APPLICATION NOTE

Characterization of biosimilars derived from PlantForm's *vivo*XPRESS® technology with Alto™

Overview

Biological drugs, or biologics, have transformed patient outcomes worldwide due to their ability to treat diseases with a higher degree of precision and specificity compared to small-molecule drugs. While biosimilars, which are highly similar copies of existing biologics, have increased affordability to biologics due to their abbreviated approval process, there still remains a need for more cost-effective and efficient methods to develop these drugs due to their complexity and increasing demand. In this application note, Alto Digital SPR was used to characterize biosimilars derived from PlantForm's *vivo*XPRESS® technology, demonstrating its ability to provide high-quality data while reducing time to answer.

Introduction

The emergence of biologics over the past several decades has heralded a new era for medicine by providing therapeutics for many serious conditions, including those with no other treatment options. Biologics are large-molecule drugs derived from natural sources such as cells and organisms, and include a wide range of products, including somatic cells, gene therapy, and recombinant therapeutic proteins.[1] Compared to conventional drugs, biologics have different and often more complex source material, structures and formulations, manufacturing processes, quality assurance, and regulatory requirements, all of which translate into a higher price tag for patients and limited accessibility.[1,2] Biosimilars offer a more cost-efficient alternative, thereby increasing accessibility to biologic therapies and improving patient outcomes.

Biosimilar development

Biosimilars were introduced to increase access for patients needing treatment with biological drugs. A biosimilar, as defined by the U.S. Food and Drug Administration (FDA), is "a biologic that is highly similar to and has no clinically meaningful differences in terms of safety, purity, and potency (safety and effectiveness) from an existing FDA-approved biologic, called a reference product."[3]

The FDA and other regulatory bodies have a recognized abbreviated process for approval of biosimilars, with the intent of increasing available treatment options, mitigating barriers to access, and fostering competition to decrease healthcare costs.[4,5] While pioneer biologics require data on safety, efficacy, and manufacturing process, biosimilars only require data to demonstrate their clinical equivalence to the reference biologic, and permit reliance on previous data where applicable.[4,5] As well, manufacturers may opt to seek the higher designation of interchangeability by demonstrating the biosimilar can reliably yield the same clinical outcome as the reference biologic in any given patient without significant risk when switching.[6] The global biosimilar market is expected to grow from $30B USD in 2021 to over $100B USD by 2030.[7] In addition, patents for over 190 biologics from top pharma companies are set to expire by 2030, with an expected loss of more than $230B for the industry.[8] This forecast, paired with the abbreviated approval process for biosimilars, presents a significant market opportunity for biosimilars. However, despite presenting considerable time and capital savings compared to reference biologics, biosimilars remain costly to develop and bring to market, requiring an average of 8-10 years and $100-$200 M.[2]

Streamlined methods for biosimilar development and manufacturing can enable new players to overcome this market deficit and meet growing demand. For example, new technologies that enable more efficient biosimilar characterization early in the pipeline could have important implications for incentivizing innovation, decreasing end-user costs, and increasing accessibility.

PlantForm's *vivo*XPRESS® technology

PlantForm's *vivo*XPRESS® is a proprietary technology that leverages genetically modified plant Nicotiana benthamiana to develop and manufacture high-value protein and antibody drugs with minimized costs and production time. The process from seed to high-value pharmaceuticals is completed within six weeks and presents several other advantages compared to conventional biomanufacturing methods, including scalability, transferability, and minimized costs.

PlantForm's *vivo*XPRESS® platform is already accelerating development of biosimilars for Lucentis® (ranibizumab) and Keytruda® (pembrolizumab), expected to be market ready by 2027 and 2028, respectively. The streamlined manufacturing process presents significant cost savings, generating biosimilars at 10% of the cost of mammalian cell fermentation technology and at 12.5% to 25% of the cost of bacterial fermentation. *Vivo*XPRESS® also reduces the production time to generate stable, optimized expression of biosimilars from up to six months to just six weeks.

Ligand binding assays for biosimilars

Ligand binding assays (LBAs) are critical to the development and manufacturing of biosimilars, as they are used to assess biosimilarity, validate the development process, and enforce quality control. These assays compare the biosimilar to the original drug by characterizing ligand binding to its target molecule to demonstrate functional similarity. They are also used to assess potential impacts of the purification process on protein structure and function, to determine whether changes in the purification method affect protein structure, and to monitor stability over storage. In addition, LBAs are used for quality control, to evaluate lot-to-lot consistency and for process validation.

LBAs with Alto Digital SPR

Streamlining the biologics development process requires introducing efficiencies at every step. Alto, the world's first digital SPR platform, offers a label-free, real-time, and automated approach for characterizing ligand binding. Alto's proprietary integration of digital microfluidics and localized SPR (LSPR) biosensors into a single disposable cartridge has revolutionized SPR by providing a more user-friendly, economical, and streamlined alternative. LBAs can be conducted with a fraction of the hands-on time and sample volume required by other techniques, representing potential cost-savings in biologics development.

Nicoya's Alto and PlantForm's *vivo*XPRESS® technology are both uniquely suited to support development of a strong biosimilar pipeline. To demonstrate their compatibility, we collaborated with PlantForm to conduct proof-of-concept LBAs on Alto using Keytruda® and pembrolizumab, with the results summarized in this application note.

Materials

- Nicoya Alto 16-Channel Instrument (ALTO16)
- Alto 16-Channel Carboxyl Cartridge (KIN-CARTCBX-16)
- Running Buffer: PBS-T (0.1% Tween 20), pH 7.4 (ALTO-R-PBST)
- Regeneration Buffer: Glycine HCl pH 1.5 (0.1% Tween 20) (ALTO-R-GLYHCl-1.5)
- Nicoya Streptavidin Coupling Kit (ALTO-R-STV-KIT)
- Ligand: PD-1
- Analytes:
  - Mammalian-based anti-human PD-1 (Keytruda®)
  - Plant-based anti-human PD-1 (pembrolizumab)

Method

This label-free SPR assay was performed using Alto, the first and only digital microfluidic (DMF) powered SPR. Alto uses a cartridge-based, gold nanostructure sensor with 16 channels (8 reference channels and 8 active channels). The experimental method was designed remotely using Alto's cloud-based user portal, the Nicosystem, which was uploaded to the instrument with the click of a button.

First, a 16-Channel Carboxyl Cartridge was loaded into Alto followed by dispensing of the cartridge fluid into the cartridge. Since Alto is an automated SPR instrument, all the reagents were pipetted into the cartridge wells. After all the reagents were loaded into the cartridge, the experiment was initiated by selecting the "Run Method" command on Alto. All subsequent steps were automated by Alto.

Normalization of sensors with high and low RI droplets was first performed. The sensors were then cleaned with 10 mM HCl for 60 s, followed by activation of 16 carboxyl sensors in the cartridge with 25 mM EDC/NHS for 5 min. The ligand (PD-1, 50 µg/mL) was suspended in 10 mM sodium acetate pH 5.5 and immobilized on the 8 active channels for 5 min. All sensors were subsequently blocked with 1 M ethanolamine for 5 min to quench the remaining active carboxyl groups.

Two analytes were tested on each cartridge, Keytruda® or pembrolizumab, by loading 2 µL of 300 nM solution per well. Tested concentrations of both analytes were 100 nM, 33.3 nM, 11.1 nM, 3.70 nM and 1.23 nM, facilitated through the automatic mixing of five 3-fold serial dilutions per analyte. The analytes were introduced in a single-cycle kinetics (SCK) format, with an association time of 180 s, without dissociation or regeneration between each sample, starting from the lowest to highest concentration. A dissociation time of 600 s was set to run after the highest concentration. The sensor surface was regenerated with a 60 s exposure of 10 mM glycine HCl, pH 1.5, which resulted in 100% regeneration. Four cycles of SCK were run and analyzed per analyte.

Upon completion of the test, binding curves were automatically fitted to a 1:1 binding model in the Nicosystem analysis software to determine kinetic and affinity constants.

Results & Discussion

Kinetic values were calculated based on the sensorgrams obtained on one cartridge with Alto, with each analyte tested across 4 lanes and 4 rounds. The data were fit to a Langmuir 1:1 binding model analyzed in the Nicoya Analysis Software. Kinetic parameters for both analytes are reported in Table 1. From this analysis, association and dissociation rate constants for Keytruda® were determined to be 1.60 x 10^6 $M^{-1}s^{-1}$ and 2.07 x 10^{-4} s^{-1}, respectively, resulting in a K_D of 1.30 x 10^{-10} M (Figure 1). Additionally, association and dissociation rate constants for pembrolizumab were determined to be 1.45 x 10^6 $M^{-1}s^{-1}$ and 1.85 x 10^{-4} s^{-1}, respectively, resulting in a K_D of 1.28 x 10^{-10} M (Figure 2). No NSB was observed, as evidenced by the lack of response in the reference channels, and full regeneration was achieved by 10 mM glycine HCl, pH 1.5 for both Keytruda® (Figure 3) and pembrolizumab (Figure 4). The data sets obtained across the 4 lanes and 4 rounds for each of Keytruda® (Figure 5) and pembrolizumab (Figure 6) demonstrate the excellent reproducibility of the data across all channels and rounds.

Table 1: Kinetic parameters measured for either Keytruda® or pembrolizumab using Alto.

	k_a(1/(M*s))	k_d (1/s)	K_D (pM)
Keytruda®	1.6 x 10^6 ± 2.8 x 10^5	2.1 x 10^{-4} ± 3.8 x 10^{-5}	128.6 ± 1.3
Pembrolizumab	1.5 x 10^6 ± 1.2 x 10^5	1.9 x 10^{-4} ± 4.6 x 10^{-5}	127.7 ± 7.5

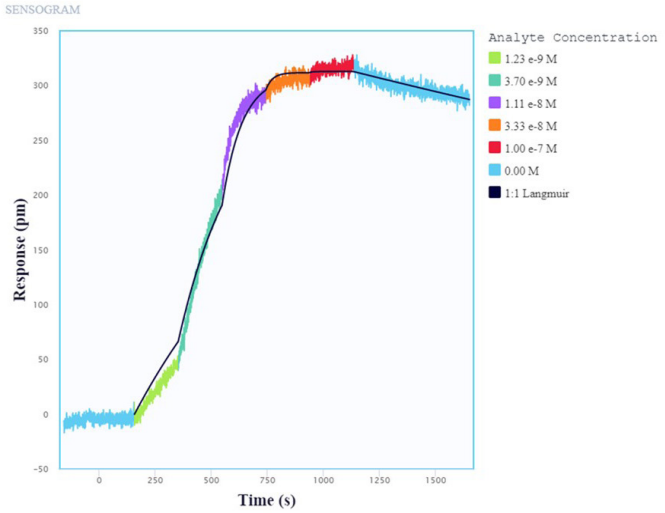

Figure 1: *Single-cycle kinetics of Keytruda® (analyte) binding to immobilized PD-1 (ligand) on Alto. Analyte was titrated from 1.23 nM to 100 nM. Black curve is the Langmuir 1:1 binding fit model analyzed in the Nicosystem analysis software.*

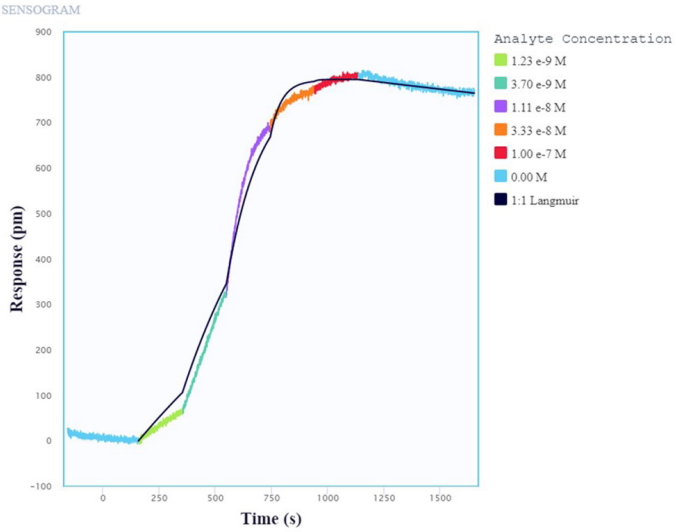

Figure 2: *Single-cycle kinetics of pembrolizumab (analyte) binding to immobilized PD-1 (ligand) on Alto. Analyte was titrated from 1.23 nM to 100 nM. Black curve is the Langmuir 1:1 binding fit model analyzed in the Nicosystem analysis software.*

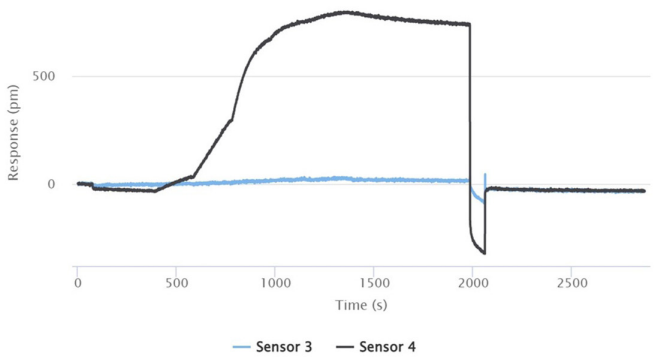

Figure 3: *Reference (blue trace) and active channel (black trace) binding data showing binding of Keytruda® to PD-1 in the active channel and lack of NSB in the reference channel. Full regeneration was achieved by 10 mM glycine HCl pH 1.5.*

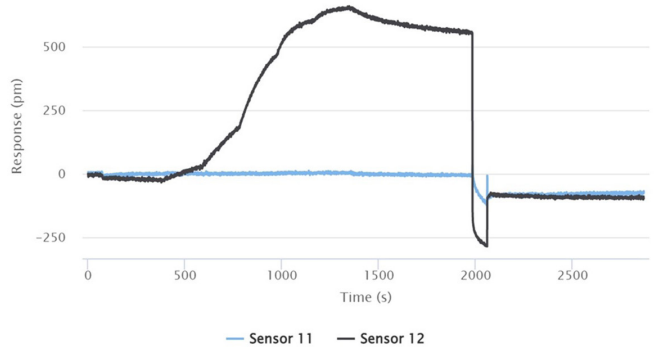

Figure 4: *Reference (blue trace) and active channel (black trace) binding data showing binding of pembrolizumab to PD-1 in the active channel and lack of NSB in the reference channel. Full regeneration was achieved by 10 mM glycine HCl pH 1.5.*

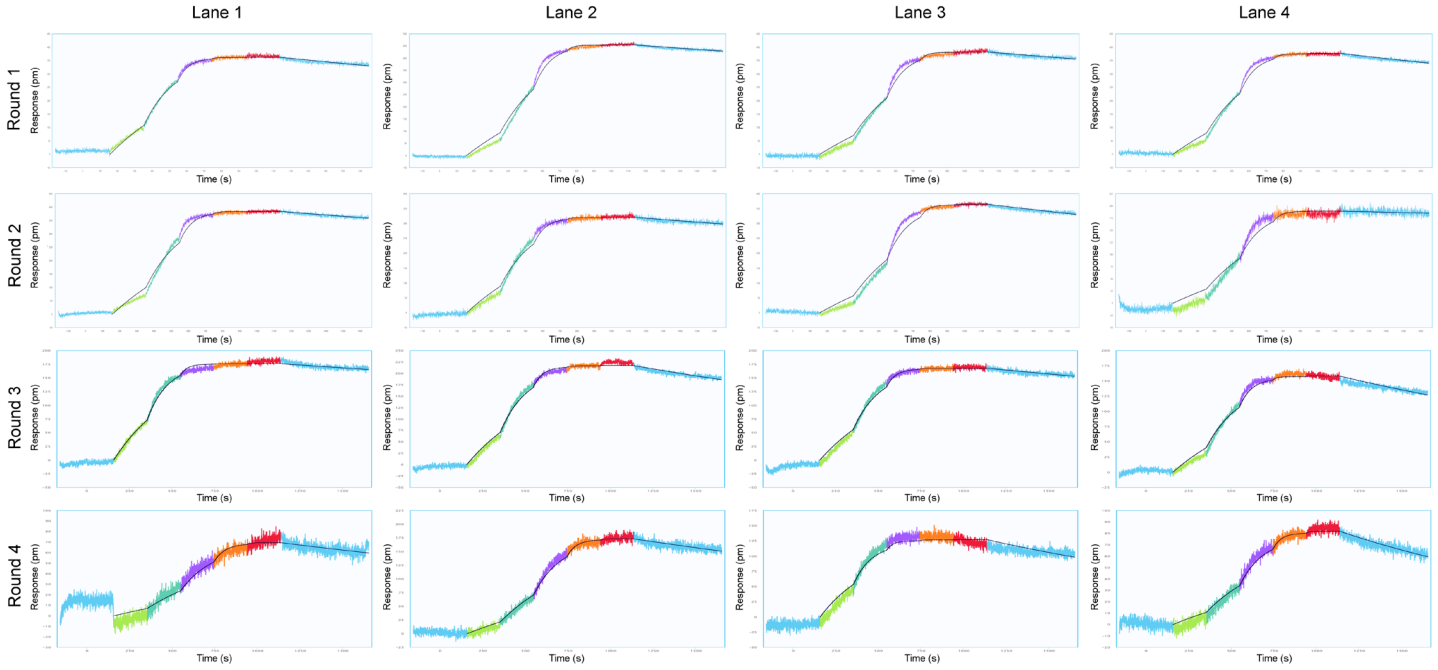

Figure 5: *Example of 16 SCK data points of Keytruda® (analyte) binding to immobilized PD-1 (ligand) on Alto. Analyte was titrated from 1.23 nM to 100 nM. Black curve is the Langmuir 1:1 binding fit model analyzed in the Nicosystem analysis software.*

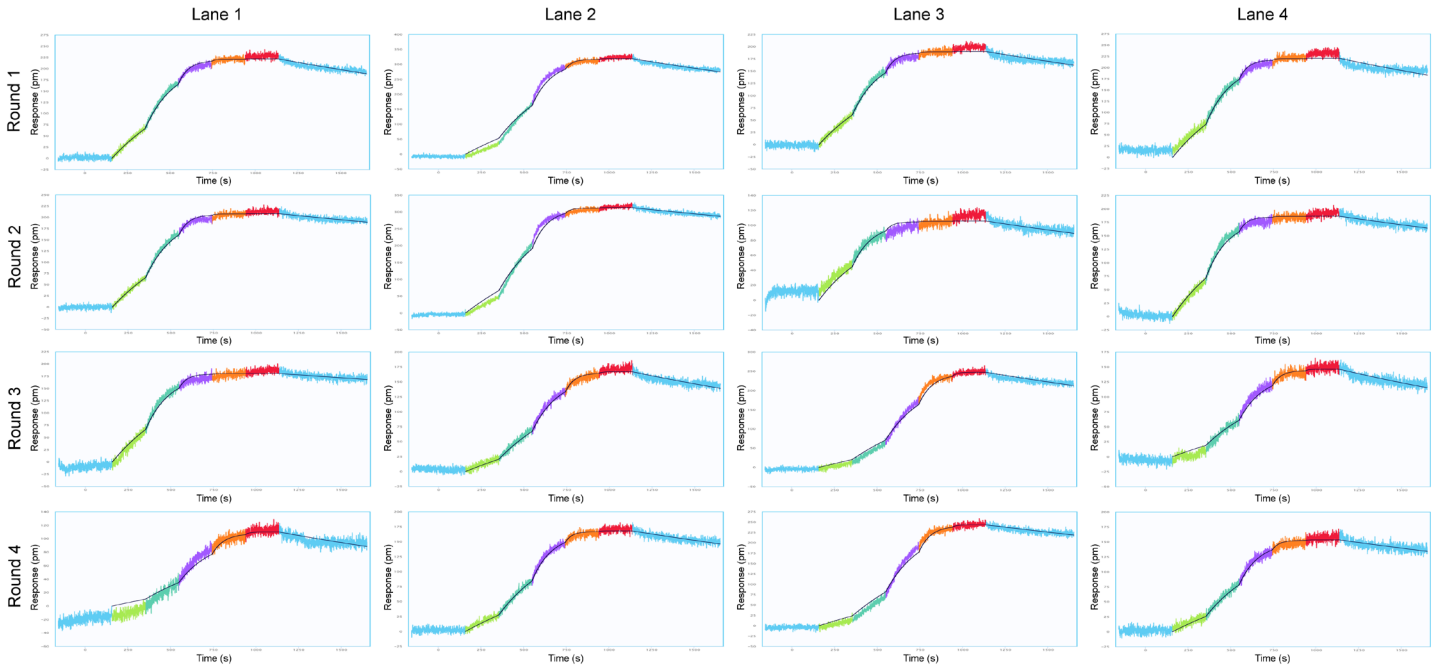

Figure 6: *Example of 16 SCK data points of pembrolizumab (analyte) binding to immobilized PD-1 (ligand) on Alto. Analyte was titrated from 1.23 nM to 100 nM. Black curve is the Langmuir 1:1 binding fit model analyzed in the Nicosystem analysis software.*

Conclusion

An analysis of multiple interactions of different antibodies against a programmed cell death-1 protein with Alto demonstrated the platform's ability to characterize high affinity interactions with robust reproducibility. All aspects of the experiment were automated by Alto using digital microfluidic technology, allowing all analysis to be conducted on a single cartridge with just a fraction of typical sample requirements, while requiring just 30 minutes of hands-on time with the instrument. The results highlight Alto's ability to provide high-quality data while reducing time to answer, proving its ability to accelerate the development of ligand binding assays (LBAs) that are critical to the development and manufacturing of biosimilars.

The biosimilar market is projected to boom over the next decade as an unprecedented number of biologics patents are set to expire, presenting a significant market opportunity for biosimilar manufacturers. Given the complexity of developing and manufacturing biological drugs, innovative players have an edge in bringing their products to market and fulfilling the growing demand. Nicoya's next-generation Alto and PlantForm's *vivo*XPRESS® technology are both uniquely suited to support the development of a strong biosimilar pipeline in the coming years.

References

1. What are "biologics" questions and answers. U.S. Food and Drug Administration. Updated February 6, 2018. Accessed January 18, 2023. https://www.fda.gov/about-fda/center-biologics-evaluation-and-research-cber/what-are-biologics-questions-and-answers
2. Halimi V, Daci A, Netkovska KA, Suturkova L, Babar ZUD, Grozdanova A. Clinical and regulatory concerns of biosimilars: A review of literature. Int J Environ Res Public Health. 2020;17(16):5800. doi: 10.3390/ijerph17165800
3. Review and Approval. U.S. Food and Drug Administration. Updated December 13, 2022. Accessed January 18, 2023. https://www.fda.gov/drugs/biosimilars/review-and-approval
4. Implementation of the biologics price competition and innovation act of 2009. U.S. Food and Drug Administration. Updated February 12, 2016. Accessed January 18, 2023. https://www.fda.gov/drugs/guidance-compliance-regulatory-information/implementation-biologics-price-competition-and-innovation-act-2009
5. Biosimilar biologic drugs in Canada: Fact sheet. Health Canada. Updated August 27, 2019. Accessed January 18, 2023. https://www.canada.ca/en/health-canada/services/drugs-health-products/biologics-radiopharmaceuticals-genetic-therapies/applications-submissions/guidance-documents/fact-sheet-biosimilars.html
6. Biosimilar and interchangeable biologics: More treatment choices. U.S. Food and Drug Administration. Updated October 12, 2021. Accessed January 18, 2023. https://www.fda.gov/consumers/consumer-updates/biosimilar-and-interchangeable-biologics-more-treatment-choices
7. Global Market Insights Inc. Biosimilars Market size to exceed $100bn by 2030. Published June 2022. Accessed January 18, 2023. https://www.gminsights.com/pressrelease/biosimilars-market
8. Dutton G. Looming patent cliff will be pharma's moment of truth. BioSpace. June 7, 2022. Accessed January 18, 2023. https://www.biospace.com/article/looming-patent-cliff-will-be-pharma-s-moment-of-truth/