High-resolution affinity datasets for assessing mutation-driven loss of efficacy and epitope-driven efficacy of Ebola therapeutics

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ABSTRACT

Introduction: Inmazeb and Ebanga are the primary FDA-approved antibody-based therapeutics for Ebolavirus (EBOV). While Zmapp was simultaneously developed against the same EBOV Zaire glycoprotein (GP), clinical trials failed, showing lower efficacy attributed to differences in epitope targeting. Current assays lack sufficient throughput and resolution to identify and predict how therapeutic candidates will be affected by mutations based on their binding affinities and epitope specificities. Herein, we utilize a novel high-resolution and high-throughput SPOC SPR assay for high-resolution affinity screening of EBOV Zaire GPs versus anti-GP antibodies.

Capability Description: SPOC synthesizes up to 1152 antibodies (mAb, Fab, scFv, VHH) on a single 1.5 sq-cm chip for surface plasmon resonance (SPR) analysis. Our proprietary Protein NanoFactory production system utilizes direct-from DNA synthesis in nanowells, with simultaneous capture-purification onto SPR chips. This enables rapid, high-throughput therapeutic discovery workflows spanning affinity ranking and maturation, epitope binning and mapping, polyreactivity, and developability analysis (thermal, pH, and oxidative stress).

Methods/Technical Approach: In this study, we synthesized on-SPOC chips anti-EBOV GP antibodies from Inmazeb, Ebanga, and Zmapp and screened against multiple Zaire strain GPs to obtain kinetics metrics (on-rate, off-rate, affinity, and residence time) and epitope bins. The preliminary data is benchmarked versus published literature and clinical efficacy data.

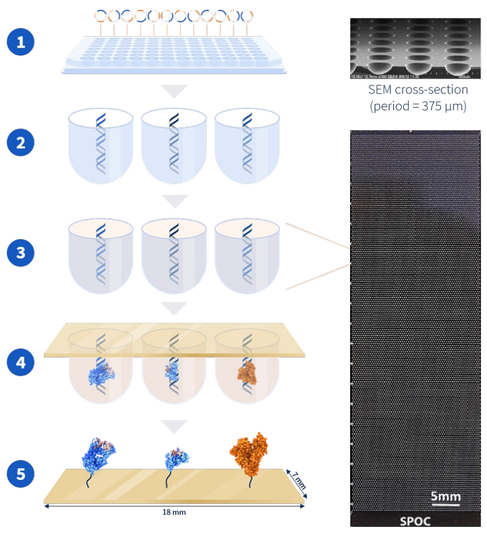
Applicability to Roles of Medical Care: SPOC supports medical countermeasure (MCM) rapid discovery for new and emerging pathogens and their variants – supporting full discovery workflows in a few weeks versus months. With high-resolution datasets, SPOC can support the development or recovery of therapeutics more resistant to mutations and better predict clinical efficacy, saving time and cost of expensive clinical trials.

Impact to Warfighter/Significance: Ultimately, SPOC envisions these high-resolution datasets supporting an AI model which has fully details contact maps between antibody therapeutics and pathogens. This will enable more advanced MCMs with mutation resistance and faster delivery to the field, saving lives.

Developmental Status of the Technology: SPOC technology is TRL8/9, available commercially with a panel of biologics discovery assays.

SENSOR-INTEGRATED PROTEOME ON-CHIP (SPOC®) TECHNOLOGY OVERVIEW

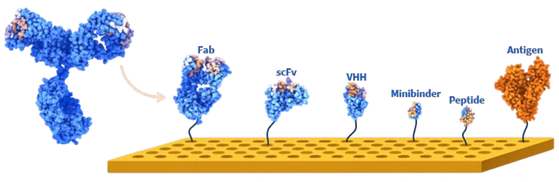
1) Protein NanoFactory™ for biosensor production



Single-step protein production, purification & capture onto biosensor chips:

1. DNA library prepared in microplates
2. DNA is dispensed in silicon nanowells
3. Nanowells filled with cell-free lysate (human, *E. coli*, other)
4. Wells sealed against biosensor chip (SPR, MALDI MS) and synthesized proteins captured as a library of '**pure protein spots**' on the chip surface
5. Chip used for bioanalysis or shipped to customer

- ✓ Rapid customization
- ✓ Reduces cost by 10x from traditional CRO methods
- ✓ 4 week turn around time, including DNA synthesis

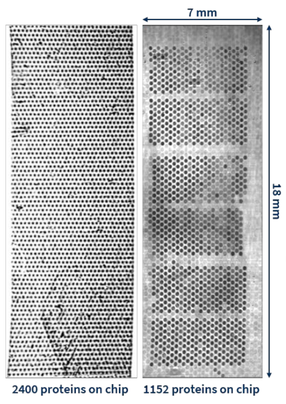


Up to 1152 on-chip produced antibodies/proteins

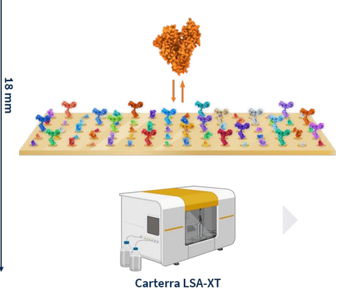
Compatible formats:

- Antibodies: scFvs, VHHs (nanobodies), Fabs, mAbs
- Peptides, mini-proteins
- Soluble/secreted proteins
- ECDs of membrane proteins
- Intracellular proteins
- Membrane proteins in nanodiscs (under development)

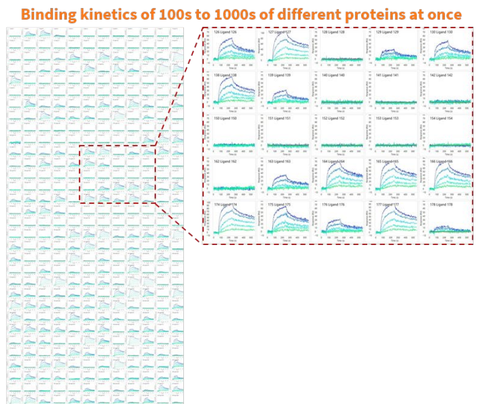
2) High-throughput kinetic screening by SPR



Simultaneous measurement of binding kinetics across the whole SPR biosensor surface. Each sensorgram = unique protein spot.



Binding kinetics of 100s to 1000s of different proteins at once

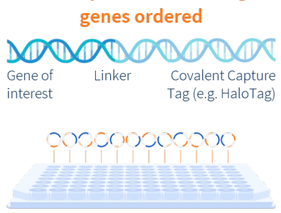


- SPOC is the only proteomics platform capable of producing **quantitative, qualitative, and kinetic (K_D, k_a, k_d, $t_{1/2}$) data in a single assay** at scale.
- Using proprietary software, SPOC can simultaneously and independently analyze binding interactions for thousands of proteins using **real-time kinetic SPR sensing to collect data within 5-30 minutes**.
- Detect biomolecules down to **500 Daltons**.

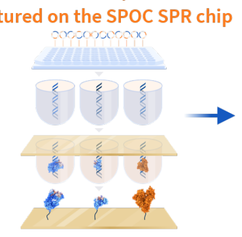
3) SPOC Workflow: From Sequence to SPR Data in 3-4 Weeks

Customer provides peptide/protein/Ab sequences

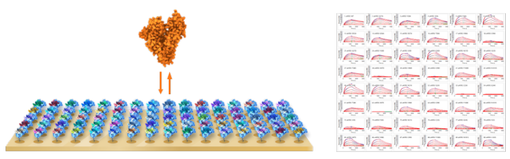
Codon-optimized, full-length genes ordered



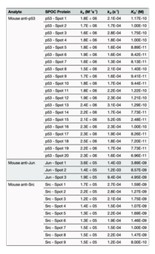
Proteins are expressed & captured on the SPOC SPR chip



All sequences are tested simultaneously in Carterra LSA-XT



Full SPR kinetics data (k_{on}, k_{off}, K_D, $t_{1/2}$, R_{max}) delivered in multiple formats, such as CSV, JSONL, Parquet, and HDF5, in addition to the raw data.



MATERIALS: ANTIBODIES & EBOV STRAINS

Antibodies – Therapeutic Candidates

Inmazeb cocktail (FDA approved, 2020)
- Atoltivimab
- Maftivimab
- Odesivimab

Zmapp cocktail (RUO)
- Cosfroviximab
- Larcaviximab
- Progaviximab

Ebanga (FDA approved, 2020)
- Ansuvimab

EBOV Strains

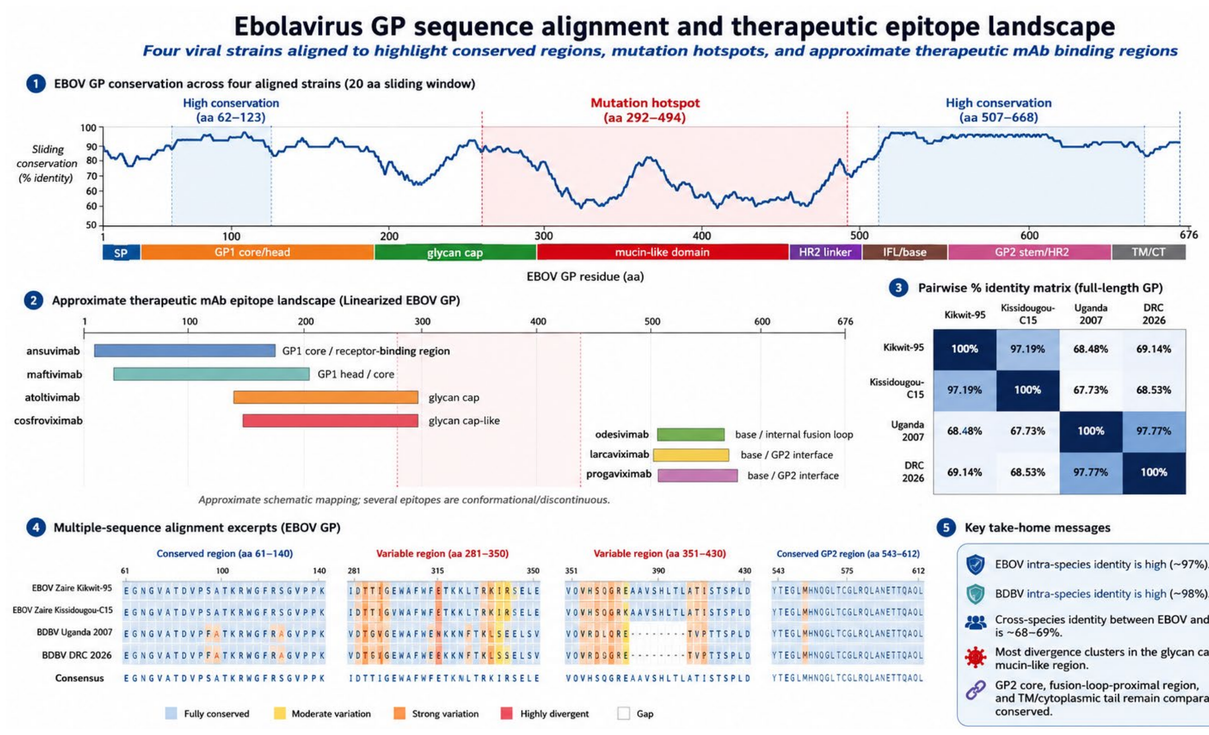
EBOV
- Zaire Kikwit-95 GP
- Zaire Kissidougou-C15 GP

BDBV
- Uganda 2007 GP
- DRC 2026, rVSV-GP

There are seven EBOV Zaire GP-targeting mAb therapeutic candidates or FDA-approved drugs that were selected for cell-free expression with SPOC's Protein NanoFactory system for SPR analysis.

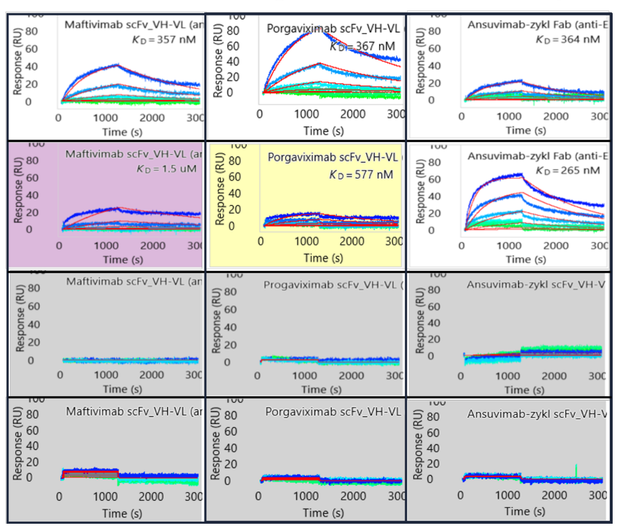
From real-world data, it is known that these drugs cannot neutralize or prevent infection from BDBV.

Our goal was to characterize binding kinetics of the seven candidates versus 2 EBOV and 2 BDBV strains to demonstrate the ability to perform rapid *in vitro* screening for loss of efficacy against emerging viruses or variants and inform novel therapeutic design.

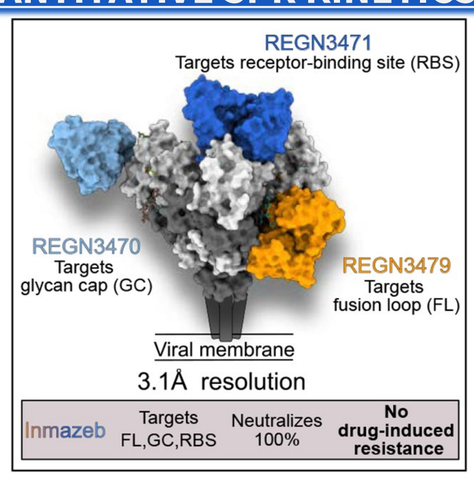


DRUG EFFICACY EVALUATION THROUGH QUANTITATIVE SPR KINETICS

(A)



(B)



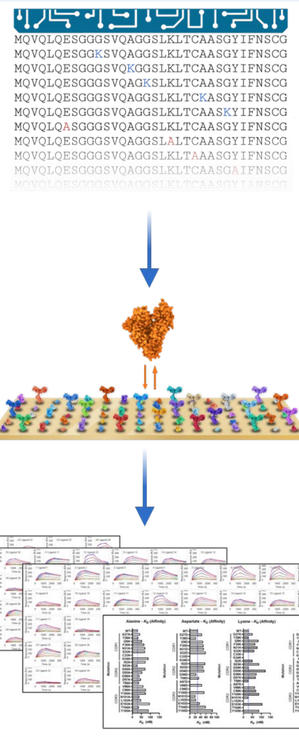
(C)

	Maftivimab (scFv) Inmazeb	Porgaviximab (scFv) Zmapp	Ansuvimab (Fab) Ebanga
Zaire, Kikwit-95	357 nM	367 nM	264 nM
Zaire, Kissidougou-C15	1.5 uM	577 nM	265 nM
BDBV, Uganda 2007	N.D.	N.D.	N.D.
BDBV, DRC 2026	N.D.	N.D.	N.D.

(A) SPR sensorgrams of 3 scFv and Fab format constructs from the 7 EBOV therapeutic candidates, one from each cocktail, with 6-concentration titration curves for affinity calculations. Grey indicates non-binder, yellow indicates sub-optimal 1:1 binding model fit, purple indicates analyte too low/slow off-rate.
(B) Structure of Inmazeb cocktail on EBOV GP demonstrates recognition of three unique epitopes to achieve full neutralization. Image taken from Rayaprolou et al, Cell Host Microbe 2023.
(C) Left, table of calculated and reported K_D values across the three functional constructs versus each strain GP. N.D. = not detected

- A total of seven constructs were expressed on-SPOC-chip as scFvs in both VH-VL and VL-VH orientations and as Fabs. All constructs were then screened against 6 concentrations for each of the four strains with regeneration in between – **enabling us to screen multiple targets on the same chip with quantitative kinetics data overnight.** Results shown in (A).
- Three of the seven constructs bind both Zaire strains with similar affinity. Affinity results shown in (B). The remaining 4 Abs are being further optimized for expression and screening on the SPOC platform.
- Our team will also be validating the EBOV and BDBV commercial GP targets versus commercially sourced mAb drug molecules.
- Of the 3 validated constructs, none exhibit cross-reactive binding to the BDBV strains. This may be due to the mutations, recombinant antigen structure, or screening conditions, which will be explored further in future assays. **The complete loss of binding affinity is therefore detectable through *in vitro* SPR screening assays of existing drug molecules immediately upon the identification of a new strain or variant, allowing proactive recognition of loss of efficacy without the need for neutralization assays. Such data can be used to train AI/ML models to predict loss of drug efficacy versus future predicted variants and proactively develop new therapeutics.**

BUILD MUTATION-RESISTANT THERAPEUTICS WITH HIGH-RESOLUTION PATHOGEN IQ DATASETS FOR AI/ML MODEL TRAINING



PathogenIQ

Library-on-library screening, amino acid resolution structural data for AI/ML model training

1. 1,152 Antibody variant library is synthesized on the SPR biosensor chip.
2. Up to 100 pathogen antigen alanine-scan variants are injected onto the surface one by one.
3. Full matrix of kinetics data are captured for all antibody variants against all antigen variants.

Max Output: 1,152 antibody variants x 100 antigen variants = **115,200 data points in a single experiment**

- Detailed structural information on how each residue affects binding, both on paratope (MCM) and epitope (pathogen).
- AI provides insights into how to develop mutation-proof therapeutics, resistant to future emerging variants.
- Fully trained AI model enables prediction of MCM loss of efficacy for new pathogens.

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We are now offering scFv/VHH/Fab libraries on SPOC chips with in-house screening services.

Contact us today to learn more about this early access program!

PUBLICATIONS

Read our pre-print on bioRxiv to learn more about antibody fragment libraries on SPOC SPR chips

Read our technology overview in Nature Communications Biology

Read our Biomolecules paper discussing detection of fast off-rate binders

Read our pre-print on bioRxiv to learn more about target mutational scanning screening with SPOC