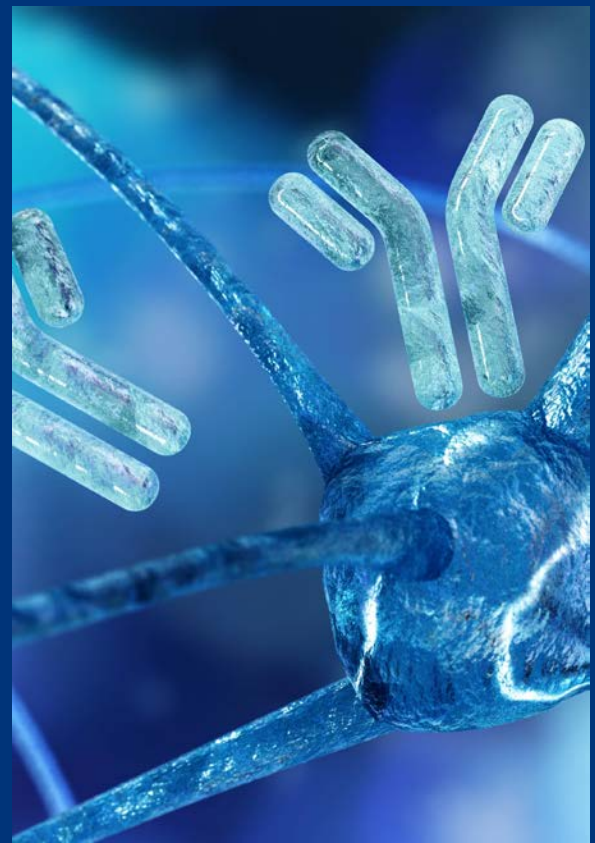
Robust Bispecific Process Development and Commercial Manufacturing Platform

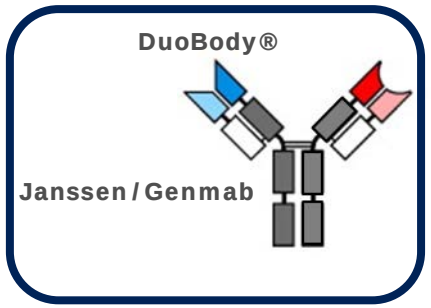
Development and Characterization challenges leading to insights for increased understanding and control of Bispecific Production

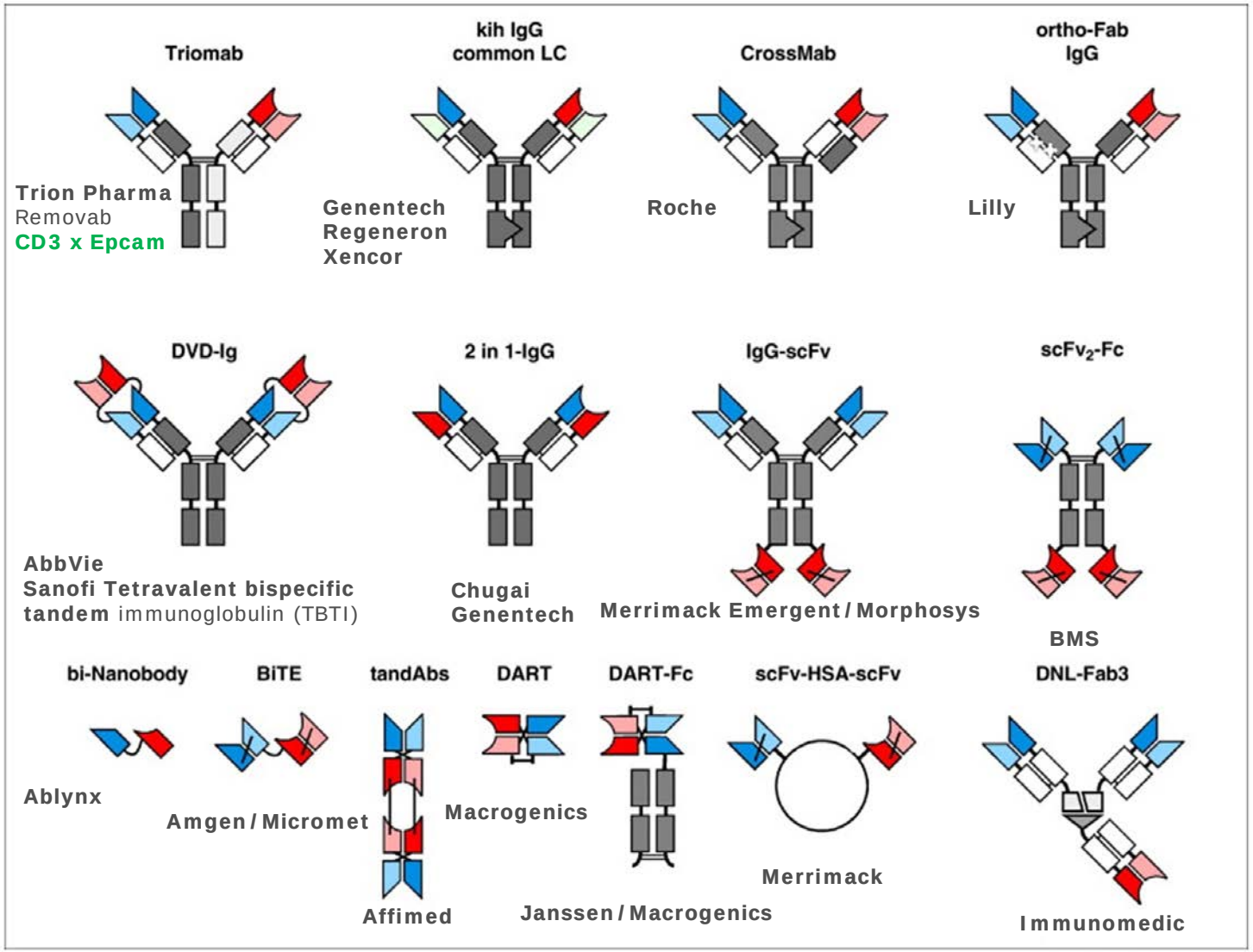


WCBP, CASSS Washington DC
January 28th, 2020

Pedro Alfonso, Ph.D.
Janssen R&D

Bispecific Agents in Clinical Trials

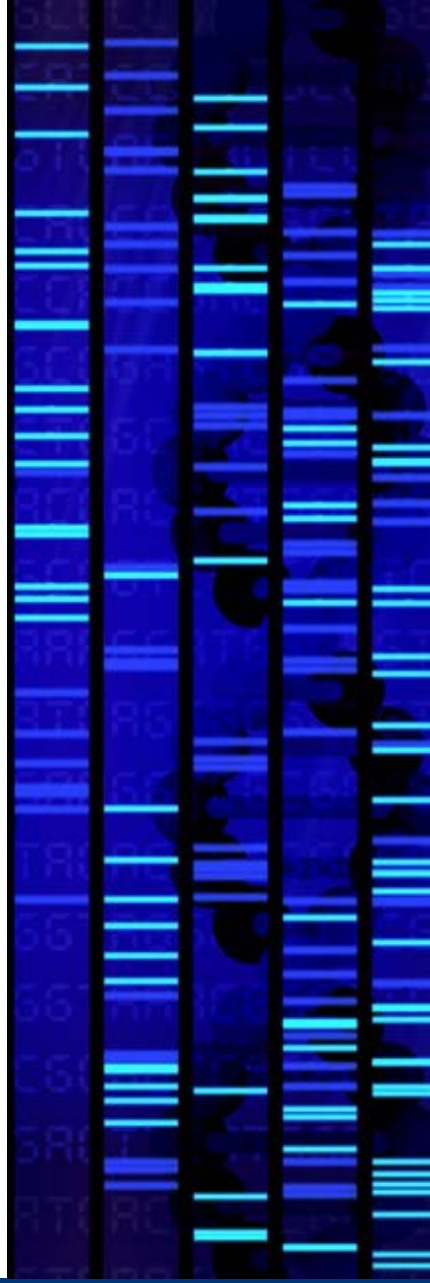




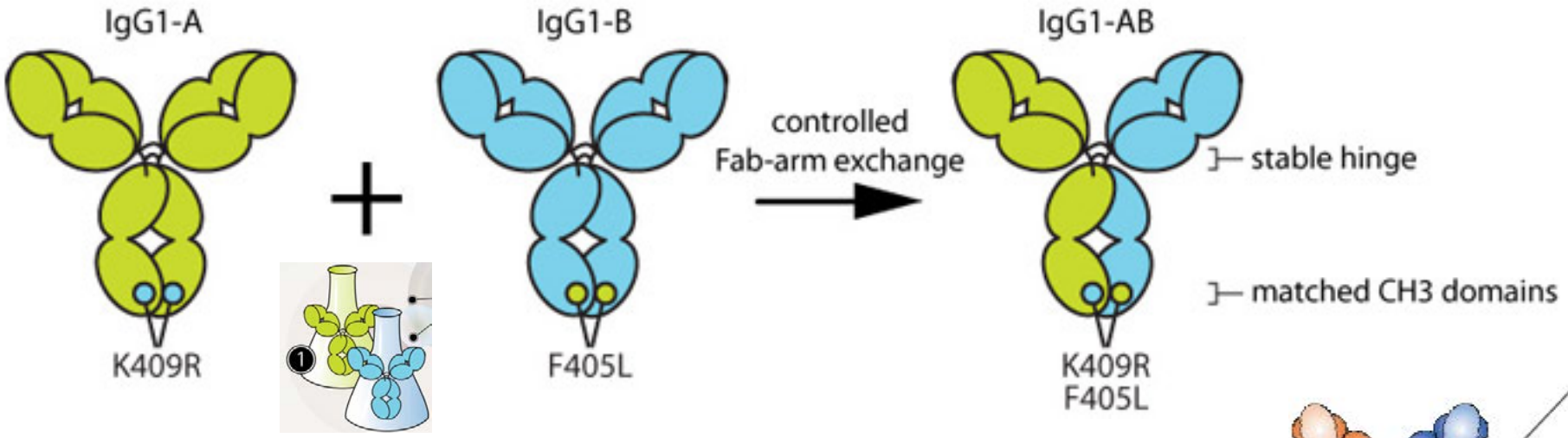
Kontermann and Brinkmann, Bispecific antibodies, Drug Discovery Today, 2015
https://www.sciencedirect.com/science/article/pii/S135964461500077X?via%3Dihub

Outline

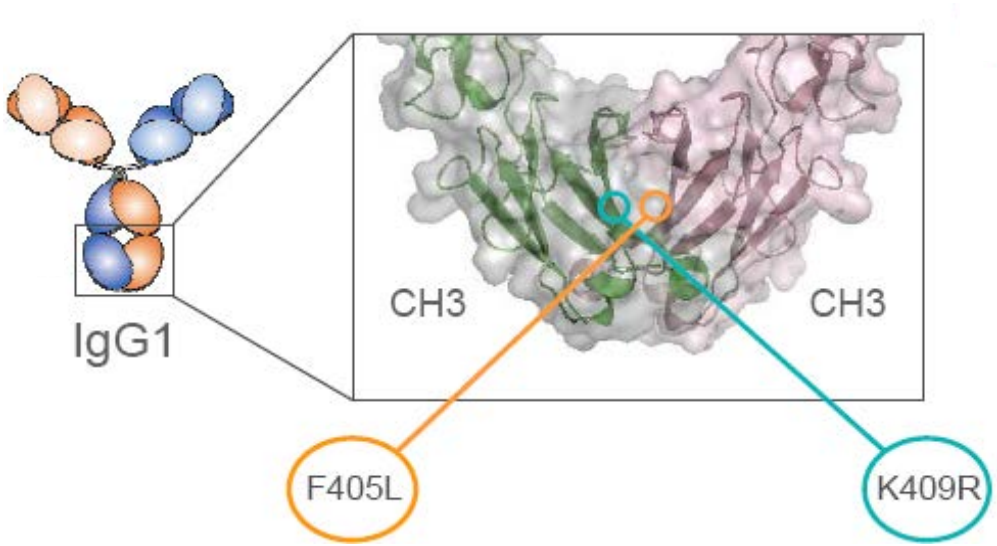
- DuoBody® platform and cFAE overview
- Kinetic Studies and ΔG
- Mechanism & Manufacturing Insight
- Characterization and Structure Function
- cFAE Model development



Overview Duobody® BsAb Technology



One bioreactor
per parental mAb

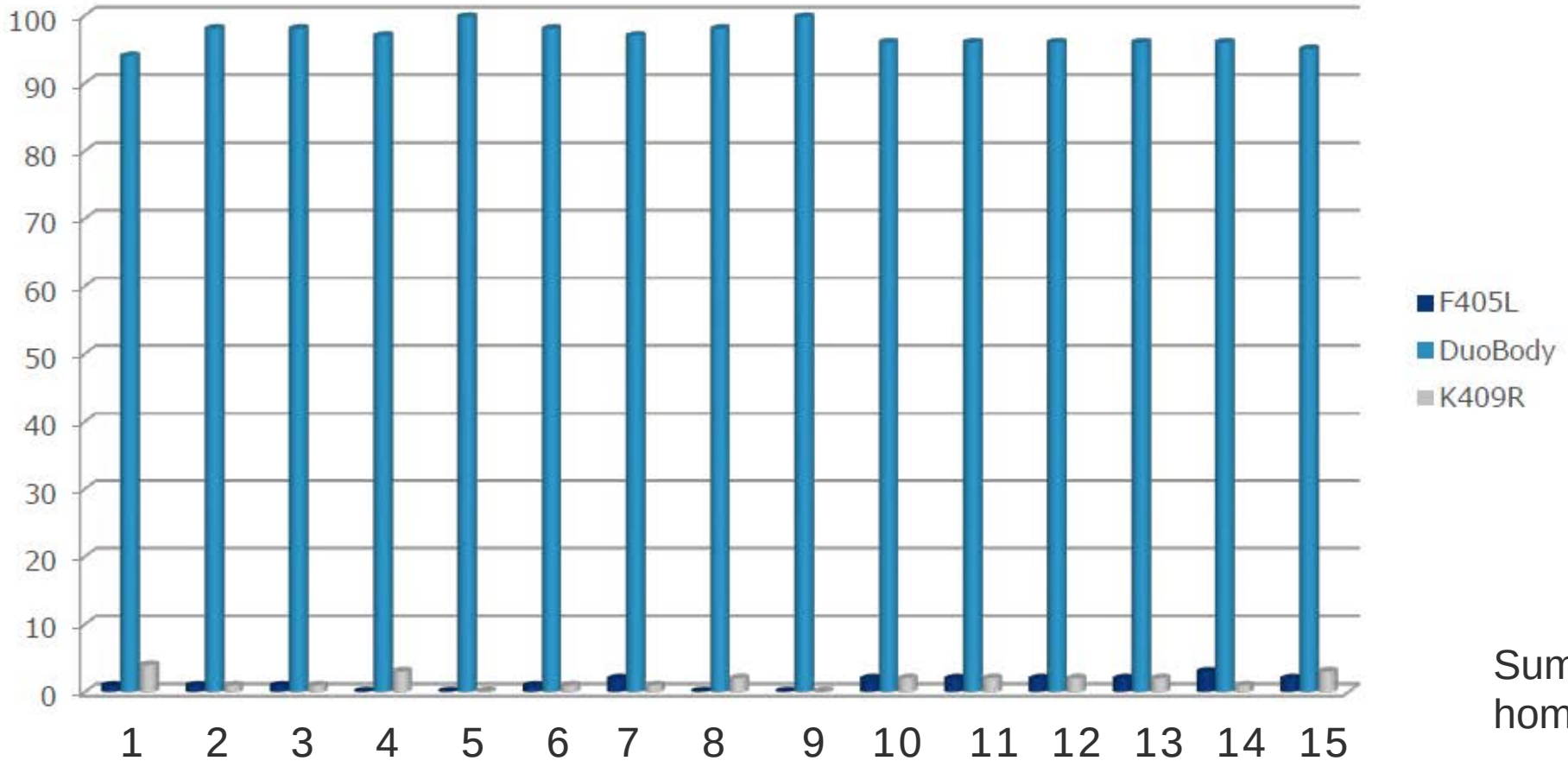


- Bispecific Ab is made by exchange of parental mAbs that contain complementary CH3 mutations
- K409R and F405L are destabilizing mutations in the CH3 interface
- The complementary mutations favor heterodimerization

Labrijn et al, PNAS 2013;110(13):5145-5150.
Gramer et al. mAbs 2013;5(6): 962–973.
Labrijn et al. Nature Protocols 2014;9(10):2450-63.

DuoBody® formation through cFAE is a robust process

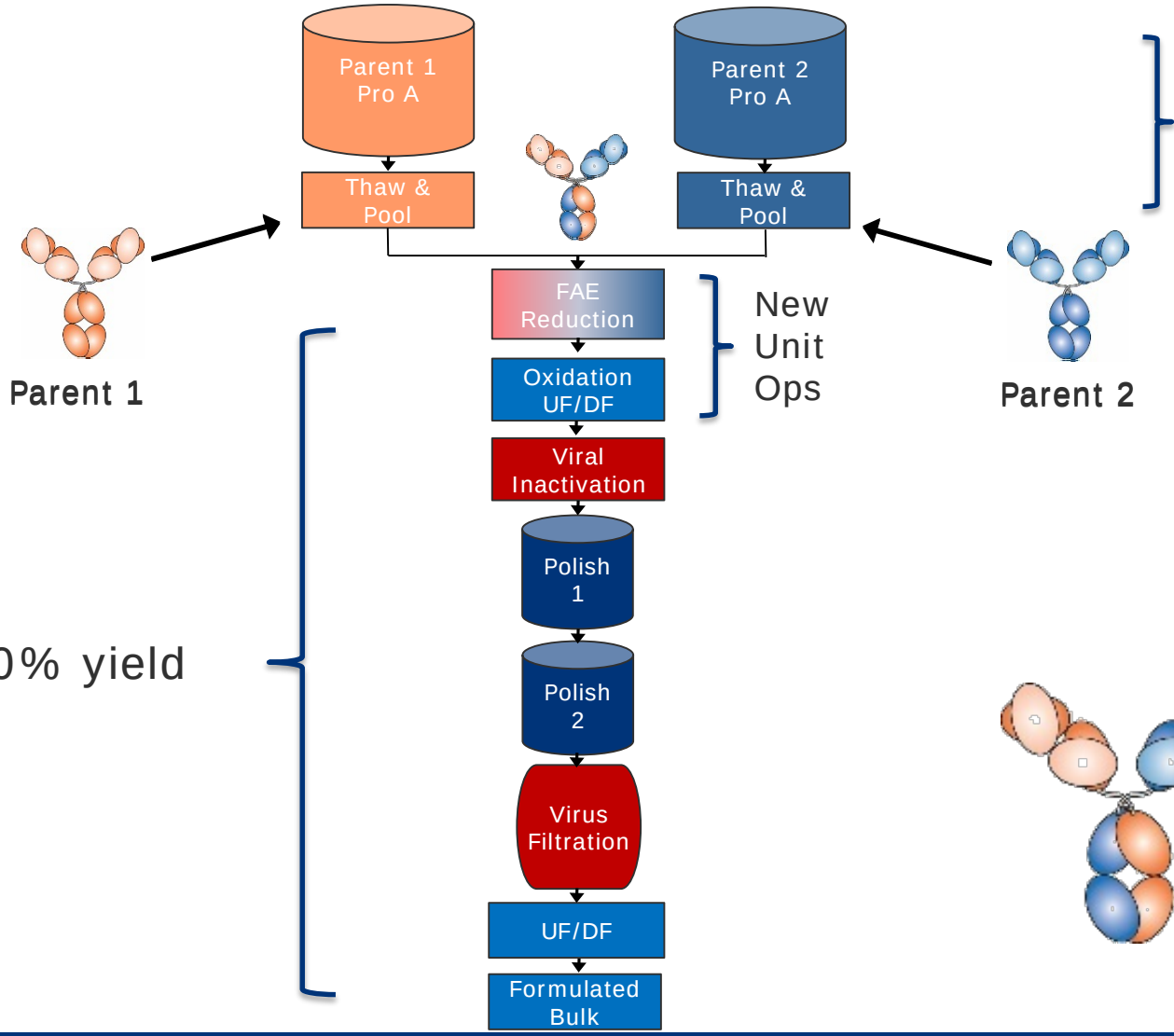
- BsAb formation is typically greater than > 90%



Sum of Parental homodimers ~ 5%

- Parental mAbs are generated separately followed by downstream *in vitro* Fab arm exchange

Bispecific Process leverages Janssen mAb platform



mAb

- ✓ 1 USP
- ✓ Continuous or Freeze/Thaw
- ✓ Virus Inactivation/Removal
- ✓ No cFAE
- ✓ Similar DSP train (no cFAE)
- ✓ One set of PTMs and CQAs

BsAb

- ✓ 2 USP
- ✓ Freeze/Thaw
- ✓ Virus Inactivation/Removal
- ✓ cFAE
- ✓ Similar DSP train after cFAE
- ✓ Two sets of PTMs and CQAs

BsAb Impurity Clearance is similar to mAb platform

BsAb | mAb

BsAb	mAb 1 %	mAb 2 %	Reducing Agent µM	HCP ng/mg	DNA pg/mg	Total Virus log Clearance
A	< 2.0	<1.0	<2	1	< 2	15.4
B	<1.0	<1.0	<1.5	1	< 2	> 18.3
C	<1.5	<1.5	<2	3	<2	16.7
D	<1.0	<1.8	<2	4	< 2	16.3
E	<1.5	<1.5	<1.5	40	< 2	>15.3
F	<1.8	<2.5	<2	<10	< 2	17.8
G	<1.5	<1.5	<1.5	1	< 2	>21.4

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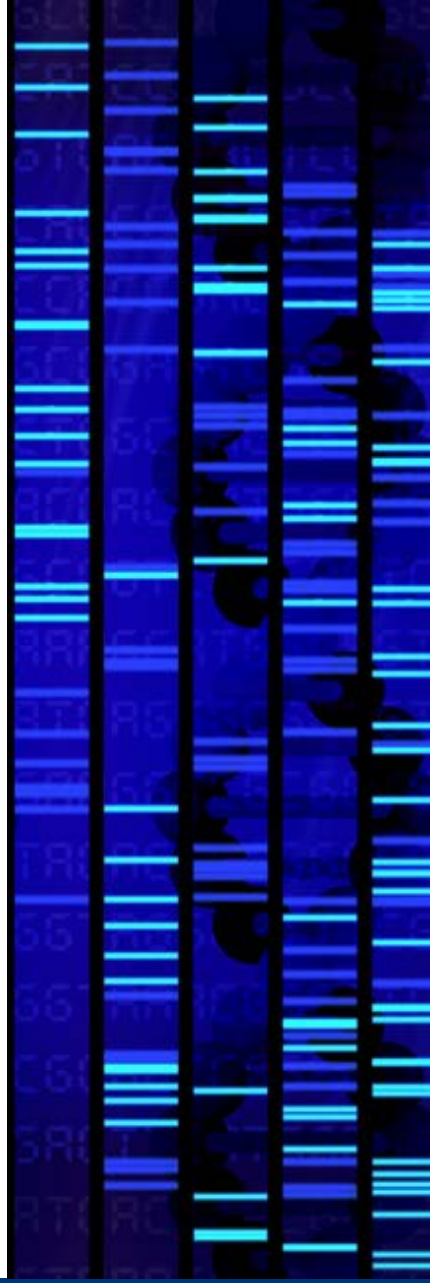
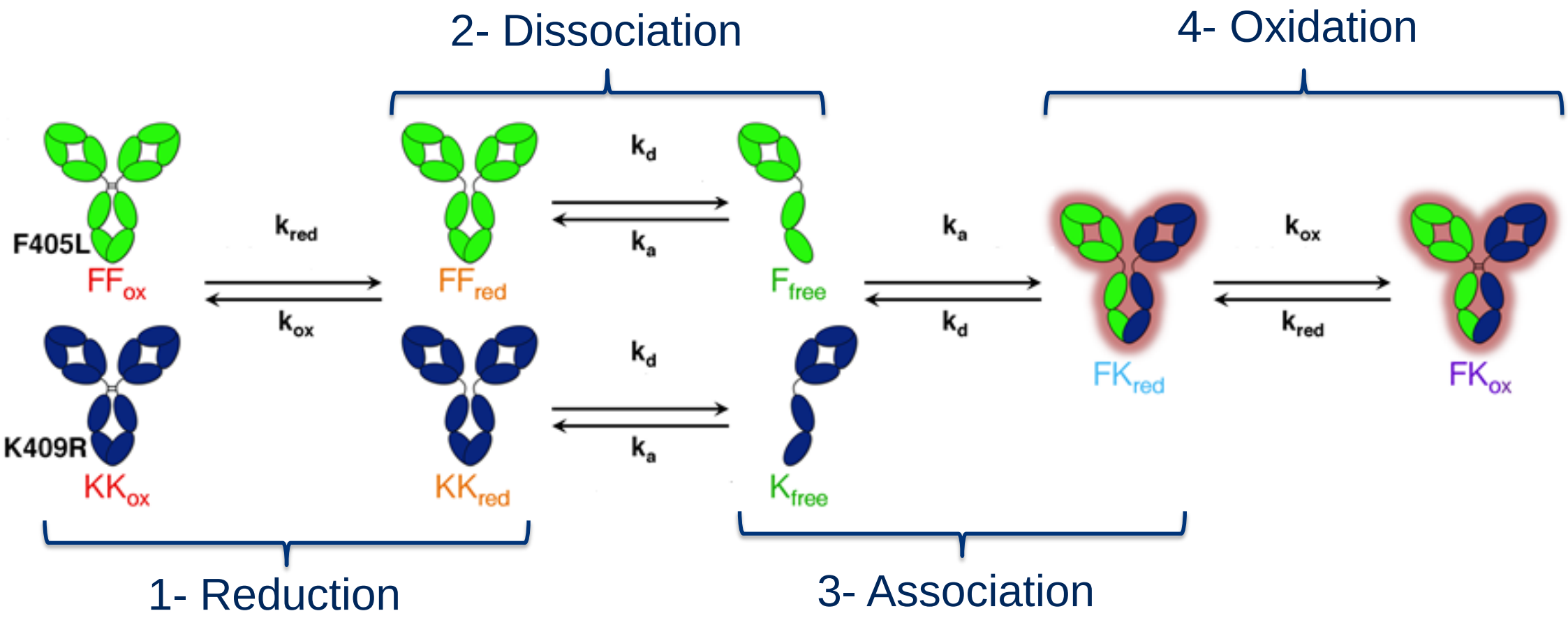
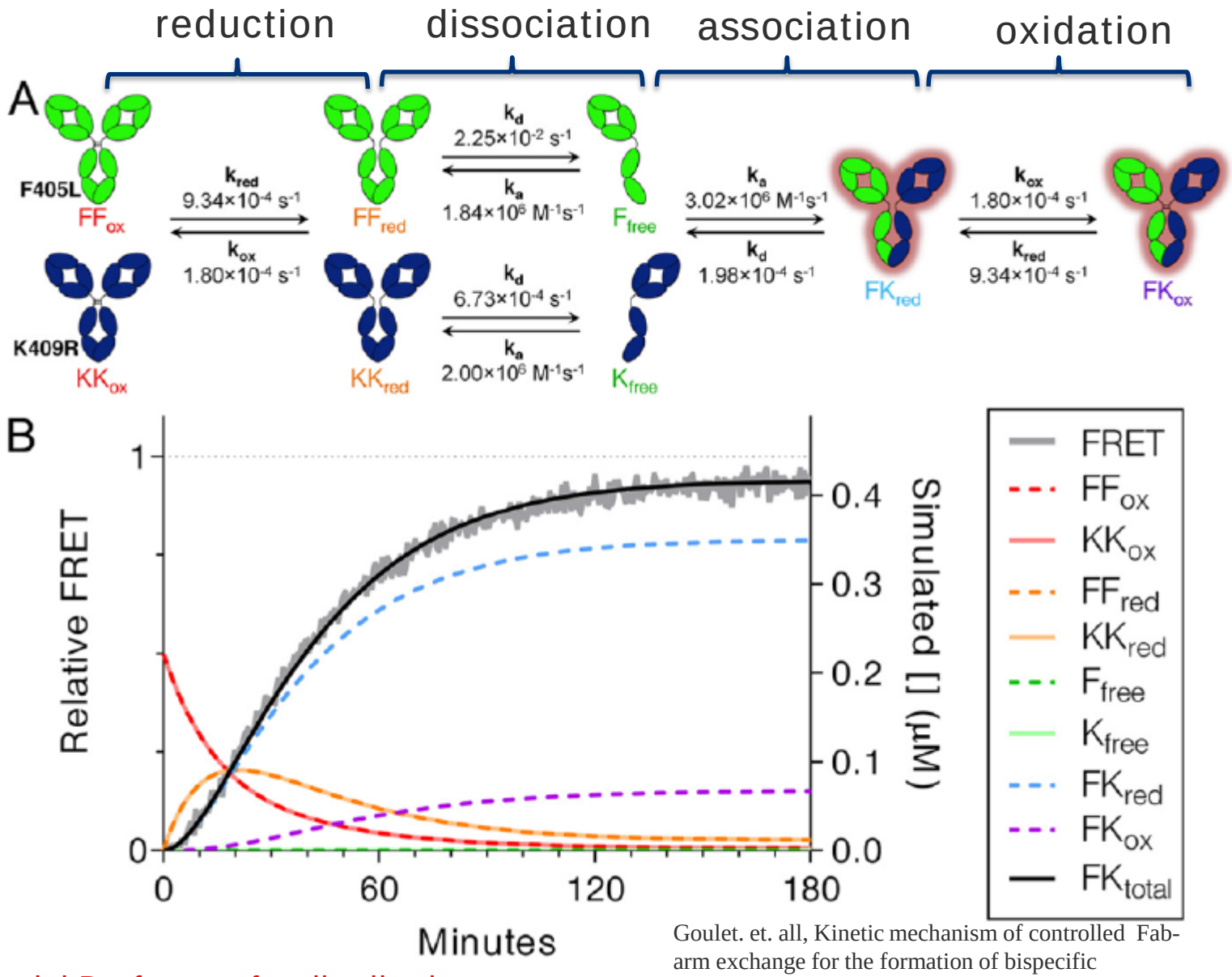


Kinetic mechanism of cFAE: 4 different steps



Goulet. et. all, Kinetic mechanism of controlled Fab-arm exchange for the formation of bispecific immunoglobulin G1 antibodies, JBC, 2018

Complete kinetic description of cFAE



Goulet. et. all, Kinetic mechanism of controlled Fab-arm exchange for the formation of bispecific immunoglobulin G1 antibodies, JBC, 2018

Fluorescence resonance energy **transfer** (**FRET**)*

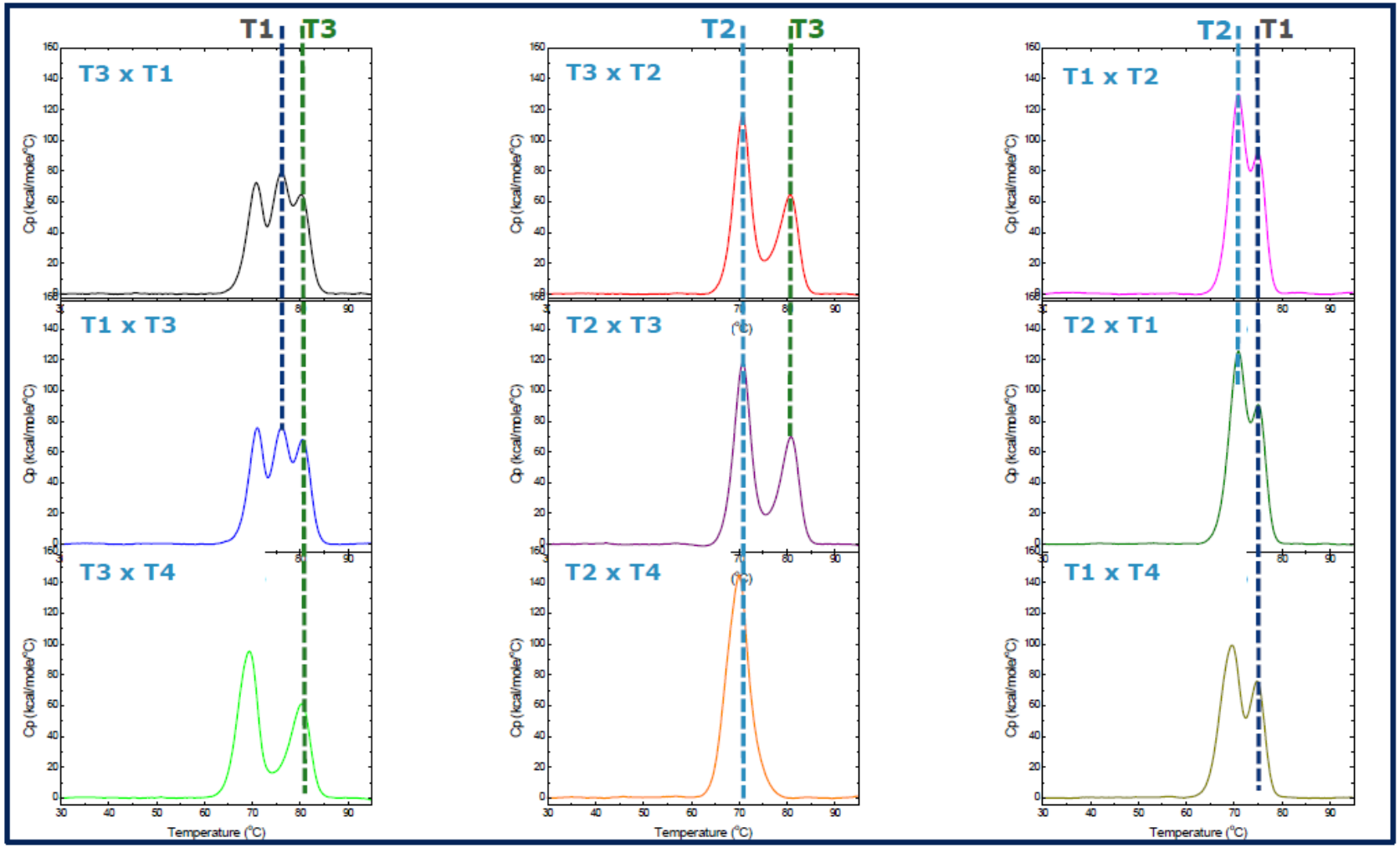
Association of parental mAbs has a negative ΔG value

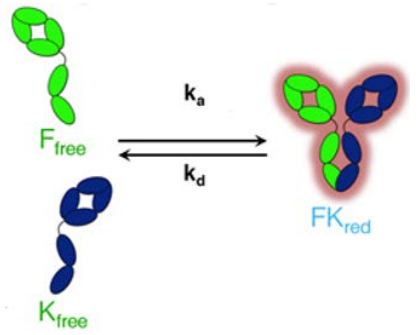
$$\Delta G = - RT \ln \frac{K_{AA}K_{BB}}{K_{AB}^2}$$

ΔG = - 27.7 kJ/mol

H bond equivalent ~5-13 KJ/mol

Tm and Enthalpies of Fabs Arms in BsAbs are conserved



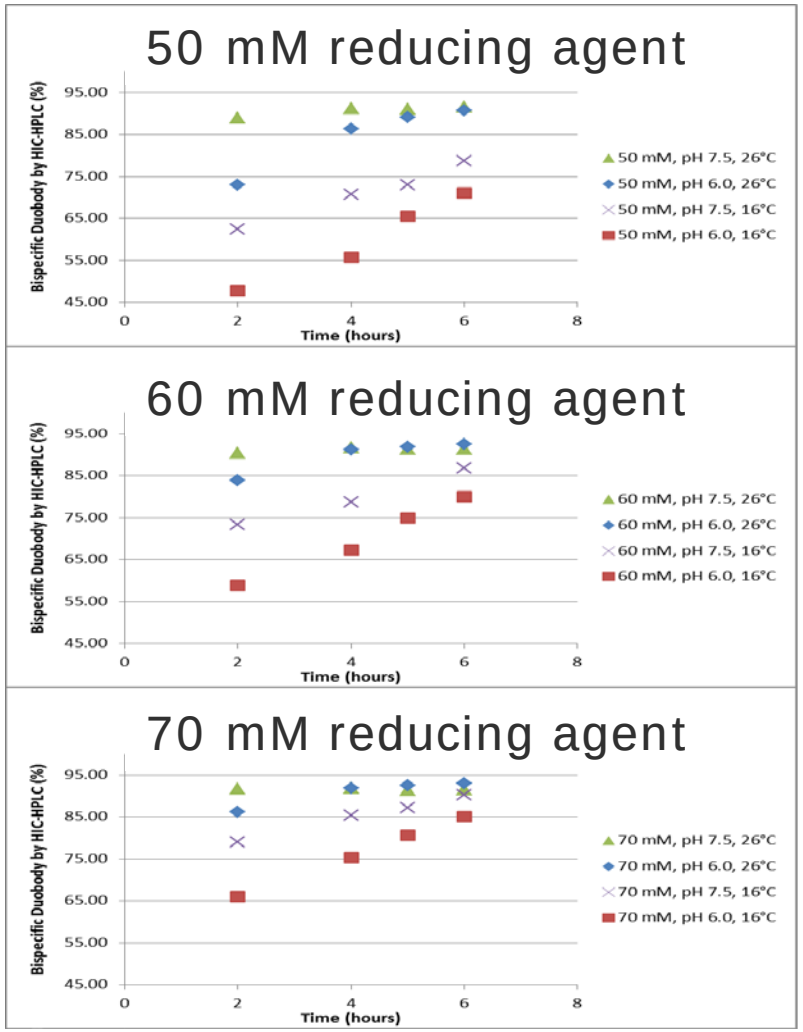


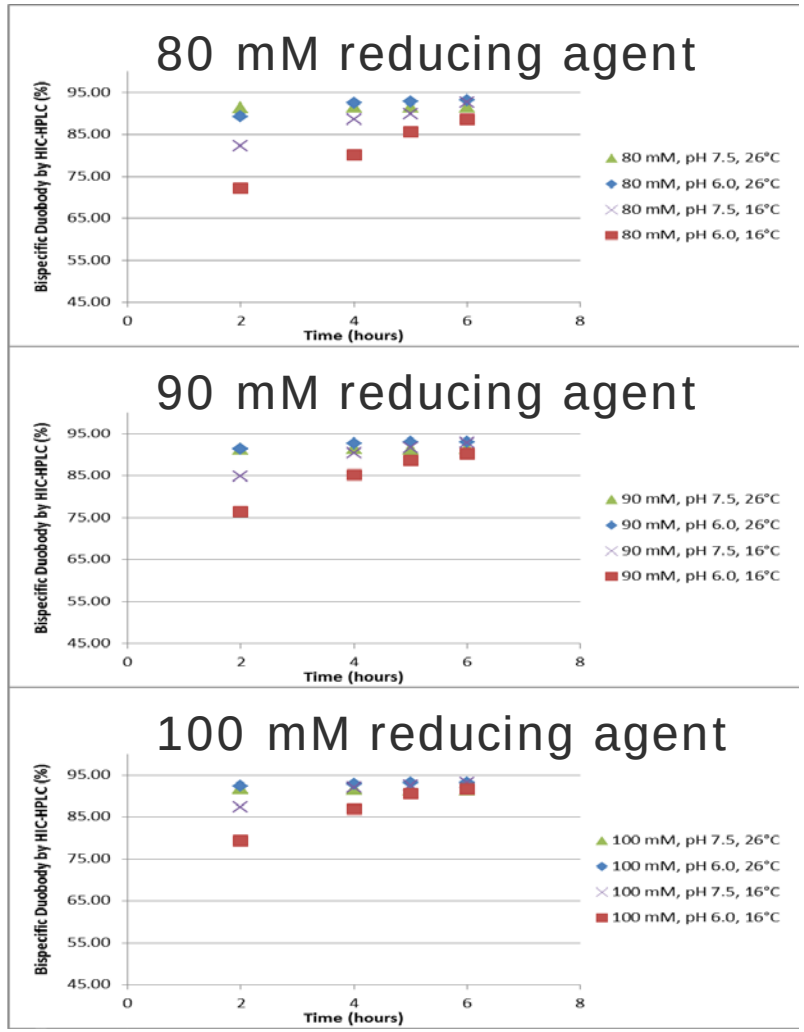
Fab domains maintain their unique folding upon BsAb formation

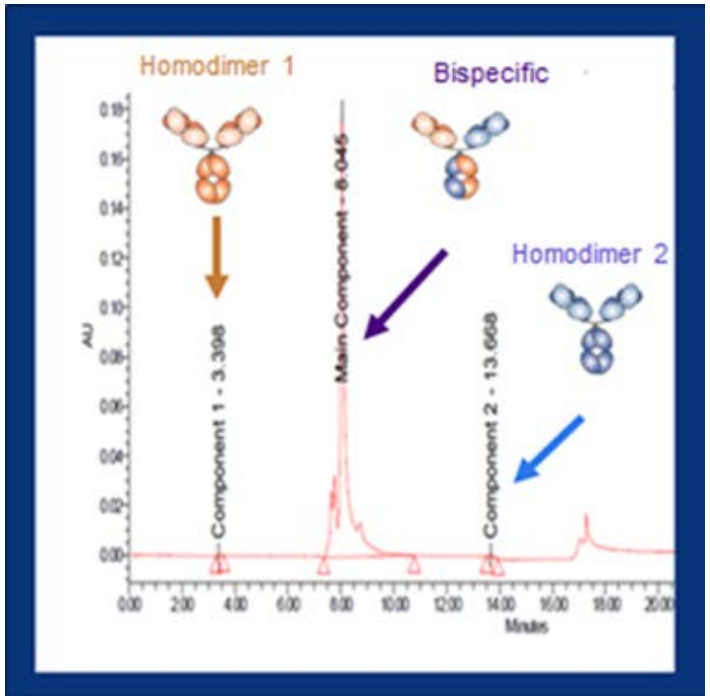
A unique structure is maintained irrespective of bispecific Ab

Mass spec data, peptide mapping, free thiol, bioactivity and other data confirm correct assembly BsAb

Parameters controlling cFAE:
pH, reducing agent concentration, temperature, time





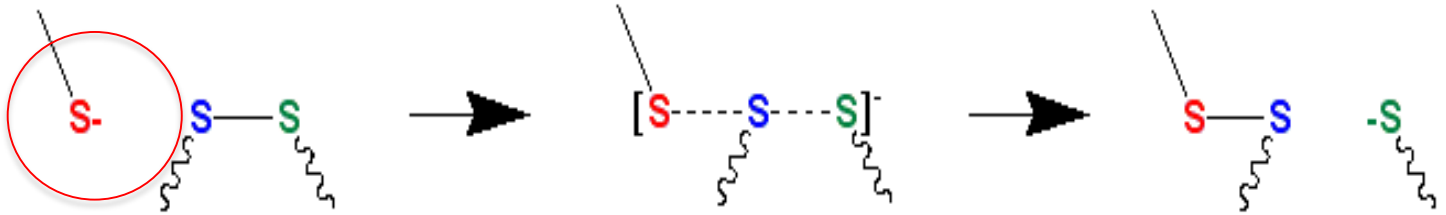


HIC HPLC assay @ T= 5 h

IgG1 BsAb

Reduction step: pH is key in hinge reduction chemistry

Thiolate attacks disulfide bond



$H_2N - R - S^-$ + $LH - S - S - HL$ ⇌ $LH - S^-$ + $H_2N - R - S - S - HL$

Reducing agent + mAb ⇌ Fab + Reducing Agent – Fab

High pH

favorable

unfavorable

Henderson-Hasselbalch Equation

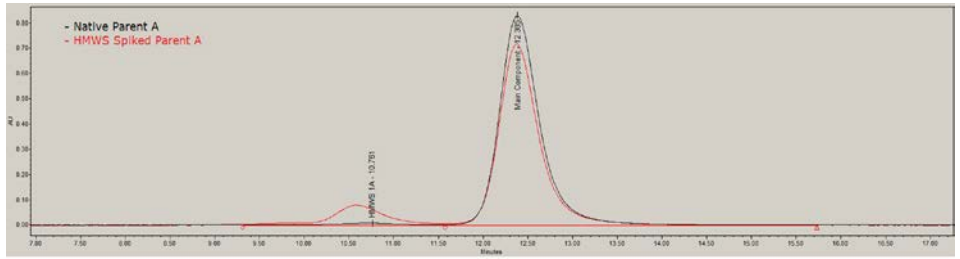
pH	$[S^-]/[SH] = I0^{(pH - pKa)}$
6	0.02
7	0.2
7.4	0.5
8	2.0

$H_2N - R - S^-$ + $H_2N - R - S^-$ ⇌ $H_2N - R - S - S - R - H2N$

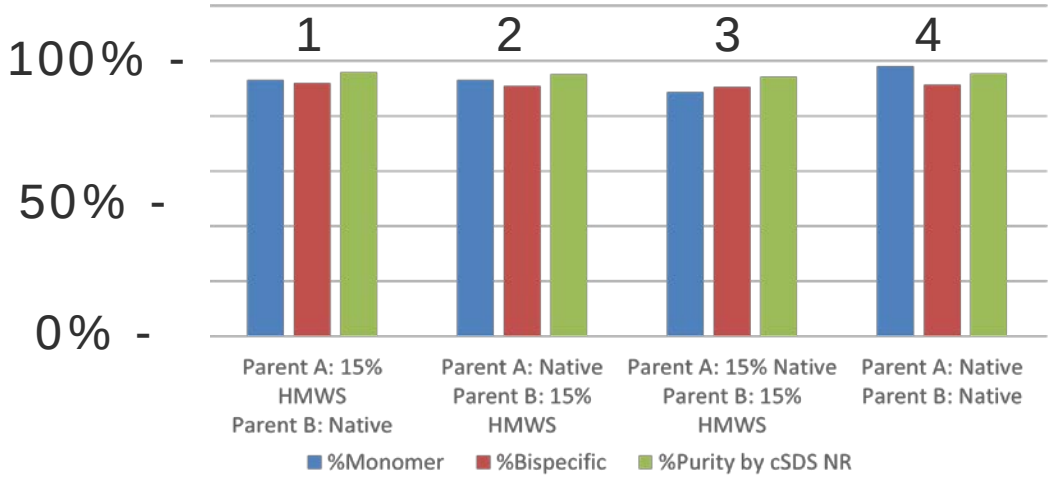
Reducing agent + Reducing agent ⇌ Dimerized Reducing Agent

Creighton, T.E., *Disulfide bond formation in proteins*, in *Methods in Enzymology*. 1984, Academic Press. p. 305-329.

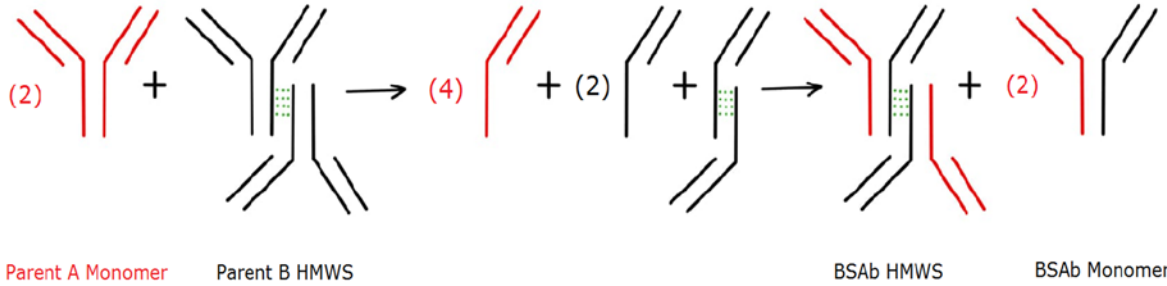
cFAE is a robust process – Minimal impact of HMWS (Dimer) on BsAb formation



Run #	Parent A HMWS	Parent B HMWS
1	15%	Native (<5%)*
2	Native (<5%)*	15%
3	15%	15%
4	Native (<5%)*	Native (<5%)*



Sample	Expected Dimer (Da)	Measured Dimer (Da) DOE Run 3
Parent A	290,936	ND
Parent B	294,112	ND
Bispecific	292,524	292,555



Minimal impact of HMWS (Dimer) on BsAb formation

- Parental HMWS entering the cFAE reaction were not observed in the product of the cFAE
- Parental mAbs containing HMWS entering the cFAE reaction resulted in the formation of both bispecific monomer and bispecific dimer, suggesting parental dimer can reduce and later recombine as bispecific dimer without dissociating into monomer
- The cFAE is a robust process that has minimal impact from the presence of HMWS on the formation of intact formation of bispecific

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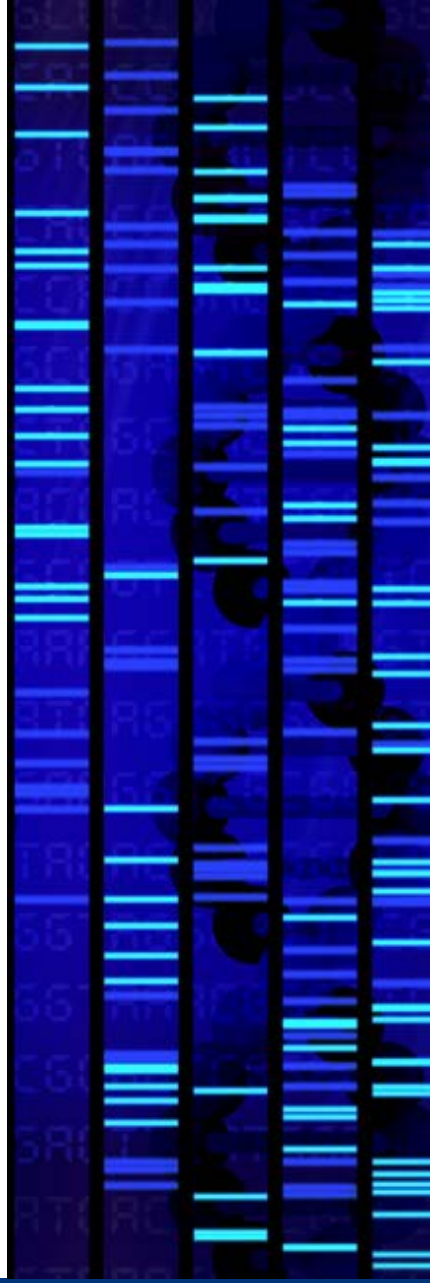
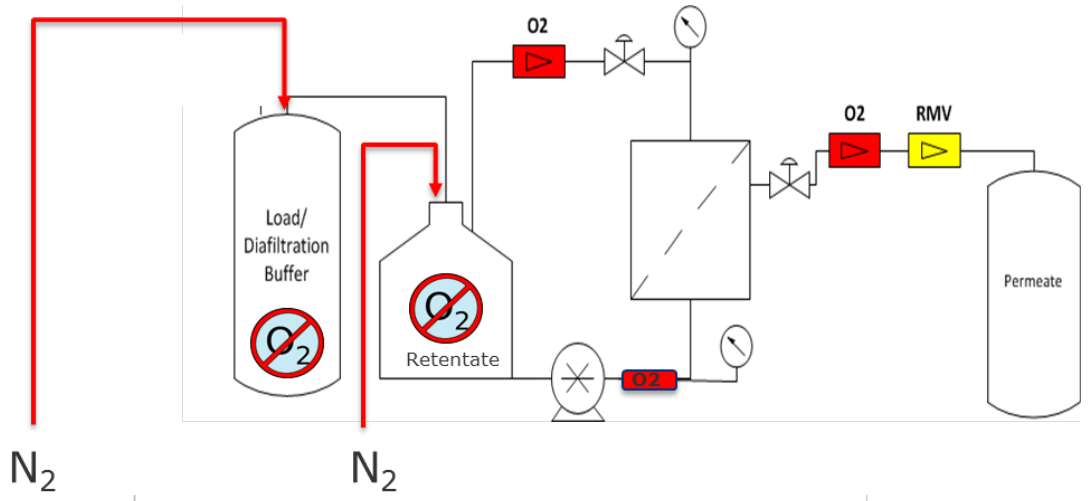
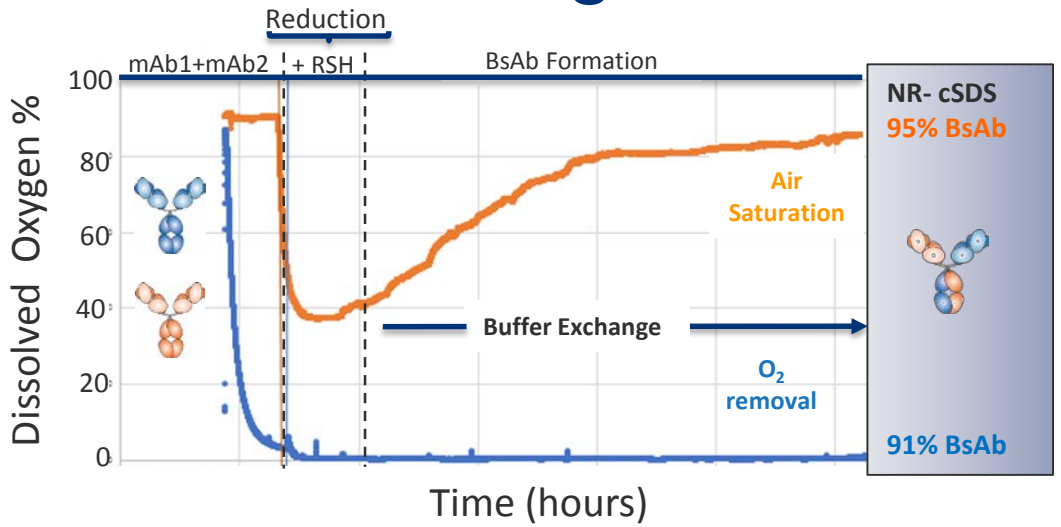
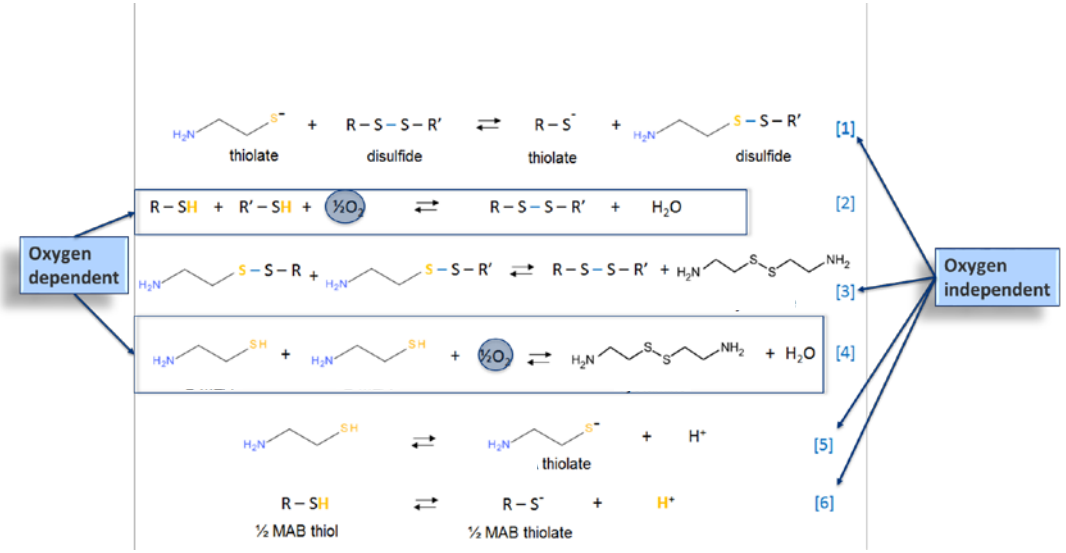


Insights in cFAE mechanism to enable control: Oxygen is not limiting at manufacturing scale





Air saturation or depleted oxygen during cFAE yields > 90% NR-cSDS BsAb



Oxygen is not limiting at scale = More options

- Multiple pathways to achieve disulfide formation of a DuoBody® BsAb
- Oxygen and free metals are not critical for DuoBody® bispecific Ab formation
- Robust manufacturing with wider bispecific design space.
- Wider design space increases the ability to fit different manufacturing plant configurations providing flexibility to the Janssen Supply Chain (JSC) enhancing our ability to deliver new drugs to patients

Bezila, Sobkow, Rao, Cohen
Cressman, Li, Capaldi

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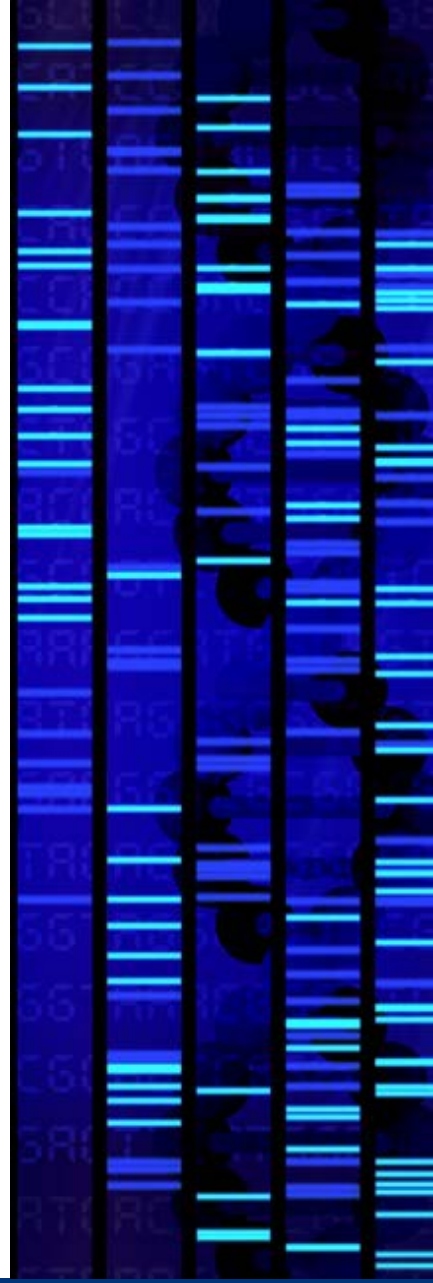


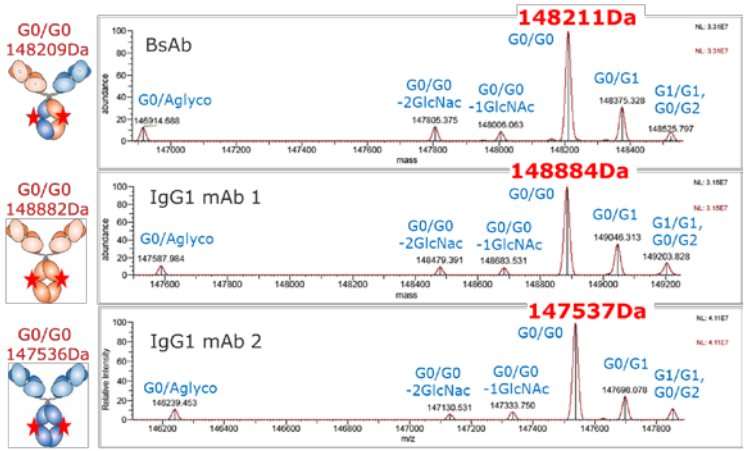
Key Challenges for DuoBody® Characterization

Bispecfics have similar properties as parental antibodies.

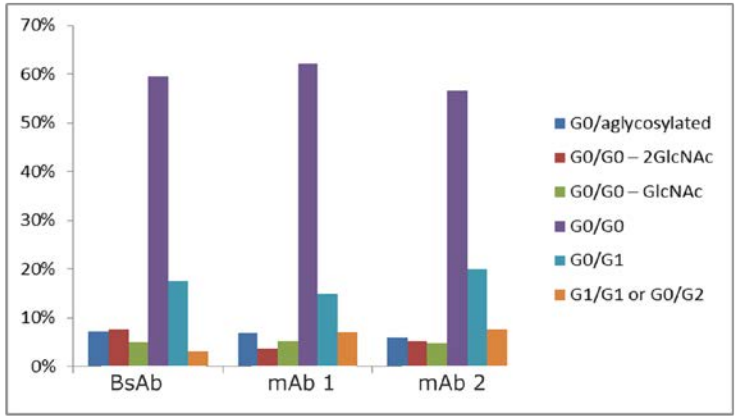
- ❑ Confirm DuoBody identity and purity
  - ➢High resolution MS analysis
- ❑ Confirm DuoBody structural integrity
  - ➢NR peptide map for disulfide linkage analysis
  - ➢Fab and Fc integrity analysis
  - ➢Free thiol analysis
  - ➢Thermal stability analysis

BsAb Identity and Purity by UPLC - High Resolution MS

BsAb UPLC-Intact Mass Analysis

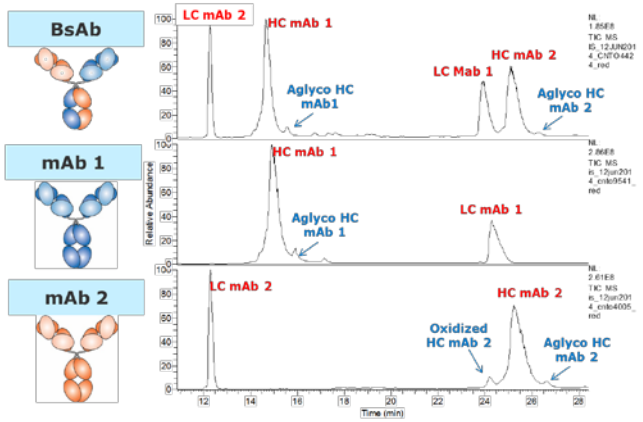


Relative Quantitation of Glycoforms by Intact Mass Analysis



Comparable glycoform profiles were observed among DuoBody and parentals

UPLC-Reduced MS Analysis Verifies Chain Composition



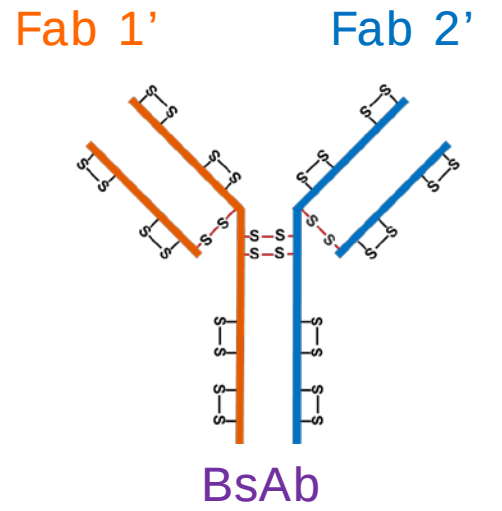
UPLC-Intact Mass Analysis Quantifies Residual Parentals and Other Impurities

BsAb Process Intermediates	LC mAb 2 (23252 Da)	Intact BsAb (148209 Da)	Intact BsAb–LC mAb1 (124971 Da)	Intact mAb 2 (148882 Da)	Intact mAb 1 (147537 Da)	LC mAb 1 (23238 Da)
FB	0.2%	99.5%	ND	ND	ND	0.3%
1xDPBS	0.2%	95.0%	ND	2.6%	1.9%	0.3%
FAE VIN	0.3%	90.8%	ND	4.9%	3.4%	0.5%
FAE	0.4%	92.0%	ND	4.1%	2.8%	0.7%
UF/DF1 DV_6	0.6%	90.8%	ND	4.6%	3.2%	0.8%
UF/DF1 DV_8	0.7%	87.4%	3.1%	4.6%	3.1%	0.9%

- Quickly identifies all impurities in one assay
- Quantifies relative abundance of all impurities by UV & MS

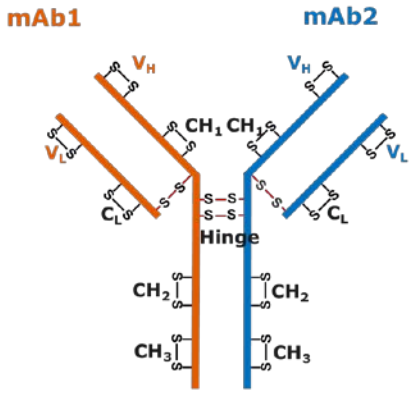
Structural Integrity Characterization

- **Non-reduced Peptide Map** – confirm disulfide bond linkage and detect disulfide scrambling
- **Fab / Fc Mass Analysis** – detect potential LC swapping
- **Free thiol analysis** – measure free thiol content
- **DSC** – evaluate thermal stability



BsAb Structural Integrity Characterization

Theoretical Disulfide Bonds in BsAb



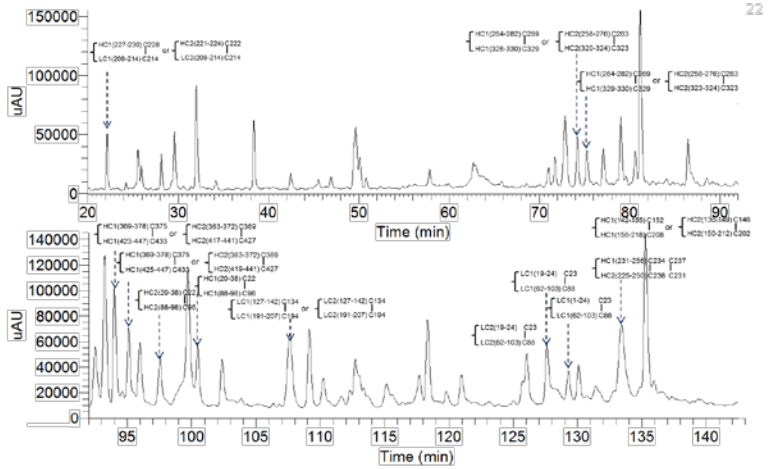
BsAb

- V_HmAb1
- V_LmAb1
- V_HmAb2
- V_LmAb2

- C_L (mAb1 or mAb2)
- LC-HC (mAb1 or mAb2)
- CH_1 (mAb1 or mAb2)
- CH_2 (mAb1 or mAb2)
- CH_3 (mAb1 or mAb2)
- Hinge

10 unique disulfide bonds

NR Tryptic Peptide Map of BsAb

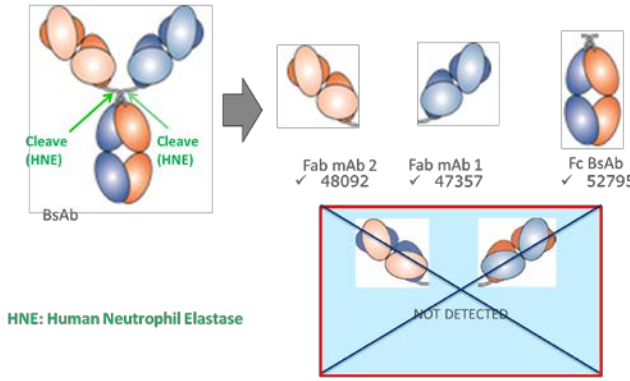


All 10 Disulfide Bonds Were Confirmed

Structural Region	Disulfide Bond	Peptide	Retention Time (min)	Expected Mass (Da)	Observed Mass (Da)	Mass Error (Da)
V_H 4005	HC1(C22)-HC1(C96)	{HC1(20-38)C22 / HC1(88-98)C96}	100.5	3378.49	3378.51	0.02
V_H 9541	HC2(C22)-HC2(C96)	{HC2(20-38)C22 / HC2(88-98)C96}	97.5	3402.48	3402.51	0.03
CH_1	HC1(C152)-HC1(C208) or HC2(C146)-HC2(C202)	{HC1(142-155)C152 / HC1(156-218)C208} or {HC2(136-148)C146 / HC2(150-212)C202}	135.3	7916.92	7916.95	0.03
LC-HC	HC1(C228)-LC1(C214) or HC2(C222)-LC2(C214)	{HC1(227-230)C228 / LC1(208-214)C214} or {HC2(221-224)C222 / LC2(208-214)C214}	22.1	1260.49	1260.49	0.00
Hinge	HC1(C234,C237)-HC1(C228,C231)	{HC1(231-256)C234 C237 / HC1(225-250)C228 C231}	133.4	5454.78	5454.84	0.06
CH_2	HC1(C269)-HC1(C329) or HC2(C263)-HC2(C323)	{HC1(264-282)C269 / HC1(329-330)C329} or {HC2(258-276)C263 / HC2(323-324)C323}	75.3	2328.10	2328.12	0.02
		{HC1(264-282)C269 / HC1(326-330)C329} or {HC2(258-276)C263 / HC2(320-324)C323}	74.2	2748.30	2748.33	0.03
CH_3	HC1(C375)-HC1(C433) or HC2(C369)-HC2(C427)	{HC1(369-378)C375 / HC1(425-447)C433} or {HC2(363-372)C369 / HC2(419-441)C427}	95.1	3844.82	3844.86	0.04
		{HC1(369-378)C375 / HC1(423-447)C433} or {HC2(363-372)C369 / HC2(417-441)C427}	94.0	4087.96	4087.98	0.08
V_L 4005	LC1(C23)-LC1(C88)	{LC1(19-24)C23 / LC1(62-103)C88}	127.6	5280.42	5280.48	0.06
		{LC1(1-24)C23 / LC1(62-103)C88}	129.3	7078.34	7078.40	0.06
V_L 9541	LC2(C23)-LC2(C88)	{LC2(19-24)C23 / LC2(62-103)C88}	126.1	5215.42	5215.48	0.06
		{LC2(1-24)C23 / LC2(62-103)C88}	125.7	7061.27	7061.35	0.08
C_L	LC1(C134)-LC1(C194) or LC2(C134)-LC2(C194)	{LC1(127-142)C134 / LC1(191-207)C194} or {LC2(127-142)C134 / LC2(191-207)C194}	107.6	3555.75	3555.78	0.03

- High mass accuracy MS and MS/MS give unambiguous peak assignments

Mass Analysis of Fab, Fc shows no evidence of LC Swapping



HNE: Human Neutrophil Elastase

Free Thiol Analysis of BsAb and IgG1 Parentals

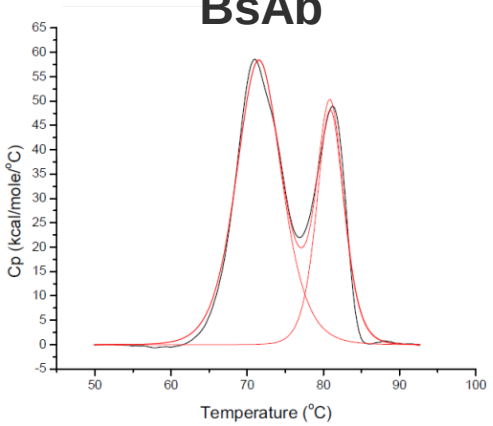
Sample	Mean Free SH/Protein (mol/mol)	SH%*
BsAb, Lot M1D03-14	0.26	0.8%
mAb 2, Lot M14D012	0.13	0.4%
mAb 1, Lot M1D23-14	0.19	0.6%

* SH% = mean free SH per protein / 32 * 100%

- Observed free thiol values for BsAb and IgG1 parentals mAb1 and mAb2 were typical for Janssen IgG1 products.

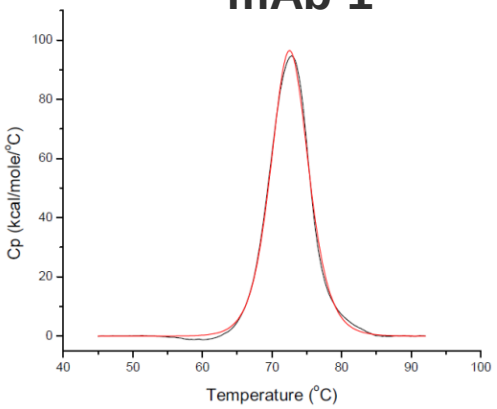
DSC Analysis of BsAb and Parentals in Formulation Buffer

BsAb



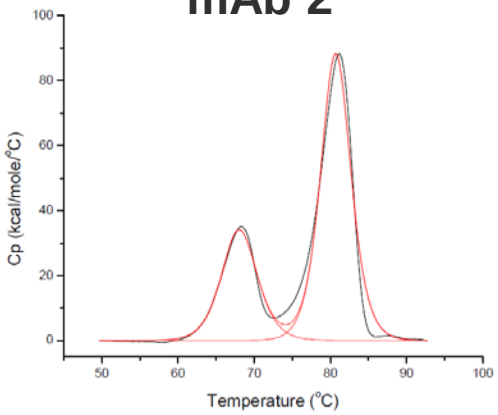
T_m1 (°C)	71.6
ΔH	4.6E+5
T_m2 (°C)	81.0
ΔH	2.2E+5

mAb 1



T_m1 (°C)	72.6
ΔH	7.0E+5

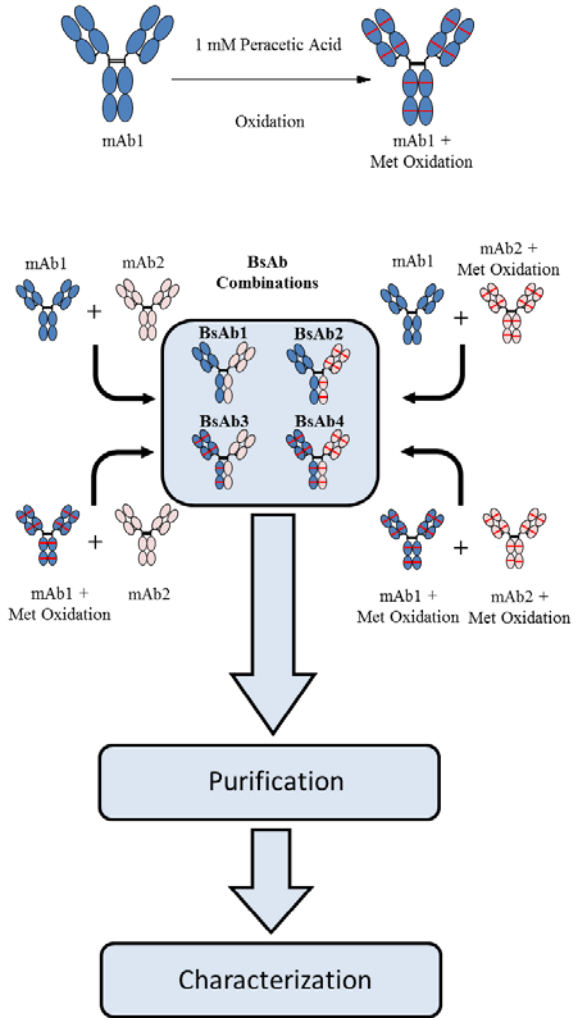
mAb 2

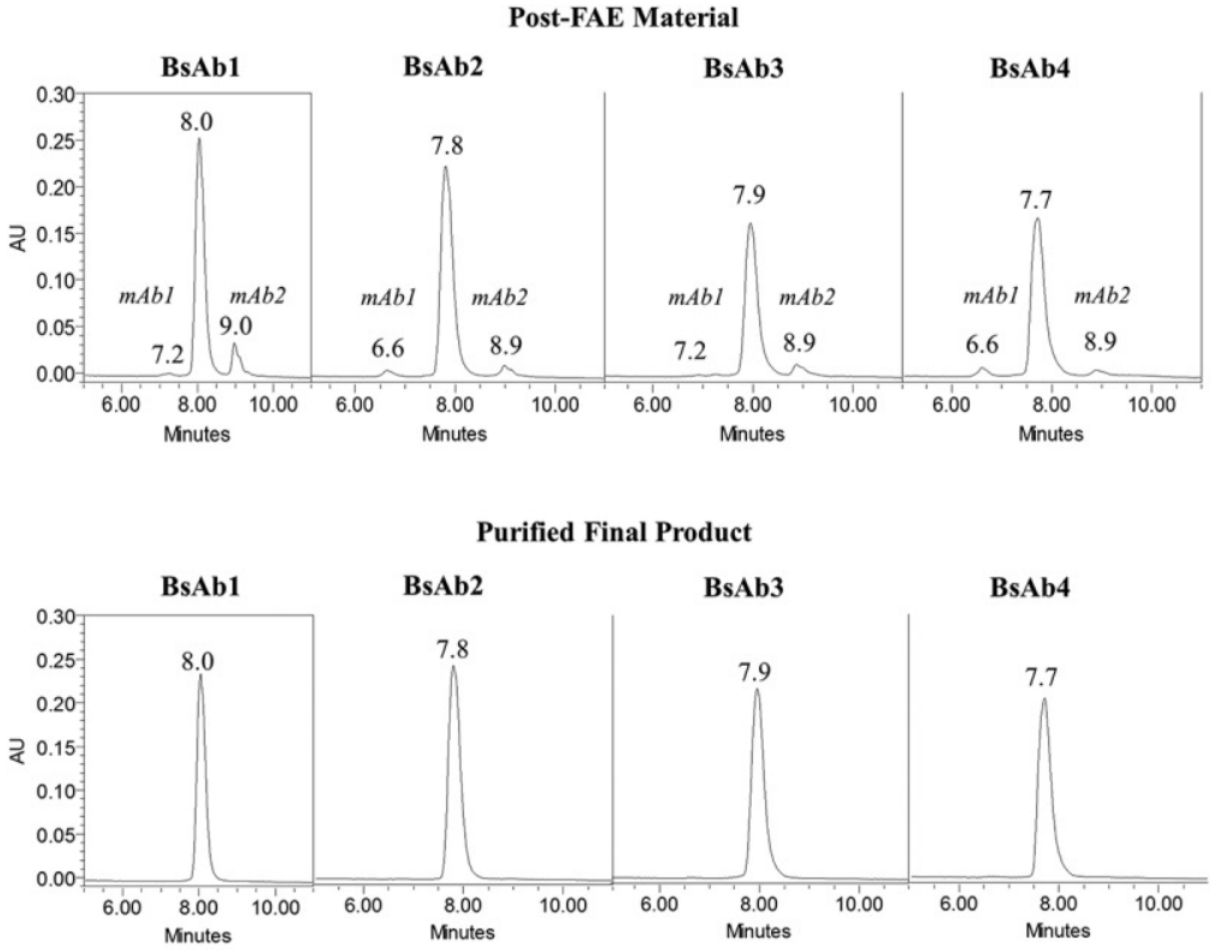


T_m1 (°C)	67.9
ΔH	2.1E+5
T_m2 (°C)	80.7
ΔH	4.4E+5

- DuoBody inherited T_ms from both parentals.

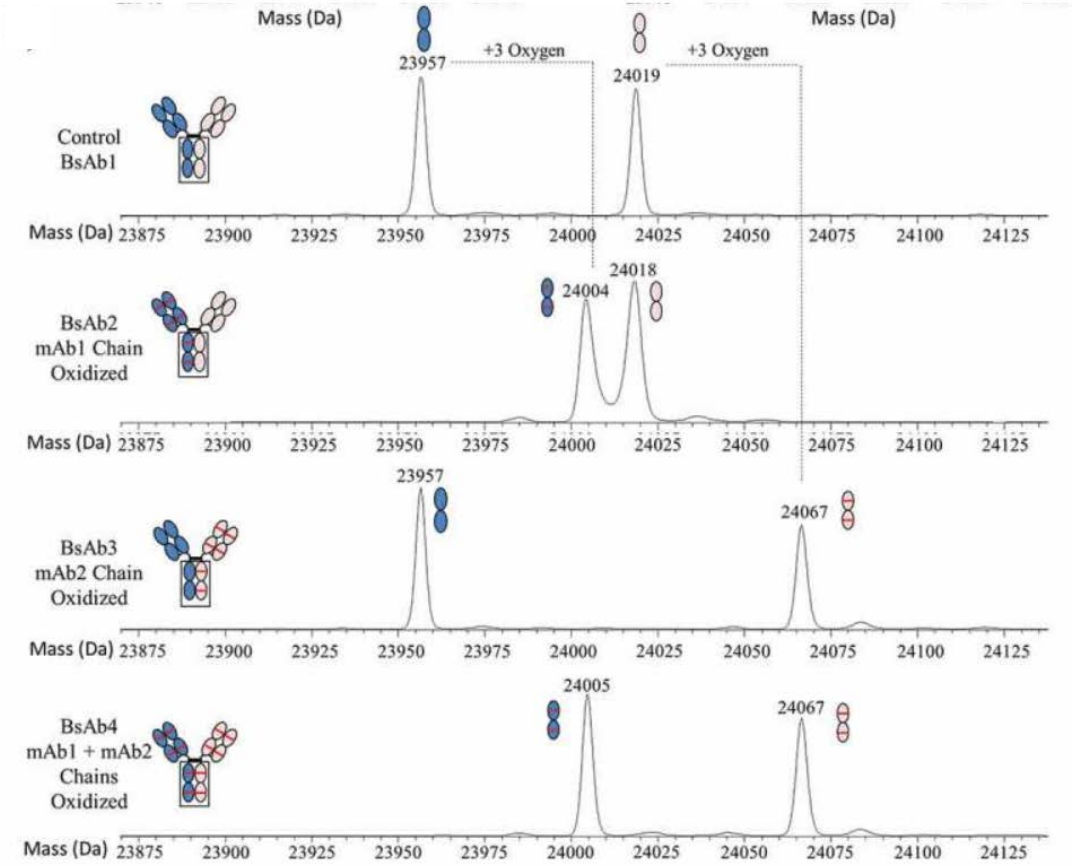
Structure–function of symmetrically and asymmetrically modified BsAb

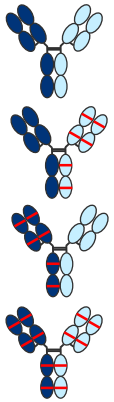




Robust assembly of BsAb in presence of Oxidized Fab Arms

Structure–function of symmetrically and asymmetrically modified BsAb





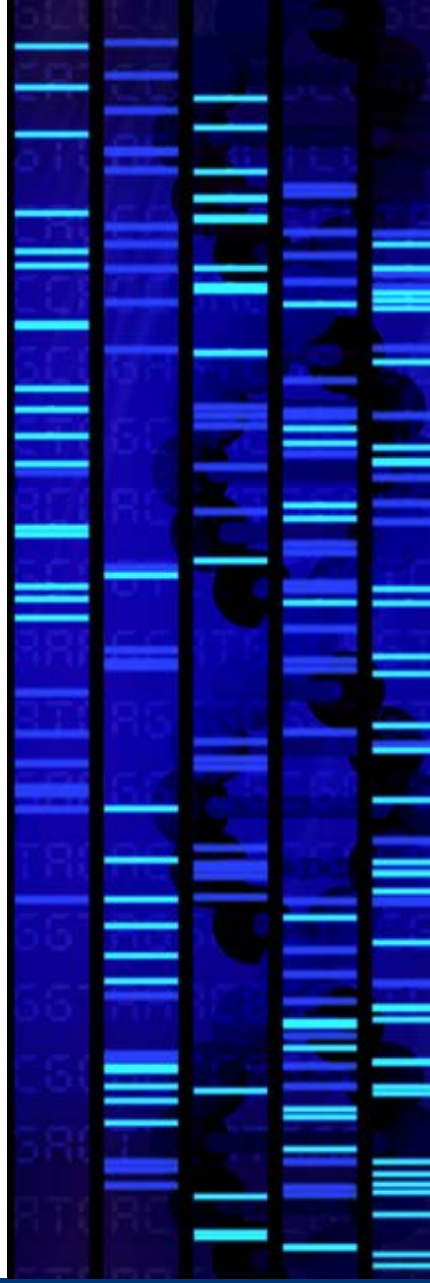
Duobody Combination			M254 Oxidation (Peptide Map)			Fc Binding Assay*			
Description	**mAb1**	**mAb2**	**mAb1**	**mAb2**	**Final Product**	**FcRn**	**FcγRI**	**FcγRII**	**FcγRIII**
BsAb1	Native	Native	3.8%	3.0%	5.7%	100%	100%	100%	100%
BsAb2	Native	Oxidized	3.8%	99.9%	56.4%	44.4%	97.6%	94.3%	102.1%
BsAb3	Oxidized	Native	99.9%	3.0%	57.6%	54.3%	120.2%	89.7%	106.4%
BsAb4	Oxidized	Oxidized	99.9%	99.9%	99.4%	8.6%	128.6%	112.6%	125.5%

**Binding is normalized relative to control*

- Asymmetrically oxidized BsAb 2 & 3 bind ~ 50% of the FcRn vs the control BsAb1
- Experimental support for 2:1 FcRn:IgG binding ratio
- FcRn can bind independently to either chain.

Outline

- DuoBody® platform and cFAE overview
- Kinetic Studies and ΔG
- cFAE Rxn Mechanism & Manufacturing Insight
- Characterization and Structure Function
- cFAE Model development

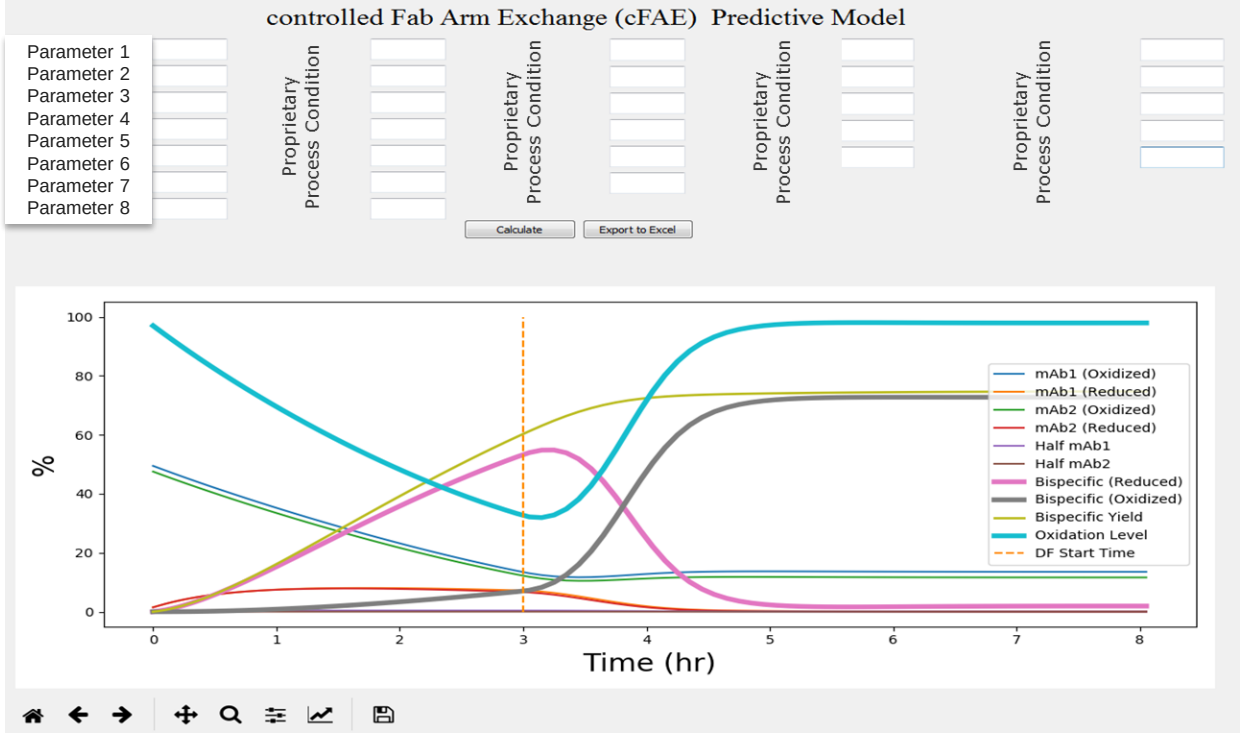


Modeling cFAE Chemistry:

- **Optimizing Process Parameters**
  - Finding the target and MORs
- **Defining a Design Space**
  - Designing DOE studies
- **Limiting the Number of experiments**
  - Avoiding unnecessary experiments
  - Reducing development time
- **Monitoring and Investigating**
  - Using the model to investigate failed batches

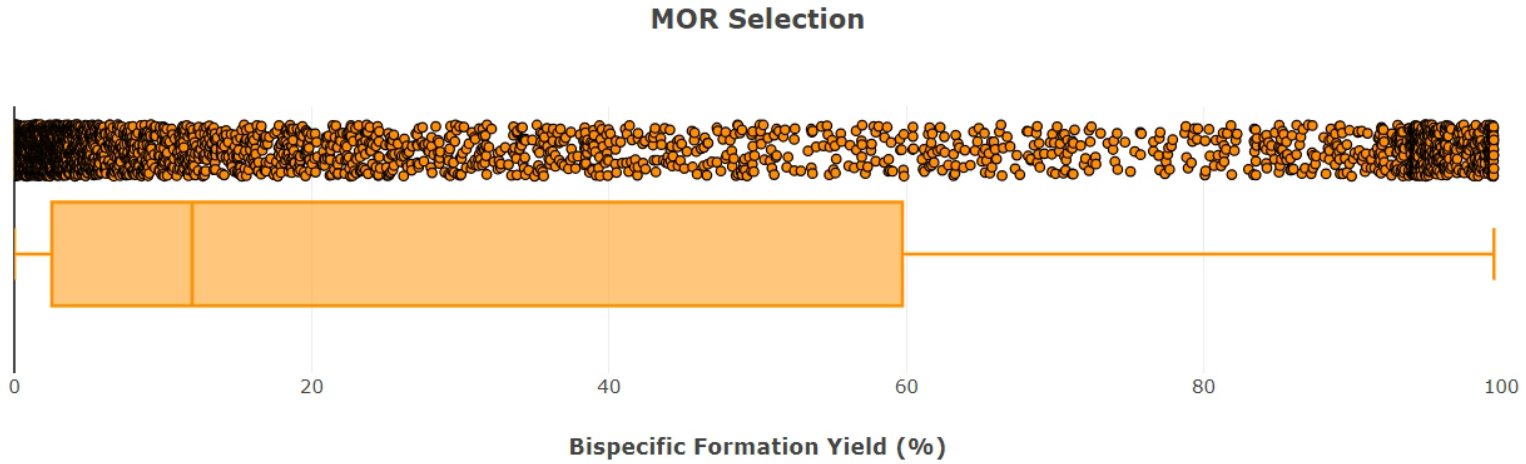
cFAE model considerations and outline

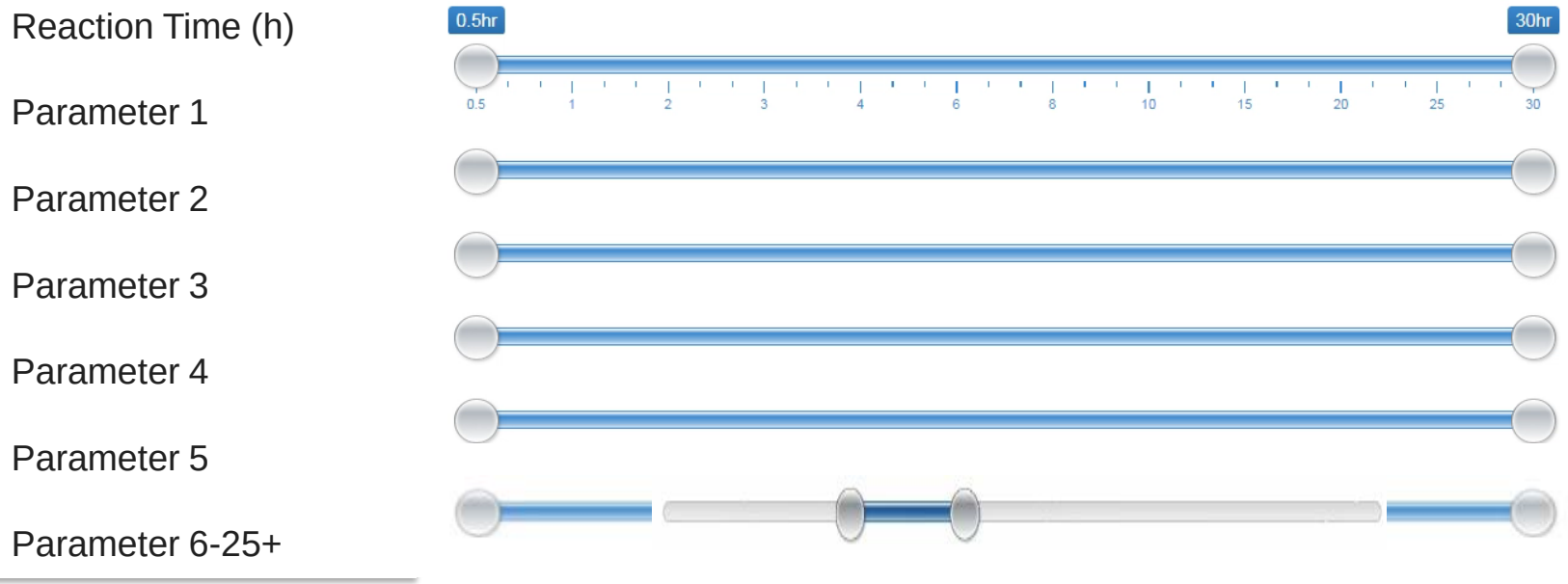
Mixed Model
- First principles
- Experimental Data
  - Lab & Manufacturing Scale
- Mechanism of Action
- Kinetic Forms
- Linear/Non-Linear behavior



Interactive web based app uses >25 parameters to predict BsAb reformation of disulfides

Using cFAE model to guide design space selection





Predictive modeling uses over 25 parameters to narrow optimized conditions

Key Messages

- Controlled Fab arm exchange (cFAE) is a robust process
- Leverage existing mAb platform & yields: 2 cell lines & USP
- cFAE understanding is key to manufacturing control
- BsAb characterization supports correct assembly and Structure/Function
- cFAE modeling optimizes development and design space
- DuoBody® technology is translated into a robust manufacturing platform for Janssen BsAbs

Thank You

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