Characterization of Biosimilars Using High-Quality Full-Length Antigens and Second-Generation Biolayer Interferometry

Harsha Agarwal[1], Anuja Patil[1], Indrani Chakraborty[1], Peter Hsueh[2], Spencer Chiang[2]
[1]Gator Bio, Palo Alto, CA, [2]ACROBiosystems, Newark, DE

INTRODUCTION

Membrane proteins, including CD20, stand at the frontier of drug discovery, offering both opportunity and challenge. As key mediators of cellular signaling and immune regulation, they comprise a large share of therapeutic targets, yet their complexity often renders them “undruggable.” CD20, a multi-pass transmembrane protein, is unstable outside native lipids, hindering structural preservation and functional assays. High-quality, full-length CD20 antigens are therefore essential for rigorous antibody assessment, from Rituximab and biosimilars to multispecifics. ACROBiosystems provides HEK293-expressed, biologically active CD20 in nanodisc, detergent, and VLP formats that maintain native conformation. Using Gator Bio’s second-generation biolayer interferometry (BLI) - a label-free, real-time platform quantifying k_{on}, k_{off}, and K_D - antibodies were quantitatively profiled for real-time binding kinetics. All CD20 formats exhibited strong, specific interactions, with the VLP format showing enhanced avidity and slower dissociation. This collaboration demonstrates an efficient, label-free workflow for high-throughput biosimilar characterization and antibody screening. The combined strengths of stabilized CD20 antigens and advanced BLI detection provide a robust, scalable solution to accelerate therapeutic antibody development and enable next-generation immunotherapies targeting CD20.

Gator Bio BLI Solutions

- Label-free, real-time kinetic analysis technology based on reflection of light on the surface of a biosensor tip: direct k_{on}, k_{off}, K_D from sensorgrams
- The shift in interference pattern plotted against time when a molecule is bound
- The change in pattern is proportional to the number of biomolecules bound
- Minimal hands-on time
- Wide applications ranging from protein-protein interactions, therapeutics development and viral
- Detergent-tolerant & format agnostic: works with nanodisc-, detergent-solubilized-, and VLP-CD20
- Allows simultaneous testing of up to hundreds of drug candidates against CD20, accelerating discovery workflows

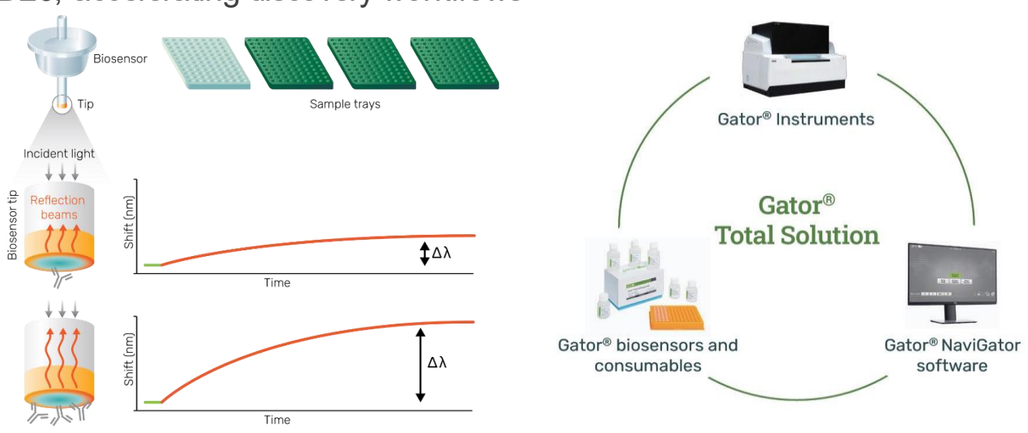


Figure 1: Gator® next-generation BLI, a versatile real-time analysis platform solution for label-free analysis

ACROBiosystems’ Solutions for Multi-Pass Transmembrane Proteins (TPs)

- Established specialized technology platforms to overcome structural challenges of multi-pass TPs.
- Provides full-length TPs with native folding to support antibody characterization and mechanism of action studies.
- Meets diverse application needs in drug development and research.

PLATFORM	PRINCIPLE	ADVANTAGES	APPLICATIONS
VLP (Virus-Like Particle)	Expresses TPs on the host cell surface using the HEK293 expression system.	Displays full-length, correctly folded multi-pass TPs	Immunization
	Converts cell surfaces into soluble lipid bilayer particles with high TP concentration	Higher abundance compared to overexpressing cells	ELISA
	Expresses TPs on the host cell surface using the HEK293 expression system.	High immunogenicity	SPR/BLI
		Size (100-300 nm) suitable for dendritic cells and phage display *in vivo*	Cell experiments
		Ideal for antibody screening and immunization	CAR detection
Detergent Micelle Platform	Transmembrane regions are stabilized in detergents (e.g., DDM/CHS) to maintain correct folding post-extraction from the membrane	TPs retain complete conformation	Immunization
		Accurate quantification possible	ELISA
		Suitable for various assays	SPR/BLI
Nanodisc Platform	Synthetic phospholipid bilayer composed of membrane scaffold proteins (MSPs) and phospholipids	Detergent-free formulation improves hydrophilicity	CAR expression tests
	TPs are integrated into the nanodisc, retaining native folding and biological activity	Retains native folding and biological activity	Immunization
		Optimized for industrial scale-up production	ELISA
		Long-term stability ensured for large-scale applications	SPR/BLI

Table 1 : Summary of full-length TPs with native folding to support antibody characterization and mechanism of action studies

Lionel Rougé, et al. Structure of CD20 in complex with the therapeutic monoclonal antibody RTX. Science. 2020

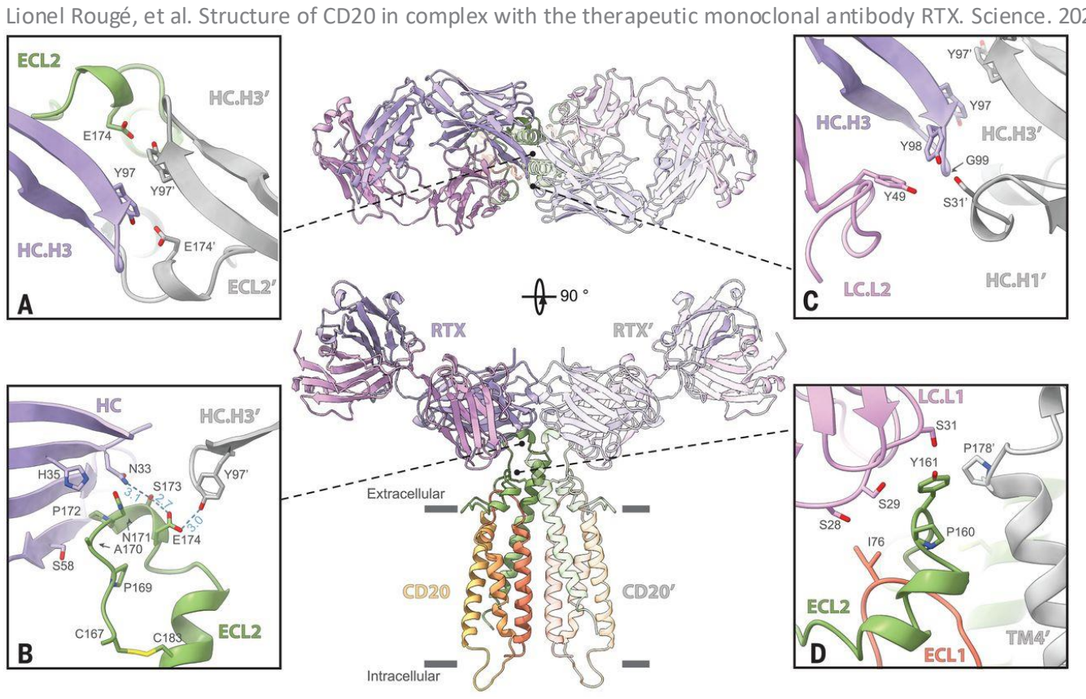


Figure 1. Key molecular binding interactions between CD20 and Rituximab (purple).

RESULTS SUMMARY

- Representative sensorgrams for the biosimilars binding to membrane proteins are shown in **Table 2**.
- With a ready-to-use system and a toolbox of biosensors available, capturing membrane proteins or antibodies of interest is straightforward.
- **SA XT biosensors:** Used to capture both nano-disc-based and detergent-based membrane proteins.
- **Anti-HFC1 sensors:** Used to capture the two full-length antibody.
- **Anti-human FAB biosensors**: Specifically used to capture Ibritumomumab, as it is an F(ab’)2 molecule.

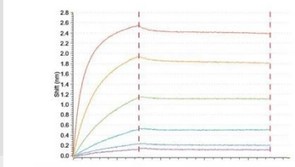
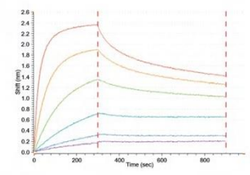
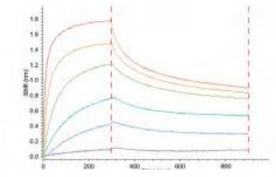
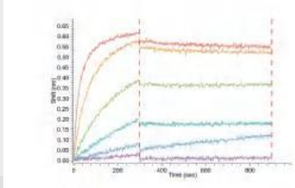
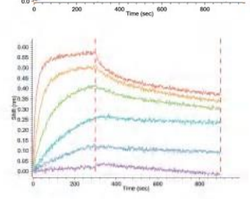
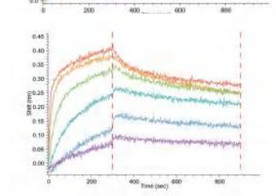
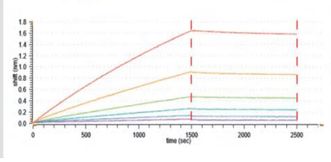
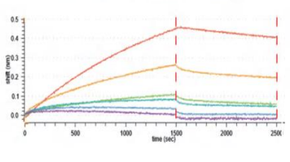
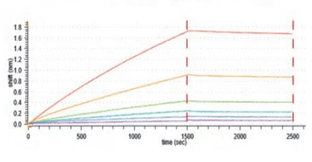
	Rituximab	Tositumomab	Ibritumomab
Biotinylated Human CD20 (Detergent)			
Biotinylated Human CD20 (Nanodisc)			
Human Full-Length CD20 (VLP)			

Table 2: Kinetic summary of binding responses against anti-CD20 monoclonal (mAb) against full-length CD20 in detergent, nanodisc, and VLP formats from ACROBiosystems.

- Nanodisc and detergent formats exhibit similar kinetic profiles.
- VLP format demonstrates an avidity profile with a much slower dissociation rate, constate.
- Robust binding across all three formats – nanodisc, detergent-solubilized, and VLP
- Screening suitability: Any of these receptor formats is suitable for screening large panels of therapeutic candidates to identify valid binders.

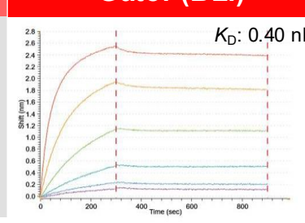
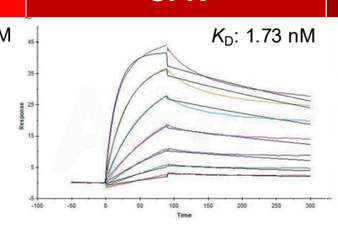
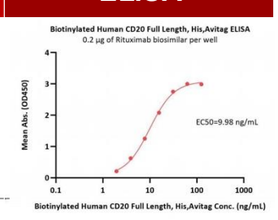
Rituximab	Gator (BLI)	SPR	ELISA
Biotinylated Human CD20 (Detergent)			

Table 3: Comparison of full-length CD20 binding to Rituximanb in different bioanalytical assay formats

CONCLUSION

- **Solutions for TPs:** CD20 is a critical therapeutic target for lymphoma, leukemia, and certain autoimmune diseases, but low expression and unstable bioactivity have hindered access to reliable, active protein for antibody and CD20 CAR-T development. ACROBiosystems provides highly active, full-length, multi-transmembrane CD20 (HEK293) that preserves both large and small ECD loops and shows high, specific binding in ELISA, SPR, and BLI, delivering dependable reagents.
- **Advanced instrumentation:** Gator Bio’s second-generation BLI platform enables label-free, high-throughput characterization with a versatile biosensor toolbox for both screening and detailed kinetics (k_{on}, k_{off}, K_D).
- **Integrated impact:** Together, ACROBiosystems’ CD20 and Gator Bio’s BLI provide robust end-to-end workflow that accelerates CD20 therapeutic discovery and engineering.