Direct Bioanalysis of ADCs using Affinity Capture-LC-HRAMS Techniques for Characterization and Quantification—a Progress Update

Rand Jenkins *et al*

EBF 8th Open Symposium
Barcelona Spain, 18 Nov 2015

EXPECT EXCELLENCE
ADVANCING DRUG DISCOVERY AND DEVELOPMENT

PPD®

Overview

1. Biotherapeutics diversity
2. Why new BA approaches are needed
3. LC-MS protein bioanalysis workflows
4. ADC characterization
5. Intact ADC quantification
6. Open questions & final thoughts

Biotherapeutics diversity

peptides

mAbs

bispecifics (bsAbs, DART®s, BiTE®s)

ADCs

prodrug/carrier conjugates (albumin, PEG)

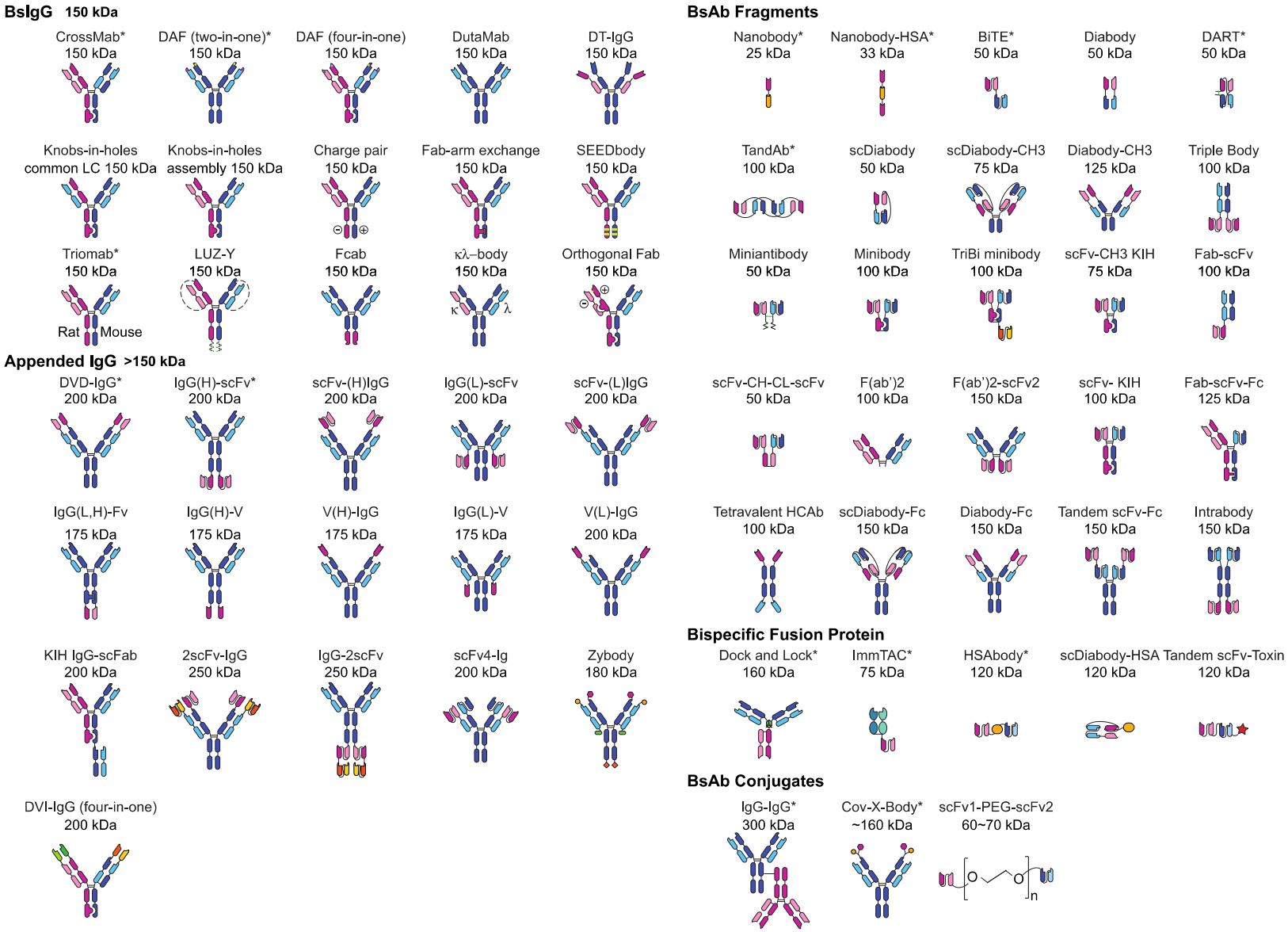
fusion proteins

messenger RNA therapeutics™

oligonucleotides (ssDNA, siRNA, mRNA)

CARs

Alternative formats for bispecifics



Christoph Spiess, Qianting Zhai, Paul J. Carter, Alternative molecular formats and therapeutic applications for bispecific antibodies, *Molecular Immunology* 67 (2015) 95–106

Antibody-Drug Conjugates

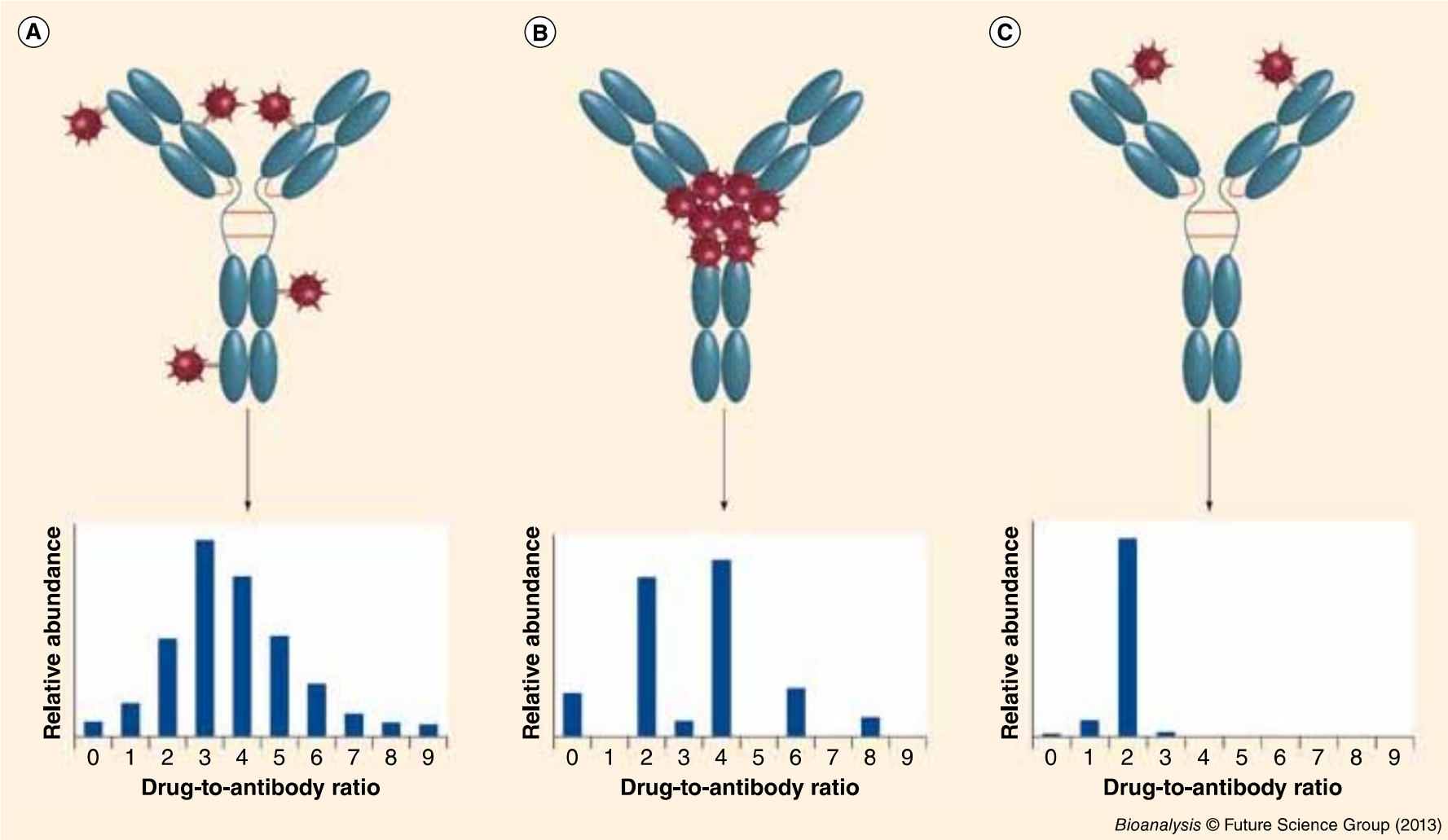
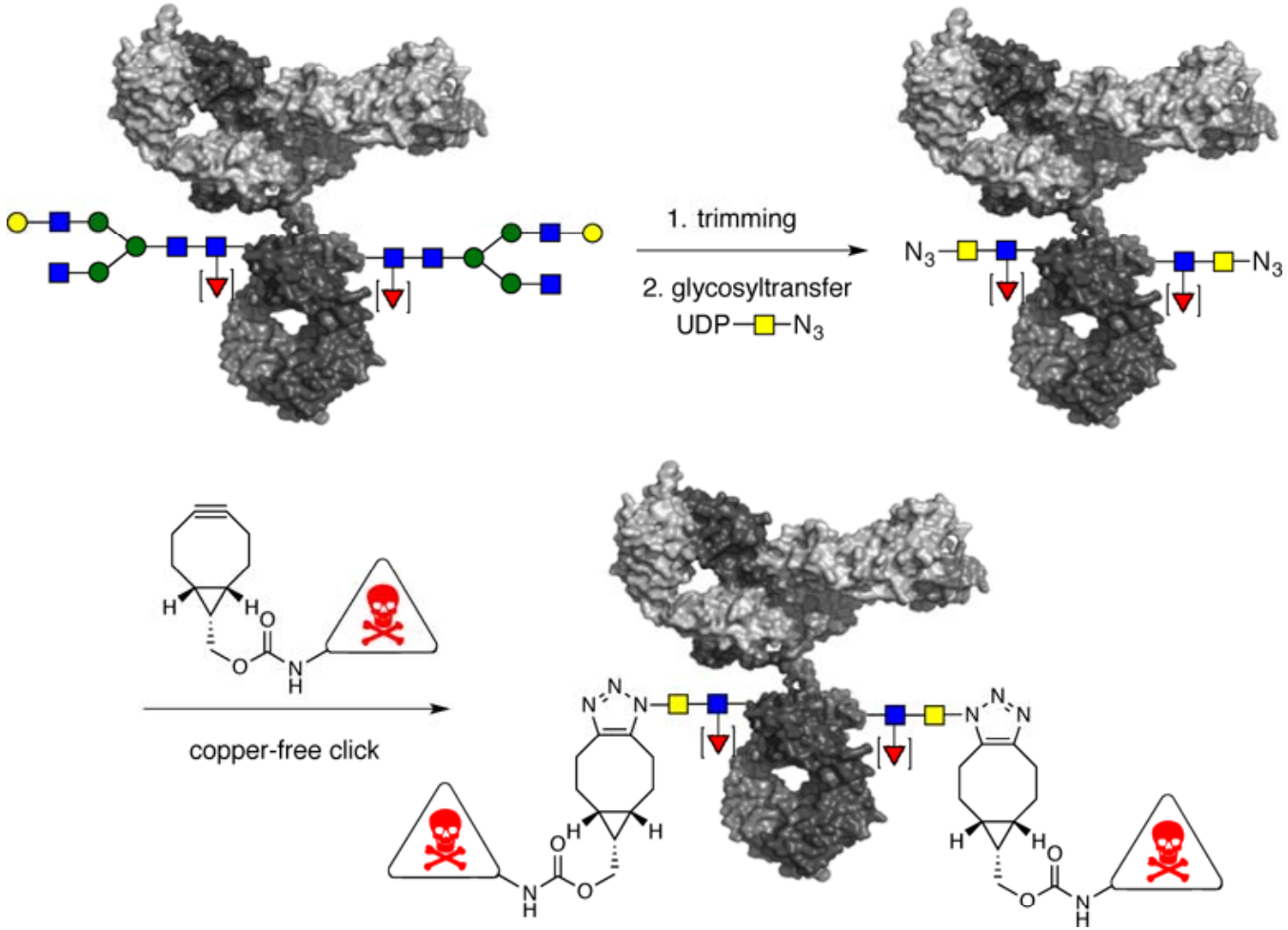


Figure 1. Antibody–drug conjugate conjugation sites and drug-to-antibody ratio heterogeneity. (A) Conjugation through lysines, **(B)** conjugation through reduced interchain disulfide bonds, and **(C)** conjugation through engineered cysteines.

Kaur S *et al*. *Bioanalysis*, (2013) 5(2), 201–226

GlycoConnect ADC: a new glycan conjugation idea



Remon van Geel *et al*, Chemoenzymatic Conjugation of Toxic Payloads to the Globally Conserved N-Glycan of Native mAbs Provides Homogeneous and Highly Efficacious Antibody−Drug Conjugates, *Bioconjugate Chem.* 2015, in press

Why new BA approaches are needed

+ Biotherapeutics innovation is producing increasingly complex molecular constructs

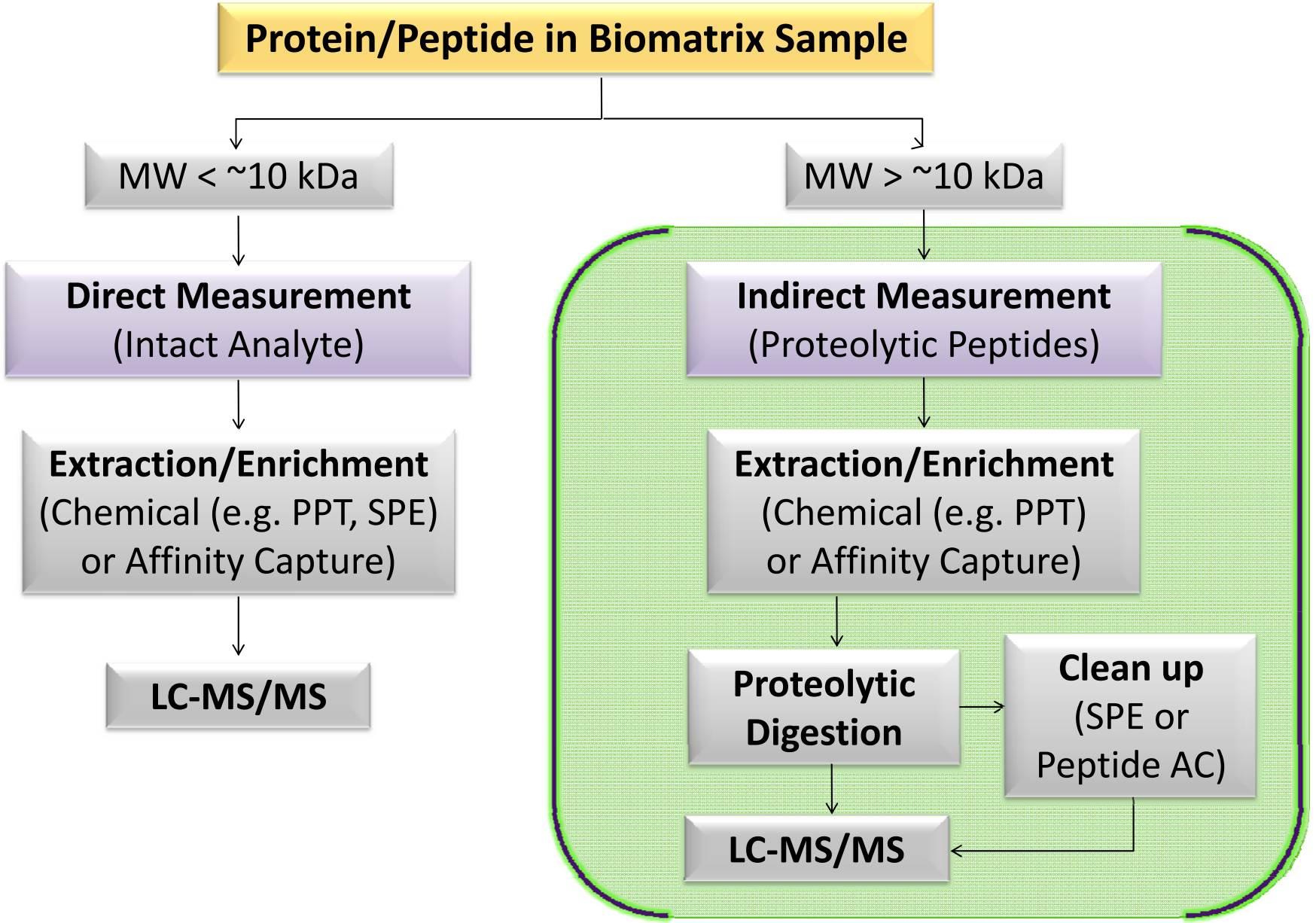
+ Their inherent heterogeneity and potential to undergo stability- and/or catabolism-related changes are driving the need for greater characterization

+ Conventional Ligand Binding Assays (LBA) are limited in their ability to provide data reflecting molecular structure (e.g. Drug Antibody Ratio (DAR) distribution for ADCs)

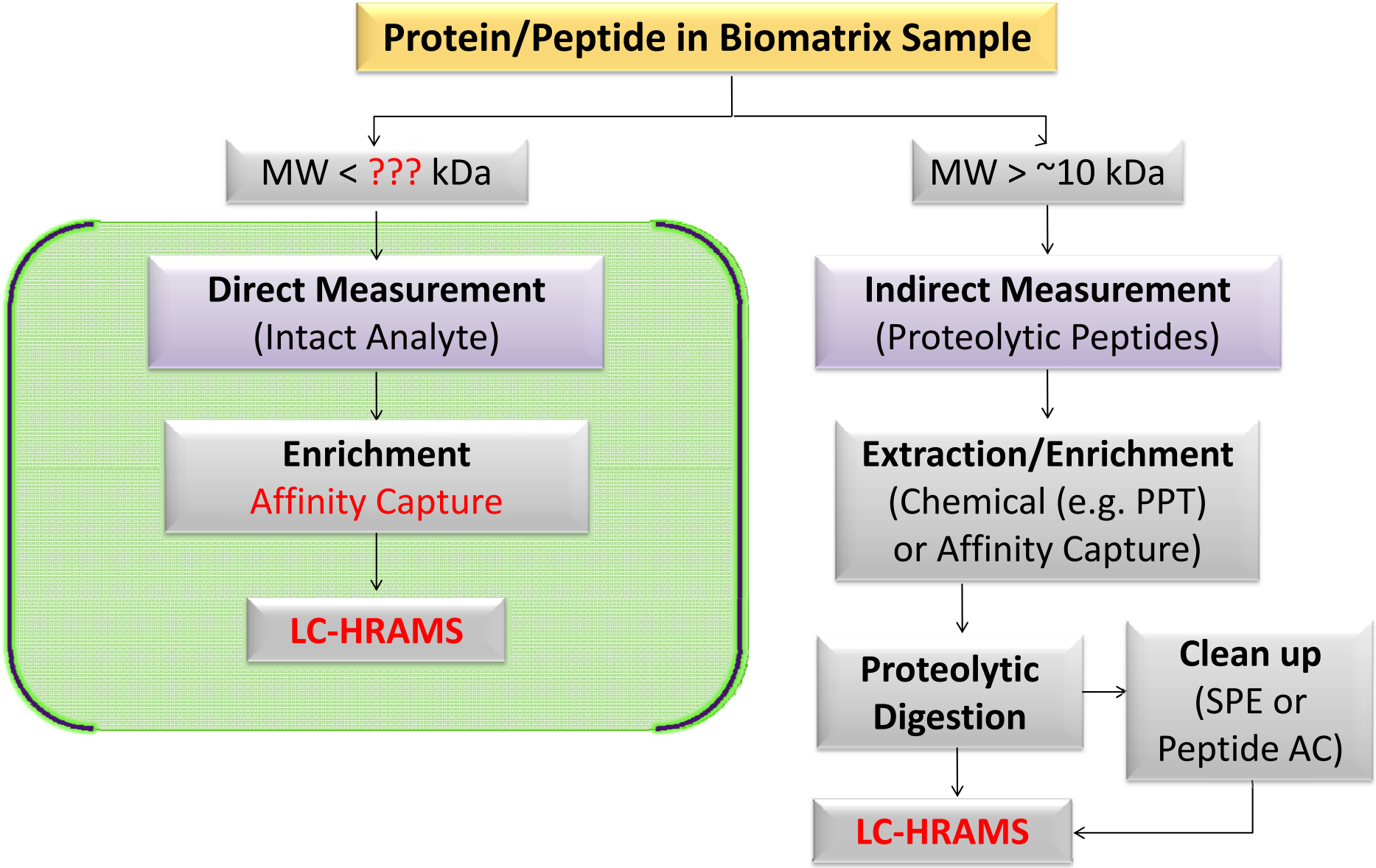
+ MS-based methods, including affinity capture-liquid chromatography-high resolution accurate mass spectrometry (AC-LC-HRAMS), are emerging as versatile tools

LC-MS protein bioanalysis workflows

Protein LC-MS/MS Bioanalysis General Strategy



Protein LC-HRAMS Bioanalysis Alternative Strategy



ADC characterization

ADC *in vivo* changes

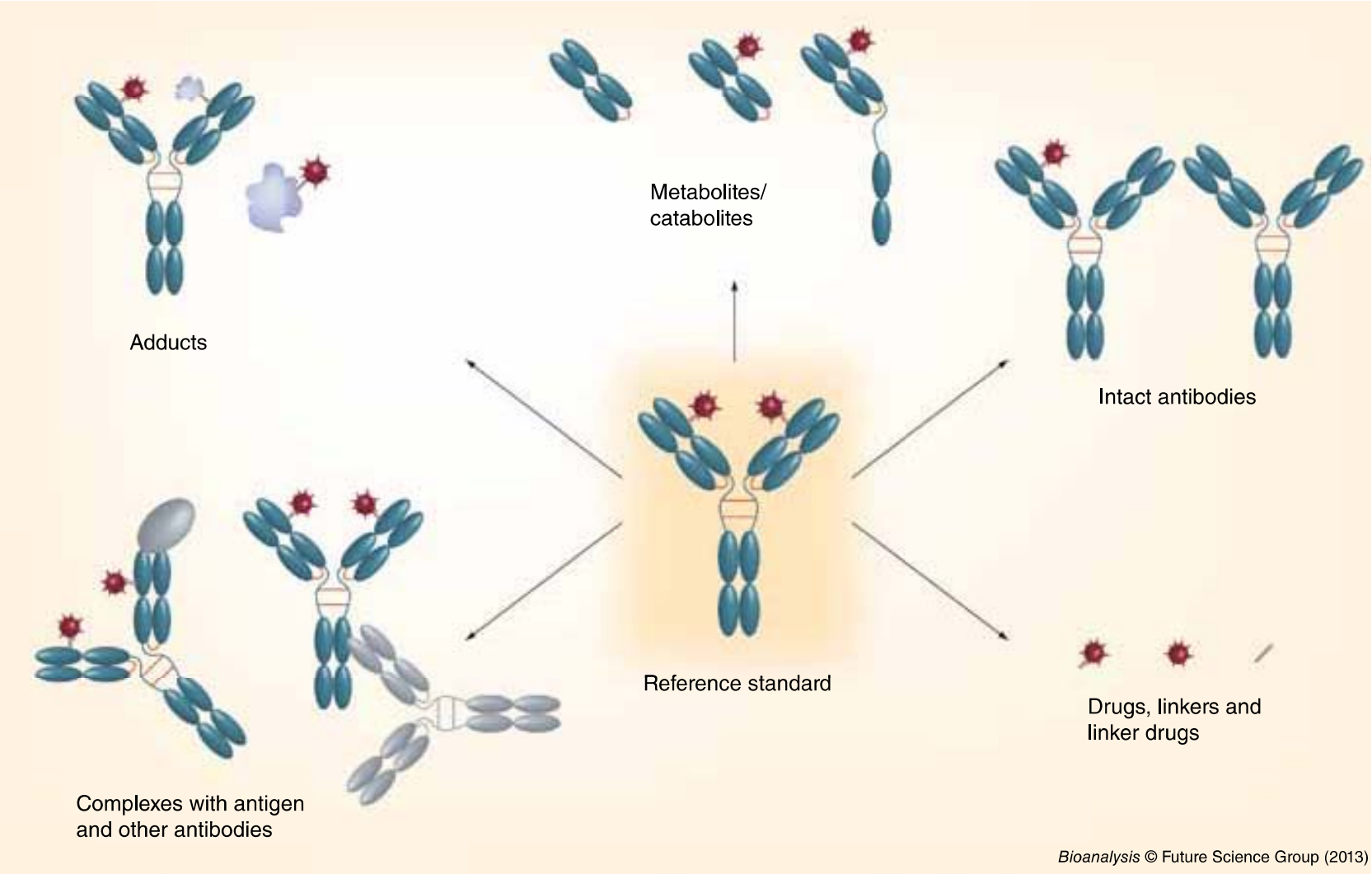


Figure 2. Antibody–drug conjugate complexity may increase *in vivo* due to catabolism or metabolism.

Kaur S *et al*. *Bioanalysis*, (2013) 5(2), 201–226

LC-MS ADC characterization *and beyond*

Assay	Technology
Drug or drug-linker metabolites *Which species exist and are important?*	LC-MS/MS
Catabolites (ADC or mAb) *Which species exist and are important?*	Affinity capture LC-HRAMS
DAR Characterization *Assess ADC stability (in vitro / in vivo)*	Affinity capture LC-HRAMS or HIC
Critical reagent DAR specificity *Assess LBA reagents*	Affinity capture LC-HRAMS or HIC

LC-MS ADC characterization *and beyond*

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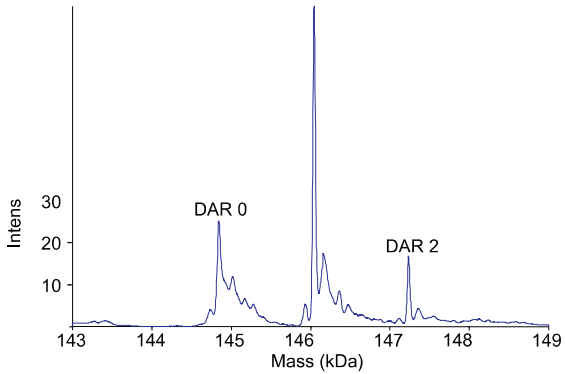
Critical reagent DAR specificity *Assess LBA reagents*	Affinity capture LC-HRAMS or HIC
Intact ADC quantification *Useful to measure individual DARs?*	**Affinity capture LC-HRAMS**

AC-LC-HRAMS to assess *intact* ADC changes

: streptavidin coated paramagnetic bead, : biotinylated capture probe,
: antibody or antibody drug conjugate (ADC)
: proteins, peptides and other species in plasma



Xu K, Liu L, Saad O, et al, Anal Biochem, 412, 56–66 (2011).

in vivo ADC characterization

lose

dose

-dose

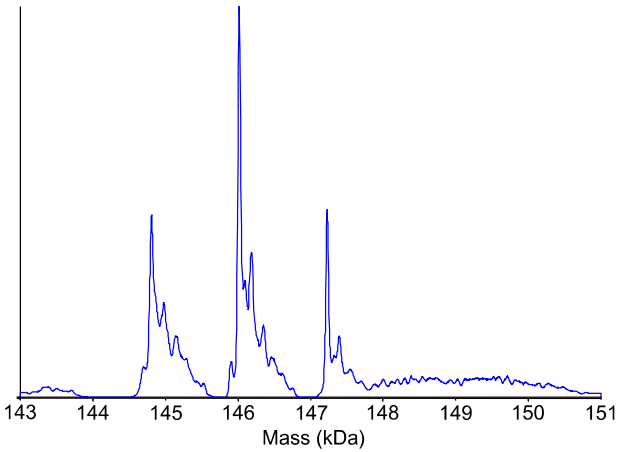
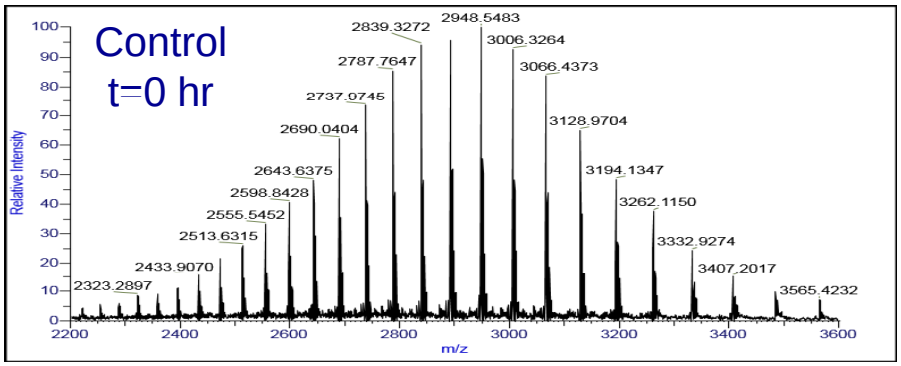
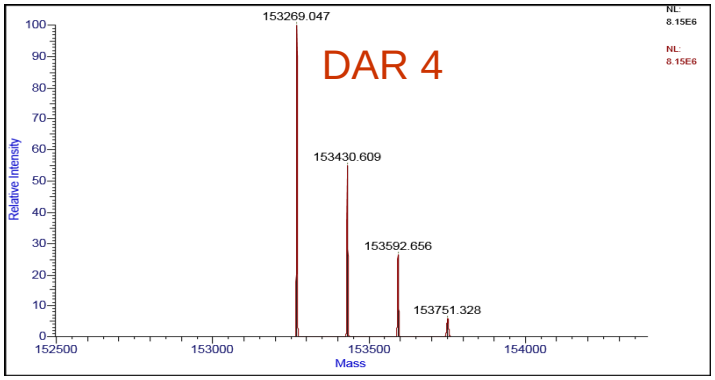


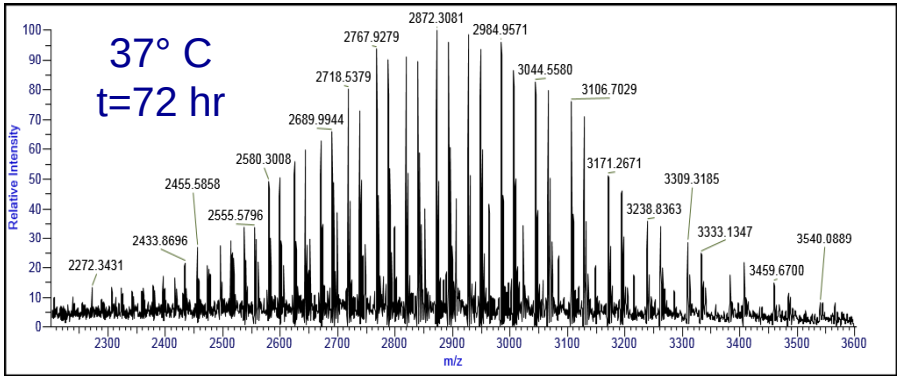
Fig.6. Drug release observed in a multiple-dose toxicokinetic study of anti-MUC16 TDC *in vivo*. Anti-MUC16 TDC (DAR 2) was administered to cynomolgus monkeys intravenously once every 3 weeks for a total of four doses. Three dose groups at 6, 10, and 20 mg/kg were evaluated. Deconvoluted mass spectra of the TDC species of a representative animal receiving the first dose of 6 mg/kg anti-MUC16 TDC show the drug release from DAR 2 to form DAR 1 and DAR 0 with time: (A) 1 day postdose; (B) 7 days postdose; (C) 21 days postdose.

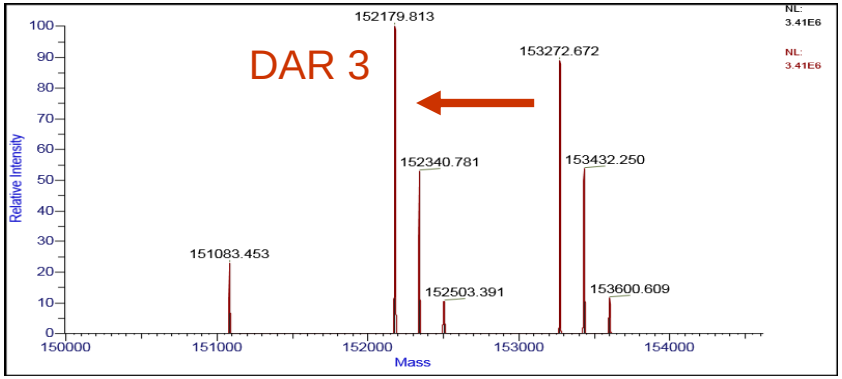
Xu K *et al*, *Anal. Biochem*, 412, 56–66 (2011)

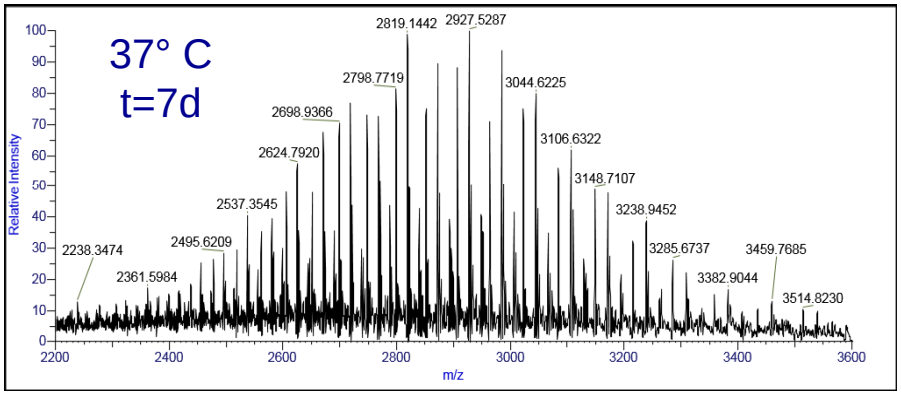
Engineered ADC (DAR4) *in vitro* plasma stability

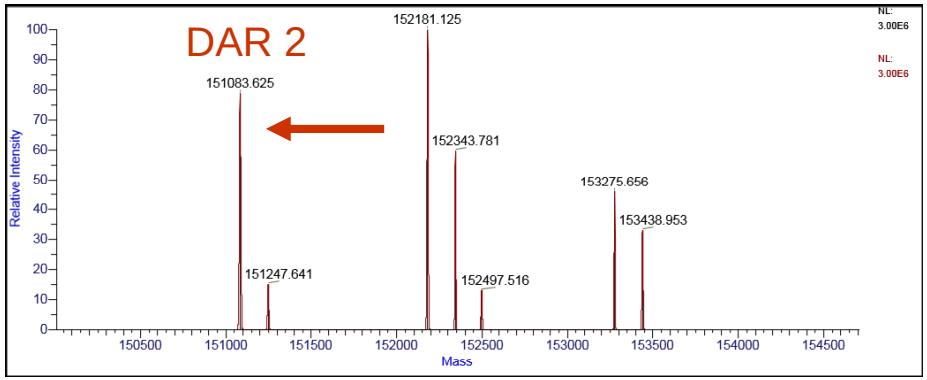












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Non-cleavable ADC with site-specific catabolism

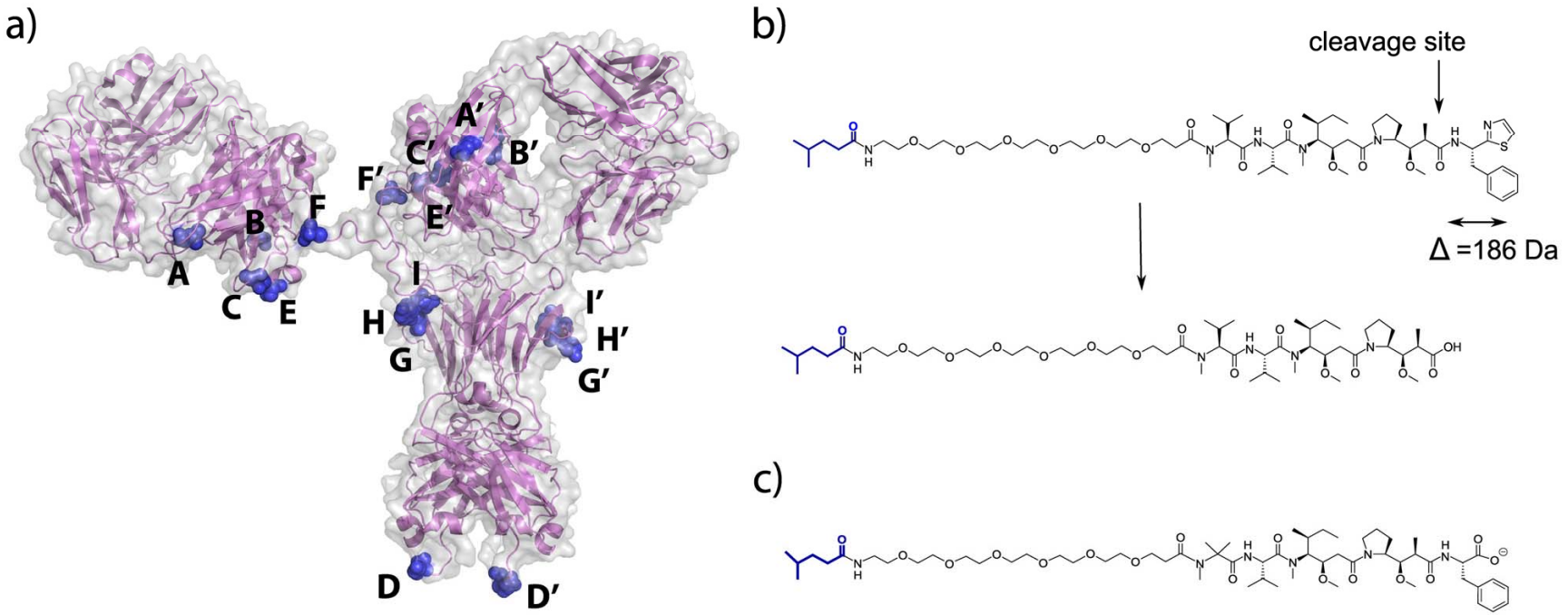


Fig 1. Stability studies of site-specific non-cleavable ADCs. a) Positions of conjugation sites on an antibody. b) Structure of the PEG6-C2-MMAD non-cleavable payload conjugated to the glutamine tag on the antibody, and its cleavage product. The glutamine residue is shown in blue. c) Structure of the PEG6-C2-Aur3377 non-cleavable payload conjugated to the glutamine tag shown in blue.

doi:10.1371/journal.pone.0132282.g001

Magdalena Dorywalska *et al*, Site-Dependent Degradation of a Non-Cleavable Auristatin-Based Linker-Payload in Rodent Plasma and Its Effect on ADC Efficacy, PLOS ONE | DOI:10.1371/journal.pone.0132282 July 10, 2015

Non-cleavable ADC with site-specific catabolism

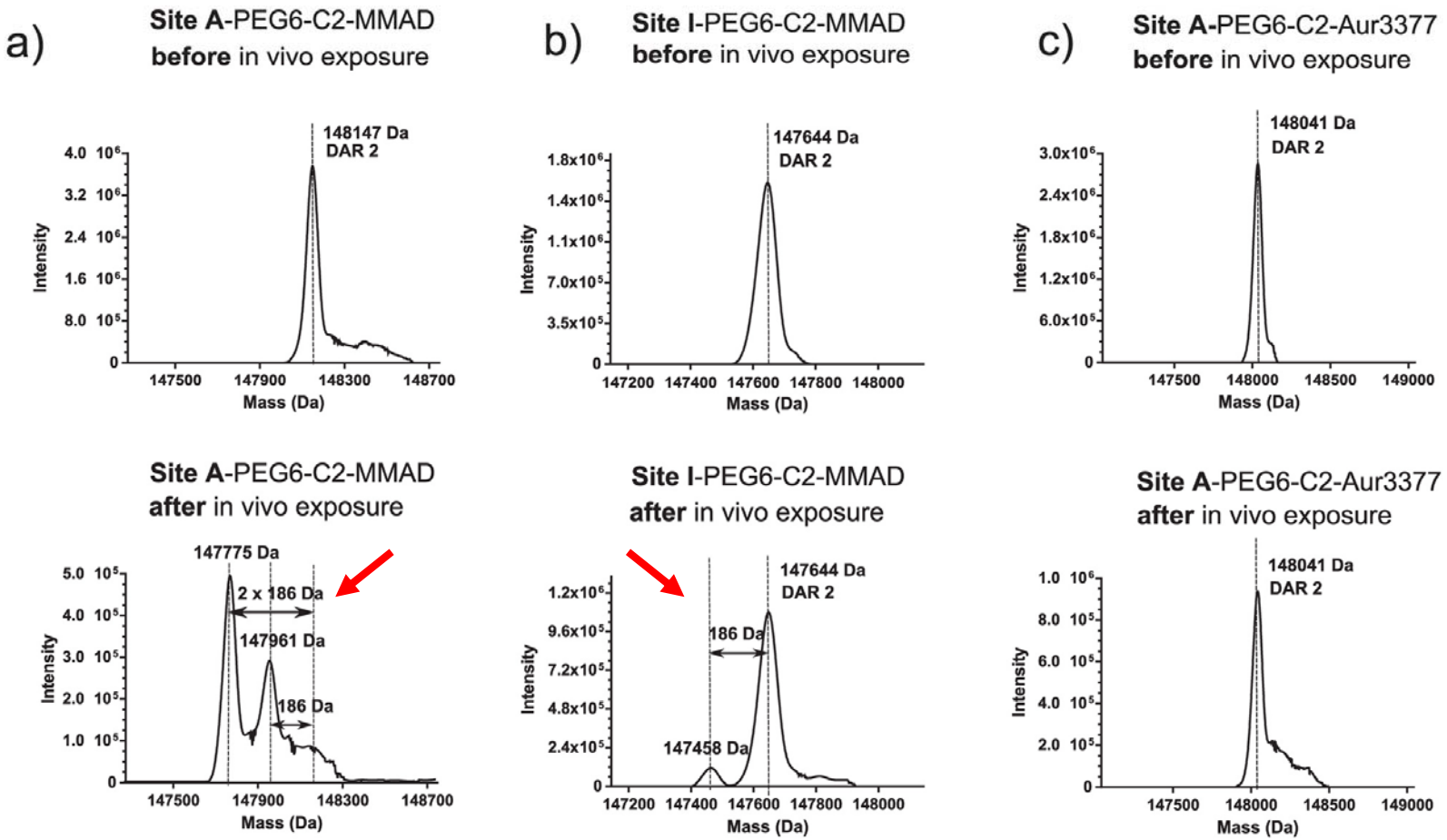


Fig 2. Mass spectrometric analysis of non-cleavable conjugates

Magdalena Dorywalska *et al*, Site-Dependent Degradation of a Non-Cleavable Auristatin-Based Linker-Payload in Rodent Plasma and Its Effect on ADC Efficacy, PLOS ONE | DOI:10.1371/journal.pone.0132282 July 10, 2015

Intact ADC quantification

LC-HRAMS quantitation data processing

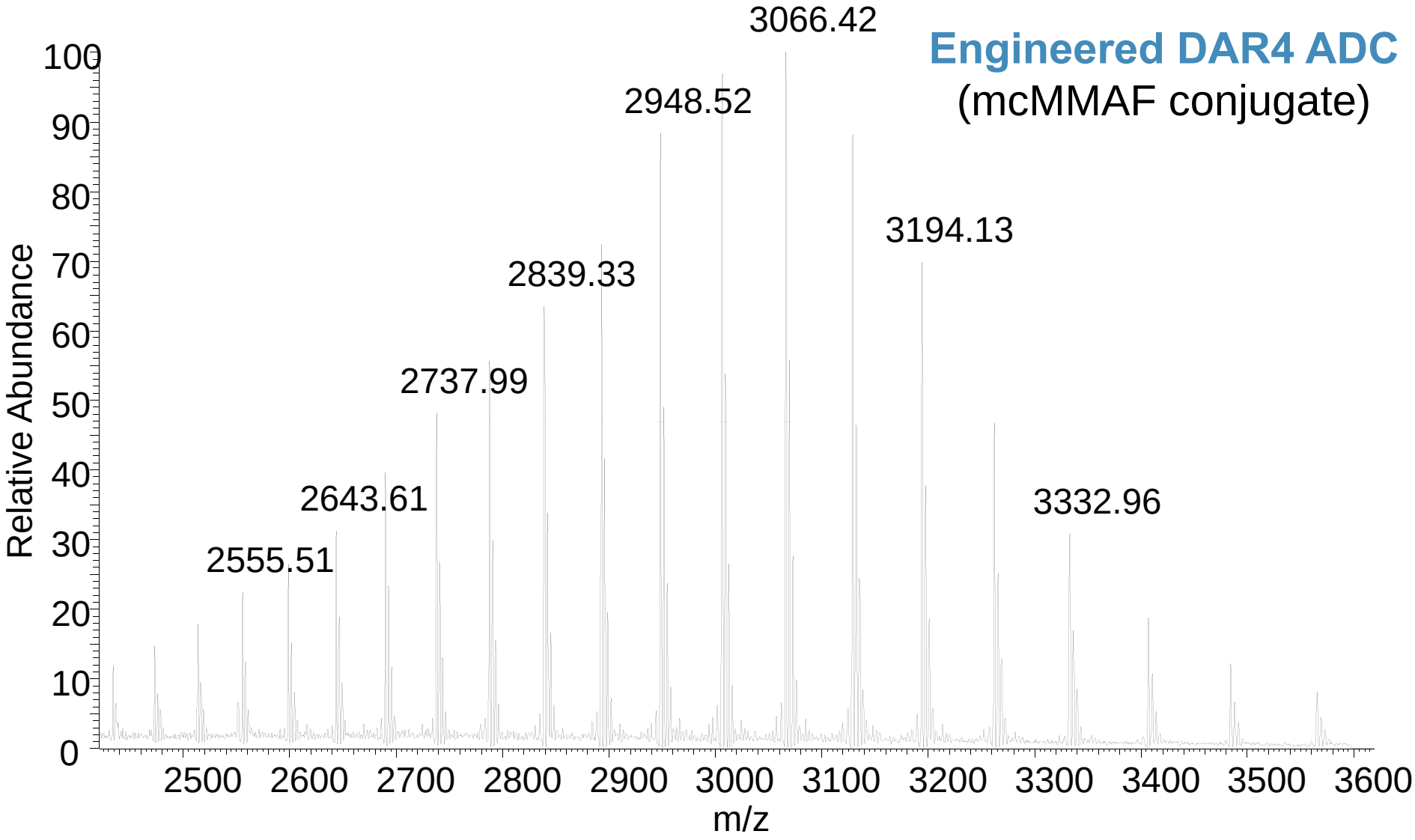
Potential options using full spectrum (FS) data acquisition

+ XICs and traditional peak integration
  - select m/z charge state(s), individual or summed
  - use narrow data extraction window (e.g. 5 mDa)
  - integrate XIC peak area

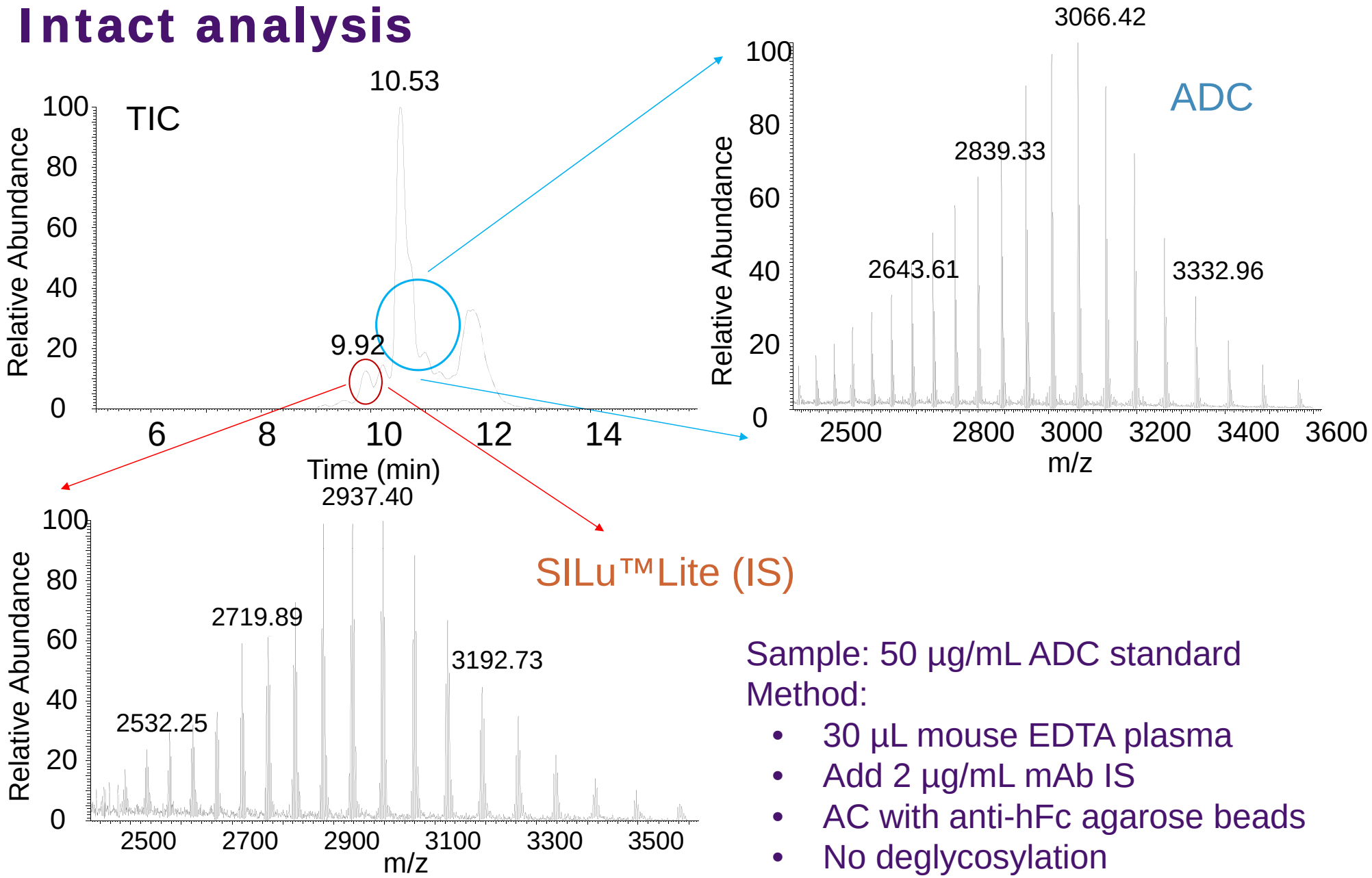
+ Deconvoluted composite spectrum
  - average/sum FS across entire chromatographic peak
  - peak area = “zero-charge” state response

+ Deconvoluted individual FS
  - generate “zero-charge” state XIC
  - integrate XIC peak area

Raw HR mass spectrum



Intact analysis



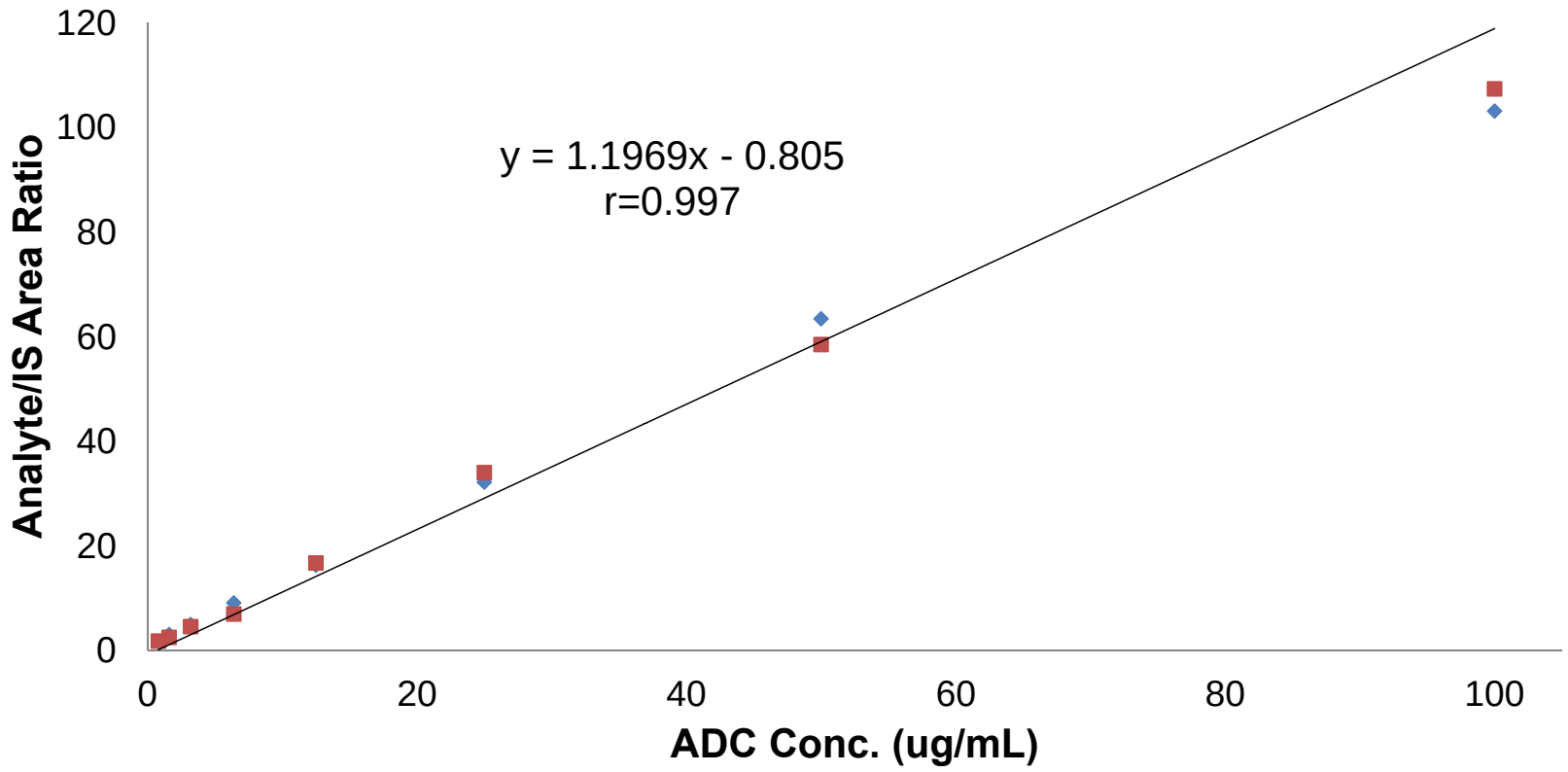
Sample: 50 µg/mL ADC standard

Method:

- 30 µL mouse EDTA plasma
- Add 2 µg/mL mAb IS
- AC with anti-hFc agarose beads
- No deglycosylation
- Elute and analyze

Summed XICs approach (with mAb IS)

Calibration Curve (XICs: m/z 2839, 2892, 2948, 3006, 3066)

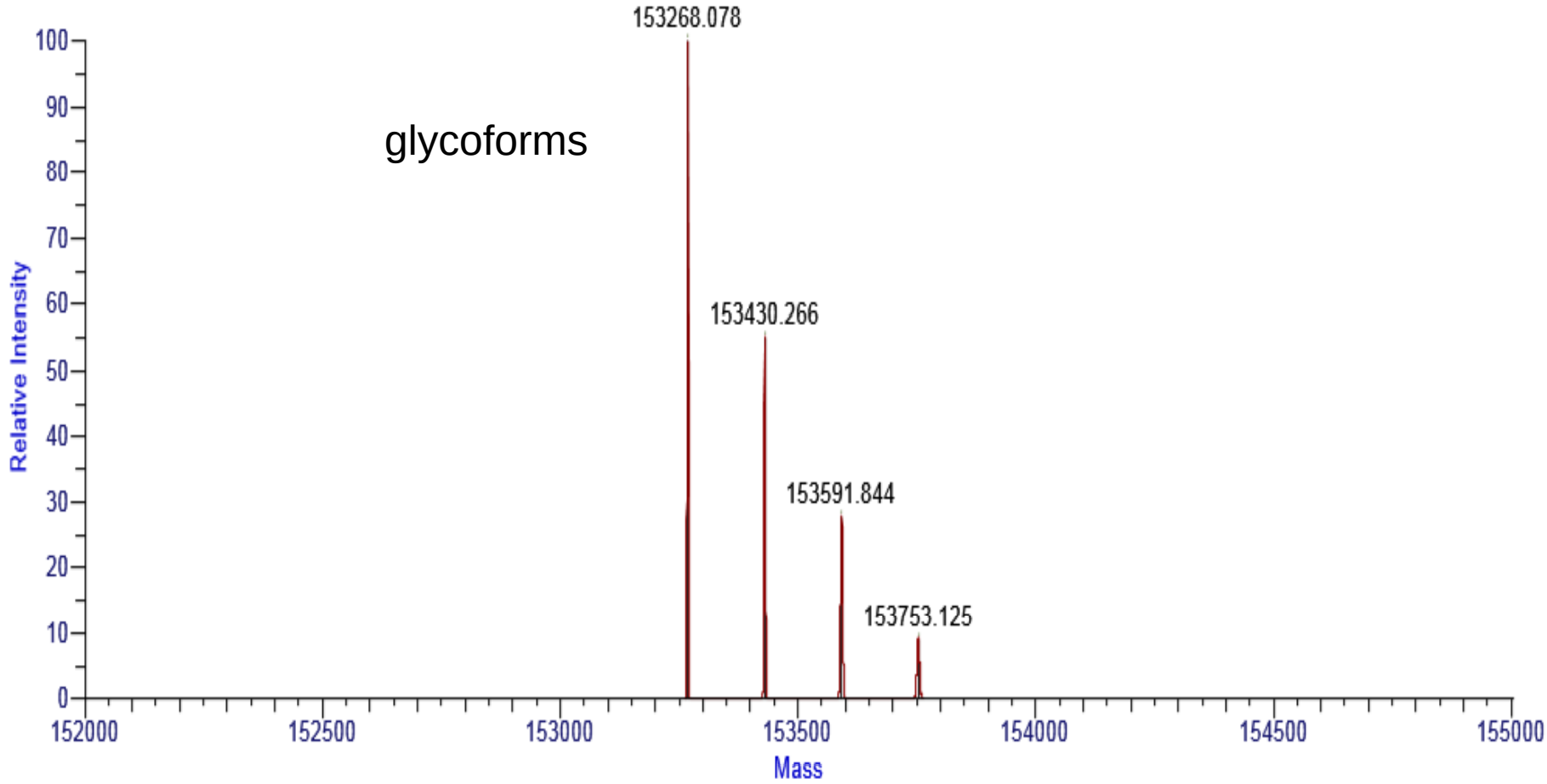


A&P - Summed XICs approach (with mAb IS)

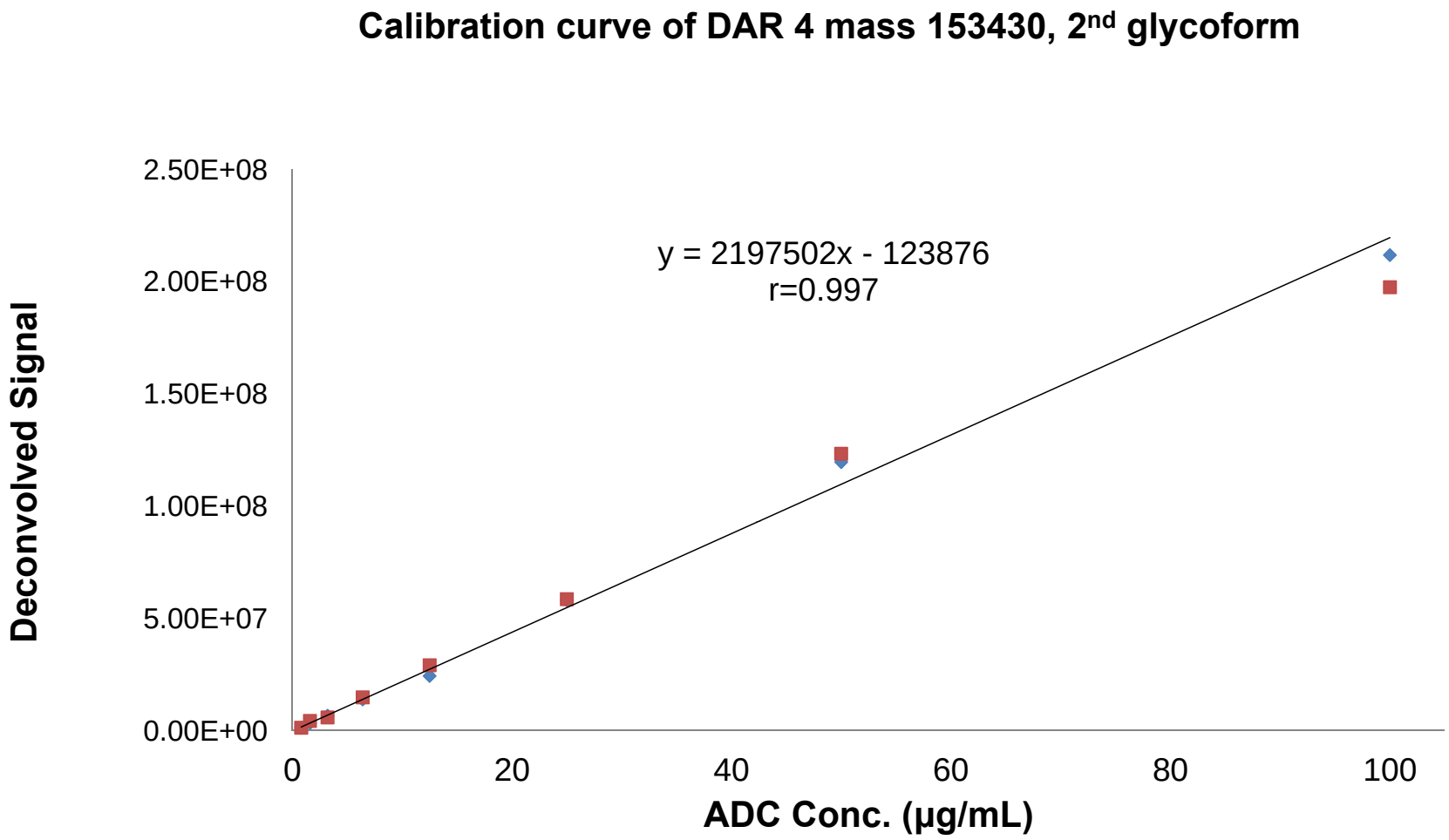
QCs	Analyte/IS	Cal. Conc. (ug/mL)	Nominal Conc. (ug/mL)	Accuracy	CV%
QC 1-1	3.00	1.83	1.6	115	11.7
QC 1-2	2.79	1.66	1.6	104	
QC 1-3	2.49	1.41	1.6	88.1	
QC 1-4	2.51	1.42	1.6	89.0	
QC 1-5	2.56	1.47	1.6	91.9	
QC 2-1	17.3	13.8	12.5	110	8.35
QC 2-2	15.2	12.0	12.5	96.3	
QC 2-3	14.9	11.8	12.5	94.1	
QC 2-4	17.8	14.2	12.5	114	
QC 2-5	16.0	12.7	12.5	101	
QC 3-1	108	89.3	100	89.3	3.01
QC 3-2	106	88.2	100	88.2	
QC 3-3	108	89.9	100	89.9	
QC 3-4	102	84.3	100	84.3	
QC 3-5	102	84.6	100	84.6	

Deconvoluted mass spectrum

Engineered DAR4 ADC
(mcMMAF conjugate)



Deconvoluted MS approach (no IS)



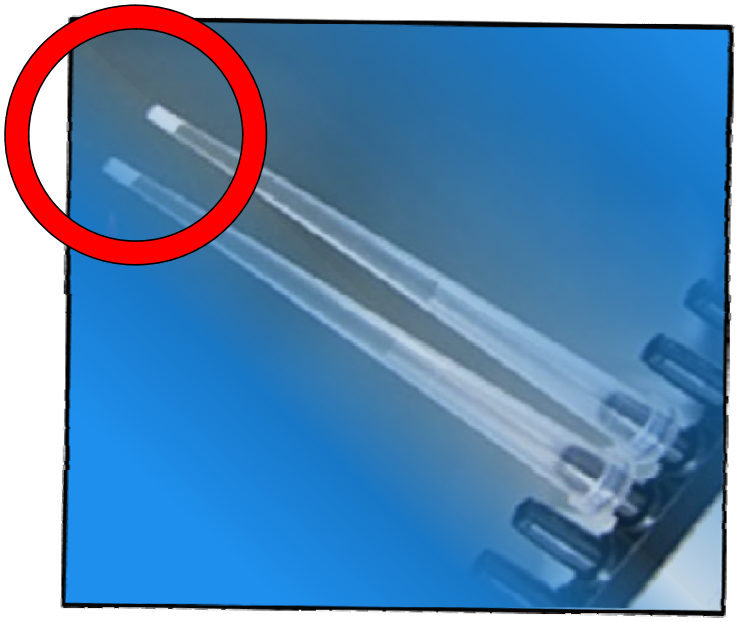
A&P - Deconvoluted MS approach (no IS)

QCs	Intensity	Cal. Conc. (ug/mL)	Nominal Conc. (ug/mL)	Accuracy	CV%
QC 1-1	3040465	1.44	1.60	90.0	12.1
QC 1-2	3171365	1.50	1.60	93.7	
QC 1-3	3384557	1.60	1.60	99.8	
QC 1-4	2532637	1.21	1.60	75.6	
QC 1-5	3566412	1.68	1.60	105.0	
QC 2-1	28494807	13.0	12.5	104.2	3.99
QC 2-2	26480701	12.1	12.5	96.9	
QC 2-3	25990923	11.9	12.5	95.1	
QC 2-4	28256266	12.9	12.5	103.3	
QC 2-5	27660784	12.6	12.5	101.1	
QC 3-1	201954923	92.0	100	92.0	2.03
QC 3-2	202279068	92.1	100	92.1	
QC 3-3	200604530	91.3	100	91.3	
QC 3-4	210591547	95.9	100	95.9	
QC 3-5	206835150	94.2	100	94.2	

MSIA D.A.R.T.'S – Efficient Analyte Capture

MSIA D.A.R.T.'S: Proprietary Affinity Technology

Described is an affinity micro-column comprising a high surface area material, which has high flow properties and a low dead volume, contained within a housing and having affinity reagents bound to the surface of the high surface area material that are either activated or activatable. The affinity reagents bound to the surface of the affinity micro-column further comprise affinity receptors for the integration into high throughput analysis of biomolecules.

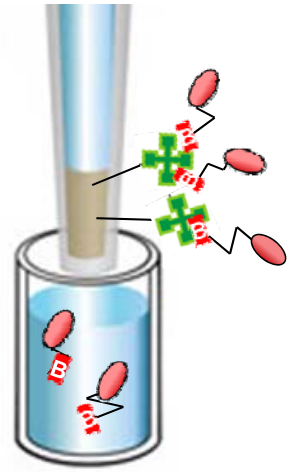


- Improved assaying performance
- Decreased matrix carryover or loss
- Decreased non-specific binding
- Flexibility in assay sample volume

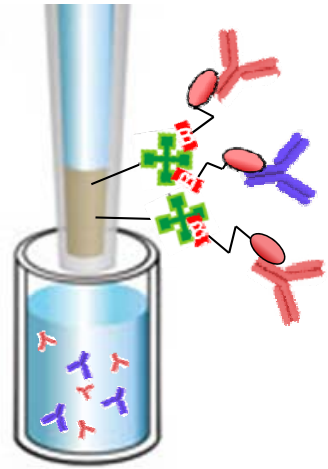
Flexible Solution: Top-Down LB-MSIA (Intact)

Evolution of the Ligand Binding Assay

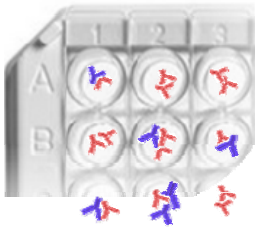
Start → Capture → Start → Inject → Data



Streptavidin – CaptureSelect

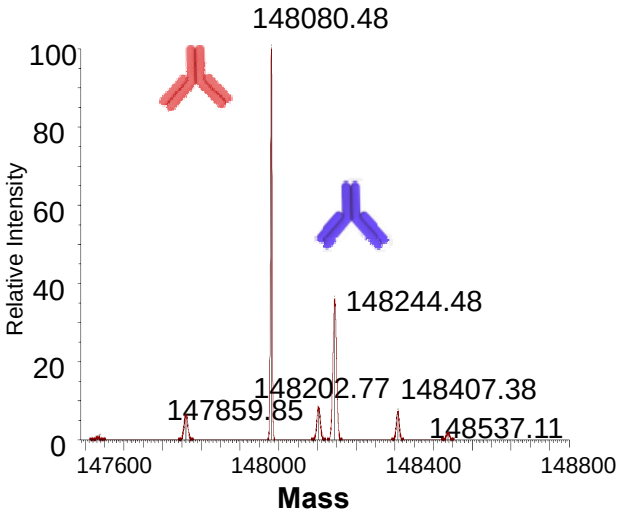


Therapeutic mAb Capture



Eluted analyte Intact Detection

HRAM MS detection



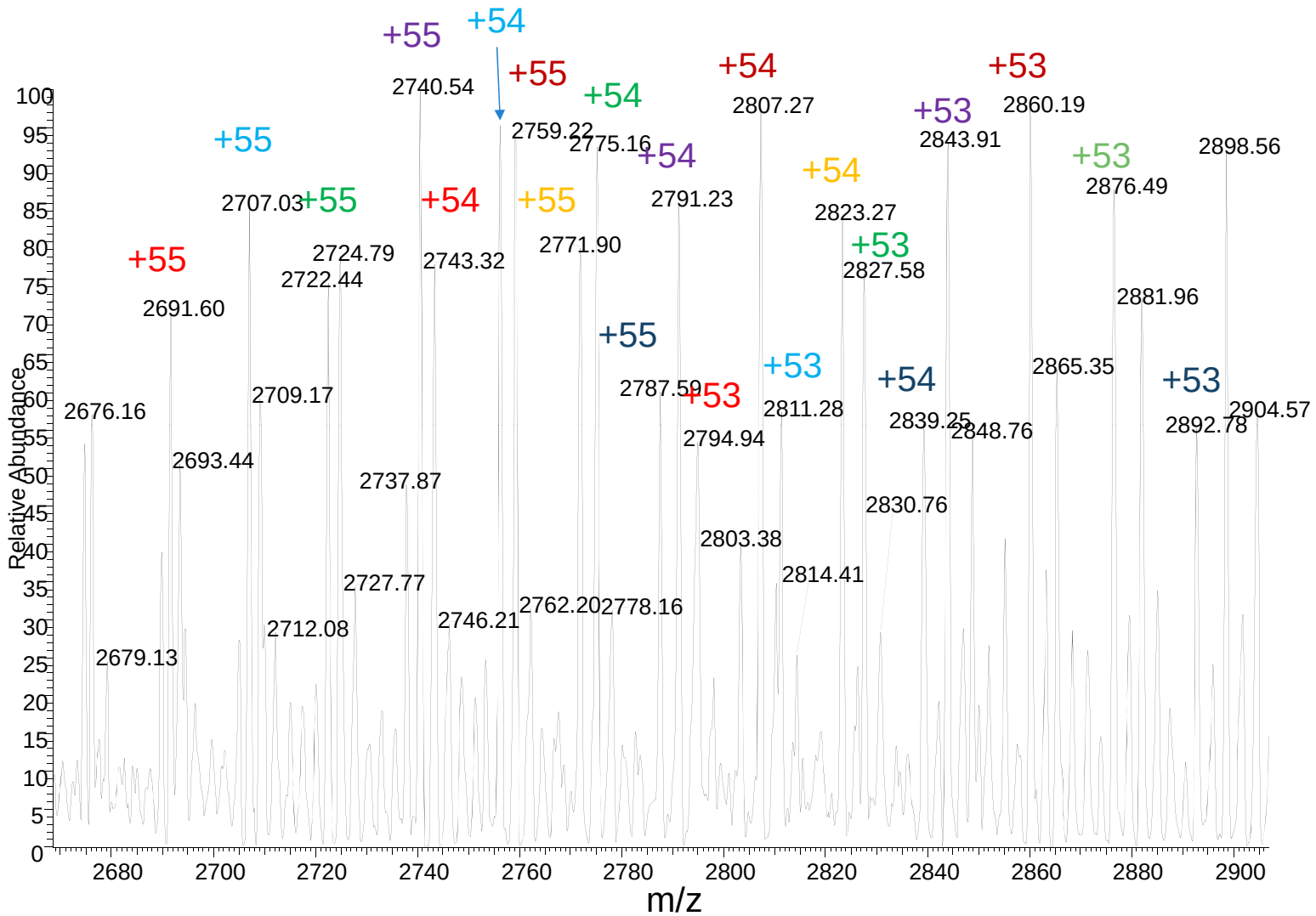
Intact mAb data content

Pre-Analytical | **Analytical - Detection**

Hybrid MS Workflow Solution

ThermoFisher SCIENTIFIC

Intact Detection
ADC masses Utilized for XIC Plots



DAR0

DAR1

DAR2

DAR3

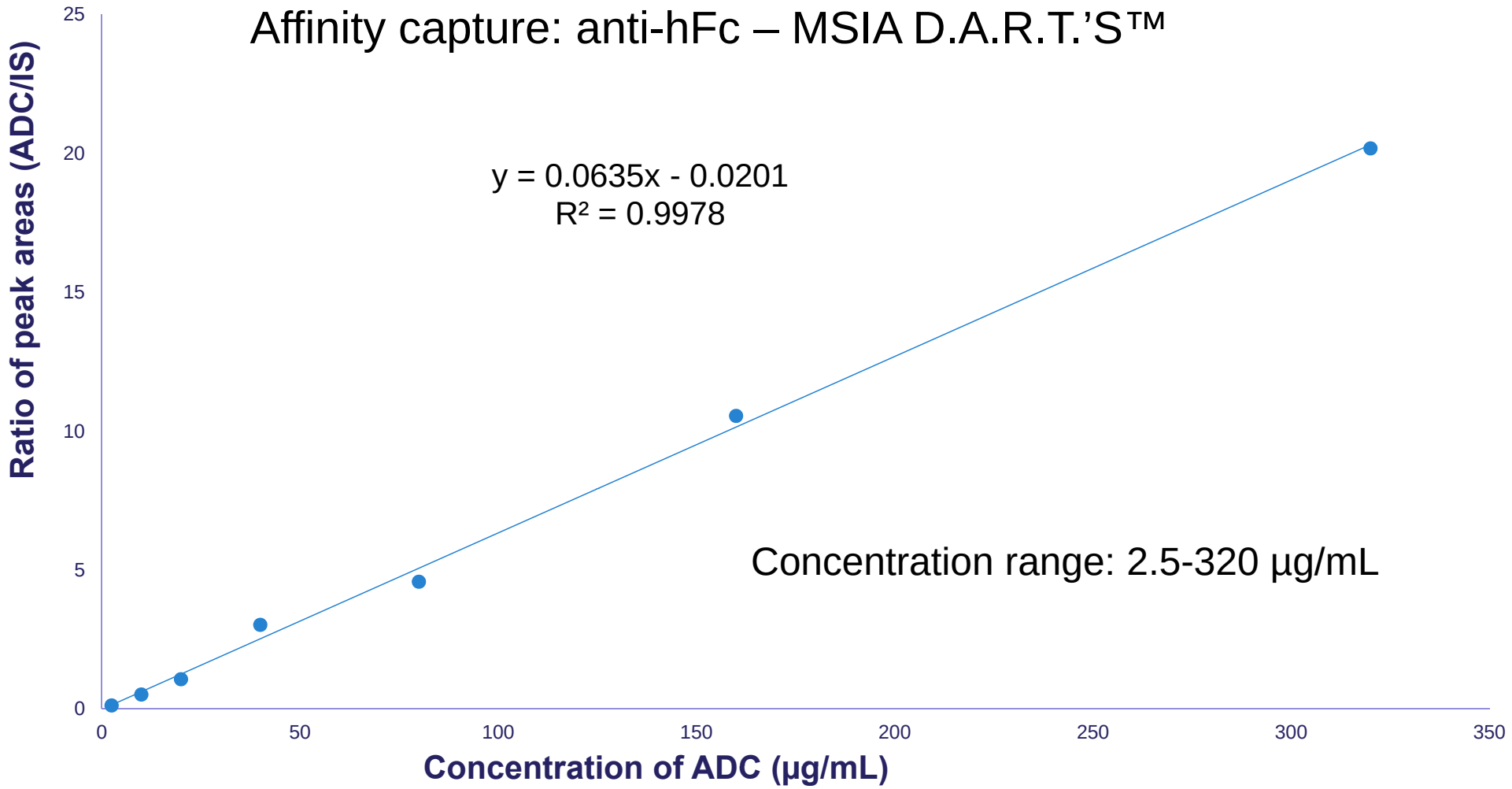
DAR4

DAR5

DAR6

ThermoFisher SCIENTIFIC

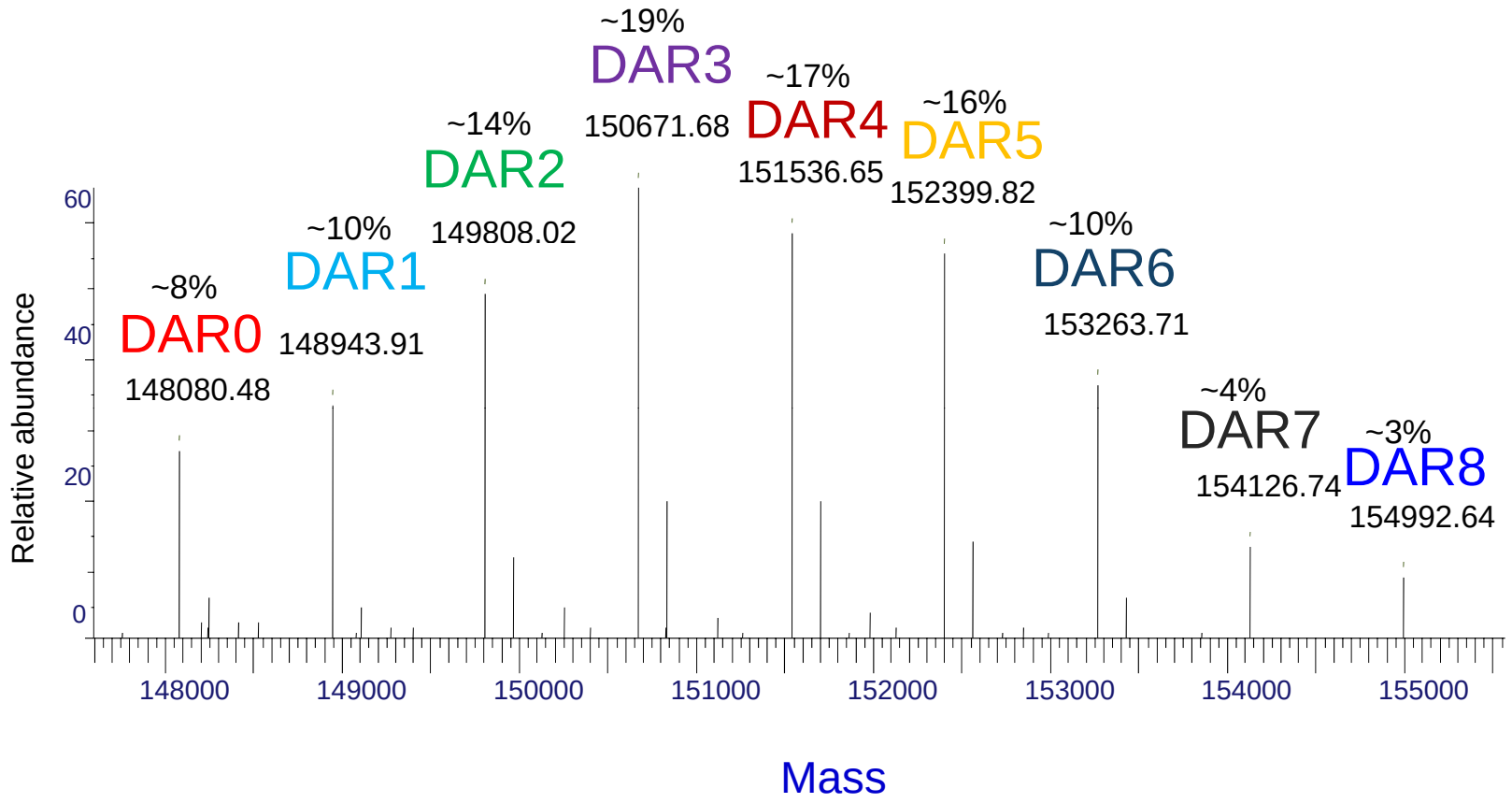
ADC Standard Curve in Rat Plasma



Curve: XIC-based Quantification

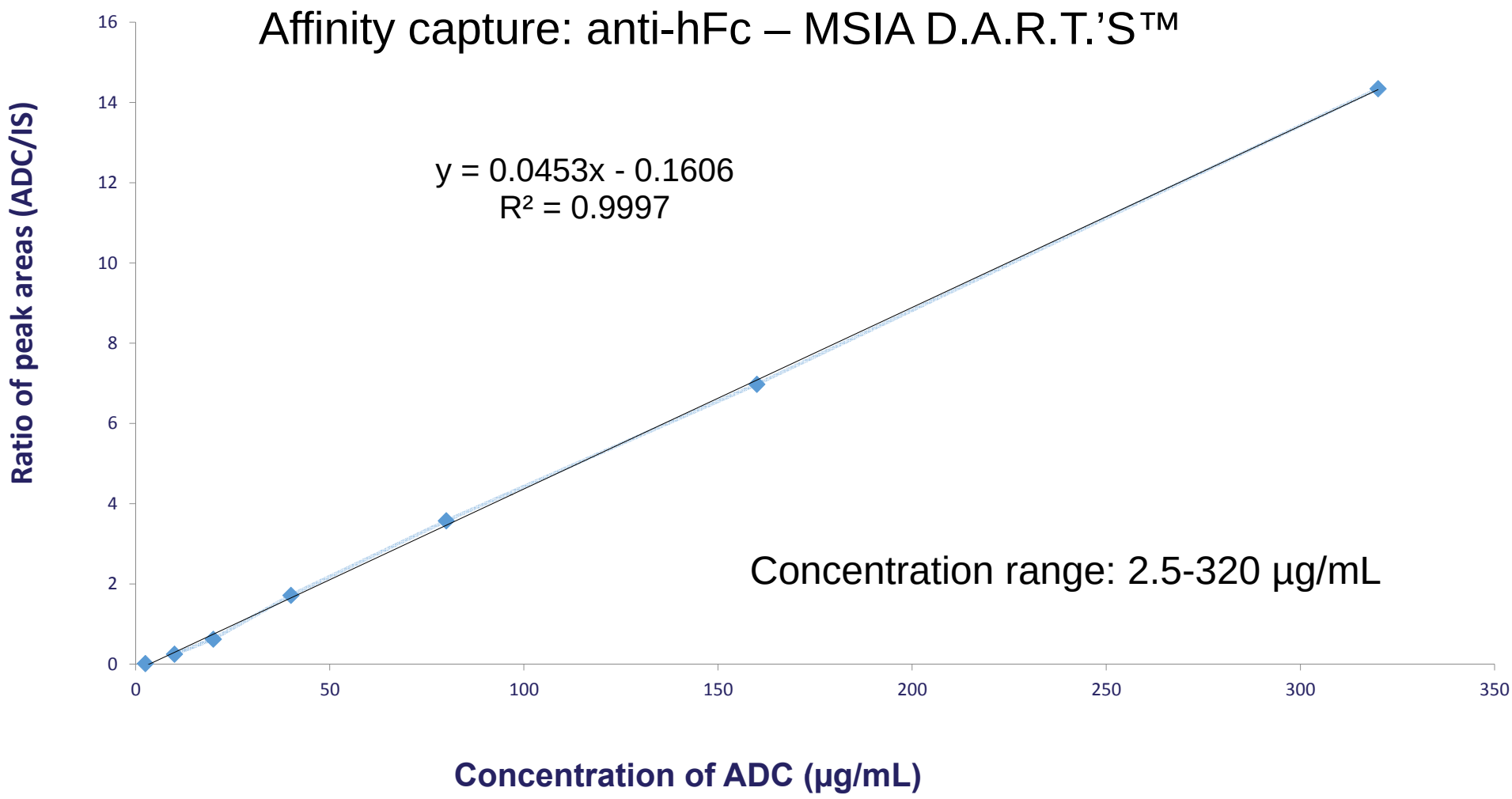
ThermoFisher SCIENTIFIC

Intact Detection
Deconvoluted Spectrum



ThermoFisher SCIENTIFIC

ADC Standard Curve in Rat Plasma



Curve: Deconvoluted-based Quantification

ThermoFisher SCIENTIFIC

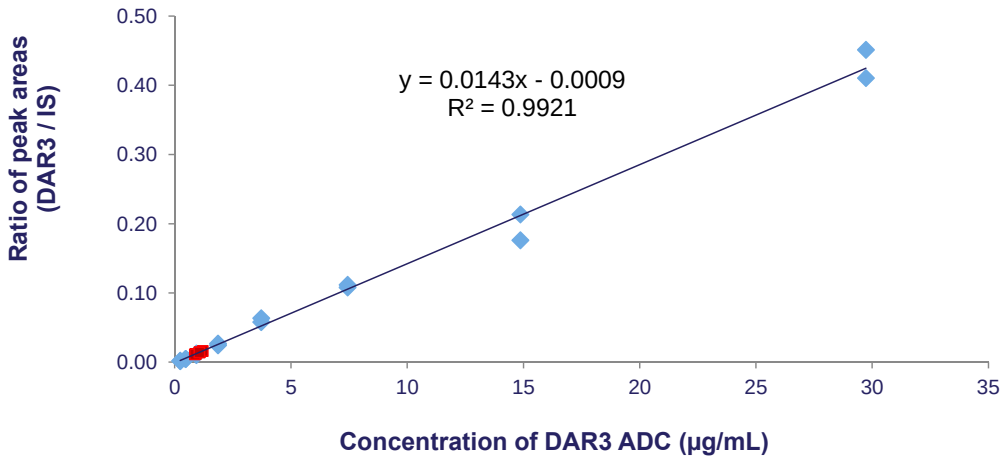
Assay Characteristics

Deconvoluted-based Quantification

Sample Concentration (µg/mL)	Average concentration (µg/mL) (n=5)	% CV (n=5)	% Accuracy (n=5)
Day 1			
5.0	4.5	7.2	-10.7
13.5	14.2	5.9	5.2
53.5	53.4	5.0	-0.1
213.5	225.6	4.9	5.4
Day 2			
5.0	5.3	23.2	5.6
13.5	13.2	15.3	-2.6
53.5	52.4	8.3	-2
213.5	237.6	3.8	11.3
Day 3			
5.0	5.4	9.4	8.5
13.5	12.5	13.1	-7.5
53.5	59.5	13.7	11.2
213.5	238	8.0	11.5

ThermoFisher SCIENTIFIC

Individual ADC DAR Species Quantification (DAR3)



Experimental Measurement

QCs: 4 levels (n=5 replicates)

Deconvolution approach

QC (µg/mL)	% CV	% Accuracy
0.43	15.5	91.7
1.02	13.5	110
6.45	3.35	86.8
27.8	4.80	93.4

Note: DAR3 theoretical concentrations based on 19% of total ADC concentration

Open questions

AC-LC-HRAMS *intact* analysis open questions

+ *Technical:*
  - Sensitivity good enough?
  - Mass resolution required to ensure specificity?
  - How to best process raw FS data?
  - Reference standards heterogeneity/species purity?
  - What about internal standards?
  - How critical is affinity capture specificity/efficiency?
  - How important is chromatography?
  - What to do with so much data?

AC-LC-HRAMS *intact* analysis open questions

+ *Biological:*
  - Multiple detected species, which are important to measure?
    - ❖ Pharmacologically active (*total*)?
    - ❖ Potentially toxic or off-target effects?
    - ❖ Potentially immunogenic?
  - PTMs or glycosylation changes possible, are they relevant?
  - Peptide modifications (e.g. deamidation, oxidation)?
  - Structural *liabilities* (e.g. terminal clipping, labile linkers)?

Final thoughts

+ Keeping up with biotherapeutics innovation is a challenge for both analytics (CMC) and bioanalysis

+ Effective discovery and development of complex biologics requires both LBA and LC-MS assay technologies

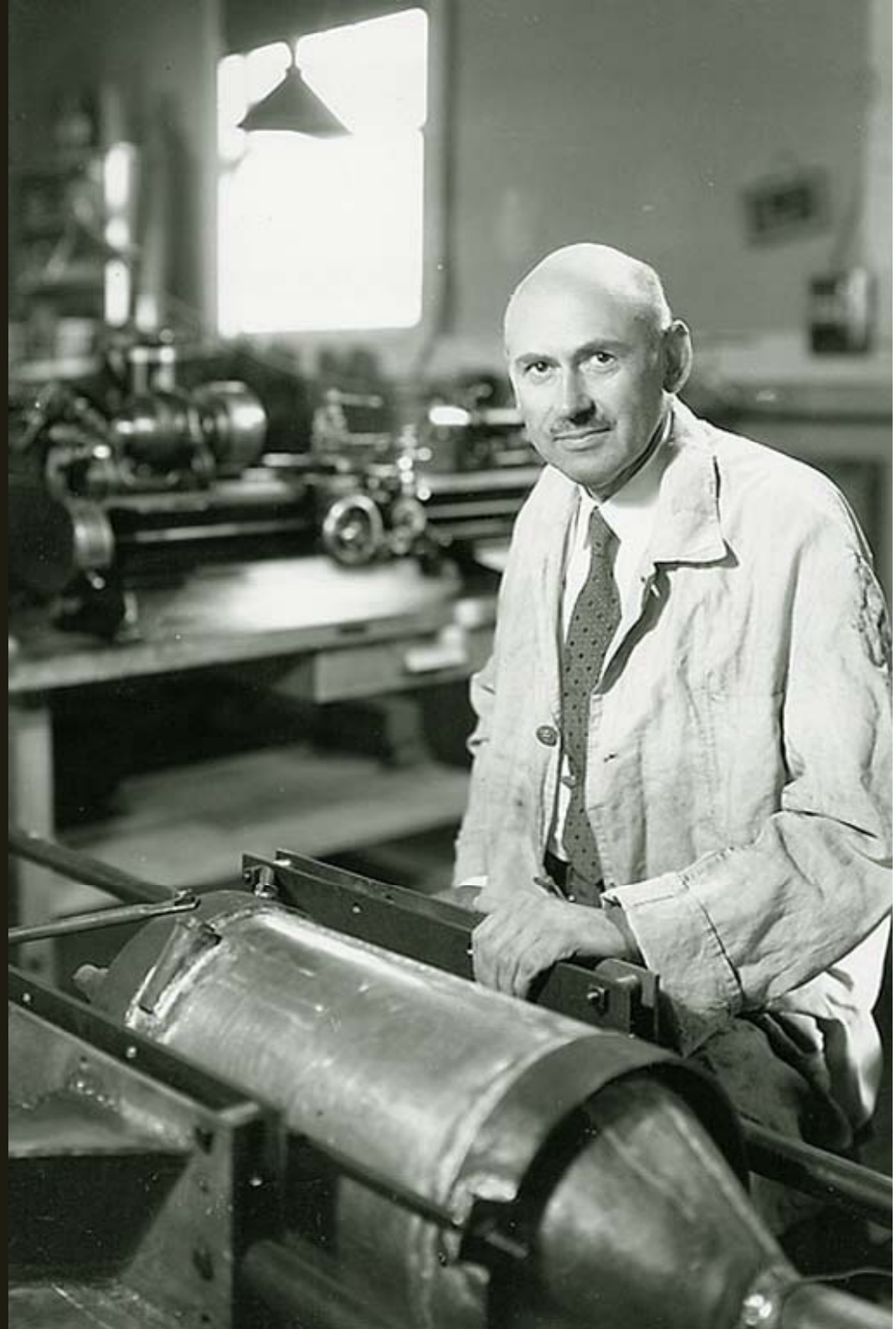
+ Inherent heterogeneity of biologics and potential dynamic changes *in vivo* require in-depth characterization

+ Regulators are aware and likely to increase their expectations

+ AC-LC-HRAMS techniques have significantly advanced, providing both characterization and sensitive *intact* quantification to aid biotherapeutics development

“It is difficult to say what is impossible, for the dream of yesterday is the hope of today and the reality of tomorrow.”

—Dr. Robert H. Goddard
(1882-1945)



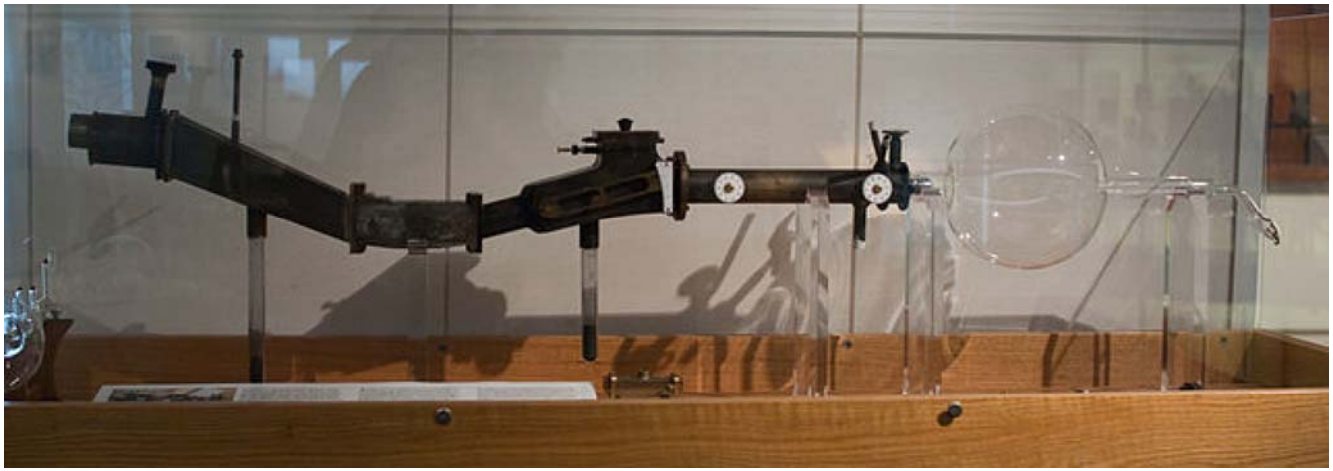
16 March 1926
Auburn, Massachusetts
USA

NASA New Horizons
19 January 2006

Pluto
from 280,000 miles
13 July 2015

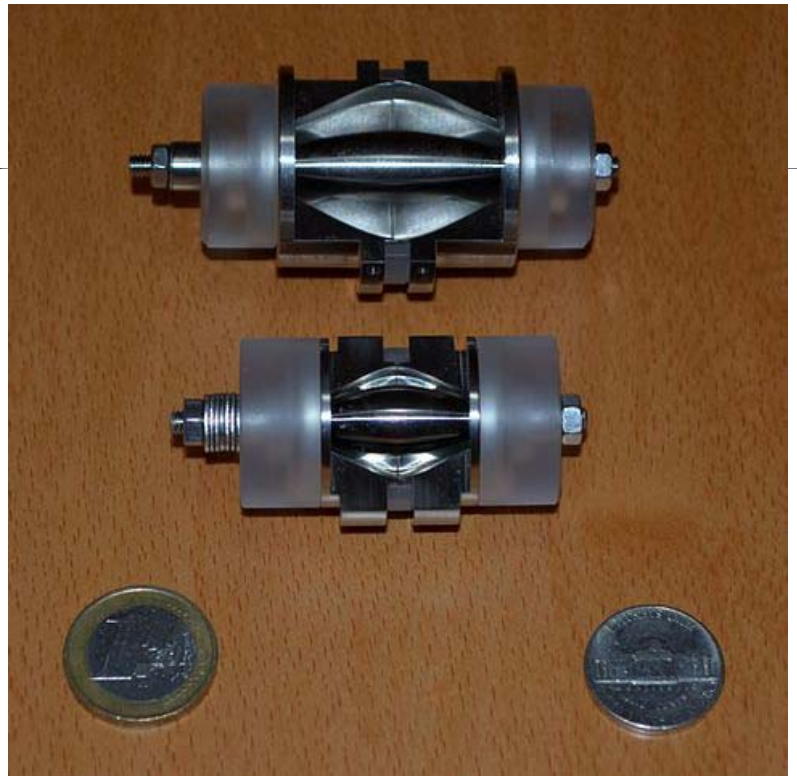
midnight EDT on 15 July 2015
(3.6 billion miles away)

Farewell to Pluto from New Horizons
looking back from 1.25 million miles

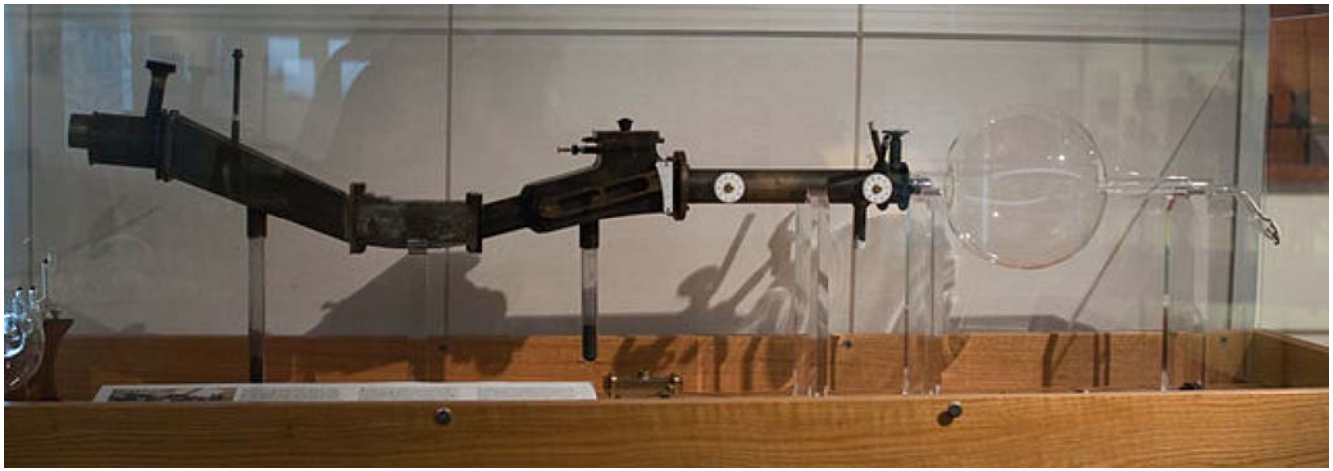


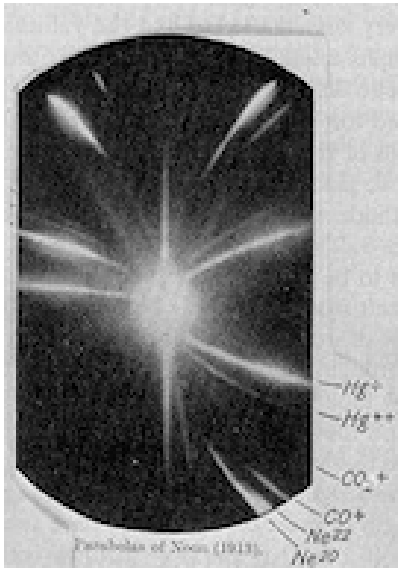
Replica of J.J. Thomson's
mass spectrometer (1912)

100 years…

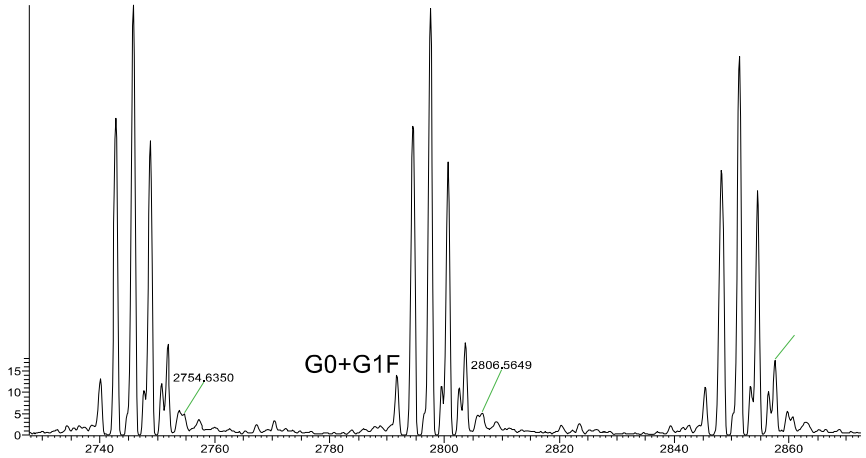


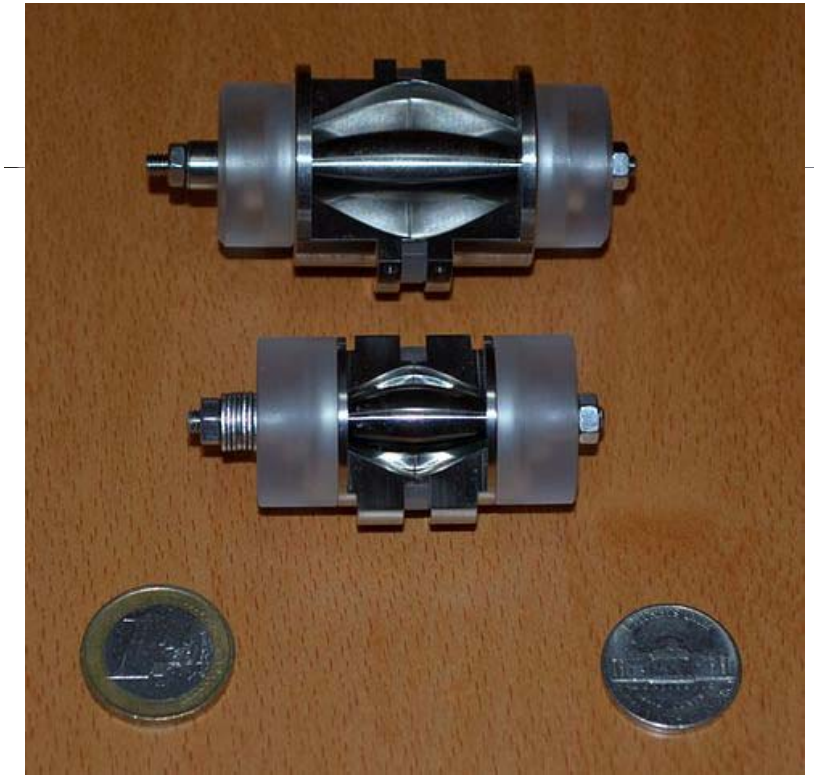
ThermoFisher Scientific
Orbitrap analyzers (2012)




Hg+
Hg++
CO2+
CO+
Ne22
Ne20

^{20}Ne, ^{22}Ne


G0+G1F
2754,6350
2806,5649
15
10
5
0
2740
2760
2780
2800
2820
2840
2860



Acknowledgements

PPD® Laboratories

- **Dongliang Zhan**
- Diego Cortes
- William Mylott
- Pat Bennett

ThermoFisher SCIENTIFIC

- Kwasi Antwi
- Jonathan Josephs
- Urban A. Kiernan
- Keeley Murphy
- Eric E. Niederkofler
- Jessica Wang

Genentech

- Keyang Xu
- Surinder Kaur

Seattle Genetics

- Shawna Hengel
- Steve Alley

…and many others

Thank You

midnight EDT on 15 July 2015

Looking back…