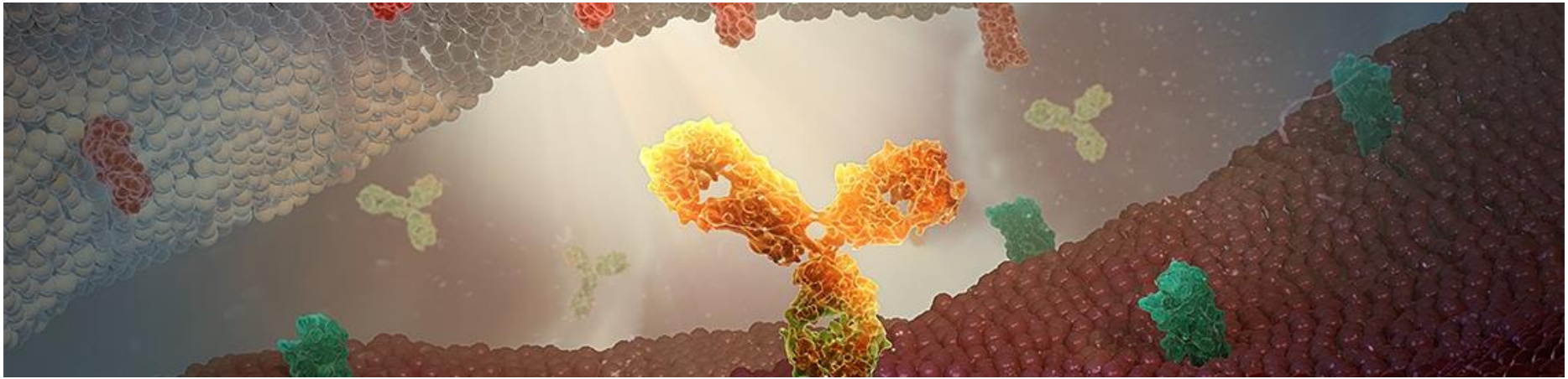
Fc engineering for improved developability and retained biological activity

M. Jack Borrok
Antibody Discovery & Protein Engineering Department
Medimmune, Gaithersburg MD, USA

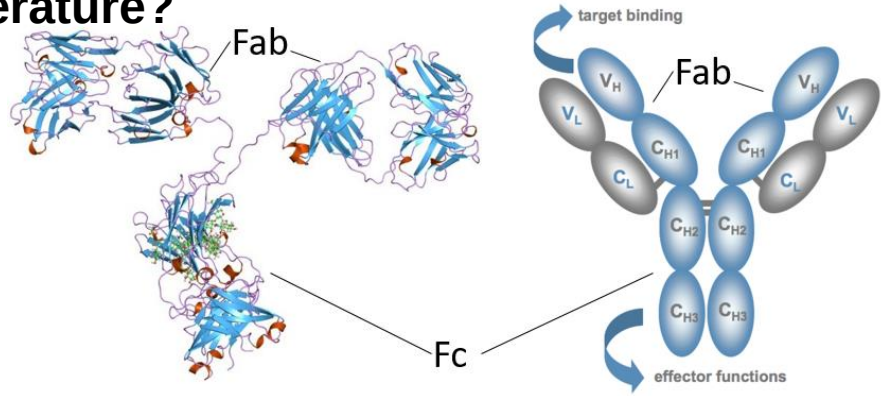
Knect 365 Antibody Engineering and Therapeutics.
Dec. 11, 2017



The value of Fc engineering

- Engineered Fc variants compose a powerful toolbox for adding value and differentiation to subsequent generations of therapeutic antibodies and Fc fusions
  - # of targets are finite, bar always rising, competition always increasing
- Many Fc modifications in literature; few in clinic
  - Almost all were designed for specific biological activity not manufacture or stability
- Fc mutations are seldom “plug and play”
- **How to generate a working Fc toolbox from the breadth of mutations/modifications described in literature?**

Check, E. Immunology: Pimp my Antibody *Nature* **446**, 964-966 (26 April 2007)



Types of Fc mutations/modifications

- Increase or decrease effector functions: Benign, immunosilenced blocking antibodies vs. potent tumor destroying antibodies (*e.g.* TM & others for decreased ADCC and CDC; Afucosylation, different mutations for enhanced ADCC)

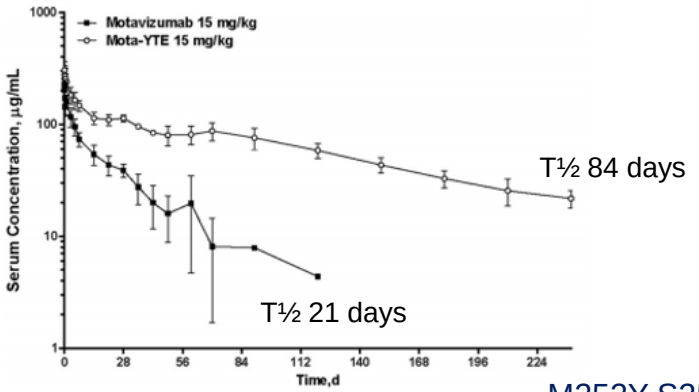
- Improved Pharmacokinetics: Half-life can be changed to hours or months depending on need (*e.g.* YTE, Xtend, many others)
  – Caveats apply

- Other Unique Fc approaches: FcγRIIb specific, C1q specific, Hexabody, IgA grafting, etc.

- **Fc engineering can help to meet ever increasing standards of efficacy, safety, dosing & superiority**

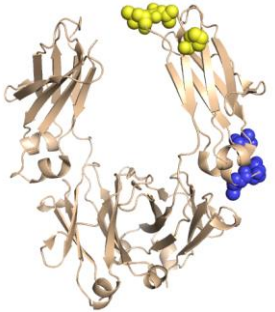
Combining mutation combinations for desired activity

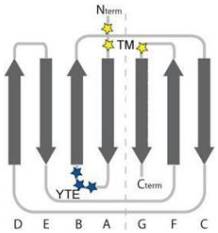
- **YTE for half-life extension**
- T½ from 28 to ~80-100 days!
- Works by enhancing affinity to FcRn



M252Y S254T T256E

- **TM for 'silencing' effector function**
- For when ADCC, ADCP, and/or CDC would be detrimental
- Many different formats/ways to achieve silencing
- TM: 2 mutations from IgG4 and 1 from murine IgG2b

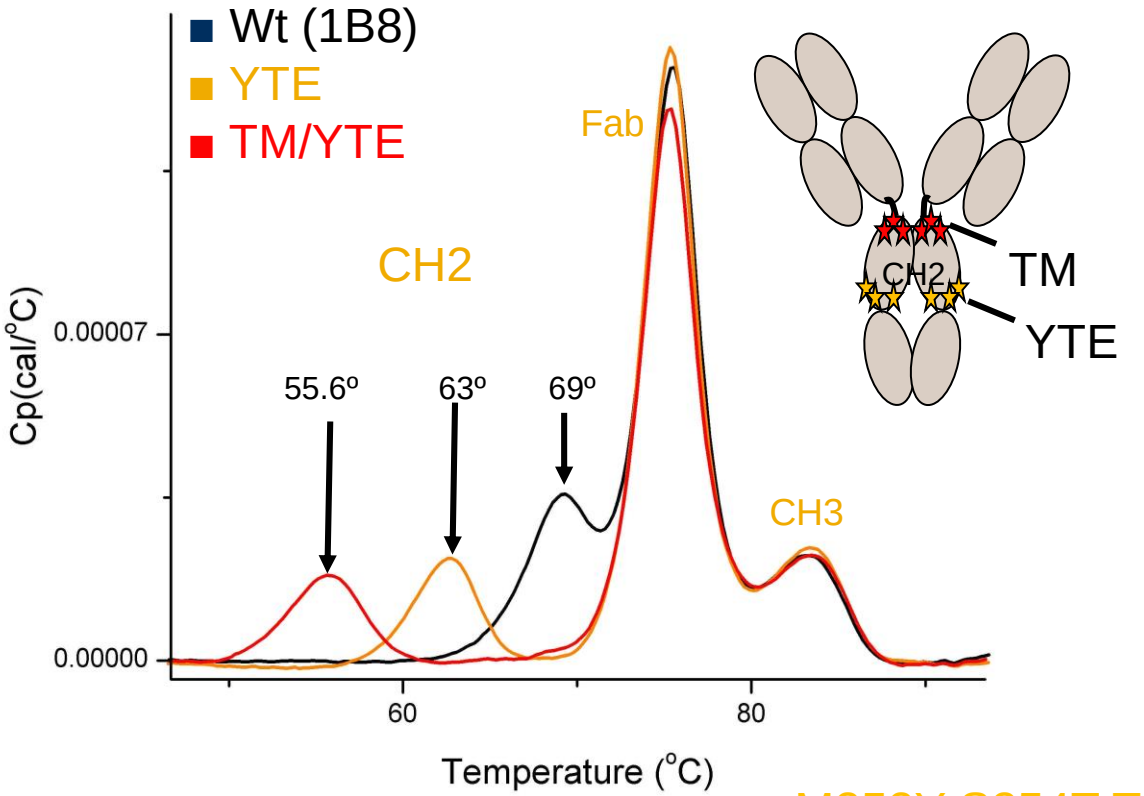




L234F L235E P331S

Both half-life extension & abolished effector function are desirable traits for 'benign blockers' (among others)

Mutations in the Fc can lower thermal stability of an antibody



- C_H2 is already lowest Tm of all Ig domains in IgG
- One mutation set (YTE) decreases Tm1, but still 'developable'
- Additional mutations can further lower Tm1 and coincide with other problems
- Different mutations affect conformational stability to different degrees

M252Y S254T T256E/L234F L235E P331S

Potential developability problems with TM-YTE

- TM-YTE combination has ~13-14ºC drop in C_H2 Tm
- Decreased monomer content in accelerated stability studies
  - Increased aggregation
- Decreased Stability with shear stress[1]
- Mutation is not necessarily unmanageable… but there is room for improvement
  - Stability ultimately dependent on Fab & formulation
  - HCLF

[1]R. Tavakoli-Keshe, J.J. Phillips, R. Turner, D.G. Bracewell **Understanding the relationship between biotherapeutic protein stability and solid–liquid interfacial shear in constant region mutants of IgG1 and IgG4**
J Pharm Sci, 103 (2) (2013), pp. 437-444

Engineering approach to make a better TM-YTE combination

- Several different possible approaches

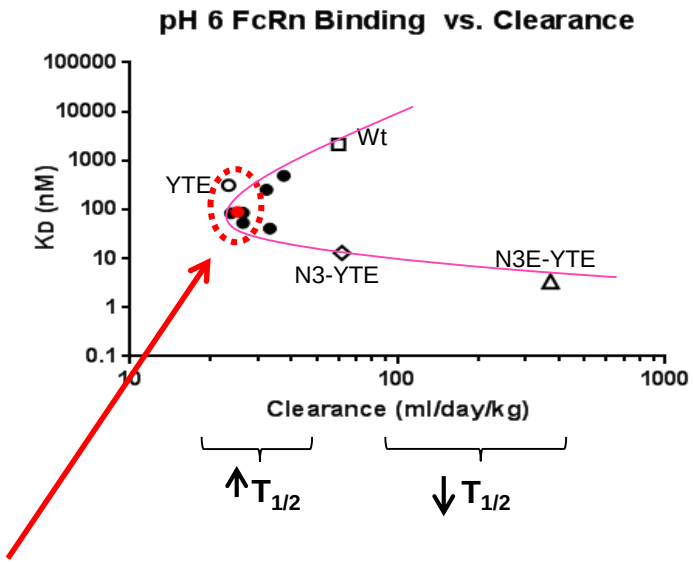
- Leave YTE unaltered
  - Developable, low/no immunogenicity, in optimal FcRn zone

- Identify most destabilizing residues in TM
  - Dissecting TM by mutagenesis

- Substitute alternate amino acids at the position(s) found to be destabilizing

- Explore mutations at different positions as needed

Why not evaluate YTE thermal stability in tandem?

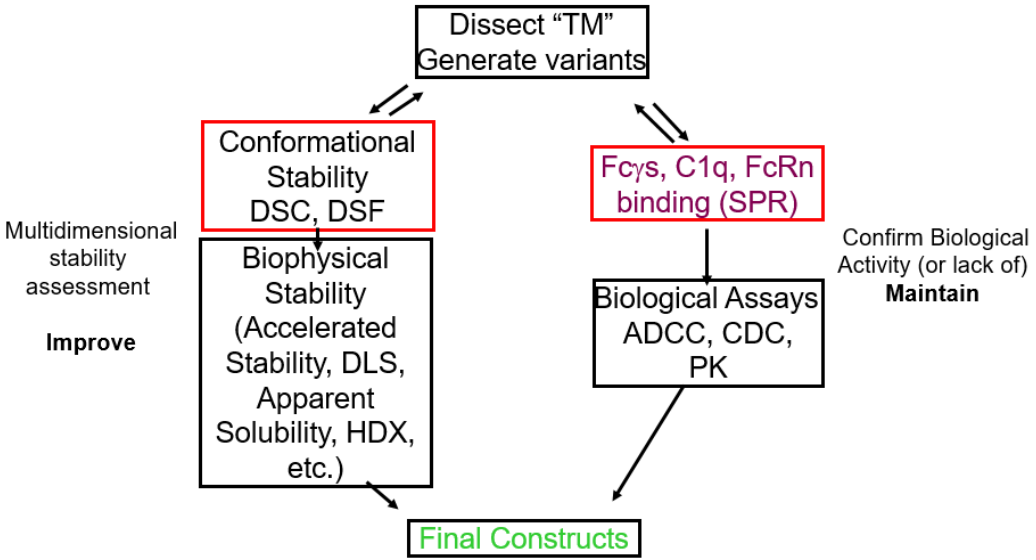


"Sweet Spot" for FcRn affinity

- Lowers T_m by ~6ºC but still quite developable
- Multiple molecules in clinic
  - Low/no ADA in multiple healthy adult phase 1 studies
- FcRn mediated Half life Extension relies on pH 6 FcRn Affinity being in a "sweet spot"

Borrok MJ *et al*. pH-dependent binding engineering reveals an FcRn affinity threshold that governs IgG recycling. J Biol Chem 2015 Feb 13;290(7):4282-90. doi: 10.1074/jbc.M114.603712. Epub 2014 Dec 23.

Engineering approach to make a better TM-YTE combination



- All single and double mutant versions of TM were made
  - Site 1 (L234F) "Neutral" on stability
  - Site 2 (L235E) ↓ 2 to 3ºC
  - Site 3 (P331S) ↓ 4 to 5ºC
- Triple mutations needed to fully knock out all Fcγ and C1q binding
  - Double mutants had residual ADCC or CDC
- Keep L234F mutation and find alternatives for other two destabilizing mutations
  - L235E & P331S

TM dissection and finding alternatives

- DSC was good starting point for assessing stability changes in individual mutants
- Destabilizing mutations in combination identified
- All amino acids tried at L235 had thermal stability improvements over glutamate
- Attempts to alter P331 met with little improvement
- Spatially adjacent site mentioned in literature K322 gave better results
- “FQQ-YTE and two others moved forward to further testing

Assessing the Thermal Stability of TM-YTE Variants to Identify More Stable Mutations

Construct/Notes	*L234*	*L235*	*P331*	*K322*	DSC T_m1 (°C)
“YTE”	–	–	–	–	62.7
“TM-YTE”	F	E	S	–	56.0
Single TM dissection mutants	F	–	–	–	63.0
	–	E	–	–	60.5
	–	–	S	–	58.7
Double TM dissection mutants	F	E	–	–	60.5
	F	–	S	–	58.5
	–	E	S	–	55.0
Identifying more stable alternatives for L235E	F	Q	–	–	63.4 (+2.9)[a]
	F	N	–	–	63.3 (+2.8)[a]
	F	A	–	–	63.3 (+2.8)[a]
	F	F	–	–	62.9 (+2.4)[a]
	F	I	–	–	63.2 (+2.7)[a]
	F	V	–	–	63.3 (+2.8)[a]
Identifying more stable alternatives for P331S	F	E	G	–	57.0 (+1.0)[b]
	F	E	A	–	57.0 (+1.0)[b]
	F	E	–	A	57.2 (+1.2)[b]
	F	E	–	E	55.7 (−0.3)[b]
	F	E	–	N	49.4 (−6.6)[b]
	F	E	–	H	53.1 (−2.9)[b]
	F	E	–	Q	58.6 (+2.6)[b]
“FQG-YTE”	F	Q	G	–	60.5 (+4.9)[b]
“FAQ-YTE”	F	A	–	Q	60.0 (+4.4)[b]
“FQQ-YTE”	F	Q	–	Q	61.3 (+5.7)[b]

All mutations are in YTE (M252Y/S254T/T256E) Fc background. T_m1 (unfolding of the C_H2 domain) as measured by DSC is reported. All DSC measurements are made in the HB20.3 IgG1 YTE background with the exception of those below the double line which are in the 1B8 IgG1 background. Dashed lines (–) indicate wild-type amino acids (italics) at those positions.

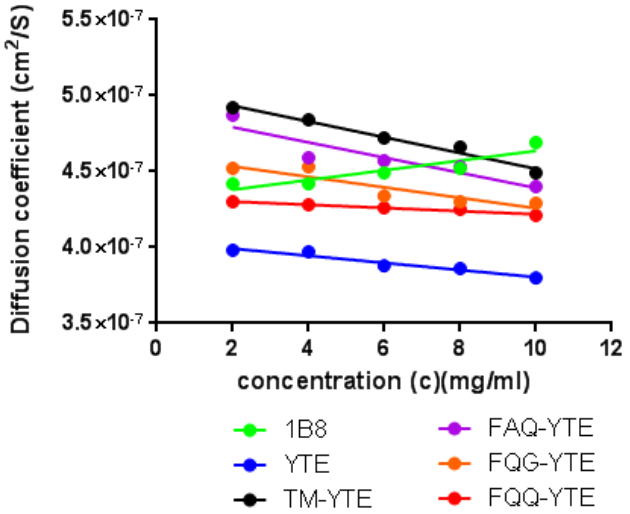
[a] Values in parentheses represent changes in T_m1 from the comparator mutation L234F/L235E-YTE.

[b] Values in parentheses represent changes in T_m1 from the comparator mutation L234F/L235E/P331S-YTE (TM-YTE) in the respective IgG1 background.

Borrok MJ et al. An “Fc-Silenced IgG1 format with extended half-life designed for improved stability. *J. Pharm Sci.* 2017 Apr;106(4)

Colloidal Stability, Apparent Solubility, Accelerated Stability

- Interaction parameter (DLS) shows improvement for FQQ-YTE compared to TM-YTE
- PEG Apparent Solubility rankings also show improvement
- Minor improvement in aggregation during accelerated stability



Summary of Colloidal and Accelerated Stability Studies With Antibody Variants

Antibody	Interaction Parameter k_D (mL/g)	Slope $\times 10^{-6}$ ($cm^2/s \cdot mL/g$) (R^2)	PEG_{midpt} (% PEG 6000)	Apparent Solubility (mg/mL) from Extrapolation	40°C Aggregation Increase/Month (%)	40°C Fragment Increase/Month (%)
1B8 IgG1	7.23	3.13 (*0.83*)	9.33	1100	0.59	2.16
YTE	−5.72	−2.31 (*0.95*)	6.70	600	0.67	2.10
TM-YTE	−10.34	−5.21 (*0.98*)	7.06	100	1.47[a]	2.10
FQQ-YTE	−2.51	−1.09 (*0.96*)	8.79	1300	1.10	2.01
FQG-YTE	−7.48	−3.45 (*0.84*)	7.08	100	0.74	2.63
FAQ-YTE	−10.24	−5.01 (*0.84*)	6.68	50	0.65	5.63[b]

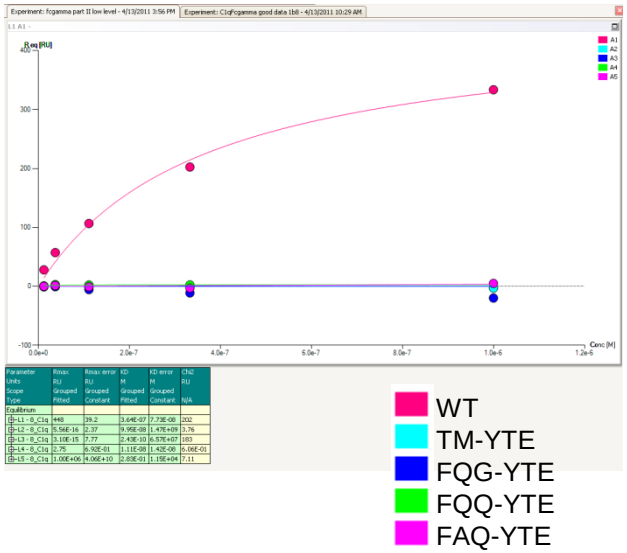
The interaction parameter (k_D) and slope values were determined by DLS. R^2 values for the slopes are shown (italics). PEG_{midpt} and apparent solubility values were derived from precipitation of antibody with PEG 6000 at various concentrations.

[a] Slope values are significantly different from IgG1, YTE, FQG-YTE ($p < 0.001$), and FAQ-YTE ($p < 0.01$).

[b] Slope values are significantly different from all other values in column ($p < 0.001$).

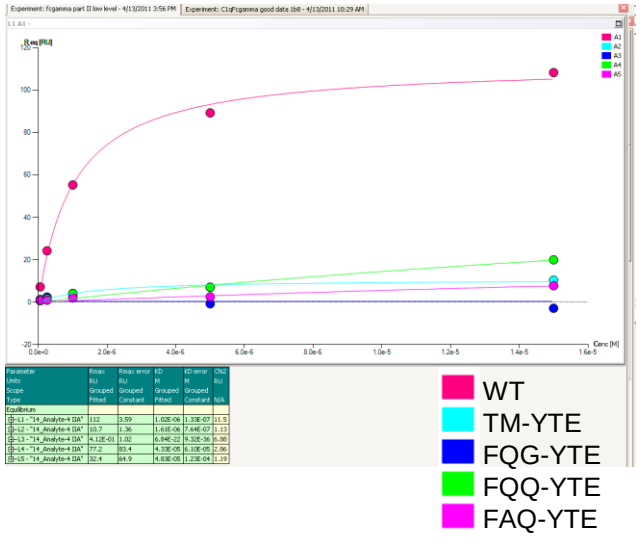
Fcγ & C1q binding abrogated similarly to TM-YTE

C1q



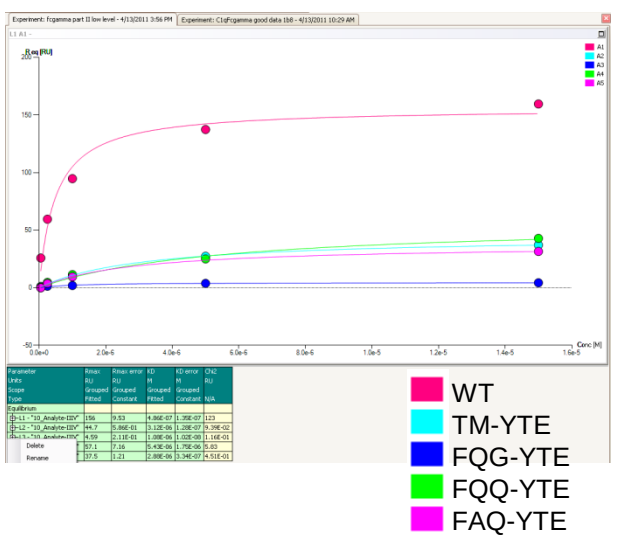
WT
TM-YTE
FQG-YTE
FQQ-YTE
FAQ-YTE

FcγRIIa



WT
TM-YTE
FQG-YTE
FQQ-YTE
FAQ-YTE

FcγRIIIa (158V)



WT
TM-YTE
FQG-YTE
FQQ-YTE
FAQ-YTE

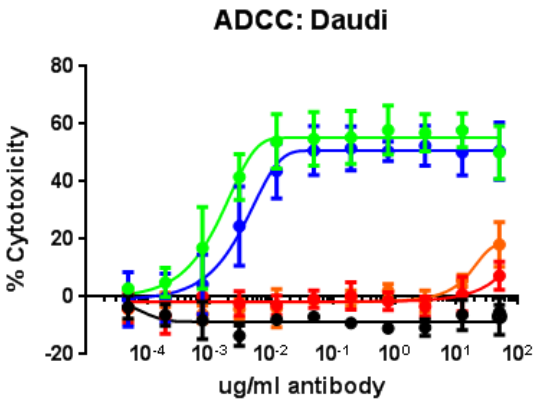
Similar Data for RIa, RIIb, IIIa (158F) also in two different IgG backgrounds

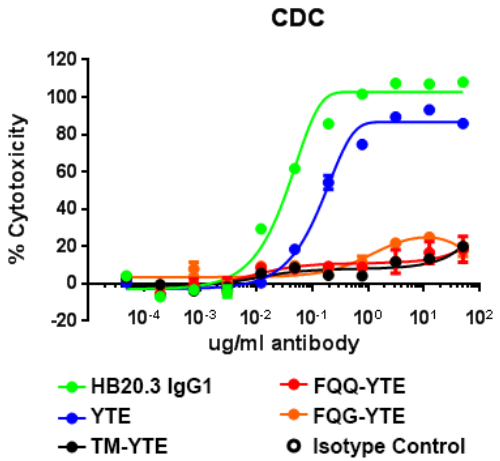
YTE-like FcRn binding at pH 6 is maintained

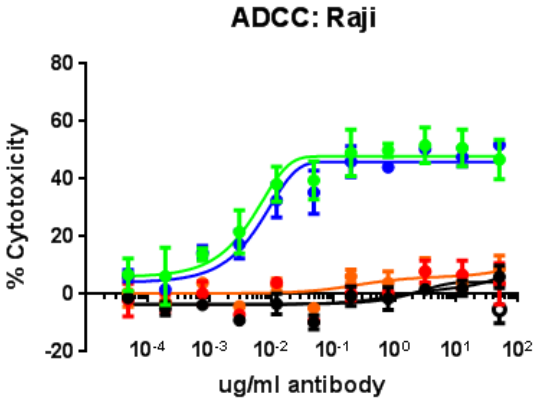
FQQ-YTE similar to TM-YTE in biological activity

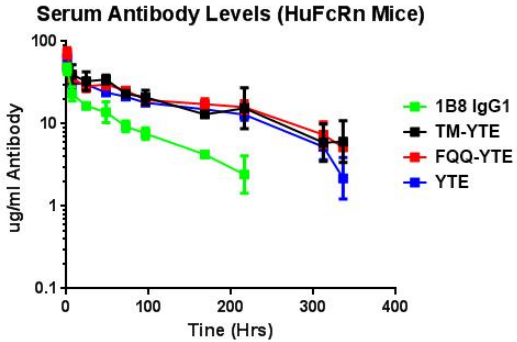
- FQQ-YTE must be as good or better than predecessor at abrogating effector function and extending half life
- SPR showed binding to Fcγ & C1q similar for all variants & TM-YTE

- Anti-CD20 (HB20.3) antibodies generated
- ADCC, CDC tested
- PK tested in HuFcRn mice model



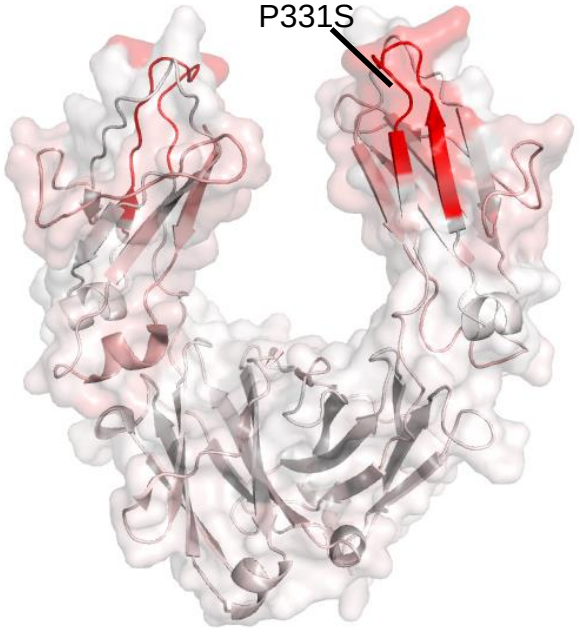




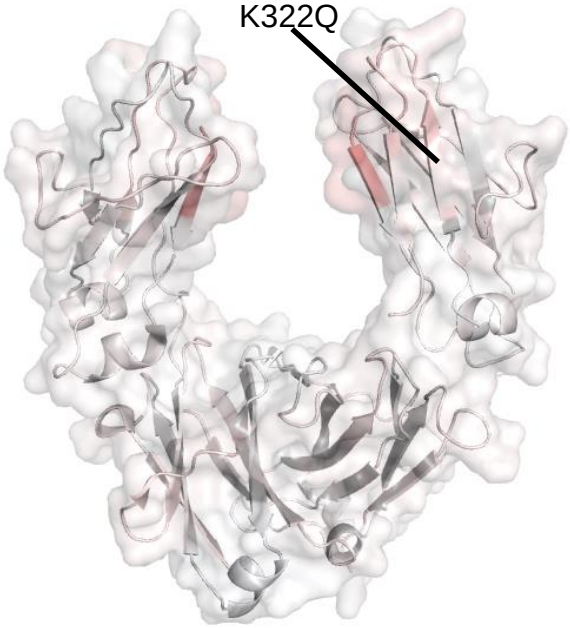


Borrok MJ et al. An “Fc-Silenced IgG1 format with extended half-life designed for improved stability. *J. Pharm Sci.* 2017 Apr;106(4)

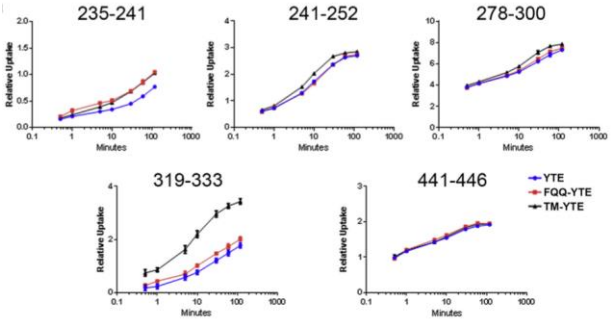
HDX differences in solvent accessibility



TM-YTE vs. YTE



FQQ-YTE vs. YTE



- HDX exchange data correlate with DSC data
- Increased conformational flexibility near P331S
- FQQ has deuterium uptake similar to YTE
  - (little change)

Borrok MJ et al. An “Fc-Silenced IgG1 format with extended half-life designed for improved stability. *J. Pharm Sci.* 2017 Apr;106(4)

Subsequent preclinical evaluation

- Evaluated head to head with other Fc formats in preclinical project
- Similar improvement (T_m & Aggregation) as with model IgG on prev. slide
- Particularly stable Fab region

Characteristic	IgG1 TM	IgG1 TM YTE	IgG1 FQQ YTE
Transient Expression	450 mg/L	522 mg/L	622 mg/L
% Monomer Loss per month (by HP-SEC) at 40 ºC	1.8 (1.0% agg; 0.7% frag)	2.7 (1.7% agg; 1.1% frag)	1.7 (1.0% agg; 0.7% frag)
Thermal stability (ºC by DSC)	63.3	55.9	61.9
Protein Concentration (by A280)	No significant changes after 4 weeks at 40°C		
Appearance	No significant change in visual appearance at 2-8°C. Increased particle formation was seen at 40°C especially with TM format, which also showed more noticeable opalescence increase. Surfactant level will be optimised to reduce particle formation		
Potency	No loss after 4 weeks at 40°C		

- Additional Derisking: Immunogenicity assessment via Episcreen™

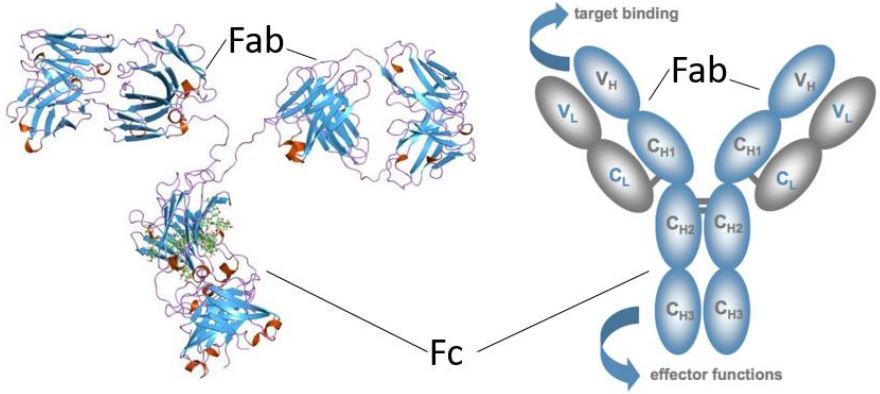
FQQ-YTE Conclusions

- TM & YTE are both clinically validated Fc mutations for Fc silencing and half-life extension respectively.
  - Both have acceptable developability profiles
- Combination of TM-YTE desirable for long lasting ‘benign blocker’ applications
  - Developability of combo is borderline, may be problematic for HCLF
- Dissection of TM revealed L235E and P331S to be destabilizing
- Screened for alternatives based on DSC
- New combinations subjected to additional stability testing
  - Multiple stability assays & biological assays in tandem
- New combination (FQQ-YTE) has superior stability profile, with equivalent biological activity (or lack)

Fc engineering & Developability Conclusions

- Engineered Fc mutations can add value and differentiation to existing Fc containing therapeutics
  - safety, tailored dosing, enhanced efficacy, etc.
- Impact on developability is often overlooked, but crucial to making viable drug products
  - Should be incorporated into engineering/screening process
  - Immunogenicity potential
- Thermal Stability good starting point for screening, but not always correlative to overall stability/ agg. propensity *etc*.
- Poorly behaved Fab can exacerbate Fc stability problems (and *vice versa*)

Check, E. Immunology: Pimp my Antibody *Nature* **446**, 964-966 (26 April 2007)



Acknowledgements

Antibody Discovery & Protein Engineering

Ping Tsui
Mike Bowen
William Dall'Acqua
Herren Wu

Development

Neil Mody
Xiaojun Lu
Megan Kuhn
Steve Bishop