Supporting Information

Table S1. MMS data repeatability of all samples in triplicates as %similarity. %Similarity is calculated by the area of overlap using the similarity plot (inverted second derivative plot) of each replicate compared to the average spectrum of the replicates.

Sample name	Sample type	Similarity (%) of replicates			Mean±SD (%)
Trastuzumab	Formulation-21mg/ml	99.95	99.96	99.93	99.95±0.01
	Formulation-5mg/ml	99.67	99.81	99.79	99.76±0.07
	PBS-5mg/ml	99.78	99.68	99.71	99.72±0.05
Pertuzumab	Formulation-30mg/ml	99.95	99.96	99.97	99.96±0.01
	Formulation-5mg/ml	99.83	99.84	99.80	99.82±0.02
	PBS-5mg/ml	99.72	99.80	99.87	99.79±0.08
Atezolizumab	Formulation-60mg/ml	99.98	99.98	99.98	99.98±0.00
	Formulation-5mg/ml	99.82	99.89	99.80	99.84±0.05
	PBS-5mg/ml	99.84	99.77	99.78	99.80±0.04
Durvalumab	Formulation-50mg/ml	99.97	99.96	99.96	99.96±0.00
	Formulation-5mg/ml	99.75	99.78	99.82	99.78±0.04
	PBS-5mg/ml	99.68	99.78	99.64	99.70±0.08
Cetuximab	Formulation-5mg/ml	99.73	99.80	99.82	99.78±0.04
	PBS-5mg/ml	99.75	99.91	99.75	99.80±0.09
Bevacizumab	Formulation-25mg/ml	99.96	99.96	99.96	99.96±0.00
	Formulation-5mg/ml	99.69	99.73	99.75	99.73±0.03
	PBS-5mg/ml	99.63	99.78	99.64	99.68±0.08

Basiliximab	Formulation-4mg/ml	99.59	99.41	99.53	99.51±0.09
	PBS-4mg/ml	99.78	99.77	99.70	99.75±0.05
Pembrolizumab	Formulation-25mg/ml	99.95	99.95	99.94	99.94±0.01
	Formulation-5mg/ml	99.57	99.69	99.80	99.69±0.11
	PBS-5mg/ml	99.77	99.77	99.81	99.78±0.02
Tocilizumab	Formulation-20mg/ml	99.93	99.90	99.94	99.92±0.02
	Formulation-5mg/ml	99.86	99.81	99.86	99.84±0.03

Table S2. CD data repeatability of all samples in triplicates as %similarity. %Similarity is calculated by area of overlap.

Sample name	**Sample type**	**Similarity (%) of replicates**			**Mean±SD (%)**
Trastuzumab	Formulation-5mg/ml	97.39	97.82	98.13	97.78±0.37
	PBS-5mg/ml	98.18	98.19	98.54	98.30±0.21
Pertuzumab	Formulation-5mg/ml	97.59	98.36	97.97	97.97±0.38
	PBS-5mg/ml	98.99	99.10	99.06	99.05±0.06
Atezolizumab	Formulation-5mg/ml	99.13	99.02	98.75	98.97±0.20
	PBS-5mg/ml	98.69	99.14	98.90	98.91±0.23
Durvalumab	Formulation-5mg/ml	98.61	98.36	98.72	98.56±0.18
	PBS-5mg/ml	98.71	98.51	98.68	98.63±0.11
Cetuximab	Formulation-5mg/ml	98.63	98.79	98.47	98.63±0.16
	PBS-5mg/ml	98.48	98.35	98.77	98.53±0.21
Bevacizumab	Formulation-5mg/ml	98.36	98.71	98.91	98.66±0.28

	PBS-5mg/ml	98.33	98.73	98.37	98.48±0.22
Basiliximab	Formulation-4mg/ml	98.44	98.52	98.19	98.38±0.17
	PBS-4mg/ml	98.48	98.28	98.48	98.41±0.12
Pembrolizumab	Formulation-5mg/ml	98.35	98.22	98.59	98.39±0.19
	PBS-5mg/ml	98.47	97.92	98.28	98.22±0.28

Figure S1. MMS data output for Pertuzumab. (A) Raw differential absorbance spectra of 30 mg/mL sample in formulation buffer, 5 mg/mL sample in formulation buffer, and 5 mg/mL sample in PBS. (B) Absolute absorbance spectra of the samples. (C) Second derivative plots of the samples.

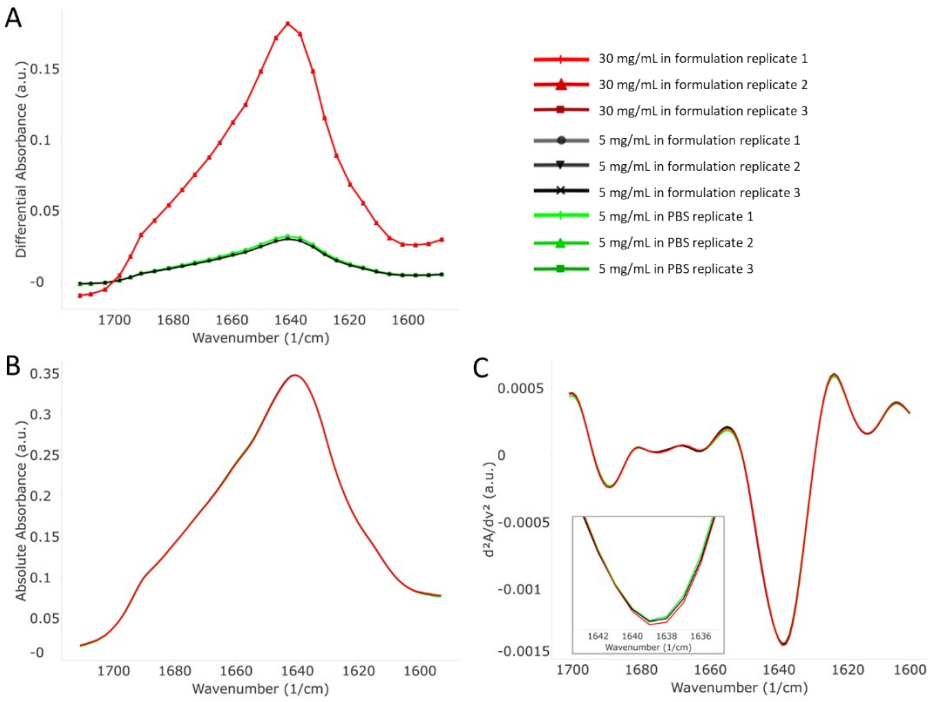

Figure S2. MMS data output for Atezolizumab. (A) Raw differential absorbance spectra of 60 mg/mL sample in formulation buffer, 5 mg/mL sample in formulation buffer, and 5 mg/mL sample in PBS. (B) Absolute absorbance spectra of the samples. (C) Second derivative plots of the samples.

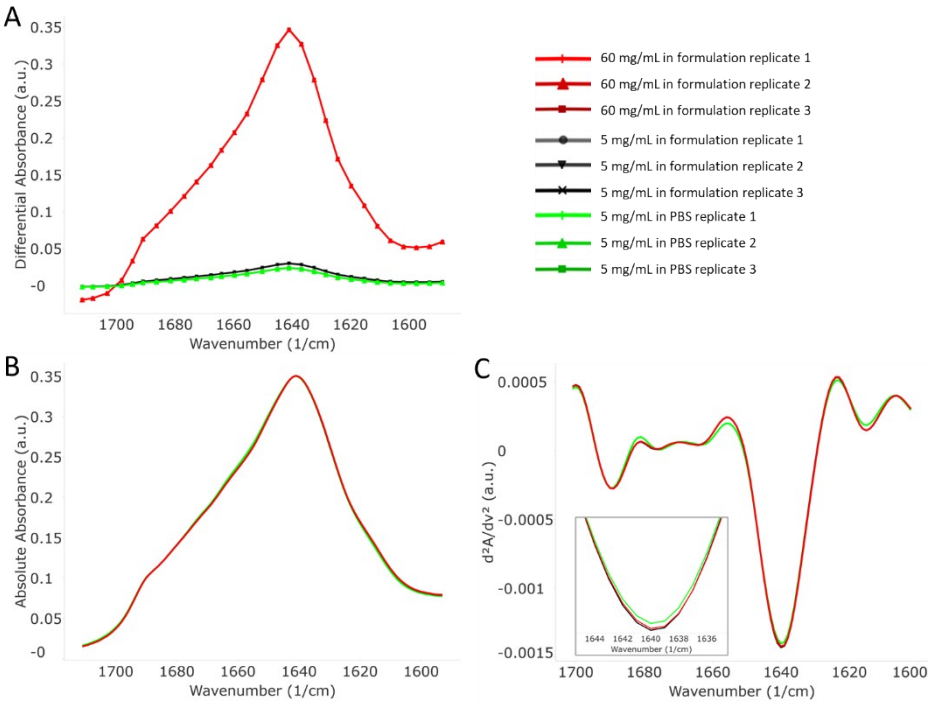

Figure S3. MMS data output for Durvalumab. (A) Raw differential absorbance spectra of 50 mg/mL sample in formulation buffer, 5 mg/mL sample in formulation buffer, and 5 mg/mL sample in PBS. (B) Absolute absorbance spectra of the samples. (C) Second derivative plots of the samples.

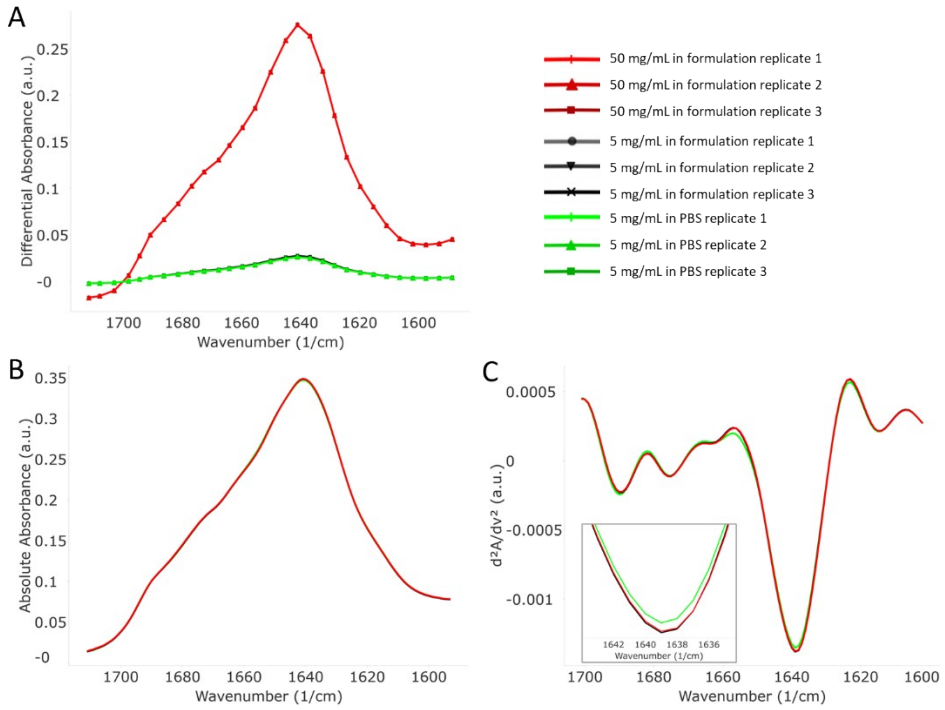

Figure S4. MMS data output for Bevacizumab. (A) Raw differential absorbance spectra of 25 mg/mL sample in formulation buffer, 5 mg/mL sample in formulation buffer, and 5 mg/mL sample in PBS. (B) Absolute absorbance spectra of the samples. (C) Second derivative plots of the samples.

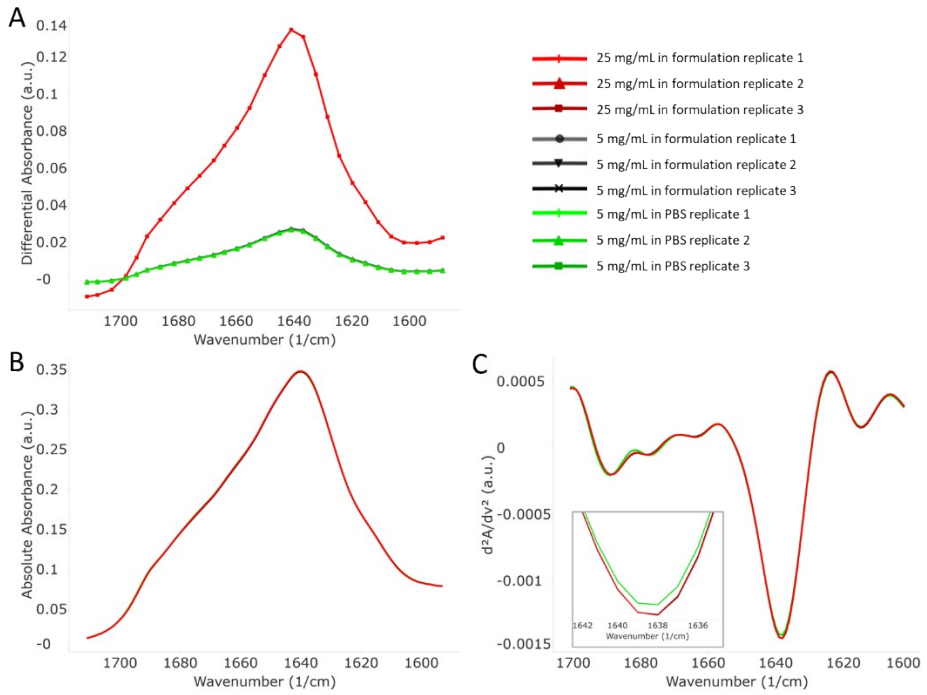

Figure S5. MMS data output for Pembrolizumab. (A) Raw differential absorbance spectra of 25 mg/mL sample in formulation buffer, 5 mg/mL sample in formulation buffer, and 5 mg/mL sample in PBS. (B) Absolute absorbance spectra of the samples. (C) Second derivative plots of the samples.

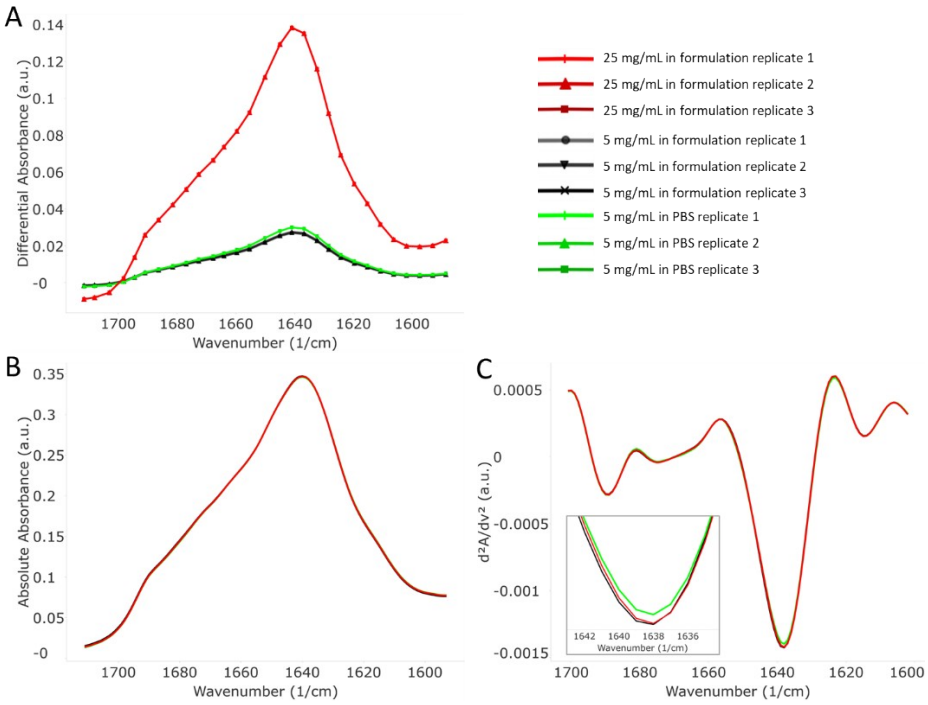

Figure S6. MMS data output for Cetuximab. (A) Raw differential absorbance spectra of 5 mg/mL sample in formulation buffer and 5 mg/mL sample in PBS. (B) Absolute absorbance spectra of the samples. (C) Second derivative plots of the samples.

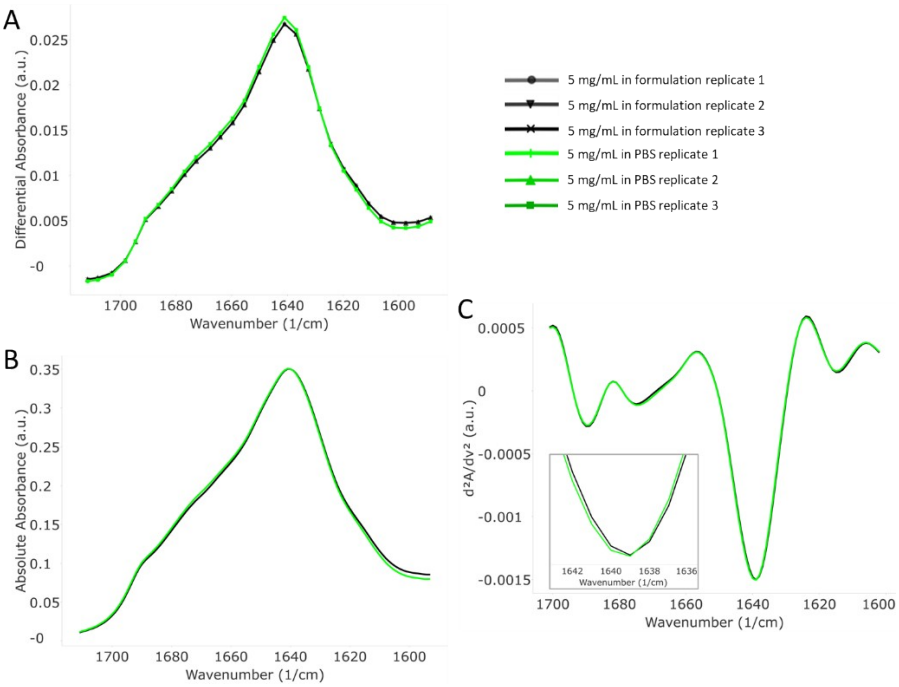

Figure S7. MMS data output for Tocilizumab. (A) Raw differential absorbance spectra of 20 mg/mL sample and 5 mg/mL sample in formulation buffer. (B) Absolute absorbance spectra of the samples. (C) Second derivative plots of the samples.

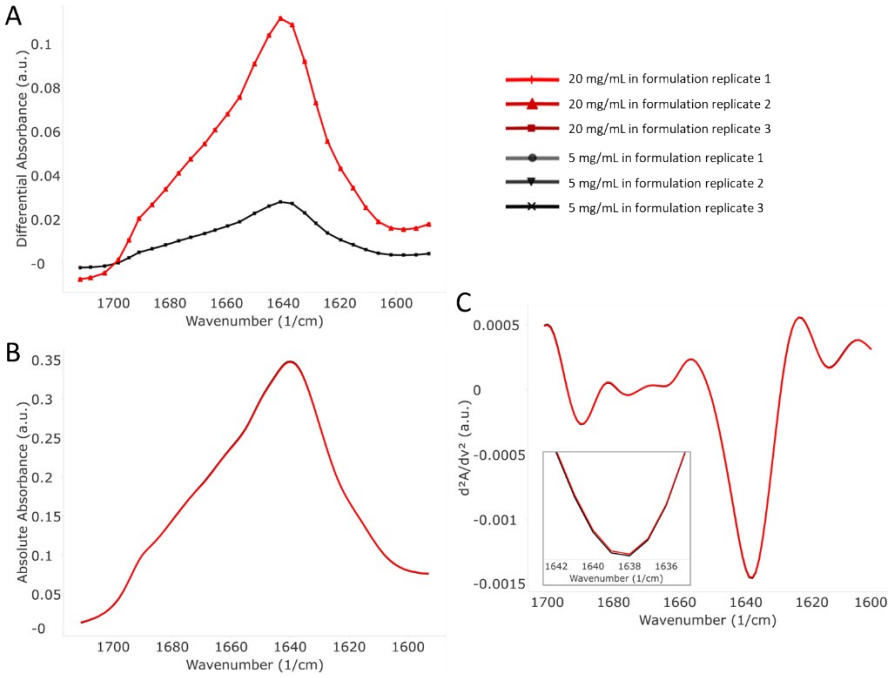

Figure S8. Second derivative plot (A) and HOS fractional contribution (B) of Trastuzumab and Pembrolizumab in formulation and PBS. There are significant differences in the secondary structure between these two mAbs. Specifically, Trastuzumab exhibits higher beta-sheet content and Pembrolizumab exhibits higher unordered content. Between the different buffers, there is a consistently small but quantifiable difference in the HOS, showing higher beta-sheet content in formulation and higher unordered content in PBS.

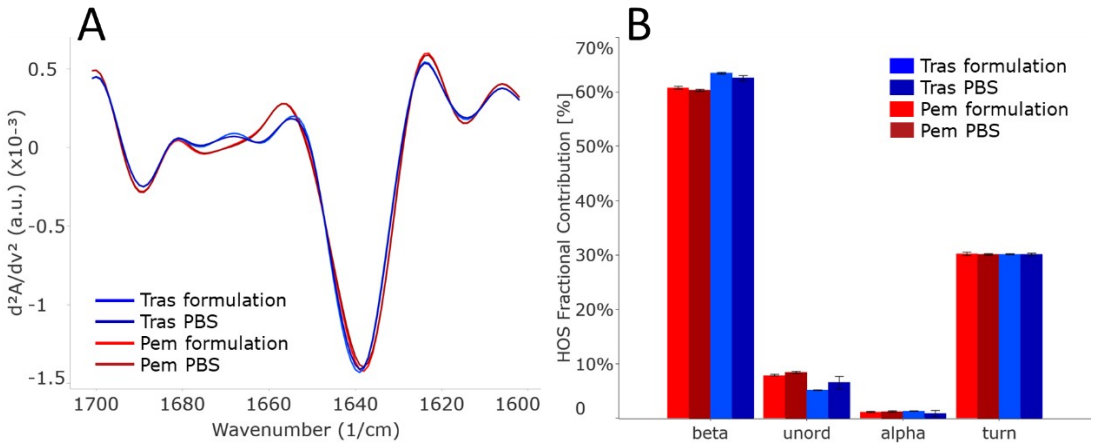

Figure S9. Thermal ramping MMS data of Trastuzumab and Pembrolizumab in PBS. (A) and (B) Differential absorbance spectra of Trastuzumab and Pembrolizumab in PBS. (C) and (D) Thermal ramp heat maps of Trastuzumab and Pembrolizumab in PBS.

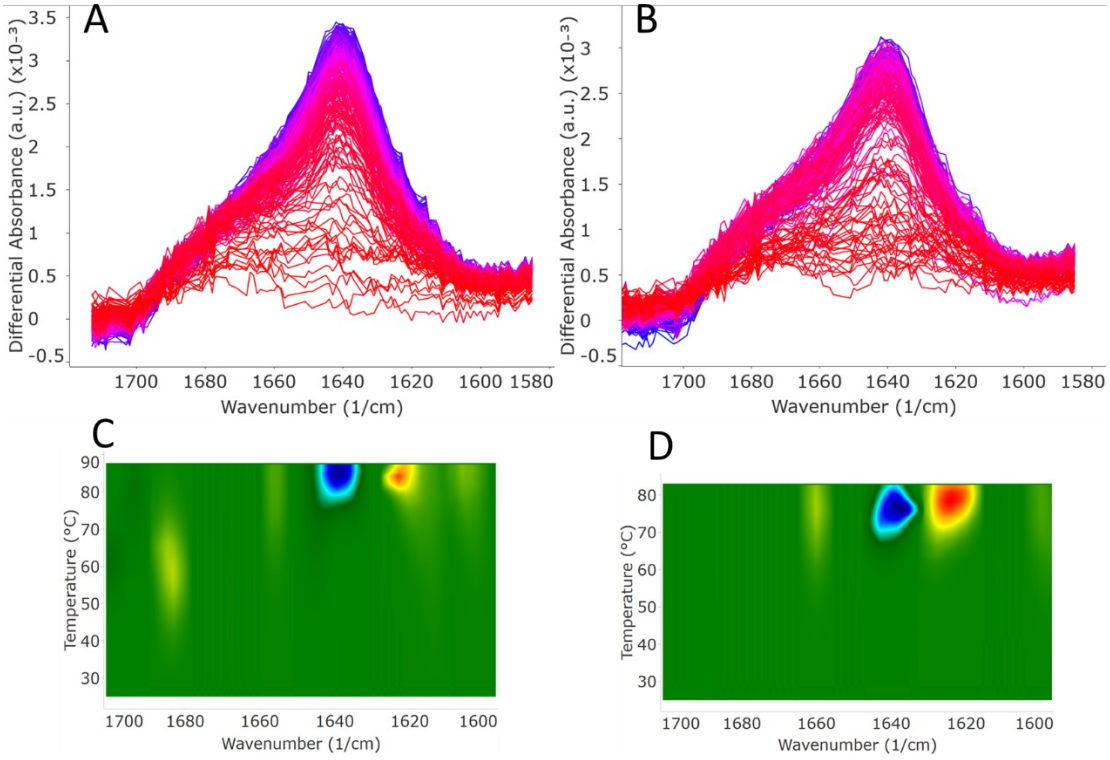

Table S3. Effect of dilution on protein structure by MMS: sample similarities before and after dilution are all above 99.5% in all mAbs.

Sample	Concentration	Repeatability (%)	Sample-to-Sample Similarity (%)
Trastuzumab	21 mg/mL	99.95 ± 0.02	Control
	5 mg/ML	99.76 ± 0.08	99.55 ± 0.21
Pertuzumab	30 mg/mL	99.96 ± 0.01	Control

	5 mg/mL	99.83 ± 0.02	99.54 ± 0.02
Atezolizumab	60 mg/mL	99.98 ± 0.00	Control
	5 mg/mL	99.84 ± 0.04	99.68 ± 0.04
Durvalumab	50 mg/mL	99.96 ± 0.01	Control
	5 mg/mL	99.79 ± 0.04	99.60 ± 0.06
Bevacizumab	25 mg/mL	99.96 ± 0.00	Control
	5 mg/mL	99.72 ± 0.03	99.68 ± 0.07
Pembrolizumab	25 mg/mL	99.95 ± 0.01	Control
	5 mg/mL	99.69 ± 0.11	99.55 ± 0.07
Tocilizumab	20 mg/mL	99.92 ± 0.02	Control
	5 mg/mL	99.85 ± 0.03	99.71 ± 0.11

Table S4. Effect of change in formulation to protein structure measured by MMS: assessment of sample-to-sample similarities shows significant structural changes after buffer-exchange from formulation to PBS.

Sample	**Buffer**	**Repeatability**	**Sample-to-Sample**	**P-value /**

		(%)	Similarity (%)	Structural Change
Trastuzumab	Formulation	99.76 ± 0.08	Control	
	PBS	99.72 ± 0.04	99.11 ± 0.14	0.009 / Yes
Pertuzumab	Formulation	99.83 ± 0.02	Control	
	PBS	99.80 ± 0.08	99.22 ± 0.14	0.004 / Yes
Atezolizumab	Formulation	99.84 ± 0.04	Control	
	PBS	99.81 ± 0.02	98.60 ± 0.08	0.001 / Yes
Cetuximab	Formulation	99.78 ± 0.05	Control	
	PBS	99.81 ± 0.09	99.22 ± 0.11	0.001 / Yes
Durvalumab	Formulation	99.79 ± 0.04	Control	
	PBS	99.70 ± 0.07	98.87 ± 0.17	0.002 / Yes
Bevacizumab	Formulation	99.72 ± 0.03	Control	
	PBS	99.69 ± 0.08	99.00 ± 0.21	0.007 / Yes
Pembrolizumab	Formulation	99.69 ± 0.11	Control	
	PBS	99.78 ± 0.02	99.38 ± 0.07	0.005 / Yes

Table S5. Effect of change in formulation to protein structure measured by CD: sample-to-sample similarities show significant structural changes after buffer-exchange from formulation to PBS.

Sample	Buffer	Repeatability (%)	Sample-to-Sample Similarity (%)	P-value / Structural Change
Trastuzumab	Formulation	97.78	Control	
	PBS	98.30	n.a.	n.a.
Pertuzumab	Formulation	97.97	Control	
	PBS	99.05	79.39	0.0019 / Yes
Atezolizumab	Formulation	98.97	Control	
	PBS	98.91	84.76	0.0006 / Yes
Cetuximab	Formulation	98.63	Control	
	PBS	98.53	95.21	0.0004 / Yes
Durvalumab	Formulation	98.56	Control	
	PBS	98.63	85.18	0.0001 / Yes
Bevacizumab	Formulation	98.66	Control	
	PBS	98.48	96.59	0.0003 / Yes
Pembrolizumab	Formulation	98.39	Control	
	PBS	98.22	88.18	0.0002 / Yes