

Control Strategies Tailored for Multispecifics

-Addressing Homodimer and Mispairing Species

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Pfizer

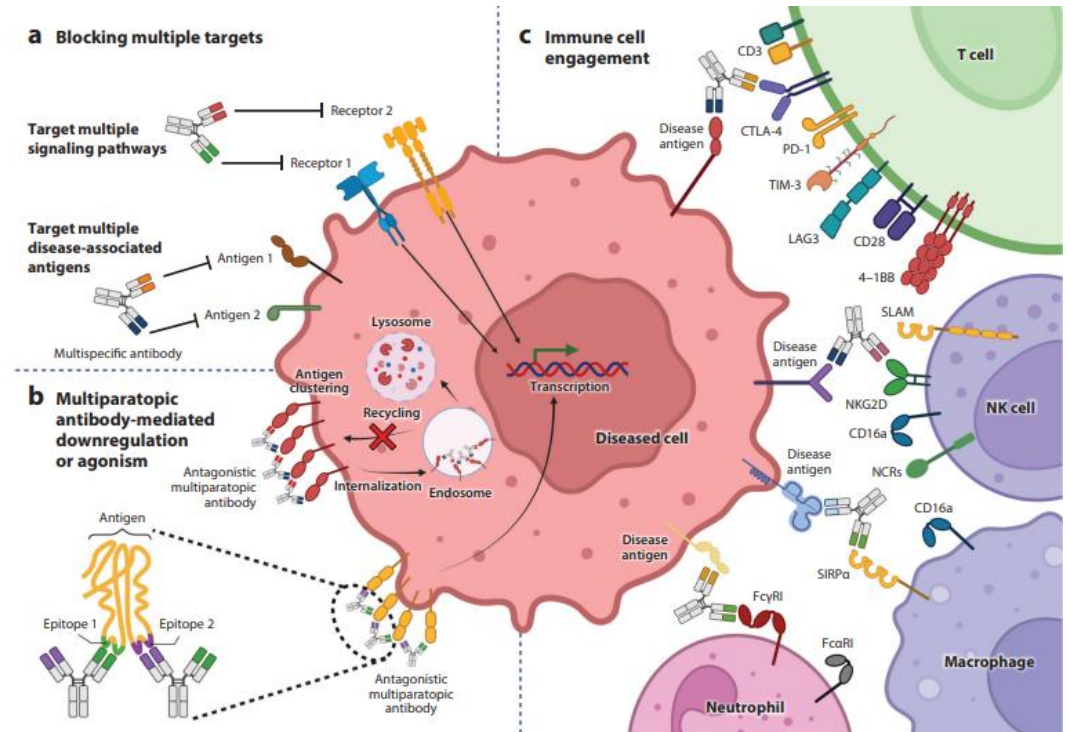
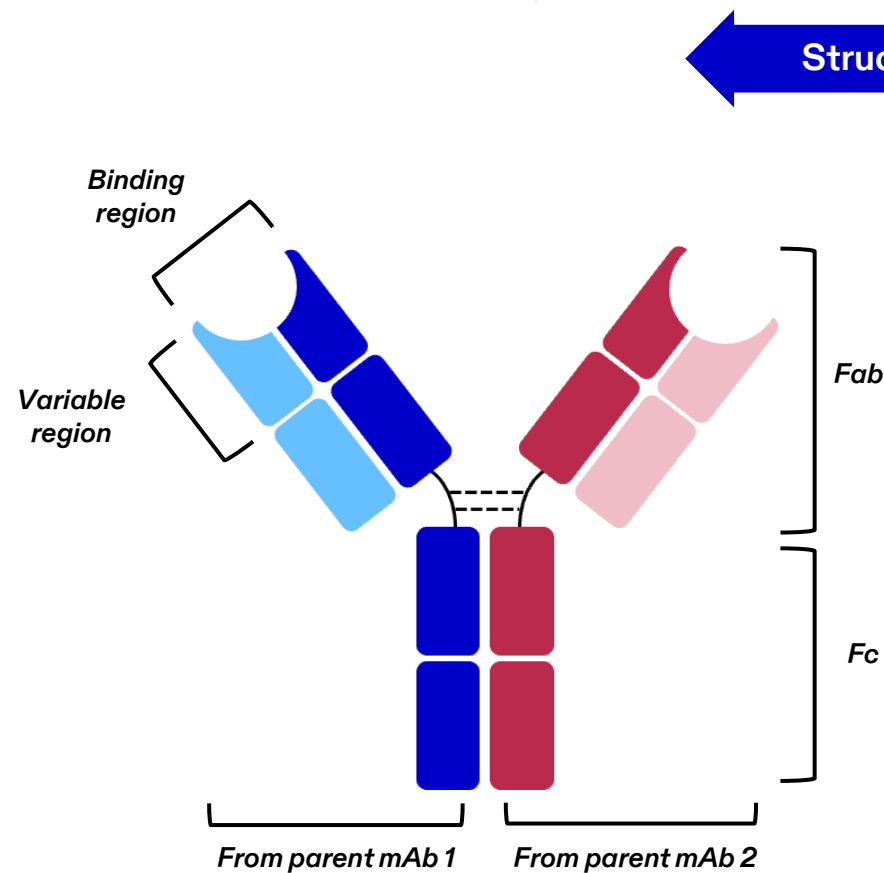


Outline

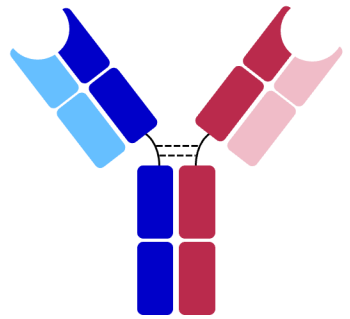
- Introduction of Multispecifics
- Unique Quality Attributes - Homodimer and Mis-pairing
- Regulatory Recommendations for Homodimer and Mis-pairing
- Introduction of Control Strategy
- Control Homodimers and Mis-pairing in Multispecifics in a Bispecific Antibody

What and Why Multispecifics

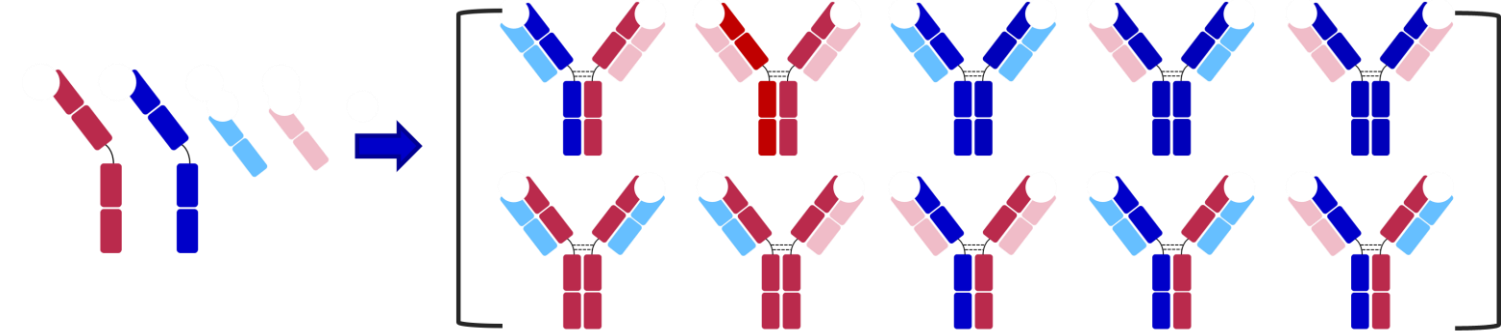
Asymmetrical molecules that recognize two (or more) different antigens, or multiple different epitopes on the same antigen.



The Pairing Problem

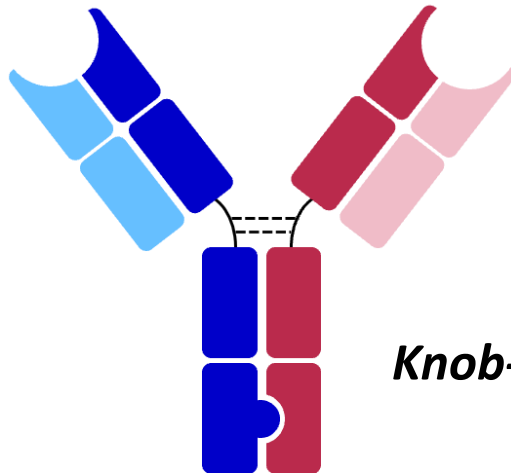


Target Bispecific

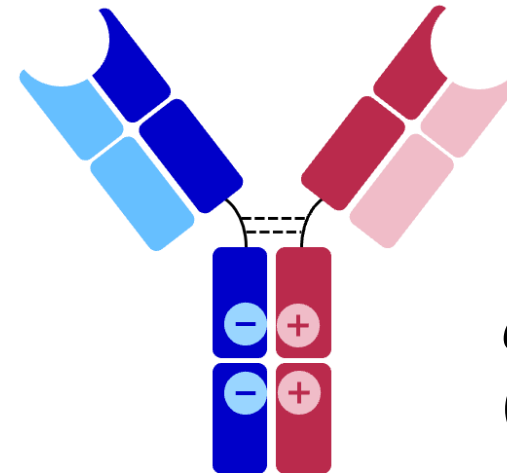


10 possible combinations

How do we promote preferred heterodimerization?



Knob-into-hole (KiH)

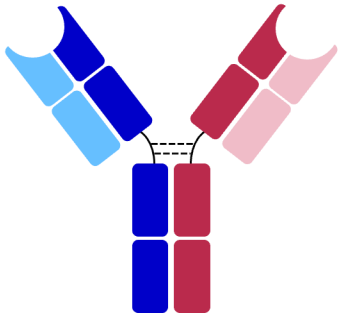


*Charge based pair
(EE/RR, EEE/RRR)*

Unique Quality Attributes

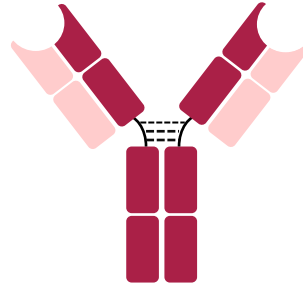
- Product Related Impurities

Bispecific antibody

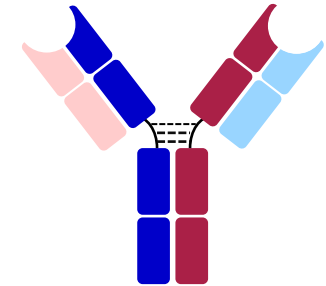
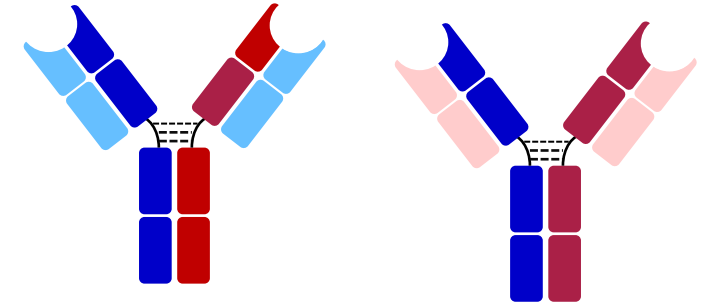


Typical antibody attributes apply - fragments, aggregate, charge heterogeneity, potency, etc.

Homodimers



Mis-pairing/Chain Swapping



Regulatory Guidance on Multispecifics



Bispecific Antibody Development Programs Guidance for Industry

May 2021

<https://www.fda.gov/regulatory-information/search-fda-guidance-documents/bispecific-antibody-development-programs-guidance-industry>

III. Scientific Considerations

A. CMC Quality Considerations

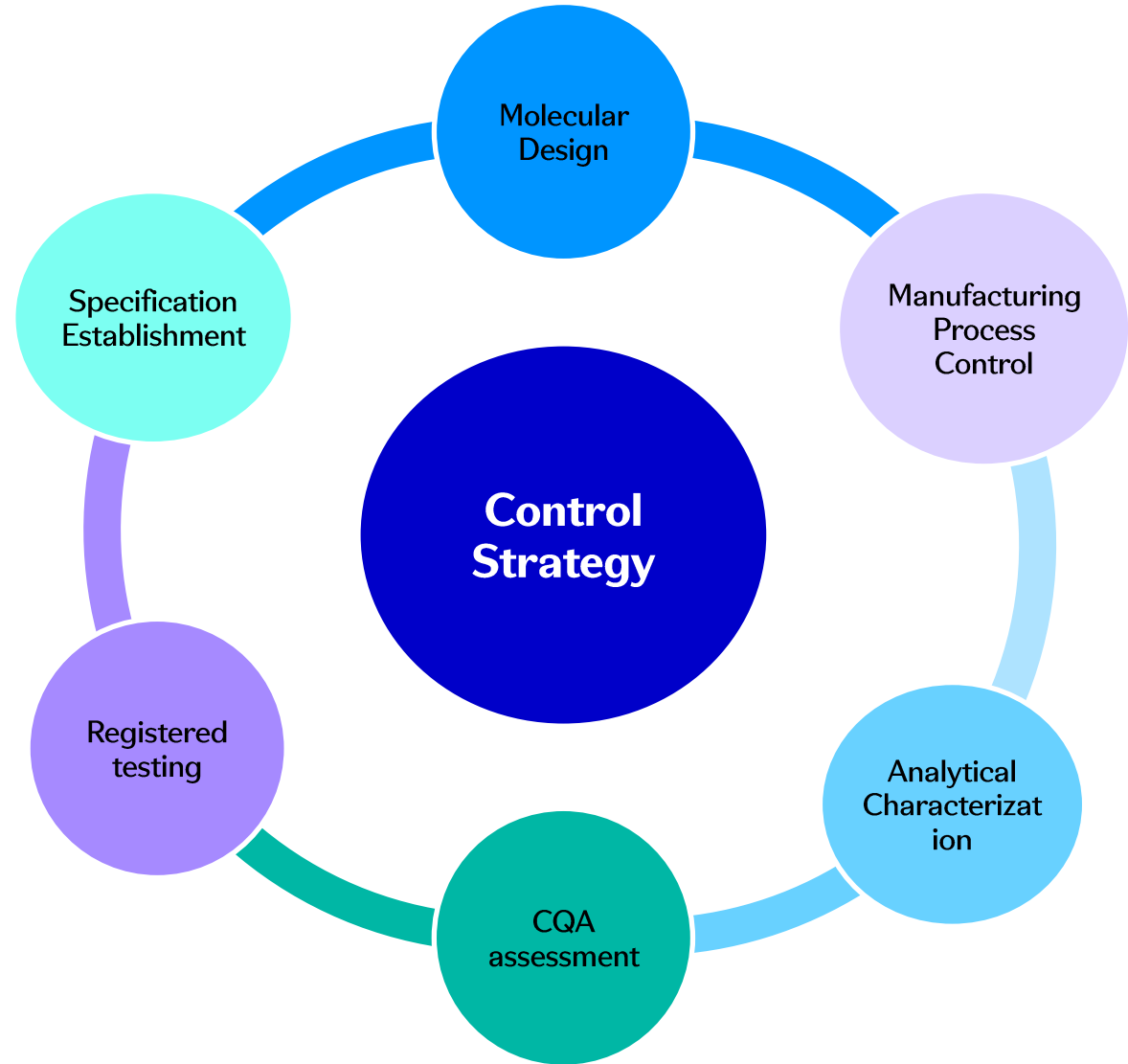
“Quality attributes that may affect pharmacology should be studied, including ... product-related impurities such as ... **homodimers**, and other **mis-paired species**...

The **relative amounts of homodimers should be assessed**. This evaluation is particularly important for effector cell engaging constructs where homodimers of the anti-CD3 or anti-Fc engaging arm may lead to **cytokine release**. Also, novel structures could potentially lead to increased immunogenicity.”

Control Strategy

- ICH Q10 definition:

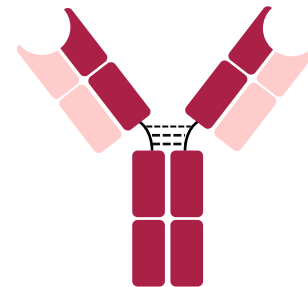
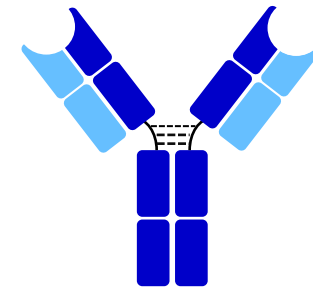
“A planned set of controls, derived from current product and process understanding that ensures process performance and product quality. The controls can include parameters and attributes related to drug substance and drug product materials and components, facility and equipment operating conditions, in-process controls, finished product specifications, and the associated methods and frequency of monitoring and control.”



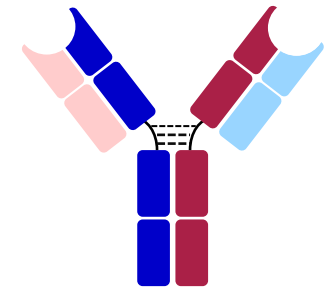
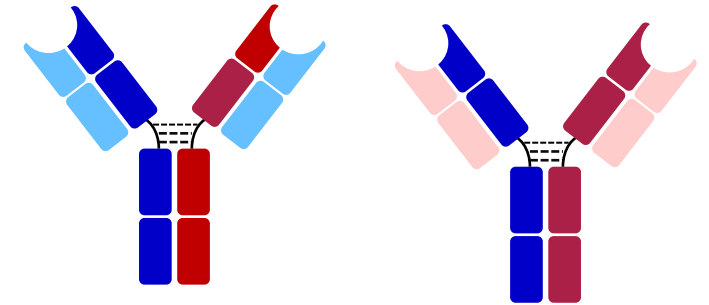
Control Homodimers and Mis-Pairing in a Bispecific Antibody



Homodimers

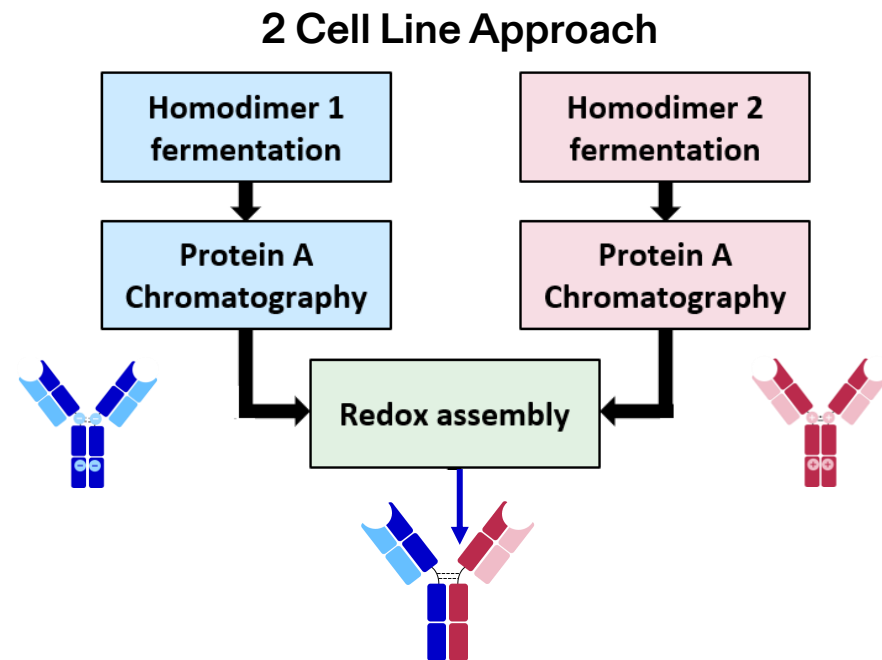
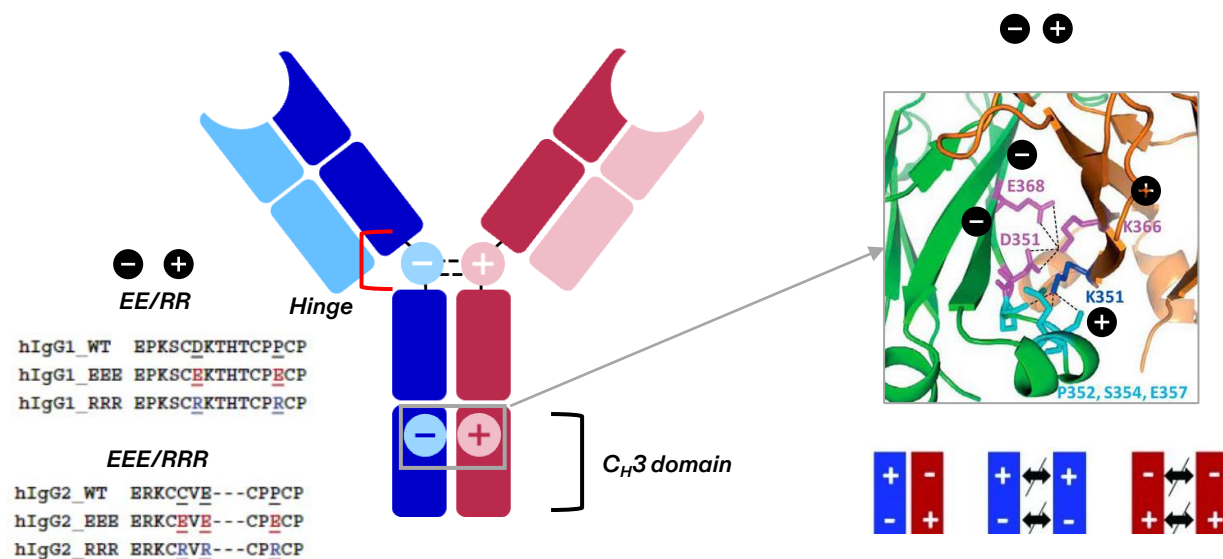


Mis-pairing/Chain Swapping



Quality by Molecular Design

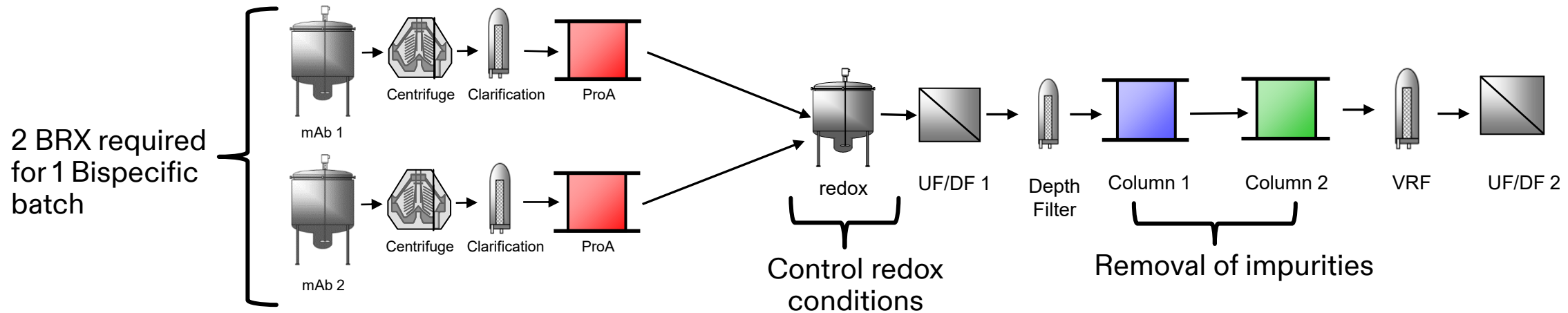
– Heterodimerization is favored by electrostatic steering



- Amino acid replacement having opposite charges, such as EEE/RRR, EE/RR.
- **Favored by opposite charge**; electrostatic interaction driven.
- Bispecific formation via in vitro redox

Manufacturing Process Control

- Remove Homodimers and Mis-Pairing



Unique considerations :

- Production of two mAbs - manufacturing timing/scheduling (sequential or parallel bioreactors)
- Unique process step – Redox step requires creative design & characterization
 - Control ratio of parent mAbs to reduce one specific homodimer
 - Control protein concentration, redox reagents, reaction time and temperature to form bispecific and minimize homodimer and mispairing
- Downstream
 - two column purification to remove potential impurities

Analytical Characterization

-Product Quality, Efficacy and Safety Assessment

Structure Characterization

- Homodimers: trace level observed in drug substance using intact analysis such as CEX, AEX and LC/MS;
- Mis-pairing: not observed in drug substance
- Homodimer product quality assessment: disulfide linkages, fragments and HMMS, assessed

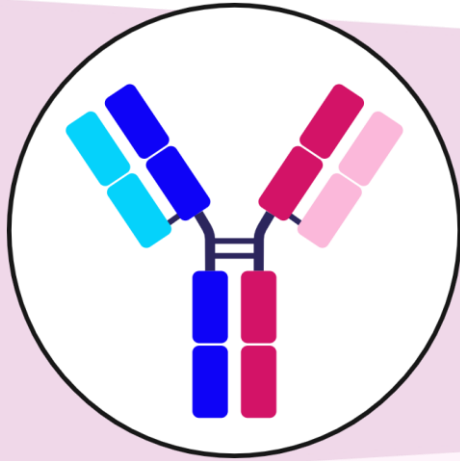
Functional Analysis

- Homodimers have no dual target binding as confirmed by the dual binding ELISA and no target cellular co-engagement as confirmed by cell-based assay
- Mis-pairing: not assessed as it is not observed in the manufacturing

Safety Assessment

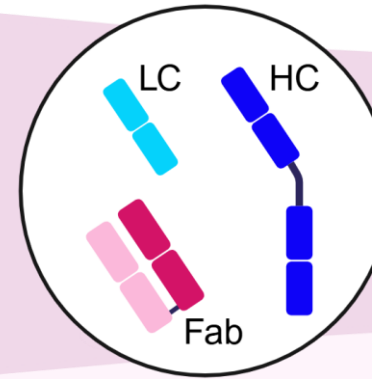
- Homodimer spiking in vitro study: bispecific containing 5% of each homodimer did not change the overall cytokine profile or magnitude, suggestive that overall safety risk has not changed.
- Mis-pairing: not assessed as it is not observed in the manufacturing

Analytical Characterization -Tools for MsAbs



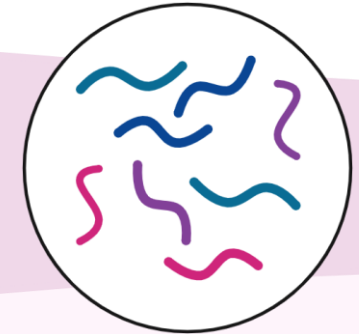
Intact level

- Size Exclusion Chromatography (SEC)
- Non-reducing Capillary Gel Electrophoresis (NR-CGE)
- Imaged capillary electrophoresis (iCE)
- Hydrophobic Interaction Chromatography (HIC), Ion Exchange Chromatography (IEX), Reverse Phase (RP)-HPLC
- **Intact LC-MS**
- Bioassay ELSA, cell based assay
- Biophysical analysis
- Spiking studies



Subunit level

- Reducing Capillary Gel Electrophoresis (R-CGE)
- Reducing Subunit LC-MS
- **Enzyme Digested Subunit LC-MS** (such as GingisKHAN, etc.)

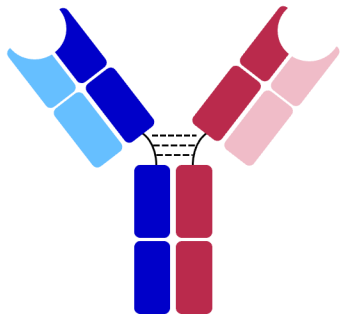
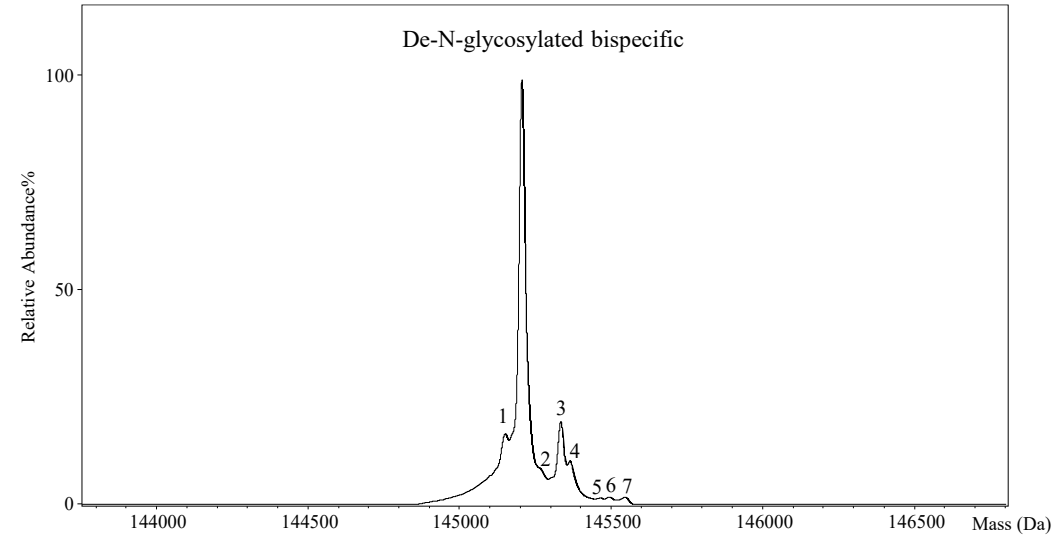
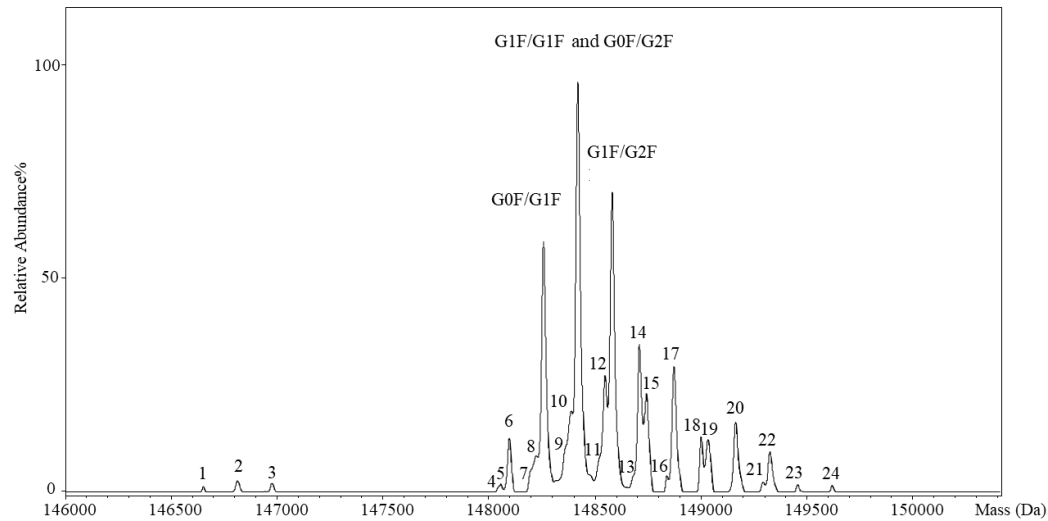


Peptide level

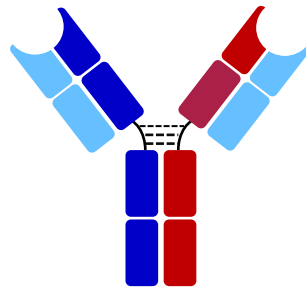
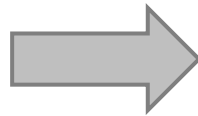
- **Reduced/alkylated peptide map LC-MS/MS**
- **Non-reduced peptide map LC-MS/MS**
- **Multi-Attribute Method (MAM)**
- **N-Glycan LC/MS Analysis**
- Peptide Map LC-UV

Intact MS Analysis

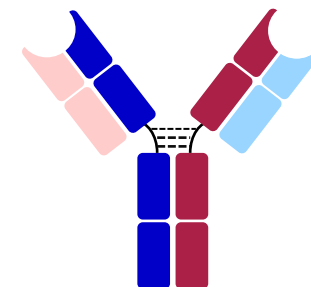
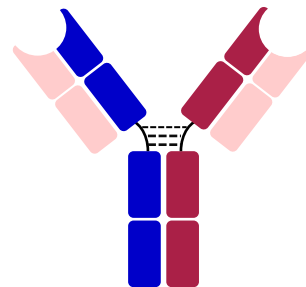
- Confirm No Misparing Species Formation



Bispecific antibody



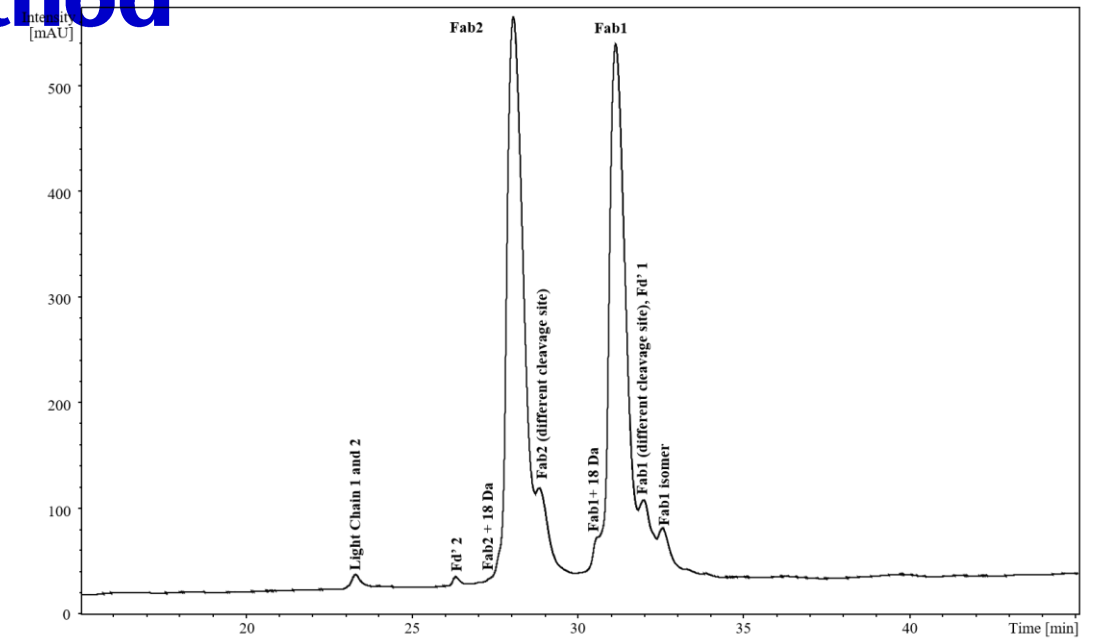
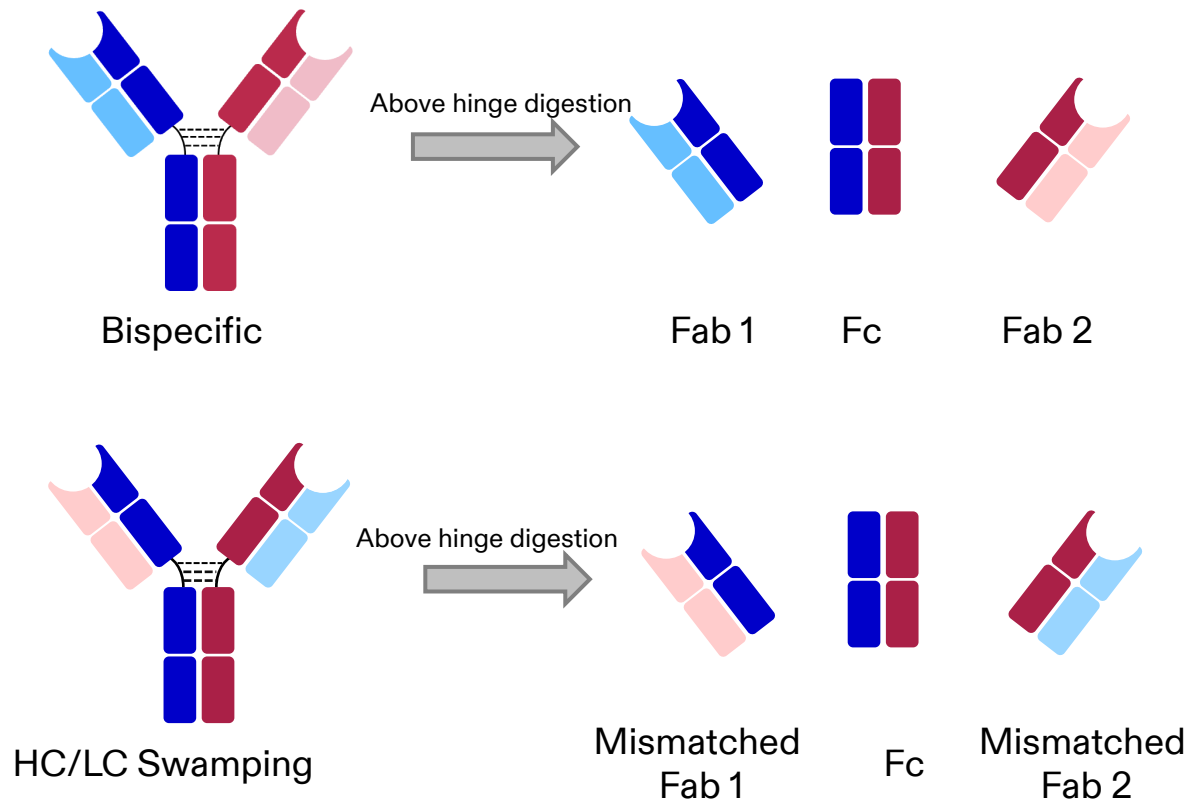
Single HC/LC Swamping
-Mass difference with target
bispecific antibody



Double HC/LC Swamping
-Same mass as target bispecific antibody
-Assumption: single swamping as
precursors to double swamping

A Sensitive Subunit LC/MS Method

-Assess HC/LC Mispairing



- Papain digestion
 - Above hinge digestion
 - Multiple cleavage sites with a primary cleavage site
 - Mild reducing condition does not reduce disulfide connection
- No Mispairing detected with this sensitive subunit method

- Confirm Disulfide Bonds and Assess Disulfide Scrambling, Mispairing

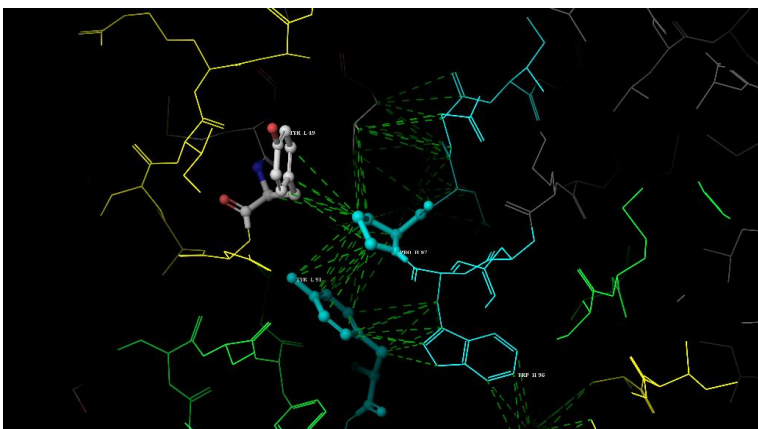
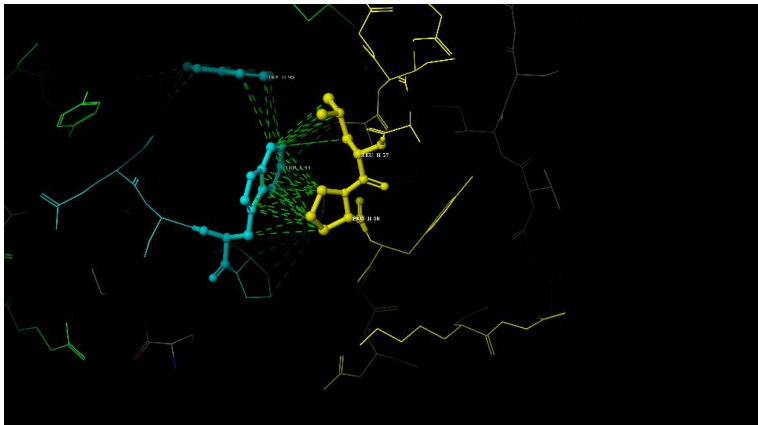
- [illegible]



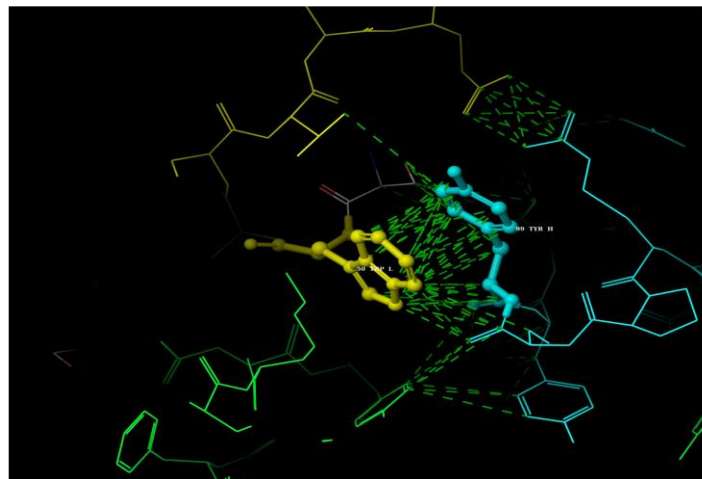
Structural Modeling Analysis

-Suggests Mispairing is Unfavored

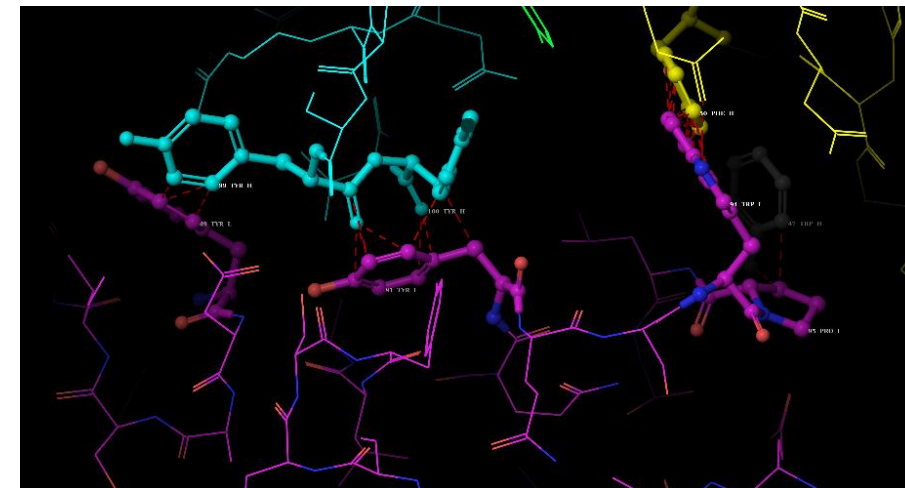
Multiple Favorable contacts in Fab 1



Multiple Favorable contacts in Fab 2



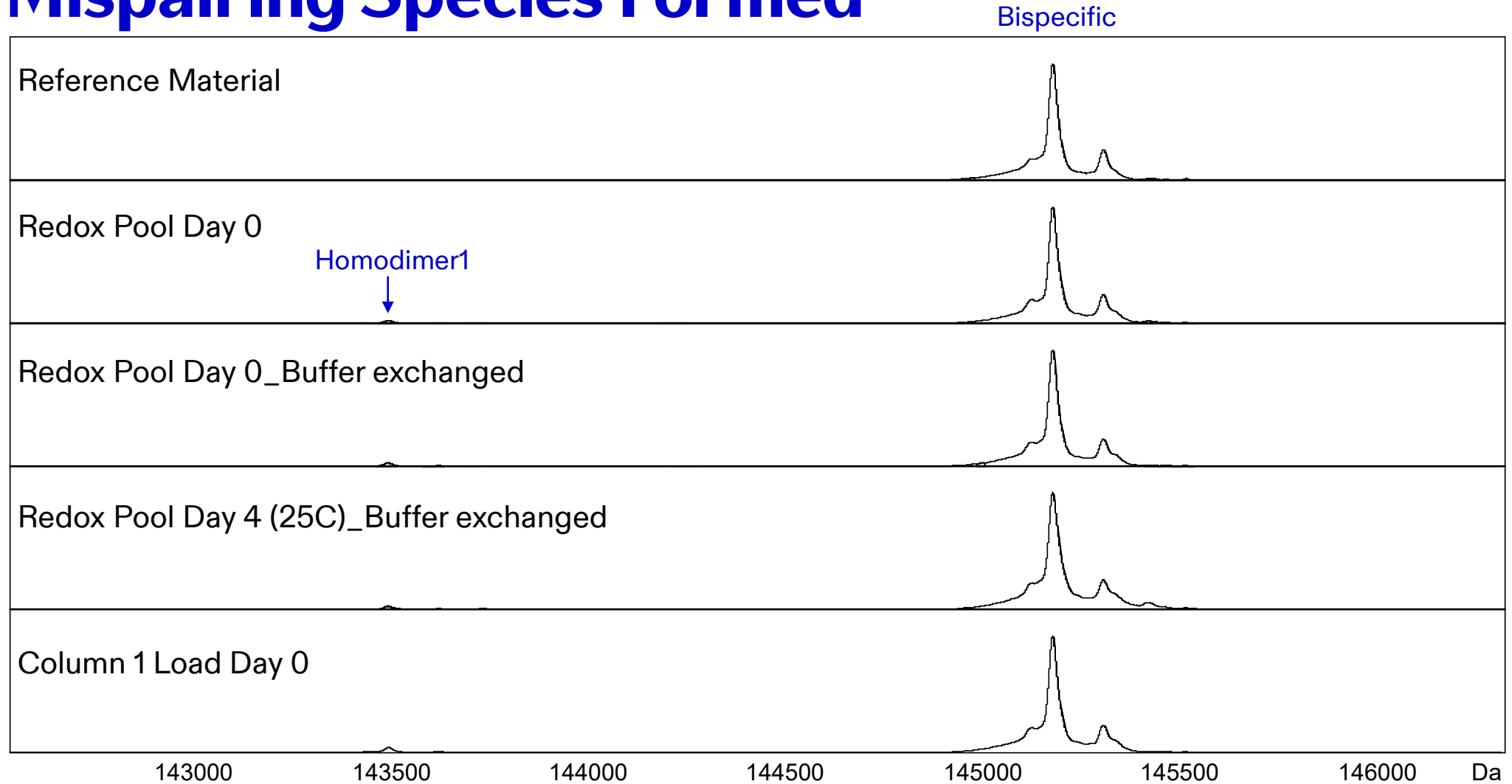
Spatial conflicts (red dots)
in simulated mispaired Fab



Red dotted lines indicate contacts closer than 0.75 times the sum of the Van Der Waals atomic radii.

Hold Time Study by De-N-Gly Intact Analysis

- No Mispairing Species Formed

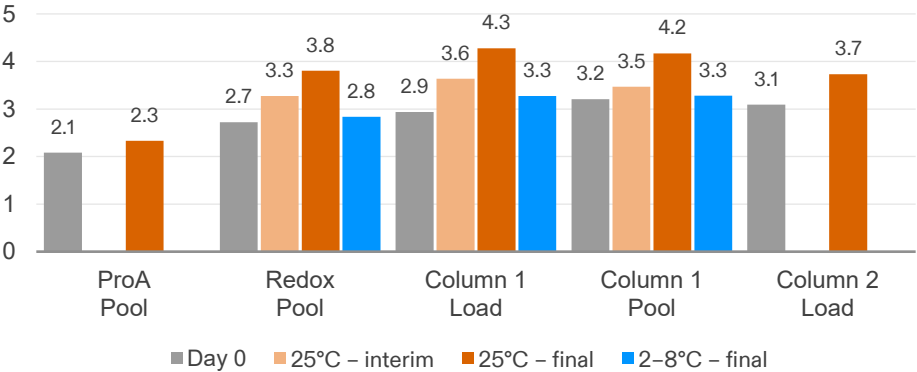


- Comparable de-N-glycosylated profiles at different hold time
- Glycosylation profiles by Intact MS analysis are also consistent (data not shown)
- Low level homodimer 1 observed
- No Mispairing species observed

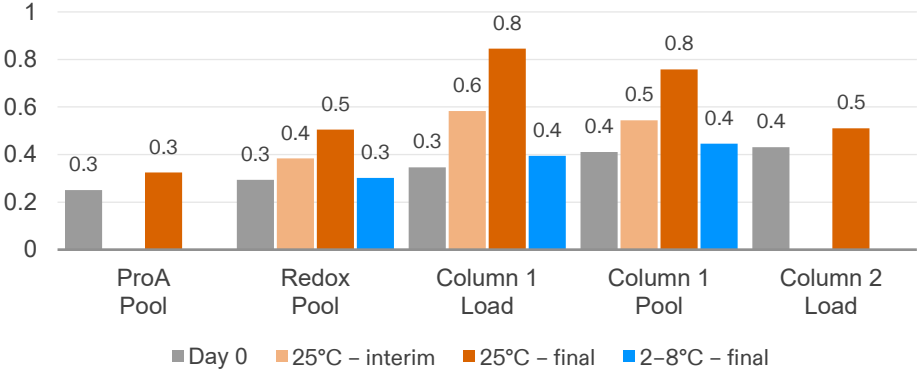
Hold Time Study by MAM

– Expected Product Quality

Fc PENNYK Deamidation (%)

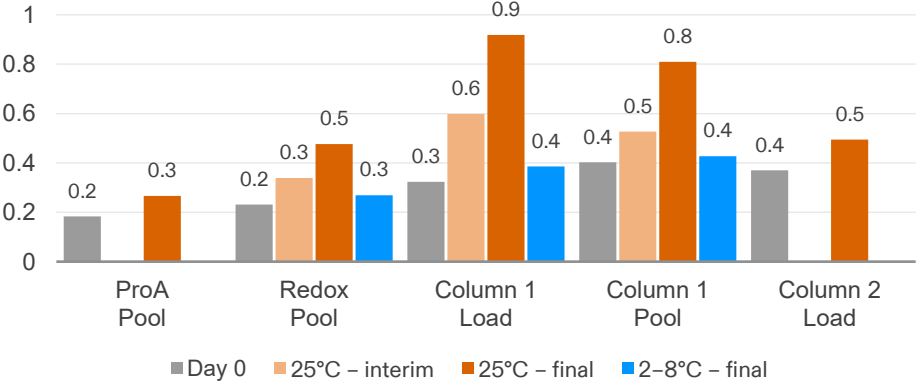


LC2 Asn Deamidation (%)

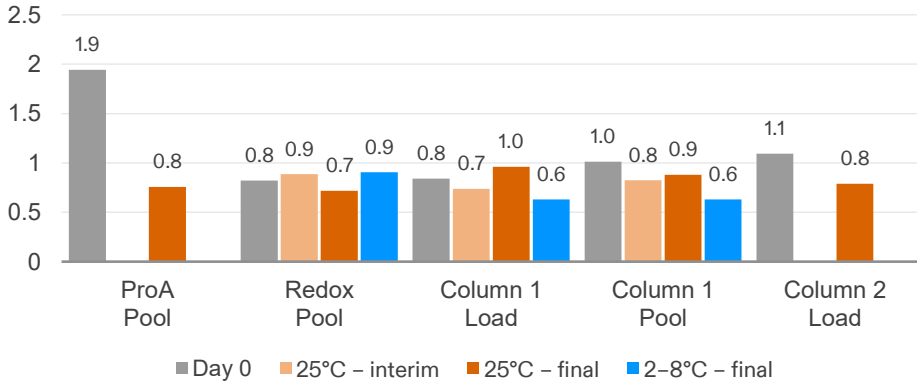


grey = Day 0; orange = 25°C hold (interim & final timepoint per step: Redox Pool/TMAE Load = Day 2 & 4, TMAE Pool = Day 1 & 3, ProA Pool/CHA Load = single timepoint); blue = 2-8°C hold (final timepoint)

HC2 Asp Isomerization (%)

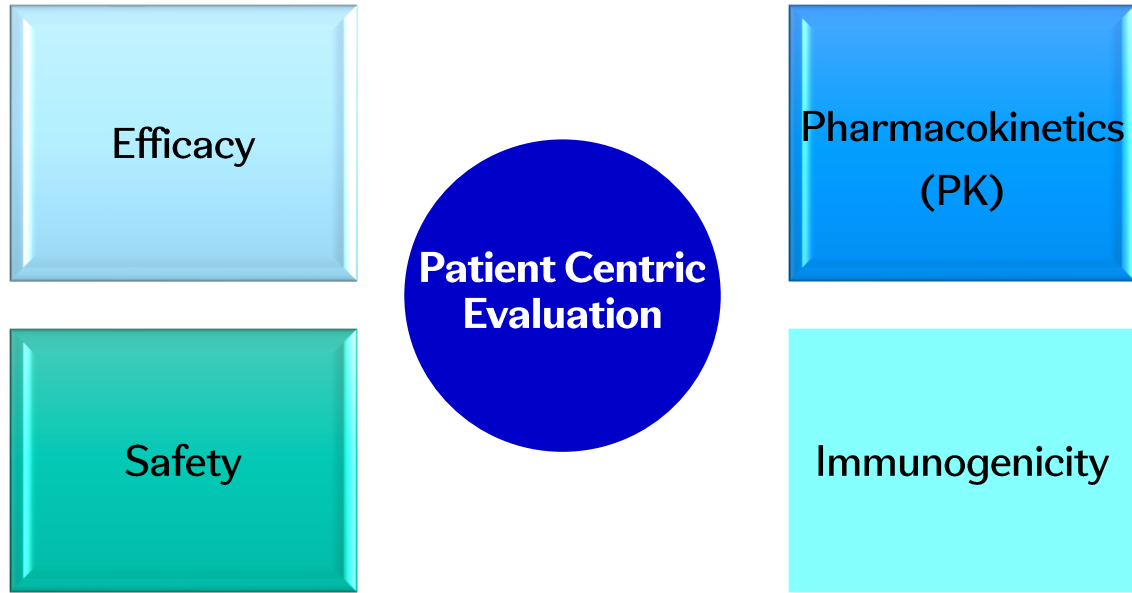


HC1 Met Oxidation (%) – no trend



Attributes remain stable at 2-8°C across all process steps; extended hold at 25°C shows a slight, low-level, time-dependent increase in deamidation/isomerization at select Fc and CDR sites — supporting defined in-process hold time limits within the control strategy.

CQA Assessment



Homodimers: CQA

- **Efficacy:** lack of the full bispecific mechanistic activity; confirmed with both dual-binding ELISA and cell-based assay
- **Safety and immunogenicity:** may have safety concern per literature; low level ($\leq 5\%$ homodimer) does not impact safety profile based on cytokine release observations in spiking study
- **PK:** not assessed

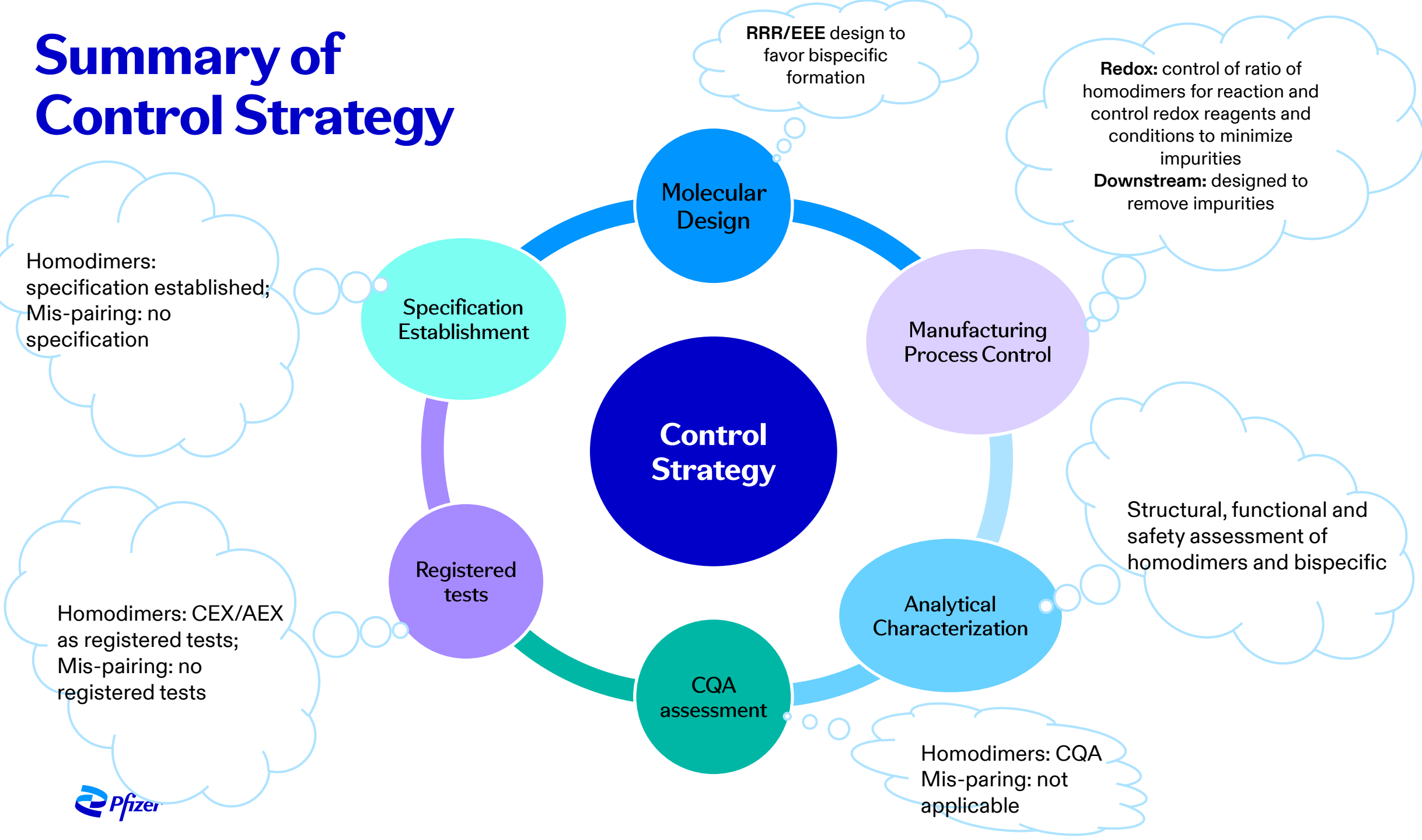
Mis-Pairing: Not Applicable

- Not observed during the manufacturing history; eliminated by molecular design and manufacturing process.
- No additional studies performed.

Registered Tests and Specification

Quality Attributes	Criticality	Registered Tests	Specification	Justifications
Homodimers	CQA	CEX/AEX	Homodimer 1: $\leq 3.0\%$ Homodimer 2: $\leq 2.0\%$	• Manufacturing capability; • Historical and clinical batches for statistical analysis; • Final acceptance criteria was slightly widen than the statistical range (tolerance interval) based on safety assessment of spiking materials with up to 5% homodimers; • Bispecific containing 5% of each homodimer did not change the overall cytokine profile or magnitude, suggestive that overall safety risk has not changed.
Mis-pairing	Not Applicable	N/A	N/A	• Not observed in manufacturing history

Summary of Control Strategy



Thank You

- **Pfizer**
 - Analytical Research & Development
 - MS data contributors: Leah Wang, Kathleen Cornelius, Thomas Powers, Yuting Huang, Bradley Bare*, Michelle English*
 - Bioprocess Research & Development
 - Pharmaceutical Research & Development
 - Pfizer Global Supply (Andover, MA; Grange Castle, Ireland; Kalamazoo, MI; Puurs, Belgium)
- CASSS MS Forum, 2026

* Previous Pfizer colleagues

