Robust heavy chain – light chain pairing solutions for generating clinical bi- and multispecific antibodies

Kyle Barlow, Heather Lynaugh, Beth Sharkey, Paul Widboom, Ross Connor, Emily Radke
Arvind Sivasubramanian

Adimab LLC, 7 Lucent Drive, Lebanon NH 03766

ADIMAB

BACKGROUND

Adimab's discovery and optimization platform excels in identifying diverse, highly specific, and developable antibody panels. In addition to discovery, Adimab has developed technology for assembling multispecific antibodies, leading to clinical development. Here we present the design and validation of orthogonal CH1-Cκ interfaces for correct heavy chain – light chain pairing.

140+ Pharma/Biotech Partners

650+ Therapeutic Programs

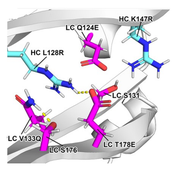
90 Clinical Advancements

6 Approved Products

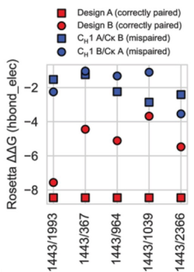
- 2009-10: **IgG discovery and optimization**
- 2011-12: **Bispecific engineering**
- 2013-14: **Foundational developability papers published**
- 2015-16: **Membrane obligate target discovery**
- 2017-18: **Isolation of novel α-CD3 antibodies**
- 2019-20: **HCAb platform**
- 2021-22: **Proprietary heterodimerization solutions for bispecifics**
- 2023-24: **TCR engineering TCRm discovery**
- 2025-26: **α-CD3 and α-TCR HCAb generation**; **Discovery from synthetic TCR library**

Designing CH1-Cκ heterodimerization[1]

CH1-Cκ interface designed computationally

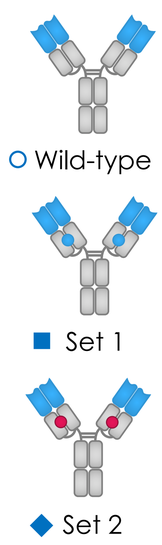


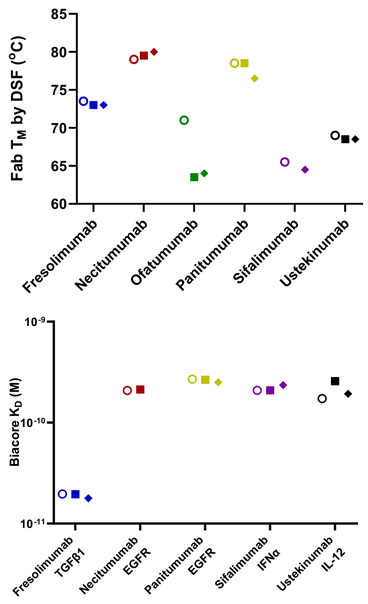
CH1-Cκ interface designs evaluated computationally



CH1-Cκ designs with greatest computed ΔΔG tested experimentally
Orthogonal sets successfully identified
Set 1: 3 CH1 and 3 Cκ mutations
Set 2: 2 CH1 and 3 Cκ mutations

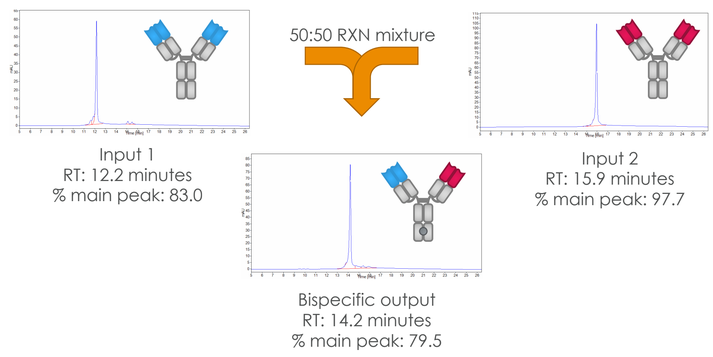
CH1-Cκ mutations do not alter target binding or biophysical properties



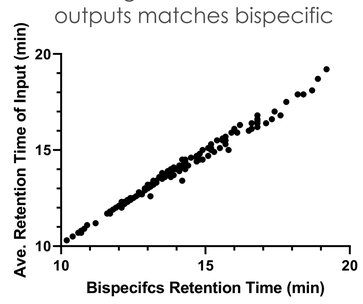


Developing multispecific antibodies at Adimab

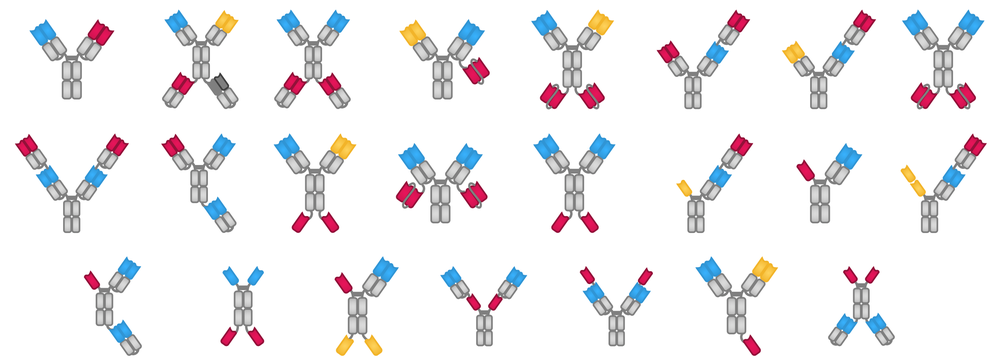
Chain exchange allows for rapid production of large multispecific panels



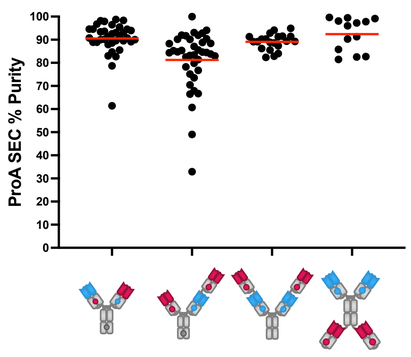
Average retention time of outputs matches bispecific



Delivery of >20 formats and >1200 samples to partners in the past two years
Screening formats leads to production in single transient transfection



Delivery of >100 multispecifics with CH1-Cκ mutations in transient CHO in the past two years
10 programs for 8 partners



34 bispecifics
6 partner programs
Mean SEC: 90.5%

36 bispecifics
2 partner programs
Mean SEC: 81.2%

12 bispecifics
3 partner programs
Mean SEC: 93.2%

14 tetraspecifics
5 bispecifics
3 partner programs
Mean SEC: 89.1%

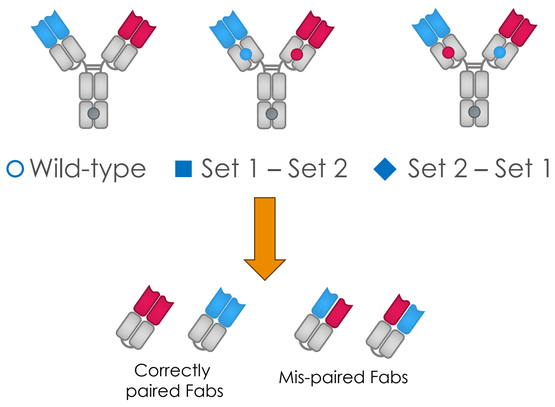
Testing CH1-Cκ heterodimerization

Heterodimerization mutations tested in five bispecific pairs

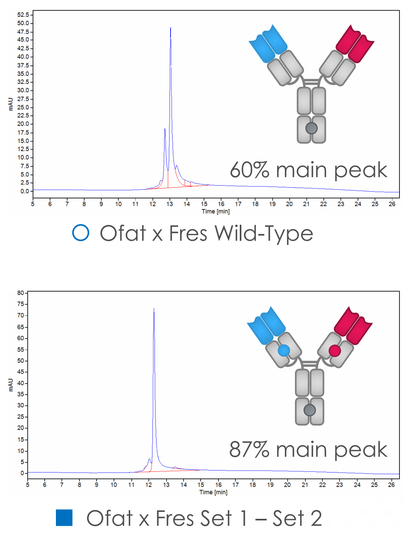
Index	Variable 1	Variable 2
◆	ofatumumab	fresolimumab
◆	necitumumab	fresolimumab
◆	necitumumab	sifalimumab
◆	ofatumumab	sifalimumab
◆	panitumumab	ustekinumab

Antibody pairs picked based on:
1. Good biophysical properties
2. HC-LC promiscuity among pairs
3. Human V-regions

Each antibody pair tested in three constructs

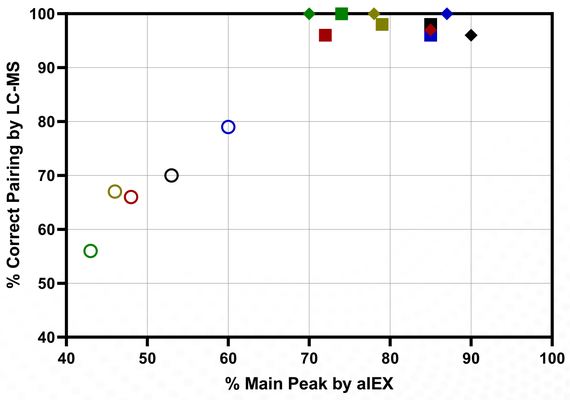


ProA purified bispecific mixture run on analytical ion exchange

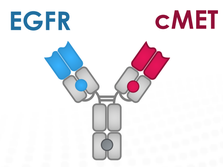


Mass spec bispecific quantitation:
1. ProA purified samples digested to Fabs
2. Fabs run on LC-MS
3. Peaks integrated for quantitation

CH1-Cκ mutations drive 100% correct pairing

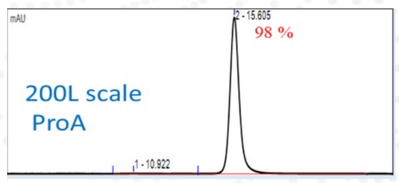


CH1-Cκ heterodimerization in clinical bispecifics



PM-1080

Adimab partner Biotheus moves PM-1080 into Phase III
Trials for colorectal cancer, nSCLC
Stable cell line titer 6 g/L
High purity after ProA column purification
No HC-LC mispair
Data from **Biotheus** poster:
AACR 2023 Annual Meeting Abstract #5675



Acknowledgments and references:
We thank our partners at NextPoint and Biotheus. All members of the Adimab scientific and engineering teams contributed to this technology.

1. Barlow et al., **MAbs** 2025 Dec;17(1). 2. Liu et al., **MAbs** 2023 Jan-Dec; 15(1).

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