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Automated immunocapture of biotherapeutics for LC-MS analysis: Comparison of the AssayMAP Bravo & KingFisher™ robotic platforms

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Bioanalysis & Immunogenicity, DMPK, Sanofi

10th EBF YSS

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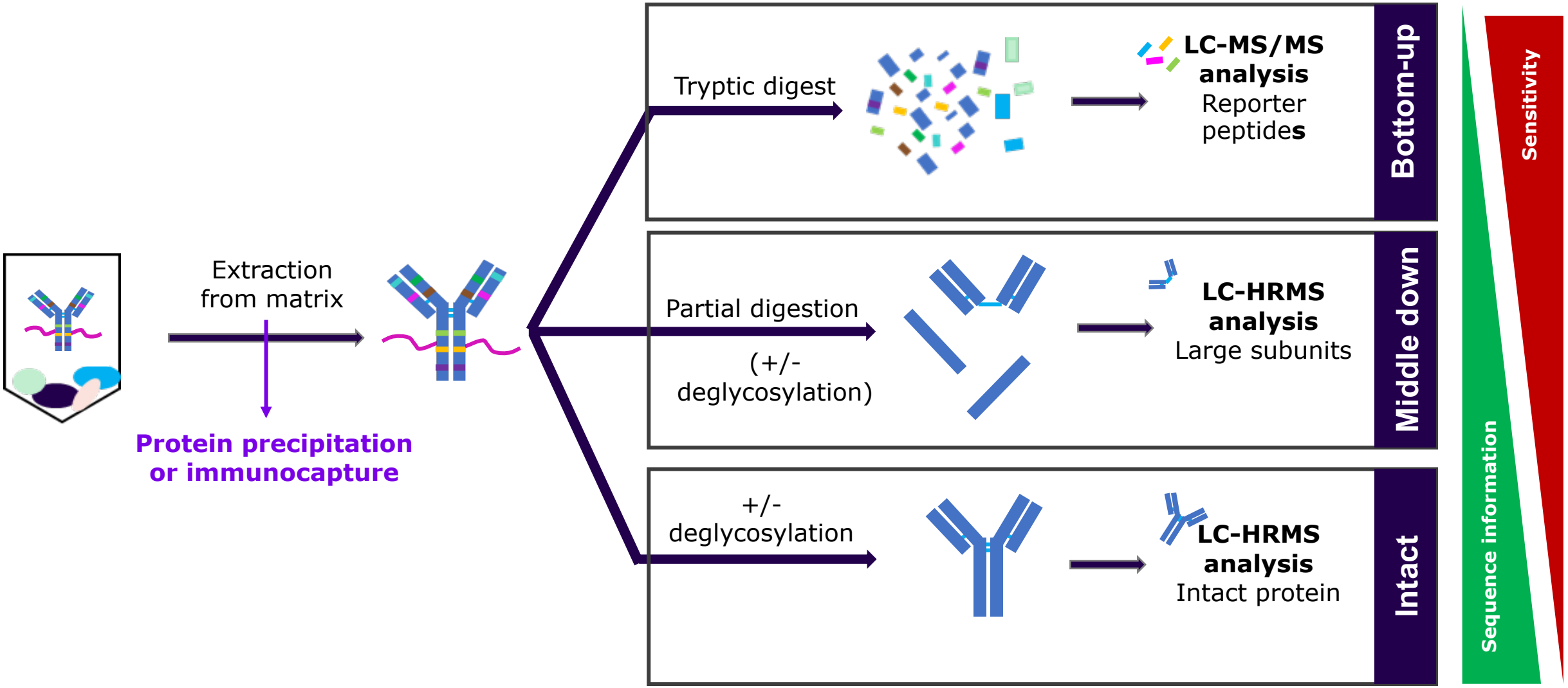
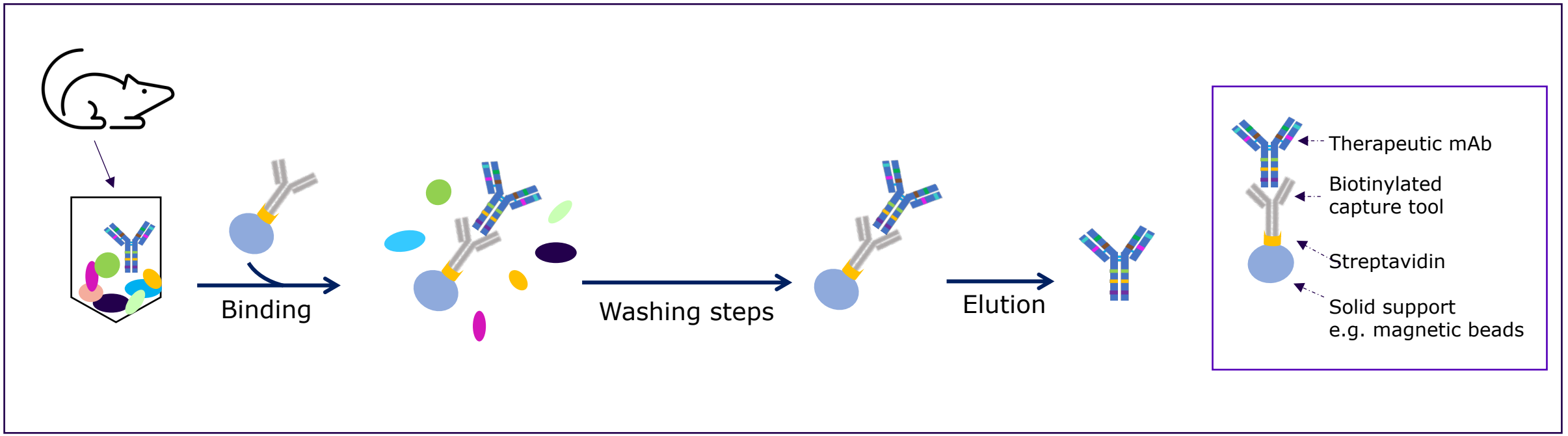
LC-MS strategies for biotherapeutics



Strategy choice depending on the question to answer, sensitivity needed, construct, matrix...

Immunocapture

- **Why an immunocapture step?**



Immunocapture → Enrichment of the biotherapeutic and reduction of the background

→ Increased sensitivity /specificity of the analysis

→ Careful choice of the capture tool (degradation product, interference…)

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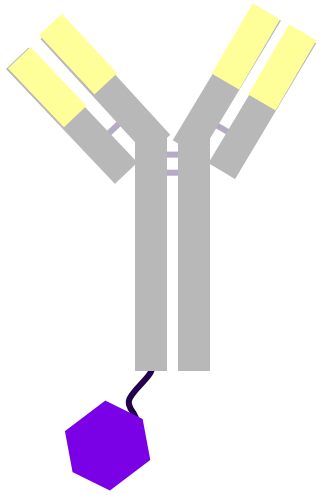
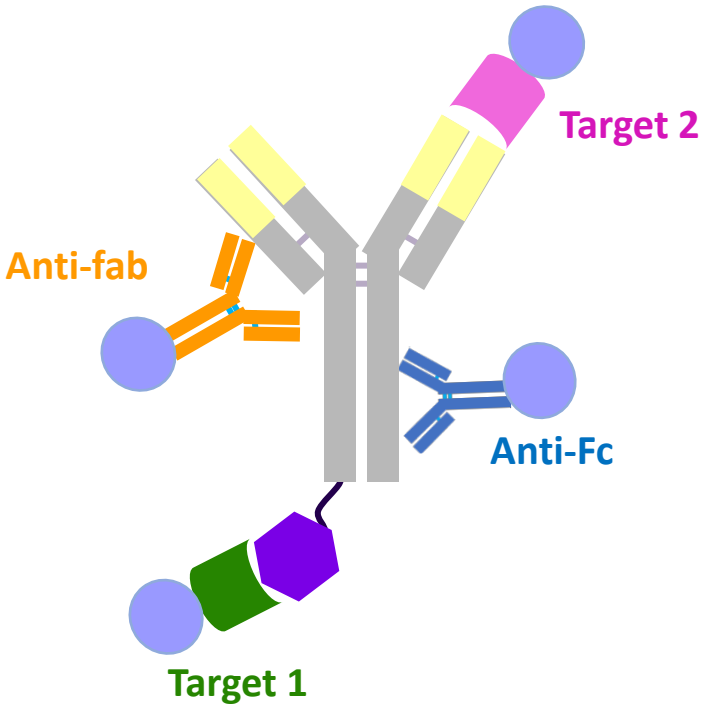
Immunocapture

- **Which immunocapture tool?**



Immunocapture

- **Which immunocapture tool?**



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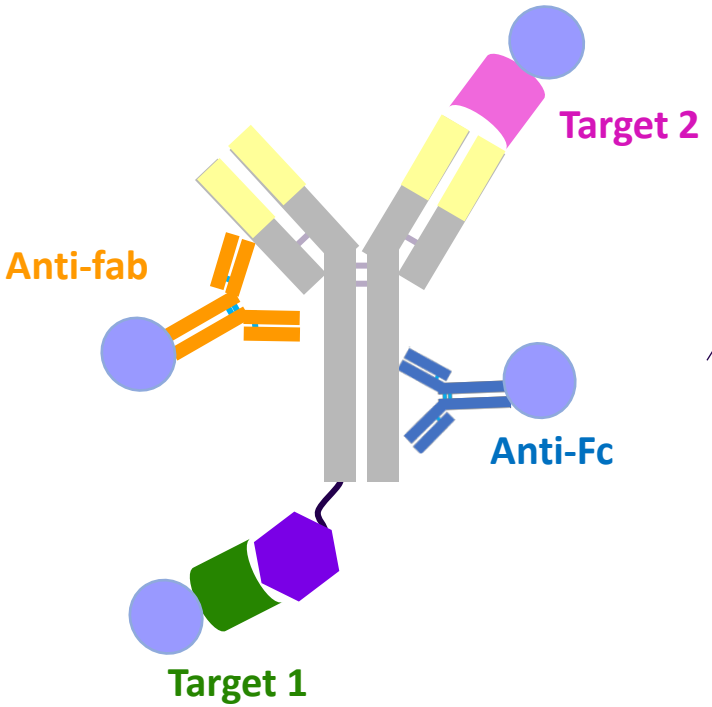
Immunocapture

- **Which immunocapture tool?**



Case 1

Anti-fab / Anti-Fc

Target 2 / Target 1

Case 2

Anti-fab / Anti-Fc / Target 2

Target 1

Case 3

Hu cells or hu IgG

Anti-fab / Anti-Fc

Target 1 / Target 2

Case 4

Anti-fab / Anti-Fc / Target 2

Target 1

Interference ?

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KingFisher™ & Bravo platforms

- **KingFisher™ Flex (ThermoFisher Scientific)**

  - „Versatile benchtop automated extraction instrument“
  - Used with magnetic beads
  - DNA, RNA, proteins, cells
  - Protein immunocapture

- **Bravo Automated Liquid Handling Platform (Agilent)**

  - “Versatile, configurable platform for sample preparation”
  - Used with AssayMAP head:
    Microchromatography for sample preparation
  - Protein immunocapture & on-cartridge reactions

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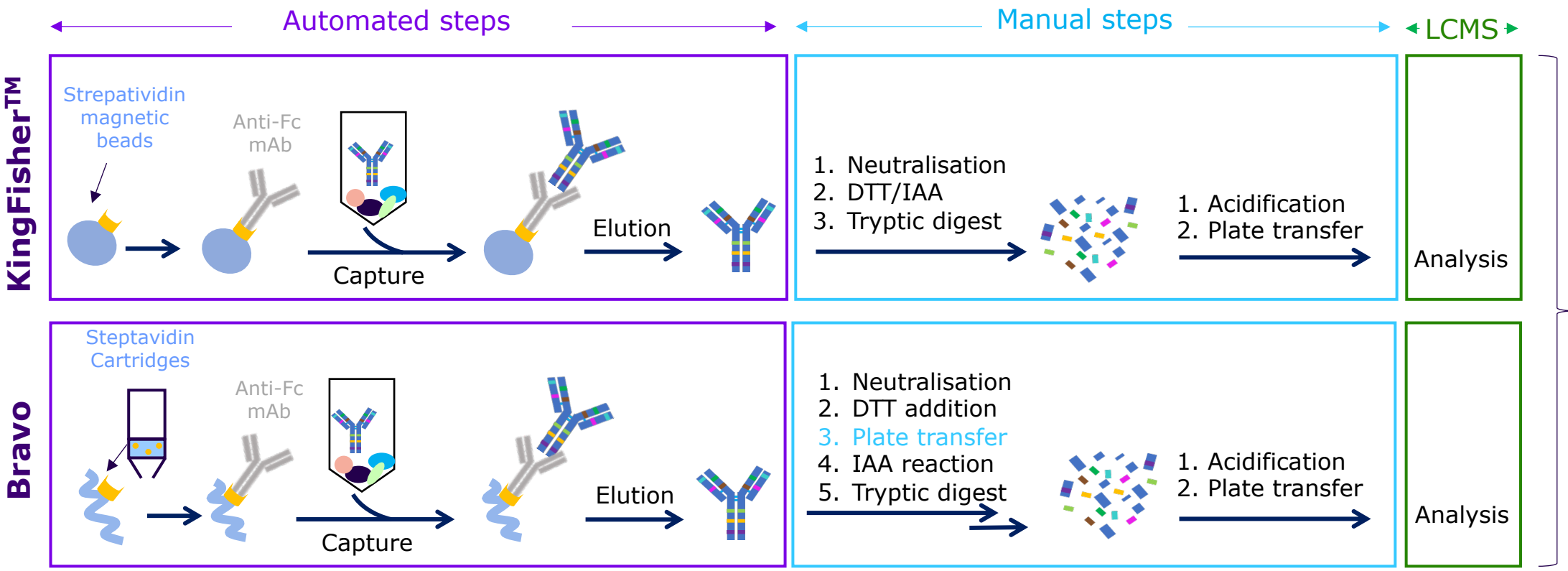
Bottom-Up Analysis

- **Processes**



Practicability: **Similar** (manual digest)

One manual step more for Bravo

- **Gathered data: 3 direct comparisons (multispecific biologics) → Preliminary observations**

Calibration in mice plasma (duplicate)
Settings: 1-2 person – 1-2 day – same samples & solutions
Instruments: MS: Sciex 6500 or 7500

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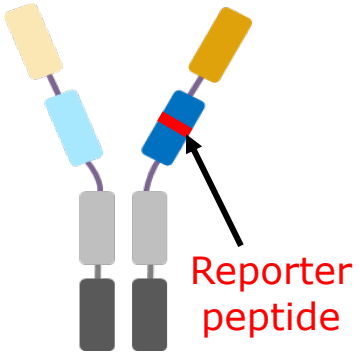
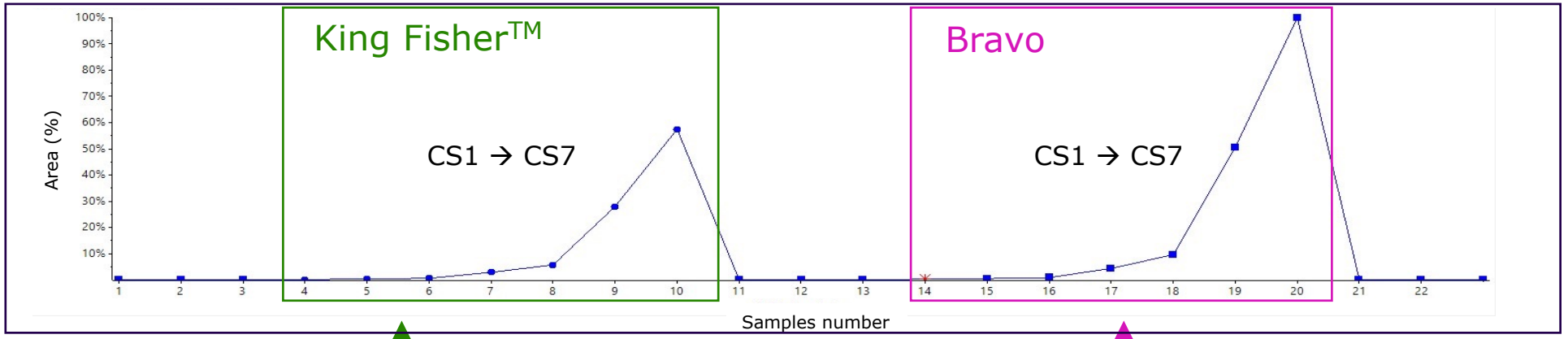
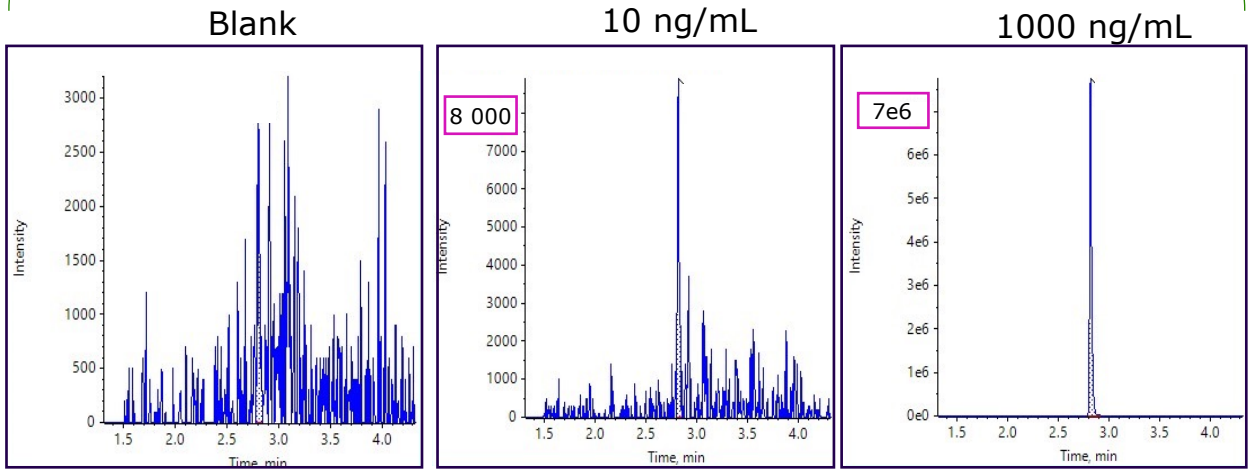
Bottom-Up Analysis

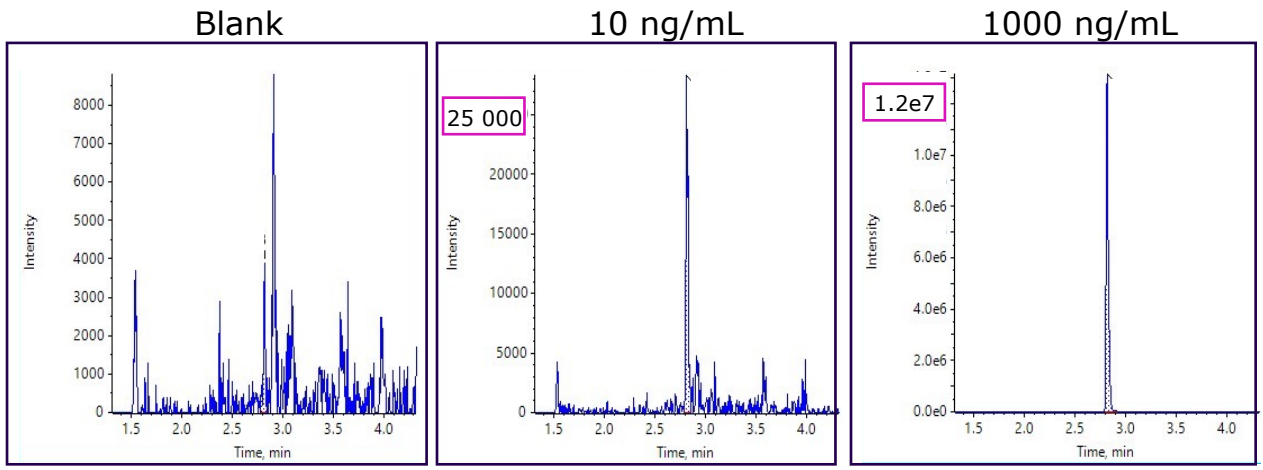
- **<u>Results</u>: No striking difference. Advantage for Bravo system on tested proteins.**

  → Bravo more robust? 1 KingFisher experiment failed.
  → Higher intensity with Bravo for most of the transitions (>85%) of protein tested.
  → Further investigations and repetitions needed



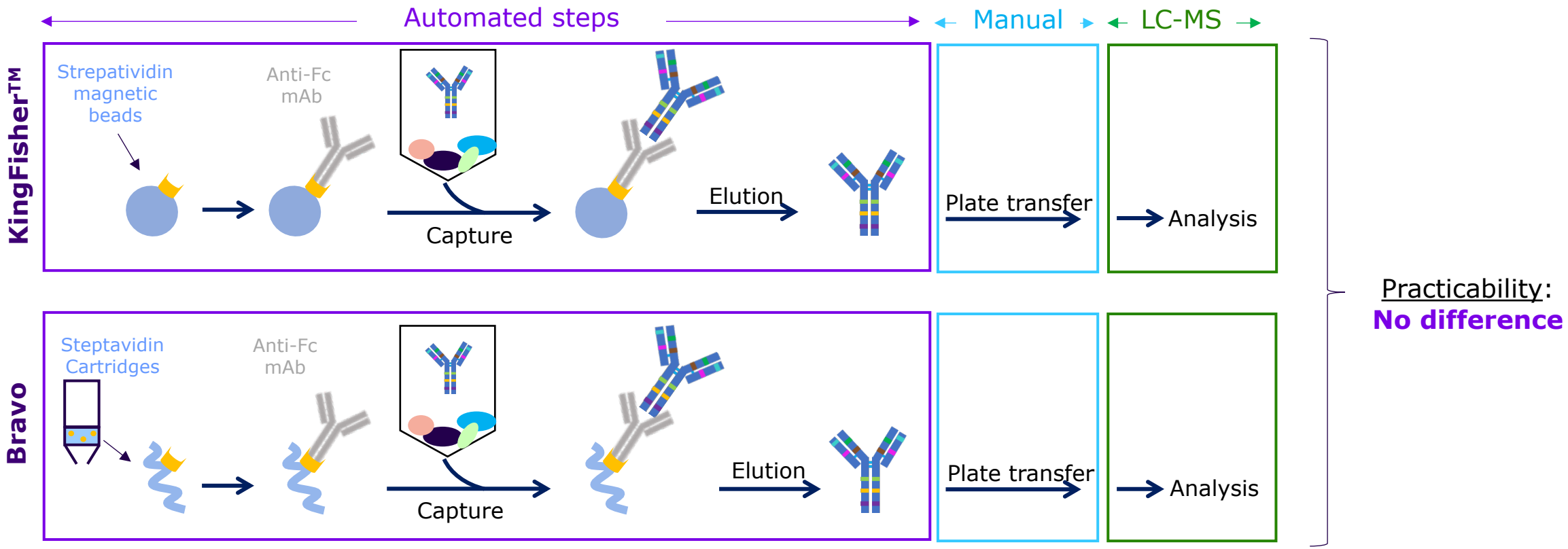






Intact Analysis

- **Processes**



- **Gathered data: 6 independant experiments**

Adalimumab (**Low & homogenous glycosylation**)
Calibration in mice/rat plasma

Settings: 1-2 persons (1 for most experiments)
Instruments: MS: Q-TOF (Sciex 6600+)

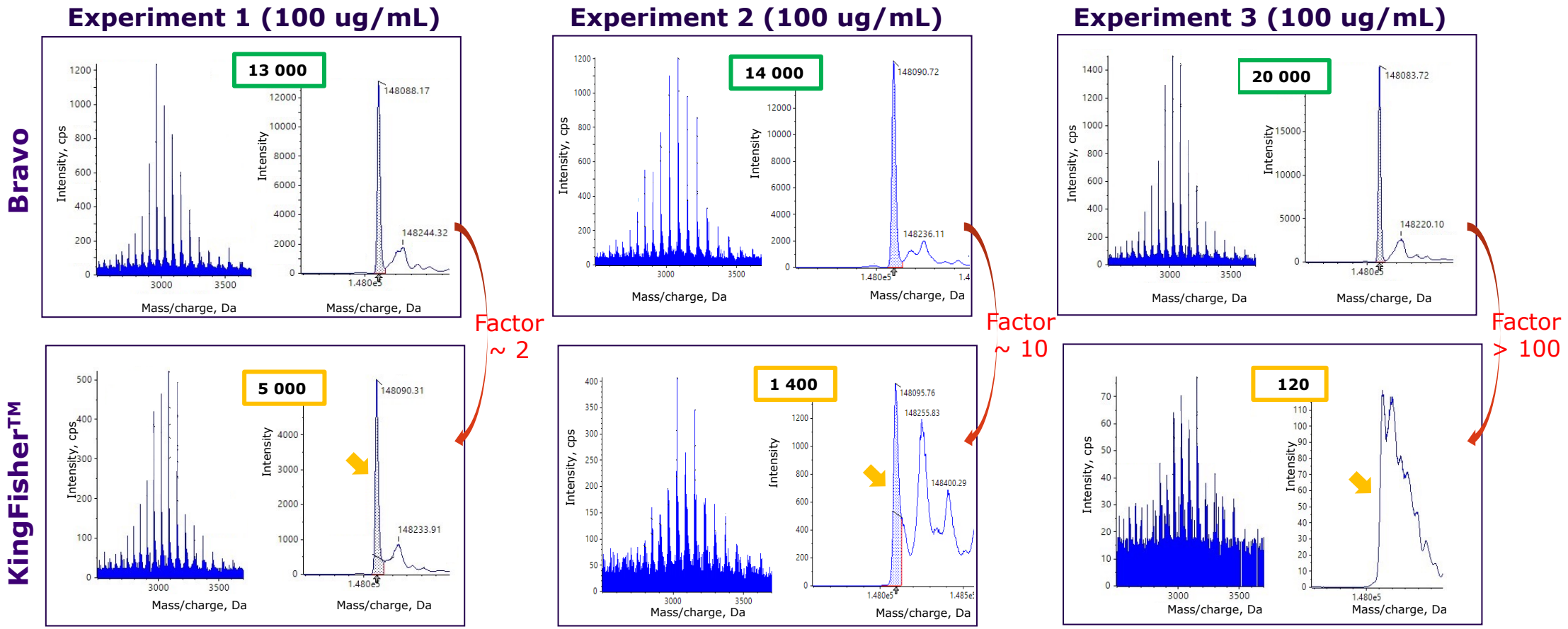
Intact Analysis

- **<u>Results</u>: Advantage for Bravo platform**

→ Higher intensity with Bravo
→ Better reproducibility (inter-experiment) with Bravo
→ Mass shift & adducts sometimes observed with KingFisher (Oxidation? Metal adduct?)

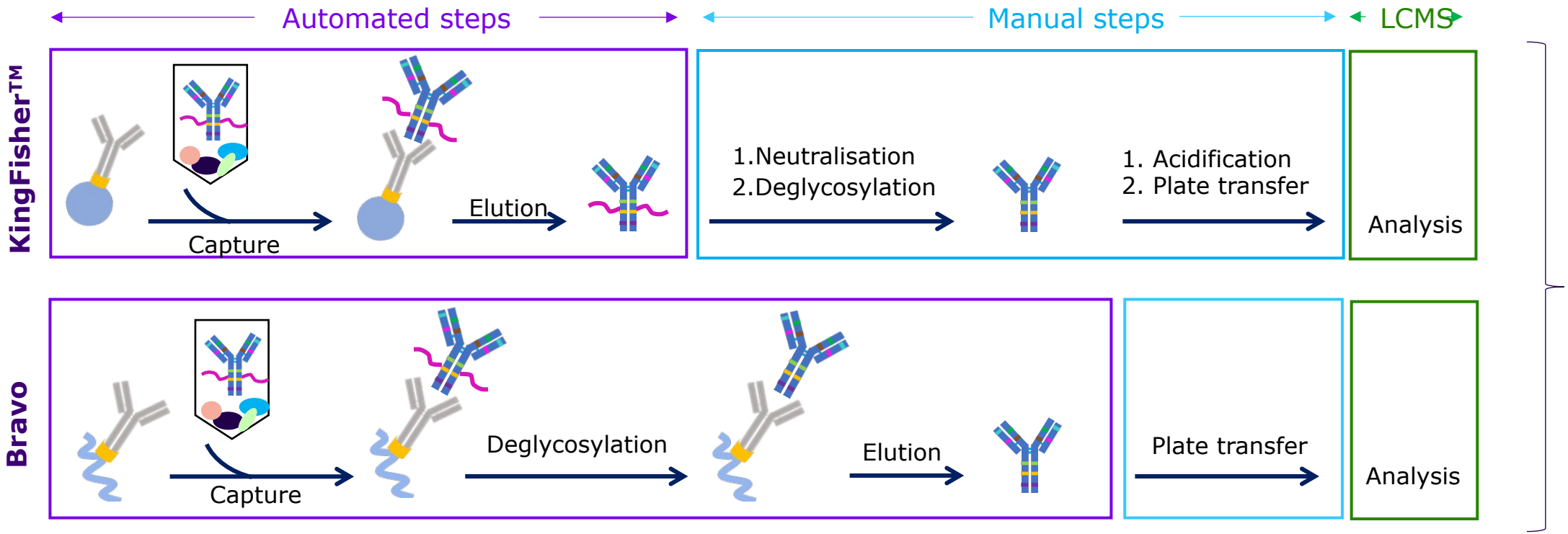
Impact LLOQ & quality of analysis

→ Variability intra-run: KingFisher™~ Bravo (Good for both platform)



Intact Analysis with deglycosylation

- **Processes**



Practicability:
Bravo >> KF
More automatized steps!

- **Gathered data: 6 direct comparisons (3 different multispecific biologics)**

Calibration in mice plasma (duplicate)
Settings: 1 person - 1 day – same samples & solutions
Instruments: MS: Q-TOF (Sciex 6600+)

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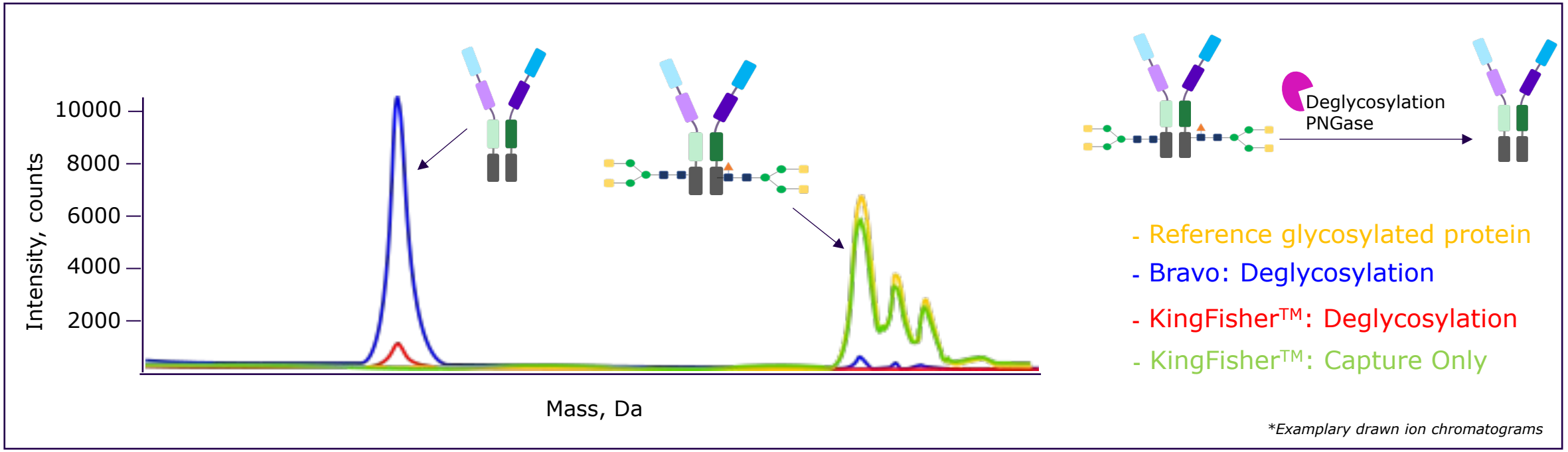
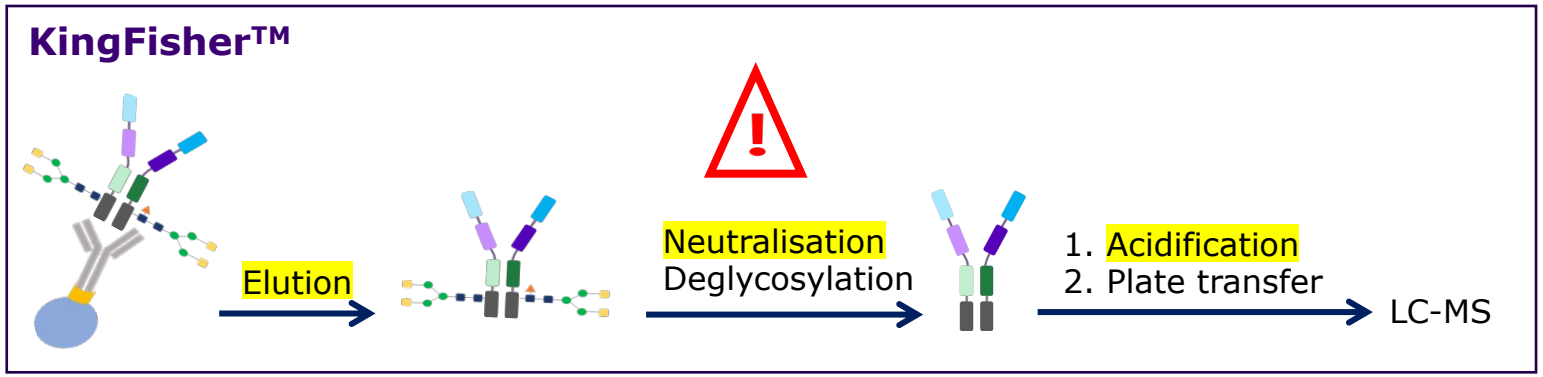
Intact Analysis with deglycosylation

- **Results: Advantage for Bravo platform**

  → Lost of protein intensity during KingFisher process.





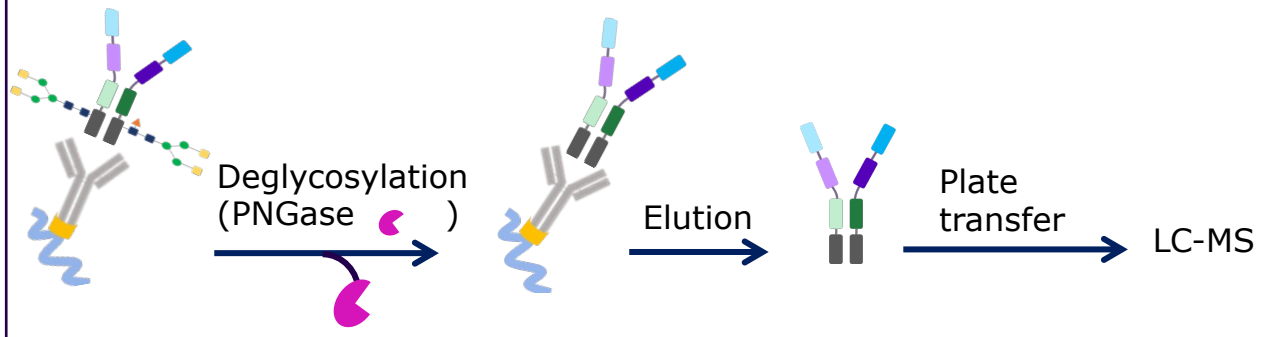
Reason for the loss of protein ?
Unfolding/Precipitation/aggregation due to acidification and change of pH?

Intact Analysis with deglycosylation

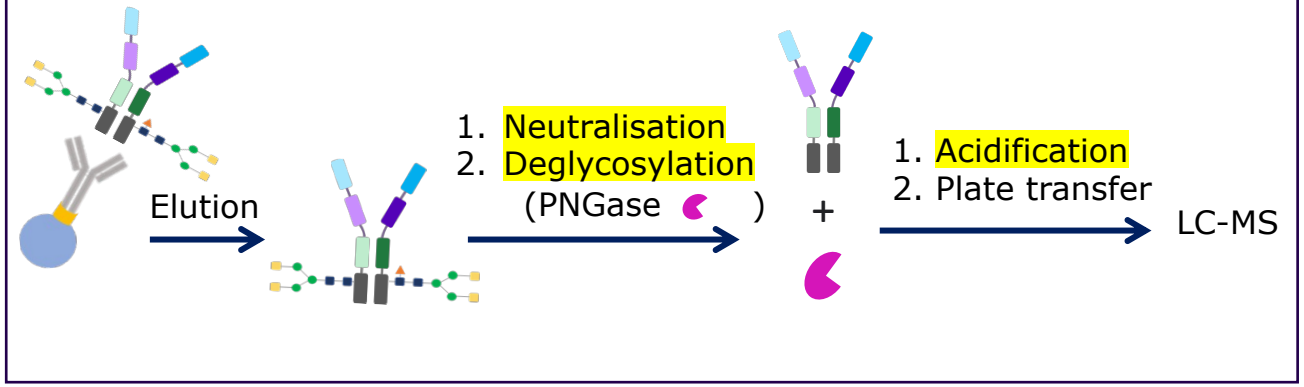
- **<u>Results</u>: Advantage for Bravo platform for all compounds tested**

  → Additional inconvenients with KingFisher™ process

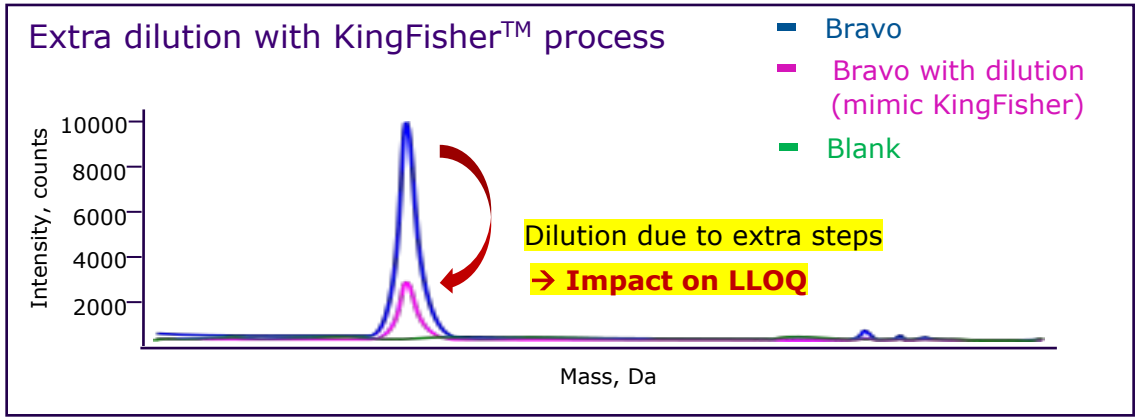
Bravo



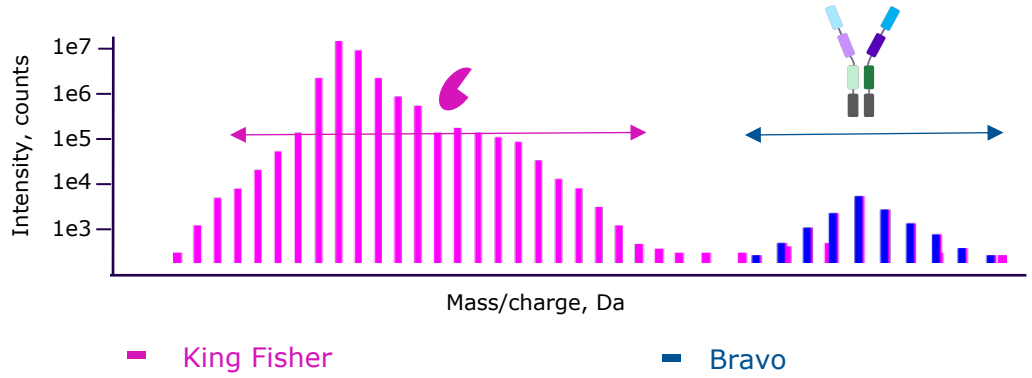
KingFisher™



Extra dilution with KingFisher™ process



MS Interference due to PNGase in the sample to inject



Coeluting PNGase: - Suppress ionization / saturate instrument detector
- Interference, especially for smaller proteins

**Examplary drawn ion chromatograms*

Conclusion

- Conclusions comparison (based on current acquired data):
  - **Advantage for Bravo** (Intact, bottom-up MS, middle-up MS)
  → Sensitivity, reproducibility, conveniency
  - Further comparison needed to draw more certain and statistical conclusions
  - Optimization of both platforms and methods possible

- Choice of the platform: **Project dependent**
  Type of method, sensitivity needed, construct, matrix, site of analysis

- Price
  - Instrument: KingFisher™ Flex (~**55 000 €**) & Bravo (~**180 000 €**)
  - Consumables: Beads (**~1 €** for **1 sample**) & Cartridge **(~10 €** for **1 sample**)

- Consumables source
  - Cartridges: Agilent ➔ Single source
  - Beads ➔ Multiple vendors (more flexibility)

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Thank you!

A big thank you to the B&I LC-MS - DMPK, Sanofi Frankfurt

Especially to: D. Fleck
A. Albrecht
M. Kamm
M. Giessler
S. Greco
K. Schroeter

Thank you to M. Dupré from B&I LC-MS - DMPK, Sanofi Paris

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