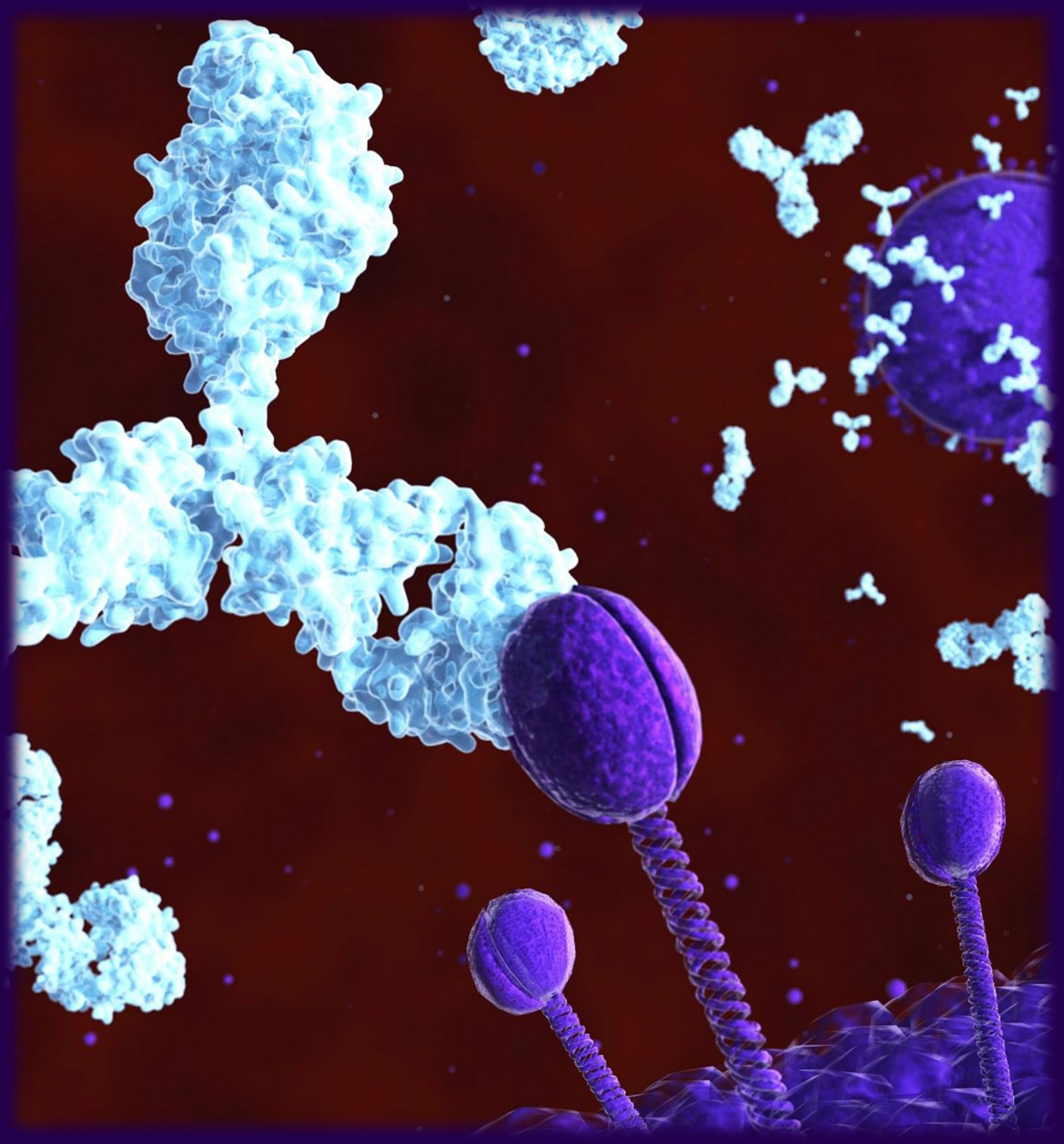

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Protein vs peptide immunocapture: the case study of the quantitation of total sBCMA by IC-LC-MS/MS

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Translational Medicine and Early Development,
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Introduction

Case study: Quantification of sBCMA

- **General background**
  - Why quantifying sBCMA?
  - Analytical challenges
- **Anti-protein vs anti-peptide immunocapture**
  - Development of the two approaches
  - Comparison of both approaches: pro and cons
- **Quantification of a biomarker by IC-LC-MS/MS**
  - Context of Use (CoU): method validation results
- **Conclusion and perspectives**

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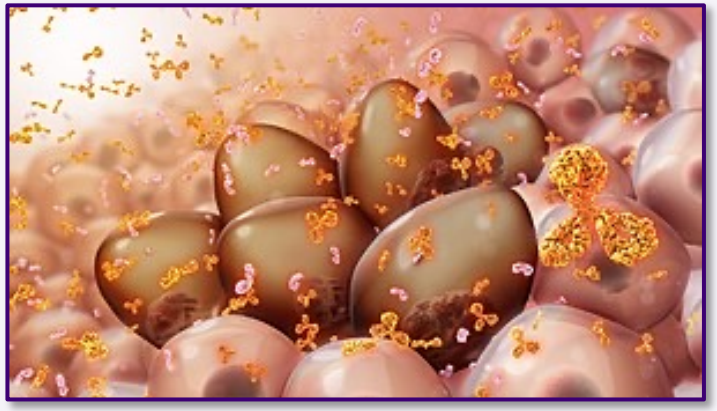
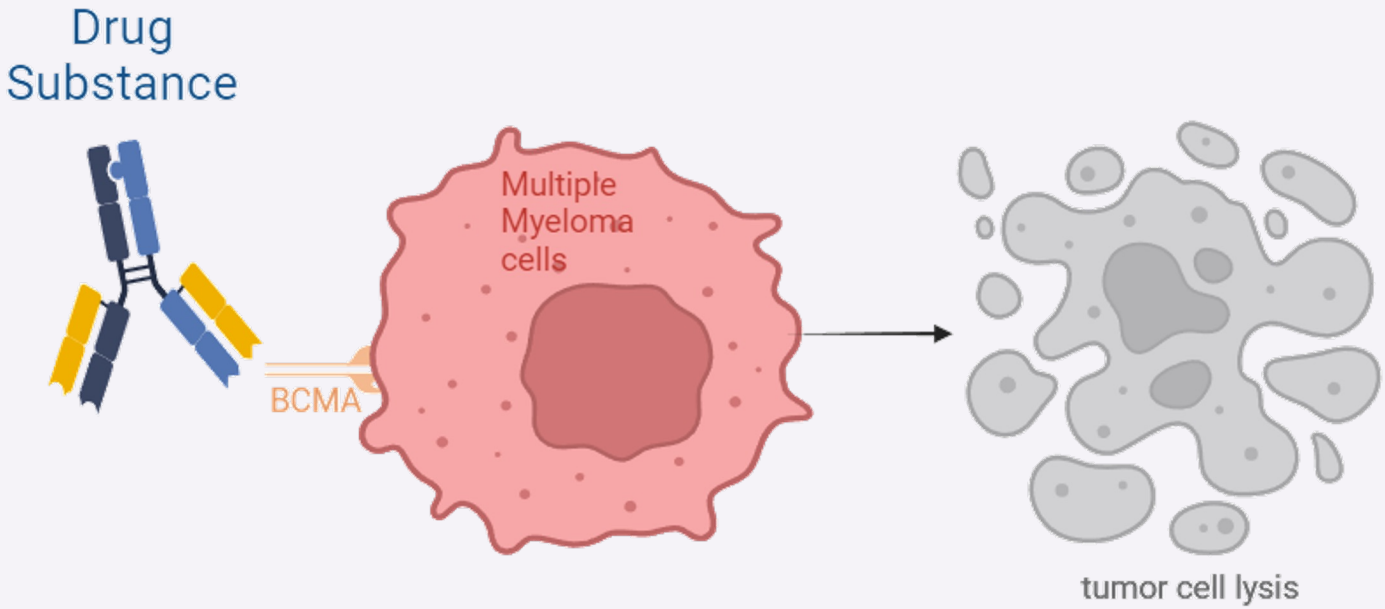
General Background

Overexpression of BCMA in MM patients

Multiple Myeloma (MM) is a disease characterized by a clonal expansion of plasma cells in Bone Marrow



Scientific Animations



- BCMA overexpressed in Multiple Myeloma cells
- BCMA-targeted therapies
- BCMA expression is usually assessed by flow cytometry or immunochemistry

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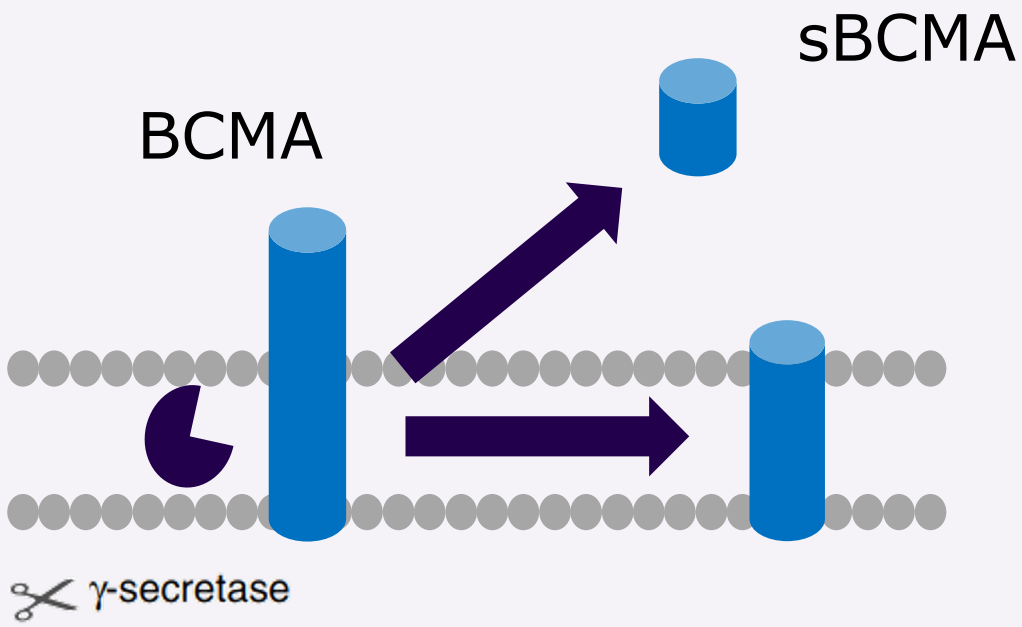
Why quantifying sBCMA?

Soluble B-cell maturation antigen



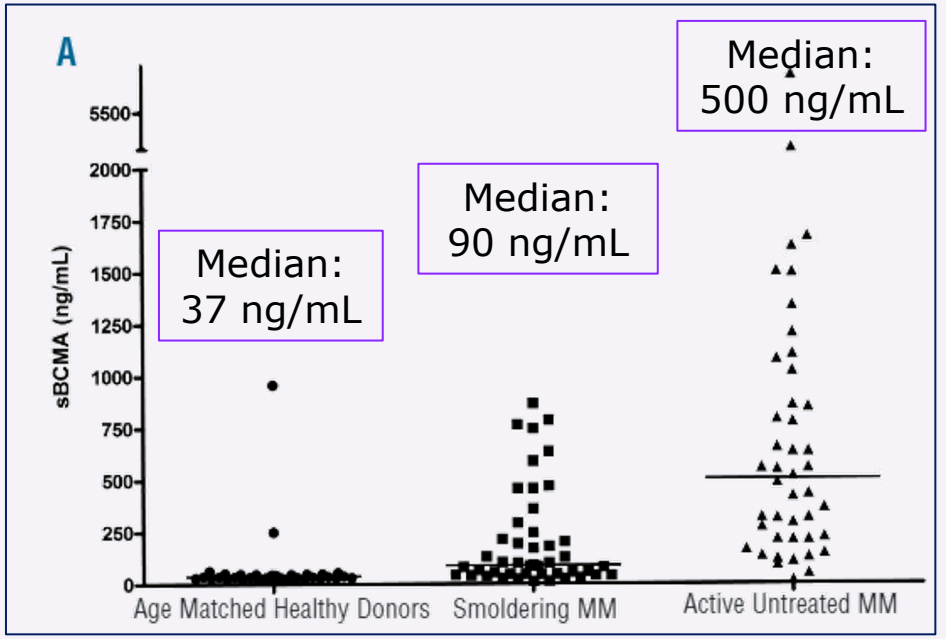
Gamma-secretase mediated shedding

1- sBCMA as a biomarker

Concentrations of sBCMA are high in MM patients, correlated with the proportion of plasma cells in bone marrow biopsies, as well as related to disease progression and treatment efficacy.

Short biomarker half-life (24-36h).

Less invasive than BMA Analysis.



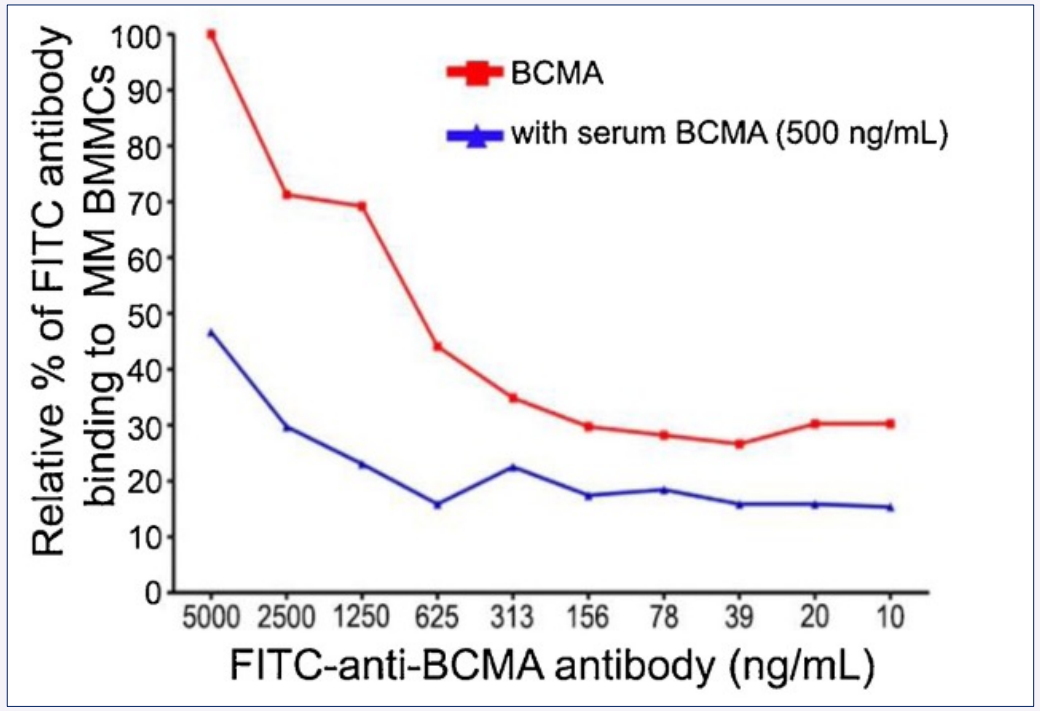
sBCMA levels Ghermezi et al, 2017

Why quantifying sBCMA?

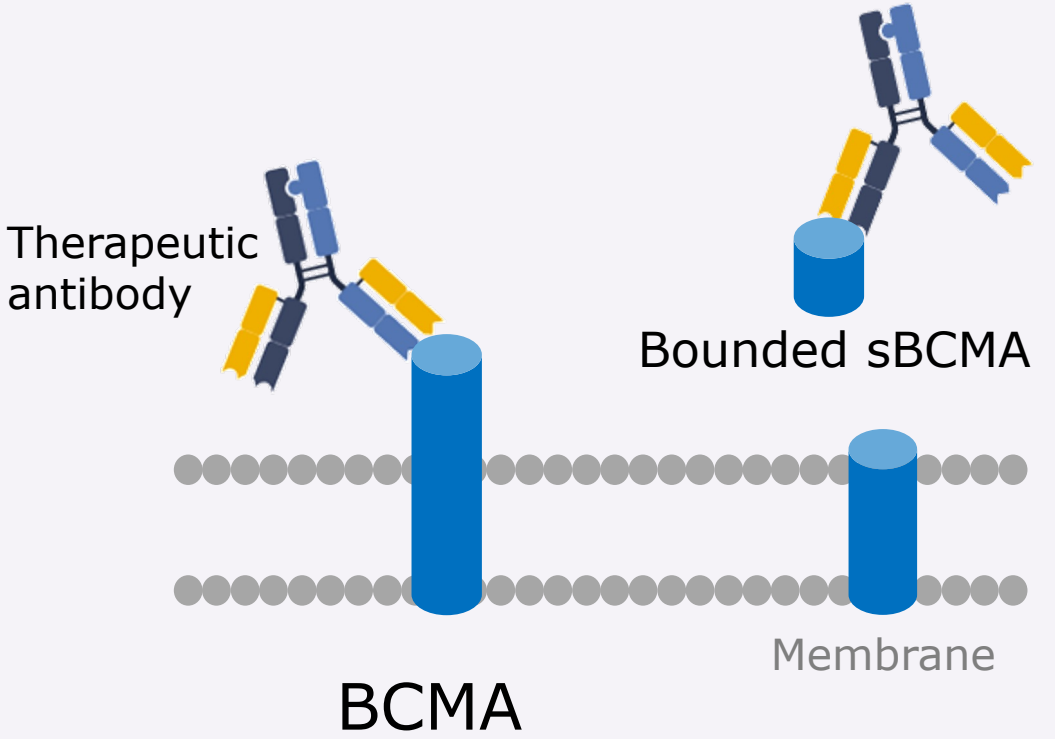
Off target of the drug substance

2- Interference of sBCMA in treatment

Reduces the efficacy of the treatment



sBCMA interference, Chen et al, 2019



Anti-BCMA/BCMA binding

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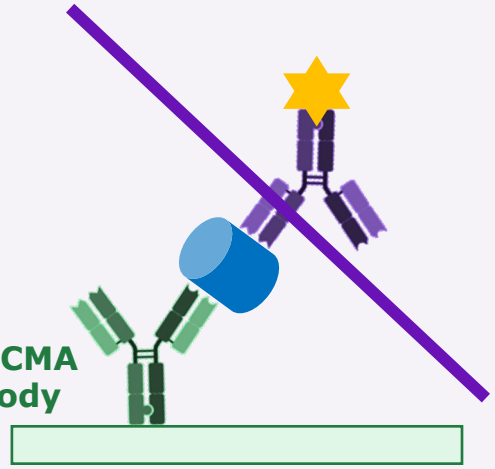
sBCMA Quantification strategies



Only Free sBCMA!

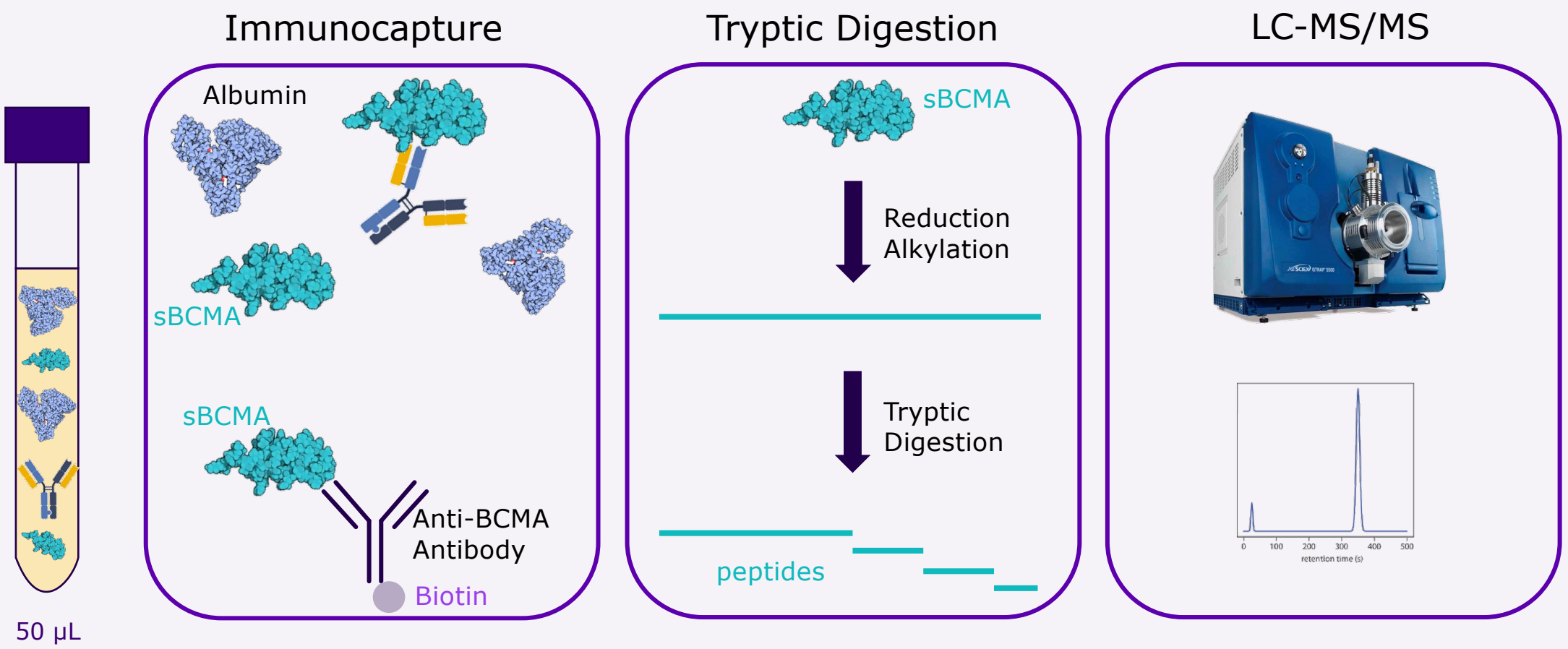
- Could be challenging to use this strategy to assess **total** sBCMA due to biotherapeutic interference
- **In MS:** Use of the bottom-up approach to perform an anti-peptide immunocapture

~~Anti-protein immunocapture~~

Anti-peptide immunocapture

Hybrid LC-MS/MS Strategy 1 – Protein Capture

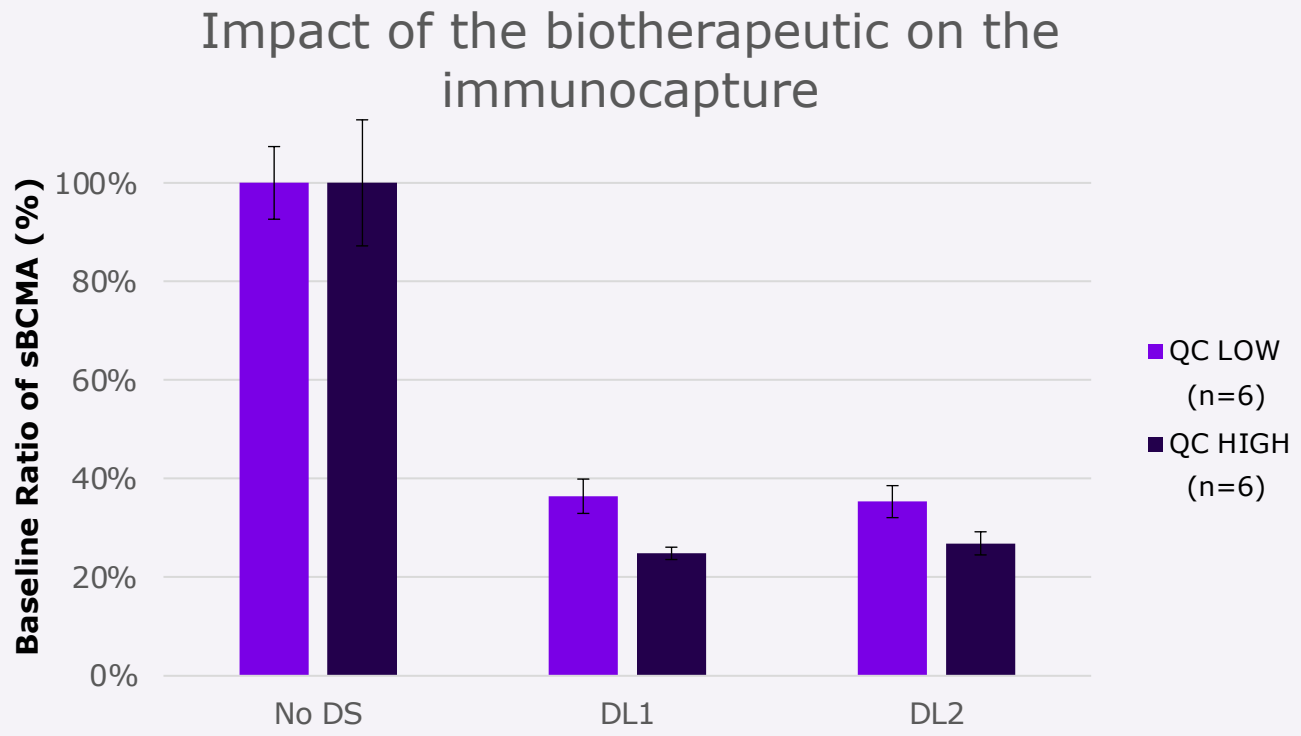
Capture with **anti-protein** antibody



Competition expected between the capture antibody and the drug

Hybrid LC-MS/MS Strategy 1 – Protein Capture

Drug substance (DS) interference



- Immunocapture recovery
- Spiking of two dose level (DL) concentrations of the BCMA-targeting drug substance (DS)
- QC LOW: endogenous level (~25 ng/mL)
- QC HIGH: Spiked at 700 ng/mL sBCMA
- Incubation 4h at 37ºC

Strong Interference of the drug substance using this approach

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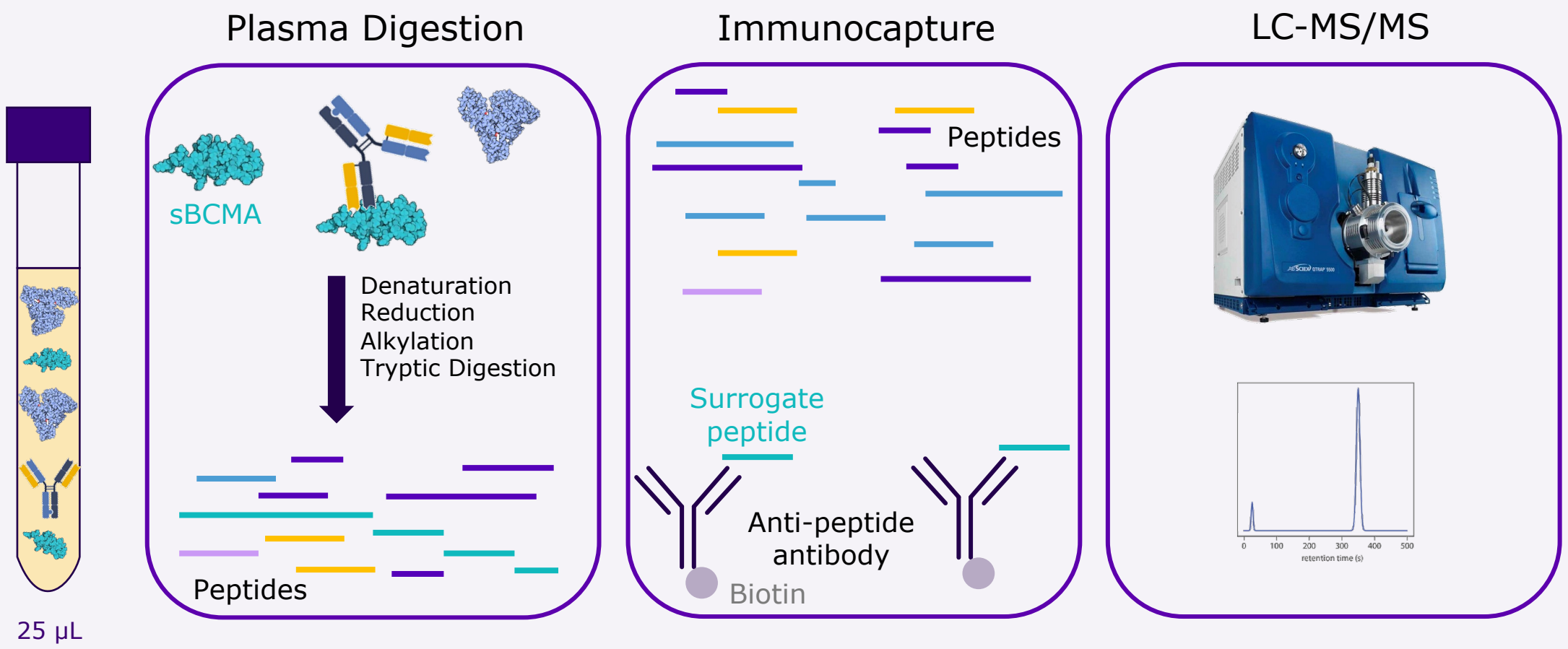
Hybrid LC-MS/MS Strategy 2 – Peptide Capture

Capture with **anti-peptide** antibody

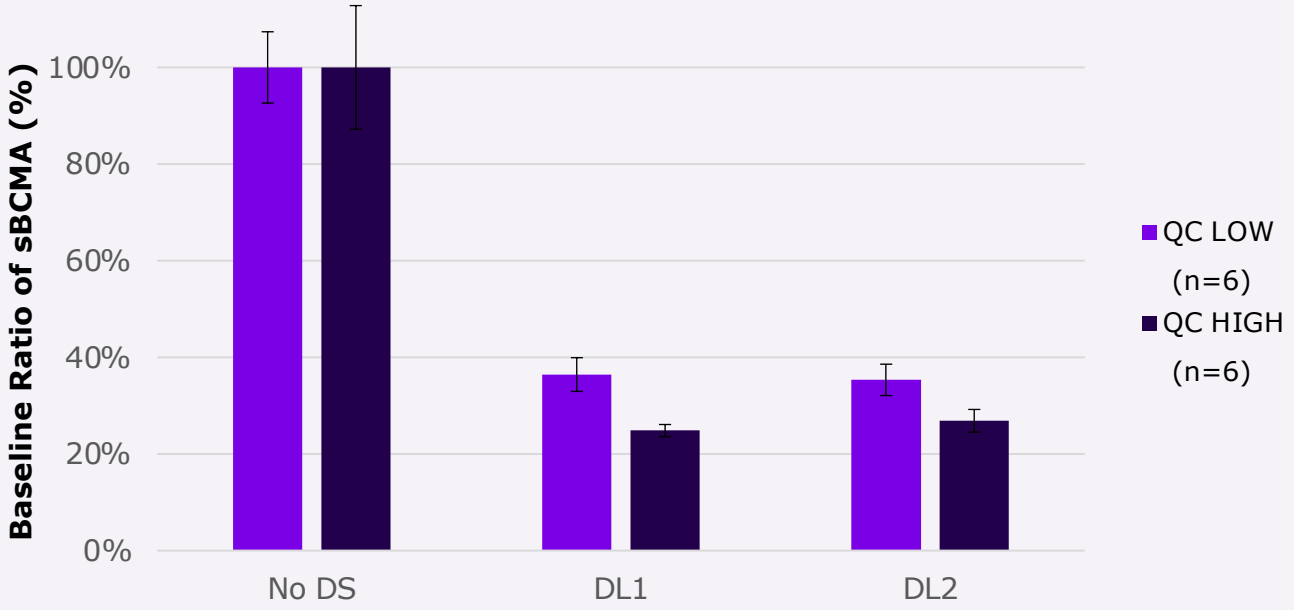


Total sBCMA ?

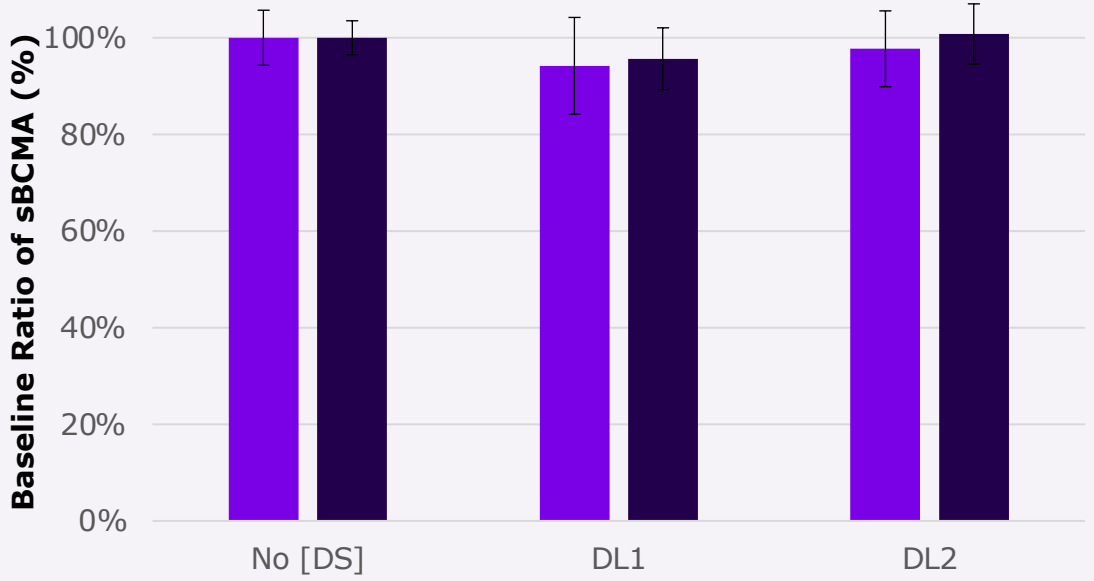
Comparison of the two approaches

Drug substance (DS) interference

Impact of the biotherapeutic on the **protein** immunocapture



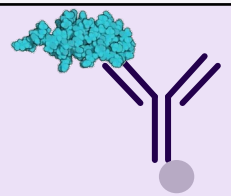
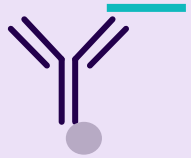
Impact of the biotherapeutic on the **peptide** immunocapture



Elimination of the drug substance interference

Comparison of the 2 strategies

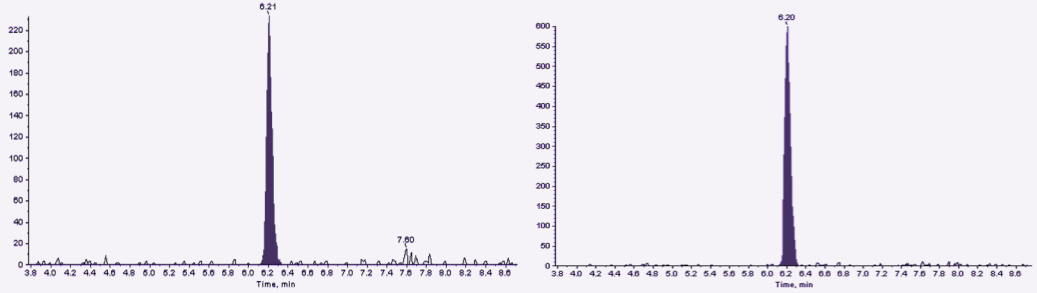
Method development

		Anti-protein Approach	Anti-peptide Approach
Critical reagent Reference standard	**Capture reagent**	 Commercial	 Custom made immunization time consuming
	Internal standard	**Heavy protein:** Unavailable and/or expensive	**Flanked heavy peptide:** Easy and cheap to produce
Process	**Digestion**	Usual protocol	**Plasma digestion:** Optimization required Denaturation step added
	Automated Immunocapture	 Streptavidin tips	 Magnetic Beads Volumes

Challenges of a Biomarker quantification by LC/MS-MS

Context of use	• What is the data used for? • Primary or exploratory BM?
Parallelism	• Need to evaluate a surrogate matrix • Endogenous vs recombinant protein
Endogenous level	• Low endogenous level – LLOQ? • Preparation of the QCs? Target determination
Internal standard	• Potential Matrix Effect in capture and/or MS between surrogate and authentic matrix
Acceptance criteria	• ± 20% with an IC step (± 25% at the LLOQ level) can be difficult to reach with multiple steps assay (variability)

Bioanalytical method validation



QC LLOQ

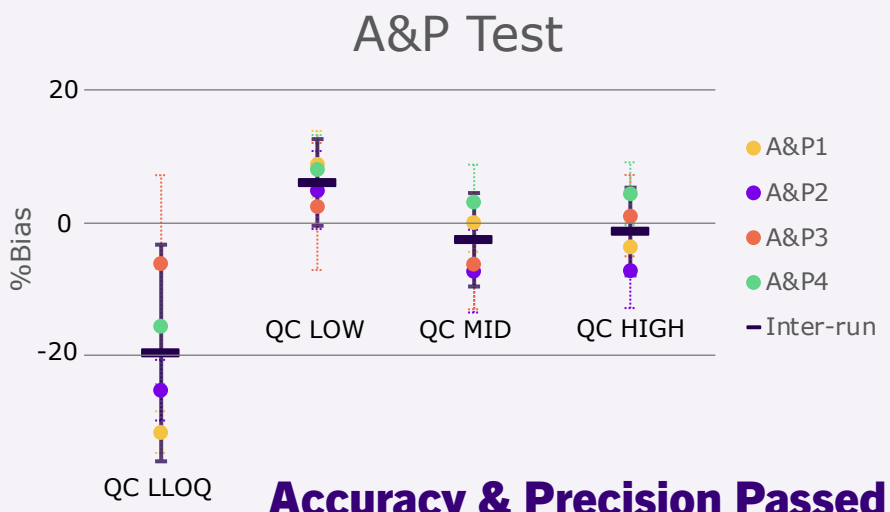
10 ng/mL

Monkey Plasma

QC LOW

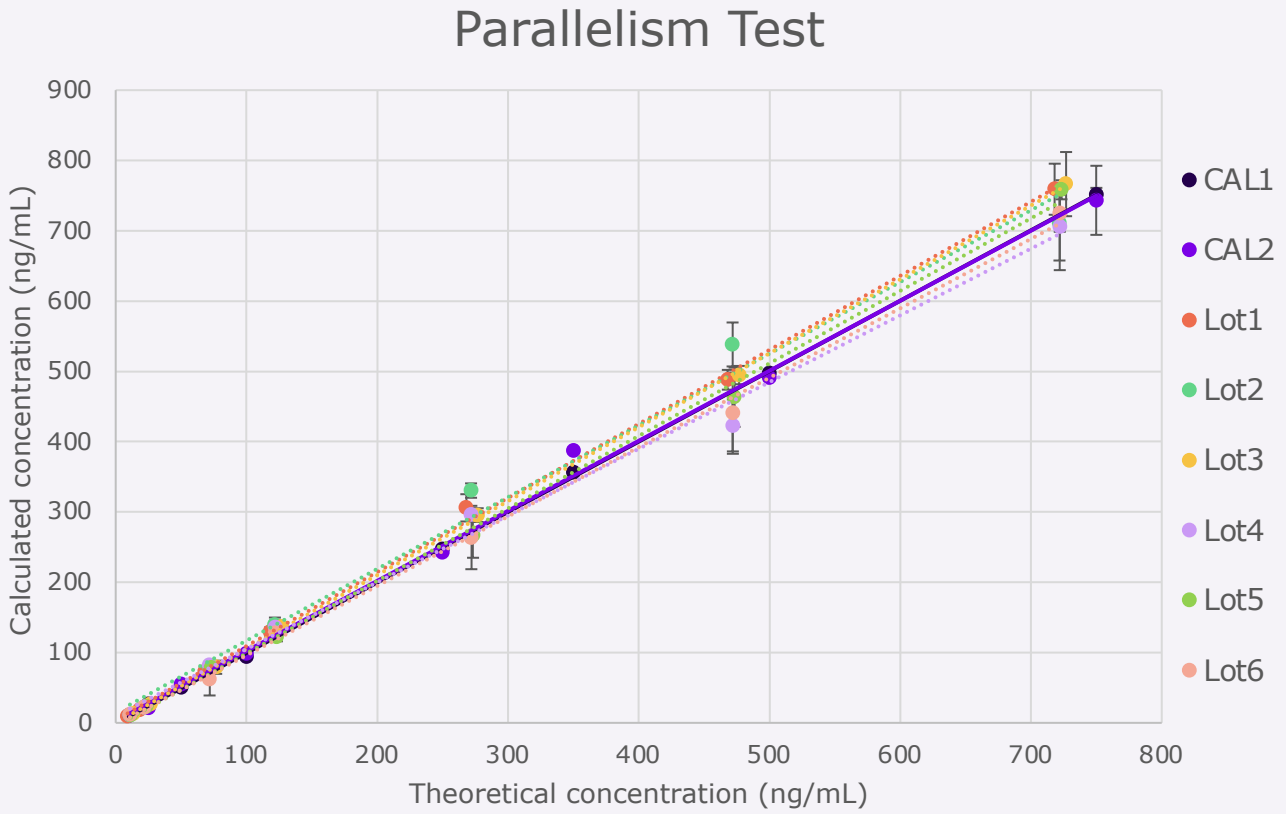
~25 ng/mL

Human Plasma



Accuracy & Precision Passed

Intra-run<20% (25% LLOQ)



Parallelism Test Passed

2 calibration curves (CAL1 & CAL2 in monkey plasma), 6 human plasma lots tested (spiking of 50, 100, 250, 450, 700 ng/ml of recombinant sBCMA, dilution by two in monkey plasma)

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Conclusion

- The method was successfully **qualified** (Context of Use validation)
- Interference with drug substance was **eliminated** with the anti-peptide immunocapture
- This bioanalytical strategy can be used:
  - In BM quantification: Biotherapeutic Interference
  - In PK measurements: ADA Interference
- Needs to be **anticipated** (delays and costs)

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Thank you

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Thank you for your attention

Mass Spectrometry Team in Montpellier, France