P_Protein Engineering and Evolution

P-1

Development of High-Throughput Screening System for the Evolution of Fucosyltransferase

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Fucosyllactose is taking up the largest proportion of Human Milk Oligosaccharide(HMO) compounds. HMOs are functional material, with prebiotic effects, immunity and brain activity effects and are generally used in the industry. We studied advancing productivity by improving fucosyllactose production enzyme, fucosyltransferase, through Random Mutagenesis and we studied the high-throughput screening method to select improved active enzyme. In previous research, we used HPLC to measure fucosyllactose. We convert the produced GDP to GTP and using single phosphate with phosphatase and then detect phosphate with malachite green. Based on this method, we discover the enzyme that has improved productivity by screening mutant library through error prone PCR.

Keywords : Fucosyllactose, enzyme evolution, HTS

P-2

Improvement of Formate-Utilizing Related Gene by Site-Directed Mutagenesis Based on the Enzyme Engineering in silico

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Formate is the simplest organic acid and reported as one of the desirable bulk chemicals regarding economic and environmental benefits. However, lots of microorganisms cannot use formate because of its toxicity and related enzymes. In this study, we used Discovery Studio with CHARMm, a molecular dynamics engine, for finding candidates of enzyme engineering. We hypothesized that the regulation of structure in the enzyme level can improve substrate uptake. First, we can build a suitable homology model based on the known sequence and structure. Using the constructed model, we are able to calculate CDOCKER Energy for estimating the stability of the enzyme when the enzyme binds to the substrate. According to the serial results, we can find the appropriate mutant candidates for improving enzyme stability. Finally, the modified enzyme structure might be show improvement of formate uptake. Based on the results, we might get a great mutant to improving formate uptake as a sole carbon source.

Keywords : Formate, CHARMm, CDOCKER

P-3

Structural and Functional Characterization of an M38 β-Aspartyl Peptidase from *Fervidobacterium islandicum* AW-1

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Isoaspartyl dipeptidase is an enzyme that catalyzes the hydrolysis of isoaspartyl-containing peptides, which can contribute to inactivation, aggregation, and aging of proteins in tissue. Previously, we found that a gene (NA23_RS08100) annotated as M38 β-aspartyl peptidase (Bap) in the keratin-degrading bacterium *Fervidobacterium islandicum* AW-1 showed the highest expression level among other proteases when cells were under starvation conditions. To investigate the role of *Fi*Bap in cells' survival especially in extreme and stressful environments, we characterized the structure and function of the NA23_RS08100 gene from *F. islandicum* AW-1. We found that the oligomeric structure of this enzyme is octamer, comprising of tetramer-of-dimers, and its apo enzyme exhibited maximal activity at 80℃ and pH 7.0 - 8.0 in the presence of Zn^{2+}. Remarkably, despite of the structural similarity with other M38 Bap homologs, *Fi*Bap structure revealed that its thermostability is provided by four salt bridges in each dimer, resulting in the sixteen ionic interactions in octamer, which is unique to thermophilic *Fi*Bap. Furthermore, metal ions such as Zn^{2+} were coordinated with two-triad metal binding motif including Asp156 on cis-peptide loop, which classifies *Fi*Bap to type-I Bap.

Keywords : M38 β-aspartyl peptidase, *Fervidobacterium islandicum* AW-1, structure

P-4

Naturally Programmed Stringent Response for High-Throughput Enzyme Engineering

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Genetic circuit-based biosensors are effective analytical tool in synthetic biology that can be applied for detection of target metabolites or *in vivo* enzyme activities. Transcription factors (TF) can be adopted as a molecular switch to express reporter signals, such as cellular fluorescence and antibiotic resistance. Herein, we demonstrate that intrinsic silent property of sigma 54 (σ^{54})-dependent TFs could control host cell state to be more appropriate state for high-throughput screening. DmpR, the phenol-dependent TF can be used for the high-throughput enzyme engineering by controlling the physiology of a host cell. Cellular (penta)tetraphosphate ((p)ppGpp) level-dependent stringent responses can improve σ^{54} TF-based biosensor activity. By controlling stringent response and optimizing assay conditions of a DmpR-based biosensor, catalytically improved tyrosine phenol lyase (TPL) was successfully obtained, thereby demonstrating practical application of this biosensor. High-throughput enzyme engineering using TF-based biosensors will be even more efficient by using this combinatorial strategy.

Keywords : Transcription factor, stringent response, biosensor

P-5

Tissue-Specific Codon Optimization for Gene Therapy

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The developments in artificial gene synthesis have led ground to the construction of synthetic bioparts [1]. Of these, recombinant protein production is one of them, as its potential application is vast and important in biotechnology especially as an emergent therapeutic paradigm with a demonstrated benefit for genetic disorders. Gene therapy promises an effective long-lasting treatment for genetic disorders but poses one of the greatest technical challenges in modern medicine due to the difficulties to deliver the normal gene to the correct tissue, as genes are selectively expressed in one tissue are often discriminated from genes expressed in other tissue based on codon bias, and cells capabilities in shutting down introduced genes. In this study, we used codon optimization for designing a target protein sequence used for gene therapy so that it will significantly be expressed in the desired tissue. We performed codon optimization using the tissues' codon usage bias, particularly, individual codon usage and codon pair usage. Information's of highly expressed genes in the tissue were collected and computed from available -omics data sources (healthy human). As a result, the frequency of each codon and codon pair distribution were determined leading to the establishment of the codon bias. Then the target protein sequence and host's reference codon bias were used as primary inputs into the codon optimization along with the appropriate design criteria's and constraints to guide the synthetic gene design, thereby producing the optimized sequence. Our results suggest that, the usage of codon bias has a significant impact on the expression. References Nandagopal N, Elowitz MB, *Science*. 333:1244-1248 (2011)

Keywords : Codon optimization, gene therapy

P-6

Wet-Corrosion-Processed TiO_2 Nanowires for Peptide Sequencing Using LDI-TOF MS

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TiO_2 nanowires synthesized from a wet-corrosion process were presented for peptide sequencing by photocatalytic reaction with UV radiation. To photocatalytic decompose peptides, the peptide sample was dropped on a target plate containing synthesized TiO_2 nanowire zones and UV-irradiated. Subsequently, the target plate was analyzed by laser desorption/ionization time-of-flight (LDI-TOF) mass spectrometry using the synthesized TiO_2 nanowires as a solid matrix. The feasibility of peptide sequencing based on the photocatalytic reaction of the synthesized TiO_2 nanowires was demonstrated using six types of peptides GHP9 (G1-H-P-Q-G2-K1-K2-K3-K4, 1006.59 Da), BPA-1 (K1-S1-L-E-N-S2-Y-G1-G2-G3-K2-K3-K4, 1394.74 Da), PreS1(F1-G-A-N1-S-N2-N3-P1-D1-W-D2-F2-N4-P2-N5, 1707.68 Da), HPQ peptide-1 (G-Y-H-P-Q-R-K, 884.45 Da), HPQ peptide-2 (K-R-H-P-Q-Y-G, 884.45 Da), and HPQ peptide-3 (R-Y-H-P-Q-G-K, 884.45 Da). The identification of three different peptides with the same molecular weight was also demonstrated by using the synthesized TiO_2 nanowires for their photocatalytic decomposition as well as for LDI-TOF mass spectrometry as a solid-matrix.

Keywords : TiO_2 nanowires, peptide sequencing, LDI-TOF

P-7

Crystal Structure of the AhpD-Like Protein DR1765 from *Deinococcus radiodurans* R1

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Deinococcus radiodurans is well known for its extreme resistance to ionizing radiation (IR). Since reactive oxygen species generated by IR can damage various cellular components, *D. radiodurans* has developed effective antioxidant systems to cope with this oxidative stress. *dr1765* from *D. radiodurans* is predicted to encode an alkyl hydroperoxidase-like protein (AhpD family), which is implicated in the reduction of hydrogen peroxide (H_2O_2) and organic hydroperoxides. In this study, we constructed a *dr1765* mutant strain (Δ*dr1765*) and examined the survival rate after H_2O_2 treatment. Δ*dr1765* showed a significant decrease in the H2O2 resistance compared to the wild-type strain. We also determined the crystal structure of DR1765 at 2.27 Å resolution. DR1765 adopted an all alpha helix protein fold representative of the AhpD-like superfamily. Structural comparisons of DR1765 with its structural homologues revealed that DR1765 possesses the Glu74-Cys86-Tyr88-Cys89-His93 signature motif, which is conserved in the proton relay system of AhpD. Complementation of Δ*dr1765* with *dr1765* encoding C86A or C89A mutation failed to restore the survival rate to wild-type level. Taken together, these results suggest that DR1765 might function as an AhpD to protect cells from oxidative stress.

Keywords : *Deinococcus radiodurans*, Alkyl hydroperoxide reductase, antioxidant system

P-8

Engineering of CYP102A1 for Regioselective Hydroxylation of Phenolic Compounds

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Phenolic compounds are a main class of secondary metabolites in plants and are divided into phenolic acids and polyphenols. These compounds from plants were reported to have pharmacological efficacy such as anti-inflammatory, anti-oxidant, and anti-carcinogenic. Cytochromes P450 (CYPs) have great potential as biocatalysts in pharmaceutical and fine chemical industries, because they catalyze diverse oxidative reactions using a wide range of substrates. Here, to develop highly active enzymes toward phenolic compounds we constructed engineered enzymes by random mutagenesis at heme domain of CYP102A1 and chimeric enzyme that was made by swapping between heme and reductase domains of CYP102A1 natural variants. To investigate whether these engineered enzymes displayed enhanced activity toward phenolic compounds, we screened activities of the engineered CYP102A1 enzymes. Among them, M371 and M16 showed the highest activity toward compound A and B, respectively. However, CYP102A1 WT could not metabolize two compounds, apparently. In addition, association constants and kinetic parameters of the engineered enzymes toward compound A and B were determined. These results suggest that engineered CYP102A1 would be useful for bioconversion of pharmaceutically important phenolic compounds

Keywords : Cytochrome P450, phenolic compounds, protein engineering

P-9

Bacterial Production of Recombinant Human Enterokinase Catalytic Subunit through Solubilizing Fusion Partners

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Hydrolase is a class of enzymes that catalyze the hydrolysis of various biochemical bonds to regulate biological processes precisely. Of them, protease is known to recognize and cleave a specific peptide sequence to control the activity of proteins and maintain homeostasis. Enterokinase, also called enteropeptidase, is mainly secreted from intestinal visceral mucosa for converting inactive pancreatic trypsinogen into active trypsin directly. Trypsin is an important activator of the digestive enzyme activation cascade. Moreover, the remarkable specificity of enterokinase for DDDDK sequence can provide a versatile molecular tool for modifying recombinant proteins in biological industries. Despite wide applications of enterokinase, however, bacterial overexpression of the enzyme as a soluble and active form remains a challenge. Herein, we overcome the limitations by using two solubilizing proteins, thioredoxin and phosphoglycerate kinase. We successfully express human enterokinase as a soluble form using bacterial system, and identify that the expressed protein has a specific proteolytic activity for substrate sequence.

Keywords : Eenterokinase, partner protein, recombinant proteins

P-10

Screening of a Single Chain Fv (scFv) with a Specific Binding Activity Towards Biotin from scFv Library on *Escherichia coli* Outer Membrane

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Biotin is a vital small molecule that is best known for binding with avidin. Despite its broad versatility in its role as a molecular linker, relatively bulky avidin had to be replaced with small molecule in nano-scale detection. This work presented the screening of a new single chain Fv (scFv) with a specific binding affinity to biotin. For the screening of scFv against biotin, a scFv library with three complementarity-determining regions (CDR) of heavy chain (VH) was expressed on the outer membrane of *Escherichia coli* using surface display technology. The scFv library was designed to have a CDR 3 region with randomized amino acid sequence (11-residues) and the diversity of library was estimated to have more than 106 clones. The targeted scFv screening was carried out using flow cytometry and the affinity constant (Kd) of scFv to biotin was estimated to be 667 μM. The specific binding of scFv to biotin was demonstrated using competition assays between biotin and biotin-like peptide HPQ. Finally, the CDR 3 region of scFv was chemically synthesized and demonstrated the biotng-binding activity using SPR biosensor.

Keywords : Single chain antibody, *E.coli*, biotin

P-11

Highly Potent Chimeric Endolysin ClyC with Improved Antibacterial Activity against *Staphylococcus aureus*

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The prevalence of multi-drug resistant *Staphylococcus aureus* with strong biofilm-forming capacity poses a serious threat to public health. Endolysin derived from bacteriophage is one of the promising solutions for antibiotic resistance problems. However, natural staphylococcal endolysins have several shortcomings such as low solubility, weak bactericidal activity, and high sequence homology among domains. To overcome these limitations, we constructed a hybrid endolysin library by swapping an enzymatically active domain (EAD) and a cell wall binding domain (CBD) of 12 natural staphylococcal endolysins. We developed a novel chimeric endolysin, ClyC, which showed enhanced lytic activity against *S. aureus* compared to its parental forms. ClyC also exhibited a strong antibacterial activity against *S. aureus* in various biomatrices such as milk, serum, and blood. Moreover, the treatment of chimeric endolysin effectively eradicated biofilms of multi-drug resistant bacteria including methicillin-resistant *S. aureus*(MRSA) and *S. epidermidis*(MRSE). Taken together, our data suggest that the chimeric endolysin ClyC can be regarded as a potential antibacterial agent against antibiotic-resistant *S. aureus*.

Keywords : *Staphylococcus aureus*, chimeric endolysin, antibiotic resistance

P-12

Development of L-Methionine Sensors by FRET-Based Protein Engineering

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Amino acids are essential nutrients not only used as protein building blocks but also involved in biochemical processes and human diseases. Quantitative analysis of amino acids in complex biological sample is an important analytical process for which many methods have been developed. In this study, a protein sensor was designed as a L-Met sensor based on fluorescence resonance energy transfer (FRET) in which a fluorescence unnatural amino acid (CouA) and YFP were used as a FRET pair. A natural Met-binding protein (MetQ) was chosen as a sensor protein, and CouA and YFP were incorporated into the protein by genetic code expansion technology and a genetic fusion. For incorporation of CouA into MetQ, four sites were screened, and R189 was selected for the best site for L-Met sensing. The sensor protein (YFP-MetQ-R189CouA) showed a large FRET signal change (2.7-fold increase) upon L-Met binding. While YFP-MetQ-R189CouA exhibited excellent FRET signal and had strong binding affinity to L-Met, the protein also recognized D-Met and L-Gln with significant FRET signal change at 50 μM concentration. To improve amino acid specificity of the sensor protein, four residues in the active site of MetQ were mutated to afford 11 mutants, of which the mutant (YFP-MetQ-R189CouA-H88F) with the H88F mutation showed no FRET signal change with D-Met and L-Gln at 50 μM concentration, retaining the maximum FRET signal change with L-Met. The optimized sensor protein was applied to determine L-Met concentration in FBS and optical purity in D- and L-Met mixture. The sensor protein and design strategy would be useful for amino acid analysis and sensor design for important biomolecules.

Keywords : L-methionine, biosensors, FRET

P-13

Construction of Engineered Organisms with an Active Transport System for Unnatural Amino Acids

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Engineered organisms with an expanded genetic code have attracted much attention in chemical and synthetic biology research. In this work, engineered bacterial organisms with enhanced unnatural amino acid (UAA) uptake abilities were developed by evolving a periplasmic binding protein (PBP) for recognition of UAAs. A FRET-based assay was used to identify a mutant PBP (LBP-AEL) with excellent binding affinity ($K_d \approx 500$ nM) to multiple UAAs from 37 mutants. Bacterial cells expressing LBP-AEL showed up to 5-fold enhanced uptake of UAAs, which was determined by genetic incorporation of UAAs into a green fluorescent protein and measuring UAA concentration in cell lysates. To the best of our knowledge, this work is the first report of engineering cellular uptake of UAAs and could provide an impetus for designing advanced unnatural organisms with an expanded genetic code, which function with the efficiency comparable to that of natural organisms. The system would be useful to increase mutant protein yield from lower concentrations of UAAs for industrial and large-scale applications. In addition, the techniques used in this report such as the sensor design and the measurement of UAA concentration in cell lysates could be useful for other biochemical applications.

Keywords : Genetic code expansion, periplasmic binding protein, unnatural amino acids

P-14

Characterization of Novel Salt-Tolerant Esterase Isolated from the Marine Bacterium *Alteromonas* sp. 39-G1

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An esterase gene, estA1, was cloned from *Alteromonas* sp. 39-G1 isolated from the Beaufort Sea. The gene is composed of 1,140 nucleotides and codes for a 41,190 Da protein containing 379 amino acids. As a result of a BLAST search, the protein sequence of esterase EstA1 was found to be identical to *Alteromonas* sp. esterase (GenBank: PHS53692). As far as we know, no research on this enzyme has yet been conducted. Phylogenetic analysis showed that esterase EstA1 was a member of the bacterial lipolytic enzyme family IV (hormone sensitive lipases). Two deletion mutants (Δ20 and Δ54) of the esterase EstA1 were produced in *Escherichia coli* BL21 (DE3) cells with part of the N-terminal of the protein removed and His-tag attached to the C-terminal. These enzymes exhibited the highest activity toward p-nitrophenyl (pNP) acetate (C2) and had little or no activity towards pNP-esters with acyl chains longer than C6. Their optimum temperature and pH of the catalytic activity were 45℃ and pH 8.0, respectively. As the NaCl concentration increased, their enzyme activities continued to increase and the highest enzyme activities were measured in 5 M NaCl. These enzymes were found to be stable for up to 8 h in the concentration of 3-5 M NaCl. Moreover, they have been found to be stable for various metal ions, detergents and organic solvents. These salt-tolerant and chemical-resistant properties suggest that the enzyme esterase EstA1 is both academically and industrially useful.

P-15

Synthesis of Engineered Quenchbodies for Detection of Periodontal Disease Biomarker MMP9

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Periodontal disease has affected more than 700 million of patients annually, and the number of patients has increased. As such, early detection of periodontitis risk prior to signs and symptoms as well as periodontal diagnostics are important. Recently, it has been revealed that salivary matrix metalloproteinase 9 (MMP9), which is a cancer related enzyme that plays important roles in the cleavage and degradation of extracellular proteins and in tumor invasion, metastasis, and angiogenesis, is one of the periodontitis biomarkers that highly presents in periodontitis patients. A Quenchbody is an antibody with site-specifically conjugated fluorophore(s) that fluoresces upon antigen-dependent removal of the fluorophore quenching. Quenchbody-based assay offers the advantages of only taking minutes to complete by eliminating washing steps and complicated working processes, which is highly time-sensitive. In this study, we have generated antibody fragments against MMP9 and converted them to Quenchbodies, for using them as periodontal disease diagnostic sensors. Especially, we have designed the antibody fragments using a protein modeling system for obtaining engineered antibodies with high antigen-binding affinity.

Keywords : Matrix metalloproteinase 9, Quenchbody, protein modeling system

P-16

Systemic Evaluation of Tumor Localization of Protein Binders with Different Affinities

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Sufficiently strong binding to tumor antigen is acknowledged as necessary for targeted cancer therapy. However, unlike *in vitro* binding, high affinity does not lead to maximal tumor accumulation in general. In this study, we evaluated the impact of binding affinity on tumor localization of EGFR-specific Repebody, a small-sized protein binder, using near-infrared fluorescence imaging. Four kinds of Repebodies with different affinities were chemically labeled with Cy5.5 dye, showing the equivalent dye-to-Repebody ratio as 2:1. We observed the resulting Repebody-Cy5.5 conjugates can specifically bind to EGFR-overexpressing cancer cells in an affinity-dependent manner. On the other hand, *in vivo* tumor localization of Repebodies did not show the proportional relationship with binding affinity. Although all Repebodies showed strong tumor accumulation, the Repebody-AC1 showing low nanomolar affinity accumulated more rapidly and remained significantly higher in tumor tissue till day 4 than others. Also, we confirmed that the highest affinity Repebody-EgH9 (K_D of 50.5 pM) was mainly localized at the perivascular region due to the binding-site-barrier effect. We concluded that an adequate target binding affinity is associated with the efficiency of *in vivo* tumor localization. These results imply that binding affinity and kinetic parameters should be considered as important factors in development of protein therapeutics and diagnostics.

Keywords : Binding affinity, repebody

P-17

Exploring Mo-Cu Dependent Carbon Monoxide Dehydrogenase from *Hydrogenophaga pseudoflava* for Application in Dissolved Carbon Monoxide Concentration Monitoring

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CO dehydrogenase (EC.1.2.5.3), a key enzyme in CO metabolism catalyzes the oxidation of CO with H_2O to yield CO_2, two protons and two electrons. CODHs found in anaerobic and aerobic bacteria are structurally different and contain different metallocofactors at their active sites albeit catalyzing the same reaction. Notably, the aerobic CODH from *Hydrogenophaga pseudoflava*, a molybdenum (Mo) and copper (Cu) dependent enzyme retains their catalytic activity when exposed to air, unlike the anaerobic CODH, nickel (Ni) and iron (Fe) dependent CODHs which are extremely sensitive to air, thus, hinder the practical applications. However, the heterologous expression of Mo-Cu CODH for high-level production has not been attempted in the past as *Escherichia coli* was initially thought to be unable to synthesize molybdopterin cytosine dinucleotide (MCD)-type Mo cofactor which is exclusively found in Mo-Cu CODH and required for CO oxidation activation. After the discovery of enzymes containing MCD in *E. coli*, it is hypothesized that it might be a suitable host for expressing recombinant Mo-Cu CODH. In this study, the expression of Mo-Cu CODH in *E. coli* was established, which resulted in active protein. Further, the practicality of CODH in dissolved CO sensing system was analyzed and characterized.

Keywords : Carbon monoxide dehydrogenase, *Hydrogenophaga pseudoflava*, dissolved CO concentration monitoring

P-18

Binding Specificity of FAD-Dependent Glucose Dehydrogenase fused to Gold Binding Peptides toward Au in Multi-Material Patterned Substrate

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The development of multi-enzyme based bioelectrochemical system is one of the most rapidly developing areas for electrochemical applications such as biosensor, bioelectrochemical system and biofuel cell. For efficient operation of device, the immobilization of enzyme to the electrode must be secured to have desired electrochemical reaction and efficiency in applications. Although some currently obtained technologies such as physical entrapment, chemical linking, and affinity binding have been used for multi-enzymes and their co-immobilization, most these techniques still remain technical drawbacks since enzymatic positioning and orientation on electrode immobilization are not highly controllable indicating that the performance of enzyme-electrode reaction is not fully satisfactory. In this study, we propose the multi-enzymes and co-immobilization strategy *via* genetic fusion of gold binding peptide (GBP) on the enzyme (i.e., enzyme tested immobilization) in order to investigate binding affinity and specificity of enzymes toward multi-material patterned electrode. It was designed multi-material electrode which consists with Au, Ti, Pt and Ag on Si wafer. The GBP candidates have been searched through literature survey. The designed electrode which contains five materials was used to see the GBP candidate's binding selectivity toward Au and binding exclusiveness toward other materials. Additionally, the selective binding of synthetic enzymes fused with GBP candidates, toward target material was also tested on multi-material patterned electrode. This study provides an initial step for orientation and position controllable co-immobilization of multi-enzymes on the electrode.

Keywords : Multi-enzyme system, enzyme co-immobilization, Gold binding peptide

P-19

Oxidative Folding of Antibody Fragments Displayed on *Escherichia coli* for Nef Antigen Detection

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Autodisplay technology enables the display of heterologous proteins and peptides on the surface of *Escherichia coli* It's competitive in that it displays ~150,000 molecules per *E. coli*, and multimerisation on the cell surface is possible. Autodisplayed proteins have been successfully applied to numerous biosensors. However, still, proteins which have disulfide bonds cannot be autodisplayed because of its structure formation prior to autotransport, so they can be only autodisplayed as a denatured form. Due to its complicate structure including disulfide bonds, antibodies have been regarded as inappropriate proteins to be autodisplayed. In this study, we tried to get autodisplayed single-chain-fragment-variant of antibodies(scFv) by using oxidant, especially glutathione and its oxidized form(GSSG). First we autodisplayed antibody fragments of anti-Nef as a denatured form, and make sure that they were refolded by treatment of GSH:GSSG with SDS-PAGE analysis. We figured out Nef peptide affinity of autodisplayed scFv is around 4 times enhanced after refolded. Finally we applied autodisplayed scFv to ELISA and SPR biosensors for Nef detection which have a dynamic range of 0.5~500 μM.

Keywords : Negative regulatory factor, surface display, refolding

P-20

Identification of Organic Hydroperoxide Specific Detoxification Enzyme in Divergent DinB Superfamily from Radiation Resistance Bacterium *Deinococcus radiodurans* R1

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Deinococcus radiodurans is a radiation resistant bacterium which is well known for extremely resistance to many oxidative-damaging agents such as hydrogen peroxide, tert-butyl hydroperoxide (tBOOH), and Cumene hydroperoxide(CHP). It is well known that *Deinococcus* species have efficient enzymatic antioxidant system to cope with oxidative stress. Unlike other bacteria, *Deinococcus* species have specific expansions of divergent DinB family homologs although their biological functions remain ambiguous. To examine their function, we constructed all of 15 DinB knock-out mutants and investigated their individual phenotypic trait in response to various stresses. Among the mutants, one knock-out mutant specifically sensitive to CHP. Moreover, its growth was also severely retarded by CHP treatment. To better understand their characteristic, we expressed CHP specific DinB protein (CsDinB) in *Escherichia coli* Purified 18 kDa of protein exhibited Bacillithiol(BSH)-conjugation activity like thiol-s-tranfserase. Moreover, GC-MS analysis showed that cumyl alcohol (CuOOH) was produced from the CHP during the reaction of CsDinB enzyme with BSH. This results indicated that *D. radiodurans* has unique ROS scavenging system using thiol-transferase and CsDinB specifically catalyzed the CHP scavenging in *D. radiodurans*.

Keywords : *Deinococcus*, DinB, oxidative stress

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Characterization of Tyr-CS: A Tyrosinase with High Activity and Stability in Organic Solvents

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Tyrosinase is an enzyme containing copper at the active site, and catalyzes the ortho-hydroxylation of monophenols(cresolase activity) and the oxidation of diphenols(catecholase activty) without any additional cofactor. However, conventional tyrosinases are mostly active only in aqueous solution, while many compounds including phenol and catechol groups have high solubility in organic solvents. Here, we present a functional tyrosinase, which showed a great initial activity and stability in organic solvents. The enzyme had high activity in pH 6-9 and 30-40℃. Even in the organic media, the enzymatic properties were similar with other tyrosinases including a commercially available mushroom tyrosinase. Overall, the high catalytic performance in the presence of organic solvents suggests that the enzyme might be efficiently used for the synthesis of industrially important catechol derivatives.

Keywords : Tyrosinase, organic solvent, high activity and stability

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Direct Electron Transfer Based Glucose Biosensor Using Flavin Adenine Dinucleotide (FAD)-Dependent Glucose Dehydrogenase Fused to a Gold Binding Peptide

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Here, direct electron transfer (DET) capable enzyme-electrode system using flavin adenine dinucleotide (FAD)-dependent glucose dehydrogenase (GDH) fused with gold binding peptide (GBP) at the catalytic subunit is described in term of glucose biosensing application to analyze electrochemical and mechanical properties of GBP fused FAD-GDH immobilized on gold electrode (GDH-GBP/Au). In the electrochemical test using cyclic voltammetry, the GDH-GBP/Au displayed significantly high and stable oxidative current from the glucose oxidation and the generated current was retained over 90% after successive potential scans. The dynamic range of GDH-GBP/Au based biosensor is 3-30 mM of glucose concentration at 30℃ and it exhibits high selectivity toward glucose in the co-presence of other interfering molecules. Additionally, the biosensor was used to measure glucose concentration in the human whole blood samples and the relative errors compared with results by conventional measurement method was less than 10%. Therefore, we suggest that GDH-GBP/Au based biosensor would be practical in various biosensing applications.

Keywords : Direct electron transfer, gold binding peptide, biosensor

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Molecular Study of Olfactory Receptor

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The olfactory nervous system recognizes and distinguishes many different chemicals in the general living environment. Insects have evolved a group of odorant-gated ion channels composed of highly-developed olfactory receptors capable of distinguishing and distinguishing between various chemicals with symbolic or evasive specificities. Recently, aphid genomes related to olfaction, including olfactory receptors and proteins, have been identified and olfactory receptors have been reported that are differentially differentiated from Drosophila. The genome of the olfactory receptor has a very conservative sequence and a systematic signaling system. A representative receptor, odorant-gated ion channels comprised of a highly conserved co-receptor (Orco) has a homotetramer channel structure with four subunits arranged symmetrically around the central hole. It has a very similar structure to the 7-transmembrane receptor present in the human body and has a very similar structural form and gating mechanism to receptors of neurotransmitters. In this study, whole cell voltage clamp recording was performed with cell expression system of OR85b gene, which is a subtype of olfactory neuro-receptor isolated from Drosophila. After the successful expression of this receptor, microbial culture extract of microorganism, a harmful insect inducer, was used to investigate whether olfactory receptor activity was regulated. The activity of the receptor was confirmed in the recording media diluted 10,000 times with the microbial culture extract. Therefore, it is possible to identify attractant or repellent substance using the olfactory receptor activity regulating system of insects. Through this study, MZ01 shows the attracting phenomenon by activating insect receptor OR85b, The results of the scientific analysis of the performance of the extracts are presented.

Keywords : Olfactory nervous system