zymeworks

Engineering a Pure and Stable Heterodimeric IgA for the Development of Multispecific Therapeutics

PepTalk 2024

Meghan Verstraete, PhD

Legal Disclaimer

This presentation includes “forward-looking statements” within the meaning of the U.S. Private Securities Litigation Reform Act of 1995 and “forward-looking information” within the meaning of Canadian securities laws, or collectively, forward-looking statements. Forward-looking statements herein include, but are not limited to, statements that relate to the potential of IgA as a neutrophil-engaging cancer therapeutic, potential development of an IgA heterodimeric Fc platform, the commercial potential of technology platforms and other information that is not historical information. Forward-looking statements can often be identified by the use of terminology such as “future,” “potential,” “progress,” “subject to,” “anticipate,” “plan,” “expect,” “estimate,” “project,” “may,” “will,” “could,” “can,” the negatives thereof, variations thereon and similar expressions, or by discussions of strategy. In addition, any statements or information that refer to expectations, beliefs, plans, projections, objectives, performance or other characterizations of future events or circumstances, including any underlying assumptions, are forward-looking. All forward-looking statements are based upon our current expectations and various assumptions. Zymeworks believes there is a reasonable basis for its expectations and beliefs, but they are inherently uncertain. Zymeworks may not realize its expectations, and its beliefs may not prove correct. Actual results could differ materially from those described or implied by such forward-looking statements as a result of various factors, including, without limitation, clinical trials may not demonstrate safety and efficacy of any of Zymeworks’ or its collaborators’ product candidates; any of Zymeworks’ or its partners’ product candidates may fail in development, may not receive required regulatory approvals, or may be delayed to a point where they are not commercially viable; regulatory agencies may impose additional requirements or delay the initiation of clinical trials; the impact of new or changing laws and regulations; and the factors described under "Risk Factors" in Zymeworks’ quarterly and annual reports and other sections of our public filings with the Securities and Exchange Commission and Canadian securities regulators.

These forward-looking statements are made only as of the date hereof, and Zymeworks undertakes no obligation to update or revise the forward-looking statements, whether as a result of new information, future events or otherwise, except as required by law.

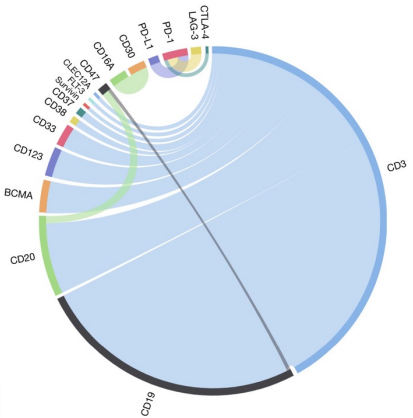
Advances in IgG protein engineering have fueled rapid development and expansion of multispecific antibody formats and their applications

Bispecific IgG antibodies have been developed with broad application for cancer immunotherapy and the treatment of other diseases

More than 30 mature commercial technology platforms have been used to develop bispecific antibodies

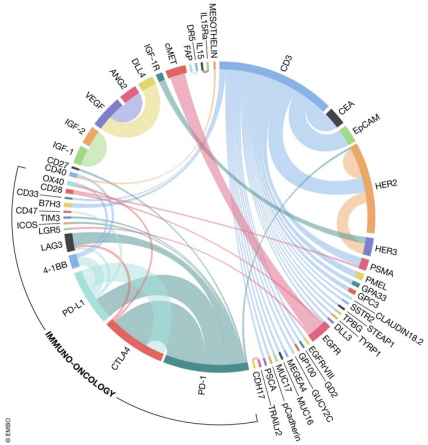
Target combinations of bispecific antibodies:

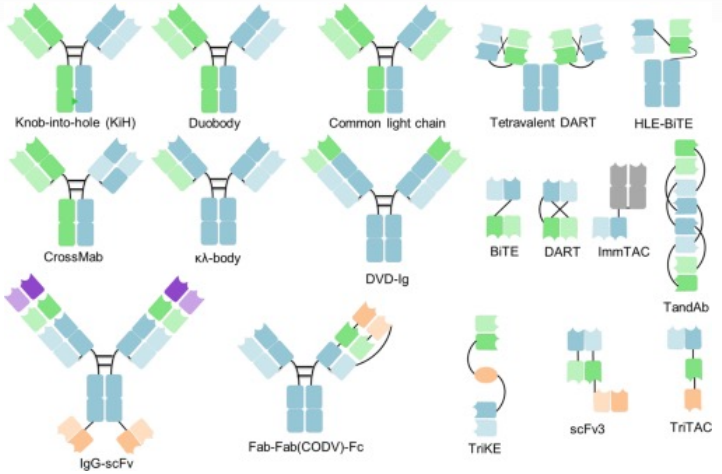
Hematological malignancies



Wang et al, EMBO Mol Med, 2021

Solid tumors

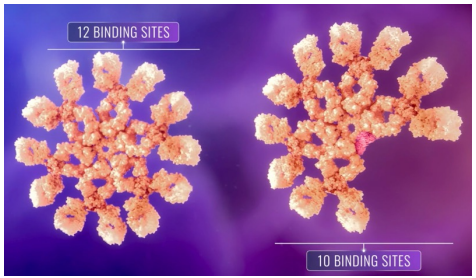




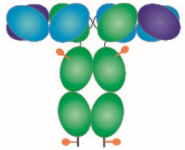
Jin et al, Nature, 2022

IgG is not the only antibody isotype that can be leveraged for therapeutic design

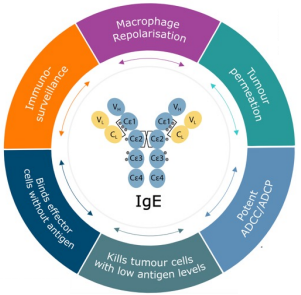
IgM for avidity and Fc-mediated effector functions (eg. complement)



IgA for Fc-mediated effector functions (eg. neutrophil activation)



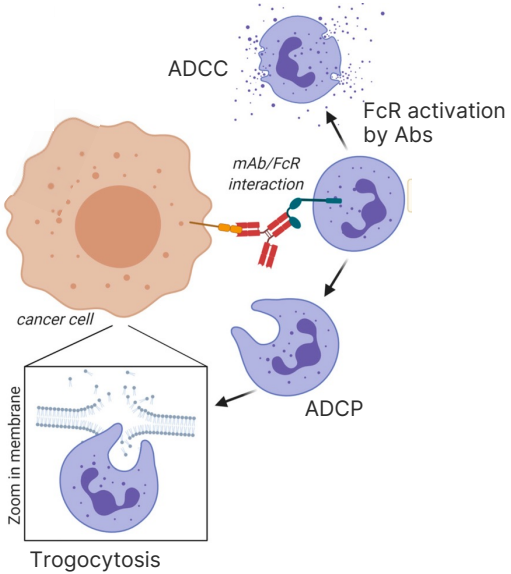
IgE for Fc-mediated effector functions (eg. macrophage repolarization)



Engineering a bispecific Fc can grant therapeutic tractability to new antibody isotypes by enabling multispecific design

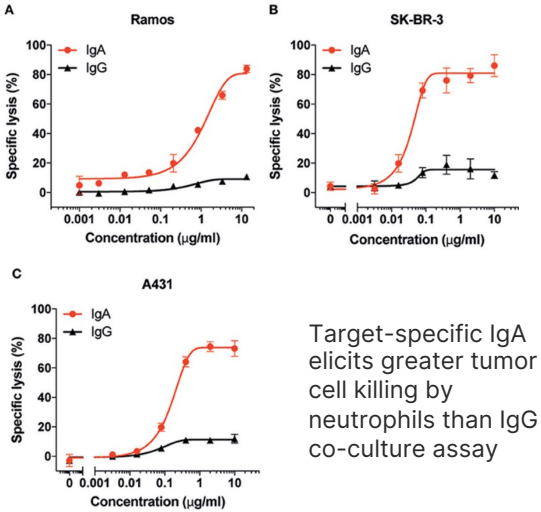
Recent work has highlighted the potential of IgA as a neutrophil-engaging cancer therapeutic

- Antibody-driven mechanisms of cytotoxicity



Adapted from Avtenyuk et al, Int J Mol Sci, 2020

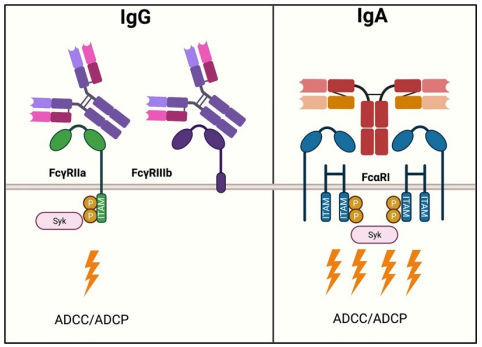
- IgA can potently activate neutrophils to kill tumor cells *in vitro* and *in vivo*



Target-specific IgA elicits greater tumor cell killing by neutrophils than IgG in co-culture assay

Brandsma et al, Front Immunol, 2019

- Differential Fc receptor engagement



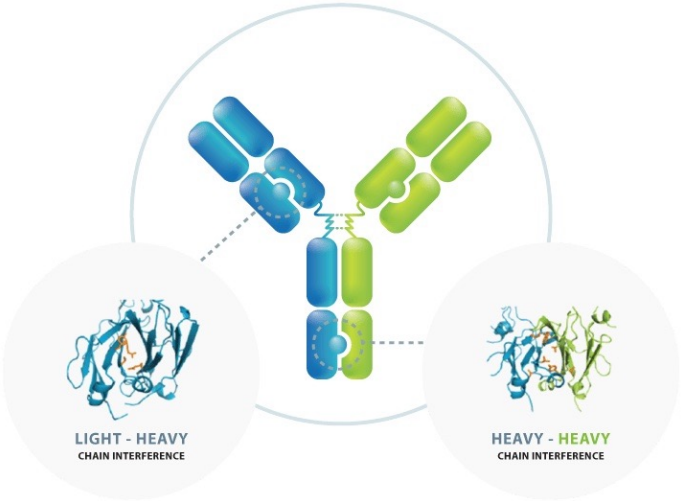
Chan et al, Front Immunol, 2022

Engineering for developability: Zymeworks' previous success engineering an IgG heterodimeric Fc

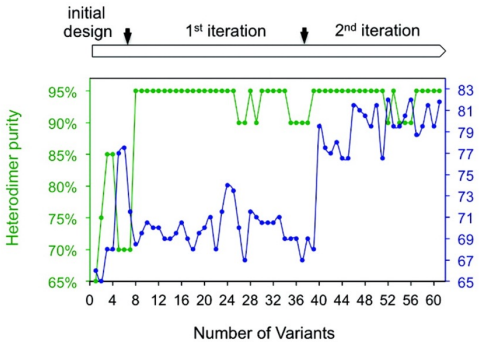
Azymetric™

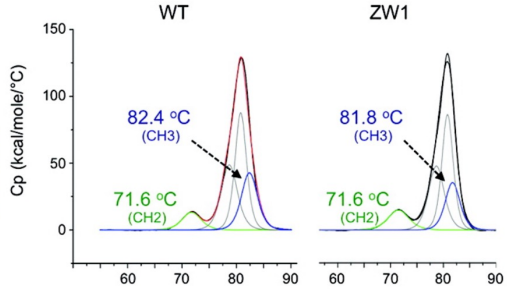
The foundation to how we build and design multi-functional and unique antibodies

Azymetric™ is an IgG heterodimeric antibody technology developed using proprietary internal tools



Developability concepts and parameters for **purity and stability** introduced early in the engineering process





Spreter Von Kreudenstein *et al*, mAbs, 2013
Spreter Von Kreudenstein *et al*, Methods, 2014

Use knowledge and tools used to develop IgG Azymetric™ to build an **IgA heterodimeric Fc platform**

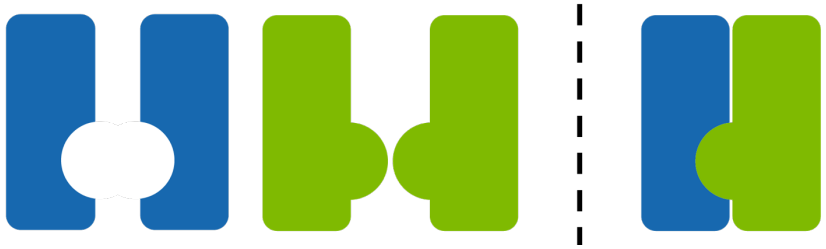
Rational design strategy used for IgA Fc interface engineering

Design Concept:

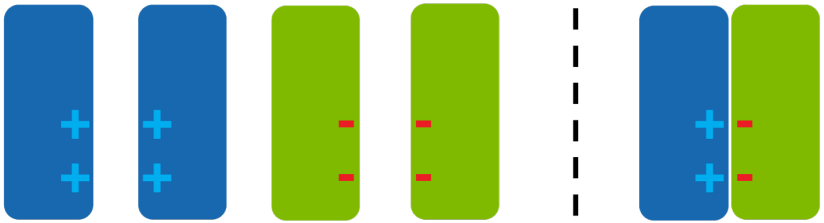
Negative Design
Homodimers Disfavoured

Positive Design
Heterodimer Favoured

1. Steric design



2. Electrostatic design



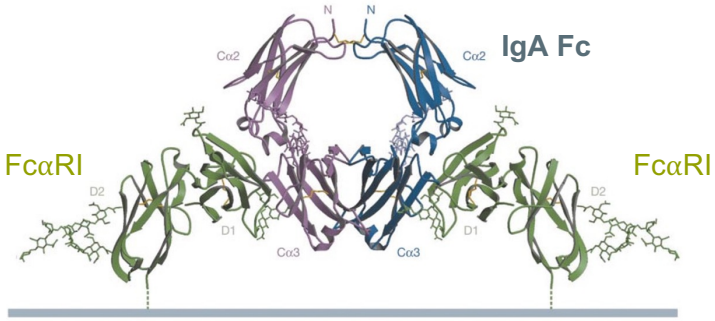
Engineer for heterodimer **purity** using negative design

Engineer for heterodimer **stability** using positive design

1. Structure Model Creation → 2. System Analysis → 3. *In silico* design → 4. Variant selection → 5. Experimental screening

Crystal structure of homodimeric (wild-type) IgA Fc used for in-depth analysis of IgA CH3-CH3 interface

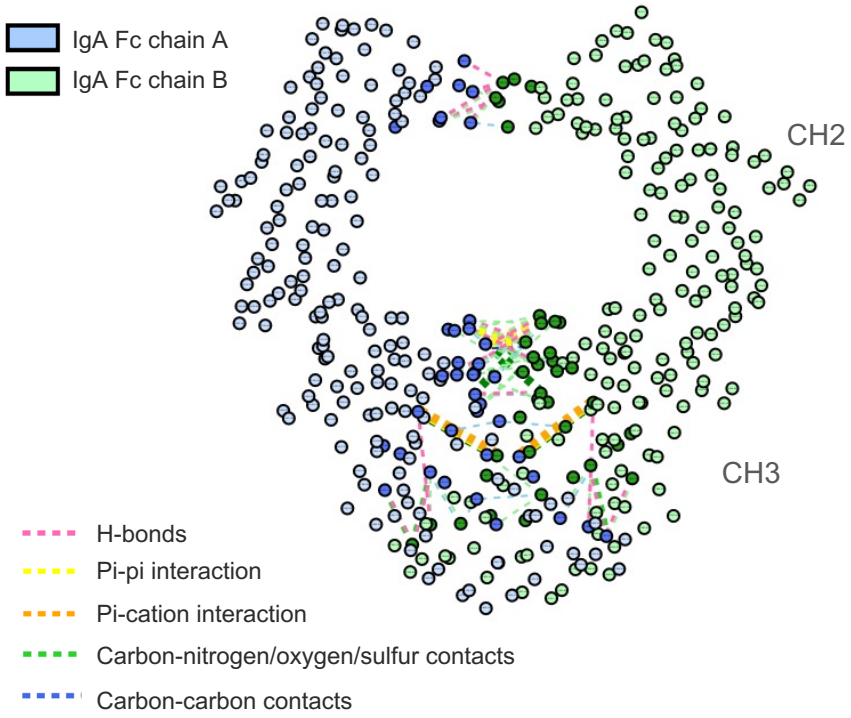
1. Structure Model Creation → 2. System Analysis



PDB ID:1OW0; from Herr et al, Nature, 2003

1. Interface analysis to predict the energetic contribution of amino acid residues at the interface and the non-bonded contacts in the structure
2. Identify core interface positions (hot spots) where introduction of mutation is predicted to increase or decrease the strength of the interface
3. Mutations were introduced and modelled computationally

IgA Fc interface contact analysis



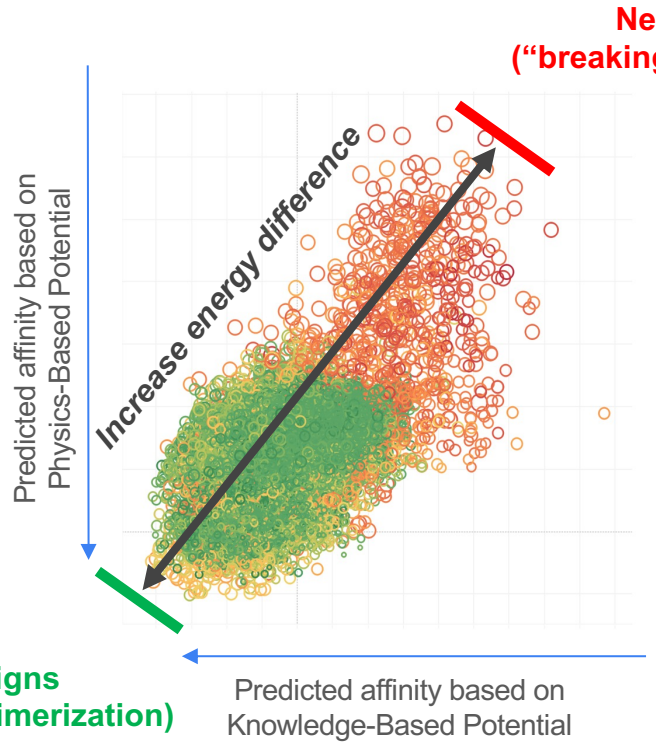
In silico tools employed to inform heterodimeric IgA Fc design

3. *In silico* design

Variants were predominately selected based on affinity metrics

Models of homodimeric and heterodimeric IgA CH3:CH3 variants were scored for affinity based on analysis of:

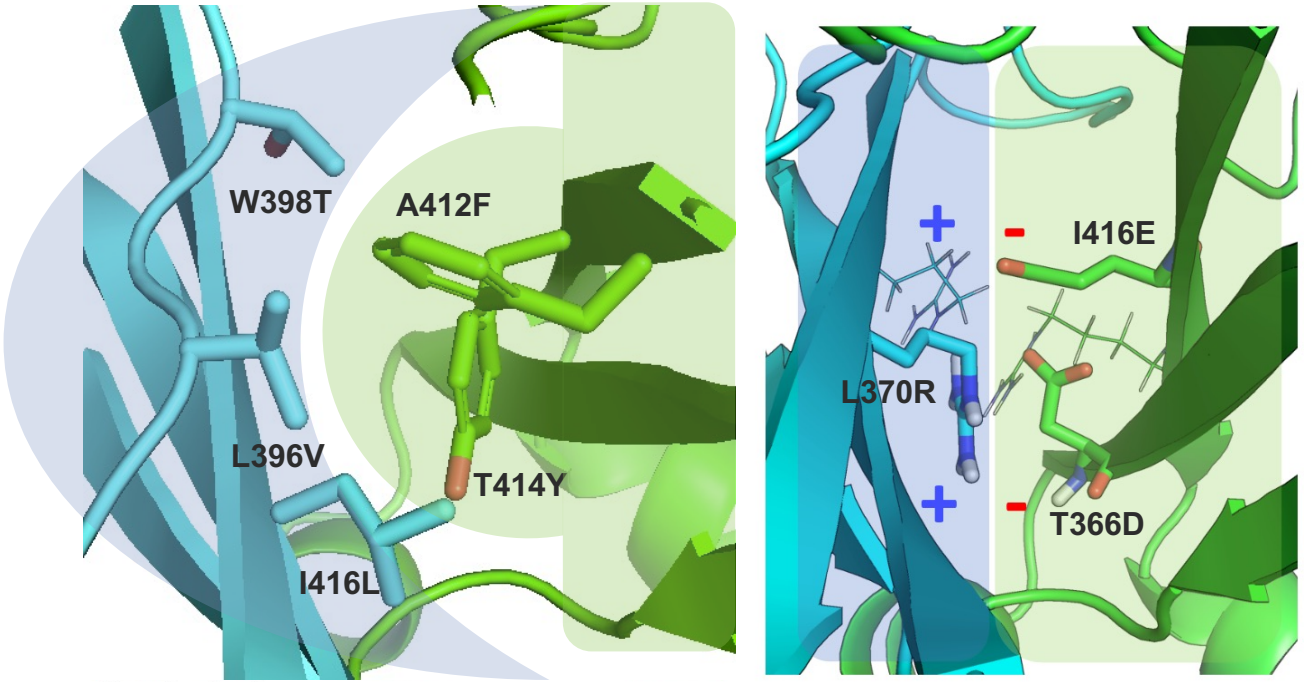
- **Knowledge-based potential:** probabilities of interactions between atoms based on known protein structures
- **Physics-based potential:** energetic contribution of residue interactions across the interface



- Top designs were selected based on the **largest energetic difference** between homodimer and heterodimer
- Thresholds on **stability metrics and clashes** were used to further narrow down the search

Example *in silico* models of steric vs electrostatic heterodimeric IgA Fc designs

4. Variant selection



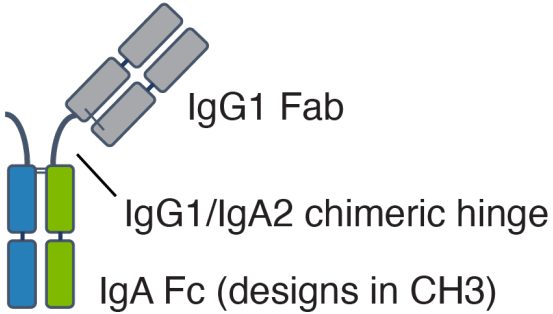
Steric design
1-3 mutations/chain

Electrostatic design
2-5 mutations/chain

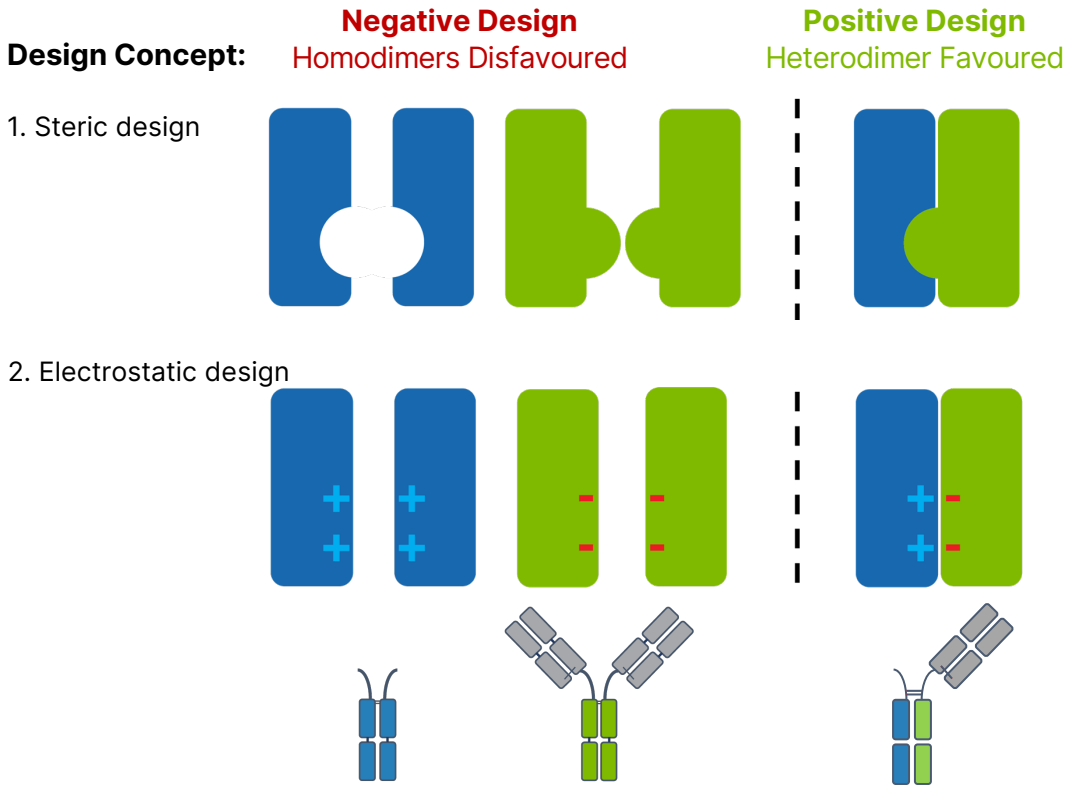
Top 12 designs selected for experimental screening

Experimental *in vitro* evaluation of IgA heterodimeric Fc designs

One-armed antibody (OAA) format used to test top-ranked designs

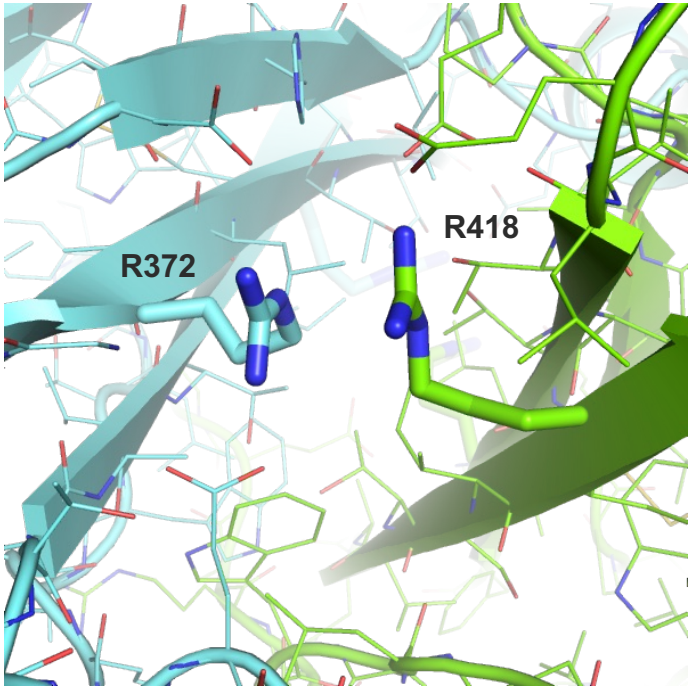


- Unique sets of mutations introduced into **IgA CH3** domains for "**chain A**" and "**chain B**"



IgA Fc variants containing electrostatic design mutations did not have any detectable protein expression

Wildtype IgA CH3:CH3 interface

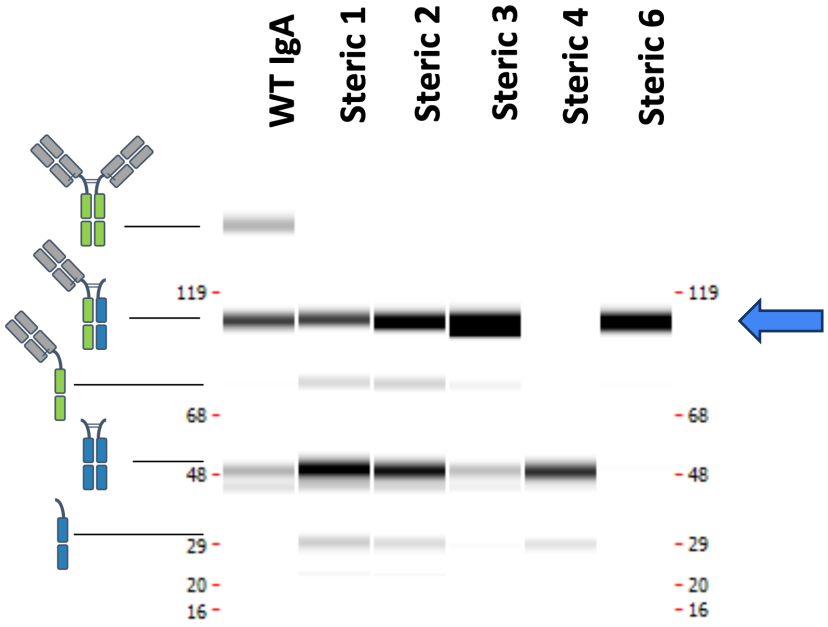


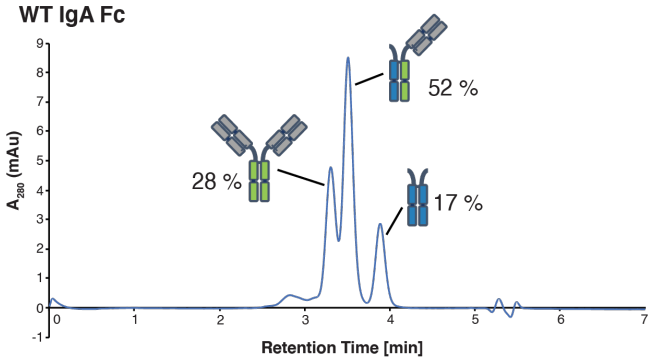
- All electrostatic designs included the replacement of the R372-R418 pi-pi interaction in the CH3:CH3 interface with a salt bridge.
- Mutations had the potential to skew the energetics of the CH3 interface to favour heterodimerization, but also carried significant risk of destabilization of the protein.

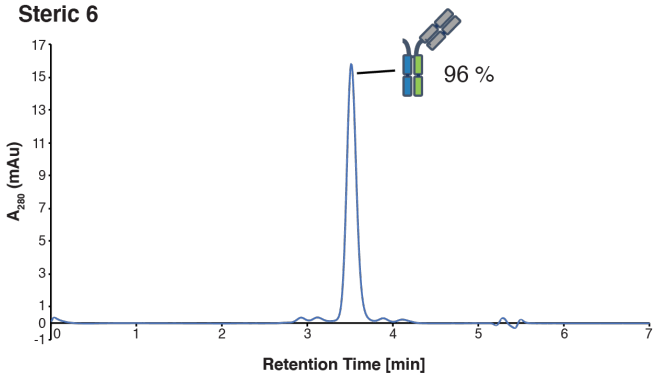
Design	Chain A Mutations	Chain B Mutations
Electrostatic 1	T366D_L370D_**R372D**_I416E	T366R_L370R
Electrostatic 2	T366E_L370E_**R372D**_I416D_ **R418E**	T366R_L370K
Electrostatic 3	T366E_**R372D**_I416D_**R418D**	T366R_L370K_I416R
Electrostatic 4	T366E_**R372D**_I416E_**R418E**	T366K_L370K_**R372K**_I416R
Electrostatic 5	**R372D**_E403D_I416D_**R418E**	L370K_**R372K**_E403R_ I416K
Electrostatic 6	**R372D**_E403D_I416D_**R418E**	L370K_**R372K**_I416K

High heterodimeric IgA Fc purity measured by CE-SDS and analytical SEC after affinity purification for steric 6 design

5. Experimental screening

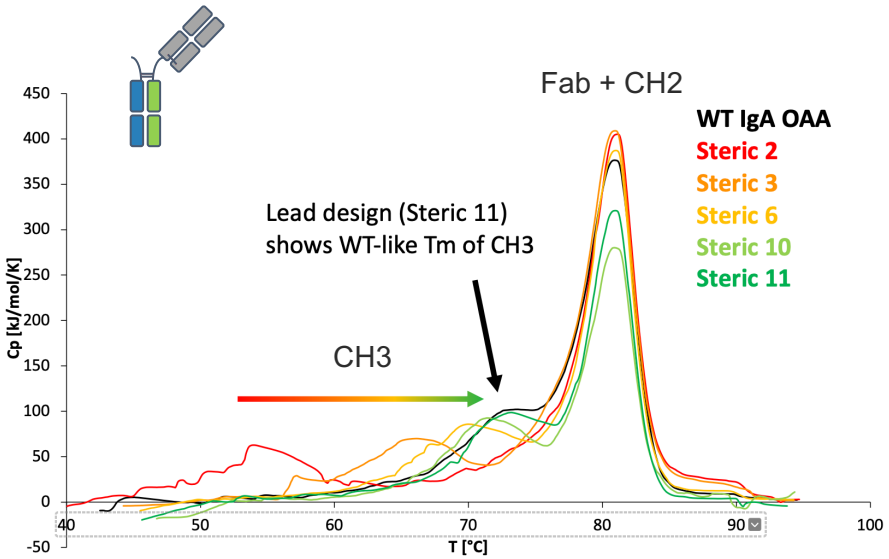






Heterodimeric IgA Fc engineered for thermal stability and high purity

Thermal stability of purified heterodimeric IgA Fc designs assessed by differential scanning calorimetry



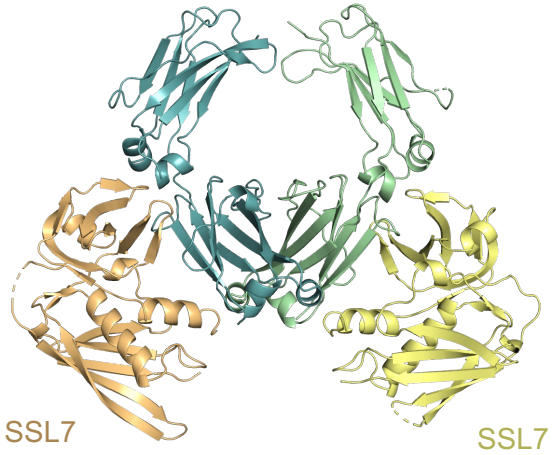
Summary of purity and stability for IgA Fc design

Design	Post affinity purification			Post prepSEC purification			
	HPLC-SEC OAA purity (%)	CE-SDS OAA purity (%)	Total yield (mg/L culture)	HPLC-SEC OAA purity (%)	CE-SDS OAA purity (%)	OAA yield (mg/L culture)	IgA CH3 Tm (°C)**
Wild-type	52	49	324	91	92	76	74.2
Steric 2	65	55	240	97	96	76	55
Steric 3	91	89	328	100	98	136	65.9
Steric 6 *	96	92	320	100	97	100	71.9
Steric 10 *	72	88	370	100	85	82	72
Steric 11 *	74	95	440	100	93	71	73.6

*Lead designs

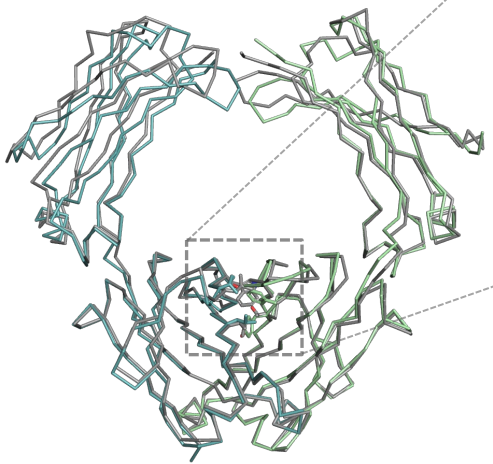
A crystal structure of the heterodimeric IgA Fc revealed that the IgA CH3 mutations do not perturb the overall IgA Fc structure and a heterodimeric IgA interface consistent with *in silico models*

Heterodimeric IgA Fc
(steric 6 design)

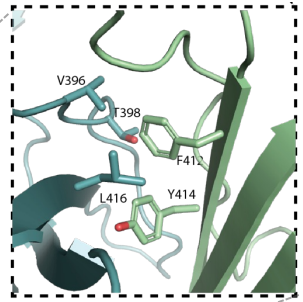


- Heterodimeric IgA Fc (steric 6) crystal structure was solved in complex with *Staphylococcus aureus* protein SSL7 (PDB ID: 7TTZ)

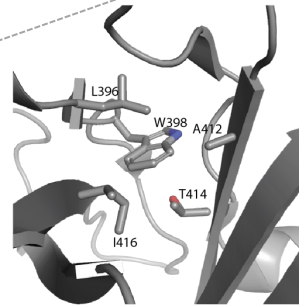
Chain A mutations	Chain B mutations
L396V, W398T, I416L	A412F, T414Y



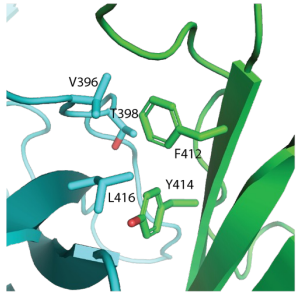
- Heterodimeric IgA Fc (blue/green) superimposed with wildtype IgA Fc (grey) have RMSD of 0.94 Å across C_α atoms in the Fc



Heterodimeric IgA Fc



Wildtype IgA Fc
(PDB ID: 2QEJ)



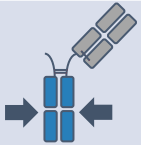
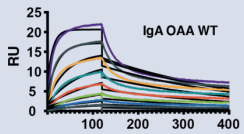
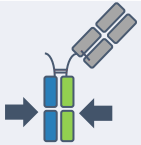
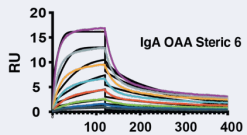
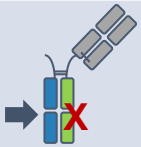
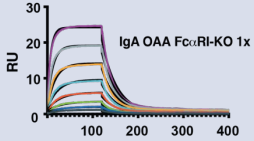
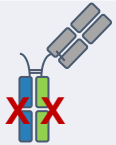
In silico model

- The RMSD of the heterodimeric IgA Fc CH3 crystal structure relative to the *in silico* model was 1.2 Å across C_α atoms (residues 345-450)

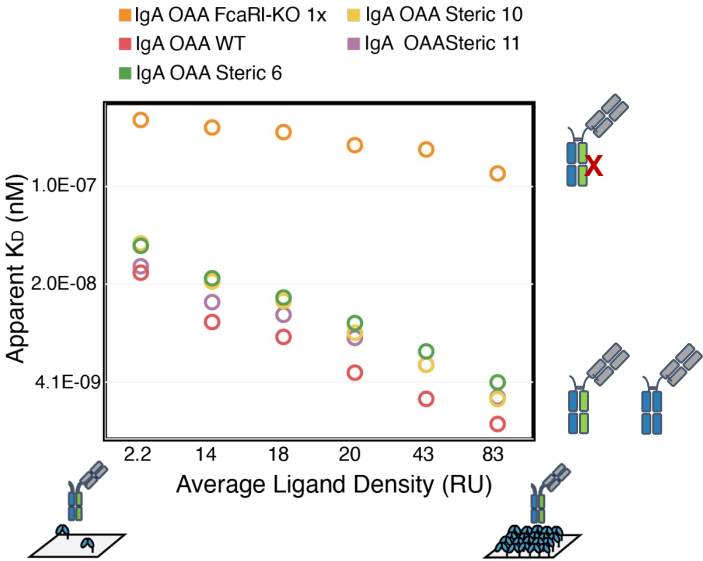
Heterodimeric IgA Fc designs show preserved binding to FcαRI by SPR

Analyte: IgA Fc variant
Ligand: FcαRI

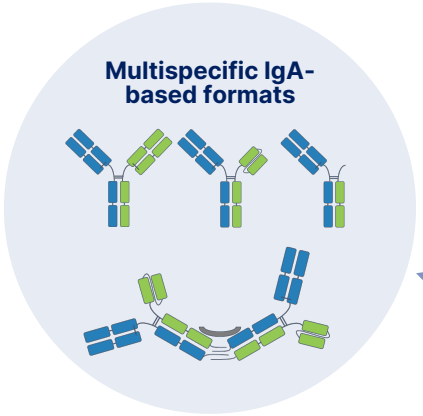
FcαRI binding site
X FcαRI binding site knockout (KO)

IgA variant	Variant diagram	Sensorgram	Kinetic parameter	Kinetic value
Wild-type IgA Fc			ka (1/Ms)	2.76E+05
			kd (1/s)	3.07E-03
			KD (M)	**1.11E-08**
Heterodimeric IgA Fc steric 6			ka (1/Ms)	6.46E+05
			kd (1/s)	1.48E-02
			KD (M)	**2.28E-08**
IgA FcαRI-KO 1x			ka (1/Ms)	1.08E+05
			kd (1/s)	2.96E-02
			KD (M)	**2.73E-07**
IgA FcαRI-KO 2x		**No binding**	ka (1/Ms)	**NA**
			kd (1/s)	
			KD (M)	

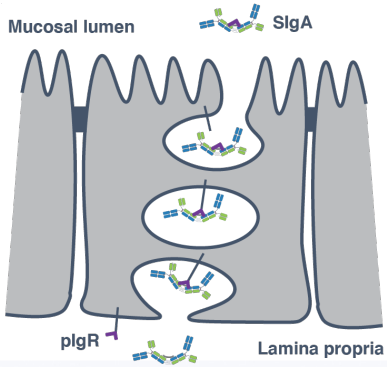
Increasing the density of FcαRI resulted in higher avidity-driven binding for wild-type and heterodimeric IgA Fc variants

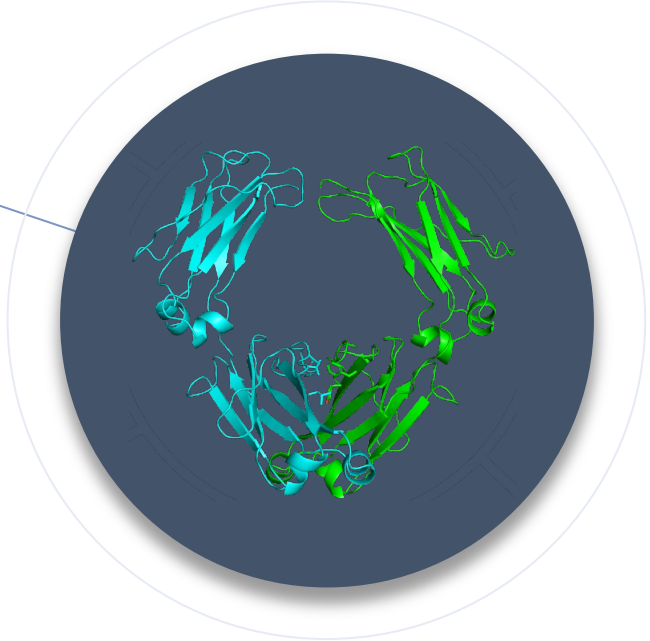


Applications of heterodimeric IgA Fc



Multispecific therapeutics targeting mucosa





Dimeric IgA specifically disables intracellular mutated oncodrivers

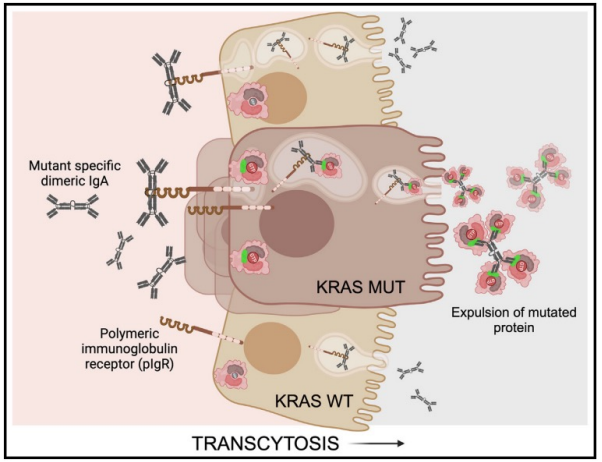
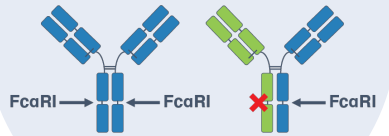


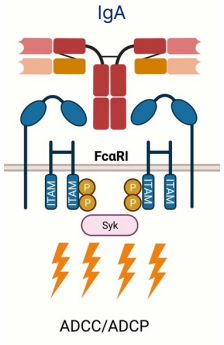
Figure from Prince and Hollmen, Immunity Previews, 2023
Biswas *et al*, Immunity, 2023
Biswas *et al*, Nature, 2021

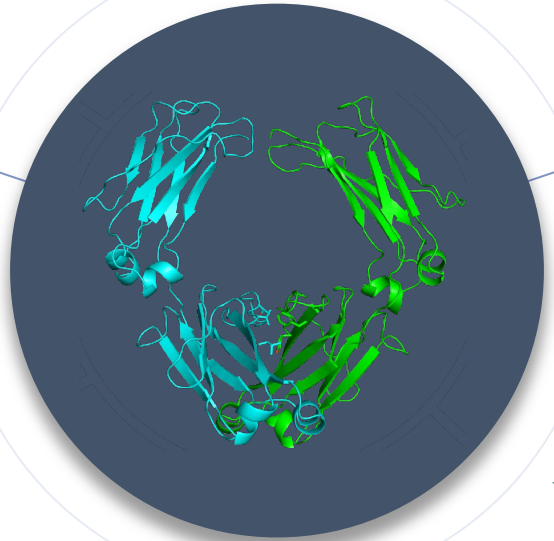
Applications of heterodimeric IgA Fc

Investigate IgA:FcαRI stoichiometry required for neutrophil activation

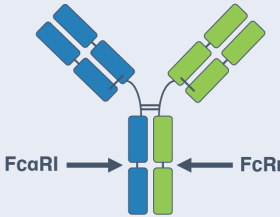


Is bivalent binding of FcαRI required for neutrophil activation?

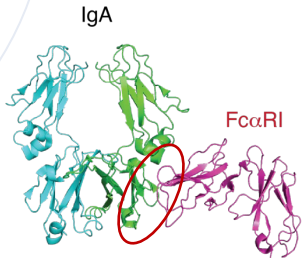




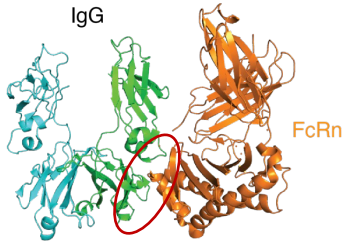
IgA half-life modulation



FcRn and FcαRI binding sites are structurally mutually exclusive



PDB ID: 1OW0



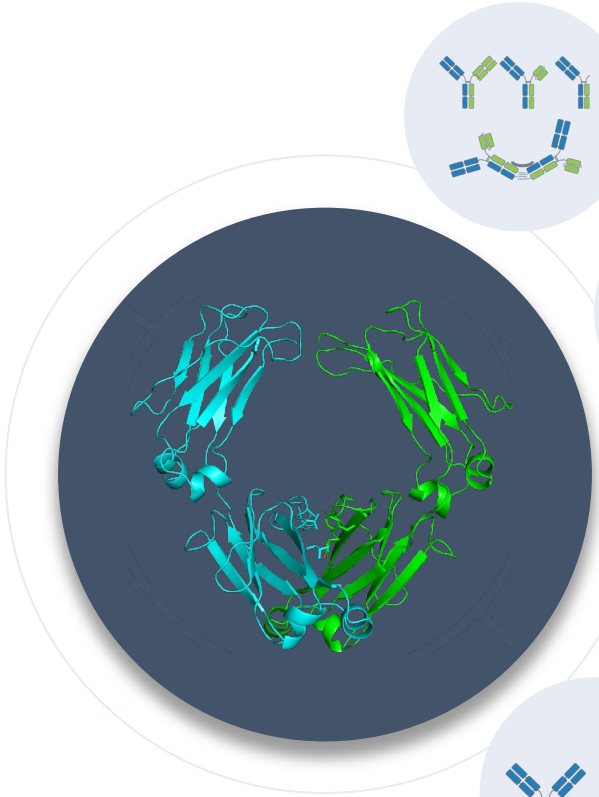
PDB ID: 1I1A

Summary

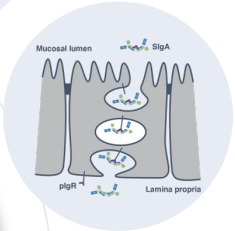
Protein Engineering
Integrating **purity and stability** design requirements yielded a **heterodimeric IgA Fc** with favorable developability characteristics

Application
A heterodimeric IgA Fc unlocks the potential to explore **multispecific IgA therapeutics** and interrogate **IgA-FcαRI biology**

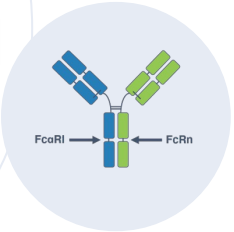
Quality by Design
Example of how introducing developability concepts and parameter early in design process can promote success in the development of **novel multispecific antibodies with new functionalities**



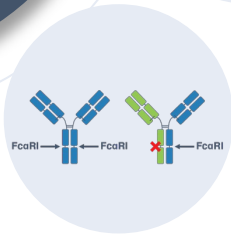
Multispecific IgA-based formats



Multispecific therapeutics targeting mucosa



IgA half-life modulation



Investigate IgA:FcαRI stoichiometry required for neutrophil activation

Acknowledgments

Zymeworks team members
(past and present)

Florian Heinkel
Eric Escobar
Thomas Spreter Von Kreudenstein
Surjit Dixit
Siran Cao
Janessa Li
Patrick Farber
Begonia Silva-Moreno
Elizabeth Stangle

University of Victoria

Martin Boulanger
Fangni Peng

MABS
2022, VOL. 14, NO. 1, e2141637 (13 pages)
https://doi.org/10.1080/19420862.2022.2141637

Taylor & Francis
Taylor & Francis Group

REPORT

OPEN ACCESS Check for updates

Engineering a pure and stable heterodimeric IgA for the development of multispecific therapeutics

Florian Heinkel[a*], Meghan M. Verstraete[a*], Siran Cao[a], Janessa Li[a], Patrick Farber[a], Elizabeth Stangle[a], Begonia Silva-Moreno[a], Fangni Peng[b], Surjit Dixit[a], Martin J. Boulanger[b], Thomas Spreter Von Kreudenstein[a], and Eric Escobar-Cabrera[a]

Photo from CBRE

Thank you