Programme Schedule
and
Abstracts

Schedule for the International Conference on Nucleic Acid Enzymes and Enzymes in Human Diseases

June 16

14:30 Registration and on-campus accommodation check-in at S.H. Ho College, till 18:30
18:30 Welcoming dinner (at Dining Hall of SH Ho College, CUHK)

June 17

8:30 Registration at 7th floor Mong Man Wai Building

9:00 Opening ceremony and group photo

9:15 Richard Roberts, New England Biolab Inc., USA
"Bacterial Methylomes" – A01 (Introduced by Kam-Bo Wong)

10:00 Coffee break

Morning session (*session co-chairs)

Nucleic Acids Enzymes (I)

10:20 Ichizo Kobayashi, University of Tokyo, Japan
"Mobility of restriction-modification systems and their domains" – A02

10:50 *Nigel Richards, Indiana University-Purdue University Indianapolis, USA
"Dissecting the functional control elements in the active site of oxalate decarboxylase" – A03

11:20 Chwan-Deng Hsiao, Institute of Molecular Biology, Academia Sinica, Taiwan
"Mutations altering the interplay between GkDnaC helicase and DNA reveal an insight into helicase unwinding" – A04

11:50 *Weiguo Cao, Clement University, USA
"Old enzymes with new function and new enzymes without old function in uracil DNA glycosylase superfamily" – A05

12:20 Hua Wang, New England Biolab Inc., USA
"The heterodimer of restriction endonuclease EcoO109I K173C and wild-type recognize asymmetric sequence and has new engineered specificity" – A06

Afternoon session

Nucleic Acids Enzymes (II)

14:00 *Geoffrey Wilson, New England Biolab Inc., USA
"Studies on the mechanism of protein-DNA sequence-specificity" – A07

14:30 *Guoliang Xu, Shanghai Institutes of Biological Sciences, China
"DNA oxidation regulates gene expression" – A08

15:00 Kin-Yiu Wong, Hong Kong Baptist University, Hong Kong

"Determining enzymatic mechanisms *in silico* by computing quantum free-energy profiles and kinetic isotope effects" – A09

15:30 Tea break and poster session

Kulshina Nadia, New England Biolab Inc., USA
"Homology/structure-guided engineering of MmeI specificity at positions 1 and 2 of the target site" – P01 (poster)

Ying Zhou, New England Biolab Inc., USA
"Tt PURE: Reconstituted *in vitro* protein synthesis at elevated temperature for studying protein translation and protein evolution" – P02 (poster)

Siu-Hong Chan, New England Biolab Inc., USA
"Increasing cleavage specificity and activity of restriction endonuclease KpnI" – P03 (poster)

Posters from local postgraduate students

16:30 Hsiu-Fang Fan, National Yang-Ming University, Taiwan
"Real-time single-molecule tethered particle motion analysis reveals mechanistic similarities and contrasts of Flp site-specific recombinase with Cre and λ Int" - A10

Enzymes and proteins in medicine (I)

17:00 Yangxiang Zhou, Hong Kong Polytechnic University
"Dissecting the β-lactamase active site: how general is the general base?" – A12

17:30 Shannon Au, Chinese University of Hong Kong, Hong Kong
"Hijacking of host sumoylation system by bacterial pore-forming toxin" – A13

June 18

<u>Morning session</u>

Restriction/modification enzymes and innovative techniques (I)

9:00 *Shuang-Yong Xu, New England Biolab Inc., USA
"Natural zinc ribbon HNH endonucleases and engineered nicking endonucleases" – A14

9:30 *Makkuni Jayaram, UTexas, Austin
"Deciphering the chemical and stereochemical mechanisms of tyrosine family site-specific recombinases using methylphosphonate modification of the scissile phosphate" – A15

10:00 Sriharsa Pradhan, New England Biolab Inc., USA
"The many facets of DNMT1 targeting and regulation in mammalian cell" – A16

10:30 Alexey Fomenkov, New England Biolab Inc., USA
"DrdVI, SmoLII and PenI: a new family of ATP-dependent, multi-subunit restriction-modification system that uses a split methyltransferase for host protection" – A17

11:00 Coffee break

11:20 *Siu-Hong Chan, New England Biolab Inc., USA
"The role of the methyltransferase domain of bifunctional restriction enzyme RM.BpuSI on cleavage activity" – A18

11:50 *Elmar Weinhold, RWTH Aachen University, Germany
"Covalent plasmid DNA-antibody conjugates for targeted cell transfection" – A19

12:20 Brian Anton, New England Biolab Inc., USA
"Mapping the landscape of bacterial DNA methyltransferases using single-molecule real-time sequencing" – A20

12:50 Deming Gou, Shenzhen University, China
"A novel real-time PCR assay of microRNAs using S-Poly(T), a specific Oligo(dT) reverse transcription primer with excellent sensitivity and specificity" – A21

<u>Afternoon session</u>

Ribosomal stalk and rRNA N-glycosidase

14:30 *Uchiumi Toshio, Niigata University, Japan
"The ribosome has multiple "Arm-like" structures to catch translation factors" – A22

15:00 Kam-Bo Wong, Chinese University of Hong Kong, Hong Kong
"Solution structures of the eukaryotic stalk P-proteins reveal structural organization of eukaryotic stalk complex and how trichosanthin is recruited to the ribosome" – A23

15:30 Qin Yan, Institute of Biophysics, China
"The regulatory mechanism of the interaction between ribosomal protein L7/L12 and L11" – A24

16:00 *Nilgun Tumer, Rutgers University, USA
"Ribosome interactions of ribosome inactivating proteins" – A25

16:30 Tea break

16:50 Pang-Chui Shaw, Chinese University of Hong Kong, Hong Kong
"The interaction of trichosanthin and maize ribosome-inactivating protein with ribosomal P protein" – A26

17:20 Xiao-Ping Li, Rutgers University, USA
"The ribosome binding surface of ricin A chain is on the opposite side of the active site cleft and is blocked by the B chain" – A27

17:50 Dong-Xu He, Jiang Nan University, China
"Anti- Herpes simplex virus type 1 and anti-tumor activity of trichosanthin" – A28

18:30 **Banquet**

June 19

Morning session

Restriction-modification enzymes and innovative techniques (II)

9:00 * Rao Desirazu, Indian Institute of Science, India
"Interaction of DprA with restriction-modification system favours genetic diversity in *Helicobacter pylori*" – A29

9:30 David Dryden, University of Edinburgh, Scotland
"The structure of the Type I DNA restriction enzymes and their control of horizontal gene transfer in *Staphylococcus aureus*" – A30

10:00 Richard Morgan, New England Biolab Inc., USA
"Rational engineering of DNA recognition: simple mutations that change Type IIL restriction enzyme specificity" – A31

10:30 Coffee break

10:50 *Michael Chan, Chinese University of Hong Kong, Hong Kong
"Application of pyrrolysine analogs for the preparation and detection of ubiquitinated and sumolyated proteins" – A32

11:20 Yu Zheng, New England Biolab Inc., USA
"DNA modification-dependent restriction enzymes for epigenetic studies" – A33

Free in afternoon

June 20

Morning session

Enzymes and proteins in medicine (II)

9:00 **Keynote -** Sen-Feng Sui, Tsinghua University, China
"Towards understanding the structure and function of NSF, a member of AAA+ ATPase family" - A35 (Introduced by Shannon Au)

9:45 *Jihong Han, Nankai University
"DNA topoisomerase II inhibitors induce macrophage ABCA1 expression and cholesterol efflux – an LXR-dependent mechanism" – A36

10:15 Nei-Li Chan, National Taiwan University, Taiwan
"Structural basis of antizyme-mediated inhibition and degradation of ornithine decarboxylase" – A37

10:45 Coffee break

11:00 *Jinsong Liu, Guangzhou Institutes of Biomedicine and Health, China

“Structure of NF-κB-inducing kinase (NIK) and structure-based inhibitor design for NF-κB-inducing kinase” – A38

11:30 Quan Hao, University of Hong Kong, Hong Kong
“Novel protein post-translational modifications revealed by the crystal structure of SIRT5” – A39

12:00 Wei-Guo Zhu, Peking University Health Science Center
“Applications of histone deacetylases inhibition in cancer research” – A40

12:30 Kong-Hung Sze, University of Hong Kong, Hong Kong
“Structural insights into the association of ubiquitin C-terminal hydrolase L1's mutations with the risk of Parkinson's disease” – A41

Afternoon session

14:00 *Wyatt W. Yue, University of Oxford, England
“High throughput structural biology of inborn errors of metabolism” – A42

14:30 Mary Waye, Chinese University of Hong Kong, Hong Kong
“Diacylglycerol kinases and their association with Dyslexia and Bipolar Disorder” – A43

15:00 *Yu Wai Chen, King’s College, University of London, England
“Ataxin-3: A disease protein looking for a function” – A44

15:30 Guang Zhu, Hong Kong University of Science and Technology, Hong Kong
“Mechanistic study of proteins that regulate cell proliferation and differentiation” – A45

16:00 Tea break

16:20 *Henry Mok, National University of Singapore, Singapore
“NMR structural characterization of human dermcidin antimicrobial peptides and its relationship with Atopic Dermatitis” – A46

16:50 Jacky Ngo, Chinese University of Hong Kong, Hong Kong
“Functional characterization of the splicing kinase SRPK2” – A47

17:20 Conclusion remarks

June 21

Departure

Abstracts

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A01

Bacterial methylomes

Richard J. Roberts

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Bacterial DNA methyltransferases (MTases) are best known as orphan enzymes such as the Dam methylase of *E. coli* or as components of restriction-modification (RM) systems. Until recently, rigorously determining the specificity of MTases has been a tedious process. When they were components of Type II restriction systems it has been assumed that the MTases would have the same specificity as the cognate restriction enzyme. For Type I and Type III RM systems specificity determination was rarely attempted. With the advent of SMRT sequencing from Pacific Biosciences this situation has changed dramatically. Now it has become very simple to determine MTase recognition sequences both by cloning individual MTases in plasmids and also by sequencing whole bacterial genomes. Many Type I RM systems have been characterized and display a larger range of sequence specificities than expected. Similarly, many novel Type III RM systems have been characterized including ones using m4C for protection. This ability to examine the methylation patterns in whole genomes offers new insights into the functioning of bacteria and has led to the discovery of several novel MTases with unexpected properties.

Mobility of restriction-modification systems and their domains

Ichizo Kobayashi

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Restriction–modification systems consist of a sequence-specific DNA methyltransferase and a restriction enzyme. They contribute to maintenance and adaptive evolution of genomes by providing a unique gene expression pattern as well as a barrier to genetic flux between lineages.

Restriction-modification systems are themselves mobile and behave as selfish mobile genetic elements. In some cases, their mobility is through symbiosis with other forms of mobile elements. In other cases, their movement is unlinked to them. Insertion of restriction- modification systems induces other genome rearrangements such as amplification and inversion. Their mobility allowed detection, through genome comparison, of a restriction enzyme family of a novel fold (half pipe). Even a domain within a protein can be the unit of mobility. In some restriction-modification systems, subunits that recognize a target DNA sequence contain mobile amino-acid sequences that can apparently move between different domains of a protein through DNA recombination (Domain Movement or DoMo).

Comparison of Type III restriction-modification systems in *Helicobacter pylori* genomes revealed that their target recognition domain (TRD) sequences are mobile, moving between different orthologous groups that occupy unique chromosomal locations. Sequence comparisons suggested that a likely underlying mechanism is movement through homologous recombination between similar DNA sequences that encode amino-acid sequence motifs conserved among Type III DNA methyltransferases. Horizontal acquisition of diverse TRD sequences was suggested by detection of homologs in other Helicobacter species and distantly related bacterial species. TRD movement represents a novel route for diversification of DNA- interacting proteins. (http://www.ims.u-tokyo.ac.jp/ikobaya/publication_en.html)

A03

Dissecting the functional control elements in the active site of oxalate decarboxylase

Kellett WF [1], Zhu W [1], Campomanes P [2], Ozarowski A [3], Rothlisberger U [2], Angerhofer A [4] and Richards NGJ [1]

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Oxalate decarboxylase (OxDC) catalyzes the Mn-dependent conversion of oxalate monoanion to carbon dioxide and formate, and therefore has potential clinical application to the treatment of kidney stones and other oxalate-related human diseases. Many questions about exactly how the metal participates in catalysis and the extent to which reactivity is controlled by protein environment remain to be resolved, however. This lecture will describe recent experimental and computational studies that reveal the role of (i) a conserved tryptophan residue (W132), and (ii) an active site loop segment in modulating the electronic properties of the catalytically important Mn center. I will show that a single hydrogen bonding interaction is critical for controlling the redox potential of the metal, the utility of "state-of-the-art" quantum mechanical/molecular mechanics (QM/MM) for predicting the EPR fine structure parameters of the protein-bound transition metal, and the importance of controlling loop dynamics for catalysis. The implications of these experiments for the likely clinical utility of OxDC will also be discussed.

Mutations altering the interplay between *Gk*DnaC helicase and DNA reveal an insight into helicase unwinding

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DNA helicase is a motor protein that unwinds and separates duplex DNA using energy derived from the hydrolysis of nucleoside triphosphates (NTPs). In different organisms, helicase has a number of different mechanisms for unwinding activity. The studies of the helicase activity in complex with individual and sets of accessory proteins will elucidate the complexity of the regulatory network within the whole replisome machinery. In this study, we use the replicative helicase of *Geobacillus kaustophilus*, a gram-positive bacterium, as a model system (*Gk*DnaC). Based on our previous crystal structure of the *Gk*DnaC-ssDNA complex, we identified several residues involved in ssDNA binding. The locations of these key residues imply that these ssDNA-interacting sites correlate with helicase translocation. To investigate the nature of unwinding by hexameric *Gk*DnaC helicase (superfamilies IV), we studied how the activity of *Gk*DnaC is modulated by specific mutations and by accessory proteins. Our results imply that a partial loss in the interaction of helicase with ssDNA leads to an enhancement in helicase activity, while their ATPase activities remain unchanged. Based on kinetic evidences from single-molecule tethered particle motion (smTPM) experiments, the unwinding rates of these *Gk*DnaC mutants were consistently faster than that of wild-type enzyme. In addition, we characterized the role of accessory proteins of *Gk*DnaC helicase, showing that the interaction with accessory proteins alters both the ATPase and the unwinding velocity of the helicase.

Old enzymes with new function and new enzymes without old function in uracil DNA glycosylase superfamily

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DNA is subject to chemical damage under normal and stressed biological conditions. A common type of DNA base damage involves deamination of cytosine (C) to uracil (U), adenine (A) to hypoxanthine (H or I), and guanine (G) to xanthine (X) and oxanine (O). DNA glycosylases play a major role in the repair of deaminated DNA damage. The uracil DNA glycosylase (UDG) superfamily consists of five families based on conserved motifs and structural similarity. Structurally, they are organized by a four-stranded beta-sheet surrounded by alpha-helices, while functionally, all of the DNA glycosylases within the superfamily studied previously are proven biochemically to be uracil DNA glycosylases. The specificities and the catalytic centers vary from family to family. In the course of studying deaminated DNA repair, we identified strong new DNA repair activity in Family 2 enzymes called mismatch-specific uracil-DNA glycosylase (MUG). More interesting, we identified a new class (family 6) of repair enzymes in the UDG superfamily that lack the conventional uracil DNA glycosylase function. This presentation will cover biochemical, mutagenesis, structural, and genetic investigations to define the specificity and catalytic function of families 2 and 6 enzymes in the UDG superfamily.

The heterodimer of restriction endonuclease EcoO109I K173C and wild-type recognize asymmetric sequence and has new engineered specificity

Hua Wang, Keith Lunnen, Daniel Heiter, Elizabeth Lang and Geoffrey Wilson

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EcoO109I is a type II restriction endonuclease that recognizes the palindromic DNA sequence: RG/GNCCY ('/' indicates the cleavage position, R=A/G, Y=T/C). EcoO109I forms homodimers and each subunit of a dimer nicks one DNA strand simultaneously. The EcoO109I and DNA co-crystal structure indicates that Lys173 is involved in the determinant of the outer base specificity. Lys173 interacts with the N7 atom of purine via a hydrogen bond (Hashimoto et al. 2005). In order to explore the sequence-recognition mechanism between restriction enzymes and purine, activities of Lys173 variants were tested. A unique phenomenon was revealed that homogeneous K173C was inactive; however, heterodimers of K173C and wild-type EcoO109I showed new engineered specificity. The other mutants: K173A, K173G and K173S had the same property and specificity. The EcoO109I heterodimers were experimentally formed by co-expressing a Lys173 mutant and the wile-type gene in one expression system, engineering to fuse a mutant and the wide-type protein through an amino acid linker, dissociating and re-associating individually purified mutant and wide-type proteins. The sequence analysis of the heterodimer proteins digested lambda DNA indicated that K173C and wild-type complex recognized DGGNCCY (D=A/G/T) and CGGNCCY at low frequency. Lys126 is at the catalytic site of EcoO109I and K126A becomes inactive (Hashimoto et al. 2005). Run-off sequencing data of K126A-K173C complex digested pUC19 substrate showed that the K173C subunit of a K126A-K173C heterodimer nicked both AGGCCCT and TGGTCCT sites on pUC19, which implied that the K173C subunit of a heterodimer functioned at the cleavage reaction, furthermore, it had a new specificity.

Studies on the mechanism of protein-DNA sequence-specificity

Wang H, Lunnen KD, Lang EM, Heiter DF and Wilson GG

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Restriction endonucleases are remarkable for the high accuracy with which they bind-to and cleave particular sequences in duplex DNA. A typical restriction enzyme efficiently recognizes and cleaves DNA at one specific sequence—AAGCTT in the case of HindIII, for example—but at none of the other >4000 sequences of this length that are different. We discuss current ideas about the molecular mechanism of sequence-recognition, and show that it depends in part on precise steric fit: on how closely the surfaces of the DNA sequence and the enzyme conform to each other.

The X-ray crystal structures of over thirty restriction enzymes bound to their recognition sequences have been solved. Using site directed mutagenesis, we changed the amino acid-base pair contacts in some of these to evaluate their exact roles in recognition. Protein-DNA hydrogen bonds, usually considered the most important determinants of specificity, are surprisingly unimportant, we found. When H-bonds were removed by substituting non-polar amino acids for polar amino acids, accurate sequence recognition continued. In contrast, when the sizes of the amino acid side chains were altered, recognition often changed. We surmise that it is the physical sizes of the contact amino acids, rather than their ability to form H-bonds, that determines which base pairs DNA-binding sites can accommodate, and which base pairs, due to steric clash, they cannot.

A08

DNA oxidation regulates gene expression

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Mammals possess the Ten-Eleven Translocation (Tet) dioxygenase gene family. All three Tet dioxygenases can catalyze the oxidation of 5-methylcytosine (5mC), the most abundant type of base modification in DNA, to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxymethylcytosine (5caC). In vitro, Tet enzymes oxidize 5mC almost completely to 5caC under physiologically relevant conditions (e.g. in the presence of 1mM ATP). However, in embryonic stem cells and other tissues, 5hmC is the predominant form among the oxidation products of 5mC. We aim to elucidate how the oxidation process in vivo is controlled by investigating proteins binding to 5hmC and 5caC DNA. Factors including small molecule cofactors of the Tet enzymes are also studied. We demonstrate that vitamin C can regulate the Tet-mediated DNA oxidation activity both *in vitro* and *in vivo*. The impact of 5mC oxidation on gene expression in development and disease will be discussed.

Determining enzymatic mechanisms *in silico* by computing quantum free-energy profiles and kinetic isotope effects

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For many biomolecules, catalytic chemical reaction steps (e.g., proton transfer, phosphorylation, cleavage of polypeptide bonds) play crucial roles in biological functions and malfunctions. In terms of computer simulations, sampling quantum free-energy profiles (based on fundamental laws in classical physics, physical chemistry, quantum and statistical mechanics) is one of the most realistic and explicit methods to unravel the reaction mechanisms in details, by painting a complete reaction path along with the biomolecular structure of transition state. This is largely because electronic density keeps redistributing throughout biochemical reactions, and thus should be handled by quantum electronic-structure theory (e.g., density-functional theory; DFT) during the free-energy samplings in phase space. Furthermore, the outstanding interplay between the experimental and computational analysis of kinetic isotope effects (e.g., computed by Feynman's path integral) can be used to accurately determine a molecular structure of the rate-limiting transition state in the atomic details.

In this introductory talk, I will demonstrate how we can use these powerful computational techniques to elucidate the reaction mechanisms of the following two important biomolecular systems:

(1) Phosphodiesterase, a protein enzyme that the well-known drug Viagra® designed to inhibit for treating erectile dysfunction.
[*FEBS Journal* 2011, 278:2579];
(2) Ribonuclease A, a protein enzyme that catalyzes RNA 2′-*O*-transphosphorylation and provides a textbook example of how biological catalysis is achieved.
[*Angewandte* 2012, 51:647]

Real-time single-molecule tethered particle motion analysis reveals mechanistic similarities and contrasts of Flp site-specific recombinase with Cre and Int

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Flp, a tyrosine site-specific recombinase coded for by the selfish 2 micron plasmid of *Saccharomyces cerevisiae*, plays a central role in the maintenance of plasmid copy number. The Flp recombination system can be manipulated to bring about a variety of targeted DNA rearrangements in its native host and under non-native biological contexts. We have performed an exhaustive analysis of the Flp recombination pathway from start to finish by utilizing single-molecule tethered particle motion (TPM). The recombination reaction is characterized by its early commitment and high efficiency, with only minor detraction from 'non-productive' and 'wayward' complexes. The recombination synapse is stabilized by strand cleavage, presumably by promoting the establishment of functional interfaces between adjacent Flp monomers. Formation of the Holliday junction intermediate poses a rate-limiting barrier to the overall reaction. Isomerization of the junction to the conformation favoring its resolution in the recombinant mode is not a slow step. The chemical steps of strand cleavage and exchange are irreversible during a recombination event. Our findings demonstrate similarities and differences between Flp and the mechanistically related recombinases λ Int and Cre. The commitment and directionality of Flp recombination revealed by TPM is consistent with the physiological role of Flp in amplifying plasmid DNA.

A11 (no presentation)

A12

Dissecting the β-lactamase active site: how general is the general base?

Xuehua Pan, Wai-Ting Wong and Yanxiang Zhao

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β-lactamases are a large family of bacterial enzymes that hydrolyze and inactivate β-lactam antibiotics with high efficiency. They are one of the major causes of antibiotic resistance and pose serious threat to public health. In-depth understanding of the catalytic mechanism of β-lactamase will help to identify novel antibiotics that can escape or resist the inactivation by these enzymes.

The catalysis process of β-lactamases is highly conserved and involves the two-step process of acylation, in which the antibiotic substrate forms an acyl adduct with the catalytic residue S70; followed by deacylation, in which the acylated substrate is hydrolyzed and released from S70 in the inactivated form.

The identity of the general base to promote the nucleophilic attack at the acylation/ deacylation step has not be finalized. Residue E166 has been suggested as the likely candidate. Furthermore some other residues such as K73, S130, K234 and R244 also facilitate the proton transfer process.

Here we report our work to explore the identity of the general base within the β-lactamase active site and its relationship to catalysis and antibiotic resistance. A series of mutations were introduced at the active site with varying degree of general base capacity. The activities of these mutant enzymes were assayed and the structures of these enzymes have been solved. We find that several mutations at residue 166 can substitute E166 to serve as the general base and K73 plays a critical role in facilitating the general base in the deacylation step. Our studies provide new details on the catalytic machinery of β-lactamases and may help the discovery of novel antibiotics.

Hijacking of host sumoylation system by bacterial pore-forming toxin

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Protein post-translational modifications (PTM) are unique mechanisms that regulate protein activity and function spatially and temporally. They play an essential role for cell viability in eukaryotes. Pathogen-mediated PTM have drawn much attention in the past decade. Increasing number of studies has demonstrated that pathogens exploit the host cellular system for advantages in infection and/or replication. Recently, it has been revealed that bacterial pore-forming toxins listeriolysin, perfringolysin and pneumolysin can trigger a proteasome-independent degradation of SUMO E2 conjugating enzyme Ubc9 leading to a global decrease of sumoylation. This process appears partially linked to a yet-to-be identified aspartyl protease, reflecting that other proteolytic mechanism(s) are involved. We have examined the effects of streptolysin and suilysin on sumoylation, and characterized the functional and structural elements of Ubc9 critical for its stability upon bacteriolysin treatment. In this talk, I will summarize our current work towards the understanding of bacteriolysin-mediated sumoylation impairement.

Natural zinc ribbon HNH endonucleases and engineered nicking endonucleases

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Many bacteriophage and prophage genomes encode an HNH endonuclease (HNHE) next to their cohesive end site and terminase genes. The HNH catalytic domain contains the conserved catalytic residues His-Asn-His and a zinc-binding site $[CxxC]_2$. An additional zinc ribbon (ZR) domain with one to two zinc-binding sites ([CxxxxC], [CxxxxH], [CxxxC], [HxxxH], [CxxC] or [CxxH]) is frequently found at the N-terminus or C- terminus of the HNHE or a ZR domain protein (ZRP) located adjacent to the HNHE. We expressed and purified 10 such HNHEs and characterized their cleavage sites. These HNHEs are site-specific and strand-specific nicking endonucleases (NEase or nickase) with 3- to 7-bp specificities. A minimal HNH nicking domain of 76 amino acid residues was identified from *Bacillus* phage γ HNHE and subsequently fused to a zinc finger protein to generate a chimeric NEase with a new specificity (12-13 bp). By site-directed mutagenesis, we generated a number of attenuated HNH nicking domains with reduced DNA nicking activities. The attenuated nicking domains have been fused to other DNA binding proteins to generate novel NEases. The identification of a large pool of previously unknown natural NEases and engineered NEases provides more 'tools' for DNA manipulation and molecular diagnostics.

Deciphering the chemical and stereochemical mechanisms of tyrosine family site-specific recombinases using methylphosphonate modification of the scissile phosphate

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The hallmark of the active site of tyrosine family site specific recombinases is a conserved catalytic pentad motif, Arg-Lys-His-Arg-His/Trp, plus the tyrosine nucleophile that attacks the scissile phosphate during the strand cleavage step of recombination. In the Flp site specific recombinase, these residues are: Arg-191, Lys-223, His-305, Arg-308, Trp-330 and Tyr-343, respectively. In the related Cre recombinase, the corresponding residues are Arg-173, His-289, Arg-292, Trp-315 and Tyr-324. Substitution of the pentad residues individually by alanine gives rise to recombinase mutants that are almost completely inactive in the chemical steps of recombination. However, charge neutralization of the scissile phosphate by methylphosphonate substitution can overcome the catalytic inactivity of the individual Arg-to-Ala mutants of both Flp and Cre. By this experimental design, we have been able to deduce the distinct strategies employed by these two recombinases to prevent abortive hydrolysis of the phosphodiester bond during recombination, despite the tremendous excess of the water nucleophile over the active site nucleophile. The methylphosphonate substitution also confers chirality on the scissile phosphate group. Reactions of stereochemically pure *R*P and *S*P forms of these substrates with specific Flp and Cre mutants reveal that the positional invariance of catalytic residues within the active site does not necessarily translate into absolute functional conservation as well.

The many facets of DNMT1 targeting and regulation in mammalian cells

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A principal mechanism of epigenetic gene regulation is modulated by post replicative DNA modification, namely methylation of cytosine residues. Three active DNA (cytosine-5) methyltransferases (DNMTs) have been identified in mammalian cells, DNMT1, DNMT3a, and DNMT3b. DNMT1 is primarily a maintenance methyltransferase, as it prefers to methylate hemimethylated DNA during DNA replication in-vitro. The mechanism by which DNMT1 is recruited into the replication fork (RF) is not yet fully understood. Two nuclear proteins, proliferating cell nuclear antigen (PCNA) and ubiquitin-like with PHD and ring finger domains 1 (UHRF1) are shown to recruit DNMT1 during S-phase to hemimethylated CpGs generated at the RF. In addition, there are other recruiter/interactor molecules that are involved in locus specific or global DNMT1 localization. Apart from targeting, a new phenomenon of DNMT1 regulation has been observed, directed by enzymatic lysine methylation, which alters DNMT1 stability. Lysine methylated DNMT1 gets ubiquitinylated and is prone to proteasome-mediated degradation, suggesting that signaling through lysine methyltransferases represents a means of DNMT1 enzyme turnover, allowing for epigenetic changes by demethylation. Furthermore, interplay between monomethylation of DNMT1 at Lys142 by SET7 and phosphorylation of DNMT1 at Ser143 by AKT1 kinase was observed. These two modifications are mutually exclusive, and structural analysis suggests that Ser143 phosphorylation interferes with Lys142 monomethylation. In mammalian cells phosphorylated DNMT1 was more stable although small amounts of methylated enzyme still remained throughout the cell cycle. Recently, we have discovered a lysine monomethylation reader PHF20L1 that is also a participant in DNMT1 stability.

In this seminar I hope to elucidate the pathways by which DNMT1 is stabilized, identify the molecules that are involved in this process, provide clues to the mechanisms of genome reprogramming and the epigenetic differences between normal and transformed cells.

Suggested reading:

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DrdVI, SmoLII and PenI: a new family of ATP-dependent, multi-subunit restriction-modification system that uses a split methyltransferase for host protection

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Restriction-modification systems have evolved highly diverse forms to accomplish their task of cutting foreign DNA, while protecting the host DNA from their endonuclease activity. Here, we describe new variants of the multi-subunit R-M system that have an unusual split methyltransferase: DrdVI, isolated from *Deinococcus radiodurans* NEB479, SmoLII, isolated from *Streptobacillus monitiformis* DSM12112 and PenI isolated from *Porphiromonas endodontales* ATCC 35406. PenI is encoded by two subunits, where endonuclease and the full-length methyltransferase with DNA recognition domain are fused together; however, the AdoMet binding domain is located on a separate subunit. SmoLII and DrdVI are encoded by three different subunits where each subunit carries separate activities: an AdoMet binding domain, a mutated second methyltransferase with DNA recognition and an endonuclease-helicase functional domain. These polypeptides were expressed and characterized.

Using classical [H^3] restriction mapping and the new single molecule real time DNA sequencing platform from Pacific Biosciences, we identified the specificities of these restriction-modification systems: DrdVI recognizes the contiguous, asymmetric six base pair sequence, 5'-GCAGCC-3'and modifies just one DNA strand, converting the lone adenine in the top strand to m6A: 5'-GC(m6A)GCC-3', while SmoLII and PenI turned out to be isoschizomers that recognize the contiguous five base pair sequence 5’-GCAGT-3’ and modify one DNA strand, converting the lone adenine in the top strand to m6A: 5’-GC(m6A)GT-3’.

Endonuclease is formed by the association of the full-length methyltransferase, containing the DNA binding domain, with the endonuclease-helicase subunit. Endonuclease activity requires ATP/dATP hydrolysis, divalent cations, and at least two oppositely oriented sites to generate DNA cleavage, which occurs at random positions.

The overall characteristics of the split methyltransferase R-M system differ from established R-M types: while the ATP requirement is similar to both Type I and Type III systems, the recognition sequence is Type III-like, the non-specific cleavage Type I-like, while the subunit composition and the split methyltransferase differs from all of these. Thus, our findings suggest that they represent a new sub-type of restriction-modification system.

The role of the methyltransferase domain of bifunctional restriction enzyme RM.BpuSI on cleavage activity

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Restriction enzyme (REase) RM.BpuSI cleaves 10 and 14 nt downstream of GGGAC at the top strand and bottom strand, respectively. It can be described as a Type IIS/C/G REase for its cleavage site outside of the recognition sequence (Type IIS), bifunctional polypeptide possessing both methyltransferase (MTase) and endonuclease activities (Type IIC) and endonuclease activity stimulated by S-adenosyl-L-methionine (SAM) (Type IIG). The presence of both MTase and REase activities on the same polypeptide poses a major dilemma to the REase, as the MTase activity of Type IIC REases can potentially modify the substrate which becomes unsusceptible for cleavage. However, it has been well documented that SAM, the co-factor for the MTase activity, can stimulate the cleavage activity of these bifunctional enzymes, although it is not clear how it is achieved. Here we report that the RM.BpuSI MTase activity only modifies unmethylated target sites with cleavage product being the preferred substrate over the uncleaved substrate. We also showed that SAM increases the V_{max} of the RM.BpuSI cleavage activity. The increase of V_{max} is independent of the MTase activity because sinefungin (SIN), an inactive analog of SAM, increases the V_{max} to the same level. The crystal structure of RM.BpuSI predicts that dramatic conformational changes are necessary for the enzyme to attend a cleavage competent conformation. The higher V_{max} suggests that SAM or SIN facilitates the conformational changes required for RM.BpuSI, and possibly other Type IIC/G REases. The MTase domain of the RM.BpuSI is hence an integral part of the enzyme for endonuclease activity.

Covalent plasmid DNA-antibody conjugates for targeted cell transfection

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Delivery of plasmid DNA into cells is commonly achieved with transfection agents. They enwrap the DNA and form cationic aggregates, which bind non-specifically to the cell surface and lead to low transfection efficiencies. To overcome these limitations we plan to equip plasmid DNA with antibody fragments, which can specifically recognize and bind to antigens on diseased cells and lead to enhanced cellular uptake and gene expression.

For the specific covalent attachment of proteins to plasmid DNA we combine Sequence-specific Methyltransferase-Induced Labeling (SMILing DNA) with SNAP-tag technology. The SNAP-tag is a mutant of the human *O*^{6}-alkylguanine-DNA alkyltransferase, which can be fused with the desired protein of interest and reacts specifically with benzylguanine derivatives.[1] Benzylguanine will be attached to DNA by SMILing DNA.[2,3] Naturally, DNA methyltransferases (MTases) catalyze the transfer of the activated methyl group from the ubiquitous cofactor *S*-adenosyl-L-methionine (AdoMet or SAM) to the exocyclic amino groups of adenine and cytosine or the C5-position of cytosine within their specific recognition sequence. DNA MTases can also be used as tools for specific labeling of DNA with synthetic cofactors. Here, *N*-adenosylaziridine cofactors carry a benzylguanine residue are synthesized and sequence-specifically coupled with plasmid DNA by DNA MTases. The benzylguanine-modified DNA is then reacted with SNAP-tag fusions to produce the desired plasmid DNA-protein (antibody fragment) conjugates.

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Mapping the landscape of bacterial DNA methyltransferases using single-molecule real-time sequencing

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DNA methyltransferases (MTases), which in microbial genomes are often paired with restriction endonucleases (REases) to form restriction-modification (R-M) systems, recognize specific DNA sequences and methylate a specific base within or near them. We have performed a comprehensive sequence clustering analysis of 961 DNA MTases, primarily from Type II R-M systems, based on local sequence similarity. The clusters are broadly consistent with previous classification schemes, but do suggest several previous classes consist of convergently evolved subgroups. In addition, the new analysis highlights specific MTases whose characterization would be of particular importance: MTases from clusters with no previously characterized members, MTases falling at the boundary between groups with different properties, and MTases whose current annotation (e.g., in REBASE) conflicts with the properties of other members of its cluster. For many years, the experimental determination of the specific sequences recognized by MTases was a difficult and time consuming process, and one undertaken only in rare cases. With the advent of single-molecule real-time (SMRT) DNA sequencing, that process has become not only significantly easier, but also more accurate, since the recognition sequence can now be determined as the consensus of many methylated sequences. We have selected 12 DNA MTases of importance based on the criteria above, cloned them, and performed SMRT sequencing to identify or confirm both the recognition sequence and methylation type. This general approach of identifying "important cases" for experimental analysis should help rapidly improve subsequent *in silico* predictions for this family of enzymes.

A novel real-time PCR assay of microRNAs using S-Poly(T), a specific oligo(dT) reverse transcription primer with excellent sensitivity and specificity

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MicroRNA (miRNA) profiling is very crucial for understanding their biological roles, and also for realizing them as biomarker of diseases. Here, we describe a novel, highly sensitive, and reliable miRNA quantification approach，termed S-poly(T) miRNA assay. In this assay, miRNAs are subjected to polyadenylation and reverse transcription with a S-Poly(T) primer that contains a universal reverse primer, a universal Taqman probe, an oligo(dT)11 sequence and six miRNA-specific bases. Individual miRNAs are then amplified by a specific forward primer and a universal reverse primer and the PCR products are detected by a universal Taqman probe. The most noteworthy feature of the S-poly(T) assay is its high sensitivity, exhibiting at least 4 times more sensitive than the stem-loop or poly(A)-based methods. A remarkable specificity in discriminating miRNAs with high sequence similarity was also obtained with this approach. Using this method, we profiled miRNAs in human pulmonary arterial smooth muscle cells (HPASMC) and identified several differentially expressed miRNAs associated with hypoxia treatment. In healthy human serum samples, we were able to detect a total of 518 miRNAs using the S-Poly(T) method. With excellent sensitivity, specificity, and high-throughput, the S-Poly(T) method provides a powerful tool for miRNA quantification and identification of disease-specific biomarkers.

The ribosome has multiple “arm-like” structures to catch translation factors

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Ribosomes from all domains of life contain multiple copies of flexible protein components, the so-called stalk, near the factor-binding center in the large subunit. The stalk proteins play a key role to recruit translation factors to the ribosomal GTPase-associated center. We have resolved crystal structure of the stalk protein complex of the archaeal ribosome. In this complex, the stalk protein aP1 forms homodimer through its N-terminal dimerization domain, and three aP1-aP1 dimers, through the N-terminal domain, bind to the C-terminal helices of another anchor protein aP0, resulting in formation of the heptameric complex $aP0(aP1)_2(aP1)_2(aP1)_2$. Our most prominent finding is the first detection of direct binding of the C-terminal sequence shared by aP0 and aP1 to aEF-1α, aEF-2, and aIF5B, irrespective of the bound nucleotides, GTP or GDP. The results suggest that seven copies of the C-terminal regions have ability to bind the factors before and after GTP hydrolysis in the ribosome. We infer that the multiple copies of aP1 may participate not only in recruitment of GTP-bound translation factors, but also in dissociation of GDP-bound factors from the ribosome, and enhance translation cycle. In the case of eukaryotes, the stalk complex is pentameric form composed of two P1-P2 heterodimers and P0 anchor, as $P0(P1\text{-}P2)_2$. It is, however, likely that the eukaryotic stalk complex has a highly conserved functional structure with archaeal stalk complex, because eukaryotic translation factors can be accessible to the archaeal stalk complex. Five copies of stalk tails presumably work cooperatively and participate in efficient translation.

Solution structures of the eukaryotic stalk P-proteins reveal structural organization of eukaryotic stalk complex and how trichosanthin is recruited to the ribosome

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Eukaryotic ribosomal stalk is responsible for binding eukaryotic-specific ribosome-inactivating proteins such as trichosanthin and domain-specific actions of translation factors. The eukaryotic stalk consists of a scaffold P0 protein binding two copies of P1/P2 heterodimers to form a $P0(P1/P2)_2$ pentameric P-complex. We have shown that P2 form homodimer in solution and P1 can dissociate P2 homodimer spontaneously to form a more stable P1/P2 1:1 heterodimer. The C-terminal domain of P0 contains two segments of spine helices that can separately bind two copies of P1/P2 heterodimer. To provide a better understanding on the function and structural organization of eukaryotic stalk, we have determined the solution structure of the N-terminal dimerization domain (NTD) of P2/P2 homodimer and P1/P2 heterodimer. Our structures suggested that the two P1/P2 heterodimers located at the spine helices of P0 is likely to be in P2/P1:P1/P2 topology. We have also determined the structure of the full-length P1/P2 heterodimer that has a C-terminal tail extend to 125Å away from the stalk. ^{15}N relaxation study reveals that the C-terminal tails are flexible, having a much faster internal mobility than the N-terminal domains. Together with biochemical evidence, we propose a model that how the stalk proteins facilitate the recruitment of trichosanthin and translation factors to the sarcin-ricin loop of eukaryotic ribosomes.

The regulatory mechanism of the interaction between ribosomal protein L7/L12 and L11

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Translational GTPases (trGTPases) regulate all phases of protein synthesis. An early event in the interaction of a trGTPase with the ribosome is the contact of the G-domain with the C-terminal domain (CTD) of ribosomal protein L12 (L12-CTD) and subsequently interacts with the N-terminal domain of L11 (L11-NTD). Here, we identified a universally conserved residue, Pro22 of L11, that functions as a proline switch (PS22), as well as the corresponding center of peptidyl-prolyl *cis-trans* isomerase (PPIase) activity on trGTPases that drives the *cis-trans* isomerization of PS22. Only the *cis* configuration of PS22 allows direct contact between the L11-NTD and the L12-CTD (1). Sequentially, we studied the structural and functional relationships between L12-CTD and L11-NTD. We found that L11-NTD interacts with L12-CTD through electronic contact of Lys-Arg residues and hydrophobic stacking. When Loop62 of L11-NTD protrudes into a cleft in L12-CTD, it leads to an open conformation of this domain and exposure of hydrophobic core. This unfavorable situation for L12-CTD stability is resolved by a chaperone-like activity of the contacting G-domain (2). Our results suggest that all trGTPases—regardless of their different specific functions—use a common mechanism including PPIase and chaperone activities for stabilizing the L11-NTD/L12-CTD interactions (1, 2).

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Ribosome interactions of ribosome inactivating proteins

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Ricin belongs to a family of plant and bacterial toxins that are collectively referred to as ribosome inactivating proteins (RIPs) due to their ability to depurinate the α-sarcin/ricin loop (SRL) of the large rRNA and inhibit protein synthesis. We examined the structural features within ricin A chain (RTA) that affect transport out of the endoplasmic reticulum (ER) into the cytoplasm and showed that glycosylation does not affect the catalytic activity of RTA, but stimulates toxicity by promoting transport of RTA out of the ER. We showed that the ribosomal stalk is the docking site for RTA on the ribosome in vivo and is critical for RTA to depurinate the SRL. RTA interacts with ribosomes via a two-step interaction model consisting of a saturable, P1/P2 protein-dependent interaction with a rapid association and dissociation rate, and a nonsaturable P1/P2-independent interaction with a much slower association and dissociation rate. Mutations in RTA which disrupted both interactions with the ribosome dramatically reduced the level of depurination in vivo and toxicity. A positively charged region in RTA, which is separate from the active site, is blocked by RTB in the ricin holotoxin. Basic residues in this region are critical for the interaction of RTA with ribosomes. Our results show that RTA uses one side of the protein to interact with the stalk and the opposite side to depurinate the SRL. These findings provide insight into how RIPs select their depurination target on the ribosome.

The interaction of trichosanthin and maize ribosome-inactivating protein with ribosomal P protein

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Ribosome-inactivating proteins (RIPs) inhibit protein synthesis by enzymatically depurinating a specific adenine residue at the sarcin-ricin loop of the 28S rRNA, which thereby prevents the binding of elongation factors to the GTPase activation centre of the ribosome. Deletion mutagenesis showed that trichosanthin, a Type I RIP, interacts with the C-terminal tail of ribosomal subunit P2, the sequence of which is conserved in P0, P1 and P2. The P2-binding site on TCS was mapped to the C-terminal domain by chemical shift perturbation experiments. By solving the crystal structure of TCS complexed to SDDDMGFGLFD, the conserved C-terminal of P proteins, we show that the N-terminal region of this peptide interacts with Lys173, Arg174 and Lys177 in TCS, while the C-terminal region is inserted into a hydrophobic pocket. This 11-mer C-terminal P peptide can be docked with ricin A, saporin and Shiga toxin, indicating that a similar interaction may also occur with other RIPs.

With the structure of TCS-P2 peptide elucidated, we also set forth to investigate the interaction between maize RIP (MOD), an atypical RIP with an internal inactivation loop, with the ribosomal protein P2. We found that the interaction is via Lys158–Lys161 in MOD, which is located in the N-terminal domain and at the base of its internal loop. Hydrophobic interaction with the ‘FGLFD’ motif of P2 is not evidenced in MOD-P2. As a result, interaction of P2 with MOD was weaker than those with TCS and Shiga toxin A, as reflected by the dissociation constants of their interaction. Despite MOD and TCS target at the same ribosomal protein P2, MOD was found 48 and 10 folds less potent than TCS in ribosome depurination and cytotoxicity to 293T cells respectively, implicating the strength of interaction between RIPs and ribosomal proteins is important for the biological activity of RIPs. Our work illustrates the flexibility on the docking of RIPs on ribosomal proteins for targeting the sarcin-ricin loop and the importance of protein-protein interaction for ribosome-inactivating activity.

The ribosome binding surface of ricin A chain is on the opposite side of the active site cleft and is blocked by the B chain

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Ricin inhibits protein synthesis by depurinating the α-sarcin/ricin loop (SRL). Ricin holotoxin is inactive in cell-free translation assays unless the disulfide bond between the A (RTA) and B (RTB) subunits is reduced. When RTA is separated from RTB, arginine residues located at the interface are exposed to the surface. Since this positively charged region, but not the active site, is blocked by RTB, we investigated if the inactivity of the holotoxin toward ribosomes is due to the steric blockage of the ribosome binding site by RTB. Ricin holotoxin did not bind ribosomes, while RTA bound ribosomes with nanomolar affinity. Mutation of arginine residues located at or near the interface of RTB identified variants, which were structurally similar to wild type RTA, but could not bind ribosomes. They depurinated free RNA as wild type RTA, but their depurination activity against intact ribosomes was reduced 20 to 50-fold. They showed reduced toxicity and depurination in vivo, demonstrating that ribosome binding is required for depurination of the SRL and toxicity. The mutated arginines have side chains behind the active site cleft, indicating that the ribosome binding surface of RTA is on the opposite side of the surface that interacts with the SRL. We propose that the interaction of RTA with the stalk proteins orients its active site towards the SRL and thereby allows docking of the SRL into the active site. This mechanistic model may be applicable to other ribosome inactivating proteins and translation factors, which interact with the P-protein stalk.

Anti-Herpes simplex virus type 1 and anti-tumor activity of trichosanthin

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Trichosanthin (TCS) is a type I ribosome-inactivating protein with wide spectrum of pharmacological activities. It shows anti-viral and anti-tumor activities but the mechanism remains unclear. In this report, our recent advances in the inhibitory activities of TCS on Herpes simplex virus type 1 (HSV-1) and tumor growth are discussed. Firstly, we found the anti-HSV-1 ability of TCS is tightly related to apoptosis pathways, TCS triggered apoptotic signaling networks in HSV-1 infected cells, including mitochondrial and p53 related pathways, to kill more infected cells than uninfected ones. Secondly, we found the non-toxic level of TCS could inhibit tumor angiogenesis by interfering vascular endothelial growth factor (VEGF) pathways. These findings give new insights on the pharmacological properties of TCS and other members of ribosome-inactivating proteins.

Interaction of DprA with restriction modification system favours genetic diversity in *Helicobacter pylori*

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Helicobacter pylori is a highly genetically diverse bacterial species. It causes gastric diseases like stomach ulcer, gastric inflammation etc. Natural transformation is a major cause of its high genetic diversity. Previously, a DNA binding protein, DprA (DNA Processing Protein A) was shown to facilitate natural transformation of Gram positive bacteria by protecting incoming ssDNA and promoting RecA loading on it. In the present study, the molecular and biochemical role of DprA has been analysed in natural transformation pathway of *Helicobacter pylori*. It was observed that HpDprA (Helicobacter *pylori* DprA) binds and protects both ssDNA as well as dsDNA from various DNA nucleases. Further investigation of role of HpDprA in protecting dsDNA from restriction enzymes was stemmed from this observation. We noticed that not only HpDprA protected dsDNA from restriction enzymes but also positively interacted with MTase *hpyAVIAM*. Greater protection from cognate restriction enzyme was observed when DNA was pre-methylated with methyltransferase in presence of HpDprA. Moreover, deletion of *hpyAVIAM* reduced the transformation efficiency due to an increase in expression of restriction enzymes in *hpyAVIAM* deletion strains. These results combined together indicate that positive interaction of HpDprA with *hpyAVIAM* may result in epigenetic regulation of restriction enzymes. Thus, HpDprA inhibits the restriction enzymes by (a) occluding the restriction enzymes by substrate (b) modifying the substrate and thus rendering it resistant to restriction enzymes and (c) regulation of restriction enzyme expression in the cell . These results indicate that HpDprA could be one of the factors that modulate the restriction modification barrier during inter-strain natural transformation in *H. pylori*.

The structure of the Type I DNA restriction enzymes and their control of horizontal gene transfer in *Staphylococcus aureus*

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The structure of the Type I DNA restriction enzymes EcoKI and EcoR124I shows them to be complex assemblies of a DNA specificity subunit, two modification methyltransferase subunits and two restriction endonuclease subunits. A complex series of conformational changes allow DNA recognition, assessment of the methylation status of the recognition sequence and a choice to be made between methylation of hemimethylated sequences or cleavage of unmethylated sequences.

A limited number of Methicillin-resistant *Staphylococcus aureus* (MRSA) clones are responsible for MRSA infections worldwide, and those of different lineages carry unique Type I restriction enzyme variants. We experimentally demonstrate that this Type I system is sufficient to block horizontal gene transfer between clinically important MRSA, confirming the bioinformatic evidence that each lineage is evolving independently. We have identified the specific DNA sequence targets for the dominant MRSA lineages CC1, CC5, CC8 and ST239.

Rational engineering of DNA recognition: simple mutations that change Type IIL restriction enzyme specificity.

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Type II restriction endonucleases (REs) robustly recognize specific DNA sequences. Most of these endonucleases have evolved to resist changes to their specificity, since altered endonuclease specificity without an identical change in methyltransferase specificity would be lethal. However, the Type IIL enzymes use a single DNA recognition domain to target both their endonuclease and modification activities, allowing rapid evolution of altered specificity. We have developed methodology that allows us to rationally engineer DNA recognition specificity in such Type IIL restriction systems. First, alignments of the RE protein sequences and of their DNA recognition sequences are formed, then the two alignments are interrogated to identify co-variation between aligned amino acid residues and the DNA base pair present at each position in the aligned recognition sequences. From such co-variation we identify amino acids positions likely to specify DNA base recognition. In the MmeI Type IIL family we have identified amino acid positions and residues specifying five of the six variable positions in the RE recognition sequences. We then alter recognition specificity by mutating the amino acids at the identified positions to those correlated with recognition of the desired new base. To date we have successfully altered specificity at five positions in the recognition sequences. Individual enzymes tolerate changes at multiple positions; for example, NmeAIII has been altered at three positions, from GCCGAG21/19 to GCGRAC21/19. The majority of the enzymes so altered have specific activity similar to that of the wild type enzymes. Recent crystallographic results confirm that the positions identified by bioinformatics are indeed contacting the specified bases. Thus using simple and predictable mutagenesis we demonstrate that it is possible to create hundreds of unique new Type II RE specificities.

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Application of Pyrrolysine Analogs for the Study of Ubiquinated and Sumoylated Proteins

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Ubiquitination and sumoylation involve the attachment of the small protein modifiers ubiquitin and SUMO, respectively, to specific lysine residues on a target protein. These posttranslational modifications lead to alterations in protein recognition and function and are important mechanisms for regulating and directing cellular processes in biology. Two challenges in the biochemical study of target proteins attached to ubiquitin and SUMO are the preparation of the modified form in large scale, and the identification of the specific lysine attachment site(s). Here, we will describe our efforts to use the pyrrolysine incorporation system to incorporate novel pyrrolysine analogs into proteins that allow for (a) the site-specific synthesis of ubiquitinated and somoylated proteins via expressed chemical ligation, or (b) the identification of the lysine attachment sites by a novel click-and-release methodology.

DNA modification-dependent restriction enzymes for epigenetic studies

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We present here a few families of DNA modification-dependent restriction enzymes which can be useful to interrogate the DNA modification status in epigenetic studies. They include the MspJI family, which recognize both 5-methylcytosine and 5-hydroxymethylcytosine (Zheng Y. et al., 2010; Horton J. et al. 2012), and the PvuRts1I family, which recognize 5-hydroxymethylcytosine or 5-glucosylmethylcytosine (Wang H. et al., 2012). Both enzymes cleave DNA at fixed distances away from the modified cytosine at the 3'-side. We used MspJI to map the methylome (Cohen-Karni D. et al. 2011) and AbaSI, a PvuRts1I homolog, to map the hydroxymethylome in mammalian cells (Sun Z. et al., 2013). We suggest these enzymes provide basis on which many future methods can build to decode the epigenomes of different organisms.

A34 (no presentation)

A35

Towards understanding the structure and function of NSF, a member of AAA+ ATPase family

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N-ethylmaleimide-sensitive fusion protein (NSF) belongs to the family of "ATPases Associated with various cellular Activities" (AAA+), which is required for vesicular transport throughout the constitutive secretory and endocytic pathways. NSF has been found in all tissues, but is most highly expressed in the nervous system where it is needed to maintain synaptic vesicle transportation and has been implicated in numerous human diseases. During membrane fusion, SNARE proteins on opposing vesicles coil together to form the SNARE complex, a highly stable four-helix bundle. After membrane fusion, the SNARE complex must be disassembled into individual SNARE proteins for reuse. NSF upon ATP hydrolysis is responsible for disassembling the SNARE complex. Without NSF, membrane trafficking ceases as the assembled SNARE complexes accumulate. The mechanism of how NSF to disassemble the stable SNARE complex, however, is still unknown. In this study, we used electron microscopy and single particle analysis to reconstruct the structures of NSF and the complex structure of NSF with its adaptor and substrate. These results provide important insight into the disassembly process of the SNARE complex by NSF.

DNA topoisomerase II inhibitors induce macrophage ABCA1 expression and cholesterol efflux - an LXR-dependent mechanism

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ATP-binding cassette transporter A1 (ABCA1) facilitates cholesterol efflux and thereby inhibits lipid-laden macrophage/foam cell formation and atherosclerosis. ABCA1 expression is transcriptionally regulated by activation ofliver X receptor (LXR). Both etoposide and teniposide are DNA topoisomerase II (Topo II) inhibitors and are chemotherapeutic medications used in the treatment of various cancers. Interestingly, etoposide inhibits atherosclerosis in rabbits by unclear mechanisms. Herein, we report the effects of etoposide and teniposide on macrophage ABCA1 expression and cholesterol efflux. Both etoposide and teniposide increased macrophage free cholesterol efflux. This increase was associated with increased ABCA1 mRNA and protein expression. Etoposide and teniposide also increased ABCA1 promoter activity in an LXR-dependent manner and formation of the LXRE-LXR/RXR complex indicating that transcriptional induction had occurred. Expression of ABCG1 and fatty acid synthase (FAS), another two LXR-targeted genes, was also induced by etoposide and teniposide. *In vivo* administration of mice with either etoposide or teniposide induced macrophage ABCA1 expression and enhanced reverse cholesterol transport from macrophages to feces. Taken together, our study indicates that etoposide and teniposide increase macrophage ABCA1 expression and cholesterol efflux that may be attributed to the anti-atherogenic properties of etoposide. Our study also describes a new function for Topo II inhibitors in addition to their role in anti-tumorigenesis.

Structural basis of antizyme-mediated inhibition and degradation of ornithine decarboxylase

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Polyamines, including spermidine, spermine, and putrescine, are positively charged small organic cations. By interacting with negatively charged nucleic acids and acidic surface patches of proteins, these compounds participate in a large number of cellular processes, ranging from functional modulations of proteins to nucleic acid metabolism and packaging. Therefore, polyamines are essential for cell growth and differentiation, and aberrant cellular polyamine level has been implicated in neoplastic transformation. L-Ornithine decarboxylase (ODC) catalyzes the first and rate-limiting step in the polyamine biosynthetic pathway, and its enzymatic activity is subjected to a tight regulation. In mammals, ODC is targeted for proteasomal degradation by interacting with antizyme (Az), a 26.5 kDa intracellular protein that nds ODC to form a non-covalent 1:1 complex. Az-binding induces a conformational change at the ODC C-terminal region, which triggers degradation via the 26S proteasome in an unique ubiquitin-independent manner. To decipher how Az recognizes and inhibits ODC, and how Az-binding promotes proteasomal proteolysis of ODC, we have obtained a crystal structure of ODC in complex with the C-terminal domain of Az. The substantial overlap between the Az-binding surface and the homodimerization interface of ODC readily explains why the formation of a catalytically active ODC dimer is blocked in the presence of Az. Moreover, a proposed proteasome-targeting region of ODC undergoes conformational changes and become surface-exposed upon Az binding, likely allows its recognition by the 26S proteasome. Additional functional implications of this structure will be presented during the meeting.

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Structure of NF-κB-inducing kinase (NIK) and structure-based inhibitor design for NIK

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NF-κB-inducing kinase (NIK) is a central component in the non-canonical NF-κB signaling pathway. Excessive NIK activity is implicated in various disorders, such as autoimmune conditions and cancers. Here, several crystal structures of truncated human NIK in complex with various small molecules will be discussed. The truncated protein is a catalytically active construct, including an N-terminal extension of 60 residues prior to the kinase domain, the kinase domain, and 20 residues afterward. The structure reveals that the NIK kinase domain assumes an active conformation in the absence of any phosphorylation. Analysis of the structure uncovers a unique role for the N-terminal extension sequence, which stabilizes helix C in the active orientation and keeps the kinase domain in the catalytically competent conformation. Our findings shed light on the long-standing debate over whether NIK is a constitutively active kinase. They also provide a molecular basis for the recent observation of gain-of-function activity for an N-terminal deletion mutant of NIK, leading to constitutive non-canonical NF-κB signaling with enhanced B-cell adhesion and apoptosis resistance. Structure-based design of inhibitors against NIK will also be discussed.

Novel protein post-translational modifications revealed by the crystal structure of SIRT5

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Sirtuins have been recognized as NAD-dependent deacetylases that regulate important biological processes, including life span, transcription, cell survival, and metabolism. Small molecules that can regulate sirtuin activity are reported to be potential therapeutic agents for treating cancer, neurodegeneration diseases, and metabolic diseases. Mammals have 7 sirtuins, SIRT1-7. Of the seven human sirtuins, only three of them, SIRT1, SIRT2, and SIRT3, have robust deacetylation activities *in vitro* and *in vivo*. The other sirtuins either have no detectable or very weak deacetylation activity *in vitro*. Here we present structural and biochemical data demonstrating that SIRT5 is an efficient desuccinylase and demalonylase *in vitro*, catalyzing the hydrolysis of succinyl lysine and malonyl lysine residues. The preference for succinyl and malonyl groups can be explained by the presence of an arginine residue (R105) and a tyrosine residue (Y102) in the acyl pocket of SIRT5, which is typically occupied by hydrophobic residues in sirtuins that exhibit strong deacetylation activity. We further show that protein lysine succinylation and malonylation exist in mammalian cells and several proteins with succinyl/malonyl lysine modifications have been identified by mass spectrometry. Our work establishes that protein lysine malonylation and succinylation are previously unrecognized reversible protein posttranslational modifications. SIRT5 is the first sirtuin demonstrated to prefer an acyl group other than acetyl, suggesting that other sirtuins showing little or no deacetylation activity may prefer to hydrolyze different acyl groups too.

Applications of histone deacetylases inhibition in cancer research

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The genome contains information in two forms, genetics and epigenetics. Epigenetics functions on cell through two main mechanisms: DNA modification and histone modification. Although histone modifications occur throughout the entire sequence, the core N-terminus of histones (called histone tails) is particularly highly modified. These modifications include acetylation, methylation, ubiquilation, phosphorylation and sumoylation. Acetylation is the most studied modification. The histone acetylation equilibrium is sustained in cells by interplay of two classes of enzymes: the histone acetylases (HAT) and histone deacetylases (HDAC). In general, HATs are associated with active gene expression, while HDACs facilitate suppression of the transcriptional activity. Accumulating evidence suggests that disruption of histone acetylation is associated with cancer development in human cancers. Histone deacetylases (HDACs) comprise a family of 18 genes that are subdivided into four classes (I-IV).

Inhibition of HDAC activity was found to be cytostatic and to arrest cells in G_1 and G_2/M phases. The realization that acetylation was frequently abnormal in cancer raises interest in developing agents for inhibition of HDAC. Up to date, six classes of HDAC inhibitors (HDACI) have recently been developed. Here I focus on Class I HDAC inhibitor depsipeptide (FK228) and its functions in cancer cells including synergistically with DNA demethylating agent to induce cell death, acetylating non-histone protein, p53, and induction of DNA demethylation. Also I will discuss that SIRT1 and SIRT2, NAD^+ dependent HDAC, are involved in negative regulating p53 activity or induction of autophagy.

Structural insights into the association of ubiquitin C-terminal hydrolase L1's mutations with the risk of Parkinson's disease

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Protein ubiquitination and deubiquitination, play important roles in many aspects of cellular mechanisms. Its defective regulation results in diseases that range from developmental abnormalities to neurodegenerative diseases and cancer. Ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) is a enzyme of 223 amino acids, which is highly abundant in brain, constituting up to 2% of total brain proteins. Although it was originally characterized as a deubiquitinating enzyme, recent studies indicate that it also functions as a ubiquitin ligase and a mono-Ub stabilizer. Down-regulation and extensive oxidative modifications of UCH-L1 have been observed in the brains of Alzheimer's disease and Parkinson's disease (PD) patients. Of importance, I93M and S18Y point mutations in the UCH-L1 gene have been reported to be linked to susceptibility to and protection from PD respectively. Hence, the structure of UCH-L1 and the effects of disease associated mutations on the structure and function are of considerable interest.

Our circular dichroism studies suggest that the S18Y point mutation only slightly perturbs the structure while a significant decrease in the α-helical content is observed in the I93M mutant. We have determined the solution structure of S18Y and mapping its interaction with ubiquitin by chemical shift perturbation approach. The electrostatic surface potential analysis reveals that the interaction between ubiquitin and UCH-L1-S18Y is primarily electrostatic in nature, with negatively charged residues on the surface of UCH-L1-S18Y interacting with the positively charged residues on the basic face of ubiquitin. Although the active site and the L8 loop in UCH-L1-S18Y adopts conformations similar to that observed in the crystal structure of UCH-L1-WT, both the altered hydrogen bond network and surface charge distributions have demonstrated that the S18Y substitution could lead to profound structural changes. In particular, the difference in the dimeric interfaces of the wild-type and the S18Y mutant has shown that mutation can significantly affect the distribution of the surface-exposed residues involved in the dimeric interface. Such observed difference might weaken the stability of the UCH-L1 dimer and hence may explain the reduced dimerization-dependent ligase activity of UCH-L1-S18Y in comparison to UCH-L1-WT.

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High throughput structural biology of inborn errors of metabolism

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Approximately 7000 inherited monogenic diseases, while individually rare, collectively afflict ~5% of the global population and present an unmet medical need in disease diagnosis, management and treatment. Proteins constitute potent therapeutic targets for a majority of rare diseases, where many disease-causing mutations are located within protein-encoding exons.

The Metabolic & Rare Disease group at the SGC explores how genetic defects lead to disease at the protein molecular level, by determining 3D structures and biochemical properties of metabolic enzymes and protein-protein complexes linked with inborn errors of metabolism (IEM) [1], a heterogeneous subset of ~400 rare diseases associated with metabolic enzyme deficiency and dysfunction. With a repertoire to date of >50 crystals structures and further 120 recombinant human proteins linked with IEM, we have created a protein-centric, translational platform to study rare diseases. Working closely with clinicians, geneticists, and drug developers, we aim to establish the structure-function relationship of IEM-associated proteins, characterize the biochemical penalties caused by pathogenic mutations and assist the development of small molecule therapeutics.

Pathogenic missense mutations often disrupt protein folding and stability that lead to aggregation, degradation and loss-of-function. Using our high throughput structural biology platform we have characterized a number of IEM-associated proteins (mitochondrial integrity [2], cobalamin metabolism [3] and glycogen synthesis [4]), and identified mutations that cause the misfolding phenotype. These disease alleles serve as starting point for the screening of ‘pharmacological chaperones’, small molecules that stabilize and rescue misfolded proteins, to generate novel therapeutic perspectives to treat these debilitating diseases.

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Diacylglycerol Kinases and Their Association With Dyslexia and Bipolar Disorder

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Diacylglycerol kinases (DGK or DGAK) belong to a family of enzymes that catalyze the conversion of diacylglycerol (DAG) to phosphatidic acid (PA). The structure of several bacteria DGK, e.g. DgkB, the soluble DAGK from *Staphylococcus aureus,* have been solved; while in the mammalian system, nine members of the DGK family have been cloned and identified.

Some of the members of diacylclycerol kinases were known for their association with diseases. The association between DGKH and psychiatric disorder has been reported (Moskvina et al, 2008; Zeng et al, 2011), and the association between DGKI and dyslexia has also been reported (Matsson et al, 2011).

DGKβ is widely distributed in the central nervous system, such as the olfactory bulb, cerebral cortex, striatum, and hippocampus. DGKβ KO mice showed behavioral abnormalities, such as hyperactivity and reduced anxiety, with impairment in Akt-glycogen synthesis kinase (GSK) 3β signaling and cortical spine formation (Kakefuda et al, 2010). Recent studies reported that the splice variant at the COOH-terminal of DGKβ was related to bipolar disorder, and the use of phosphatidylcholine has also been shown to be somewhat helpful in the treatment of bipolar depression; however, its efficacy is controversial. This talk will include recent findings on the evidence of association of DGK with diseases, and also a description of a case study showing the apparently beneficial effect of a moderate daily dose of phosphatidylcholine supplementation. Our preliminary results suggest that the use of phosphatidylcholine might be useful for some cases of insomnia and hypomania for bipolar disorder patients.

A44

Ataxin-3: A disease protein looking for a function

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Ataxin-3 is a 360-residue protein which is implicated in the inherited neurodegenerative disease spinocerebellar ataxia type 3 (SCA3). On mutation, an otherwise harmless glutamine repeat (polyQ) tract is abnormally lengthened, leading to fibril formation inside the nuclei of affected neurons. Sequence comparison revealed that the protein consists of a conserved N-terminal domain, named Josephin (Jph), and a variable C-terminal domain (CTD) which harbors the polyQ tract. Two or three ubiquitin-interaction motifs (UIM) are identified in the CTD, although not much can be said about its functions because of a lack of homology. This is an example of the "structural genomics" approach where researchers worked backwards from structural studies in order to understand native cellular functions. With solution NMR spectroscopy, the structure of Jph was determined, revealing that it is a deubiquitination enzyme. Several studies have helped to locate multiple ubiquitin binding sites on the protein. It is now known that Ataxin-3 is closely associated with components of the cellular ubiquitin-proteasome system (UPS). Ironically, this protein, which is implicated in the misfolding disease SCA3, has a native role in the mechanism that is supposed to assist in clearing the cell of misfolded and unwanted proteins. The structural studies leading to the current understanding of Ataxin-3 will be reviewed.

Mechanistic study of proteins that regulate cell proliferation and differentiation

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Proper organ development requires the precise regulation of both the total number of cells and the types of cells. During cell proliferation, Cdt1 mediated loading of DNA helicase (Mcm2-7) to replication origins is required for DNA replication. And Hox gene activation is necessary for embryonic cell differentiation. It has been shown that cell proliferation and differentiation are mutually regulated through the cell cycle-regulator Geminin and the homeodomain-containing transcription factors Hox. To understand the molecular mechanism involved, we determined the solution structures of Geminin-Hox and Cdt1-Mcm6 complexes by nuclear magnetic resonance (NMR) spectroscopy and conducted biochemical study to delineate the structural basis of this regulation. In addition, we found that histone H4-K20 methyltransferase SET8 is a new cell-cycle regulator and plays an important role in the developmental program of metazoans.

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NMR structural characterization of human dermcidin antimicrobial peptides and its relationship with atopic dermatitis

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Atopic dermatitis (AD) is an itchy, chronic inflammatory skin condition complicated by frequent skin infections, particularly those due to *Staphylococcus aureus*. In Singapore, the one-year period prevalence of AD is 20.8%. Emerging evidence suggests that inflammation in AD results from inherited and acquired insults to both chemical and permeability skin barriers. The antimicrobial peptide dermcidin (DCD) is constitutively expressed in sweat glands and serves as a chemical skin barrier; however, DCD is significantly suppressed in cases of AD. We found that DCD peptides are unstructured in solution but formed helical conformations in the presence of SDS and POPG, but not in POPC. We have determined the NMR structure of DCD-1L peptide in SDS and showed that it is comprised of three α-helices connected by flexible turns and forming an "L-shaped" molecule. The structure agrees with an ionic channel model on lipid bilayer with the cationic N-terminus participating in a toroidal pore. Residues that are essential for the interaction between DCD-1L and the lipid vesicle formed by POPG are being determined. Human ribonuclease 7 (RNase7) was found to be antimicrobial due to the presence of cluster of cationic residues on the surface. Single nucleotide polymorphism (SNP) on residues of RNase7 was found to be highly correlated with the development of AD. Structural and activity studies on wild type and SNP mutants are being performed to determine the correlation between SNPs of RNase7 and onset of AD.

Functional characterization of the splicing kinase SRPK2

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Pre-mRNA splicing is a critical step in gene expression and the generation of protein diversity in higher eukaryotes. Serine-arginine (SR) proteins represent a family of splicing factors that play important auxiliary roles in both constitutive and alternative pre-mRNA splicing. The C-terminal domains of SR proteins contain consecutive RS dipeptides that could be extensively phosphorylated and their phosphorylation states (hypo- and hyperphosphorylation) govern multiple facets of SR protein activity including subcellular localization, splice site selection, mRNA transport, and translation. One key kinase family that phosphorylates SR proteins is the SR protein kinase (SRPK) family. SRPKs are constitutively active kinases that localize predominantly in the cytoplasm and could translocate into the nucleus. A member of the family, SRPK2, has recently been identified as a key player during spliceosome assembly. Furthermore, it has been implicated in cell cycle regulation and HBV viral infection.

SRPK2 is highly related to SRPK1, a well-studied SRPK, in sequence of kinase cores and *in vitro* properties, yet it is shown to have distinct substrate specificity and physiological function *in vivo*. Understanding the molecular basis of the substrate specificity of SRPK2 is thus essential for exploring its different biological roles. In this study, we investigate the molecular basis for distinct phosphorylation mechanisms of SRPK2 for SR-containing proteins. Furthermore, we will also provide new evidence that SRPK2, besides regulating pre-mRNA splicing, also plays a role in the transcriptional activation by means of serum-response element.

P01

Homology/structure-guided engineering of MmeI specificity at positions 1 and 2 of the target site

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MmeI is a type IIG restriction enzyme from *Methylophilus methylotrophus* [1]. It recognizes the sequence 5'-TCCRAC-3', and cuts ~20 bp downstream from the recognition site. It cuts the two DNA strands at one site simultaneously. MmeI is an unusual restriction enzyme in that it contains the methylation and restriction domains in a single protein molecule, which is comprised of 919 amino acids. MmeI methylates only one DNA strand for host protection, specifically, the adenine in the top strand of the target site: 5'-TCCRAC-3'.

We have previously demonstrated that the target site recognition specificity in positions 3, 4, and 6 can be rationally altered by mutating specific pairs of amino acid residues [2]. These residues were identified based on covariation of amino acid residues with DNA base recognition in multiple sequence alignments within the MmeI family. In the current work we explored homology and structure guided engineering of this enzyme to modify its specificity in positions 1 and 2 of the target site. Our findings suggest that these positions are less amenable to engineering than positions 3, 4 and 6. We did not obtain active mutants with altered specificity at position 1. We were able to alter specificity, however, from recognition of C to M (A or C) at position 2.

References:

1. Morgan RD, et al. MmeI: a minimal Type II restriction-modification system that only modifies one DNA strand for host protection. (2008) *Nucleic Acids Res*, 36:6558-6570.
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Tt PURE: Reconstituted i*n vitro* protein synthesis at elevated temperature for studying protein translation and protein evolution

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Cell-free protein synthesis has become the center of attention as a rapid and high throughput technology to obtain proteins from their genes in the post genomic era. The reconstituted protein synthesis system has significantly reduced levels of nuclease and protease activities, thus is preferred to the cell-extract-based system in ribosome display and compartmentalization methods for *in vitro* protein evolution. However, all cell-free systems, including PURE system, conduct protein synthesis at an optimal temperature range of 30-37°C, therefore limiting their applications at more elevated temperatures. We present here the development of the reconstituted protein synthesis system with purified components from *Thermus thermophilus* (Tt PURE). The Tt PURE is capable of translating natural mRNA into active full-length proteins at temperatures ranging from 37 to 65°C and with yields up to 60 µg/ml. Using such a defined *in vitro* system, we demonstrated a minimal set of components that are sufficient for translating active proteins at high temperatures, the functional compatibility of key translation components between *T. thermophilus* and *E. coli*, and the functional conservation of a number of resurrected ancient elongation factors. Our effort may facilitate *in vitro* biochemical studies of protein translation and ribosome functions, and provide an effective platform for engineering thermostable proteins such as vaccines and industrial enzymes.

Increasing cleavage specificity and activity of restriction endonuclease KpnI

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Restriction endonuclease KpnI is a HNH superfamily endonuclease requiring divalent metal ions for DNA cleavage but not for binding. The active site of KpnI can accommodate a diverse set of metal ions of varying atomic radii for DNA cleavage. While Mg2+ ion beyond 500 µM mediates promiscuous activity, Ca2+ suppresses the promiscuity activity. Here we report that a conservative mutation of the metal-coordinating residue D148, to glutamate results in the elimination of the Ca2+-mediated cleavage but imparting high cleavage fidelity with Mg2+. High cleavage fidelity of the mutant D148E is achieved through better discrimination of the target site at the binding and cleavage steps. Biochemical experiments and molecular dynamics simulations suggest that the mutation inhibits Ca2+-mediated cleavage activity by altering the geometry of the Ca2+-bound HNH active site. Although the D148E mutant reduces the specific activity of the enzyme, we identified a suppressor mutation that increases the turnover rate to restore the specific activity of the high fidelity mutant to the WT level. Our results show that the active site plasticity in coordinating different metal ions is related to KpnI promiscuous activity and tinkering the metal ion coordination is a plausible way to reduce promiscuous activity of metalloenzymes.

Poster Presentation by Local postgraduate Students

PS1. The role of MT1-MMP in regulating lineage progression of chondrocytes

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PS2. Identification of inhibitors targeting influenza A nucleoprotein through structure-based virtual screening

Lo CY[1], Choi ST[1], Li TW[2] , Ngo CK[1], Wan CC[3], Poon LM[2] and Shaw PC[1]
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PS3. The Genetics of SELEX

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PS4. Sox2 regulates hair pigmentation

Ho B[1], Cheng C[1], Leung K[1], Lovell-Badge R[2], Rizzoti K[2] and Cheah KSE[1]
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PS5. Structure of UreG/UreF/UreH complex reveals how urease accessory proteins facilitate maturation of *Helicobacter pylori* urease

Fong YH[1], Wong HC[1], Yuen MH[1], Lau PH[1], Chen YW[2] and Wong KB[1]
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PS6. Crosstalk between inorganic polyphosphate and interleukin - 11 in osteoblasts

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PS7. Structural characterization of human ribosomal stalk proteins

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PS8. Characterization of heterogeneous nuclear ribonucleoprotein C (hnRNP C), a potential telomerase inhibitor, on telomerase function

Tam CYF[1], Cheung DHC[1], Kung HF[2], Huang JJ[3] and Shaw PC[2]
[1]Biochemistry Programme and Centre for Protein Science and Crystallography, [2]School of Biomedical Sciences, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China, [3]Laboratory of Tumor and Molecular Biology, Beijing Institute of Biotechnology, Beijing, China

PS9. Structure-function study of the ligand-binding domain of an Arabidopsis Vacuolar Sorting Receptor

Luo F[1], Shen JB[2], Fong YH[1], Zeng YL[2], Jiang LW[2] and Wong KB[1]
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PS10. Severe acute respiratory syndrome Coronavirus M-protein induces cell death through disruption of PDK1/Akt signaling cascade

Li L, Chen ZF, Tsoi H and Chan HYE
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PS11. Metabolic changes in cancer cells in response to hypoxic stress

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PS12. Characterizing the function of the N-terminal of influenza B virus nucleoprotein

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PS13. An investigation on the anti-tumor and immunomodulatory effects of conjugated fatty acids

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PS14. Exploiting aptamer binding properties as a novel diagnostic technology for malaria

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