The Versatility of Q-TOF HRMS in Bioanalysis

From Small Peptides Quantitation to Protein Complexes Characterization

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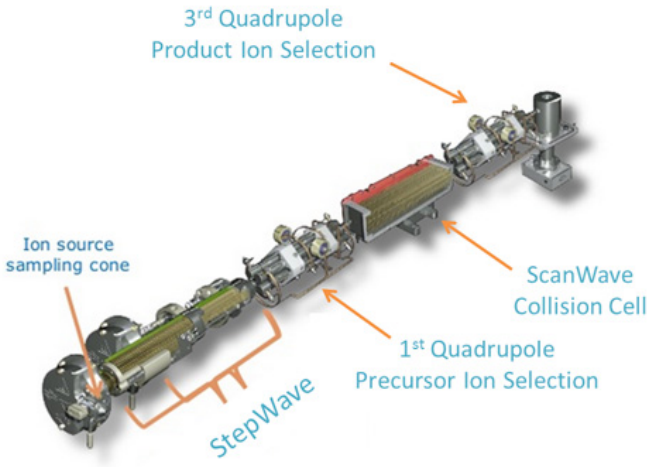
The Sensitive and Robust Quantitation of Intact Peptide Hormones Using Q-TOF HRMS

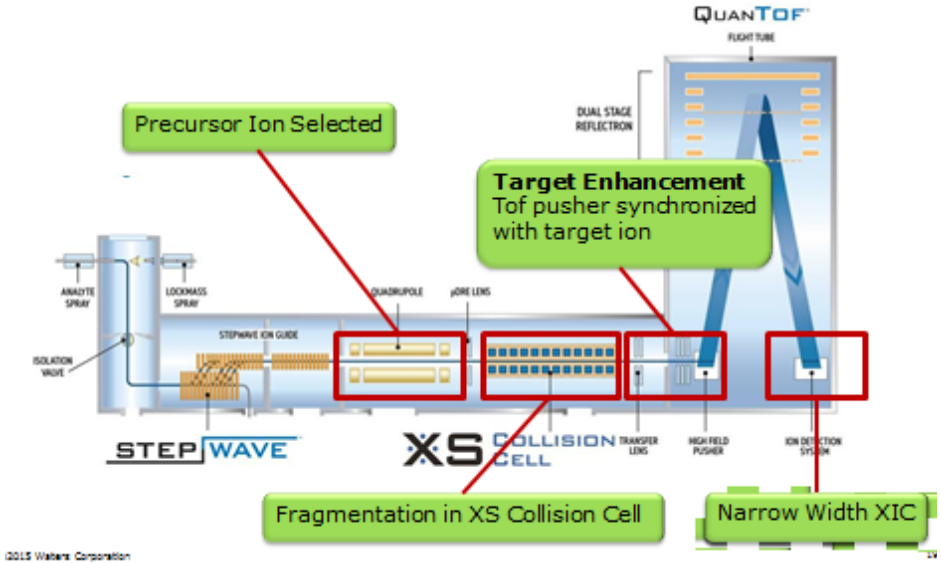
Z Chen *et al.* ***Bioanalysis*** (2019) 11(13), 1275–1289

Comparison of Two Mass Spectrometers on Protein Quantitation

QQQ (Waters Xevo TQ-XS)



Q-TOF (Waters Xevo G2-XS)



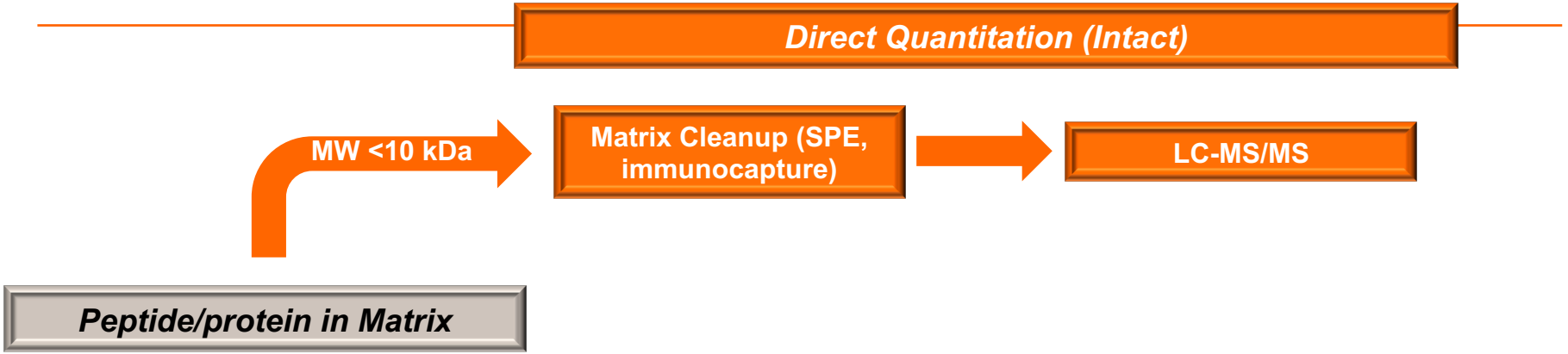
Images from: https://proteomics.research.chop.edu/instruments.php http://blog.waters.com/wp-content/uploads/2015/03

Optimal MRM Methods are Similar in QQQ and Q-TOF

Hormone	M.W.	Precursor Ion	Product Ion	CE in QQQ	CE in Q-TOF
Angiotensin II	1046	523.7760 (2+)	784.4098 (b6, 1+)	19	19
Endothelin-1	2492	831.3429 (3+)	1144.4747 (b20, 2+)	20	20
Endothelin-3	2643	661.5337 (4+)	1163.4775 (b19, 2+)	18	17
ANP	3080	616.8918 (5+)	584.2767 (b27+H_2O, 5+)	24	26
IAPP	3903	976.7201 (4+)	883.7466 (b25, 3+)	31	35
CRF	4757	793.7496 (6+)	883.2783 (y38, 5+)	21	22
Insulin	5807	1162.5286 (5+)	345.2107 (y3 from chain B, 1+)	44	44
PTH	9424	725.9220 (13+)	770.8134 (y82, 12+)	19	19

- Optimal MRM methods are the same in buffer and matrix (plasma).

Workflow for Small Peptide/Protein Quantitation using LC-MS



- **Sample preparation**
  - Spike hormone mixture (10 pg/mL – 1000 ng/mL) into 50 µL of human plasma, two sets of calibration standards. Three levels of QCs in four replicates.
  - After SPE cleanup (Waters HLB), dry and reconstitute elution. Inject 15 µL to LC/MS.
- **LC/MS method**
  - iKey microflow: 2.5 min of trapping time (15 µL/min) and 12 min of analytical time (3 µL/min, 8 min gradient 1-70% B + 4 min wash and re-equilibrate)
  - For TOF-MRM data, integration over 0.05 Da mass extraction window.

Quantitation Performance for Seven Intact Hormones Using QQQ or TOF-MRM

		ET-1	ET-3	ANP	IAPP	CRF	INS	PTH
QQQ	r^2	0.9926	0.9937	0.9903	0.9880	0.9907	0.9887	0.9885
	LLOQ (ng/ml)	0.01	0.01	0.05	0.1	0.5	0.1	2
	HLOQ (ng/ml)	≥1000	≥1000	≥1000	≥1000	≥1000	500	≥1000
	Linear range (Log_{10})	≥5.0	≥5.0	≥4.3	≥4.0	≥3.3	≥3.7	≥2.7
Q-TOF	r^2	0.9779	0.9759	0.9716	0.9810	0.9932	0.9877	0.9848
	LLOQ (ng/ml)	0.02	0.05	0.5	0.5	1	0.05	20
	HLOQ (ng/ml)	50	200	500	≥1000	≥1000	200	≥1000
	Linear range (Log_{10})	3.4	3.6	3.0	≥3.3	≥3.0	3.6	≥1.7

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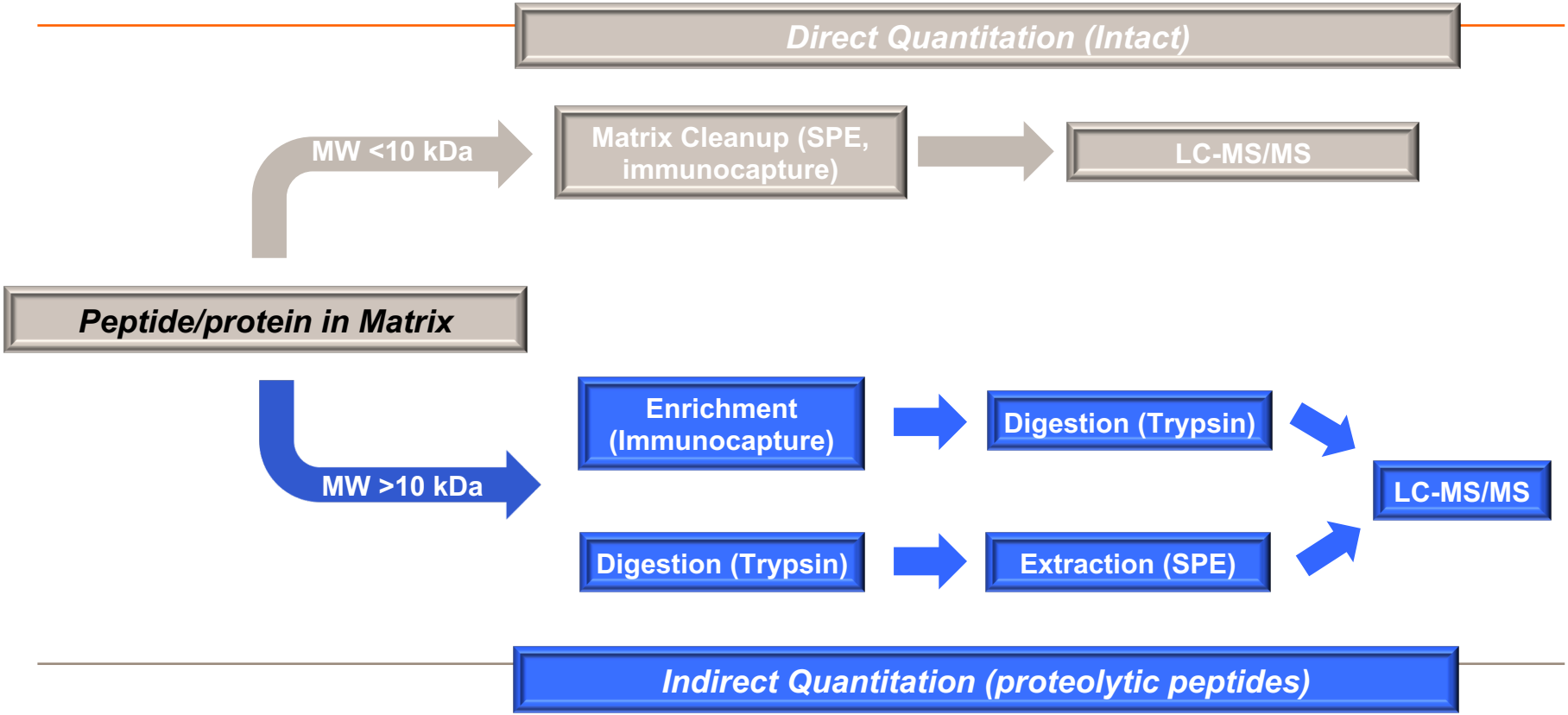
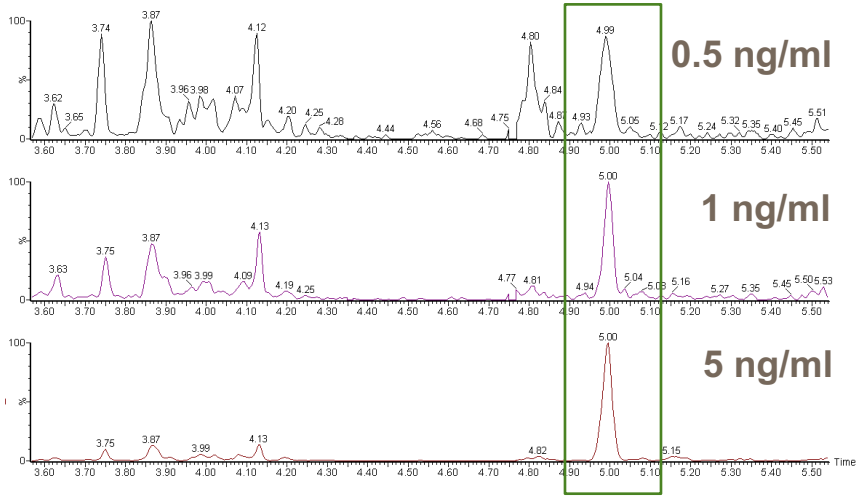
Workflow for Large Peptide/Protein Quantitation using LC-MS

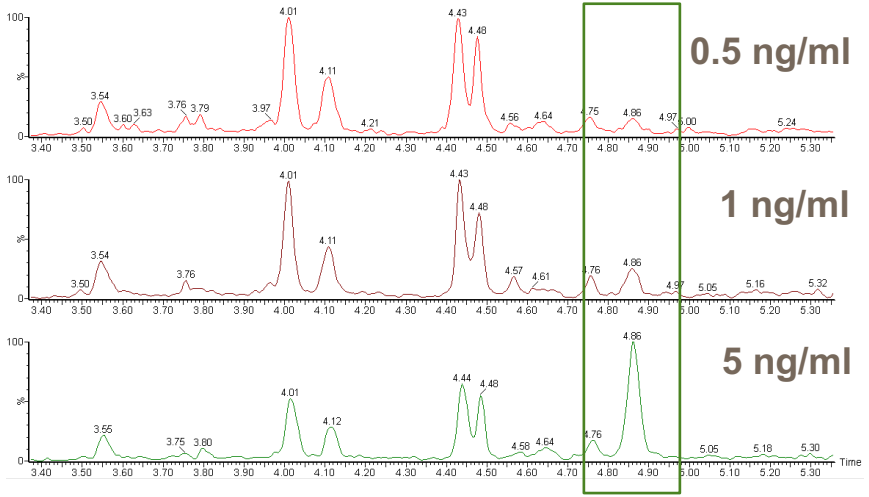


Higher Selectivity of Q-TOF Decreases Endogenous Interferences

TOF-MRM
(0.05 Da Mass Extraction Window)



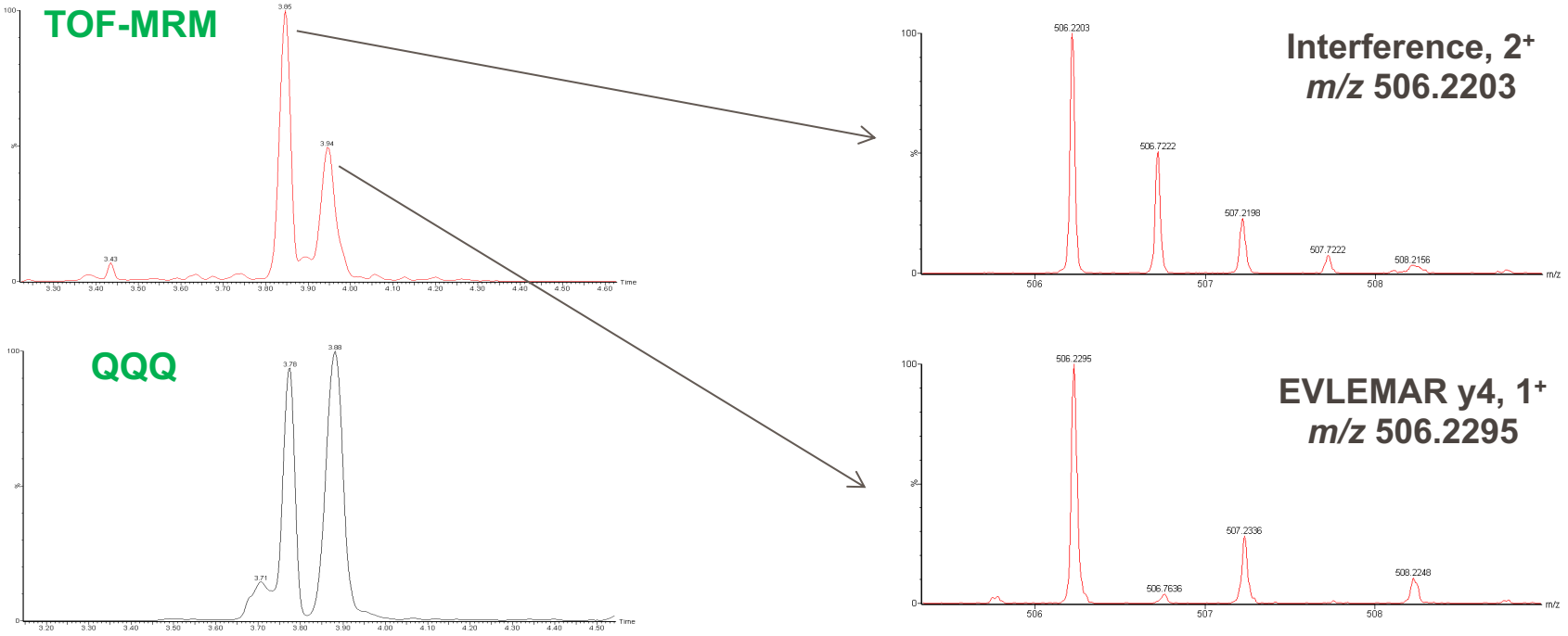
QQQ



Parathyroid hormone (1): SLGEADKADVNVLTK ($MH3^{+}$ → $y13^{++}$)

Some Interference Issues Cannot be Solved by Higher Selectivity of HRMS

Chromatogram Optimization is Still Necessary in Some Cases



Corticotropin-releasing hormone: EVLEMAR ($MH2^+$ → y4)

Conclusion

- Quantitation Performance of Q-TOF HRMS compared to QQQ
  - Both instruments can accurately quantitate peptides up to ~ 10 kDa in a multiplexed manner with ***at least 3-4 orders of magnitude*** of linear dynamic range
  - Compared to Q-TOF, QQQ provides ***slightly*** better sensitivity and wider dynamic range.
  - Higher selectivity of ***Q-TOF may decrease endogenous interferences*** leading to reduced chromatograph run time and higher confidence of assay development.

- Data Processing
  - TOF-MRM function reduces file size to the appropriate level for continuous bioanalytical use: ***~40 MB with TOF-MRM*** and ~0.8 MB with QQQ.
  - Processing time is ***NOT*** significantly increased with TOF-MRM data with TargetLynx.

Native HRMS Analysis of Noncovalent Protein Complexes up to 450 kDa

Z Chen *et al.* ***Bioanalysis*** (2020) 12(19), 1353–1362

Why Native MS and What Is the Limitation?

Benefits of Native MS

- Buffers for conventional intact protein analysis usually contain strong acids to denature proteins and destroy noncovalent protein–protein interactions.
- In native MS, proteins or noncovalent complexes are retained in their native state in the gas phase, and thus the integrity of proteins is not disrupted.
- Direct analysis of noncovalent protein interactions has great potential to elucidate the pharmacology/toxicology of mAbs and target engagement.

Technical Limitation

- Instrumentation modifications are usually required to transmit large ions in the native state with high *m/z*.

Native MS Analysis of mAb

- **Sample:** GSK mAb (145.9 kDa), buffer exchanged (3x with Vivaspin 30 kDa MWCO) into 50 mM ammonium acetate pH 6.8 (final concentration of 2 mg/mL)

- **Instrument and injection mode:** Waters Synapt G2-S in ESI+/Sensitivity/MS mode with Z-Spray ion source, direct infusion with Hamilton syringe pump at 10 µL/min

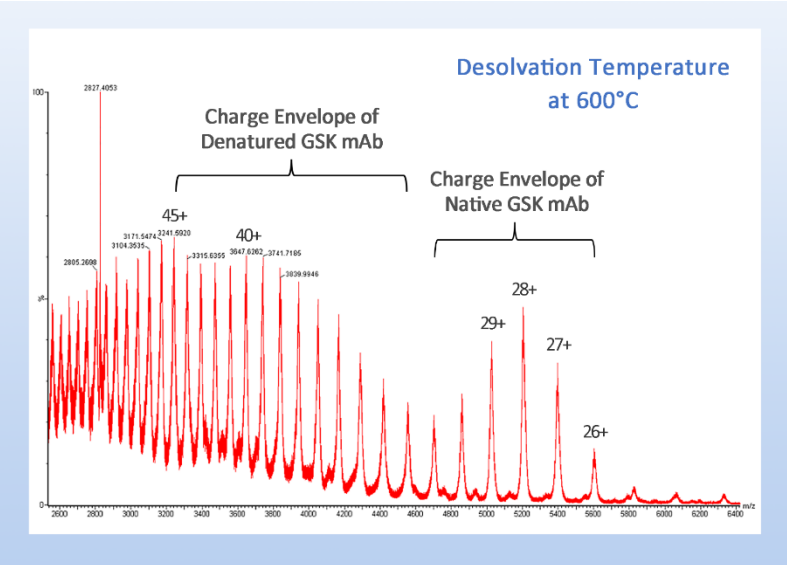
- Exploration of instrumental settings
  - Infusion flow rate: 5, 10, 15, 20 µL/min
  - Capillary Voltage: 2.5, 3.0, 3.5 kV
  - Sample Cone: 50, 100, 150 V
  - Source Offset: 50, 105
  - Source Temperature: 60,100,120 °C

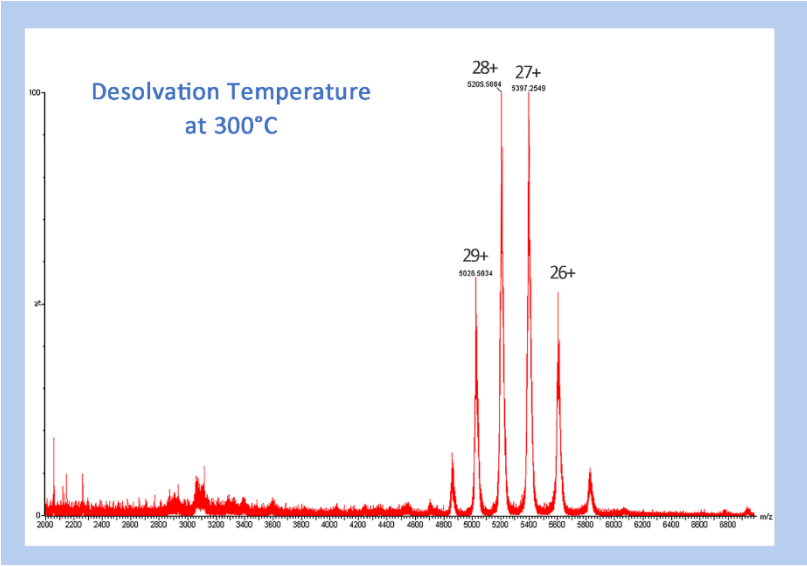
Showed limited impact on peak intensity or system robustness, but no observable influence on native MS

  - **Desolvation Temperature**: 200, 300, 600 °C

Mass Spectrum of GSK mAb at Different Desolvation Temperatures

– MS conditions: Infusion flow rate 10 µL/min, capillary voltage 2.6 kV, source temperature 80 °C, sample cone voltage 30 V, source offset 30, desolvation gas flow 200 L/hr.





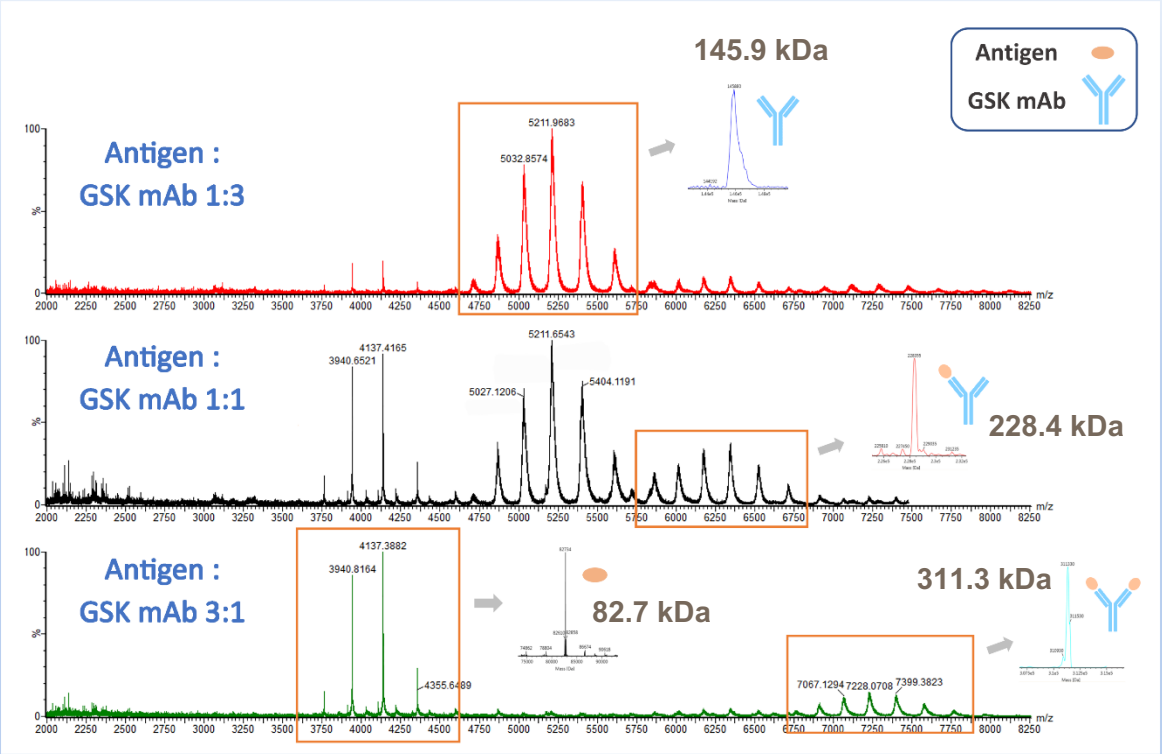
Native MS Analysis of Antigen-Antibody Complex

- Samples: GSK mAb (145.9 kDa) and its target protein antigen (82.7 kDa)

- Prepare 10 μM GSK mAb solution and 10 μM antigen solution in PBS; incubate two solutions with three different mole ratios at 3:1, 1:1, 1:3 at 37 °C for 2 hr.

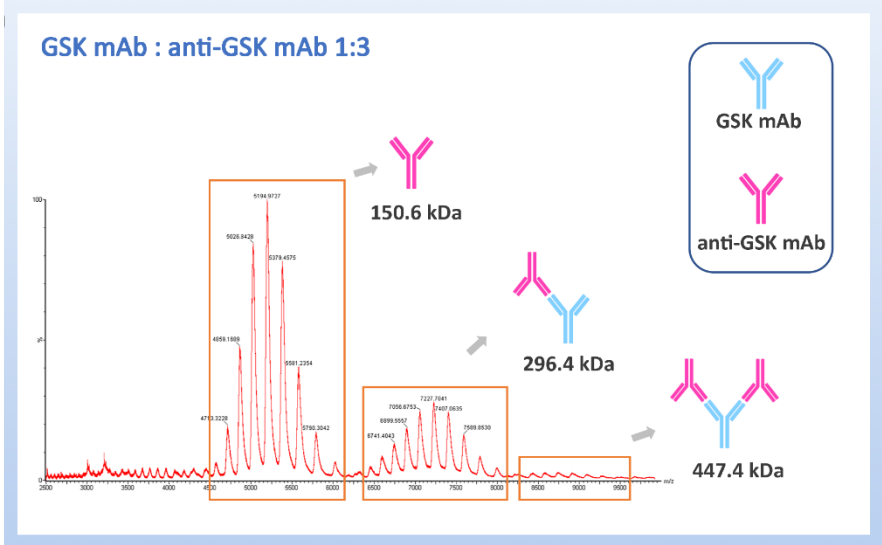
- After incubation, the solution was buffer exchanged (3x with Vivaspin 30 kDa MWCO) into 50 mM ammonium acetate pH 6.8. Final protein concentration is ~ 0.5 mg/ml.

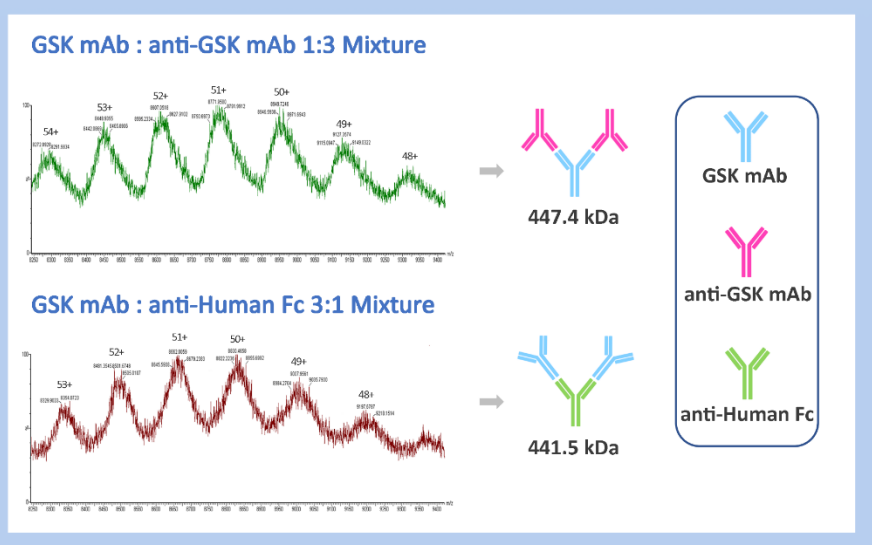
Native MS Analysis of Antigen-Antibody Complex



- Native MS can provide an accurate qualitative analysis of protein complex consistent with subunit stoichiometry.
- Non-covalent complexes could not be observed under denaturing conditions.
- Native MS parameters optimized by non-covalent complexes or single protein were consistent.

Native MS Analysis of Antibody-ADA Complex





- Protein complexes (up to ~450 kDa) with similar components but slightly different masses can be distinguished.
- Detection of CsI signals proved that *m/z* 9000 is the practical upper limit of this commercial Q-TOF without modification.

Conclusion

- It is feasible to use a commercially available Q-TOF HRMS and ESI ion source without any modification to perform native MS analysis.
- Desolvation temperature is a very important parameter for native MS.
- *In vitro* noncovalent binding of GSK mAb with its target and ADAs was characterized, and the results were consistent with the subunit stoichiometry of these protein assemblies.
- A molecular weight around 450 kDa for native MS analysis, is the upper detection limit of this commercial instrument under the default configuration.
- Future direction may focus on *in vivo* studies that process samples in biological matrices without compromising the compatibility with native MS.

Acknowledgements

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