# The 17th Symposium
# of Japan Consortium for Glycobiology and Glycotechnology

## Prospects of Glycoscience and Glycoengineering
## –Variation and Universality of Glycans: Deciphering glycan code and manipulation of functions

**October 29 – 30 at the Main Hall of Shimadzu Corporation Kyoto**

## Program on October 29 (Tue), 2019

**Opening Address**
**Kazunari Akiyoshi (Kyoto University)**

**President Address**
**Tamao Endo (JCGG President)**

### Session 1　Bird's-Eye View of Glycan Functions
**Chair: Koichi Fukase (Osaka University)**

### How to think about origin of saccharides and their recognition in life systems
**Jun Hirabayashi (AIST)**

Important to note, only few component saccharides, i.e., D-Glc, D-Man and D-Gal are utilized in nature among possible 16 aldohexoses. This observation implies that the first living organisms could make use of a relatively small number of simple saccharides that had been sufficiently available on the prebiotic earth. In this lecture, the author reviews structural and metabolic features of naturally occurring saccharides on the basis of classic glycochemistries and discusses how these basic chemistries and resultant component saccharides affect the current recognition systems in biology.

References

1) 阿武宮美子, 瀬野信子：糖化学の基礎. 1984, 講談社サイエンティフィック
2) Hirabayashi J: On the origin of elementary hexoses. Q Rev Biol 71(3), 365-80, 1996.

## Complex carbohydrates and signal transduction
Koichi Furukawa (Chubu University)

A number of studies on dynamic changes in complex carbohydrates depending on development, cell/tissue differentiation and malignant transformation have been performed, leading to the idea that individual glycosylation patterns are implicated in the regulation of our body and homeostasis. They are now considered to generate and/or modulate cell signals at membrane microdomains named lipid rafts. However, how they generate/modulate cell signals to determine the phenotypes and fates of cells remains to be clarified. Here, I would introduce results of our studies on the regulatory mechanisms of cell signals with complex carbohydrates based on the artificial manipulation of glycosyltransferase genes in cancers and nervous systems. In particular, "glycosignals" generated with interaction of glycosphingolipids and their recognizing molecules at lipid rafts in cancer cells and nervous tissues will be summarized, expecting efficient application of those results in the therapeutics.

References

1) Ohmi Y, Kambe M, Ohkawa Y, Hamamura K, Tajima O, Takeuchi R, Furukawa K, Furukawa K: Differential roles of gangliosides in malignant properties of melanomas. PLoS One 13:e0206881, 2018

2) Furukawa K, Ohmi Y, Ohkawa Y, Bhuiyan RH, Zhan P, Tajima 0, Hashimoto N, Furukawa K: New era of cancer-associated glycosphingolipids. Cancer Sci 110, 1544-51, 2019

3) Hashimoto N, Ito S, Tsuchida A, Bhuiyan RH, Okajima T, Yamamoto A, Furukawa K, Ohmi Y, Furukawa K: The ceramide moiety of disialoganglioside (GD3) is essential for GD3 recognition by the sialic acid-binding lectin SIGLEC7 on the cell surface. J Biol Chem 294, 10833-45, 2019

## General functions of N-glycans on proteins
Yasuhiro Kajihara (Osaka University)

Oligosaccharides of protein surface play important roles in many biological events. In order to evaluate the function of oligosaccharides dependent on glycosylation positions, glycoproteins having homogeneous oligosaccharides are essential. Recently, chemical and semisynthetic methods successfully generate homogeneous glycoproteins. Homogeneous sialyloligosaccharides and high mannose type oligosaccharides are isolated from egg yolk and used for solid phase

peptide synthesis (SPPS). SPPS and native chemical ligation yield target glycosyl polypeptides and subsequent folding experiments afford correctly folded homogeneous glycoproteins.

In this presentation, I would discuss the contribution of chemical characteristic nature of oligosaccharides to the stability and biological activity of glycoproteins.

References
1) Murakami M et al.: Chemical synthesis of erythropoietin glycoforms for insights into the relationship between glycosylation pattern and bioactivity. Sci Ad 2016, DOI: I0.1126/sciadv.1500678.
2) Kiuchi T et al.: Monitoring of glycoprotein quality control system with a series of chemically synthesized homogeneous native and misfolded glycoproteins. J Am Chem Soc 140, 17499-507, 2018, DOI: 10.1021/jacs.8b08653.

## Glycan's strategies in life: Heterogeneity and field formation

Ken Kitajima (Nagoya University)

Glycans ubiquitously occur in all organisms, occupying a large volume due to their hydration. They are synthesized by adding a monosaccharide onto a nonreducing terminus residue one by one by a series of glycosyltransferases. Since each glycosylation step is not complete because of its sensitivity to multiple circumstantial factors, resultant glycan structures are inevitably perturbed to show "heterogeneity". In addition, glycans can easily change their structures by a single nucleotide alteration in a glycosyltransferase gene, which should be a convenient strategy when rapid structural changes are necessary at evolutional processes. Glycans are recognized by their interacting molecules to show their functions, and specific lectin-glycan interactions are a molecular base for how glycans are functional in various interactions. Therefore, heterogeneity of glycan structures would be disadvantageous to the specific interaction. In my presentation, I will discuss how intrinsic properties of glycan-mediated interaction compensate the drawback of heterogeneity.

References
1) Sato C, Hane M: Mental disorders and an acidic glycan-from the perspectiveof polysialic acid (PSA/polySia) and the synthesizing enzyme, ST8Sia2. Glycoconj J 35, 353-373, 2018
2) Sato C, Kitajima K: Sialic acids in neurology. Adv Carbohydr Chem Biochem 76,

1-64. 2019
3) Adachi T, Sato C, Kishi Y, Totani K, Murata T, Usui T, Kitajima K: Membrane microdomains from early gastrula embryos of medaka, *Oryzias latipes*, are a platform of E-cadherin- and carbohydrate-mediated cell-cell interactions during epibody. Glycoconj J 26, 285-99, 2009.

## Short Presentation of Sponsor Corporations
**Chair Eriko Hagiya (Mizutani Foundation for Glycoscience)**

Agilent Technologies, GlycoTechnica Ltd., Shimadzu Corporation, Toray Research Center, FUSHIMI Pharmaceutical Co., Tokyo Chemical Industry Co., Waters Corporation, Fujifilm Wako Pure Chemical Corporation

## Luncheon Seminar (sponsored by GlyTech. Inc.)
**The science of sugar oxazolines**
**Shin-ichiro Shoda (Tohoku University)**

The oxazoline derivatives of sugars are a class of valuable and versatile intermediates in carbohydrate chemistry. Several methods for preparation of sugar oxazolines that are suitably protected are introduced. Protected sugar oxazolines have been widely used as glycosyl donors for the synthesis of various kinds of glycosyl compounds. A brief history and the chemistry of unprotected sugar oxazolines is described. In the past few years, our research group has developed protection-free chemo-enzymatic process for synthesis of glycosyl compounds such as oligosaccharides and glycoproteins through unprotected sugar oxazolines as reactive intermediates based on the concept of "*direct anomeric activation*". In this seminar, the one-step preparation of sugar oxazolines by using formamidinium-type dehydrating agents is presented.

## Session 2　Synthetic and Analytical Technologies Driving Glycan Research
**Chair Keiko Shimamoto (Life Bioorganic Research Institute)**

### Leading glycoscience by chemical synthesis
**Yukisige Ito (RIKEN)**

Glycan chains of various glycoconjugates are known to play pivotal roles in biological systems. Given their structural complexity diversity, chemical synthesis is

expected to be powerful in understanding functions of glycan chains at molecular level. However, synthesis of glycan chains is more problematic than other biomolecules such as oligopeptides and oligonucleotides, because high efficiency, especially in terms of precise stereocontrol is difficult to achieve. In addition, glycan containing molecules having biological relevance often have highly complex structures. To remove these problems, various studies have been conducted, among which highly significant contributions have been made by researchers in various countries. This talk aims to summarize recent advances in the synthesis of complex glycans, glycolipids and glycoproteins, along with development of methodologies, in order for emphasizing the role of synthetic organic chemistry in glycoscience.

References
1) Ito Y, Takeda Y, Seko A, Izumi M, Kajihara Y: Sem Cell Dev Biol 41, 90-8, 2015
2) 伊藤幸成：有機合成化学協会誌 74, 206-18, 2016
3) 伊藤幸成：有機合成化学協会誌 76, 59-65, 2018

## Addressing a grand challenge in glycan synthesis; seeing for a method of facile massive synthesis of glycans

Hiromune Ando (Gifu University)

It is undoubted that the synthesis of structurally fine glycans and their functionalized analogs as probes is of great importance to the elucidation of the biological function of glycans. Our research group has long been seeking for a practical and robust method for the synthesis of sialic acid containing glycans, particularly focusing on solving the long-standing issue of α-selective sialylation. Very recently, we have succeeded in fully stereoselective α-glycosidation of sialic acid for the first time. A simple modification of sialic acid enabled to completely exclude the formation of β-glycoside during glycosidation reaction. Thereby, α-selective glycosidation of sialic acid becomes the most facile and promising among glycosidations, which would allow for expanding the scope of automated glycan synthesis. The lecture will intend to share the detail of the α-sialylation study and our latest research outcomes.

References
1) Ando H et al.: A synthetic challenge to the diversity of gangliosides for unveiling their biological significance. J Synth Org Chem Jpn 75, 1162-70, 2017
2) Komura N et al.: Raft-based interactions of gangliosides with a GPI-anchored

receptor. Nat Chem Biol 12, 402-10, 2016
3) Komura N et al.: Constrained sialic acid donors enable selective synthesis of α-glycosides. Science 364, 677-80, 2019

## Chair Katsunori Tanaka (RIKEN)

### Toward understanding of glycan structure-function relationship
### Yoshiki Yamaguchi (Tohoku Medical and Pharmaceutical University)

Our knowledge is still limited on the physiological functions of glycans. But it is likely that many glycans and glycoconjugates express their functions through glycan-protein interactions at many physiological events. Thus, 3D structural analyses are of considerable importance for understanding glycan-dependent mechanisms. Solution NMR methodology has a great potential in determining primary and tertiary structure of glycans and the interaction mode(s) with the binding molecules. Here I will introduce our experiences in the NMR analysis of glycans and glycoconjugates.

References
1) Nagae M, Yamaguchi Y: Adv Exp Med Biol 1104, 119-47, 2018
2) Kanie Y, Yamaguchi Y, Hayashi A, Uzawa J, Hatakeyama M, Hidaka Y, Toda N, Nakamura S, Kanie O: Carbohydr Res 473, 104-14, 2019
3) Uzawa J, Shimabukuro J, Suzuki T, Imamura A, Ishida H, Ando H, Yamaguchi Y: Magn Reson Chem 56, 836-846, 2018

### Elucidation of glycan function by microscopic observation
### Kazuya Kabayama (Osaka University)

In recent years, live imaging of immune cells has been desired. However, in the case of floating cells, behavioral analysis is not easy due to defocusing and movement out of the observation area. Therefore, we established a nano-wrapping technology that uses ultra-thin films of polylactic acid to keep floating cells in the observation area. Then, we visualized calcium oscillation, transferrin endocytosis, and antibody adhesion of floating cells. Furthermore, we have succeeded in observing receptor-dependent internalization of the innate immunity ligand by the settings of the optical device. In addition, we have established a microfluidic device system as a tool for closely stimulating drugs to target cells. Recently, we confirmed

that cell membrane fluidity is altered by volatile anesthetic treatment, so we report the reverified data using this device. We evaluate the biological phenomena by "correctly" visualizing the dynamics of glycolipids and cell membrane proteins using such optical techniques.

References
1) Zhang H, Aoki T, Hatano K, Kabayama K, Nakagawa M, Fukase K, Okamura Y: Porous nanosheet wrapping for live imaging of suspension cells. J Mater Chem B 6, 6622-8, 2018, selected Hot Papers in 2018
2) Arai Y, Yokoyama K, Kawahara Y, Feng Q, Ohta I, Shimoyama A, Inuki S, Fukase K, Kabayama K, Fujimoto Y: Time-lapse monitoring of TLR2 ligand internalization with newly developed fluorescent probes. Org Biomol Chem 16, 3824-30, 2018
3) Ono J, Fushimi S, Suzuki S, Ameno K, Kinoshita H, Shirakami G, Kabayama K: Effect of the volatile anesthetic agent isoflurane on lateral diffusion of cell membrane proteins. FEBS Open Bio 8, 1127-34, 2018

## Special Lecture
## Chair Koichi Kato (Institute for Molecular Science)

### A critical role of carbohydrate structure in the physical chemistry of antibodies
Kouhei Tsumoto (The University of Tokyo)

Understanding the principles on how N-glycosylation modulates those properties is important for the molecular design, manufacturing, process optimization and quality control of therapeutic antibodies. In this study, we have separated a model therapeutic antibody into three fractions according to the composition of the N-glycan by using a novel FcγRIIIa chromatography column. Notably, Fc galactosylation was a major factor influencing the affinity of IgG-Fc to the FcγRIIIa immobilized on the column. Each antibody fraction was employed for structural, biological and physicochemical analysis, illustrating the mechanism by which galactose modulates the affinity to FcγRIIIa. In addition, we discuss the benefits of the FcγRIIIa chromatography column to assess the heterogeneity of the N-glycan.

References
I) Assessing the heterogeneity of the Fc-glycan of a therapeutic antibody using an engineered Fey receptor Illa-Immobilized column. Sci Rep 8(1), 3955, 2018

2) Glycosylation of IgG-Fc: a molecular perspective. Int Immunol 29(7), 311-7, 2017
3) Structural analysis of Fc/FcγR complexes: a blueprint for antibody design. Immunol Rev 268(1), 201-21, 2015
4) Structural basis for binding of human IgG1 to its high-affinity human receptor FcγR1. Nature Commun 6, 6866, 2015

## Special Lecture

### Chair Tamao Endo (Tokyo Metropolitan Institute of Gerontology)

### Dynamics of function and regulation of the endoplasmic reticulum

Kazutoshi Mori (Kyoto University)

The endoplasmic reticulum (ER), where newly synthesized secretory and transmembrane proteins are folded and assembled, has the ability to discriminate folded proteins from unfolded proteins and controls the quality of synthesized proteins. Only correctly folded molecules are allowed to move along the secretory pathway, whereas unfolded proteins are retained in the ER.

The ER contains a number of molecular chaperones and folding enzymes (ER chaperones hereafter), which assist productive folding of proteins, and therefore newly synthesized proteins usually gain correct tertiary and quaternary structures quite efficiently. Yet unfolded or misfolded proteins even after assistance of ER chaperones are retrotranslocated back to the cytosol, ubiquitinated and degraded by the proteasome. This disposal system is called ER-associated degradation (ERAD). Thus, the quality of proteins in the ER is ensured by two distinct mechanisms, productive folding and ERAD, which have opposite directions.

Under a variety of conditions collectively termed ER stress, however, unfolded or misfolded proteins accumulate in the ER, which in turn activates ER stress response or Unfolded Protein Response (UPR).

I will talk on the mechanism, evolution and physiological importance of the UPR and ERAD as well as its involvement in development and progression of various diseases.

## Program on October 30 (Wed), 2019

### Session 3　Frontiers of Material Glycan Science
**Chair Toshiyuki Inazu (Tokai University)**

### Artificial synthesis of human milk oligosaccharides
**Shin-ichiro Shoda (Tohoku University)**

A human milk oligosaccharide, lacto-N-tetraose (LNT), has gathered a lot of interest due to its health-promoting effects in the intestine of breastfed infants. A chemo-enzymatic microflow system for synthesis of LNT has been developed. First, lacto-N-biose (LNB) was converted to the corresponding oxazoline derivative, LNB-oxa, by using direct anomeric activation technique. The resulting activated glycosyl donor was reacted with lactose catalysed by a mutated lacto-N-biosidase (LNBase), giving rise to LNT. One-pot chemo-enzymatic synthesis of LNT proceeded under lower temperature. This protection-free chemo-enzymatic technique is a promising tool for preparation of various kinds of human milk oligosaccharides.

References

1) Shoda S: Proc Japan Acad Ser B 93, 125, 2017
2) Noguchi M et al.: J Org Chem 74, 2210, 2009

### Visualization of vascular function at cellular and tissue level using 3D tissue model
**Yukiko T. Matsunaga (The University of Tokyo)**

Inhibiting or normalizing pathological angiogenesis is a therapeutic strategy that has been extensively studied and already brought up clinically with approved drugs. However, most experimental assays for drug development rely on 2D cell culture models, which fail to mimic sprouting from a parent vessel. We have developed a microvessel-on-a-chip which enables the study of drugs targeting a specific pathway of angiogenesis. Microvessels were prepared using human umbilical vein endothelial cells (HUVEC) within a collagen gel scaffold. It was revealed that the technology enables to contribute to improve the discovery of promising anti-angiogenic molecules and provide a convenient tool to assess fundamental questions about mechanisms at both tissue and cellular level during angiogenesis.

References

1) Pauty J, Usuba R et al.: A vascular endothelial growth factor-dependent sprouting

angiogenesis assay based on an in vitro human blood vessel model for the study of anti-angiogenic drugs. EBioMedicine 27, 225-36, 2018
2) Usuba R, Pauty J, Soncin F, Matsunaga YT: EGFL7 regulates sprouting angiogenesis and endothelial integrity in a human blood vessel model. Biomaterials 197, 305-16, 2019

### Trial production vehicle utilizing CNF
### Arimitsu Usuki (Koyo University)

Cellulose nano fiber (CNF) is a new class of bio-based materials with characteristics such as high strength, low thermal expansion, low density, and is produced from various sources of cellulose sources such as plants. CNF is carbon neutral and renewable material. Nano Cellulose Vehicle (NCV) Project started in October 2016. It is expected that the reduction of weight of vehicle leads to better energy efficiency which reduces emission of carbon dioxide ($CO_2$) from vehicles. Contribution to global warming countermeasures is expected. Program of the project covers evaluation/verification of material constants of CNF related materials, moldability of CNF nano composite materials, structural element models such as plate and pipes, performance of CNF based parts and components, and model vehicles. Example of parts are intake manifolds, door trims, engine hoods. Initial evaluation results indicate the use of CNF based materials has advantage with respect to the reduction of weight of automotive parts.

## Session 4　Future of Science Covering Glycan-Related Diseases
### Chair Shogo Oka (Kyoto University)

### Genetic disorders related glycan catabolism
### Tadashi Suzuki (RIKEN)

Glycans on proteins/lipids are known to play pivotal roles in various cellular processes. The biosynthesis/processing pathway for glycans has been well characterized in mammalian cells, while there are issues remaining to be clarified concerning aspects of their catabolism/degradation. While the molecular mechanism of their lysosomal degradation pathway has been well studied, especially with related to genetic disorders collectively called lysosomal storage diseases, evidence also suggested that there are also "non-lysosomal" degaradation pathway. In this lecture, I will focus on degradation of asparagine-linked (N-linked) glycans and summarize our current knowledge for their lysosomal/non-lysosomal

degradation. We will also overview human genetic disorders caused by the defect of these processes.

References
1) Suzuki T: Mol Aspects Med 59, 89-93, 2016
2) Suzuki T et al.: Gene 577, 1-7, 2016
3) Suzuki T: Trends Glycosci Glycotechnol 31, SE55-6, 2019

## Glycopathology in diabetes
Jin-ichi Inokuchi and Hirotaka Kanoh (Tohoku Medical and Pharmaceutical University)

GM3 ganglioside in human serum is composed by a variety of fatty acids including long-chain (LCFA) and very long-chain (VLCFA). GM3 by itself had no effects on TLR4 activation; however, VLCFA-GM3 synergistically and selectively augmented TLR4 activation by LPS/HMGBl, and in contrast, LCFA- and unsaturated VLCFA-GM3 suppressed TLR4 activation. Serum VLCFA-GM3 increased significantly and LCFA-GM3 decreased sharply in metabolic disorders. Artificial intelligence based approaches revealed that GM3 species are significantly related to the disease symptoms. GM3 interacted with extracellular regions of TLR4/MD-2, and modulated dimerization/oligomerization. VLCFA-GM3 also increased in the adipose tissue of obese mice, and GM3-synthase knockout ameliorated early pathogenesis of metabolic disorders. The increase of VLCFA-GM3 was attenuated in TLR4-mutant mice, implying a feedback loop from TLR4 to GM3. Our findings suggest that VLCFA-GM3 is a risk factor for TLR4-mediated disease progression.

References
1) Veillon et al.: PLosOne 10(6):e0129645, 2015
2) Kanoh et al.: in revision
3) Nitta et al.: Glycobiology 29, 260-8, 2019

## Cancer and glycosylation
Eiji Miyoshi (Osaka University)

In this symposium, I would like to see research of cancer and glycosylation from a higher perspective. It is well known fact that oligosaccharide structure is dramatically changed in carcinogenesis. Increases in branching structure, sialylation

and fucosylation are the representative glycosylation in cancer. GnT-V and ST6Gal1 are the most well known glycosyltransferases involved in cancer glycosylation. There are a variety of glyco-biomarkers for cancer. The most important issue in biomarker research is to analyze molecular mechanism underlying the increase of glyco-biomarkers in sera of cancer patients. Next generation glycan antibody, which recognizes both characteristic oligosaccharide structure and peptides is a bona-fide tool for cancer research with glycosylation. Combination of glyco-biomarkers and other markers with AI system can bring novel stream of biomarker study.

References.

I ) Capurro M, Wanless IR, Sherman M, Deboer G, Shi W, Miyoshi E, Filmus J: Glypican-3: a novel serum and histochemical marker for hepatocellular carcinoma. Gastroenterology 125(1), 89-97, 2003

2) Moriwaki K, Noda K, Furukawa Y, Ohshima K, Uchiyama A, Nakagawa T, Taniguchi N, Daigo Y, Nakamura Y, Hayashi N, Miyoshi E: Deficiency of GMDS leads to escape from NK cell-mediated tumor surveillance through modulation of TRAIL signaling. Gastroenterology 137(1),188-98, 2009

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## Luncheon Seminar (sponsored by Sumitomo Bakelite Co.)

### Development of a novel platform for mucin analysis

**Akihiko Kameyama (AIST)**

Many of studies aim to discover disease-biomarkers by comparing the proteomes of clinical specimens. However, mucins are hardly detected in these studies because of their inability to enter a polyacrylamide gel due to their large molecular sizes, highly glycosylated, and resistance to proteases. Different strategies from conventional proteomics may be required for discovery of mucin biomarkers. Here I introduce a novel platform for mucin characterization, supported molecular matrix electrophoresis (SMME) that is a membrane electrophoresis using hydrophilic polymer soaking into the porous membrane of polyvinylidene difluoride (PVDF) as the separation medium. Besides, I also introduce "eliminative oximation", an O-glycan liberation method from glycoproteins. This method gives O-glycan oximes

that can be labeled with a fluorescent group by a conventional reductive amination. The glycan recovery rate of this method is as much as that of gas-phase hydrazinolysis, but the peeling rate is one fifth. We believe that the method would be quite useful as a general technique of O-glycan liberation for glycomics, glycobiology research, and the biopharmaceutical industry.

References
1) Matsuno YK, Saito T, Gotoh M, Narimatsu H, Kameyama A: Anal Chem 81, 3816-23, 2009
2) Kameyama A, Thet Tin WW, Toyoda M, Sakaguchi M: Biochem Biophys Res Commun 513, 186-192, 2019

## Special Lecture
**Chair Shoko Nishihara (Soka University)**

### Integrative understanding of life. Why is glycan research important?
**Kenji Kadomatsu (Nagoya University)**

We have proposed Human Glycome Project (HGP) to the Science Council of Japan. Combined with genomics and proteomics, glycomics would provide a new stage to truly understand life. Most proteins are modified by glycans. Lipids are also. Glycans give diversity and individuality to proteins and lipids, and consequently to cells. Moreover, glycans are beyond the control of the central dogma, and most sensitively reflect phenome. Glycome shows a huge diversity (e.g., more than SNPs) and is not directly influenced by other omics. Integration of glycome and other omics may contribute to our understanding of diseases and developing of new therapies. We can now initiate Human Glycome Project, since technologies for glycoscience have strikingly developed. For the success of Human Glycome Project, we need to set up a condition in which the data are fully presented to the public and shared by scientists worldwide toward the goal of national legacy to be a base to rapidly develop science for the well-being of people and society.

## Session 5　Frontiers of Extracellular Vesicles or Exosomes Research
**Chair Jun Hiorabayashi (AIST)**

### Special lecture
### The evolutionary history of extracellular vesicles (or exosomes)

## Kiyotaka Shiba (Cancer Institute)

In this presentation, I review the research history regarding extracellular vesicles (EVs), which originated sometime around the 1950s. I will discuss what is still needed to further advance EV research and the direction in which it is headed. Generated from cells via distinct routes, EVs are comprised of a heterogeneous population. Through circular interaction between cells, the nature of intercellularly exchanged EVs is dynamically and instantly evolved. Novel methods to differentiate heterogeneous populations of EVs are urgently needed, including accurate and easy-to-use equipment for categorizing and quantifying specific subclasses of EVs. We must also explore new strategies to understand biological communication among heterogeneous entities.

References
1) Valadi H et al.: Nat Cell Biol 9, 654-659, 2007
2) Thery C et al.: J Extracell Vesicles 7, 1535750, 2018

## Functional analysis of glycans of exosomes in biological responses and control

## Kazunari Akiyoshi (Kyoto University)

Extracellular vesicles (EVs) such as exosomes are released from various cells and play an important role in cellular communications relating to various diseases. Exosomes can be used as biomarkers for diagnosis, prognosis, and determining the cell state. Although proteomics or genomics of EVs have been extensively studied, little is known about details of surface glycans on EVs. We develop biosciences in exosomal carbohydrates related to structural and functional diversity of exosomes, and also specific interaction with tissues or cells. For example, we proposed that an evanescent field fluorescence-assisted (EFF) lectin array method is a powerful tool for functional analysis of glycan on EV surface and also biomarker discovery.

References
1) Shimoda A et al.: Sci Rep 6, 18346, 2016
2) Seo N et al.: Nature Communications 9, 435, 2018
3) Shimoda A et al.: Sci Rep 9, 11497, 2019

## Characterization of blood exosomes and medical applications
Hiroaki Tateno (AIST)

Glycans are one of the major building blocks of exosomes. However, their roles and applications have not been completely explored. We analyzed the glycome of exosomes derived from human induced pluripotent stem cells (hiPSCs) using high-density lectin microarray. We demonstrated that the characteristic glycan signature of hiPSCs are retained by exosomes derived from them. Similar results were also obtained for exosomes derived from cultures of human pancreatic cancer cell lines. We further analyzed the glycome of exosome derived from serum of various diseases including pancreatic cancer and mental disorders. A particular exosome was found to be increased upon the onset of various diseases such as cancers and mental disorders, which might be applied for the early detection of these diseases.

References

1) Saito S, Hiemori K, Kiyoi, Tateno H: Glycome analysis of extracellular vesicles derived from human induced pluripotent stem cells using lectin microarray. Sci Rep 8(1), 3997, 2018.

## Exosomes for medical use
Hiroshi Shiku (Mie University)

Exosomes are involved in pathogenesis of a variety of diseases including cancer. In addition to basic research of exosomes in cancer, their usefulness in diagnosis and therapy is widely challenged.

We investigated whether exosomes derived from activated $CD8^+$ T cells modulate tumor progression including tumor growth, invasion, and metastasis by affecting tumor microenvironment. Intratumoral administration of $CD8^+$ T cell exosomes resulted in the attenuation of tumor growth, and strong inhibition of tumor invasion and metastasis with disappearance of tumor stromal cells including mesenchymal stem cells (MSCs) and cancer-associated fibroblasts (CAFs).

These findings prompt us to explore use of T cell derived exosomes for cancer therapy.

References

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## Closing
## Kazunari Akiyoshi (Kyoto University)