SUPPORTING INFORMATION

In vitro functional quality characterization of NOTA-modified somatropins

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SUPPORTING INFORMATION DESCRIPTION

Table S1: SEC column comparison

Specifications	Column 1	Column 2
Column	BioSep-SEC-S 2000	YMC-Pack Diol-120
Recommended MW range	1,000 – 300,000 Da	5,000 – 100,000 Da
Dimensions	7.8 (i.d.) x 300 mm	4.6 (i.d.) x 150 mm
Pore size	145 Å	120 Å
Particle size	5 µm	3 µm
Surface area	Info not available	330 m^2/g
Recommended pH Range	2.5-7.5	5.0-7.5
Chemical modifications	Deactivated silica	1,2-dihydroxypropane derivatised silica
Carbon load (%C)	Info not available	7%

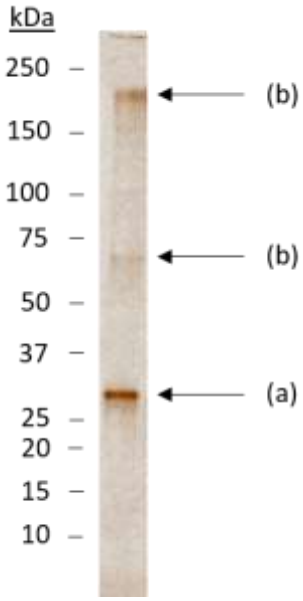


Figure S1: SDS-PAGE analysis of used hGHBp. On the left the molecular weight of the marker is indicated. (a) hGHBp and (b) higher molecular weight impurities or hGHBp oligomers.

A 12% Criterion™ TGX™ Precast Midi Protein Gel, 18 well (Bio-Rad Laboratories nv, Belgium) was used for the SDS-PAGE analysis of the hGHBp sample (1 µg). The molecular weight marker 'Precision plus protein all blue standards' (Bio-Rad Laboratories) was used to estimate the molecular weight of the compounds. Compounds were visualized with the fluorescent Sypro Ruby gel stain after gel fixation and scanned using a Versadoc imaging system (Bio-Rad Laboratories). Next to hGHBp (a), also larger impurities or hGHBp oligomers were detected (b).

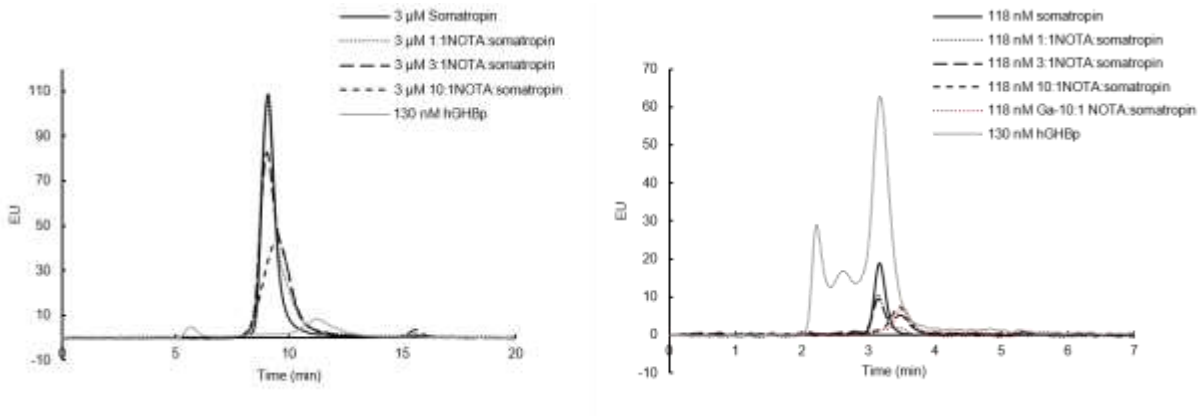


Figure S2: Overlay chromatogram of the single protein injections. Left: column 1, right: column 2.

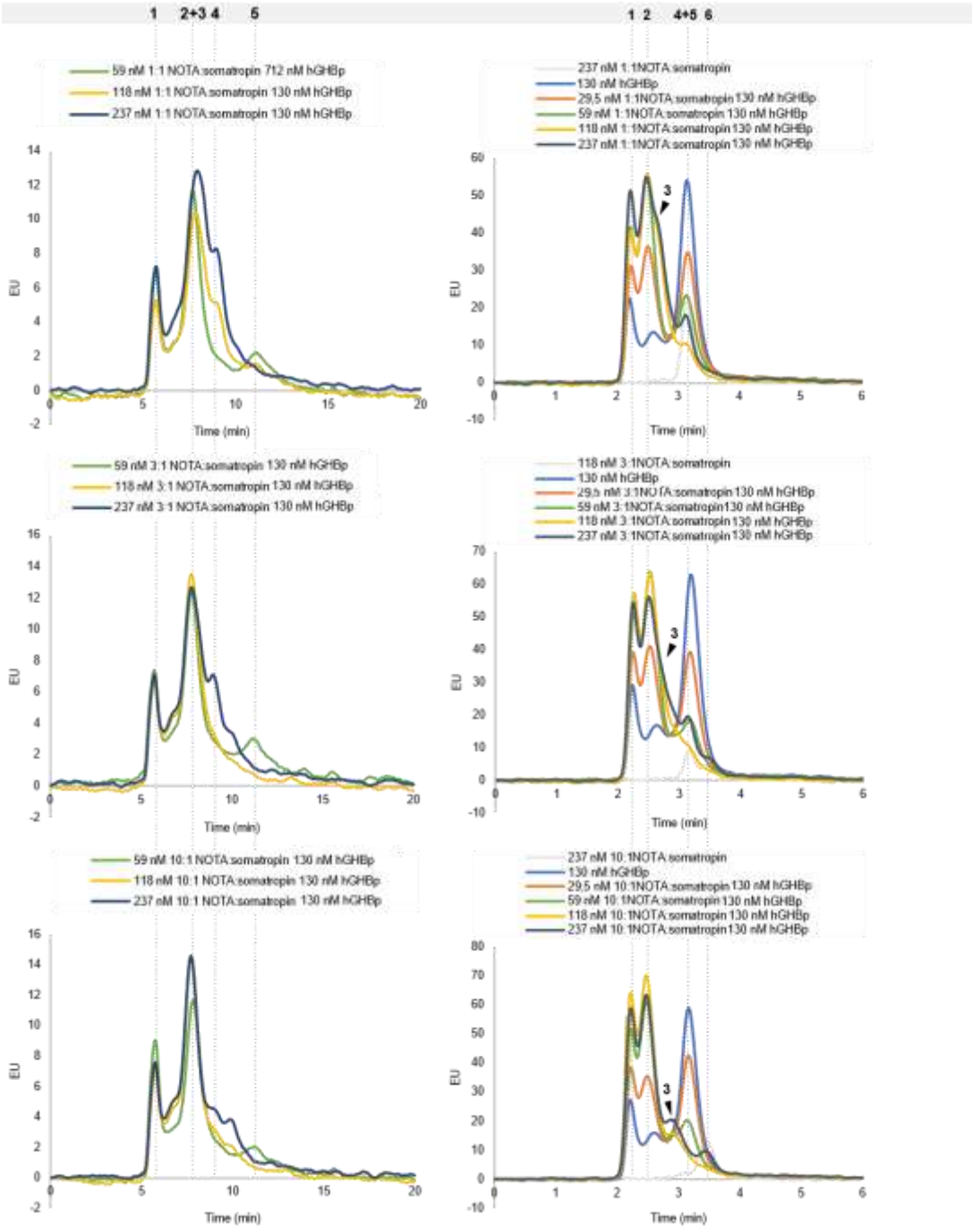
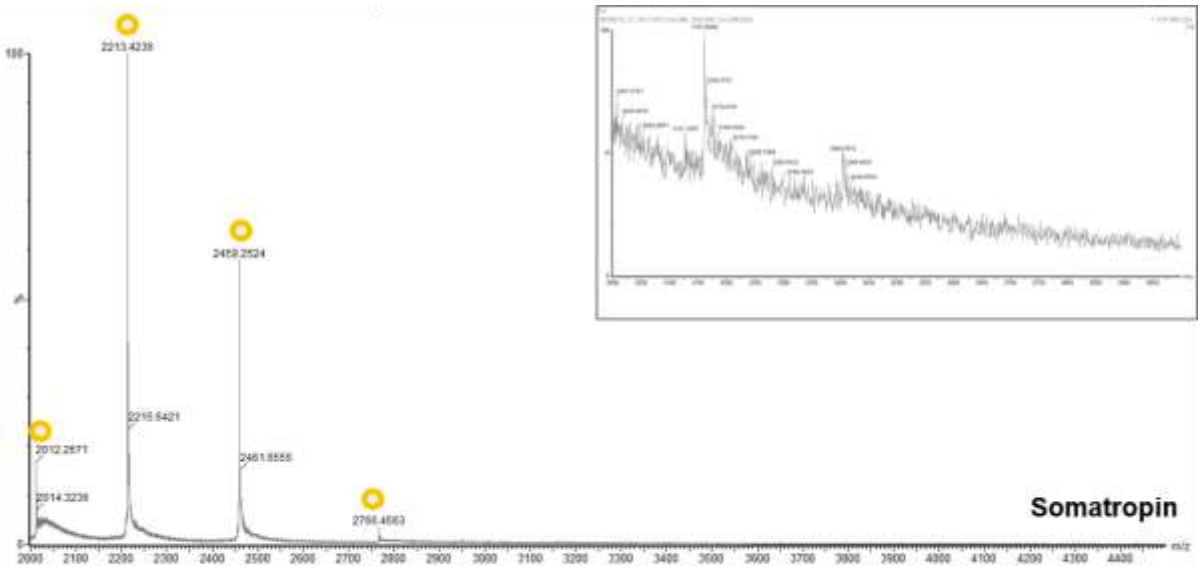


Figure S3: Overlay chromatograms of the stoichiometric analysis of NOTA-modified somatropin and hGHBp on column 1 (right) and column 2 (left). (1): Impurity from hGHBp sample, (2): 1:2 analyte:hGHBp, (3): 1:1 analyte:hGHBp, (4): analyte, (5): hGHBp and (6): analyte with higher NOTA-substitution degree.

A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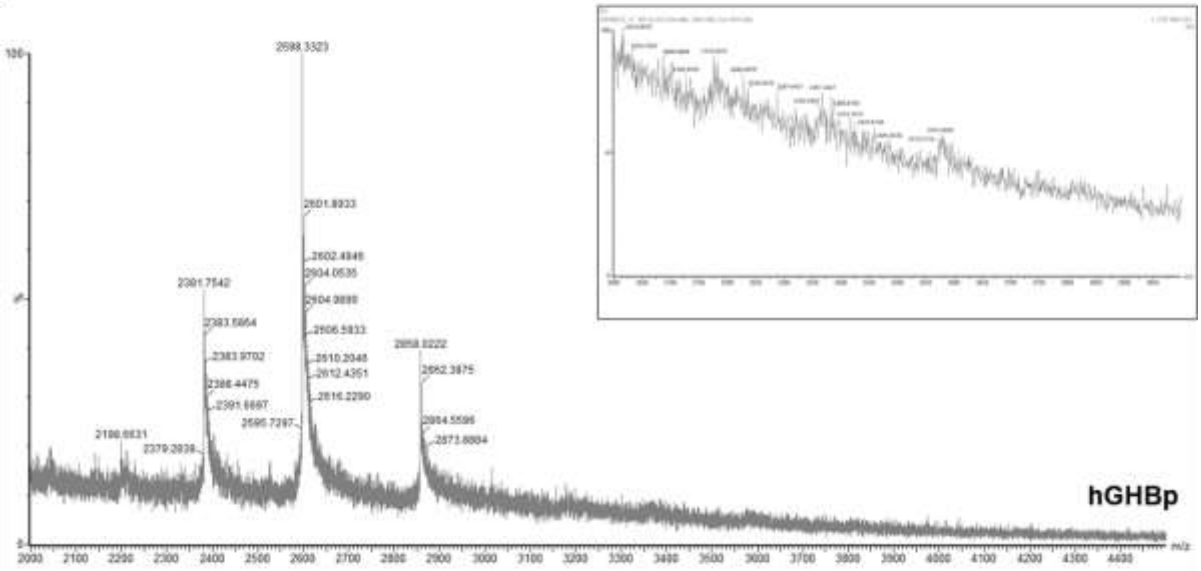
2215.5421
2012.2671
2461.8555
2014.3239
2766.4663
Somatropin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2601.8933
2602.4846
2604.0535
2604.9899
2606.5833
2610.2048
2612.4261
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2381.7542
2383.5964
2383.9702
2386.4475
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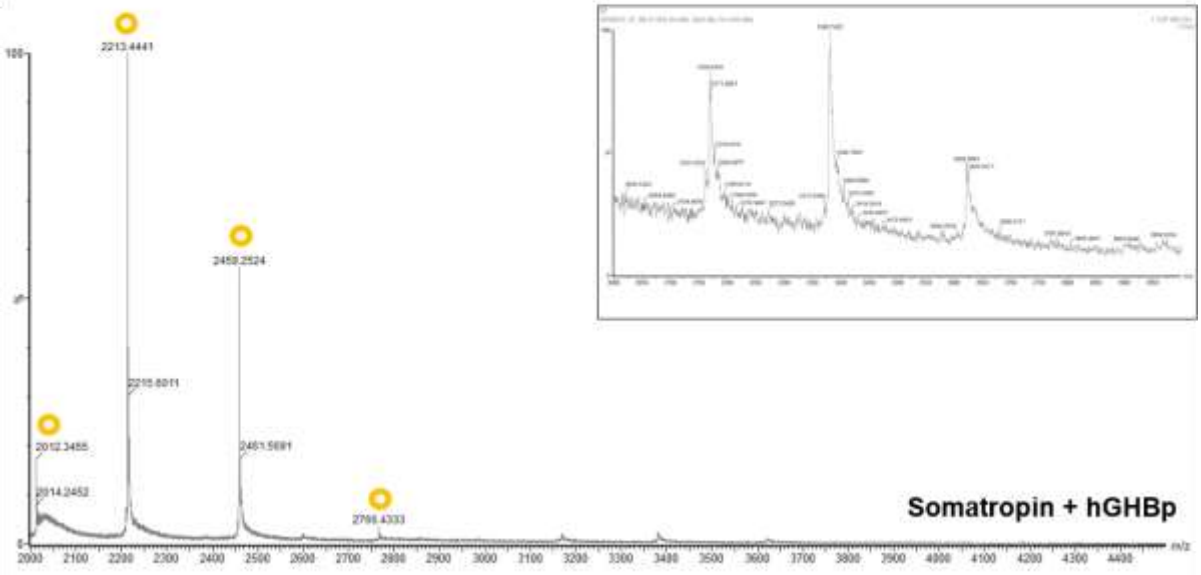
2662.3875
2664.5596
2673.6804
hGHBp


2213.4441
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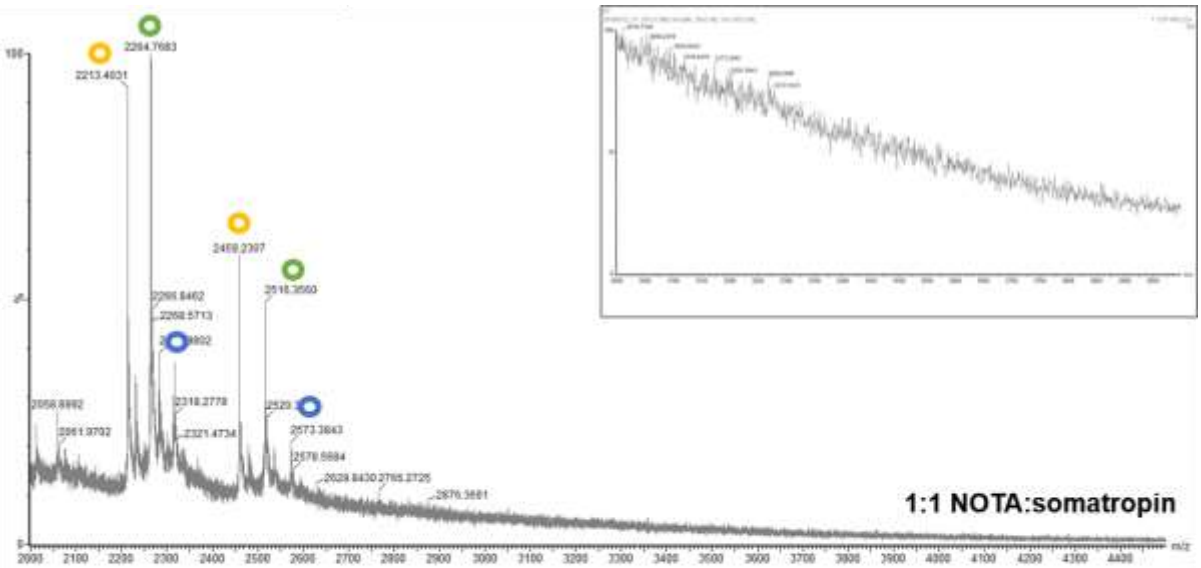
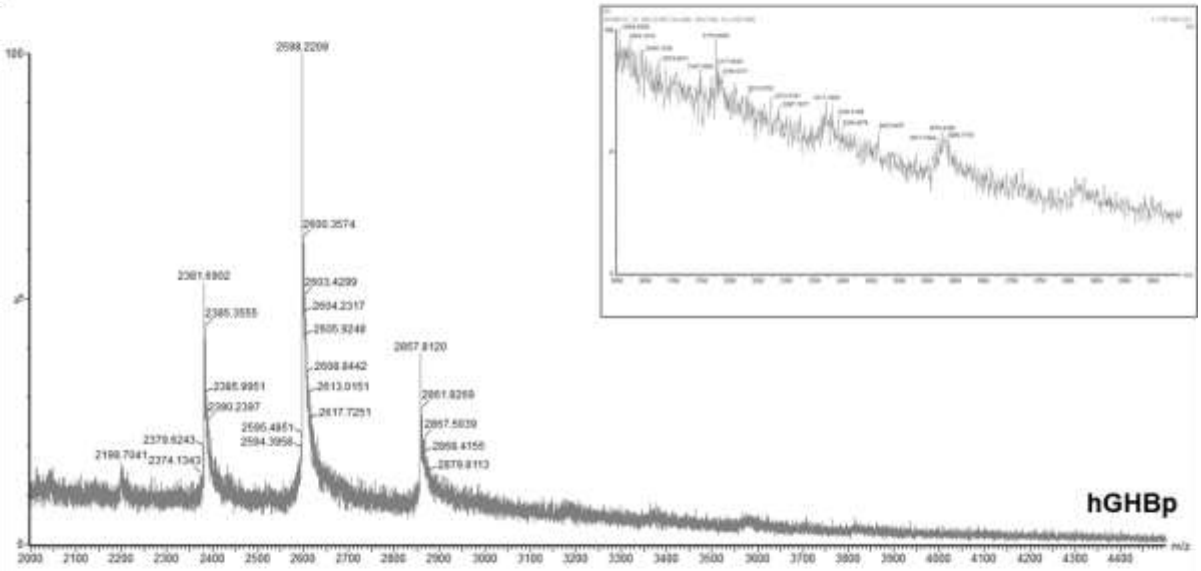
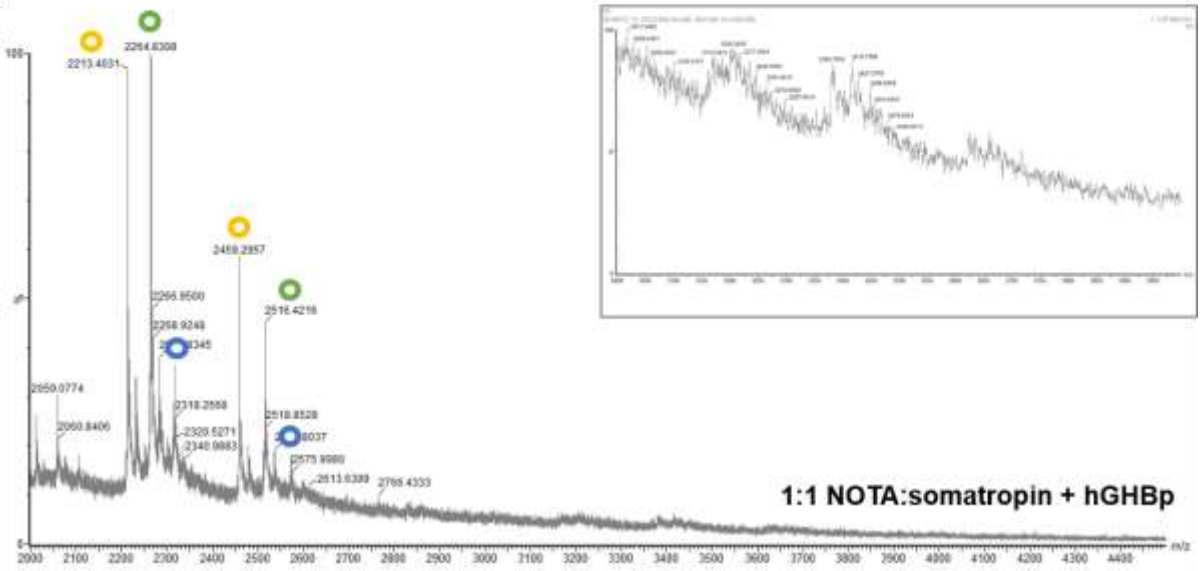
2215.6011
2012.3455
2461.5691
2014.2452
2766.4333
Somatropin + hGHBp

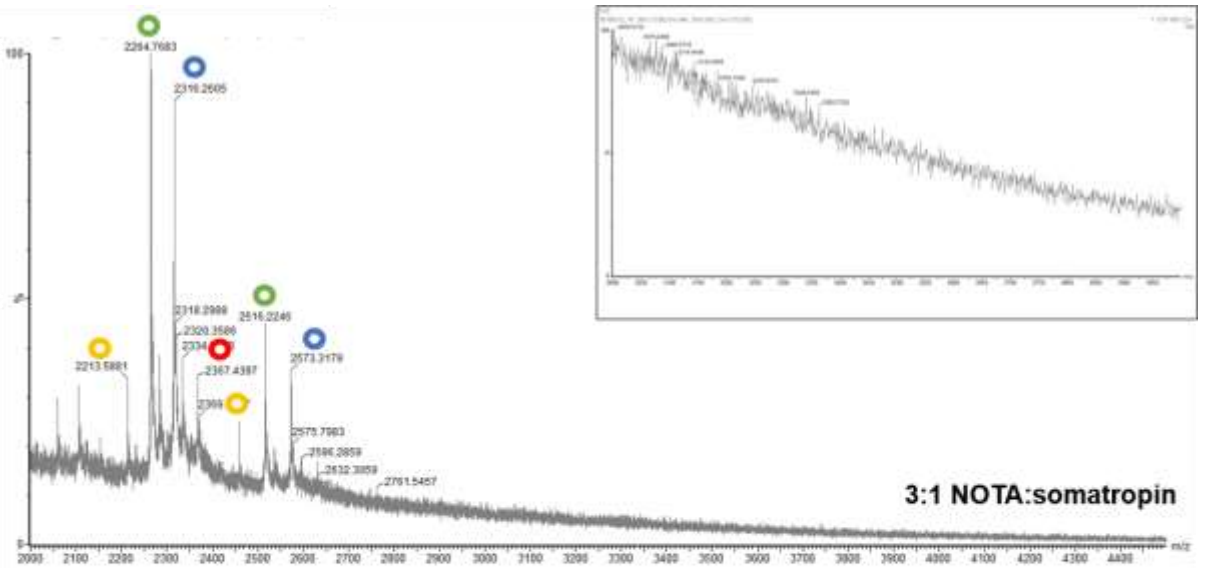
B







C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2516.2246
2320.3586
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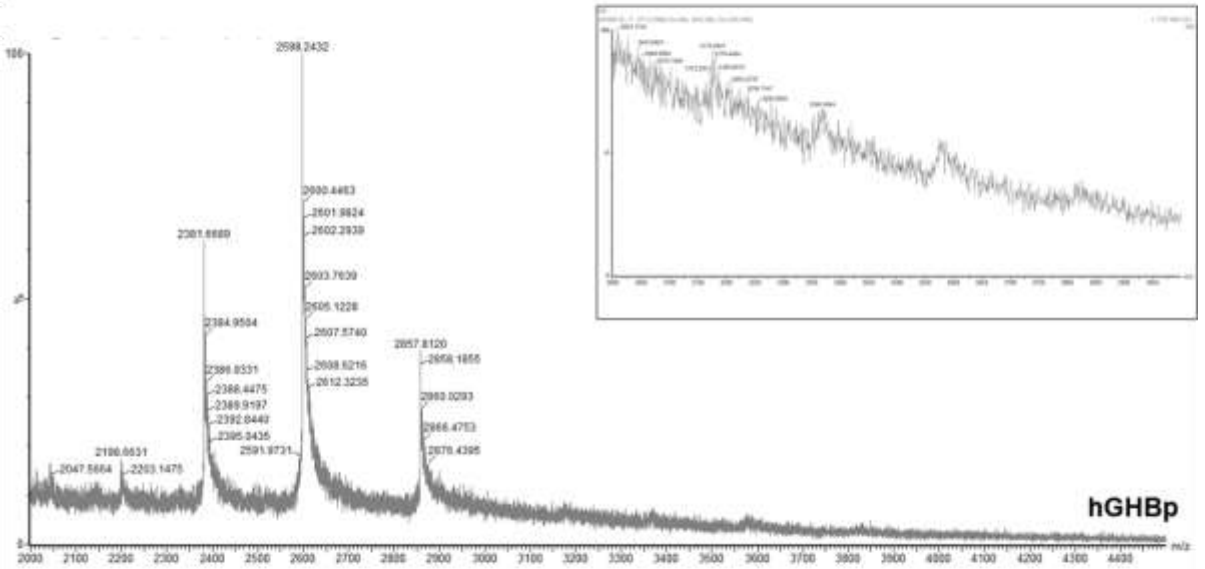
2367.4387
2575.7983
2586.2859
2632.3859
2791.5457
3:1 NOTA:somatropin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2600.4483
2601.9824
2602.2939
2381.6689
2603.7609
2605.1228
2384.9594
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2608.6216
2386.8331
2612.3235
2388.4475
2389.9197
2392.8449
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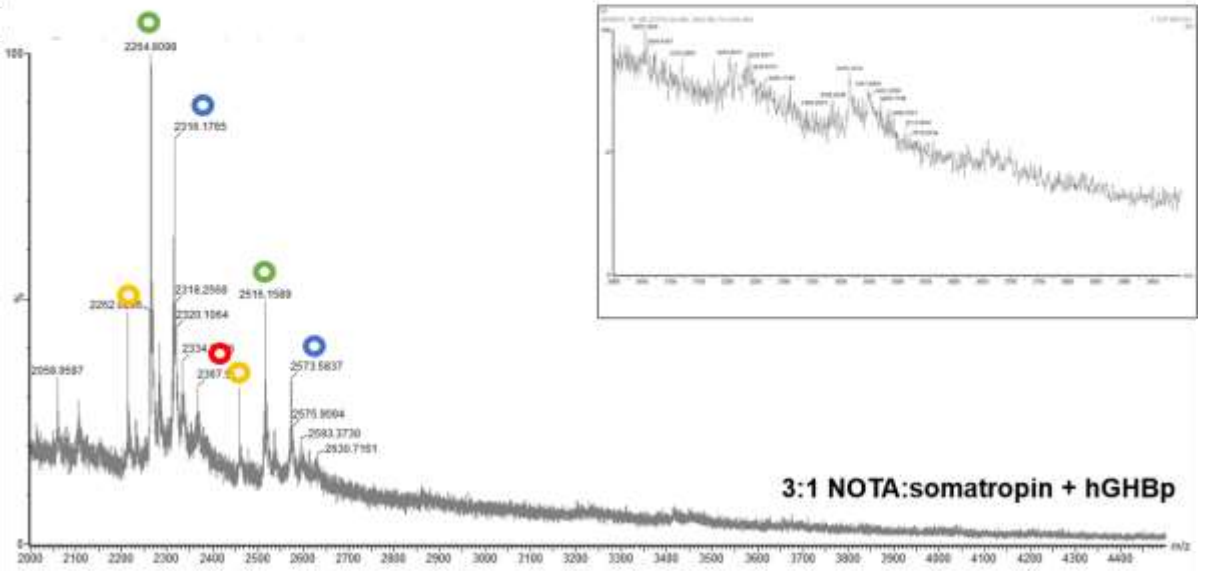
2591.9731
2199.0631
2047.5664
2253.1475
2666.4753
2676.4395
hGHBp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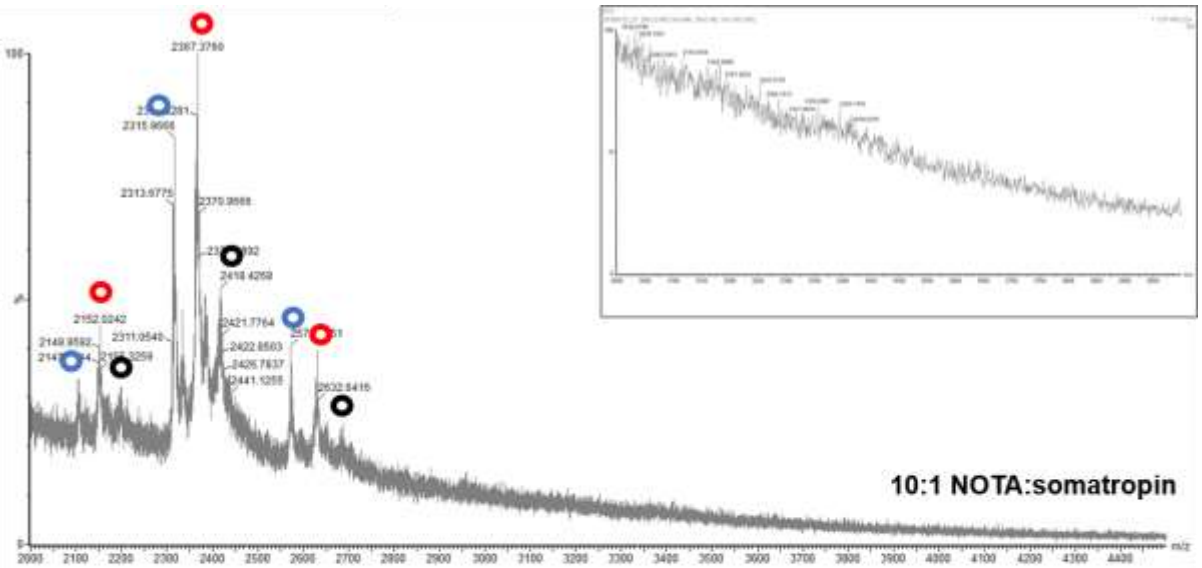
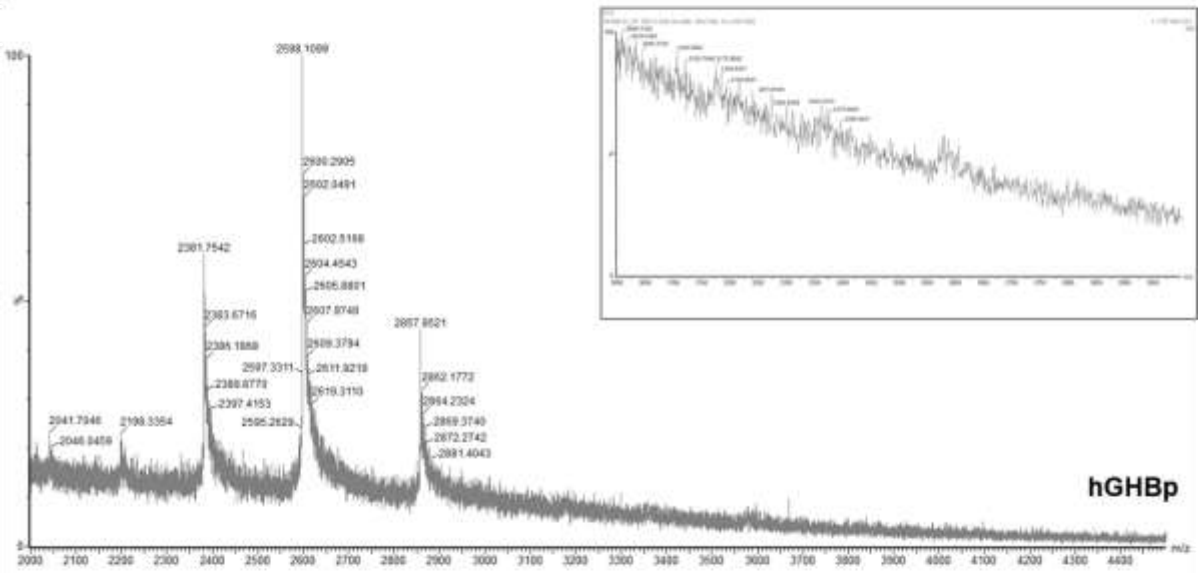
2318.2568
2516.1589
2320.1064
2573.5837
2575.9004
2593.3730
2630.7151
3:1 NOTA:somatropin + hGHBp

D





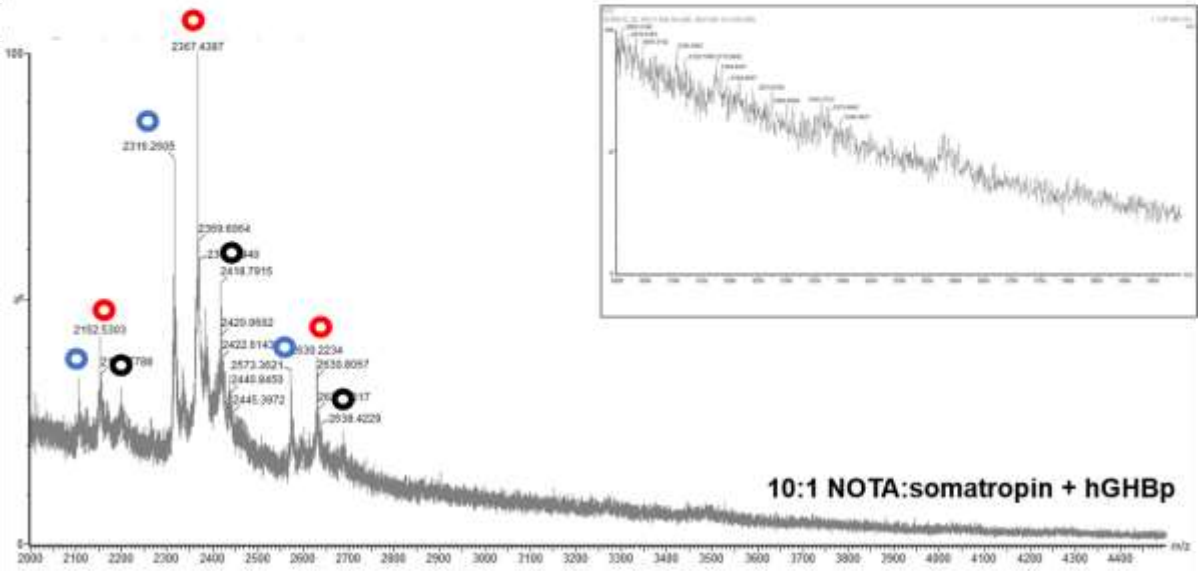


Figure S4: MS spectra of the standard and sample single proteins and complexes. (A) Somatropin analyte, (B) 1:1 NOTA:somatropin analyte, (C) 3:1 NOTA:somatropin analyte and (D) 10:1 NOTA:somatropin analyte. Insets: zoom on region between 3000-4000 m/z. Colour codes for substitution degree species, orange: unmodified somatropin, green: 1 NOTA on somatropin, blue: 2 NOTAs on somatropin, red: 3 NOTAs on somatropin and black: 4 NOTAs on somatropin.

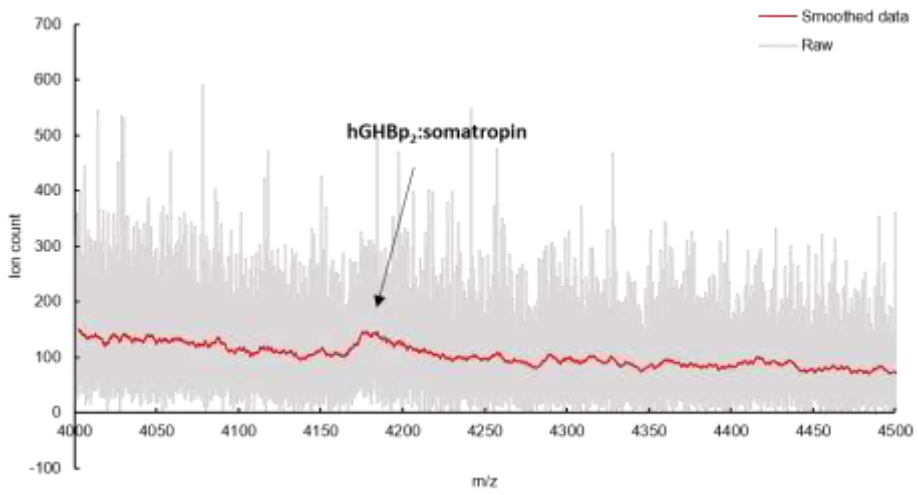


Figure S5: MS spectrum of hGHBp$_2$:somatropin (z=19).