MAASTRICHT . THE NETHERLANDS
IMSC 2022

The 24th International Mass Spectrometry Conference

Targeted deconvolution feature extraction and annotation via time-domain transient modelling in Orbitrap FTMS for biopharma applications

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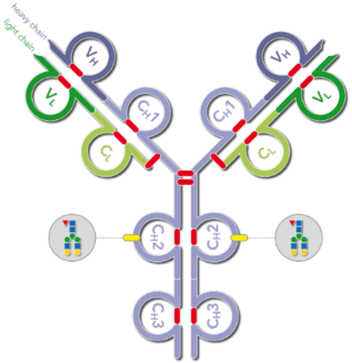
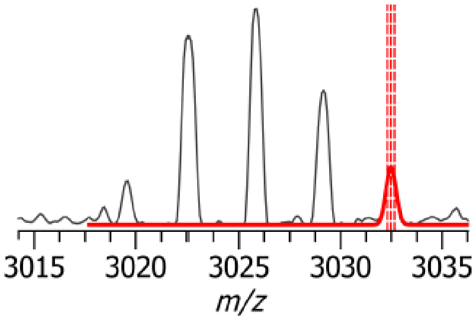




Talk #379 on August 30, 2022 at 12:20

Spectroswiss

Deconvolution in the Structural Analysis of mAbs / ADCs

Intact mass
proteoform
heterogeneity

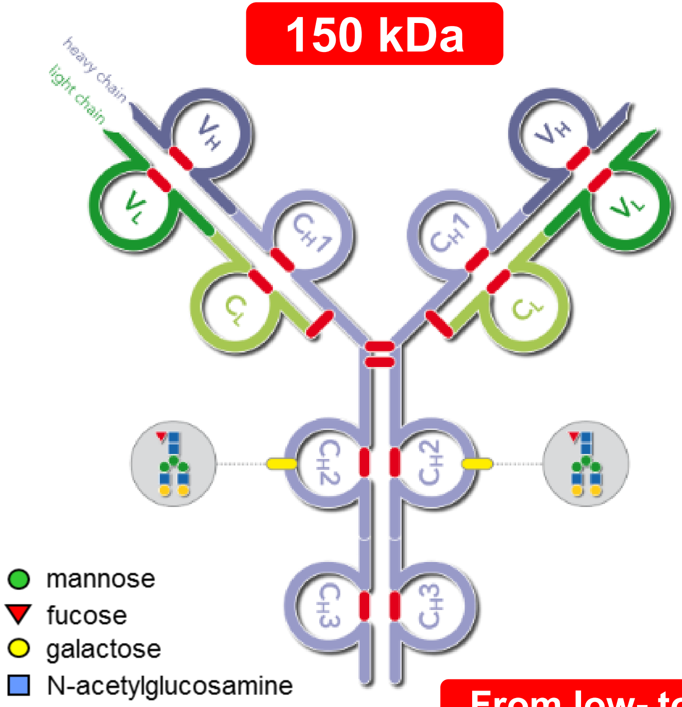
Middle-up:
subunit analysis for
proteoform heterogeneity

Middle-down MS/MS:
amino-acid level
analysis of subunits

50-80%

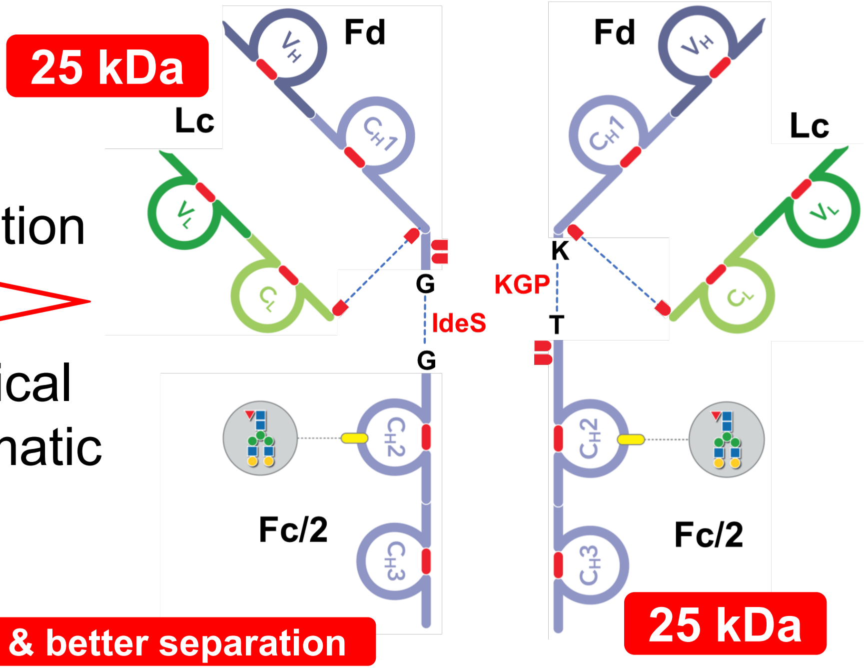
Top-Down MS/MS:
amino-acid level
analysis of mAbs

30-35%



mAb
decomposition

- chemical
- enzymatic



From low- to high-resolution & better separation

Intact Mass Measurements of mAbs: Low-Resolution Workflow

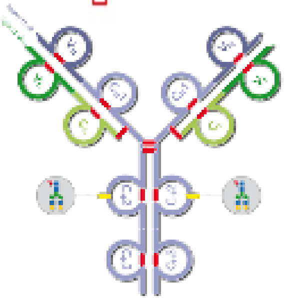
MS

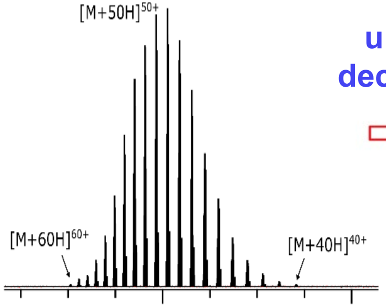
mass spectra

low-res

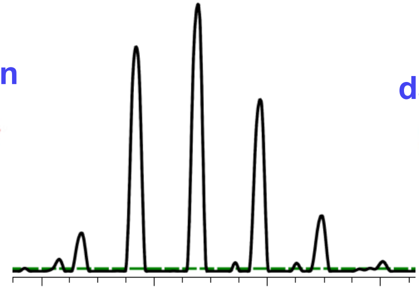
native or denaturing

Direct
RPLC
SEC
HIC
CE





untargeted deconvolution



unknown features

matching deconvolved data & database

+ other (many) untargeted deconvolution approaches

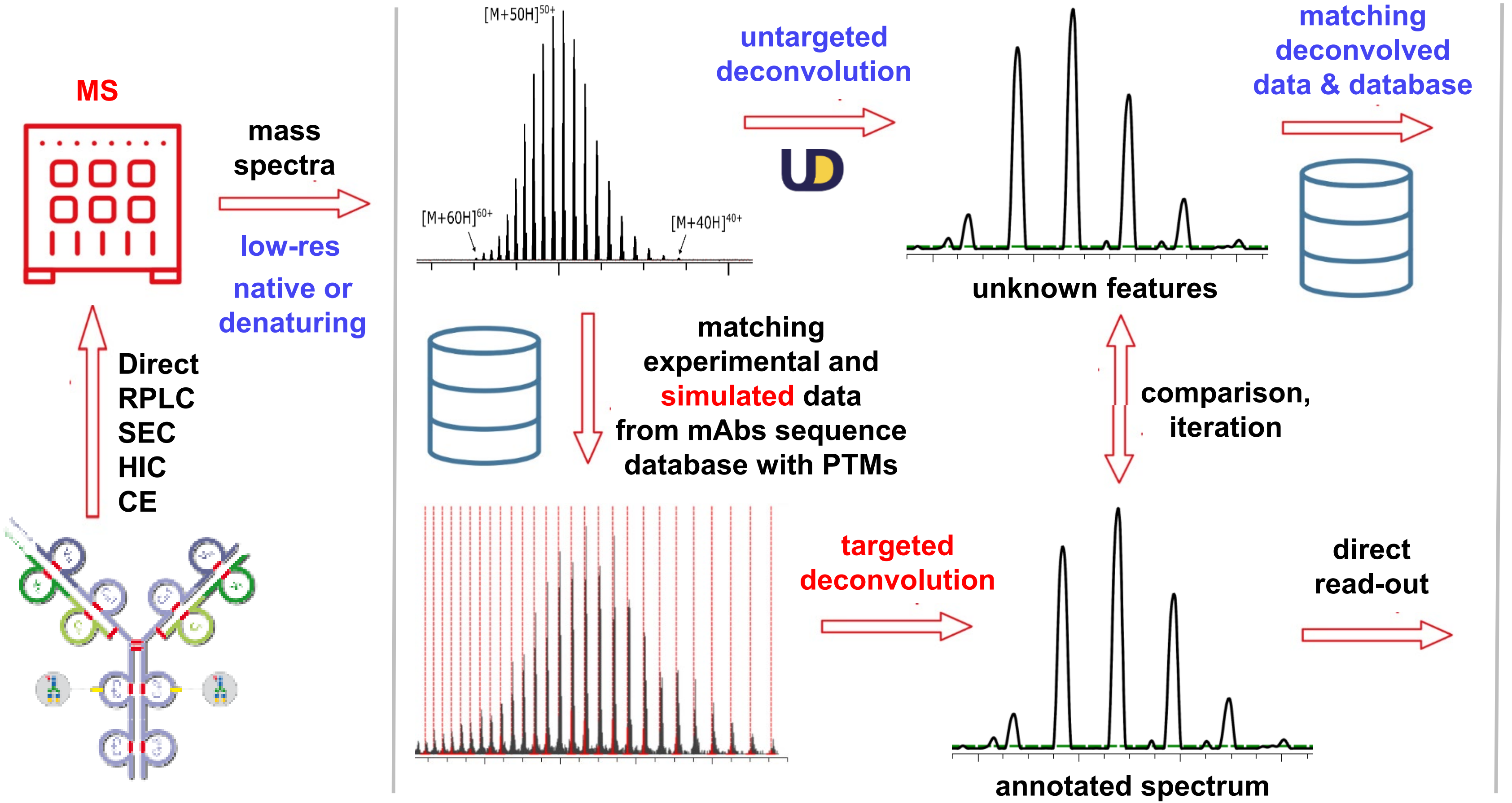
deconvolution artefacts
parameters selection
complex algorithms
automation
speed

REPORT

Annotation
Relative %

Untargeted deconvolution (including sliding windows) is the conventional approach

Intact Mass Measurements of mAbs: Low-Resolution Workflow



MS

mass spectra

low-res

native or denaturing

Direct
RPLC
SEC
HIC
CE

untargeted deconvolution

unknown features

matching deconvolved data & database

matching experimental and simulated data from mAbs sequence database with PTMs

comparison, iteration

targeted deconvolution

annotated spectrum

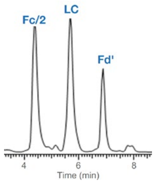
direct read-out

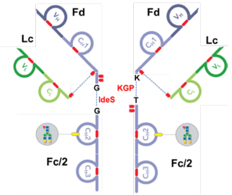
REPORT

Annotation
Relative %

Can targeted deconvolution complement or replace the untargeted approach?

Middle-Up of mAbs Subunits: High/Low-Resolution Workflow

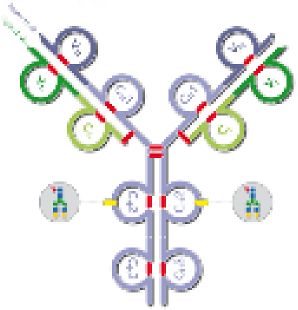


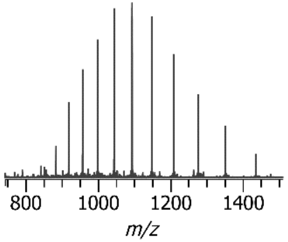


RPLC MS

high-res
low-res

subunits:
reduction,
enzymes
(IdeS, KGP)

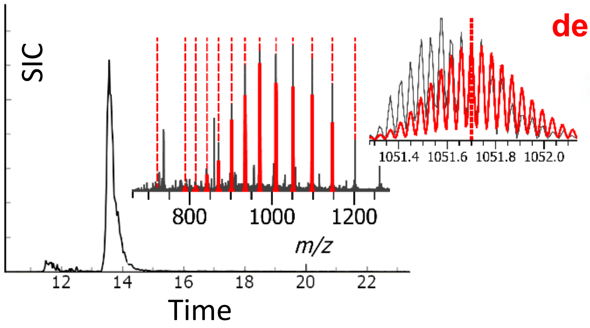




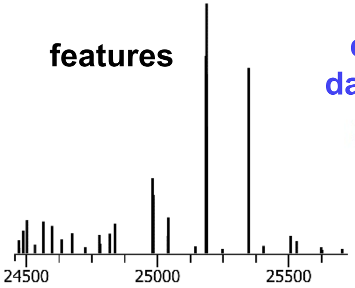
untargeted deconvolution

FLASHdeconv and others

matching experimental data & database



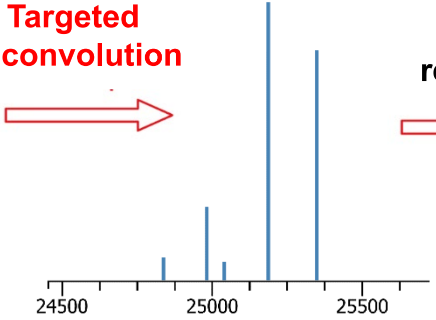
features



matching deconvolved data & database

comparison, iteration

Targeted deconvolution



annotated spectrum

direct readout

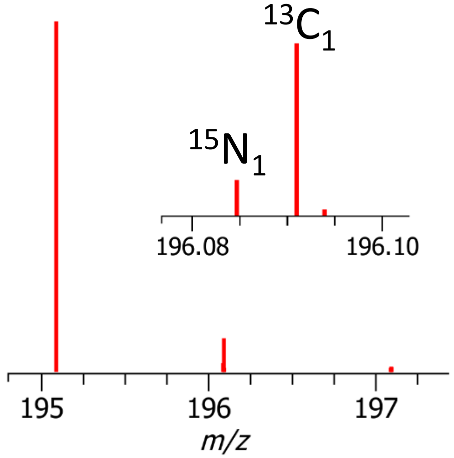
REPORT

Annotation
Relative %

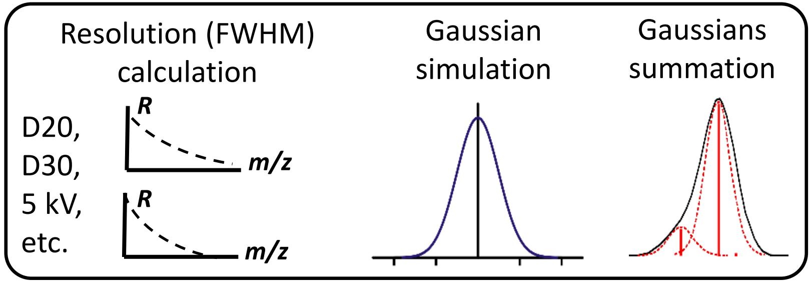
Targeted deconvolution assumes simulation of mass spectra: using what approach?

FTMS Data Simulation Approaches

Isotopic distribution calculation from elemental composition



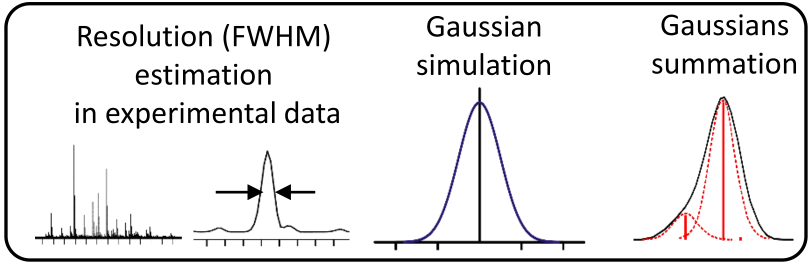
ChemCalc
IsoSpec2
EnviPat
…


Resolution (FWHM) calculation

D20,
D30,
5 kV,
etc.

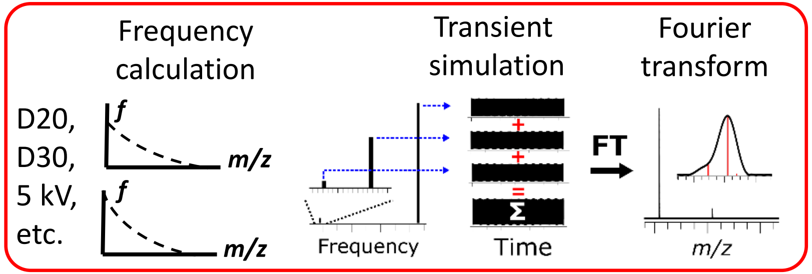
Gaussian simulation

Gaussians summation


Resolution (FWHM) estimation in experimental data

Gaussian simulation

Gaussians summation

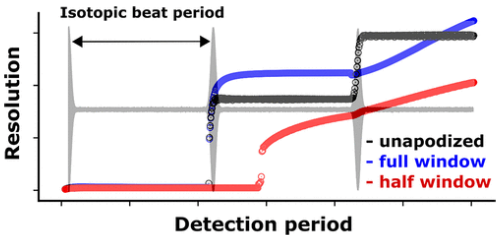

Frequency calculation

D20,
D30,
5 kV,
etc.

Transient simulation

Fourier transform

Isotopic beats of mAbs?



Nagornov *et al.*, *JASMS* 2022

Peak interference?

Computational speed?

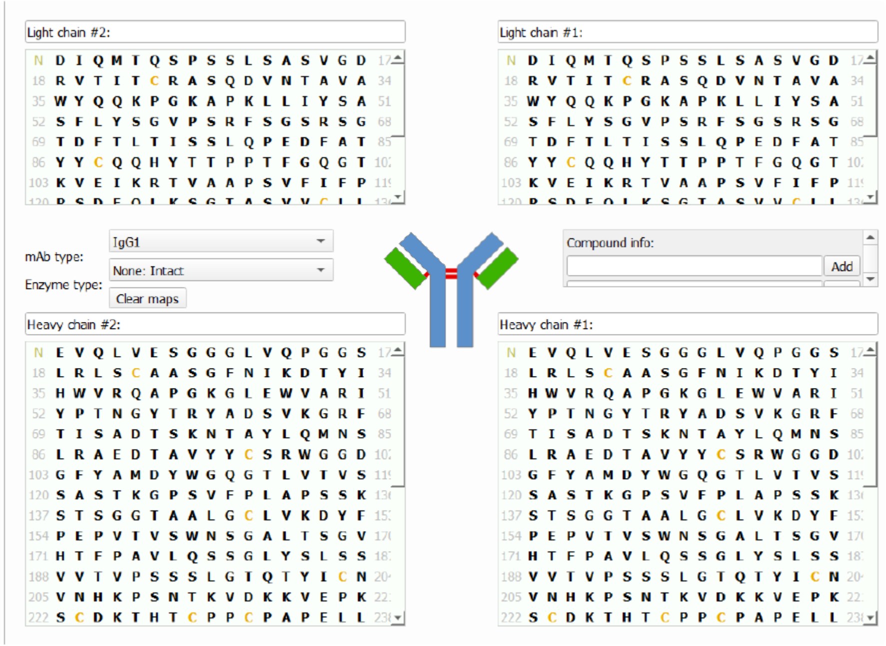
Nagornov et al.: Small proteins: *JASMS 2020, 31, 1927–1942;* Large proteins: *JASMS 2022, 33, 1113–1125*

Profile Library Simulations for mAbs: Low Resolution

Spectroswiss

- Automatically determine FTMS instrument model and settings from metadata
- Specify a database of target compounds (from small molecules to proteins)
- Suggest adducts, modifications, charge carriers, and charge states

SIMULATE



Light chain #2:

```
N   D I Q M T Q S P S S L S A S V G D 17
18  R V T I T C R A S Q D V N T A V A 34
35  W Y Q Q K P G K A P K L L I Y S A 51
52  S F L Y S G V P S R F S G S R S G 68
69  T D F T L T I S S L Q P E D F A T 85
86  Y Y C Q Q H Y T T P P T F G Q G T
103 K V E I K R T V A A P S V F I F P
```

Light chain #1:

```
N   D I Q M T Q S P S S L S A S V G D 17
18  R V T I T C R A S Q D V N T A V A 34
35  W Y Q Q K P G K A P K L L I Y S A 51
52  S F L Y S G V P S R F S G S R S G 68
69  T D F T L T I S S L Q P E D F A T 85
86  Y Y C Q Q H Y T T P P T F G Q G T
103 K V E I K R T V A A P S V F I F P
```

mAb type: IgG1

Enzyme type: None: Intact

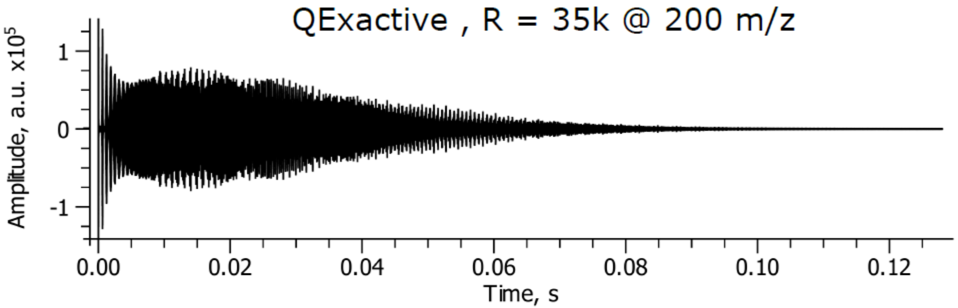
Compound info:

Heavy chain #2:

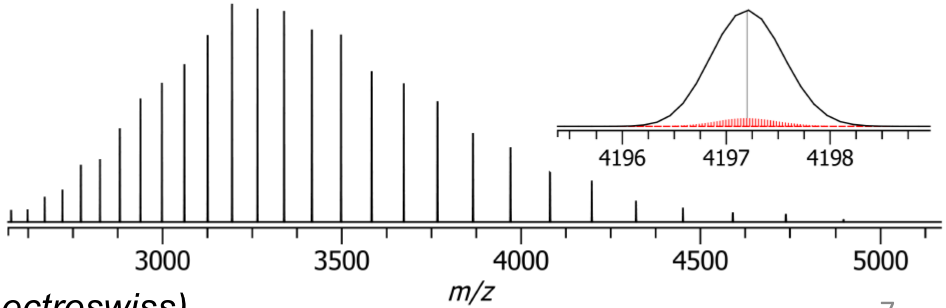
```
N   E V Q L V E S G G G L V Q P G G S 17
18  L R L S C A A S G F N I K D T Y I 34
35  H W V R Q A P G K G L E W V A R I 51
52  Y P T N G Y T R Y A D S V K G R F 68
69  T I S A D T S K N T A Y L Q M N S 85
86  L R A E D T A V Y Y C S R W G G D
103 G F Y A M D Y W G Q G T L V T V S
120 S A S T K G P S V F P L A P S S K
137 S T S G G T A A L G C L V K D Y F
154 P E P V T V S W N S G A L T S G V
171 H T F P A V L Q S S G L Y S L S S
188 V V T V P S S S L G T Q T Y I C N
205 V N H K P S N T K V D K K V E P K
222 S C D K T H T C P P C P A P E L L
```

Heavy chain #1:

```
N   E V Q L V E S G G G L V Q P G G S 17
18  L R L S C A A S G F N I K D T Y I 34
35  H W V R Q A P G K G L E W V A R I 51
52  Y P T N G Y T R Y A D S V K G R F 68
69  T I S A D T S K N T A Y L Q M N S 85
86  L R A E D T A V Y Y C S R W G G D
103 G F Y A M D Y W G Q G T L V T V S
120 S A S T K G P S V F P L A P S S K
137 S T S G G T A A L G C L V K D Y F
154 P E P V T V S W N S G A L T S G V
171 H T F P A V L Q S S G L Y S L S S
188 V V T V P S S S L G T Q T Y I C N
205 V N H K P S N T K V D K K V E P K
222 S C D K T H T C P P C P A P E L L
```



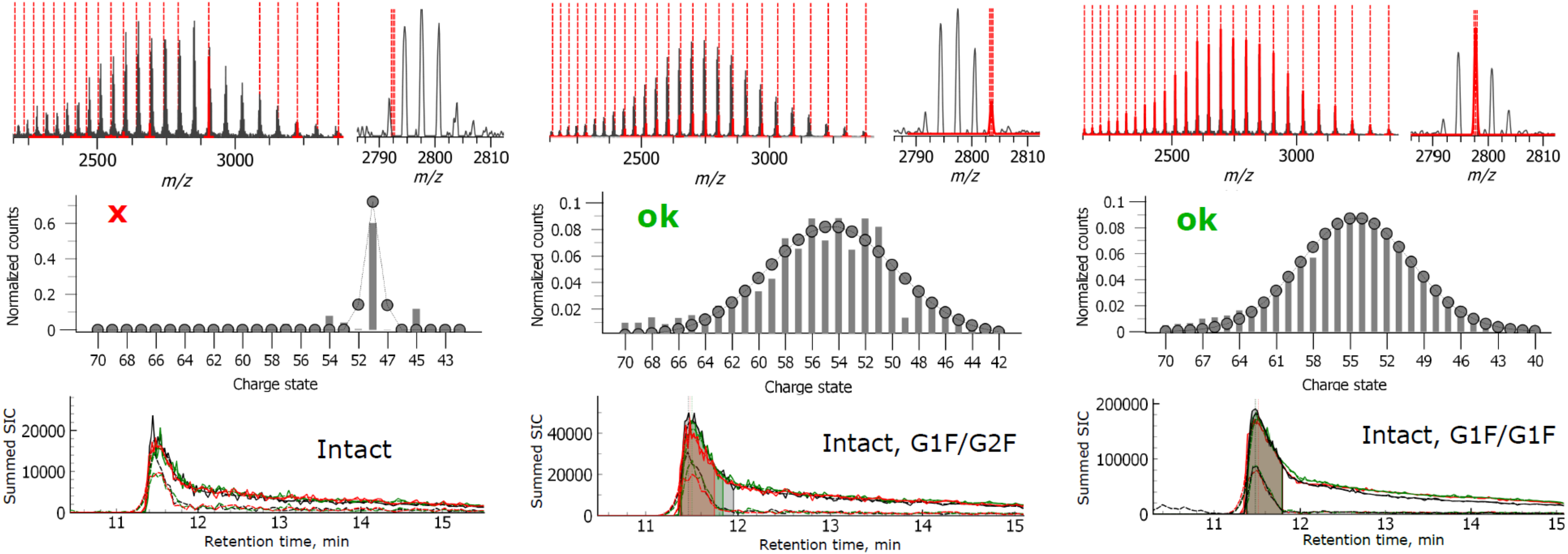
QExactive , R = 35k @ 200 m/z



Implementation example: Peak-by-Peak BioPharma (Spectroswiss)

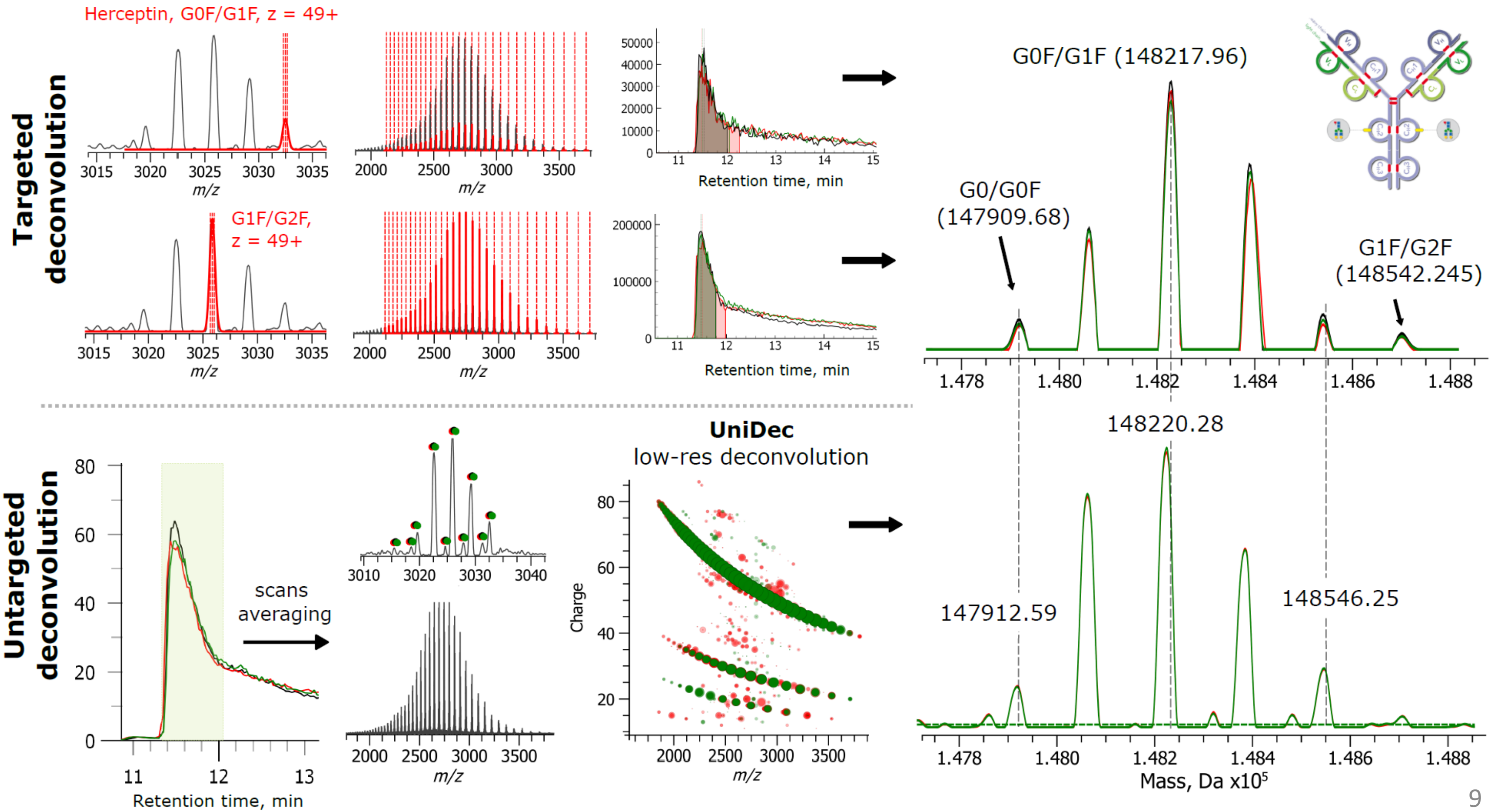
Correlate Experimental and Simulated Data: Low-Res

- Isotopic envelopes are compared for each charge state (no deconvolution, similarity)
- Charge state distributions are used to filter out the false positives
- Selected Ion Current (SIC) chromatograms show proteoform-specific elution periods



Implementation example: Peak-by-Peak BioPharma (Spectroswiss)

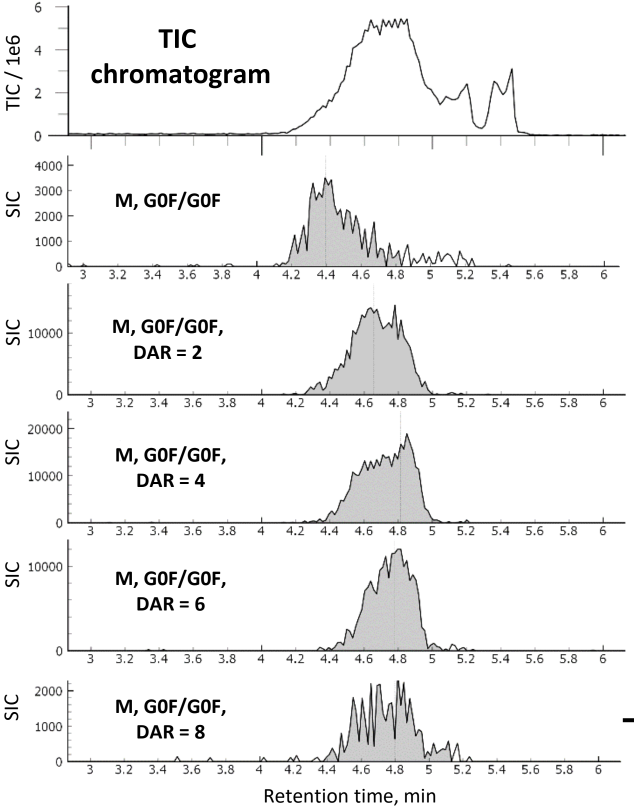
Targeted & Untargeted Deconvolution: Low Resolution



Drug-to Antibody Ratios (DARs) for Antibody Drug Conjugates (ADCs)

Spectroswiss

Proteoform-level SICs

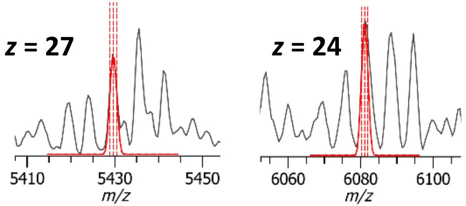


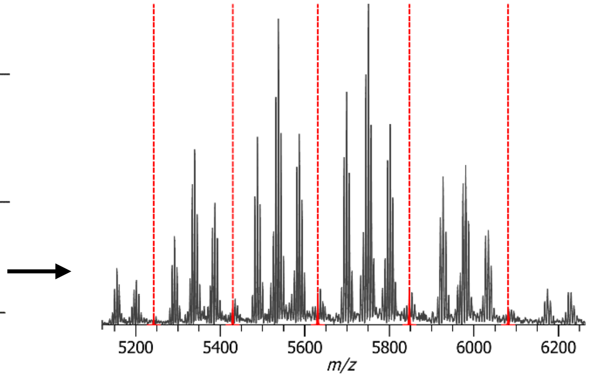
SiLu mAb ADC (Sigma)

DAR reported: 4.0 +/- 0.8

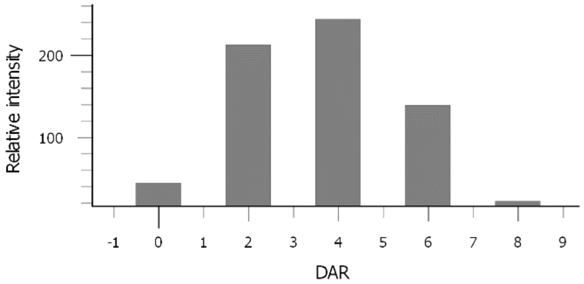
QExactive HF, R = 15k, native

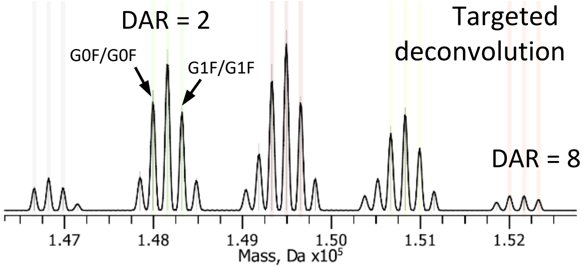
M, G0F/G0F, DAR = 8

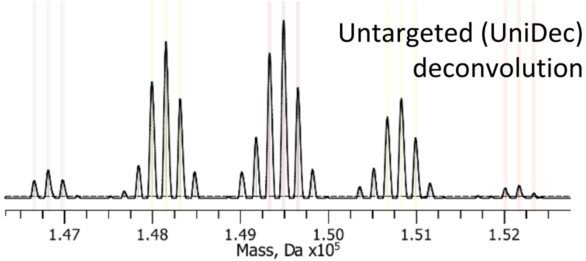




DAR: 3.6 via SIC peak areas of G0F/G0F, G0F/G1F, G1F/G1F







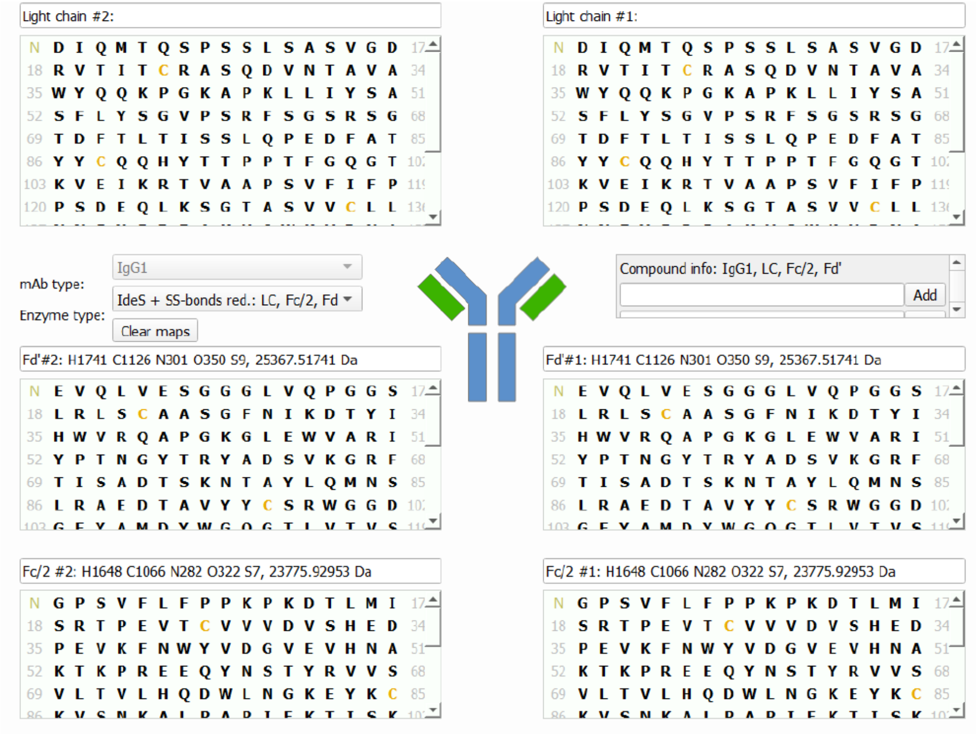
Targeted approach eliminates the need to perform a sliding window-based deconvolution

Profile Library Simulations for Subunits: High-Res

Spectroswiss

- Isotopically resolved envelopes

SIMULATE



Light chain #2:

```
N   D I Q M T Q S P S S L S A S V G D
18  R V T I T C R A S Q D V N T A V A
35  W Y Q Q K P G K A P K L L I Y S A
52  S F L Y S G V P S R F S G S R S G
69  T D F T L T I S S L Q P E D F A T
86  Y Y C Q Q H Y T T P P T F G Q G T
103 K V E I K R T V A A P S V F I F P
120 P S D E Q L K S G T A S V V C L L
```

Light chain #1:

```
N   D I Q M T Q S P S S L S A S V G D
18  R V T I T C R A S Q D V N T A V A
35  W Y Q Q K P G K A P K L L I Y S A
52  S F L Y S G V P S R F S G S R S G
69  T D F T L T I S S L Q P E D F A T
86  Y Y C Q Q H Y T T P P T F G Q G T
103 K V E I K R T V A A P S V F I F P
120 P S D E Q L K S G T A S V V C L L
```

mAb type: IgG1

Enzyme type: IdeS + SS-bonds red.: LC, Fc/2, Fd

Compound info: IgG1, LC, Fc/2, Fd'

Fd'#2: H1741 C1126 N301 O350 S9, 25367.51741 Da

```
N   E V Q L V E S G G G L V Q P G G S
18  L R L S C A A S G F N I K D T Y I
35  H W V R Q A P G K G L E W V A R I
52  Y P T N G Y T R Y A D S V K G R F
69  T I S A D T S K N T A Y L Q M N S
86  L R A E D T A V Y Y C S R W G G D
```

Fd'#1: H1741 C1126 N301 O350 S9, 25367.51741 Da

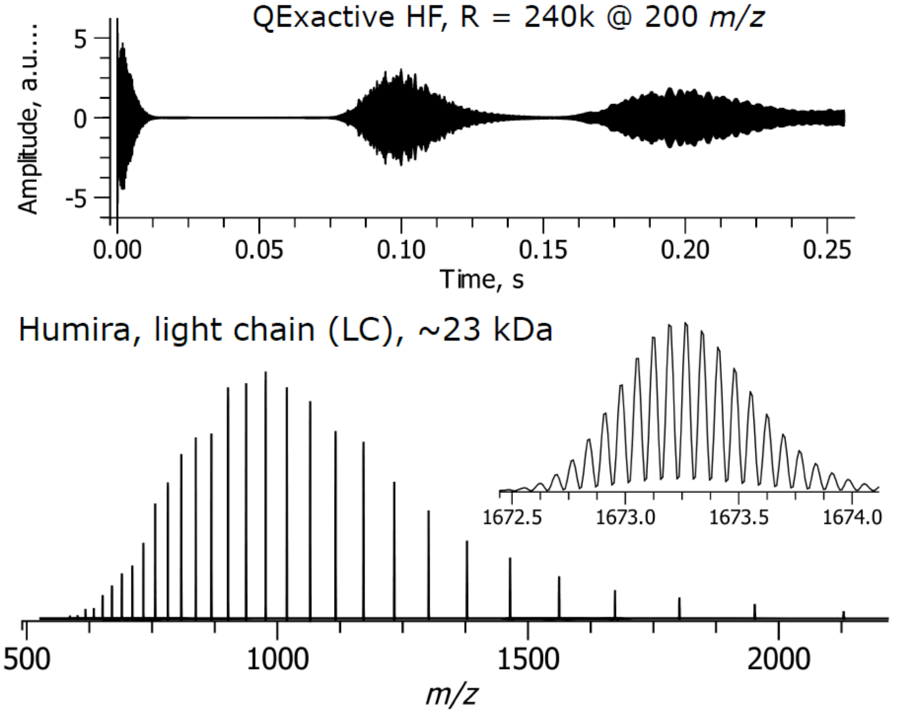
```
N   E V Q L V E S G G G L V Q P G G S
18  L R L S C A A S G F N I K D T Y I
35  H W V R Q A P G K G L E W V A R I
52  Y P T N G Y T R Y A D S V K G R F
69  T I S A D T S K N T A Y L Q M N S
86  L R A E D T A V Y Y C S R W G G D
```

Fc/2 #2: H1648 C1066 N282 O322 S7, 23775.92953 Da

```
N   G P S V F L F P P K P K D T L M I
18  S R T P E V T C V V V D V S H E D
35  P E V K F N W Y V D G V E V H N A
52  K T K P R E E Q Y N S T Y R V V S
69  V L T V L H Q D W L N G K E Y K C
```

Fc/2 #1: H1648 C1066 N282 O322 S7, 23775.92953 Da

```
N   G P S V F L F P P K P K D T L M I
18  S R T P E V T C V V V D V S H E D
35  P E V K F N W Y V D G V E V H N A
52  K T K P R E E Q Y N S T Y R V V S
69  V L T V L H Q D W L N G K E Y K C
```



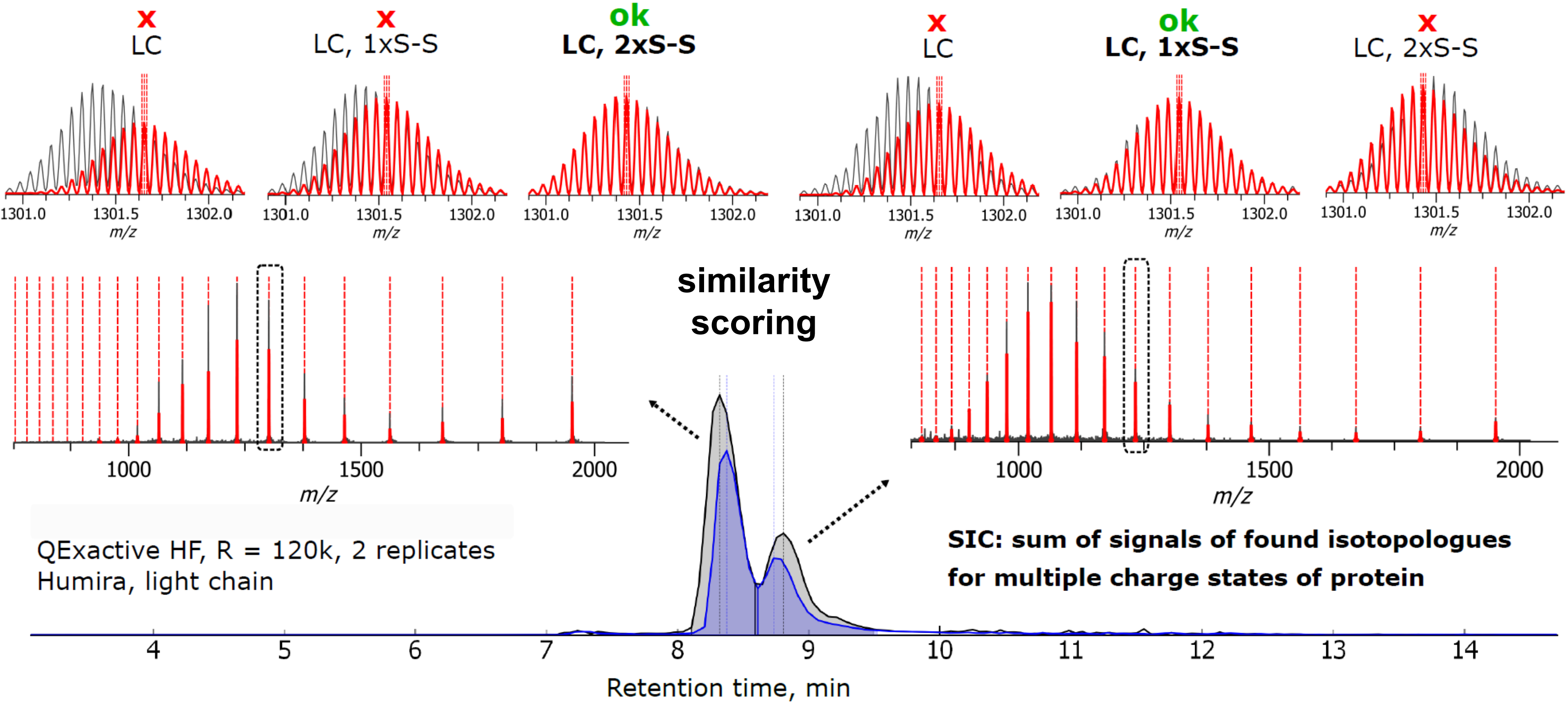
QExactive HF, R = 240k @ 200 m/z

Humira, light chain (LC), ~23 kDa

Implementation example: Peak-by-Peak BioPharma (Spectroswiss)

Correlate Experimental and Simulated Data: High-Res

- Analysis of ~25 kDa mAb light chains (incomplete S-S bond reduction)



similarity scoring

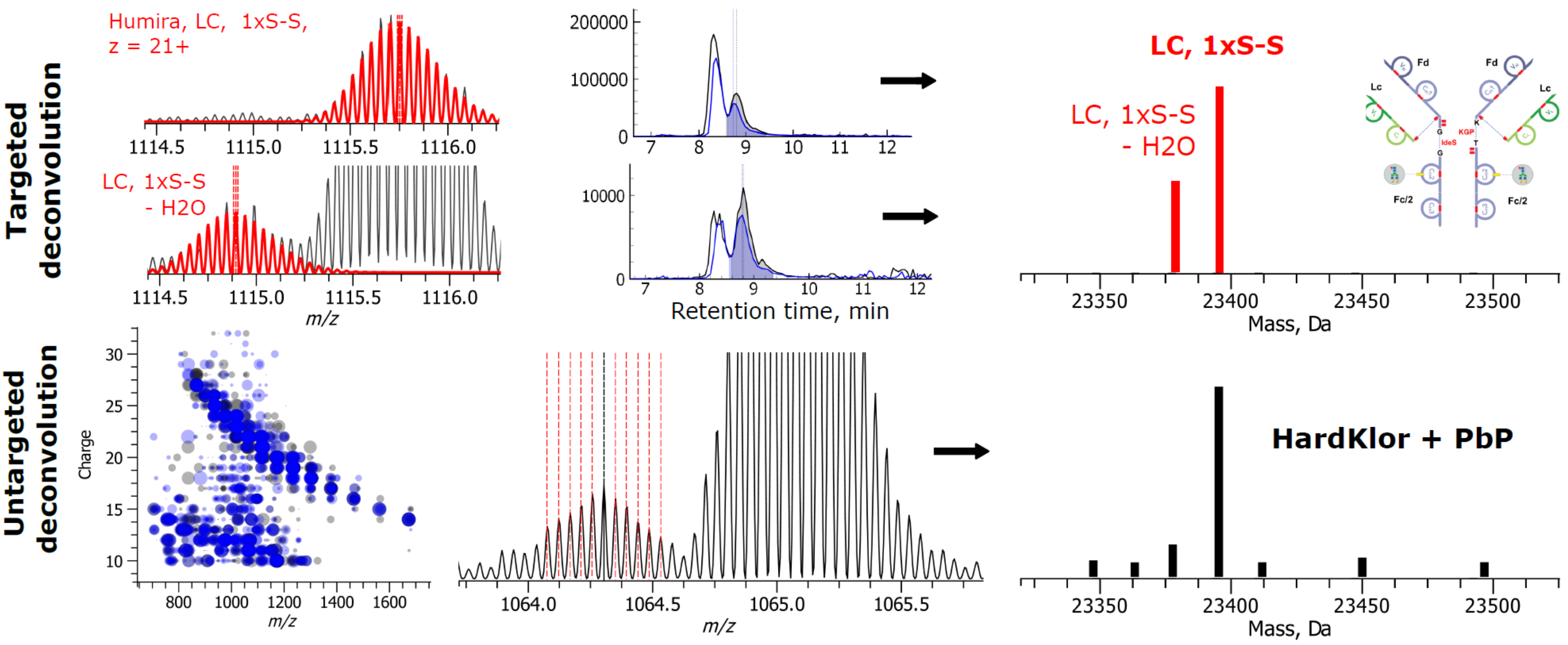
QExactive HF, R = 120k, 2 replicates
Humira, light chain

SIC: sum of signals of found isotopologues for multiple charge states of protein

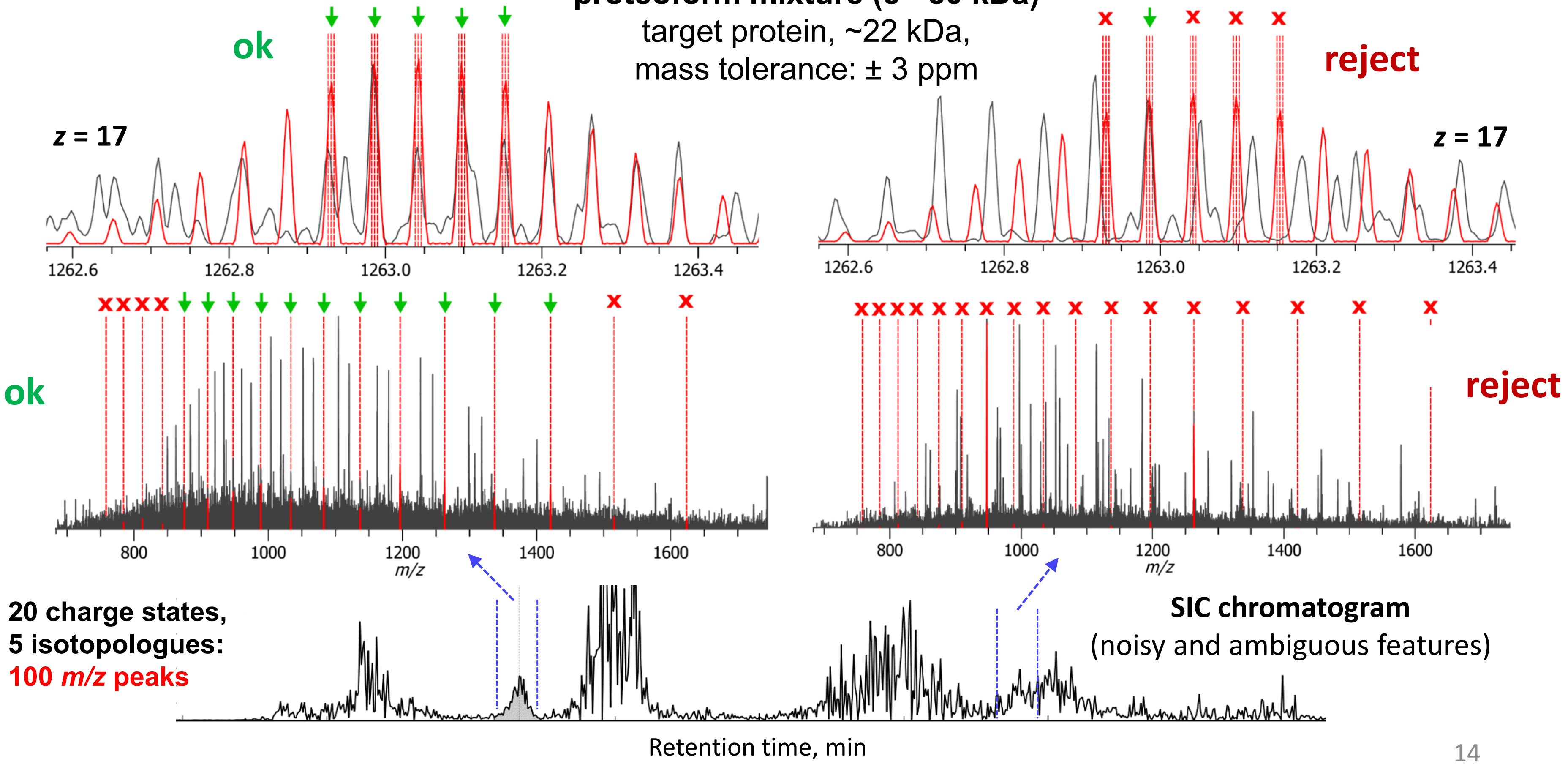
Implementation example: Peak-by-Peak BioPharma (Spectroswiss)

Targeted & Untargeted Deconvolution: High Resolution

- Untargeted deconvolution of the isotopically-resolved MS data: FLASHDeconv, Hardklor, ...



Targeted Deconvolution: High Resolution & Low Abundance



proteoform mixture (5 - 50 kDa)
target protein, ~22 kDa,
mass tolerance: ± 3 ppm

ok

***z* = 17**

reject

***z* = 17**

ok

reject

20 charge states,
5 isotopologues:
100 *m/z* peaks

SIC chromatogram
(noisy and ambiguous features)

Retention time, min

Conclusions & Outlook

- Targeted deconvolution based on FTMS time-domain transient modelling: a new tool
- The developed approach is succesfully benchmarked for biopharma applications
- An easy to understand approach, facile manual curation, and suitable for automation
- Approach appears useful for intact, subunit, and peptide level analysis of mAbs/ADC
- High speed of calculations: computationally-efficient python-based implementation
- Accuracy of isotopic envelopes modelling importance: do we need transients?
- Targeted and untargeted deconvolution: solo or duet?
- Other MS application areas: analysis of proteoform mixtures, metabolomics, imaging
- Other MS/MS application areas: targeted bottom-up, middle-down, top-down

Powerful computational approaches & resources create new opportunities in MS

Thank you!

Spectroswiss

TOP SPEC

Project number:
829157