A3 Foresight Program
日中韓フォーサイト事業

Institute of
SCIENCE TOKYO

JSPS

EARTH - LIFE SCIENCE INSTITUTE

The 1st A3 Foresight meeting

Program booklet

Date: January 19th (Mon.)-21st (Wed), 2026

Venue: Incubation CANVAS TOKYO

Contents

1. General Information

Date

January 19th (Mon.)-21st (Wed), 2026

Venue

Incubation CANVAS TOKYO, Tokyo, Japan

Homepage

English:

https://a3foresight.elsi.jp/2025/12/14/announcing-the-1st-a3-foresight-onsite-meeting/

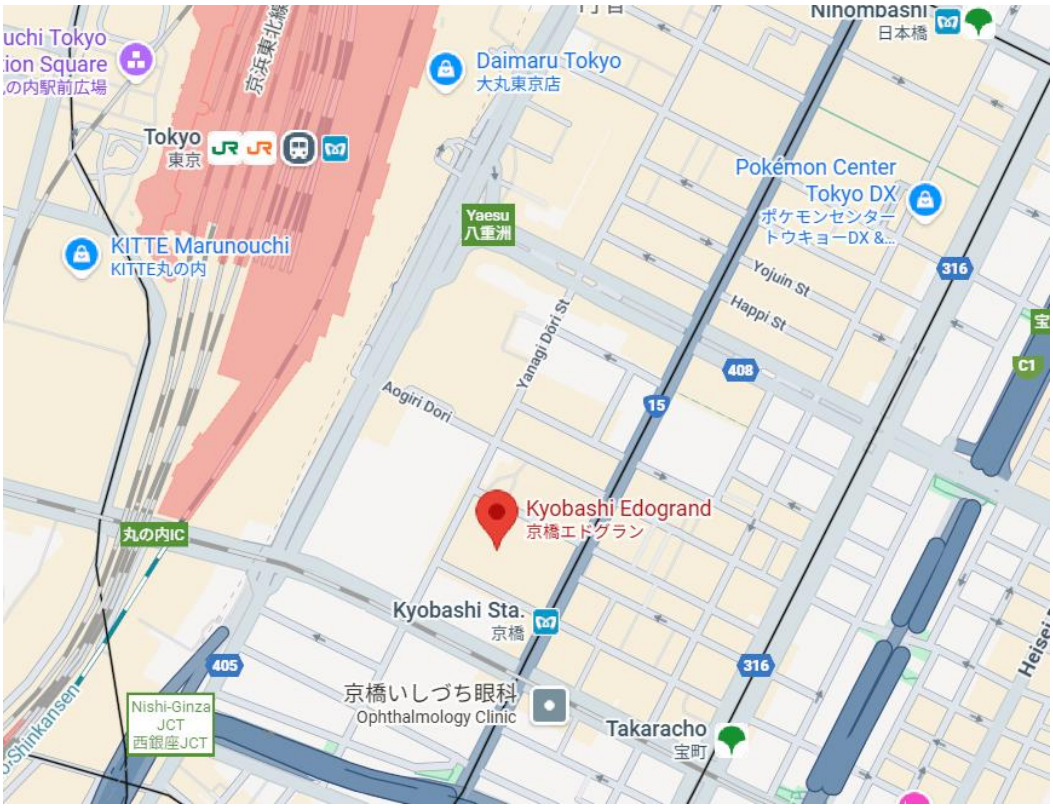
Japanese:

https://a3foresight.elsi.jp/ja/2025/12/14/announcing-the-1st-a3-foresight-onsite-meeting/

2. Venue

Address: 〒104-8377 29F, Kyobashi Edogrand, 2-2-1 Kyobashi, Chuo-ku, Tokyo 104-0031, Japan

HP: https://incubation-canvas.tokyo/



3. Program

Please come to the conference site at least 15 min before the session starts.

You will receive a 2D barcode needed to enter the building that will be sent individually by Incubation CANVAS TOKYO.

January 19		January 20		January 21	
		10:00	P5: Kwanwoo Shin	10:00	P13: Tomoaki Matsuura
		10:30	P6: Ryuji Kawano	10:30	P14: Doseok Kim
		11:00	P7: Jingjing Zhao	11:00	P15: Kwang-Hwan Jung
		11:30	S1: Byungsoo Kim	11:30	P16: Wenxia Xu
		11:42	S2: Eunjin Kim		
		11:54	S3: Qudan Agnes Park	12:00	R3: Sang Ho Lee
		12:06	***poster +lunch***	12:20	R4: Shin-Gyu Cho
				12:40	Concluding remarks
		14:00	P8: Myung Chul Choi		
		14:30	P9: Masahiro Takinoue		
15:00	Welcome from the chair/PIs	15:00	P10: Shubin Li		
15:15	P1: Xiaojun Han	15:30	S4: Saebom Yun		
		15:42	S5: Sungwoo Park		
15:45	P2: Norikazu Ichihashi	15:54	S6: Tror Seangly		
		16:06	***Coffee break***		
16:15	***Coffee break***	16:30	P11: Wataru Aoki		
16:45	P3: Hyomin Lee	17:00	P12: Sungho Jang		
17:15	P4: Kazunori Matsuura	17:30	S7: Yeansonn Setha		
		17:42	S8: Yoojin Kim		
17:45	R1: Hyunjin Lee	17:54	S9: Seunghyun Ryu		
		18:06	**Banquet + poster**		
18:05	R2: Hironori Sugiyama	20:00	**closing**		
18:25	**closing**				

4. Information for participants

1) Registration desk open at **2 pm on the 19th**.

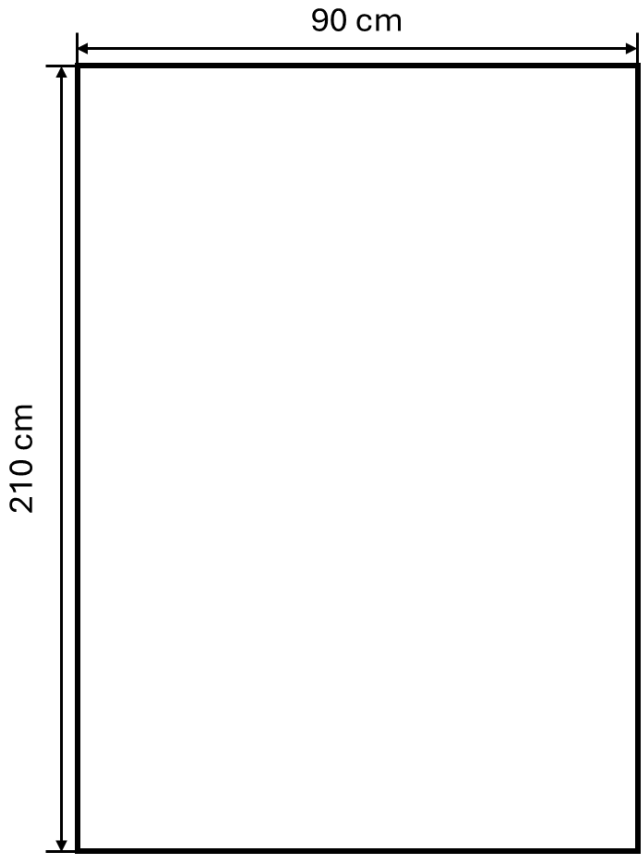
2) Oral presentation

Time for presentation (presentation + Q&A):

- P: 25+5 min
- R: 17+3 min
- S: 10+2 min

3) Poster presentation

- The size of the poster boards is 90 cm x 210 cm. Please design your posters to fit the boards.



- For poster presenters, please put on your posters on the boards according to your poster number by **9:30am on the 20th** and take off by **19:30pm**.
- Presentation time: ODD: **12 pm – 1 pm**, EVEN, **1 pm – 2 pm**.
- Poster numbers:

No.	**Presenter**
Pos 01	Chaeeon Lim
Pos 02	Hasaeam Cho
Pos 03	Jaehyeok Lee
Pos 04	Jiajue JI
Pos 05	Juncheol Lee
Pos 06	Junyoung Jung
Pos 07	Kentaro Shoji
Pos 08	Koshi MAEKAWA
Pos 09	Masahito Ishikawa
Pos 10	Nanami Takeuchi
Pos 11	Nayoun Kim
Pos 12	Seng Sreynith
Pos 13	Seunghyun Ryu
Pos 14	Shin-Gyu Cho
Pos 15	Shoko Fujita
Pos 16	Soei Watari
Pos 17	Sohyun Nam
Pos 18	Yuishin KOSAKA
Pos 19	Hirotake UDONO
Pos 20	Zugui PENG
Pos 21	Yuto Ueda
Pos 22	Kaito Seo
Pos 23	Hiroka Sugai
Pos 24	Wancheng Zhang

4) Payment for beverages for the January 20 banquet will be accepted in cash only. (Please note that no change will be provided.

5) The central Co-working Space on the Incubation CANVAS TOKYO floor will not be available, except during the banquet on Tuesday, January 20, from 18:00. At all other times, please use the seminar rooms only.

6) Lab tour

We provide three lab tours to those who would like to participate as follows:

A. Earth-Life Science Institute (ELSI), January 21th, 15:00pm~
B. The University of Tokyo, January 22th, 9:00am~
C. Tokyo University of Agriculture and Technology, January 22th, 11:00am~

   The tour at Tokyo University of Agriculture and Technology (TUAT) will include a 15-minute lab tour of the Kawano Lab, followed by a self-guided visit to the TUAT Museum (approximately 20 minutes).

   Please note that the museum does not offer English-guided tours, and most of the exhibit descriptions are written in Japanese. We therefore recommend that you keep your phone with you for translation if needed.

* More details regarding to the lab tours will be announced later.

5. Abstracts

5.1 Abstract – Oral presentation

Day 1 (19th Jan., Monday)

P1.

Construction of functional artificial cells

Xiaojun Han,
School of Chemistry and Chemical Engineering, Harbin Institute of Technology

Life begins with cells. Artificial cells are cellular like structures that can mimic some (or all) of cell structures and functions. Building artificial cells from the bottom up helps to reveal the working mechanism of cells and provide a theoretical basis for the origin of life. Targeting the key issues in this field, we carried out following projects in recent years.[1] Par system was reconstituted into GUVs to realize the even plasmids segregation and inheritance mimicry.[2] The mechanism of the influence of osmotic pressure on vesicular deformation was clarified to construct a divisible artificial "eukaryotic cell".[3]Glycolysis metabolic pathway was rebuilt inside an artificial cell for the synthesis of amino acid.[4] We developed a method to construct non spherical organelles[5] to mimic chloroplast grana capable of capturing light energy. The artificial cells capable of carbon fixation [8] and NO production [9] were built. We built spatial coded artificial tissues using magnetic [6,7] and acoustic fields. These tissues possessed the vasodilation and muscle contraction functions. [10,11].

References:

[1] X. Han, et. al. Adv. Mater. 2021, 33, 2002635. [2] X. Han*, et. al. Nat. Commun., 2024, 15, 4956. [3] X. Han*, et. al. J. Am. Chem. Soc. 2017, 139, 9955. [4] X. Han*, et. al. J. Am. Chem. Soc. 2024, 146, 21847−21858. [5] X. Han*, et. al. Adv. Mater. 2018, 30, 1707482. [6] X. Han*, et. al. Nat. Commun. 2020, 11, 232. [7] X. Han*, et. al. Nat. Commun. 2022, 13, 2148. [8] X. Han*, et. al. Angew. Chem. Int. Ed. 2025, 64, e202421827. [9] X. Han*, et. al. Adv. Mater. 2025, 37, 2500242. [10] X. Han*, et. al. Adv. Mater. 2022, 34, 2204039. [11] X. Han*, et. al. Nat. Commun. 2023, 14, 7507.*

P2.

Toward a self-regenerative in vitro central dogma

◯Norikazu Ichihashi[1]

[1] The University of Tokyo,

The central dogma is the core of living systems that allows replication of genetic information, expression of RNA and proteins, and growth of the entire system. The in vitro construction of the central dogma is a major challenge in the field of bottom-up synthetic biology. We are building an in vitro central dogma in a simpler scheme than natural ones to make a self-regenerative system. In the meeting, I will share three of our recent progresses. First, we have constructed a simple in vitro DNA replication scheme that requires only two genes (phi29 DNA polymerase and Cre recombinase) for continuous replication. We reported a circular DNA encoding the two genes that replicates continuously by expressing the genes (1). The continuous replication also allows Darwinian evolution of the DNA. Second, we reported the sustained regeneration of 20 aminoacyl-tRNA synthetases, one of the main components in the translation system, from DNA in the reconstituted transcription/translation system (PURE system). We succeeded in maintaining the original level of translation even after 20 rounds of serial dilution with the aaRS-omitted PURE system (2). Third, we reported the expression of the minimal but sufficient tRNA set from a single polycistronic DNA template in the tRNA-free PURE system using a novel tRNA array method (3). While these results bring us a step closer to establishing an in vitro central dogma, much remains to be done. I look forward to collaborating with researchers around the world.

References:

1. Okauchi, H., Ichihashi, N., Continuous cell-free replication and evolution of artificial genomic DNA in a compartmentalized gene expression system, ACS Synthetic Biology, 10, 3507–3517 (2021)

2. Hagino, K., Masuda, K., Shimizu, Y., Ichihashi, N., Sustainable Regeneration of 20 Aminoacyl-tRNA Synthetases in a Reconstituted System Toward Self-Synthesizing Artificial Systems. Sicence Advances, in Press (2025)

3. Miyachi, R., Shimizu, Y., Ichihashi, N., Simultaneous in vitro expression of minimal 21 transfer RNAs by tRNA array method, Nature Communications, 16, 7418 (2025)

P3.

Surfactant-Mediated Water Transport in Artificial Cell-Like Double Emulsions

Hyomin Lee[1]

[1]Pohang University of Science and Technology (POSTECH), KOREA

Artificial cell models require both compartmentalization and controllable exchange of materials with the surrounding environment. Water transport across the oil shell is particularly critical in double emulsions (W/O/W), as osmotic imbalances can rapidly alter inner volume, destabilize interfaces, and compromise encapsulated reactions or cargo. Here, we use droplet microfluidics to generate monodisperse artificial cell-like double emulsions and investigate how mixed surfactant composition governs water transport and interfacial stability. By systematically varying surfactant type and concentrations in the oil phase, we quantify osmotic volume dynamics of the inner compartment and relate transport behavior to shell integrity over time. The results establish practical design rules for decoupling stability from permeability enabling double emulsions that remain structurally robust while exhibiting tunable, predictable water exchange. This surfactant-based strategy provides a simple and broadly applicable route to engineer artificial cell-like compartments for controlled microenvironments, sustained encapsulation, and reaction-enabled protocell systems.

References

[1] Eunseo Kim, Jongsun Yoon, Jong Dae Jang, Heemuk Oh, Hyomin Lee, Mixed Surfactants for Stabilizing and Controlling Water Transport in Double Emulsions. *Sci. Adv.*, **2025**, 11, 26, eadv7043.

P4.

Dynamic deformation of giant liposome by designed peptides

◯Kazunori Matsuura, Yingbing Liang, Miu Hirahara, Yasunari Takaki, Hiroshi Inaba

Tottori University

Spatio-temporal cytoskeleton polymerization and depolymerization control cellular dynamics such as deformation and migration. In this study, we developed artificial cytoskeleton based on spiropyran/merocyanine (SP/MC)-modified peptide nanofibers which can be induced local deformation of phase-separated GUV by photocontrol of the polymerization and depolymerization. We synthesized SP/MC-modified β-sheet-forming peptide (FKFEC$^{SP/MC}$KFE) and confirmed the photo-controlled conversion between β-sheet (SP form) to random coil (MC form) by CD spectra, TEM, and CLSM imaging. Interestingly, upon visible light irradiation on spherical GUVs encapsulating the MC form peptides, the photoisomerization to the SP-form induced a dramatic morphological change to worm-like vesicles.[1] The membrane fluidity of GUV significantly affects the aspect ratio after GUV deformation by photoisomerizing the peptide nanofiber. We prepared phase-separated GUVs consisting of the liquid disorder (L_d) phase (DOPC and DGS-Ni-NTA) and the liquid order (L_o) phase (DPPC). FKFEC^{MC}KFE and FKFEC^{MC}KFEHHHHHH peptides were encapsulated into the phase-separated GUVs. It was confirmed that the peptides were localized at the L_d phase by the coordination of His-tag and Ni-NTA lipids. Surprisingly, photoisomerization of the nanofibers from the MC-form to SP-form induced local growth at the L_d phase and local deformation of GUV.[2]

References: [1] Y. Liang, S. Ogawa, H. Inaba, K. Matsuura, *Front. Mol. Biosci*., **10**, 1137885 (2023); [2] Y. Liang, Y. Takaki, H. Inaba, K. Matsuura, *Langmuir*, **41**, 15173 (2025)

R1.

Individual characterization and one-pot reaction of 1-deoxynojirimycin biosynthetic enzyme

Hyunjin Lee[1, 2] and Sungho Jang[1, 2]

[1]Incheon National University, Incheon 22012, Korea.

[2]Research Center for Bio Materials & Process Development, Incheon National University, Incheon 22012, Korea.

1-Deoxynojirimycin (1-DNJ) is an iminosugar derived from fructose-6-phosphate (F6P) and is proposed to be biosynthesized through an aminotransferase, a phosphatase, and an oxidoreductase. In this study, we expressed and purified three putative biosynthetic enzymes-GabT1, Yktc1, and GutB1-from *Bacillus velezensis* K26 and investigated their biochemical properties and functional roles using an in vitro one-pot assay. When the three enzymes were incubated with F6P, LC-MS analysis identified a dominant product corresponding to mannojirimycin (MJ)-dehydrate (m/z 162), while 1-deoxymannojirimycin (1-DMJ) and 1-deoxynojirimycin (1-DNJ) were not detected. These results demonstrate that GabT1, Yktc1, and GutB1 act sequentially to convert F6P into MJ-dehydrate under cell-free conditions, indicating that the downstream conversion of MJ to 1-DNJ requires additional enzymatic activities, such as epimerization and reduction. Overall, this study provides direct biochemical evidence for the initial steps in the 1-DNJ biosynthetic pathway and establishes a foundation for future elucidation of the complete iminosugar biosynthetic pathway.

References

1. H Lee et al. 1-Deoxynojirimycin core biosynthetic enzymes: Individual characterizations and enzymatic one-pot synthesis of its biosynthesis intermediates. Under review.
2. H Lee et al. 1-Deoxynojirimycin-producing bacteria: production, opimization, biosynthesis, biological activities. *Biotechnol. Bioprocess Eng*. 2024. 29:981-992
3. H Lee et al. Genome analysis of 1-deoxynojirimycin (1-DNJ)-producing Bacillus velezensis K26 and distribution of Bacillus sp. harboring a 1-DNJ biosynthetic gene cluster. *Genomics.* 2021. 113:647-653

R2.

Uphill molecular transport across the liposomal membrane and recent study on GPCR engineering

◯Hironori Sugiyama[1], Tomoaki Matsuura[1]

[1]ELSI, Science Tokyo

Liposomes are closed phospholipid bilayers that form cell-like compartments by separating inner and outer aqueous phases with a hydrophobic membrane barrier. While this barrier helps retain encapsulated components for constructing biochemical systems, it also limits the supply of small molecules across the membrane. Here, using an automated microfluidic observation platform, we show that liposomes can accumulate small molecules, including PEG and ATP derivatives, without any assist of membrane proteins, relying only on lipid membranes [1,2].

In addition, we present our recent GPCR engineering project targeting A2AR and ETR. Starting from *in vitro* directed evolution [3], the workflow is now integrated with *in silico* design using protein language model-based algorithms to accelerate variant selection and optimization.

References:

[1] Sugiyama et al., Commun. Chem., 2020; [2] Sugiyama et al., Langmuir, 2022; [3] Fukasawa et al., chemRxiv, 2025

Day 2 (20th Jan., Tue.)

P5.

Extracellular Matrix-Coated Vesicles as a Biomimetic Model of Membrane-Matrix Interplay

Kwanwoo Shin[1]
[1] Department of Chemistry and Institute of Biological Interfaces, Sogang University

Artificial membrane systems have enabled powerful studies of lipid dynamics and bilayer mechanics, yet they lack the structural complexity of living cells, where membranes are embedded within an extracellular matrix (ECM). Here, a biomimetic platform is presented that integrates fibronectin (FN) and collagen type I (COL) onto the surface of giant unilamellar vesicles (GUVs) to investigate ECM-induced modulation of membrane properties. ECM coating imparts distinct, protein-specific effects on vesicle curvature, mechanical resilience, and lipid diffusivity. FN promotes vesicle budding and membrane softening, while COL induces rugged membrane topographies and mechanical stiffening. Furthermore, ECM proteins reshape the geometry and stability of phase-separated lipid domains, mimicking curvature heterogeneity observed in cell membranes. Strikingly, vesicle budding events observed in FN-coated GUVs resemble exosome-like release, suggesting that ECM identity not only dictates membrane mechanics but may also regulate vesicle biogenesis. This system captures essential mechanobiological interactions between the ECM and the plasma membrane in the absence of transmembrane linkers. The findings provide a tunable platform for studying ECM–membrane coupling and ECM-vesicle interplay with relevance to exosome modeling, offering new directions for engineering responsive synthetic cells and advancing extracellular vesicle biology

References: K. Shin et. al, Extracellular Matrix-coated vesicles as a biomimetic model of membrane-matrix interplay, Small Methods, doi.org/10.1002/smtd.202501785 (2025)

P6.

Construction of artificial cell-membrane systems using microfluidics

◯Ryuji Kawano

Tokyo University of Agriculture and Technology, Japan

Biological nanopores are protein assemblies that form nanometer-sized channels in lipid bilayer membranes and enable the selective transport of ions and molecules. The practical use of nanopore-based single-molecule DNA sequencing has shown that membrane proteins can be repurposed for artificial and engineering applications beyond their natural biological roles. In this presentation, I introduce our approach to artificial membrane transport systems based on designed nanopores combined with microfluidic technologies. By tailoring pore structures and charge properties of nanopores and integrating them into lipid bilayers formed in microfluidic devices, we aim to control molecular transport across membranes in a reproducible manner.

References: Z. Peng. *et. al.* ***ACS Nano*** (2025), S. Takiguchi. *et. al.* ***Small*** (2025), J. Ji *et. al.* ***Lab Chip*** (2025), S. Takiguchi. *et. al.* ***Chem.Soc.Rev.*** (2025), Z. Peng. *et. al.* ***Lab Chip*** (2024), Y Numaguchi. *et. al.* ***PNAS Nexus*** (2024), S. Fujita. *et. al.* ***ACS Nano*** (2023), K. Shimizu. *et. al.****Natrue Nano*** (2022).

P7.

Investigation of artificial cells containing the Par system for bacterial plasmid segregation and inheritance mimicry

Jingjing Zhao[1], Xiaojun Han[1*]

[1]State Key Laboratory of Urban-rural Water Resource and Environment, Heilongjiang Provincial Joint Laboratory of Molecular Science (International Cooperation), School of Chemistry and Chemical Engineering, Harbin Institute of Technology, Harbin 150001, China

A crucial step in life processes is the transfer of accurate and correct genetic material to offspring. During the construction of autonomous artificial cells, a very important step is the inheritance of genetic information in divided artificial cells. The ParMRC system, as one of the most representative systems for DNA segregation in bacteria, can be purified and reconstituted into GUVs to form artificial cells. In this study, we demonstrate that the eGFP gene is segregated into two poles by a ParM filament with ParR as the intermediate linker to bind ParM and *parC*-eGFP DNA in artificial cells. After the ParM filament splits, the cells are externally induced to divide into two daughter cells that contain *parC*-eGFP DNA by osmotic pressure and laser irradiation. Using a PURE system, we translate eGFP DNA into enhanced green fluorescent proteins in daughter cells, and bacterial plasmid segregation and inheritance are successfully mimicked in artificial cells. Our results could lead to the construction of more sophisticated artificial cells that can reproduce with genetic information.

References: Jingjing Zhao, Xiaojun Han*. Nature Communications, 2024, 15(1): 4956.

S1.

Regulation of Microtubule Structural Framework by Luminal Tau

Byungsoo Kim[1], Myung Chul Choi[1]
[1]Department of Bio and Brain Engineering, KAIST, Daejeon 34141, Korea

Abnormal tau aggregation in the human brain is a hallmark pathology of Alzheimer's Disease (AD). Tau plays a crucial role in the regulation of microtubule dynamics in neuronal axons, and its binding to microtubule surface controls its structural integrity. Recently, evidence that full length tau binds not only to the outer surface, but also to the luminal surface of microtubule has been steadily accumulated. Extra-luminal tau has been suggested to interact with microtubule-associated proteins (MAPs) that bind to the outer surface and regulate the microtubule structural framework. In this study, we characterize microtubule luminal tau by cryo-electron tomography and its regulation of microtubule lattice. Our findings suggest the evidence of full-length luminal tau and its complementary role of microtubule structural regulation.

References: Kar, S., Fan, J., Smith, M.J. *et al. EMBO J* **22**, 70–77 (2003). Siahaan, V., Tan, R., Humhalova, T. *et al. Nat Chem Biol* **18**, 1224–1235 (2022). Biswas, S., Grover, R., Reuther, C. *et al. Nat. Phys.* **21**, 1616–1628 (2025). Inaba, H. et al. Chem. - Eur. J., 24, 14958−14967 (2018).

S2.

Gene Delivery to Cell-Free Protein Expression System via Lipid Nanoparticle

Eunjin Kim[1], TROR Seagnly [1], Chang Ho Kim[1], Soo Ryeon Ryu[1], Kwanwoo Shin[1]*

[1]*Department of Chemistry and Institute of Biological Interfaces, Sogang University, Republic of Korea*

Giant unilamellar vesicles (GUVs) are promising chassis for building artificial cells, but it remains challenging to integrate gene delivery, cell-free expression, membrane insertion of a newly synthesized membrane protein, and functional energy transduction within a single compartment. Here, we present an integrated strategy in which a cell-free protein synthesis (CFPS) system is encapsulated inside GUVs, while lipid nanoparticles (LNPs) deliver DNA encoding green-light-absorbing proteorhodopsin (GPR) into the vesicle lumen. Upon LNP-mediated DNA delivery, the encapsulated CFPS rapidly expresses GPR, which subsequently relocates and inserts into the GUV membrane, forming a functional light-driven proton pump. Using a pH-sensitive fluorescent reporter, we verify that green-light illumination triggers proton translocation across the membrane, producing a measurable pH change consistent with GPR activity. This work demonstrates a sequential, single-vesicle workflow—DNA delivery → in situ synthesis → membrane targeting/insertion → proton pumping—and provides a modular platform for constructing energy-harvesting artificial cells whose membrane functions can be programmed by externally supplied genetic information.

References: 1. Stephan Hirschi, et al. “Cryo-EM structure and dynamics of the green-light absorbing proteorhodopsin” Nature communications 12(2021): 4107

2. Jayna Hammond, et al. “Membrane Fusion-Based Drug Delivery Liposomes Transiently Modify the Material Properties of Synthetic and Biological Membranes” Small 21(2025): 24080389

3. Rafaela R.M. Cavalcanti, et al. “Efficient liposome fusion to phase-separated giant vesicle” Biophysical jounal 122(2023): 2099-2111

S3.

Anti-Cancer Drugs as Treatment for Tauopathies by Regulating Microtubule Lattice Structure and Mechanical Function

◯Qudan Agnes PARK[1], Juncheol LEE[1], Junichi TANAKA[2], Myung Chul CHOI[1]

[1]Department of Bio and Brain Engineering, KAIST, [2]Department of Chemical, Biology and Marine Science, University of the Ryukyus

The structural and mechanical properties of microtubules (MTs) play an essential role in intracellular trafficking with the support of tau. Tau, a key protein in neuronal development, is known to stabilize MT dynamics. Anti-cancer drugs, paclitaxel (PTX) and laulimalide (LMD), suppress cell division by also stabilizing MTs. Though both drugs are MT stabilizers, they bind to distinct sites: PTX binds to the MT lumen, and LMD binds to the MT external surface. However, it is yet to be understood how tau and these drugs change the MT lattice structure and its mechanical function. It has also been suggested that these drugs could be applied in treating tauopathies, although one of the main challenges has been the inability to cross through the blood-brain barrier (BBB). We present an experimental demonstration of the regulatory roles of PTX and LMD in tau-bound MT structures and further develop a drug carrier to enable these anti-cancer drugs to cross the BBB.

P9.

Molecular computation for non-equilibrium artificial cells

◯Masahiro Takinoue[1,2]

[1]Laboratory for Chemistry and Life Science, Science Tokyo, Japan

[2]Research Center for Autonomous Systems Materialogy (ASMat), Science Tokyo, Japan

Living systems exhibit intelligent behaviors, such as environmental sensing, decision-making, memory storage, information learning, adaptation, and evolution. Recently, the construction of intelligent systems based on molecules and materials has been actively studied[1]. In particular, the integration of the molecular computing concept[2] with micro- to macro-scale material systems is attracting attention for the creation of life-like intelligent systems. In this presentation, we show molecular computation systems[3] integrated with biomolecular condensates[6–8] under non-equilibrium reactions based on nucleic acid hybridization and strand displacement. In addition, we will present a new type of nucleic acid condensate[9] based on string-like DNA nanostructures for non-equilibrium artificial cells.

References: [1] C. Kaspar, et al., *Nature* 2021, 594 (7863), 345–355. [2] L. M. Adleman, *Science* 1994, 266 (5187), 1021–1024. [3] J. Gong et al., *Adv. Funct. Mater.* 2022, 32 (37), 2202322. [4] H. Udono et al., *ACS Nano* 2024, 18 (24), 15477–15486. [5] T. Maruyama et al., *Nat. Commun.* 2024, 15 (1), 7397. [6] K. Matsuura et al., *Biomacromolecules* 2007, 8 (9), 2726–2732. [7] B.-J. Jeon, et al. *Soft Matter* 2018, 14 (34), 7009–7015. [8] Y. Sato et al., *Sci. Adv.* 2020, 6 (23), eaba3471. [9] H. X Chai et al., *JACS Au* 2025, 5 (7), 3249–3261.

P10.

Construction of synthetic communities containing artificial cells and metabolic mimicking regulation

◯Shubin Li[1], Xiaojun Han[1]

[1]Harbin Institute of Technology

Constructing a synthetic community system helps scientist understand the complex interactions among species in a community and its environment. Herein, a two-species community is constructed with species A (artificial cells encapsulating pH-responsive molecules and sucrose) and species B (*Saccharomyces cerevisiae*), which causes the environment to exhibit pH oscillation behaviour due to the generation and dissipation of CO_2. In addition, a three-species community is constructed with species A′ (artificial cells containing sucrose and G6P), species B, and species C (artificial cells containing NAD and G6PDH). The solution pH oscillation regulates the periodical release of G6P from species A′; G6P then enters species C to promote the metabolic reaction that converts NAD to NADH. The location of species A′ and B determines the metabolism behavior in species C in the spatially coded three-species communities with CA′B, CBA′, and A′CB patterns. The proposed synthetic community system provides a foundation to construct a more complicated microecosystem.

References: Li, S.; Zhao, Y.; Wu, S.; Zhang, X.; Yang, B.; Tian, L.*; Han, X*. *Nat. Commun.* **2023**, **14**: 7507.

S4.

Development of RNA-based artificial gene regulators for detection of metformin and imidazole propionate, type-2-diabetes-related molecules

Saebom Yun[1] and Sungho Jang[1,2]

[1]Incheon National University, Incheon 22012, Korea.

[2]Research Center for Bio Materials & Process Development, Incheon National University, Incheon 22012, Korea.

Type 2 diabetes mellitus (T2DM) is a highly prevalent metabolic disorder, representing more than 90% of diabetes cases. Metformin is widely prescribed as a first-line therapy for T2DM and activates adenosine monophosphate-activated protein kinase (AMPK) to suppress hepatic gluconeogenesis. However, recent studies have shown that imidazole propionate (ImP), a gut microbiota-derived metabolite, inhibits metformin efficacy by inducing inhibitory phosphorylation of AMPK through Akt activation. To address this limitation, we present RNA-based artificial gene regulators separately responsive to metformin or ImP. These regulators were designed to combine structure-switching RNA aptamers with small transcription-activating RNA (STAR). RNA aptamers were enriched using capture-SELEX, followed by integration into STAR-containing plasmids and transformation into *Escherichia coli*. Fluorescence-activated cell sorting was used for *in vivo* selection to enrich gene regulators that modulate gene expression in response to metformin or ImP. The developed regulators will enable monitoring of metformin and ImP, identification of ImP-producing microbes, and discovery of ImP-degrading enzymes, ultimately contributing to engineered live biotherapeutic products for improved T2DM treatment.

References

1. Koh, Ara, et al. "Microbial imidazole propionate affects responses to metformin through p38γ-dependent inhibitory AMPK phosphorylation." *Cell metabolism* 32.4 (2020): 643-653.
2. Chappell, James, et al. "Computational design of small transcription activating RNAs for versatile and dynamic gene regulation." *Nature communications* 8.1 (2017): 1051.
3. Boussebayle, Adrien, Florian Groher, and Beatrix Suess. "RNA-based Capture-SELEX for the selection of small molecule-binding aptamers." *Methods* 161 (2019): 10-15.

S5.

Mg^{2+}-Triggered Intervesicular Lipid Nanotubes with Internal Actin as Mimicry of Tunneling Nanotubes

Sungwoo Han Park[1], Sang Ho Lee[1], Chengying Yin[2], Liangfei Tian[2], and Kwanwoo Shin[1*]
[1]Department of Chemistry & Institute of Biological Interface, Sogang University, Korea

[2]Department of Biomedical Engineering, Zhejiang University, China

Actin is a key cytoskeletal protein in formation and maintenance of cellular membrane tubes. Among these, tunnelling nanotubes (TNTs) are actin-filled intercellular membrane tubes that facilitate direct material exchange, yet their formation mechanisms remain poorly understood. Here, we demonstrate Mg^{2+}-triggered formation of lipid nanotubes with internal actin (AT-LNTs) as a biomimetic TNT model. We confirmed TNT-like bundled architecture and recapitulated unwiring processes from AT-LNTs. Upon Mg^{2+} exposure, vesicles containing Mg^{2+} ionophore and actin monomers rapidly generated intervesicular AT-LNT connections. Mg^{2+} simultaneously induced actin polymerization and bundling to exert protrusive forces while electrostatically recruiting actin to membrane via charge modulation. Systematic variation of lipid phase, membrane tension, [Mg^{2+}], [Actin], and [ionic strength] yielded distinct morphologies, highlighting mechanisms by membrane energetics and actin-membrane interactions. Furthermore, we demonstrated solute transport along AT-LNTs and direct connection to live cells, suggesting potential for direct targeted delivery.

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S6.

Cell-Free Protein Expression System in a Single Liposome to Create Artificial Cells with Energy Harvesting Capabilities

Seangly Tror[1], Chang Ho Kim[1], Soo Ryeon Ryu[1], Shin-Gyu Cho[2], Kwang-Hwan Jung[2], and Kwanwoo Shin[1*]

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Cell-free protein expression (CFPE) has attracted significant attention in biotechnology in recent years, as it enables high-throughput protein synthesis using mRNA or DNA in an in vitro environment free of living cells or microorganisms. This system can also be applied to produce proteins within artificially constructed liposomal compartments, offering a powerful platform for understanding cellular metabolism and advancing the development of regenerative artificial cells.[1] In this context, our objective was to synthesize proteorhodopsin (PR), an energy-harvesting protein, through cell-free expression to create an artificial cell capable of generating its own energy within the liposomal space. We confirmed that our system successfully synthesized the PR protein within a single liposome and induced its translocation to the phospholipid membrane, demonstrating the viability of this approach. Finally, we verified the functional activity of PR by demonstrating its ability to pump protons across the membrane upon green light illumination, thereby generating a proton gradient which is a key energy source for cellular processes. The CFPE model in a single liposome has the potential to evolve into an artificial eukaryotic cell-like model with comparable biological properties, including transcription and translation, as well as metabolic functions similar to natural cells.[1]

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P11.

Ribosome biogenesis in vitro

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Ribosome biogenesis is pivotal in the self-replication of life. In *Escherichia coli*, three ribosomal RNAs and 54 ribosomal proteins are synthesized and subjected to cooperative hierarchical assembly facilitated by numerous accessory factors. Realizing ribosome biogenesis in vitro is an essential goal to understand the self-replication of life and creating artificial cells; however, this has not been realized because of its complexity. We have reported the first successful ribosome biogenesis in vitro. Specifically, we developed a highly specific and sensitive reporter assay for the detection of nascent ribosomes. The reporter assay allowed for combinatorial and iterative exploration of reaction conditions for ribosome biogenesis, leading to the simultaneous, autonomous synthesis of both small and large subunits of ribosomes in vitro through transcription, translation, processing, and assembly in a single reaction space. Our achievement represents a critical step toward revealing the fundamental principles underlying the self-replication of life and creating artificial cells.

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P12.

Development of nucleic acid-based gene regulators for metabolic engineering and molecular diagnosis

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Synthetic biology aims at providing solutions for the challenges of modern society and pave new ways to understand life. Realizing the true potential of synthetic biology is dependent on our ability to regulate gene expression. Nucleic acids offer new opportunities for developing artificial gene regulators based on their programmability, versatility, low genetic footprint, and economic production both in vivo or in vitro. In this talk, I am going to introduce examples of artificial gene regulators developed through rational design and artificial evolution. Specifically, an efficient development process for RNA-based inducible gene regulators and their application to sensing of small molecules will be presented. Additionally, a rationally-designed nucleic acid-based molecular program for rapid diagnosis of pathogens called SENSR will be discussed.

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S7.

Light-Driven Proton Pumping in Artificial Organelles: A Sustainable Bioenergy Conversion

Setha Yeansonn[1], Seangly Tror[1], Chang Ho Kim[1], Soo Ryeon Ryu[1], Meng An Chheng[2], Kwanghwan Jung[2], Kwanwoo Shin[1*]

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The bottom-up construction of functional artificial cells represents a major challenge in chemistry and synthetic biology, requiring the incorporation of efficient energy-transducing modules. Proteorhodopsin (PR), a light-driven proton pump, is an attractive candidate for solar-powered energy generation in minimal cell systems. Here, we report the successful reconstitution of PR into a lipid-based artificial cell model using a detergent-mediated incorporation strategy. Reconstitution was systematically validated by UV–visible spectroscopy, which revealed the characteristic PR absorbance maximum at 525 nm in the proteoliposome population. In addition, confocal fluorescence microscopy directly confirmed PR integration into giant lipid vesicles, showing clear colocalization of the protein with the vesicle membrane. This well-defined reconstitute system establishes a robust platform for subsequent functional studies. Future investigations will exploit this platform to demonstrate light-driven proton translocation and to quantify the resulting transmembrane proton gradient (ΔpH). Successful implementation of this functionality would enable downstream applications, including co-reconstitution with F0F1-ATP synthase for light-driven ATP synthesis, substrate transport and the development of self-sustaining artificial cells and solar-powered nanoreactors.

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S8.

Development of a cell-free biosensor system for the measurement of plastic monomers based on one-pot transcription and ligation

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To address the rapid increase in plastic waste and the resulting environmental pollution, enzymatic depolymerization of plastic into monomers is emerging as a promising sustainable solution. This strategy depends on the discovery of high-performance enzymes, which requires high-throughput screening technologies for accurate detection of plastic monomer. Conventional whole-cell biosensors(WCBs), however, are limited by low cell membrane permeability, and cytotoxicity. To overcome these challenges, this study focuses on developing a cell-free biosensor platform where gene expression components are exposed in solution, making it highly adaptable for sensing diverse chemical compounds. Specifically, we integrate the SENSR(sensitive splint-based one-pot isothermal RNA detection), a cell-free biosensor for sensitive and quantitative detection of pathogen RNA, with plastic monomer-responsive allosteric transcription factors (aTFs). The presence of target monomer, the aTFs regulate the transcription of a fluorescence-activating RNA aptamer, resulting in the production of a fluorescent signal. Here, we present a next-generation cell-free biosensor that enables highly sensitive and quantitative detection of plastic monomers, thereby overcoming the limitations of both WCBs and conventional cell-free biosensor.

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S9. & Pos 13.

Competition of Tau and Paclitaxel Regulates Microtubule Structure

◯Seunghyun RYU[1], Stuart FEINSTEIN[2], Youli LI[2], Cyrus SAFINYA[2], Myung Chul CHOI[1]

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Microtubules (MTs) are a major component of the eukaryotic cytoskeleton, and their architecture is highly regulated by a number of MT-targeted chemotherapeutic agents, such as paclitaxel (PTX), as well as microtubule-associated proteins, such as Tau. We examined the binding ability of PTX and each of the six alternatively spliced isoforms of human wild-type Tau to MTs, as well as their effects upon MT structure. While the extent of PTX and Tau bound to MTs was assayed by high-performance liquid chromatography and co-sedimentation/Western blotting, respectively, the radial size of MTs was determined by synchrotron small-angle X-ray scattering. Our results suggest that all Tau isoforms initially bind to the well-studied binding sites on the outer surface of MTs, followed by binding to a less-well understood binding site within the MT lumen in an isoform-specific manner. Differential use of the luminal Tau binding site of MTs further raises the possibility of isoform-specific action in fetal versus adult nervous systems.

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Day 3 (21st Jan., Wed.)

P13.

Artificial cells that respond to environmental oscillations

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One of the important properties that an artificial cell should have is the ability to respond to environmental changes[1]. The external environmental changes can be broadly classified into chemical and physical changes. We recently reported the development of two novel synthetic riboswitches that respond to two different chemicals, respectively, and switch the protein expression in a reconstituted cell-free protein synthesis (CFPS) system[2]. We encapsulated the CFPS system and DNA-templated encoding reporter genes regulated by each riboswitch in artificial cells and showed that gene expression is switchable (ON/OFF) by external chemicals. We have also succeeded in creating artificial cells that respond to changes in their physical environment. Artificial cells with different lipid compositions were prepared, and when exposed to freezing and thawing (F/T)[3,4], a selective enrichment of the lipid was observed. Moreover, selection acting on lipid composition was propagated to the encapsulated genetic material. We conclude that membrane lipid composition can be a direct target of selection for grown vesicles under an icy environment, leading to indirect but concurrent enrichment of compartmentalized genetic molecules, independent of their encoded information.

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P14.

Anomalous Increase of Sum-frequency Signal From Cationic Langmuir Monolayer Upon Salt Addition

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Ions in water near the interface creates an electrical double layer, in which counterions are attracted while co-ions are repelled from the interface. Adding salt to subphase water is always expected to decrease the electric field in the electric double layer as increased counterions screen the surface charge more effectively. We discovered a phenomenon contrary to this common belief from cationic Langmuir monolayer upon addition of fluoride salts. Langmuir monolayer consisting of DPTAP (1,2-dipalmitoyl-3-trimethylammonium-propane chloride) at different concentrations of fluoride salts (LiF, NaF, and KF) in the subphase water were investigated by sum-frequency vibrational spectroscopy. The OH signal from interfacial water molecules increased upon addition of NaF and LiF, reached a maximum signal (twice larger than that from pure water) at ~ 100 mM, and decreased afterwards. This phenomenon was explained by the action of Li^+ and Na^+ co-ions that binds effectively with the ubiquitous bicarbonate (HCO_3^-) ions at neutral pH and remove these counterions that initially screen the positively charged surface of DPTAP molecules. By contrast, the sum-frequency signal decreased monotonically with KF addition, indicating the K^+ co-ion cannot capture the bicarbonate counterions effectively.

P15. & Pos 14.

Expression, purification, and applications of membrane protein for artificial cell

Authors:

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Abstract:

Rhodopsins are light-sensing seven-transmembrane proteins whose structural and photochemical versatility underpins diverse applications ranging from optogenetics to bio-photonic energy conversion. Yet their intrinsic hydrophobicity and membrane-integration requirements complicate high-yield expression and functional purification. Building upon a cell-free and cell-based protein production framework optimized for large-scale expression under simplified reaction conditions, we developed an efficient method for rhodopsin expression, detergent-based solubilization, and functional purification and reconstitution. Established strategies from microbial expression systems to yeast guide our optimization, including protocols for producing properly folded variants suitable for functional studies in artificial cells. Following expression, rhodopsin was extracted using optimized detergent mixtures and subsequently reconstituted into stabilizing micellar or lipid environments, enabling reliable chromophore binding and verification of native-like absorbance characteristics. The resulting purified protein supports a broad range of downstream applications, including spectral tuning analysis, mechanistic studies of phototransduction, engineered ion-transport systems, and the development of light-driven biochemical modules. This work demonstrates a streamlined rhodopsin production that enhances accessibility to functional membrane protein preparations and accelerates their integration into synthetic biology and photo-bioengineering platforms.

P16.

Application of Alendronate Carbon Dots in Biomedical Field

◯Wenxia Xu , Xiaojun Han

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Carbon dots are a new type of fluorescent nanoparticles with a diameter less than 10 nm. Due to their excellent biocompatibility, rich surface functional groups and high specific surface area, they are widely used in biomedical engineering. We studied a series of new carbon dots derived from alendronate. These carbon dots all have good biocompatibility, photoluminescence properties and bone-targeting ability. We used the microwave method to synthesize ALEN-CDs with polyethylene glycol and alendronate as precursors. ALEN-CDs can inhibit the differentiation and maturation of osteoclasts, and exert immunomodulatory effects by inhibiting the polarization of macrophages to the pro-inflammatory M1 phenotype and promoting their polarization to the anti-inflammatory M2 phenotype. Moreover, compared with alendronate, large doses of ALEN-CDs do not cause bisphosphonate-related osteonecrosis of the jaw, which may be related to the regulation of M2 macrophage polarization. In addition, in vivo studies have shown that ALEN-CDs can significantly reduce bone loss and improve osteoporosis in castrated mice. Proteomic analysis indicates that ALEN-CDs may regulate the bone immune microenvironment by affecting mitochondrial metabolism, especially oxidative phosphorylation. In conclusion, ALEN-CDs can directly inhibit osteoclast differentiation and regulate the bone immune microenvironment, and may be a promising drug for the treatment of osteoporosis.

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R3.

Divalent Cation Regulation of Actin Filamentary Bundles and Networks

◯Sang Ho Lee[1], Chang Ho Kim[1], Soo Ryeon Ryu[1], Kwanwoo Shin[1]

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The regulation of actin filamentary bundles and networks by divalent cations, especially calcium (Ca^{2+}) and magnesium (Mg^{2+}), plays a pivotal role in the structural and functional dynamics of eukaryotic cells. Actin filaments, crucial for cytoskeletal support, external stress resistance, and cellular motility, are significantly influenced by G-actin concentration and the presence of Mg^{2+}. Advanced imaging techniques, such as TEM, confocal, and STED microscopy, are employed to elucidate the effects of varying G-actin and Mg^{2+} concentrations on stable actin network bundle formation, particularly effective at 17mM Mg^{2+}. While TEM provides a detailed yet narrow view of actin filament structure, confocal microscopy offers a broader perspective, crucial for understanding large-scale actin network organization. Additionally, the use of a flow system allows for the observation of actin network formation in a dynamic and continuous manner, surpassing the limitations of static batch conditions. This comprehensive approach not only highlights the role of Mg^{2+} in actin-ATP interactions and hydrolysis but also opens new avenues in disease modeling, therapeutic applications, and the development of artificial cell models, enhancing our understanding of cellular mechanisms.

R4.

High-Yield E. coli CFPS Optimization Enables Rapid Carbonic Anhydrase Screening

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Cell-free protein synthesis (CFPS) is a key enabling technology for artificial cell research, where high expression yield under defined conditions is essential for robust prototyping. Here, we established an *E. coli* extract–based CFPS platform and optimized it for maximum protein production using sfGFP as a quantitative reporter. To improve practicality and reduce reagent cost, we implemented a maltodextrin/hexametaphosphate (HMP)–driven energy regeneration scheme and systematically tested major workflow and reaction parameters [1]. Maximal expression was obtained by simplifying common steps/additives: run-off reaction and lysate dialysis were omitted (clarified lysate used directly), and PEG-8000 was excluded due to reduced protein synthesis. Strain comparison indicated that BL21 (DE3) provided the best performance in our setup. The optimized CFPS condition supported robust expression even at low plasmid DNA inputs (down to 2 nM, 4 ng/μL), enabling cost-effective screening and DNA limited implementations.

Using this optimized CFPS workflow as a rapid screening front-end, we next investigated carbonic anhydrases (Cas), ubiquitous metalloenzymes that catalyze $CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$ and contribute to inorganic carbon balance and pH homeostasis across organisms [2]. CFPS-based screening identified promising CA candidates, and a theta-class CA was selected for downstream validation. The selected enzyme was subsequently expressed and purified in *E. coli*, and its enzymatic activity was confirmed by activity assays. While light-driven CO_2 fixation in artificial cells remains a future objective, these results establish a practical pipeline linking high-yield CFPS optimization to enzyme discovery/validation and motivate future integration of CA with compartmentalized cell-free systems, potentially in combination with light-powered ATP modules and RuBP/Rubisco-based carbon fixation.

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5.2 Abstract - Poster Presentation

Pos 01.

Overexpression of $\alpha_5\beta_1$ Integrin in HEK293T Cell Enhances Cell–Cell and Cell–ECM Interactions Enabling Multi-Cell Layer Fabrication

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Cell–cell and cell–extracellular matrix (ECM) interactions are essential for maintaining the structural and functional integrity of tissues. The integrin receptor $\alpha_5\beta_1$, a principal fibronectin-binding molecule, plays a pivotal role in mediating these adhesive interactions. In this study, we investigated how overexpression of $\alpha_5\beta_1$ integrin enhances intercellular and cell–ECM adhesion using human embryonic kidney (HEK293T) cells as a model system. HEK293T cells were genetically modified to overexpress $\alpha_5\beta_1$ integrin via viral transduction, followed by selection using fluorescence-activated cell sorting (FACS). Successful overexpression was confirmed by flow cytometry analysis, western blot and immunocytostaining. Functional assays demonstrated that $\alpha_5\beta_1$ integrin overexpression significantly enhanced cell aggregation and cell–ECM adhesion properties compared to control cells. Furthermore, as an application of enhanced adhesion, $\alpha_5\beta_1$ integrin-overexpressed cells exhibited improved structural organization in multi-cell layer assembly, suggesting their potential utility in scaffold-free tissue engineering approaches. These findings highlight the critical role of $\alpha_5\beta_1$ integrin in regulating adhesive properties and its promise as a molecular target for advancing cell-based tissue construction.

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Pos 02.

Human tau-microtubule structural phase transitions regulated by ionic strength

◯Hasaeam Cho[1], Hanjoon Nho[1], Juncheol Lee[1], Sang Yeop Lee[1], Shina Min[1], Kyeong Sik Jin[2], Herbert P. Miller[3], Leslie Wilson[3], Stuart C. Feinstein[3], Cyrus R. Safinya[3], Myung Chul Choi[1]

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Human tau is one of the microtubule-associated proteins (MAPs) and mostly found in the axons of neurons. Tau is highly soluble, a random-coil-like intrinsically disordered protein (IDP), and a polyampholyte with a specific distribution of both positive and negative charges. Tau proteins bind to the microtubules (MTs) in neurons and stabilize their structures. Neuronal MTs form bundles that maintain the cytoskeleton. In contrast, MT structures in other human cells are dynamic, such as spindle fibers during mitosis. While the binding architecture of tau and MT is unclear, it is known that electrostatic interactions drive their binding. The positively charged microtubule-binding-region (MTBR) of tau has an affinity for the negatively charged MT surface. Also, tau is related to numerous neurodegenerative disorders collectively termed ‘tauopathies’, including Alzheimer’s disease, progressive supranuclear palsy, and frontotemporal dementia with Parkinsonism linked to chromosome 17. Here, we present human tau-microtubule binding models and structural phase transitions regulated by ionic strength.

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Pos 03.

Tubulin NanoStructures as Drug Delivery Platform

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We introduce Tubulin NanoStructures (TNSs) as a drug delivery platform for microtubule-targeting agents (MTAs) in cancer therapy. A range of MTAs, including docetaxel, laulimalide, and monomethyl auristatin E, bind to specific drug-binding pockets on tubulin and, in the presence of poly(L-lysine), cooperatively induce conformational changes in tubulin protofilaments. This synergistic interaction drives the self-assembly of tubulin into TNSs, leading to increased drug-loading efficiency and enhanced physiological stability. TNSs effectively induced apoptosis in cancer cells and selectively accumulated in tumors, resulting in significant inhibition of tumor growth in xenograft mouse models. Collectively, this work establishes a new concept of using a drug's target protein as a carrier and suggests the potential of TNSs as a versatile platform for the targeted delivery of diverse therapeutics.

Pos 04.

Construction of liposome-based biological systems with DNA computing for autonomous molecular recognition and response

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Bottom-up approaches for constructing biological systems within lipid membranes (liposomes) have attracted considerable attention in synthetic biology as platforms for elucidating the principles underlying self-responsive artificial cells [1]. In this study, we constructed liposome-based artificial cellular systems incorporating DNA computing circuits using a microfluidic fabrication strategy[2]. Cell-sized liposomes were generated through a controlled droplet-to-liposome transformation process, enabling stable encapsulation of functional DNA reaction networks. Streptolysin O (SLO) embedded in the lipid membrane facilitated the transport of external molecular signals into the liposome interior, where they were processed by internal DNA computing circuits to trigger autonomous molecular responses. This system demonstrates a closed, membrane-confined process from signal input to internal information processing and response generation. Our results highlight a design principle for artificial cells capable of programmable molecular responses and provide insight into the construction principles of self-responsive artificial cellular systems.

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Pos 05.

Programmable Self-assembly: Controlling Tubulin Architecture via Molecular Switches

◯Juncheol Lee[1], Eunsun Song[1], Byungsoo Kim[1], Chaeyeon Song[1], Hanjoon Nho[1], Hasaeam Cho[1], Sanghoon Lee[2], Gayoung Kim[2], Kipom Kim[2], Myung Chul Choi[1]*

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Tubulins are protein building blocks intrinsically preprogrammed to assemble into cytoskeletal microtubules (MTs). MTs undergo dynamic structural transitions essential for cellular functions, driven by 2D orthogonal in-plane conformational modes of tubulin. We controlled higher-order tubulin assemblies by using cationic polymers as molecular switches that electrostatically engage tubulin and modulate its 2D shape. Synchrotron small-angle X-ray scattering (SAXS) and transmission electron microscopy (TEM) resolved the nanoscale architectures of the tubulin-based nanotubes. These findings provide the insight into the design of anticancer drug-delivery systems and suggest potential routes towards templates for metallization and molecular machines. Here, we report our latest findings in tubulin nanotechnology.

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Pos 06.

Synergistic Anti-Inflammatory and Antioxidant Effect via Targeted Co-Delivery of Dual Therapeutic Proteins

◯Junyoung Jung[1], Giyoong Tae[1]

[1]School of Materials Science and Engineering, Gwangju Institute of Science and Technology

Effective treatment of inflammatory diseases often requires simultaneous suppression of both excessive immune responses and oxidative stress. Here, we introduce a targeted co-delivery platform designed to address both pathological axes using a combination of an anti-inflammatory cytokine and an antioxidant enzyme. These dual therapeutic proteins were co-encapsulated in a target-specific peptide-conjugated nanocarrier. In an inflammatory animal model, the targeted co-delivery of an anti-inflammatory cytokine and an antioxidant enzyme showed markedly improved therapeutic outcomes compared to monotherapies. These include enhanced suppression of inflammatory cytokines, reduced oxidative damage, and improved restoration of tissue homeostasis. Notably, the cooperative interaction between the proteins led to superior efficacy that was not achievable with either agent alone. To the best of our knowledge, this is the first report demonstrating the co-delivery of an anti-inflammatory cytokine and an antioxidant enzyme as a synergistic therapeutic strategy, offering a new avenue for protein-based combinational nanomedicine.

References: Jung *et al.*, *Bioactive Materials*, **2025**, 52, 604-622

Pos 07.

Expression control in the PURE system using T7 promoter variants.

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One of the key challenges in bottom-up synthetic biology is constructing a self-regenerating system *in vitro*. Our recent study successfully regenerated all 20 aminoacyl-transfer RNA synthetases (AARSs) in the PURE system by optimizing the concentration of each template DNA[1]. However, this approach precludes coupling with DNA replication, which is essential for realizing evolutionary processes. Therefore, it is necessary to integrate these genes into a single template DNA using an alternative, concentration-independent expression control method.

In this study, we implemented expression control at the transcriptional level by utilizing a library of T7 promoters comprising all native T7 promoter sequences and their variants[2]. The activity of each T7 promoter was characterized by quantifying the mRNA levels of a downstream reporter gene via RT-qPCR. We will present our findings, including the latest results.

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[2] Komura, Ryo, Wataru Aoki, Keisuke Motone, Atsushi Satomura, and Mitsuyoshi Ueda. 2018. "High-Throughput Evaluation of T7 Promoter Variants Using Biased Randomization and DNA Barcoding." *PloS One* 13 (5): e0196905.

Pos 08.

Quantitative Analysis of the Dynamics of Gel-in-GUV System in Response to Osmolality

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Living cells change their volume in response to osmotic pressure, which simultaneously affects membrane morphology as well as the internal molecular density, diffusion of molecules, and the kinetics of associated biochemical reactions[1]. To investigate the relationship between the changes in cytoplasmic physical properties and membrane shape transformations, a constructive approach using simplified models based on artificial cells is effective. The Gel-in-GUV system, an artificial cell model encapsulating a hydrogel, mimics molecular crowding in the cytoplasm and exhibits swelling and shrinking behavior in response to osmotic pressure. In PEG-hydrogel–encapsulated GUVs used in this study, we found that variations in gel shrinkage, PEG concentration, and the size of encapsulated molecules lead to differences in the number and size of membrane buds formed, as well as in the spatial distribution of the encapsulated molecules. This system, which allows precise control of membrane morphology and the intravesicular environment, not only contributes to a deeper understanding of the relationship between membrane dynamics and intramembrane molecular behavior but also holds potential for the creation of artificial organelles and self-replicating artificial cells.

References: 1. Mourão MA, Hakim JB, Schnell S. Connecting the dots: the effects of macromolecular crowding on cell physiology. *Biophys J*. 2014;107(12):2761-2766. doi: 10.1016/j.bpj.2014.10.051

Pos 09.

Artificial bacterial cells forming a biofilm-like structure

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Biofilms are living assemblies of microorganisms that enhance stress tolerance and material conversion efficiency through high-density organization. Our research group aims to reconstruct biofilms composed of artificial cells. As an initial step, we sought to develop controllable self-aggregating liposomes. Previously, we constructed artificial cells capable of nonspecific adhesion by combining benzylguanine (BG)–modified liposomes with a SNAP-tag–fused adhesive nanofiber protein, AtaA1. In the present study, to achieve selective liposome aggregation, we employed nanobody–antigen pairs developed by Glass *et al*.[2], each fused to a SNAP tag. Mixing liposomes modified with the SNAP-nanobody or antigen resulted in antigen–antibody–mediated aggregation, which was confirmed by flow cytometry and confocal laser scanning microscopy.

References: [1]*J Am Chem Soc*. 141, 48, 19058-19066 (2019), [2]*Cell*, 174, 3, 649-658 (2018)

Pos 10.

miRNA-Triggered Antisense Oligonucleotide Synthesis in Liposomes

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Theranostics integrates diagnostic and therapeutic functions within a single carrier to enable personalized cancer treatment. Here, we report a lipid vesicle-based molecular robot that autonomously performs molecular diagnosis and antisense oligonucleotide synthesis inside a single liposome. Designed for small-cell lung cancer, this system detects the biomarker miR-20a and triggers in situ synthesis of the antisense oligonucleotide Oblimersen via an isothermal DNA computing reaction encapsulated in a giant unilamellar vesicle (GUV).[1] A pore-forming protein, streptolysin-O (SLO), facilitates miR-20a transport across the lipid membrane. Oblimersen synthesis persisted for over three hours and was confirmed using a fluorescent molecular beacon. These results demonstrate an integrated liposomal system that combines molecular diagnosis with targeted therapeutic molecule synthesis, with future evaluation planned in HeLa cells.

References: 1. Takiguchi et al., *Small*, 21, 34, 2501560 (2025).

Pos 11.

Development of a Flexible and Biocompatible Electrochemical Sensor for Continuous Liver Enzyme Monitoring

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Liver enzymes such as AST are widely used to assess liver function and to support early diagnosis and disease monitoring, but conventional blood tests are invasive and unsuitable for continuous measurement. To address this, we developed a flexible, biocompatible electrochemical sensor integrated with a hollow microneedle (ECS-HMN) platform for on-skin, continuous AST sensing. Laser-induced graphene electrodes were transferred onto PDMS and functionalized with Cu nanoparticles, Nafion, and a GelMA hydrogel, enabling stable detection under physiological conditions (LOD 4.96 U/L) and month-long performance. Hollow microneedles fabricated by SLA 3D printing enabled continuous ISF sampling, and negative-pressure tests on hydrogel skin models confirmed stable operation when ≥100 μL of ISF was collected, demonstrating feasibility for wearable liver monitoring.

References: Vivaldi, F. M.; Dallinger, A.; Bonini, A.; Poma, N.; Sembranti, L.; Biagini, D.; Salvo, P.; Greco, F.; Di Francesco, F. ACS Appl. Mater. Interfaces 2021, 13(26), 30245–30260; Rathinam, K.; Singh, S. P.; Li, Y.; Kasher, R.; Tour, J. M.; Arnusch, C. J. Carbon 2017, 124, 515–524; Chinnappan, R.; Mir, T. A.; Alsalameh, S.; Makhzoum, T.; Alzhrani, A. Diagnostics 2023, 13(18), 2967.

Pos 12.

Single-Nozzle Extrusion-Based 3D Bioprinting Technique: Fabricate Hierarchical Structure in Tissue Engineering

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Gradient hydrogel fabrication provides a powerful approach for mimicking the spatial heterogeneity of native tissues, which are crucial for replicating diverse tissue interfaces. Challenges remain in fabricating functionally graded scaffolds using a single-nozzle system capable of both continuous and discontinuous hierarchical gradient transitions. In this study, we introduce a single-nozzle extrusion 3D bioprinting technique, termed single-nozzle active mixing with on-printing refueling, which enables both continuous and discontinuous transitions. Notably, we emphasize the single-nozzle discontinuous transition, which has not been previously demonstrated. To test the functional gradient of hydrogel, a human ear cartilage model was fabricated with ink composed of methacryloyl, sodium alginate, and polyethylene glycol diacrylate (PEGDA), where the local elasticity of the hydrogel was controlled by altering the concentration of PEGDA from 0%v/v to 10%v/v. The printed ear cartilage scaffold showed an elasticity difference from 9.37 to 120 kPa depending on PEGDA concentrations. Moreover, scanning electron microscopy of the gradient scaffold interfaces revealed composition-dependent pore sizes: 5–9 μm in the non-PEGDA region and 3–5 μm in the 10% PEGDA-containing region. This new technique shows great potential for engineering complex tissue interfaces, disease modeling, and next-generation biomedical applications.

(1) Jadhav, S.; Kusekar, S.; Belure, A.; Digole, S.; Mali, A.; Cheepu, M.; Mugale, M.; Alkunte, S.; Kim, D. Recent Progress and Scientific Challenges in Wire-Arc Additive Manufacturing of Metallic Multi-Material Structures. *Journal of Manufacturing and Materials Processing* **2025**, *9* (8), 284.

Pos 15.

Cell-free synthesis of peptide nanopores

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Here we present studies on the synthesis of peptides and proteins that form nanometer-scale pore structures (nanopores) in bilayer lipid membranes using a cell-free protein synthesis (CFPS) system. Nanopores are most commonly used for single-molecule detection, known as nanopore sensing. In the context of artificial cells, they are used to transport molecules or information between the exterior and interior of lipid bilayer vesicles [1]. To enable straightforward synthesis of these nanopores, we employ a CFPS platform, specifically the PURE system. Recently, by optimizing the liposomal lipid composition in liposome-supplemented CFPS, we have successfully synthesized and characterized a highly aggregative peptide nanopore SVG28 [2]. In addition to this work, we will introduce several enabling technologies, such as CFPS of hydrophilic peptide nanopores [3] and the design of novel *de novo* nanopore proteins.

References: [1] Z. Peng *et al., Lab Chip* (2024)

[2] K. Shimizu *et al., Nat. Nanotechnol.* (2022)

[3] S. Fujita *et al., ACS Nano* (2023)

Pos 16.

Development of microtubule binding-tetrameric protein that control bundling of intracellular microtubules

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Microtubules are tubular cytoskeletons that are formed by polymerization of tubulin dimers, and involve in various cell functions such as cell division and migration. Specifically, microtubules form axon by bundling microtubules in neuron[1)]. Herein, we focused on microtubule binding domain of microtubule-associated protein MAP4 (MAP4m) and tetrameric fluorescent protein Azami-Green (AG) for artificial bundling of microtubules. We developed a microtubule bundling protein (MAP4m-AG) which fused MAP4m and AG. By using MAP4m-AG, we achieved bundling and stabilization of intracellular microtubule, and regulation of cell functions.

References: 1). C. Conde, A. Cáceres, *Nat. Rev. Neurosci*., 10, 319 (2009).

Pos 17.

Fibronectin-Coated Nanoliposomes with Heparin-Hemin for Enhanced Ulcerative Colitis Therapy

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Ulcerative colitis, an inflammatory bowel disease, involves mucosal inflammation, excessive ROS accumulation, and ECM degradation. Current immunosuppressive therapies fail to address ROS damage and ECM depletion. We developed a dual-functional nanotherapeutic combining heparin-conjugated hemin nanozymes (Hep-H) with fibronectin-coated liposomes (FN-SUVs). Hep-H provides catalase and superoxide dismutase mimetic activities for ROS scavenging, while FN-SUVs restore ECM integrity and prevent bacterial adhesion. Three conjugation strategies were evaluated; Hep-H encapsulation within FN-SUVs demonstrated superior ROS scavenging and excellent biocompatibility. This platform simultaneously eliminates ROS and restores ECM, enabling comprehensive tissue repair beyond conventional therapies.

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Sahu, A.; Min, K.; Jeon, S. H.; Kwon, K.; Tae, G. Self-Assembled Hemin-Conjugated Heparin with Dual-Enzymatic Cascade Reaction Activities for Acute Kidney Injury. *Carbohydrate Polymers* **2023**, *316*, 121088–121088

Pos 18.

Ribosome biogenesis in vitro

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Ribosome biogenesis is pivotal in the self-replication of life. In *Escherichia coli*, three ribosomal RNAs and 54 ribosomal proteins are synthesized and subjected to cooperative hierarchical assembly facilitated by numerous accessory factors. Realizing ribosome biogenesis in vitro is an essential goal to understand the self-replication of life and creating artificial cells; however, this has not been realized because of its complexity. We have reported the first successful ribosome biogenesis in vitro. Specifically, we developed a highly specific and sensitive reporter assay for the detection of nascent ribosomes. The reporter assay allowed for combinatorial and iterative exploration of reaction conditions for ribosome biogenesis, leading to the simultaneous, autonomous synthesis of both small and large subunits of ribosomes in vitro through transcription, translation, processing, and assembly in a single reaction space. Our achievement represents a critical step toward revealing the fundamental principles underlying the self-replication of life and creating artificial cells.

References: Kosaka, Y., Miyawaki, Y., Mori, M. et al. Autonomous ribosome biogenesis in vitro. Nat Commun 16, 514 (2025). https://doi.org/10.1038/s41467-025-55853-7

Pos 19.

RNA liquid condensate operating AND logic gate

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DNA droplets, artificial liquid-like condensates of well-engineered DNA sequences, allow the critical aspects of phase-separated biological condensates to be harnessed programmably, such as molecular sensing and phase-state regulation. In contrast, their RNA-based counterparts remain less explored despite more diverse molecular structures and functions ranging from DNA-like to protein-like features. Here, we design and demonstrate computational RNA droplets capable of two-input AND logic operations. We use a multibranched RNA nanostructure as a building block comprising multiple single-stranded RNAs. Its branches engaged in RNA-specific kissing-loop (KL) interaction enables the self-assembly into a network-like microstructure. Upon two inputs of target miRNAs, the nanostructure is programmed to break up into lower-valency structures that are interconnected in a chain-like manner. We optimize KL sequences adapted from viral sequences by numerically and experimentally studying the base-wise adjustability of the interaction strength. Only upon receiving cognate microRNAs, RNA droplets selectively show a drastic phase-state change from liquid to dispersed states due to dismantling of the network-like microstructure. This demonstration strongly suggests that the multistranded motif design offers a flexible means to bottom-up programming of condensate phase behavior. Unlike submicroscopic RNA-based logic operators, the macroscopic phase change provides a naked-eye-distinguishable readout of molecular sensing. Our computational RNA droplets can be applied to in situ programmable assembly of computational biomolecular devices and artificial cells from transcriptionally derived RNA within biological/artificial cells.

References: "Programmable computational RNA droplets assembled via kissing-loop interaction." *ACS nano* 18.24 (2024): 15477-15486.

Pos 21.

RNA replicators fused with parasites in evolutionary experiments

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It is thought that at the origin of life, self-replicating organic matter evolved and complexified, thereby giving rise to primitive organisms. In our laboratory, a translation-coupled RNA replication system has been constructed, which consists of RNA encoding RNA polymerase and a cell-free translation system encapsulated in water-in-oil droplets [1]. Long-term evolutionary experiments using this system as a model for self-replicating organic matter have confirmed the diversification up to five lineages through host-parasite competition and the formation of a cooperative replication network [2-3].

In the present study, the RACE method was used to read the terminal sequences, and we found that self-replicating RNA became longer than expected and that “extra” sequences originated from the short parasitic RNA. This suggests that self-replicating RNA fused with parasitic RNA in the evolutionary experiment, and that replicators may acquire new functions by fusion with parasites.

References: [1] N. Ichihashi, *et al.*, 2013 [2] T. Furubayashi, *et al.*, 2020 [3] R. Mizuuchi, *et al.*, 2022

Pos 22.

Toward Genetically Encoded Membrane Synthesis in Artificial Cells using Lysophospholipid Acyltransferases

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In this study, we aim to construct an artificial cell capable of synthesizing membrane molecules using genetically encoded enzymes, enabling the evolutionary optimization of membrane synthesis.

Previous studies by Bhattacharya et al. (2019, 2021) achieved efficient vesicle growth and division using chemically modified lysophospholipids that react with adenylated fatty acids to form phospholipids. Inspired by this design, we focused on lysophospholipid acyltransferases (LPLATs), membrane protein that catalyze the same reaction biologically. We constructed a sequence similarity network of LPLATs to identify candidate enzymes and performed cultivation assays to detect phosphatidylcholine (PC) production. Among ten newly tested enzymes, two novel LPLATs exhibiting PC production were identified. We are currently characterizing the acyl-chain specificity of the produced phosphatidylcholine and evaluating their suitability for in vitro expression toward artificial cell reconstruction.

Pos 23.

Synthetic Biomolecular Condensates with Anisotropic Morphology

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Biomolecular self-assembly provides a fundamental framework for understanding molecular mechanisms in biology and for guiding the rational design of functional materials systems. Such assemblies exhibit a wide range of structural organizations, from fluidic architectures, such as phase-separated droplets, to highly ordered structures, including fibrils. Here, we focus on cell-sized biomolecular condensates with anisotropic morphologies that integrate both fluidic and structurally ordered properties. These condensates are formed through hierarchical supramolecular assemblies of chemically defined peptides and oligonucleotides. In this poster presentation, we report the design strategies, assembly dynamics, and structural characteristics of these systems. These findings suggest the possibility that synthetic biomolecular condensates may be developed as foundational materials for artificial cell systems.

Reference: H. Sugai *et al*. *ChemRxiv* DOI: 10.26434/chemrxiv-2025-xh4zs

Pos 24.

Toward More Stable Artificial Cells using Gel-in-Giant Unilamellar Vesicle System

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Giant unilamellar vesicles (GUVs) have been widely utilized as models of artificial cells yet possessing obvious limitations due to their simplicity and low stability under complex conditions to mimic natural cells. In this study, we present a gel-in-GUV system (GiG), in which a polyethylene glycol (PEG) hydrogel is encapsulated as artificial inner cellular environment and covalently linked to the lipid bilayer to mimic the binding between cell membrane and cytoskeleton. This GiG system exhibits enhanced stability against detergents and mechanical stress, while exhibiting benign ability of substance exchange via osmolarity variation and incorporation of membrane proteins. Henceforth, this system offers valuable insights into the design of more robust and complex artificial cell models.