A Holistic Strategy to Characterize the *In Vivo* Stability of Novel Modalities using Affinity Capture Coupled to LC-MS or CE-Based Methods

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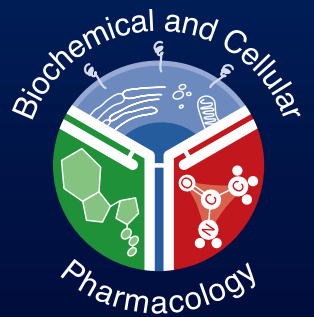
CE Pharm, October 1st 2020



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Outline

- Background
  - LM BCP MS group overview
  - Novel large molecule modalities
  - Biotransformation
- Analytical workflow for intact stability analysis
  - Affinity capture + LC-MS
  - CE LIF
  - CE Western blot
- Summary

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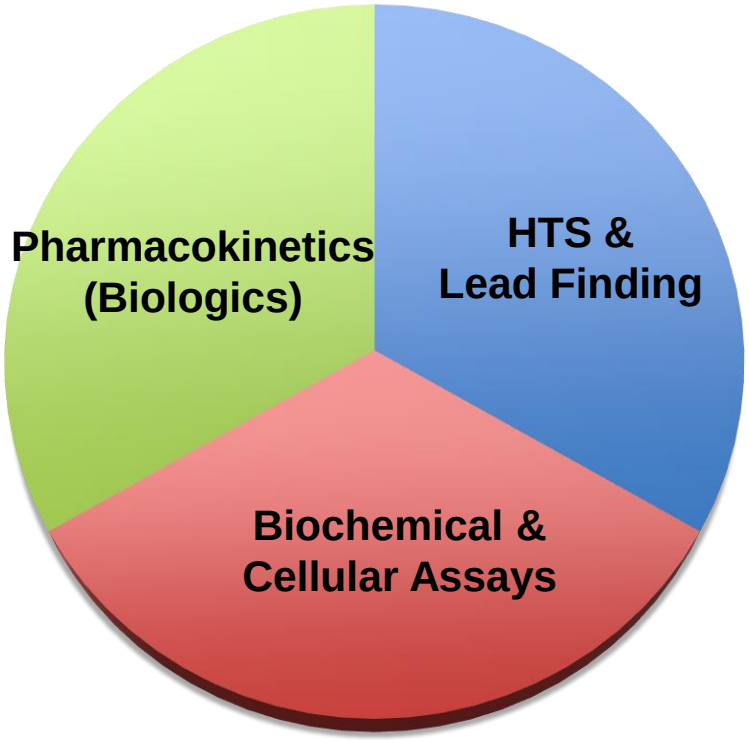
Large Molecule Biochemical and Cellular Pharmacology Mass Spec (LM BCP MS) group

Target ID and Validation → Identify/ Optimize Lead → Clinical Trials → Market



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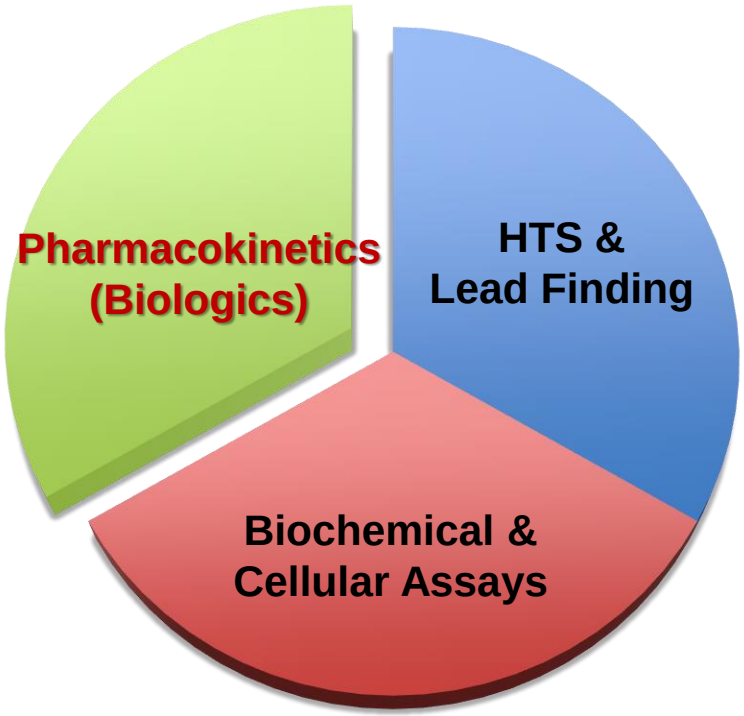

Pharmacokinetics (PK):

- Biotransformation:
  - Intact stability (clipping)
  - Amino acid level modification
  - Chemical stability of conjugates
- Biodistribution:
  - Total drug concentration in circulation
  - In tissue

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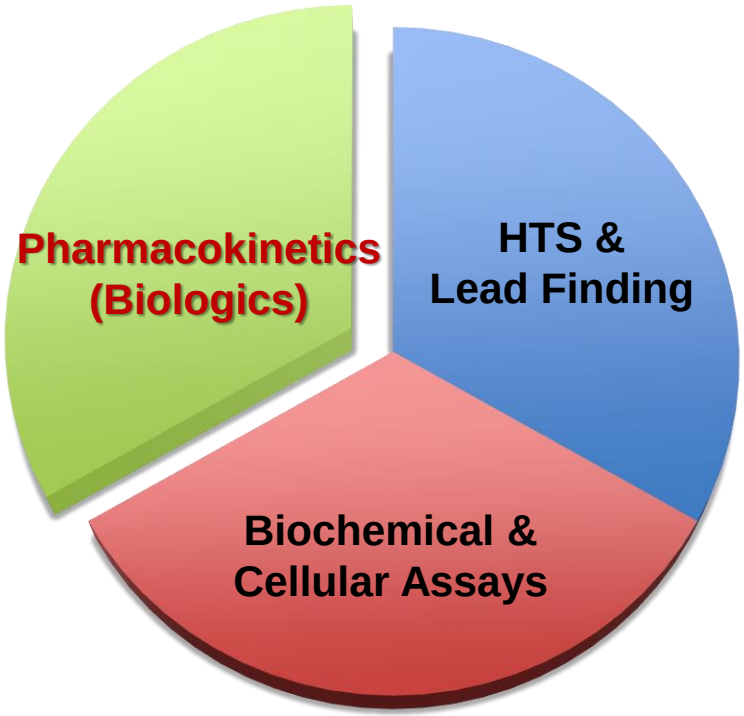

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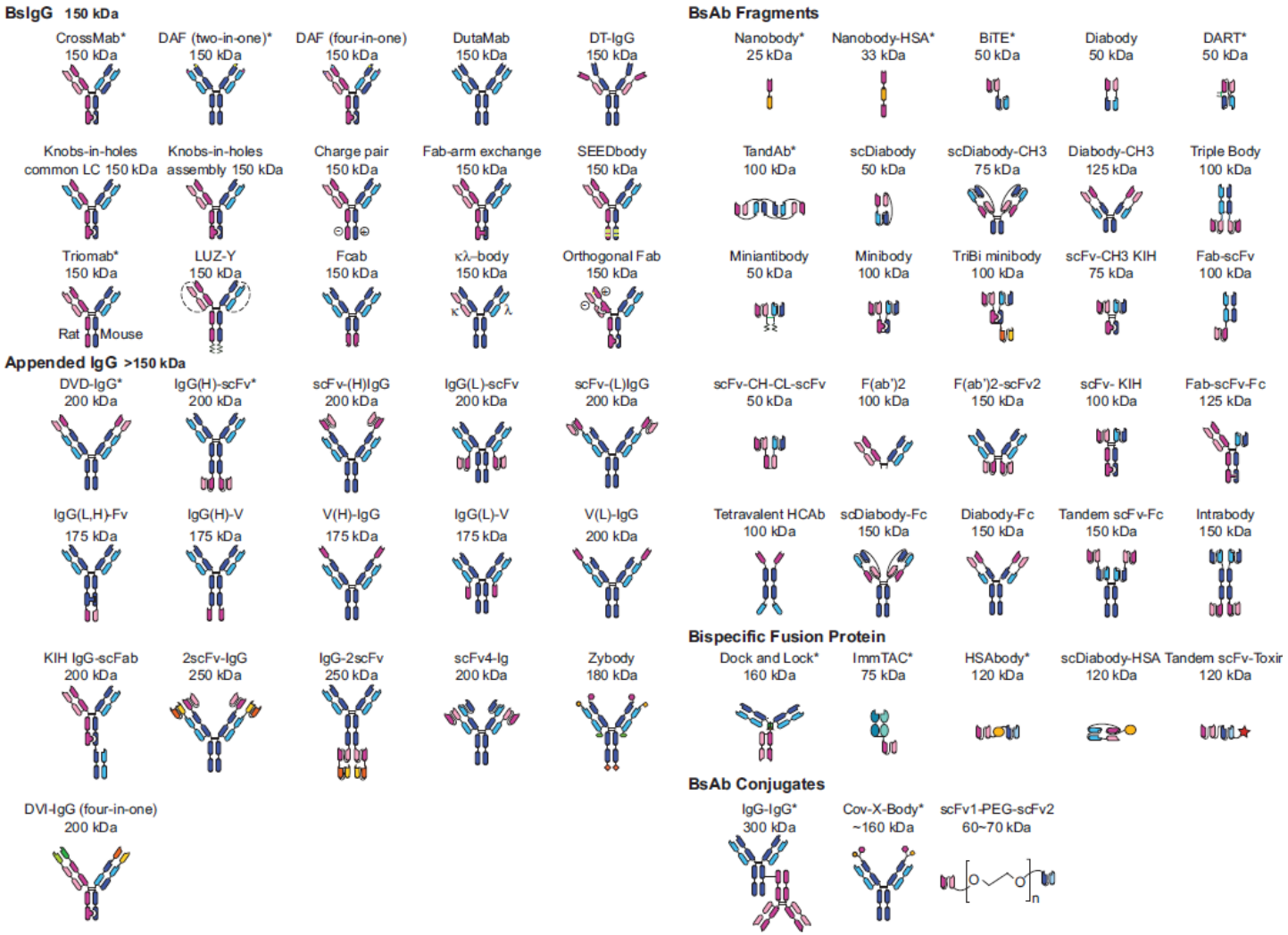
Emerging new modalities aim to modulate challenging targets



C. Spiess, Q. Zhai, P.J. Carter, *Molecular Immunology*, 67, 2015, 95-106.

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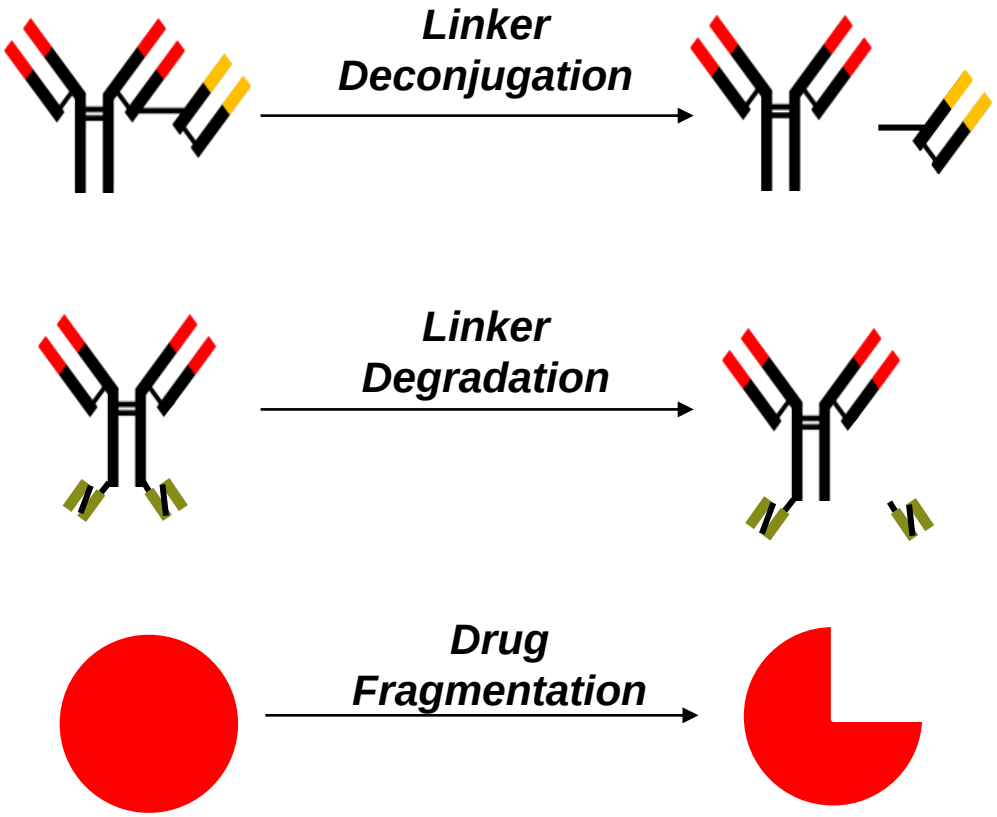
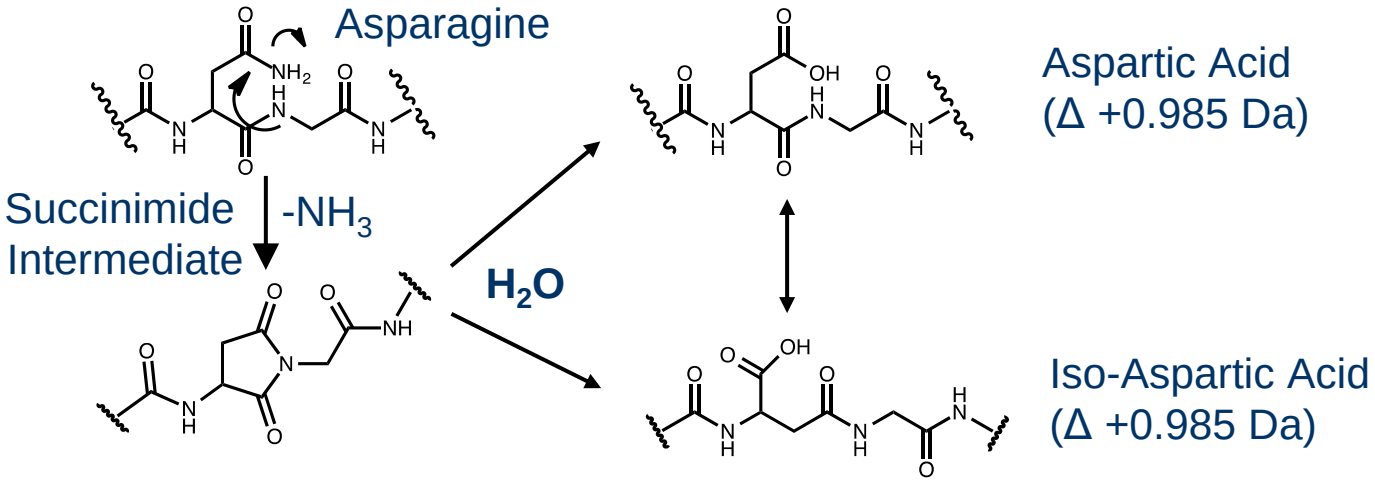
Biotransformation – *in vivo* intact stability

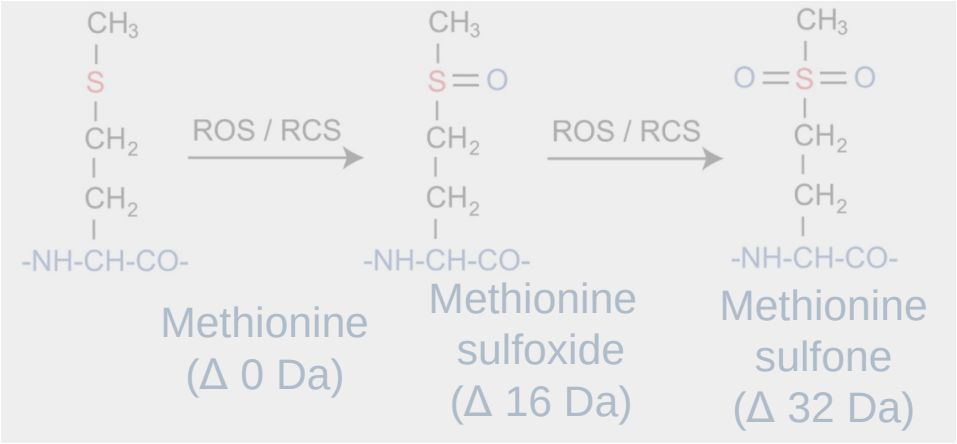


Biotransformation – *in vivo* amino acid level modifications

Deamidation – N, Q, Isomerization – D, E



Oxidation – M,W



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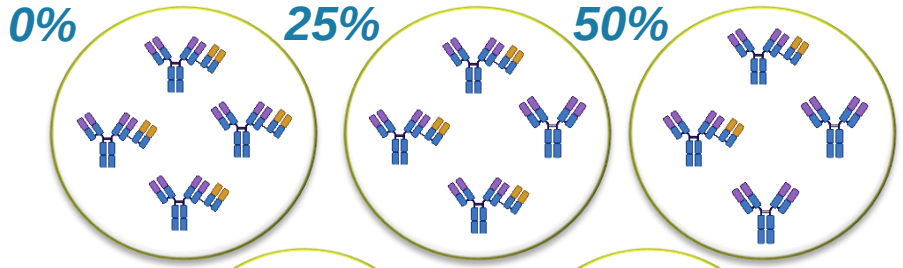
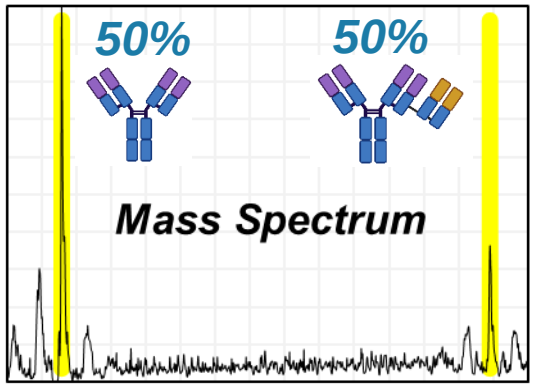
Affinity capture + LC-MS workflow for intact stability analysis

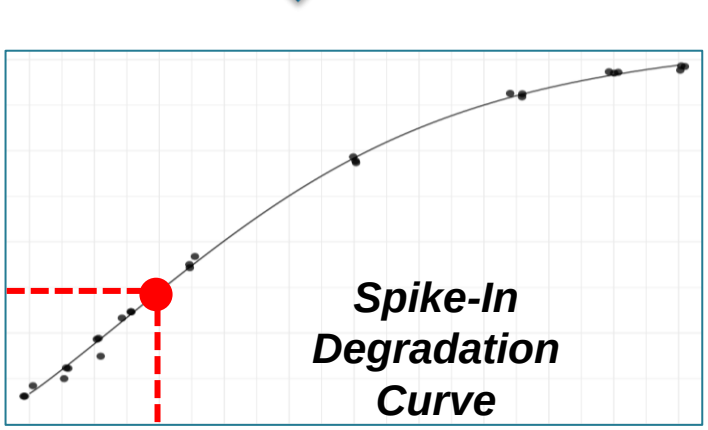


Mix of Intact and degraded species

Affinity Capture



Calibration curve

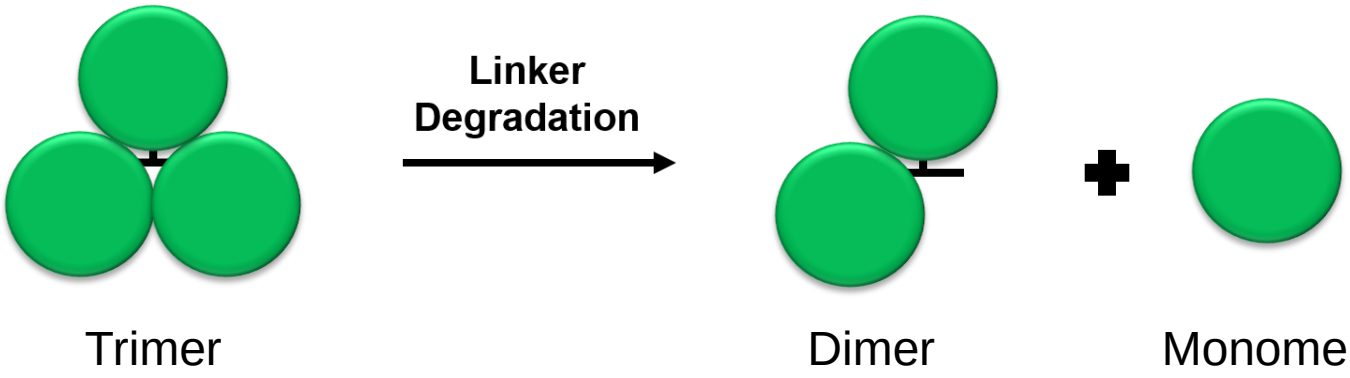


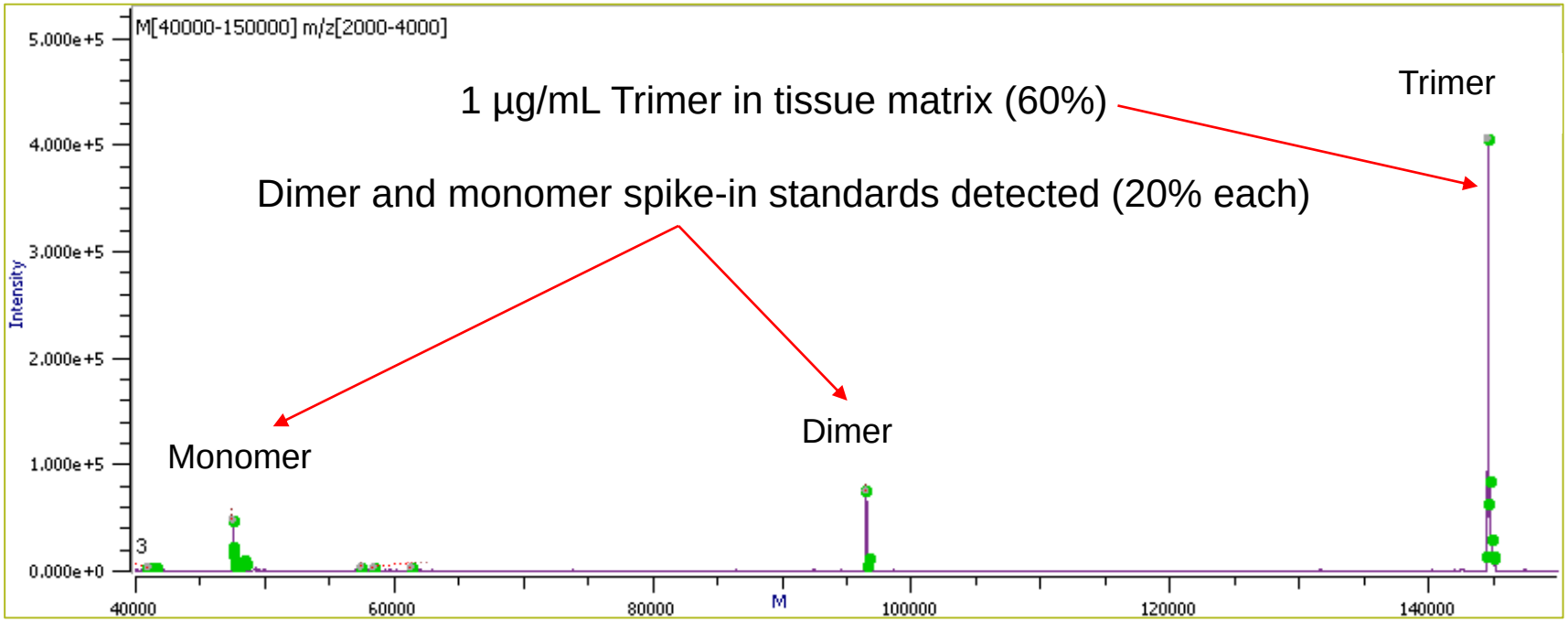
Quantification

Relative Degradation in vivo / ex vivo

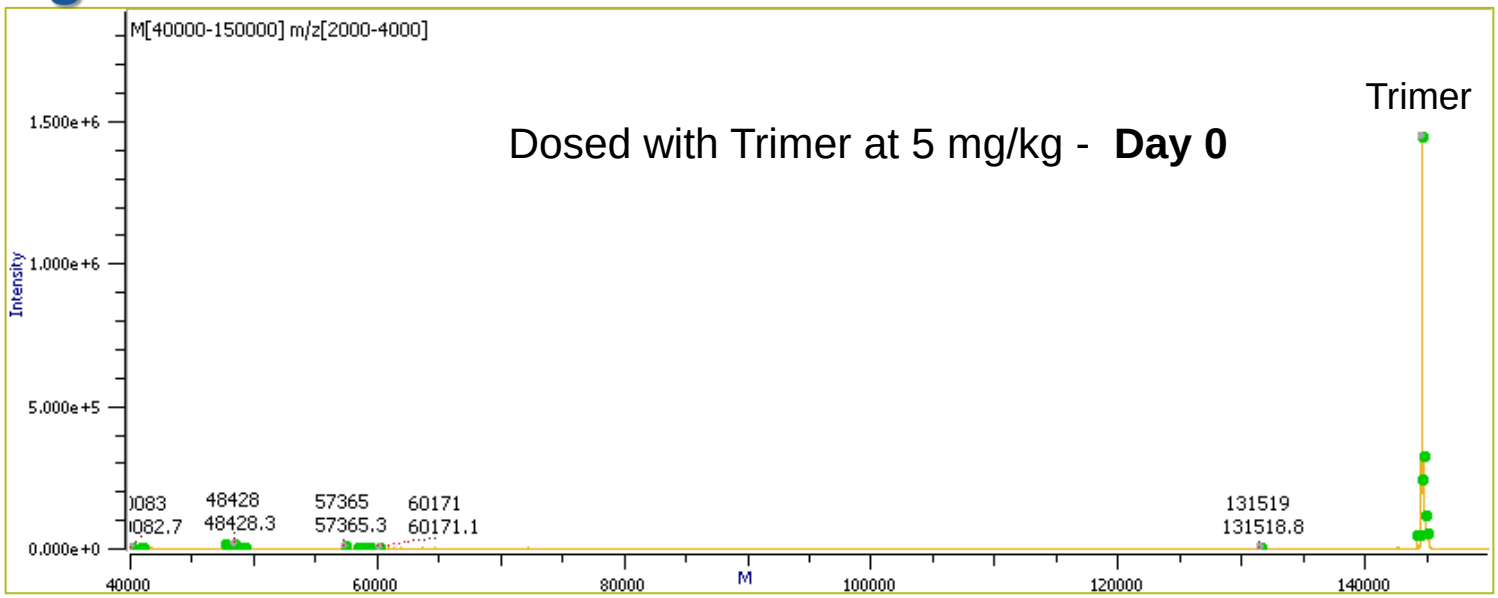
Wenjing Li, *et al. Anal. Chem. 2020, 92, 10, 6839-6843*

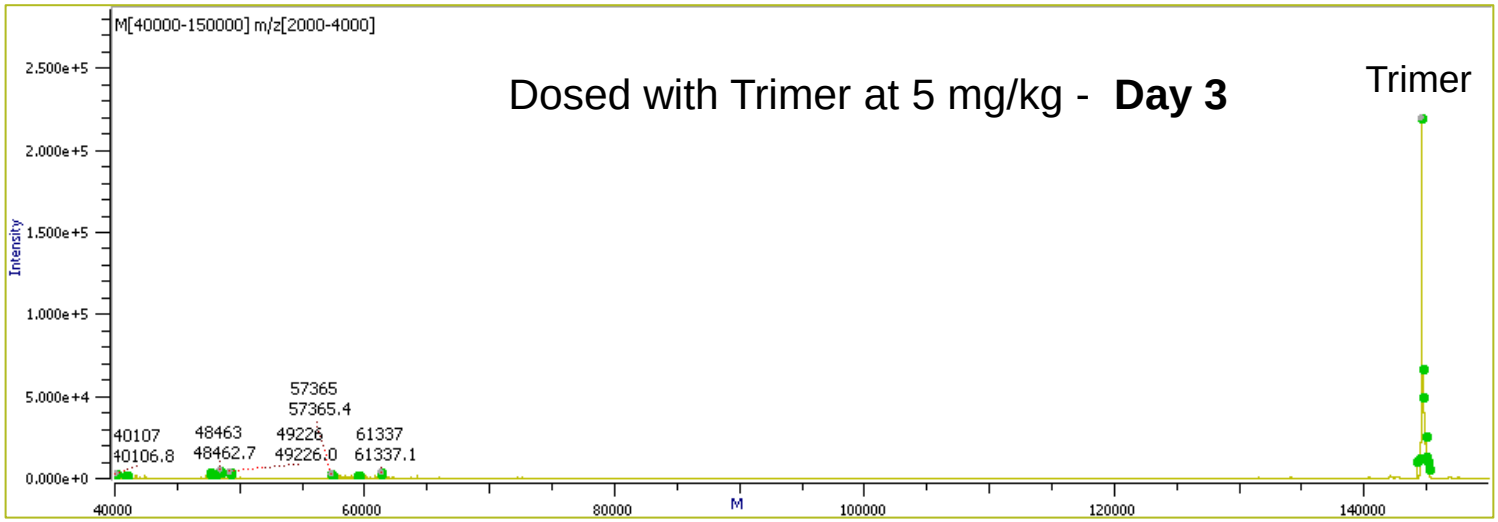
Case study of a trimeric molecule stability in tissue





PK samples from Day 0 vs Day 3 showed no detectable degradation





No degradation products were observed up to Day 3

Comparison of bioanalytical tools for *in vivo* clipping characterization and quantitation

	LC-MS	CE-SDS LIF	CE-Western Blot
Specificity	High		
Sensitivity	Medium with MW bias		
Relative quantitation ability	Standards and calibration curve required		
Resolution	Single amino acid resolution		
Robustness	Medium		

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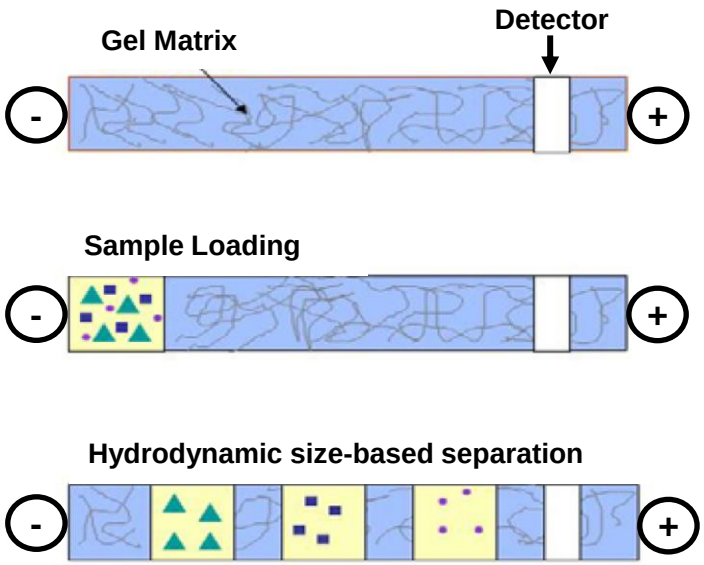
Capillary Electrophoresis SDS Laser Induced Fluorescence (CE – SDS LIF)

- Calibration curve – LIF signal is directly quantitative



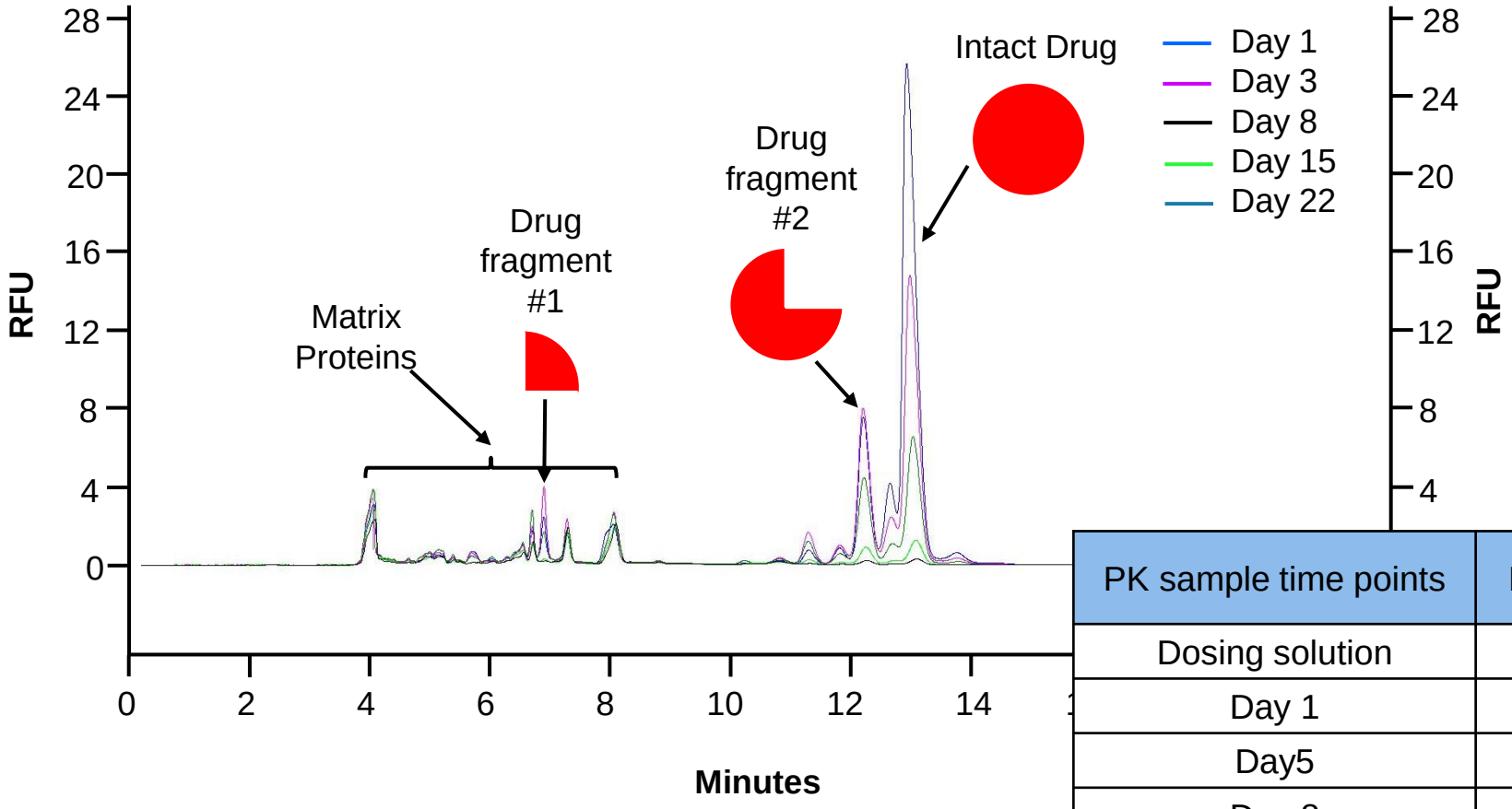
Fundamental steps of CE-SDS separation

Sciex PA800 plus

1. Denature samples with SDS (and DTT for reduced CE-SDS)
2. Fluorescently label samples with FQ dye
3. Signal detection at 600 nm upon excitation at 488 nm

Salas-Solano *et al. Anal. Chem.* 2006, 78, 6583-6594
Michels *et al. Electrophoresis,* 2012, 33, 815-826

Case study of a multimeric drug in a PK study



PK sample time points	Intact%
Dosing solution	80%
Day 1	71%
Day5	57%
Day 8	50%
Day 15	50%
Day 22	49%

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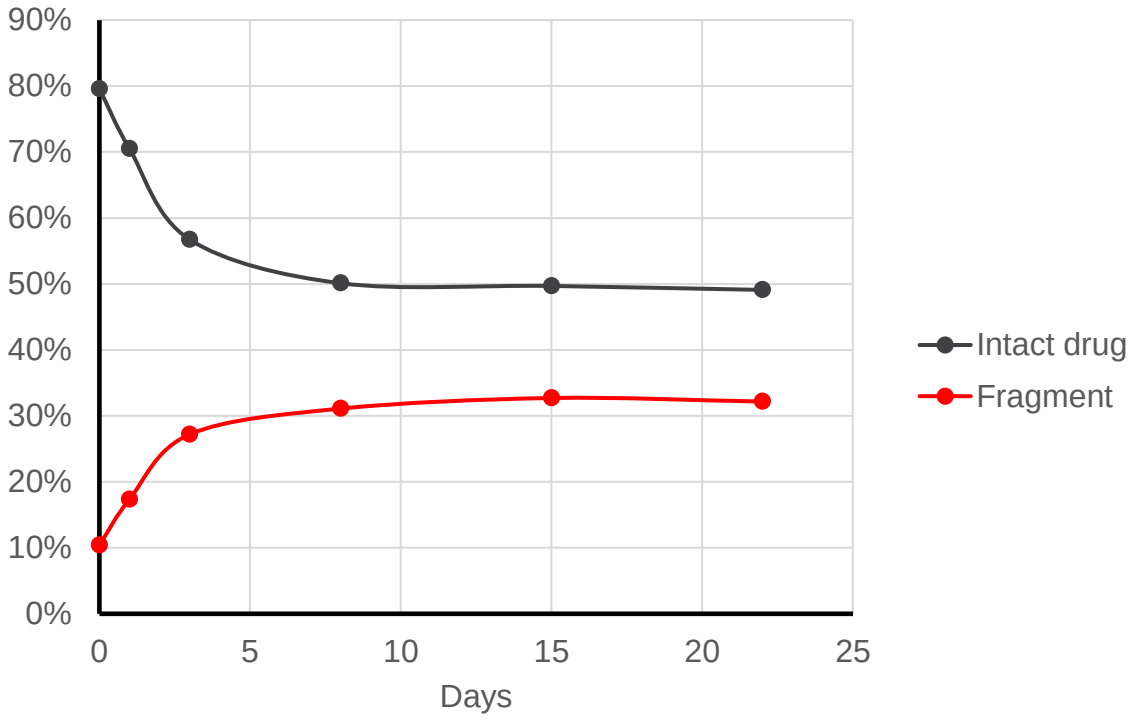

- Fragmentation of intact drug was significant in the first ~8 days after single dose injection
- The relative percentages of intact and fragmented drugs remained the same after Day 8

Comparison of bioanalytical tools for *in vivo* clipping characterization and quantitation

	LC-MS	CE-SDS LIF	CE-Western Blot
Specificity	High	Low	
Sensitivity	Medium with MW bias	Medium non-biased	
Relative quantitation ability	Standards and calibration curve required	LIF signal directly quantitative	
Resolution	Single amino acid resolution	Chain level resolution	
Robustness	Medium	High	

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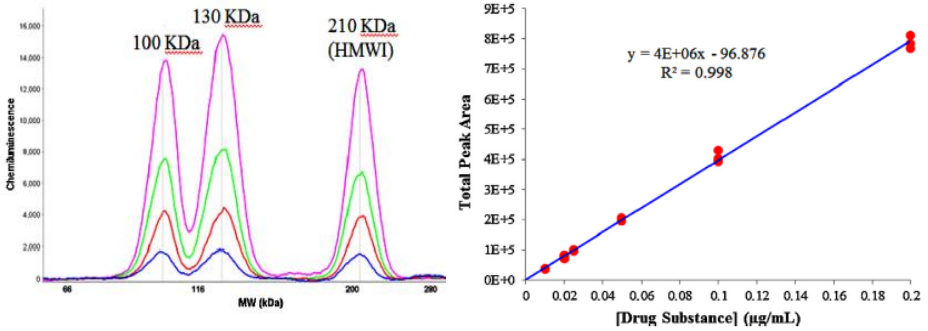
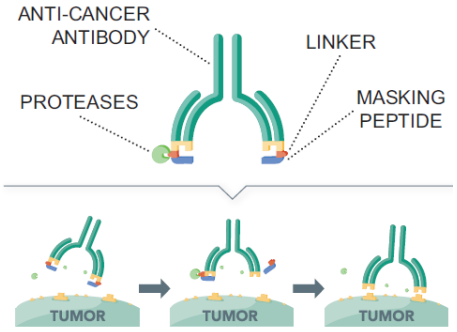
Rationale for biotransformation

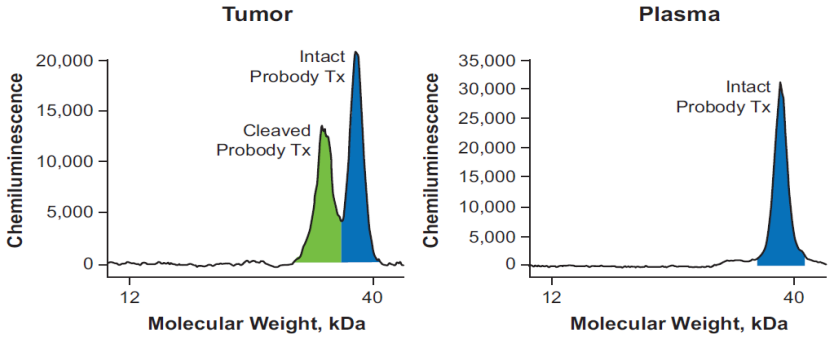
Size-based separation for clipping



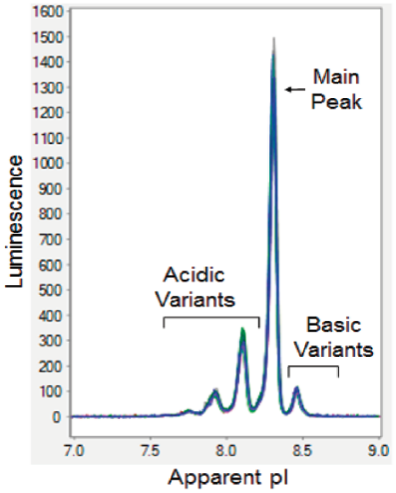
Xu *et al*. **J Phar Biomed Anal** (2012)

Poster TPS3107 presented by CytomX Therapeutics, **ASCO** 2017



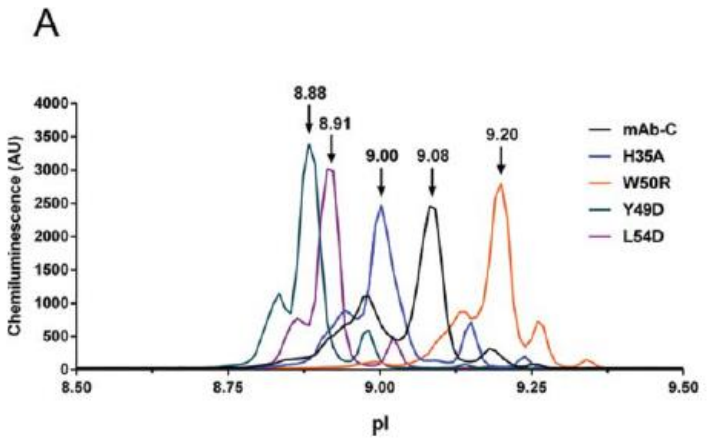


Charge-based separation for deamidation



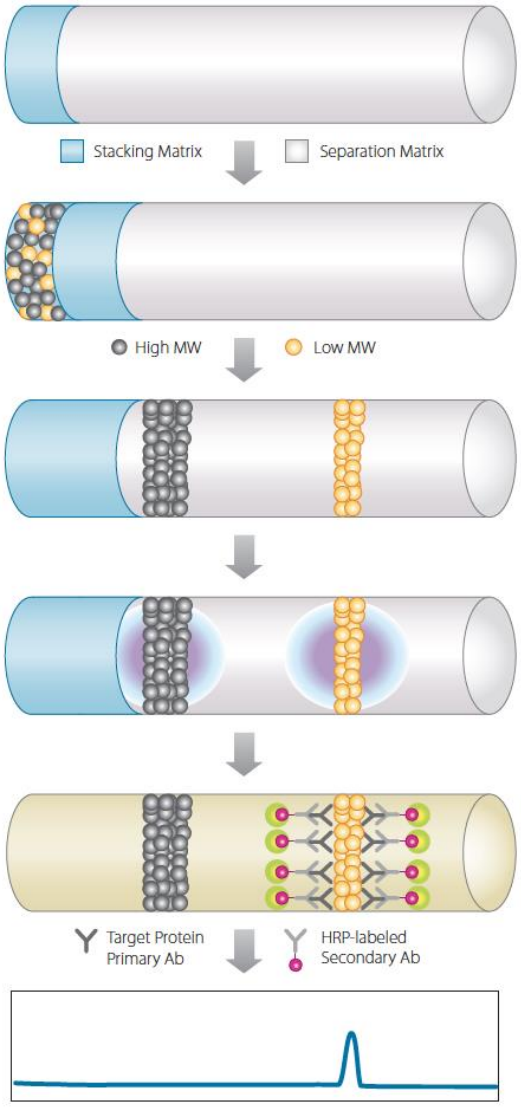
- 12 injection overlays showed high repeatability
- Limit of detection (S/N = 3) : 6 ng/mL (2 pg/capillary)
- Dynamic range: 12.5 ng/mL – 2 µg/mL
- Samples directly loaded from cell culture supernatant

Michels *et al*. **Analytical Chemistry** (2012)



Geoghegan *et al*. **mAbs** (2016)

BACKGROUND ON CE WESTERN BLOT



Load matrices (up to 12 channels)

Load samples
(reduced and denatured)

Separate based on MW (or pI)

Immobilize via UV

Immunoblot

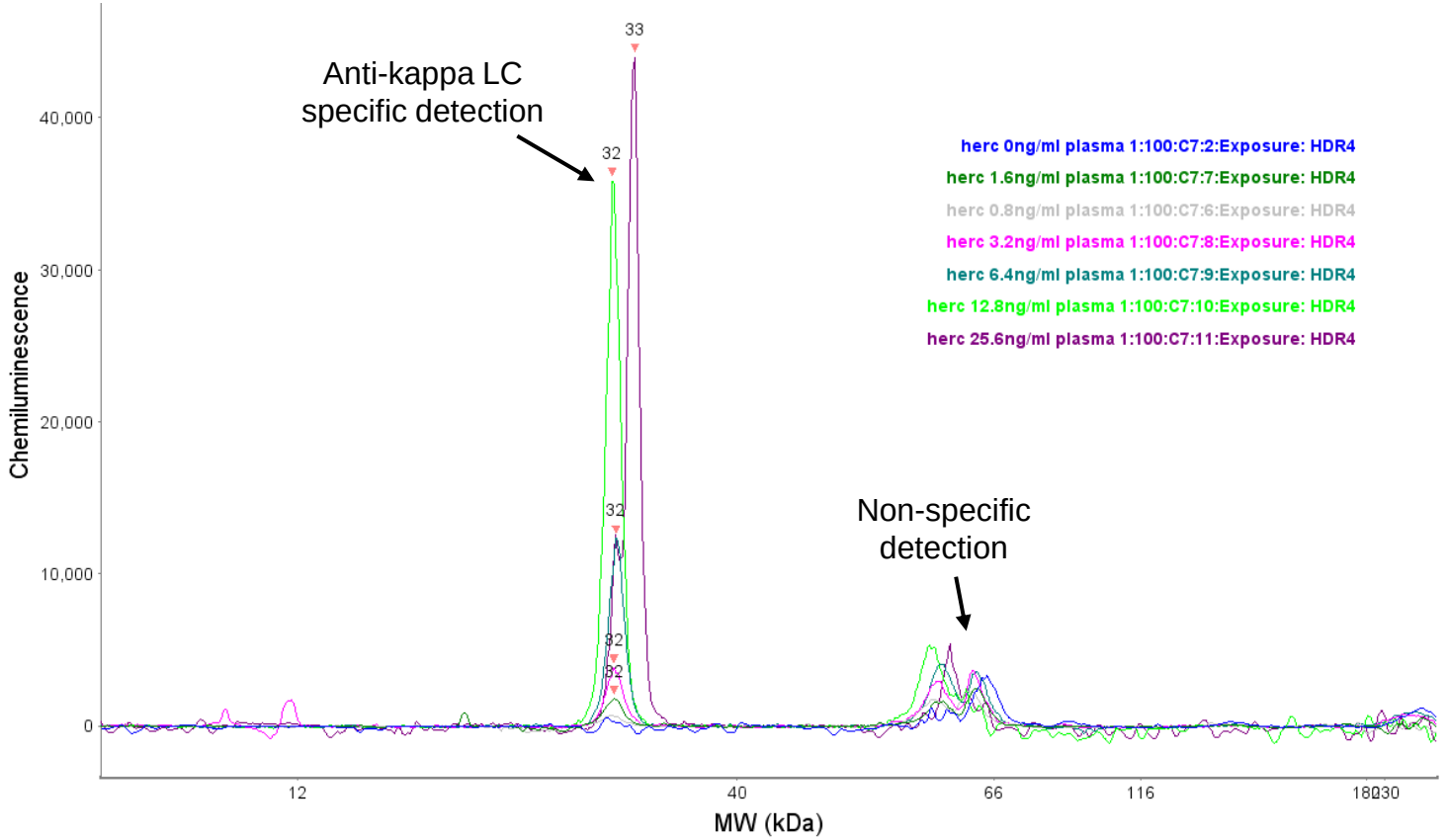
Quantitate

PeggySue:
Automated Multiplex Western Blot System

https://www.proteinsimple.com/sally_sue_video.html

LC- or HC-specific primary antibodies allow highly specific and sensitive detection of clipping events

Herceptin spiked in diluted C57BL/6 plasma @ 0 – 25.6 ng/mL



- *In vivo* samples can be directly analyzed without affinity capture after dilution
- Although sensitive, the dynamic range of CE Western is narrow 1~2 orders of magnitude

Comparison of bioanalytical tools for *in vivo* clipping characterization and quantitation

	LC-MS	CE-SDS LIF	CE-Western Blot
Specificity	High	Low	High
Sensitivity	Medium with MW bias	Medium non-biased	High non-biased
Relative quantitation ability	Standards and calibration curve required	LIF signal directly quantitative	Quantitative in a narrow dynamic range
Resolution	Single amino acid resolution	Chain level resolution	Chain level resolution
Robustness	Medium	High	Medium

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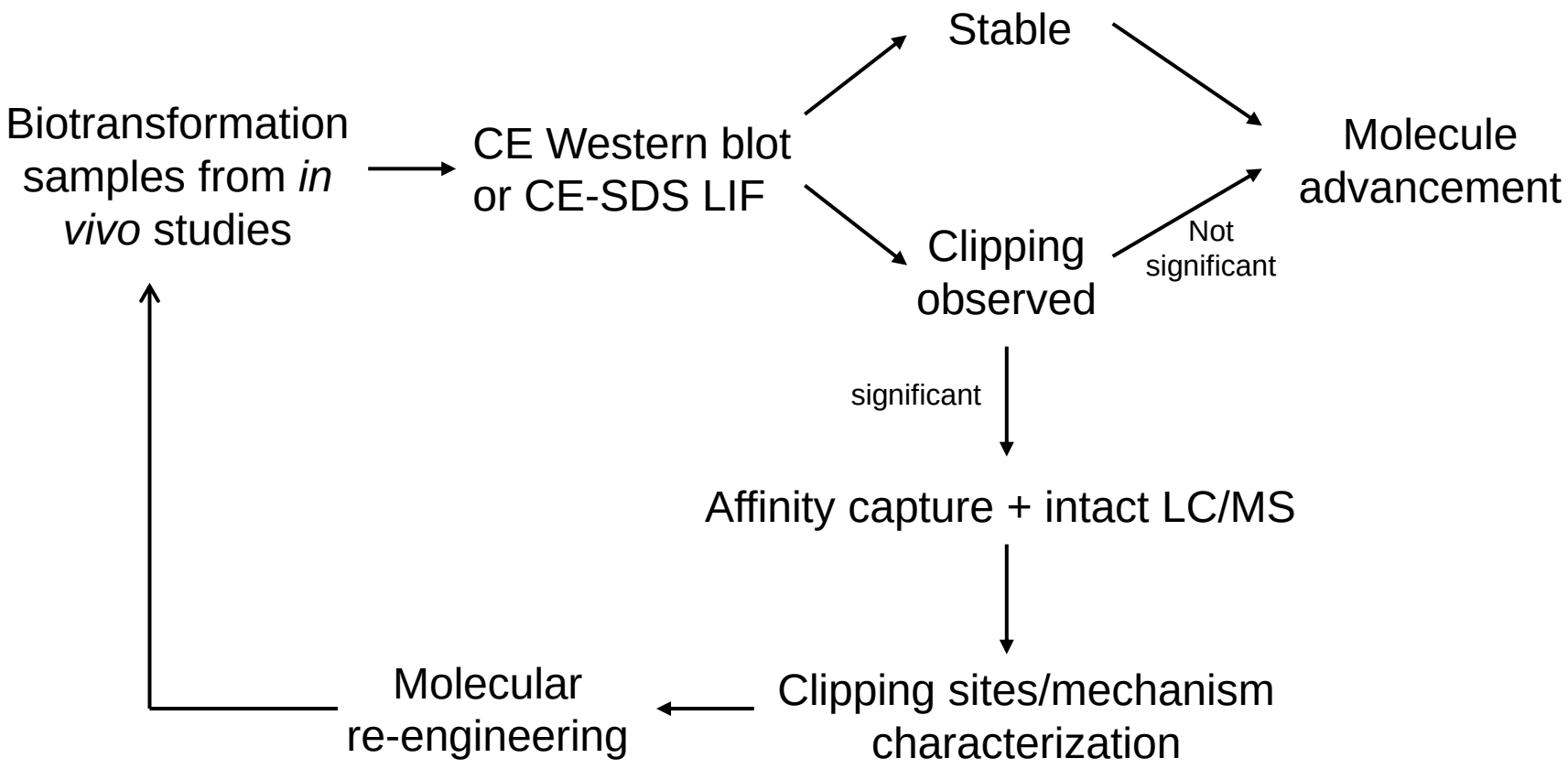
In vivo intact stability triaging strategy for novel modalities



Future directions

- **Evaluate the connection and difference between**
  - In circulation vs at site of action
  - healthy vs disease tissues
- **Characterize the mechanisms of *in vivo* clipping**
  - Enzymatic proteolysis
  - Chemical hydrolysis
  - *In vitro* system to recapitulate clipping *in vivo*
- **Explore charge-based CE Western for other types of biotransformation**
  - Deamidation
  - Glycosylation
  - Other charge-altering modifications

Acknowledgment

- **Biochemical and Cellular Pharmacology**
  - John Tran
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  - Dennis Price
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  - Rachel Loui
  - Jose Meza
  - Stephanie Diamond
  - Brinker Gildersleeve


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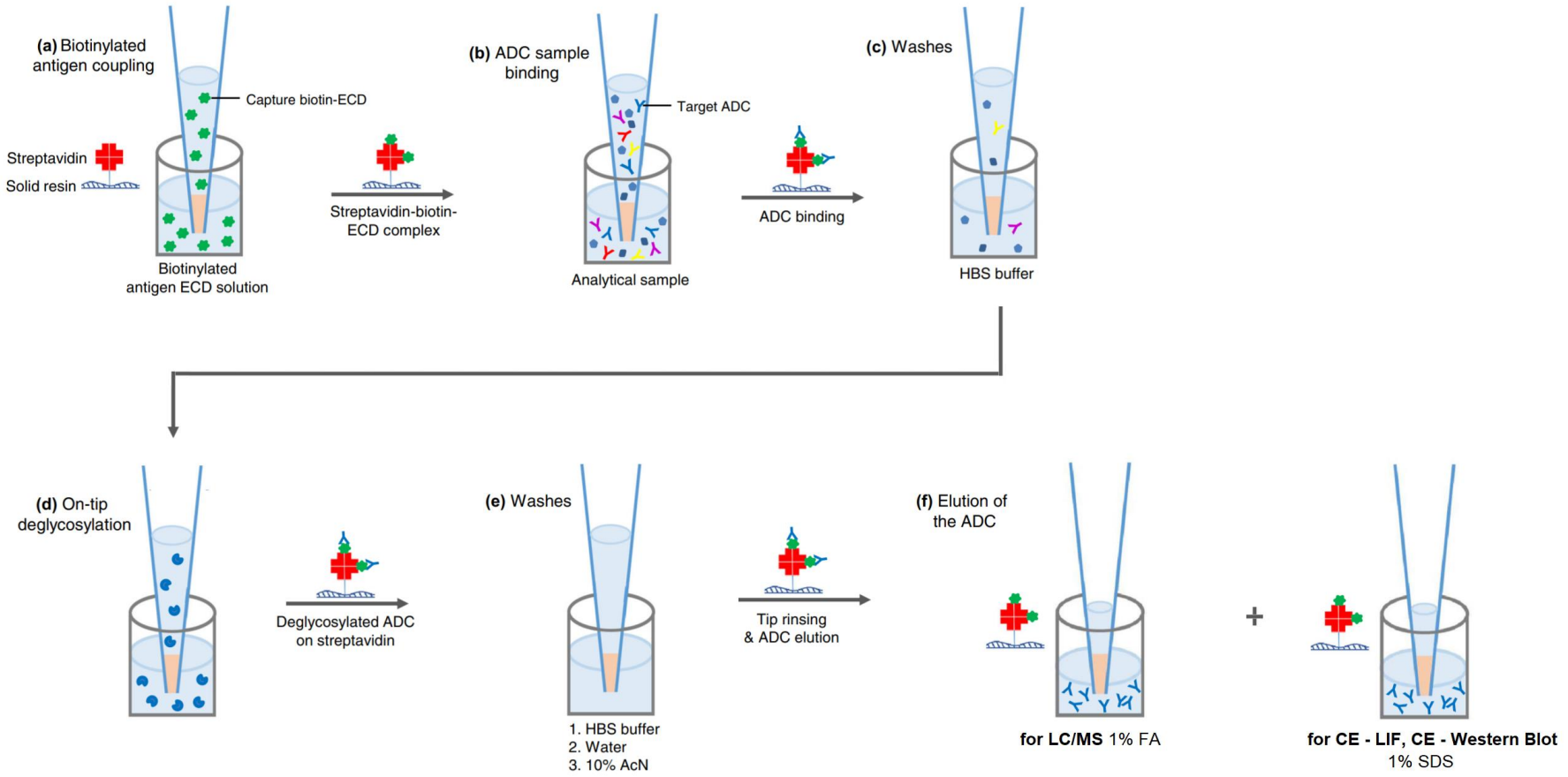
AFFINITY CAPTURE ON ASSAYMAP



Adapted from Li KS, *et al*. 2018

Comparison of various affinity capture approaches

		Pros	Cons
Bead based		• Compatible with dirty matrix; not prone to clogging • Compatible with various elution methods • Semi-automated	• Bead pellets to bottom of well; incomplete bead removal • Consumes more starting materials, reagents and labware • Time-consuming
Plate based		• Amenable to automation • High throughput (>>1 plate) • Fast	• Well-to-well variability • Method dev needed for on-plate degly and/or digestion • Dead volume
Tip based	MISA tip*	• Amenable to automation • Fast • Ease of use	• Tip binding capacity • Prone to introducing bubbles • Requires custom automation • Not compatible with on-tip digestion
	AssayMap	• Automated • Fast (1-2 plates) • Ease of use • Highly veritable utilities	• Temperature control (>37˚C) • Tips are expensive consumables (reusable after testing) • Integrability with custom automation

*Li KS, *et al*. J Am Soc Mass Spectrom. 2018 Jul;29(7):1532-1537