Predicting biophysical properties of multispecific antibodies

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ADIMAB

BACKGROUND

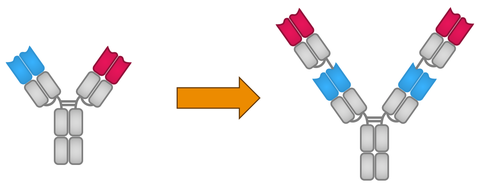
Adimab has produced over 2,700 multispecific antibodies for our partners over the last four years. Over 2,200 were produced by our chain exchange process, and ~500 from our standard CHO transfection.

Comparing biophysical data from the input IgGs to the resulting multispecifics allows for prediction of polyreactivity and hydrophobicity. Factors such as valency and location of Fab alter the influence of the component variable regions

How does location and number of additional binding arms affect polyreactivity and hydrophobicity of multispecifics?

2+2 N-term Fab

2+1+1 C-term scFv



Polyreactivity is measured by binding to the polyspecificity reagent (PSR)

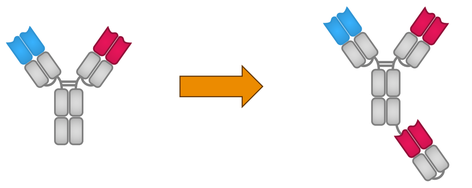
Binding is normalized on a scale of 0-1
Higher number indicates higher polyreactivity

How does the number of each binding arm (valency) affect polyreactivity?

1+1 IgG-like

2+1 C-term Fab

2+2 N-term Fab

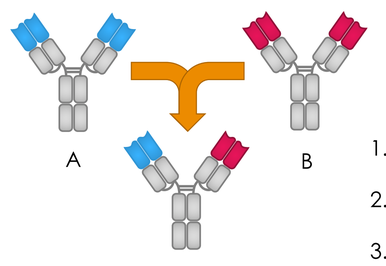


Hydrophobicity is measured by hydrophobic interaction chromatography (HIC)

Longer retention time (RT) indicates a more hydrophobic molecule

How does the location of a Fab (N or C-term) affect hydrophobicity?

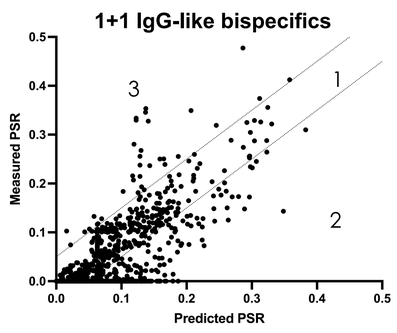
Binding arm valency influences multispecific polyreactivity prediction

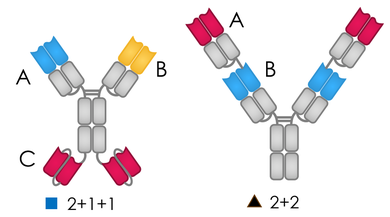


PSR binding measured for over 1,100 1+1 IgG-like bispecifics

$$\text{Predicted PSR} = \frac{A + B}{2}$$

1. 732 (65%) have measured PSR within +/- 0.05 of input average PSR
2. 361 (32%) have measured PSR less than -0.05 of input average
3. 35 (3%) have measured PSR greater than 0.05 of input average





2+1+1 Example 1:

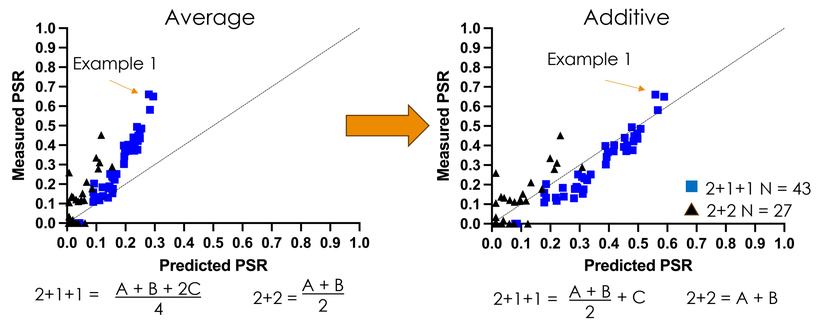
Average:

$$\frac{A + B + 2C}{4} = \frac{0.35 + 0.21 + 2(0.28)}{4} = 0.28$$

Additive:

$$\frac{A + B}{2} + C = \frac{0.35 + 0.21}{2} + 0.28 = 0.56$$

When a binding arm is present **twice**, PSR is additive, not an average

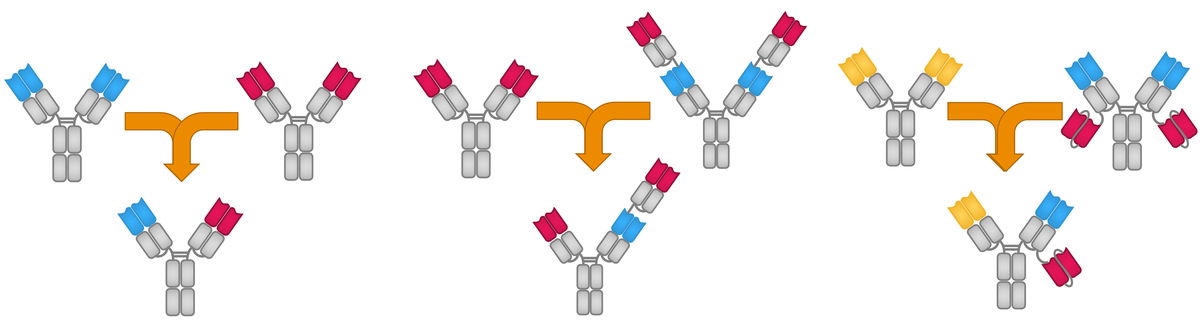


$2+1+1 = \frac{A + B + 2C}{4}$ $2+2 = \frac{A + B}{2}$

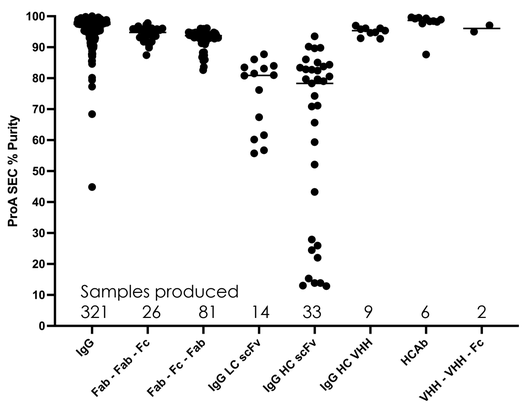
$2+1+1 = \frac{A + B}{2} + C$ $2+2 = A + B$

How does Adimab produce large panels of multispecifics?

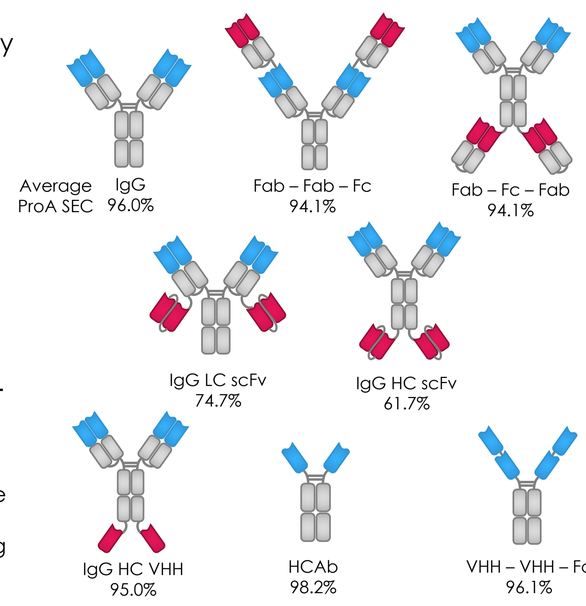
Chain exchange allows for multiple specificities, valencies and formats



Chain exchange input ProA SEC % purity

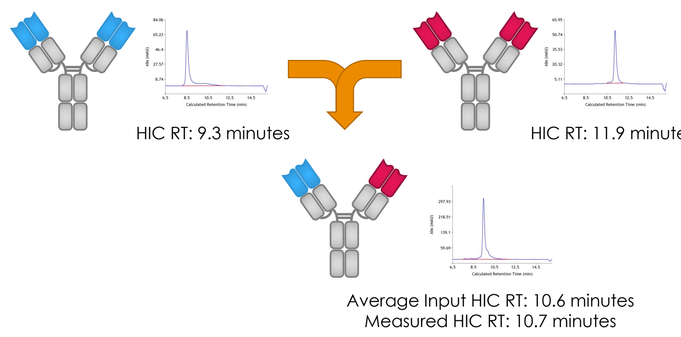


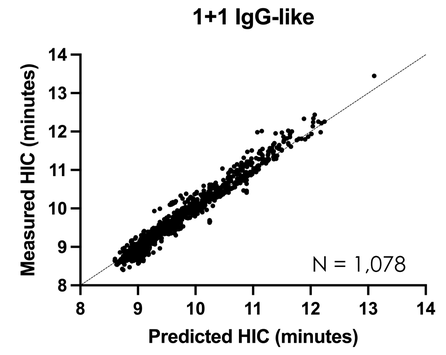
Fab and VHH based chain exchange input have good purity after protein A purification
High MW aggregates removed through polishing before chain exchange reaction



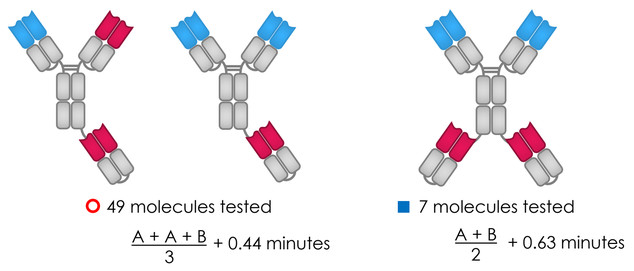
Predicting multispecific hydrophobicity based on input mAbs

Average of input mAb HIC RT predicts 1+1 IgG-like bispecifics





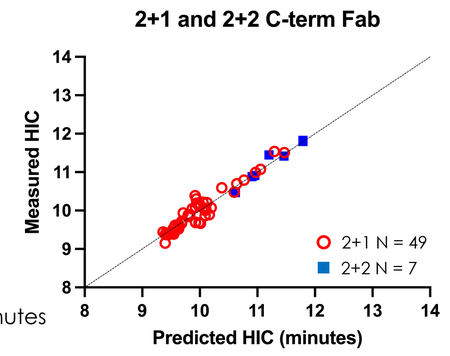
Average of input mAbs predicts HIC RT of bispecifics with C-term Fabs



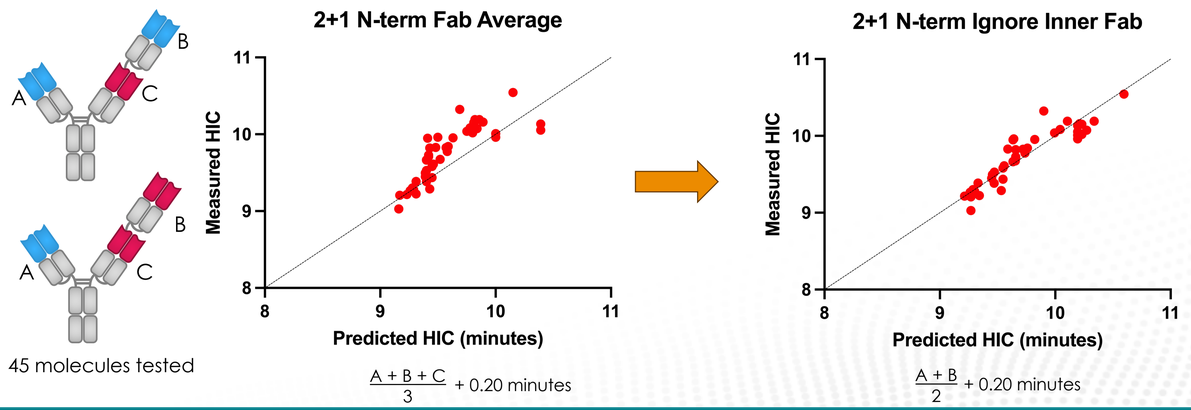
$\frac{A + A + B}{3}$ + 0.44 minutes

$\frac{A + B}{2}$ + 0.63 minutes

Larger molecules have longer retention time;
Prediction requires adding time based on size: 2+1 C-term add 0.44 minutes



Ignoring inner Fab of 2+1 N-term bispecifics improves prediction

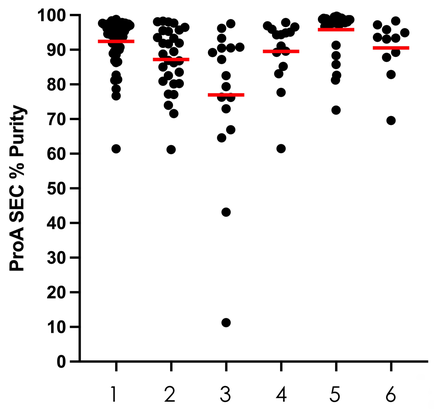


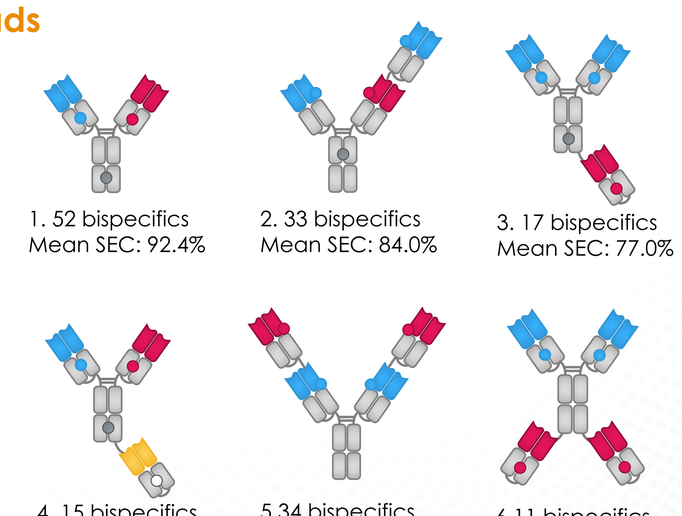
$\frac{A + B + C}{3}$ + 0.20 minutes

$\frac{A + B}{2}$ + 0.20 minutes

After screening with chain exchange, CH1-Cκ heterodimerization is applied to therapeutic leads

CHO production ProA SEC % purity





1. 52 bispecifics
Mean SEC: 92.4%

2. 33 bispecifics
Mean SEC: 84.0%

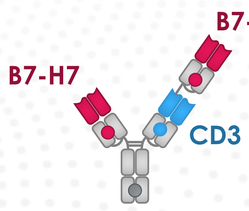
3. 17 bispecifics
Mean SEC: 77.0%

4. 15 bispecifics
Mean SEC: 89.6%

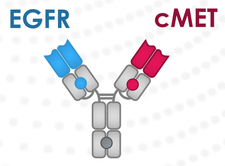
5.34 bispecifics
Mean SEC: 95.8%

6.11 bispecifics
Mean SEC: 90.5%

6 partner clinical programs use Adimab CH1-Cκ heterodimerization[1]

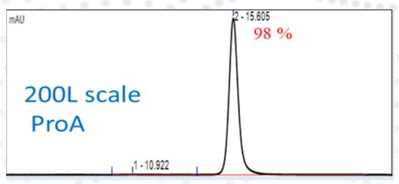


NPX372
Adimab CD3 binder[2]



PM-1080

Adimab partner Biotheus (Hansoh) moves PM-1080 into Phase III



Stable cell line titer 6 g/L
No HC-LC mispair

Acknowledgments and references:

We thank our partners at NextPoint and Biotheus for sharing data on their programs. All members of the Adimab scientific and engineering teams contributed to this technology.

1. Barlow et al., **MAbs** 2025 vol. 17: 2479531. 2. Liu et al., **MAbs** 2023 15: 2189974.

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