P1-001

Mechanism and Kinetics of Pore Formation in Membranes by Water-soluble Peptides

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How antimicrobial peptides form pores in membranes is of interest as a fundamental membrane process. However, the underlying molecular mechanism, which has potential applications in therapeutics, nonviral gene transfer and drug delivery, has been in dispute. We have resolved this mechanism by observing the time-dependent process of pore formation in individual giant unilamellar vesicles (GUVs) exposed to a melittin solution. Individual GUV first expanded its surface area at constant volume and then suddenly reversed to expanding its volume at constant area. The area expansion, the volume expansion and the point of reversal all match the results of equilibrium measurements performed on peptide-lipid mixtures. The mechanism includes a negative feedback that makes peptide-induced pores stable with a well-defined size, contrary to the suggestion that peptides disintegrate the membrane in a detergent-like manner.

P1-002

Mutagenesis and Structural Studies of the Substrate Binding Cavity of Truncated *Fibrobacter succinogenes* 1,3-1,4-β-D-Glucanase

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1,3-1,4-β-D-Glucanases (EC 3.2.1.73) specifically hydrolyze β-1,4-glycosidic bonds located prior to β-1,3-glycosidic linkages in lichenan or β-D-glucans. It has been suggested that the truncated *Fibrobacter succinogenes* 1,3-1,4-β-D-glucanase (TFsβ-glucanase) can accommodate five glucose rings into its active site upon enzyme-substrate interaction, and that about 20 residues are involved in substrate binding. In this study, 12 mutant enzymes were created by mutating the residues Gln70, Asn72, Gln81, and Glu85 proposed to bind with substrate subsites +1 and +2, and the catalytic properties of these mutants determined. The most significant change in catalytic activity was seen on mutation of Gln70, with a 193-fold and 321-fold lower k_{cat}/K_m in mutants Q70A and Q70I, respectively, than in the wild-type enzyme. The comparative energy ΔG_b value and catalytic efficiency were well correlated in individual enzymes. The crystal structure of mutant E85I was determined at 2.2 Å resolution. Glu85 is proposed to bind with substrate subsite +2 of carbohydrate via hydrogen bond. Further analysis of the E85I mutant structure revealed that the catalytic residue Glu-60 shifted away because the loop located at the concave site moved approximately 2 Å from its position in the native enzyme complex. Mutagenesis, kinetic and structural studies reveal that residues surrounding the active site of TFsβ-glucanase indeed play an important role in its catalytic function.

P1-003

Crystal Structure of FlgD from *Xanthomonas campestris*: Insights into the Hook Capping Essential for Flagellar Assembly

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The bacterial flagellum is a very complicated nano-structure assembled from more than 40 gene products. It comprises the basal body, hook, filament, and other labile structures such as motor, switch, and export apparatus. The flagellum is sequentially assembled from simpler to more complex structures. In a swimming cell, flagella rotate at high speed in the order of 300 Hz. However, it is important to note that flagella are attached on an immobile basal body. Hence an intermediate substructure is necessary for a cell to withstand the enormous torsional force caused by the highly rotating flagella. Such a “buffer” is served by a hook substructure of around 55 Å. However, to form such an intermediate hook structure, a cap is necessary to prevent the leakage of hook monomers into the medium. The scaffolding protein FlgD is believed to be the protein to form the hook cap structure. Besides its obvious role as a cap, it also serves the active roles of productive hook monomer polymerization, and determination of correct hook length, along with the help of FliK protein. The importance of hook capping is clearly revealed in the *flgD* mutant, which cannot continue flagellar biogenesis when basal body is completed.

The first crystal structure of a hook-capping protein FlgD from the plant pathogen *Xanthomonas campestris* has been determined using X-ray crystallography. The monomer comprises 221 amino acids with a MW of 22.7kD, but the disordered N-terminus is cleaved for up to 75 residues during crystallization automatically. The final core structure reveals a novel hybrid comprising a tudor-like domain interdigitated with a fibronectin type III domain. In the crystal, the monomers form an annular pentamer of dimers of pseudo five-fold symmetry, due to different dimer-dimer interactions employed. The resulting asymmetrical star-like complex has a outer dimensions of approximately 110 Å x 90 Å x 65 Å, and a shortest diameter of approximately 20 Å in the center. The outer dimensions of the atomic *Xanthomonas* hook-capping FlgD complex turn out to be very similar to those of the *Salmonella* filament cap complex observed by electron microscopy. This atomic hook cap structure may help understand hook protein – cap protein interactions, hook protein insertion, and hook length control, the three features that are crucial for understanding the bacterial flagellar biogenesis.

P1-004

Crystal Structure of DFA0005 Complexed with α-Ketoglutarate: A Novel Member of the ICL/PEPM Superfamily from Alkali-tolerant *Deinococcus ficus*

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The pale-pink alkali-tolerant *Deinococcus ficus* (DF strain CC-FR2-10T) is a novel bacterium isolated from the rhizosphere of the sacred tree Ficus religiosa L. It is found to possess high degrading power for food and organic waste at pH 10 and is being applied to industrial usage. It contains one chromosome of 2801970 bp, and three mega-plasmids of 642681, 395118, 311736 bp, respectively. The total genome sequence has been recently completed (Lin *et al*, manuscript in preparation), and a structural genomics project is followed to study the structures of proteins encoded in this genome and to study the alkali adaptation mechanism exhibited by this alkali-tolerant bacterium compared with their non-alkaline counterparts.

The crystal structure of the DFA0005 protein complexed with α-ketoglutarate (AKG) from an alkali-tolerant bacterium *Deinococcus ficus* has been determined to a resolution of 1.62 Å. The monomer forms an incomplete α7/β8 barrel, with a protruding α8 helix that interacts extensively with another subunit to form a stable dimer of two complete α8/β8 barrels. The dimer is further stabilized by four glycerol molecules situated at the interface. One unique AKG ligand binding pocket per subunit is detected. Fold match using the DALI and SSE servers identifies DFA0005 as belonging to the isocitrate lyase / phosphoenolpyruvate mutase (ICL/PEPM) superfamily. However, further detailed structural and sequence comparison with other members in this superfamily and with other families containing AKG ligand indicate that DFA0005 protein exhibits considerable distinguishing features of its own and can be considered a novel member in this ICL/PEPM superfamily.

P1-005

The Structure of UMP kinase/GTP Complex from *Xanthomonas campestris* Reveals a Novel GTP-binding Site and Provides Structural Insights into the Allosteric Mechansim by GTP

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Uridylate kinase (UMPK) is a key enzyme in pyrimidine biosynthesis. It catalyzes the ATP dependent phosphorylation of UMP to UDP, which is further phosphorylated by nucleotide diphosphate (NDP) kinase to UTP. UTP then serves as a precursor for RNA biosynthesis. UDP can also be reduced to dUDP, leading to the synthesis of dTTP and dCTP that serve as precursors for DNA biosynthesis. Due to its importance in nucleic acid biosynthesis, UMPK is ubiquitous in every living organism, including bacteria, archaea, and eukarya. However, UMPKs of bacterial origin are very different from the UMPKs of eukaryotic origin. In general, the eukaryotic enzymes have a broader substrate preference, exhibiting dual specificity toward UMP or CMP, while the enzymes of bacteria origin exhibit a more dedicated UMP-specific activity, and appears to be essential for bacterial growth. Thus, bacterial UMPKs may represent potential targets for developing antibacterial drugs. Furthermore, the regulation of prokaryotic UMPKs seem to be rather complex. Usually UMPKs are stimulated by GTP and inhibited by UTP, which make sense as the need for pyrimidine nucleotide biosynthesis will be high when the supply of purine nucleotides is high, and low when the end-product of the pyrimidine nucleotide accumulates to a high level. But the mechanism by which GTP stimulate UMPKs appear to be different from organism to organism.

We have successfully determined the tertiary structures of apo-form and GTP-bound form of UMPK from the plant pathogen *Xanthomonas campestris* using crystals grown under a strong magnetic field by X-ray diffraction methodology. The flexible ATP-binding and UMP-binding loop structures were clearly defined in our apo-form structure. A novel GTP-binding site located in the central hole of the hexameric interface in the UMPK/GTP complex structure is also observed. Detailed structural analyses between the apo-form and the GTP-bound form reveal significant loop shifting accompanying GTP binding, which may be correlated with its allosteric control mechanism.

P1-006

XC1028 from *Xanthomonas campestris* Adopts a PilZ Domain-like Structure Yet with Trivial c-di-GMP Binding Activity

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Cyclic di-GMP (c-di-GMP) has emerged as an important secondary messenger, controlling a variety of cellular processes, mainly in biogenesis of extracellular components, flagella and pili, and exopolysaccharide synthesis in diverse bacteria. It is thus proposed to be a key regulator in bacterial evolution, during its transition from a single-cellular and motile entity to a multicellular and sessile entity. Since bacterial motility is often correlated with bacterial pathogenecity, the study of c-di-GMP turnover and its interaction with receptors are important biochemical issues.

Significant progress in elucidating the process of c-di-GMP turnover has been achieved in the past few years; the synthesis of c-di-GMP is generally believed to be carried out by cyclases encoded in the GGDEF domain-containing proteins, and its degradation by c-di-GMP specific phosphodiesterases encoded in the EAL and HD-GYP domain-containing proteins. However the molecular mechanisms of c-di-GMP action remain essentially unknown, due to the lack of identified c-di-GMP receptors. Recently, PilZ domain (Pfam PF07238) was proposed to be the c-di-GMP binding target from a bioinformatics study, which was also experimentally confirmed in the PilZ domain-containing proteins VCA0042 in *Enterobacteria* and PA4608 in *Vibrio cholerae*.

The c-di-GMP receptor protein in *Vibrio cholerae* has been determined by NMR method (1YWU), and that from *Enterobacteria* (1YLN) by X-ray crystallography. A co-crystal structure of PilZ/c-di-GMP has also been reported (2RDE), providing more structural insight into the conformational changes brought about by c-di-GMP binding. The PilZ domain was found to contain five highly conserved amino acid residues RxxxxR, D/NxSxxG that are proposed to be involved in c-di-GMP binding.

XC1028 from *Xanthomonas campestris* was annotated as a type IV pillus assembly protein. Its crystal structure has been determined to a resolution of 2.5 Å using the MAD approach. Although it bears little sequence similarity with the PilZ domain from *Vibrio cholerae* (1YWU determined by NMR method), it does adopt a similar tertiary structure, yet with some significant differences in the architecture. Discovery of novel member of PilZ domain proteins will provide more impetus to the study of c-di-GMP actions, important in controlling bacterial pathogenicity.

P1-007

High Resolution XcRecX Crystal Structure Provides Structural Insights into the Inhibition Mechanisms Against RecA Functions

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The bacterial RecA is a multifunctional protein, possessing a variety of activities such as ATP hydrolysis (as an ATPase), LexA repressor cleavage (as a coprotease), homologous DNA strand exchange (as a recombinase), and SOS responses when assembled on ssDNA to form a helical nucleoprotein filament. Although homologous recombination plays important roles in maintaining the structural and functional integrity of a genome, proteins associated with this activity need to be tightly controlled in order to prevent unnecessary DNA exchanges that may be lethal. Recently, a plethora of proteins that exhibit regulatory function of the RecA activities, including RecABC, RecF, RecO, RecR, DinI, RdgC, and UvrD proteins etc. have been discovered. However, regulatory proteins affecting the RecA functions are not completely understood, and new members are being added to this regulatory network each year.

RecX is a recent addition to this network. It was first described in 1993 in *Pseudomonas aeruginosa* as an open reading frame (ORF) located downstream of *recA*. Since then, many *recX* homogoues have been found in other microorganisms, including *Xanthomonas campestris*. *RecX* is usually located downstream of *recA*, and RP-PCR experiments indicated that *recX* is co-transcribed with *recA* in *E. coli*, *M. tuberculosis*, *S. lividans*, and *T. ferrooxidans*. In *X. campestris*, on the contrary, the *recX* gene and the other two genes *recA* and *lexA* that are important in the SOS regulatory system, are individually transcribed from their own promoters, although they are situated in the same *lexA-recA-recX* operon. These indicate that the regulation mechanism of the SOS system in *X. campestris* may be very different from that in other bacterial species. Until to date, the structural information of RecX is still rare, although RecX has been shown to function as potent inhibitor against the various RecA activities, such as the ATP hydrolysis, LexA repressor cleavage, homologous DNA strand exchange, and RecA filament capping *in vitro*. To better understand the function of RecX, we have, in the present report, determined the crystal structure of RecX from *Xanthomonas campestris* to a high resolution of 1.6 Å. The structure reveals that XcRecX adopts a novel tandem repeat of three-helix bundle. The docking study of RecX with RecA was also performed to reveal the possible inhibition mechanism of RecX toward the various activities of the RecA filament.

P1-008

Structural insights into TDP-43 in nucleic acid binding and protein assembly

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Mutations, deletions, misfolding and aggregation of protein molecules are related to numerous genetic and sporadic diseases. TAR DNA-binding protein 43 (TDP-43) is such a protein that its function and aggregation is directly linked to the common lethal genetic disease of cystic fibrosis (CF), and two neurodegenerative disorders: frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS). TDP-43 was firstly characterized as a transcriptional factor bound to the TAR DNA to repress the transcription of HIV-1 gene. Subsequently, TDP-43 was identified as a RNA-binding protein and plays a role as a splicing repressor in the splicing of CFTR (cystic fibrosis transmembrane conductance regulator) gene. Recently, it was further identified as the major disease protein of the pathogenic inclusions in the brains cells of FTLD-U and ALS patients. To address the molecular basis of TDP-43 in DNA/RNA binding and protein assembly, we characterized its nucleic-acid binding activities by filter binding assays and determined the crystal structure of a truncated TDP-43 in complex with a 10-mer DNA at a resolution of 1.65 Å. We found that TDP-43 binds both single-stranded and double-stranded DNA/RNA, and it prefers to bind TG-rich DNA and UG-rich RNA sequences. The crystal structure of the truncated TDP-43/DNA complex revealed the structural basis for its preference in TG/UG-rich sequences. Most interestingly, we show here that the RRM2 of TDP-43, the fragments identified in TDP-43 inclusions, may assemble into a “supęr β-helix” structure, implicating its possible role in forming non-amyloid fibril aggregates in the neurodegenerative diseases of TDP-43 proteinopathies.

P1-009

RNA degradation by Escherichia coli polynucleotide phosphorylase

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RNA degradation is an important biological process in regulating mRNA levels in cells. In eukaryotes, a protein complex, exosome, plays a major role for mRNA degradation in cytoplasm. The bacterial trimeric polynucleotide phosphorylase (PNPase) shares similar sequence and domain organization and bears similar function in mRNA degradation to that of eukaryotic exosome. We determined the crystal structure of *E. coli* PNPase at a resolution 2.7 Å. PNPase, containing two RNase PH domains and a S1/KH domain, forms a trimer. The six RNase PH domains of the trimeric PNPase fold into a ring-like structure containing a central channel for RNA binding and degradation. This ring-like architecture of bacterial PNPase is similar to those of archeal and human exosomes, supporting the hypothesis that a common mechanism for RNA degradation is used in all kingdoms of life. We also found that the wild-type PNPase produces a final end product of 4-nucleotide RNA. However, a R106A mutant and a S1/KH domain truncated PNPase produce different RNA end products, indicating that R106 located inside the channel and S1/KH domain are likely involved in RNA recognition.

P1-010

Structural and Functional Analyses of XC5397 from *Xanthomonas campestris*: A Gluconolactonase Important in the Glucose Secondary Metabolic Pathways

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In living cells, most of the consumed glucose is catabolized via glycolysis to produce pyruvate, which in turn is oxidized via the citric acid cycle to generate ATP. However, some secondary metabolism pathways for glucose are present to produce compounds crucial for cell survival, for examples, the pathways that produce pentose phosphates and ascorbic acids (Vitamin C). XC5397 from *Xanthomonas campestris* is annotated as a gluconolactonase from the UniProtKB/Swiss-Prot (http://us.expasy.org/sprot/) database. It bears significant sequence identities to a variety of bacterial gluconolactonases, such as Q4UZ89 (97.7%), Q3BY55 (81.3%), Q5GUU3 (80.0%), Q1NHQ3 (43.8%), A1G3J1 (42.4%), A4X2N5 (40.9%). Interestingly, it is also classified as belonging to the SMP-30/Gluconolaconase/LRE-like superfamily that contains many mammalian sequences. The SMP-30 (Senescence Marker Protein 30) is a recently characterized protein that was found to act as an “anti-aging” factor in mice.

Gluconolactonase was first identified more than 50 years ago. However, until to date, no tertiary structure of any gluconolactoase has yet been published. In the present manuscript, we report the first crystal structure of a bacterial gluconolactonase determined to a resolution of 1.68 Å using X-ray diffraction. It comprises a dimer of two identical subunits of molecular weight 35 kDa (305 residues each). The subunit folds into well recognized six-bladed β propeller domain. However, it has a characteristic long N-terminal sequence of forty-three residues that wraps around the outermost β strands of blades 5, 6, and 1 to further stabilize the “Velcro” closure of the propeller domain. Each monomer also contains two well bound calcium ions in the central water-filled tunnel. One calcium ion is situated at the “top” of the propeller, coordinating directly with the side chain oxygen atoms of Glu48, Asn134, Asn191, and Asp242, and is possibly involved in the catalysis reaction. The two subunits interact extensively through a number of loop residues to form a distinct arch-type dimer, stabilized by four H-bonds, four salt bridges, and two disulfide bonds, and many bridging water molecules. Two loosely bound calcium ion was also detected in the interface. Enzyme assay using several γ- and δ-lactones with 5-, and 6-carbon indicates that XC5397 is a gluconolactonase specific for D-glucono-δ-lactone.

P1-011

Crystal Structure of Pollen Group 4 Allergen from Cynodon dactylon (Cyn d 4)

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Allergic diseases such as atopic dermatitis, rhinitis and asthma afflict approximately 20% of the world population. Airbone grass pollen has been considered as one of the most important sources of outdoor allergens. In warm temperate and sub-tropical climates, bermuda grass (*Cynodon dactylon*) is one of the dominant allergen sources. Cyn d 4, identified as a glycoprotein and the first flavinylated allergen, is one of the major allergens in bermuda grass pollen. Here we have determined the crystal structure of Cyn d 4 isolated directly from grass pollen at 2.15-Å resolution. The FAD cofactor is cross-linked to Cyn d 4 via the C^6 atom and the 8α-methyl group of the isoalloxazine ring with Cys^{177} and His^{113}, respectively. A structural similarity search by DALI revealed that Cyn d 4 belongs to the vanillyl-alcohol oxidase flavoprotein family, in which a detailed comparison illustrates the structural conservation as well as the divergence between the members. The protein is composed of an N-terminal FAD-binding domain and a C-terminal substrate-binding domain. The FAD-binding domain is much more superimposable than the substrate-binding domain perhaps because of similar FAD recognition but distinct substrate binding. Interestingly, these flavoenzymes utilize many backbones for the FAD binding, and hence the sequences of the corresponding residues are divergent but their spatial positions are convergent in four regions. In contrast, the highly diverse architectures of the substrate-binding sites are needed for the various substrates ranging from a small lactate to the bulky cholesterol and long chain oligosaccharides. Compared with other members, Cyn d 4 possesses an open, large and hydrophobic binding pocket, revealing the characteristics of its substrate.

P1-012

Complex Structures of a Potent Antimicrobial Drug Target RibG Insights into the substrate specificity, catalytic mechanism and inhibitor design

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Bacterial RibG composed of an N-terminal deaminase and a C-terminal reductase, is a potent target for antimicrobial agents because it catalyzes the two consecutive deamination and reduction steps in the riboflavin biosynthesis, and because it is the only protein to date to share high structure homology to the pharmaceutically important dihydrofolate reductase. Reducing reagents such as dithiothreitol were needed in substrate preparation, perhaps prevention against the compound oxidation. The complex structures of *Bacillus subtilis* RibG at 2.56-Å resolution reveals distinct interaction networks and hence may be useful in guiding drug design. Upon the substrate binding to the deaminase domain, significant conformational changes were induced in two loops moving toward for interaction with the ribosyl and phosphate moieties, respectively. The phosphate forms hydrogen bonds with Asn^{23}, His^{49}, His^{76}, Lys^{79}, and Thr^{80}, while the ribose contacts with Asp^{101} and Asn^{103}. Mutational analyses displayed that Glu^{51} and Lys^{79} involved in the proton transfer and phosphate binding, respectively, are essential for deaminase activity. Interestingly, a new conserved amino-binding hole is identified for facilitation of the substrate binding, intermediate stabilization and product release. Unexpectedly, in the reductase domain, the electron density map demonstrates a ribitylimino intermediate bound at the active site. The pyrimidine ring forms hydrogen bonds with Lys^{151}, Ser^{167}, Ile^{170} and Thr^{171}, the ribityl group with Asp^{199} and Glu^{290}, while the phosphate with Arg^{183}, Ser^{202}, Leu^{203}, and Arg^{206}. Lys^{151} has been evolved to ensure specific recognition of the deaminase product through its amino group interacting with the carbonyl moiety, but repelling the substrate amino group. Glu^{290}, instead of previously proposed Asp^{199}, may assist in the proton transfer in the reduction reaction.

(1) Chen S.C., *et al., J. Biol. Chem.,* **281,** 7605-7613 (2006).

P1-013

Structural and functional insights into human Tudor-SN, a key component linking RNA interference and editing

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Human Tudor-SN is involved in the degradation of hyper-edited inosine-containing microRNA precursors, thus linking the pathways of RNA interference and editing. Tudor-SN contains four tandem repeats of staphylococcal nuclease-like domains (SN1 to SN4) followed by a tudor and C-terminal SN domain (SN5). Here we showed that Tudor-SN requires tandem repeats of SN domains for its RNA binding and cleavage activity. The crystal structure of a 64-kD truncated form of human Tudor-SN further shows that the four domains, SN3, SN4, tudor and SN5, assemble into a crescent-shaped structure. A concave basic surface formed jointly by SN3 and SN4 domains is likely involved in RNA binding, where citrate ions are bound at the putative RNase active sites. Additional modeling studies provide a structural basis for Tudor-SN's preference in cleaving RNA containing multiple I·U wobble-paired sequences. Collectively these results suggest that tandem repeats of SN domains in Tudor-SN function as a clamp to capture RNA substrates.

P1-014

Glucooligosaccharide Oxidase from *Acremonium strictum*: Crystal Structure, 6-*S*-Cysteinyl, 8α-*N1*-Histidyl FAD and Enzyme Mechanism

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Glucooligosaccharide oxidase (GOOX) from *Acremonium strictum* catalyzes the oxidation of a variety of carbohydrates to the corresponding lactones with high selectivity for cello- and maltooligosaccharides. The crystal structures of GOOX alone or with substrates including 5-amino-5-deoxy-cellobiono-1,5-lactam, cellobiose, cellotriose, cellotetraose, and lactose, show the substrate preference with an open carbohydrate-binding groove allowing the accommodation of higher oligosaccharides. The complex structures also display the preference of a β-D-glucosyl residue at the reducing end with the conserved Y429 acting as a general base to abstract the OH^1 proton in concert with the H^1 hydride transfer to the flavin N^5. Moreover, the first known bi-covalent flavinylation, a 6-*S*-cysteinyl, 8α-*N1*-histidyl FAD, is demonstrated which is cross-linked to C130 and H70. Single mutants of H70A/S/C/Y and C130A still retain the covalent FAD, indicating that flavinylation at these two sites is independent. The wild-type protein exhibits a midpoint potential of +126 mV. The H70A, H70C and C130A mutants with a redox potential of +70 mV, +106 mV and +61 mV, respectively, still possess lower activity, but the k_{cat} values reduced to about 2%, 32%, and 5%, while the flavin reduction rate to 0.6%, 9% and 14%, compared to the wild-type GOOX. The C130A crystal structure provides direct evidence for a novel function of 6-*S*-cysteinylation in assistance of substrate binding. Finally, the double H70A/C130A replacement abolishes the covalent linkage, FAD binding and enzyme activity. The wild-type enzyme is more heat and guanidine-HCl resistant than the mutants. Therefore, the bi-covalent flavin linkage enhances not only the cofactor binding, the redox potential, the substrate binding but also the stability of the protein structure.

(1) Huang *et al*. (2005) *J. Biol. Chem.* **280**, 38831-38838

(2) Huang *et al*. (2008) manuscript in submission

P1-015

Crystal structure of the human FOXO3a-DBD/DNA complex suggests the effects of post-translational modification

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FOXO3a is a transcription factor of the FOXO family. The FOXO proteins participate in multiple signaling pathways, and their transcriptional activity is regulated by several post-translational mechanisms, including phosphorylation, acetylation and ubiquitination. Because these post-translational modification sites are located within the C-terminal basic region of the FOXO DNA-binding domain (FOXO-DBD), it is possible that these post-translational modifications could alter the DNA-binding characteristics. To understand how FOXO mediate transcriptional activity, we report here the 2.7 Å crystal structure of the DNA-binding domain of FOXO3a (FOXO3a-DBD) bound to a 13-bp DNA duplex containing a FOXO consensus binding sequence (GTAAACA). Based on a unique structural feature in the C-terminal region and results from biochemical and mutational studies, our studies may explain how FOXO-DBD C-terminal phosphorylation by protein kinase B (PKB) or acetylation by cAMP-response element binding protein (CBP) can attenuate the DNA-binding activity and thereby reduce transcriptional activity of FOXO proteins. In addition, we demonstrate that the methyl groups of specific thymine bases within the consensus sequence are important for FOXO3a-DBD recognition of the consensus binding site.

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P1-016

Crystal structure of CRN-4: implications for domain function in apoptotic DNA degradation

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Programmed cell death (apoptosis) is an essential biological process for development, maintenance of appropriate cell number and defense against virus infection. One of the major biochemical features of apoptosis is chromosome DNA fragmentation. Cell death related nuclease 4 (CRN-4) is one of the apoptotic nucleases involved in DNA degradation in *Caenorhabditis elegans*. CRN-4 contains a N-terminal DEDDh exonuclease domain and a C-terminal domain of unknown function. To reveal the molecular basis of DNA fragmentation in apoptosis, we analyzed CRN-4's biochemical properties and determined its crystal structures in apo-form, Mn^{2+}-bound active form and Er^{3+}-bound inactive form. CRN-4 is a dimer with both metal-dependent DNase and RNase activities. It prefers to cleave double-stranded over single-stranded DNA, with optimal DNase activity in apoptotic salt conditions and pH ranges. Comparison of the Mn^{2+}-bound and Er^{3+}-bound CRN-4 structures revealed the geometry of the functional nuclease active site in the N-terminal DEDDh domain. The C-terminal domain bound to a structural zinc ion, termed the Zn-domain, and folded into a novel mixed α/β structure, containing basic surface residues ideal for RNA/DNA recognition. Site-directed mutagenesis further confirmed the catalytic residues in the N-terminal DEDDh domain and the DNA-binding residues in Zn-domain. The structural comparison of CRN-4 to a number of dimeric DEDDh family nucleases, PARN, TREX2 and RNase T, further demonstrated that CRN-4 both dimerizes and interacts with DNA in a unique way. Combining all these data, we suggest a structural model where DNA is bound at the Zn-domain and cleaved at the DEDDh nuclease domain in CRN-4 when the cell is triggered for apoptosis.

P1-017

Crystal structures of the 70-kDa heat shock proteins in domain disjoining conformation

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The 70-kDa heat shock proteins (Hsp70s) are highly conserved ATP-dependent molecular chaperones composed of an N-terminal nucleotide binding domain (NBD) and a C-terminal protein substrate binding domain (SBD) in a bilobate structure. Interdomain communication and nucleotide-dependent structural motions are critical for Hsp70 chaperone functions. Our understanding of these functions remains elusive due to insufficient structural information on intact Hsp70s that represent the different states of the chaperone cycle. We report here the crystal structures of DnaK from *Geobacillus kaustophilus HTA426* bound with ADP-Mg^{2+}-Pi at 2.37Å and the 70-kDa heat shock cognate protein from *Rattus norvegicus* bound with ADP-Pi at 3.5 Å. The NBD and SBD in these structures are significantly separated from each other, and they might depict the ADP-bound conformation. Moreover, a Trp reporter was introduced at the potential interface region between NBD and the interdomain linker of GkDnaK to probe environmental changes. Results from fluorescence measurements support the notion that substrate binding enhances the domain-disjoining behavior of Hsp70 chaperones.

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P1-018

Crystal structure of the *Streptomyces sioyaensis* endo-1,3-β-glucanase at 1.5 Å resolution

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The 1,3-β-glucanase (E.C.3.2.1.39) is classified as member of the family 16 glycosyl hydrolases which hydrolyzes the internal 1,3-β-D-glucosidic linkages in 1,3-β-D-glucans, a major component of cell wall in bacteria and therefore are thought to play an important role of cell defense in plant against the fungal invasion. The microorganisms (eg. Streptomyces) also produce β-1,3 glucanases to suppress the growth of other bacteria and were often used in biotechnological process as anti-fungal reagent.

The crystal structure of the catalytic domain of the endo-1,3-β-gludanase (273 a.a.) from *Streptomyces Sioyaensis* was solved by using the SAD phasing method. The crystal diffracts X-ray beyond 1.5 Å resolution limit and complete native diffraction data containing 38,844 unique reflections with an overall Rmerge of 4.5% and a completeness of 99% were collected on an in-house R-axis IV++ image plate system. The crystal symmetry belongs to an orthorhombic system with unit cell parameters, a=39.514, b=75.962, and c=79.656 Å. The space groups is $P2_12_12_1$ with one molecule per asymmetric unit and the solvent content and the Matthews coefficient (VM) are 40.4% and 2.1 $Å^3$/Da respectively. Currently, the structure is on the structure refinement process. The Rtest is 18% and the Rfree is 21.3% respectively. The enzyme has an overall beta sandwich fold similar to the structure of a *bacillus* 1,3-1,4-β-glucanase. However, the cleft of the active site was narrow on one end which suggested the preference binding specificity for 1,3-β-linkage glucans of the enzyme.

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P1-019

Crystal Structure of Carboxynorspermidine Decarboxylase from *Helicobacter pylori*

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Polyamines, such as putrescine, spermidine and spermine, are essential for the regulation of cell proliferation and differentiation in most organisms. In addition to their roles in cell growth, polyamines function in biofilm formation and motility in bacteria. Norspermidine is a polyamine with similar structure to the common spermidine, and being researched for its use as an antitumor medicine in cancer treatment. Carboxynorspermidine decarboxylase (CANSDC) belongs to pyridoxal 5'-phosphate (PLP)-dependent decarboxylases, and catalyzes the decarboxylation of carboxynorspermidine to norspermidne in norspermidine synthesis. The enzymatic activity of CANSDC is not clear and there is no any structural information of CANSDC available either. Carboxynorspermidine decarboxylase from *Helicobacter pylori* (HpCANSDC), encoded by the *nspC* gene, was expressed and purified with a molecular weight of 47.3 kDa. HpCANSDC shows a low sequence homology with other decarboxylases and lacks their characteristic signature sequence. HpCANSDC was crystallized using ammonium sulfate as a precipitate and the crystal was diffracted to a resolution of 2.2 Å with a space group, $P2_1$. The structural phase was determined by multiwavelength anomalous dispersion (MAD) using seleno-methionine derivative. The overall structure of HpCANSDC consists of two domains, a TIM-like β/α barrel and a β-barrel domain. HpCANSDC forms as a dimer in both the solution and crystal states.

P1-020

The ssDNA Binding Mode of Single-Stranded DNA Binding Protein from Helicobacter pylori

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Single-stranded DNA binding protein (SSB) plays an important role in DNA metabolism, such as DNA replication, repair and recombination. The N-terminal domain of SSB functions as ssDNA binding domain by forming an oligonucleotides/ oligosaccharides binding (OB) fold and the highly conserved acidic sequence of the C-terminal tail plays a crucial role in protein-protein interaction. The crystal structure of C-terminal truncated SSB (residues 1-134) from Helicobacter pylori (HpSSB134) bound to poly(dT)35 has been determined to 2.3 Å resolution. In the HpSSB134-poly(dT)35 crystal, two strands of poly(dT)35 bind to HpSSB134 tetramer. The ssDNA wraps around HpSSB134 by the electrostatic and hydrophobic stacking interactions. To determine the ssDNA binding of HpSSB134 in solution, we used fluorescence titration and gel mobility shift assay to detect the formation of ssDNA-SSB complex. We determined that the binding length of ssDNA for HpSSB134 is 25-30 nucleotides in solution. In conclusion, HpSSB134 functions as a 25-30 nucleotides binding mode in solution, and we also observed two strands of poly(dT)35 binding with HpSSB134 in crystal. Various SSB binding modes may each function selectively in replication, recombination and repair processes.

P1-021

Crystal Structure of the Outer Membrane Protein LipL32 from *Leptospira shermani*

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Tubulointerstitial nephritis is a cardinal renal manifestation caused by pathogen leptospira that most accumulate in the proximal tubules. The outer membrane protein (OMP) from pathogen leptospira is able to induce the inflammatory response in injured renal tubule cells through the Toll-Like Receptor pathway. LipL32 from *Leptospira shermani*, a 32-kD lipoprotein, is one kind of the OMP of and the major pathogenic component, which is absent in the nonpathogenic leptospira. Several evidences indicate that LipL32 is lipid modified at N-terminal cysteine in a manner similar to that of other prokaryotic lipoproteins. LipL32 shows a low sequence identity with other OMPs and the recombinant LipL32 has purified in our laboratory. LipL32 was crystallized using tacsimate as a precipitate and the crystal was diffracted to a resolution of 2.2 Å with a space group, $P4_32_12$. The structural phase of LipL32 was determined by multiwavelength anomalous dispersion (MAD) using seleno-methionine derivative. The crystal structure of LipL32 belongs to an atypical jelly-roll fold with a β-sandwich architecture. The overall structure of LipL32 contains an N-terminal hairpin β-strand, a β-sandwich core with eight antiparallel β strands, and a α-helix in the C-terminus. The protruded N-terminal hairpin β-strand might involve in the protein-protein interaction. The possible lipid binding site of LipL32 might be at the binding cavity made up by the flexible N-terminal loop, the hairpin β-strand, and two β strands from β-sandwich core.

P1-022

Crystal Structure of *Sulfolobus Solfataricus* RadA: a New Conformation and its Implication in dsDNA Binding

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Homologous recombination is a universal mechanism for repairing DNA double strand breaks (DSBs) and injured DNA replication forks. RecA family proteins play a central role in homologous recombination, forming the RecA–ssDNA presynaptic nucleoprotein filament to facilitate the search for homologous double strand DNA (dsDNA) and promote the DNA strand pairing reaction. These proteins include the prokaryotic RecA, archaeal RadA, and eukaryotic Rad51 and Dmc1. Other than the prokaryotic RecA, the archaeal RadA and eukaryotic Rad51 are functionally and structurally more related to each other, and consequently, archaeal RadA offers an applicable model for studying eukaryotic recombination system. Here we report the orthorhombic crystal structure of the *Sulfolobus solfataricus* RadA protein at a resolution of 2.9 angstroms, proving the existence of the left–handed helical filament. The asymmetric unit of our structure consists of two chains: Chain A is similar to the protomer of the left–handed RadA filament in previous studies; the C-terminal domain (CTD) of chain B resemble chain A's but its N-terminal domain (NTD) shows a different conformation as a result of the flexible loop between NTD and CTD. The NTD has been proposed to include the dsDNA binding site. By superimposing various structures and comparing the torsion angles of different RecA family proteins, we identified two loop residues, Asp70 and Arg72, that may regulate the flexibility of NTD and thus influence the dsDNA binding activity of *Sso*RadA. Subsequent biochemical analyses confirmed that these two residues are crucial to homologous recombination. Altogether, our structural and biochemical observations suggest a molecular mechanism of DNA binding to the RecA family proteins, which involves an entire length of the NTD–CTD hinge.

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P1-023

Structural analysis of the α-fucosidase and inhibitor complexes with a wide range of inhibition constants from *micro* to *pico* molar

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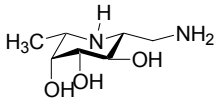
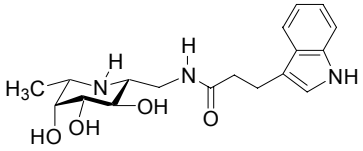
α-Fucosidase is involved in the removal of nonreducing terminal L-fucose residues that are connected to oligosaccharides via α1,2-, α1,3-, α1,4-, or α1,6-linkages. This enzyme plays an important role in many biological events, such as inflammation, infection and cancer. Glycosidase inhibitors have shown great medicinal and pharmaceutical values as exemplified by the therapeutic treatment of influenza virus and non-insulin-dependent diabetes. Previously, we discovered the picomolar, slow tight-binding inhibitors against the α-fucosidase from *Thermotoga maritima* by a rapid screening for an optimal aglycon attached to 1-aminomethyl fuconojirimycin. The time-dependent inhibition displays the progressive tightening of enzyme-inhibitor complex from a low nanomolar K_i to a picomolar K_i* value. In order to gain insight into the interactions between α-fucosidase and the inhibitors and its mechanism, we have determined the crystal structures of protein:inhibitor complexes of Core1 (Ki = Ki* = 16.3 ± 2.5 nM, see below), F10 (Ki* = 0.469 ± 0.14 pM) and F10-2C-O (Ki* = 231.4 ± 7.20 pM) using the molecular replacement method and applying the published α-fucosidase structure as the model.

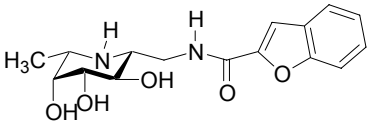
The structural results revealed that two loops of amino acids 46~65 and 266~275, which were missing in the previously published structures, can be clearly seen in the protein complexes with all the inhibitors of Core1, F10 and F10-2C-O. These were due to extra interactions of the inhibitors with the residues in both loops. The closure of these two loops in the protein:in hibitor complexes can explain why these inhibitors showed much higher affinity than fucose to α-fucosidase. Also, F10 show higher affinity than Core1 and F10-2C-O to α-fucosidase, because its flexibility of the tail to fit into the catalytic pocket and thus have more hydrophobic interactions and a tighter binding. In summary, these data describe the detailed interactions between the inhibitors and α-fucosidase and thus are useful for developing potent inhibitors in the future.



Core1



F10



F10-2C-O

P2-001

Rapid Data Collection of Multidimensional NMR Experiments by Projection NMR Spectroscopy

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The data collection time for a typical three-dimensional triple resonance experiment can take up several days of NMR spectrometer time. This can cause a problem for a shared NMR facility with limited number of spectrometers; and also for proteins with limited stability. One approach to shorten the data collection time is the projection-reconstruction procedure, proposed by Kupče and Freeman[1]. In this approach (projection reconstruction-NMR abbreviated as “PR-NMR”), only several 2D sub-spectra are required in order to reconstruct a full 3D spectrum. We have established a suit of 3D triple resonance experiments for sequential backbone resonance experiments. ^{15}N-semi constant time scheme has also been implemented to enhance digital resolution; options for TROSY and deuterium decoupling are also available. Substantial amount of time saving for data collection has been achieved for several proteins from our facility users. In addition, we have also implemented 3D, 4D and 6D automated projection spectroscopy (APSY). In addition to time saving, these rapid high dimensional experiments can also be useful for proteins with limited stability or for spectral with high degree of overlap.

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P2-002

NMR Structure and Backbone Dynamics of Streptopain: Insight into Diverse Substrate Specificity

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Streptopain (streptococcal pyrogenic exotoxin B; SPE B) is a cysteine protease expressed by the pathogenic bacterium *Streptococcus pyogenes*. In order to study the effect of the allelic polymorphism on the protease activity of SPE B, the D9N, G163S, G163S/A172S and G239D mutant proteins have been expressed in *E. coli* and purified to homogeneity. The activities of D9N, G163S, and G163S/A172S mutants were 1-2 folds less than that of wild-type protein. Interestingly, the G239D mutant was ~12-fold less active. However, the G239 residue is located in the C-terminal loop (R223-Q245), and the S230-G240 region cannot be observed in X-ray structure. To understand the role of the C-terminal loop, we used NMR spectroscopy to determine 3D structures and backbone dynamics of 28-kDa mature SPE B and its inhibitor complexes. Unlike X-ray structure of 42-kDa zymogen SPE B, we observed NOEs between the residues in the catalytic loop (V192 and A196) and the residues in the C-terminal loop (A231 and A238) of 28-kDa mature SPE B. The catalytic loop (G188-F197) and the X-ray unobservable region (S230-G240) of 28-kDa mature NMR structure were found to be close to each other. Dynamics analysis of SPEB and the SPE B/inhibitor complexes showed that the catalytic and C-terminal loops were the most flexible regions with low order parameter values of 0.5-0.7, and they exhibited the motion on the ps/ns timescale. These findings suggest that the flexible C-terminal loop of SPE B may play an important role in controlling the substrate binding, resulting in its broad substrate specificity.

P2-003

Reversible oxidation of PmrD from *Klebsiella penumoniae*, implications of a novel mechanism for a redox-sensitive protein in two-component signal transduction

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In bacteria, the two-component signal-transduction system is the most common system for sensing environmental signals and transducing the information into the cell [1]. The small basic protein PmrD, a polymyxin B resistance protein, is the only protein known to be able to connect PmrA/PmrB and PhoP/PhoQ two-component systems [2]. We found that the recombinant PmrD of *Klebsiella penumoniae* (KP-PmrD) initially contains both oxidized and reduced forms, and the reduced form is nearly converted into the oxidized form within two weeks in the air. The CD spectra revealed the secondary structure and the melting temperature are somewhat different between the two forms. Both mass analysis and NMR data confirmed the formation of a disulfide bridge at Cys17 and Cys35 for the oxidized form. Dramatic chemical-shift changes between the two forms were observed, further revealing their structural difference. A detailed comparison of secondary structures between the two forms showed that the major conformational difference is located at C-terminal α-helix. Reversible oxidation of redox-sensitive proteins has been shown to regulate protein phosphorylation in signal transduction and gene expression [3]. The existence of both reduced and oxidized forms of PmrD has not been reported previously, and the observations of the redox-sensitive behavior for PmrD will be discussed in the poster.

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P2-004

Structures and Molecular Interaction Studies of Ferrous Iron Transport Proteins in *Klebsiella Pneumonia*e

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Klebsiella pneumoniae is an enteric gram-negative bacillus causing liver abscess and metastatic meningitis, the most common community-acquired bacterial diseases in Taiwan. Iron acquisition system is one of the most important pathogenic factors from *K. pneumoniae.* Ferrous iron transport proteins including *FeoA*, *FeoB* and *FeoC* play a specific role for acquiring Fe^{2+} from surroundings or their hosts. *FeoB* has been postulated to be a GTPase-coupled ferrous iron permease whereas the roles of *feoA* and *feoC* are unknown. In the present studies, we report the three-dimensional NMR structures of *FeoA* and FeoC and the X-ray crystal structure of the N-terminal domain of *FeoB (*N*FeoB*, residues 1-267*)*. The structure of *NFeoB* confirms its role as a GTPase. Using a variety of triple resonance NMR experiments, partial backbone assignments have been achieved for the N*FeoB*, and complete assignments of ^{1}H, ^{15}N, and ^{13}C resonances in *FeoA* and *FeoC* have been accomplished. Based on NMR chemical shift perturbation and other physico-chemical experimental results, the molecular interactions among the Feo component proteins of *K. pneumoniae* have been investigated and the specificities have been screened as well. The results reveal that recombinant *Feo* proteins are in a biologically active conformation.

P2-005

Structural Characterization of Apolipoprotein E C-terminal domain

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Human apolipoprotein E (apoE) is a polymorphic protein of 299 amino acids with a molecular mass of ~34 kDa. It is composed of two independently folded domains (N-terminal and C-terminal domain) separated by a hinge region. The 22-kDa N-terminal domain (residues 1-191) and the 10-kDa C-terminal domain (residues 216-299) of apoE are responsible for receptor binding and lipid binding, respectively. The APOE gene has three major alleles, ε2, ε3 and ε4, which encode three major isoforms, apoE2, apoE3, and apoE4, respectively. The three isoforms differ from one another only at residue 112 and 158, but have marked differences in their biological functions. ApoE has been known to play a key role in the transport of plasma cholesterol and lipoprotein metabolism. It is a major determinant in cardiovascular disease. ApoE is also highly associated with late-onset familial and sporadic Alzheimer's disease (AD). The lipid- and receptor-binding abilities of apoE are isoform-specific, suggesting that structural characteristics of apoE isoforms may play important roles in their biochemical functions. The three-dimensional structures of the N-terminal domain of apoE isoforms have been solved by x-ray crystallography, but the three-dimensional structure of apoE C-terminal domain is limited. In this study, we characterized the structural properties of apoE C-terminal domain in the presence and absence of lipid by NMR spectroscopy. The structural model of apoE c-terminal domain will be presented.

P2-006

Solution Structure and DNA-binding Properties of the Three Cys_2His_2 Zinc Finger Domains of Tzfp

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The testis zinc finger protein (Tzfp) binds to the upstream flanking sequence of the Aie1 (aurora-C). The mouse *Tzfp* gene contains a N-terminal BTB/POZ domain and three C-terminal C_2H_2 zinc fingers. The zinc finger domain of Tzfp (TZD) binds to the TGTACAGTGT at the Aie1 promoter with high specificity. The interaction between Tzfp and Aie1 promoter may play an important role in the biological function. To gain insight into the transcriptional mechanism, we have applied a variety of biophysical experiments for structural studies on zinc finger domain.

Circular dichroism results clearly revealed that the TZD is coordinated by zinc and the secondary structure is significantly induced. Without zinc or treating with EDTA, the TZD is unfolded. By using heteronuclear multi-dimensional NMR experiments and structure calculation, the solution structure of TZD was determined, which contained a conserved ββα structure with very flexible orientations. Besides, the TZD was complexed with three different lengths of synthesized DNAs (10,12, and 16 mers), which all contain a core motif of (TGTACAGTGT). By comparing the HSQC spectra between free TZD and it's complex, we found that many residues located in all regions of TZD showed chemical-shift perturbations, suggesting that TZD wrap duplex DNA completely. Surface plasmon resonance (SPR) assay indicated that TZD in complex with different lengths of DNA has similar K_D value, $1.2x10^{-8}$, $1.3x10^{-8}$, $3.4x10^{-8}$,respectively. On the other hand, in gel shift assay, the 16-mer DNA-TZD complex obviously showed band shift but the 16-mer mutant (16m5FR) did not, which is in good agreement with NMR result showing there is no significant shift change for the mutant. In summary, our study clearly indicates that TZD binds to Aie1 promoter with high specificity and that binding affinities of TZD with different lengths of DNA containing TGTACAGTGT are similar.

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P2-007

Structural Study of Myb1 DNA-Binding Domain Complexed with the Sequence-Specific Consensus DNA in Trichomonas vaginalis

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Trichomonas vaginalis is the causative agent of trichomoniasis which is one of the most common sexually transmitted diseases, and also independently associated with a variety of adverse health consequences, such as increasing human immunodeficiency virus (HIV) transmission, chronic prostatitis, and cervical cancer, etc. The *ap65-1* (an iron-inducible virulence and adhesion protein gene) promoter contains two Myb recognition elements (MREs) in *T. vaginalis*. Myb1 interacts with A-AACGAT spanning MRE-1/MRE-2r, and ATCG in MRE-2f, and it critically regulates multifarious *ap65-1* transcription via differential selection of multiple promoter sites upon environmental changes. To further gain insight into DNA binding characteristics of *tv*Myb1, we have applied a number of biophysical techniques on *tv*Myb1$_{35\text{-}141}$, R2R3 DNA binding domain of *tv*Myb1, as well as their complexes with MREs. CD spectrum clearly indicated that *tv*Myb1$_{35\text{-}141}$ is a typical α-helical conformation and similar as the Myb-related R2R3 domain. The equilibrium constants of *tv*Myb1$_{35\text{-}141}$ with MRE-1/MRE-2r and MRE-2f derived from surface plasmon resonance experiments are 1.23×10^{-9} M and 1.24×10^{-9} M, respectively. We have finished the NMR resonance assignments of *tv*Myb1$_{35\text{-}141}$ and the complex of *tv*Myb1$_{35\text{-}141}$ with DNAs. Moreover, the NMR chemical shift perturbations were used to map the binding interface of *tv*Myb1$_{35\text{-}141}$ with MRE-1/MRE-2r, or with MRE-2f, including residues V36, F38, T39, N69, Q72, E75, N78, Y80, N82, L85, R86, T87, N110, S120, N122, N123, and N126. On the basis of the shift perturbation results, we generated the complex model by using HADDOCK, the model shows that *tv*Myb1$_{35\text{-}141}$ recognizes the major groove of DNA with the residues on α3 in R3 domain, binds the vicinal minor groove with loop between R2 and R3 domain, and contacts the adjacent flexible flank of DNA with the N-terminal L1 in R2 domain. These biophysical studies should provide valuable information for further understanding how *tv*Myb1 regulates *ap65-1* gene.

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P2-009

Vapreotide-SDS micelle interactions via NMR spectroscopy

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Nuclear Magnetic Resonance spectroscopy has been applied to investigate the interaction between vapreotide and SDS micelle. Base on the analysis of chemical shift deviation, NOE cross-peaks and coupling constants, the backbone conformation of vapreotide, under micellar environment, was β-strand at N-terminal, D-Phe^{1}–D-Trp^{4} and partially bend structures at C terminal, Val^{6}~Trp^{8}, with aβ-turn centered at D-Trp^{4}-Lys^{5}. According to temperature coefficient and hydrogen deuterium exchange rate for backbond amide proton of each residue suggest the existence of a hydrogen bond formed between Val^{6} NH and Tyr^{3} C=O hydrogen bond. The close proximity between the side chains of D-Trp^{4} and Lys^{5} is confirmed by the upfield shift observed for the Lys^{5} NH resonances, which may be caused by the ring current D-Trp^{4} aromatic side chain. The association constant of vapreotide-micelle complex is estimated by a two-state approximation based on the diffusion coefficients for vapreotide in aqueous and micellar solutions, respectively. The diffusion coefficient is measured by PFG-NMR spectroscopy. The binding constant is about 6.06×10^{2} M^{-1}, which implied that the interaction between vapreotide and SDS micelle was spontaneous under current condition. The interaction between vapreotide and SDS micelle is also monitored by the blue shift and attenuation of fluorescence signal of peptide upon the introduction of micelle. The correlation between the relative positions in the aromatic side chain network of the peptide, base on the current conformation, and its receptor selectivity is further discussed.

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P2-010

Expression in *Pichia pastoris* and Backbone Dynamics of Margatoxin and Agitoxin2; Implications of Their Functional Differences

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Margatoxin (MgTX) and agitoxin 2 (AgTX2) are alpha-class scorpion toxins which possess a similar architecture. Although they share a common fold and high degree of sequence similarity, AgTX2 has higher potency towards Kv1.3 and shaker channels. To study their function and dynamics relationships, we expressed MgTX and AgTX2 in *Pichia pastoris*. Based on our NMR analysis, both MgTX and AgTX2 expressed in *P. pastoris* possess the same structures as those of native proteins. The global correlation time (te) for two toxins was found to be similar time scales (~1.86ns). Order parameters (S^2) data suggest that helix, loop2, strand 2, β-turn and strand 3 are rigid in both the toxins. Strand1 in MgTX and loop1 in AgTX2 are flexible. However, closer examination of backbone motions of conserved and key residues revealed the differences over ps-ns to us-ms time scale motions in both the toxins. Most of the conserved and key residues showed substantially rigid motions with minor exceptions. The N-terminus of strand2, turn structure and C-terminus of strand3 are flexible in AgTX2 but the corresponding backbones of MgTX are rigid. The main interacting surfaces, beta hairpin (strand2-turn-strand3), are predominantly rigid in both the toxins. Comparison of dynamics properties of MgTX and AgTX2 with other members of scorpion toxins, charybdotoxin (ChTX), suggests that differences over ps-ns and μs-ms time scale motions along the protein sequence may play an important role in their specificity. The results thus not only provide the first direct evidence that highly disulfide-bonded scorpion toxins can be expressed in *P. pastoris* with the correct fold but also the dynamics data of two toxins furnish new information about their backbone dynamics.

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P2-011

Roles of structure and structural dynamics in antibody recognition of allergen proteins: A NMR study on *Blomia tropicalis* major allergen

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Blo t 5 is a 14KDa major allergen from *Blomia tropicalis* mites, and shows strong IgE reactivity with up to 90% of the asthmatic and rhinitis patients' sera. Results from NMR investigation on ^{13}C ^{15}N isotopically enriched Blo t 5 illustrated that Blo t 5 tumbles slower compared to globular proteins of its size, and exhibits short transverse relaxation time (*T2*) of ~55ms. The solution structure of Blo t 5 as solved by NMR, comprises of three long α-helices, forming an up and down coiled-coil bundle with left-handed twist, and is the 1st major allergen identified with triple helical bundle fold. Classical NMR methods were used to study Blo t 5 interaction with the Fab' of specific monoclonal antibody, mAb 4A7. The Chemical shift perturbation values are deduced after assigning Blo t 5 amides in complex with Fab'. The mAb epitope comprises of two closely spaced surfaces, I and II, connected by a sharp turn, bearing critical residues Asn46 and Lys47 on one surface, and Lys54 and Arg57 on the other. Epitope localization was confirmed by site directed mutagenesis using Blo t 5 mutants. This discontinuous epitope overlaps with the human IgE epitope(s) of Blo t 5. Furthermore, ^{15}N relaxation and Modelfree analysis were used to study backbone dynamics of Blo t 5. Epitope surface II of Blo t 5 experiences conformational exchange on ms-μs range and it is also the interface of stronger interaction with the antibody. This slow motion is probably attributed to the unfavorable burial of the acidic Glu108 residue in the hydrophobic core. This unique motion is suspected as an important protein dynamics in allergen-antibody recognition. In summary, we demonstrated that the mapping of conformational epitopes of monoclonal antibody is an effective tool for identifying critical residues recognized by polyclonal human IgEs, and the knowledge gained here is critical for the design of hypoallergenic Blo t 5 variants for safe and effective immunotherapy.

P2-012

NMR Solution Structure of KP-TerD, a Tellurite Resistance Protein from *Klebsiella pneumoniae*

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Klebsiella pneumoniae belongs to the proteobacteria and the bacterium shares genetic phenogeny with other closely related species of the family Enterobacteriaceae, such as *E. coli, Salmonella, Shigella, and Yersinia*. *K. pneumoniae* is an enteric gram-negative bacillus causing hospital-required infections and infections in debilitated or immunocompromised patient. KP-TerD was isolated from *K. pneumoniae* plasmid and contains 200 amino-acid residues (including 8 extra amino acids His-tag at the C terminal region) and the molecular weight of 21.5 kDa. We have successfully expressed and purified unlabeled and isotopically labeled KP-TerD, and carried out a variety of biophysical experiments. In this poster, we will report the conformational change at different pH values as well as the secondary structure at neutral pH for KP-TerD based on NMR and CD techniques. KP-TerD shows heat reversible character and the melting temperature is 81.2℃. Interestingly, we found that polyethylene glycol (PEG20000) can definitely improve NMR quality, particularly enhancing the 3 D spectra. KP-TerD is composed of 13 βstands and 2 α helices. KP-TerD is quite stable and shows the same NMR spectra even after three years storage at 4 ℃. Three unusual chemical shifts in the upfield (L41, N64) and downfield (G77) are primarily due to the ring-current effect. From the HD exchange rate study, there are more than 70 residues (>35%) with slowly exchanged amino protons, and they are hydrogen bonded or located in the hydrophobic core region. The tertiary NMR solution structure of KP-TerD was determined on the basis of 1893 distance restrains, 48 hydrogen bonds, 103 dihedral angle restrains, and the backbone RMSD is 0.65 angstrom. This structural study may provide valuable information for drug development for combating *K. pneumoniae* -associated disease.

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P2-013

Characterization of peanut skins anthocyanin by NMR

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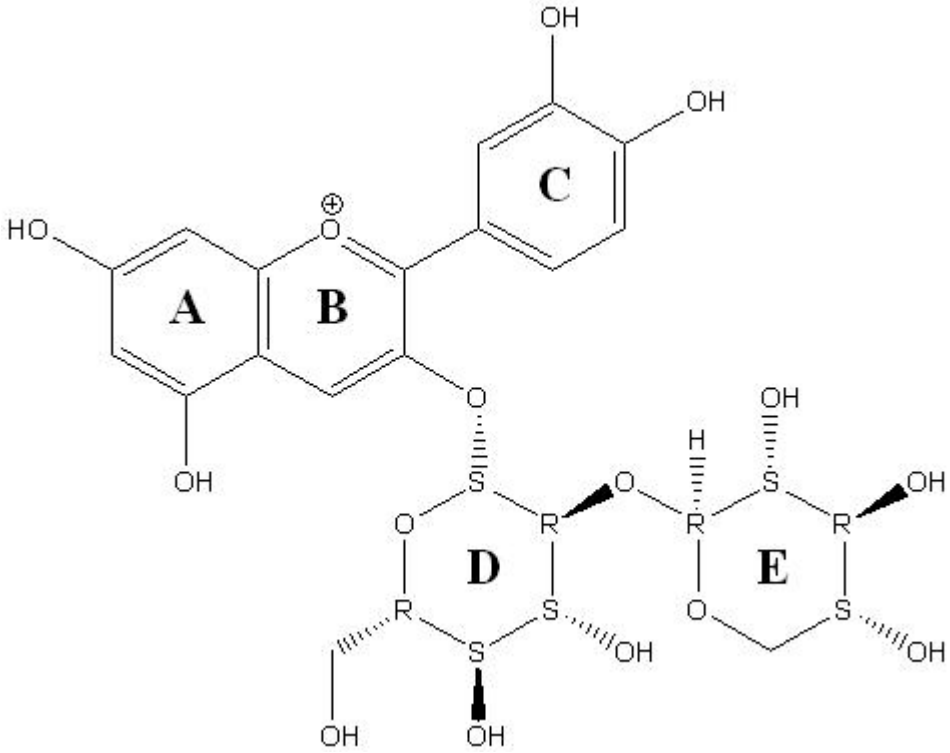
The structure (including the absolute configuration) of 1-Benzopyrylium,2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2-O-β-D-xylopyranosyl-β-D-glucopyranosyl)oxy] (**1**, shown at below), isolated from peanut (*Arachis hypogaea L.*) skin, was studied by high-resolution multi-NMR spectroscopy. The assignments of ^{13}C and ^{1}H signals of **1** were determined by DEPT, 2D COSY unambiguously. Both the anthocyanin structure (A, B, and C) and the side chain sugar structure (D and E) of **1** were elucidated from the ^{1}H-^{13}C 2D HMBC and 2D HMQC. The absolute configuration about the chiral carbons (designated by R and S) was determined by 2D NOESY. The detail will be presented and discussed. (Acknowledgement to the High-Field Biomacromolecular NMR Core Facility supported by the National Research Program for Genomic Medicine and Academia Sinica, Taiwan, ROC)



1-Benzopyrylium,2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2-O-β-D-xylopyranosyl-β-D-glucopyranosyl)oxy] (**1**)

P2-014

DNA Recognition Mechanism of Myb2 Protein Derived from Trichomonas vaginalis

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Trichomonas vaginalis (T. vaginalis), an anaerobic, single celled, parasitic flagellated protozoan, is the causative agent of human trichomoniasis, the most common nonviral sexually transmitted infections (STIs) in the world. Cytoadherence, a crucial part for T. vaginalis to cause an infection, has been reported to involve multiple surface adhesion proteins. The ap65-1 gene was the member of ap65 (adhesion protein 65) multigene family encoding multiple homologous 65-kDa proteins. Recently, novel transcription factors, Myb1 and Myb2 proteins, were found to be involved in the transcriptional regulation of AP65-1 in T. vaginalis. MRE-1/MRE-2r (which overlap) and MRE-2f, which were found to be the multiple Myb recognition elements, regulate multifarious ap65-1 expression, inferring the involvement of Myb-like transcription factors in the transcription machinery of the parasite

The full-length Myb2 protein encoded by the myb2 gene was found to interact with specific sequence contexts spanning MRE-2r and MRE-2f. The truncated Myb2 protein, Myb2x, spanning amino acid sequence 40-156, has been found to retain similar DNA binding affinity. We have investigated the interaction of Myb2x protein with MRE-2r and MRE-2f by the biophysical methods. Moreover, we are determined the structures and dynamics of Myb2x protein, Myb2x-MRE-2r complex, and Myb2x-MRE-2f complex by NMR. The results allow us to pinpoint the molecular mechanism involved in Myb2/DNA interactions.

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P2-015

Structural study of the Thr^{35}-phosphorylated Inhibitor-1

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Protein phosphatase-1 (PP1) is one of the major mammalian serine/threonine protein phosphatases that regulate a large number of cellular functions in many tissues, such as neuronal signaling. PP1 is specifically inhibited by three intrinsic unstructure and acid-, heat-stable protein inhibitors, including inhibitor-1(I-1), DARPP-32 and inhibitor-2. I-1 shares a highly identity in the N-terminal region of 50 residues with DARPP-32. This region contains two inhibitory subdomains that are required for inhibition of PP1. When Thr-35 of I-1 is phosphorylated by cAMP-dependent protein kinase (PKA), it will be converted into a potent inhibitor of PP1. In order to understand the molecular mechanism of the interactions between PP1 from the structural point of view, we performed NMR structural studies of phospho-inhibitor-1. We have prepared ^{13}C and ^{15}N labeled phospho-I-1 for NMR studies. The effect of the phosphorylation on the structure of inhibitor-1 will be presented.

P2-016

The Structure and Dynamics Studies of the Histidine-Containing Phosphotransfer Protein B in *Pseudomonas aeruginosa* PAO1

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Previous study has demonstrated that histidine-containing phosphotransfer protein B (HptB) is an intermediary component for multistep phosphorelay system in Pseudomonas aeruginosa PAO1. The hybrid sensor PA1611 carries out specific signaling phosphate, though HptB, to the cognate regulator PA3346. As assessed by phenotypic changes in the HptB deletion mutant, the pathway is likely to be involved in the regulation of flagellar activity, the chemotaxis response, twitching motility, and biofilm formation in the bacteria. The HptB contains a Cys residue resulting in the formation of intermolecular disulfide bond under oxidative condition. Circular dichroism spectroscopic data suggest that the secondary structure of HptB is predominantly alpha-helical. The three-dimensional structure and the dynamic behavior of HptB are currently under investigation by NMR spectroscopy.

P2-017

Structural Basis of *Neisseria meningitidis* Ag473 Proteins, the Membrane-associated Proteins with different Tandem Repeats (EAVTEAK)

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Neisseria meninggitidis (NM) is an obligate human pathogen that inhabits the upper respiratory tract, from which it occasionally disseminates, causing disease. Invasion results in bacteremia, with possible progression to sepsis, meningitis, and death (1). Recently studies had identified a newly-found lipoprotein Ag473, with 7-amino-acid (EAVTEAK) tandem repeats, is expressed on surface of all NM strained examined. It has important functions for the association between CPS and bacterial cell. Its expression levels increase while entering post-infection and prolong phase. All results suggest Ag473 would be a potential vaccine for immunotherapy (2).

The 3D structure of Ag473 is not solved yet, and little is known about the function of the tandem repeats of this membrane protein. Our goal is to determine the structure of Ag473 and understand the structural insights about Ag473 proteins of the four types of tandem repeats. All Ag473 proteins with different repeats have been cloned and over-expressed. Circular Dichroism (CD) data were collected for all purified proteins to compare their thermo-stabilities and secondary structures in different pH values. To further understand property of this membrane protein, we intend to gain some aspect of solvent-protein interaction from CD results collected in different solvents. NMR chemical shift perturbation experiments would be carried out for all Ag473 proteins to map the location of epitope and to elucidate the molecular mechanism of Ab-Ag interaction. The comparison of various tandem repeats of Ag473 should be able to provide more structural information of the antibody recognition for vaccine development. Structure determination of Ag473 using NMR technique is undergoing. Some preliminary data will be discussed in this poster.

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P3-001

The N-terminal domain of *Escherichia coli* RecA binds double-stranded DNA and promotes strand exchange

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Escherichia coli RecA is the founding member of the RecA protein family, which also includes archaeal RadA and eukaryotic proteins, Rad51 and Dmc1. These proteins bind to single-stranded DNA at a DSB site to form a presynaptic nucleoprotein filament, align this presynaptic filament with homologous sequences in another double-stranded DNA segment, promote DNAstrand exchange and then dissociate. RadA, Rad51, and Dmc1 each contains a similar N-terminal domain (NTD) for dsDNA binding. In contrast, the NTD of RecA was considered only to mediate RecA polymerization for almost 15 years. To solve this long standing puzzle, we present new evidences that RecA NTD is structurally conserved to those of RadA, Rad51 and Dmc1. Mutant analysis further reveals that RecA NTD is also required for dsDNA binding and that this activity is essential for promotion of the strand exchange reaction.

(1) C.D. Lee *et al.* An improved SUMO fusion protein system for effective production of native proteins. *Protein Science* (In press)

(2) C.D. Lee *et al* The N-terminal domain of *Escherichia coli* RecA binds double-stranded DNA and promotes strand exchange (manuscript in preparation)

P3-002

An Aβ40 mutant forms an amyloid-like aggregate and is able to attenuate the cytotoxicity of Aβ40

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The residue immediately preceding each glycine in the 40 amino acid Aβ40 (beta 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 (V24, I32, and V36) 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. Moreover, the mutant peptide V24P, when mixed with Aβ40, can attenuate the cytotoxicity of Aβ40.

P3-003

Structure based alanine scanning of the *Vigna radiate* defensin 1

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Plant defensin is a family of proteins that contain the cysteine-stabilized αβ motif. Proteins of the family exhibit low sequence homolog and high conserved three-dimensional structure. By multiple sequence alignment, the highly conserved spots were analyzed. In this study, a structure guided alanine scanning was perform on the *Vigna radiate* defensin 1 (VrD1) to probe the hot spots that affect the structure stability and biochemical function of the protein. The secondary structure was detected with circular dichroism spectrometry and the secondary structure was well formed in all mutants, even in the absence of the first pair of disulfide bond. It has been reported that the VrD1 is able to inhibit the *Tenebrio molitor α*-amylase (TMA) activities. Alanine substitution of the five residues, M4, K6, G9, W10 and L14, will totally abolish the enzyme inhibition activity of the VrD1. The G9 and W10 may be related to the conformation of the unique 3_{10} helix and the helix was suggested to be important in the enzyme inhibition activities of VrD1. The M4, K6, L14 and D16 may participate in the interaction of the VrD1 and the target enzyme. The loop 3 of the VrD1 has been showed as the major functional loop in enzyme inhibition activities and the positive charge of the loop was important to the enzyme inhibition ability of the VrD1. Two positively charged residues, the K7 and R26, may regulate the enzyme inhibition ability of the VrD1. In general, the mutants with alanine substitution on the structure region expressed lower TMA inhibition abilities than the wild type. The results indicated that not only the surface electrostatics but also the fine tune of structure are important to the enzyme inhibition of VrD1.

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P3-004

The Functional Roles of The Deaminase-Reductase Domain Fusion in RibG

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Bacterial RibG, composed of an N-terminal deaminase and a C-terminal reductase, is a potent target for antimicrobial agents because it catalyzes the two consecutive deamination and reduction steps in the riboflavin biosynthesis, and because it is the only protein to date to share high structure homology to the pharmaceutically important dihydrofolate reductase. The crystal structure of RibG from *Bacillus subtilis* (*Bs*RibG) suggests that fusion of the deaminase-reductase domains is for formation of a stable tetramer but not a substrate transport channel. However, RibGs from *T. martitima* and *E. coli* exist as a dimer. Our structure comparison and mutational analysis showed that these distinct oligomerization states may be resulted from different domain orientations and inter-domain interactions. In order to approach the functional role of the tetramer in *Bs*RibG, dimer variants are generated through disruption of the deaminase interfaces. The interface residues involved in hydrogen bonds or great hydrophobic contacts are selected, and more than ten mutants are generated. All the mutants display a lower melting temperature (Tm) by 3-7 ℃, implying that the deaminase interfaces make a significant contribution for the thermal stability. Upon the guanidine hydrochloride-induced unfolding, all the mutants exhibit a three-state unfolding process, whereas the wild-type protein displays a two-state process. Ultracentrifugation analyses of the recombinant proteins in the presence of various concentrations of guanidine hydrochloride demonstrate the appearance of a dimer intermediate in the mutants but not in the wild-type enzyme. In addition, the dimer variants possess a comparable activity to wild type. Therefore, together with structure studies, the present results suggest that the domain fusion is crucial for stabilization of the protein structure.

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P3-005

Change of phospholipid bilayer induced by the hydrolytic products of phospholipase A2

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Phospholipase a2 (PLA2) is an enzyme which catalyze the hydrolysis of the sn-2 bond in various phospholipids and generate a fatty acid (FA) and a lysophospholipid (lyso-PC). In pure phosphatidylcholine membranes, a lag phase of low initial PLA2 activity is always appearance. However, after this initial lag phase, a sudden increase can occur in the enzyme activity. Much evidence suggests that this abrupt increase in PLA2 activity is due to the accumulation of hydrolytic products molecules.

For understanding of the molecular mechanism of membrane perturbation by the hydrolytic products, dimyristoyl phosphatidylcholine (DMPC) mixed with Lyso-PC and FA in different molar ratio was done to mimic the hydrolytic process. These studies demonstrated that the lamellar structure still be maintained when hydrolytic products below 50% and the distance of repeat unit of lamellar bilayer and phase transition temperature were increased by differential scanning calorimetry and X-ray diffraction methods. Deuterium labeled FA separated the vibration frequency of FA and DMPC/Lyso-PC in infrared spectroscopy. It reveals the more detail structural information on phospholipids membranes. Finally, we used molecular dynamics simulations to address the role of hydrolytic products of PLA2 and explain experimental observations.

P3-006

Two-dimensional binding model of heparin by utilizing a β-sheet presenting miniature protein

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Glycosaminoglycans (GAGs) are polysaccharides with structural and functional diversity. These molecules have been demonstrated to be involved in a wide range of biological activities such as cell adhesion, cell mobility and cell proliferation through interacting with various cellular proteins, such as protease, cytokines, growth factors, adhesion molecules and so on. There are six classes of GAGs, including heparin sulfate (HS), heparin, chondroitin sulfate (CS), dermatan sulfate (DS), keratin sulfate (KS) and hyaluronic acid (HA); of all the GAG family members, heparin is the most well studied and has been used clinically as an anticoagulant since 1935. Recent experiments have shed light on the physiological significance of heparin which is essential for the storage of specific granule proteases in mast cells. The details of the interaction between heparin and positively charged proteins are not known except the electrostatic interactions seem to play an important role in this biomolecular association. With the characterization of more heparin-binding proteins, it was understood that the binding epitopes can also be defined by sequentially remote residues that form an optimal binding surface. However, none of these studies, such as the early studied key structural motifs of heparin-binding sequences XBBXBX and XBBBXXBX, have completely addressed the binding pattern for heparin-protein interactions. Therefore, we use a structure-guided approach, termed “protein grafting”, to elucidate the protein-heparin binding pattern. We targeted the heparin utilizing a thermal stable domain, B1 domain of IgG-binding protein G, because of its firmly packed conformation to accomplish spatial binding information. Through the planer four-beta-sheet scaffold design of B1 domain, the number and position of the positive amino acids within were verified by applying fluorophore-assisted carbohydrate electrophoresis analysis (FACE). The results reveal that the specific spacing and favored residue (arginine) seemed to be critical for heparin binding process.

P4-001

Atomic Force Microscopy of pH Dependent Morphological Changes of *Escherichia coli* Flagella

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Flagella contribute to the virulence of pathogenic bacteria through chemotaxis, adhesion to and invasion of host surfaces. Previous investigations pointed out that a reduction in urethra pH level with a concomitant may reduce the adherent activity of the bacteria. It was also observed that the swarming ability of *Proteus* was inhibited by an alkaline condition of the medium. As flagella are the key factor for the adhesion and mobility of the bacteria, environmental pH may affect these bacterial abilities via affecting the bacterial flagella.

In this study, we firstly tested swarming ability of *E.coil* in pH 7, pH 6 and pH 8. The results showed that swarming ability of *E.coil* was indeed affected by the environmental pH. The best swarming ability of *E.coil* was observed in pH 7, and the worst was in pH 8. Atomic force microscope (AFM) was then applied to characterize the morphology of bacteria flagella in different pH environments. Different diameters of flagella were measured in different pH by the AFM. The diameters of the flagella were 76.83±3.37 nm, 43.66±1.28 nm, and 27.36±0.72 nm in pH 7, pH 6and pH 8, respectively. These measurements were also confirmed by transmission electron microscopy (TEM). Our results indicated a strong correlation between flagella diameters and swarming ability of *E.coli* in different pH environments.

P4-002

Investigating Sterilization Effect of Photocatalyst Carbon-Doped TiO_2 on *Escherichia coli* using Atomic Force Microscopy

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Titanium dioxide (TiO_2) has recently been applied as a disinfectant to prevent bacteria caused diseases. The antibacterial activity of photocatalyst TiO_2 substrates is induced originally by UV irradiation. Recent reports have shown that carbon-doped TiO_2 substrates were able to exhibit photocatalytic activities under visible-light illumination. In this study, atomic force microscopy (AFM) was applied to observe the mechanism of sterilization effect of carbon-doped TiO_2 upon *Escherichia coli*. The AFM results indicate that the roughness of *E. coli* surface was greatly increased by the TiO_2 treatments. In addition, during the treatments, the destruction of the *E. coli* initiated from forming holes at the apical terminus of the bacteria and the holes enlarged until the cells were totally flattened. We also found that the log phase *E. coli* was the most easily affected by the photocatalyst compared with the lag phase and the stationary phase bacteria.

P4-003

Molecular mechanism of plant G-protein system and its signaling regulatory protein, AtRGS1

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Heterotrimeric G-protein system, a well studied subject, serves many important roles in signal transduction and physiologies of mammalian and yeast. Upon ligand activation, GPCR (G-protein coupled receptor) could lead a conformational change in Gα subunit and trigger the replacement of GDP for GTP in Gα. The Gα-GTP, the activated form of Gα, can then initialize a series of signal cascading inside the cells. The signal would be terminated through intrinsic GTPase enzyme activity of Gα which hydrolyzes GTP into GDP and restore Gα to ground state. The GTPase activity of Gα could be further accelerated by a regulator of G-protein signaling (RGS) protein which interacts with Gα and turns off the Gα signaling state. In *Arabidopsis thaliana*, it has been reported that RGS domain of AtRGS1 could accelerate GTPase activity of Arabidopsis Gα subunit, AtGPA1, but little was known about their interaction mechanism. Here, we use two kind of fluorescent probes, BODIPYTR-GTP and Lucifer Yellow, to investigate the function and interaction between these two proteins. Through these two fluorescent probes, we can depict the molecular mechanism of AtRGS1 and AtGPA1 and determine the binding affinity of these two proteins.

P4-004

The role of phenylalanine residues in somatostatin

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When somatostatin (SRIF-14) moves into the micellar environment from aqueous solution, not only blue shift, but also the intensity changes of fluorescencec signal can be observed. Blue shift is an evidence to indicate that tryptophan residue of SRIF-14 locates in the hydrophobic environment of SDS micelles. Results from previous study show that, when lysines (4^{th} , 9^{th}) of SRIF-14 were replaced by alanine, fluorescence quench can be still observed upon the introduction of SDS micelles. In this work four alanine substituted somatostatin analogues (SRIF-14 $F^6 \rightarrow A$ 、SRIF-14 $F^7 \rightarrow A$ 、SRIF-14 $F^{11} \rightarrow A$ 、 SRIF-14 $F^7F^{11} \rightarrow A$) and pharmaceutical active fragment (NH_2-Phe-Phe-Trp-Lys-Thr-Phe-$CONH_2$) are prepared. Upon moving into micellar environment from aqueous solution, blue shift ofλ_{max} in fluorescence spectra can be observed for all peptides, showing that the tryptophan residue for all the peptide was also in the hydrophobic environment of SDS micelles. The fluorescence intensity of SRIF-14 $F^7 \rightarrow A$ 、SRIF-14 $F^{11} \rightarrow A$ and SRIF-14 $F^7F^{11} \rightarrow A$ and the active fragment were quenched upon the introduction of SDS micelles. This observation is similar to that for native somatostatin. On the contrary, the fluorescence intensity of SRIF-14 $F^6 \rightarrow A$ increased upon associating with SDS micelles. These results suggest that the unusual quench effect of native somatostatin fluorescence signal upon interacting with SDS micelle could be resulted from the closely proximation between the side chain of Trp^8 and Phe^6.

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P4-005

Developing of a new drug screening strategy using fluorescently modified RGS (Regulator of G-protein signaling protein) and G-alpha proteins

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Heterotimeric G-protein consists of Gα and Gβγ subunits, involving the signal transferring from GPCR (G protein coupled receptor) to cell inside. RGS (regulator of G protein signaling protein) proteins function as GAPs (GTPase accelerating proteins) by interacting and stimulating the intrinsic GTPase activity of Gα, which consequently accelerate the turning off the signal transition in G-protein system. In resent years, the importance of RGS proteins is escalating, especially in cardiovascular and nerve systems, and many pharmacological studies are focus on the GAP activity of RGS that potentially can reduce side effects or enhance drug efficiency. The need to have a high throughput drug screening method to detect GAP activity efficiencies is obvious. Here we report a method to measure the GAP activity of RGS using Lucifer yellow modified Gαt protein and RGS9-box (the GAP functional domain of RGS9) to real-time monitoring the interaction between them. A total of more than 100% fluorescence increase upon Gα activation can be observed and it is confirmed to be practically useful for reagent screening purpose. Setup of 96 multi-well Multilabel Reader will be further demonstrated for the efficiency of this reagent screening strategy.

P4-006

Early Detection for Human Oral Cavity Cancer Utilizing the Synchrotron-based Infrared Microspectroscopy

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This paper employs synchrotron-based infrared microspectroscopy (SR-IMS) associated with a kinetic study of wax absorptivity for screening and early detection of human oral cavity cancer. The infrared spectral image of peak ratio of 1030 cm^{-1} (glycogen) / 1080 cm^{-1} ($\nu_s PO^{2-}$) revealed that the average of ratio values was lower in the layer of epithelial within tissue than that of others in tissue section, on the contrary, the average of ratio value for epithelial was higher. We proposed that the peak ratio of 1030 cm^{-1} (glycogen) / 1080 cm^{-1} ($\nu_s PO^{2-}$) can be a biomarker for cancer screening. On the preliminary results of kinetic study for wax absorptivity of oral epithelia carcinoma cell (OCEM-1), we found the absorbance of wavenumbers at 2924 cm^{-1} and 2852 cm^{-1} for beeswax-treated OCEM-1 was increased obviously after immersion treatment of xylene for 5 seconds. It was thought that beeswax prefer attaching carcinoma cell but paraffin, moreover, we also found the same tendency for beeswax absorptivity in human oral cavity cancer tissue section. Hence, we proposed that the absorptivity for polarity of wax could be employed to be excellent signpost for screening and the purpose of early detection of cancer.

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P4-007

Can paraffin be a marker for colorectal cancer screening?

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This paper utilizes Synchrotron-based infrared microspectroscopy (SR-IMS) associated with linear discriminant analysis (Linear Discriminant Analysis, LDA) for differentiating and screening normal and malignant colorectal cells. The results of LDA analysis presented three definite classifications for normal human fibroblast, CCD, and human carcinoma cells, SW-403 and SW-480, based on the infrared absorption spectra of 3500-2800 cm^{-1}. And the grouping behavior for carcinoma cells was more diffused and heterogeneous than that of fibroblast, which was condensed and uniform. On the other hand, the tolerance region of LDA analysis for paraffin-treated colon cells after the treatment of 5 seconds xylene immersion obviously showed a differentiating behavior from the fibroblast, however, the tolerance region of LDA analysis for carcinoma cells were barely affected by xylene treatment in the spectra of 3000-2800 cm^{-1}. Furthermore, the results of the kinetic behavior for the ability of absorbing wax for colon cells revealed that CCD was prefer absorbing non-polar wax, paraffin ($C_{25}H_{52}$), i.e., the absorbance at 2921 cm^{-1} ($\nu_{as}CH_2$) and 2852 cm^{-1} ($\nu_{s}CH_2$) were more intense than that of carcinoma cells, SW-403 and SW-480. On the contrary, the absorption for $\nu_{as}CH_2$ and $\nu_{s}CH_2$ of cancer cells was stronger than that of fibroblast as using polar wax, beeswax ($C_{46}H_{92}O$) and amino acid wax ($C_{38}H_{72}N_2O_2$, N, N'-Ethylenebis) to be absorptive material. Results demonstrated that the polarity of cell membrane for normal and carcinoma cells was opposite to each other, and it would differentiate malignant cells from normal cells for employing wax with varied polarity to be a marker to screen cells in the future.

P4-008

Expression of Six Photoreceptors in *Haloarcula marismortui* Unveiled Unique Photosensing Features

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Microbial photoreceptors play important roles when responding to environmental stimuli in microbes and a total of more than one hundred microbial photoreceptors has been identified. So far, four distinct functions have been identified, including bacteriorhodopsin (BR) functions as outward proton pump, halorhodopsin (HR) serves as an inward chloride pump, sensory rhodopsin I (SRI) mediates both attract and repellent signaling, and sensory rhodopsin II (SRII) triggers repellent signaling toward UV light. According to the genomic project of the *Haloarcula marimortui*, there are six predicted photoreceptors, most numbered photoreceptors in a single archaeon. This study will address two questions: A) the maximum absorbance distribution of those six photoreceptors and, B) the possible transducer of the proposed sensory rhodopsin I. We successful cloned and over-expressed all six photoreceptors with *E. coli* system, and concluded: i) a unique distribution pattern of maximum absorbance of those six photoreceptors, and ii) identified a new type of sensory other than the four well known ones; and iii) two photoreceptors as proton pumps were confirmed (see the other two posters from our lab for details). This is the first time a single microbe found to have two proton pumps. Also, the missing two-transmembrane region in the predicted transducer of sensory rhodopsin I was proved to actually have two-transmembrane region.

P4-009

A new type of sensory rhodopsin, HmSRM, in *Haloarcula marismortui*

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Photoreceptors play important roles in responding to environmental stimuli like light and they are essential for surviving in microbes. Ion pumps and photosensory are two main types of microbial rhodopsin among known microbial photoreceptors. Proton pump, like bacteriorhodopsin (BR) in *Halobacteria salinarum*, pumps out protons to create a proton gradient for eventually energy generation, while photosensory rhodopsins, on the other hand, mediate repellent or attract signal in response to different wavelength of light. Sensory rhodopsin I (SRI) mediates both attract/repellent responses together with its cognate partner protein, HtrI, while sensory rhodopsin II (SRII) is solely responsible for repellent response when working together with its cognate transducer, HtrII.

The genome project for *Haloarcula marismortui* predicted the existence of six photoreceptors, a most abundance and variety among any single archaeon. Overexpression and absorbance measurements of those six photoreceptors concluded them as three ion pumps, two sensory rhodopsins and one unknown type rhodopsin. Here, we propose that this function-unknown protein as a new type of modulatory photoreceptor based on the results that this HmSRM can switch between ground state and M-intermediate state when exposed to different light wavelength. Also, we cloned and identified the HmSRM cognate partner transducer, HmHtrM, which has two trans-membrane regions followed by a very short cytoplasmic region containing a HAMP domain in the C-terminus. Finally, we propose HmSRM modulates photosensory signal for *H. marismortui* by changing the ratio of Ground/M-intermediate state in response to different wavelength of light.

P4-010

Ion Pump Photoreceptors in *Haloarcula marismortui*: One Chloride Pump and Two Different Photoreceptors Function As Proton Pumps in A Single Archaeon

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Microbial photoreceptors play important roles in sensing environmental light stimulation and harvesting light energy. Four different distinct functions of photoreceptors were identified in *Halbacteria salinarium*, including i) proton pump, like bacteriorhodopsin (BR), ii) chloride pump, found in halorhodopsin (HR), iii) light attract/repellent sensing, like sensory rhodopsin I (SRI), and iv) repellent signaling, found in sensory rhodopsin II (SRII). Here, we report a single archaeon, *Haloarcula marismortui*, containing two proton pumps among six authentic photoreceptors. Among them, BR I (*bop*) and BR II (*xop*1) are outward proton ion pump and HR (*hop*) is a inward chloride pump. Their functionalities were measured with a computer driven ion/pH meter with three digital accuracy and the illumination of light were performed with corresponding wavelength LED or a green laser. A light illumination-dependent increase in both proton or chloride pumps among three photoreceptors were observed.

P5-001

A strategy for efficient site-specific FRET-dye labeling of ubiquitin

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To study conformational changes within a single protein molecule, sp-FRET (single pair fluorescence resonance energy transfer) is an important technique to provide distance information. However, incorporating donor and acceptor dyes to the same protein molecule is not an easy work. Here, we report a strategy for the efficient double-labeling of a protein on a solid support. An ubiquitin mutant with two Cys mutations, one with high solvent accessibility and the other with low solvent accessibility, was constructed. The protein was bound to magnetic beads and reacted with the dyes. The first dye reacted with the side-chain of the Cys with the high solvent accessibility and the second with the other Cys under partially denaturing conditions. Using this method, we can easily label two dyes in a site-specific way on ubiquitin with a satisfied yield. The labeling sites for donor and acceptor dyes can be easily swapped.

P5-002

Morphological characterization of amyloid beta aggregates fibril in different environmental conditions

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Alzheimer'disease (AD) is a neurodegerative disease and the commonest form of dementia. One of the hallmarks of Alzheimer'disease is the self-aggregation of amyloid β peptide (Aβ) in extracellular amyloid plaques. Theses Aβ aggregates are toxic to neuron, and the toxicity induced by Aβ is highly dependent on environmental conditions. The examination of environmental factors accelerating Aβ formation and growth is an important key to unravel etiology of AD. Thus, in the present study, we investigated the process of Aβ aggregation and morphology of Aβ aggregates under different pH, concentration, incubation time and temperature. Several biophysical techniques, including Atomic force microscopy (AFM), fourier transform infrared (FTIR) spectroscopy and other spectroscopies, were used to visualize the morphology, secondary structure, and aggregation process. Results show that the morphology of Aβ aggregates form a rod-like shape under pH6.5-7.5, 4℃ and 48hrs. On the other hand, the morphology of Aβ aggregate shows a disk-like shape under disk-like under pH3.5-4.5, 37℃ and 4 weeks. The secondary structure of Aβ in nucleation state contains 56% β-sheet and 44% random coil at pH7.0 and 63% β-sheet and 37% random coil at pH4.0. Turbidity assay indicates that the aggregation of Aβ42 in rod-like fibril is more rapid than Aβ42 in disk-like fibril.

P5-003

Single Molecule Imaging of *E. coli* Undecaprenyl Pyrophosphate Synthase (UPPs) Interacting with Artificial Cell Membrane

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Undecaprenyl pyrophosphate synthase (UPPs) catalyzes eight consecutive condensation reactions of farnesyl pyrophosphate (FPP) with isopentenyl pyrophosphate (IPP) to generate C_{55} undecaprenyl pyrophosphate (UPP), which is a lipid carrier to mediate the synthesis of bacterial cell wall peptidoglycans. UPPs is highly soluble when expressed in *E. coli*, but its product, UPP, is membrane bound. It is unknown how the cytosolic protein transfers its product to bacterial cell membrane. In order to address the question, we employed single molecule imaging technology to observe the dynamics of UPPs in different artificial cell membranes in the absence and presence of different ligands. The results showed that during the synthesis of UPP, wild-type UPPs was adhered to DPPC artificial membrane, which has no net charge. The mutant UPPs including D26A and $S^{83}(Ala)_5$ were also adhered to DPPC artificial membrane in the presence of the substrates, even though the activities of these mutant UPPs are 10^3~10^4 fold lower compared to the wild-type UPPs. Moreover, without the substrates, UPPs was adhered to DMPG/DMPE artificial membrane, which has negative charge. The conformational change of UPPs, resulting from binding of different ligands, and the net charge of the membrane determine whether UPPs is adhered to the bacterial cell membrane.

P5-004

The use of membrane nanotubes to examine ligand-receptor binding strengths

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We assume cell membrane nanotubes (tethers) result from specific ligand-receptor bonds. The lifetimes of theses bonds can be estimated by measuring the length of the tethers. Here we use AFM to pull nanotubes out of a cell, then characterize integrin $\alpha_2\beta_1$- collagen binding and Concanavalin A (ConA)- cell surface binding. Experimental results show that tether formation is non-specific to the $\alpha_2\beta_1$ ligand-receptor interaction. On the other hand tether formation is inhibited by addition of extra ConA coverage on cell surface.

P5-005

Photoinduced Fluorescence Enhancement in Single Colloidal Quantum Dots

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Colloidal CdSe semiconductor QDs (also referred to as semiconductor nanocrystals) have attracted much attention due to excellent fluorescence properties, such as high quantum yields, size-dependent emission spectra, and good photostability.[1,2] Because of high surface to volume ratio, fluorescence properties are variable and sensitive to their surface molecules,[3] local environments,[4-6] and light illumination.[7-8]

In this work, photoinduced fluorescence enhancement (PFE) in colloidal CdSeTe/ZnS core/shell quantum dots (QDs) was investigated by monitoring ensemble fluorescence and single-QD fluorescence blinking behavior upon illumination. Ensemble fluorescence was increased in air and in vacuum, but with different enhanced factors. At the single-QD levels, the fluorescence intensity was also enhanced for some individual QDs. Relatively long on-times, high quantum yields within the on-times, and multi-level on-states were found in fluorescence blinking time traces. Based on our experimental results, we suggest that the origin of PFE phenomenon *from single-QD viewpoint* is attributed to the contributions of both surface passivation by photo-induced charged carriers and formation of the neutral core/charged shell QD states.

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P5-006

Imaging tight-turn DNA triplexes on mica by atomic force microscopy in liquid

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DNA triplexes with 'tight turns', i.e., sharp turns without any intervening bases, are visualized using atomic force microscopy (AFM) in liquid. Our AFM images allow the structure of the smallest DNA triplex (18 Triad) and duplex to be observed and characterized in the first time. Our previous studies revealed that the sequence 5′-TCTCTCCTCTCTAGAGAG-3′ was able to form intramolecular triplex helix in slightly acidic solutions by folding into a "paperclip" structure with a tight turn structure[1,2]. In this study, we observed a similar triplex formation of the longer oligomer sequence under similar conditions. The most constant dimension of the DNA triplexes is their relative heights ~ 1.5 nm and their lengths vary between 30 - 40 nm (without calibration for tip radius) under slightly acidic condition (pH 6.0). Whereas, in the buffer solution with pH 8.0, we observed a diffuse "tail", which may corresponds to the unstructured residues (the single strand) at one end of DNA duplex. The above conclusion can be confirmed by CD observations. Namely, CD spectrum of the oligomer 5′-(CT)9(TC)9(AG)9-3′ at pH 6.0 shows a characteristic triplex negative band at 210 nm but not in 8.0. This approach to molecular visualization could serve as a useful tool for the investigation of structures in DNA and other bioploymers, as well as studies of the molecular mechanisms of DNA replication at the single-molecular level.

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P5-007

Single Molecule Tug-of-War of DNA through 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 caused by confinement-induced entropic recoiling forces (1, 2). The dimension of nanoslits is of 50 nm in depth, 10 μm in width, and 2-4 μm in length. 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 from one micro regime to another. Surprisingly, our preliminary results suggest the probabilities of which end of a DNA polymer will take precedence do not depend on the initial lengths of DNA exposed in both micro regimes. A theoretical approach of deriving entropic recoiling force in the single molecule tug-of-war scenario is also proposed.

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P5-008

Studies on the *in vivo* and *in vitro* Dynamics of the Bacterial MinD Cytoskeleton

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The cell division in *Escherichia coli* requires the septal machinery to be precisely placed at the middle of a cell. The dynamic pole-to-pole oscillation of the Min system is critical to the division site placement and consequently prevents the inadequate division which results in the production of unequal-sized daughter cells. The MinD cytoskeleton is the central participant of this process. The mechanism of oscillation has been proposed to underlie cycles of rapid polymerization and depolymerization of the MinD protein filaments through the MinE-regulated ATP binding and hydrolysis in MinD[1].

Our goals are to characterize MinD dynamics both *in vivo* and *in vitro* through: (1) analysis of MinD oscillation patterns in *E. coli* when cells are confined in microfluidic channels of defined shapes, and (2) *in vitro* reconstruction of MinD protein filaments undergoing the dynamic processes of polymerization and depolymerization. We have currently entrapped and reshaped *E. coli* cells within micro-chambers. Moreover, we have observed that purified MinD can interact with liposome and form extended filamentous structures in our *in vitro* system. Fluorescence and total internal reflection (TIRF) microscopy will be used to further study the MinD oscillation patterns *in vivo* and protein polymerization and depolymerization processes *in vitro*.

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P6-001

Cooperative transport in a potassium ion channel

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Our current understanding of ion permeation through the selectivity filter of the potassium channel is based on the concept of a multi-ion transport mechanism (1). The details of this concerted movement, however, are not well understood. Our study on the ion permeation processes in the KcsA ion channel (2)(3) using manipulated molecular dynamics simulations (4) provides new insights about the multi-ion transportation mechanism. It is shown that ion translocation is based on the collective hopping of ions and water molecules which is mediated by the flexible charged carbonyl groups lining the backbone of the channel pore. In particular, there is strong evidence for pairwise translocations where one ion and one water molecule form a bound state. We suggest a physical explanation of the observed phenomena employing a simple lattice model. It is argued that the water molecules can act as rectifiers during the hopping of ion-water pairs.

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P6-002

8a from SARS-CoV: Experiments and molecular dynamics simulations

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Many genomes of viruses encode small membrane spanning proteins which are proposed to alter membrane permeability for ions and small molecules. These channel or pore forming proteins are getting into the focus for antiviral therapy since they are essential for some of the viruses. One of the common themes of the mechanism of function of the proteins is to self-assemble to form the functional form.

We present a study on the open reading frame (orf) 8a membrane protein encoded in structural region of Human Severe Acute Respiratory Syndrome Coronavirus (SARS-CoVs). The full length 8a protein is 39 residues long and contains a single transmembrane (TM) domain. Full length protein is synthesized using solid phase peptide synthesis and reconstituted into artificial lipid bilayers. The bilayer recordings show ion channel activity. In silico studies with a 22 amino acid TM domain are done to assess conformational space of the monomeric 8a helix. With this monomeric helix homooligomeric helical bundle models are built and embedded in a fully hydrated 1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphatidylcholine (POPC) bilayer. Results of both experimental channel recordings and computational modeling indicate SARS 8a to act as a channel forming protein.

P6-003

Viral channel forming proteins: p7 from HCV forms channels in artificial bilayers.

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With approximately 170 million infected people, HCV is the major health problem worldwide. One of its membrane proteins, p7, has been discovered to show channel activity in vitro experiments [1]. Channel activity is due to the assembly of the protein and leads to a change in electrochemical gradient across the membrane. This task is assumed to be essential for the life cycle of HCV, since p7-deletion mutants cannot survive. However, the detailed role of the p7 protein from hepatitis C virus (HCV) in the virus life cycle is still unknown. It has been shown recently that p7 can be inhibited by long-alkyl-chain iminosugar derivatives [1].

The p7 protein has two hydrophobic transmembrane (TM) domains, TM1 and TM2 as suggested by secondary structure prediction programs [2]. In this study peptides comprising of $TM1_{13-23}$ and $TM2_{36-58}$ have been synthesized to discover potential ion channel activity. In addition to the synthesis, we also expressed full length p7 and tested for channel activity. Computational models have been built in order to flank the experimental activities and analyze p7 on a molecular level.

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P6-004

Amyloid-β 40 monomer alters the fibrils and oligomers population of Amyloid-β 42 by stabilizing the oligomers of Amyloid-β 42

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Alzheimer's disease (AD), the most common neurodegenerative disease, is estimated to affect 20 million people world wide. Amyloid-β (Aβ) is the main component in the senile plaques found in AD patients. Different lengths of Aβ peptides mainly 40 and 42 are clipped from amyloid precursor protein by β and γ-secretases. The subsequent aggregation is a protein misfolding event related to progressing of dementia. It is gradually believed that the neurotoxic species are the Aβ oligomers but not fibrils, however, its assembly, toxicity, and aggregation mechanism are still controversial. Clinical and biochemical evidences showed that Aβ42 aggregates more aggressively than Aβ40, and elevated ratio of Aβ42 /40 in cerebrospinal fluids promotes early onset of AD. Moreover, higher Aβ40 level favors cerebral amyloid angiopathy, whereas, higher Aβ42 level favors formation of parenchymal plaques. To elucidate the aggregation mechanism of Aβ42, we first characterized the initial population differences in Aβ40 and 42. The results showed that Aβ42 initially populates as a monomer and a soluble oligomeric form but not Aβ40. The Aβ42 oligomer dissociates into trimers in SDS-PAGE indicating the oligomer is a trimer multiples. Then, we characterized the aggregation pattern of Aβ42 and demonstrated that at least four species exist during aggregation. The Aβ42 oligomers and fibrils co-exist until the end of aggregation while the amount of fibrils increase through time. In the presence of one-tenth population of Aβ40, Aβ42 aggregation pattern was altered and showed a steady population of soluble oligomers without increasing the amount of fibrils. From our study, we suggest Aβ40 monomer plays a role in changing Aβ42 aggregation and prefers the existence of Aβ42 soluble oligomers. By manipulating the ratio of Aβ40 and 42, we can further examine the possible aggregation mechanism existed in AD.

P6-005

Coupling in FitzHugh-Nagumo model

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Firing pattern can be modulated by the complex network effect through coupling and characteristic time scales in oscillations. The abnormal spiking frequency can lead to some serious diseases in nerves system. By considering the fundamental coupling in a system of two FitzHugh-Nagumo excitable elements in the presence of noise, the detail dynamic properties are investigated. Coupling an intrinsically faster element to a slower one not only increases the frequency of slower element, but can also lead to the novel effect of further enhancing the frequency of the faster element. These dynamic properties are modulated by coupling strength and noise intensity.

P6-006

Conformational Stability of β-Amyloid and Its Familial Mutants

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β-Amyloid (Aβ) was highly demonstrated the causative factor in Alzheimer's disease (AD). The conformational and assembly change during Aβ aggregation is the crucial process contributing the neurotoxicity. It was shown that soluble oligomers of Aβ rather than monomers or fibrils, are the major player in neuron degeneration. Hence, the result provokes the immediate importance of understanding how early stage folding affects aggregation of Aβ in order to prevent toxicity. To reveal the early stage folding of Aβ, several $Aβ_{40}$ familial mutants including A21G (Flemish), E22G(Arctic), E22K(Italian), E22Q(Dutch), and D23N(Iowa) were investigated. We first examined the native and unfolded Aβ conformation by circular dichroism spectroscopy. All native Aβ and its variants were random coil like, however, a helical like residual structure was shown after subtracting the native and unfolded spectra. By using extrinsic fluorescence probe that binds to the hydrophobic cluster on the protein surface, we are able to examine equilibrium folding of Aβ prior to its aggregation. Preliminary urea and guanidine hydrochloride denaturation data show that Aβ wild type has similar stability with A21G, whereas all other mutants are significantly destabilized, especially E22 mutations. The fitting on denaturation curves of all Aβ adopts a two-state mechanism. In aggregation assays using thioflavin T to monitor cross β fibril formation, we found A21G does not aggregate in our experimental time, whereas E22G, E22K, E22Q, and D23N aggregate much faster than the wild type. The fibril formation is affected by the presence of denaturants and may indicate the residual structural changes among Aβ and the variants. Overall, our study provides thermodynamic parameters of the early stage Aβ and may contribute to understand the aggregation mechanism and physiological significance of Aβ amyloidosis.

P6-007

Homology modeling and docking analysis of hERG (human ether-a-go-go-related gene) potassium channel

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hERG (human ether-a-go-go-related gene) forms the channel which mediates the rapidly activating delayed rectifier K^+ current (I_{Kr}), and is responsible for the phase 3 repolarization of cardiac cells. hERG potassium channel can be blocked by some drugs, important examples are cisapride (a gastrointestinal prokinetics), terfenadine (an anti-arrhythmic drugs), sertindole (an antipsychotics), and also some cardiovascular drugs (such as dronedarone, amiodarone). Drug-induced hERG channel blockade is linked to long QT syndrome (LQTS), resulting in an increased risk of ventricular arrhythmia, namely, *torsades de pointes* (TdP), that may cause syncope and sudden death. Thus, hERG is now considered as an important predictor of proarrhythmic activity, and it is now a routine practice in the pharmaceutical industry to test compounds for hERG channel activity during the drug development process.

Due to the absence of a crystal structure for hERG, the homology modeling technique provides us an alternative method to investigate the structural basis of blocking mechanism. With the help of hERG homology models, we can perform the study of the drug/protein docking analysis. A series of hERG potassium channel blockers were used as ligands to docking into the hERG homology model (the model was derived from bacterial KcsA channel). From these result of docking analyses, we may identify important amino acid residues likely to be involved in the blocker-channel interaction. A combination of receptor homology models and ligand-docking analysis may provide the best hope of truly prediction of the potential hERG-binding affinity of new chemical entities.

P6-008

Multiscale Simulations of the molecular motor

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The ATP synthase F_0F_1 enzyme which is embedded in the mitochondrial membrane is one of essential molecular motors that have received high attention recently. One portion called F_0 which works like a rotary motor is a membrane-spanning component. Another portion called F_1 where hydrolysis or generation of ATP is a soluble component. Based on the available NMR data [1] of individual components, we have conducted molecular dynamics simulations of F_0 part in the POPC bilayer with all-atom and coarse-grained model for hundreds of nanoseconds. The goal of our work is to investigate the motion of F_0 at different protonation states of key residues, i.e., ASP-61 of the c-subunit and ARG-210 of the a-subunit, and under electric fields with bilayer axial directions. The motions and properties of POPC lipids which are different from pure bilayer ones are another key point we paid attention to.

The flip-flop motions of some POPC lipids are observed of systems with electric fields. With equilibrium MD, the F_0 protein and lipids are steadier. Refer to different dipole moments of c-subunit after simulations, dipole moment is one important indicator to understand the effect caused by electric fields.

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P7-001

NETWORK ANALYSIS OF PROTEIN-PROTEIN INTERFACES

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Network analysis is a powerful tool for modeling and analyzing dynamics of complex systems. Protein-protein interaction is a common mechanism responsible for functioning of numerous physiological processes in the cell, metabolic networks, signaling pathways and cell adhesion are some of many examples of protein-protein interaction. For understanding more insights into principles governing the interaction of proteins, we modeled protein complex structures as networks [1,2] and studied the characteristics of protein-protein interfaces using a structurally non redundant set of 948 two chain protein complexes derived from PDB. Our results show that contact density of 20 different amino acids both at interior as well as at interfaces does not vary significantly. Amino acid composition of interface residues is also similar to that of whole protein. From interface network we identified all structurally important building blocks of protein interfaces, called motifs and used network parameters to explore the packing strength of interface residues. Finally we extended our study towards comparison of whole interface network with interior protein network.

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P7-002

Mononuclear versus Binuclear Metal-Binding Sites in Metalloenzymes: Metal-Binding Affinity and Selectivity from PDB Survey and DFT/CDM Calculations

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Binuclear metal centers in metalloenzymes are involved in a number of hydrolytic, hydration, isomerization, and redox processes. Despite the growing number of studies elucidating their structure, properties, and function, questions regarding certain aspects of the bimetallic proteins' biochemistry still remain; e.g., (i) What are the general characteristics of binuclear sites found in 3D structures such as the range of metal-metal distances and the most common ligand bridging the two metal cations? (ii) How does the presence of a metal cation in one of the binuclear sites affect the metal binding affinity/selectivity of the other site? (iii) How do the characteristics and metal binding affinity/selectivity of *bi*nuclear sites compare with those of their *mono*nuclear counterparts? Here we address these questions by combining a Protein Data Bank survey of binuclear sites with density functional theory (DFT) coupled with continuum dielectric method (CDM) calculations. The results reveal that for homo-binuclear sites, the metal separation depends on the metal's charge and the electron-accepting ability, and Asp^-/Glu^-, bidentately bound to the two cations, is the most common bridging ligand. They also reveal that Mg^{2+} occupying one of the binuclear sites attenuates the metal binding affinity, but enhances the selectivity of its neighboring site, compared to the corresponding mononuclear counterparts. These findings are consistent with available experimental data. The weak metal binding of one of the binuclear sites would enhance the metal cofactor mobility in achieving the transition state, whereas the enhanced selectivity of Mg^{2+}-Mg^{2+} centers helps protecting the binding site against unwanted substitutions by transition metal ions, which are generally stronger Lewis acids compared to Mg^{2+}.

P7-003

3D-SARST — an efficient protein structural database search tool

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The amounts of known protein structural data is growing so rapidly that fast and accurate structure similarity search tool is in a strong demand. We have developed a structure similarity search tool called SARST (Structural similarity search Aided by Ramachandran Sequential Transformation) that is able to perform extremely rapid database search with accuracy comparable to CE (Combinatorial Extension) by using a linear encoding methodology. Now we aim to modify the linear encoding strategy of SARST by integrating more protein structural information to improve its accuracy.

SARST linearly encode protein structures by utilizing a Ramachandran map organized by nearest neighbor clustering. Traditionally, Ramachandran map is a two-dimensional (2D) plot displaying the distribution of dihedral angles (φ, ψ) of residues. Different regions on this map represent different secondary structural preferences of backbone local structures; however, structural information can be lost in the process of transforming the three-dimensional (3D) protein structure into the 2D map. Our speculation is that, if we can extend the Ramachandran plot into a 3D map by adding an extra axis describing another structural property of backbone conformation, more structural information can be preserved in the transformation processes and thus improves the performance of SARST. Hence, we call the new search tool developed based on this speculation 3D-SARST.

3D-SARST, adopting the advantage of SARST, is a rapid database search tool with reasonable compromise of accuracy. Although we have not found a suitable condition to make it generally outperform SARST, we do find that 3D-SARST can achieve higher accuracy for various structural classes under specific situations According to the results, we can firstly determine the structural class of the query protein and then use 3D-SARST running under appropriate condition and parameter settings for that class to increase the accuracy of database searching. This two-step strategy has improved the precision of SARST by 4%, making it a little more accurate than CE., As the amount of protein structural data increases ever rapidly nowadays, we suppose that an efficient database search engine such as 3D-SARST can be valuable in many post-genomic researches fields.

P7-004

PLID: the Protein-Ligand Interaction Database

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The Protein-Ligand Interaction Database (PLID) is a collection of information related to the interactions between proteins and ligands. Structures of the protein-ligand complexes were obtained from the Protein Databank (PDB), and more than 12,000 literatures have been inspected to retrieve the binding affinity information. Currently PLID collects more than 1480 non-redundant PDB entries, all of which contains inhibitory constants (K_i's) or dissociation constants (K_D's), and the references to the assay measurements. PLID also includes many molecular descriptors of these complexes, and the curated ligand files and protein files with predicted protonation states based on the pH values in the inhibition or binding assays, which can help for more accurately describing the structure-activity relationships. PLID can be queried by PDB ID, protein name, protein sequence, protein EC number, ligand name, ligand similarity, ligand substructure, SMILES string, range of ligand molecular weight, range of inhibitory constant, range of resolution, etc. Possible applications of this database include the prediction of drug binding sites, design of new scoring functions for protein-ligand interactions, comparison of receptor-based and ligand-based pharmacophore models, and construction of the quantitative structure-activity relationships of some focused sets of compounds. PLID can be accessed freely at http://plid.ibms.sinica.edu.tw/.

P7-005

Physical Basis of Structural and Catalytic Zn-Binding Sites in Proteins

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Zn^{2+}, an element that is essential to all life forms, can play a catalytic or a solely structural role. Previous works have shown that Zn^{2+} binds preferentially to water molecules and His in *catalytic* sites, but to Cys^- in *structural* sites, but the molecular basis for the observed ligand preference is unclear. Here, we show that the different Zn^{2+} roles are also reflected in the different bond distances to Zn^{2+} in structural and catalytic sites. We reveal the physical basis for the observed differences between structural and catalytic Zn sites: In most *catalytic* sites, *water* is found bound to Zn^{2+} as it transfers the least charge to Zn^{2+} and is less bulky compared to the protein ligands, enabling Zn^{2+} to serve as a Lewis acid in catalysis. In most *structural* sites, however, ≥2 *Cys^-* are found bound to Zn^{2+}, as Cys^- transfers the most charge to Zn^{2+} and reduces the Zn charge to such an extent that Zn^{2+} can no longer act as a Lewis acid; furthermore, steric repulsion among the bulky Cys(S^-) prevents Zn^{2+} from accommodating another ligand. Based on the observed ligand preference and Zn-ligand distance differences between structural and catalytic Zn sites, we present a simple method for distinguishing the two types of sites and for verifying the catalytic role of Zn^{2+}. Finally, we discuss how the physical bases revealed aid in designing potential drug molecules that target Zn proteins.

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P7-006

CPSARST – Circular Permutation Search Aided by Ramachandran Sequential Transformation

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Circular permutation (CP) of a protein can be visualized as if the original amino- and carboxyl-termini were linked and new ones created elsewhere. Circular permutants usually retain native structures and biological functions. This interesting property has made CP useful in many protein engineering and folding research fields. Although there have been many circular permutants found in well-known protein families, efficient database search tools are not available yet because of the complicated rearrangement nature of CP. Here we report CPSARST (Circular Permutation Search Aided by Ramachandran Sequential Transformation), to be a novel and efficient circular permutant search method. It features with (1) describing three-dimensional structures as one-dimensional text strings, (2) duplicating the query structure and (3) working through a “double filter-and-refine” strategy. When tested with engineered circular permutants, CPSARST successfully retrieved all the natural proteins with accurate permutation site predictions. Its ability to identify natural circular permutations is also comparable to other structure-based CP-detecting methods. The speed of CPSARST is thousands of times as high as related algorithms. Its high efficiency makes routine database searches achievable. Several novel CP relationships have been detected by CPSARST and reported in this article. In this post-genomics era, when the amount of protein structural data increases exponentially, CPSARST can provide a new way to rapidly detect novel relationships among proteins and help to reveal how Nature achieves protein evolutionary and functional diversity by using circular permutation.

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P7-007

SLITHER : a web server for generating contiguous conformations of substrate molecules entering into deep active sites of proteins or migrating across membrane transporters

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Many proteins use a long channel to guide the substrate or ligand molecules into the well-defined active sites for catalytic reactions or for switching molecular states. Specific substrates of membrane transporters can migrate to another side of cellular compartment. SLITHER is a web server that can generate contiguous conformations of a molecule along a curved tunnel inside a protein, as well as the binding free energy profile along the predicted pathway. SLITHER adopts an iterative docking scheme[1,2], combining with a puddle-skimming procedure, i.e., to repeatedly elevate local potential energies of the global minima identified in the previous stage, thereby determine the contiguous binding modes of substrates inside the protein. In contrast to the HOLE program[3] that is widely used to determine the geometric dimensions in the ion channels, SLITHER can be applied to predict the conformation of substrate or ligand molecules along the inner channel of proteins.

SLITHER can be accessed at http://bioinfo.mc.ntu.edu.tw/slither/.

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P7-008

Deriving protein dynamical properties from weighted protein contact number

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It has recently been shown that in proteins the atomic mean-square displacement (or B-factor) can be related to the number of the neighboring atoms (or protein contact number), and that this relationship allows one to compute the B-factor profiles directly from protein contact number. This method, referred to as the protein contact model, is appealing, since it requires neither trajectory integration nor matrix diagonalization. As a result, the protein contact model can be applied to very large proteins and can be implemented as a high-throughput computational tool to compute atomic fluctuations in proteins. Here, we show that this relationship can be further refined to that between the atomic mean-square displacement and the weighted protein contact-number, the weight being the square of the reciprocal distance between the contacting pair. In addition, we show that this relationship can be utilized to compute the cross-correlation of atomic motion (the B-factor is essentially the auto-correlation of atomic motion). For a nonhomologous dataset comprising 972 high-resolution X-ray protein structures (resolution <2.0 A and sequence identity <25%), the mean correlation coefficient between the X-ray and computed B-factors based on the weighted protein contact-number model is 0.61, which is better than those of the original contact-number model (0.51) and other methods. We also show that the computed correlation maps based on the weighted contact-number model are globally similar to those computed through normal model analysis for some selected cases. Our results underscore the relationship between protein dynamics and protein packing. We believe that our method will be useful in the study of the protein structure-dynamics relationship.

P7-009

Structural prediction of the adenosine A_{2A} receptor and comparison of its resting state and active state conformations

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The activation of human adenosine A_{2A} receptor has recently been identified as a candidate target for designing therapeutics for the Huntington disease. Adenosine A_{2A} receptor belongs to the GPCR, rhodopsin-like superfamily. It consists of 7 transmembrane α-helices, and each is about 25 residues in length. Nowadays, the three-dimensional structure of adenosine A_{2A} receptor obtained from experimental methods is still unavailable. The determination of membrane protein structures via experimental approaches remains a major challenge in the field of structural genomics. An alternative approach to building a molecular model of a protein is from homology modelling procedure. We used bovine rhodopsin crystal structure (PDB code: 1U19, 2.2Å) as homology modelling template to construct the adenosine A_{2A} receptor (1-301) except its c-tail using **MODELLER9v1**. In the alignment step, we aligned all the rat adenosine receptor family sequences (A_1, A_{2A}, A_{2B}, and A_3) with rhodopsin sequence to take evolutionary relationship into account and improve the alignment accuracy by using **CLUSTALW**. The exceptional long c-tail structure of adenosine A_{2A} receptor (302-410) was modeled from best model of **TASSER-Lite** web server using the fold-recognition approach. After the full-length adenosine A_{2A} receptor structure has been constructed, we put the receptor into lipid-water environment to run 2-ns molecular dynamics simulation refinement using **AMBER9**. We also made the pharmacophore model of adenosine A_{2A} receptor agonist and antagonist, respectively, using **Catalyst®**. Then, the most potent agonist and antagonist conformations which fitted its pharmacophore model best were selected. The chosen agonist and antagonist conformations were docked with adenosine A_{2A} receptor using **Autodock3**. Finally, we made 15-ns molecular dynamics simulations of receptor only, receptor with an agonist, and receptor with an antagonist, to analyze the receptor-ligand binding interactions. The detailed knowledge of the binding locations will help to design more potent inhibitors for this receptor.

P7-010

A novel free energy evaluation scheme based on energetic decomposition of molecular dynamics simulations

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Accurate methods for predicting the affinity of a small molecule with a protein or other biomolecule play a crucial role in computational drug design. This is because these predictions can be valuable for lead generation or lead optimization. Nowadays there are many computational methods to evaluate the binding affinity, such as empirical scoring function, free energy perturbation (FEP), thermodynamic integration, end-point methods, and so on. The linear interaction energy (LIE) method is one of the free energy calculation methods for evaluation of binding affinity and it is more economical than other free energy methods, such as the FEP and the MM-PBSA method, yet the dynamic effect of the protein-ligand complex is still included.

The LIE method was first introduced by Åqvist et al. and this method is a semi-empirical method and uses only the initial and final states (i.e., end-points) of the binding process of one ligand, the ligand in waters and the ligand in complex with solvated protein. The free energies are calculated as sum of intermolecular electrostatic interactions and intermolecular van der Waals interactions between the ligand and its surroundings. However, in their studies, simulations were carried out in a solvated 20 Å sphere and the protein atoms out of the simulation sphere were fixed. The long range interactions were not treated correctly, and the sampling of the conformational space may be flawed.

In our studies, the protein was solvated in a solvated cubic box and the periodic boundary condition was applied. Simulations were performed using the program GROMACS 3.3.1 and the GROMACS force field parameter set. Non-bonded cutoff was 0.9 Å and the particle mesh Ewald method was used to treat the long-range Coulomb interactions. Proteins were used the program PDB2PQR to predict protonation states and ligands were prepared using PRODRG to generate the topologies and assign partial charges. We selected nine FK506 binding protein (FKBP) complexes, 1D7H, 1D7I, 1D7J, 1FKB, 1FKF, 1FKG, 1FKH, 1FKI, and 1J4R, to calculate the binding free energies using the LIE formulation. Besides, we decomposed energies from simulations into different energy terms, such as electrostatic interactions between the ligand and the protein, electrostatic interactions between the ligand and the solvent etc, to delineate these energy terms on the binding free energies. We also used different cutoff radius to get these terms and found that some of these terms have large change with the cutoff radius. Our approach will provide a more robust evaluation of protein-ligand interactions.

P7-011

Prediction of Protein Structure by Accelerated Sampling Technique

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Molecular dynamics simulation is a general method of protein structure prediction, but how to get the native state of protein structure from the unstable state is still a crucial and challenging problem. Simulated Annealing method usually makes protein structure trap in the energy local minima and it is difficult to find the energy global minima. In our simulation, we find Simulated Annealing is indeed unable to find the native state.

Replica Exchange Molecular Dynamics (REMD) (1) method is another choice to implement the simulation, and it was showed that Replica Exchange Molecular Dynamics method can get better result for finding the native state of mini-protein (2). In our research, we use Replica Exchange Molecular Dynamics method with the secondary structure distance restraint to find the native state of a 108-residue protein *thioredoxin*. We choose several initial structures with the different temperature to start the REMD simulation. We find the results are better than that with Simulated Annealing method. Finally, we also combine Self-Guided Langevin Dynamics method and Replica Exchange Molecular Dynamics method to implement the simulation and get more ideal results.

Our results reveal Replica Exchange Molecular Dynamics method is not only suitable to find the native of mini-protein but it can apply to the protein with 108-residue as *thioredoxin*.

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P7-012

GenePathway Viewer: a web server for visualizing gene expression levels and correlated genes in biological pathways

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GenePathway Viewer (http://bioinfo.mc.ntu.edu.tw:8080/GenePathway) is a web server that can be used to visualize gene expression levels and correlated genes on the maps of the biological pathways. GenePathway Viewer, different from other existent similar pathway viewers, is facilitated directly by the web service provided by the KEGG API and will acquire the most up-to-date pathway information in the KEGG database. Web service is an emergent powerful technology identified by a Universal Resource Identifier (URI), whose public interfaces and bindings are defined by XML. A distinct feature of web service is that the constituent software components, which are communicated via the Simple Object Access Protocol (SOAP), can be loosely coupled, in contrast to the more traditional client-server models that are very tightly coupled. On the other hand, GenePathway Viewer is also a meta-server that can combine various resources on gene identification and gene annotation information. With these integrated features, GenePathway Viewer will help expedite the understanding of gene functions and their correlations or causal relationships upon different medicinal treatments.

P7-013

Pathway Analysis based on Gene Expression Profiles from Huntington's Disease Brain

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Huntington's disease is a neurological disorder associated with dysfunction and degeneration of the basal ganglia. The abnormal CAG repeat in the *HD* gene leads to the characteristic motor and cognitive symptoms with a mid-life onset. Striatum was found to be the tissue with earliest noted changes and most severe damages. We used the GenePathway Viewer (http://bioinfo.mc.ntu.edu.tw:8080/GenePathway) to analyze the microarray data on the pubic database to detect the important genes that are involved in the Huntington's disease and other neurodegenerative diseases. Our GenePathway Viewer is a web server that can be used to visualize gene expression levels and correlated genes on the maps of the biological pathways. GenePathway Viewer, different from other existent similar pathway viewers, is facilitated directly by the web service provided by the KEGG API and will acquire the most up-to-date pathway information in the KEGG database. On the other hand, GenePathway Viewer is also a meta-server that can combine various resources on gene identification and gene annotation information. With these integrated features, GenePathway Viewer can be used to expedite the understanding of gene functions and their correlations or causal relationships upon different medicinal treatments. The aim of this analysis was to provide Huntington's disease pathology on the molecular level. Four brain regions were investigated, which are caudate nucleus, motor cortex, cerebellum, and prefrontal association cortex. In the current pathway analysis, only the microarray data for the caudate nucleus was used because greatest number and magnitude of differentially expressed genes were found in this tissue. mRNA levels in laser capture micro-dissected neurons was measured to confirm that the mRNA changes are not due to cell loss alone. The GenePathway Viewer can successfully identify relevant pathways for Huntington's disease using the microarray data. These pathways provide a direct visualization for the roles of various differently expressed genes in the proteomic maps. The pathways obtained in this analysis may be further integrated for providing a systems biological view of Huntington's disease.

P7-014

Determination of Conformational Degrees of Freedom of Small Compounds by Quantum Chemical Calculations

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The determination of conformational degrees of freedom of small compounds plays a key role for the computer-based prediction of the three dimensional structure of protein–ligand complexes which is often referred to as molecular docking. Currently several popular docking scoring function consider the term about number of rotatable bonds (Ntor) in ligand, such as Autodock (1), Cerius2/LUDI, SYBYL/F-Score, G-Score, ChemScore and so on. And further, Ntor holds the balance in above scoring functions cause of large weighted parameter. It is easier to predict rotatable bond with simply small compound by topology of molecular, some approach of popular molecular descriptor prediction programs such as PRODRG (2), OpenBabel, Q-mol and so on. But in some complicated compounds which are drug-like compounds including more rings and resonance type bonds, it is delicate to predict rotatable bond accurately. The determination is only to observe molecular structure by experience.

The proposed calculating number of rotatable bonds algorithm may be decomposed into two ways. First one, we obtain the bond order from Wiberg bond index matrix in the NAO basis via quantum chemical software Gaussian03 and NBO 5.0 (3) calculating. Then the information about bond order is embedded in modified Autotors program for outputting pdbq format which includes root, branch, leaf for description of conformational change. The second one, quantum chemical calculating is used to each bond with different rotation degree for analysis of energy statistics. Finally, we have developed these automatically programs for docking and virtual screening proposes.

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P7-015

DS-SARST: 3D Domain Swapping Search Aided by Ramachandran Sequential Transformation

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Three-dimensional (3D) domain swapping is a mechanism for forming protein quaternary structures from their monomers. It is defined as two or more protein chains exchanging part of their identical structure domain to form intertwined dimer or higher oligomers. 3D domain swapping is considered that it can occur under appropriate conditions in any protein with an unconstrained terminus. The term “3D domain swapping” was first used to describe the dimeric structure of diphtheria toxin in 1994. After that, the data of domain-swapped proteins has greatly expanded.

Because sequence comparison has shown that 3D domain-swapped partners often share minor sequence similarity and traditional rigid structural comparison methods, such as CE (Combinatorial Extension) and DALI (Distance Matrix Alignment), are unable to detect their real structural similarities, it is difficult to detect 3D domain swapping from protein structure databases. Here we present DS-SARST (3D Domain Swapping Search Aided by Ramachandran Sequential Transformation) to be an efficient database search tool.

DS-SARST utilizes the linear encoding algorithm RST to transform 3D protein structures into one-dimensional structural strings, the flexible properties of which in describing backbone conformations can be applied to the rapid detection of 3D domain swapping. Furthermore, we use a “double filter-and-refine” strategy to improve its accuracy. We suppose that DS-SARST can be an efficient structural database search tool useful in detecting novel 3D domain swapping cases and offering sufficient clues for their evolutionary relationships.

The symposium is regularly attended by about 300 Biophysicists in Taiwan, in particular students and young biophysicists. Your participation will help to make this an outstanding symposium. We look forward to seeing you in this meeting.

P9-001

L8/I9 Regulate Dimerization of *S. cerevisiae* Geranylgeranyl Pyrophosphate Synthase Through Multiple Interactions

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Geranylgeranyl pyrophosphate synthase (GGPPs) catalyzes a condensation reaction of farnesyl pyrophosphate (FPP) with isopentenyl pyrophosphate (IPP) to generate C_{20} geranylgeranyl pyrophosphate (GGPP), which is a precursor for carotenoids, chlorophylls, geranylgeranylated proteins, and archaeal ether linked lipids. The 3-D structure of GGPPs from *Saccharomyces cerevisiae* is composed entirely of fifteen α-helices joined by connecting loops and is arranged with α-helices around a large central active-site cavity. Distinct from other known structures of *trans*-prenyltransferases, the N-terminal 17 amino acids (9-amino acid helix A and the following loop) of this GGPPs protrude from the helix core into the other subunit and contribute to the tight dimer formation. In previous study, we have known that L8 and I9 were essential in dimerization. The double mutant L8G/I9G became a monomer. In order to investigate possible pathways by which L8G/I9G caused the disruption of this GGPPs from dimer to monomer, we examined the 3-D structure, performed site-directed mutagenesis studies and obtained explanation from MD simulation. Single mutation of D145K, M167G, or N101G involved in one pathway and E134A or R175A involved in the other pathway did not result in the disruption of dimer to monomer, whereas double mutation of M167G/N199A involved in both pathways resulted in a mixture of dimer and monomer. This represents a novel case in which the amino acids L8 and I9 at N-terminus are far away from the dimer interface (~26 Å), but significantly contribute to the dimer formation through multiple interactions.

P9-002

Multiple Nucleic Acid Binding Sites of SARS-CoV Nucleocapsid Protein

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Coronavirus nucleocapsid proteins contain a blend of structural and unstructured regions. The structural regions of SARS-CoV nucleocapsid protein have been shown to bind nucleic acid, whereas the roles of the unstructured regions are largely unknown. Here we present evidence that these unstructured regions also contribute towards nucleic acid binding, and the SARS-CoV nucleocapsid protein is a “sticky” molecule. Among the unstructured regions, the central linker between the two structural domains plays an important role in modulating the binding affinity towards nucleic acids. Bioinformatics analyses show that although the linker is not well conserved at the sequence level among coronaviruses, biophysical and biochemical predictors reveal conserved features that are found across all groups of coronaviruses. Implications on the RNA-binding mechanism of the coronavirus nucleocapsid proteins will be discussed.

P9-003

Studying the Folding Kinetics of an Antifreeze Protein RD1 by Using Photolabile Caging Strategy and Laser Flash Photolysis

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In order to understand the intrinsic principle of protein folding, early events of folding process have to be systematically explored. Here we proposed a photo-triggered caging-strategy to analyze the whole folding protein on a nanosecond time-scale. Our target protein is an antifreeze protein, RD1. It is small (7-kDa), compact, and consists of many secondary structure. Taking advantage of the existence of a small cavity in the center of the protein. We change Ala-7 of RD1 to Cys (designated RD1-A7C) by site-directed mutagenesis. In order to create the unfolded state of protein, we add a photolabile cage group, 4-(bromomethyl)-6,7-dimethoxycoumarin, to the residue Cys-7 (designated RD1-A7C-DMC) around the cavity. The bulky size of cage can hinder the hydrophobic packing and unfold the protein. A pulse UV laser (~10^{-12} s) is used to break the photolabile cage and to initiate the refolding of the protein toward its native state. We monitor the refolding process by using photoacoustic calorimetry (PAC) or photothermal beam deflection (PBD). The time resolution is greatly improved by the use of PAC (from nanosecond to a few microseconds) and PBD (from microsecond to several milliseconds).

In the future, by the use of nuclear magnetic resonance spectroscopy (NMR) and circular dichroism spectroscopy (CD), we can confirm the structural alteration of RD1-A7C-DMC before and after photolysis. After de-convolution of PAC, the time constant of the refolding process could be obtained.

P9-004

Different Fluorescent dyes affect transition behavior observed in DPPC Giant Unilamellar Vesicles

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Since phospholipids make up the backbone for cell membranes, a Giant Unilamellar Vesicle (GUV) composed of various lipids is a well-controlled system to study membranes. To track GUVs, we utilize fluorescence microscopy, also a common method in cell biology to distinguish cellular compartments. Adding minute amount of molecular probes is generally thought to have negligible affect on the organic molecules. However, in our experiments, pure DPPC GUVs observed with different fluorescent dyes have different transition patterns. Isotherm of DPPC monolayers shows that pure DPPC has two distinct phases, LC phase and gel phase, and this phase transition is characterized by two observations: change in density and domain formation. We show a consistency in transition patterns of 2-D monolayers and 3-D GUVs. In GUVs incorporated with NBD-PC dye, GUV undergoes volume decrease; while, DPPC GUVs visualized with Rhodamine-B dye burst before transition can be completed. These results lead us to propose a model to explain these differences: permeability of the lipid hydrocarbon chain is a dominant factor to consider if a GUV can tolerate volume change, thus completing phase transition. Thus DPPC-GUVs incorporated with NBD allow better permeability to water because of the asymmetrical hydrocarbon region of NBD; and DPPC-GUVs visualized with Rhodamine, is less permeable to water because Rhodamine has symmetrical hydrocarbon tails like that of DPPC. To verify this hypothesis, we test osmotic response of these two different systems, and experiments seem to adhere to proposed model.

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P9-005

Nonadditive interactions in protein folding: the Zipper model of cytochrome c

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Hydrogen exchange (HX) experiments [M. M. G. Krishna et al. J. Mol. Biol. 359, 1410, (2006)] reveal that folding-unfolding of cytochrome c occurs along a defined pathway in a sequential, stepwise manner. The simplified zipper-like model involving nonadditive coupling is proposed to describe the classical “on pathway” folding-unfolding behavior of cytochrome c. Using free energy factors extracted from HX experiments, the model can predict and explain cytochrome c behavior in spectroscopy studies looking at folding equilibria and kinetics. The implications of the proposed model are discussed for such problems as classical pathway vs. energy landscape conceptions, structure and function of a native fold, and interplay of secondary and tertiary interactions.

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P9-006

Investigation of Anti-cancer Effect of Benzenesulfonamidoindolinone Derivative J-3944 in Human Non-small Cell Lung Cancer A549 Cells

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The aim of this study is to investigate the anticancer mechanism of J-3944, benzenesulfonamide, against the non-small cell lung cancer A549. J-3944 is a benzenesulfonamidoindolinone, a small molecule designed and synthesized in our laboratory as an anticancer agent. J-3944 exhibited potent inhibitory activity against various human cancer cell lines, especially the invasive human lung cancer A549 cell line (GI_{50} = 0.07 M). The flow cytometric cell cycle analysis of J-3944 on A549 cell lines showed G2/M arrest. The TUNEL assay confirmed an apoptosis. The confocol microscope showed that J-3944 resulted in shorter microtubule scatter around nucleus in A549 cell. The microtubule assembly assay and tubulin competition-binding SPA assay revealed J-3944 as a microtubule inhibitor and through binding to the colchicines binding site. Results of Western blotting showed an increased expression of some mitotic marker protein like MPM-2 and cyclin B1 during 3 to 12 hr indicating that cells entered and blocked at mitotic phase. The expression of p53 was significantly increased after treatment. The pro-apoptotic proteins of Bcl-2 family, PUMA、Bad、Bax, were induced by p53. Meanwhile, the expression of anti-apoptotic protein of Bcl-2 family: Mcl-1 was decreased. The net-effect of those Bcl-2 family proteins caused mitochondrial outer membrane permeabilization and subsequently the release of cytochrome *c*. Further, the activation of caspase-3 and 9 triggered apoptosis. Survivin played a dual role in up-regulated when cell arrested at M phase because it participated in the spindle formation and down-regulated following mitotic arrest indicating its IAPs function. We also found that increased expression of active cleaved form of cathepsin B might be involved in apoptosis. The lysosome integrity analyzed by flow cytometry revealed that the activated cathepsin B may result from the lost of lysosome membrane integrity.

P9-007

Thermodynamic parameters of protein folding

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It has been suggested both experimentally and theoretically that the characteristics of protein folding kinetics could be quite simple. Nowadays, there are many protein folding models which are based on simple ideas. For example, “Kinetic Ising Model” with the mean field approximation has been applied to protein folding problems. It assumes that a protein is composed of a topological collection of structural units. Experimentally, hydrogen exchange (HX) experiments have revealed that cytochrome c (Cyt *c*) folds in a sequential way. They have shown that Cyt *c* protein consists of five cooperative folding-unfolding units, called foldons. Actually both structural units and foldons describe the same concept.

In this study, we proposed a thermodynamic treatment on the temperature dependence of protein folding. It is used to calculate the thermodynamic parameters from experimental data such as DSC, UV/Vis, CD, etc. We derive the mathematical formulas analytically with proper approximations. Besides, our treatment requires the deconvolution of DSC data. The deconvolution process is based on the protein foldon theory and some physical constraints. We have calculated a series of thermodynamic proteins including Cyt *c*, lysozyme, etc. Our results agree quite well with other experiments. It suggests that we can apply our treatment to calculate thermodynamic parameters of other proteins.

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P9-008

Model for Cell Differentiation: From Single Cells to Multi-cellular Organism

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We propose a model for cell differentiation and carcinogenesis. By distinguishing different stages of cell differentiation and its interaction energies, we simulate cell aggregation and organogenesis during the processes of embryogenesis with Monte Carlo simulations. This model can also describe the process of carcinogenesis and other tumorigenesis including both benign and malignant tumors. Even dysfunctions and aging events can also be incorporated. We hope this model will provide further insights toward cancer formations.

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P9-009

Measuring the elasticity of human leucocytes by utilizing Home-made AFM

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Atomic force microscopy (AFM) has been developed an important tool for quantifying mechanical properties of molecules and cells in biological field. Recently, AFM is utilized measuring elastic and viscoelastic properties of blood cells. Here we built a AFM system to measure and compare the deformability of human promyelocytic leukemia (HL-60) cells with HL-60 by Garlic oil treatment. In this study the cells are well described by an elastic model based on Hertzian mechanics from force-displacement curves. And we demonstrated that the elastic modulus of HL-60 and HL-60 by Garlic oil treatment are about 607 Pa and 2258 Pa, respectively. The result indicated the elastic modulus of HL-60 is increased by Garlic oil treatment.

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