Medical Proteomics Laboratory
疾患プロテオミクスラボラトリー

Professor	Mutsuhiro Takekawa, M.D., Ph.D.	教　授	博士(医学)	武　川　睦　寛
Project Professor	Kouhei Tsumoto, Ph.D.	特任教授	博士(工学)	津　本　浩　平
Associate Professor	Masaaki Oyama, Ph.D.	准教授	博士(医学)	尾　山　大　明
Senior Assistant Professor	Makoto Nakakido, Ph.D.	講　師	博士(生命科学)(大学院工学系研究科)	中木戸　　誠
Assistant Professor	Ryo Matsunaga, Ph.D.	助　教	博士(生命科学)(大学院工学系研究科)	松　長　　遼
Project Assistant Professor	Hiroshi Sagara, Ph.D.	特任助教	博士(医学)	相　良　　洋

The mission of our laboratory is to develop advanced technologies for integrative proteomic analyses from a physicochemical, structural and systems biology point of view. Currently, we mainly focus on functional protein-protein interaction networks related to a variety of diseases including cancer and infection. We are also engaged in collaborative researches regarding mass spectrometry and electron microscopy, which have made a substantial contribution to many scientific achievements.

<Group I>

1. Integrative analysis of cancer cell signaling networks by high-resolution proteomics and systems biology

Post-translational modifications (PTMs), such as phosphorylation, ubiquitination and acetylation, are known to be widely involved in the regulation of various biological processes through extensive diversification of each protein function at the cellular network level. Previous functional analyses of cancer cell signaling under a variety of experimental conditions revealed many of the key molecules and their associated protein modifications in relation to each type of cancer. In order to systematically discover critical modulators from diversified signaling molecules, we have developed a high-resolution mass spectrometry-based proteomics platform for integrative identification and quantification of multiple post-translational modifications from various types of cancer cells.

1-1. High-resolution proteomic analysis of EGF-regulated ubiquitination dynamics in human cancer cells

Hiroko Kozuka-Hata, Tomoko Hiroki, Aya Kitamura, Aiko Aizawa, Naoaki Miyamura, Kouhei Tsumoto, Jun-ichiro Inoue and Masaaki Oyama.

Protein ubiquitination is one of the most prevalent post-translational modifications (PTMs) and plays critical roles in regulating protein degradation, signal transduction and DNA repair in cooperation with other PTMs such as phosphorylation and acetylation. Recent mass spectrometry-based proteomics coupled with efficient enrichment technologies for each type of the modified peptides has enabled us to identify precise modification sites and measure their quantitative changes on a global scale. Our previous lysine-modification proteomic analyses of thirteen representative human cancer cell lines led us to identify thousands of ubiquitination (Ub) and acetylation (Ac) sites in total and revealed that their system-wide modification status was mutually different at the cellular network level. In this study, we further applied SILAC (Stable Isotope Labeling by Amino acids in Cell culture) for quantitative description of EGF-dependent lysine-modification site dynamics in HeLa cells in a time-resolved manner. Through integration of large-scale SILAC-encoded data on six time points upon EGF stimulation, we successfully quantified approximately 1,000 kinds of Ub-sites as well as 700 kinds of Ac-sites and found that one-third of these

Ub-modified molecules, including several EGF signaling effectors, were subjected to downregulation by proteasomal inhibition.

1-2. Proteome-wide analysis of lysine acetylation and ubiquitination reveals critical signaling regulation in cancer cells

Hiroko Kozuka-Hata, Aya Kitamura, Tomoko Hiroki, Aiko Aizawa, Kouhei Tsumoto, Jun-ichiro Inoue and Masaaki Oyama.

Post-translational modifications (PTMs), such as phosphorylation, ubiquitination and acetylation, are known to be widely involved in the regulation of various biological processes through extensive diversification of each protein function at the cellular network level. Previous functional analyses of cancer cell signaling under a variety of experimental conditions revealed many of the key molecules and their associated protein modifications in relation to each type of cancer. In order to systematically discover critical modulators from diversified signaling molecules, we have developed a high-resolution mass spectrometry-based proteomics platform for integrative identification and quantification of multiple post-translational modifications from various types of cancer cells. Our large-scale proteomic analysis enabled us to identify more than 5,000 kinds of ubiquitinated sites and 1,600 kinds of acetylated sites from representative human cancer cell lines, leading to identification of approximately 900 novel lysine modification sites in total. Very interestingly, 236 lysine residues derived from 141 proteins were found to be modified with both ubiquitination and acetylation. As a consequence of the subsequent motif extraction analyses, glutamic acid (E) was found to be highly enriched at the position (-1) for the lysine acetylation sites, whereas the same amino acid was relatively dispersed along the neighboring residues of the lysine ubiquitination sites.

1-3. System-wide perturbation of the proteome and phosphoproteome dynamics in glioblastoma stem cells through mTOR signaling inhibition

Hiroko Kozuka-Hata, Tomoko Hiroki, Ryo Koyama-Nasu, Kouhei Tsumoto, Jun-ichiro Inoue, Tetsu Akiyama and Masaaki Oyama.

As glioblastoma is the most common and aggressive brain tumor with poor prognosis, systematic elucidation of signaling networks causally linked to the tumorigenesis is very crucial for developing more effective treatments for this intractable cancer. In our previous study, we applied a high-resolution mass spectrometry-based proteomics technology in combination with SILAC quantitative methods to understand EGF-dependent phosphoproteome dynamics in patient-derived glioblastoma stem cells. We demonstrated that the phosphorylation levels of the representative mTOR signaling molecules such as RPS6 and PRAS40 were dramatically up-regulated upon EGF stimulation. As EGFR signaling has been reported to play a pivotal role in regulating the maintenance of cancer stem cells, we next carried out mTOR inhibitor-dependent signaling perturbations to unravel stemness-related pathways at the network level.

In the present study, we identified a total of 3,726 proteins including 49 up-regulated and 436 down-regulated factors by Torin 1 treatment. Interestingly, we found that one of the well-known cancer stem cell markers was significantly down-regulated through mTOR signaling inhibition. Our in-depth phosphoproteome analysis also led to identification of 6,250 unique phosphopeptides derived from 2,221 proteins and unveiled a variety of dynamic changes regarding phosphorylation levels of cancer and neural stem cell markers in a comprehensive manner. The integrative view of the mTOR inhibitor-dependent proteome and phosphoproteome dynamics in glioblastoma stem cells presents us with further prospects towards understanding previously unrecognized regulations at the system level.

1-4. System-level analysis of CagA-dependent signaling network dynamics by Helicobacter pylori infection

Hiroko Kozuka-Hata, Masato Suzuki, Kotaro Kiga, Shinya Tasaki, Jun-ichiro Inoue, Tadashi Yamamoto, Chihiro Sasakawa and Masaaki Oyama.

The signal transduction system within a cell regulates complex biological events in response to bacterial infection. The previous analyses of cell signaling in Helicobacter pylori-infected gastric epithelial cells have revealed that CagA, a major virulence factor of Helicobacter pylori, is delivered into cells via the type IV secretion system and perturbs signaling networks through the interaction with the key signaling molecules such as SHP-2, Grb2, Crk/Crk-L, Csk, Met, and ZO-1. Although the biological activity of tyrosine-phosphorylated CagA has intensively been studied, system-wide effects of the virulence factor on cellular signaling have yet to be analyzed. Here we performed time-resolved analyses of phosphoproteome and CagA-interactome in human gastric AGS cells by CagA-positive/negative Helicobacter pylori infection. Our highly sensitive nanoLC-MS/MS analyses in combination with the Stable Isotope Labeling by Amino acids in Cell culture (SILAC) technology defined CagA-dependent perturbation of signaling dynamics along with a subset of CagA-associated possible modulators on a network-wide scale. Our result indicated that the activation level of the phosphotyrosine-related signaling molecules in AGS cells was

suppressed overall in the presence of CagA during Helicobacter pylori infection. As Helicobacter pylori infection plays pivotal roles in the progression of gastric diseases including carcinogenesis, a comprehensive and fine description of the signaling dynamics would serve as a fundamental platform to theoretically explore for the potential drug targets through analyzing the regulatory mechanisms at the system-level.

2. Mass spectrometry-based annotation of the human short ORFeome

Masaaki Oyama, Hiroko Kozuka-Hata, Sumio Sugano, Tadashi Yamamoto and Jun-ichiro Inoue.

In parallel with the human genome projects, human full-length cDNA data has also been intensively accumulated. Large-scale analysis of their 5'-UTRs revealed that about half of these had a short ORF upstream of the coding region. Experimental verification as to whether such upstream ORFs are translated is essential to reconsider the generality of the classical scanning mechanism for initiation of translation and define the real outline of the human proteome. Our previous proteomics analysis of small proteins expressed in human K562 cells provided the first direct evidence of translation of upstream ORFs in human full-length cDNAs (Oyama et al., Genome Res, 14: 2048-2052, 2004). In order to grasp an expanded landscape of the human short ORFeome, we have performed an in-depth proteomics analysis of human K562 and HEK293 cells using a two-dimensional nanoLC-MS/MS system. The results led to the identification of eight protein-coding regions besides 197 small proteins with a theoretical mass less than 20 kDa that were already annotated coding sequences in the curated mRNA database. In addition to the upstream ORFs in the presumed 5'-untranslated regions of mRNAs, bioinformatics analysis based on accumulated 5'-end cDNA sequence data provided evidence of novel short coding regions that were likely to be translated from the upstream non-AUG start site or from the new short transcript variants generated by utilization of downstream alternative promoters. Protein expression analysis of the *GRINL1A* gene revealed that translation from the most upstream start site occurred on the minor alternative splicing transcript, whereas this initiation site was not utilized on the major mRNA, resulting in translation of the downstream ORF from the second initiation codon. These findings reveal a novel post-transcriptional system that can augment the human proteome via the alternative use of diverse translation start sites coupled with transcriptional regulation through alternative promoters or splicing, leading to increased complexity of short protein-coding regions defined by the human transcriptome (Oyama et al., Mol Cell Proteomics, 6: 1000-1006, 2007).

3. Shotgun proteomics deciphered age/division of labor-related functional specification of three honeybee (*Apis mellifera* L.) exocrine glands

Toshiyuki Fujita, Hiroko Kozuka-Hata, Yutaro Hori, Jun Takeuchi, Takeo Kubo and Masaaki Oyama.

The honeybee (*Apis mellifera* L.) uses various chemical signals produced by the worker exocrine glands to maintain the functioning of its colony. The roles of worker postcerebral glands (PcGs), thoracic glands (TGs), and mandibular glands (MGs) and the functional changes they undergo according to the division of labor from nursing to foraging are not as well studied. To comprehensively characterize the molecular roles of these glands in workers and their changes according to the division of labor of workers, we analyzed the proteomes of PcGs, TGs, and MGs from nurse bees and foragers using shotgun proteomics technology. We identified approximately 2000 proteins from each of the nurse bee or forager glands and highlighted the features of these glands at the molecular level by semiquantitative enrichment analyses of frequently detected, gland-selective, and labor-selective proteins. First, we found the high potential to produce lipids in PcGs and MGs, suggesting their relation to pheromone production. Second, we also found the proton pumps abundant in TGs and propose some transporters possibly related to the saliva production. Finally, our data unveiled candidate enzymes involved in labor-dependent acid production in MGs.

4. In-depth proteomic analysis of drug-responsive signaling pathway elements in human cancer cells

Wei QI, Aya Kitamura, Naoaki Miyamura, Tomoko Hiroki, Aiko Aizawa, Kazuki Mori, Hiroko Kozuka-Hata and Masaaki Oyama.

Abnormal expression of histone deacetylases (HDACs) in human cancer cells was reported to be associated with angiogenesis, migration, chemotherapy resistance as well as cell differentiation and apoptosis in a wide range of previous studies. Therefore, clinical use of HDAC inhibitors has been discussed as a new therapeutic approach against cancer for a long period. In 2006, suberoylanilide hydroxamic acid (SAHA), a pan-inhibitor targeting HDACs and also known as Vorinostat, was approved by the US Food and Drug Administration (FDA) for the treatment of cutaneous T-cell lymphoma. In addition to the anticancer activity against hematologic cancers, SAHA also shows a significant antitumor effect on solid tumors through inducing apoptosis, arresting cell cycle or elevating radiation sensitization. In order to unveil

the underlying complex mechanism, we used human HeLa cells as the model platform for analyzing SAHA-responsive elements on a proteomic scale. According to the experimental pre-evaluation through western blotting for acetylated histone H3 and microscopic observation of cell growth under a variety of drug-perturbed conditions, we determined to treat cultured cells with SAHA for 24 h to perform an in-depth quantitative proteomic analysis of SAHA-responsive elements in human HeLa cells. After SAHA treatment, the cells were lysed, trypsin-digested and analyzed by high-resolution nanoflow liquid chromatography- tandem mass spectrometry. As a result of ultra-deep proteomic analysis by Orbitrap Eclipse Tribrid system coupled with Ultimate3000 RSLCnano liquid chromatography technology, a total of 5,135 proteins was identified using Proteome Discoverer software. Approximately 8 % of the identified proteins were found to be differentially regulated with more than two-fold changes in response to SAHA treatment by Label Free Quantification (LFQ). The subsequent pathway analysis based on Kyoto Encyclopedia of Genes and Genomes (KEGG) indicated that cell cycle and anti-apoptotic pathway elements including p27 and HO-1 were prominently correlated with SAHA-dependent regulation in human HeLa cells.

5. Stress-dependent cell stiffening by tardigrade tolerance proteins that reversibly form a filamentous network and gel

Akihiro Tanaka, Tomomi Nakano, Kento Watanabe, Kazutoshi Masuda, Gen Honda, Shuichi Kamata, Reitaro Yasui, Hiroko Kozuka-Hata, Chiho Watanabe, Takumi Chinen, Daiju Kitagawa, Satoshi Sawai, Masaaki Oyama, Miho Yanagisawa and Takekazu Kunieda.

Tardigrades are able to tolerate almost complete dehydration by entering a reversible ametabolic state called anhydrobiosis and resume their animation upon rehydration. Dehydrated tardigrades are exceptionally stable and withstand various physical extremes. Although trehalose and late embryogenesis abundant (LEA) proteins have been extensively studied as potent protectants against dehydration in other anhydrobiotic organisms, tardigrades produce high amounts of tardigrade-unique protective proteins. Cytoplasmic-abundant heat-soluble (CAHS) proteins are uniquely invented in the lineage of eutardigrades, a major class of the phylum Tardigrada and are essential for their anhydrobiotic survival. However, the precise mechanisms of their action in this protective role are not fully understood. In the present study, we first postulated the presence of tolerance proteins that form protective condensates via phase separation in a stress-dependent manner and searched for tardigrade proteins that reversibly form condensates upon dehydration-like stress. Through a comprehensive search using a desolvating agent, trifluoroethanol (TFE), we identified 336 proteins, collectively dubbed "TFE-Dependent ReversiblY condensing Proteins (T-DRYPs)." Unexpectedly, we rediscovered CAHS proteins as highly enriched in T-DRYPs, 3 of which were major components of T-DRYPs. We revealed that these CAHS proteins reversibly polymerize into many cytoskeleton-like filaments depending on hyperosmotic stress in cultured cells and undergo reversible gel-transition in vitro. Furthermore, CAHS proteins increased cell stiffness in a hyperosmotic stress-dependent manner and counteract the cell shrinkage caused by osmotic pressure, and even improved the survival against hyperosmotic stress. The conserved putative helical C-terminal region is necessary and sufficient for filament formation by CAHS proteins, and mutations disrupting the secondary structure of this region impaired both the filament formation and the gel transition. On the basis of these results, we propose that CAHS proteins are novel cytoskeleton-like proteins that form filamentous networks and undergo gel-transition in a stress-dependent manner to provide on-demand physical stabilization of cell integrity against deformative forces during dehydration and could contribute to the exceptional physical stability in a dehydrated state.

<Group II>

Biomolecular recognition is based on collective and specific non-covalent interactions between discrete biological molecules. Our laboratory studies various protein systems, for instance, antibody-antigen and protein-ligand complexes, to understand quantitatively how these coordinated non-covalent interactions contribute to their specific recognition in biological and artificial systems. We seek to elucidate the molecular mechanisms by which biological molecules obtain high-specificity and affinity from multiple angles using advanced instrumentation. To produce functional molecules with higher performance and better properties, we aim to build a solid foundation from which to develop drugs that modulate specific interactions between biomolecules and ultimately to understand the principles of molecular interactions in our lives.

.

1. Current advances in biopharmaceutical informatics: guidelines, impact and challenges in the computational developability assessment of antibody therapeutics

Khetan R, Curtis R, Deane CM, Hadsund JT, Kar U, Krawczyk K, Kuroda D, Robinson SA, Sormanni P, Tsumoto K, Warwicker J and Martin ACR.

Therapeutic monoclonal antibodies and their derivatives are key components of clinical pipelines in the global biopharmaceutical industry. The availabil-

ity of large datasets of antibody sequences, structures, and biophysical properties is increasingly enabling the development of predictive models and computational tools for the "developability assessment" of antibody drug candidates. Here, we provide an overview of the antibody informatics tools applicable to the prediction of developability issues such as stability, aggregation, immunogenicity, and chemical degradation. We further evaluate the opportunities and challenges of using biopharmaceutical informatics for drug discovery and optimization. Finally, we discuss the potential of developability guidelines based on *in silico* metrics that can be used for the assessment of antibody stability and manufacturability.

2. B cell-intrinsic TBK1 is essential for germinal center formation during infection and vaccination in mice

Lee MSJ, Inoue T, Ise W, Matsuo-Dapaah J, Wing JB, Temizoz B, Kobiyama K, Hayashi T, Patil A, Sakaguchi S, Simon AK, Bezbradica JS, Nagatoishi S, Tsumoto K, Inoue JI, Akira S, Kurosaki T, Ishii KJ and Coban C.

The germinal center (GC) is a site where somatic hypermutation and clonal selection are coupled for antibody affinity maturation against infections. However, how GCs are formed and regulated is incompletely understood. Here, we identified an unexpected role of Tank-binding kinase-1 (TBK1) as a crucial B cell-intrinsic factor for GC formation. Using immunization and malaria infection models, we show that TBK1-deficient B cells failed to form GC despite normal Tfh cell differentiation, although some malaria-infected B cell-specific TBK1-deficient mice could survive by GC-independent mechanisms. Mechanistically, TBK1 phosphorylation elevates in B cells during GC differentiation and regulates the balance of IRF4/BCL6 expression by limiting CD40 and BCR activation through noncanonical NF-κB and AKTT308 signaling. In the absence of TBK1, CD40 and BCR signaling synergistically enhanced IRF4 expression in Pre-GC, leading to BCL6 suppression, and therefore failed to form GCs. As a result, memory B cells generated from TBK1-deficient B cells fail to confer sterile immunity upon reinfection, suggesting that TBK1 determines B cell fate to promote long-lasting humoral immunity.

3. Development of an Outward Proton Pumping Rhodopsin with a New Record in Thermostability by Means of Amino Acid Mutations

Yasuda S, Akiyama T, Kojima K, Ueta T, Hayashi T, Ogasawara S, Nagatoishi S, Tsumoto K, Kunishima N, Sudo Y, Kinoshita M and Murata T.

We have developed a methodology for identifying further thermostabilizing mutations for an intrinsically thermostable membrane protein. The methodology comprises the following steps: (1) identifying thermostabilizing single mutations (TSSMs) for residues in the transmembrane region using our physics-based method; (2) identifying TSSMs for residues in the extracellular and intracellular regions, which are in aqueous environment, using an empirical force field FoldX; and (3) combining the TSSMs identified in steps (1) and (2) to construct multiple mutations. The methodology is illustrated for thermophilic rhodopsin whose apparent midpoint temperature of thermal denaturation Tm is ~91.8 °C. The TSSMs previously identified in step (1) were F90K, F90R, and Y91I with ΔTm ~5.6, ~5.5, and ~2.9 °C, respectively, and those in step (2) were V79K, T114D, A115P, and A116E with ΔTm ~2.7, ~4.2, ~2.6, and ~2.3 °C, respectively (ΔTm denotes the increase in Tm). In this study, we construct triple and quadruple mutants, F90K + Y91I + T114D and F90K + Y91I + V79K + T114D. The values of ΔTm for these multiple mutants are ~11.4 and ~13.5 °C, respectively. Tm of the quadruple mutant (~105.3 °C) establishes a new record in a class of outward proton pumping rhodopsins. It is higher than Tm of Rubrobacter xylanophilus rhodopsin (~100.8 °C) that was the most thermostable in the class before this study.

4. Development of a high-throughput method to screen novel antiviral materials

Nakakido M, Tanaka N, Shimojo A, Miyamae N and Tsumoto K.

Respiratory infectious diseases pose a serious threat worldwide, and novel antiviral materials are highly demanded. Photocatalytic nanoparticles have been developed to inhibit indirect transmission of pathogens by acting as surface coating materials. During development of such antiviral materials, researchers use bacteriophages as model viruses due to their safety and experimental efficiency. Screening methods are used to identify potential antiviral materials, and better screening technologies will accelerate the discovery of antiviral treatments. In this study, we constructed a novel platform to evaluate antiviral activity of surface coating materials using the M13 bacteriophage and phagemid system derived from phage display technology. The evaluation results generated by this system for the two tested antiviral materials were comparable to those for the materials tested on the Qβ bacteriophage and influenza virus using traditional screening methods. The experimental system developed in this study provides rapid and effective screening and can be applied to the development of novel antiviral materials.

5. Oligo(N-methylalanine) as a Peptide-Based Molecular Scaffold with a Minimal Structure and High Density of Functionalizable Sites

Yokomine M, Morimoto J, Fukuda Y, Shiratori Y, Kuroda D, Ueda T, Takeuchi K, Tsumoto K and Sando S.

Functionalizable synthetic molecules with nanometer sizes and defined shapes in water are useful as molecular scaffolds to mimic the functions of biomacromolecules and develop chemical tools for manipulating biomacromolecules. Herein, we propose oligo(N-methylalanine) (oligo-NMA) as a peptide-based molecular scaffold with a minimal structure and a high density of functionalizable sites. Oligo-NMA forms a defined shape in water without hydrogen-bonding networks or ring constraints, which enables the molecule to act as a scaffold with minimal atomic composition. Furthermore, functional groups can be readily introduced on the nitrogens and α-carbons of oligo-NMA. Computational and NMR spectroscopic analysis suggested that the backbone structure of oligo-NMA is not largely affected by functionalization. Moreover, the usefulness of oligo-NMA was demonstrated by the design of protein ligands. The ease of synthesis, minimal structure, and high functionalization flexibility makes oligo-NMA a useful scaffold for chemical and biological applications.

6. Biophysical Characterization of the Contribution of the Fab Region to the IgG-FcγRIIIa Interaction

Kosuge H, Nagatoishi S, Kiyoshi M, Ishii-Watabe A, Terao Y, Ide T and Tsumoto K.

The cell-surface receptor FcγRIIIa is crucial to the efficacy of therapeutic antibodies as well as the immune response. The interaction of the Fc region of IgG molecules with FcγRIIIa has been characterized, but until recently, it was thought that the Fab regions were not involved in the interaction. To evaluate the influence of the Fab regions in a biophysical context, we carried out surface plasmon resonance analyses using recombinant FcγRIIIa ligands. A van't Hoff analysis revealed that compared to the interaction of the papain-digested Fc fragment with FcγRIIIa, the interaction of commercially available, full-length rituximab with FcγRIIIa had a more favorable binding enthalpy, a less favorable binding entropy, and a slower off rate. Similar results were obtained from analyses of IgG1 molecules and an IgG1-Fc fragment produced by Expi293 cells. For further validation, we also prepared a maltose-binding protein-linked IgG1-Fc fragment (MBP-Fc). The binding enthalpy of MBP-Fc was nearly equal to that of the IgG1-Fc fragment for the interaction with FcγRIIIa, indicating that such alternatives to the Fab domains as MBP do not positively contribute to the IgG-FcγRIIIa interactions. Our investigation strongly suggests that the Fab region directly interacts with FcγRIIIa, resulting in an increase in the binding enthalpy and a decrease in the dissociation rate, at the expense of favorable binding entropy.

7. Mirror-image streptavidin with specific binding to L-biotin, the unnatural enantiomer

Suganuma M, Kubo T, Ishiki K, Tanaka K, Suto K, Ejima D, Toyota M, Tsumoto K, Sato T and Nishikawa Y.

The streptavidin-biotin system is known to have a very high affinity and specificity and is widely used in biochemical immunoassays and diagnostics. However, this method is affected by endogenous D-biotin in serum sample measurements (biotin interference). While several efforts using alternative high-affinity binding systems (e.g., genetically modified streptavidin and biotin derivatives) have been attempted, these efforts have all led to reduction in affinity. To solve this interference issue, the enantiomer of streptavidin was synthesized, which enabled specific binding to L-biotin. We successfully obtained a functional streptavidin molecule by peptide synthesis using D-amino acids and an in vitro folding technique. Several characterizations, including size exclusion chromatography (SEC), circular dichroism spectra (CD), and heat denaturation experiments collectively confirmed the higher-order enantiomer of natural streptavidin had been formed with comparable stability to the natural protein. L-biotin specific binding of this novel molecule enabled us to avoid biotin interference in affinity measurements using the Biacore system and enzyme-linked immunosorbent assay (ELISA). We propose the enantiomer of streptavidin as a potential candidate to replace the natural streptavidin-biotin system, even for in vivo use.

8. Experimental Comparison of Bond Lifetime and Viscoelastic Relaxation in Transient Networks with Well-Controlled Structures

Katashima T, Kudo R, Naito M, Nagatoishi S, Miyata K, Chung UI, Tsumoto K and Sakai T.

We demonstrate an experimental comparison of the bond lifetime, estimated using surface plasmon resonance (SPR), and the viscoelastic relaxation time of transient networks with well-controlled structures (dynamically cross-linked Tetra-PEG gel). SPR and viscoelastic measurements revealed that the temperature dependences of the two characteristic times are in agreement, while the viscoelastic response is delayed with respect to the lifetime by a factor of 2-3, dependent on the network strand length. Polymers cross-linked by temporary interactions form transient

networks, which show fascinating viscoelasticity with a single relaxation mode. However, the molecular understanding of such simple viscoelasticity has remained incomplete because of the difficulty of experimentally evaluating bond lifetimes and heterogeneous structures in conventional transient networks. Our results suggest that bond dissociation and recombination both contribute to the macromechanical response. This report on direct bond-lifetime-viscoelastic-relaxation time comparison provides important information for the molecular design of transient network materials.

9. Antibody recognition of complement factor H reveals a flexible loop involved in atypical hemolytic uremic syndrome pathogenesis

Yokoo T, Tanabe A, Yoshida Y, Caaveiro JMM, Nakakido M, Ikeda Y, Fujimura Y, Matsumoto M, Entzminger K, Maruyama T, Okumura CJ, Nangaku M and Tsumoto K.

Atypical hemolytic uremic syndrome (aHUS) is a disease associated with dysregulation of the immune complement system, especially of the alternative pathway (AP). Complement factor H (CFH), consisting of 20 domains called complement control protein (CCP1-20), downregulates the AP as a cofactor for mediating C3 inactivation by complement factor I. However, anomalies related to CFH are known to cause excessive complement activation and cytotoxicity. In aHUS, mutations and the presence of anti-CFH autoantibodies (AAbs) have been reported as plausible causes of CFH dysfunction, and it is known that CFH-related aHUS carries a high probability of end-stage renal disease. Elucidating the detailed functions of CFH at the molecular level will help to understand aHUS pathogenesis. Herein, we used biophysical data to reveal that a heavy-chain antibody fragment, termed VHH4, recognized CFH with high affinity. Hemolytic assays also indicated that VHH4 disrupted the protective function of CFH on sheep erythrocytes. Furthermore, X-ray crystallography revealed that VHH4 recognized the Leu1181-Leu1189CCP20 loop, a known anti-CFH AAbs epitope. We next analyzed the dynamics of the C-terminal region of CFH and showed that the epitopes recognized by anti-CFH AAbs and VHH4 were the most flexible regions in CCP18-20. Finally, we conducted mutation analyses to elucidate the mechanism of VHH4 recognition of CFH and revealed that VHH4 inserts the Trp1183C-CP20 residue of CFH into the pocket formed by the complementary determining region 3 loop. These results suggested that anti-CFH AAbs may adopt a similar molecular mechanism to recognize the flexible loop of Leu1181-Leu1189CCP20, leading to aHUS pathogenesis.

10. Structure and role of the linker domain of the iron surface-determinant protein IsdH in heme transportation in Staphylococcus aureus

Valenciano-Bellido S, Caaveiro JMM, Morante K, Sushko T, Nakakido M, Nagatoishi S and Tsumoto K.

Staphylococcus aureus is a major cause of deadly nosocomial infections, a severe problem fueled by the steady increase of resistant bacteria. The iron surface determinant (Isd) system is a family of proteins that acquire nutritional iron from the host organism, helping the bacterium to proliferate during infection, and therefore represents a promising antibacterial target. In particular, the surface protein IsdH captures hemoglobin (Hb) and acquires the heme moiety containing the iron atom. Structurally, IsdH comprises three distinctive NEAr-iron Transporter (NEAT) domains connected by linker domains. The objective of this study was to characterize the linker region between NEAT2 and NEAT3 from various biophysical viewpoints and thereby advance our understanding of its role in the molecular mechanism of heme extraction. We demonstrate the linker region contributes to the stability of the bound protein, likely influencing the flexibility and orientation of the NEAT3 domain in its interaction with Hb, but only exerts a modest contribution to the affinity of IsdH for heme. Based on these data, we suggest that the flexible nature of the linker facilitates the precise positioning of NEAT3 to acquire heme. In addition, we also found that residues His45 and His89 of Hb located in the heme transfer route toward IsdH do not play a critical role in the transfer rate-determining step. In conclusion, this study clarifies key elements of the mechanism of heme extraction of human Hb by IsdH, providing key insights into the Isd system and other protein systems containing NEAT domains.

11. Addition of arginine hydrochloride and proline to the culture medium enhances recombinant protein expression in Brevibacillus choshinensis: The case of RBD of SARS-CoV-2 spike protein and its antibody

Matsunaga R and Tsumoto K.

Brevibacillus choshinensis is a gram-positive bacterium that is known to efficiently secrete recombinant proteins. However, the expression of these proteins is often difficult depending upon the expressed protein. In this study, we demonstrated that the addition of arginine hydrochloride and proline to the culture medium dramatically increased protein expression. By culturing bacterial cells in 96-well plates, we were able to rapidly examine the expression conditions and easily scale up to 96 mL of culture for production. Although functional expression of the recep-

tor binding domain (RBD) of the SARS-CoV-2 spike protein without any solubility-enhancing tag in bacterial strains (including Escherichia coli) has not been reported to date, we succeeded in efficiently producing RBD which showed a similar CD spectrum to that of RBD produced by eukaryotic cell expression systems. Furthermore, RBD from the omicron variant (B.1.1.529) was also produced. Physicochemical analyses indicated that omicron RBD exhibited markedly increased instability compared to the wild-type. We also revealed that the Fab format of the anti-SARS-CoV-2 antibody C121 can be produced in large quantities using the same expression system. The obtained C121 Fab bound to wild-type RBD but not to omicron RBD. These results strongly suggest that the Brevibacillus expression system is useful for facilitating the efficient expression of proteins that are difficult to fold and will thus contribute to the rapid physicochemical evaluation of functional proteins.

12. Ladder observation of bovine serum albumin by high resolution agarose native gel electrophoresis

Tomioka Y, Nakagawa M, Sakuma C, Nagatoishi S, Tsumoto K, Arakawa T and Akuta T.

A commercially available bovine serum albumin (BSA) was examined by agarose native gel electrophoresis using two different agarose sources, UltraPure and MetaPhor agarose. While UltraPure agarose up to 5 % showed no clear separation of BSA oligomers, MetaPhor agarose clearly demonstrated oligomer bands above 4 %, indicating that the latter agarose has greater molecular sieving effects and is hence characterized to have high resolution for size differences, as probed by a greater slope of Ferguson plot. Physical properties are different between two agaroses. In general, UltraPure agarose has physical strength, while MetaPhor agarose is considerably fragile, but MetaPhor agarose solution is less viscous so that even 10 % gel can be made. Cause of oligomers was shown to be not associated with inter-chain disulfide bonds, but is due to association of native or native-like molecules.

13. Correction: Antibody recognition of complement factor H reveals a flexible loop involved in atypical hemolytic uremic syndrome pathogenesis

Yokoo T, Tanabe A, Yoshida Y, Caaveiro JMM, Nakakido M, Ikeda Y, Fujimura Y, Matsumoto M, Entzminger K, Maruyama T, Okumura CJ, Nangaku M and Tsumoto K.

No abstract available

14. Residue-based program of a β-peptoid twisted strand shape via a cyclopentane constraint

Kim J, Kobayashi H, Yokomine M, Shiratori Y, Ueda T, Takeuchi K, Umezawa K, Kuroda D, Tsumoto K, Morimoto J and Sando S.

N-Substituted peptides, such as peptoids and β-peptoids, have been reported to have unique structures with diverse functions, like catalysis and manipulation of biomolecular functions. Recently, the preorganization of monomer shape by restricting bond rotations about all backbone dihedral angles has been demonstrated to be useful for de novo design of peptoid structures. Such design strategies are hitherto unexplored for β-peptoids; to date, no preorganized β-peptoid monomers have been reported. Here, we report the first design strategy for β-peptoids, in which all four backbone dihedral angles (ω, ϕ, θ, ψ) are rotationally restricted on a per-residue basis. The introduction of a cyclopentane constraint realized the preorganized monomer structure and led to a β-peptoid with a stable twisted strand shape.

15. Nucleic acid-triggered tumoral immunity propagates pH-selective therapeutic antibodies through tumor-driven epitope spreading

Furuya G, Katoh H, Atsumi S, Hashimoto I, Komura D, Hatanaka R, Senga S, Hayashi S, Akita S, Matsumura H, Miura A, Mita H, Nakakido M, Nagatoishi S, Sugiyama A, Suzuki R, Konishi H, Yamamoto A, Abe H, Hiraoka N, Aoki K, Kato Y, Seto Y, Yoshimura C, Miyadera K, Tsumoto K, Ushiku T and Ishikawa S.

Important roles of humoral tumor immunity are often pointed out; however, precise profiles of dominant antigens and developmental mechanisms remain elusive. We systematically investigated the humoral antigens of dominant intratumor immunoglobulin clones found in human cancers. We found that approximately half of the corresponding antigens were restricted to strongly and densely negatively charged polymers, resulting in simultaneous reactivities of the antibodies to both densely sulfated glycosaminoglycans (dsGAGs) and nucleic acids (NAs). These anti-dsGAG/NA antibodies matured and expanded via intratumoral immunological driving force of innate immunity via NAs. These human cancer-derived antibodies exhibited acidic pH-selective affinity across both antigens and showed specific reactivity to diverse spectrums of human tumor cells. The antibody-drug conjugate exerted therapeutic effects against multiple cancers in vivo by targeting cell surface dsGAG antigens. This study reveals that intratumoral immunological reactions propagate tumor-oriented immunoglobulin clones and demonstrates a new therapeutic modality for the universal treatment of human malig-

nancies.

16. PRELP Regulates Cell-Cell Adhesion and EMT and Inhibits Retinoblastoma Progression

Hopkins J, Asada K, Leung A, Papadaki V, Davaapil H, Morrison M, Orita T, Sekido R, Kosuge H, Reddy MA, Kimura K, Mitani A, Tsumoto K, Hamamoto R, Sagoo MS and Ohnuma SI.

Retinoblastoma (RB) is the most common intraocular pediatric cancer. Nearly all cases of RB are associated with mutations compromising the function of the RB1 tumor suppressor gene. We previously demonstrated that PRELP is widely downregulated in various cancers and our in vivo and in vitro analysis revealed PRELP as a novel tumor suppressor and regulator of EMT. In addition, PRELP is located at chromosome 1q31.1, around a region hypothesized to be associated with the initiation of malignancy in RB. Therefore, in this study, we investigated the role of PRELP in RB through in vitro analysis and next-generation sequencing. Immunostaining revealed that PRELP is expressed in Müller glial cells in the retina. mRNA expression profiling of PRELP-/- mouse retina and PRELP-treated RB cells found that PRELP contributes to RB progression via regulation of the cancer microenvironment, in which loss of PRELP reduces cell-cell adhesion and facilitates EMT. Our observations suggest that PRELP may have potential as a new strategy for RB treatment.

17. Analysis of bovine serum albumin unfolding in the absence and presence of ATP by SYPRO Orange staining of agarose native gel electrophoresis

Tomioka Y, Nakagawa M, Sakuma C, Kurosawa Y, Nagatoishi S, Tsumoto K, Arakawa T and Akuta T.

An attempt was made to specifically stain unfolded proteins on agarose native gels. SYPRO Orange is routinely used to detect unfolded protein in differential scanning fluorimetry, which is based on the enhanced fluorescence intensity upon binding to the unfolded protein. We demonstrated that this dye barely bound to the native proteins, resulting in no or faint staining of the native bands, but bound to and stained the unfolded proteins, on agarose native gels. Using bovine serum albumin (BSA), it was shown that staining did not depend on whether BSA was thermally unfolded in the presence of SYPRO Orange or stained after electrophoresis. On the contrary, SYPRO Orange dye stained protein bands in the presence of sodium dodecylsulfate (SDS) due to incorporation of the dye into SDS micelles that bound to the unfolded proteins. This staining resulted in detection of new, intermediately unfolded structure of BSA during thermal unfolding. Such intermediate structure occurred at higher temperature in the presence of ATP.

18. Human antibody recognition and neutralization mode on the NTD and RBD domains of SARS-CoV-2 spike protein

Otsubo R, Minamitani T, Kobiyama K, Fujita J, Ito T, Ueno S, Anzai I, Tanino H, Aoyama H, Matsuura Y, Namba K, Imadome KI, Ishii KJ, Tsumoto K, Kamitani W and Yasui T.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes coronavirus disease 2019 (COVID-19). Variants of concern (VOCs) such as Delta and Omicron have developed, which continue to spread the pandemic. It has been reported that these VOCs reduce vaccine efficacy and evade many neutralizing monoclonal antibodies (mAbs) that target the receptor binding domain (RBD) of the glycosylated spike (S) protein, which consists of the S1 and S2 subunits. Therefore, identification of optimal target regions is required to obtain neutralizing antibodies that can counter VOCs. Such regions have not been identified to date. We obtained 2 mAbs, NIBIC-71 and 7G7, using peripheral blood mononuclear cells derived from volunteers who recovered from COVID-19. Both mAbs had neutralizing activity against wild-type SARS-CoV-2 and Delta, but not Omicron. NIBIC-71 binds to the RBD, whereas 7G7 recognizes the N-terminal domain of the S1. In particular, 7G7 inhibited S1/S2 cleavage but not the interaction between the S protein and angiotensin-converting enzyme 2; it suppressed viral entry. Thus, the efficacy of a neutralizing mAb targeting inhibition of S1/2 cleavage was demonstrated. These results suggest that neutralizing mAbs targeting blockade of S1/S2 cleavage are likely to be cross-reactive against various VOCs.

19. Molecular basis for thermal stability and affinity in a VHH: Contribution of the framework region and its influence in the conformation of the CDR3

Kinoshita S, Nakakido M, Mori C, Kuroda D, Caaveiro JMM and Tsumoto K.

The camelid single domain antibody, referred to VHH or Nanobody, is considered a versatile tool for various biotechnological and clinical applications because of its favorable biophysical properties. To take advantage of these characteristics and for its application in biotechnology and therapy, research on VHH engineering is currently vigorously conducted. To humanize a camelid VHH, we performed complementarity determining region (CDR) grafting using a humanized VHH currently in clinical trials, and investigated the effects of these changes on the biophysical properties of the resulting VHH. The chimeric

VHH exhibited a significant decrease in affinity and thermal stability and a large conformational change in the CDR3. To elucidate the molecular basis for these changes, we performed mutational analyses on the framework regions revealing the contribution of individual residues within the framework region. It is demonstrated that the mutations resulted in the loss of affinity and lower thermal stability, revealing the significance of bulky residues in the vicinity of the CDR3, and the importance of intramolecular interactions between the CDR3 and the framework-2 region. Subsequently, we performed back-mutational analyses on the chimeric VHH. Back-mutations resulted in an increase of the thermal stability and affinity. These data suggested that back-mutations restored the intramolecular interactions, and proper positioning and/or dynamics of the CDR3, resulting in the gain of thermal stability and affinity. These observations revealed the molecular contribution of the framework region on VHHs and further designability of the framework region of VHHs without modifying the CDRs.

20. Repression of the PRELP gene is relieved by histone deacetylase inhibitors through acetylation of histone H2B lysine 5 in bladder cancer

Shozu K, Kaneko S, Shinkai N, Dozen A, Kosuge H, Nakakido M, Machino H, Takasawa K, Asada K, Komatsu M, Tsumoto K, Ohnuma SI and Hamamoto R.

Background: Proline/arginine-rich end leucine-rich repeat protein (PRELP) is a member of the small leucine-rich proteoglycan family of extracellular matrix proteins, which is markedly suppressed in the majority of early-stage epithelial cancers and plays a role in regulating the epithelial-mesenchymal transition by altering cell-cell adhesion. Although PRELP is an important factor in the development and progression of bladder cancer, the mechanism of PRELP gene repression remains unclear.

Results: Here, we show that repression of PRELP mRNA expression in bladder cancer cells is alleviated by HDAC inhibitors (HDACi) through histone acetylation. Using ChIP-qPCR analysis, we found that acetylation of lysine residue 5 of histone H2B in the PRELP gene promoter region is a marker for the de-repression of PRELP expression.

Conclusions: These results suggest a mechanism through which HDACi may partially regulate the function of PRELP to suppress the development and progression of bladder cancer. Some HDACi are already in clinical use, and the findings of this study provide a mechanistic basis for further investigation of HDACi-based therapeutic strategies.

Keywords: Bladder cancer; Extracellular matrix proteins; Gene expression; H2BK5ac; HDACi; PRELP.

21. Performance Comparison of Spectral Distance Calculation Methods

Oyama T, Suzuki S, Horiguchi Y, Yamane A, Akao K, Nagamori K and Tsumoto K.

Circular dichroism (CD) spectroscopy is a widely used technique for assessing the higher-order structure (HOS) of biopharmaceuticals, including antibody drugs. Since the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use established quality control guidelines, objective evaluation of spectral similarity has been required in order to assess structural comparability. Several spectral distance quantification methods and weighting functions to increase sensitivity have been proposed, but not many reports have compared their performance for CD spectra. We constructed comparison sets that combine actual spectra and simulated noise and performed a comprehensive performance evaluation of each spectral distance calculation method and weighting function under conditions that consider spectral noise and fluctuations from pipetting errors. The results showed that using the Euclidean distance or Manhattan distance with Savitzky-Golay noise reduction is effective for spectral distance assessment. For the weighting function, it is preferable to combine the spectral intensity weighting function and the noise weighting function. In addition, the introduction of the external stimulus weighting function should be considered to improve the sensitivity. It is crucial to select the weighting function based on the balance between spectral changes and noise distributions for robust, sensitive antibody HOS similarity assessment.

<Group III>

1. Development of new methods for analyzing neural circuits in the retina

Neural circuits in the central nervous system are the basis of various higher-order brain functions. It is also true in case of retina. In the retina, six main classes of neural cells connect systematically to make up complex neural circuits. Characteristics of the retinal neural cell functions have been examined mainly by the electrophysiological methods and models of cell connectivity have been proposed. Morphological studies of the actual neural connection, which constitute the physiological properties of retinal neurons, have been desired. Until recently the only method to reveal the three-dimensional (3D) connectivity of actual neural cells morphologically was to collect ultrathin serial sections and observe them in transmission electron microscope (TEM). But the technical

difficulties discouraged us from such a troublesome procedure. Recent progress in scanning electron microscope (SEM) equipment allowed us to develop a new method to observe ultrathin TEM sections in SEM (thin section scanning electron microscopy: TSSEM). To observe thin TEM sections, we have developed new sample staining methods to enhance electron contrast. To collect huge number of serial sections stably and efficiently, we have been developing new equipment and techniques. By using this equipment, it became possible to collect more than 1000 serial sections of less than 30 nm thickness much easier. We have analyzed about 500 serial thin sections of zebrafish retinal outer plexiform layer by this method and succeeded in tracing thin processes of bipolar cells into the photoreceptor terminals.

Aside from getting 3D information, TSSEM method provide us precise information of much wider areas of thin sections more effectively and more easily. Such studies are currently in progress.

2. Collaborative and supportive works as electron microscope core-laboratory

This group is also engaged in collaborative researches using electron microscope. We offer supports for the research projects those need electron microscopic analysis. The services available in this group are the conventional thin section transmission electron microscopy, immuno-electron microscopy, negative staining techniques and scanning electron microscopy. By using these individual technique or combination of some of these we can offer direct visual evidence that cannot be acquired by other methods. This year, 19 projects in 12 laboratories were performed as core-laboratory works.

a. Thin section transmission electron microscopy

Thin section transmission electron microscopy is the most widely used technique to observe the inner structure of cells and tissues. In this method, samples are fixed and embedded in epoxy resin, thin sections with about 70 nm thickness are cut and observed in the electron microscope. In case of immuno-electron microscopy, thin sections are obtained by similar procedure and the antigen epitopes exposed on the surface of the sections are marked by sequential reaction with appropriate primary antibodies and colloidal gold labeled secondary antibodies. This year, thin section electron microscopy and those combined with immuno-electron microscopy were used in many collaborative works.

a-1. Ultrastructural analysis of entry and assembly of Herpes Simplex Virus

We have been performing several studies with research groups in Dr. Kawaguchi[1]'s laboratory: [1]Division of Molecular Virology, Department of Microbiology and Immunology, regarding the infection/replication processes of herpes simplex virus (HSV). Thin section electron microscopy has been used to analyze the function of viral proteins in trans-nuclear membrane processes of the newly formed viruses. By analyzing the virus forming processes in some mutant host cells, we could analyze viral proteins as well as candidate host molecules those may be involved in the trans-nuclear process of the HSV.

a-2. Roles of membrane lipids in development and maintenance of photoreceptor outer-segment

We have been performing several studies also with research groups in Dr. Watanabe[2]'s laboratory: [2]Department of Retinal Biology and Pathology, Graduate School of Medicine, The University of Tokyo. This year, we analyzed the composition of phospholipids in individual cell types in developing mouse retina under physiological and pathological conditions and checked with electron microscopic data. With the combination of cell sorting and mass spectrometry analysis, most of phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) in retina are included in photoreceptor cells. When compared with the electron microscopic data in pathological conditions, PC and PE composition are dramatically changed before photoreceptor cell degeneration are apparent, suggesting that changes in PC and PE composition in photoreceptor cells may lead to the photoreceptor degeneration. (ref. Hamano *et al*) Another work regarding the composition of phospholipids in the photoreceptor cells and photoreceptor degeneration are also performed and revealed that the perturbation of mitochondrial functions in regard with PC synthesis defect are the main cause of photoreceptor death. (ref. Nagata *et al*) Another project about the structure of retina and retinal capillaly is also running with Dr. Watanabe's laboratory.

Some other collaborative research works using thin section electron microscopy and/or immuno-electron microscopy were performed with Dr. Sasou[3], [3]Division of Mucosal Immunology, Dr. Eguchi[4], in [4]Division of Genetics, Dr. Nakahara[5] in [5]Department of Life Science Dentistry, The Nippon Dental University and so on.

b. Negative staining techniques

Negative staining techniques are simple and quick method to observe the morphology of the macro-molecules. This year, negative staining techniques were used to analyze exosomes in collaboration with Dr. Hayashi[6] in [6]Division of Vaccine Science, Laboratory of Adjuvant Innovation. The same techniques are also used in the research with Dr. Shibata[7], and Dr. Maeda[7], in [7]Research Organization for Nano and Life In-

novation, Waseda University.

c. Conventional scanning electron microscopy

Conventional scanning electron microscopy is a technique used to examine the surface structure of the cells, tissues or other non-biological materials. Scanning electron microscopy combined with thin section transmission microscopy were used in collaborative work with Dr. Ishikawa[8], [8]Laboratory of Reproductive Systems Biology, about the structure of young and aged mouse oocyte zona pellucida. Scanning electron microscopy are also used in the observation of the surface structure of electrochemically active microorganisms with Dr. Kobayashi[9], [9]Frontier Research Center for Energy and Resource (FRCER), Graduate School of Engineering, The University of Tokyo.

Publications

<Group I>

Maeda F, Kato A, Takeshima K, Shibazaki M, Sato R, Shibata T, Miyake K, Kozuka-Hata H, Oyama M, Shimizu E, Imoto S, Miyano S, Adachi S, Natsume T, Takeuchi K, Maruzuru Y, Koyanagi N, Jun A, and Yasushi K. Role of the Orphan Transporter SLC35E1 in the Nuclear Egress of Herpes Simplex Virus 1. J Virol, 96: e0030622, 2022.

Tanaka A, Nakano T, Watanabe K, Masuda K, Honda G, Kamata S, Yasui R, Kozuka-Hata H, Watanabe C, Chinen T, Kitagawa D, Sawai S, Oyama M, Yanagisawa M, and Kunieda T. Stress-dependent cell stiffening by tardigrade tolerance proteins that reversibly form a filamentous network and gel. PLoS Biol, 20: e3001780, 2022.

Ohe S, Kubota Y, Yamaguchi K, Takagi Y, Nashimoto J, Kozuka-Hata H, Oyama M, Furukawa Y, and Takekawa M. ERK-mediated NELF-A phosphorylation promotes transcription elongation of immediate-early genes by releasing promoter-proximal pausing of RNA polymerase II. Nat Commun, 13: 7476, 2022.

Miyashita R, Nishiyama A, Qin W, Chiba Y, Kori S, Kato N, Konishi C, Kumamoto S, Kozuka-Hata H, Oyama M, Kawasoe Y, Tsurimoto T, Takahashi TS, Leonhardt H, Arita K, and Nakanishi M. The termination of UHRF1-dependent PAF15 ubiquitin signaling is regulated by USP7 and ATAD5. Elife, in press.

秦裕子、尾山大明、井上純一郎「プロテオミクスの現状と課題―ゲノミクスおよび疾患との関連について―」医用工学ハンドブック、エヌ・ティー・エス、31-37、2022.

<Group II>

Khetan R, Curtis R, Deane CM, Hadsund JT, Kar U, Krawczyk K, Kuroda D, Robinson SA, Sormanni P, Tsumoto K, Warwicker J, and Martin ACR. Current advances in biopharmaceutical informatics: guidelines, impact and challenges in the computational developability assessment of antibody therapeutics. MAbs, 14: 2020082, 2022.

Lee MSJ, Inoue T, Ise W, Matsuo-Dapaah J, Wing JB, Temizoz B, Kobiyama K, Hayashi T, Patil A, Sakaguchi S, Simon AK, Bezbradica JS, Nagatoishi S, Tsumoto K, Inoue JI, Akira S, Kurosaki T, Ishii KJ, and Coban C. B cell-intrinsic TBK1 is essential for germinal center formation during infection and vaccination in mice. J Exp Med, 219: e20211336, 2022.

Yasuda S, Akiyama T, Kojima K, Ueta T, Hayashi T, Ogasawara S, Nagatoishi S, Tsumoto K, Kunishima N, Sudo Y, Kinoshita M, and Murata T. Development of an Outward Proton Pumping Rhodopsin with a New Record in Thermostability by Means of Amino Acid Mutations. J Phys Chem B, 126: 1004-1015, 2022.

Nakakido M, Tanaka N, Shimojo A, Miyamae N, and Tsumoto K. Development of a high-throughput method to screen novel antiviral materials. PLoS One, 17: e0266474, 2022.

Yokomine M, Morimoto J, Fukuda Y, Shiratori Y, Kuroda D, Ueda T, Takeuchi K, Tsumoto K, and Sando S. Oligo(N-methylalanine) as a Peptide-Based Molecular Scaffold with a Minimal Structure and High Density of Functionalizable Sites. Angew Chem Int Ed Engl, 61: e202200119, 2022.

Kosuge H, Nagatoishi S, Kiyoshi M, Ishii-Watabe A, Terao Y, Ide T, and Tsumoto K. Biophysical Characterization of the Contribution of the Fab Region to the IgG-FcγRIIIa Interaction. Biochemistry, in press.

Suganuma M, Kubo T, Ishiki K, Tanaka K, Suto K, Ejima D, Toyota M, Tsumoto K, Sato T, and Nishikawa Y. Mirror-image streptavidin with specific binding to L-biotin, the unnatural enantiomer. Sci Rep, 12: 9568, 2022.

Katashima T, Kudo R, Naito M, Nagatoishi S, Miyata K, Chung UI, Tsumoto K, and Sakai T. Experimental Comparison of Bond Lifetime and Viscoelastic Relaxation in Transient Networks with Well-Controlled Structures. ACS Macro Lett, 11: 753-759, 2022.

Yokoo T, Tanabe A, Yoshida Y, Caaveiro JMM, Nakakido M, Ikeda Y, Fujimura Y, Matsumoto M, Entzminger K, Maruyama T, Okumura CJ, Nangaku M, and Tsumoto K. Antibody recognition of complement factor H reveals a flexible loop involved in atypical hemolytic uremic syndrome pathogenesis. J Biol Chem, 298: 101962, 2022.

Valenciano-Bellido S, Caaveiro JMM, Morante K, Sushko T, Nakakido M, Nagatoishi S, and Tsumoto K. Structure and role of the linker domain of the iron

surface-determinant protein IsdH in heme transportation in Staphylococcus aureus. J Biol Chem, 298: 101995, 2022.

Matsunaga R and Tsumoto K. Addition of arginine hydrochloride and proline to the culture medium enhances recombinant protein expression in Brevibacillus choshinensis: The case of RBD of SARS-CoV-2 spike protein and its antibody. Protein Expr Purif, 194: 106075, 2022.

Tomioka Y, Nakagawa M, Sakuma C, Nagatoishi S, Tsumoto K, Arakawa T, and Akuta T. Ladder observation of bovine serum albumin by high resolution agarose native gel electrophoresis. Int J Biol Macromol, 215: 512-520, 2022.

Yokoo T, Tanabe A, Yoshida Y, Caaveiro JMM, Nakakido M, Ikeda Y, Fujimura Y, Matsumoto M, Entzminger K, Maruyama T, Okumura CJ, Nangaku M, and Tsumoto K. Correction: Antibody recognition of complement factor H reveals a flexible loop involved in atypical hemolytic uremic syndrome pathogenesis. J Biol Chem, 298: 102262, 2022.

Kim J, Kobayashi H, Yokomine M, Shiratori Y, Ueda T, Takeuchi K, Umezawa K, Kuroda D, Tsumoto K, Morimoto J, and Sando S. Residue-based program of a β-peptoid twisted strand shape via a cyclopentane constraint. Org Biomol Chem, 20: 6994-7000, 2022.

Furuya G, Katoh H, Atsumi S, Hashimoto I, Komura D, Hatanaka R, Senga S, Hayashi S, Akita S, Matsumura H, Miura A, Mita H, Nakakido M, Nagatoishi S, Sugiyama A, Suzuki R, Konishi H, Yamamoto A, Abe H, Hiraoka N, Aoki K, Kato Y, Seto Y, Yoshimura C, Miyadera K, Tsumoto K, Ushiku T, and Ishikawa S. Nucleic acid-triggered tumoral immunity propagates pH-selective therapeutic antibodies through tumor-driven epitope spreading. Cancer Sci, 114: 321-338, 2022.

Hopkins J, Asada K, Leung A, Papadaki V, Davaapil H, Morrison M, Orita T, Sekido R, Kosuge H, Reddy MA, Kimura K, Mitani A, Tsumoto K, Hamamoto R, Sagoo MS, and Ohnuma SI. PRELP Regulates Cell-Cell Adhesion and EMT and Inhibits Retinoblastoma Progression. Cancers (Basel), 14: 4926, 2022.

Tomioka Y, Nakagawa M, Sakuma C, Kurosawa Y, Nagatoishi S, Tsumoto K, Arakawa T, and Akuta T. Analysis of bovine serum albumin unfolding in the absence and presence of ATP by SYPRO Orange staining of agarose native gel electrophoresis Anal Biochem, 654: 114817, 2022.

Otsubo R, Minamitani T, Kobiyama K, Fujita J, Ito T, Ueno S, Anzai I, Tanino H, Aoyama H, Matsuura Y, Namba K, Imadome KI, Ishii KJ, Tsumoto K, Kamitani W, and Yasui T. Human antibody recognition and neutralization mode on the NTD and RBD domains of SARS-CoV-2 spike protein. Sci Rep, 12: 20120, 2022.

Kinoshita S, Nakakido M, Mori C, Kuroda D, Caaveiro JMM, and Tsumoto K. Molecular basis for thermal stability and affinity in a VHH: Contribution of the framework region and its influence in the conformation of the CDR3. Protein Sci, 31: e4450, 2022.

Shozu K, Kaneko S, Shinkai N, Dozen A, Kosuge H, Nakakido M, Machino H, Takasawa K, Asada K, Komatsu M, Tsumoto K, Ohnuma SI, and Hamamoto R. Repression of the PRELP gene is relieved by histone deacetylase inhibitors through acetylation of histone H2B lysine 5 in bladder cancer. Clin Epigenetics, 14: 147, 2022.

Oyama T, Suzuki S, Horiguchi Y, Yamane A, Akao K, Nagamori K, and Tsumoto K. Performance Comparison of Spectral Distance Calculation Methods. Appl Spectrosc, 76: 1482-1493, 2022.

<Group III>

Hirano M, So Y, Tsunekawa S, Kabata M, Ohta S, Sagara H, Sankoda N, Taguchi J, Yamada Y, Ukai T, Kato M, Nakamura J, Ozawa M, Yamamoto T, and Yamada Y. MYCL-mediated reprogramming expands pancreatic insulin-producing cells. Nat Metab, 4: 254-268, 2022.

Nagata K, Hishikawa D, Sagara H, Saito M, Watanabe S, Shimizu T, and Shindou H. Lysophosphatidylcholine acyltransferase 1 controls mitochondrial reactive oxygen species generation and survival of retinal photoreceptor cells. J Biol Chem, 298: 101958, 2022.

Hamano F, Kuribayashi H, Iwagawa T, Tsuhako A, Nagata K, Sagara H, Shimizu T, Shindou H, and Watanabe S. Correction: Mapping membrane lipids in the developing and adult mouse retina under physiological and pathological conditions using mass spectrometry. J Biol Chem, 298: 102427, 2022. Erratum for: J Biol Chem, 296: 100303, 2021.