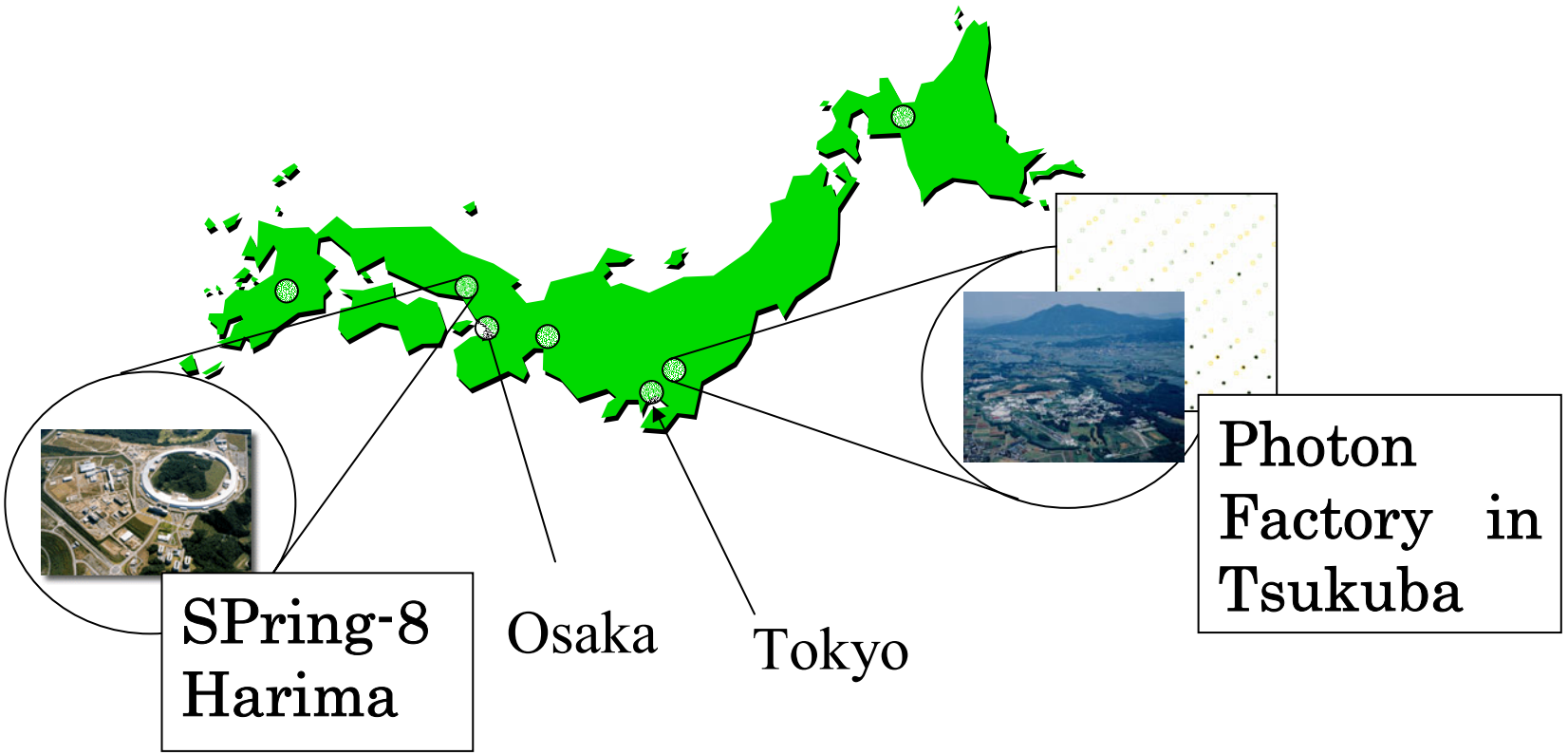
Synchrotron based structural genomics project targeted to protein transport and posttranslational modification

Soichi Wakatsuki

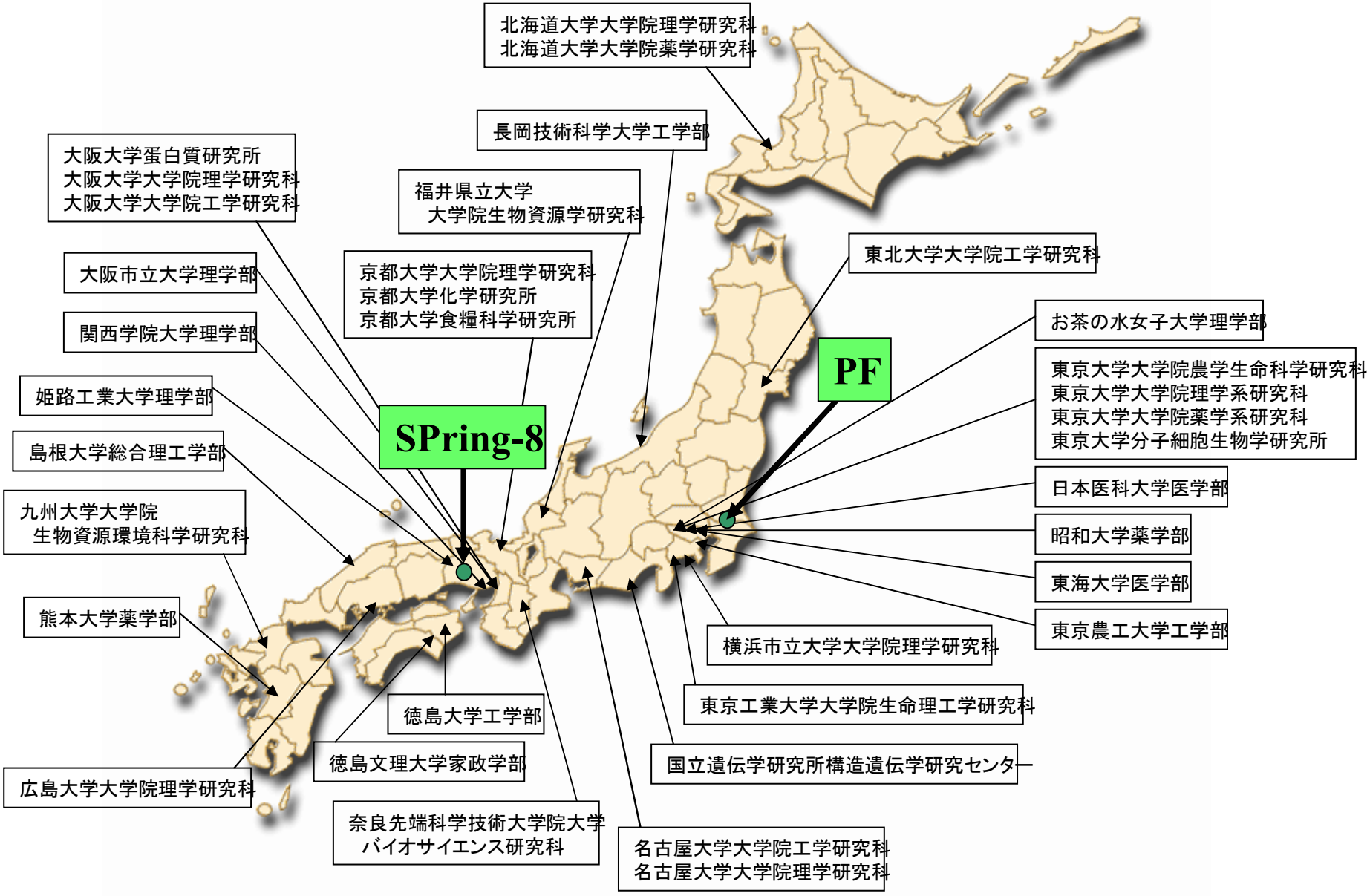
Photon Factory, IMSS, KEK, Tsukuba, Japan

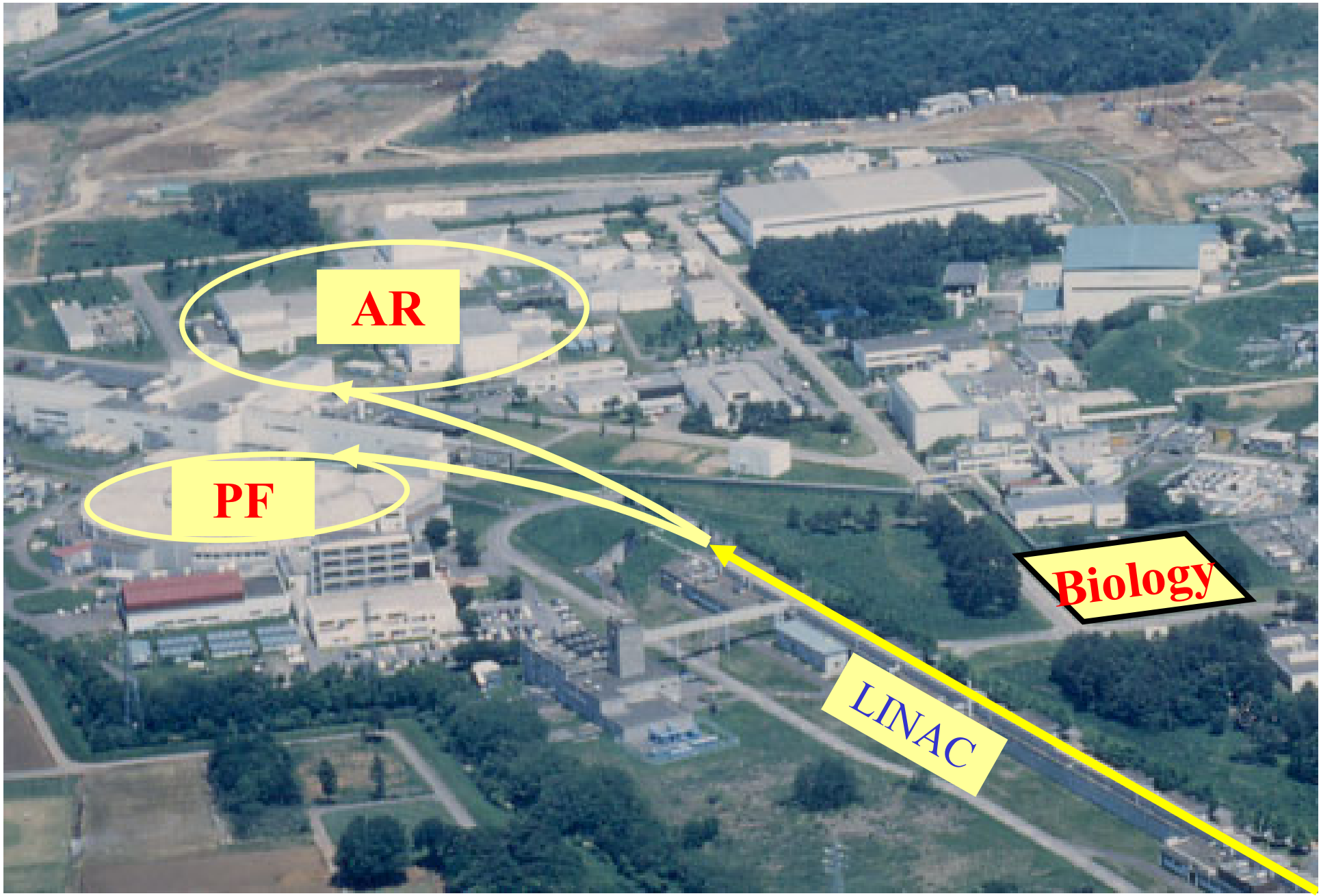
Outline

1. New national project of Protein 3000
2. Target oriented structural genomics on posttranslational modification and transport of proteins
3. A case study of human GGA proteins in the vesicle transport of lysosomal proteins modified with mannose 6-phosphate
4. Beam line development and high-throughput R&D


SPring-8
Harima
Osaka
Tokyo
Photon
Factory in
Tsukuba

Two synchrotron facilities and PX groups in Japan

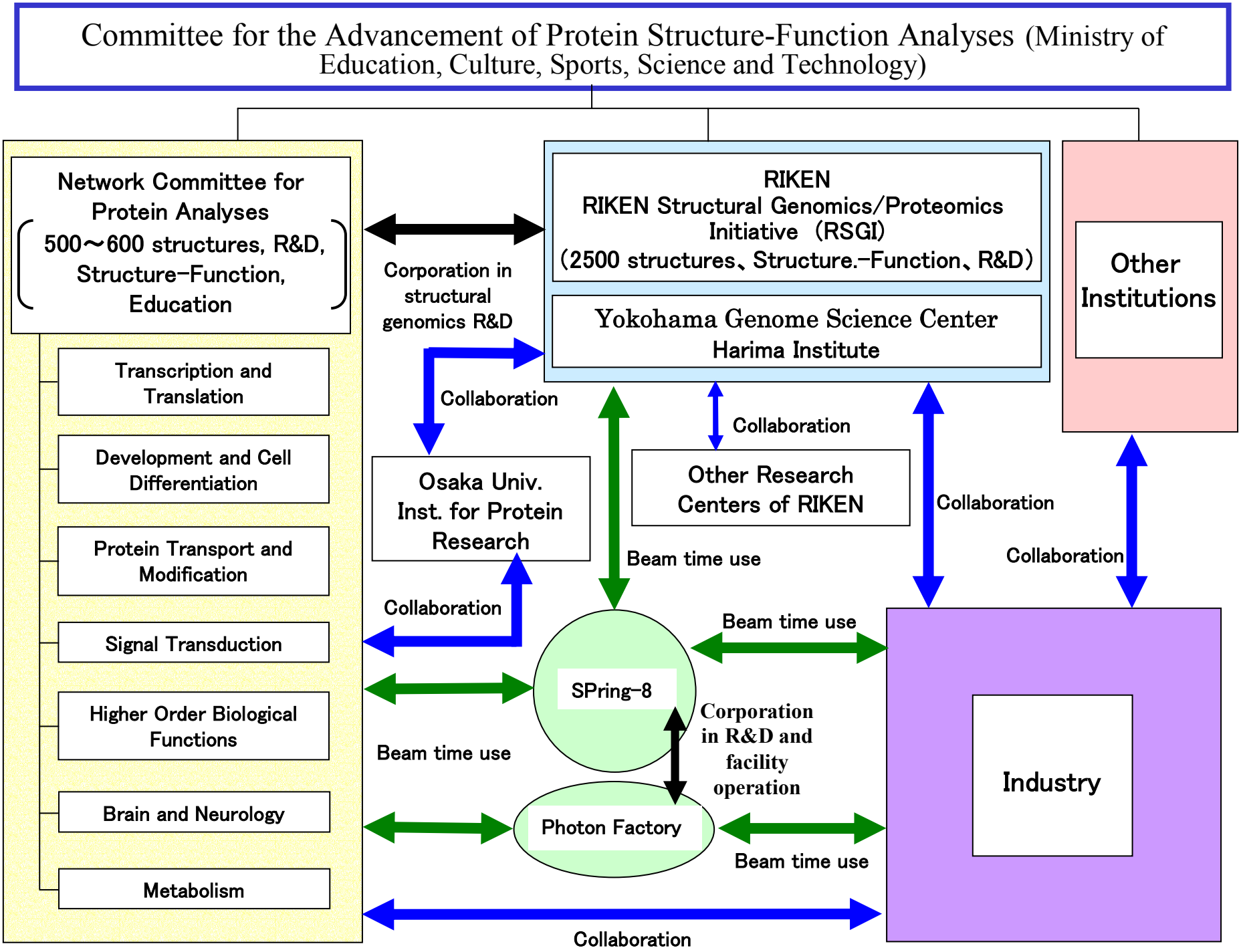



AR
PF
Biology
LINAC

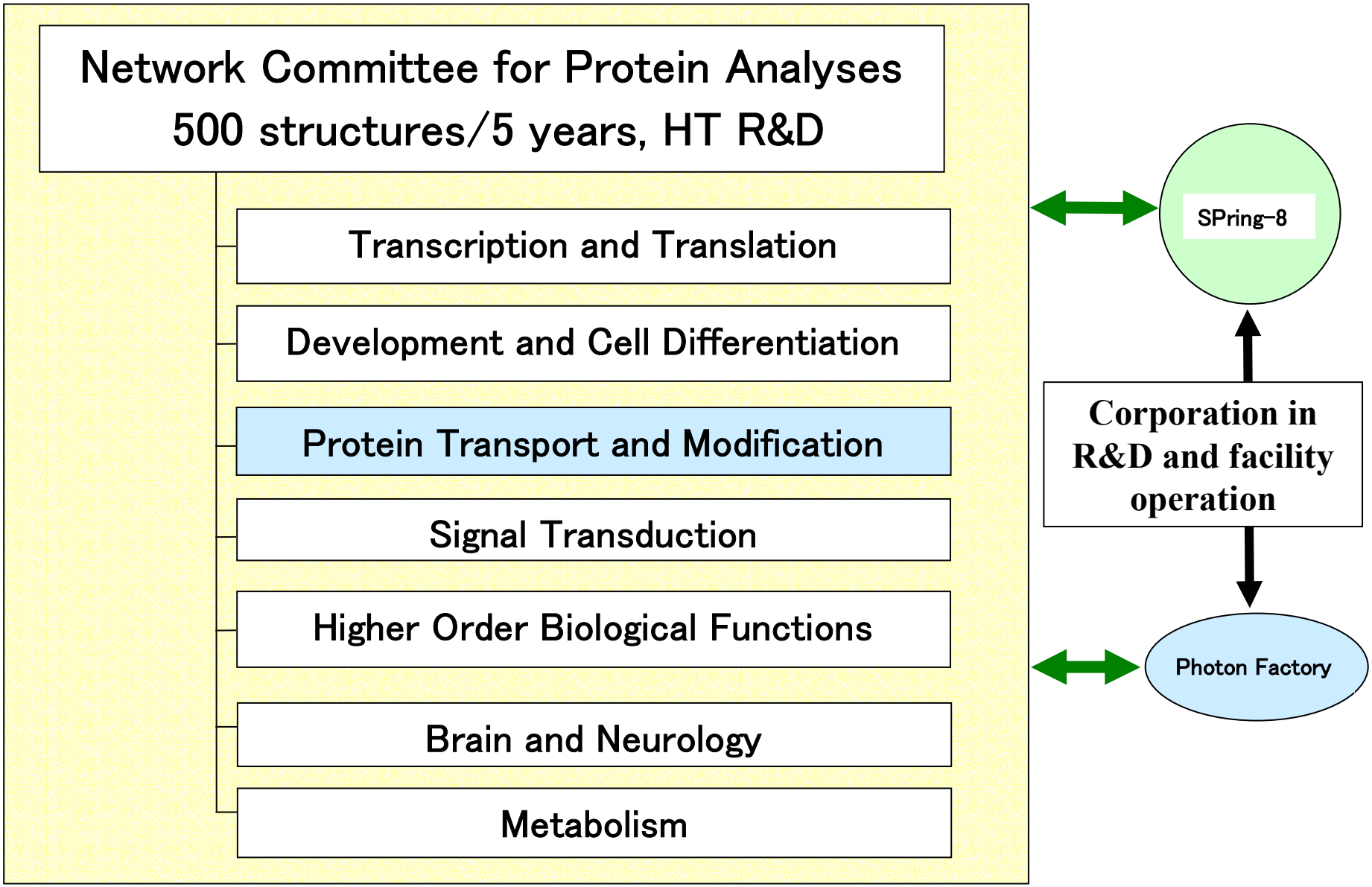
National project of determining 3000 structures in 5 years from FY 2002

The proposal of the Japanese universities

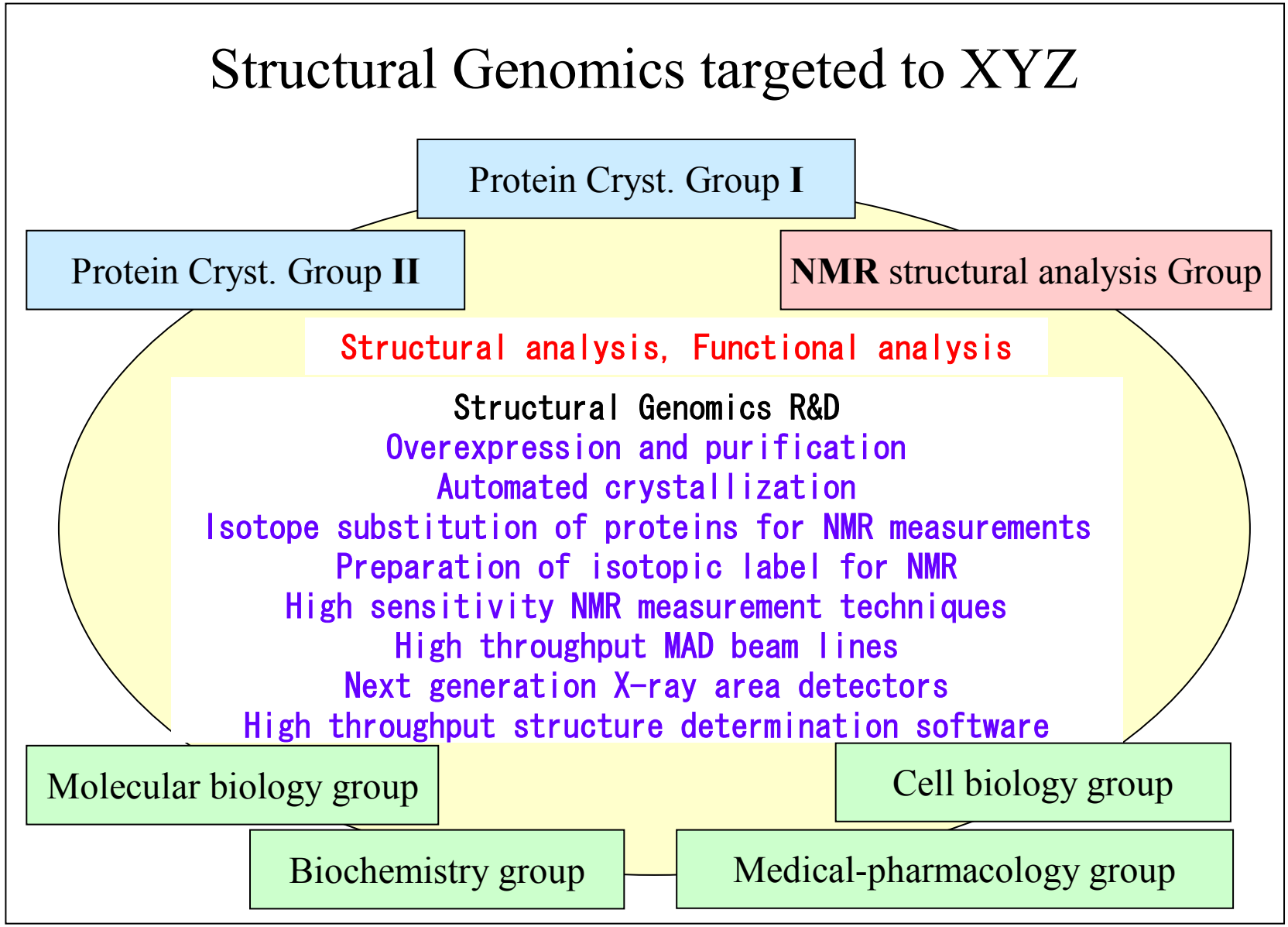
- Initiative of the university researchers
- Strong collaboration with biochemistry, molecular and cell biology, medicine, pharmacology departments of the universities
- Key technology developments
- Increase the overall efficiency of structure determination in Japan by a factor of 10
- Determining 500 biologically important protein structures
- Education of next generation structural biologists


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
Osaka Univ. Inst. for Protein Research
Other Research Centers of RIKEN
Collaboration
Collaboration
Beam time use
Collaboration
Beam time use
SPring-8
Beam time use
Corporation in R&D and facility operation
Photon Factory
Beam time use
Industry
Collaboration

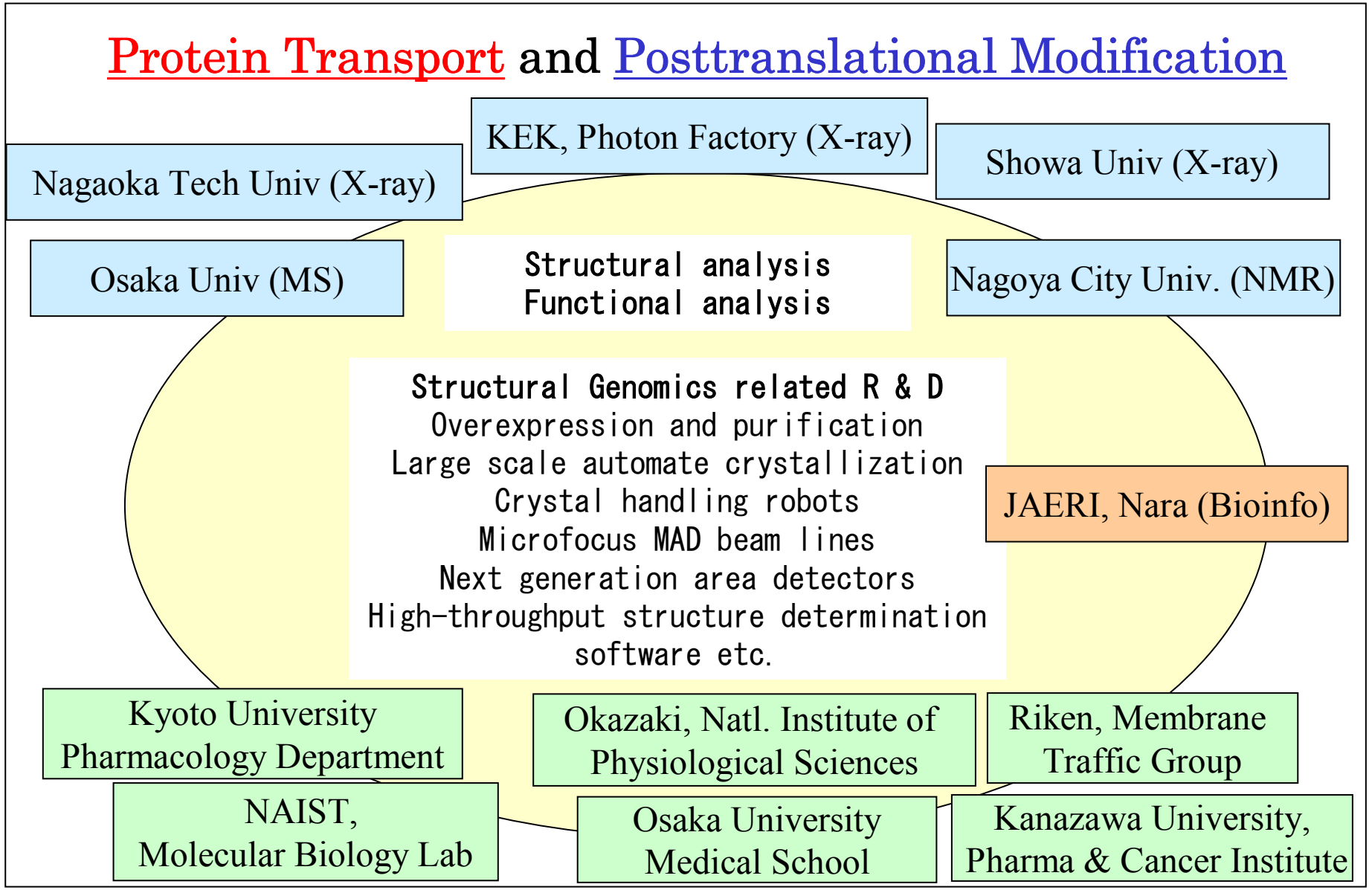
Target oriented structural genomics consortia of universities and national institutes



Concept of a S.G. Research Consortium

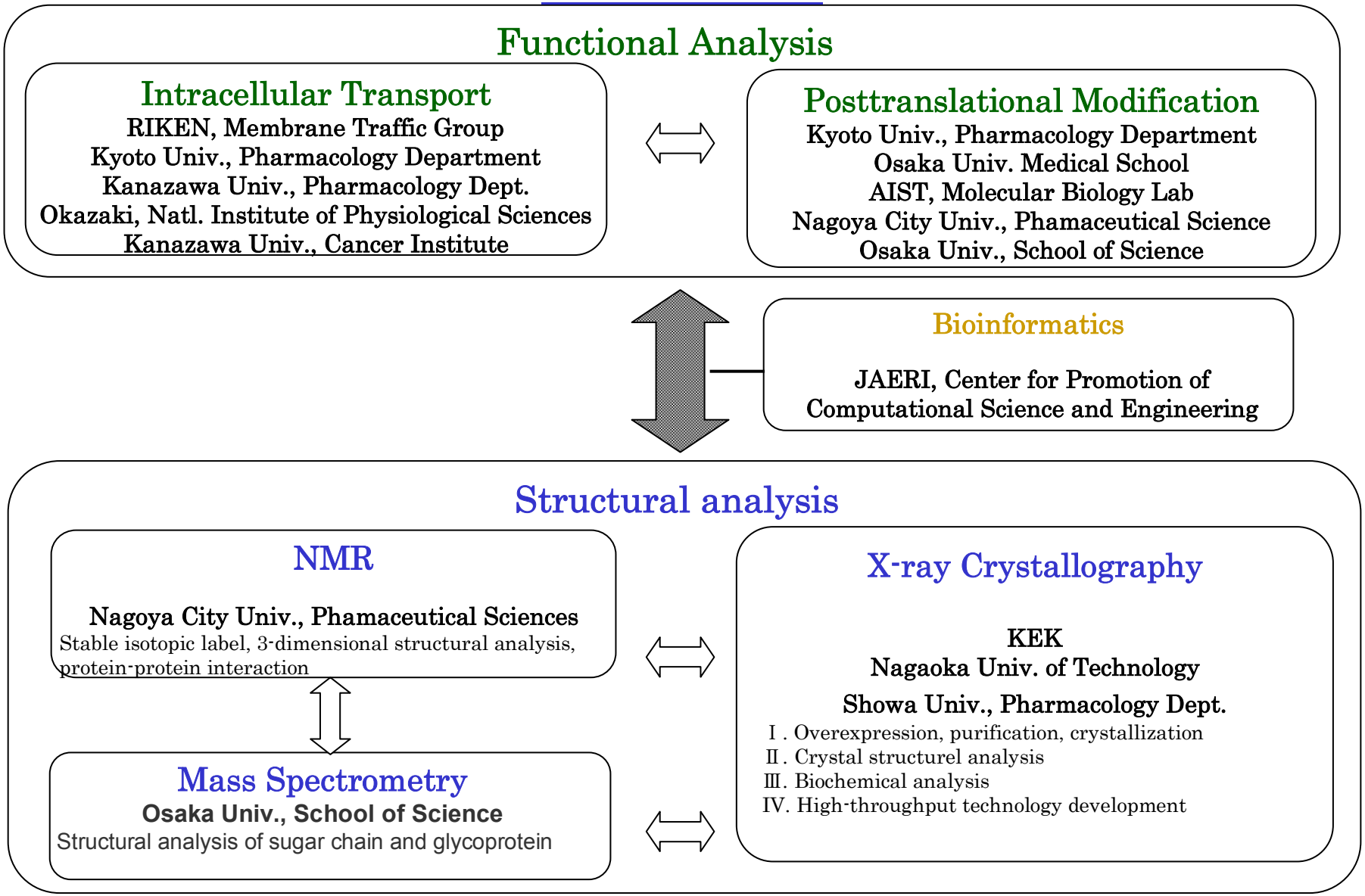


Tsukuba Structural Biology Consortium



Proposal of a Target Oriented Structural Genomics Consortium: Intracelluar Transport and Posttranslational Modification

Leader: Soichi Wakatsuki (KEK)



Core facilities (1)

New technology development for high-throughput protein crystallography

(1) New MAD beam lines

Mamoru Suzuki, Noriyuki Igarashi, Naohiro Matsugaki (KEK, PF)

(2) Robotics

Masahiko Hiraki, Minoru Nagai (KEK, PF),
Kenkichi Tanikawa, Kohtaro Ohba (AIST)

(3) New generation detector

Wataru Mochizuki (NHK)

(4) Software

Yuri Gaponov, Noriyuki Igarashi, Masahiko Hiraki (KEK, PF)

Center for protein expression and structural analysis (KEK, PF)

(1) Protein expression, purification, biochemical analysis and crystallization

Ryuichi Kato, Masato Kawasaki (KEK, PF)

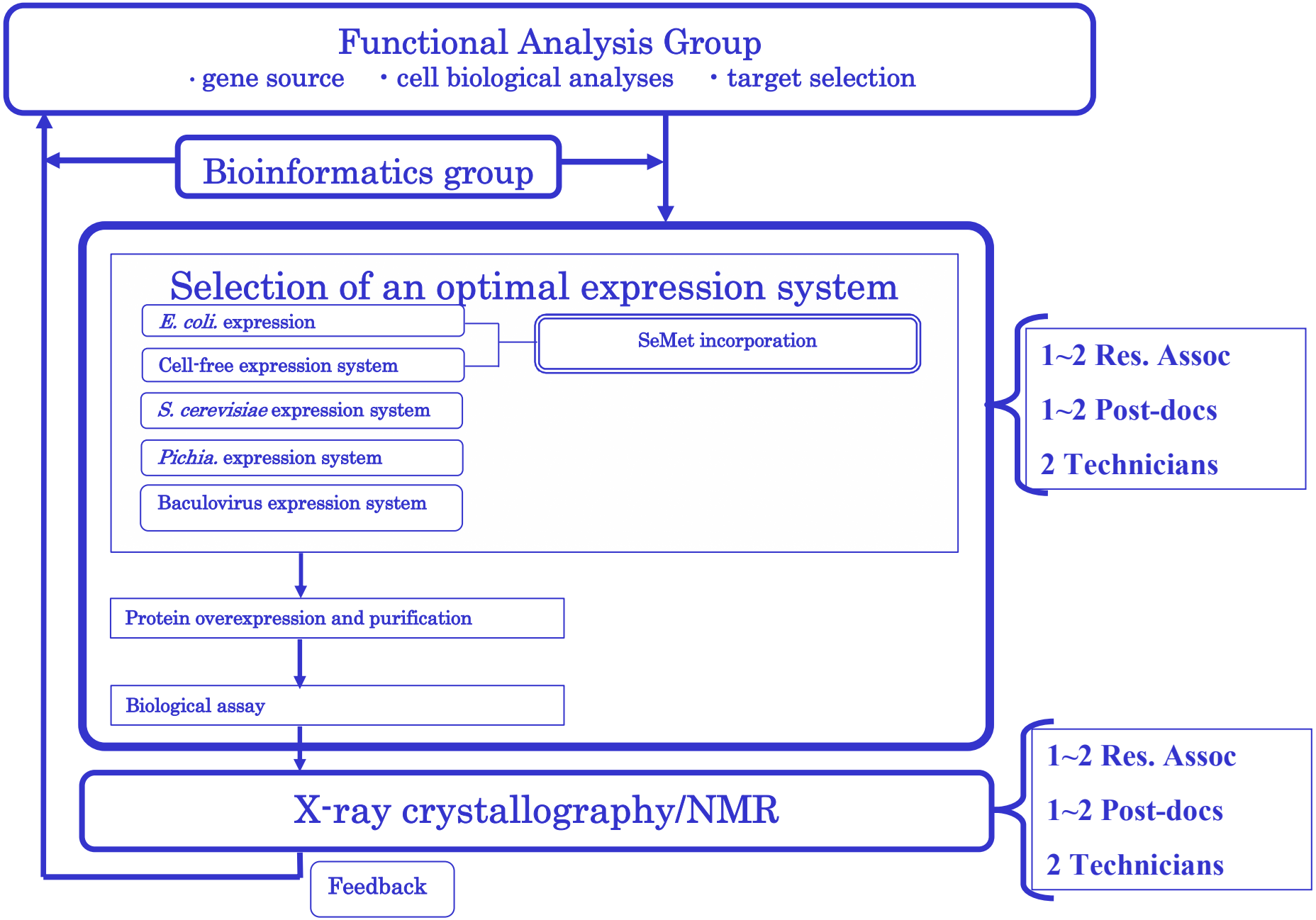
- Postdoc — Technician
- Postdoc — Technician
- Postdoc — Technician
- Postdoc — Technician

(2) Data collection and structural analysis

Mamoru Suzuki, Noriyuki Igarashi, Naohiro Matsugaki (KEK, PF)

- Postdoc — Technician
- Postdoc — Technician

Flowchart of functional and structural analysis team (total of 4)



Core facilities (2)

X-ray crystallographic structural analysis

Faculty of Pharmaceutical Sciences, Kyoto University (Hiroaki Kato)

Nagaoka University of Technology（Takamasa Nonaka）

Showa University（Noabutada Tanaka）

NMR structural analysis

Nagoya City University（Koichi Kato）

Functional analysis : post-translational modification

Faculty of Pharmaceutical Sciences, Kyoto University (Toshisuke Kawasaki)

Glycosyltransferases expressed in nervous system

: GlcAT-P, GlcAT-S

Faculty of Medicine, Osaka University (Naoyuki Taniguchi)

Glycosyltransferases for carcinogenesis and immune system

: GnT-III, GnT-V

Research Center for Glycoscience, National Institute of Advanced Industrial Science and Technology (Yoshifumi Jigami)

Metabolic system of sugar nucleotide : Och1, YND1

Nagoya City University (Koichi Kato)

Quality control and transport of proteins : Fcγ receptor II

Faculty of Science, Osaka University (Sumihiro Hase)

Glycosyltransferases expressed in early embryo : FucT1, FucT2

Core facilities (3)

Functional analysis : intracellular protein transport

Faculty of Pharmaceutical Sciences, Kanazawa University (Kazuhisa Nakayama)

Transport between Golgi and lysosome : GGA, BIG

Cancer Research Institute, Kanazawa University (Hiroshi Ohno)

Intracellular protein transport : AP complexes

The Institute of Physical and Chemical Research (Akihiko Nakano)

Vesicle transport : Sar1p, Sec12p

Center for Integrative Bioscience, Okazaki National Research Institute (Masayuki Murata)

Visualization of protein transport of semi-intact cells

Faculty of Pharmaceutical Sciences, Kyoto University (Hiroaki Kato）

Peroxisome protein import : Pex19p, PMP70

Bioinformatics

Center for Promotion of Computational Science and Engineering, Japan Atomic Energy Research Institute (Kei Yura）

TLO

Tsukuba Liaison (Akira Tasaki)

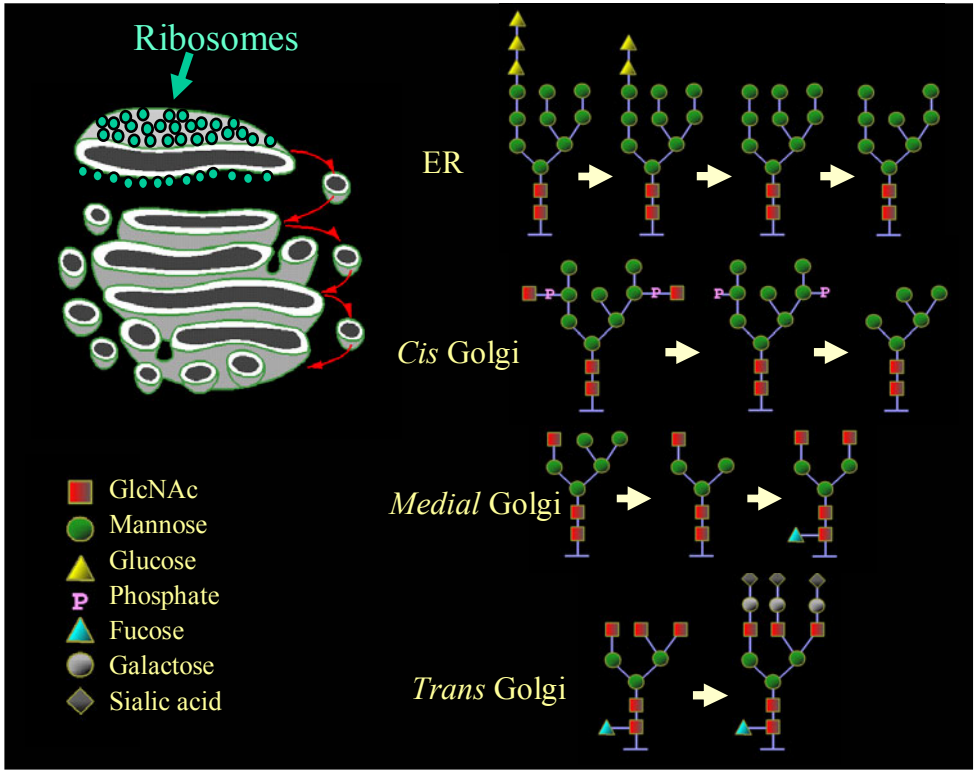
Target oriented structural genomics!

(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.

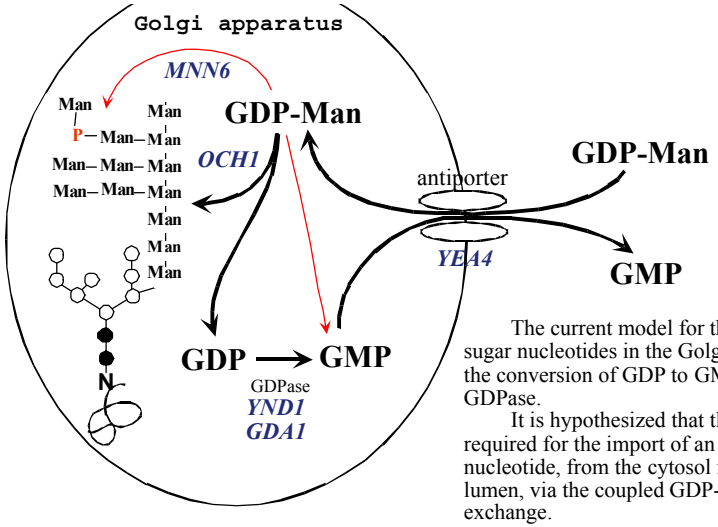


Application of 3-D structure of glycosyltransferases

Development of new drugs for regulation of glycosyltransferase activity

- Control of tumor metastasis
- Control of inflammation
- Organ xeno-transplantation
- Highly active glycoprotein drugs
- Defense against infective diseases

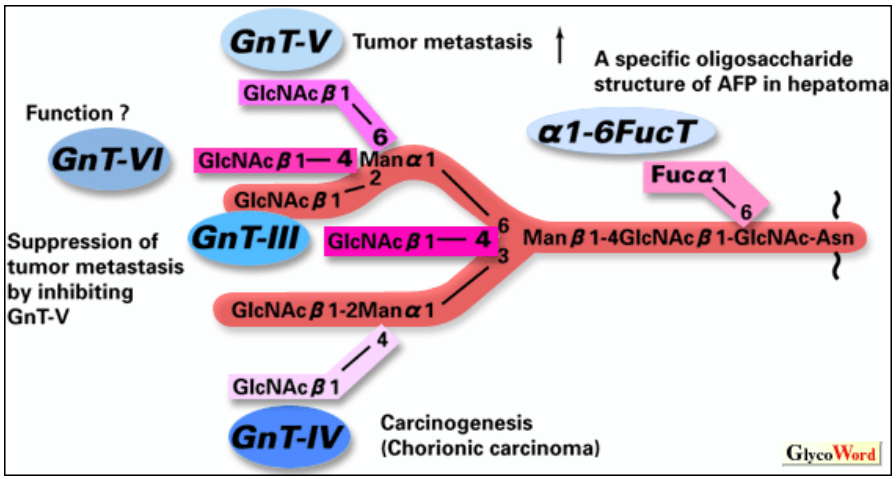
The current model of GDP-mannose transport and utilization in the Golgi-apparatus



The current model for the utilization of sugar nucleotides in the Golgi lumen emphasize the conversion of GDP to GMP by lumenal GDPase.

It is hypothesized that this reaction is required for the import of an additional sugar nucleotide, from the cytosol into the Golgi lumen, via the coupled GDP-Man/GMP exchange.

N-acetylgulcosimine transfereses

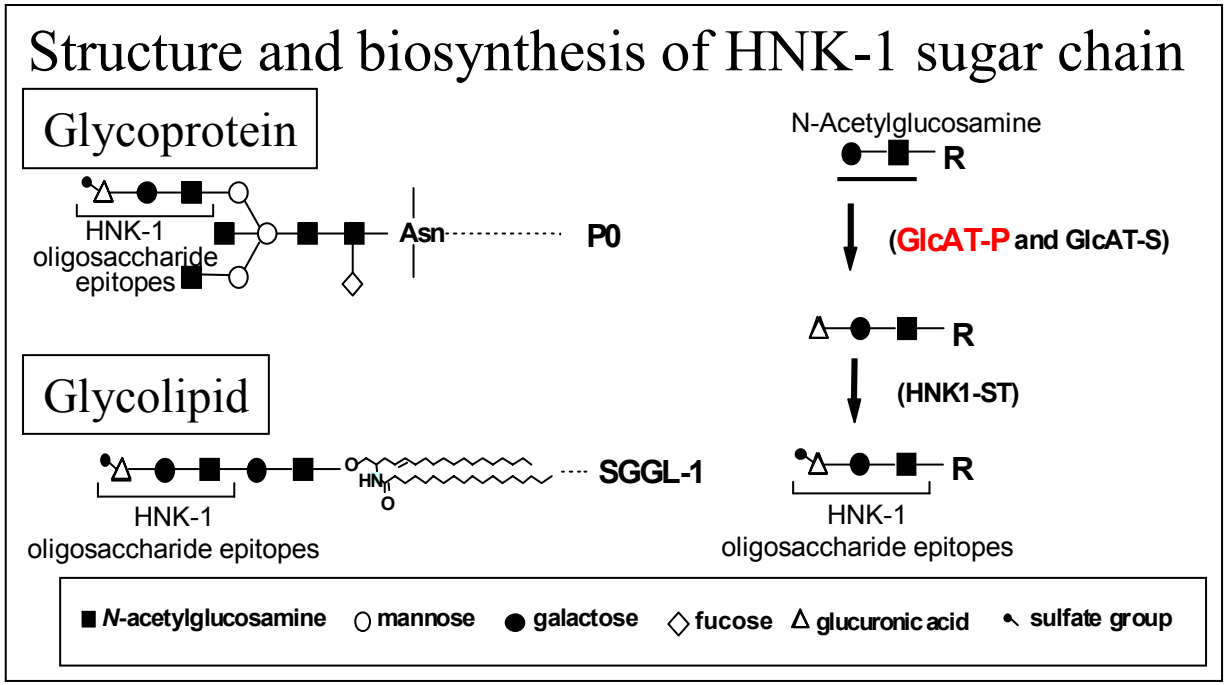


HNK-1 Carbohydrate Epitope (CD57)

- is recognized by HNK- 1 (human natural killer -1) monoclonal antibody
- is found in insect and human cells, and is localized in nerve adhesion molecules (NCAM, P0, L1, MAG etc.) and neuroglycolipids （SGGL-1,-2).
- is expressed in specific phase and position during development and growth of nerve tissues.
- is used as marker of nerve development.

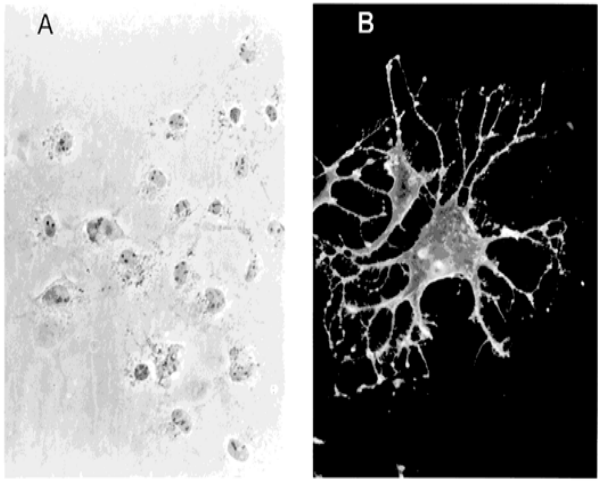
GlcAT-P, S :

enzymes required for the synthesis of HNK-1 oligosaccharide epitopes



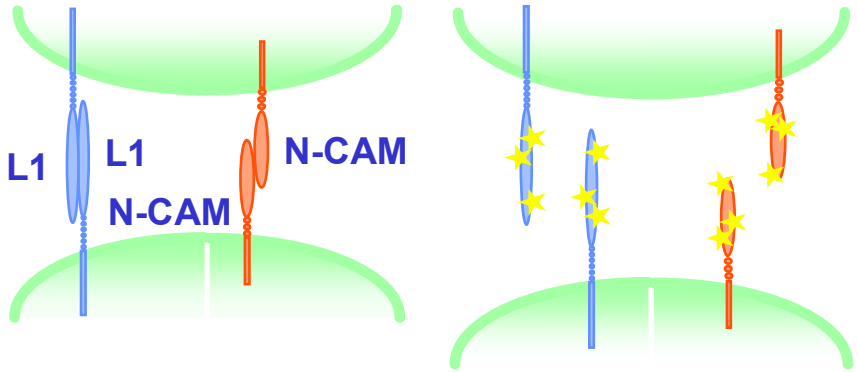
Changes of cells which express HNK-1 sugar chain antibody

Elongation of neurite

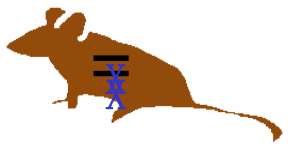


Inhibition of cell adhesion

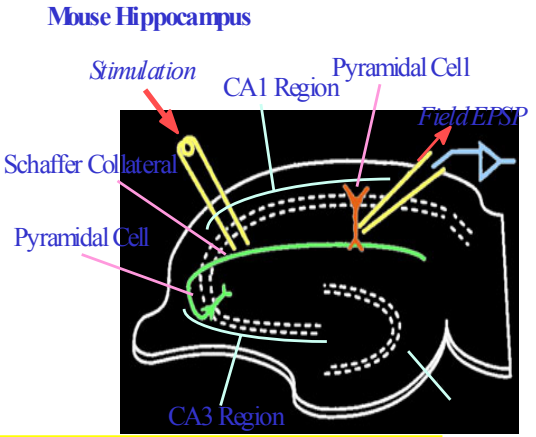
HNK-1 negative cell HNK-1 positive cell



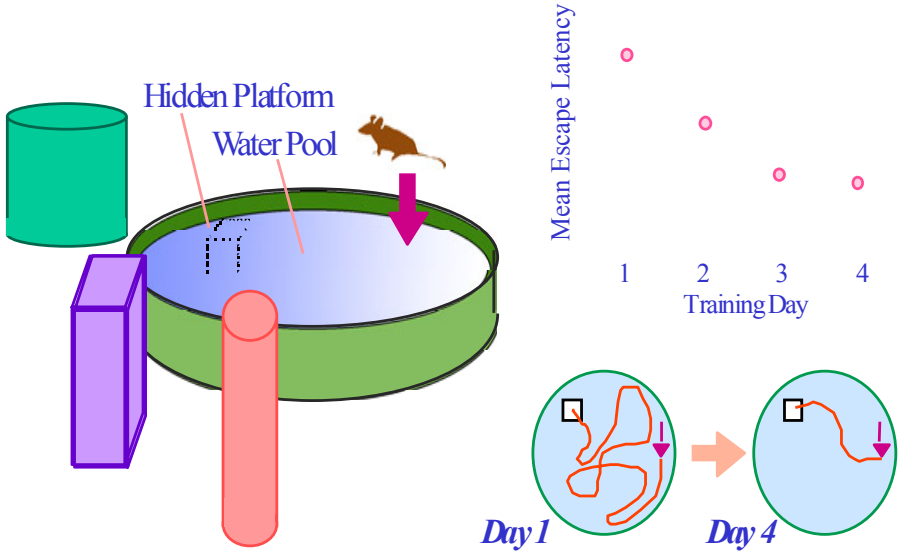
GlcAT-P KO mouse (defective of HNK-1 oligosaccharide synthesis)



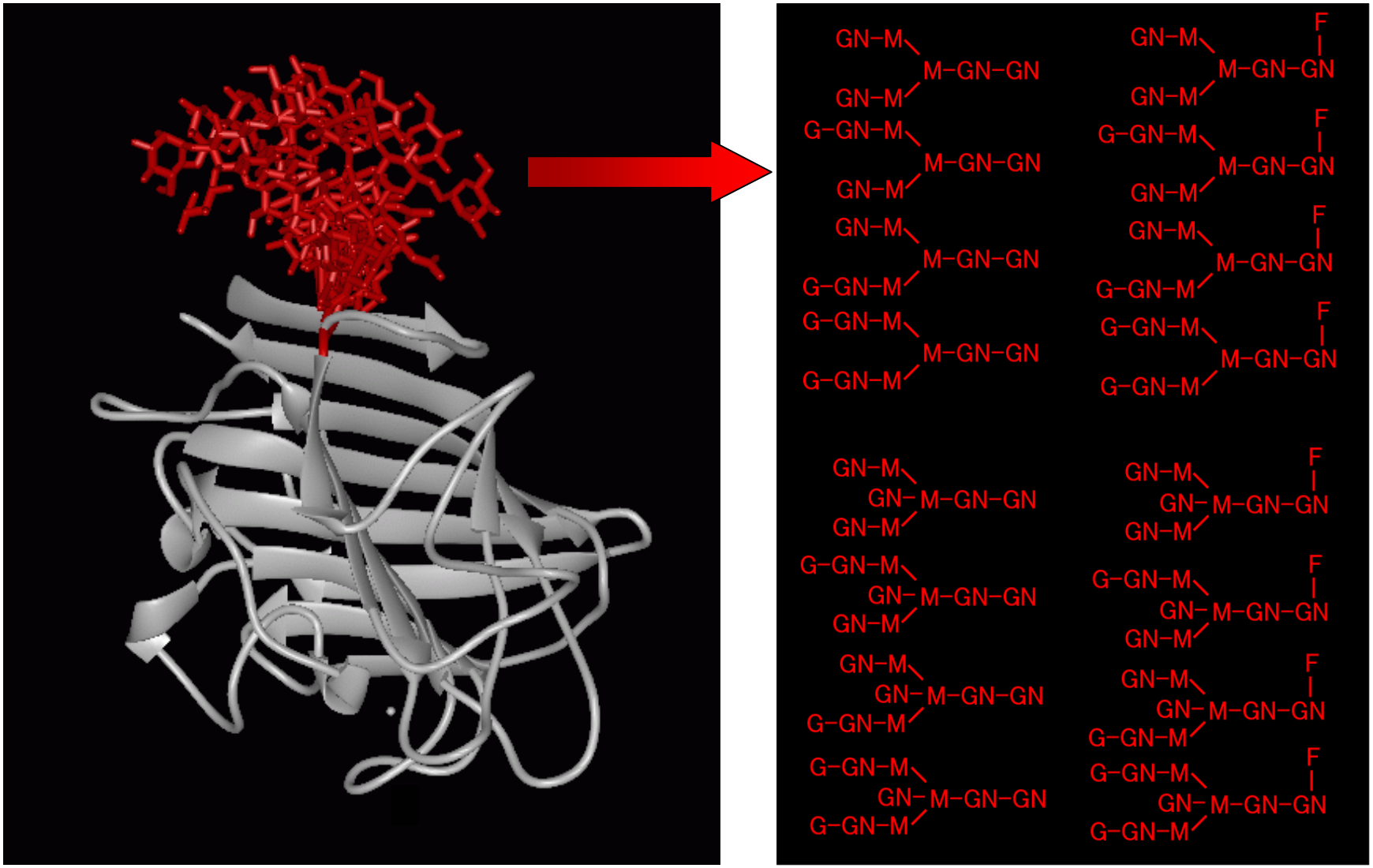
Decrease of long term potentiation (LTR) at hippocampus CA1



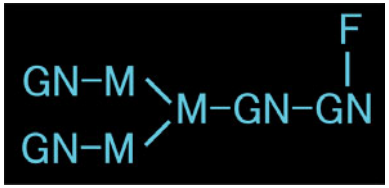
Difficulty of space recognition

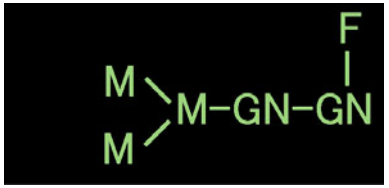


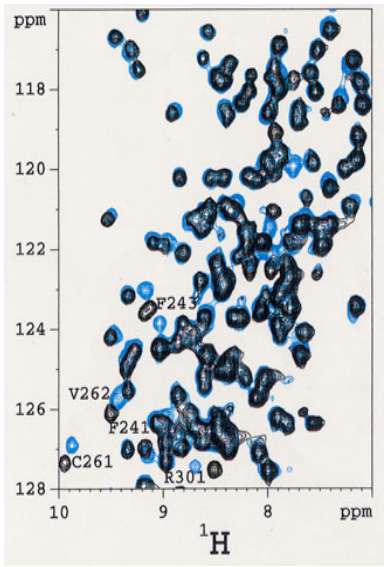
Microheterogeneity and high mobility of oligosaccharides → Difficulty of structural analysis

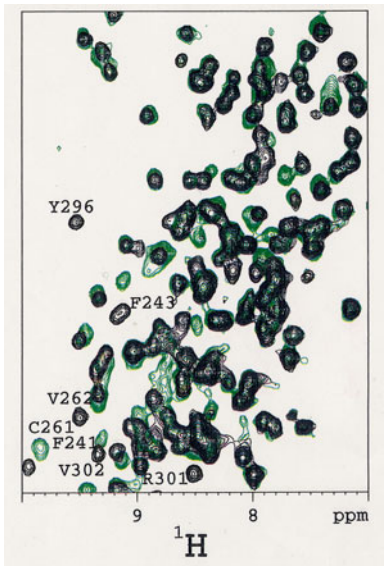


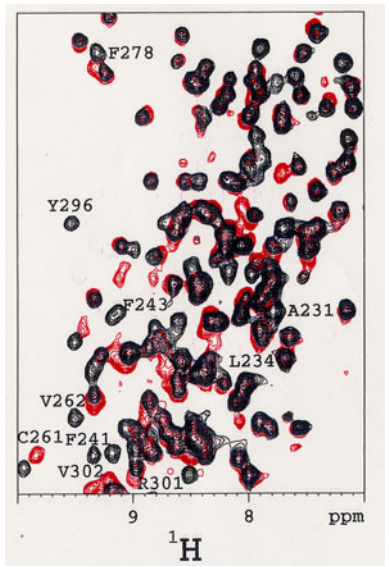
Analysis of dynamics of glycoproteins by NMR

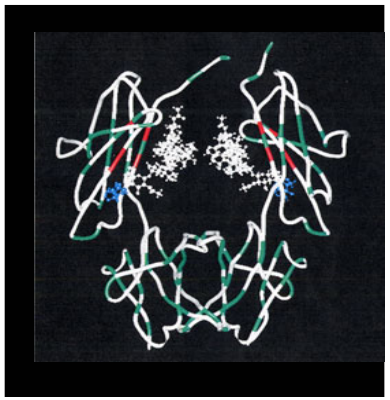


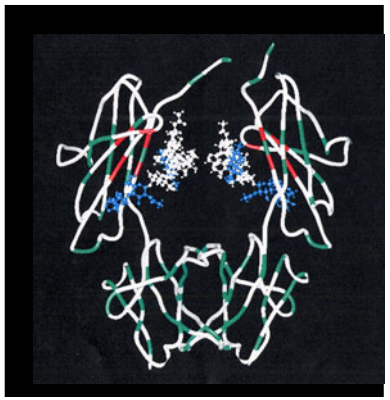


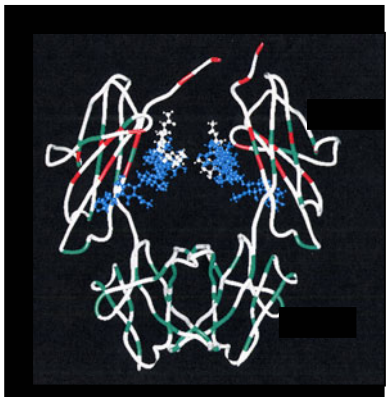












Atomic level observation of dynamics of sugar chains by NMR

Construction of protein expression system

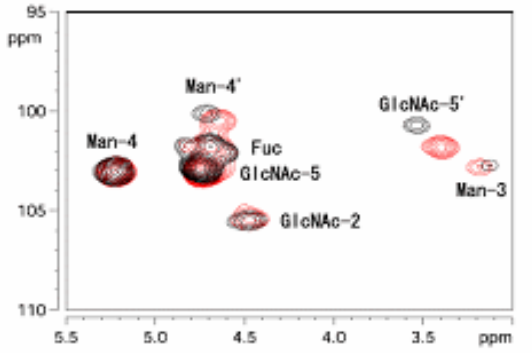
Control of glyco-genes

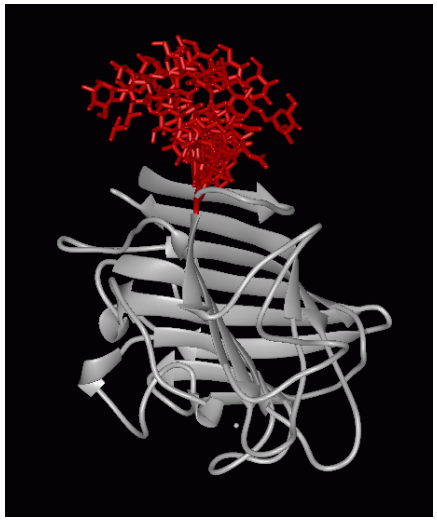
Functional analysis

Multi-dimensional HPLC mapping procedure

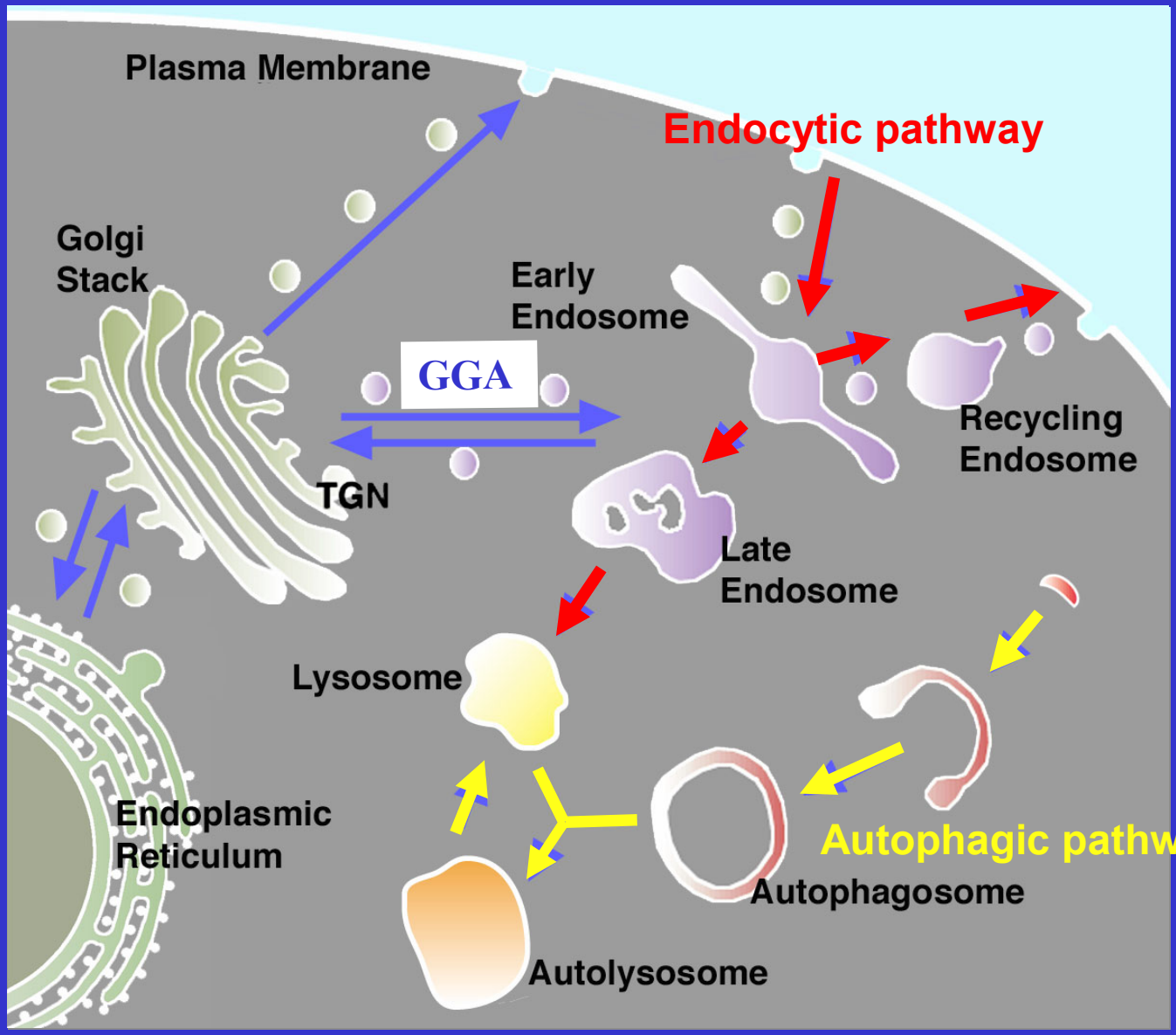
Three dimensional structural analysis by NMR

^{13}C,^{15}N-labeled precursors

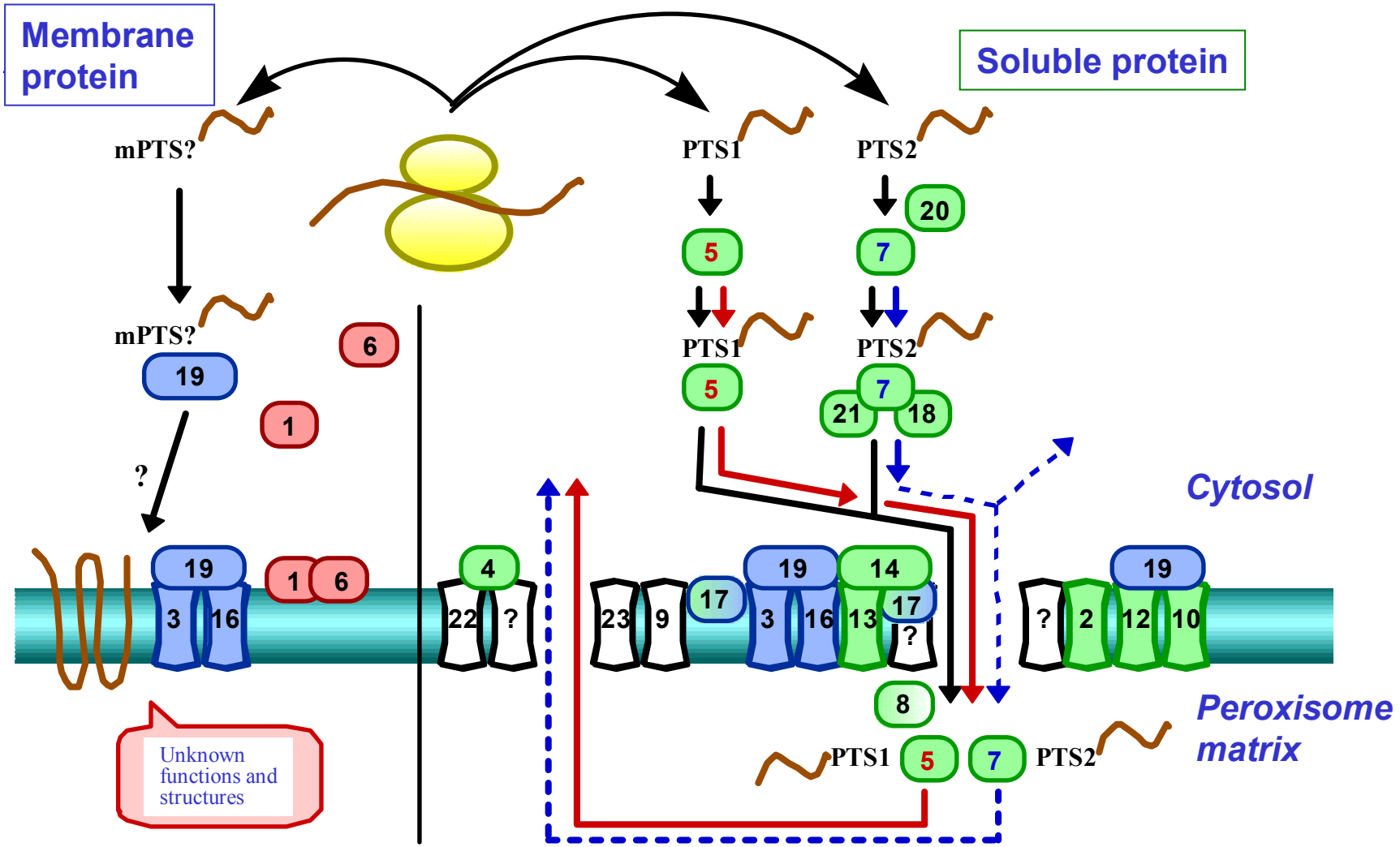




Lysosomal function depends on membrane traffic

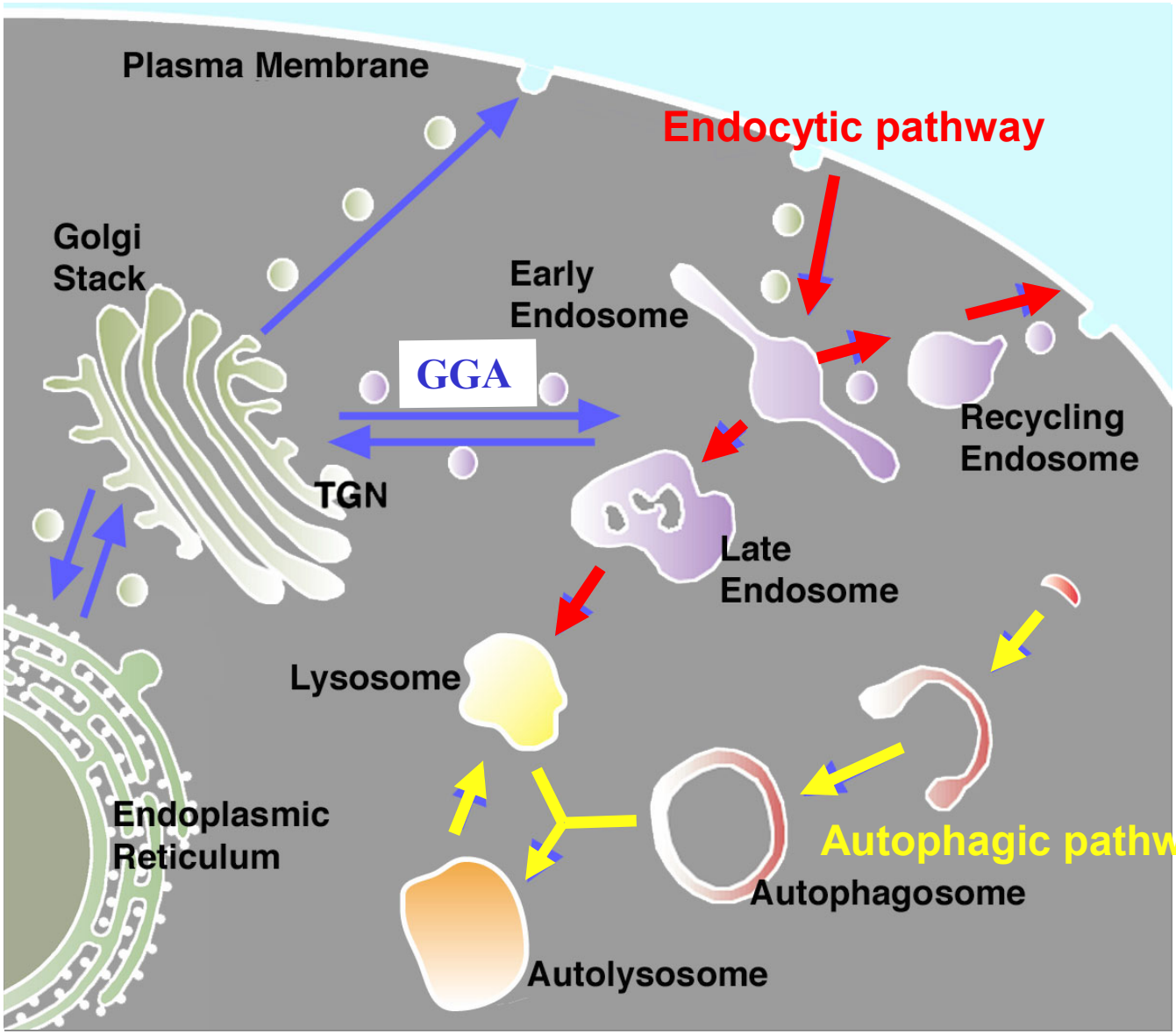


Structural and functional analysis of peroxisome inserted membrane protein and related protein transport system (Pex3p, Pex6p, Pex19p, ABC transporter PMP70)

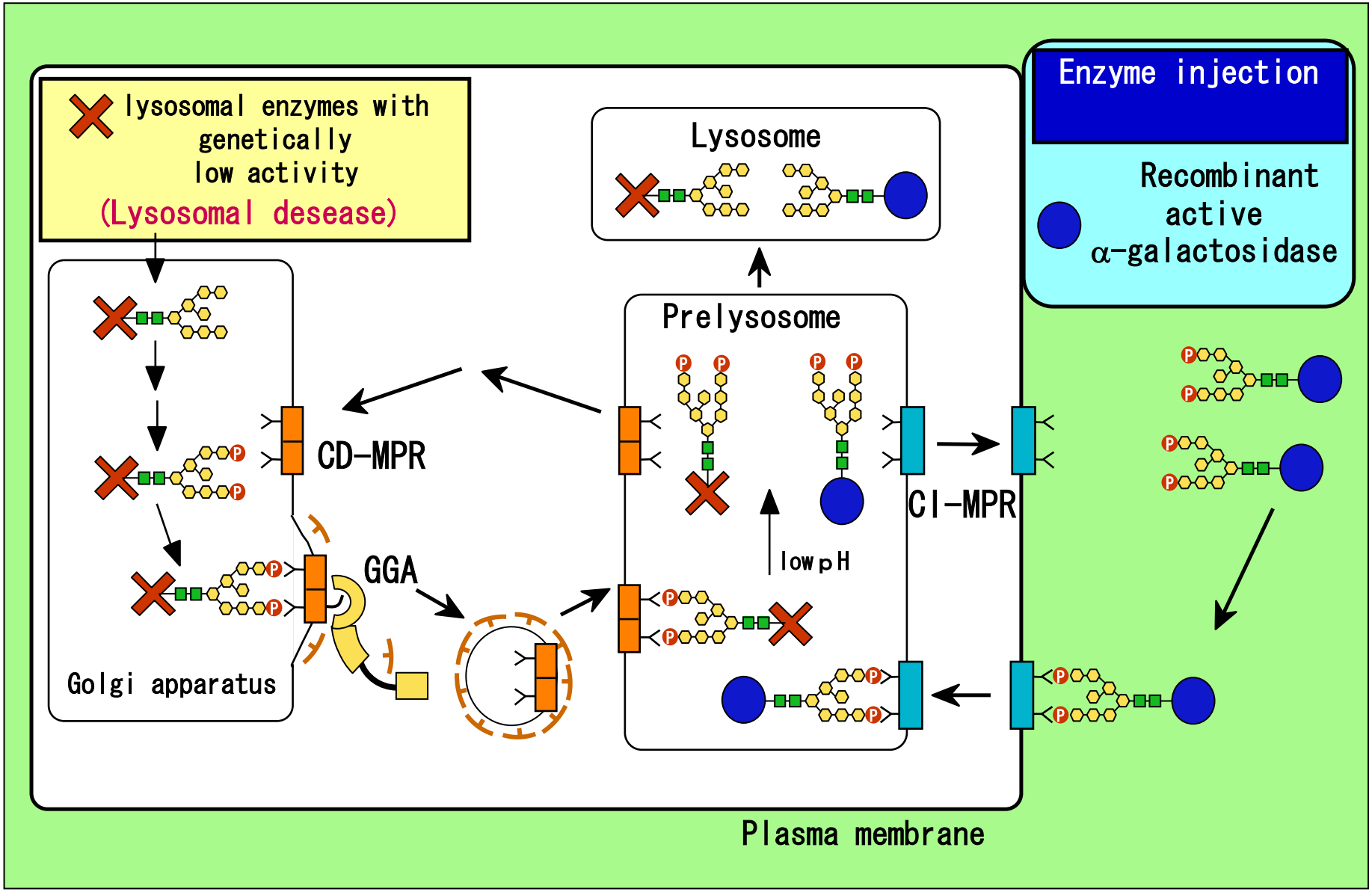


Photon Factory Structural Biology Building
(completed in April 2001)

Lysosomal Function Depends on Membrane Traffic

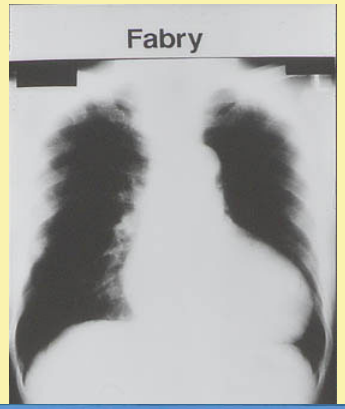


Treatment of lysosomal deseases

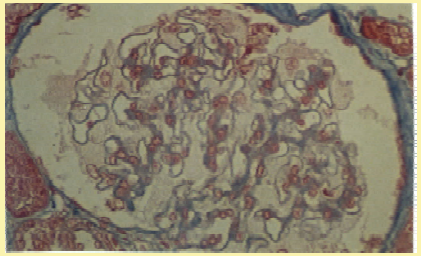


Fabry Disease and Enzyme Replacement Therapy

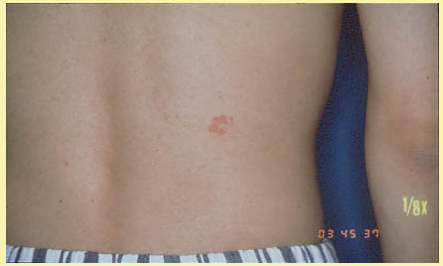
Fabry disease：A disease caused by mutation of α-galactosidase gene, which degrades enzymatic activity of the hydrolase in lysosome leading to accumulation of glycolipids



Enlargement of the heart

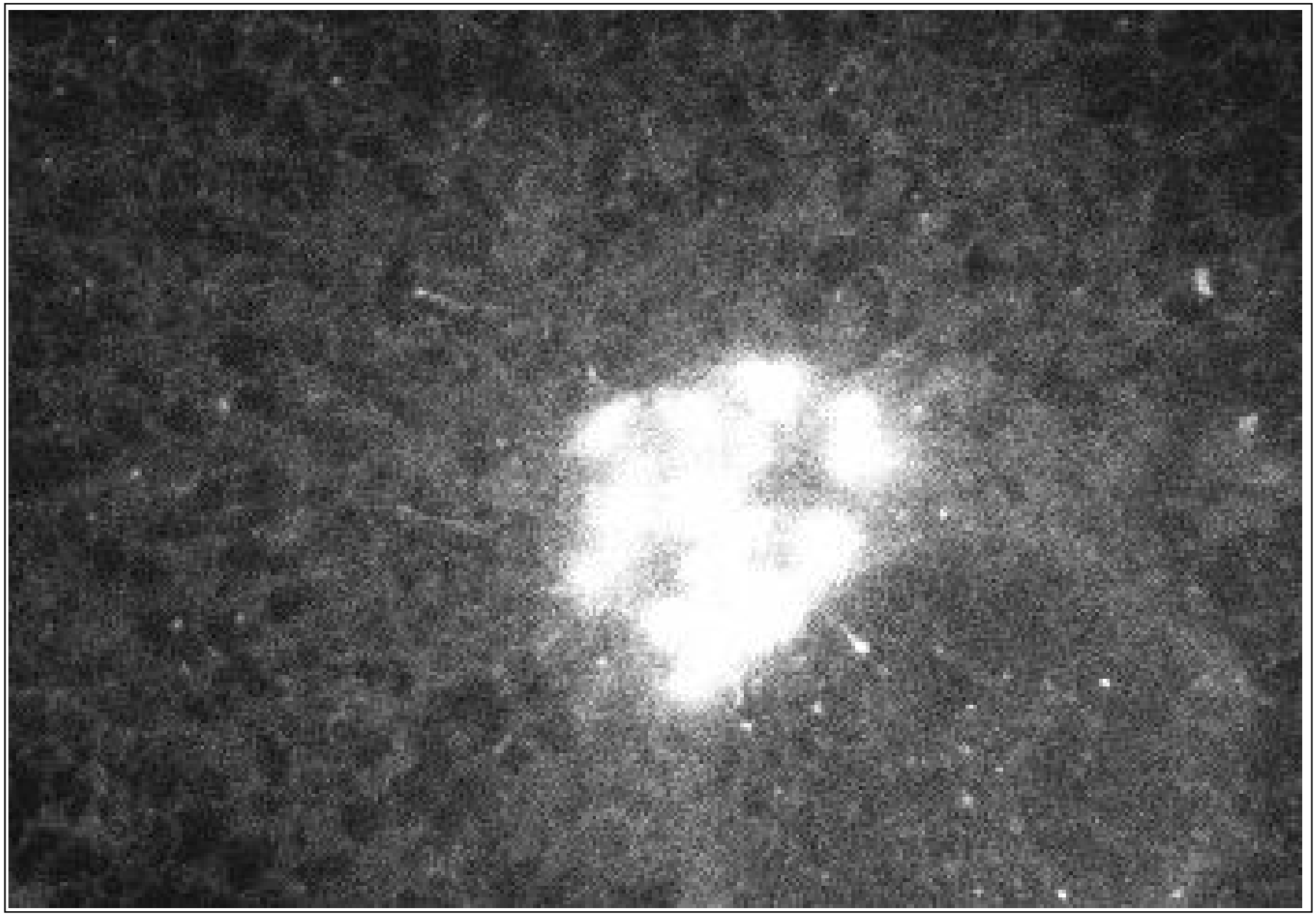


Accumulation of glycolipids in glomerulus

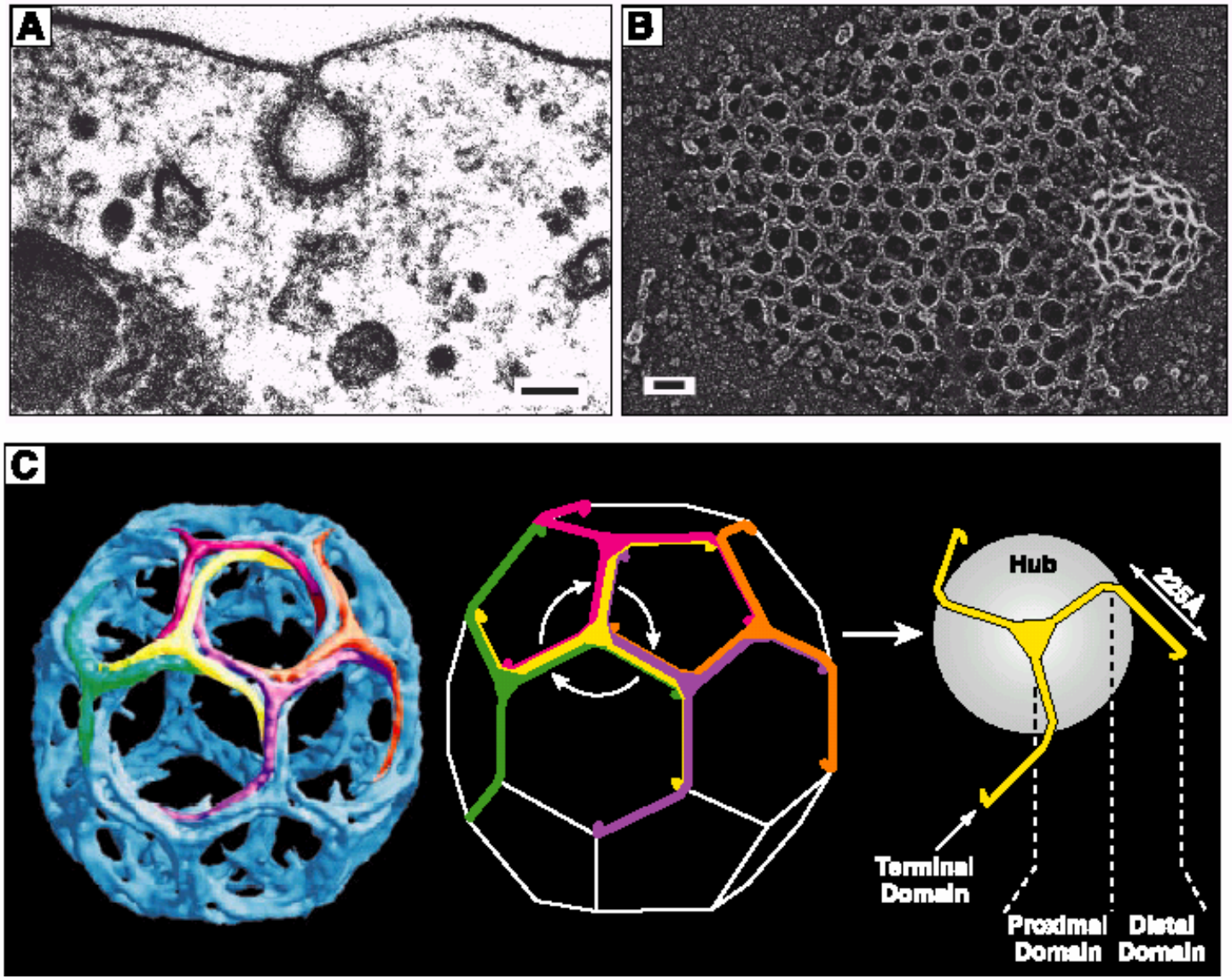


Angiokeratoma

Vesicle transport from the ER to the Golgi apparatus



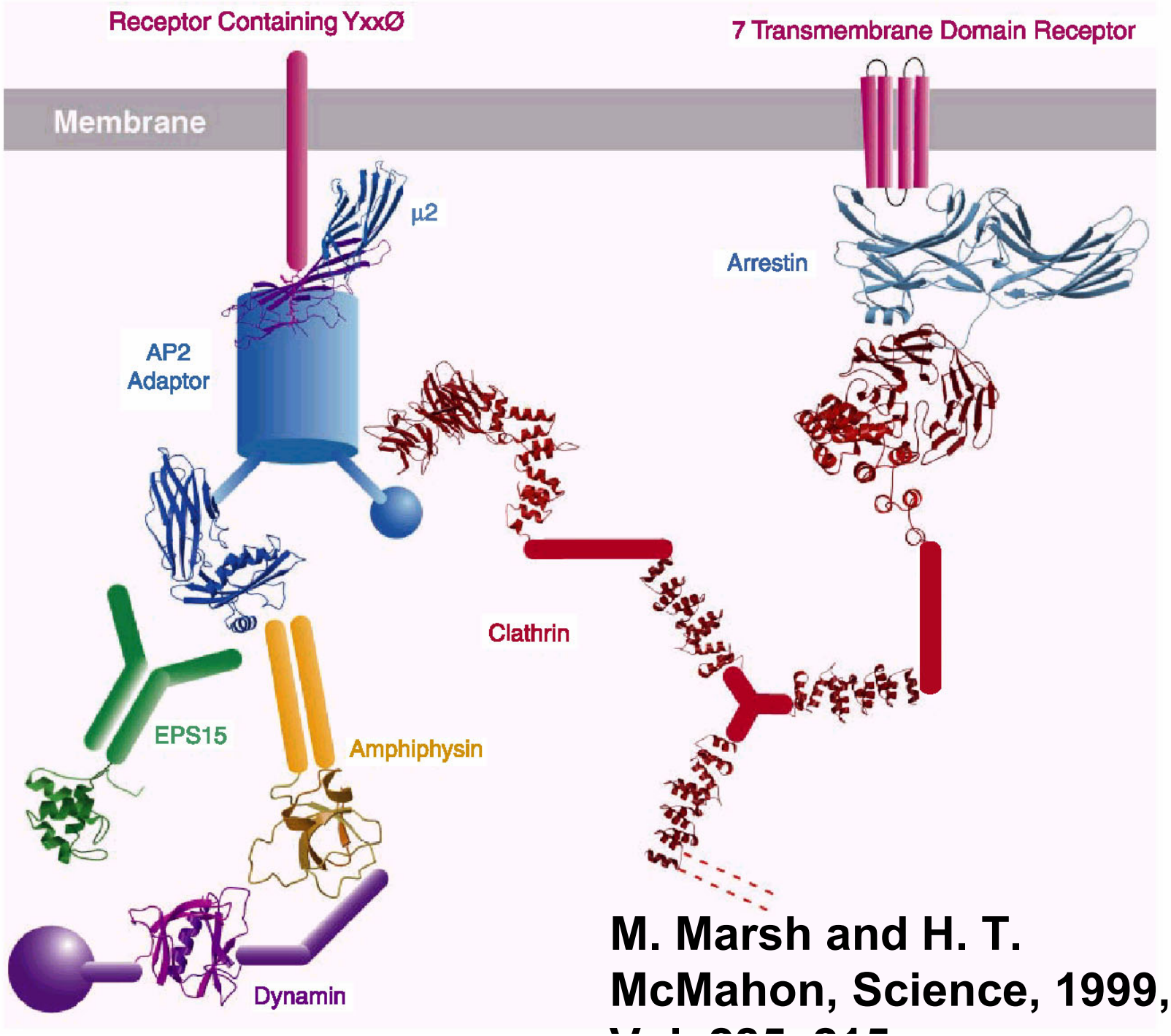
Lippincott-Schwartz, J. (1998) MBC 9, 1617



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

Clathrin movie by Allison Bruce, Harvard University

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



M. Marsh and H. T. McMahon, Science, 1999,

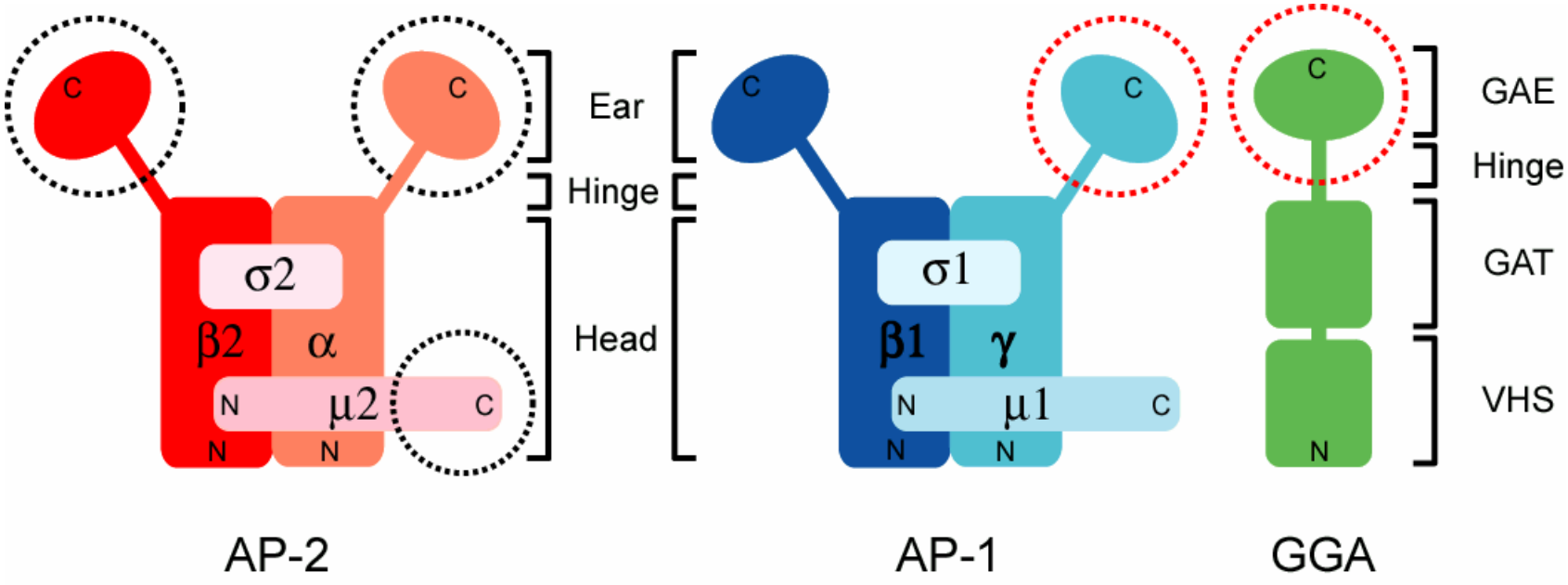
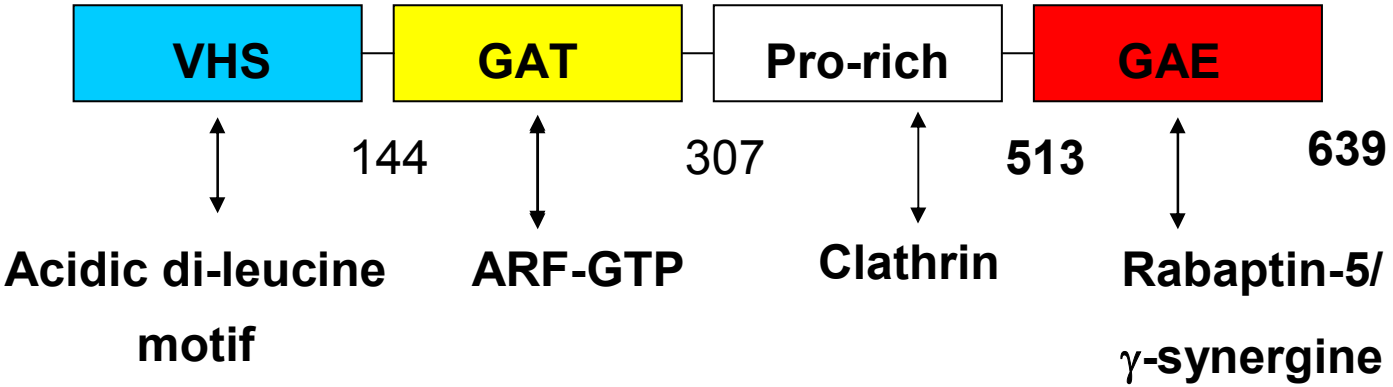


Figure 1 (Nogi *et al.*)

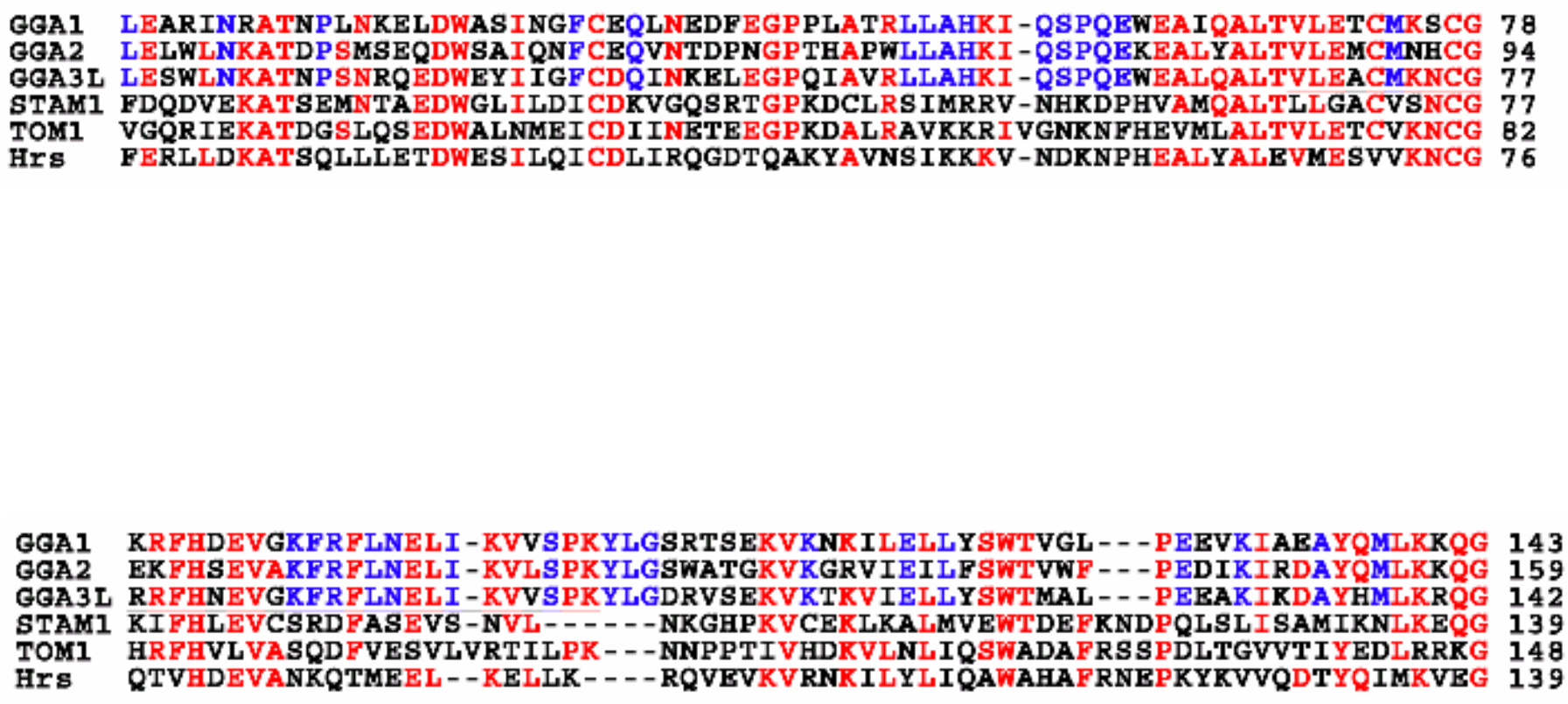
Schematic representation of the domain structure of GGA1

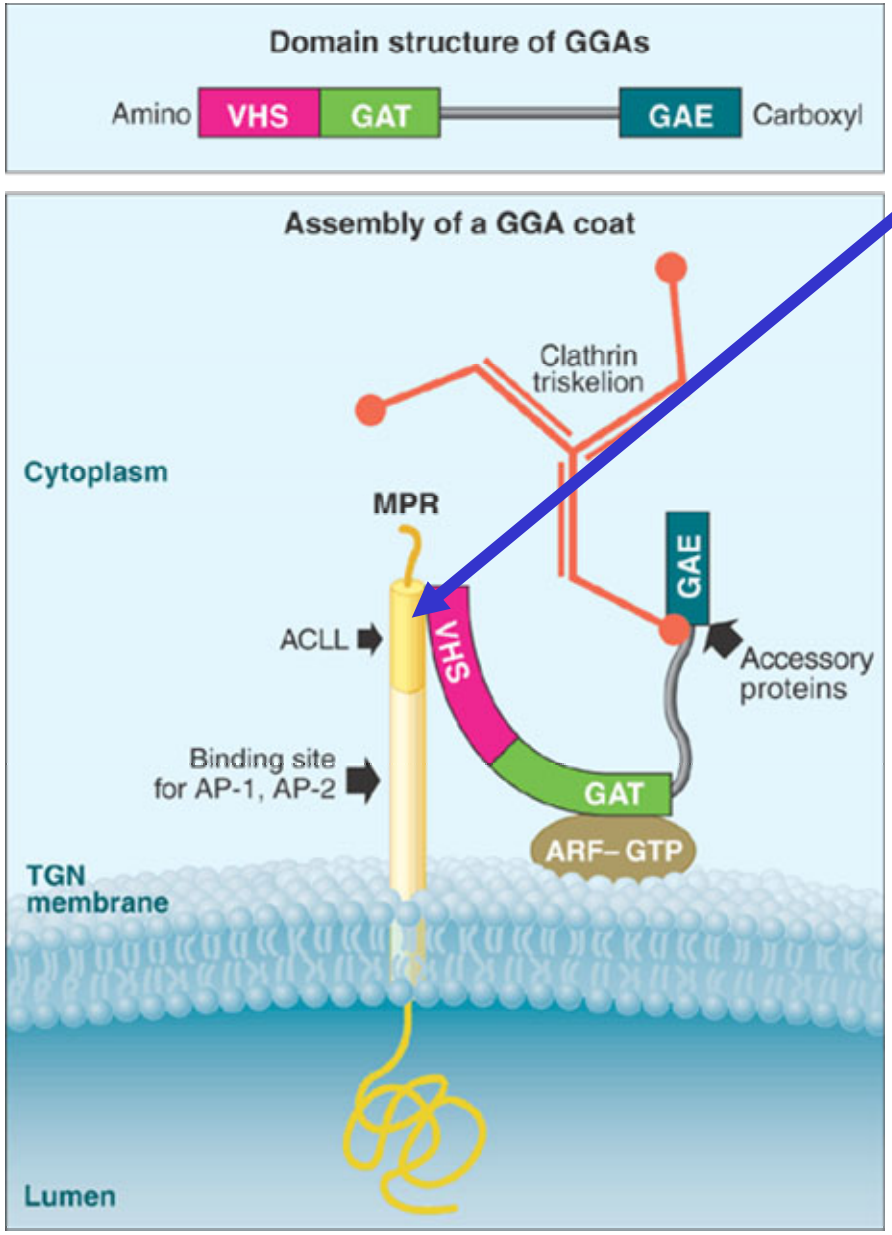


VHS: Vps27p/Hrs/STAM Domain

GAT=GGAH: GGA Homology Domain

GAE=AGEH: Adaptor g Ear Homology Domain


GGA1 LEARINRATNPLNKELDWASINGFCEQLNEDFEGPPLATRLLAHKI-QSPQEWEAIQALTVLETCMKSCG 78
GGA2 LELWLNKATDPSMSEQDWSAIQNFCEQVNTDPNGPTHAPWLLAHKI-QSPQEKEALYALTVLEMCMNHCG 94
GGA3L LESWLNKATNPSNRQEDWEYIIGFCDQINKELEGPQIAVRLLAHKI-QSPQEWEALQALTVLEACMKNCG 77
STAM1 FDQDVEKATSEMNTAEDWGLILDICDKVGQSRTGPKDCLRSIMRRV-NHKDPHVAMQALTLLGACVSNCG 77
TOM1 VGQRIEKATDGSLQSEDWALNMEICDIINETEEGPKDALRAVKKRIVGNKNFHEVMLALTVLETCVKNCG 82
Hrs FERLLDKATSQLLLETDWESILQICDLIRQGDTQAKYAVNSIKKKV-NDKNPHEALYALEVMESVVKNCG 76
GGA1 KRFHDEVGKFRFLNELI-KVVSPKYLGSRTSEKVKNKILELLYSWTVGL---PEEVKIAEAYQMLKKQG 143
GGA2 EKFHSEVAKFRFLNELI-KVLSPKYLGSWATGKVKGRVIEILFSWTVWF---PEDIKIRDAYQMLKKQG 159
GGA3L RRFHNEVGKFRFLNELI-KVVSPKYLGDRVSEKVKTKVIELLYSWTMAL---PEEAKIKDAYHMLKRQG 142
STAM1 KIFHLEVCSRDFASEVS-NVL------NKGHPKVCEKLKALMVEWTDEFKNDPQLSLISAMIKNLKEQG 139
TOM1 HRFHVLVASQDFVESVLVRTILPK---NNPPTIVHDKVLNLIQSWADAFRSSPDLTGVVTIYEDLRRKG 148
Hrs QTVHDEVANKQTMEEL--KELLK----RQVEVKVRNKILYLIQAWAHAFRNEPKYKVVQDTYQIMKVEG 139



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

FEBS 26327 FEBS Letters, on line publication on 9 July 2002 FEBS Letters 524 (2002) 183–187

Memapsin 2 (β-secretase) cytosolic domain binds to the VHS domains of GGA1 and GGA2: implications on the endocytosis mechanism of memapsin 2

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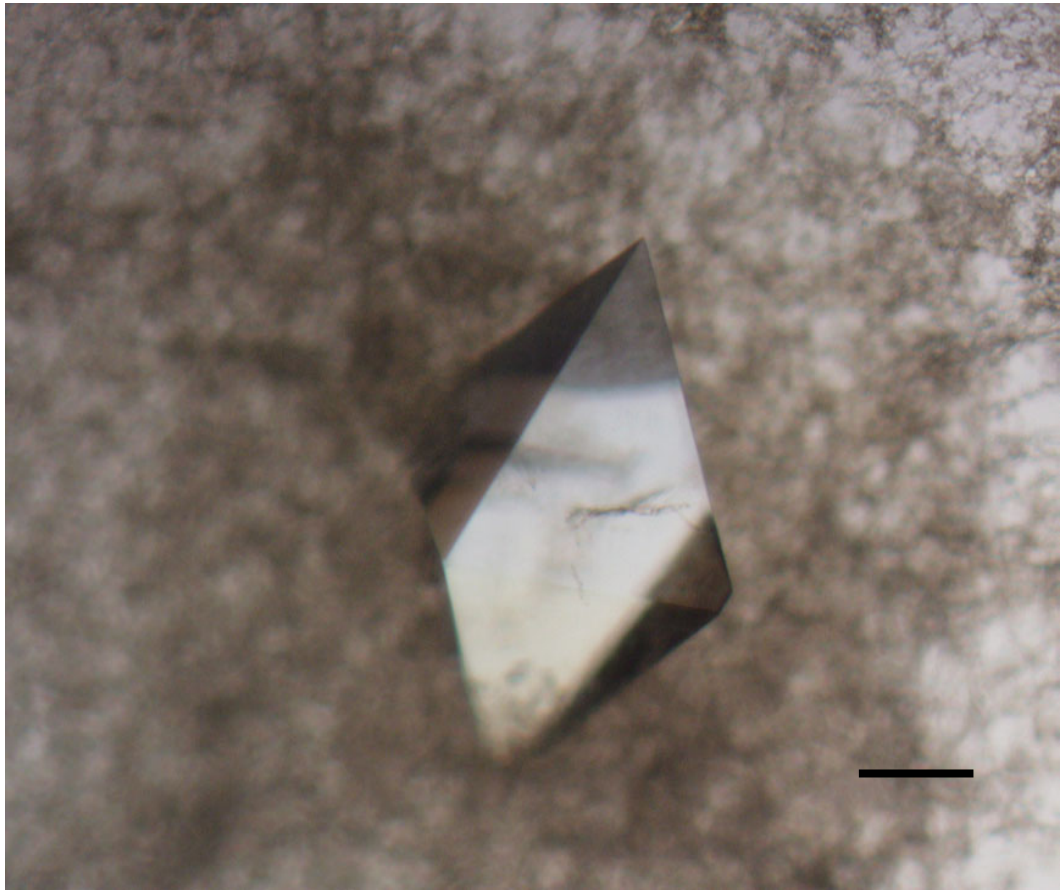
[b] *Zapaq, Inc., Oklahoma City, OK 73104, USA*
[c] *Department of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, USA*

Protein source	Peptide name	Sequence
A. Alignment of C-terminal sequences[a]		
Memapsin 2	M2	-CLRQQHDDFADDISLLK
Mannose-6-phosphate receptor		
Cation-Independent	CI-MPR	-CTKLVSFHDDSDEDLLHI
Cation-Dependent		-DDQLGEESEERDDHLLPM
Sortilin		-TNKSGYHDDDSDEDLLE
LRP3		-PPCSPMLEASDDEALLVC
Memapsin 1	M1	-CQRRQRDPEVVNESSLVRHRWK

Pi

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**

Crystallization of Native VHS domain of human GGA1 and complex with M6PR peptide

	Native	**Complex**
Protein conc.:	**13 mg / ml**	**9 mg / ml**
Precipitant:	**17 % (w/v) PEG3350, 0.2 M KH_2PO_4**	**14 % (w/v) PEG3350, 0.2 M NH_4I**
Buffer:	**0.1 M Tris-HCl (pH 7.5)**	**0.1 M Tris-HCl (pH 7.5)**
G1S : peptide:	**-**	**1:5**
Temperature:	**20 ºC**	**20 ºC**
		M6PRpeptide: SFHDDSDEDLLHI

Crystallographic Data of Native VHS domain of human GGA1 and complex with M6PR peptide

	Native	Complex
Crystal system:	Tetragonal	Orthorhombic
Space group	$P4_32_12$	$P2_12_12_1$
Cell dimensions:	*a* = 55.12, *c* = 105.51 Å	*a* = 55.2, *b*= 65.9, *c* = 101.6 Å
Number of molecule:	1 / asymmetric unit	2 / asymmetric unit
*V*m:	2.39 $Å^3$ / Da	2.57 $Å^3$ / Da
Solvent content:	48.4 %	52.1 %

Diffraction Data Collection Statistics of Native VHS domain of human GGA1 and complex with M6PR peptide

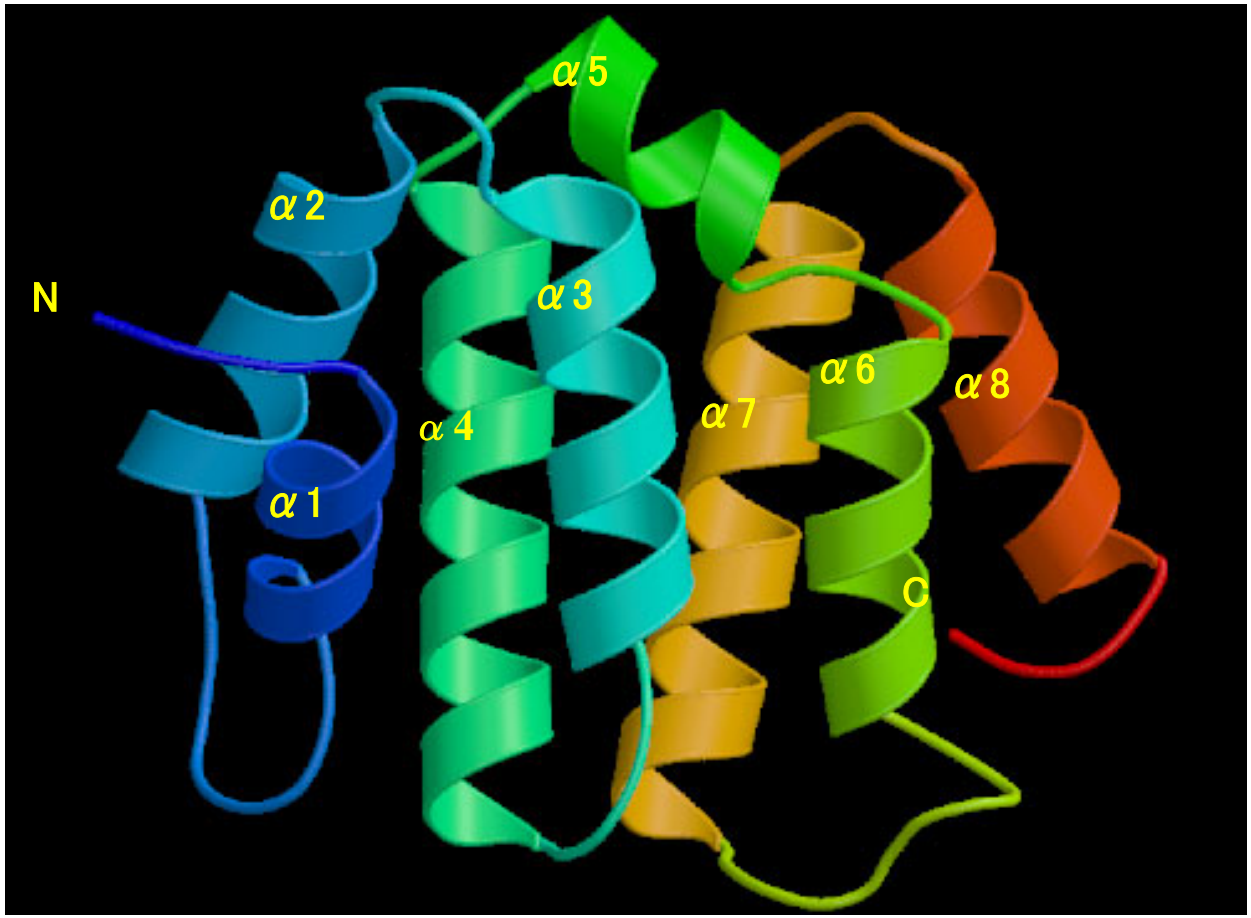
	Native		Complex	
X–ray source:	Synchrotron PF-BL6B		ALS 5.0.2	
Wavelength:	1.0 Å		1.0 Å	
Temperature:	Room temperature		100 K	
Resolution:	15 – 2.1 Å		30 –2.0 Å	
Total reflections:	39,125		167,862	
Unique reflections:	9,580		25,975	
Completeness:	95.6 %	(89.4)	99.6 %	(99.3)
R_{merge} (I):	4.4 %	(28.8)	6.7 %	(35.9)
I / sigma:	25.8	(4.7)	7.1	(1.9)

Values in parentheses are for the highest resolution shell; (2.17 – 2.1 Å) for the native and (2.11 –2.0 Å) for the complex

Refinement Statistics of Native VHS domain of human GGA1 and complex with M6PR peptide

	Native	Complex
Resolution range (Å)	15.0 – 2.1	30 - 2.0
Number of reflections (completeness)		24,632 (99.7%)
Number of non – hydrogen atoms (protein)		2,246
Number of non – hydrogen atoms (peptide)	-	103
Number of Water molecules		206
Number of iodide Ion molecules	-	6
R_{total} (%)	20.3	22.7
R_{work} (%)		22.5
R_{free} (%)	26.1	26.0
Average *B* - value (Å^2)		38.85
R.m.s. deviation from ideal values		
Bond length (Å)	0.039	0.011
Bond angle （°）	3.128	1.313

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



Protein preparation started on 23 April, 2001

Structure solved on 28 May, 2001

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

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

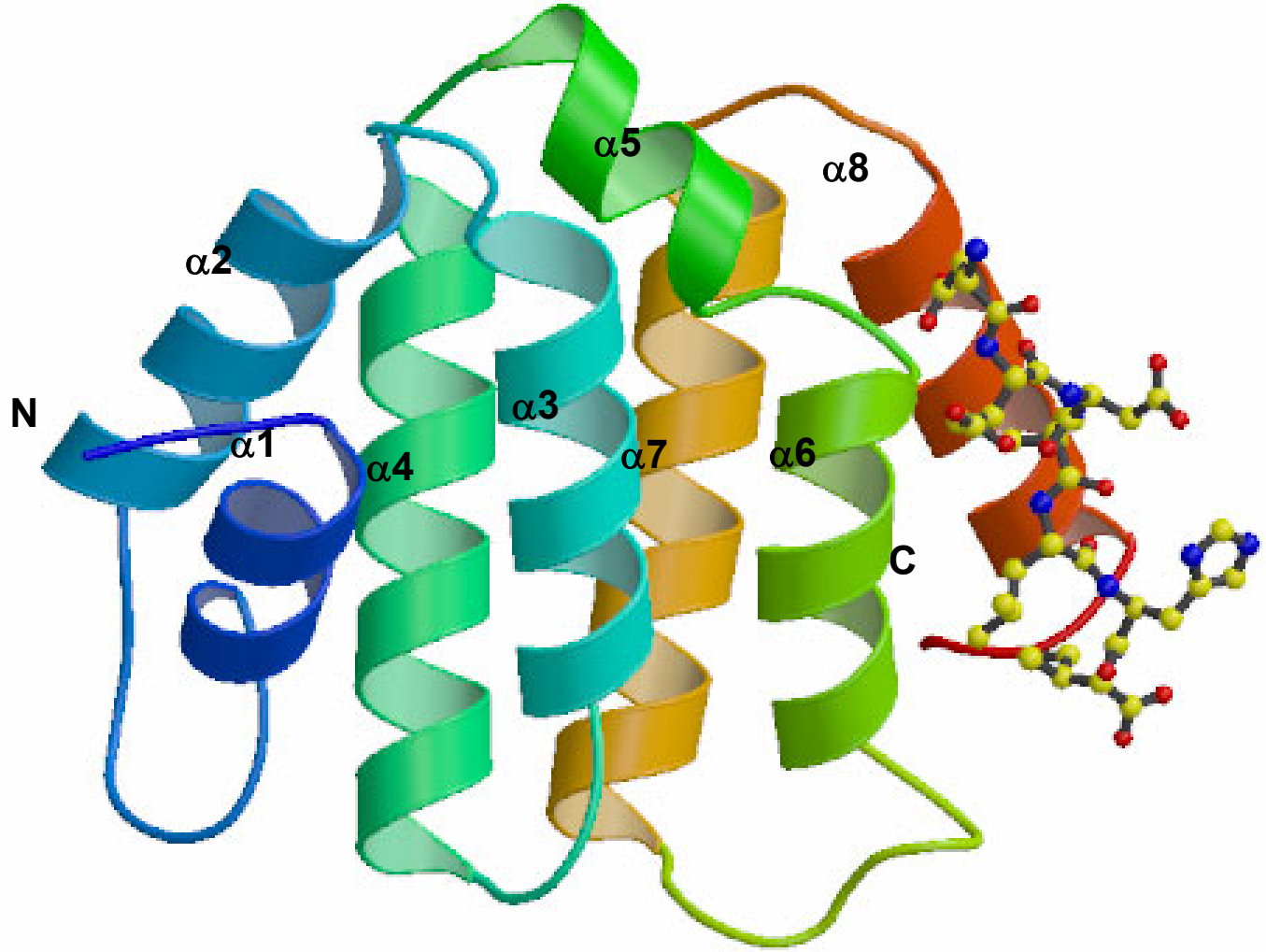
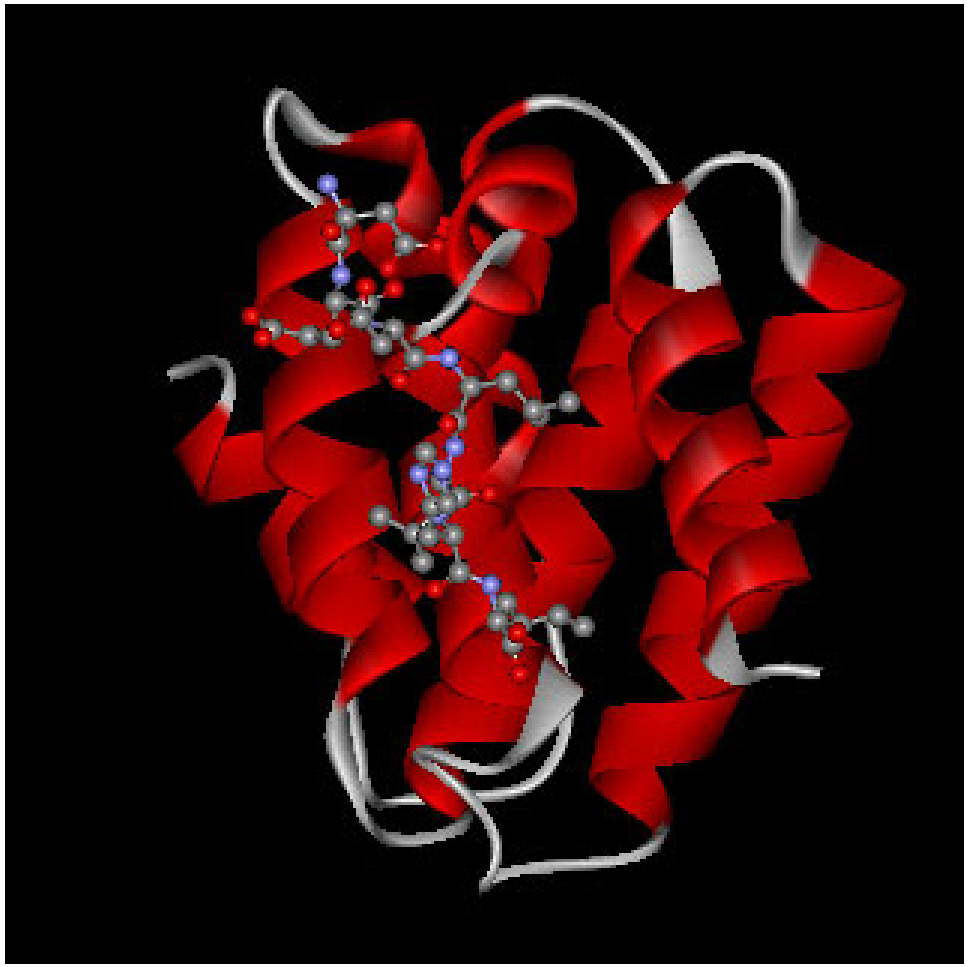


Fig.1
Ribbon diagram of VHS domain of human GGA1 complex with M6PR peptide. The peptide molecule is shown as a ball-and-stick model colored according to atom type (nitrogen, blue; carbon, yellow; oxygen, red).



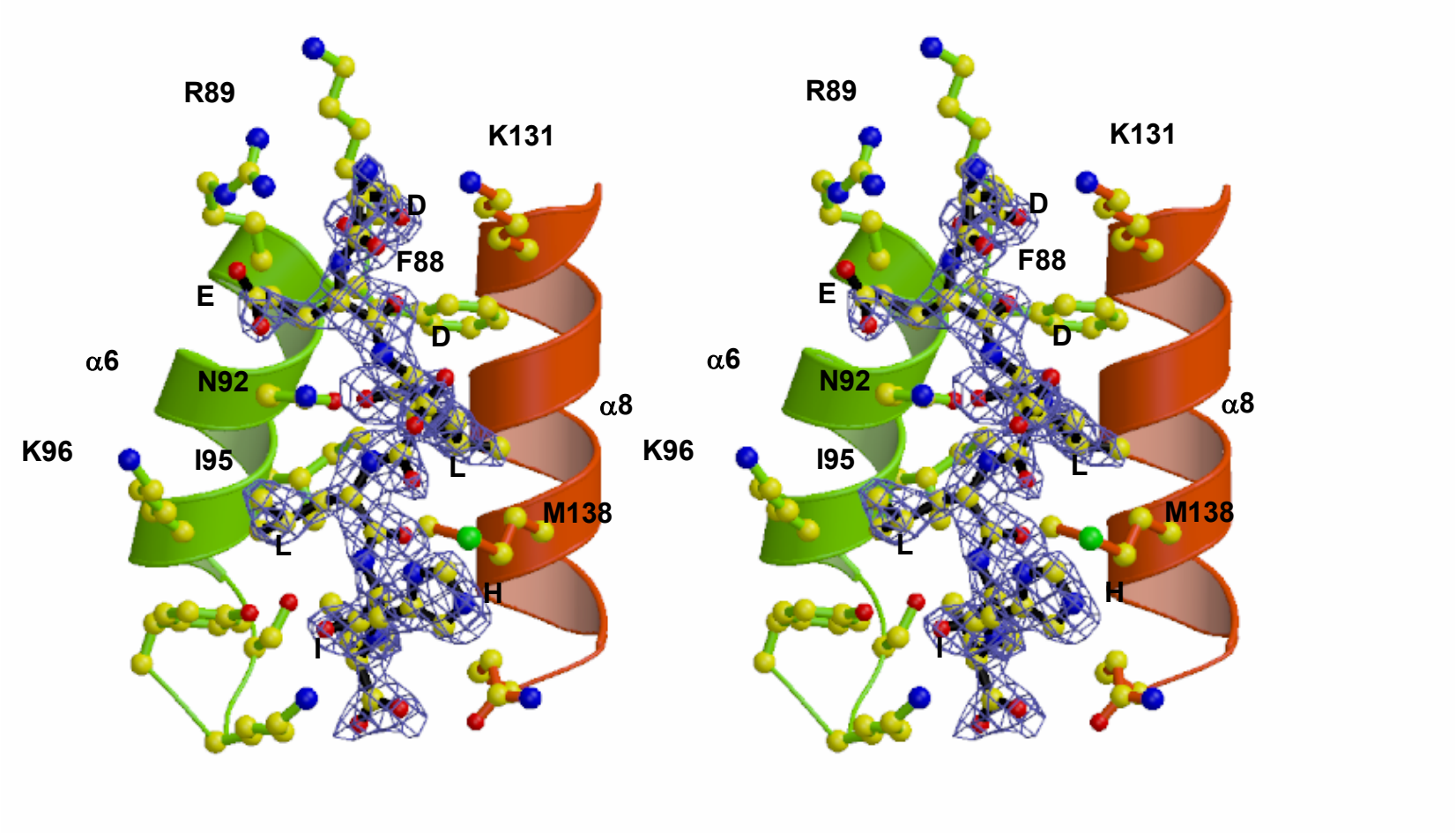
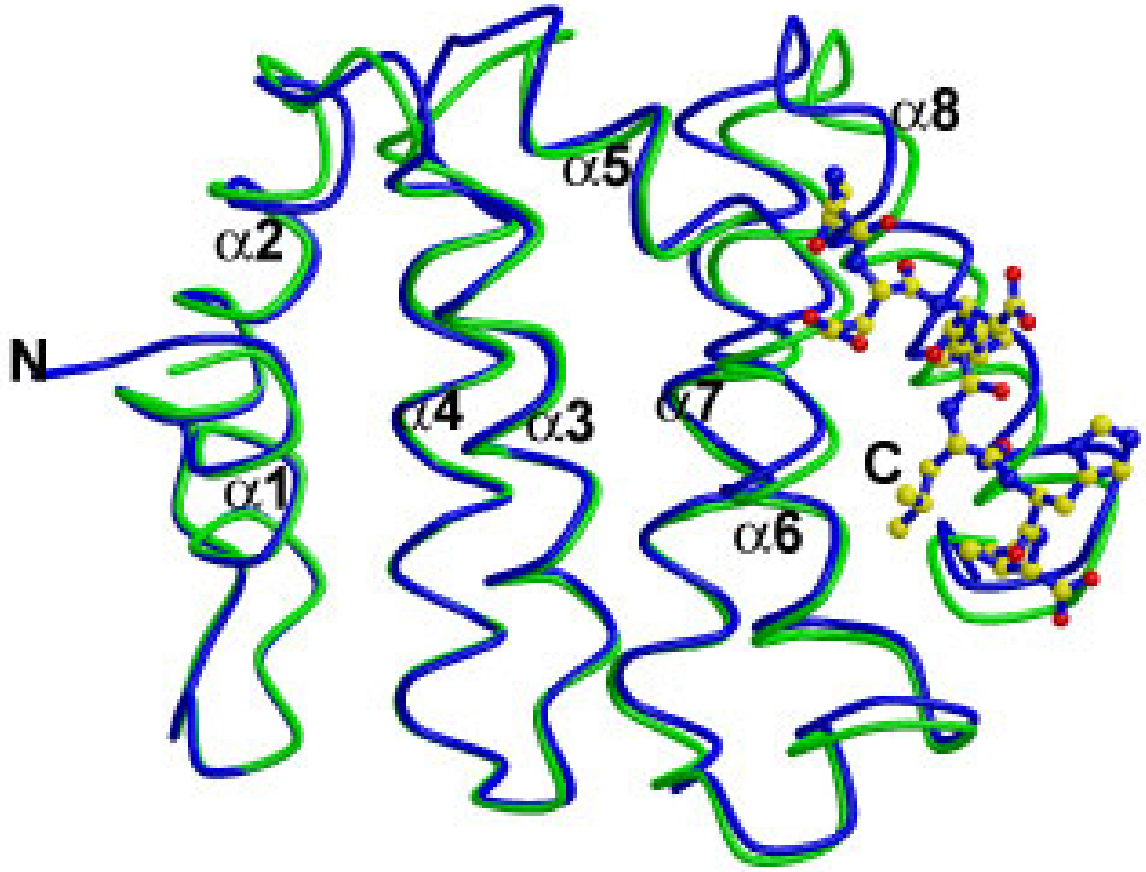
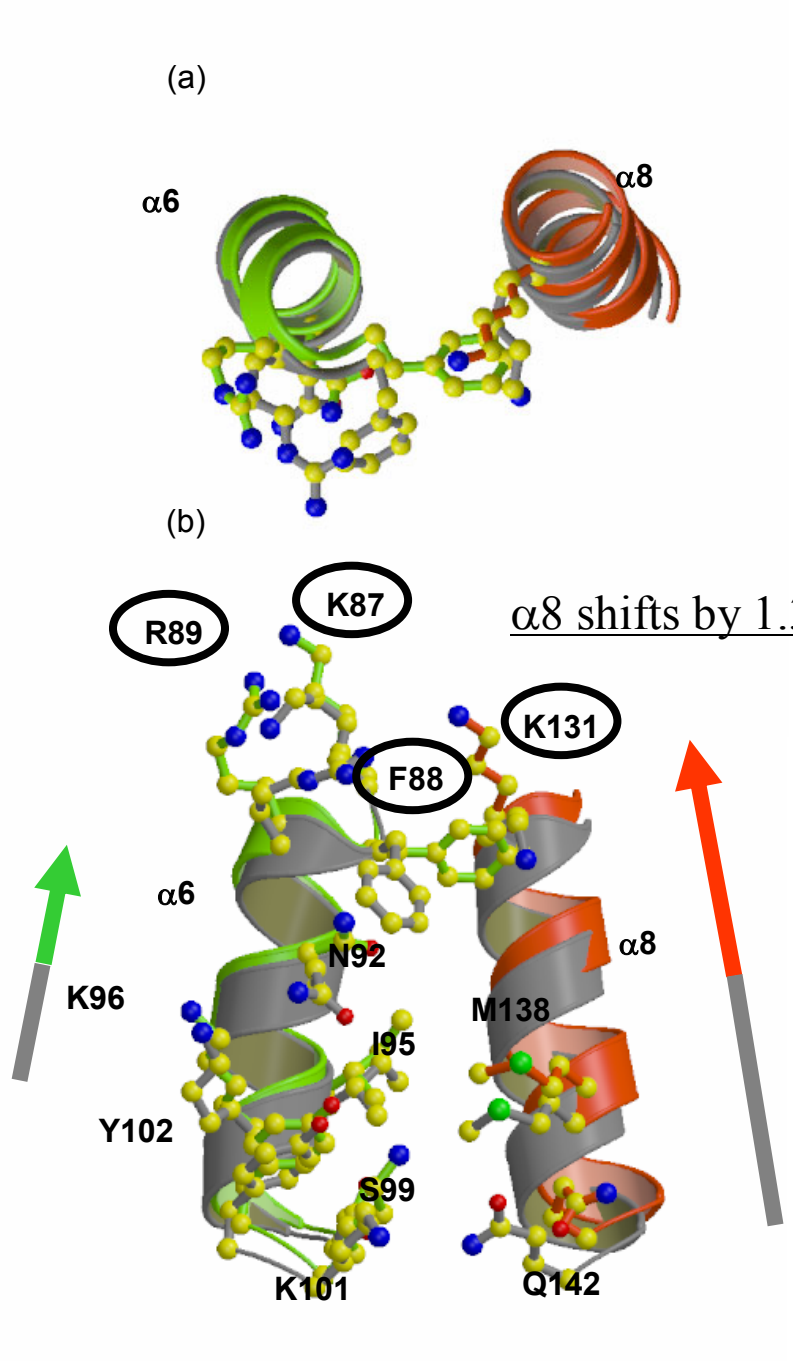


Fig. 3
Stereo view of the omit Fo – Fc electron density map of M6PR peptide (chain C). The map is contoured at 3.0 σ. The peptide molecule is shown as a ball-and-stick model colored by atom type (nitrogen, blue; carbon, yellow; oxygen, red) and bond color is shown as black. The protein (chain A) residues, which interaction with the peptide (chain C) is shown as a ball-and-stick model.



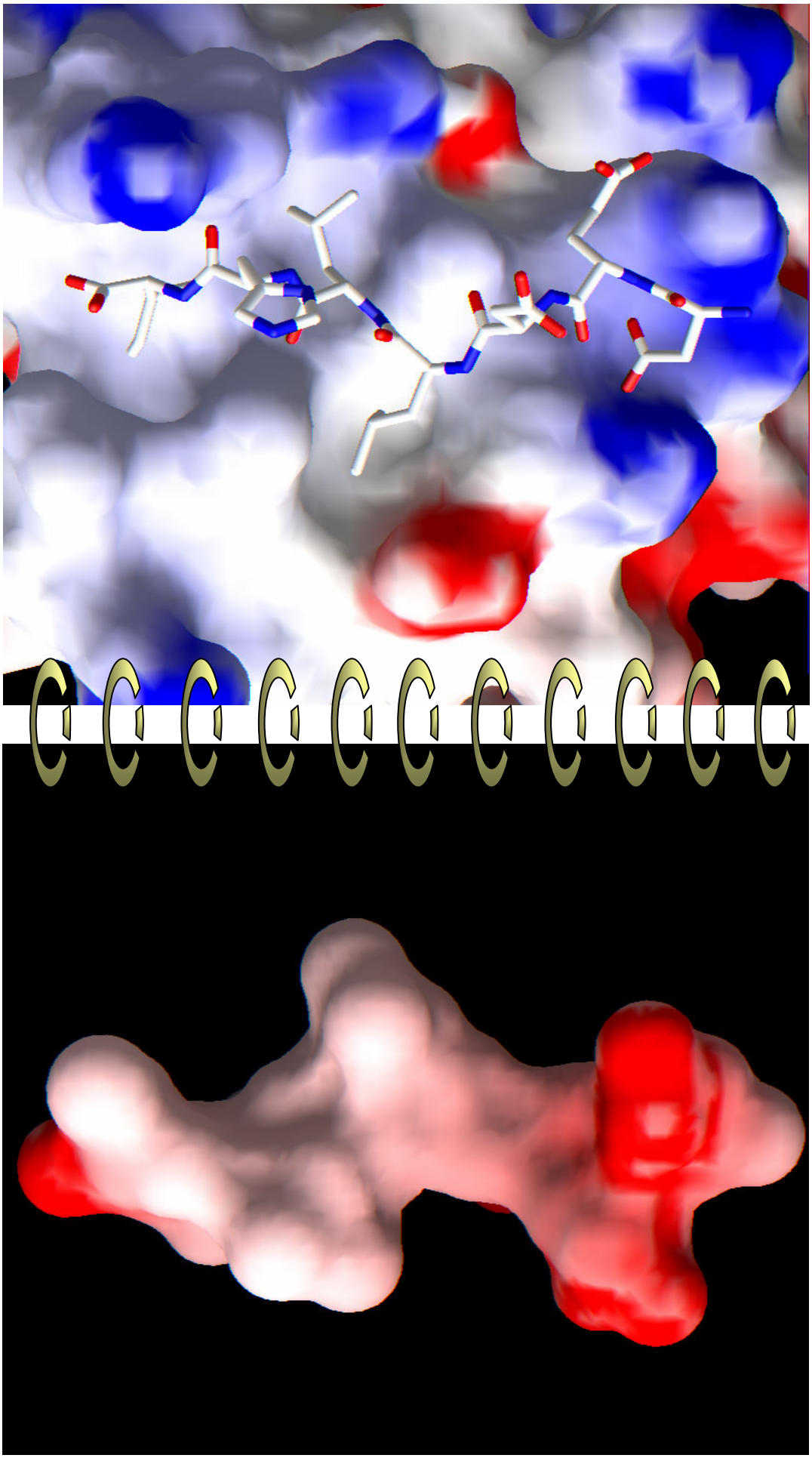
Superposition of the **native GGA1 VHS domain (green)** and **its complex with the ACLL peptide (blue)**. The peptide molecule is shown as a ball-and-stick model.

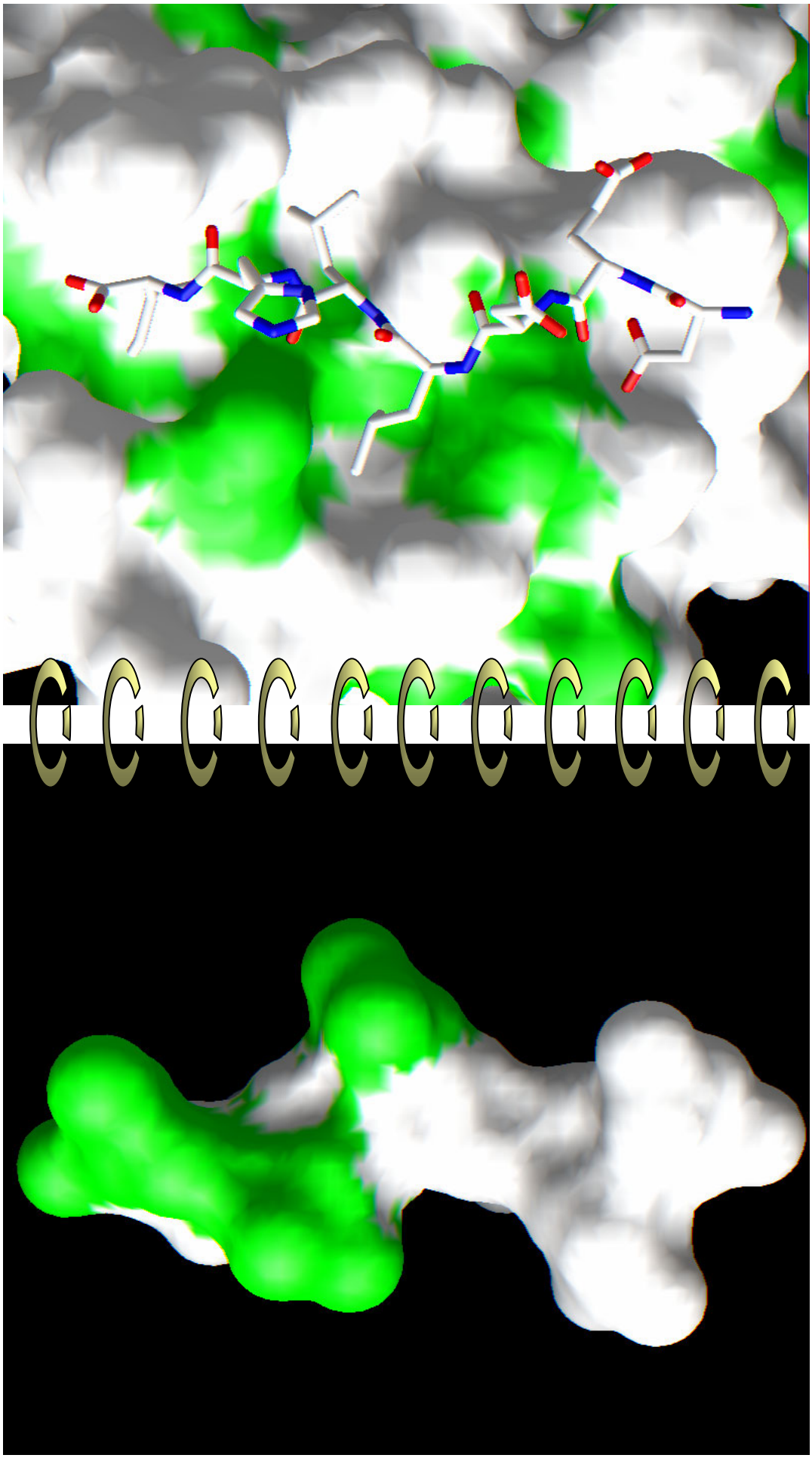
Structural changes of the GGA-VHS are rather small upon binding of the MP6R signal peptide



Summary on GGA1 VHS complexed with CI-M6PR

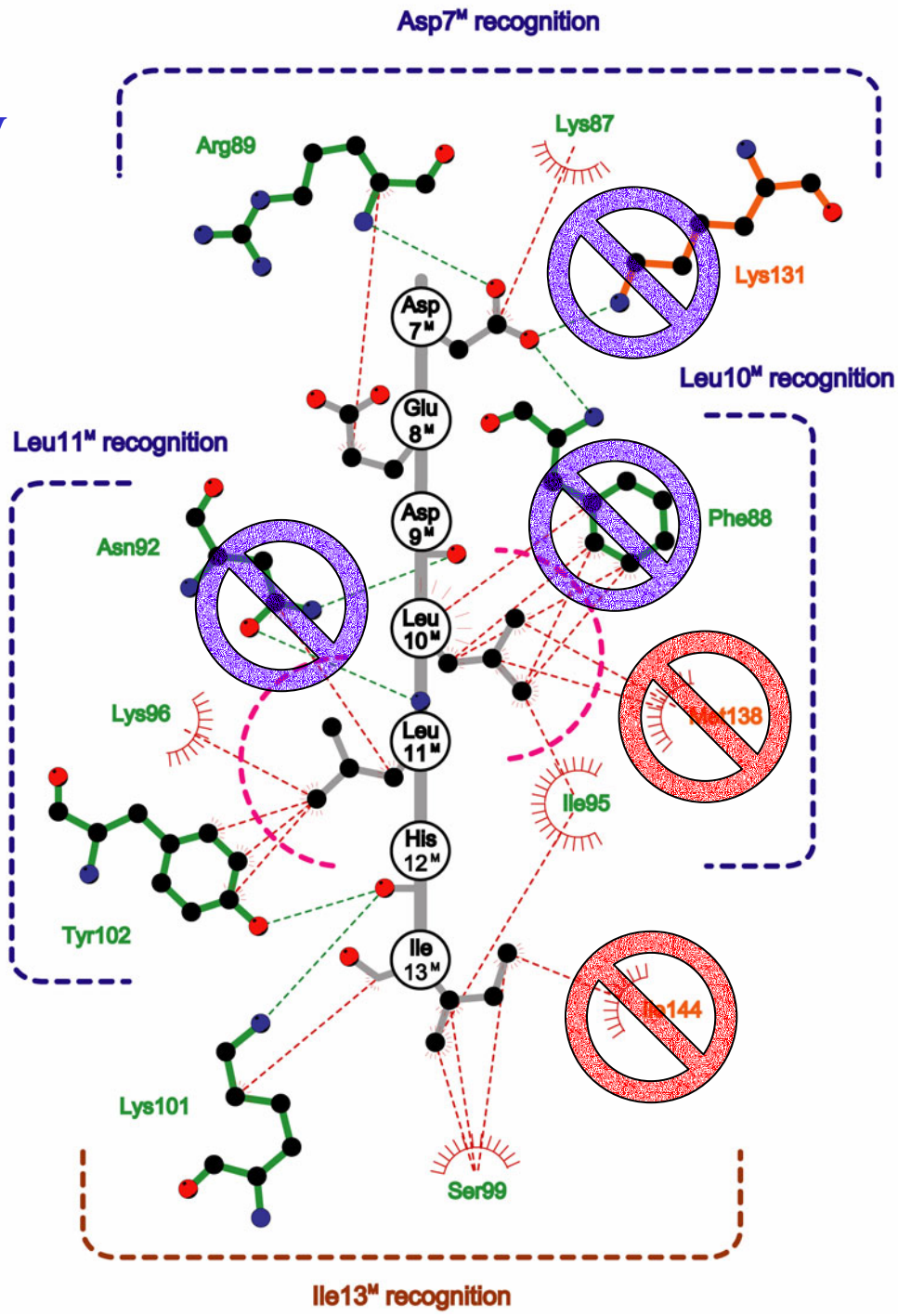
- Similar to Tom1 and Hrs, GGA1 VHS domain forms a super helix with 8 α-helices.
- There are no drastic changes in the superhelical structure upon binding of the cation independent mannose 6-phosphate receptor (CI-M6PR) C-terminal peptide except for the linear movement of Helix 8 by 1.3 Å towards the N-terminal end of the helix and upward flipping of Lys87, Phe88, Arg89, Asn92, and Lys131.
- Helices 6 and 8 and adjoining loops are responsible for recognition of the acidic dileucine motif of M6PR C-terminal peptide.
- The $Asp7^{M}$ of CI-M6PR is mainly recognized by basic residues of the protein whereas $Leu10^{M}$ and $Leu11^{M}$ find themselves in tight packing with hydrophobic residues and hydrophobic parts of Tyr102 and Lys96.
- CI-MPR specific recognition is achieved by the interaction between $His12^{M}$ and $Ile13^{M}$ to the adjoining loops of Helices 6 and 8.
- Lys101 of the C-terminal loop of Helix 6 is expected to play a crucial role in recognizing glutamic acid at the C-terminal end of sotilin.
- Taken together, the structure of GGA1 VHS domain in complex with CI-M6PR shows the intricate recognition of M6P receptor in the vesicle transport of soluble proteins destined for lysosomes in the cell. This is the first example of protein-protein interaction of a VHS domain.


C C C C C C C C C C C



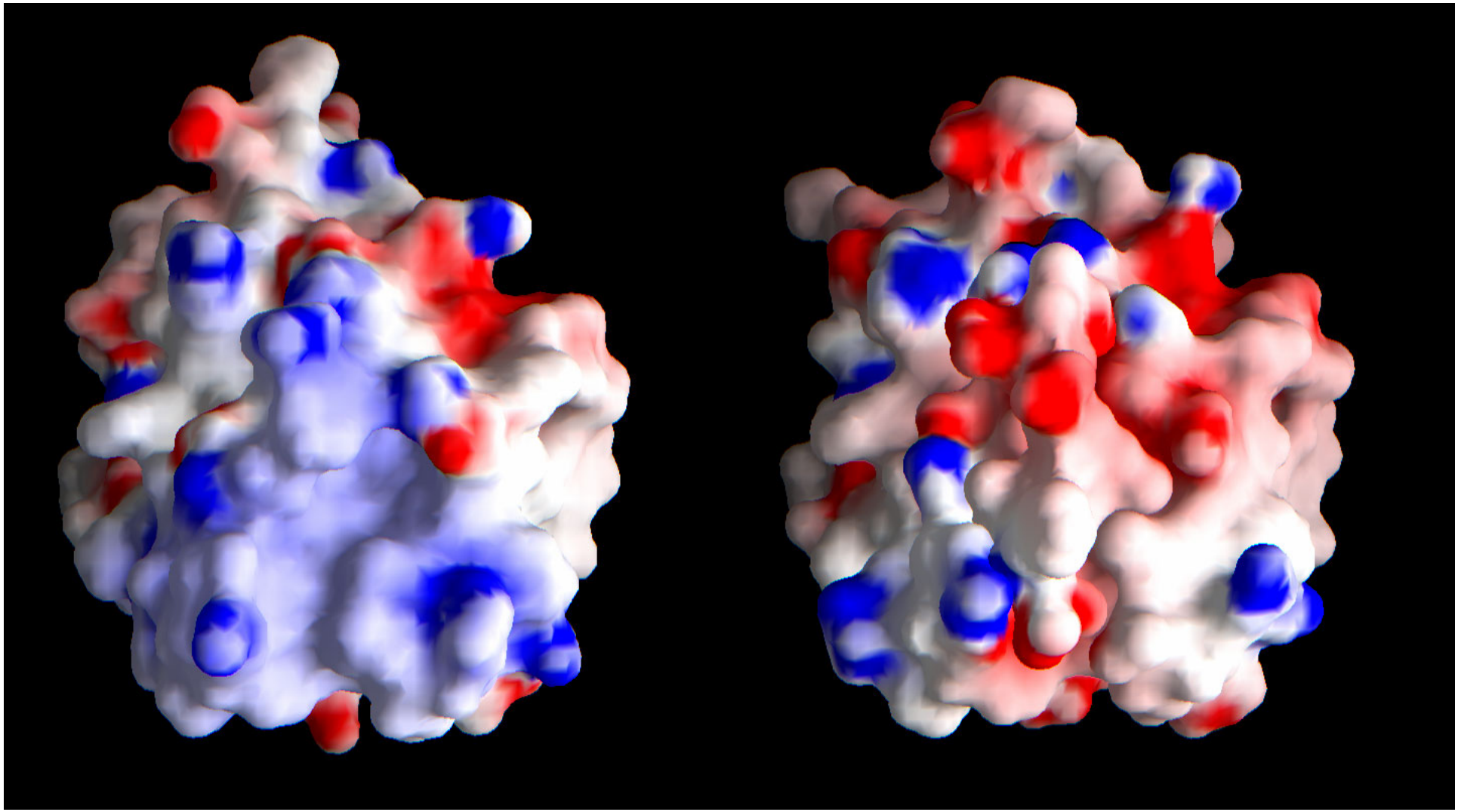
M6PR recognition by GGA1-VHS

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



VHS domain only

VHS domain + M6PR peptide



GGA1-VHS M6PR complex structure: Acknowledgement

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H. Takatsu & K. Nakayama

ALS, Berkeley, CA, USA

T. Earnest

Photon Factory Structural Biology Group

T. Shiba, T. Nogi, N. Matsugaki, M. Suzuki, N. Igarashi, M. Kawasaki, & R. Kato

Diffraction Data Collection Statistics of Native VHS domain of human GGA1 and complex with M6PR peptide

	Native		Complex	
X–ray source:	Synchrotron PF-BL6B		ALS 5.0.2	
Wavelength:	1.0 Å		1.0 Å	
Temperature:	Room temperature		100 K	
Resolution:	15 – 2.1 Å		30 –2.0 Å	
Total reflections:	39,125		167,862	
Unique reflections:	9,580		25,975	
Completeness:	95.6 %	(89.4)	99.6 %	(99.3)
R_{merge} (I):	4.4 %	(28.8)	6.7 %	(35.9)
I / sigma:	25.8	(4.7)	7.1	(1.9)

Values in parentheses are for the highest resolution shell; (2.17 – 2.1 Å) for the native and (2.11 –2.0 Å) for the complex

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*

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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*

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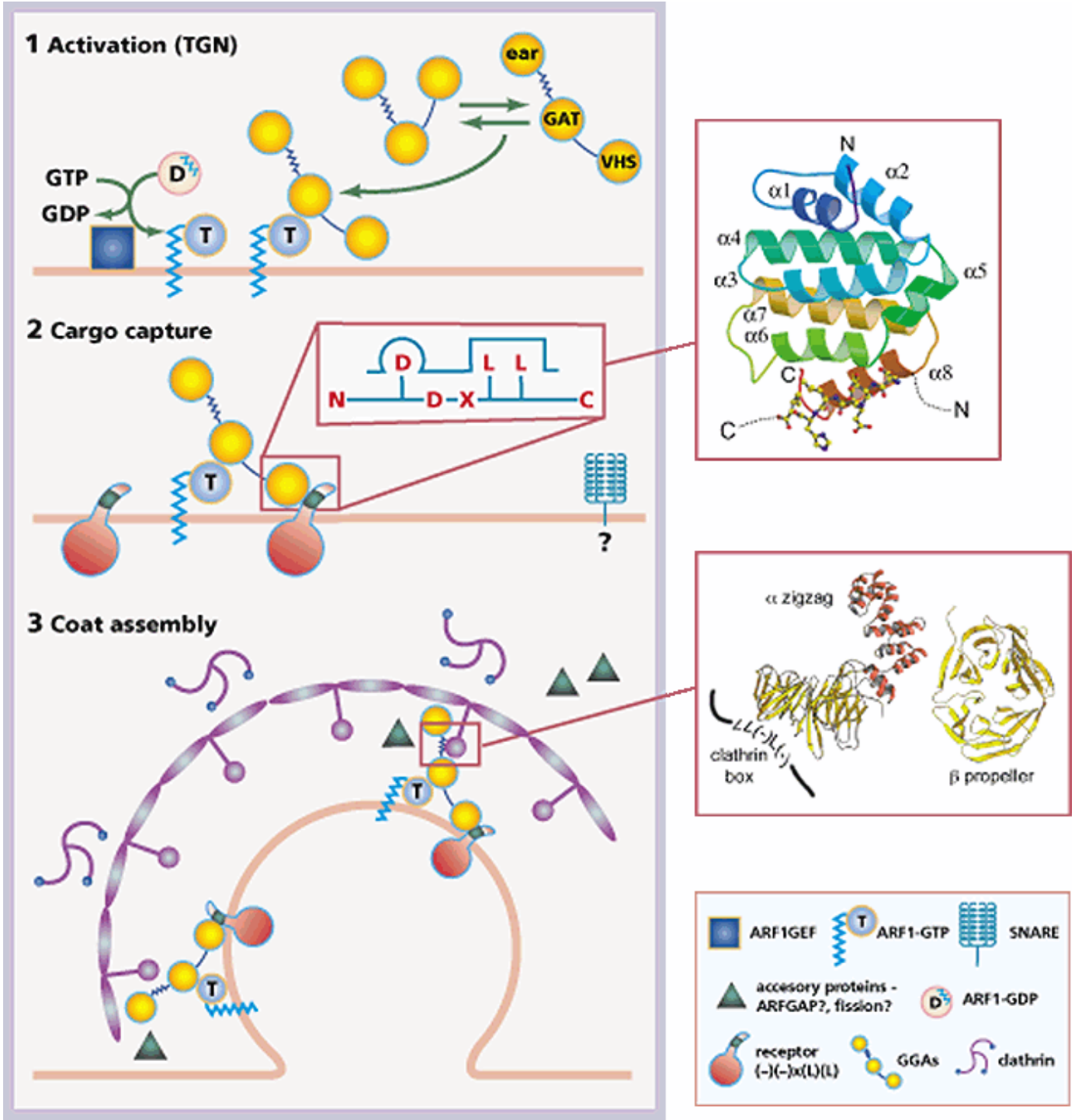
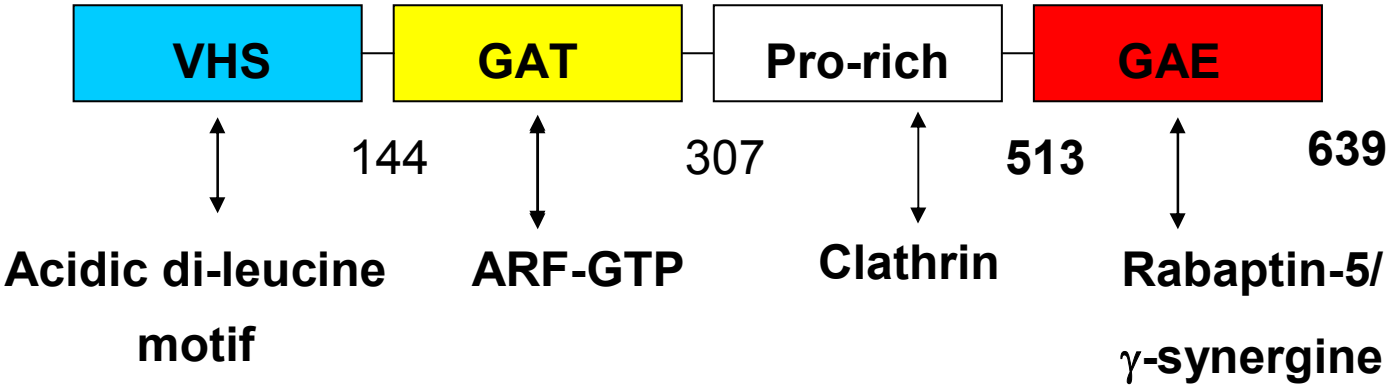
‡ *These authors contributed equally to this work*

pp 937-941

"Single-handed recognition of a sorting traffic motif by the GGA proteins", T. Kirchhausen, *Nature Structural Biology*, April 2002 Vol. 9 pp 241 – 244.



Schematic representation of the domain structure of GGA1



VHS: Vps27p/Hrs/STAM Domain

GAT=GGAH: GGA Homology Domain

GAE=AGEH: Adaptor g Ear Homology Domain

Residues 677-822 of human GGA1 (γ1-ear domain) were expressed in *E. coli*

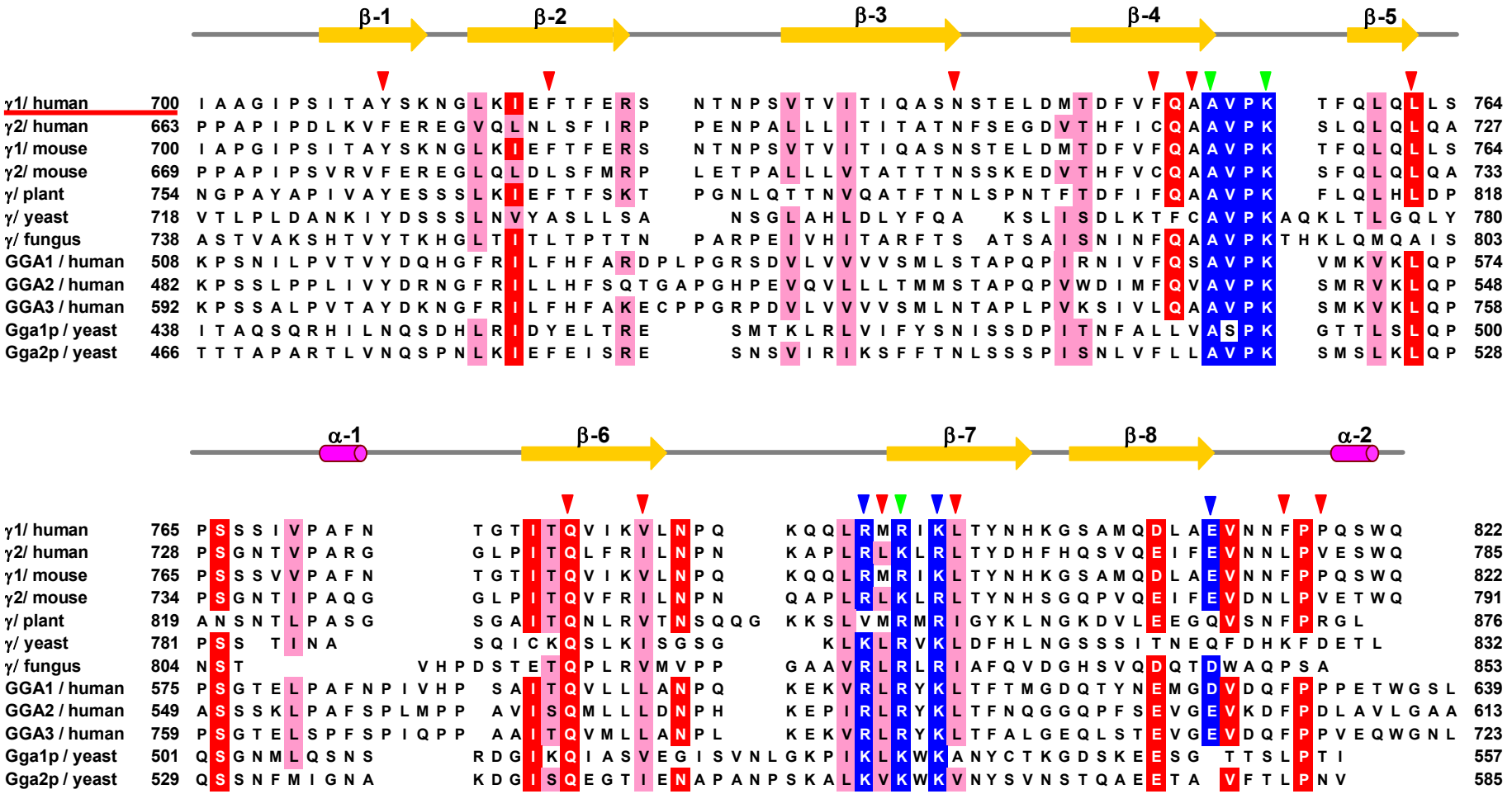


Figure 2 (Nogi *et al.* ***Nature Structural Biology***, *vol. 9, 527, July 2002*)

Table 1 Crystallographic data

Data set	Native	$KAu(CN)_2$ for SIRAS	SeMet for MAD		
			Remote	Peak	Edge
Crystal data					
Space group	$P4_32_12$	$P4_32_12$	$P4_32_12$		
Cell dimensions					
$a = b$ (Å)	62.13	62.24	62.06		
c (Å)	147.87	147.58	147.20		
Data collection statistics					
Beam line	BL-44XU, SPring-8	BL-44XU, SPring-8	BL-6A, PF	BL-6A, PF	BL-6A, PF
Wavelength (Å)	0.900	0.900	0.9600	0.9778	0.9785
Resolution range (Å)	57.0-1.8	57.0-1.8	40.0-2.6	40.0-2.6	40.0-2.6
Outer resolution shell (Å)	1.89-1.80	1.89-1.80	2.73-2.60	2.73-2.60	2.73-2.60
Observations	372969	214570	129917	129874	129501
Unique reflections	27518	27823	9485	9476	9476
Completeness (%)	99.2 (99.2)	99.9 (100.0)	99.9 (99.3)	99.9 (99.2)	99.9 (99.2)
I/σ	7.0 (2.7)	4.9 (1.6)	7.3 (2.5)	7.9 (2.8)	7.7 (2.7)
R_{sym} (%)	6.4 (21.9)	9.3 (34.3)	9.6 (30.7)	8.7 (27.3)	9.0 (28.2)
R_{anom} (%)		4.4 (13.5)	4.5 (9.3)	5.4 (9.8)	2.9 (7.4)

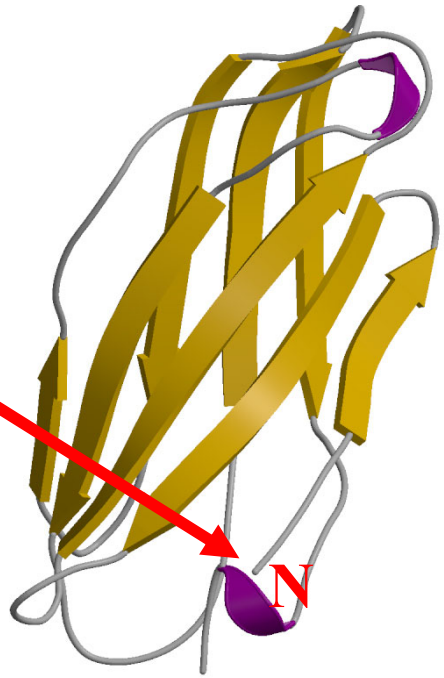
Phasing statistics					
	Numer of sites	1	Numer of sites	6	
	R_{cullis} (centric/acentric)	0.72 / 0.84	Refined $\Delta f'$	-3.793	-7.098	-8.829
	Phasing power (centric/acentric)	0.93 / 1.08	Refined f''	3.402	4.521	1.562
	FOM	0.34	FOM	0.55		
	FOM after DM	0.86	FOM after RESOLVE	0.64		

Refinement statistics	
Resolution (Å)	20.0-1.8
Outer resolution shell (Å)	1.85-1.80
R_{work} (%)	22.6 (27.1)
R_{free} (%)	24.7 (31.7)
Number of non-H atoms	
Protein	1898
Water	134
Rmsd from ideality	
Bonds (Å)	0.009
Angles (deg.)	1.35
Average B-factors (Å^2)	
Protein	23.98
A-monomer	19.97
B-monomer	27.92
Water	27.84

The First MAD experiment using the new set-up of BL6A

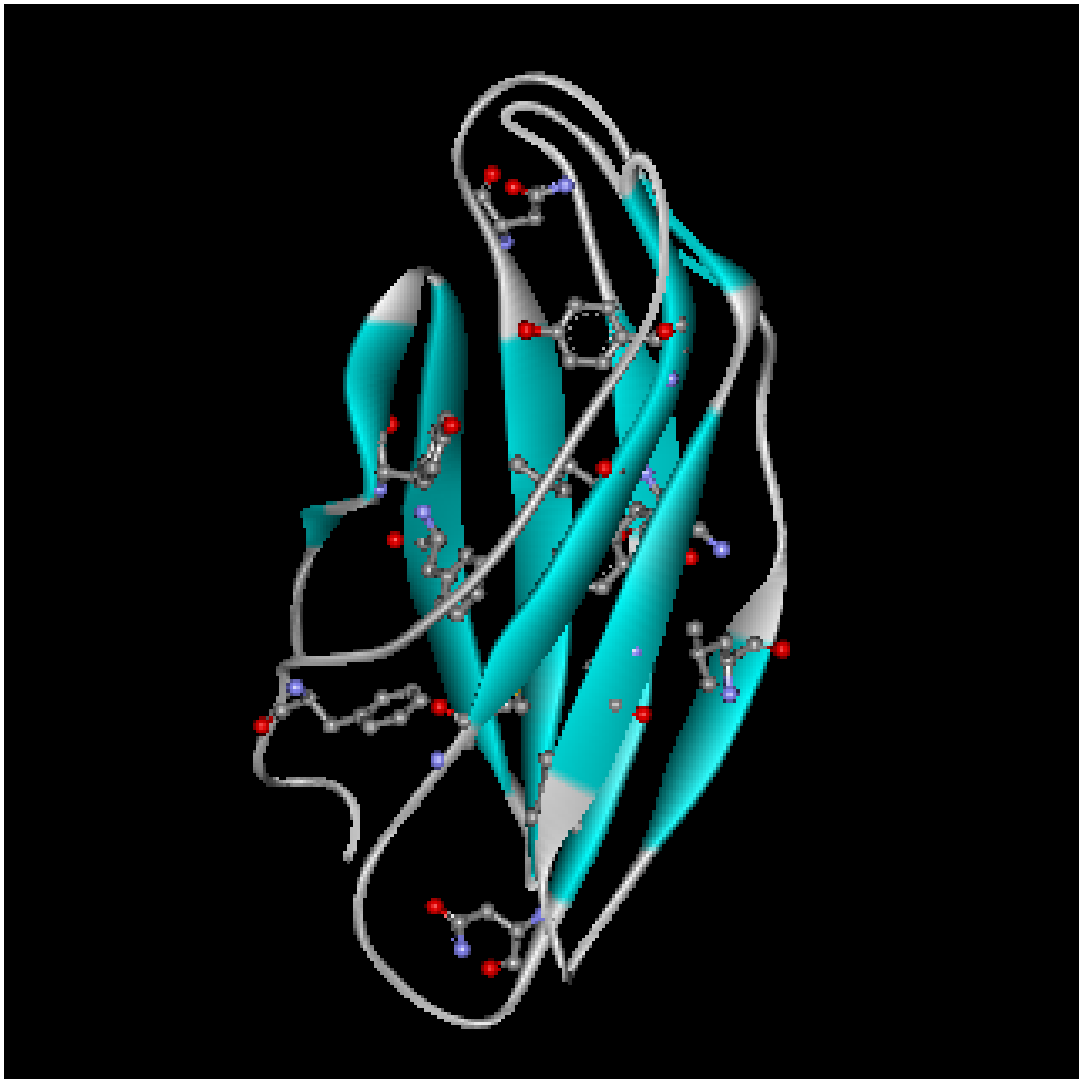
SeMet for MAD Remote	Peak	Edge	
$P4_32_12$			
62.06 147.20			
BL-6A, PF 0.9600 40.0-2.6 2.73-2.60 129,917 9,485 99.9 (99.3) 7.3 (2.5) 9.6 (30.7) 4.5 (9.3)	BL-6A, PF 0.9778 40.0-2.6 2.73-2.60 129,874 9,476 99.9 (99.2) 7.9 (2.8) 8.7 (27.3) 5.4 (9.8)	BL-6A, PF 0.9785 40.0-2.6 2.73-2.60 129,501 9,476 99.9 (99.2) 7.7 (2.7) 9.0 (28.2) 2.9 (7.4)	
Numer of sites Refined $\Delta f'$ Refined f'' FOM FOM after RESOLVE	6 -3.793 3.402 0.55 0.64	 -7.098 4.521	 -8.829 1.562

First 23 a.a. are not seen in the map



The first structure determined of a transport protein from a Se-MAD experiment using the new set-up of BL6A（T. Nogi et al., *Nature Structural Biology vol. 9, 527, July 2002*）

Binding assay using gamma-1 ear mutants



Isolated mutations; hydrophobic residues

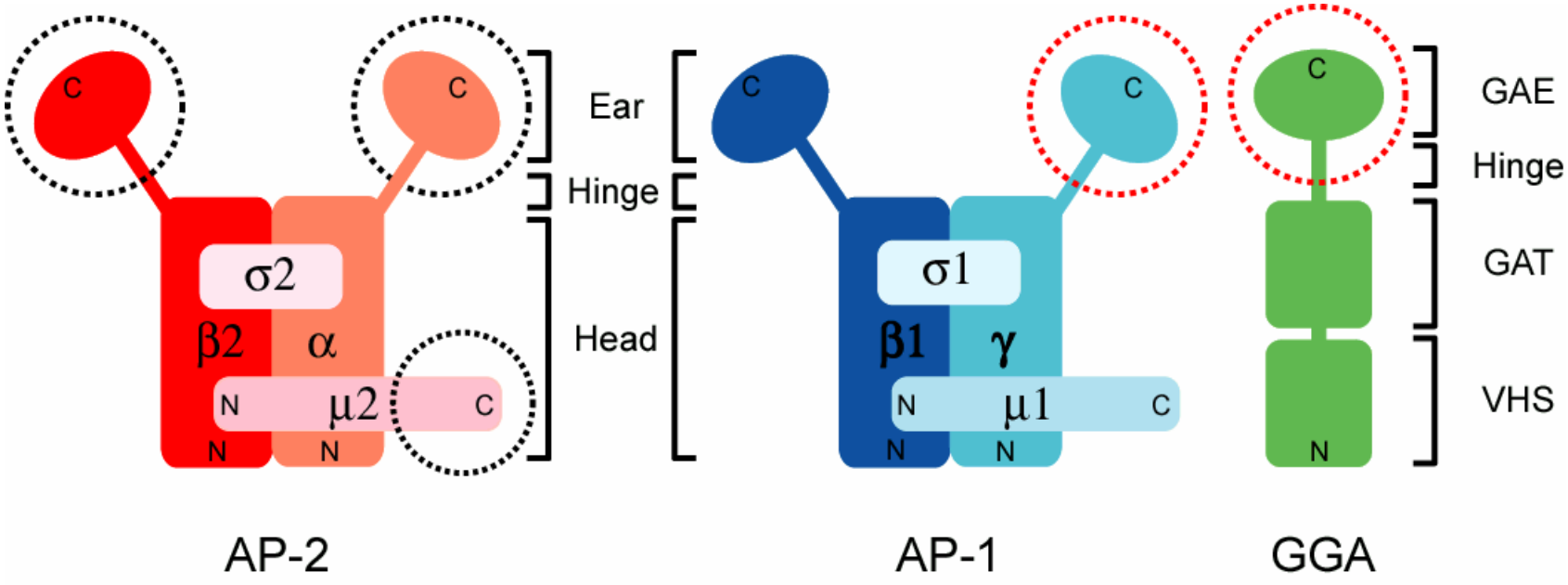
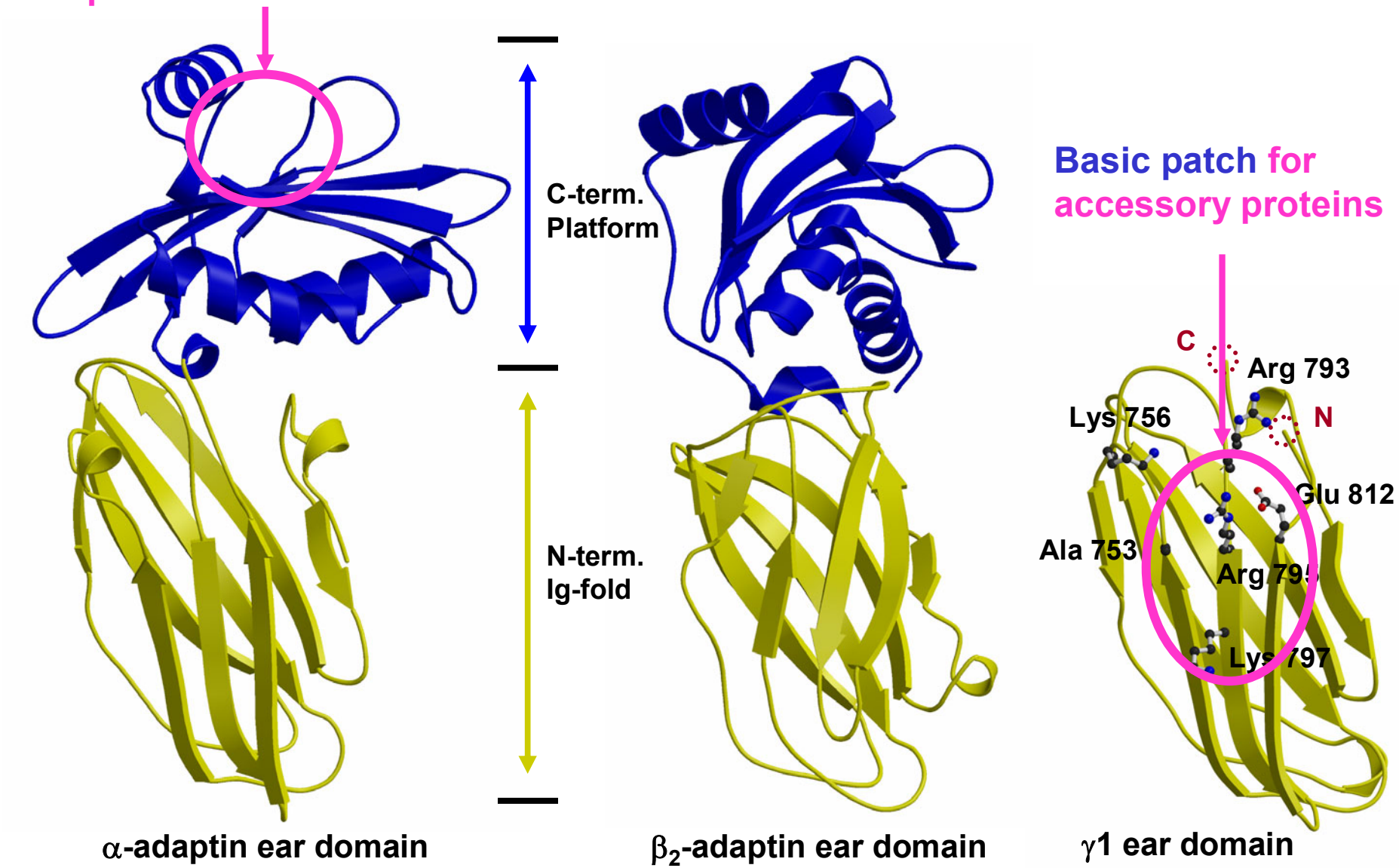
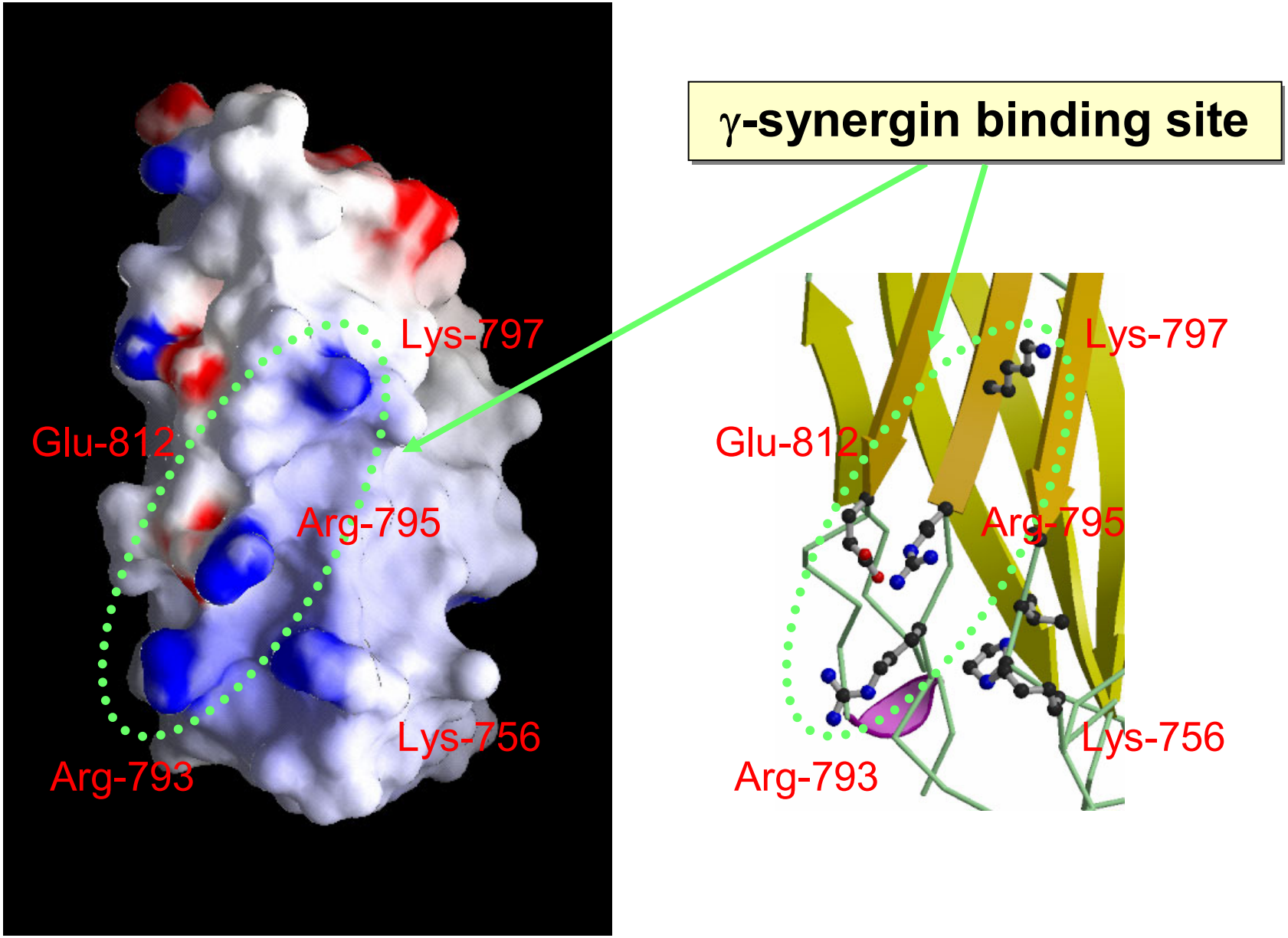


Figure 1 (Nogi *et al. Nature Structural Biology vol. 9, 527, July 2002*)

Ear domain structurs




γ-synergin binding site
Lys-797
Glu-812
Arg-795
Lys-756
Arg-793
Lys-797
Glu-812
Arg-795
Lys-756
Arg-793

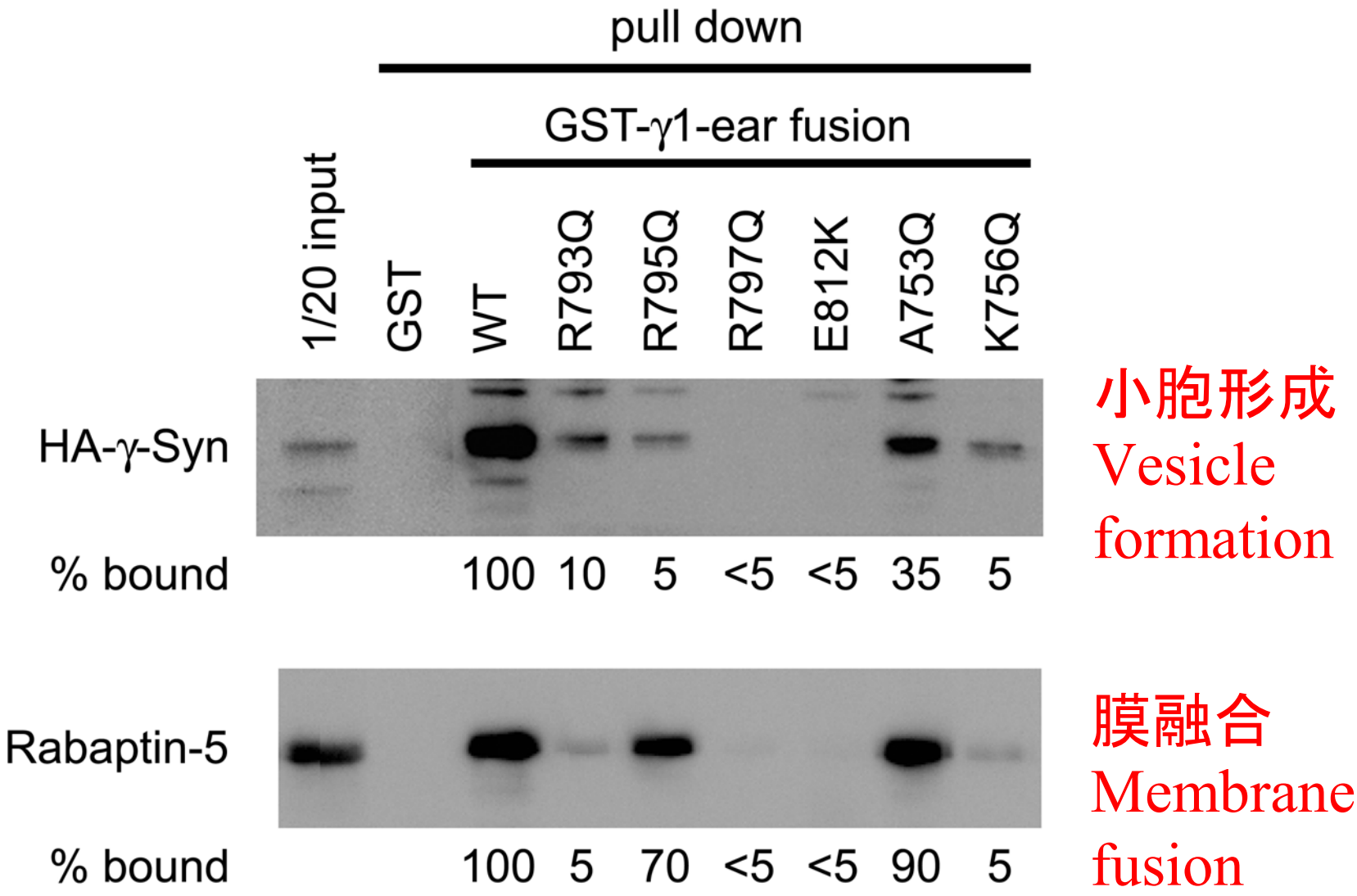


Figure 3 (Nogi *et al. Nature Structural Biology vol. 9, 527, July 2002*)

CD-MPR lumenal domain with a Lysosomal Enzyme, β-Glucuronidase Roberts et al. Cell, Vol. 93, 639–648, 1998

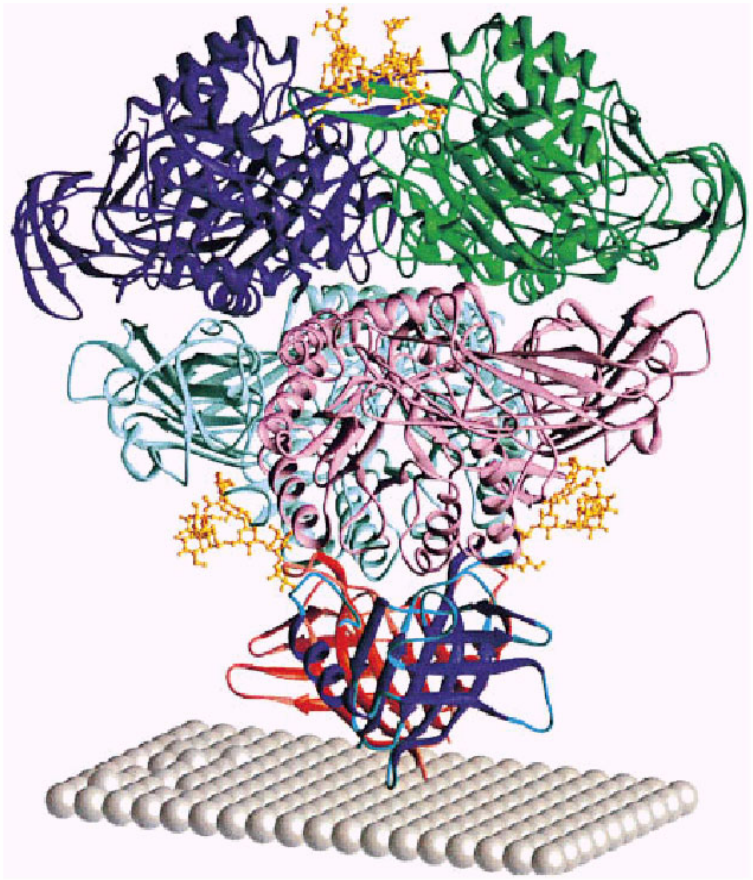
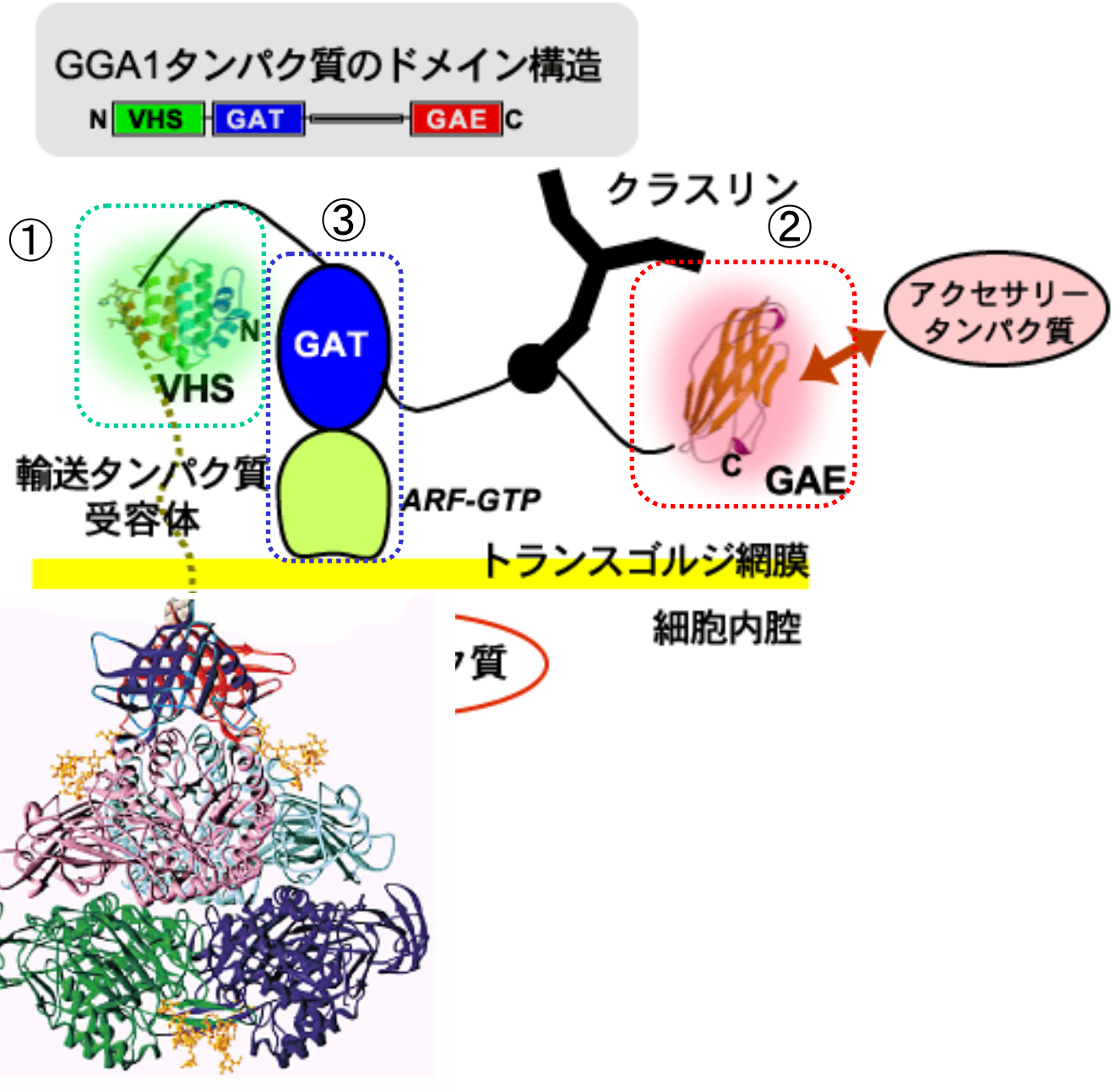


Figure 7. Molecular Modeling of a Complex between the CD-MPR and a Lysosomal Enzyme, β-Glucuronidase

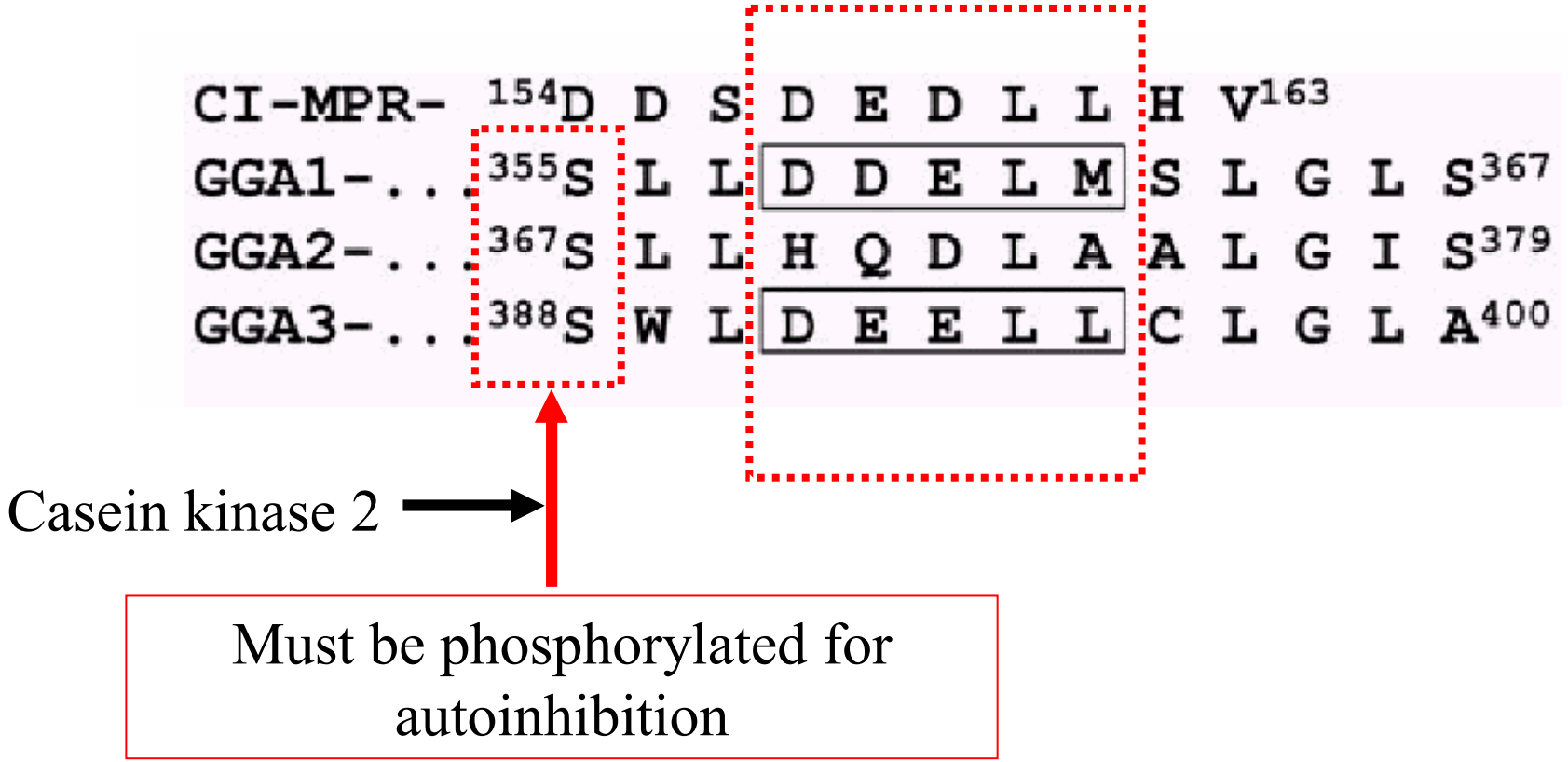
The refined structure of the CD-MPR and the published coordinates of β-glucuronidase (PDB accession code 1BHG) were used. The CD-MPR dimer (blue/cyan and red/rose ribbons) are shown in the same orientation as in Figure 4A. The tetrameric β-glucuronidase protein is shown. The oligosaccharide attached to Asn-173 is also shown (gold ball-and-stick model). The model was generated by overlapping the terminal mannose residue of the β-glucuronidase oligosaccharide with the CD-MPR Man-6-P substrate. The location of the membrane is also indicated (gray spheres).


GGA1タンパク質のドメイン構造
N
VHS
GAT
GAE
C
クラスリン
①
③
②
N
VHS
GAT
C
GAE
アクセサリー
タンパク質
輸送タンパク質
受容体
ARF-GTP
トランスゴルジ網膜
細胞内腔

Balraj Doray, Kerry Bruns, Pradipta Ghosh, and Stuart A. Kornfeld

Autoinhibition of the ligand-binding site of GGA1/3 VHS domains by an internal acidic cluster-dileucine motif

PNAS vol. 99, pp.8072-8077. (11 June 2002)



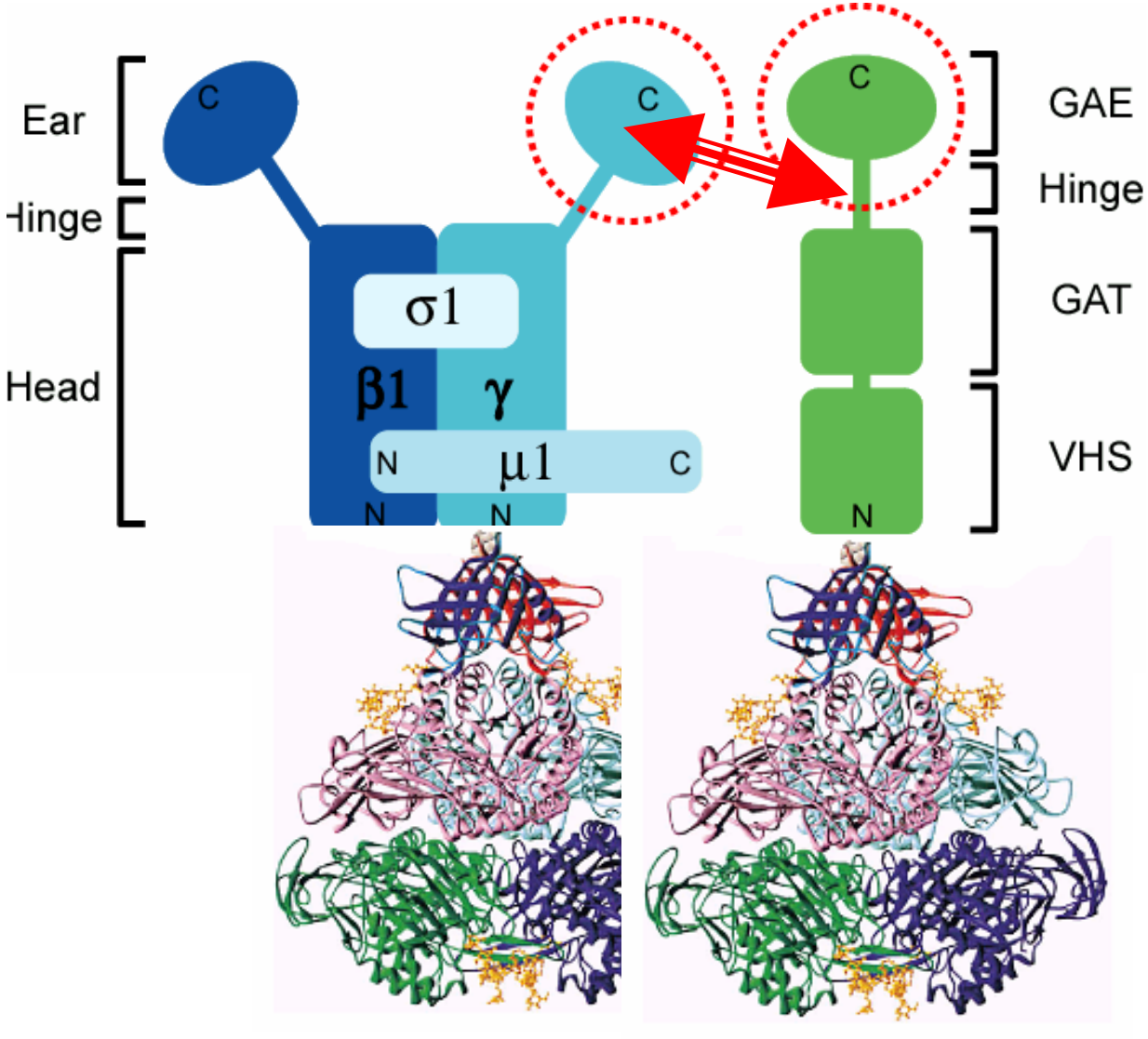
Cooperation of GGAs and AP-1 in Packaging MPRs at the Trans-Golgi Network

Balraj Doray,[1]*† Pradipta Ghosh,[1]* Janice Griffith,[2]
Hans J. Geuze,[2] Stuart Kornfeld[1]‡

The Golgi-localized, γ-ear–containing, adenosine diphosphate ribosylation factor–binding proteins (GGAs) are multidomain proteins that bind mannose 6-phosphate receptors (MPRs) in the Golgi and have an essential role in lysosomal enzyme sorting. Here the GGAs and the coat protein adaptor protein–1 (AP-1) were shown to colocalize in clathrin-coated buds of the trans-Golgi networks of mouse L cells and human HeLa cells. Binding studies revealed a direct interaction between the hinge domains of the GGAs and the γ-ear domain of AP-1. Further, AP-1 contained bound casein kinase–2 that phosphorylated GGA1 and GGA3, thereby causing autoinhibition. This could induce the directed transfer of the MPRs from GGAs to AP-1. MPRs that are defective in binding to GGAs are poorly incorporated into AP-1–containing clathrin-coated vesicles. Thus, the GGAs and AP-1 interact to package MPRs into AP-1–containing coated vesicles.

Science, vol 297, 1700, 6 September 2002

GGA1 hinge region interacts with the γ-ear domain of AP-1



Doray,,, Kornfeld, Science, vol 297, 1700, 6 September 2002

Model for the assembly of C