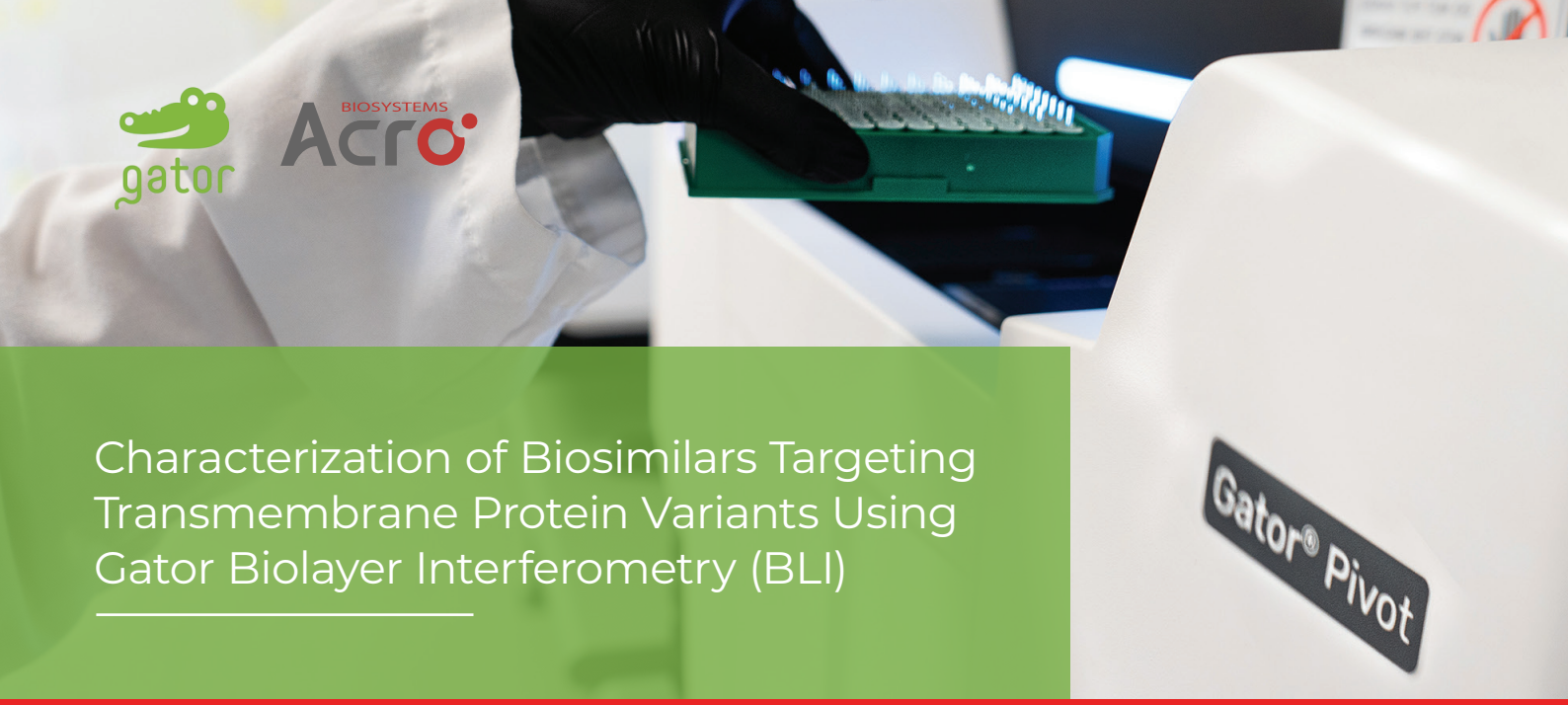

Characterization of Biosimilars Targeting Transmembrane Protein Variants Using Gator Biolayer Interferometry (BLI)

Introduction

Transmembrane proteins (TPs) play essential roles in maintaining cellular function, serving as gatekeepers and signal transducers across the phospholipid bilayer. These proteins facilitate the transport of ions and molecules, mediate intracellular signaling, and regulate vital processes such as metabolism, cellular activity, and differentiation. Structurally, TPs can be categorized as either alpha-helical or beta-barrel, and topologically as single-pass or multi-pass based on the number of times they span the membrane and the orientation of their N- and C-termini. Given their pivotal role in cellular communication and disease pathology, TPs are important therapeutic targets—especially in conditions where membrane protein dysfunction contributes to disease progression.

Studying TPs outside of their native environment remains technically challenging. When removed from the membrane, TPs often lose stability and may aggregate or denature in aqueous environments. Traditional approaches use detergents to solubilize these proteins by mimicking the membrane's hydrophobic environment. Detergent micelles—spherical assemblies formed above a detergent's critical micelle concentration—surround and stabilize hydrophobic regions of TPs. However, excessive or inappropriate detergent use can disrupt native protein conformation, potentially altering function or binding behavior.

To address these limitations, alternative membrane mimetics have emerged. Virus-like particles (VLPs) and nanodiscs offer more biologically relevant environments for membrane protein study. VLPs are self-assembling, non-infectious particles that mimic viral membranes. They enable high-density, multivalent display of full-length TPs within a lipid bilayer, preserving native orientation and structure. Nanodiscs, by contrast, are small, discoidal lipid bilayers encircled by membrane scaffold proteins or synthetic polymers. These systems offer a defined, detergent-free lipid environment that closely resembles the native membrane, making them ideal for functional and structural characterization.

Biolayer Interferometry (BLI) is a label-free technique well-suited for characterizing interactions with transmembrane protein targets. By measuring association and dissociation rates in real time, BLI provides valuable kinetic insight into antibody or drug binding—critical for screening and optimizing biotherapeutic candidates. Its high-throughput efficiency further accelerates drug discovery workflows, making it a vital tool in the development of next-generation therapeutics.

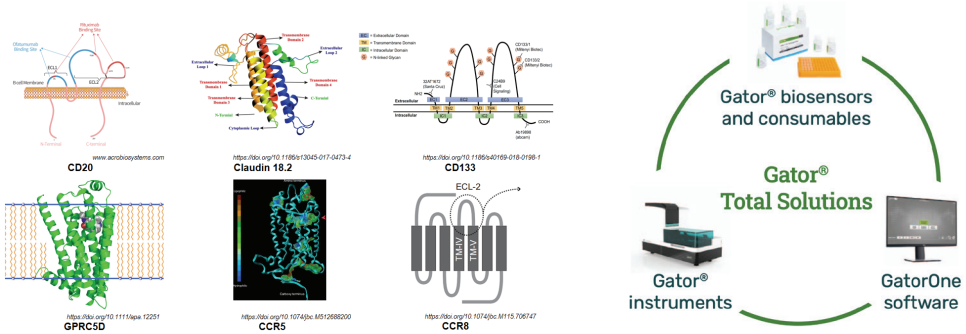

Figure 1. *Gator® next-generation Biolayer Interferometry offers a versatile real-time analysis platform solution for label-free analysis.*

In this application note, we demonstrate how the Gator Bio BLI platform, paired with ready-to-use biosensors, enables kinetic characterization of two widely studied transmembrane proteins—CD20, a four-pass TP, and GPRC5D, a seven-pass G-protein coupled receptor—from ACROBiosystems. These targets were prepared in three different presentation formats: detergent micelles, VLPs, and nanodiscs, to evaluate how presentation method impacts kinetic readouts (Figure 1).

Materials and Methods

Three membrane receptor formats were provided by ACROBiosystems: Detergent Micelle, VLP, and nanodisc. These formats were selected to assess how mimetics influence binding kinetics in BLI-based assays. Tables 1 and 2 summarize the reagents used, including the specific transmembrane protein constructs from ACROBiosystems and the biosimilar antibodies tested against each presentation format.

Table 1. CD20 reagents used to support antibody characterization and mechanism of action studies.

No.	Category	Reagent	Vendor	Cat #
1	TP: CD20 Detergent	Biotinylated Human CD20 / MS4A1 Full Length Protein, His,Avitag™ (SPR verified)	ACROBiosystems	CD0-H82E5
2	TP: CD20 Nanodisc	Biotinylated Human CD20 / MS4A1 Full Length Protein, His,Avitag™	ACROBiosystems	CD0-H82E3
3	TP: CD20 VLP	Human CD20 Full Length Protein	ACROBiosystems	CDP-H52P6
4	Biosimilar	Human CD20 (Research Grade Rituximab Biosimilar) Antibody	R&D Systems	MAB9575
5	Biosimilar	Human CD20 (Research Grade Tositumomab Biosimilar) Antibody	R&D Systems	MAB10440
6	Biosimilar	Human CD20 F(ab')2 (Research Grade Ibritumomab Biosimilar) Antibody	R&D Systems	MAB11054-FAB2

Table 2. GPRC5D reagents used to support antibody characterization and mechanism of action studies.

No.	Category	Reagent	Vendor	Cat #
1	TP: GPRC5D Detergent	Biotinylated Human GPRC5D Full Length Protein, Flag,His,Avitag™	ACROBiosystems	GPD-H52D6
2	TP: GPRC5D Nanodisc	Biotinylated Human GPRC5D Full Length Protein, Flag,His,Avitag™	ACROBiosystems	GPD-H82D4
3	TP: GPRC5D VLP	Human GPRC5D Full Length Protein	ACROBiosystems	GPD-H52P7
4	Biosimilar	Talquetamab Biosimilar – Anti-CD3E, GPRC5D mAb	Proteogenix	PX-TA1632
5	Biosimilar	Forimtamig Biosimilar – Anti-GPRC5D and CD3 mAb	Proteogenix	PX-TA2067

The Gator® Prime instrument was used to perform all kinetic binding assays in this study; however, any Gator Bio BLI platform is compatible with this workflow. A set of specialized biosensors (Table 3) was employed to ensure accurate and reliable detection of molecular interactions across all transmembrane protein formats.

Table 3. Biosensors used in the TP-mAb kinetic studies

Part Number	Probe Name	Binding Specificity
PN# 160003	Anti-Human IgG Fc (HFC)	Human IgG Fc regions
PN# 160013	Anti-Human FAB (FAB)	Human antibody Fab regions
PN# 160029	SA XT	Streptavidin-biotin interactions

To maintain optimal assay conditions, **K Buffer (PN# SKU 120011, Gator Bio)** was used as the running buffer throughout the experiment, providing stability and reproducibility across all steps. The assay workflow is outlined in Figure 2 and detailed in Table 4.

The biosensor probe was first pre-wetted in K Buffer (Step 1), followed by baseline establishment in the same buffer (Step 2). Once a stable baseline was achieved, the probe was loaded with the ligand or sample of interest (Step 3). A subsequent wash step (Step 4) removed any unbound or non-specific components. The loaded probe was then immersed in the analyte solution to initiate the binding phase (Step 5), during which a real-time nm shift signaled the association of the analyte with the immobilized ligand. Finally, the probe was transferred back into K Buffer (Step 6) to monitor the dissociation phase, providing kinetic insights into complex stability.

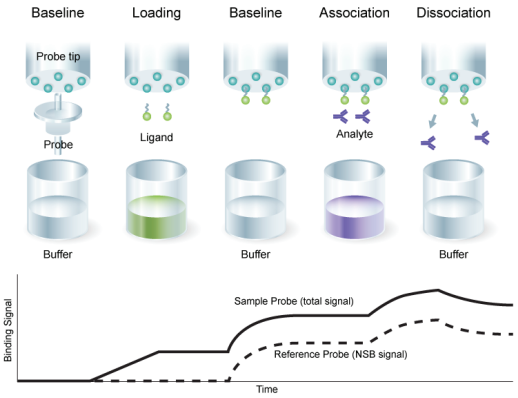

Figure 2. *Steps for the BLI assay. After pre-wetting the probe, a baseline is established. The probe is then loaded with ligand and the baseline is established again. The analyte is then added and allowed to associate with the ligand on the probe which is measured in real-time. The last step enables the dissociation of the ligand-analyte complex to be measured in real-time by moving the probe into the appropriate buffer solution.*

Table 4. Steps in the assay

No	Step	Reagent	Time (sec)	RPM
1	Pre-wet	K buffer	300	Shaker A:0; Shaker B: 1000
2	Baseline	K buffer	120	1000
3	Loading	mAb/ TPs	200	1000
4	Baseline	K buffer	60	1000
5	Association	TPs/mAb	300	1000
6	Dissociation	K buffer	600	1000

Transmembrane proteins engineered with affinity tags—such as His-tags or Avi-tags—can be directly and directionally immobilized onto compatible biosensor surfaces, enabling efficient assay setup and consistent orientation. For untagged proteins, an alternative strategy involves immobilizing an antibody or binding partner onto a pre-functionalized biosensor, followed by capturing the transmembrane protein through its native binding interaction.

Depending on the assay design, either the membrane protein or the biosimilar antibody can serve as the ligand. In some experiments, the tagged transmembrane protein is immobilized onto an SA-XT biosensor (Step 3) and probed with the biosimilar antibody as the analyte (Step 5). In other cases, the biosimilar is first captured via its Fc or Fab region, and the membrane protein format is introduced as the analyte. The choice of biosensor is determined by the tag and format of the transmembrane protein.

Gator Bio offers a broad range of biosensor options optimized for these applications. Additionally, most transmembrane proteins from ACROBiosystems are available with His-tags, Avi-tags, or dual tags, making them highly compatible with label-free BLI workflows.

Results and Discussion

Binding response, Robustness, and Comparison across formats

To evaluate the suitability of various membrane protein presentation strategies for BLI-based kinetic analysis, we examined the binding behavior of anti-CD20 three biosimilar monoclonal antibodies and anti-GPRC5D (two biosimilar monoclonal antibodies) across three distinct TP formats: detergent micelle, nanodiscs, and VLP. Schematic representations of each TP format and their defining biophysical characteristics are in Figure 3.

Nanodiscs constitute a synthetic lipid bilayer system stabilized by membrane scaffold proteins (MSP1D1), which mimic the native lipid environment and confer enhanced structural integrity and monodispersity. This format supports functional reconstitution of integral membrane proteins while maintaining their conformational stability. In contrast, VLPs are formed through self-assembly and budding process that incorporates membrane proteins into nanoscale particles. This process preserves the native orientation and multimeric architecture of the embedded proteins, thereby providing a biologically relevant presentation ideal for affinity screening of therapeutic antibodies.

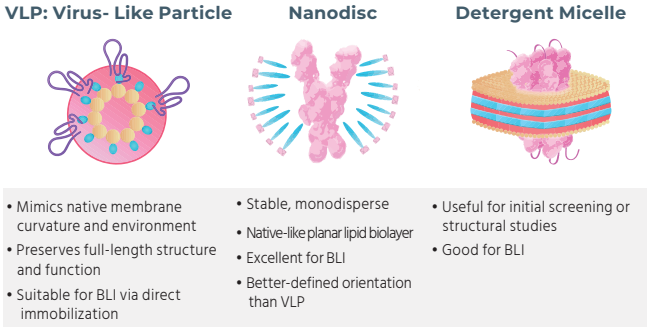

Figure 3. *Three different transmembrane receptor formats were used in this study; virus-like particles (VLP), nanodiscs, and detergent micelle. Generally, detergents can be used to solubilize membrane proteins to provide a means to isolate and study them independently. However, due to their hydrophobicity, protein conformation can often be lost. The VLP and nanodisc formats facilitate solubilization in addition to providing a more native biological environment that can help to maintain the three-dimensional structure of membrane proteins.*

Sensorgrams obtained from the Gator® Prime BLI system for all antibody–TP interactions are shown in Figures 4 and 5. The assay workflow allowed flexibility in experimental configuration; either the TP construct or the biosimilar antibody could be immobilized as the ligand, depending on the experimental design. SA-XT biosensors demonstrated robust performance in capturing both detergent micelle- and nanodisc- membrane proteins, while Anti-HFC1 and Anti-human FAB biosensors selectively bound full-length IgG and F(ab')$_2$ fragments, respectively.

All three TP formats exhibited strong and reproducible binding to the evaluated biosimilars. Notably, both detergent micelle and nanodisc formats yielded highly comparable kinetic profiles across all antibody interactions, suggesting that the mode of membrane mimicry had minimal impact on the integrity of the antigenic epitope or the accessibility of the binding interface.

However, the VLP format produced a distinct kinetic signature characterized by markedly slower dissociation rate constants (k_off), indicative of enhanced avidity. This phenomenon likely results from the high-density and repetitive display of membrane proteins on the VLP surface, which promotes multivalent binding interactions. Such avidity-driven effects are advantageous for high-affinity antibody screening but may mask subtle kinetic differences that are resolvable in monomeric formats such as nanodiscs or detergent micelles.

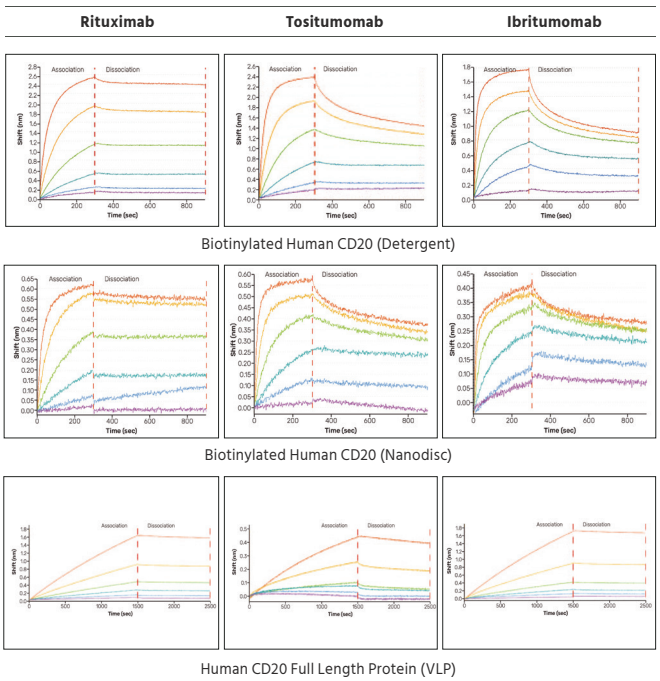

Figure 4. *Sensorgrams for the binding of three biosimilars (Rituximab, Tositumomab, and Ibritumomab) to three CD20 membrane formats (detergent micelle, nanodisc, and VLP). Robust and reproducible binding is observed in all cases with similar results for detergent micelle and nanodisc formats. The VLP formats show a slower dissociation indicating enhanced stability for the antibody-antigen binding.*

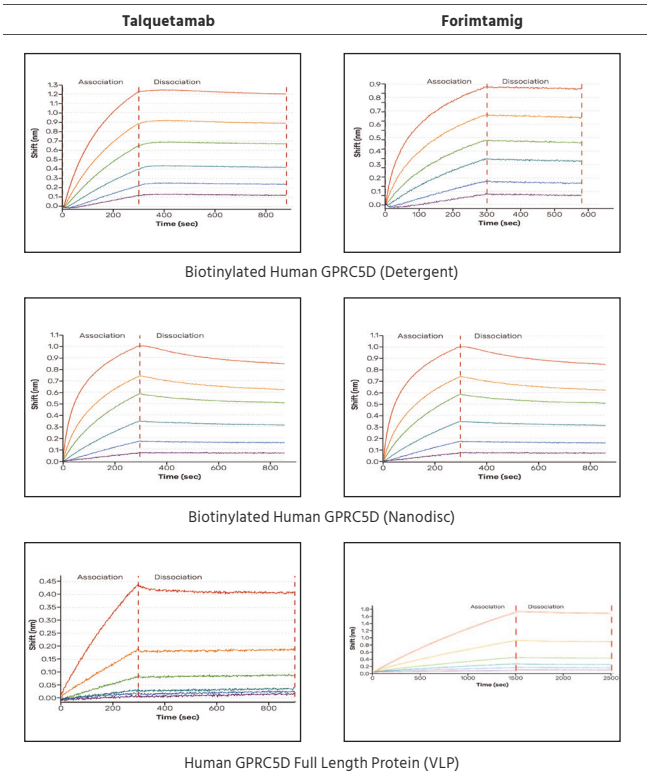

Figure 5. *Sensorgrams for the binding of two biosimilars (Talquetamab and Forimtamig) to three GPRC5D membrane formats (detergent micelle, nanodisc, and VLP). Robust and reproducible binding is observed in all cases with similar results for detergent micelle and nanodisc formats. The VLP formats show a slower dissociation indicating enhanced stability for the antibody-antigen binding.*

In BLI experiments, inverted signals can sometimes occur due to factors such as a changes in sample refractive index, buffer composition, or variations in ligand density on the sensor surface. These artifacts manifest as apparent negative binding responses, stemming from shifts in the interference pattern unrelated to molecular interaction. In the context of transmembrane proteins, specific causes of inverted signals may include differences in formulation buffer, particle size, or protein composition. As illustrated in Figure 6, the interaction between Forimtamig and GPRC5D in the VLP format produced an inverted raw sensorgram. Importantly, adjusting assay conditions—such as switching to an alternative format like GPRC5D in nanodiscs—can resolve this issue and yield standard binding curves. Moreover, the Gator Bio software is equipped to detect and computationally correct signal inversion, as demonstrated by the processed data for the same interaction shown in Figure 5.

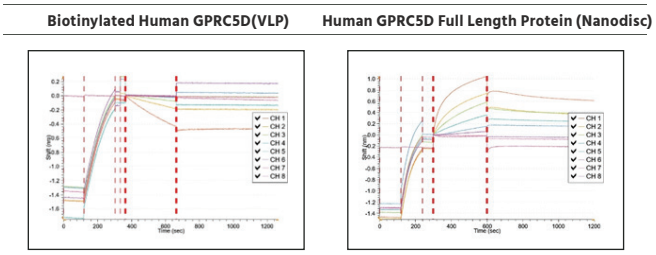

Figure 6. *Binding raw data for the Forimtamig biosimilar with GPRC5D shows an inverted association and dissociation signal when the VLP format is used (left). In contrast, the nanodisc format (right) displays the expected signal orientation.*

Signal Inversion and Data Correction in BLI Analysis

While inverted signals can present a challenge in data interpretation, the advanced analytics provided by the Gator Bio system ensure that these artifacts do not compromise the integrity of kinetic results. The software automatically corrects baseline shifts and normalizes signal orientation, enabling accurate quantification of association and dissociation kinetics. This feature is especially critical when working with membrane proteins, whose structural and compositional variability can introduce optical complexities during sensorgram acquisition.

Comparative Assessment of BLI Versus Other Bioanalytical Platforms

To contextualize the performance of the BLI assay, we conducted a comparative analysis of full-length CD20 binding to Rituximab across BLI, surface plasmon resonance (SPR), and enzyme-linked immunosorbent assay (ELISA) platforms (Figure 7). While SPR is recognized for its sensitivity and precise kinetic resolution, and ELISA for its high-throughput and ease of use, BLI offers a compelling combination of real-time kinetic measurement, flexible ligand orientation strategies, and operational simplicity. These features make BLI particularly well-suited for screening large antibody panels or evaluating membrane protein interactions in a label-free format.

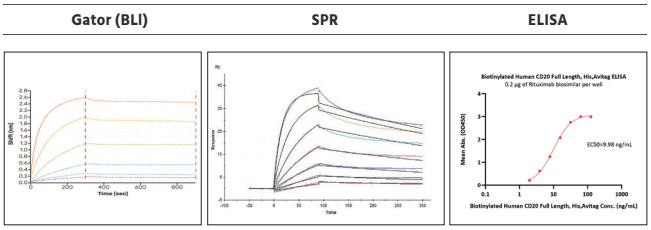

Figure 7. *Comparison of three different assays, BLI, SPR, and ELISA, for studying binding kinetics of Rituximab with detergent-solubilized CD20 membrane protein.*

CONCLUSION

The generation of full-length, biologically active multi-pass transmembrane proteins by ACROBiosystems represents a significant advancement in addressing long-standing challenges in the development of antibody-based therapeutics and CAR-T cell constructs. By employing HEK293 expression systems and preserving native protein topologies, including both large and small extracellular loops, these constructs exhibit high conformational fidelity and consistent bioactivity across assay platforms.

In parallel, the Gator Bio biolayer interferometry (BLI) platform demonstrated robust analytical performance, offering both flexibility in assay configuration and high sensitivity for kinetic characterization. The use of diverse biosensor types enabled the systematic interrogation of various receptor formats—detergent-solubilized, nanodisc-reconstituted, and virus-like particle (VLP)-displayed—revealing strong and reproducible binding profiles across all formats. These results affirm the suitability of each format for high-throughput screening applications and detailed kinetic studies.

The synergy between ACROBiosystems' engineered transmembrane targets and Gator Bio's BLI instrumentation provides a versatile and scalable solution for accelerating the discovery and optimization of therapeutic antibodies. This platform supports the identification of high-affinity candidates targeting complex membrane proteins implicated in malignancies such as lymphoma and leukemia, as well as autoimmune disorders, ultimately enabling more efficient development of next-generation biologics.

About Gator Bio, Inc.

Gator Bio, Inc. is a pioneering life sciences company dedicated to providing analytical solutions that accelerate the development of therapeutics and diagnostics. Gator instruments and biosensors enable real-time analysis of biomolecular interactions, providing critical information on affinity, kinetics, concentration, epitope binning, and more. Gator Bio's analytical capabilities significantly enhance the characterization of drug candidates and viral vector analytics, delivering greater value in drug development and gene therapy applications. The company is headquartered in Palo Alto, California.

About ACROBiosystems

ACROBiosystems Group, founded in 2010 and listed in 2021, is a biotechnology company aimed at becoming a cornerstone of the global biopharmaceutical industry by providing life science tools and business model innovation. ACROBiosystems spans across the globe and maintains over twelve offices, research & development centers, and production bases across the United States, Europe, and Asia. Numerous partnerships with well-known enterprises such as Pfizer, Novartis, and other academic institutes have been established. Through the continuous development of new technologies and tools, ACROBiosystems is dedicated towards helping their customers overcome challenges from drug discovery to commercialization through innovative life science tools and solutions.