Control Strategies Tailored for Multispecifics

Wenqin Ni

Pfizer

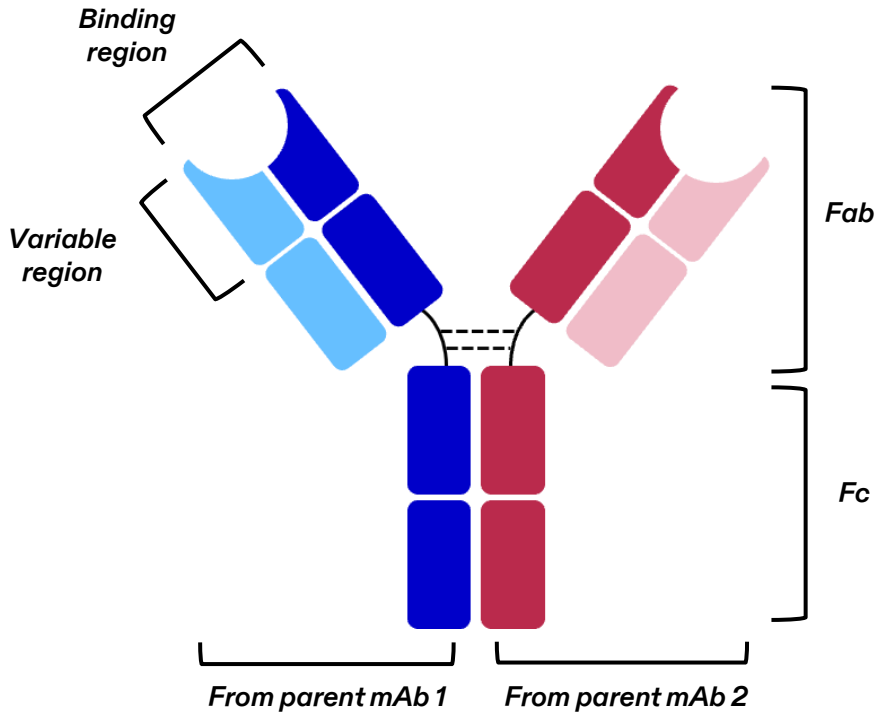
Outline

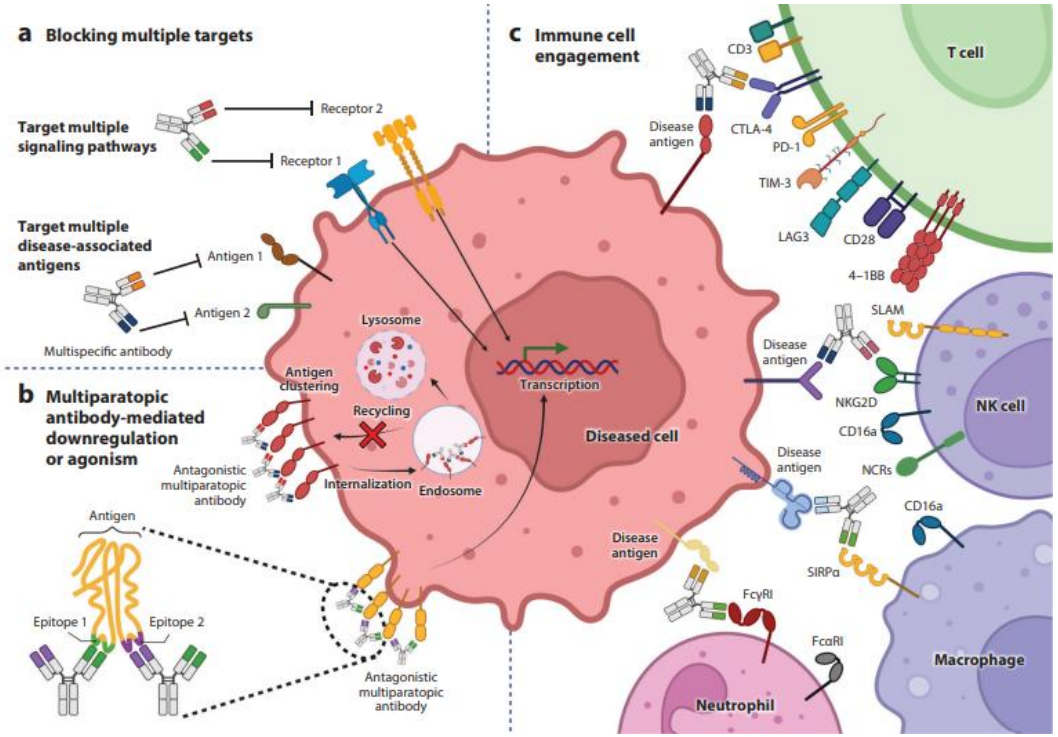
- Introduction of Multispecifics
- Unique Quality Attributes - Homodimer and Mis-pairing
- Regulatory Recommendations for Homodimer and Mis-pairing
- Introduction of Control Strategy
- Case study 1: Control Homodimers and Mis-pairing in Multispecifics in a Bispecific Antibody to Support Commercial Process
- Case study 2: Control Homodimers in a Bispecific Fusion Protein to Support Clinical Manufacturing

What and Why Multispecifics

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

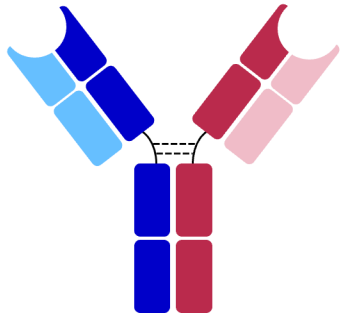
Structure | **Mechanism of Action**



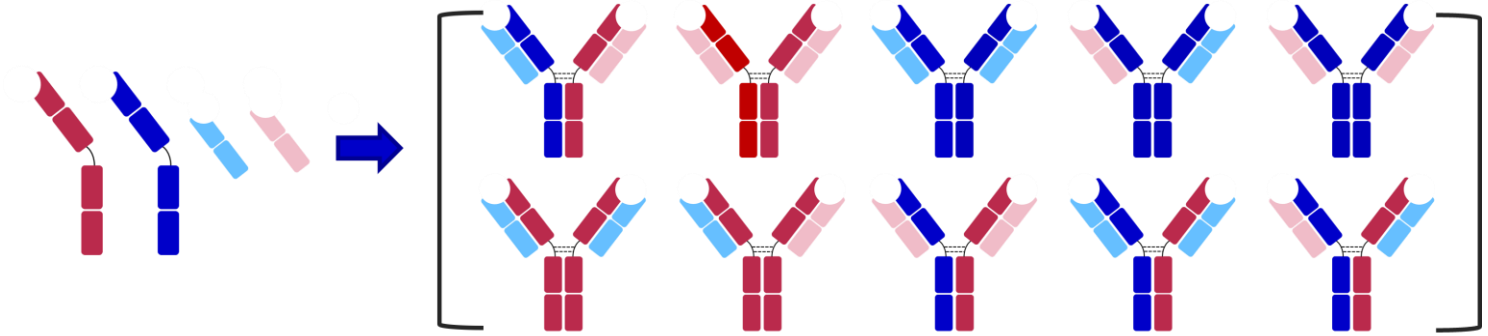


Annu. Rev. Chem. Biomol. Eng. 2024. 15:105–38

The Pairing Problem

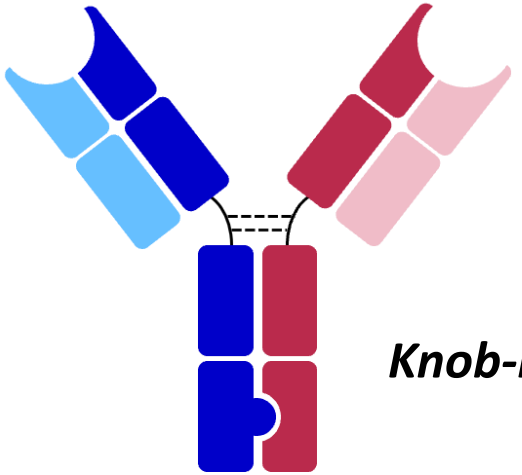


Target Bispecific

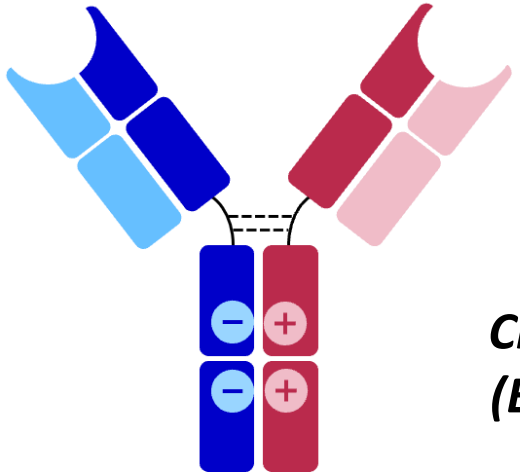


10 possible combinations

How do we promote preferred heterodimerization?



Knob-into-hole (KiH)

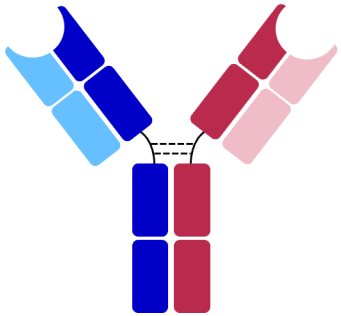


Charge based pair (EE/RR, EEE/RRR)

Unique Quality Attributes

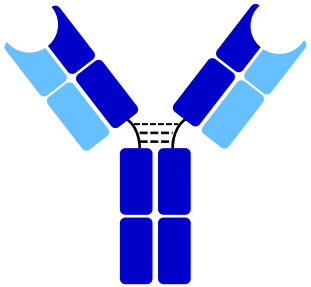
– Product Related Impurities

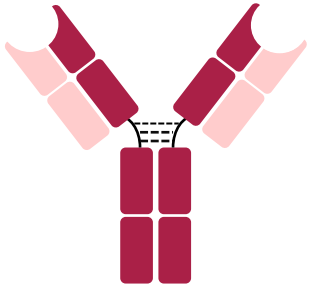
Bispecific antibody



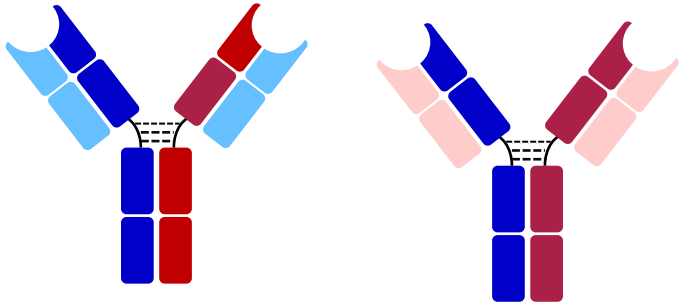
Typical antibody attributes apply - fragments, aggregate, charge heterogeneity, potency, etc.

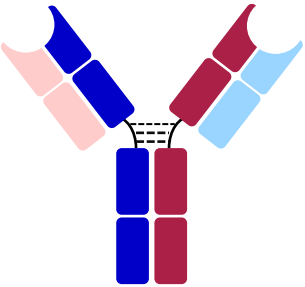
Homodimers





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

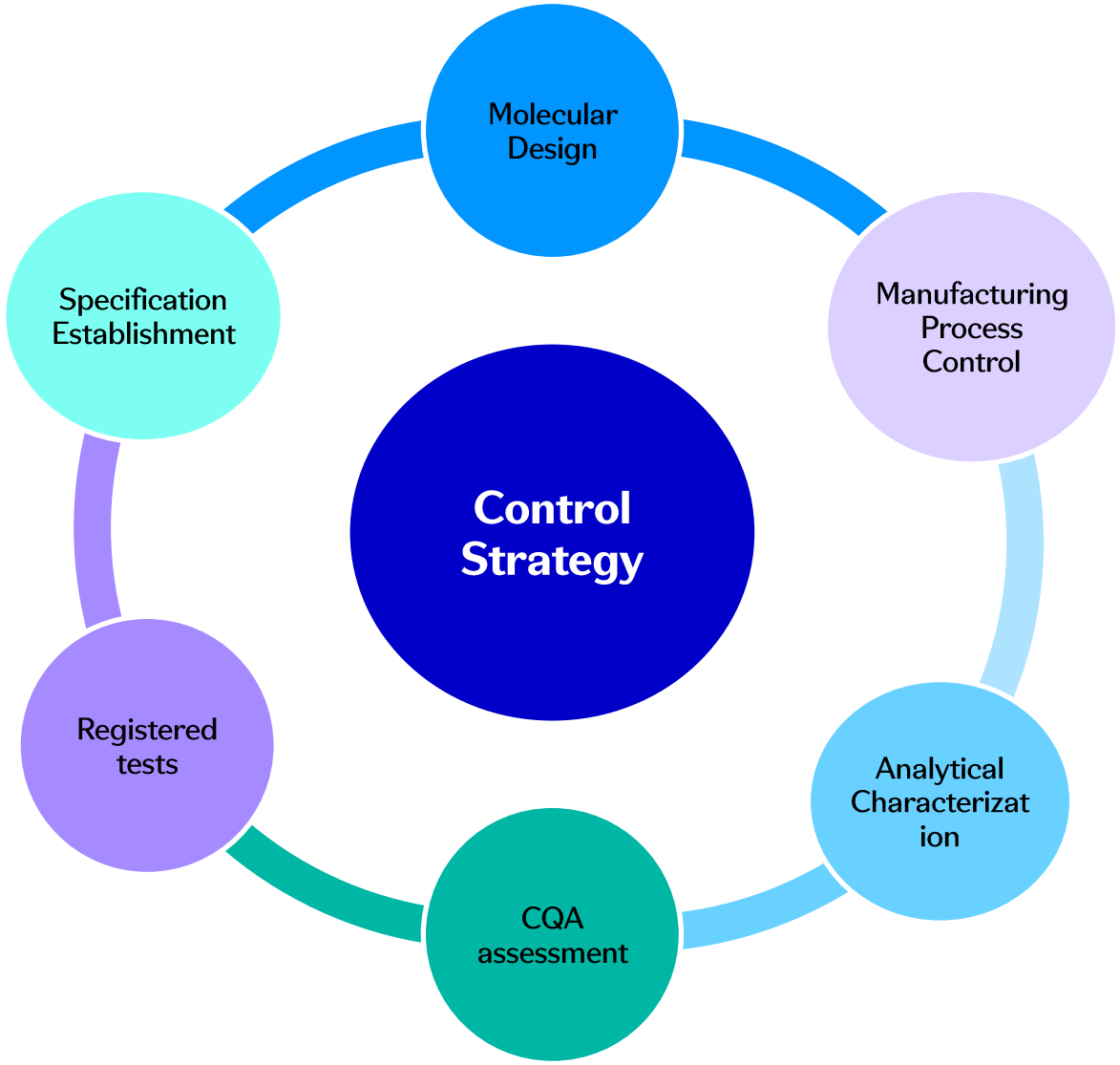
A. CMC Quality Considerations

"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:

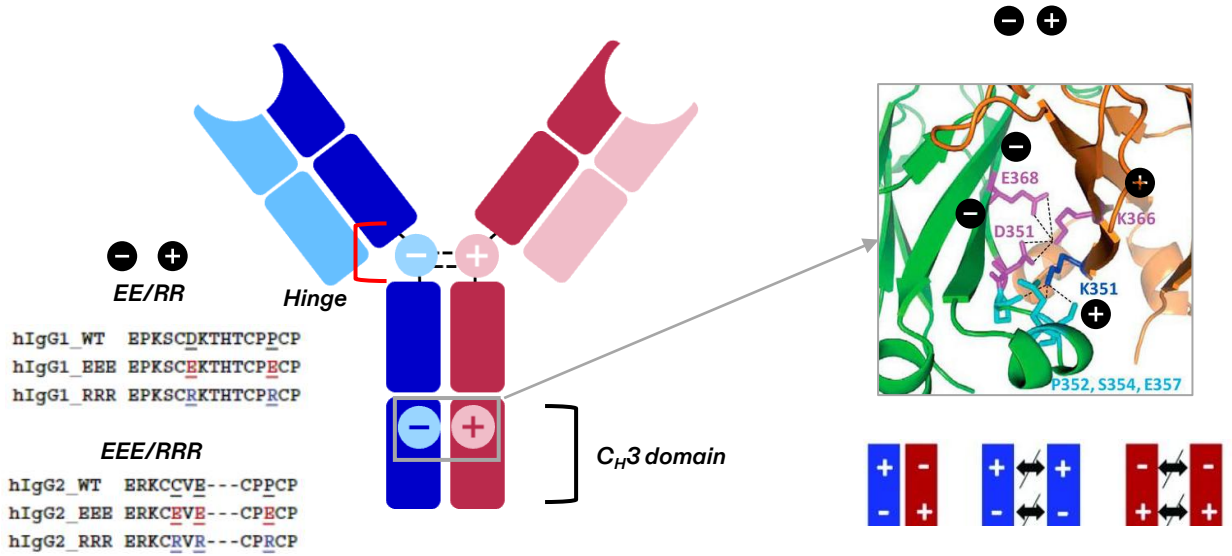
"A planned set of controls, derived from current product and process understanding that ensures process performance and product quality. The controls can include **parameters** and **attributes** related to drug substance and drug product **materials** and **components**, **facility** and **equipment**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

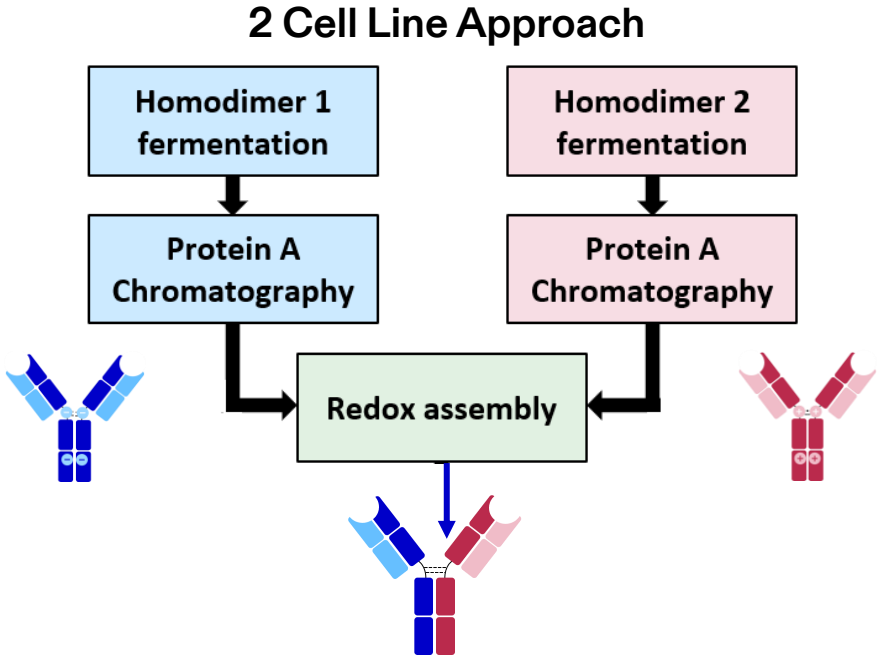


Case Study 1: Control Homodimers and Mis-Pairing in a Bispecific Antibody to Support Commercial Process

Quality by Molecular Design

– Heterodimerization is favored by electrostatic steering

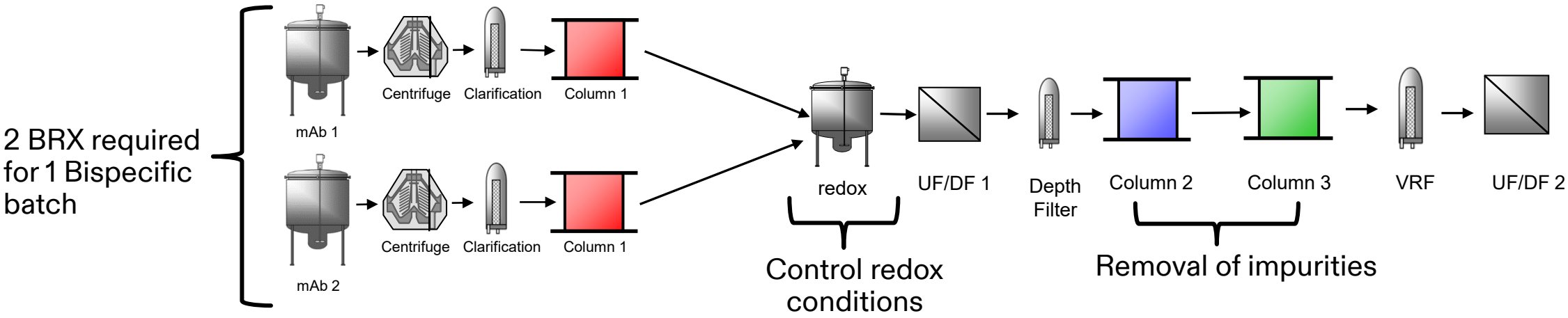




- Amino acid replacement having opposite charges, such as EEE/RRR, EE/RR.
- **Favored by opposite charge**; electrostatic interaction driven.
- Bispecific formation via in vitro redox

Williams et al, Biopharmaceutical processing, 2018
Strop et al, J Mol Biol. 2012
Van Gils and Sanders, J Biol Chem, 2017

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;
- Homodimer product quality assessment: disulfide linkages, fragments and HMMS, assessed
- Mis-pairing: not observed in drug substance

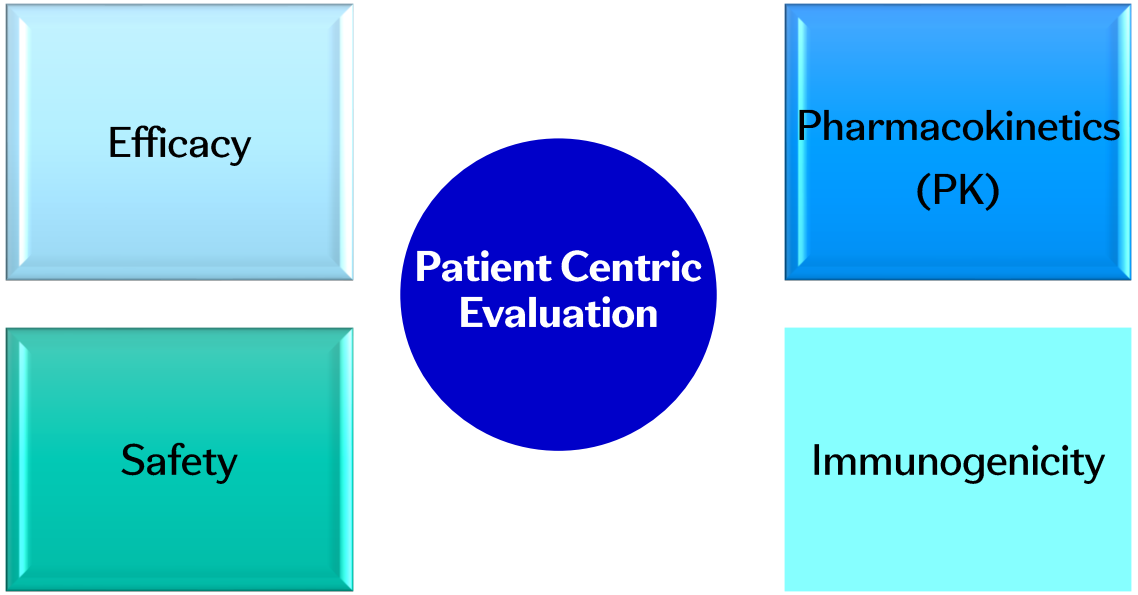
Functional Analysis

- 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

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 (≤5% homodimer) does not impact safety profile based on cytokine release observations in spiking study
- **PK:** not assessed

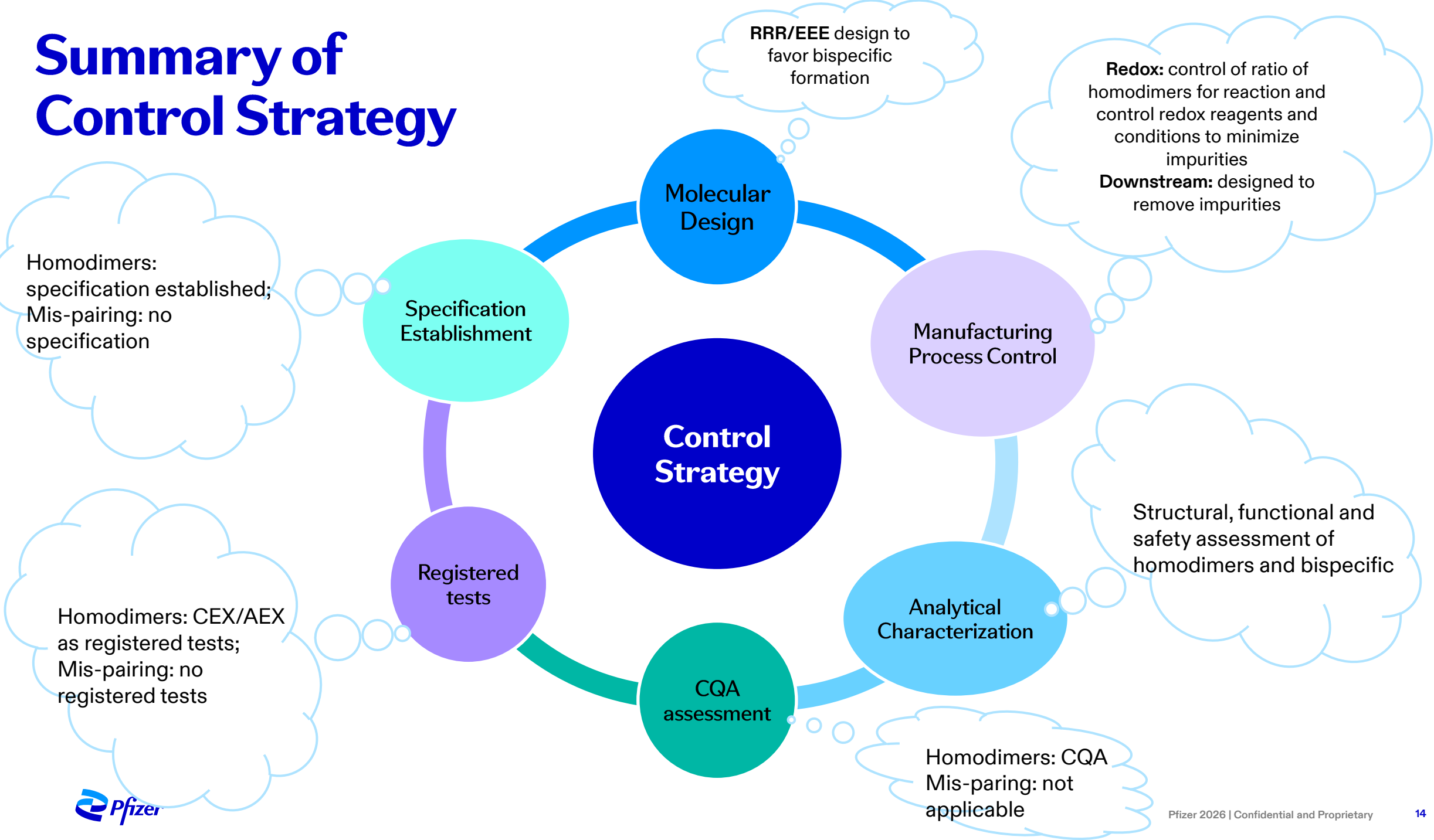
Mis-Pairing: Not Applicable

- 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: ≤ 3.0% Homodimer 2: ≤ 2.0%	• Manufacturing capability; • Historical and clinical batches for statistical analysis; • Final acceptance criteria was slightly wider than the statistical range (tolerance interval); • 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

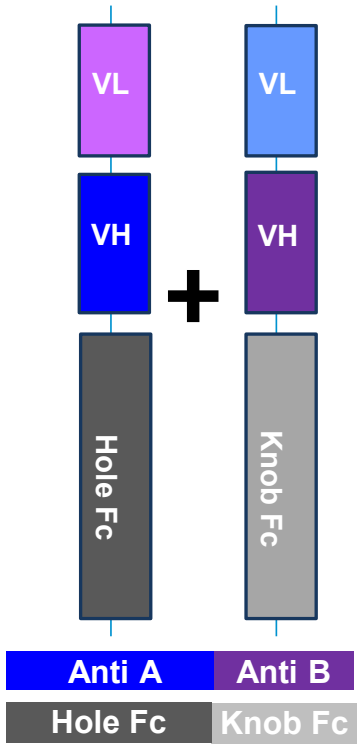
Summary of Control Strategy

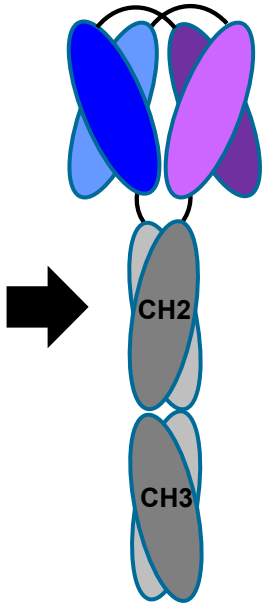


Case Study 2: Control Homodimers in a Bispecific Fusion Protein to Support Clinical Manufacturing

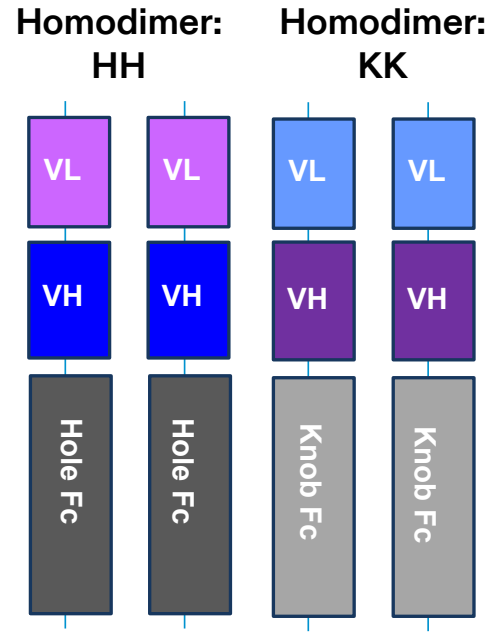
Quality by Molecular Design

– Heterodimerization is driven by knob into hole design



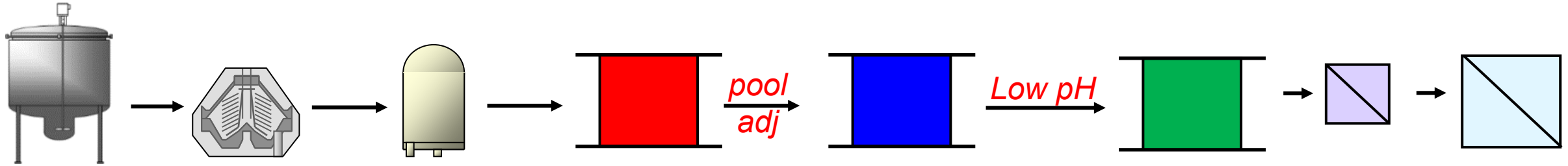


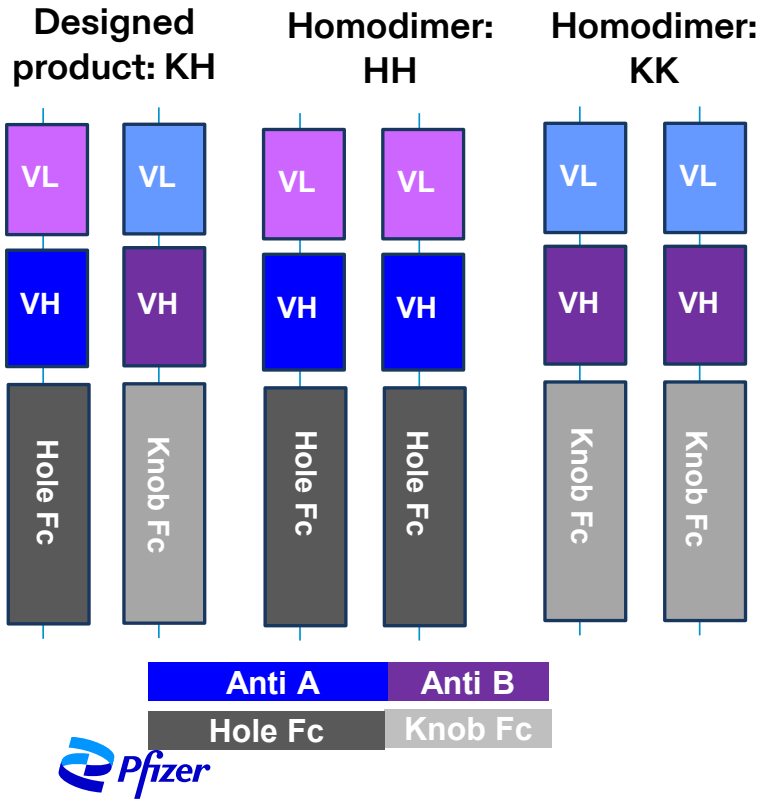
- A diabody like structure, Fc with knob-into-hole structure
- Heterodimerization is driven by knob-into-hole;
- Amino acid replacement to generate knob and hole:
  - Fc-knob: Y349C and T366W
  - Fc-hole: S354C, T366S, L368A, and Y407V
- Each homodimer is not expected to fully bind either target, as each lacks one half of the Fab by design;
- One cell line manufacturing



Modified from Discovery and optimization of a novel anti-GUCY2c x CD3 bispecific antibody for the treatment of solid tumors, Root AR, mAbs, 2021, VOL. 13, NO. 1

Manufacturing Process Control
– Remove Homodimers During Purification





Unique considerations:

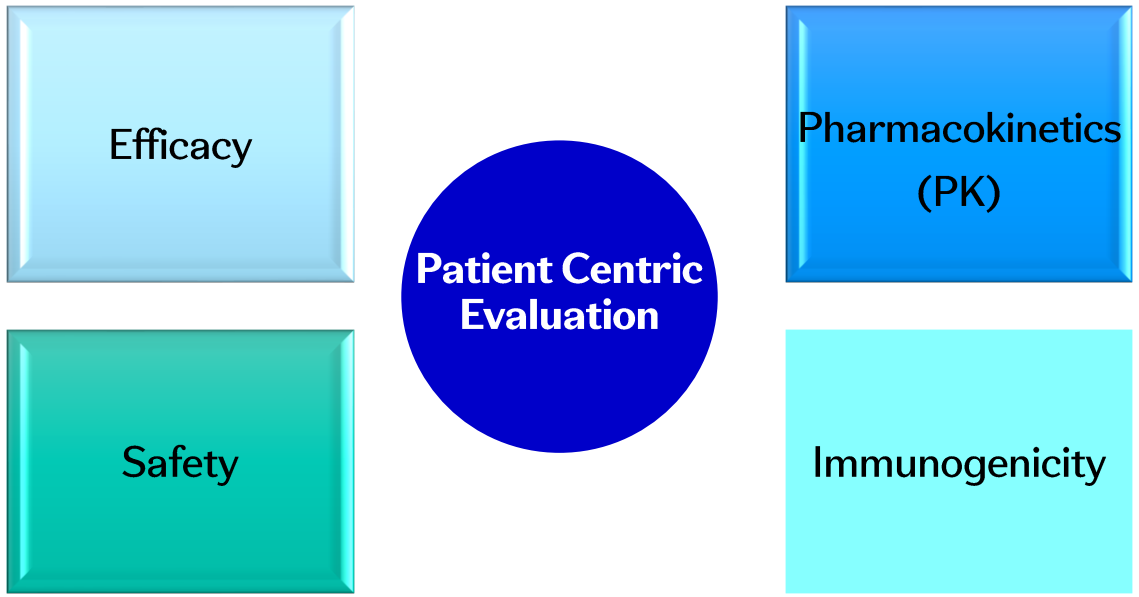
- HH homodimer is not stable, easily convert to HMMS
- Hold at low pH for certain time to convert HH to HMMS
- Filtration and column 2 and 3 are designed to remove HH and HMMS
- KK is in trace level

Analytical Characterization

- Characterize HH and KK in bispecific with non-reducing peptide mapping LC/MS based on molecular weight difference for different disulfide linkages in HK, HH and KK
- KK is in trace level during manufacturing
- HH is in various level in the manufacturing process and trace level in the final DS
- HH is not stable; if present, it will convert to HMMS
- HIC method is further developed to quantitate HH

Products	Disulfide Linkages
HK	Hole: -----Y----C------K Knob: -----C----S-----K
HH	Hole: -----Y----C------K Hole: -----Y----C------K
KK	Knob: -----C----S-----K Knob: -----C----S-----K

CQA Assessment



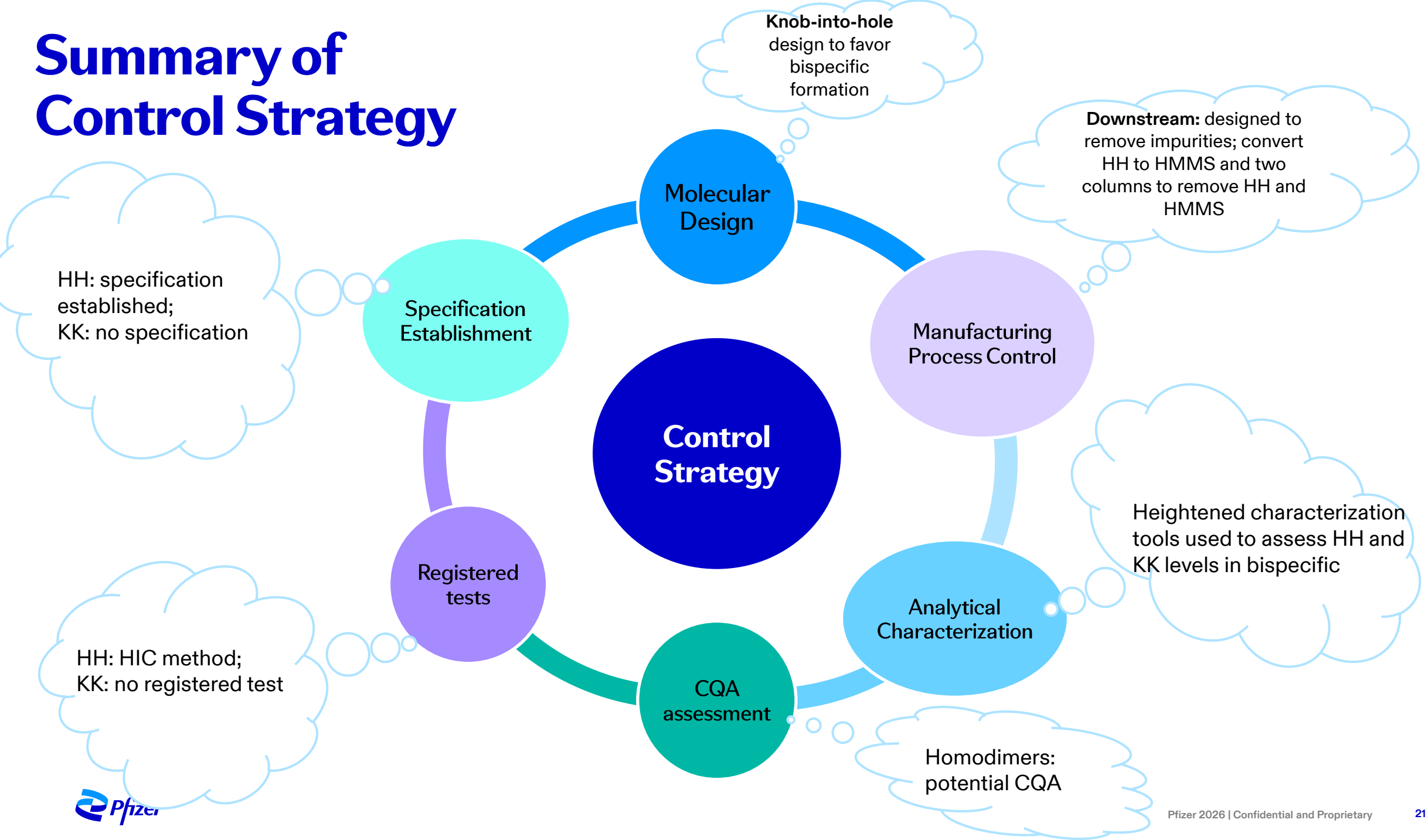
HH and KK Homodimers: potential CQA

- **Efficacy:** expect to lack the full bispecific mechanistic activity and individual target binding activity
- **Safety and immunogenicity:** low safety concern per molecular design

Registered Tests and Specification

Quality Attributes	Criticality	Registered Tests	Specification	Justifications
HH	Potential CQA	HIC	Control main species and report HH	• Manufacturing capability, downstream purification is robust to remove HH; • Historical lots in the clinical usage; • For ultimate commercial process, may consider process validation for removal vs. routine testing
KK	Potential CQA	N/A	N/A	• Not expected to generate KK in manufacturing; • Only trace level observed • Characterization tool (non-reducing peptide mapping) is used to monitor KK during development

Summary of Control Strategy



Thank You

- **Pfizer**
- •Analytical Research & Development
- •Bioprocess Research & Development
- •Pharmaceutical Research & Development
- •Pfizer Global Supply (Andover, MA; Kalamazoo, MI; Grange Castle, Ireland; Puurs, Belgium)

- **CMC Strategy Forum North America 2026**