JOINT EVENT

20th Global Congress on Biotechnology

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Keynote Forum

Day 1

Peter J F Henderson, J Biotechnol Biomater 2018, Volume 8
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Peter J F Henderson

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Kinetic and molecular dissection of coupled ion-substrate membrane transport proteins

The Mhp1 Na^+, -hydantoin membrane symport protein from *Microbacterium liquefaciens* is a paradigm for the nucleobase-cation-symport, NCS-1, family of transport proteins found widely in archaebacteria, bacteria, yeasts and plants. Their metabolic roles include the capture by cells of nitrogen compounds and vitamins from the environment. Mhp1 is also a structural model for the huge range of '5-helix-inverted-repeat' superfamily of proteins, because, unusually, crystal structures are available for its open-outwards, occluded, and open-inward conformations. Here we accomplish a detailed dynamic model of the partial reactions in an alternating access cycle of membrane transport derived from substrate binding studies to the purified Mhp1 protein by combining novel mass spectrometry, stopped-flow and steady state kinetic analyses and mutagenesis. The mechanism of coupling substrate transport to the Na^+, -gradient is revealed during a sequence of mostly reversible kinetic steps that explain how transfer of substrate across the membrane is affected by changes in conformational states. The AceI H^+/substrate antiport protein from Acinetobacter baumannii is a paradigm for the proteobacterial antimicrobial compound efflux (PACE) family of drug efflux proteins found dispersed throughout the Proteobacteria. AceI contributes to the resistance of Acinetobacter baumannii towards the widely used antiseptic, chlorhexidine. Currently there is little structural information about the PACE family of transport proteins, but progress towards understanding the recognition of substrates and cations by AceI and its homologues will be discussed.

Figure 1: Scheme for the coupled transport of Na+ and hydantoins by Mhp1.

Recent Publications

1. Shimamura T, Weyand S, Beckstein O, Rutherford N G, Hadden J M et al. (2010) Molecular basis of alternating access membrane transport by the sodium-hydantoin transporter Mhp1. Science. 328(5977):470-473.
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3. Calabrese A N, Jackson S M, Jones L N, Beckstein O, Gsponer J (2017) Topological dissection of the membrane

transport protein Mhp1 derived from cysteine accessibility and mass spectrometry. Anal. Chem. 89(17):8844-8852.

4. Hassan K A, Jackson S M, Penesyan A, Patching S G, Tetu S G et al. (2013) Transcriptomic and biochemical analyses identify a novel family of chlorhexidine efflux proteins. Proc Natl. Acad. Sci. USA. 110(50):20254-20259.
5. Hassan K A, Liu Q, Henderson P J F, Paulsen I T (2015) Homologs of the Acinetobacter baumannii AceI transporter represent a new family of bacterial multidrug efflux systems. mBio. 6(1):e01982-14. Pg.1-5.

Biography

Peter J F Henderson is a Professor of Biochemistry and Molecular Biology in the University of Leeds. He obtained his BSc in 1965 and PhD in 1968, both in Biochemistry, at the University of Bristol. After Postdoctoral training at the Enzyme Institute, Madison, University of Wisconsin and in the Department of Biochemistry at Leicester, he became a University Lecturer in 1973. In 1975 he moved to the Department of Biochemistry at Cambridge, where he became Reader in Molecular Biology of Membranes in 1990. He has held Visiting Professorships in Japan, Canada and Australia. He was Scientific Director of the European Membrane Protein (EMeP) consortium 2003-2008, Coordinator of the European Drug Initiative for Channels and Transporters (EDICT) 2008-2012 and held Leverhulme Trust Emeritus Research Fellowships in 2001-2002 and 2014-2017. He has published over 200 scientific papers in the fields of Membrane Transport, Enzyme Kinetics and Structural Biology.

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Magali Remaud Simeon, J Biotechnol Biomater 2018, Volume 8
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Magali Remaud Simeon

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Mixing enzyme discovery with engineering for sucrose-derived bioproducts: The case of GH13 and GH70 polymerases

The exploration of the natural diversity, through data mining, functional genomics and/or metagenomics is an efficient mean to discover enzymes showing new functions or improved performances. These approaches can be further completed or run in parallel with semi-rational protein engineering based on structure/function studies or directed molecular evolution inspired from nature. Which of these alternatives are the best ones, in terms of effort, rapidity and efficiency? This is an open question to which a definite answer can be hardly formulated a priori. For illustration, we will take a few examples from our most recent work on glucansucrases from GH13 and GH70 families. These enzymes are naturally very efficient transglucosylases. They use sucrose as substrate and catalyze polymerization of its glucosyl units as a main reaction. Depending on their specificity, structures varying in size as well as in glycosidic linkage types can be obtained, thus giving access to an interesting panel of biopolymers. A campaign of genome sequencing and data mining allowed the isolation of atypical enzymes with new product specificities. In particular, a hyper efficient polymerase producing a gel-like polymer and, in contrast an enzyme synthesizing directly from sucrose a polymer of well-controlled low molar mass could be characterized. Structure-function studies combined with mutagenesis assays allowed us to decipher some of the molecular mechanisms behind the control of the polymer size and enzyme processivity. Another key property of these catalysts is coming from their ability to glucosylate a broad spectrum of hydroxylated molecules. Computational protein design, structurally-guided engineering and also random approaches such as neutral evolution was implemented for a fine tuning of their acceptor specificity toward non-natural acceptors such chemically protected disaccharides for vaccinal applications, polyol, flavonoids, or various chemicals. These various approaches will be described and discussed with regard to the engineering objectives.

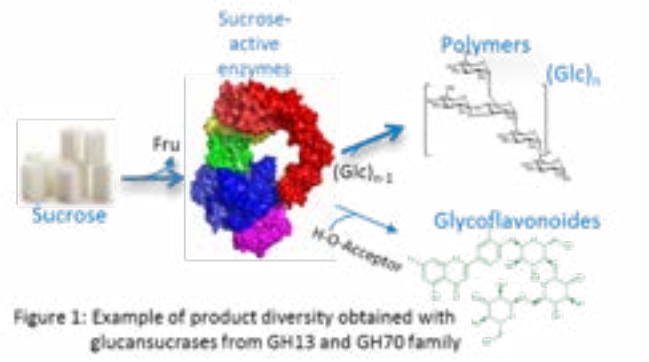

Figure 1: Example of product diversity obtained with glucansucrases from GH13 and GH70 family

Recent Publications

1. Claverie M et al. (2017) Investigations on the determinants responsible for low molar mass dextran formation by DSR-M dextransucrase. ACS Catal. 7(10):7106-7119.

2. Vuillemin M et al. (2017) A dextran with unique rheological properties produced by the dextransucrase from Oenococcus kitaharae DSM 17330. Carbohydr. Polym. 179:10-18.
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4. Salamone S et al. (2015) Programmed chemo-enzymatic synthesis of the oligosaccharide component of a carbohydrate-based antibacterial vaccine candidate. Chem. Comm. 51(13):2581-2584.
5. Verges A et al. (2015) Computer-aided engineering of a transglucosylase for the glucosylation of an unnatural disaccharide of relevance for bacterial antigen synthesis. ACS Catalysis. 5(2):1186-1198.

Biography

Magali Remaud Simeon is Professor at the National Institute of Applied Sciences of Toulouse and is head of the Catalysis and Enzyme Molecular Engineering group of the "Laboratoire d'Ingénierie des Système Biologiques and Procédé (LISBP). She received her PhD in Biochemistry from the University of Toulouse and was Post-Doc at the University of Pennsylvania. She has co-authored more than 150 papers and is co-inventor of 22 patents. Her research activities focus on Enzyme Engineering for white biotechnology, green chemistry, health, food/feed industries and synthetic biology. They cover enzyme structure/activity relationship studies, kinetic resolution, evolution combining both rational and combinatorial approaches, and applications to the synthesis of glycans, glycoconjugates and various synthons of interest. Her work is currently focused on the search and generation of enzymes displaying new specificities and improved catalytic properties. Her objective is to open new trajectories for biomass transformation. To this end, she specifically targets the integration of tailored enzymes in chemo-enzymatic cascades, new metabolic pathways or enzyme-based processes.

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Sergey Suchkov, J Biotechnol Biomater 2018, Volume 8
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Sergey Suchkov

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Proteolytic abzymes as translational tools of the newest generation to be exploited for biodesign and bioengineering

Catalytic Abs (catAbs) are multivalent im-munoglobulins (Igs) with a capacity to hy-drolyze the antigenic (Ag) substrate. In this sense, proteolytic Abs (Ab-proteases) repre-sents Abs to provide proteolytic effects. Abs against myelin basic protein/MBP with pro-teolytic activity exhibiting sequence-specific cleavage of MBP is of great value to monitor demyelination whilst in multiple sclerosis. The activity of Ab-proteases was first regis-tered at the subclinical stages, 1-2 years prior to the clinical illness and the activity of the Ab-proteases revealed significant correlation with scales of demyelination and the disabil-ity of the patients as well. So, the activity of Ab-proteases and its dynamics tested would confirm a high subclinical and predictive (translational) value of the tools as applicable for personalized monitoring protocols. Ab-proteases directly affecting remodeling of tissues with multilevel architectonics (for in-stance, myelin) are of tremendous value. By changing sequence specificity one may reach reduction of a density of the negative proteo-lytic effects within the myelin sheath and thus minimizing scales of demyelination. Ab-proteases can be programmed and re-programmed to suit the needs of the body metabolism or could be designed for the de-velopment of new catalysts with no natural counterparts. Further studies are needed to secure artificial or edited Ab-proteases as translational tools of the newest generation to diagnose, to monitor, to control and to treat and rehabilitate multiple sclerosis patients at clinical stages and to prevent the disorder at subclinical stages in persons at risks.

Recent Publications

1. Gabibov A A, Paltsev M A and Suchkov S V (2011) Antibody-associated proteol-ysis in surveillance of autoimmune de-myelination: clinical and preclinical is-sues. Future Neurology 6(3):303-305.
2. D Kostyushev, I Tsarev, D Gnatenko, M Paltsev and S Suchkov (2011) Myelin-associated serological targets as applica-ble to diagnostic tools to be used at the preclinical and transient stages of multiple sclerosis progression. Open J Immunology 1(3):80-86.
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4. Ponomarenko N A, Durova O M, Voro-biev I I, Belogurov A A, Telegin G B, et al. (2005) Catalytic activity of autoanti-bodies toward myelin basic protein corre-lates with the scores on the multiple scle-rosis expanded disability status scale. Immunol. Lett. 103(1):45-50.
5. Ponomarenko N A, Durova O M, Voro-biev I I, Aleksandrova E S, Telegin G B, et al. (2002) Catalytic antibodies in clini-cal and experimental pathology: human and mouse models. Journal of Immuno-logical Methods 2002(269):197-211.

Biography

Sergey Suchkov graduated from Astrakhan State Medical University and awarded with MD, then in 1985 maintained his PhD at the I M Sechenov Moscow Medical Academy and in 2001, he maintained his Doctorship Degree at the Nat Inst of Immunology, Russia. From 1987 through 1989, he was a senior Researcher, Koltzov Inst of Developmental Biology. From 1989 through 1995, he was a Head of the Lab of Clinical Immunology, Helmholtz Eye research Institute in Moscow. From 1995 through 2004, he was a Chair of the Dept. for Clinical Immunology, Moscow Clinical Research Institute. He has been trained at: NIH; Wills Eye Hospital, PA, USA; Univ. of Florida in Gainesville; UCSF, S-F, CA, USA; Johns Hopkins University, Baltimore, MD, USA. He was an Exe Secretary-in-Chief of the Editorial Board, Biomedical Science, an international journal published jointly by the USSR Academy of Sciences and the Royal Society of Chemistry, UK. At present, he is a Chair, Dept. for Personal-ized and Translational Medicine, I M Sechenov First Moscow State Medical University. He is a member of the New York Academy of Sciences, USA; American Chemical Society (ACS), USA; American Heart Association (AHA), USA; EPMA (European Association for Predictive, Preventive and Personalized Medicine), Brussels, EU; ARVO (American Association for Research in Vision and Ophthalmology); ISER (International Society for Eye Re-search); PMC (Personalized Medicine Coalition), Washington, USA.

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David Rabuka, J Biotechnol Biomater 2018, Volume 8
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David Rabuka

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Developing site-specifically modified ADCs using a chemoenzymatic approach

We have developed the SMARTag™ technology platform, which enables precise, programmable, site-selective chemical protein modification. Leveraging the target sequence of formylglycine generating enzyme (FGE), we chemoenzymatically modify proteins to generate a precisely placed aldehyde functionality that can be chemically elaborated. Subsequently, novel ligation chemistry is employed that exploits this "aldehyde tag" site. We will present recent data on our novel protein modification platform and its application to generating novel bioconjugates, including ADCs, utilizing our new conjugation chemistries and linkers. The application of these chemistries to generate site-specifically modified bioconjugates with improved efficacy and safety profiles will be presented. Additionally, we will highlight the progress in developing conjugates with a focus on preclinical studies as well as highlight our progress in cell line development and manufacturing by using this chemoenzymatic approach.

Biography

David Rabuka received a PhD in Chemistry at the University of California, Berkeley as a Chevron Fellow in the Lab of Carolyn Bertozzi. His research included developing and applying the SMARTagTM platform technology to cell surface modification. Prior to joining Bertozzi's lab, he worked at the Burnham Institute synthesizing complex glycans followed by Optimer Pharmaceuticals, where he focused on the development of glycan and macrolide based antibiotics. He was CSO, President and Co-founder of Redwood Bioscience, where he developed novel protein conjugation methods and biotherapeutic applications such as antibody-drug conjugates. Redwood Bioscience was acquired by Catalent Pharma Solutions in Oct 2014, where he has continued to apply the SMARTag™ technology with various collaborators and partners as a Global Head of R&D. He graduated with a Double Honors BS in Chemistry and Biochemistry from the University of Saskatchewan, where he received the Dean's Science Award, and holds an MS in Chemistry From the University of Alberta. He has authored over 45 major publications, as well as numerous book chapters and holds over 30 patents.

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Sergey Suchkov

I M Sechenov First Moscow State Medical University, Russia

Principles of profiling as applicable to the infrastructure of continuous education system to impact for having drug design to suit an innovative model of translational pipeline

Personalized medicine (PM) as the healthcare of the future represents an innovative model for advanced healthcare and robust platform for relevant industrial branches for diagnostics and pharmaceutics. However, rapid market penetration of new technologies demands the implementation of reforms not only in biopharma, but also in education. Therefore, the problem of the updated education of specialists in bioengineering, drug design and affiliated fields is becoming particularly urgent, and it requires significant revision of newer programs and curricula to be updated. Modernization and integration of widely accepted standards require consolidation of both the natural and medical sciences that may become the conceptual basis for the biopharma education. The main goal of this training is to provide development of novel multifaceted approaches to build academic schools for future generations. So, a higher, secondary and primary education as a trio should be integrated into the circuit. Based on current trends and own experience, we have made the first steps towards reshuffling the canonical educational tandem "School-University" and restructuring of specialized groups (with targeted disciplines) to get the mentees to be involved into having the existing healthcare system advanced and stepped forward. Moreover, non-canonical approach has been used to create a team of young researchers and biopharma students which has been recognized as The International Research Team of Youngsters under the aegis of EPMA (Brussels, EU) and ISPM (Tokyo, Japan). The integration of the primary and secondary education provides: 1. development in the chosen direction; and 2. optimization of the jointly set activity of a student and the teacher within a pair or a tandem (mentor-mentee). The above-mentioned has pre-determining value, because under the disintegration of the world community expressed the competition in quality of the scientific intellect dramatically increases. The same occurs in the areas of quality of all of three segments of the educational process, i.e., pre-college (secondary school), university and graduate.

Biography

Sergey Suchkov graduated from Astrakhan State Medical University and awarded with MD, In 1985 he completed his Ph.D. at I M Sechenov Moscow Medical Academy and in 2001, maintained his Doctorship Degree at the Nat Inst of Immunology, Russia. From 1987 through 1989, he was a senior Researcher, Koltzov Inst of Developmental Biology. From 1989 through 1995, he was a Head of the Lab of Clinical Immunology, Helmholtz Eye Research Institute in Moscow. From 1995 through 2004, he was a Chair of the Dept. for Clinical Immunology, Moscow Clinical Research Institute. He has been trained at: NIH; Wills Eye Hospital, PA, USA; Univ. of Florida in Gainesville; UCSF, S-F, CA, USA; Johns Hopkins University, Baltimore, MD, USA. He was an Exe Secretary-in-Chief of the Editorial Board, Biomedical Science, an international journal published jointly by the USSR Academy of Sci-ences and the Royal Society of Chemistry, UK. At present, he is a Chair, Dept. for Personalized and Translational Medicine, I M Sechenov First Moscow State Medical University. He is a member of the New York Academy of Sciences, USA; American Chemical Society (ACS), USA; American Heart Association (AHA), USA; EPMA (European Association for Predictive, Preventive and Personalized Medicine), Brussels, EU; ARVO (American Association for Research in Vision and Ophthalmology); ISER (International Society for Eye Re-search); PMC (Personalized Medicine Coalition), Washington, USA.

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Ibrahim Abdulhalim

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Plasmonic biosensors on demand: Tunable penetration depth, compactness, ultrahigh sensitivity and enhanced spectroscopies

Evanescent wave optical biosensors allow specific sensing by using a surface binding layer which enhances the capture of specific bio-entities within the nanoscale neighborhood to the sensor surface. However, this evanescence region is sometimes too small at the scale of few tens of nanometers which prevents obtaining monotonic signal versus concentration when the bio-entities are larger than the optical penetration depth. The purpose of this study is to describe methods for sensing both small (molecules, viruses, etc.) and large bioentities (cells, large molecules) using plasmonic sensors with tunable penetration depth. During the last few years, we have been developing different structural and system configurations for improving the performance of plasmonic biosensors based on improving the reading method and enhancing the local electromagnetic (EM) field further for the purpose of improving the sensitivity and lowering the detection limit based on SPR, SERS and SEF. The structural improvements include: (i) planar thin metal films combined with dielectric films, (ii) periodic metallic structures on planar substrate, (iii) nanosculptured thin films prepared by the glancing angle deposition technique. (iv) long range self-referenced plasmonic configurations, and lately, (v) combination of nanostructures with thin metal films for coupling of extended surface plasmons (ESP) to localized surface plasmons (LSP). The system improvements include: (i) diverging beam approach in the angular mode, (ii) polarimetric spectral mode, (iii) image and signal processing. Particularly, we have shown recently that even much higher enhancement of the EM fields is obtained by exciting the LSPs through extended surface plasmons generated on a semi-infinite metallic film surface. Biotechnology applications will be presented for sensing biomolecules and cells in water and in blood. In spite of the technological advances in optics, the need for developing molecular binding layer to improve the specificity is still in demand from the biotechnology community.

Biography

Ibrahim Abdulhalim is a Professor at the Electro-optical Engineering Unit at Ben-Gurion University of the Negev. He worked in academic institutions and companies such as the OCSC in University of Colorado Boulder, the ORC at Southampton University, the Thin Films Center of the University of Western Scotland, in KLA-Tencor, Nova and GWS Photonics. He has published over 200 articles, two books, 10 chapters and has 20 patents. He is a fellow of IoP and SPIE and an Associate Editor for the *Journal of NanoPhotonics* and for the *Journal of Imaging*.

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Jennifer A Littlechild, J Biotechnol Biomater 2018, Volume 8
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Jennifer A Littlechild

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Thermophilic enzymes with applications for industrial biocatalysis

There is an increasing demand for new enzymes with enhanced performance and/or novel functionalities that provide savings in time, money and energy for industrial processes in the areas of high value chemical production and other white biotechnology applications. Only a small proportion of nature's catalysts have been utilised for industrial biotechnology. The number of enzymes explored to date remains within the range of 1-2% of known biodiversity. A problem with using enzymes for industrial biocatalysis reactions is often their stability under the harsh conditions employed. The use of naturally thermostable enzymes isolated from hot environments are more stable to high temperatures, extremes of pH and exposure to organic solvents. The projects HOTZYME and THERMOGENE have identified hydrolase and transferase enzymes of industrial interest isolated from high temperature environments around the world. These have been isolated from thermophilic bacterial and archaeal genomes and metagenomes. A selection of these novel thermostable enzymes including cellulases, carboxylesterases, lactonases, epoxide hydrolases, transketolases, hydroxymethyl transferases and transaminases have been characterized both biochemically and structurally. Transaminase enzymes have received special attention for the production of chiral amines which are important building blocks for the pharmaceutical industries. These enzymes catalyse the reversible transfer of an amino group from a donor substrate onto a ketone/aldehyde or sugar acceptor molecule. They can be subdivided into 6 classes. The less studied class 4 (branched chain) (R) selective, class 5 (S) selective and class 6 (sugar) enzymes have been identified. An example of the archaeal class 4 enzyme from *Archaeoglobus fulgidus*; a thermostable class 5 archaeal transaminase from *Sulfolobus solfataricus* and class 6 sugar transaminase from *A. fulgidus*. Two new enzymes with interesting substrate specificity and stereo-selectivity have been discovered which have already been demonstrated at industrial scale for the production of new chiral chemical building blocks.

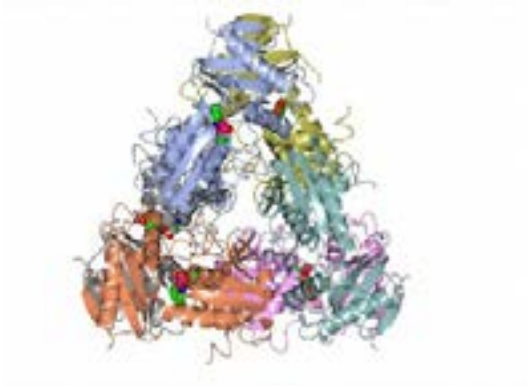

Figure 1: Hexameric structure of branched chain transaminase from A. fulgidus. An inhibitor bound to the cofactor pyridoxal phosphate at the active sites shown in spheres.

Biography

Jennifer A Littlechild is an Emeritus Professor of Biological Chemistry and has established the Henry Wellcome Centre for Biocatalysis at Exeter University in 2003. Her research studies involve the structural and mechanistic characterisation of a range of enzymes from thermophilic bacteria and archaea that have industrial applications. She has published over 200 publications in refereed high impact journals and presented her research work internationally. She has coordinated EU related project THERMOGENE and was a partner in a consortium grant HOTZYME. In UK she is funded from BBSRC and Innovate UK. These grants involve both large industrial companies and SME enterprises. She has supervised over 40 PhD students and acts as External Examiner for other PhD and Masters Students. She is the UK representative and Vice Chair of the European Section of Applied Biocatalysis and a Member of EU advisory committees for Industrial Biotechnology.

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Kam Bo Wong

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How urease accessory proteins coupled GTP hydrolysis/binding to nickel delivery to urease?

Urease is a nickel-containing metalloenzyme that catalyzes the hydrolysis of urea into ammonia and carbon dioxide. This enzymatic reaction, which produces the acid-neutralizing ammonia, is essential for the survival of *Helicobacter pylori* in human stomach. In *Helicobacter pylori*, nickel ions delivery for urease maturation is assisted by four urease accessory proteins, UreE, UreF, UreG and UreH. Specific protein-protein interactions among these urease accessory proteins are essential for the control of binding/release of nickel along the metal delivery pathway. We have previously determined the crystal structures of UreF/UreH and GDP-bound-UreG/UreF/UreH complexes. Upon binding of UreH, the C-terminal residues of UreF are induced to form an extra helix and a loop structure stabilized by Arg-250. These conformational changes facilitate the recruitment of UreG to the UreG/UreF/UreH complex, which is essential to urease maturation. Recently, we have determined the crystal structure of the nickel/GTP-bound UreG dimer, which reveals how GTP hydrolysis induces conformational changes that induce dissociation of UreG from the UreG/UreF/UreH complex and the release of nickel to the urease.

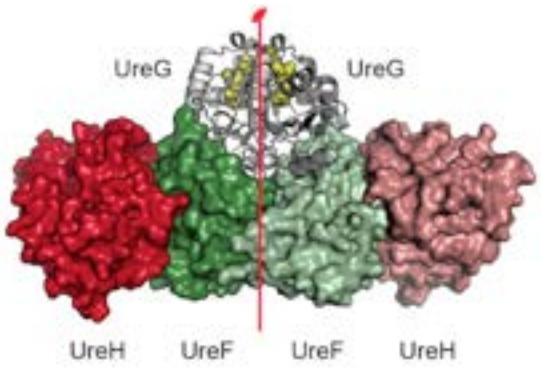

Figure : Crystal structure of UreG/UreF/UreH complex.

Biography

Kam Bo Wong obtained his BSc and MPhil from the Chinese University of Hong Kong. He then pursued his PhD degree in the laboratory of Prof. Alan Fersht at the University of Cambridge. After Postdoctoral training in the University of Washington and University of Cambridge, he joined the Chinese University of Hong Kong in 1999, where he is now a Professor at the School of Life Sciences. His research interests are on the structure-function studies of proteins. His research group uses multi-disciplinary techniques, including protein engineering, biophysical characterization, computational methodologies, and structure determination by NMR and X-ray crystallography, to study how proteins function on the atomic and molecular levels.

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