DIVERSA

Discovering and Optimizing the Best from Nature

Comprehensive Optimization of Antibody Therapeutics by Directed Evolution

Monoclonal Antibody Therapeutics for BEID Workshop

March 29, 2006

Diversa Platforms

DISCOVERY

EVOLUTION

MARKETS

ALTERNATIVE ENERGY

HEALTH & NUTRITION

INDUSTRIAL PROCESSES

DIVERSA Pharma
Strategic Partnerships

MERCK

Antibody optimization

MEDAREX

Enhanced antibody functionality

syngenta

Multi-project relationship

XOMA

Multi-project relationship

Bio-Defense Grants

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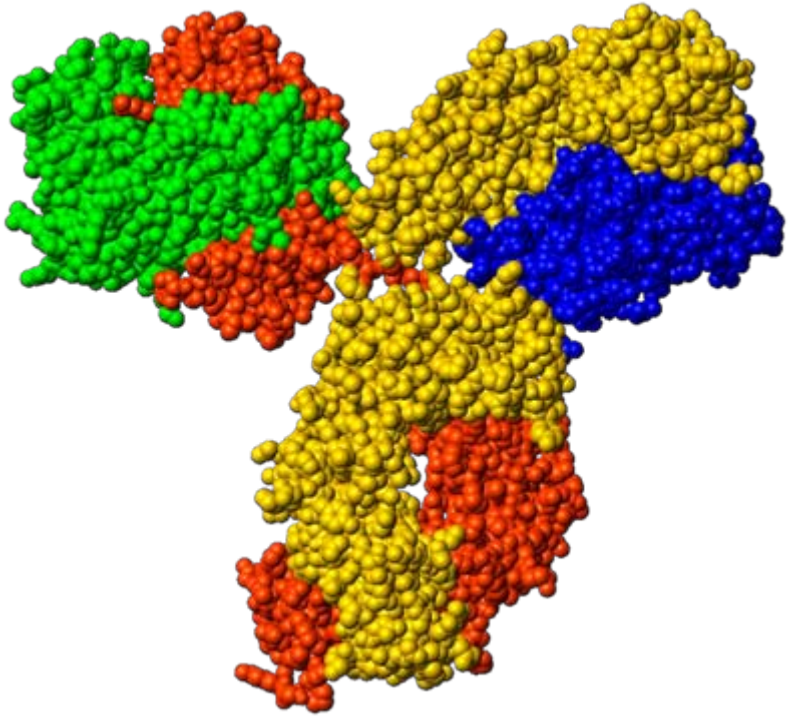
Antibody Optimization

Antibody Optimization

DirectEvolution® Technologies



- Affinity
- Functional Activity
- Stability (thermal, pH, protease)
- Expression
- Effector Functions
- Immunogenicity
- Solubility
- Serum Half-Life
- Conjugates

Diversa's Technology Platforms For Antibody Optimization

DirectEvolution® Technologies

GSSM™ Technology

GeneReassembly™ Technology

MedEv™ Platform

Human Framework Reassembly™ Platform

- Comprehensive and rapid
- Broad evolution capability

Improving the Properties of a Lead Antibody

*Patents Pending
**Patented

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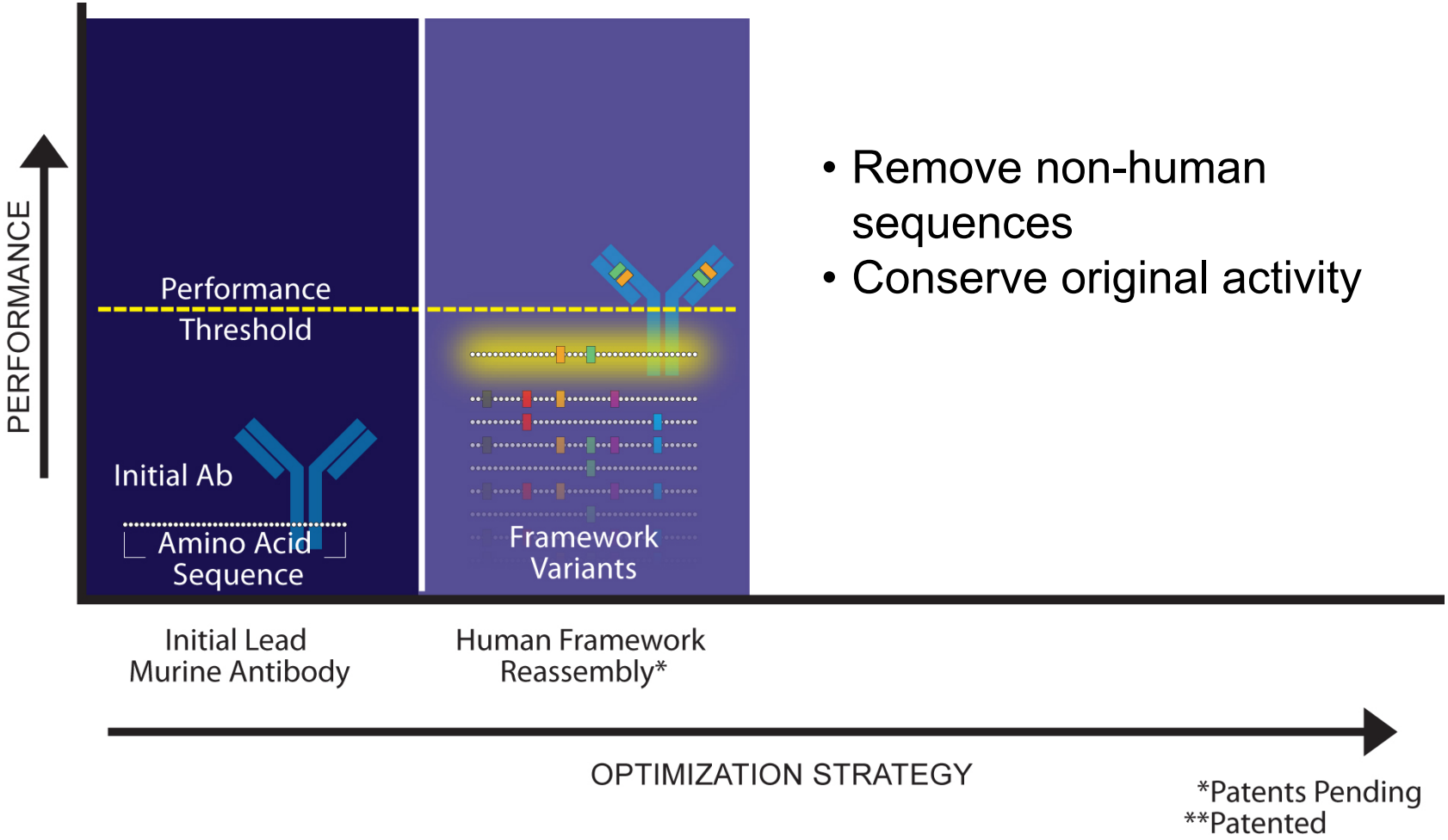
Improving the Properties of a Lead Antibody



- Remove non-human sequences
- Conserve original activity

*Patents Pending
**Patented

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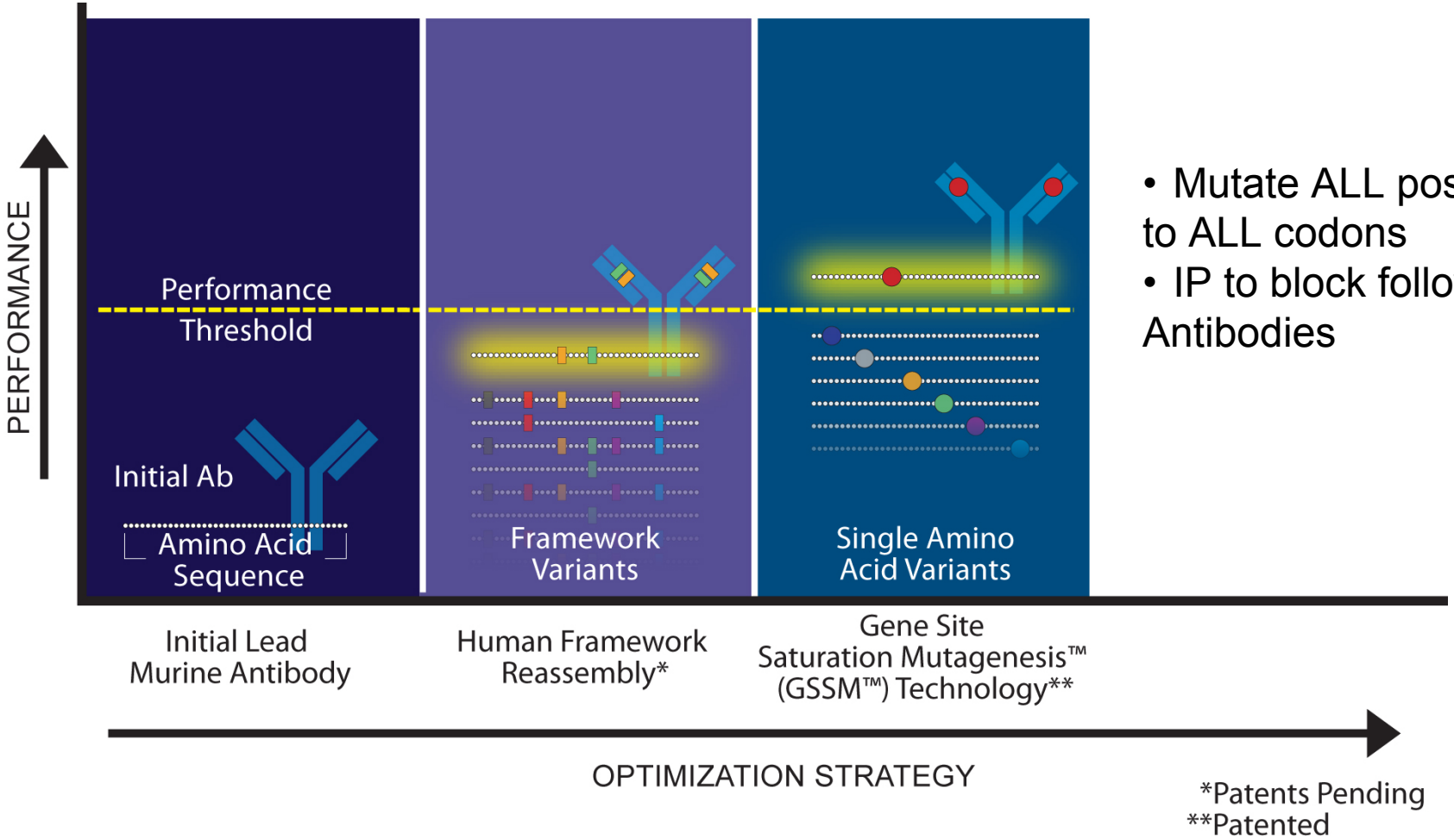
Improving the Properties of a Lead Antibody



- Mutate ALL positions to ALL codons
- IP to block follow-on Antibodies

*Patents Pending
**Patented

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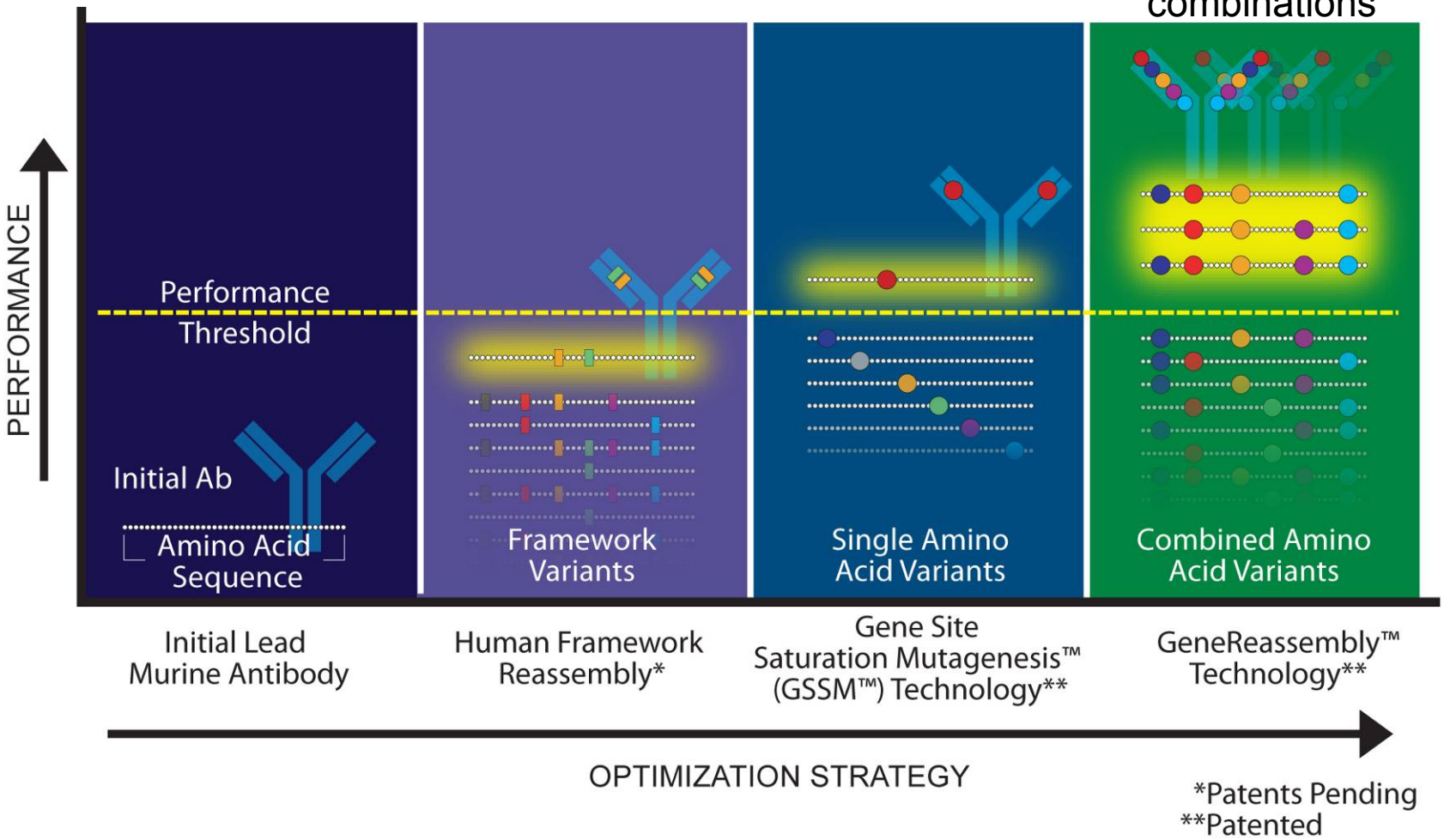
Improving the Properties of a Lead Antibody

•Optimizes GSSM combinations



*Patents Pending
**Patented

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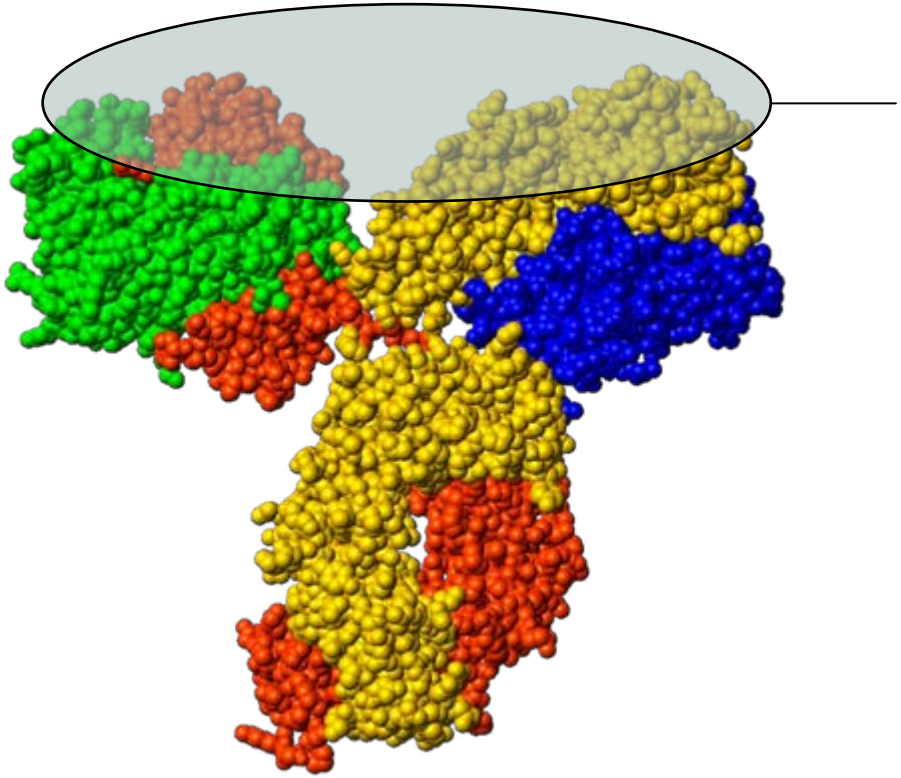
Antibody Optimization Examples



GSSM / GeneReassembly

- Antigen Specificity

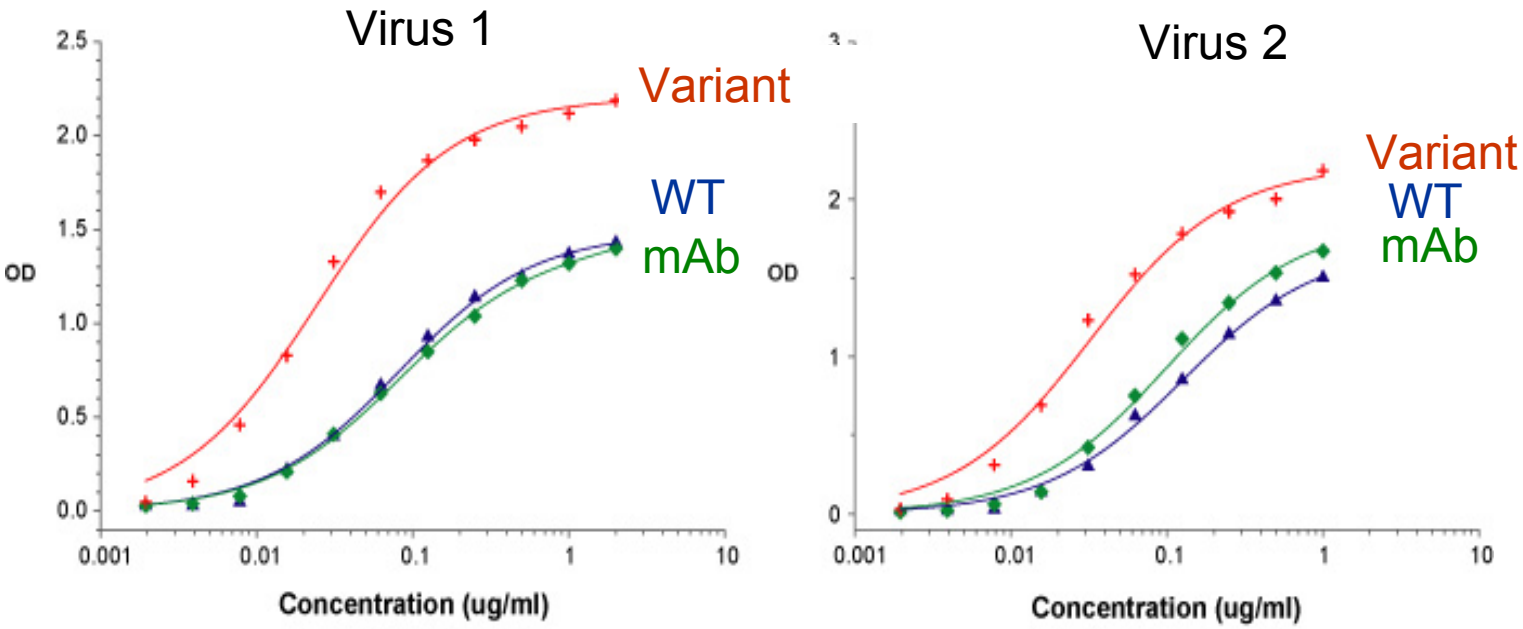
Optimization of an Anti-Viral Antibody

Goal:

- Improve Diagnostic Detection Sensitivity of a Pan Specific Anti-Viral Antibody
- Funded by NIH grant 1 U01 AI056422-01

Optimization of an Anti-Viral Antibody

The Up-variant recognizes several viral isolates and shows better binding than the parent antibody



WT: anti-viral antibody IgG chimera
mAb: corresponding murine antibody
Variant : mutations at position 37 (HC) and position 49 (LC)

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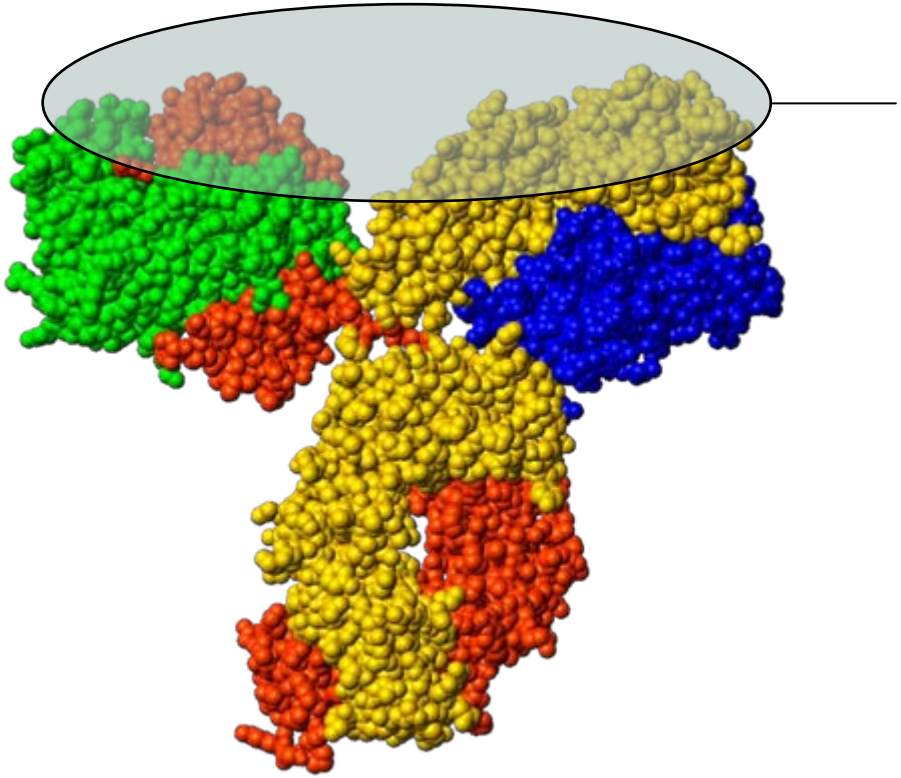
Antibody Optimization Examples



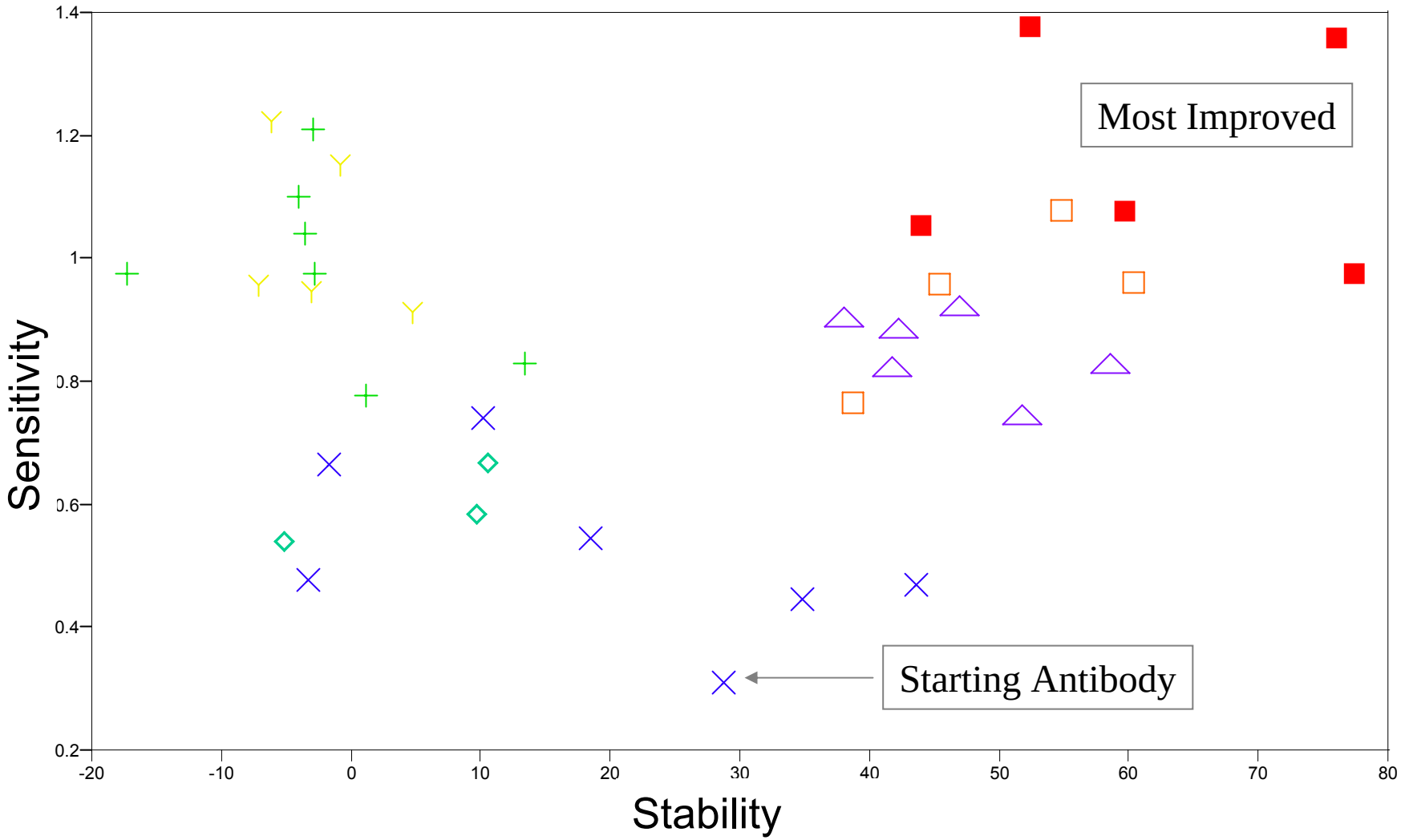
GSSM / GeneReassembly

- Affinity
- Thermal Stability
- Expression

Antibody Optimization
Affinity

Variant	Mutations	ka	kd	BIAcore (K_D)		Sapidyne (K_D)
Parental Ab		9.1×10^{5}	8.0×10^{-4}	8.8×10^{-10}	880 pM	286 pM
Div1	1	8.7×10^{5}	2.03×10^{-4}	2.34×10^{-10}	234pM	20 pM
Div2	3	7.0×10^{5}	2.8×10^{-4}	4.0×10^{-10}	400pM	
Div3	5	1.36×10^{6}	2.45×10^{-5}	1.8×10^{-11}	18pM	
Div4	5					2.5 pM
Div5	6					2.1 pM
Div6	5					1.3 pM
Div7	7	8.8×10^{5}	9.7×10^{-6}	1.1×10^{-11}	11pM	940 fM
Div8	5					670 fM

Simultaneous Evolution for Multiple Properties



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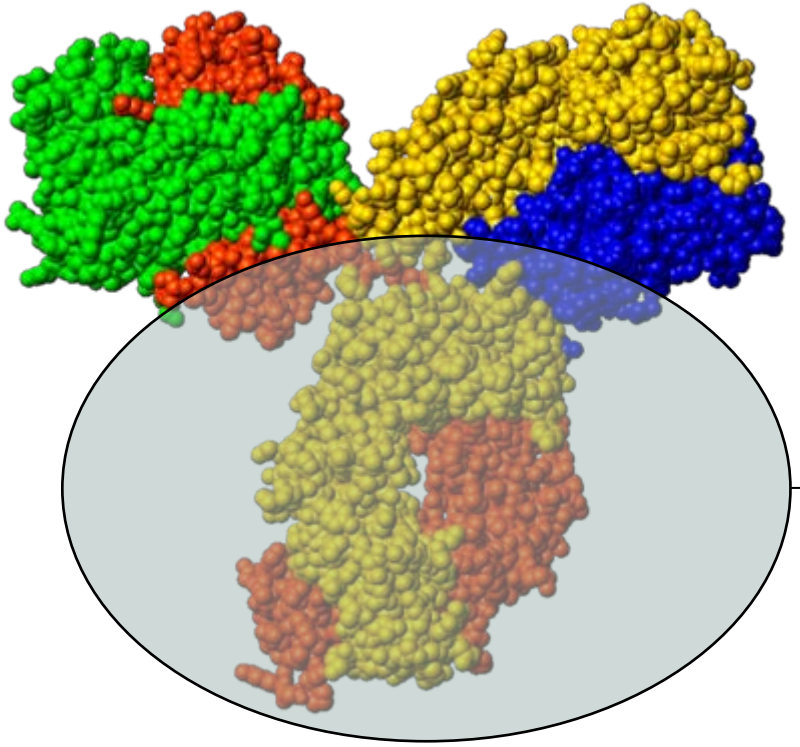
Antibody Optimization Examples



Stability to pH and Proteases

Antibody Optimization
GI Stability

GOAL

- Optimized, gut-stable antibody for oral delivery
  - Resistant to degradation by gastric and intestinal fluids (in vitro)
  - Retention of biological activity following oral dosing (in vivo)

Antibody Optimization
GI Stability

GI Stability Program	
Collaborator	syngenta
Goal	Generation of a gut stable, orally-delivered, therapeutic antibody
Target	Undisclosed

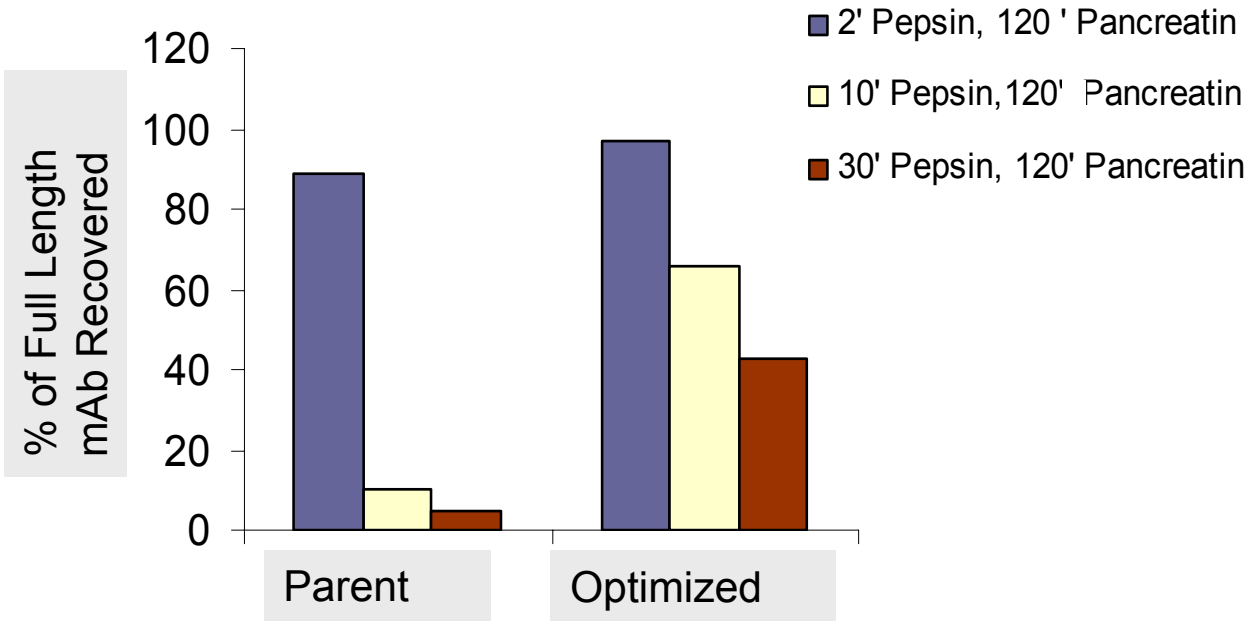
Antibody Optimization
GI Stability

Program

- Create library of IgG variants
- Screen for in vitro resistance to simulated gastric and intestinal fluids
- Test lead candidate in vivo

GI Stability - In vitro studies

Parental vs. optimized mAb in simulated gastric and intestinal fluids



- Antibodies were incubated with pepsin at pH 3 for 2', 10' and 30' followed by pancreatin digestion for 120'

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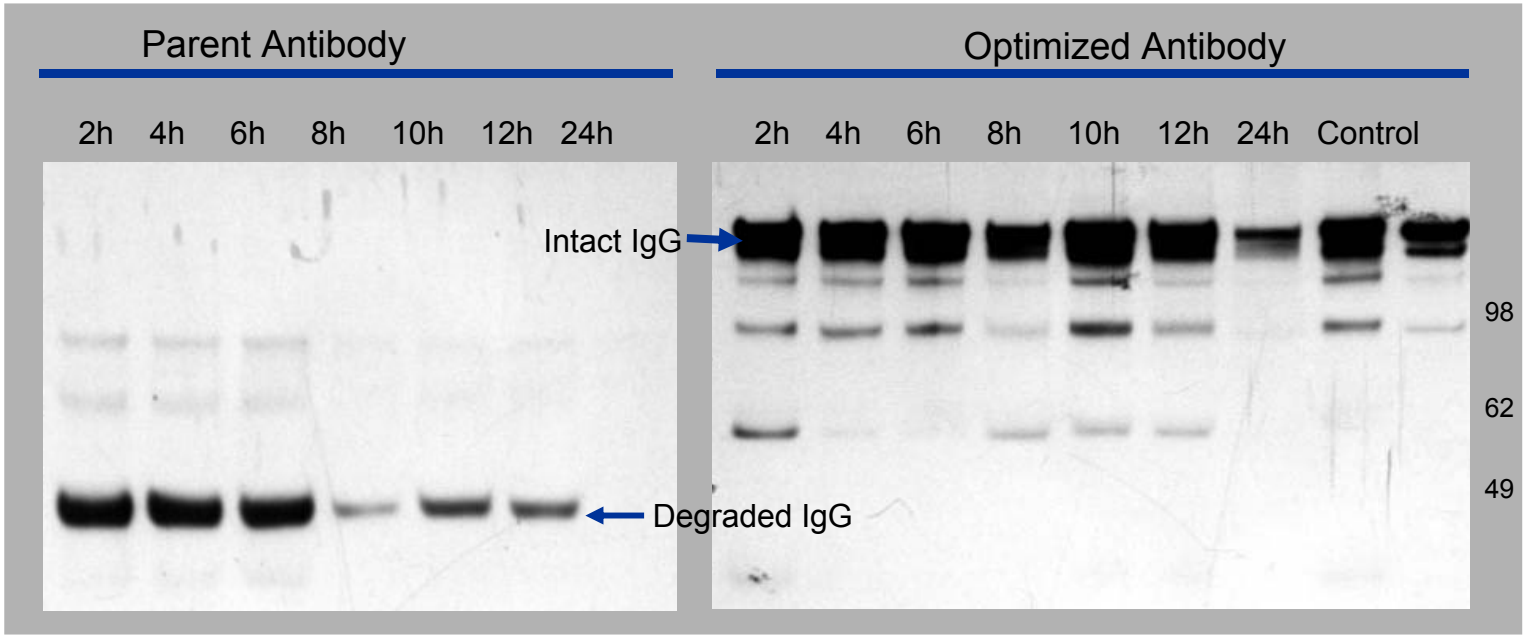
GI Stability - In vivo Studies

Optimized IgG survived transit through the GI tract



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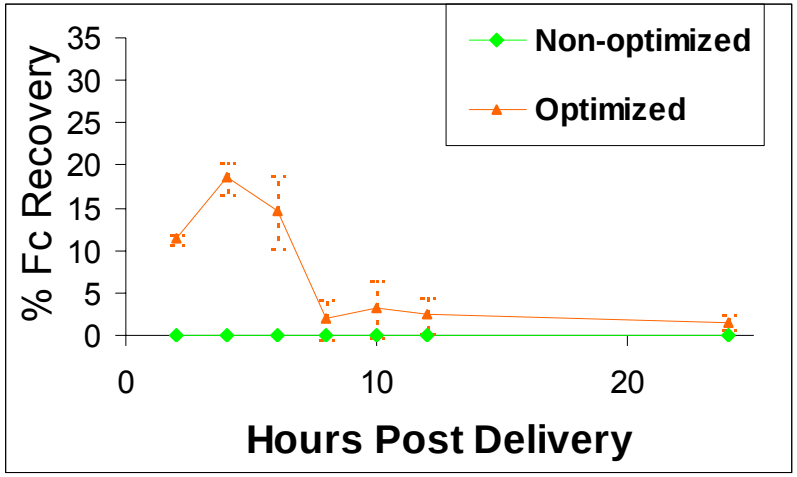
GI Stability - In vivo Studies

Time course of Antibody Recovery from Mice Feces
Mice were fed with 2.5 mg Antibody (pH 7.4)



The optimized antibody was recovered in fecal samples following transit in the GI tract

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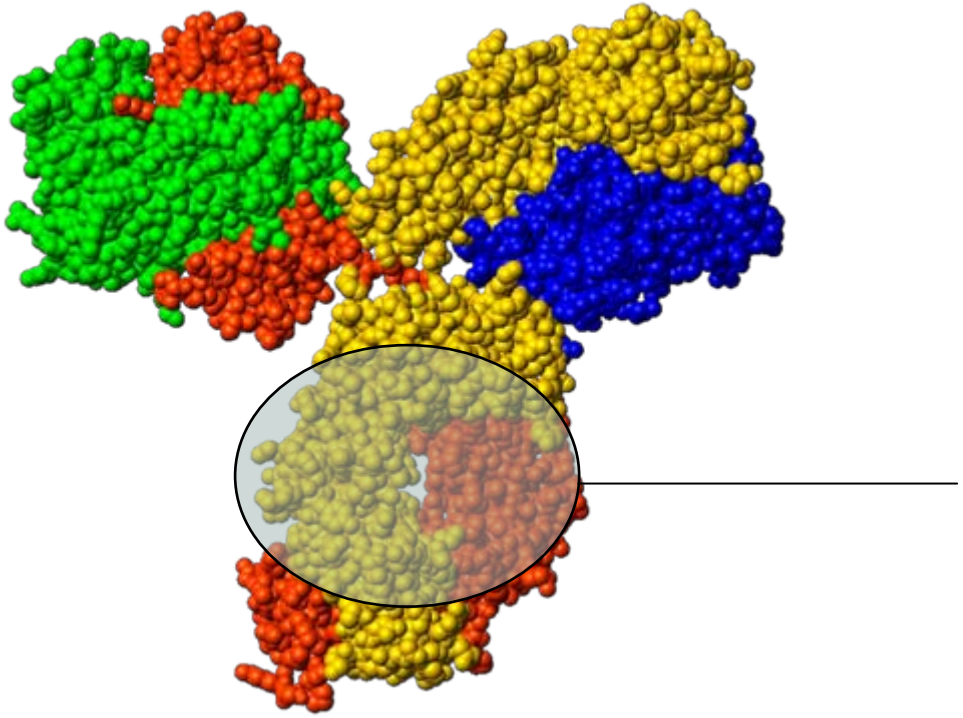
GI Stability - In vivo Studies

<u>Cumulative</u> Recovery from Mice Feces for 24 h after Oral Delivery
Mice were fed with 2.5 mg Antibody (pH 7.4)

	%Intact	%Fab
Optimized	55 ± 4	100 ± 9
Parent	0	65 ± 6

About 50% of the optimized antibody was recovered in fecal samples following oral administration and transit through the GI tract

Antibody Enhancement Opportunities
Fc Optimization

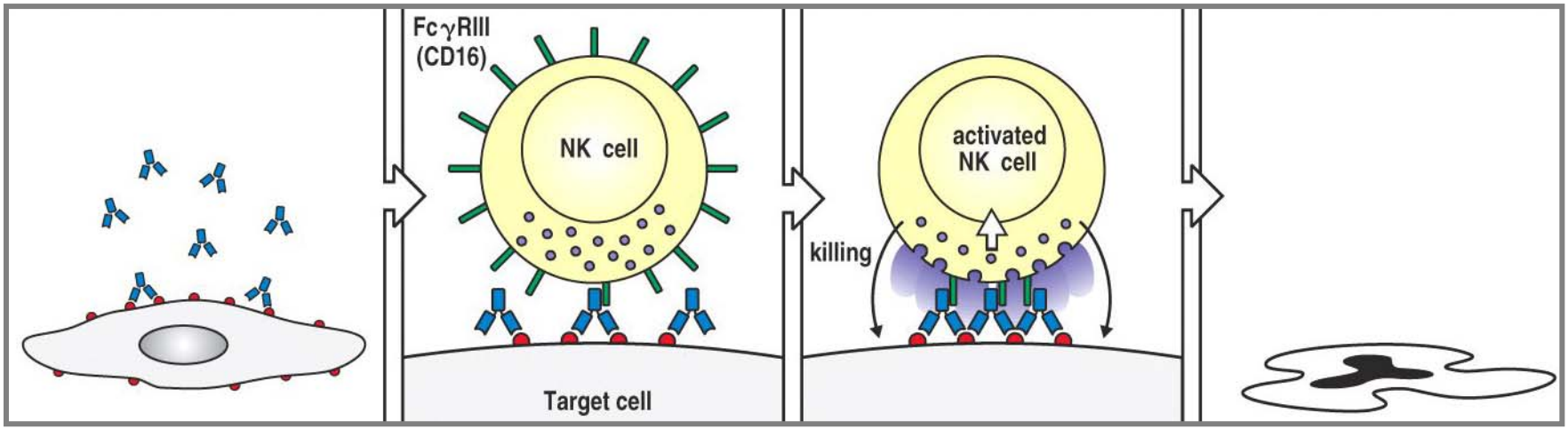


GSSM / GeneReassembly

- Pharmacokinetics
- Complement
  - Lysis (CDC)
- Cellular Fc Receptors
  - Lysis (ADCC)
  - Phagocytosis

Effector Functions Example
ADCC

ADCC
Antibody Dependent Cell Cytotoxicity



Binding | Recruitment of immune cells | Killing | Clearance

From Immunobiology, 6th Edition

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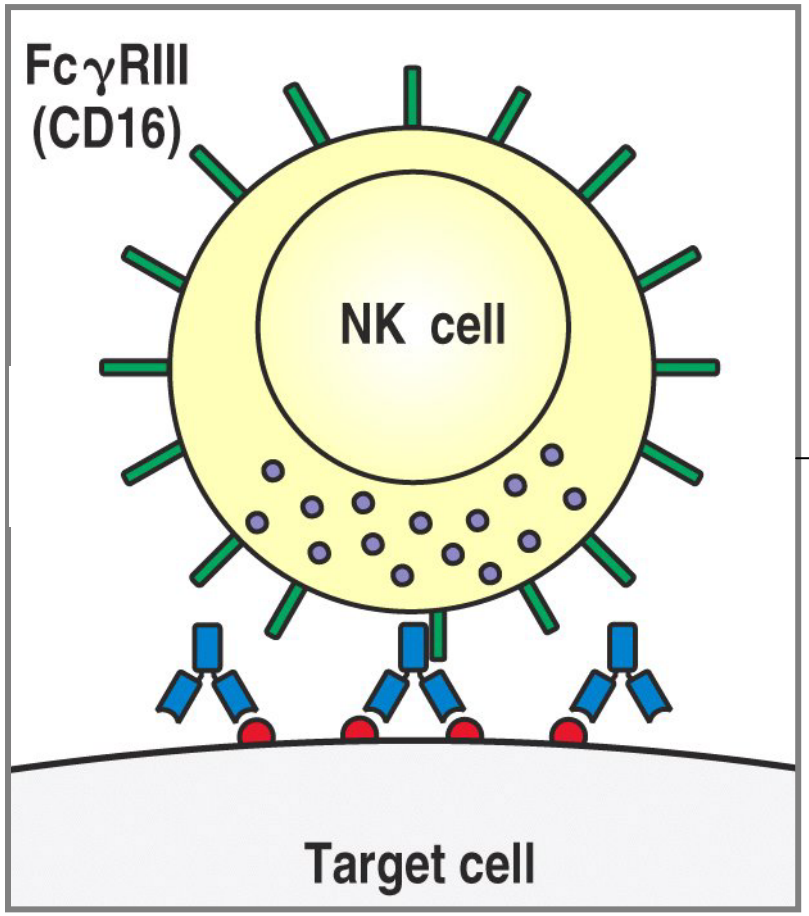
Effector Functions Example
ADCC



From Immunobiology, 6th Edition

Application

- Increase tumor cell clearance by ADCC

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Fc Optimization Program
ADCC

Fc Optimization Program	
Collaborator	MEDAREX
Goal	Potency/efficacy enhancement via ADCC
Target	Oncology

Fc Optimization Program Workflow

MEDAREX

- Create library of Fc variants
  - DirectEvolution® Technologies
    - GSSM™ and Gene Reassembly™
- Fc variants screened as IgG
  - Profile binding properties of Fc variants in a panel of Fc receptors
  - Immune function assays

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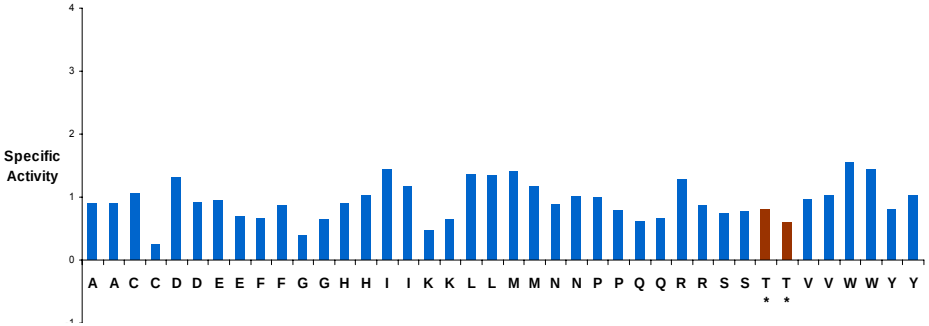
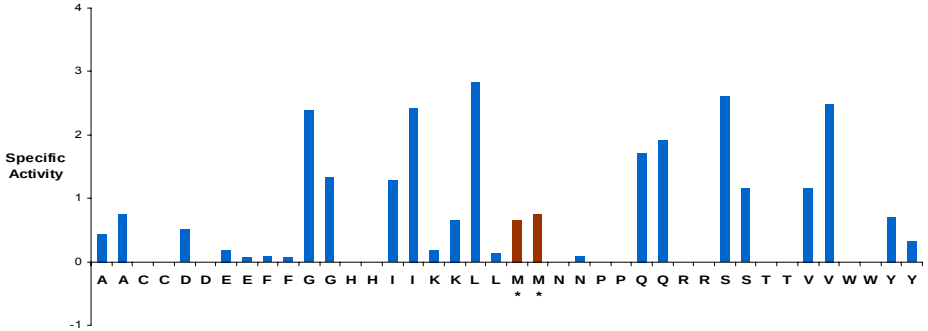
Fc Functional Map

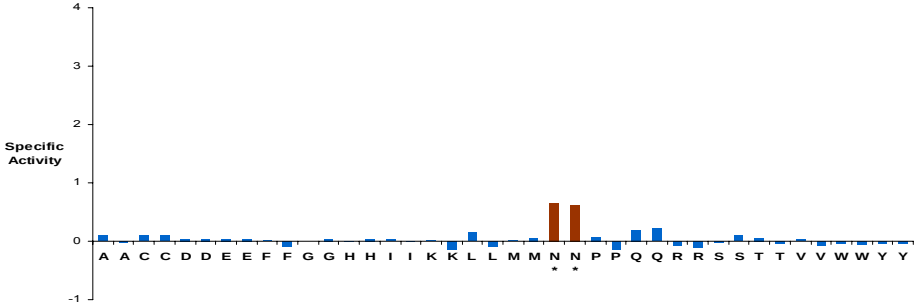
Permissive Position



Improved Position

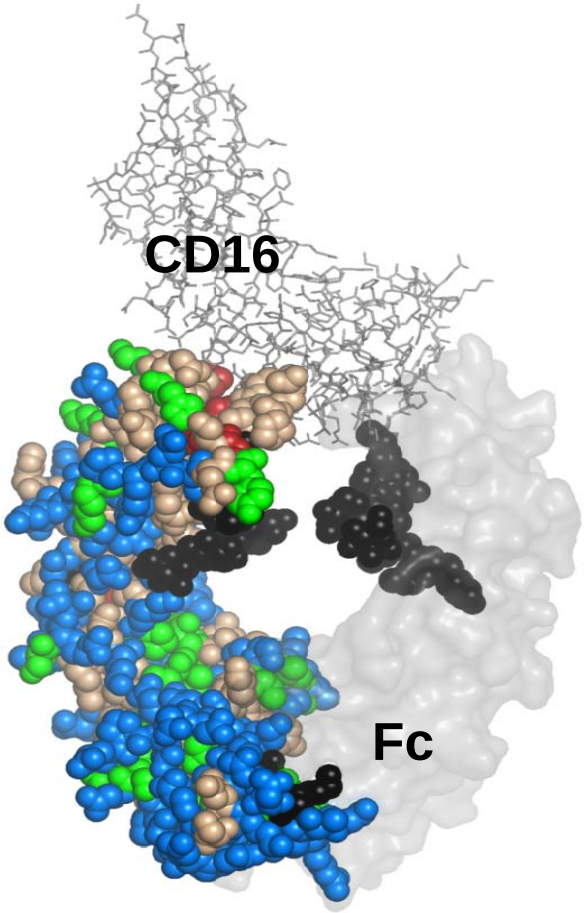


Restrictive Position



Relative Specific Activity = Functional ELISA / Quant. ELISA

Position Mapping of Residues with Enhanced Binding to FcγRIII

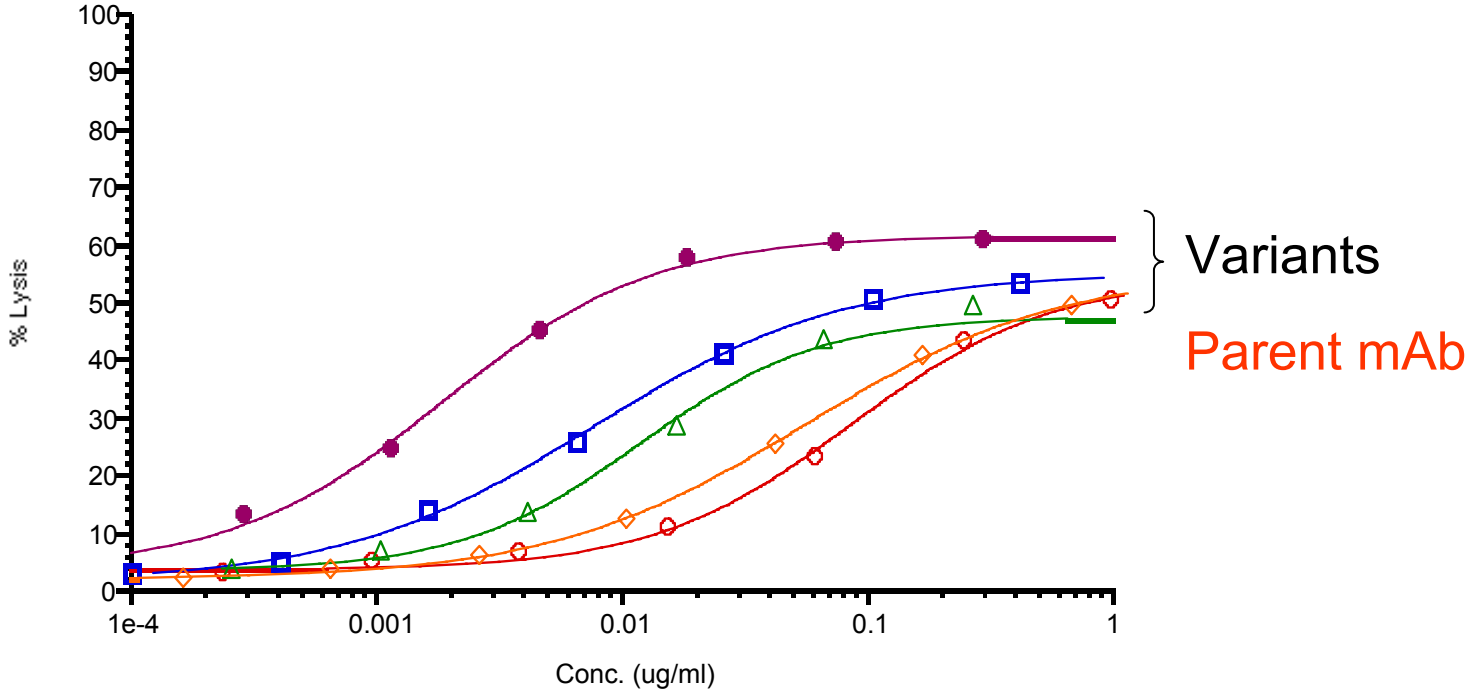


- Hit
- Tolerant
- Limited
- Intolerant

- **Permissive positions are surface residues**
- **Intolerant positions are contact residues**

Efficacy and Potency of Parent vs. Fc Variant mAbs in ADCC Assays

Tumor killing assays measure the % lysis of tumor cells in presence of mAbs and immune cells



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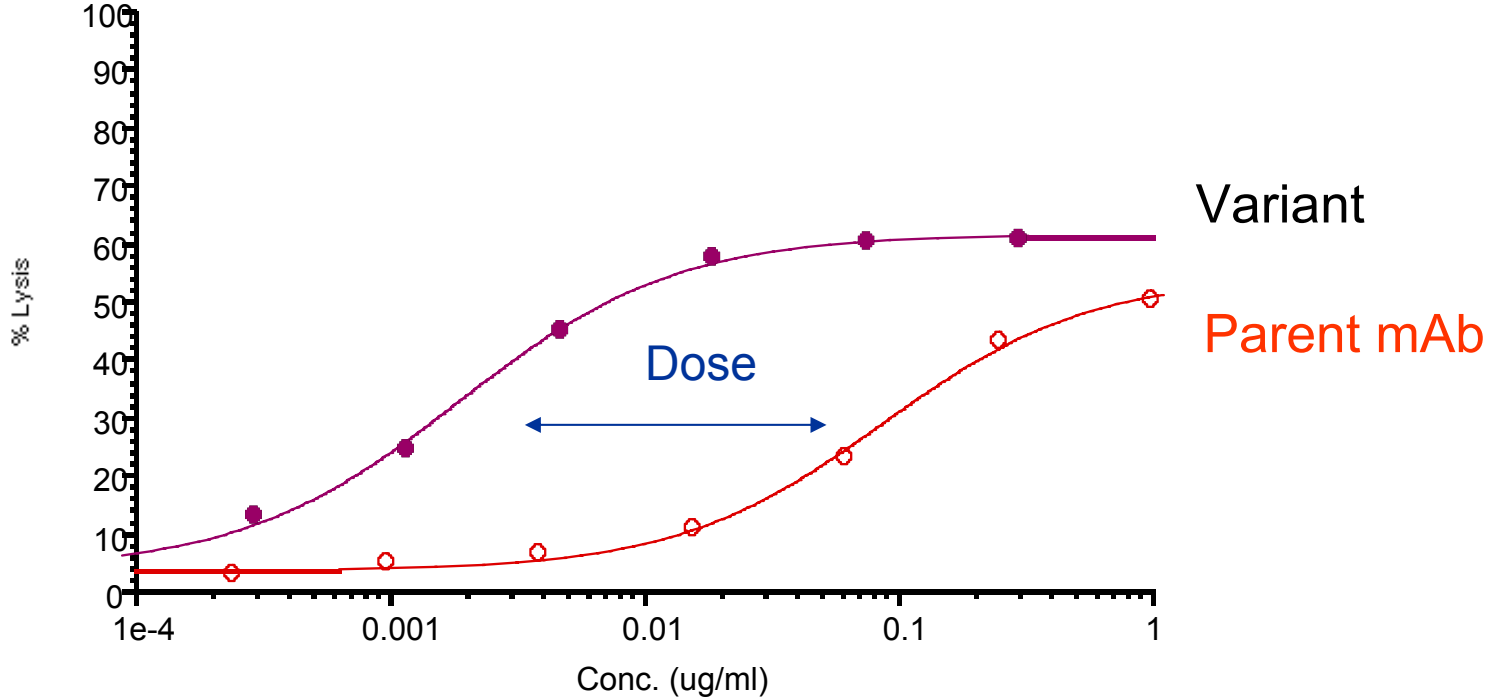
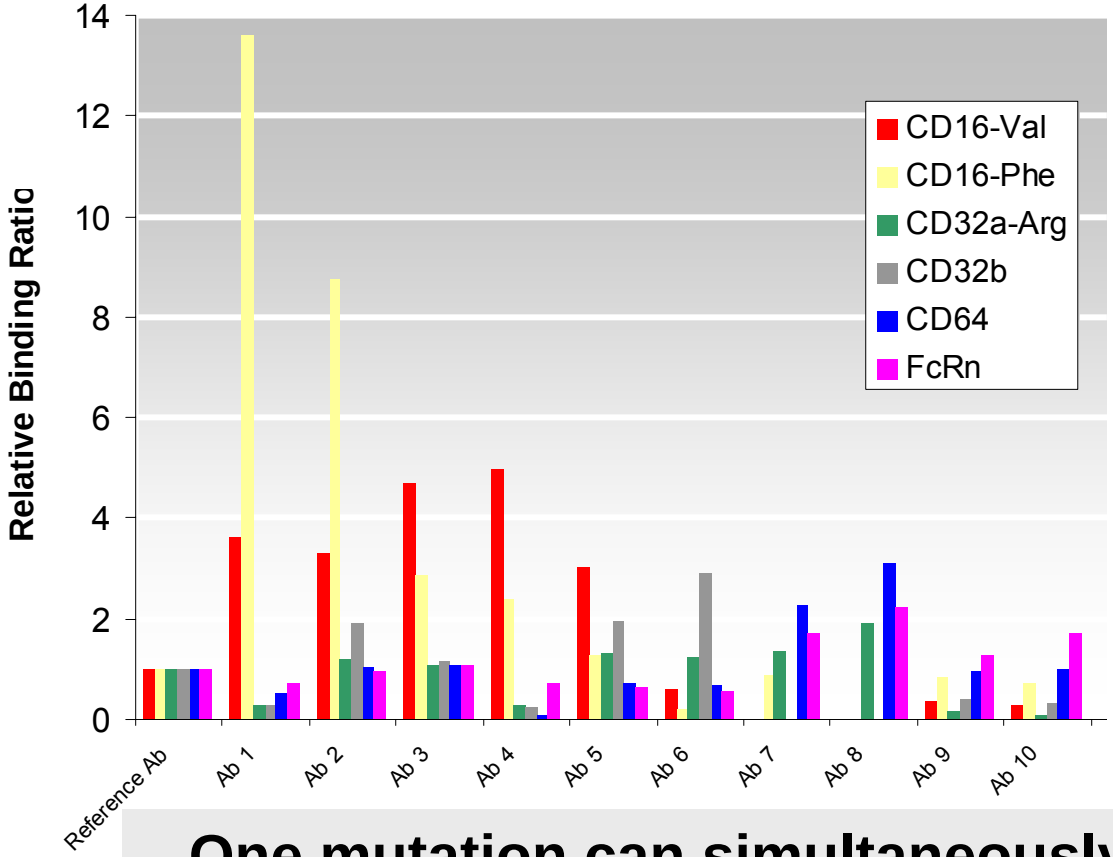


Variants are more efficacious and more potent than the parental mAb

Binding Proprieties of Fc Variants to Panel of Fc Receptors



One mutation can simultaneously increase binding to one receptor and decrease it to another one

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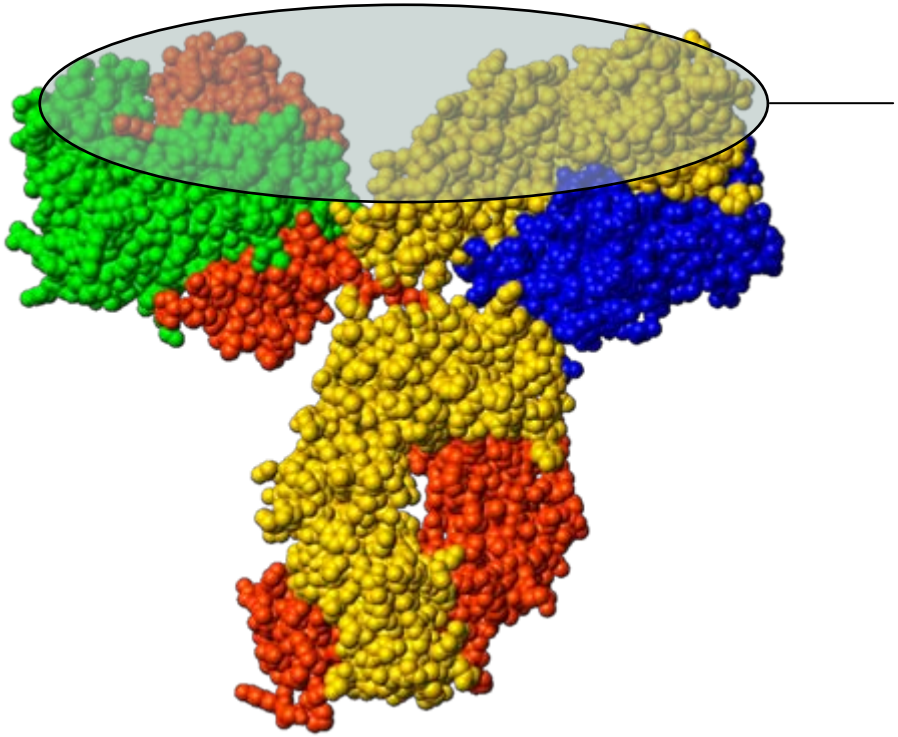
Design Intended Effector Functions

The antibody structure can be designed to fit an intended therapeutic application

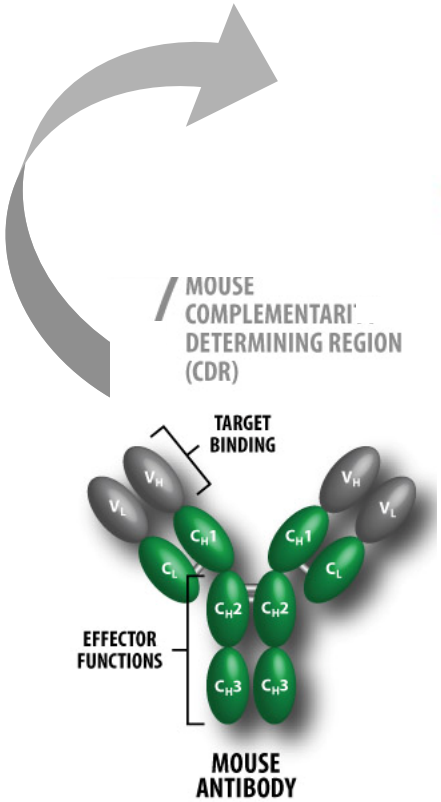
Effector function	Anti-tumor Ab	Anti-infective Ab	Conjugated Ab
ADCC	X		
CDC	X	X	
Phagocytosis		X	
IgG half-life	X	X	X
Increased binding	CD16,CD32aC D64,C1q, FcRn	CD32a, CD64, C1q, FcRn	
Decreased binding	CD32b		CD16,CD64 CD32a, CD32b, C1q, FcRn

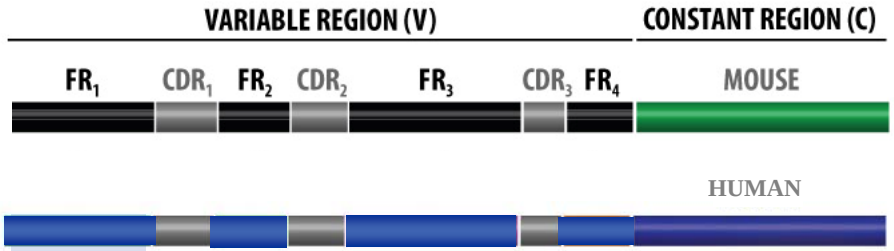
Antibody Optimization Examples



Human Framework Reassembly

Traditional Antibody Humanization





FR - HUMAN FRAMEWORK REGION
CDR - COMPLEMENTARITY DETERMINING REGION

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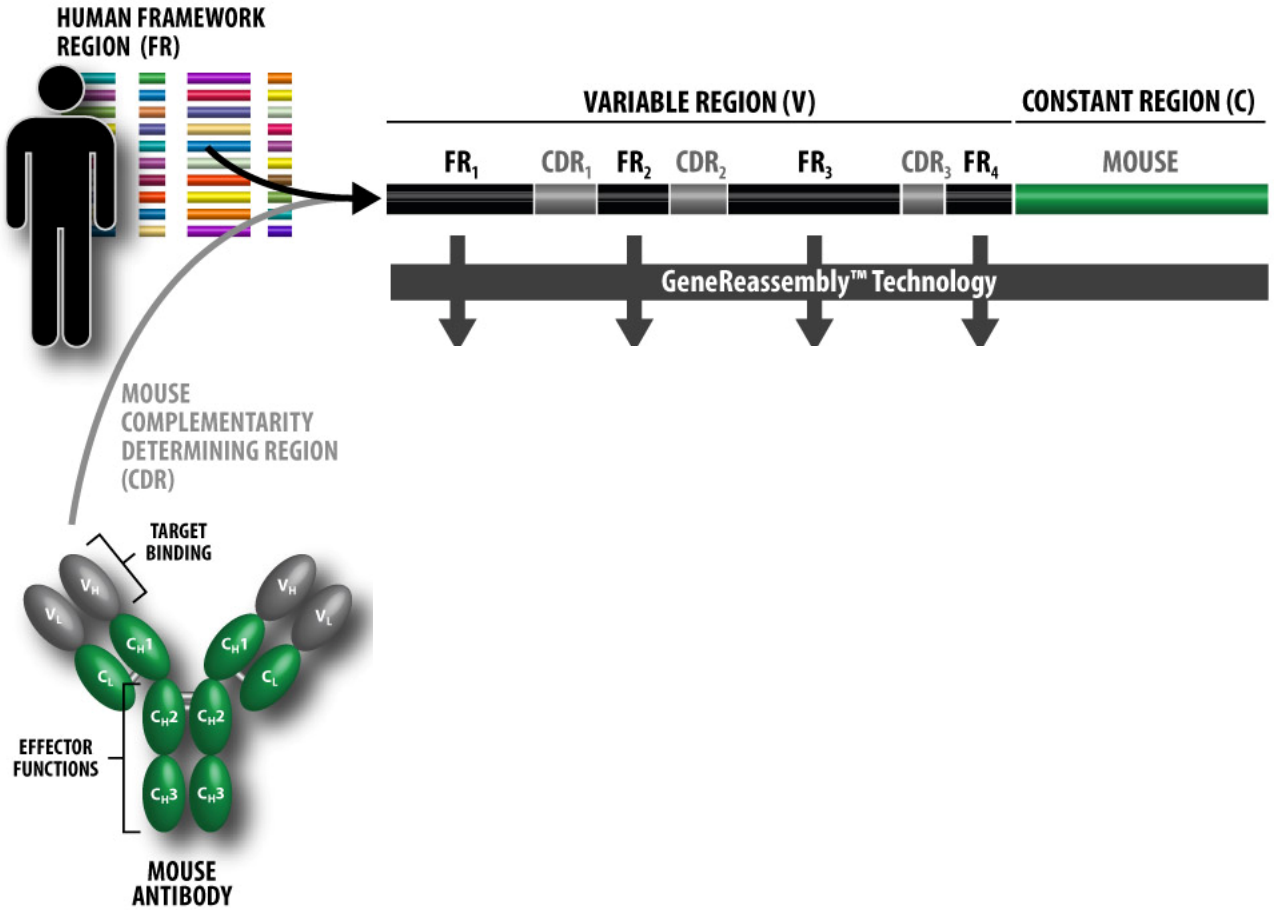
Human Framework Reassembly



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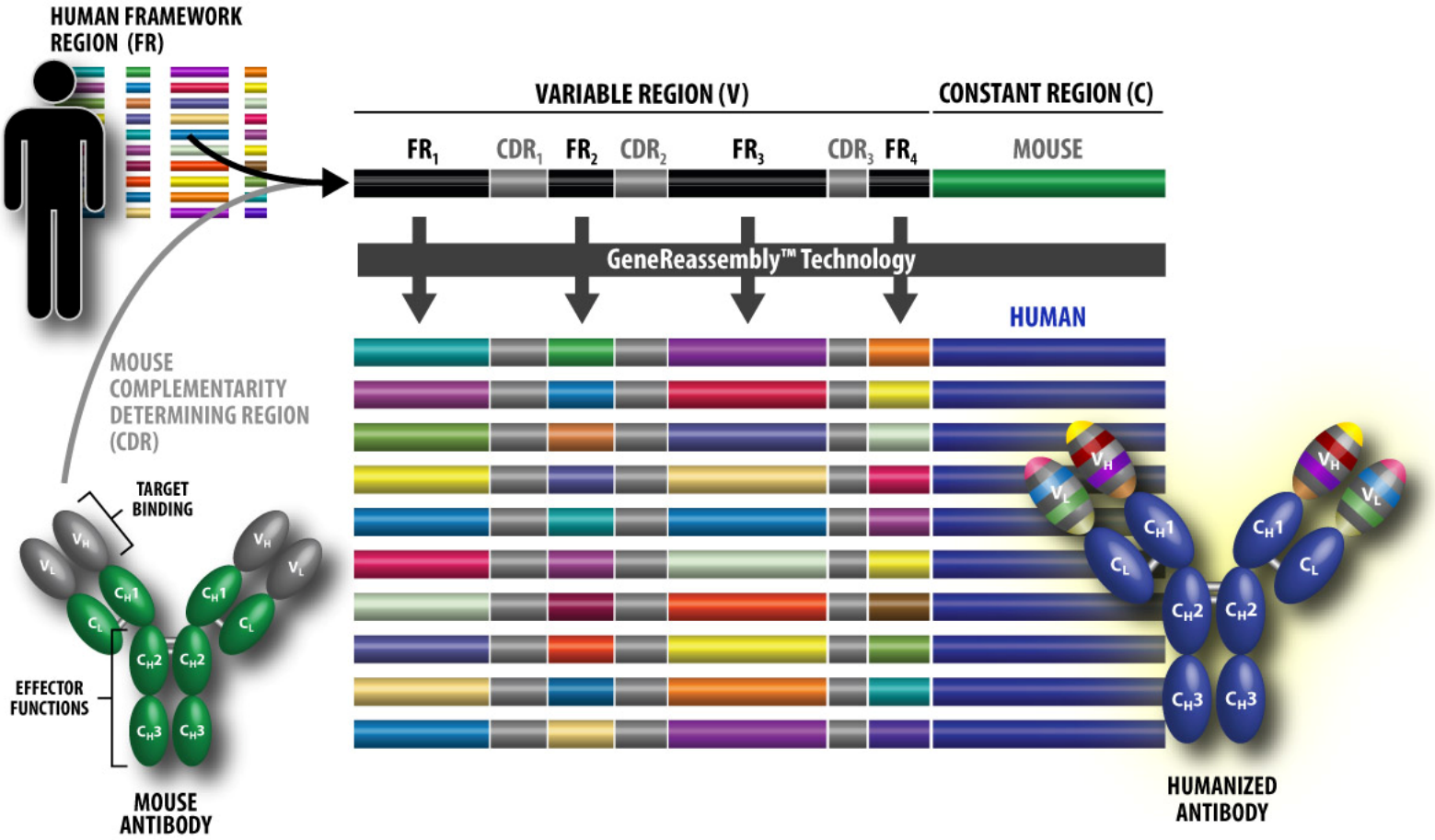
Human Framework Reassembly



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Human Framework Reassembly

- Example
  - Library built as Fab format

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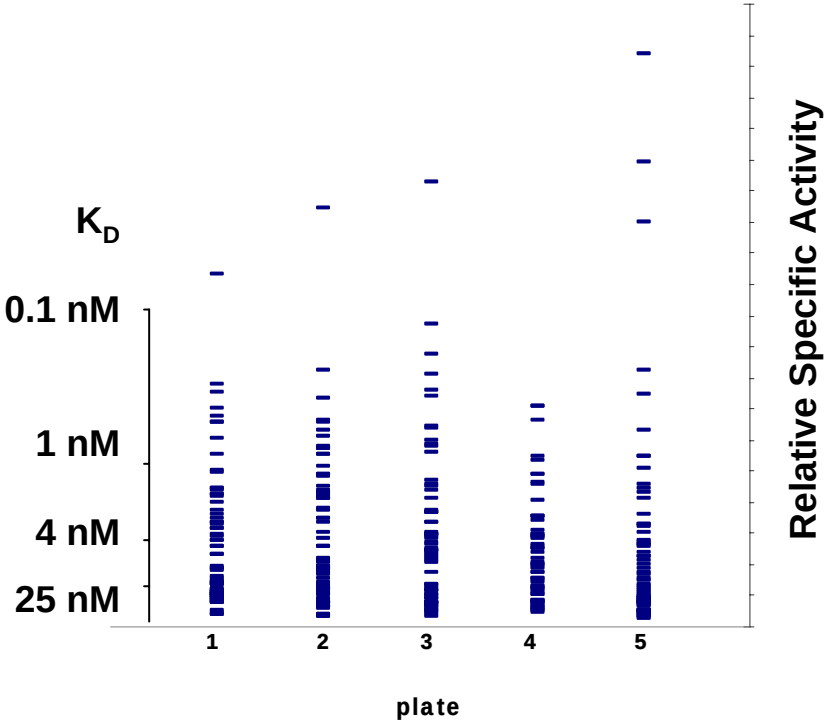
Affinity Distribution for Reassembled Abs



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Human Framework Reassembly

- Create functional antibodies with fully human frameworks
- Advantages
  - Rapid process (~ 3 to 4 months)
  - Approach doesn’t require any structural information
  - Backmutations are not required

Diversa Antibody Optimization

Properties	Programs
Properties	
Expression	*
Affinity	*
Stability	*
Human FR Reassembly	*
Class Switch	*
Protease Stability	*
Pan-Specificity	*
Effector Functions	
Enhanced ADCC	*
Decreased ADCC	*
Enhanced CDC	*
Phagocytosis	*

Summary

- Ability to humanize murine antibodies
- Ability to optimize antibodies for multiple characteristics
- Ability to optimize Fc and effector functions
- Ability to optimize therapeutic proteins