

Supporting Information

MiS-MALDI: Microgel-Selected detection of protein biomarkers by MALDI-ToF mass spectrometry

Eleonora Cerasoli,^a Paulina D. Rakowska,^a Adrian Horgan,^a Jascindra Ravi,^a

Melanie Bradley,^b Brian Vincent^b and Maxim G. Ryadnov^{a,}*

^aNational Physical Laboratory, Teddington, TW11 0LW, UK, ^bSchool of Chemistry,

Cantock's Close, University of Bristol, Bristol, BS8 1TS

Table S1. Peptides analysed by MS-MALDI.

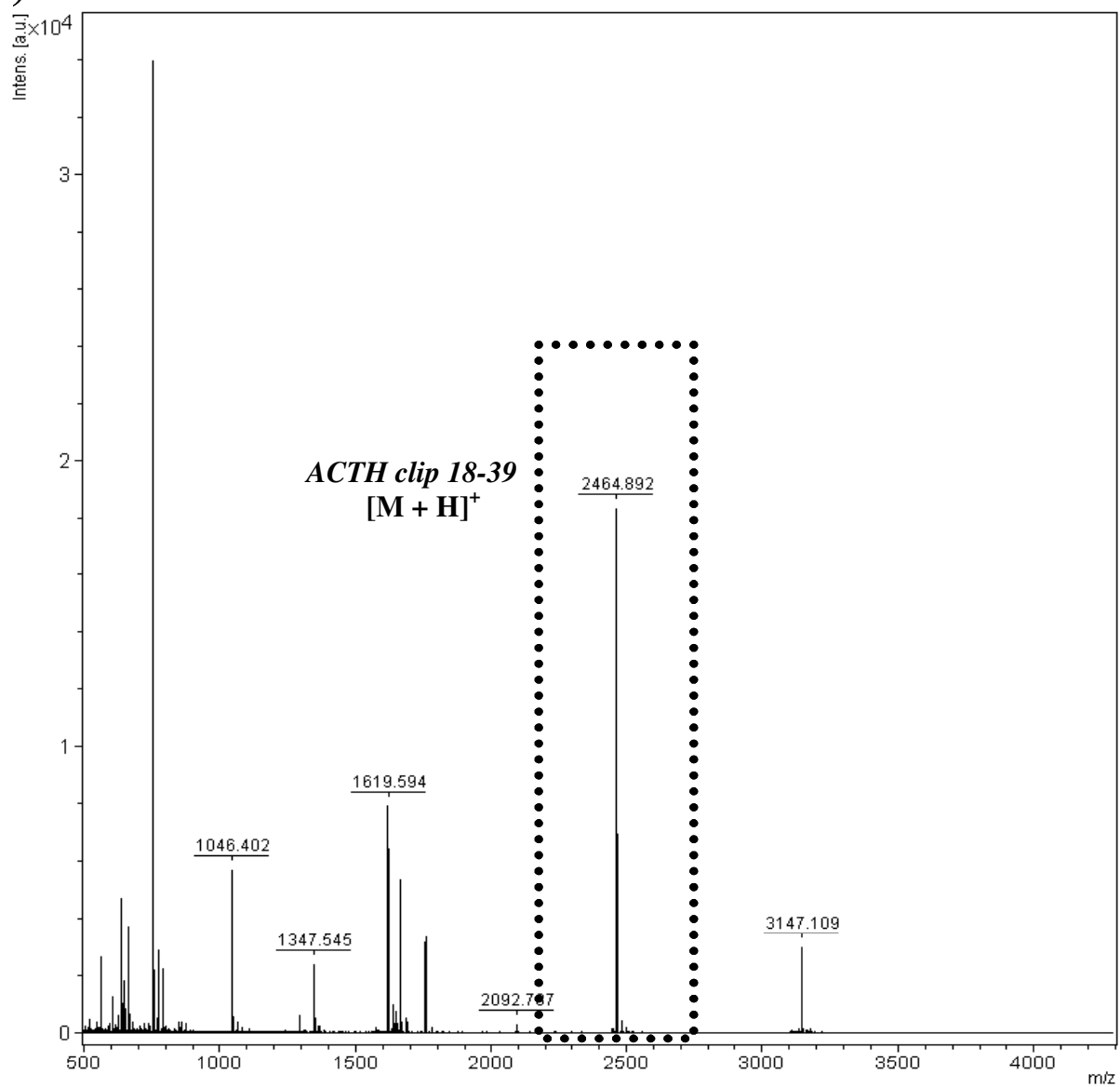
Peptide calibrant	$[M+H]^+$, m/z		Sequence	Theoretical pI	Captured by microgel	Theoretical R_H (nm)
	calc.	found				
Angiotensin II	1046.2	1046.5	DRVYIHPF	6.74	No	0.61
Angiotensin I	1296.5	1296.5	DRVYIHPFHL	6.92	No	0.66
Substance P	1348.6	1347.7	RPKPQQFFGLM	11.00	No	0.68
Bombesin	1620.8	1619.8	Pyr ^a -QRLGNQWAVGHLM	9.76	No	0.73
ACTH clip 1-17	2093.4	2093.1	SYSMEHFRWGKPVGKKR	10.45	No	0.82
gp41 ₆₅₉₋₆₇₁	1616.8	1616.8	ELLELDKWASLWN	4.14	Yes	0.73
ACTH clip 18-39	2465.7	2464.9	RPVKVYPNGAEDESAEAFPLEF	4.25	Yes	0.88
Somatostatin 28	3150.6	3149.5	SANSPAMAPRERKAGCKNFFWKFTSC	9.85	No	0.97
Coiled coil strand A•	2301.6	2302.2	ac-YGKEIAALEQEIAALEQEIAA-am	3.98	Yes	0.85
Coiled coil strand B•	2160.4	2161.1	ac-LEQENAALEKEIAALEQGY-am	3.98	Yes	0.83
Coiled coil strand AB	4361.3	4361.7	ac-YGQKIAALKEKVAALKQKIAALKQKNAALKEKIAALKEKA-am	10.15	No	1.12

^a pyrrolidone carboxylic acid (Pyr)

Table S2. Series of proteins analysed by MiS-MALDI used to verify the selectivity towards charge and size.

<i>Protein</i>	<i>[M+H]⁺, m/z</i>		<i>Theoretical pI</i>	<i>Captured by microgel</i>	<i>Theoretical R_H, (nm)</i>
	<i>calc.</i>	<i>found</i>			
RNAse B	14700	14895	9.6	No	1.88
EDQM hGH	22124	22135	4.9	Yes	2.14
Ovalbumin	42000	40081	4.5 – 5.1	No	2.97
mAb	150000	146935	5.5	No	5.07
Aldolase (subunits)	158000 (~40000)	- (~38006)	6.6 6.7 – 8.2	No	5.18

a)



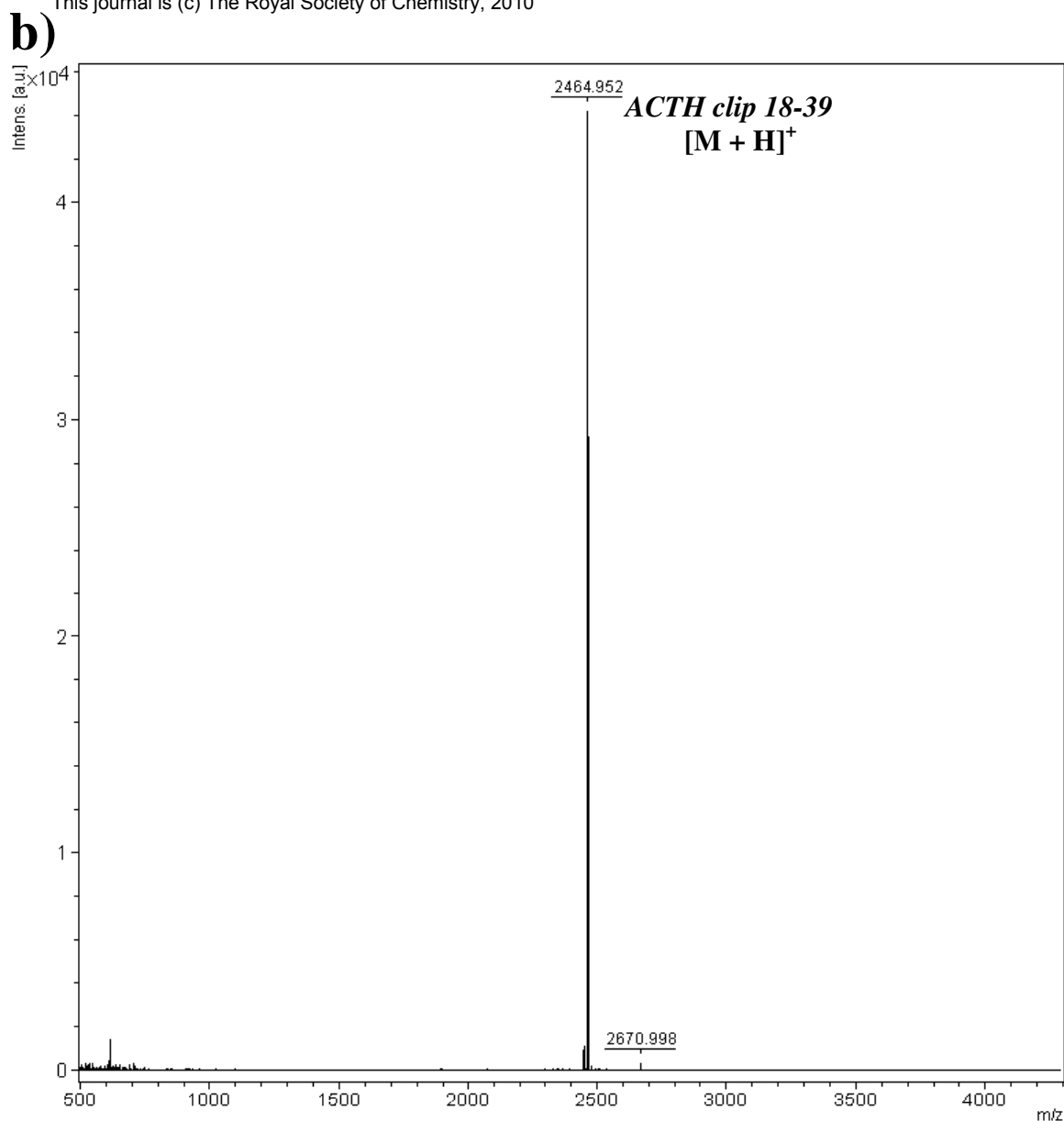
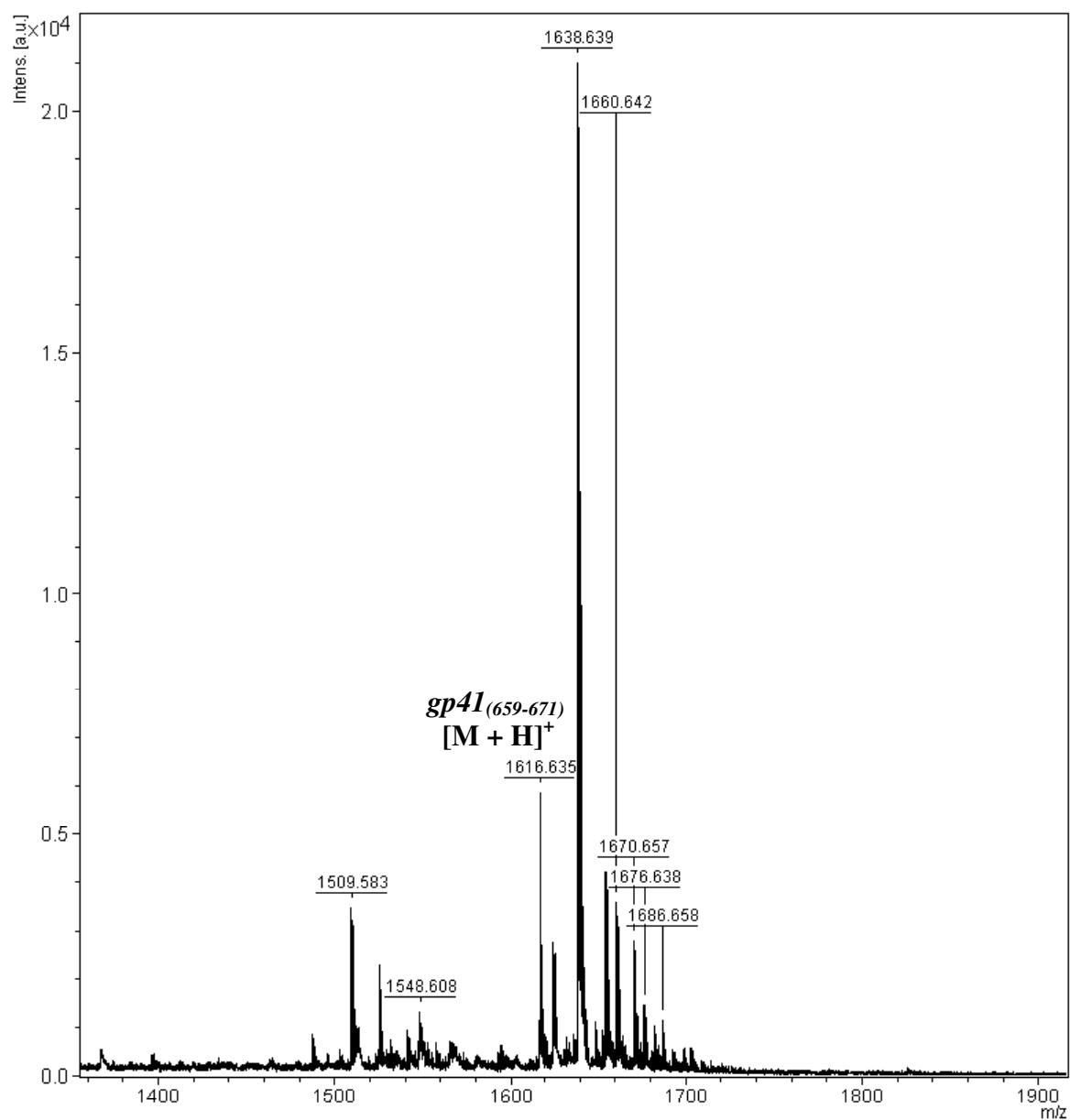


Figure S1. MALDI-ToF mass spectra of peptide calibrants obtained from solution (**a**) and from the microgel (**b**). Only ACTH clip 18-39 peptide (m/z 2465) was captured and detected in the pellet.

a)



b)

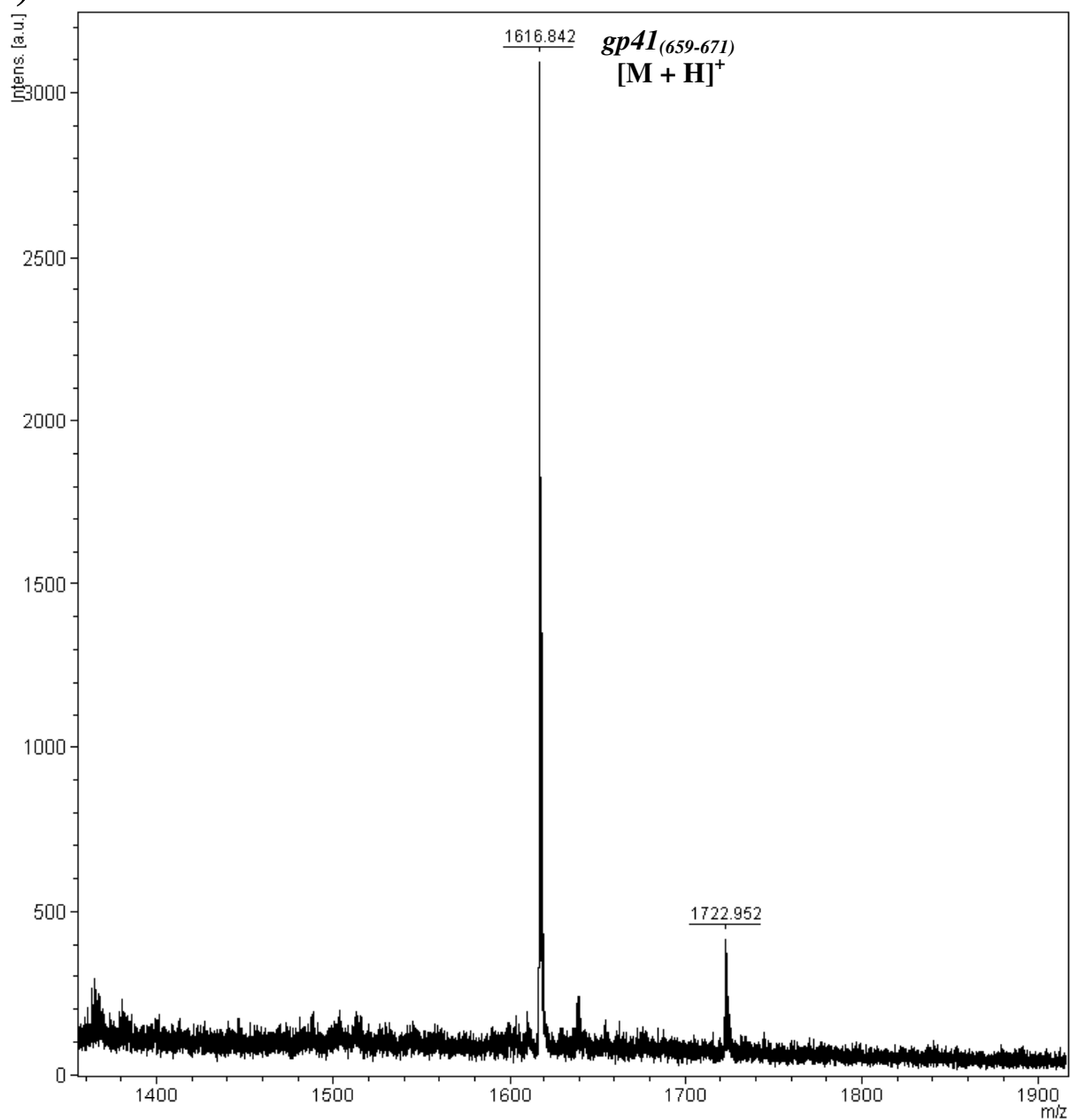


Figure S2. MALDI-ToF mass spectra for gp41₍₆₅₉₋₆₇₁₎ recorded from solution (a) and the microgel (b).

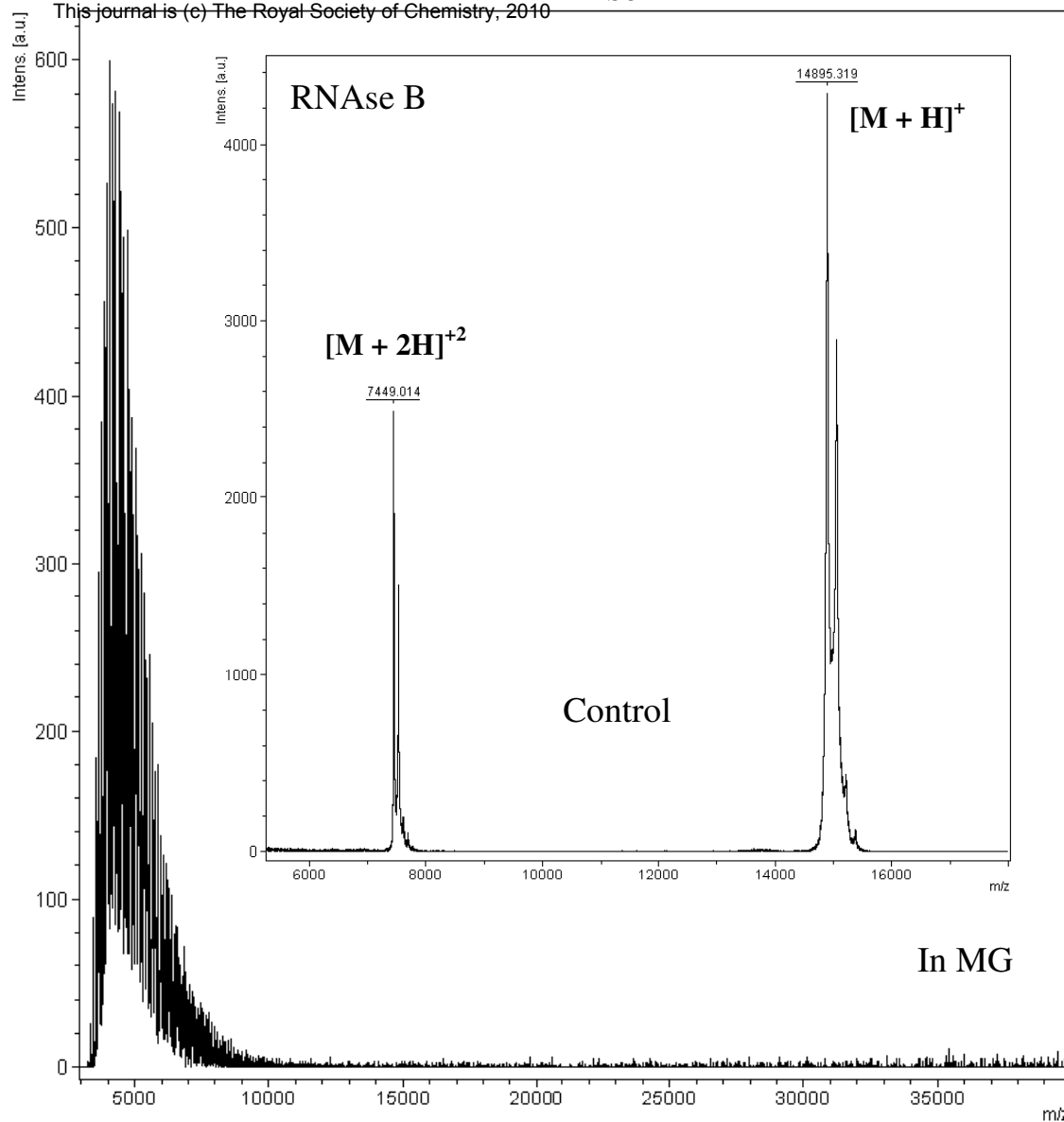


Figure S3. MALDI-ToF mass spectra of RNase B obtained from the microgel and from solution (inset) showing the charge-exclusion of the protein from the microgel.

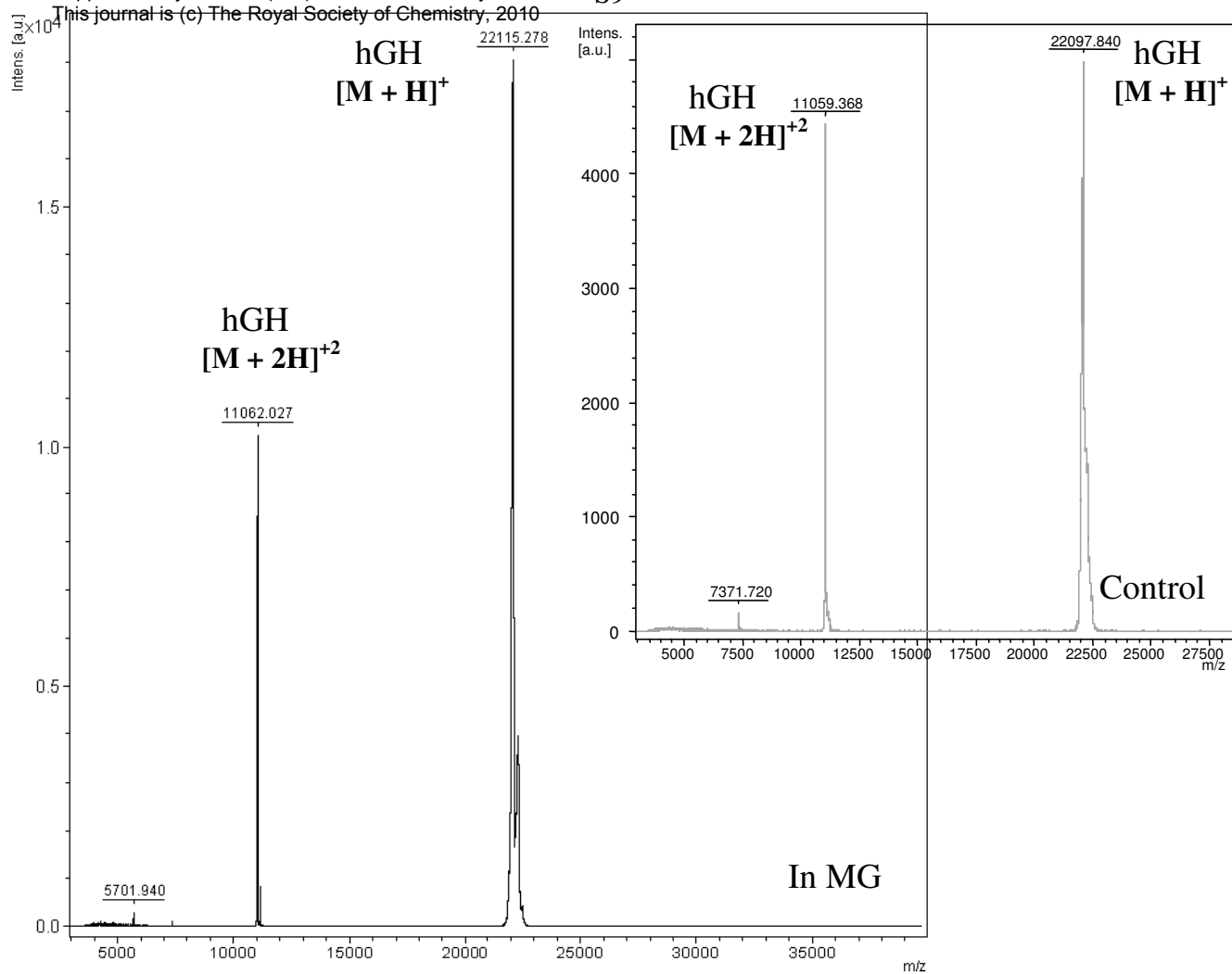


Figure S4. MALDI-ToF mass-spectra of hGH obtained from the microgel and from solution (inset) showing the inclusion of the protein in the microgel.

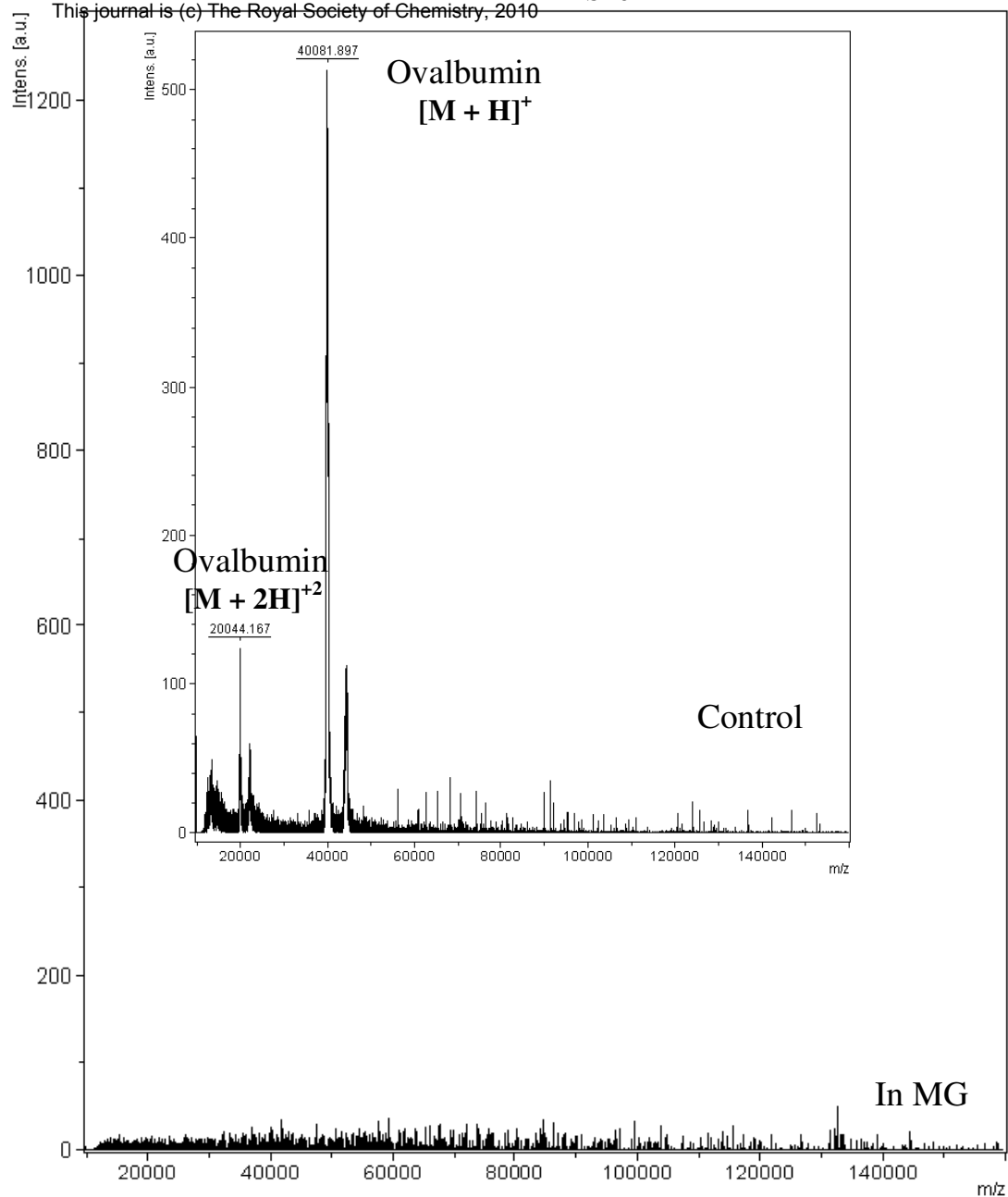


Figure S5. MALDI-ToF mass spectra of ovalbumin obtained from the microgel and from solution (inset) showing the size-exclusion of the protein from the microgel.

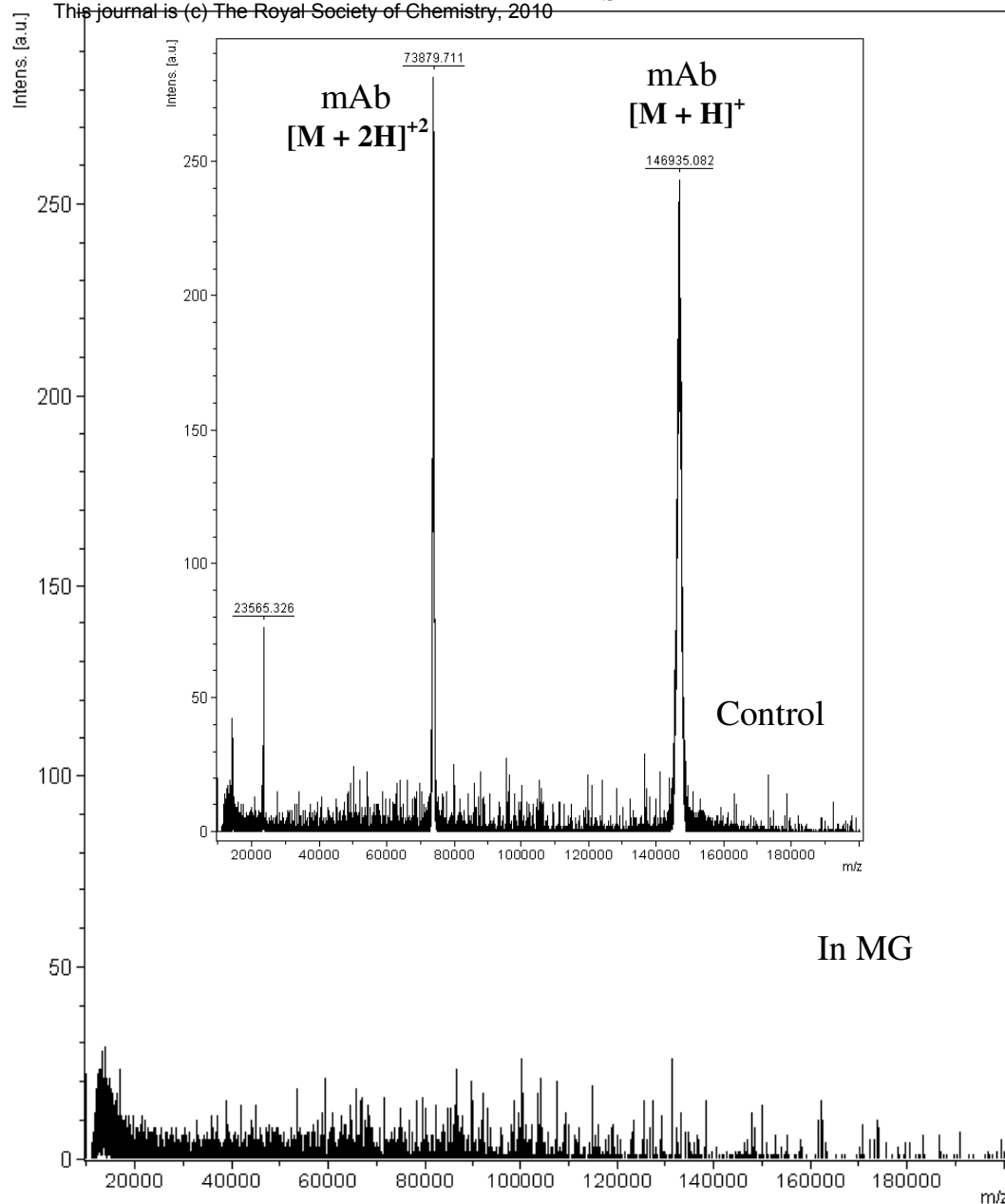


Figure S6. MALDI-ToF mass spectra of a monoclonal antibody (mAb) obtained from the microgel and from solution (inset) showing the size-exclusion of the protein from the microgel.

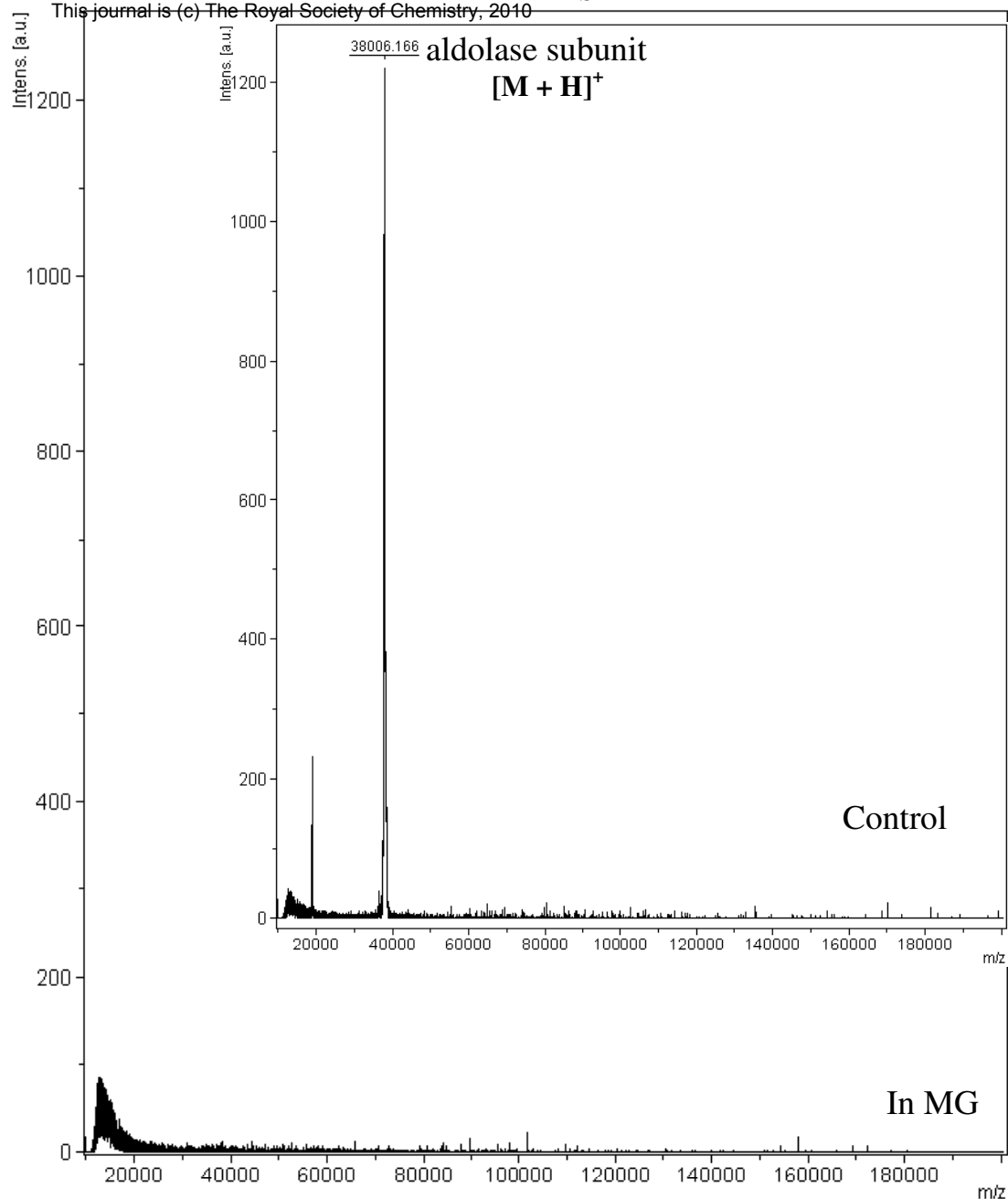
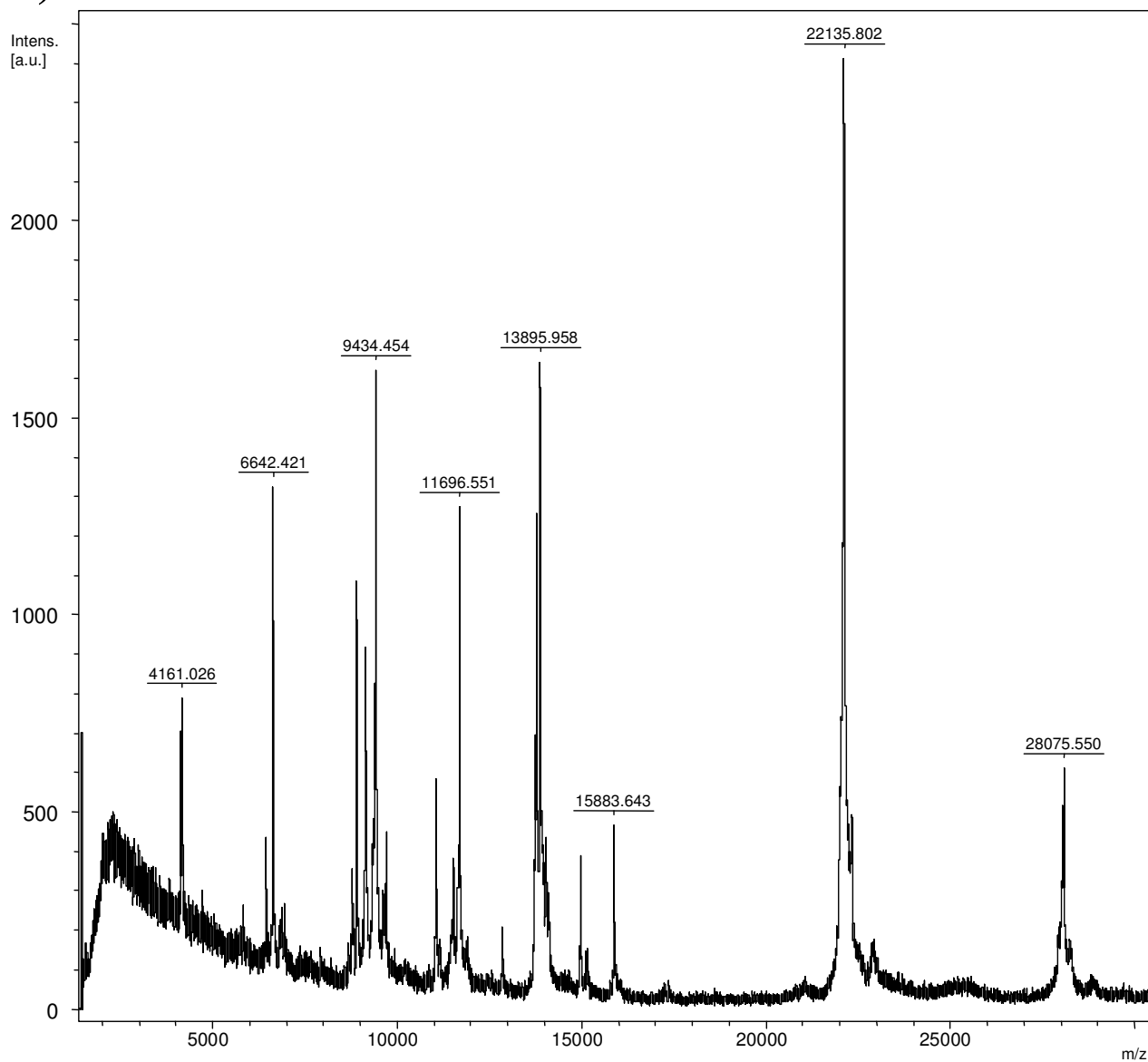
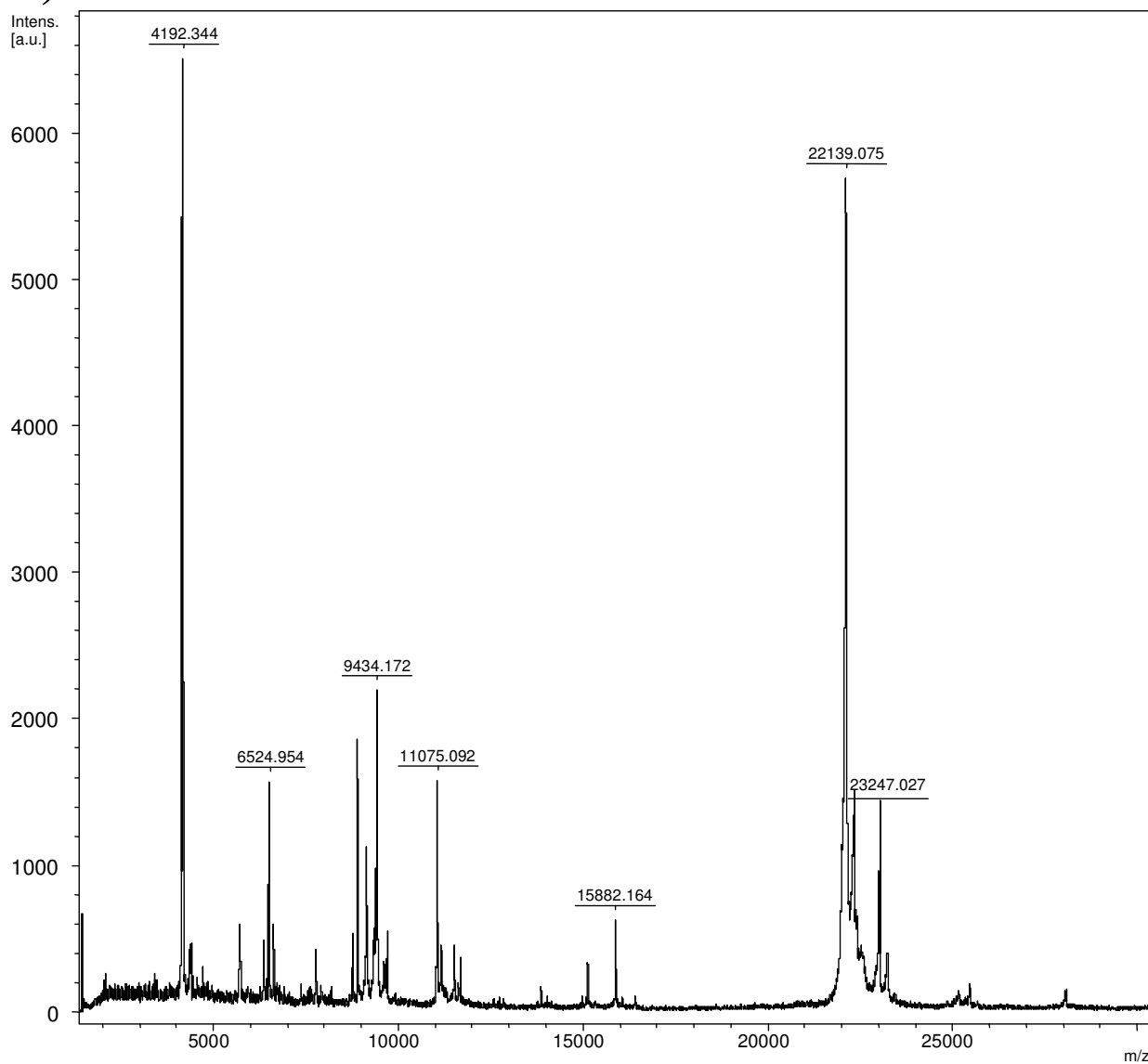


Figure S7. MALDI-ToF mass spectra of aldolase subunit obtained from the microgel and from solution (inset) showing the size-exclusion of the protein from the microgel.

a)



b)



c)

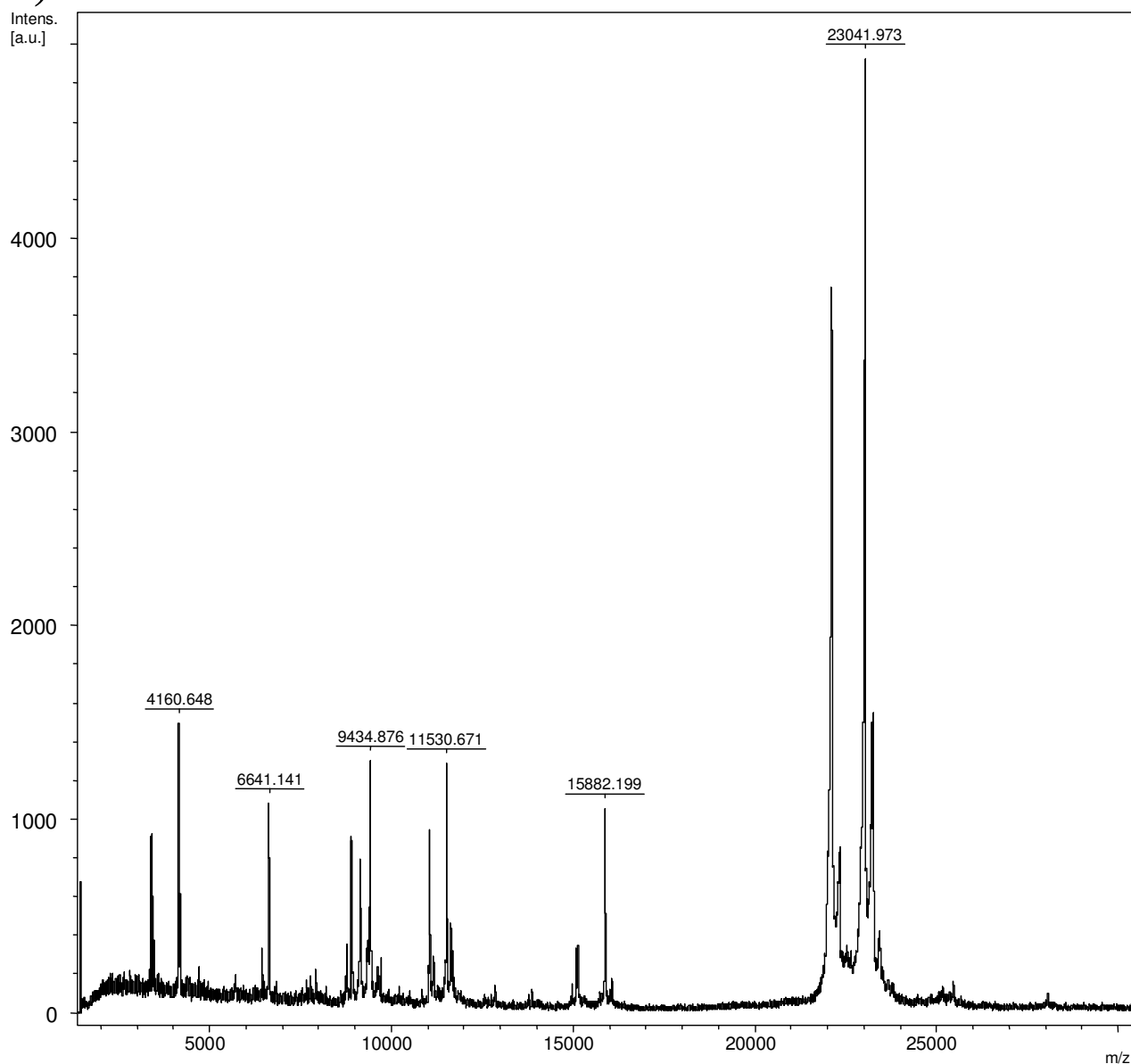
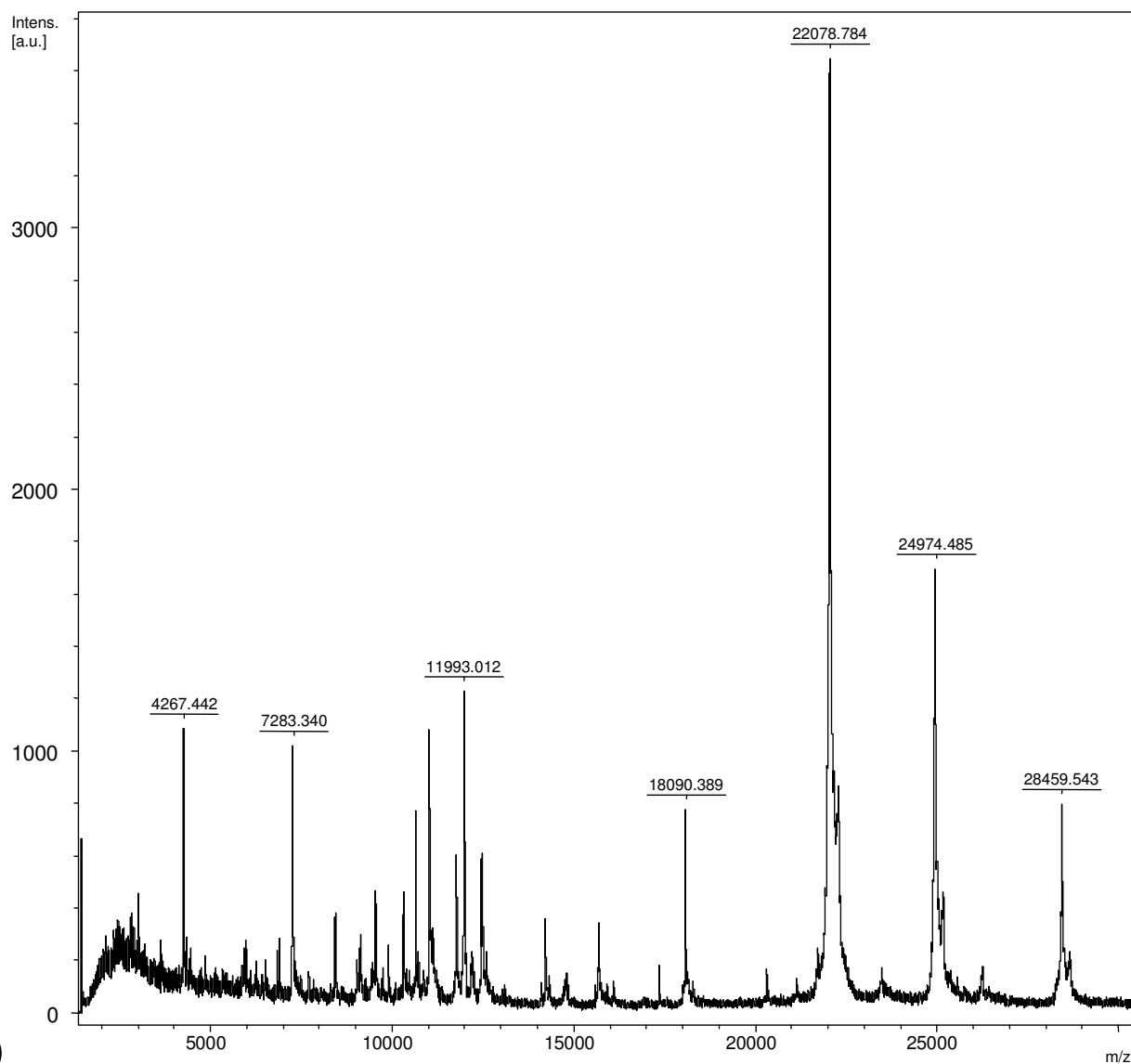


Figure S8. MALDI-ToF mass spectra of a tenfold-diluted serum with hGH spiked at 100 $\mu\text{g/mL}$ (**a**), its microgel-captured fraction (**b**), and microgel-captured fraction of undiluted serum with hGH spiked at 100 $\mu\text{g/mL}$ (**c**).



a)

b)

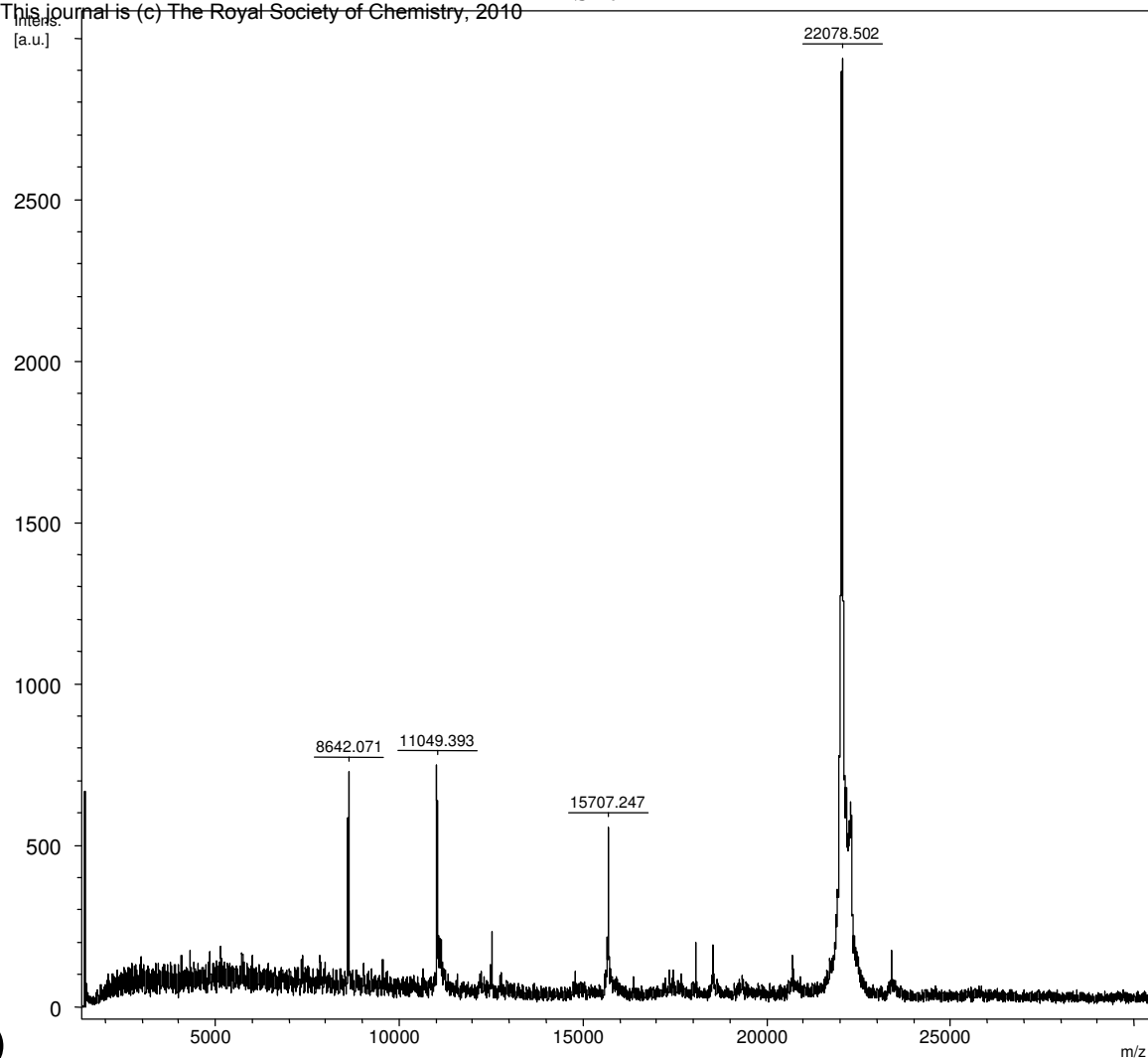


Figure S9. MALDI-ToF mass spectra of crude *E. coli* periplasm containing over-expressed hGH (a), and its microgel-captured fraction (b).

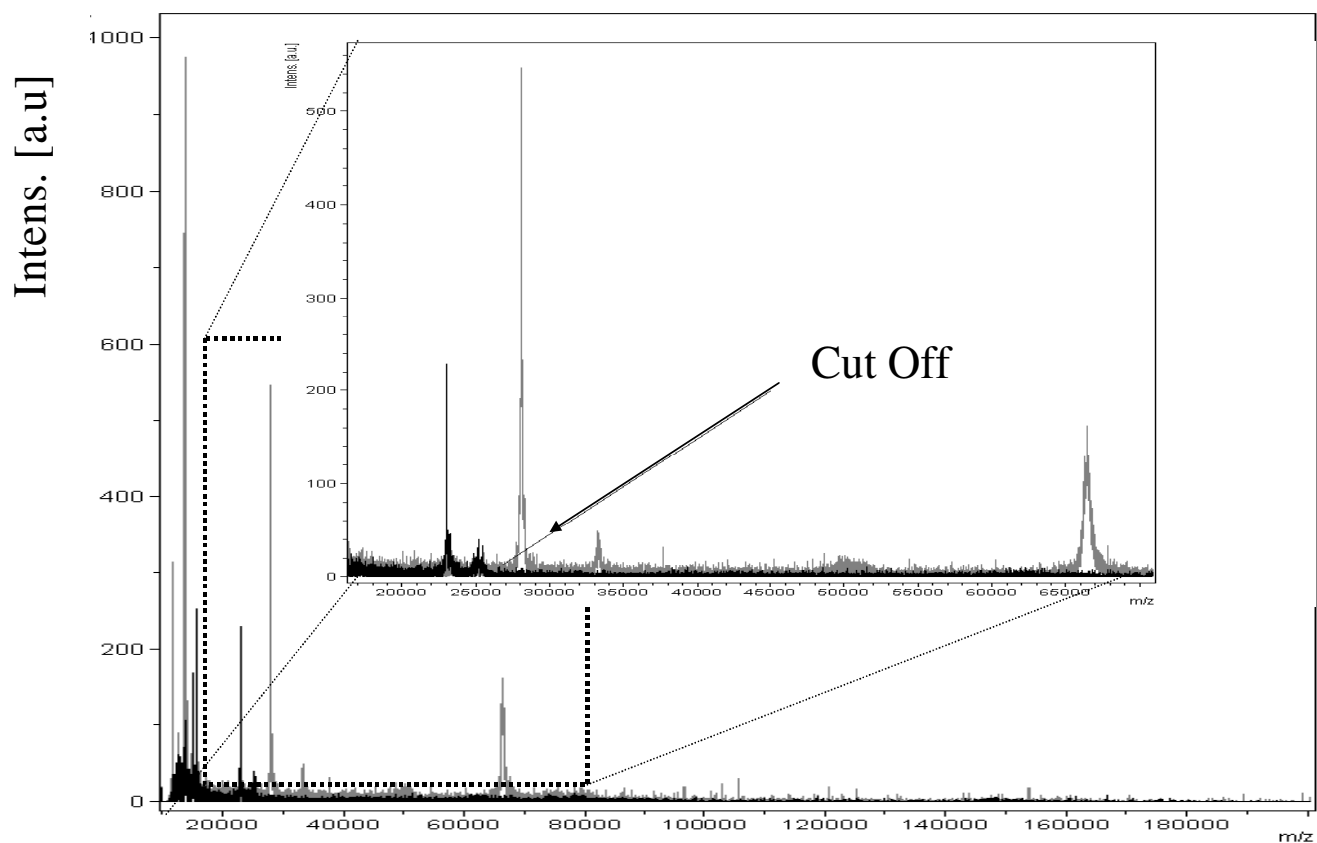


Figure S10. MALDI-ToF mass spectra of a tenfold-diluted serum obtained from the microgel (black) and from solution (grey) showing the size-selective capture of plasma proteins with MW <30 kDa (inset).

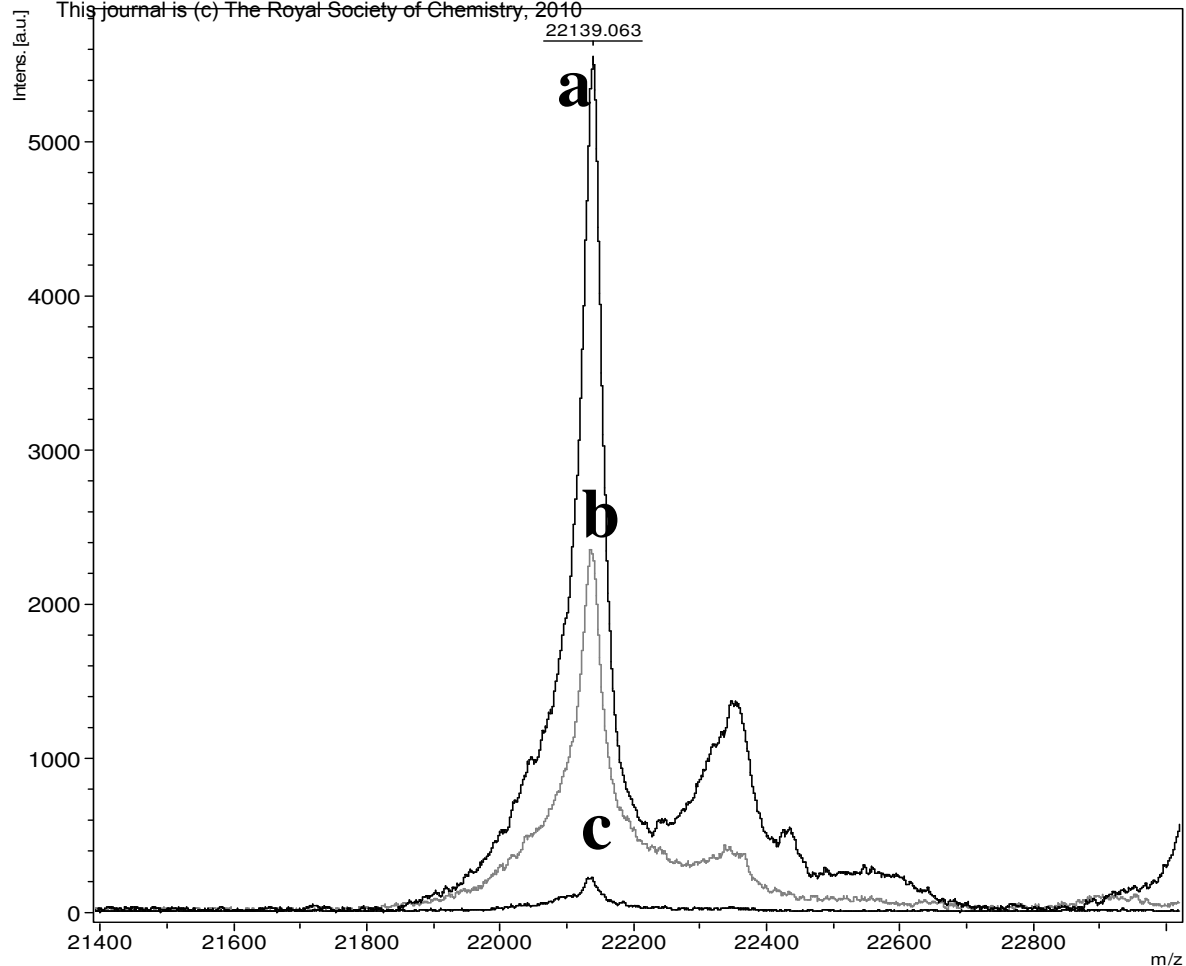


Figure S11. Concentrating effect of MiS-MALDI. Mass-spectra of hGH obtained from the microgel (a), from solution before incubation (b) and from the supernatant after incubation (c).

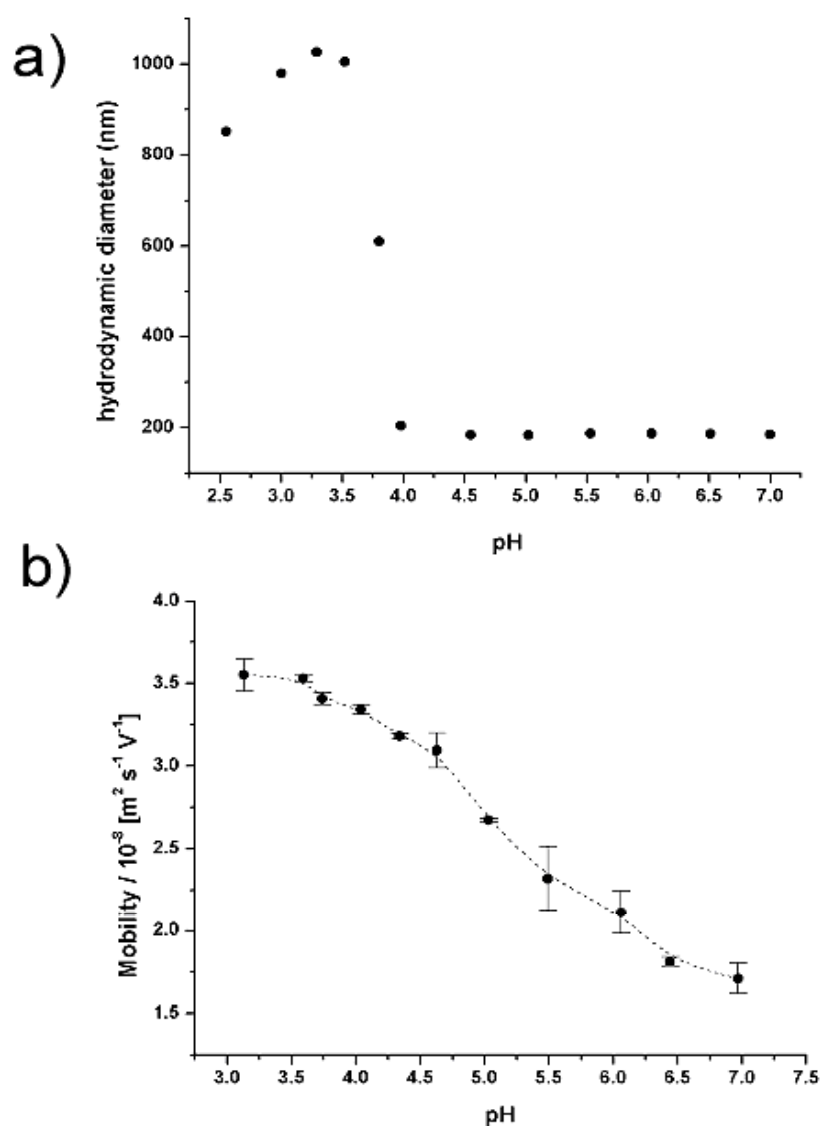


Figure S12. Hydrodynamic diameter (a) and electrophoretic mobility (b) of 1%-x-linked PVP/PEGMA MG as a function of pH.