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

PepTalk 2024

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These forward-looking statements are made only as of the date hereof, and Zymeworks Inc. 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 has 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

Solid tumors

Wang et al, EMBO Mol Med, 2021

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)
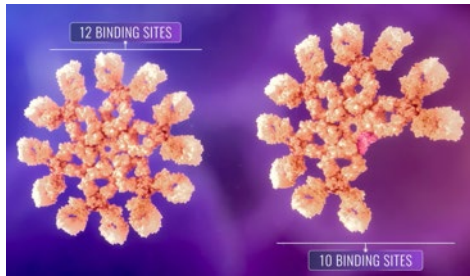
IgA for Fc-mediated effector functions (eg. neutrophil activation)
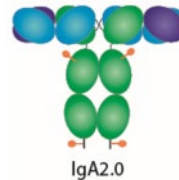
IgE for Fc-mediated effector functions (eg. macrophage repolarization)
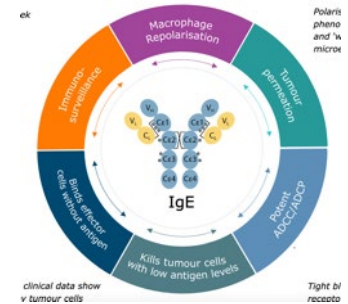
Engineering a bispecific Fc can grant therapeutic tractability to new antibody isotypes by enabling multispecific design

fully heterodimeric | VHH-Fab | one-armed | common light chain | scFv-Fab | scFv-scFv | 2+1 bispecific

Recent Work has Highlighted the Potential of IgA as a Cancer Therapeutic

- Neutrophils are a large untapped effector cell population that could be harnessed to kill tumor cells.

- Utility of IgA to engage neutrophils in vitro and in vivo to kill cancer cells has been demonstrated

- IgA can potently activate neutrophils via binding to the FcαRI receptor

ADCC

FcR activation by Abs

mAb/FcR interaction

cancer cell

ADCP

Zoom in membrane

Trogocytosis

A — Ramos — IgA / IgG
B — SK-BR-3 — IgA / IgG
C — A431 — IgA / IgG
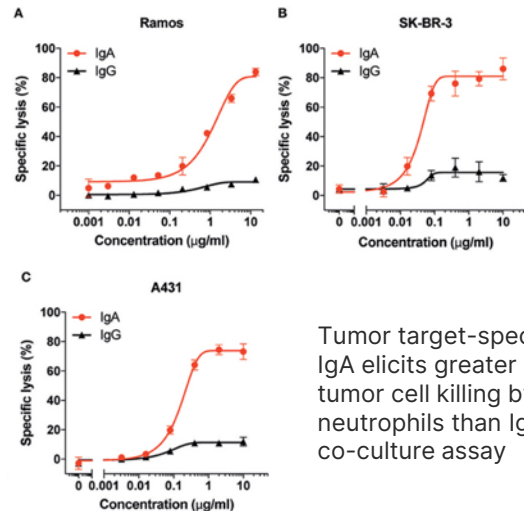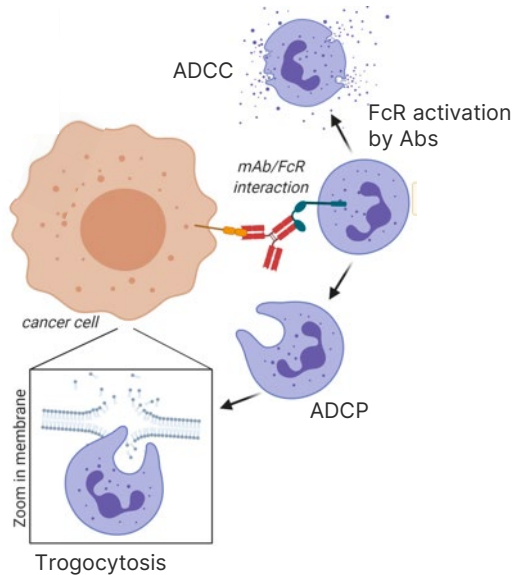
Tumor target-specific IgA elicits greater tumor cell killing by neutrophils than IgG in co-culture assay
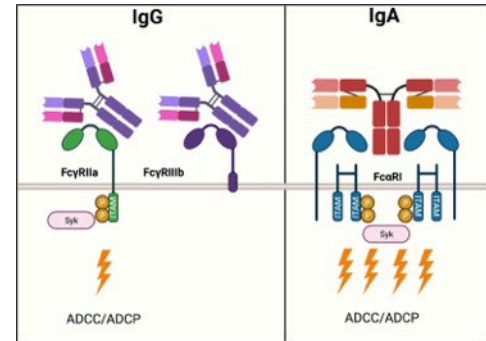
IgG / IgA
FcγRIIa   FcγRIIIb   FcαRI
Syk
ADCC/ADCP   ADCC/ADCP

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

Brandsma et al, Front Immunol, 2019

Chan et al, Front Immunol, 2022

Azymetric™

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

Azymetric™ is a best-in-class IgG heterodimeric antibody technology developed using proprietary internal tools
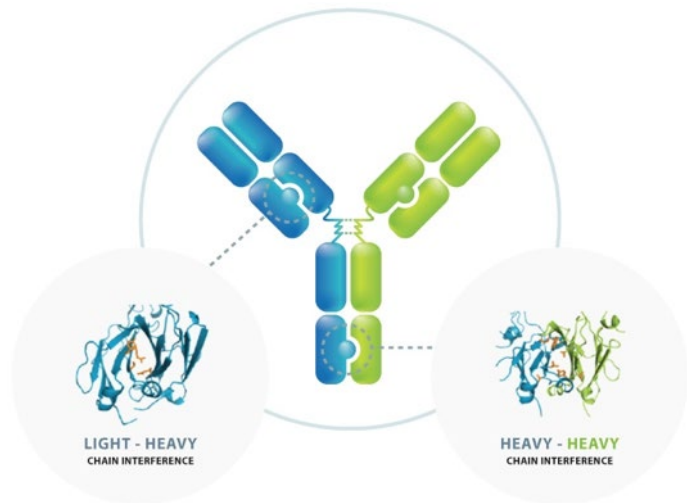
Developability concepts and parameters for **purity and stability** introduced early in the engineering process
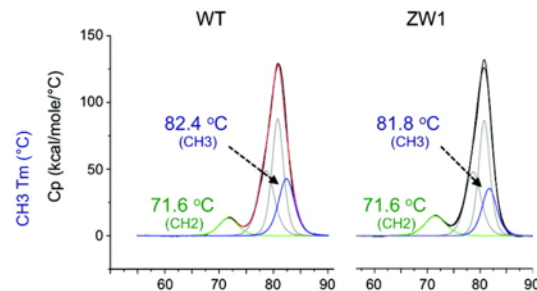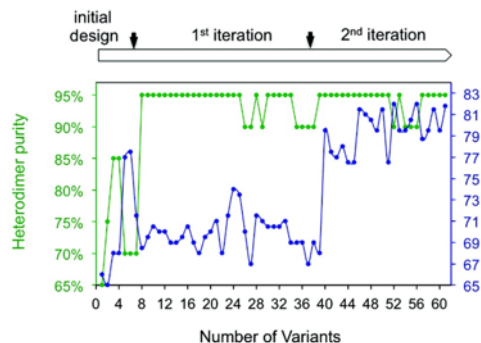

Spreter Von Kreudenstein *et al*, mAbs, 2013
Spreter Von Kreudenstein *et al*, Methods, 2014

LIGHT - HEAVY
CHAIN INTERFERENCE

HEAVY - HEAVY
CHAIN INTERFERENCE

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

Engineer for heterodimer **purity** using negative design
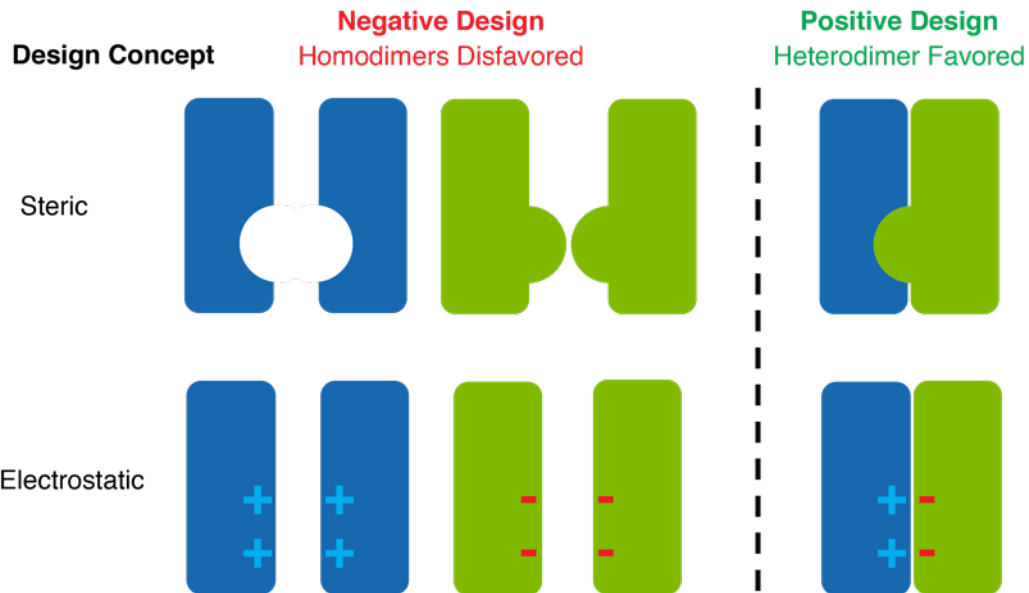
Engineer for heterodimer **stability** using positive design

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

IgA Fc

FcαRI

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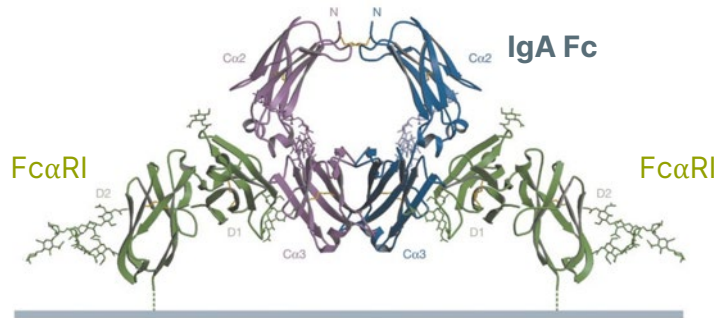
PDB ID:1OW0; from Herr et al, Nature, 2003

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

IgA Fc chain A
IgA Fc chain B

CH2

CH3
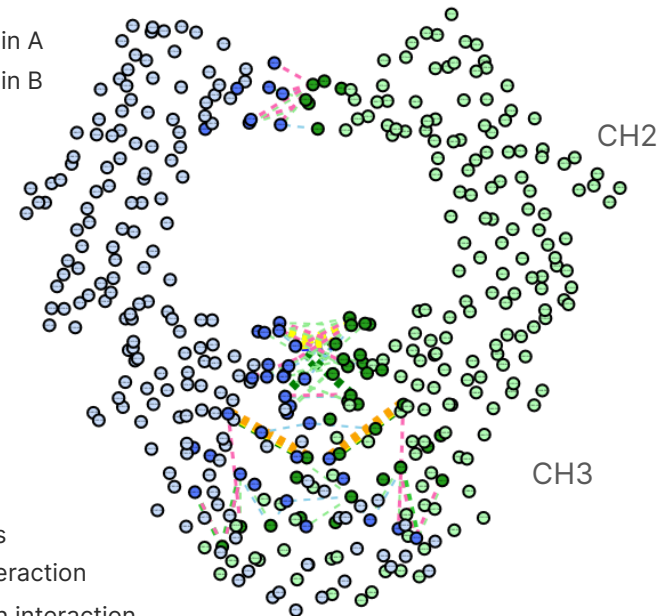
---- H-bonds
---- Pi-pi interaction
---- Pi-cation interaction
---- Carbon-nitrogen/oxygen/sulfur contacts
---- Carbon-carbon contacts

In Silico Tools Employed to Inform Heterodimeric IgA Fc Design

3. *In silico* design

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

- **Knowledge-based potential:** interface amino acid contacts (hot spots)
  - H bonds/pi interactions/vdW
  - Shell analysis

- **Physics-based potential:** energetic contribution of residue interactions across the interface
  - Coulumb (charged interactions)
  - Leonard Jones (packing interactions/hydrophobics)
  - Desolvation energy

Variants were predominately selected based on affinity metrics

Negative designs ("breaking" homodimerization)

Increase energy difference

Predicted affinity based on Physics-Based Potential

Positive designs ("rescuing" heterodimerization)

Predicted affinity based on Knowledge-based Potential

- 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
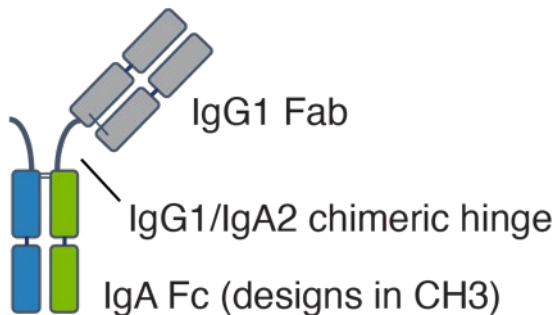
4. Variant selection

Top 12 designs selected for experimental screening
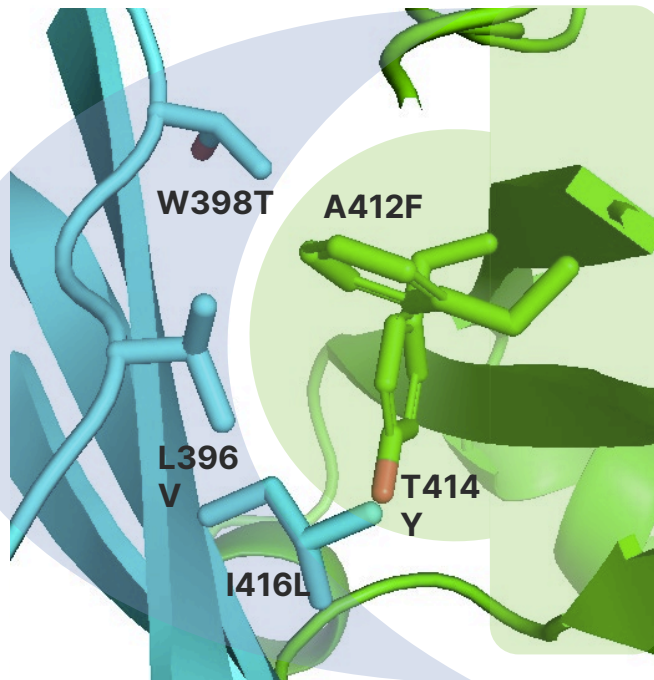
Steric design
1-3 mutations/chain

W398T   A412F
L396V   T414Y
I416L

Electrostatic design
2-5 mutations/chain

I416E
L372R
T366D

Experimental *In Vitro* Evaluation of IgA Heterodimeric Fc Designs

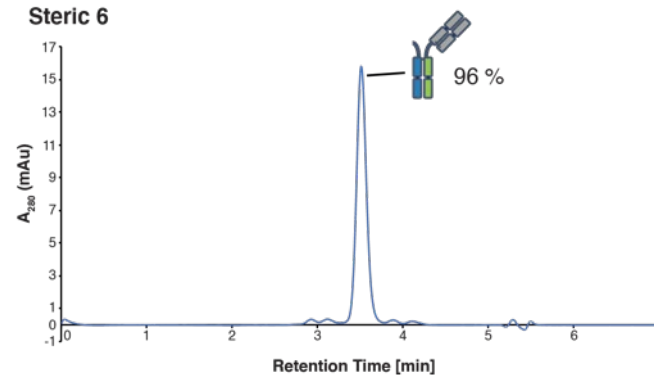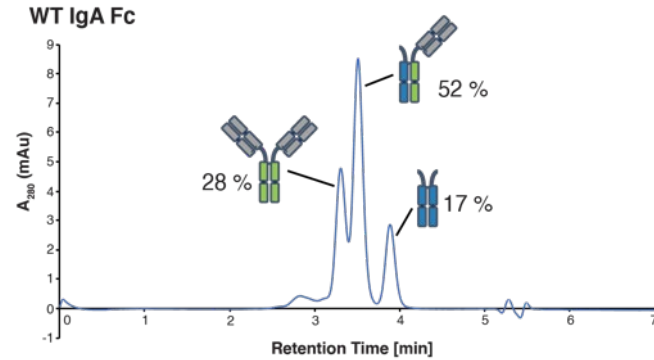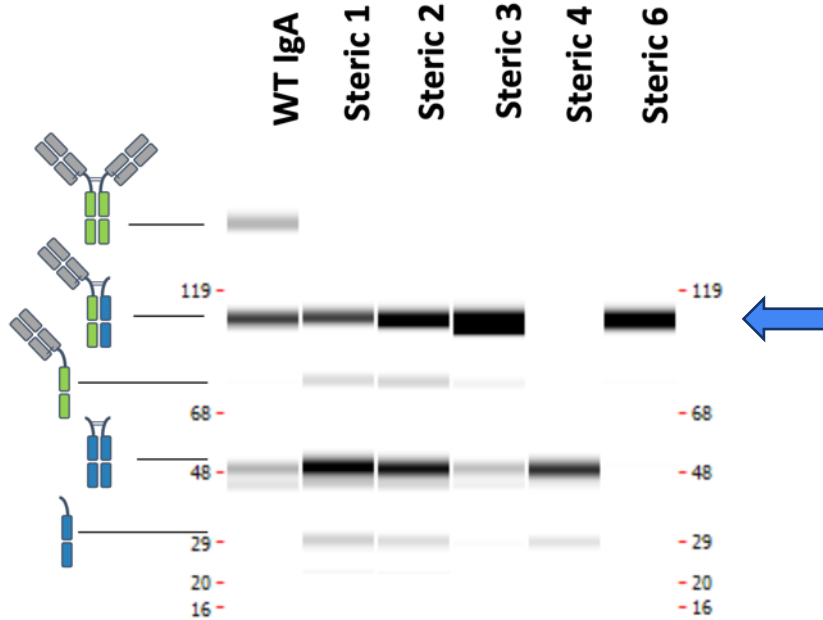One-armed antibody (OAA) format used to test top-ranked designs

IgG1 Fab

IgG1/IgA2 chimeric hinge

IgA Fc (designs in CH3)

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

Design Concept

Negative Design
Homodimers Disfavored

Positive Design
Heterodimer Favored

Steric

Electrostatic

High Heterodimeric IgA Fc Purity Measured by CE-SDS and Analytical SEC after Affinity Purification

Heterodimeric IgA Fc Engineered for Thermal Stability and High Purity

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

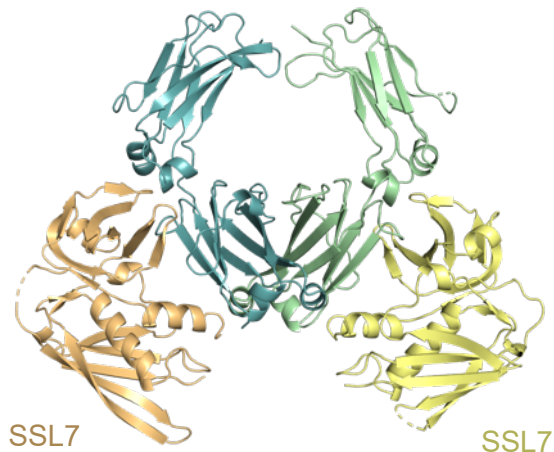Summary of purity and stability for IgA Fc design
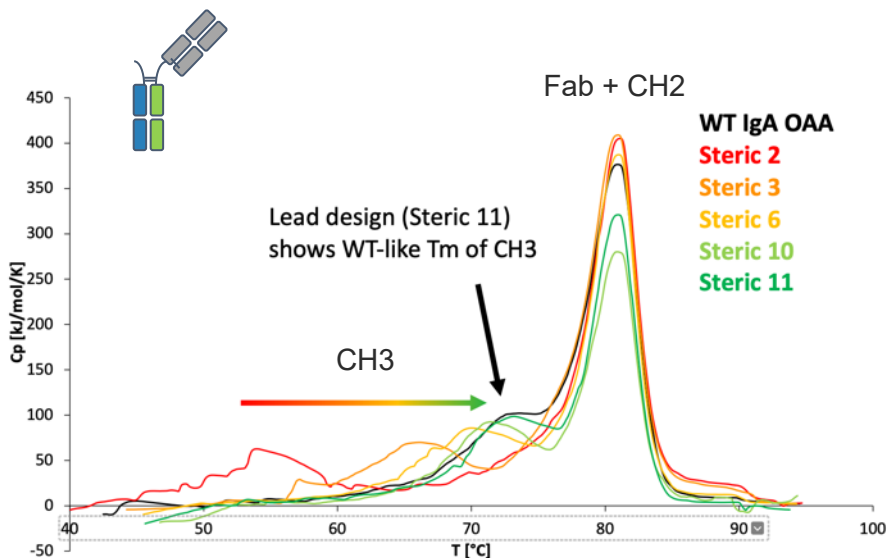
| Design	Post affinity purification			Post prepSEC purification			
	HPLC-SEC OAA purity (%)	CE-SDS OAA purity (%)	Total yield (mg/L culture)	HPLC-SEC OAA purity (%)	CE-SDS OAA purity (%)	OAA yield (mg/L culture)	IgA CH3 Tm (°C)**
Wild-type	52	49	324	91	92	76	74.2
Steric 2	65	55	240	97	96	76	55
Steric 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*
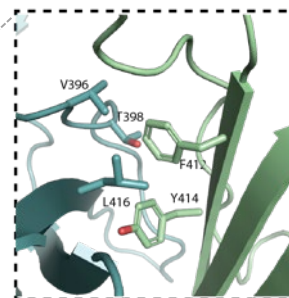
Heterodimeric IgA Fc
(steric 6 design)

SSL7   SSL7

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

Heterodimeric IgA Fc

In silico model

Wildtype IgA Fc
(PDB ID: 2QEJ)

- Heterodimeric IgA Fc (steric 6) crystal structure was solved in complex with *Staphylococcus aureus* protein SSL7 (PDB ID: 7TTZ)
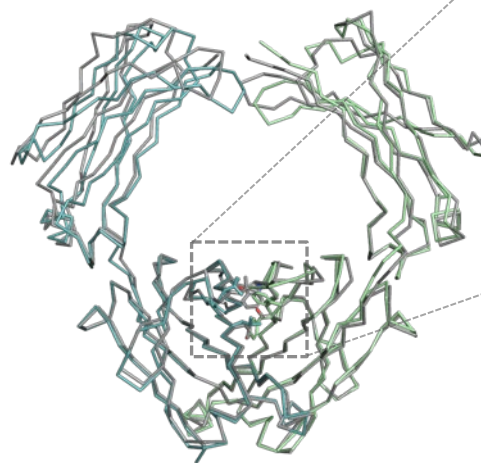
- Heterodimeric IgA Fc (blue/green) superimposed with wildtype IgA Fc (grey) have RMSD of 0.94 Å across C_α atoms in the Fc
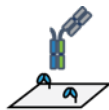
- The RMSD of the heterodimeric IgA Fc CH3 crystal structure relative to the *in silico* model was 1.2 Å across C_α atoms (residues 345-450)

Analyte: IgA Fc variant
Ligand: FcαRI

➡ FcαRI binding site
✗ FcαRI binding site knockout (KO)

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

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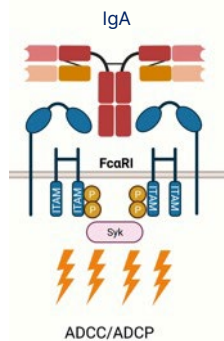

Legend:
- ■ IgA OAA FcaRI-KO 1x
- ■ IgA OAA WT
- ■ IgA OAA Steric 6
- ■ IgA OAA Steric 10
- ■ IgA OAASteric 11

Plot: Apparent K_D (nM) vs Average Ligand Density (RU)
Y-axis: 1.0E-07, 2.0E-08, 4.1E-09
X-axis: 2.2, 14, 18, 20, 43, 83

Applications of Heterodimeric IgA Fc

Investigate IgA:FcαRI stoichiometry required for neutrophil activation

FcαRI ← FcαRI   ✗ ← FcαRI
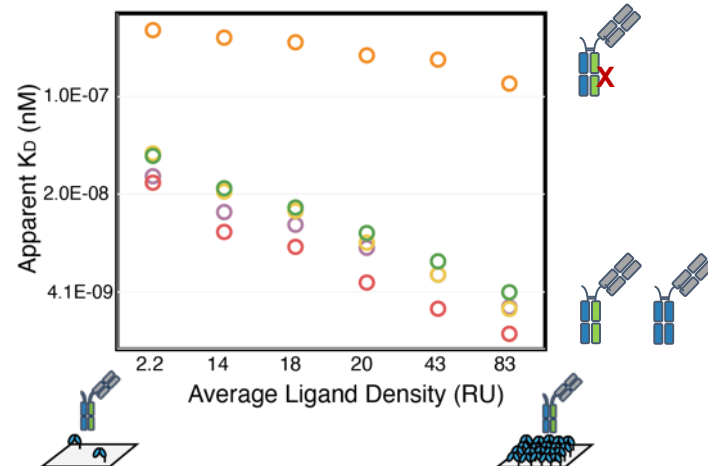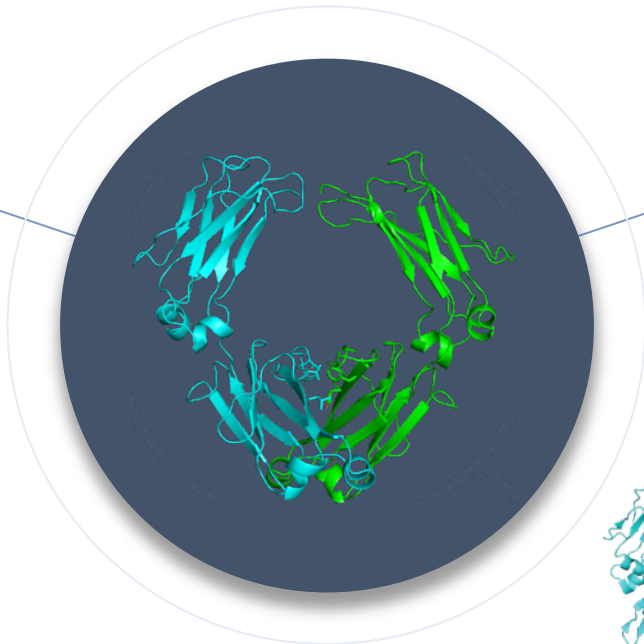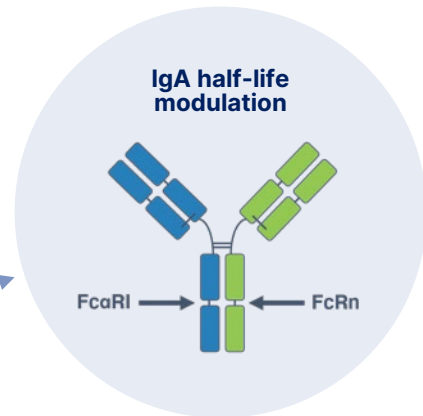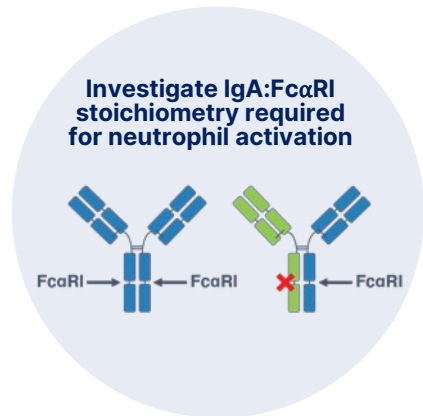
Is bivalent binding of FcαRI required for neutrophil activation?

IgA

FcαRI

ITAM ITAM P P P P ITAM ITAM

Syk

ADCC/ADCP

IgA half-life modulation

FcαRI →   ← FcRn

FcRn and FcαRI binding sites are structurally superimpose

IgA   IgG

FcαRI   FcRn
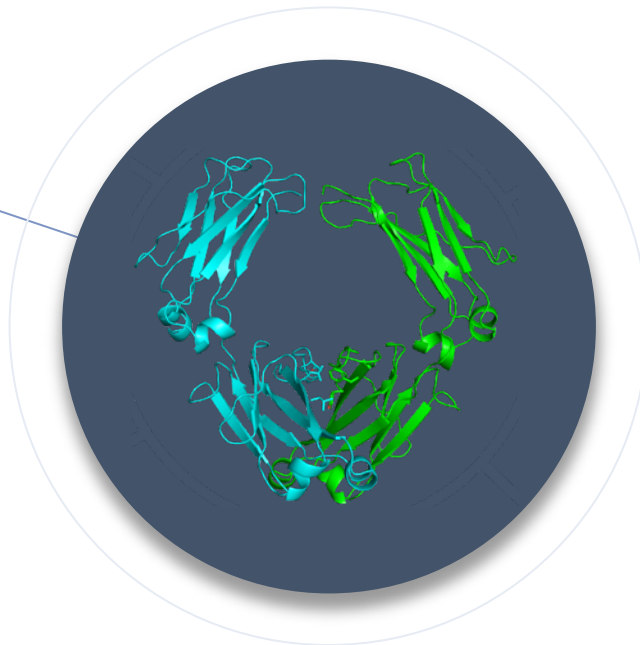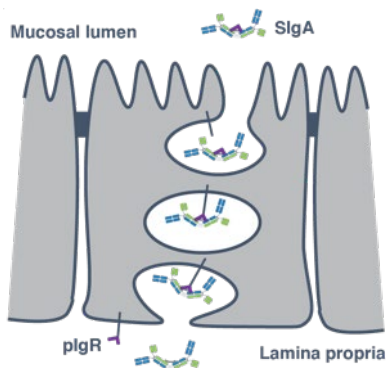
PDB ID: 1OW0   PDB ID: 1I1A
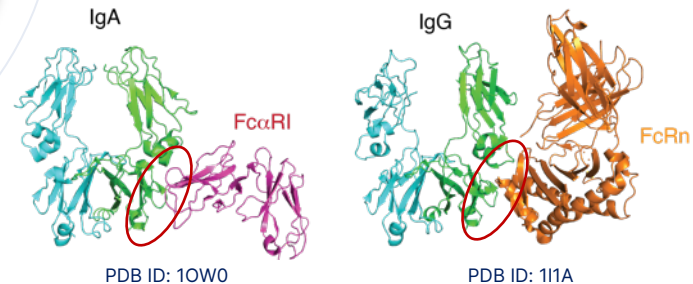
Applications of Heterodimeric IgA Fc

Multispecific IgA-based formats
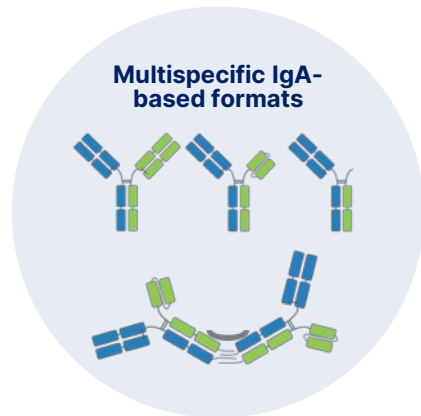
Dimeric IgA specifically disables intracellular mutated oncodrivers

Multispecific therapeutics targeting mucosa

Mucosal lumen

SIgA

pIgR

Lamina propria

Mutant specific dimeric IgA

Polymeric immunoglobulin receptor (pIgR)
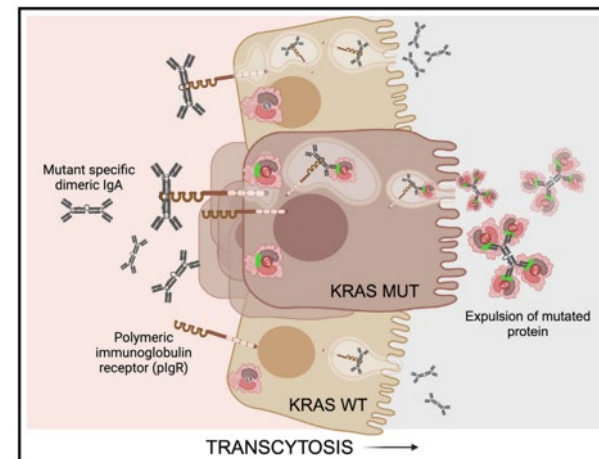
KRAS MUT

Expulsion of mutated protein

KRAS WT

TRANSCYTOSIS

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

Summary

Protein Engineering
Integrating **purity and stability** design requirements yielded a **heterodimeric IgA Fc** with favorable developability characteristics
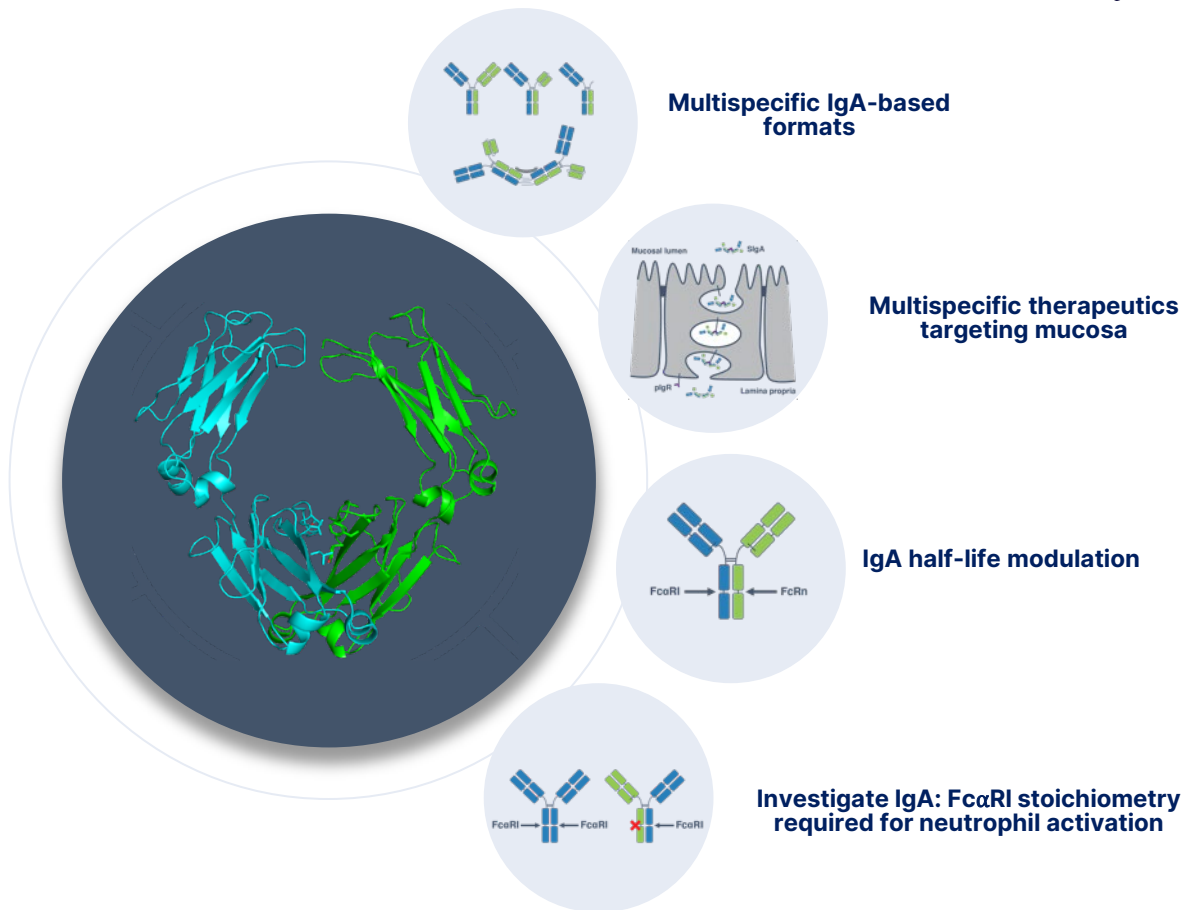
Application
A heterodimeric IgA Fc unlocks the potential to explore **multispecific IgA therapeutics** and interrogate **IgA-FcαRI biology**

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
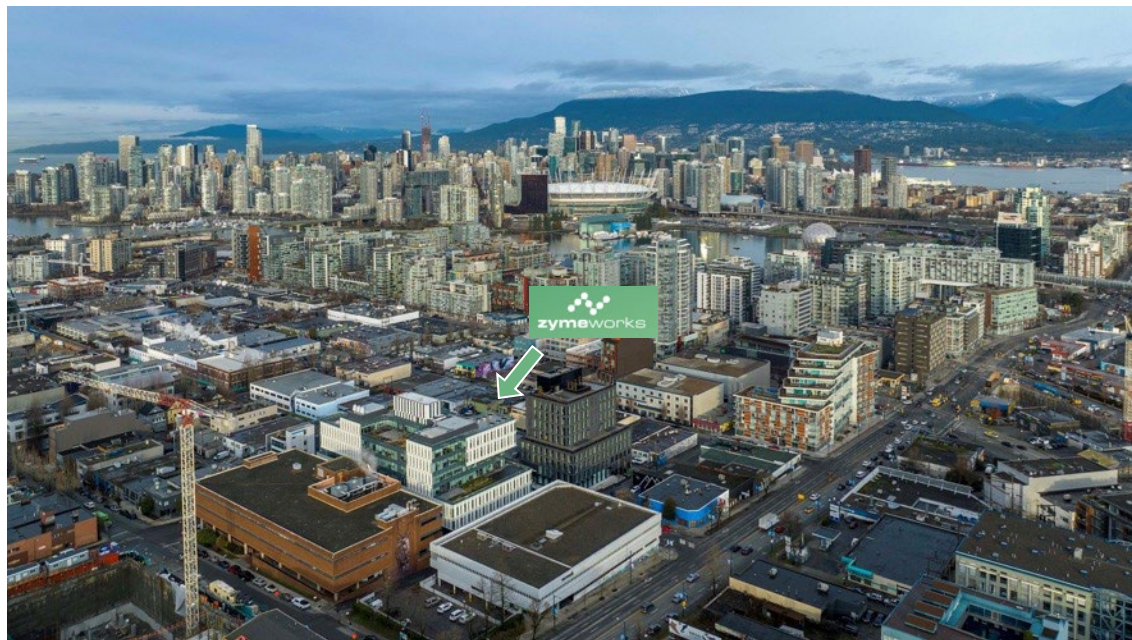
Photo from CBRE

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

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REPORT

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

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Thank you