

論文の内容の要旨

論文題目 Structure-based Computational Design and Pose Prediction of Nanobodies
(構造ベースインシリコVHH抗体設計と結合ポーズの予測)

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Substantial growth in antibody drug development in the pharmaceutical industry is foreseeable due to the advantage over small molecule drugs in terms of, for example, specificity, biodegradability, and non-toxic metabolites from degradation. Out of the known antibody fragments in different molecular sizes, recently the nanobody, which is a heavy-chain only antibody from camelid species, has drawn considerable attention in antibody drug research due to its superior properties over the larger antibody fragments, such as high thermostability and versatility in choice of expression systems for production.

Until now, several experimental methods in antibody drug screening, for example, the classical animal immunization techniques and the more recent directed evolution methods (e.g. molecular displays), have been some of the standard practices in developing antibody drugs with good affinity and specificity to the target of interest. However, there are drawbacks to these experimental methods. For instance, one major disadvantage is the inability to rationally design antibodies to target a specific epitope of interest, where such control is often desirable because the functional alteration to the pharmaceutical targets is usually epitope-dependent. Moreover, the precise control of epitopes by rational antibody design minimizes off-target toxicity by avoiding binding to epitopes that inhibit other normal functions of the target.

In this study, we have explored the applications of computational nanobody design, which is an emerging technique in rational antibody design, on two pharmaceutically important targets, one targeting ELMO1-RhoG interaction, which is a key protein-protein interaction in signaling cancer cell migration and another targeting S2 of SARS-CoV-2, in an attempt to develop a broad-spectrum antibody drug effective to SARS-CoV-2 mutant strains and SARS-related CoVs.

In the computational nanobody design targeting ELMO1-RhoG interaction, we have applied the dock-and-design approach by repurposing known nanobody structures from the PDB to bind ELMO1 on its interface with RhoG, which could theoretically quench the downstream signaling of cancer cell migration through the ELMO1/DOCK180 pathway, which normally induces actin polymerization and cell membrane protrusion for cell movements. We improved the selection of initial nanobody poses by applying an *in cerebro* guided optimization of antibody mode in PatchDock that used two positions on the CDR loops as distance constraints

in nanobody-antigen docking, which lead us to initial poses with improved visual resemblance to known nanobody-antigen poses. We have adopted a new approach in pose selection we termed "pose-selection-by-design," which selected poses that generated binding energy funnels with good resemblance to the deep, funnel-shaped binding energy landscape commonly observed in protein-protein interactions. From our first batch of 20 designs tested for binding to ELMO1 by SPR binding assay, we have obtained one potential hit, nano-79, which showed weak binding to ELMO1. Based on nano-79 as an initial hit, we performed a second-round design (Figure 1) to explore additional sequence variations that potentially improve binding affinity to our target. We have successfully obtained a set of designs which showed improved binding overall, with the best binder exhibiting a dissociation constant of 2 μ M to ELMO1.

During the current COVID-19 pandemic, due to the frequent emergence of SARS-CoV-2 mutant strains worldwide, there is a need to develop therapeutics that are tolerant to potential mutation escape of the SARS-CoV-2 variants. Currently, the majority of the spike-targeting antibodies developed bind at the RBD or its surrounding residues on S1. However, in general, RBD on S1 represents a relatively variable epitope compared with the S2 ectodomain. An antibody drug that targets a conserved epitope on S2 that is functionally important to the cell fusion and entry mechanism of SARS-CoV-2 could deliver a promising antibody drug that possesses a broad-spectrum neutralizing effect to the circulating and the to-be emerged mutant strains of SARS-CoV-2. We focused on one conserved structural epitope on S2 of SARS-CoV-2 (Figure 2), which contains the proteolytic cleavage site S2' and is proximal to HR1 in its pre-fusion state, implying the functional importance of this epitope to the dissociation of S1 from S2, which is essential to the S-mediated host membrane fusion of SARS-CoV-2. We designed 21 nanobody structures that potentially bind to the epitope through an overall similar design approach as in the ELMO1-RhoG nanobody design. Preliminary result from SPR binding assay showed our designs did not bind SARS-CoV-2 S with dissociation constant less than 5 μ M, which needs further examination to improve their binding affinity.

Computational antibody design is still a relatively new technique in antibody drug development. There is a need for further methodological optimization to increase the hit rate of generating a binder with a detectable affinity for further affinity maturation. In structure-based computational antibody design, one of the difficulties lies in the pose selection from a large number of alternative poses generated by antibody-antigen docking, which directly affects the success of subsequent design simulations. Conceptually, designing native-like poses should have a better chance of developing a binder than designing poses far from the native. Followed by the two studies of computation nanobody design, we have explored the application of machine learning to improve the pose selection of nanobodies. With the calculation of features that consisted of a contact profile (e.g. CDR loop contacts) and an energy profile calculated by

InterfaceAnalyzer from Rosetta and AnalyseComplex from FoldX, we have trained a binary classifier with the implementation of a gradient-boosted decision tree model, XGBoost, which can distinguish native-like from non-native-like poses with a given nanobody-antigen complex structure. To benchmark the performance of our binary classifier, we are currently comparing the performance of our model to ClusPro, the current state-of-the-art protein-protein docking algorithm, and DOVE, a competing method that distinguishes native and non-native protein-protein complex structures. Our model successfully ranked native-like nanobody poses with a significantly higher ranking than ClusPro (Figure 3), demonstrating the potential application of our nanobody pose prediction model to improve accuracy in native pose prediction of nanobody from protein-protein docking algorithms.

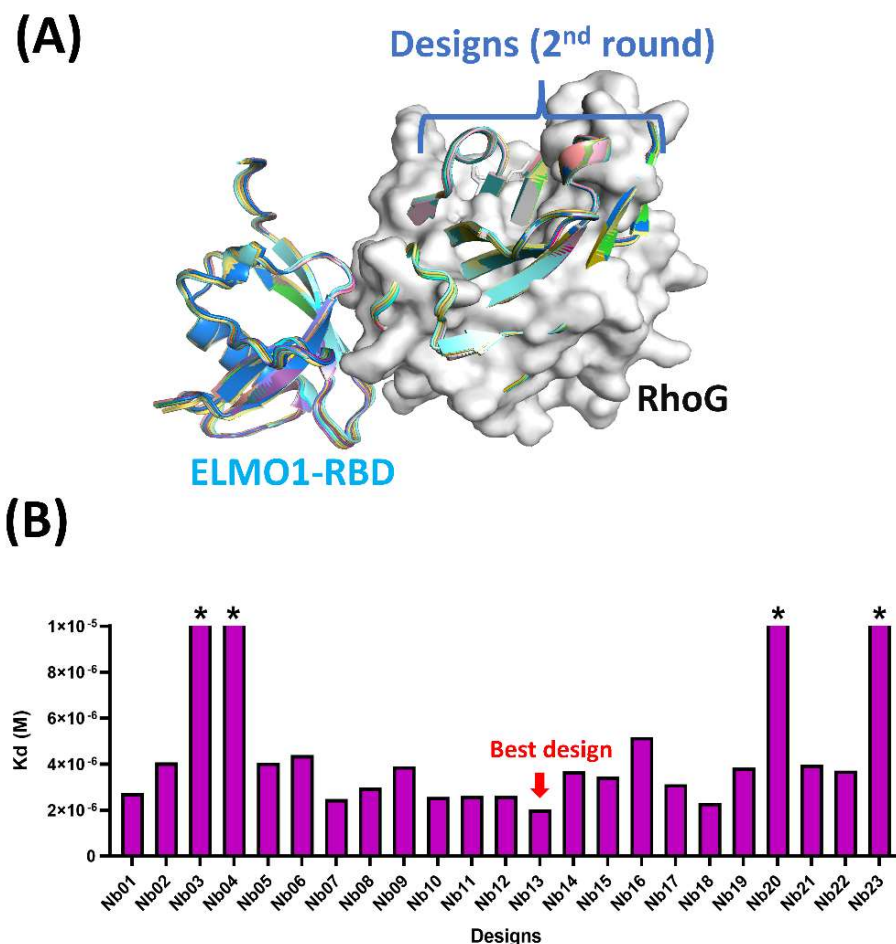


Figure 1. Major results from the computational design of nanobody targeting ELMO1-RhoG interaction. (A) Binding poses of ELMO1-RBD-targeting nanobody from second round design. (B) Dissociation constant (Kd) calculated from SPR single-cycle measurement. Asterisks represents designs with Kd larger than 1e-5 M.

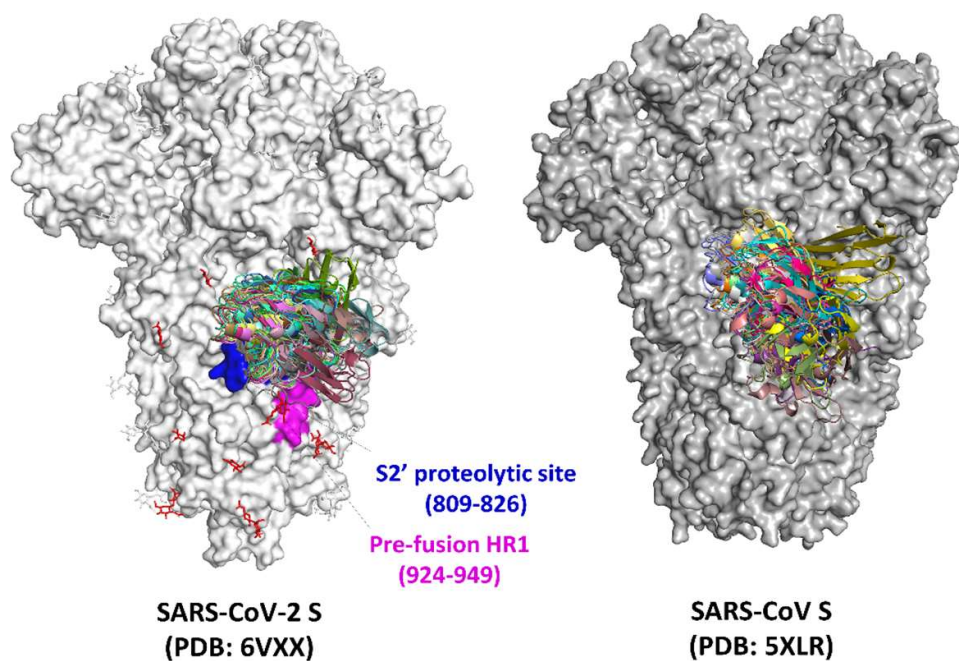


Figure 2. Binding poses of nanobody designs targeted to the selected S2 epitope of SARS-CoV-2 (left) and SARS-CoV (right).

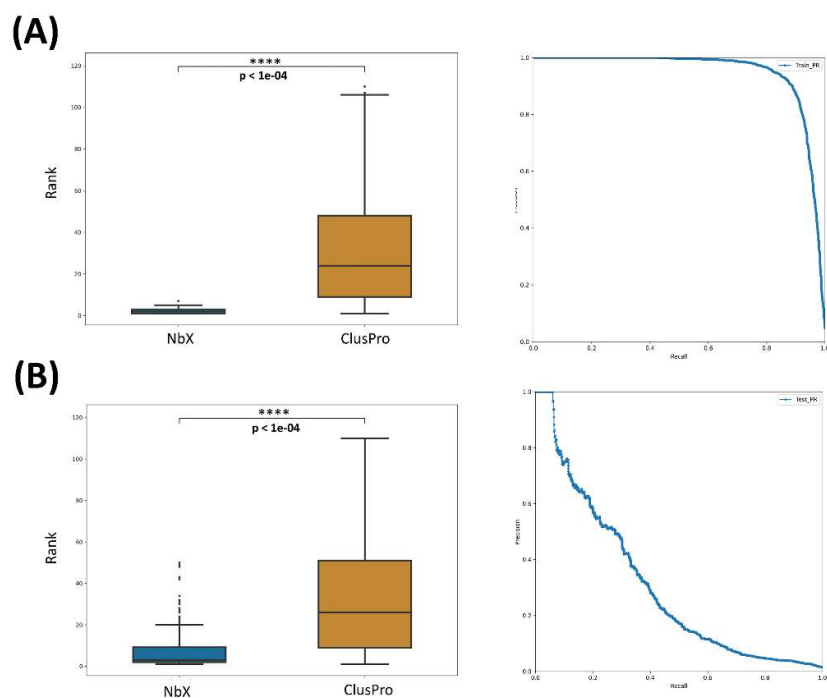


Figure 3. Prediction performance comparison between the nanobody pose prediction model (NbX) and ClusPro on the 5-fold validated prediction of (A) training set and (B) test set.