Clayton Biotechnologies

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Clayton Foundation for Research
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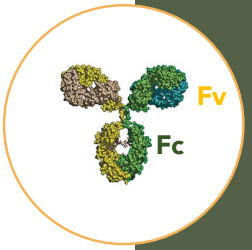
University of Texas at Austin

Engineered Fc Antibodies for Enhanced Serum Half life.

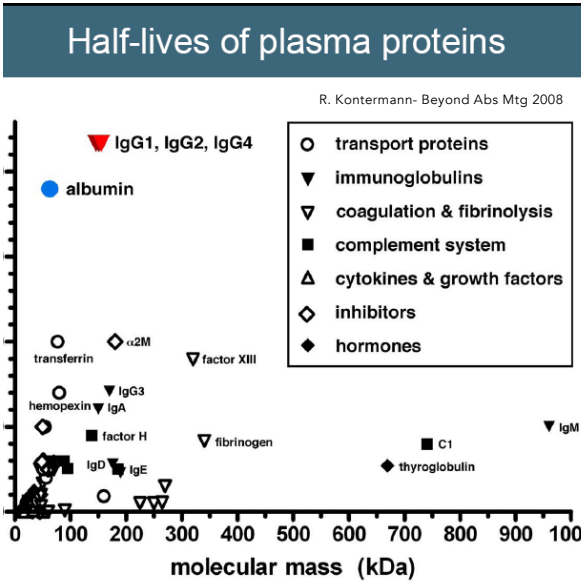
Fc engineered Aglycosylated Antibodies
Enhanced Serum Half-Life.

- **DHS Fc domains confer extended half life to IgG antibodies by enhanced pH-dependent binding to FcRn.**
- **DHS technology** refers to 3 amino acid mutations in the Fc domain
- DHS antibodies have **higher binding to FcRn** at **endosomal pH** (5.8).
- DHS antibodies do not bind to FcRn at serum pH (7.4).
- This property confers **longer half life** in **humanized mouse models** (several models were used).
- 3rd Party evaluation – **DHS overcomes developability issues** and ongoing **NHP studies** are promising
- **Differentiated** from the "YTE" Fc domains (Medimmune) and "LS" Fc domains (Xencor) because YTE and LS bind to FcRn at serum pH (7.4).
- **Glycosylated or aglycosylated** Fc domain.
- Strong **patent** position.

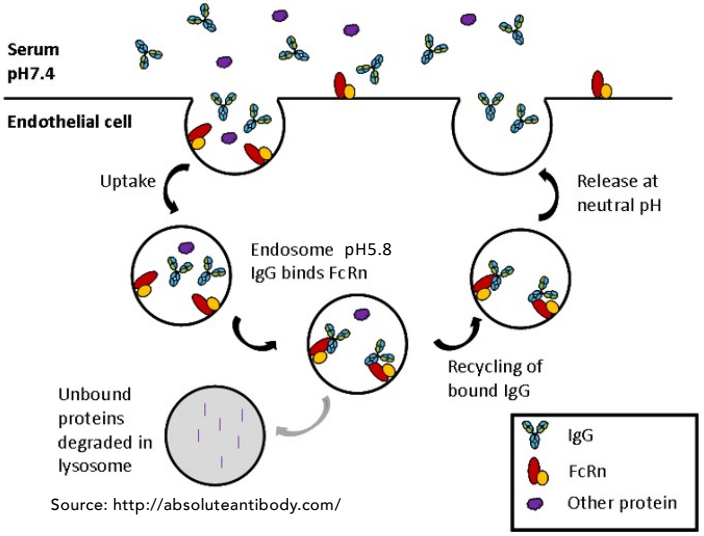


Why enhance serum half-life?

- **Therapeutic antibodies serum half-life** of a few days up to 3-4 weeks.
- **Longer half-life of mAbs** compared to other biologic therapeutics due to their **ability to bind to FcRn**
- Increasing half-life enhances **therapeutic window** for an antibody drug
  - Allows to **dose with less drug**
  - **Decrease the frequency** of patient administration
  - **Increased efficacy for drugs** that require long exposure for effective activity.



FcRn recycling of IgG antibodies is pH dependent.

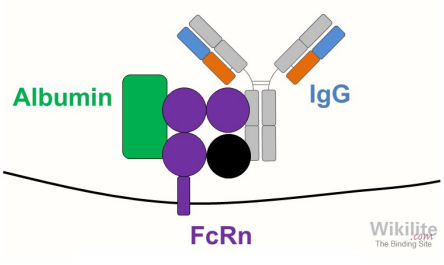


Source: http://absoluteantibody.com/

Improving IgG PK:

- Enhanced affinity to FcRn at endosomal pH (5.8)
- Minimal or no binding at serum pH (7.4)

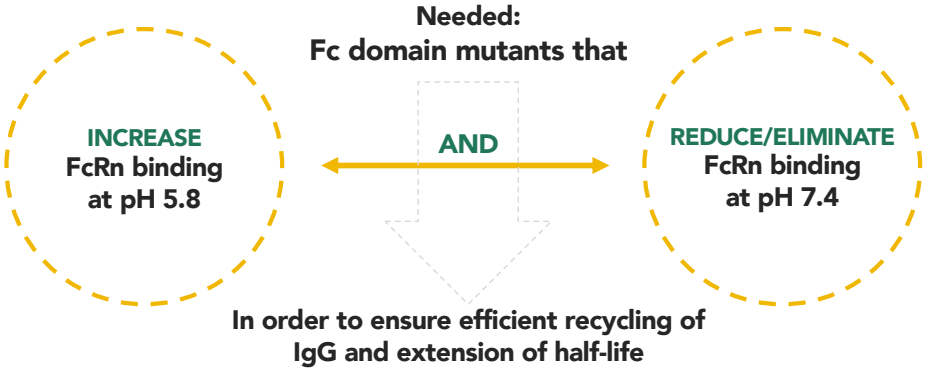
Antibodies with increased serum half-life by increased binding to FcRn.



- **YTE Fc domains** (Medimmune) and **LS Fc domains** (Xencor) bind with **increased affinity to FcRn**.
- YTE mutation in the Fc domain of motavizumab (mota-YTE) decreased clearance by 71% to 86% and increased serum half-life 2- to 4-fold (up to 100 days) compared with the parent antibody, motavizumab in studies with healthy adult subjects (MedImmune).
- While both YTE and LS have increased binding affinity to FcRn at pH 5.8, they also have increased binding affinity at pH7.4.

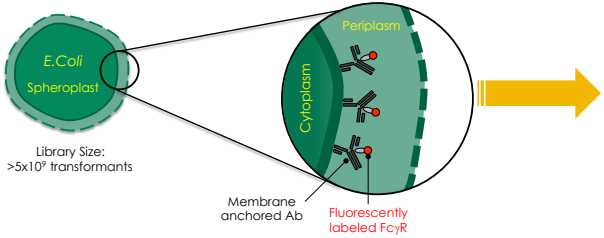
Problem: While Fc mutations that increase FcRn binding at pH 5.8 should increased serum half-life significantly, **if the binding affinity is also increased at pH 7.4, this will accelerate the elimination of the antibody** because it cannot be released from the endosome.

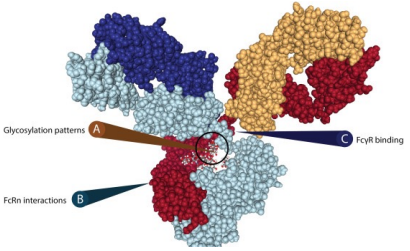
Fc engineered Aglycosylated Antibodies with Enhanced Serum Half-Life.



Proprietary Platform for Engineering the Fc domain of Antibodies.

- **The innovation** behind this **unique technology platform** stems from **Prof. George Georgiou's** laboratory at The University of Texas at Austin. The platform allows the identification of specific Fc engineered entities that have improved functional properties.
- **The technology** is based on a **proprietary bacterial display system** containing a **large library of antibody Fc mutants**. The bacterial display system allows for both positive and negative selection strategies to be applied.





DHS

Enhanced binding at pH 5.8

No binding at pH7.4

https://doi.org/10.1016/j.ijbiomac.2018.07.141

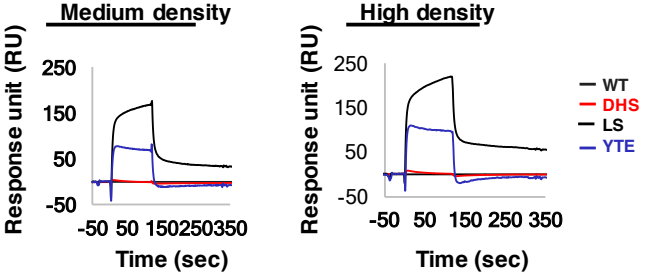
Optimization of pH-dependent binding.

DHS does not show any binding response for FcRn at pH 7.4 and has enhanced affinity for FcRn than wild type at pH 5.8.

hFcRn Binding at pH 5.8

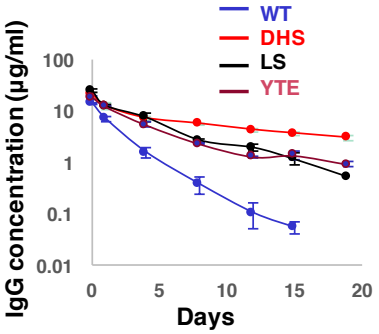
	K_D (nM)	Fold
WT-IgG1	550 ± 46	-
DHS-IgG1	111 ± 20	5.0
LS-IgG1	55 ± 3.2	10.0
YTE-IgG1	23 ± 1.0	23.9

hFcRn Binding at pH 7.4



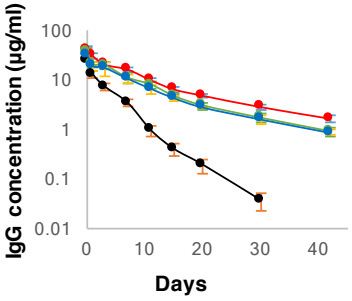
Pharmacokinetics in Tg272 hFcRn mice

(n=10, 2 mg/kg i.v.).



	Clearance (ml/day/kg)	β phase $T_{1/2}$ (h)	$AUC_{inf.}$ (µg*days/ml)	Vss (ml/kg)
WT	0.58 ± 0.17	49.3 ± 8.8	41.2 ± 23.6	1.11 ± 0.15
DHS	0.061 ± 0.003	336 ± 49.5	326.6 ± 17.9	0.28 ± 0.01
YTE	0.099 ± 0.004	107 ± 14.3	200.7 ± 9.4	0.34 ± 0.01
LS	0.111 ± 0.028	204.3 ± 17.4	178.7 ± 12.5	0.42 ± 0.01

Pharmacokinetics - “Scarlet” (FcγRKI, FcRnKI, β2mKI, IgG1KI) (n=10, 2mg/kg).



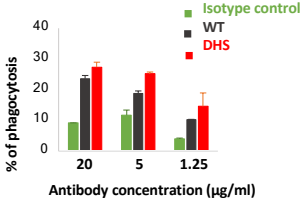
	Clearance (ml/day/kg)	β phase $T_{1/2}$ (h)	$AUC_{inf.}$ (µg*days/ml)	Vss (ml/kg)
WT	0.141 ± 0.014	98.4 ± 0.1	146.2 ± 15.9	0.40 ± 0.04
DHS	0.026 ± 0.001	345.0 ± 5.0	771.6 ± 10.6	0.11 ± 0.01
YTE	0.034 ± 0.004	302.9 ± 18.9	597.5 ± 37.5	0.16 ± 0.02
LS	0.036 ± 0.005	292.5 ± 24.9	549.4 ± 43.4	0.17 ± 0.02

DHS does not diminish effector function

Relative Affinities to Fc receptors (measured by SPR)

	FcγRI	FcγRIIa$_{H131}$	FcγRIIa$_{R131}$	FcγRIIb	FcγRIIIa$_{F158}$	FcγRIIIa$_{V158}$	C1q
WT	1	1	1	1	1	1	1
DHS	0.8	0.8	1.2	0.9	2.4	0.9	1.7
YTE	0.7	0.4	0.4	0.4	0.3	0.2	1

ADCP (M1-macrophages)

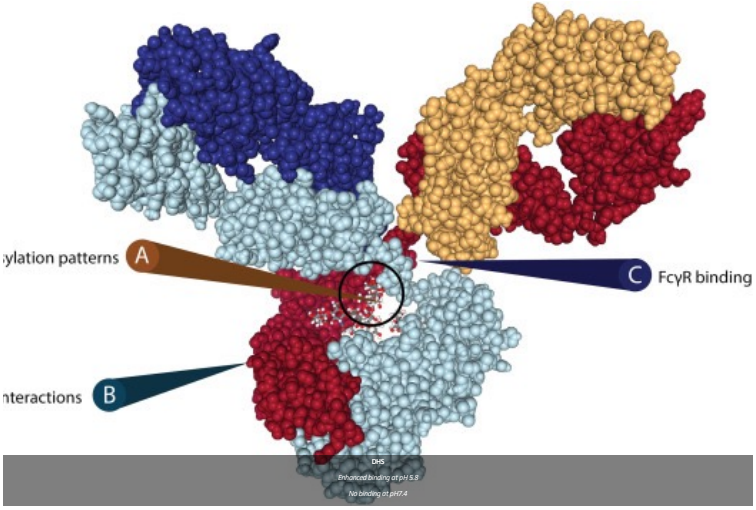


Summary of data

Our **DHS Fc domain** was engineered specifically for **higher affinity to FcRn at endosomal pH** and a **very low affinity for the high density FcRn at neutral pH**, thereby resulting in **superior serum half life**.

- Enhanced affinity for FcRn at endosomal pH 5.8 and no detectable binding to FcRn at serum pH 7.4.
- **Maintains FcγR binding activities and effector functions** (in IgG1, IgG2, IgG3, and IgG4 formats).
- Also exists in an aglycosylated format with no effector function.
- **Significantly slower serum clearance than wild type IgG1 (**slower clearance compared to LS and YTE).
- **Excellent pharmacokinetics** in hFcRn transgenic mice also expressing hFcRs and hIgG1.

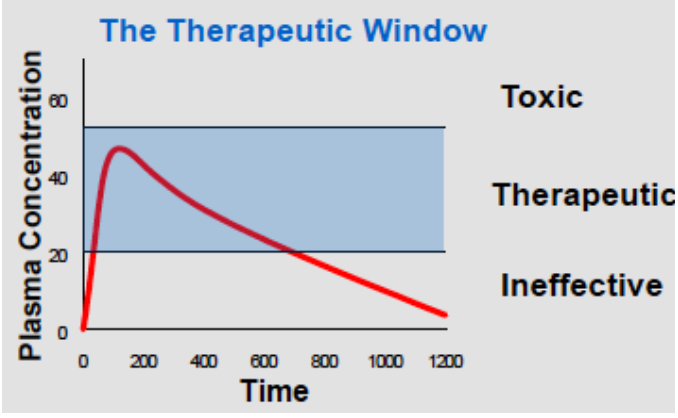
DHS Fc Domain



DHS Fc domain

- **Increased binding** to FcRn **endosomal pH.**
- **Very low affinity** for the high density FcRn at **serum pH**.
- Result: **superior serum half life.**
- Engineered for maintaining or superior **ADCC and ADCP activity.**
- Also exists in format with **no ADCC** activity.

DHS to Enhance Therapeutic Window for your Antibody Drug



- ✓ Allows to **dose with less drug** while maintaining or improving efficacy of the drug.
- ✓ **Decrease the frequency** of patient administration
- ✓ **Increased efficacy for drugs** that require long exposure for effective activity.
- May help **overcome formulation and aggregation issues** (reduced concentration)
- Allows for **subcutaneous administration**
- Works either as a **glycosylated or aglycosylated** antibody.
- Strong patent position.

Pipeline of Novel Fc domains.

Fc domains selectively increase or decrease binding to individual activating or inhibitory FcγRs or C1q, with a dramatic effect on the effector function of the antibody.

Fc Domain	Fc ID	Biochemical Feature	Main Optimized Function
Immune Inhibitor	**CHL13/21**	Selective binding to **FcγRIIb** with or without ITIM activity	Increased agonistic activity for TNF-sFR Ligands; Blocks activation of immune cells
Cytotoxic	**Fc1004**	Increased binding ratio **FcγRIIa / FcγRIIb**	Increased ADCP by monocytes/macrophages
	TH409	Selective binding to **FcγRIIIa**	Increased ADCC. ADCP
	IgGA.3	Binding to **FcaRI** as well as the **FcγR**	Neutrophil recruitment - Highly potent ADCC and ADCP mediated cell killing
Dendritic Cell Activating	**Fc5**	Selective binding **FcγRI**	Dendritic cell activation
Complement Activating	**Fc801/802**	Selective binding to **C1q**	Increased CDC and opsonization
	Fc805	IgG1-like binding to **activating FcγRs**; increased binding to **C1q**; no binding to inhibitory **FcγRIIB**	Increased ADCC. ADCP & CDC

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Thank you for your attention