Comparing Potential Bispecific Formats of Trastuzumab and a Humanized OKT3

Ed Horton Ph.D.

Antibody Society Webinar 8/3/23

absolute biotech

Our Brands

Our mission is to serve as “antibody curators” for customers worldwide

absolute biotech

absolute antibody

We are experts in antibody engineering and recombinant antibody technology.

LSBio

We are leaders in IHC validation, offering a comprehensive catalog of antibodies, proteins and ELISAs.

kerafast

We make unique laboratory-made research tools easily accessible to the global scientific community.

exalpha

We offer antibodies, reagents and kits at the cutting edge of the research life sciences market.

Nordic MUbio

We develop high quality antibody and flow cytometry reagents according to strict ISO 9001 guidelines.

everest

We are specialists in anti-peptide and antigen affinity purified goat polyclonal antibodies.

absolute antibody LSBio kerafast exalpha Nordic MUbio everest

Our Reagents

absolute biotech

We offer a wide selection of off-the-shelf antibodies, reagents and kits:

Product Types

- Primary & Secondary Antibodies
- ELISA & Assay Kits
- IHC Antibodies & Reagents
- Cell Lines & Proteins
- Flow Cytometry Buffers

Key Research Areas

- Cancer & Immunotherapy
- Infectious Disease & Virology
- Immunology & Inflammation
- Allergy
- Neuroscience

Antibody Highlights

- Recombinant Antibodies
- Engineered Antibodies
- Immunohistochemistry-Validated Antibodies
- Goat Polyclonal Antibodies

absolute antibody LSBio kerafast exalpha Nordic MUbio everest

Absolute Antibody - Overview

- Company vision
  - To make recombinant engineered antibodies more accessible to the wider community, particularly those in research and diagnostics.
  - Manufacturing and R&D based in UK
- Founded in 2012
  - Part of LSBio in 2021
  - Now a global company as Absolute Biotech
- Core technologies
  - High-throughput hybridoma sequencing
  - Recombinant expression and purification
  - Antibody engineering
  - Humanization

Our Expertise

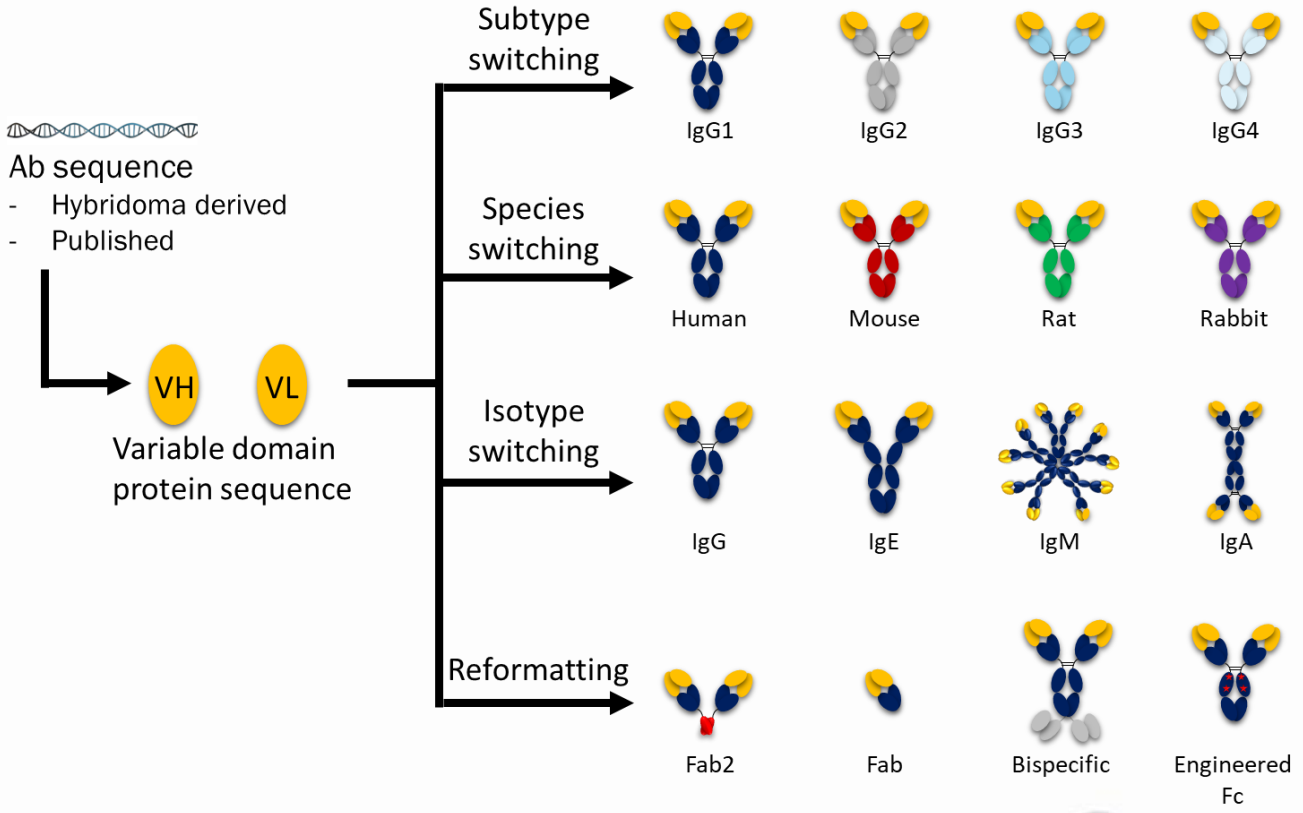
absolute biotech

Recombinant Antibodies

- We offer a wide range of catalog recombinant antibodies, in addition to custom recombinant antibody manufacturing
- We have completed 21,000+ recombinant antibody production runs

Antibody Engineering

- Our catalog antibodies are available off-the-shelf in different species, isotypes, subtypes, and formats
- We can custom engineer antibodies into any format
- We have manufactured 180+ different formats

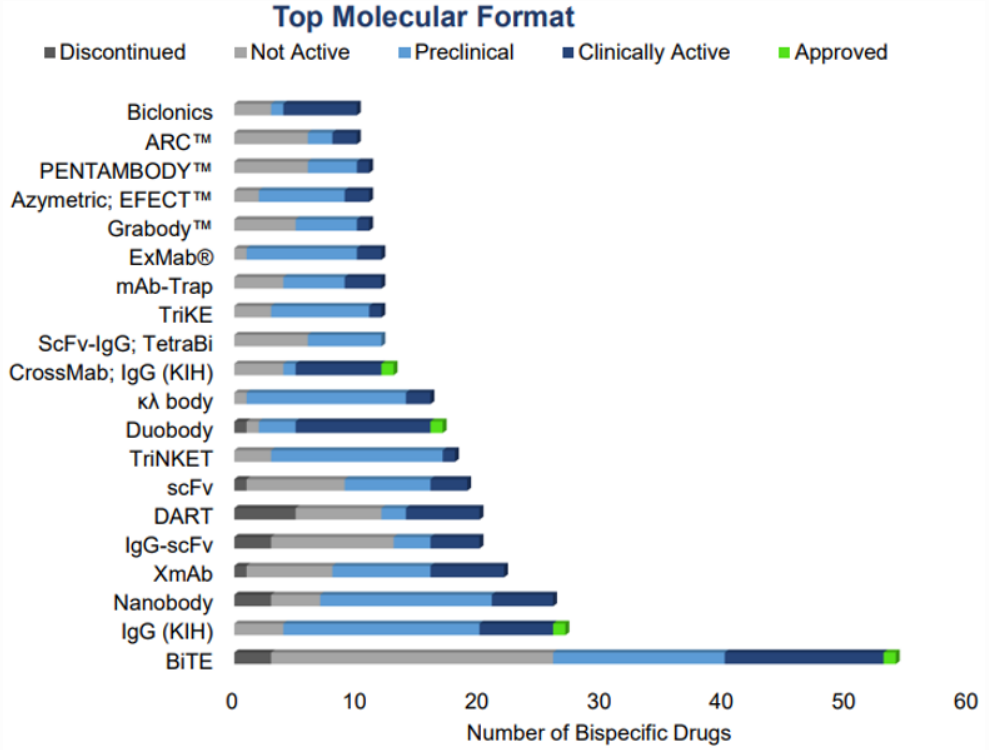


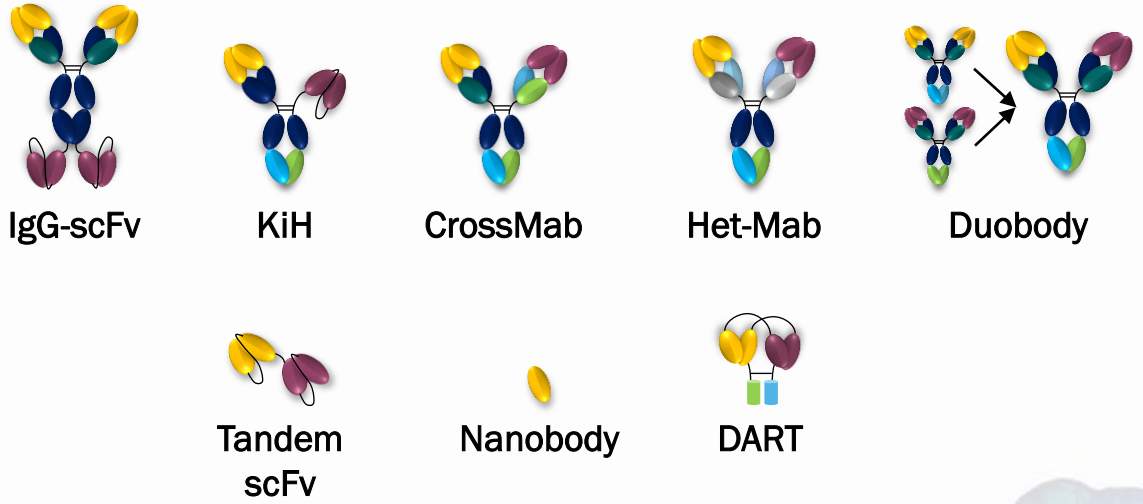
absolute antibody LSBio kerafast exalpha Nordic MUbio everest

Bispecific therapeutics landscape

- 7 marketed; 5 recent approvals in 2022-23
- More than 20 technology platforms; 349 molecules in clinical development

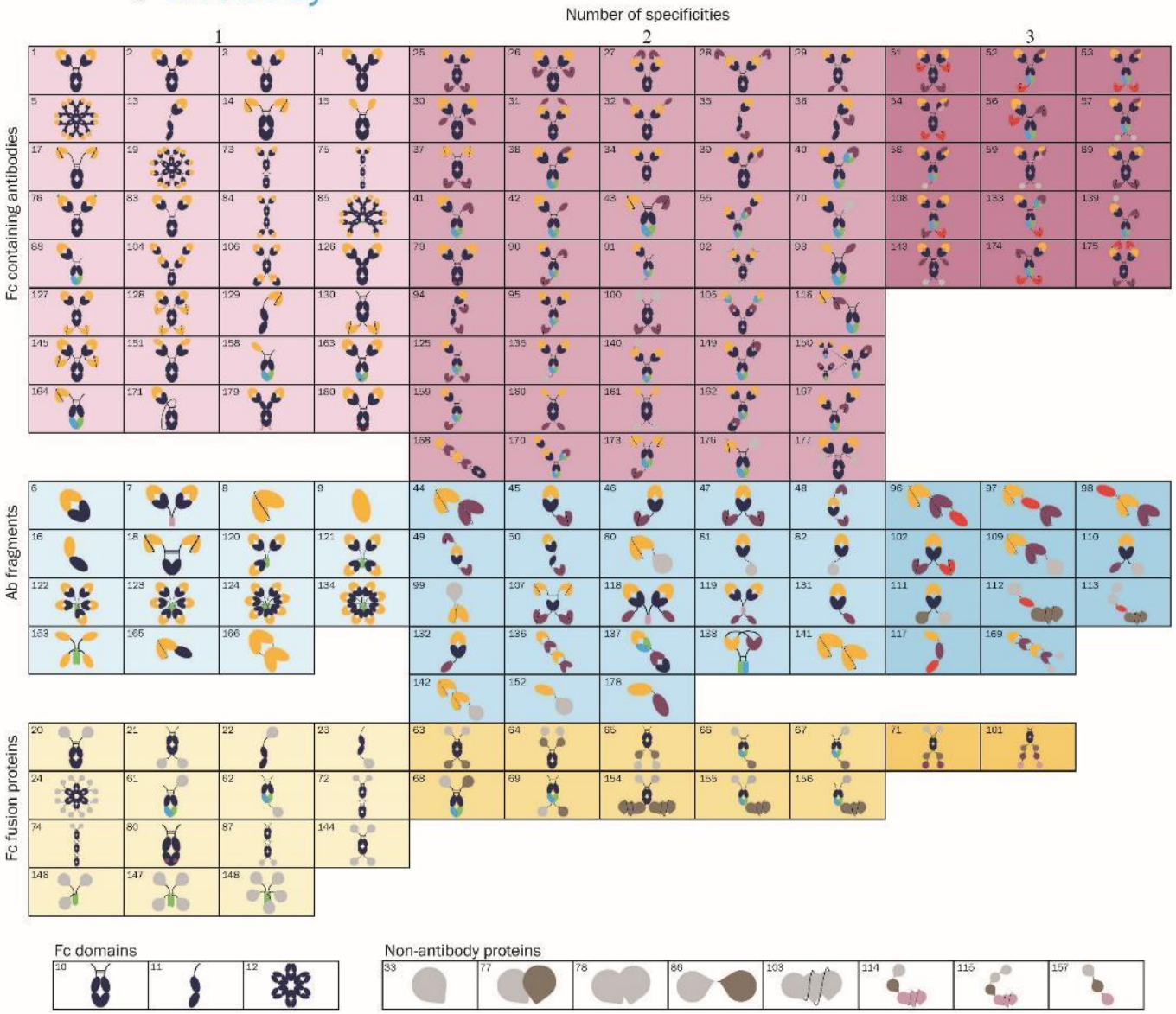
Source - Beacon Bispecific: mid-year review – Hanson Wade 2022





absolute antibody

Periodic Table of Antibodies



absolute biotech

Antibody Engineering for everyone!

>200 formats and counting...

absolute antibody · LSBio · kerafast · exalpha · Nordic MUbio · everest

Case study: Her2-CD3 bispecifics

HEXpress™ | absolute biotech

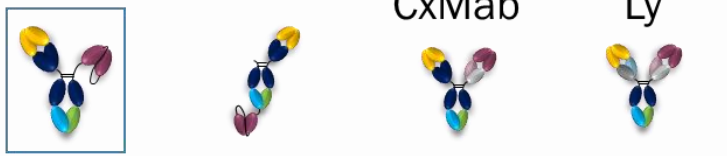
2:2 (Fc null)

bAb1 bAb2 bAb3 bAb4 bAb5

2:1 (Fc null)

bAb6 bAb7 bAb8

1:1 (Fc null)



bAb9 bAb10 CxMab bAb11 Ly bAb12

2:2 (Ab fragments)

bAb13

2:1 (Ab fragments)

bAb14

1:1 (Ab fragments)

bAb15 bAb16 bAb17

R&D controls

Her2 IgG cAb1

hOKT3 IgG cAb2

hOKT3 scFv-Fc cAb3

hOKT3 Fc-scFv cAb4

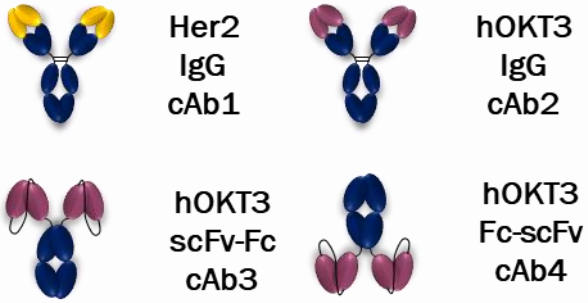
Sanquin
In collaboration

Samples for In-vitro assay

absolute antibody | LSBio | kerafast | exalpha | Nordic MUbio | everest

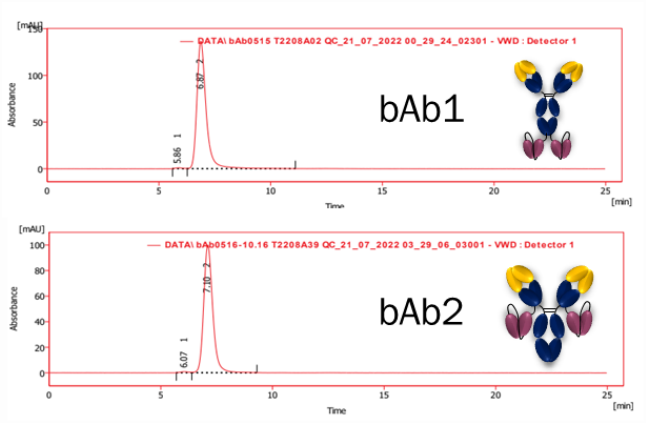
Parental antibodies – Trastuzumab and OKT3

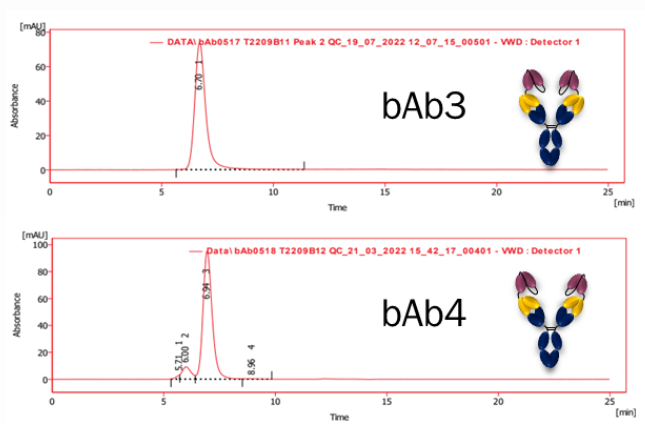
AbAb ID	Description	Yield	Monomer % (ProtA)	Final yield (pilot)
cAb1	Her2 IgG	100 mg/L	87%	5 mg
cAb2	hOKT3 IgG	87 mg/L	94%	7 mg
cAb3	N-term hOKT3 scFv-Fc	62 mg/L	86%	2 mg
cAb4	C-term Fc-hOKT3scFv	162 mg/L	71%	7.5 mg

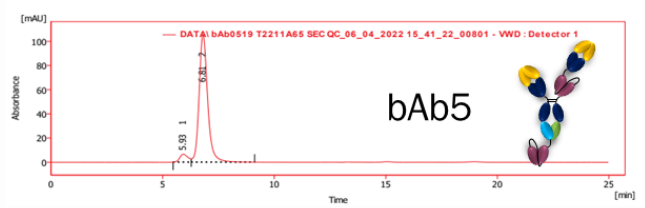


- Parental monoclonals have comparable expression
- Human IgG1 with LALA mutations to reduce FcR binding
- Selected CD3 as the scFv in bAb designs to facilitate tuning of potency; provide a modular approach to be used with other antigen targets
- Humanized OKT3 to create stable scFv

2:2 Bivalent Fc-null bispecific designs



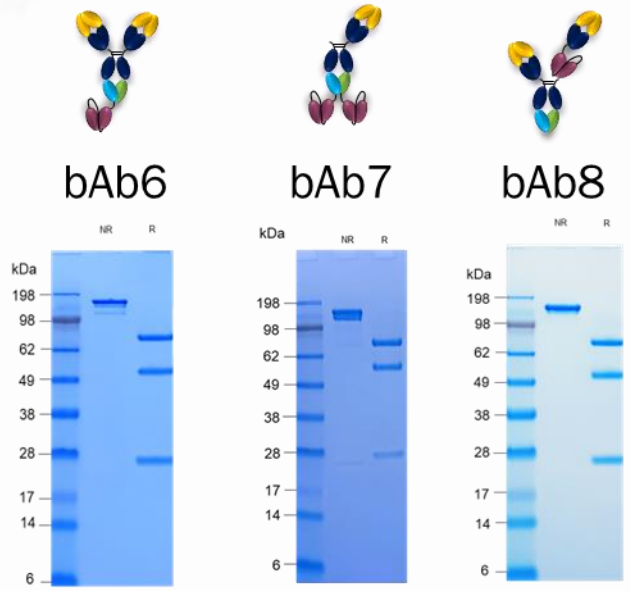


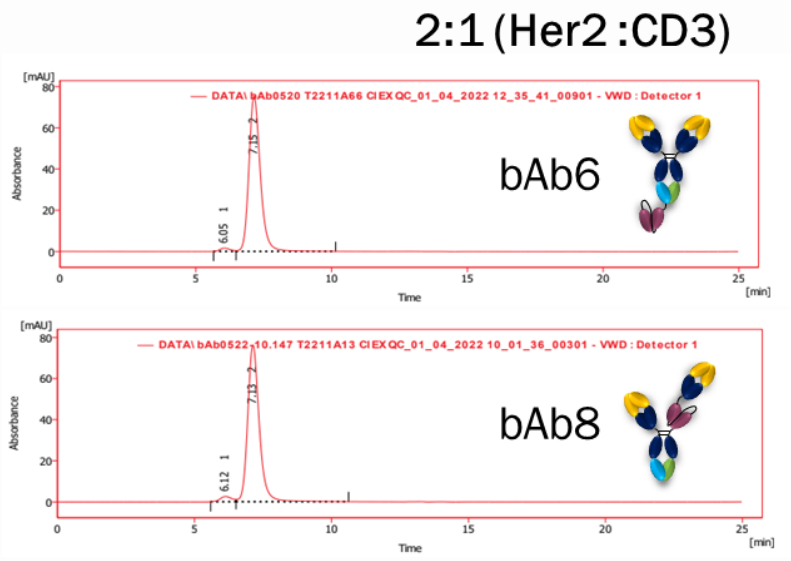


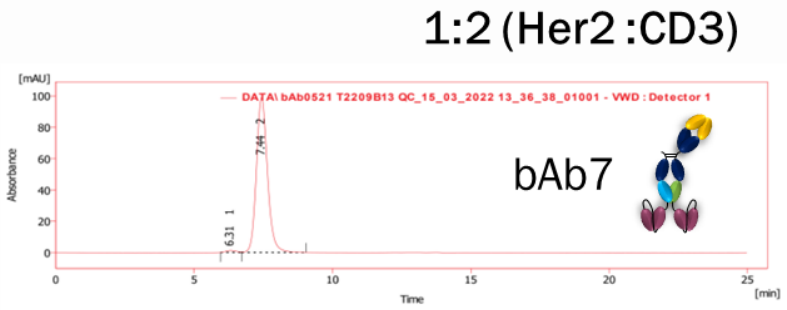
AbAb ID	Description	Yield	Final yield	Final monomer %	Average EC50 (nM)
bAb1	2:2 C-term HC-scFv	80 mg/L	38 mg	99%	0.37
bAb2	2:2 C-term LC-scFv	90 mg/L	37 mg	99%	0.55
bAb3	2:2 N-term scFv-HC	200 mg/L	50 mg	100%	0.57
bAb4	2:2 N-term scFv-LC	80 mg/L	27 mg	99%	0.91
bAb5	2:2 N&C scFv-KiH HC	14 mg/L	6 mg	94%	0.29

- Bivalent bispecifics with different hOKT3 scFv placements have dramatic effects on expression and aggregate levels and ultimately the final purified yield.
- bAb1 is one of the most widely used bispecific formats.

2:1 Fc-null bispecific designs



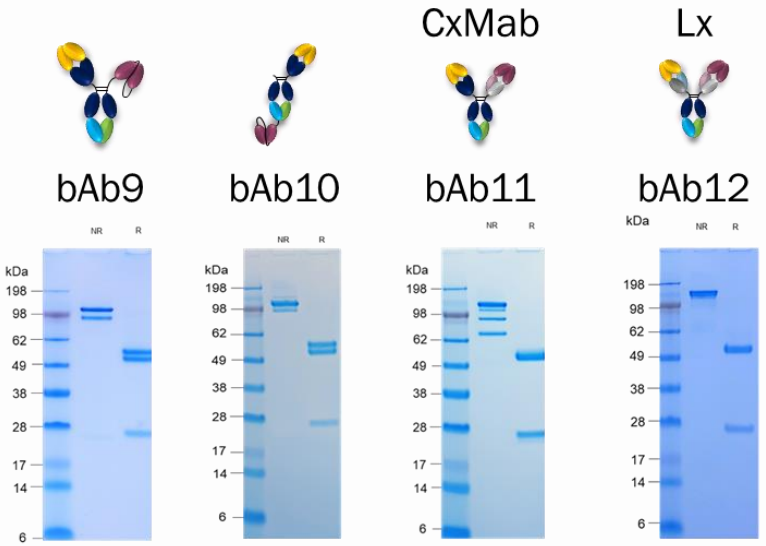


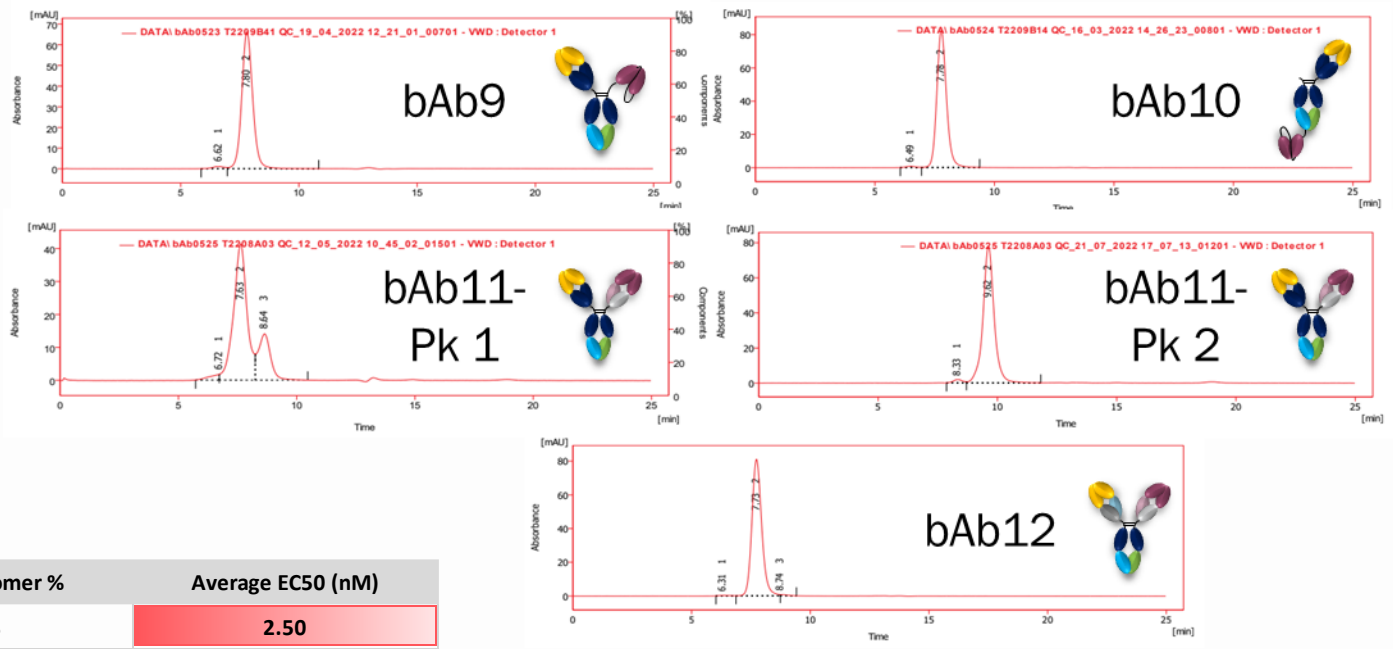


AbAb ID	Description	Yield	Final yield	Final monomer %	Average EC50 (nM)
bAb6	2 Fab; 1 C-term KiH-scFv	79 mg/L	39 mg	98.20%	0.46
bAb7	1 Fab; 2 C-term KiH-scFv	52 mg/L	26 mg	98.90%	1.92
bAb8	2 Fab; 1 N-term scFv-KiH	27 mg/L	12 mg	98.30%	0.28

- Bispecifics with heterodimeric Fc were produced at high quality and readily with different hOKT3 scFv placements.
- Unsurprisingly, the addition and placement of scFv can impact expression as observed in these examples. More IgG like constructs tend to express better.

1:1 Fc-null bispecific designs

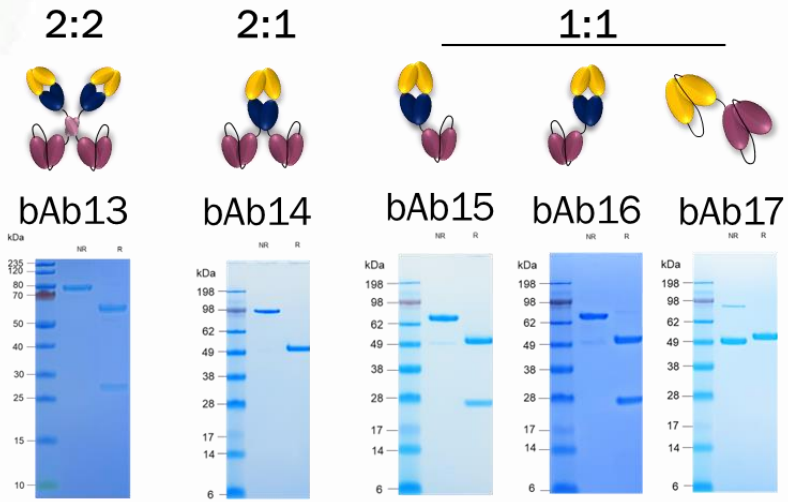


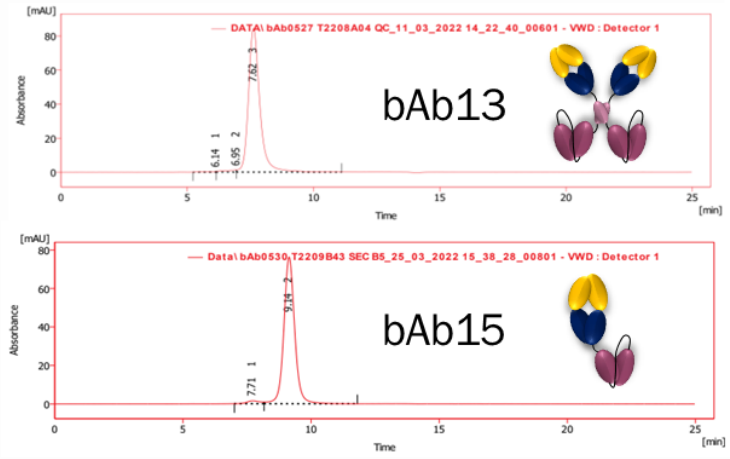


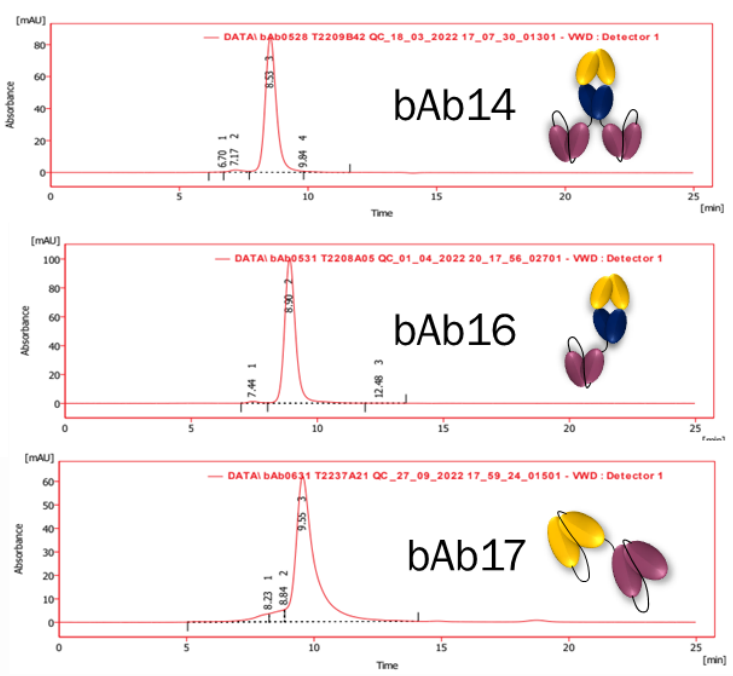
AbAb ID	Description	Yield	Final yield	Final monomer %	Average EC50 (nM)
bAb9	1:1 Fab:scFv KiH	83 mg/L	37 mg	98%	2.50
bAb10	1:1 N-Fab; C-scFv KiH	80 mg/L	40 mg	99%	2.49
bAb11	1:1 X-mab	4 mg/L	4 mg; 0.6 mg	75%; 98%	0.87
bAb12	1:1 Ly	120 mg/L	60 mg	99%	0.92

- 1:1 heterodimeric bispecifics were successfully produced. Stable interfaces can produce high-quality monovalent bispecific in high yield as demonstrated by bAb12 vs the domain swapped bispecific bAb11.

Non-Fc bispecifics (Fab-scFv)







AbAb ID	Description	Yield	Final yield	Final monomer %	Average EC50 (nM)
bAb13	2:2 Fab-scFv	19 mg/L	19 mg	98%	0.13
bAb14	1 Fab;2 C-term scFv	60 mg/L	30 mg	97%	1.14
bAb15	1:1 Fab; C-term HscFv	50 mg/L	25 mg	98%	0.71
bAb16	1:1 Fab; C-term LscFv	18 mg/L	17 mg	98%	0.87
bAb17	1:1 Tandem scFv	26 mg/L	13 mg	92%	# confirmed by direct HRP

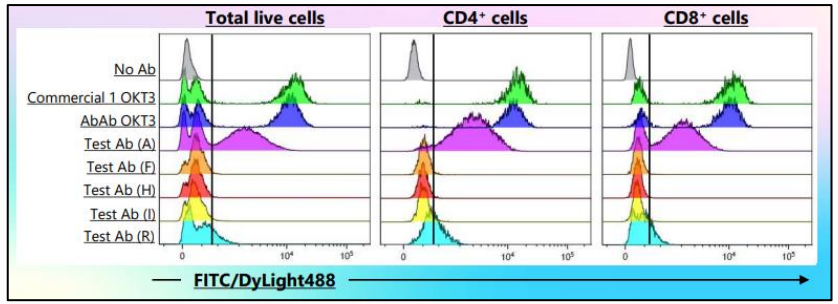
- Non-Fc bispecifics using Fab-scFv or tandem scFv designs were produced successfully. A novel dimerization domain was used to create the Fab2 design. Interestingly, scFv attached to the C-terminal of the Fab heavy chain produced higher yield than the placement at the C-terminal of the light chain.

Conclusions

- At AbAb, we have produced over 200 different engineered antibody formats. We systematically built and evaluated 17 bispecific designs using 2 well-studied monoclonals.
- We found that Fc containing formats and the placement of scFv can influence the expression dramatically. Valency and steric accessibility can tune binding potency.
- Knob-in-hole mutations in Fc have been used widely for heterodimeric Fc designs. Well-designed interfaces between heavy chain and light chain are critical for success.
- Functional characterizations are on-going.

In collaboration

Sanquin



- We are a global company for the full breadth of antibody-related reagents, services and expertise
- Our custom antibody services enable researchers to:
  - Sequence and express their antibodies to protect against loss, mutation or contamination, ensuring long-term supply
  - Engineer their antibodies to improve utility in research, diagnostic and therapeutic applications
- Looking for technology collaborations – expanding our offerings

Advance with Absolute Confidence

www.absolutebiotech.com