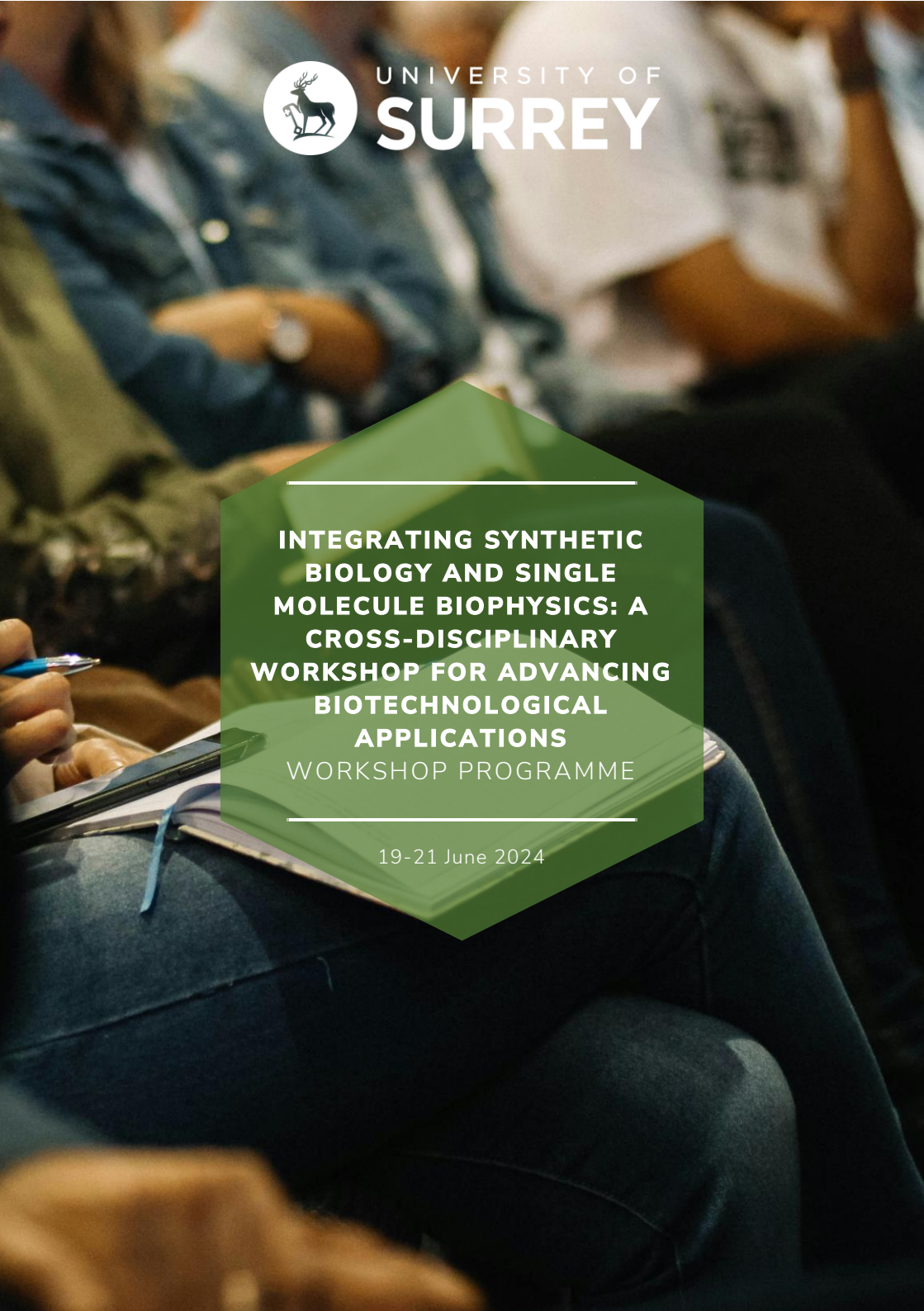

UNIVERSITY OF SURREY

INTEGRATING SYNTHETIC BIOLOGY AND SINGLE MOLECULE BIOPHYSICS: A CROSS-DISCIPLINARY WORKSHOP FOR ADVANCING BIOTECHNOLOGICAL APPLICATIONS

WORKSHOP PROGRAMME

19-21 June 2024

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INTRODUCTION

With recent international efforts on reducing carbon emissions, the transition to green industry is required more than ever. Synthetic biology is a relatively new field, where existing biological parts are reconfigured to perform new functions by taking engineering approach. It has the potential to transform current industrial procedures and reduce carbon emissions. For example, synthetic biology could be used to develop new procedures in making fuel, food, and everyday materials with low carbon footprint. However, the performances of newly designed synthetic circuits are difficult to predict. To realise the full potential of synthetic biology, we need to develop better ways to predict the performance of synthetic genetic circuits.

Single molecule biophysics allows us to study the behaviour of individual molecules with a goal of better understanding biological systems. While single molecule biophysics been often used with the combination of genome engineering, we believe the field can be greatly enhanced by taking key concepts in synthetic biology such as high throughput experiments or machine-learning based approaches. By bringing world-leading experts in single molecule biophysics and synthetic biology together, we aim to develop breakthrough cross-disciplinary research ideas that can advance both fields.

Organising committee:
Dr Wooli Bae, University of Surrey
Dr Cherlhyun Jeong, KIST, Korea and
Professor Won-Ki Cho, KAIST, Korea

Administrative support:
Ms Louise Jones, Institute of Advanced Studies, Ms Natalia Romero Diaz, University of Surrey and Mrs Samia Bakhtawar, University of Surrey

PROGRAMME

DAY 1 – WEDNESDAY 19 JUNE

Innovation for Health Building, Room 02 IFH 01

(BST)	
08.00 – 09.00	Registration & Morning Refreshments
09.00 – 09.30	Welcome
09.30 – 10.30	**Keynote Speaker: Synthetic Biology** - Dr Rodrigo Ledesma Amaro, Imperial College London
10.30 – 11.00	Coffee Break
11.00 – 12.30	**Session 1-1: Synthetic Biology 1** - Professor Hiroki Ueda, University of Tokyo - Professor John McCarthy, University of Warwick - Flash Talks
12.30 – 14.00	Group Photo & Lunch
14.00 – 15.30	**Poster Session 1: Synthetic Biology**
14.30 – 15.00	Coffee Break
15.30 – 17.30	**Session 1-2: In Vitro Synthetic Biology** - Dr Yuval Elani, Imperial College London - Dr Tomislav Plesa, University of Cambridge - Dr Vahid Shahrezaei, Imperial College London - Professor Jung-Hyun Na, Sungshin Women's University
17.30 – 18.30	Break
From 18.30	Dinner at the White House, Guildford

DAY 2 – THURSDAY 20 JUNE

Innovation for Health Building, Room 02 IFH 01

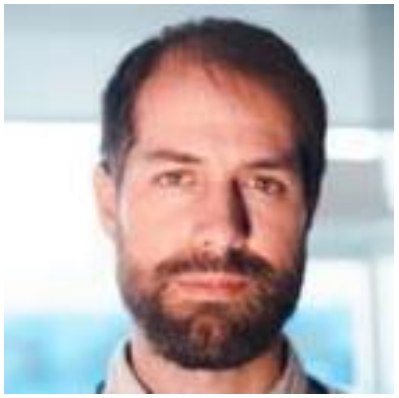
(BST)	
08.30 – 09.30	**Keynote Speaker: Single Molecule** - Professor Jong-Bong Lee, Postech
09.30 – 10.30	**Session 2-1: Single Molecule 1** - Professor Jae-Hyung Jeon, Postech - Professor Min Ju Shon, Postech
10.30 – 11.00	Coffee Break
11.00 – 12.00	**Session 2-2: Single Molecule 2** - Professor Inwha Baek, Kyunghee University - Professor Won-Ki Cho, KAIST
12.00 – 14.00	Lunch, **Poster Session 2: Single Molecule** & Coffee Break
From 14.00	Excursion and Dinner Including Poster Award and Closing Remark

DAY 3 – FRIDAY 21 JUNE

Alan Turing Building, Room 30 BB 03

(BST)	
09.00 – 10.30	Optional Event for Early Career Researchers Including University Tour

INVITED SPEAKERS



Dr Rodrigo Ledesma Amaro

Rodrigo Ledesma-Amaro, a renowned researcher, obtained his PhD from the University of Salamanca, focusing on the systems metabolic engineering of A. gossypii for producing vitamins, nucleosides, and lipids. His work has produced numerous research papers and industrial patents, including those used by BASF. After his PhD, he moved to France and conducted his postdoctoral research in the oleaginous yeast Yarrowia lipolytica, focusing on producing compounds, using low-cost carbon sources, and facilitating product recovery. Rodrigo has experience in organizing conferences, teaching courses, and supervising students and researchers.

Professor Hiroki R. Ueda

Hiroki R. Ueda graduated from the Faculty of Medicine, the University of Tokyo in 2000, and obtained his Ph.D. in 2004 from the same university. He was appointed as a team leader in RIKEN in 2003. He became a full professor at the Graduate School of Medicine, the University of Tokyo in 2013. He is also currently appointed as a team leader in the RIKEN Centre for Biosystems Dynamics Research (BDR), and an affiliate professor in the Graduate School of Information Science and Technology at the University of Tokyo and Osaka and Tokushima University. In 2016, he found the first sleep-promoting kinases, CaMKIIalpha and CaMKIIbeta, and proposed the phosphorylation hypothesis of sleep that phosphorylation-dependent regulation of Ca2+-dependent hyperpolarization pathway underlies the regulation of sleep homeostasis in mammals. In 2018, he also found the first essential genes of REM sleep, muscarinic receptors M1 and M3. To accelerate these studies, he also invented whole-brain and whole-body clearing and imaging methods called CUBIC as well as the next-generation mammalian genetics such as Triple-CRISPR and ES-mice methods for one-step production and analysis of KO and KI mice without crossing.

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Professor John McCarthy

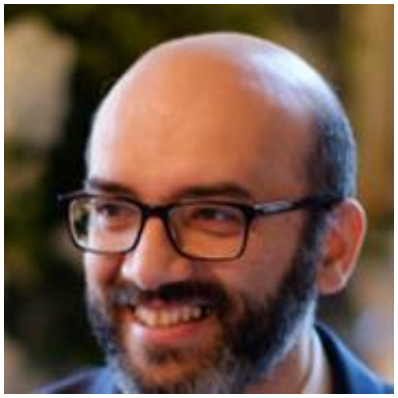
John McCarthy has held various positions, including director at Warwick Integrative Synthetic Biology Centre, head of Life Sciences School, BBSRC Professorial Research Fellow, chair of JIC Governing Council, and founding associate editor of OUP Integrative Biology journal. The McCarthy group specializes in molecular systems biology of posttranscriptional gene expression, studying the structural and behavioural features of key molecular components in cellular machinery involved in mRNA translation and degradation. They have developed new tools for studying RNA-protein interactions, fluorescence techniques, and gene expression stochasticity. Their current projects focus on gene expression noise, metabolic pathways optimization, and bioengineering of next-generation posttranscriptional devices and circuitry.

Dr Yuval Elani

Yuval Elani is a UKRI Future Leaders Fellow and Senior Lecturer in the Department of Chemical Engineering at Imperial College London. hE co-Directs the membrane biophysics platform and founder and Co-Director of the fabriCELL centre for synthetic cell research. Yuval studied Natural Sciences as an undergraduate (Cambridge, 2009) followed by a PhD in the Institute of Chemical Biology (Chemistry, Imperial College, 2015). After his PhD he held a series of fellowships working on various topics in biochemical engineering. He leads a diverse group of c. 25 researchers working on frontier research in applied biotechnology, including soft matter particle manufacture, microfluidics, bio-membrane engineering and synthetic biology.

Dr Tomislav Plesa

Dr Tomislav Plesa's research includes analysing, characterizing, and applying mathematical models in the context of biology. Tomislav is particularly interested in synthetic biology - an interdisciplinary field of science that aims to design biochemical systems that behave in a desired manner. From a biological perspective, his work is focused on elucidating the design principles of living systems and investigating some of the major challenges that are faced when biochemical systems are designed, controlled, and experimentally implemented. From a mathematical perspective, he focuses on constructing deterministic and stochastic dynamical systems that can be realized using biological substrates. He completed his doctoral research in the Mathematical Institute at the University of Oxford in 2018, and postdoctoral research in the Department of Bioengineering at Imperial College London in 2021, before joining Peterhouse as a lecturer and fellow.



Dr Vahid Shahrezaei

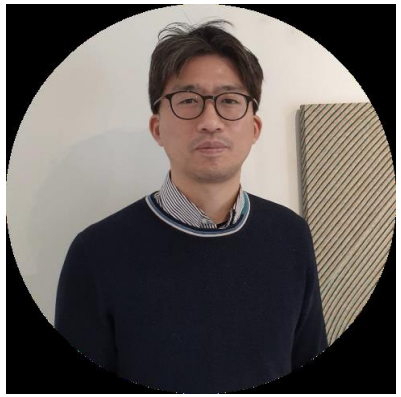
Dr Vahid Shahrezaei is a Reader in Biomathematics. Before joining Imperial he was a Postdoctoral Fellow in the Center for Nonlinear Dynamics at McGill University in Montreal. He did his PhD at Simon Fraser University, Vancouver. He is spending a sabbatical year at the Crick Institute in 2023-2024. The main area of Dr Shahrezaei's research is Computational Molecular Systems Biology. He is interested in studying design principles that enable cells to function robustly, in spite of significant inherent stochasticity and environmental noise. To this end, a combination of analytical and computational methods is used to investigate the temporal, spatial and stochastic dynamics of biochemical networks. In recent years Shahrezaei's group has developed methods for the analysis of single-cell RNA-sequencing data and simulation-based inference methods for biochemical networks.

Assistant Professor Jung-Hyun Na

Dr Na's career reflects a relentless pursuit of harnessing antibodies for drug development. During his Master program, he isolated highly specific antibodies for explosive detection. This work played a key role in the development of an innovative explosive biosensor. His PhD research explored a novel E.coli based protein expression system for the ligand-gated ion channel, serotonin receptor 3A. paving the way for more efficient protein research. Recognizing the importance of protein structure, Dr Na delved into structural biology, mastering X-ray crystallography and protein modeling. This expertise led to a breakthrough: resolving the first structure of an archaeal-like chaperonin, a discovery published in Nature Communications. Transitioning to a Principal Investigator, Dr Na's success continued. He secured a £2 million licensing deal for an antibody targeting a cancer-linked receptor, showcasing the commercial potential of his research. His impactful contributions were further recognized by the 4th Korea Biopharmaceutical Award.

Professor Jong-Bong Lee

Professor Jong-Bong Lee completed his Ph.D. in Physics from Brandeis University, MA in 2004; MS in Physics from Sogang University, Korea in 1996; BS in Physics and Mathematics in 1993. He has held various positions, including Visiting Professor at Rice University, Head of IBIO at POSTECH, and Chair of Division of Biological Physics at the Korean Physical Society. They have also served on the National Research Foundation of Korea and Harvard Medical School.



Assistant Professor Jae-Hyung Jeon

Jae-Hyung Jeon is currently an associate professor at the Department of Physics, Pohang University of Science and Technology, South Korea, and the executive director of the Asia Pacific Center for Theoretical Physics. Previously, he was the KIAS assistant professor at the Korea Institute for Advanced Study (2014--2016) and had postdoc experiences at the Technical University of Munich & Tampere University of Technology from 2008 to 2014.

Assistant Professor Min Ju Shon

Dr Min Ju Shon is an Assistant Professor at POSTECH, specializing in biophysical techniques for nanoscale measurements at the single-molecule level. He completed his bachelor's degree in Chemistry at Seoul National University, followed by a Ph.D. in Physical Chemistry from Harvard University in 2014. His doctoral research focused on developing and applying new techniques for trapping and manipulating single biomolecules. After earning his doctorate, Dr. Shon held postdoctoral positions at KAIST and Seoul National University, where he further developed his expertise in biophysical tools and biochemical methods, notably utilizing high-resolution single-molecule imaging and magnetic tweezers. In 2020, he joined POSTECH as an Assistant Professor in the Department of Physics and the School of Interdisciplinary Bioscience and Bioengineering. His current research focuses on studying protein mechanics in synaptic systems and probing interactions between membrane proteins.

Professor Inwha Baek

Professor Inwha Baek has a diverse academic background, including a Ph.D. in Biological and Biomedical Sciences from Harvard University, a M.S. in Biochemistry from Seoul National University, a B.S. in Pharmacy from South Korea, and a postdoctoral fellowship from Rockefeller University in New York. They have also received scholarships and full-tuition scholarships for their academic pursuits. She is an Assistant Professor at Kyung Hee University, South Korea, College. of Pharmacy since 2023.

Assistant Professor Won-Ki Cho

Dr Won-Ki Cho is currently an associate professor in Department of Biological Sciences at Korea Advanced Institute of Science and Technology (KAIST). Won-Ki has studied eukaryotic transcription using super-resolution imaging techniques during post-doc at MIT. Won-Ki's major research interest is understanding principles of chromatin organization and collective behaviours of gene expression regulatory components using fluorescence microscopy techniques in living cells.

ABSTRACTS

Keynote Speaker: Synthetic Biology: Sustainable Bioproduction in Yeast: Engineering Metabolism in Microbial Cells and Microbial Production

Rodrigo Ledesma Amaro

Metabolic engineering allows us to manipulate microorganisms to produce chemicals, fuels, food, materials, and pharmaceuticals in a more sustainable way. Yeasts are considered ideal hosts for production at scale due to their robustness and ease of engineering. In this talk, I will cover the recent advances in synthetic biology to produce molecules of interest, with particular emphasis on food and food ingredients. Some of these tools include overcoming heterogeneity problems using single-cell studies. Other solutions are related to the creation of synthetic microbial communities and harnessing the division of labour to achieve more efficient production.

Whole-Body/Organ Imaging with Single-Cell Resolution by Tissue-Clearing Methods CUBIC

Hiroki R. Ueda

State-of-the-art tissue clearing methods provide unprecedented, high-quality optical access to very large biological specimens from individual organs to entire intact animals. When combined with light-sheet microscopy and image informatics, currently available tissue-clearing methods including a hydrophilic tissue-clearing method CUBIC, allow us to perform whole-brain and whole-body imaging with sub-cellular resolution. In addition, the dilapidation of biological specimens by tissue-clearing methods enables whole-organ staining with specific antibodies, which can be applied even to human biology and pathology. When combined with tissue clearing, these labelling methods allow us to systematically extract structural and functional information of complex mammalian organs. In this talk, I will discuss how tissue-clearing and its related technologies have been successfully integrated to create new biological insights and provide a perspective for future opportunities in organism-level synthetic biology and systems biology.

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Multi-Level Generation of Gene Expression Stochasticity

John McCarthy

It has been estimated that >76% of the total cellular energy budget in exponentially growing Saccharomyces cerevisiae is dedicated to protein synthesis which, in turn, is closely linked to growth capacity and thus organism competitiveness. Indeed, it is reasonable to assume that gene expression pathways in this yeast and its ancestral lineage would have been subject to strong evolutionary pressures over 100s of millions of years. Stochasticity in these pathways, and cellular metabolism overall, can be expected to exert significant influence over the (quantitative) interrelationships between selective forces and system evolution. However, our understanding of the underpinning mechanistic principles is limited. Much of the research on gene expression noise, and the resulting cell-to-cell heterogeneity, has focused on the transcription process, whereby it has been widely assumed that translation kinetics affect primarily/solely the amplitude of transcriptional bursts, rather than constituting a separate source of noise. In contrast, we have extended our research to include the characterization of noise-generating post-transcriptional processes. This work has yielded several novel findings, including evidence that the unwinding/folding of mRNA structure can generate translational stochasticity, ribosomal scanning stochasticity can create cell-to-cell heterogeneity in a nutritional stress response mechanism, and limiting translation capacity constrains the degree to which aging can increase gene expression noise. This presentation will report how we have combined analytical and computational approaches to elucidate our observations in these areas and will also consider how we might address technical constraints that currently limit progress in this field.

Microfluidic and Bio-Membrane Technologies for the Design and Construction of Synthetic Cells

Yuval Elani

Synthetic cells (SynCells) are bioinspired micromachines constructed from molecular building blocks, mimicking the form and function of biological cells. They are increasingly used as both simplified cell models and engineered microdevices, offering broad applications in industrial and clinical biotechnology. Despite their

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promise, SynCells are structurally simplistic, primarily consisting of spherical liposomes, unlike their biological counterparts. Given that form and function are intertwined, this lack of architectural complexity restricts the development of sophisticated behaviours. In this talk, I will discuss our recent efforts to overcome these limitations by employing microfluidic assembly lines for SynCell production, enabling the creation of a wide repertoire of SynCell architectures. We harness this increased complexity to create a new generation of SynCells with biomimetic behaviours, most notably those capable of detecting external stimuli (including as temperature, light, and magnetic fields) and initiating a biochemical response. Additionally, we have recently expanded our toolkit to access the nano-regime, allowing us to construct nano-organelles for multi-stage release of different payloads at defined time points and to develop attolitre bio-reactors for in situ biochemical synthesis.

Systematic Design and Control of Chemical Systems

Tomislav Plesa

Problems arising in biology are often direct: given a chemical system, the task is to deduce some of its dynamical properties. Conversely, in the field of synthetic biology, and the sub-field of DNA computing in particular, the defining problems are inverse in nature [1]: given a dynamical property, the task is to design a chemical system that displays the property. Alternatively, given a chemical system, the task may also be to introduce additional molecular species to control properties of the original system. In this talk, I will present mathematical methods for systematic design and control of chemical systems. Focus will be placed on the methods suitable for well-mixed chemical systems containing some species in lower copy-numbers [2,3], when the dynamics is stochastic and modelled with the Gillespie stochastic simulation algorithm. I will demonstrate the mathematical methods by constructing relatively simple chemical systems displaying pre-defined dynamics and various noise-induced phenomena. Also presented will be a blueprint for an experimental implementation of some of the chemical systems via dynamic nucleic acid nanotechnology.

Bayesian Model Discovery for Reverse-Engineering Biochemical from Data

Vahid Shahrezaei

In systems and synthetic biology, we typically have mathematical models with uncertain model structure and parameters that need to be constrained by experimental data. These mathematical models do typically not have analytical likelihood functions available but can be simulated. I discuss in this talk, recent work from my group in the area of simulation-based inference parameter inference and model discovery applied to biochemical reaction networks.

Single Domain Antibodies and their Potential for Therapeutic Applications

Jung-Hung Na

The conventional view of antibodies as molecules consisting of heavy and light

Continued ►

chains was changed by the identification of heavy chain-only antibodies in the adaptive immune system of camelids and cartilaginous fish. This discovery has led to the development of a new category of antibody fragments known as single domain antibodies (sdAbs). Due to their inherent favourable properties, such as high affinity and specificity for cryptic epitopes, high physicochemical stability, high solubility, small size, and easy engineering, SdAbs are emerging as promising antigen-binding receptor candidates in various fields of research and medicine. In this talk, I will introduce the characteristics of sdAbs and their potential applications in therapeutic fields.

Keynote Speaker: Single Molecule: Unraveling the Intricacies of DNA Mismatch Repair: Insights from Single-Molecule Studies

Jong-Bong Lee

DNA mismatch repair (MMR) corrects DNA base-pairing errors that can occur during DNA replication and recombination. MMR catalyzes strand-specific DNA degradation and resynthesis by dynamic molecular coordination of sequential downstream pathways. The temporal and mechanistic order of molecular events is essential to ensure interactions in MMR over long distances on DNA. However, the detailed mechanism of MMR has been poorly understood. Real-time observation of individual biomolecules and their complex going through reactions enables us to observe transient intermediate states, multiple pathways, and the fluctuation of the molecular properties. Thus, the removal of ensemble averaging can elucidate the salient features of the molecular mechanism in MMR. Using a variety of cutting-edge single-molecule methods, we have successfully explored the stochastic orchestration of proteins involved in the reactions of DNA mismatch repair. I will present how we have developed and applied single-molecule techniques to reveal the undisclosed mechanism of MMR.

Hi-C-Based Modeling of Target Search of DNA-Binding Proteins: Theory and Application

Jae-Hyung Jeon

The target search problem of DNA-binding proteins is biologically relevant and a timely subject. In this talk, I briefly introduce the history of biological target search problems dating from the seminal paper by Riggs et al., J. Mol. Bio. (1970). After the short overview of the field, I present a new target search modeling and theoretical study that considers the information on the three-dimensional folding structure of DNA inside a nucleus. Our approach combines the first-passage theory with the Hi-C data. Our model enables us to calculate the mean target search time (first passage time) and mean recurrence time for DNA-binding proteins on specific DNA sites. It is revealed that the target search dynamics highly depend on the values of the DNA association and dissociation constants. Surprisingly, the target search time exhibits two distinct behaviours depending on whether the Hi-C structure is relevant.Our theory of the recurrent search quantitatively explains the in vivo transcription rate profile for E. coli DNAs.

Single-Molecule Approaches to Study Membrane Protein Interactions

Min Ju Shon

The in vitro investigation of membrane proteins requires meticulous reconstitution of the lipid bilayer environment to preserve the proteins' native structure and function. In this context, I will present our recent efforts to develop a versatile platform for analysing membrane proteins and their interactions, particularly at the single-molecule level. Utilizing quantitative fluorescence-based imaging, we assessed interactions between proteins and membrane-bound vesicles, revealing pronounced cooperativity among membrane proteins in recruiting lipid vesicles from diverse origins. Additionally, we observed that some membrane-bound proteins can form molecular condensates, indicating their potential to act as nanoscale hotspots for eliciting biochemical responses. Then, to further study membrane protein interactions while maintaining their lateral diffusion, I will introduce a new free-standing lipid bilayer system as a platform for observing these interactions. Using SNARE proteins, which mediate membrane fusion, as a model system, we demonstrate the effectiveness of this approach in providing deeper insights into membrane protein dynamics.

Single-Molecule Imaging of RNA Polymerase II Transcription

Inhwa Baek

In eukaryotes, RNA polymerase II (Pol II) transcribes messenger RNAs (mRNAs) and noncoding RNAs. For Pol II to successfully synthesize an RNA transcript, transcription initiation, elongation, and termination factors need to be recruited to and released from Pol II in a timely manner. However, the association and dissociation kinetics of these factors are poorly characterized partly because past Pol II transcription studies utilized ensemble assays, which can only reveal the averaged behaviour of individual molecules. To investigate the temporal dynamics of Pol II transcription, in vitro multi-wavelength single-molecule fluorescence microscopy was implemented. Our single-molecule microscopy experiments reveal several unexpected features. During transcription activator-dependent Pol II transcription, initial recruitment of Pol II and some general transcription factors, TFIIE and TFIIF, does not require the core promoter. Instead, they first interact with enhancer-bound activators, with dwell times on the order of a few seconds. More interestingly, multiple Pol II complexes can simultaneously bind a single enhancer, creating a localized Pol II cluster. This Pol II cluster could potentially explain the mechanism underlying transcription bursting. In contrast to other factors, TFIIH association with DNA is dependent on the core promoter. In sum, our kinetic measurements lead to a new branched model for activator dependent Pol II preinitiation complex assembly that has so far been masked by ensemble assay.

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Topological Formation of Transcriptional Condensates Relative to Subnuclear Compartments

Won- Ki Cho

Mammalian genome is organized into a hierarchical structure at multiple scales in a cell nucleus. At the most macroscopic scale, individual chromosomes are segregated into own separated intranuclear regions, which is so called chromosome territories. On the other hand, recent intensive studies using live-cell imaging techniques have characterized behaviors of subnuclear compartments, exemplified with transcriptional condensates and nuclear speckles. They are functionally specialized non-membrane bound organelles, mostly follow liquid properties. However, how the genome structure and subnuclear organelles are spatially organized in the nucleus has not been elucidated. We found that chromatin looping by CTCF, a chromatin architectural protein, acts as an architectural prerequisite for the assembly of phase-separated transcriptional condensates. Moreover, we observed a layered structure of transcriptional and splicing condensates near the nuclear matrix discovered by super-resolution imaging of RNA polymerase II, nuclear speckles, and Scaffold attachment factor A (SAF-A, a nuclear matrix associated proteins).

POSTER PRESENTATIONS

Microfluidic Technologies for Artificial Cell Membrane Engineering

Marcus Fletcher

Engineering biological devices that can emulate capabilities of living cells from molecular building blocks is a longstanding goal of synthetic biology. In recent years great progress has been made in recapitulating sophisticated cytosolic processes from purified components, such as the ability to regulate protein synthesis. In contrast, artificial cell membranes lag behind in their capabilities due to a poor understanding of how to engineer membrane complexity, for example, by introducing functional membrane proteins. Among the many outstanding challenges, one is the lack of precision techniques to systematically explore the multidimensional spaces of membrane composition parameters like lipid content. In consequence, relationships between critical membrane processes, e.g. protein binding and inserting, and lipid composition are poorly characterized. To alleviate this need, we present the development of suite of microfluidic tools to build, manipulate artificial cells with different membrane composition and quantify their biophysical properties at the single artificial cell level. Advances in our microfluidic tool-kit includes devices for on-the-fly variation of artificial cell lipid composition and multiplexed perfusion over immobilized artificial cell

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populations. Furthermore, we add to our compositional probes by developing a DNA based optical sensor for assaying membrane surface charge.

Developing the Principles for Designing Custom Genomes Using the Yeast Minimal Cell Cycle

Anastasiya Malyshava

The eukaryotic cell cycle, a pivotal biological process, has been extensively studied and mathematically modelled in recent decades. Despite concerted efforts, identifying the minimal gene set essential for orderly cell cycle progression remains elusive. Synthetic biology, renowned for genetic engineering applications, also provides a pathway for addressing fundamental biological queries through "learning from building." The Synthetic Yeast Genome (Sc2.0) project exemplifies this by synthesising Saccharomyces cerevisiae's genome with changes that advance our understanding of eukaryotic genomes. Expanding from Sc2.0's groundwork, we aim to pioneer synthetic yeast genomes that are minimal, modular, and reprogrammable. As a proof-of-concept, we constructed a synthetic genome module housing nine of the key cell cycle genes. Employing CRISPR, we systematically deleted these genes from their native loci and reinserted them together as a synthetic gene cluster. While individually non-essential, the combined absence of all nine genes renders this synthetic module indispensable. Through Cre/loxP-mediated recombination, we investigated the gene combinations necessary for yeast cell cycle progression. Cre recombinase facilitated targeted gene deletions between intergenic loxP sites within the module, and rapidly generated diverse strains with combinatorial cluster deletion profiles, covering all potential combinations. Using flow cytometry sorting, we developed a way to isolate hundreds of viable deletion combinations and developed the Pool of Long Amplified Reads (POLAR) sequencing technique to enable the analysis of gene deletion frequency and gene content combinations for hundreds of strains with different cell cycle modules. These experimental findings were compared to computational models of the cell cycle and get us closer to understanding the minimal gene content for this function. Upon pioneering this work, we now envisage a future where genome designers can predict gene sets necessary for specialised tasks and can then synthetically arrange these genes on chromosomes and design intergenic regions to regulate their gene expression appropriately.

Tracing tracrRNA: Unveiling its Crucial Role in CRISPR/Cas9 Mechanisms

Jeongmin Lee

The trans-activating CRISPR RNA (tracrRNA) is fundamental to the CRISPR/Cas9 system, forming guide RNA with crRNA. Despite its known importance in crRNA maturation and Cas9 RNP-mediated DNA cleavage, the exact function of tracrRNA scaffolds remains unclear. In this investigation, we generated five tracrRNA variants by removing specific scaffolds, including Stem loops 1, 2, and 3, and the Linker. Using a new single-molecule assay, we directly observed target binding and cleavage processes guided by Cas9 RNP.

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Our findings underscore the vital role of the Linker in initiating R-loops and highlight the significance of Stem loop 2 in identifying PAM-distal mismatches within target DNA. Furthermore, we explored cleavage efficiency by adding tracrRNA segments, indicating that maintaining the integrity of Stem loops 2 and 3 is crucial for potent Cas9 activity. We believe that these results deepen our understanding of Cas9 functionality and offer insights into its detailed mechanism from target binding to cleavage.

Exploring the Biophysics of Tau–DNA Interactions at the Single-Molecule Level

Celine Park

Tau, known primarily as a microtubule-binding protein, is also found in the nucleus where it binds to DNA. Recent investigations have focused on its role in stabilizing DNA and chromosomes, but the biophysical understanding of its molecular mechanisms, particularly regarding tau's phase separation properties, remains limited. In this study, we used in vitro single-molecule assays to show that tau interacts with DNA to form co-condensates, significantly altering the mechanical properties of DNA. Our findings indicate that tau can wet the DNA strand in low-salt conditions, effectively condensing and stiffening the DNA. At high concentrations, tau also forms droplet-shaped condensates on DNA, like its interaction with microtubules. Notably, these condensates are mobile and may act as nucleation sites for microtubule growth. This study reveals previously unknown effects of Tau-DNA condensation and suggests that these interactions could influence microtubule dynamics during mitosis.

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