

## SUPPORTING INFORMATION

# Benefits and Limitations of High-Resolution Cyclic IM-MS for Conformational Characterization of Native Therapeutic Monoclonal Antibodies

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**Table S1. Voltages used to set the direction of the ions in the multifunction array region of the SELECT SERIES Cyclic IMS spectrometer for each type of cIM event, with the default settings (top) and the settings adapted for native IM-MS (bottom). The voltages changed for analysis in native conditions are highlighted in red.**

cIM mobility event	Voltages in the multifunction array region (V)				
	Pre-Array Gradient	Pre-Array Bias	Array Entrance	Array Wave Height	Array Offset
<i>Default</i>					
Inject / Reinject from Pre-Store	85	70	10	2	45
Separate	85	70	30	0	70
Eject and Acquire	85	70	50	25	45
<i>Native IM-MS</i>					
Inject / Reinject from Pre-Store	95	85	10	8	70
Separate	95	80	30	0	75
Eject and Acquire	85	70	50	25	60










**Table S2. Determination of correction factors to derive drift times from raw arrival times obtained at 1 and 5 passes on the cIM-MS instrument for charge state 27+.**

		Number of passes			Mean time per pass (ms)	Applied correction factor (ms)	Number of passes	
		1	4	5			1	5
		$t_{A \text{ main peak}}$ (ms)					$t_{A \text{ main peak}}$ (ms)	$t_D$ (ms)
<b>IgG1</b>	trastuzumab	60.9	202.1	250.3	47.2	13.7	47.2	236.6
	elotuzumab	61.0	203.9	250.7	47.5	13.5	47.5	237.2
	ofatumumab	61.5	204.6	253.1	47.8	13.7	47.8	239.4
<b>IgG4</b>	nivolumab	60.2	201.2	245.8	46.7	13.5	46.7	232.3
	reslizumab	60.3	202.2	246.9	47.0	13.5	46.8	233.4
	natalizumab	62.2	206.9	255.0	48.1	14.1	48.1	240.9
<b>IgG2</b>	panitumumab	62.6	208.5	257.3	48.6	14.0	48.6	243.3
	denosumab	63.3	210.8	260.8	49.3	14.0	49.3	246.7
	eculizumab	63.5	212.3	261.8	49.6	13.9	49.6	247.9



$$\begin{aligned}
 & \downarrow \\
 & = t_A @ 1 \text{ pass} - \text{Mean time per pass} \\
 & \downarrow \\
 & = \frac{t_A @ n \text{ passes} - t_A @ 1 \text{ pass}}{n - 1}
 \end{aligned}$$

**Table S3. Intensities obtained on the cIM-MS instrument for extracted ATDs of 27+ charge states. (A) Intensities at 1 and 5 passes for the different mAbs presented in Figures 1C and 2A-F. (B) Intensities at 1 and 2 passes for CIU experiments on trastuzumab, corresponding to Figure 3E.**

**(A)**

		Passes	Replicate 1	Replicate 2	Replicate 3	Average	Standard Deviation	Ion losses
IgG1	trastuzumab IgG1	1p	9.8E+04	9.8E+04	9.6E+04	9.7E+04	1.4E+03	 -89%
		5p	1.1E+04	1.1E+04	1.1E+04	1.1E+04	5.8E+01	
	elotuzumab IgG1	1p	1.5E+05	1.6E+05	1.6E+05	1.6E+05	2.1E+03	 -91%
		5p	1.1E+04	1.4E+04	1.6E+04	1.3E+04	2.1E+03	
IgG4	ofatumumab IgG1	1p	7.6E+04	5.8E+04	7.9E+04	7.1E+04	1.1E+04	 -87%
		5p	1.0E+04	8.2E+03	1.0E+04	9.5E+03	1.1E+03	
	nivolumab IgG4	1p	6.7E+04	7.0E+04	7.1E+04	6.9E+04	1.8E+03	 -90%
		5p	6.8E+03	7.0E+03	7.2E+03	7.0E+03	2.0E+02	
reslizumab IgG4	1p	6.4E+04	6.6E+04	6.6E+04	6.6E+04	1.1E+03	 -85%	
	5p	9.9E+03	1.0E+04	1.0E+04	1.0E+04	1.5E+02		
IgG2	natalizumab IgG4	1p	7.8E+04	8.3E+04	8.7E+04	8.3E+04	4.3E+03	 -78%
		5p	1.8E+04	1.8E+04	1.8E+04	1.8E+04	5.8E+01	
	panitumumab IgG2	1p	1.0E+05	1.1E+05	1.1E+05	1.1E+05	4.2E+03	 -86%
		5p	8.5E+03	8.5E+03	8.7E+03	8.5E+03	1.7E+02	
denosumab IgG2	1p	7.0E+04	7.3E+04	7.5E+04	7.3E+04	2.1E+03	 -92%	
	5p	1.0E+04	1.0E+04	1.1E+04	1.0E+04	1.5E+02		
eculizumab IgG2/4	1p	1.2E+05	1.2E+05	1.2E+05	1.2E+05	2.0E+03	 -86%	
	5p	1.7E+04	1.7E+04	1.7E+04	1.7E+04	3.1E+02		

**(B)**

		Trap CV	Passes	Replicate 1	Replicate 2	Replicate 3	Average	Standard Deviation	Ion losses
trastuzumab	80 V	1p		1.7E+04	1.8E+04	1.7E+04	1.7E+04	4.0E+02	 -49%
		2p		8.9E+03	8.6E+03	9.1E+03	8.8E+03	2.5E+02	
	180 V	1p		1.1E+04	1.2E+04	1.2E+04	1.2E+04	5.2E+02	 -53%
		2p		6.9E+03	4.1E+03	5.5E+03	5.5E+03	1.4E+03	

**Table S4. Reproducibility of Gaussian fittings at 1 and 5 passes for the different mAbs shown in Figure 2.** Centroids and amplitudes are given for each Gaussian feature based on three technical replicates of measurements. Additionally, for nivolumab, measurements were performed on two individual preparations, on different days. Colors used for Gaussian fits correspond to those of Figure 2. When reported in black, Gaussian fittings were not considered to be significant (*i.e.* no major contribution to  $r^2$ ). Main contributions are highlighted in light grey.

## 1 PASS

IgG1s				
trastuzumab	Centroid (ms)	47.2 ± 0.1	49.2 ± 0.1	-
	Amplitude (%)	97 ± 1	38 ± 1	-

elotuzumab	Centroid (ms)	47.4 ± 0.1	49.1 ± 0.1	-
	Amplitude (%)	91 ± 2	58 ± 1	-

ofatumumab	Centroid (ms)	47.6 ± 0.1	49.3 ± 0.1	-
	Amplitude (%)	94 ± 1	40 ± 1	-

IgG4s				
nivolumab Preparation #1	Centroid (ms)	46.4 ± 0.1	48.1 ± 0.1	49.5 ± 0.1
	Amplitude (%)	86 ± 1	69 ± 1	28 ± 1

reslizumab	Centroid (ms)	46.5 ± 0.1	48.1 ± 0.1	49.3 ± 0.2
	Amplitude (%)	91 ± 3	65 ± 6	34 ± 6

natalizumab	Centroid (ms)	47.4 ± 0.1	48.9 ± 0.1	50.2 ± 0.1
	Amplitude (%)	83 ± 2	67 ± 1	29 ± 1

IgG2s				
panitumumab	Centroid (ms)	47.4 ± 0.1	49.0 ± 0.1	-
	Amplitude (%)	65 ± 2	83 ± 1	-

denosumab	Centroid (ms)	47.1 ± 0.1	49.3 ± 0.1	51.2 ± 0.3
	Amplitude (%)	57 ± 1	97 ± 2	17 ± 4

eculizumab	Centroid (ms)	47.5 ± 0.1	49.7 ± 0.1	51.2 ± 0.3
	Amplitude (%)	25 ± 6	94 ± 3	26 ± 3

## 5 PASSES

IgG1s												
trastuzumab	Centroid (ms)	219.7 ± 0.6	223.9 ± 1.2	228.5 ± 1.3	234.0 ± 0.4	237.3 ± 0.2	240.5 ± 0.1	244.4 ± 0.5	248.2 ± 0.1	252.4 ± 0.8	-	
	Amplitude (%)	6 ± 1	10 ± 2	14 ± 1	67 ± 7	73 ± 1	64 ± 1	42 ± 2	28 ± 6	10 ± 2	-	

elotuzumab	Centroid (ms)	-	-	227.0 ± 0.3	233.8 ± 0.1	237.3 ± 0.2	240.8 ± 0.3	244.5 ± 0.4	248.4 ± 0.3	252.7 ± 0.5	-
	Amplitude (%)	-	-	8 ± 5	50 ± 7	79 ± 3	67 ± 3	48 ± 2	25 ± 2	8 ± 1	-

ofatumumab	Centroid (ms)	-	224.1 ± 1.0	229.0 ± 0.5	235.2 ± 0.1	238.3 ± 0.5	241.6 ± 0.7	245.1 ± 1.0	248.9 ± 1.3	253.1 ± 1.4	256.7 ± 0.7
	Amplitude (%)	-	5 ± 1	9 ± 1	54 ± 4	68 ± 10	72 ± 3	47 ± 2	30 ± 5	12 ± 4	7 ± 1

IgG4s												
nivolumab Preparation #1	Centroid (ms)	217.1 ± 0.2	222.3 ± 0.1	227.9 ± 0.1	231.2 ± 0.1	235.0 ± 0.1	238.9 ± 0.1	242.6 ± 0.1	246.2 ± 0.1	250.5 ± 0.2	255.9 ± 0.3	
	Amplitude (%)	9 ± 1	15 ± 1	45 ± 1	81 ± 2	77 ± 3	74 ± 1	67 ± 2	42 ± 1	19 ± 1	9 ± 1	

nivolumab Preparation #2	Centroid (ms)	217.5 ± 0.3	222.6 ± 0.2	228.3 ± 0.1	231.6 ± 0.3	235.3 ± 0.5	239.3 ± 0.6	243.1 ± 0.7	246.4 ± 0.2	250.7 ± 0.4	256.0 ± 0.6
	Amplitude (%)	9 ± 1	15 ± 1	53 ± 5	79 ± 4	78 ± 3	77 ± 1	66 ± 3	37 ± 5	16 ± 5	9 ± 1

reslizumab	Centroid (ms)	-	222.1 ± 0.4	228.2 ± 0.2	231.7 ± 0.4	235.7 ± 0.2	239.5 ± 0.1	243.2 ± 0.2	246.9 ± 0.1	251.5 ± 0.1	256.0 ± 0.3
	Amplitude (%)	-	7 ± 1	38 ± 4	69 ± 3	80 ± 3	78 ± 3	65 ± 4	38 ± 8	14 ± 3	9 ± 1

natalizumab	Centroid (ms)	225.5 ± 1.4	230.3 ± 0.8	235.7 ± 0.4	239.0 ± 0.5	242.6 ± 0.4	246.4 ± 0.3	250.2 ± 0.3	254.2 ± 0.7	258.5 ± 0.4	-
	Amplitude (%)	9 ± 1	13 ± 1	44 ± 8	76 ± 1	75 ± 3	72 ± 3	57 ± 1	31 ± 3	10 ± 2	-

IgG2s												
panitumumab	Centroid (ms)	-	225.0 ± 1.1	230.1 ± 0.2	234.3 ± 0.2	237.9 ± 0.4	241.7 ± 0.4	245.1 ± 0.2	248.8 ± 0.2	253.3 ± 0.2	-	
	Amplitude (%)	-	9 ± 2	24 ± 7	47 ± 10	68 ± 5	70 ± 6	72 ± 13	31 ± 6	9 ± 3	-	

denosumab	Centroid (ms)	-	225.5 ± 0.2	231.6 ± 0.1	236.7 ± 0.2	240.6 ± 0.4	244.7 ± 0.3	248.4 ± 0.4	251.3 ± 0.4	254.9 ± 0.1	259.6 ± 0.1
	Amplitude (%)	-	11 ± 4	34 ± 6	53 ± 3	50 ± 1	61 ± 9	81 ± 2	63 ± 8	23 ± 9	11 ± 1

eculizumab	Centroid (ms)	228.0 ± 1.6	233.2 ± 1.2	236.9 ± 0.9	241.1 ± 0.7	244.7 ± 0.2	248.1 ± 0.2	251.8 ± 0.1	256. ± 0.6	260.6 ± 0.6	-
	Amplitude (%)	7 ± 1	15 ± 1	35 ± 2	39 ± 2	61 ± 9	77 ± 5	53 ± 6	25 ± 2	9 ± 2	-

**Table S5. %RSDs between technical replicates of mAbs at 1 and 5 passes on the cyclic instrument, based on parameters used by the US FDA and illustrated in Figure S1 (DOI: 10.1007/s13361-016-1369-1). (A) Evaluation of %RSDs between technical replicates of measurements. AF = asymmetry factor. (B) Evaluation of %RSDs on technical replicates of preparations (two distinct preparations of nivolumab analyzed on two different days).**

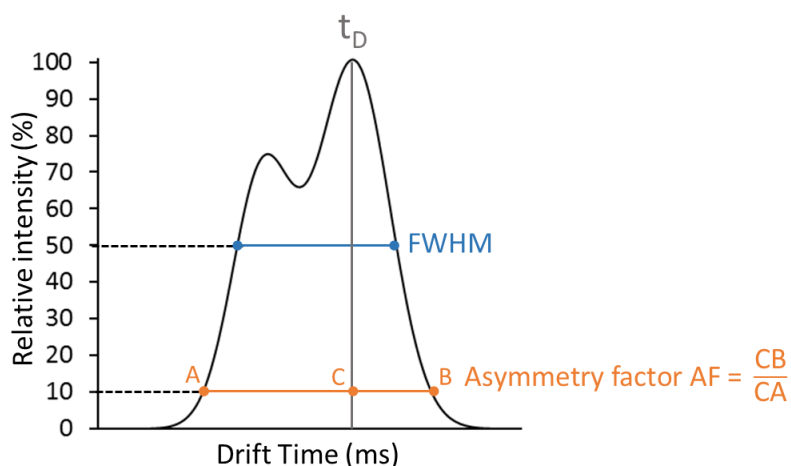
	<b>1 PASS</b>	Technical replicates (n = 3)			%RSD		
		$t_{D \text{ main peak}}$ (ms)	FWHM (ms)	AF	$t_{D \text{ main peak}}$ (ms)	FWHM (ms)	AF
		<b>Values reported by FDA</b>			<b>1.4</b>	<b>7.0</b>	<b>9.0</b>
<b>IgG1s</b>	trastuzumab	47.2 ± 0.1	2.5 ± 0.1	2.2 ± 0.1	0.1	0.6	2.8
	elotuzumab	47.5 ± 0.2	3.2 ± 0.1	1.8 ± 0.2	0.3	0.5	8.7
	ofatumumab	47.8 ± 0.1	2.7 ± 0.1	1.9 ± 0.1	0.2	2.5	6.3
<b>IgG4s</b>	nivolumab	46.7 ± 0.1	3.9 ± 0.1	2.1 ± 0.1	0.2	0.4	3.9
	reslizumab	46.8 ± 0.2	3.8 ± 0.1	1.9 ± 0.1	0.3	0.3	6.1
	natalizumab	48.1 ± 0.1	3.6 ± 0.1	1.4 ± 0.1	0.3	0.7	2.1
<b>IgG2s</b>	panitumumab	48.6 ± 0.1	3.1 ± 0.1	0.7 ± 0.1	0.1	1.3	1.6
	denosumab	49.3 ± 0.1	3.8 ± 0.1	0.7 ± 0.1	0.2	3.5	5.2
	eculizumab	49.6 ± 0.1	2.5 ± 0.1	0.8 ± 0.1	0.1	1.6	1.1

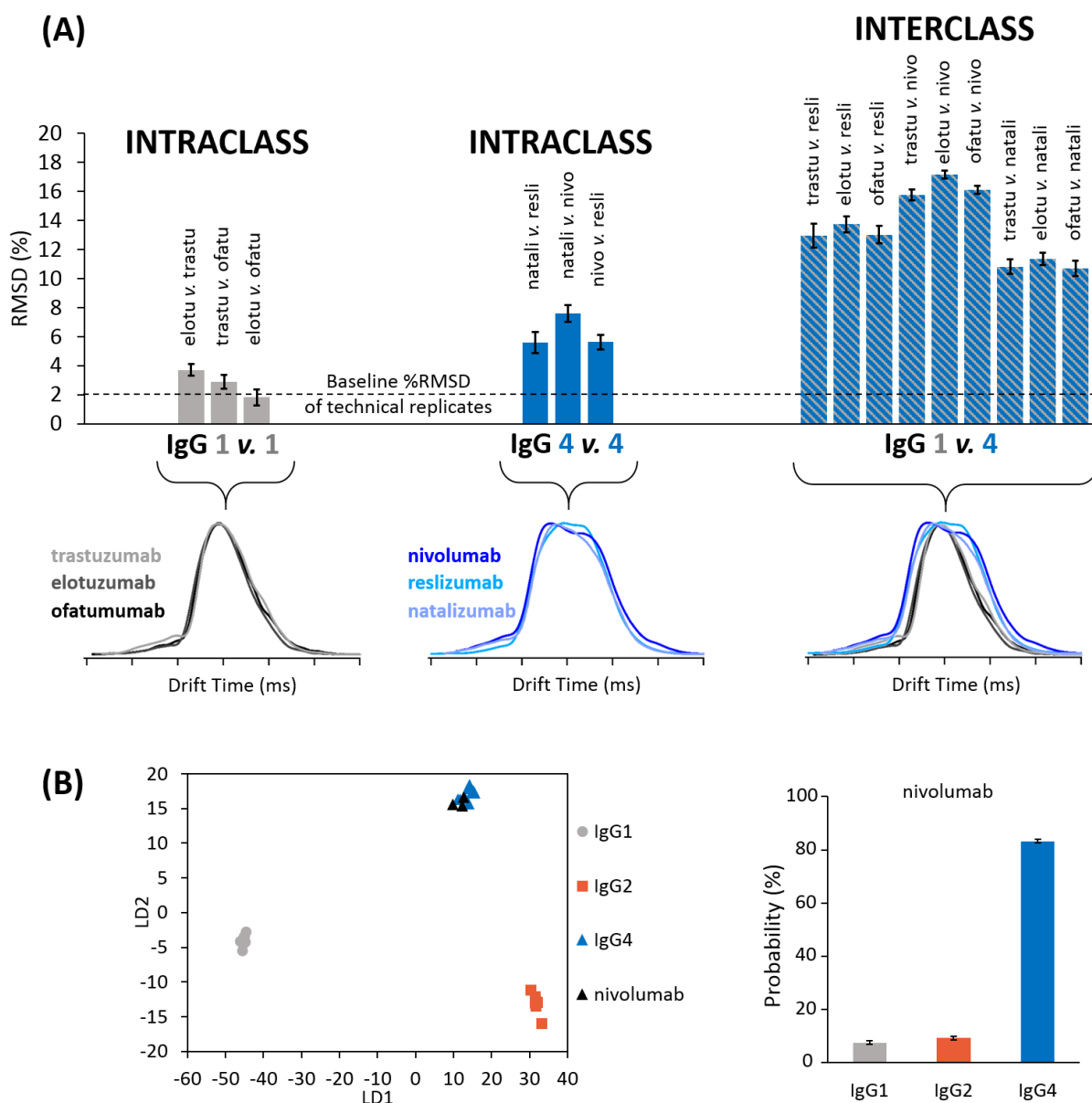
	<b>5 PASSES</b>	Technical replicates (n = 3)			%RSD		
		$t_{D \text{ main peak}}$ (ms)	FWHM (ms)	AF	$t_{D \text{ main peak}}$ (ms)	FWHM (ms)	AF
		<b>Values reported by FDA</b>			<b>1.4</b>	<b>7.0</b>	<b>9.0</b>
<b>IgG1s</b>	trastuzumab	236.6 ± 0.2	12.8 ± 0.1	1.2 ± 0.1	0.1	0.1	1.3
	elotuzumab	237.2 ± 0.5	12.7 ± 0.1	1.7 ± 0.1	0.2	0.5	2.5
	ofatumumab	239.4 ± 0.2	12.9 ± 0.1	1.7 ± 0.1	0.1	0.4	3.6
<b>IgG4s</b>	nivolumab	232.3 ± 0.4	18.5 ± 0.1	1.7 ± 0.1	0.2	0.5	0.7
	reslizumab	233.4 ± 0.4	18.0 ± 0.1	1.3 ± 0.1	0.2	0.6	6.0
	natalizumab	240.9 ± 0.5	17.6 ± 0.2	1.3 ± 0.1	0.2	1.0	6.8
<b>IgG2s</b>	panitumumab	243.3 ± 0.2	14.7 ± 0.3	0.6 ± 0.1	0.1	0.8	1.4
	denosumab	246.7 ± 0.1	21.6 ± 0.1	0.6 ± 0.1	0.1	0.6	0.6
	eculizumab	247.9 ± 0.2	12.0 ± 0.2	0.7 ± 0.1	0.1	1.4	4.9

	<b>5 PASSES</b>	Technical replicates from 2 different preparations (n = 3 each)			%RSD		
		$t_{D \text{ main peak}}$ (ms)	FWHM (ms)	AF	$t_{D \text{ main peak}}$ (ms)	FWHM (ms)	AF
		<b>Values reported by FDA</b>			<b>1.4</b>	<b>11.4</b>	<b>3.4</b>
<b>IgG4</b>	nivolumab	232.4 ± 0.1	18.7 ± 0.3	1.7 ± 0.1	0.1	1.3	0.7



**Figure S1. Parameters used to evaluate the reproducibility of cIM profiles.**



**Figure S2. Intra- and interclass variations. (A)** Evaluation of RMSDs between cIM profiles of mAbs at 5 passes for charge state 27+. IgG1s are represented in grey and IgG4s in blue. ATDs were aligned on the same drift time centroid to avoid a mass bias and better illustrate differences between the two subclasses. **(B)** Linear determinant analysis of ATDs at 5 passes for charge state 27+, showing intraclass differences. Independent measurements of nivolumab (IgG4) not used to build the categorization method are taken to assess IgG subclass determination based on ATDs.

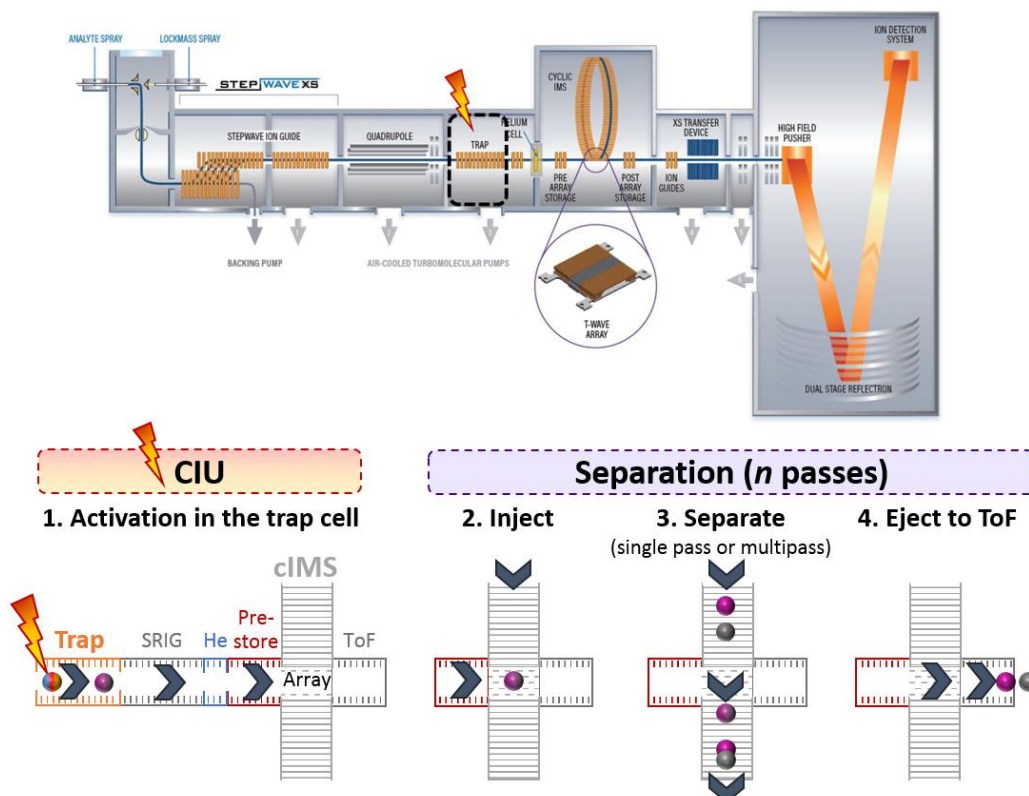


Figure S3. Principle of CIU experiments performed in the trap cell of the cIM-MS instrument.

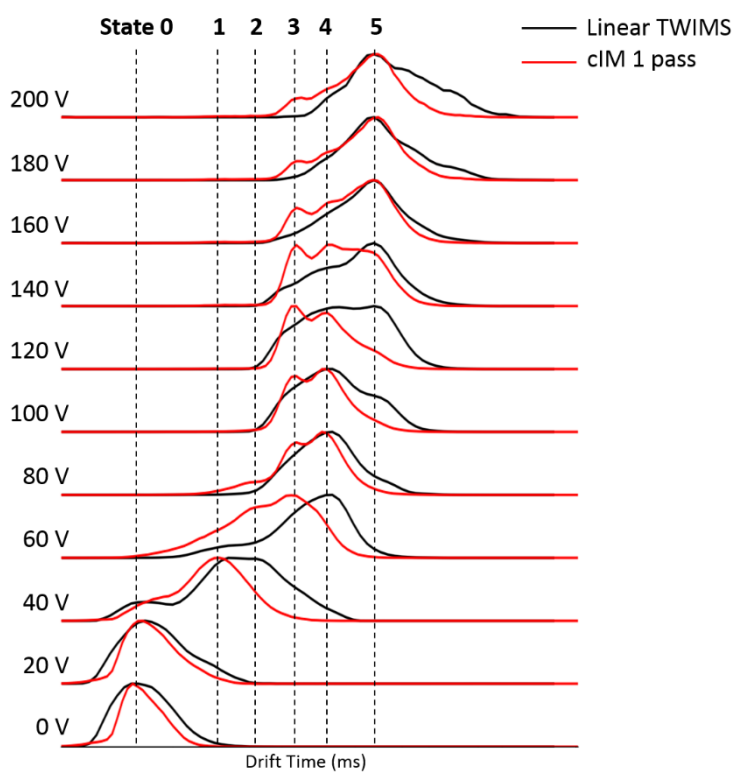
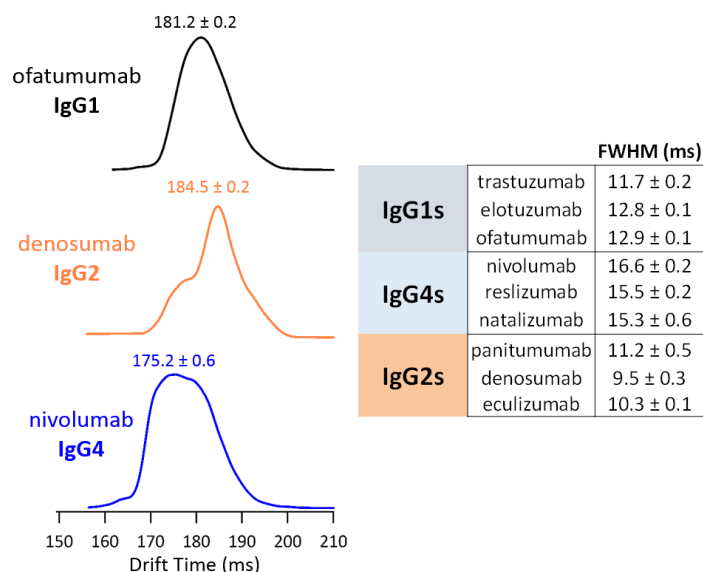
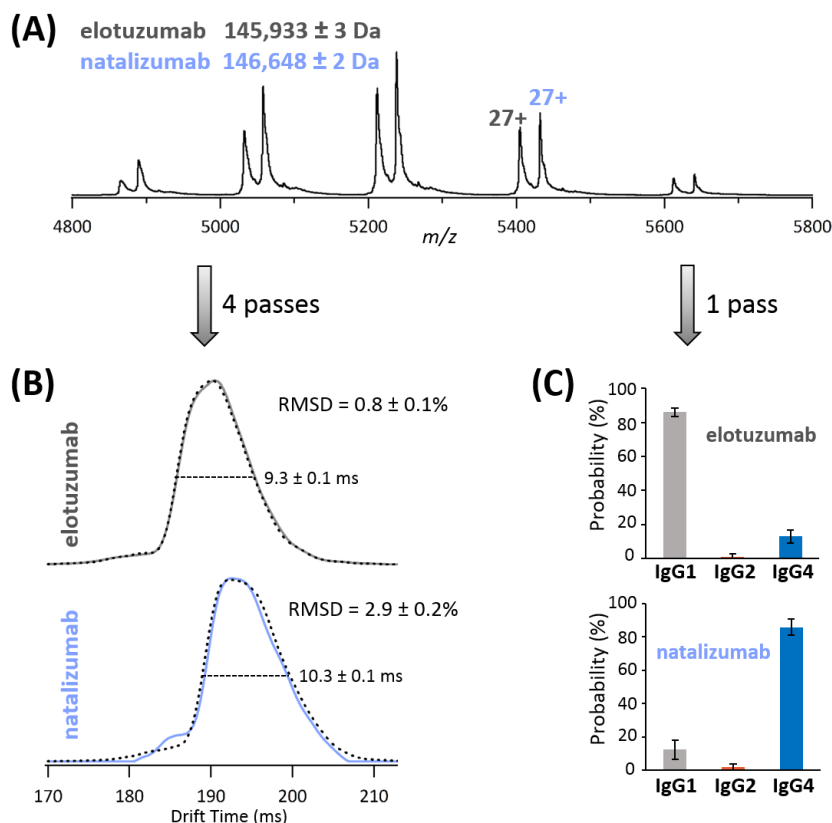


Figure S4. Comparison of CIU plots obtained for intact deglycosylated nivolumab using a linear versus cyclic TWIMS cell. Stacked ATDs of the 27+ charge state were plotted using ORIGAMI<sup>ANALYZE</sup> (Migas *et al.*, *Int J Mass Spectrom* **2018**, 427, 20 – 28).

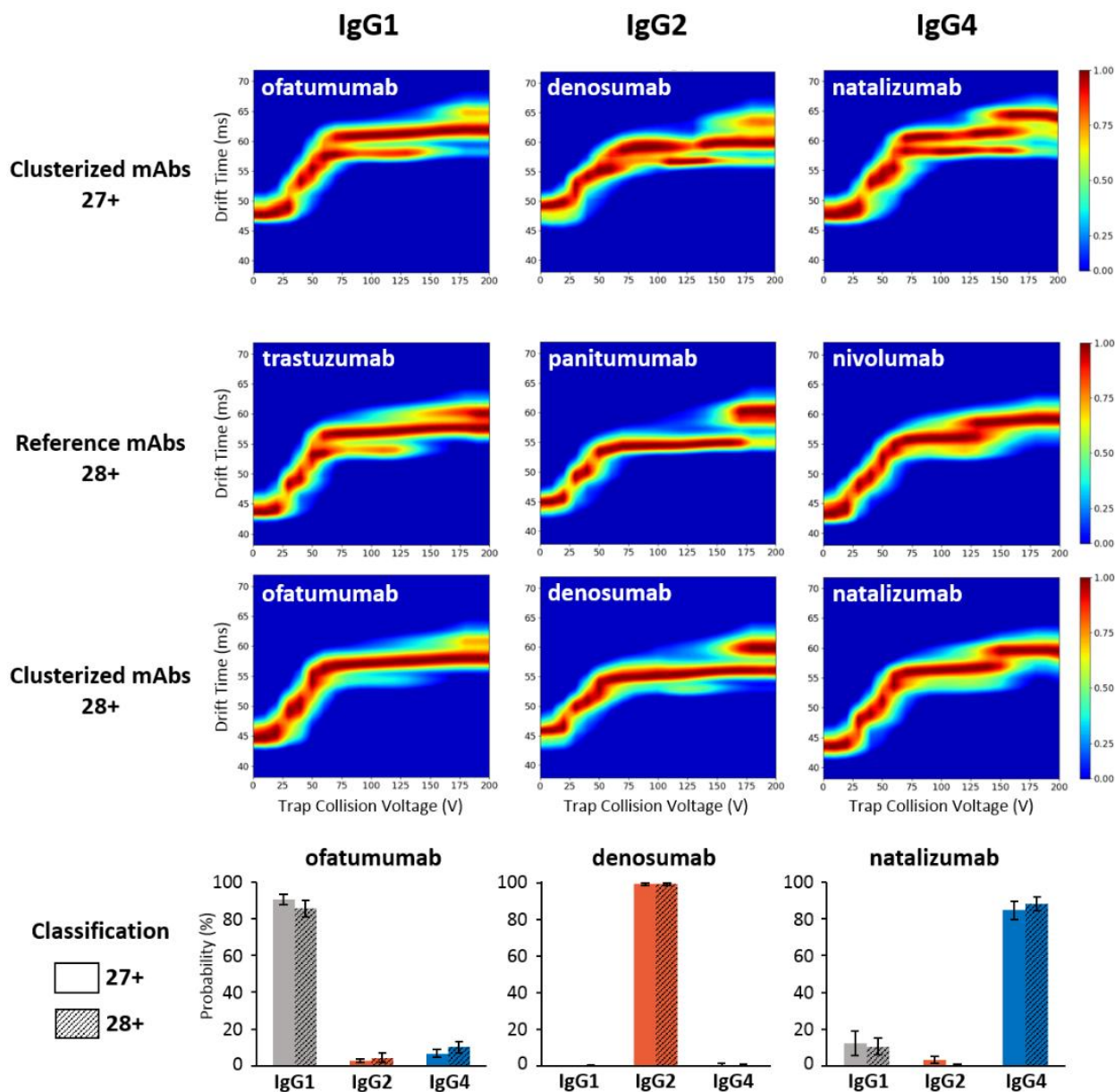




**Figure S5. cIM-MS experiments for charge state 28+ after 4 passes.** ATDs were extracted for three mAbs from different IgG subclasses. FWHMs are reported for each mAb studied in the main text.



**Figure S6. Analysis of a mixture containing two different mAbs on the cIM-MS instrument, including targeted scheduled CIU subclassification.** (A) Native MS spectrum of a mixture of intact deglycosylated elotuzumab (IgG1) and natalizumab (IgG4). (B) Extracted ATDs at 4 passes for the 27+ charge state. Solid lines represent ATDs extracted from each individual sample, whereas dotted lines correspond to ATDs extracted from the mixture. FWHMs are given for the mixture. RMSDs are calculated for cIM profiles of individual *versus* mixed samples of each mAb. (C) Classification for the 27+ charge state of elotuzumab and natalizumab obtained from targeted scheduled CIU experiments at 1 pass. Only the most discriminating CVs (125 – 155 V using 5 V steps) were acquired.



**Figure S7. CIU fingerprints and IgG classification scores for charge states 27 and 28+.** Intact deglycosylated ofatumumab (IgG1), denosumab (IgG2), and natalizumab (IgG4) are used as test data (*i.e.* clusterized mAbs) to show the efficiency of our classification method that was built based on three reference mAbs (trastuzumab – IgG1, panitumumab – IgG2, nivolumab – IgG4). For charge state 27+, trap CVs used for classification are 125 to 155 V (5 V steps) and for charge state 28+, 100 to 140 V (5 V steps). Similar subclassification scores are obtained for charge states 27+ and 28+.