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Application Note

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An Innovative Approach in Diabetes Research: The Role of Biosensor Technology in Advancing GLP-1 Analog Development

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Abstract

Glucagon-like peptide-1 (GLP-1) is a hormone that plays a crucial role in glucose metabolism and has garnered significant attention in the field of endocrinology and diabetes management. Discovered in the 1980s, GLP-1 is an incretin hormone, meaning it is released after eating and stimulates insulin secretion. It is produced in the intestinal L-cells and released in response to nutrient ingestion.

Globally there are over 650 million adults with excess adiposity, presenting numerous health challenges for the individuals and associated costs for healthcare systems. The physiological effects of GLP-1 are multifaceted, making it a potent target for therapeutic interventions, particularly in the treatment of type 2 diabetes mellitus (T2DM) and adiposity-based chronic disease.

Regulatory-approved analogues of GLP-1 that are currently used in treatment act through the same mechanisms and are responsible for regulating insulin and glucagon secretion. Analysis of GLP-1 analogues can often be challenging due to poor ionization and fragmentation due to the inherent large peptide structure. Therefore, a highly sensitive and robust platform is essential for the quantification of GLP-1 analogs.

This application note highlights the ease of assay design and setup offered by the dip-and-read format on Sartorius' Octet® Biolayer Interferometry (BLI) systems. This approach allows for the determination of binding kinetics and affinity of GLP-1 and its analogues Victoza® (Liraglutide), Trulicity® (Dulaglutide) and Ozempic® (Semaglutide), and facilitates the discussion of their structure-function properties.

Introduction

Glucagon-like peptide-1 (GLP-1) is a hormone that plays a crucial role in glucose metabolism and has garnered significant attention in the field of endocrinology and diabetes management. Discovered in the 1980s, GLP-1 is an incretin hormone, which means it is released after eating and stimulates insulin secretion.

The initial product GLP-1 (1–37) is derived from tissue-specific posttranslational processing of the proglucagon peptide. GLP-1 (1–37) is susceptible to amidation and proteolytic cleavage, resulting in the generation of two active versions: GLP-1 (7–36) and GLP-1 (7–37) (Figure 1A), both of which are biologically active.

GLP-1 exerts its effects through the GLP-1 receptor, which is expressed in various tissues, including the pancreas, brain, heart, and gastrointestinal tract. The primary actions of GLP-1 include:

- **Stimulation of Insulin Secretion:** GLP-1 enhances glucose-dependent insulin secretion from the pancreatic β cells. This means that insulin is released in response to elevated blood glucose levels, helping to lower blood sugar.
- **Inhibition of Glucagon Secretion:** GLP-1 suppresses the release of glucagon, a hormone that increases blood glucose levels by promoting gluconeogenesis and glycogenolysis in the liver.
- **Slowing Gastric Emptying:** By delaying gastric emptying, GLP-1 helps regulate the rate at which glucose enters the bloodstream, thereby preventing postprandial spikes in blood glucose levels.
- **Reduction of Appetite and Food Intake:** GLP-1 acts on the central nervous system to promote satiety and reduce appetite, contributing to weight loss.

GLP-1 Analogs: Therapeutic Applications

Due to degradation by ubiquitous endogenous enzymes, dipeptidyl peptidase IV (DPP-IV) and neutral endopeptidases (NEP), GLP-1 (7–37) has a half-life of approximately 2 minutes. Therefore, given the beneficial effects of GLP-1 (7–37) discussed previously, researchers have developed GLP-1 (7–37) analogs designed to mimic the action of natural GLP-1 (7–37) but with a longer half-life, making them more suitable for clinical use.

Some well-known GLP-1 analogs include, Victoza® (Liraglutide), Trulicity® (Dulaglutide), and Ozempic® (Semaglutide).

Victoza® (Liraglutide, 3,751 Da): Victoza® (Figure 1B) shares a 97% sequence homology with native GLP-1 (7–37). Victoza® contains a lysine-to-arginine substitution at position 34 and a C-16 fatty acid chain (palmitic acid) is then

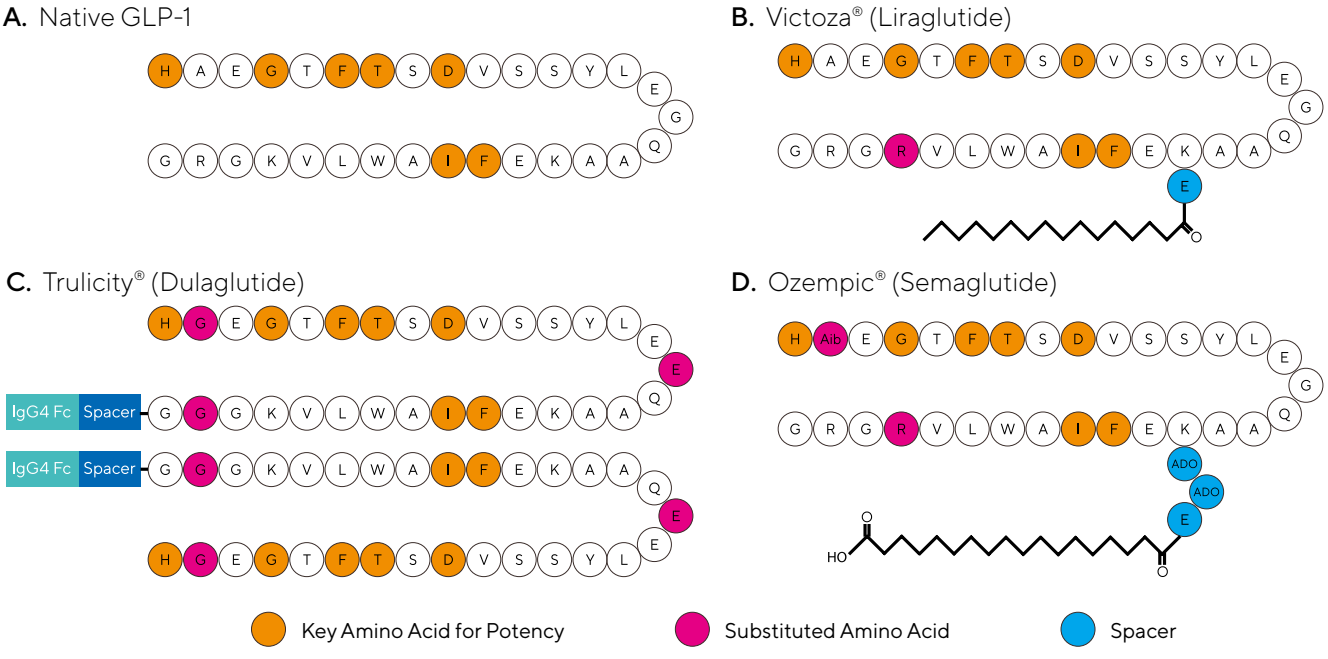

Figure 1: Peptide sequences and molecular structures of FDA approved GLP-1 receptor agonists. Active GLP-1 (7 – 37) contains two α-helices from amino acid position 13–20 and 24–35 separated by a linker region. Adapted from Yu et al.[3]

attached via a glutamic acid spacer, to the remaining lysine at position 26 [1]. The self-association of Victoza® delays absorption, increases plasma protein binding, and increases stability against degradation by DPP-IV and NEP. This results in a plasma half-life of approximately 13 hours, requiring once-daily injection [2].

Trulicity® (Dulaglutide, ~63 kDa): Trulicity® consists of two identical disulfide-linked chains (Figure 1C). Each chain contains an N-terminal GLP-1 (7–37) analog that shares a 90% sequence homology with native GLP-1 and is covalently linked to the Fc portion of a modified human immunoglobulin G4 (IgG4) heavy chain by a small peptide spacer [4]. Amino acid substitutions in the native GLP-1 (7–37) region decrease degradation by DPP-IV and NEP. Additional modifications were made in an area with a potential T-cell epitope and in the areas of the IgG4 Fc part of the molecule responsible for binding the high-affinity Fc receptors and half-antibody formation. This results in a plasma half-life of approximately 120 hours, requiring once-weekly injection [2].

Ozempic® (Semaglutide, 4113 Da): Ozempic® (Figure 1D) shares a 94% sequence homology with native GLP-1 (7–37). Ozempic® contains a lysine-to-arginine substitution at position 34 and an alanine-to-α-aminobutyric acid (Aib) substitution at position 8. In addition, a C-18 di-acid fatty chain (stearic acid) is attached via a glutamic acid and two 8-amino-3,6-dioxaoctanoic acid (ADO) spacer to the remaining lysine at position 26 [2,5]. The amino acid substitutions and addition of C-18 di-acid fatty chain result in an increase in albumin binding, which is the main protraction mechanism of Ozempic®, while also shows a decrease in degradation by DPP-IV. This results in a plasma half-life of approximately 120 hours, requiring once-weekly injection [2].

Although there is a large amount of structural and functional information about how GLP-1 and GLP-1 analogs exert their effects, there is a lack of information regarding how structural changes, including amino acid substitution, dimerization, and the addition of fatty acid chains, affect the kinetics and affinity of binding to the GLP receptor.

Here, we demonstrate how the Octet® BLI system can be used to assess how changes in structural analogs of GLP-1 (7–37) can affect binding to the GLP receptor. This study can facilitate further drug development using structural and functional changes.

Materials and Methods

Material	Supplier	Product Number
10X Kinetics Buffer (KB)	Sartorius	18-1105
Octet® SAX2 Biosensors	Sartorius	18-5136
Octet® Evaporation Covers	Sartorius	19-0081
96-well, black, flat bottom microplate	Greiner Bio-One	655209
1X PBS	Gibco	14190-094
Victoza® (Liraglutide)	Novo Nordisk	Supplied by WEP Clinical
Trulicity® (Dulaglutide)	Eli Lilly	Supplied by WEP Clinical
Ozempic® (Semaglutide)	Novo Nordisk	Supplied by WEP Clinical
Human GLP-1 Recombinant Protein (7 – 37)	PeproTech	130-08
Recombinant Human GLP1R Protein (His & Avitag™)	SinoBiological	13944-H49H-B

Table 1: Materials required for GLP1R binding assay.

Methods

Octet® SAX2 Biosensors (18-5136), which are preimmobilized with Streptavidin, were hydrated for at least 10 minutes at room temperature prior to use in 10X KB in a 96-well, black, flat bottom microplates (Greiner Bio-One, 6552091). All assays were performed at 25 °C.

The Octet® BLI assay followed a general assay flow of:

Assay Step	Step Name	Time (s)	Shake Speed (RPM)
1	Baseline	60	1,000
2	Loading	Analyte Specific	1,000
3	Baseline	60	1,000
4	Association	Analyte Specific	1,000
5	Dissociation	Analyte Specific	1,000

Table 2: General Octet® BLI assay flow for GLP1R binding assay

Ligand Preparation

Recombinant AviTag™ biotinylated Human GLP1R was prepared to a final concentration of 2.5 µg/mL in 10X KB (GLP-1 (7–37), Ozempic®and Victoza®) and 1.0 µg/mL (Trulicity®).

Analyte Preparation

GLP-1 (7–37), Victoza®, Trulicity®, Ozempic® were diluted in 10X Kinetics Buffer to a final concentration of 10,000 nM GLP-1 (7–37), 250 nM Ozempic®, 1,000 nM Victoza® and 50 nM Trulicity®. A 2-fold dilution series was used in all assays. A reference sample of 10X KB was used in all assessments in order to correct for baseline drift. Double reference subtraction was performed by performing replicate steps using ligand-unloaded biosensors.

Analysis was performed using an Octet® BLI R8 system using Octet® BLI Discovery and Analysis Studio Software version 13.1.0.25. Data was fitted using Octet® Analysis Studio software 13.1.0.38 to a global 1:1 model.

Results and Discussion

As discussed in the introduction, GLP-1 (7–37) has a half-life of approximately 2 minutes, and this is reflected in the observed binding to the GLP receptor. As shown in Figure 2, GLP-1 (7–37) exhibits fast association and dissociation kinetics (Table 3). Although degradation by endogenous enzymes is not a factor in biophysical assays, it is clear to see that endogenous GLP-1 (7–37) does not occupy the GLP receptor for extended periods.

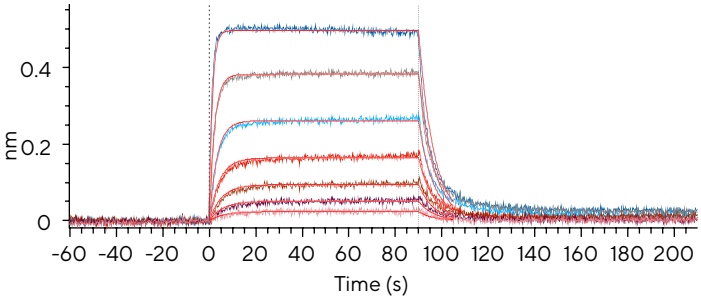

Figure 2: Binding of GLP-1 (7–37) to the GLP Receptor.

Analyte	k_a [$M^{-1}s^{-1}$]	k_d [s^{-1}]	K_D [nM]	R^2
GLP-1 (7–37)	$5.85 * 10^4$	$1.54 * 10^{-1}$	2627	0.9969
Victoza® (Liraglutide)	$1.09 * 10^4$	$2.98 * 10^{-3}$	272	0.9912
Trulicity® (Dulaglutide)	$1.72 * 10^6$	$4.40 * 10^{-3}$	2.6	0.9934
Ozempic® (Semaglutide)	$8.83 * 10^4$	$8.61 * 10^{-3}$	97.4	0.9903

Table 3: Comparison of kinetics and affinity of GLP-1 (7–37) and structural analogs.

Victoza® is administered once daily in patients, and the main difference to GLP-1 (7–37) is the addition of a C-16 fatty acid chain (palmitic acid), which increases stability against degradation by DPP-IV and NEP. In addition, lysine at position 34 is substituted to arginine in order to ensure the fatty acid chain attaches at the correct location. As shown in Figure 3, Victoza® exhibits an affinity for the GLP receptor that is approximately 10-fold higher than that of GLP-1 (7–37). Analysis of the kinetics shows that this increased affinity is primarily due to a decrease in dissociation from the GLP receptor (Table 3).

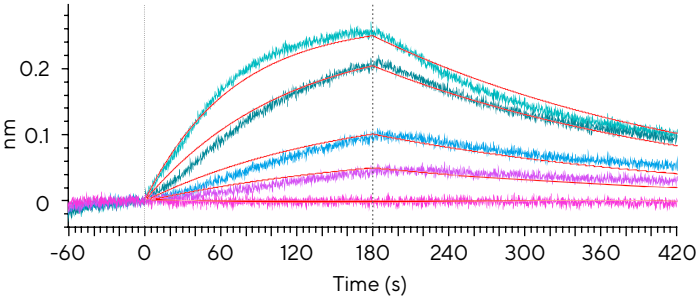

Figure 3: Binding of Victoza® (Liraglutide) to the GLP Receptor.

Unlike Victoza®, Trulicity® is modified through key amino acid substitutions that stabilize the helical region of the GLP-1 (7–37) peptide through substitution of Gly 22 to Glu, which has been shown to cause an 8-fold increase in potency [6]. In addition, Trulicity® does not contain a fatty acid chain but is dimerized through the addition of an

IgG4 Fc region. With a half-life of 120 hours; Trulicity® is administered once a week.

As shown in Figure 4 and Table 3, Trulicity® displays a 1000-fold increase in affinity compared to GLP-1 (7–37).

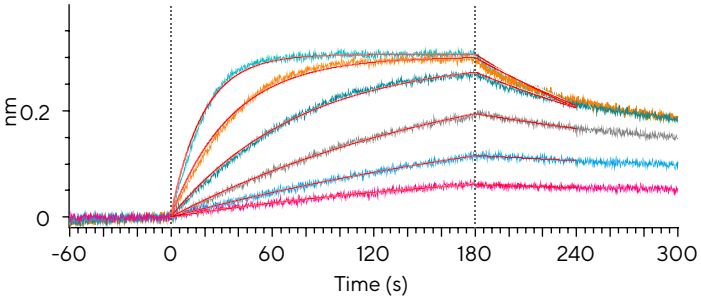

Figure 4: Binding of Trulicity® (Dulaglutide) to the GLP Receptor.

Ozempic®, contains an alanine-to-α-aminobutyric acid (Aib) substitution at position 8, in addition to the lysine-to-arginine substitution at position 34. A C-18 di-acid fatty chain (stearic acid) is attached to the remaining lysine. Although Ozempic® has the longest half-life of the GLP-1 analogs tested here (168 hours), the observed affinity is only 20-fold higher than native endogenous GLP-1 (7–37) (Figure 5 and Table 3).

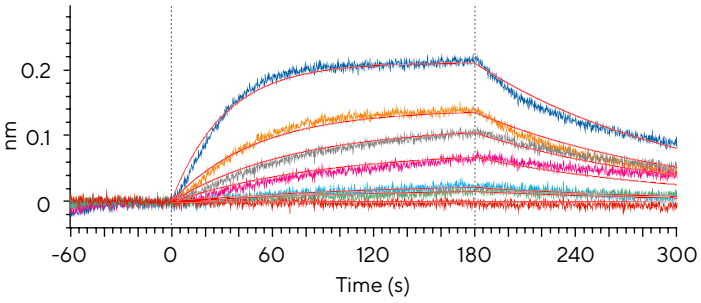

Figure 5: Binding of Ozempic® (Semaglutide) to the GLP Receptor.

As discussed in the introduction, native GLP-1 (7–37) exhibits a short half-life of approximately 2 minutes, which is reflected in the low affinity interaction with the GLP receptor (Figure 2). As shown by Victoza®, Trulicity® and Ozempic®, a range of affinities for the interaction with the GLP receptor are observed, but these affinities are not directly correlated with the half-life of the molecule. For example, although Ozempic® is known to have the longest half-life in the human body (~168 hours), its affinity for the GLP receptor is approximately 50-fold lower than that of Trulicity® (Dulaglutide) (half-life of 120 hours), with affinities of 97.4 and 2.6 nM, respectively. Therefore, the kinetics and affinity observed here indicate that receptor occupancy is only a small factor in the improved half-life compared to endogenous GLP-1 (7–37), and there are multiple paths to achieving extended duration. It is clear that the increased albumin binding through amino acid substitution and the addition of a C-18 di-acid fatty chain, which decreases degradation by endogenous enzymes, is the major factor in the protraction of Ozempic® while amino acid substitution and dimerization are responsible for and the subsequent increase in the observed half-life when compared to endogenous GLP-1 (7–37).

Conclusions

Biophysical interaction plays a crucial role in drug discovery, providing valuable insights into the molecular dynamics and binding affinities of potential drug candidates. However, it does not encompass the entire picture. Orthogonal techniques and *in vivo* studies offer critical information that complement biophysical data, ensuring a comprehensive understanding of drug efficacy and safety. Despite this, biophysical methods are often favored for their speed and efficiency in screening potential drug candidates, allowing researchers to identify promising compounds before committing to extended and costly *in vivo* assessments.

This application note provides a comprehensive overview of the biophysical interaction of GLP-1 and its analogs binding to the GLP receptor. The development of GLP-1 analogs continues to evolve, with novel formulations such as oral GLP-1 analogs and combination therapies with other antidiabetic agents being explored.

Octet® BLI systems provide a label-free, real-time technology that is ideal for biophysical drug development techniques, such as amino acid substitutions, where aggregation issues may arise. Techniques like surface plasmon resonance (SPR) often suffer from assay artifacts caused by aggregation, leading to challenges such as incomplete dissociation of the analyte from the receptor or increased non-specific binding. These artifacts can compromise the integrity of subsequent sample assessments within the assay. In contrast, Octet® BLI biosensors eliminate these issues by utilizing individual sensor surfaces for each assessment, ensuring precise and reliable data without the need for replicate assays. This approach not only enhances the accuracy of biophysical measurements but also streamlines the drug development process, making it more efficient and effective.

Next page: References and contact info >

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