

The 69th ASMS Conference, Philadelphia, PA

Proteoform Peak Integration Facilitates DAR Estimation from Orbitrap Mass Spectra of Complex ADCs

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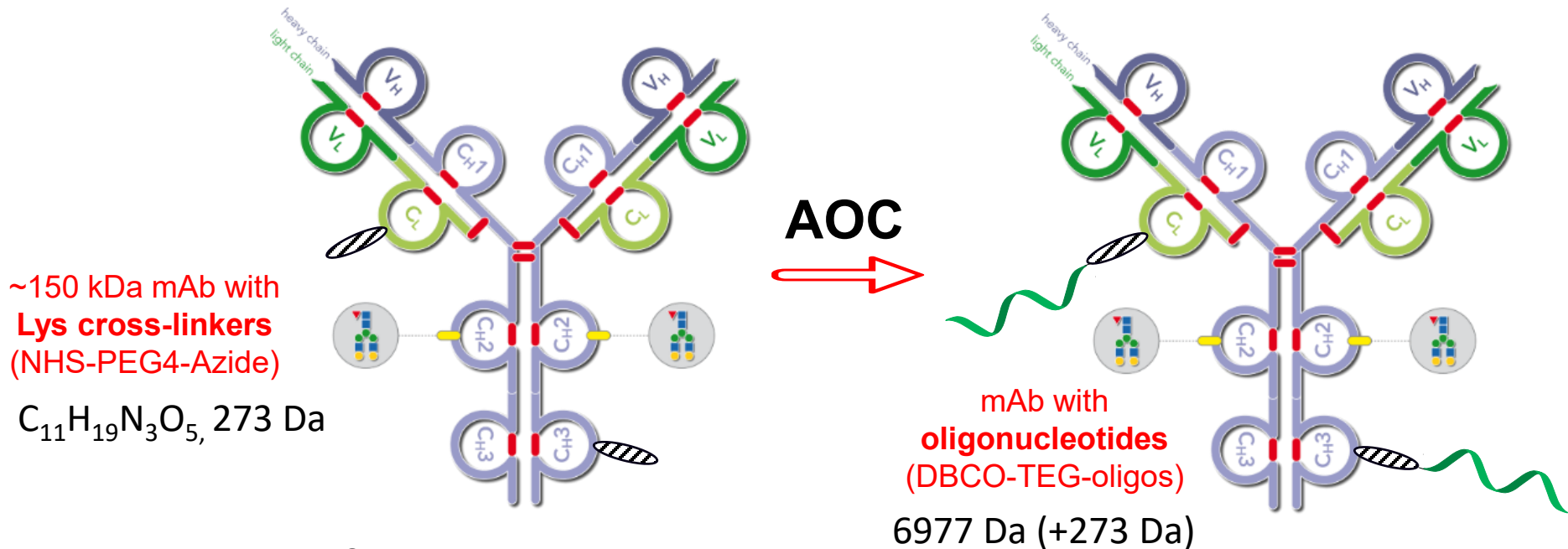
November 2, 2021; TOH pm – 03:50

Antibody-Oligonucleotide Conjugate (AOC)

Oligonucleotides – **selectively** arrest protein production by targeting specific genes

AOCs: Jain et al., *J. Clin. Med.* 2021 Wagner et al., *Bioconj. Chem.* 2019

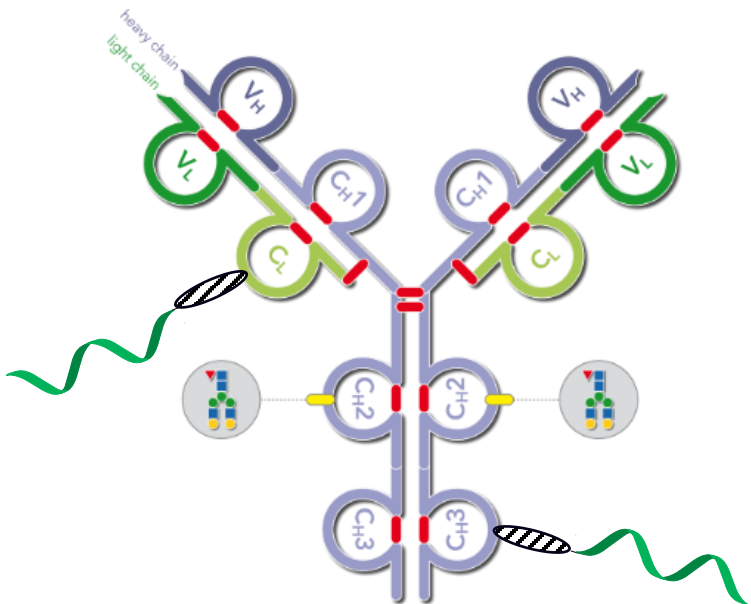
Sample: a trastuzumab-oligonucleotide conjugate with ~7 kDa per payload (size & ionization)



Nagornov et al., *Anal Chem*, 2021, 12930

Objective: To Estimate the Drug-to-Antibody Ratio

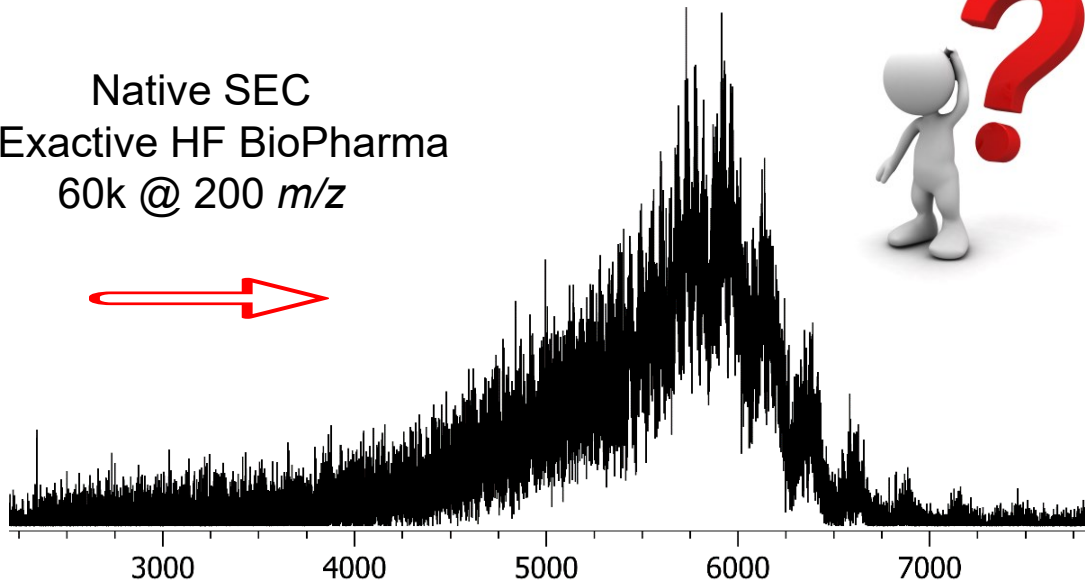
Drug-to-antibody ratio (**DAR**) is the average number of drugs conjugated to an antibody



trastuzumab conjugated
with oligonucleotides

SEC: size-exclusion chromatography

Native SEC
Q Exactive HF BioPharma
60k @ 200 m/z



unresolved proteoforms

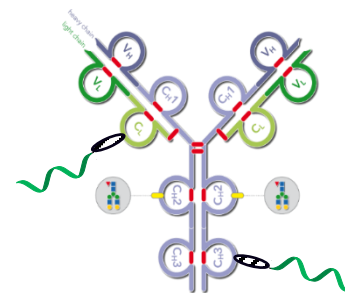
- Sample preparation (deglycosylation)
- Data analysis algorithms

Approaches to Heterogeneity in Native MS
Prell et al., Chem. Rev., 2021

Addressing Mass Spectrum Complexity

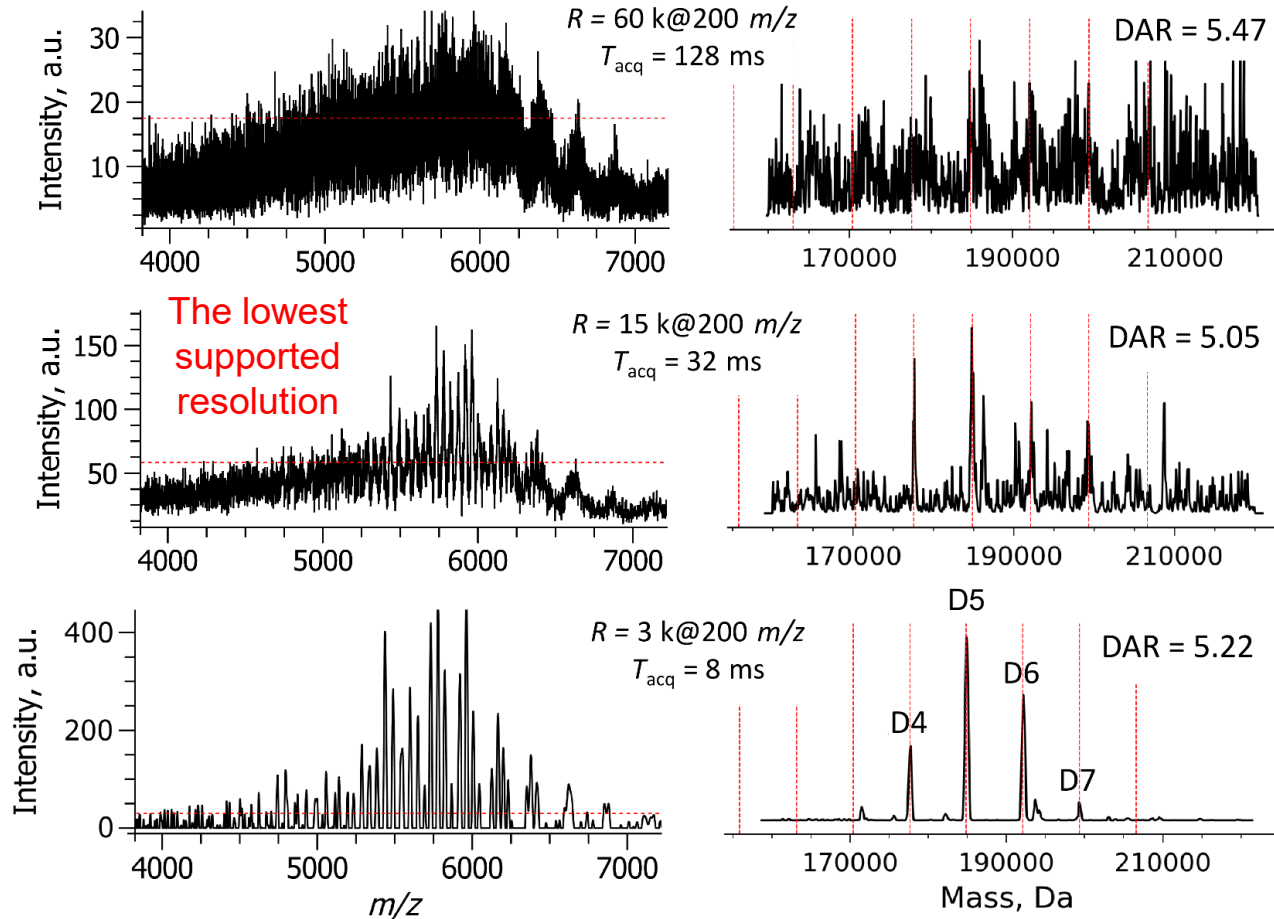
resolution

Sample:
Intact AOC



Experiment:
Native conditions
SEC-Orbitrap
(Q Exactive HF)

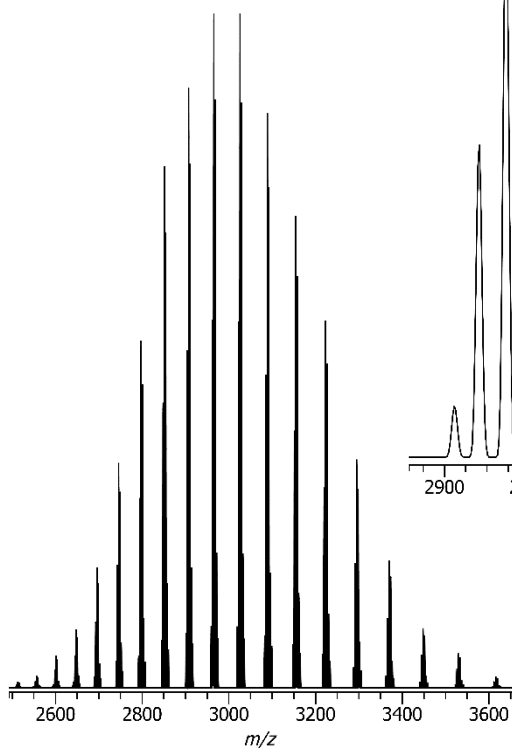
Anal Chem, 2021, 12930



The AOC Sample Complexity

mAb glycoforms
(charge states)

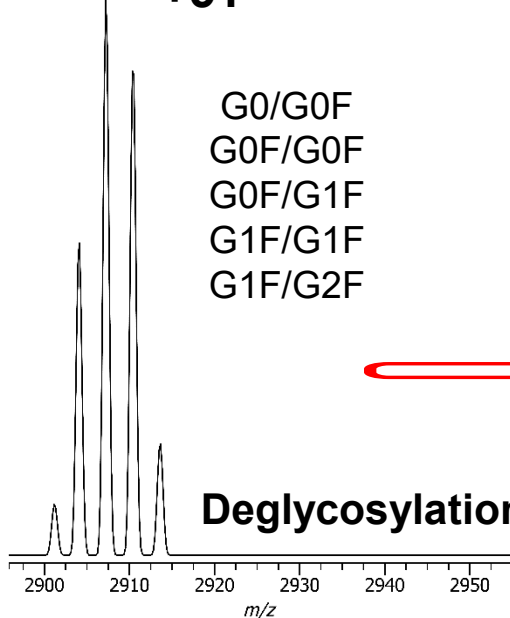
+51



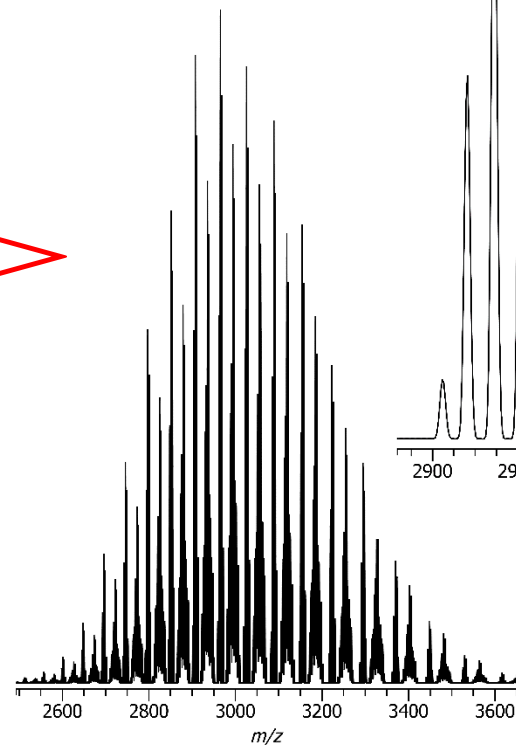
+51

G0/G0F
G0F/G0F
G0F/G1F
G1F/G1F
G1F/G2F

Deglycosylation?

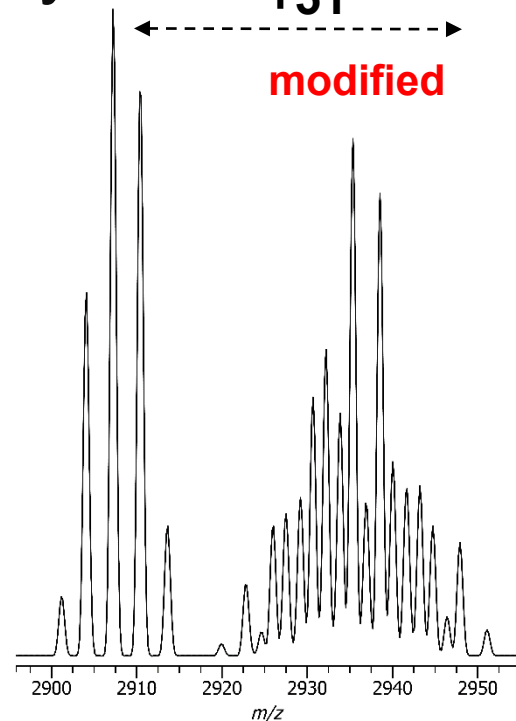


+ cross-linkers
(4-8 cross linkers)



+51

modified



FTMS**SIMULATOR**

Q Exactive HF
15k @ 200 m/z

WP051

JASMS 2020, 1927

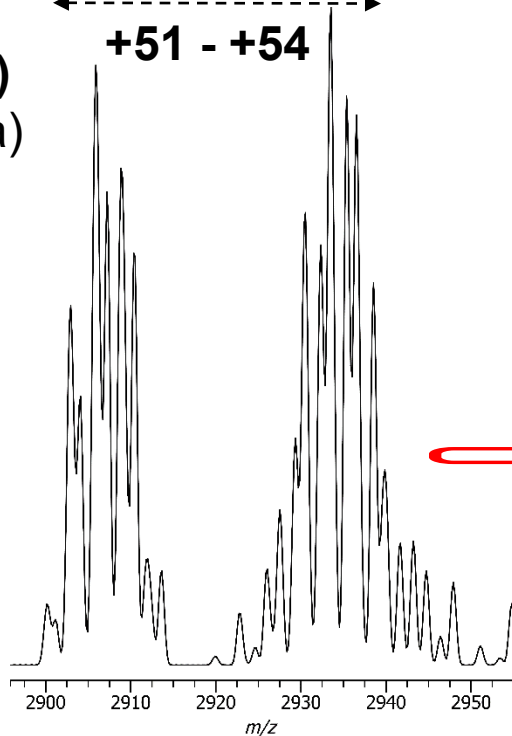
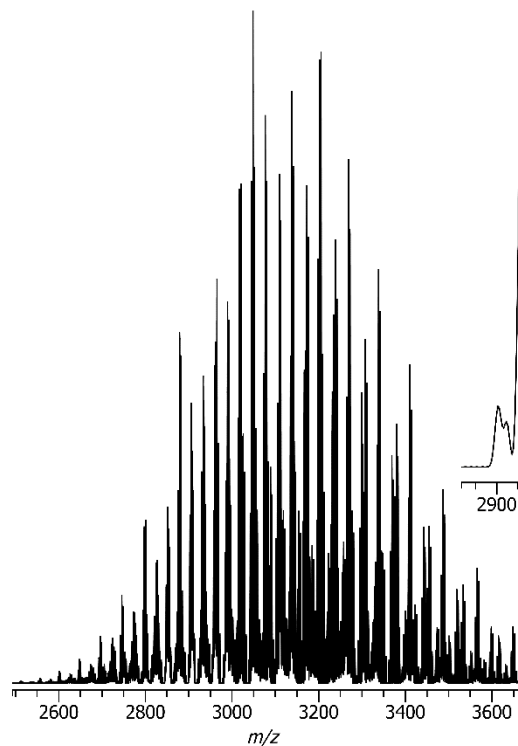
The AOC Sample Complexity

+ payload (drugs)
(4-8 drugs, 1.2 kDa)

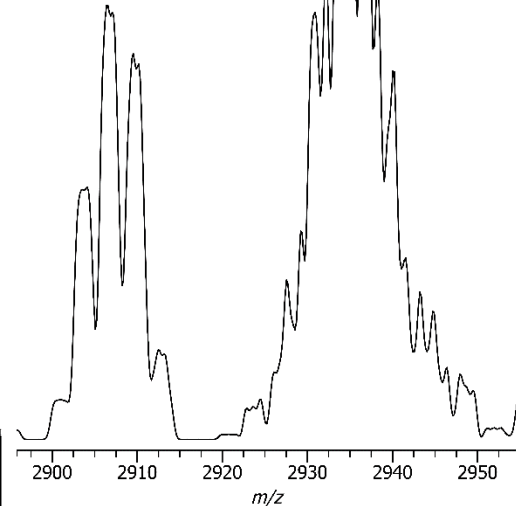
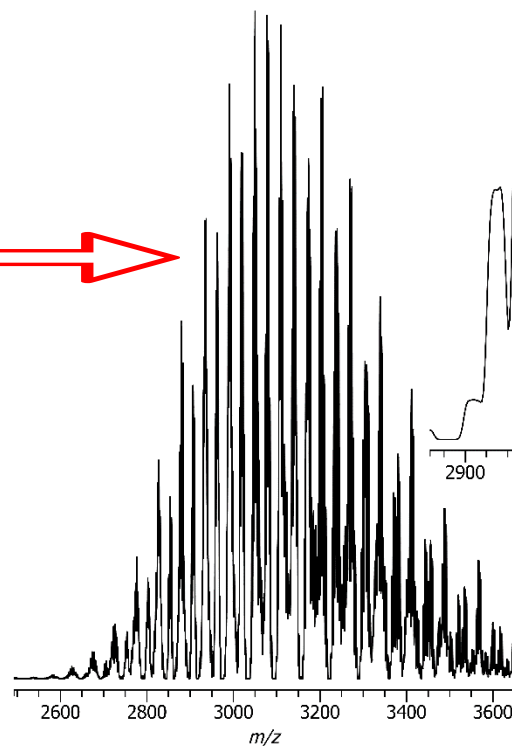
+51 - +54

+ modifications
(16, 32, 44, ... Da)

+51 - +54



FTMSIMULATOR
Q Exactive HF
15k @ 200 m/z



WP051

JASMS 2020, 1927

Solution: Proteoform Peak Integration

R
resolution

Isotopes
(fine structure)



Isotopologues
(monoisotopic mass)

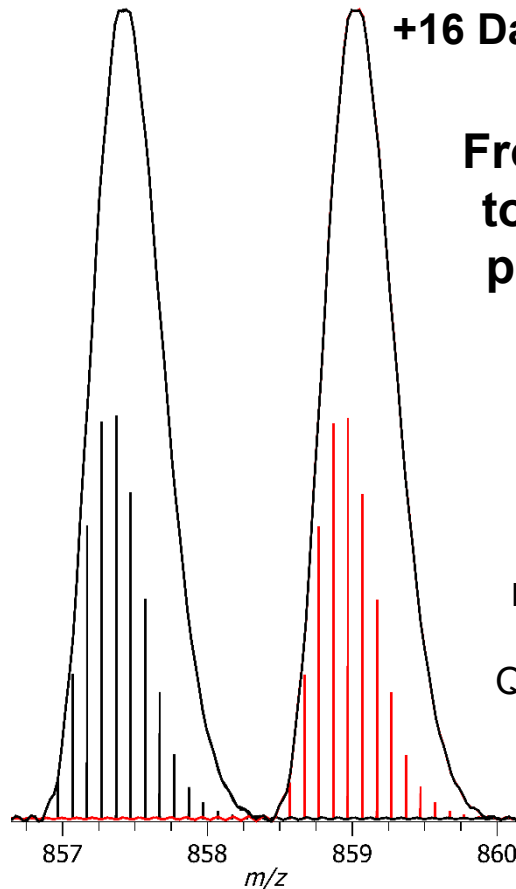


Proteoforms
(average mass)



**Integrated
Proteoforms**

$R = 1.5k$



oxidized ubiquitin,
+16 Da, $[M+10H]^{10+}$

From resolved
to **integrated**
proteoforms

$R \downarrow$

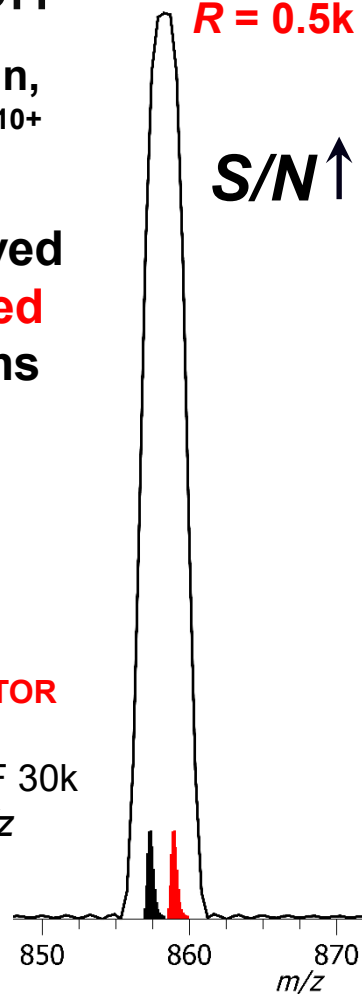


FTMS**SIMULATOR**

Q Exactive HF 30k
@ 200 m/z

$R = 0.5k$

$S/N \uparrow$

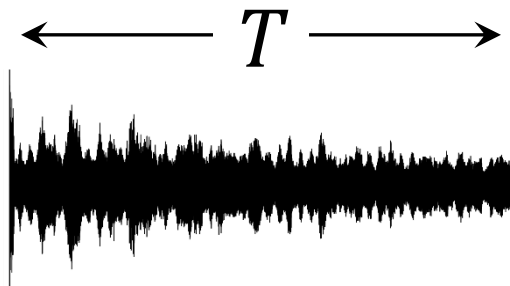


How to Reduce Resolution to Integrate Proteoforms?

- directly via lower resolution mass spectra – limit of 15k @ m/z 200 (Q Exactive HF)
- via truncation of the **time-domain transients**, e.g., using an add-on DAQ system

time-domain
transient

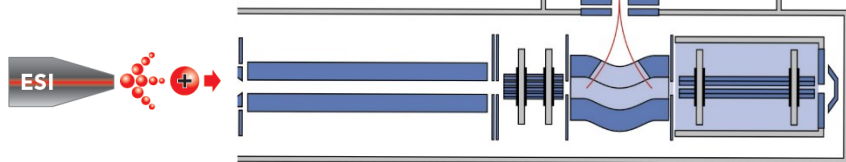
$$R \sim T$$



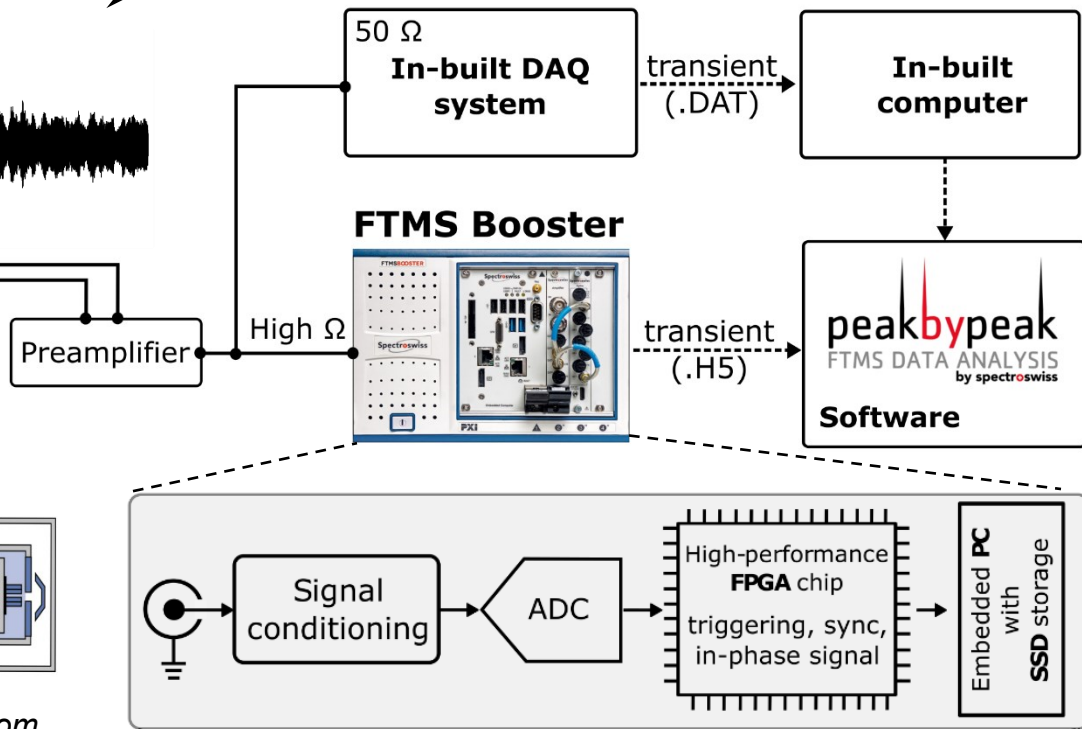
Anal Chem, 2021, 12930

JASMS 2020, 257

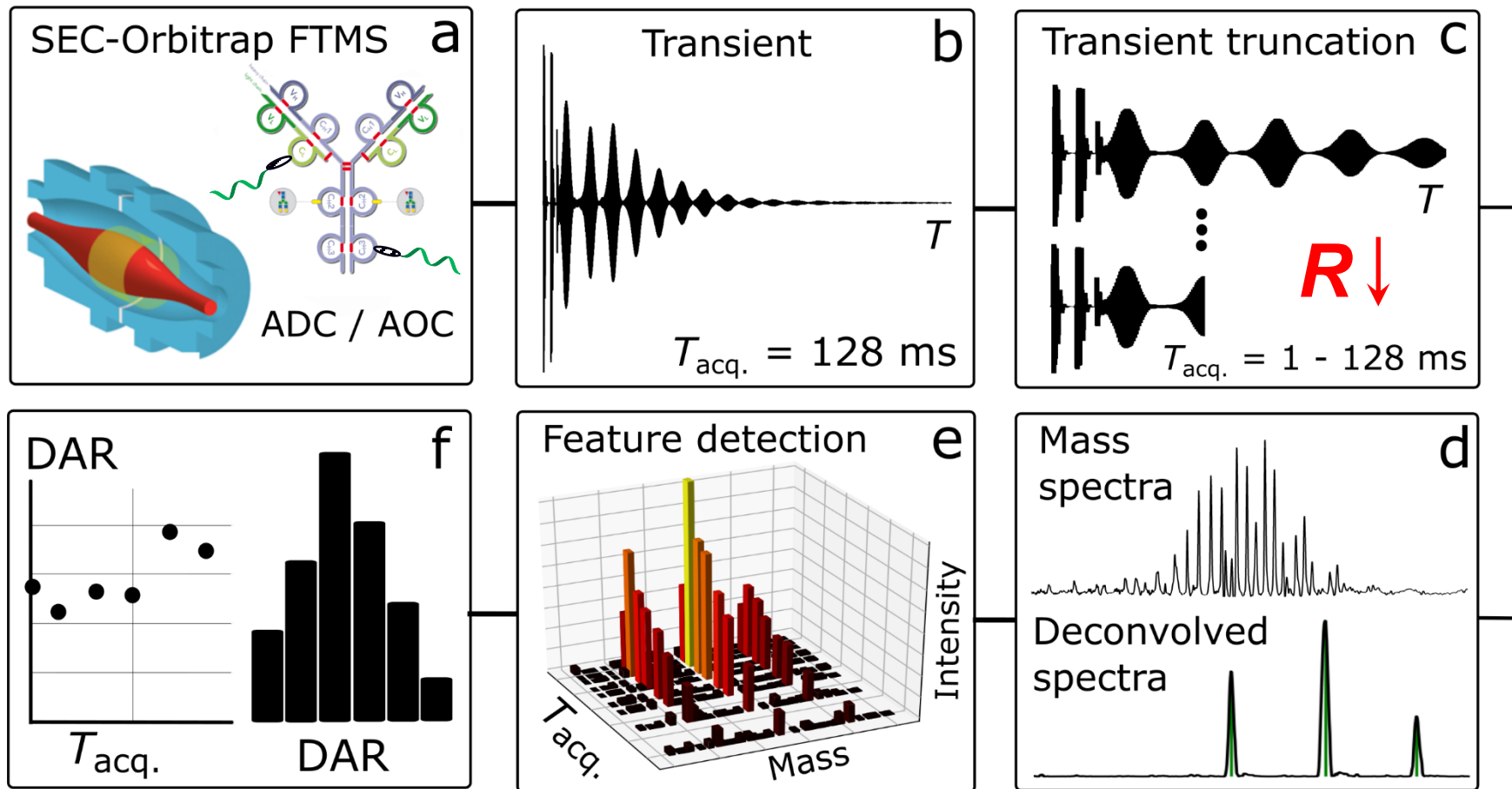
JASMS 2021, 1224



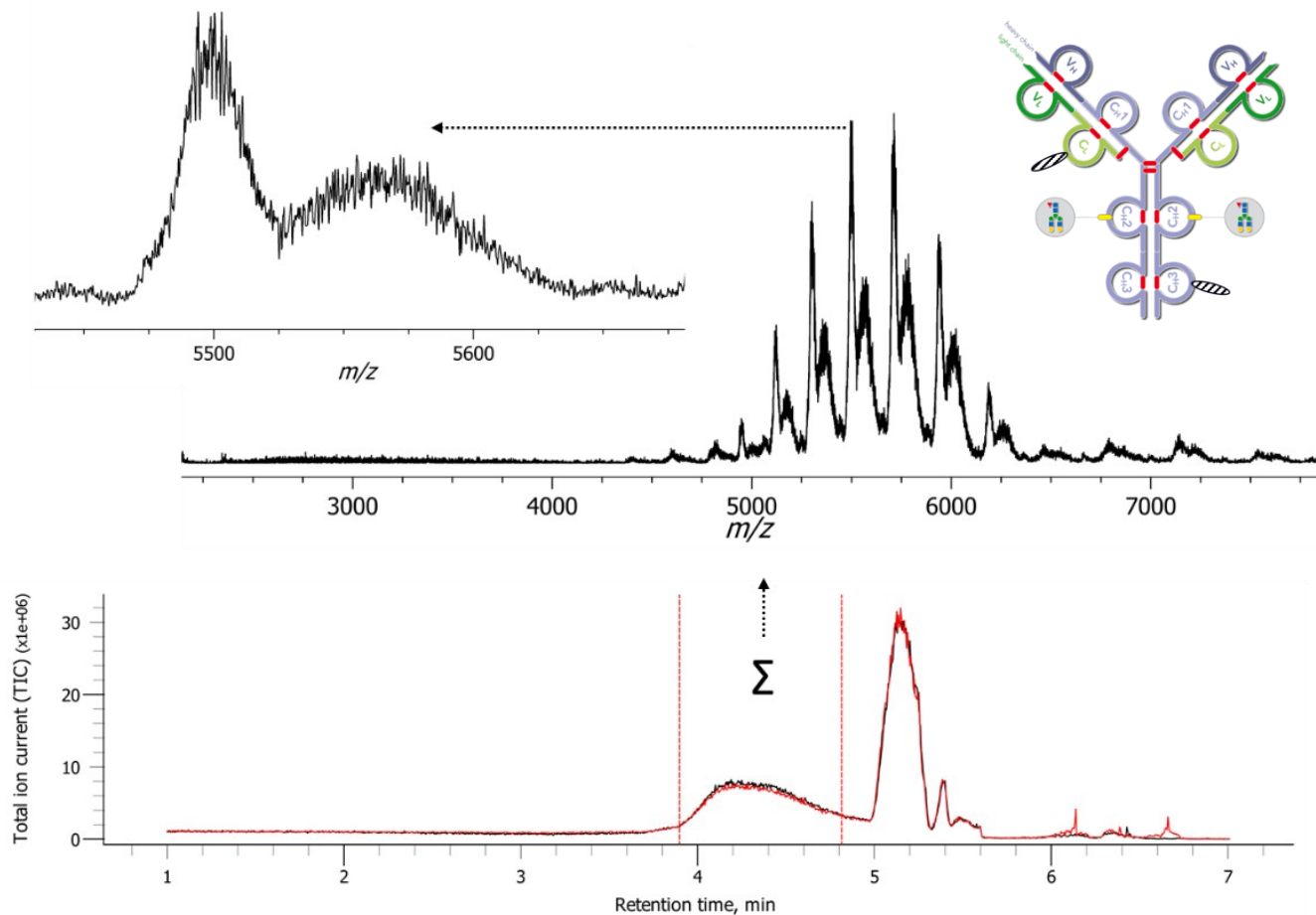
QE schematics: www.planetorbitrap.com



Proteoform Peak Integration Workflow



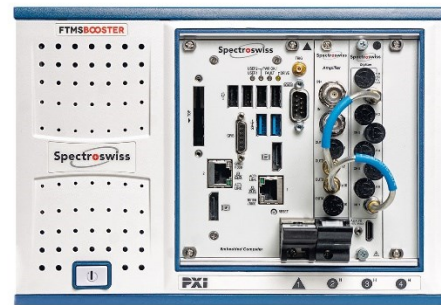
Analysis of an Antibody-Crosslinker Conjugate



Native mode analysis of trastuzumab modified with cross-linkers

Sample separation with SEC (20 ug per infusion)

FTMS: a Q Exactive HF BioPharma. Settings: AGC 5e6, resolution 60k at m/z 200 (128 ms)

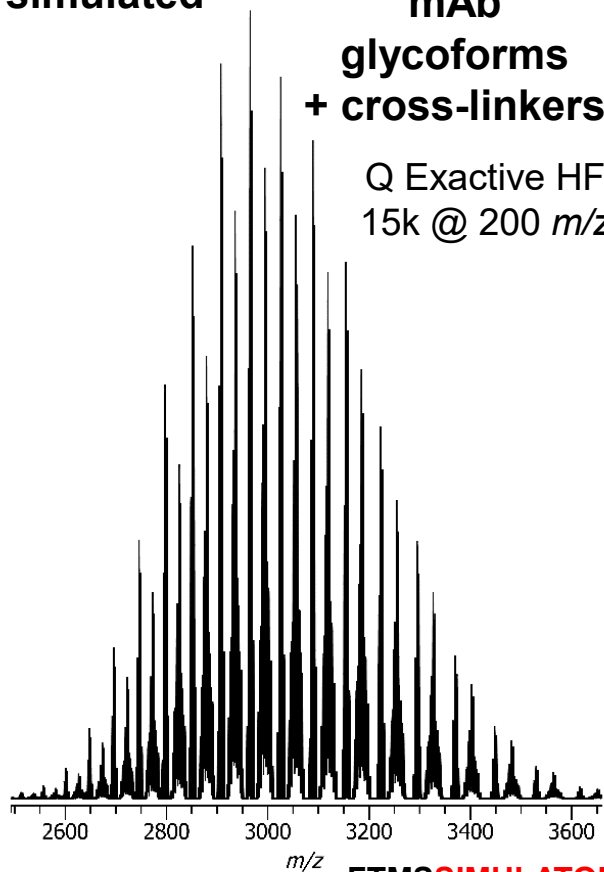


Analysis of an Antibody-Crosslinker Conjugate

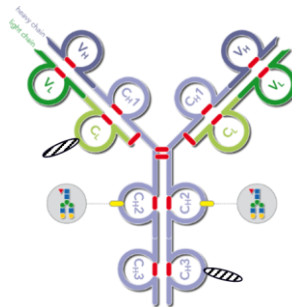
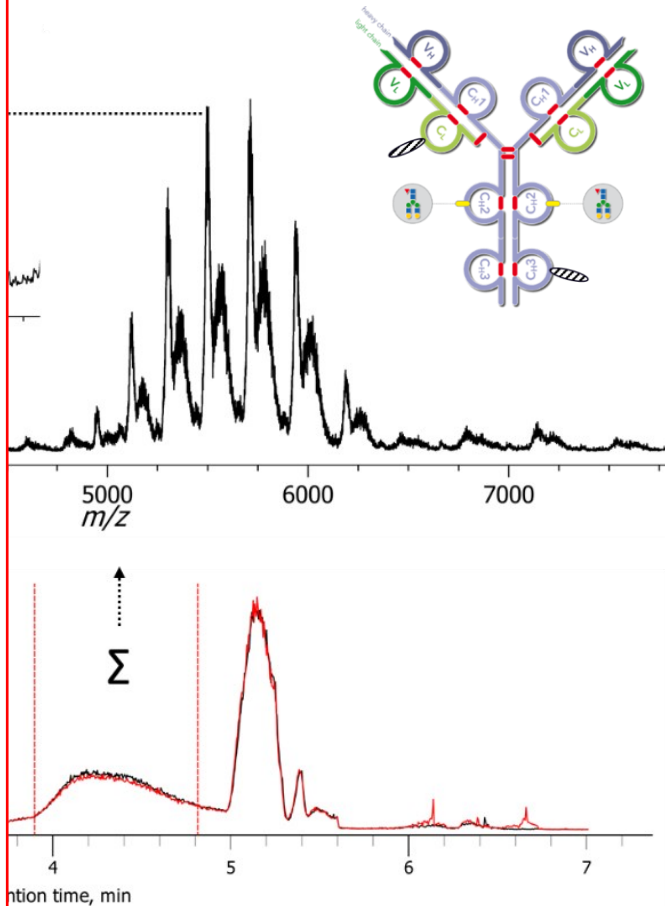
simulated

mAb
glycoforms
+ cross-linkers

Q Exactive HF
15k @ 200 m/z



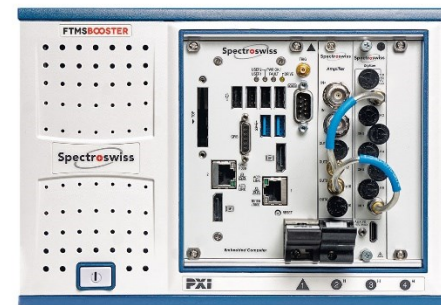
FTMSIMULATOR



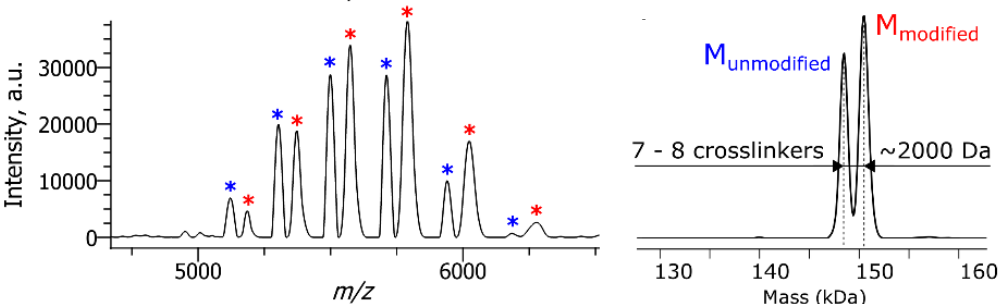
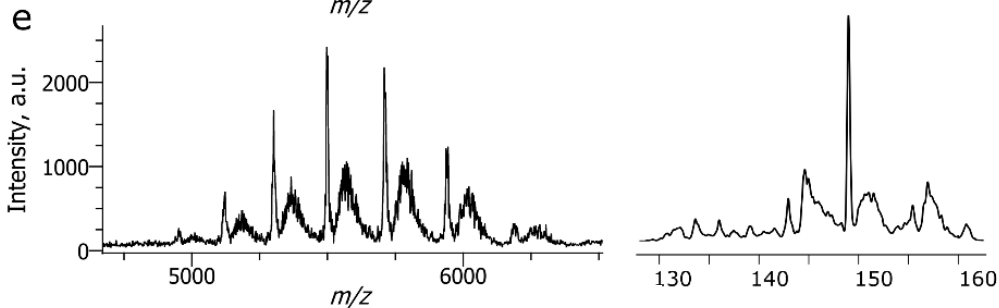
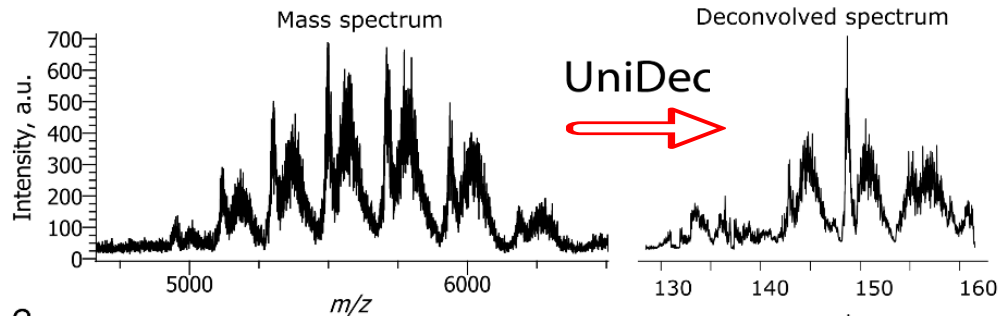
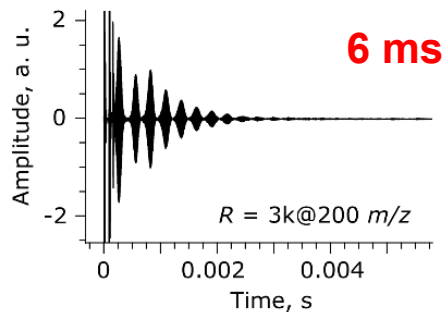
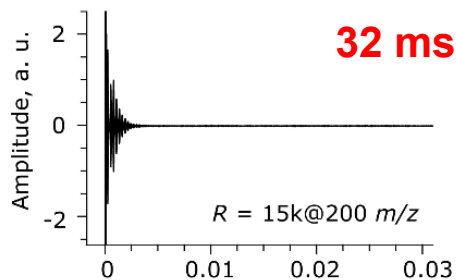
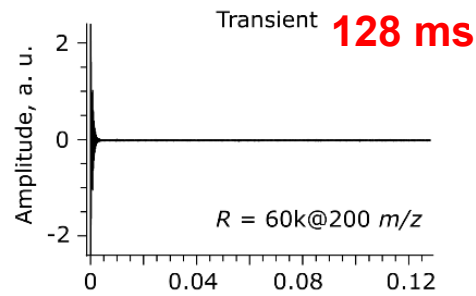
Native mode analysis of
trastuzumab modified with
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BioPharma. Settings:
AGC 5e6, resolution
60k at m/z 200 (128 ms)



Analysis of an Antibody-Crosslinker Conjugate



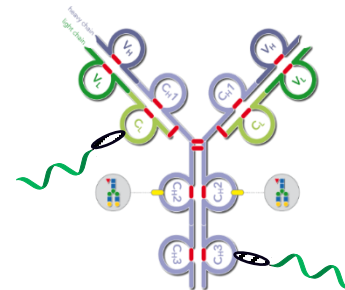
mAb sample:
trastuzumab
(Herceptin)

cross-linkers:
NHS-PEG4-Azide
 $C_{11}H_{19}N_3O_5$
MW = 273.29 Da

Experiment:
Native conditions
SEC-Orbitrap
(Q Exactive HF)

Transients:
FTMS Booster
Peak-by-Peak

resolution

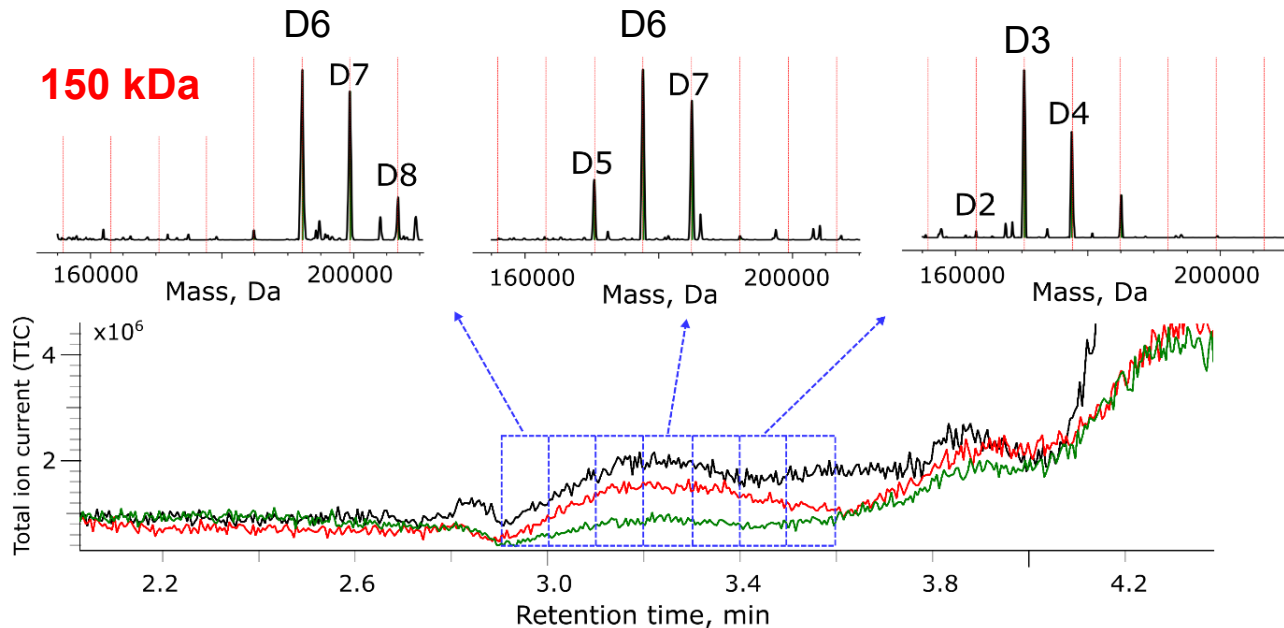


Experiment:
Native conditions
SEC-Orbitrap
(Q Exactive HF)

Transients:
FTMS Booster
Peak-by-Peak

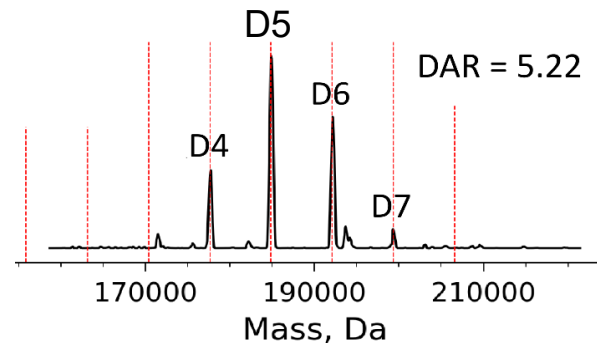
Analysis of an Intact AOC Sample: Sliding Window

Application of a sliding window approach to LC-MS analysis of AOCs



$$\text{DAR} = \frac{\sum_{n=0}^m (I_n \times n)}{\sum_{n=0}^m I_n}$$

Integrated approach



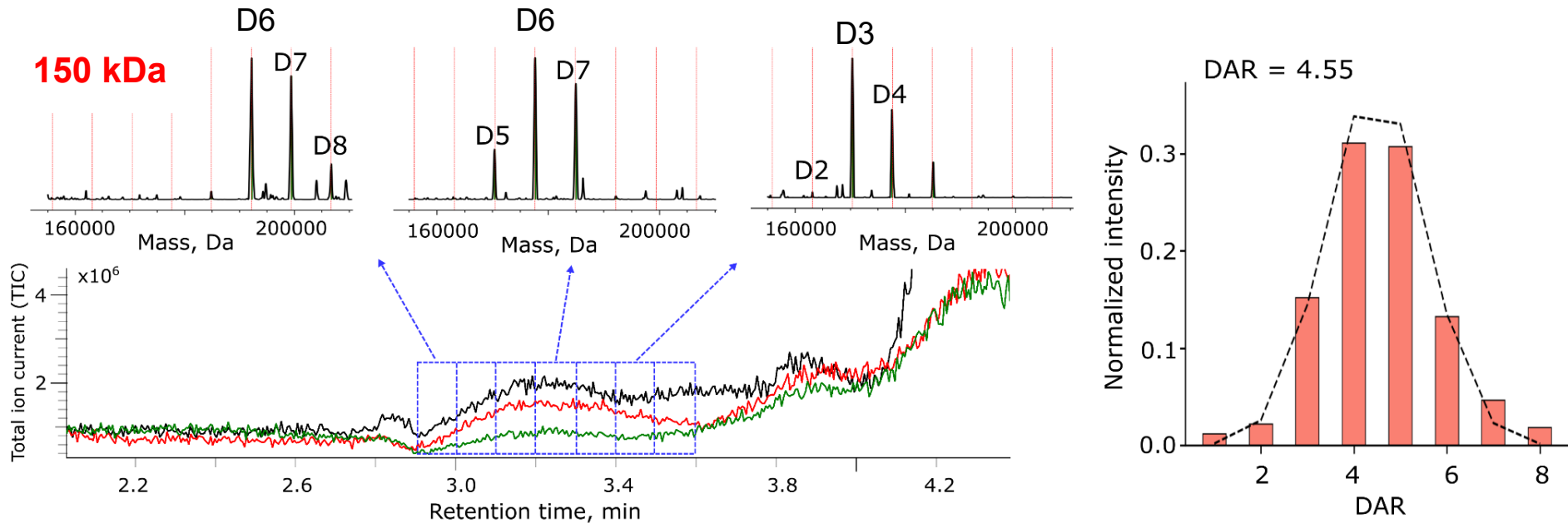
DARs: Integrated approach (**5.22**) vs. Sliding window approach (**4.55**)

Sliding window approach: ADC – Thermo PO72283; mAbs – *Eur J Pharm Biopharm* 2021 158: 83

What if the AOC sample complexity is reduced?

Analysis of an Intact AOC Sample: **Sliding Window**

Application of a sliding window approach to LC-MS analysis of AOCs



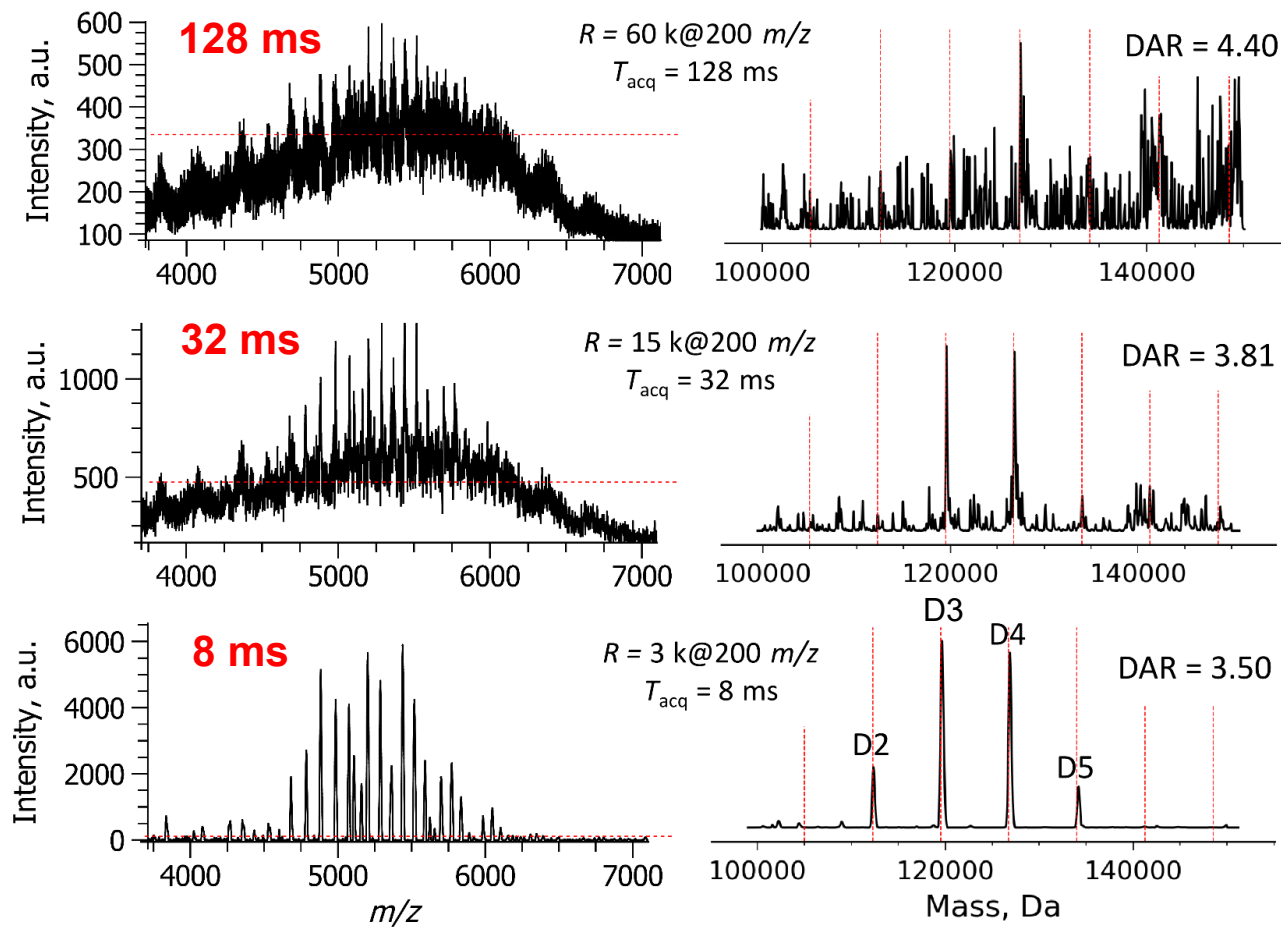
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Sliding window approach: ADC – Thermo PO72283; mAbs – *Eur J Pharm Biopharm* 2021 158: 83

What if the AOC sample complexity is reduced?

Analysis of an Antibody Subunit-Drug Conjugate

resolution



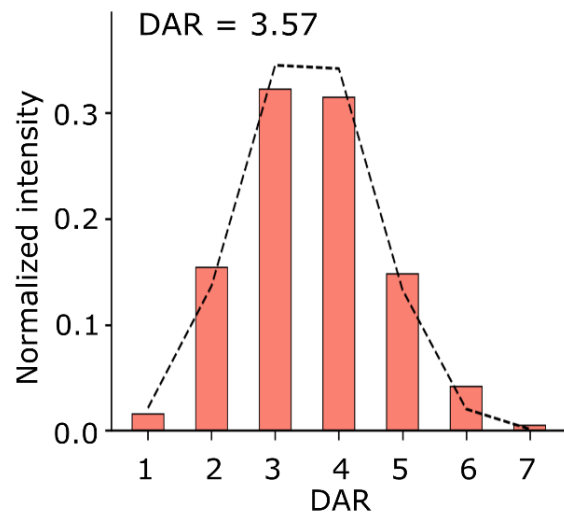
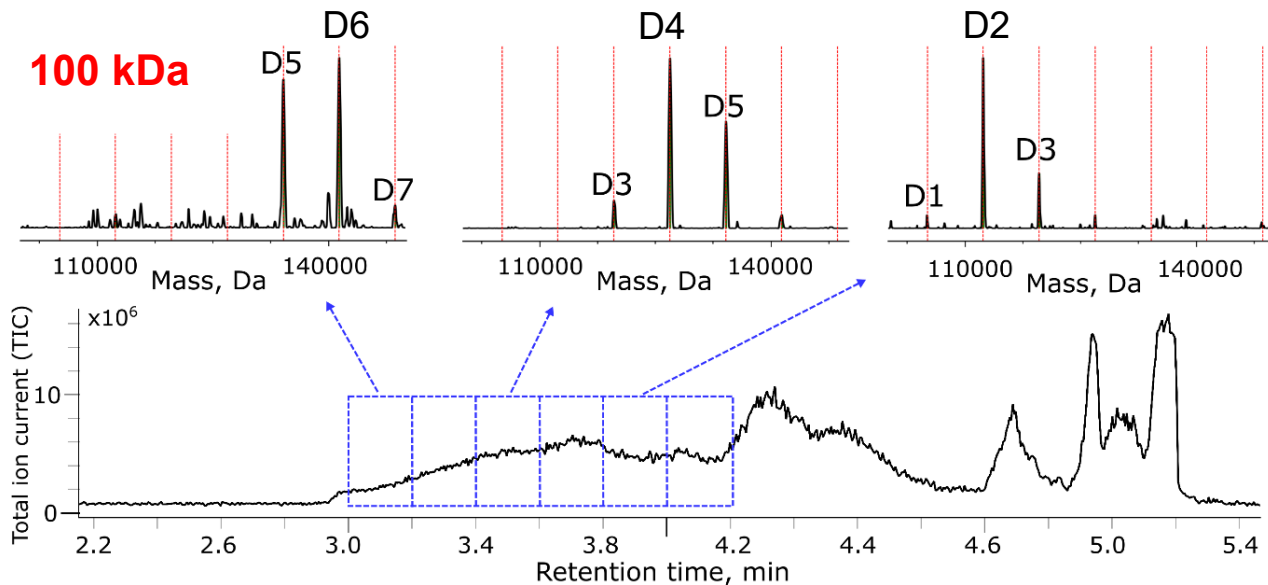
AOC sample:
IdeS-derived
F(ab)₂ subunit of
trastuzumab
(~100 kDa) with
oligonucleotides
of ~7 kDa each

Experiment:
Native conditions
SEC-Orbitrap
(Q Exactive HF)

Transients:
FTMS Booster
Peak-by-Peak

Analysis of an Antibody Subunit-Drug Conjugate

Application of a sliding window approach to LC-MS analysis of ADCs

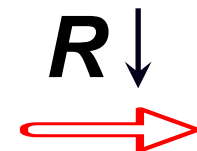
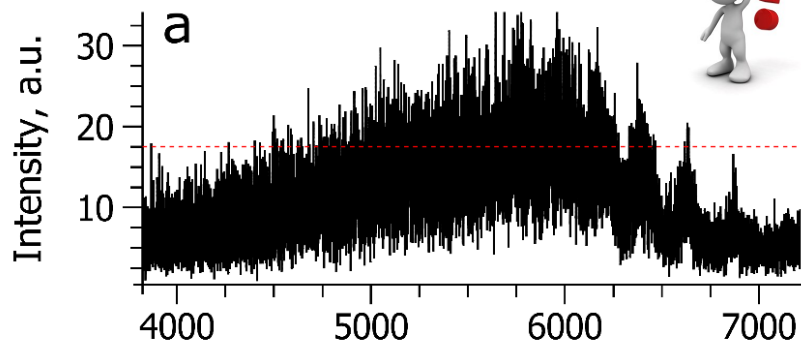


DAR estimate: Integrated approach (**3.50**) vs. Sliding window approach (**3.57**)

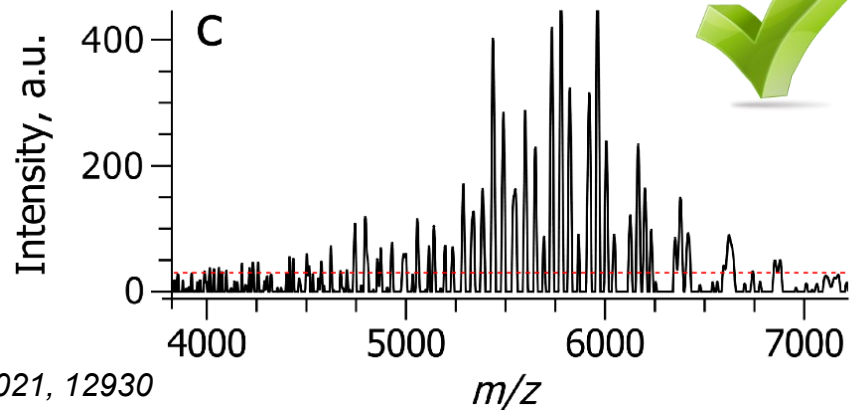
The revealed DARs are in line with the anticipated and simulated values

Conclusions

“resolved” proteoforms



integrated proteoforms



Anal Chem, 2021, 12930

- Proteoform peaks can be integrated by **significant** resolution reduction in Orbitrap FTMS
- **Proteoform integration shows utility for DAR analysis on complex ADC/AOC samples**
- **Sliding window** approach applied to ADC/AOC LC-MS data increases analysis accuracy
- Resolution reduction by user-controlled time-domain transient truncation is readily available
- Practical implementation: method automatization and optimization; comparison with TOF MS
- Other use cases in the analysis of heterogenous samples, different ADC/AOC complexity?

Resolution – Use with Caution! Sometimes Less is More.

Thank you!

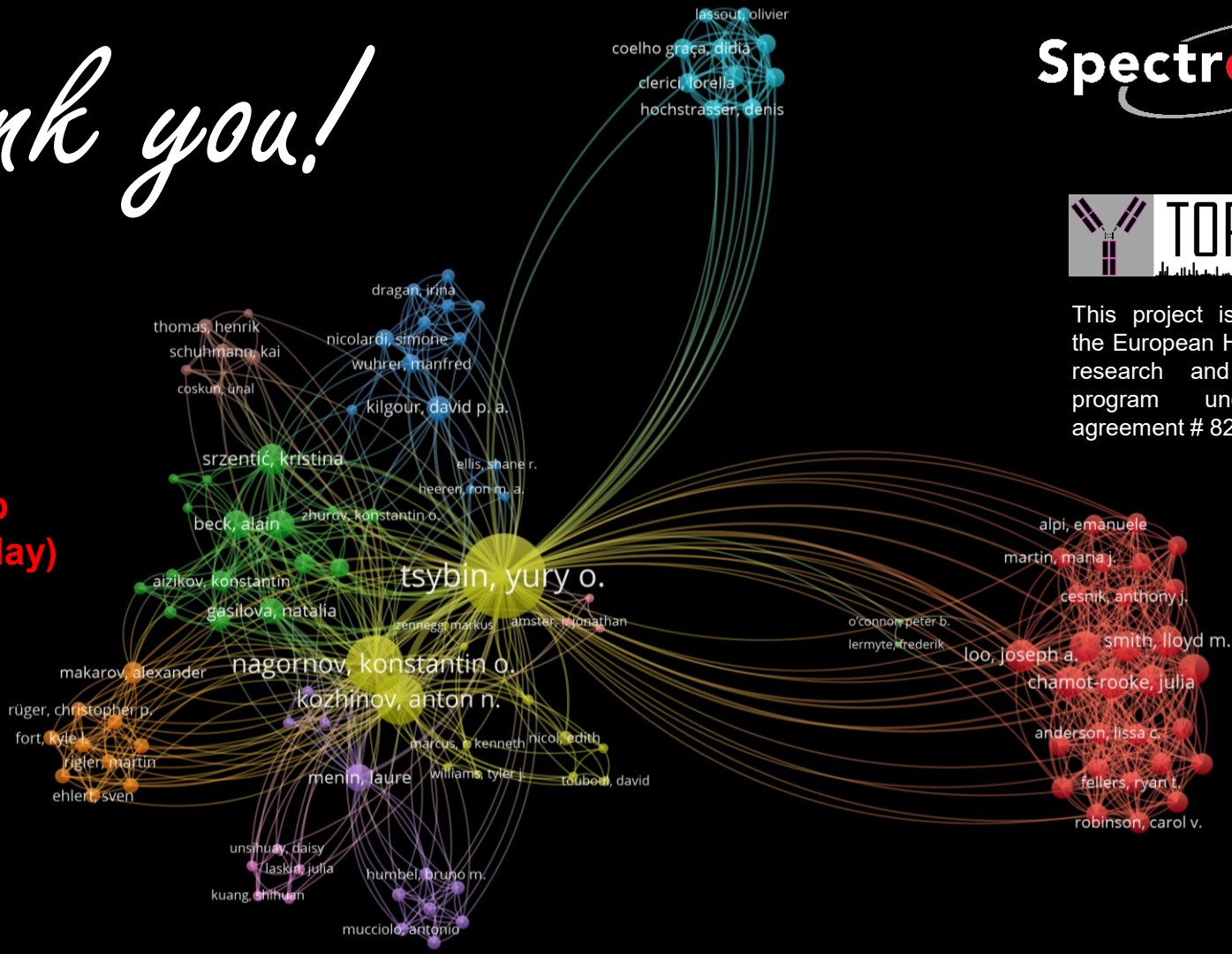
WP051

ThP120

FTMS

Workshop

(Wednesday)



Spectro+swiss



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