Mass Spectrometry Coupled to Ion Mobility for Antibody-Based Product Characterization

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OUTLINE

- **Native MS and IMS-MS : basics**

- **TOPIC 1 : IMS-MS to monitor conformational heterogeneity/homogeneity**
  - Qualitative IMS-MS data interpretation for batch to batch comparison
  - For S-S bridge assessment

- **TOPIC 2 : IMS-MS to monitor dynamics of conformational changes**
  - Fab Arm Exchange of IgG4
  - Drug conjugation for ADC formation

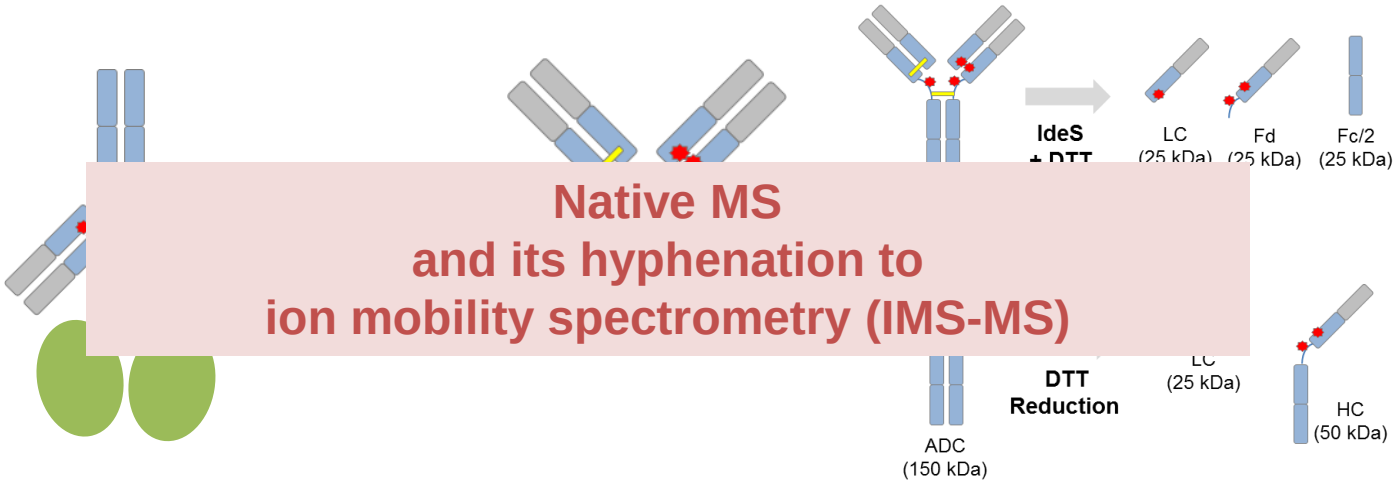
- **TOPIC 3 : IMS-MS to distinguish between mAb isotypes/subclasses**
  - Middle level *v.* intact level IMS-MS CIU
  - SEC-CIU
  - High resolution CIU-cIMS

- **TOPIC 4 : IMS-MS for the characterization of complex mAb formats**
  - The multispecific case-study

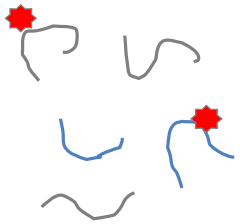
- **Concluding remarks**

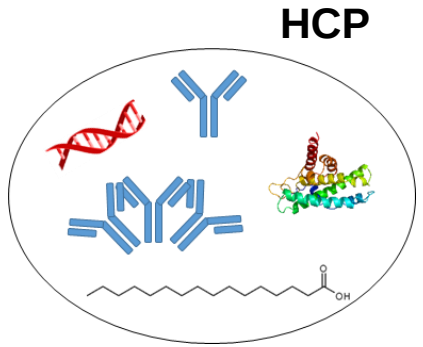
Multi-level therapeutic protein characterization

Higher Order Structure Aggregates ADC/Ag complexes (>150 kDa)	Top level (intact ADC) (150 kDa)	Middle level (fragment/subdomain) (25-100 kDa)	Bottom level (peptide mapping) (700-7000 Da)	"Impurities"



Native MS
and its hyphenation to
ion mobility spectrometry (IMS-MS)



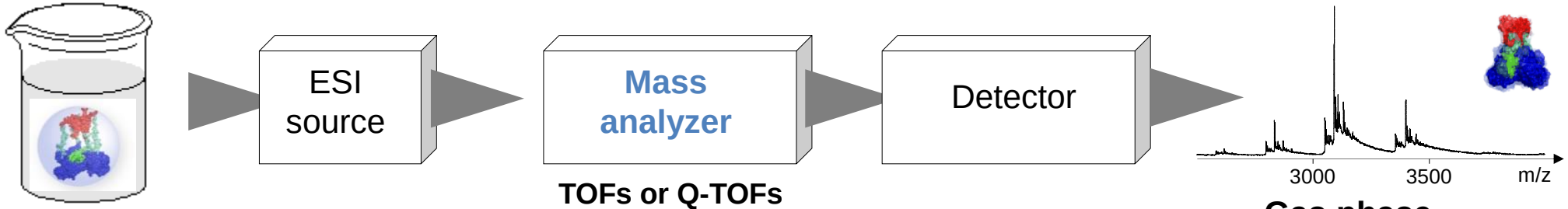


(SEC)- Native MS IMS-MS HDX-MS	(SEC)-Native MS IMS-MS Top-down MS	LC-MS Middle-down MS	Enzymatic digestion (nano)LC-MS/MS	LC-SRM LC-MS/MS

Beck A et al. Expert Rev Proteomics. 2016 Feb;13(2):157-83.

Native Mass Spectrometry

Native MS also « MS in non-denaturing conditions » gives information on assemblies maintained by noncovalent interactions



Solution

TOFs or Q-TOFs + Orbitraps

Gas phase

Sample preparation step = desalting step

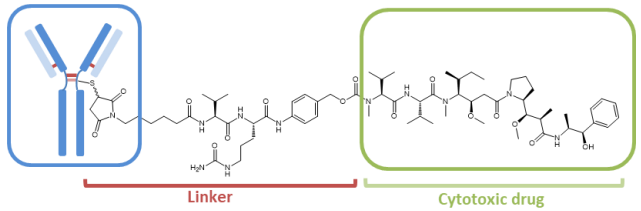
- Buffer exchange
- Use of volatile aqueous buffer at neutral pH
- Ammonium buffer (0-1 M) with controlled pH

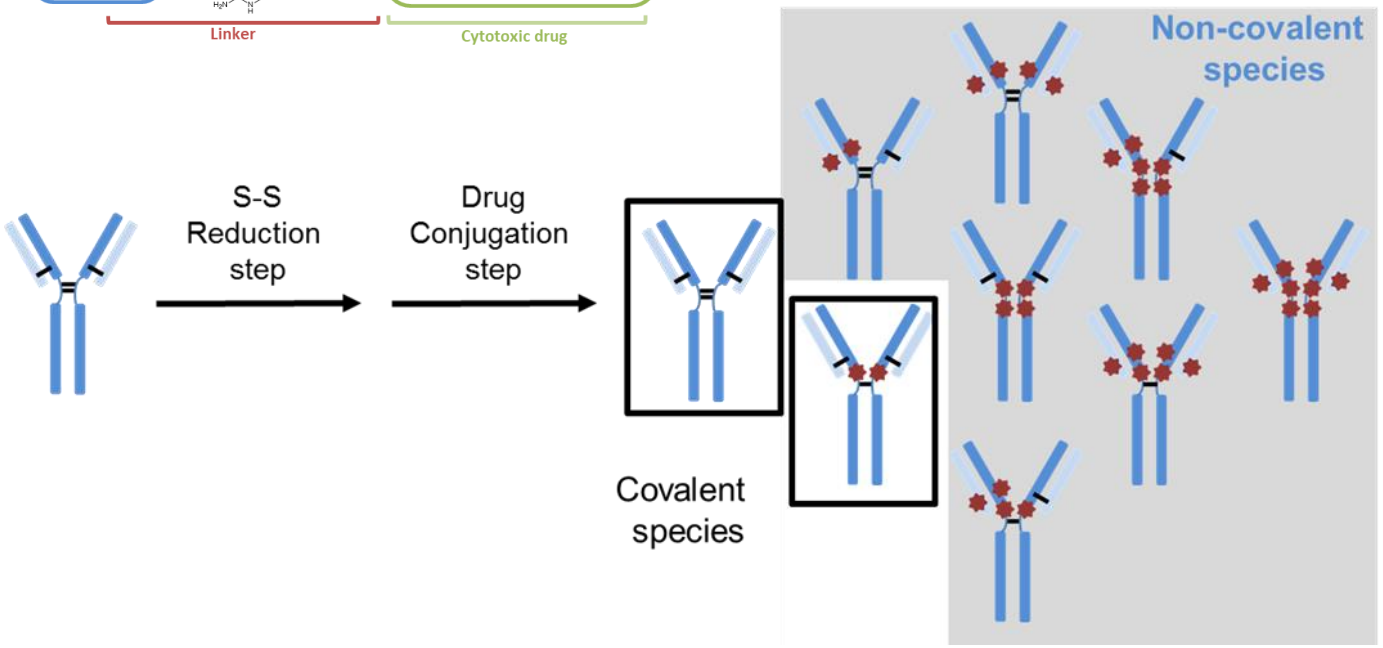
Data interpretation

- Binding stoichiometries
- Binding specificity
- Solution affinities
- Dynamics of assembly/disassembly

Native MS is less « destructive » than denaturing MS

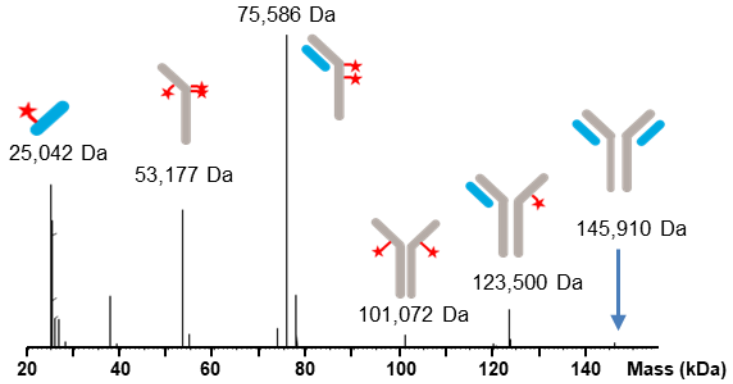
- Example of cysteine linked ADC



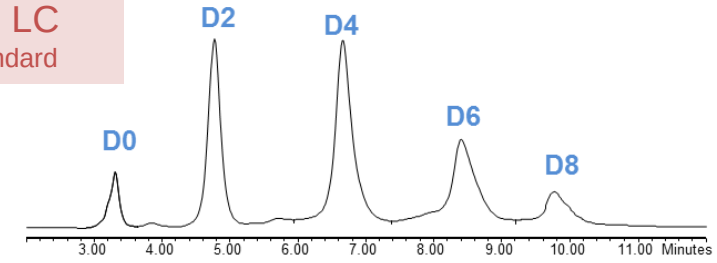


Denaturing conditions

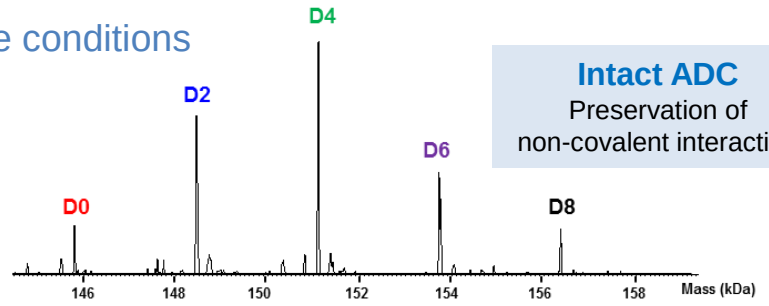
ADC fragments
Non covalent
interaction dissociated



Native LC
gold standard



Native conditions



Intact ADC
Preservation of
non-covalent interaction

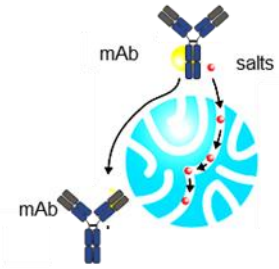
Native MS analysis allows cysteine-linked ADC characterization through intact DAR mass measurements

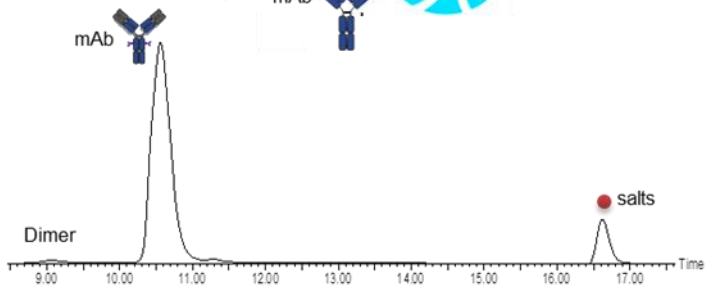
Debaene F. et al. Anal Chem. 2014, 86(21):10674-83.

Towards automated native MS: online SEC coupled to native MS (SEC-nMS)

Fast, Efficient & Automated sample preparation

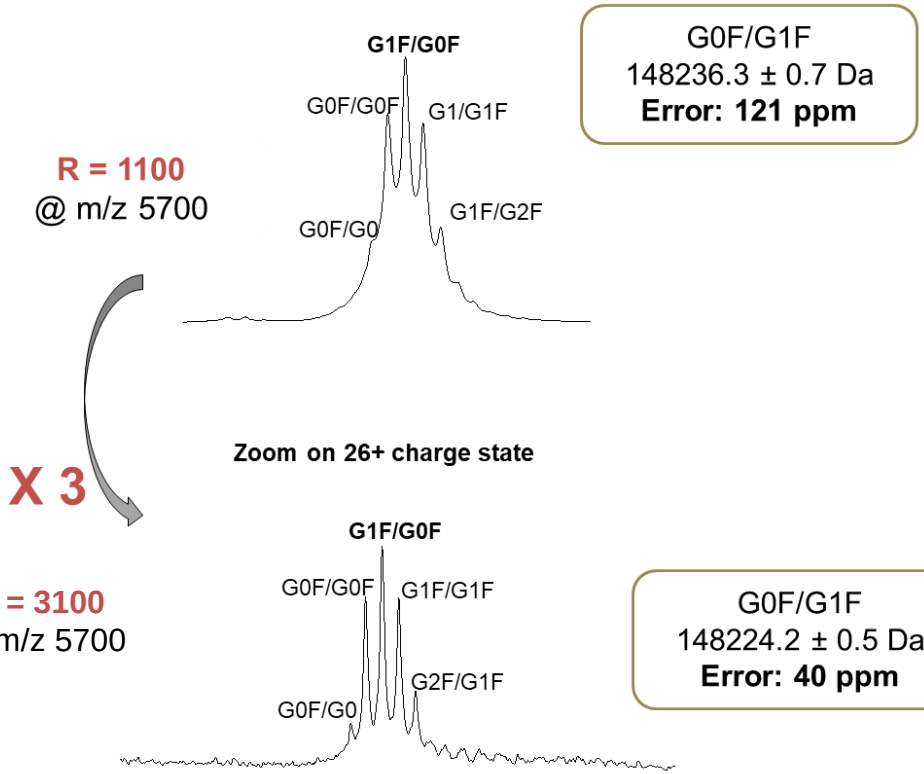
Size Exclusion Chromatography (SEC)
Online sample preparation (desalting)





SEC allows separation of non-volatile salts (100 Da) from mAbs (150 kDa)

SEC-native (IMS)-MS affords more efficient desalting than manual desalting

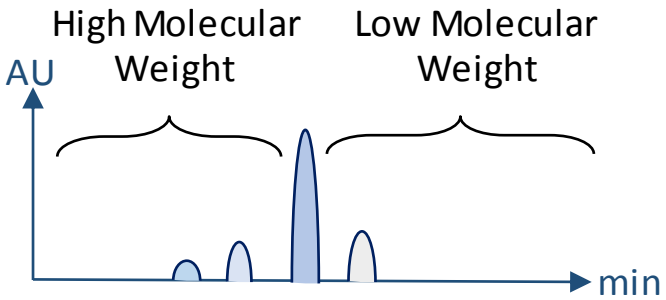


- Improved desalting efficiency
- Improved mass resolution, accuracy and sensitivity
- Increase in throughput 3-5 min/run on 3-5 cm cartridges
- Automated LC-native MS

Ehkirch et al. J. Chrom B 2018;1086:176-183

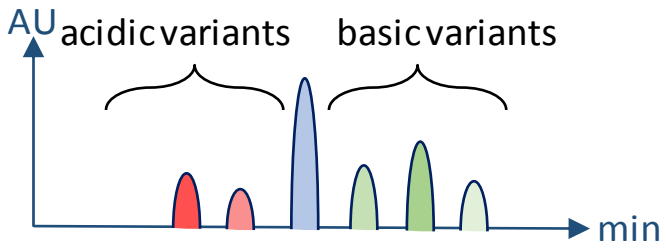
Online non-denaturing LC-native MS for mAb analyses

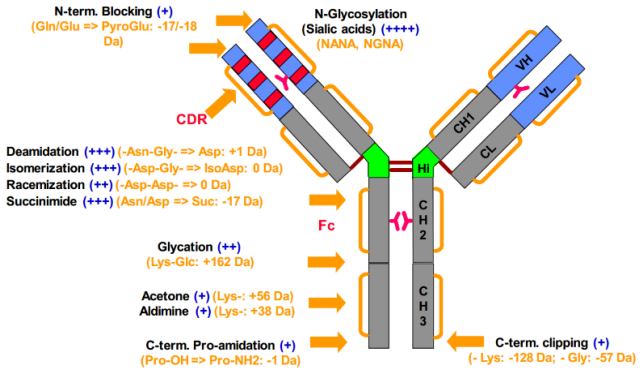
SEC – native MS for size variants



mAb aggregation
mAb degradation products
mAb size variant profile (CQA)
Forced degraded studies

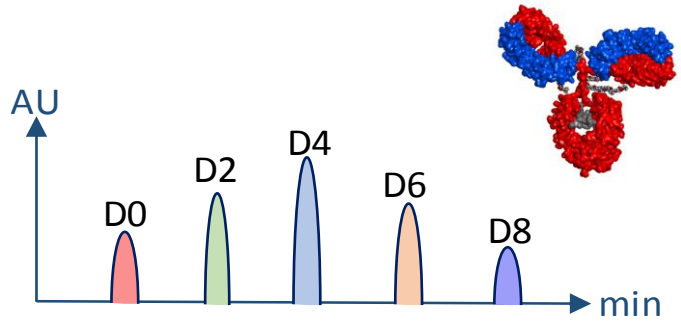
IEX – native MS for charge variants

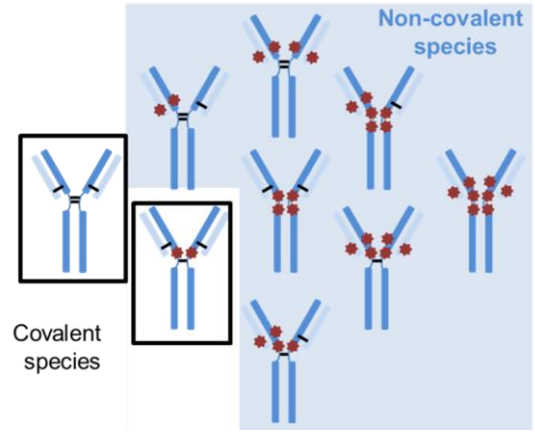




mAb charge variants profile (CQA)

HIC – native MS for hydrophobic variants



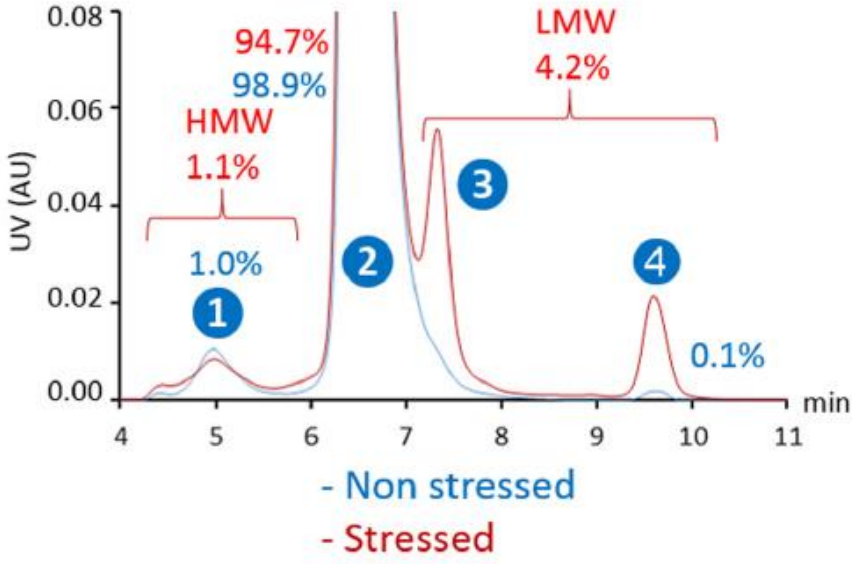


Cys- ADC DLD
mAb CQA

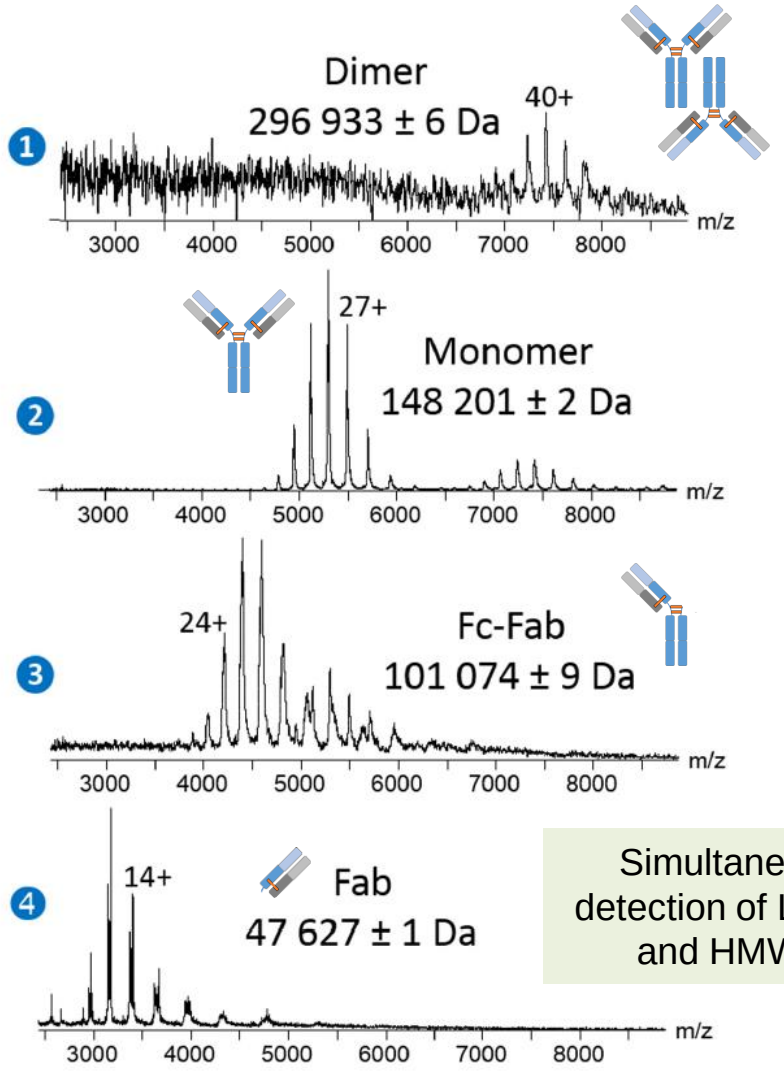
Online SEC-native MS for size variant analysis

SEC – native MS
for size variants

NISTmab (2 months thermally stressed at 37°C)
100 mM $AcONH_4$ pH 6.8
BEH 200Å, 4.6 x 150 mm, 1.7 µm
Vc: 180 V; Pi: 6 mbar



SEC-UV allows separation and quantification of mAb size variants



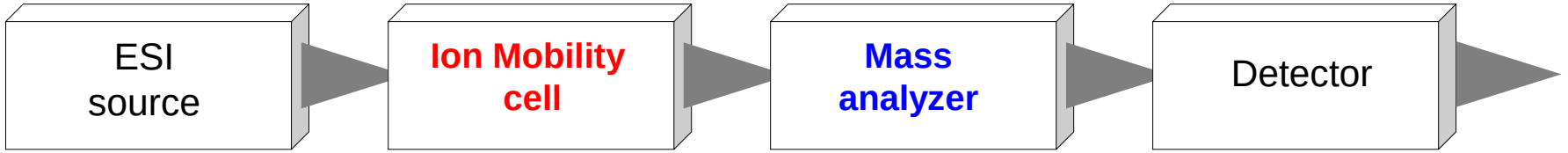
Simultaneous detection of LMWS and HMWS

Native MS allows identification of each size variant

Ehkirch et al. J Chrom. B 2018

Native Ion Mobility MS (IMS-MS) for conformational characterization

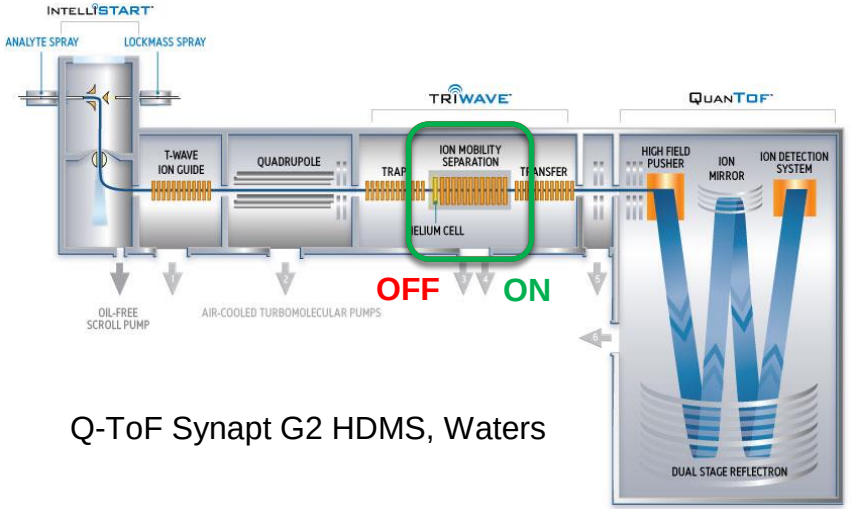
- Native IMS-MS: simultaneous measurement of conformations and masses in non-denaturing conditions



Instrumental setting optimizations

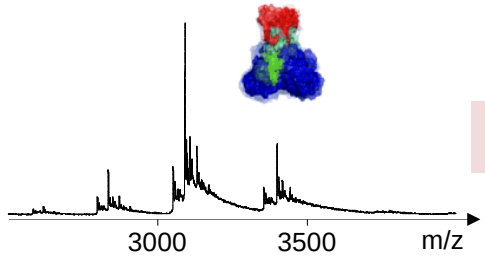
Data interpretation

- Conformation of biomolecules
- Conformational changes



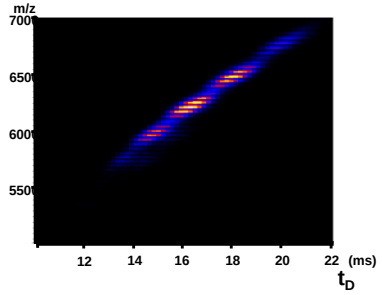
Q-ToF Synapt G2 HDMS, Waters

IMS OFF: native MS mode



➔ Binding characteristics

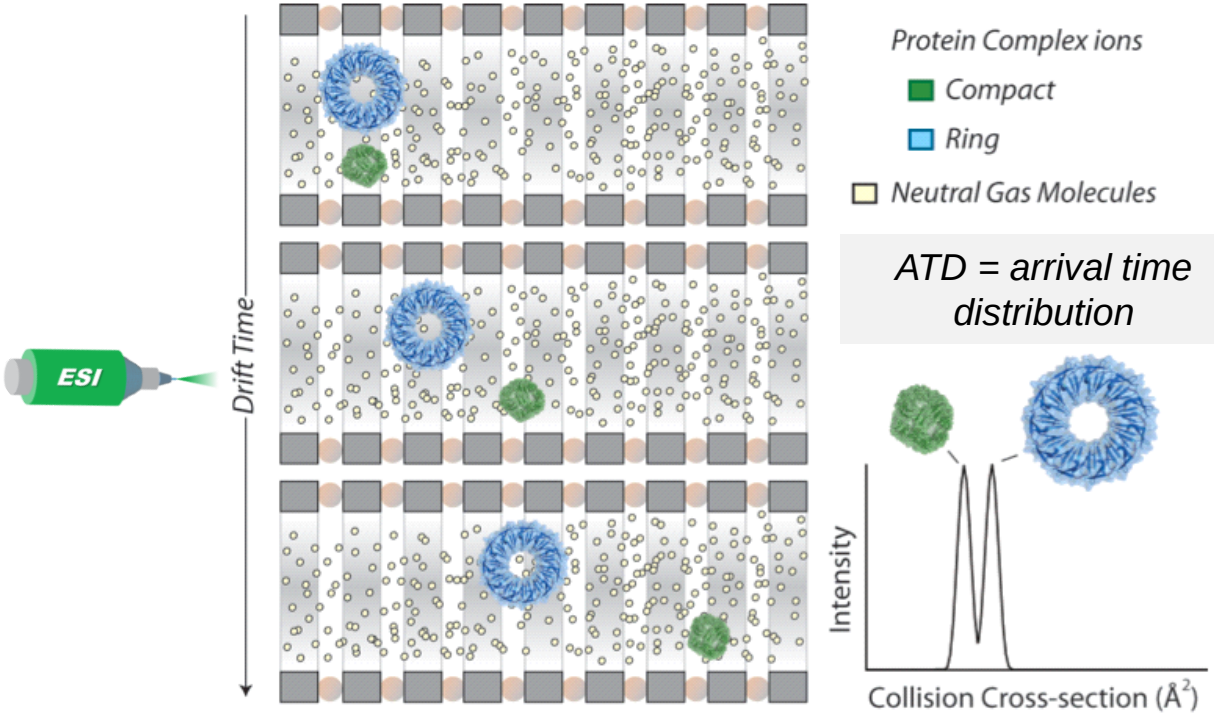
IMS ON: native IMS-MS mode



➔ Conformational characteristics

Ion Mobility brings an additional level of conformational characterization

IMS-MS principles and CCS calculations

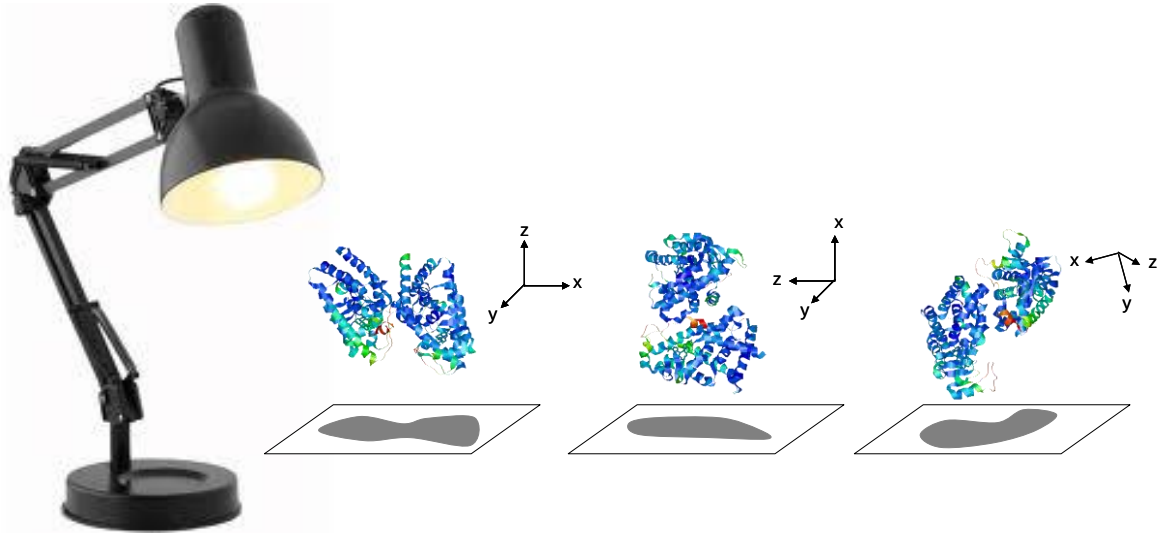


ATD = arrival time distribution

- IMS ion separation according to Shape/Size (Mass) and Charge
- IMS can distinguish different conformations

- From drift time values, **collision cross sections (CCS)** can be calculated.
- These CCS values represent the **effective area for the interaction between an individual ion and the neutral gas** through which it is travelling.

$$\Omega = \frac{3e}{16N} \times \frac{T}{273.15} \times \frac{760}{P} \sqrt{\frac{2\pi}{k_b T}} \times \sqrt{\frac{1}{M_{gas}} + \frac{1}{M_{ion}}} \times \frac{z \times tD \times E}{L}$$

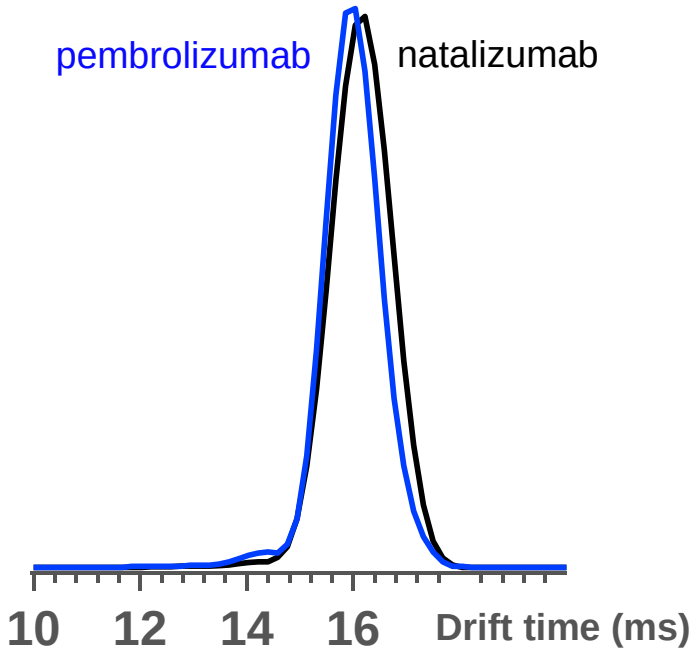


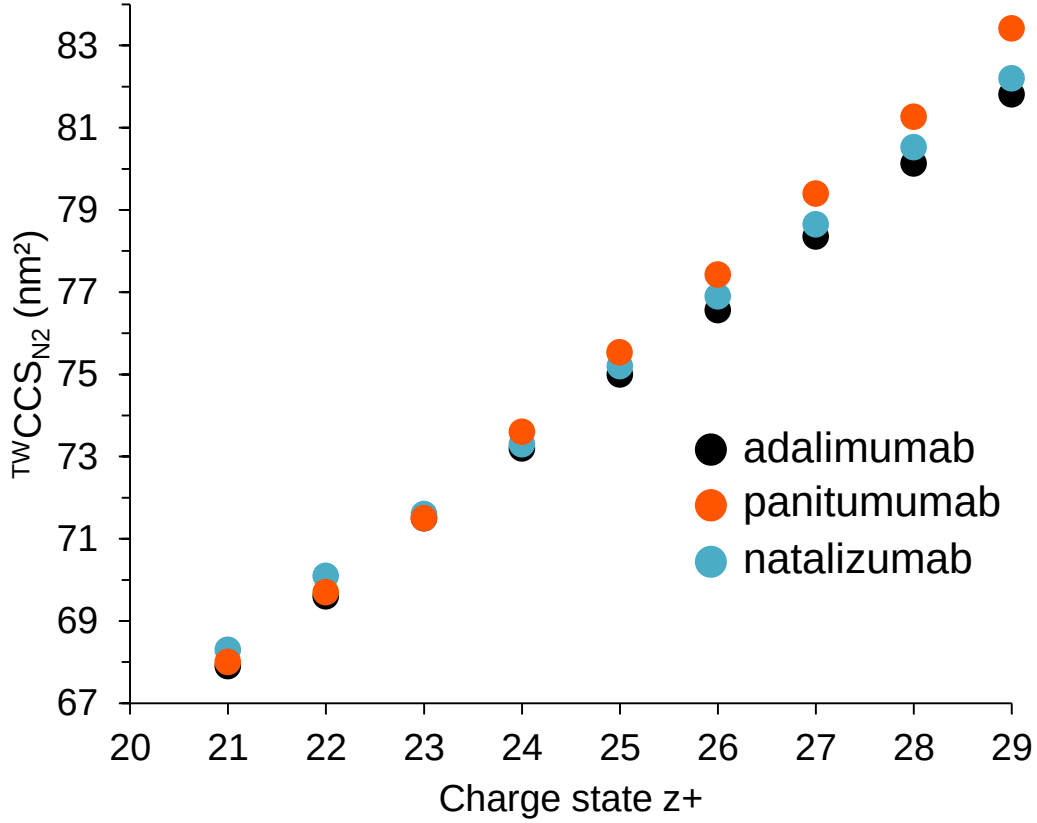
a 2D representation of a 3D structure !

Hilton and Benesch, 2012 J R Soc Interface
D'Atri et al, 2017 J Sep Sci

CCS measurements are often not enough to distinguish conformers

- Especially true for mAb-based products due to
  - Inherent mAb conformational heterogeneity → flexibility and broad ATD peaks
  - Low 1st generation IMS cell resolutions

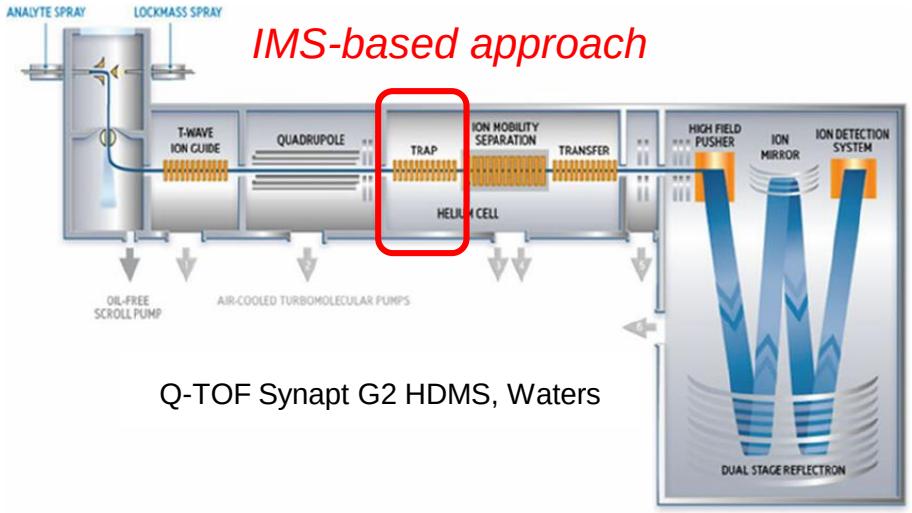




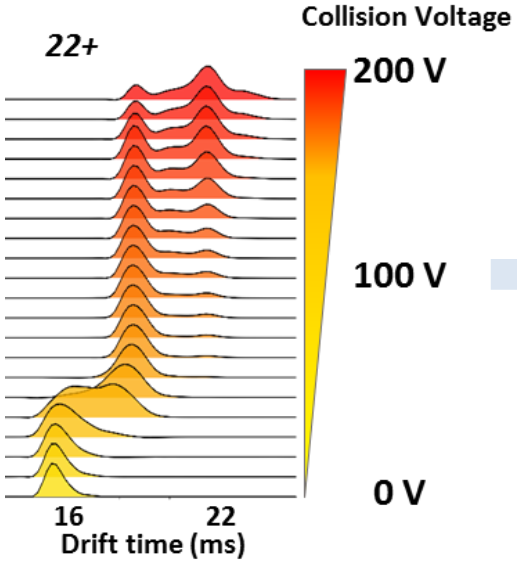
- No ATD separation
- CCS differences fall within the uncertainty of the technique (ΔCCS<2%)

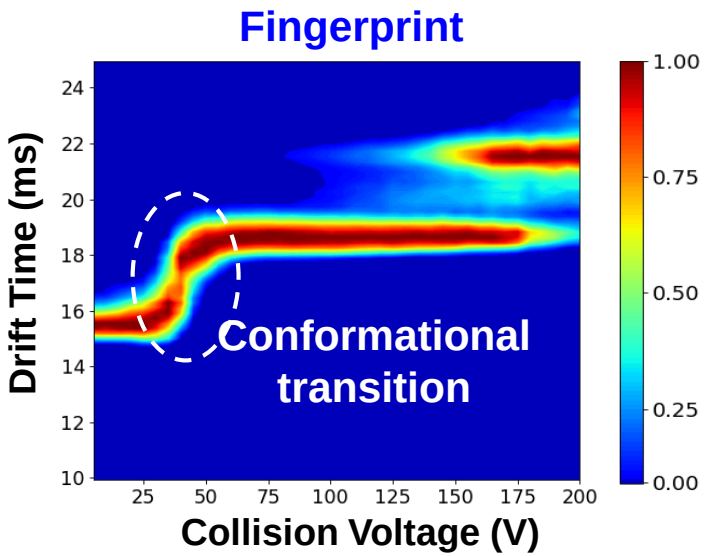
Botzanowski et al., Anal Chem. 2020;92(13):8827-8835

Alternative IMS-MS approaches to circumvent lack of resolution of IMS cells:
Collision Induced Unfolding - CIU



Q-TOF Synapt G2 HDMS, Waters





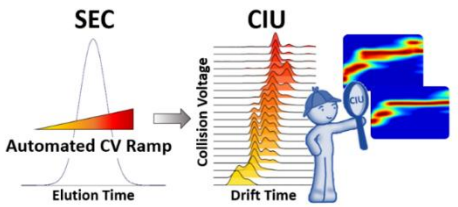
- Trap collision voltages increased
- Ions collide with the trap gas = **Activation**

CIU = Unfolding patterns

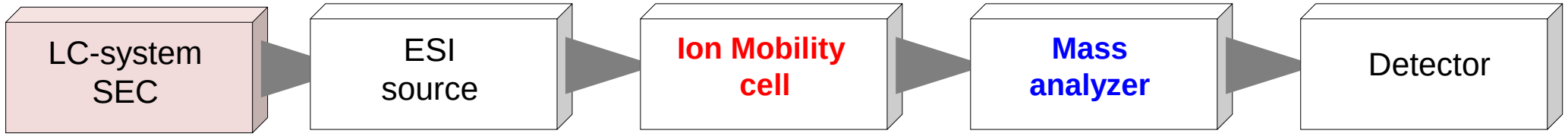
*CIUSuite 2 software: Polasky et al., *Anal Chem*, 2019

Rabuck JN et al. Anal Chem. 2013, 85(15):6995.
Niu S et al. Curr Opin Chem Biol. 2013, 7(5):809.

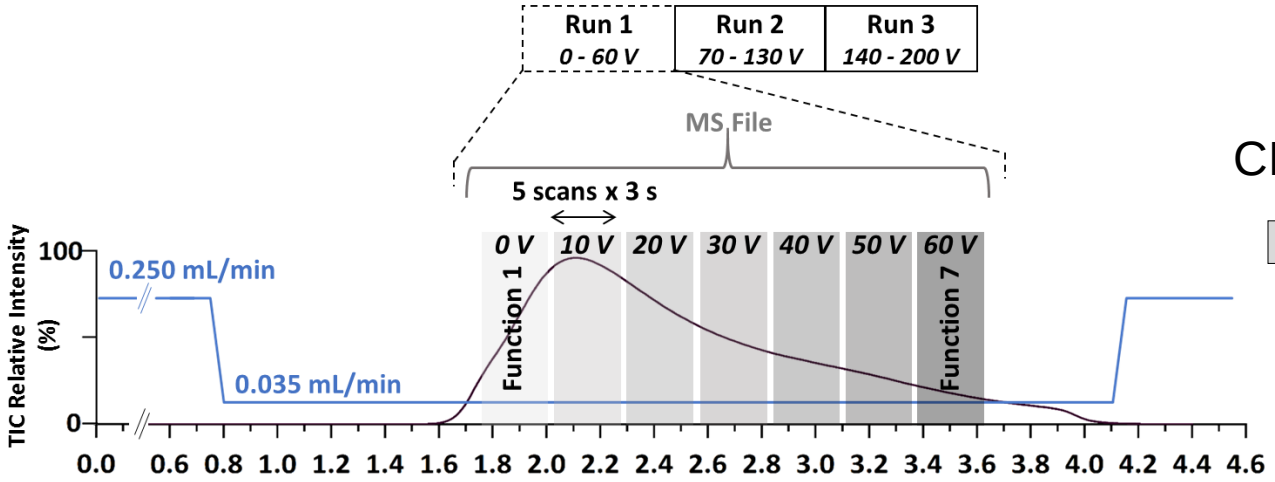
Alternative IMS-MS approaches to circumvent lack of resolution of IMS cells: Collision Induced Unfolding - CIU



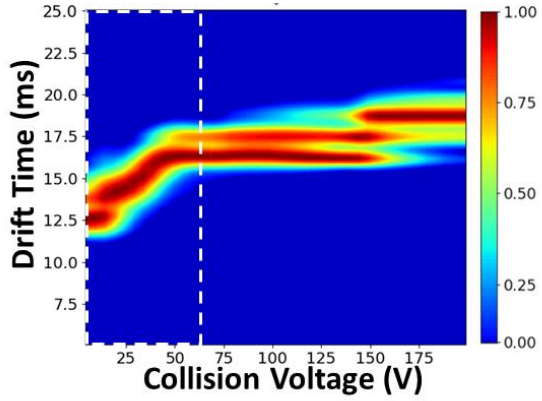
SEC-CIU-IMS: a fully automated process from sample preparation to data interpretation



Instrumental setting optimizations



CIUSuite 2



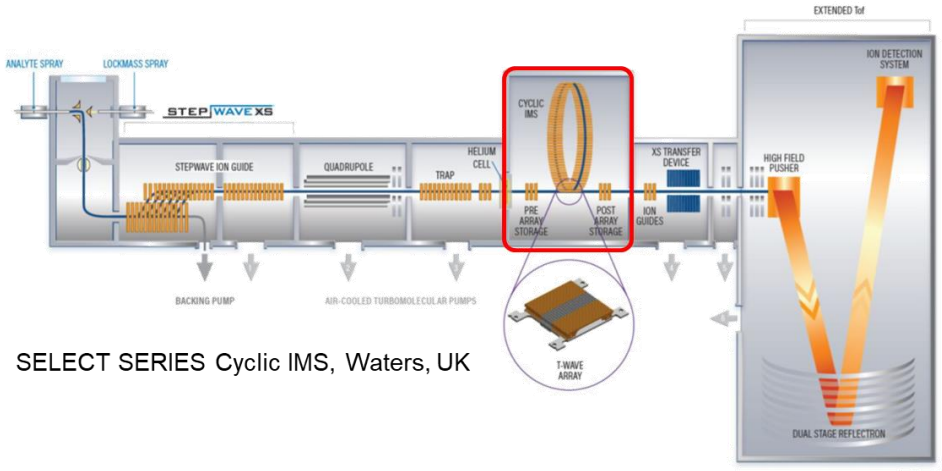
45 min
(1 mAb in triplicate)

Fast and efficient online desalting
+ Overall time process divided by 3

Deslignière et al., *Anal Chem*, 2020

Alternative IMS-MS approaches to circumvent lack of resolution of IMS cells: high resolution IMS-MS

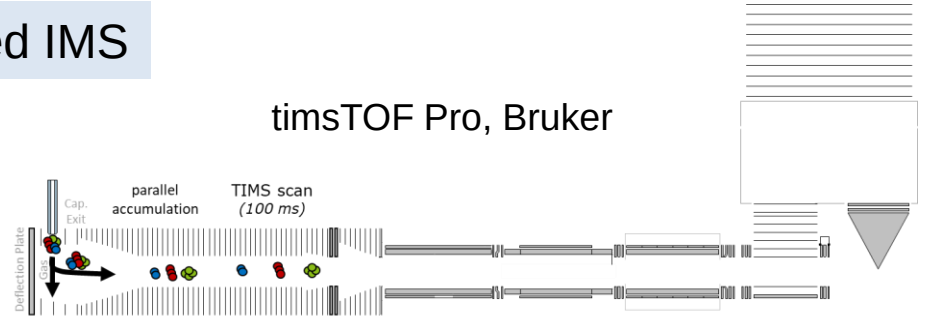
Cyclic IMS

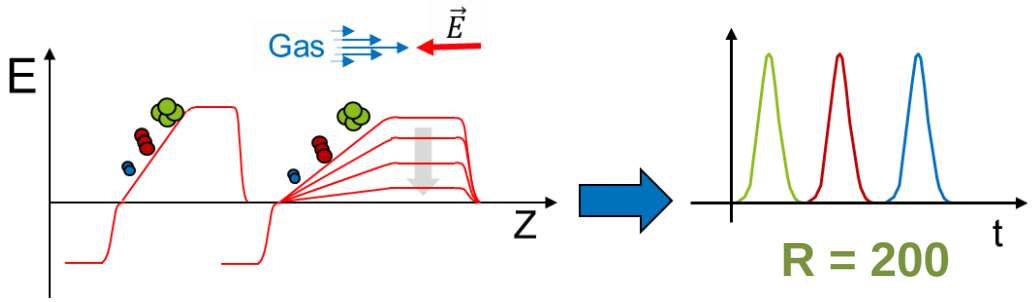


SELECT SERIES Cyclic IMS, Waters, UK

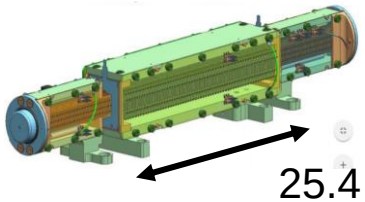
Trapped IMS

timsTOF Pro, Bruker



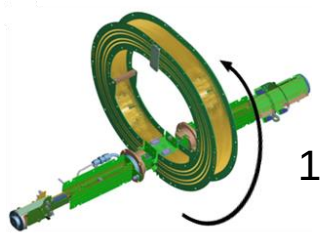


Linear TWIMS cell
(Synapt G2 HDMS)



R = 40 (Ω/ΔΩ)

Cyclic TWIMS cell



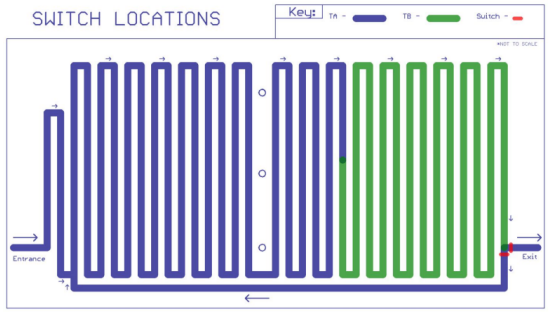
1 pass = 98 cm

$R_{1\ pass}$= 78

$R_{16\ passes}$ = 350

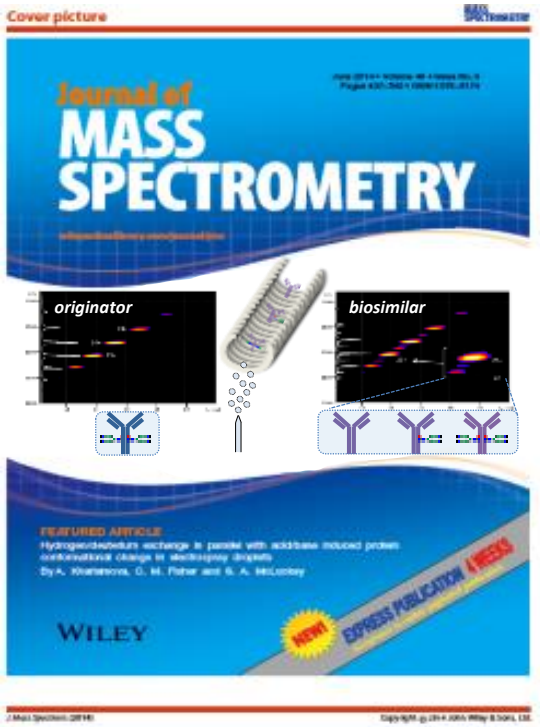
SLIM device
(Structures for Lossless Ion Manipulations)

13.5m separation in T-Waves (serpentines)



Giles et al., Anal Chem 2019

Nagy et al., Anal Chem 2020



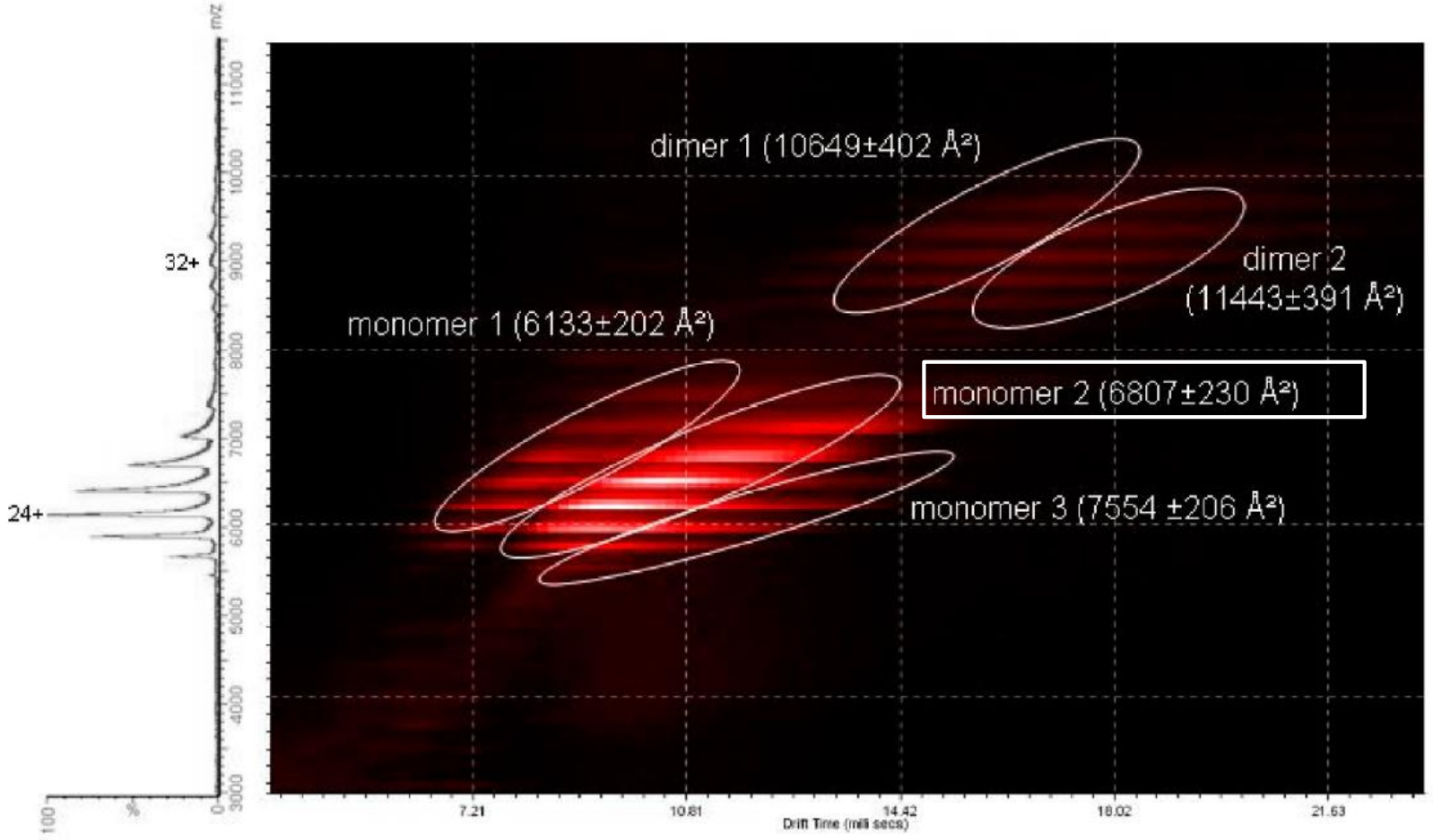
Topic 1

IMS-MS to monitor conformational heterogeneity/homogeneity

- Qualitative IMS-MS data interpretation for batch to batch comparison
- For S-S bridge assessment

Native IMS-MS for global conformational characterization of mAb-products

- IMS-MS analysis of a commercial rhGH-specific antibody



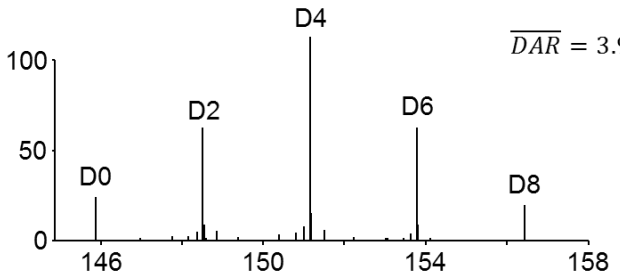
80% of monomer 2

- 3 monomer conformations (CCSs: 6100 - 7600 Å²)
- 2 dimers (CCSs: 10600–11500 Å²)

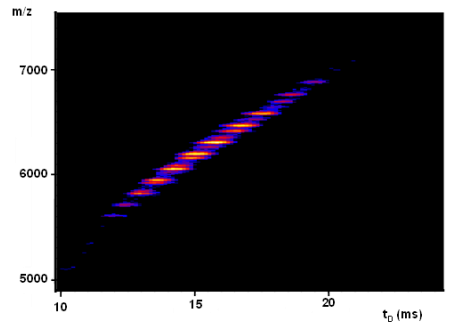
Pritchard et al. Anal. Chem. 2013, 85, 7205

Native IMS-MS to tackle conformational heterogeneity of drug binding within ADCs

Brentuximab Vedotin (Cys-ADC)

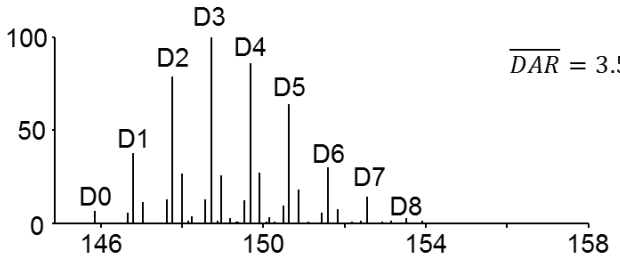


$\overline{DAR} = 3.9 \pm 0.1$

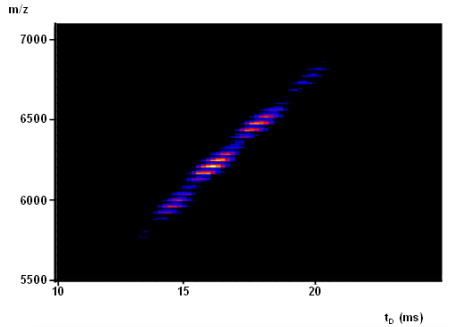


Heterogeneous +

T-DM1 (Lys-ADC)

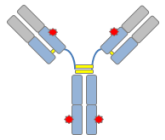


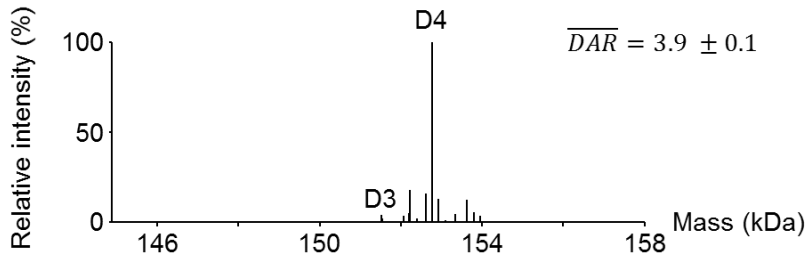
$\overline{DAR} = 3.5 \pm 0.1$



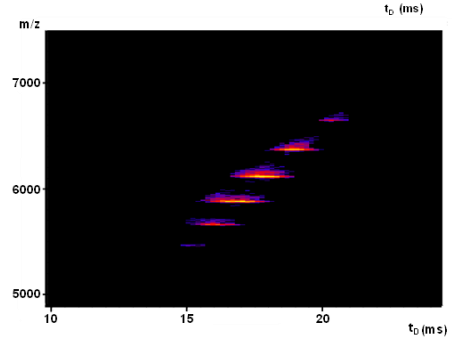
Heterogeneous ++

Site-specific DAR4





$\overline{DAR} = 3.9 \pm 0.1$



Homogeneous ++

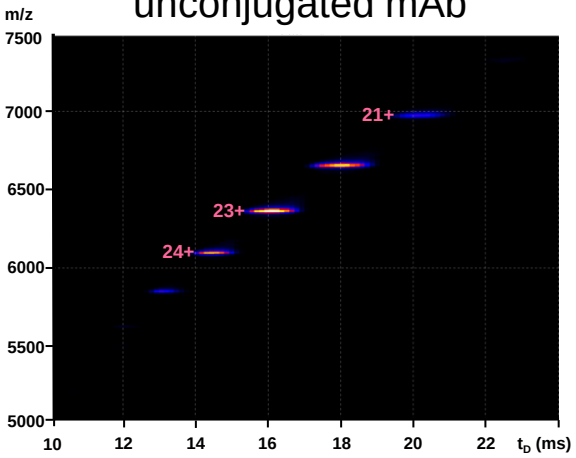
- Site-specific ADCs are more homogeneous than 1st generation ADCs.
- IMS-MS can provide a snapshot of ADC homogeneity/heterogeneity without extensive data interpretation.

Botzanowski et al. mAbs. 2017, 9,801.

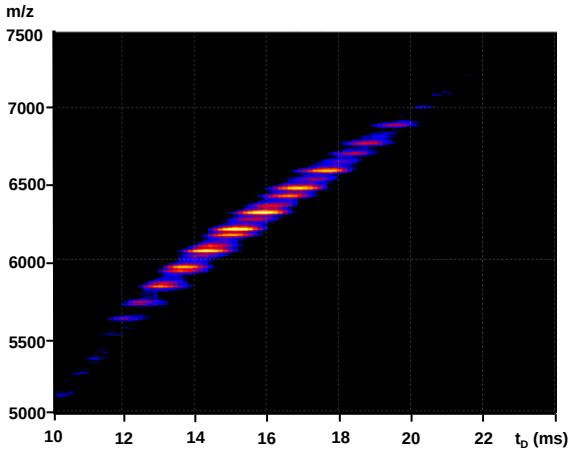
Cys-ADC: Native IMS-MS of intact cysteine-linked ADC

- Native IMS-MS provides a snapshot of Adcetris heterogeneity of conjugation

deglycosylated unconjugated mAb



deglycosylated Adcetris

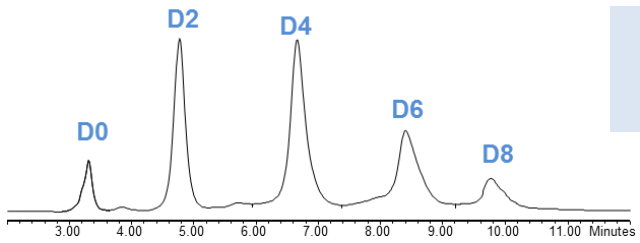


Synapt G2, 3 µM,
$AcONH_4$ 150 mM pH7.4
Vc = 80v, Pi = 6mbar, Bias 25,
WH = 40 V, WV = 923m/s,
He = 130 mL/min, N_2 = 45mL/min

Extraction and Integration of Arrival Time Distribution (ATD) of each drug load species

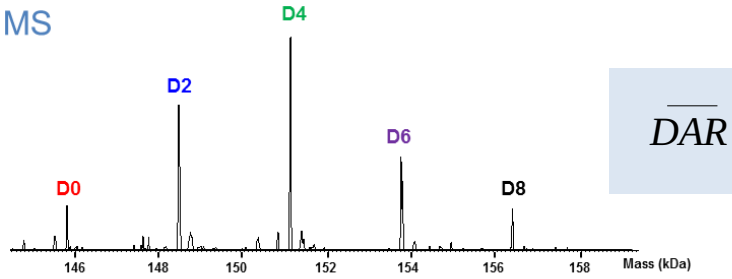
- CQAs can also be obtained by native IMS-MS

HIC



$\overline{DAR}$ = 4.0

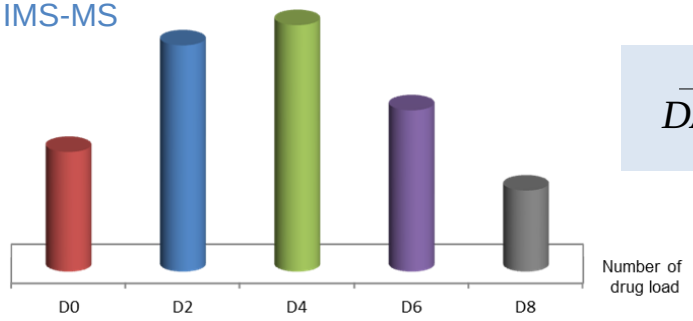
Native MS



$\overline{DAR}$ = 3.9 ± 0.1

n=3

Native IMS-MS



$\overline{DAR}$ = 3.7 ± 0.1

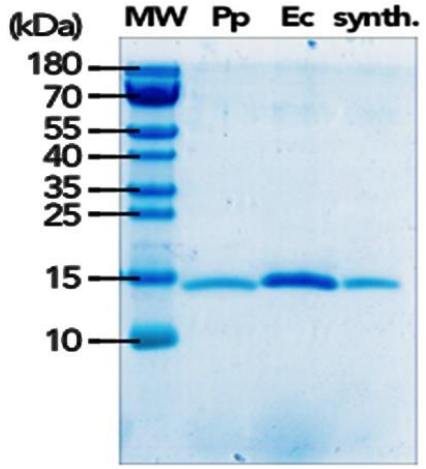
n=3

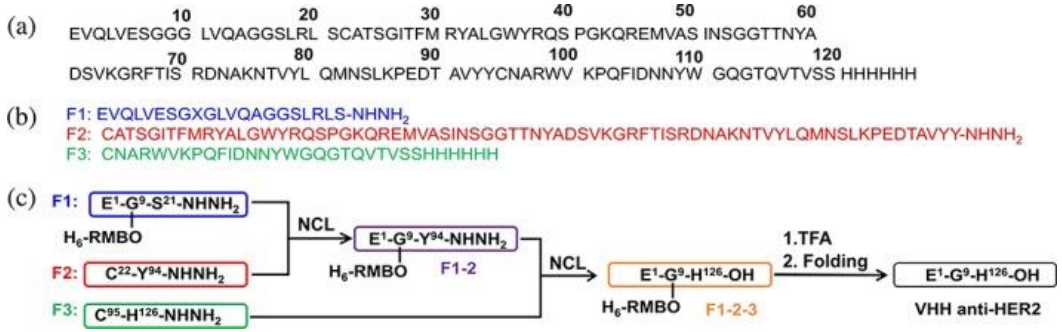
IMS-MS can provide also quantitative data for DLD distribution and average DAR calculations

Debaene F et al. Anal Chem. 2014, 86(21):10674-83.

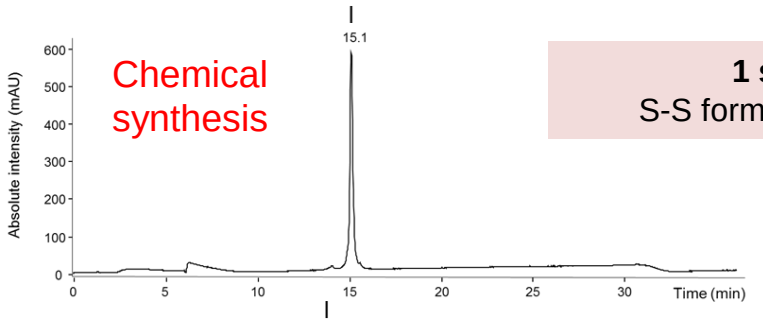
Native IMS-MS for batch comparability studies of VHH samples

- Comparability study of 3 different anti-HER2 VHH batches:
  - from *Pichia pastoris (*Pp*)*
  - from *Escherichia coli* (Ec)
  - from chemical synthesis (synth)

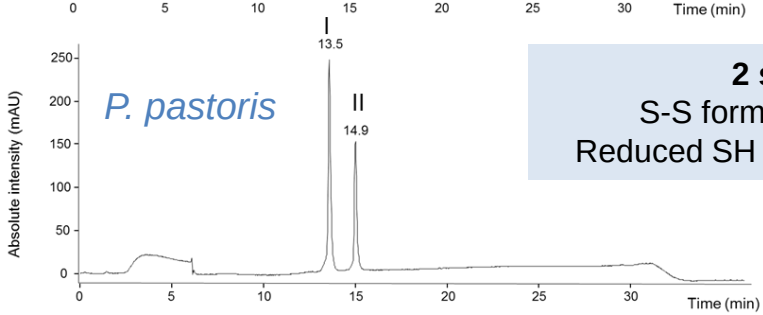




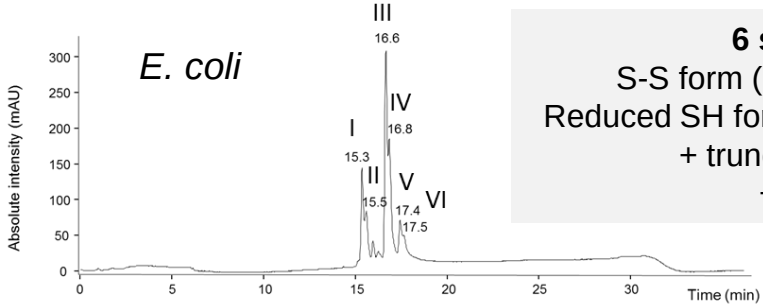
LC-MS to assess purity and homogeneity of S-S bond formation



1 species
S-S form (14 022.7 Da)



2 species
S-S form (14 166.9 Da)
Reduced SH form (14 168.9 Da)



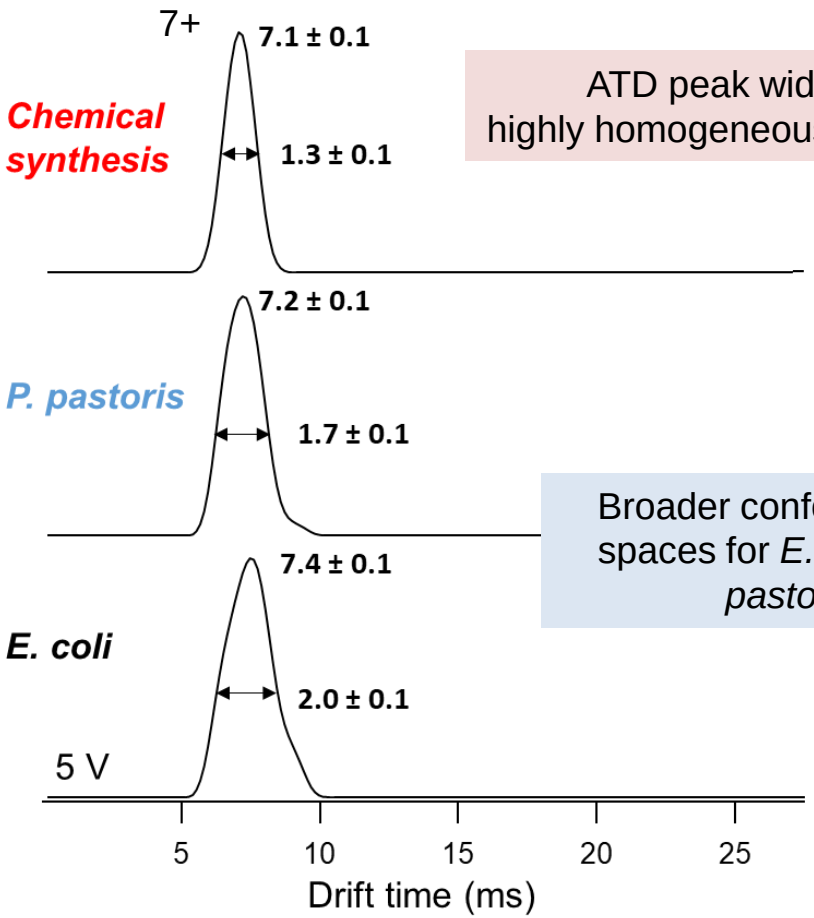
6 species
S-S form (14 153.9 Da = I)
Reduced SH form (14 155.9 Da = III)
+ truncated forms
+ fMet

Purity and homogeneity ranking from LC-MS:
Synth > *P. pastoris* > *E.coli*

Hartmann L et al. Protein Sci. 2019, 28(10):1865-1879.

Native IMS-MS for batch comparability studies of VHH samples

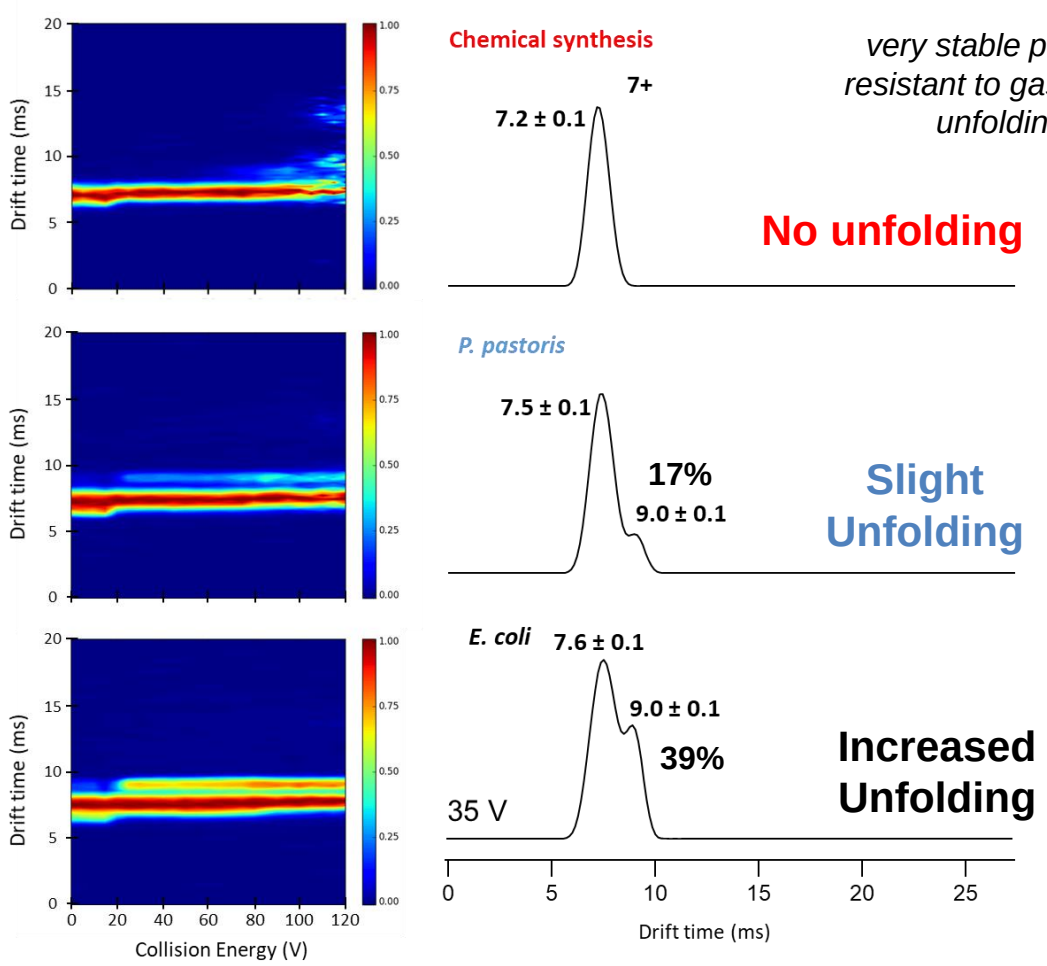
Native IMS-MS for conformational characterization of anti-HER2 VHH batches



ATD peak width reveals highly homogeneous synthetic VHH

Broader conformational spaces for *E.coli* and *P. pastoris*

CIU experiments for gas-phase conformational stability of anti-HER2 VHH batches



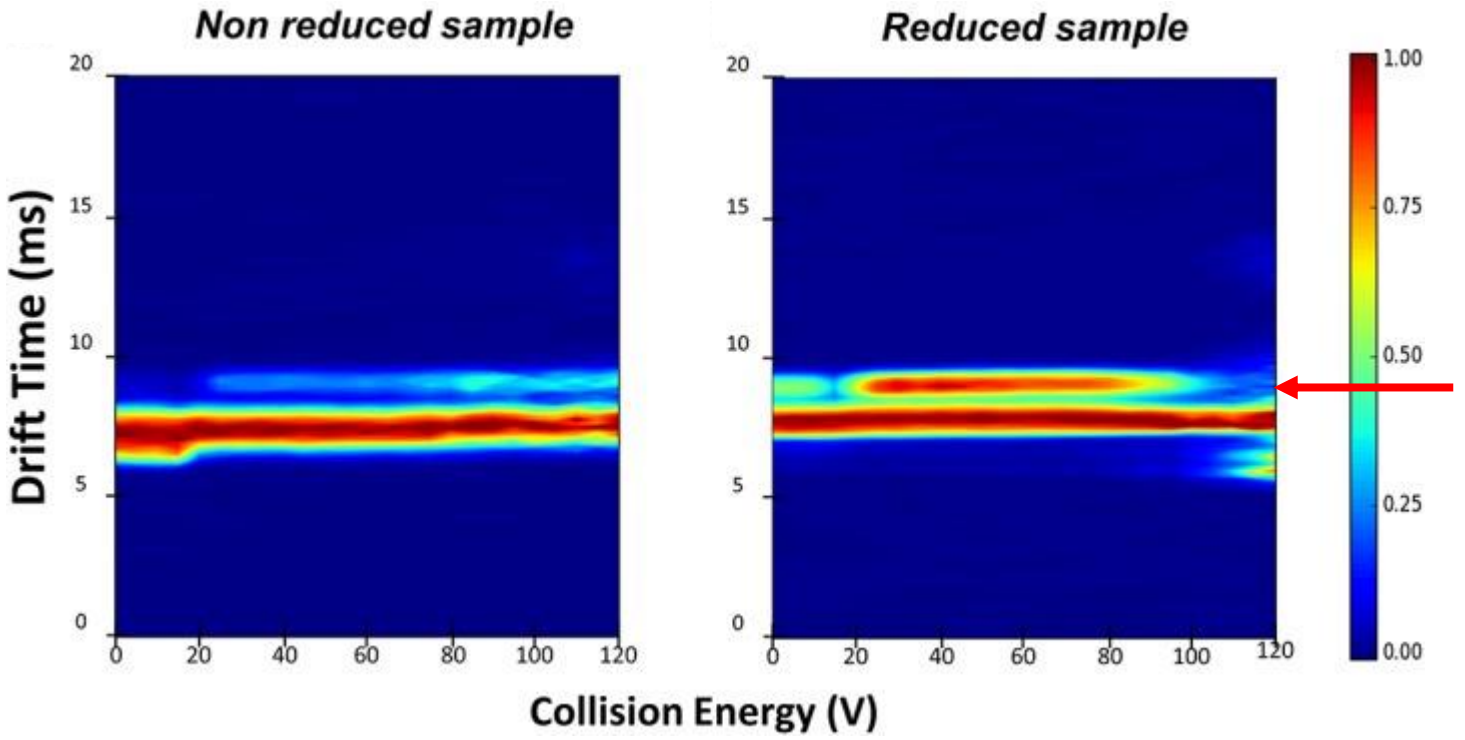
very stable product resistant to gas phase unfolding

Ranking of resistance towards gas phase unfolding:
Synth > *P. pastoris* > *E.coli*

Hartmann L et al. Protein Sci. 2019, 28(10):1865-1879.

Native IMS-MS for batch comparability studies of VHH samples

CIU experiments of P. pastoris +/- DTT reduction

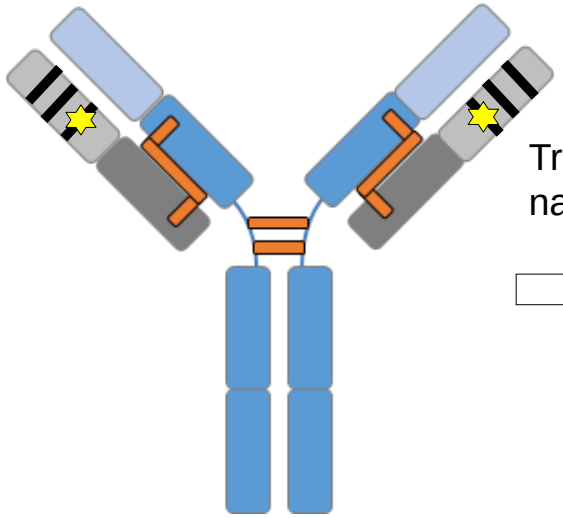


a stronger intensity of the extended conformation is observed after full reduction

Differences in gas phase behavior between chemical synthesis and *P. pastoris*/*E.coli* can be attributed to the presence of reduced VHH forms in both samples

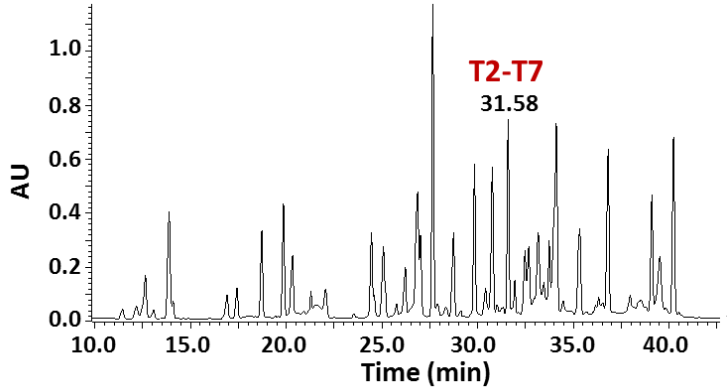
Hartmann L et al. Protein Sci. 2019, 28(10):1865-1879.

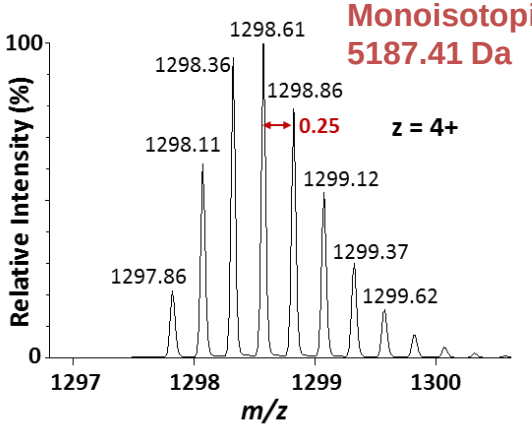
High Resolution cIMS-MS to Assign Additional Disulfide Bridge Pairing



Additional Cys in CDR3 of light chain in IgG4

Trypsin digestion
nanoLC-MS/MS





Identification of a dipeptide of the CDR3 of the light chain with 2 additional disulfide bonds involving Cys91/100/23/88

Positional isomer 1

23
VTITCR
91 100
FSGSGSGTDFTLTISSLQPEDVATYYCAGCGSGTADDVCAFGGGTK
88

Positional isomer 2

23
VTITCR
88 100
FSGSGSGTDFTLTISSLQPEDVATYYCAGCGSGTADDVCAFGGGTK
91

Positional isomer 3

23
VTITCR
88 91
FSGSGSGTDFTLTISSLQPEDVATYYCAGCGSGTADDVCAFGGGTK
100

Can IMS-MS provide separation of disulfide peptide structural isomers?

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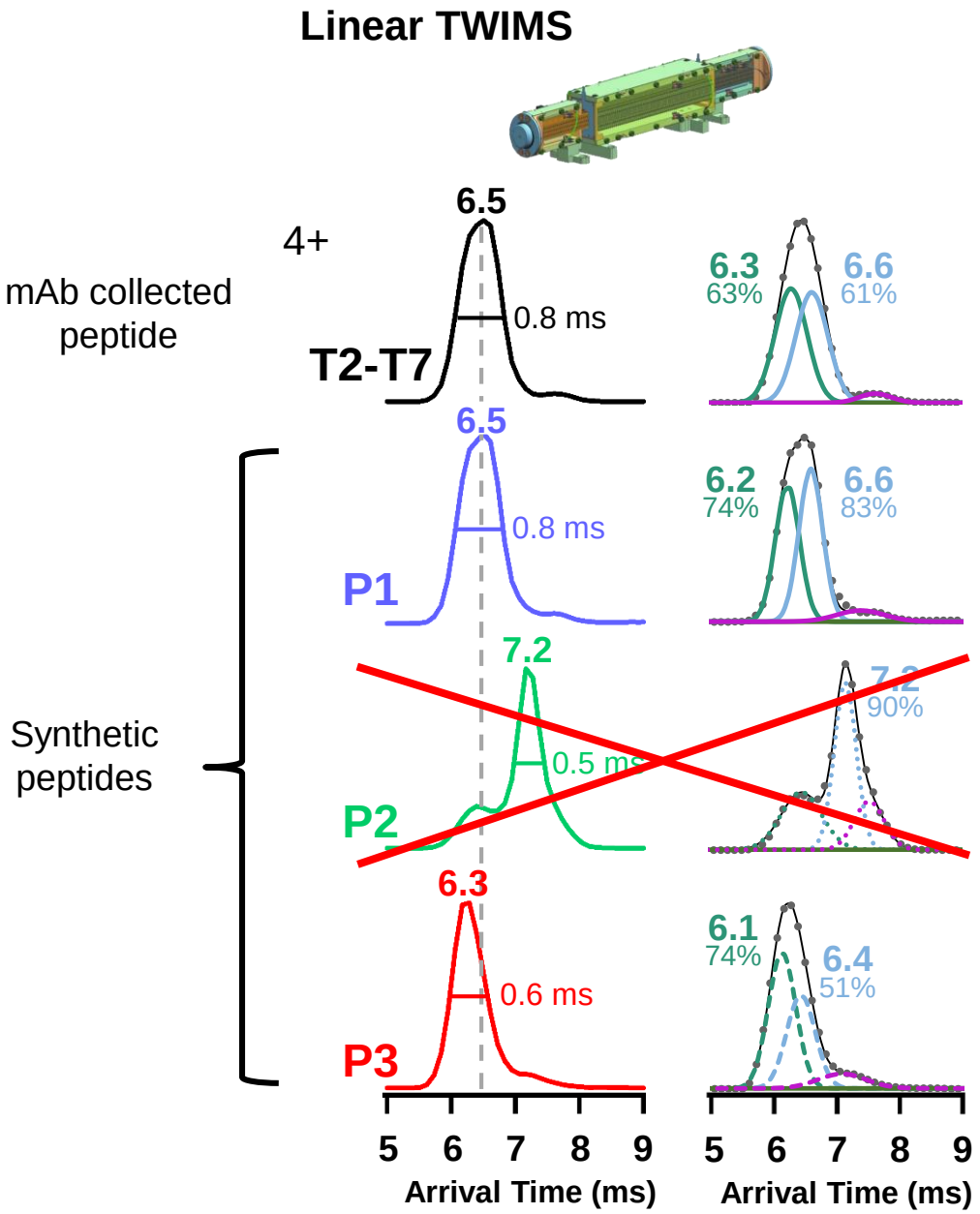
Classical IMS-MS to Assign Additional Disulfide Bridge Pairing

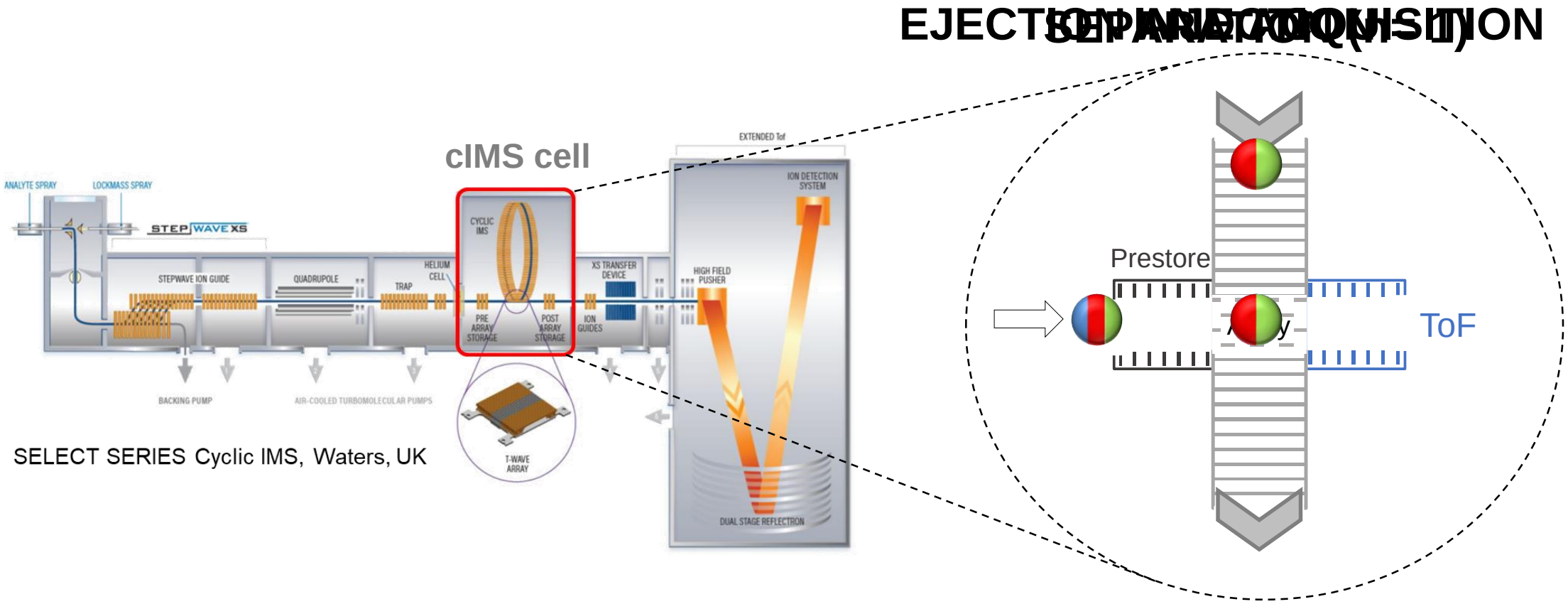


Based on arrival time and ATD profile, IMS-MS results might suggest that:

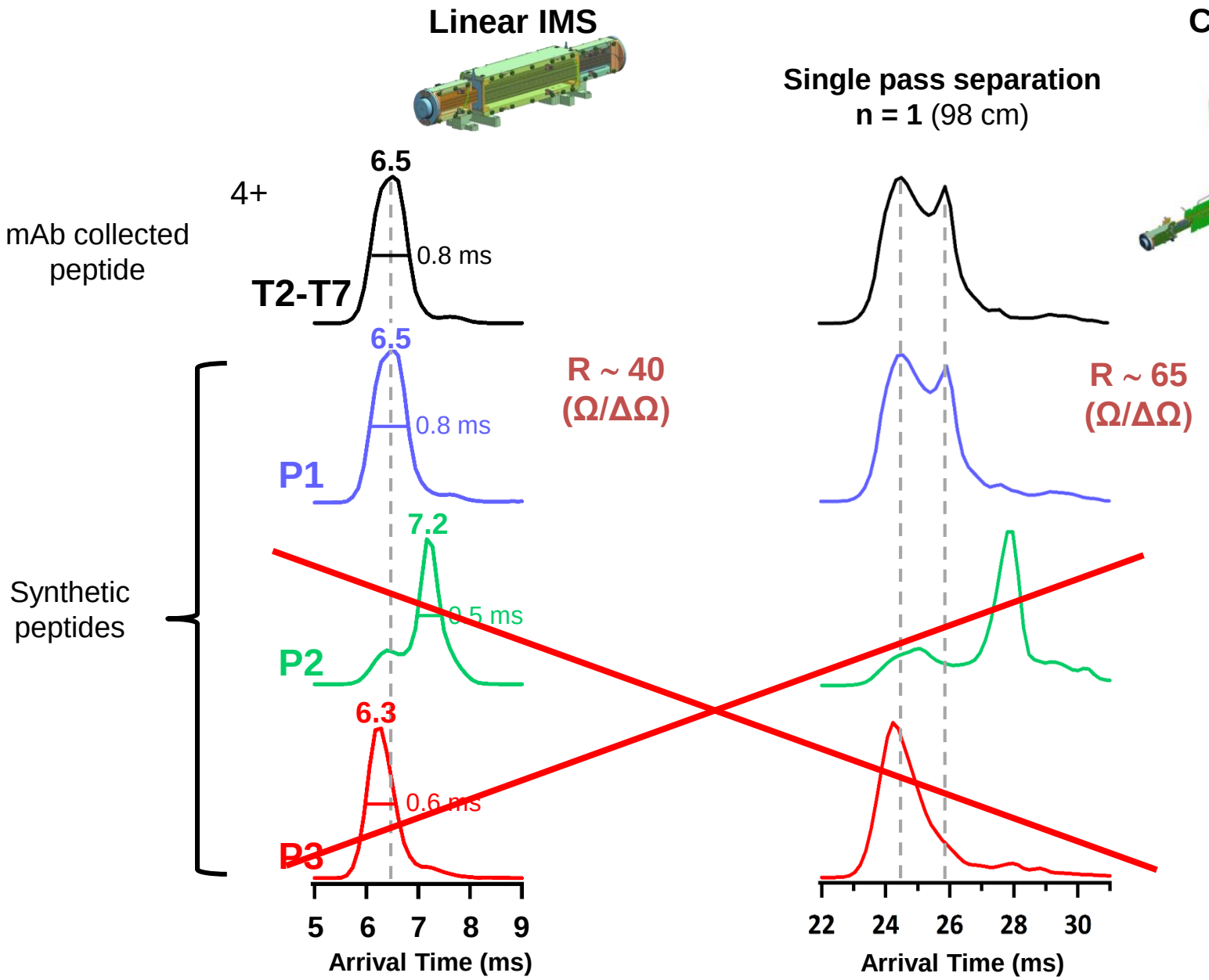
- **T2-T7 is similar to P1 or P3**
- P2 can be discarded
- P3 cannot be definitively ruled out (different peak shapes but very close ATDs + same number of conformers after Gaussian fitting)
- P1 and P3 also co-elute in rpLC

Can high-resolution IMS-MS provide better separation of disulfide peptide isomers?

Single pass cIMS separation



High Resolution cIMS-MS to Assign Additional Disulfide Bridge Pairing

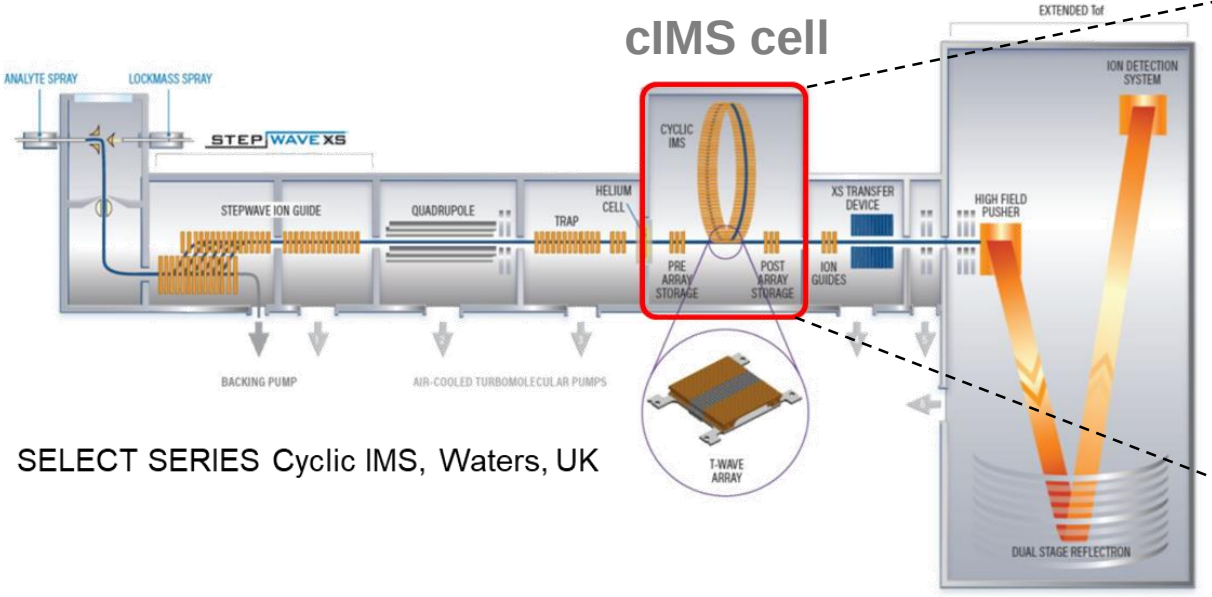


Based on arrival time and ATD profile, cIMS-MS results confirm that:

- **T2-T7 is similar to P1 or P3**
- P2 can be discarded
- P3 can be ruled out

Benefits of cIMS over linear TWIMS to detect multiple conformers are already clear after one pass

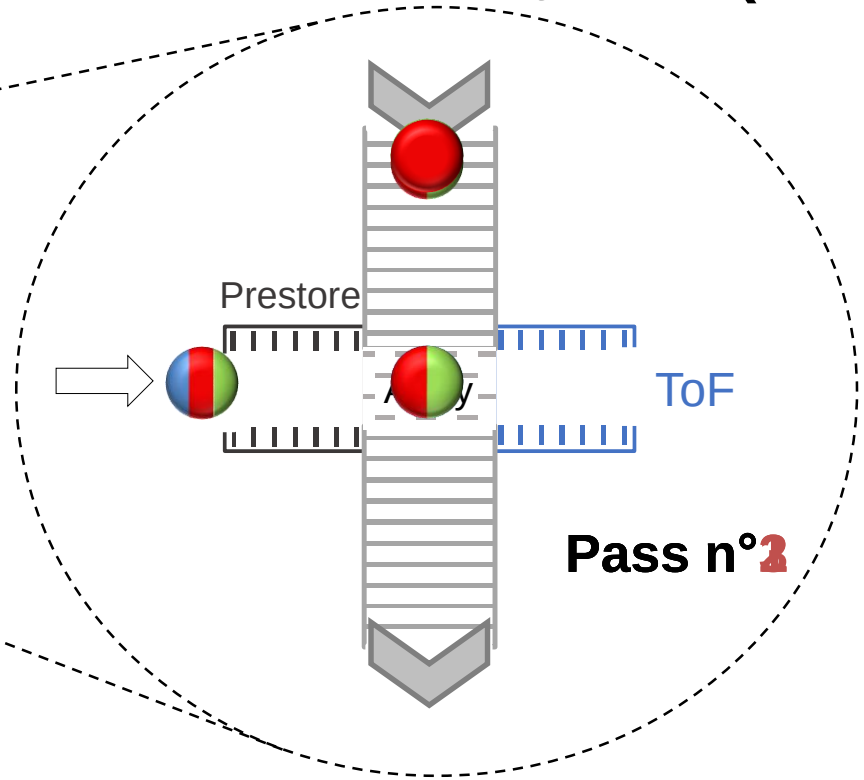
Multipass cIMS separation



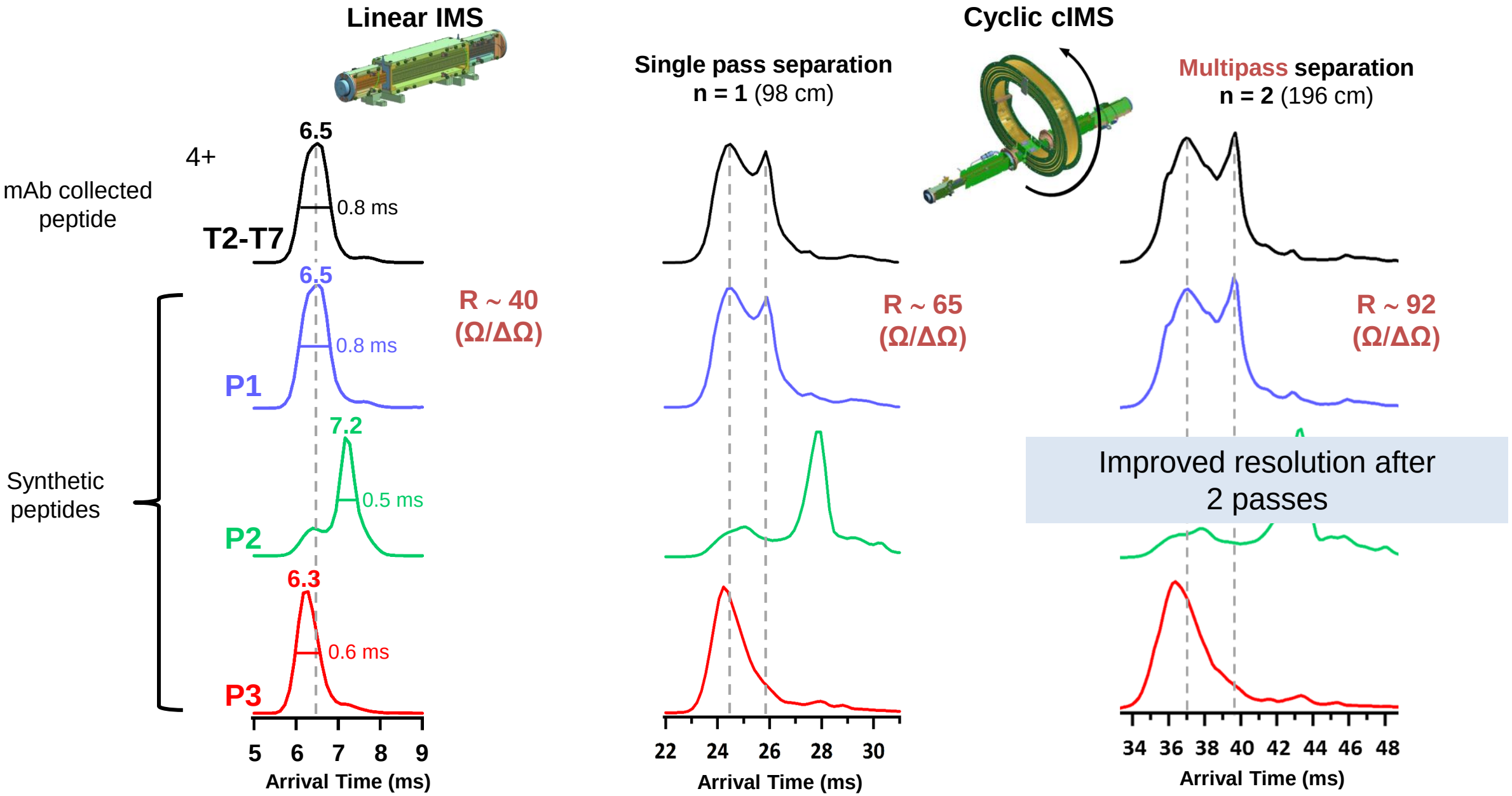
cIMS cell

SELECT SERIES Cyclic IMS, Waters, UK

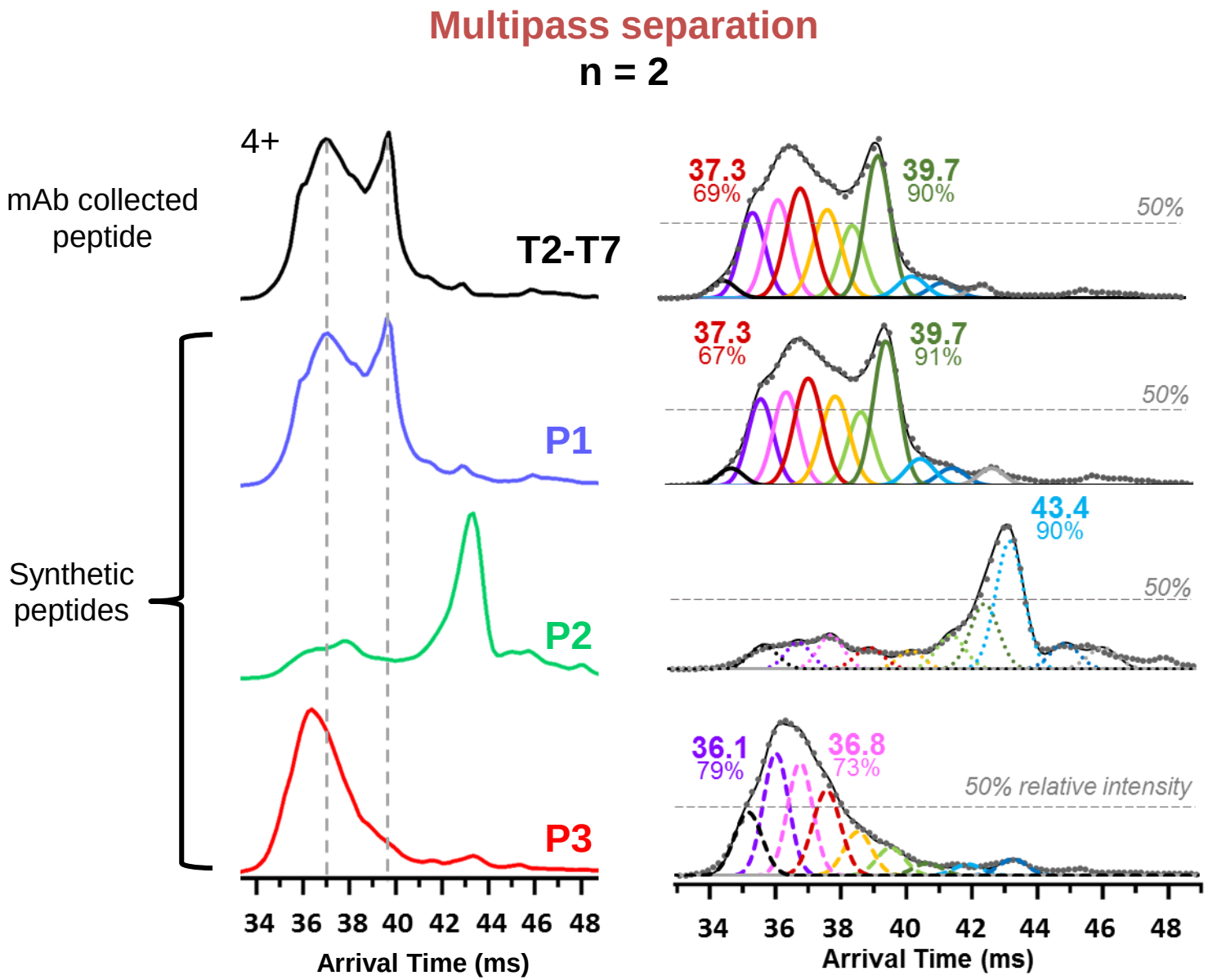
MULTIPASS SEPARATION (n = 3)



High Resolution cIMS-MS to Assign Additional Disulfide Bridge Pairing



High Resolution cIMS-MS to Assign Additional Disulfide Bridge Pairing



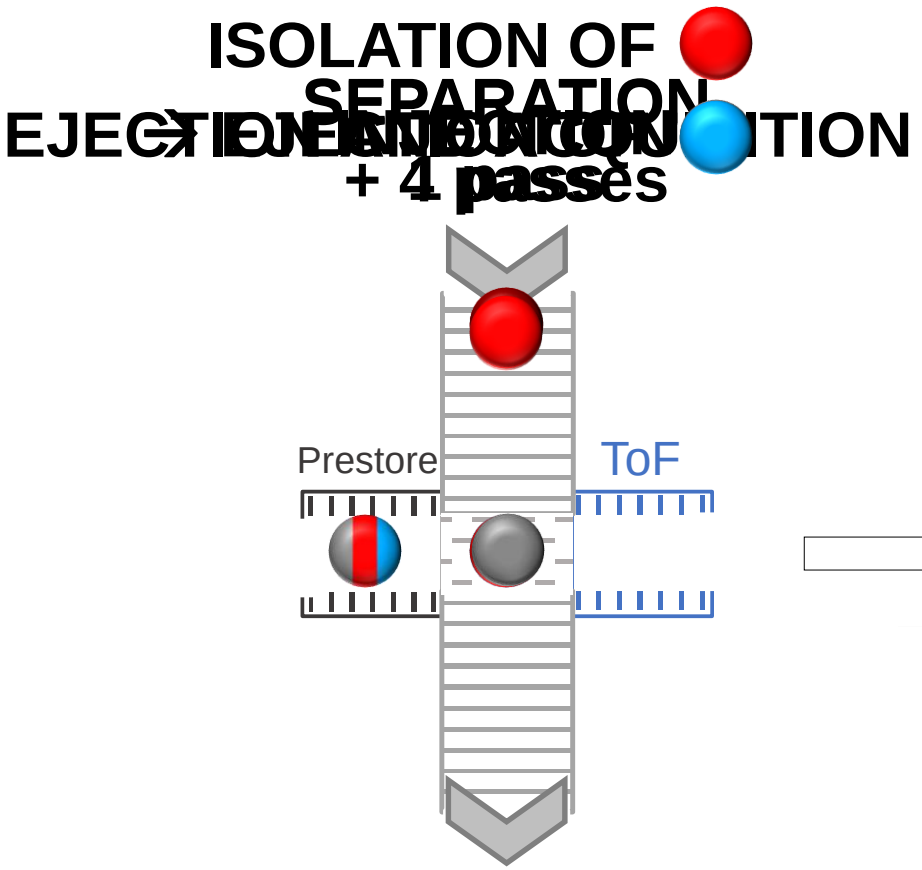
Based on arrival time and ATD profile, cIMS-MS results confirm that :

- **T2-T7 is similar to P1 or P3**
- P2 can be discarded
- P3 can be ruled out

Benefits of **multipass cIMS-MS** :
- Increased resolution
- Detection of new conformational features

Deslignière et al. JASMS 2021 online

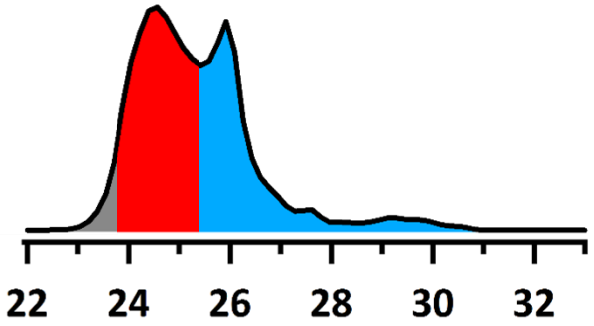
High Resolution cIMS-MS: Isolation experiment

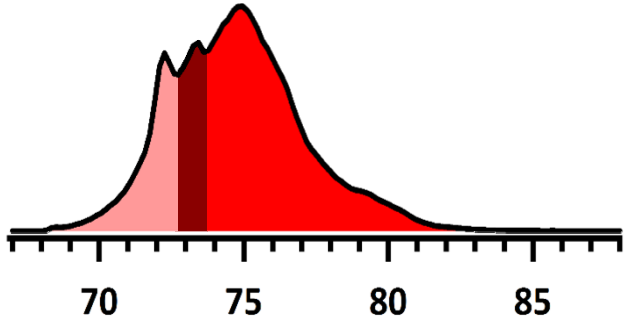


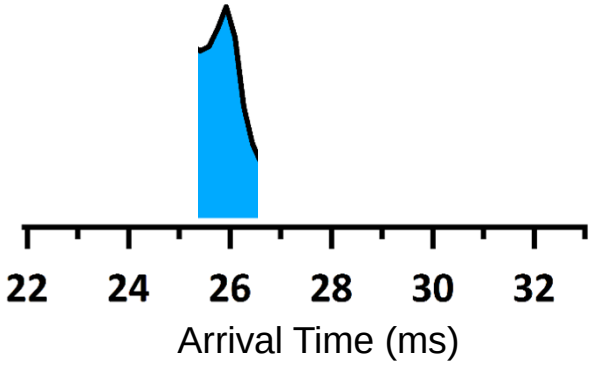
n = 1

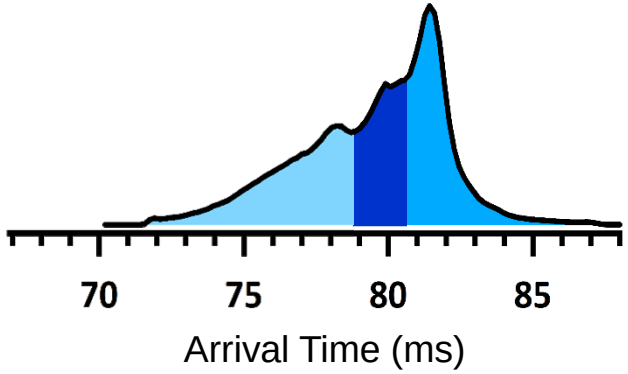
Total: n = 5

R ~ 145
(Ω/ΔΩ)



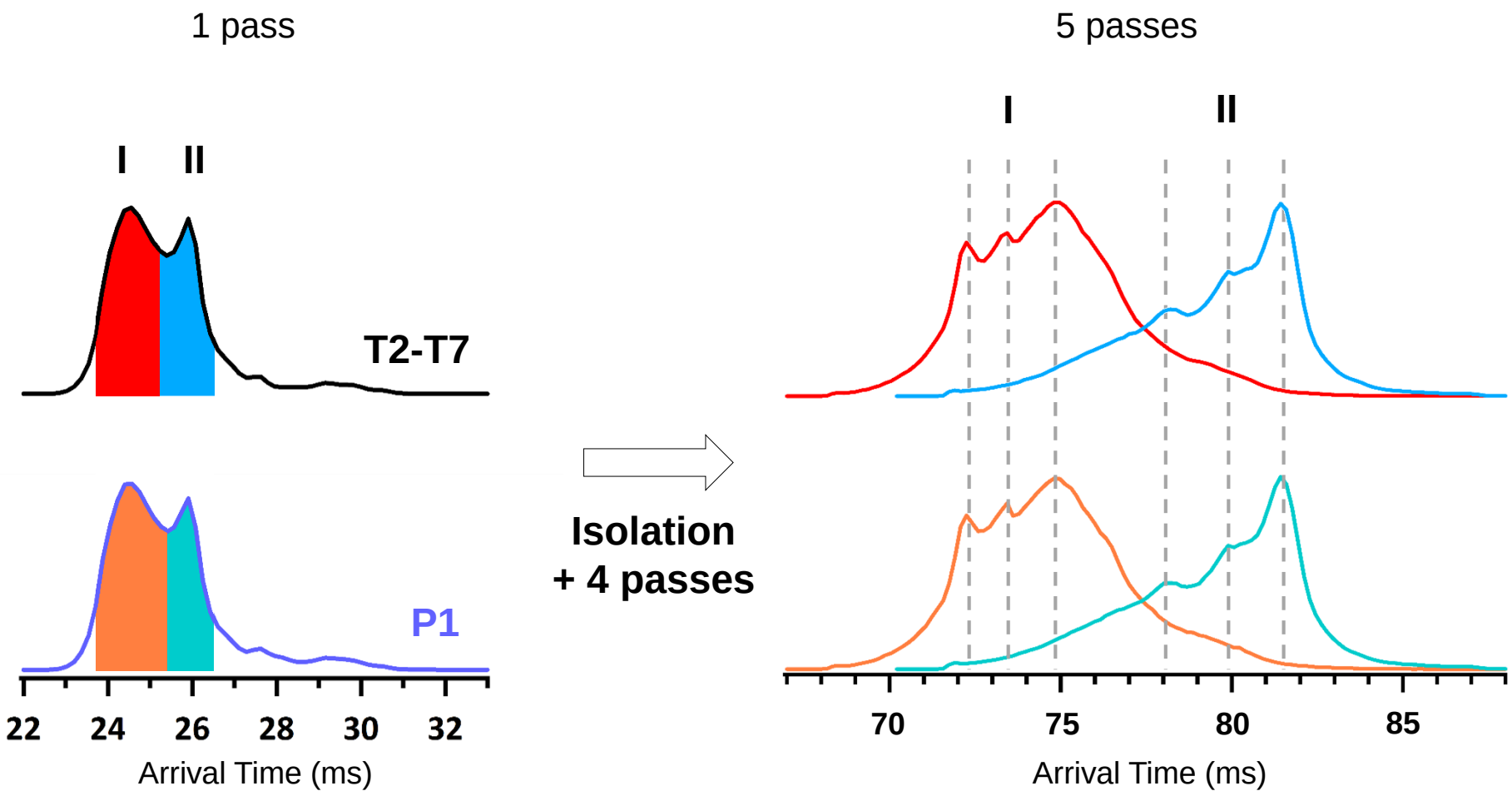






Deslignière et al. JASMS 2021 online

High Resolution cIMS-MS: Isolation experiment



After n=5, strictly identical isolated-ATD profiles were obtained for T2-T7 and P1, strengthening data obtained after n=2

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High Resolution cIMS-MS to Assign Additional Disulfide Bridge Pairing: Conclusions

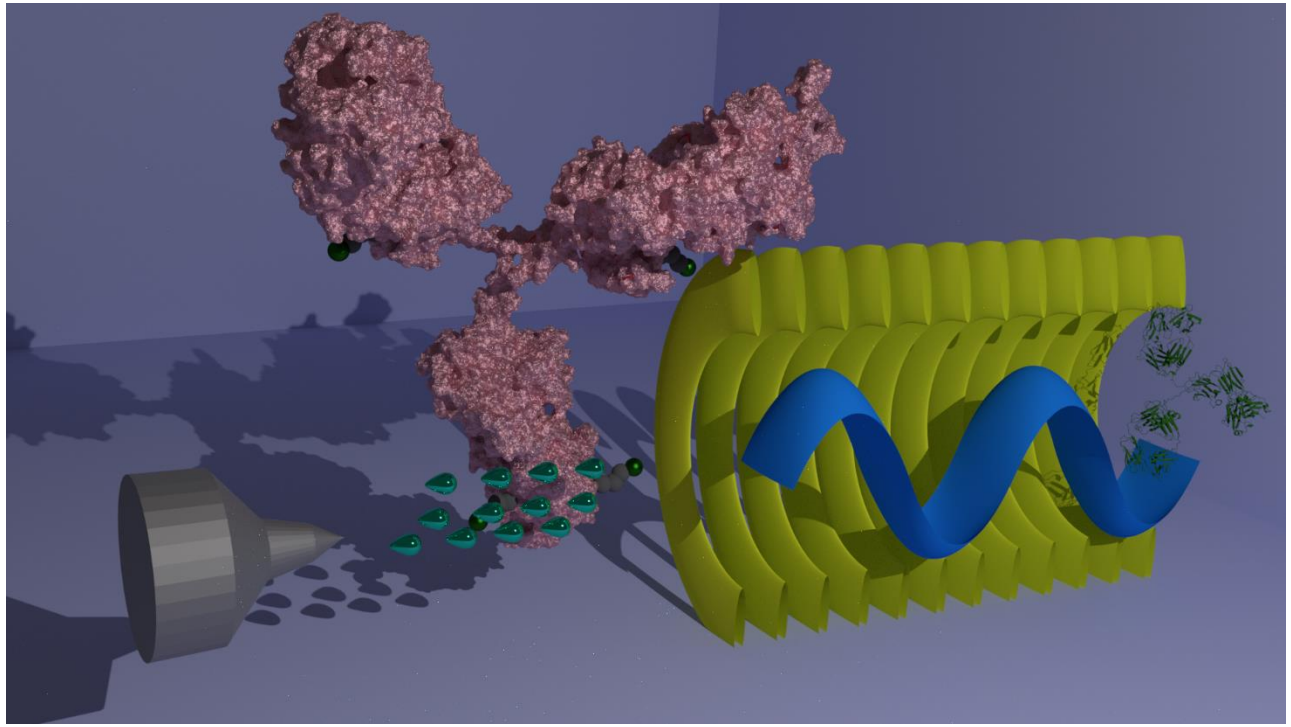
- **Linear TWIMS IMS-MS**: a high similarity between T2-T7 and P1 can be hypothesized, but P3 conformer cannot be definitively ruled out

- **High-resolution cyclic IMS-MS** allowed increased confidence in IMS profile to definitively conclude that T2-T7 disulfide bond connectivities resemble to P1 ones
  This is obvious after **one pass but even strengthened after multiple passes** through the cIMS device

1. A **comparison between ATDs** obtained on the cIMS device for the mAb-collected peptide and synthetic peptides provided **unequivocal determination of disulfide bridges.**

2. **Benefits of higher IMS resolutions** obtained on a cyclic instrument over linear TWIMS are clearly illustrated.

3. Potential of high-resolution IMS for **rapid and unambiguous profiling of disulfide pairings in biotherapeutics**



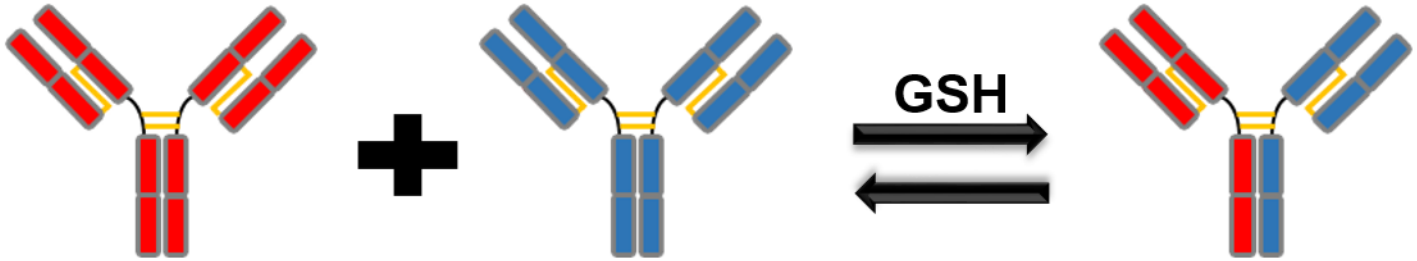
Topic 2

IMS-MS to monitor dynamics of conformational changes

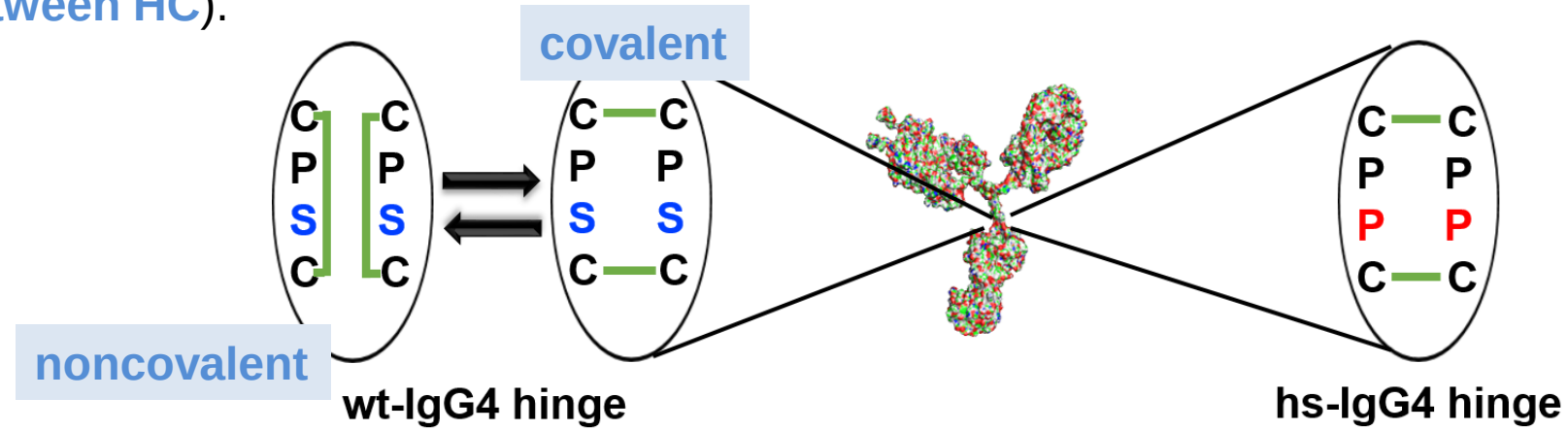
- Fab Arm Exchange of IgG4
- Drug conjugation for ADC formation

Dynamics and real-time native MS and IMS-MS to monitor bispecific mAb formation through Fab Arm Exchange (FAE)

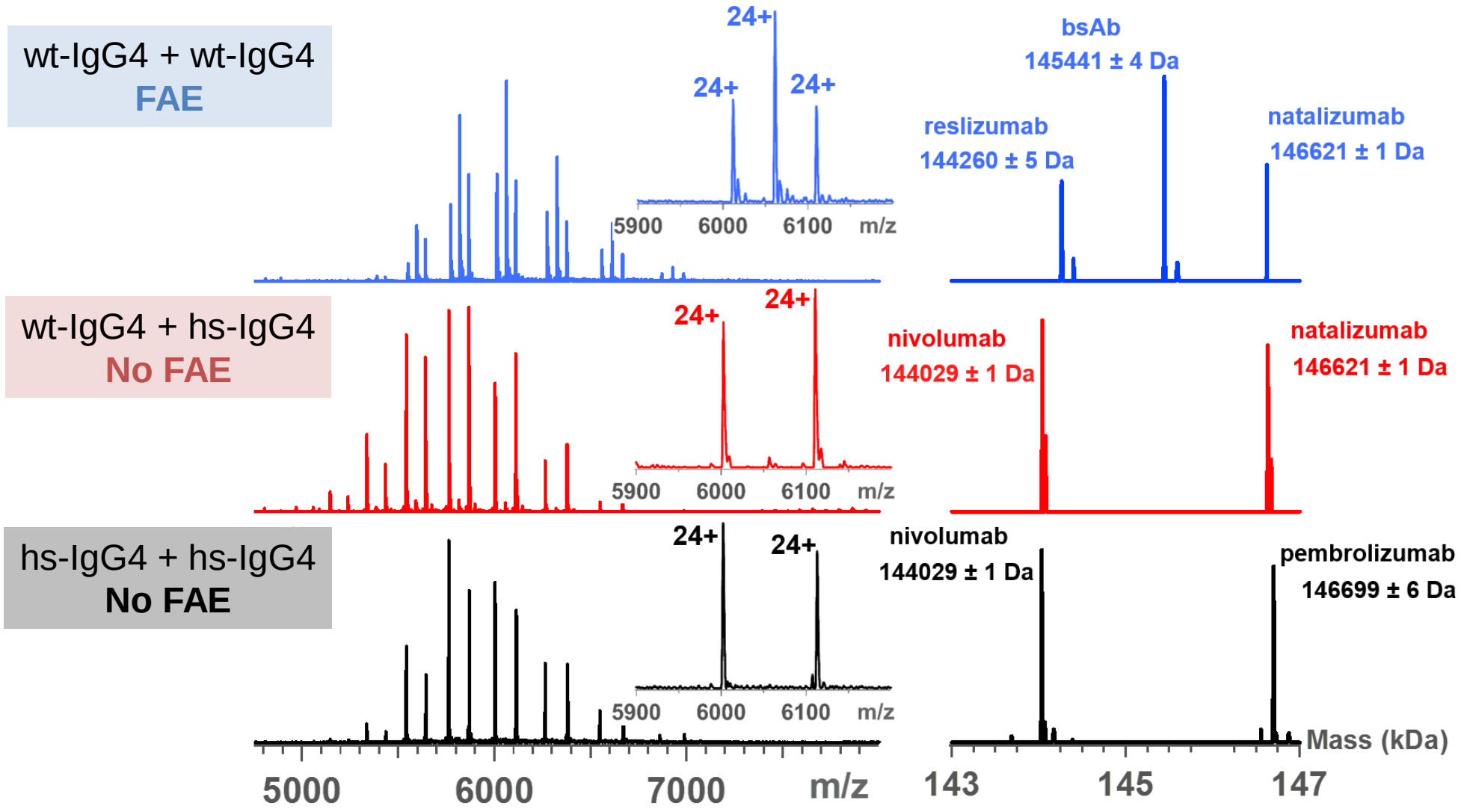
- In vivo, IgG4s can exchange half molecules by a dynamic process called **Fab-arm exchange (FAE).** In vitro, the FAE process can be mimicked by a reaction with glutathione (GSH)



- Serine 228 in IgG4 introduces flexibility in the core hinge region. Besides the usual disulfide bonds connecting two heavy chains (**covalent between HC**), intrachain disulfide bonds may form instead (**only non-covalent bonds between HC**).



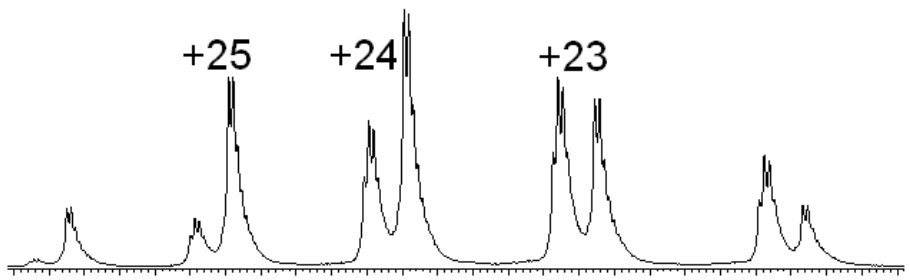
Native MS to monitor bispecific mAb formation after FAE

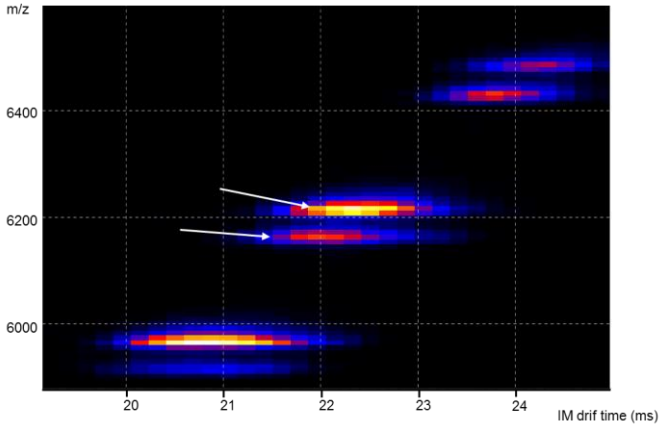


Debaene F. et al. Anal Chem. 2013 85, 20, 9785.
Hernandez-Alba et al. Anal Chem. 2018, 90(15):8865-8872.

Real-time native IMS-MS to monitor bispecific mAb formation

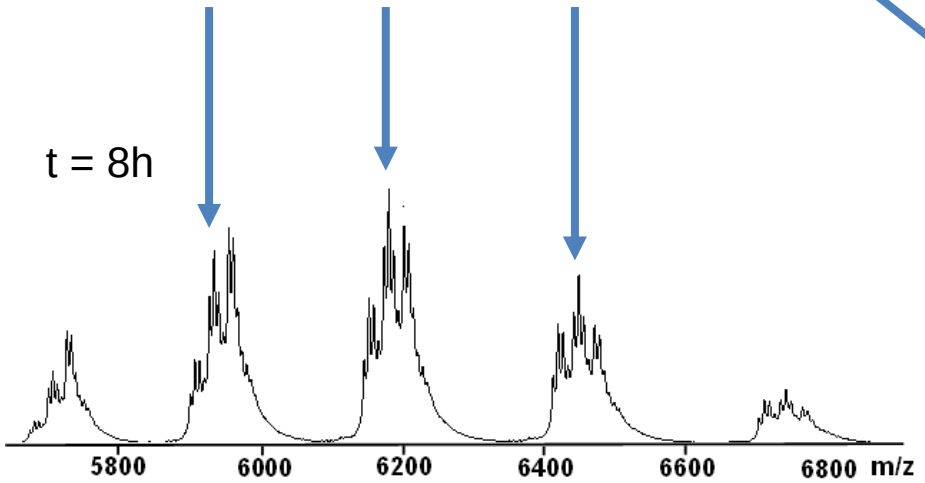
t = 0

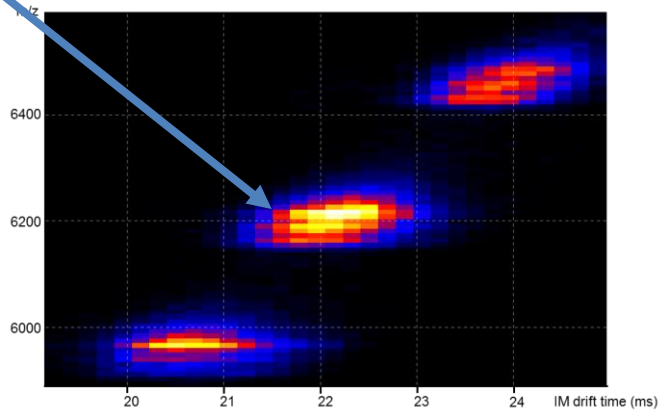


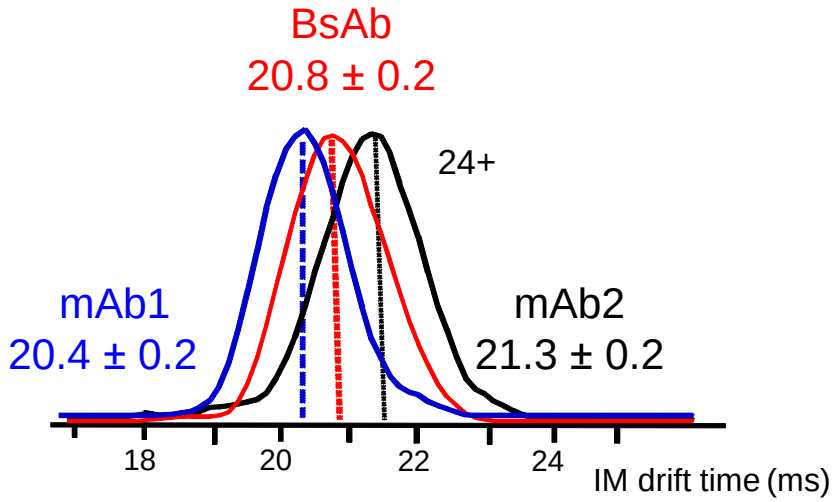


Real-time monitoring of bsAb formation

t = 8h



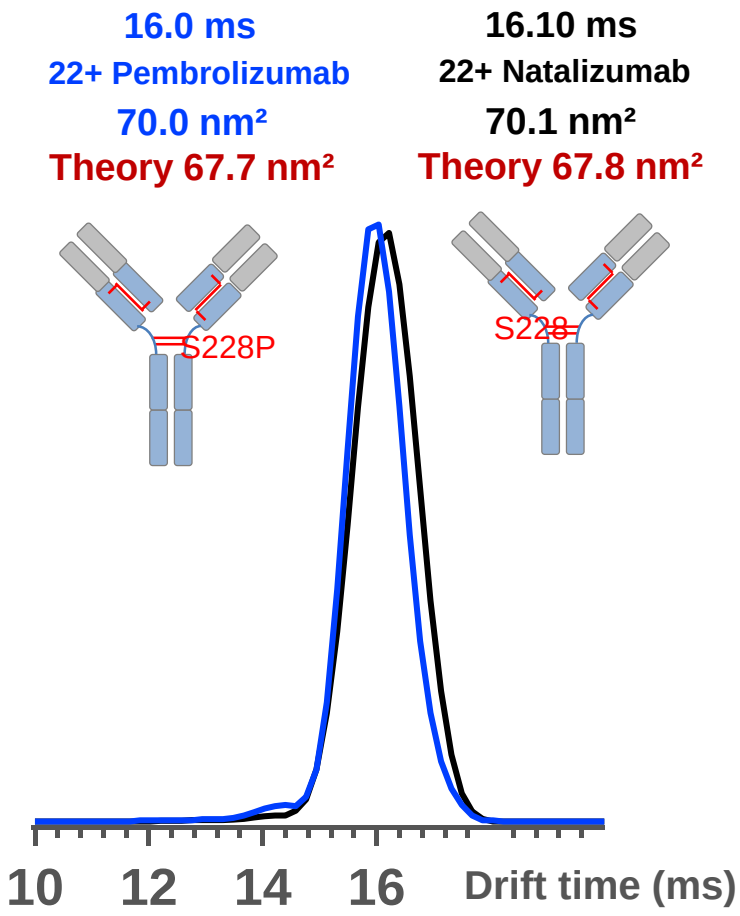




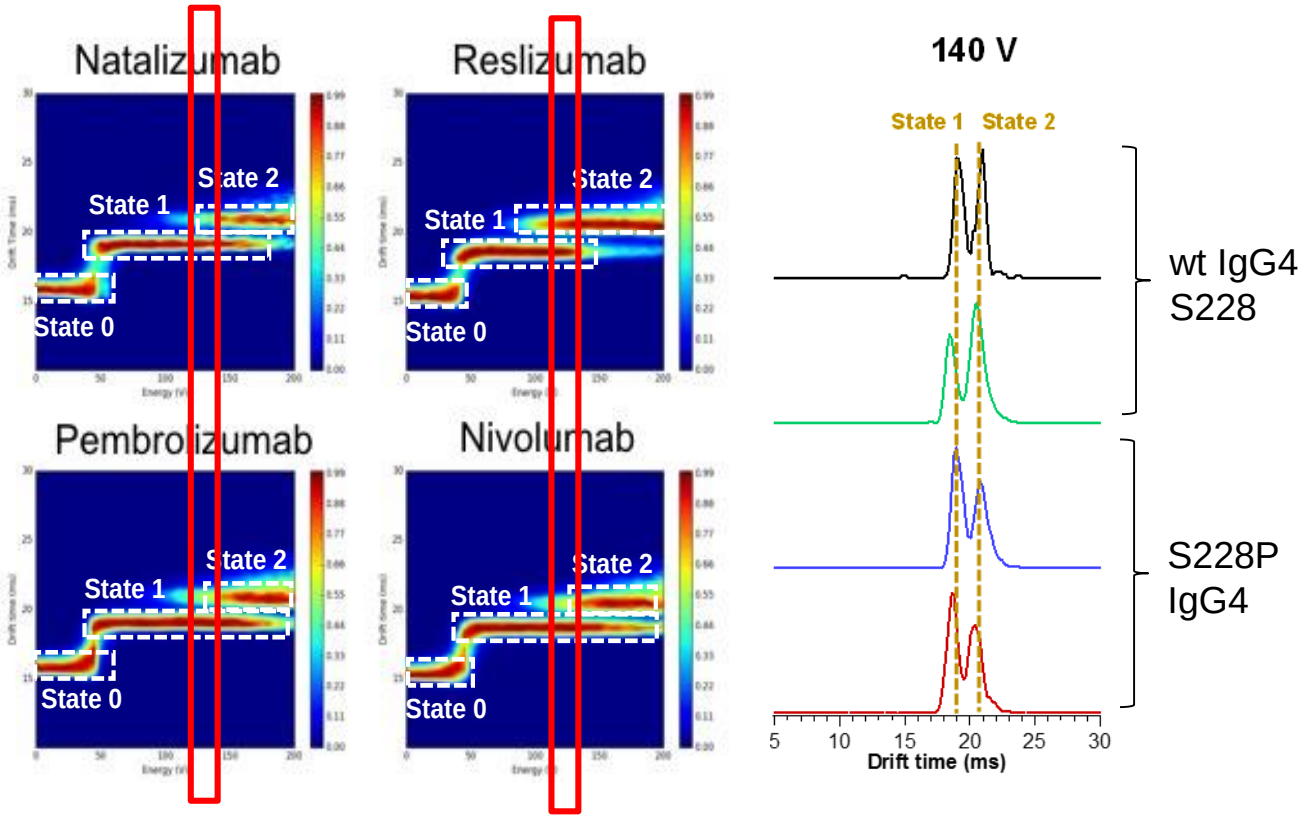
bsAb has an intermediate conformation between mAb1 and mAb2

Debaene F. et al. Anal Chem. 2013 85, 20, 9785.

Native MS and CIU to distinguish wt and stabilized IgG4



No separation of wt and stabilized IgG4 by IMS-MS



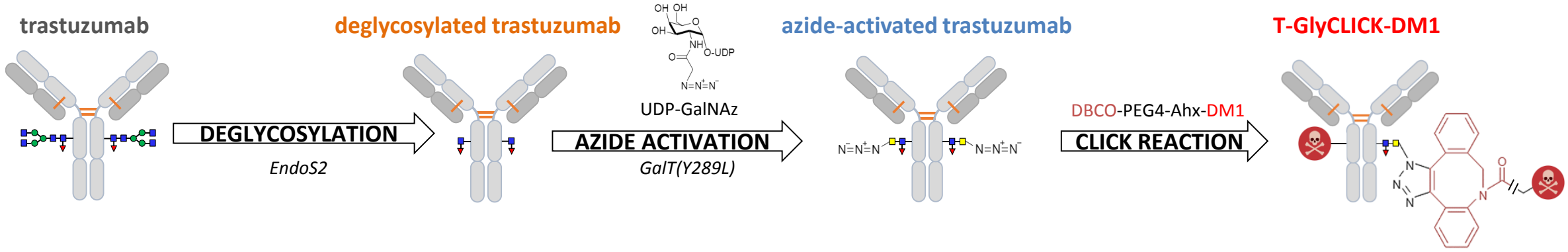
CIU reveals subtle differences in unfolding patterns:
@140V State 2 is the most abundant for wt IgG4 while state 1 stays the major one for S228P IgG4

wt IgG4 are more prone to gas phase unfolding than hinge stabilized IgG4

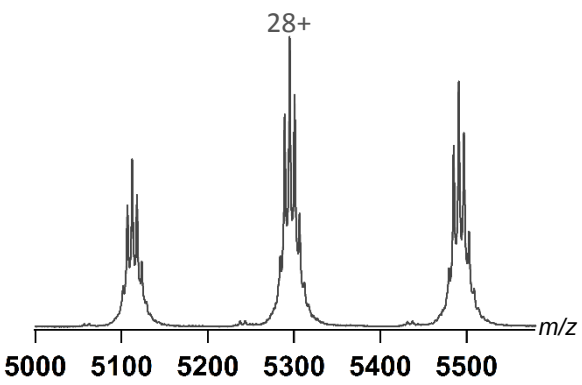
Hernandez-Alba et al. Anal Chem. 2018, 90(15):8865-8872.

Native MS and CIU to monitor site-specific ADC formation

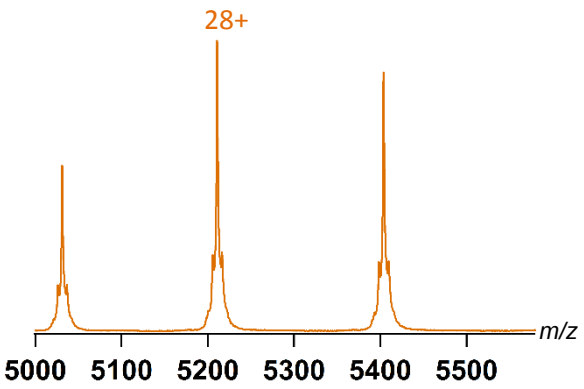
Site-specific ADC on sugars: monitoring of reactions



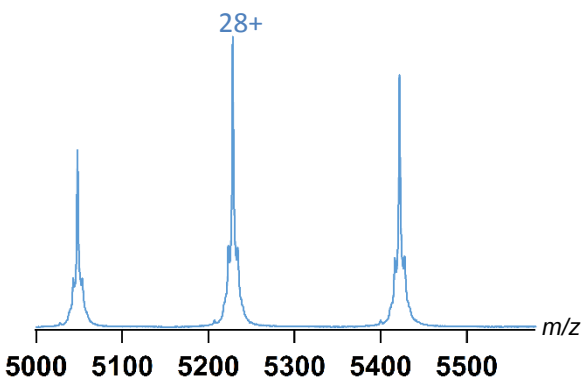
148 228 ± 2 Da (G1F/G0F)



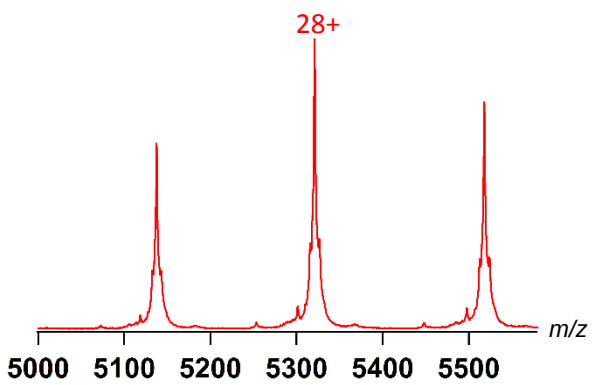
145 875 ± 1 Da



146 372 ± 2 Da



148 960 ± 3 Da

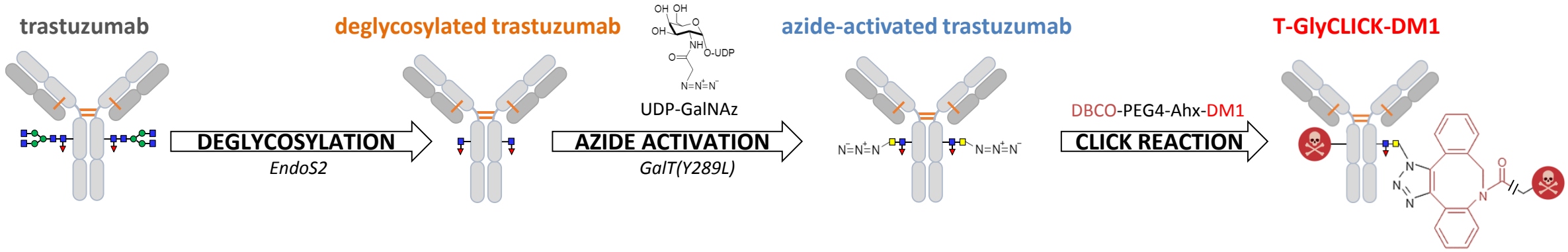


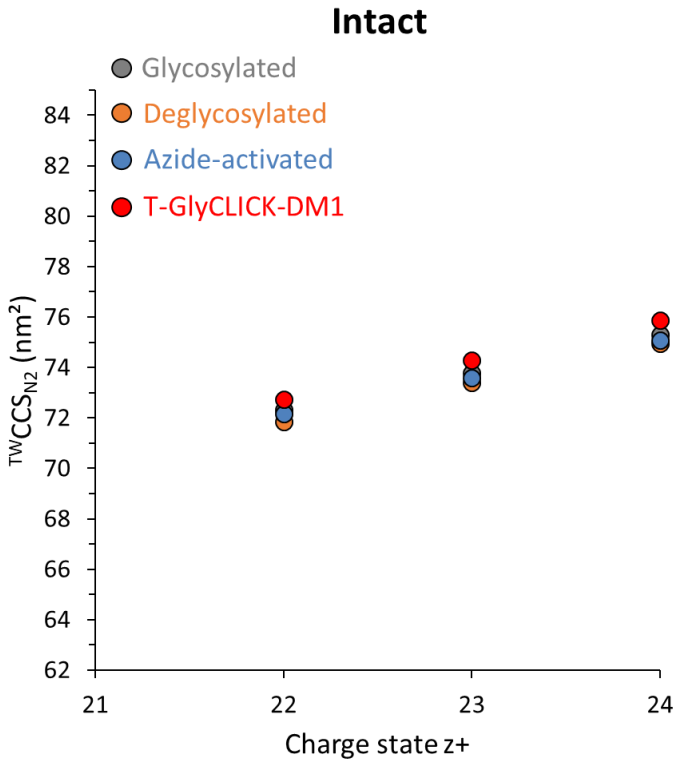
in Collaboration with Genovis (Sweden)

Deslignière E et al. Pharmaceuticals 2021, 24;14(6):498.

Native IMS-MS fails in monitoring conformational changes induced upon drug conjugation

The chemical conjugation process does not drastically affect the overall global conformation of the mAb

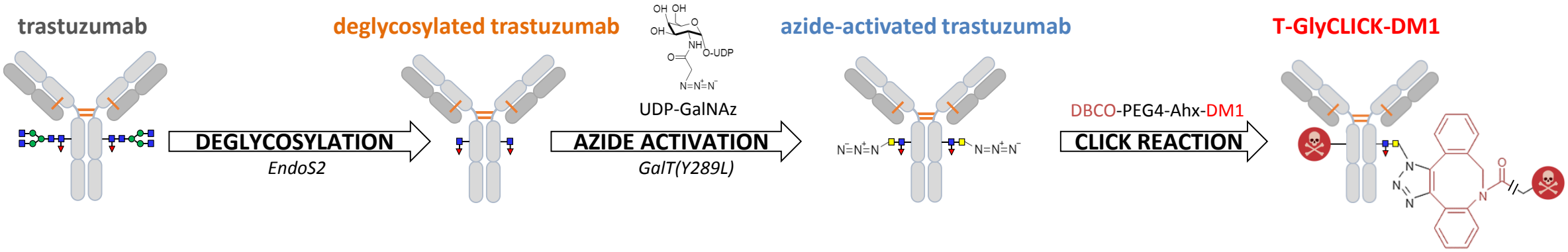


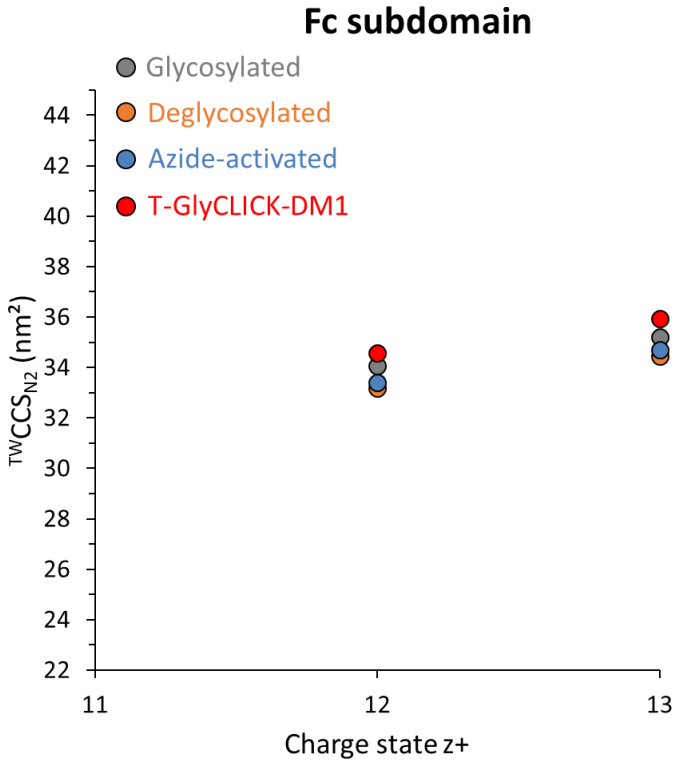


only very slight CCS differences (< 1.3%) that fall within the error of the IMS measurement (2%) might be observed

Native IMS-MS fails in monitoring conformational changes induced upon drug conjugation

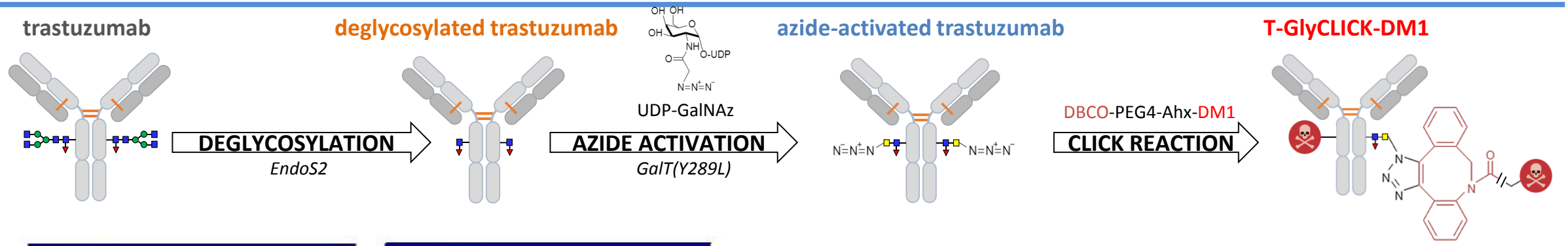
The chemical conjugation process does not drastically affect the overall global conformation of the mAb

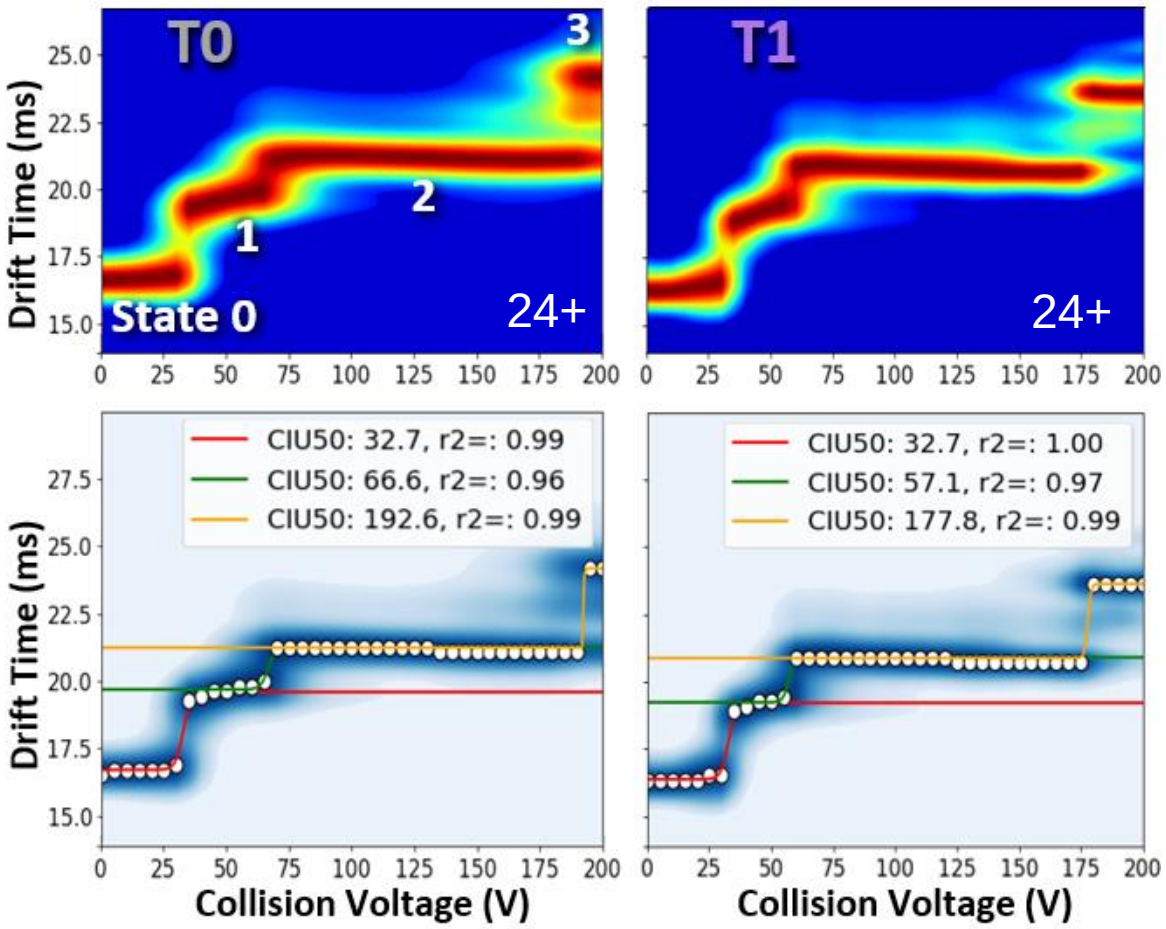




only very slight CCS differences (< 1.3%) that fall within the error of the IMS measurement (2%) might be observed

Native MS to monitor site-specific ADC formation



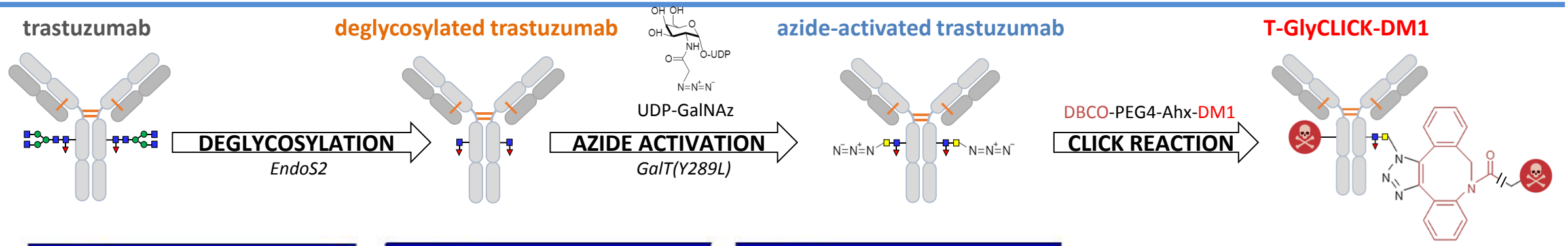


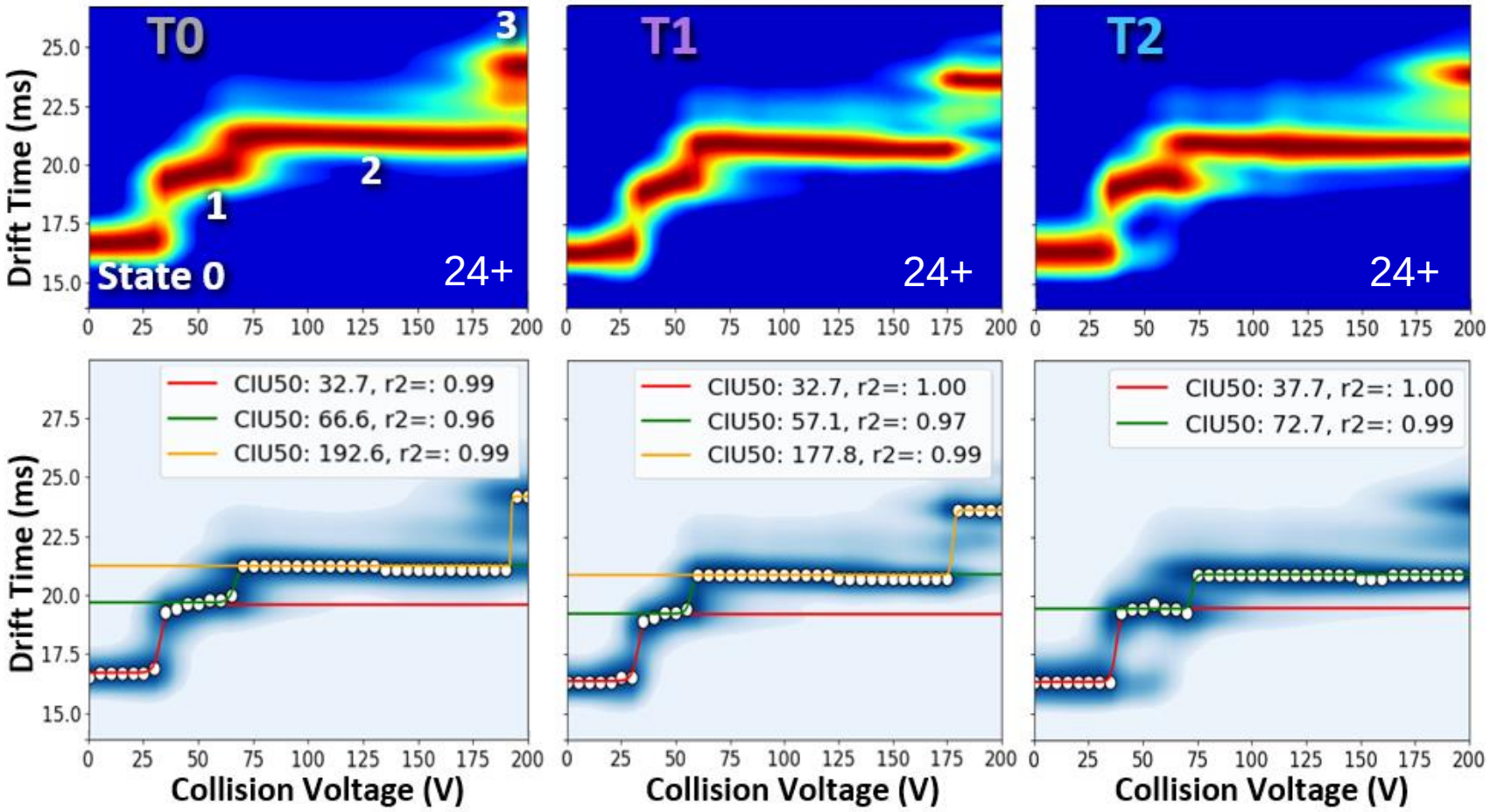
- Similar CIU fingerprints before and after deglycosylation
- Still 3 transitions after deglycosylation

- 1st transition: similar voltages
- 2nd transition: lower CIU50 values (57.1 V) than for glycosylated T0 (66.6 V)
- 3rd transition: lower voltage also (177.8 V for T1, but only at 192.6 V for T0)

Deglycosylated trastuzumab T1 is more prone to unfolding than its glycosylated counterpart

Native MS to monitor site-specific ADC formation



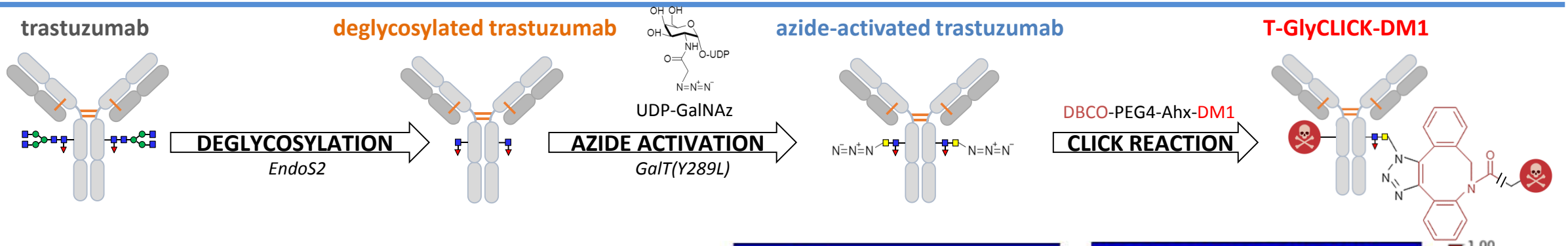


- Similar CIU fingerprints

- 1st transition: higher voltages than T1
- 2nd transition: higher voltages than T1

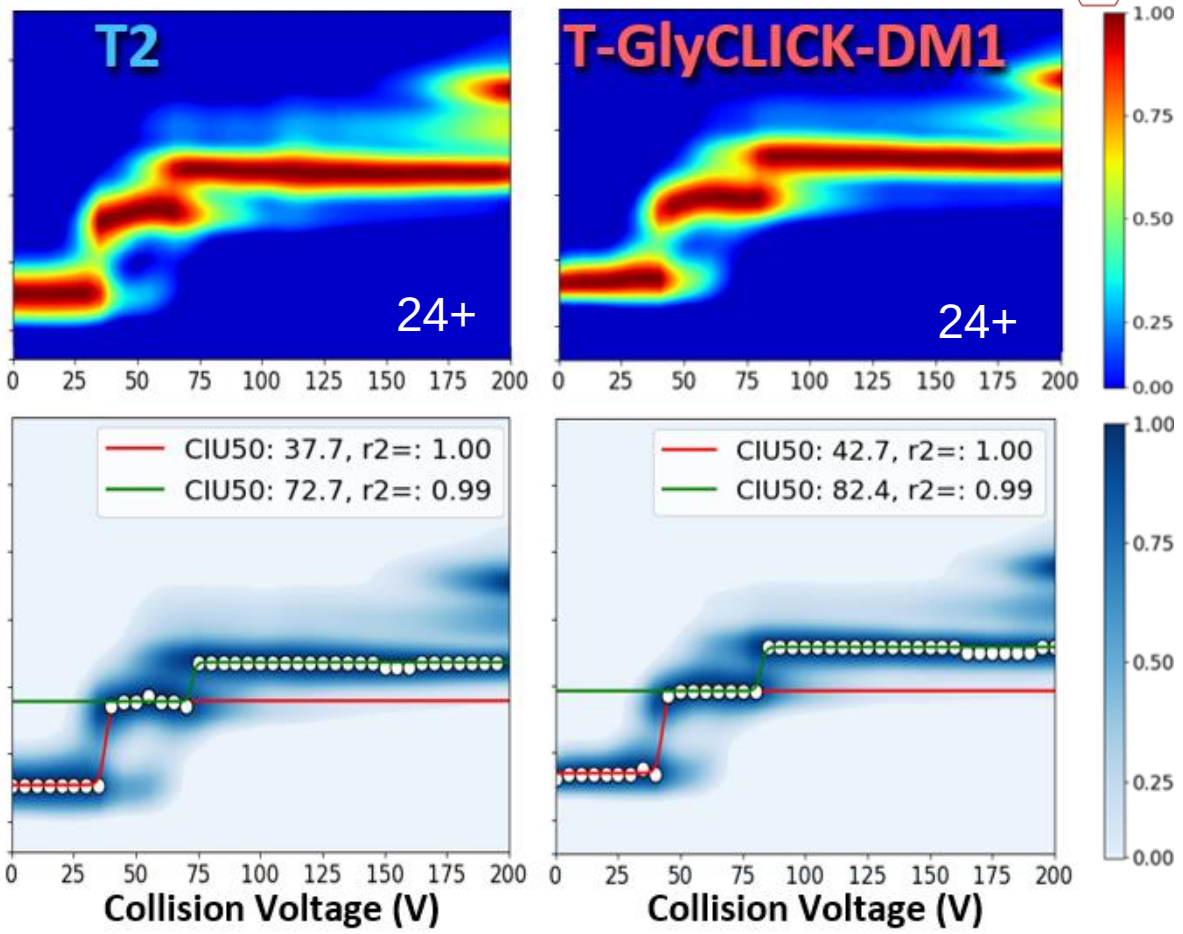
In favor of gas-phase stabilization upon azide activation

Native MS to monitor site-specific ADC formation



- The 2 conformational transitions occur at higher CIU50 values (42.7 and 82.4 V) than the other reaction compounds

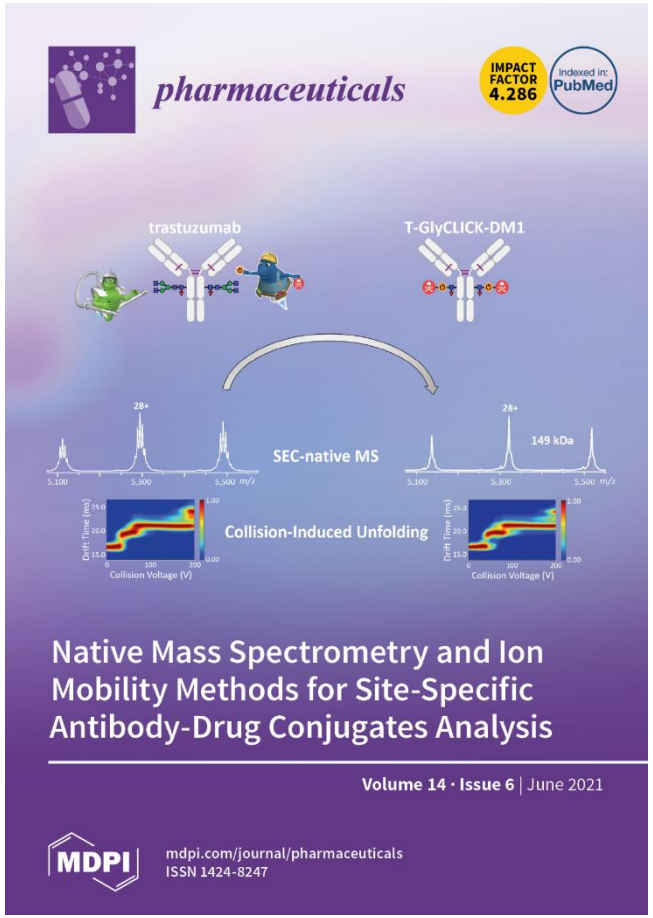
The click chemistry step mostly contributes to the increased resistance to unfolding of T-GlyCLICK-DM1



Native IMS-MS strategies to monitor ADC formation

- Drug conjugation reinforces the overall stability of the mAb towards gas-phase unfolding, as reported for:

a DAR2 site-specific oligo-ADC engineered using the GlyCLICK technology (Genovis)



Deslignière et al. Pharmaceuticals 2021;14(6):498

a DAR4 site-specific therapeutic ADC (Catalent Biologics)

MABS
2017, VOL. 9, NO. 5, 801–811
https://doi.org/10.1080/19420862.2017.1316914
Taylor & Francis
Taylor & Francis Group

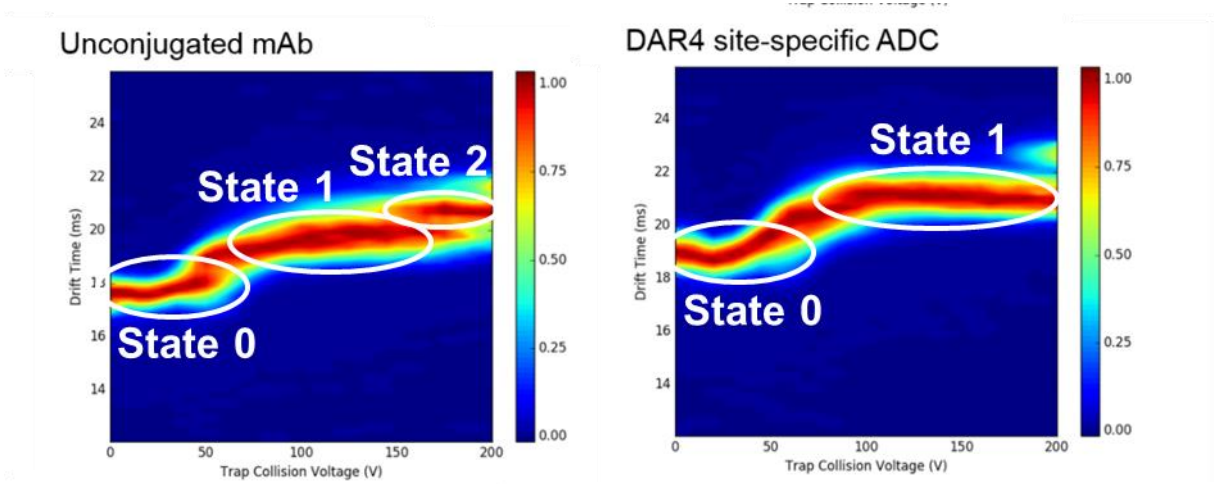
REPORT

Check for updates

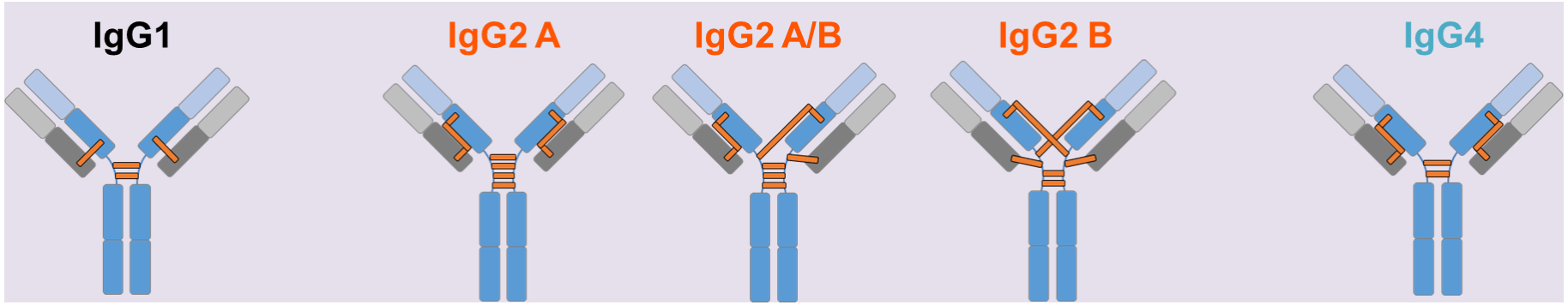
Insights from native mass spectrometry approaches for top- and middle- level characterization of site-specific antibody-drug conjugates

Thomas Botzanowski[a,*], Stéphane Erb[a,*], Oscar Hernandez-Alba[a], Anthony Ehkirch[a], Olivier Colas[b], Elsa Wagner-Rousset[b], David Rabuka[c], Alain Beck[b], Penelope M. Drake[c], and Sarah Cianférani[a]

[a]Laboratoire de Spectrométrie de Masse BioOrganique, Université de Strasbourg, CNRS, Strasbourg, France; [b]Centre d'Immunologie Pierre-Fabre (CIPF), Saint-Julien-en-Genevois, France; [c]Catalent Biologics West, Emeryville, CA, USA



Botzanowski et al. MAbs. 2017, 9,801.



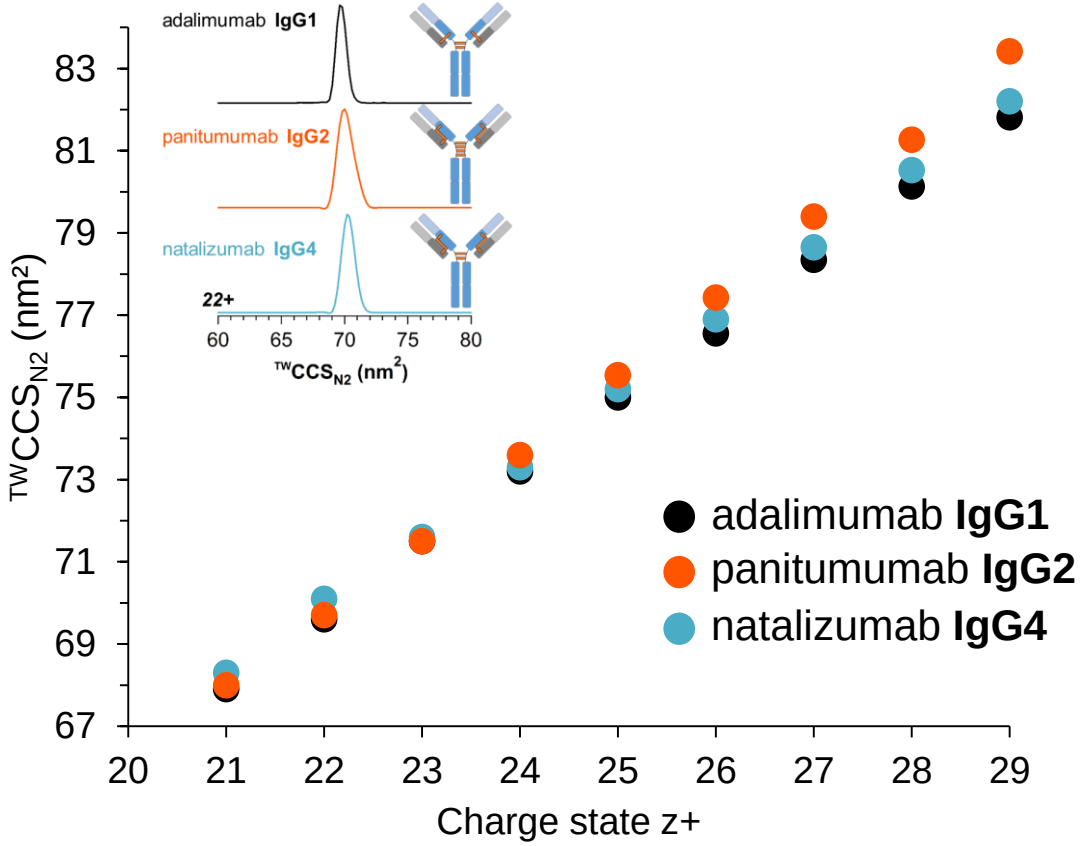
Topic 3

IMS-MS to distinguish between mAb isotypes/subclasses

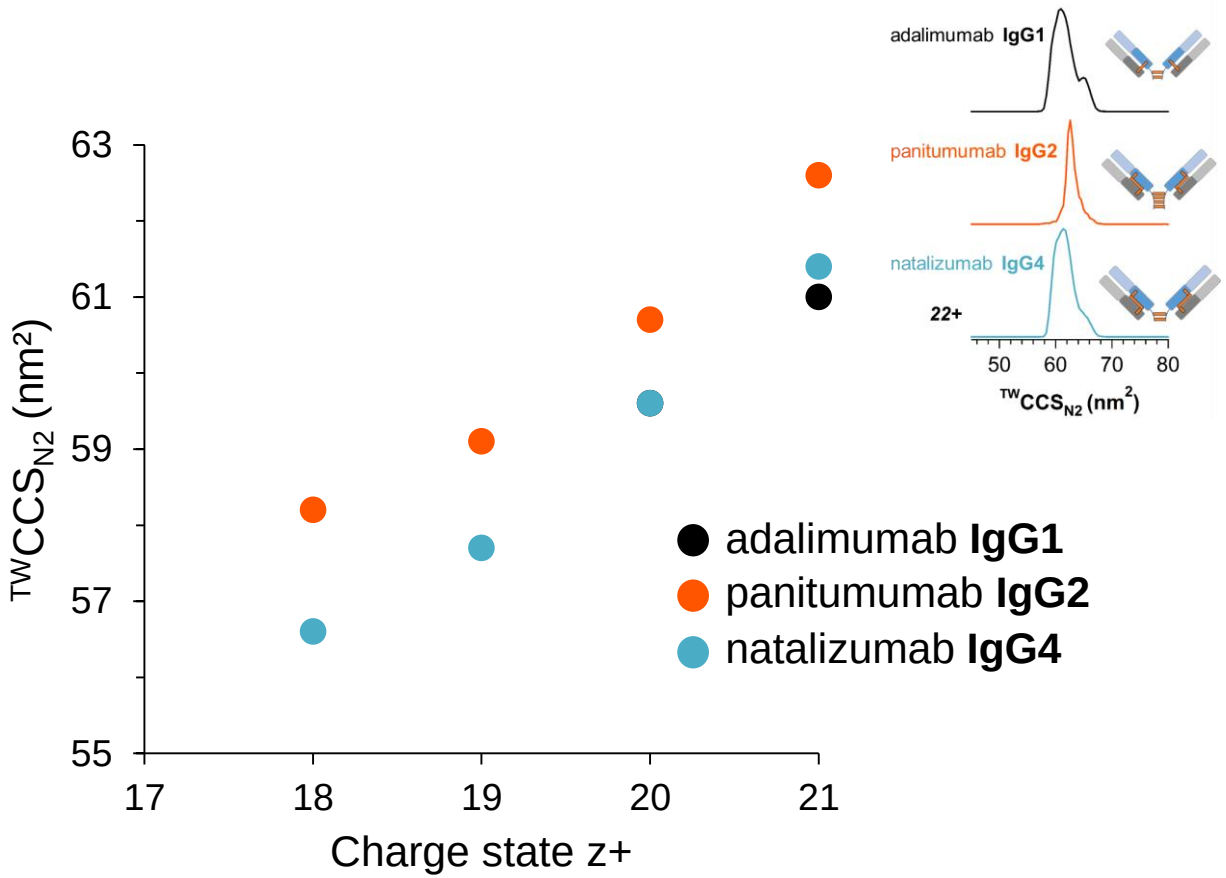
- Middle level *v.* intact level IMS-MS CIU
- SEC-CIU
- High resolution CIU-cIMS

CCS measurements to distinguish mAb isotypes/subclasses

Intact level



Middle level – F(ab')$_2$ region

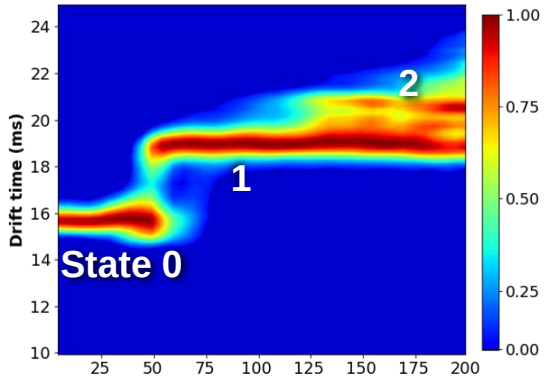


- CCS measurements do not allow mAb isotype classification neither @intact (150 kDa) nor @middle (50 kDa) levels
- CCS differences fall within the uncertainty of the technique (ΔCCS < 2%)

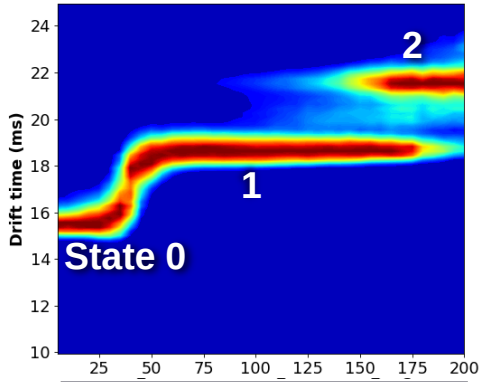
Botzanowski et al., *Anal Chem*, *2020*

Intact-level CIU Isotype classification is not straighforward

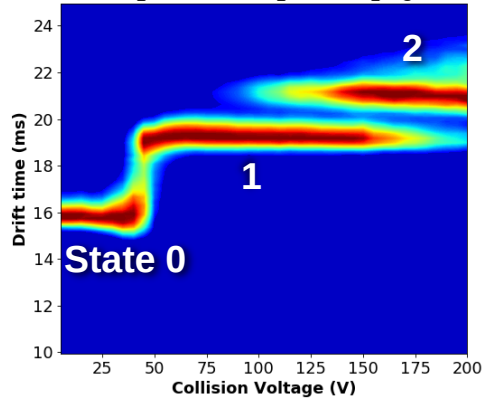
Ref IgG1



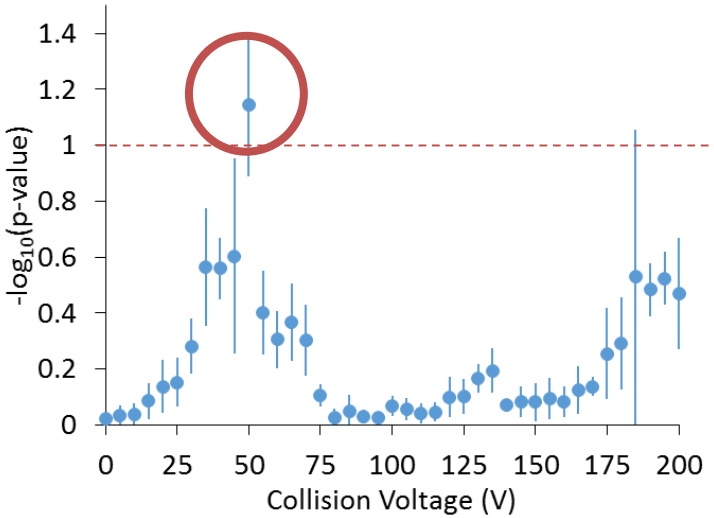
Ref IgG2



Ref IgG4

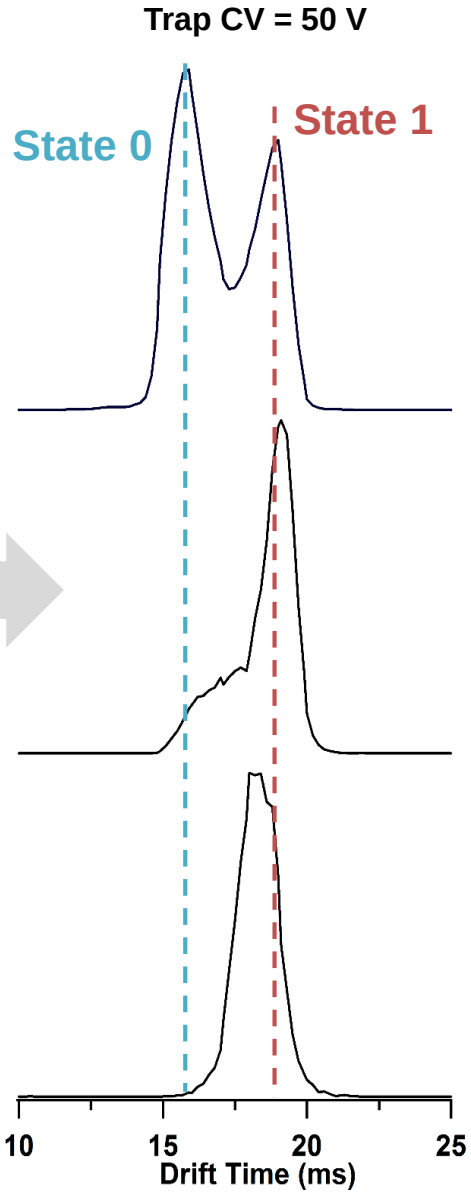


The UFS* plot exhibits few diagnostic CVs



*Univariate Feature Selection plot

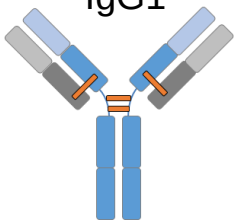
mAb isotypes are not easily distinguished by intact CIU

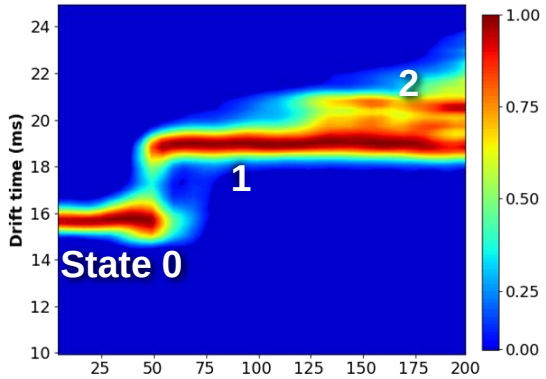


Botzanowski et al., *Anal Chem*, 2020

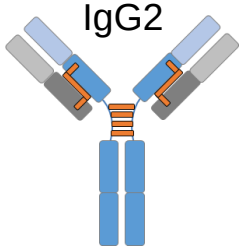
Middle-level CIU provides more accurate isotype classification

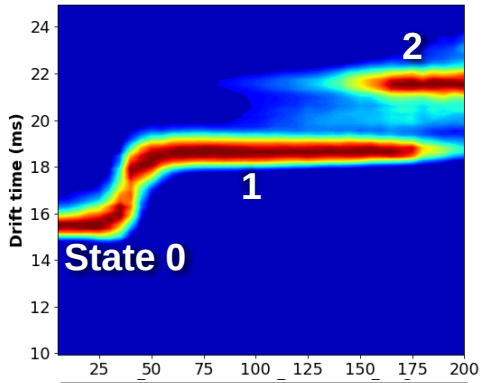
Ref IgG1



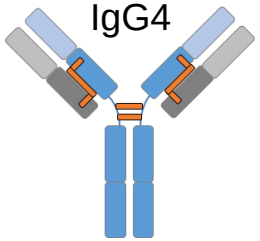


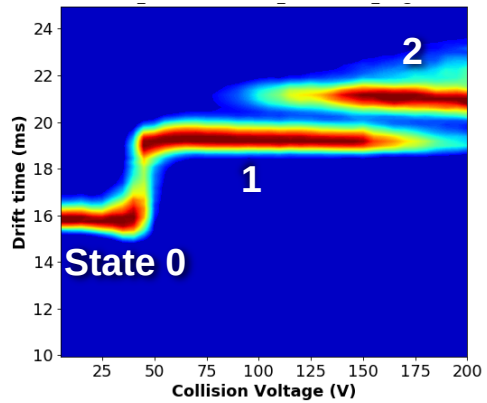
Ref IgG2



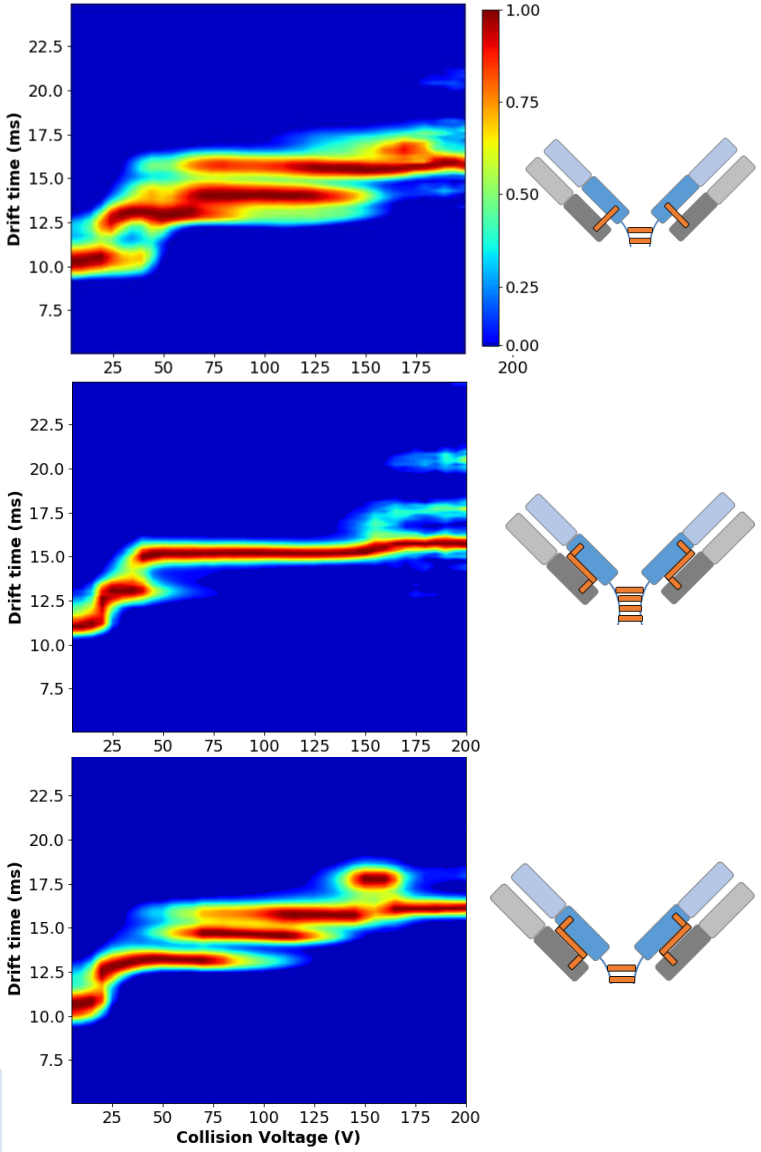


Ref IgG4





IdeS (Genovis) digestion

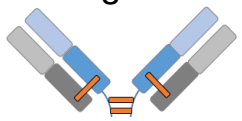


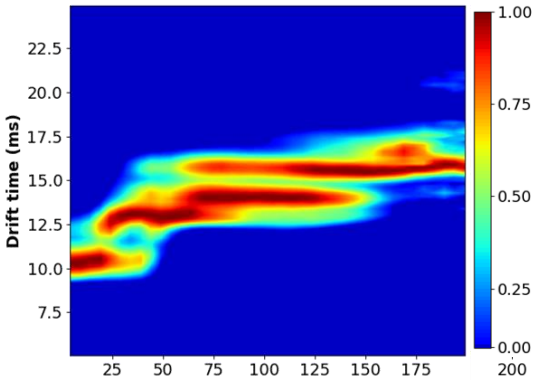
F(ab')$_2$ CIU fingerprints are obviously different between mAb isotypes

Botzanowski et al., *Anal Chem*, 2020

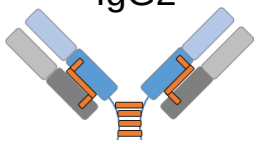
Middle-level CIU provides more accurate isotype classification

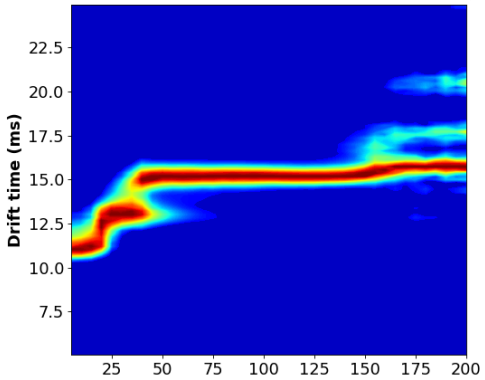
Ref IgG1



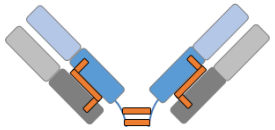


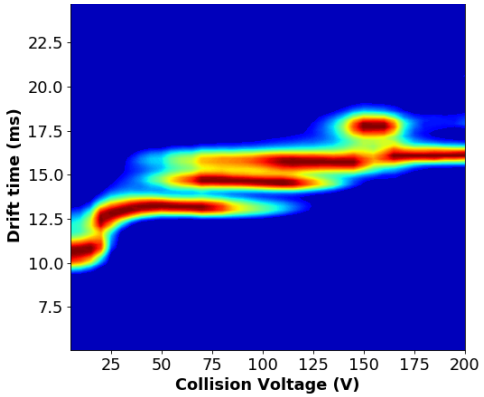
Ref IgG2

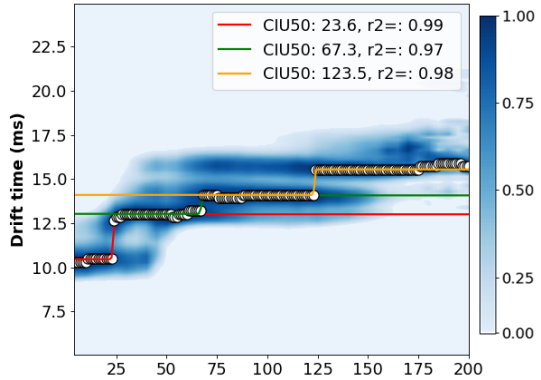


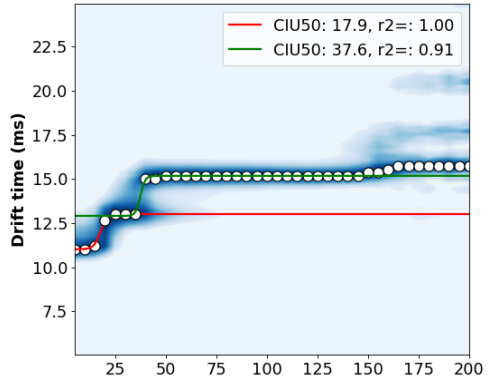


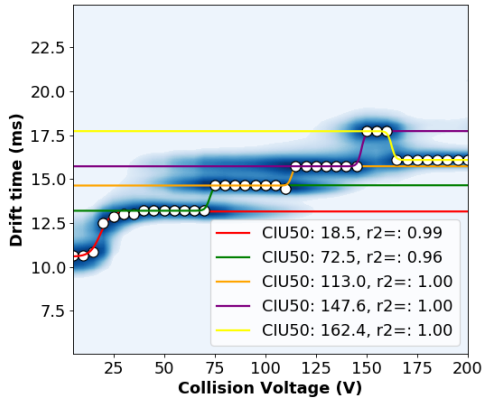
Ref IgG4









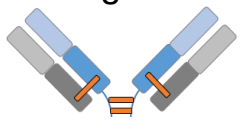


$F(ab')_2$ CIU fingerprints exhibit differences:
- In the number of transitions
- In CIU50 values

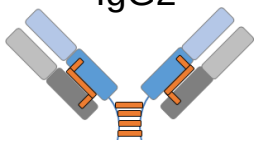
Botzanowski et al., *Anal Chem*, 2020

Middle-level CIU provides more accurate isotype classification

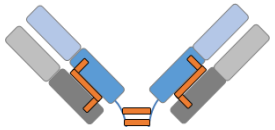
Ref IgG1

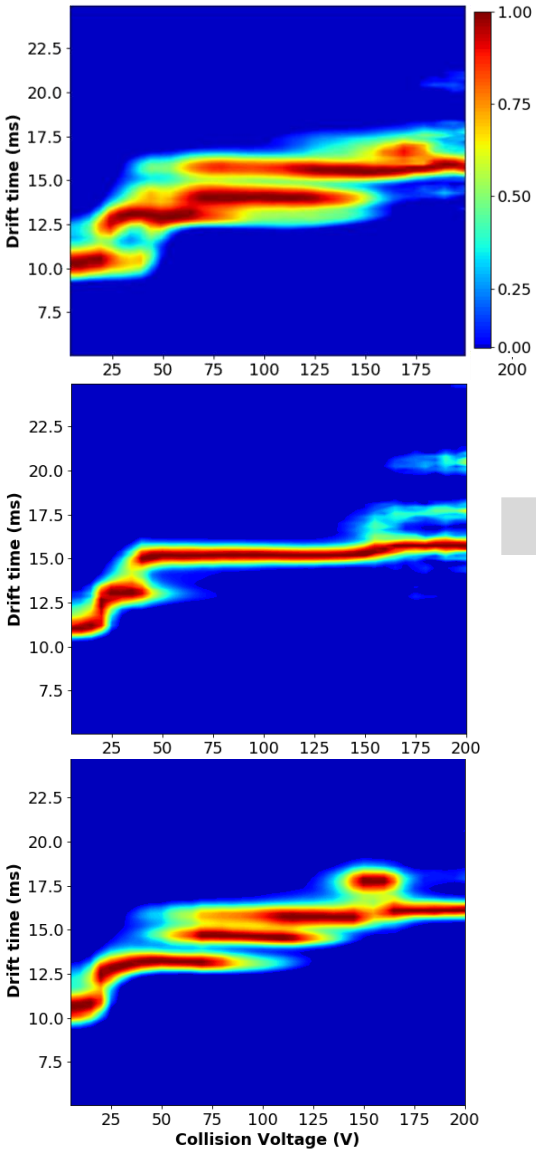


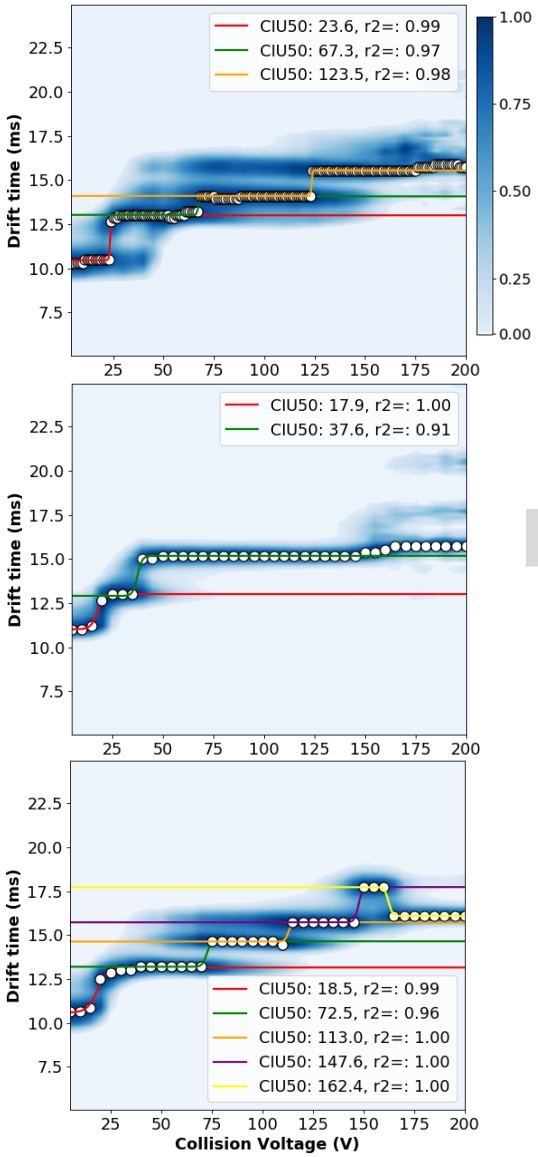
Ref IgG2



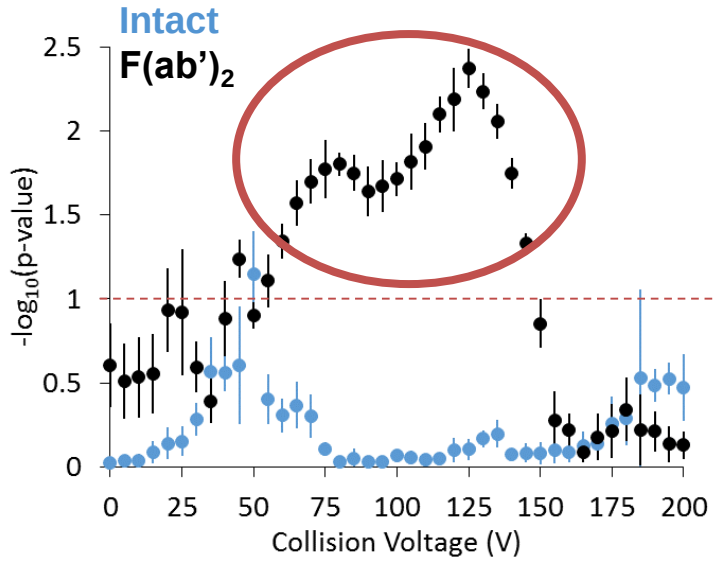
Ref IgG4







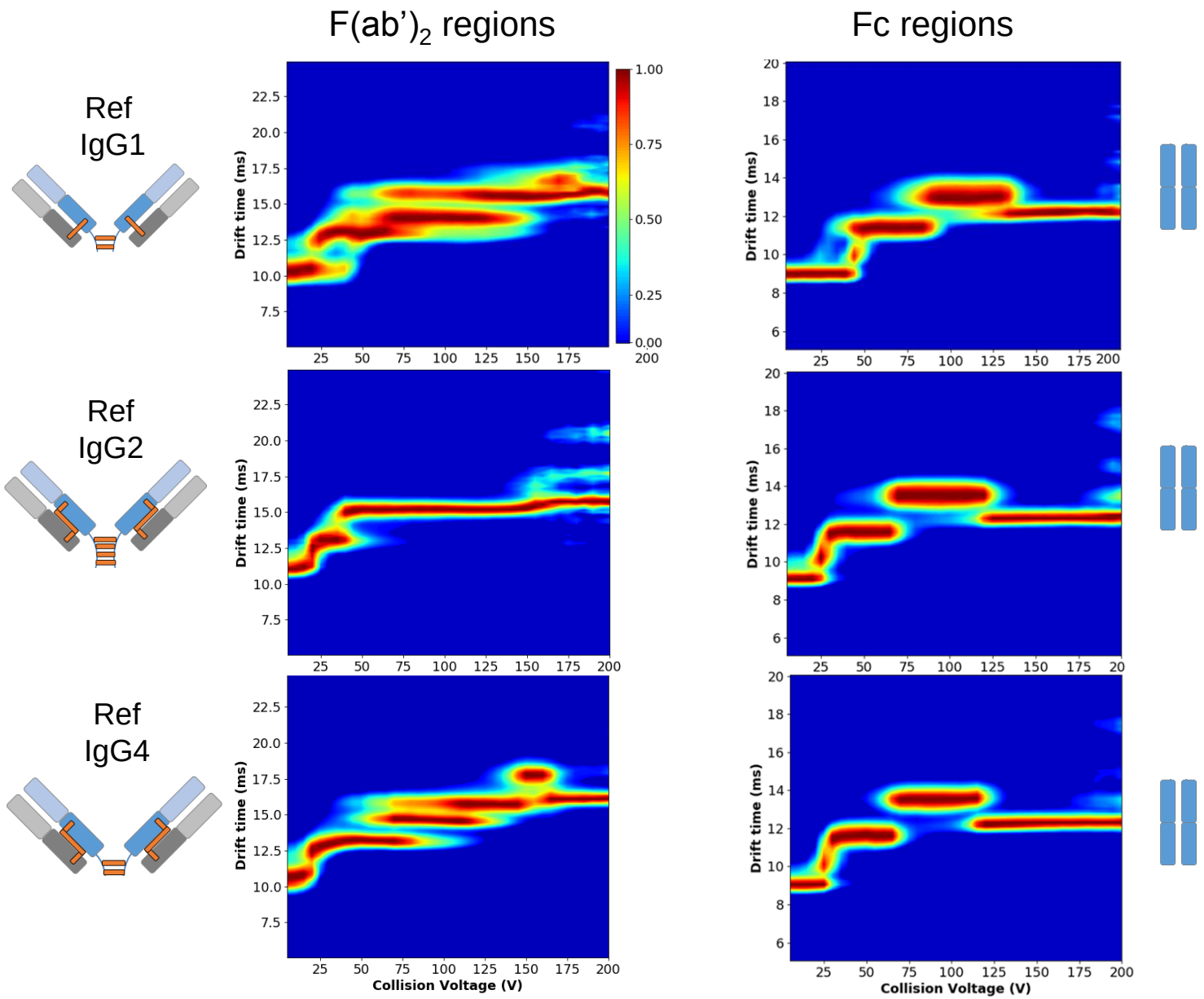
The UFS* plot exhibits several diagnostic CVs



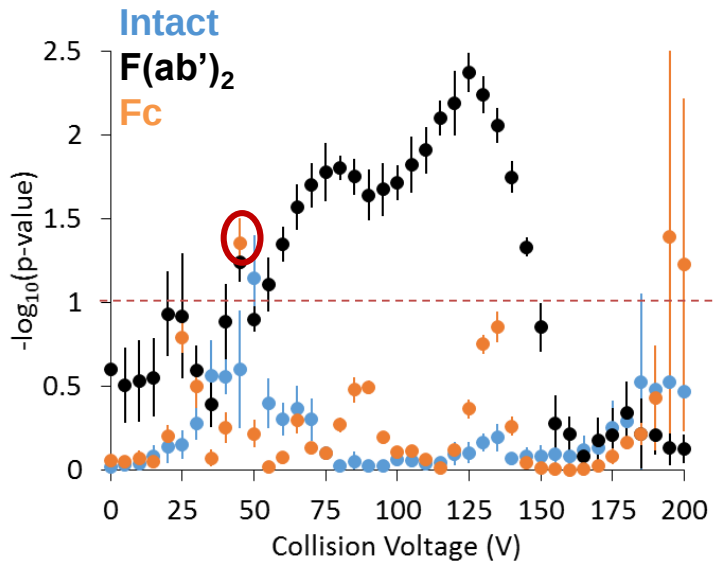
Higher differentiation scores for F(ab')$_2$ regions

Botzanowski et al., *Anal Chem*, 2020

Middle-level CIU provides more accurate isotype classification



The UFS* plot exhibits few diagnostic CVs for Fc

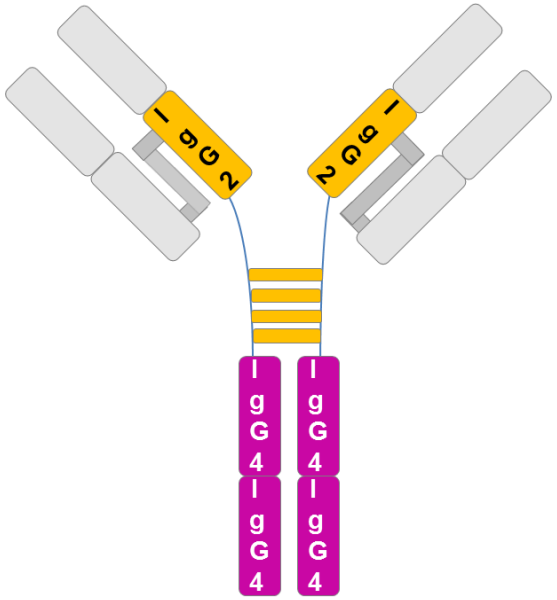


As expected Fc regions are less informative (higher homology of sequence in Fc regions)

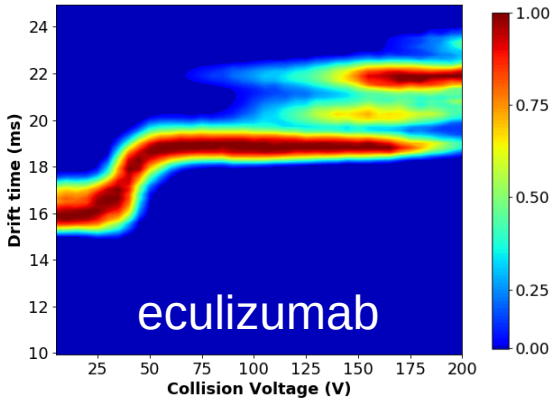
Botzanowski et al., *Anal Chem*, 2020

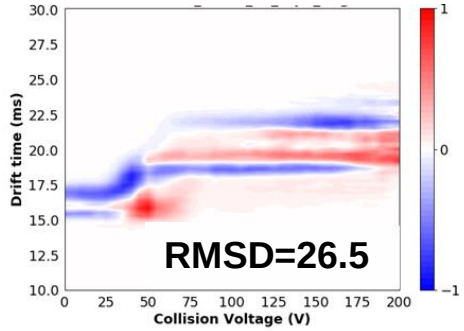
Middle-level CIU provides more accurate isotype classification: the eculizumab case study

Eculizumab: a composite IgG2/IgG4 mAb

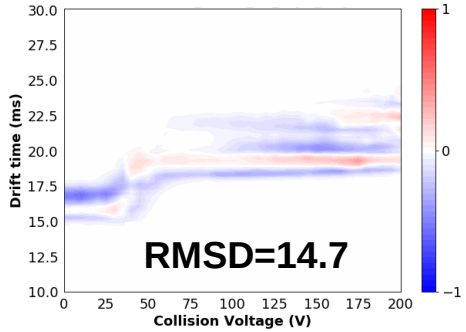


Intact-level CIU fails in detecting the hybrid format of eculizumab

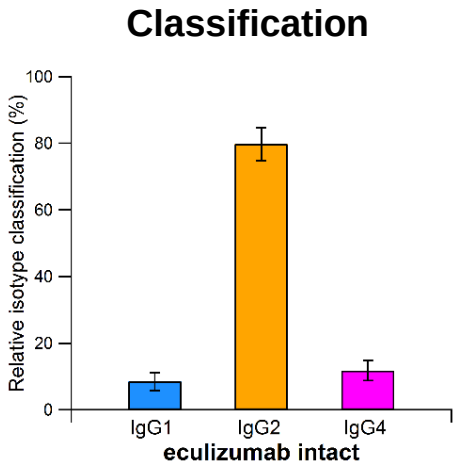


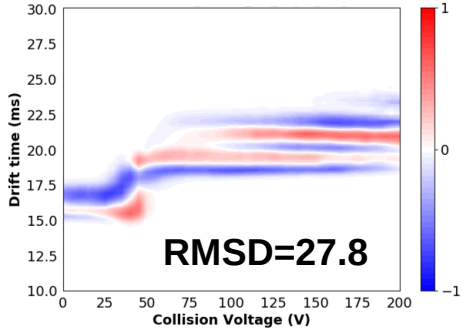


IgG1 *vs* eculizumab



IgG2 *vs* eculizumab



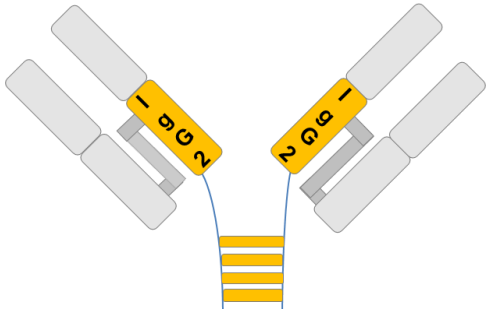


IgG4 *vs* eculizumab

Botzanowski et al., *Anal Chem*, 2020

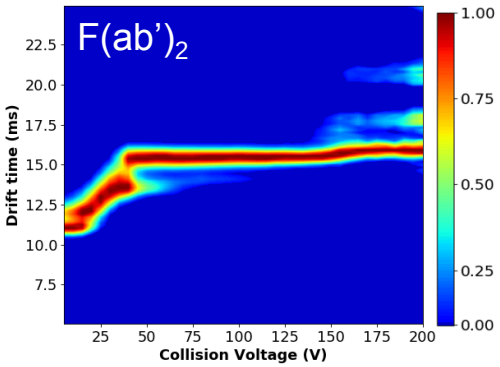
Middle-level CIU provides more accurate isotype classification: the eculizumab case study

Eculizumab: a composite IgG2/IgG4 mAb

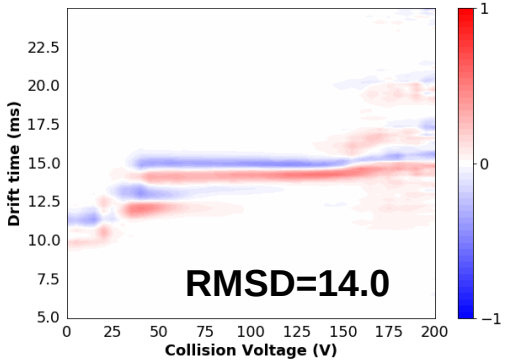


IdeS digestion

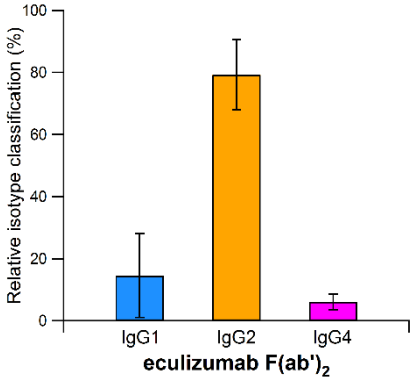
Middle-level CIU allows precise classification of both domains

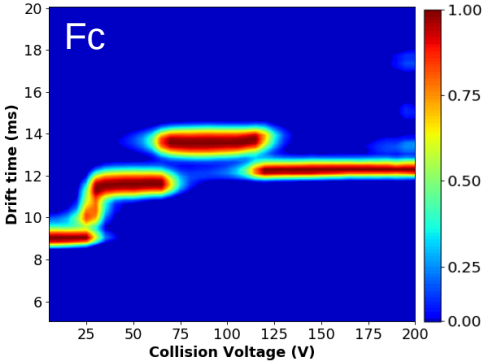


IgG2 *vs* eculizumab

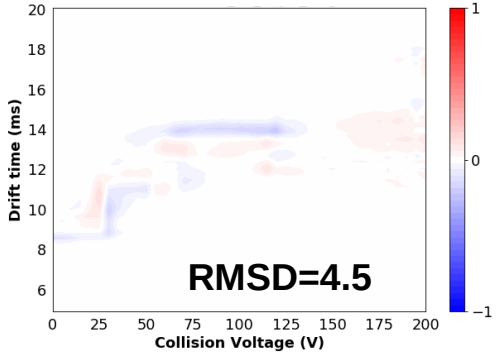


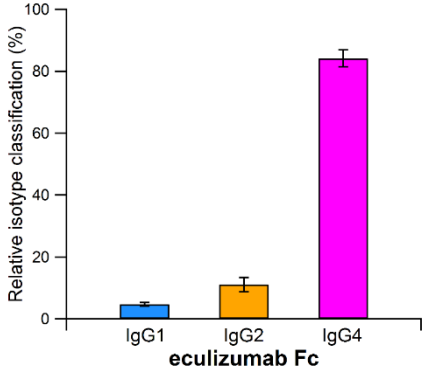
Classification





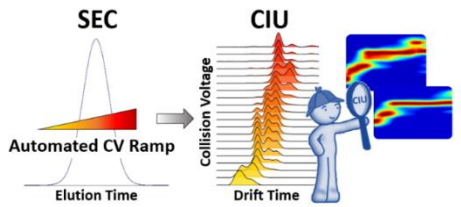
IgG4 *vs* eculizumab



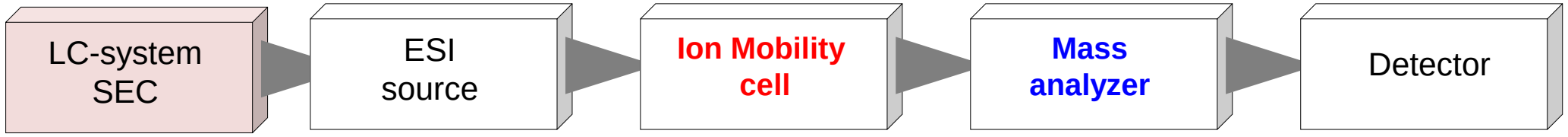


Botzanowski et al., *Anal Chem*, 2020

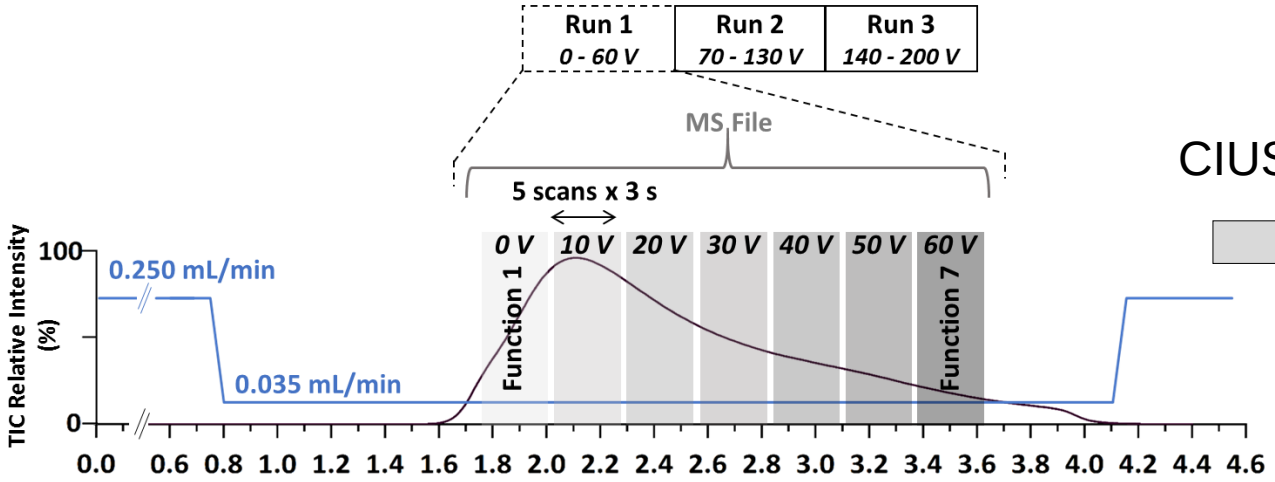
Alternative IMS-MS approaches to circumvent lack of resolution of IMS cells: Collision Induced Unfolding - CIU



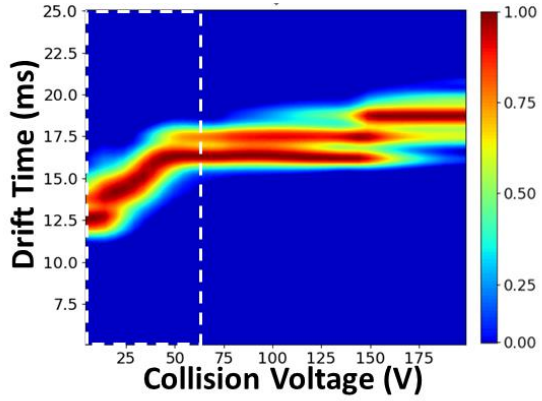
SEC-CIU-IMS: a fully automated process from sample preparation to data interpretation



Instrumental setting optimizations



CIUSuite 2



45 min
(1 mAb in triplicate)

Fast and efficient online desalting
+ Overall time process divided by 3

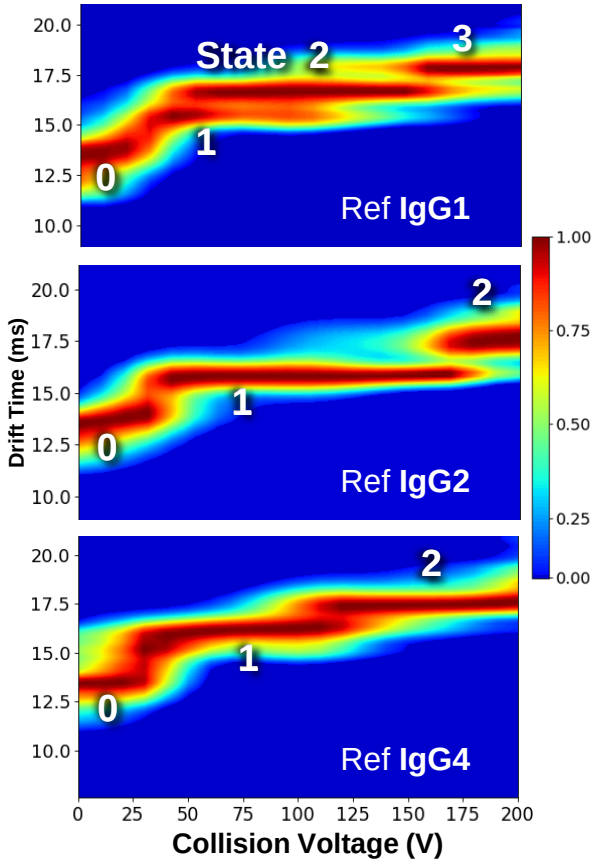
Deslignière et al., *Anal Chem*, 2020

SEC-CIU allows fast mAbs IgG subclasses differentiation

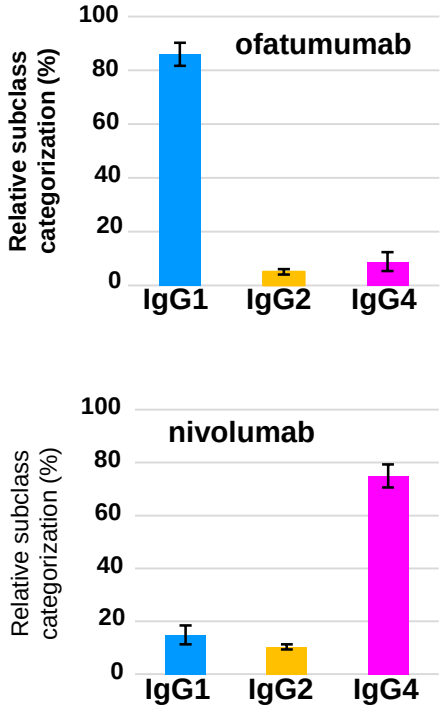
SEC-CIU detects subtle differences between mAbs isotypes

Intact

Reference mAbs

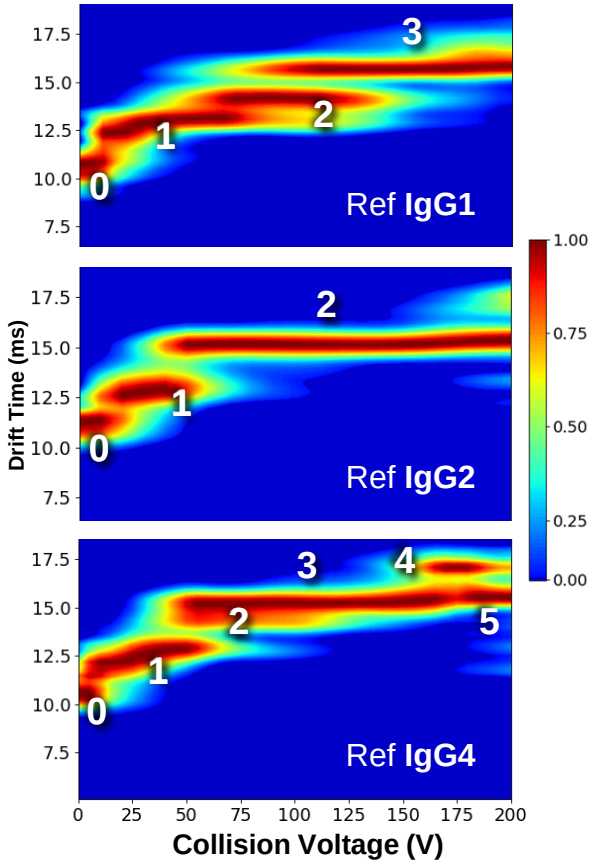


Classification

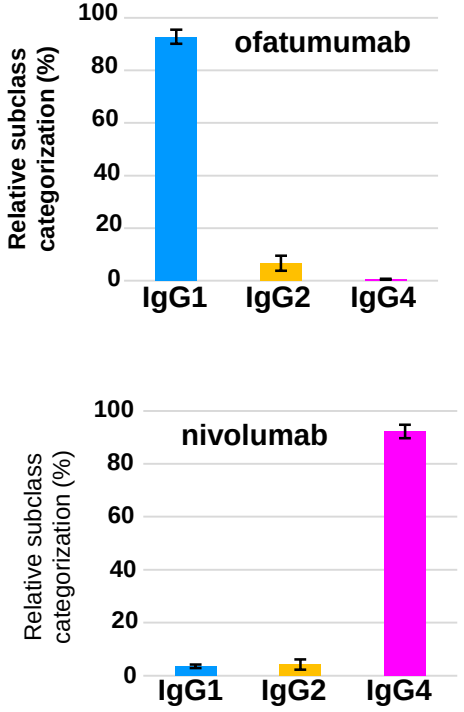


Middle F(ab')2 fragment

Reference mAbs



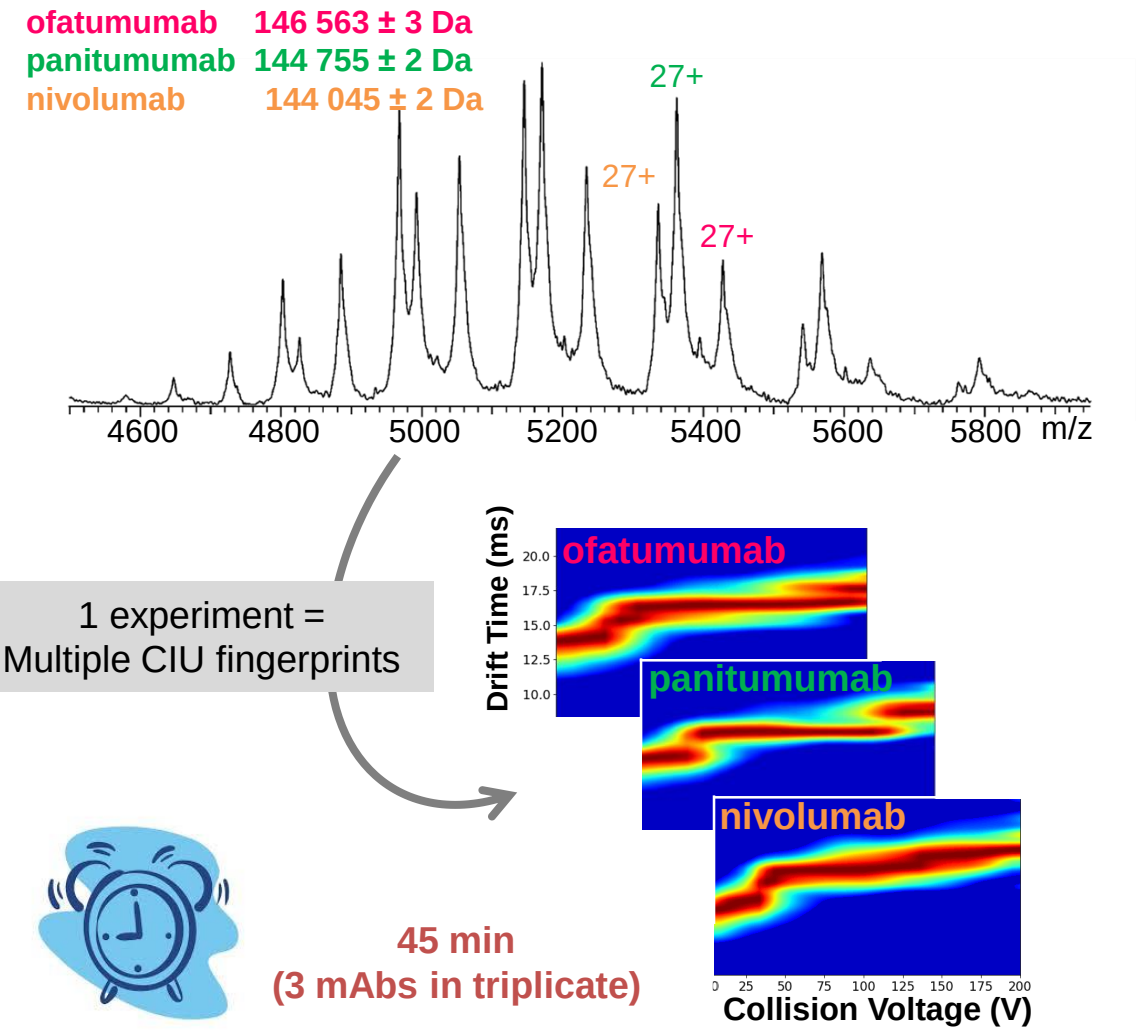
Classification



Deslignière et al., *Anal Chem*, 2020

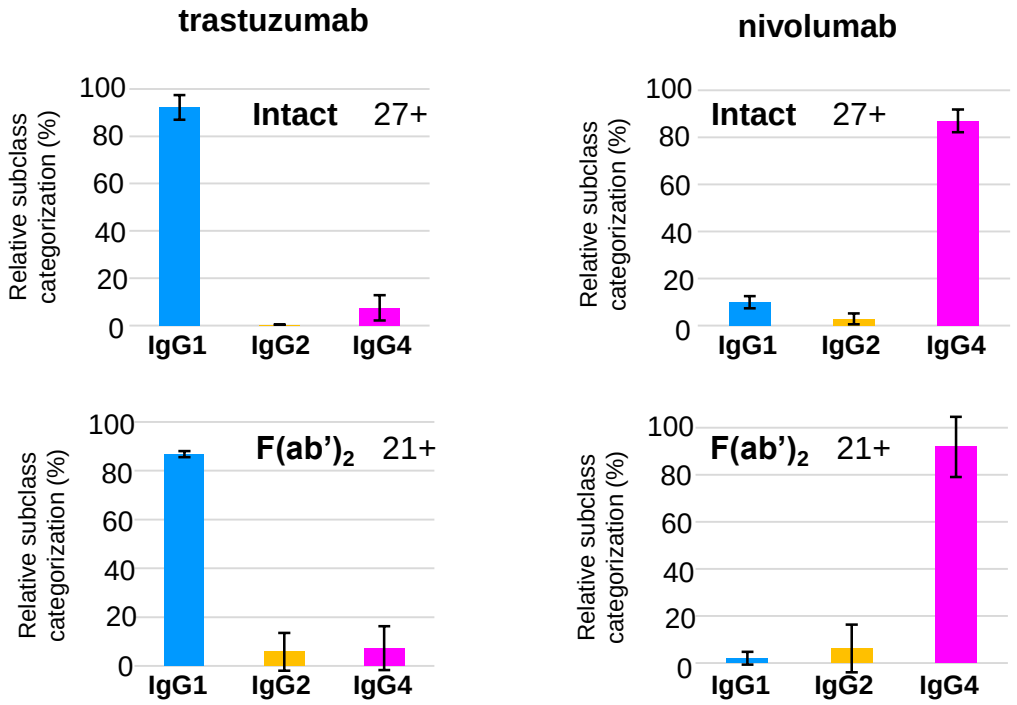
Tricks to improve SEC-CIU throughput

Multiplexed SEC-CIU mAbs analysis



Targeted SEC-CIU for fast-mAb classification

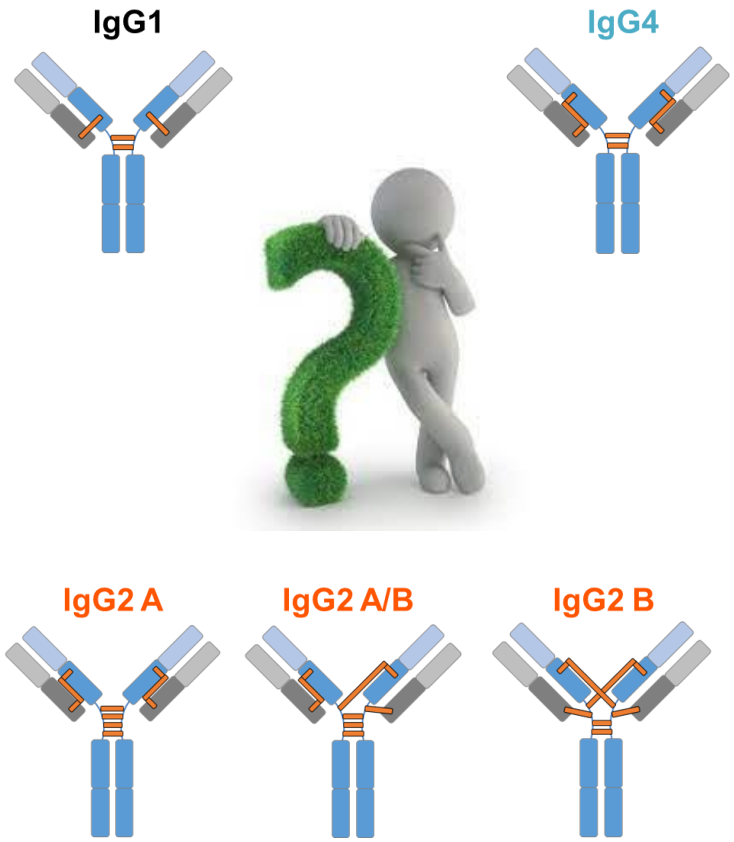
10 min / 7 CVs
(1 mAb in triplicate)



Acquisition of the most diagnostic CVs only

Deslignière et al., *Anal Chem*, 2020

IMS-MS based approaches for IgG subclass categorization: conclusions

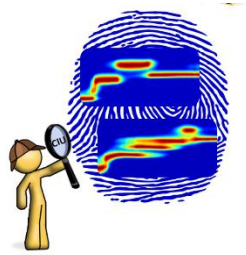


From CCS calculations

- not easy neither for intact- nor middle-level mAb analysis
- inherent mAb flexibility
- lack of IMS resolution

From CIU data

- Possible on 1st and 2nd generation TWIMS instruments
- Automation through SEC coupling



@ intact level

✓ Clear benefit of high-resolution

× Tricky with linear TWIMS

@ middle level

✓ Clear benefit of high-resolution with more informative CIU fingerprints

✓ Good results also on linear TWIMS

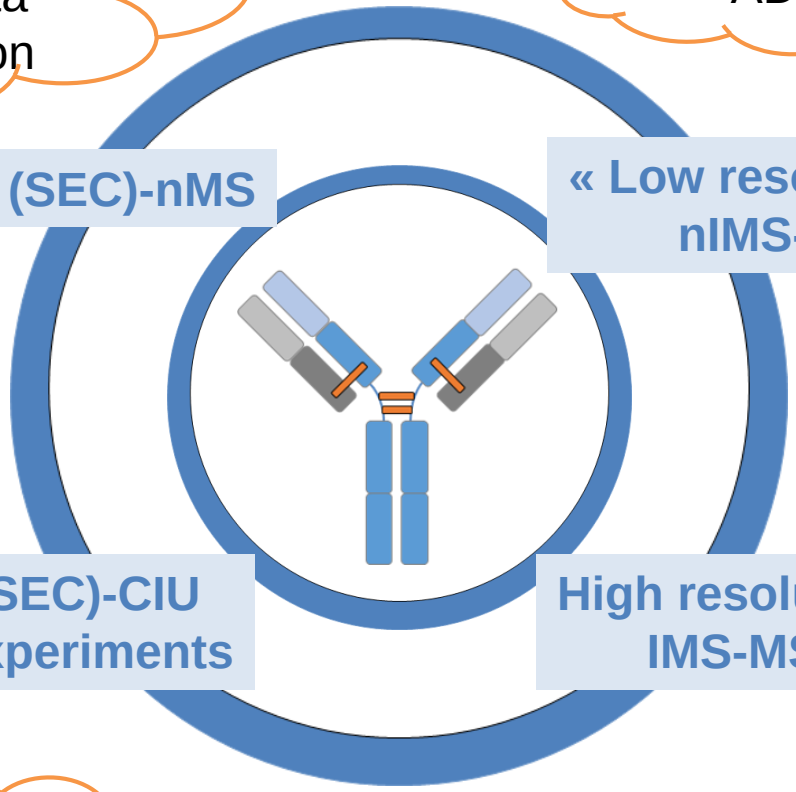
… waiting for CCS measurements on high resolution IMS instrumentations

Topic 4

IMS-MS for the characterization of complex mAb formats

- Engineered new generation multispecific mAb

Concluding remarks: where do we stand with IMS-MS for mAb characterization?



Qualitative or quantitative IMS-MS data interpretation

Drug conjugation ADC

Mutispecific mAbs

Conformational purity/homogeneity

VHH/nanobodies

Differences in disulfide bridging

Assessment of therapeutic similarity (biosimilars, MAM)

Subtype/Isotype classification

Manufacturing Process Monitoring

Thanks !

The LSMBO team

Thanks !

The structural MS team

Evolène DESLIGNIERE
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Hélène DIEMER
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