# The 7th Symposium
# of Japan Consortium for Glycobiology and Glycotechnology

## Development of Medical Glyco-Biomarkers and Resolution of Sugar Chain Functions

**December 8 - 9, 2009 at Senri Life Science Center (Osaka)**

**Program December 8 (Monday), 2009**

**Opening Address**
**Yoshitaka Nagai (President, JCGG)**

### Invited Lecture 1
**Chair : Yasuhiro Kajiwara (Osaka University)**

**Recent status of Biosimilar**
**Teruhide Yamaguchi (National Institute of Health Sciences)**

Recently, the expiration of patents and/or data protection for the first major group of originator's biotechnology products has ushered in an era of products that are designed to be 'similar' to a licensed originator product. These products rely, in part, for their licensing on prior information regarding safety and efficacy obtained with the originator products. The clinical experience and established safety profile of the originator products should contribute to the development of similar biotechnology products (namely biosimilar/follow-on biologic products). In this symposium, I would like to introduce the main issues to be discussed during the development of follow-on biologics guideline. I also will discuss to evaluate the comparability between the follow-on biologics and the originator product as a reference product.

### Session 1 Analytical Methods for Oligosaccharide Structure of Glycoproteins
**Chair : Akihiko Kameyama (AIST)**

**Mass spectrometry in development of glycosylated biopharmaceuticals**
**Nana Kawasaki (National Institute of Health Science)**

Many glycoproteins are approved for clinical use. Carbohydrate moieties in biopharmaceuticals affect their physicochemical properties, thermal stability, reactivity with their receptors, circulating half-life, and safety, and it is therefore crucial to analyze glycosylation in detail. Additionally, glycan tests that allow us to

confirm the consistency of glycosylation are needed to ensure the safety and efficacy of biopharmaceuticals. On the other hand, for scientific, safety-related, and economic reasons, there have been some attempts to change the manufacturing process for existing biopharmaceuticals and develop biosimilar/follow-on biologic products. Changes in cell substrates and manufacturing processes may cause the alteration of glycosylation, which defines glycosylated biopharmaceuticals. Therefore, a comparative test is needed against the existing control drug. Here I would like to discuss the application of mass spectrometry in glycosylation analysis and a comparative test of glycosylated biopharmaceuticals.

## Structural analysis of glycoprotein directed towards understanding the Golgi function

**Osamu Kanie (Mitsubishi Kagaku Institute of Life Science)**

Glycans composed of ca ten monosaccharides attached to proteins and ceramide create a diverse molecular species, which plays important biological functions. The structural investigation of such molecules are thus important. We are developing new methods to tackle with unsolved problems. In this symposium, a combined use of mass spectrometry and atomic microscopy is proposed, and a method to discriminate structural isomers by energy-resolved mass spectrometry will also be presented. Chaoptin is a large glycoprotein specifically present at Drosophila eye. After detailed MS analysis, glycoforms at individual linkage positions spread over 13 sites were determined. Since crystallographic analysis of Chaoptin have not achieved, its three dimensional structure is not known. We wanted to obtain information regarding relationship between glycoform and the structure of peptide backbone, thus we used an atomic force microscopy (AFM) to obtained overall structural images of single individual molecules. AFM revealed that horseshoe-shaped structure. Combining data from MS analysis, we showed the glycans present at the exterior of the protein is highly processed. On the contrary, high-mannose type glycans are reserved at interior of the protein

References
1) Kanie Y et al.: PLoS One 4, e5434, 2009
2) Suzuki K et al.: Anal Chem 79, 9022-9, 2007

## Session 2 Efficient Chemical Synthesis of Oligosaccharides Sustaining Glycobiology

**Chair : Toshiyuki Inazu (Tokai University)**

## Glycoscience research stemmed on organic synthesis

Yukishige Ito (RIKEN)

Importance of organic synthesis has been demonstrated in various fields of biological sciences. Our study has aimed to 1) develop original synthetic methodologies, 2) their applications to the synthesis of glycan probes, and 3) precise analysis of various carbohydrate recognizing proteins. This talk will aim to demonstrate the advantage of organic synthesis-based approaches for detailing our understanding on biological roles of glycoproteins.

## Synthesis of sialosides: biological recognition at molecular and cellular levels, and biomedical application

Makoto Kiso (Gifu University)

Sialic acid-containing glycans (sialoglycans), which are the components of gangliosides and the glycans of glycoproteins, have the receptor functions of bacterial toxins and viruses, and play important roles in the basic and dynamic biological events such as cell-recognition and signaling, differentiation and proliferation, oncogenesis, inflammation, immune response, neural functions, and so on. They are also the target molecules against neuraminidases to be related to a various biological processes and diseases. We present here the novel, efficient chemical synthesis of gangliosides and analogues toward the structure-function studies and medicinal applications

References

1) 木曽　真, 安藤弘宗, 石田秀治：医薬応用に向けた糖鎖合成技術の活用―シアル酸グルコリル化反応の改良と共通構造から展開するガングリオシドの系統合成の探求、臨床糖鎖バイオマーカーの開発―糖鎖機能の解明とその応用(成松久編)遺伝子医学MOOK11、(株)メディカルドゥ, pp95-103, 2008

2) Imamura A, Ando H, Ishida H, Kiso M: Ganglioside GQ1b: Efficient total synthesis and the expression to synthetic derivatives to elucidate its biological roles. J Org Chem 74, 3009-23, 2009

3) Abdu-Allah H, Watanabe K, Hayashizaki K, Takaku C, Tamanaka T, Takematsu H, Kozutsumi Y, Tsubata T, Ishida H, Kiso M: Potent small molecule mouse CD22-inhitors: Exploring the interaction of the residue at C-2 of sialic acid scaffold. Bioorg Med Chem Lett 19, 5573-5, 2009

## Synthesis of oligosaccharides library based on new glycosylation

Takashi Takahashi (Tokyo Institute of Technology)

In this report, we present development of new glycosidation methods and its applications to the synthesis of the biologically important oligosaccharides and

oligosaccharide libraries. Oligosaccharides play important roles in many biological events on cell surface. The use of chemically pure synthesized oligosaccharides would be desirable in terms of study for elucidation of structure-activity relationships. Recent development of carbohydrate chemistry involving efficient glycosylation reactions allows for the synthesis of complex oligosaccharides. We reported on the efficient synthesis of oligosaccharides involving oligosialic acids using 4N,5O-carbonyl protected thiosialosides. The sialyl donors enable α-selective glycosidation without use of acetonitrile as a solvent. In addition, combinatorial chemistry allows one to prepare the carbohydrate libraries. We have developed one-pot glycosylation involving sequential multiple glycosidations in one-pot as a methodology for the synthesis of carbohydrate libraries. A combinatorial library related to Lewis X oligosaccharide was prepared by one-pot glycosylation utilizing a solution-phase automated synthesizer. We also reported on a polymer- assisted deprotection of protected oligosaccharides involving deprotection of solid-supported protected sugars, followed by release of the fully deprotected oligosaccharides from resin.

References
1) Tanaka H, Iwata Y, Takahashi D, Adachi M, Takahashi T: J Am Chem Soc 127, 1630, 2005
2) Tanaka H, Ishida T, Matoba N, Tsukamoto H, Yamada H, Takahashi T: Angew Chem Int Ed 45, 6349, 2006
3) Tanaka H, Nishiura Y, Takahashi T: J Am Chem Soc 130, 17244, 2008

## JCGG Luncheon Seminar 1 (sponsored by Ohtsuka Chemical Co.)

### Analysis of N-, O-, and glycosaminoglycans in cells by a sensitive and rapid screening methods

Kazuaki Kakeihi (Kinki University)

We developed the sensitive and rapid methods for the analysis of N- and 0-glycans as well as glycosaminoglycans in cultured cells, and applied the methods to characterize the cancer cells by comprehensive analysis of glycans. We found that comprehensive analysis of glycans led to the following results: 1) confirmation of the original tissues from the glycans in spread tumor tissues; 2) measurement of differentiation of cancer cells.

References
1) Yamada K et al.: Comparative studies on the structural features of O-glycans between leukemia and epithelial cell lines. J Proteome Res 8, 521-37, 2009
2) Kamoda S et al.: Rapid and sensitive screening of N-glycans as 9-fluorenyl methyl

derivatives by high-performance liquid chromatography: A method which can recover free oligosaccharides after analysis. J Proteome Res 4, 146-52, 2005

## Session 3 Synthesis of Homogeneous Glycoproteins

**Chair : Yasuhiro Kajiwara (Osaka University)**

### Synthesis of glycoproteins having homogeneous oligosaccharides

**Yasuhiro Kajiwara (Osaka University)**

Complex type oligosaccharide chains on the protein surface concern with protein conformation, dynamics, protein traffic king and the glycoprotein lifetime in blood. The preparation methods of homogeneous glycoproteins have developed by use of expression method and chemical approaches. We have also examined synthesis of homogeneous glycoproteins having human complex type oligosaccharide in order to evaluate oligosaccharide functions toward proteins. We have synthesized several small glycoproteins (amino acids 40-80 residues), erythropoietin analogue (amino acids 166 residues). In order to synthesize these glycoproteins, polypeptide sequence of the target glycoprotein were divided into several segments and these were synthesized by Fmoc-SPPS. After prepared both glycopeptide-thioester and peptide having cysteine residue at the N-terminal, these were coupled by repetitive Native Chemical Ligation (NCL). After construction of the glycosylated polypeptide chain, we examined folding experiments and evaluated effect of oligosaccharide during protein folding process. In this presentation, I would like to show several synthetic examples of homogeneous glycoproteins along with circumstances of glycoprotein preparations in worldwide.

References

1) Ledford H: Nature 449, 259, 2007; Ledford H: Nature 449, 274- 6, 2007
2) Walsh G, Jefferis R: Nat Biotechnol 24, 1241-52, 2006
3) Hamilton SR, Davidson RC, Sethuraman N, Nett JH, Jiang Y, Rios S, Bobrowicz P, Stadheim TA, Li H, Cho1 B-K, Hopkins D, Wischnewski H, Roser J, Mitchell T, Strawbridge R, Hoopes J, Wildt S, Gerngross TU: Science 313, 1441-3, 2006
4) Amano K, Chiba Y, Kasahara Y, Kato Y, Kaneko M, Kuno A, Ito H, Kobayashi K, Hirabayashi J, Jigami Y, Narimatsu H: Proc Nat Acad Sci 105, 3232-7, 2008
5) a) Umekawa M, Huang W, Li B, Fujita K, Ashida A, Wang L-X, Yamamoto K: 283, 4469-79, 2008; b) Huang W, Li C, Li B, Umekawa M, Yamamoto K, Zhang X, Wang L-X: J Am Chem Soc 131, 2214-23, 2009
6) Rich JR, Withers SG: Nat Chem Biol 5, 206-15, 2009
7) Dawson PE, Muir TW, Clark-Lewis I, Kent SBH: Science 266, 776 - 9, 1994
8) Kochendoerfer GG et al.: Science 299, 884 - 7, 2003
9) Hojo H, Matsumoto Y, Nakahara Y, Ito E, Suzuki Y, Suzuki M, Suzuki A, Nakahara

Y: J Am Chem Soc 127, 13720-25, 2005
10) Macmillan D, Bertozzi CR: Angew Chem Int Ed 43, 1355 – 9, 2004.
11) Hackenberger CPR, Friel CT, Radford SE, Imperiali B: J Am Chem Soc 127, 12882-9, 2005
12) Yamamoto N, Tanabe Y, Okamoto R, Dawson PE, Kajihara Y: J Am Chem Soc 130, 501-10, 2008
13) Hirano K, Macmillan D, Tezuka K, Tsuji T, Kajihara Y: Angew Chem Int Ed DOI: 10.1002/ anie.200904376, 2009

## Progress in the synthesis of O-glycans and glycoproteins
Yoshiaki Nakahara (Tokai University)

Recent advances in synthetic technology have made possible access to such complex molecules as glycoprotein. Discussed are our studies on chemical and chemo-enzymatic synthesis of glycopeptides carrying O-glycans and their segment condensations.

## Efficient addition and remodeling of sugar chain by single enzyme
Kenji Yamamoto (Kyoto University)

Endo-β-N-acetylglucosaminidase from *Mucor hiemalis* (Endo-M) acts on the N,N'-diacetylchitobiose moiety in the N-linked sugar chain of glycoproteins and catalyzes not only the hydrolysis reaction but also the transglycosylation reaction that transfers the releasing sugar chain to an acceptor having hydroxyl residue. We established the chemo-enzymatic synthesis of a glycopeptides using the trans-glycosylation activity of Endo-M. This method consists of the chemical synthesis of N-acetylglucosaminyl peptide and the transglycosylation of N-linked sugar chain from oligosaccharide donor to an N-acetylglucosaminyl peptide by Endo-M. We could add the sialo-complex-type oligosaccharides to bioactive peptides such as calcitonin and also to the glutamine residue of Substance P neuropeptide by this method. We also could exchange the high-mannose type oligosaccharides in glycoprotein/glycopeptides to the complex types by the transglycosylation of Endo-M. Nevertheless, since Endo-M is inherently glycohydrolase, its application for synthetic purpose has been hampered by low transglycosylation activity and by the issue product hydrolysis. Therefore, we attempted to obtain the mutants with superior transglycosylation and less hydrolysis activity. The site-directed mutagenesis on residues in the catalytic region of Endo-M was carried out, and two interesting mutants were discovered. The Y217F mutant was found to possess much enhanced transglycosylation activity and much diminished hydrolytic activity. The other mutant, N175Q, acts like a glycosynthase and was able to take the highly active sugar oxazolines (the transition state mimics) as donor substrates for

transglycosylation. The usefulness of the novel mutants was exemplified by the efficient synthesis of various glycopeptides with potent functions.

## Glycoprotein production by glycosyltransferases and yeast expression system

Yasunori Chiba (AIST)

Protein therapeutics and biosimilar drugs (follow-on biologics), such as antibodies and cytokines, is the largest class of new drug candidates being developed by pharmaceutical companies. Although most of these glycoproteins are produced in mammalian cells, there is concern that heterogeneity of glycan structure affects its *in vivo* activity. Therefore we should establish a standardization method for analysis of glycan structure of the biologics, and produce glycans and glycoproteins as reference standards. Moreover, methods for homogeneous glycan (glycoprotein) production must overcome the heterogeneity problem. We have engineered the yeast cells capable of producing mammalian-type glycans by the deletion of the genes responsible for the yeast-specific modification of sugar chains and the introduction of the genes required for production of mammalian-type N- and 0-glycans. Several glycoproteins for therapeutics have been produced *in vivo* by the yeast system and the recombinant protein. We have also succeeded in producing a large amount of human glycosyltransferases as recombinant proteins that are useful for *in vitro* creation and modification of the glycans. Combination of our technologies may provide us homogeneous glycoproteins applicable for protein therapeutics.

References

1) Kuroda K, Kobayashi K, Kitagawa Y, Nakagawa T, Tsumura H, Komeda T, Shinmi D, Mori E, Motoki K, Fuju K, Sakai T, Nonaka K, Suzuki T, Ichikawa K, Chiba Y, Jigami Y: Appl Environ Microbiol 74, 446-53, 2008

2) Akeboshi H, Kasahara Y, Tsuji D, Itoh K, Sakuraba H, Chiba Y, Jigami Y: Glycobiology 19, 1002-9, 2009

3) Amano K, Chiba Y, Kasahara Y, Kato Y, Kaneko MK, Kuno A, Ito H, Kobayashi K, Hirabayashi J, Jigami Y, Narimatsu: Proc Natl Acad Sci USA 105, 3232-7, 2008

## Session 4　Chemical Glycobiology and Imaging

**Chair : Fumino Manabe (RIKEN)**

## Chemical approaches toward understanding and application of glycan-processing in the ER

Kiichiro Totani (Seikei University)

In the rough endoplasmic reticulum (ER), most of newly generated proteins are modified by high-mannose-type glycan. It has been becoming clear that ER glycoprotein quality control system regulates each process for the folding acceleration, the folding check, the transportation and the degradation, by oligosaccharide-tag on the protein surface. We chemically synthesized a series of endoplasmic reticulum types of high-mannose-glycan related molecular probes. With these synthetic probes, quantitative oligosaccharide specificity analysis for calnexin (CNX)/calreticulin (CRT) cycle, the central machinery in the glycoprotein quality control, was carried out in detail.

Our data show that glycoproteins are introduced to the CNX/CRT cycle as G1M9 forms. The client glycoproteins would be gradually released from CNX/CRT with protein maturation followed by Glc'ase II mediated deglucosylation to give M9 forms. Reglucosylation of the resulting M9 with UDP-Glc:glycoprotein glucosyltransferase (UGGT) was found to accelerate by CNX/CRT-trapping of the produced G1M9. The glycan-recognizing motif of UGGT and glucosidase II was also elucidated by inhibition experiments. Moreover, we examined the relationship between oligosaccharide-processing profiles in the ER and folding diseases.

References

1) Totani K et al.: Biochemistry 48, 2933, 2009
2) Totani K et al.: J Biol Chem 281, 31502, 2006
3) Totani K et al.: J Am Chem Soc 130, 2101, 2008

## Molecular imaging medicinal chemistry by PET in RIKEN CMIS

Hisashi Doi (RIKEN)

Positron emission tomography (PET) is a powerful non-invasive molecular imaging technology for the investigation of *in vivo* biochemistry, especially in the human organ. The need for the development of new PET probes has grown with the increase in its use for diagnosis in medicine and for drug development processes. During the current paradigm shift in drug discovery, molecular imaging will become a core research area to further advance "evidence-based medicine". Our chemistry groups are working to develop general synthetic methodologies for short-lived PET probes, which will be fundamental for promoting PET research. In particular, our research is focused on developing PET probe syntheses for drug candidates and novel chemical labeling reactions with the aim of broadening the spectrum of potential applications of organic chemistry in life science research. As one of our main projects, we are striving toward introducing $^{11}C$ into carbon frameworks of bioactive organic compounds by developing rapid C-[$^{11}C$]methylation reactions based on carbon-carbon bond formation, focusing on the methyl group as the minimum carbon substituent. We have already succeeded in developing several

cross-coupling reactions between organotin or organoboron compounds and [$^{11}$C]methyl iodide in the presence of palladium catalyst. These reactions are attracting worldwide attention as groundbreaking synthesis for introducing the [$^{11}$C]methyl group into a carbon framework in the very short time of 5 min. In this symposium, we would like to introduce the RIKEN original methodologies for PET probe syntheses and our ongoing progress of medicinal chemistry based on PET, which will contribute to the drug discovery and development.

## Chemistry-based labeling and engineering on proteins and cell surfaces: application to non-invasive imaging of oligosaccharides

Katsunori Tanaka (Osaka University)

New labeling probes of fluorescences and [$^{68}$Ga]-DOTA, as the positron emission nucleus for PET, through rapid 6$\pi$-azaelectrocyclization were designed and synthesized, (£)-ester aldehydes. The high reactivity of these probes enabled the labeling of lysine residues in peptides, proteins, and even the amino groups on the cell surfaces at very low concentration (~$10^{-8}$ M) within a short reaction time (~10 min) to result in "selective" and "non- destructive" labeling of the more accessible amines.

The first MicroPET of glycoproteins, [$^{68}$Ga]-DOTA-orosomucoid and asialoorosomucoid successfully visualized the differences in the circulatory residence of glycoproteins, in the presence or absence of the sialic acids. On the other hand, dynamic trafficking of the lymphatic cells to the spleen in the nude mice, was clearly visualized by the *in vivo* fluorescence imaging. The present electrocyclization protocol is also applicable to the engineering of the proteins and/or the cell surfaces by the oligosaccharides; the chemically engineered lymphatic cells by N-glycan successfully target the tumor tissue implanted in the BALB/c nude mice. New N-glycan clusters and their *in vivo* dynamics, significantly affected by their glycan structures, will also be reported.

References

1) (a) Tanaka K, Masuyama T, Hasegawa K, Tahara T, Mizuma H, Wada Y, Watanabe Y, Fukase K: Angew Chem Int Ed 47, 102, 2008 (b) Tanaka K, Fukase K: Org Bioml Chem 6, 815, 2008 (c) Tanaka K, Fukase K, Org Chem 5, 153, 2008 (d) Tanaka K, Fujii Y, Fukase K: ChemBioChem 9, 2392, 2008

2) Tanaka K, Minami K, Tahara T, Fujii Y, Siwu ERO, Nozaki S, Onoe H, Yokoi S, Koyama K, Watanabe Y, Fukase K: submitted

3) Tanaka K, Siwu ERO, Minami K, Hasegawa K, Nozaki S, Kanayama Y, Koyama K, Chen W, Paulson JC, Watanabe Y, Fukase K: submitted

## Invited Lecture 2

### Chair : Koichi Fukase (Osaka University)

### Sugar and proteins: Strategies in synthetic biology

**Benjamin G. Davis (University of Oxford)**

Sugars and Post-Translational Modifications are critical biological markers that modulate the properties of proteins. Our work studies the interplay of proteins, sugars and modifications.

Synthetic Biology's development at the start of this century may be compared with Synthetic Organic Chemistry's expansion at the start of the last; after decades of isolation, identification, analysis and functional confirmation, the future logical and free-ranging redesign of biomacromolecules offers tantalizing opportunities. This lecture will cover 3 emerging areas in our group:

(i) New methods are required[1-8]: despite 80-years- worth of non-specific, chemical modification of proteins, precise methods in protein chemistry remain rare. The development of efficient, complete, chemo- and regio-selective methods, applied in benign aqueous systems to redesign the structure and function of proteins will be presented.

(ii) Bioconjugate Applications[3,9-11]: * preparative biocatalysis * drug delivery * selective protein degradation * nanomolar inhibitors of bacterial interactions * gene delivery vehicles * probes of *in vivo* function and non-invasive pre-symptomatic disease diagnosis.

(iii) Sugar Protocell Design[12-13]: Use of encapsulated autocatalytic sugar proto-metabolisms allowing communication with natural cells.

References

1) Davis BG: Chem Rev 102, 579-601, 2002; Gamblin DP, Scanlan EM, Davis BG: Chem Rev 109, 131- 63, 2009

2) Davis BG: Science 303, 480-2, 2004

3) van Kasteren SI, Kramer HB, Jensen HH, Campbell SJ, Kirkpatrick J, Oldham NJ, Anthony DC, Davis BG: Nature 446, 1105-9, 2007

4) van Kasteren SI, Kramer HB, Gamblin DP, Davis BG: Nat Protoc 2, 3185-94, 2007

5) Bernardes GJL, Grayson EJ, Thompson S, Chalker JM, Errey JC, Oualid ElF, Claridge TDW, Davis BG: Angew Chem Int Ed 47, 2244-7, 2008

6) Bernardes GJL, Chalker JM, Errey JC, Davis BG: J Am Chem Soc 130, 5052-3, 2008

7) Lin YA, Chalker JM, Floyd N, Bernardes GJL, Davis BG: J Am Chem Soc 130, 9642-3, 2008

8) Chalker JM, Wood CM, Davis BG: J Am Chem Soc ASAP

9) Robinson MA, Charlton ST, Garnier P, Wang X, Davis SS, Perkins AC, Frier M, Duncan R, Savage TJ, Wyatt DA, Watson SA, Davis BG: Proc Natl Acad Sci USA 101, 14527-32, 2004

10) Fleming C, Maldjian A, Da Costa D, Penny P, Noble RC, Cameron NR, Davis BG: Nat Chem Biol 1, 270-4, 2005
11) van Kasteren SI, Campbell SJ, Serres S, Anthony DC, Sibson NR, Davis BG: Proc Natl Acad Sci USA 106, 18-23, 2009
12) Cronin L, Krasnogor N, Davis BG, Alexander C, Robertson N, Steinke JHG, Schroeder SLM, Khlobystov AN, Cooper G, Gardner PM, Siepmann P, Whitaker BJ, Marsh D: Nat Biotech 24, 1203-6, 2006
13) Gardner PM, Winzer K, Davis BG: Nat Chem 1, 377-83, 2009

## Program December 8 (Tuesday), 2009

### Invited Lecture 3

**Chair : Toshisuke Kawasaki (Ritsumeikan University)**

### Discovery, mechanism and impact of the unfolded protein response

**Kazutoshi Mori (Kyoto University)**

Proteins must be correctly folded and assembled to fulfill their functions as assigned by genetic code. All living cells have developed systems to counteract unfolding or misfolding of proteins. A typical example of such a homeostatic response is triggered when unfolded proteins are accumulated in the endoplasmic reticulum (ER). Eukaryotic cells cope with ER stress by attenuating translation generally to decrease the burden on the folding machinery, by inducing transcription of ER-localized molecular chaperones and folding enzymes (ER chaperones) to augment folding capacity, and by inducing components of ER-associated degradation (ERAD) machinery to enhance degradation capacity. These translational and transcriptional controls are collectively termed the unfolded protein response (UPR). An ER stress signal is sensed and transmitted across the membrane by a transmembrane protein(s) in the ER. Interestingly, the number of such functional sensors/transducers ubiquitously expressed has increased with evolution, namely one (Ire1) in *S. cerevisiae*, two (Ire1 and Pek1) in C. elegans and *D. melanogaster*, and three (IRE1, PERK and ATF6) in mammals. Accordingly, mammalian cells are able to cope with ER stress in a more sophisticated manner. I will summarize discovery, mechanism and impact of the UPR in my lecture.

References

1) Bukau B, Weissman J, Horwich A: Molecular chaperones and protein quality control. Cell 125, 443-51, 2006
2) Mori K: Tripartite management of unfolded proteins in the endoplasmic reticulum. Cell 101, 451-4, 2000

3) Ron D, Walter P: Signal integration in the endoplasmic reticulum unfolded protein response. Nat Rev Mol Cell Biol 8, 519-29, 2007
4) 森和俊: 特集：波及する小胞体ストレスの概念. 実験医学 23, 2760-5, 2005
5) Reimold AM et al.: An essential role in liver development for transcription factor XBP-1. Genes Dev 14, 152-7, 2000
6) Reimold AM et al.: Plasma cell differentiation requires the transcription factor XBP-1. Nature 412, 300-7, 2001.
7) Yamamoto K et al.: Transcriptional induction of mammalian ER quality control proteins is mediated by single or combined action of ATF6alpha and XBPl. Dev Cell 13, 365-76, 2007
8) 浦野文彦: 特集： Protein Homeostasis を解明する小胞体ストレスと疾患. 実験医学, 27, 482-526, 2009
9) Harding HP et al.: Diabetes mellitus and exocrine pancreatic dysfunction in Perk$^{-/-}$ mice reveals a role for translational control in secretory cell survival. Mol Cell 7, 1153-63, 2001
10) Delepine M et al.: EIF2AK3, encoding translation initiation factor 2-alpha kinase 3, is mutated in patients with Wolcott-Rallison syndrome. Nat Genet 25, 406-9, 2000

## Invited Lecture 4

**Chair : Koichi Furukawa (Nagoya University)**

### Epigenetic regulation through nuclear glycosylation

**Shigeaki Kato (The University of Tokyo)**

Dynamic regulation of post-translational modification on histone tail is essential for determination of cell fate. During differentiation, temporal activation of selective histone modifying enzymes is considered to allow lineage-specific conversion of chromatin structure and transcriptional outcome, resulting in acquisition of cell identity. In this study, we focused on the molecular mechanism, by which retinoic acid (RA) signaling induces granulocytic differentiation of HL60 cells.

MLLS was first identified by affinity purification from undifferentiated HL60 cells using nuclear RA receptor alpha (RARα), and characterized as a ligand-dependent RARα co-activator. MLLS harbors H3K4 dimethyltransferase activity, and its HKMT-active complex was found to contain additional enzyme O-linked N-acetyl-glucosamine transferase (OGT), suggesting a direct role for O-GlcNAc modification in HKMT activity. MLLS was itself targeted by OGT and catalytically activated by O-GlcNAc modification at T440 in its catalytic SET domain. Furthermore, we found that O-GlcNAc modification of MLLS facilitated RA-induced granulopoiesis of HL60 cells, and that up-regulation of cellular O-GlcNAc modification dramatically restored the potency of RA-resistant HL60-R2 cells into granulocytes. Hence, these findings

demonstrated that nuclear MLLS O-GlcNAcylation triggers cell lineage determination of HL60 cells through HKMT activity.

Reference
1) Fujiki R et al.: Nature 459, 455-9, 2009

## Session 5 Recent Progress of Glycobioloy-1

**Chair : Naoyuki Taniguchi (Osaka University)**
**(Core Research for Evolutional Science and Technology: CREST funded by Japan Science and Technology Agency)**

### Clarification of the biological functions of sugar chains and the use of this knowledge in applied technologies

**Naoyuki Taniguchi (Osaka University)**

### Glycoanalytical methods and their applications to cancer glycomics

**Miyako Nakano (Macquarie University)**

Glycosylation is a crucial event that dominates post- or co-translational modifications to proteins. Analysis of glycans linked to proteins is important for precise understanding of the functions of glycoproteins. In particular, analysis of glycans on protein therapeutics such as antibody pharmaceuticals is essential in terms of their quality control. Therefore high-throughput and highly sensitive methods for glycan analysis are required. Three examples of these methods will be given. ① We have developed a rapid (5 hours) method including release of glycans, derivatization with Fmoc and CE-ESI MS analysis. To validate our method, we analyzed glycans derived from α-fetoprotein, a tumor marker of hepatocellular carcinoma. ② Site-specific N-glycan structures of haptoglobin in sera obtained from patients with pancreatic cancer or chronic pancreatitis were analyzed using LC-ESI MS. We found tetra-antennary N-glycans containing a Lewis Y-type fucose only at the Asn211 site in pancreatic cancer patients. ③ We have investigated the alteration of glycan structures on the cell membrane proteins of leukaemia cells that have acquired drug-resistance. The change is related to a reduced expression of α2-6 sialylated glycans.

Thus, the ability of doing detailed glycan analysis has provided information on a diverse range of different types of cancer.

References
1) Nakano M, Kondo A, Kakehi K, Taniguchi N: Drug Discovery Today. Technology 3, 39-47, 2006

2) Nakano M, Higo D, Arai E, Nakagawa T, Kakehi K, Taniguchi N, Kondo A: Glycobiology, 19, 13-43, 2009
3) Nakano M, Nakagawa T, Ito T, Kitada T, Hijioka T, Kasahara A, Tajiri M, Wada Y, Taniguchi N, Miyoshi E: Int J Cancer 122, 2301-9, 2008

## Regulation of GPI-anchored protein transport from the ER by GPI-glycan remodeling

Morihisa Fujita (Osaka University)

Glycosylphosphatidylinositol (GPI) anchoring of proteins is one of the highly conserved posttranslational modifications in eukaryotes. Approximately 150 proteins are attached to the cell surface via GPIs, which are essential for embryonic development, immune responses and neurogenesis in mammals. GPI biosynthesis and attachment to proteins are carried out on the endoplasmic reticulum (ER) membrane. However, how GPl-anchored proteins (GPI-APs) are trafficked from the ER is poorly understood, but the GPI moiety has been postulated to function as a signal for sorting and transport. Here, we established mutant cell lines that were selectively defective in transport of GPI-APs from the ER to the Golgi. We identified a responsible gene, designated *PGAP5* (post-GPI-attachment to proteins 5). PGAP5 belongs to a dimetal-containing phosphoesterase family, and catalyzed the remodeling of glycan moiety of GPI-APs: removal of a side-chain ethanolamine-phosphate attached to the second mannose of GPI. PGAP5 appears to function at the ER-exit site, and its catalytic activity is prerequisite for the efficient exit of GPI-APs from the ER. Our data demonstrate that GPI glycan acts as an ER-exit signal and further suggest that glycan remodeling mediated by PGAP5 regulates GPI-AP transport in the early secretory pathway.

References

1) Fujita M, Maeda Y, Ra M, Yamaguchi Y, Taguchi R, Kinoshita T: GPI-glycan remodeling by PGAP5 regulates transport of GPI-anchored proteins from the ER to the Golgi. Cell 139, 352-65, 2009
2) Fujita M, Kinoshita T: Structural remodeling of GPI anchors during biosynthesis and after attachment to proteins. FEBS Letters in press
3) Fujita M, Jigami Y: Lipid remodeling of GPI-anchored proteins and its function. Biochim Biophys Acta 1780(3) 410-20, 2008

## Importance of N-glycosylation on integrin α5β1 for its biological function

Tomoya Isaji (Tohoku Pharmaceutical University)

The N-glycosylation of integrin $\alpha 5\beta 1$ is thought to play important roles in cell spreading, cell migration, ligand binding as well as dimer formation, but the

underlying mechanism remains unclear. The functions of the N-glycans on α5β1 subunit were investigated with using sequential site-directed mutagenesis. We found that sites 3,4,5 on α5β1 is important for cell spreading, site 5 on α5 is a most important site for its expression on the cell surface. Removal of the N-glycosylation sites on the I-like domain of β1subunit (β1-Δ4-6) resulted in a decrease in the expression levels and blocked hetero-dimeric formation as well as cell spreading. Furthermore, the activities of cell spreading could be observed in β1 subunit carrying on only I-like domain (β1-S4-6). To investigate which N-glycosylation site is the most important for the biological function and regulation, we characterized the α5-S3,4,5, α5-S3,5 and α5-S4,5 mutants in detail. We found that site-4 on β-propeller domain of α5 subunit is a key site that can be specifically modified by N-acetylglucosaminyltransferase III (GnT-III). These results, taken together, strongly suggest that N-glycosylation of β-propeller domain on the α5 subunit and I-like domain on β1 subunit are the most important sites for its biological functions such as cell spreading and cell surface expression.

## JCGG Luncheon Seminar 2 (sponsored by Immuno-Biological Laboratories Co.)

### Glycans and Disease: CNS disease and cancer
### Yasuhiro Hashimoto (Fukushima Medical University)

Idiopathic normal pressure hydrocephalus (iNPH) is caused by abnormal metabolism of cerebrospinal fluid (CSF), which is mainly produced by choroid plexus in the lateral ventricles. We found that a certain glycoprotein (X-1) in CSF was decreased in iNPH patients compared with controls. X-1 was purified to apparent homogeneity and subjected to lectin microarray analysis. The N-glycan structure of X-1 was distinct from that of serum glycoproteins. To examine whether X-1 is produced in choroid plexus, X-1 extracted from the tissue was purified by immuno-precipitation and then characterized by lectin affinity column chromatography. The result indicated that X-1 extracted from choroid plexus has similar N-glycan as that of CSF, again suggesting that X-1 is secreted from choroid plexus and could be diagnostic marker for iNPH. Siglec-7, a sialic acid binding immunoglobulin like lectin, predominantly transduces inhibitory signals through cytosolic immunoreceptor tyrosine-based inhibitory motifs (ITIMs). Here, we report that clustering of Siglec-7 with a specific F(ab')2 reduced a certain cellular function. Interestingly, a truncated Siglec-7 lacking the cytosolic ITIM domain still reduced the function, suggesting that the ITIMs are not essential for the inhibitory signaling. The critical region for the signaling was mapped to four amino acids on membrane-proximal C2-set domain.

## Session 6　Recent progress of Glycobiology 2

**Chair : Tadashi Suzukii (RIEKEN)**

**(Osaka University Global COE Program: Frontier Biomedical Science Underlying Organelle Network Biology)**

### Chemical Synthesis of oligosaccharides on ganglioside and analysis of interactions using NMR technique

**Shinya Hanashima (RIKEN)**

Glycans on cell surface proteins and lipids play key roles in several biological events involving cell adhesions by interacting with the carbohydrate-recognition proteins or the other carbohydrates. We have investigated such carbohydrate-protein interactions as well as carbohydrate-carbohydrate interactions using NMR techniques. To achieve the purpose, chemical synthesis supports to obtain homogeneous oligosaccharides.  In first part, we describe the synthesis of the starfish ganglioside AG2 pentasaccharide and binding study between AG2 pentasaccharide and siglec-2. AG2 pentasaccharide was synthesized with efficient sialylation strategy using novel sialic acid building block. Further binding study between AG2 pentasaccharide having unique inner sialic acid and recombinant siglec-2 was then performed. Based on $^{1}$H-NMR titration and TR-NOESY experiments, AG2 exhibited binding to human siglec-2. In contrast, mouse siglec-2 exhibited no binding evidence to AG2 pentasaccharide by NMR experiments. In second part, we report the observation of the hydroxy protons on Lewis X trisaccharide in water using $^{13}$C-NMR isotope effects, and further investigation about Lewis X-$Ca^{2+}$-Lewis X interaction by evaluating proton exchanging rates on the sugar hydroxy groups. From the novel NMR experiment, the five hydroxy groups at Fuc(C2), Fuc(C4), Gal(C2), GlcNAc(C6), and Gal(C6) were potentially involved in the Lewis X-$Ca^{+2}$-Lewis X interaction.

References

1) Hanashima S, Yamaguchi Y, Ito K, Sato K: Tetrahedron Lett 50, 6150-3, 2009

2) Hanashima S, Sato Y, Ito Y, Yamaguchi Y: J Org Chem 4215-20, 2009

3) Hanashima S, Suetake T, Kato K, Yamaguchi Y: in preparation

### Transport mechanism and catabolic pathway for free N-linked ologosaccharide

**Yoshimi Haga (RIKEN)**

During N-glycosylation of proteins, significant amounts of free unconjugated glycans are also generated in the lumen of the endoplasmic reticulum (ER). These ER-derived free glycans are translocated into the cytosol by a putative transporter on the ER membrane for further processing. However, the molecular nature of the

transporter remains to be determined. Here, we report the establishment of a novel assay method for free oligosaccharide transport from the ER lumen using chemically synthesized fluorescence-labeled N-glycan derivatives. In this method, fluorescence-labeled glycan substrates were encapsulated inside mouse liver microsomes, followed by incubation with the cytosol and a fluorescence-quenching agent (anti-fluorophore antibody). The rate of substrate efflux was then monitored in real time by the decrease in the fluorescence intensity. The present data clearly demonstrated that the oligosaccharide transport activity under the current assay conditions was both ATP- and cytosol-dependent. The transporter activity was also found to be glycan structure-specific, because free glucosylated glycans were unable to be transported out of the microsomes. This new assay method will be a useful tool for identifying the transporter protein on the ER membrane.

References

1) Cacan R, Hoflack B, Verbert A: Fate of oligosaccharide-lipid intermediates synthesized by resting rat-spleen lymphocytes. Eur J Biochem 106, 473-9, 1980
2) Suzuki T, Funakoshi Y: Free N-linked oligosaccharide chain: Formation and degradation. Glycoconj J 23, 291-302, 2006
3) Haga Y, Totani K, Ito Y, Suzuki T: Establishment of a real-time analytical method for free oligosaccharide transport from the ER to the cytosol. Glycobiology 19, 987-94,2009

## Session 7 Oligosaccharide and Disease
## Chairperson : Shoko Nishihara (Soka University)

### A possibility of sorting machinary of fucosylated glycoproteins in hepatocytes as the mechanism of an increase in AFP-L3 in sera of patients with hepatocellular carcinoma

Tsutomu Nakagawa (Osaka University)

Fucosylated alpha-fetop rotein (AFP) is a highly specific tumor marker for hepatocellular carcinoma (HCC). Up-regulation of Fut8 activity and GDP-fucose levels are involved in the production of fucosylated AFP in patients with HCC. To understand the molecular mechanism in more details, we examined the effect of fucosylated oligosaccharides on the secretion of glycoproteins into bile. We found that glycoproteins in bile were strongly fucosylated compared with those in serum and that levels of glycoproteins were quite low in bile of Fut8 deficient mice as compared to wild type mice. We next analyzed the oligosaccharide structures of glycoproteins in bile and serum of LEC rats during hepatocarcinogenesis. Lectin microarray and HPLC analyses suggested that the selective secretion of fucosylated glycoproteins into bile might be partially disrupted in the liver with HCC although

the sorting mechanism was retained in the liver with chronic hepatitis. These results suggested that fucosylation is a possible signal for the secretion of glycoproteins into bile and a disruption in the sorting mechanism might involve an increase in fucosylated AFP in sera of patients with HCC. Furthermore, we investigated the sorting mechanism of glycoproteins, including AFP, in a polarized human hepatoma cell line HepG2

## The role of N-glycans of ErbB family

**Motoko Takahashi (Hokkaido University)**

Sugar chains play a role in a variety of biological events by affecting the physico-chemical properties of glycoproteins. The ErbB family consists of four members, and each member contains around 10 potential glycosylation sites in their extracellular domains. The study with glycosylation-deletion mutants indicated that N-glycan on Asn420 of EGFR suppresses ligand-independent spontaneous oligomerization. The N-glycan on Asn418 of ErbB3, which corresponds to Asn420 of EGFR, also regulates both the homodimerization and heterodimerization of ErbB3, and the N-glycan appears to be involved in transforming activity. It is assumed that those N-glycans might be involved in maintaining the inactive form of the receptors. On the other hand, studies using GnT-III transfectants revealed that modification of N-glycan affects the rate of EGFR endocytosis; the endocytosis of EGFR is upregulated and downstream Erk phosphorylation is increased in the GnT-111 overexpressing HeLaS3 cells. It is considered that the interaction between lectin-like molecules and specific N-glycan of EGFR is involved in control of endocytosis. Recent studies revealed the significance of endosomal signaling, supporting the functional importance of regulation of endocytosis by receptor glycosylation. In conclusion, it is suggested that N-glycosylation controls ErbB signaling by various mechanisms.

References

1) Sato Y, Takahashi M, Shibukawa Y, Jain SK, Hamaoka R, Miyagawa J, Yaginuma Y, Honke K, Ishikawa M, Taniguchi N: Overexpression of N-acetylglucosaminyl-transferase III enhances the EGF-induced phosphorylation of ERK in HeLaS3 cells by upregulation of the internalization rate of the receptors. J Biol Chem 276, 11956-62, 2001

2) Yokoe S, Takahashi M, Asahi M, Lee SH, Li W, Osumi D, Miyoshi E, Taniguchi N: The Asn418-linked N-glycan of ErbB3 plays a crucial rule in preventing spontaneous heterodimerization and tumor promotion. Cancer Res 67, 1935-42, 2007

3) Takahashi M, Yokoe S, Asahi M, Lee SH, Li W, Osumi D, Miyoshi E, Taniguchi N: N-Glycan of ErbB family plays a crucial role in dimer formation and tumor promotion. Biochim Biophys Acta 1780, 520-4, 2007

## Polysialic acid and schizophrenia

Ken Kitajima (Nagoya University)

Polysialic acid (polySia) is present on neural cell adhesion molecule (NCAM) in brain in a spaciotemporally regulated manner. PolySia has an "anti-adhesive effect" due to its bulky polyanionic nature. Due to this nature, it regulates cell-cell/matrix interactions. We have recently shown that polySia functions as a "reservoir molecule" for BDNF and dopamine that are deeply involved in neural functions and diseases. Two polysialyltransferases, ST8SiaII/STX and ST8SiaIV/PST, are responsible for the synthesis of polySia. Recently, a relationship between promoter region of SNPs of the ST8SiaII/STX and schizophrenia has been reported. In the present study, we focus on two SNPs in ORF region of STX. We measured activities of a wild-type and two mutant enzymes found in schizophrenia patients. We also analyzed the polySia-NCAM products for the amount of Sia and polySia by chemical and immunochemical methods. We further examined how polySia-NCAM-Fc synthesized by these mutant enzymes bound to BDNF and dopamine. Enzymatic activities of mutant enzymes were weaker than that of the wild-type *in vitro* and *in vivo* and the affinities toward BDNF and dopamine were dramatically reduced. All these data suggest that polySia might be abnormal in its expression and quantity in brains of schizophrenia patients, and that such abnormal polySia structures might disrupt their restoration function of neural molecules that are deeply involved in mental activities such as BDNF and dopamine.

References
1) Kanato Y, Kitajima K, Sato C: Glycobiology 12, 1044-53, 2008
2) Isomura R, Kitajima K, Sato C: BMB p161, 2008
3) Yabe U, Kitajima K, Shimoda Y, Watanabe K, Sawada M, Sato C: Glycoconj J 22, 245, 2005

# Session 8　Recent progress of Glycobiology and Glycotechnology

**Chair : Jun Hirabayashi (AIST)**
**(National Institute of Advanced Industrial Science and Technology (AIST) and Glyco-Innovation and Industrial Technology (GLIT))**

## Glycoproteome analysis technology and its applications

Hiroyuki Kaji (AIST)

Protein glycosylation concerns in widespread of biological events by regulating protein folding, localization, interaction, and stability. To accelerate uncovering of molecular and cellular functions of glycan on protein, it is important to know, (1) what kinds of glycoproteins are present in biological samples of interest, (2) which

potential sites are actually glycosylated, and (3) what sugar motifs are attached on there. Therefore, we developed a method to identify large numbers of glycoproteins and to determine actual glycosylation site in high throughput manner, which was named Lectin-IGOT LC/MS method. The method was applied to protein extract of *C. elegans*, mouse tissues, and currently human cancer secretions such as culture media and body fluids, and allowed identification of about 800, 1600, and 1000 glycoproteins, respectively. Using the experimental data as resource, we are now constructing a glycoprotein database, GlycoProtDB, and partial data of the worm are already available at JCGGDB; http://jcggdb.jp/database.html). These large set of data may provide insight into the mechanism of regulation for protein glycosylation and glycan processing.

References
1) Kaji H et al.: Nat Biotechnol 21(6), 667-72, 2003
2) Kaji H et al.: Mol Cell Proteomics 6(12), 2100-9, 2007
3) Narimatsu H et al.: FEBS J 2009 in press

## Multiplex lectin-antibody sandwich immunoassay system for development of hepatic disease specific glyco-biomarker

Atsushi Kuno (AIST)

Hepatitis B and C virus (HBV, HCV) infections are prevalent health problems, affecting 2 billion and 170 million people, respectively, worldwide. As about 80% of cirrhosis patients contracted HCC within the past 10 years, 90% of HCC cases in Japan originated from chronic hepatitis caused by HCV infection. It is evident that the best way of monitoring the progression of hepatitis is to establish an accurate serological method for quantitative evaluation of fibrosis. Consequently, a great variety of noninvasive methods, including the FibroScan and FibroTest, have been developed. However, liver biopsy, an invasive and high-risk procedure, is widely regarded as the "gold standard" for defining liver disease status. In brief, alternative convenient serological biomarkers are still needed for frequent monitoring. On the basis of our premise that tissue-specific glycoproteins with disease-specific glyco-alteration are the most promising serological glyco-markers, we focused on the abundant serum glycoproteins, which are mainly synthesized in the liver and undergoe glyco-alteration, as a potential marker for predicting liver cirrhosis. We statistically estimated the relationship of each lectin signal obtained using the lectin microarray analysis with the progression of fibrosis using histopathological evidence and selected indicator lectins most relevant to the progression of this disease.

References
1) Kuno A, Uchiyama N, Koseki-Kuno S, Ebe Y, Takashima S, Yamada M, Hirabayashi

J: Evanescent-field fluorescence-associated lectin microarray: a new strategy for gycan profiling. Nat Methods 2, 851-6, 2005
2) Kuno A, Kato Y, Matsuda A, Kaneko MK, Ito H, Amano K, Chiba Y, Narimatsu H, Hirabayashi J: Focused differential glycan analysis with the platform antibody-associated lectin profiling (ALP) for glycan-related biomarker verification. Mol Cell Proteomics 8, 99-108, 2009
3) Narimatsu H, Sawaki H, Kuno A, Kaji H, Ito H, Ikehara Y: A strategy for discovery of cancer glyco-biomarkers in serum using newly developed technologies for glycoproteomics. FEBS J in press

## Cellular glycomics and endogeneous lectins
Hiroaki Tateno (AIST)

All cells in nature are covered with a dense and complex array of glycans. Each cell type expresses a different set of glycans. The structures of cell surface glycans change depending on cell differentiation and during malignant transformation. Therefore cell surface glycans are often called "cell signature" or "cell face". In many organisms, however, only little or no information is available about glycan structures. In order to analyze cell surface glycans of living cells, we have developed a multiplex assay system of cell surface glycome of living cells using lectin microarray. Using the system, we have successfully analyzed cellular glycome of mammalian cells, fungi, and bacteria. For the screening of novel lectins, we have also developed glycoconjugate microarray. Using the system, we have reported novel specificities of several lectins such as Mincle, a *C. elegans* C-type lectin, and C21orf63. Recently, we found that Langerin expressed on Langerhans cells shows dual specificity to mannosylated and sulfated glycans via a single C-type CRD. We are now searching glycan-binding activity and specificity of lectin-like molecules encoded in human genome.

References
1) Kuno A, Uchiyama N, Koseki-Kuno S, Ebe Y, Takashima S, Yamada M, Hirabayashi J: Nat Methods 2, 2005
2) Tateno H, Uchiyama N, Kuno A, Togayachi A, Sato T, Narimatsu H, Hirabayashi J: Glycobiology 17, 1138-46, 2007
3) Tateno H, Hori A, Uchiyama N, Yabe R, Iwaki J, Shikanai T, Angata T, Narimatsu H, Hirabayashi J: Glycobiology 18, 789-98, 2008

## JCGGDB-Released services and plans for the final year
Toshihide Shikanai (AIST)

As part of Life Science Integrated Database project by MEXT, we are integrating

glycoscience-related databases cooperating with DB owner research institutes and universities. As initial outcomes, keyword or glycan structure search has been released across GlycoScience DBs, and next, a platform for data exchange at integrated search will be built up toward the final year. Now we are preparing prototype of the integrated search, developing basic technologies and organizing the information.

Additionally, some more DBs are under construction. Resource DB providing resource information required for research, online experimental protocols for researchers specializing in proteomics as well as glycomics (joint research with Ritsumeikan University), glycan synthesis DB supporting how to obtain desired glycans based on standard preparation (joint research with Noguchi Institute), inhibitor DB for the information on glycogene activity inhibition and glycoprotein, glycan-associated disease DB, and pathogen adherence to carbohydrate DB. These DBs will be released upon completion and incorporated into the integrated DB. During the final year, registration system will be developed for each DB to allow researchers to enter their own information.

For the future, we are building infrastructure to link with more DBs including areas peripheral to glycoscience and user-friendly, intuitive interface for researchers specializing in broader area.

## Closing Remarks