

審 査 の 結 果 の 要 旨

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The thesis submitted by Tam Chun Lai centers on the computational structure-based nanobody design. It consists of three separate but related projects. The first project is the design of nanobodies targeting the ELMO1-RhoG interaction with potential application of treating cancer metastasis. The second project is the design of nanobodies targeting the conserved S2 domain of the SARS-CoV-2 spike protein. The computationally designed nanobodies have all been experimentally tested and their binding affinities to their respective protein targets verified. The third project is the development of a new method for the selection of the best binding pose of a nanobody among many candidate poses derived from docking simulations. This nanobody pose prediction method uses machine learning to capture key features that determine the interactions between a nanobody and its antigen. This method was benchmarked with two state-of-the-art methods, namely ClusPro and DOVE, and shown to have superior performance in the selection of the best binding pose.

This thesis consists of three chapters covering the three projects mentioned above. These three chapters are: Chapter 1 – Nanobody design targeting ELMO1-RHO G interaction; Chapter 2 – Nanobody design targeting SARS-CoV-2 S2; Chapter 3 – Nanobody pose prediction. Each chapter is divided into three sections, namely, 1) introduction; 2) materials and methods; 3) results and discussion.

In chapter 1, the role of ELMO1-RhoG interaction in cancer metastasis is reviewed, which laid out the rationale for the discovery of inhibitors of ELMO1-RhoG interaction as a means to treat cancer metastasis. The advantages of computationally designing nanobodies that disrupt ELMO1-RhoG interaction is described. After the selection of available nanobody structures as potential starting points for the computational design, a commonly used protein-protein docking method, PatchDock, was used to screen the entire collection of nanobodies against ELMO1. A novel concept of “pose selection by design” was introduced in the selection of the best binding pose of nanobodies. Followed by Rosetta design protocol on the interface, the designed nanobodies were further evaluated by MD simulations and their binding energies were calculated. Multiple criteria were combined for the selection of nanobody candidates for experimental validation. One weak binding nanobody was identified by SPR analysis, and a second round of computational nanobody design was conducted and finally 23 candidates were selected and experimentally validated by SPR. There are several nanobodies with binding affinities of single digit micromolar have been discovered.

In chapter 2, the rationale for the design of nanobodies targeting the conserved S2 domain of the spike protein in SARS-CoV-2 was laid out. It was to avoid the mutational escape of most other antibodies that bind to the variable RBD of the spike protein. Using a

similar computational strategy as for the ELMO1 binding nanobodies, a total of 21 candidate nanobodies were designed. These nanobodies were expressed and purified. Their binding to the spike protein was measured by SPR. It was found that none of these designs had a detectable K_d lower than 5 μM , which was the highest concentration of the nanobodies used in the experiment. The possible reasons for the failure to design detectable binding nanobodies were explained. One possibility is the inaccuracies in the energy functions used in docking and the insufficient exploration of the conformational flexibility of the CDR loops. The other is that the use of the spike protein with four proline mutations in order to stabilize the protein for expression and experimental handling might have caused interference with the nanobody binding since two of the mutated prolines are located at the nanobody binding site.

In chapter 3, a computational method aimed at improving the selection of the binding poses of nanobodies proposed by protein-protein docking was developed. A feature set that characterizes the interface between the nanobody and its antigen was calculated. A machine learning tool, XGBoost, was used to map the feature set to a probability of the pose being native-like. This nanobody pose selection method was benchmarked against two of state-of-the-art methods, ClusPro and DOVE, and found to be of superior performance in terms of the ranking of the nanobody binding poses.

A thesis examination meeting was conducted online via ZOOM on July 7, 2021 with all the thesis committee members in attendance. The candidate has demonstrated good understanding of the subject matter presented in his thesis and satisfactorily answered questions from the committee members. The committee members have suggested some changes in order to improve the thesis. The candidate has incorporated these recommendations and submitted a revised thesis, which the committee has considered to be satisfactory.

なお、本論文は、白水 美香子、新野 睦子、松本 武久との共同研究であるが、論文提出者が主体となって分析及び検証を行ったもので、論文提出者の寄与が十分であると判断する。

よって本論文は博士（科学）の学位請求論文として合格と認められる。

以上英文 742 単語、和文 112 字