In Vitro Assay to Predict Human IgG Half-Life

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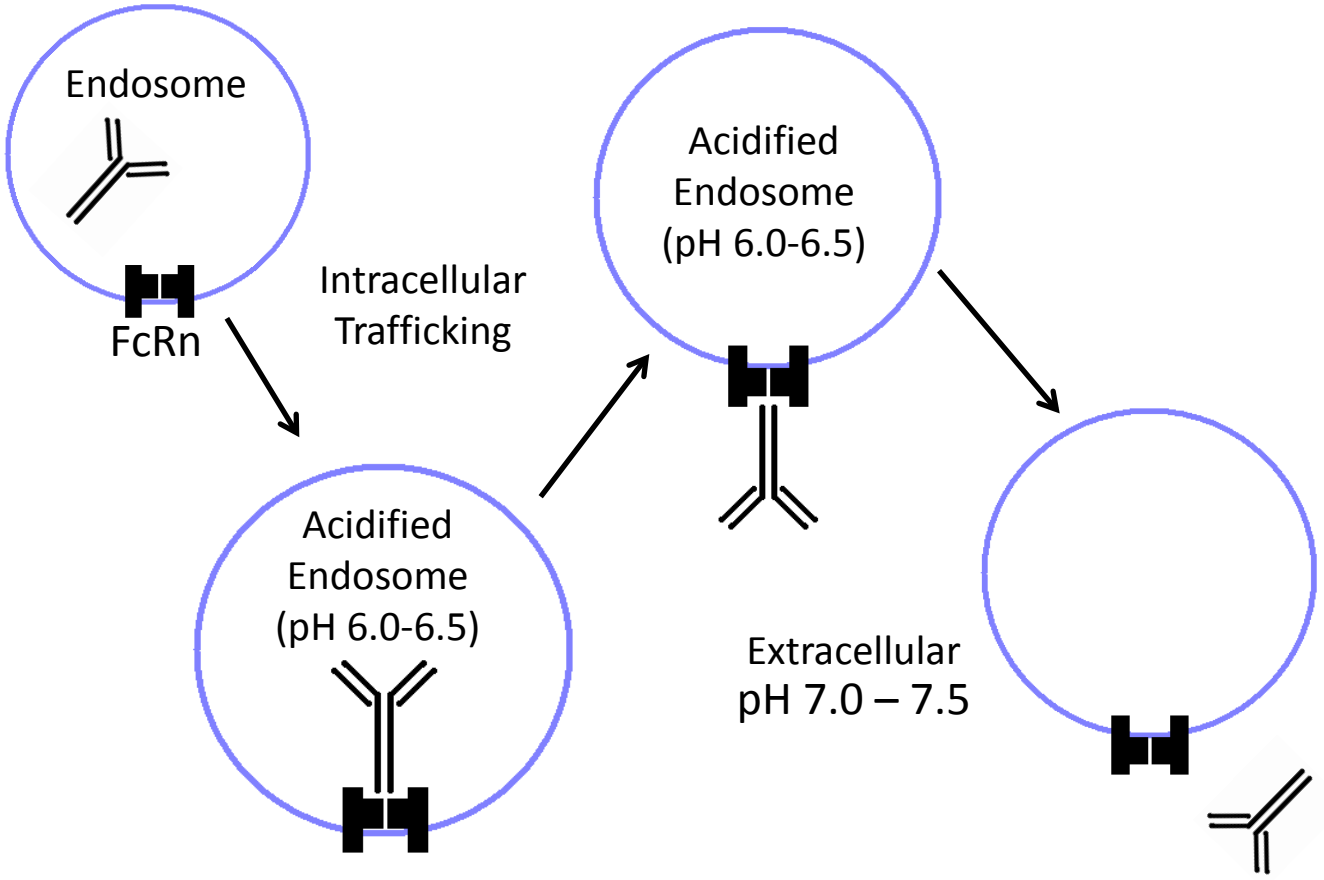
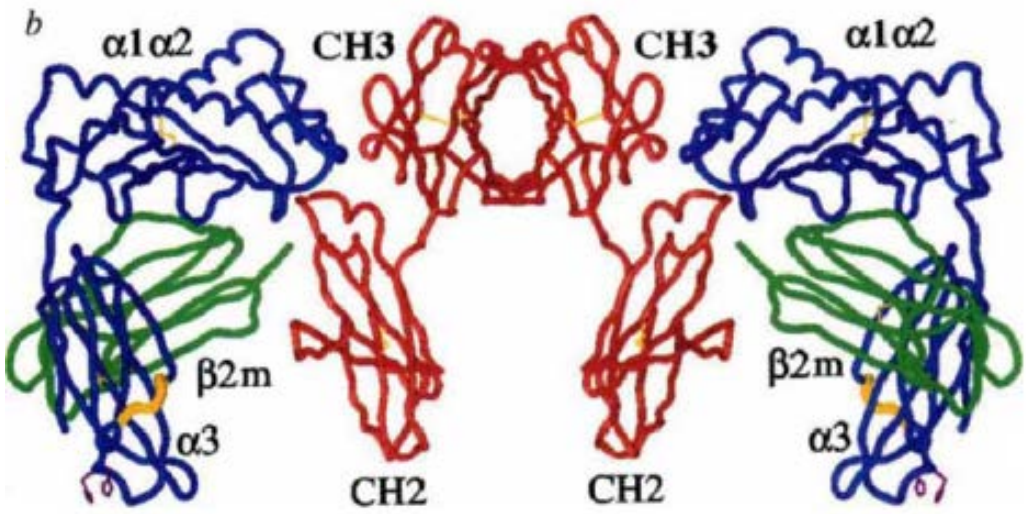
Clinical Relevance of Antibody Half-Life

- For therapeutic mAbs, longer half-life enhances:
  - Safety
  - Efficacy
  - Cost
  - Convenience
- For diagnostic mAbs, shorter half-life is usually desired.
- Lack of cost effective, high-throughput assays to predict human IgG half-life
  - Implement during screening and candidate selection stages of discovery

Mechanism IgG Half-Life





Burmeister et al, Nature 1997

Assessing IgG-FcRn Interactions

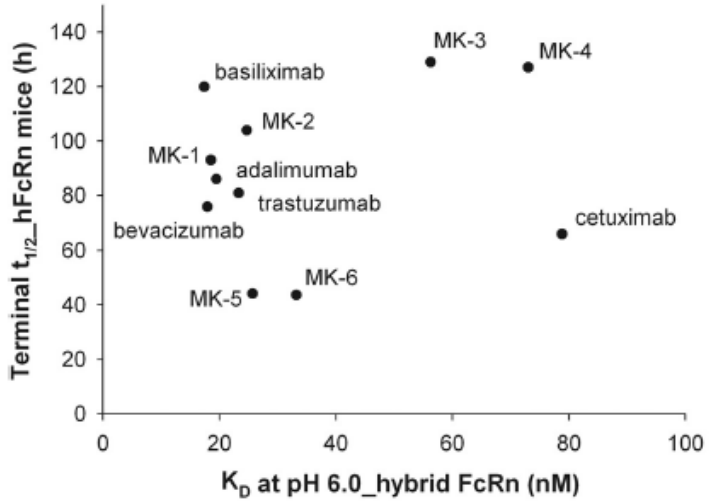
Wang et al., Drug Metab Dispos 2011

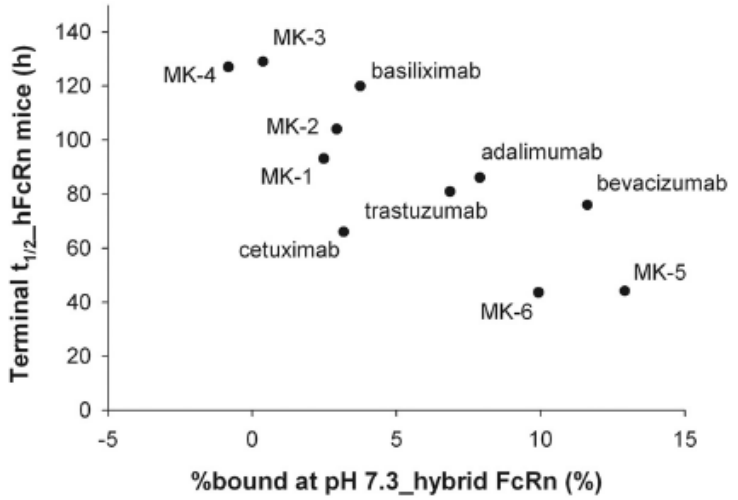
TABLE 1

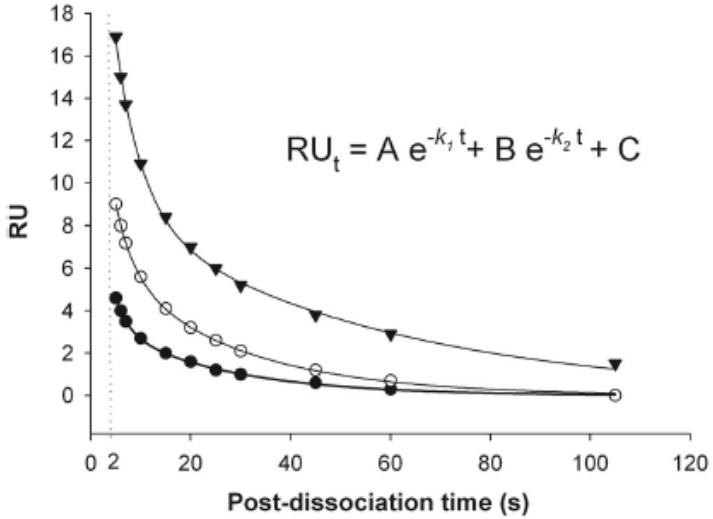
In vitro human FcRn binding and dissociation parameters of MK-1 to MK-6

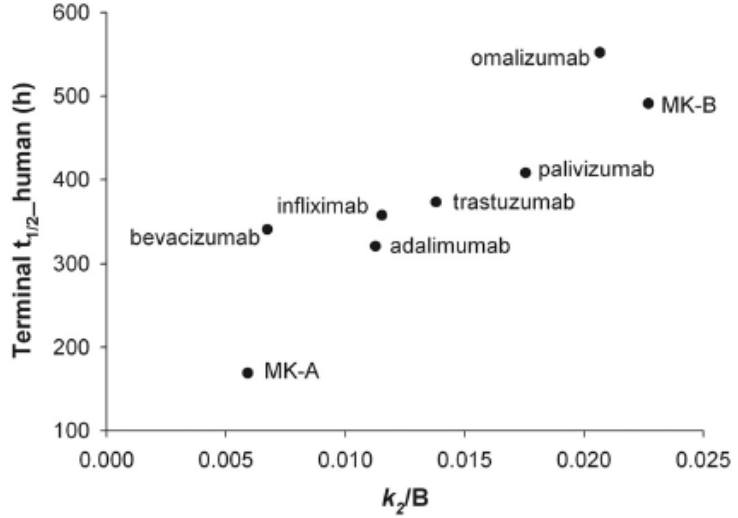
Compounds	MK-1	MK-2	MK-3	MK-4	MK-5	MK-6
Isotype	IgG1	IgG2	IgG1	IgG2	IgG1	IgG2
Fab[a]	A	A	B	B	C	C'
K_D (nM) at pH 6.0	56.3	73.0	18.5	24.7	25.7	33.2
%bound at pH 7.3 (%)	0.4	0.0	2.5	2.9	12.9	9.9

[a] MK-1 and MK-2 have an identical Fab domain A, MK-3 and MK-4 have an identical Fab domain B, and MK-5 and MK-6 have closely related, but slightly different, Fab domains C and C'.





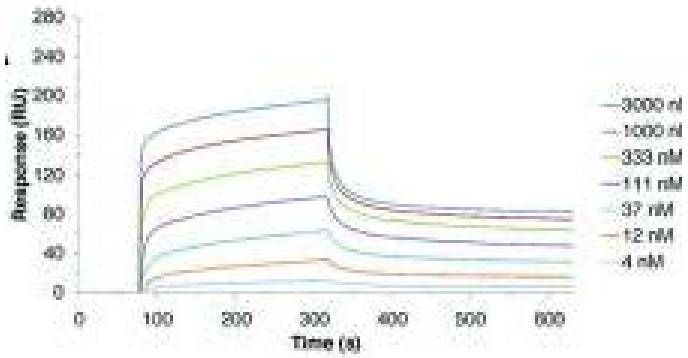


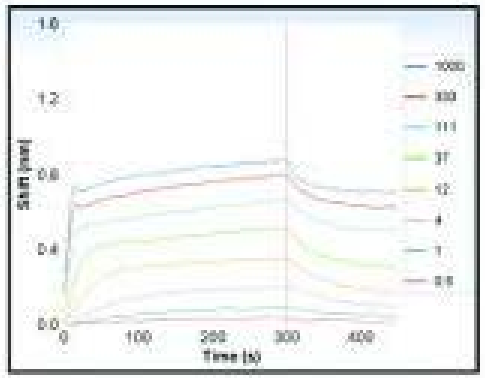


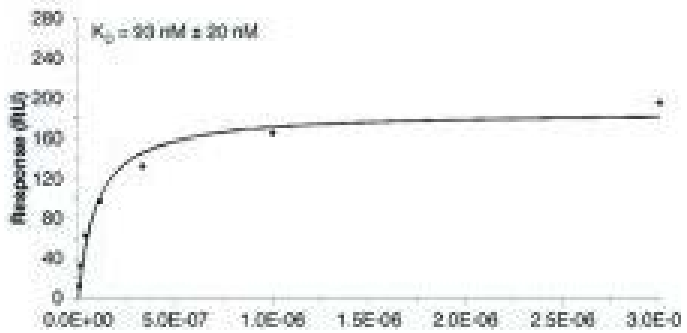
IgG-FcRn Interaction on SPR vs BLI Neuber et al., mAbs 2014

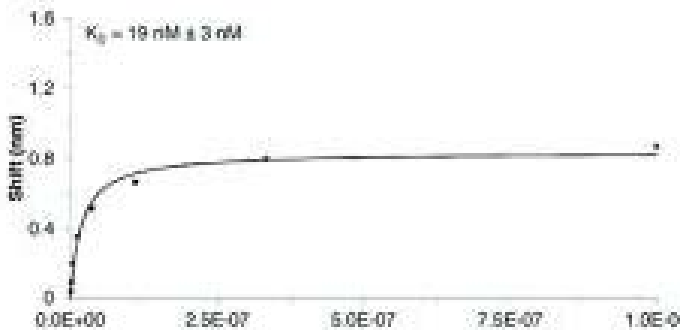
SPR

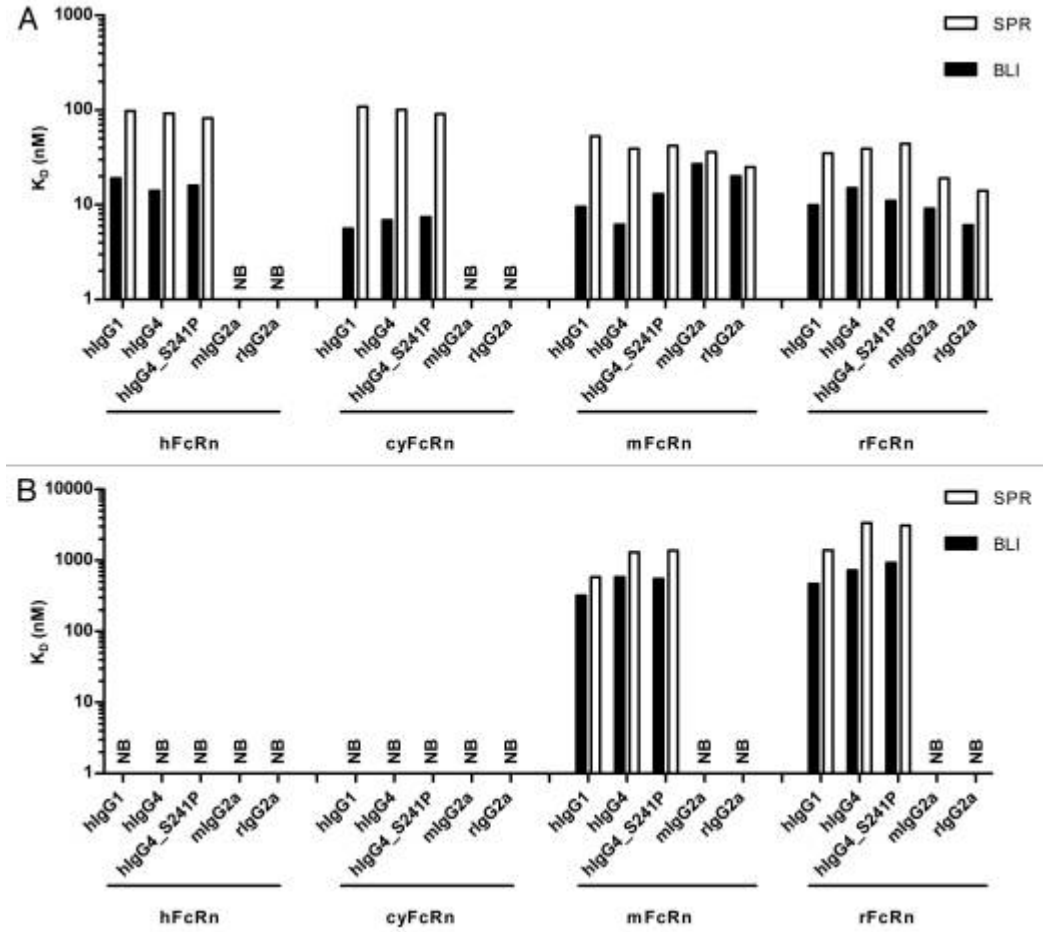
BLI











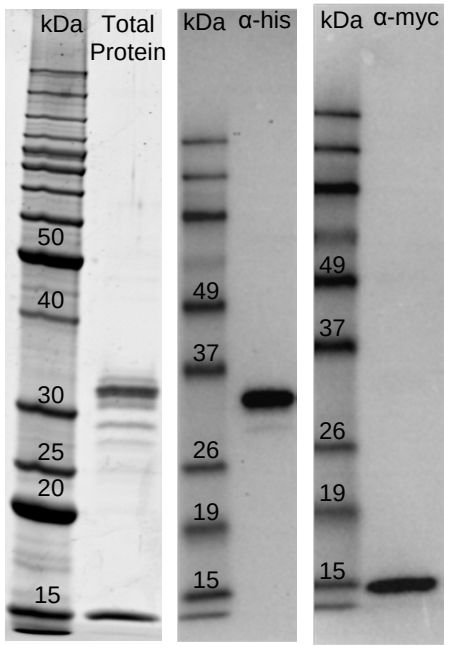
BLI Assay for Half-Life Prediction Souders et al., mAbs 2015

- Extend current knowledge to combine:
  - Association at acidic pH
  - Dissociation at physiological pH
  - On and off rates (rather than equilibrium constants)

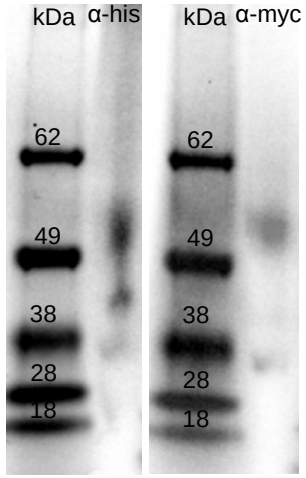
- Implement assay to screen for potential therapeutic mAbs with high half-life and investigate sequence or post-translational modifications that may enhance half-life.

Production of High-Quality Reagents

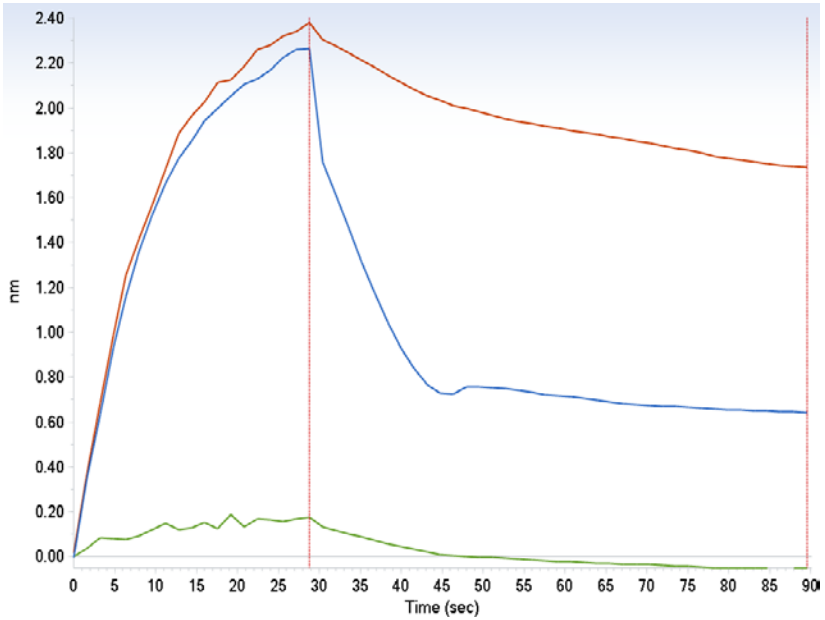
FcRn heterodimer purified from stable cell line producing αFcRn-His and β_2M-Myc



Reduced, denatured



Native

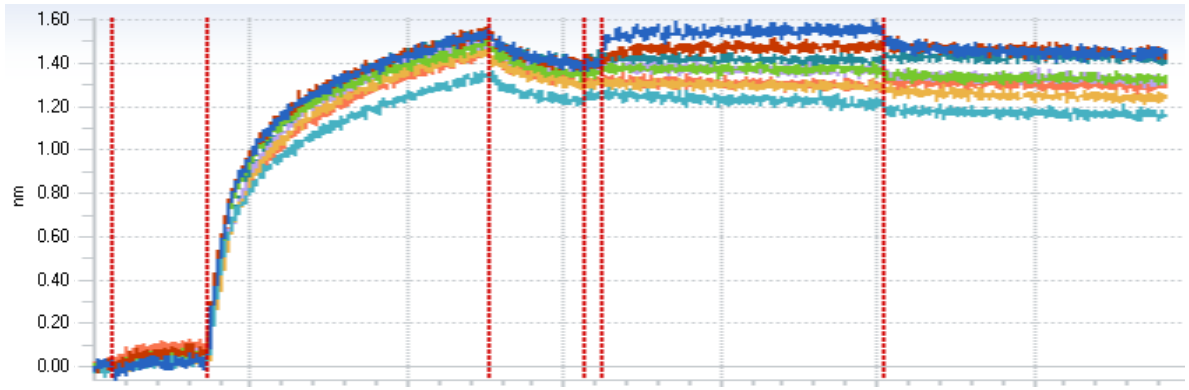


Condition			
Association pH	6.0	6.0	7.5
Dissociation pH	7.5	6.0	7.5
K_{on} (1/s)	0.110	0.105	negligible
K_{off} (1/s)	0.101	0.020	N/A

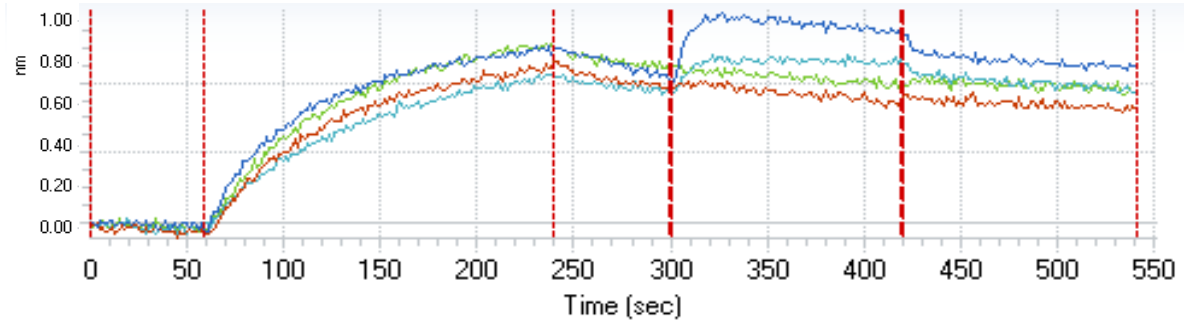
Souders et al., mAbs 2015

Assay Development Biosensor Selection

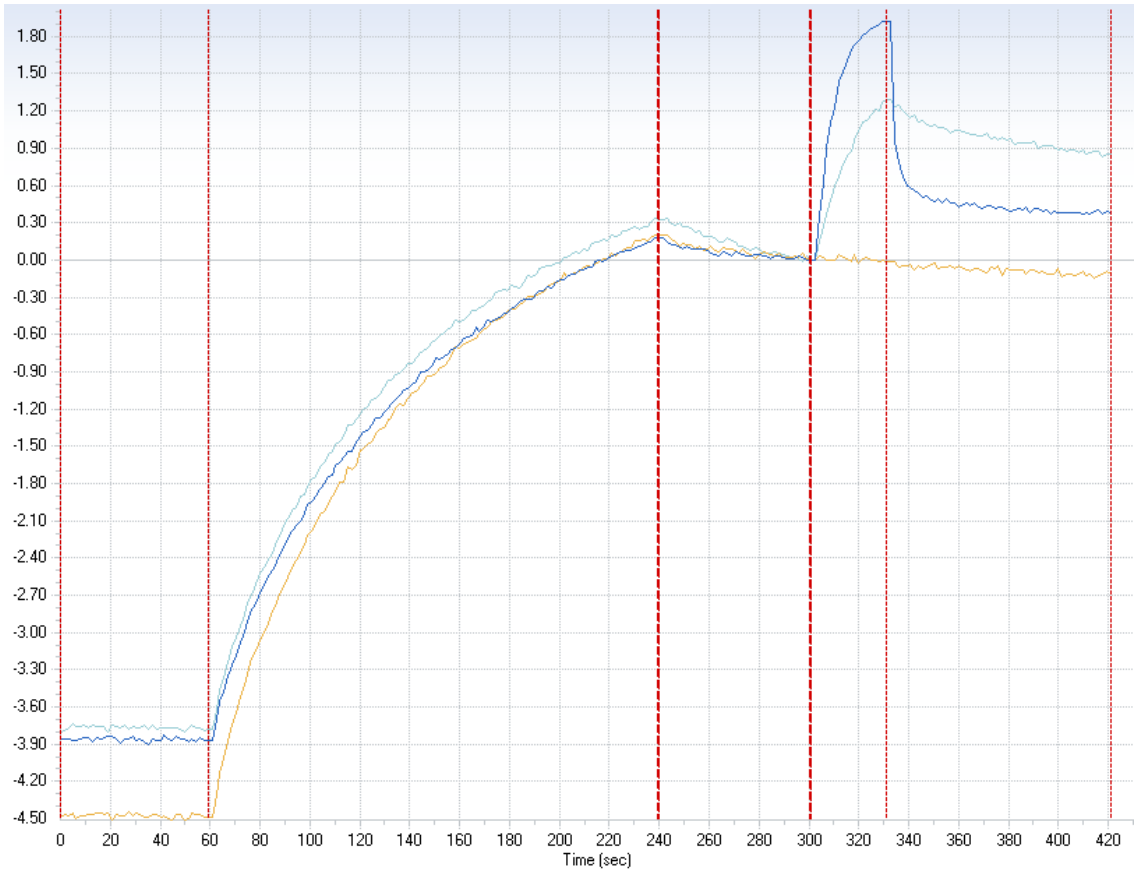
Amine Reactive Biosensors



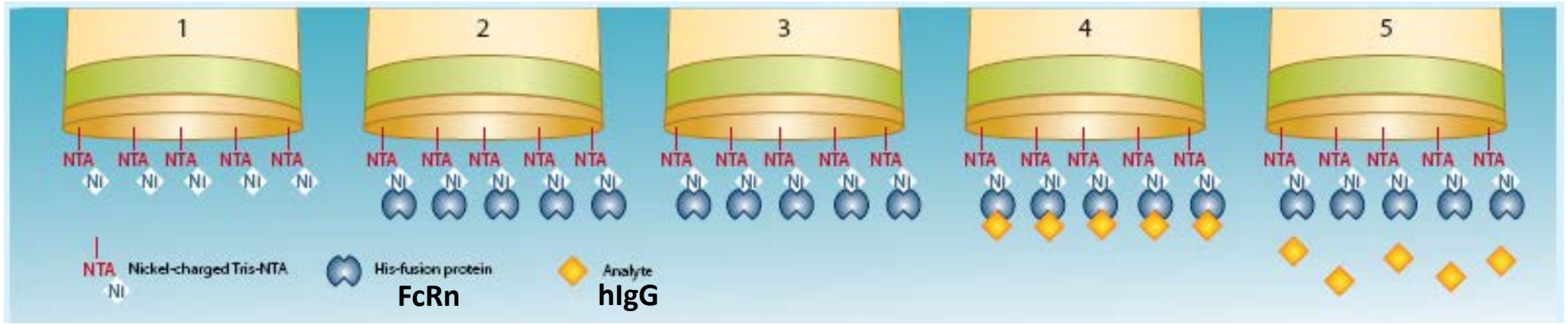
Anti-Penta-His Biosensors



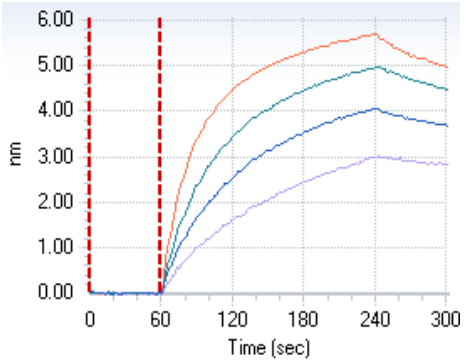
Ni-NTA Biosensors



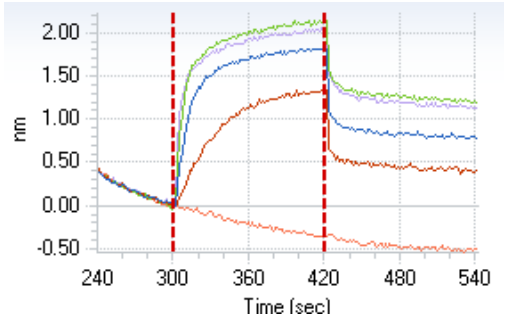
Assay Development Concentration Scouting



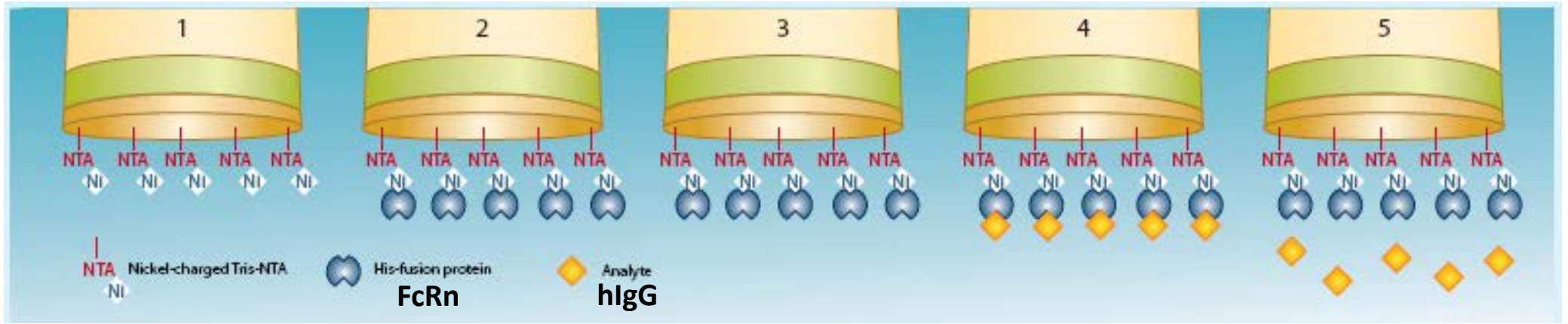
Loading Concentration



Association Concentration



Assay Development pH Scouting



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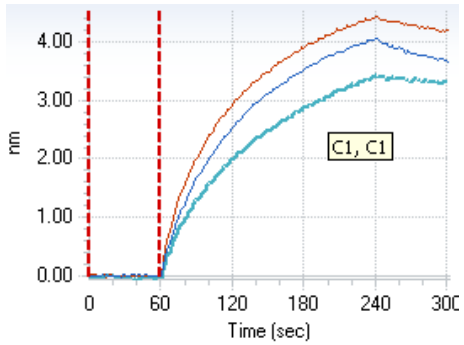
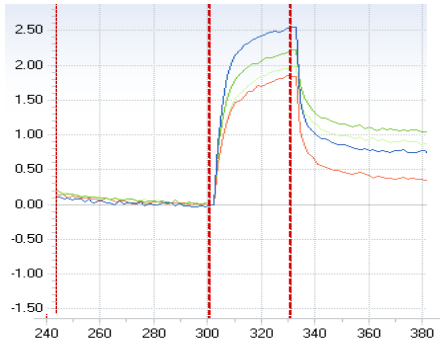
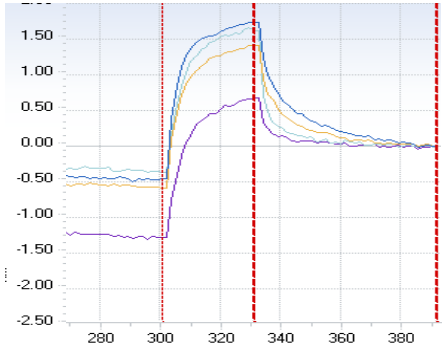


Association pH

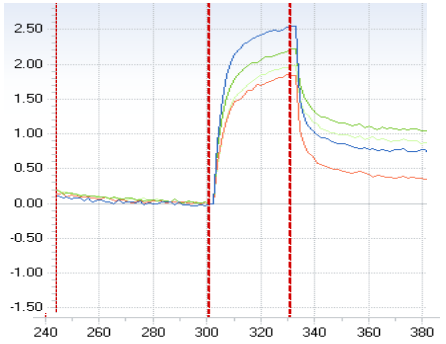


Dissociation pH

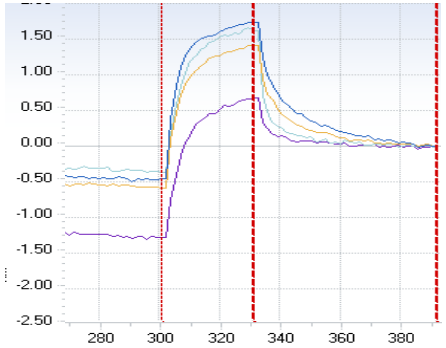


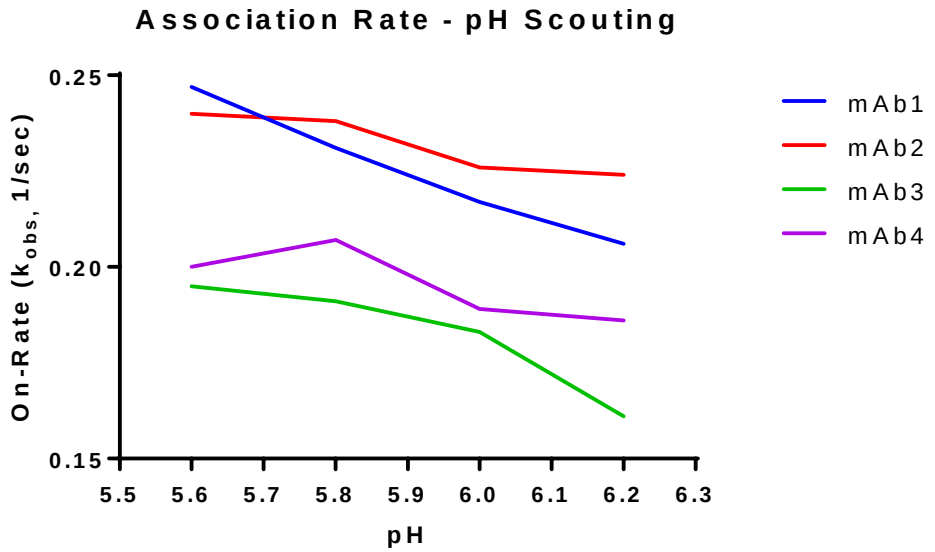
Assay Development pH Scouting

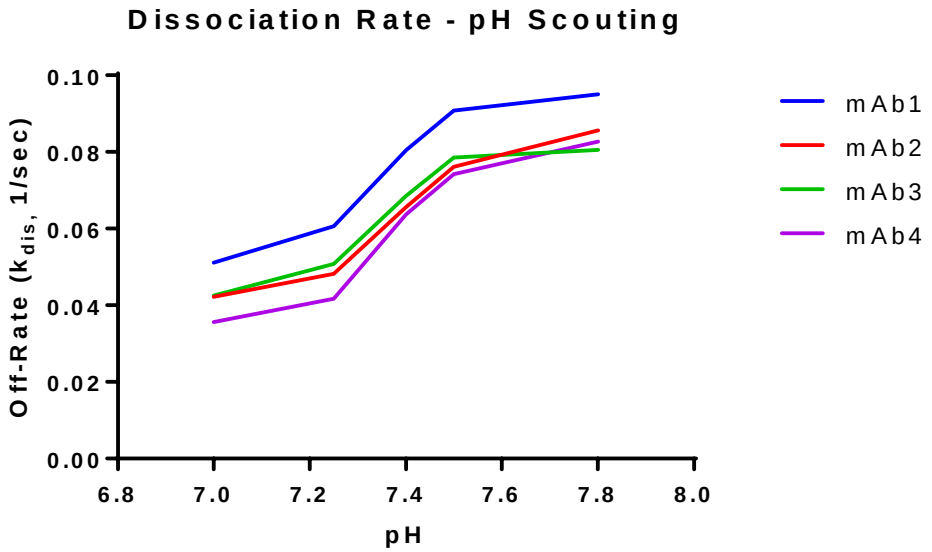
Association pH



Dissociation pH



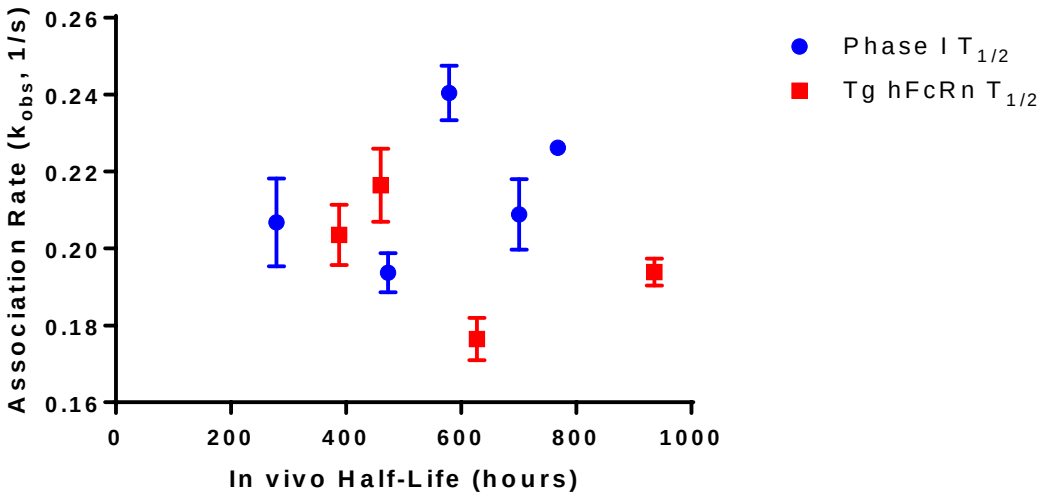


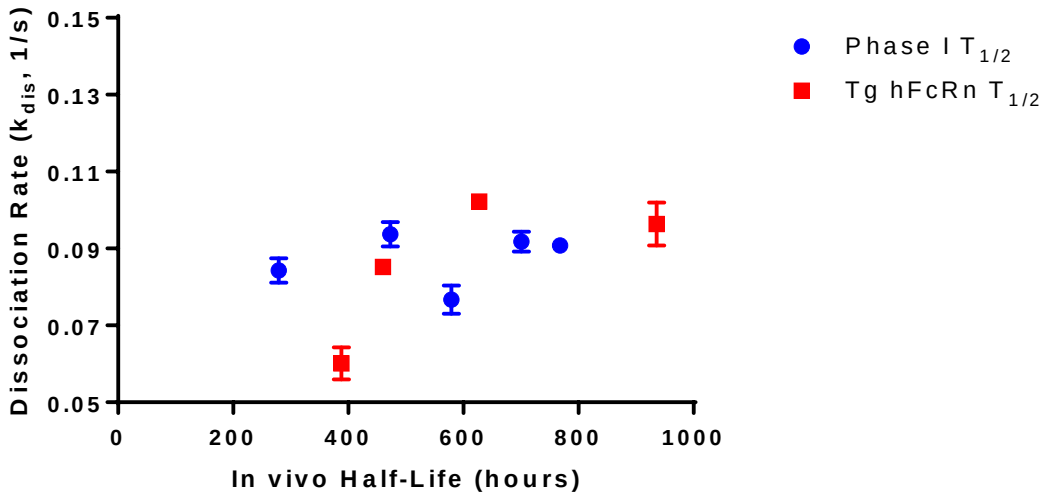


Predictive Model

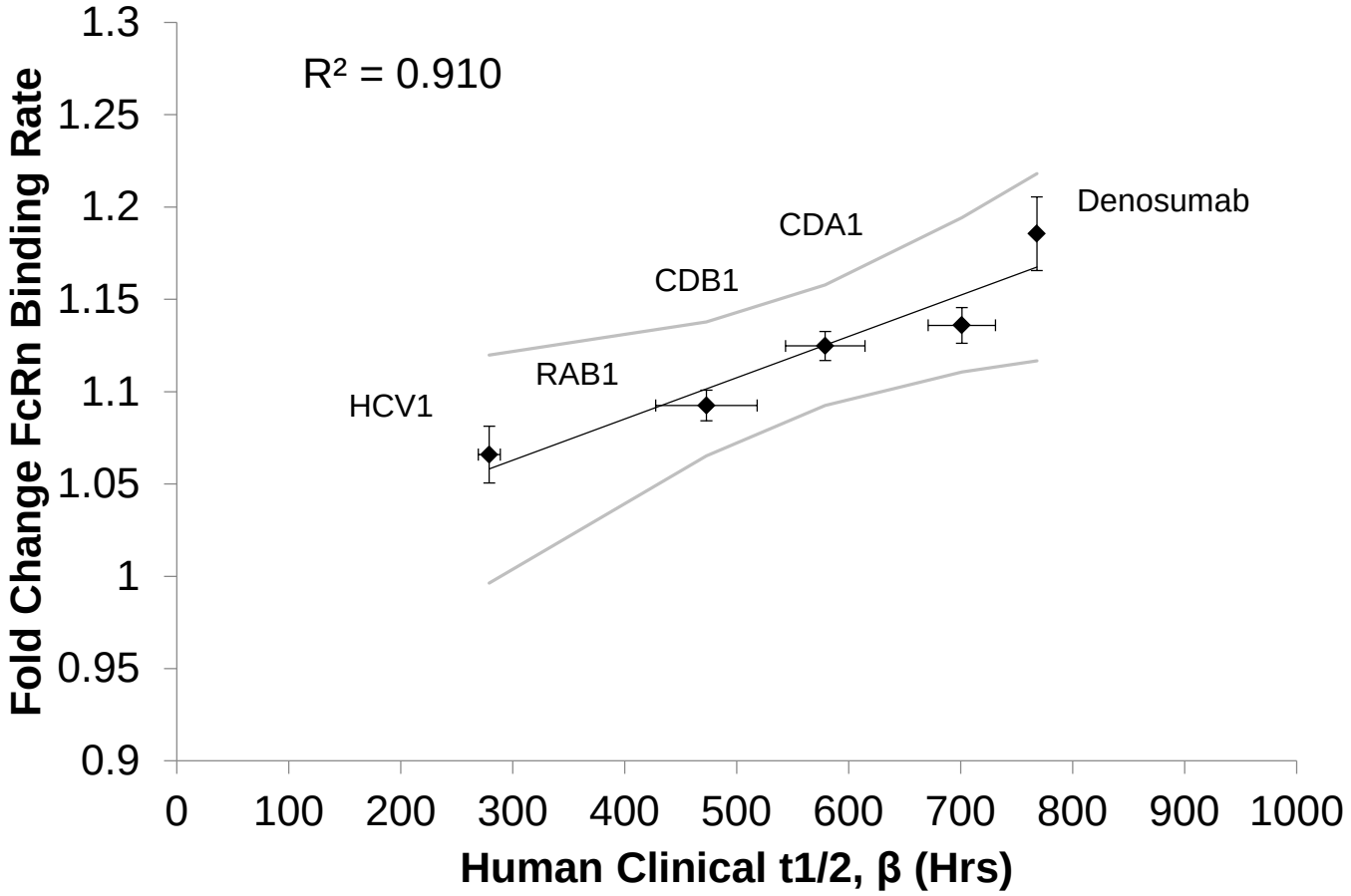
Independent Association and Dissociation Rates

mAb	Target	Phase I $T_{1/2\beta}$ (Days)
Denosumab	RANKL	32.0
Actoxumab (CDA1)	C. difficile Toxin A	29.2
Bezlotoxumab (CDB1)	C. difficile Toxin B	24.1
RAB1	Rabies Virus Glycoprotein	19.7
HCV1	Hep C Virus Glycoprotein	11.6





Predictive Model Combining Association and Dissociation Rates



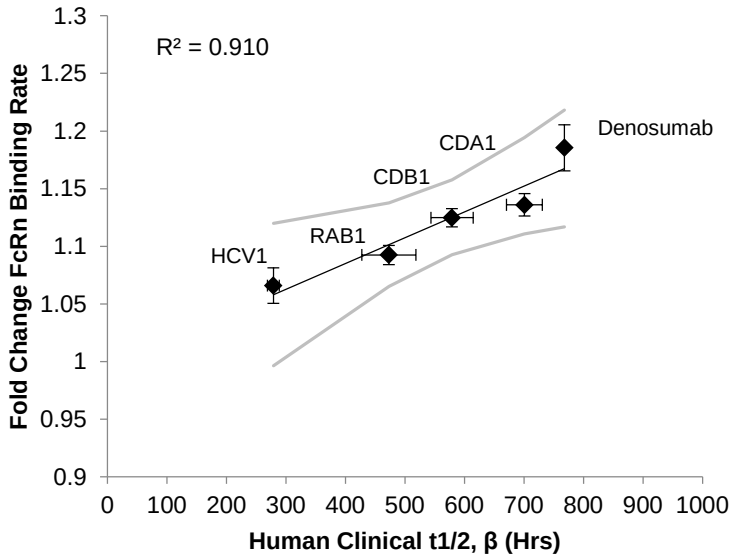
$$\text{Fold Change Association Rate} = \frac{K_{obs}}{K_{obs\,(}\,of\ slowest\ on{-}rate)}$$

$$\text{Fold Change Dissociation Rate} = \frac{K_{dis}}{K_{dis\,(}\,of\ slowest\ off{-}rate)}$$

$$\text{FcRn Binding Rate} = \frac{Fold\ Change\ Assoc. + Fold\ Change\ Dissoc.}{2}$$

Souders et al., mAbs 2015

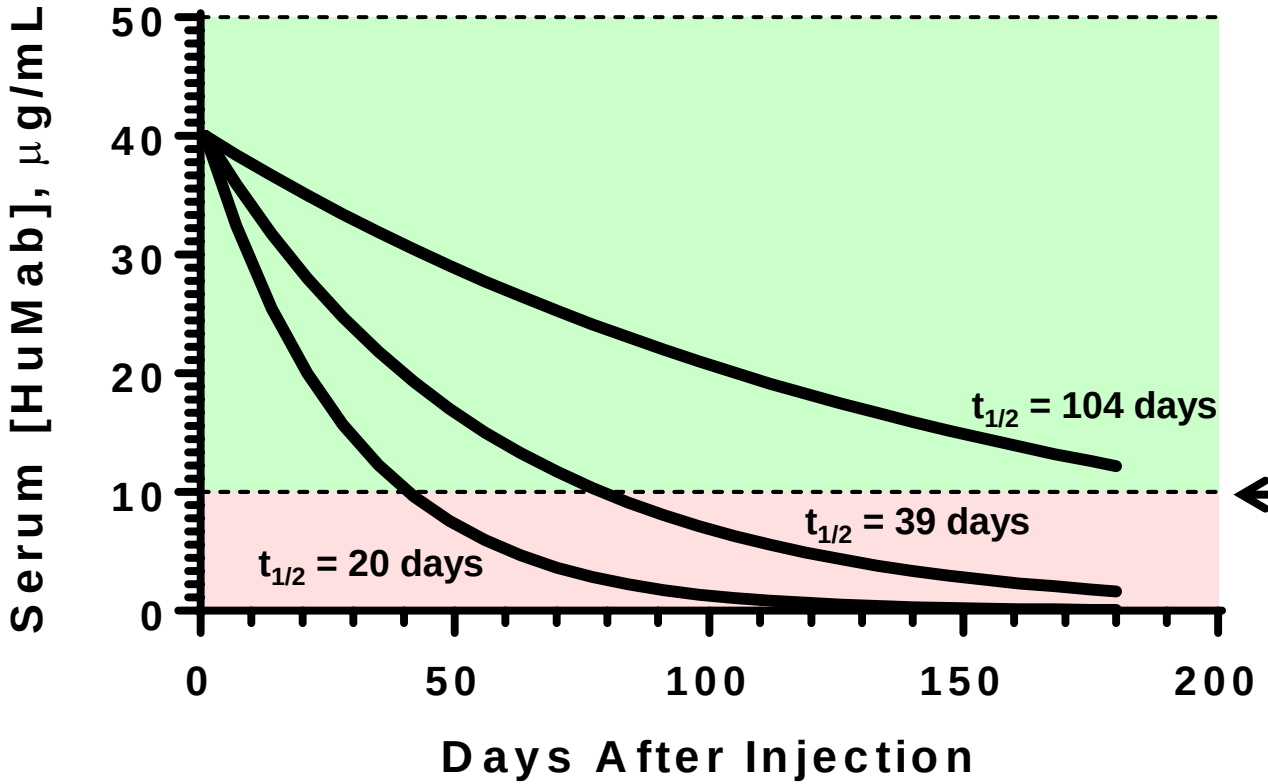
Predicting IgG Human Half-Life Preclinical Therapeutic mAbs



mAb	$T_{1/2}$ (Hours)
CDA1	**700.8**
mAb1-1	639.4
mAb1-2	629.6
mAb2-1	612.7
CDB1	**579.4**
mAb1-3	548.0
RAB1	**473.0**
mAb3-1	451.1
mAb2-2	439.5
mAb2-3	395.6
mAb2-4	378.5
HCV1	**279.0**
mAb4-1	124.2

Predicting IgG Human Half-Life

Therapeutic mAbs for PrEP

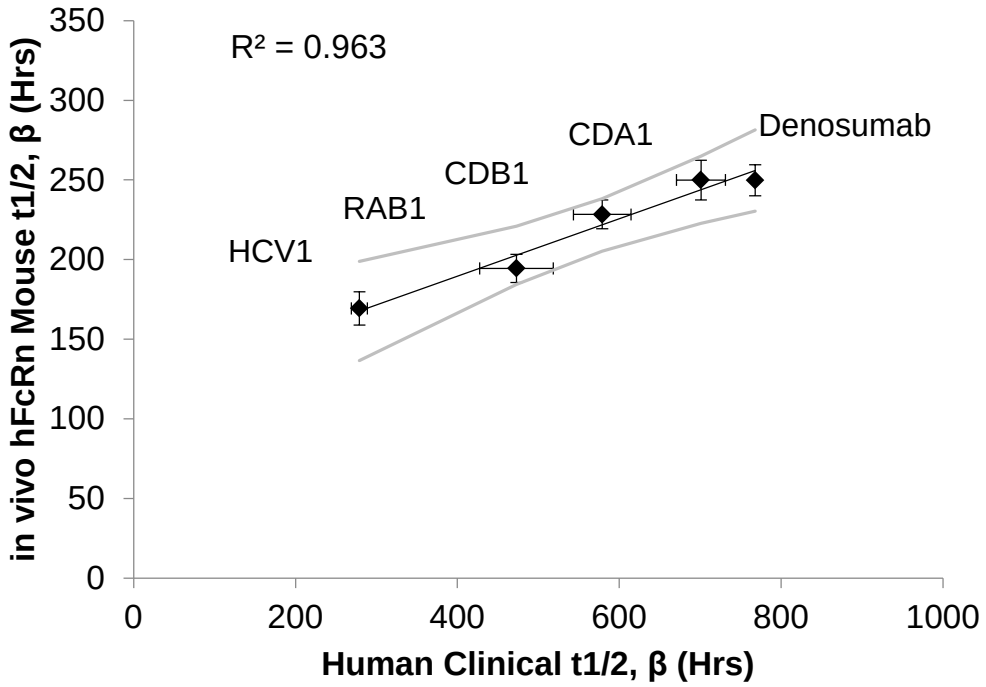


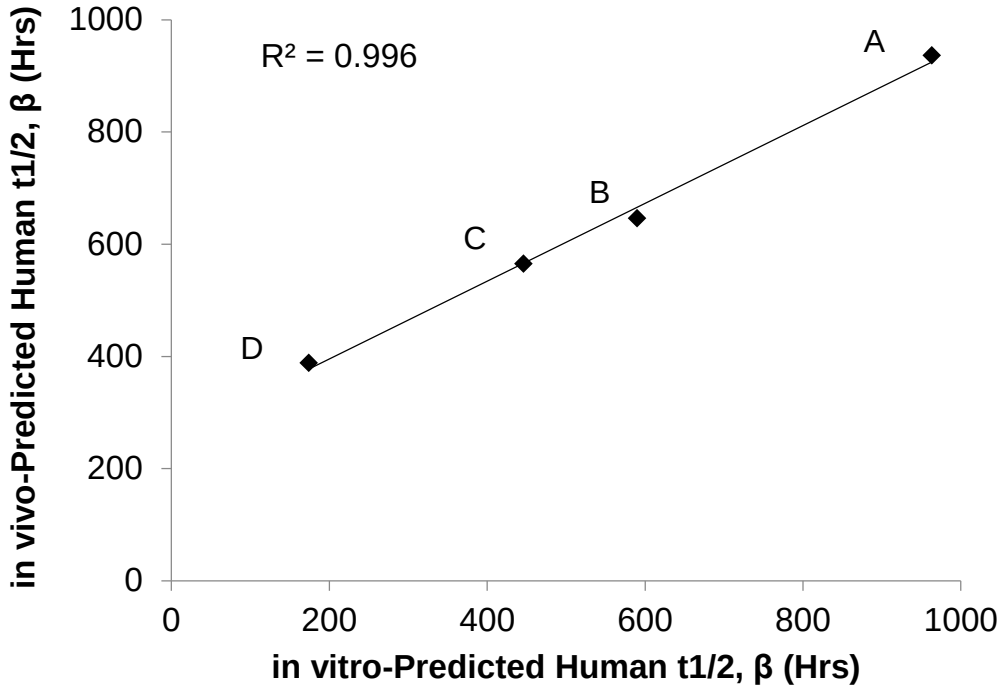
mAb	$T_{1/2}$ (Hrs)
A	963
B	590
C	446
D	174

Souders et al., mAbs 2015

Predicting IgG Human Half-Life

Therapeutic mAbs for PrEP

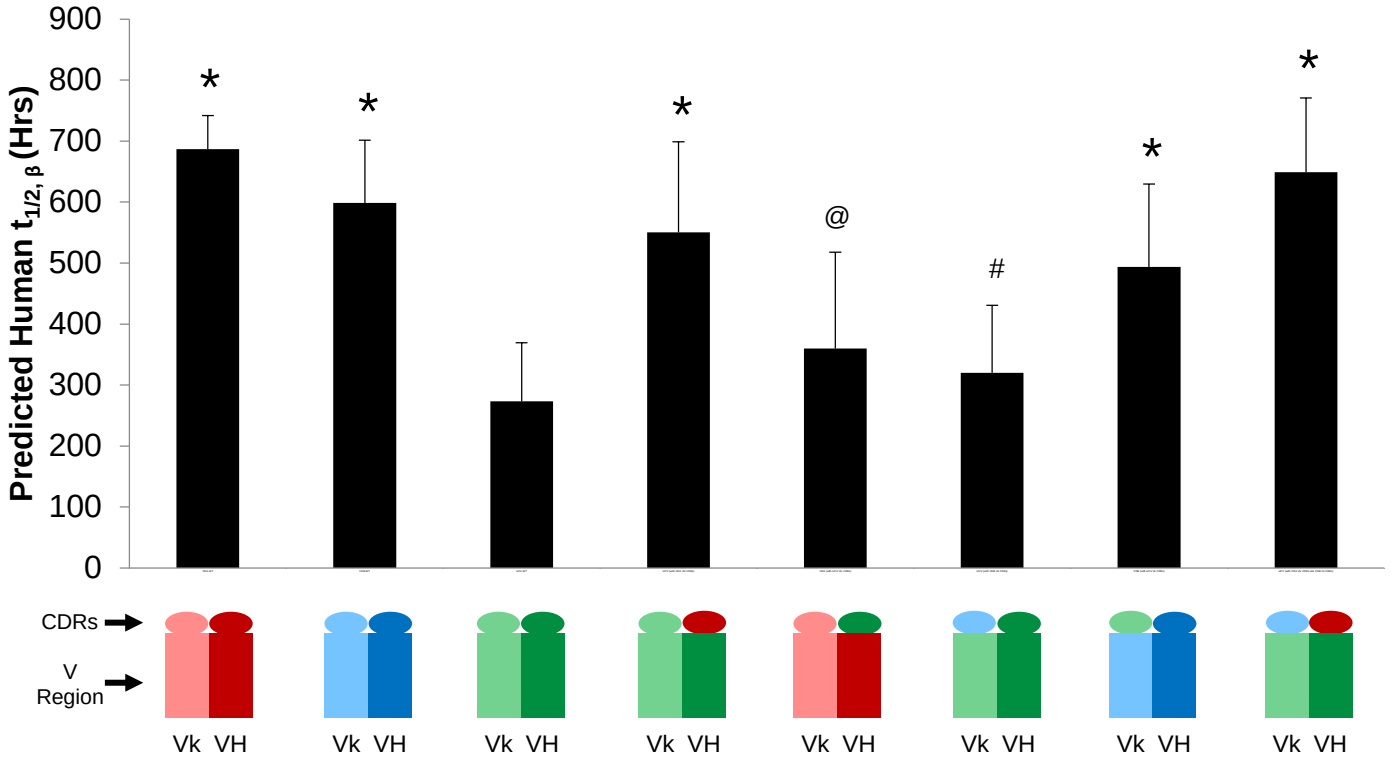


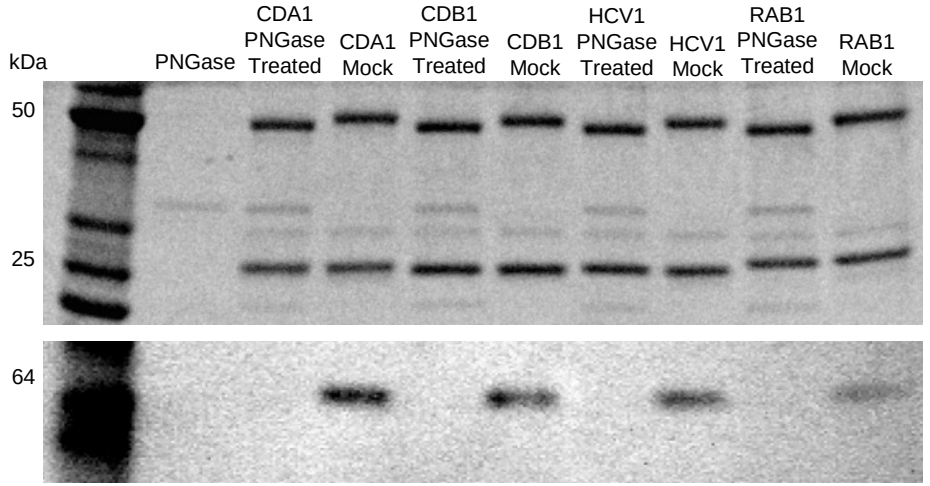


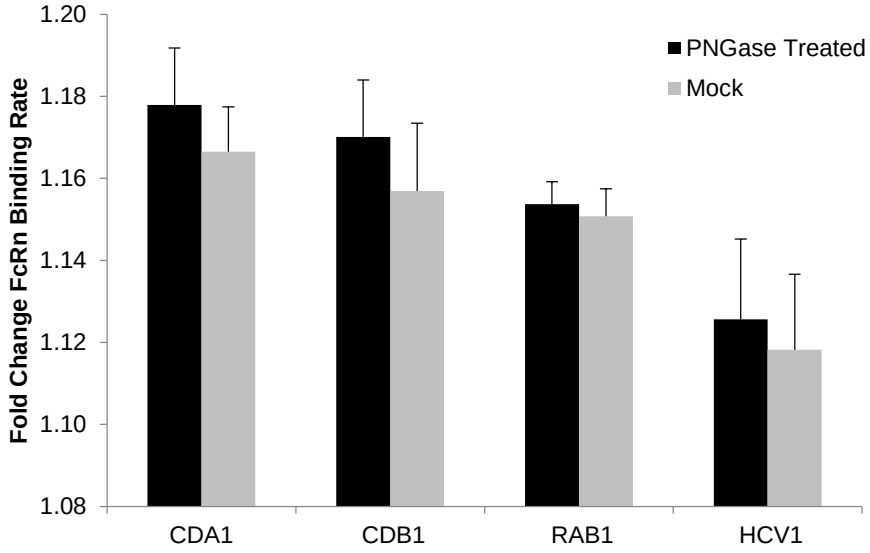
mAb	in vivo model (Hrs)	in vitro model (Hrs)
A	936	963
B	646	590
C	565	446
D	388	174

Souders et al., mAbs 2015

Predicting Half-Life Modifications V Region Sequence & Glycosylation







Souders et al., mAbs 2015

Summary

- Optimal half-life is important for clinical success of mAbs
- BLI-based assay to predict human IgG half-life that considers:
  - Both association and dissociation at relevant pH
  - On and off –rate
- Confirmed model accuracy with hFcRn transgenic mice
- Useful for screening early stage preclinical candidates and investigating IgG modifications.