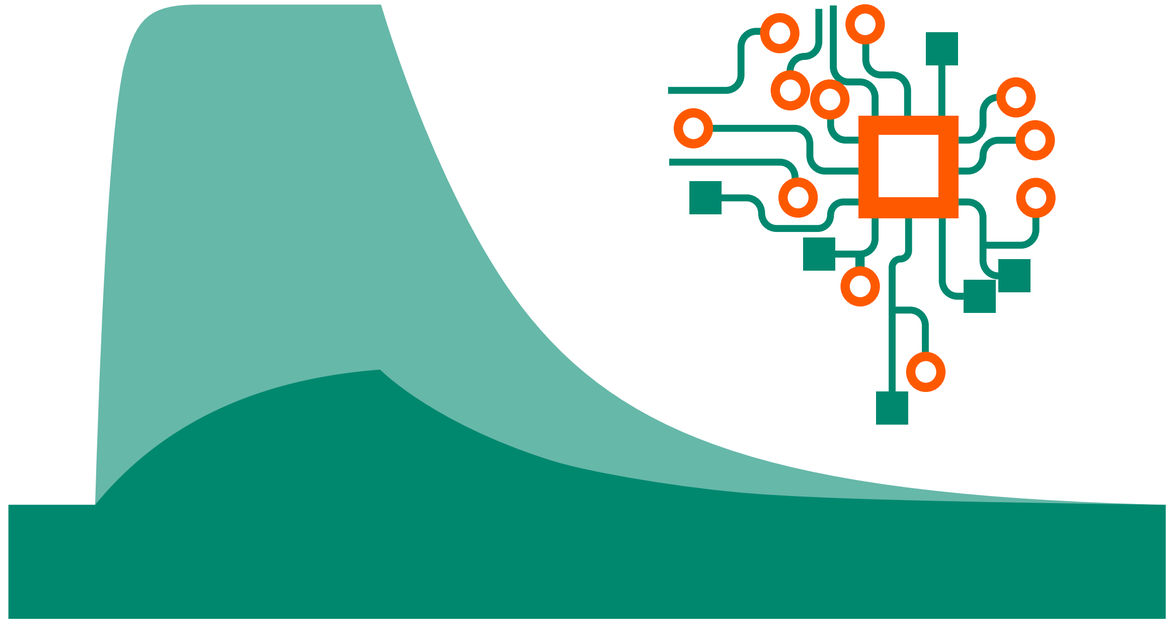
Biacore™ application guide

Biacore Intelligent Analysis™ software



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Introduction

Biacore™ surface plasmon resonance (SPR) instruments enable characterization of potential binders to protein targets at high throughput. To support this high throughput workflow, Biacore Intelligent Analysis™ software (BIA) applies a type of artificial intelligence called machine learning to analyze large quantities of data with minimal input from the user.

Biacore Intelligent Analysis software extension includes all features for running and evaluating binding level screens and affinity screens as you are familiar with from Biacore Insight Extended Screening extension. In addition, BIA also provides input on whether to trust the results as wells as suitable settings for analysis of each compound. This saves time, provides confidence and consistency during evaluation, minimizes human bias, and reduces the risk of selecting leads with intrinsic problems such as non-specific binding and aggregation. Rejection of data always comes with a rationale, to ensure transparency.

If you do not agree with the prediction model, you can easily override it and train it with your own preferences. This makes it better adapted to your data, needs and interpretations in future evaluations. In other words, the more you use BIA, the better it becomes at helping you to interpret data and to decide upon next step.

All descriptions in this application guide assume that Biacore Intelligent Analysis software extension is active.

Terminology

Term	Meaning
Affinity screen	A concentration series assay to select potential binders based on estimated affinity to the target.
BIA	Short for Biacore Intelligent Analysis software, a Biacore Insight Software extension that employs machine learning to support the user in the evaluation of binding level screens and affinity screens.
Binding level screen	A single-concentration assay to identify potentially interesting compounds binding to the target as well as poorly behaving compounds by assessing response level and binding curve shape.
Binder prediction	Biacore Intelligent Analysis for binding level screen evaluation.
K_D	The equilibrium dissociation constant, describing binding affinity.
Machine learning	A type of artificial intelligence that involves the training of algorithms to perform tasks without explicit instructions.
Prediction model	A machine learning algorithm. In BIA, it primarily predicts how well the evaluated results can be trusted.
Quality prediction	Biacore Intelligent Analysis for affinity screen evaluation.

Related documents

Documentation	Main contents
Biacore Insight Evaluation Software User Manual	Detailed instructions for using Biacore Insight Evaluation Software to evaluate Biacore SPR results.
Application guide: Fragment and small molecule screening	General information on how to perform fragment and small molecule binding level screen and affinity screen runs and how to evaluate the results.
Application guide: Solvent correction	Information on how to perform solvent correction.

All documentation is available for download from cytiva.com/biacore.

How Biacore Intelligent Analysis software support your work

BIA supports two different types of Biacore screening assays, binding level screen and affinity screen.

For binding level screens, BIA compares the properties of the measured data with properties of the data used for training, to predict if it is of high or low quality (Fig 1A). Signs of low quality may be binding to the reference surface, having atypical dissociation, or sensorgram artefacts (Fig 1B). Low quality binders may show visible binding but may for other reasons not be trusted.

For affinity screens, BIA predicts from which part of the sensorgram to extract the response, excludes sensorgrams of poor quality, and predicts whether to use an affinity model with constant R_{max} or fitted R_{max}. Finally, it predicts whether the data should be accepted or rejected based on, for example, binding profile, any binding to the reference surface, or if the concentrations were too high or too low for an accurate affinity estimation (Fig 1C).

Incorporating BIA into your daily work has many advantages and is recommended in a multitude of situations such as:

- When time needed to evaluate the data manually becomes a bottleneck in your workflow, BIA can significantly speed up data interpretation.
- When a team contains members of different experience: more experienced team members can embed their experience in the prediction models through training, which any team members can then apply to the data.
- To drive consistency in data evaluation across projects and teams.

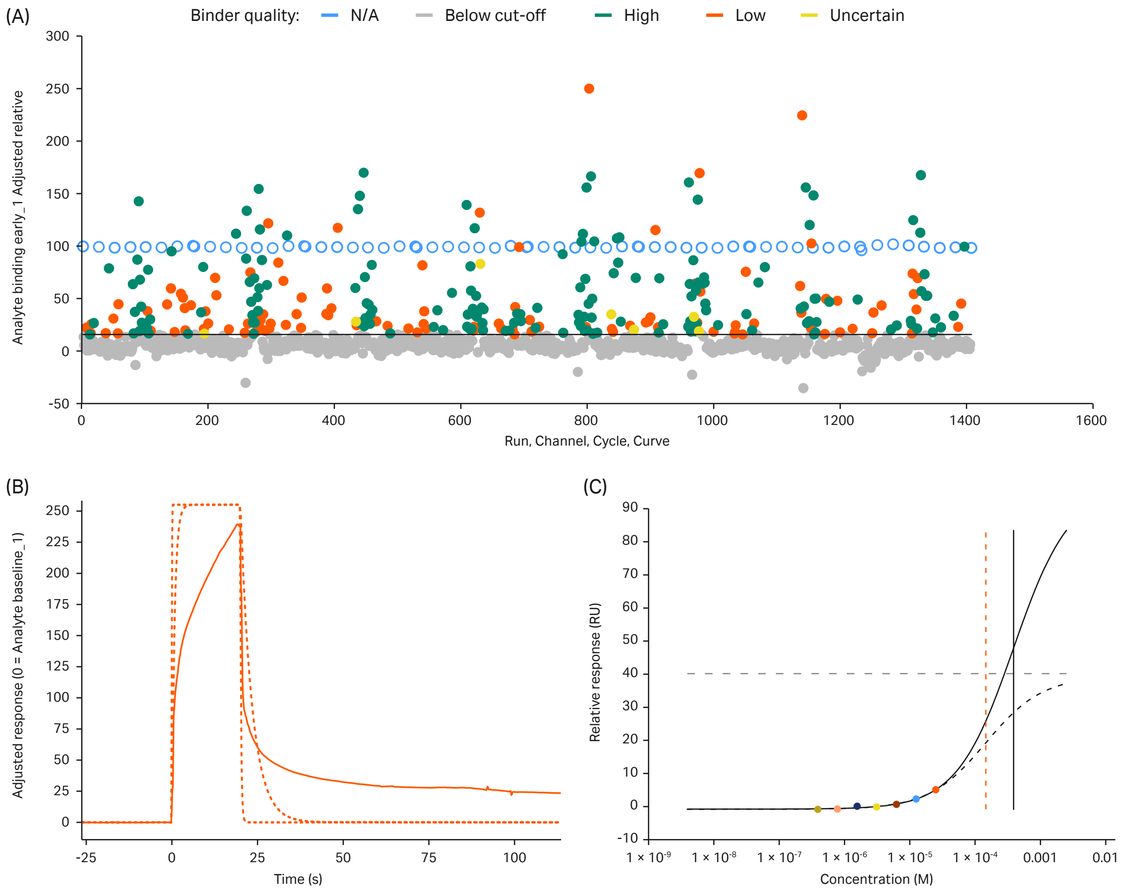

Fig 1. Examples of results from Biacore Intelligent Analysis software. A) Overview of binding level screen results, colored by predicted quality. B) Example of a measured binding level screen sensorgram (solid line) compared to two ideal fragment binding profiles (dashed lines). This fragment was predicted to be of low quality because of its positive slope during injection and the atypical dissociation. C) Example of an affinity screen series that was rejected since the concentrations were too low for an accurate K_D estimation.

Tips for Biacore Intelligent Analysis software

- The predefined run methods for fragment binding level screen and fragment affinity screen contain the necessary components for BIA (see *Requirements for BIA*, for more information).
- Train your model to better adapt it to your data, needs and quality interpretations.
- If the pretrained prediction models included in Biacore Intelligent Analysis software do not predict your type of data well, it can be better to create your own prediction model and base it on an empty model than on the pretrained model.
  Note: *The amount of data that a model is based on determines how robust it will be, but also how much data it takes to tweak it by retraining. The pretrained models are based on significant amounts of data to gain robustness.*
- Refer to the application guide *Fragment and small molecule screening with Biacore systems* for general information on how to set up a fragment binding level screen and an affinity screen.
- Refer to *Biacore Insight Evaluation Software User Manual* for detailed information on how to use the software.

Workflow

Prediction using a predefined evaluation method

Follow the steps below to evaluate binding level screen or affinity screen data with BIA.

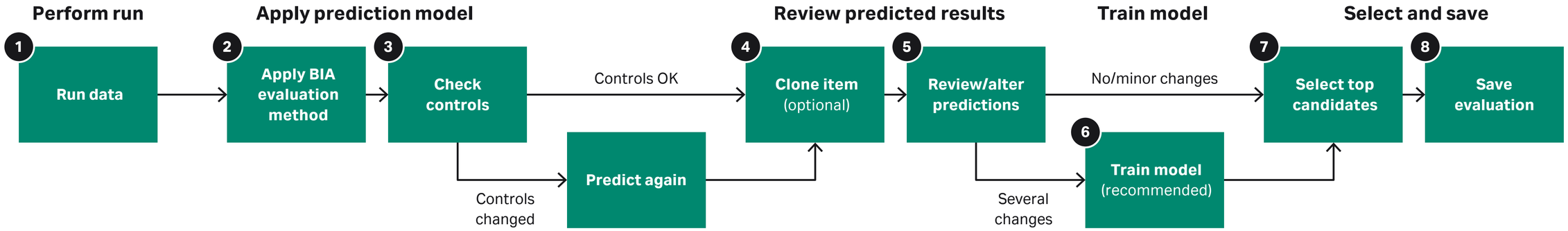

Fig 2. Overview of the workflow when using a predefined BIA evaluation method.

Step	Action
1	Select one or several binding level screen or affinity screen runs in Biacore Insight Evaluation Software.
2	Apply a BIA evaluation method. This opens the data, evaluates the results, and performs the prediction.
3	Globally exclude any controls with deviating curve shape or amplitude (see *Exclude deviating controls*). If any controls are excluded, perform an additional prediction (see *Prediction without a predefined evaluation method*).
4	**Optional step.** Clone the item containing the evaluated and predicted results before proceeding to the review process in step 5. This makes it possible to later compare the original predictions with the end results.
5	Review the predicted results. Change classifications, quality or acceptance state if suitable. See *Review the results,* for more information.
6	**Recommended step.** If a significant number of changes were made in step 5, train a new version of the prediction model to customize it to your type of data and definition of quality.
7	Identify the compounds of interest. Suggested strategy: • Binding level screen: Filter the plot table to only see binders with high predicted quality and sort on binding levels from high to low. • Affinity screen: Select the result table tab with accepted compounds and sort on K_D values from low to high.
8	Save the evaluation.

Prediction without a predefined evaluation method

Sometimes, it is not possible to apply a BIA evaluation method to obtain quality predictions, such as when the run data does not contain sufficient information or when you want to perform a prediction of data that has already been evaluated without prediction. In these cases, predictions can be performed directly in the evaluation once all requirements for BIA are fulfilled (see *Requirements for BIA*), as described in the workflows for binding level screen and affinity screen.

Binding level screen

Step	Action
1	Globally exclude any positive or negative controls with deviating curve shape or amplitude (see *Exclude deviating controls*).
2	Create a new Plot item.
3	Plot the adjusted response of **Analyte binding early_1**. Recommended adjustments are blank subtraction, molecular weight adjustment and adjustment for controls. ***Note:*** *The pretrained model for fragment binding level screen that comes with the extension may not be suitable for data that are not adjusted according to these recommendations.*
4	Set a boundary that is not based on ranking.
5	Select a prediction model and perform the prediction from the ***Binder prediction*** settings.
6	Perform steps 4–8 described in *Prediction using a predefined evaluation method.*

Affinity screen

Step	Action
1	Globally exclude any controls with deviating curve shape or amplitude (see *Exclude deviating controls*).
2	Create a new Affinity item.
3	Select a prediction model and perform the prediction from the ***Quality prediction*** settings.
4	Perform steps 4–8 described in *Prediction using a predefined evaluation method.*

Requirements for BIA

There are some components that are required or highly recommended to include when setting up runs for BIA. These are presented in Table 1 and are also included in the predefined run methods for fragment binding level screen and fragment affinity screen.

The components must have been defined correctly in the run setup for the predefined BIA evaluation method to create all items. Missing information can be added later in the ***Variables*** and ***Properties*** workspaces of Biacore Insight Evaluation Software, but this requires manual creation of evaluation items and application of prediction models (see *Prediction without a predefined evaluation method*).

Table 1. Components definition

Component	Purpose	Details
Binding level screen		
Positive control[1]	Define level for stoichiometric binding and to correct for ligand activity decline	Should be injected at a high enough concentration to reflect maximum binding activity. If a positive control is missing, the theoretical R_{max} used for defining stoichiometric binding is calculated by the software provided that the immobilization level and ligand molecular weight is available from the run or are entered in the ***Properties*** workspace. This option assumes a ligand activity of 100% and that the ligand is immobilized and not captured.
Negative control[1]	Primarily for blank subtraction, potentially also to define cut-off level	Should be prepared in a sample like manner and preferably not be running buffer.
Molecular weight information	Molecular weight adjustment of binding level for samples and controls.	Stoichiometry is ignored by the prediction model if molecular weight adjustment is off. If molecular weight adjustment is on, samples without molecular weight information will not receive any quality or classification predictions.
Affinity screen		
Positive control[1]	Correct for ligand activity decline	Can be the same compound as the R_{max} control.
R_{max} control or ligand level + molecular weight information	Calculate expected R_{max} for fitting with constant R_{max} and to find super and sub stoichiometric binders for series with a fitted R_{max}	The R_{max} control is ideally a concentration series of up to $15\times K_D$ but can be a single concentration of $10\text{-}20\times K_D$. If an R_{max} control is missing, the expected R_{max} value is calculated by the software provided that the immobilization level and ligand molecular weight is available from the run or are entered in the ***Properties*** workspace. This option assumes a ligand activity of 100% and that the ligand is immobilized and not captured.
Molecular weight information	Molecular weight adjustment of expected R_{max}	Molecular weight must be in the same unit for all compounds.
Concentration series	Plot dose response curves for affinity estimation	Each series must contain multiple non-zero concentrations and a zero concentration.

[1]Not required to perform a prediction but highly recommended for accurate results.

Exclude deviating controls

Controls play a large role in BIA, which makes it important to remove any controls that behave unexpectedly. A negative control with unusually high binding leads to too low blank subtracted responses, and deviating positive or R_{max} controls affect the definition of stochiometric binding. Control responses can be viewed in plots to get a quick overview. Select points to see their corresponding sensorgrams, to further investigate the cause of the deviation (Fig 3).

Perform another prediction once the controls have been removed.

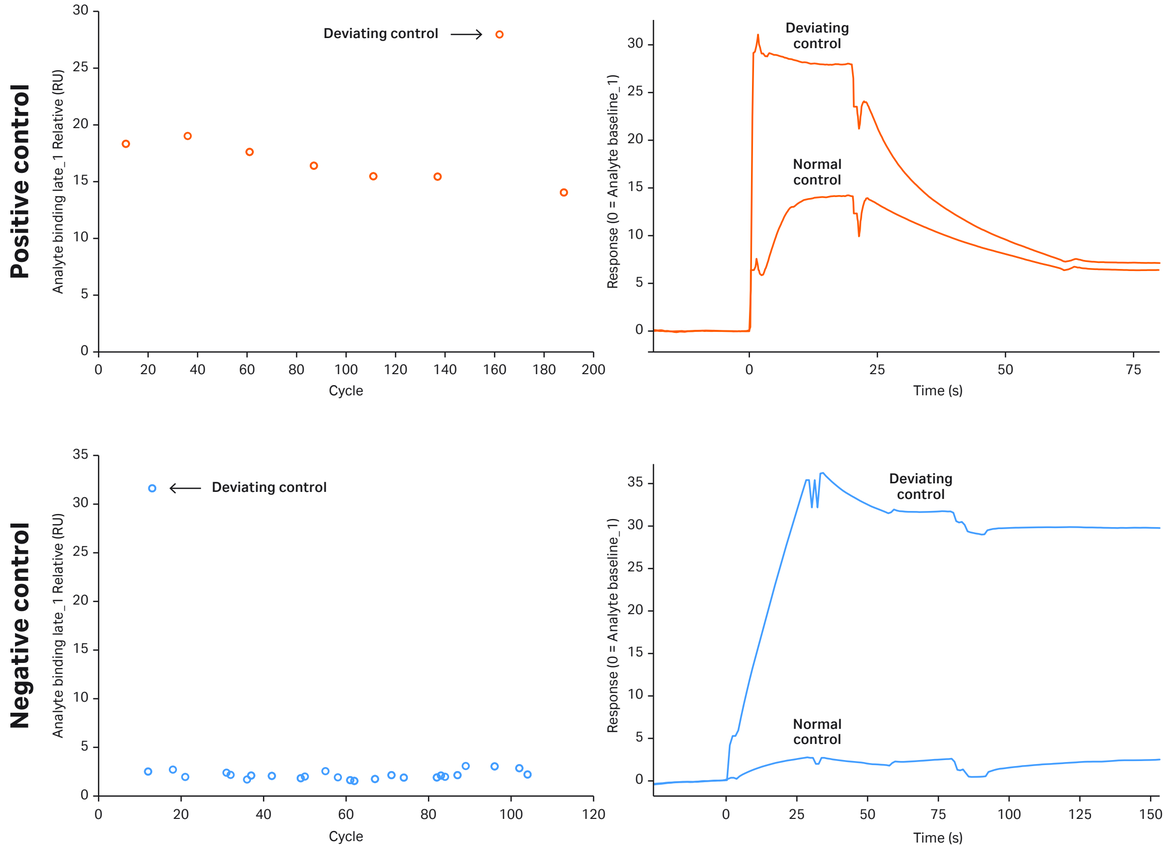

Fig 3. Examples of controls that should be excluded. The plots of the positive and negative controls each show one deviating control with higher response. Their corresponding sensorgrams were compared with sensorgrams from non-deviating controls, which showed clear differences in binding behavior.

Review the results

It is recommended to review the results of the prediction, to ensure a high quality of the evaluation. In particular:

- Go through all results that the prediction model was uncertain of and classify them based on your interpretation. It is possible to display only these results in the tables.
- For the other results, edit the predicted settings, classifications or quality/acceptance state if you do not agree with the predictions.

When you have a model that you trust, the data classified as uncertain might be the only data needing attention, saving huge part of the evaluation time. Changes to predictions are primarily done in the ***Classification*** panel and apply to selected data. The following sections contain information about the data interpretation and review process that is specific for binding level screen respectively affinity screen.

Note: *All manual changes are discarded if you perform another prediction.*

Tip: *Manual changes can be incorporated into a new version of the prediction model version by training it after the review.*

Review binding level screens

The result table displays information about the binding level of each compound. With the predefined BIA evaluation method, the levels are displayed as % of the positive control level and are blank subtracted and adjusted by molecular weight and ligand activity changes.

Additionally, the result table contains predicted information about the quality of the binder:

- Binder quality: The predicted quality of the binder, which is set to High, Low, Uncertain (not possible to determine if it is High or Low) or Below cut-off. Below cut-off is set for compounds with binding levels below a defined cut-off. These compounds do not obtain any other quality information and are not included in prediction model training.
- High quality certainty (%): The predicted certainty that the fragment is of high quality. Values close to 100% corresponds to a high predicted quality, while values close to 0% corresponds to a low predicted quality.
- Binder classification: A description of the behavior of the fragment. A fragment predicted to be of high quality does not necessarily have any classifications, while all fragments predicted to be of low quality have at least one classification that act as a rationale.

The measured sensorgrams (solid lines) are displayed together with ideal binding profiles (dashed lines) and are adjusted in the same way as the plot data (Fig 4).

Definitions of the classifications and how to review them are presented in Table 2.

Table 2. Classification definitions

Classification	Definition	Review strategy
Atypical dissociation	During the dissociation phase: the sensorgram signal is significantly below the baseline at any time, there is a drift or an offset in the signal, and/or the dissociation is slow and the signal remains above the baseline for the duration of the dissociation phase (Fig 4A, 4B).	Compare the measured and ideal sensorgrams or investigate the ***Dissociation response*** or ***Dissociation slope*** table columns.
Baseline difference	Large difference in the baseline levels between the current cycle and the next cycle.	Investigate the column ***Baseline difference*** in the table.
Binding to reference	The remaining binding to the reference surface after the analyte injection is significant.	Display the reference flow cell sensorgram and hide the adjusted sensorgram in the chart settings.
Irregular injection	During the analyte injection: the sensorgram slope changes significantly, and/or has spikes or irregularities (Fig 4C).	Compare the measured and ideal sensorgrams.
Negative slope	The sensorgram has a significant negative slope during the analyte injection (Fig 4A).	Compare the measured and ideal sensorgrams or investigate the ***Association slope*** table column.
Positive slope	The sensorgram has a significant positive slope during the analyte injection (Fig 4B).	Compare the measured and ideal sensorgrams or investigate the ***Association slope*** table column.
Super stoichiometric	The binding level is much higher than expected. This classification is only possible if the samples have been molecular weight adjusted.	Compare the binding level of the fragment with the binding level of the positive control in the plot.

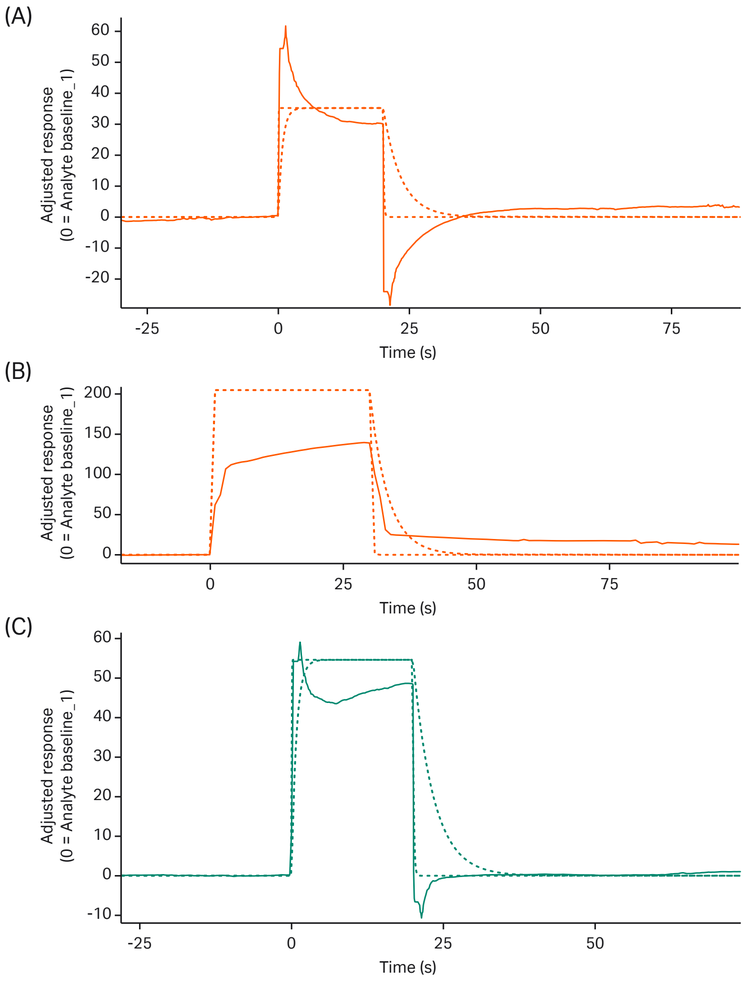

Fig 4. Classification examples of measured curves (solid line), with ideal profiles for comparison (dashed lines): A) Negative slope during injection and an atypical dissociation, B) Positive slope instead of quickly reaching steady state, and an atypical dissociation, C) Irregular injection with some spikes.

Review affinity screens

For affinity screens, BIA starts by predicting the ***Affinity range position*** from which the response is gathered, marked with bars on the sensorgrams. Sensorgrams with deviating profile are then excluded. These are visible as grey dotted lines in the ***Sensorgrams*** panel.

Dose response curves calculated from an affinity model with constant R_{max} (dashed line) or a fitted R_{max} (solid line) are both visible in the chart, with corresponding vertical lines representing their K_D values and the estimated R_{max} presented as a dashed horizontal line (Fig 5). BIA predicts which affinity model that is the most suitable for each data series and sets the ***Rmax type*** accordingly. The K_D corresponding to the selected affinity model is presented in the result table and visualized as a blue vertical line in the graph, or red if all measured concentrations are below or above K_D.

Note: *The constant R_{max} model can only be used for samples with known molecular weight.*

Once the ***Affinity range position*** has been set, poor sensorgrams have been excluded and the ***Rmax type*** selected, BIA predicts the quality of the results. This information is presented in the result table:

- Classification: A description of the behavior of the series and the K_D estimation.
- Acceptance state: Describes whether the estimated K_D value was accepted, rejected or if it was uncertain if it should be accepted or rejected. The acceptance states have separate table tabs; series move to a different tab when their acceptance state is changed. The acceptance state of a series is based on the collective information of all its classifications. For severe classifications such as sub/super stoichiometric, a single classification is sufficient for a rejection. During this review process, classifications and acceptance state can be set independently.
- Acceptance certainty: The predicted certainty that the K_D estimation should be accepted.

When reviewing the predictions, follow the same order as how BIA operates, starting with the ***Affinity range position*** and ending with classifications and acceptance state. Any changes to the ***Affinity range position***, sensorgram exclusion or ***Rmax type*** causes a re-fit, which sometimes motivates changes to the classifications or acceptance states.

Definitions and review strategies of the fit settings and classifications are presented in Table 3. Right-click on a sensorgram to exclude or include it. All other changes can be done from the ***Classification*** panel. Supporting columns can be included from the table settings.

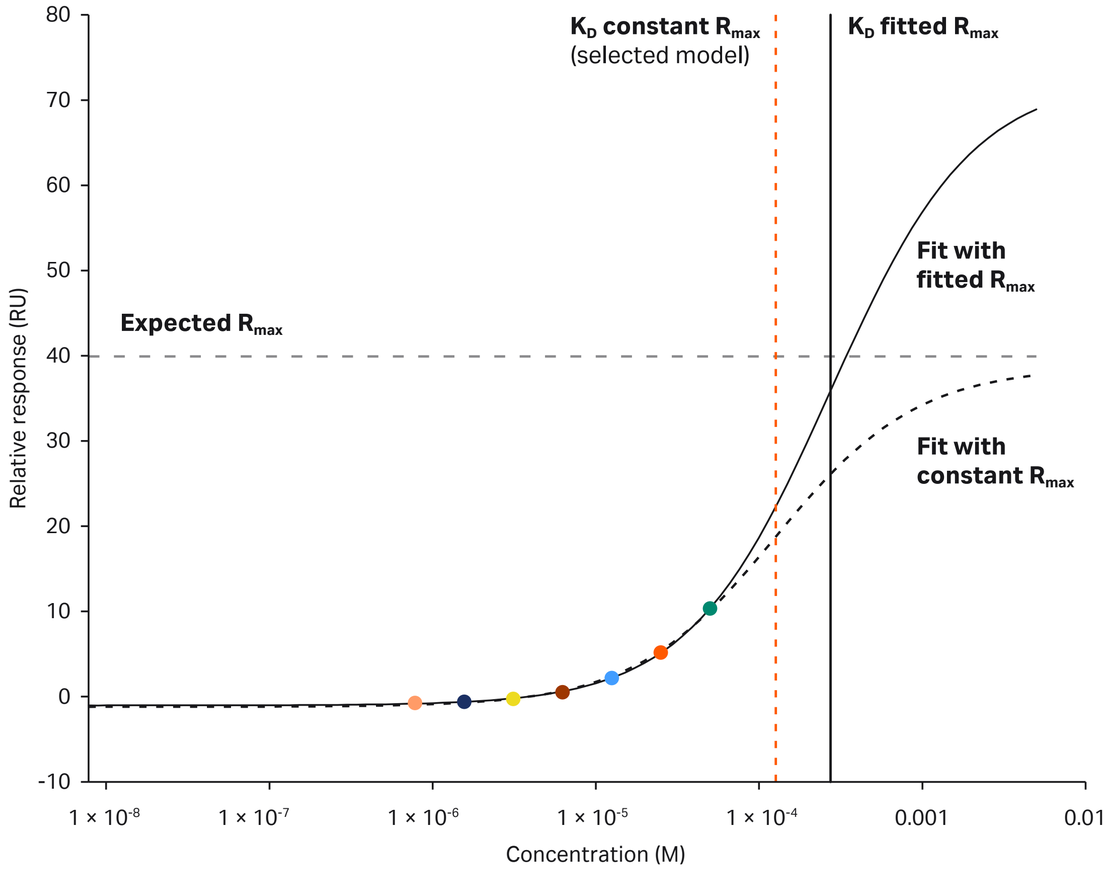

Fig 5. Example of affinity screen results. Both the affinity model fit with constant R_{max} (dashed line) and the one with fitted R_{max} (solid line) are displayed. This series received the ***Constant Rmax*** classification, which made the vertical K_D line for constant R_{max} (dashed) red. If the measured concentration range (colored points) would have covered K_D, the line would have been blue.

Table 3. Classification settings

Setting or classification	Definition	Review strategy
Affinity range position	The position of the response range used for calculating the affinity, either early or late during the analyte injection. With the default settings, these correspond to 6 s after injection start (early) or 5 s before injection end (late). Default settings can be changed in the ***Affinity range*** settings.	Choose a position where: • Steady-state has been reached, visible as a signal plateau • There are no signal disturbances
R_{max} type	The type of R_{max} setting used for calculating the affinity, either constant or fitted.	Use the model that best fulfills the following criteria: • Reasonable value of R_{max} in relation to expected R_{max} • The measured concentration range covers or is close to the estimated K_D value • The model follows the data points well
Atypical/artifact	The majority of the sensorgrams show one or several of the following: significant drift, large offsets, signals below baseline during the dissociation phase, the dissociation is slow and shows no sign of reaching the baseline, negative response.	Investigate the sensorgrams.
Baseline difference	Large difference in the baseline levels between the current cycle and the next cycle.	Investigate the column ***Baseline difference*** in the result table.
Binding to reference	The remaining binding to the reference surface after the analyte injection is significant.	Investigate the response levels after injection in the ***References*** tab of the ***Fit details*** panel. If only one or a few concentrations shows binding to the reference: Consider excluding their corresponding reference subtracted sensorgrams instead of classifying/rejecting the whole series.
Concentrations above K_D	The analyte concentration range is high in relation to the affinity.	Are all points on the dose-response curve far to the right of the vertical K_D line? Use lower concentrations if repeating the run.
Concentrations below K_D	The analyte concentration range is low in relation to the affinity.	Are all points on the dose-response curve far to the left of the vertical K_D line? Use higher concentrations, if possible, if repeating the run.
Large offset	The offset of the fitted curve is large in comparison to expected R_{max}.	Compare the offset and the R_{max} value in the ***Parameters*** tab of the ***Fit details*** panel.
Low binding	The binding responses are very low.	Investigate the sensorgram or dose response curve.
Poor fit	The fitted curve fits poorly to the data points.	Compare the positions of the data points with the fitted dose response curve.
Sub stochiometric	The series has a fitted R_{max} much lower than the expected R_{max}.	Investigate the ***Rmax/Expected Rmax*** table column or corresponding levels in the chart.
Super stoichiometric	The series has a fitted R_{max} much higher than the expected R_{max}.	Investigate the ***Rmax/Expected Rmax*** table column or corresponding levels in the chart.
Too few concentrations	The number of data points in the dose-response curve are few.	The number of non-zero, non-excluded, concentrations should be at least three. Some fits require more.

Train and create prediction models

BIA comes with pretrained models for fragment binding level screen and affinity screen. Although they have been trained by Biacore SPR scientists, they do not guarantee perfect predictions of your specific data, in particular if the data you generate differ much from the data the models were trained for, if you perform different adjustments, or if you have other experience with certain binding behavior that makes you want to accept or reject differently. Because of this, BIA offers the possibility to customize the prediction models through training. Different strategies are recommended depending on situation, as described in Table 4 and summarized in Figure 6.

Table 4. Model prediction scenarios

Situation	Recommendation
You agree with the predictions of the pretrained model (Fig 6A).	Use the pretrained model as it is.
You somewhat agree with the predictions of the pretrained model (Fig 6B).	Use the pretrained model and train it with new input. The pretrained model is robust and changes slowly upon training, since a large amount of data was used in its initial training. ***Note:*** *There is no risk of overwriting the original pretrained models since only copies of them are used for prediction and training.*
You do not agree much with the predictions of the pretrained model (Fig 6C).	Create a new model in the ***Prediction model*** workspace and base it on the ***Empty model*** that is included with the extension. It must be trained before it can be used for prediction. The empty model quickly adapts to your preferences.

Tip: *If you are working with compounds behaving very differently, it can be good to create and train several prediction models, one for each type of behavior.*

Training a model typically reduces the number of uncertain results in future predictions. Every time a model is trained, a new version of it is saved. The new version is based on the latest version in combination with any changes you have made to the predictions. Compounds that are below the cut-off level (binding level screen) or set to ***Uncertain*** are not included in the training.

Tip: *A model is only good at recognizing behaviors it has been trained on. Aim at training with data containing many different binding behaviors to make the model more general.*

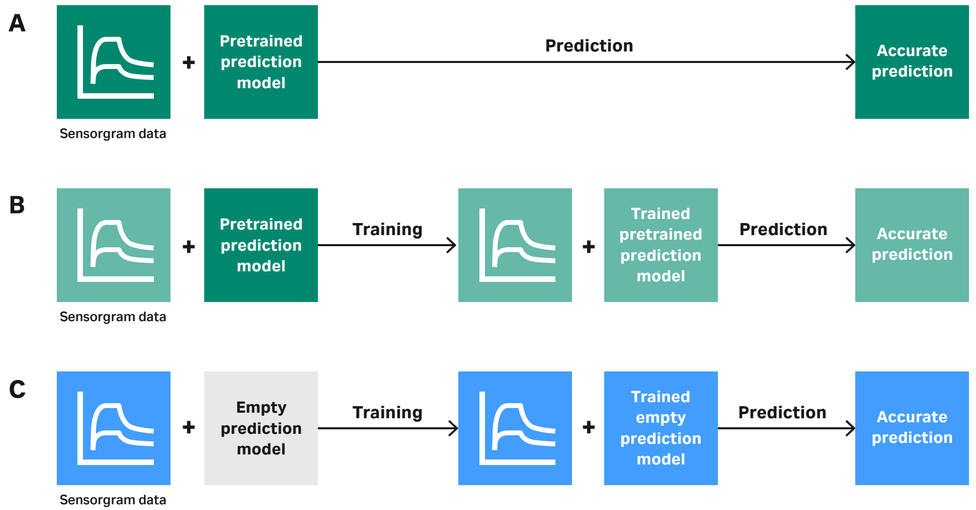

Fig 6. Different strategies for working with the prediction models depending on the situation. A) If you agree with the pretrained model, use it as it is. B) If you like to improve the pretrained prediction model you can continue its training with your own data. C) If you do not agree with the predictions from the pretrained model start with an empty model instead and train it with your own data until you reach a satisfactory prediction quality.

How the pretrained prediction models were created

This section describes how the pretrained BIA prediction models were originally created. The same approach may be used when training other models, to confirm that they perform as expected.

To train the model, data was divided into a training and a validation set. The training set was annotated by an expert who assigned classifications and acceptance states to each sensorgram or sensorgram series. This input was used for training of the model, which was then applied to the validation set. The predictions of the validation set were examined by an expert and if the results were not satisfactory, the model continued to be trained with new data until it correctly predicted the expected results of the validation set (Fig 7).

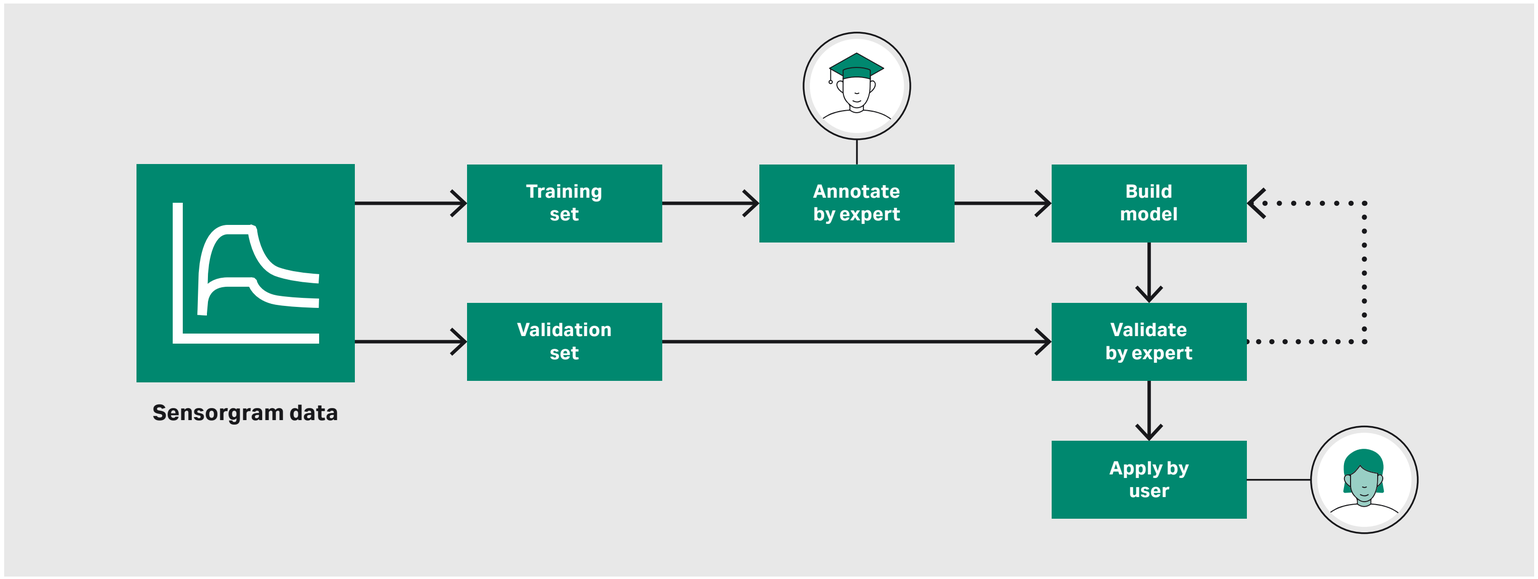

Fig 7. Strategy for training prediction models.

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