Social Cooperation Research Program

Project Division of Advanced Biopharmaceutical Science

先進的バイオ医薬品学社会連携研究部門

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Various types of antibodies have been approved for therapeutic use and are currently being examined in clinical trials. In recent years, the development of new technologies for the discovery and optimization of high-potency antibodies have enabled the rational design of specific and stable antibodies with desired biological properties. Biophysical analyses of therapeutic antibodies, particularly those of protein interaction and stability, are critical for biopharmaceutical development and represent an essential step toward the design of next-generation antibodies. The development of analytical methods with quantitative and highly sensitive evaluation of antigen interaction, protein stability, and biological function of an antibody, therefore, remains a major challenge for pharmaceutical companies. In this division, we study the biophysical properties of various antibodies to propose a new strategy for the development of highly efficient next-generation antibodies.

1. Generation of antagonistic biparatopic anti-CD30 antibody from an agonistic antibody by precise epitope determination and utilization of structural characteristics of CD30 molecule

Akiba H, Ise T, Satoh R, Abe Y, Tsumoto K, Ohno H, Kamada H, Nagata S.

Background: CD30 is a member of the tumor necrosis factor receptor superfamily. Recently, blocking CD30-dependent intracellular signaling has emerged as potential strategy for immunological regulation. Development of antibody-based CD30 antagonists is therefore of significant interest. However, a key challenge is that the bivalent form of natural antibody can crosslink CD30 molecules, leading to signal transduction even in the absence of specific ligand, CD153. Biparatopic antibodies (BpAbs) offer a solution, using two different variable fragments (Fvs) to bind distinct epitopes on a single antigen molecule. BpAbs format is an attractive alternative of natural antibody by potentially avoiding unwanted crosslinking and signaling induction

2. Secific recognition mechanism of an antibody to sulfated tyrosine and its potential use in biological research

Ujiie K, Nakakido M, Kinoshita S, Jose Caaveiro M M, Entzminger, C J Okumura, Maruyama, Miyauchi k, Matano T, Tsumoto K

Post-translational modification of proteins is a crucial biological reaction that regulates protein functions by altering molecular properties. The specific detection of such modifications in proteins has made significant contributions to molecular biology research and holds potential for future drug development applications. In HIV research, for example, ty-

rosine sulfation at the N-terminus of C-C chemokine receptor type 5 (CCR5) is considered to significantly enhance HIV infection efficiency. However, antibodies specific to sulfated CCR5 still need to be developed. In this study, we successfully generated an antibody that specifically recognized the sulfated N-terminal peptide of CCR5 through rabbit immunization and panning via phage display using a CCR5 N-terminal peptide containing sulfate modification. We used various physicochemical methods in combination with molecular dynamics simulation to screen for residues that could be involved in recognition of the sulfated peptide by this antibody. We also confirmed that this antibody recognized the sulfated full-length CCR5 on the cell surface, which suggested it should be useful as a research tool that could lead to the development of novel therapeutics. Although the antibody binding did not inhibit HIV infection, it could be also described as sulfation site-specific binding, beyond sulfation-specific binding.

3. The pericellular function of Fibulin-7 in the adhesion of oligodendrocyte lineage cells to neuronal axons during CNS myelination

Yamada M, Sasaki B, Yamada N, Hayashi C, Tsumoto K, de Vega S, Suzuki N

Myelin is an electrical insulator that enables saltatory nerve conduction and is essential for proper functioning of the central nervous system (CNS). It is formed by oligodendrocytes (OLs) in the CNS, and during OL development various molecules, including extracellular matrix (ECM) proteins, regulate OL differentiation and myelination; however, the role of ECM proteins in these processes is not well understood. Our present work is centered on the analyses of the expression and function of fibulin-7 (Fbln7), an ECM protein of the fibulin family, in OL differentiation. In the expression analysis of Fbln7 in the CNS, we found that it was expressed at early postnatal stage and localized in the processes of OL precursor cells (OPCs), in the inner region of myelin, and in axons. The functional analysis using recombinant Fbln7 protein (rFbln7) revealed that rFbln7 promoted OPC attachment activity via β1 integrin and heparan sulfate receptors. Further, rFbln7 induced the adhesion to neurites and the differentiation of OLs. Altogether, our results show that Fbln7 promotes the adhesion between OLs and axons and OL differentiation.

4. FASTIA: A rapid and accessible platform for protein variant interaction analysis demonstrated with a single-domain antibody.

Matsunaga R, Tsumoto K

Antibodies are critical tools in medicine and research, and their affinity for their target antigens is a key determinant of their efficacy. Traditional antibody affinity maturation and interaction analyses are often hampered by time-consuming steps such as cloning, expression, purification, and interaction assays. To address this, we have developed FASTIA (Fast Affinity Screening Technology for Interaction Analysis), a novel platform that integrates rapid gene fragment preparation, cell-free protein synthesis, and bio-layer interferometry with non-regenerative analysis. Using this approach, we can analyze the intermolecular interactions of over 20 variants over 2 days, requiring only the parent protein expression plasmid and basic equipment. We have demonstrated the ability of FASTIA to discriminate between single-domain antibody variants with different binding affinities using the anti-HEL VHH antibody D2-L29, and mapped the results to the crystal structure to identify key interaction sites. FASTIA provides results comparable to those obtained using traditional methods. Our system bypasses the need for genetic engineering facilities and can be easily adopted by laboratories, accelerating the protein engineering and optimization processes. In addition, FASTIA is applicable to other protein-protein interactions, making it a versatile tool for studying molecular recognition. FASTIA facilitates efficient affinity maturation, protein engineering, and analysis of protein-protein interactions. This provides a rapid and accessible route for improving antibodies and a broader understanding of protein interactions.

5. Affinity-stability trade-off mechanism of residue 35 in framework region 2 of VHH antibodies with β-hairpin CDR3

Yamamoto K, Nagatoishi S, Matsunaga R, Nakakido M, Kuroda D, Tsumoto K.

Single-domain V_HH antibodies are promising therapeutic and diagnostic tools. The third complementarity-determining region (CDR3) is usually the most critical region for antigen recognition by V_HH antibodies. When CDR3 adopts a short and extended β-hairpin conformation, framework region 2 (FR2) often interacts directly with the antigen. However, the importance of these interactions in antigen recognition remains unclear. In this research, we investigated the role of FR2 residues in V_HH antibodies with β-hairpin CDR3s. We found that several FR2 residues, particularly at positions 35 and 37, are critical for high-affinity antigen binding. Notably, a trade-off was observed: introducing a charged residue at position 35 enhanced binding affinity but reduced thermal stability. These findings provide insights into optimizing FR2 in single-domain antibodies to improve their functionality for diagnostic and therapeutic applications.

6. Mitochondrial complexity is regulated at ER-mitochondria contact sites via PDZD8-FKBP8 tethering

Nakamura K, Aoyama-Ishiwatari S, Nagao T, Paaran M, Obara CJ, Sakurai-Saito Y, Johnston J, Du Y, Suga S, Tsuboi M, Nakakido M, Tsumoto K, Kishi Y, Gotoh Y, Kwak C, Rhee HW, Seo JK, Kosako H, Potter C, Carragher B, Lippincott-Schwartz J, Polleux F, *Hirabayashi Y.

Mitochondria-ER membrane contact sites (MERCS) represent a fundamental ultrastructural feature underlying unique biochemistry and physiology in eukaryotic cells. The ER protein PDZD8 is required for the formation of MERCS in many cell types, however, its tethering partner on the outer mitochondrial membrane (OMM) is currently unknown. Here we identify the OMM protein FKBP8 as the tethering partner of PDZD8 using a combination of unbiased proximity proteomics, CRISPR-Cas9 endogenous protein tagging, Cryo-electron tomography, and correlative light-electron microscopy. Single molecule tracking reveals highly dynamic diffusion properties of PDZD8 along the ER membrane with significant pauses and captures at MERCS. Overexpression of FKBP8 is sufficient to narrow the ER-OMM distance, whereas independent versus combined deletions of these two proteins demonstrate their interdependence for MERCS formation. Furthermore, PDZD8 enhances mitochondrial complexity in a FKBP8-dependent manner. Our results identify a novel ER-mitochondria tethering complex that regulates mitochondrial morphology in mammalian cells.

7. Design of Aromatic Interaction Networks in a Protein Cage Modulated by Fluorescent Ligand Binding

Hishikawa Y, Suzuki T, Maity B, Noya H, Yoshizawa M, Asanuma A, Katagiri Y, Abe S, Nagatoishi S, Tsumoto K, Ueno T.

Dynamic behavior of proteins, such as orientation changes of aromatic residues, plays an important role in controlling biomolecular functions. Protein design that can precisely control such dynamic behavior at the atomic level is a challenging issue. The study reports the development of a system capable of orientational changes of aromatic side chains upon ligand binding. Aromatic pockets are constructed on the inner surfaces of protein cages to bind polycyclic aromatic fluorescent molecules to the targeted position by π-π stacking interactions. X-ray crystal structural analysis indicated the cooperative orientation changes of the aromatic clusters around the pocket triggered by the ligand binding. A comparison of various ligands shows that the movement of aromatic clusters can be controlled depending on the ligand structures. Fluorescence quantum yield and fluorescence lifetime are enhanced due to isolation of the fluorescent molecules in an aromatic pocket. These findings provide an understanding of the unique molecular behavior and fluorescence properties of ligands due to the assembly of aromatic residues and a guideline for developing dynamically controlled supramolecular biomaterials.

8. Antiviral potential of proton-type zeolite

Kimura Y, Takemoto M, Nakamura N, Ichinohe T, Nakakido M, Iyoki K, Ohta S, Miyamae N, Egami Y, Sato K, Okubo T, Tsumoto K, Wakihara T.

This study reports the potent antiviral ability of proton-type zeolites without the aid of any metal cations. Antiviral activities of zeolites with different topologies and chemical compositions are investigated using M13 phage and influenza virus. Proton-type zeolites exhibit excellent antiviral activity, equivalent to 99% inactivated. Antiviral tests using a centricon device suggest that direct contact of viruses on the external surface of zeolites is required to inactivate the viruses. This discovery is of importance in an interdisciplinary research field covering both zeolite science and virological science and shed light on the possibility of the development of low-cost and environmentally friendly antiviral zeolites.

9. Accelerating antibody discovery and optimization with high-throughput experimentation and machine learning

Matsunaga R, Tsumoto K.

The integration of high-throughput experimentation and machine learning is transforming data-driven antibody engineering, revolutionizing the discovery and optimization of antibody therapeutics. These approaches employ extensive datasets comprising antibody sequences, structures, and functional properties to train predictive models that enable rational design. This review highlights the significant advancements in data acquisition and feature extraction, emphasizing the necessity of capturing both sequence and structural information. We illustrate how machine learning models, including protein language models, are used not only to enhance affinity but also to optimize other crucial therapeutic properties, such as specificity, stability, viscosity, and manufacturability. Furthermore, we provide practical examples and case studies to demonstrate how the synergy between experimental and computational approaches accelerates antibody engineering. Finally, this review discusses the remaining challenges in fully realizing the potential of artificial intelligence (AI)-powered antibody discovery pipelines to expedite therapeutic development.

10. The Structural Fingerprint of Therapeutic Monoclonal Antibodies Determined Using a Combination of Near-UV Circular Dichroism and Statistical Approach for Comparative Analysis

Kiyoshi M, Oyama T, Shibata H, Suzuki S, Higuchi Y, Tsumoto K, Ishii-Watabe A.

quality characteristics in terms of efficacy and safety. However, the HOS of therapeutic monoclonal antibodies has been assessed using low- to medium-resolution spectroscopy. Furthermore, because the analysis of the obtained spectra has been performed by visual evaluation in many cases, their specificity, structural similarity, and statistical significance have not been thoroughly discussed. Therefore, there is an increasing need for advanced, statistically comparable, and highly specific analytical methods for the HOS of therapeutic monoclonal antibodies. Herein, we describe an analytical method for the HOS of therapeutic monoclonal antibodies using a powerful combination of near-UV circular dichroism (CD) and statistical analysis. The obtained near-UV CD spectra of 14 therapeutic monoclonal antibodies were employed for principal component analysis. Moreover, the spectral data were converted into Euclidean distances to perform equivalence tests and significance tests. The results clearly demonstrated that each antibody had a unique near-UV CD spectrum, like a structural fingerprint. All antibodies were judged to be not equivalent to other antibodies and were also judged to be significantly different from other antibodies. Moreover, the equivalence tests were performed on several lots of antibodies, and each lot of the antibodies were judged to be equivalent. We believe that our methods are useful for identity testing and also for comparative analysis of HOS of therapeutic monoclonal antibodies.

11. Development of a small compound that regulates the function of a maltodextrin-binding protein of Streptococcus pyogenes by multi-faceted screenings

Yamawaki T, *Nakakido M, Nagatoishi S, Caaveiro JMM, Kuroda D, Aikawa C, Nakagawa I, *Tsumoto K.

Group A Streptococcus (GAS) are gram-positive bacteria that cause various symptoms. The treatment of GAS infections currently relies on antibiotics, but new treatment options are needed due to the spread of antibiotic resistance. To develop novel treatment methods that circumvent the generation of antibiotic resistance, we used virtual screening followed by several biophysical-based screening methods to identify antibacterial compounds that target SPs0871, which is a maltodextrin-binding protein that is involved in carbohydrate catabolism in GAS. We narrowed down the list of compounds in the library via multi-step screening and finally isolated a compound that bacteriostatically inhibited the growth of GAS. Together with our previous study showing that an anti-SPs0871 variable heavy domain of heavy chain antibody, which completely blocked ligand binding, did not suppress bacterial growth, our results provide guidelines for designing an antistreptococcal therapeutic.

12. Developing FGF2 Mutants with Selectively Reduced Heparan Sulfate Affinity to Explore their Impact on FGFR1 Signaling

Okada Y, Eguchi A, Kuroda D, Tsumoto K, Ueki R, Sando S.

Fibroblast growth factor 2 (FGF2)regulates signal transduction by forming complexes with its receptors, FGF receptors (FGFRs), and heparan sulfate (HS), playing a crucial role in biological systems. Although HS has been suggested to modulate FGF/FGFR signaling as a coreceptor, multiple hypotheses exist regarding how HS affects FGF/FGFR signaling and the mechanism remains unclear. Herein, to highlight the role of FGF2/HS interaction in FGF2/FGFR1 signaling, FGF2 mutants with reduced HS-binding affinity are rationally designed through in silico analysis. These FGF2 mutants exhibit reduced HS affinity by more than two orders of magnitude while maintaining binding affinity to FGFR1. In addition, these mutants retain their thermal stability. Cellular assays using the FGF2 mutant suggest that, contrary to previous reports, the contribution of the FGF2/HS interaction in FGF2/FGFR1 signaling may be limited. The mutant FGFs that specifically alter the interaction with HS, achieved in this study, would contribute to an understanding of the role of FGF/HS interaction in FGF/FGFR signaling.

13. PRELP functions via multiple interactions with intrinsically weak affinity relying on ECM anchoring and remodeling

Kosuge H, Nakakido M, Susana de Vega, Ohnuma S, Tsumoto K

A small leucine-rich repeat proteoglycan PRELP is responsible for various biological functions. Here, to quantitatively assess the ligand binding of PRELP and its relevance to physiological activities, we validated the premise that PRELP multi-specifically binds to TGFβ1, IGFI-R, and p75NTR with relatively weak, micromolar range of affinities using surface plasmon resonance analysis. Results of a direct binding assay using N-terminal-truncated PRELP and chimeric PRELP and a dual injection assay to evaluate the binding regions and competitiveness suggested that PRELP interacts with the ligands via different but

partially overlapping regions in the leucine-rich repeat domain. RNA-seq analysis revealed that PRELP greatly promotes gene expression of various extracellular matrix (ECM) components in A549 lung carcinoma cells, also at micromolar concentration. Since we reasoned that ECM anchoring contributes to an increase of apparent local concentrations of PRELP required for the weak affinity interactions, we validated the direct binding and co-localization of PRELP with ECM proteins using ELISA analysis and immunofluorescence staining. Results of this study suggest that PRELP modulates multiple interactions with intrinsically weak binding affinities through the anchoring to ECM proteins and also promotes the ECM protein expression to maintain the preferred environment to exert the molecular functions.

14. Improving the solubility of single domain antibodies using VH-like hallmark residues

Uto Y, Nakakido M, Yokoo T, Jorge Fernandez-Perez, Kevin Entzminger, Maruyama T, C. J. Okumura, Kuroda D, Jose M. M. Caaveiro, Tsumoto K

Single domain antibodies (sdAbs) can be generated from variable regions of heavy-chain antibodies, which lack light chain and CH1 region. They have attracted attention due to their small size and molecular characteristics. Hydrophilic hallmark amino acids at framework region 2 (FR2) are key residues involved in the solubility of sdAbs. Nevertheless, previous studies reported that several sdAbs with human VH-like hydrophobic hallmark residues were soluble in a monomeric state and suggested that solubility also depends on the amino acid sequences in the complementarity-determining region. In this study, we obtained two sdAbs (sdAb A and B) with VH-like hallmark residues and low solubility from an alpaca immune library. We introduced VHH-like mutations (V37Y, G44E, L45R, W47L) into the hallmark residues in FR2 of both sdAb A and B. We were able to prepare sdAb A as a monomer without an additive in the buffer, but sdAb B was polydispersed when arginine was not added to the buffer. We also predicted the hydrophobicity of the sdAb B surface by spatial aggregation propensity calculations and identified W99 as the residue responsible for its low solubility. Subsequently, we obtained the sdAb B mutant as a monomer by introducing the W99A mutation. We characterized the engineered sdAbs using structural, physicochemical, and biophysical analyses and found that the solubility-improved sdAbs retained their functionality. Our findings can be applied to improving the solubility of sdAbs even in the absence of structural information.

15. The hook-like adaptor and cargo-binding (HAC) domain in the kinesin-2 tail enables adaptor assembly and cargo recognition

Jiang X, Danev R, Ichinose S, Niu B, Ohtsuki S, Yanagisawa H, Nagatoishi S, Tsumoto K, Hirokawa N, Kikkawa M.

Intracellular transport relies on motor proteins such as kinesins to deliver cargo along microtubules, yet how they recognize cargo remains unclear. Here, we present high-resolution cryo-electron microscopy structures of the heterotrimeric kinesin-2 complex (KIF3A/KIF3B/KAP3) bound to the cargo protein APC. Our findings reveal a previously uncharacterized KIF3 tail hook-like motif, termed the "HAC" domain, which mediates binding to both KAP3 adaptor and APC cargo. Within this domain, the KIF3A helical regions ensure cargo specificity, while a β-hairpin and KIF3B provide structural support. Biochemical and neuronal experiments confirm its functional importance. Notably, the HAC/KAP3 structure resembles hook-like architectures seen in kinesin-1 and dynein, suggesting a shared cargo recognition framework. These findings also shed light on kinesin-2 cargo specificity and offer a structural framework for understanding related neuronal transport mechanisms.

16. Megalin-Mediated Direct and Indirect Pathways for the Development of Contrast-Induced Proximal Tubule Epithelial Cell Injury

Goto S, Hosojima M, Kabasawa H, Takemoto K, Aoki H, Komochi K, Kobayashi R, Sugita N, Endo T, Kuwahara S, Tanaka T, Kaseda R, Nagatoishi S, Yoshida Y, Narita I, Yamamoto S, Tsumoto K, Hirayama Y, Saito A

Megalin is a multiligand endocytic receptor in proximal tubule epithelial cells (PTECs) that facilitates the reabsorption of certain nephrotoxic drugs. Notably, cilastatin competes with these drugs for binding to megalin. Patients with chronic kidney disease are at increased risk of contrast-induced (CI) acute kidney injury, in which iodine-mediated PTEC toxicity is a crucial pathogenic factor. This study investigated the interplay between megalin and CI-PTEC injury, along with the protective effect of cilastatin, utilizing electron probe microanalysis, a mouse CI-PTEC injury model, quartz crystal microbalance analysis, and binding assays with magnetic beads immobilized with iomeprol, a water-soluble nonionic contrast medium (CM). Electron probe microanalysis revealed the reduced renal uptake of CMs in the kidney cortex of kidney-specific conditional megalin-knockout mice compared with controls. In the CI-PTEC injury model using kidney-specific conditional or mosaic megalin-knockout mice, a significant re-

duction of vacuolization was observed in PTECs in the cortex compared with controls in the former, while co-expression of megalin and kidney injury molecule-1 was observed in the latter. These findings indicate that megalin is involved in the development of CI-PTEC injury. Cilastatin inhibited the renal uptake of CMs and ameliorated CI-PTEC injury in wild-type mice. Of note, two distinct pathways of renal CM uptake were delineated, both of which cilastatin inhibited: the direct binding of CMs to megalin and an indirect route involving transcobalamin-2, an endocytic megalin ligand, functioning as a CM carrier. Collectively, megalin governs the direct and indirect pathways in the development of CI-PTEC injury, and cilastatin effectively impedes both.

17. Development of a VHH that inhibits the binding of neuronal pentraxin 2 to a postsynaptic glutamate receptor, AMPAR

Yokoo T, Nakakido M, Matsuda K, Caaveiro JMM, Fernandez-Perez J, Yuzaki M, Tsumoto K.

Neurons connect to each other via synapses to form neural circuits. Rece nt research has shown that neuropsychiatric disorders and neurological disorders, such as autism spectrum disorders and Alzheimer's disease, are synaptic diseases caused by abnormalities of synapses. Synaptic organizers are molecules responsible for synapse formation. Neuronal pentraxin 2 (NP2) is a synaptic organizer and a secreted protein that is expressed mainly in the hippocampus and cerebellum, and it contributes to synaptic plasticity. NP2 forms clusters with its family proteins, NP1 and neuronal pentraxin receptor, and binds to postsynaptic amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-type receptors. In recent years, research has revealed the disease relevance of NP2. For example, it can be a biomarker of Alzheimer's disease, and its overexpression in the peripheral nervous system has been reported to cause chronic itch. However, the mechanism of NP2 function has not been well described at the molecular level. In this study, we developed a variable domain of a heavy-chain antibody (VHH) against NP2 to elucidate its molecular mechanism of action and to regulate its function of NP2. The obtained VHH N1 showed high specificity and affinity to NP2, and its binding mechanism was elucidated by X-ray crystallography. Furthermore, VHH N1 inhibited the binding of NP2 to amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-type receptors, and this inhibitory activity was confirmed in cells. These results provide useful insights into the molecular mechanism of NP2 function and highlight the potential application of VHH N1 as a detection agent for NP2 or as a therapeutic agent for chronic itch.

18. Solubilization and refolding of inclusion bodies by detergents

Arakawa T, Akuta T, Ejima D, Tsumoto K.

Recombinant proteins play many important roles in development of biological reagents and biopharmaceuticals. Here, we will mainly review refolding of recombinant proteins when expressed in inclusion bodies, although strategies to enhance soluble expression are described as an alternative to refolding inclusion bodies. These strategies include, but not limited to, adding chemical chaperones in cell culture media, modifying cell lysis buffer and using solubility-enhacing fusion tags. Another solubility enhancement was to generate lipid complex for membrane proteins that form insoluble proteins without lipid. Among various solubilization and refolding technologies, those using denaturant, alkaline pH and pressure are also desribed, while we focus on solubilization and refolding using detergents, which are effective and cost-friendly. Sodium dodecylsulfate, lauroyl-glutamate, sarkosyl and cetyltrimethylammonium have been extensively used, as summarized in this review. Slow or step-wise removal of denaturants or ionic detergents used to solubilize appears to play a critical role in successful refolding by maintaining the solubility of proteins during refolding. In alkaline refolding, slow pH adjustment also helps maintain protein solubility. In pressure refolding, small amount of guanidine hydrochloride assisted refolding.

19. Structural basis for heme binding by the Shr protein from Streptococcus pyogenes

Seki K, Senoo A, Nagatoishi S, Yanaka S, Nakakido M, Tsumoto K, Caaveiro JMM.

Streptococcus pyogenes causes a range of infectious diseases. In an era of increasing antibiotic resistance, new antimicrobial strategies targeting virulence factors, rather than essential survival mechanisms, are being explored. A key virulence factor in S. pyogenes is the bacterial iron acquisition system, because iron is essential but limited in the host due to sequestration by proteins like hemoglobin. The bacteria S. pyogenes possesses the Shr protein that acquires heme from host hemoglobin and transfers it to Shp, a membrane proximity protein. Shr comprises multiple domains, including two NEAr-Transporter (NEAT) domains that directly bind to heme. While structural information of NEAT domains from other bacteria are available, the structure of NEAT domains from Shr remains unknown. In this study, crystal structures of Linker-NEAT1 and NEAT2 domains were determined to 2.35 Å resolution and 2.66 Å resolution, respectively. Structural and mutational analyses revealed that methionine residues play a key role in heme binding, which seems to be a characteristic of heme-binding

proteins from S. pyogenes, but not of NEAT domains from other gram-positive species. These findings enhance our understanding of heme acquisition in S. pyogenes and may guide novel therapeutic approaches.

20. myCRP, Cysteine-Rich Protein in the Yesso Scallop Mizuhopecten yessoensis, Is a Novel High-Molecular Weight Cd-Binding Protein

Zheng Z, Namikawa Y, Awaji M, Kato Y, Iimura K, Nakakido M, Tsumoto K, Negishi L, Kurumizaka H, Homma-Takeda S, Suzuki M.

Heavy metal bioaccumulation in marine organisms is a critical concern for the oceanic environment and food safety. Scallops, as filter-feeding bivalves, accumulate cadmium (Cd) in their midgut gland to toxic concentrations that may pose risks to human health. Despite extensive research on Cd bioaccumulation in the Yesso scallop Mizuhopecten yessoensis, a major Cd-binding protein has not yet been functionally characterized. In this study, we used high-performance liquid chromatography coupled with inductively coupled plasma mass spectrometry (HPLC-ICP-MS) to isolate Cd-binding proteins, and identified myCRP, a novel high-molecular-weight Cd-binding protein. myCRP contains a cysteine-rich sequence, resembling metallothionein Cd-binding motifs, and is expressed exclusively in the midgut gland. Cd-binding assays demonstrated that myCRP binds Cd in a dose-dependent manner. Immunostaining revealed the presence of a small cluster of myCRP-containing cells in the connective tissue surrounding digestive tubules in the midgut gland. Synchrotron radiation X-ray fluorescence (SR-XRF) analyses showed the distribution of Cd throughout the entire midgut gland tissue. We conclude that myCRP is partly responsible for Cd binding in the midgut gland. Our findings clarify a new mechanism of Cd-binding in marine organisms from both biological and molecular perspectives.

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