Surrogate potency assays with SPR—two CQA determined in only one assay

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Introduction

An array of potency assays is needed to measure and monitor critical quality attributes (CQA) of antibody therapeutics. We present how Biacore™ surface plasmon resonance (SPR)-based assays can be used as surrogate potency assays to facilitate comparability and biosimilar studies (1). A capture-based assay that can be expanded to include estimates of relative potency for several CQAs in a single experiment is described. We also demonstrate how *Sensorgram comparison* complements dose response curves to ensure compliance with kinetic properties.

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.

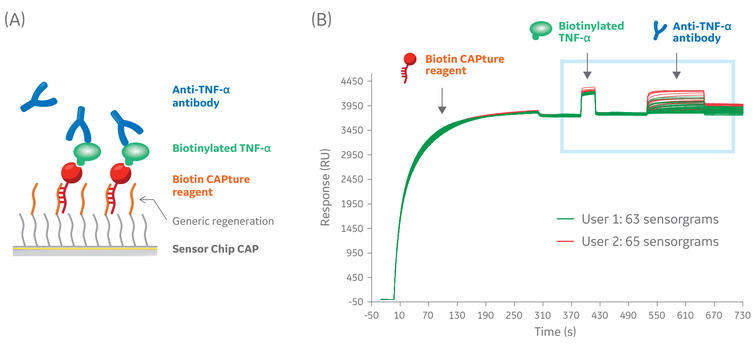


Fig 1. (A) The sensor chip surface was functionalized by injection of a streptavidin oligo conjugate Biotinylated TNF-α was then captured to the surface followed by antibody binding. (B) Overlay plot showing more than 120 cycles from two different users, illustrating the robustness of the assay. After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

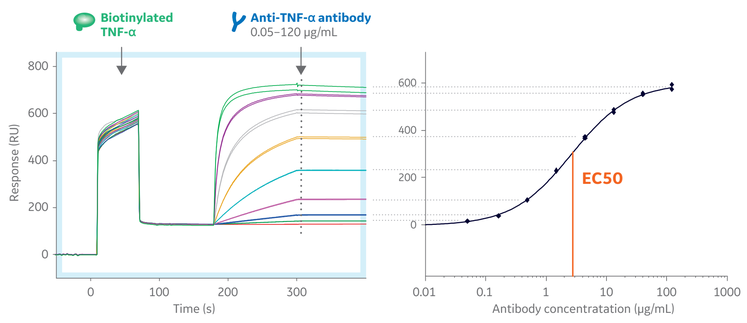
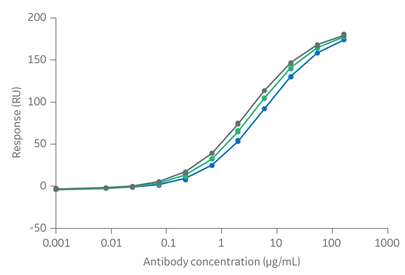


Fig 2. Construction of a potency curve. Responses taken after end of antibody injections (dashed vertical line) were plotted against concentration and a four-parameter fitting of data was used.

The assay is fit for purpose



Sample relative conc. (%)	EC50 (µg/mL)	Relative potency (%)
100 (reference)	4.23	100.0
130	3.30	128.1
70	5.90	71.7

Fig 3. Varying nominal concentrations shows that EC50 values are linear with respect to concentration.

Results

Potency data for two CQA obtained in one experiment

Antibody and Fcγ receptor bind in sequence resulting in two potency curves from the same experiment.

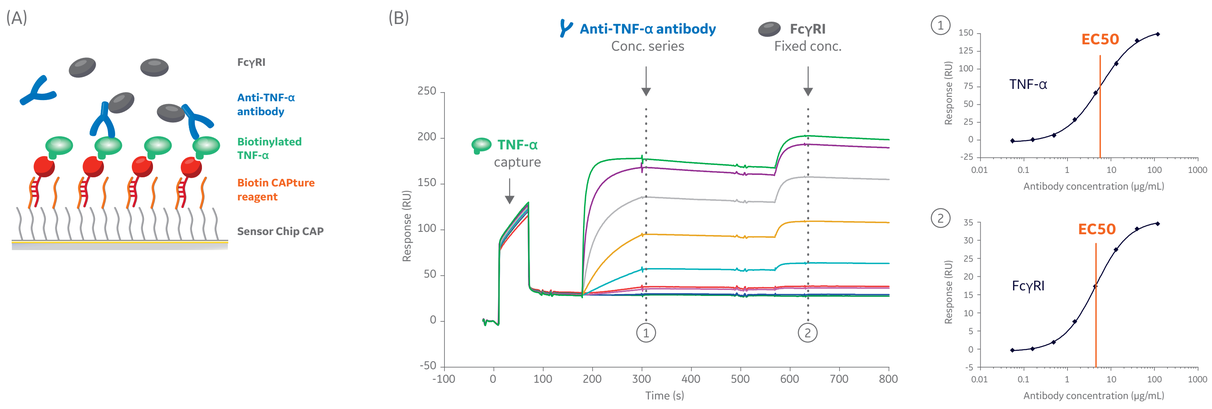


Fig 4. (A) Anti-TNF-α antibody and FcγRI bind in sequence. (B) Responses (dashed lines) were plotted against antibody concentration. Potency curves for antibody binding to both TNF-α and FcγRI were obtained in the same experiment.

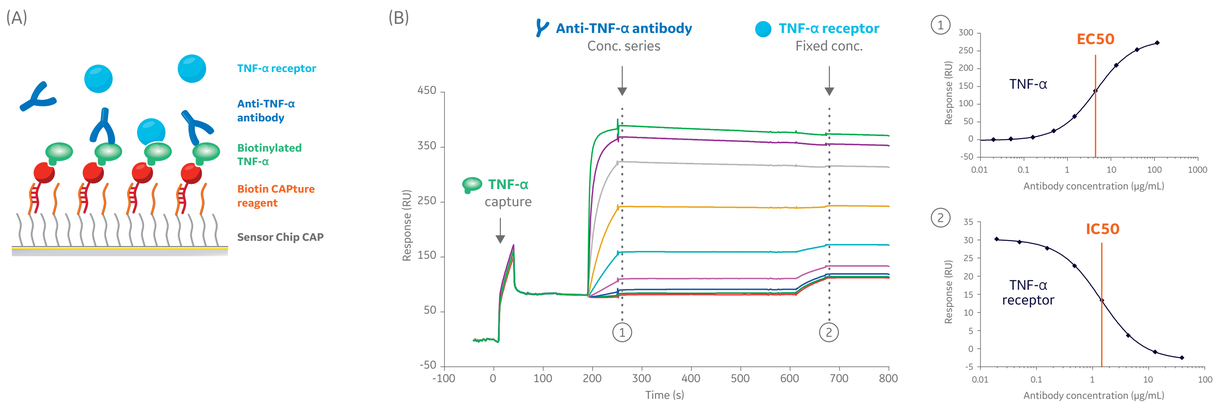


Fig 5. (A) Anti-TNF-α antibody and TNF-α receptor compete for binding to captured TNF-α. (B) Responses (dashed lines) were plotted against antibody concentration. Two potency curves were obtained from the same experiment as was the case in Figure 4. Here, however, TNF-α receptor binding resulted in an inhibition curve and an IC50 value (curve 2).

Dose-response curves from Biacore assay, ELISA, and other techniques do not provide the whole story

Curve shifts depend not only on concentration but also on kinetic parameters

Simulated data demonstrate how kinetic properties can shift the potency curve. Data mimics the stable binding between anti-TNF-α antibody and TNF-α with rate constants: $k_a = 1.6 \times 10^6\ M^{-1}\ s^{-1}$; $k_d = 8.5 \times 10^{-5}\ s^{-1}$.

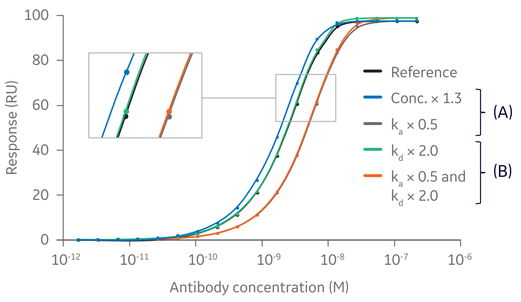


Fig 6. Potency curve shifts depend on concentration and kinetic parameters. (A) Differences in concentration and in association rate shift the curve and EC50. (B) Changing the dissociation rate does not shift the curve.

Differences in dissociation rates can be missed for stable binders

Sensorgram comparison complements potency data

By prolonging the dissociation time for a selected concentration during potency analyses, differences in dissociation can be detected. *Sensorgram comparison* (2) describes this: a comparison window is defined by the user, allowing for some variation of the reference. Samples falling within the window are accepted (Similarity score = 100%), while samples falling outside need further analysis, and may be ranked according to decreasing similarity percent.

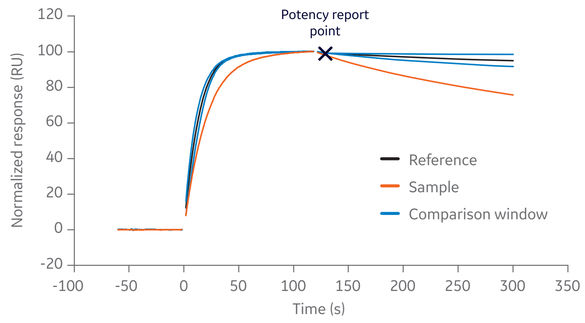


Fig 7. Potency assay complemented with *Sensorgram comparison*. The sample (red curve) obtains similar potency response (×) as the reference but appears *outside* the comparison window when dissociation is prolonged. Kinetic profiles for reference and sample differ.

Differences in kinetic profiles can be detected

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, bispecifics.
- Dose-response curves for Biacore assay, ELISA, and other techniques give limited information on similarity, especially for strong binders. By including the Biacore software functionality *Sensorgram comparison* in potency data evaluation, differences in kinetics can be detected.

References

1. Karlsson R. *et al.* Kinetic and specific concentration information strengthens confidence in relative potency analyses of antibody critical quality attributes. *J. Pharm. Anal.* Submitted for publication (2017).
2. Karlsson R. *et al.* Comparison of surface plasmon resonance binding curves for characterization of protein interactions and analysis of screening data. *Anal. Biochem.* **502**, 53–63 (2016).

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