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ダイジェスト

JB REVIEW

Senso-immunology: the past, present, and future

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Pain and mechanical stimulation are thought to be alarm systems that alert the brain to physical abnormalities. When we experience unpleasant feelings in infected or traumatized tissues, our awareness is directed to the afflicted region, prompting activities such as resting or licking the tissue. Despite extensive research into the molecular biology of nociceptors, it was unclear whether their role was limited to the generation and transmission of unpleasant feelings or whether they actively modulate the pathogenesis of infected or traumatized tissues. Recently, it has become clear how the sensory and immune systems interact with one another and share similar receptors and ligands to modify the pathogenesis of various diseases. In this paper, we summarize the mechanisms of crosstalk between the sensory and immune systems and the impact of this new interdisciplinary field, which should be dubbed 'senso-immunology,' on medical science.

Keywords: immunology, infection, mechanical stimuli, pain, physiology

Structural insights into endothelin receptor signalling

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Endothelins and their receptors, type A (ET_A) and type B (ET_B), modulate vital cellular processes, including growth, survival, invasion and angiogenesis, through multiple G proteins. This review highlights the structural determinations of these receptors by X-ray crystallography and cryo-electron microscopy, and their activation mechanisms by endothelins. Explorations of the conformational changes upon receptor activation have provided insights into the unique G-protein coupling feature of the endothelin receptors. The review further delves into the binding modes of the clinical antagonist and the inverse agonists. These findings significantly contribute to understanding the mechanism of G-protein activation and have potential implications for drug development, particularly in the context of vasodilatory antagonists and agonists targeting the endothelin receptors.

Keywords: endothelin, G-protein-coupled receptor, structural biology, vascular system

REGULAR PAPER
BIOCHEMISTRY
Protein Structure

Structural insights into a bacterial *β*-glucosidase capable of degrading sesaminol triglucoside to produce sesaminol: toward the understanding of the aglycone recognition mechanism by the C-terminal lid domain

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The sesaminol triglucoside (STG)-hydrolyzing *β*-glucosidase from *Paenibacillus* sp. (PSTG1), which belongs to glycoside hydrolase family 3 (GH3), is a promising catalyst for the industrial production of sesaminol. We determined the X-ray crystal structure of PSTG1 with bound glycerol molecule in the putative active site. PSTG1 monomer contained typical three domains of GH3 with the active site in domain 1 (TIM barrel). In addition, PSTG1 contained an additional domain (domain 4) at the C-terminus that interacts with the active site of the other protomer as a lid in the dimer unit. Interestingly, the interface of domain 4 and the active site forms a hydrophobic cavity probably for recognizing the hydrophobic aglycone moiety of substrate. The short flexible loop region of TIM barrel was found to be approaching the interface of domain 4 and the active site. We found that n-heptyl-*β*-D-thioglucopyranoside detergent acts as an inhibitor for PSTG1. Thus, we propose that the recognition of hydrophobic aglycone moiety is important for PSTG1-catalyzed reactions. Domain 4 might be a potential target for elucidating the aglycone recognition mechanism of PSTG1 as well as for engineering PSTG1 to create a further excellent enzyme to degrade STG more efficiently to produce sesaminol.

Keywords: glycoside hydrolase family 3, sesaminol triglucoside, *β*-glucosidase

Abbreviations: STG, sesaminol triglucoside; PSTG1, STG-hydrolyzing *β*-glucosidase from Paenibacillus sp; GH3, Glycoside

Hydrolase Family 3; TIM, Triosephosphate isomerase, Fn-III, Fibronectin type III; 2-SDG, 2-*O*-(*β*-D-glucopyranosyl)-*β*-D-glucopyranosylsesaminol; 6-SDG, 6-*O*-(*β*-D-glucopyranosyl)-*β*-D-glucopyranosylsesaminol; SMG, *β*-D-glucopyranosylsesaminol; HTG, n-Heptyl-beta-D-thioglucopyranoside; OTG, n-Octyl-*β*-D-glucoside; pNP-*β*-Glc, *p*-Nitrophenyl-*β*-D-glucopyranoside

Hydrogen bonds connecting the N-terminal region and the DE loop stabilize the monomeric structure of transthyretin

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Transthyretin (TTR) is a homo-tetrameric serum protein associated with sporadic and hereditary systemic amyloidosis. TTR amyloid formation proceeds by the dissociation of the TTR tetramer and the subsequent partial unfolding of the TTR monomer into an aggregation-prone conformation. Although TTR kinetic stabilizers suppress tetramer dissociation, a strategy for stabilizing monomers has not yet been developed. Here, we show that an N-terminal C10S mutation increases the thermodynamic stability of the TTR monomer by forming new hydrogen bond networks through the side chain hydroxyl group of Ser10. Nuclear magnetic resonance spectrometry and molecular dynamics simulation revealed that the Ser10 hydroxyl group forms hydrogen bonds with the main chain amide group of either Gly57 or Thr59 on the DE loop. These hydrogen bonds prevent the dissociation of edge strands in the DAGH and CBEF *β*-sheets during the unfolding of the TTR monomer by stabilizing the interaction between *β*-strands A and D and the quasi-helical structure in the DE loop. We propose that introducing hydrogen bonds to connect the N-terminal region to the DE loop reduces the amyloidogenic potential of TTR by stabilizing the monomer.

Keywords: denaturation < protein, folding < protein, molecular dynamics < methods, NMR < methods, transthyretin

Protein Interaction and Recognition

Inter-domain interaction of ferredoxin-NADP+reductase important for the negative cooperativity by ferredoxin and NADP(H)

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Ferredoxin-NADP$^+$ reductase (FNR) in plants receives electrons from ferredoxin (Fd) and converts NADP$^+$ to NADPH. The affinity between FNR and Fd is weakened by the allosteric binding of NADP(H) on FNR, which is considered as a part of negative cooperativity. We have been investigating the molecular mechanism of this phenomenon and proposed that the NADP(H)-binding signal is transferred to the Fd-binding region across the two domains of FNR, NADP(H)-binding domain and FAD-binding domain. In this study, we analyzed the effect of altering the inter-domain interaction of FNR on the negative cooperativity. Four site-directed FNR mutants at the inter-domain region were prepared, and their NADPH-dependent changes in the K_m for Fd and physical binding ability to Fd were investigated. Two mutants, in which an inter-domain hydrogen bond was changed to a disulfide bond (FNR D52C/S208C) and an inter-domain salt bridge was lost (FNR D104N), were shown to suppress the negative cooperativity by using kinetic analysis and Fd-affinity chromatography. These results showed that the inter-domain interaction of FNR is important for the negative cooperativity, suggesting that the allosteric NADP(H)-binding signal is transferred to Fd-binging region by conformational changes involving inter-domain interactions of FNR.

Keywords: protein–protein interaction, negative cooperativity, inter-domain interaction, ferredoxin-NADP$^+$ reductase, ferredoxin

Abbreviations: cytc, cytochrome c; Fd, ferredoxin; FNR, ferredoxin-NADP$^+$ reductase

MOLECULAR BIOLOGY

Gene Expression

Cooperative DNA-binding activities of Chp2 are critical for its function in heterochromatin assembly

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Heterochromatin protein 1 (HP1) is an evolutionarily conserved protein that plays a critical role in heterochromatin assembly. HP1 proteins share a basic structure consisting of an N-terminal chromodomain (CD) and a C-terminal chromoshadow domain (CSD) linked by a disordered hinge region. The CD recognizes histone H3 lysine 9 methylation, a hallmark of heterochromatin, while the CSD forms a dimer to recruit other chromosomal proteins. HP1 proteins have been shown to bind DNA or RNA primarily through the hinge region. However, how DNA or RNA binding contributes to their function remains elusive. Here, we focus on Chp2, one of the two HP1 proteins in fission yeast, and investigate how Chp2's DNA-binding ability contributes to its function. Similar to other HP1 proteins, the Chp2 hinge exhibits clear DNA-binding activity. Interestingly, the Chp2 CSD also shows robust DNA-binding activity. Mutational analysis revealed that basic residues in the Chp2 hinge and at the N-terminus of the CSD are essential for DNA binding, and the combined amino acid substitutions of these residues alter Chp2 stability, impair Chp2 heterochromatin localization and lead to a silencing defect. These results demonstrate that the cooperative DNA-binding activities of Chp2 play an important role in heterochromatin assembly in fission yeast.

Keywords: chromatin, chromosomes, DNA binding, fission yeast, heterochromatin, HP1

BIOTECHNOLOGY

Biomaterials

Characterization of K-binding factor involved in water-soluble complex of menaquinone-7 produced by Bacillus subtilis natto

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Vitamin Ks are expected to contribute bone and cardiovascular health. Especially, menaquinone-7 has a higher bioavailability and a longer half-life than other vitamin Ks in the human body. However, their low water-solubility limits their application. On the other hand, *Bacillus subtilis natto* produces a water-soluble complex, which comprises menaquinone-7 and peptides. The peptide named K-binding factor (KBF) has been reported as the main component of the complex. In the present, the structural characteristics of KBF were studied. Mass spectrometry showed significant peaks at $m/z = 1050$, while the previous PAGE suggested that molecular weight of KBF was ~3 k. Amino acid analysis revealed that the 1k peptides were the various combinations of nine amino acids, among which Asx, Glx, Val, Leu and Met were found to be the most abundant. The peptides could serve as detergent properties. The 1k peptides could be isolated by reverse-phase high performance liquid chromatography. The bundle of three 1 k detergent-like peptides would participate to the micelle structure containing menqauinone-7 inside. In conclusion, a basic unit of KBF would be the ~1 k peptides, and the three basic unit assemble to the ~3 k bundle, then the bundle-form a water-soluble micelle including menqauinone-7 inside.

Keywords: Bacillus subtilis natto, K-binding factor, menaquinone-7, solubilization, vitamin K. Abbreviations: CV, column volume; DLS, dynamic light scattering; FPLC, Fast protein liquid chromatography; KBF, K-binding factor; MK-7, menaquinone-7; natto-MK-7, a water-soluble complex of menaquinone-7; RH, hydrodynamic radius

Gene and Protein Engineering

Analysis of thermostability for seven Phe to Ala and six Pro to Gly mutants in the Fab constant region of adalimumab

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To identify amino acids that play important roles in the structural stability of Fab, seven phenylalanine residues in the Fab constant region of the therapeutic antibody adalimumab were subjected to alanine mutagenesis. Six Fab mutants, H:F130A, H:F154A, H:F174A, L:F118A, L:F139A and L:F209A, showed decreased thermostability compared with wild-type Fab. In contrast, the $T\mathbf{m}$ for the L:F116A mutant was 1.7°C higher than that of wild-type Fab, indicating that the F116 residue was unfavorable for Fab thermostability. Six proline mutants, H:P131G, H:P155G, H:P175G, L:P119G, L:P120G and L:P141G, were also prepared to investigate the effect of proline residues adjacent to mutated phenylalanine residues. The thermostability of the H:P155G and L:P141G mutants in particular was significantly reduced, with decreases in $T\mathbf{m}$ of 5.0 and 3.0°C, respectively, compared with wild-type Fab. The H:P155 and L:P141 residues have a *cis* conformation, whereas the other mutated proline residues have a *trans* conformation. H:P155 and L:P141 had stacking interactions with the H:F154 and L:Y140, respectively, at the interface between the variable and constant regions. It is suggested that the interactions of the aromatic ring with a *cis*-form proline at the interface between the variable and constant regions is important for stability of Fab.

Key words: adalimumab, constant region, Fab, stacking interactions, thermostability

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ダイジェスト

JB REVIEW

Toward a high-resolution mechanism of intrinsically disordered protein self-assembly

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Membraneless organelles formed via the self-assembly of intrinsically disordered proteins (IDPs) play a crucial role in regulating various physiological functions. Elucidating the mechanisms behind IDP self-assembly is of great interest not only from a biological perspective but also for understanding how amino acid mutations in IDPs contribute to the development of neurodegenerative diseases and other disorders. Currently, two proposed mechanisms explain IDP self-assembly: (1) the sticker-and-spacer framework, which considers amino acid residues as beads to simulate the intermolecular interactions, and (2) the cross-β hypothesis, which focuses on the β-sheet interactions between the molecular surfaces constructed by multiple residues. This review explores the advancement of new models that provide higher resolution insights into the IDP self-assembly mechanism based on new findings obtained from structural studies of IDPs.

Keywords: amyloid fibrils, intrinsically disordered proteins, liquid–liquid phase separation, membraneless organelles, neurodegenerative diseases

Evolutionary implications from lipids in membrane bilayers and photosynthetic complexes in cyanobacteria and chloroplasts

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In biomembranes, lipids form bilayer structures that serve as the fluid matrix for membrane proteins and other hydrophobic compounds. Additionally, lipid molecules associate with membrane proteins and impact their structures and functions. In both cyanobacteria and the chloroplasts of plants and algae, the lipid bilayer of the thylakoid membrane consists of four distinct glycerolipid classes: monogalactosyldiacylglycerol, digalactosyldiacylglycerol, sulfoquinovosyldiacylglycerol, and phosphatidylglycerol. These lipids are also integral components of photosynthetic complexes such as photosystem II and photosystem I. The lipid-binding sites within the photosystems, as well as the lipid composition in the thylakoid membrane, are highly conserved between cyanobacteria and photosynthetic eukaryotes, and each lipid class has specific roles in oxygenic photosynthesis. This review aims to shed light on the potential evolutionary implications of lipid utilization in membrane lipid bilayers and photosynthetic complexes in oxygenic photosynthetic organisms.

Keywords: chloroplasts, cyanobacteria, glycerolipids, photosystems, thylakoid membrane

REGULAR PAPER
BIOCHEMISTRY
Biochemistry General

Direct binding of calmodulin to the cytosolic C-terminal regions of sweet/umami taste receptors

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Sweet and umami taste receptors recognize chemicals such as sugars and amino acids on their extracellular side and transmit signals into the cytosol of the taste cell. In contrast to ligands that act on the extracellular side of these receptors, little is known regarding the molecules that regulate receptor functions within the cytosol. In this study, we analysed the interaction between sweet and umami taste receptors and calmodulin, a representative Ca^{2+}-dependent cytosolic regulatory protein. High prediction scores for calmodulin binding were observed on the C-terminal cytosolic side of mouse taste receptor type 1 subunit 3 (T1r3), a subunit that is common to both sweet and umami taste receptors. Pull-down assay and surface plasmon resonance analyses showed different affinities of calmodulin to the C-terminal tails of distinct T1r subtypes. Furthermore, we found that T1r3 and T1r2 showed the highest and considerable binding to calmodulin, whereas T1r1 showed weaker binding affinity. Finally, the binding of calmodulin to T1rs was consistently higher in the presence of Ca^{2+} than in its absence. The results suggested a possibility of the Ca^{2+}-dependent feedback regulation process of sweet and umami taste receptor signaling by calmodulin.

Keywords: calmodulin, cytosol, sweet taste, taste receptor type 1, umami taste

Protein Structure

The stability of NPM1 oligomers regulated by acidic disordered regions controls the quality of liquid droplets

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The nucleolus is a membrane-less nuclear body that typically forms through the process of liquid–liquid phase separation (LLPS) involving its components. NPM1 drives LLPS within the nucleolus and its oligomer formation and inter-oligomer interactions play a cooperative role in inducing LLPS. However, the molecular mechanism underlaying the regulation of liquid droplet quality formed by NPM1 remains poorly understood. In this study, we demonstrate that the N-terminal and central acidic residues within the intrinsically disordered regions (IDR) of NPM1 contribute to attenuating oligomer stability, although differences in the oligomer stability were observed only under stringent conditions. Furthermore, the impact of the IDRs is augmented by an increase in net negative charges resulting from phosphorylation within the IDRs. Significantly, we observed an increase in fluidity of liquid droplets formed by NPM1 with decreased oligomer stability. These results indicate that the difference in oligomer stability only observed biochemically under stringent conditions has a significant impact on liquid droplet quality formed by NPM1. Our findings provide new mechanistic insights into the regulation of nucleolar dynamics during the cell cycle.

Keywords: Intrinsically disordered region, liquid droplets, MD simulation, nucleolus, phosphorylation

Abbreviations: FRAP, fluorescence recovery after photobleaching; GC, granular component; IDR, intrinsically disordered region; LLPS, liquid–liquid phase separation; MD simulation, molecular dynamics simulation; NPM1, nucleophosmin 1; rRNA, ribosomal RNA

Protein Interaction and Recognition

Binding profile of quinonoid-dihydrobiopterin to quinonoid-dihydropteridine reductase examined by in silico and in vitro analyses

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Quinonoid dihydropteridine reductase (QDPR) catalyses the reduction of *quinonoid*-form dihydrobiopterin (qBH2) to tetrahydrobiopterin (BH4). BH4 metabolism is a drug target for neglected tropical disorders because trypanosomatid protozoans, including *Leishmania* and *Trypanosoma*, require exogenous sources of biopterin for growth. Although QDPR is a key enzyme for maintaining intracellular BH4 levels, the precise catalytic properties and reaction mechanisms of QDPR are poorly understood due to the instability of *quinonoid*-form substrates. In this study, we analysed the binding profile of *q*BH2 to human QDPR in combination with *in silico* and *in vitro* methods. First, we performed docking simulation of *q*BH2 to QDPR to obtain possible binding modes of *q*BH2 at the active site of QDPR. Then, among them, we determined the most plausible binding mode using molecular dynamics simulations revealing its atomic-level interactions and confirmed it with the *in vitro* assay of mutant enzymes. Moreover, it was found that not only *q*BH2 but also *quinonoid*-form dihydrofolate (qDHF) could be potential physiological substrates for QDPR, suggesting that QDPR may be a bifunctional enzyme. These findings in this study provide important insights into biopterin and folate metabolism and would be useful for developing drugs for neglected tropical diseases.

Keywords: molecular dynamics, pteridine reductase, *quinonoid*-dihydropteridine reductase, tetrahydrobiopterin

Abbreviations: AAAH, aromatic aminoacid hydroxylase; BH2, dihydrobiopterin; BH4, tetrahydrobiopterin; DHFR, dihydrofolate reductase; NADH, nicotinamide adenine dinucleotide; NAM, nicotinamide; MD, molecular dynamics; PT, pterin; PTR1, pteridine reductase 1; qBH2; *quinonoid* dihydrobiopterin; qDHF, *quinonoid* dihydrofolate; QDPR, *quinonoid* dihydropteridine reductase; SDR, short-chain dehydrogenase/reductase; THF, tetrahydrofolate

Biochemistry of Proteolysis

Calpain-3 not only proteolyzes calpain-1 and -2 but also is a substrate for calpain-1 and -2

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Calpain is an intracellular cysteine protease that cleaves its specific substrates in a limited region to modulate cellular function. Calpain-1 (C1) and calpain-2 (C2) are ubiquitously expressed in mammalian cells, but calpain-3 (C3) is a skeletal muscle-specific type. In the course of calpain activation, the N-terminal regions of all three isoforms are clipped off in an intramolecular or intermolecular fashion. C1 proteolyzes C2 to promote further proteolysis, but C2 proteolyzes C1 to suspend C1 proteolysis, indicating the presence of C1–C2 reciprocal proteolysis. However, whether C3 is involved in the calpain proteolysis network is unclear. To address this, we examined whether GFP-tagged C3:C129S

(GFP-C3:CS), an inactive protease form of C3, was a substrate for C1 or C2 in HEK cells. Intriguingly, the N-terminal region of C3:CS was cleaved by C1 and C2 at the site identical to that of the C3 autoproteolysis site. Furthermore, the N-terminal clipping of C3:CS by C1 and C2 was observed in mouse skeletal muscle lysates. Meanwhile, C3 preferentially cleaved the N-terminus of C1 over that of C2, and the sizes of these cleaved proteins were identical to their autoproteolysis forms. Our findings suggest an elaborate inter-calpain network to prime and suppress proteolysis of other calpains.

Keywords: autolysis, calpain, calpain-3, LGMD2A/R1, skeletal muscle

MOLECULAR BIOLOGY

Molecular Biology General

Inhibition of Sirt2 Decreases ApoE Secretion in Astrocytes and Microglial Cells

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Amyloid-β (Aβ) accumulation caused by an imbalance of the production and clearance of Aβ in the brain is associated with the development of Alzheimer's disease (AD). Apolipoprotein E (ApoE) (the strongest genetic risk factor) enhances Aβ clearance, preventing Aβ deposition. Sirtuin 2 (Sirt2) is an NAD^+-dependent histone deacetylase and its inhibition has been reported to ameliorate memory impairment in AD-like model mice. However, the role of Sirt2 in ApoE secretion is unknown. Here, we found that inhibition of Sirt2 activity in primary cultured astrocytes and BV2 cells decreased ApoE secretion, resulting in the accumulation of intracellular ApoE and inhibiting extracellular Aβ degradation. However, the reduction of Sirt2 protein level by Sirt2 siRNA decreased ApoE protein level, which ultimately reduces ApoE secretion. In addition, the knockdown of Sirt2 in the HEK293-APP cells also decreased levels of intracellular ApoE leading to reduction of its secretion, which is accompanied by increased Aβ levels without altering APP and APP processing enzymes. Our findings provide a novel role of Sirt2 in ApoE secretion.

Keywords: Alzheimer's disease, amyloid-β, apolipoprotein E, glial cells, Sirt2

Abbreviations: AD, Alzheimer's disease; ABCA1, ATP-binding cassette protein A1; ADAM10, A disintegrin and metalloproteinase domain-containing protein 10; Aβ, Amyloid-beta; APP, Amyloid precursor protein; ApoE, Apolipoprotein E; BACE1, β-site amyloid precursor protein cleaving enzyme 1; IDE, Insulin degrading enzyme; NEP, Neprilysin; PS1, Presenilin 1; Sirt2, Sirtuin 2

BIOTECHNOLOGY

RNA Technology

Selection of aptamers using β-1,3-glucan recognition protein-tagged proteins and curdlan beads

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RNA aptamersare nucleic acids that are obtained using the systematic evolution of ligands by exponential enrichment (SELEX) method. When using conventional selection methods to immobilize target proteins on matrix beads using protein tags, sequences are obtained that bind not only to the target proteins but also to the protein tags and matrix beads. In this study, we performed SELEX using β-1,3-glucan recognition protein (GRP)-tags and curdlan beads to immobilize the acute myeloid leukaemia 1 (AML1) Runt domain (RD) and analysed the enrichment of aptamers using high-throughput sequencing. Comparison of aptamer enrichment using the GRP-tag and His-tag suggested that aptamers were enriched using the GRP-tag as well as using the His-tag. Furthermore, surface plasmon resonance analysis revealed that the aptamer did not bind to the GRP-tag and that the conjugation of the GRP-tag to RD weakened the interaction between the aptamer and RD. The GRP-tag could have acted as a competitor to reduce weakly bound RNAs. Therefore, the affinity system of the GRP-tagged proteins and curdlan beads is suitable for obtaining specific aptamers using SELEX.

Keywords: aptamer, curdlan, βGRP, SELEX

Abbreviations: AML1, acute myeloid leukaemia 1; βGRP, β-1,3-glucan recognition protein; GST, glutathione S-transferase; His-tag, poly histidine tag; HTS, high-throughput sequencing; MBP, maltose-binding protein; RD, Runt domain; RUNX1, RUNX family transcription factor 1; SELEX, systematic evolution of ligands by exponential enrichment; SPR, surface plasmon resonance