Structure and Dynamics of a Site-Specific Labeled Human Fc Fragment with Tuned Effector Functions

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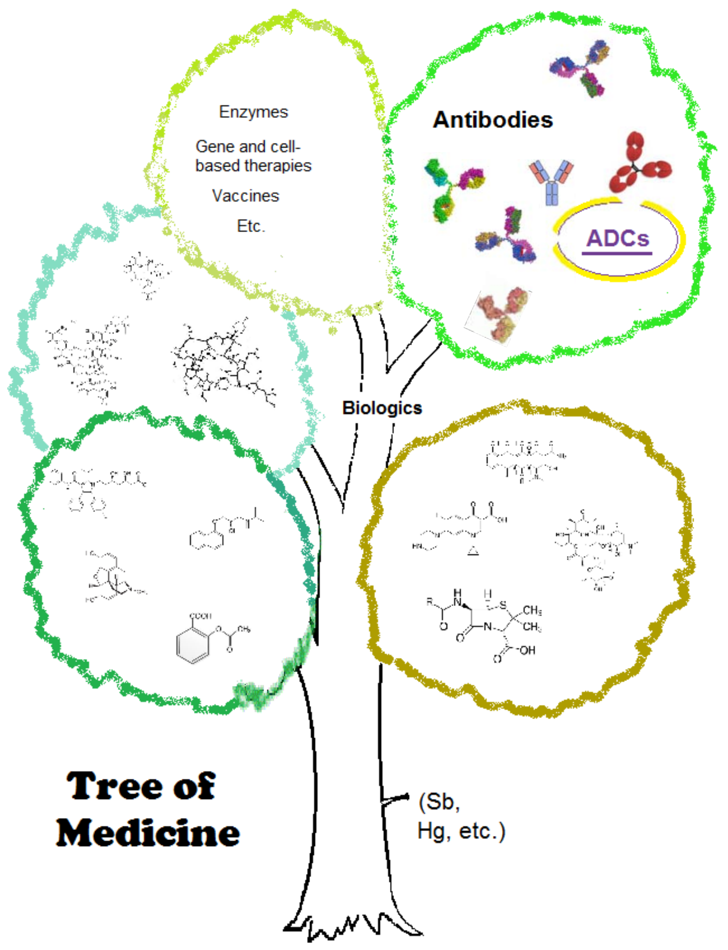
Evolution of Antibody-Based Medicines

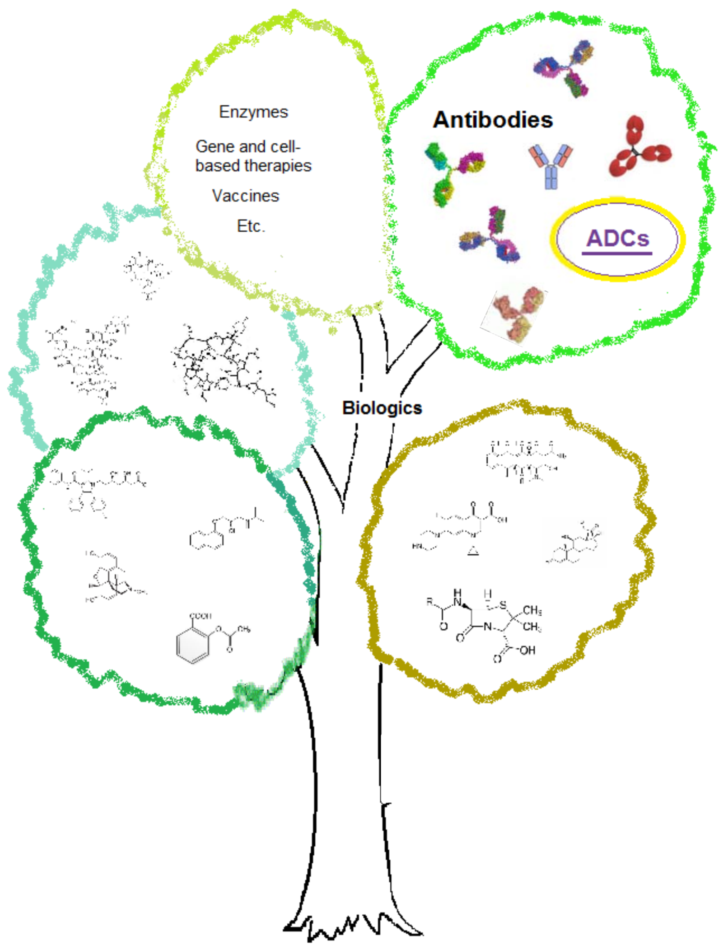


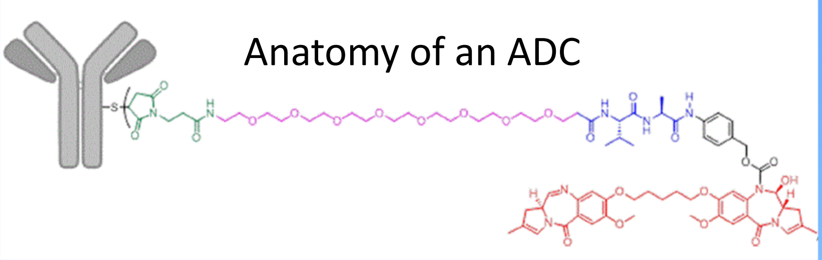
Top 10 Drugs by Sales for 2017, $85 Billion: Seven are Biologics, Six are mAbs

Product	2017 Sales	2017 vs 2016	Use	Type
Humira (Adalimumab)	$18,427 M	14.6%	Anti-inflammatory	mAb
Rituxan (Rituximab)	$9,238 M	2.0%	Anti-Cancer	mAb
Revlimid (Lenalidomide)	$8,187 M	17.4%	Anti-Cancer	Small molecule
Enbrel (Etanercept)	$7,885 M	-11.1%	Autoimmune diseases	Protein/IgG
Herceptin (Trastuzumab)	$7,441 M	3.4%	Anti-Cancer	mAb
Eliquis (Apixaban)	$7,395 M	46.3%	Anticoagulant	Small molecule
Remicade (Infliximab)	$7,152 M	-13.1%	Autoimmune diseases	mAb
Avastin (Bevacizumab)	$7,096 M	-1.4%	Anti-Cancer	mAb
Xarelto (Rivaroxaban)	$6,589 M	11.3%	Anticoagulant	Small molecule
Eylea (aflibercept)	$6,034 M	9.4%	Macular degeneration	Fusion protein

Quality control and reproducibility → need for data and standards → NIST mAb

Antibody-Drug Conjugates




Anatomy of an ADC

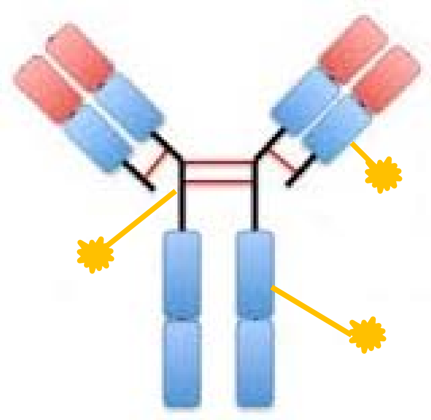
Where to attach?

Trend is toward…

*carefully chosen specific site(s)

*protected locations

*carefully controlled conjugation



Structure of this Talk

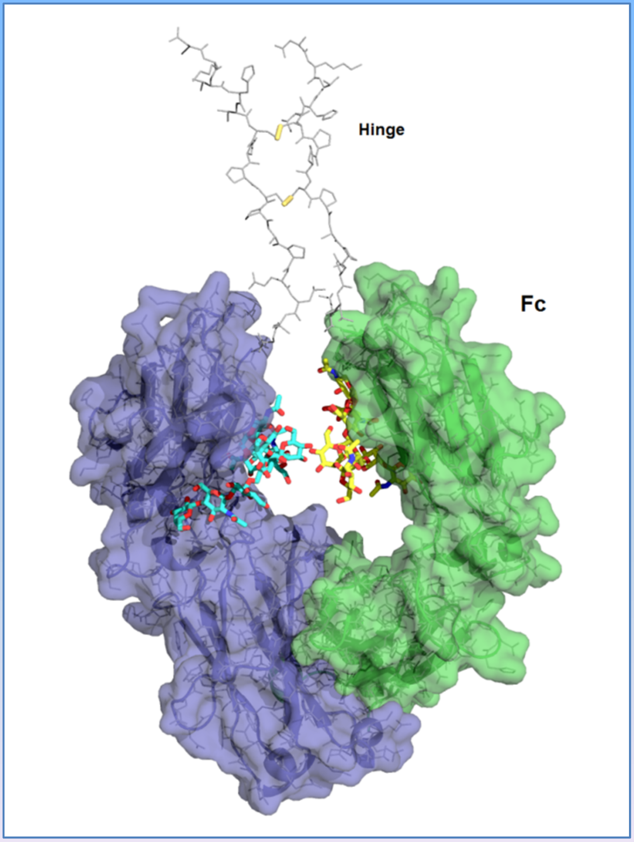
1. **Intro to ADC insertion mutant Fc_C239i**

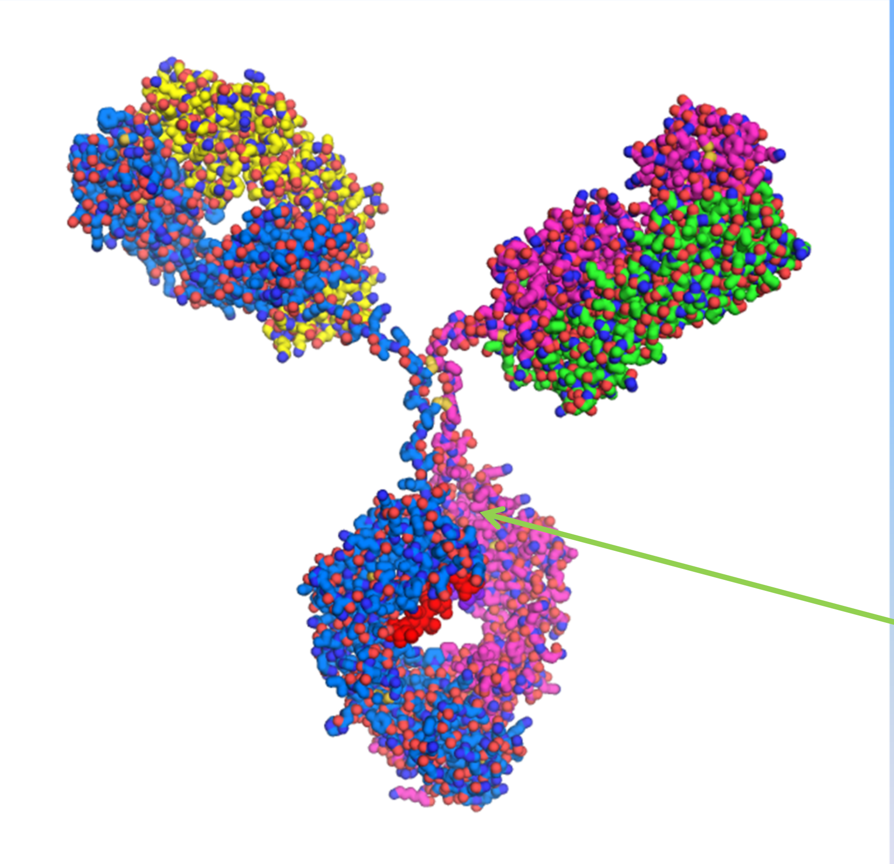
2. Fc_C239i crystal structures
3. Dynamics
4. Receptor interactions
5. Conclusions

IgG1 heavy chain sequence:

-- hinge -- Fc…
Fab…….CPPCPAPELLGGPSVF…

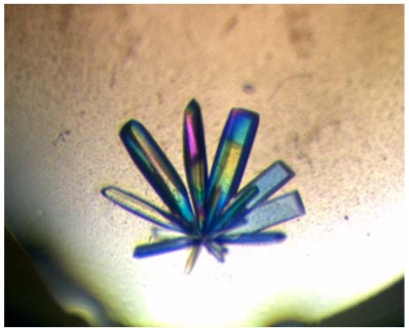
Cys inserted after Ser239





Cys inserted here,
after Ser 239

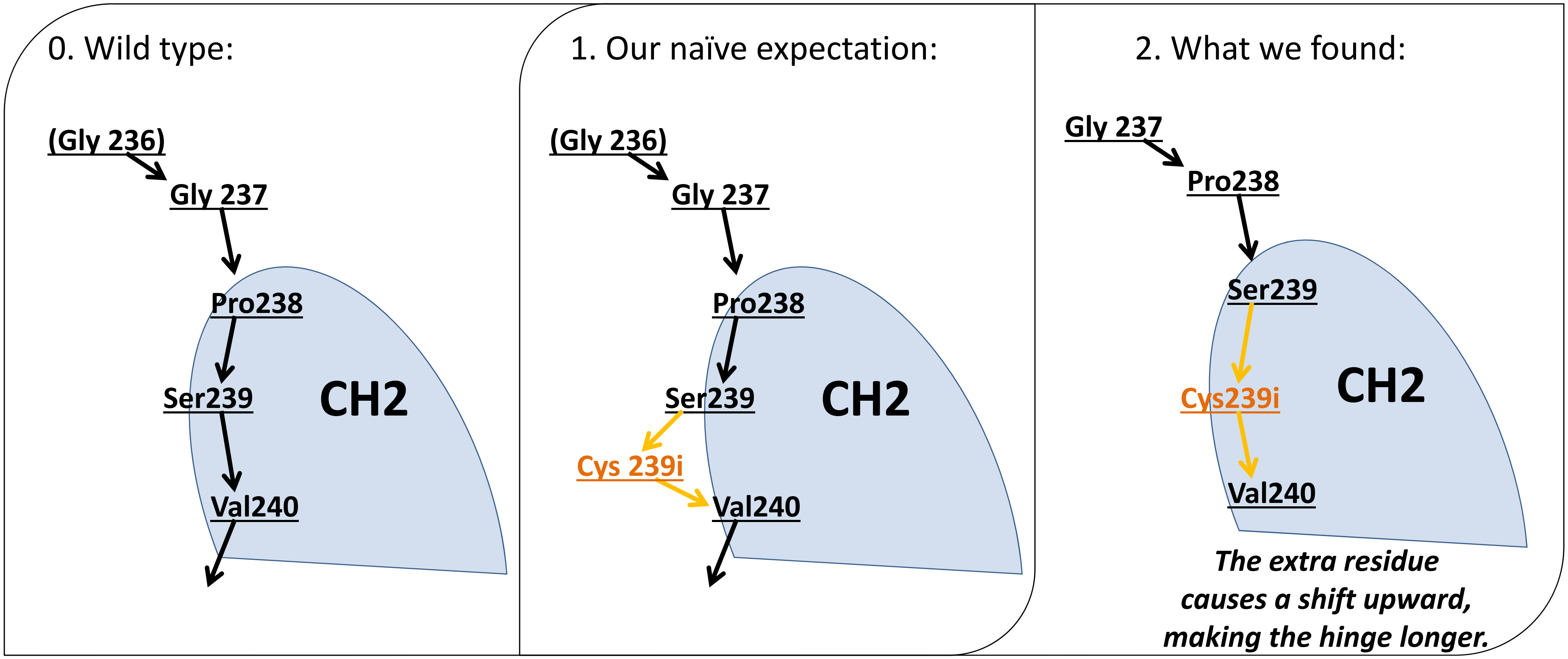
Crystals of the Fc fragment with the C239i mutation.



Diffraction statistics for Fc wt and two Fc_239i adduct structures.
All are in space group 19 with unit cell approximately 50, 80, 135 Å

	#non-H atoms	resolution	Rsym	refined R/Rf	rmsd-ideal
Fc wt(5vgp)	3765	2.1 A	0.09	0.20/0.25	0.013
Fc239i+cys	3652	2.3 A	0.07	0.22/0.27	0.014
Fc239i+maleim	3628	2.6 A	0.10	0.26/0.31	0.010

How does the inserted Cys affect the local structure?



0. Wild type:

(Gly 236) → **Gly 237** → **Pro238** → **Ser239** → **Val240** →

CH2

1. Our naïve expectation:

(Gly 236) → **Gly 237** → **Pro238** → **Ser239** → **Cys 239i** → **Val240** →

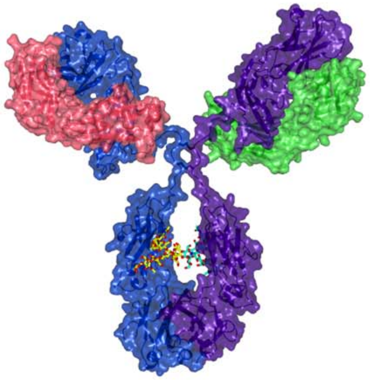
CH2

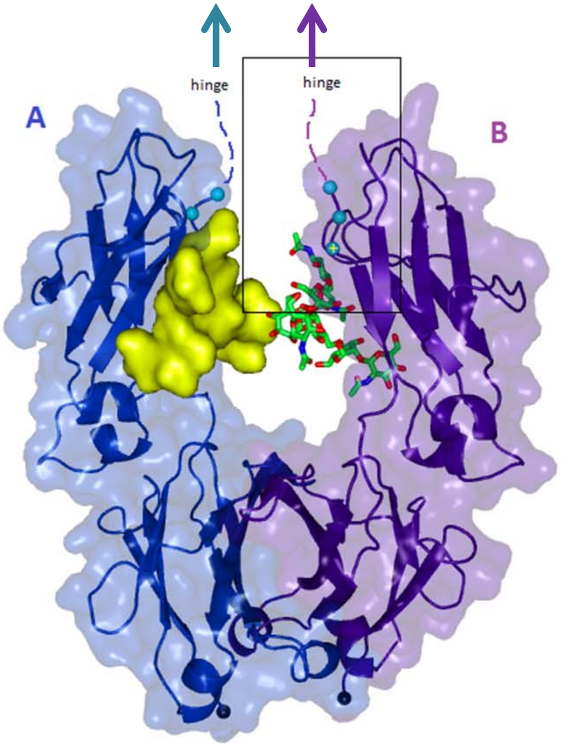
2. What we found:

Gly 237 → **Pro238** → **Ser239** → **Cys239i** → **Val240**

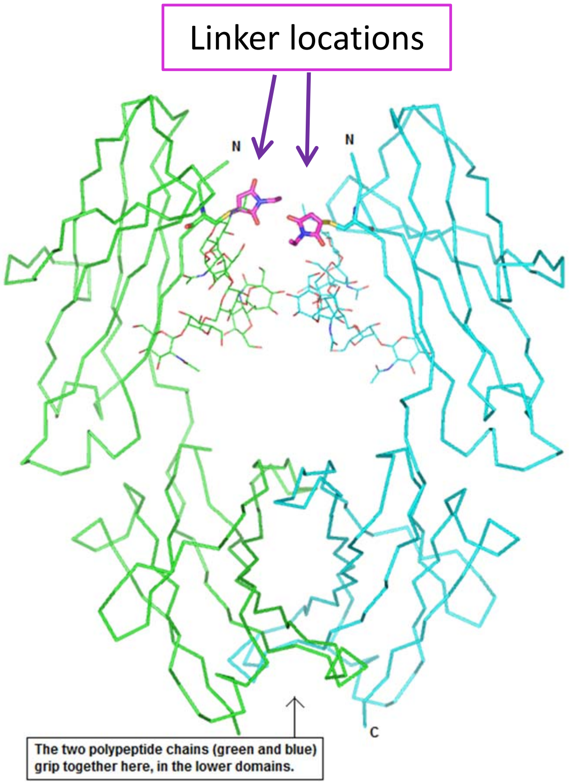
CH2

The extra residue causes a shift upward, making the hinge longer.





The extra Cys structurally replaces Ser239,
forcing 239 and 240 upward
and extending the hinge by 1 residue.

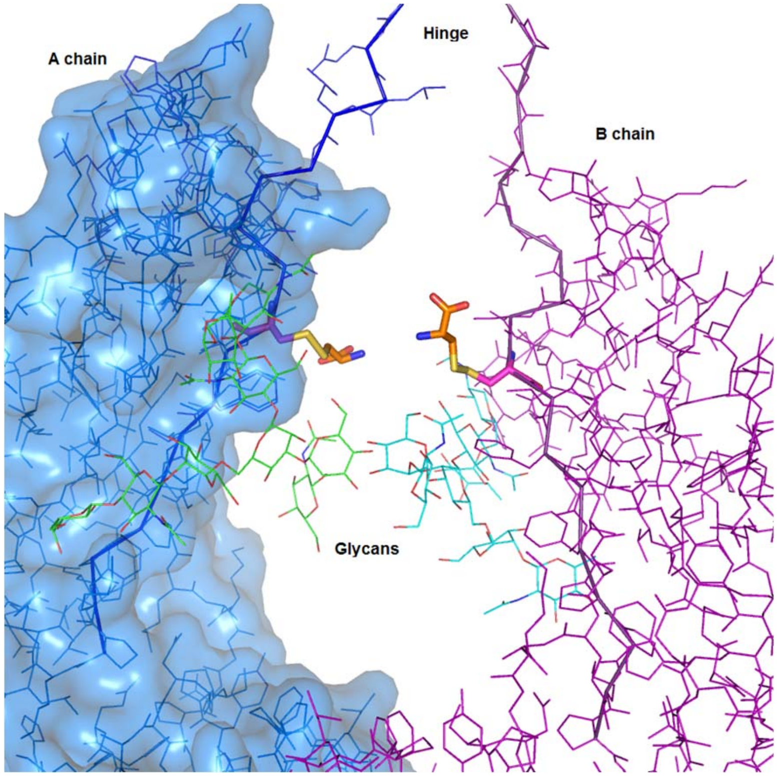


Linker locations

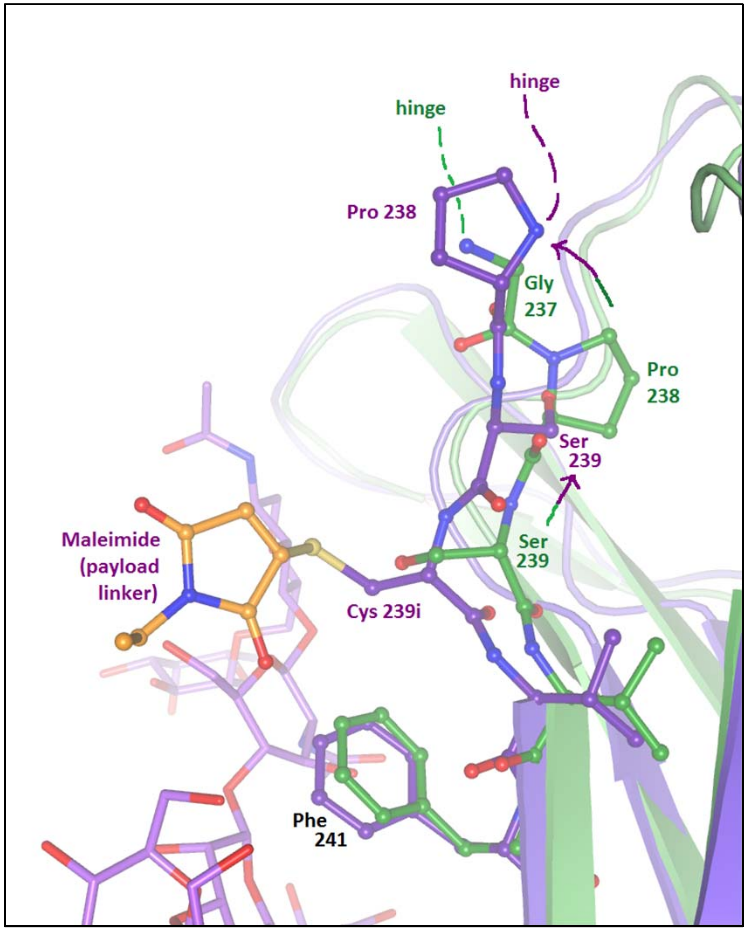
The two polypeptide chains (green and blue) grip together here, in the lower domains.

Adducts attached to Cys239i:

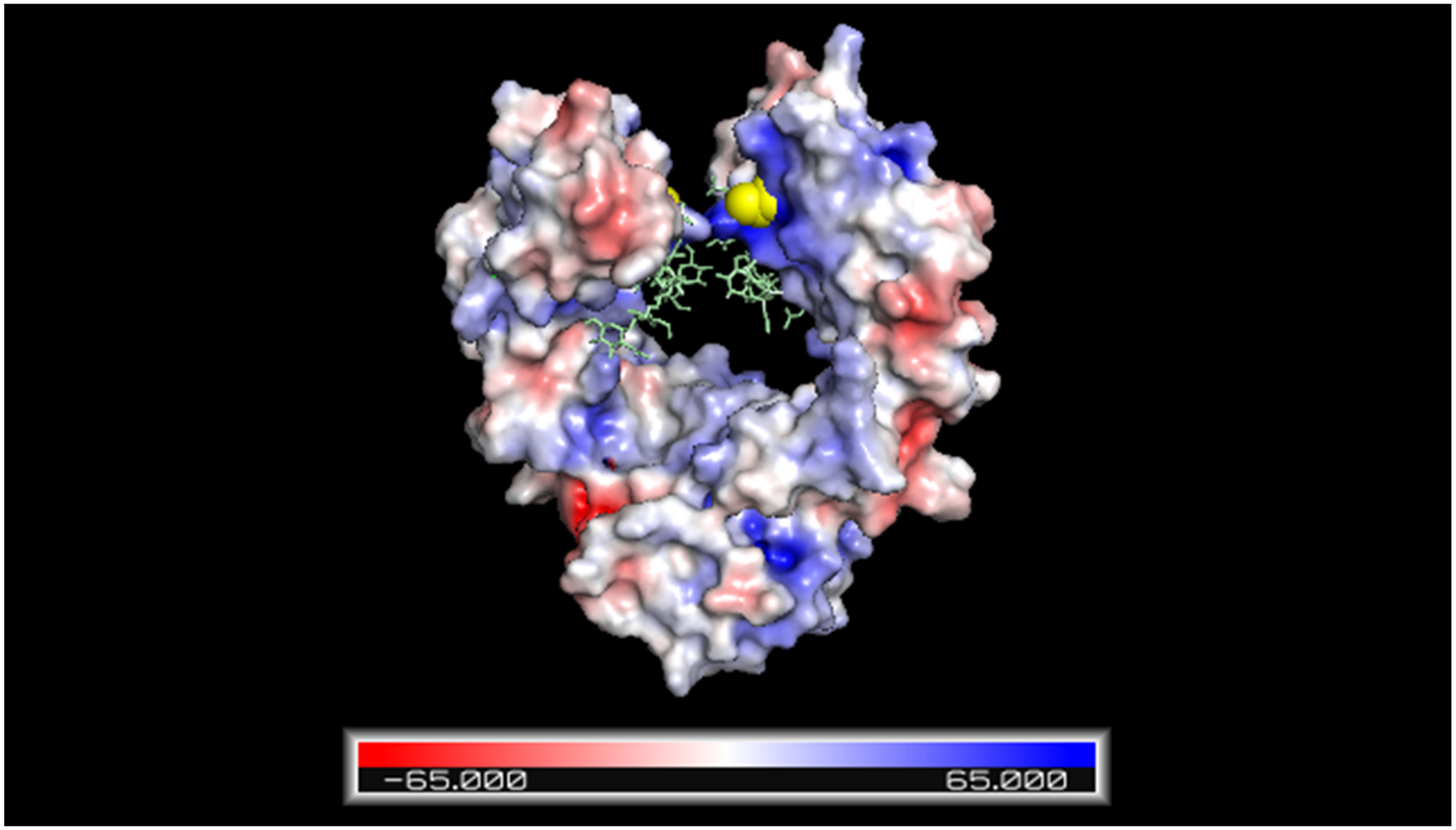
1. extra Cysteine



2. Maleimide



Local electrostatics may affect linker stability



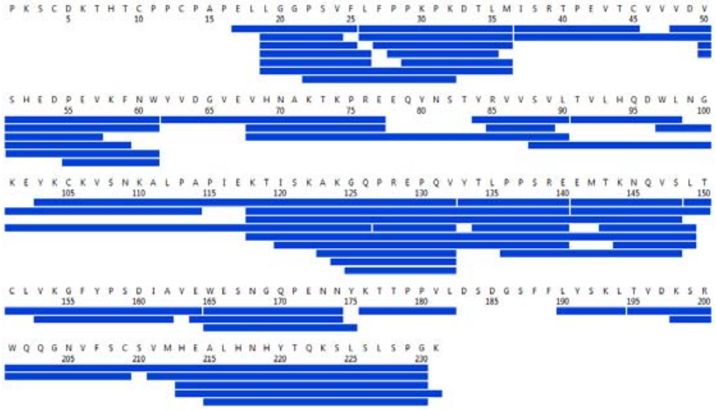
Structure of this Talk

1. Introduce Fc_C239i -> Done
2. Crystal structures of proto-linker adducts -> Done

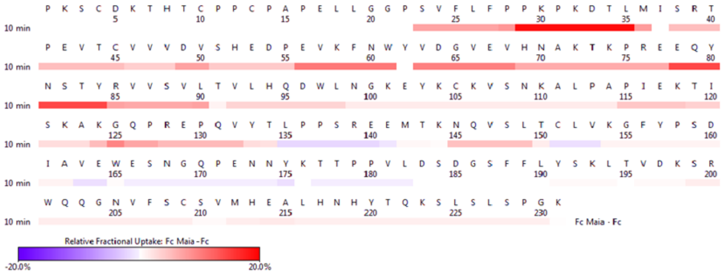
3. Dynamics
4. Receptor interactions
5. Conclusions

Hydrogen-Deuterium Exchange

HDX coverage and heat maps

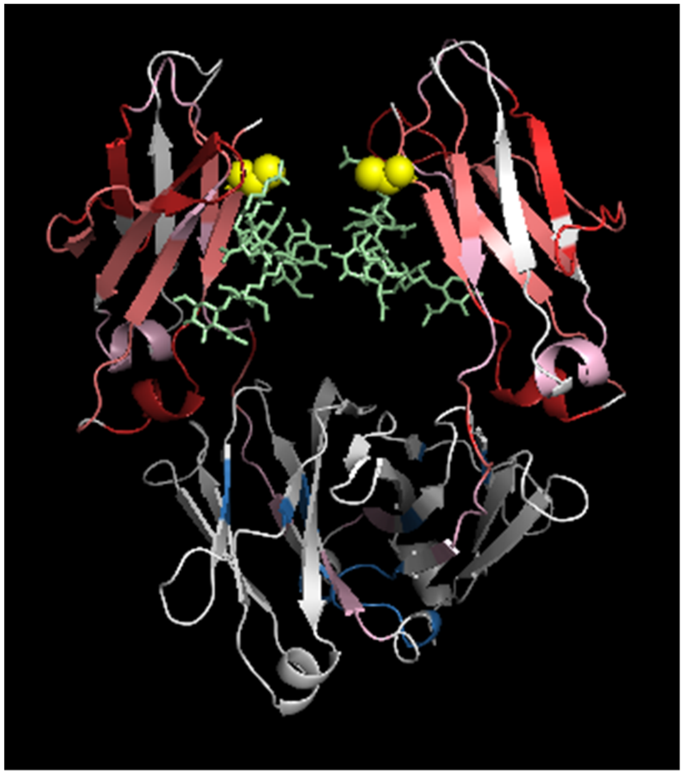


Total: 56 Peptides, 90.0% Coverage, 3.58 Redundancy



Two ways to measure dynamics in Fc_239i

(both are normalized to wild-type)

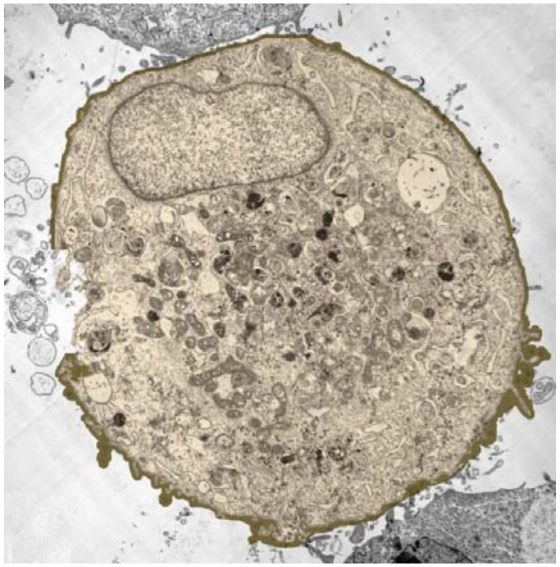


Dynamics by HDX

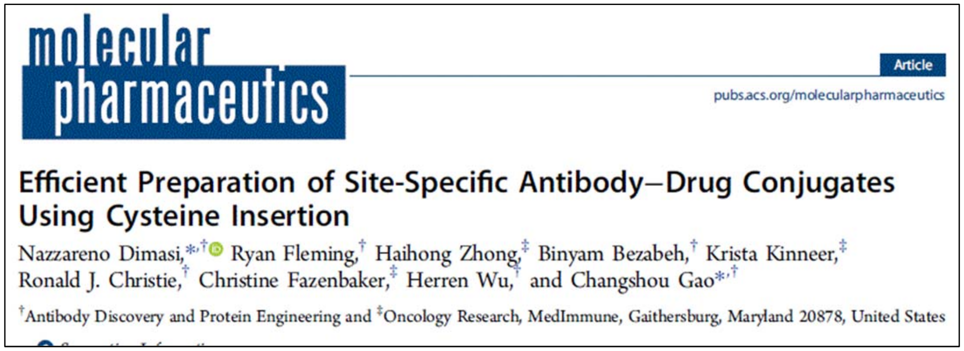
Dynamics by diffraction

Receptor Interactions

FcRn, FcR-gamma, C1q



Extensive measurements reported in 2017 paper:



molecular pharmaceutics

Article

pubs.acs.org/molecularpharmaceutics

Efficient Preparation of Site-Specific Antibody–Drug Conjugates Using Cysteine Insertion

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Ronald J. Christie,[†] Christine Fazenbaker,[‡] Herren Wu,[†] and Changshou Gao[*,†]

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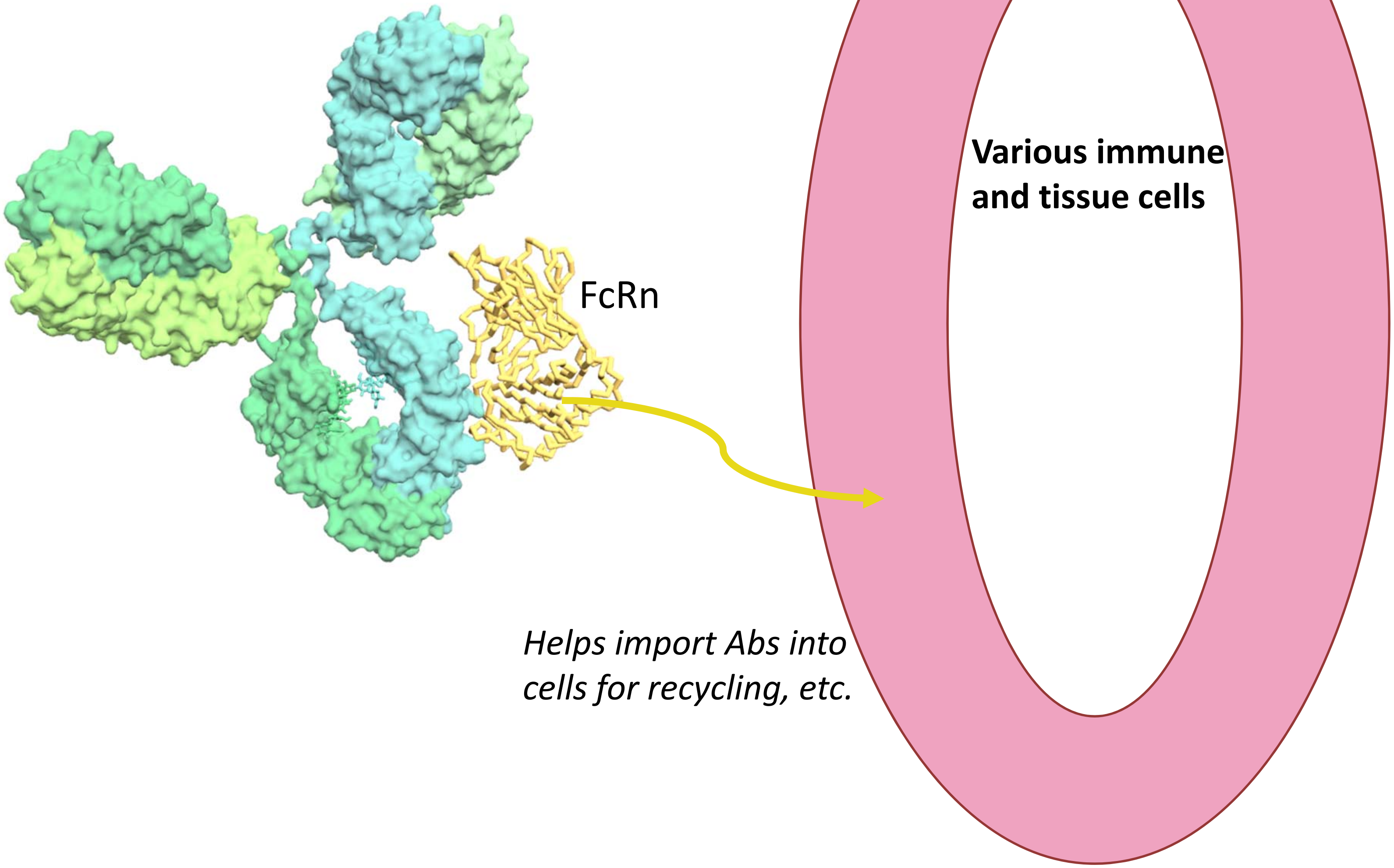
receptor:	**FcRn**	**FcR-gamma**	**C1q**
binding:	**+**	**-**	**-**

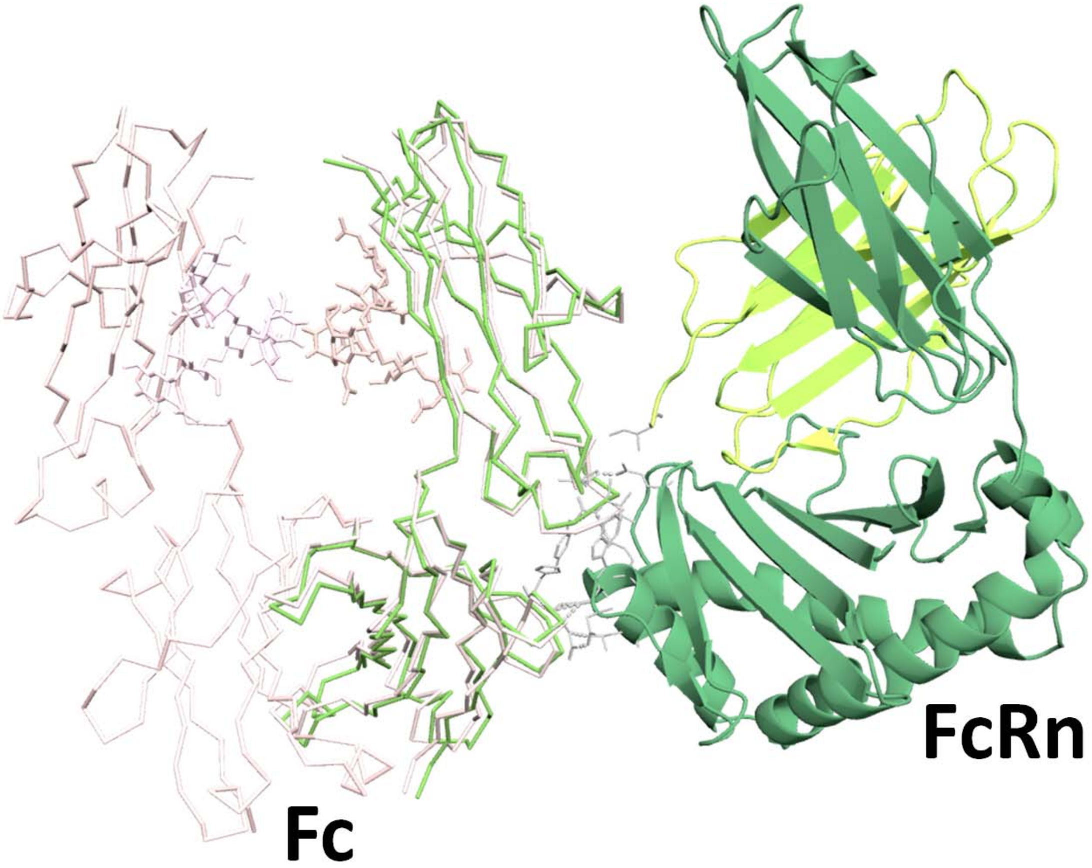
Biological role of FcRn

helps protect, preserve, recycle, distribute Abs.

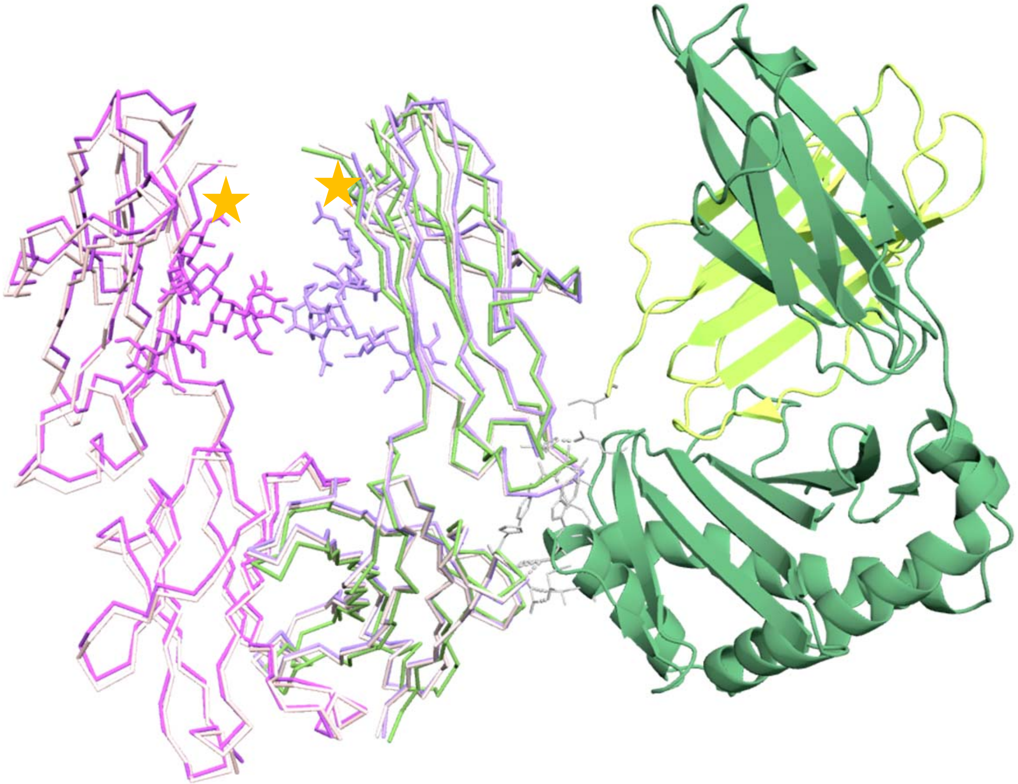


Helps import Abs into cells for recycling, etc.

Wild-type Fc binding to FcRn (PDB: 4N0U)



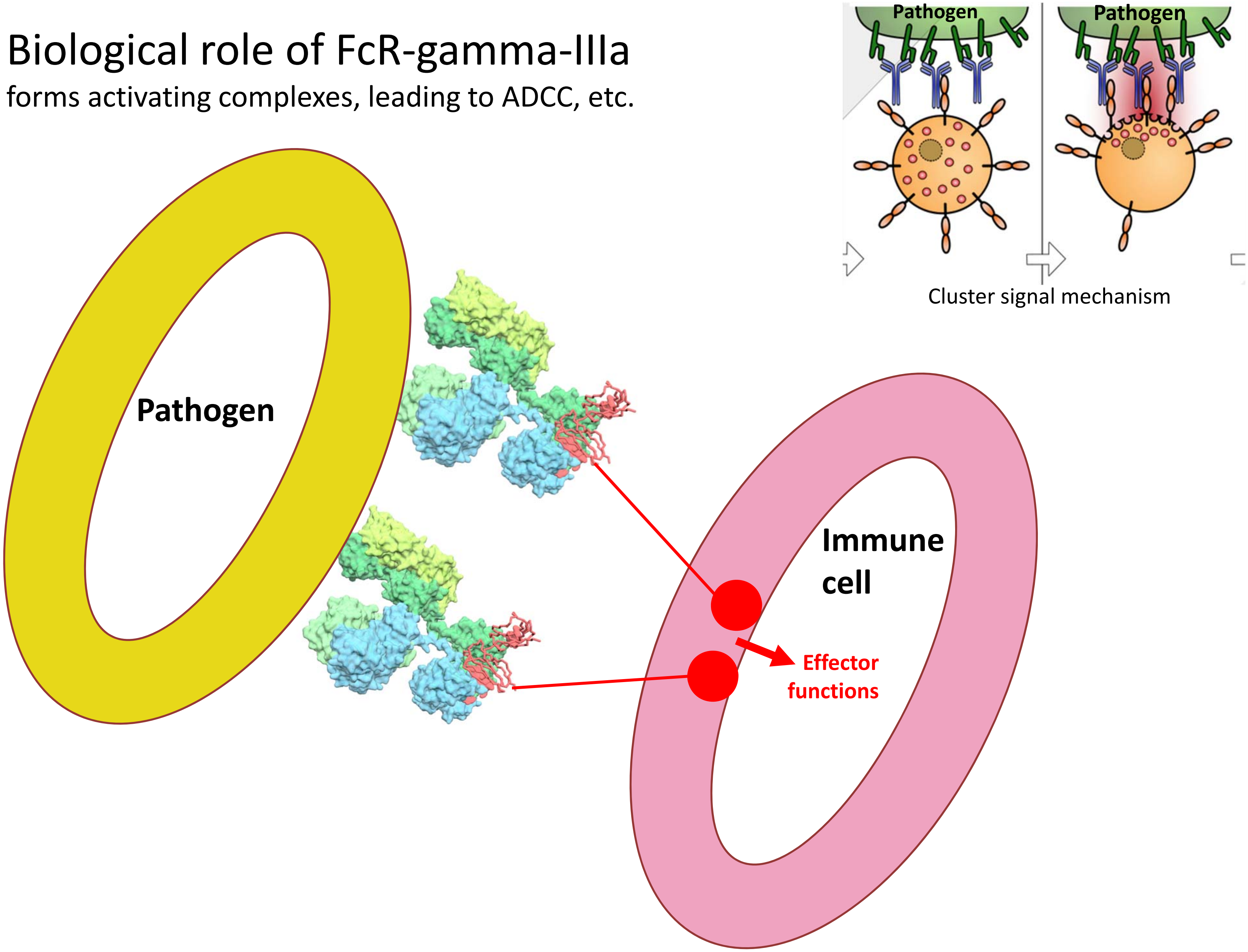
Model of Fc_239i binding to FcRn



The binding interface is far from the mutation.

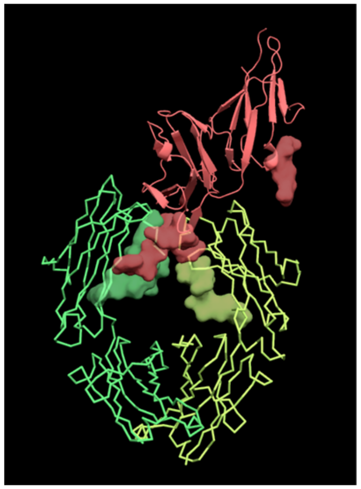
Biological role of FcR-gamma-IIIa

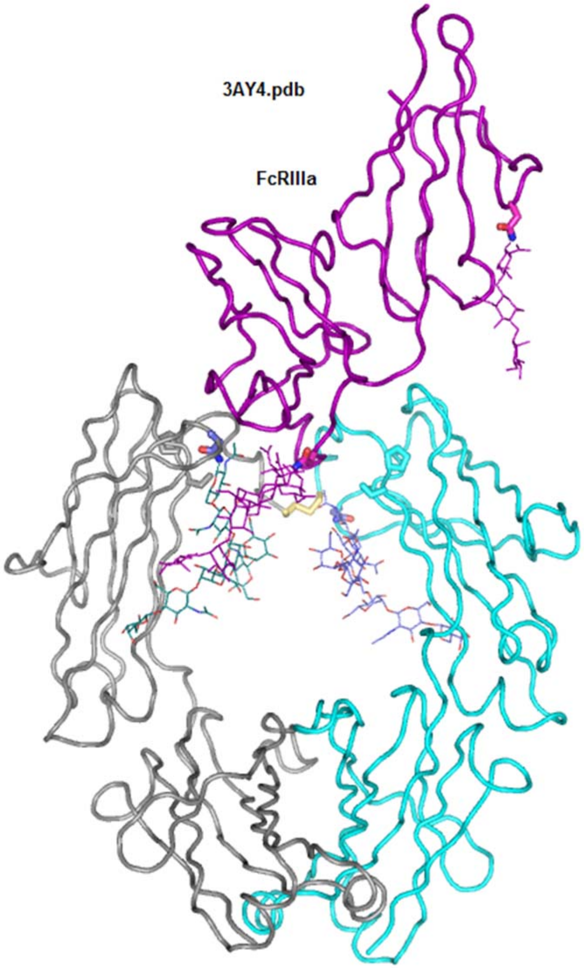
forms activating complexes, leading to ADCC, etc.

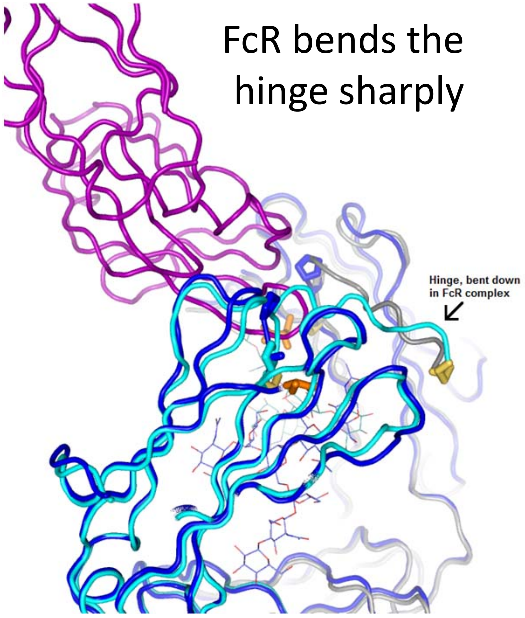


Cluster signal mechanism

Wild type Fc in complex with FcRIIIa complex (PDB: 3AY4



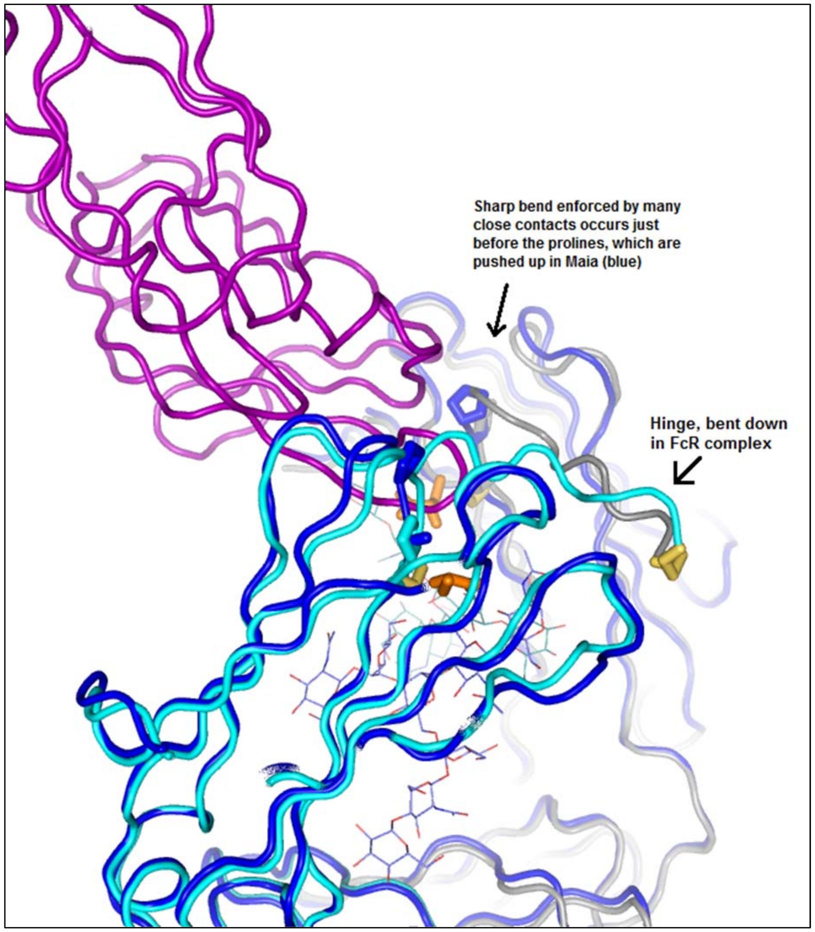




FcR bends the hinge sharply

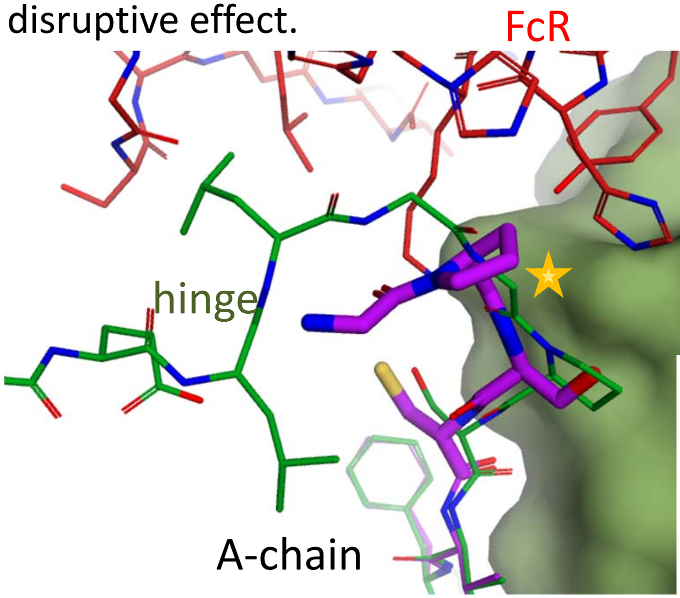
Fc_239i is sterically blocked from binding to FcRIII.

Shifting Pro 238 in place of Gly 237 has a strong disruptive effect.

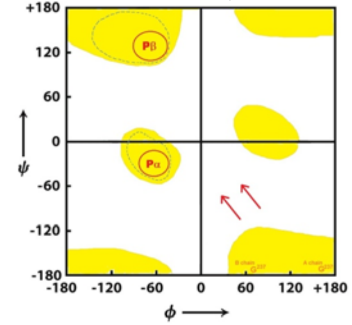


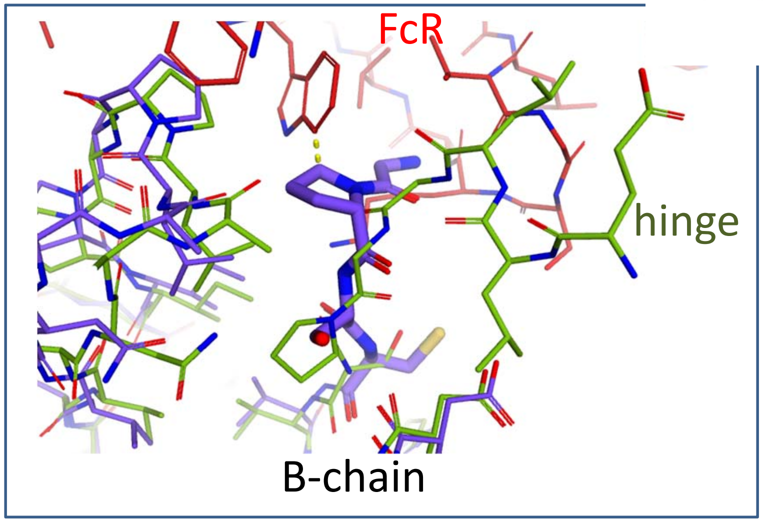
Sharp bend enforced by many close contacts occurs just before the prolines, which are pushed up in Maia (blue)

Hinge, bent down in FcR complex



Pro can't do what Gly can.





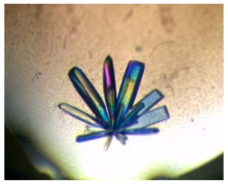
Conclusions

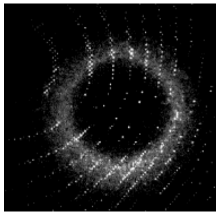
1. The ADC-engineered Fc fragment Fc_C239i crystal structure shows the molecular basis for several observed favorable properties, including:
    - general protein stability and conjugate stability (sheltered site)
    - non binding of Fc-gamma receptors, so that ADCC is not induced
    - normal binding to FcRn

2. Key features appear to be:
    - the sheltered-but-accessible conjugation site near to the FcR interface
    - the specific secondary structure at the start of the Fc: ...GGPS(C)...

3. Structure at 2.3 Angstrom resolution is deposited as 6xxx.pdb

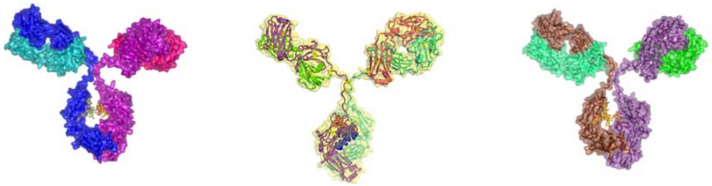
Structure and Dynamics of a Site-Specific Labeled Human Fc Fragment with Tuned Effector Functions





Collaborators:

Chris McCullough	MedImmune/NIST
Joomi Ahn	MedImmune
Robert Brinson	NIST
Nazzareno Dimasi	MedImmune



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