Structure Guided Optimization of Antibody Fc and Fab Domains for Improved Pharmacokinetic Properties

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Antibody Engineering For Improved PK

Background: Antibodies are a preferred treatment modality, particularly in cancer and autoimmune diseases, with more than 50 approved antibodies and more than 500 molecules in various stages of clinical development. The success of antibodies in disease intervention is, in part, due to their high level of specificity, relative safety, and long circulating half-life. The circulating half-life of immunoglobulin G (IgG), roughly 10-21 days depending on IgG isotype and attributes of the variable region, is attributed to association with the neonatal Fc receptor (FcRn) leading to antibody recycling and minimal endosomal degradation. FcRn plays a key role in serum IgG homeostasis as well as in placental transfer of IgG molecules from mother to fetus. Following pinocytosis, the acidic environment of the early endosome allows for binding of IgG (as well as albumin) to FcRn, which provides protection from degradation and facilitates trafficking of IgG back to the extracellular environment, where the molecules dissociate back into circulation upon exposure to physiological pH.

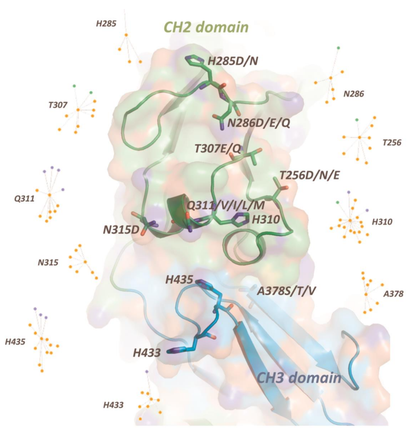
Abstract: The FcRn salvage pathway is the primary driver for the long serum half-life of IgG and albumin molecules. However, despite having the same Fc domain, antibodies can vary widely in their pharmacokinetic (PK) properties. Multiple factors have been proposed that explain the observed differences: target-mediated disposition, rate of fluid phase of pinocytosis, polyreactivity, immunogenicity, etc. The emerging model of the IgG-FcRn interaction, in which the Fab region is facing the endosomal membrane, offers an additional explanation for variation in PK properties between antibodies with identical Fc domains. To improve the pharmacokinetic properties and enhance half-life, both the Fc and Fab domains can be engineered to optimize their interaction with FcRn. We present a structure and network-based framework to interrogate the engagement of IgG with multiple Fc receptors simultaneously. Using this framework, we identified features that govern Fc-FcRn interactions and identified multiple distinct pathways for enhancing FcRn binding in a pH-specific manner while maintaining effector function. Additionally, using a model antibody, we applied structure guided engineering to identify multiple pathways to optimize Fab domain for improved PK properties. The combination of Fc engineering and Fab optimization provided significant increase in half-life in a human FcRn mouse model over the parental molecule and highlights the importance of optimizing both domains for improved PK properties.

Rational Fc Designs for Improved FcRn Binding

Selection of Residues to Engineer Enhanced FcRn Binding

We identified four sets of Fc residues which, when mutated, we predicted would alter the FcRn interaction and slow the dissociation rate. These include residues that make direct contact with FcRn, as well as peripheral and non-surface exposed residues that have the potential to modify the interaction surface and/or influence the dynamics of the 250-helix.

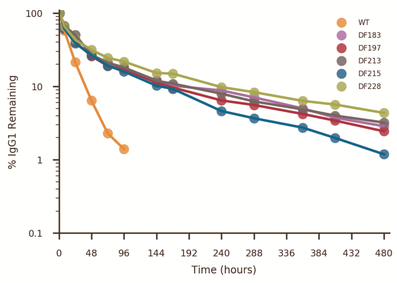
Design of Fc Variants for Enhanced FcRn Binding



- First, we identified the network of interactions mediated by each residue of interest.
- Specific sets of mutations were rationally designed to enhance both hydrophobic interactions within the core of the Fc-FcRn interface and polar and electrostatic interactions at its periphery.
- The impact of each mutation on its interaction network was assessed and, when needed, additional mutations were introduced to maintain the stability of the protein.
- Charge complementarity was introduced at non-contact residues near the protein-protein interface to help reduce the k_{off} rate.

PK of Engineered Fc Domains

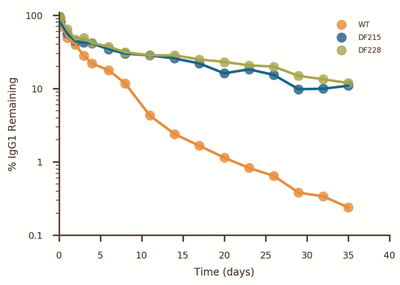
Pharmacokinetics in Tg276 Mice Expressing Human FcRn



Fc	Half-life (hours)	Cmax (µg/mL)	AUCinf (hr•µg/mL)	Clearance (mL/hr/kg)
WT	22	29.3	513.9	3.89
YTE	193	26.3	1682.5	1.19
LS	167	30.6	1731.3	1.16
DF183	145	24.9	1689.3	1.18
DF197	170	22.0	1385.5	1.44
DF213	184	21.4	1546.1	1.29
DF215	220	27.3	1588	1.26
DF228	207	20.1	1741.3	1.15

- **Five lead Fc designs showed significantly improved PK in Tg276 mice that express human FcRn, with DF215 increasing half-life by 10-fold.**

Pharmacokinetics in Cynomolgus Monkeys

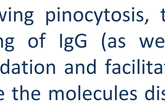


Fc	Half-life (hours)	Cmax (µg/mL)	AUCinf (hr•µg/mL)	Clearance (mL/hr/kg)
WT	4.78	219.9	658	7.64
DF215	15.09	278.5	2988	1.72
DF228	18.77	306.2	3908	1.32

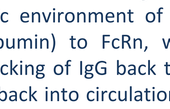
- **Fc designs DF215 and DF228 showed a significant improvement in in PK in cynomolgus monkeys with > 3-fold improvement.**

Modeling IgG Binding to Membrane FcRn

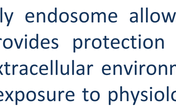
Y conformation

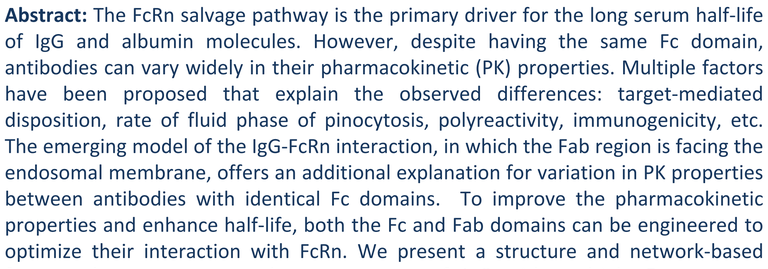


Y/T conformation



T conformation





Detailed above is the current best-fit model of IgG-FcRn complex formation. The "standing-up" orientation of Fc-FcRn engagement is shown as a schematic diagram (top) and molecular diagram (bottom). The IgG-FcRn complex is modelled in the context of membrane bilayer bound FcRn (mFcRn) to illustrate the severe steric clash presented by the membrane bilayer with the Fab domains of the bound IgG in a Y-shaped conformation and mixed Y/T conformation.

- **The model of IgG-mFcRn complex in which IgG adopts a T-shaped is the only conformation that does not impose a severe steric clash between the Fab domains and the membrane bilayer.**
- **Only the standing up T-conformation of IgG supports FcRn binding to IgG and HSA simultaneously.**
- **The model provides an additional explanation for how the Fab domain can impact engagement with membrane FcRn and PK properties.**

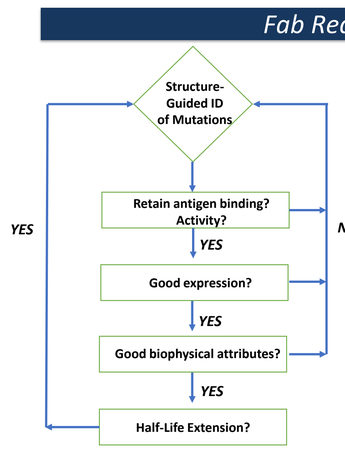
Screening for Enhanced FcRn Binding

Designation	Mutations	Biolayer Interferometry: Fold Increase K_d	Fold Increase K_{on}	Fold Decrease K_{off}	Surface Plasmon Resonance: k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	K_d (nM)
WT	–	NA*	NA	NA	NA	NA	825
YTE**	M252Y/S254T/T256E	8.5	2.3	3.6	NA	NA	NA
LS**	M428L/N434S	9.2	2.6	3.6	NA	NA	NA
DF045	T256D/T307R/Q311V	5.9	2.3	2.6	NA	NA	NA
DF219	T256D/N315D/A378V	7.9	2.8	2.8	NA	NA	NA
DF171	T256D/N286D/T307R/Q311V	8.5	2.5	3.1	NA	NA	NA
DF197	H285N/T307Q/N315D	9.1	2.9	3.0	$1.59x10^6$	$3.28x10^{-2}$	21
DF186	T256D/T307R/Q311V/A378V	9.5	2.9	3.1	NA	NA	NA
DF186	T256D/T307R/Q311V/A378V	9.5	2.9	3.1	NA	NA	NA
DF216	H285D/Q311V/A378V	9.6	3.4	2.8	NA	NA	NA
DF223	T256D/H285D/A378V	10.3	3.7	2.7	NA	NA	NA
DF183	T256D/Q311V/A378V	11.0	3.2	3.2	$1.93x10^6$	$4.02x10^{-2}$	21
DF227	T256D/H285D/N286D/T307R/A378V	11.6	3.6	3.2	NA	NA	NA
DF228	T256D/H286D/T307R/Q311V/A378V	12.4	3.2	4.0	$1.39x10^6$	$1.42x10^{-2}$	10
DF215	T307Q/Q311V/A378V	12.4	3.4	3.7	$1.37x10^6$	$2.70x10^{-2}$	19
DF213	H285D/T307Q/A378V	13.2	3.8	3.3	$0.80x10^6$	$1.92x10^{-2}$	24
DF229	T256D/H285D/T307R/Q311V/A378V	14.0	3.7	3.7	NA	NA	NA

- **More than 150 designed Fc mutants were recombinantly expressed as full length IgG and evaluated for binding to human FcRn by biolayer interferometry. More than 10 distinct variants had greater than a 5-fold increase in K_d as compared to IgG with a native Fc domain, with many of these lowering k_{off} at pH 6.0 by more than 2.5-fold.**
- **Lead Fc variants DF183, DF197, DF213, DF215 and DF228 were further characterized by surface plasmon resonance and exhibited greater than 30-fold affinity enhancement to FcRn at pH 6.0, primarily driven by a significantly slower k_{off} rate.**
- **Enhancement of FcRn binding affinity was achieved by mutation of centrally-located Fc-FcRn interface residues to improve hydrophobic interactions, and mutation of peripheral residues to improve electrostatic and polar interactions.**
- **The Fc residues T256, T307, H285, N286 and N315 located on the periphery of the interface were mutated to polar and charged amino acids to complement the positively charged N-terminal region of the FcRn β-domain.**
- **Additionally, Q311 located at the Fc-FcRn interface, were mutated to hydrophobic residues. However, aromatic amino acids were avoided to minimize the impact on thermal stability.**

Fab Re-engineering For Improved PK

Fab Redesign Strategy

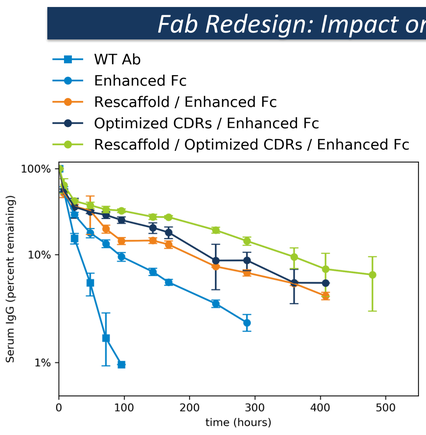


Step 1:
Selection of optimal scaffold.

Step 2:
Re-engineer CDR loops to minimize liabilities (e.g. large hydrophobic or charged patches).

Step 3:
Iterative design approach to incorporate optimal CDRs and scaffold designs that improve PK and retain functionality.

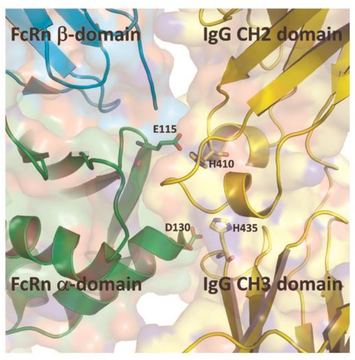
Fab Redesign: Impact on PK in Tg276 Mice



- **Addition of Enhanced Fc (DF215) for improved PK.**
- **Antibody resurfacing (framework optimization) further improved PK.**
- **Potential liabilities in LCDR1 and HCDR3 loops were identified and re-engineered.**
- **Combination of enhanced Fc, resurfacing, and CDR redesign significantly improved PK. properties of parental antibody.**
- **Multiple independent paths towards improved PK were identified.**

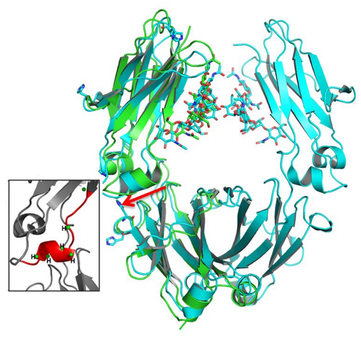
Structural Analysis of Fc:FcRn Interaction

Fc:FcRn Interface



Structural view of human IgG CH2-CH3 domains (yellow) in complex with human FcRn α (green) and β2M (cyan) domains. The pH specific binding is driven by the protonation of His310 and His435 (shown as sticks) on IgG and their interaction with Glu115 and Asp130 (shown as sticks) on the FcRn α domain.

Fc pH Conformation



Crystal structures of the Fc domain at pH 4.0 and pH 6.5 show lateral movement of the 250-helix of the CH2 domain in response to pH change. Additionally, deuterium exchange studies revealed enhanced exchange rates on the 250-helix at lower pH, further supporting the pH specific conformation change in this region.

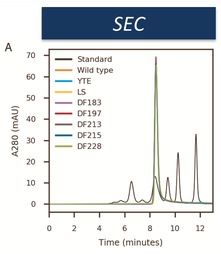
- **The pH sensitivity of the Fc:FcRn interaction is crucial to the IgG salvage pathway, as binding occurs within the acidic endosome and release back into solution occurs at physiological pH.**
- **The affinity of the Fc:FcRn at pH 6.0 is weak (~ 600 nM); however, the rate of association (k_{on} ~ 10^5 $M^{-1}s^{-1}$) is typical of an antibody:antigen interaction, while the rate of dissociation is much faster (k_{off} ~ 0.1 s^{-1}) .**
- **Rational engineering to slow the k_{off} rate at pH 6.0 could significantly improve the binding kinetics of Fc:FcRn.**

Biophysical Properties and Effector Function

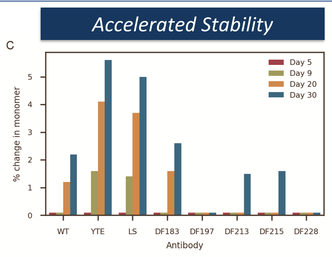
Thermal Stability

Variant	Tm (°C)
Wild type	68.9
YTE	61.7
LS	68.0
DF183	64.0
DF197	68.4
DF213	70.2
DF215	69.2
DF228	66.7

SEC



Accelerated Stability



- **Lead Fc designs have minimal impact on the thermal stability of the CH2 domain.**
- **All lead Fc designs have a typical monomeric profile, a retention time comparable to wild type Fc on SEC, and minimal aggregation.**
- **All lead Fc designs had comparable stability to wild type in a 30 day accelerated stability study.**

Designation	FcγRIIIa EC_{50} (nM)	ADCC EC_{50} (nM)	C1q EC_{50} (nM)	CDC EC_{50} (µg/mL) **
Wild Type	537.5	6.1	22.6	0.72
YTE	1014.8	15.0	21.5	> 20
LS	191.6	4.8	21.4	0.35
DF183	87.2	3.9	16.5	0.1
DF197	97.4	8.3	46.2	2.54
DF213	139.4	4.0	20.7	0.27
DF215	142.0	2.7	21.9	0.14
DF228	154.5	2.7	21.0	0.22

Lead Fc variants were tested with the Rituximab Fab domain for binding to FcγRIIIa and C1q by ELISA. Additionally, they were tested for ADCC and CDC activity in cell based assays to confirm that they retain both binding to the receptor and functionality.

- **All lead Fc designs maintain binding to FcγRI, IIa, IIb, IIIa, C1q and TRIM21 with similar or stronger binding affinity than wild type Fc.**
- **All lead Fc designs have similar or higher levels of ADCC and CDC activity as compared to wild type Fc.**
- **Binding to FcRn at pH 6.0 has been significantly improved without sacrificing stability or functionality of the Fc domain.**

Conclusions

- **We developed a structure- and network-based approach to identify molecular features governing Fc-FcRn interactions and used this approach to identify multiple Fc variants that enhance FcRn binding at endosomal pH.**
- **Incorporation of these Fc variants on control IgGs significantly decreased clearance rates (by up to 83%) and enhanced elimination half-life of antibodies (>3.9 fold) in cynomolgus monkeys and human FcRn transgenic mice.**
- **The lead variants maintain the desired effector functions of the antibody such as ADCC and CDC and are thermally stable.**
- **Additionally, multiple paths for Fab domain engineering to improve PK properties were identified by framework optimization and CDR redesign to remove problematic sites.**
- **The combination of Fc and Fab domain engineering can be used to identify lead drug candidates with the optimal PK properties.**
- **We anticipate this approach will be used to engineer the next class of antibody based therapeutics that will be used as prophylactics or to treat chronic conditions with minimal dosing and an extended therapeutic window.**

Visterra