様式 Form 7
（外国人招へい研究者）

Fellowship ID：BR240204

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独立行政法人日本学術振興会理事長　殿
To: President, Japan Society for the Promotion of Science

研究活動報告書
Research Report

１．受入研究者/ Host researcher

受入研究機関・部局・職 Name of Host Institution, Department and Title	東京大学・生産技術研究所・准教授
受入研究者氏名 Host Researcher's Name	金　秀炫

２．外国人招へい研究者/ Fellow

所属研究機関・部局・職 Name of Institution, Department and Title	IEMN, UMR CNRS8520, Reseracher
外国人招へい研究者氏名 Fellow's Name	**BACCOUCHE Alexandre Raymond Maher**

３．採用期間/ Fellowship Period

2024 年　9 月　25 日　～　2024 年　10 月　14　日　20　日（Days）　か月（Months）

４．研究課題/ Research Theme

Coupling CRISPR based transcriptional regulators to artificial protein synthesis towards *in vitro* gene regulatory networks

５．研究活動報告/ Research Report

（1）研究活動の概要・成果/ Summary of Research Results

During this 20-day research visit, we successfully conducted preliminary experiments on implementing molecular program-based control over protein synthesis in abiotic conditions, using cell-free protein synthesis (CFPS) and the PURE system. The focus of the research was to evaluate and optimize the compatibility, performance, and interaction of the molecular program with the protein synthesis process. In particular, we redirected the previously developed smart RNA guides for conditional control of CRISPR-Cas activity (the main result of the JSPS fellowship in 2017) toward the inhibition of in-vitro translation of a synthetic gene coding for four proteins:

- UnaG is a green fluorescent protein (480/510 nm) derived from the Japanese eel (Anguilla japonica). It requires the cofactor bilirubin for fluorescence and this interaction is characterized by high specificity and affinity, with a dissociation constant (Kd) of approximately 98 pM
- smURFP is a near-infrared fluorescent protein (640/660 nm) derived from Trichodesmium erythraeum IMS101. It requires the cofactor biliverdin for fluorescence and is known for its rapid maturation.
- HiGFP is a green fluorescent protein (480/510 nm) derived from Hydra viridissima and miRFP670 is a near-infrared fluorescent protein (65/670 nm) derived from Rhodopseudomonas palustris.

（注）採用期間終了後３ヶ月以内に提出
※　(Note) Submit the form within 3 months after the expiration of fellowship.
※　様式１に記載された情報を基に確認しますので、部局名等の名称含め、内容に誤りが無いか必ずご確認ください。

- miRFP670 has an excitation maximum at approximately 645 nm and an emission maximum at around 670 nm. This protein also requires biliverdin as cofactor for fluorescence

The workflow of this project laid as follows: first, the preparation of the DNA from the synthetic gene (gblock, IDT) by PCR amplification. After quantification, we assembled a fraction of PCR product with the PURE components (including ribosomes, tRNAs, aminoacyl-tRNA synthetases, translation factors, nucleotides, energy sources, and necessary salts) to synthesize the target protein. At first, PCR and agarose gel analyses were conducted on these synthetic genes. Initial experiments revealed that only UnaG produced the best detectable fluorescence signal, indicating successful expression and functionality. Subsequent work investigated the influence of temperature changes on protein folding for unresponsive proteins. We then tested the compatibility of different buffer systems to ensure they supported both the activity of the molecular program and the efficiency of protein production. The results showed that the selected buffers provided the necessary stability and consistency for both components to function effectively. Additionally, we worked on tuning the production rates to achieve a balance between the molecular program's output and the synthesis rate of the target protein. By optimizing these rates, we reduced bottlenecks and improved overall system performance.

Further experiments targeted molecular program interactions with synthetic gene expression. Guide RNAs were transcribed in vitro to target specific regions of the UnaG gene (promoter, RBS, and codons 10-16) and were combined with Cas9 before introducing the complex into PURE reactions. However, no significant effects on protein synthesis were observed, likely due to the high efficiency of the PURE system. To address this, experiments shifted focus to destabilizing reaction conditions and enhancing CRISPR-Cas9 activity by increasing concentrations. Results suggest the need for a comprehensive redesign of RNA guides and modifications to the synthetic gene for improved outcomes.

Networking objectives were also successfully addressed. Meetings with Dr. Tony Z. Jia (Tokyo Institute of Technology) focused on collaborations in prebiotic chemistry, particularly molecular programming within the context of the origin of life. Discussions with Dr. Pauline Robbe (RIKEN) explored integrating synthetic biology and bioinformatics for cancer research, emphasizing early detection strategies. Additionally, meetings with Pr. Yutetsu Kuruma and Pr. Nomaki Hidetaka (JAMSTEC) examined biomineralization systems in synthetic biology and their relevance to origin-of-life studies.

（２）主な研究発表（雑誌論文、学会、集会、知的財産権等）/ Main Research Publications

During this research visit, I prepared and submitted two grant applications.

The first application, in collaboration with Pr. Soo Hyeon Kim, focuses on developing artificial cell systems for the concentration and isolation of circulating tumor cells (CTCs). This project leverages synthetic vesicle-based systems to capture and isolate CTCs from blood samples, facilitating improved diagnostics and cancer monitoring. The grant is part of the international funding initiative "Welcoming Internationals to Lille" (WILL), a France 2030 program designed to foster international collaborations at the University of Lille. The WILL program supports 14 international research chairs over four years, promoting high-level cooperation between foreign senior researchers and Lille's academic teams, including CNRS, Inserm, and Centrale Lille. This project integrates expertise in synthetic biology and microfluidics to address clinical challenges in oncology.

The second grant application is a collaboration with Pr. Yutetsu Kuruma at JAMSTEC and supported by IEMN through a FEDER-funded project. This FEDER initiative focuses on enhancing regional research infrastructure in collaboration with international research institutions and supporting projects with high innovation potential. The call emphasizes research and development in synthetic and nanotechnologies for emerging applications, with priorities on sustainable energy, health technologies, and biomimetic systems. Our project aims to harness cell-free protein synthesis and RNA nanotechnology within artificial systems to simulate complex biochemical processes found in underwater organisms, exploring applications ranging from the origin of life studies to innovative bioremediation solutions. This collaboration combines JAMSTEC's expertise in marine biology with IEMN's cutting-edge nanotechnology facilities to deliver transformative research outcomes.

（３）その他/ Remarks

None

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