2018 Nanjing International DMPK Symposium – Workshop
2018南京国际药代会议学习班
June 29, 2018，星期五

Workshop I
Bioanalysis of Protein Therapeutics and Antibody-Drug Conjugates by LC-MS
色质联用在蛋白制品和抗体-药物偶合药物生物分析中的应用

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https://www.amgenscience.com/the-shape-of-drugs-to-come/

Workshop I

Strategy and technologies in protein therapeutics and ADC biotransformation

蛋白质和抗体-药物偶合药物生物转化分析的策略和技术

宿殿 （基因泰克/罗氏）

- **HRMS platforms**

高分辨质谱技术和平台

- **HRMS for intact protein quantitation and profiling**

高分辨质谱技术在完整蛋白药物定性和定量分析的应用

- Top-down and middle-down approaches

  自顶向下和自中向下的方法

- **Immuno-affinity LC-HRMS for ADC biotransformation**

免疫-高分辨色质杂化分析在药物偶合药物生物转化分析中的应用

- Top-down and middle-down approaches

  自顶向下和自中向下的方法

D. Su

HRMS platforms
高分辨质谱技术和平台

- **HRMS (high resolution mass spectrometry)**

  - **Mass accuracy: measures exact mass; mass error <5 ppm.**
  - **Mass resolution: indicates a platform's ability to distinguish peaks;**
  - **Platforms:**

- **Time-of-flight MS (TOF MS)**
- **Orbitrap MS**
- **Fourier transform ion cyclotron resonance (FT-ICR) MS**

Ghaste, M, et al. Int. J. Mol. Sci. 17:E816, 2016.

HRMS platforms
高分辨质谱技术和平台

Mass Spectrometer	Resolving Power (FWHM)	Mass Accuracy (ppm)	Sensitivity (g)	Speed (Hz)	Dynamic Range
Quadrupole	5K	50	10^{-15} (SRM)		10^7
Linear Ion Trap	10K	50	10^{-15}		10^5
Time of Flight	**30K-50K**	**<1**	**10^{-15} (full scan)**	**10-10^4 (in theory)**	**10^6**
Orbitrap	**100K-1M**	**<1**	**10^{-15} (full scan)**	**10-40**	**10^5**
FT-ICR	**1M-10M**	**0.25-1**	**10^{-12} (full scan)**	**0.001-10**	**10^5**

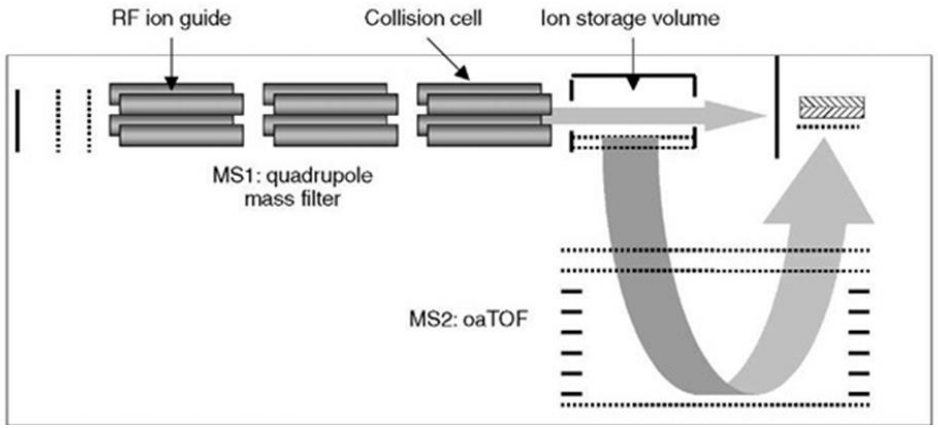
Ghaste, M, et al. Int. J. Mol. Sci. 17:E816, 2016.

HRMS platforms
高分辨质谱技术和平台

Time-of-flight MS (TOF MS): Measures time to calculate m/z

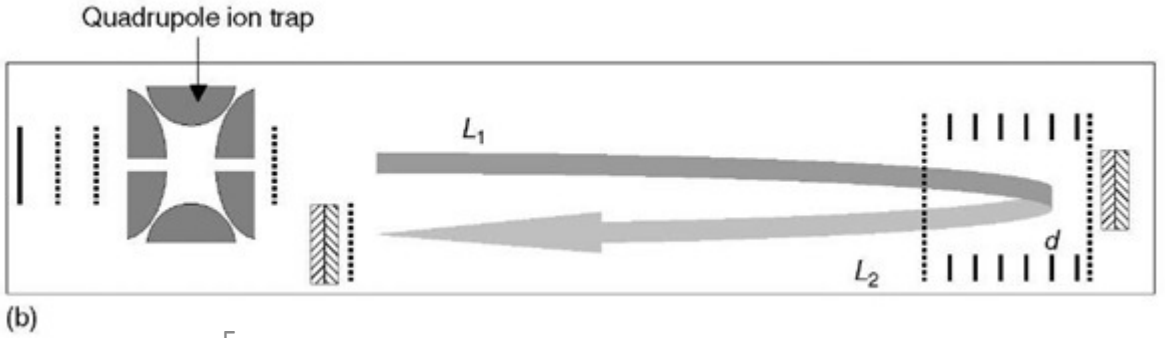
Agilent Technologies SHIMADZU BRUKER AB SCIEX Waters

- **QTOF MS:**



- **Qualitative and quantitative**
- **Excellent for MS/MS analysis**
- **Ideal intact protein analysis (** high-resolution, accurate-mass data on proteins (MW. hundreds of kDa)
- **SWATH: data-independent acquisition (DDA) strategy**
- ***> 3,000 proteins or 15,000 peptides***
- ***Coefficients of variation (CV) <20%***
- ***> 4 orders of biological dynamic range***

- **Ion-trap TOF MS:**



HRMS platforms
高分辨质谱技术和平台

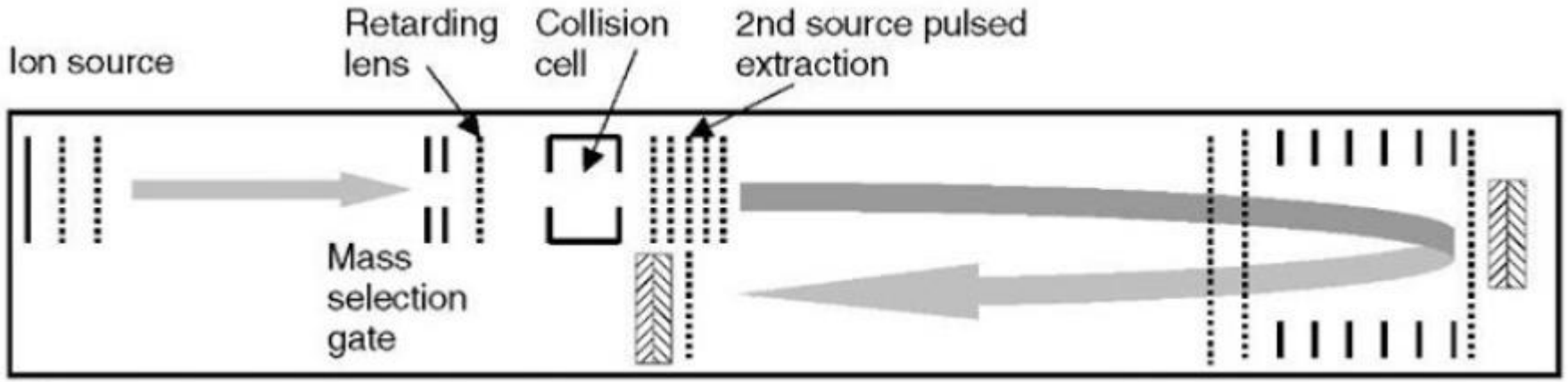
Time-of-flight MS (TOF MS)

Agilent Technologies SHIMADZU BRUKER AB SCIEX Waters

- **TOF/TOF:**

  - **Two reflectrons**

  - **A linear, pulsed extraction first analyzer, a mass selection gate at (or near) the space-velocity focus point, a collision chamber, and a second reflectron mass analyzer**



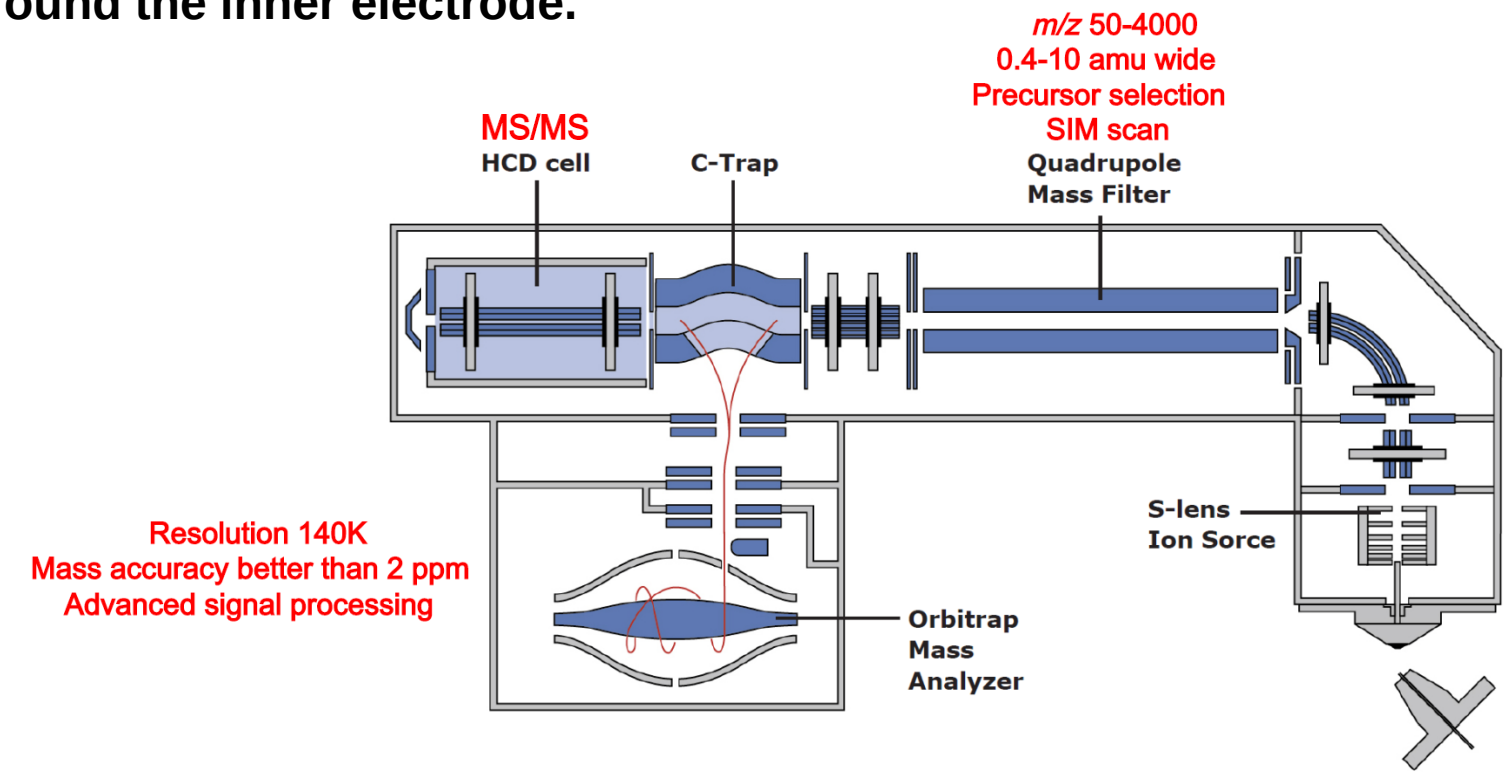
HRMS platforms
高分辨质谱技术和平台

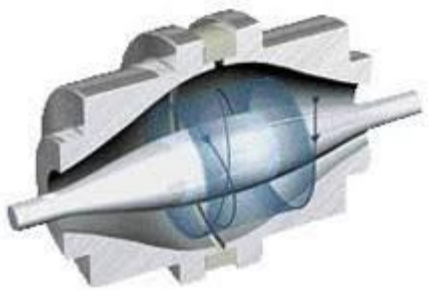
Orbitrap MS

Thermo SCIENTIFIC

Measures frequency and then provides m/z using a Fourier transform

- **Orbitrap:** ions are trapped between an outer electrode and an inner electrode, where the ions orbit around the inner electrode.





Q Exactive Plus Orbitrap

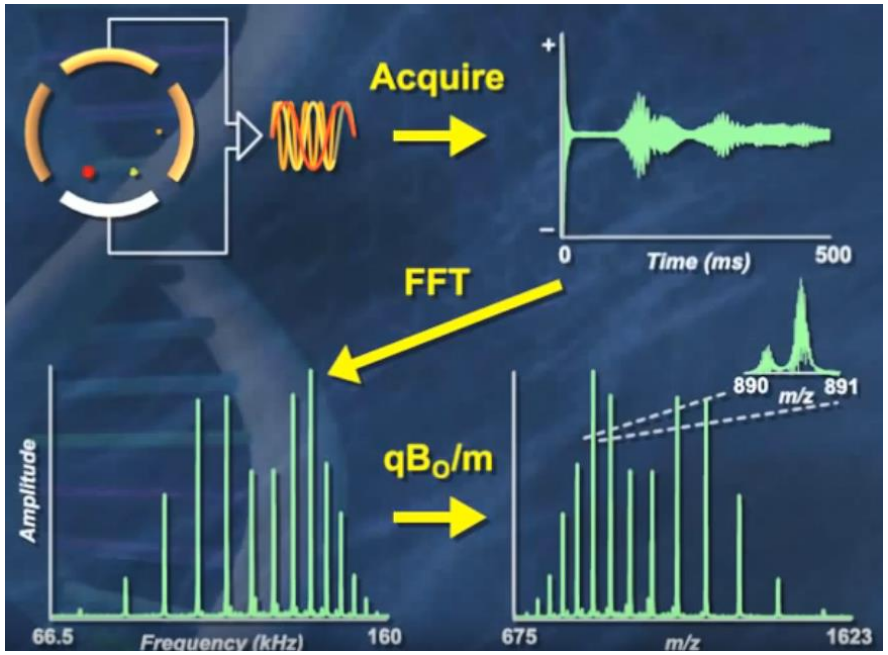
HRMS platforms
高分辨质谱技术和平台

FT-ICR MS

BRUKER Thermo SCIENTIFIC

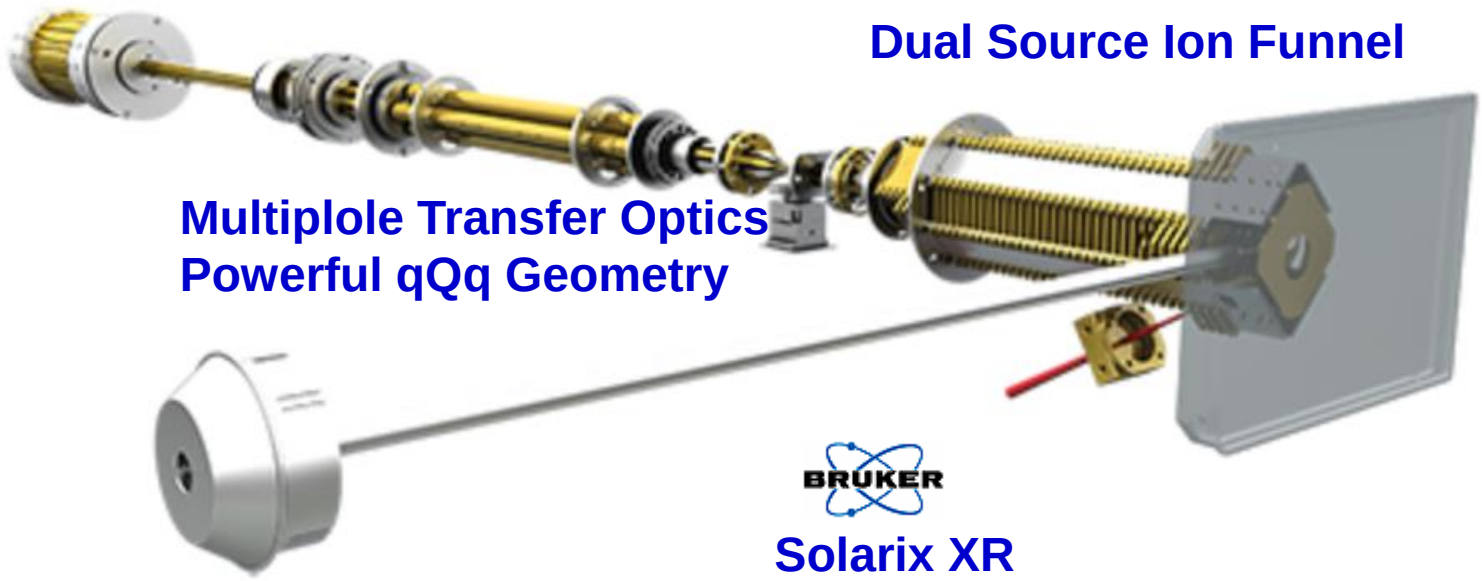
Measures frequency and then provides m/z using a Fourier transform

- **FT-ICR(Fourier-transform ion cyclotron resonance): ions form, cool, focus, and accumulate, move to a Penning trap, which stores the ions and excites them to their cyclotron frequencies.**



Paracell Detector

Dual Source Ion Funnel



Multiplole Transfer Optics
Powerful qQq Geometry

BRUKER

Solarix XR

HRMS for intact protein quantitation and profiling
高分辨质谱技术在完整蛋白药物定性和定量分析的应用

Why the intact MS approach?

- **Limitations of the LC-MS/MS approach using surrogate peptides:**

  - Not directly measure the protein isoform
  - Potentially miss biotransformation information
  - Negatively be affected by sample preparation artifacts
  - Require longer sample preparation time
  - Require time and efforts to select surrogate peptides
  - Require extensive evaluations on digestion efficiency and reproducibility
  - Require optimization of MRM methods

HRMS for intact protein quantitation and profiling
高分辨质谱技术在完整蛋白药物定性和定量分析的应用

Why the intact MS approach?

- **Benefits of the intact MS approach:**

  - Identification and quantitation of catabolites
  - Comprehensive understanding of circulating biotherapeutic forms, biotransformations, glycoforms and post-translational modifications (PTMs).
  - Higher throughput with reduced sample pre-treatment

HRMS for intact protein quantitation and profiling
高分辨质谱技术在完整蛋白药物定性和定量分析的应用

Challenges of the intact MS approach

- **Sensitivity:** multiple charge states
- **Specificity:** interference from endogenous proteins
- **Chromatography:** limited separation efficiency, analyte heterogeneity
- **Data analysis:** Single or multiple charge states? Deconvoluted spectrum?

HRMS for intact protein quantitation and profiling
高分辨质谱技术在完整蛋白药物定性和定量分析的应用

Strategies for improving the intact MS approach

- Maximizing enrichment by IA by using best capturing antibody for improving mass spectrometry S/N?
- Optimizing extraction window for quantitation using extracted ionextracted-ion chromatogram (XIC or EIC)?
- Optimization of chromatographic conditions for intact proteins
- Subunits quantification?
- Summing charge states/isotope signals?
- Deglycosylation?
- Charge state coalescence with DMSO
- Others…

HRMS for intact protein quantitation and profiling
高分辨质谱技术在完整蛋白药物定性和定量分析的应用

Case study 1

analytical chemistry

Article

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Generic Hybrid Ligand Binding Assay Liquid Chromatography High-Resolution Mass Spectrometry-Based Workflow for Multiplexed Human Immunoglobulin G1 Quantification at the Intact Protein Level: Application to Preclinical Pharmacokinetic Studies

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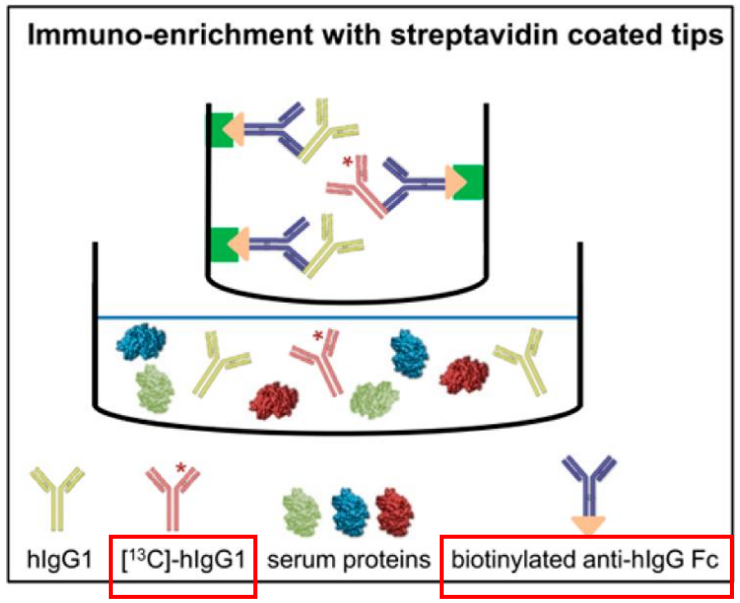
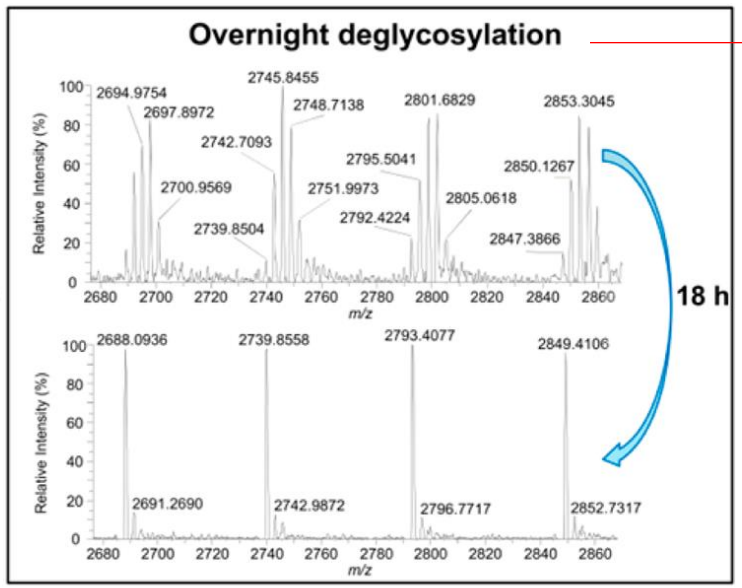
Case study 1

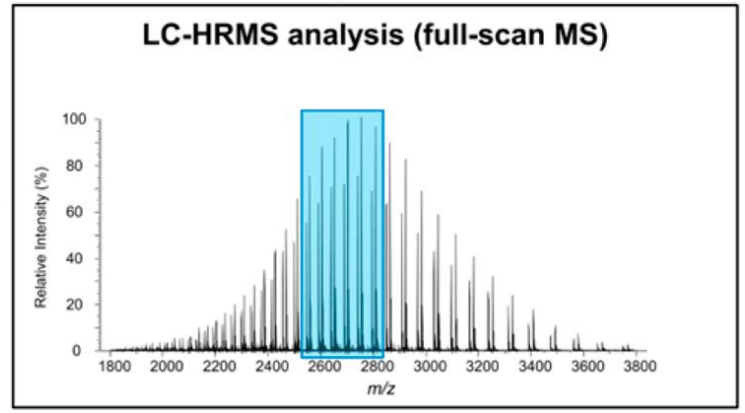


hIgG1 elution by acid dissociation

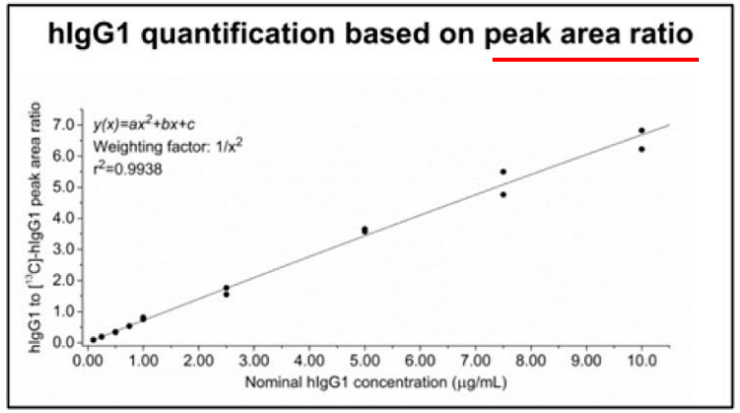


Optional (inactive vs. active glycan forms)

Data acquisition



XIC of six most intense charge states with a defined MXW



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Case study 1

- **LC**
  - Dionex UltiMate 3000 LC (Thermo Fisher Scientific)
  - 60 µL injection
  - The monolithic **ProSwift RP-4H** column (1 mm × 250 mm) @ **70 °C**
  - The mobile phases consisted of 0.1% FA in water (A) and 0.1% FA in ACN (B)
  - Flow rate: **200 µL/min**
- **MS**
  - **Q-Exactive hybrid quadrupole orbitrap** (Thermo Fisher Scientific)
  - MS **resolution @ 17 500** at full width at half-maximum at m/z 200
  - **inject time: 150 ms**

- **Data processing:**
  - XICs of 6 most intense charge states:m/z 2543−2545 (56+) to m/z 2952−2954 (49+)
  - Mass extraction window (MXW): 2 m/z units +/-5 ppm
  - Area under the curve (AUC): using summed XICs
  - Deconvolution: Protein deconvolution software 4.0 (Thermo Fisher Scientific) using the ReSpect algorithm for isotopically unresolved spectra
  - Input MS: m/z 2400−3400 covering in total 18 charge states
  - Output MS: 142−146 kDa
  - Noise rejection confidence interval of 95%

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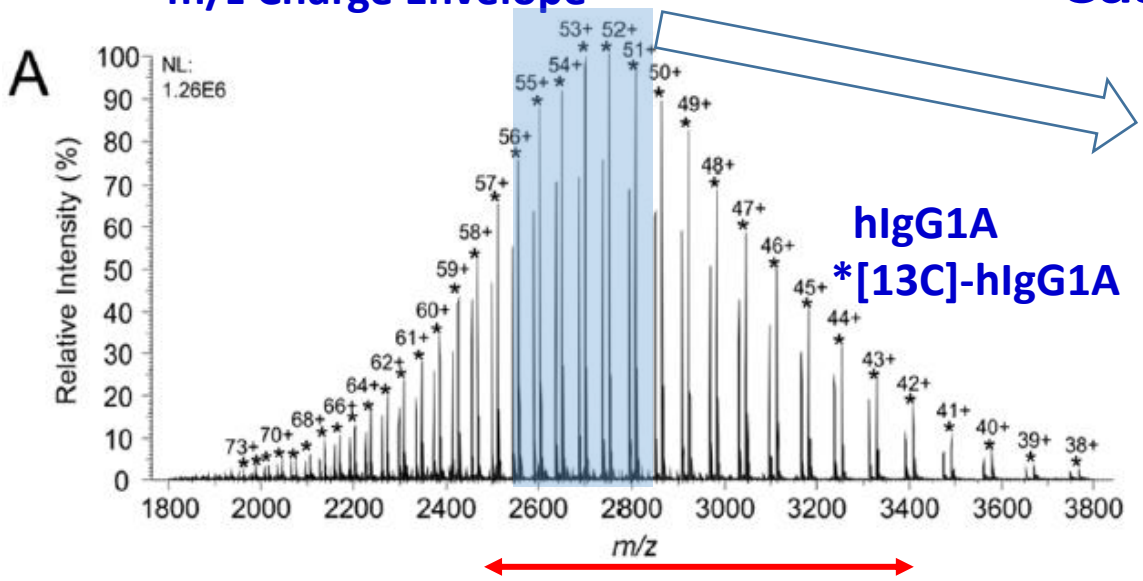
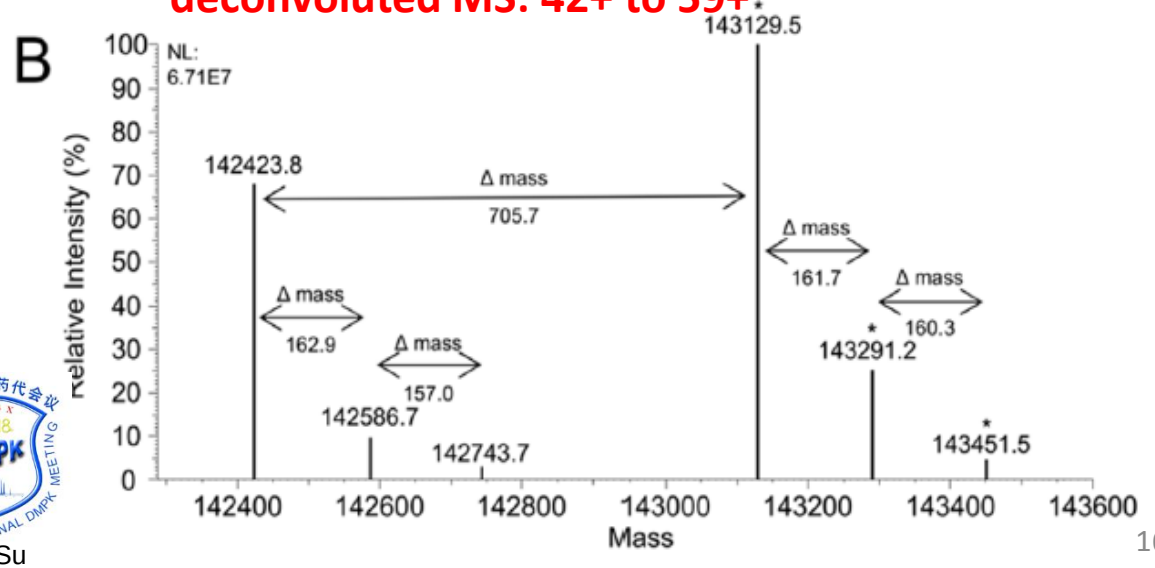
Case study 1

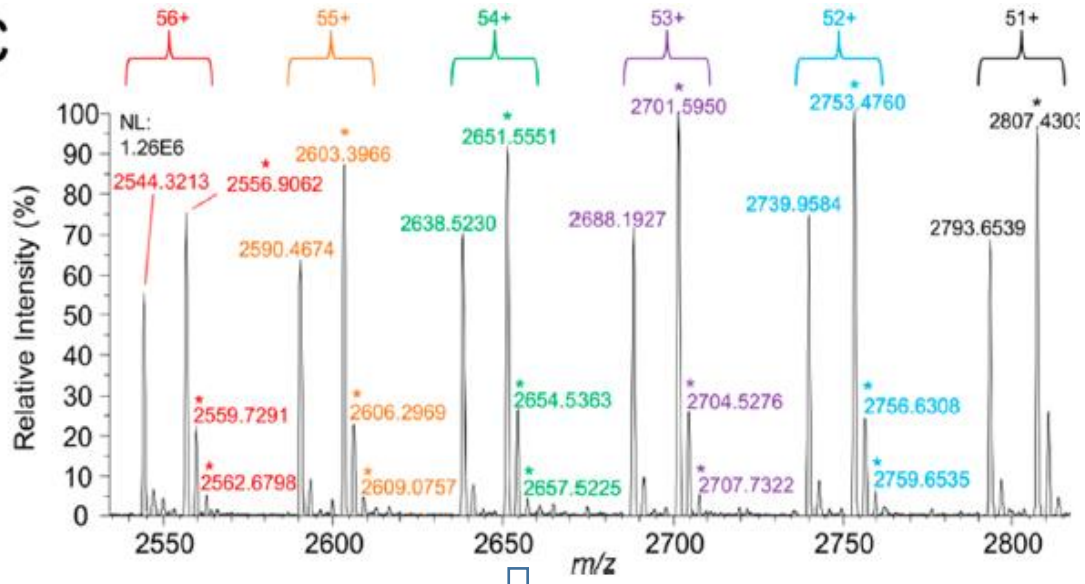
m/z Charge Envelope



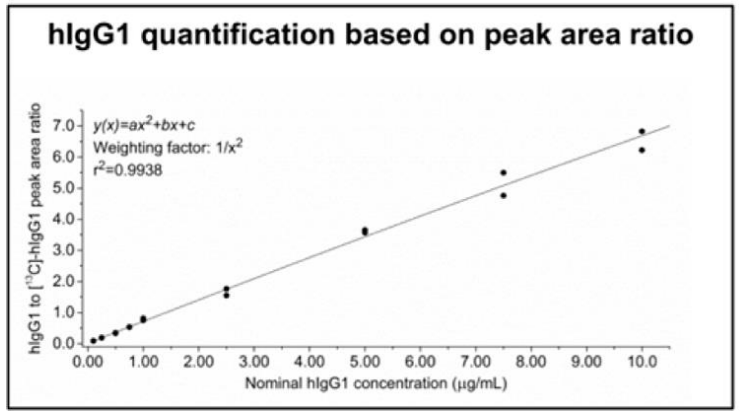
deconvoluted MS: 42+ to 59+



XIC: 51+ to 56+



Sum peak area of XICs



LLOQ: 0.100 µg/mL
ULOQ :10.0 µg/mL

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HRMS for intact protein quantitation and profiling
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Case study 1

Table 1. Accuracy and Precision Data Obtained in QC Samples Spiked with hIgG1A Based on the Peak Area Ratio Using the XIC Approach with Different Numbers of Charge States and a MXW Width of 2 m/z Units

			nominal QC concentration in rat serum (μg/mL)			
			8.00	5.00	0.250	0.100
	no. of charge states		intraday accuracy and precision (n = 3)			
3	3 (51+ to 53+)	mean concentration (μg/mL)	8.70	5.44	0.273	0.109
	r^2 = 0.9891	intraday accuracy (% bias)	8.7	8.7	9.4	9.0
		intraday precision (% CV)	3.2	11.3	8.4	1.9
6	6 (51+ to 56+)	mean concentration (μg/mL)	8.09	5.45	0.265	0.112
	r^2 = 0.9932	intraday accuracy (% bias)	1.1	9.0	5.9	12.3
		intraday precision (% CV)	3.6	3.5	4.1	6.7
9	9 (48+ to 56+)	mean concentration (μg/mL)	8.51	5.55	0.289	0.115
	r^2 = 0.9876	intraday accuracy (% bias)	6.4	11.0	15.6	15.4
		intraday precision (% CV)	6.1	3.9	3.4	7.7
18	18 (42+ to 59+)	mean concentration (μg/mL)	8.32	5.53	0.291	0.119
	r^2 = 0.9928	intraday accuracy (% bias)	4.0	10.6	16.6	19.2
		intraday precision (% CV)	6.2	1.0	1.6	3.3

Acceptance criteria: ±20% (±25% @LLOQ /ULOQ)

- **The number of charge states did not impact the assay's accuracy and precision.**
- **Inaccurate data @LLOQ with a higher number of charge states used for the XIC approach.**

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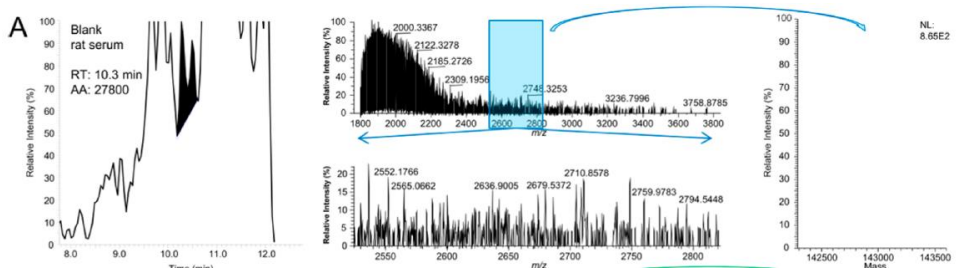
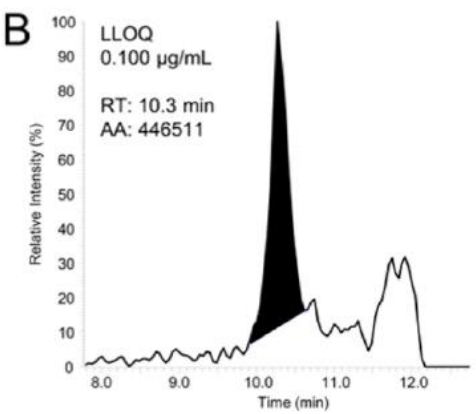
Case study 1

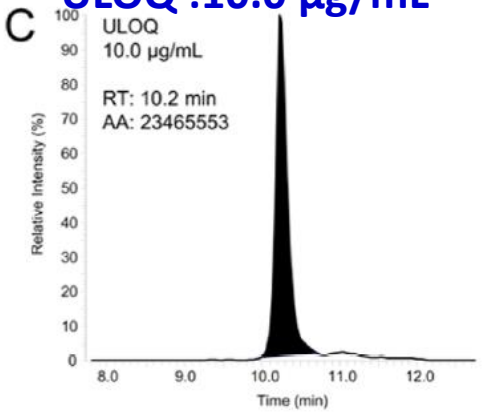
Blank



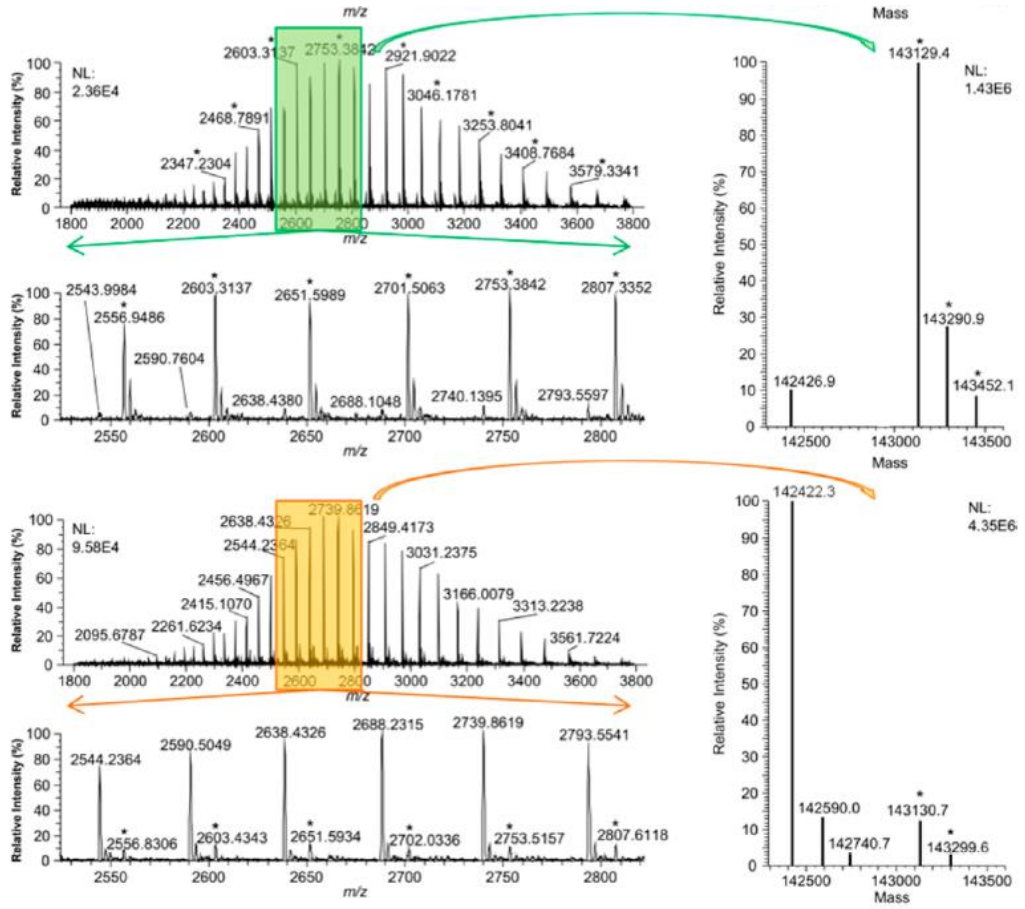
LLOQ: 0.100 µg/mL



ULOQ :10.0 µg/mL



6 charges



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Case study 1

Table 2. Summary of Hybrid LBA-LC–HRMS Method Validation for Intact hIgG1A Quantification in Rat Serum

parameter	results
selectivity: three blank batches ($n = 3$)	hIgG1A: ≤3.0%, [^{13}C]-hIgG1A: ≤0.3%
contribution of signal	[^{13}C]-hIgG1A to hIgG1A: 12.8%, hIgG1A to [^{13}C]-hIgG1A: 13.0%
linearity ($n = 3$), $y = ax^2 + bx + c$, $1/x^2$ weighting	0.100−10.0 μg/mL, $r^2 = 0.9919 \pm 0.0027$
carry-over (blank after ULOQ sample)	hIgG1A: <LLOQ, [^{13}C]-hIgG1A: 0.0% of response in zero sample
accuracy (% bias) and precision (% CV)	intraday ($n = 3$): −2.7 to 16.0% bias, 1.3 to 11.7% CV
QC at 0.100, 0.250, 5.00, and 8.00 μg/mL	interday ($n = 9$): −0.1 to 9.3% bias, 6.1 to 8.7% CV
dilution linearity (300 μg/mL, 50-fold, $n = 5$)	mean bias of 2.9% with precision of 8.6% CV
reproducibility	97% of incurred samples ($n = 30$) met acceptance criterion of ±20%

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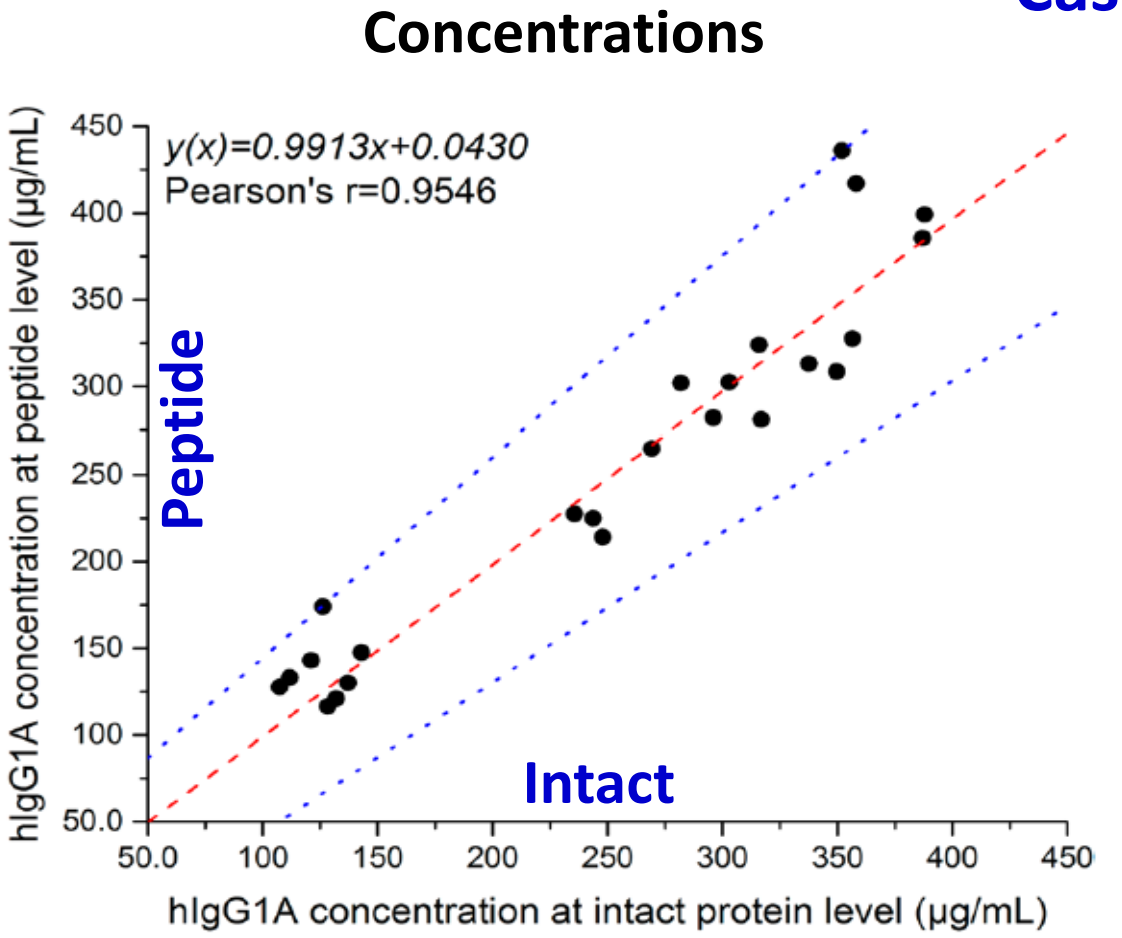
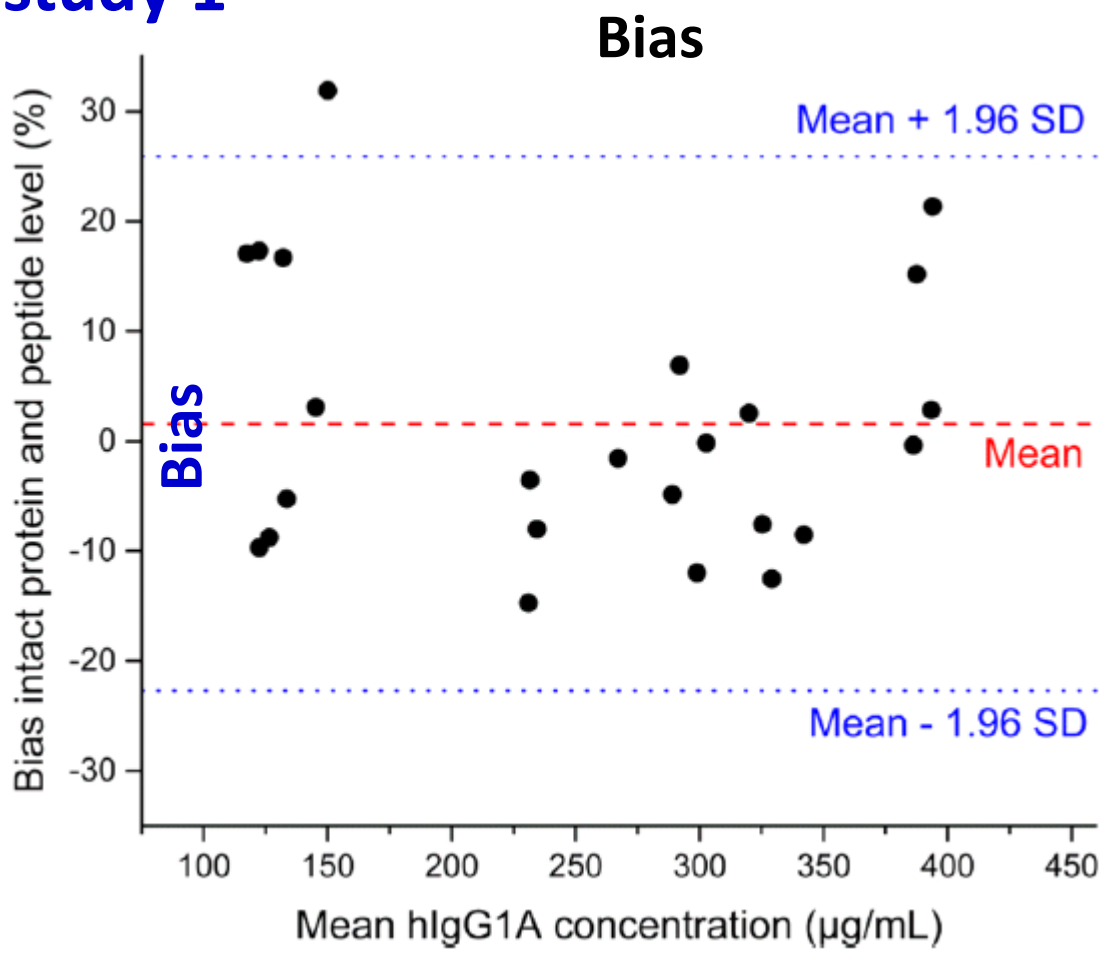
Case study 1





- **Hybrid LBA-LC–HRMS Intact MS: equivalent results relative to LC–MS/MS @ peptide level**

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HRMS for intact protein quantitation and profiling
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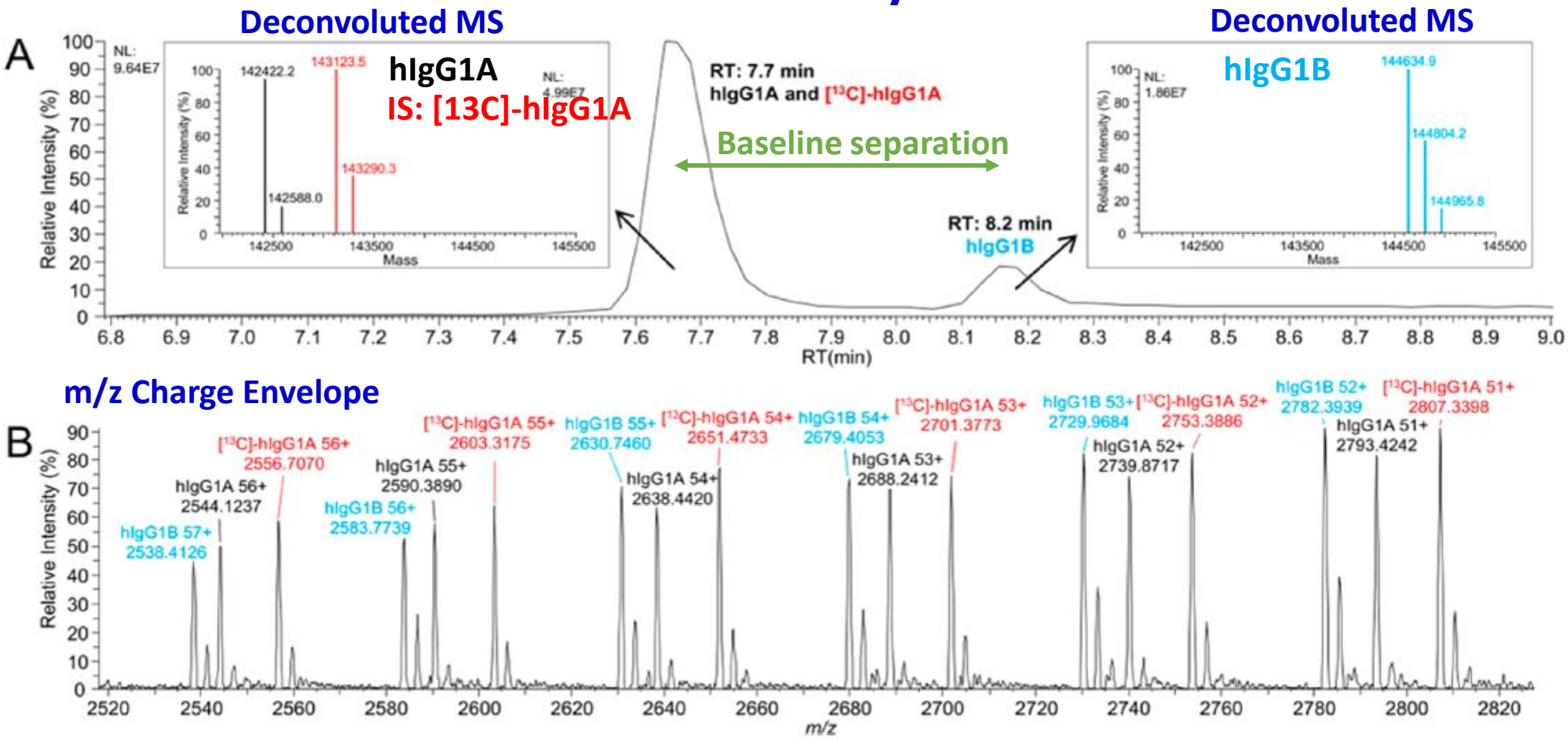
Case study 1



- **Multiplexing quantification of hIgG1A and hIgG1B in rat serum**

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HRMS for intact protein quantitation and profiling
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Case Study 2A

Bioanalysis

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A whole-molecule immunocapture LC–MS approach for the *in vivo* quantitation of biotherapeutics

Aim: Large-molecule biotherapeutic quantitation *in vivo* by LC–MS has traditionally relied on enzymatic digestion followed by quantitation of a 'surrogate peptide' to infer whole-molecule concentration. MS methods presented here measure the whole molecule and provide a platform to better understand the various circulating drug forms by allowing for variant quantitation. **Results:** An immunocapture LC–MS method for quantitation of a biotherapeutic monoclonal antibody from human plasma is presented. Sensitivity, precision and accuracy for each molecular portion are presented along with an example of glycoform variant quantitation. **Conclusion:** The method is presented as a basic platform to be further developed for Good Practice (GxP) applications, critical quality attribute analysis or general understanding of molecular forms present as required for the wide range of drug development processes.

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Kellie *et al.* Bioanalysis, 2016, 8 (20), 2103-2114

HRMS for intact protein quantitation and profiling
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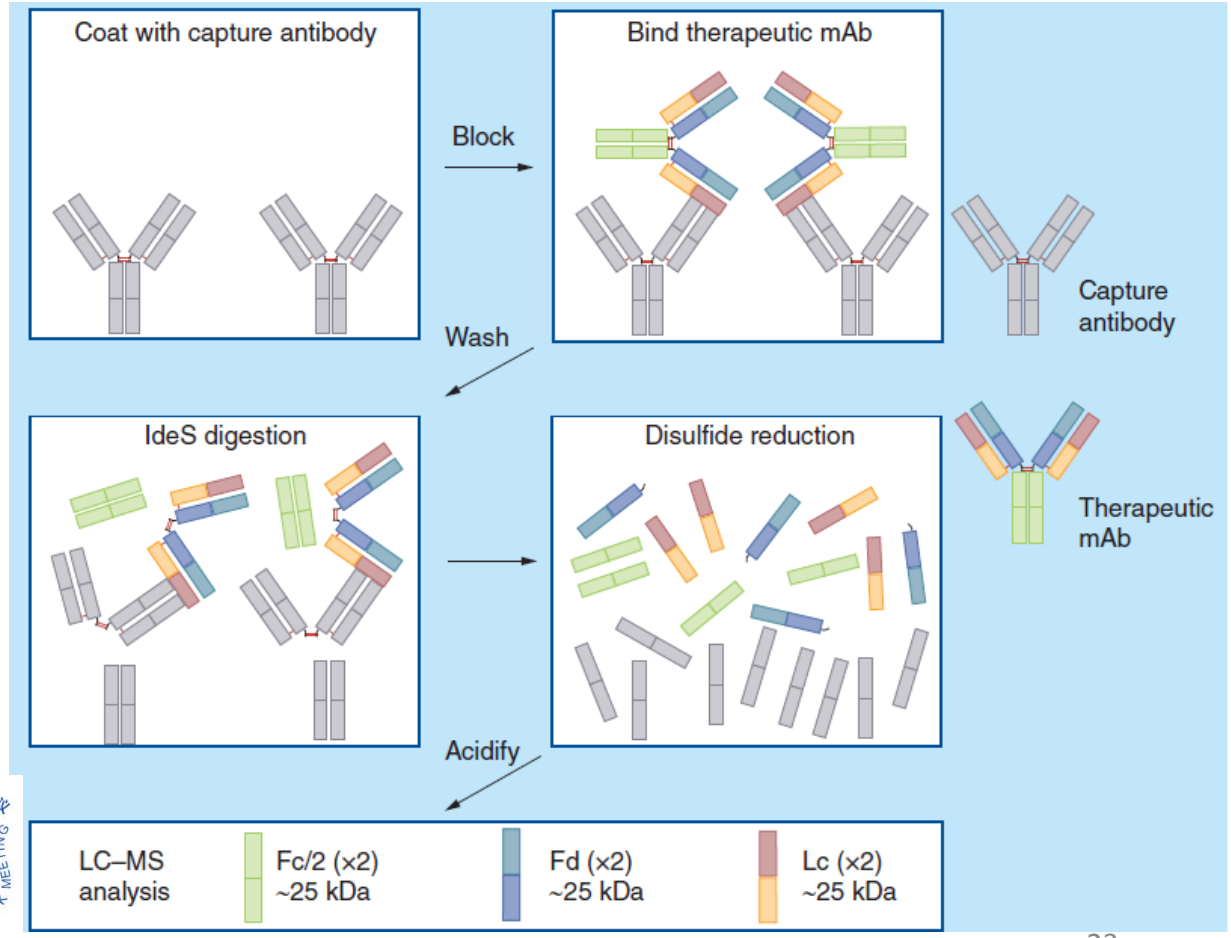
Case study 2A

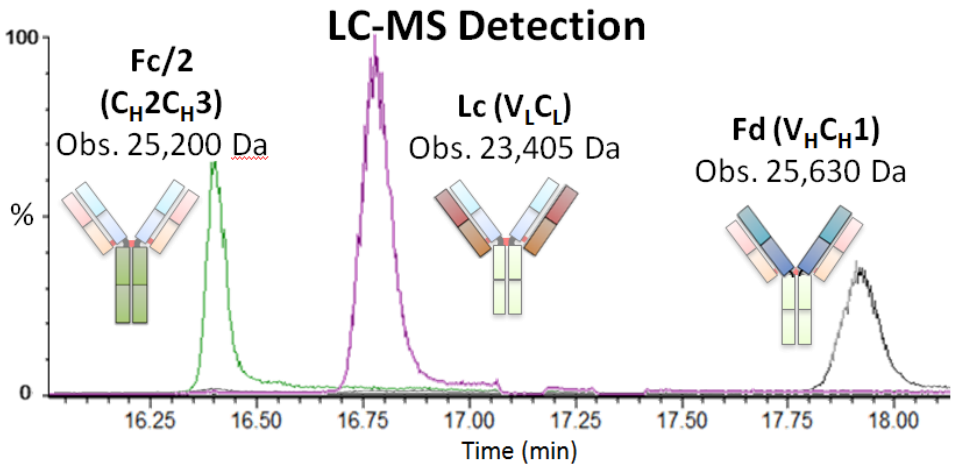
- **The immunocapture, elution and reduction steps prior to LC–MS analysis**

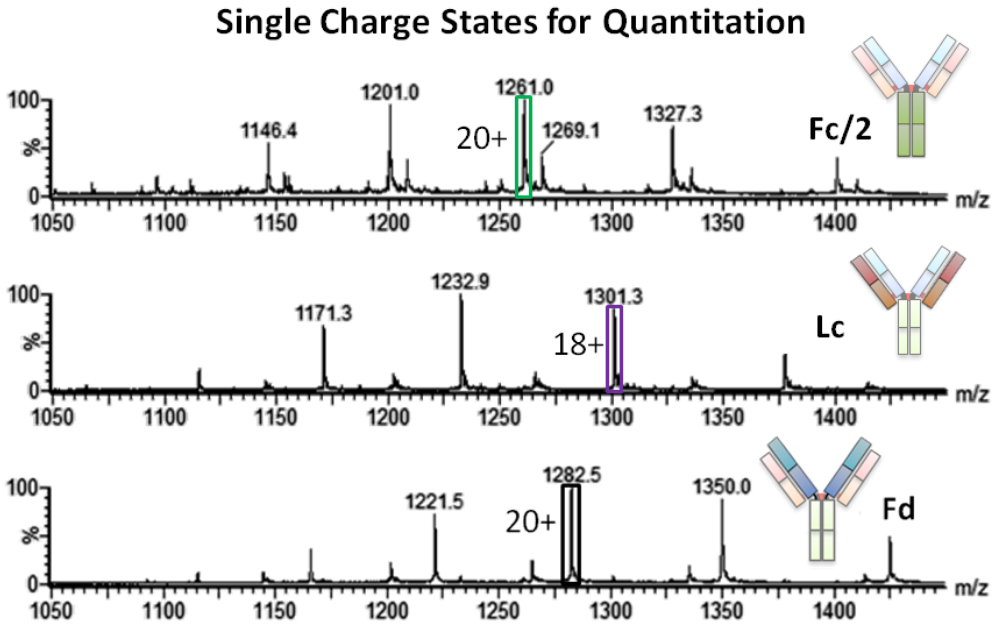


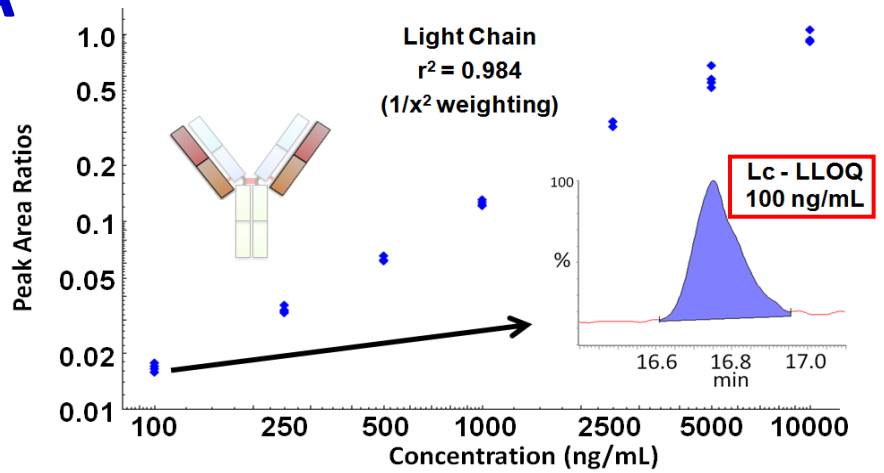
- **LC :**
  - **Waters M Class Acquity LC system**
  - An iKEY BEH PrST **C4 column** (150 µm X 50 mm, 300 Å, 1.7 µm)
  - LC buffer A: 0.1% FA in water; buffer B: 60:40 ACN:IPA w/ 0.1% FA.
  - 3 µL injection
  - Run time of 22 min at **65°C**
  - Flow: 2 µl/min @ 90% A @ 0-4 min; **0.75 µl/min** @70% A @ 4.5 min; 50% A @ 18 min; 20% A @ 18.5 min; 90% A @ 19.5 min; 2 µl/min@ 90% A @20 -22 min
- **MS:**
  - **Waters Synapt G2-Si Q-TOF**
  - ESI+ with an ionKey source

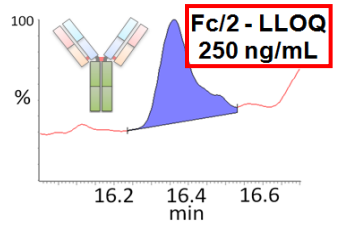
Kellie *et al.* Bioanalysis, 2016, 8 (20), 2103-2114

HRMS for intact protein quantitation and profiling
高分辨质谱技术在完整蛋白药物定性和定量分析的应用
Case study 2A









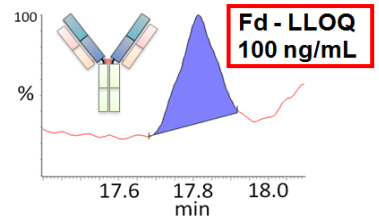


Table 1. Linearity, precision and accuracy results from immunocapture LC–MS method for a biotherapeutic monoclonal antibody.

	Fc/2		Lc		Fd	
r^2 value	0.982		0.984		0.980	
Conc. (ng/ml)	% bias	%CV	% bias	%CV	% bias	%CV
10,000	-9.0	11.1	-17.6	7.0	-11.6	8.5
5000	9.0	5.1	0.3	12.5	-3.0	2.2
2500	6.8	9.7	11.8	3.2	0.2	5.0
1000	-1.5	7.5	4.9	3.2	9.6	5.3
500	-9.6	7.0	2.0	3.4	8.2	10.2
250	4.3	2.7	-0.3	4.6	-1.5	10.0
100	NQ	NQ	-1.1	7.0	-2.4	24.2

Fd: Fab heavy chain; Lc: Fab light chain; NQ: Not quantifiable.

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HRMS for intact protein quantitation and profiling
高分辨质谱技术在完整蛋白药物定性和定量分析的应用
Case study 2A

- **Relative glycoform quantitation on the Fc/2 region**
- **Quantification is performed on single charge state data in the m/z domain**

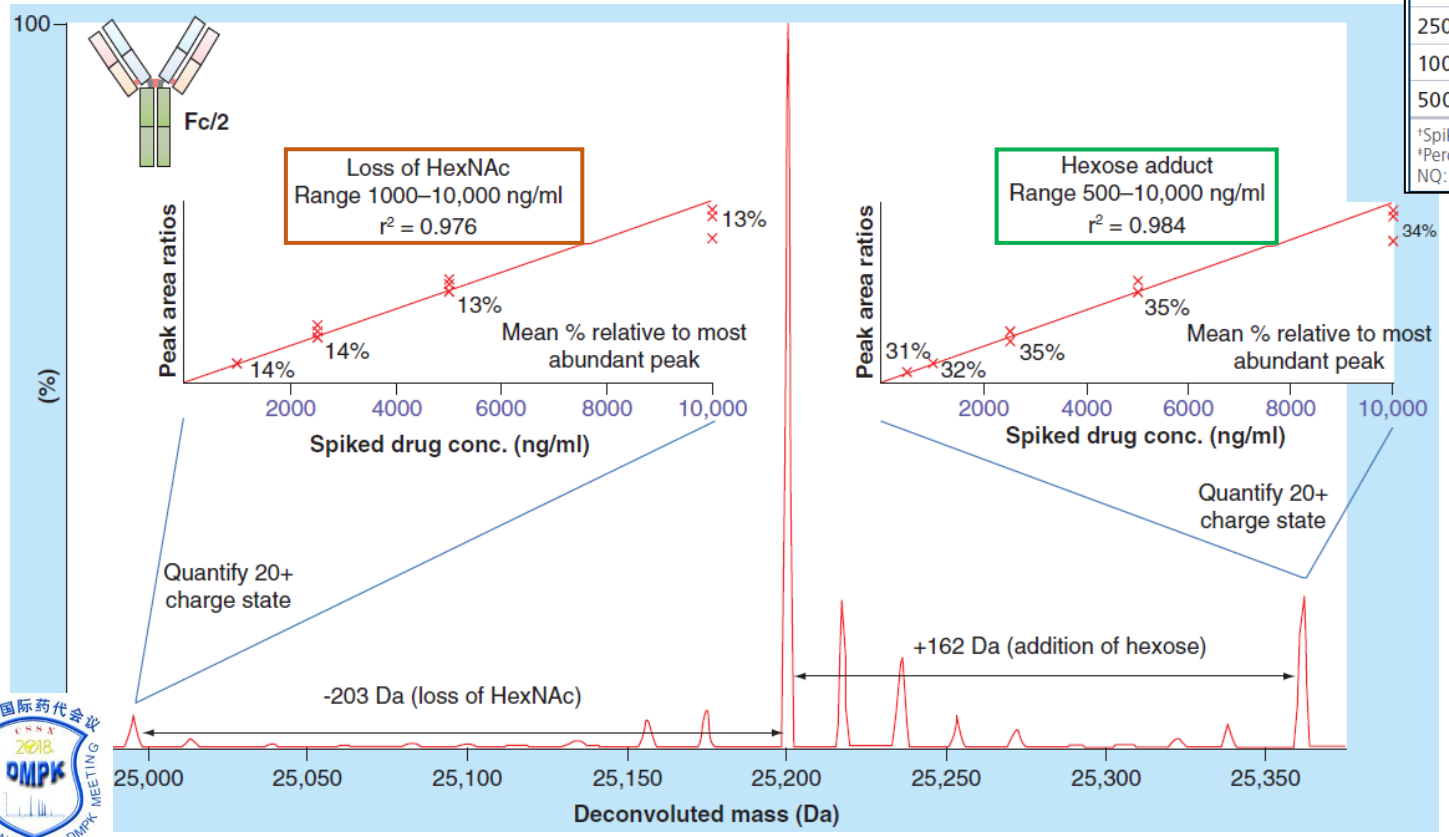

Table 2. Relative glycoform quantitation from Fc/2.

	Loss of HexNAc		Adduct of hexose	
r^2 value	0.976		0.984	
Conc. (ng/ml)†	% abundance‡	%CV	% abundance‡	%CV
10,000	12.9	3.8	34.0	6.4
5000	13.3	2.5	34.6	2.8
2500	14.2	9.1	34.6	3.0
1000	14.2	9.1	32.1	4.8
500	NQ	NQ	31.4	8.1

†Spiked stock drug concentration.
‡Percent relative abundance compared with most abundant Fc peak at 20+ charge state.
NQ: Not quantifiable.

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HRMS for intact protein quantitation and profiling
高分辨质谱技术在完整蛋白药物定性和定量分析的应用
Case study 2B

Bioanalytical Challenge

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Bioanalysis

Toward best practices in data processing and analysis for intact biotherapeutics by MS in quantitative bioanalysis

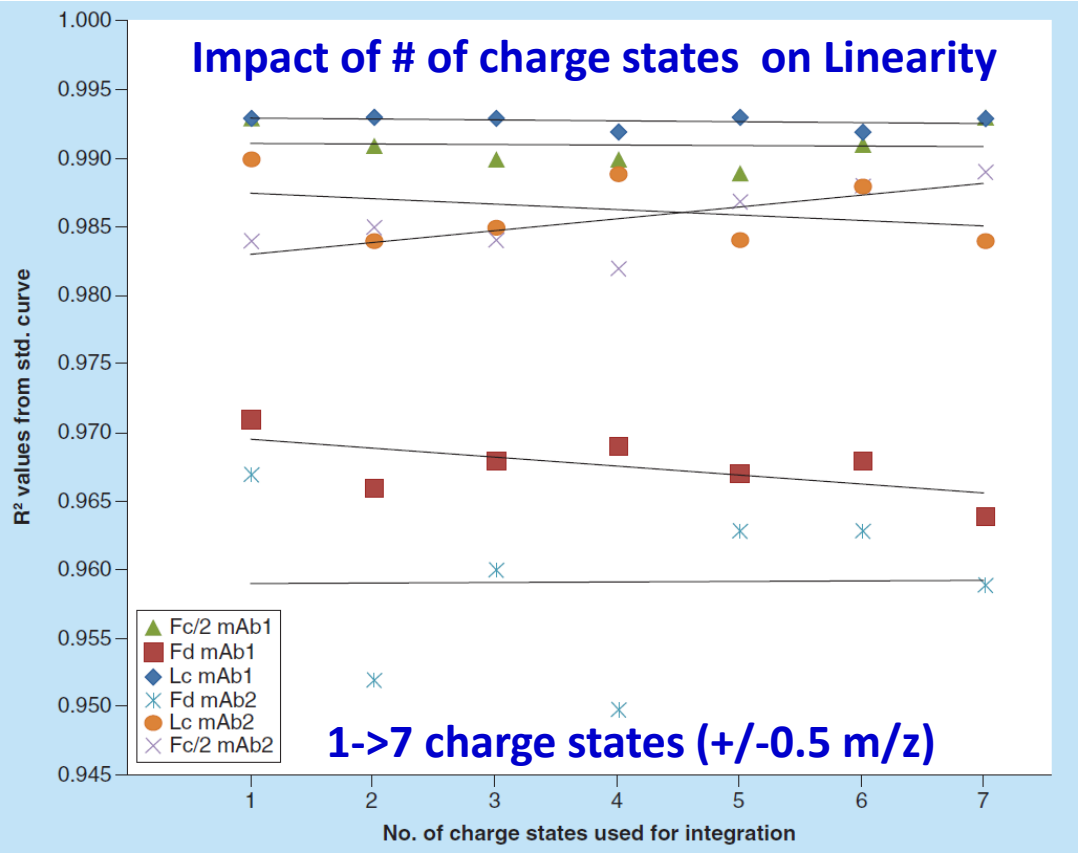
John F Kellie*,[1], Jonathan R Kehler[1], Molly Z Karlinsey[1] & Scott G Summerfield[12]

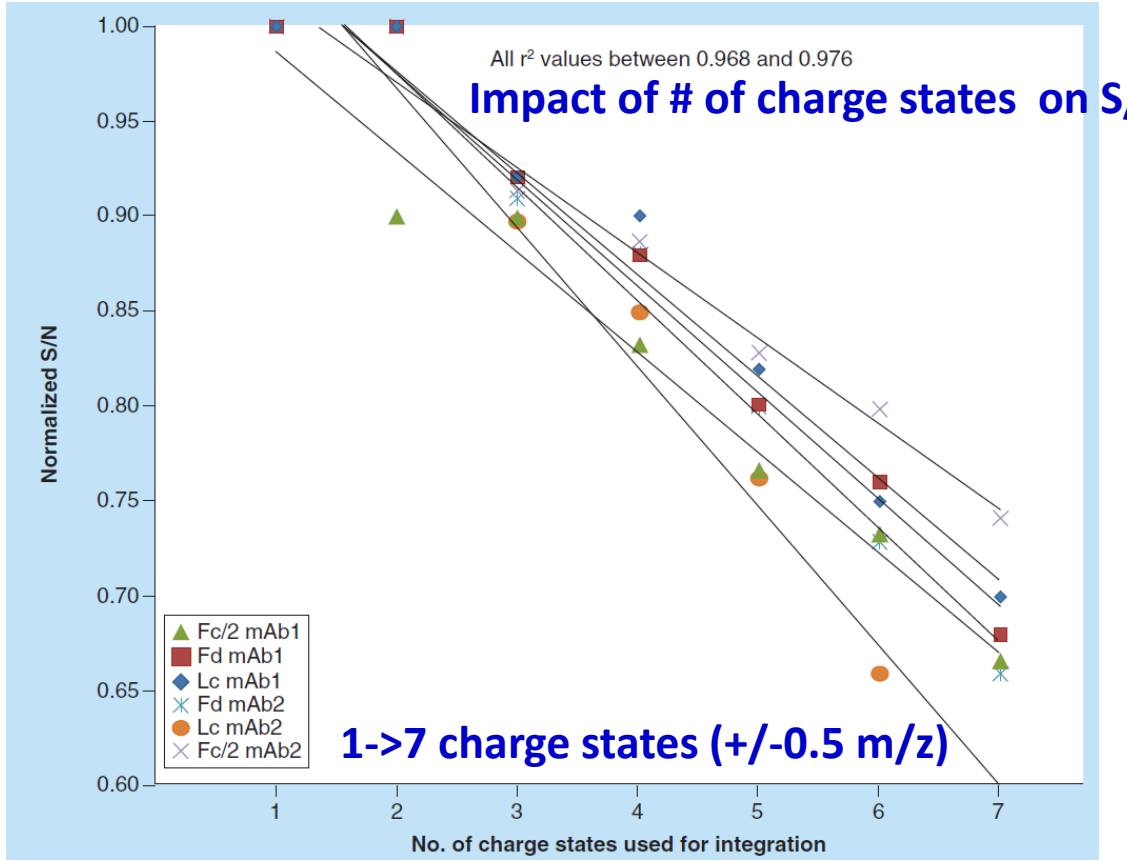
[1]Bioanalysis, Immunogenicity & Biomarkers, In vitro/In vivo Translation Platform, R&D Platform Technology & Science, GSK, 709 Swedeland Rd. King of Prussia, PA, 19460, USA

[2]Bioanalysis, Immunogenicity & Biomarkers, In vitro/In vivo Translation Platform, R&D Platform Technology & Science, GSK, David Jack Centre for R&D, Park Road, Ware, Hertfordshire SG12 0DP, UK

* Author for correspondence: john.x.kellie@gsk.com

HRMS for intact protein quantitation and profiling
高分辨质谱技术在完整蛋白药物定性和定量分析的应用
Case study 2B





Lc: mAb Light Chain; Fc: mAb Fragment Crysrallizable; Fd: mAb Fc Degraded; dAb: Domain Antibody.

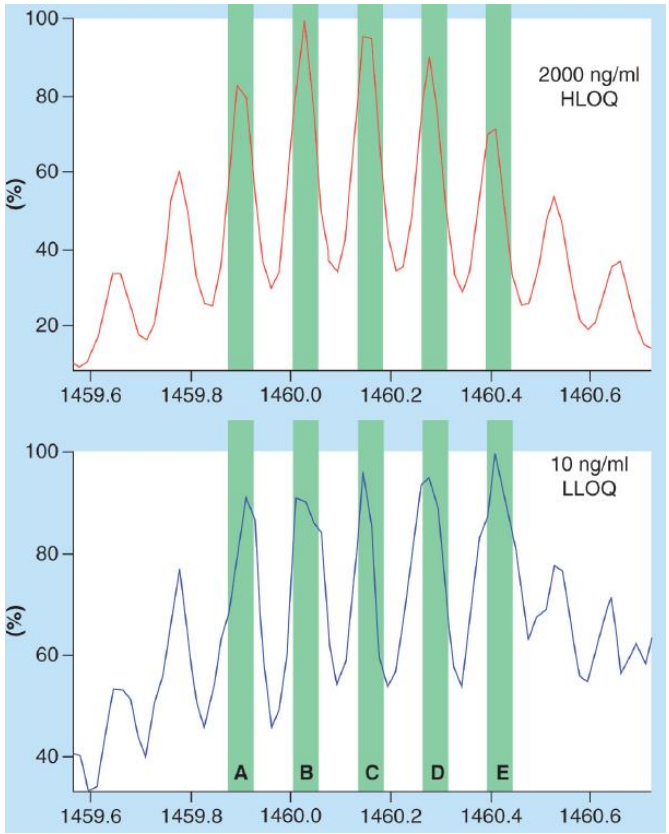
- Equivalent linearity observed regardless of number of charge states used for EIC integration
- Decrease in observed S/N at the LLOQ for each subunit as the number of charge states utilized increases

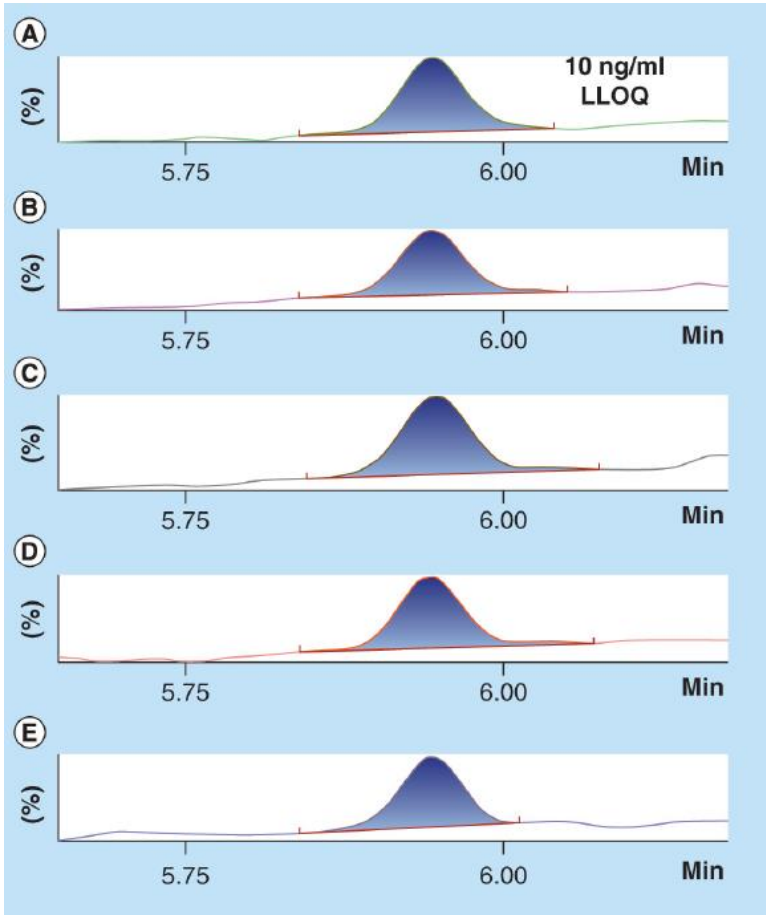
D. Su

Bioanalysis (2017) 9(23), 1883–1893

HRMS for intact protein quantitation and profiling
高分辨质谱技术在完整蛋白药物定性和定量分析的应用
Case study 2B

Multiple isotope quantitation of a domain antibody





- The isotopes for a single charge state are shown with a 0.05-m/z extracted-ion chromatogram integration width for the 8+ charge state.
- Extracted ion chromatograms from each isotope within the charge state that are summed to give total intensity.

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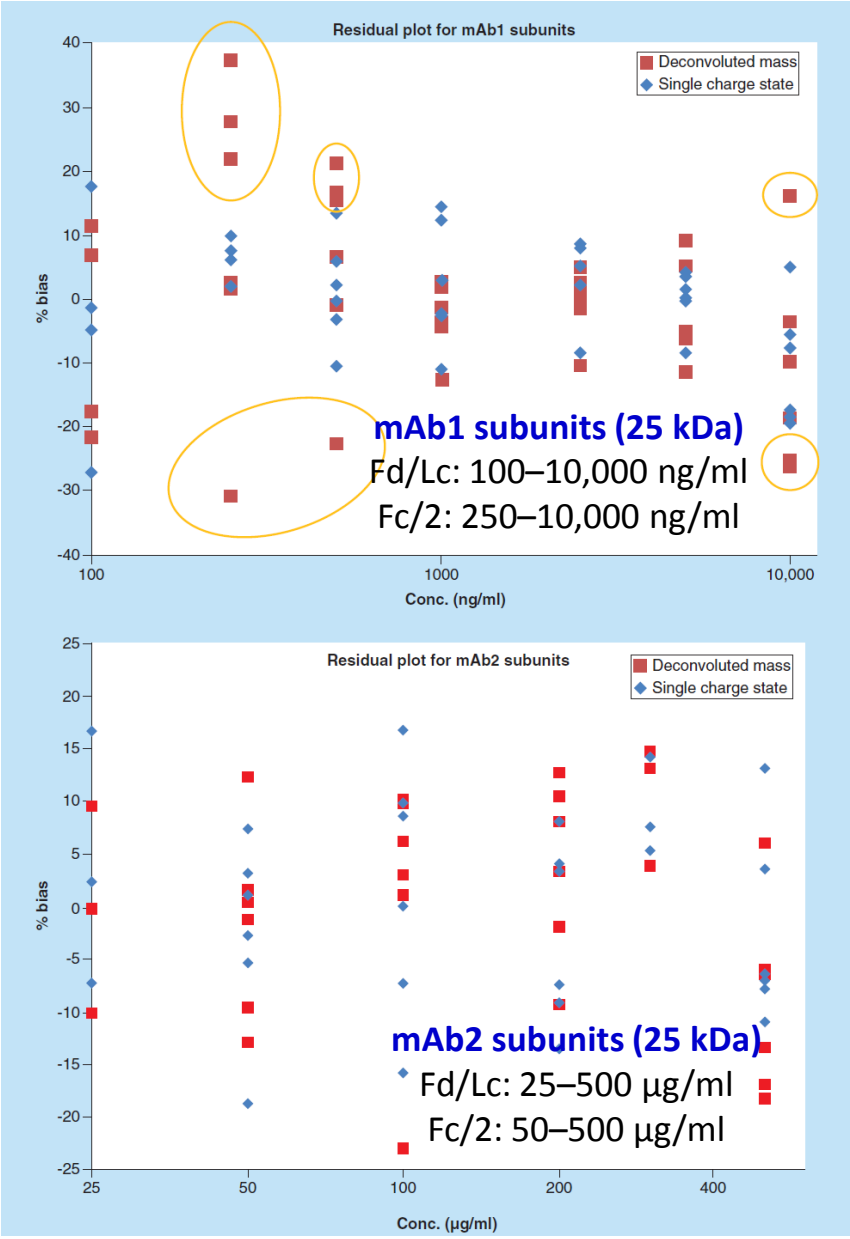
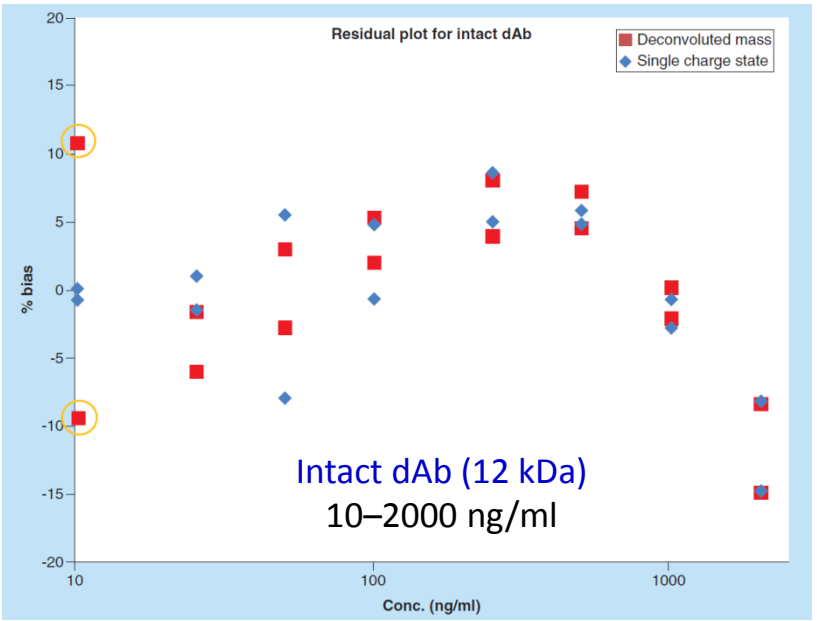
HRMS for intact protein quantitation and profiling
高分辨质谱技术在完整蛋白药物定性和定量分析的应用

Case study 2B



Higher %bias values of the **deconvoluted mass** relative to the **use of a single charge state** for the LLOQ is circled in orange



- **The charge state method demonstrates more accurate quantitation at the LLOQ than the deconvoluted mass approach.**

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HRMS for intact protein quantitation and profiling
高分辨质谱技术在完整蛋白药物定性和定量分析的应用
Case study 2B

Table 2. R^2 values observed for duplicate reference standard curves in plasma from subunits of two monoclonal antibodies and an intact domain antibody according to a single charge state area, sum of three charge state areas or deconvoluted mass peak height.

Mode	Lc mAb1	Fd mAb1	Fc/2 mAb1	Lc mAb2	Fd mAb2	Fc/2 mAb2	dAb
1 Charge state	0.993	0.971	0.993	0.989	0.976	0.984	0.995
3 Charge states	0.993	0.968	0.990	0.985	0.960	0.984	0.996
Deconvoluted mass	0.950	0.912	0.777	0.981	0.975	0.983	0.979

Lc: mAb Light Chain; Fc: mAb Fragment Crysrallizable; Fd: mAb Fc Degraded; dAb: Domain Antibody.

- **Based on the data presented here at 12–25 kDa, a recommendation for intact or large mass quantitation is to take single or a few high abundant, selected charge states (depending on molecular mass).**

Bioanalysis (2017) 9(23), 1883–1893

HRMS for intact protein quantitation and profiling
高分辨质谱技术在完整蛋白药物定性和定量分析的应用
Case study 3

Quantitative analysis of intact apolipoproteins in human HDL by top-down differential mass spectrometry

Matthew T. Mazur[a,2], Helene L. Cardasis[a], Daniel S. Spellman[a], Andy Liaw[b], Nathan A. Yates[a], and Ronald C. Hendrickson[a,1]

[a]Department of Proteomics and [b]Biometrics Research, Merck Research Labs, 126 E. Lincoln Avenue, P.O. Box 2000, Rahway, NJ 07065

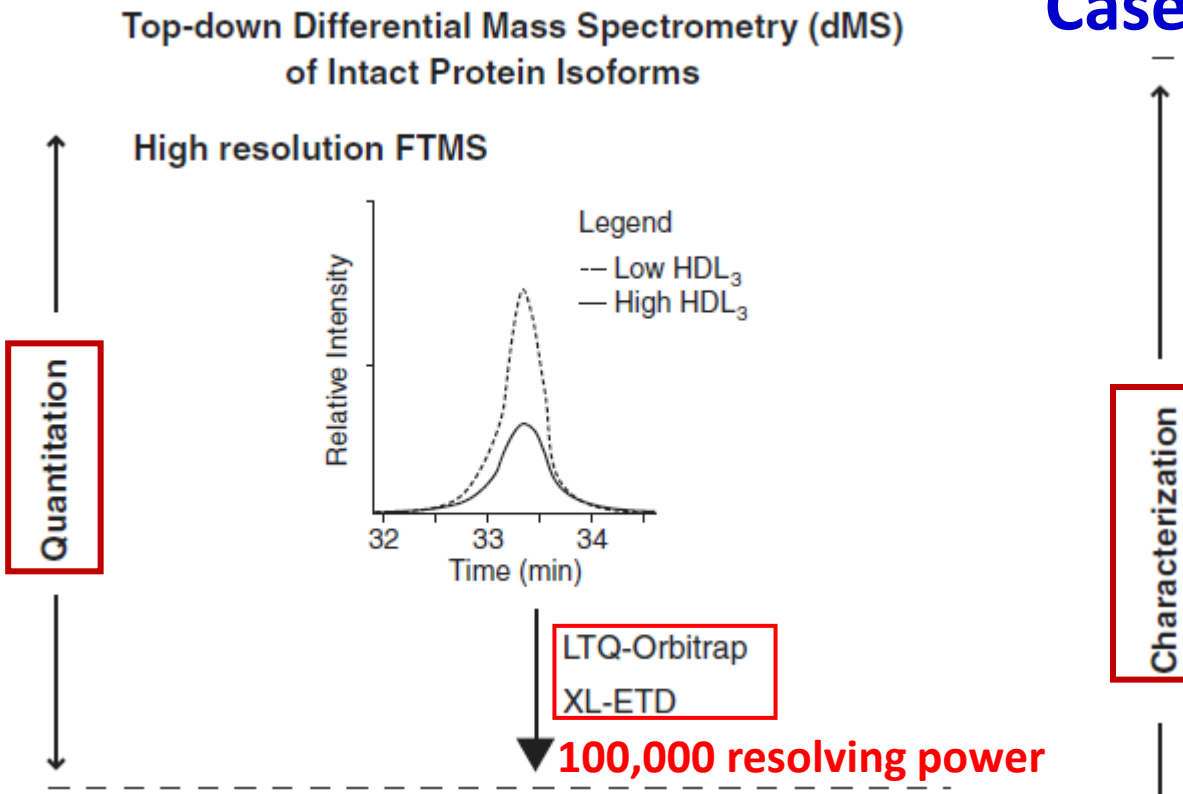
Edited by Fred W. McLafferty, Cornell University, Ithaca, NY, and approved February 17, 2010 (received for review September 22, 2009)

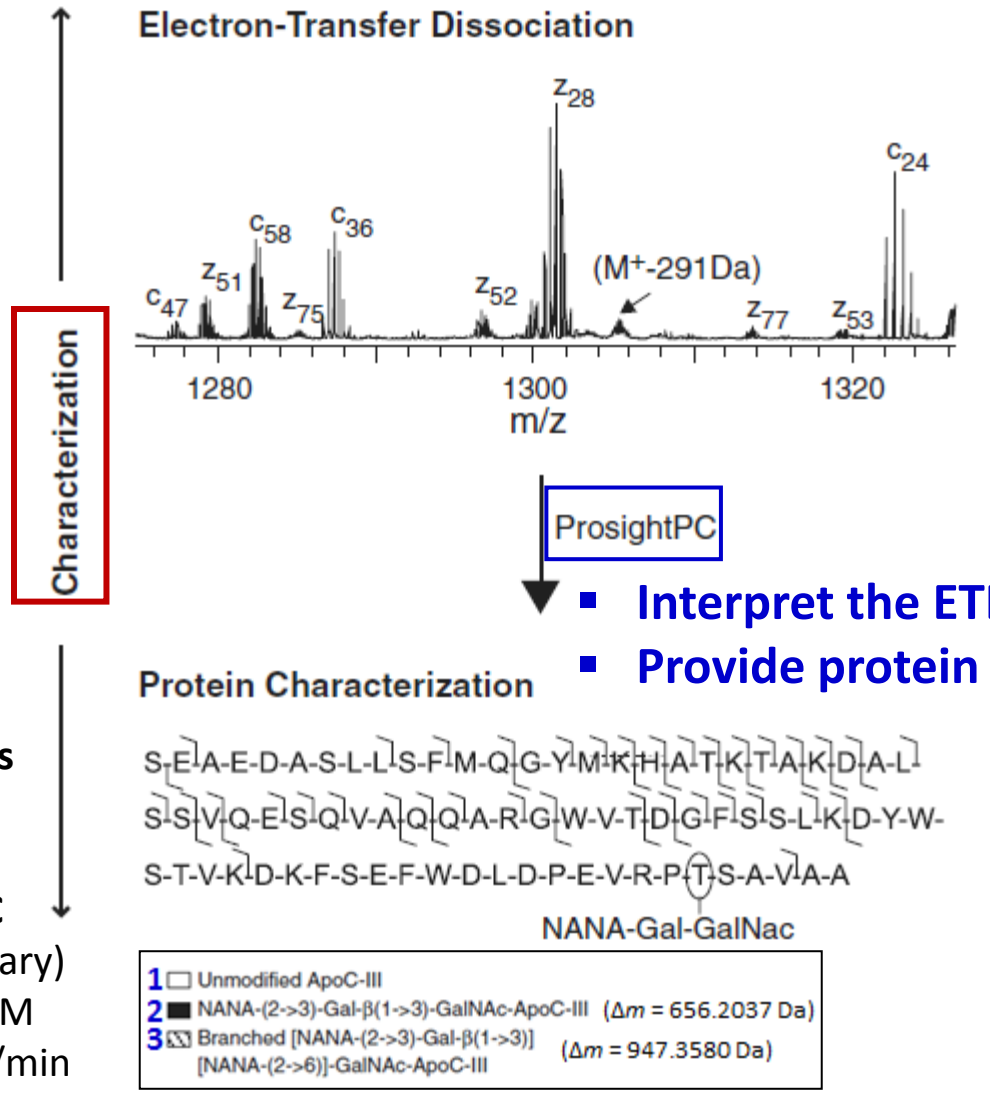
- **Identified the protein species at 9415.45 Da as an O-glycosylated form of apolipoprotein C-III [NANA-(2 → 3)-Gal-β(1 → 3)-GalNAc, +656.2037 Da], a protein associated with coronary artery disease**

PNAS April 27, 2010. 107 (17) 7728-7733

HRMS for intact protein quantitation and profiling
高分辨质谱技术在完整蛋白药物定性和定量分析的应用

Case study 3



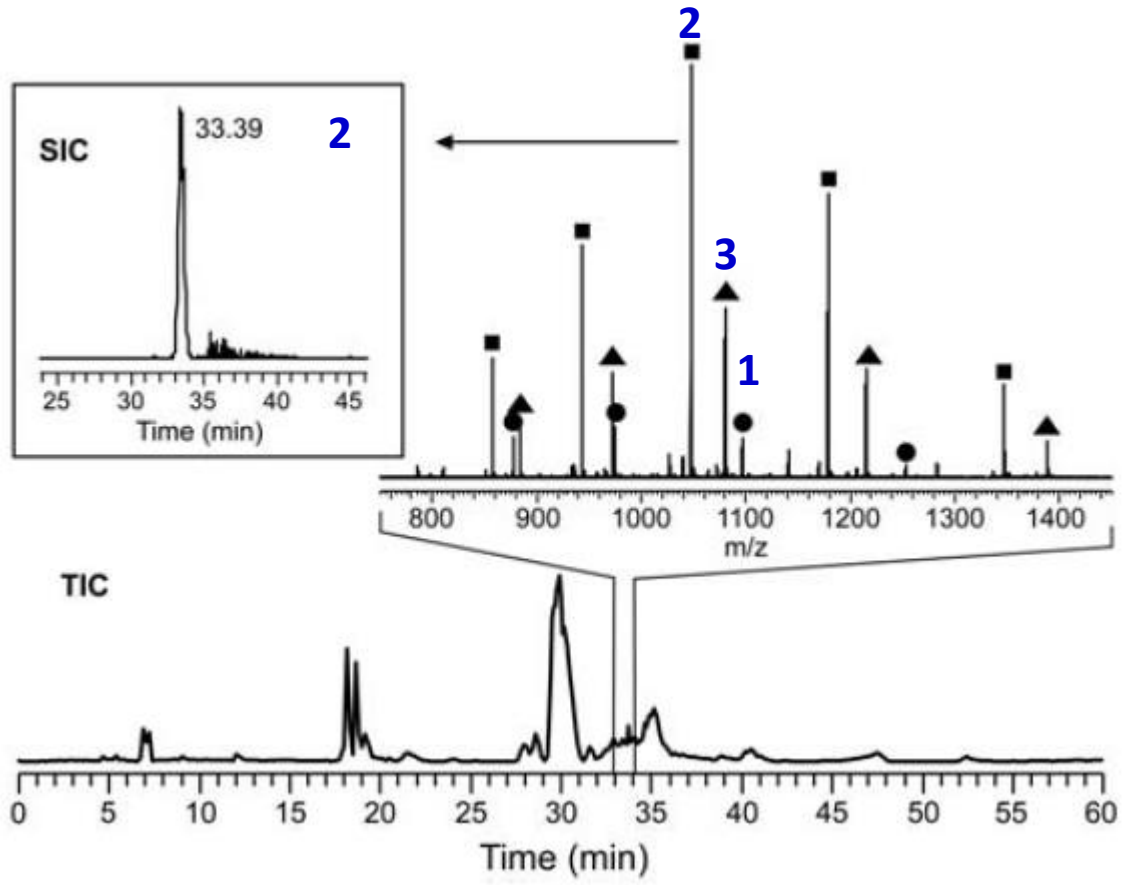


- **Interpret the ETD spectra**
- **Provide protein ID and PTMs**

- Reverse-phase nano-HPLC coupled to a **LTQ-FT hybrid mass spectrometer (ThermoFisher).**
- 1-µL aliquot inject onto a Biobasic C8 trap column (1 cm × 75 µm, New Objective) and eluted from a **nano-LC column** packed with POROS R1 media (6 cm × 75 µm capillary) using a binary gradient increasing from 10% solvent B (0.1 M acetic acid in acetonitrile) to 70% solvent B at a rate of 2%/min (Agilent 1100 series).

PNAS April 27, 2010. 107 (17) 7728-7733

HRMS for intact protein quantitation and profiling
高分辨质谱技术在完整蛋白药物定性和定量分析的应用
Case study 3

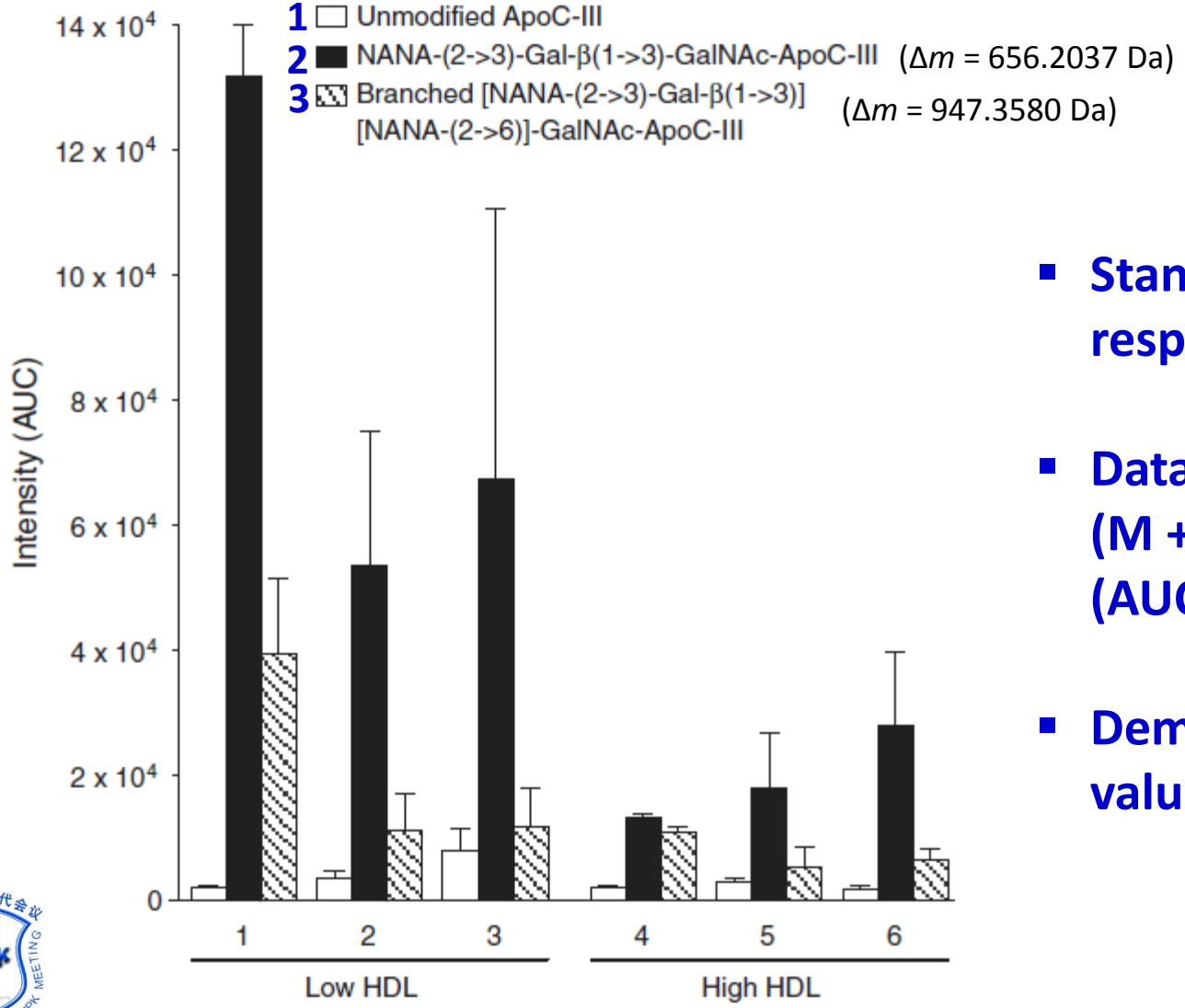


1 Unmodified ApoC-III
2 NANA-(2->3)-Gal-β(1->3)-GalNAc-ApoC-III (Δm = 656.2037 Da)
3 Branched [NANA-(2->3)-Gal-β(1->3)] [NANA-(2->6)]-GalNAc-ApoC-III (Δm = 947.3580 Da)

Fig. 2. Total ion current (TIC) chromatogram from LC-MS analysis of HDL_3. High-resolution ESI-mass spectrum of eluting apolipoprotein C-III (33.0–33.8 min, 46 scans averaged) showing the unmodified (•), NANA-(2 → 3)-Gal-β(1 → 3)-GalNAc-Thr^{94} (■), and branched [NANA-(2 → 3)-Gal-β(1 → 3)] -[NANA-(2 → 6)]-GalNAc-Thr^{94} (▲) protein forms. (*Inset*) Selected ion chromatogram (SIC) for the $(M + 9H^+)^{9+}$ ion of NANA-(2 → 3)-Gal-β(1 → 3)-GalNAc-Thr^{94} modified apolipoprotein C-III at m/z 1047.1678.

PNAS April 27, 2010. 107 (17) 7728-7733

HRMS for intact protein quantitation and profiling
高分辨质谱技术在完整蛋白药物定性和定量分析的应用
Case study 3



- Standard curve with a linear instrument response 100 nM -100 µM
- Data points : summed $(M + 11H)^{11+}$ to $(M + 7H)^{7+}$ and plotted as area under curve (AUC) with a linear regression ($R^2 = 0.999946$)
- Demonstrates an average fold change of 4.7 (p-value 0.017)

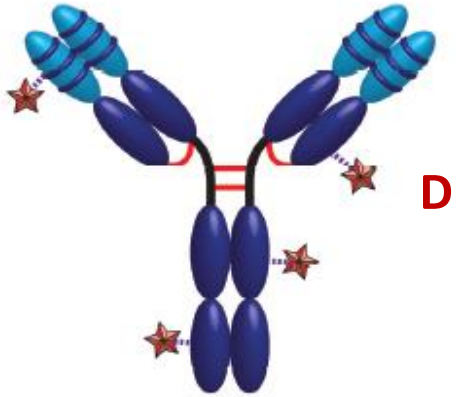
D. Su

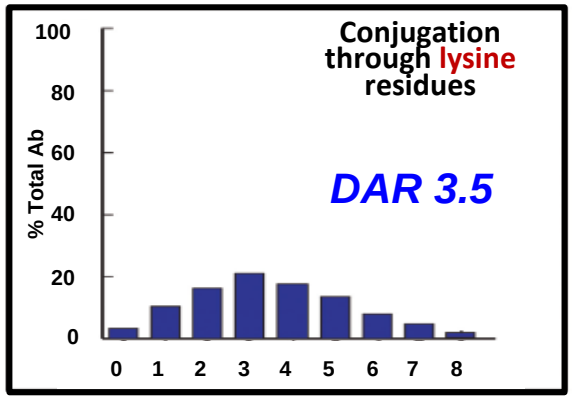
PNAS April 27, 2010. 107 (17) 7728-7733

HRMS for intact protein quantitation and profiling
高分辨质谱技术在完整蛋白药物定性和定量分析的应用

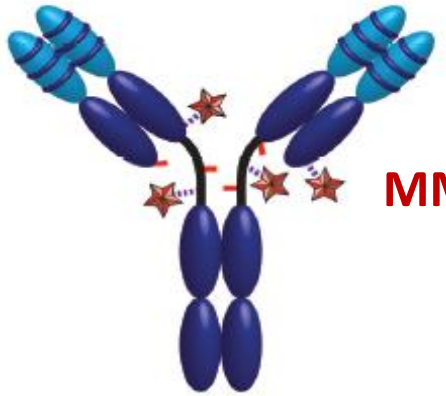
Antibody-Drug Conjugates (ADCs)

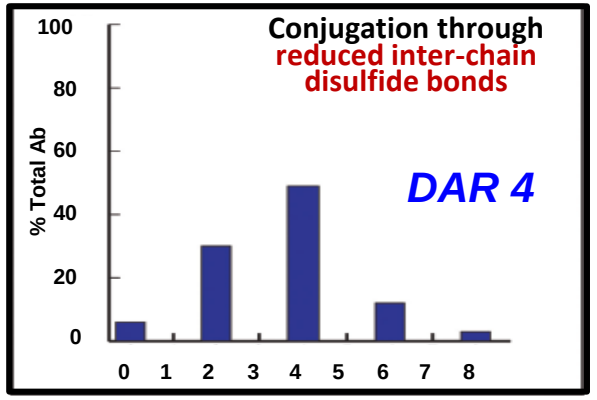
Antibody drug conjugate (ADC)



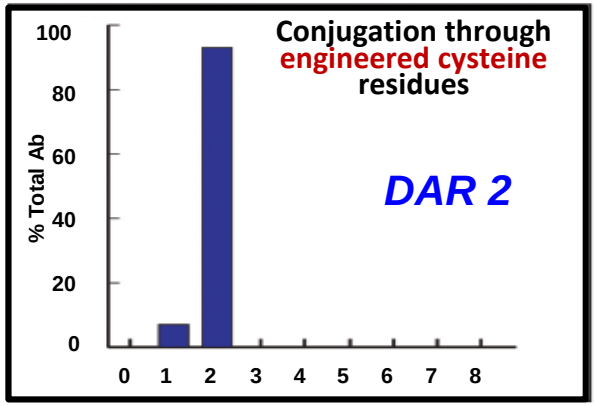


Antibody drug conjugate (ADC)





THIOMAB™ antibody drug conjugate (TDC)



"DAR" = Drug-Antibody Ratio

Panowski, S., *et al. mAbs,* 2014, *6*, 1.

Junutula J, Raab H, Clark S, *et al. Nat. Biotechnol., 2008; 26:925-32*

D. Su

HRMS for intact protein quantitation and profiling
高分辨质谱技术在完整蛋白药物定性和定量分析的应用

Case Study 4: ADCs DAR Profiling by Native MS

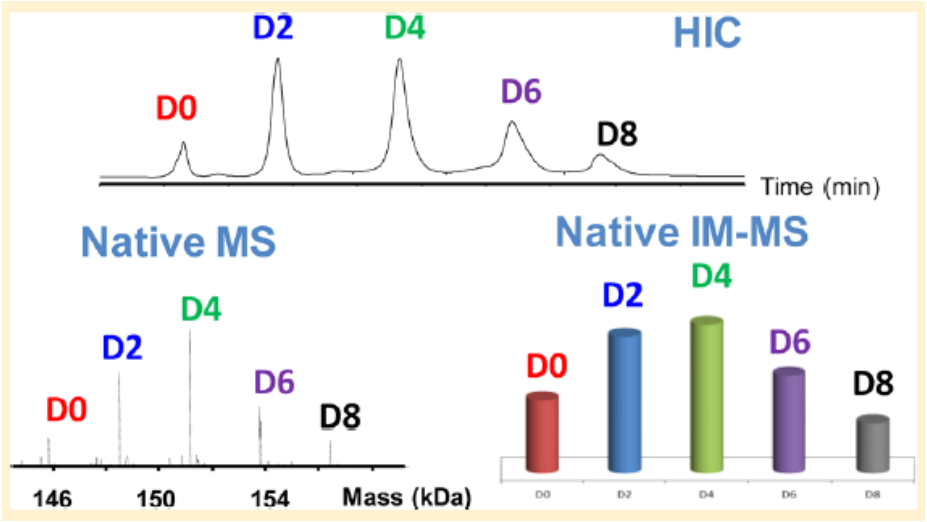
Innovative Native MS Methodologies for Antibody Drug Conjugate Characterization: High Resolution Native MS and IM-MS for Average DAR and DAR Distribution Assessment

François Debaene,[†,‡,§] Amandine Bœuf,[†,∥] Elsa Wagner-Rousset,[∥] Olivier Colas,[∥] Daniel Ayoub,[∥,⊥] Nathalie Corvaïa,[∥] Alain Van Dorsselaer,[‡,§] Alain Beck,*[,∥] and Sarah Cianférani*[,‡,§]

[‡]BioOrganic Mass Spectrometry Laboratory (LSMBO), IPHC, Université de Strasbourg, 25 rue Becquerel, 67087 Strasbourg, France

[§]IPHC, CNRS, UMR7178, 67087 Strasbourg, France

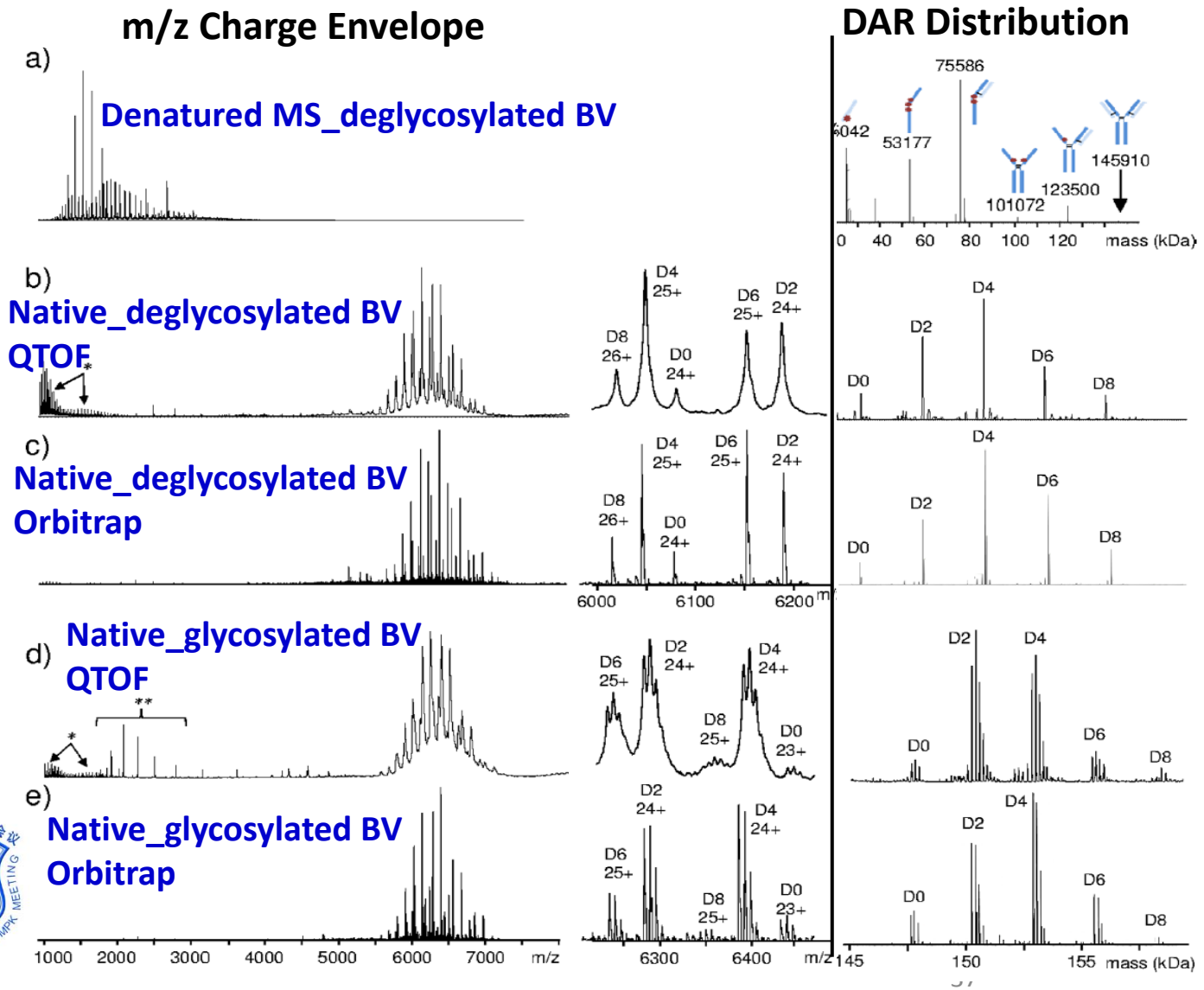
[∥]Centre d'Immunologie Pierre-Fabre (CIPF), 5 Av. Napoléon III, BP 60497, 74164 Saint-Julien-en-Genevois, France



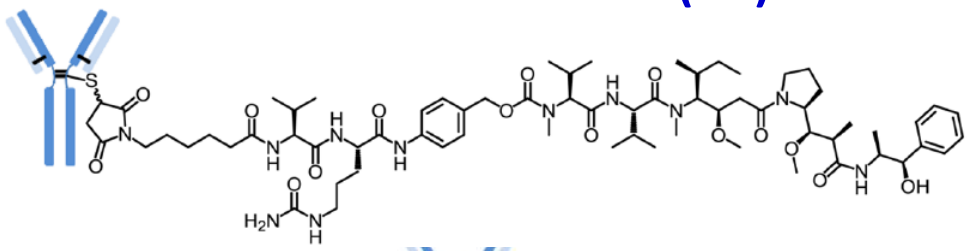
Anal. Chem. 2014, 86, 10674−10683

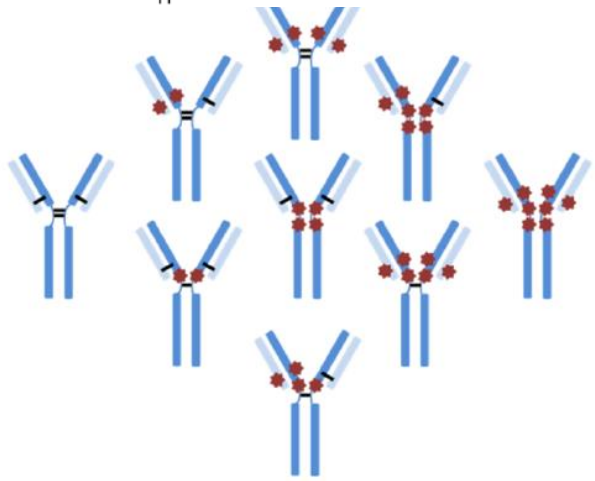
HRMS for intact protein quantitation and profiling
高分辨质谱技术在完整蛋白药物定性和定量分析的应用

Case Study 4: ADCs DAR Profiling by Native MS



Brentuximab Vedotin (BV)





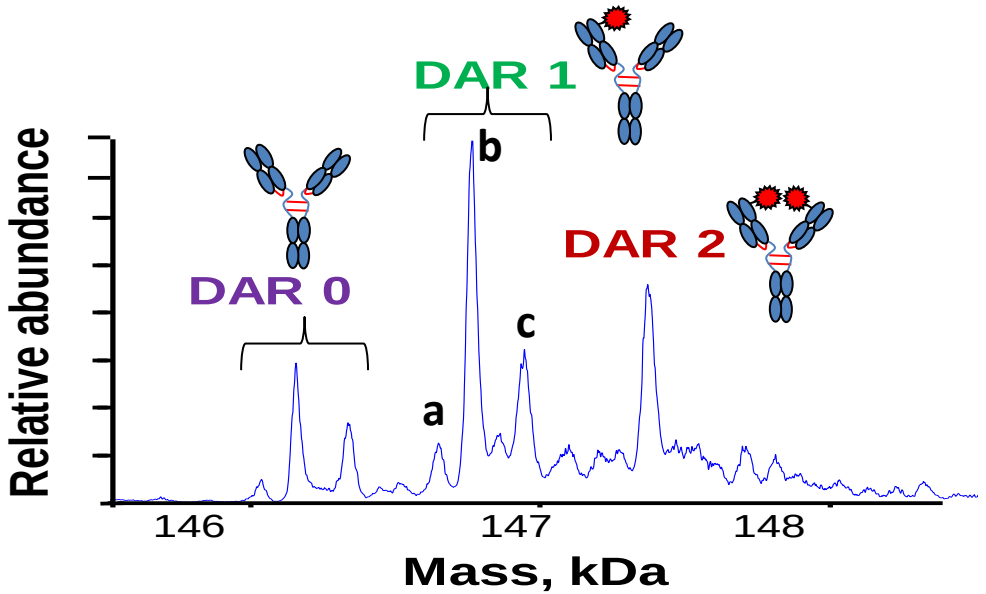
$$\overline{DAR} = \frac{\sum_{0}^{8} nA_{DAR_n}}{\sum_{0}^{8} A_{DAR_n}} = 4.0$$

Anal. Chem. 2014, 86, 10674−10683

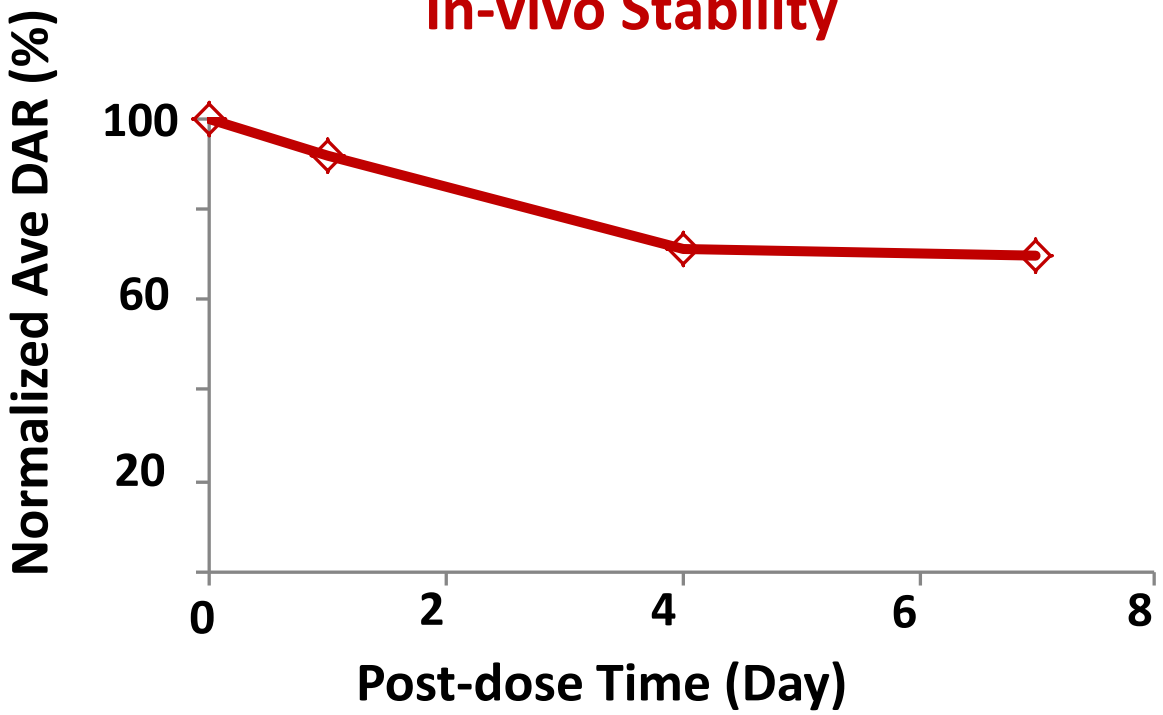
D. Su

HRMS for intact protein quantitation and profiling
高分辨质谱技术在完整蛋白药物定性和定量分析的应用
Comprehensive ADC Catabolite ID and DAR profiling

Catabolite ID & DAR distribution



In-vivo Stability



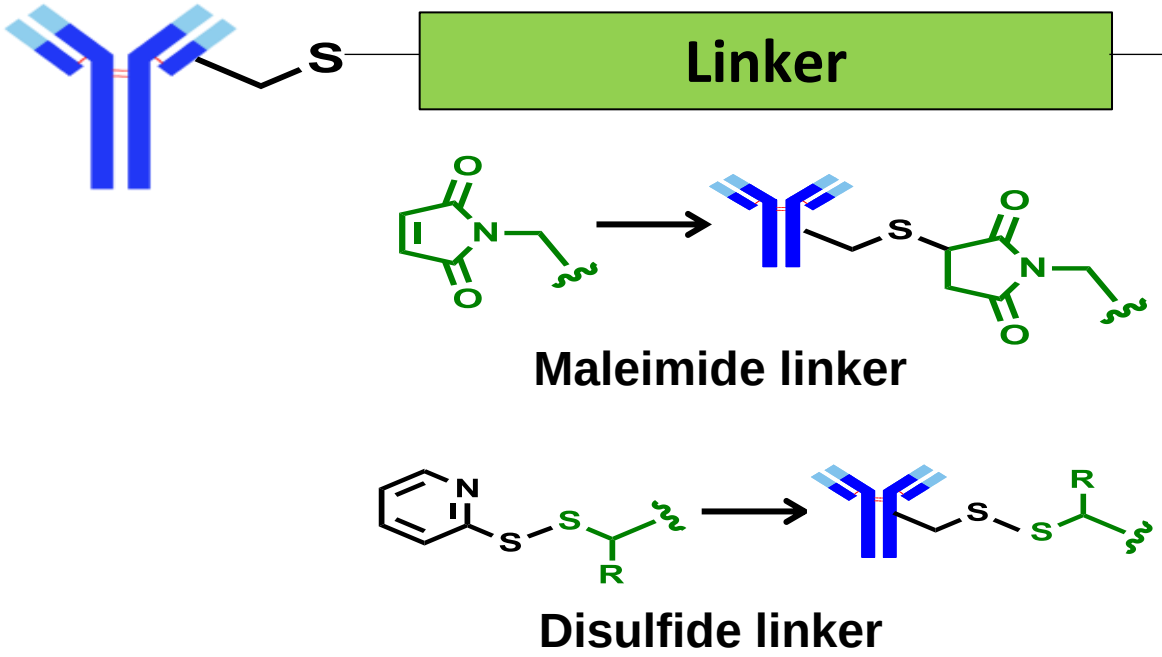
ADC: Antibody-drug Conjugates; DAR: drug-antibody ratio

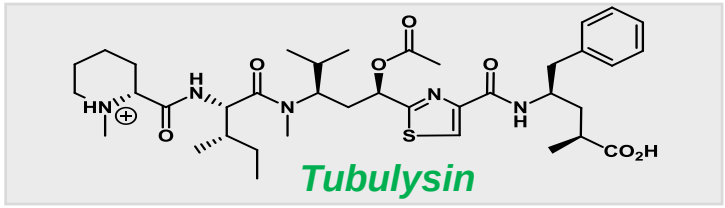
Average DAR=∑ (%peak area × number of conjugated drugs)/100

- Various DAR species (DAR0, DAR1, DAR2) and components (a, b, c)
- The stability data help to understand ADC efficacy/toxicity profiles and optimize drug design

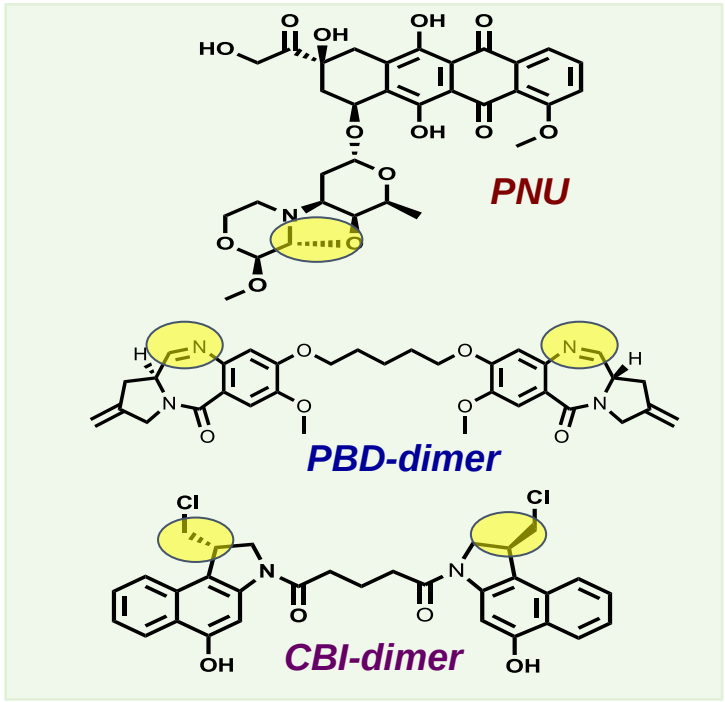
Immuno-affinity LC-HRMS for ADC biotransformation
免疫-高分辨色质杂化分析在药物偶合药物生物转化分析中的应用

Next-generation ADCs





tubulin binder



DNA binder/cross-linker

- **Diversified MOAs**

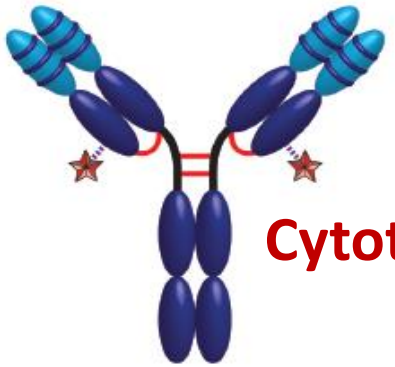
ADC: Antibody-drug Conjugates

PBD = Pyrrolo-BenzoDiazepine
CBI = Cyclopropa-Benz-Indol-4-one

Genentech
A Member of the Roche Group

Immuno-affinity LC-HRMS for ADC biotransformation
免疫-高分辨色质杂化分析在药物偶合药物生物转化分析中的应用

Next-generation ADCs



mAb — Protector / Carrier

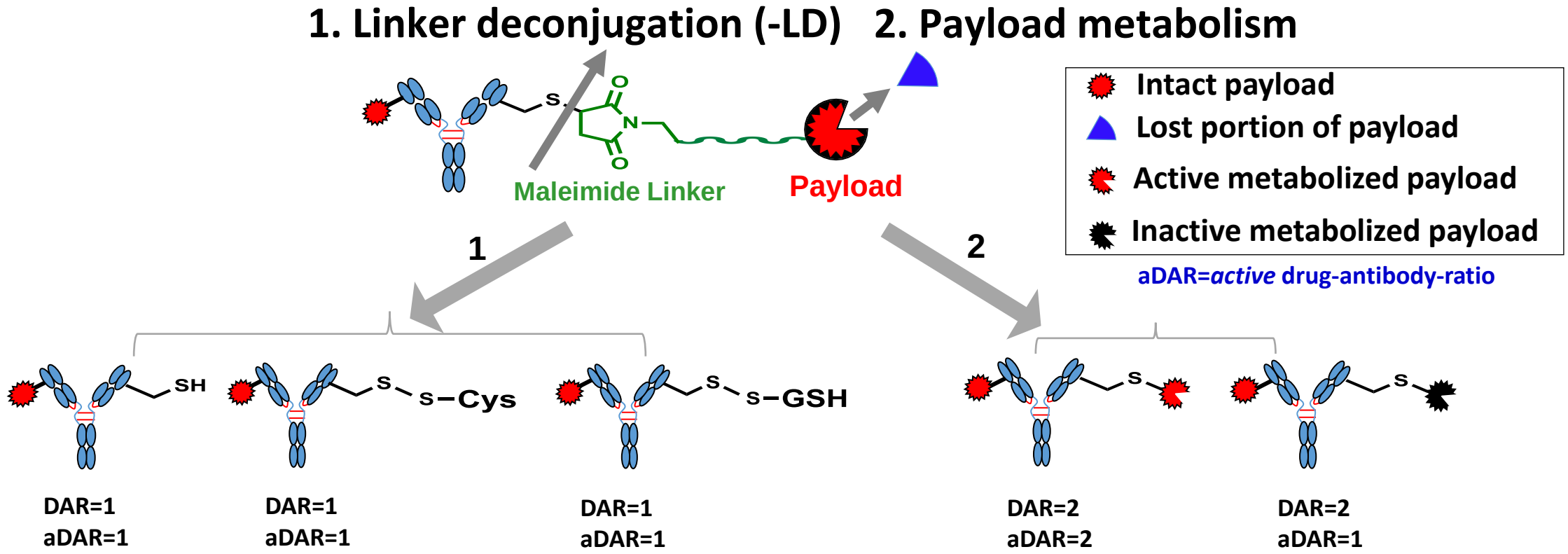
Cytotoxic drug/Payload

"Prodrug"

ADC: Antibody-drug Conjugates; DAR: drug-antibody ratio

Immuno-affinity LC-HRMS for ADC biotransformation
免疫-高分辨色质杂化分析在药物偶合药物生物转化分析中的应用

Complexity of ADC Biotransformation



- **Deconjugation (pathway 1)** leads to deactivation/loss of the payload and possible formation of antibody-cys/GSH adduct.
- **Payload metabolism (pathway 2)** results in either active (in red) or inactive (in black) payload corresponding to different changes to aDAR.

Su D et al., Bioconjugate Chem. 2018, 29, 1155−1167

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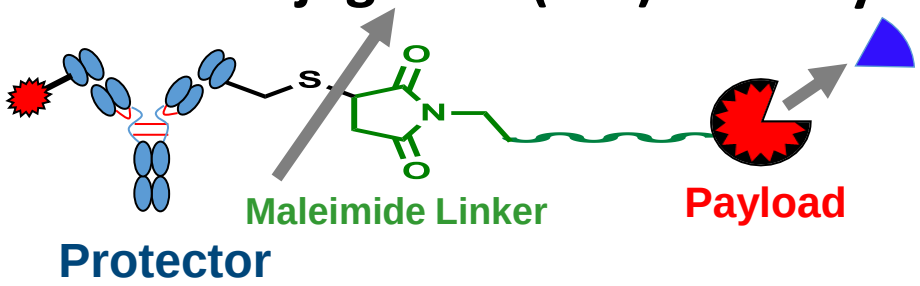
Immuno-affinity LC-HRMS for ADC biotransformation
免疫-高分辨色质杂化分析在药物偶合药物生物转化分析中的应用

Complexity of ADC Biotransformation

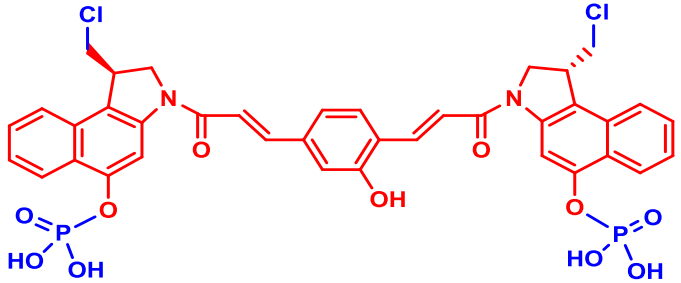
1. Linker deconjugation (-LD) **2. Payload metabolism**



- Intact payload
- Lost portion of payload
- Active metabolized payload
- Inactive metabolized payload

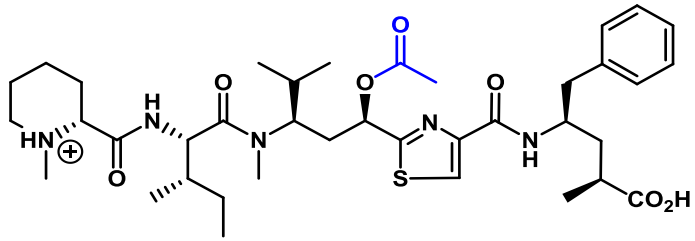
aDAR=*active* drug-antibody-ratio

Phosphate hydrolysis -> active payload



CBI dimer (phosphate prodrug) aDAR-

Ester hydrolysis -> inactive payload



Tubulysin aDAR ↓

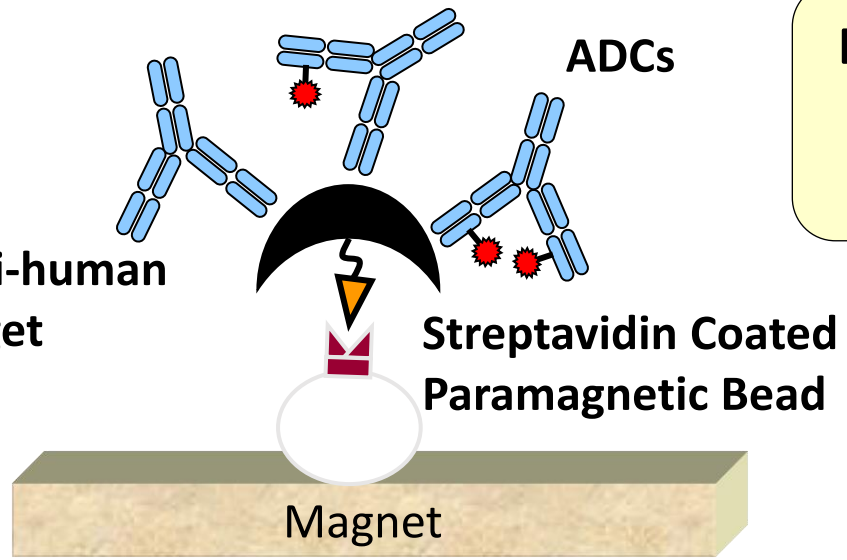
- **Hypothesis: solvent accessibility or steric hindrance has a significant impact on ADC instability (e.g, deconjugation and payload metabolism)**
- **Modulating parameters: conjugation sites, linkers, payloads, *etc***

Curr Pharmacol Rep (2018) 4: 45-63
Su D et al., Bioconjugate Chem. 2018, 29, 1155−1167

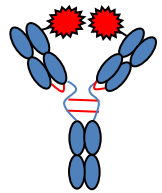
Immuno-affinity LC-HRMS for ADC biotransformation
免疫-高分辨色质杂化分析在药物偶合药物生物转化分析中的应用

Affinity Capture LC-MS Assays

(15 µL/min @ 70 °C on Sciex 5600)



Deglycosylation with PNGase F for overnight → **LC-MS**



ADC (~150kDa)

Remove Fc fragments with IdeS for 1 hour → **LC-MS**

F(ab')2 (~100 KDa)

- **Enhanced sensitivity (3-5 folds) and resolution (2-3 folds)**
- **For analysis of LOW-DOSE, LABILE, and COMPLEX site-specific ADCs with linker-drug conjugated in the Fab region.**

Xu K et al., Anal. Biochem., 412, 56-66, (2011)

S. Kaur, K. Xu and O. Saad, European patent 2277044, issued 17 June 2015

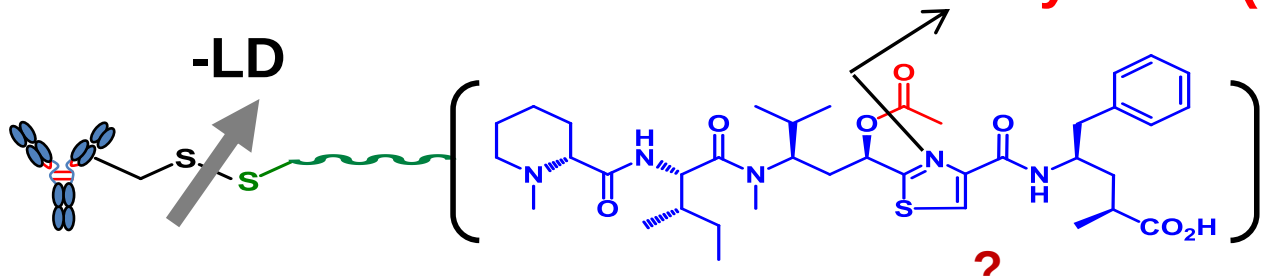
S. Kaur, K. Xu and O. Saad, United States patent S 8541178, issued 24 Sept 2013

Su D et al., Anal. Chem. 2016, 88, 11340-11346

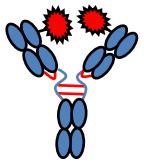
Immuno-affinity LC-HRMS for ADC biotransformation
免疫-高分辨色质杂化分析在药物偶合药物生物转化分析中的应用

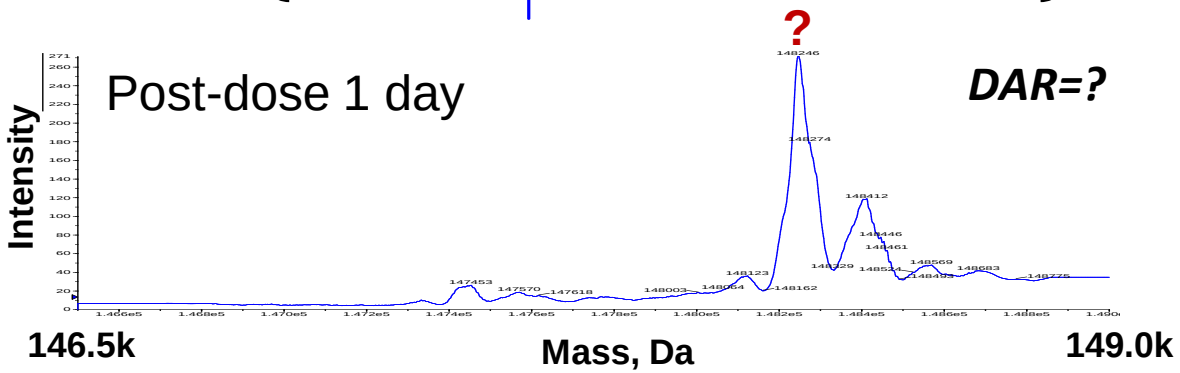
Affinity Capture LC-MS F(ab')2 Assay

TDC-L1
(Tubulysin, disulfide linker)

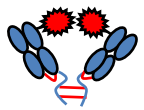


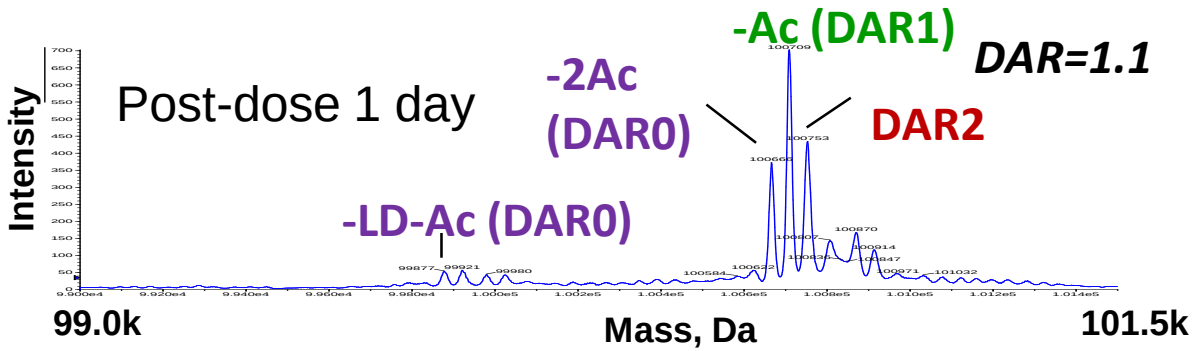
PNGase F digestion
37^{0}C overnight





IdeS digestion
37^{0}C 1 h





- **Resolved peaks with Δm=43 Da at the non-reduced level**

Su D et al., Anal. Chem. 2016, 88, 11340-11346
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Immuno-affinity LC-HRMS for ADC biotransformation
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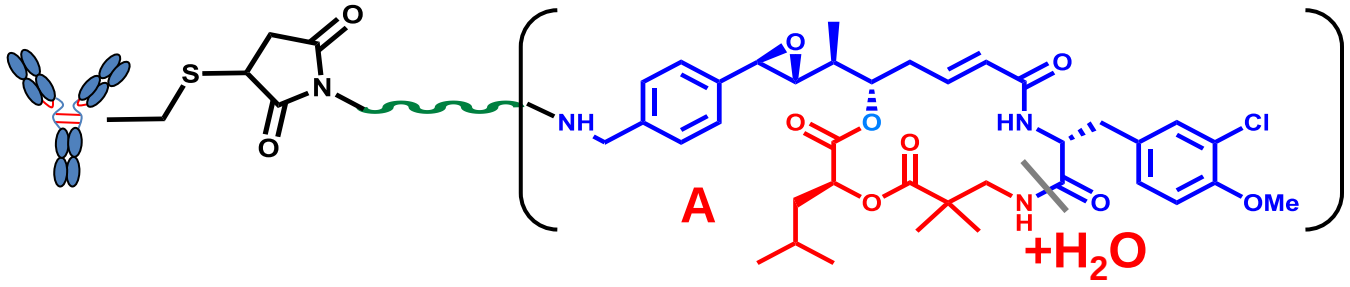
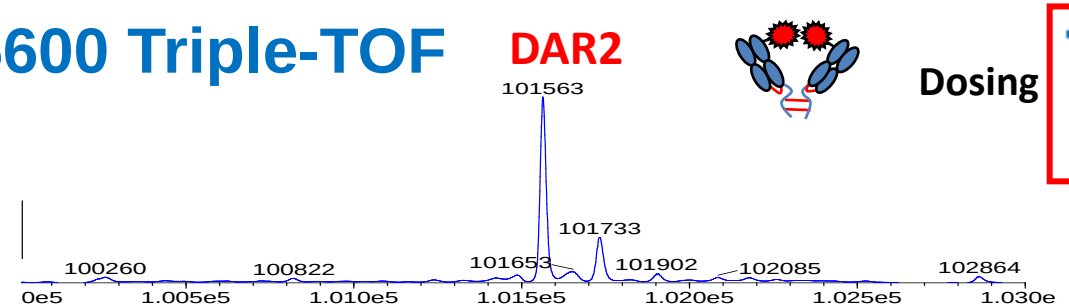
Affinity Capture LC-MS F(ab')2 Assay

TDC-L2

(Cryptophycin, maleimide linker)

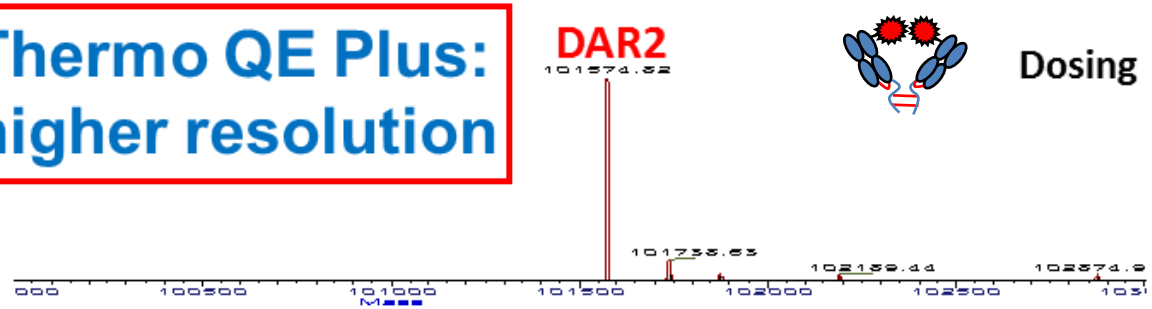


5600 Triple-TOF

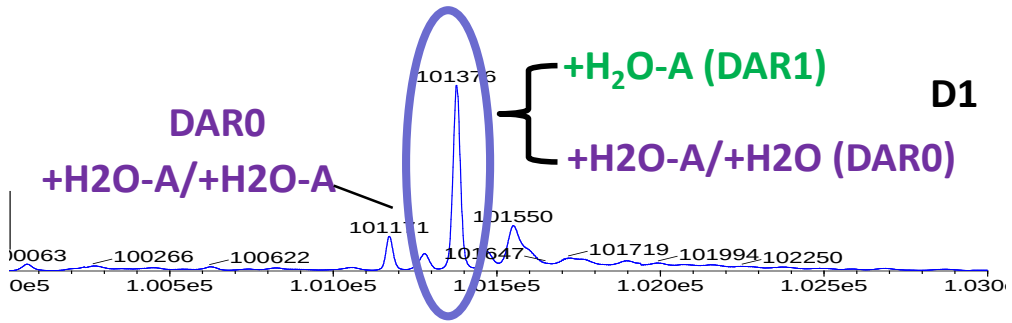


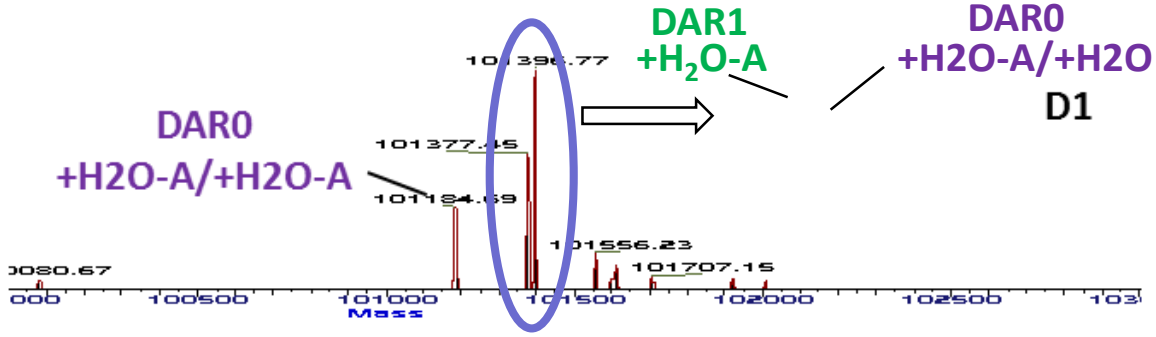
Dosing

Thermo QE Plus: higher resolution



Dosing

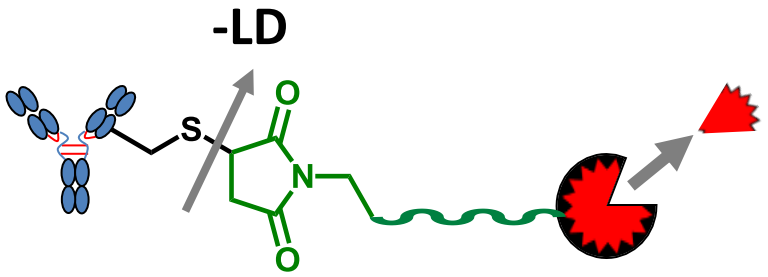




- **Resolved peaks with Δm=18 Da at the non-reduced level**

Immuno-affinity LC-HRMS for ADC biotransformation
免疫-高分辨色质杂化分析在药物偶合药物生物转化分析中的应用
Affinity Capture LC-MS F(ab')2 Assay

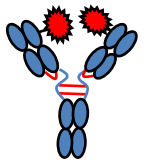
TDC-L3
(CBI dimer, maleimide linker)

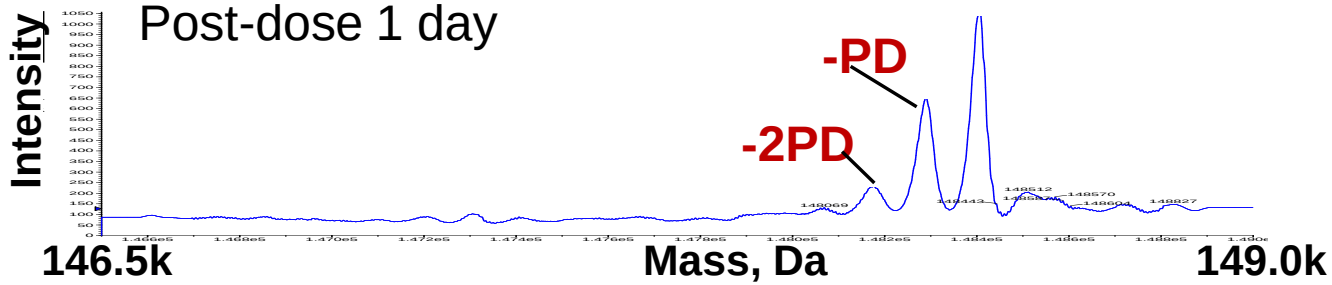


Partial drug loss (-PD) due to payload metabolism

PNGase F digestion

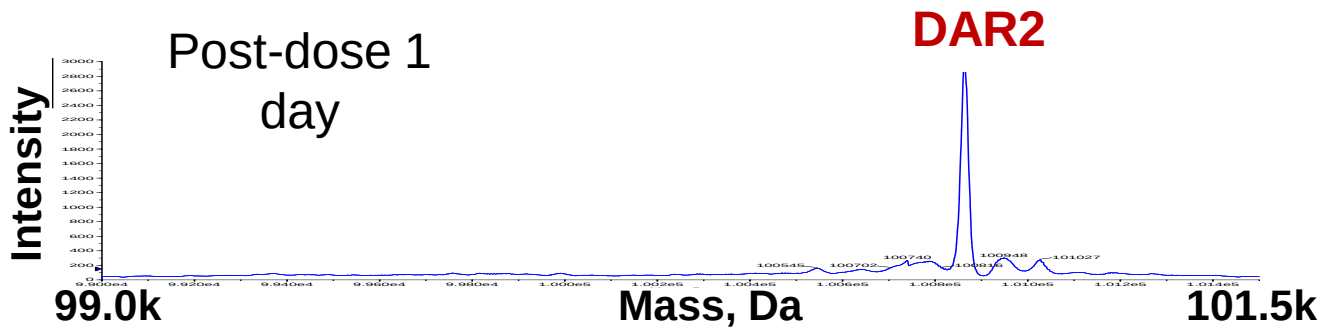
37^{0}C overnight





IdeS digestion

37^{0}C 1 h



- **Reduced sample process time: < 7 hr**

Su D et al., Anal. Chem. 2016, 88, 11340-11346
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Immuno-affinity LC-HRMS for ADC biotransformation

免疫-高分辨色质杂化分析在药物偶合药物生物转化分析中的应用

Case Study 5: Affinity LC-MS to Assist in Addressing ADC Payload Metabolism

Bioconjugate Chemistry

Article

Cite This: *Bioconjugate Chem.* 2018, 29, 1155−1167

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Modulating Antibody−Drug Conjugate Payload Metabolism by Conjugation Site and Linker Modification

Dian Su,*[,†,#] Katherine R. Kozak,[†,#] Jack Sadowsky,[†] Shang-Fan Yu,[†] Aimee Fourie-O'Donohue,[†] Christopher Nelson,[†] Richard Vandlen,[†] Rachana Ohri,[†] Luna Liu,[†] Carl Ng,[†] Jintang He,[†] Helen Davis,[†] Jeff Lau,[†] Geoffrey Del Rosario,[†] Ely Cosino,[†] Josefa dela Cruz-Chuh,[†] Yong Ma,[†] Donglu Zhang,[†] Martine Darwish,[†] Wenwen Cai,[‡] Chunjiao Chen,[‡] Hongxiang Zhou,[‡] Jiawei Lu,[‡] Yichin Liu,[†] Surinder Kaur,[†] Keyang Xu,[†] and Thomas H. Pillow[†]

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[‡]Wuxi Biologics, 288 Fute Zhong Road, Waigaoqiao Free Trade Zone, Shanghai 200131, China

Immuno-affinity LC-HRMS for ADC biotransformation

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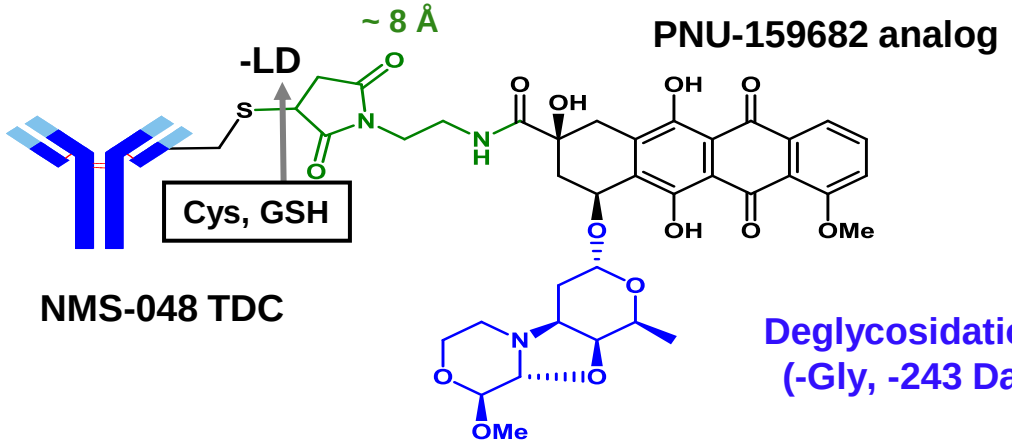
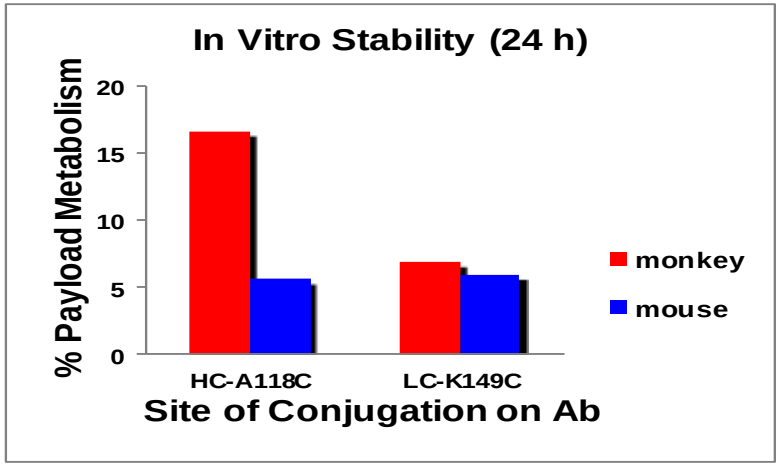
Case Study 5: Affinity LC-MS to Assist in Addressing ADC Payload Metabolism

A

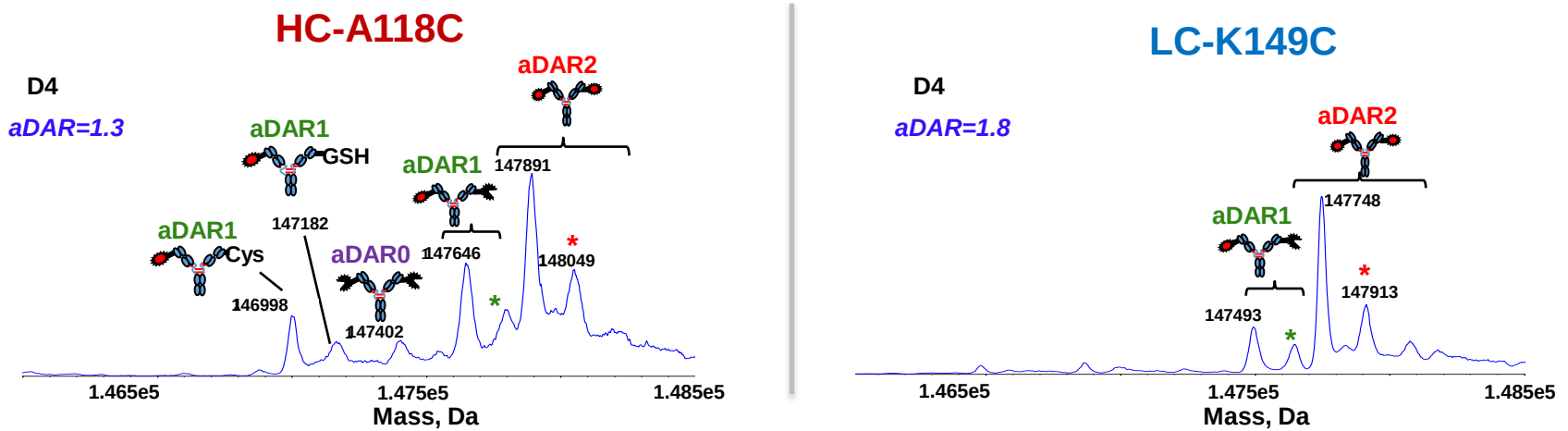


B



C

In vivo stability: Intact MS profiles



MS peaks labeled with *represent the glycated TDC species of the corresponding (color coded) aDAR value

- **Deconjugation(-LD) and payload metabolism(-Gly): A118C > K149C**

Su D et al., Bioconjugate Chem. 2018, 29, 1155−1167

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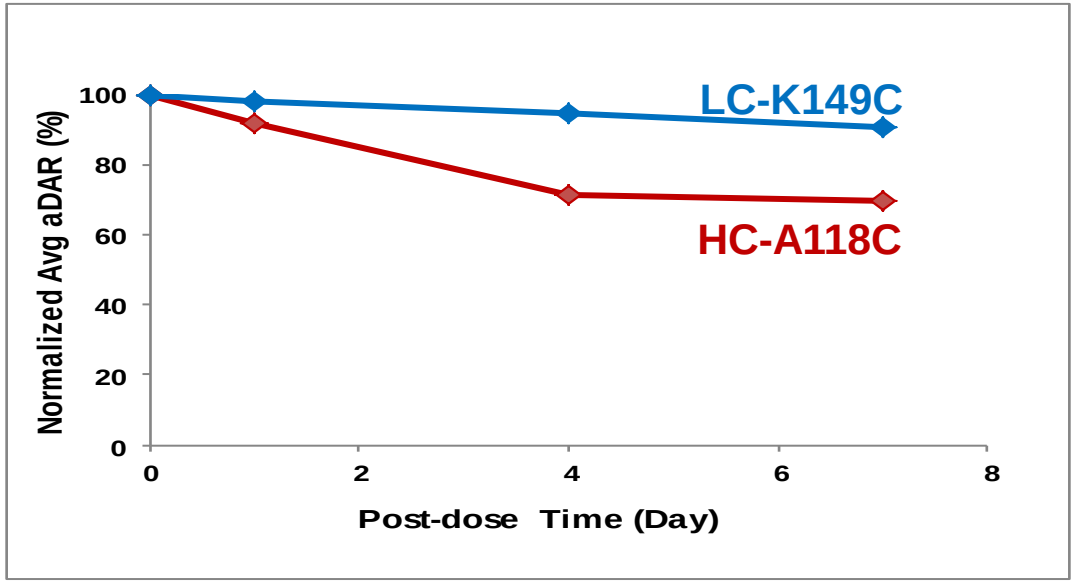
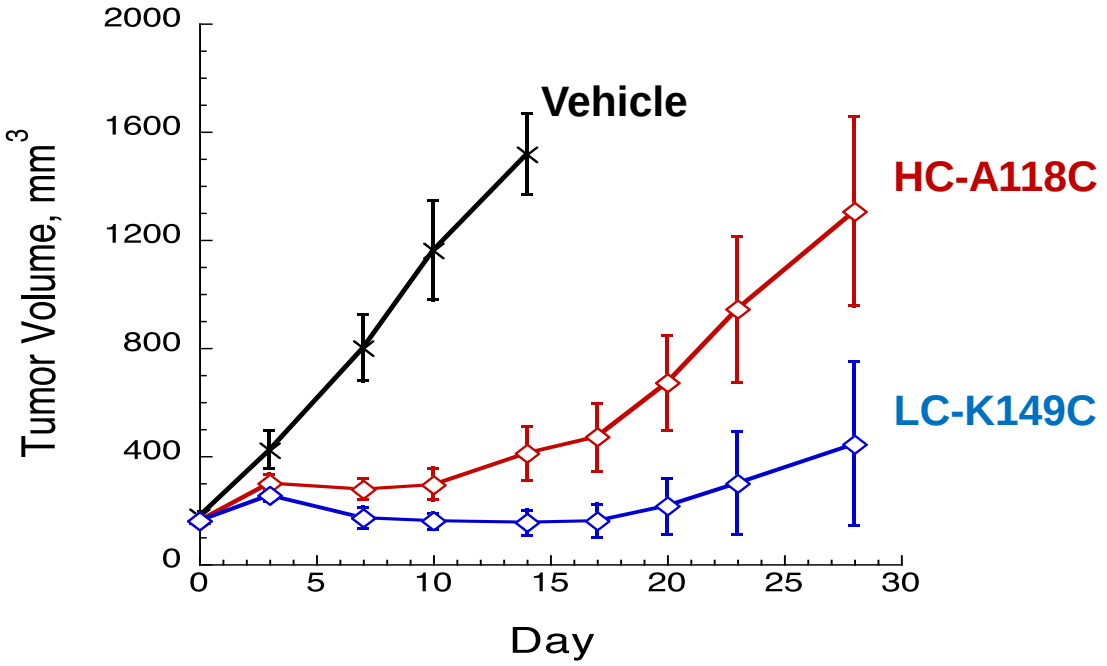
Immuno-affinity LC-HRMS for ADC biotransformation
免疫-高分辨色质杂化分析在药物偶合药物生物转化分析中的应用
Case Study 5: Affinity LC-MS to Assist in Addressing ADC Payload Metabolism

D In vivo stability: aDAR changes



aDAR=*active* drug-antibody-ratio

E In vivo efficacy



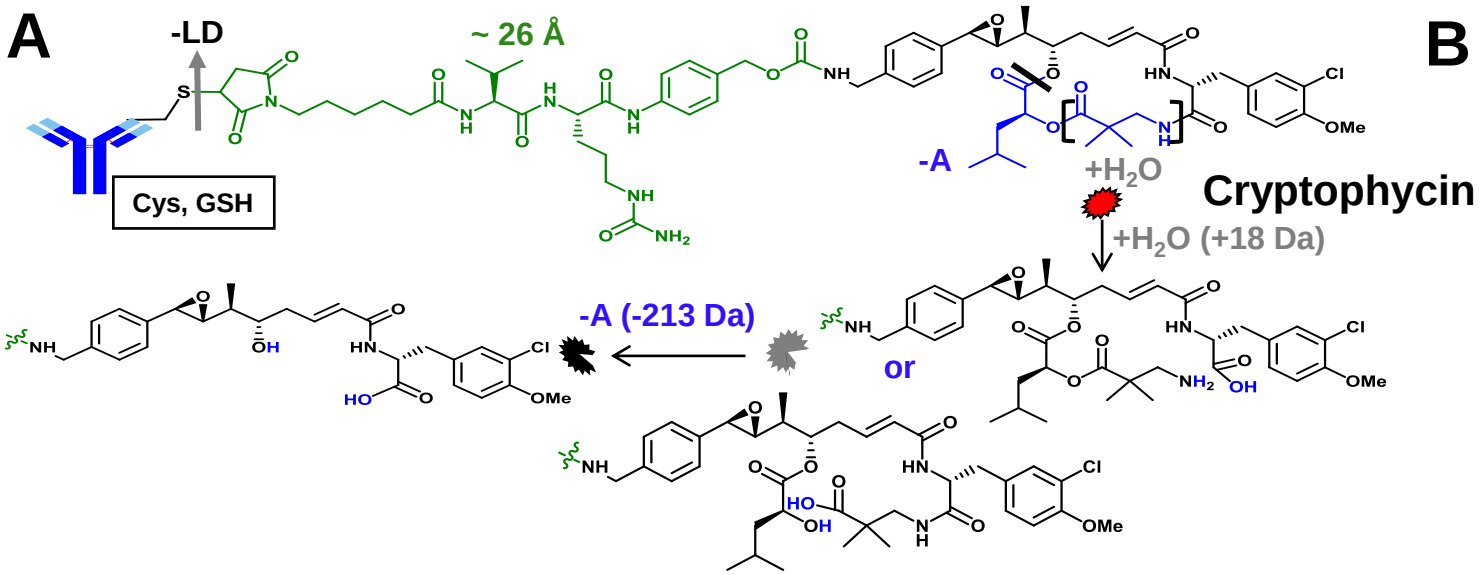
- **In vivo stability and efficacy in mouse: A118C < K149C**

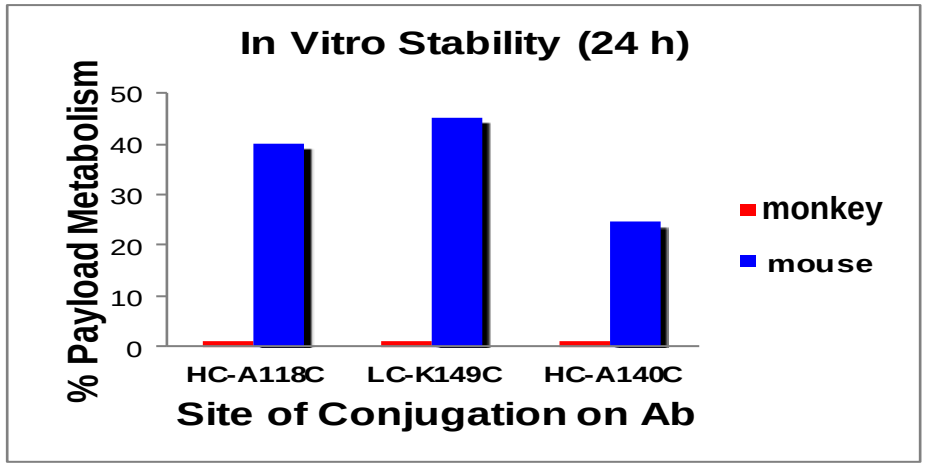
Su D et al., Bioconjugate Chem. 2018, 29, 1155−1167

Immuno-affinity LC-HRMS for ADC biotransformation

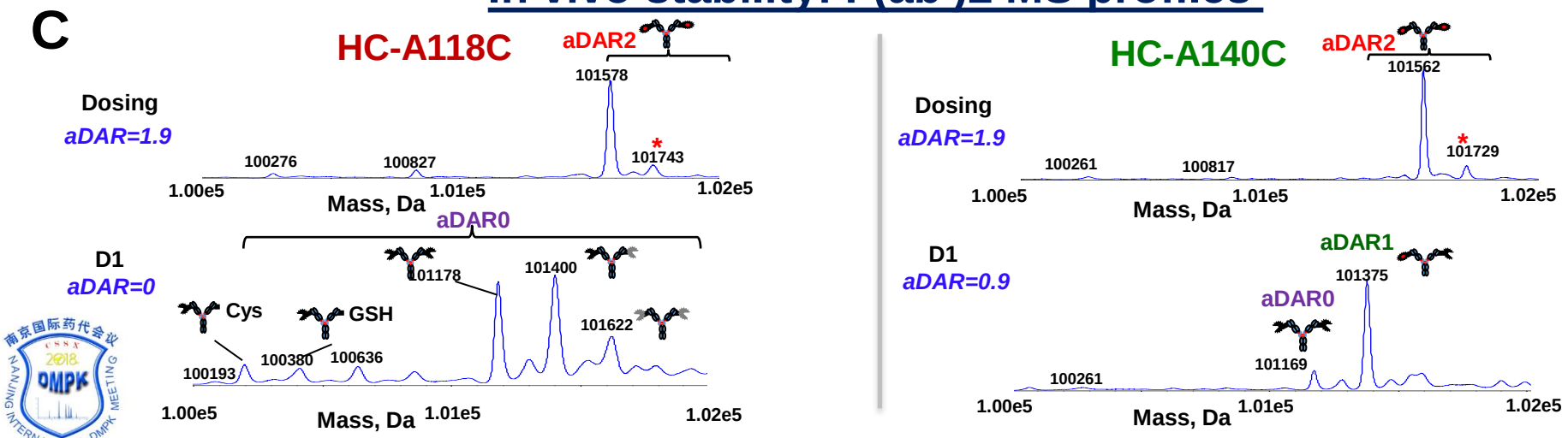
免疫-高分辨色质杂化分析在药物偶合药物生物转化分析中的应用

Case Study 5: Affinity LC-MS to Assist in Addressing ADC Payload Metabolism





In vivo stability: F(ab')2 MS profiles



Color:
aDAR2
aDAR1
aDAR0

- Intact cryptophycin
- -A+H_2O (inactive)
- +H_2O (inactive)

aDAR=*active* drug-antibody-ratio

MS peaks labeled with *represent the glycated TDC species of the corresponding (color coded) aDAR value

- **Payload metabolism: A118C~K149C > A140C**

D. Su

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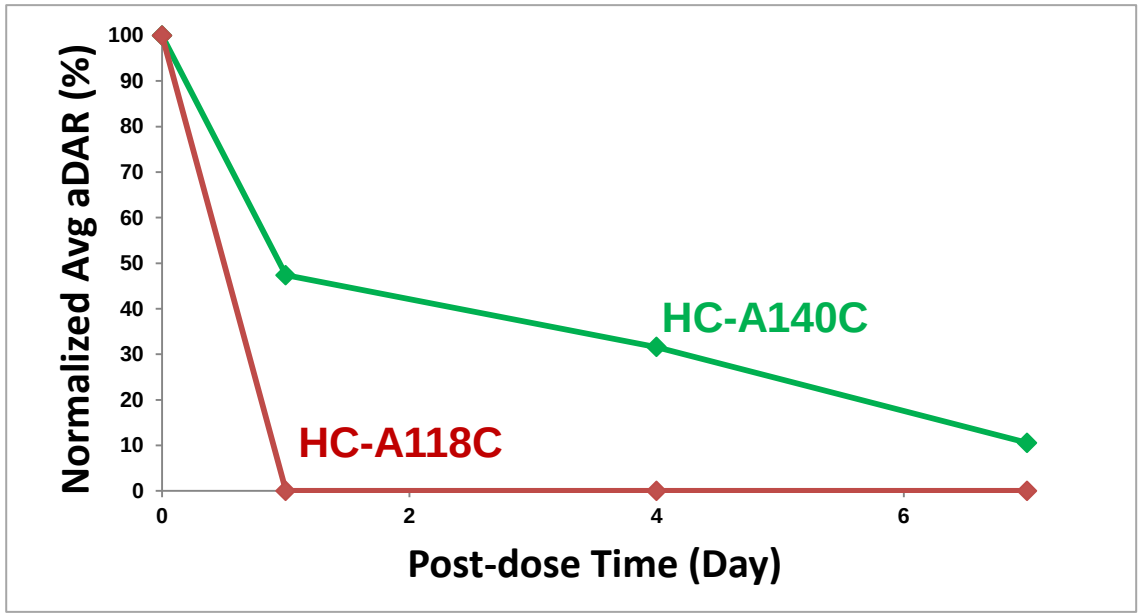
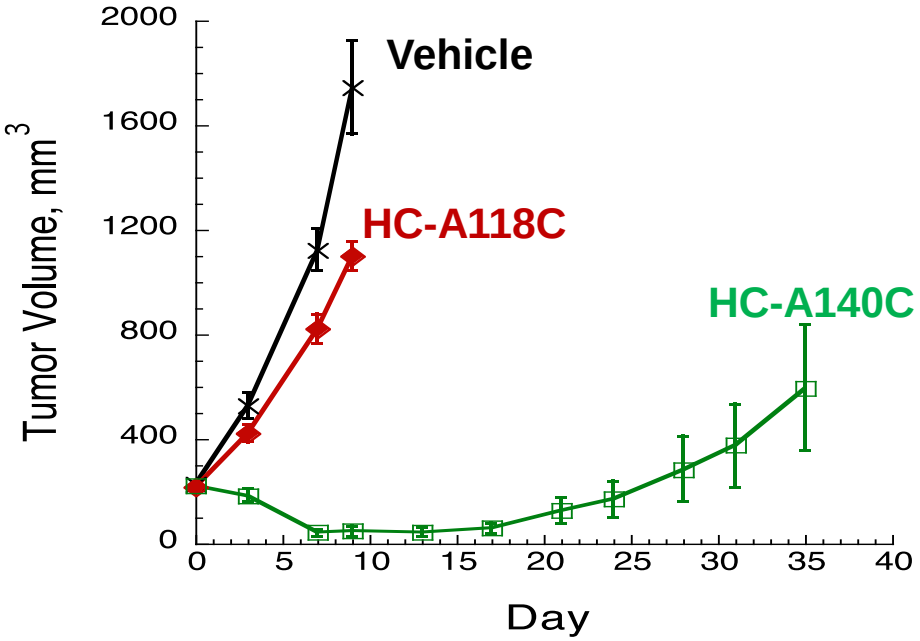
Immuno-affinity LC-HRMS for ADC biotransformation
免疫-高分辨色质杂化分析在药物偶合药物生物转化分析中的应用
Case Study 5: Affinity LC-MS to Assist in Addressing ADC Payload Metabolism

D In vivo stability: aDAR changes



aDAR=*active* drug-antibody-ratio

E In vivo efficacy



- **In vivo stability and efficacy in mouse: A118C < A140C**

Su D et al., Bioconjugate Chem. 2018, 29, 1155−1167

Immuno-affinity LC-HRMS for ADC biotransformation

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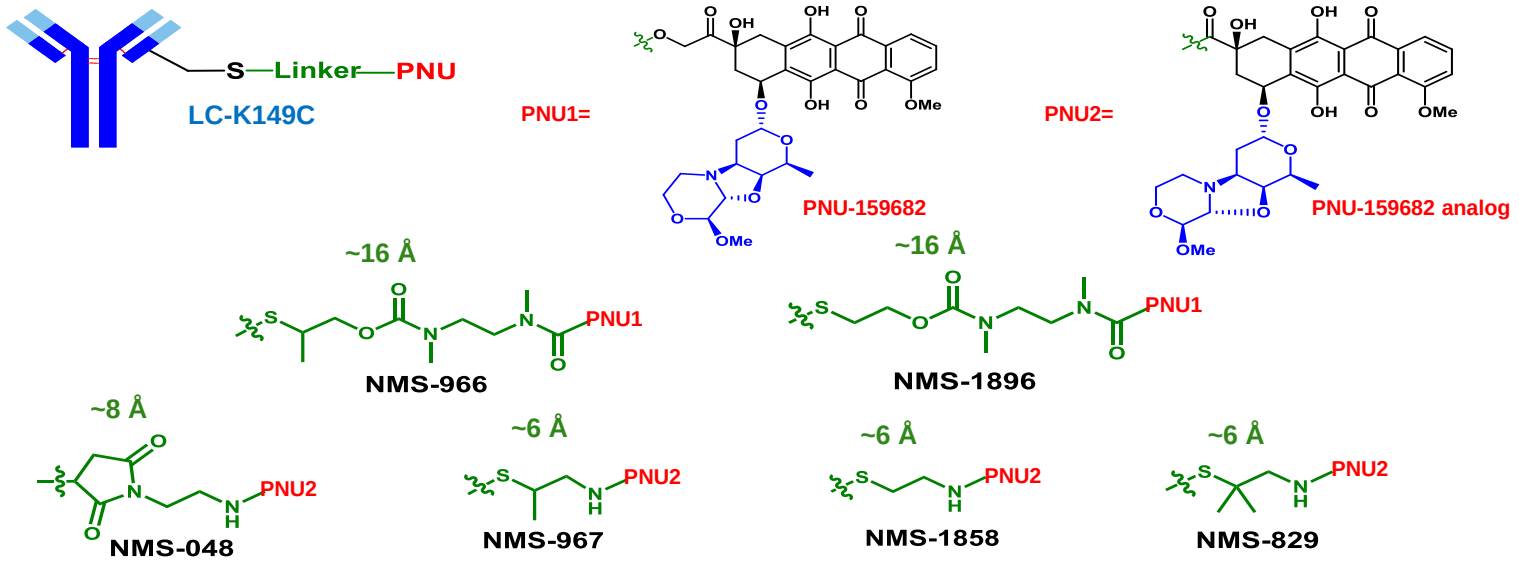
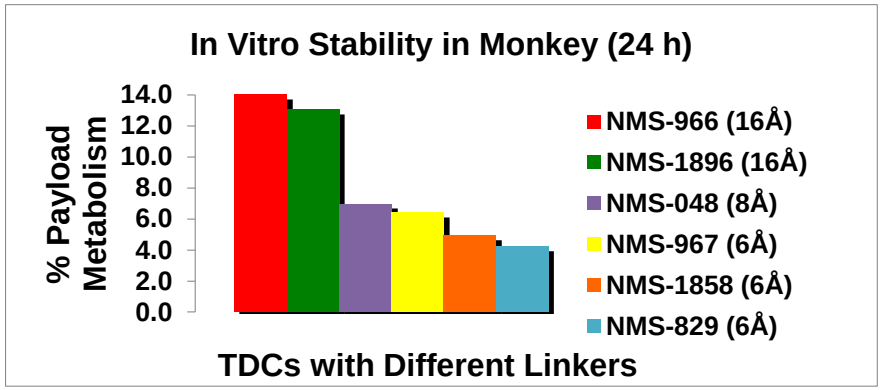
Case Study 5: Affinity LC-MS to Assist in Addressing ADC Payload Metabolism

A

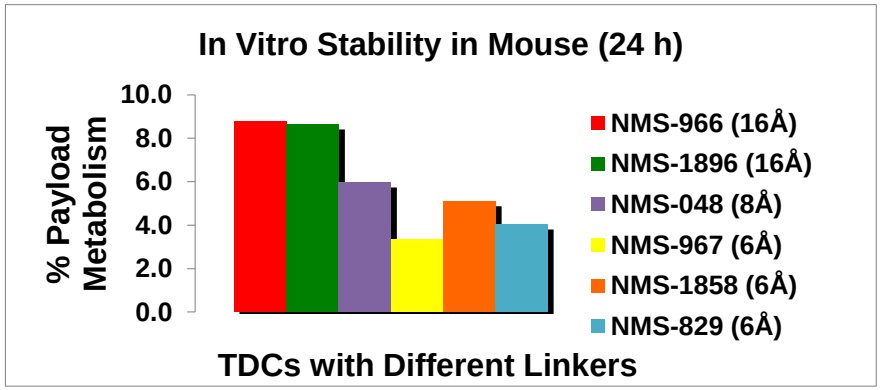


In vitro stability results of PNU (analog) conjugates by affinity capture LC-MS

B



C



- **Payload metabolism was improved by using shorter linkers**

Su D et al., *Bioconjugate Chem.* 2018, 29, 1155−1167
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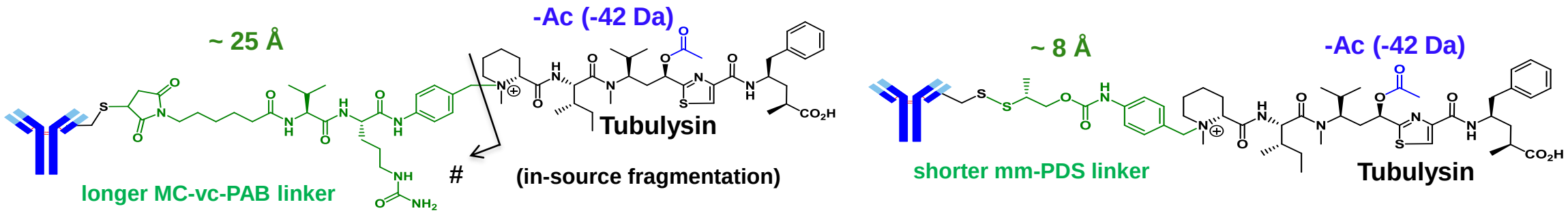
Immuno-affinity LC-HRMS for ADC biotransformation

免疫-高分辨色质杂化分析在药物偶合药物生物转化分析中的应用

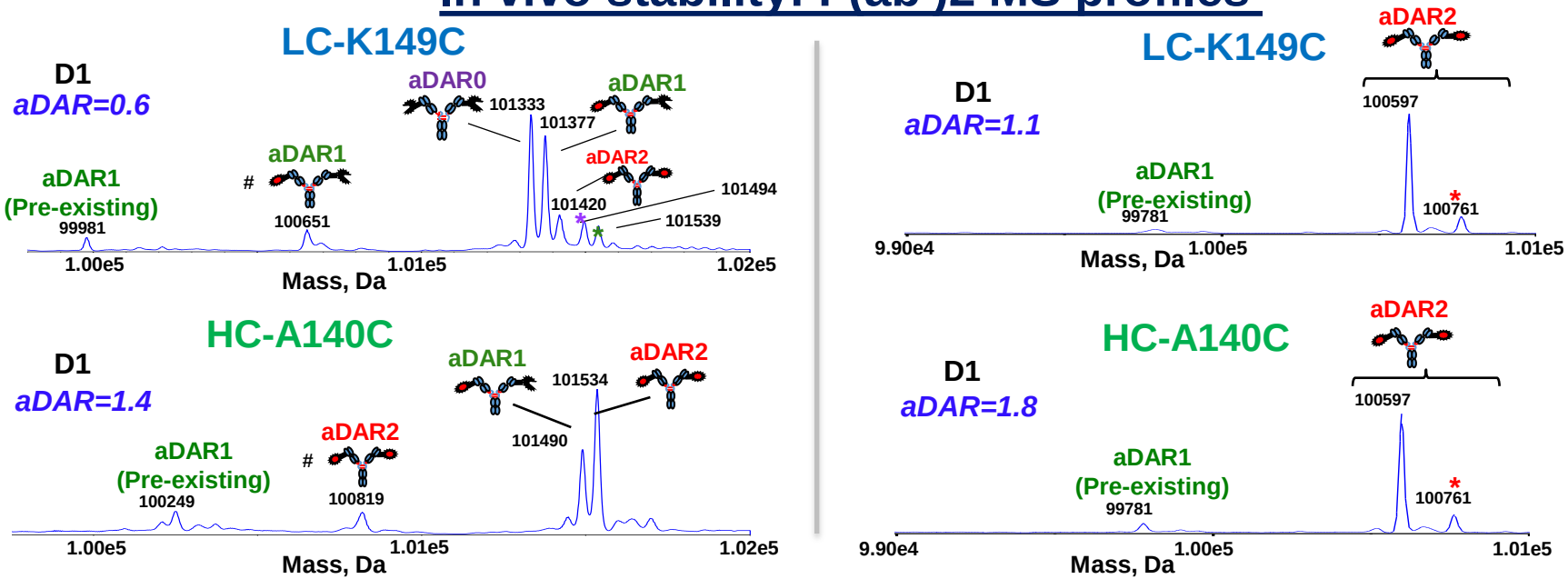
Case Study 5: Affinity LC-MS to Assist in Addressing ADC Payload Metabolism

A



In vivo stability: F(ab')2 MS profiles

B



MS peaks labeled with *represent the glycated TDC species of the corresponding (color coded) aDAR value

Payload metabolism was addressed by witching the conjugation site from K149C to A140C and using a shorter linker

Su D et al., Bioconjugate Chem. 2018, 29, 1155−1167

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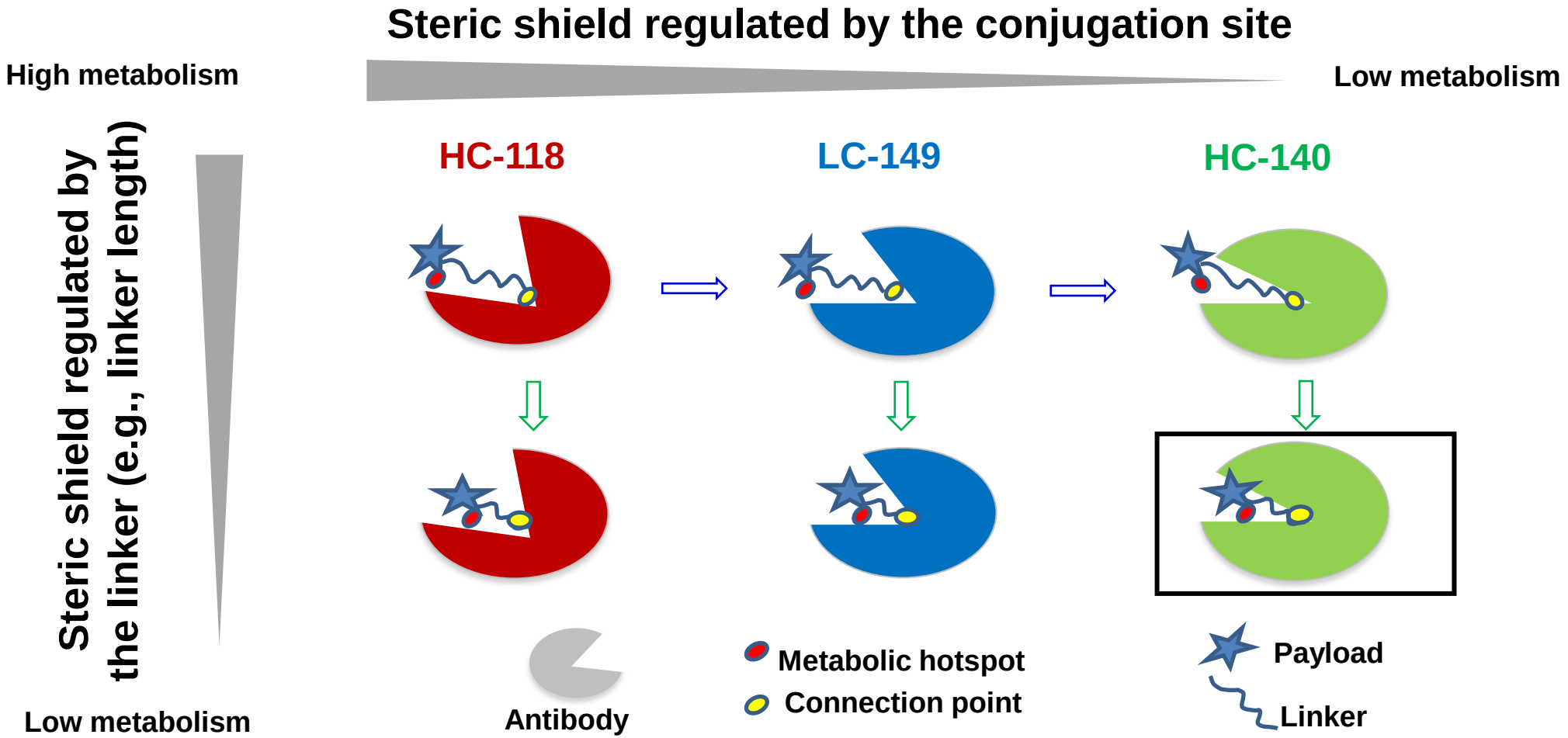
Immuno-affinity LC-HRMS for ADC biotransformation

免疫-高分辨色质杂化分析在药物偶合药物生物转化分析中的应用

Case Study 5: Affinity LC-MS to Assist in Addressing ADC Payload Metabolism



- Payload metabolism can be addressed by using less-accessible sites (e.g., K149, A140) and/or optimized linkers (e.g., a shorter linker)

Su D et al., Bioconjugate Chem. 2018, 29, 1155−1167

Workshop I

Strategy and technologies in protein therapeutics and ADC biotransformation

蛋白质和抗体-药物偶合药物生物转化分析的策略和技术

Summary

- **HRMS platforms**
  高分辨质谱技术和平台
  TOF, orbitrap, FT-ICR

- **HRMS for intact protein quantitation and profiling**
  高分辨质谱技术在完整蛋白药物定性和定量分析的应用
  Data processing, sample preparation, sensitivity/accuracy/precision, top/middle-down approach

- **Immuno-affinity LC-HRMS for ADC biotransformation**
  免疫-高分辨色质杂化分析在药物偶合药物生物转化分析中的应用
  Appropriate assays, catabolite/metabolite ID, DAR profiling and their roles in optimizing ADC drug design, top/middle-down approach

Workshop I

Strategy and technologies in protein therapeutics and ADC biotransformation

蛋白质和抗体-药物偶合药物生物转化分析的策略和技术

Acknowledgements

- 顾哲明
- 张东鲁 (Genentech)
- Steven Wang
- 王坚 (Bristol-Myers Squibb)
- Fabio Garofolo (Angelini Pharma)
- Genentech DMPK department, BAS department and LSR ADC team
- 南京国际药代会议筹备组