Antibody Engineering & Therapeutics Europe

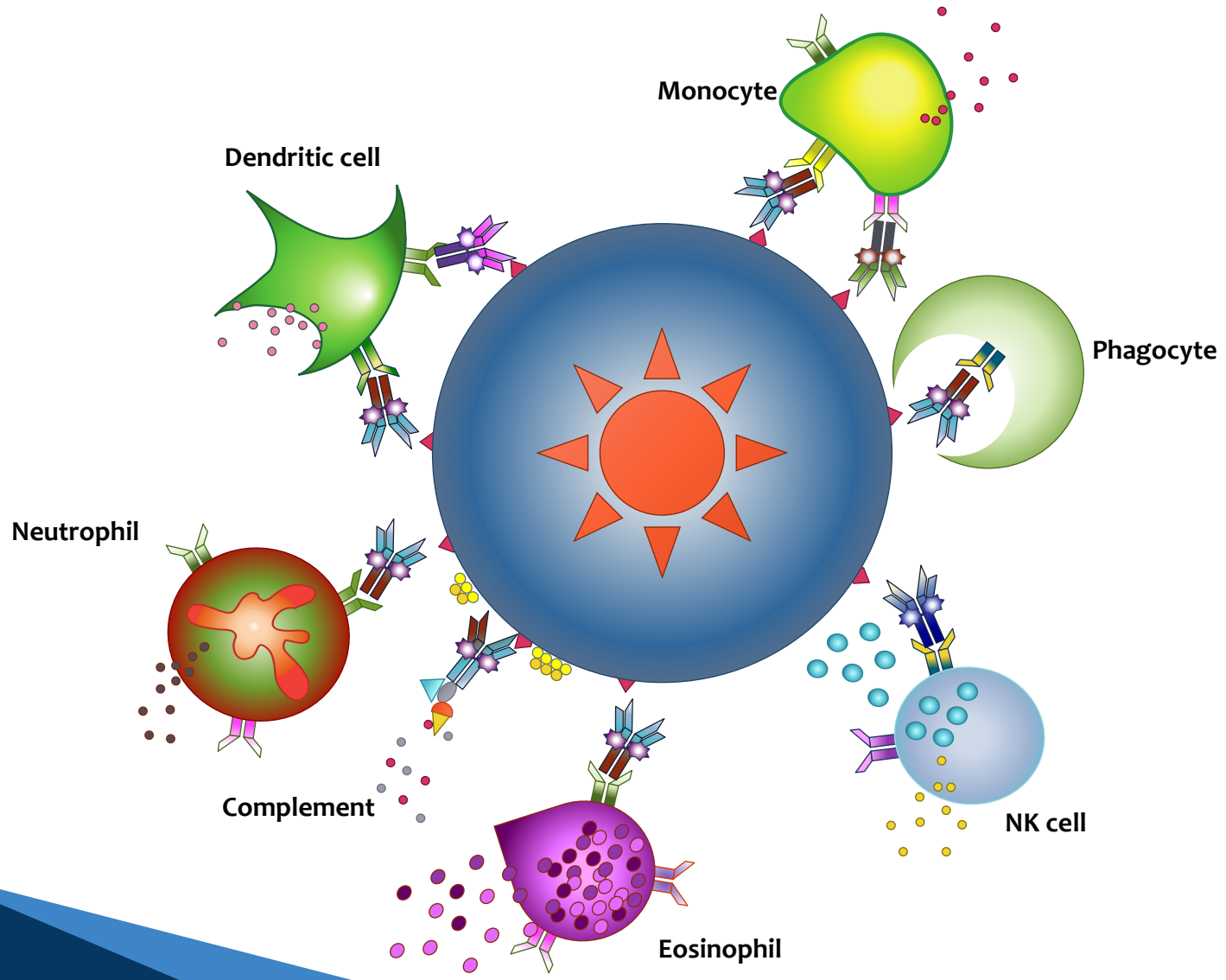
Comprehensive High Throughput Fc Effector Function Profiling as a Strategy for Improved Efficacy and Safety of Antibody Therapeutics

Shashi Jatiani, PhD
Director, Research
SeromYx Systems, Inc.

Scientific Briefing 2
05 June 2024

SeromYx SYSTEMS

The physiologic immune complex is tripartite



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Robust Fc effector function assays exist

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

A versatile high-throughput assay to characterize antibody-mediated neutrophil phagocytosis

Christina B. Karsten[a,1], Nickita Mehta[a,1], Sally A. Shin[a], Thomas J. Diefenbach[a], Matthew D. Slein[a], Wiktor Karpinski[a], Edward B. Irvine[a,b], Thomas Broge[a], Todd J. Suscovich[a], Galit Alter[a,*]

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[b] Harvard T.H. Chan School of Public Health, 677 Huntington Ave, Boston, MA 02115, USA

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Research paper

A high-throughput, bead-based, antigen-specific assay to assess the ability of antibodies to induce complement activation☆

Stephanie Fischinger[a,b], Jonathan K. Fallon[a], Ashlin R. Michell[a], Thomas Broge[a], Todd J. Suscovich[a], Hendrik Streeck[b], Galit Alter[a,*]

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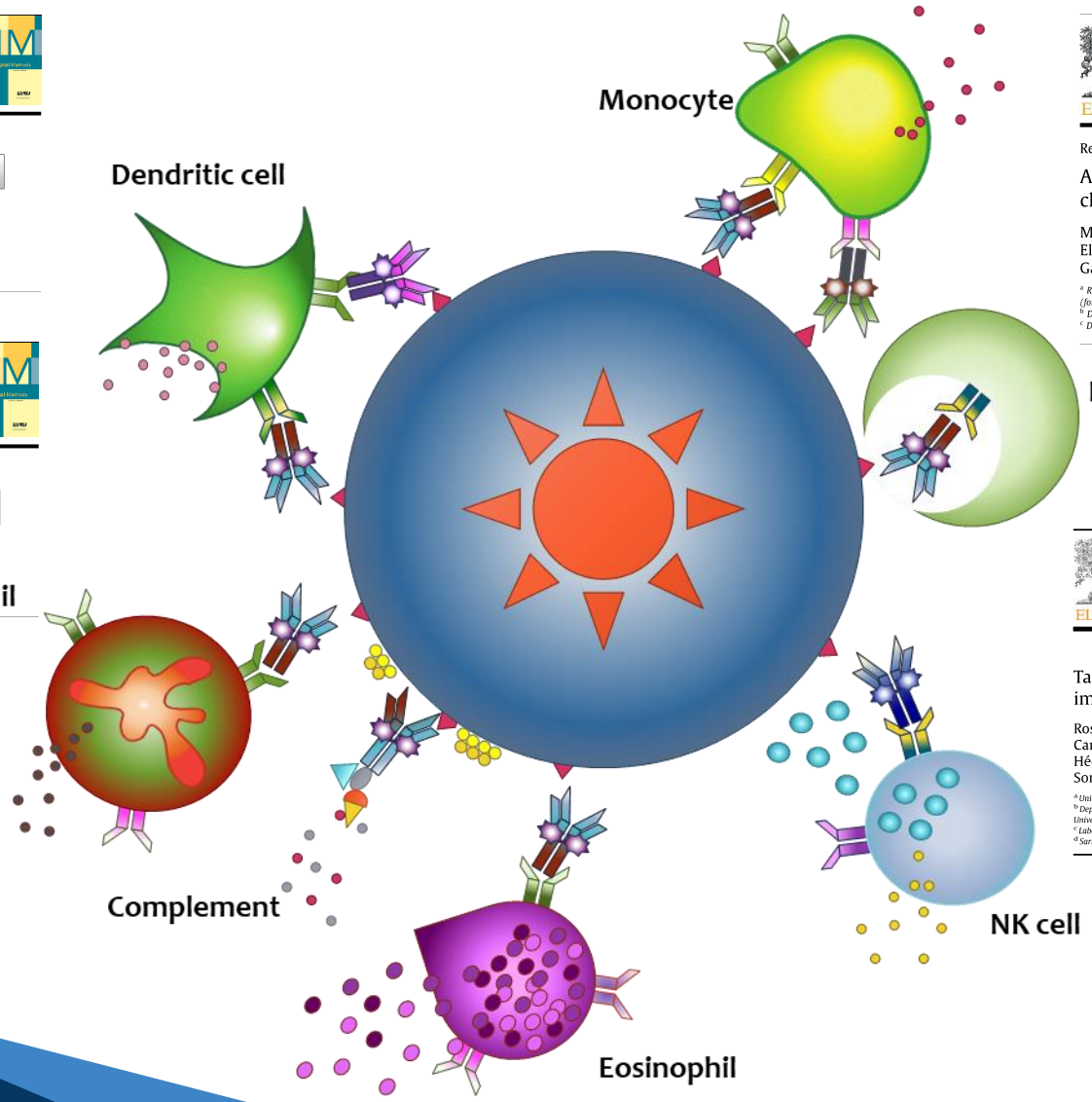
[b] University of Duisburg-Essen, Essen 47057, Germany



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Journal of Immunological Methods

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Research paper

A robust, high-throughput assay to determine the phagocytic activity of clinical antibody samples

Margaret E. Ackerman[a,*], Brian Moldt[b,1], Richard T. Wyatt[b], Anne-Sophie Dugast[a], Elizabeth McAndrew[a], Stephen Tsoukas[a], Stephanie Jost[a], Christoph T. Berger[a], Gaia Sciaranghella[a], Qingquan Liu[a], Darrell J. Irvine[a,c], Dennis R. Burton[a,b], Galit Alter[a]

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[b] Department of Immunology and Microbial Science and IAVI Neutralizing Antibody Consortium, The Scripps Research Institute, La Jolla, CA, United States
[c] Departments of Biological Engineering and Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, MA, United States

Contents lists available at ScienceDirect

Biotechnology Reports

journal homepage: www.elsevier.com/locate/btre

Taking advantage of a high-throughput flow cytometer for the implementation of an ADCC assay for regulatory compliance

Rosa Camacho-Sandoval[a,1], Alexis Jiménez-Uribe[a,1], Alejandra V. Tenorio-Calvo[a], Carlos A. López-Morales[a], Leslie Muñoz-García[a], Alejandra Montes-Luna[a], Héctor Leonardo García-Xolalpa[d], Marco Velasco-Velázquez[b], Lenin Pavón[c], Sonia Mayra Pérez-Tapia[a,*], Emilio Medina-Rivero[a,*]

[a] Unidad de Desarrollo e Investigación en Bioprocesos, Escuela Nacional de Ciencias Biológicas. Instituto Politécnico Nacional, Ciudad de México, Mexico
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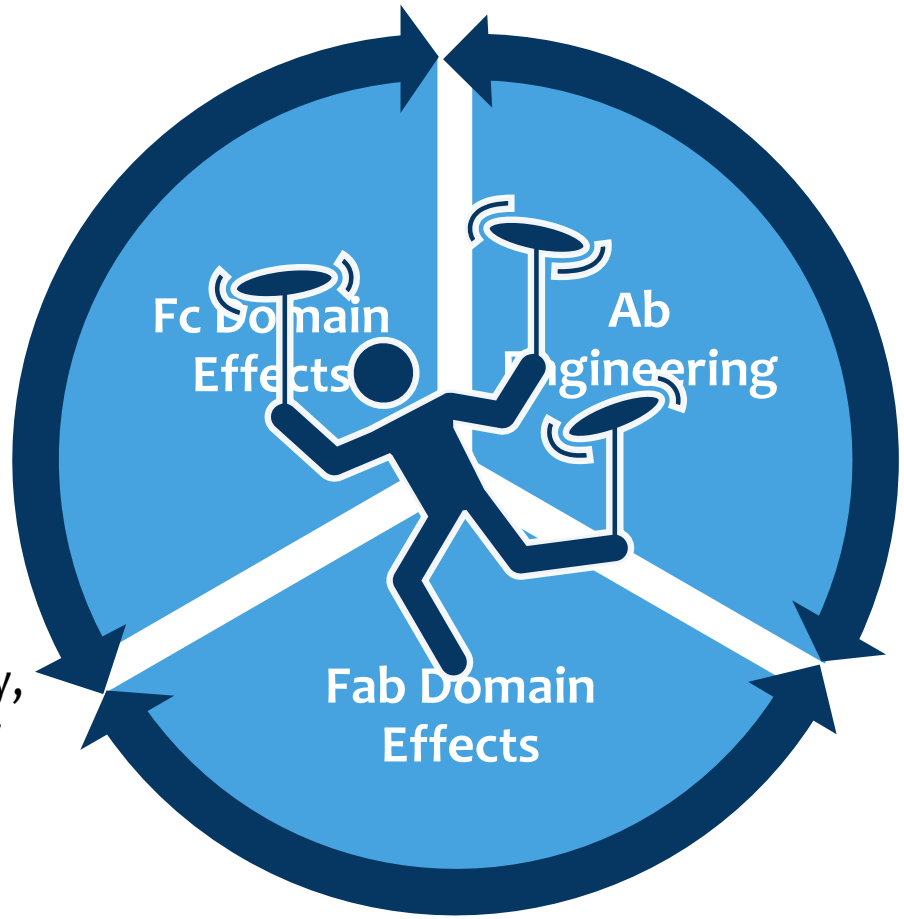
Understanding, optimizing and predicting function is a delicate balancing act

Fc Domain Effects

- Isotype/Subclass
- Glycosylation

Fab Domain Effects

- Affinity
- Valency
- Epitope specificity, binding geometry
- Fab/Fc allostery



Ab Engineering

- Affinity maturation
- Targeting multiple epitopes
- Modulating antibody valency

- Driving IgG self-assembly
- Tuning effector function by target location
- Modifying complement binding
- Modifying FcγR binding
- Extending half-life

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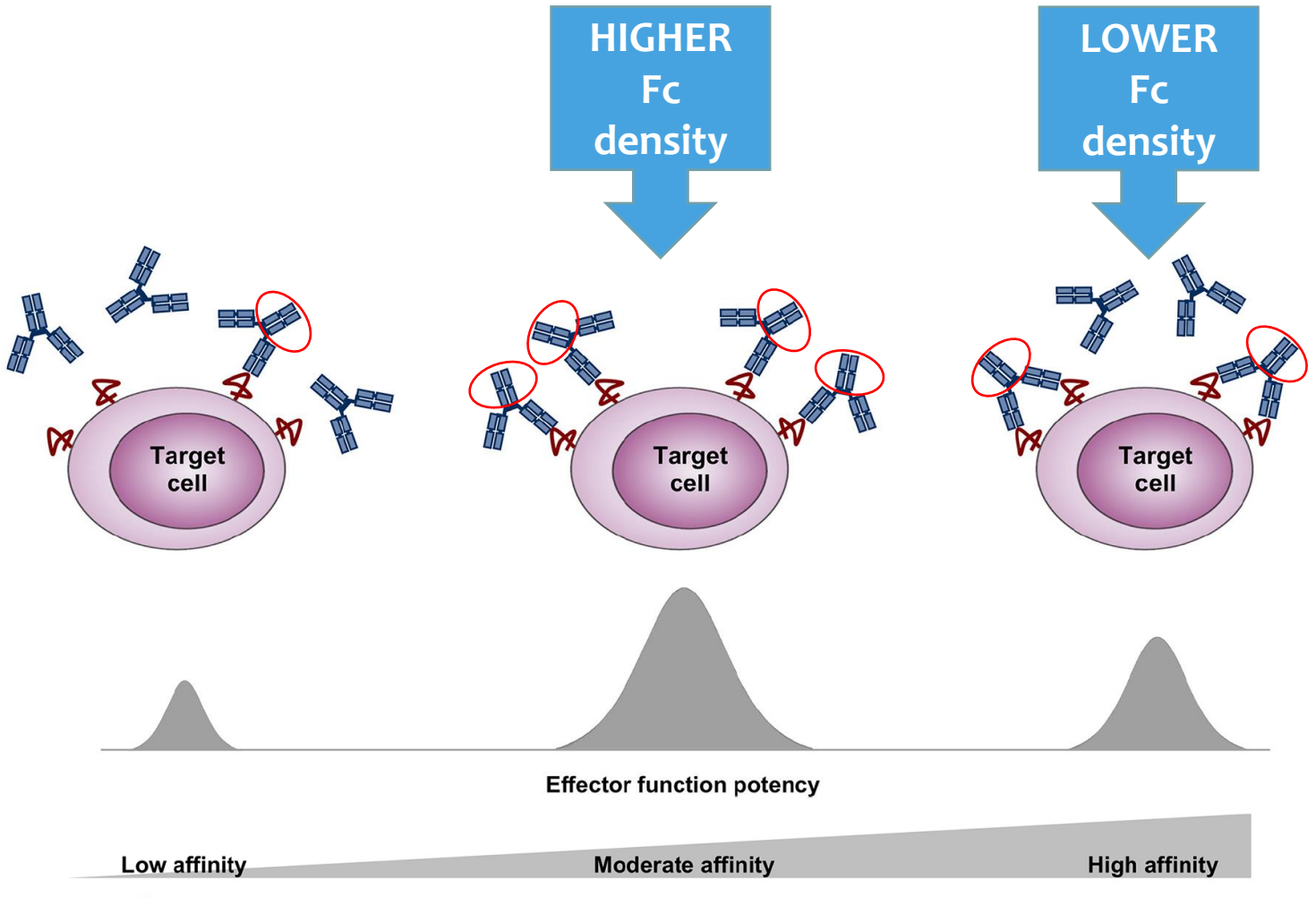
Fc effector function is nuanced

More than just bipartite mAb-Antigen affinity



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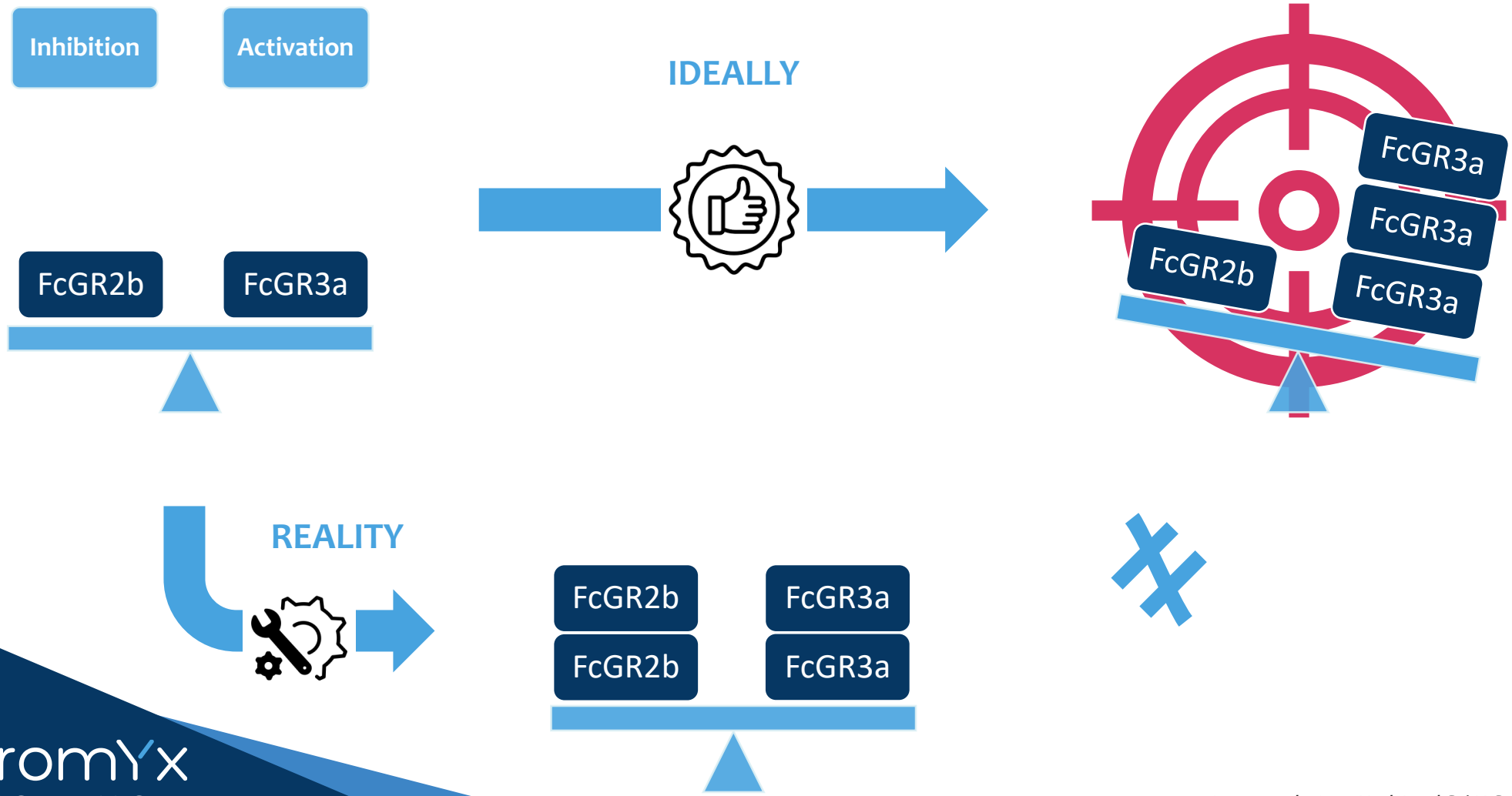
Mazor et al. PLoS One. 2016;11(6):e0157788.
Wang et al. MAbs. 2020;12:1690959.

Fc modifications that target specific FcγRs can inadvertently impact binding to other FcγRs and product efficacy



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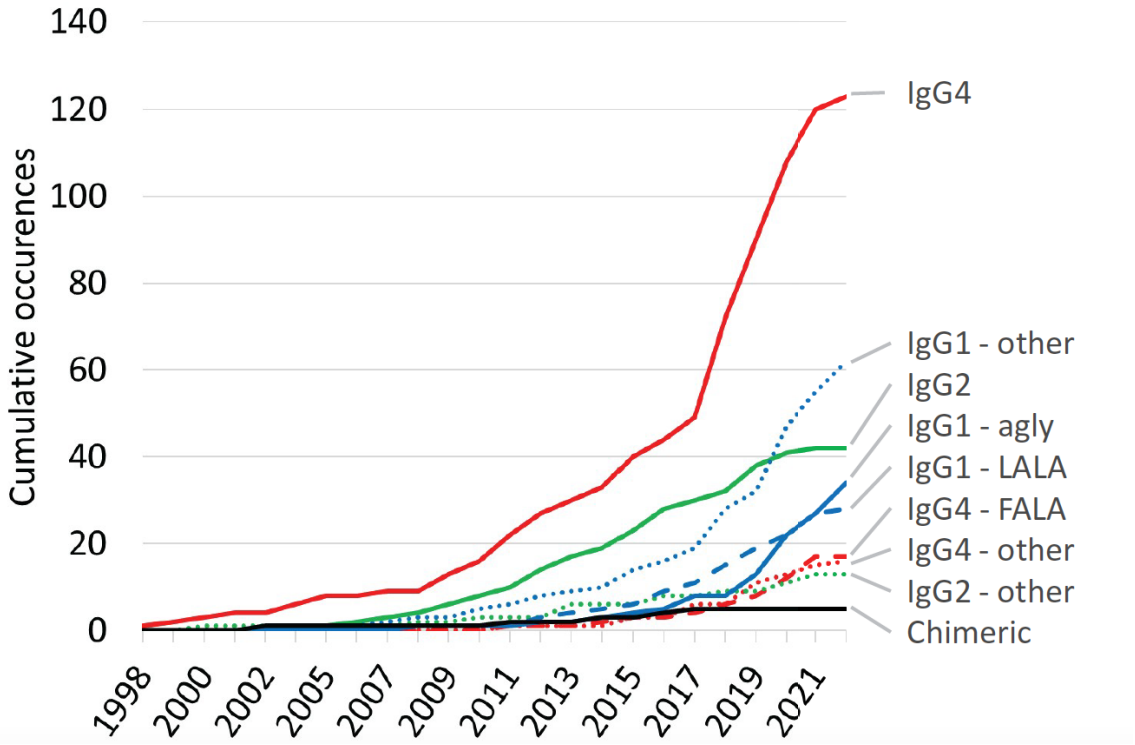
Lazar et al., Proc Natl Acad Sci U S A. 2006;103:4005-10.

Fc effector function silencing is a major therapeutic focus



- 339/756 (44.8%) of clinical stage Abs are designed for reduced effector function
  - 36.2% IgG4
  - 12.4% IgG2
  - 51.4% contain mutations to reduce FcγR binding
- Includes 49 different Fc variants designed to reduce effector function
- Increased trend since 2007 to develop antibody-based products with reduced effector function

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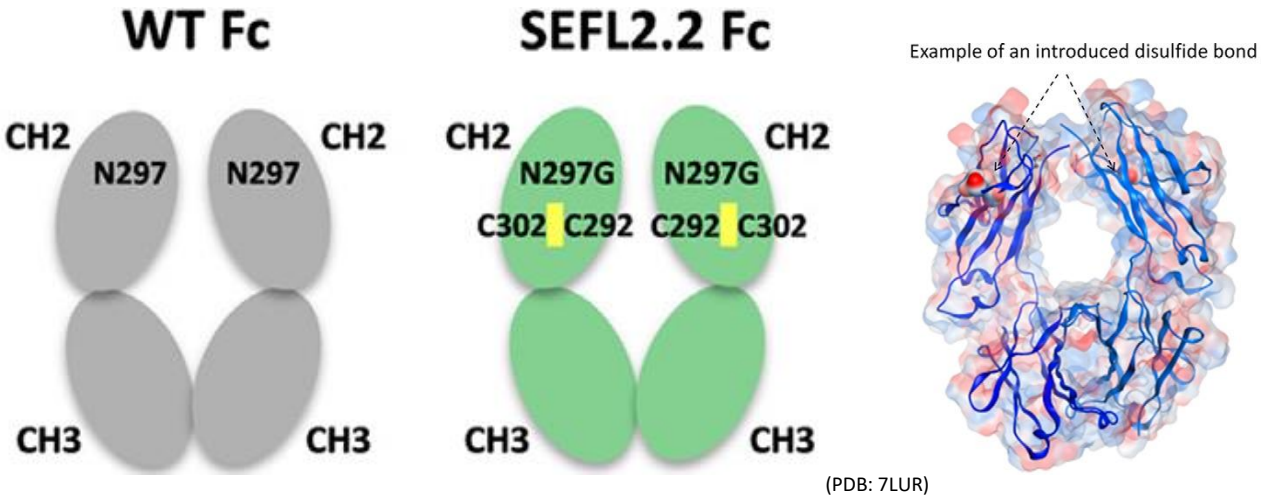
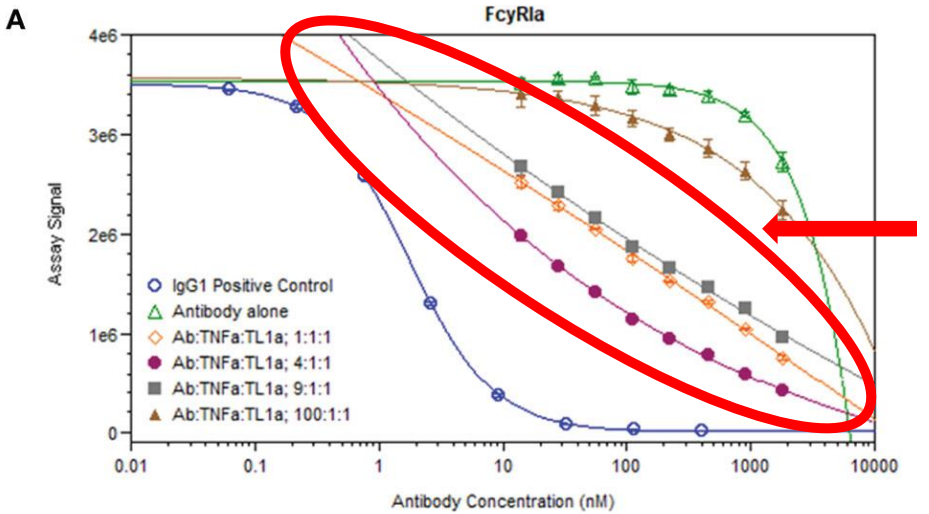
Wilkinson I, Hale G. MAbs. 2022;14:2123299.

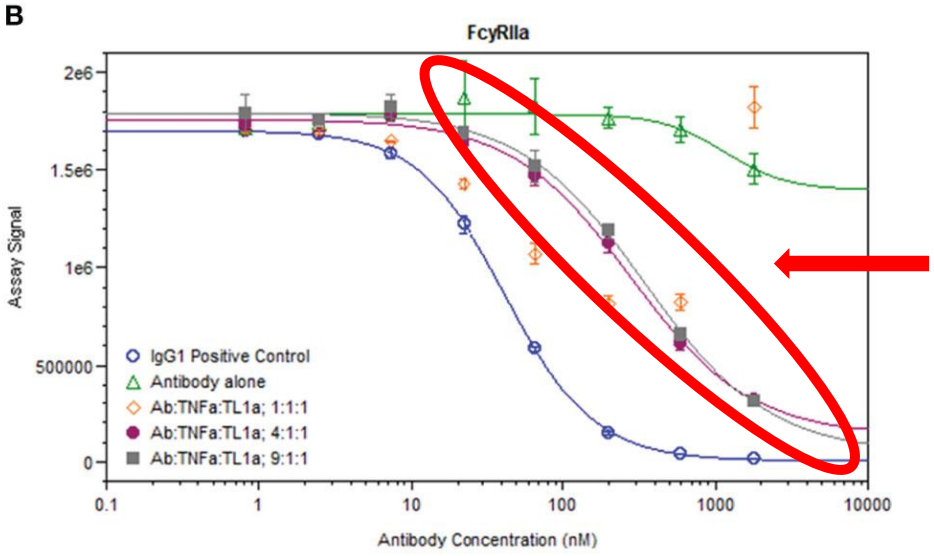
Silencing mutations may not always prove effective

The need for tripartite binding to truly assess silencing



SEFL: stable effector functionless Fc (Amgen)
Complete Fc silencing attempted via stabilizing the non-glycosylated N297G variant by introducing a novel engineered disulfide bond at a solvent inaccessible CH2 location.





Estes B et al. iScience. 2021;24:103447.
Jacobsen FW et al. J Biol Chem. 2017;292:1865-1875.
Kroenke MA et al. Front Immunol. 2021;12:782788.

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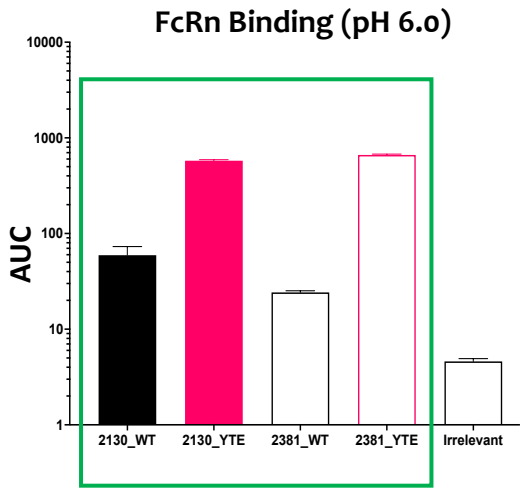
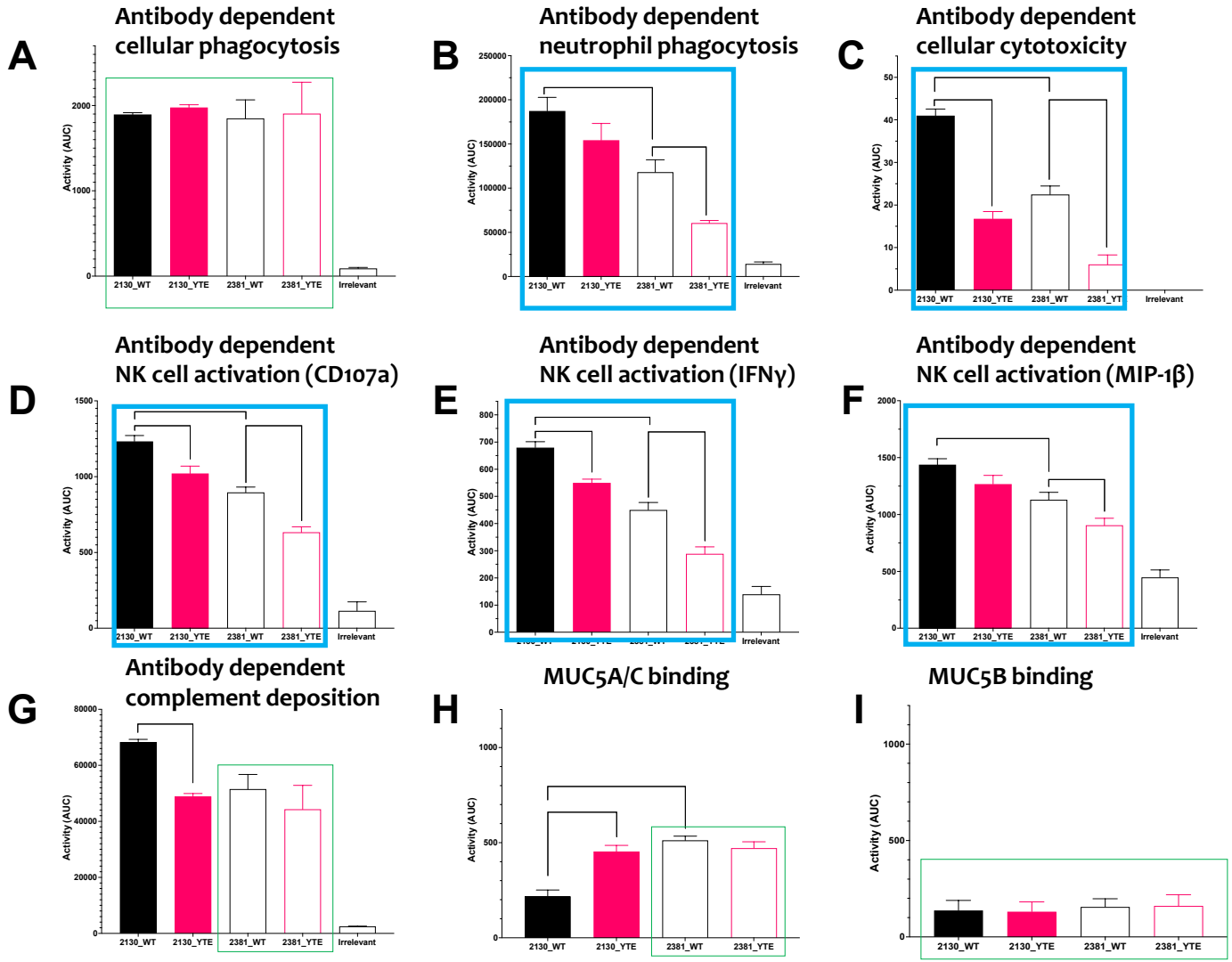
Anti-SARS-CoV-2 Spike mAbs engineered for t=1/2 extension:

Unintended reductions in ADCC, NK cell activation and ADNP





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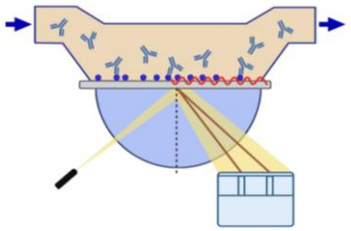
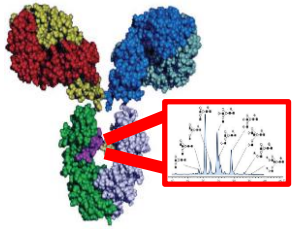
House et al., PLoS One. 2022;17:e0267796.

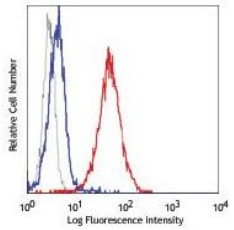
SeromYx Platform: Broad characterization of therapeutic antibody effector function



Surface Plasmon Resonance



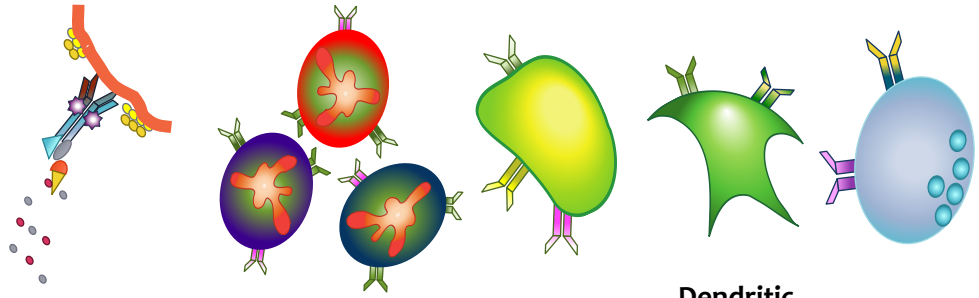
Glycan Analysis



Fc-receptor Binding

Biophysical assays

SPR, Antibody Fc receptor binding, Antibody glycosylation



Complement | Granulocytes | Monocyte | Dendritic cells | NK cell

10 functional assays

ADCP, ADCC, CDC, ADNKA, ADEP, ADBP, ADCD, ADNP, ADDCP, ADMB

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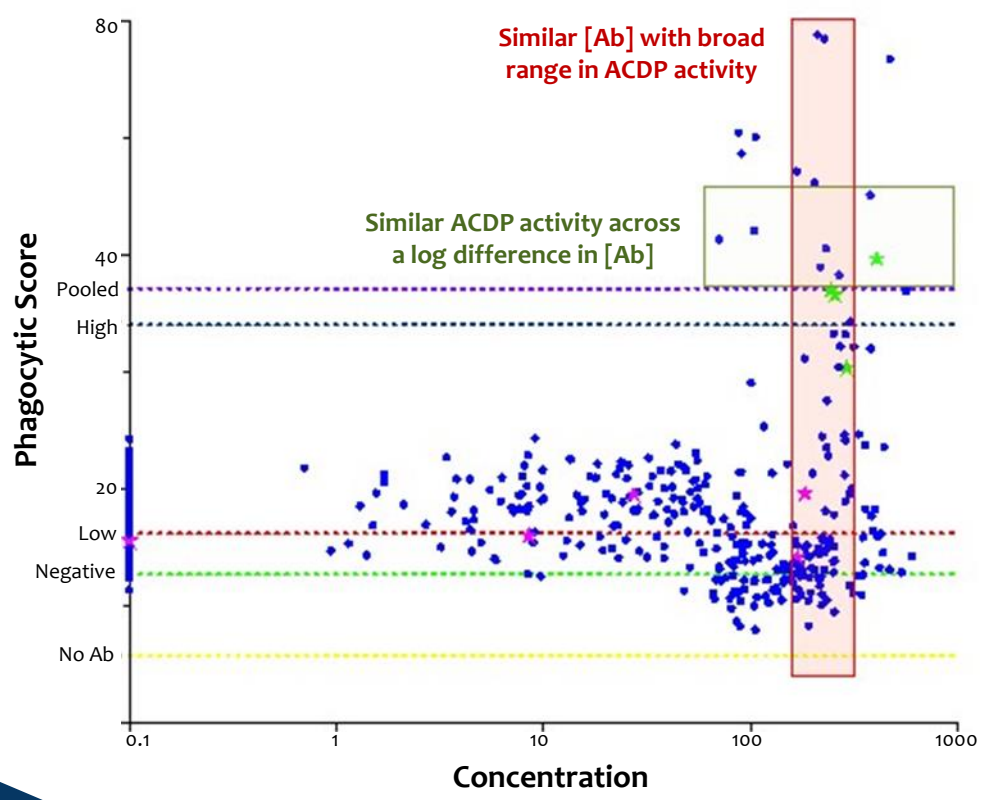
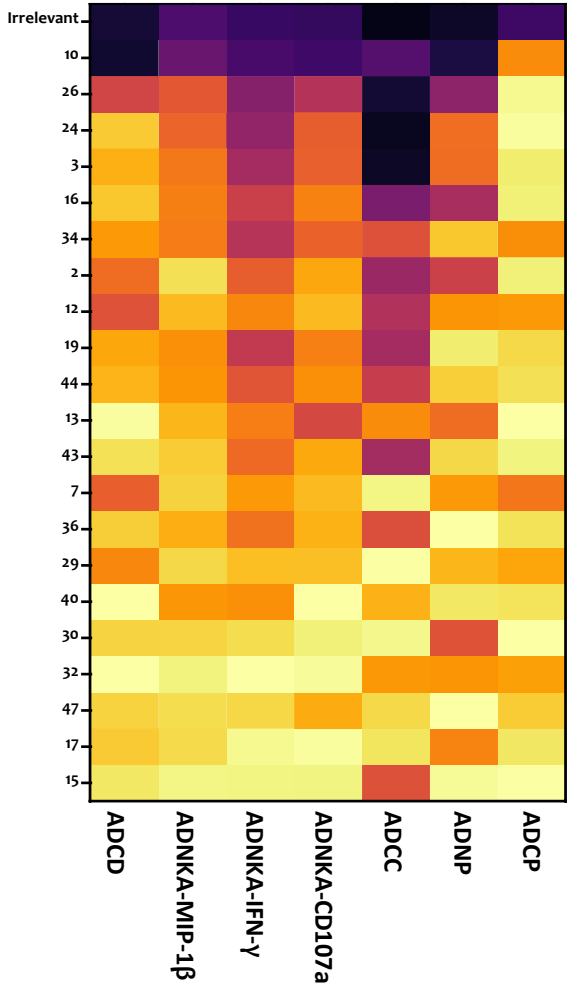
Constant Fc: Variations in Effector Function

A mAb is more than the sum of its independently assessed Fab and Fc

Phagocytic activity of 598 mAbs with the same Fc but targeting different epitopes of a single target antigen



Functions of a panel of clinical mAbs containing an identical Fc



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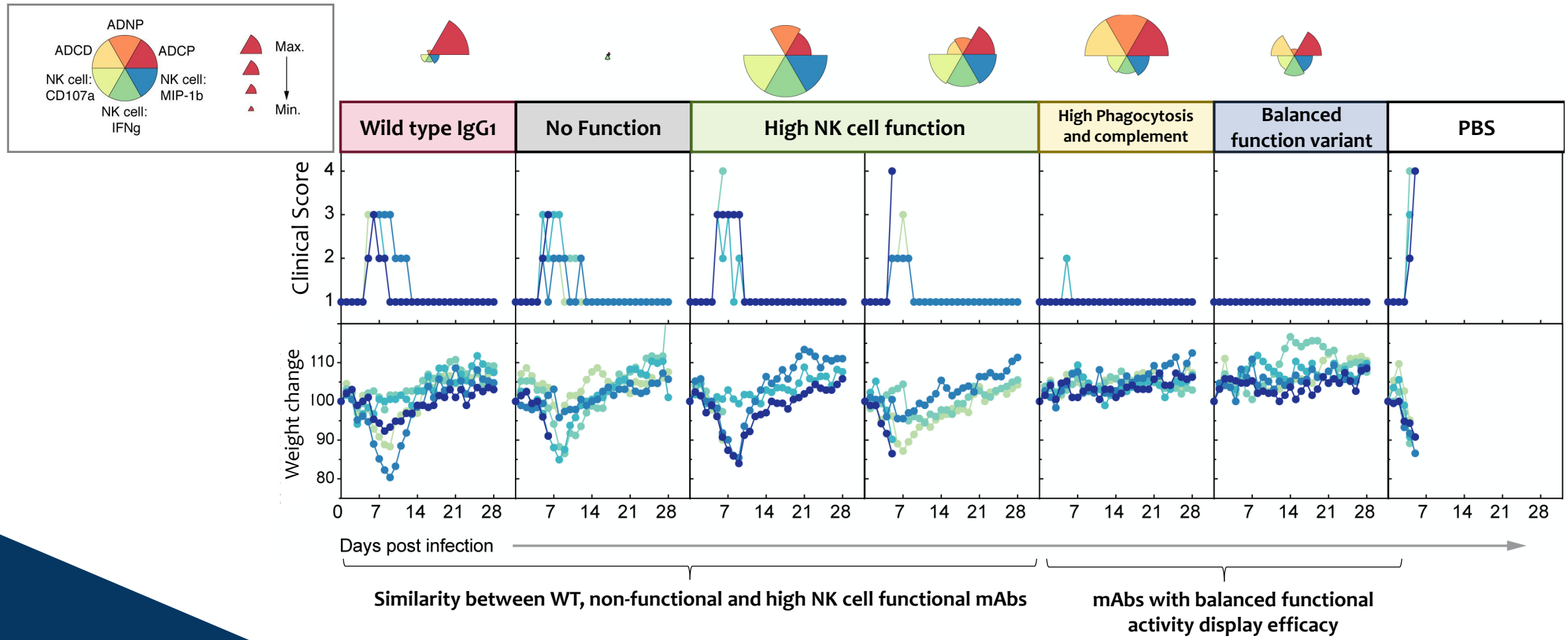
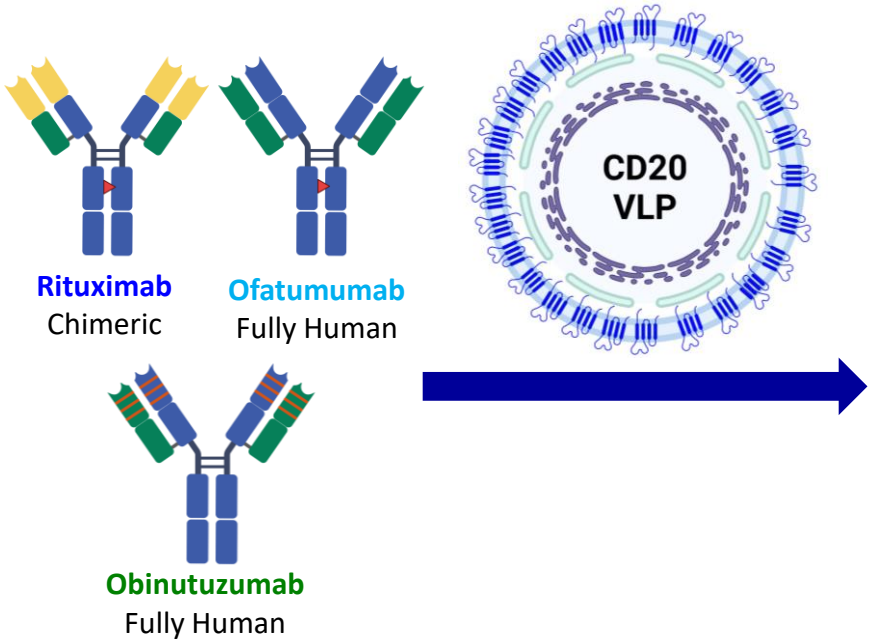
Constant Fab: Optimizing treatment efficacy

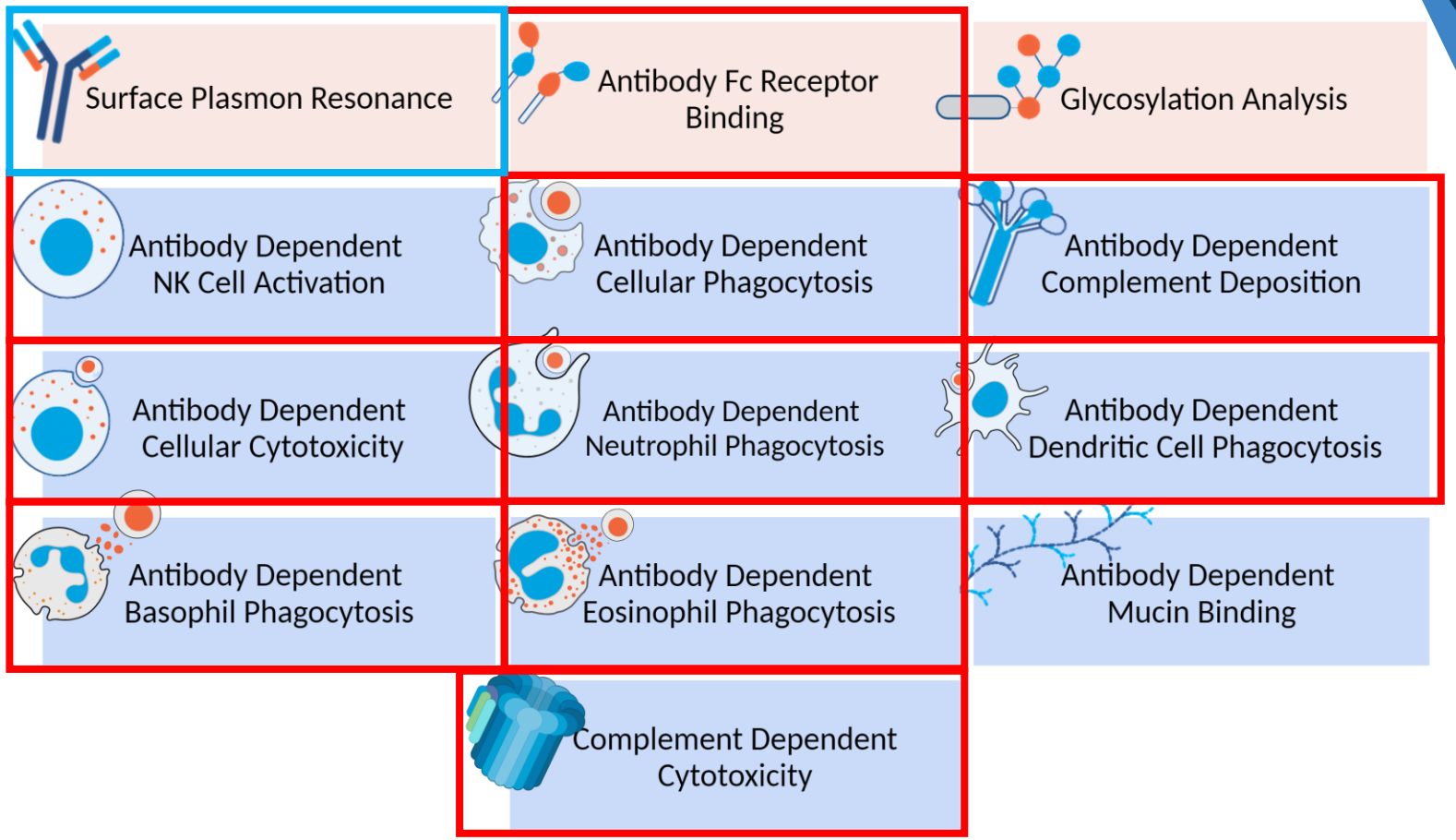
A case for broader effector function profiling



Gunn et al, Immunity. 2021;54:815-828.e5.

Anti-CD20 mAbs: Broader Fc Effector Function Characterization



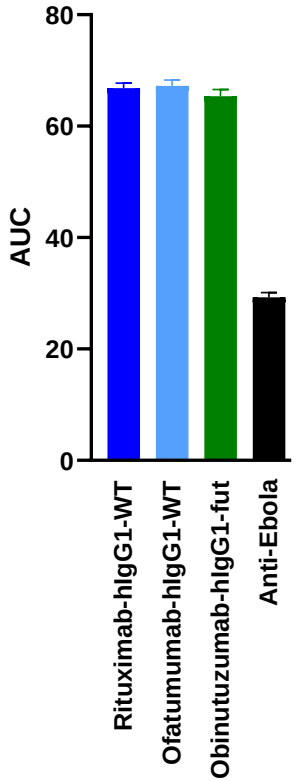


SeromYx SYSTEMS

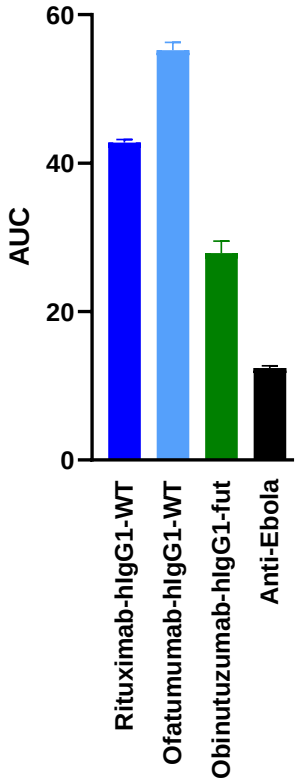
Preliminary results:

SeromYx Platform Recapitulates known Fc Effector Functions of anti-CD20 mAbs

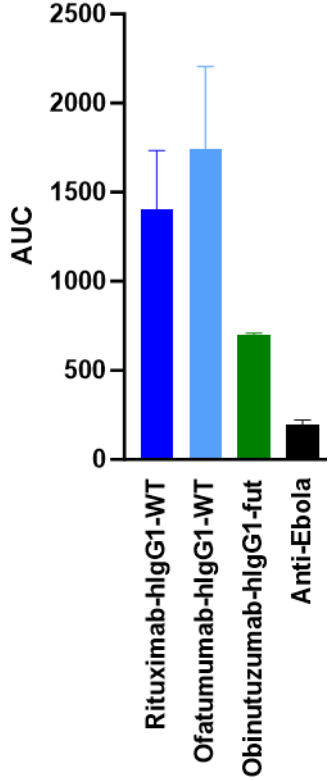
Antibody Dependent Cellular Phagocytosis (ADCP)



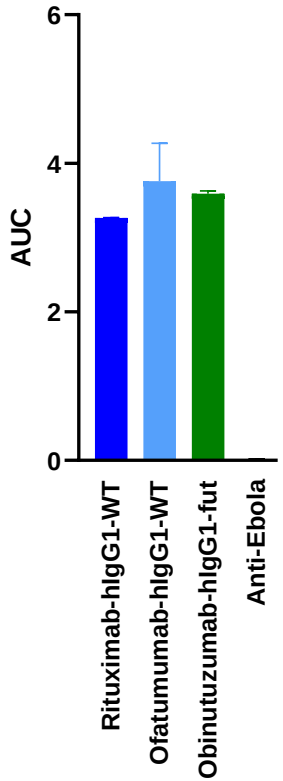
Antibody Dependent Complement Deposition (ADCD)



Complement-Dependent Cytotoxicity (CDC; Raji cells)



Antibody Dependent Cellular Cytotoxicity (ADCC; Raji cells)



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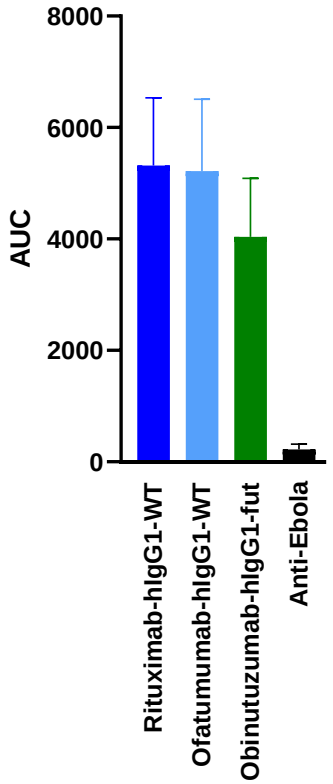
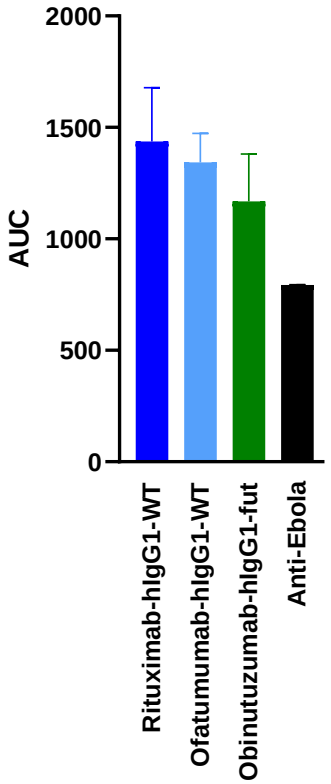
Preliminary results:

SeromYx Platform Uncovers Novel Fc Effector Functions of anti-CD20 mAbs

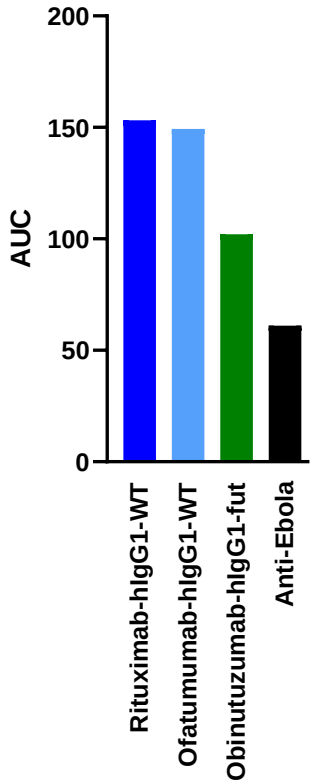
Antibody Dependent Neutrophil Phagocytosis (ADNP)



Antibody Dependent Eosinophil Phagocytosis (ADEP)



Antibody Dependent Dendritic Cell Phagocytosis (ADDCP)



N=1 due to donor failure.

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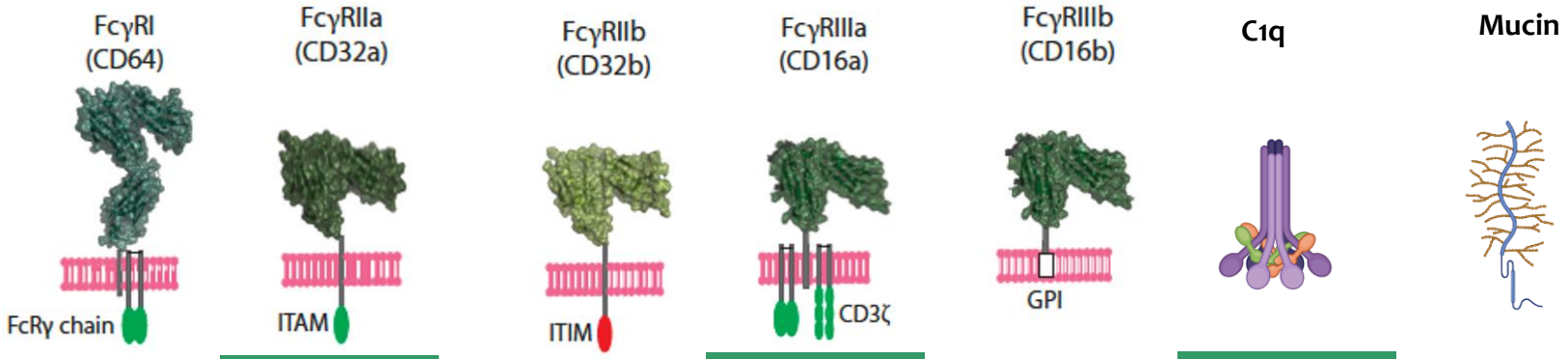
Fc Effector Function Landscape

Vast reduction of the range of Fc functions antibodies induce



Monocytes
ADCP

Industry Standard

NK Cells
ADCC

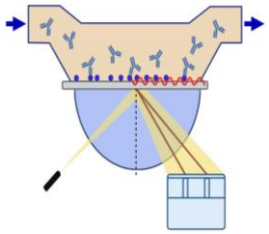
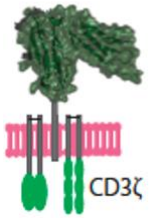
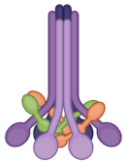
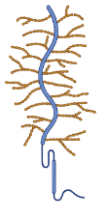
Industry Standard

Complement
CDC

Industry Standard

Adapted from Delidakis et al., Annu Rev Biomed Eng. 2022;24:249-274.

SeromYx Effector Function Platform

SPR	FcγRI (CD64)	FcγRIIa (CD32a)	FcγRIIb (CD32b)	FcγRIIIa (CD16a)	FcγRIIIb (CD16b)	C1q	Mucin
							
Binding Assay	Binding Assay	Monocytes ADCP Basophils ADBP Eosinophils ADEP DCs ADDCP Binding Assay	Binding Assay	NK Cells ADCC Binding Assay	Binding Assay	Complement CDC ADCD Binding Assay	Mucin ADMB

Adapted from Delidakis et al., Annu Rev Biomed Eng. 2022;24:249-274.

Critical attributes integrated into the assays

- **Robustness:** extensive development and optimization of each assay component
- **Assay quality:**
  - **Fit-for-purpose**
  - **Qualification**
  - **Validation**
- **Adaptability**: variety of antigens and sample matrices –Never found an antigen we can't work with, but antigen quality is critical.
- **High-throughput:** 1000s of samples in a single experimental run

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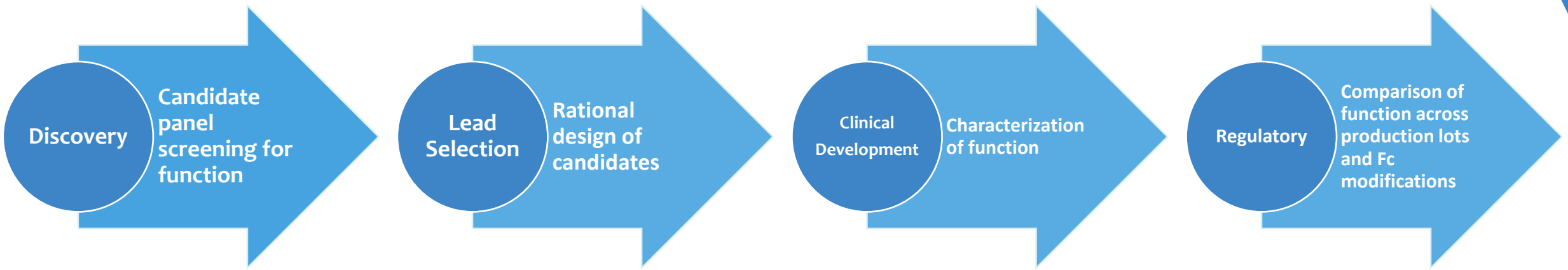
Antigen specific characterization: Added value throughout the mAb discovery and development process



- Finding the right mAb with the desired mix of function.

- Fc specificity
- Improved effector function
- Safety and efficacy

- IND enabling

- BLA enabling

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Please take a copy of our White Paper

View from the Fc: Five Rules for mAb Development Risk Reduction

Understanding your product and avoiding nasty surprises in mAb development

1. Fc Functions: Three is not a Crowd
2. Screening: a mAb is more than the Sum of its Parts
3. More Screening: Nature vs. Nurture
4. Engineering: “Design In” vs. “Measure Out”
5. Mimicking Life: as Physiological as Possible

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- Piers Whitehead
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- Brianna Dougherty

- Will Graham
- Amanda Gross
- Max Halpern
- Thomas Linnekin
- Alexander Morrill
- Jeffrey Parsons
- Tom Shneer

Thank you!

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SeromYx SYSTEMS

Figures for White Paper

Figure 1: SeromYx Platform Uncovers Novel Fc Effector Functions for approved anti-CD20 mAbs

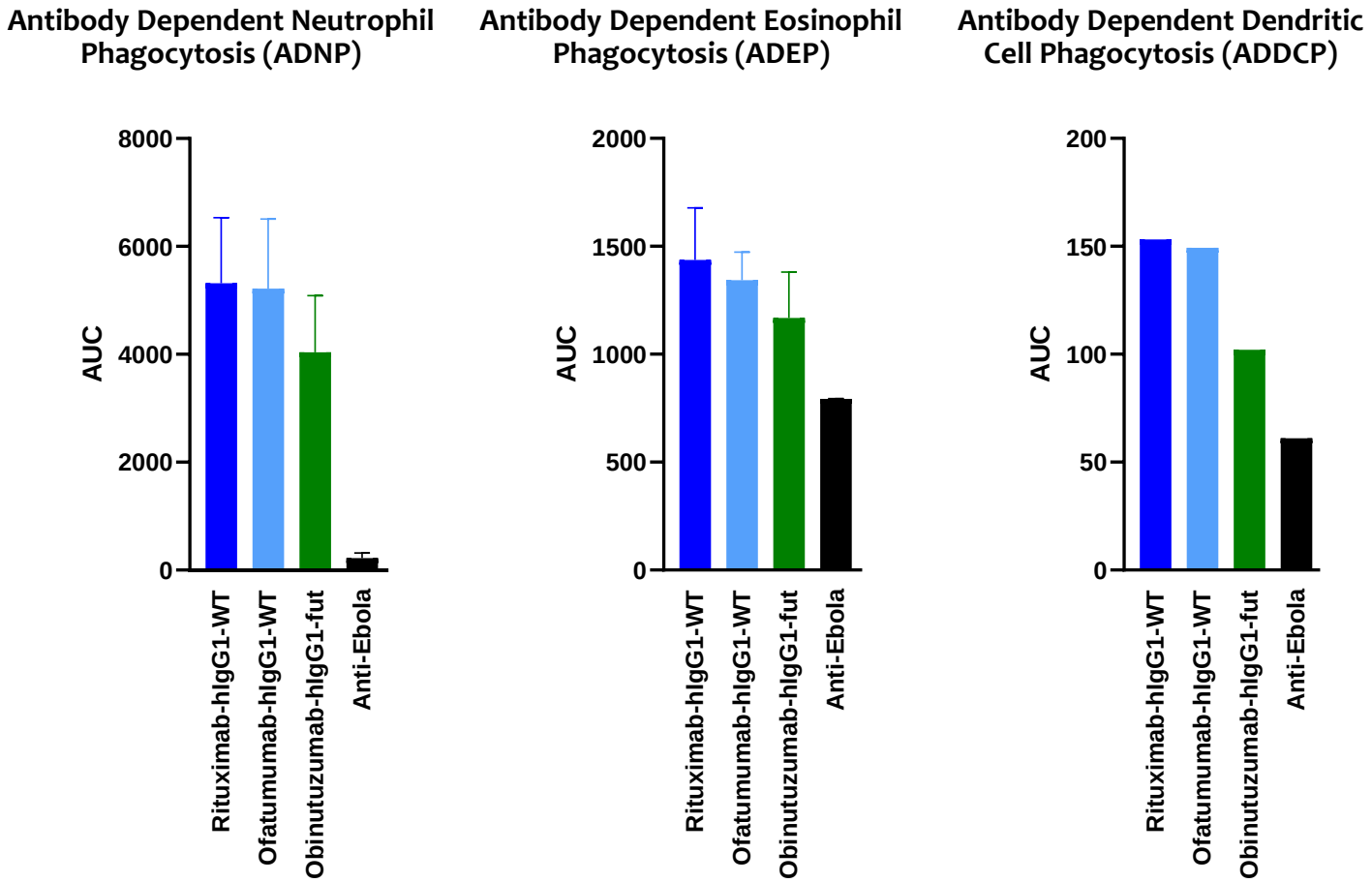

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Figure 2: Epitope-specific variations in Fc effector functions on a constant Fc backbone.

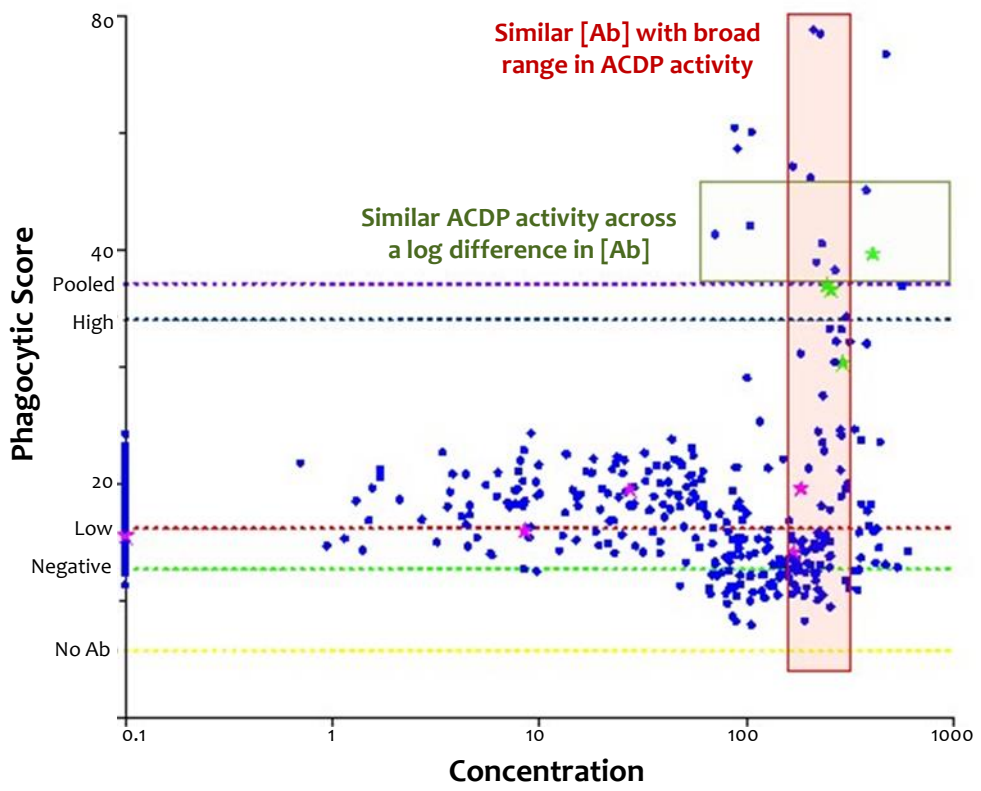

Figure 3: Unintended reductions in ADCC, NK cell activation and ADNP triggered by Fc engineering aimed at half-life extension.

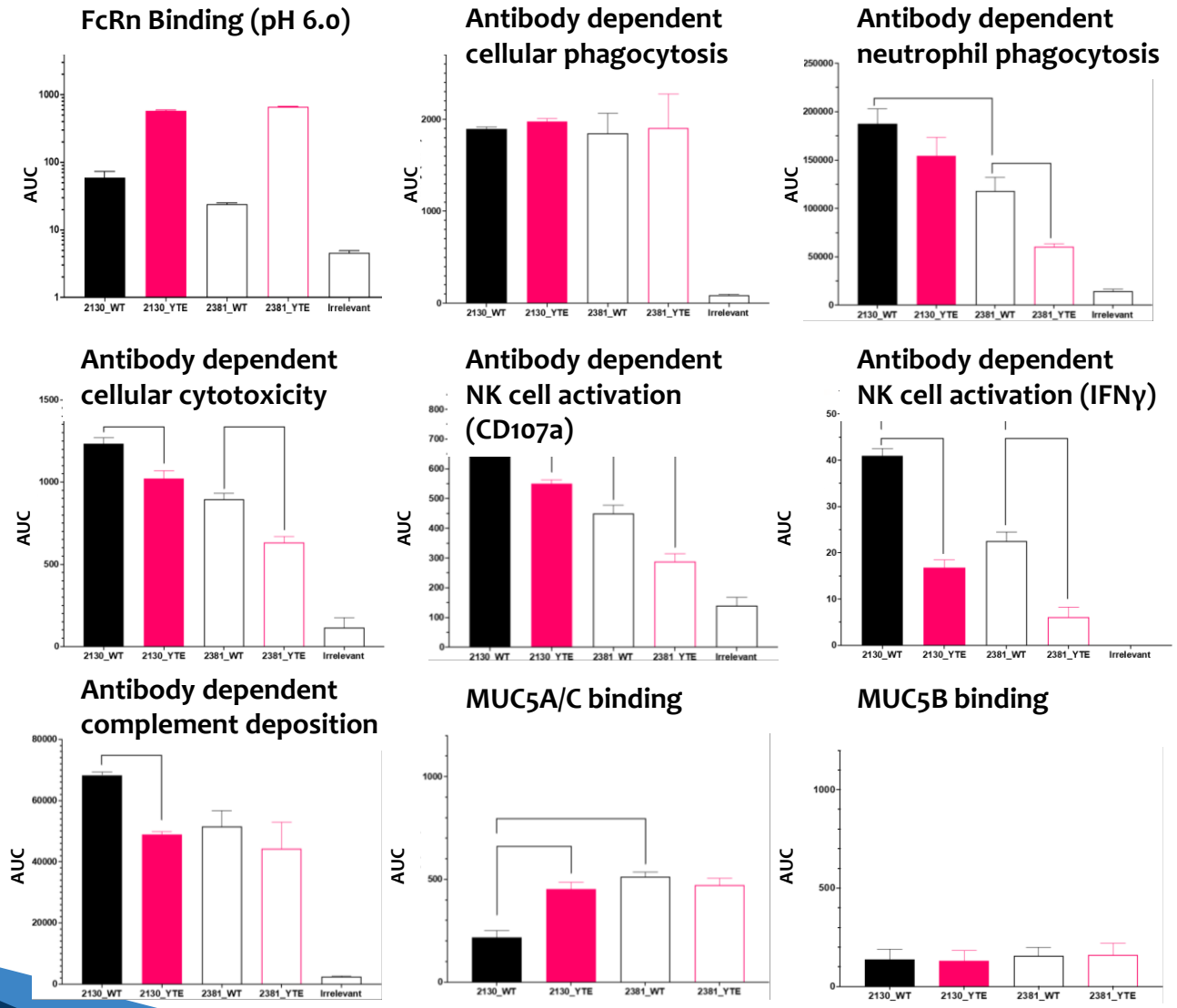

Figure 4: SeromYx Platform offers the broadest array of robust Fc function assays.

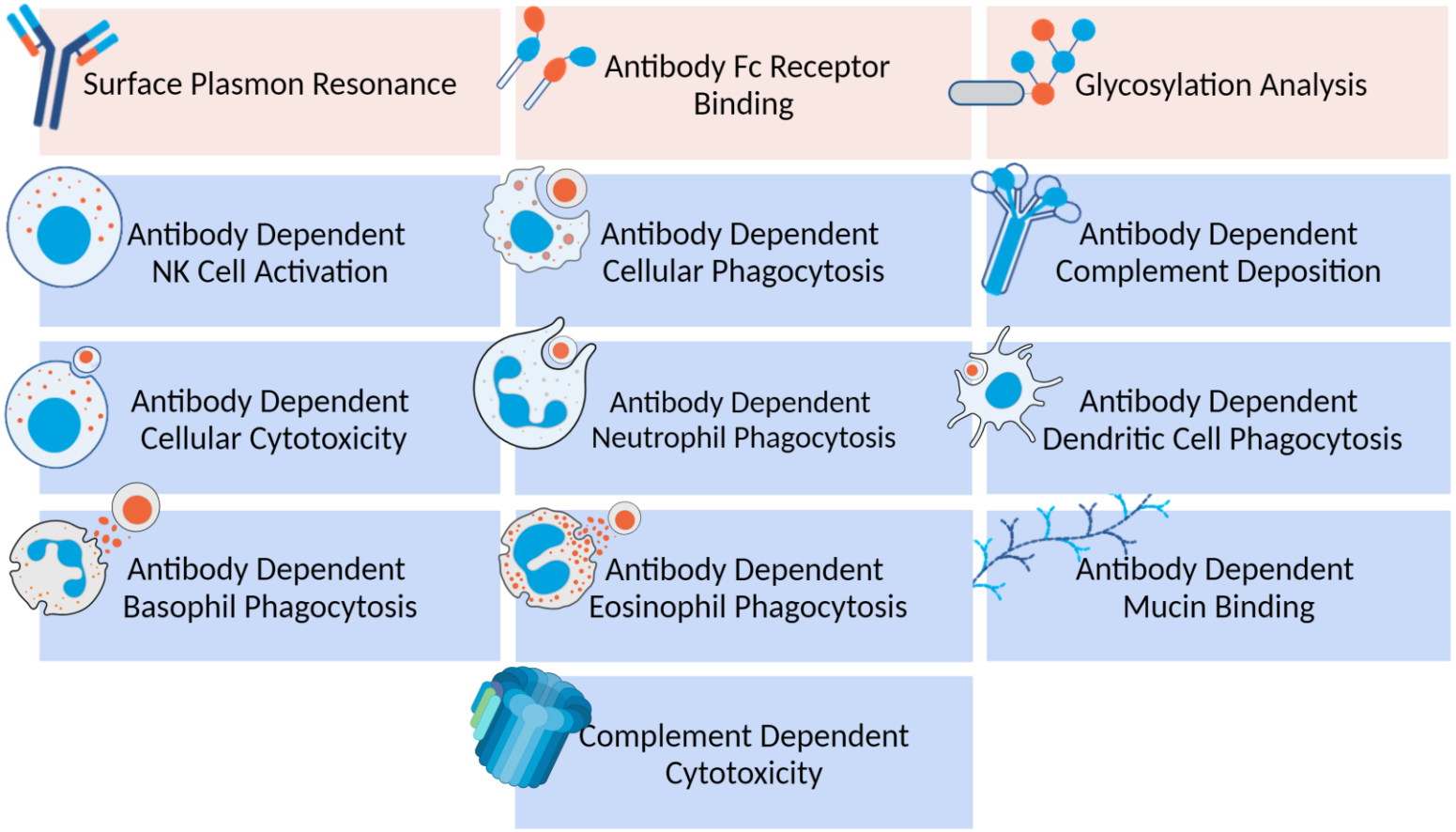

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Figure 5: SeromYx Platform adds value throughout the mAb discovery and development process.