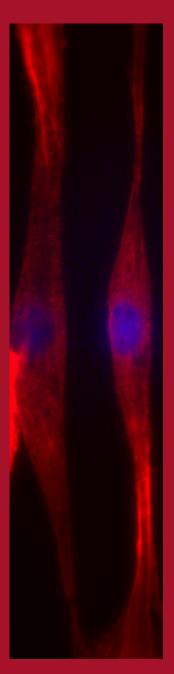
IUPAB / ABA BIOPHYSICS SATELLITE MEETING 2025

Pioneering Biophysical Tools and Structural, Single-Molecule, and Atomic Biophysics in South-East Asia

19-20 July 2025 | Kuala Lumpur, Malaysia





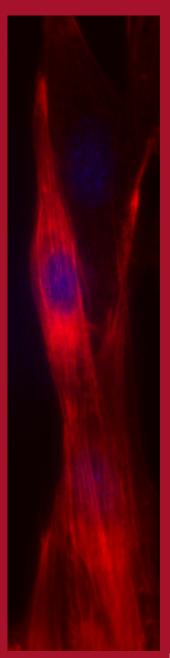












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Cover Image Credit:
Microscopy images by Mohd Khairul Akma bin Darwis, MSc candidate at Universiti Sains Malaysia. REF52-Fucci cells (purple) on circular micropatterned islands. C2C12 cells (red, actin cytoskeleton) aligned on 0° ridge topography. Images were acquired at the Max Planck Institute for Medical Research and supported by the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 872869.

WELCOME MESSAGE

Welcome to you all at this Biophysics satellite meeting to the IUPAC congress in Kuala Lumpur this last week. The meeting has been sponsored jointly by the International Union of Pure and Applied Biophysics (IUPAB.ORG), and the Asian Biophysics Association (ABA), with the intention of continuing the mission of IUPAB to facilitate the enlargement of the global biophysics' community, and to facilitate future interactions between to various countries across South-East Asia.

Previously, IUPAB has facilitated the establishment of the Latin American Federation of Biophysics (LAFEBS) and more recently since 2021, the Society of African Biophysical Societies (SABS), with more than 12 new groups or societies across the African continent. The Asian Biophysics Association is under the new Presidency of Taka Nishizaka, who, with Ruchi Anand and Martina Havenith are also IUPAB Councillors and are all here at the meeting - please do talk to them. ABA and IUPAB are seeking to widen still further the community in South-East Asia and beyond. Both senior researchers and early career scientists, interested and practising biophysics, can gain much from joint projects, mobility of researchers and common use of facilities and instrumentation, with the prime aim of pushing forward the frontiers of this fascinating cross-, multi- and transdisciplinary area of science.

Representatives from more than 10 nations are here, and some have newly established societies, such as Vietnam in 2024, and some are welcomed as returning to IUPAB in 2024 as adhering societies such as Korea and Singapore. Of course, the strong and wellestablished societies of Japan, India and Australia are thriving and give superb models for biophysical societies and, in many cases, the destination for young researchers to gain experience and further their biophysics interests.

We very much hope that you all, students and faculty alike who are based in this fascinating part of the world, will find new opportunities to engage with the global community of biophysics in whatever way is appropriate. We hope that this meeting, over two days, will be the kick-start to future meetings, personnel exchanges and joint projects. IUPAB is here to facilitate and help any cross-border activity that is appropriate, and we look forward to promoting more activities in the future and bringing a major IUPAB congress back to the region very soon.

Anthony Watts, on behalf of IUPAB



Past-President



President



Manuel Prieto Anthony Watts Angela Gronenborn President-elect



Ron Clarke Secretary



Christina Sizun Treasurer

ORGANISING & SCIENTIFIC COMMITTEES

Organising Committee

Chair (International): Martina Havenith-Newen, Ph.D.

International Union for Pure and Applied Biophysics (IUPAB) Councillor Professor, Physical Chemistry, Ruhr University Bochum, Germany.

Co-Chair (International): Takayuki Nishizaka, Ph.D.

President, Asian Biophysics Association (ABA);

IUPAB Councillor;

Professor, Department of Physics, Faculty of Science,

Gakushuin University, Japan.

Chair (Local): Siti Hawa Ngalim, Ph.D.

Senior Lecturer, Department of Biomedical Science, Advanced Medical and Dental Institute, Universiti Sains Malaysia, Malaysia.

International Scientific Committee

Frances Separovic, Ph.D.

Professor Emeritus of Biophysical Chemistry The University of Melbourne, Australia

Peter Török, Ph.D.

Professor of Optical Physics Nanyang Technological University, Singapore

Winston Zhao Ziqing, Ph.D.

Assistant Professor of Biophysics National University of Singapore, Singapore

Anthony Watts, Ph.D.

Professor of Biochemistry University of Oxford, United Kingdom

Chalermpol Kanchanawarin, Ph.D.

Associate Professor of Biophysics Suranaree University of Technology, Thailand

Jihye Seong, Ph.D.

Professor of Biophysics Ewha Womans University, Korea

Huy Quang Tran, Ph.D.

Senior Researcher in Biophysics Vietnam Academy of Science and Technology, Vietnam

Nei-Li Chan, Ph.D.

Professor of Structural Biology National Taiwan University, Taiwan

Local Scientific Committee

Siti Hawa Ngalim, Ph.D.,

Senior Lecturer in Nanomedicine Universiti Sains Malaysia

Che Azurahanim Che Abdullah, Ph.D.

Assoc. Prof. in Bionanotechnology Universiti Putra Malaysia

Nusakinah Suardi, Ph.D.

Associate Professor in Medical Physics Universiti Sains Malaysia

Normi Mohd Yahaya, Ph.D.

Assoc. Prof. in Molecular and Structural Biology Universiti Putra Malaysia

Local Student Committee

Mohd Khairul Akma Bin Darwis

Master's degree student Universiti Sains Malaysia

Zenab Aldurrah

Doctorate student Universiti Sains Malaysia

Sunthara Murthi A/L Anamalai

Doctorate student Universiti Sains Malaysia

PROGRAMME INFORMATION

The Biophysics Satellite Meeting aims to highlight the vibrant biophysics research taking place across South-East Asia. Researchers representing biophysics communities from eight countries in the region will deliver lectures on their work and serve as ambassadors to foster networking, research collaborations, researcher mobility, and the planning of future regional conferences.

This satellite meeting is supported by IUPAB (iupab.org) and ABA (asian-bp.org), building on ABA's previous activities and helping to encourage the establishment of new biophysics societies and communities. In attendance are three IUPAB Councillors and the President of ABA.

PROGRAMME AT-A-GLANCE

Day 1: Saturday, 19 July 2025

1330	Registration open, USM@KL
1400 - 1620	Session 1 - Theme: Novel Tools in Biophysics
1620	Refreshment Break
1630 - 1720	Flash Poster Presentations (1-2 slides; 2 mins/talk) Session Chairs: Martina Havenith, Prof. Ruchi Anand
1720 - 1830	Mixer at Poster Session
1830	From USM@KL to the Dinner Venue at Bronx V, Level 14, Berjaya Hotel Times Square Hotel
1900 - 2100	Dinner
2100	Leave & Disembark at Suria, KLCC

Day 2: Sunday, 20 July 2025

0900 - 1220	Session 2 - Theme: Structural biology
1220	Light Lunch
1330 - 1530	Session 3 - Theme: Single Molecule and Atomistic Biophysics
1530	Refreshment break
1540 - 1620	General Discussion with Participants: Way Forward and Future Planning for Biophysics in South-East Asia
1620	Best Poster Award
1630	Closing Remark

PROGRAMME (Day 1: Saturday, 19 July 2025 - afternoon)

-	
1330	Registration open Venue: Level 20, USM@KL
1400	 Opening Remarks Anthony Watts (President, IUPAB) Takayuki Nishizaka (President ABA; IUPAB Councillor) Siti Hawa Ngalim (Chair, Local Organizing Committee)
	Session 1 - Theme: Novel Tools in Biophysics Session Chair: Ruchi Anand, India
1420	Announcing the Oscar for Best Supporting Actor: Water! Invited Speaker 1: Martina Havenith, Germany
1455	Direct Visualization of Dynamics in Archaeal and Bacterial Motility Machinery Invited Speaker 2: Takayuki Nishizaka, Japan
1530	Cell Adhesion and Signal Crosstalk as Tunable Tools: Bridging Biophysics and Bone Health Invited Speaker 3: Siti Hawa Ngalim, Malaysia
1605	Rational Design and Computational Screening of Peptide Inhibitors Targeting BLEG-1, an Evolutionary Divergent B3 Metallo-β-lactamase Selected Short Talk 1: Muhammad Hakimi Aqil bin Mohd Hussin, Malaysia
1620	Refreshment Break
1630	Flash Poster Presentations (1-2 slides; 2 mins/talk) Session Chairs: Martina Havenith, Ruchi Anand
	Novel tools
	Fluorescent Biosensing on the Move Flash Talk 1/ Poster P-01: Chittanon Buranachai, Thailand
	Penetration Assessment of 810 nm Light for Transcranial Photobiomodulation Using In-Vitro Models Flash Talk 2/ Poster P-02: Nursakinah Binti Suardi, Malaysia
	Development of the Scaffold's Mechanical Properties

Lipid Bilayer Order Profile as a Representation of Pressure Balance: Bilayer Response to Solvation of an Amphiphilic Solute Flash Talk 4/ Poster P-04: Adriana Sturcova, Czech Republic

Flash Talk 3/ Poster P-03: Witchukorn Phuthong, Thailand

Investigation Method

PROGRAMME (Day 1: Saturday, 19 July 2025 - afternoon, cont'd.)

Dynamic Regulation of Protein Liquid-Liquid Phase Separation by Gold Nano-Butterfly

Flash Talk 5/ Poster P-05: Tomohiro Nobeyama, Japan

Bioinformatic Analysis of Mesenchymal Stem Cell Derived-Exosome Gene Cargo in Wound Healing

Flash Talk 6/ Poster P-06: Karthikeyan Linu Mithran, Malaysia

Exploring the ceRNA network for key biomarkers in Epstein-Barr virus-associated nasopharyngeal carcinoma cell line (C666) Flash Talk 7/ Poster P-07: Navaneetha Krishnan Kottai Rajan, Malaysia

Silent Strokes: A Quantitative Investigation on How Small Vessel Disease Rewires the Aging Brain

Flash Talk 8/ Poster P-08: Niraj Kumar Gupta, India

Engineering BMP-7 Peptides for Surface Functionalization of Stainless-Steel Implants: A Biophysical Approach to Bone Regeneration

Flash Talk 9/ Poster P-09: Sunthara Murthi A/L Anamalai, Malaysia

Single Molecule and Atomistic

In Silico Evaluation of Novel Metformin Derivatives as α-Glucosidase Inhibitors: A Structure-Based Drug Design Approach Flash Talk 10/ Poster P-10: Nor Akmalyati Binti Sulong, Malaysia

Structural Biology

Discerning the Enzymatic Properties, Structural Modulations and Binding Affinities of a Promiscuous BLEG-1 B3 Metallo- β -Lactamase towards Chemically Distinct Substrates

Flash Talk 11/ Poster P-11: Normi Binti Mohd Yahaya, Malaysia

Exploring the biophysical and structural characteristics of membrane acid phosphatase (LdMAcP) for therapeutic development against Leishmaniasis

Flash Talk 12/ Poster P-12: Jyotisha, India

Effect of 532 nm, 0.1W Laser Irradiation on Zebrafish Larvae Caudal Fin Regeneration

Flash Talk 13/ Poster P-13: Najlaa' Ilani Fitri Binti Mohamad Khomizal, Malaysia

405 nm Laser Toxicity Assessment on the Viability and Development of Zebrafish Embryos

Flash Talk 14/ Poster P-14: Umairah Binti Mohd Zaki, Malaysia

PROGRAMME (Day 1: Saturday, 19 July 2025 - afternoon cont'd.)

Probing Phase Separation-Mediated Intranuclear Organization and Dynamics of Brg1 in Single Human Cells

Flash Talk 15/ Poster P-15: Ng Woei Shyuan, Singapore

1720 Mixer (with light refreshments) at Posters

1830 Dinner

- For all participants. Please bring your Meeting ID.
- Food for for participants with dietary restrictions (gluten-free, beef-free, and vegetarian) are available.
- Move together from USM@KL to the restaurant (~10 mins with shuttle van)

Venue: Bronx V, Level 14, Berjaya Hotel Times Square Hotel

2100 Event adjourn

- Leave together from the restaurant. Disembark at Suria@KLCC (~10 mins with shuttle van).
- Participants return to own accommodations nearby.

PROGRAMME (Day 2: Sunday, 20 July 2025 - morning)

	Session 2 - Theme: Structural biology Session Chair: Takayuki Nishizaka
0900	The Necessity of Complementary Techniques for Studying Complex Samples – Nucleic Acids, Membrane Systems, Bioactive Products Invited speaker 4: Alison Rodger, Australia
0935	Structural and Dynamic Impact of Inhibitors on Nucleic Acid- Binding Proteins Invited Speaker 5: Yu-Yuan Hsiao, Taiwan
1010	Refreshment break
1045	The structures and functions of bacterial pore-forming toxins from Bacillus thuringiensis Invited Speaker 6: Chalermpol Kanchanawarin, Thailand
1120	Ribosomal Methyltransferase Mediated Antibiotic Resistance Invited Speaker 7: Ruchi Anand, India
1205	Structural basis for lipid-regulated activation of vacuolar anion channel ALMT9 Selected Short talk 2: Sangho Lee, Korea
1220	Light lunch

PROGRAMME (Day 2: Sunday, 20 July 2025 - afternoon)

	Session 3 - Theme: Single Molecule and Atomistic Biophysics Chair : Siti Hawa Ngalim
1330	Visualizing, Quantifying and Mapping Chromatin-based Intranuclear Dynamics in Live Human Cells, One Molecule at a Time Invited Speaker 7: Winston Zhao, Singapore
1405	Single-Molecule Biophysics of Nucleic Acid Motor Proteins and Cellular Mechanobiology in Phagocytosis Invited Speaker 8: Gwang Rog Lee, Korea
1440	Exploring Atomic Conformational Dynamics and Actin-Crosslinking Function of Alpha- actinin Using High-Speed AFM, AFMfit, and MD Simulations (SimHS-AFMfit) Invited Speaker 9: Kien Xuan Ngo, Vietnam
1515	Stall force measurement of the kinesin-3 motor KIF1A using a programmable DNA origami nanospring Selected Short Talk 3: Kumiko Hayashi, Japan
1530	Refreshment break
1540	General Discussion with Participants: Way Forward and Future Planning for Biophysics in South-East Asia
	Discussion leads – • Takayuki Nishizaka (IUPAB/ABA) • Martina Havenith (IUPAB) • Ruchi Anand (IUPAB) • IUPAB Executives will be online
1620	Best Poster Award
1630	Closing Ceremony

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O-01

Rational Design and Computational Screening of Peptide Inhibitors Targeting BLEG-1, an Evolutionary Divergent B3 Metallo-\(\beta\)-lactamase

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The increasing prevalence of antimicrobial resistance (AMR) represents a major global health challenge particularly due to spread of metallo-β-lactamases (MBLs) particularly subclass B3 enzymes such as BLEG-1, where clinical inhibitors are scarce. MBLs belong to class B of β -lactamases enzyme that hydrolyze β -lactam antibiotics including carbapenems, rendering bacteria resistant to these drugs. They are primarily found in Gram-negative bacteria and require zinc ions for catalytic activity. BLEG-1 which is an evolutionary divergent MBL from Bacillus lehensis G1 has been biochemically validated as a functional B3 MBLs with ampicillin as a preferred substrate. In this study, the combination of experimental and computational approaches were used to identify and evaluate peptidebased inhibitors against BLEG-1. In vitro enzymatic assays showed that two designed peptides inhibited BLEG-1 activity by over 50% at submicromolar concentrations (IC₅₀ = 0.90 μ M and 0.50 μ M, respectively) with near complete inhibition at 10–20 μ M. Isothermal titration calorimetry (ITC) analyses indicated enhanced binding properties of the peptides when compared to ampicillin. The docked peptide-protein complexes revealed that Peptide 7.3 bound close to the the BLEG-1 active site while Peptide 8.4 bound directly at the center of active site itself. To expand on these findings, a new set of rationally designed peptides was computationally modelled and docked against BLEG-1 using AutoDock Vina. Six peptides exhibited binding energies more favourable than ampicillin (-6.0 kcal/mol) with values ranging from -6.8 to -8.2 kcal/mol, suggesting stronger and stable interactions. This integrated computational-experimental pipeline highlights the potential of rationally engineered peptides to inhibit subclass B3 MBLs such as BLEG-1. These findings lay the groundwork for future in vivo evaluation and structural optimization of MBL inhibitors as lead inhibitors to combat AMR.

O-02

Structural basis for lipid-regulated activation of vacuolar anion channel ALMT9

Yeongmok Lee¹, Elsa Demes-Causse², Jaemin Yoo¹, Seo Young Jang³, Seoyeon Jung¹, Justyna Jaslan², Geum-Sook Hwang³, Jejoong Yoo¹, Alexis De Angeli², Sangho Lee^{1*}

Vacuolar ALMTs control anion accumulation in plant cells. In guard cells, they regulate stomata aperture. The activation of vacuolar ALMTs depends on voltage and cytosolic malate, but the underlying molecular mechanisms remain elusive. Here we report the CryoEM structures of ALMT9 from Arabidopsis thaliana (AtALMT9), a malate-activated vacuolar anion channel, in plugged and unplugged lipid-bound states. In all these states, membrane lipids interact with the ion conduction pathway of AtALMT9. We identify two unplugged states presenting two distinct pore width profiles. Combining structural and functional analysis we identified conserved residues involved in ion conduction and in the pore lipid interaction. Molecular dynamics simulations revealed a peculiar anion conduction mechanism in AtALMT9. We propose a voltage-dependent activation mechanism based on the competition between pore lipids and malate at the cytosolic entrance of the channel.

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³ Korea Basic Research Institute, Republic of Korea

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O-03

Stall force measurement of the kinesin-3 motor KIF1A using a programmable DNA origami nanospring

Nobumichi Takamatsu¹, Hiroko Furumoto², Takayuki Ariga³, Mitsuhiro Iwaki⁴, Kumiko Hayashi^{1*}

DNA origami technology is a method for designing and constructing nanoscale structures using DNA, and it is being applied across various fields. This technology was advanced by developing the nanospring (NS), a fluorescently visible molecular spring that quantifies forces through its extension and has been used to measure myosin-generated forces. This study aims to measure the force exerted by the kinesin-3 motor protein KIF1A, mutations of which cause KIF1A-associated neurological disorder (KAND) and are associated with reduced force and motility. Unlike kinesin-1, KIF1A detaches easily under perpendicular loads, which can occur in optical tweezers experiments. By applying force parallel to the microtubule using the NS, we were able to precisely measure the stall force even for KAND mutants, for which such measurements are typically challenging. This result highlights the potential of the NS as a new tool for force spectroscopy in biophysics.

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Fluorescent Biosensing on the Move

Kittirat Phooplub^{1,2}, Sirirat Ouiganon^{1,2}, Chongdee Thammakhet-Buranachai^{1,2,3}, Panote Thavarungkul^{1,2,3,4}, Proespichaya Kanatharana^{1,2,3}, Apinya Prachugsorn⁵, Phuvadol Thanakiatkrai^{5,6}, Thitika Kitpipit^{5,6}, Watcharin Loilome^{7,10}, Narong Khuntikeo^{8,10}, Wittaya Ngeontae^{9,10}, Chittanon Buranachai^{1,2,4*}

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- Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Khon Kaen University, Khon Kaen, Thailand
- ¹⁰ Cholangiocarcinoma Research Institute, Khon Kaen University, Khon Kaen, Thailand

Modern technologies are offering more practical and economical solutions to recurring challenges, such as ones found in food safety, healthcare and environment. A conventional approach to tackle these problems is through a routine monitoring of, e.g. pathogens or contaminants, but it often requires well-equipped laboratories and well-trained personnels. Therefore, alternative methods or tools are getting more attention and progressing rapidly. Biosensors are among emerging technology that can complement conventional analysis techniques thanks to their exceptional performances, ease of operation and portability potential. To join in, the first part of this presentation illustrates how a DNA-based fluorescent biosensor capable of isothermal signal amplification at room temperature via the catalyzed hairpin assembly (CHA) reaction can be used with the asymmetric direct polymerase chain reaction (daPCR) to detect food frauds with high selectivity and sensitivity. Our method may help mitigate frauds, especially in areas with limited access to standard testing facilities. The second part of the presentation involves the development of portable fluorescence detection devices based on smartphone, single-board computers and electronic components widely commercially available. Our devices perform comparably to costly commercial equipment but with a fraction of the price and complexity. Sample applications include mobile health monitoring and on-site sensing.

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Penetration Assessment of 810 nm Light for Transcranial Photobiomodulation Using In-Vitro Models

Nursakinah Suardi*, Nurbatrisha Mohamad Shariff, Puteri Nur Ain Megat Hasbuddin, Mcleod Andy Jomi, Ahmad Fairuz Omar

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Photobiomodulation (PBM) therapy is emerging as a non-invasive approach for neuromodulation in neuropsychiatric disorders, offering potential benefits in enhancing brain function and alleviating symptoms through targeted light stimulation. However, its effectiveness is constrained by limited light penetration and the need for precise dosing due to its biphasic dose-response nature. This study aims to characterize power output of 810 nm near-infrared LEDs light from the Thinkfast PBM Device and compare the penetration depth and light intensity using in-vitro tissue models representing different scalp thicknesses. The power output was evaluated across frequencies ranging from 4 Hz to 200 Hz in both single and cluster LED configurations. Results showed a 6.80% variation in single LED output and 3.43% variation in cluster LED output across frequencies. The cluster LED consistently exhibited over 70% higher power than the single LED, supporting its potential for achieving greater penetration, which is critical for neuromodulation targets located beneath the scalp. To simulate scalp conditions, excised rabbit skin samples at two thicknesses (0.78 mm and 1.91 mm) were used, both representing hair covered regions. These values approximate human scalp thickness, which ranges from 1.25 mm (prefrontal) to 1.48 mm (parietal). Measurements were taken at three regions: prefrontal, temporal, and parietal. At 0.78 mm, penetration ranged from 52.81% to 58.20%, with the highest transmission intensity in the temporal site (85.45%). With increased thickness (1.91 mm), penetration dropped to 32.55%-40.31%, though transmission intensity remained high up to 79.53% in the temporal region. With the transmission values remained within a range (32–58%), it is sufficient to support transcranial delivery, particularly to superficial cortex. These findings show potential for effective transcranial photobiomodulation in neuropsychiatric applications.

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Development of the scaffold's mechanical properties investigation method

Witchukorn Phuthong¹, Chanati Chantarachotechatchawan², Kitiphat Sinthiptarakoon³, Bralee Chayasombat⁴

I will present our in-process methodology for spatially resolving the three-dimensional mechanical properties of biomaterial scaffolds by combining X-ray microcomputed tomography (X- μ CT) with Atomic Force Microscopy (AFM) force spectroscopy. To demonstrate, we are mechanically characterizing a scaffold composed of fish gelatin and soy protein, emphasizing spatial variations in stiffness and adhesion—key parameters for tissue engineering. X- μ CT was employed to capture detailed scaffold morphology, while AFM provided high-resolution measurements of local mechanical properties. By integrating these modalities, the proposed approach would enable precise mapping of the relationship between structural features and localized mechanical behavior. Furthermore, with deep learning, we are interpolating and extrapolating mechanical data across unsampled scaffold regions, enhancing model accuracy and predictive capability. We hope that our integrative framework would offer a powerful toolset for the design of scaffolds with spatially controlled mechanical properties to advance biomaterials research.

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Lipid Bilayer Order Profile as a Representation of Pressure Balance: Bilayer Response to Solvation of an Amphiphilic Solute

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The ordering of the hydrocarbon chains organized in a lipid bilayer can be evaluated by the use of the average deuterium order parameter $\langle S \rangle$ as obtained from NMR spectroscopy. Various theoretical and empirical models related the order parameter and the average chain geometry – in the empirical models, the values of $\langle S \rangle$ were determined by NMR spectroscopy, while the geometry of the chain was determined by X-ray diffraction.

Recently, two different approaches were used to express the lipid bilayer structure (i.e. the average area per hydrocarbon chain and properties derived from it) as a function of order parameter $\langle S \rangle$ and these two approaches resulted in the same mathematical description. Such agreement indicated that the order parameter is, i.a., a representation of bilayer pressure, while the model relates the inter- and intra-bilayer forces (pressures) to bilayer elastic properties.

The model was used to analyze the response of 1,2-dimyristoyl-sn-glycero-3-phosphatidylcholine (DMPC)/water bilayers to solvation of a small amphiphilic molecule benzyl alcohol. Various binding sites were confirmed within the bilayer, and it was shown that benzyl alcohol molecules partition between them in a dynamic and hydration-dependent way.

It was also shown that benzyl alcohol molecules are solubilized by the bilayer until the DMPC hydrocarbon chains adopt certain limiting values of the order parameter. Such limit to the chain disordering most likely reflects a more general property – the limiting chain order profile is reached just before the solute phase-separates and the solute/bilayer system ceases to exist as a single phase.

Dynamic Regulation of Protein Liquid-Liquid Phase Separation by Gold Nano-Butterfly

Tomohiro Nobeyama^{1*}, Koji Takata², Tatsuya Murakami², Yoichi Yamada³, Kentaro Shiraki³

¹ Kyoto University, Japan

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Proteins undergo drastic assembly and disassembly, in particular the formation/dissolution of condensates. Recent studies have shown that protein droplets formed by liquid-liquid phase separation of proteins play a critical role in gene coding, RNA transcription, cell metabolism and the generation of pathogenic protein assemblies. Droplets are reaction fields that can bundle multiple enzymes and reactants for efficient sequential reactions or alter the physical properties of proteins, as in amyloidosis. Regulation of the formation/deformation of this important reaction field would be the target of nextgeneration cell engineering methodology. In this study, we achieved regulation of droplet formation/deformation by creating butterfly-shaped gold nanoparticles (GNBs). The GNBs were designed to stabilize the nanoscale precursors of droplets by pinching them by the wings and let them mature into droplets. In addition, the photothermal effects of GNBs can dissolve the droplets when irradiated with a near-infrared laser. We embodied the concept by applying GNBs to poly-L-lysin/ATP, human immunoglobulin G and lysozyme system. In addition, we were able to upregulate the enzymatic activity of enzymes in GNBinduced droplets. Our results have paved the way for a first attempt to control droplet formation/deformation dynamics with nanodevices.

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Bioinformatic Analysis of Mesenchymal Stem Cell Derived-Exosome Gene Cargo in Wound Healing

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Mesenchymal stem cell (MSC)-derived exosomes have demonstrated strong regenerative capacity in tissue repair, particularly in wound healing. However, the bioinformatic integration of their gene cargo with wound healing-associated genes remains underexplored. This study aimed to identify critical exosome gene targets associated with wound healing by integrating gene sets from MSC-derived exosomes and wound healing databases using a systems biology approach.

Wound healing-related genes were obtained from NCBI Gene dataset, and exosome genes from MSC were retrieved via the ExoCarta database. Common genes were identified and used to construct a protein-protein interaction (PPI) network using STRING. Cytoscape was employed to analyze network topology and identify hub genes based on node degree. Enrichment analysis was done using Enrichr to identify the biological processes (GO) and pathways (KEGG).

A total of 743 wound healing-related genes and 3,746 genes associated with MSC-derived exosomes were retrieved from respective databases. Comparative analysis identified 243 common genes, which were subjected to protein-protein interaction network construction. Subsequent network analysis revealed 10 hub genes, including FN1, AKT1, and MMP2, as key regulators within the wound healing gene network. GO terms such as positive regulation of cell migration, inflammatory response, phosphatase binding and growth factor stimulus were highly enriched. The key pathways included AGE-RAGE signaling in diabetic complications, FoxO signaling and relaxin signaling pathway emphasizing their roles in tissue regeneration, angiogenesis, and ECM remodeling.

This integrative bioinformatics approach highlights essential MSC-derived exosome genes and pathways involved in wound healing, providing novel insight into exosome-mediated tissue repair and informing potential therapeutic strategies in regenerative medicine.

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Exploring the ceRNA network for key biomarkers in Epstein-Barr virusassociated nasopharyngeal carcinoma cell line (C666)

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Nasopharyngeal carcinoma (NPC) is one of the most common head and neck cancers in Malaysia. Epstein-Barr virus (EBV) is one of the major etiological factors for NPC. While EBV-related genomic alterations have been extensively studied, the role of non-coding RNAs in NPC remains underexplored. The aim of this study is to identify key regulatory genes and oncogenic pathways through the construction of a ceRNA network in EBV-associated NPC.

In this study, we analysed gene expression profiles from EBV-positive (C666) and EBV-negative (CNE) NPC cell lines using the GEO dataset GSE181906. Differentially expressed mRNAs and circRNAs were identified using the limma package via GEO2R, while miRNAs were analysed using edgeR via the Galaxy platform. Cutoffs were set at $|\log_2 FC| \ge 2$ for circRNAs and mRNAs, and $|\log_2 FC| \ge 1.5$ for miRNAs, with p < 0.05.A competing endogenous RNA (ceRNA) network was constructed by integrating DEcircRNAs, DEmiRNAs, and DEmRNAs. circRNA-miRNA interactions were predicted using CircBank, and miRNA-mRNA interactions using TargetScan and miRDB. The top 10 DEcircRNAs and their associated DEmiRNAs and DEmRNAs were selected for network construction. Protein-protein interaction (PPI) analysis was performed to identify hub mRNAs, and a core ceRNA network was derived using the CytoHubba plugin in Cytoscape. Functional enrichment analysis (GO and KEGG) was performed using the Enrichr tool.

We identified 746 DEcircRNAs, 193 DEmiRNAs, and 998 DEmRNAs. The core ceRNA network comprises 10 circRNAs, 22 miRNAs and 10 hub mRNAs including ROBO1, PAK1, PAK2, and MAPK14. Among these, ROBO1 was highly connected within the core ceRNA network. KEGG analysis revealed that ROBO1 was enriched in the axon guidance pathway, PAK1 and PAK2 in ErbB and Ras signaling pathways, and MAPK14 and PAK1 in the MAPK pathway.

These findings highlight key regulatory axes and suggest potential biomarkers and therapeutic targets in EBV-associated NPC.

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Silent Strokes: A Quantitative Investigation on How Small Vessel Disease Rewires the Aging Brain

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While investigating brain structural health with aging, T2-FLAIR MR images revealed that a substantial subset (>70%) of cognitively-normal (CN) subjects exhibited significant cerebral small-vessel disease (CSVD) load. Observed as Periventricular (PVWMH) and Deep White Matter Hyperintensities (DWMH), their accumulation may disrupt brain health. We examined whether PVWMH and DWMH exhibit differential trajectories and thresholds beyond which structural, microstructural, functional and cognitive disruptions emerge.

WMHs were segmented using k-nearest neighbor approach in T2-FLAIR scans from NACC (N=389) and ADNI (N=382) cohorts. Segmentation of T1-weighted images provided 174 neuroanatomical volume/thickness parameters. Diffusion tensor imaging assessed fractional anisotropy (FA), and resting-state fMRI enabled seed-based Default-Mode Network (DMN) connectivity analysis. CN individuals were stratified into PVWMH (Q1: ≤0.93 mL; Q4: >6.12 mL) and DWMH (Q1: ≤0.92 mL; Q4: >2.75 mL) quartiles. Cognitive domains examined included attention, executive-function, memory, and language.

PVWMH increased exponentially with age, twice as rapidly as DWMH. Cognitive performance remained intact below a PVWMH volume of 2.3mL. Above this threshold, significant impairments in executive-function, attention and semantic-memory were observed (p<0.01), alongside atrophy in precentral-gyrus, rostral middlefrontal gyrus, lingual-gyrus, and nucleus-accumbens. FA reductions were noted in the corpus callosum, SLF, and internal-capsule. Notably, CSF Neurofilament light-chain (NfL) levels increased significantly. DMN connectivity showed anterior-posterior reductions with high PVWMH load. PVWMH-related cognitive deficits are mediated via specific structural-atrophy. A PVWMH threshold >2.3mL delineates a critical inflection point for covert cognitive and neurobiological decline in aging. Stratifying aging individuals by PVWMH burden may refine clinical and neuroimaging aging frameworks.

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Engineering BMP-7 Peptides for Surface Functionalization of Stainless-Steel Implants: A Biophysical Approach to Bone Regeneration

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Peptide-coated 316L stainless steel (SS) discs were evaluated using human bone marrowderived mesenchymal stem cells (MSCs) to determine cellular response at the biointerface. Fluorescence microscopy (phalloidin/DAPI) and quantitative analysis demonstrated that Peptide 1 coating enhanced MSC adhesion and spreading compared to Peptide 2 and noncoated SS surfaces. Cells exhibited elongated morphology, organized actin cytoskeleton, and intact nuclei, highlighting strong substrate interaction and viability. Additionally, proliferation studies showed sustained viability with Peptide 1 coating up to Day 7, demonstrating a 7.9% increase over non-coated controls, confirming ISO-related biocompatibility and highlighting surface chemistry's role in bone regeneration strategies. The integration of biomolecular signals with engineered surfaces represents a promising frontier in biomaterials design. Bone morphogenetic protein 7 (BMP-7) is a potent osteoinductive factor acting through Smad and MAPK pathways in MSCs, yet its application is limited by cost and instability. In this study, we adopt a biophysics-guided strategy to synthesize and characterize BMP-7-mimetic peptides for functionalization of 316L SS implants. Three BMP-7-derived peptides were synthesized using solid-phase peptide synthesis with DOPA for metal adhesion and Ahx spacers to maintain bioactivity. Fluorescein-labeled analogues aided surface-binding analysis. Peptides 1 and 2 showed high purity (>90%) and accurate mass, while Peptide 3 was excluded due to poor yield. Surface modification with solvent cleaning enhanced bioreactivity, confirmed by XPS (increased N 1s, O 1s) and AFM (uniform topography). Contact angle analysis verified increased hydrophilicity. This peptide-functionalized SS platform demonstrates a scalable, bioinspired, cost-effective approach for orthopedic applications while reducing reliance on growth factors, with further biological characterizations ongoing to confirm bone regeneration potential.

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In Silico Evaluation of Novel Metformin Derivatives as α -Glucosidase Inhibitors: A Structure-Based Drug Design Approach

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Type 2 Diabetes Mellitus (T2DM) remains a significant global health concern, and α -glucosidase is a proven therapeutic target for reducing postprandial glucose spikes. Although metformin is widely used in T2DM management, it lacks direct α -glucosidase inhibitory activity. This study applies a structure-based computational approach to design and assess novel metformin derivatives with potential inhibitory effects on α -glucosidase. A focused library of metformin derivatives was developed and screened using molecular docking against α -glucosidase (PDB ID: 2ZE0). Top-performing compounds were evaluated through 100 ns molecular dynamics simulations using AMBER20 to determine complex stability and dynamic behavior. Analyses included RMSD, RMSF, hydrogen bond occupancy, and MM-GBSA binding free energy. Results revealed that several derivatives exhibited higher binding affinities and greater stability than metformin, indicating potential as lead compounds. This work underscores the promise of computational drug design in repurposing and optimizing existing antidiabetic scaffolds for enhanced multifunctional activity.

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Discerning the Enzymatic Properties, Structural Modulations and Binding Affinities of a Promiscuous BLEG-1 B3 Metallo- β -Lactamase towards Chemically Distinct Substrates

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Metallo-β-lactamases (MBLs) are class B β-lactamases that are significantly linked to antibiotic resistance by catalyzing the hydrolysis of a broad-spectrum of clinically prevalent β-lactam antibiotics. Recently, evolutionary studies of MBLs have gained momentum to gain better insights on their substrate and catalytic evolutions. BLEG-1, locally identified from Bacillus lehensis G1, is an evolutionary diverged MBL of B3 subclass with similar overall protein structure and active site architecture to both B3 MBL and glyoxalase II (GLXII). In this study, its kinetic properties, secondary structural changes and molecular binding affinities towards two chemically distinct substrates, i.e. ampicillin (substrate for MBL) and SLG (substrate for GLXII), were investigated respectively to gain better insights into key aspects of its dual functionalities. BLEG-1 in vitro steady-state kinetic analysis revealed that it has similar magnitude of activities towards both ampicillin and SLG. Circular dichroism (CD) analysis of the secondary structures of BLEG-1 and its substrate complexes showed structural modulations in which more helical structures were formed when in interaction with ampicillin, as compared to when SLG is present. The binding association constants (Kd) computed from CD results indicated that the binding affinities of BLEG-1 towards both substrates were similar, with the Kd value towards ampicillin is significantly comparable to the Kd determined previously using nano-isothermal titration calorimetry. These results highlight that BLEG-1 can recruit both ampicillin and SLG at equal magnitude, thus contributing to its dual functionality. This lays valuable foundation for the evolutionary studies and design of inhibitors against BLEG-1 and its homologs.

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Exploring the biophysical and structural characteristics of membrane acid phosphatase (LdMAcP) for therapeutic development against Leishmaniasis

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Leishmaniasis, caused by leishmanial parasites, remains a significant global health concern. Despite continuous efforts, no effective vaccines are available, and current chemotherapeutic approaches are limited by cost, toxicity, administration complexity, and rising drug resistance. This necessitates the identification of novel therapeutic targets. Among emerging candidates, parasite-derived protein phosphatases are of particular interest due to their role in host-pathogen interactions and parasite survival. Membranebound acid phosphatase (LdMAcP), a member of the histidine acid phosphatase (HAcP) family, is implicated in virulence by supporting adaptation to acidic environments and facilitating phosphate acquisition within host cells. Hence, LdMAcP was successfully cloned, expressed, and purified to homogeneity. Biophysical characterization confirmed its stable conformation, with secondary structure analysis revealing a dominance of α -helices and fluorescence quenching, indicating buried tryptophan residues in hydrophobic regions, supporting the protein's stable conformation. The enzyme retained activity across a wide temperature range, with optimal performance under acidic conditions, mimicking the intracellular environment of host macrophages. Structural analysis revealed that LdMAcP adopts a conserved α/β fold characteristic of HAcP enzymes. Docking studies showed that sanguinarine (inhibitor) binds more strongly to the active site than pNPP (substrate), driven mainly by hydrophobic interactions. Molecular dynamics simulations indicated enhanced stability of the LdMAcP-ligand complexes compared to the apo form. Moreover, binding analysis indicated that the LdMAcP-sanguinarine complex had a higher affinity and more negative binding energy than the LdMAcP-pNPP complex. These findings highlight LdMAcP's potential as a novel therapeutic target for L. donovani, paving the way for selective inhibitor development.

Effect of 532 nm, 0.1W Laser Irradiation on Zebrafish Larvae Caudal Fin Regeneration

Najlaa Ilani Fitri Mohamad Khomizal^{1*}, Mohd Hamzah Mohd Nasir¹, Nursakinah Suardi², Umairah Mohd Zaki²

Regeneration is a vital biological process, yet the mechanisms that enhance tissue repair remain insufficiently understood. This study investigates the effects of laser irradiation on the regeneration of caudal fin tissue in zebrafish larvae (Danio rerio), a well-established model for tissue regeneration research. A low-power diode-pumped solid-state (DPSS) laser emitting at 532 nm with a power output of 0.1 W was used to examine the impact of varying exposure durations (1, 5, 10, 15, and 30 minutes) on the regeneration process. The regeneration progress was quantified by measuring the length of the caudal fin using calibrated ImageJ software at 24, 48, and 72 hours post-amputation (hpa). Additional experiments explored the effects of double laser irradiation with a 1-hour interval between exposures to evaluate whether multiple treatments could enhance regenerative outcomes. Statistical analysis, including one-way ANOVA, was performed to determine significant differences among treatment groups. The results demonstrated that laser exposure significantly promoted caudal fin regeneration (p < 0.001), with optimal enhancement observed at specific exposure times. However, repeated laser irradiation produced an inhibitory effect, likely due to cumulative stress or overstimulation of cellular pathways which require further investigation. These findings indicate that low-power laser treatment may serve as a non-invasive strategy to stimulate tissue regeneration. This study provides new insights into the use of photobiomodulation for enhancing regenerative processes and underscores the importance of optimizing laser parameters to achieve therapeutic benefits while minimizing potential adverse effects. Further investigation is recommended to explore the molecular mechanisms underlying the observed regenerative responses.

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405 nm Laser Toxicity Assessment on the Viability and Development of Zebrafish Embryos

Umairah Mohd Zaki¹, Nursakinah Suardi¹ and Mohd Hamzah Mohd Nasir²

Photobiomodulation therapy, previously known as low-level laser therapy (LLLT), has gained increasing attention for its non-invasive application in treating various medical conditions. Among the wavelengths utilized, the 405 nm violet laser has demonstrated notable antimicrobial activity by effectively inactivating bacteria and viruses, in addition to its ability to ablate soft tissue with high precision. These attributes have positioned it as a promising tool in clinical and therapeutic settings. Despite its growing use, the biological safety of 405 nm laser exposure, particularly during early development stage, remains insufficiently studied. As a form of non-ionizing radiation, the 405 nm laser does not possess enough photon energy to cause direct DNA damage. However, concerns remain regarding its potential indirect effects, such as oxidative stress or interference with normal cellular processes. Therefore, the present study evaluates the developmental toxicity of 405 nm laser irradiation using zebrafish embryos as the experimental model. Zebrafish embryos are widely recognized for their rapid development, transparency, and suitability for cost-effective in vivo toxicity screening. In this study, embryos were exposed to the 405 nm laser at a fixed power output of 100 mW for durations of 1, 2, 4, 6, 8, and 10 minutes. Viability and morphological changes were monitored at 0, 24, 48, 72, and 96 hours post-fertilization (hpf) using microscopy. The results showed a clear dose-dependent response, where longer exposure durations led to reduced embryo viability. Notably, morphological malformations appeared at 8 minutes of exposure, and complete mortality was observed at 10 minutes. These findings suggest that, although the 405 nm laser holds therapeutic potential, it may exert toxic effects at higher doses. This highlights the importance of establishing safe dosage limits, especially as laser technologies continue to expand in clinical and biomedical use.

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Probing Phase Separation-Mediated Intranuclear Organization and Dynamics of Brg1 in Single Human Cells

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SWI/SNF chromatin remodelers are a key family of multi-subunit complexes that regulates genome access inside the cell. Central to SWI/SNF activity is the core ATPase/translocase subunit BRG1 common to all major subtypes of the remodeler family, with mutations implicated in diverse human cancers and overexpression associated with the upregulation of multiple oncogenes. The underlying mechanism for the spatio-temporal organization of SWI/SNF remodeler dynamics remains unclear. In light of the existence in BRG1 of extensive intrinsically disordered regions (IDRs) known to mediate the liquid-liquid phase separation (LLPS) of a wide range of biomolecules implicated in diverse intracellular processes, we hypothesize that LLPS of BRG1 represents a key mechanism employed by the cell to dynamically regulate SWI/SNF remodeler organization and activity in space and time. This thesis therefore aims to investigate the phase-separation potential of BRG1 by systematically characterizing its behaviors both in cellulo and in vitro using a multidisciplinary approach, as well as attempts to reveal the biological implications of such phase-separation-mediated organizational dynamics of the SWI/SNF remodeler complex.

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