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YabXnization platform: A monoclonal antibody heterologization server based on rational design and artificial intelligence-assisted computation

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ABSTRACT

The application of antibody therapeutics is promising in the field of immunotherapy. While, heterologization should be done in most cases before applying the therapeutic antibodies into bodies, e.g., humanization, caninization and felinization for human beings, canine and feline, respectively. Here we report YabXnization, the platform which realizes antibody heterologization on the basis of rational design and artificial intelligence (AI)-assisted computation. YabXnization provides two ways for heterologization: traditional CDR-grafting and backmutation-based rational design; and AI-assisted fusion computational design. Taking humanization as example, both of the two ways first find the proper template for heavy and light chains with CDR-grafting followed. For rational design, bioinformatics analysis-based backmutation is then conducted. For AI-assisted computational design, the backmutation and humanness evaluation are implemented through evolutionary computation framework with DeepForest-based humanness evaluation model and the distance to the previously found human template as objective functions. Finally, the top *K* heterologized antibodies can be provided by YabXnization platform. We examined the platform with 18 antibodies to be heterologized, in which 10 for humanization, 6 for caninization and 2 for felinization, respectively. The heterologized antibodies were measured by indirect ELISA and BLI(Octet)/SPR(Biacore) binding affinity measurement methods. Test results show a 90% success rate with the binding affinity loss of heterologized antibodies within an order of magnitude compared to the corresponding chimeric antibodies. It even shows an increase in the binding affinity on some of the heterologized antibodies. The platform can be reached through https://www.genscript.com/tools/yabxnization-service.

1. Introduction

Antibodies recognize antigens with high affinity and specificity, making them widely used in the fields of biopharmaceuticals and immunotherapy, including cancer treatment, immunomodulatory diseases, and infectious disease treatment. Since the first monoclonal antibody drug was approved by the U.S. Food and Drug Administration (FDA) in 1986, the market for antibody drugs has shown exponential growth. In 2018, six of the top 10 best-selling drugs are monoclonal antibodies, and it is estimated that by 2025, the market size of monoclonal antibodies alone will reach $300 billion [1]. By November 2023, almost 200 antibody therapeutics have either been approved for marketing or are under regulatory review in at least one country (www.antibodysociety.org/antibodytherapeutics-product-data). Furthermore, as of October 2023, company clinical pipelines featured over 1,100 antibody therapeutics in Phase 1, Phase 1/2, or Phase 2 trials, with nearly 70% of these aimed at cancer treatment [2]. In those commercially approved and under clinical studied therapeutic antibodies, most of them are generated from monoclonal antibody. Currently, cell fusion and hybridoma technology are the most reliable methods for preparing monoclonal antibodies with mice, rats, sheep, rabbits and other animals used for immunization. However, the monoclonal antibodies obtained by these methods are of species-specified origin. To develop animal-derived monoclonal antibodies into antibody drugs for disease treatment or prevention, especially for human use, heterologization must be carried out to reduce the anti-antibody reaction caused by heterologous antibodies.

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Taking humanization as an example, the process of replacing part of the sequence of a non-human antibody (i.e., of animal origin such as murine) with a human antibody sequence to make it more like a human antibody in order to reduce or eliminate the immunogenicity, is called antibody humanization [3]. As a routine requirement in today's antibody engineering, antibody humanization mainly includes the following methods: chimeric method [4], CDR-grafting [3], SDR-grafting [5], resurfacing method [4], reproduction based on CDR or SDR-grafting cell lineage humanization [6,7], and so on. The differences in the above humanization methods and their corresponding representative antibody drugs can be found in the literature [4]. Traditional humanization mainly relies on manual operations, including rational design and reverse mutation site analysis on the basis of experience. At present, there are many computer-assisted humanization programs, including the random forest-based Hu-mAb [8], the ProtBert (a natural language processing pre-trained model)-based BioPhi [9], the multivariate Gaussian model-based statistical model [10], the CDR-grafting-based online humanization tool Tabhu [11], etc. In many cases of the traditional humanization, the operation with CDR-grafting and backmutation is not enough and further optimization is necessary. However, nearly all the traditional humanization methods stop their steps after the backmutation operation, and resign the humanization results (keeping the binding affinity of the humanized antibody to specific antigens, and making the humanness as high as possible) to fate.

Still taking humanization as an example, the next step of antibody engineering after humanization is humanness evaluation. The World Health Organization (WHO) and the American Medical Association (AMA) have respectively launched the International Nonproprietary Name (INN) and United States Adopted Name (USAN) projects to standardize the nomenclature of antibody drugs, and recommend the use of IMGT/DomainGapAlign [12] to measure and analyze the degree of humanization of antibody drugs. The IMGT/DomainGapAlign can determine the degree of homology between the humanized antibody and the antibody-coding genes of the human germ cell lineage. Earlier, USAN project experts recommended that the humanization degree of VH and VL of monoclonal antibody drugs need to be no less than 85% before -zumab and -umab can be used in drug naming. However, this has brought a lot of controversy because the humanization degree of many approved antibody drugs labeled as 'humanized' is actually not higher than 85% [4,13,14]. What's more, the IMGT/DomainGapAlign only considers antibody-coding genes of the human germline lineage, while contains no somatic hypermutation. Accordingly, ≥85% is not considered as a hard standard at present. To evaluate the humanness degree, the full length heavy/light chain of the humanized antibody V region should be compared with the human antibodies [13,14], with the use of Blastp program.

As a representative of AI, deep learning has been used for antibody humanization, such as ProtBert based BioPhi [9], and the LSTM based humanization degree measurement model [15]. However, there are several unavoidable problems in applying deep learning to computational biology: **(1)** the model can perform well only when training with massive data; **(2)** the hyperparameters of the models need to be systematically screened and set, which is cumbersome; **(3)** low interpretability; **(4)** high cost when accelerating calculations with GPU or TPU [16]. DeepForest [17] is a deep model inspired by deep neural networks (DNN) and designed using multi-granularity cascaded random forests which does not rely on the back-propagation algorithm. Compared with deep learning based on neural networks, the advantages of DeepForest include: **(1)** more stable and better learning performance when processing data of different data scale, fast training, and reduced computing costs; **(2)** no complex hyperparameter settings; **(3)** the tree structure of the deep forest has better interpretability than the neural network, which is beneficial to mining and explain biological implications.

In view of the problems existing in deep learning in antibody humanization, and for the purpose of further optimizing the humanized antibody with CDR-grafting, we propose here an antibody humanization method based on CDR-grafting, deep forest-based humanness evaluation model, and multi-population genetic algorithm (MGA)-based backmutation optimizer. After traditional CDR-grafting operation, the evolutionary computation is conducted under the MGA framework with the constructed humanness evaluation model and the distance to previously found template as objective functions. Besides, we expend the humanization application to canine and feline, called caninization and felinization, respectively, and integrate those 3 heterologization methods into one platform which named YabXnization. YabXnization provides two ways for heterologization: traditional CDR-grafting and backmutation based rational design; and AI-assisted fusion computational design. The overall workflow of YabXnization platform is shown in Fig. 1. The user should first provide 4 groups of information to be filled in the blanks: the 'Task Name' (job name given by user), 'Top *K*' (the number of total heterologized antibodies, maxim 25), 'Target Sequences' (heavy/light V-region sequence), and 'Template' (the template used in heterologization, optional); then select 4 groups of given options: 'Xnization' (humanization, caninization and felinization), 'Origin Species' (the species of target antibody), 'Design Mode' (AI design or rational design), and 'Computing Mode' (speed first or accuracy first); by clicking the 'Submit' button, the selected heterologization will start running in the backend, and a notification email will be sent to the user once the heterologization is finished; Finally, the top *K* heterologized antibodies can be downloaded with an extra TXT file for manually review.

2. Materials and methods

2.1. Dataset

2.1.1. Dataset used for constructing the humanness evaluation models

There are over 2.5 billion antibody sequences in the Observed Antibody Space, OAS [18,19] database and 15,996 variable regions in The Structural Antibody Database, SAbDab [20] (as of January in 2024). The dataset used for constructing the humanness evaluation models were obtained from the OAS and SAbDab, which were divided into 4 parts: human heavy chains, human lights chains, non-human heavy chains and non-human light chains, namely, Human_H (98,456,731 items), Human_L (6,097,114 items), NonHuman_H (31,382,063 items) and NonHuman_L (2,067,941 items), respectively. All heavy and light chains were numbered by ANARCI [21] with specific numbering schema (e.g., IMGT) and marked CDR regions with specific CDR marking rules (e.g., North) [22]. And the heavy and light chains were aligned to the same length with gaps in the sequences filled by 'X' at the same time, respectively.

2.1.2. Dataset used for constructing the template database

The aligned heavy and light chain sequences described in section 2.1.1 were also used for constructing the human template database. Here we also describe the construction process of the template database used for caninization from the international ImMunoGeneTics information system, IMGT (https://www.imgt.org/). The IGHV (55 itmes), IGHD (6 items), IGHJ (6 items), IGKV (40 items), IGKJ (5 items), IGLV (122 items), and IGLJ (9 items) corresponded amino acid sequences of canine species were first downloaded from IMGT reference directory in FASTA format (IG and TR) (https://www.imgt.org/vquest/refseqh.html#VQUEST). Then, the V-region antibody heavy chain sequences were combined from IGHV, IGHD and IGHJ (IGKV, IGKJ, IGLV and IGLJ for light chain) according to the mechanism to produce antibody diversity based on the recombination of V(D)J gene. The complete heavy chain template database contains 2,310 sequences including IGHV+IGHJ (55*6 = 330) and IGHV+IGHD+IDHJ (55*6*6 = 1,980). The complete light chain template database contains 1,298 sequences including IGKV+IGKJ (40*5 = 200) and IGLV+IGLJ (122*9 = 1,098).

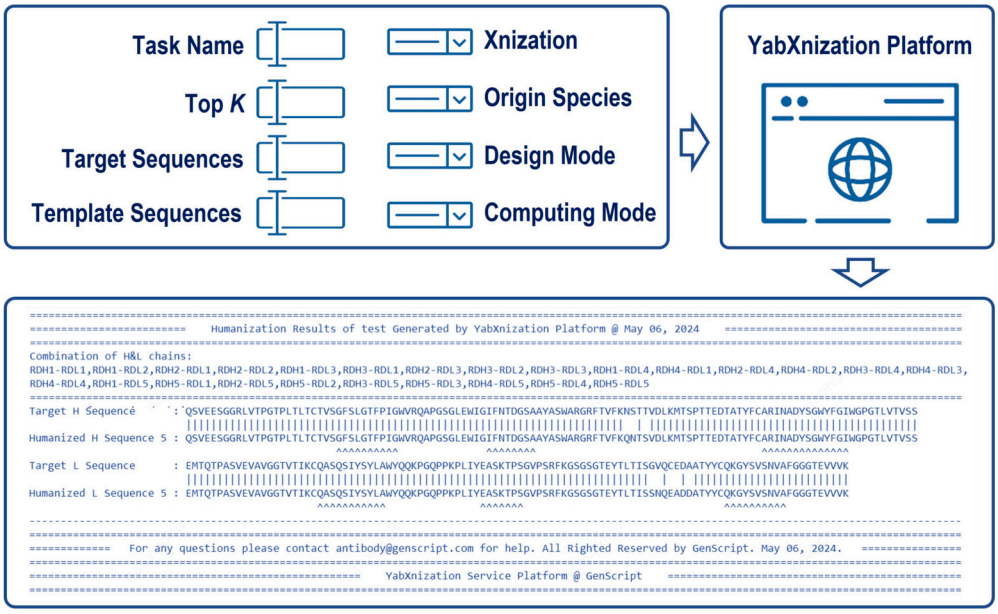

Fig. 1. The overall workflow of YabXnization platform.

2.1.3. Dataset used for heterologization testing

We have developed 18 clones of antibodies against several antigens through MonoRab^{TM} rabbit monoclonal antibody customized service (https://www.ge-nscript.com.cn/custom-rabbit-monoclonal-antibody-generation.html) for testing, in which 10 of them are for human, 6 for canine and 2 for feline.

2.2. CDR-grafting

CDR-grafting [3] is a classical and important technique for heterologization. It first finds out the CDRs in both target sequence (the antibody heavy/light chain sequences to be heterologized) and template sequence (the antibody heavy/light chain sequences from the target species to be heterologized to). The CDRs in the target sequence are then grafted to the corresponding positions in the template sequence. The newly generated sequence is called heterologized sequence and is used for the next steps of operations. The CDR-grafting has been implemented in the Python package AbNumber (https://abnumber.readthedocs.io/) which was used in our method.

2.3. Backmutation for rational design

Compared to original antibody, extensive sequence modifications within the framework regions (FR) often result in the reduction or even a loss of binding affinity [23,24]. This effect may be attributed to critical framework positions within the antibody framework sequence, which stabilize the overall protein structure or the VH/VL interface [25], contact the antigen directly [26] or establish the Vernier zone [27] by providing a suitable physico-chemical environment for a proper conformational ensemble of the CDR loops. Accordingly, with only CDR-grafting is always not sufficient for most cases of heterologization, and several critical positions within the framework regions have to be backmutated to the wild-type amino-acids in the original species. The amino acids should be backmutated to its original amino acids should satisfy following conditions: **(1)** the positions which are highly conserved; **(2)** the positions which are close to CDR regions; **(3)** the positions which are highly important to the stability of the antibody.

2.4. DeepForest-based humanness evaluation model

The humanness evaluation models for heavy and light chains were constructed separately with the use of DeepForest [17], and the humanness evaluation was conducted separately for heavy and light chains as well. The DeepForest was implemented with the use of Python deep-forest package (https://github.com/kingfengji/gcForest) with its default parameters. For heavy and light chains, the DeepForest humanness evaluation models were trained on the aligned 873,179 (590,740 for human + 282,439 for non-human) heavy and 3,940,707 (3,658,268 for human + 1,240,765 for non-human) light chains, respectively. When making evaluation of a given antibody, the heavy and light chains were first numbered and aligned to a certain length with the ANARCI under specific numbering schema (e.g., IMGT) and with gaps in the sequences filled by 'X' at the same time, then input to the constructed models. The probability of the given chain to be predicted as human could be given by the model. What to be noted is that no matter what kind of species the origin is, our proposed model can make an accurate distinguishment. The constructed model can be used for both rational design as humanness evaluator for guiding the selection of humanized sequences; and for AI-assisted computational design as one of the objective functions of the evolutionary computation for guiding the searching process.

2.5. Evolutionary computation-based heterologization optimization

Evolutionary computation has been successfully applied for solving the biology problem, such as GenSmart^{TM} codon optimization tool https://www.ge-nscript.com/gensmart-free-gene-codon-optimization.html [28] and molecular docking [29]. Genetic algorithm (GA) [30] is a classical representative of evolutionary computation, and was adopted in this work. Two objective functions were used here: the previous constructed humanness evaluation model, and the distance (e.g., the distance calculated from the sequence alignment-based substitution scoring matrix, PHAT or PAM, etc. [31]) to the previous found human template. The humanness evaluates how similar the optimized antibody sequence to human, the distance calculates how far the optimized antibody sequence to the selected human template. The goal of the evolutionary computation is for making the optimized antibody sequence more similar to human but not too far from the selected template sequence.

2.6. Binding affinity measurement methods

2.6.1. ELISA relative affinity ranking

Enzyme-Linked Immunosorbent Assay (ELISA) is a widely used technique for detecting and quantifying antigens or antibodies in a sample.

When using ELISA to determine the relative affinity of antibodies, the process generally involves comparing the binding strengths of different antibodies to a specific antigen under standardized conditions. Here are the steps and considerations for performing a relative affinity ranking using ELISA:

(1) Preparation of antigen and antibodies: Coat a microtiter plate with a known concentration (1 μg/ml, 100 μl/$well$, with the use of Phosphate Buffered Saline, PBS, pH 7.4) of the antigen and prepare a range of dilutions (from original concentration to approximately 0, take 1/30 times as step, totally 12 steps) of the antibodies you wish to compare;

(2) Binding Assay: Incubate the antigen-coated wells with the different antibody dilutions, then wash the wells (with the use of PBS containing 0.05% Tween-20) to remove unbound antibodies;

(3) Detection: Add a secondary antibody that is specific to the primary antibodies and is conjugated to an enzyme (e.g., HRP-Horseradish Peroxidase), then wash the wells (with the use of PBS containing 0.05% Tween-20) again to remove unbound secondary antibodies;

(4) Substrate addition: Add a substrate that the enzyme can convert into a detectable signal (e.g., a colorimetric change);

(5) Signal measurement: Measure the signal using a plate reader;

(6) Data analysis: Plot the absorbance values against the concentrations of antibodies and determine the concentration at which each antibody reaches half-maximal binding (EC50);

(7) Relative affinity ranking: Compare the EC50 values of the different antibodies. A lower EC50 indicates higher affinity, as it means that a lower concentration of antibody is required to achieve half-maximal binding.

2.6.2. BLI binding affinity measurement

Biolayer Interferometry (BLI) is a label-free technology for measuring biomolecular interactions in real-time. It is widely used to determine binding affinities, kinetics, and concentration of analytes. BLI operates on the principle of detecting changes in the interference pattern of light reflected from a biosensor surface when molecules bind to it. Here are the steps and considerations for performing a binding affinity measurement using BLI:

(1) Sensor selection: Choose an appropriate biosensor type based on the nature of the ligand (e.g., Protein A, Ni-NTA for His-tagged proteins. HIS1K (Sartorius Cat. No. 18-5120, Lot. No. 2209010411) were used here);

(2) Ligand immobilization: Immobilize the ligand (e.g., an antibody or protein) onto the biosensor surface;

(3) Baseline establishment: Place the ligand-immobilized biosensors into a buffer (10 mM Tris, 150 mM NaCl and 2 mM $CaCl_2$, pH 7.5 (GenScript, Lot. No. 20231229P0006)) solution to establish a baseline signal;

(4) Analyte binding: Dip the biosensors into wells containing different concentrations of the analyte (e.g., an antigen or another protein) to observe the association phase: the binding of the analyte to the ligand on the sensor surface will cause a change in the interference pattern;

(5) Dissociation measurement: Transfer the sensors back into wells containing only the buffer to observe the dissociation phase: the decrease in signal over time indicates the dissociation of the analyte from the ligand;

(6) Kinetic analysis: Analyze the association and dissociation curves to calculate the kinetic rate constants (k_{on} for association, k_{off} for dissociation);

(7) Affinity determination: Calculate the equilibrium dissociation constant (K_D) using the formula: $K_D = k_{on}/k_{off}$;

(8) Global fitting: Using the software provided with BLI instrument (Octet^{RED}384) to perform global fitting of the kinetic data from multiple analyte concentrations to obtain accurate kinetic and affinity constants.

2.6.3. SPR binding affinity measurement

Surface Plasmon Resonance (SPR) is also a widely used technique for measuring binding affinities and kinetics of biomolecular interactions. SPR measures changes in the refractive index near a sensor surface when biomolecules bind to it. These changes affect the resonance angle of surface plasmons, which is detected as a shift in the SPR signal. Here are the steps and considerations for performing a binding affinity measurement using SPR:

(1) Surface functionalization: Coat the SPR sensor surface with a ligand;

(2) Ligand immobilization: Immobilize the ligand on the sensor chip (Series S Sensor Chip CM5 from Cytiva with Cat. No. BR-1005-30) using appropriate coupling chemistry;

(3) Baseline establishment: Flow a running buffer (diluted from 10× HBS-EP+ buffer) over the sensor surface to establish a stable baseline;

(4) Analyte injection: Inject the analyte (e.g., antigen or protein) at various concentrations over the sensor surface: the binding of the analyte to the immobilized ligand changes the refractive index, which is detected as an SPR signal;

(5) Buffer flow: After the association phase, flow the running buffer over the sensor surface to observe the dissociation of the analyte from the ligand;

(6) Kinetic analysis: Analyze the association and dissociation curves to calculate the kinetic rate constants (k_{on} for association, k_{off} for dissociation);

(7) Affinity determination: Calculate the equilibrium dissociation constant (K_D) using the formula: $K_D = k_{on}/k_{off}$;

(8) Global fitting: Using the software provided with SPR instrument (Biacore T200 from Cytiva with Cat. No. 28975001) to perform global fitting of the kinetic data from multiple analyte concentrations to obtain accurate kinetic and affinity constants.

3. Results

3.1. Web server

The YabXnization web server we developed now can provide three kinds of heterologization: humanization, caninization and felinization. For humanization, YabXnization provides two ways of design: rational design and AI-assisted computational design. Fig. 2 shows a snapshot of the job submission page of the web server. As described in the Introduction, there are 4 blanks should be filled by user: 'Task Name', 'Top K', 'Target Sequences', and 'Template', the meaning of which are: the user defined task name, how many heterologized antibodies should be output, the heavy and light chains to be heterologized, and the template heavy/light chains which are going to be used in the heterologization, respectively; And 4 options should be selected by user: 'Xnization', 'Origin Species', 'Design Mode', and 'Computing Mode', the meaning of which are: what kind of heterologization the user is going to do, origin species of the provided sequence to be heterologized, rational design or AI-assisted design, and speed or precision first when doing the heterologization, respectively. In all the 4 blanks, 'Template' is optional to be filled, and other blanks are all mandatory. In all the 4 options, only 'Design Mode' can be selected by user when doing humanization, for caninization and felinization, 'Design Mode' is set to rational design by default and could not be changed at moment. With all blanks filled and options selected, by clicking the 'Submit' button, the selected heterologization will start running in the backend, and a notification email will be sent to the user once the heterologization is finished. Finally, the top K heterologized antibodies can be downloaded from the YabXnization platform with an extra TXT file for manually review.

3.2. Performance of humanness evaluation model

In the AI-assisted computation method for humanization, the successfulness is highly related to the humanness evaluation model since which is the objective function of the evolutionary computation. Accordingly, we tested the performance of humanness evaluation model

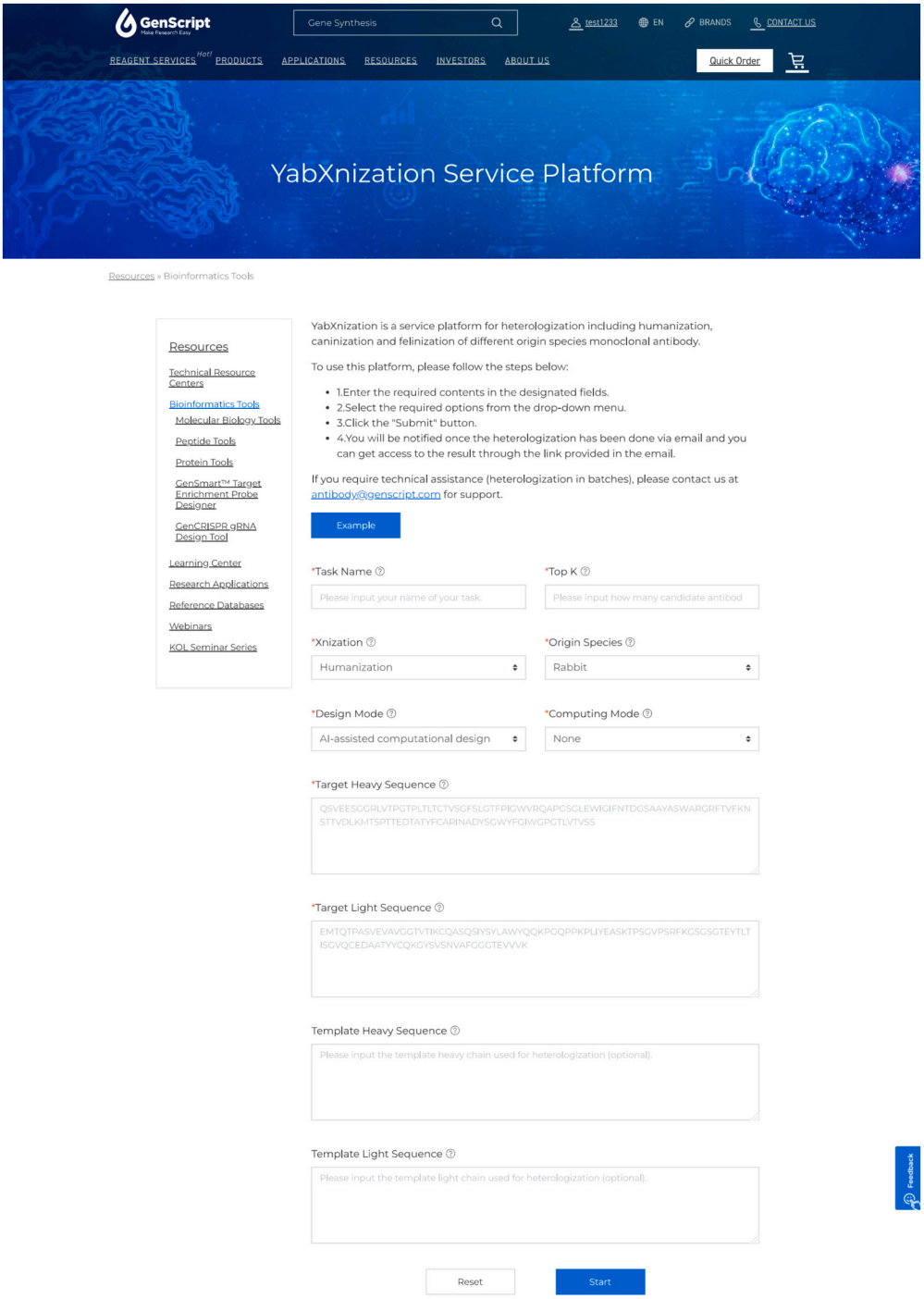

Fig. 2. The snapshot of the job submission page of the web server.

by 5-fold cross-validation with down sampling of original dataset (due to the extremely large amount). In the test, 620,000 samples for heavy chain and 550,000 samples for light chain were used for 5-fold cross-validation, and other 620,000 samples for heavy chain and 410,000 samples for light chain were used for independent test. For avoiding the information leakage in the cross-validation, the sequences with sequence similarity higher than 90% (for heavy chain) and higher than 95% (for light chain) would not appear in the training and testing datasets at the same time when splitting the data. The 5-fold cross-validation results and independent test results for heavy and light chains are depicted in Figs. 3(a)-(f) and 3(g)-(l), respectively. The indicators achieve nearly 1.000 in terms of AUROC (area under receiver operating characteristic curve) and AUPRC (area under precision-recall curve), which show that the classification performance of the constructed models is considerably good.

For avoiding the sampling bias and the effect of sample number, we use different amount scale samples from 1,000 to 100,000 with 5,000 as increasements for constructing the model, and use an independent test set including 200,000 samples for testing. The corresponding test results are summarized in Tables 1 and 2, which shows that the performance increase with the sample number used in training, but the increasement is not that obvious. Thus, we can draw a conclusion that there exists no sampling bias in the constructing process of the model, and the constructed model performs well with the given down-sampled samples.

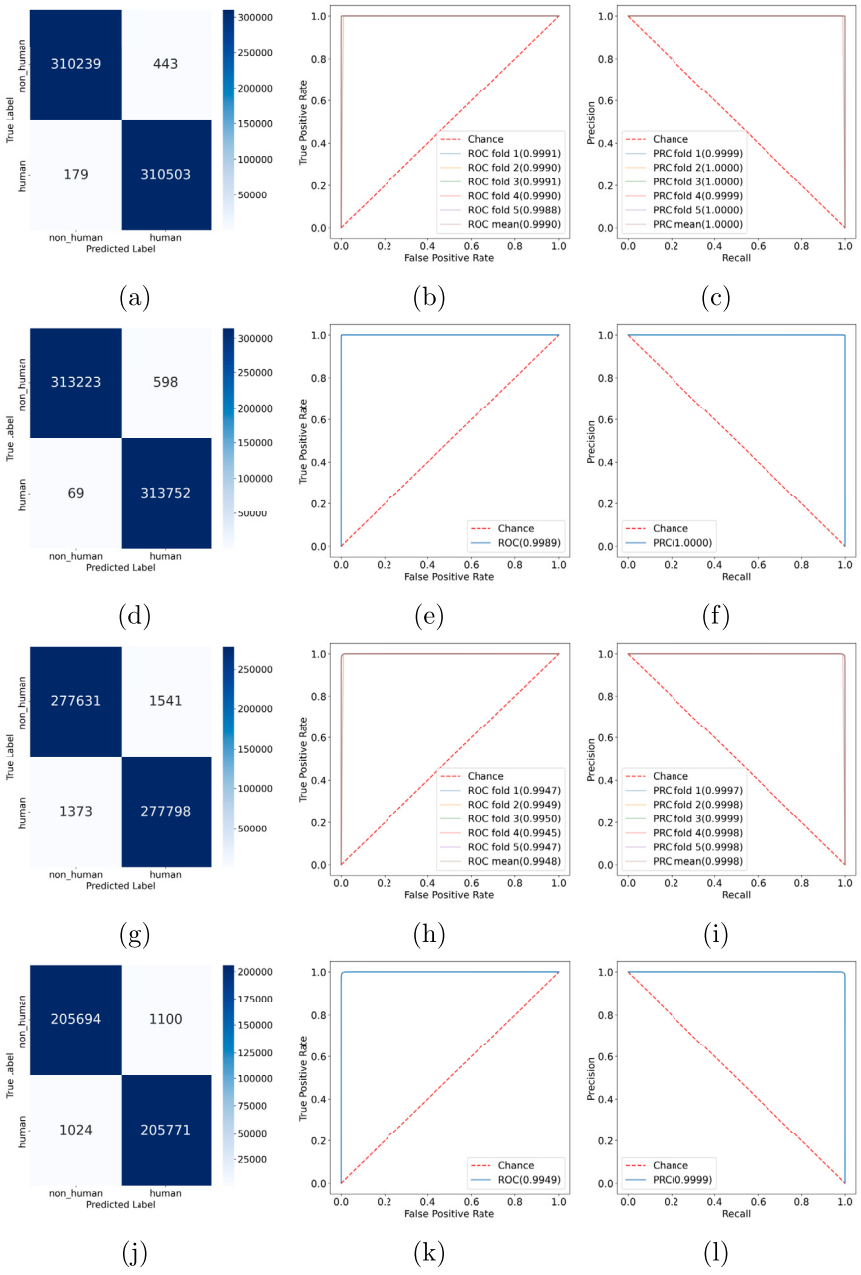

Fig. 3. The 5-fold cross-validation and independent test results. (a)-(c) for heavy chain 5-fold cross-validation, (d)-(f) for heavy chain independent test. (g)-(i) for light chain 5-fold cross-validation, (j)-(l) for light chain independent test. The figures at first, second and last column are confusion matrix, ROC plot and PRC plot, respectively.

Table 1
Test results on different number of samples for building the model (heavy chain).

Nsample	*TP*	*TN*	*FP*	*FN*	*ACC*	*F1*	*AUROC*	*AUPRC*
1E03	998934	991950	8050	1066	0.9954	0.9954	0.9954	0.9998
5E03	999213	996195	3805	787	0.9977	0.9977	0.9977	0.9999
1E04	999335	997576	2424	665	0.9984	0.9984	0.9984	0.9999
5E04	999666	998676	1324	334	0.9991	0.9991	0.9992	1.0000
1E05	999670	999301	699	330	0.9995	0.9994	0.9995	1.0000

Table 2
Test results on different number of samples for building the model (light chain).

Nsample	*TN*	*TP*	*FN*	*FP*	*ACC*	*F1*	*AUROC*	*AUPRC*
1E03	992696	990099	9901	7304	0.9914	0.9914	0.9914	0.9995
5E03	993825	993404	6596	6175	0.9936	0.9936	0.9936	0.9997
1E04	994810	993881	6119	5190	0.9943	0.9943	0.9943	0.9997
5E04	995177	995348	4652	4823	0.9953	0.9953	0.9953	0.9999
1E05	995266	995911	4089	4734	0.9956	0.9956	0.9956	0.9999

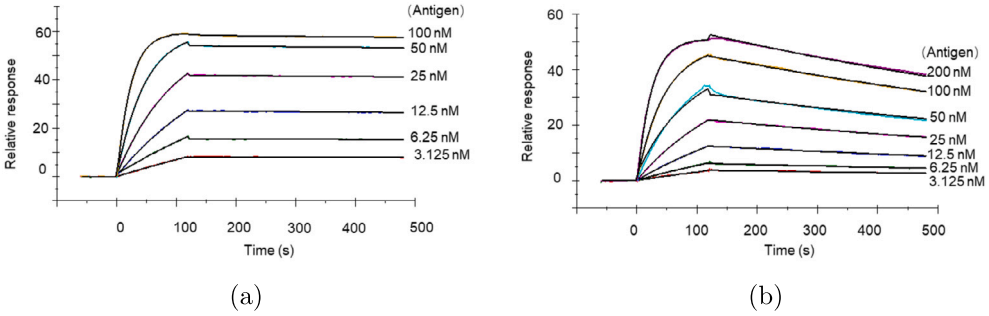

Fig. 4. The rational design-based humanization results (mAb5). (a) the SPR biding affinity measurement of original rabbit monoclonal antibody, which shows 1.11E-10 binding affinity. (b) the SPR binding affinity measurement of humanized antibody, which shows 4.46E-09 binding affinity.

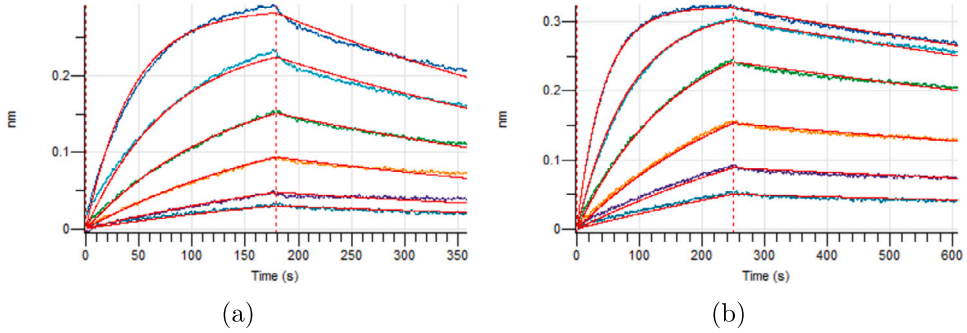

Fig. 5. The humanization results based on AI-assisted computational design (mAb2). (a) the BLI biding affinity measurement of original rabbit monoclonal antibody, which shows 9.28E-09 binding affinity. (b) the BLI binding affinity measurement of humanized antibody, which shows 2.28E-09 binding affinity.

Table 3
Binding affinity test results on 10 test cases of humanization.

Case ID	KD(M)/ EC50(ng/ml) before humanization	KD(M)/ EC50(ng/ml) after humanization	Binding Affinity Increase(↑)/ Decrease(↓)/ Unchanged(≈)	Design Mode Rational Design (RD)/ AI Design (AI)
mAb1	1.77E-08	8.73E-09	success, ≈↑ 1.03x	AI
mAb2	9.28E-09	2.28E-09	success, ↑ 3.07x	AI
mAb3	3.796 (EC50)	2.383 (EC50)	success, ≈↑ 0.59x	AI
mAb4	8.836 (EC50)	144.7 (EC50)	fail, ↓ 15.8x	RD
mAb5	1.11E-10	4.46E-09	success, ↓ 39.18x	RD
mAb6	1.15E-09	4.34E-09	success, ↓ 2.77x	RD
mAb7	1.37E-09	4.77E-11	success, ↑ 27.72x	RD
mAb8	5.99E-10	1.54E-09	success, ≈↓ 1.57x	AI
mAb9	7.96E-10	1.35E-09	success, ≈↓ 0.70x	AI
mAb10	1.39E-10	1.61E-09	success, ↓ 10.58x	AI

Table 4
Binding affinity test results on 8 test cases of caninization/felinization.

Case ID	KD(M)/EC50(ng/ml) before caninization /felinization	KD(M)/EC50(ng/ml) after caninization /felinization	Binding Affinity Increase(↑)/ Decrease(↓)/ Unchanged(≈)
mAb11	2.03E-09	1.17E-09	success, ≈↑ 0.74x
mAb12	8.97E-10	3.09E-08	fail, ↓ 33.45x
mAb13	0.09 (EC50)	146.5 (EC50)	fail, ↓ 1626.78x
mAb14	4.49E-08	7.71E-07	success, ↓ 16.17x
mAb15	1.18E-08	1.21E-08	success, ≈↓ 0.03x
mAb16	1.19E-08	3.89E-07	success, ↓ 31.69x
mAb17	7.25E-10	2.64E-10	success, ≈↑ 1.75x
mAb18	8.223 (EC50)	92.72 (EC50)	fail, ↓ 10.28x

3.3. Case study for heterologization

3.3.1. Humanization

YabXnization platform provides two ways for humanization, traditional CDR-grafting and backmutation based rational design; and AI-assisted fusion computational design. We have conducted 10 groups of humanization test and the corresponding results are summarized in Table 3. Columns 2-3 list the binding affinity values of each test mAbs before and after humanization. Column 4 presents the information of binding affinity change with the marks "↑", "↓" and "≈" which represents increase, decrease and approximately unchanged, respectively. Column 5 shows the design mode used for the reported clone (the best one of all designed clones) of each mAbs.

According to the succuss definition (delivery standard of our heterologization services): the binding affinity losses of heterologized antibodies are all within an order of magnitude compared to the corresponding chimeric antibodies, YabXnization platform achieves an 90% success rate on humanization. Here we take 4-1BB agonist antibody as example for showing the capability of YabXnization platform in doing humanization. The SPR binding affinity measurement results for original antibody and humanized antibody are depicted in Figs. 4(a) and 4(b), respectively. Also, we show an example which use AI-assisted fusion computational design in Fig. 5. Different from general humanization, test results show an increase of the binding affinity of humanized antibody with the use of AI-assisted computational design.

3.3.2. Caninization and felinization

Currently, YabXnization platform can provide only rational design for caninization and felinization due to the small amount of existing canine and feline antibodies which have been sequenced and stored in public databases. We have conducted 8 groups of caninization/felinization test and the corresponding results are summarized in Table 4 in which mAb11 - mAb16 are canine and mAb17 - mAb18 are feline. Similar to Table 3, columns 2-3 list the binding affinity values of each test mAbs before and after caninization/felinization. Column 4 presents the information of binding affinity change with the marks "↑", "↓" and "≈" which represents increase, decrease and approximately unchanged, respectively.

According to the success definition (delivery standard of our heterologization services): the binding affinity losses of heterologized antibod-

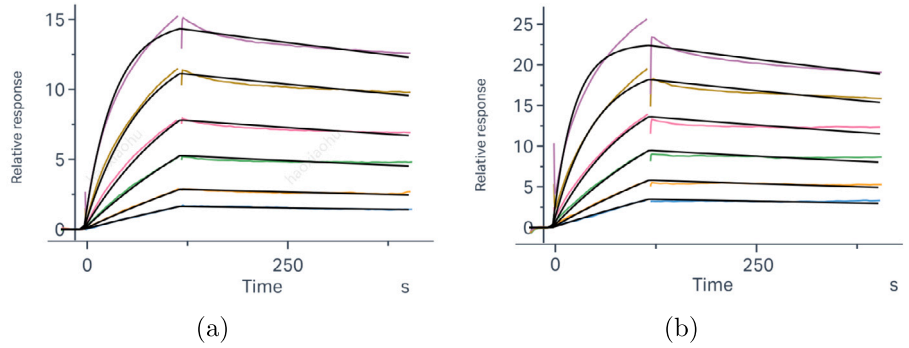

Fig. 6. Test results for caninization (mAb11). (a) the SPR binding affinity test results of corresponding chimeric antibody, which shows 2.03E-09 binding affinity. (b) the SPR binding affinity test results of caninized antibody, which shows 1.54E-09 binding affinity.

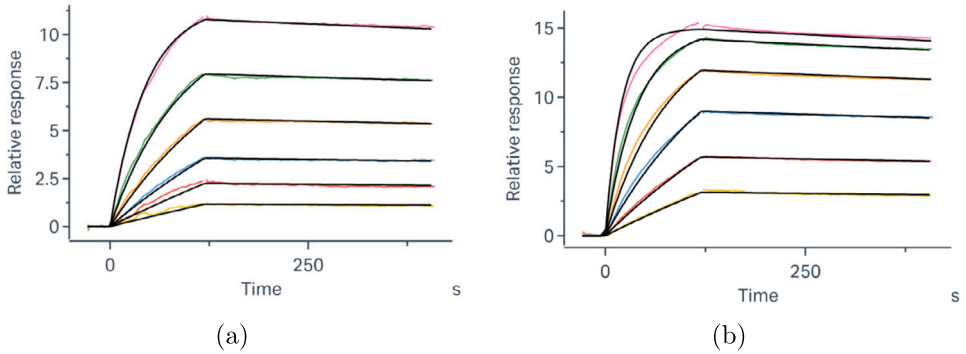

Fig. 7. Test results for felinization (mAb17). (a) the SPR binding affinity test results of corresponding chimeric antibody, which shows 7.25E-10 binding affinity. (b) the SPR binding affinity test results of felinized antibody, which shows 2.64E-10 binding affinity.

ies are all within an order of magnitude compared to the corresponding chimeric antibodies, YabXnization platform achieves an 62.5% (5/8) success rate on caninization/felinization. Here we show two example results for caninization and felinization in Fig. 6 and Fig. 7. Both figures depict only the SPR binding affinity test results of first candidate caninized/felinized antibody (there are 15 candidates for each antibody to be caninized/felinized in our test in total, most of those candidates with similar binding affinity while some of them with binding affinity slightly decreased) and its corresponding chimeric antibody in the figure. Test results show an increase of binding affinity in both cases, which indicates that our method has the capability to maintain the binding affinity in caninization and felinization.

3.3.3. Binding affinity measurement bias analysis

In this work, the binding experiments were conducted once as is usually done in the production process. The main considerations are listed as follows: (1) When doing the binding affinity measurement of the heterologized antibody, the corresponding chimeric antibody is measured together for getting the relative change, and also for eliminating the batch effects of the instrument as much as possible. (2) In all of the three kinds of testing methods (ELISA, BLI and SPR), the indicators which show the confidence of the experimental results would be reported together with the binding affinity, they are R^2 (range from 0 to 1, the higher the better) for ELISA, χ^2 (range from 0 to 1, the lower the better) and R^2 (range from 0 to 1, the higher the better) for BLI, and R_{max} (the max response unit, should be smaller than the theoretical values) for SPR. All those indicators in the test cases indicate high confidence of the binding experiments. Multiple binding experiments would be conducted only if required by the customer, or the initial binding experiments had low confidence indicators.

However, multiple binding experiments would help to eliminate the bias or errors of the instrument and could provide a more rigorous explanation of the binding affinity change in a statistical way. Accordingly, two extra antibodies (mAb-ex1 mAb-ex2) were selected as examples for conducting multiple times (totally 3 times) of binding experiments. The corresponding test information is summarized in Table 5, including the mean and std (standard division) of the tested binding affinity. The association and dissociation kinetic curves of the binding affinity test are plotted in Fig. 8. The std values listed in the table show the stability of the BLI binding affinity measurement.

Table 5
Binding affinity test results on 2 test cases of humanization.

Case ID	mAb-ex1	mAb-ex2
KD(M) before - 1	1.90E-09	1.47E-08
KD(M) before - 2	2.46E-09	3.16E-08
KD(M) before - 3	2.53E-09	2.45E-08
mean±std KD(M) before Humanization	2.30E-09±2.82E-10	2.36E-08±6.90E-09
KD(M) after - 1	2.51E-09	4.77E-08
KD(M) after - 2	2.78E-09	6.05E-08
KD(M) after - 3	2.86E-09	4.56E-08
mean±std KD(M) after Humanization	2.72E-09±1.50E-10	5.13E-08±6.60E-09
Binding Affinity Change Incre(↑)/Decre(↓)/Unchan(≈)	success, ≈↓ 0.18x	success, ≈↓ 1.17x

4. Discussion

In this work, we introduced a webserver named YabXnization which provides monoclonal antibody heterologization, specifically, humanization, caninization and felinization. YabXnization provides two modes for humanization for selection: rational design and AI-assisted computational design. As described in previous section, YabXnization achieves an 90% success rate on humanization, and 62.5% success rate on

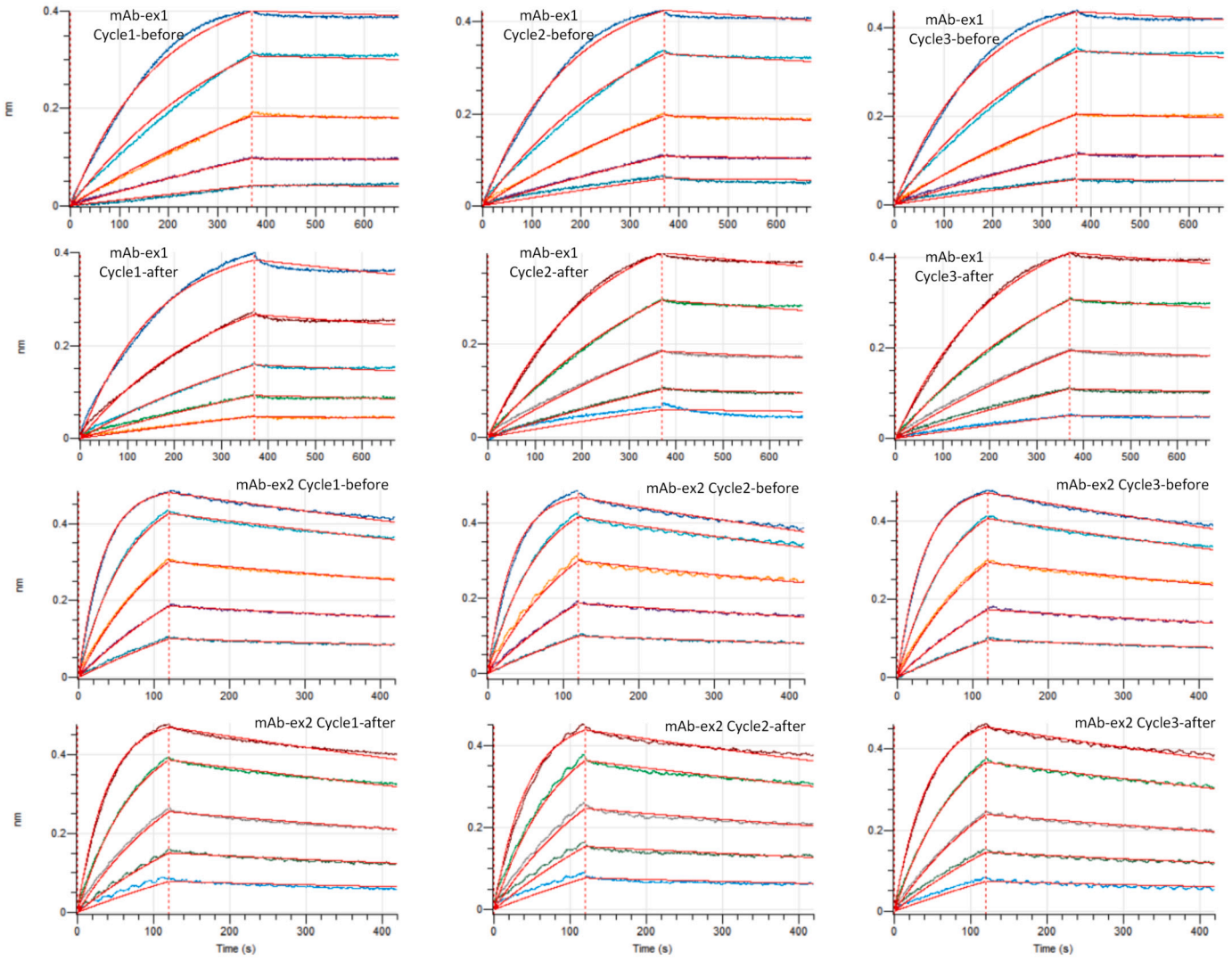

Fig. 8. The association and dissociation kinetic curves of the binding affinity test using the Octet^{RED} 384 with AHC2 biosensor for mAb-ex1 and mAb-ex2.

caninization/felinization. The user can easily get access to the server and conveniently use the service with few parameters to be filled or selected.

We summarize several key points here that affect the success of monoclonal antibody heterologization. **1.** A proper template antibody is the first step for doing heterologization, the quality of the template directly affects the successfulness, which means enough number of human/canine/feline antibodies should be collected. **2.** The key amino acids in the framework region of origin antibody sequences which may have important functions (e.g., essential for keeping the whole structure) should be exactly found out and kept in the heterologization process. **3.** Accurately modeling of the antibody structure is important for finding those previous mentioned amino acids. **4.** The combination of heavy and light chains may also affect the binding of the heterologized antibody. As the success rate described in previous section, the success rate is 90% on humanization while only 62.5% on caninization/felinization. We have billions of human antibodies in hand while only hundreds of canine antibodies and several feline antibodies. This is also the reason why we can provide AI-assisted computational design for humanization but currently only offer rational design for caninization and felinization. For further improving the success rate, we are considering to use the AIGC-based method for humanization, which could mine the billions of human antibodies in a smarter and more AI way. As for the caninization and felinization, we are planning to generate more specific antibodies and further build our own larger canine and feline antibody database.

CRediT authorship contribution statement

Xiaohu Hao: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Dongping Liu:** Writing – review & editing, Visualization, Validation, Software, Formal analysis. **Long Fan:** Writing – review & editing, Supervision, Software, Resources, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors are current employees of GenScript and may hold shares in GenScript Corporation. Methods and compositions described in this manuscript are the subject of one or more pending patent applications (one of the patent granted in China: ZL202311160298.8).

Data availability

The data used in this work are all attached in the manuscript or can be found in the reference papers. We apologize that the source code could not be released at moment due to the policy of GenScript Co., Ltd.

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