ICBE Asia 2020

10th International Conference on Biomolecular Engineering

JANUARY 7-9, 2020 | COPTHORNE KINGS HOTEL, SINGAPORE

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TIPS FOR A SUCCESSFUL MEETING

Say **hello** to everyone.
You might make someone's day.

Introduce yourself to people you don't know.
They may be your next good friends.

Stop and **smile**.
You will brighten the room considerably.

Be **understanding**.
Everybody makes mistakes.

Help those with less experience.
We were all novices at some point.

Respect others.
We all have something valuable to contribute.

Value staff and volunteers.
They are here for you.

Be **kind**.
You will never like everybody, but you can be cordial to all.

Enjoy the meeting!
You can have fun while sharing,learning and networking.

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WELCOME ADDRESS

Welcome!

A warm welcome to the 2020 International Conference on Biomolecular Engineering (ICBE) brought to you by the Society for Biological Engineering (SBE), an AIChE Technological Community and hosted at the Copthorne Kings Hotel, Singapore.

ICBE Asia 2020 brings together researchers to advance the understanding and application of molecular biology, biophysical chemistry, metabolic engineering, cellular and tissue engineering, biomaterials and synthetic biology. In this conference, engineers, scientists from academia and industry will gather to discuss the use of biology and biomolecular engineering to further research and discover new innovations in the health sector. The conference's plenary lecture topics provide deeper understandings into the key areas, which include: protein and peptide engineering, biomolecular engineering and self-assembly, and biomolecular analytics and characterizations.

The protein and peptide engineering session will focus on new tools and platforms developed to enable engineering of proteins and peptides. Technologies could include, but are not limited to, those for evolutionary design or synthesis.

The biomolecular engineering and self-assembly session will focus on advances in biomolecular design rules and engineering tools across various length scales to achieve super molecular structures, leading to applications in therapeutics and bio catalysis.

The biomolecular analytics and characterizations session will showcase advances in analytical tools that enable investigations at molecular level and its implications on the bulk properties which are relevant for translation into industrial scales.

A lot of work has gone into making this conference a success. We extend our thanks to the contributions of our expert steering committee and the dedication of the staff here at SBE and AIChE in realizing the conference. Our distinguished speakers who made the conference an exciting one; our thanks to them.

Before we close, we would like to thank each of you for attending ICBE 2020 and bringing your expertise and enthusiasm to the meeting. You have the vision, knowledge and experience to help us pave our way into the future. You are truly our greatest asset today and tomorrow, and we could not accomplish what we do without your support and leadership. Throughout this conference, we ask you to stay engaged, keep proactive, and help us shape the future of biomolecular engineering. We hope your experience is a pleasant, educational, and inspiring one.

Sincerely,

Conference Chairs of ICBE 2020

Sierin Lim
Nanyang Technological University (NTU)
Singapore

Susanna Su Jan Leong
Singapore Institute of Technology

TECHNICAL PROGRAM

Tuesday, January 7, 2020	
8:30 – 9:30 AM	**Registration**
9:30 – 9:45 AM	**Opening Ceremony**
9:45 – 10:30 AM	**Plenary Lecture I: Peptide/Protein Engineering** **583624: James P. Tam, Nanyang Technological University, Singapore. Plenary Talk: Superglue from Nature**
10:30 – 11:00 AM	Break
11:00 AM – 12:30 PM	**Technical Session 1: Enzyme Engineering**
11:00 – 11:30 AM	591024: Mark Howarth, University of Oxford, United Kingdom. Invited Talk: Spy and Snoop Peptide Superglues to Empower Vaccines, Biomaterials and Antibodies
11:30 – 11:50 AM	588543: Daniel T. Peters, Newcastle University, United Kingdom. Engineering the Plague Capsular Antigen (Caf1) Protein: A Thermally Reformable Protein Polymer
11:50 AM – 12:10 PM	588649: Bunyarit Meksiriporn, King Mongkut's Institute of Technology Ladkrabang, Thailand. An Engineered Survival-Selection Strategy for Synthetic Binding Scaffolds Specifically Targeting Post-Translationally Phosphorylated Proteins
12:10 – 12:30 PM	588529: MengJun Fang, ZheJiang University, China. Improving the Productivity of 5-Hydroxy-L-Tryptophan in Escherichia coli By Combinational Evolution of Several Key Enzymes and Co-Enzymes
12:30 – 2:00 PM	Lunch
2:00 – 3:40 PM	**Technical Session 2: Protein Engineering and Formulation**
2:00 – 2:30 PM	588858: Alois Jungbauer, BOKU, Austria. Invited Talk: Digital Twins of Continuous Integrated Biomanufacturing Processes
2:30 – 3:00 PM	590928: Jakob Buecheler, Novartis, Switzerland. Invited Talk: A Closer Look into Freeze-Thaw Processes for Therapeutic Protein Formulations
3:00 – 3:20 PM	588845: Donald Belcher, The Ohio State University, USA. Evaluating Chemo-Sensitizing Potential of Oxygen Delivery Facilitated By Transfused Polymerized Hemoglobins on Vascularized Solid Tumors
3:20 – 3:40 PM	590838: Tae Hyeon Yoo, Ajou University, Korea, Republic of (South). Site-Specific Modification of IgG Via a Simple Photocrosslinking Reaction
3:40 – 5:00 PM	**Poster Session and Reception**
Wednesday, January 8, 2020	
9:00 – 9:30 AM	**Registration**
9:30 – 11:00 AM	**Technical Session 3: Cellular Engineering**
9:30 – 10:00 AM	591063: Paul Freemont, Imperial College London, United Kingdom. Invited Talk: In Vitro Synthetic Biology Using Cell Free Systems to Prototype Parts and Pathways to Enzymatic Conversion in a Test-Tube
10:00 – 10:20 AM	588534: Xixian Chen, BioTrans, A*STAR Singapore, Singapore. Integrating Enzyme and Metabolic Engineering for Apocarotenoids Production
10:20 – 10:40 AM	584898: Kathakali Sarkar, Saha Institute of Nuclear Physics, Homi Bhabha National Institute, India. Synthetic Genetic Devices for Decoding and Encoding Chemical Signals in Living Bacterial Cells
10:40 – 11:00 AM	584867: Deepro Bonnerjee, Saha Institute of Nuclear Physics, Homi Bhabha National Institute, India. Synthetic Genetic Devices for Higher Order Artificial Information Processing in Living Cells: Towards a Cellular Robotics Platform
11:00 – 11:30 AM	Break
11:30 – 11:50 AM	**Rapid Fire Poster Presentations**
11:30 – 11:40 AM	589975: Muen Chung, RWTH Aachen, SeSaM-Biotech, Germany. Display of Functional Nucleic Acid Polymerase on Escherichia coli Surface and Its Application in Directed Polymerase Evolution
11:40 – 11:50 AM	583968: Young-Kee Kim, Hankyong National University, Korea, Republic of (South). Effect of Nutrient Supplement on Syngas Fermentation With Clostridium Autoethanogenum
11:50 AM – 1:30 PM	Lunch
1:30 – 2:15 PM	**Plenary Lecture II: Biomolecular Engineering & Self-assembly** **588708: Samuel Stupp, Northwestern University, USA. Plenary Talk: "Biomimetic Structures for Regenerative Medicine"**
2:15 – 3:25 PM	**Technical Session 4: Self-assembly**
2:15 – 2:45 PM	588467: Stefan Salentinig, University of Fribourg, Switzerland. Invited Talk: Lipid-Peptide Co-Assemblies for Multifunctional Biointerfaces
2:45 – 3:05 PM	583836: Andrew Care, Macquarie University, Australia. Bioengineering Prokaryotic Nanocompartments into Photosensitizing Nanoparticles

TECHNICAL PROGRAM

3:05 – 3:25 PM	588655: Rana Gamal, University of Garden City, University of Medical Sciences and Technology, Sudan. Green Synthesis of Gold Nanoparticles from Camellia sinensis and Its Application Against UTI Bacteria
3:25 – 4:30 PM	**Poster Session**
6:30 – 8:30 PM	Ticketed Dinner
Thursday, January 9, 2020	
9:00 – 9:30 AM	**Registration**
9:30 – 10:15 AM	**Plenary Lecture III: Biomolecular Analytics and Characterizations**
	591152: Daniel Fletcher, UC Berkeley, USA. Plenary Talk: Cell Surface Topography: How Protein Size can Alter Organization and Signaling at Cell-Cell Interfaces
10:15 – 10:45 AM	Break
10:45 AM – 12:05 PM	**Technical Session 5: Analytics**
10:45 – 11:15 AM	591007: Shee-Mei Lok, Duke-NUS Medical School, USA. Invited Talk: Zika virus and its antibody complex structures
11:15 – 11:35 AM	588640: Masamichi Ikeguchi, Soka University, Japan. Invited Talk: Time-Resolved Small-Angle X-Ray Scattering for Monitoring the Biomolecular Assembly Process
11:35 AM – 12:05 PM	590379: Yu Jing, Nanyang Technological University, Singapore, Singapore. Invited Talk: Explore the Adhesion Mechanisms of Mussel Adhesive Proteins
12:05 – 12:25 PM	**Technical Session 6: Applications**
12:05 – 12:15 AM	584563: Julie Lake, University of California, Berkeley, USA. Converting Fatty Acids to Alkanes with Blue Light
12:15 – 12:25 AM	588537: Hwa Jun Cha, Osan university, Korea, Republic of (South). Ip Is Decreased Melanogenesis Using Inhibition of PKA
12:25 PM – 12:45 PM	**Closing Remarks**

Poster Session

Poster #	Abstract #: **Name**, *Affiliation*, Poster Title
1	584439: **Hyeon Nae Jeon**, *Yonsei University, Bioinformatics and Molecular Design Research Center (BMDRC), Korea, Republic of (South)*. Development of Computer-Aided Rational Protein Engineering Toolkit (CARPET)
2	584530: **Arjun Bhagwat**, *University of British Columbia (Vancouver), Canada*. Studying Performance of Microbial Fuel Cell Inoculated with Shewanella Oneidensis MR-1 for Copper Recovery
3	584563: **Julie Lake**, *University of California, Berkeley, USA*. Converting Fatty Acids to Alkanes with Blue Light
4	585287: **Hyun Jong Lee**, *Gachon University, Korea, Republic of (South)*. The Comparison of MSC Secretome Effects on Corneal Wound Healing Depending on the Cell Culture Environments
5	587829: **Tsutomu Tanaka**, *Kobe University, Japan*. Metabolic Engineering to Improve 1,5-Diaminopentane Production from Cellobiose Using Bgl-Secreting Corynebacterium glutamicum
6	587838: **Ryosuke Fujiwara**, *Kobe university, Japan*. Parallel Metabolic Pathway Engineering" for the Bioproduction of Shikimate Pathway Derivatives
7	588537: **Hwa Jun Cha**, *Osan university, Korea, Republic of (South)*. Ip Is Decreased Melanogenesis Using Inhibition of PKA
8	589291: **Junchul Kim**, *Seoul National University, Korea, Republic of (South)*. CXCR4 Regulates Branching Morphogenesis in Developing Epithelial Organ
9	590581: **Takumi Kuwata**, *Soka University, Japan*. Salt-Induced Assembly Kinetics of Archaeoglobus Fulgidus Ferritin Monitored By Time-Resolved Small-Angle X-Ray Scattering
10	590697: **Jong Deog Kim**, *Chonnam Natational University, Korea, Republic of (South)*. Theasaponin E1 Attenuate Amyloid Beta (Aβ) Proteins and Hyperphosphorylated Tau in Sweapp N2a and Shy-5Y Cells By Down or up-Regulation of the Associated Signaling Molecules and Enzymes
11	590756: **Dima Abu Alhawa**, *Texas A&M University at Qatar, Qatar*. Solar Spectrum Characterization, Impact on Human Health and Mitigation Using Innovative Materials
12	590784: **Ying Xie**, *Nanyang Technological University, Singapore*. Polarisome Scaffolder Spa2-Mediated Macromolecular Condensation of Aip5 for Actin Polymerization
13	590881: **Sharon Chee**, *p53LAB (A*STAR), Singapore*. Selection of Peptides Inhibiting p53 Function
14	591232: **Shuhei Noda**, *Riken, Japan.* Microbial Platform to Synthesize Valuable Bulk Chemicals in Escherichia coli
15	591046: **Ayokunmi Oyeleye**, *Universiti Putra Malaysia, Malaysia*. Loop Motions and Dynamics in the Catalytic Domain of GH Family 19 Chitinases Are Essential for Binding to Complex Substrates

Plenary Speaker Biographies

Daniel Fletcher
University of California, Berkeley

Daniel Fletcher, Ph.D. is the Purnendu Chatterjee Professor of Bioengineering and Biophysics at UC Berkeley, where his research explores organizational principles of the cell membrane and cytoskeleton, mechanotransduction in cancer and infectious diseases, and development of biomedical technologies for global health. He received a B.S.E. from Princeton University, a D.Phil. from Oxford University as a Rhodes Scholar, and a Ph.D. from Stanford University as an NSF Graduate Research Fellow. After a postdoctoral fellowship in the Stanford University School of Medicine, he began his faculty career in the Department of Bioengineering at UC Berkeley, where he continues to teach courses on optics, microscopy, and cell mechanics. Prof. Fletcher's research has received numerous awards, including an NSF CAREER Award, a Tech Laureate Award from the San Jose Tech Museum, and a "Best of What's New" designation by Popular Science magazine. He has served as a White House Fellow in the Office of Science and Technology Policy, was elected to the American Institute for Medical and Biological Engineering (AIMBE), and previously held the Lester John and Lynne Dewar Lloyd Distinguished Professorship at UC Berkeley. Prof. Fletcher's mobile phone-based biomedical technologies, known as "CellScope", are being tested in multiple countries for disease diagnostic applications and have been supported by the Bill and Melinda Gates Foundation, USAID, and other sources. He and his laboratory are known for development of new optical and force microscopy tools to study cell mechanics, and for innovative research on the membrane and cytoskeletal structures that animate cell movements, work supported by the NIH, NSF, and DOE. Prof. Fletcher is also Deputy Director of the Physical Biosciences Division of Lawrence Berkeley National Laboratory; a faculty affiliate of the Department of Molecular and Cell Biology, the Blum Center for Developing Economies, the California Institute for Quantitative Biosciences (QB3), the Center for Information Technology Research in the Interest of Society (CITRIS), and the Center for Emerging and Neglected Diseases (CEND); as well as a member of the Bioengineering, Biophysics, and Nanoscale Science and Engineering Graduate Groups at UC Berkeley.

Samuel Stupp
Northwestern University

Samuel Stupp is Board of Trustees Professor of Materials Science and Engineering, Chemistry, Medicine, and Biomedical Engineering at Northwestern University. He directs at Northwestern the Simpson Querrey Institute and the Energy Frontiers Research Center for Bio-Inspired Energy Science funded by the Department of Energy. Professor Stupp is a member of the National Academy of Engineering, the American Academy of Arts and Sciences, the Spanish Royal Academy, and the National Academy of Inventors. He is a fellow of the American Physical Society, the Materials Research Society, the Royal Society of Chemistry, and the National Academy of Inventors. His awards include the Department of Energy Prize for Outstanding Achievement in Materials Chemistry, the Materials Research Society Medal Award, the American Chemical Society Award in Polymer Chemistry, the American Chemical Society Ronald Breslow Award for Achievement in Biomimetic Chemistry, the International Award from The Society of Polymer Science in Japan, and the Royal Society Award in Soft Matter and Biophysical Chemistry. He has received honoris causa doctorates from Eindhoven Technical University in the Netherlands, the University of Gothenburg in Sweden, and the National University of Costa Rica.

James P. Tam
Nanyang Technological University, Singapore

James P. Tam is currently the Lee Wee Nam Professor and the Director of the Synzymes and Natural Products Center (SYNC) in School of Biological Sciences. He served as the Founding Dean of the School of Biological Sciences, the Founding Director of Biological Research Center and the Founding Director of the double-degree program in Biomedical Science and Chinese Medicine at Nanyang Technological University, Singapore. He

received his Ph.D. in Medicinal Chemistry from the University of Wisconsin, Madison, USA and held appointments as Associate Professor at The Rockefeller University, USA (1982-1991), Professor at Vanderbilt University, USA (1991-2004) and The Scripps Research Institute, USA (2004-2008). He invented peptide dendrimers as protein mimetics in the 1980s for immunologics. His recent research work focuses on the discovery, design and development of bio-therapeutics for healthy ageing, particularly ultra-stable, orally-active peptide biologics from medicinal plants. Professor Tam has published more than 330 papers in these areas of research. He received major awards for his seminal contributions in chemistry and biology of peptide sciences.

Invited Speaker Biographies

Paul Freemont
Imperial College London

Professor Paul Freemont is the co-founder of the Imperial College Centre for Synthetic Biology and Innovation (2009) and co-founder and co-director of the National UK Innovation and Knowledge Centre for Synthetic Biology (SynbiCITE; since 2013) and Director of the London BioFoundry (since 2016) at Imperial College London. He is also currently the Head of the Section of Structural Biology in the new Department of Infectious Diseases at Imperial. He was previously the Head of the Division of Molecular Biosciences at Imperial (2005-2012), Head of the Imperial College Centre for Structural Biology (2000-2005) having joined Imperial from Cancer Research UK London Research Institute (now known as the Crick Research Institute) where he was a Principle Investigator and Head of Group. In 2019, he led the establishment of the Global Biofoundry Alliance (GBA) comprising 23 institutions on four continents aimed at building and sharing open technology platforms for synthetic biology and currently the chair of the GBA. His research interests span from understanding the molecular mechanisms of human diseases and infection to developing synthetic biology foundational tools for specific applications including automation. His research group has pioneered the use of cell free extract systems for synthetic biology prototyping and biosensor applications and he is the author of over 240 scientific publications (H-index 74). He is an elected member of European Molecular Biology Organization and Fellow of the Royal Society of Biology, Royal Society of Chemistry and Royal Society of Medicine and is an Honorary Fellow of the Royal College of Art. He was a co-author of the British Government's UK Synthetic Biology Roadmap and was a recent member of the Ad Hoc Technical Expert Group (AHTEG) on synthetic biology for the United Nations Convention for Biological Diversity (UN-CBD). He has also appeared regularly on radio and television broadcasts on the subject of synthetic biology and has successfully co-supervised Imperial iGEM teams since 2006.

Alois Jungbauer
University of Natural Resources and Life Sciences, Vienna (BOKU), Austria

Professor Alois Jungbauer received his PhD in Food Technology and Biotechnology from BOKU. He serves as a professor at the Department of Biotechnology at BOKU. He teaches Protein Technology and Downstream Processing and Bioprocess Engineering and is study director of the Ph.D. program Bioprocess Engineering. He also acts as area head of Bioprocessing Engineering and Deputy Director of Research in the Austrian Centre of Industrial Biotechnology. He is currently working in the field of bioprocess engineering of proteins, plasmids and viruses. He has published 340 papers on recombinant protein production, bioseparation and advanced materials for bioprocess engineering, 17 patents and 12 book contributions and recently a monograph entitled "Protein Chromatography, Process Development and Scale Up". He is executive editor of Biotechnology Journal. He acts also as the vice president of research of the European Society of Biochemical Engineering Science.

Shee-Mei Lok
Duke University-National University of Singapore Medical School

Dr. Shee-Mei Lok is a Professor in the Emerging Infectious Disease program, Duke-NUS (Duke University-National University of Singapore) Medical School. She was a National Research Foundation Fellow in year 2009 to 2014 and is now a NRF Investigator. She is a structural virologist specializing in X-ray crystallography and cryo-electron microscopy. Her research focuses on the structural changes of flavivirus and alphavirus during its infection cycle and the effect of anti-viral therapeutics on the virus particle. She obtained her PhD from NUS in 2004 specializing on x-ray crystallography. She did her post-doctoral training in Purdue University under the supervision of Prof Michael Rossmann from 2004 till 2009. She served as visiting Assistant Professor in Baylor College of Medicine in the year 2010 in the laboratory of Prof Wah Chiu.

INVITED SPEAKER BIOGRAPHIES

Mark Howarth
Oxford University

Mark Howarth is the Professor of Protein Nanotechnology in Oxford University Department of Biochemistry, where he has been a principal investigator since 2007. In 2017 he was awarded the Royal Society of Chemistry Norman Heatley Award. He did postdoctoral studies at MIT with Alice Ting, where he developed monovalent streptavidin and single molecule probes for tracking neurotransmitter receptors. His doctoral work was with Tim Elliott at Southampton University investigating MHC class I-peptide quality control. His current work is on innovating ultra-stable protein interactions for applications in synthetic biology, enzyme stabilization and vaccines.

Jakob Buecheler
Novartis

Jakob Buecheler joined Novartis in January 2019 as part of the Advanced Manufacturing and Technologies group with the focus on Drug Product Technologies. He joined from Merck KGaA, where he was working on Antibody-Drug Conjugates as part of his PhD with the focus on degradation pathways and formulation aspects. He holds a BSc. in Biology from the University of Mainz, Germany and a MSc. in Microbiology from the University of Tuebingen, Germany. He further had research engagements at the University of Queensland, Australia and the Nanyang Technological University, Singapore in the field of Bioengineering as well as at Hoffmann-La Roche, Switzerland at the Department of Pharmaceutical Development.

ORAL ABSTRACT SUBMISSIONS

PLENARY LECTURE I: PEPTIDE/PROTEIN ENGINEERING

Plenary Talk: Superglue from Nature.

James P. Tam
School of Biological Sciences, Synzymes and Natural Products Center, Nanyang Technological University, Singapore, Singapore

Over the past 25 years, major advances are ligation chemistries to form peptide or non-peptide bonds between molecules. These advances are enabled by the development of novel chemical ligation methods and expansion of genetic codons as well as the discovery of inteins and peptide ligases. Of particular interest to our laboratory are the discoveries of plant-derived ligases, which are superglue from nature. These ligases or superglue enable bonding between peptides, peptide-to-protein, and protein-to-protein, without protecting groups, activating agents and under aqueous conditions, and with exquisite selectivity. Here, I will present our work in ligation chemistry to enable site-specific bonding of chemicals, polymers, peptides and proteins to form new compounds under physiological conditions. In particular, I will discuss our work on the Asx-specific peptide ligases such as the butelases, which were discovered in NTU campus and which act as superglue for labeling proteins and live cells as well as precision biomanufacturing industrial enzymes and therapeutics under environmental friendly conditions.

TECHNICAL SESSION 1: ENZYME ENGINEERING

Invited Talk: Spy and Snoop Peptide Superglues to Empower Vaccines, Biomaterials and Antibodies.

Mark Howarth
University of Oxford, Oxford, United Kingdom

A special feature of *Streptococcus pyogenes* enables spontaneous isopeptide bond formation within certain cell-surface proteins. We engineered this system to generate an irreversible peptide-protein interaction (SpyTag/SpyCatcher). This system is rapid, genetically-encodable and specific in diverse biological environments. Latest advances include accelerating to infinite affinity and a toolbox of modules for rapidly controlling protein architectures. Cyclizing enzymes using SpyTag conferred resilience to boiling, for biotransformation and nutrition. SpyTag and the related superglue SnoopTag allow programmable synthesis of multi-functional teams or biomaterials, to modulate precisely cancer cell signaling. Virus-like particles (VLPs) are nano-assemblies with many attractive features for vaccination. However, decorating VLPs with antigens by genetic fusion or chemical modification is often unsuccessful. We demonstrated complete reaction to SpyCatcher-VLPs after mixing with SpyTag linked to a range of malaria antigens and cancer targets. Spy-VLPs efficiently induced antibody responses after only a single immunization and in the absence of adjuvant. Modular assembly using SpyTag should accelerate vaccine development against a range of human and veterinary diseases.

Engineering the Plague Capsular Antigen (Caf1) Protein: A Thermally Reformable Protein Polymer.

Daniel T. Peters[1], *Gema Dura*[2], *Helen Waller*[1], *Adrian Yemm*[1], *Neil D. Perkins*[1], *Mark A. Birch*[3], *David A. Fulton*[2], *and Jeremy H. Lakey*[1]
(1)Biosciences Institute, Newcastle University, Newcastle Upon Tyne, United Kingdom, (2)School of Natural and Environmental Sciences, Newcastle University, Newcastle upon Tyne, United Kingdom, (3)Department of Surgery, Cambridge University, Cambridge, United Kingdom

Caf1 is a proteinaceous polymer produced by *Yersinia pestis* bacteria that helps them to evade phagocytosis. Individual 15 kDa Caf1 subunits associate non-covalently to form long (MDa, µm), highly thermostable polymers. Caf1 polymers can be combined with cross-linkers to form hydrogels of tuneable porosity and stiffness, are free from animal products, and are simple to produce and purify. Additionally, cells adhere poorly to the material,

making it “non-stick”. Through mutagenesis, this phenotype can be reversed, allowing different bioactivities to be engineered into the protein. These properties make Caf1 a uniquely interesting biomaterial to develop for biomedical applications.

Here, we engineer Caf1 mutants containing signals involved in osteogenesis, and combine these to form a “mosaic” Caf1 polymer that can trigger the early stages of bone formation when used as a scaffold for bone marrow stromal cells. We next demonstrate that denatured Caf1 polymers can self-assemble into oligomers, including mosaic oligomers, allowing us to control the composition of bioactive subunits within the material. Finally, we show that we can use this property to create thermally reformable Caf1 hydrogels, providing a simple and effective route towards cell encapsulation within the 3D material. We anticipate that these developments will increase Caf1’s utility as a biomaterial for use in 3D cell culture, as well as other applications.

Evaluating Chemo-Sensitizing Potential of Oxygen Delivery Facilitated By Transfused Polymerized Hemoglobins on Vascularized Solid Tumors.

Donald Belcher
Department of Chemical and Biomolecular Engineering, The Ohio State University, Columbus, OH

A major constraint in chemo-and radio-therapeutic cancer treatment is inadequate oxygenation of solid tumors. Consequently, alleviating hypoxia in solid tumors is considered a promising target for improving the efficacy of anti-cancer therapeutics such as chemotherapy. Our group has synthesized variable molecular weight (MW) Polymerized human hemoglobin (PolyhHb) with low (T-state) and high (R-state) oxygen (O_2) affinities for use as RBC substitutes. These O_2 carriers can be transfused to increase solid tumor oxygenation and improve the efficacy of anti-cancer therapeutics. In this study, we analyzed the biophysical properties of the synthesized PolyhHbs. Three-dimensional computational models of blood flow and O_2 transport in the tumor micro-environment were used to examine how dosage and type of PolyhHb impacted tumor O_2 delivery. To compliment this study, an animal study was performed by periodically delivering PolyhHb in the T- and R-state. Decreases in the apparent viscosity resulting from PolyhHb exchange transfusion may result in significant changes in flow distributions throughout the tumor micro-circulatory network. The difference in wall shear stress implies that PolyhHb may have a more significant effect on capillary beds. The increased O_2 flux and decreased pO_2 drop per unit length indicates that both PolyhHbs are suited to deliver O_2 under hypoxic conditions. Both T- and R-state PolyhHb exchange transfusion may lead to elevated O_2 delivery at low $pO_{2,in}$. In the experimental mouse model, HBOC transfusion led to decreased angiogenesis, tumor growth, and hypoxic gene expression.

Improving the Productivity of 5-Hydroxy-L-Tryptophan in Escherichia coli By Combinational Evolution of Several Key Enzymes and Co-Enzymes.

MengJun Fang[1]*, HaiJiao Wang*[1]*, ZhiNan Xu*[2]*, and Lei Huang*[2]
(1)ZheJiang University, HangZhou, China, (2)College of Chemical and Biological Engineering, ZheJiang University, HangZhou, China

In our previous work, one combined biosynthetic pathway was engineered to produce 5-hydroxy-L-tryptophan (5-HTP) with glycerol as carbon source in *E. coli*. In the present work, the low specific bioactivity of tryptophan hydroxylase and low stability of two co-enzymes (pterin-4α-carbinolamine dehydratase (PCD) and dihydropteridine reductase (DHPR)) were identified to be two factors which seriously hinders further enhancement of 5-HTP production. A gene-circuit based high-throughput screening method was developed to evolve the above proteins. One mutant of TPH-8 was screened out to have 1.5-fold bioactivity improvement, and the mutants of PCD 94 or DHPR 109 were also obtained to have obviously improved stabilities. By combining the evolution of all the three proteins, the titer of 5-HTP was improved 3.7 fold compared with the original strain in the shake flask, and no L-trp was left which would make the purification of 5-HTP from the culture medium much easier . The present work would pave one new road for the bioproduction of 5-hydroxy-L-tryptophan in industry.

TECHNICAL SESSION 2: PROTEIN ENGINEERING AND FORMULATION

Digital Twins of Continuous Integrated Biomanufacturing Processes.

Alois Jungbauer
Department of Biotechnology, BOKU, Vienna, Austria

A lot of industries already realized Industry 4.0 and Industry 5.0 technologies into their manufacturing. This enables smart automation and model-based control strategies. In biopharmaceutical industry digitalization is still not common. The product is mainly performed in batchwise manner and the focus is on in-process and release analytics, which are executed off-line and time consuming. The imminent potential for process optimization control and predictive maintenance cannot be fully utilized without manufacturing in continuous mode and a digital representations of bioprocesses. Models for bioprocesses can be based on understanding of underlying biophysical mechanism e.g. heat/mass balance, adsorption/desorption kinetics or steric hindrance or similar and combined with computational fluid dynamics. Such mechanistic models are available to describe and predict chromatography, filtration, and centrifugation. Strategies will be shown how to convert a batch into a continuous integrated bioprocess and the benefits and challenges will be discussed such as reduction of floor space, high productivity but also the problem of propagation of process disturbances will be addressed. For a continuous end to end antibody manufacturing process the residence time distribution model will be shown and how process disturbance propagate. Finally, a roadmap will be presented how we could arrive at a digitized or virtual bioprocess.

A Closer Look into Freeze-Thaw Processes for Therapeutic Protein Formulations.

Jakob Buecheler
Novartis, Basel, Switzerland

Freezing and thawing are essential processes of the life cycle for a therapeutic protein. They enable stability during transport or storage as well as decoupling of DS and DP shelf-life for most Biologics Bulk Drug Substances (BDS).

Reports and publications about the impact of freezing and thawing on the stability of therapeutic proteins are contradictory. Some link a fast freezing process with an increase in protein degradation, others report the reverse.

Protein stress factors during freezing include an increase in interfaces (such as ice to liquid or to air), but also cold denaturation or cryoconcentration could lead to degradation. Furthermore, it has been reported that cryo-protectants, which should stabilize the protein in frozen state, can crystalize during frozen storage and can cause aggregate formation.

The importance of the thawing process is often being underestimated, as a 300% increase in protein concentration is not uncommon due to stratification effects and can cause degradation. Additionally, various studies show that slow and apparent mild thawing conditions at low temperatures can lead to gelation or precipitation due to protein-protein interactions.

An Engineered Survival-Selection Strategy for Synthetic Binding Scaffolds Specifically Targeting Post-Translationally Phosphorylated Proteins.

Bunyarit Meksiriporn[1], *Morgan R. Baltz*[2], *Erin Stephens*[3], *Allen Jiang*[4], *Dujduan Waraho*[5], *Hyeon Cheol Lee*[6], *and Matthew P. DeLisa*[7]
(1)Biology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand, (2)Chemical & Biomolecular Engineering, Cornell University, Ithaca, NY, (3)Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY, (4)Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA, (5)Biological Engineering, King Monkut's University of Technology Thonburi, Bangkok, Thailand, (6)Reseach center, ForBioKorea co, ltd, Seoul, NY, Korea, Republic of (South), (7)Robert Frederick Smith School of Chemical and Biomolecular Engineering, Cornell University, Ithaca, NY

Protein phosphorylation plays an important role in the regulation of protein function and many cellular processes. Aberrant phosphorylation has been shown to be a cause of cell death as well as maligination. As such, there is an urgent need for affinity reagents that target phospho-modified sites on individual proteins for either immunodetection or immunotherapy; however, generating such reagents remains a significant challenge. Here, we described a genetic selection strategy termed PhLI-TRAP (*ph*ospho-functional *l*igand-binding *i*dentification by *T*at-based *r*ecognition of *a*ssociating *p*roteins) for routine laboratory isolation of phospho-specific designed ankyrin repeat proteins (DARPins) by linking in vivo affinity capture of a phosphorylated target protein with antibiotic resistance of *Escherichia coli* cells. The assay is validated using an existing panel of DARPins that selectively bind the nonphosphorylated (inactive) form of extracellular signal-regulated kinase 2 (ERK2) or its doubly phosphorylated (active) form (pERK2). PhLI-TRAP was successfully used to improve the affinity of a phospho-specific DARPin for its cognate pERK2 antigen as well as to reprogram the specificity of the same parental DARPin for binding to noncognate ERK2. Importantly, by linking antibiotic resistance with phospho-epitope binding in the cytoplasm of E. coli cells, the PhLI-TRAP method eliminates the need for purification or immobilization of the phosphoprotein target and only requires selective plating of bacteria on solid medium to uncover productive binders. Collectively, these results establish our genetic selection as a useful and potentially generalizable protein engineering tool for studying phospho-specific binding proteins and customizing their affinity and selectivity.

Site-Specific Modification of IgG Via a Simple Photocrosslinking Reaction.

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Conjugation of antibody has expanded its applications in therapeutics and diagnostics, and various methods have been developed based on chemical or enzymatic reactions. However, majority of them have focused on synthetic molecules such as small molecules, nucleic acids, or synthetic materials, but site-specific conjugation of antibody with protein cargos has been rarely demonstrated so far. In this presentation, I describe a PEptide-DIrected Photocrosslinking (PEDIP) reaction for site-specific conjugation of IgG with protein using an Fc-binding peptide and a photoreactive amino acid analogue, and demonstrate this method by developing an immunotoxin composed of a Her2-targeting IgG (trastuzumab) and an engineered *Pseudomonas* exotoxin A (PE24). The ADP-ribosylation of eukaryotic elongation factor-2 by the bacterial toxin inhibited the ribosomal translation of protein, and the trastuzumab-PE24 conjugate exhibited the cytotoxicity toward Her2-overexpressing cell lines. The PEDIP reaction can also be applied for many other types of cargo with slight modifications of the method.

TECHNICAL SESSION 3: CELLULAR ENGINEERING

Invited Talk: *In Vitro* Synthetic Biology Using Cell Free Systems to Prototype Parts and Pathways to Enzymatic Conversion in a Test-Tube.

Paul S. Freemont
UK Dementia Research Institute Care Research and Technology Centre, Imperial College London, London, United Kingdom

Cell-free transcription/translation systems (known as CFPS or TX-TL) have recently been re-evaluated as a promising platform for enabling synthetic biology research and applications. In particular CFPS has been shown to provide a reproducible prototyping platform for regulatory elements where measurements in vitro are in part consistent with similar measurements in vivo. The advantage of being non-GMO allows rapid automated assays for characterizing parts and genetic circuit designs for pathway engineering, natural product discovery and biosensor designs and field implementation. My lab has been interested in exploring cell free extracts from non-model organisms and I will present our most recent work on cell-free extract systems. I will also present our recent studies on combining in vitro geno-chemtic strategies to allow rapid access to xenobiotic compounds which may provide improved therapeutic activity. By focusing on the violacein biosynthesis pathway and using seven different

substrate analogues, we have been able to generate new to nature analogues of violacein. Further new derivatives were also generated from brominated analogues via Suzuki-Miyaura cross-coupling reaction directly using the crude extract without prior purification. This approach shows that biosynthesized natural products can be chemically derivatized in cell extracts.

Integrating Enzyme and Metabolic Engineering for Apocarotenoids Production.

Xixian Chen, *Sudha Shukal, and Congqiang Zhang*
*BioTrans, A*STAR Singapore, Singapore, Singapore*

Metabolic engineering aims to balance intracellular pathways and increase the precursor supply. However, some heterologous enzymes are not evolved to support high flux. To remove the limitation, the catalytic properties of rate-limiting enzymes must be enhanced. Previously, we have demonstrated the use of fusion-partners to improve the expression of carotenoid cleavage dioxygenase 1 (CCD1) in *Escherichia coli*. Here, we further engineered CCD1 whose intrinsic promiscuity and low activity limited the production of α-ionone in *Escherichia coli*. Site-directed mutagenesis was carried out to mutate three structural elements of CCD1: an active site loop, η-helices and α-helices. Furthermore, mutated CCD1 was fused with lycopene epsilon-cyclase to facilitate substrate channelling. Collectively, these methods improved α-ionone concentration by > 2.5-fold as compared to our previously optimized strain. Lastly, the engineered enzyme was used in conjunction with metabolic engineering strategy to further boost α-ionone concentration by another 20%. This work deepens our understanding of CCD1 catalytic properties and proves integrating enzyme and metabolic engineering can be synergistic for higher microbial production yield.

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Synthetic Genetic Devices for Decoding and Encoding Chemical Signals in Living Bacterial Cells.

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Forward engineering of synthetic gene circuits has made higher order information processing feasible to be performed in living cells. One of the next challenges in Synthetic biology is to create artificial decision-making signal processing system with more complex human-defined function and real world application. Here, we develop biological genetic circuits which are analogous to electronic 2-to-4 decoder and 4-to-2 priority binary encoder at the population level in living *E. coli* using synthetic logic gates, regulated by synthetic promoters containing binding sites for several transcription factors (TFs). We consider extracellular chemicals as inputs such as Isopropyl β-D-1thiogalactopyranoside (IPTG), anhydrotetracycline (aTc), n-acyl homoserine lactone (AHL) & arabinose and various fluorescent proteins as outputs. We have also used one mutated version of λ repressor CI: frame-shifted CI which we built and characterized in our lab. We have designed the component circuits based on the electronic decoder and encoder circuit conventions, constructed and characterized to get expected logic behaviors, studied their mathematically predictable properties and established agreement between their mathematical models and experimental outputs in terms of fluorescent protein expression.

Synthetic Genetic Devices for Higher-Order Artificial Signal Processing and Computation in Living Cells.

Deepro Bonnerjee[1,2] *and Sangram Bagh*[3]
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Bhabha National Institute, Mumbai, India, (3)Biophysics and Structural Genomics, Saha Institute of Nuclear Physics, Kolkata, India

Biology is a complex arena that involves a multitude of inputs and outputs occurring at the right levels, time and combination to achieve the fine tuning called life. These interaction networks implement robust genetic circuitries created over time. Today, Synthetic Biology demands Synthetic Genetic networks that can function involving larger sets of inputs and outputs and perform more complex decision-making regimes. Despite the plethora of synthetic circuits already designed, we are still far behind the genetic complexity of nature and thus far from attaining the full potential of engineering genomes and cellular robotics. Towards this, we have been trying to develop a few complex genetic computational circuits- namely a biological 1-2 multiplexer (input selector) and a 2-1 de-multiplexer (output selector) amongst others- conceived from electronic circuit design principles. Attaining desired regulation through external chemical inducers, transcription factors and translational control, these circuits have required designing and characterizing libraries of synthetic Promoters and RBS's. Fine tuning of the transcriptional-translational regulatory machinery has culminated in satisfactory Boolean response. Furthermore, we have also explored how minimalistically can one increase the input-output complexities of a synthetic gene circuit through developing a 3 input-3 output combinatorial genetic system. It orthogonally senses three small molecule signals as inputs and responds through three fluorescent proteins in accordance with input logical states. Consensus of the Mathematical models and experimental validations of the systems show good predictability. These circuits may be integrated in future into larger circuits and are stepping stones to more complex application-oriented genetic networks.

RAPID FIRE POSTER PRESENTATIONS

Display of Functional Nucleic Acid Polymerase on *Escherichia coli* Surface and Its Application in Directed Polymerase Evolution.

Muen Chung
Biotechnology, RWTH Aachen, Aachen, Germany; SeSaM-Biotech, Aachen, Germany

DNA polymerase-based techniques such as PCR and DNA sequencing have become indispensable for modern molecular biology. These techniques foster an expanding field of applications, including clinical diagnostics, drug screening, forensic research and archaeology. To tailor the properties of a polymerase for the desired application, various directed evolution strategies have been employed, such as phage display, compartmentalized self replication and primer extension assay. Here we report, to the best of our knowledge, the first high-throughput screening method for DNA polymerase based on cell surface display (CSD). CSD separates the polymerase from cytosolic materials which might interfere with the selection of the polymerase for desired activities. A DNA polymerase is displayed on *Escherichia coli (E.coli)* cell surface using an inactivated esterase autotransporter. The activity of the displayed polymerase is then detected by a dye that intercalates into elongated DNA and emits more fluorescence. Using this method, we evolved Klenow fragment of *E. coli* DNA polymerase I to incorporate NTP and C2'-OMe modified oligonucleotides. The CSD-based screening method provides a novel alternative for directed evolution of DNA polymerase.

Effect of Nutrient Supplement on Syngas Fermentation With *Clostridium Autoethanogenum*.

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(1)Chemical Engineering, Hankyong National University, Anseong, Korea, Republic of (South), (2)Chemical Engineering, Hankyong National University

The syngas fermentation process is attracting attention as a technology to produce bioethanol from lignocellulosic biomass based syngas or waste gas, but feasibility improvement is required for industrial utilization. The cost of the culture media required for fermentation is a big part of overall fermentation cost, and the yeast extract, which is widely used as nutrient supplement, is a relatively expensive ingredient used to make basal medium for cultivation of acetogenic bacteria. In this study, we investigated the effect of corn steep liquor, malt extract, and

vegetable extract as a low cost nutrient substitute for yeast extract on microbial growth and product formation during syngas fermentation. We verified that corn steep liquor, malt extract, and vegetable extract could replace yeast extract in syngas fermentation. Moreover, the use of malt extract and vegetable extract resulted in a significant improvement in ethanol productivity.

PLENARY LECTURE II: BIOMOLECULAR ENGINEERING & SELF-ASSEMBLY

Plenary Talk: "Biomimetic Structures for Regenerative Medicine".

Samuel Stupp
Northwestern University, Evanston, IL

Due to shifting demographics, one of the grand challenges for science in this century is to create strategies to regenerate parts of the human body in order to achieve longer “healthspans”. Ideally, designed bioactive materials could act as extracellular matrices with finite half-life that traffic signals in dynamic fashion. This in fact would mimic the function of natural matrices as tissues develop or repair after injury, and requires molecular design of soft materials to directly activate signaling pathways. An important feature of the ideal biomaterials is the nature of internal dynamics across scales, thus imitating the non-static nature of living matter. This lecture will describe a broad platform of *supramolecular biomaterials* built with a toolbox self-assembling peptide amphiphiles, glycans, and nucleic acids that has been validated by pre-clinical models of neural and musculoskeletal regeneration. These systems can be crafted as highly effective growth factor mimics with longer half lives than proteins, and the tunable dynamics of their non-covalently bonded molecules can have a profound effect on biological efficacy. The lecture will also describe systems in which biological signals can be switched on and off through external cues, and others in which reversible self-assembly of superstructures modulates the phenotype of brain cells.

TECHNICAL SESSION 4: SELF-ASSEMBLY

Invited Talk: Lipid-Peptide Co-Assemblies for Multifunctional Biointerfaces.

Stefan Salentinig[1]*, Mahsa Zabara*[2]*, and Mark Gontsarik*[2]
(1)University of Fribourg, Fribourg, Switzerland, (2)Empa, Switzerland

Amphiphilic lipids and peptides can self-assemble to form a diversity of structures with functionality on multiple length scales. Directing the self-assembly and structure formation provides opportunities for the design of functional biointerfaces for various applications including medical therapies and food products.[1]

This presentation demonstrates the co-assembly of selected food lipids with peptides into functional biointerfaces that kill bacteria and promote wound healing.[2] We further show that external variables including pH, humidity, enzymes and temperature can be used to tailor the biointerface morphology and trigger the antibacterial activity on demand.[3,4,5] These materials are sustainable, biodegradable and food grade. The mechanisms at play underlying the self-assembly structure formation and its dynamic transformations with external triggers are studied using highly contemporary biophysical and computational methods including in situ time-resolved synchrotron SAXS and GISAXS, bio-AFM, cryo-TEM and online confocal Raman microscopy. Additional antibacterial assays on a broad range of both gram negative and positive bacteria strains, together with toxicity assays bridge the colloidal structure and composition to the biological activity.

The detailed insights into dynamic self-assembly in bio-mimetic materials and their characterisation on multiple length scales may provide essential knowledge for the comprehensive design of functional biointerfaces in form of nanoparticles and coatings.

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Bioengineering Prokaryotic Nanocompartments into Photosensitizing Nanoparticles.

Dennis Diaz[1], Xavier Vidal Asensio[2], Anwar Sunna[1], and ***Andrew Care[1]***
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Photodynamic therapy (PDT) is a selective and non-invasive cancer treatment. To destroy tumour cells, PDT relies on photosensitizers that are activated by light to convert the oxygen within tumour cells into toxic reactive oxygen species (ROS) that induce cell death. The fluorescent proteins KillerRed (KR) and mini-Singlet Oxygen Generator (mSOG) are unique biological photosensitizers that produce ROS when irradiated with light. Herein, encapsulins (ENC), a class of protein-based nanoparticles found in prokaryotes, were engineered to encapsulate KR or mSOG variants. All photosensitizer-loaded ENCs were produced in *Escherichia coli* and were ~30 nm in size, monodisperse and fluorescent. KR (Type I photosensitizer) generates ROS under green/yellow light. Upon activation with green light, KR-loaded ENC (KR-ENC) produced similar amounts of ROS as free KR, while unloaded ENC generated no ROS. We also visualised the rapid internalisation of KR-ENC by human brain cancer cells, confirming ENC's feasibility as a nanocarrier for functional biological photosensitizers. mSOG (Type II photosensitizer) produces singlet oxygen ($^1O_2$) upon blue light irradiation. mSOG variants, mSOG-1 and mSOG-2, previously engineered for enhanced $^1O_2$ generation were loaded into ENC. All mSOG-loaded ENCs produced $^1O_2$ under blue light activation, with a mSOG-1-loaded ENC variant (mSOG-1-ENC) shown to be the most effective. Next, we evaluated the PDT killing effect of mSOG-1-ENC in a cell model of lung cancer. mSOG-1-ENC displayed no cytotoxicity in the dark, however, when activated with blue light, it caused a ~25% reduction in cancer cell viability. These results show light-activated mSOG-1-ENC's photosensitizing capacity and its ability to mediate PDT.

Green Synthesis of Gold Nanoparticles from Camellia sinensis and Its Application Against UTI Bacteria.

Rana Gamal
Biomedical Engineering, University of Medical Sciences and Technology, khartoum, Sudan

Green synthesis of gold nanoparticles (AuNPs) using plant extracts is one of the most promising approaches for obtaining environment friendly nanomaterials for biological applications.

In this study, AuNPs were synthesized using black tea extract as reducing, capping and stabilizing agent. A single-step green bottom-up method was deployed to synthesize constrained gold nanoparticles of different of sizes. Ultraviolet visible spectrophotometry (UV-vis) and Scanning electron microscopy (SEM) were used to inspect the formation, size and shape of the AuNPs. The antioxidant and antibacterial activities of biosynthesized AuNPs were investigated by using the DPPH chemical reagent and two microorganisms, Gram negative Escherichia coli (E. coli) and Gram-positive Staphylococcus aureus (S. aureus).

It was confirmed that the AuNPs have bacteriostatic effect on the Gram-negative E.coli and Gram-positive S.aureus bacteria, which was due to the physio-chemical properties of AuNPs that depends strongly on their shape and size.

In conclusion, Green synthesis of AuNPs has been successfully investigated and proposed as a simple, reproducible, low-cost, nontoxic and ecofriendly method. The characteristic studies proved that the formed AuNPs had uniform spherical shapes. Furthermore, the synthesized AuNPs exhibited antibacterial activity.

PLENARY LECTURE III: BIOMOLECULAR ANALYTICS AND CHARACTERIZATIONS

Plenary Talk: Cell Surface Topography: How Protein Size can Alter Organization and Signaling at Cell-Cell Interfaces.

Daniel A Fletcher
Department of Bioengineering, University of California, Berkeley, Berkeley, CA

Membrane interfaces formed at junctions between cells are often associated with characteristic patterns of membrane protein organization, such as in epithelial tissues and between cells of the immune system. The size of cell surface proteins is emerging as a critical property that can directly affect cell-cell interface formation and contribute to spatial arrangement of membrane proteins at junctions, as well as their downstream signaling. This talk will describe a new method for characterizing cell surface protein size that enables nanometer-scale height measurements. With this information, we use in vitro systems based on giant unilamellar vesicles and live immune cells to show that fluid membrane interfaces linked by adhesion proteins can drive segregation of non-adhesive proteins, directly affecting macrophage signaling during receptor-mediated target recognition. Results from these studies support a model in which cell surface protein size plays a key role in mediating cell-cell communication and function.

TECHNICAL SESSION 5: ANALYTICS

Invited Talk: Zika Virus and its Antibody Complex Structures.

Shee-Mei Lok
Duke University-National University of Singapore, Singapore, Singapore

ZIKV is a member of the family *flaviviridae*, other members in the same family are West Nile, dengue, yellow fever viruses. ZIKV was previously thought to cause only a mild febrile disease characterized by fever, rash, joint pain and red eye, in only 20% of the infected humans. However, in the recent outbreaks in South America and French Polynesia, reports of an increased incidence of Guillain-Barré syndrome (GBS) in adults and microcephaly in foetuses associated with the disease sounded the global alarm. In addition to spreading by the bite of an infected mosquito, ZIKV can also be spread by sexual intercourse suggesting that the transmission could be faster than DENV. Therefore, there is an immediate need to develop therapeutics and vaccines. To produce a safe vaccine and therapeutics, more basic understanding of ZIKV is required. Here we will discuss the high resolution ZIKV structure and the development of antibody therapeutics and vaccine development using structural information obtained by cryo-electron microscopy.

Invited Talk: Time-Resolved Small-Angle X-Ray Scattering for Monitoring the Biomolecular Assembly Process.

Masamichi Ikeguchi
Department of Bioinformatics, Soka University, Hachioji, Japan

The small-angle X-ray scattering (SAXS) is a useful technique to know the assembly state of biomolecules. We can obtain the molecular weight of the assembled complex from the forward scattering intensity, that is, we can know how many molecules are included in the complex. The scattering profile also provides the information about the shape of the complex. The development of a photon-counting PILATUS detector made it possible to observe two-dimensional scattering images at every few milliseconds. Using this detector and a stopped-flow apparatus, we followed the assembly reactions of *Escherichia coli*ferritin A (EcFtnA) and its mutants by the time-resolved SAXS (TR-SAXS) method. EcFtnA forms a cage-like structure that consists of 24 identical subunits and dissociates into dimers at acidic pH. The dimer maintains native-like secondary and tertiary structures and is able to reassemble into a 24-mer when the pH is increased. The reassembly reaction was induced by pH jump and reassembly was followed by TR-SAXS. Time-dependent changes in the forward scattering intensity and in the radius of gyration suggested the existence of a significant population of intermediate oligomers during the assembly reaction. The initial reaction was a mixture of second- and third-order reactions (formation of tetramers and hexamers) from the protein-concentration dependence of the initial velocity. The time-dependent change in SAXS profile could be explained by a simple model in which only tetramers, hexamers, and dodecamers were considered as intermediate.

Invited Talk: Explore the Adhesion Mechanisms of Mussel Adhesive Proteins.

Jing Yu
School of Materials Science and Engineering, Nanyang Technological University, Singapore, Singapore, Singapore

The rapid and robust adhesion of marine mussels to diverse solid surfaces in wet environments is mediated by the secreted mussel adhesive proteins which are abundant in a catecholic amino acid, *L*-3,4-dihydroxyphenylalanine (Dopa). Over the last two decades, enormous efforts have been devoted to the development of synthetic mussel-inspired adhesives with water-resistant adhesion and cohesion properties by modifying polymer systems with Dopa and its analogues. Using the surface forces apparatus (SFA), we systematically explored the adhesion mechanism of various mussel foot proteins. Our SFA results show that mussels achieve strong interfacial binding via balancing a variety of covalent and noncovalent interactions including oxidative cross-linking, electrostatic interaction, metal-catechol coordination, hydrogen bonding, hydrophobic interactions and π–π/cation-π interactions. The SFA can also measure the adhesion and interfacial tension of complex coacervates formed by mussel foot proteins (Mfps). The insights obtained from the understanding of the interaction mechanisms between individual Mfp and disparate substrates provide critical guidance for the design of next-generation wet adhesive materials.

TECHNICAL SESSION 6: APPLICATIONS

Converting Fatty Acids to Alkanes with Blue Light.

Julie Lake[1] *and Samuel Curran*[1,2]
(1)University of California, Berkeley, Berkeley, CA, (2)Joint BioEnergy Institute, Emeryville, CA

The production of fatty-acid derived fuels and chemicals by microbial metabolism offers a renewable alternative to petroleum-based fuels on the market. Microbial hydrocarbon biosynthesis in particular is a target for metabolic engineering efforts due the potential use of alkanes and alkenes as drop-in transportation fuels. Here, we explore the development of an *Escherichia coli* strain engineered for increased fatty acid production and conversion of fatty acids to alkanes by a light-driven enzyme. This photoenzyme, named fatty acid photodecarboxylase (FAP), is native to microalgae *Chlorella variabilis* and has been shown to directly decarboxylate fatty acids to their corresponding C_{n-1} alkanes in a reaction catalyzed by blue light. By deleting the *fadD* and *fadE* genes responsible for fatty acid degradation and expressing FadR and 'TesA proteins for increased free fatty acids, we were able to show increased alkane production by the FAP enzyme *in vivo*. Our findings suggest that the FAP enzyme could be useful for the production of biodiesel-like alkanes and further investigation into other light-regulated metabolic pathways is warranted.

Ip Is Decreased Melanogenesis Using Inhibition of PKA.

Hwa Jun Cha
Beauty & Cosmetics, Osan university, Osan, Korea, Republic of (South)

Melanogenesis is essential UV protection mechanism in skin. However, excessive melanogenesis causes hyperpigmentation deceases, such as a melasma, freckle, etc. Therefore, through depigmentation agents, it is necessary to regulate melanogenesis. In this study, we identified noble whitening agent and their molecular mechanism. Firstly, we show that IP led to decrease melanin contents in a dose dependent manner in B16F10. The decrease of melanin synthesis was occurred by IP mediated decreasing Tyrosinase activity and expression. In general, Tyroainase expression is regulated by Microphthalmia-associated transcription factor (MITF) in a-MSH treated B16F10. Thus, we identified change of MITF activity in IP treated B16F10. IP down-regulated MITF activity and also MITF expression in a-MSH treated B16F10. Next, we identified CREB activity and phosphorylation in IP-treated B16F10, because CREB activity is implicated in MITF expression in a-MSH mediated melanogenesis. As shown our result, IP decreased CREB activity and phosphorylation in B16F10. Additionally, as CREB is

phosphorylated by PKA, we showed PKA activity using phosphor-PKA. Phosphor-PKA was up-regulated by IP in B16F10. Overall, IP regulates melanin synthesis using regulation of PKA-CREB-MITF-Tyrosinase axis.

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POSTER SESSION

Development of Computer-Aided Rational Protein Engineering Toolkit (CARPET).

Hyeon Nae Jeon[1], ***Hocheol Lim***[1,2], *Sungbo Hwang*[1,2], *Jongwan Kim*[1,2], *and Kyoung Tai No*[1,2]
(1)Department of Biotechnology, Yonsei University, Seoul, Korea, Republic of (South), (2)Bioinformatics and Molecular Design Research Center (BMDRC), Seoul, Korea, Republic of (South)

Protein engineering is varying the structure of a protein to develop valuable proteins. Protein side-chain mutation is fundamental to protein engineering processes, but the astronomical number of all possible protein sequences makes protein engineering difficult. Thus, computer-aided protein engineering is required to explore all possible protein sequence space and to guide the effective protein design. A number of different approaches have been suggested and applied to prediction of protein properties, such as enzyme reactivity, binding affinity, substrate/product selectivity, thermal/pH stability, and solubility, through sequence-based machine learning algorithms, knowledge-based potential functions, all-atom molecular mechanics based calculations, and all-atom quantum mechanics based calculations. Here, we suggest a new Computer-Aided Rational Protein Engineering Toolkit (CARPET) which predicts protein properties via sequence-based algorithms and structure-based algorithms, and helps rational enzyme modification. In CARPET, it starts from phylogenetic analysis via structural superimposition for inter-species comparison. The good score mutations are selected from the suggested mutation pool based on prediction models of thermal stability and solubility. Free Energy Perturbation (FEP) and Thermodynamic Integration (TI) are used to calculate more accurate thermal/pH stability. Finally, fragment molecular orbital (FMO) method is used to calculate calculate residue-residue interactions in enzyme to predict protein properties. Through collaborative manners in our new platform, we can analyze the experimental mutation results, design putative mutation pool, predict the properties of suggested mutation, and finally pick out the best-score mutations.

Studying Performance of Microbial Fuel Cell Inoculated with Shewanella Oneidensis MR-1 for Copper Recovery.

Arjun Bhagwat
Faculty of Applied Science, University of British Columbia (Vancouver), Vancouver, BC, Canada

Conventional methods of extracting metals involve fragmentation and processing of ores through toxic chemicals. Along with the high cost and energy for processing low grade ores, dealing with immense amount of waste rock is a formidable challenge for the mining industry. Copper at high concentrations becomes anthropogenic and can be particualarly be found in acid mine drainage (AMD). Bio-electrochemical systems provide low operating costs and are a sustainable alternative for many industrial applications. Microbial fuel cells (MFC) have advanced from electricity production to wastewater treatment. Species of bacteria known as exoelectrogen can transfer electrons from their extracellular structures such as pili and flavins. These bacteria have a very well-defined metabolic pathways leading to terminal electron acceptors. Shewanella Oneidensis MR-1 has been one of the most widely studied exoelectrogen. As part of my undergraduate thesis, I am studying the kinetics of electron transport in a two-chamber microbial fuel cell inoculated with Shewanella Oneidensis MR-1 under lactate substrate and copper sulphate solution at cathode. The study also explores recovery of copper at carbon neutral cathode. The maximum power density achieved by the cell was 13 mW/m^2 with 40% removal of copper from catholyte. Furthermore, I am working on genetically engineering the strain by overexpressing genes encoding for proteins playing pivotal role in electron transfer to boost extracellular electron transfer, thereby generating higher voltage and increasing rate of copper recovery. I look forward to more development on applications of MFCs and inspire research in scaling up bio-electrochemical systems to meet industry demands.

Converting Fatty Acids to Alkanes with Blue Light.

Julie Lake[1] *and Samuel Curran*[1,2]
See Technical Session 6: Applications in Oral Abstracts

The Comparison of MSC Secretome Effects on Corneal Wound Healing Depending on the Cell Culture Environments.

Hyun Jong Lee
Chemical and Biological Engineering, Gachon University, Seongnam, Korea, Republic of (South)

The therapeutic effects of secreted factors (secretome) produced by human mesenchymal stem cells (MSCs) were evaluated depending on the cell culture environments. The cells were cultured on standard 2D culture conditions and 3D electrospun fiber scaffolds, and the secretomes were collected. The goal was to determine whether culturing MSCs within a 3D, extracellular matrix-like environment would improve the therapeutic.

The secretome of MSCs cultured on 2D substrates and 3D electrospun fiber scaffolds showed substantial compositional differences. The relative amounts of factors such as HGF and ICAM-1 were increased over 5 times in 3D cultures compared to 2D cultures. Cell viability of corneal fibroblasts sustained metabolic activity for 6 days when co-cultured with MSCs grown on the electrospun fiber scaffolds in the absence of serum. A scratch-based wound healing assay showed 95% closure at 48 hours in corneal fibroblast monolayers co-cultured with MSCs grown on electrospun fibers, while the control group only exhibited 50% closure at the same time point. When electrospun fibers with seeded MSCs were applied to an *ex vivo* rabbit corneal organ culture system, it promoted re-epithelialization. Immunostaining of the corneas showed that the expression of alpha-SMA was lower compared to the injured cornea without treatment. In addition, keratan sulfate expression was higher in the treated group compared to the untreated.

MSCs cultured on three-dimensional, electrospun fiber scaffolds facilitate wound healing effects on corneal fibroblasts *in vitro* and on explanted corneas in an organ culture model through differential secretome profiles compared to MSCs cultured on 2D substrates.

Metabolic Engineering to Improve 1,5-Diaminopentane Production from Cellobiose Using Bgl-Secreting *Corynebacterium glutamicum*.

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Microbial production of 1,5-diaminopentane from renewable feedstock is a promising and sustainable approach for the production of polyamides. We constructed a β-glucosidase (BGL)-secreting *Corynebacterium glutamicum* and successfully used this strain to produce1,5-diaminopentane from cellobiose and glucose. First, *C. glutamicum* was metabolically engineered to produce l-lysine, a direct precursor of 1,5-diaminopentane, followed by the co-expression of l-lysine decarboxylase and BGL derived from *Escherichia coli* and *Thermobifida fusca* YX (Tfu0937), respectively. This new engineered *C. glutamicum* strain produced 27 g/L of 1,5-diaminopentane from cellobiose in CGXII minimal medium using fed-batch cultivation. The yield of 1,5-diaminopentane was 0.43 g/g glucose. These results demonstrate the feasibility of1,5-diaminopentane production from cellobiose or cellooligosaccharides using an engineered *C. glutamicum* strain.

"Parallel Metabolic Pathway Engineering" for the Bioproduction of Shikimate Pathway Derivatives.

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Glucose and xylose are the major components of lignocellulosic biomass, and the effective use of both sugars contributes to improving the efficiency of bioproduction. Here we propose a method termed “parallel metabolic pathway engineering” (PMPE) for producing shikimate pathway derivatives from glucose–xylose co-substrate. In this method, we seek to use glucose mainly for target chemical production and xylose for supplying essential metabolites for cell growth. Glycolysis and the pentose phosphate pathway (PPP) are completely separated from the tricarboxylic acid (TCA) cycle. To recover cell growth, a xylose catabolic pathway that directly flows into the

TCA cycle is introduced. We focused on the Dahms pathway which is a xylose catabolic pathway in *Caulobacter crescentus*, an oligotrophic bacterium. The Dahms pathway directly produces pyruvate and glyoxylate from xylose without glycolysis and PPP. To prove our concept, we produced *cis*,*cis*-muconic acid (MA) using the PMPE *Escherichia coli* strain. MA, a shikimate pathway derivative, is a valuable compound that is a precursor of important chemical compounds such as adipic acid and terephthalic acid, and its production has recently attracted attention. We modified the metabolic pathway of *E. coli* so that only glucose could be used for MA production, and we introduced the Dahms pathway to restore cell growth. As a result, we produce 4.09 g/L of MA using the PMPE *E. coli* strain with a high yield (0.31 g/g of glucose). The "PMPE" strategy will contribute to the development of clean processes for producing various valuable chemicals from lignocellulosic resources.

Ip Is Decreased Melanogenesis Using Inhibition of PKA.

Hwa Jun Cha

See Technical Session 6: Applications in Oral Abstracts

CXCR4 Regulates Branching Morphogenesis in Developing Epithelial Organ.

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The CXC Chemokine receptor type 4 (CXCR4), a member of 7-transmembrane receptor family, is known to facilitate migration of immune cells as well as stem cell homeostasis. Not only that, CXCR4 has gained extensive attention due to its ambivalent augmentation effects on the development of organs. Many studies have been conducted on the contributions of CXCR4 to organogenesis and, specifically, to neurogenesis and angiogenesis. However, the function of CXCR4 in the early branching stages of embryo submandibular gland is yet to be explored. In this study, we investigated the relations between CXCR4 and glandular branching morphogenesis. AMD3100, a potent CXCR4 inhibitor, was applied to embryo submandibular glands extracted from E13 and E14 embryos. E14 eSMGs showed a retarded growth compared to control group whereas the branching and expansion were nearly abolished in E13 eSMGs. Both control and AMD3100-treated groups, however, showed no significant difference in their expressions of cleaved caspase-3. Analysis of mRNA sequencing data revealed considerable increase in the expressions of acinar and ductal progenitor as well as of differentiation marker genes. Collectively, our results show that CXCR4 regulates the differentiation of acinar and ductal cells in glandular branching morphogenesis.

Salt-Induced Assembly Kinetics of *Archaeoglobus Fulgidus* Ferritin Monitored By Time-Resolved Small-Angle X-Ray Scattering.

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Ferritin is a protein cage composed of 24 identical subunits with octahedral 4-3-2 symmetry. *Archaoglobus fulgidus* ferritin (AfFtnWT) has a unique "open structure" shifting from 4-fold symmetry to tetrahedral 2-3 symmetry forming 4 triangular pores with 52Å edges in the shell. It has been shown that the replacements of Lys-150 and Arg-151 to alanines dramatically change the assembly of AfFtn. The mutant K150A/R151A (AfFtnAA) assembled into a conventional octahedral cage, in which Ala-150 and Arg-151 were located at 4-fold symmetry axis. Furthermore, AfFtnWT and AfFtnAA exist as subunit dimers having native-like secondary and tertiary structures at low salt concentrations. They can reversibly reassemble into respective 24mers when salt concentration is increased. In this study, the assembly reactions of AfFtnWT and AfFtnAA were induced by NaCl-concentration jump and monitored by time-resolved small-angle x-ray scattering (TR-SAXS). The results indicated that the assembly rate of AfFtnAA is 25 times faster than that of AfFtnWT of 445mM NaCl. Although the detailed analysis of the assembly reaction was hampered by the unknown large aggregate formation at higher protein concentrations

under the low salt conditions, the kinetic curve of AfFtnAA was similar to that of *Escherichia coli* ferritin (EcFtnA), indicating that AfFtnAA assembles into 24mer via the mechanism similar to that previously elucidated for EcFtnA.

Theasaponin E1 Attenuate Amyloid Beta (Aβ) Proteins and Hyperphosphorylated Tau in Sweapp N2a and Shy-5Y Cells By Down or up-Regulation of the Associated Signaling Molecules and Enzymes.

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In present study we investigated the therapeutic effects of green tea seed isolated theasaponin E1on attenuating Alzheimer by ameliorating the Aβ and tau level in SweAPP N2a cells and SHY-5Ycells. For reducing Aβ, the inhibition of β, γ-secretases and acetylcholinesterase and activation of α-secretase (ADAM10), neprilysin and insulin degrading enzyme (IDE) was investigated. In order to determine the inhibitory or activating effects of theasaponin E1 on tau, we targeted the signaling proteins involved in tau phosphorylation and dephosphorylation (Gsk-3β, Cdk5, MARK, Jnk, Akt, CaMKII AMPK/ERK, PP1, PP2-A and PP2-B). investigation was done with fluorometric assays, RT-PCR, Western blotting and ELISA respectively. Theasponin E1 was extracted and purified from green tea seed extract via HPLC. Cultured cells after treating with various concentrations were then processed for extraction of RNA, proteins and cell lysate which was then used for fluorometric assays, RT-PCR, Western blotting and ELISA respectively. Gen specific primers were used for amplification of the target genes and specific antibody for each terget were used for expression and quantification of the proteins. Our results demonstrated that theasaponin E1 significantly reduced Aβ concentration by activation of α-Secretase (over expression of ADAM10) and neprilysin. Activities of β secretase and γ-secretase were reduced in dose-dependent manner due to down-regulation of BACE1, Presenilin (PS1) and Nicastrin (NCT) respectively. NCT inhibition was lower as compared to PS1 also theasaponin E1 significantly reduce the activities of acetylcholinesterase. Theasaponin E1 effectively inhibit tau phosphorylation in dose dependent manner via downregulating Gsk-3β, Cdk5, MARK, Jnk, CaMKII.

Solar Spectrum Characterization, Impact on Human Health and Mitigation Using Innovative Materials.

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Solar radiation, depending on the degree of exposure, is a key factor on the manufacturing of vitamin-D, especially the UVB portion of light (290-320 nm). However, excessive exposure to solar UV-B irradiance has detrimental effects to human health, since it is a genotoxic stressor, and cause unrepairable DNA damage, skin cancer etc. Surprisingly, Vitamin-D deficiency and metabolic disorders are common in Middle Eastern countries (especially Qatar) and have been linked to inadequate exposure to sunlight, regardless of the more than 9-hours average sunshine per year. Beyond the biological complexity of the above phenomena other environmental factors, as well, have a significant impact.

Efforts in this study focus on the quantification of the threshold exposure time needed in Qatar to produce vitamin-D. In other words, to create a model relating the threshold UV exposure time and the production of vitamin-D based on literature and local data. To achieve this, we assembled an experimental setup to characterize solar irradiance and spectral composition (intensity per wavelength). Measurements collected from a pyranometer and spectrometer were combined to obtain graphs of the energy and wavelength of spectrum throughout the day. In parallel, by conducting a literature review of the implications of UV irradiation, a list of different biomarkers was identified as causing significant health impacts, such as cyclobutane pyrimidine dimers (CPD) and 8-oxo-dG. Here, we present the preliminary results of our work and the python-model we developed to utilize local atmospheric data (through AERONET), our measurements, and a literature Vitamin-D synthesis model.

Polarisome Scaffolder Spa2-Mediated Macromolecular Condensation of Aip5 for Actin Polymerization.

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Polarized fungal growth requires the highly-coordinated assembly of actin cable aligning along the mother-daughter axis. For directional actin polymerization during polarized growth, actin cable nucleator formin and formin nucleation promoting factors maintain a condensed-zone at the bud tip. A macromolecular complex called polarisome plays essential roles in regulating these actin-binding proteins in a spatial-temporal manner throughout the cell cycle progression. However, the protein constituents in the polarisome and their intra- or inter-molecular interactions for the dynamic regulation of protein functions in actin assembly and polarized cell growth remain elusive. Recently, we have identified a previously uncharacterized actin-interacting protein 5 (Aip5), which directly interacts with formin and Spa2, the scaffolder of the polarisome complex. Through the cell biology, biochemical, and structural biology studies, we have revealed that by interacting with formin, Aip5, independently or synergistically nucleates actin filament assembly. Furthermore, using both in vivo cell imaging and in vitro protein reconstitution experiments, we found that the intrinsically disordered region of Aip5 plays essential roles in regulating the protein function through phase separation mechanism, in a Spa2- and actin-dependent manner, during stress adaptation. The macromolecular condensation of Aip5 provides a few new mechanisms underlying the regulation of actin polymerization during polarized fungal growth or stress adaptation.

Selection of Peptides Inhibiting p53 Function.

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As “Guardian of the Genome”, the p53 tumour suppressor plays a major role in determining cell fate. Mutant forms of p53 are commonly seen in cancer, and can drive tumour development through gain of function activities. The wild-type state of p53 is responsible for chemotherapy-associated side effects. Agents modulating p53 function are therefore of significant interest. We have used yeast-display to select for Avian Pancreatic Peptide (APP) derivatives that bind specifically to p53. The lead selectant shows on-target association with p53 as measured by pull-down assays, both *in vitro* and in cells. Furthermore, it is able to inhibit the activity of wild-type p53 in cells. This peptide is a useful tool for disrupting p53 function and is currently being further engineered to enhance its function.

Microbial Platform to Synthesize Valuable Bulk Chemicals in Escherichia coli.

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A synthetic metabol*ic* pathway suitable for the production of chorismate derivatives was designed in *Escherichia coli*. An L-phenylalanine-overproducing *E. coli* strain was engineered to enhance the availability of phosphoenolpyruvate (PEP), which is a key precursor in the biosynthesis of aromatic compounds in microbes. Two major reactions converting PEP to pyruvate were inactivated. Using this modified *E.coli* as a base strain, we tested our system by carrying out the production of salicylate, a high-demand aromatic chemical. The titer of salicylate reached 11.5 g/L in batch culture after 48 h cultivation in a 1-liter jar fermentor, and the yield from glucose as the sole carbon source exceeded 40% (mol/mol). In this test case, we found that pyruvate was synthesized primarily via salicylate formation and the reaction converting oxaloacetate to pyruvate. In order to demonstrate the generality of our designed strain, we employed this platform for the production of each of 7 different chorismate derivatives. Each of these industrially important chemicals was successfully produced to levels of 1-3 g/L in test tube-scale culture. In addition, by extending chorismate pathway, we successfully achieved maleate production,

which is one of significant dicarboxylic acids as well as succinate and malate. A novel synthetic pathway of maleate was constructed in our base strain, and the production reached 7.1 g/L. This is the first report about maleate production using genetically engineered micro-organisms.

Differences in Loop Motions and Dynamics in the Catalytic Domains of GH Family 19 Chitinases Revealed By MD Simulation.

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Plants produce GH family 19 chitinases as a means of defence against invading fungal pathogens. This family of chitinases has been identified and characterized in a few bacterial species including *Streptomyces griseus* and *Streptomyces coelicolor*. Reports have shown that bacterial chitinases require a chitin binding domain for their inhibitory properties against fungi while their plant counterparts in classes I and II of GH family 19, do not require same. Here, we have identified some distinct loop motions in a 20 ns molecular dynamics simulation that suggests that some conformational changes may be triggered by loops in the catalytic domain of plant chitinases which may be essential for recognition and binding to complex or crystalline chitin substrates such as is present in fungal cell walls. In the bacterial chitinases however, the catalytic domain undergoes fewer flexible movements. We propose that such minimal flexibility which might be optimal for binding to chitooligosaccharides as revealed in previous reports, is not sufficient for binding to crystalline chitin. Currently, studies are underway to experiment the influence of redesigning the catalytic domain of *Streptomyces griseus* chitinase C (ChiC) towards enhancing flexibility in the catalytic domain and subsequently improving its hydrolytic and antifungal properties.

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