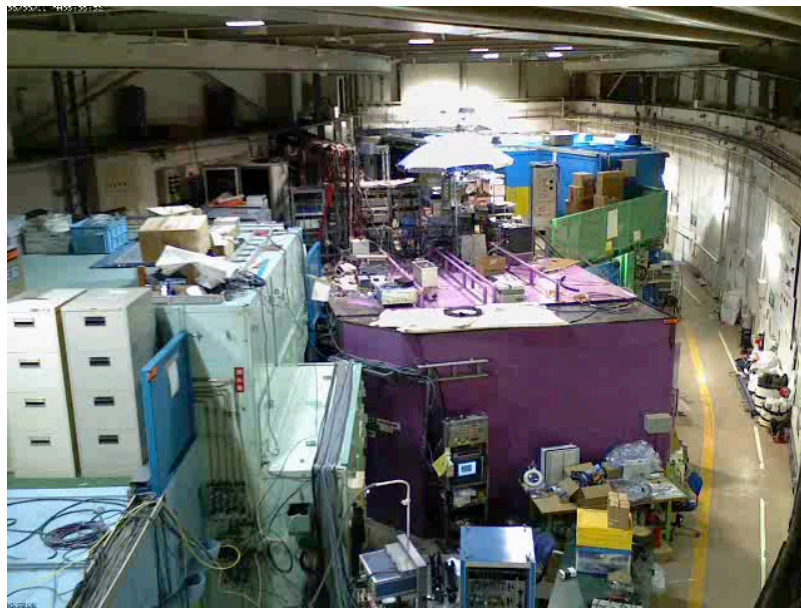
Industrial Use and Collaborations between KEK and Industry (~8% of beamtime)



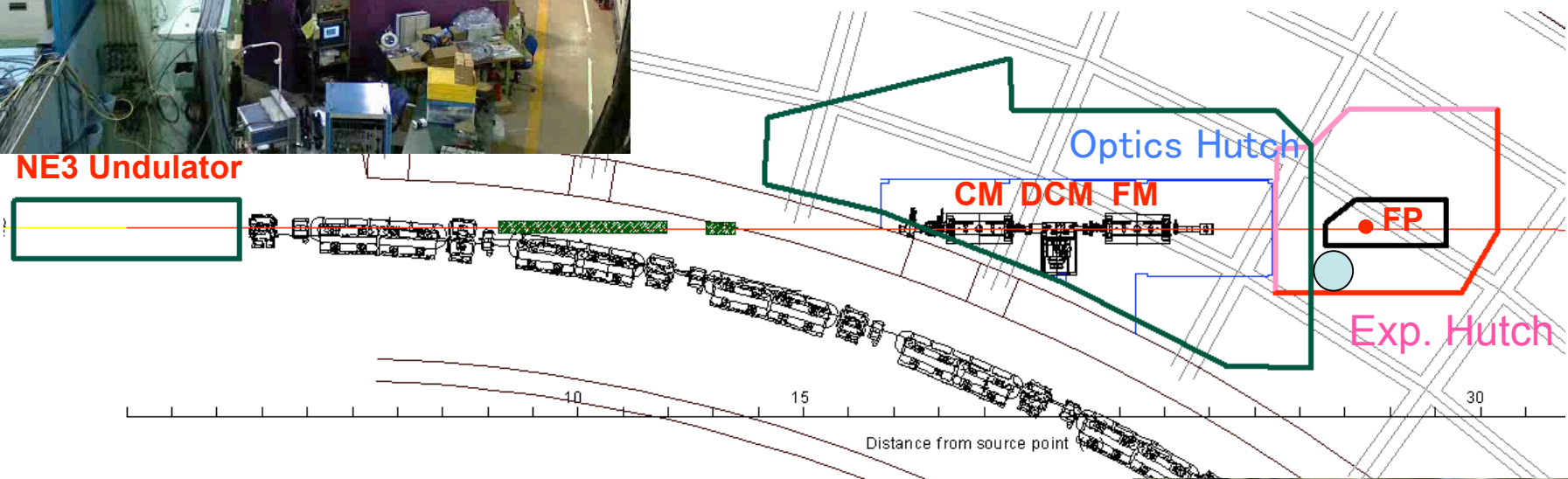
Astellas Pharma Beam Line: PF-AR NE3

New

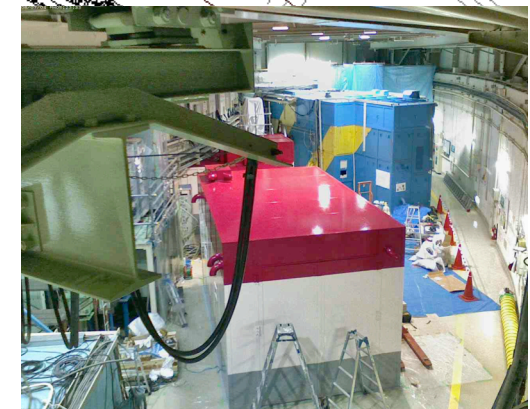
Available from Apr. 2009



NE3 Undulator



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

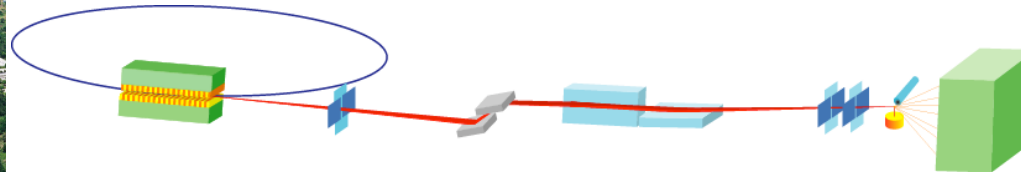


MEXT: Target Protein Project (2007-2011)

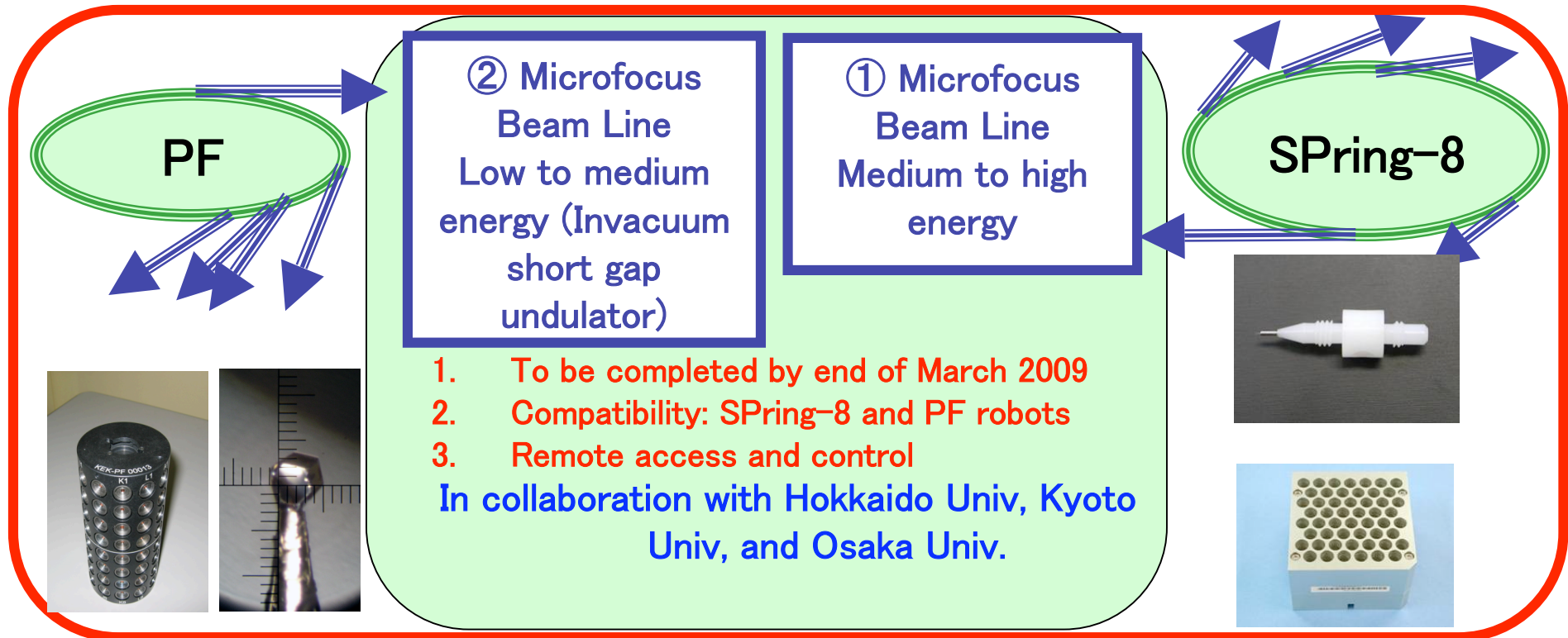
Photon Factory



SPring-8



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



Collaboration outside of Japan

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

Acknowledgements

SPring-8

Masaki Yamamoto's group

Hokkaido University

Isao Tanaka

Kyoto University

Kunio Miki

Osaka University

Atsushi Nakagawa

Mamoru Suzuki

Nagoya University

Nobuhisa Watanabe

Financial Supports

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

Protein 3000 Project (MEXT)

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

Target Protein Research
Program (MEXT)

Structural Biology Research Center, Photon Factory, KEK

Soichi Wakatsuki Professor, Director of Structural Biology Research Center

Structural Biology

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

Ex members:

Tadashi Satoh (Post Doc Fellow)
Leonard Chavas (Post Doc Fellow)

+++

Beamline & Robotics

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Naohiro Matsugaki (Res. Assoc.)
AR-NW12, BL1
Masashiko Hiraki (Res. Assoc.) Robotics
Yusuke Yamada (Research Assoc.) NE3
Yurii Gaponov (Researcher) Programming
Kumiko Sasajima (System Engineer)
Shokei Watanabe (Mechanical Engineer)
Nobuo Honda (System Engineer)
Kazutaka Demura (Beamline Assistant)
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16 October 2008



Thank you for your attention.

6.5GeV
PF-AR

2.5 GeV PF

