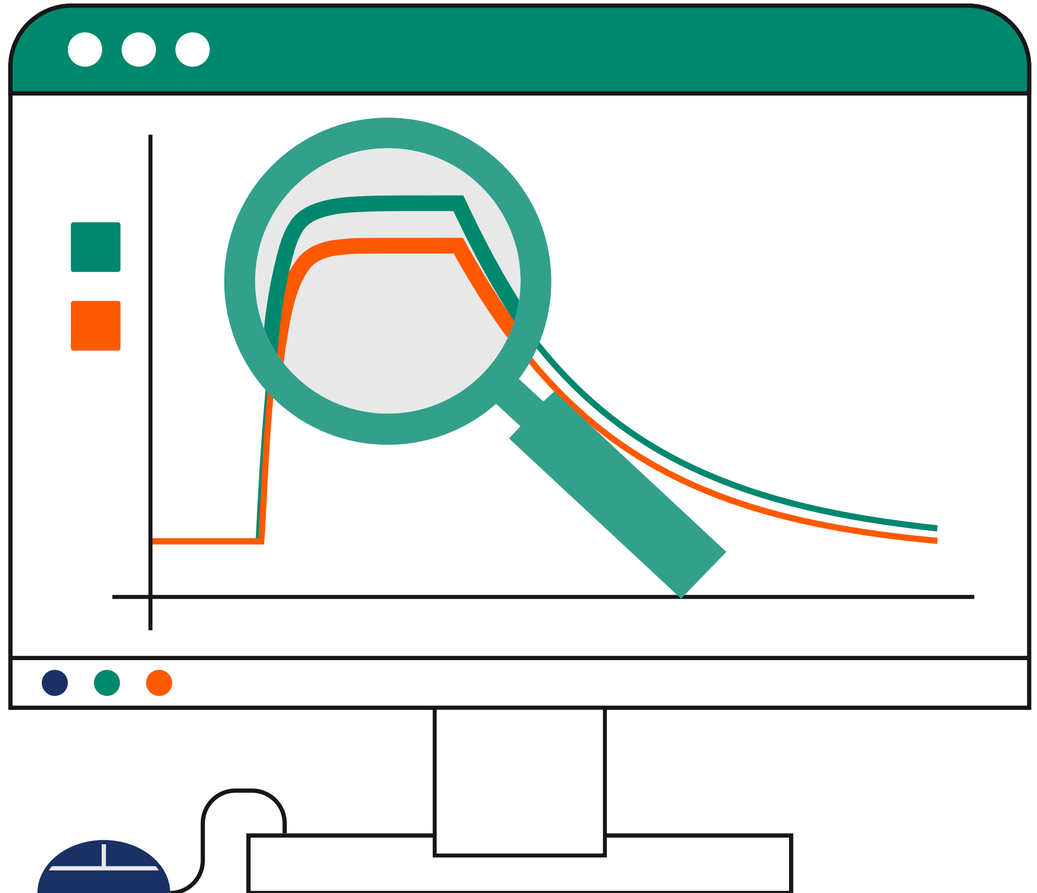
Application guide

Biacore™ *Sensorgram comparison* tool



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Contents

1. Scope of this application guide

Biologic drug development increasingly involves complex molecules such as monoclonal, bispecifics, multispecific, and other engineered antibody formats, where binding behavior can be heterogeneous, multivalent, or difficult to describe using traditional interaction models. At the same time, regulatory expectations, particularly for biosimilars, place strong emphasis on comparative analytical assessment (CAA) and the ability to demonstrate similarity to reference products across multiple functional attributes. These trends highlight the need for robust, model-free approaches that directly compare interaction data and sensitively detect changes in binding behavior across biosimilars, lots, variants, and development stages (1).

In this context, ***Sensorgram comparison*** enables quantitative comparison of full binding sensorgrams using the ***Sensorgram comparison*** functionality in Biacore™ Insight software (2). The scope of this guide is to describe the run setup and evaluation of Biacore SPR experiments designed for comparability assessment. It provides recommendations for preparing runs, handling standards, comparison settings, and assessing the similarity of samples relative to reference standards through model-free, curve shape–based evaluation.
The guide provides an application-centric overview of how ***Sensorgram comparison*** supports comparability, and characterization workflows across Biacore 1 series and Biacore 8 series systems, including use within a GxP environment (3, 4).

2. Abbreviations

Abbreviation	Meaning
SCK	Single-cycle kinetics
MCK	Multi-cycle kinetics
SC	Single concentration
MI	Multiple injections
SS	Similarity score
SD	Standard deviation
RU	Response units
Ab	Antibody
Ag	Antigen
Fc	Flow cell
GxP	Good practice (regulated) workflows

3. Terminology

Term	Meaning
k_a	Association rate constant, units $M^{-1}s^{-1}$, governs the rate at which a complex is formed.
k_d	Dissociation rate constant, units s^{-1}, governs the rate at which a complex dissociates.
K_D	Equilibrium dissociation constant, units M, describes the strength of the interaction.
R_{max}	Maximum analyte binding capacity of the surface (RU).
Standard/reference	Sensorgrams that are used to define the expected binding behavior and natural variability against which sample sensorgrams are compared.
Samples	Sensorgrams generated from test conditions that are evaluated against the reference standards.
Controls	Assay runs included controls to verify correct assay performance and provide reference points.
Sensorgram comparison	A method for comparing binding curves to assess how closely a sample's sensorgram matches a reference (standard) sensorgram.
Similarity score	A quantitative measure (0–100%) describing how closely a sample sensorgram matches the expected binding behavior defined by the similarity range created from the standard's behavior.
Similarity range	The acceptable deviation range (window of variation) around the average/median of standards sensorgrams.
Normalization	A scaling step that adjusts each sensorgram to a common response level (100 RU) so that similarity assessment focuses on curve shape rather than absolute response units.
Biosimilars	Biological medicinal products that are highly similar to an already approved reference biologic, with no clinically meaningful differences in safety, purity, and potency
GxP	A regulated framework of good practice guidelines (such as GLP, GMP, and GCP). In this context, ***Sensorgram comparison*** within Biacore Insight Software is designed to meet 21 CFR Part 11 requirements and supports controlled methods and auditable evaluation steps to ensure data integrity and compliance.

4. Introduction

Sensorgram comparison is a kinetic interaction model-free, full-curve comparison approach in Biacore Insight Software. It assesses whether sample sensorgrams match the binding behavior defined by a set of standards, producing an objective and quantitative similarity score (0–100%). ***Sensorgram comparison*** complements conventional kinetics and affinity by detecting curve-shape differences that may be difficult to capture with fitting alone.

4.1 When to use *Sensorgram comparison?*

Sensorgram comparison enables direct, visual comparison of sensorgrams without relying on kinetic interaction model-fitting or report point analysis.

- It is particularly useful in comparability and similarity assessments, such as biosimilars, lot-to-lot evaluations, reagent changes, variants, and stability or stress studies, where detecting subtle shifts in sensorgram shape is critical.
- ***Sensorgram comparison*** is also well suited for interactions that are complex or heterogeneous, including multivalent, sequential, or conformationally driven binding events, as well as cases with very slow dissociation where robust kd fitting becomes impractical.
- In screening assays, allows rapid ranking based on overall binding profile shape and off-rate behavior.
- ***Sensorgram comparison*** is recommended when traditional kinetic or report point analyses are insufficient to capture full binding behavior, particularly for modern biologics and complex interactions that challenge the limits of fitting model-based approaches.

4.2 What can you measure with *Sensorgram comparison*?

With ***Sensorgram comparison***, you can measure how closely sample binding curves follow the behavior of a chosen reference or standard, either across the entire sensorgram or within specific phases, such as association, dissociation, or both. Set a comparison window (similarity range) and get a similarity score that reflects how many data points stay inside that window and how far any points fall outside it. You do not need any kinetic model or curve fitting, so the focus stays on clear, objective evidence to decide whether your samples behave like the reference (similar) or show clear signs of being different (dissimilar).

4.3 Quantifying comparability using the similarity score

Similarity in sensorgrams is quantified using a term called similarity score, which provides a numerical measure (0–100%) of how closely a sample curve follows the expected binding behavior defined by the similarity ranges (limits) created from the standards (Fig 1). The score is calculated by combining two contributions. First, time points of the sample that fall inside the upper and lower limit curves are assigned full value (100%), indicating alignment with the expected range. Second, time points that fall outside the window reduce the score, with the reduction proportional to the distance between the observed point and the nearest limit curve. Together, these factors provide a direct, kinetic interaction model-free assessment of how similar or dissimilar each sample is compared with the reference.

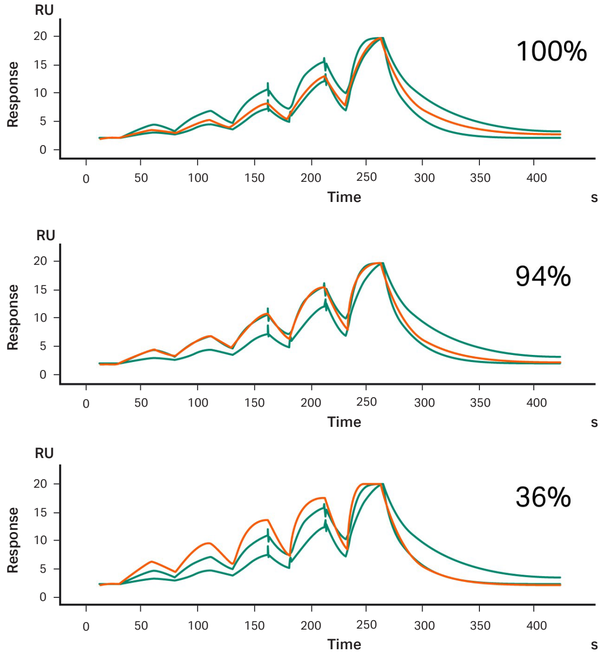

Fig 1. Representation of similarity scoring (0–100%) based on the overlay of sample sensorgram (orange curve) within the defined similarity limits (green curves).

4.4 How does *Sensorgram comparison* work

Study and establish standards limits

- Establish experimental variation of standards using diferent batches, operators, instruments, etc.

Run the samples with same assay paramaters used for standards

- Include both positive and negative controls to verify assay consistency and strengthen confidence.

Direct overlay of sample sensograms

- Objective comparison of entire binding profile.
- No curve fitting and report point analysis.

Quantitative assesment of similarity

- Expessed as a % relative to standard.
- Evaluates interactions difficult to model kinetically.

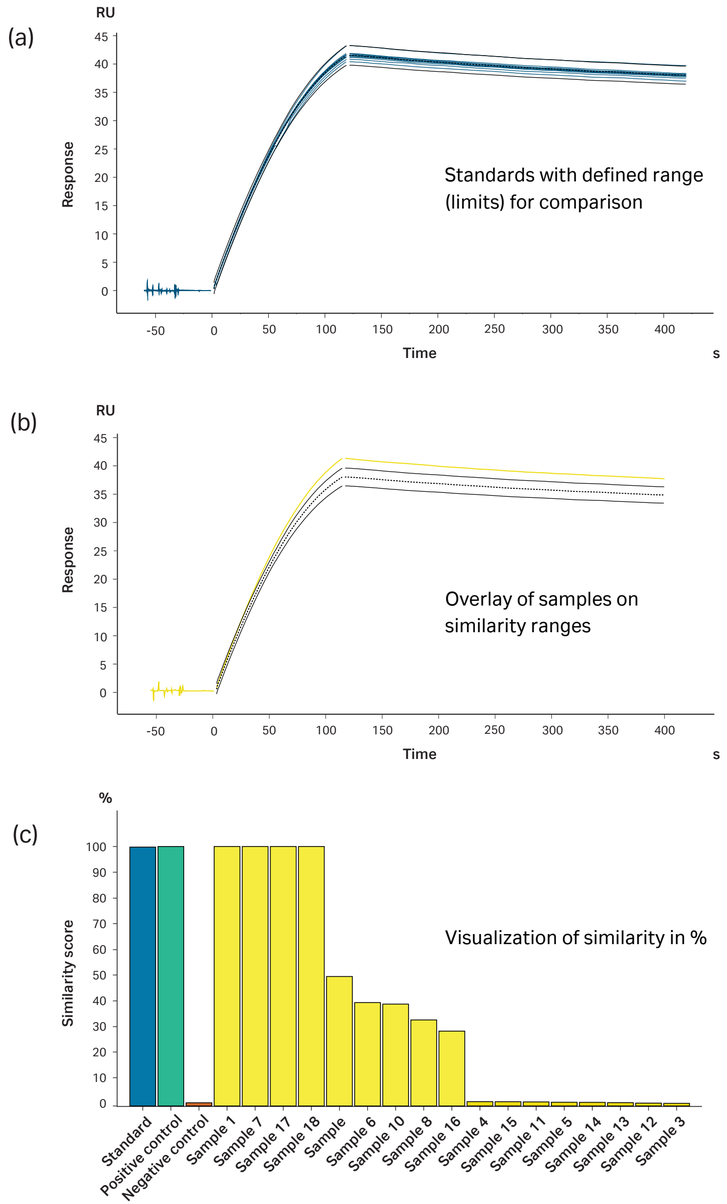

Fig 2. Biacore ***Sensorgram comparison*** workflow illustrating how samples are compared with standards to assess similarity. (a) Standards with defined range limits for comparison. (b) Overlay of samples on similarity range. (c) Visualization of similarity in %.

5. Assay formats

5.1 What assay formats are supported with *Sensorgram comparison*?

Sensorgram comparison supports evaluation of data from four types of Biacore assay formats, providing flexibility to match the method to the experimental design.

- Single-cycle kinetics (SCK) is used when all concentrations are injected in one continuous cycle, whereas multi-cycle kinetics (MCK) is used when each concentration is run in a separate cycle.
- Single concentration (SC) assays are suitable for rapid comparison of binding curve shapes across multiple samples, without running a full concentration series.
- For more complex workflows, multiple injection (MI) assays can be used, where two or more injection types, such as analyte injections, ***Dual,*** and enhancement steps are combined within a single cycle. This approach supports comparison of binding behavior across multi-step interaction events.

5.2 Single-cycle kinetics assay format

An SCK run is configured by defining a sequence of analyte concentrations (from low to high) that are injected one after another over the same ligand surface, followed by a final dissociation phase. The resulting binding curve represents the entire concentration series in one continuous sensorgram (Fig 3). This approach is preferred when regeneration conditions are difficult to establish or when binding is too strong, such as cases with very slow dissociation

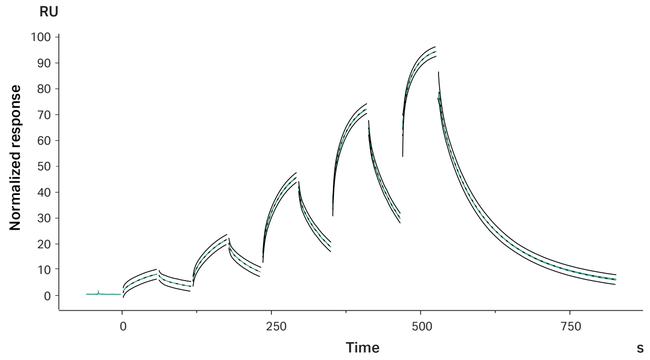

Fig 3. SCK ***sensorgram comparison*** to the similarity ranges defined from the standards.

Biacore Insight control software provides predefined run method templates that support the setup of SCK experiments, with or without a capture step and help ensure a consistent assay structure. After the run is complete, the SCK sensorgram is evaluated using a predefined evaluation method matching the assay format, allowing direct comparison of the complete binding profile.

5.3 Multi-cycle kinetics assay format

MCK is used when each analyte concentration is injected in a separate cycle. This format requires surface conditioning or regeneration for every concentration.

In the ***Sensorgram comparison*** software item, MCK experiments are supported for both direct binding and capture-based assay formats using the predefined run methods. The concentration series may be run in either low to high order or in any random order.

On Biacore 8 series systems, MCK must be run in serial mode (concentration by cycle). Parallel MCK (concentration by channel) is not supported for ***Sensorgram comparison***.

During MCK analysis, MCK cycles are automatically concatenated on a common (fictive) X-axis, from low concentration to high concentration, to enable the comparison (Fig 4).

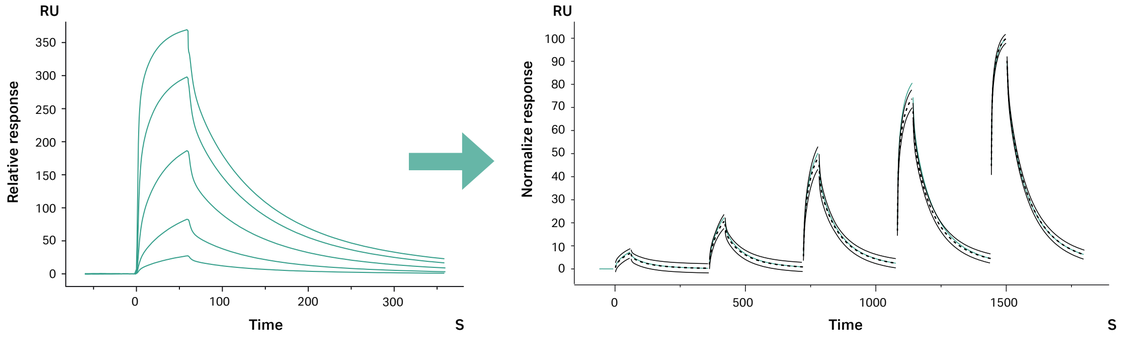

Fig 4. During evaluation, ***Sensorgram comparison*** concatenates multi cycle kinetics (MCK) cycles from the lowest to the highest concentration to create a continuous interaction profile resembling a single cycle kinetics (SCK) sensorgram. This combined profile is then used for the similarity comparison.

5.4 Single concentration assay format

Single concentration (SC) assays are set up by defining a single analyte concentration for each cycle using the analyte injection command (Fig 5).

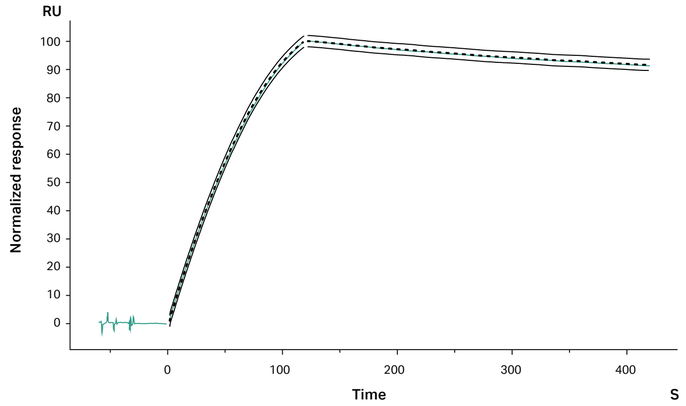

Fig 5. SC ***sensorgram comparison*** to the similarity ranges defined from the standards.

In ***Sensorgram comparison***, the SC workflow is designed to accommodate situations where sample and standard concentrations may be similar or may differ, provided that all other experimental parameters remain consistent across runs. The resulting sensorgrams are evaluated directly according to their shape, offering a rapid and flexible way to compare binding behavior without requiring a full concentration series like SCK and MCK.

5.5 Multiple injection assay format

Multiple injection (MI) assays support interaction workflows where several injection types are combined within a single cycle (Fig 6). MI runs may include analyte, ***Dual,*** and enhancement injections, and may also incorporate wash or wait steps between injections as required by the assay design.

The first injection in the cycle defines the control type for the entire MI sensorgram. For example, if the first injection is assigned as a standard, blank, positive control, or negative control, the complete MI curve inherits that designation. For blanks, a concentration of zero is assigned in the first injection.

During evaluation, ***Sensorgram comparison*** reports similarity for the entire MI interaction profile as well as individual similarity results for each injection segment

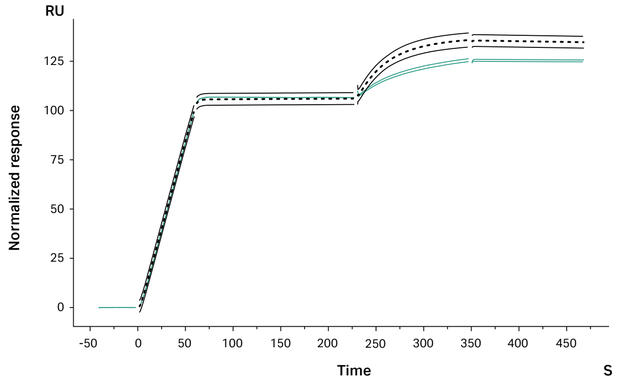

Fig 6. MI sensorgrams showing two sequential analyte injections, compared against the similarity ranges defined from the standards.

MI assays are particularly suitable for workflows involving sequential binding events, such as bispecific molecule assessment or primary-to-secondary interaction studies (for example, antigen binding followed by Flow channel receptor binding).

MI is also useful when different injection types must be combined to capture the full binding mechanism of the interaction.

6. Run setup

6.1 Run setup for standards

Standards should be defined during run setup or defined from variable table during evaluation, to enable ***Sensorgram comparison*** evaluation.

A dedicated control type, standard, is available for this purpose in Biacore Insight control software. A large number of standard cycles is recommended, ideally representing a broad range of natural variability, including differences arising from:

- different reagent lots
- different sample preparations
- different operators
- different instruments
- different run instances or dates
- variations in capture or immobilization levels

Incorporating this diversity helps establish a representative comparison window and supports generation of robust, statistically confident similarity ranges (upper and lower limit curves). During the evaluation, standards can also be saved within an evaluation method to enable consistent reuse across future analyses.

6.2 Run setup for samples and controls

Maintaining identical assay parameters for standards, samples, and controls is important for valid ***Sensorgram comparison***. This includes:

- same data collection rate
- same association and dissociation times
- same injection commands
- same flow rate
- temperature:
  - Maintain the same temperature across standards, controls and samples.
  - Comparison across different temperatures is also supported to study temperature-related stress effects on binding.

Including appropriate controls in the run setup is recommended to increase confidence in both the assay performance and the evaluation results. Controls help verify correct setup, monitor assay stability, and support interpretation of similarity outcomes.

6.3 Concentration considerations for *Sensorgram comparison*

Understanding concentration requirements for standards and samples is important, as ***Sensorgram comparison*** supports different application scenarios.

a. Standards (analyte) concentration considerations

- Standards should be defined with the same analyte concentration.
- Different concentrations for standards are treated as separate standard groups.
- The software allows multiple standard groups within the same assay to analyze different binding profiles.

b. Sample (analyte) concentration considerations

For best comparison results, it is recommended to keep sample concentrations similar to standards. Small dilution differences can often be handled by the normalization feature in ***Sensorgram comparison.***

For kinetic-based applications (SCK and MCK):

- The analyte concentration should be the same between standards and samples.
- This ensures kinetic binding curve comparability under the same conditions.

For screening-based applications (SC and MI):

- Sample concentrations can be the same or different.
- A concentration value must still be entered in the software (can be approximate).
- This value is used only for data organization and does not affect similarity scoring.
- This flexibility supports applications such as antibody screening and degradation studies.
- For larger concentration differences (e.g., in screening applications), curve shape can be affected due to saturation or mass transport effects. In such cases, dissociation phase comparison (similar to off-rate ranking) is more appropriate.

c. Ligand capture or immobilization level

- It is recommended to maintain similar immobilization or capture levels (RU) for both standards and samples.
- Minor variations in ligand levels on the sensor surface are handled through normalization, enabling comparison based on curve shape.

6.4 Recommendations for standards and samples run setup in Biacore 1 series SPR systems (6 Fc)

- Use pairwise flow cell combinations Fc2–1, Fc4–3, Fc6–5.
- Avoid serial mode across all six flow cells (risk of analyte depletion).
- Run standards on one or multiple paired flow cells.
- Include blank cycles to correct buffer and bulk effects.
- Samples from any flow cell pair can be compared.
- For immobilized ligands, generate standards on all three flow cell pairs when using one at the time, to capture surface variability and to improve the sample coverage across all 3 flow cell pairs.

6.5 Recommendations for standards and samples run setup in Biacore 8 series SPR systems (8 channels, 16Fc)

- Run standards across multiple channels (all eight or a subset).
  - Leverage the high-throughput capacity of Biacore 8 series systems to generate larger standard sets from different preparations or reagent batches within a single experiment.
  - It also helps in capture assay related variations, such as capture level differences, baseline drift, etc.
  - Include blank injections in each channel for baseline correction.
  - During analysis, standards are combined across channels if all assay parameters match.
- Samples can be run across all eight channels.
- Place positive and negative controls in any channel or cycle.
- Use Biacore 8 series systems for multiplexing when needed.
  - Use multiple channels to include standards, controls, and blanks within the same run to capture natural variability under identical conditions.
  - When possible, allocate several channels to replicate standards to build robust comparison windows and improve confidence in similarity limits.
  - It also allows evaluation of multiple standards and their matching samples in same run.
  - Each channel can also be used with different immobilized ligands (e.g., two ligands on separate flow cells per channel, total 16Fc). These ligands are handled as independent standard groups, and comparisons are performed separately for each group.

7. *Sensorgram comparison* analysis

7.1 Evaluation (analysis) workflow

- Open the run, select ***Sensorgram comparison*** from Biacore Insight evaluation software home view.
- Perform QC checks (inspection of sensorgram, baseline, references, and capture levels).
- Verify blanks, grouping, and sample details.
- Choose comparison settings (Fig 7):
  - injection phase.
  - comparison approach algorithm (SD, min-max, or % dev).
  - normalization.
  - minimal allowed range.
  - response level cut-off.
  - automatic rejection.
- Review sensorgrams using comparison and deviation charts for each group.
- Review similarity scores in the bar chart and details table.
- Export results (PPT, Excel, or PDF) and optionally save an evaluation method for reuse.

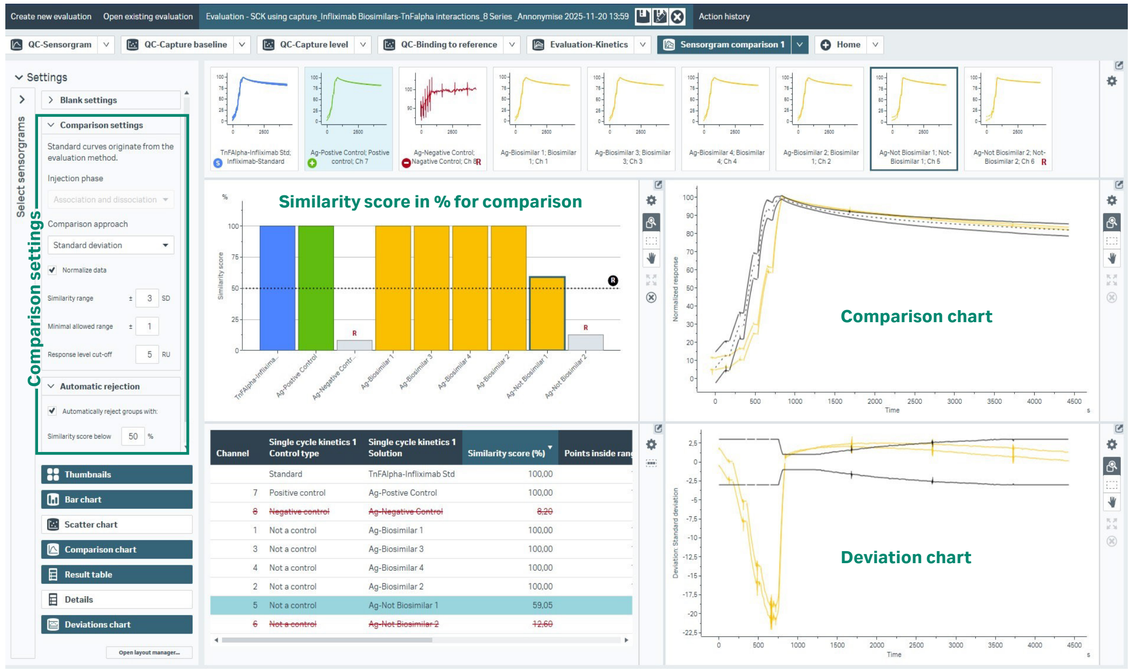

Fig 7. ***Sensorgram comparison*** analysis workflow in Biacore Insight evaluation software.

7.2 Visual inspection of sensorgrams (QC checks)

Although ***Sensorgram comparison*** evaluation is automated, a manual QC review is recommended before accepting results.

Quality control checks

- QC sensorgrams: inspect raw sensorgrams for overall shape, artifacts, or unexpected behavior.
- QC baseline: confirm a stable baseline with minimal drift.
- QC capture levels (as applicable): verify consistent capture or immobilization level(s) across standards and samples.
- QC binding to reference: ensure there is no unexpected binding on the reference surface.

After basic QC checks

- Verify blanks are correctly identified and used for blank subtraction.
- Review default comparison settings (SD=3, Normalization=ON)
- Check sensorgram overlays (comparison chart) to visualize and compare overall curve shape.
- Review deviation chart to detect subtle differences not visible in overlays. This is like the residual plot in a kinetics evaluation.

7.3 Comparison settings

Comparison setting	Description	Allowed values	Applications
Injection phase	Defines which part of the sensorgram is included in the similarity analysis.	• Association + dissociation (default) • Association only • Dissociation only	Use when specific binding phases carry most of the analytical value or when one phase contains artifacts.
Comparison approach	Software calculates an average curve and builds similarity ranges (upper and lower limits) using the selected algorithm.	• Standard deviation (SD) (default) • Max/min • % deviation	Choose based on expected variability and desired strictness of the similarity window.
Normalize data	Scales curves to compensate for capture or immobilization differences and focuses the evaluation on sensorgram shape. Available for SD and max/min approaches.	Yes/no	Useful when absolute RU levels differ but shape comparison is required (e.g., capture assays).
Minimal allowed range	Prevents the similarity window from becoming unrealistically narrow when standards overlap very closely. Apply only when the natural variation is smaller than the set minimum.	Default = 0 RU, User defined (e.g., ±1 RU)	Use when standards show very small variation to maintain meaningful comparison limits.
Response level cutoff	Excludes sensorgrams with responses below a minimum threshold, preventing low-signal curves from influencing similarity.	Default = 1 RU, User defined	Useful for removing poor quality or non-binding curves from analysis.
Automatic rejection	Automatically flags groups whose similarity score falls below the selected threshold. Rejected groups are marked “R” in the bar chart.	Default = 0, User defined threshold	Enables automated identification of dissimilar or failing sensorgrams during screening or comparability studies.

7.4 Which comparison algorithm?

Binding curves can be compared using different statistical approaches depending on the nature of the interaction, the level of assay variability, and the intended application. In some cases, variability follows relatively symmetric and predictable patterns, while in other cases it may be asymmetric or scale differently across the binding profile. To address these different scenarios, ***Sensorgram comparison*** provides multiple comparison approaches- standard deviation, min/max, and percentage deviation, which allow similarity ranges (limits) to be tailored to the behavior of the reference data and the goals of the comparability analysis.

Standard deviation (SD)

The standard deviation approach generates similarity limits that are symmetrical around the average curve (Fig 8).

This method assumes that the underlying variation in the standards follows a normal distribution, unlike the min/max approach which makes no distribution assumptions. In practice, the standard deviation approach is often a suitable starting point when there is no clear reason to expect non-normal variability in the standard set.

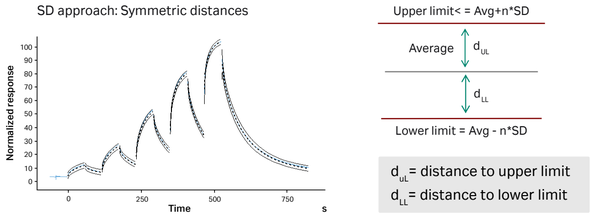

Fig 8. SD method of comparison.

Useful for establishing assay performance or comparing product or reagent stability over time, for example, quality control of production batches.

Max/min

In the min/max approach, the median curve is used as the central reference, and the upper and lower limits are defined using either the maximum and minimum standard curves or the distance between these curves and the median (Fig 9).

The distance from each limit curve (max − median or median − min) can also be scaled by applying a curve factor (CF) to widen or narrow the comparison window as needed.Useful in screening applications to describe a limit for a quick selection of leads. Useful when interactions are heterogeneous or have outliers.

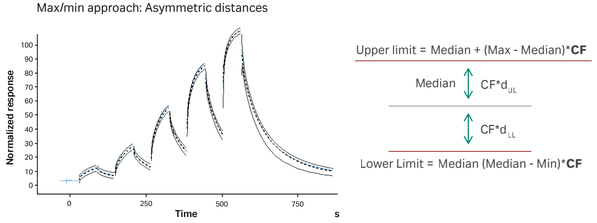

Fig 9. Max/min method of comparison.

Percentage deviation

In the percentage deviation approach, the similarity limits are generated by applying a user defined percentage deviation to the average or median curve (Fig 10).

The upper and lower limit curves are created by adding and subtracting a defined percentage from the reference curve. This approach creates limits that scale with the signal level, making it useful when variability naturally increases with response levels.

It also provides a practical way to define a reasonable comparison window (similarity ranges) when only a small number of standard runs are available or when the standard curves overlap very tightly. Suitable for early stage development or situations where only a limited number of standards are available, such as early screening workflows.

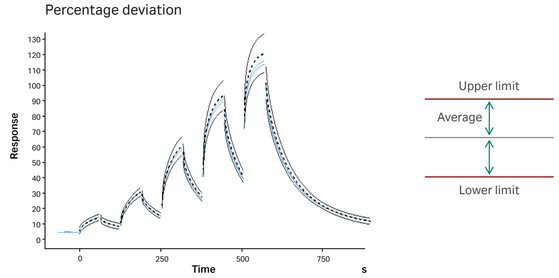

Fig 10. Percentage deviation method of comparison.

7.5 Handling standards

Standards can be applied in two ways, from the run file or from a saved evaluation method, providing flexibility based on application needs.

Standards applied from the same run file

Using standards present in the same run is recommended when assay-to-assay variation is expected, or when experimental conditions may shift between sessions. In these cases, include the standards along with samples to reflect the conditions of the current experiment and define a comparison window that best represents on the day variability.

This approach is suited for applications such as:

- assays with high capture or immobilization variability
- antibody or binder screening campaigns
- early-stage assay development and optimization

When standards are included in the run, ***Sensorgram comparison*** automatically detects the standard cycles, generates a default similarity range, and evaluates samples against these conditions.

Standards compiled in an evaluation method

Standards can also be gathered from separate runs, quality checked, and saved within an evaluation method for consistent reuse. This option is suited for established assays where stability is demonstrated and where a fixed comparison window is preferred

This approach is commonly used for:

- lot-to-lot comparability
- stability and stress testing studies
- validated or routine workflows requiring consistent reference behavior.

Once saved, the standards remain locked within the evaluation method, ensuring reproducible and traceable similarity assessment across future analyses.

When an evaluation method contains saved standards, those standards take precedence during ***Sensorgram comparison***, and any standards present in the run file are ignored. This ensures that regulated procedures use the validated and locked standard set defined in the evaluation method. To evaluate using run file standards instead, a new ***Sensorgram comparison*** item must be created.

7.6 Apparent stoichiometry

Apparent stoichiometry provides an estimate of the relative amount of analyte binding in relation to the ligand level.

It is calculated when molecular weights for both ligand and analyte are defined, using the highest analyte response and the immobilized or captured ligand level.

The value is shown per curve in the details table and as a group average in the results table.

It is not calculated when molecular weights are missing and is not available for multiple injection assays. Apparent stoichiometry is useful for revealing differences in binding capacity that may not be visible when data are normalized, providing an additional check of assay consistency and sample comparability.

8. Analysis in GxP-regulated environment

Sensorgram comparison can be used in GxP regulated environments where data integrity and traceability are required. Within Biacore Insight Software, the evaluation workflow follows controlled and auditable steps, ensuring that similarity assessments are performed using validated methods and locked standards. The software is designed to meet 21 CFR Part 11 requirements, supporting electronic records, audit trails, and controlled evaluation methods. Together, these features enable ***Sensorgram comparison*** to be applied confidently in regulated applications such as lot-to-lot comparability, release testing, and stability or stress study evaluations.

8.1 Lock standards in run method

- In regulated workflows, standards may need to be run regularly and locked to prevent modification by routine users.
- Biacore Insight control software allows locking assay steps in a published GxP procedure (Fig 11).
- To secure standards in a GxP procedure:
    - place all standard cycles into a separate assay step.
    - lock this step in the published procedure to prevent changes by non privileged users.
    - lock this step in the published procedure to prevent changes by non privileged users.
    - locked standard step can be scheduled repeatedly within the assay workflow.
    - routine users can execute the locked standards but cannot modify them, ensuring full GxP traceability.

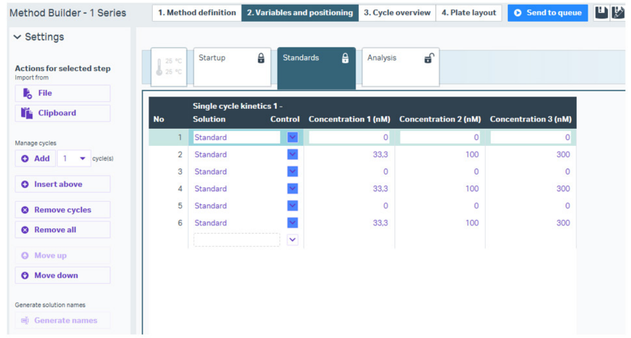

Fig 11. Run set up showing the locked standards in Biacore Insight control software.

- This approach maintains strict control of reference material behavior while preserving operational flexibility.

8.2 Handling standards in regulated evaluations

- Saved standards are automatically applied during evaluation when using a published GxP procedure.
    - These standards are locked and cannot be edited or removed by routine users.
    - Any modifications to standards or evaluation settings in regulated mode require appropriate user privileges and documentation.
- If the regulated evaluation method does not contain standards, the run must include standards.

9. Applications

9.1 Batch-to-batch monitoring

Sensorgram comparison can be used to monitor consistency across manufacturing batches by directly comparing the shape of binding curves over time. This approach helps identify shifts in binding profiles that may arise from changes in raw materials, process adjustments, storage conditions, temperature variation, etc.

By evaluating new sample sensorgram against a defined similarity range built from representative standards, ***Sensorgram comparison*** provides a clear, quantitative view of whether new batches follow the expected binding behavior or show early signs of drift. This makes it well suited for routine batch release, trending analysis, and long-term product quality monitoring (Fig 12).

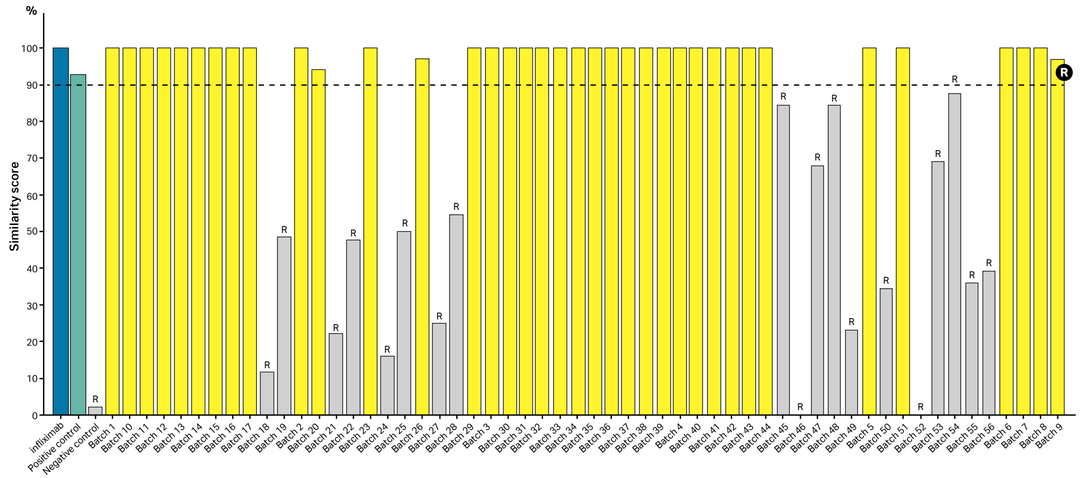

Fig 12. Illustrative example showing comparison across multiple batch samples to illustrate how ***Sensorgram comparison*** can be applied to batch-to-batch analysis. A similarity score above 90% is used as the acceptance criterion for batch release. In the ***Sensorgram comparison*** bar chart, batches that fail to meet the criterion are clearly visible and automatically flagged as rejected.

9.2 Biosimilars comparability

Sensorgram comparison can support biosimilar development by directly evaluating how closely a biosimilar candidate matches the reference product across key binding functions, such as target antigen and Fc-related interactions.

Figure 13 shows the biosimilar comparison against the originator molecule using ***Sensorgram comparison***. The analysis produced high similarity scores, demonstrating strong comparability, supporting regulatory confidence, and helping accelerate biosimilar development.

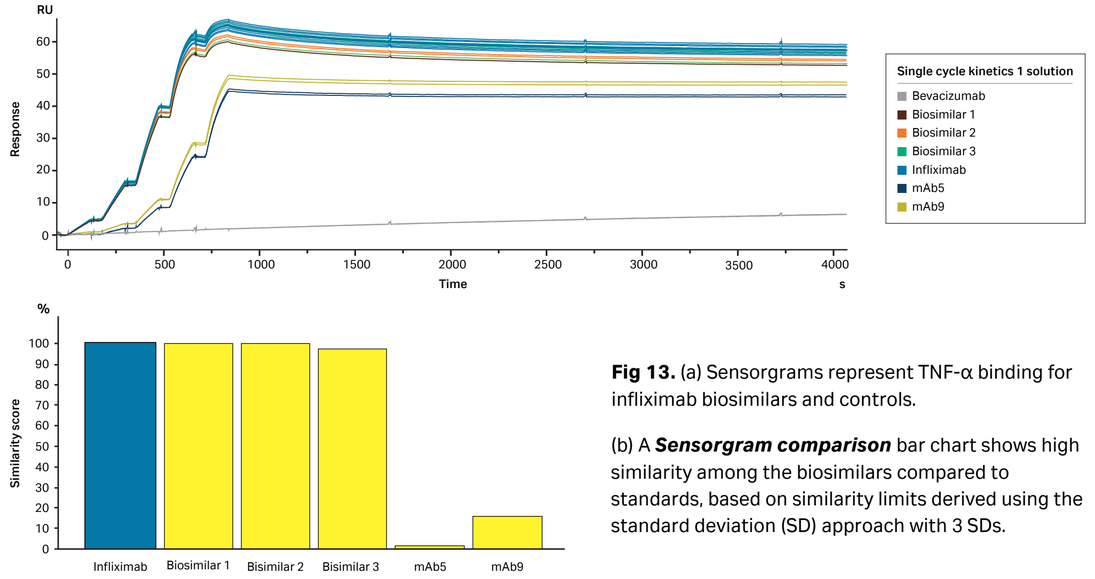

Fig 13. (a) Sensorgrams represent TNF-α binding for infliximab biosimilars and controls.

(b) A ***Sensorgram comparison*** bar chart shows high similarity among the biosimilars compared to standards, based on similarity limits derived using the standard deviation (SD) approach with 3 SDs.

By comparing full sensorgram shapes rather than relying only on fitted kinetic constants, ***Sensorgram comparison*** is particularly effective when heterogeneous or complex binding behavior makes traditional fitting approaches less reliable. The method can also detect subtle differences in binding profiles across variants providing additional evidence for similarity.

9.3 Multi-specific interaction characterization

Sensorgram comparison can support the characterization of multi-specific interactions by evaluating two or more binding events within a single sensorgram using a multiple injection format (Fig 14). This allows primary target binding to be assessed alongside secondary interactions such as FcγR, FcRn, or complement/enhancement binding, even when the individual interactions show heterogeneous behavior.

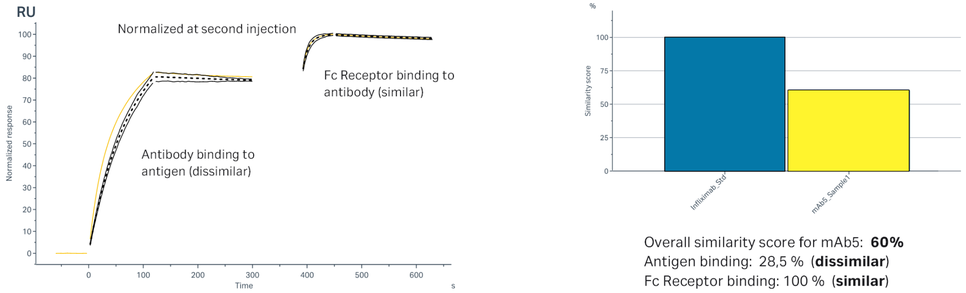

Fig 14. ***Sensorgram comparison*** applied to a multiple injection workflow for antigen-antibody and Fc receptor binding. Two critical quality attributes are evaluated within a single sensorgram.

By providing both an overall similarity score for the full interaction profile and per segment similarity values for each injection, ***Sensorgram comparison*** makes it possible to detect changes in one functional attribute while confirming that others remain unaffected. This approach can be extended to more than two injections, enabling a broader fingerprint analysis of complex molecules, including bispecifics and other multi-specific formats.

9.4 High-throughput screening data analysis

Sensorgram comparison can be applied to large-scale screening studies to rapidly identify candidates with desirable binding profile characteristics (Fig 15). By selecting standards that represent preferred binding behaviors, such as rapid-on and slow-off, or rapid-on and rapid-off profiles, the method enables direct comparison of screening sensorgrams against predefined reference shapes.

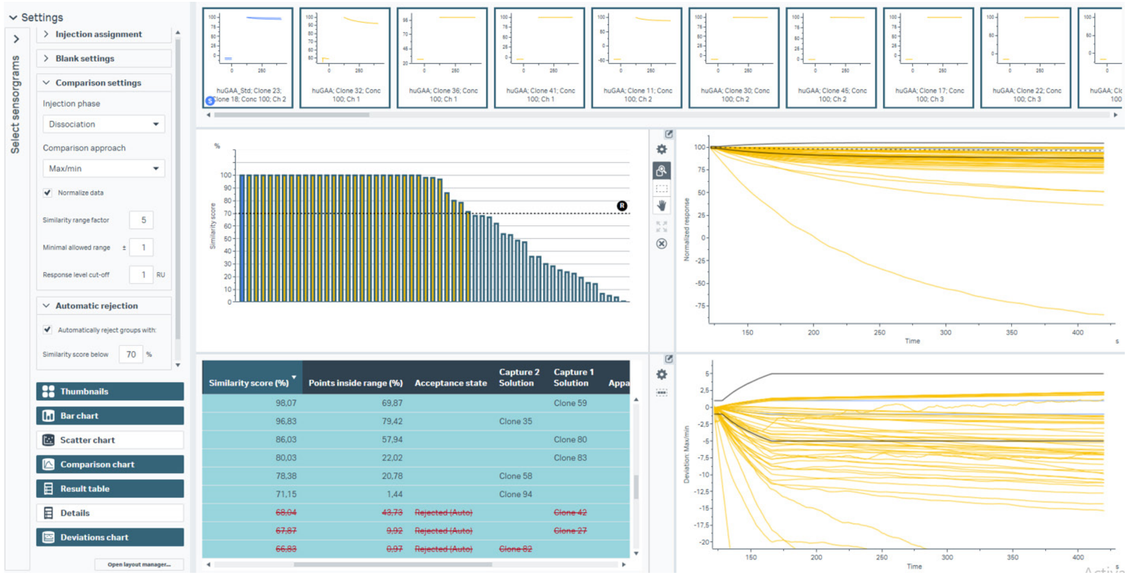

Fig 15. Workflow showing the screening of hybridoma samples evaluated using ***Sensorgram comparison***. A min/max algorithm was applied to define the acceptable window for the dissociation phase. The bar chart provides a clear overview that enables rapid ranking of binders based on their off rate behavior without requiring kinetic curve fitting.

Using evaluation methods with saved standard profiles, together with comparison (overlay) plots for quick visualization, allows consistent selection and annotation of curves across screening campaigns.

When combined with single concentration assays and similarity score ranking, this approach supports rapid triage of large screening data to highlight candidates with favorable dissociation or curve shape properties.

10. General considerations / best practices

10.1 Assay-related best practices

To obtain reliable ***Sensorgram comparison*** results, assay design must support stable and reproducible sensorgram shapes

- Use well-purified, activity-verified reagents to ensure consistent binding profiles.
- Select a sensor chip and surface attachment method that produce stable and repeatable sensorgrams suitable for comparison.
- Define standards that capture the expected variability of the assay, including appropriate blanks and reference surfaces.
- Keep all critical assay parameters identical between standards and samples.
- Include replicates when feasible to confirm consistency of sensorgram shape.

10.2 Analysis-related best practices

These practices help ensure accurate ***Sensorgram comparison*** scoring and interpretation.

- Choose appropriate variation limits based on intended use for tighter limits for QC type decisions, and broader limits for screening applications.
- Confirm that normalization improves shape comparison without obscuring real differences.
- Avoid extremely narrow SD based limits when standards show very low variation, use % deviation or minimal allowed range (small RU buffer) as needed.
- Check blanks and references for unwanted effects, exclude disturbed regions when appropriate.
- Use comparison and deviation charts to detect subtle issues.

10.3 Interpreting *Sensorgram comparison* results

- Always review the similarity score together with the comparison (overlay) plot and deviation chart.
- Lower scores point to differences in curve shape or phase behavior, inspect where and why deviations occur.
- For borderline or unexpected results, evaluate blanks, normalization, potential artifacts, or repeat critical samples.

Note (limitations):

- Comparison of samples or standards across different Biacore system platforms (for example, Biacore 1 series versus Biacore 8 series SPR systems) is not supported. The software will display a warning indicating that cross series data evaluation is not valid.
- The injection types ***Capture, ABA, Poly*** command, and ***Inject and elute*** are not supported in Biacore Insight Software v7.0 for ***Sensorgram comparison.***

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