The assembly pathway of a conserved kinase-kinesin complex in regulating motile cilia function

WU Yonglin (BSc – Biochemistry)
Advisor: Dr. HE Mu (School of Biomedical Science)

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ABSTRACT

Motile cilia are whip-like organelles in the cell membranes playing an important role on body-fluid circulation, such as the ciliated epithelium in trachea to transport mucus for expectoration. Dysfunctional motile cilia can lead to mucus accumulation, resulting in diseases such as Primary Ciliary Dyskinesia (PCD), which is associated with lower respiratory tract infections. In molecular studies, cilia are constructed by nine microtubule doublets, and the central pair of microtubules endows the cilia to become motile. Abnormal central pairs of microtubules makes abnormal motile cilia beating speed and direction.

To study for therapeutics of PCD, untying the motile ciliogenesis mechanism and pathway becomes important. Scientists have previously found and verified that KIF27 and STK36 bind with each other, while knocking either of the proteins lead to misconstruction of the central pair of microtubules. With the information, I hypothesized that the KIF27 forms a complex with STK36 to generate and regulate the central pair of microtubules in motile cilia to regulate the directional beating flow, however the docking site(s) of the two proteins remains unclear.

In the research project, I aim to investigate specific binding site of STK36 docking with KIF27. I found that the central domain of KIF27 has multiple binding sites with STK36. The study may help to understand how the KIF27-STK36 complex construct and regulate the motile cilia and hence study therapeutics of PCD. The docking prediction by Alphafold also helps me to generate chimeric plasmids of KIF27 to further investigate the binding regions.

INTRODUCTION

Preliminary studies have shown that the STK36 has a functional kinase domain in the N-terminus that involves in protein bindings, and our previous studies have shown that the central part of KIF27 (331-718 aa) binds with the full-length STK36 (Fig 1). However, the stability and functions of the domains of KIF27 and STK36 have not been fully clarified, and which challenge on constructing for stable protein truncations.

In the project, I applied Alphafold (an advanced Artificial Intelligence technique to predict protein structures) to predict and visualize the possible STK36 structures. Based on the results, I designed and constructed stable STK36 truncations to investigate for protein binding sites with the KIF27 (331-718aa). And we also studied the amino acids involve in bindings.

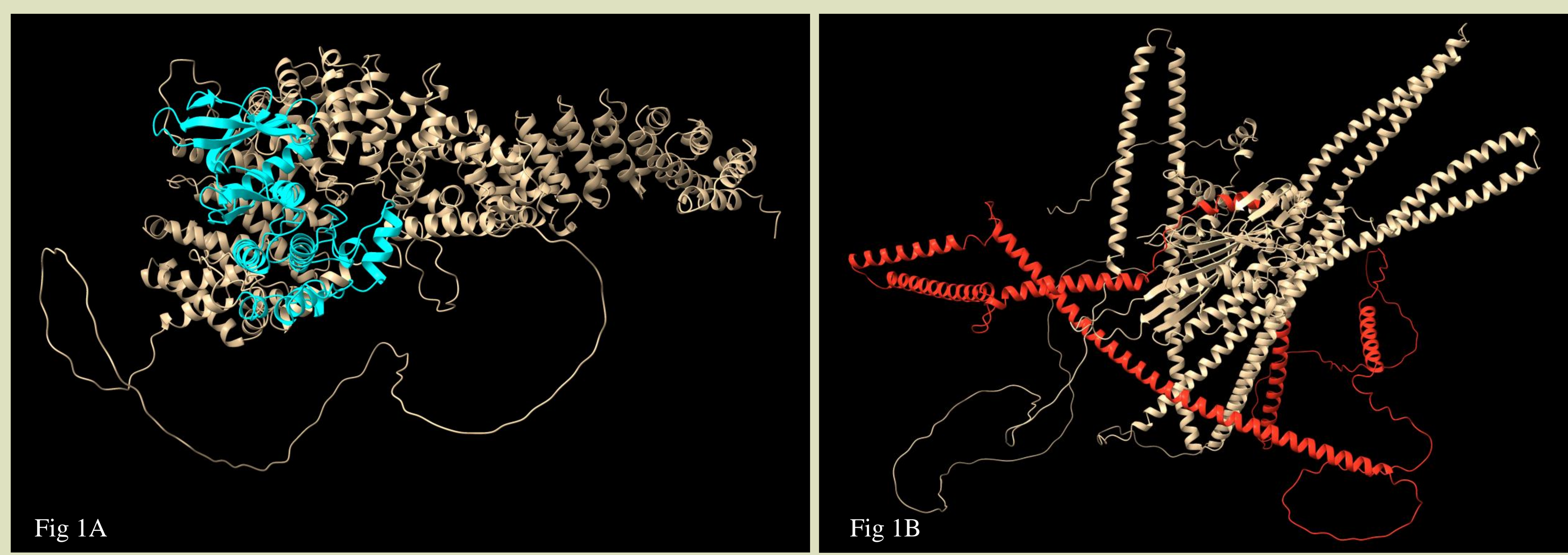
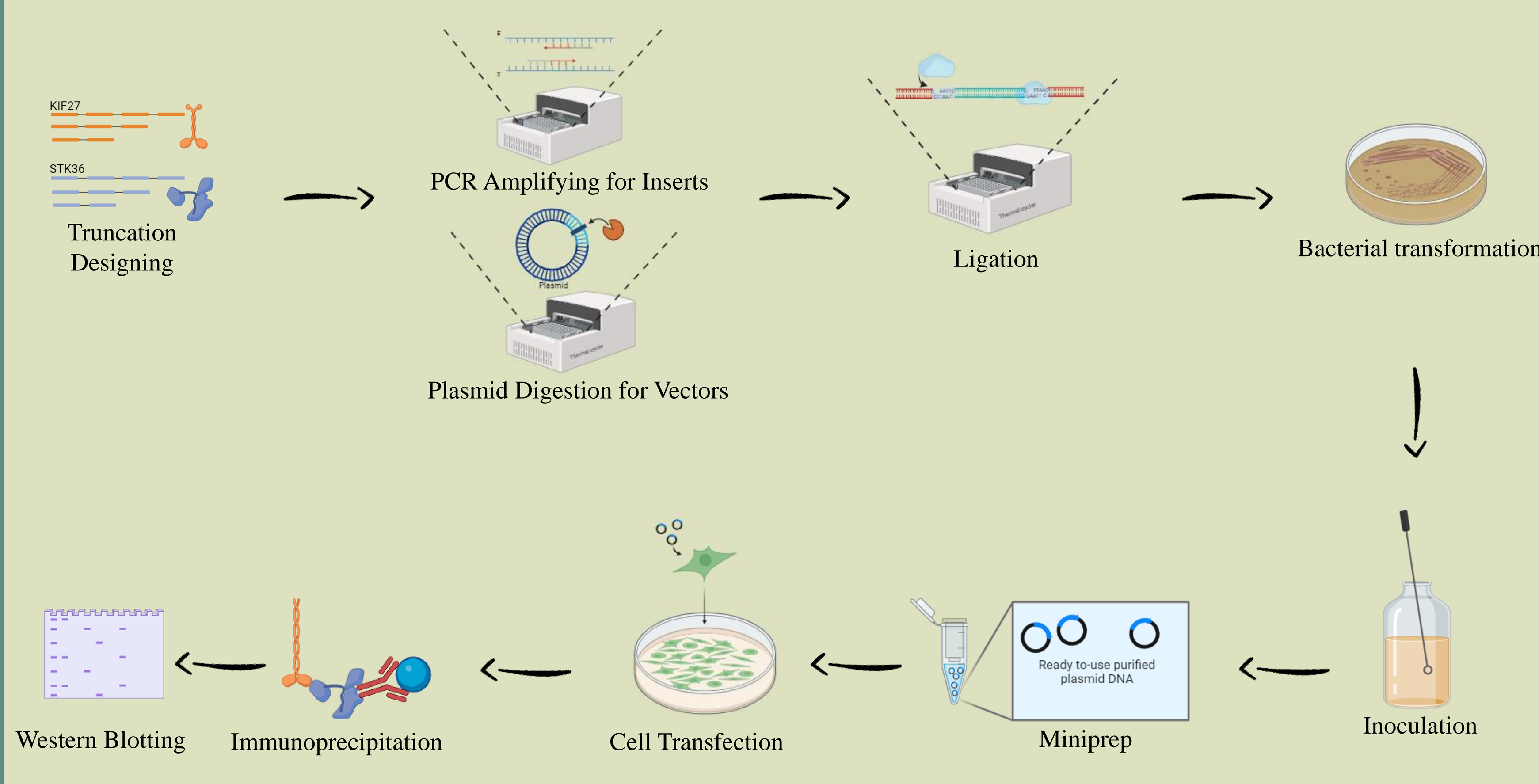


Fig 1: A) Alphafold-predicted structure of STK36, cyan indicating the kinase domain; B) Alphafold-predicted structure of KIF27, red indicating the 331-718 amino acids (The central domain).

METHDOLOGY



RESULTS

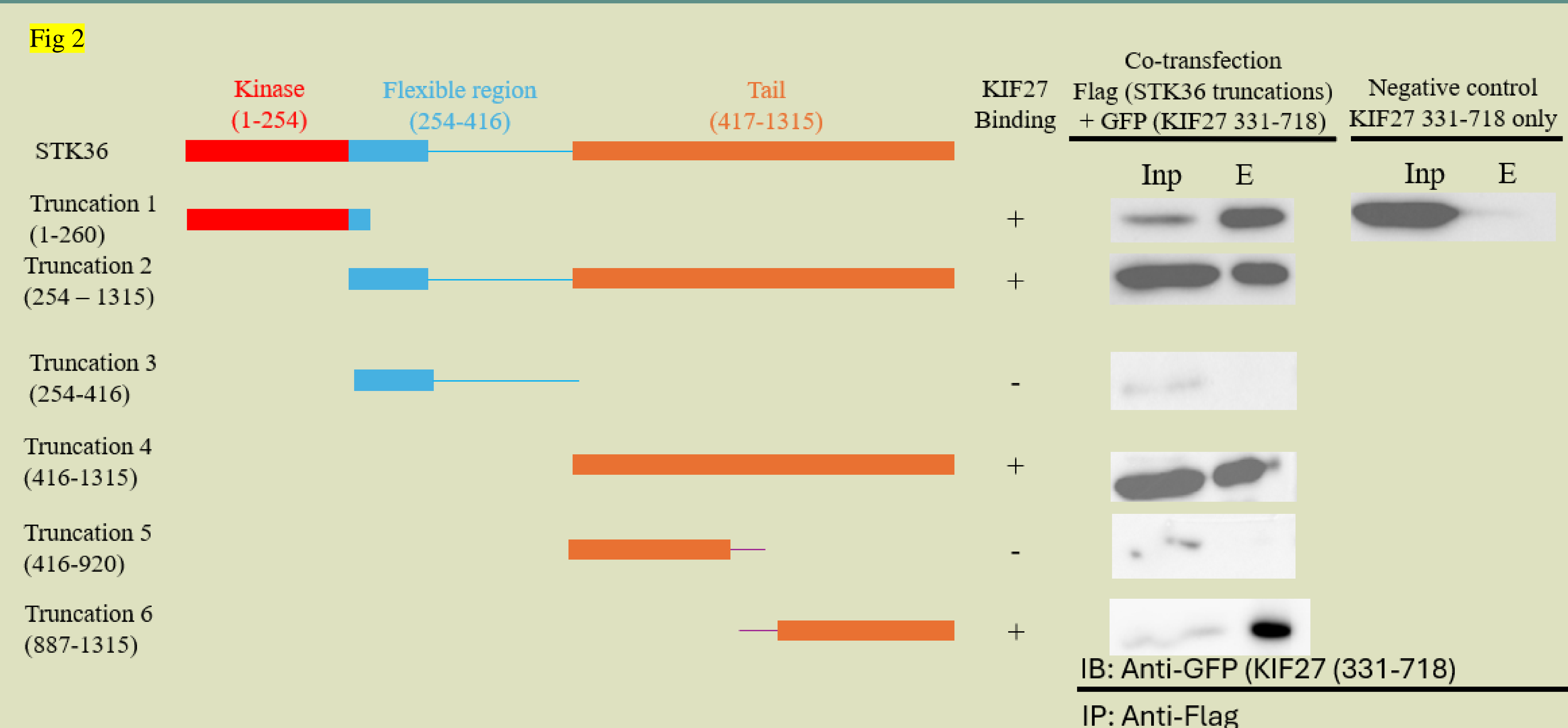


Fig 2: The truncation map of STK36 truncations designed with the demonstration of structural analysis of the AlphaFold-predicted STK36 shown on Fig 1A.
Fig 2 cont.: The corresponding protein-protein interaction testing results when co-immunoprecipitated.

RESULTS

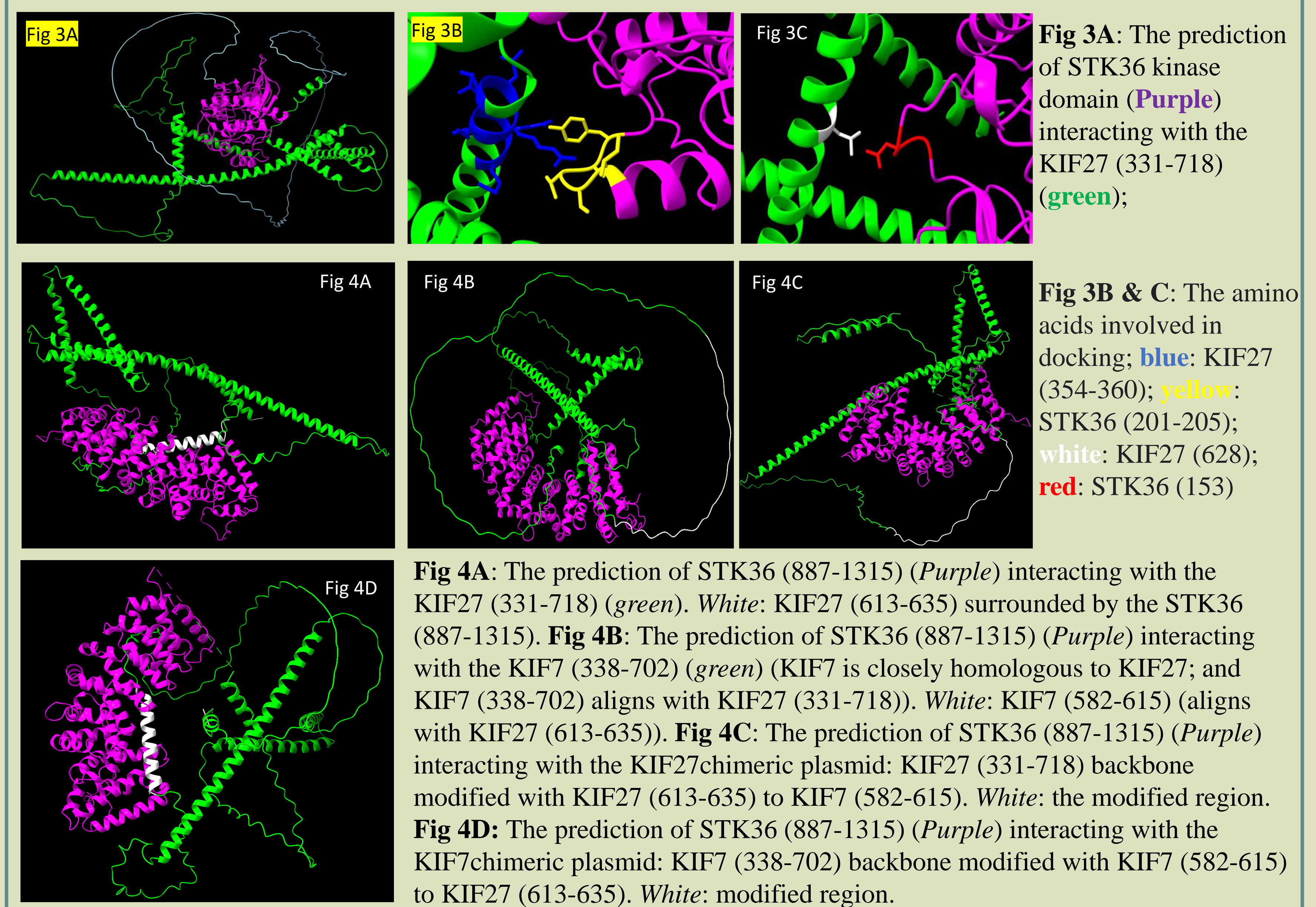


Fig 3A: The prediction of STK36 kinase domain (**Purple**) interacting with the KIF27 (331-718) (**green**);

Fig 3B & C: The amino acids involved in docking; **blue**: KIF27 (354-360); **yellow**: STK36 (201-205); **white**: KIF27 (628); **red**: STK36 (153)

Fig 4A: The prediction of STK36 (887-1315) (*Purple*) interacting with the KIF27 (331-718) (*green*). *White*: KIF27 (613-635) surrounded by the STK36 (887-1315). **Fig 4B**: The prediction of STK36 (887-1315) (*Purple*) interacting with the KIF7 (338-702) (*green*) (KIF7 is closely homologous to KIF27; and KIF7 (338-702) aligns with KIF27 (331-718)). *White*: KIF7 (582-615) (aligns with KIF27 (613-635)). **Fig 4C**: The prediction of STK36 (887-1315) (*Purple*) interacting with the KIF27chimeric plasmid: KIF27 (331-718) backbone modified with KIF27 (613-635) to KIF7 (582-615). *White*: the modified region. **Fig 4D:** The prediction of STK36 (887-1315) (*Purple*) interacting with the KIF7chimeric plasmid: KIF7 (338-702) backbone modified with KIF7 (582-615) to KIF27 (613-635). *White*: modified region.

DISCUSSION

The experiments showed that the KIF27 (331-718 aa) has multiple binding sites with STK36, including STK36 kinase domain and STK36 (887-1315 aa). To validate the results, similar biomolecular fluorescent complementation can be applied to repeat the experiments. The alternative experiments may further lower the interference of unspecific proteins on Western Blot. Smaller binding regions (fig 3&4) can be verified with Alphafold protein interaction prediction followed with Site-Directed Mutagenesis. Transfecting the two STK36 domains with KIF27 together could reveal how the domains are regulated in the binding mechanism.

Functional tests can be performed to further prove my hypothesis of KIF27-STK36 complex contributing to motile ciliogenesis and regulation of microtubules prospectively. To test how the STK36 domains apply the KIF27 with special functions and features, my truncations can be purified to dock with KIF27 to perform gliding assays, motility assay and kymograph assay to study which and how the STK36 domains are applied on KIF27 to affect the microtubules integrity and to affect the bindings stability and motility of KIF27 on microtubules in vitro. Furthermore, the truncations can be applied to the mouse models or human organoids to further model how the KIF27-STK36 complex functions in the animal microenvironment for PCD therapeutics research in vivo. Moreover, the STK36 truncation – KIF27 complex can be extracted to perform proteomics research to further examine how the complex functions on the motile ciliogenesis pathway with other cilia proteins physically.

To summarize, my preliminary protein docking results pace the prospective future studies of the ciliogenesis pathway and microtubule regulation under the functions of the KIF27-STK36 complex.

REFERNCE

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ADKNOWLEDGE

My appreciation to the research opportunities from Dr. HE, the mentorship from Ms. PARK Hyunji Hailey and other lab members.