論文の内容の要旨

Robotic *in vitro* selection of functional cyclic peptides for diverse target proteins
（ラボオートメーションの環状ペプチド創薬への展開）

氏名：　西尾　洸祐

Macrocyclic peptides are an attractive therapeutic modality due to their favorable features and have the potential to target classically ‘undruggable’ biological processes. The RaPID (Random non-standard Peptide Integrated Discovery) system has previously been shown to be a reliable platform for the de novo discovery of macrocyclic peptides that bind to a wide variety of proteins with high affinities and specificities. As it is typical for other *in vitro* selection systems, the RaPID system workflow requires researchers to perform laborious repetitive tasks. In order to streamline peptide drug discovery allowing researchers to focus on intellectual work, I have semi-automated the RaPID system using a humanoid robot, named Senta-kun. As benchmark experiments, in this thesis, four RaPID selections were performed against therapeutically relevant proteins namely TET1, cMET, Smurf2 and Akt2 to demonstrate the feasibility of performing automated RaPID system using the robot.

In chapter 1, I discuss the advantages and disavdantages of macrocyclic peptides over other modalities such as small molecules and biologics in terms of properties suitable for drugs as well as other comprehensive drug discovery technologies focusing on macrocyclic peptides. Through in depth comparisons, the superior features of the RaPID system amongst these drug discovery technologies highlight the past successes of macrocyclic peptides discovered with the RaPID system. Moreover, the general significance, current situation, and challenges for the future of laboratory automation are discussed.

In chapter 2, the specifications of the robot used in this study and its compatibilities with the RaPID system are described. The setup process of the robot and optimization process for performing each step of the RaPID system are discussed in detail. Notably, experimental considerations, pitfalls and the know-how gained from the successful setup of the robot for the RaPID system are illustrated.

Chapter 3 describes how the RaPID system was performed against two proteins, TET1 and cMET. Manual selection and automated selection are compared as a benchmark experiment to demonstrate the viability of the RaPID system using the robot. In addition, to reveal the effects of experimental conditions on the RaPID system outcomes, the same conditions between manual and robotic selections were used for TET1 and different conditions were used for cMET. As a result, similar peptide sequences were identified between the manual and robotic selection against TET1, whereas the majority of the peptide sequences discovered from the cMET selections was strongly varied from each other. All peptides tested in this study exhibited binding activity for the target protein. Moreover, three peptides, TiP1, 2, 3, that were confirmed to bind TET1 also exhibited TET1 inhibitory activity.

In chapter 4, the RaPID system was solely performed by the robot against a new target, Smurf2, to show that it is possible to conduct the RaPID system with only using the robot, resulting in the successful discovery of four peptides that bind Smurf2. Furthermore, the RaPID system workflow was applied to the affinity maturation of a known peptide inhibitor as a new application of the RaPID system. This experiment was conducted using the protein target, Akt2, and its peptide inhibitor, Pakti-L1. The result suggested that Pakti-L1 is a local maximum inhibitor, having the strongest binding affinity compared to other peptides binding via the same motif. It also resulted in the identification of a mutant with comparable activity as Pakti-L1.

Chapter 5 summarizes the results and provides an overall conclusion to the aims described in chapter 1. In this study, the RaPID system, which consists complicated multi-steps and requires precision, has been semi-automated using a humanoid robot and this system has successfully been used to discover new functional macrocyclic peptides for protein targets. The combination of laboratory automation and the RaPID system will enable us to more efficiently and productively conduct macrocyclic peptide drug discovery. In addition to the conclusions of my work, future prospects for laboratory automation and macrocyclic peptide drug discovery are also presented.