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Quantification of next generation biotherapeutics: Recent case studies demonstrating clear advantage of LC-MS over LBA.

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9th EBF YSS

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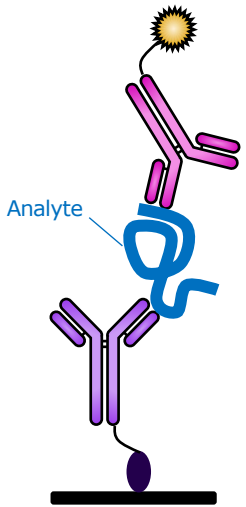
Introduction: Which bioanalytical strategy?

- Bioanalytical support for **early discovery** project for **biotherapeutics**.
- Complementarity of LBA and LC-MS-based approaches. **Which strategy for which project or compound?**

LBA

- High throughput
- Sensitive
- More robust regarding matrix effects

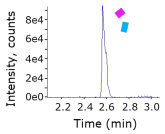


- Requires 2 affinity tools
  → Time & cost
- Multiple assays needed to prove protein integrity

LC-MS

- Faster method development
  → 0 to 1 affinity tools
  → Multiplexing ability
- Easier proof of in vivo integrity
- Less selectivity issues

- Lower throughput
- Usually, lower sensitivity



- **Presentation of 3 recent case studies for which LC-MS/MS had advantages over LBA**

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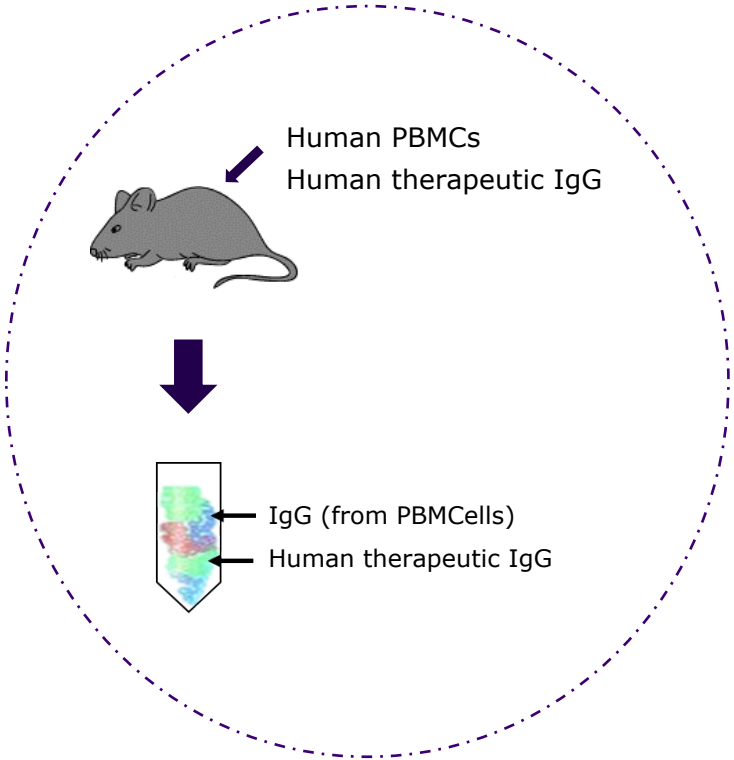
Study Case 1: Matrix Interference



Pharmacology study

- In-vivo model using mice pre-treated with human PBMCells (Peripheral Blood Mononuclear Cells)
- IgG background (PBMC) → Interferences with therapeutic human IgG

Bioanalytical request & challenges

- Exposure control: Quantification of therapeutic IgG in plasma
- Challenge of the complex matrix (PBMCells interference)
- Assay choice:
  - LBA: Complex du to selectivity issue
    Need of specific, time-consuming & expensive tools
  - LC-MS/MS chosen for exposure control
    Specific signature peptides for the therapeutic IgG

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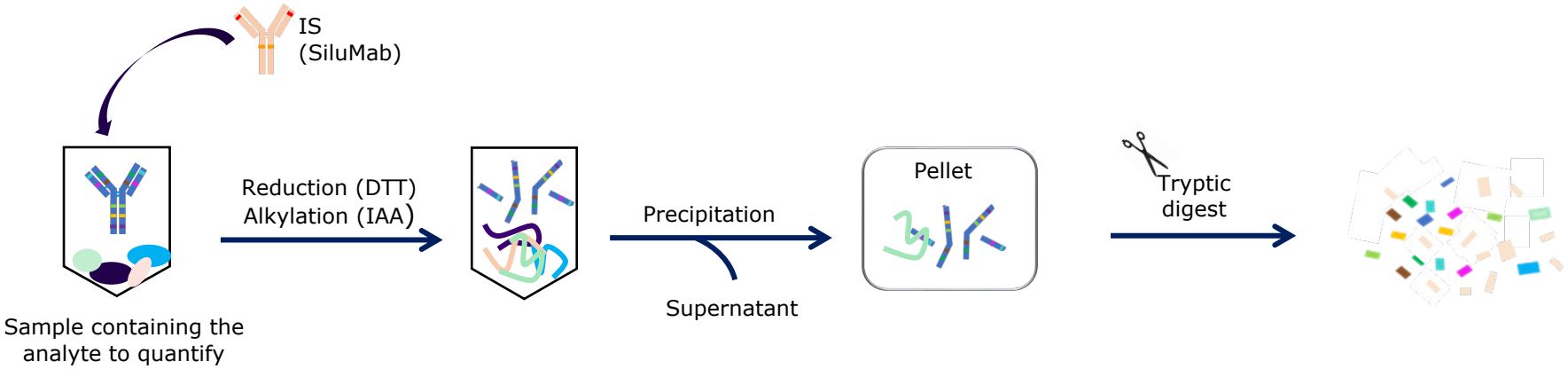
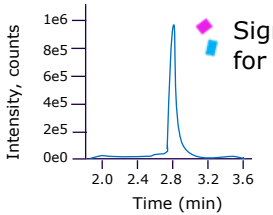
Study Case 1: LC-MS Based Strategy





Signature peptides: Specific for the therapeutic IgG

- Protein precipitation + bottom-up LC-MS/MS
- IgG interference overcomed by the capture free assay & the high selectivity of LC-MS/MS

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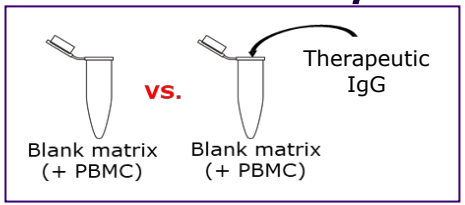
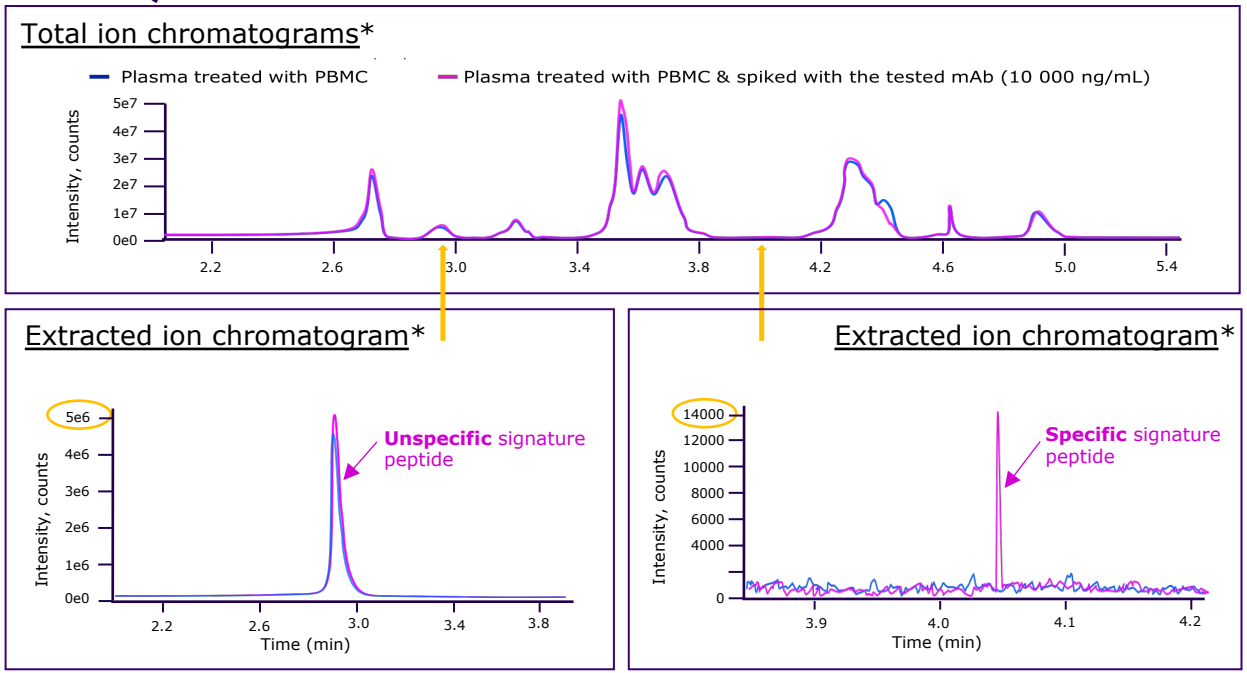
Examplary drawn chromatogram

Study Case 1: Challenging bioanalysis





- Specific signature peptides ?
  Peptides present in the therapeutic IgG but not in the IgG background.

- Results: - Only 2 specific peptides available
  - Low intensity for the specific ones

**Examplary drawn ion chromatograms*

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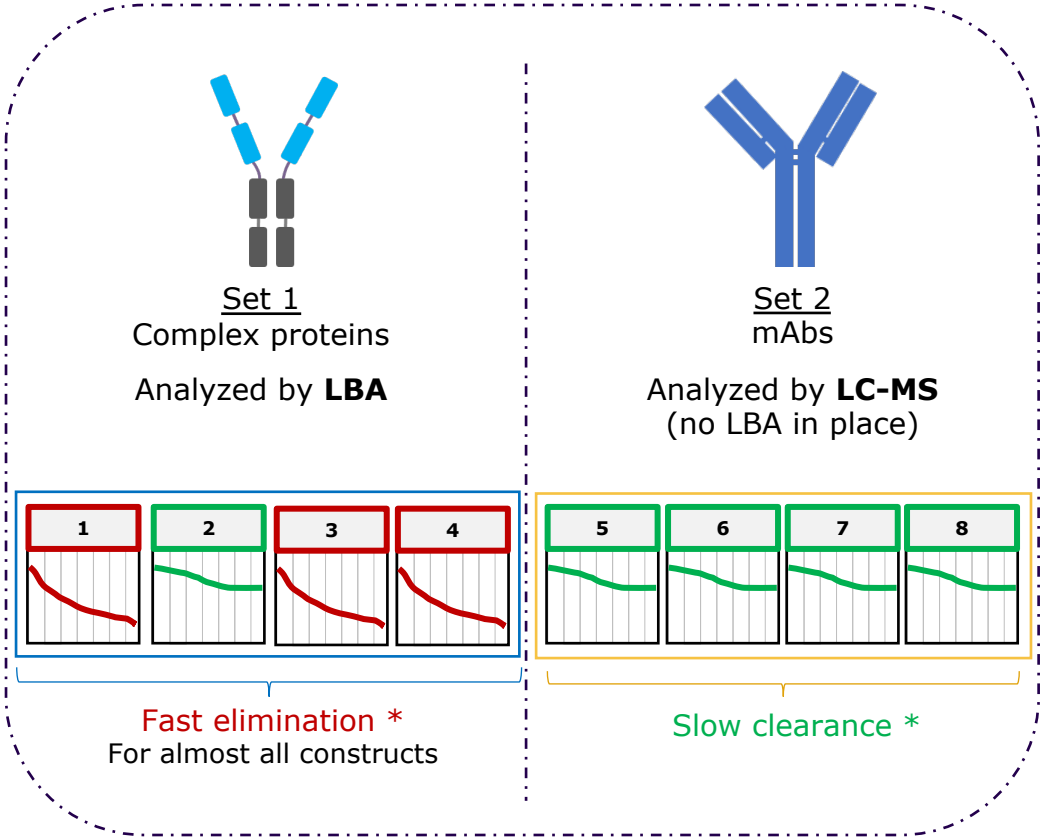
Study Case 2: Confirmation of clearance and in vivo integrity



Study and initial results

- *In vivo* mice PK studies with 2 sets of constructs
- Quantification of the construct in plasma samples

Bioanalytical request

- LC-MS/MS re-analysis of the set 1
  (initially analyzed by LBA)

→ Validation of LBA results?

→ Investigation *in vivo* integrity
Monitoring of multiple signature peptides

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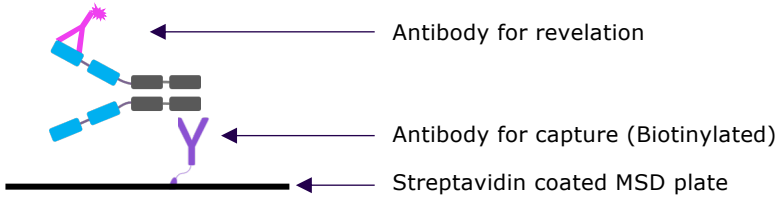
** Examplary drawn PK profiles*

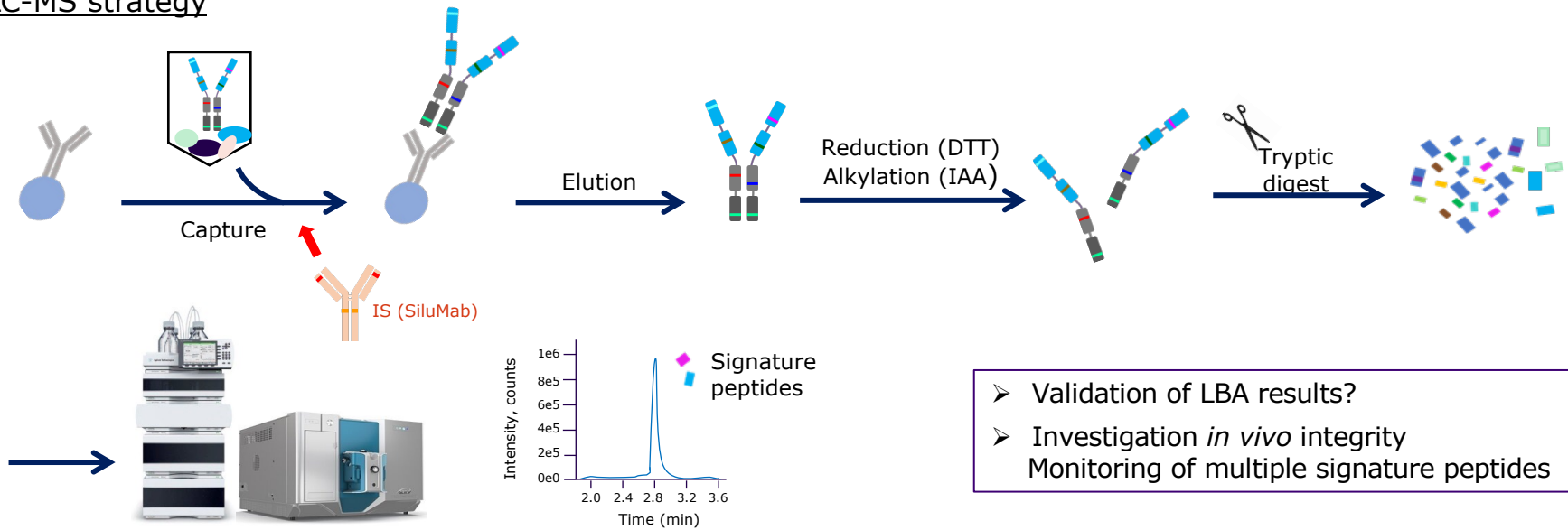
Study Case 2: LBA and LC-MS strategies

LBA strategy



- Assay used for LBA quantification
- No information about integrity

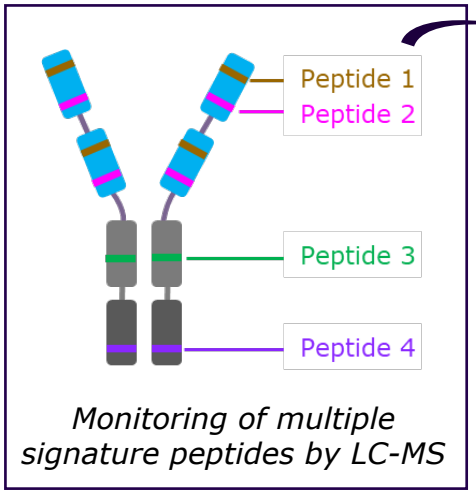
LC-MS strategy



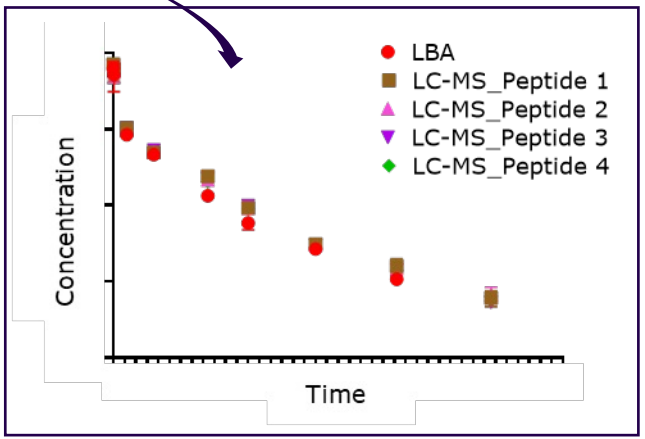
- Validation of LBA results?
- Investigation *in vivo* integrity
  Monitoring of multiple signature peptides

Examplary drawn chromatogram

Study Case 2: Results. *Example Compound 4*

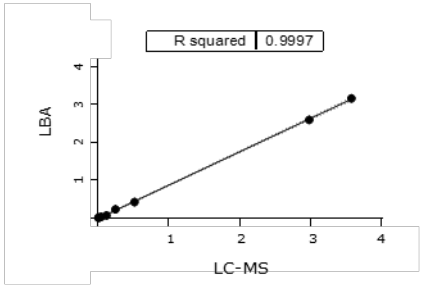


Monitoring of multiple signature peptides by LC-MS



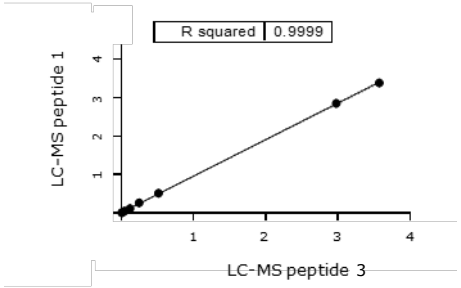
- Good correlation between LBA & LC-MS
  → **Validation of LBA results**

*Correlation LBA / LC-MS (mean of 2 animals) **



- Superimposition of all signature peptides
  → **Proof of in vivo integrity**

*Correlation Peptide 1 / Peptide 3 (mean of 2 animals)**



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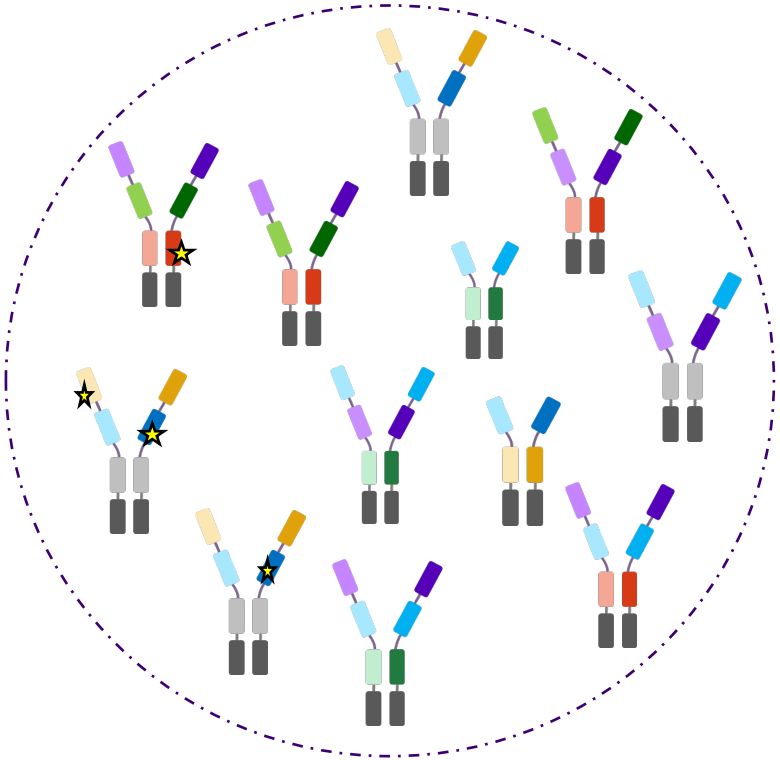
** arbitrary numbers*

Study Case 3: Multiplexing ability, integrity & proof of identity



Study: Optimization of complex multi-specific formats

- Study with **12** different complex multi-specific constructs
- 12 *in vivo* PK studies (TG32-SCID mice)

Bioanalytical request & challenges

- Rapid quantification of the compounds in serum samples
- Search for a method applicable to all constructs
- Monitoring *in vivo* integrity
- Considerations:
  - Number of constructs
  - Low sample volumes
  - Complexity of formats

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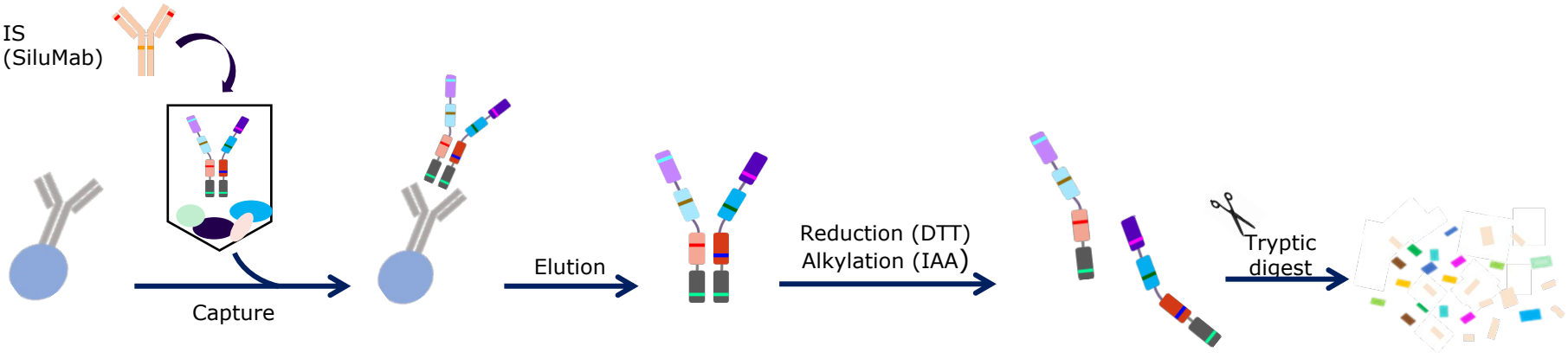
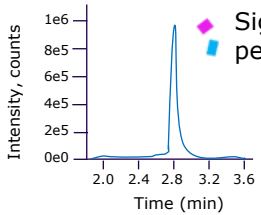
Study Case 3: LC-MS Based Strategy

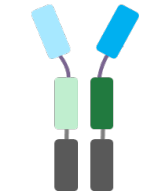




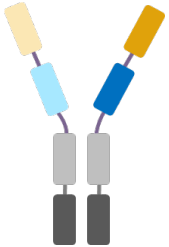
- Immunoaffinity + Bottom-up LC-MS
- Multiplexing ability
- Quantification at peptide level
  → Investigate *in vivo* integrity
  → Distinguish similar constructs

Examplary drawn chromatogram

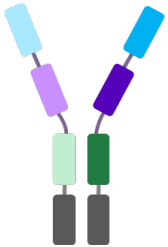
Study Case 3: Multiplexing Ability



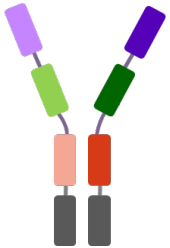
Compound 1



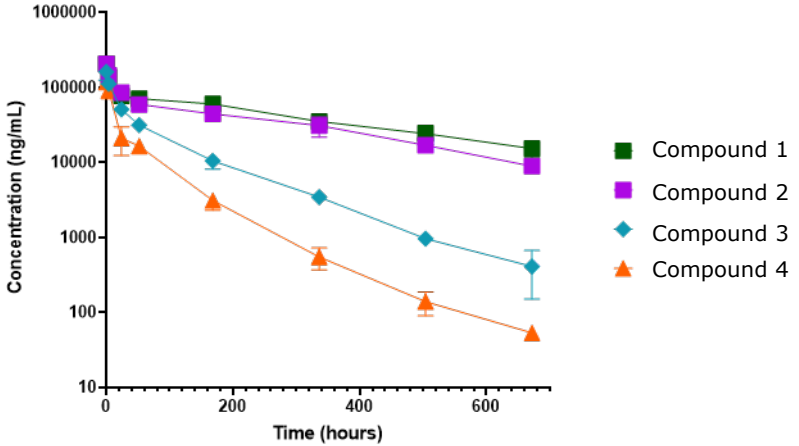
Compound 2



Compound 3



Compound 4



- One single method for different series of compounds
- Capture & LC-MS detection:
  - → No need of specific affinity tools
  - → Generic sample preparation & adaptation of signature peptides
  - → Short development time

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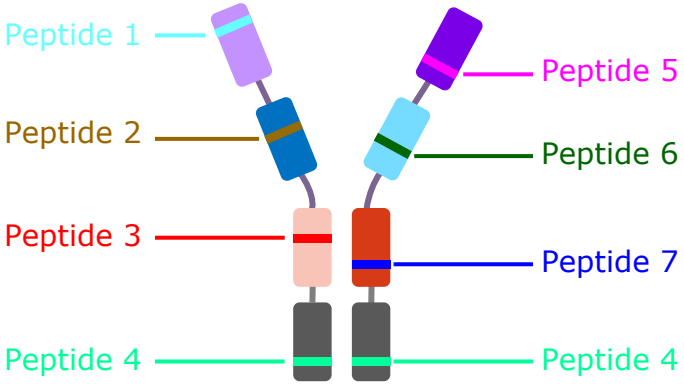
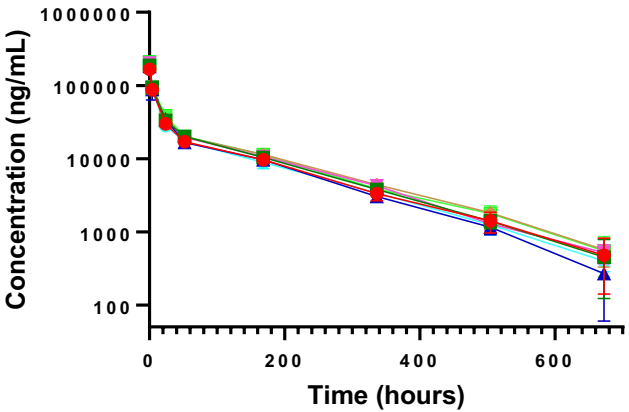
Study Case 3: Proof of in vivo integrity





- Simultaneous monitoring of multiple signature peptides
  → **Superimposition of all signature peptides** → **Proof of *in vivo* integrity**
- Investigation and successful proof of *in vivo* integrity for 12 different constructs
- Advantage of LC-MS over LBA:
  → 1 single assay by LC-MS/MS vs. multiple assays by LBA

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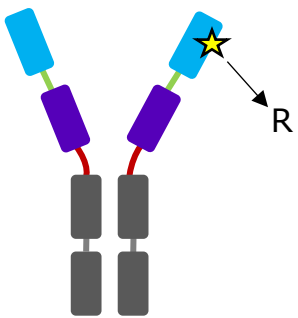
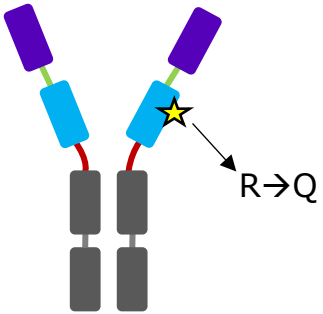
Study Case 3: Investigation of potential mix-up. *Example 1*

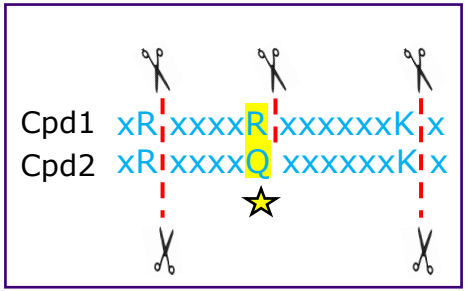
- Unexpected early research PK results
- Check for potential mix-up ?
- Challenging analysis:
  - Similar constructs
  - Domains inverted
  - Single point mutation
- LC-MS to distinguish constructs
  Point mutation → removes one trypsin cleavage site → Different peptides found by LC-MS/MS
- Retrospective proof that studies were conducted with right compounds: **No mix-up!**

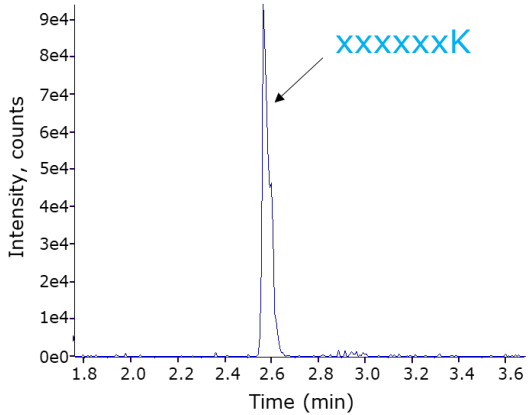


Compound 1

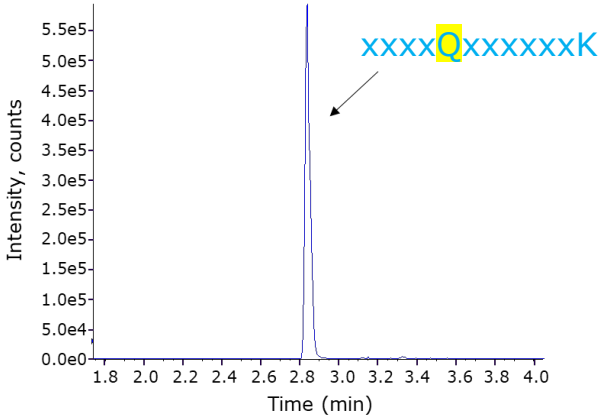


Compound 2





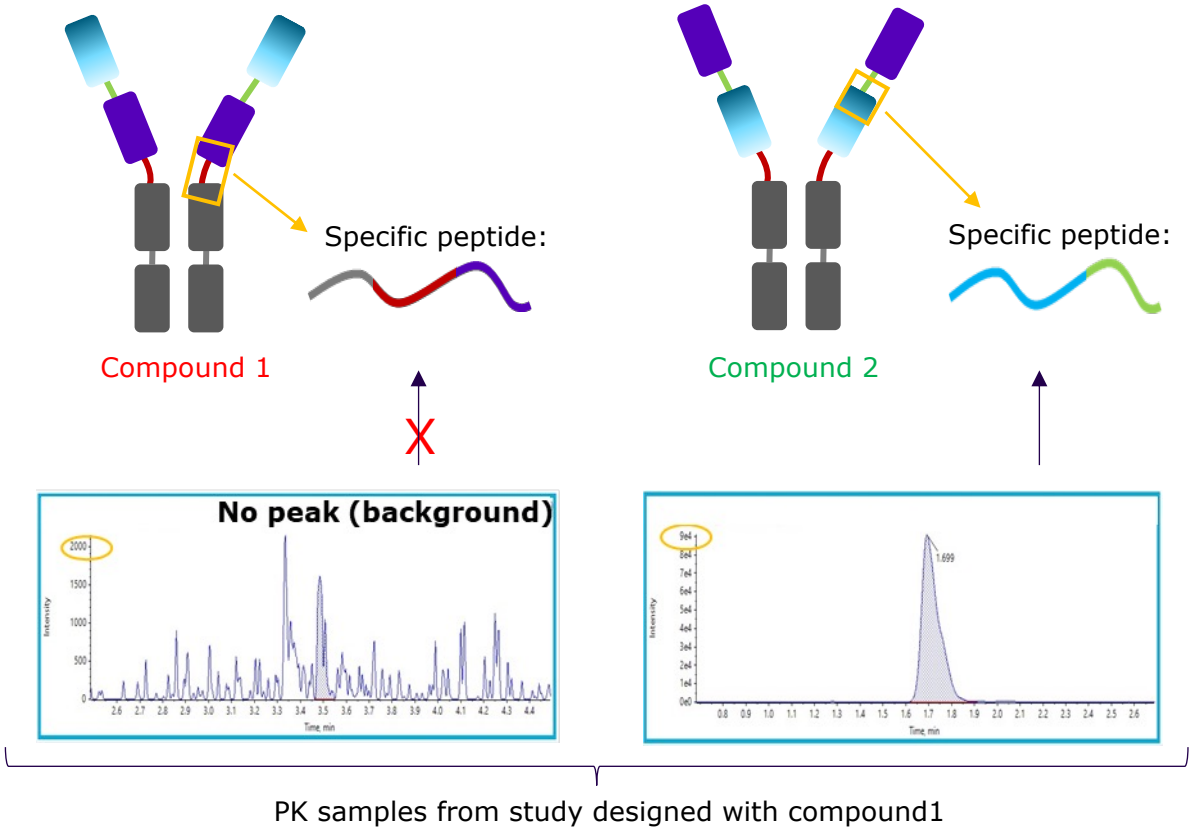
Sample from „Cpd 1 study"



Sample from „Cpd 2 study"

Study Case 3: Investigation of potential mix-up. *Example 2*

- Unexpected early research PK results
- Check for potential mix-up ?
- Challenging analysis:
  - Similar constructs
  - Same domains (inverted)
  - **NO** point mutation
- LC-MS advantage over LBA:
  LC-MS to distinguish constructs
  → Specific peptides
- Retrospective **proof of mix-up**:
  - Study made with compound2 and not compound1
  - Rescue of the results:
    Keep the data but attributed to compound2



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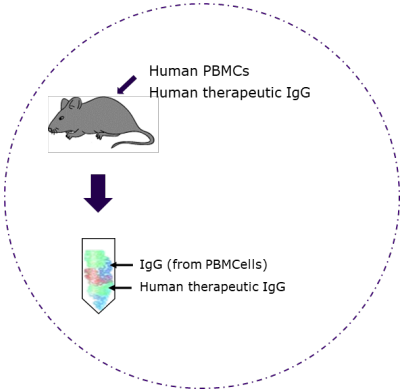
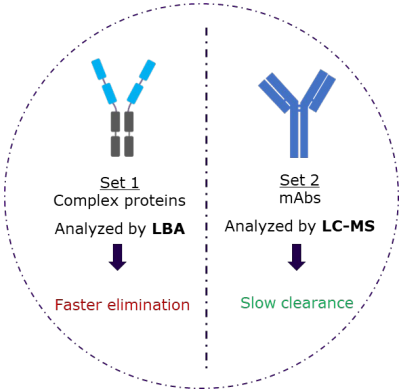
Conclusion

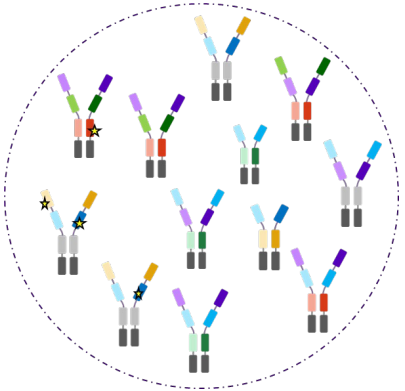
CASE STUDY 1
Matrix Interference



CASE STUDY 2
Confirmation of clearance &
in vivo integrity



CASE STUDY 3
Multiplexing ability,
integrity & proof of identity



- Complemetarity of LBA & LC-MS based approaches for large molecule analysis
- Attractivity of LC-MS based approaches for the analysis of large molecules
  - Proof of *in vivo* integrity for complex proteins
  - Overcome selectivity issues and complicated matrices
  - Multiplexing ability
  - Retrospective proof of identity of compound in samples
- Importance to consider all project aspects to choose the bioanalytical method

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Thank you!

B&I LC-MS, DMPK, Sanofi
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F.J. Mueller
M. Kamm
S. Greco
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LMR, Sanofi
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Thank you

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