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**Clayton Foundation for Research**

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# ***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).
- 3<sup>rd</sup> 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.

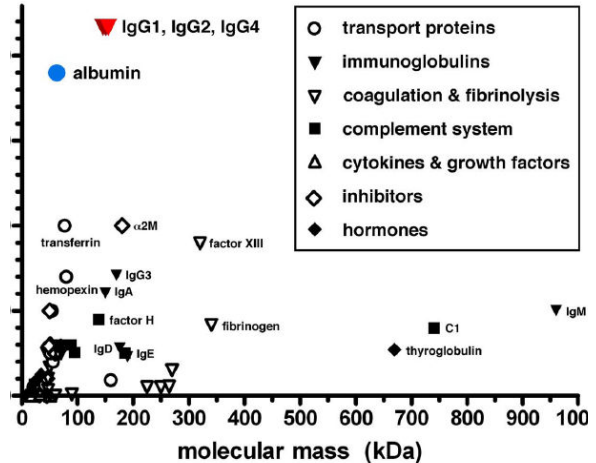


# Why enhance serum half-life?

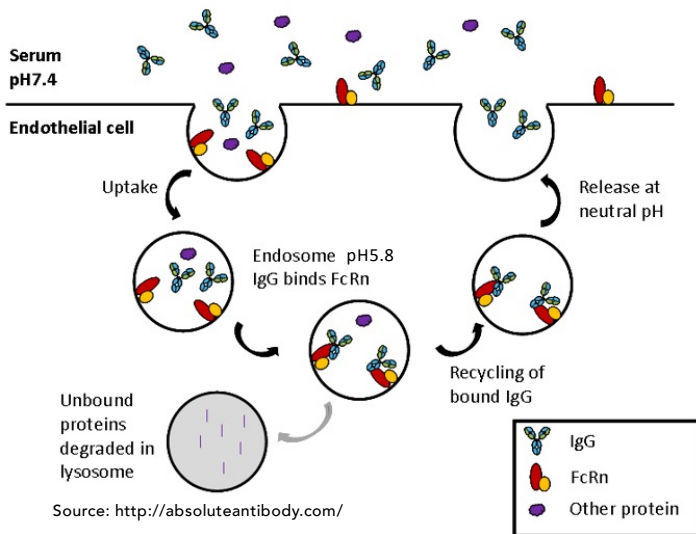
- Therapeutic antibodies serum half-life of a few days up to 3-4 weeks.
- Longer half-life of mAbs compared to other biologic therapeutics due to their ability to bind to FcRn
- Increasing half-life enhances therapeutic window for an antibody drug
  - Allows to dose with less drug
  - Decrease the frequency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

## Half-lives of plasma proteins

R. Kontermann- Beyond Abs Mtg 2008



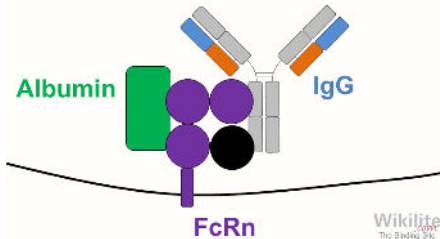
# FcRn recycling of IgG antibodies is pH dependent.



## Improving IgG PK:

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

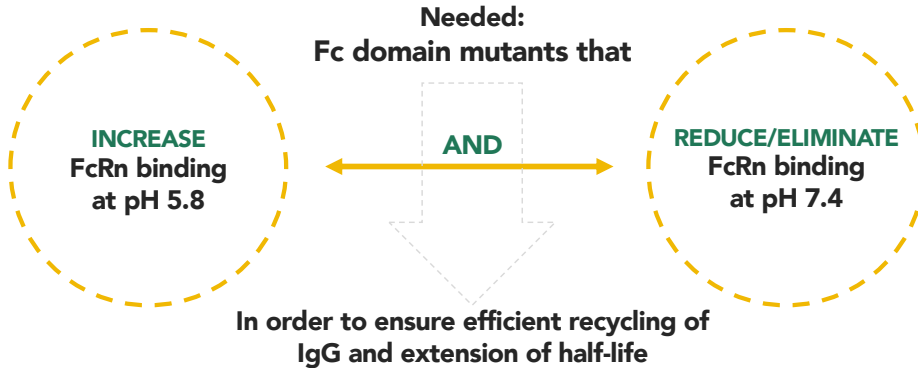
# Antibodies with increased serum half-life by increased binding to FcRn.



- **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 pH 7.4.

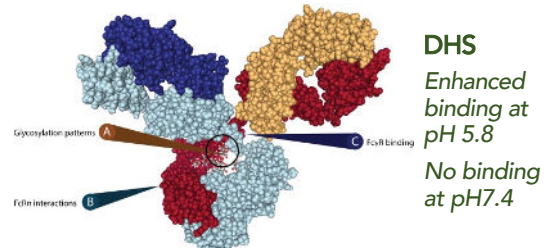
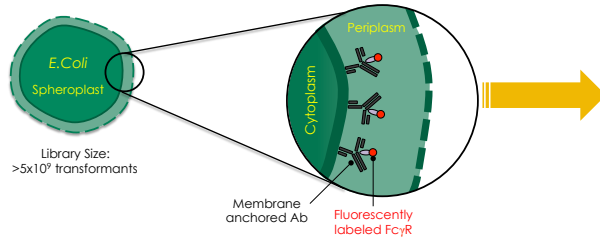
**Problem:** While Fc mutations that increase FcRn binding at pH 5.8 should increase serum half-life significantly, **if the binding affinity is also increased at pH 7.4, this will accelerate the elimination of the antibody** because it cannot be released from the endosome.

# 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.



<https://doi.org/10.1016/j.jbiomac.2018.07.141>

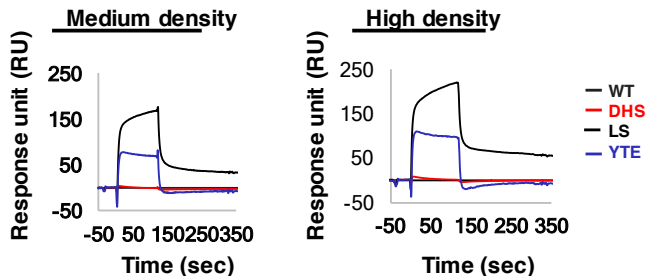
# Optimization of pH-dependent binding.

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

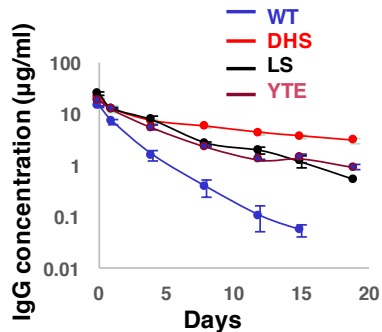
	$K_D$ (nM)	Fold
WT-IgG1	$550 \pm 46$	-
<b>DHS-IgG1</b>	<b><math>111 \pm 20</math></b>	<b>5.0</b>
LS-IgG1	$55 \pm 3.2$	10.0
YTE-IgG1	$23 \pm 1.0$	23.9

## hFcRn Binding at pH 7.4



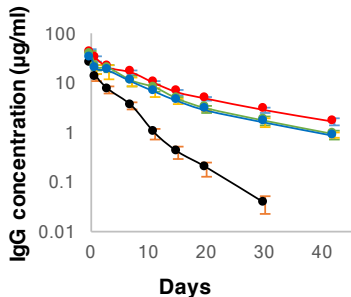


# Pharmacokinetics in Tg272 hFcRn mice (n=10, 2 mg/kg i.v.).



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

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



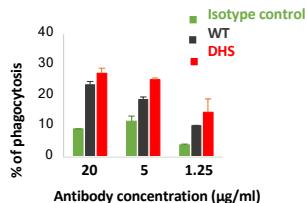
	Clearance (ml/day/kg)	β phase T <sub>1/2</sub> (h)	AUC <sub>inf.</sub> (µg*days/ml)	Vss (ml/kg)
WT	0.141 ± 0.014	98.4 ± 0.1	146.2 ± 15.9	0.40 ± 0.04
DHS	0.026 ± 0.001	345.0 ± 5.0	771.6 ± 10.6	0.11 ± 0.01
YTE	0.034 ± 0.004	302.9 ± 18.9	597.5 ± 37.5	0.16 ± 0.02
LS	0.036 ± 0.005	292.5 ± 24.9	549.4 ± 43.4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

# DHS does not diminish effector function

Relative Affinities to Fc receptors (measured by SPR)

	FcγRI	FcγRIIa <sub>H131</sub>	FcγRIIa <sub>R131</sub>	FcγRIIb	FcγRIIIa <sub>F158</sub>	FcγRIIIa <sub>V158</sub>	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)

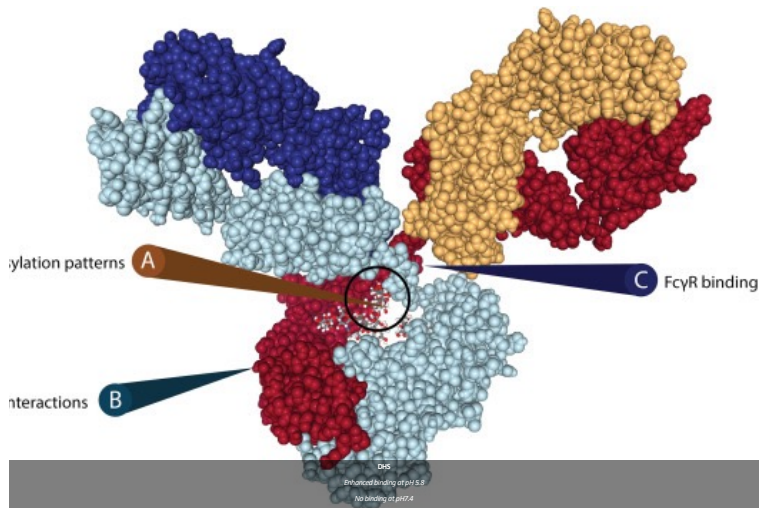


# Summary of data

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.

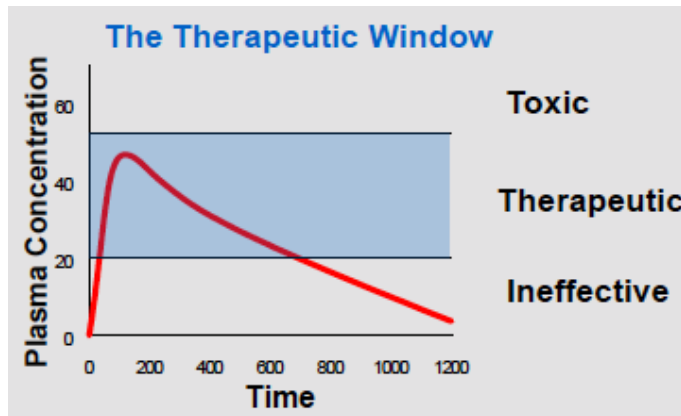
# DHS Fc Domain



## 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 ADCCP 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 <b>FcγRIIb</b> with or without ITIM activity	Increased agonistic activity for TNF-sFR Ligands; Blocks activation of immune cells
Cytotoxic	Fc1004	Increased binding ratio <b>FcγRIIa / FcγRIIb</b>	Increased ADCP by monocytes/macrophages
	TH409	Selective binding to <b>FcγRIIIa</b>	Increased ADCC. ADCP
	IgGA.3	Binding to <b>FcαRI</b> as well as the <b>FcγR</b>	Neutrophil recruitment - Highly potent ADCC and ADCP mediated cell killing
Dendritic Cell Activating	Fc5	Selective binding <b>FcγRI</b>	Dendritic cell activation
Complement Activating	Fc801/802	Selective binding to <b>C1q</b>	Increased CDC and opsonization
	Fc805	IgG1-like binding to <b>activating FcγRs</b> ; increased binding to <b>C1q</b> ; no binding to inhibitory <b>FcγRIIB</b>	Increased ADCC. ADCP & CDC



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**Thank you for your  
attention**

