Supporting information

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The challenge to quantify proteins with charge train isoforms

Supporting information

Figure S1

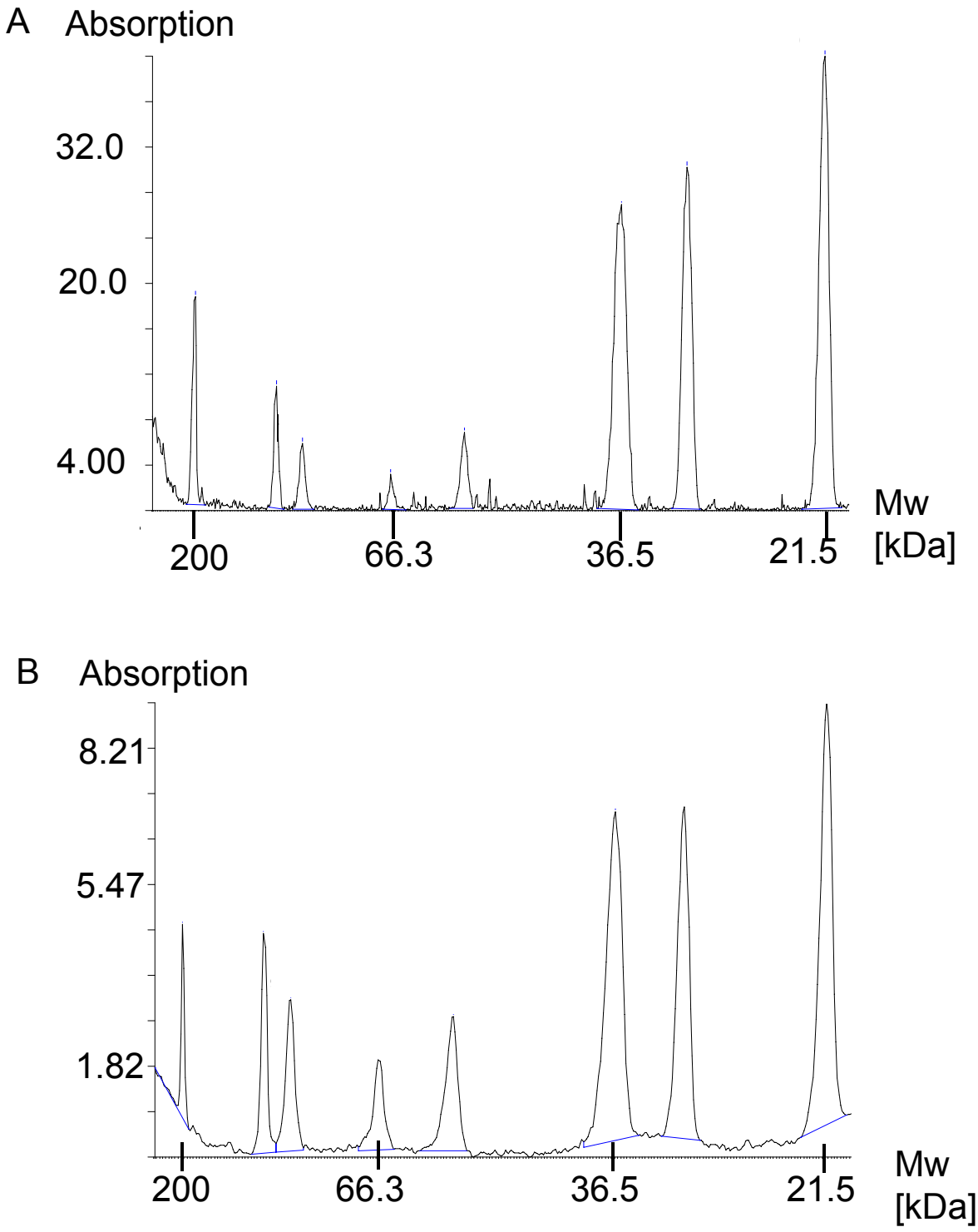

Figure S1. VIS (A) and NIR-VIS (B) detection of the same 1D gel band containing myosin (200 kDa), β-galactosidase (116.3 kDa), phosphorylase b (97.4 kDa), BSA (66.3 kDa), glutamic dehydrogenase (55.4 kDa), lactate dehydrogenase (36.5 kDa), carbonic anhydrase (31.0 kDa) and trypsin inhibitor (21.5 kDa) in the concentration of 0.023 µg/µl – 0.068 µg/µl.

Figure S2

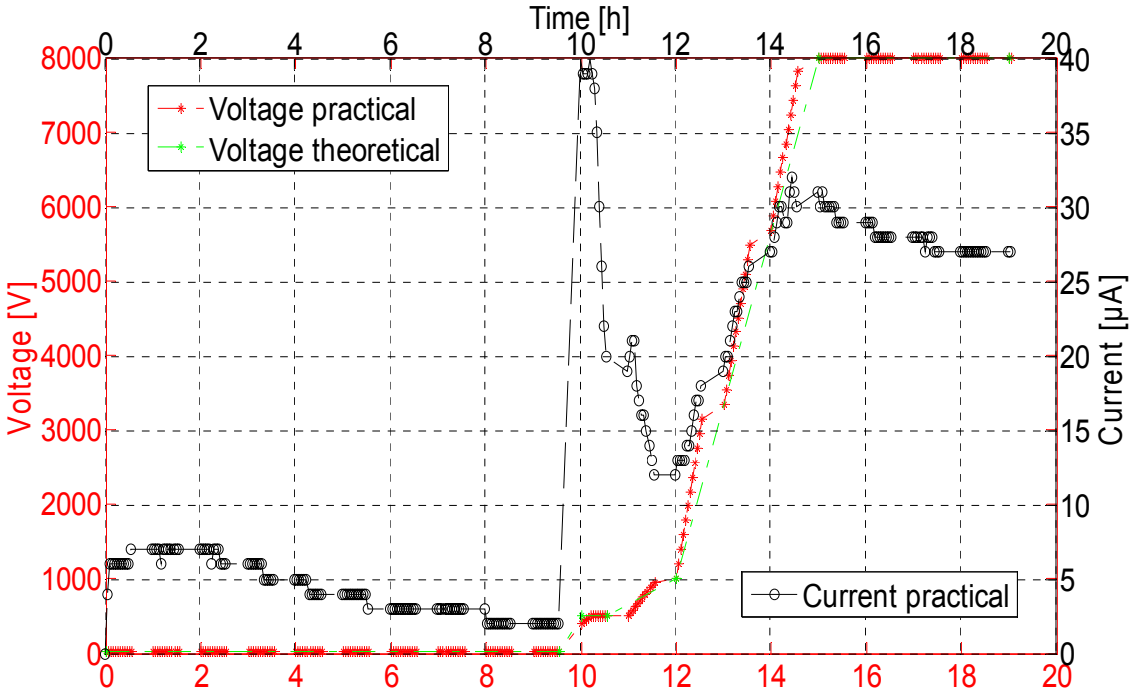

Figure S2. Representative voltage and current profile during IEF.

Figure S3

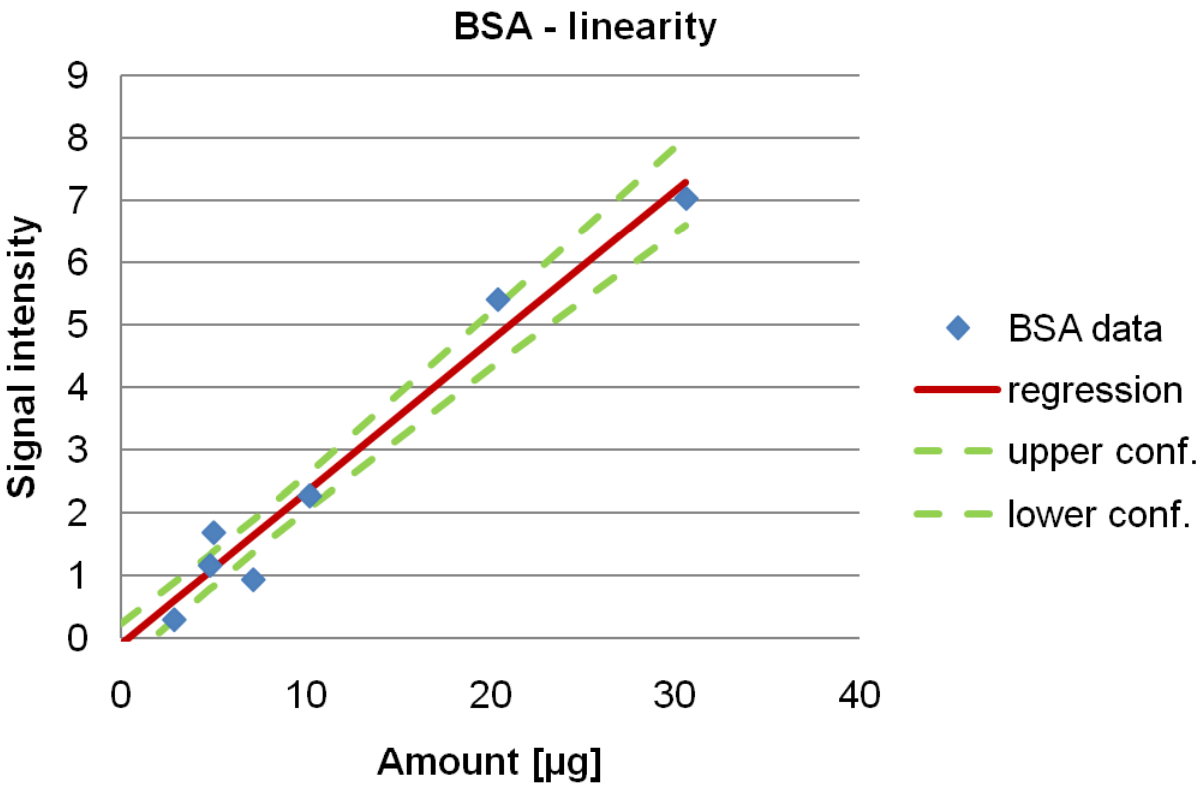

Figure S3. Regression plot for BSA with 95% confidence interval, R^2=0.9704.

Figure S4

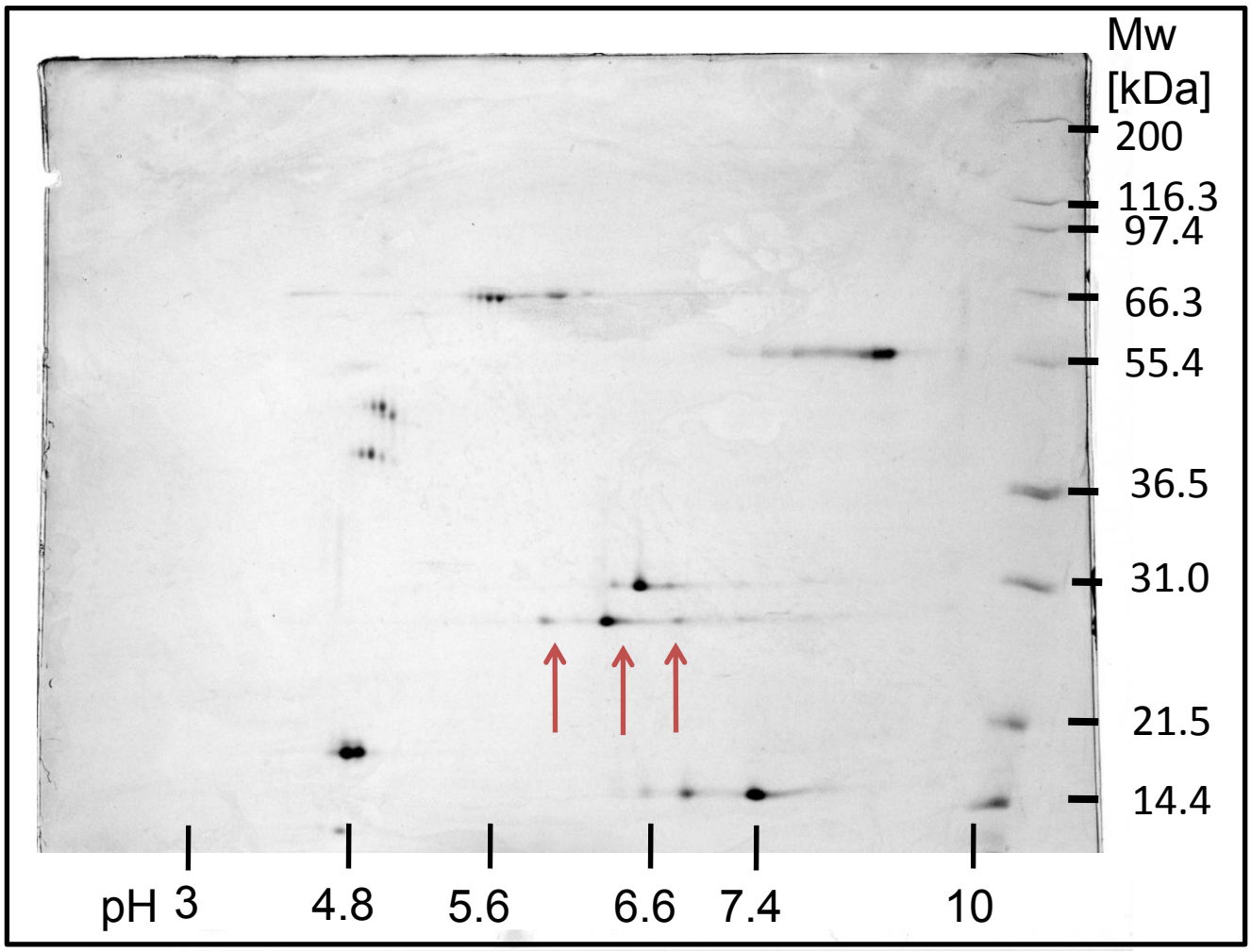

Figure S4. By using 200 kVh for IEF the light chain of matuzumab could be separated into three well resolved distinct spots (red arrows).

Figure S5

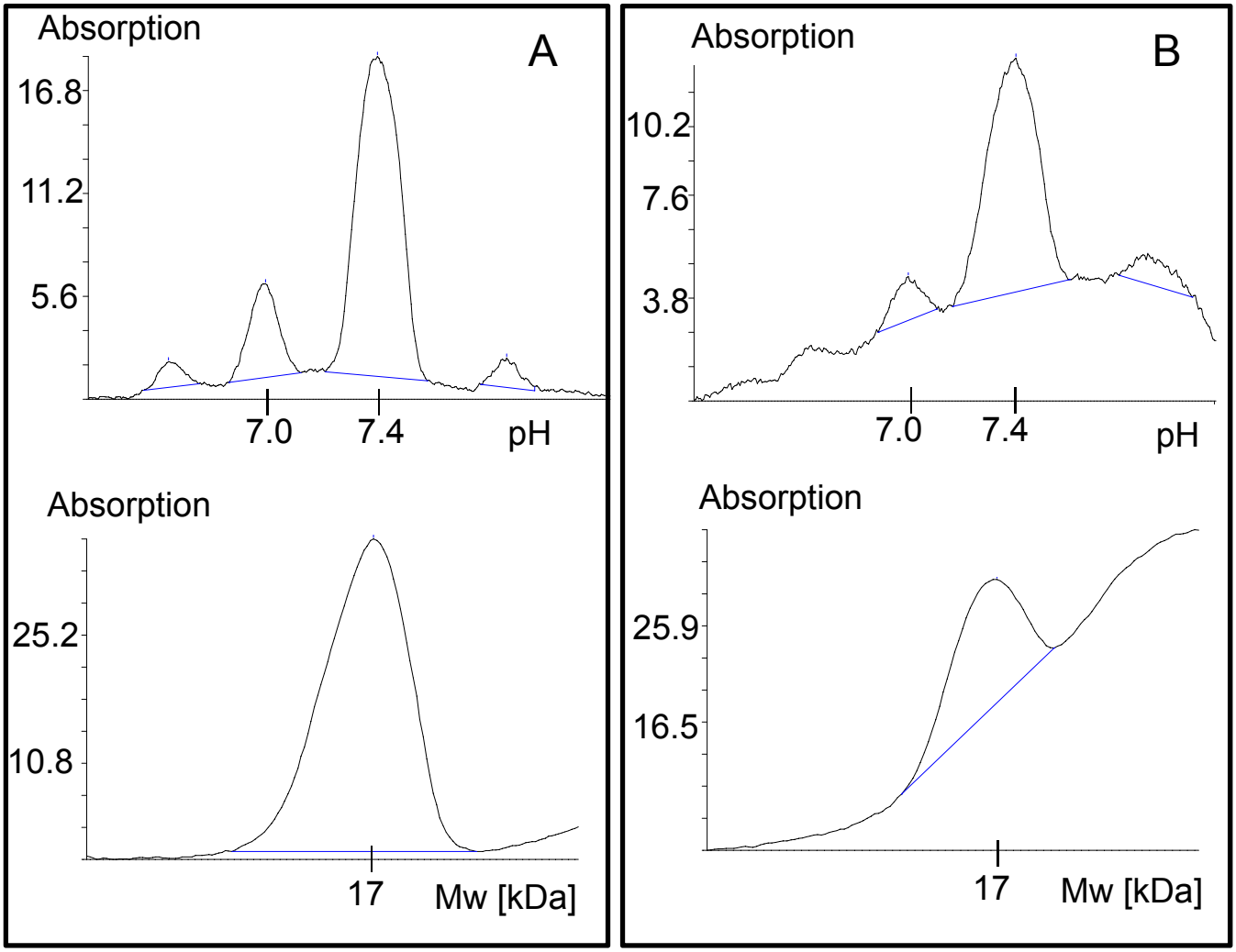

Figure S5. High background staining in the low molecular weight range leads to the high %RSD of myoglobin.

Table S1. $\%RSD_{pooled}$ by CISS and Delta2D, using integration method I), II) and III); the theoretical pIs were calculated using Expasy pI/Mw tool.

Protein	pI theo.	pI exp.	Integration with CISS			Integration with Delta2D	
Integration method			I) Spot-wise [$\%RSD_{pooled}$]	II) Spot-wise, summed [$\%RSD_{pooled}$]	III) Whole charge train [$\%RSD_{pooled}$]	I) Spot-wise [$\%RSD_{pooled}$]	II) Spot-wise, summed [$\%RSD_{pooled}$]
BSA n = 24	5.60	5.5-5.8	15.1	11.3	8.6	9.45	6.34
			11.3			6.14	
			11.5			7.00	
			13.3			8.80	
Oval-bumin, charge train 1 n = 24	5.19	4.8-5.15	22.5	17.8	10.0	18.3	8.7
			12.9			13.2	
			14.3			12.7	
						25.1	
Oval-bumin, charge train 2 n = 24			9.34	8.4	10.8	18.5	8.87
			7.17			13.3	
			9.46			9.41	
						18.5	
Myo-globin n = 18	7.36	7.0	18.5	20.0	16.8	25.3	16.1
		7.4	21.8			14.8	
β-lacto-globulin n = 24	4.83	4.9	-	-	12	14.5	10.0
						15.4	
		5.0	-	-		15.6	
						11.4	

Table S2. Software-dependant precision: CISS and Delta2D, using integration method II), the signal intensity is calculated by the spot-wise integration along the molecular weight axis and then summed together.

Protein	**CISS** II) Spot-wise, summed [$\%RSD_{pooled}$]	**Delta2D** II) Spot-wise, summed [$\%RSD_{pooled}$]	F_0	F_{crit}	**F-test**	**Results**
BSA n = 24	11.3	6.34	3.178	2.124	$F_0 > F_{crit}$	significant difference
Ovalbumin, charge train 1 n = 24	17.8	8.70	4.183	2.124	$F_0 > F_{crit}$	significant difference
Ovalbumin, charge train 2 n = 24	8.40	8.87	1.121	2.124	$F_0 < F_{crit}$	no significant difference
Myoglobin n = 18	20.0	16.1	1.545	2.203	$F_0 < F_{crit}$	no significant difference
β-lacto-globulin n = 24	-	10.0	-	-	-	-

Table S3. Software- and method-dependant precision: CISS using integration method III) quantitation of the whole spot group by integration along the pI axis; Delta2D using integration method II) the signal intensity is calculated by the spot-wise integration along the molecular weight axis and then summed together.

Protein	**CISS** III) Whole charge train [%RSD_{pooled}]	**Delta2D** II) Spot-wise, summed [%RSD_{pooled}]	F_0	F_{crit}	**F-test**	**Results**
BSA n = 24	8.60	6.34	1.841	2.124	$F_0 < F_{crit}$	no significant difference
Ovalbumin, charge train 1 n = 24	10.0	8.70	1.320	2.124	$F_0 < F_{crit}$	no significant difference
Ovalbumin, charge train 2 n = 24	10.8	8.87	1.482	2.124	$F_0 < F_{crit}$	no significant difference
Myoglobin n = 18	16.8	16.1	1.090	2.203	$F_0 < F_{crit}$	no significant difference
β-lacto-globulin n = 24	12.0	10.0	1.438	2.124	$F_0 < F_{crit}$	no significant difference