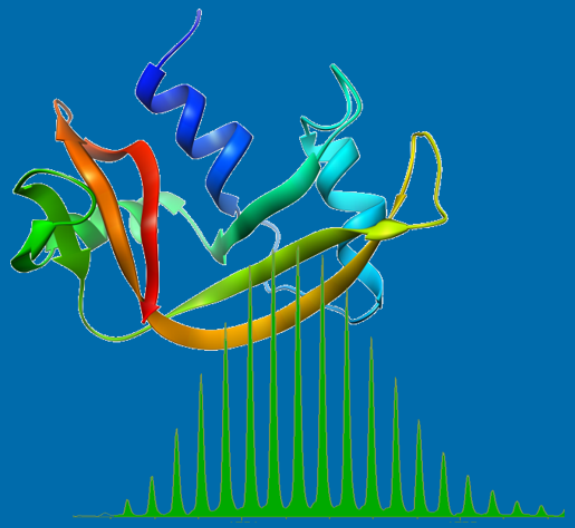
Intact mass analysis



MFPL & IMP Mass Spectrometry facilities

Markus Hartl & Karl Mechtler, June 14 2016

Intact protein mass analysis – why bother?

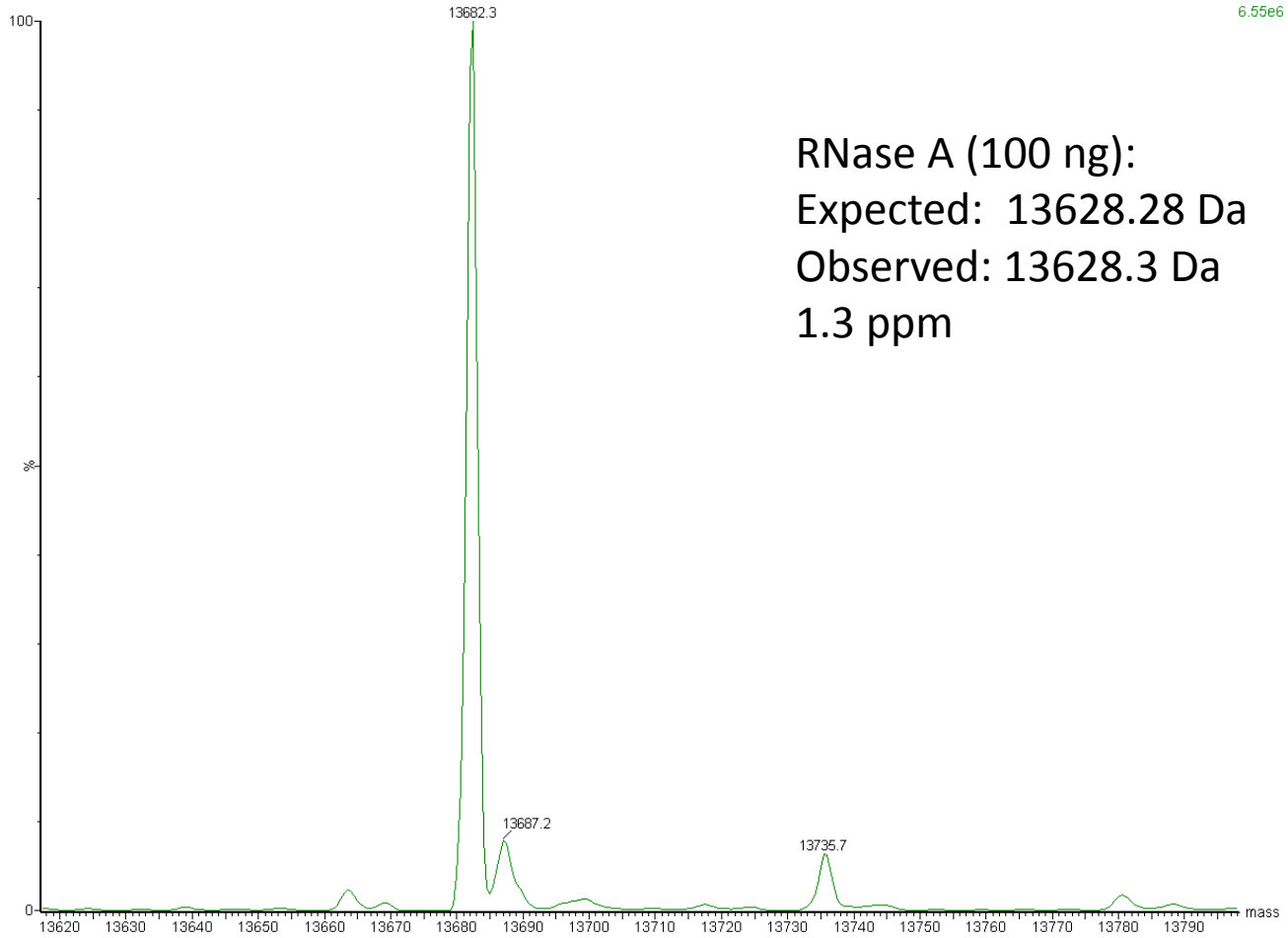
Goal: accurate **mass determination of purified intact proteins**

Main applications under **denaturing** conditions:

- Quality control of expressed proteins
- Determination of modifications and quantitative readout on their stoichiometry (no site-localisation though)

Examples (deliberately not perfect!)

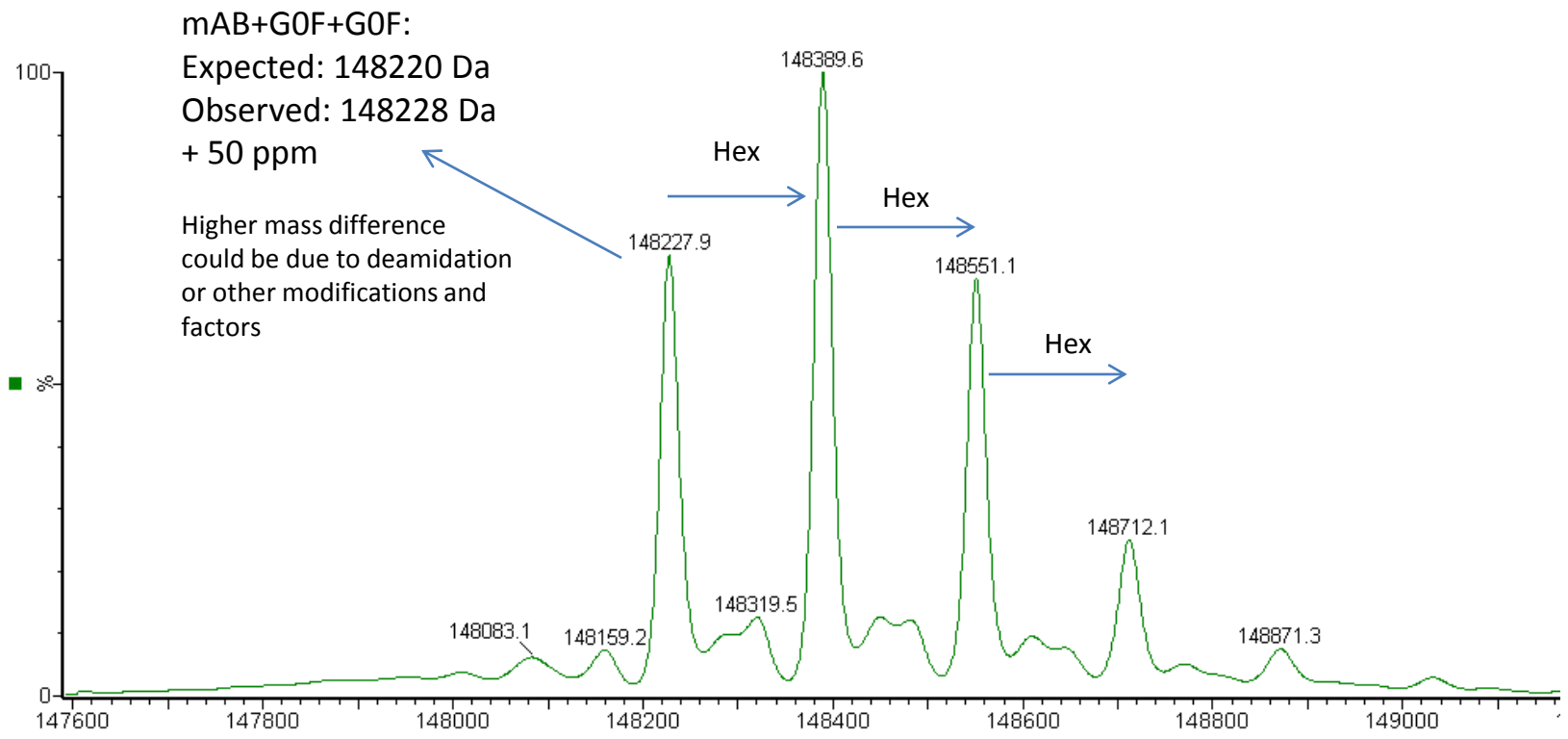
Denaturing intact protein MS: Quality control



RNase A (100 ng):
Expected: 13628.28 Da
Observed: 13628.3 Da
1.3 ppm

Denaturing intact protein MS: PTMs

250 ng intact monoclonal antibody (Waters standard), with four main glycoforms



Instrumentation: Waters Synapt G2Si HDMS

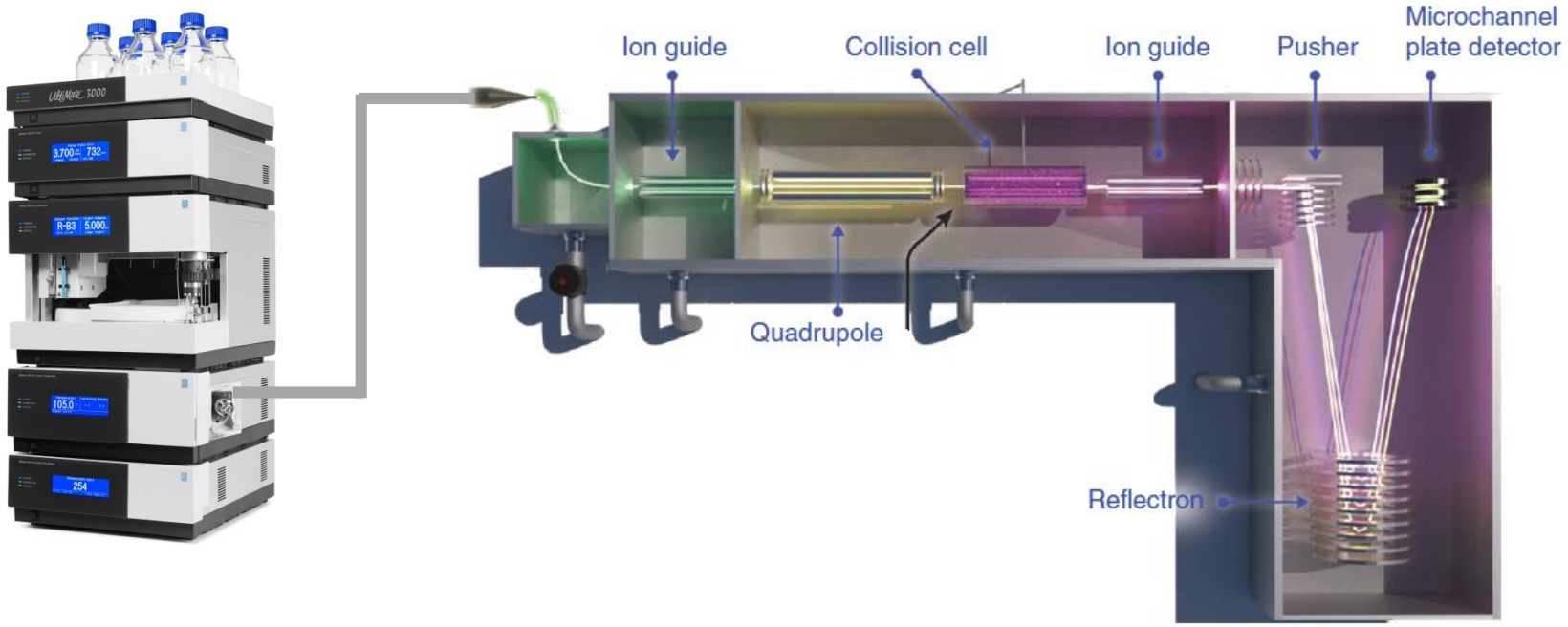
Time-of-Flight (TOF) mass spectrometer

Resolution: 10.000 - 60.000
Accuracy: <1 ppm (100-900 m/z),
In our hands: ca. 1-50 ppm for intact proteins, depending on size, modifications, etc.

Additional Ion-mobility drift cell:
separates proteins (or complexes) based on their diameter (under evaluation, not offered as service yet)

Routine setup (denaturing conditions)

- Micro-flow LC with C4-column, gradient of 0.1 % formic acid and acetonitrile, used for online desalting and protein separation
- Coupled via electrospray ionisation to MS



Picture (modified): Laganowksy et al., Nature Protocols, 2013

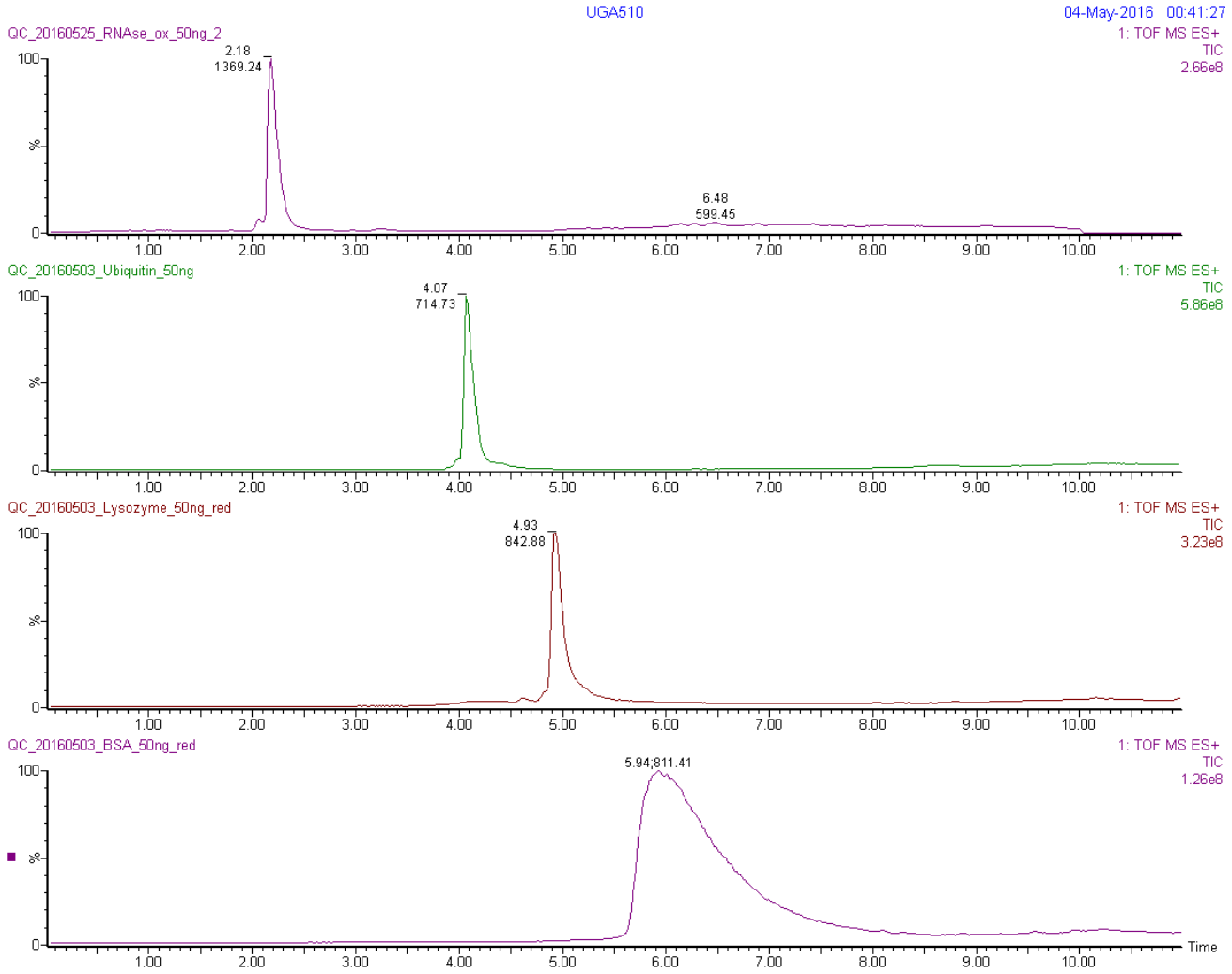
Results: protein separation on C4 column

RNase A
13.7 kDa

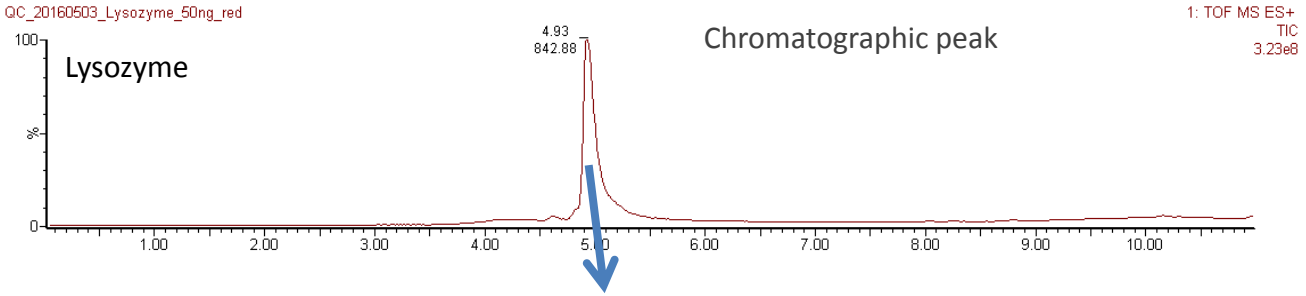
Ubiquitin
8.6 kDa

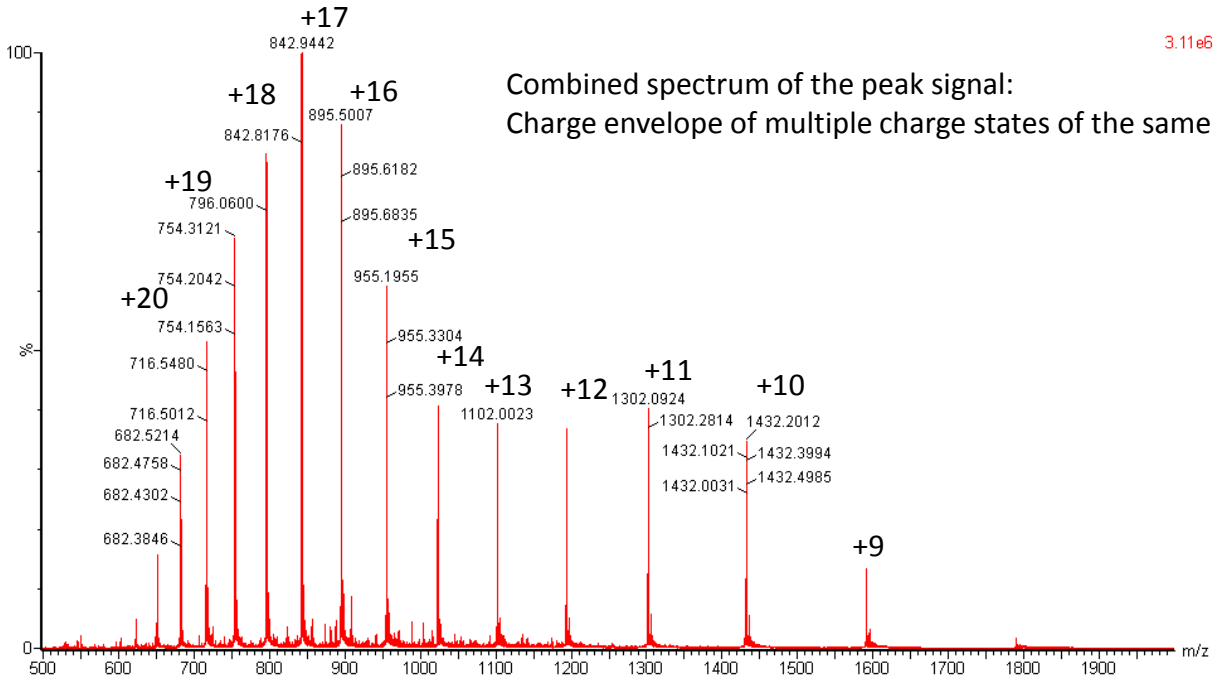
Lysozyme
14.3 kDa

BSA
66.4 kDa



Results: data processing



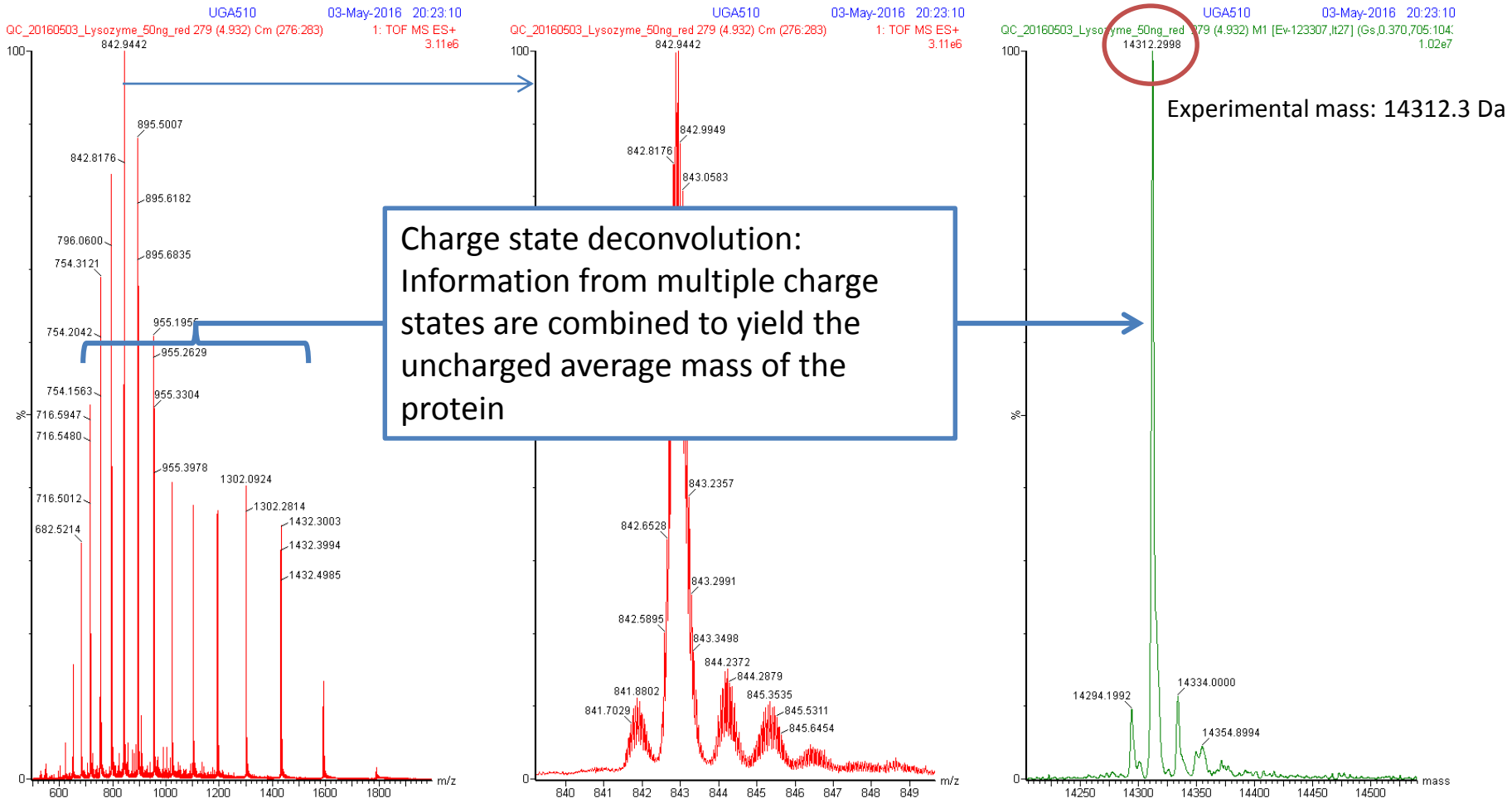


Combined spectrum of the peak signal:
Charge envelope of multiple charge states of the same protein

Results: data processing

Charge envelope

Charge state: z = 17



Charge state deconvolution: Information from multiple charge states are combined to yield the uncharged average mass of the protein

Experimental mass: 14312.3 Da

Consequences for results

Accuracy of the measurement is influenced by:

- Protein size & number of positive residues (-> charge states) and accuracy & resolution of the instrument
- Small modifications, as for example deamidation or disulfide brigdes, can shift the mass several Daltons or produce overlapping signals which lead to seemingly inaccurate masses.
- Adduct formation (salts!) and background from other proteins or chemicals can produce overlapping signals which hamper mass determination.

Sample requirements for denatured proteins

- Min. 10 µL of 0.5-1 mg/mL purified protein(s)
  (or 0.1-1 pmol as a rough guideline; we do inject less eventually but prefer to have some buffer)
- Standard biological buffers such as TRIS, HEPES or PBS are compatible.
- Glycerol < 5%, most salts, and reducing agents are acceptable. Nevertheless, try to keep the concentration of all these agents as low as possible (especially salts).
- NO detergents (SDS, Triton, etc.) and stabilizers such as PEG!
- When the protein is lyophilized, submit dry, or reconstitute in 5% organic solution (acetonitrile or methanol) with 0.1% formic or acetic acid.
- Samples will be reduced by default, unless you specify a reason against it.

Service and submission

Service offered:

- intact mass determination under denaturing conditions

Submission:

- Please fill out the **submission sheet for intact mass** and send it via email.

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