ABSTRACTS

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SOLUTION STRUCTURE OF THE DIMERIZATION DOMAIN OF THE EUKARYOTIC STALK P1/P2 COMPLEX REVEALS THE STRUCTURAL ORGANIZATION OF EUKARYOTIC STALK COMPLEX

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The lateral ribosomal stalk is responsible for the kingdom-specific binding of translation factors and activation of GTP hydrolysis during protein synthesis. The eukaryotic stalk consists of the scaffold P0 protein which binds two copies of P1/P2 hetero-dimers to form a P0(P1/P2)2 pentameric P-complex. The structure of the eukaryotic stalk is currently not known. To provide a better understanding on the structural organization of eukaryotic stalk, we have determined the solution structure of the N-terminal dimerization domain (NTD) of P1/P2 hetero-dimer. Helix-1, 2 and 4 from each of the NTD-P1 and NTD-P2 form the dimeric interface that buries 2200 Å2 of solvent accessible surface area. In contrast to the symmetric P2 homo-dimer, P1/P2 hetero-dimer is asymmetric. Three conserved hydrophobic residues on the surface of NTD-P1 are replaced by charged residues in NTD-P2. Moreover, NTD-P1 has an extra turn in helix-1, which forms extensive intermolecular interactions with helix-1 and 4 of NTD-P2. Truncation of this extra turn of P1 abolished the formation of P1/P2 heterodimer. Systematic truncation studies suggest that P0 contains two spine-helices that each binds one copy of P1/P2 heterodimer. Modeling studies suggest that a large hydrophobic cavity, which can accommodate the loop between the spine-helices of P0, can be found on NTD-P1 but not on NTD-P2 when the helix-4 adopts an “open” conformation. Based on the asymmetric properties of NTD-P1/NTD-P2, a structural model of the eukaryotic P-complex with P2/P1:P1/P2 topology was proposed.

INCREASED STRUCTURAL FLEXIBILITY AT THE ACTIVE SITE OF A FLUOROPHORE-CONJUGATED β-LACTAMASE DISTINCTIVELY IMPACTS ITS BINDING TOWARD DIVERSE CEPHALOSPORIN ANTIBIOTICS

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The Ω-loop of β-lactamases was found important in the kinetics and substrate profile of the binding of β-lactam antibiotics to these enzymes. It is next to the active site and is posed as steric hindrance towards binding of bulky substrates, such as oxyimino-cephalosporins. It has been shown that mutating certain residues on the Ω-loop would enhance the structural flexibility of the Ω-loop and expand the hydrolytic activity of β-lactamases. However, there are no such structural demonstrations on the relationship between flexibility of the Ω-loop and the substrate profile of β-lactamases. We herein report an engineered β-lactamase (PenP_E166C) that has been conjugated with an environmentally sensitive fluorophore, badan (b), near its active site in order to probe the environmental changes upon binding of diverse substrates. Our results show that this engineered β-lactamase (namely PenP_E166Cb) has improved the binding rate of and significant changes in fluorescence signal upon binding with oxyimino-cephalosporins that have large side chains, but they have little effect on non-oxyimino-cephalosporins. Our structural studies reveal that the Ω-loop undergoes a conformational change to accommodate the binding of bulky oxyimino-cephalosporins, but no such changes were observed in the binding of non-oxyimino-cephalosporins. This helps to explain the increased flexibility of Ω-loop and the improved binding rate of the enzyme. Mutational study correlates the structural flexibility of the Ω-loop with the substrate-specific fluorescence profile. The oxyimino-cephalosporin-specific fluorescence profile of our engineered β-lactamase also demonstrates the possibility of designing substrate-selective biosensing systems.

INTERACTION BETWEEN HYDROGENASE MATURATION FACTORS HYPA AND HYPB IS REQUIRED FOR [NIFE]-HYDROGENASE MATURATION

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The active site of [NiFe]-hydrogenase contains nickel and iron coordinated by cysteine residues, cyanide and carbon monoxide. Metal chaperone proteins HypA and HypB are required for the nickel insertion step of [NiFe]-hydrogenase maturation. How HypA and HypB work together to deliver nickel to the catalytic core remains elusive. Here we demonstrated that HypA and HypB from *Archaeoglobus fulgidus* form 1:1 heterodimer in solution. Based on the crystal structure of *A. fulgidus* HypB, 14 mutants were designed to map the HypA binding site on HypB. Our results showed that truncation of the N-terminal residues of *A. fulgidus* HypB abolished the interaction with HypA. Consistent with this observation, we demonstrated that two conserved residues, Leu-78 and Val-80, located at the N-terminus of the GTPase domain of *Escherichia coli* HypB were required for HypA/HypB interaction. We further showed that L78A and V80A mutants of HypB failed to reactivate hydrogenase in an *E. coli ΔhypB* strain. Our results suggest that the formation of the HypA/HypB complex is essential to the maturation process of hydrogenase. The HypA binding site is in proximity to the metal binding site of HypB, suggesting that the HypA/HypB interaction may facilitate nickel transfer between the two proteins.

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EVOLUTION CHARACTERISTICS OF PROTEIN MODIFICATION IN *ESCHERICHIA COLI*

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Protein modification which regulates activities of cells in the translation level is an epigenetic modification. *E. coli* is the most representative prokaryotic model organism. Previous studies have demonstrated various types of protein modifications in the cell, but the characteristics of protein modification in the evolution are not largely known. Using 2-DE based proteomics, 1088 protein spots were detected in *Escherichia coli* BW25113. They protein spots represented 688 unique protein entries, suggesting a plenty of protein modification. GO classification analysis showed that the higher the identity of sequence in the evolution, the more modification number of the proteins. In the same category, proteins with the more modification numbers were usually located in physiologically important hub, whereas proteins with the less modification numbers trended to be placed in secondary node. These findings indicate that protein modification is related to evolution and thus contributes to construction of stable hub and variable node.

STRUCTURAL STUDIES ON THE HEPATITIS B CORE ANTIGEN PARTICLE TREATED WITH NUCLEASE

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Hepatitis B virus (HBV) is a widespread human pathogen without effective healing methods. Our previous structural study has confirmed the key role of charge-balance on the RNA encapsidation of HBV (Liu et al., 2010). To testify the relationship between charge-balance and capsid stability (Chua et al., 2010; Newman et al., 2009), we used cryo-electron microscopy (cryoEM) technique to resolve the 3D structure of Hepatitis B core antigen (HBcAg)-164 particles after digested by micrococcal nuclease. The results indicate that the breakdown of charge-balance will lead to the reducing of RNA content in capsid, as well as the weaker of the stability of capsid architecture. This interesting structural change induced by electrostatic interaction may provide a possible way for developing anti-HBV drug in the future.

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STRUCTURAL SPECIALIZATIONS OF A2, A FORCE-SENSING DOMAIN IN THE ULTRALARGE VASCULAR PROTEIN VON WILLEBRAND FACTOR

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von Willebrand factor (vWF) is a plasma glycoprotein that is expressed by endothelial cells and megakaryocytes as large multimers of varying sizes. Larger multimers bind circulating platelets tighter, and also more readily undergo conformational changes in response to the hemodynamic forces. vWF main functions is to mediate interactions between platelets, and between platelets and the walls of blood vessels. Both types of interaction are vital in maintaining the balance between bleeding and clotting. The lengths of Von Willebrand factor (VWF) concatamers correlate with hemostatic potency. After secretion in plasma, length is regulated by hydrodynamic shear force-dependent unfolding of the A2 domain, which is then cleaved by a specific protease. The 1.9 Å crystal structure of the A2 domain demonstrates evolutionary adaptations to this shear sensor function. Unique among VWA domains, A2 contains a loop in place of the α4 helix, and a cis-proline. The central β4-strand is poorly packed, with multiple sidechain rotamers. The Tyr-Met cleavage site is buried in the β4-strand in the central hydrophobic core, and the Tyr structurally links to the C-terminal α6-helix. The α6-helix ends in two Cys residues, which are linked by an unusual vicinal disulfide bond that is buried in a hydrophobic pocket. Simulations show that the rigid 8-membered disulfide ring raises the force barrier for the initial step in unfolding, the disruption of the α6-helix. These features may narrow the force range over which unfolding occurs, and may also slow refolding. Von Willebrand disease mutations, which presumably lower the force at which A2 unfolds, are illuminated by the structure. The present structures provide the first atomic insight into the folding of the protein and its conformational stability of the ADAMTS13-cleaved site, as well as have played an important role of identifying the contribution of the key residues in vWF disease type II A.

ASI11-A07

SEARCH OF INHIBITORS THAT TARGET HIV PRE-MRNA SPLICING TO OVERCOME DRUG RESISTANCE

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Human immunodeficiency virus (HIV) is a retrovirus that cause acquired immunodeficiency syndrome (AIDs). Expression of the integrated HIV-1 provirus is largely dependent on the host's splicing factors such as serine-arginine rich proteins (SR proteins). Alternative splicing factor/splicing factor 2 (ASF/SF2), a prototypic SR protein that is essential for pre-mRNA splicing, has been shown to play critical roles during HIV-1 pre-mRNA splicing and replication. ASF/SF2, like other SR proteins, is phosphorylated by SR protein-specific kinases (SRPKs) at its C-terminal arginine/serine (RS) domain, which governs its localization and metabolism. Structural and functional studies of SRPK1 in complex with ASF/SF2 has revealed that a docking groove on SRPK1 that is distal to the active site interacts strongly with a docking motif and the RS domain of ASF/SF2, leading to high affinity binding as well as regulating the mechanism of phosphorylation. We propose that by blocking this interaction, we can interfere the phosphorylation of ASF/SF2 and inhibit its activity during splicing of HIV-1 pre-mRNA.

We have adopted structure-based *in silico* screening method to identify potential inhibitors that bind to the docking groove of SRPK1 to block the binding and phosphorylation of ASF/SF2. Currently, over 500,000 compounds have been screened using the program AutoDock VINA and 50 potential candidates of inhibitor have been selected. In vitro kinase assays showed that six compounds exhibit inhibitory activity against the phosphorylation of ASF/SF2. Biochemical analyses are underway to evaluate the effects of these inhibitors on the splicing of HIV-1 mRNA.

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ASI11-A08

APTAMERS AGAINST *P. FALCIPARUM* HISTIDINE RICH PROTEIN 2 AS A NEW APPROACH TO MALARIA DIAGNOSIS

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Malaria is a life-threatening infectious disease caused by the protist Plasmodium, of which the species *Plasmodium falciparum* is the most severe. Approximately half of the world's population is at risk of malaria and most of the malaria cases occur in the developing world, where inadequate access to diagnostic tools leads to misdiagnosis or overuse of anti-malaria drugs. Although antibody-based rapid diagnostic tests have been developed they continue to have a number of problems, therefore point of care malaria diagnostics is an ongoing challenge. We are developing a new approach for malaria diagnosis by using aptamer technology. Here, we describe the purification of *P. falciparum* histidine rich protein 2 (HRP2), a diagnostic marker of *P. falciparum* infection. We also describe the selection and characterisation of aptamers against HRP2 carried out by Systematic Evolution of Ligands by Exponential Enrichment (SELEX). This work lays a foundation for the further development nucleic acid aptamers for malaria diagnosis.

ASI11-A09

STUDY ON THE INTERACTION BETWEEN DNA-BINDING DOMAIN (DBD) OF HUMAN ANDROGEN RECEPTOR (AR) AND SWIRM DOMAIN OF LYSINE-SPECIFIC DEMETHYLASE 1 (LSD1)

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Gene regulations of prokaryotes and eukaryotes are different. Prokaryotic gene regulation requires simply binding of regulatory proteins to help with or avoid forming transcription complex. For eukaryotes like humans, however, their regulation needs “chromatin remodeling” with the association of regulatory proteins involving the opening up of DNA-histone protein complex chromatin and unwinding DNA. Without remodeling, RNA polymerases responsible of the transcription process cannot get access and perform transcription.

Lysine-specific demethylase 1 is one of the chromatin remodeling enzymes. It can demethylate specifically the N-tail mono- or di-methylated K4 residue on histone H3 by oxidation (Y. Shi et al., 2004). LSD1 has three known domains: FAD-binding domain, demethylase domain and SWIRM domain. Till now, the exact function of SWIRM domain of LSD1 is still unclear, although its solution structure has been reported.

In 2005, Metzger’s group found that the LSD1 SWIRM domain could bind the N-terminus, the DNA-binding domain (DBD) and the ligand-binding domain (LBD) of androgen receptor (AR) by performing a GST-pull down assay (Metzger et al., 2005); and shows that SWIRM domain has the strongest interaction with DBD among the domains of AR. Therefore, my study is to investigate the interaction between AR-DBD and SWIRM-LSD1 domains by NMR spectroscopy.

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ASI11-A10

STRUCTURAL BASIS FOR RNA-BINDING AND HOMO-OLIGOMER FORMATION BY INFLUENZA B VIRUS NUCLEOPROTEIN

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Influenza virus nucleoprotein (NP) is the major component of the viral ribonucleoprotein complex, which is crucial for the transcription and replication of the viral genome. We have determined the crystal structure of influenza B virus NP to a resolution of 3.2 Å. Influenza B NP contains a head, a body domain and a tail loop. The electropositive groove between the head and body domains of influenza B NP is crucial for RNA binding. This groove also contains an extended flexible charged loop (aa. 125-149). The two lysine clusters at the first half of this loop were shown to be crucial for binding RNA. Influenza B NP forms a crystallographic homo-tetramer by inserting the tail loop into the body domain of the neighboring NP molecule. A deeply buried salt bridge R472-E395 and a hydrophobic cluster at F468 are the major driving forces for the insertion. The analysis of the influenza B virus NP structure and function and comparisons with influenza A virus NP provide insights into the mechanisms of action and underpin efforts to design inhibitors for this class of proteins.

INTERACTION BETWEEN MAIZE RIBOSOME-INACTIVATING PROTEIN AND RIBOSOMES

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Ribosome-inactivating proteins (RIPs) represent a group of N-glycosidases which can cleave the N-glycosidic bond of adenine at 23S and 28S ribosomal RNA (rRNA) of ribosome and subsequently lead to a halt of protein synthesis and cell death.

Regardless to the universal rRNA target, the highly conserved catalytic residues and consensus tertiary structure of RIPs, the activity of RIPs is highly deviated. It is known that interacting with ribosomal proteins is required before RIPs elicit their direct action on rRNA. Here we hypothesize the interaction between RIPs and ribosome is correlated to the activity of RIPs.

In this study, we compared the catalytic activity of three RIPs: maize RIP (MOD), trichosanthin (TCS) and ricin A chain (RTA) and the nature of the interaction with their common ribosomal interacting partner, the stalk protein P2. Using pull-down assay and surface plasmon resonance, we found that MOD interacts with P2 chiefly through electrostatic interaction whilst TCS and RTA via a combination of electrostatic and hydrophobic forces. MOD-P2 is the weakest pair as revealed from its highest binding affinity constant (K_D) of 1 µM. TCS interacts with P2 relatively stronger at a K_D of 0.61 µM but is weaker than RTA-P2 which has a K_D of 0.24 µM. The pattern is also coincident with their *N*-glycosidase activity on rat liver ribosome and cytotoxicity on JAR and 293T cells. We conclude that RIPs despite targeting at the same ribosomal protein, the nature of the interaction is the crucial factor of the strength of their interaction and activity.

SCREENING OF ANTIVIRALS FOR INFLUENZA A NUCLEOPROTEIN BY VIRTUAL SCREENING

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The ever-changing nature of influenza virus by antigenic variations challenges the development of an effective influenza drug. The identification of drug-resistant strains from the recent outbreak of 2009 H1N1 pandemic again reminds us the importance of developing new antiviral agents. Nucleoprotein (NP) is a key component in the ribonucleoprotein (RNP) complex. Being structurally and functionally important to the viral replication cycle makes NP an attractive drug target.

Virtual screening is becoming an effective paradigm in drug discovery. We adopted structured-based virtual screening as our platform to discover novel inhibitors targeting the influenza A NP e. Out of the 9526 natural products, 15 virtual hits were selected. Chemical 7 and Chemical 16 were found to be inhibitory to the RNP polymerase activity. Two analogues of Chemical 16 were also found to suppress the RNP polymerase activity. Their inhibitory effect on viral replication was then proved by plaque reduction assay. Further optimization of these chemicals will lead to useful anti-influenza agents.

IDENTIFICATION OF NOVEL PINX1 INTERACTING PARTNER, NUCLEOPHOSMIN AND THEIR ROLE IN TELOMERASE REGULATORY PATHWAY

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Telomeres, located at the ends of the linear chromosomes, are for maintaining genomic stability and preventing end-to-end fusion of chromosomes. It becomes shortened due to the end replication problem in mitosis. Telomerase is responsible for maintaining the length of the telomeric DNA and its activity is absent in most of the cells, except germline, stem cells and cancerous cells. Thus, telomerase is believed to play an important role in both aging and cancer. PinX1 has been found as a potent telomerase inhibitor. It is shown to directly interact with the hTERT and hTR domains in telomerase, as well as TRF-1, which is a telomeric protein involved in regulating the length of telomere inside the cell. PinX1 is also shown to facilitate the nucleolar localization of telomerase. Recent research showed that both overexpression and silencing of PinX1 cause telomere shortening. The mechanism of how PinX1 regulates the telomere maintenance in cells is still not clearly elucidated. The present study focuses on characterizing the PinX1 interacting partners in order to understand the pathway involved in telomere maintenance. Around 40 potential interacting partners were identified by pull down and mass spectrometry. From the potential candidates, nucleophosmin (NPM) was confirmed to interact with PinX1 by immunoprecipitation and in vitro pull down assay. Nucleophosmin was shown to attenuate the inhibitory effect of PinX1 on telomerase. Besides, NPM and PinX1 were found to be co-localized together in HeLa cell by immunofluorescence. These suggest NPM/PinX1 interaction is involved in telomerase regulatory pathway and may be important for telomerase activation.

INTERACTION STUDY OF FLAGELLAR PROTEIN COMPLEX FLIS/HP1076/FLAB OF *HELICOBACTER PYLORI*

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The bacterial flagellum is a highly complex macromolecular structure that its assembly requires precise coordination of both gene expression and protein interactions. More than fifty proteins including structural components of the filament, hook, basal body, motor and export apparatus, and proteins in sensory and regulatory systems are involved. Export chaperone is one of the regulatory proteins which binds to flagellar molecules to prevent premature polymerization in cytosol or to facilitate their export by docking to export apparatus. In *E. coli* and Salmonella, the three export chaperones identified each shows to have specific substrate. However, in *Helicobacter pylori*, only one export chaperone FliS is identified up-to-date. Previous yeast two-hybrid analysis revealed a number of FliS interacting partners in *H. pylori* proteome, suggesting that in addition to its export chaperone activity, FliS may exhibit different biological functions in *H. pylori*. Recently, we identified an uncharacterized protein HP1076 which displays co-chaperone like activity that binds and stabilizes FliS. Here, we report the physical interaction of FliS, HP1076 and the C-terminal end of flagellin molecule (FlaBc) and preliminary crystallographic study of protein complex FliS/HP1076/FlaBc. Atomic details of the crystal structure would provide insight to understand the structural and functional diversity of FliS.

IN-SILICO SCREENING FOR INHIBITORS BLOCKING THE ASSEMBLY OF INFLUENZA A VIRUS POLYMERASE COMPLEX

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Influenza virus has always been a major threat to humankind, causing several pandemics in the past century, and killing thousands of people annually. Currently, much strains of Influenza virus had developed resistant against the FDA-approved anti-influenza drugs, thus it is essential to develop new drugs against this virus. The Influenza RNA-dependent RNA polymerase consists of three subunits - PA, PB1 and PB2. By blocking the protein-protein interactions among these subunits, the viral RNA polymerase complex would fail to assemble, thereby inhibiting Influenza virus replication. The co-crystal structure of PA-C terminal and PB1-N terminal was resolved recently. It was shown that PB1 binds to PA by inserting a short helix into a hydrophobic core of PA, and the residues at the interacting interface are well conserved within type A Influenza. Therefore, the PA-PB1 interface might be a good site for drug inhibition. We employed virtual screening technique to identify small molecules that most likely would block the PA hydrophobic core. Compound databases were archived from ZINC (UCSF) and commercial vendors (e.g. SPECS) and then virtually docked to the PA hydrophobic core by AutoDock 4.0 and AutoDock Vina. Top results were then subjected to post-screening evaluation, including visual inspection by molecular visualization software (e.g. PyMOL), prediction of drug-likeness by Lipinski's rule and consensus docking. After post-screening analysis, we selected ~150 potential hit compounds for primary screening, which involves the cytotoxicity assay and the ribonucleoprotein activity assay. In vitro assay and whole virus assay would be employed for further validation. The identification of hit compounds provides the basis for future optimization and lead compound development against Influenza virus.

CRYSTAL STRUCTURE OF NDM-1 REVEALS A COMMON B-LACTAM HYDROLYSIS MECHANISM

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Metallo-β-lactamases (MBLs) hydrolyze most β-lactam antibiotics, and bacteria containing this kind of enzyme pose a serious threat to the public health. The newly identified New Delhi MBL (NDM-1) is a new member of this family that shows tight binding to penicillin and cephalosporins. The rapid dissemination of NDM-1 in clinically relevant bacteria has become a global concern. However, no clinically useful inhibitors against MBLs exist, partly due to the lack of knowledge about the catalysis mechanism of this kind of enzyme. Here we report the crystal structure of this novel enzyme in complex with a hydrolyzed ampicillin at its active site at 1.3-Å resolution. Structural comparison with other MBLs revealed a new hydrolysis mechanism applicable to all three subclasses of MBLs, which might help the design of mechanism based inhibitors.

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ESTABLISHMENT OF NATURAL PRODUCTS DATABASE FOR NOVEL CXCR4 ANTAGONIST DISCOVERY

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With the rapid advances in personal computing power, virtual drug screenings has become increasingly popular. While there are numerous databases for synthetic compounds, there are few natural product databases that are specifically for *in silico* docking study. To facilitate virtual docking on natural compounds, we have established our in-house Natural Products Database, which contains approximately 8,000 naturally occurring chemicals so far. Most of them are documented in Traditional Chinese Medicines. Specific chemicals from various species have been collected in PDB format by CORINA web service, and converted to PDBQT and MOL2 format by AutoDock Tools software. Subsequently AutoDock vina program was used to rank the members of our natural products database, containing experimentally validated chemokine receptor type 4 (CXCR4) inhibitors. The binding energy predictions were highly correlated with experimentally results in our cell based assay. Novel core structure CXCR4 antagonists were identified by this virtual screenings, including edgeworoside, mulberrofuran, genkwanine and perrottetin. Edgeworosides (Edgeworoside A, B and C) are anti-inflammatory agents and are isolated from *Edgeworthia chrysantha* Lindl. and *Daphne bholua*. Mulberrofuran (Mulberrofuran F, G and P) are isolated from Morus, showing moderate cytotoxic activities against human cancer cell lines. Genkwanine are isolated from *Daphne genkwa* and contains a large family compounds, including Genkwanine A~O and i~v. This set of chemicals showed potent inhibitory activity against endothelium cell HMEC and cytotoxic activities. None of above compounds has been reported to have CXCR4 inhibition activity, thus providing an opportunity to develop novel CXCR4 antagonists. The *in vitro* validation will be conducted in further study.

ASSEMBLY OF THE PREACTIVATION COMPLEX FOR UREASE MATURATION IN *HELICOBACTER PYLORI*: CRYSTAL STRUCTURE OF THE UREF-UREH PROTEIN COMPLEX

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Colonization of *Helicobacter pylori* in the acidic environment of the human stomach depends on the neutralizing activity of urease. Activation of apo-urease involves carboxylation of lysine-219 and insertion of two nickel ions. In *H. pylori*, this maturation process involves four urease accessory proteins: UreE, UreF, UreG and UreH. It is postulated that the apo-urease interacts with UreF, UreG and UreH to form a pre-activation complex that undergoes GTP-dependent activation of urease. Crystal structure of the UreF/UreH complex reveals conformational changes in two distinct regions of UreF upon complex formation. Firstly, the flexible C-terminal residues of UreF become ordered, forming an extra helix α10 and a loop structure stabilized by hydrogen bonds involving R250. Secondly, the first turn of helix α2 uncoils to expose a conserved residue, Y48. Substitution of R250A or Y48A in UreF abolishes the formation of the heterotrimeric complex of UreG/UreF/UreH, and abolishes urease maturation. Our results suggest that the C-terminal residues and helix α2 of UreF are essential to the recruitment of UreG for the formation of the pre-activation complex. Molecular weight of UreF/UreH complex determined by static light scattering was 116 $\pm$ 2.3 kDa, which is consistent with the quaternary structure of a dimer of heterodimers observed in the crystal structure. Taking advantage of the unique 2-fold symmetry observed in both the crystal structures of *H. pylori* urease and the UreF/UreH complex, we proposed a topology model of the pre-activation complex for urease maturation.

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STRUCTURAL CHARACTERIZATION AND ANTI-HIV-1 ACTIVITIES OF ARGININE/GLUTAMATE-RICH POLYPEPTIDE LUFFIN P1 FROM THE SEEDS OF SPONGE GOURD (*LUFFA CYLINDRICA*)

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Luffin P1, the smallest ribosome-inactivating peptide from the seeds of *Luffa cylindrica* was found to have anti-HIV-1 activity in HIV-1 infected C8166 T-cell lines and be able to bind with HIV Rev Response Element. Nuclear magnetic resonance spectroscopy revealed that the Luffin P1 comprises a helix-loop-helix motif, with the two alpha helices tightly associated by two disulfide bonds. Based on our findings, we conclude that unlike the well-studied ribosome-inactivating proteins, which exert their action through N-glycosidase activities, Luffin P1 demonstrates a novel inactivation mechanism probably through the charge complementation with viral or cellular proteins. Our work also provides a new scaffold for the design of novel inhibitors from a simple helical motif.

CONFORMATIONAL FLEXIBILITY OF FLIG PROVIDES STRUCTURAL INSIGHTS FOR MOTOR SWITCHING AND COUPLING MECHANISM

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Flagellar rotation is controlled by a reversible rotary motor. Motor switching is regulated by the binding of phosphorylated CheY to FliM that triggers the conformational change of FliG and alter its binding with stator MotA4B2. Binding of CheY-P is highly cooperative to the switch response. A recent hallmark study explains the switch mechanism by a conformational spread model and shows that the presence of conformational heterogeneity among subunits is critical to both allosteric mechanism and to the cooperativity of the motor. However, the structural information to understand the basis of conformational heterogeneity remains limited. Here we report FliG structures from H. pylori with distinct conformations from previously reported FliG. We uncovered at least three key flexible regions that allow FliG to exist as multiple conformations. We argue that the local conformational changes conferred by the flexible loops are independent, thus intensifying the possible conformations of FliG states. The “dominant” conformation is instead stabilized by stochastic interactions with FliM which depends of the concentration of CheY-P. In the presentation, the switching mechanism based on the structural information of chemotaxis and switch proteins from H. pylori will be discussed.

EXPRESSION, PURIFICATION AND CRYSTALLISATION OF THE PLASMODIUM FALCIPARUM GTP: AMP PHOSPHOTRANSFERASE

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Malaria is an infectious disease caused by the parasite *Plasmodium*. It is widespread in tropical and subtropical regions affecting millions of life. In the five *Plasmodium* species accounting for human infection, *P. falciparum* is the most virulent causing the majority of infections in Africa and is responsible for most severe disease and mortality. Adenylate kinases (AK) are phosphotransferase enzymes that catalyses the interconversion of adenine nucleotides thereby playing an important role in energy metabolism. They are highly active enzymes that catalyze the magnesium-dependent reaction ATP + AMP $\Leftrightarrow$ 2(ADP) and act as a sensor for changes in cellular metabolism. In *P. falciparum*, three isoforms of AK (*Pf*AK) including *Pf*AK1, *Pf*AK2 and *Pf*GAK, have been identified. *Pf*GAK exhibits a substrate preference for GTP and AMP, and does not accept ATP as a substrate.

To understand the structural basis of GTP:AMP binding to the *Pf*GAK and its enzymatic activity, we have cloned and expressed the *Pf*GAK in *E. coli*. The protein has been purified using 2 steps chromatography. The specific activity of recombinant *Pf*GAK was determined to be 250 mmol min^{-1} mg^{-1}. Crystallisation conditions of *Pf*GAK were screened and hexagonal crystals were obtained under the condition of 2M ammonium sulphate and 0.1M Tris-HCl, pH 8.0. X-ray diffraction data for *Pf*GAK was collected to 3.0Å and the space group of the crystal was determined to be $P3_121$ or $P3_221$.

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CHARACTERIZATION AND STRUCTURE DETERMINATION OF THE CDT1 BINDING DOMAIN OF HUMAN MINICHROMOSOME MAINTENANCE (MCM) 6

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The minichromosome maintenance (Mcm) 2-7 complex is the replicative helicase in eukaryotic species, and it plays essential roles in the initiation and elongation phases of DNA replication. During late M and early G1, the Mcm2-7 complex is loaded onto chromatin to form pre-replicative complex (pre-RC) in a Cdt1-dependent manner. However, the detailed molecular mechanism of this loading process is still elusive. In this study we demonstrate that the previously uncharacterized C-terminal domain of human Mcm6 is the Cdt1 binding domain (CBD), and present its high-resolution nuclear magnetic resonance (NMR) structure. The structure of CBD exhibits a typical "winged-helix" fold that is generally involved in protein-nucleic acid interaction. Nevertheless, the CBD failed to interact with DNA in our studies indicating that it is specific for protein-protein interaction. The CBD-Cdt1 interaction involves the helix-turn-helix (HTH) motif of CBD. The results reported here provide insight into the molecular mechanism of Mcm2-7 chromatin loading and pre-RC assembly.

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ASI11-A23

MOLECULAR DOCKING OF CYSTEINE PROTEASE INHIBITOR FROM *ASCARIS LUMBRICOIDES* WITH CATHEPSINS

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Cathepsins, located in lysosome, belong to the protease family and contain a lot of members, such as serine protease (catehpsin A, G), aspartic protease (cathepsin D, E), and cysteine protease (cathepsin B, L, S). They play important roles in the regulation of various biological processes and are involved in some diseases such as Alzheimer's disease, cancer, and autoimmune disease.

Cystatins are a class of Cysteine protease inhibitors (CPIs), which can naturally and inversely bind with the cysteine protease active pocket. CPI super-family can be divided into three subfamilies. Type 1, about 100 amino acid residues and without signal peptide, is also called stefin (stenfin A, B). Type 2, about 120 amino acid with signal peptide and two internal disulfate bonds, contains cystatin C, D, F and CEW. The third type is a multiple type2-domain class. The cysteine protease inhibitor from *Ascaris lumbricoides* (*Al*-CPI) can regulate the immune response of its host (human) by inhibiting the human cathepsin proteases. In this work, *Al*-CPI was used as ligand and docked with different cathepsin proteases. The docking results were compared to the enzymatic activity experiment.

ASI11-A24

MOLECULAR DOCKING OF FLAVONOIDS TO ESTROGEN-RELATED RECEPTOR SUGGESTS MECHANISM FOR THEIR INVERSE AGONIST ACTIVITY

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A variety of flavonoids are considered to be important in cancer prevention. Their impact on estrogen signaling has been proposed as one of the potential mechanisms. They might act as estrogen agonists or antagonists for estrogen-related receptor (ERR) through blockage of interactions between ERR and coactivator peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α). In this study, a molecular docking study was performed to examine the binding structures of flavonoids with ERR, using three different docking programs (AutoDock, AutoDock Vina, and LigandFit). Three programs generated consistent result. Docking result showed that flavonoids could bind to ligand binding domain (LBD) of ERR in their inactivated forms only, while there is steric interference between flavonoids and the side chain of residues of ERR-LBD in their activated forms suggesting that flavonoids will not bind to the activated form. Our current result is in agreement with previous functional study, it also provides a structural insight into the inverse agonist activity for flavonoids against ERR.

ASI11-A25

CRYSTAL STRUCTURE OF HUMAN DIHYDROXYACETONE KINASE, A NEGATIVE REGULATOR OF MDA-5

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Dihydroxyacetone kinases (DAKs) are a family of evolutionarily conserved proteins. In bacteria, DAK is involved in the phosphorylation of dihydroxyacetone producing dihydroxyacetone phosphate, an intermediate for the synthesis of pyruvate via glycerol fermentation pathway. In mammals, DAK is an bifunctional enzyme, with both FMN cyclase and ATP-dependent dihydroxyacetone kinase activities. It was recently determined that this enzyme specifically interacts with MDA-5, an RNA helicase protein that is a cytoplasmic viral RNA sensor in the innate immunity pathway. This interaction results in the specific inhibition of MDA-5 mediated type I IFN signaling pathway and innate immune response. In the present study, we have expressed, purified and crystallised human DAK. The structure was solved by molecular replacement. Similar to the previously determined *Citrobacter freundii* DAK, the human DAK is homodimeric and showed swapping in the two enzymatic domains. The determination of this structure would provide insights to the mechanism of its interaction with the innate immunity RNA helicase protein MDA-5.