Supporting Information

Intact Protein Mass Spectrometry of Cell Culture Harvest Guides Cell Line Development for Tri-specific Antibodies

Fateme Tousi^{1*}, Yan Jiang¹, Sharmila Sivendran¹, Yvonne Song¹, Susan Elliott²§, Anthony Paiva², Anders Lund¹⁺, Karen Albee¹, Karen Lee¹

Corresponding Author

* Email: Fateme.tousi@sanofi.com

ORCID

Fateme Tousi: 0000-0002-4041-9274

Table of Contents

Figure S1. Raw and deconvoluted mass spectra obtained by SEC-LC-MS analysis of (a) clarified harvest and (b) ProA purified anti-CD38 TCE showing comparability of the two methods. Annotations on the deconvoluted spectra show the experimental masses and %intensities of each species.

Figure S2. Ranking anti-HER2 TCE clones based on (a) percentage of correct tsAb mass (purity) and (b) productivity measured by titer for the same clones.

Figure S3. Effect of different growth conditions on chain mispairing and half antibody levels in anti-CD38 TCE clones grown under (a) and (b) two different fed-bacth conditions in ambr15 bioreactor and (c) batch culture condition in spin tubes.

Figure S4. Effect of different growth conditions on chain mispairing levels in anti-HER2 TCE clones grown under (a) fed-batch in ambr15 bioreactor (b) batch culture condition in spin tubes.

Figure S5. Evaluating the performance of the SEC column by running a reduced mAb sample (a) at the beginning and (b) at the end of a queue of 50 clarified cell culture harvest samples tested for mispairing by intact protein mass spectrometry. First peak at RT=5.30 minute is the heavy chain and the second peak at RT=6.65 minute is the light chain.

Table S1. Relative levels of mis-paired and reference masses and precision results in triplicate analysis of different column loads for anti-CD38 TCE and anti-HER2 TCE harvest samples.

¹Department of Bioanalytics, Biologics Development, Sanofi, Framingham, Massachusetts 01701, United States

² Cell Line Development, Biologics Development, Sanofi, Framingham, Massachusetts 01701, United States

[§] Current affiliation: Rubius Therapeutics, Cambridge, Massachusetts 02139, United States

[†] Current affiliation: Synlogic Inc., Cambridge, Massachusetts 02142, United States

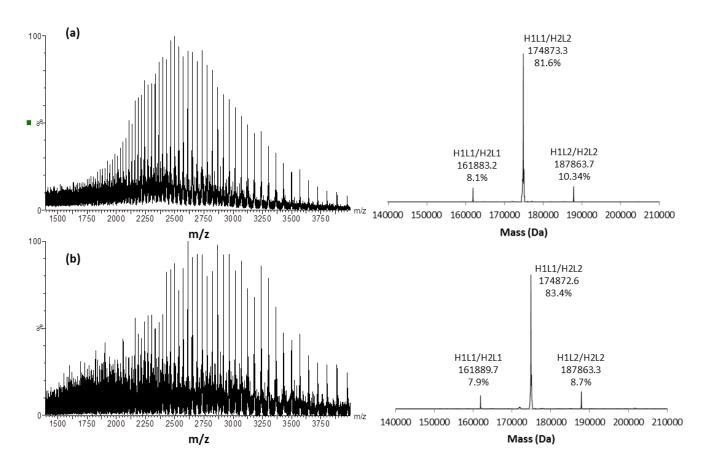


Figure S1. Raw and deconvoluted mass spectra obtained by SEC-LC-MS analysis of (a) clarified harvest and (b) ProA purified anti-CD38 TCE showing comparability of the two methods. Annotations on the deconvoluted spectra show the experimental masses and %intensities of each species.

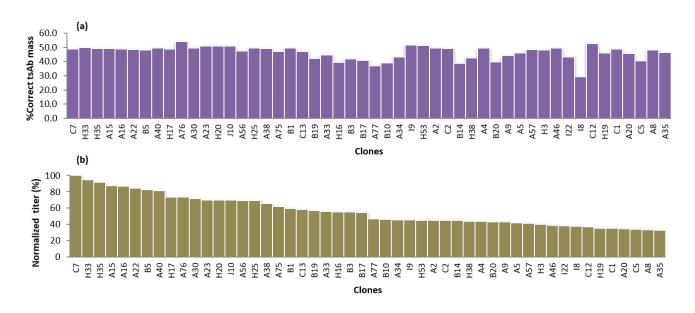


Figure S2. Ranking HER2 TCE clones based on (a) percentage of correct tsAb mass (purity) and (b) productivity measured by titer for the same clones.

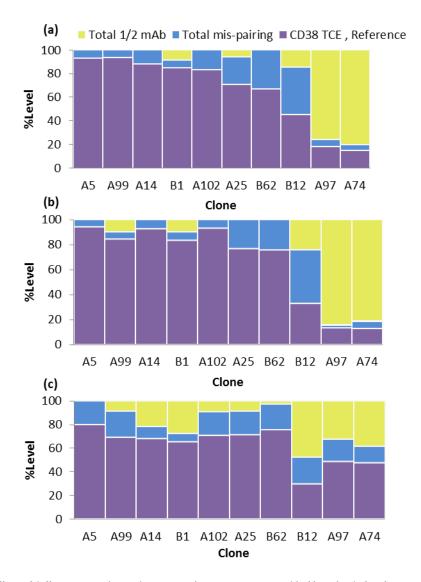


Figure S3. Effect of different growth conditions on chain mispairing and half antibody levels in anti-CD38 TCE clones grown under (a) and (b) two different fed-bacth conditions in ambr15 bioreactor and (c) batch culture condition in spin tubes.

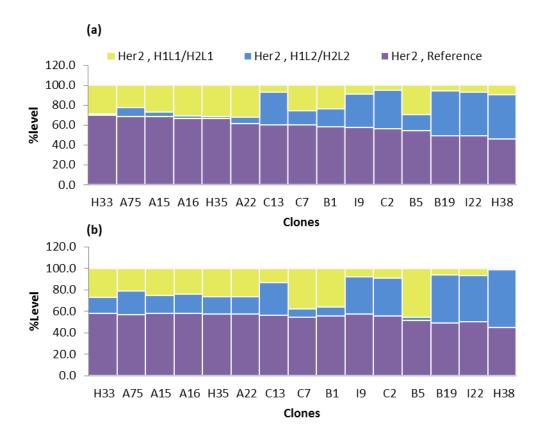


Figure S4. Effect of different growth conditions on chain mispairing levels in anti-HER2 TCE clones grown under (a) fed-batch in ambr15 bioreactor (b) batch culture condition in spin tubes.

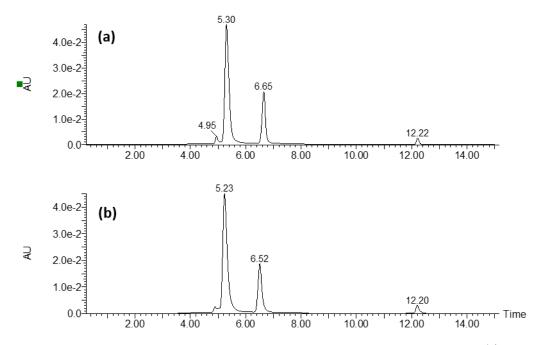


Figure S5. Evaluating the performance of the SEC column by running a reduced mAb sample (a) at the beginning and (b) at the end of a queue of 50 clarified cell culture harvest samples tested for mispairing by intact protein mass spectrometry. First peak at RT=5.30 minute is the heavy chain and the second peak at RT=6.65 minute is the light chain.

Table S1. Relative levels of mis-paired and reference masses and precision results for triplicate analysis of anti-CD38 TCE and anti-HER2 TCE harvest samples at different column loads.

			Ref. mass		H1L1/H2L1		H1L2/H2L2	
tsAb construct	Harvest injection volume (μL)	Column load (µg protein)	Mean	%RSD	Mean	%RSD	Mean	%RSD
anti-HER2 TCE	10	4.4	57.9	0.5	26.0	0.7	16.2	1.1
	20	8.7	55.7	0.4	27.0	1.8	17.3	2.8
	30	13.1	55.9	0.8	26.9	1.7	17.2	2.0
	40	17.4	55.8	1.0	27.0	1.0	17.1	2.6
	50	21.8	57.5	3.5	25.8	6.3	16.7	2.3
anti-CD38 TCE	10	11.0	83.3	0.7	6.3	2.6	10.4	4.3
	20	22.0	83.0	1.2	6.6	10.4	10.2	3.1
	30	33.0	81.9	0.7	6.8	9.3	10.0	6.4
	40	44.0	82.7	0.7	7.0	6.0	10.0	6.2
	50	55.0	82.8	0.9	7.2	8.8	10.0	6.5