Biacore SPR-based surrogate potency assays with streamlined PLA and EC50 analysis facilitate comparability studies

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Introduction

An array of potency assays is needed to measure and monitor critical quality attributes (CQA) of antibody therapeutics. Biacore™ surface plasmon resonance (SPR)-based assays can be used as surrogate potency assays to facilitate comparability and biosimilar studies (1).

An SPR-based capture assay that can be expanded to include estimates of relative potency for several CQAs in a single experiment is described.

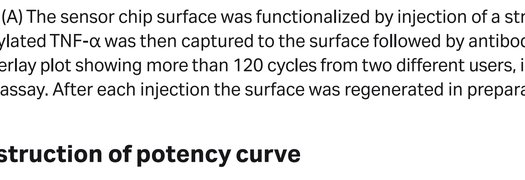
Parallel line analysis (PLA) and EC50 calculations are often used to quantitate similarities of the dose-response curves. So far, PLA and EC50 analysis of Biacore data have been handled using external software. The potency data in this study, generated in Biacore T200, were easily evaluated using Biacore Insight Evaluation Software for PLA and EC50 curve analysis, without the need to export to Microsoft® Excel® or similar software.

Surrogate potency assay

Setup and robustness: exemplified for anti-TNF-α antibodies

Biacore Biotin CAPture Kit was used rather than covalent coupling, minimizing assay development time.

(A)



(B)

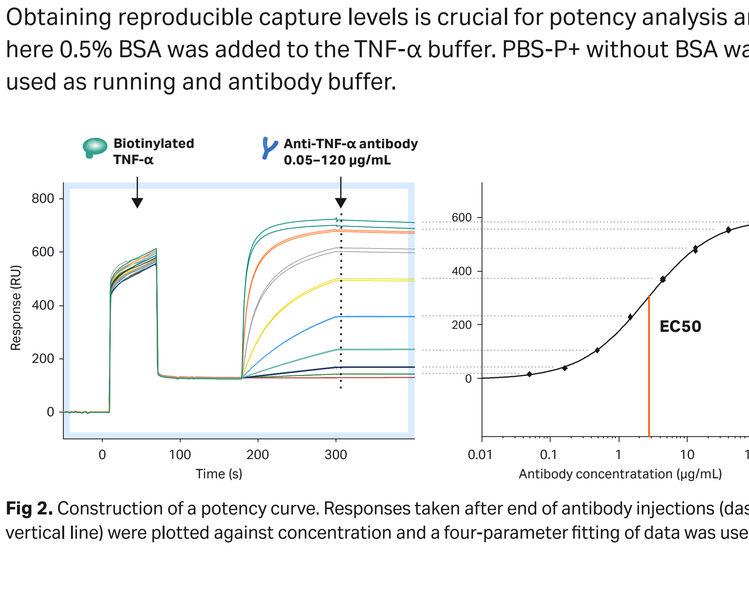


Fig 1. (A) The sensor chip surface was functionalized by injection of a streptavidin oligo conjugate Biotinylated TNF-α was then captured to the surface followed by antibody binding.
(B) Overlay plot showing more than 120 cycles from two different users, illustrating the robustness of the assay. After each injection the surface was regenerated in preparation for a new injection.

Construction of potency curve

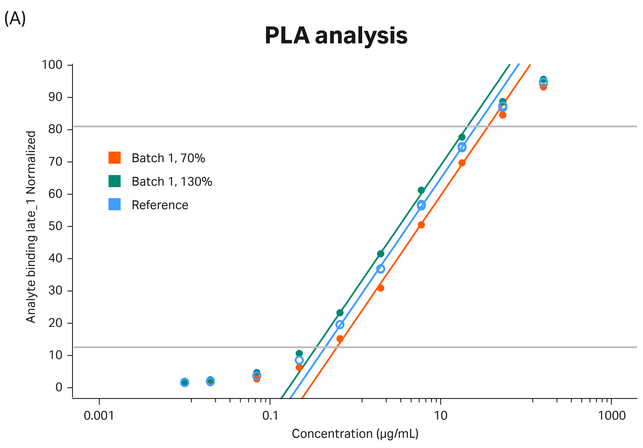
Obtaining reproducible capture levels is crucial for potency analysis and here 0.5% BSA was added to the TNF-α buffer. PBS-P+ without BSA was used as running and antibody buffer.

Fig 2. Construction of a potency curve. Responses taken after end of antibody injections (dashed vertical line) were plotted against concentration and a four-parameter fitting of data was used.

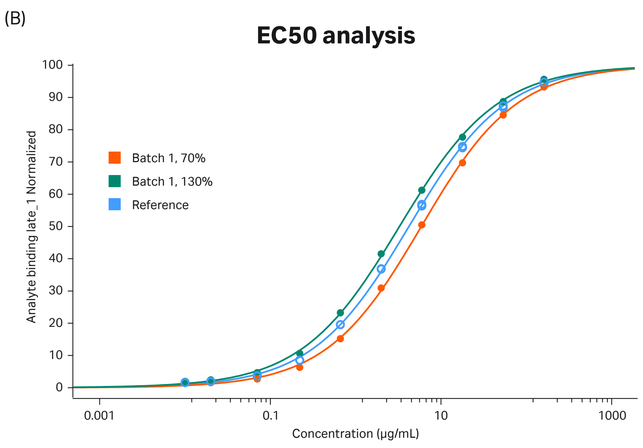
Results

Linearity test using anti-TNF-α antibodies with 70% to 130% nominal concentrations proved the surrogate potency assay was fit for purpose. PLA and EC50 analysis resulted in similar relative potency values.

(A)



(B)



(C)

PLA results

Control	Sample	Relative potency	95% confidence low	95% confidence high
Reference	Batch 1, 70%	72.89	68.03	78.07
Reference	Batch 1, 130%	127.1	120.6	134.0

EC50 results

Control	Sample	Relative potency	95% confidence low	95% confidence high
Reference	Batch 1, 70%	71.64	65.54	78.30
Reference	Batch 1, 130%	129.1	119.3	139.7

Fig 3. Curve analysis of Biacore T200 data using Biacore Insight Evaluation Software. (A) PLA analysis by linear fit to the linear part of the response vs logarithmic concentration assuming a common slope. (B) EC50 analysis based on four-parameter equation fitted to response vs concentration. (C) The results table includes 95% confidence interval for PLA and EC50, which statistically defines with 95% probability that the relative potency is within calculated low and high limits.

Potency data for two CQA obtained in one experiment

The potency assay was then extended to look at several CQA in a single sensorgram. Binding of antibody and Fcγ receptor in sequence resulted in two EC50 curves from the same experiment.

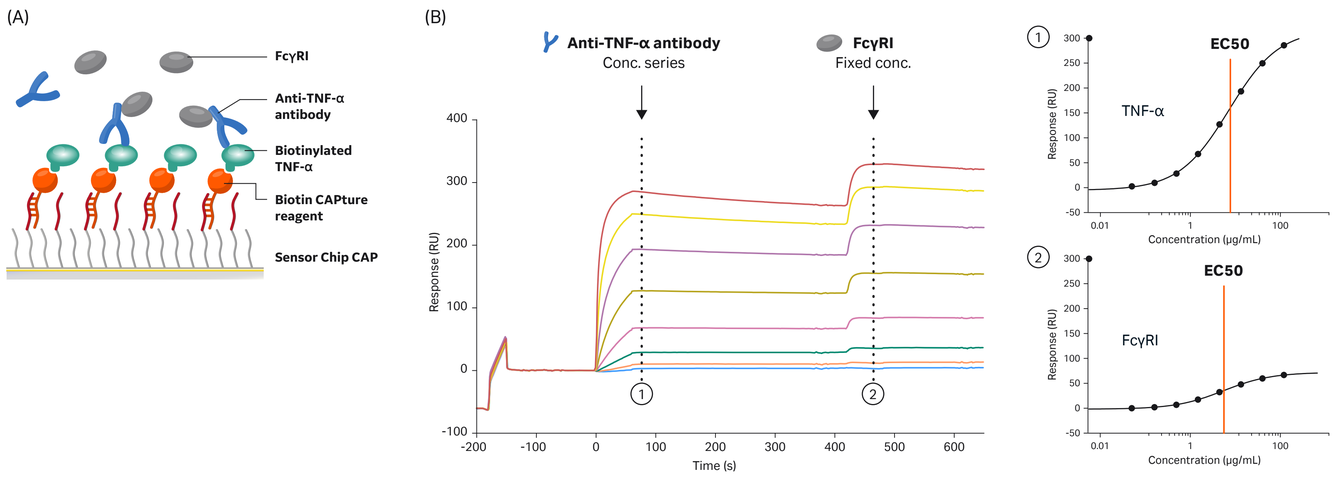


Fig 4. (A) Anti-TNF-α antibody and FcγRI bind in sequence. (B) Responses (dashed lines) were plotted against antibody concentration. By selecting which report point to set on the y-axis (1 or 2) in Biacore Insight Evaluation Software, potency curves for antibody binding to both TNF-α and FcγRI were obtained in the same experiment, saving both sample and analysis time.

Conclusions

- A Biacore SPR-based surrogate potency assay is described. The assay uses a capture format, which minimizes time for assay development.
- The assay can be extended to obtain potency data for at least two CQA in the same experiment, and is applicable also to, for example, bispecific antibodies.
- Surrogate potency assays performed in Biacore 8K, Biacore 8K+, and Biacore T200 can easily be evaluated using Biacore Insight Evaluation Software with application-specific software tools for PLA and EC50 determinations.

References

Karlsson, R. *et al.* Surrogate potency assays: Comparison of binding profiles complements dose response curves for unambiguous assessment of relative potencies. *J. Pharm. Anal.* **8**, 138–146 (2018).

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