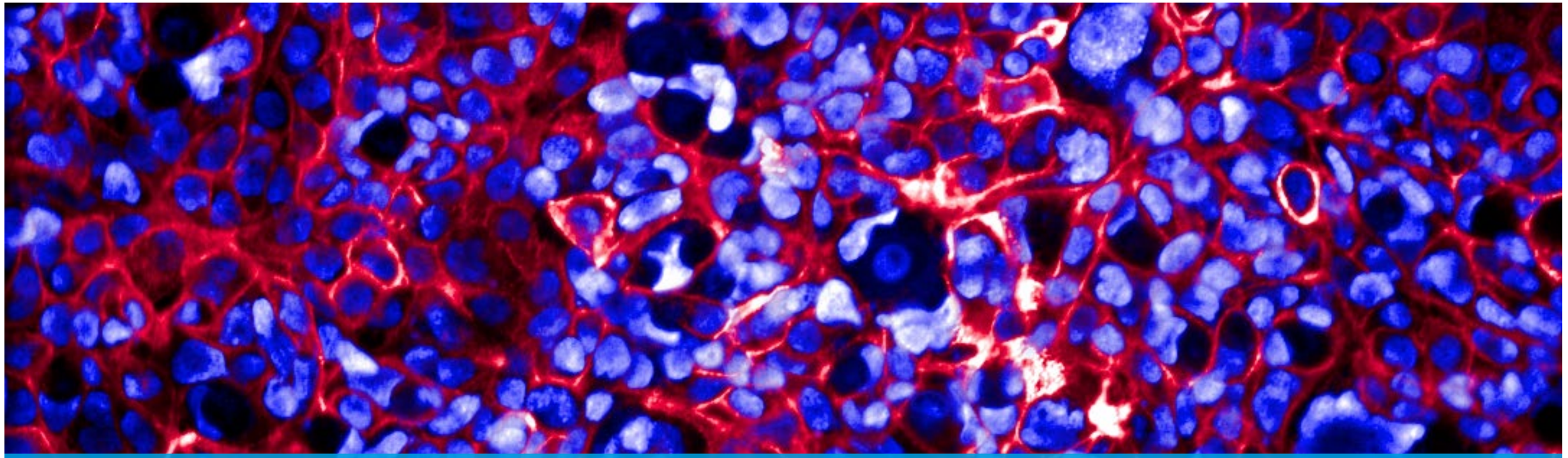

Biophysical Characterization of "Stapled" Single Chain Antibodies for Multispecific Biotherapeutics

Steven Jacobs
Sr. Director
Biophysics & Molecular Attributes, Therapeutics Discovery
Janssen R&D

Tumor cells expressing 'danger signal', a mechanism for anti-cancer activity by natural killer cells

Credit: Xiefan Lin-Schmidt, Exploratory Biology, Therapeutics Discovery

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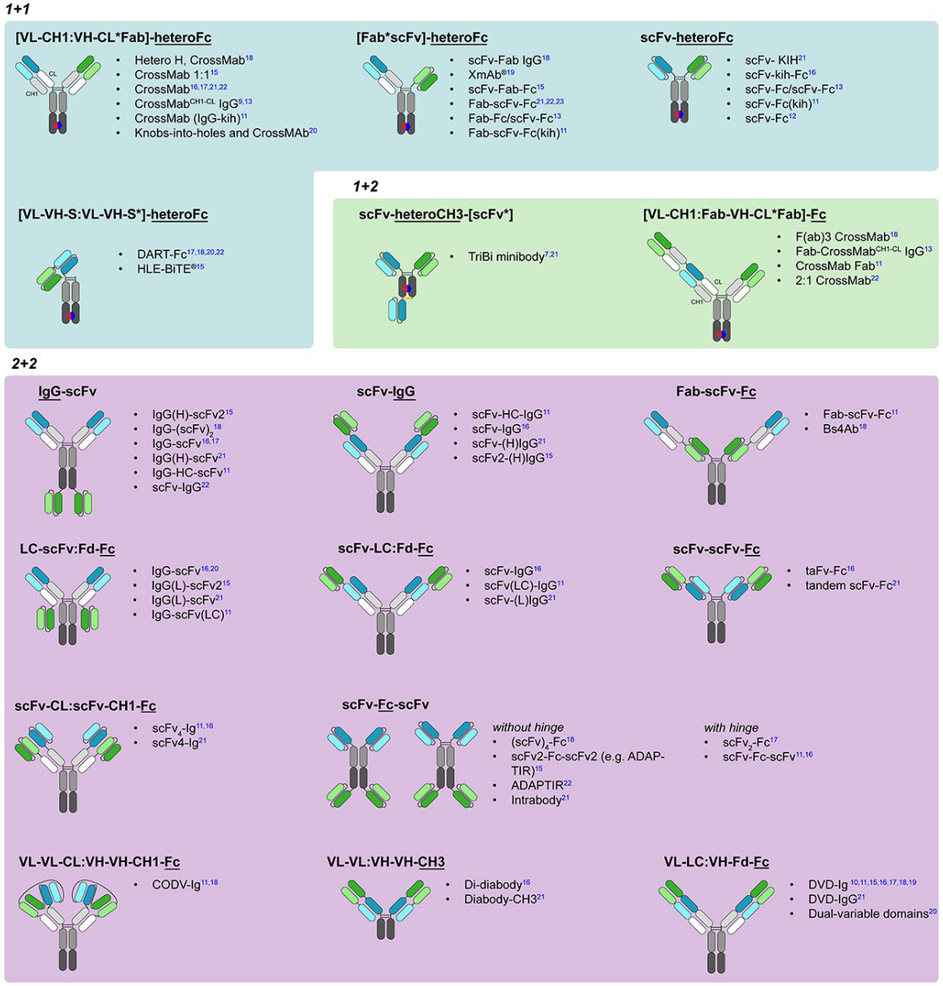
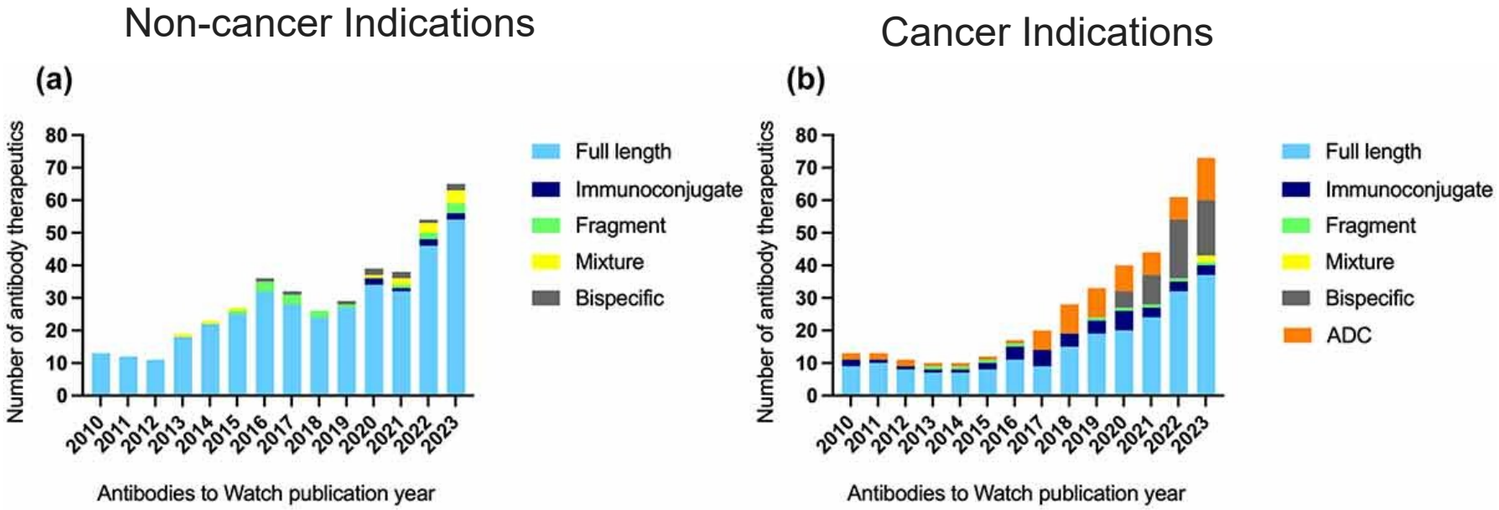
Increasing Emphasis on Multispecific Antibody Formats in Clinical Development and Discovery

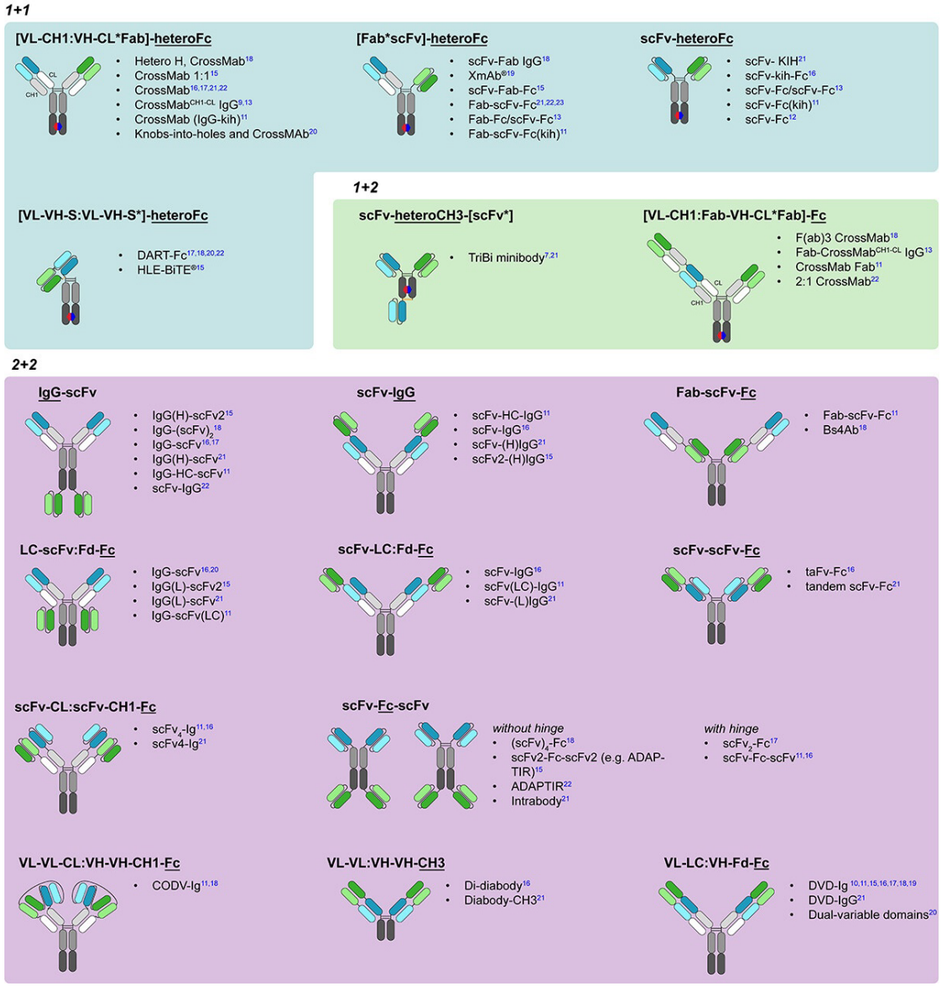


Biswas et al. 2023 https://doi.org/10.1080/19420862.2023.2207232



Kaplon et al. 2023 https://doi.org/10.1080/19420862.2022.2153410

Increasing Reliance on Subcutaneous Delivery and High API Concentrations Challenges Multispecific Formats



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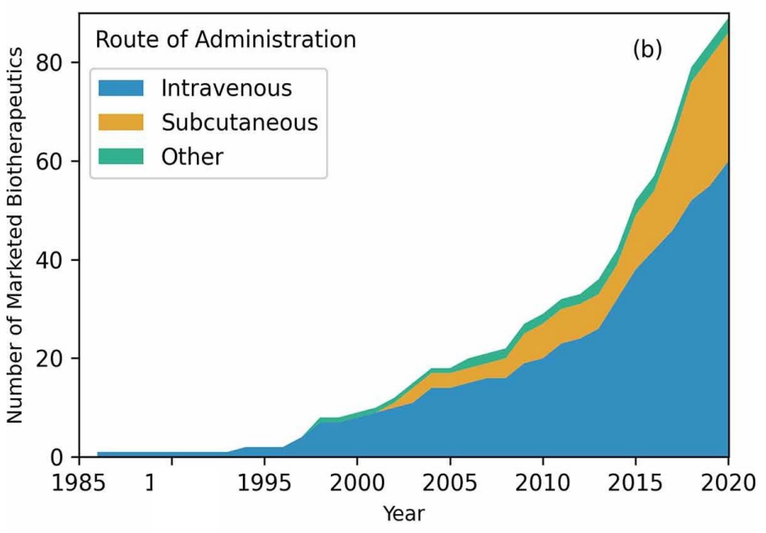
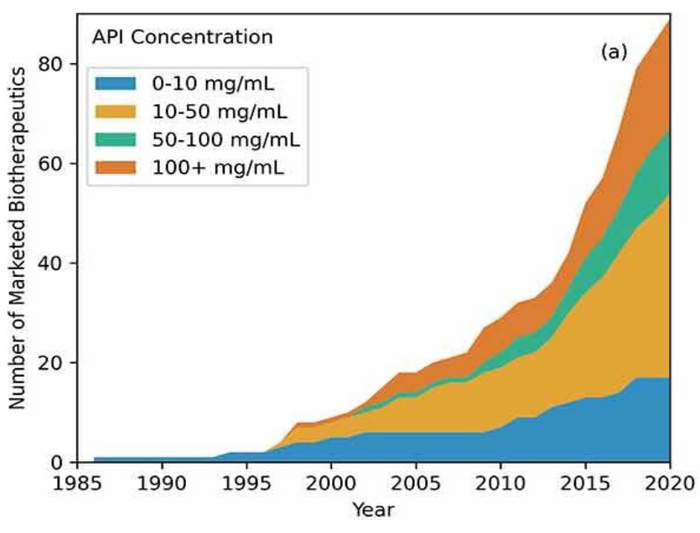
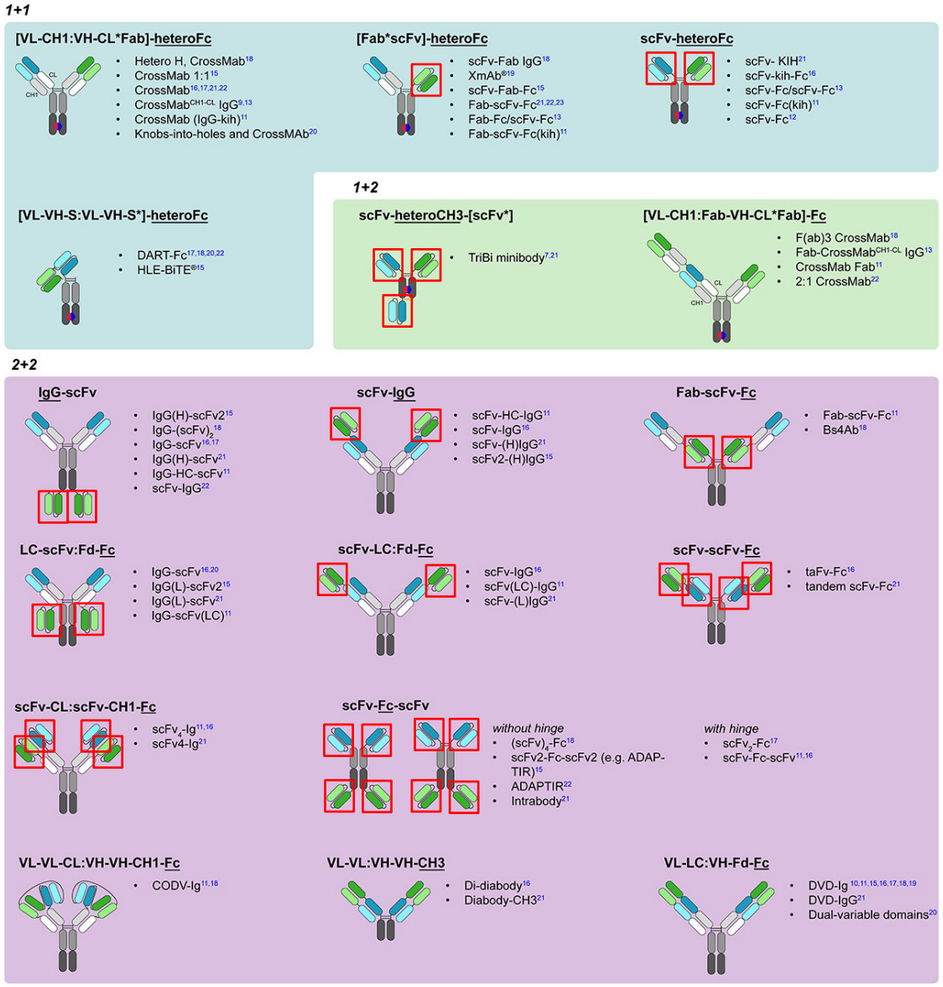




Martin et al 2023 https://doi.org/10.1080/19420862.2023.2191301

Many Novel Multispecific Antibody Formats Rely on Single Chain Antibody (scFv) Building Blocks



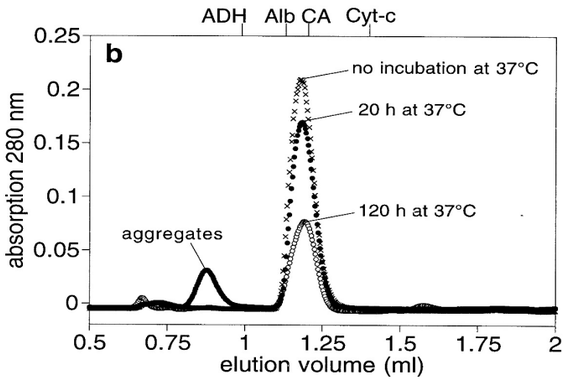
Biswas et al. 2023 https://doi.org/10.1080/19420862.2023.2207232

Engineering and optimization of scFv molecules, a 25-year-old problem:

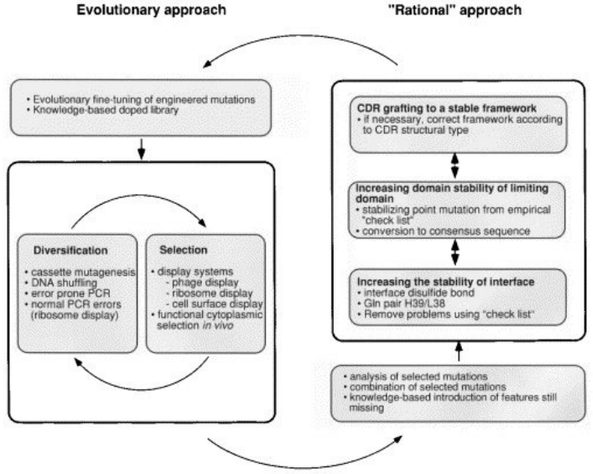
Review › Immunotechnology. 1997 Jun;3(2):83-105. doi: 10.1016/s1380-2933(97)00067-5.

New protein engineering approaches to multivalent and bispecific antibody fragments

A Plückthun [1], P Pack



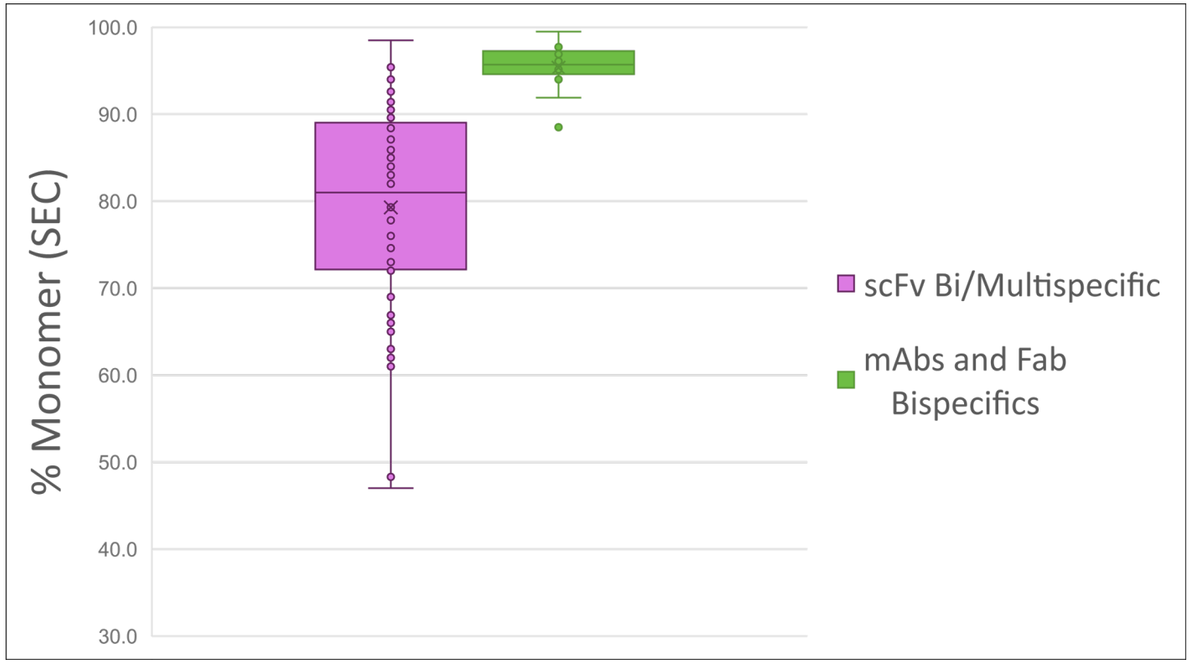
Wörn & Pluckthun, 1999. DOI: (10.1021/bi9902079)



scFv Containing Molecules Are Consistently More Prone to Aggregation Than Fab-based Molecules at Higher Concentrations

Stability after 40°C Incubation @ > 50 mg/mL

Janssen internal results



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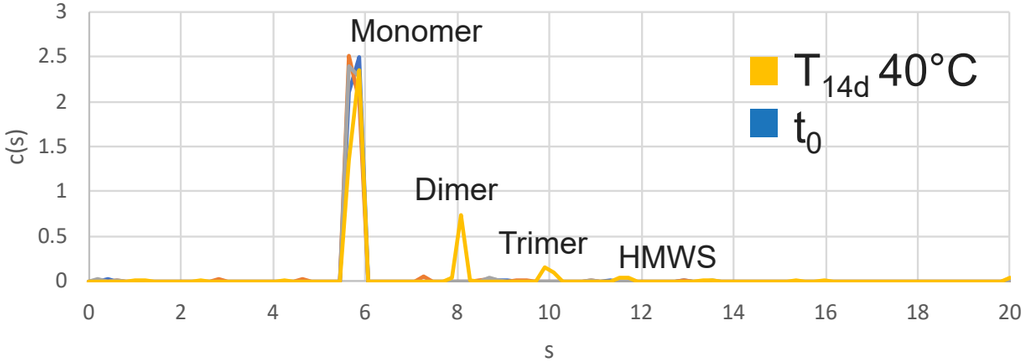

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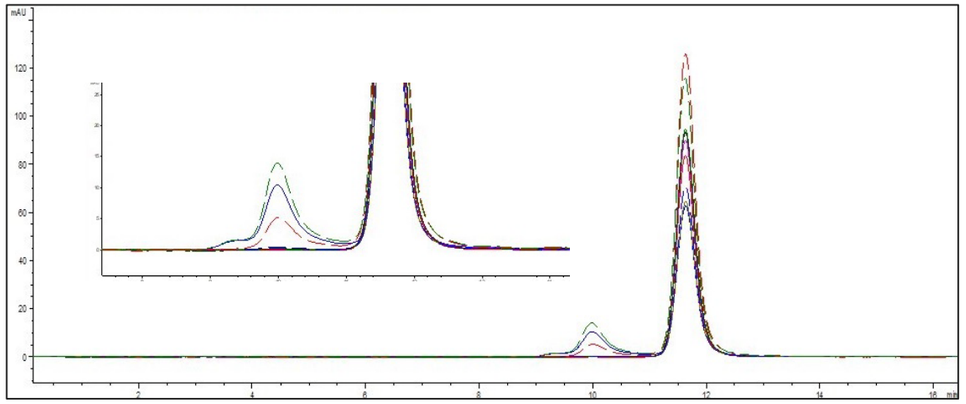
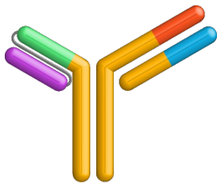
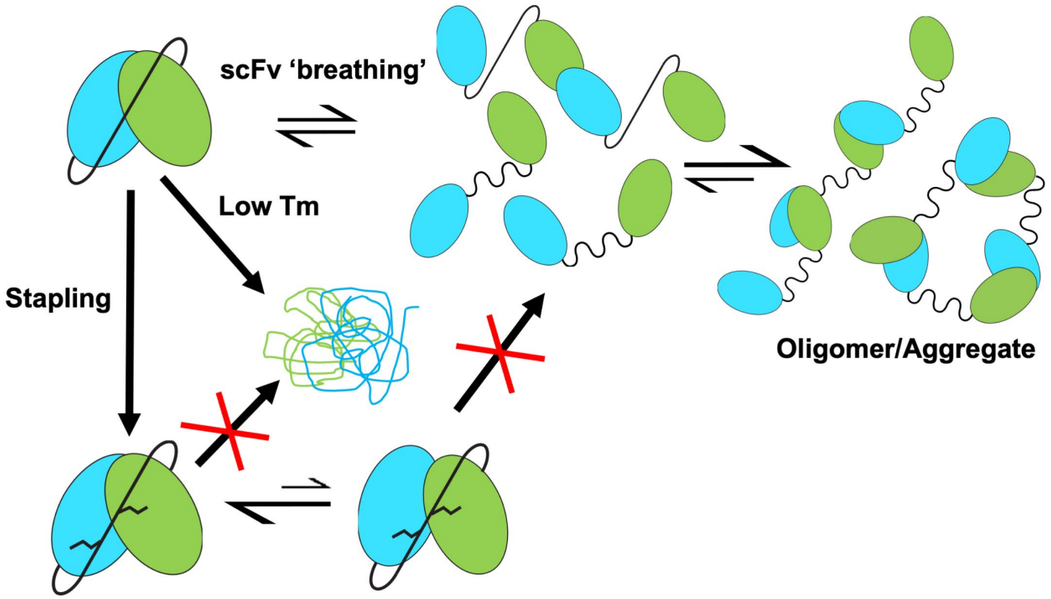


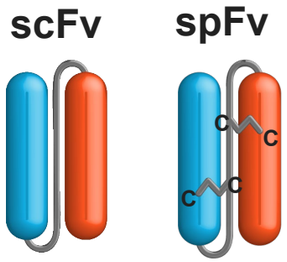


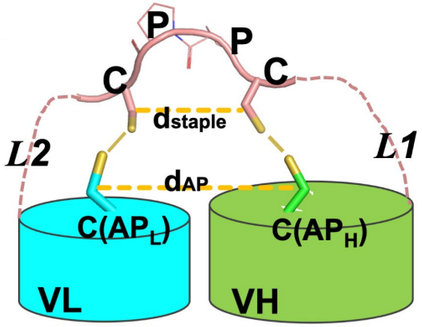
“Stapling” of scFv Fragments To Stabilize Against Concentration and Temperature Induced Aggregation

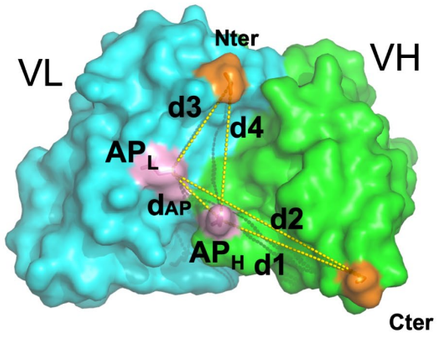
Hypothesis:



Solution: Stapled scFv



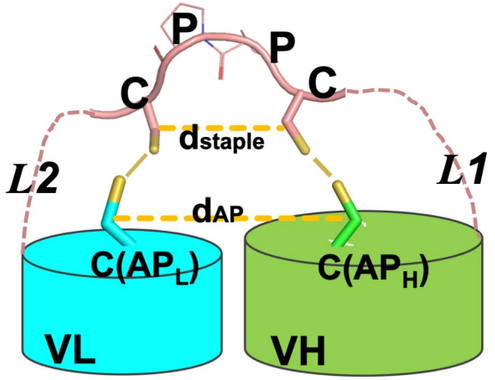


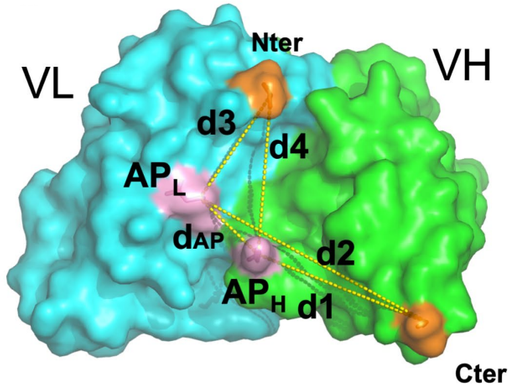


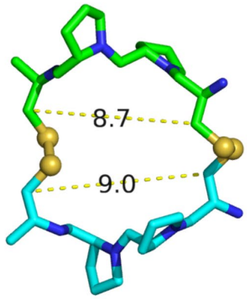
Design goals:

- Prevent scFv breathing and resultant aggregation
- Universal solution for all scFv molecules
- Retain biological potency and critical quality attributes
- No disulfide mispairing or unwanted side products

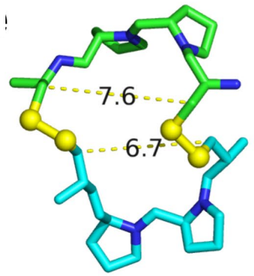
Location of Anchor Positions and Rigid Linker Region Designed To Prevent Disulfide Scrambling



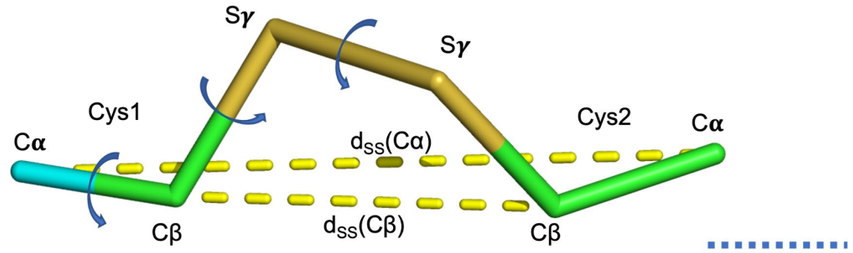


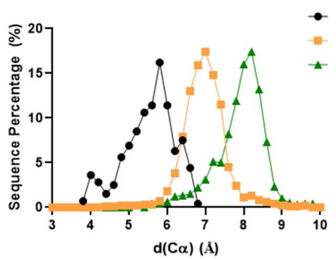


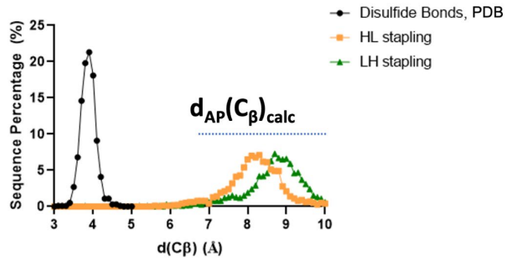
Human IgG
'CPPC' hinge

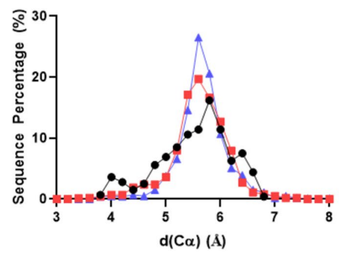


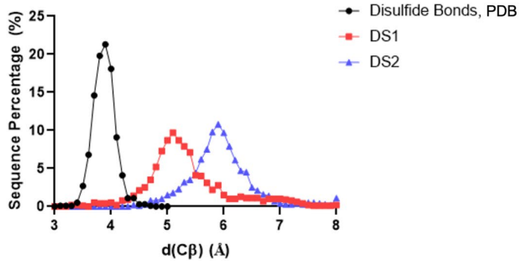
Mouse IgG2a
hinge CPPC





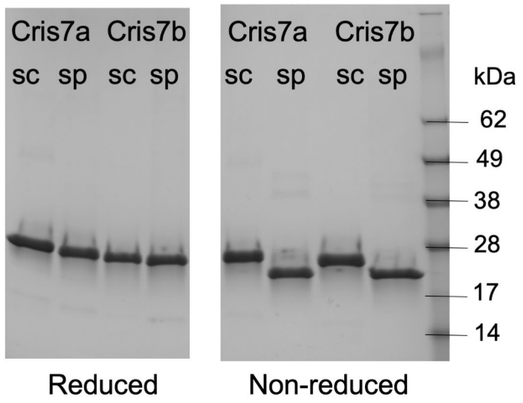


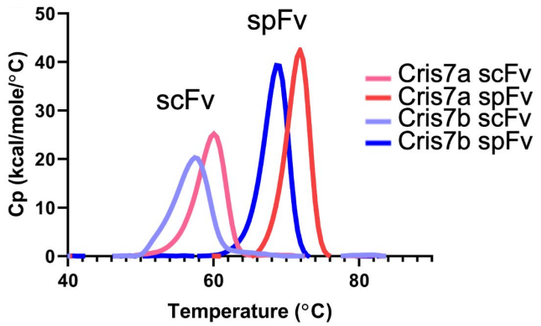




Stapling Increases Conformational Stability Through Disulfide Bond Formation

Melting Temperature Increase by ~ 12 ℃

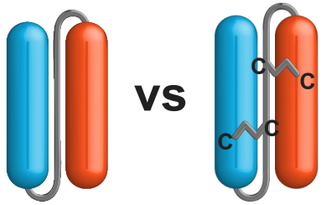




Differential Scanning Calorimetry

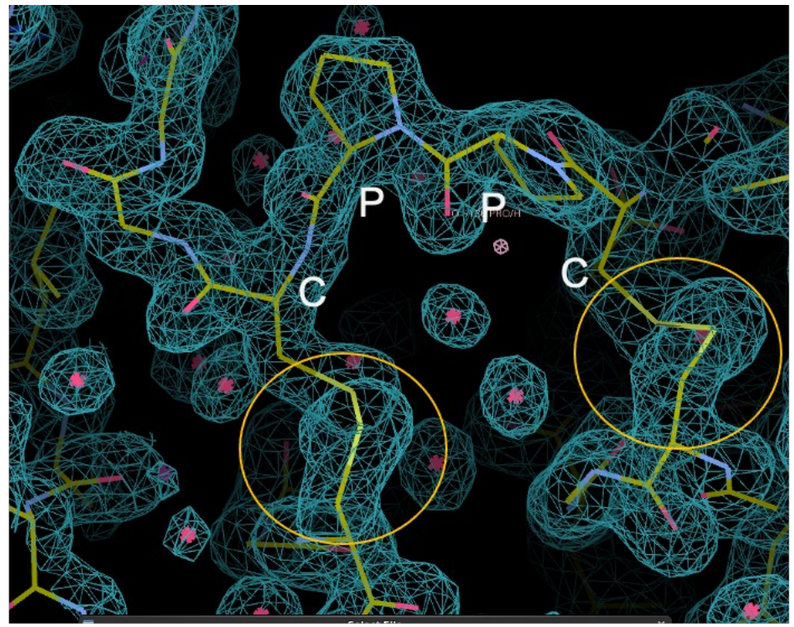
Tm increases are independent of orientation and linker length

Molecule name	Orientation	scFv	spFv	ΔTm	ΔΔH kcal/mol	spFv Linker Length
Glk2 (κ)	LH	57.9	**68.6**	10.7	18.5	18
	HL	57.3	**64.7**	7.5	10.5	14
CAT2200 (λ)	LH	57.2	**68.8**	11.6	52.3	17
	HL	55.9	**67.4**	11.5	54.1	17
scFv1 (κ)	LH	59.7	**71.6**	11.9	53	18
	HL	57.0	**68.6**	11.5	64	18



Structural Validation

X-ray crystallography



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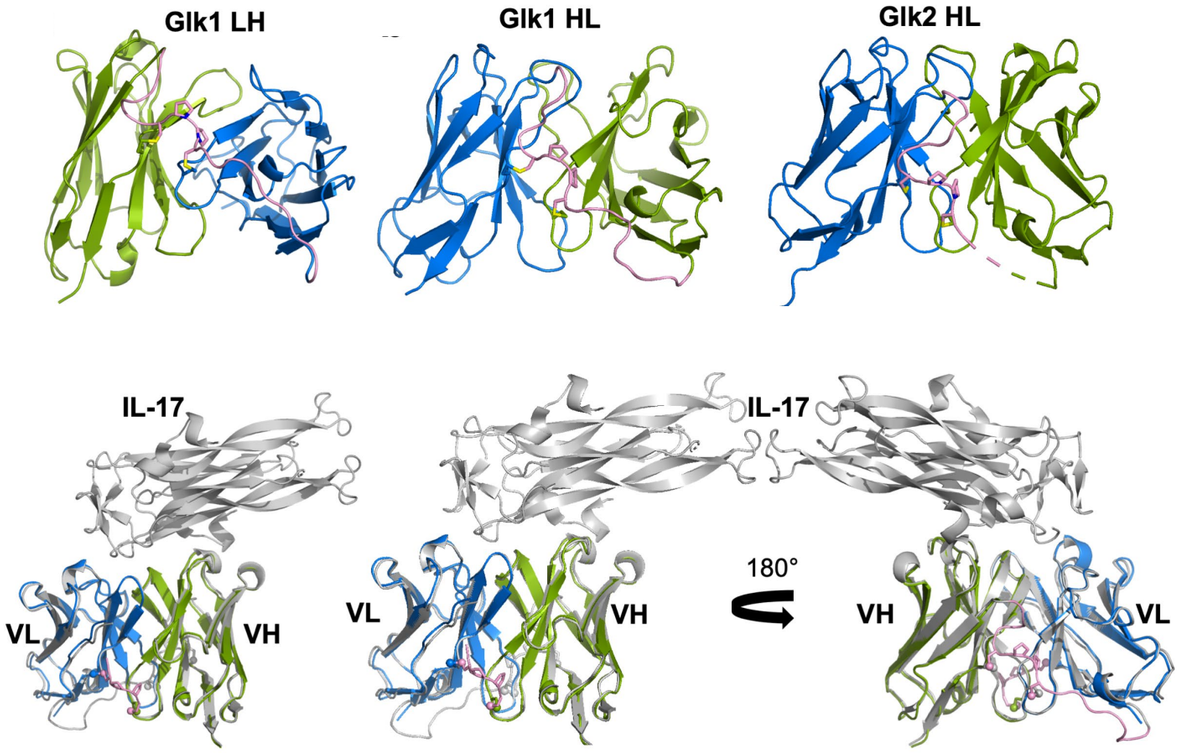
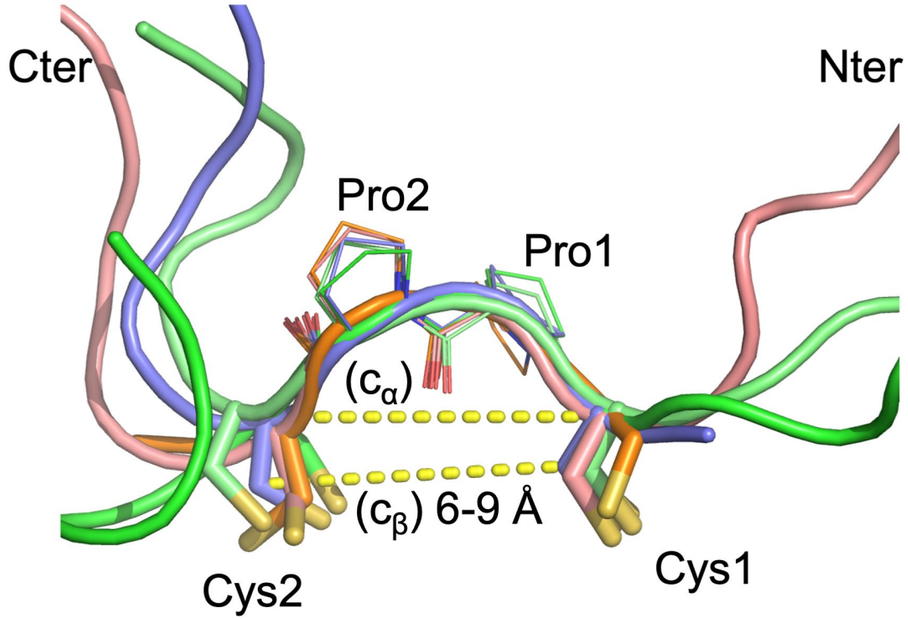
spFv Staple Forms a Consistent Structure Within Multiple Antibodies

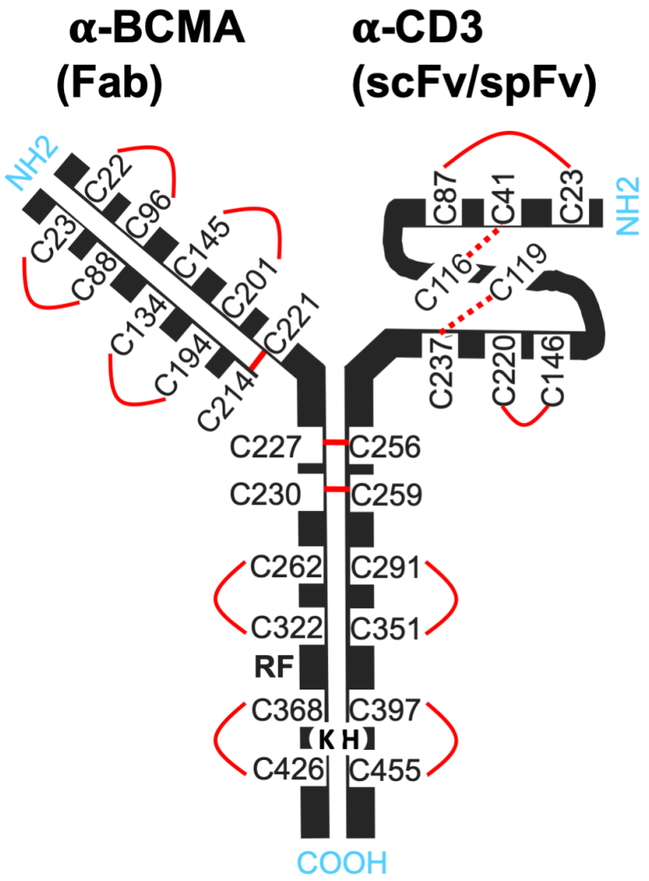


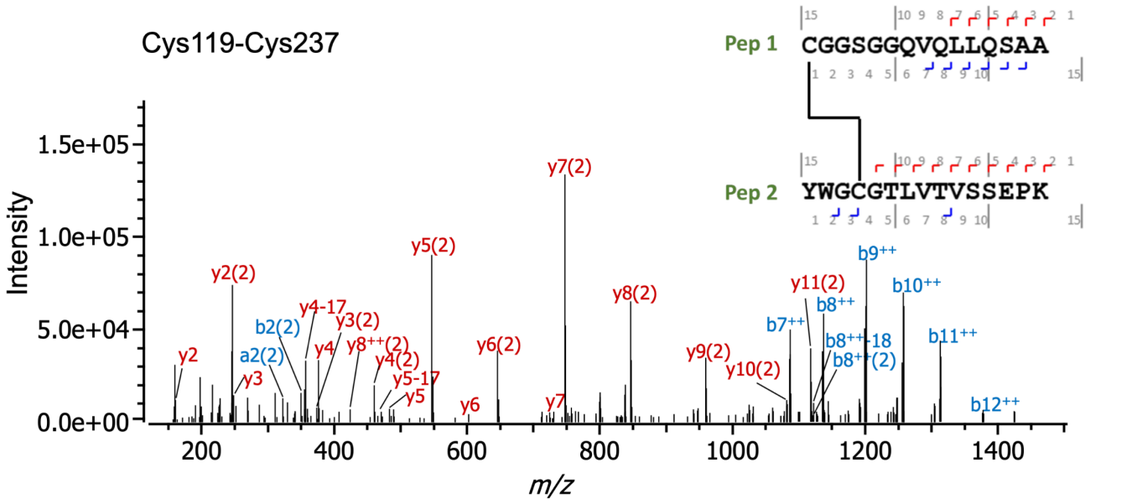
spFv HL unbound (blue, green) vs. scFv LH bound (silver)

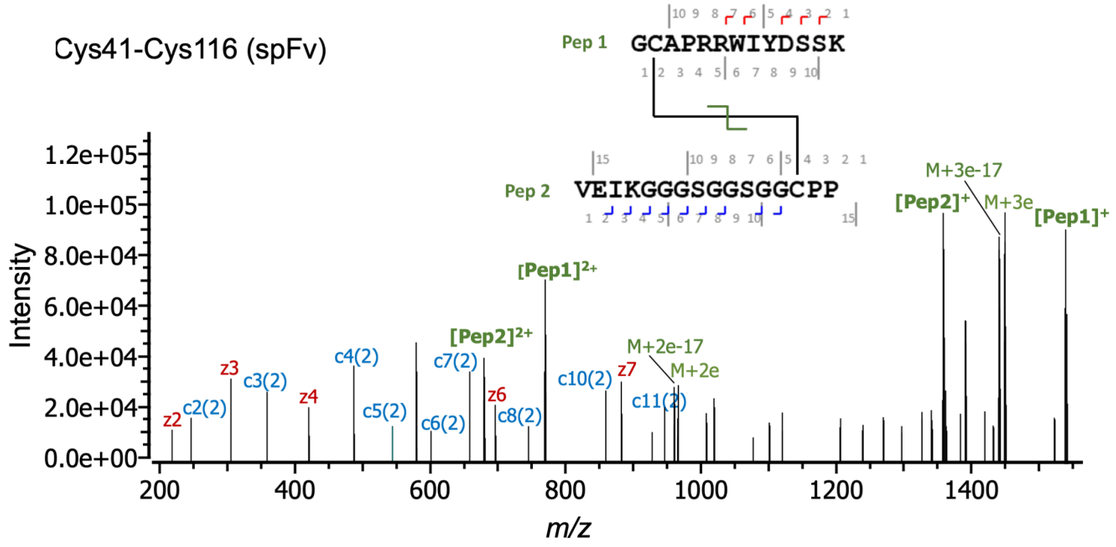
spFv HL unbound (blue, green) vs. spFv LH bound (silver)



spFv Forms Proper Disulfides in Bispecific Antibody Format

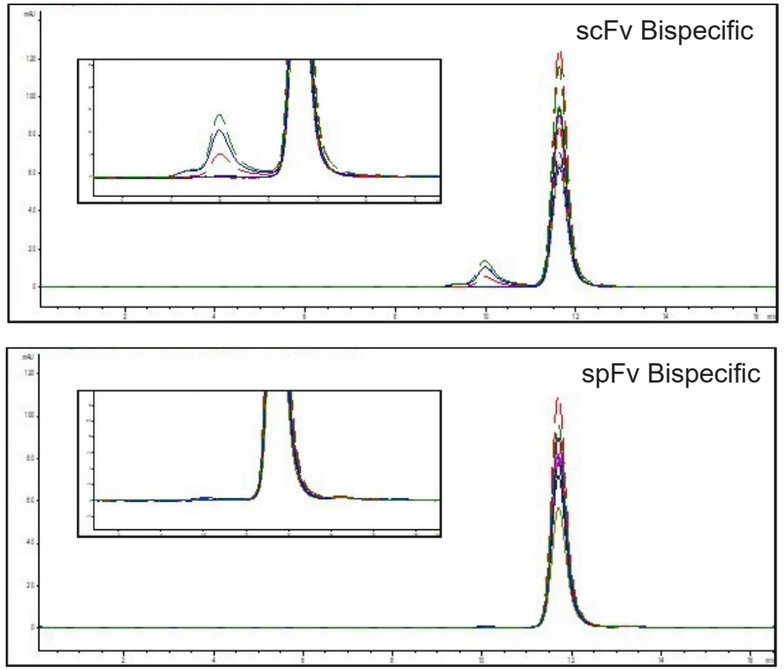


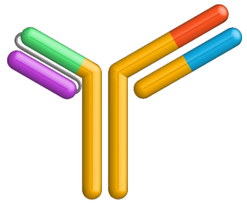


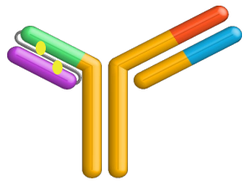


Bispecific Aggregation at High Concentrations is Alleviated by Stapling

150 mg/mL thermal stress







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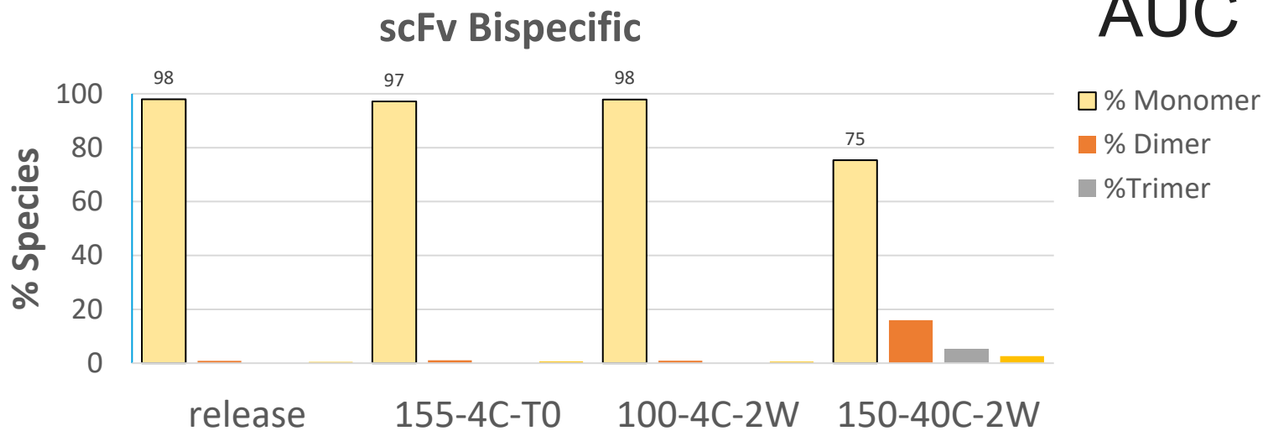
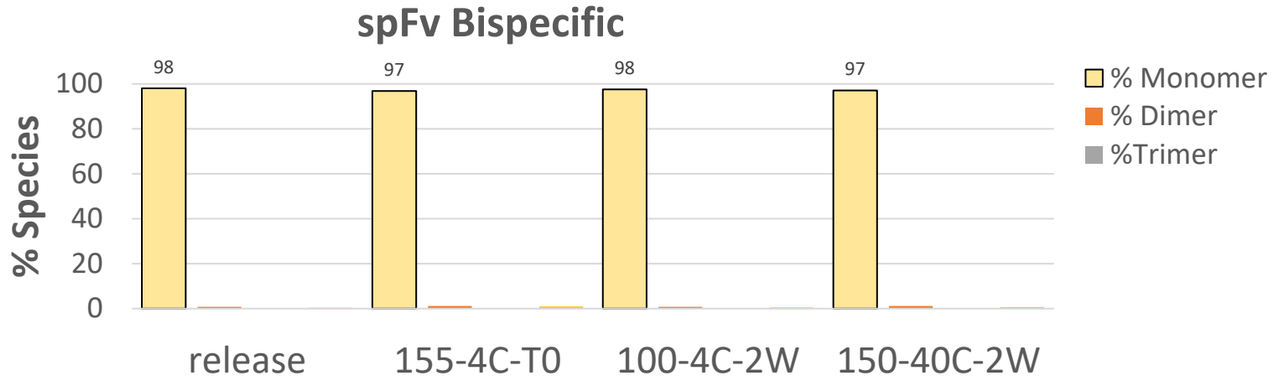




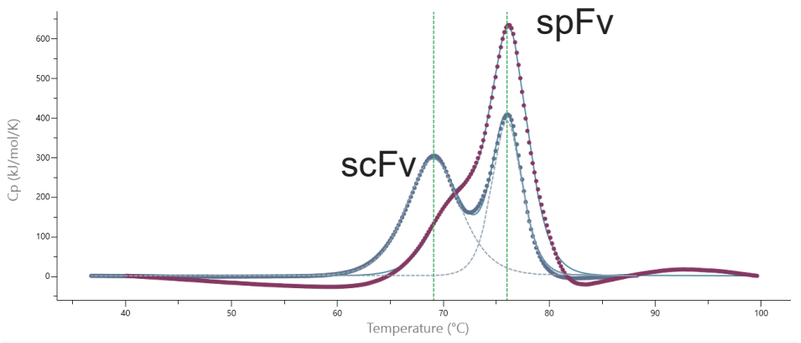
Viscosity 100 mg/mL:

scFv Bispecific = 3.6 cP

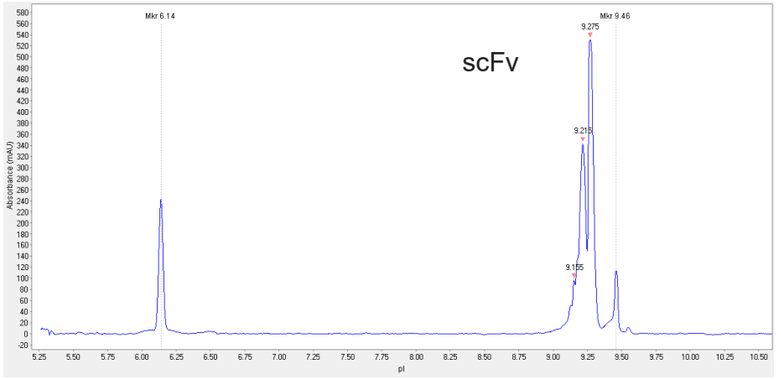
spFv Bispecific = 2.9 cP

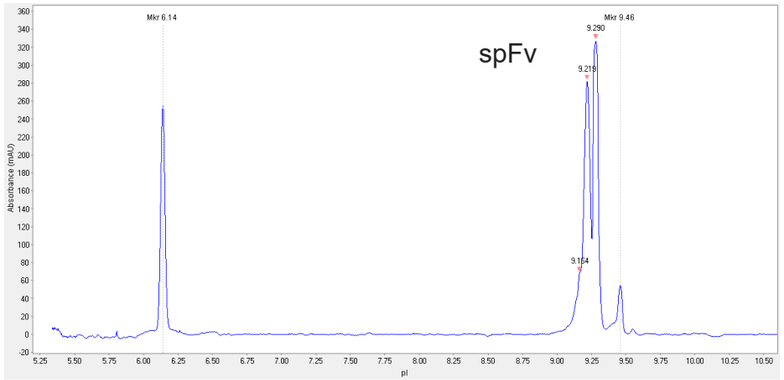
Improvements to Aggregation Without Compromising Other Critical Attributes of a Bispecific

Conformational Stability

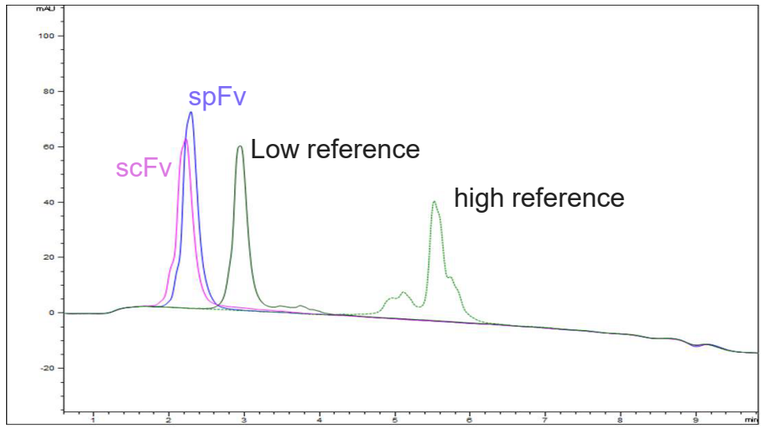


Charge Profile

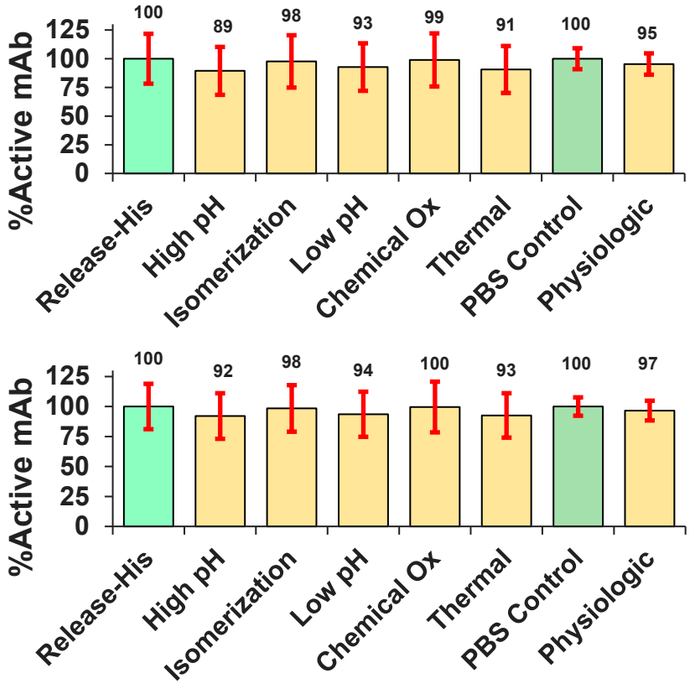




Surface hydrophobicity

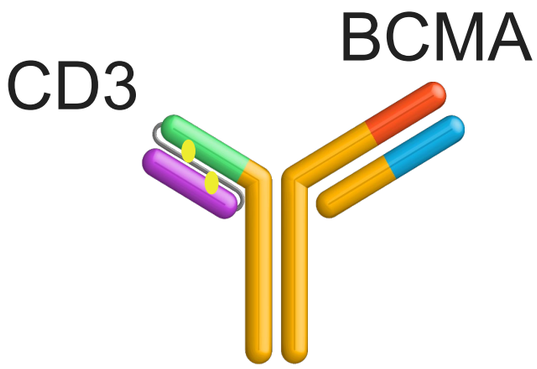


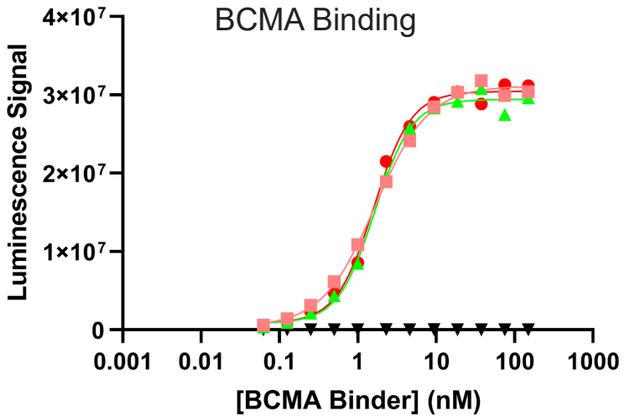
PTM/Behavior under stress

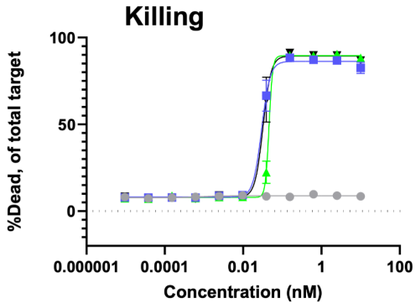


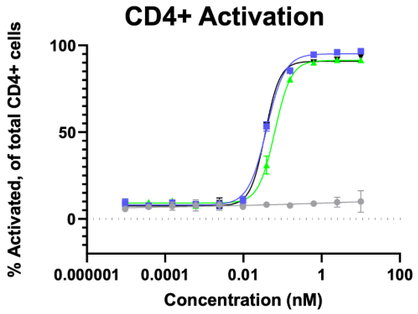
- Identical PTM profile by peptide mapping

spFv Retains Biological Potency of scFv Parent Bispecific Molecule



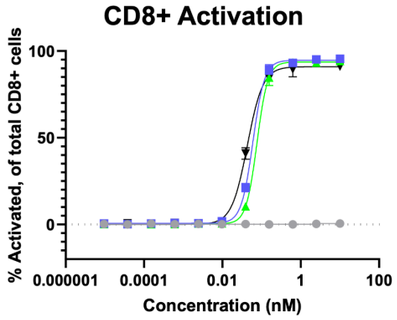




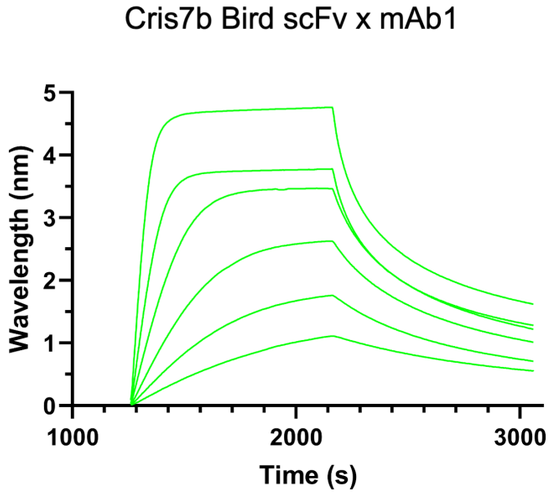


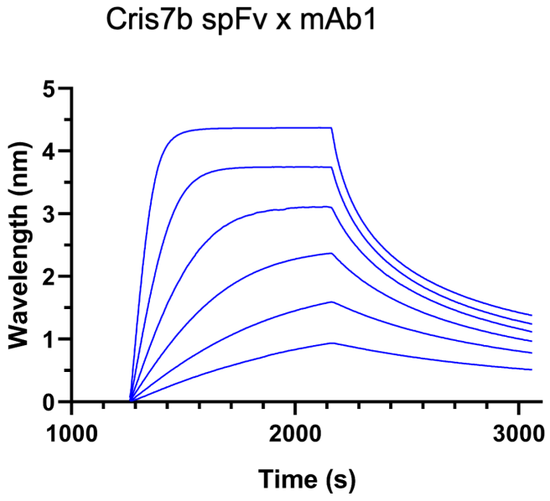
scFv Bispecific

spFv Bispecific



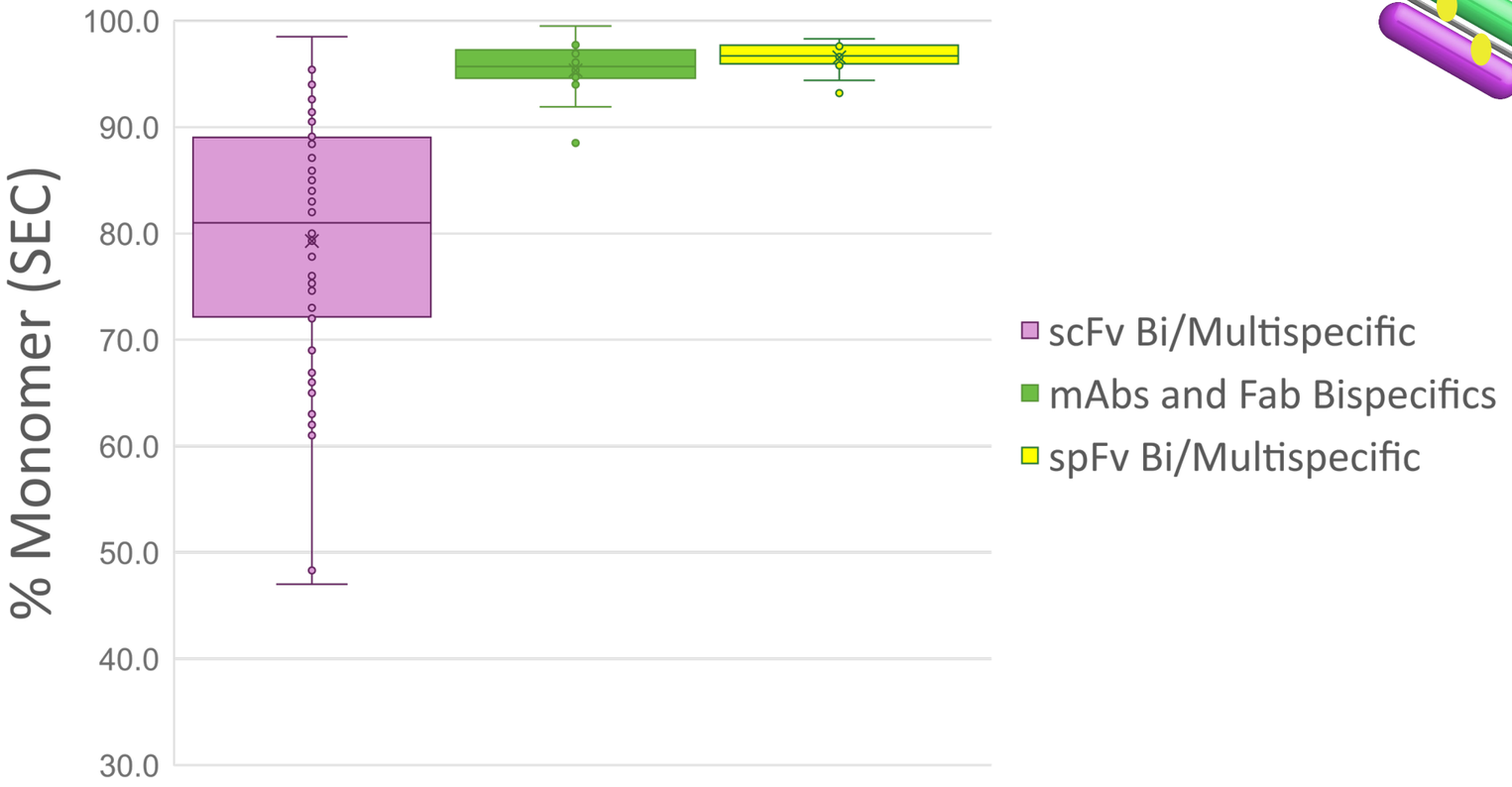
CD3 Binding

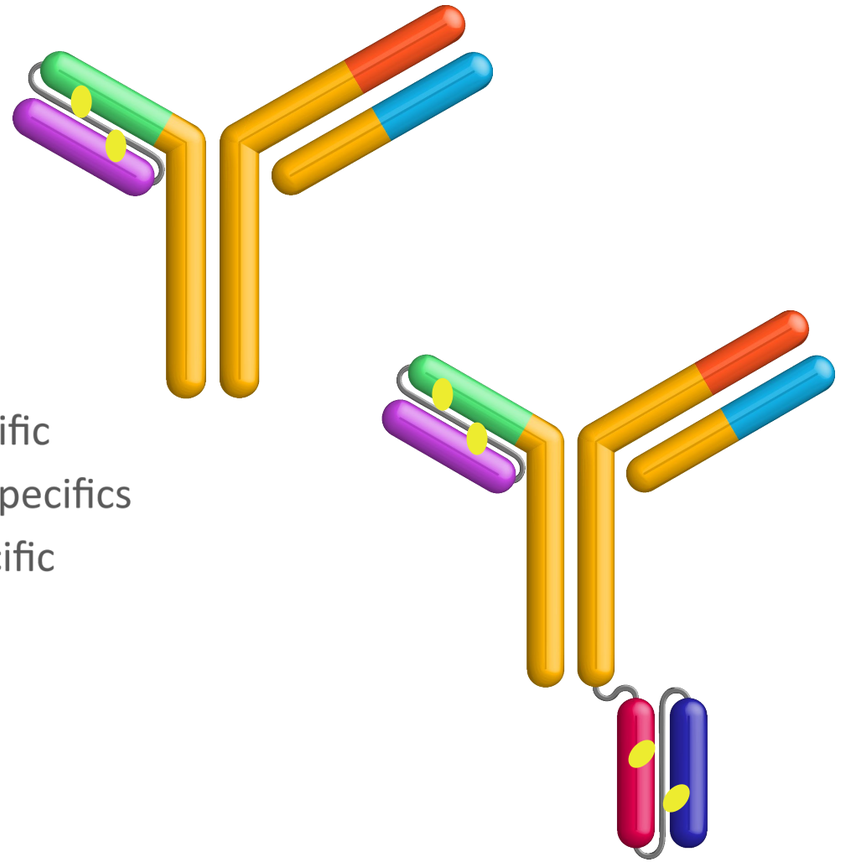




Stapling Consistently Improves Thermal Induced Aggregation of scFv Molecules in Complex Formats

Stability after 40°C Incubation @ > 50 mg/mL





Acknowledgements

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Sam Wu
Fang Yi
Samantha Heyne
Natasha Kozlyuk
Rob Davidson
Brian Del Rosario
Adam Zwolak
Neeraj Kohli
Tun Liu
Bingyuan Wu
Robin Ernst
Sagit Hindi

Mike Feldkamp
Eilyn R. Lacy
James Testa
Elsa Gorre
Andrew Mahan
Alexis Gervais
Anthony Armstrong
Elsie Samakai
And many others!

Former Janssen:
Lauren Boucher
Alexey Teplyakov
Chichi Huang