



Development, Qualification and Understanding Spike Protein in COVID- 19 Vaccine using Capillary Western Technology

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CE Pharm 2023
26Sep2023

Agenda

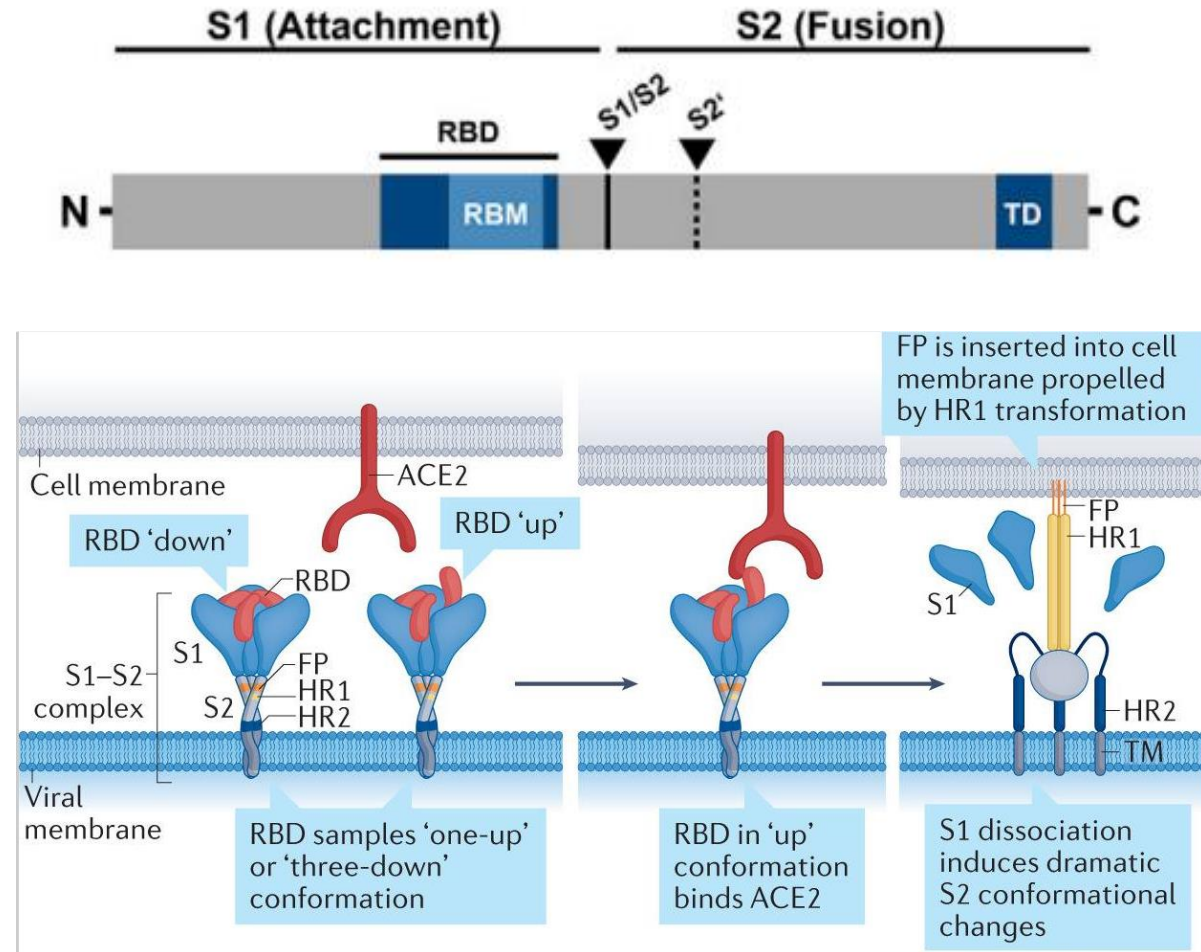


- Background of SARS-CoV-2
- Background of V590 COVID-19 program
- Overview of Simple Western™ Technology
- Development of Quantitative Spike Protein (S Protein) Simple Western™ assay
- Qualification of S Protein Simple Western™ assay
- Applications of S protein simplewestern assay to characterize V590 manufacturing process including process intermediates and drug substance as well as understanding V590 stability

SARS-CoV-2 Background



- Positive-sense single-stranded RNA virus
- S protein mediates cell binding and fusion

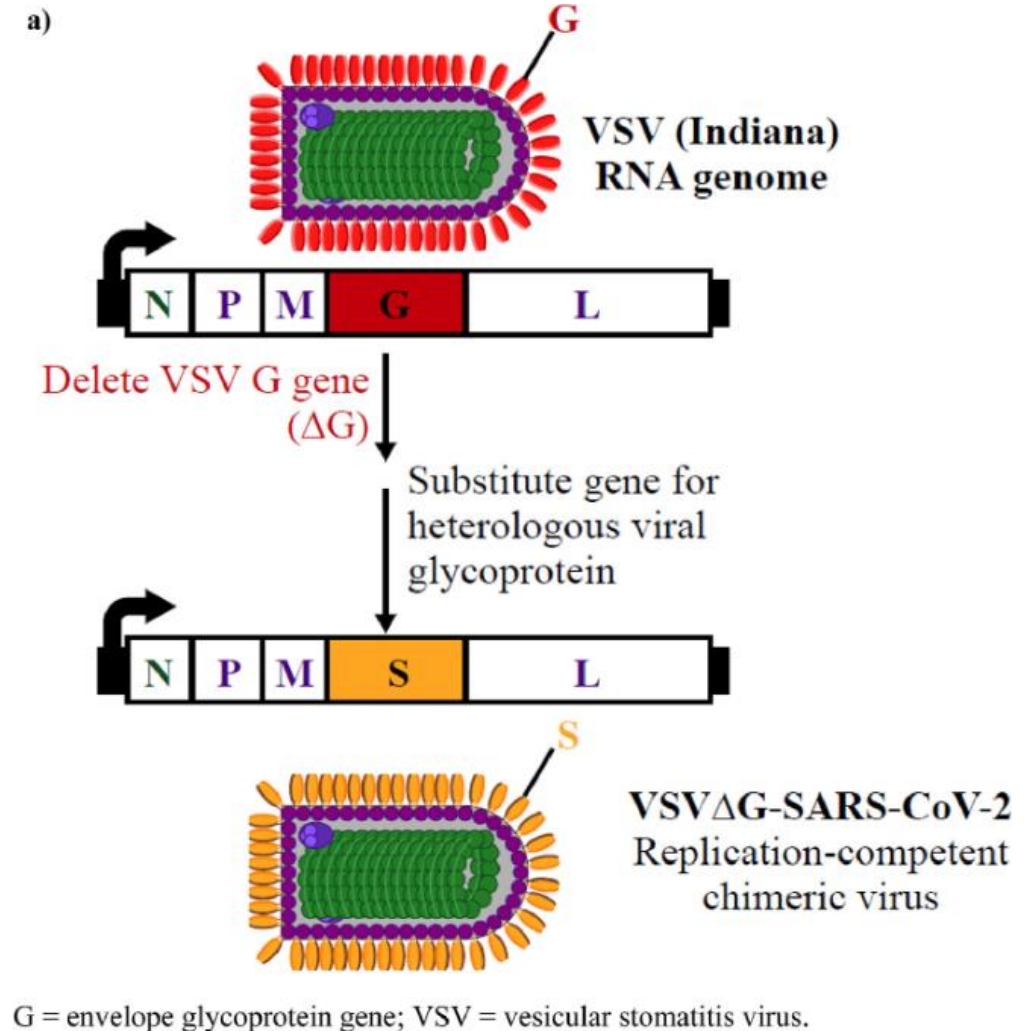


V590 COVID-19 Vaccine Platform Background

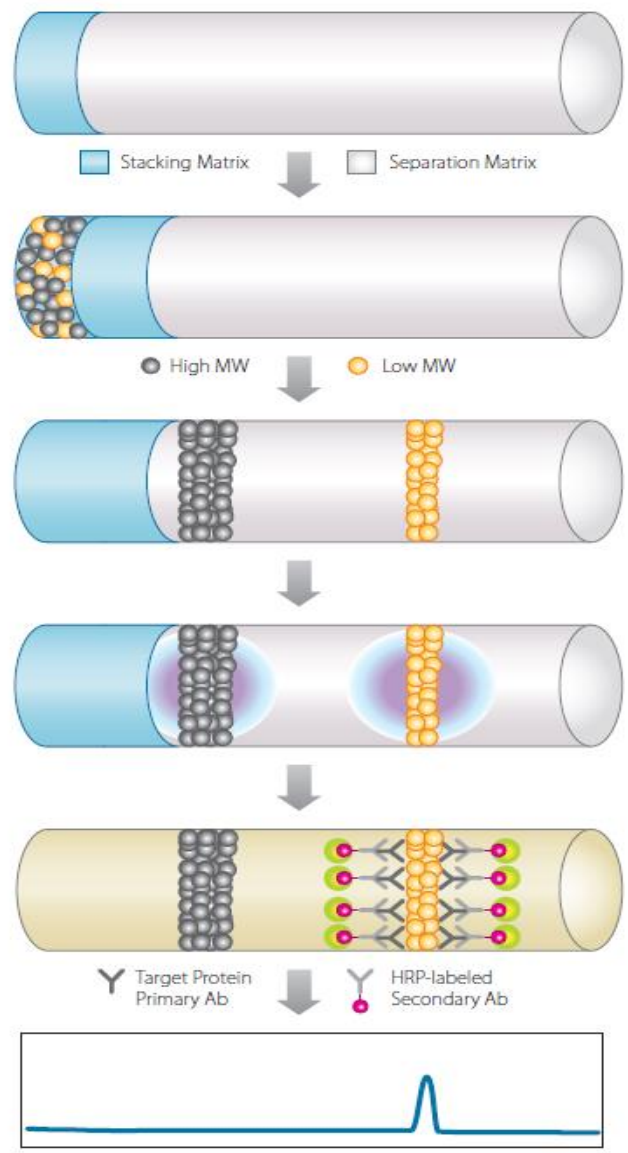
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- Recombinant vesicular stomatitis virus (rVSV)
- Same chimeric vaccine technology used in Ebola Vaccine (ERVEBO®)



Overview of Simple Western™ Technology



1 5 10 15 20 25

A B C D E F

7 8 9 10 11 12 13 14 15 16 17 18 19

A B C D E F

- Biotinylated Ladder, 5 μ L
- Prepared Samples, 3 μ L
- Antibody Diluent (2 or Milk-Free), 10 μ L
- Primary Antibody, 10 μ L
- Streptavidin-HRP or NIR, 10 μ L
- Secondary Conjugate, 10 μ L
- Luminol-Peroxide Mix, 15 μ L (for chemiluminescence only)
- Wash Buffer 500 μ L/compartments

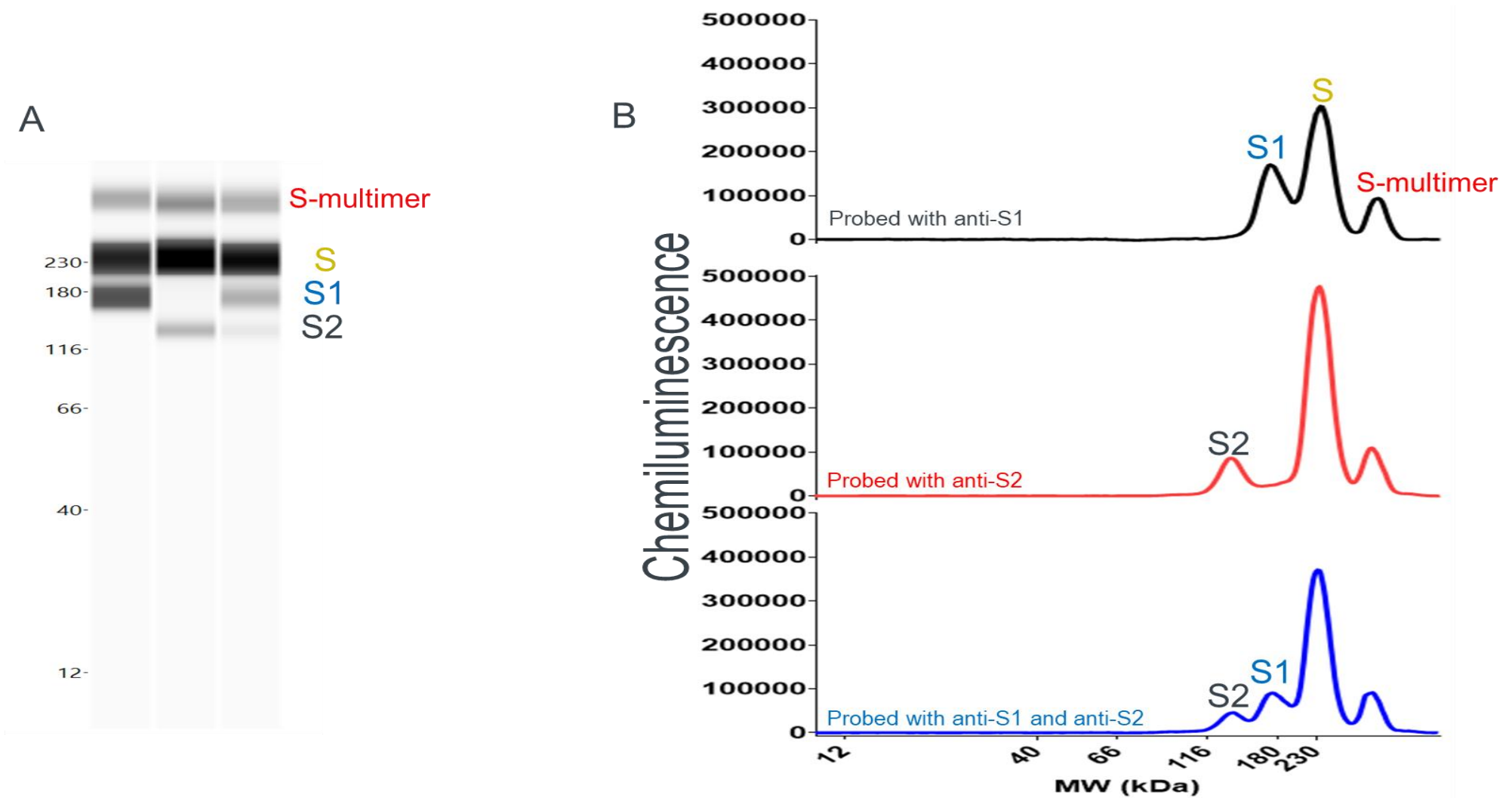
Evaporation sensitive

Peel off immediately before placing in instrument

proteinsimple

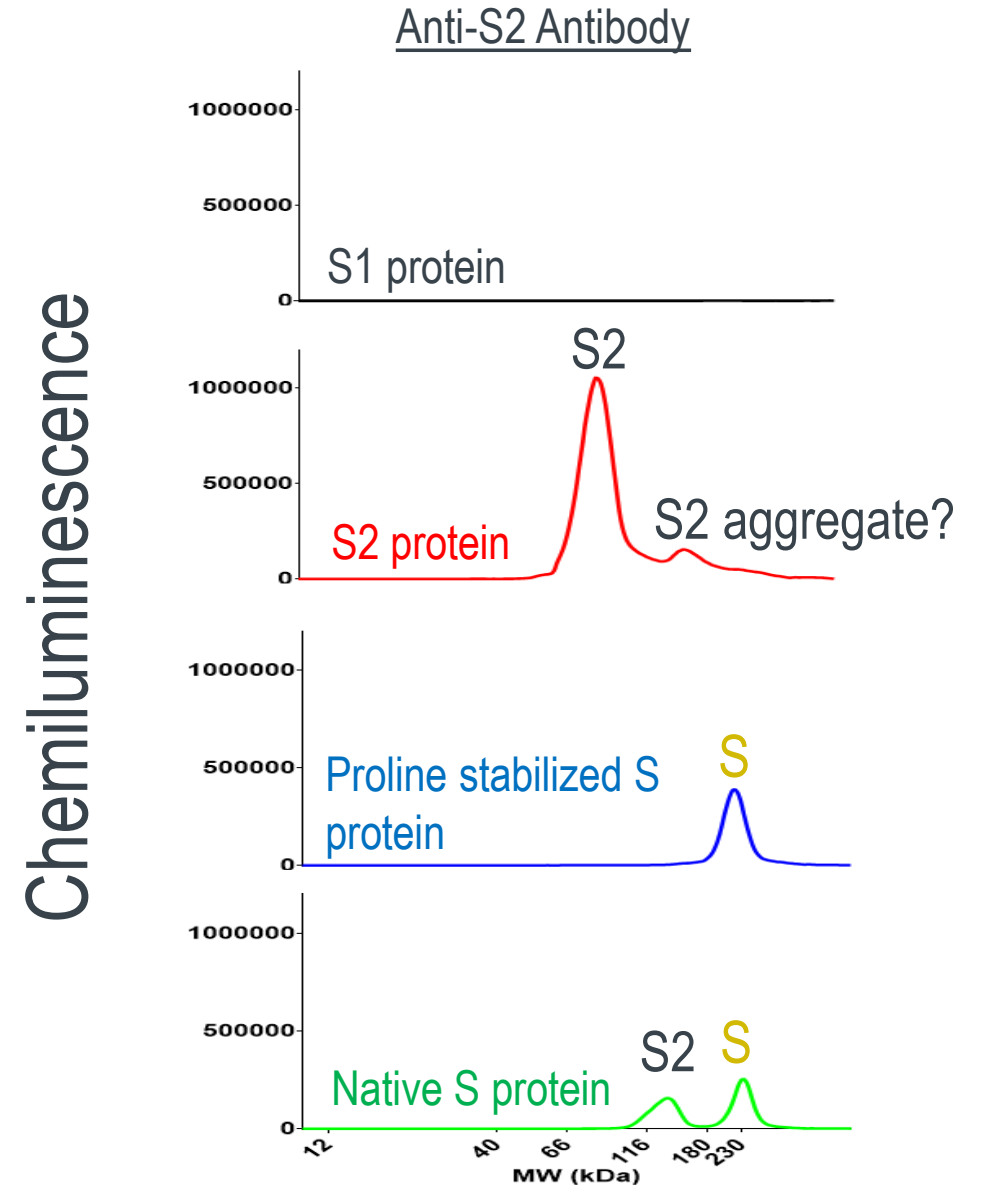
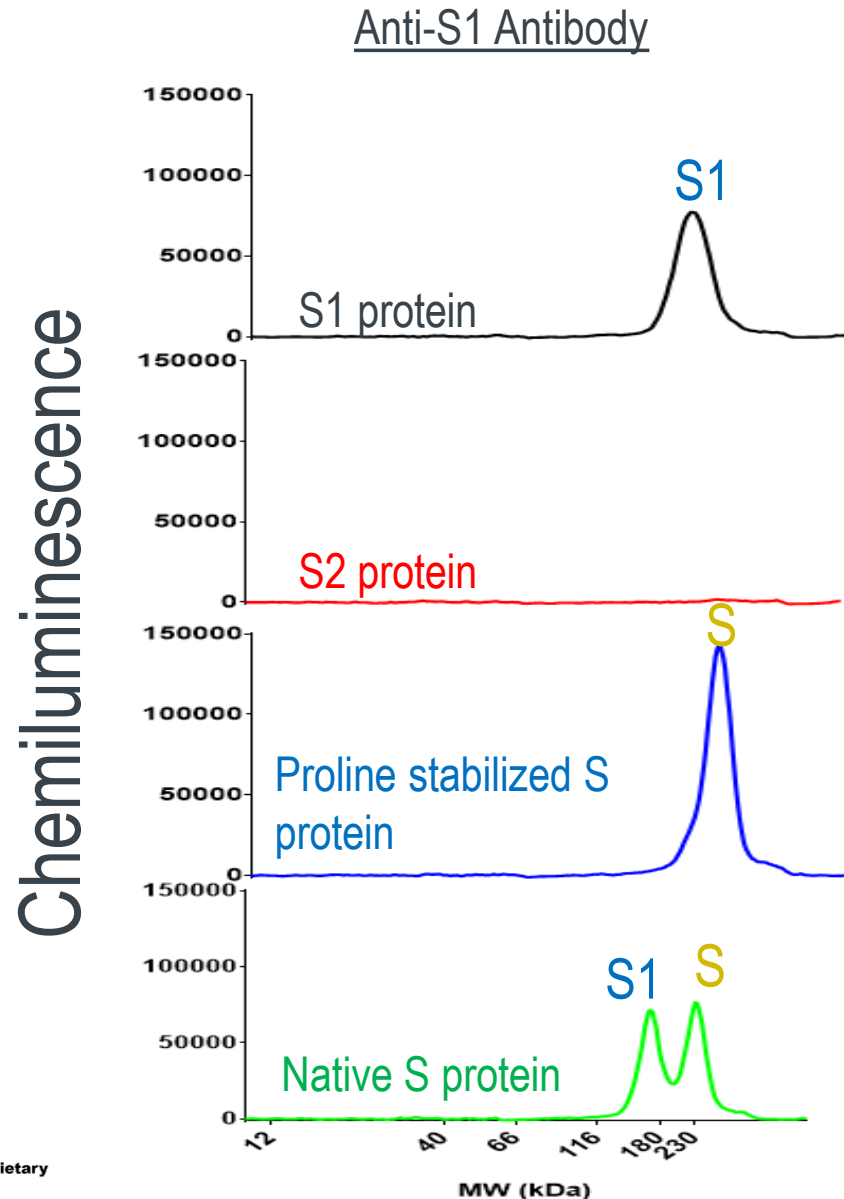
Antibody Screening against V590 Drug Substance

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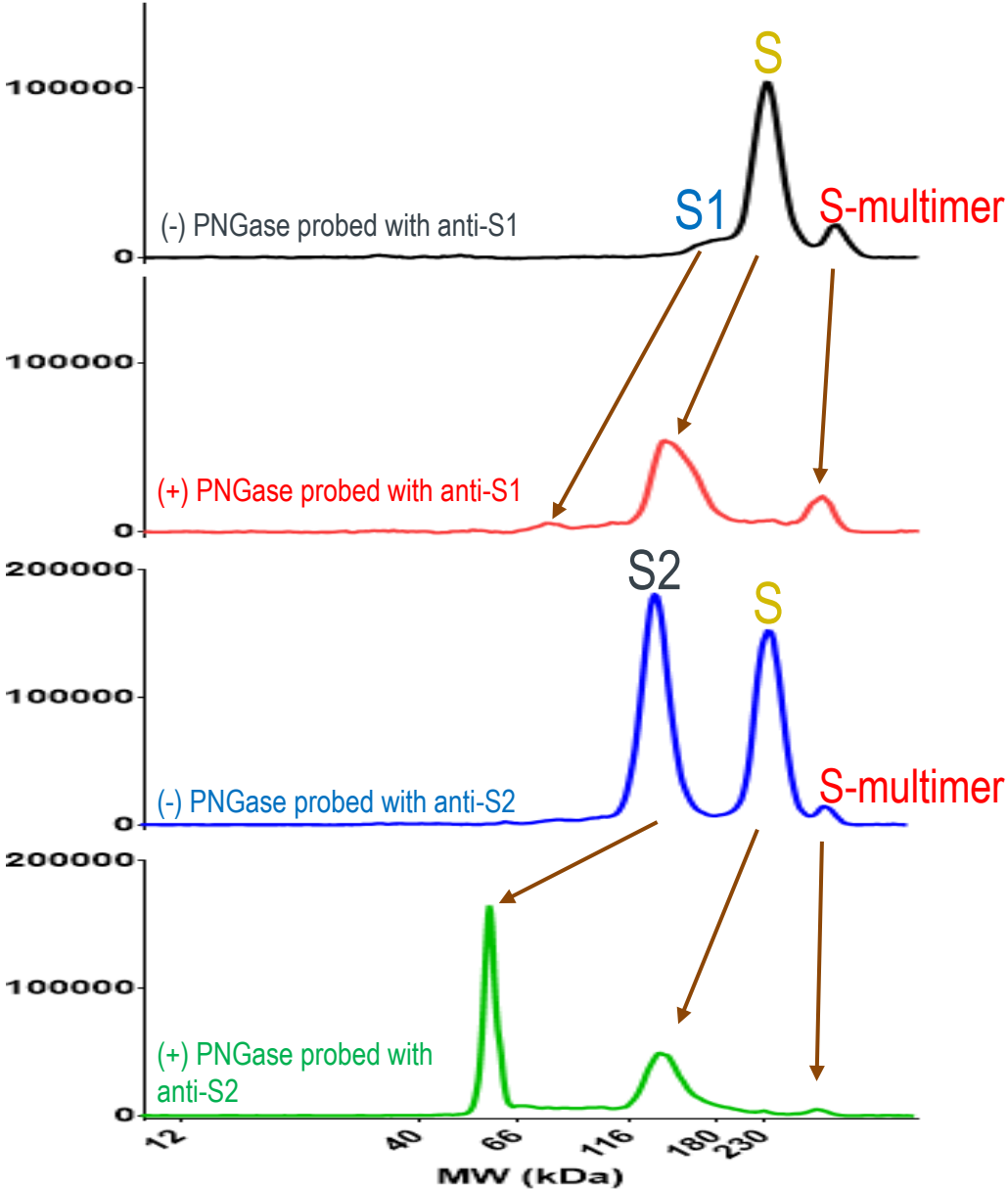
Antibody Screening against S Protein and Subunits

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S Protein Molecular Weight Confirmation

Chemiluminescence



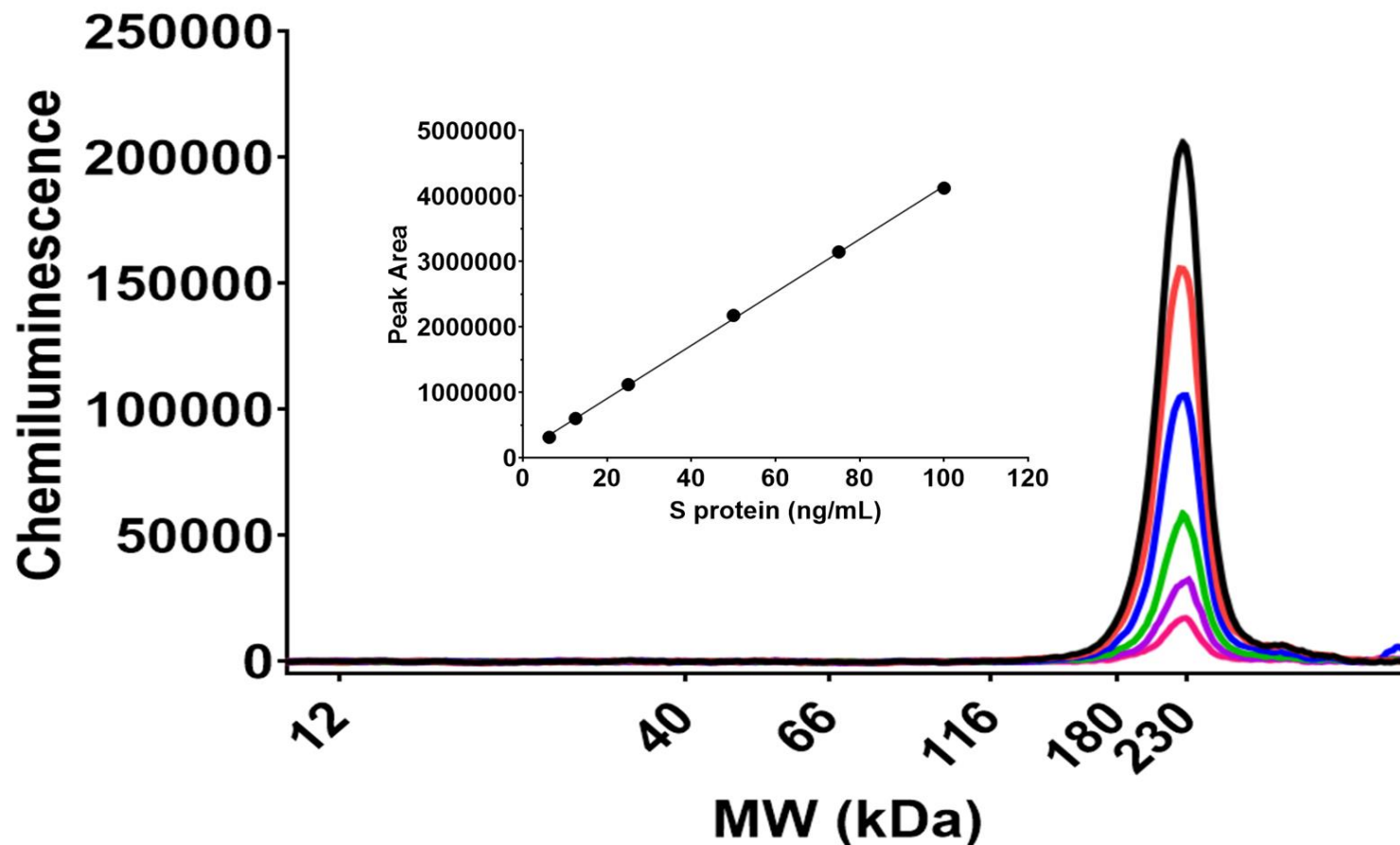
Peak Identity	Glycosylated V590 MW-Control (kDa)	Deglycosylated V590 MW-PNGase Treated (kDa)	Approximate Deglycosylated Theoretical MW (kDa)	# N-linked Glycan Sites
S Protein	227	136	135	22
S1 Subunit	167	72	75	13
S2 Subunit	135	60	64.5	9

Standard Curve using Recombinant S protein as Reference Standard

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- Reference Standard: Proline stabilized recombinant S protein
- Linear region: 6.25-100ng/mL
- $R^2 > 0.99$



Summary of S Protein Simple Western™ Assay Qualification Design



- Intermediate Precision
 - 4 days
 - 2 analysts
 - 2 instruments
 - Wes and Jess models
 - 2 primary antibody lots
- Accuracy
 - 4 spike levels
- Linearity
 - 6 levels evaluated
- Repeatability
 - Difficult to assess true repeatability

Run	Day	# Reportable Results	Parameter(s) Evaluated
1	1	6	Intermediate Precision, Specificity
2	2	6	Intermediate Precision
3	3	6	Intermediate Precision
4	4	6	Intermediate Precision
5	5	12	Accuracy
6	6	18	Linearity, Repeatability

Summary of S Protein Simple Western™ Assay Qualification Results

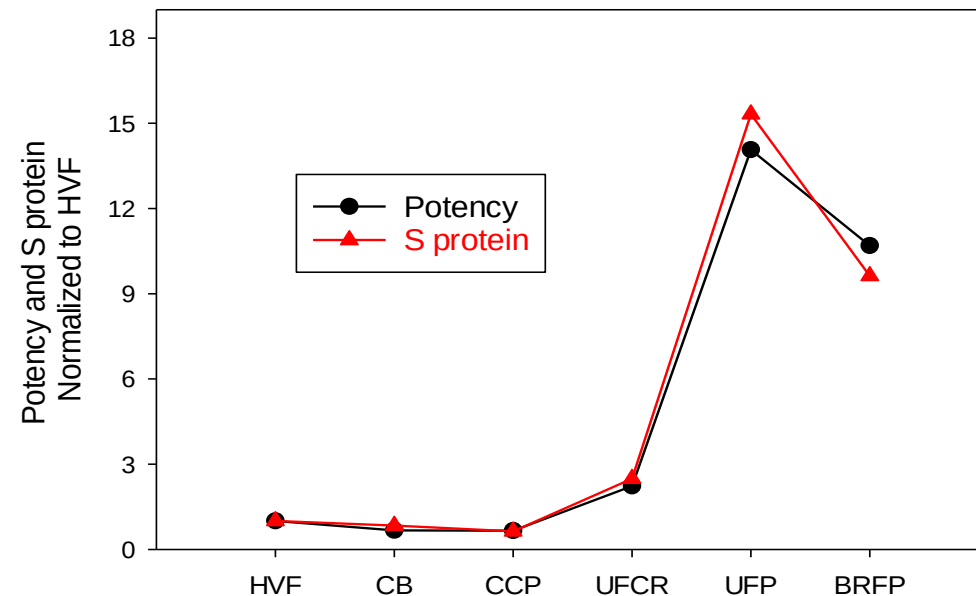
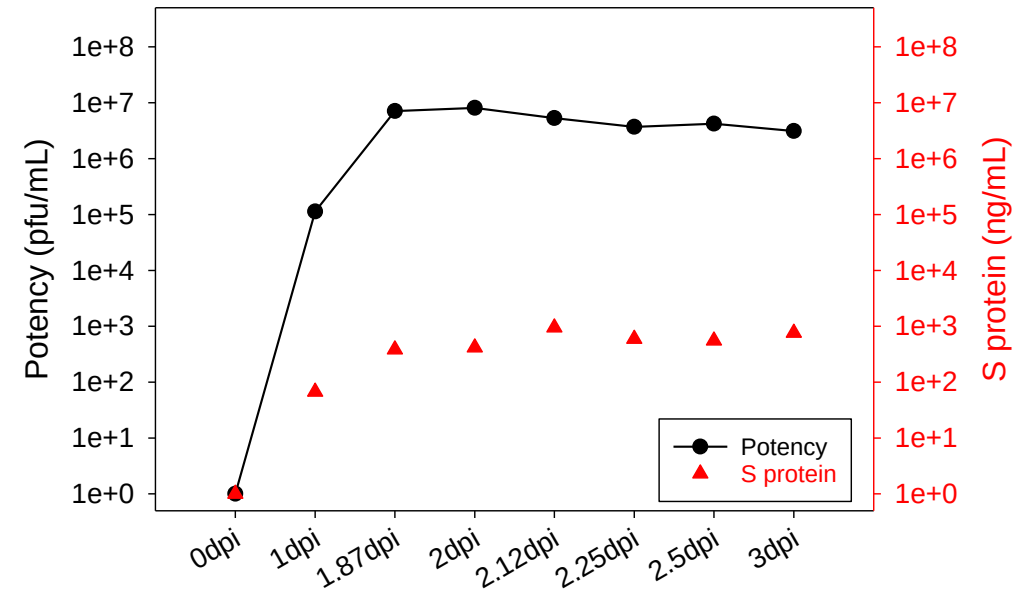
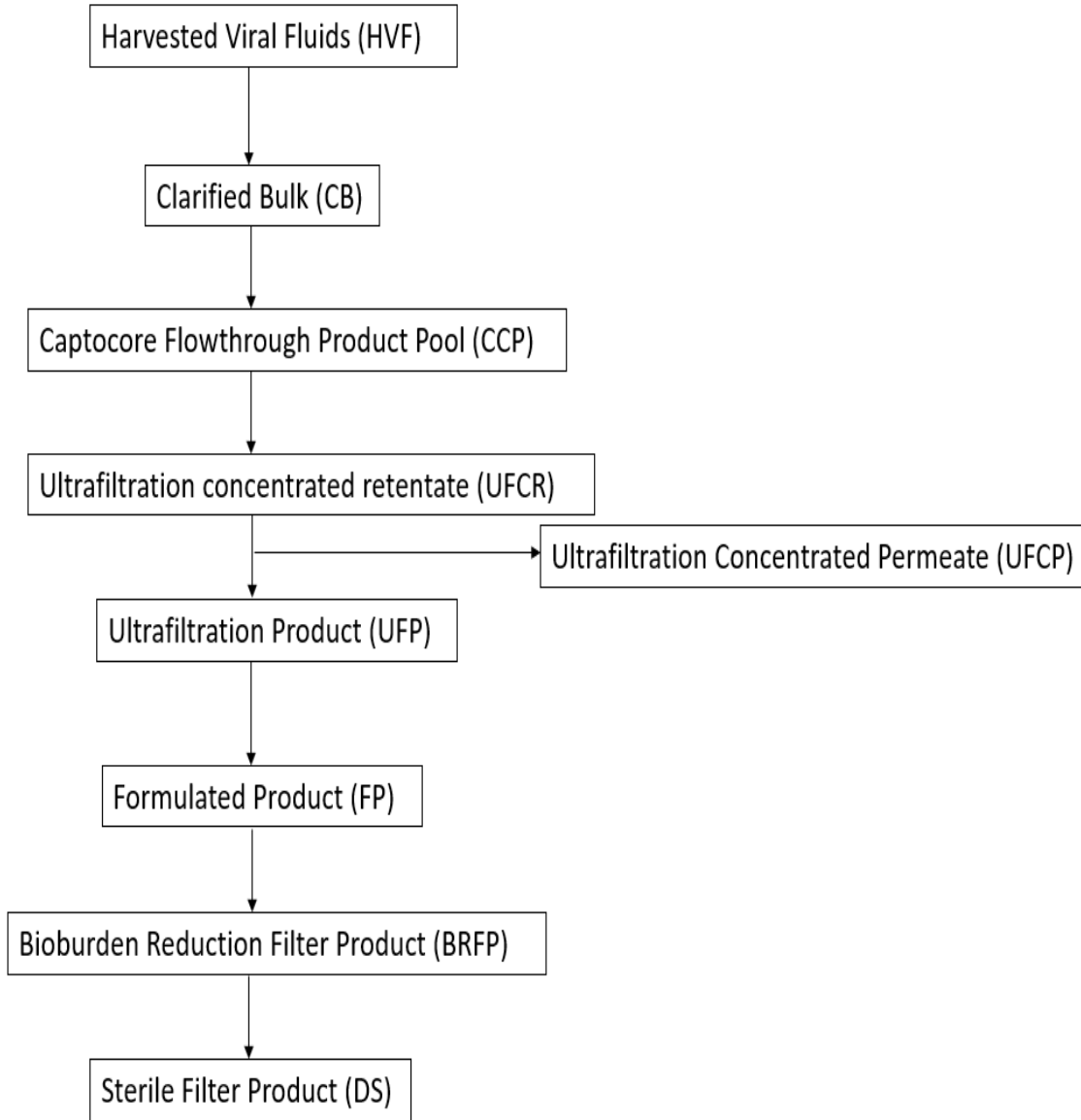
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Performance Characteristic	Results	
Repeatability	12.3% Relative Standard Deviation (RSD)	
Intermediate Precision (including day, analyst, instrument and primary antibody lot)	13.8% RSD	
Linearity	$R^2=0.997$	
	% Proportional Bias= 6.62%	
Accuracy	Spike Level (ng/mL)	Average % Recovery
	6.25 ng/mL	62%
	12.5 ng/mL	84%
	50 ng/mL	102%
	100 ng/mL	95%
Specificity	No peaks present in Drug Substance or Drug Product placebo sample electropherogram greater than or equal to the 6.25 ng/mL standard	
Range	6.25 ng/mL-100 ng/mL	
Limit of Quantitation (LOQ)	6.25 ng/mL	

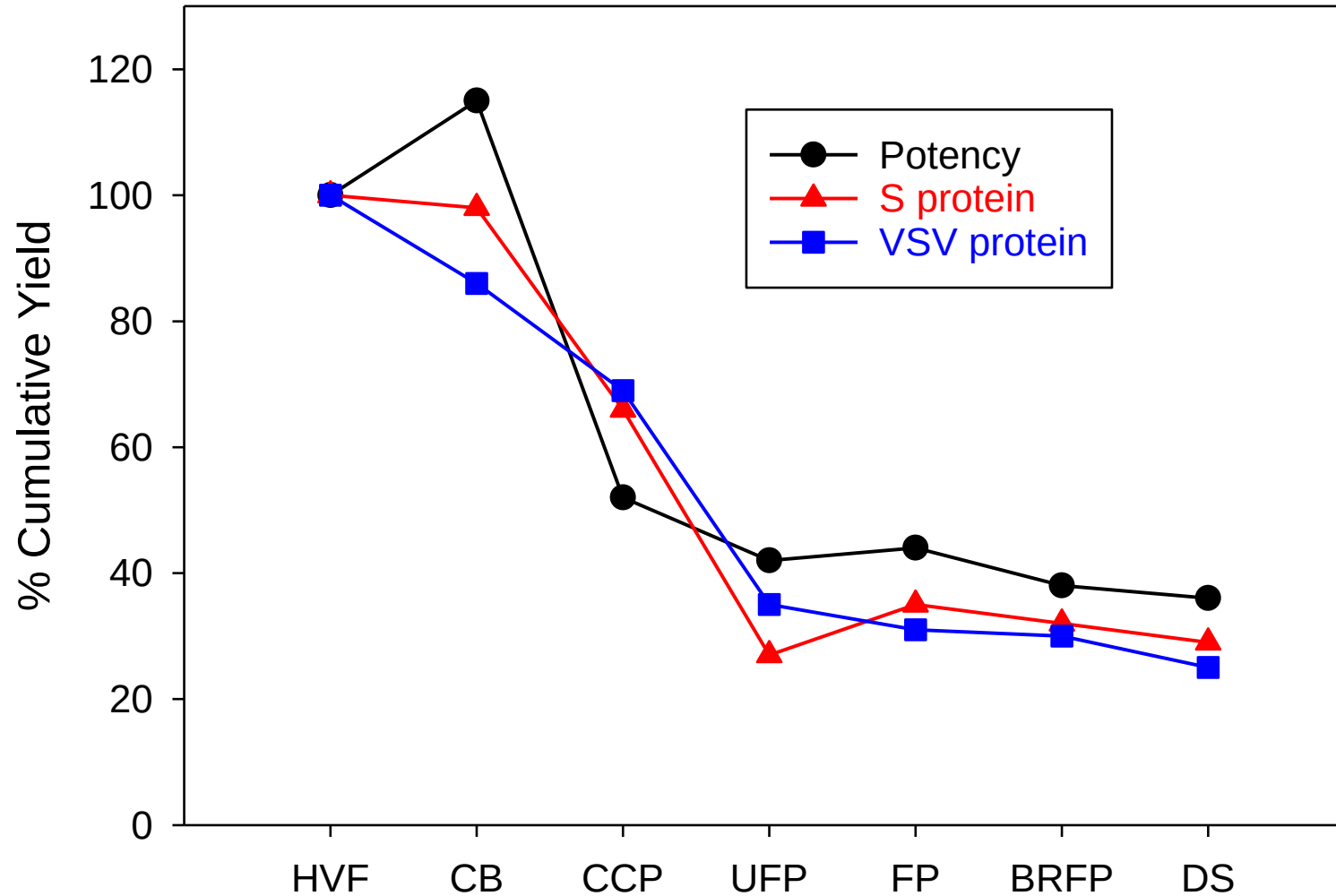
Correlation of S Protein Concentration and Potency across V590 Manufacturing Process

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Correlation of S Protein Concentration and Potency across V590 Manufacturing Process (Continued)

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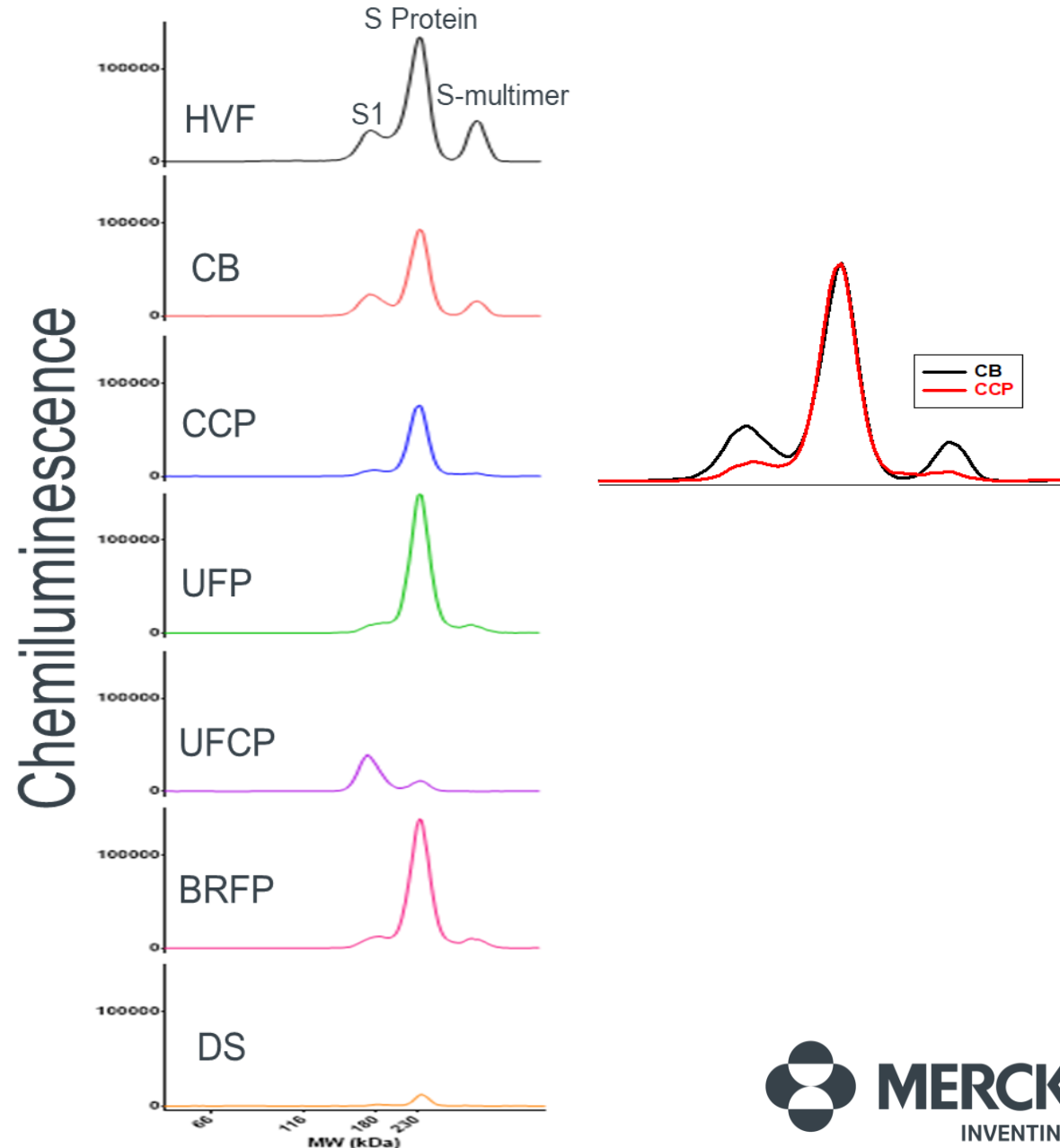


Qualitative S Protein Profiling of Process Intermediates

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- S protein profiling for process intermediates
- Captocore 700 chromatography

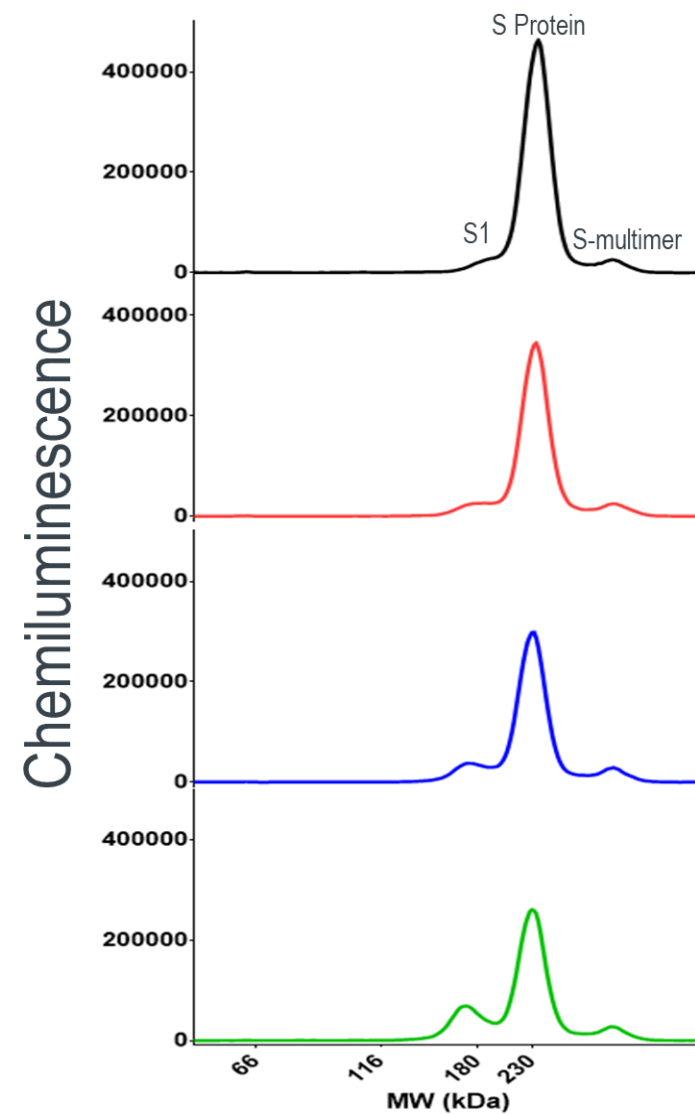
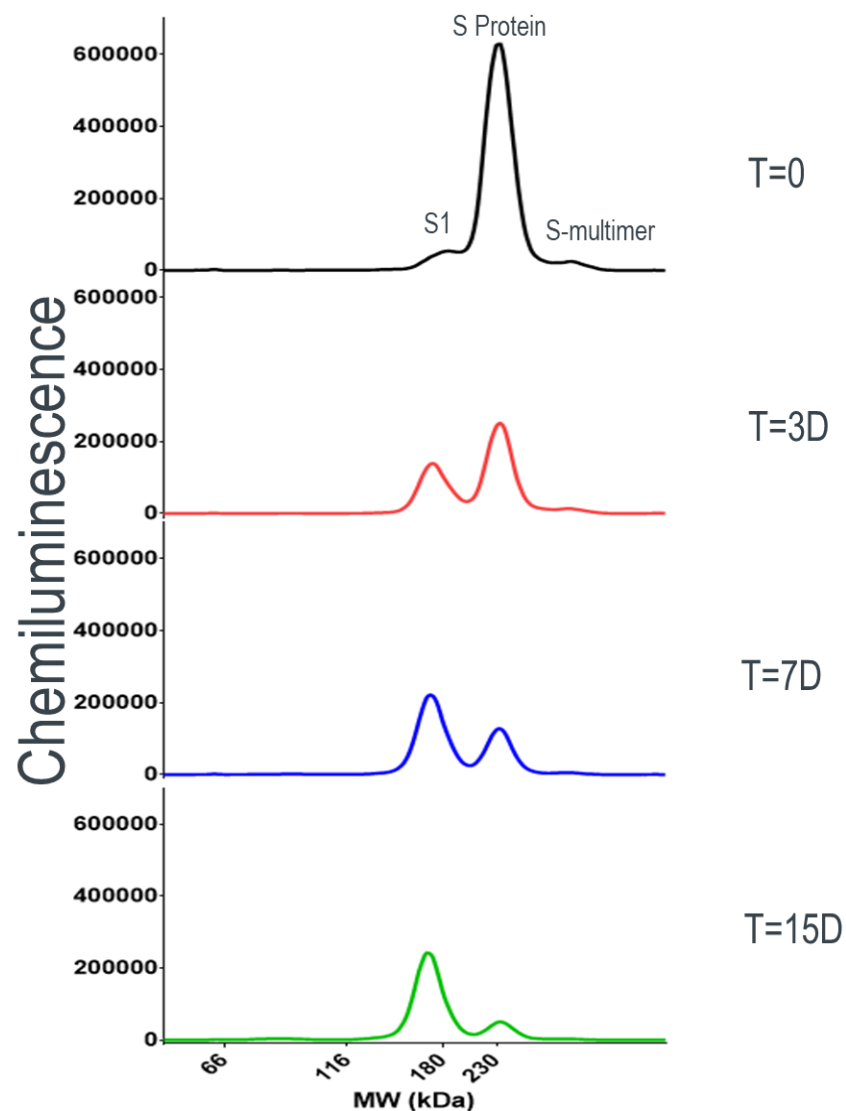


Qualitative S Protein Profiling to Understand Stability of the V590 Drug Substance between Two Different Processes

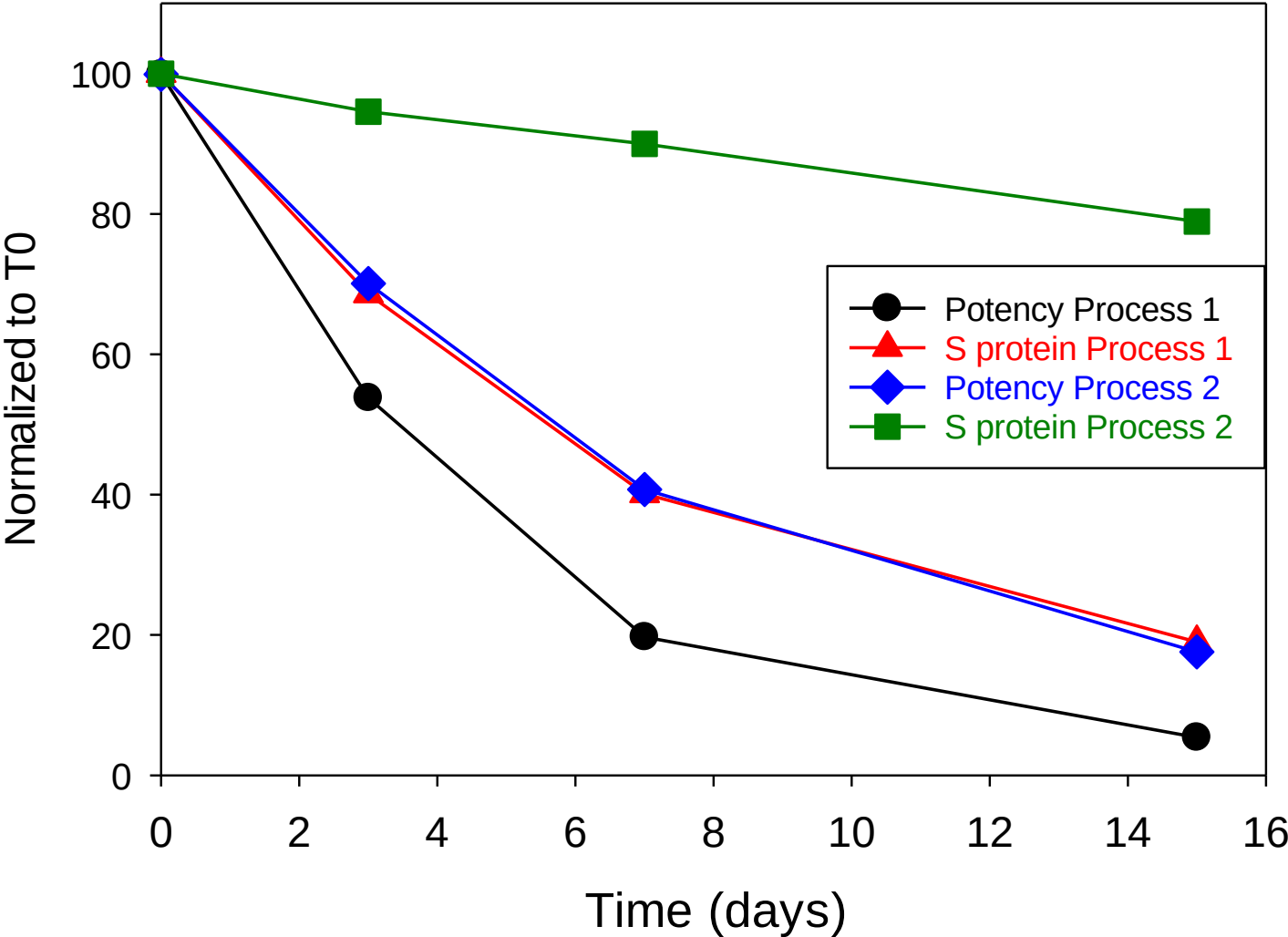
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- Stability study
@ 25°C for
15 days

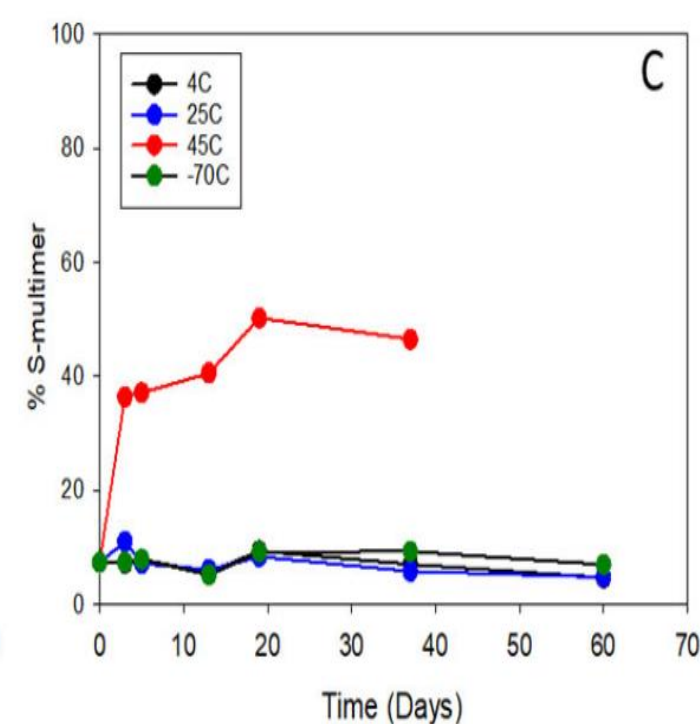
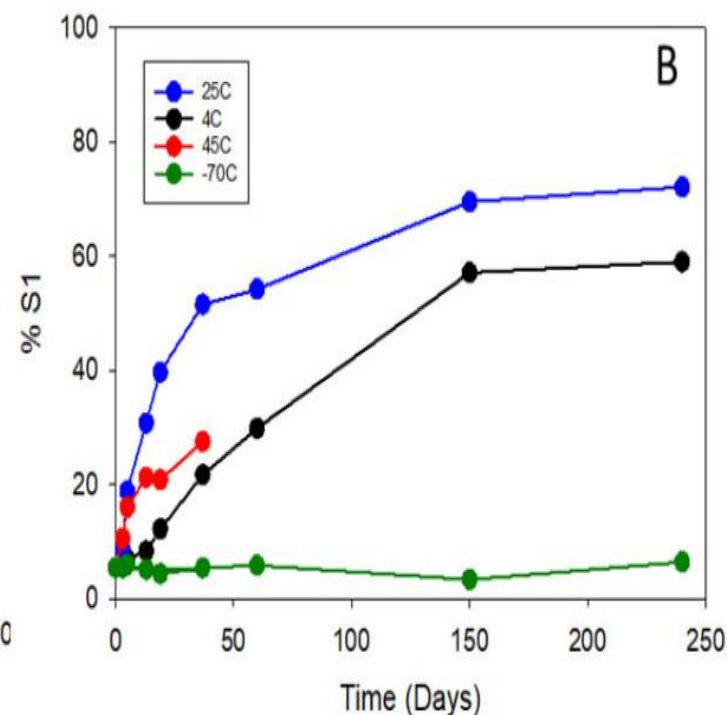
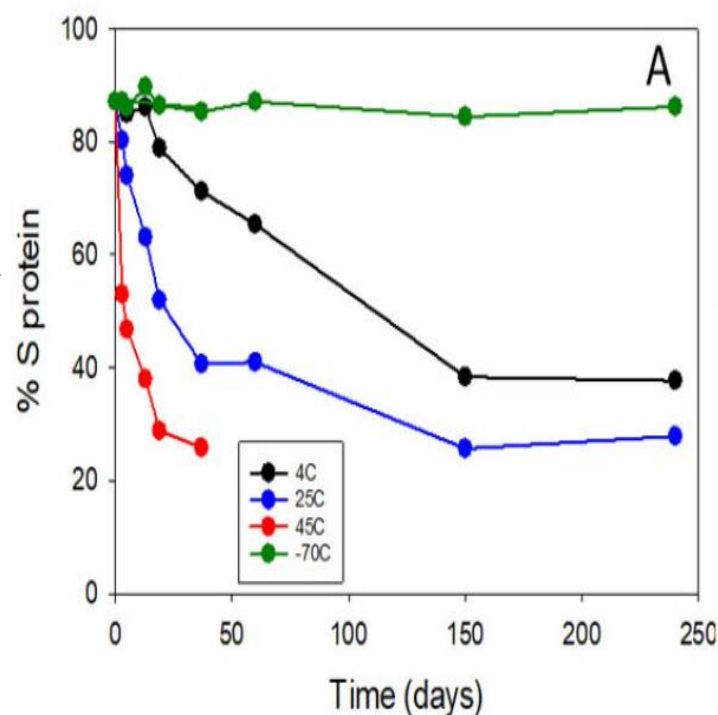


Understanding Stability of the V590 Drug Substance (Continued)



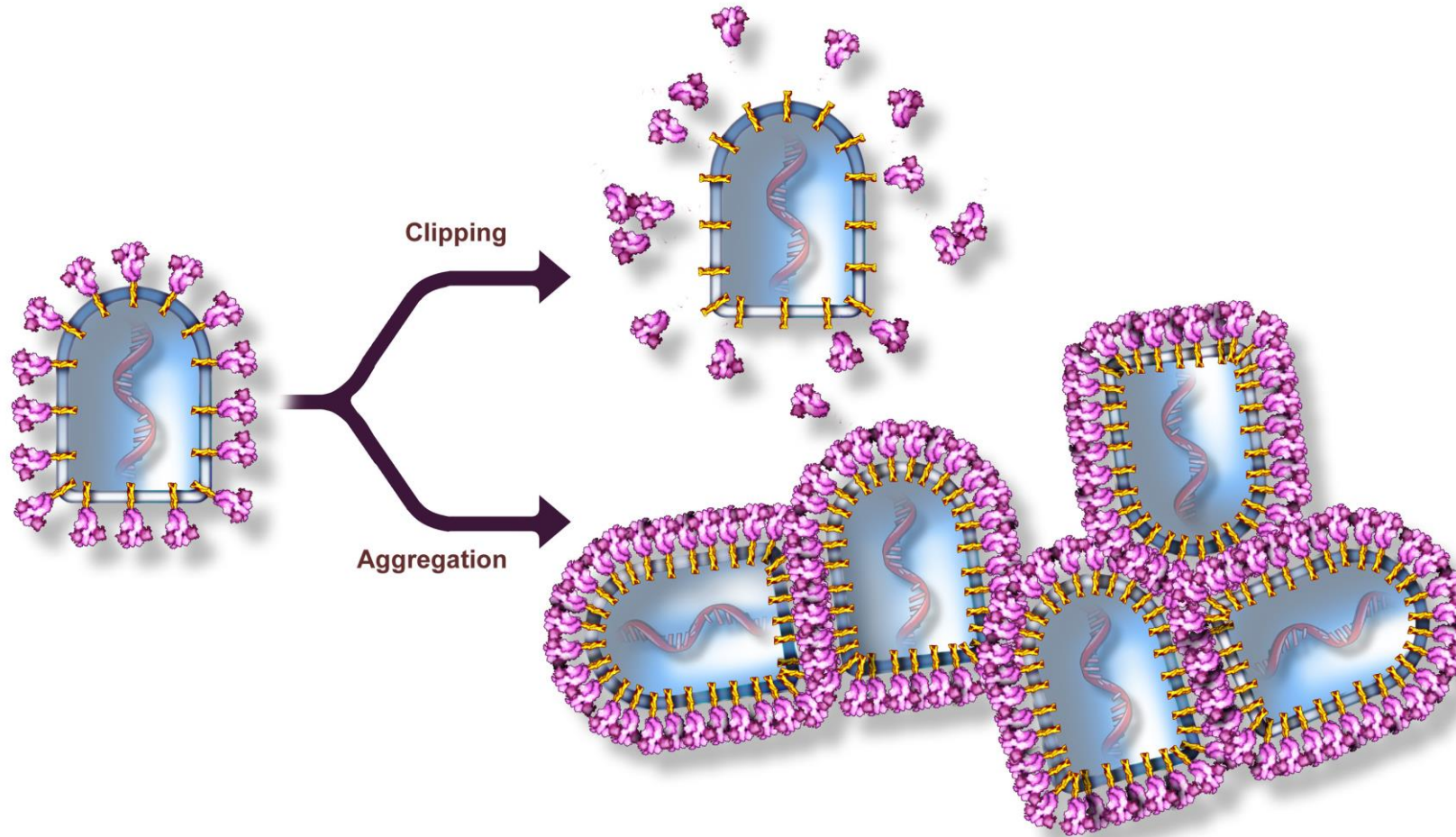
Qualitative S Protein Profiling to Understand Stability of the V590 Drug Substance (continued)

- Additional stability study from -70°C to 45°C



S Protein Degradation Mechanism Hypothesis

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Conclusions

- S protein Simple Western™ assay successfully developed and phase appropriately qualified
- S protein measurements correlated well with potency data (possible link?)
- Qualitative S protein profile provided additional stability information about V590 DS
- S protein degradation mechanism hypothesis

Acknowledgements

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- Nisarg Patel

References

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2. Jackson, Cody B et al. “Mechanisms of SARS-CoV-2 entry into cells.” *Nature reviews. Molecular cell biology* vol. 23,1 (2022): 3-20. doi:10.1038/s41580-021-00418-x
3. Robbins, Jonathan A et al. “Safety and immunogenicity of intramuscular, single-dose V590 (rVSV-SARS-CoV-2 Vaccine) in healthy adults: Results from a phase 1 randomised, double-blind, placebo-controlled, dose-ranging trial.” *EBioMedicine* vol. 82 (2022): 104138. doi:10.1016/j.ebiom.2022.104138
4. Simple Western Size Assay Development Guide. P/N 042-889 Revision 1, March 2014
5. Gillespie, Paul F et al. “Understanding the Spike Protein in COVID-19 Vaccine in Recombinant Vesicular Stomatitis Virus (rVSV) Using Automated Capillary Western Blots.” *ACS omega* vol. 8,3 3319-3328. 10 Jan. 2023, doi:10.1021/acsomega.2c06937



Backup Slides



#	Name	Manufacturer / Catalog number	Species/ Ab Type	Simplewestern Signal
1	Anti-SARS-CoV-2 Spike antibody	Genetex/GTX135356	Rabbit/pAb	++
2	Anti-SARS-CoV-2 Spike/RBD antibody	Sino Biological/40592-T62	Rabbit/pAb	+++++
3	Anti-SARS-CoV-2 Spike antibody	Sino Biological/40150-R007	Rabbit/IgG mAb	--
4	Anti-SARS-CoV-2 Spike (17aa peptide central region 550-600aa)	ProSci/3223	Rabbit/pAb	--
5	Anti-SARS-CoV-2 Spike (20aa peptide C-term region S protein)	ProSci/3525	Rabbit/pAb	--
6	Anti-SARS-CoV-2 Spike antibody (Clone AM001414)	Active Motif/91361	Human/mAb	--
7	Anti-SARS-CoV-2 (Covid-19) Spike/RDB antibody (Clone AM004414)	Active Motif/91345	Human/mAb	--
8	Anti-SARS-CoV-2 (Covid-19) Spike/RBD antibody (Clone AM005415)	Active Motif/91347	Human/mAb	--
9	Anti-SARS-CoV-2 (Covid-19) Spike antibody (Clone AM006415)	Active Motif/91351	Human/mAb	--
10	Anti-SARS-CoV-2 (Covid-19) Spike antibody (Clone AM032414)	Active Motif/91359	Human/mAb	--
11	Anti-SARS-CoV-2 (Covid-19) Spike antibody	Invitrogen/PA581795	Rabbit/pAb	+++
12	Anti-SARS-CoV-2 (Covid-19) Spike antibody	Novus/NB100-56578	Rabbit/pAb	+++
13	Anti-SARS-CoV-2 (Covid-19) Spike antibody 3A2	Abcam/AB272420	Mouse/mAb	--
14	Anti-SARS-CoV-2 (Covid-19) Spike antibody 1A9	Genetex/GTX632604	Mouse/mAb	--
15	Anti-SARS-CoV-2 (Covid-19) Spike antibody AM002414	Active Motif/91349	Human/mAb	--
16	Anti-SARS-CoV-2 (Covid-19) Spike antibody AM009105	Active Motif/91337	Human/mAb	--
17	Anti-SARS-CoV-2 (Covid-19) Spike antibody AM043105	Active Motif/91341	Human/mAb	--
18	Anti-SARS-CoV-2 (Covid-19) Spike antibody (N-term)	Genetex/GTX135360	Rabbit/pAb	--
19	Anti-SARS-CoV-2 (Covid-19) Spike antibody (centre)	Genetex/GTX135384	Rabbit/pAb	+
20	Anti-SARS-CoV-2 (Covid-19) Spike antibody (C-term)	Genetex/GTX135385	Rabbit/pAb	+
21	Anti-S1	MP/08720301	Mouse/mAb	--
22	Anti-S neutralizing	Sino Biological/40591-MM43	Mouse/mAb	--
23	Anti-S neutralizing	Sino Biological/40592-MM57	Mouse/mAb	+
24	Anti-S1 (Fc human, Fab mouse)	Sino Biological/40150-D003	Chimera/mAb	--
25	Anti-S (AM038105)	Active Motif/91339	Human/mAb	--
26	Anti-S2	MP/720411	Mouse/mAb	--
27	Anti-S2	Sino Biological/40590-T62	Rabbit/pAb	+++++

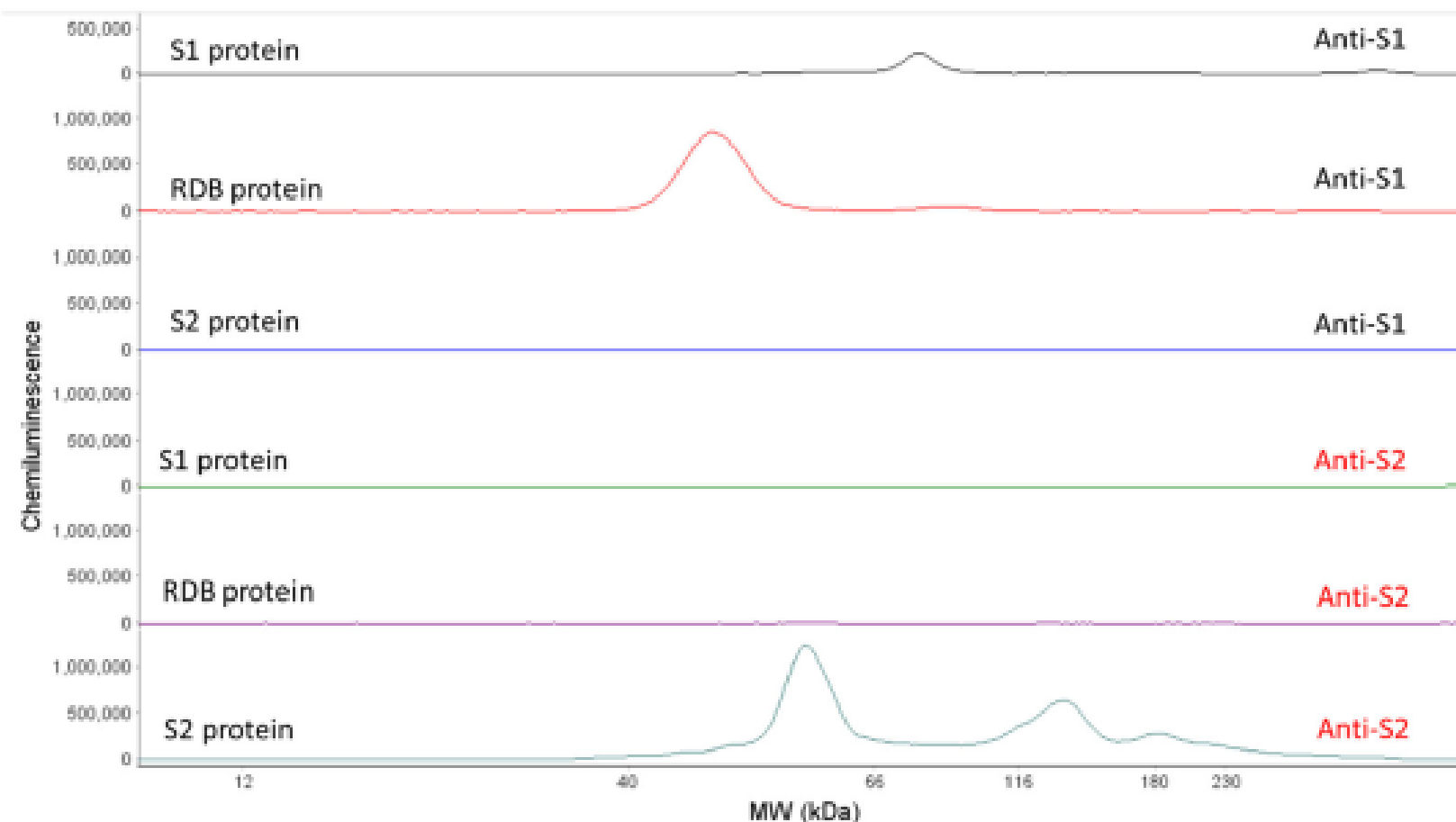
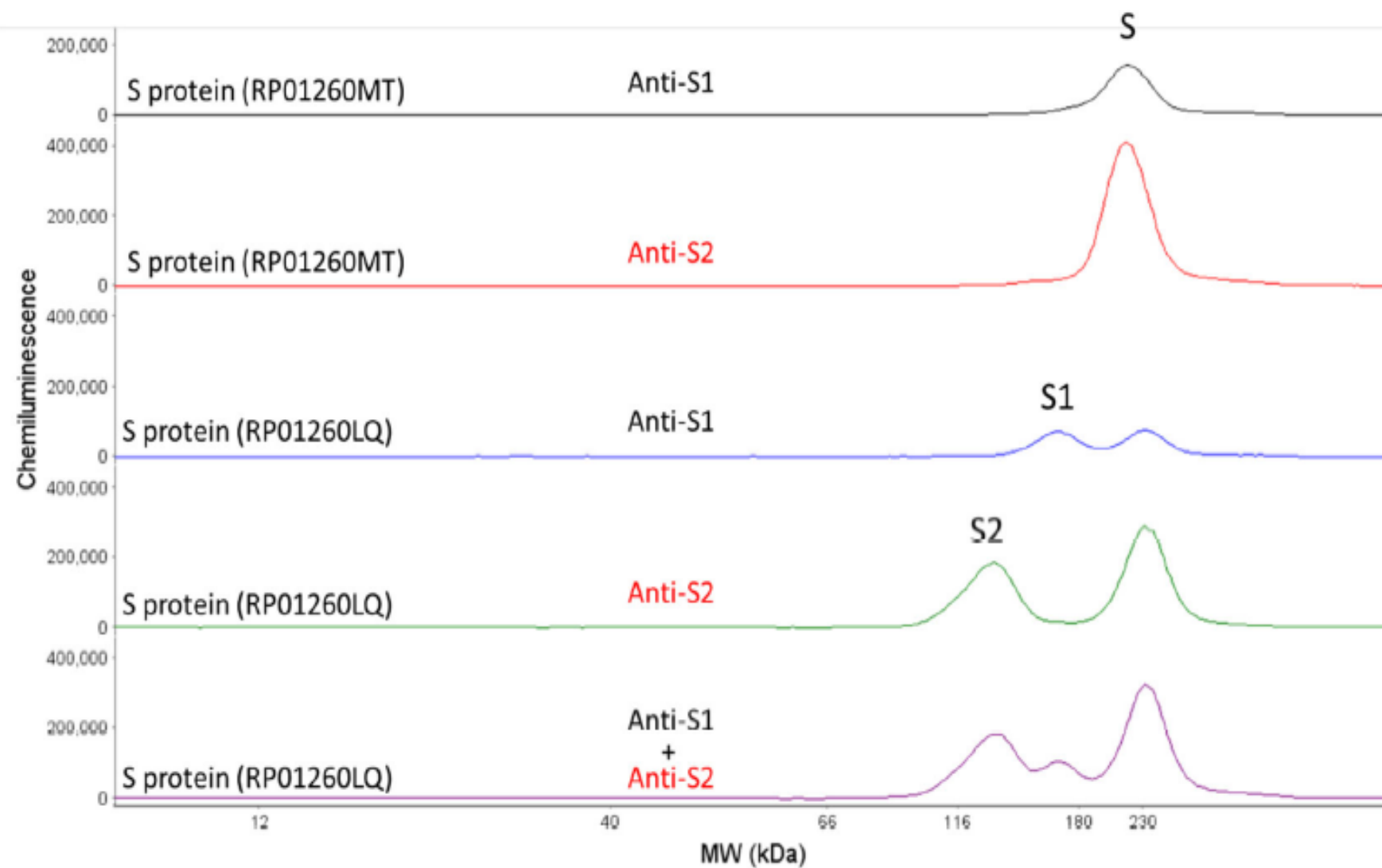


Figure S3A: Electropherograms of S1, RBD, and S2 recombinant proteins probed with two rabbit pAbs: Anti-S1 and Anti-S2.



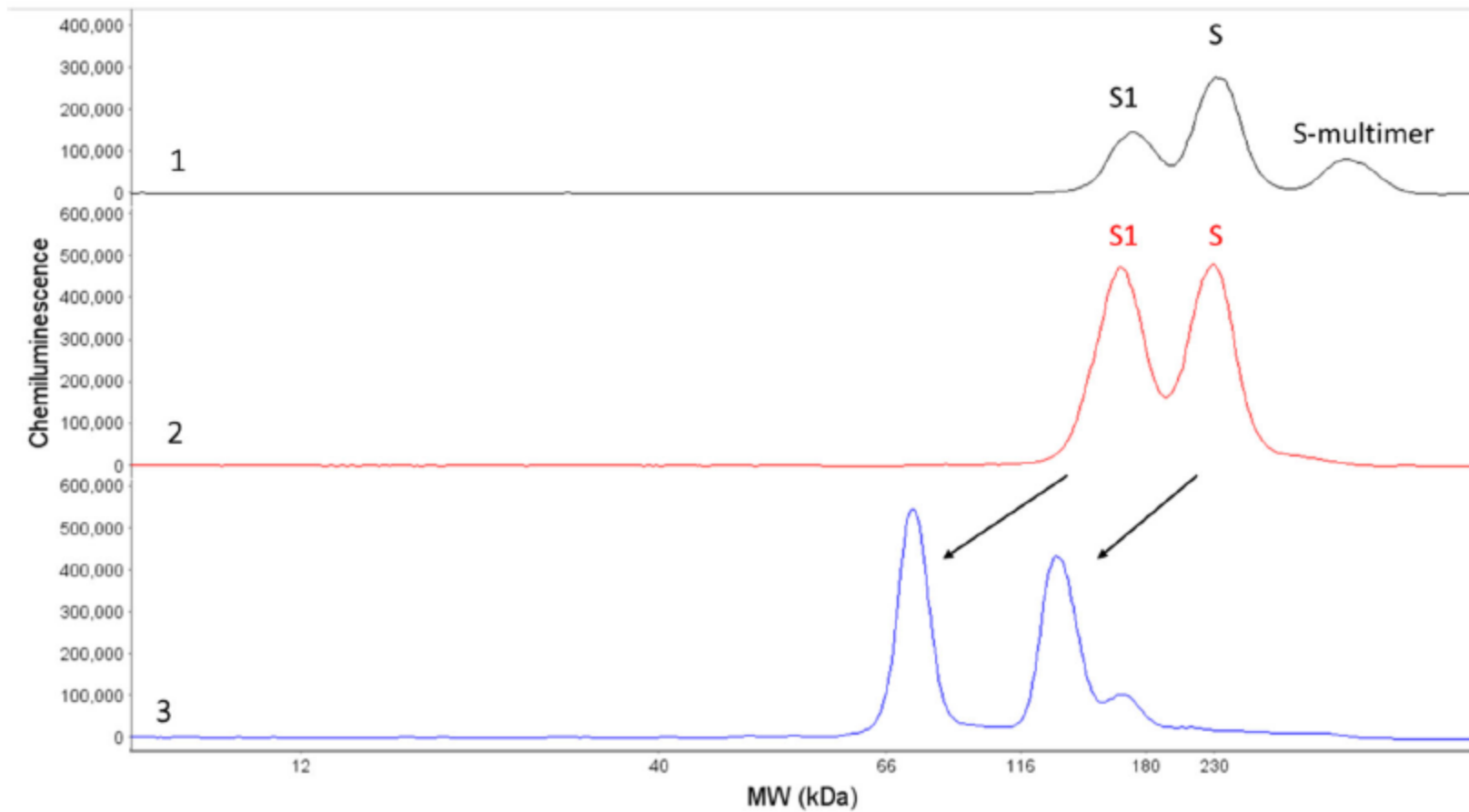


Figure S4A: Electropherograms of glycosylated V590 HVF sample (trace 1), glycosylated recombinant wild type S protein (trace 2), and PNGase F-treated recombinant wild type S protein (trace 3) probed with anti-S1 rabbit pAb. Note the extra shoulder in trace 3 at 170kDa is likely due to incomplete PNGase F digestion of S1.

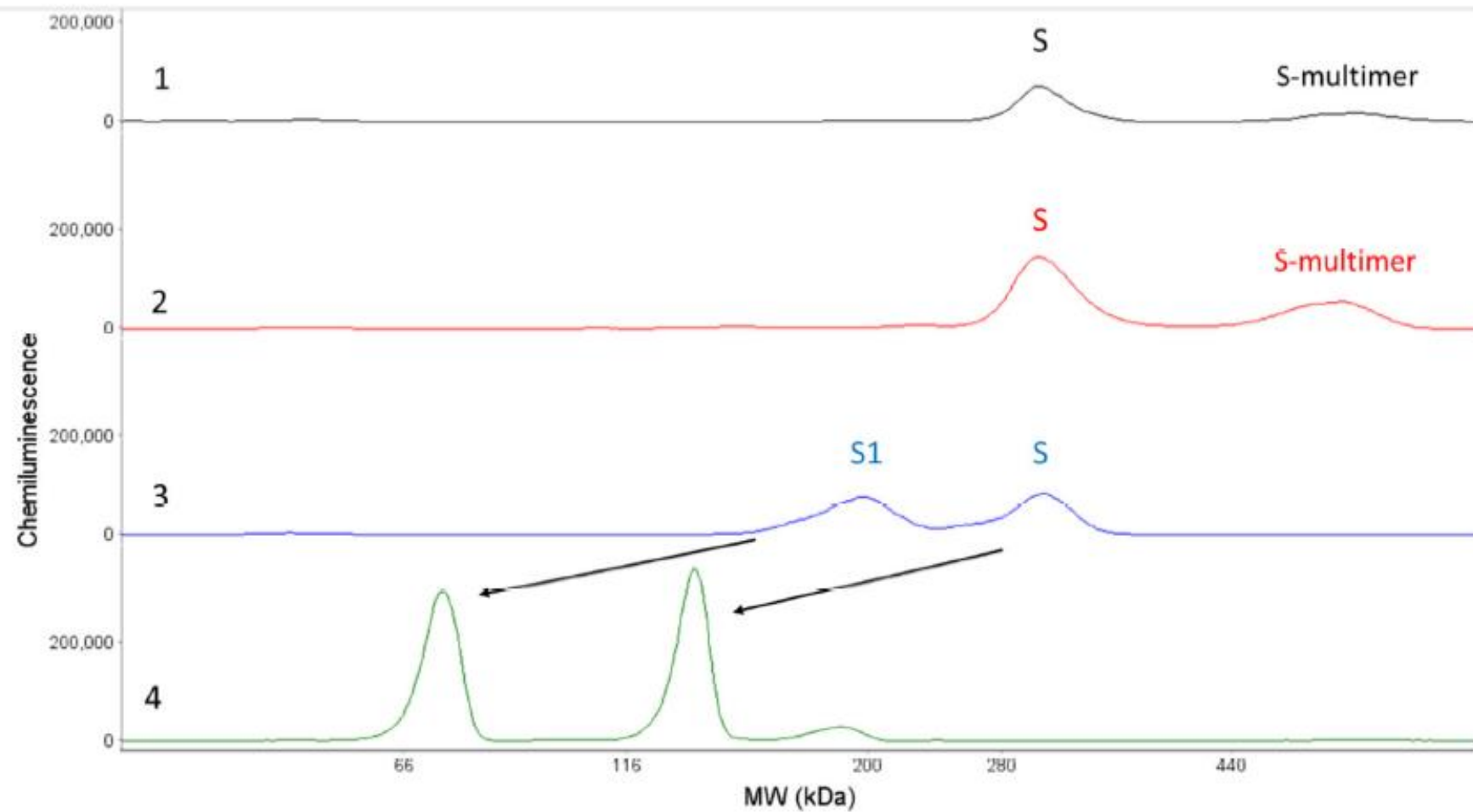
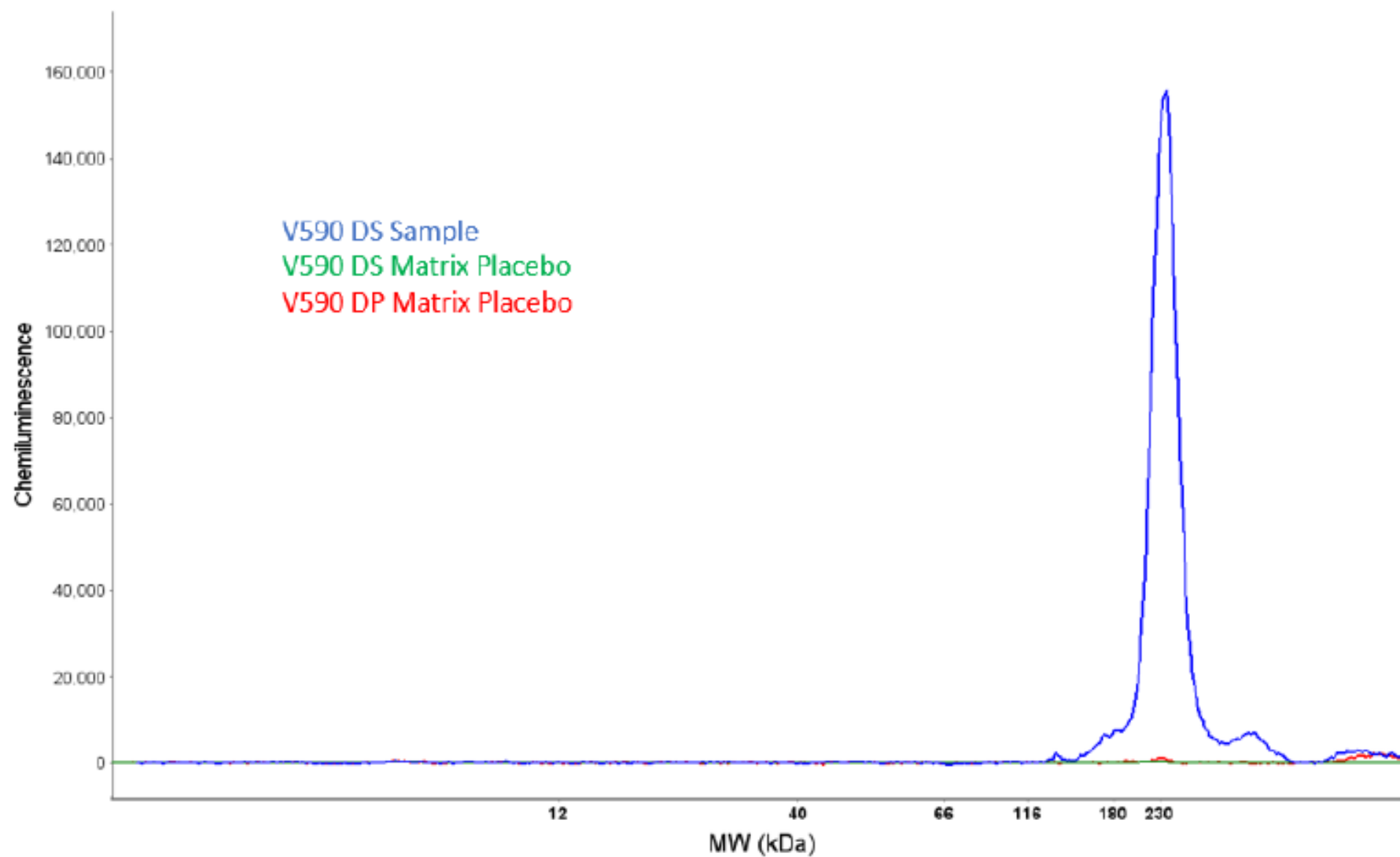


Figure S4B: Electropherograms of glycosylated V590 CB sample probed with anti-S1 (trace 1), anti-S2 (trace 2); glycosylated recombinant wild type S protein (trace 3) and PNGase F-treated recombinant wild type S protein (trace 4) were probed with anti-S1.



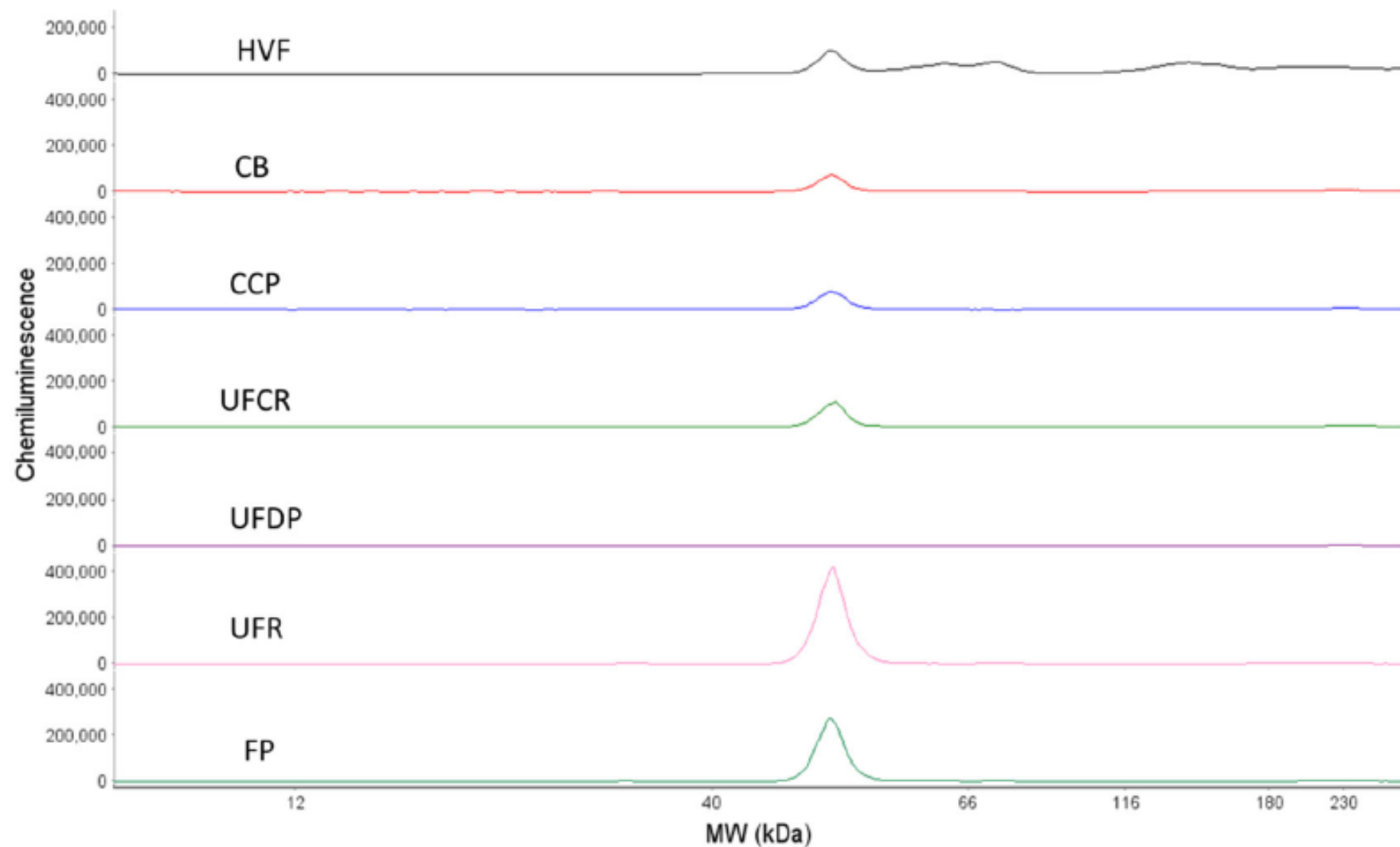


Figure S6A: Electropherograms of various purification process intermediates monitored by rVSV protein.

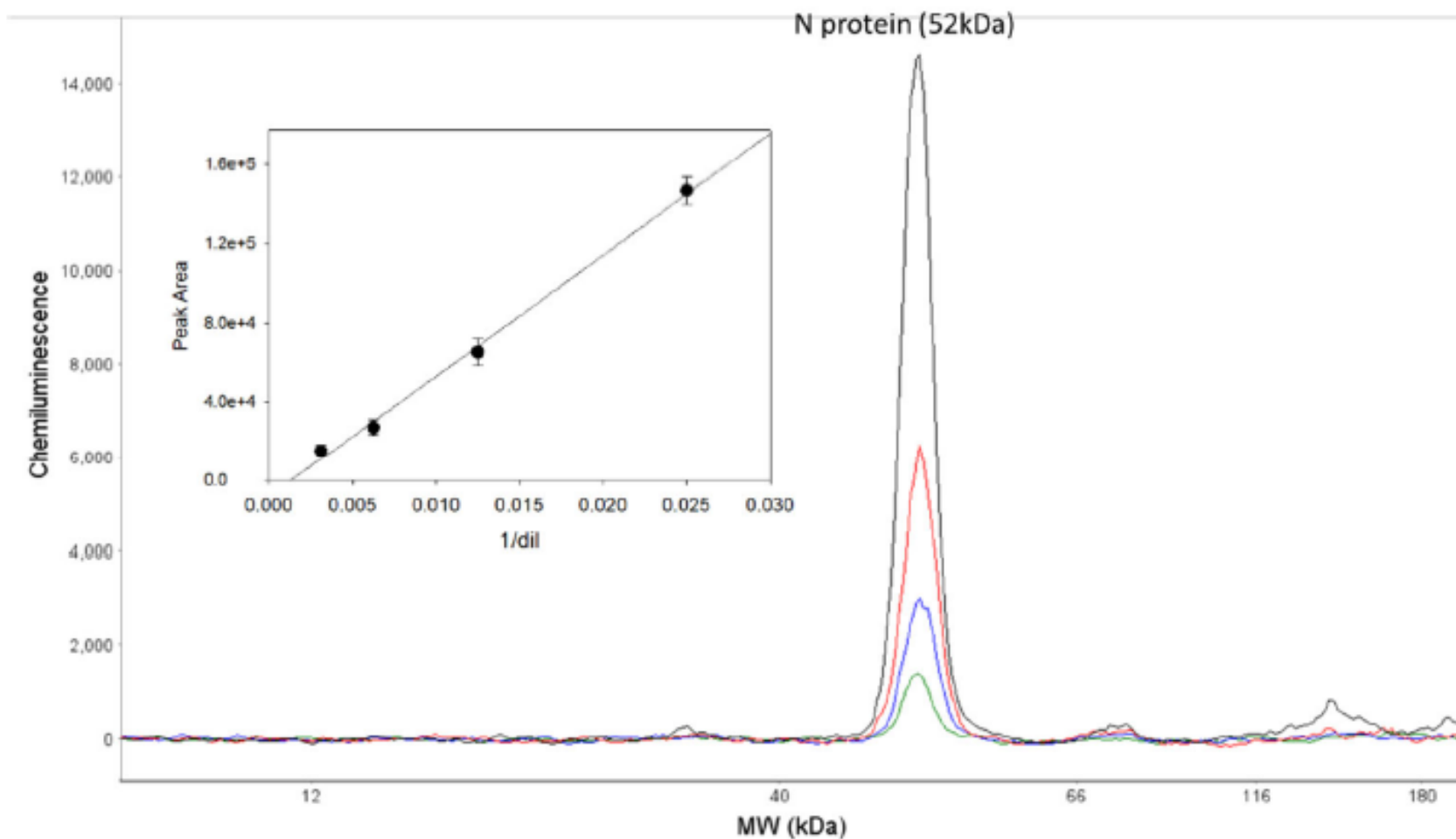
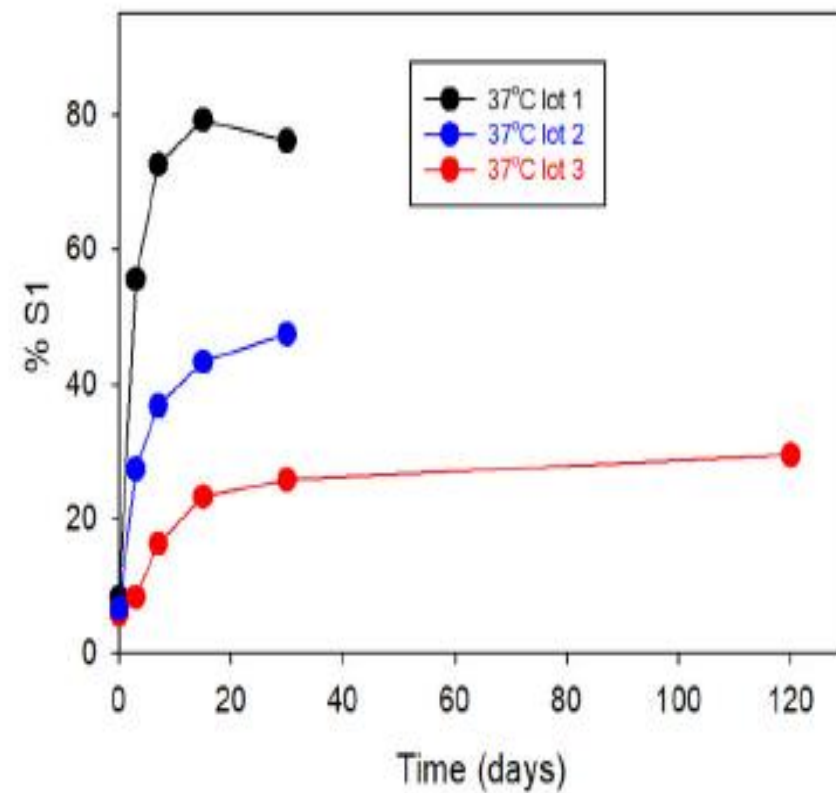
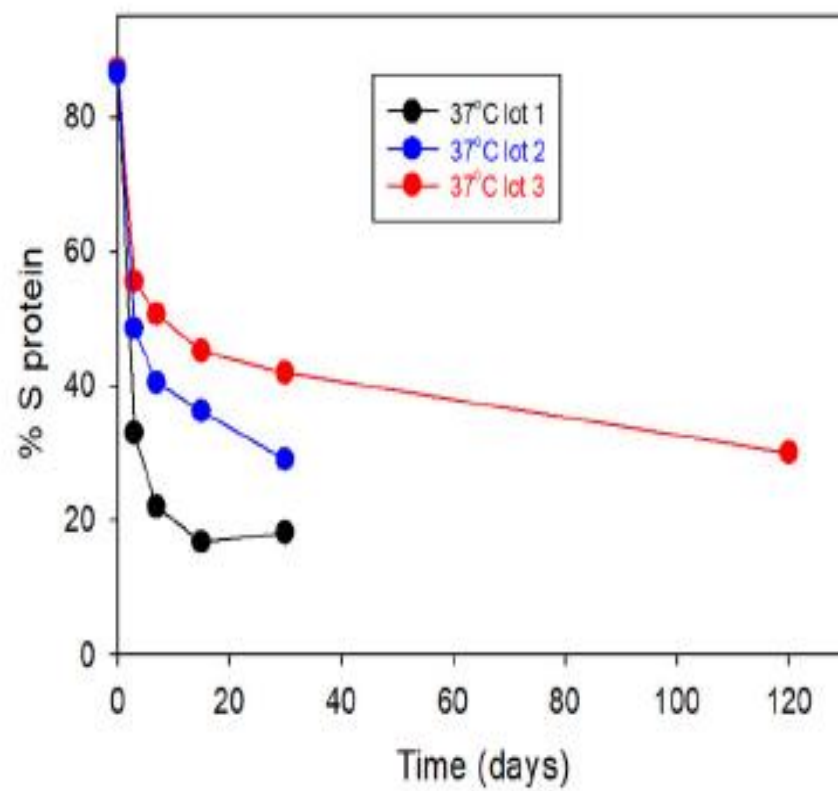


Figure S6B: Linearity dilution of rVSV protein in V590 UFP from 40x to 320x. All samples were tested within the linear range of the sample dilutional linearity.



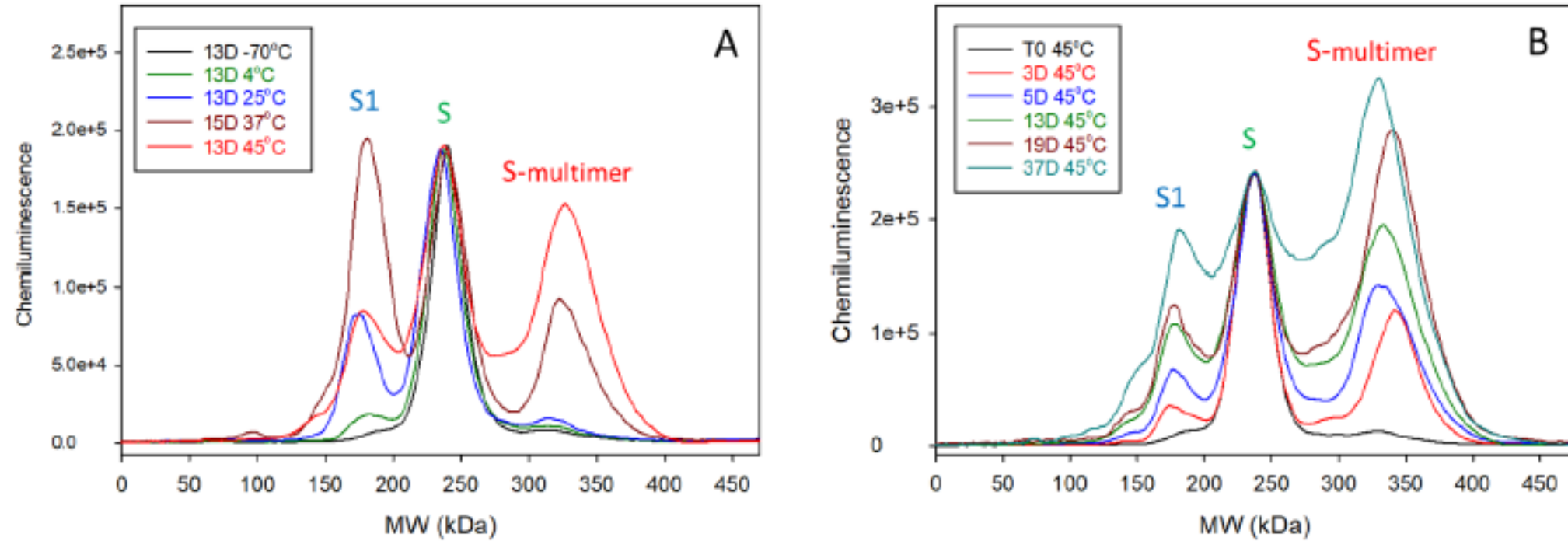


Figure S9: (A) Electropherograms of V590 DS S protein degradation at various temperatures monitored at 13 days except for 37°C at 15 days; (B) Electropherograms of V590 DS S protein degradation from 0 days to 37 days at 45°C. All data were normalized to S peak.