I-1

Galactose Encapsulated Multifunctional Magnetic Nanoparticle for Liver Cancer Cell Internalization

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Magnetic nanoparticles (MNPs) have been developed as both contrast reagent (in MRI) and drug carrier. To develop the multifunctionalized MNPs, we incorporated fluorescent and targeting molecules on the particle surface. By controlling the galactose/Cy3 ratio, we achieved the synthesis of fluorescent MNPs with different galactose density on the surface. We took the advantage of large surface area to volume ratio of metal nanoparticle and demonstrated that metal nanoparticle can serve as a good multivalent carbohydrate ligand carrier.

To study the specific targeting of galactose containing MNPs, HepG2 cell, the liver cancer cell line, was chosen as target cell due to the expression of galactose binding protein, the asialoglycoprotein receptor (ASGP-R), on its cell surface. Hela cell without ASGP-R was chosen as the negative control. The ligand proteins (ASGP-R) are efficiently degraded from the circulation by receptor-mediated endocytosis, then digested in lysosomes while the ASGP-R is recycled to the cell surface.

According to the ASGP-R structure, we designed 3 kinds of MNPs: T-Gal-l-Cy3@MNP, T-Gal-s-Cy3@MNP, and Gal-Cy3@MNP. The results of HepG2 cell uptake showed that T-Gal-s-Cy3@MNP is a better ligand to interact with ASGP-R. Our results indicate that multivalent effect is not only attracting ligands closer to receptor but also aligning ligands to receptor liked structure. Our galactose encapsulated MNPs can specific target the HepG2 cell through the receptor-mediated endocytosis process. In addition, there is no any cyto-toxicity for all the surface modified MNPs. We create a multifunctional nanoprobe platform including fluorescent, magnetic property and target ligand (galactose). Our MNPs can both be a potential drug carrier and molecular image in bio-application.

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I-2

Shuttling Device for Field Cycling Experiment on NMR Relaxation Study in Biological Systems

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Relaxation mechanisms include dipolar-dipolar (DD) interaction and chemical shift anisotropy (CSA) effect. Conventional molecular motion analysis only applies relaxation measurement of longitudinal relaxation rate (R_1), transverse relaxation rate (R_2) and Nuclear Overhauser effect (NOE) at fixed field. However, these approaches couldn't distinguish the dynamics contribution from DD interaction and CSA effect. Fortunately, measuring relaxation at various fields could overcome this difficulty. Molecular motion contributed by CSA could be obtained by measuring R_1 variation at different magnetic fields. Therefore, field cycling experiment should provide valuable information for studying molecular motion in biological systems.

We have set up a field cycling apparatus at a 600MHz NMR spectrometer. By shuttling samples vertically to the desired heights inside the magnet for relaxation and back to the central position for detection, one can measure relaxation at lower fields with high resolution. The round trip shuttling time from the central position to the top of magnet is about 0.16s, which is suitable for measuring relaxation rate in the range up to $10s^{-1}$.

In this poster we will present the details of field cycling apparatus and its applications on molecular motion studies. In the preliminary studies, we have observed the molecular motions influenced by CSA on an ocatamer DNA and single amino acids by measuring ^{31}P and ^{13}C field dependent R_1, respectively.

I-3

Synergy between CPS Virulent Factors and Antibiotics Resistance via Gene Networking in *Klebsiella*

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Bacterial pathogenicity is often contributed by multiple virulent factors, but little is known about the relationship between them. While investigating the molecular basis of pathogenicity of a primary pyogenic liver abscess (PLA) *Klebsiella pneumoniae* strain NTUH-K2044, we identified synergy between capsular polysaccharide (CPS) associated virulent factors and multidrug resistance. First, previously unknown fucose acetylation was identified and shown to be important for receptor binding, cytokine expression, and virulence to mice. Then systematic analyses of *cps* gene deletion mutants uncovered unprecedented silencing of transcriptions of up to 52 adjacent and upstream genes, including 6 multidrug efflux genes, when fucose-related genes *wcaI*, *wcaG*, or *atf* was deleted. Complementation plasmids did not restore the K1 serotype or the silenced genes, and polar effects were ruled out experimentally. The mutants showed enhanced sensitivity to antibiotics. The results demonstrate multiple causes for pathogenicity linked by unprecedented gene networking.

I-4

Direct Immobilization of Unmodified Carbohydrate Microarray through Boronate Formation

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Carbohydrate microarray is expanding rapidly for the frontier of glycomics that is revolutionizing the study of carbohydrate-protein interactions and the elucidation of their specificities in biomedical sciences. Most of the methods employed to immobilize carbohydrate microarrays rely on the modified carbohydrates on the properly derivative surfaces [1]. This strategy heavily relies on labor-intensive and time-consuming synthetic routes for modified carbohydrates. However, modification-free strategies such as photoactive random immobilization, free reducing end based immobilization through hydrazine- or aminooxy- coated surfaces, and physical adsorption were restricted respectively by synthesis of photoactive group as well as requirement of photo-reactor, availability of free reducing end, and size dependent retention ability.

We have developed a simple and direct immobilization technique that involves a one-step, covalent attachment of diverse unmodified carbohydrates to the boronic acid glass surface which has been employed for achieving glyco-targeting. The results demonstrate that this immobilization method offer better retention of carbohydrates through boronate formation over non-covalent attachment; and is applicable to the rapid evaluation of carbohydrate-mediated biomolecular interactions and the determination of quantitative binding affinities between carbohydrates and proteins.

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I-5

Total Synthesis of *Mycobacterium Tuberculosis* Phosphatidylinositol Dimannoside

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Tuberculosis (TB), the most devastating infectious diseases in the world, has recently been declared as a “global health emergency” by WHO. *M. tuberculosis,* a causative pathogen of TB is covered by thick glycocalyx that is critical for the integrity of bacteria. Of the major components of mycobacterial cell envelope, phosphatidylinositol mannosides and lipoarabinomannan’s (LAMs) [1] have been shown to exert profound physiological effects and emerged as the most potent nonpeptidic molecules to modulate the host immune response. LAMs contain a phosphatidyl *myo*-inositol (PI) anchor that is extended by mannosyltransferases to phosphatidylinositol mono-, di-, tri-, and tetramannosides. PIM_4, a key biosynthetic intermediate can be further mannosylated to obtain higher PIMs and lipomannan (LM), which after subsequent arabinosylation generates LAM. PIMs and their analogues will be important molecules to establish a structure-activity relationship of these lipoglycans and their effect on the immunopathogenesis of tuberculosis. Herein, we will present a new, straightforward and convenient synthesis of PIM_2 [2].

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I-6

Use of Foamyviral Gene Delivery System for Reporter Genes of Optical Imaging *in Vitro*

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Foamyvirus is a member of the retroviridae and exists in cats and chimpanzees [1]. The virus is suggested of having neither pathogenicity nor transmission in humans and documented as a useful and safe vehicle for gene delivery [2-3]. In the molecular imaging category, foamyviral-based methods were still lack of investigation. In this study, we applied foamyviral-mediated transduction of reporter genes as a novel approach for non-invasive molecular imaging. Foamyviral vectors from pΔΦ were constructed with reporter genes, luciferase 2 and infrared fluorescent protein, respectively. These constructs were named pΔΦ-Luc2 and pΔΦ-IFP-IRES-mCherry. Our preliminary results showed the double reporter genes expressed in packaging cells, but the infection efficiency revealed a low yielding of fluorescent cells comparing to literature documented and lentiviral infection. To increase the titers, we collected virus lysates and were further subjected to ultracentrifugation. Besides, serum starvation were also treated 24 hours prior to the infection, which enhanced the gene transduction efficiency as well.

For future study, we tend to apply the established cells or virus lysates into living animals for tracking and localizing the distribution in vivo. Thus, we were looking for improving the virus titer and building up constructs with PET imaging probe to approach our goal. Thus, molecular imaging can be achieved by a novel and safer gene delivery vehicle, foamyvirus.

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I-7

In Vitro Cellular Uptake Mechanism of Biocompatible Fluorescent Nanodiamonds

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Recent increase in the use of nanoparticles for biomedical applications shows that nanotechnology plays a cutting-edge role in various applications. Among various nanoparticles, fluorescent nanodiamonds (FNDs) are insulator-based nanoparticles whose fluorescence originates from the point defect centers in the crystal lattice (1, 2). These FNDs hold several outstanding properties such as facile surface functionalization, high photo-stability, biocompatibility, which procures the name "Diamond: the biomaterial of the 21st century" (3-7).

Deeper insights into the cellular uptake mechanism of these nanoparticles should allow us to manipulate the efficacy of nanoparticle internalization and may even enable targeting the cargo to specific sub-cellular compartments. Therefore, it is crucial to understand the mechanism of cellular uptake and intracellular trafficking. As a preliminary work, we have elucidated the uptake mechanism of FNDs (size ~ 100 nm) using a battery of pharmacological inhibitors. The FND uptake is mainly through an energy dependent, clathrin mediated endocytosis.

In addition, studies on the nanodiamond-labeled cells showed that FNDs are non-toxic as revealed by MTS, cell proliferation and clonogenic assays. We have also showed that 3T3-L1 preadipocytes and 489.2 late osteo-progenitors can be efficiently labeled with FNDs without detectable cytotoxicity and detrimental effect on their *in vitro* adipogenic and osteogenic differentiation potentials respectively. These observations extend the feasibility of using FNDs in stem cell research and other biological applications such as *in vivo* quiescent stem cell tracking, bio-imaging and as a vehicle for targeted drug and gene delivery.

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I-8

In Vivo Imaging and Toxicity Studies of Fluorescent Nanodiamonds in *C. elegans*

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A current trend in fluorescent probe technology is to expand the role of fluorophores that emit light in the far red and near infrared region. The negatively charged nitrogen-vacancy defect center, $(N\text{-}V)^-$, in type Ib diamond is one of such fluorophores and has drawn much attention in recent years. The center exhibits some distinct fluorescence features such as extended red emission at ~700 nm and is highly photostable, i.e. no photoblinking and photobleaching. These properties, along with the diverse surface functionalizability and non-toxic nature of the nanomaterial, have made fluorescent nanodiamond (FND) a promising candidate among other conventional markers, i.e. organic dyes, fluorescent proteins, and quantum dots, for biological applications.

We explore in this work the possibilities of using FNDs for long-term bioimaging *in vivo*. We choose *Caenorhabditis-elegans (C. elegans)* as our *in vivo* system because of its size amenable to optical microscopy, short life cycle, rapid growth, apparent simplicity and well defined behaviors. FNDs are incorporated into wild-type *C. elegans* by feeding them directly with FND solution. In our primary observations with bright-field and epifluorescence microscopies, the FND particles remain with in the lumen of the *C*. *elegans* and could image the whole digestive system of the organism for several days. With proper surface fictionalization with BSA protein and dextran, we find FNDs absorbed into the intestinal cells from lumen by endocytosis.

We investigated the toxicity of FND particles in *C. elegans* by examining longevity, brood size, and stress response. We found that life span and brood size were not altered in FND-treated animals.In addition, we assayed oxidative stress response by examining nuclear translocation of DAF-16 and expression of GCS-1; no stress response was detected after treating FND particles. Our results show FND is nontoxic and does not cause any oxidative stress to the organism

The second choice of incorporating FND particles into the organism is by microinjection into the syncytial gonads of gravid hermaphrodites according to the standard protocol. Later on due to the cytoplasmic streaming in *C* elegan's gonad, FNDs would get embedded in the embryos. Eventually, FNDs are dispersed within the newly hatched worms. Thus we could image the developmental stages of the whole organism effectively.

I-9

SIDSNOMS, A Proteome-wide Approach towards Comprehensive Site-specific Identification of S-Nitrosylome

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S-nitrosylation, specifically targeting on cysteine residues by nitric oxide (NO) species, is a post-translational modification that mediates important NO-based signaling in biological systems. Due to its labile nature, *S*-nitrosylation is challenging to direct detection by mass spectrometry (MS). Here, we develop a powerful SIDSNOMS, site-specific identification of S-nitrosylation (SNO) by mass spectrometry approach, towards comprehensive site-specific identification of *S*-nitrosylome. By in-depth comprehensive identifying S-nitrosylated proteins, combining subcellular fractionation and SDS-PAGE fractionation, our preliminary results showed site-specific identification of 577 unique *S*-nitrosylated peptides (Mascot score ≥ 32 where $p < 0.05$) corresponding to 380 *S*-nitrosylated proteins in *in-vivo* SNAP/L-cysteine-treated mouse endothelial cells. Among them, some identified *S*-nitrosylated proteins and sites have been reported in previous literatures. Ontologic analysis using Ingenuity™ and Metacore™ revealed 91 proteins possess enzyme regulator activity, and 28 transporters, *et. al*. For instance, *S*-nitrosylation of Cys-98 of endothelial nitric oxide synthase (eNOS) regulates its conformational change and enzyme activity. Moreover, Cys-215 protects PTP1B against oxidation-induced permanent inactivation. Most interestingly, sarcoplasmic reticulum Ca^{2+} ATPase (SERCA), which regulates the uptake of cytosolic Ca^{2+} thereby relaxing vascular smooth muscle by nitric oxide in a cyclic GMP independent manner, was also identified by our method. Using the identified nitrosylated sites, we deduced 12 potential motifs of *S*-nitrosylation, including a previously reported K(x/K)xxxxC and DxxxC motif. Taken together, we demonstrated that the SIDSNOMS is an effective proteomic approach not only to comprehensively explore *S*-nitrosylated proteins, but also to unambiguously identify the *S*-nitrosylated sites, that allow better understanding the effect of the NO at the molecular level.

I-10

Nanoprobe-based Immobilized Metal Affinity Chromatography for Enhanced Enrichment of Multiply Phosphorylated Peptides

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Magnetic nanoparticle (MNP) has rapidly evolved as a sensitive affinity probe for phosphopeptide enrichment[1]; however, the specificity and performance on proteome scale still leave rooms for improvement. Taking advantages of easy magnetic separation and flexible surface modification on MNP[2], we developed a surface-blocked nanoprobe-based immobilized metal ion affinity chromatography (NB-IMAC) method to increase purification specificity and enhanced enrichment of multiply phosphorylated peptides. Titanium (IV) ion charged nitrilotriacetic acid-conjugated MNP (Ti^{4+}-NTA-PEG@MNP) showed unbiased extraction of both mono- and multiply phosphorylated peptides from dilute β-casein (2×10^{-10} M). By MNP surface blocking with low molecular weight polyethylene glycol and controlling pH and concentration of acetic acid in loading buffers, our NB-IMAC allowed rapid and specific one-step enrichment. Without chemical derivation or pre-fractionation, we identified 1283 phosphopeptides with nearly 80% purification specificity by using 400 μg of Raji B cells. Most interestingly, the high ligand amount on MNP may enhance enrichment of multiply phosphorylated peptides by facilitating multivalent interaction between neighboring phosphates and Ti^{4+}-NTA-PEG@MNP; multiply phosphorylated peptides comprised as high as 31% of identified phosphopeptides. Furthermore, NB-IMAC complements micro-scale IMAC, as only 32.0% of mono- and 28.8% of multiply phosphorylated peptide identifications overlapped. Notably, NB-IMAC enrichs two-fold of multiply phosphorylated peptides compared to micro-scale IMAC.

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I-11

A Fluorogenic Substrate for High-Throughput Screening Compounds that Promote the Production of the Aβ-Degrading Enzyme Neprilysin in Cells

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Neprilysin (NEP) has been singled out as the most promising candidate for use in the degradation of Aβ as a therapy for Alzheimer's disease. In this study, a quenched fluorogenic peptide substrate containing the first seven residues of the Aβ peptide plus a C-terminal Cys residue was synthesized to detect neprilysin activity. A fluorophore was attached to the C-terminal Cys and its fluorescence was quenched by a quencher linked to the N-terminus of the peptide. When this peptide substrate was degraded by an endopeptidase, fluorescence was produced and proved to be a sensitive detection system for endopeptidase activity. Our results showed that this assay system was extremely sensitive to NEP and insulin degrading enzyme, but insensitive, or much less sensitive, to other Aβ-degrading enzymes. Moreover, on the basis of our findings, a cell-based assay system was developed for the high-throughput screening of chemicals that are able to enhance NEP production in human SH-SY5Y cells.

I-12

Enzymatic Synthesis of UDP-galactose by Glucose-1-phosphate Thymidylyltransferase and the Application in LacNAc Synthesis

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Carbohydrates on the cell surface play crucial roles in cell-cell recognition, viral invasion and many biological processes. It is need to have enough amount of carbohydrates for the study and understand how carbohydrates modulate these biological processes. Although many chemical methods have been developed to synthesize complex carbohydrates, all of them are heavily relyed on labor-intensive and time-consuming synthetic routes which significantly slow down the progress of glycobiology. In contrast, enzymatic synthesis may circumvent the drawbacks in organic synthesis. For example, glycosyltransferases have been extensively used to synthesize oligosaccharides. However, the donors for glycosyltransferases, sugar nucleotides, are expensive. Although large-scale production of oligosaccharides *in vivo* has been reported,[1] the multiple enzymatic synthesis of complex carbohydrate remain to be solved. To simplify the enzymatic synthesis, the synthesis of UDP-galactose from galactose-1-phosphate by direct transformation with a single enzyme is an attractive approach. In this poster, we describe the synthesis of UDP-galactose by a wild-type bacterial thymidylyltransferase (RmlA) which is used for the synthesis of TDP-glucose in nature.[2]

Using IMPACT system, the RmlA was easily purified by a single chromatographic step and obtained with good production yield (64 mg/L). In general, the UDP-galactose is synthesized by galactose-1-phosphate uridyltransferase or UDP-glucose-4-epimerase. In our approach, the RmlA was used to yield UDP-galactose using galactose-1-phosphate and UTP as reagents. The recombinant enzyme (RmlA) requires divalent cation for its activity and the optimal conditions is 10 mM Mn^{2+} in 50 mM Tricine buffer (pH 7.5). In one hundred milligram-scale synthesis of UDP-galactose, the yield is 95%. Moreover, in combination with β-1,4-galactosyltransferase, LacNAc was obtained in one-pot synthesis.

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I-13

Design of a Suicide Peptide Inhibitor to Trap Aβ40 and Attenuate Its Cytotoxicity

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The residue immediately preceding each glycine in the 40 amino acid Aβ40 (β amyloid 1-40) peptide (S8, V24, I32, and V36) was individually replaced by D-form proline (^{D}Pro). The resulting ^{D}P-G sequence (the ^{D}Pro residue and the following Gly residue) was designed as a "structural clip" to force the formation of a bend in the peptide, as this sequence has been reported to be a strong promoter of β-hairpin formation. The mutated peptides (V24P, I32P, and V36P) no longer formed an amyloid fibril structure, although they still went through a coil-to-β structural conversion. At a low peptide concentration, a random coil structure was formed, while, at a high peptide concentration, a non-fibril β-structure was formed. The converted β-structure can be converted back to random coil structure by simple dilution. Interestingly, Thioflavin T and Congo red, the dyes usually employed in amyloid detection and quantification, were able to bind to this β-structure. We concluded that these Aβ40 mutants form a new amyloid-like aggregate. Interestingly, the mutant peptide V24P, when mixed with Aβ40, can attenuate the cytotoxicity of Aβ40. A series of V24P derivatives were further designed and synthesized. First, we removed the N-terminal hydrophilic part of V24P to produce a peptide named V24P(10-40) and removed the C-terminal hydrophobic part of V24P to produce another peptide named V24P(1-28). A similar amyloid-like β-aggregate was formed in 30 μM V24P(10-40). This peptide has an even better effect on attenuating Aβ40 toxicity than V24P. Another three shorter peptides were designed: V24P(13-36), V24P(16-33), V24P(19-30). They don't have significant effect on decreasing Aβ40 toxicity, suggesting the integrity of the hydrophobic segment on both sides of ^{D}Pro-Gly turn is important.

I-14

The Strcutural Analysis of BaMV Using Single Particle Method by TEM

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Filamentous plant viruses, which make up almost half of the plant virus genera, may be classified broadly into the rigid (rod-shaped) and the flexible viruses. Tobacco mosaic virus (TMV), a rod-shaped virus, was the first virus to be subjected to structural studies using x-ray diffraction back in 1936 [1]. Previous research showed that TMV can form highly oriented sols, in which the long axes of the rod-shaped particles are oriented to within 1° of each other [2]. The structure of tobacco mosaic virus (TMV) has been determined by fiber diffraction method at resolution of 2.9Å [3]. On the other hand, the flexible filamentous viruses, including potexviruses such as Potato virus X (PVX) could not form highly orientated sols to generate high-quality fiber diffraction patterns [4]. It is proven that Bamboo mosaic virus (BaMV), one member of potexviruses, can accommodate VP1 of foot-and-mouth disease virus (FMDV) in its coat protein and act as expression vector for the vaccine production [5]. This BaMV based vector system has great potential for large-scale, inexpensive production of vaccines and other therapeutically and biotechnologically useful peptides. To achieve these goals, the knowledge of the BaMV structure will be required for designing suitable insertions.

Previous research showed that the N terminal of BaMV-CP can be deleted up to 35 a.a. without affecting the replication of BaMV (unpublished data). Interestingly, more deletions on N terminal of BaMV-CP can transform its flexible filamentous shape to rigid (rod-shaped) filament, which is very similar to TMV (unpublished data). The structures of three BaMV-CP mutants with different N terminal deletions of 0, 15 a.a. and 35a.a., will be studied using single-particle method by TEM [6]. The differences between these three mutants may be the key to answer why the deletions at N terminal of BaMV-CP could affect the shape of BaMV.

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I-15

Prostate-Specific Antigen Detection Using Nanoprobe-Based Affinity Mass Spectrometry

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Prostate-specific antigen (PSA) as a widely used biomarker of prostate cancer is a glycoprotein with a molecular mass of 28430 Da. Under normal conditions, PSA present at the levels < 4 ng/mL and can be detected by immunoassay in serum. The concentration of PSA elevates in the presence of prostate cancer and in other prostate disorders. Taking advantage of efficient affinity extraction by surface-functionalized magnetic nanoparticles (MNPs) and accurate MALDI-TOF MS readout, we present a nanoprobe-based immunoassay for simultaneous enrichment and isolation of PSA from human serum. The nanopraticles were modified with antibody to preconcentrate targeted biomarker and could be applied for direct MALDI-TOF MS analysis without an additional elution step, thereby avoiding sample loose from uncompleted elution efficiency. Our result indicated that the approach can be used to rapidly screen low-level targeted biomarker within 1 hr in complex mixture. With the ongoing analysis of PSA in human serum, this nanoprobe-based immunoassay holds promise of rapid screening for early diagnosis of disease.

I-16

Critical Role of Arginine 518 of the Lon Protease α-Domain from *Brevibacillus Thermoruber* WR-249 in DNA-Binding Specificity

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The multi-functional, homo-oligomeric, ATP-dependent Lon protease is conserved in prokaryotes and eukaryotic organelles. Here we examined the DNA-binging activity of the Lon protease from *Brevibacillus thermoruber, Bacillus subtilis,* and *Escherichia coli*. Surface plasmon resonance and isothermal titration calorimetry showed that a double-stranded DNA fragment: 5'-CTGTTAGCGGGC-3' (ms1) binds to the α-domain of the Lon protease. Five mutants of the α-domain from *Br. thermoruber* carrying single mutations (R537A, R546A, R553A, K580A and R584A) were constructed and showed only 1.2–2.0-fold lower DNA binding affinity; one mutant, R518A, displayed 26-fold lower affinity, i.e., Arg 518 of the α-domain from *Br. thermoruber* plays a critical role in the DNA-binding specificity.

I-17

Development of a Nanoscale Electronic Bar Code Reader of Transcriptional Factor Binding Stes on DNA

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The study of individual transcriptional factors (TFs), which are proteins that assist in the process of transcription of genetic information from DNA, and in particular the identification of the transcription factor binding sites (TFBSs) is helpful for understanding the mechanism of transcriptional regulatory networks and gene regulation. Abundant genomic data exists thanks to over two decades of experimentation in this field, however a better understanding of the actual TFBSs would require an ideal technology capable to probe un-sheared DNA, thus allowing whole genome coverage and providing direct readings without sophisticated biochemical manipulations, keeping in mind the need of an overall improvement of the current techniques in terms of resolution and data analysis, thus allowing fast throughput.

The development of electronic nano-instrumentation opens a possibility for the improved study of the TF binding sites, inspired after the theoretical report that nm-scale electrodes embedded on solid state nanopores, are an excellent way to measure lateral or transverse ionic currents and could provide unique blockade current signals characteristic of DNA that translocates through such structures [1]. The confinement-induced stretching effect provided by nanofluidic channels can overcome the challenge of the random coil configuration and random motion present in the current nanopore technique, thus stretching the DNA strand into a linear chain while the molecules are driven electrophoretically into the nanochannel. This, in combination with the use of an embedded nanogap detector into the nanochannel [2], offers the possibility to measure the electrical conduction perpendicular to the DNA backbone as it moves through the gap and observe electrical signals characteristic of the DNA-protein complex translocation events.

For such a task we have developed on a chip, by means of e-beam nanolithography and liftoff procedures, a gold nanoscale electronic detector as small as 10 nm across electrode tips, and current efforts are put into trying to embed it on an already fabricated 50-150 μm long, 50 nm wide, and 30nm deep nanochannel coupled to a microfluidic loading device with a world-micro-nanofluidic interface and DNA pre-stretching post arrays. We will present current fabrication results and a layout on preliminary ionic current blockade characterization experiments in artificial genetically engineered molecular constructs as reported by [3] that could allow the future direct mapping of TF binding sites on unsheared single genomic DNA molecules, as well as a better comprehension of the DNA-protein complex polymer dynamics on this device.

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I-18

High-speed Proteomic Signature by Informatics-Assisted Label-free Quantitation

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Although mass spectrometry (MS) is one of the principal technologies for biomarker discovery, bottlenecks are present referring to relatively scarce amounts of biomaterials and low throughput. Due to the complexity of proteome and incompatible low acquisition speed of MS, additional time-consuming fractionation step is a critical prerequisite to enable protein profiling at low abundant levels. Here we report a high-speed label-free quantitation strategy combining LC alignment and informatics tool towards large-scale multiplexed proteomic profiling, allowing high throughput biomarker discovery. For alignments across LC-MS/MS run with different LC gradients by using IDEAL-Q software, the strategy utilizes long LC gradient for protein identification and short LC gradient for quantitation. Base on this algorithm, A total number of 5678 peptides was quantified in 40-min LC-MS/MS runs with a standard deviation less than 0.3 (SD=0.3 and CV <30% at 95% confidence level) in replicate cell lysate. Furthermore, we also applied this approach to phosphoproteomic study. High linearity on quantitation working curve can be achieved (r2=0.991) over 200-fold dynamic range using a standard phosphoprotein β-casein. On the proteome scale, high quantitative accuracy (log2 ratio=0±0.27) and precision (CV=13.6%) were routinely obtained for 850 quantified phosphopeptides in 40-min LC-MS/MS. Furthermore, we apply this swift label-free quantitation strategy for Tof MRM biomarker validation.

I-19

Three-dimension Collagen-Chitosan Scaffold for Osteoblast Proliferation and Differentiation

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The purpose of this study was to fabricate a three-dimensional (3D) porous scaffold and evaluate both biofunctions for proliferation and differentiation of human osteoblast-like MG-63 cells cultured in the scaffold. Compare to *in vitro* cultivation of cells on 2D dish conventional, we used polymeric materials plasticity to fabricate 3D porous scaffolds in 24 well cell culture plate by removing ice crystal through freeze-dry process. In our study, we used chtiosan and collagen as the scaffold materials, and then formed a biomimetic scaffold by cross-link reaction. Collagen is one of the key extracellular matrix of animal tissue, especially in connective tissue. Extracellular matrix usually provides structural support to the animal cells in addition to performing various other important functions. Collagen was extracted and purified from swim bladder, and indentified as type II collagen. Collagen could improve cell adhesion on chitosan surface. The porous size of scaffolds was 141±11 μm through freeze process at the temperature of -20 degree Celsius. Collagen-chitosan complex scaffolds had more effect of cells spreading than chitosan scaffold by SEM detection. The result of cell proliferation showed complex scaffolds enhanced proliferation maybe via promoting cell both adhesion and spreading, and it continued for the fourth day. The result of cell differentiation showed cells cultured in complex scaffolds expressed more ALP activity at first day. The summary, complex scaffolds would be more benefic cell adhesion, spreading, proliferation and differentiation. It may be used as a desirable method that evaluation functional foods that keeping cell in good performance.

I-20

Functional Characterization and Molecular Mechanisms of Eosinophil Cationic Protein–Heparin Interaction

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Eosinophil cationic protein (ECP) is a heparin-binding cytotoxic ribonuclease found in the specific granules of human eosinophil. It causes damage of epithelial cells, correlates with airway inflammation in asthma, and functions in immune system to be against pathogens such as bacteria and virus. Recent studies revealed that the cytotoxic activity of ECP toward Beas-2B human bronchial epithelial cells depended on binding to the cell surface glycosaminoglycan (GAGs), specifically heparan sulfate proteoglycans (HSPGs). The aim of this study was to locate the essential heparin binding motif on ECP and the essential ECP binding unit on heparin. Heparin/heparan sulfate (HS) binding motif has been reported to be mainly correlated with positive charges and aromatic residues. Site-directed mutagenesis, synthetic peptides, and synthetic heparin oligosaccharides were designed for biochemical and biophysical binding assays. Single point ECP mutants were used for identification of heparin binding residues by cell-enzyme-linked immunosorbent assay (ELISA), isothermal titration calorimeter (ITC) and affinity column chromatography. It was found that mutants R22A, R34A, R36A, K38A, R45A, R73A, R77A, R101A and R104A showed significantly decreased HS binding activity. Synthetic peptides derived from a novel heparin binding motif demonstrated strong polysaccharide binding affinities and blocked ECP binding to the cells. Such binding motif appeared to be located outside the RNA binding domain, strongly indicating that the nucleic acid binding site and heparin binding site were different on this unique eosinophil ribonuclease. In addition, five heparin derivatives with different modifications were used for determining the specificity of heparin binding activity of ECP. The results indicated that the *N*-sufation on heparin predominantly contributed in ECP binding. These results provide new evidence into the structure and function correlation between ECP and HS, which in turn may facilitate further design for anti-asthma strategy.

I-21

Thermo- and Chemical- Responsive Surface Grafted Poly (β-Benzyl-L- Aspartate) Films

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In this work, we utilized surface-initiated vapor deposition polymerization (SI-VDP) technique[1] to fabricate polypeptide thin films. We demonstrated a new smart surface based on synthetic polypeptide, poly (β-Benzyl-L-Aspartate), monolayer. Since the PBLA is a unique polypeptide in that it was found to be able to adopt several secondary conformations, right-handed 3.6_{13} helix (α_R-helix), left-handed 3.6_{13} helix (α_L-helix), 4.0_{13} helix (ω-helix) and β-sheet.[2] By changing the environmental parameters such as temperature and organic solvent, the conformation can be switched from one to another. We found that the conformational versatility of PBLA is quite suitable to be used as the stimuli-responsive material to detect the change of the environmental condition. The surface-tethered PBLA monolayers can respond to several chemical vapors reversibly and repeatedly between left-handed α-helix and right-handed α-helix, or from left-handed α-helix to β-sheet. The proof of the conformational sensitivity was confirmed by Fourier transform infrared spectrometry and circular dichroism. Furthermore, the covalent immobilization gives the monolayers excellent stability in fabrication process which offers potential applications in biosensors and molecular devices.

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I-22

Development of Potent and Selective N-Acetyl-β-Glucosaminidase Inhibitors for the Treatment of Osteoarthritis

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Human N-acetyl-β-glucosaminidase (Hex) isozymes are important drug targets because they are directly linked to osteoarthritis [1,2,3] and lysosomal storage disorders [4,5]. We report the discovery of GlcNAc-type iminocyclitols as potent and selective Hex B inhibitors. The most potent inhibitor had a *K*i of 0.69 nM against human Hex B and was 2.5 × 10^5 times more selective for Hex B than for a similar human enzyme O-GlcNAcase. The inhibitors were shown to diminish the degradation of extracellular matrix in osteoarthritis.

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I-23

Molecular Dynamics Insight into the Role of Tertiary (Foldon) Interactions on Unfolding in Cytochrome c

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Previously, we have performed constant temperature Molecular Dynamics simulations (MD) of Cytochrome c (Cyt c) to investigate the macroscopic behavior of the foldons (Tsai et al., *Chem. Phys. Lett.* 2009). The results showed that the tertiary interactions in Cyt c play an important role in the stabilization of the N- and C-terminal helices. We also observed the macroscopic behavior of a black foldon in the process of thermal unfolding.

In this poster, we present our new MD results, which demonstrate more foldon behavior and the interplay between them. In addition, we examine the dynamics of each foldon in Cyt c by using (ϕ, ψ) of each amino acid in the corresponding foldon as a structural unit. We attempt to relate the structural information given by the MD simulations to the one-dimensional generalized Ising Model. This investigation provides the detail dynamics of Cyt c in terms of coarse-grained descriptions of the conformational space - (ϕ, ψ) space. Also, our study may provide an insight in designing a suitable phenomenological model in the Cyt c folding problem.

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I-24

Reshaping Biological Oscillator during Bacterial Cell Division with Micro/Nano-fluidic Devices

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Successful binary fission in *Escherichia coli* (*E. coli*) relies on remarkable oscillatory behavior of the MinCDE protein system to determine the exact division site; disability of such a biological oscillator in *E. coli* by genetic deletion of minCDE locus has been reported to perturb septum positioning, and form *mini* cells with void genetic materials inside, in addition to another large daughter strand with two chromosomes [1]. The current models to explain this fascinating spatiotemporal regulation on dynamic pattern formation of MinDE proteins in cells are all based on the physical scheme of reaction-diffusion, that is, protein-membrane and protein-protein interactions, and following proteins diffusion between cell poles [2]. Although not fully understood, geometric factors like the elongated shape of a bacterium play a crucial role in oscillating orientation of membrane-associated MinD proteins. In the present study, bacteria were cultured, confined and reshaped in various types of pre-defined PDMS microfluidic chambers, equipped with nutrient supplies distributed through nanofludic channels across microfluidic regions, to mimic negative curvature of cell poles owing to chamber geometry. *In vivo* fluorescence imaging was utilized to detail dynamics of the biological oscillator, as well as to reveal the filamentous structure of MinD protein assembly on the inner surface of a cell membrane. Here, the curvature effect due to microfluidic confinement on the oscillating patterns of MinD proteins and new pole allocation in dividing daughter cells, were characterized and compared to those under division-inhibited condition, where aztreonam was applied to prevent septum formation. The study elaborates sub-cellular biophysics in bacterial cell division through micro/nano-fluidic manipulations and *in vivo* fluorescence imaging.

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I-25

Constructing of Unidirectional Polypeptide Based Molecular Diode

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Surface-grafted polypeptide monolayer has distinct alpha-helical structures, which displays a large dipole moment parallel to the helical axis. Such peptides should be responsive to an applied electric field. Herein, we succeeded in constructing large area and well-aligned alpha-helical polypeptide film and measuring the electrical behaviors by conductive AFM. We synthesized alpha-helical poly (γ-benzyl-L-glutamate) (PBLG) thin film on gold coated substrate by vapor deposition polymerization and demonstrated a simple solvent quenching approach to promote oriented PBLG aggregates. In the solvent quenching experiments, we found that the solvent quenching efficiency is correlated to the molecule weight and surface density. With higher molecule weight and surface density, we could get the better solvent quenching efficiency. The measurements of the electrical behaviors of the unidirectional polypeptide chains were performed by using conductive AFM. The polypeptide chain aggregates were trapped between the Cr/Pt tip and gold coated substrate. Interestingly, in the conductivity measurements, the current-voltage curves indicated there is a directional dependence of electron transmission through the polypeptide chains, which is connected with the electric field generated by the molecular dipole of the helix. The current-voltage curves were highly asymmetry that the recorded currents for negative bias are up to 122 times larger than the positive bias in the range of the instrument limitation. Besides, we also found that the electrical behaviors of the polypeptide chains also influenced by the tilt angle and molecular weight of the polypeptide chains. The smaller tilt angle to the normal surface and the higher molecular weight of the polypeptide chains, the higher rectifying effect the polypeptide is.

I-26

Site-specific Immobilization of Enzyme on Magnetic Nanoparticle for Practical Applications

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Enzyme holds great promise to catalyze chemical reactions and widely applied on organic synthesis and detection. To achieve recycled usage of enzymes and broaden their applications in organic synthesis, magnetic nanoparticles (MNPs) are attractive materials to serve as a support for enzyme immobilization and facilitate the separation by applying an external magnet. However, to maintain the enzyme activity after immobilization, immobilization strategies for various properties of proteins are important and challenging tasks. Herein, we describe a hydrophilic-linker for immobilization of secreted and membrane bound enzymes.. The activity comparison of free and immobilized enzymes are also discussed.

In this poster, the secreted enzymes such as sialyltransferase (from *Pasteurella multocida*) and CMP-sialic acid synthetase (from *Neisseria meningitides*) were chosen to be expressed by intein expression system. By affinity chitin bead purification and MESNA cleavage, the resulting enzymes with thioester at their C-terminus were obtained. Cysteine-polyethylene glycol-functionalized MNP (Cys-PEG-MNP) was then prepared and used to site-specifically and covalently immobilize the hydrophilic enzymes through native chemical ligation (NCL). With the increment of the PEG linker length, the activities of immobilized enzymes become higher in comparison with free parent enzymes. In contrast, sialyltransferase from *Neisseria gonorrheae*, the membrane bound enzyme, was also immobilized on the MNP through the same way. Nevertheless, this enzyme was unstable after immobilization on MNPs using above strategy. It may be because the surface properties of Cys-PEG-MNP affect the membrane bound enzyme resulting in less stability. Whereas, when this enzyme was modified with biotin-labeled cysteine at C-terminus by NCL followed by assembling on streptavidin functionalized MNP (Strep-MNP), the activity showed slightly improvement. Overall, the results demonstrated that the property of MNP affects the immobilized enzyme activity.

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I-27

Single Molecule Tug-of-War and Confinement-induced Entropic Recoiling of DNA in 50 and 100 nm Nanoslits

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We built two microchannels bridged by a nanoslit to observe tug-of-war of single DNA molecules through micro/nano/micro fluidic interfaces; furthermore, we investigate the retraction of single DNA molecules in nanoslit caused by confinement-induced entropic recoiling forces. The dimension of nanoslits is of 50, 100 nm in depth, 10 μm in width, and 2, 3, 4, 10, 20, 30 μm in length; the depth of microchannel is 2 μm. By applying electric field, one end of DNA polymer may be moved through a nanoslit where two free ends of DNA straddle two micro/nano interfaces and form a tug-of-war scenario as field is off. Due to opposing entropic recoiling forces along a DNA polymer at two micro/nano interfaces, the tug of war between two ends of a DNA polymer across a nanoslit could last from seconds to minutes, and then retract stochastically from one micro regime to the other. We studied the DNA tug-of-war phenomenon and entropic recoiling for the first time in nanoslits of different channel length and depth. Apart from the previous approaches, we propose an alternative theoretical model of deriving single molecule entropic recoiling force in both the tug-of-war scenario and retracting recoiling scenario. The forces in 50 nm nanoslits deduced from these two scenarios are consistent to be around 1 pN.

I-28

Role of α-L-Fucosidase in the Infection of *H. pylori* to Gastric Cancer Cells

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Infection of *Helicobacter pylori* is present in about one-half of the global human population. Previous studies determine *H. pylori* as the primary cause of gastritis, duodenal ulcer and gastric cancer. However, how host cells interact with H. pylori, and whether these interactions correspond to different type of gastric diseases are still poorly understood. By using fluorescent-labeled fucose-containing glycoconjugates, here we present the first observing that *H. pylori* could uptake L-fucose from gastric cancer cells, and human α-L-fucosidase 2 (FUCA2) is secreted only under co-culture conditions (i.e., host cells infected with *H. pylori*). Furthermore, the depletion of FUCA2 by RNA interference shows that this enzyme might be essential for *H. pylori* adhesion, in particular to the gastric cancer– and duodenal ulcer–specific strains. Additionally, FUCA2 significantly enhance the expression of Lewis x antigen in *H. pylori*, which is critical for the adhesion of *H. pylori* and served as a defense strategy to escape from host surveillance. These findings demonstrate an important connection between FUCA2 and the pathogenicity of *H. pylori*, and also reveal that FUCA2 is a potential target for clinical diagnosis and therapeutic intervention of *H. pylori*–related diseases.

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I-29

Construction of the Endoglucanase-β-Glucosidase Fusion Protein as a Bifunctional Cellulase for Efficient Glucose Production

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Cellulase system is an instrument of microbes to degrade cellulosic material as their nutrition. This system involves three major enzymes, endoglucanases, exoglucanases and β-glucosidases, which have in common a specificity for 1,4-β-glucans, but differ in their modes of action. Enzymatic hydrolysis of cellulose to glucose through the catalysis of cellulase system is an important source of biofuel [1].

In this study, we found the combination of a β-glucosidase CtbglA or CcbglA encoded by the *bglA* gene of *C. thermocellum* or *C. cellulovorans* and an endoglucanase CtCel9It encoded by *cel9I* gene from the former strain were able to degrade CMC, an alternative cellulase substrate, into glucose as major product [2, 3]. These hybrid enzymes had the optimum endoglucanase activity at pH 6.0 and 50 ^{o}C similar to that for CtCel9It and the optimal β-glucosidase activity at pH 6.0 and 70 ^{o}C for CtbglA-CtCel9It, but lower temperature 40 ^{o}C for CcbglA-CtCel9It. However, CcbglA-CtCel9It had a T_m 7.3 ^{o}C higher than that of CcbglA (56 ^{o}C), indicating its higher stability. TLC analysis and HK glucose assay also revealed that CcbglA-CtCel9It had a higher efficiency than two individual enzymes together in producing glucose, possibly through substrate channeling of cellobiose.

In the study presented here, we show the hybrid enzyme CcbglA-CtCel9It with higher T_m and efficiency in cellulolytic activity compared to the mixture of two individual enzymes through the potential protein-protein interaction and cellobiose-channeling effect. This more thermostable and highly efficient bifunctional enzyme may be useful in industrial application to convert cellulose to glucose.

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I-30

Ditopic Complexation of Calcium Cation and Selenite Anion by Pirenoxine and Its Implication in Cataract Prevention

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Pirenoxine (PRX), 1-Hydroxy-5-oxo-5H-pyrido[3,2] phenoxazine -3-carboxylic acid, was first introduced in 1958 in Japan and now is widely used clinically in Asia as an anti-cataractogenic agent. However, there is little scientific evidence of its efficacy. Based on the previous studies, it is well known that excess presence of calcium or selenite causes lens crystalline turbidity. This work aimed to investigate whether pirenoxine interacts with these two ions approached by UV spectra, NMR and ITC. The results indicated that pirenoxine binds selenite and chelates calcium ions. More precisely, selenite may bind predominantly on benzoquinone and aromatic rings of PRX. The 3-carboxylate, β-ketoimine, and β-hydroxylimine groups in PRX were primary considered to have calcium chelation property. PRX with ditopic recognition properties was then demonstrated to ameliorate lens turbidity induced by selenite or calcium ions. These results may provide a rationale for using pirenoxine as an agent for prevention of cataract formation. Further in vivo study is warranted.

I-31

Coumarin Derivatives as Anti-cataract Agents

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In vitro assay was established to evaluate the anti-cataract activity of coumarin derivatives. Coumarin derivative 2 was shown to prevent γ-crystallin from UV-induced turbidity. Several modifications of derivative 2 were synthesized and evaluated by protecting porcine γ-crystallin from UV damage. Comparison of the activities of coumarin and derivatives 3-8 revealed that derivative 6 was the most potent anti-cataract activity among them. From these results, the 5-hydroxyl group on coumarin plays an important role in anti-cataract activity. Absorption spectra also showed that coumarin derivative 6 has strong absorption peak at 254 nm which may provide a rationale for anti-UV activity of this compound.

Compound 1: coumarin
Compound 2: 5,7-dihydroxy-6-(4-hydroxy benzoyl)- 4-phenyl-2H-chromen-2-one
Compound 3: 5,7-dihydroxy-6-(4-methylbenzoyl)- 4-phenyl-2H-chromen-2-one
Compound 4: 7-hydroxy-2-oxo-4-phenyl-2H-chromen-5-yl-4-cyanobenzoate
Compound 5: 6-benzoyl-2-oxo-4-phenyl-2H-chromene-5,7-diyl diacetate
Compound 6: 6-benzoyl-5,7-dihydroxy-4-phenyl-2H-chromen-2-one
Compound 7: 5,7-dihydroxy-4-phenyl-2H-chromen-2-one
Compound 8: 6-benzoyl-4-ethyl-5,7-dihydroxy-2H-chromen-2-one

I-32

Membrane Activities of Living Cells Characterized by 3D Optical Tracking and Wide-Field Profilometry

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Optical microscopy is crucial for the research in cell biology. However, because of the transparency of cell membranes, many interesting dynamical features on the membranes are invisible by using conventional optical microscopy. On the other hand, atomic force microscopy (AFM) becomes one of the most popular techniques for studying the samples in the nanometer scale. But the frame rate of AFM is still limited by the tip scanning mechanism, especially in the physiological environment. Although current AFM instruments have been demonstrated to study bio-molecular processes in real time [1], the scanning area is still limited in a few hundreds of nanometers square. This is insufficient for the studies on whole cells. Here, we purpose an advanced optical profilometry, "non-interferometric wide-field optical profilometry" (NIWOP) [2], to overcome the restrictions of the axial resolution and image acquisition rate of current techniques. The axial resolution of the NIWOP has been demonstrated to about 20 nm in the physiological environment [3]. The lateral resolution can also be improved to about 120 nm after the image restoration process [4]. Moreover, by combining the polynomial-fit Gaussian weight (PFGW) algorism [5], the lateral positioning accuracy can be further improved to about 20 nm. The image acquisition rate is about 2 Hz in a 140×140 μm^2 area. This technique is very suitable for the study of the membrane dynamics and 3D nano-particle tracking.

In this work, we demonstrate the applications of four individual issues by using the NIWOP technique, such as quantification of active membrane waves, label-free observation of filopodium activities, laser-induced membrane activities and 3D tracking of liposomes and gold nanoparticles. The recent results of these works will be presented in detail on our poster.

Because the NIWOP technique is non-contact, easy to operate, and compatible to physiological environments, we believe that this technique will become a useful tool for the research in cell membrane activities.

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I-33

Cooperative Lactonization, Electrochemical Lactonization, and C–H···O Hydrogen Bond in OligoSialic Acid Lactone

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Sialyl lactonization of tri-sialic acid by acidic catalysis in glacial acetic acid and electrochemistry in acidic water media were observed with cooperative property. The cooperative stabilization in the biopolymer has been ascribed to a structural driving force from conventional hydrogen bonds. The formation of OSA/PSA and subsequent lactonization reduces the number of exocyclic torsional degrees of freedom from four to two, and the number of hydroxyl groups from five to two, and therefore the numbers of possible conventional hydrogen bond in the final lactone product are greatly reduced. Consequently, C–H···O hydrogen bonds arise from the more rigid lactone product, which can be considered to be a main force driving cooperative lactonization. One pair of ^{3}J-correlation through the C–H···O hydrogen bond in tri-sialic acid lactone was studied by LR-COSY, and this evidence was applied to support the C–H···O hydrogen bonding cooperativity and cooperative lactonization in oligosialic acid.

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I-34

Chemical Rescue of Proton Relay System in an Inactive Mutant of Human Cytosolic Aminopeptidase P

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Aaminopeptidase P (AMPP) is a metalloprotease that cleaves the N-terminal amino acid residue from a polypeptide, provided that the second residue is proline (1,2). We use bradykinine as susbstrate, the Km and *k*cat value of human cytosolic wild type AMPP (hcAMPP) were 141.8 μM and 9.5 s^{-1}, respectively. The *k*cat values were changed to 1.8 s^{-1} and 0.8 s^{-1}, if we replace Tyrosine 527 with phenylalanine (Y527F) and replace arginine 535 with alanine (R535A) of hcAMPP respectively. And exogenous guanidines can restore activity of R535A mutant which enhance the rate of the mutant 28-fold and the turnover number for the guanidine-rescue R535A mutant is 90% that of wild type. The addition of guanidine to the R535A mutant has little effect on Km values, and the rescue effect is therefore attributed to an increase in kcat. The rescue effect are guanidine hydrochloride >methyl-guanidine> aminoguanidine > N-ethyl-guanidine when the concentration of guanidines less then10 mM. But there were no activated effects on wild type or Y527F mutant. These results indicated that R535 residue can participate in the hydrogen bond network during catalysis process. Progressively, we monitor the quaternary structural change of hcAMPP by AUC, the wild type hcAMPP was major in dimmer form but more easily dissociated on R535A mutant. This result indicated that Arginine 535 residue not only play a role on catalysis but also on quaternary structure stability.

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I-35

Label-Free and Real-Time Biosensing Arrays Using Surface Plasmon Resonance on Gold Nanoslits

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Recently, much attention is paid in designing and fabricating novel surface plasmon resonance (SPR) biosensors [1,2] for sensitive chemical, biological and environmental sensing. The common SPR biosensors utilize the attenuated total internal reflection (ATR) method to excite surface plasmon resonance on a thin gold film coated on a prism. These prism coupler-based SPR sensors are known very sensitive to surface environmental changes. The refractive index resolution is on the order of 10^{-5} for 0.2% intensity resolution. However, the ATR setup is too bulky to be applied for high-throughput and chip-based detections, such as DNA microarrays and protein microarrays. In this study, we combined the advantages of SPR detection (label-free, surface sensitive) and microarray (high throughput) to fulfill the crucial requirements for kinetic studies of DNA-DNA and protein-protein interactions.

In order to fulfill these requirements, we present a novel chip-based biosensor using surface plasmon resonance on gold nanoslit array. The bulk sensitivity was tested by detecting glycerin/water mixtures. The surface sensitivity was verified by the BSA and anti-BSA interactions and DNA-DNA hybridizations. In the experiment, gold nanoslit arrays had 600 nm period, 120 nm height and widths ranging from 30 to 200 nm. The area of each metallic nanoslit array was 150 μm×150 μm, similar to the spot size of DNA microarrays. A transverse magnetic polarized wave in these gold nanostructures generated resonant surface plasmons at a wavelength of about 825 nm in water environment. The bulk intensity sensitivity at 850 nm wavelength was measured by changing glycerin/water mixtures with different the refractive indexes. The results show that the bulk sensitivity was increased with decreasing slit width. The highest refractive index resolution will be 5×10^{-5} for 35-nm-wide slit if the intensity resolution is 0.2%. Besides, in order to demonstrate the advantage of high-throughput and real-time detection, the 842-nm-wavelength LED was used as a light source and the transmission light from nanoslits was detected by a CCD array. By detecting the BSA and anti-BSA interactions and DNA-DNA hybridization, the surface sensitivity was confirmed.

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I-36

A Sensor-Compatible Bonding Process to Seal Biofunctionalized Nanochannels

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We demonstrate the compatibility of a sealing process with the use of pre-functionalized surfaces for the fabrication of biofunctional nanofluidic channels. This method relies on the permanent bond between a plasma-treated polysilsesquioxane (PSQ) layer and a glass chip bearing nanochannels [1]. We have used this technique to seal 600 nm deep nanoslits previously grafted with probe DNA and we have confirmed the biological functionality of the probes using fluorescent-labeled complementary oligonucleotides.

The as-fabricated functionalized nanoslits exhibit a reduced diffusion time and a small Péclet number at relatively low flow rate (≈10 nL/min), thus permitting a 100% adsorption efficiency of target molecules [2]. We have experimentally observed that all the target molecules encountering the sensing area are captured by the probes and that the flowing solution gets depleted. In this convection-limited configuration the biofunctionalized nanoslit acts as a molecular filter. Besides, because the reaction is not diffusion-limited, a direct observation of the hybridization events is envisioned at higher flow rates, and so is the fast real-time detection of molecules.

This PSQ-sealing process is foreseen to enable the packaging of future lab-on-chip devices with biofunctional sensing elements integrated into nanoscale channels, offering advantages inherent to the reduction of diffusion time. Possible applications of biofunctional nanoslits include the ultra-fast detection of reagents in small samples and the purification of biological solutions.

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I-37

Hydrolysis of Rice Straw by Cellulolytic, Xylanolytic and Ligninolytic Enzymes

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Cellulolytic and xylanolytic enzymes were famous enzyme groups for breaking down cellulose and hemicellulose, main structure of plant cell wall, into monosaccharide. However, those enzymes were obstructed interaction on their substrates by lignin that also was the main structure. Thus, hydrolysis of biomass by only cellulase and xylanase produced low yield of monosaccharides. Laccase was ligninolytic enzyme that could destroy lignin structure. The pretreatment of rice straw with laccase from *Rhus vernificera* was carried out before hydrolysis with cellulolytic enzyme from *Aspergillus niger* and xylanolytic enzyme from *Trichoderma viride* for increasing the yield. The synergistic effect between those enzymes also was carried out at optimized condition, pH 5.5 and 37ºC. The single enzyme, 150 U cellulase and 100 U xylanase /g rice straw could produce highest reducing sugar yield, 219.5 and 85.5 mg/g rice straw, respective. For pretreatment of rice straw before hydrolysis by 150U cellulase/ g rice straw, 150 U laccase/ g rice straw was optimized concentration that could increase yield of reducing sugar from 219.5 mg/ g rice straw to 343 mg/g rice straw. When those lignocellulolytic enzymes were used for hydrolysis of rice straw, they could produce reducing sugar about 445 mg/ g rice straw. When 1 g of rice straw was completely hydrolysis, it contained about 512 mg of sugar. The result indicated that synergistic of cellulolytic, xylanolytic and ligninolytic enzyme could enhance conversion of rice straw to sugar. The latter sugar could be applied to produce biofuel such as bioethanol and hydrogen.

I-38

Investigations on Ubiquitin by spFRET

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Single-molecule detections have been known for the potential to provide additional information beside ensemble measurements. Population heterogeneities and synchronous (or asynchronous) reaction pathways pertaining to conformational dynamics and molecular interactions are veiled by ensemble averaging. On the other hand, FRET (Förster Resonance Energy Transfer) serves as a distance ruler in close proximity (~ 1 nm to ~ 10 nm), and dynamic changes of protein structure can be thus recorded accurately. We have established optical methods to observe ubiquitin mutants, m[C]q/S65C, either immobilized on the cover-slip surface or in free solution. Conformational dynamics and population heterogeneity are observed in our experiments, which suggest the existence of protein-dye interactions.

At the same time, a continuous-flow micro-fluidic mixer with mixing dead time down to μsec is also under development. The long-term objective is to cooperate FCS (fluorescence correlation spectroscopy), TCSPC (time-correlated single photon counting), microscopy, micro-fluidic mixer and FRET to investigate the population evolution and structural variations of ubiquitin along the kinetics of biomolecules.

I-39

The Stretching Dynamics and Transport of DNA Molecules along Sidewalls of a Quasi-Two-Dimensional Nanoslit

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We report unusual phenomena of the like-charge attraction between DNA molecules and the sidewall of a 2d glass nanoslit with height, h, less than the Kuhn length L_k of the molecules. DNA molecules are stretched while diffusing along the wall. Scaling analysis reveals that the wall-bounded DNA molecules exhibit one-dimensional (1d) dynamics. The modified de Gennes model shows better fit with the data down to 30 nm than the Odijk model, which includes the bending energy when the confined dimension is smaller than L_k. We attribute this discrepancy to the partially bound confinement. In contrast, the scaling analysis of DNA molecules far from the sidewall exhibit 2d behaviors, which can be described by de Gennes and Odijk model for $h > L_k$ and $h < L_k$, respectively. We further apply the unusual property of attraction to trap and stretch DNA molecules around circular posts in a nanoslit. DNA molecules can be transported from post to post by applied electric field and exhibit trapping-escaping movement.

I-40

A Simple Magnetic Nanoprobe-Based Approach for Selective Purification and Identification of Protein Complexes in Colorectal Cancer Cells

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Isolation and purification of high quality protein complexes using affinity purification and mass spectrometric characterization is the most common approach to study protein-protein interactions. Conventional co-immunoprecipitation, however, suffers from drawbacks of antibody contamination, non-specific purification and time-consuming procedure. By taking advantage of highly-efficient extraction of antibody-conjugated magnetic nanoparticles (Ab@MNPs) and comprehensive LC-MS/MS identification, we present herein a rapid and efficient immunoprecipitation method for selective isolation of protein complexes. Compared to microscale polymeric beads, *e.g.* Dynabead®, Ab@MNPs immunoprecipitation showed advantages of antibody-free purification and superior isolation specificity of target protein, allowing unbiased protein identification by LC-MS/MS. By applying a pre-clearing step and proper selection of washing buffer, non-specific copurification of other abundant protein was effectively minimized. We utilized this approach to enrich β-catenin protein complex in SW480 colorectal cancer cells, and identified several β-catenin-associated proteins participating in cell mobility, E-cadherin signaling pathway, and transcriptional controls. Combining the advantages of Ab@MNPs, such as the flexibility for conjugating biomolecular probes and increased homogeneity for immunocomplex formation, this approach provides a new alternative for efficient enrichment and identification of protein complex compatible for mass spectrometric analyses.

I-41

Sequential Gel-assisted Digestion for Concomitant Analysis of Phosphorylated and Glycosylated Membrane Proteome

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Alterations in glycan and phosphorylation profiles of membrane proteins have been correlated with certain disease state. More importantly, 90% of therapeutics protein drugs are glycosylated membrane proteins; phosphorylation is generally considered the most direct evidences for the perturbed signaling pathway in disease. However, it is still a great challenge to analyze the glycosylated or phosphorylated membrane proteins due to the facts that membrane proteins are highly hydrophobic, low abundant and bear heterogeneous glycosylation and phosphorylation. To facilitate sensitive and efficient characterization of glycosylated and phosphorylated membrane proteins, we proposed a strategy integrating sequential gel-assisted digestion[1], IMAC purification, and iTRAQ quantitation to provide concomitant analysis of membrane proteins and their glycosylation and phosphorylation sites.

The pilot study of glycosylated membrane proteomics was demonstrated on HeLa cells. To identify the glycosylated membrane proteins, we quantitatively compared the profiles of membrane proteins obtained after digestion by trypsin alone or sequential digestion with PNGase F and trypsin. Compared to the tryptic peptides, increased level of peptides in the latter case may arise from the de-glycosylated peptides after treatment with PNGase F. The preliminary results identified 1857 proteins ($p<0.05$, Mascot score>39) and 1297 proteins were successfully quantified by Multi-Q[2]. Among these proteins, 963 proteins were annotated as membrane or membrane-associated proteins. Further analysis of the up-regulated peptides (ratio>2) revealed that 488 peptides from 119 proteins matched the consensus sequence Asn-Xxx-Ser/Thr with mass shift of 1 Da. Molecular function classification of these proteins based on Gene Ontology indicated 20 transporters and 31 receptors among these glycosylated membrane proteins. Moreover, the cleavage of N-glycans by PNGase F from cell surface proteins contributed a 1.25-fold increase of peptides yield, suggesting that steric interference of glycans on the cell membrane may be removed by de-glycosylation and further enhanced detection of peptides with glycosylation or near the glycosylation sites.

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I-42

Cells-on-a-Chip: A Replenishment Cell Culture Substrates in Microfluidic Systems

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A novel cell investigated platform that utilizes fluid properties of synthetic supported lipid bilayer (SLB) membranes as “replenishing substrates” in a microfluidic device will be presented in this research. Traditionally, culture of adherent cells was carried out onto extracellular matrix (ECM) materials, which provide a backbone for cell adhesion, proliferation, or differentiation [1]. These ECM components were adsorbed on the surfaces, hence being immobilized. To replace adhesive materials and detach cells provide new opportunities for understanding fundamental biological research of cell interfaces. For detaching adherent cells, the substrates may also have to be treated with digestive enzymes like trypsin or be scraped off, which can affect the cells viability, phenotype, metabolism or protein expression [2]. The feasibility of this approach to replace adhesive surface materials with both temporal and spatial resolutions will be reversed. The biological behavior and cell-cell and cell-matrix interaction of cells stimulation can be observed in these replaced methods in the microfluidic cellular chip.

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I-43

Extracellular Matrix Protein-Conjugated Membrane-Mimetic Surfaces for Studying Cell-Biomaterial Interactions

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It is conceivable that surface modification by engineered biomimetic materials provides a strategy for studying cell-cell and cell material interactions, which can lead to advances in biomedical research and development such as biosensing, drug screening, and tissue engineering. Our current work describes a bio-functionalized surface containing extracellular matrix protein and nano-sized lipid vesicles. Fabrication of the model system starts with formation of supported lipid bilayer on a planar substrate, followed by immobilization of liposomes and fibronectin conjugation. Layers between bilayer, liposomes, and fibronectin are coupled via biotin-streptavidin binding specificity. Characterization of the model platform as well as liposome-cell interactions was performed using various surface techniques including quartz crystal microbalance with dissipation monitoring (QCM-D), atomic force microscopy (AFM), and confocal fluorescence microscopy. The QCM-D results reveal kinetics of each surface modification step on oxygen plasma- pretreated SiO_2 quartz. According confocal fluorescence images, it appears that liposome concentration increases with increasing biotin bilayer concentration within supported lipid bilayer and becomes saturated when biotin concentration is larger then 2 mol%, which is also evidenced by an AFM image showing highly packed liposomes. When culturing human cervical carcinoma (HeLa) cells on the model surface, we found cell adhesion is comparable to that on the conventional tissue culture polystyrene. In the absence of surface fibronectin, cells remain non-adherent. Confocal images suggest that fluorescently labeled immobilized liposomes be locally reorganized during cell adhesion and migration, and even uptake by cells. When using doxorubicin-encapsulated liposomes, we observed HeLa cell population decreased significantly (more than 90% within 24 hours of incubation), indicating drug release and possible liposome uptake by cells. We believe our model constructs provide a platform that has potentials for controlled cell adhesion as well as cell-based sensing and therapeutics.

I-44

Layer-by-Layer Polypeptide Polyelectrolyte for Efficient Selection, Enrichment, and Maintenance of Stem Cells in Vitro

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Enrichment of stem cell population is one of the most important steps in facilitating stem cell research. Fluorescence-activated cell sorting (FACS) derives are widely used for isolation and enrichment of stem cells from primary cells; however, FACS is not practical for applying because of the process is too complicated and the difficulty to remove antigen-antibody on cell membranes afterwards [1]. An alternative strategy would be to identify a suitable culture condition, for example, suitable culture plate, that can selectively enrich a particular cell type from the primary cell culture.

In recent years, polyelectrolyte multilayer (PEM) films are made by using a layer-by-layer (LbL) technique to deposit polycationic and polyanionic materials alternately through non-stochiometric electrostatic interactions. The ability to form controlled layered structures and couple functional materials in the films has also made LbL popular for a variety of biological applications such as antimicrobial coating, immunogenicity, drug delivery, and cellular culture.In this work, two polypeptides, commercial poly-L-lysine (PLL) (positively charged) and poly-L-gltumatic acid (PLGA) (negatively charged) are selected and applied to construct physically coating PEM films (c-form). Previously, using PLL/PLGA PEM films for specific cells adhesion were widely studied [2]. It is first time we use polypeptides to construct a tunable and biocompatible PEM films as compared to extra-cellular matrix (ECM) coating, for example, collagen IV and laminin, for selection, enrichment, and maintenance of mouse fetal liver stem/progenitor cells (FLSPCs) *in vitro*

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I-45

Cell Interaction with Polyelectrolyte Multilayers Conjugated on Supported Lipid Bilayers

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There is considerable interest in using supported lipid bilayers as models for potential biotechnological applications such as protein adsorption, vesicle formation, and sensing. The interaction of marcomoleculars with lipid membranes is important in membrane physics. Liver transplantation may provide a successful treatment for human liver diseases, however, the shortage of the donor cells result in the limitation of the therapy. Fetal liver stem/progenitor cells (FLSPC) have an enormous potential for the basic materials for liver transplantation since they have the capacity for self-renewal and bi-potential differentiation ability. In our previous study, we have developed a culture system to select and expand liver stem cell based on utilizing Poly vinyl alcohol (PVA). In this study, the cell interactions of FLSPC spheroids on lipid bilayer conjugated polyelectrolyte multilayer films were determined and the response of charge polyelectrolyte conjugated on the supported lipid bilayers was characterized. These FLSPC spheroids on PVA substrate was determined by immunocytochemistry and reverse transcription-polymerase chain reaction (RT-PCR) analysis. Besides, the colony numbers and maintenance ratios of FLSPC spheroids on lipid bilayers conjugated polyelectrolyte multilayer films were determined at several time points. In addition, colony morphologies on the materials at different time points were also investigated. Furthermore, the response of polyelectrolyte multilayer films on supported lipid bilayers were characterized by confocal microscopy, quartz crystal microbalance with dissipation, and atomic force microscopy. We demonstrated that supported lipid bilayers conjugated highly tuneable polyelectrolyte multilayer films were suitable for maintenance of fetal liver stem/progenitor cells. Domain formation in lipid bilayer membranes can occur through electrostatic interactions between charged lipids and oppositely charged polyelectrolytes. These results may provide a better physiological approach for selection and maintenance of liver stem/progenitor cells for future biomedical applications and the information on lipid bilayers membrane physics.

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Nanoscale Molecular Traps for Ultrafast Protein Preconcentration: 10^5-Fold Enhancement in 20 Seconds

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Early disease detection poses a great challenge in health care and medical diagnostics, mainly due to the difficulty of sensing rare number of protein biomarkers associated with a disease in its early developmental stage. Protein enrichment or preconcentration may be an effective approach towards a highly sensitive and rapid biosensing for early disease diagnostics and general protein assay analysis. Various techniques have been developed in recent years to achieve protein preconcentrations [1-4]. Among these approaches, 10^3−10^6-fold preconcentration of proteins has been demonstrated using ion exclusion-enrichment effect caused by electrical double layer overlapping at a micro-to-nanofluidic interface [1-3]. However, this approach for protein preconcentration and collection takes tens of minutes to several hours. It is thus desirable to develop faster methods to achieve a high degree of preconcentration.

In this study, we constructed nanoscale molecular traps composed of dielectric constrictions for ultrafast protein preconcentration, a scheme based on our previously developed electrodeless or insulator-based dielecrophoresis (DEP), where we demonstrated effective DNA and cell trapping [5, 6]. The chip, capable of supplying stable electric field, was designed and fabricated by NEMS (Nano-ElectroMechanical Systems) techniques including photolithography, wet and dry etching, electron beam lithography and lift-off process on a fused silica substrate, consisting of microchannels (few μm's deep) and nanochannels (100's nm deep) with embedded nanoconstrictions serving as molecular traps. The whole device, with integrated micro- and nanofluidic channels were completed by a room-temperature and low-pressure sealing process recently developed by our group [7]. Here we report effective and ultrafast protein trapping using these devices by exploiting both positive and negative DEP effects. The highlight of our findings is that protein concentration may be enhanced by >10^5-fold in less than 20 seconds, 2~3 orders of magnitude faster than previous studies [1-4]. Our approach may find practical applications in a micro-total analysis system for early disease diagnostics or protein assays in general, especially when the available specimen amounts are limited.

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