

# MERIT long-term overseas deployment report

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Location: Technology university of Eindhoven (TU/e), Netherlands

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Research project: Protein-ligand weak interaction analysis by confinement within hollow-cages

## 1. Research

The host, Prof. Luc Brunsveld's laboratory, focuses on analyzing the interactions between 14-3-3 protein and peptide ligands. By combining my expertise in protein encapsulation within hollow cages, they worked on analyzing weak interactions between proteins and ligands within these complexes (Fig. 1).

The encapsulation of the 14-3-3 protein into the hollow cage was achieved as expected. The fluorescence polarization (FP) assay was attempted as a method to assess the interaction with the ligand. However, the evaluation was difficult due to the aggregation and absorption of the complex itself. Additionally, it was not possible to determine whether the encapsulated protein maintained its structure and bound to the ligand. Therefore, we evaluated the structure and stability of the caged 14-3-3 protein by circular dichroism (CD) and differential scanning fluorimetry (DSF) measurements. The results showed that the structure of the 14-3-3 protein was denatured in acetonitrile, making it difficult to analyze its interaction with the ligand.

We also carried out interaction analysis with the ligand of lysozyme, which has been found to retain its structure. FP assay and DSF measurements showed that it indeed interacted with ligand. However, a detailed evaluation of the dissociation constants and other specifics was not achieved. In the future, we will continue to evaluate the interaction between ligands and caged proteins, including other proteins, and aim to discuss the dissociation constants and other specifics.

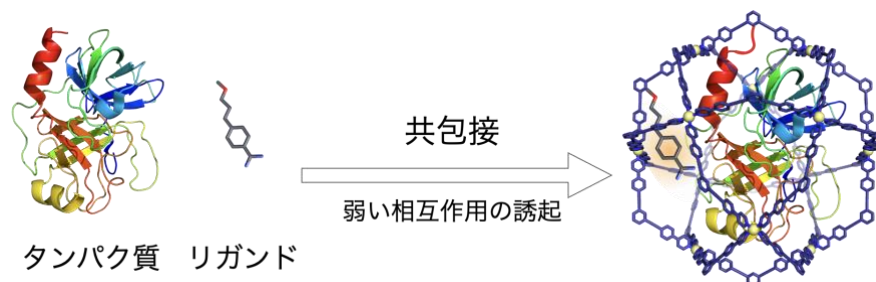


Fig.1 Protein-ligand weak interaction analysis by co-encapsulation of protein and ligand within cage.

## 2. Life in Netherlands

The host laboratory had about 30 students, many of whom were from outside the Netherlands. During the weekly meetings, there were not only lab discussions but also departmental meetings, providing the opportunity to learn about research in a variety of fields. Compared to Japan, I noticed that people here maintained a better work-life balance, focusing and working efficiently without staying late into the night. Attending international conferences and hearing from world-renowned researchers were also valuable experiences. Additionally, the laboratory organized tennis and table football tournaments, in which I was allowed to participate (Fig. 2). I brought my own racket, which made it easier to engage in activities outside of research and communicate with others. After the tennis tournaments, I even had the chance to play tennis privately with lab members, which was

a rewarding experience. Weekends were spent by traveling around Europe. I attended a tennis tournament in a neighboring town and watched the French Open (Fig. 3). I also enjoyed the unique European atmosphere while watching events like EURO 2024. Traveling in Europe was a great opportunity to experience the diverse cultures of various countries, as it was easy to visit different places for leisure.

## 3. Acknowledgements

I am very grateful to Prof. Luc Brunsveld, who accepted and supervised my stay, as well as to Dr. Yan Ni and Ms. Edmee Vandenboorn for their discussions and guidance, and to everyone in the laboratory. Additionally, I deeply appreciate my supervisor, Prof. Makoto Fujita, for respecting my wishes. I am also very thankful to Assistant Prof. Takahiro Nakama for his invaluable help and discussions about my research while I was studying abroad. This dispatch was made possible by financial support from the Japan Society for the Promotion of Science (JSPS), and I would like to express my gratitude for their support.



Fig.2 Tennis tournament in lab



Fig.3 Roland Garros