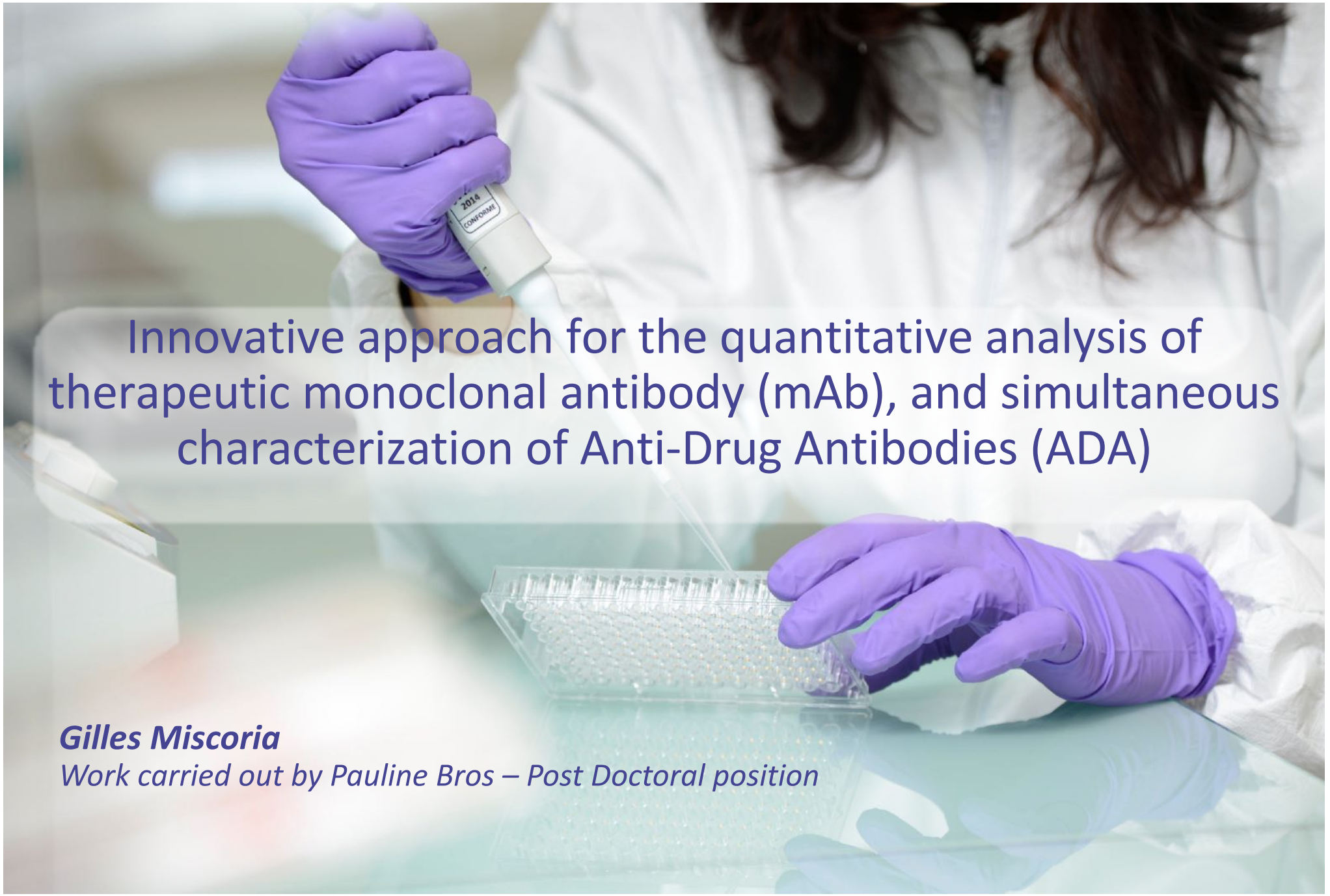


Innovative approach for the quantitative analysis of therapeutic monoclonal antibody (mAb), and simultaneous characterization of Anti-Drug Antibodies (ADA)

Gilles Miscoria

Work carried out by Pauline Bros – Post Doctoral position

Context and aim

Immunogenicity observed in humans is of major safety concern

Efficacy

- Change of PK parameters
- Loss of efficacy (nAb)
- Interferences in Bioanalytical methods
- Jeopardize predictions of PK/PD model

Safety

- Immunologic reaction
- Severe loss of treatment efficacy
- Cross reactivity with endogenous compound
- Induced autoimmune reaction

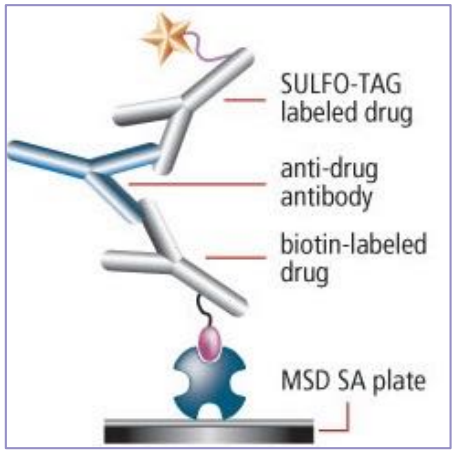
In the immunogenicity world, analytical gold standards are LBA and CBA

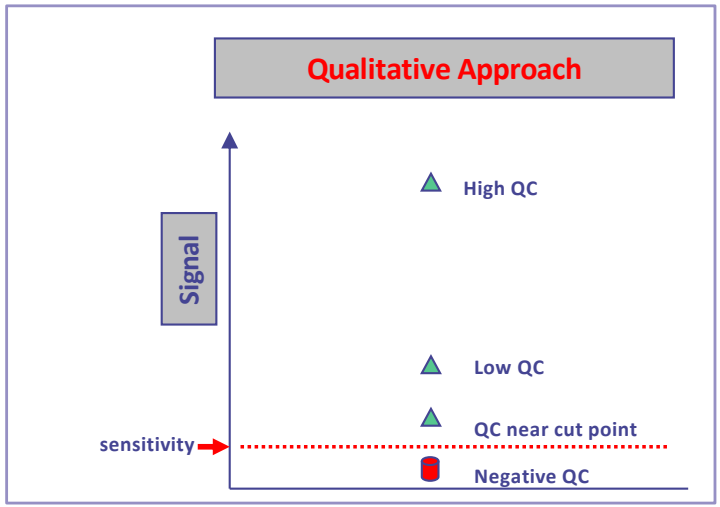
Monitoring immunogenicity by LBA

Bridging immunoassay with or without first step acid dissociation

- *Qualitative evaluation of ADA is based on Cut-point approach*
  1st step : Screening test = positive or negative signal for ADA (given some false positive)
  2nd step : Confirmatory test = (ex :competitive approach with excess of drug)
  3rd step : Titration of positive by serial dilution of the sample to fall under cut-point
  4th step : Neutralizing ADA (using CBA or LBA)

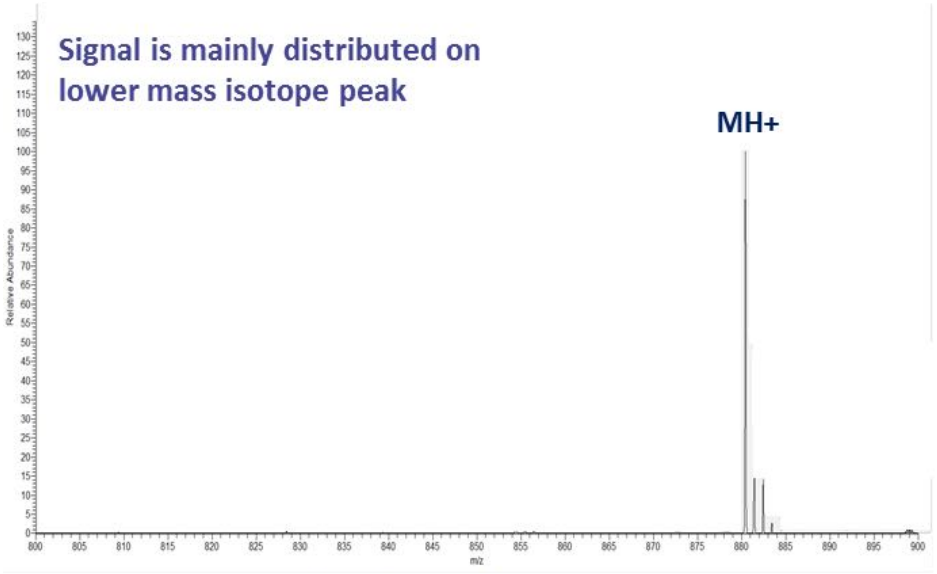
- ***Qualitative approach – No "gold" standard ADA** (Rabbit polyclonal or monoclonal ADA is only "representative")*
- ***Possible interferences in ADA assay** (ex : free drug tolerance, soluble target…)*



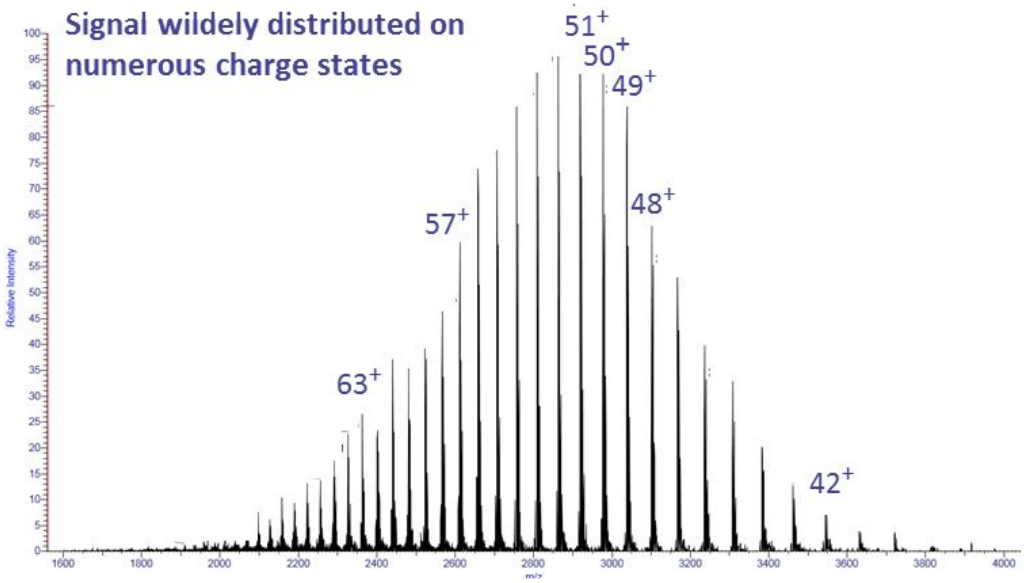


Guideline on immunogenicity assessment of biotechnology-derived therapeutic proteins, April 2008; EMEA

Can MS be used for large molecule analysis ?



ESI+ mass spectrum of small peptide
Thermo Q-Exactive+ instrument



ESI+ mass spectrum of intact monoclonal IgG_1
Thermo Q-Exactive+ instrument

Why MS doesn't fit with large molecule – Sensitivity issue ?

10 ng small molecule (ex : MW 500)

→ 20 pmole

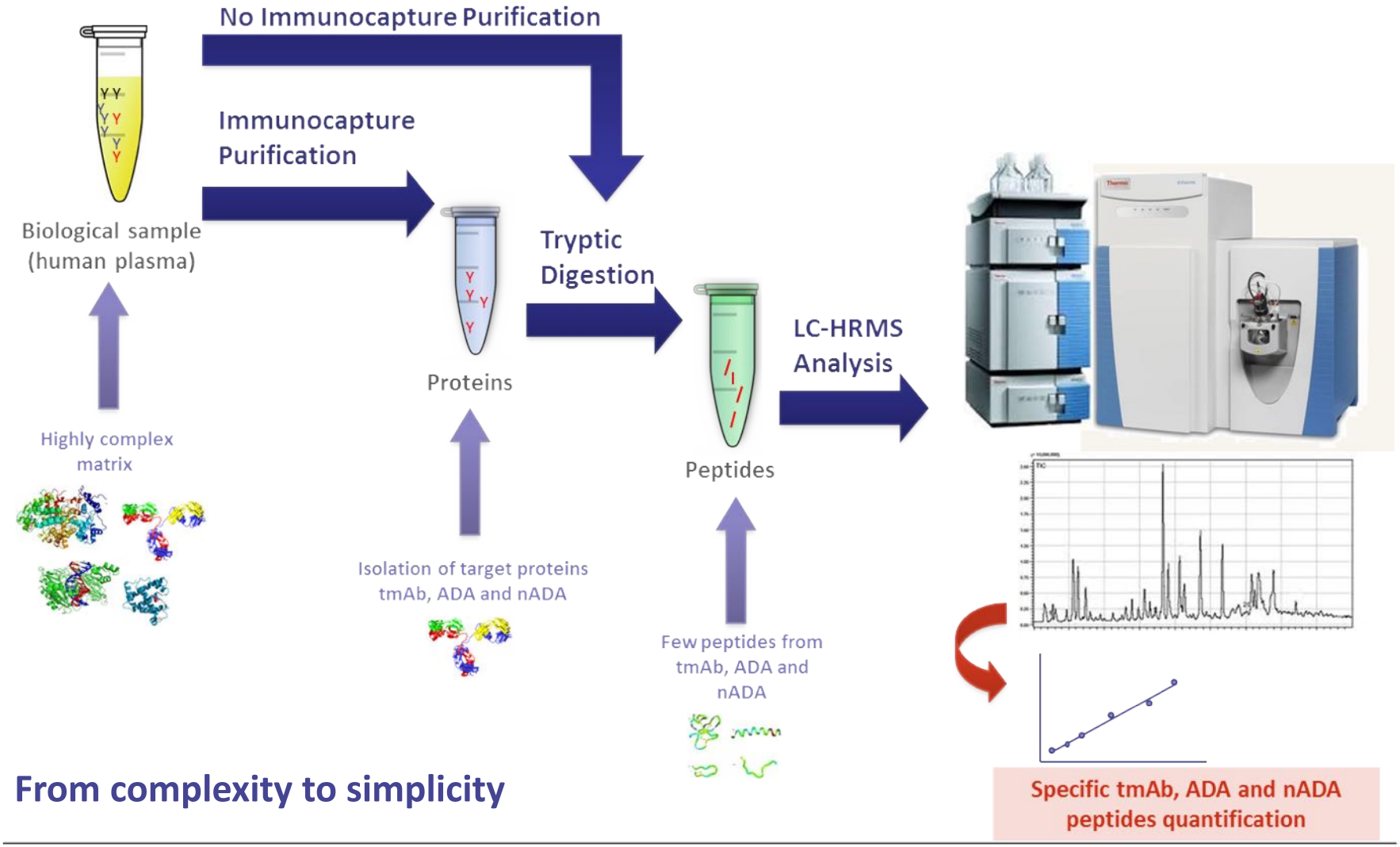
10 ng TmAb (ex : IgG1 MW: 150 KDa)

→ 0.067 pmole

300 fold less molecules !

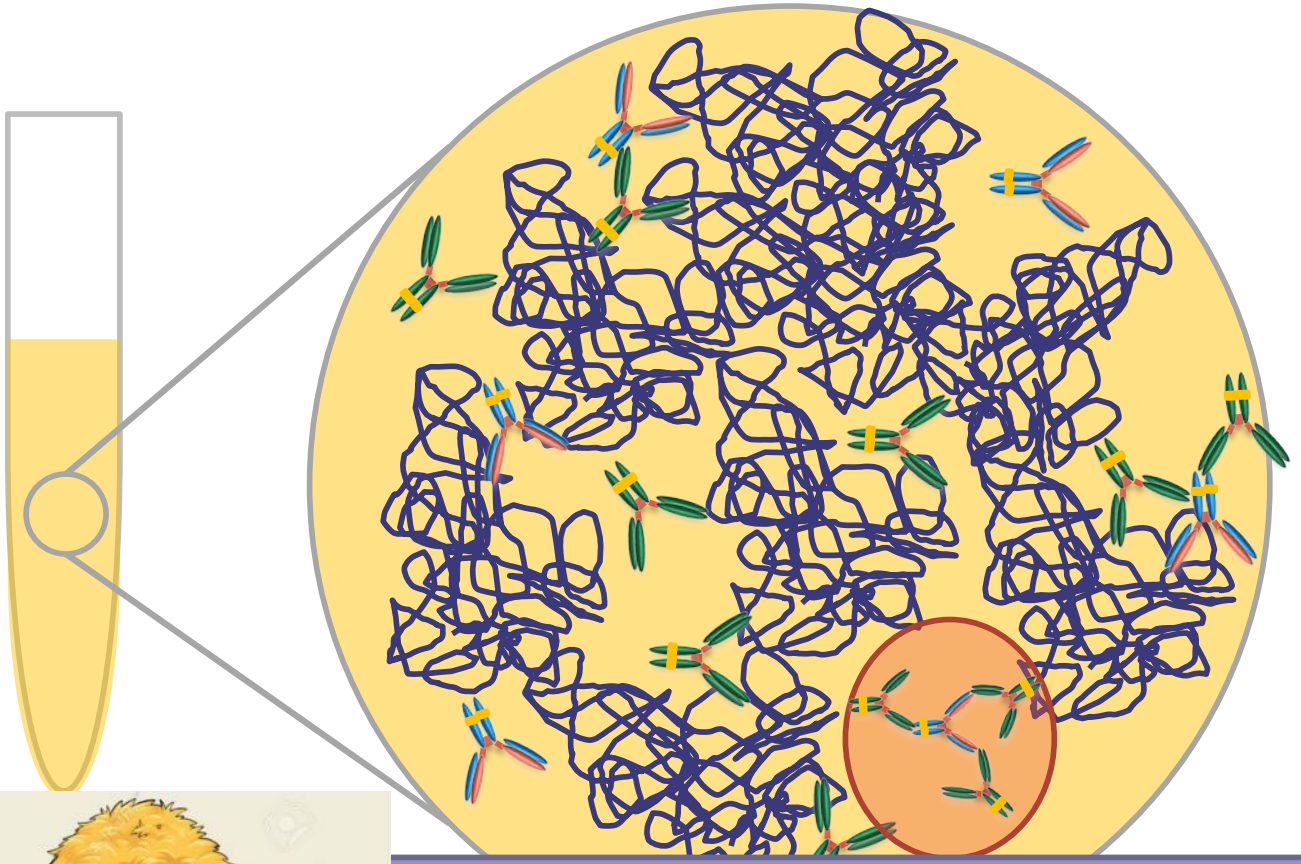
TmAb : Therapeutic monoclonal antibody

LC-MS/MS analytical process strategy
From the protein to peptide



From complexity to simplicity

Plasma is not an easy matrix



Albumin (~ 60%)
mg/mL

Endogenous immunoglobulins
mg/mL

Free TmAb
µg/mL

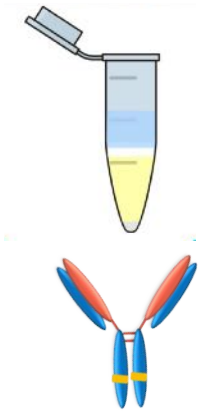
Anti-Drug Antibody (ADA)
µg/mL → ng/mL

Neutralizing ADA (nADA)

Efficient sample preparation and
And so many other proteins and endogenous compounds !
sitive/specific analysis method is crucial!

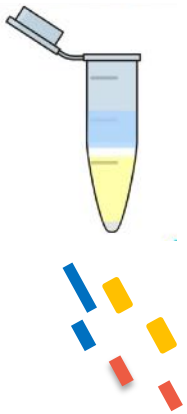
Application – Real example from a preclinical study
Total Form of TmAb (*h-IgG$_1$*)

Quantification of **total TmAb** using a specific and intense signature peptide.



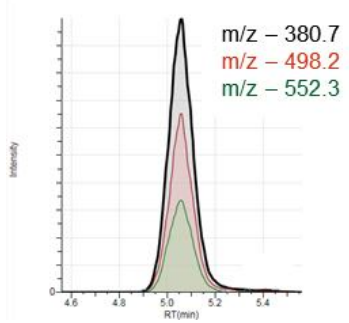
Denaturation
80°C, 10 min

Trypsin digestion
37°C Overnight



LC-MS/MS analysis

Precursor ion (m/z)	Product ions (m/z)
Ex : 630.35	380.6 498.2 552.3



Biological sample : Plasma
- *Intact TmAb and ADA*
- *No internal standard added**

Digested biological sample
- *TmAb, ADA peptides and all matrix peptides*

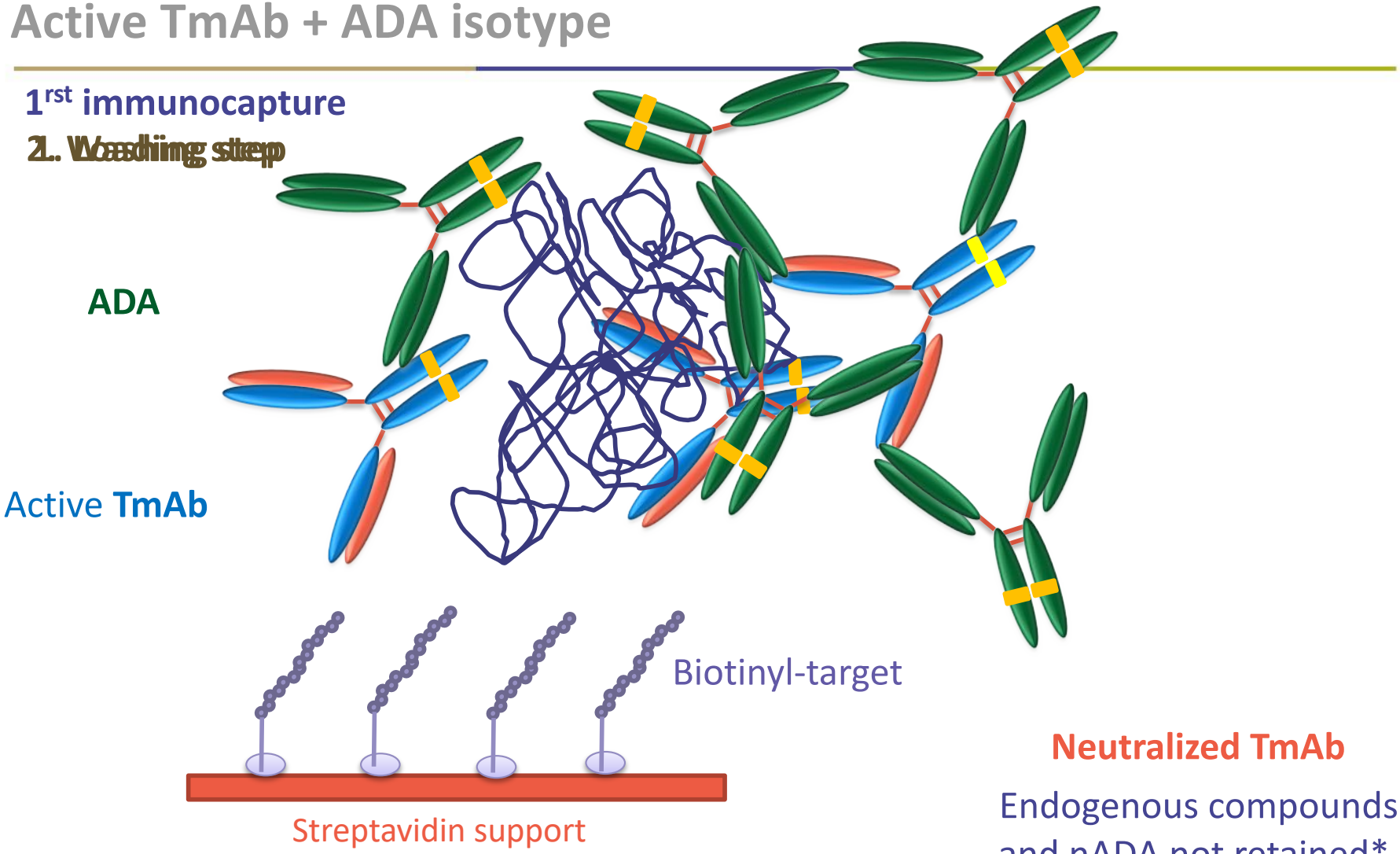
TmAb Signature peptide (as PRM signal)
LOQ : 200 ng/mL
Linear range of calibration : 0.2 µg/mL – 20 µg/mL
Assay variability in acceptance criteria < 20%

** No stable isotope labeled internal standard*

- **Concentration of Total TmAb**

Efficient sample preparation 1/3

Active TmAb + ADA isotype



Neutralized TmAb

Endogenous compounds and nADA not retained*

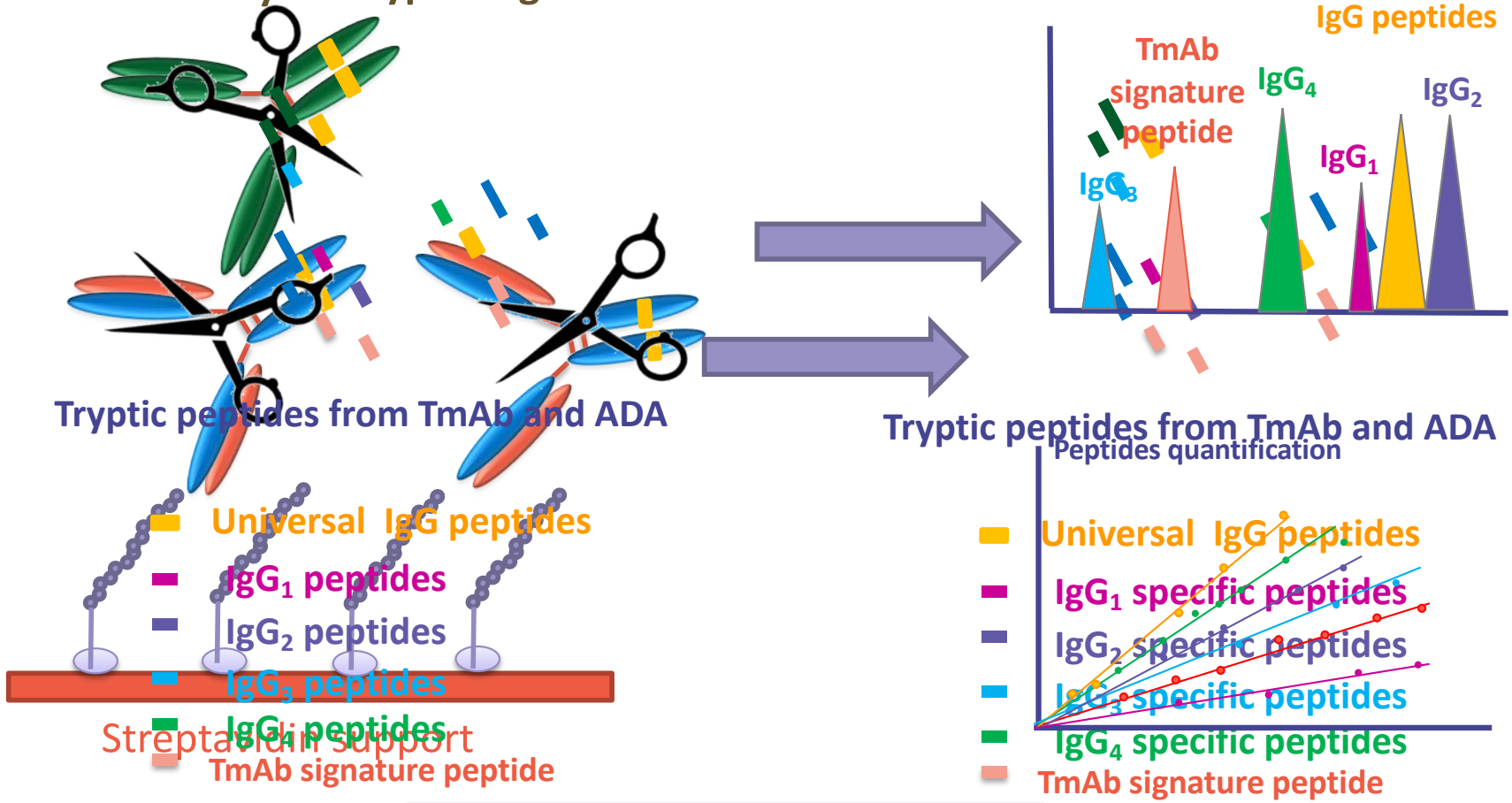
* Conserved fraction for further experiments

Efficient sample preparation 2/3

Active TmAb + ADA isotype

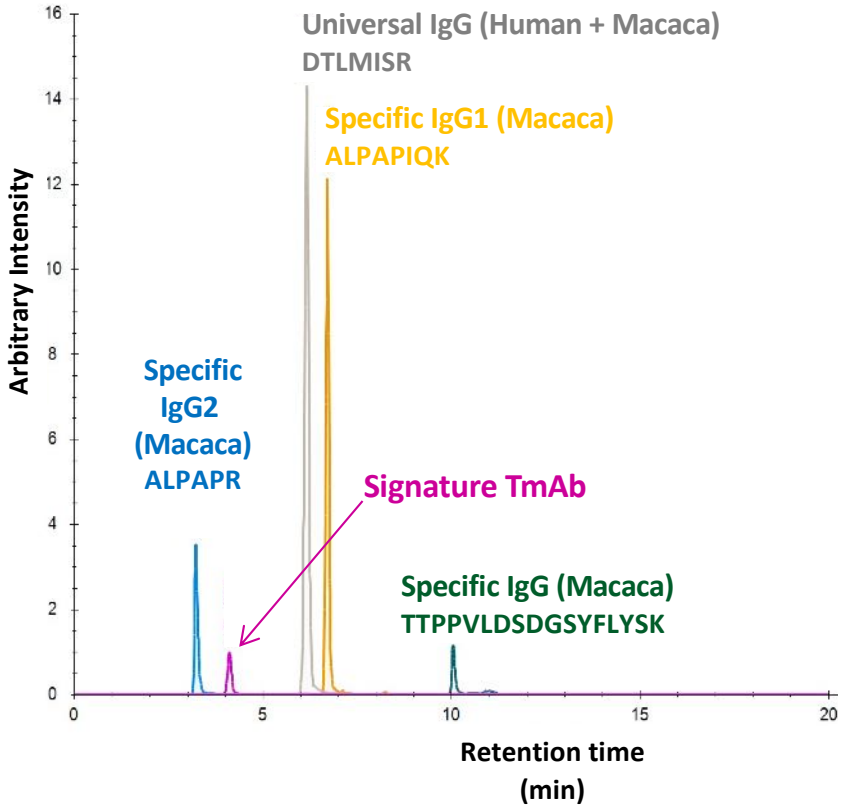
1rst immunocapture

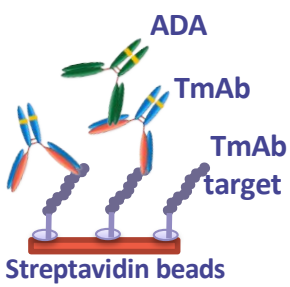
2. Acid dissociation + Tryptic digestion



- Concentration of active form of TmAb
- ADA isotype

Application – Real example from a preclinical study 3/3
Active TmAb + ADA detection (isotype)





Acid dissociation
Trypsin digestion

LC-MS/MS analysis

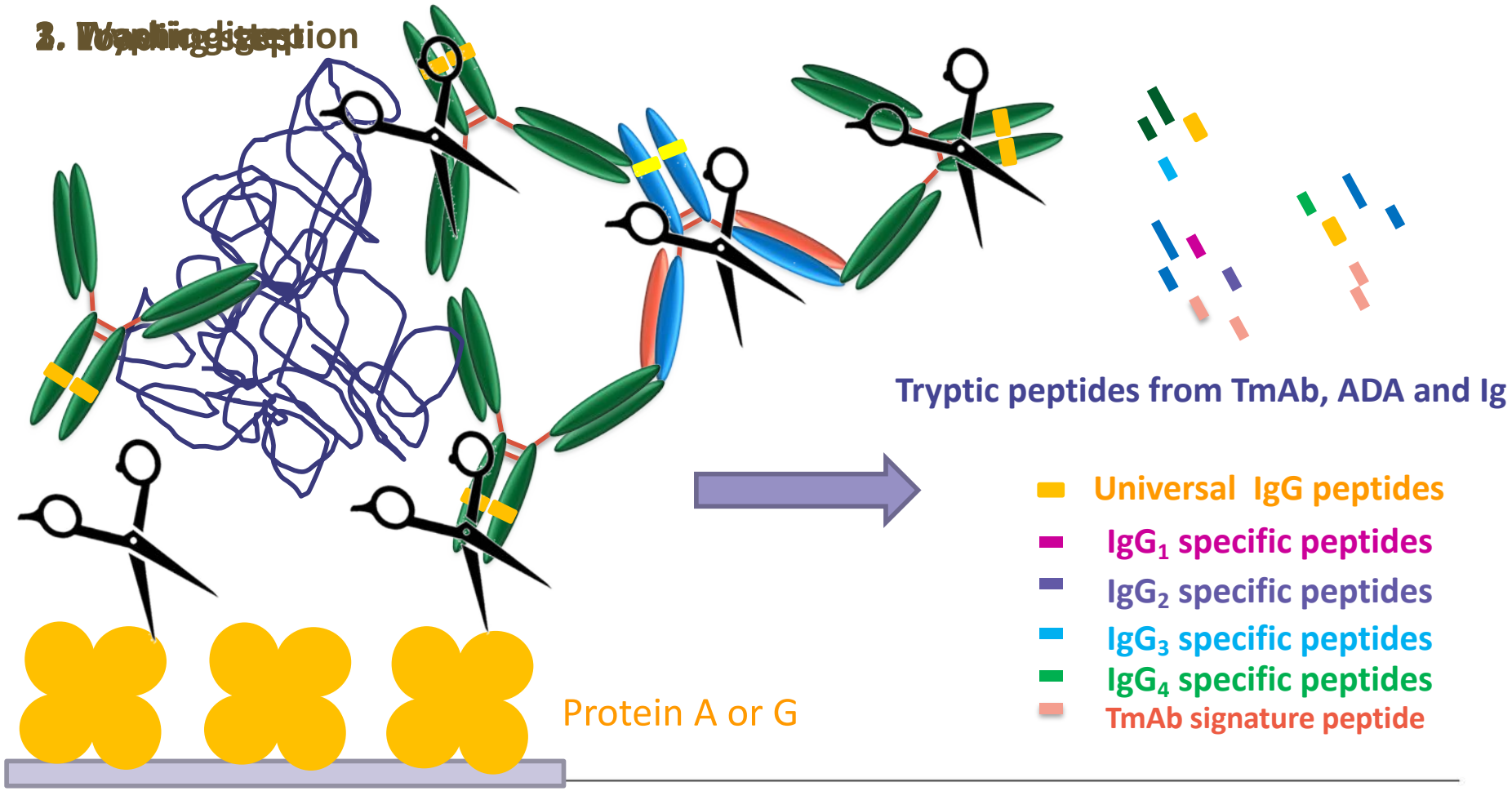
Immuno-capture step carried out using *biotinyl-TmAb target*.

- **True quantification of TmAb (mAb h-IgG1) through signature peptide signal → active form of TmAb**
- **Some ADA detected as IgG1 and IgG2 isotypes**
- **Quantification of ADA possible through their specific peptides**
- **Evaluation of ADA/TmAb ratio**

Efficient sample preparation 1/2

Neutralized TmAb form (on unretained fraction*)

2nd immunocapture to purify matrix



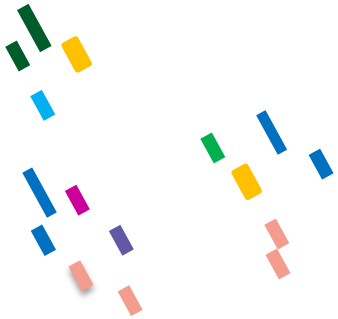
* Conserved fraction from 1rst immunocapture

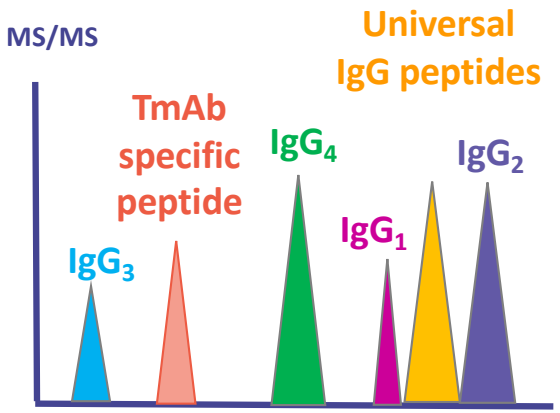
Efficient sample preparation 2/2

Neutralized TmAb form

2nd immunocapture
(on unretained fraction)

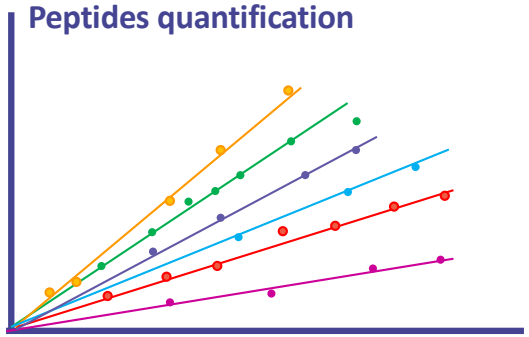
4. LC-HRMS analyse





Tryptic peptides from TmAb and ADA

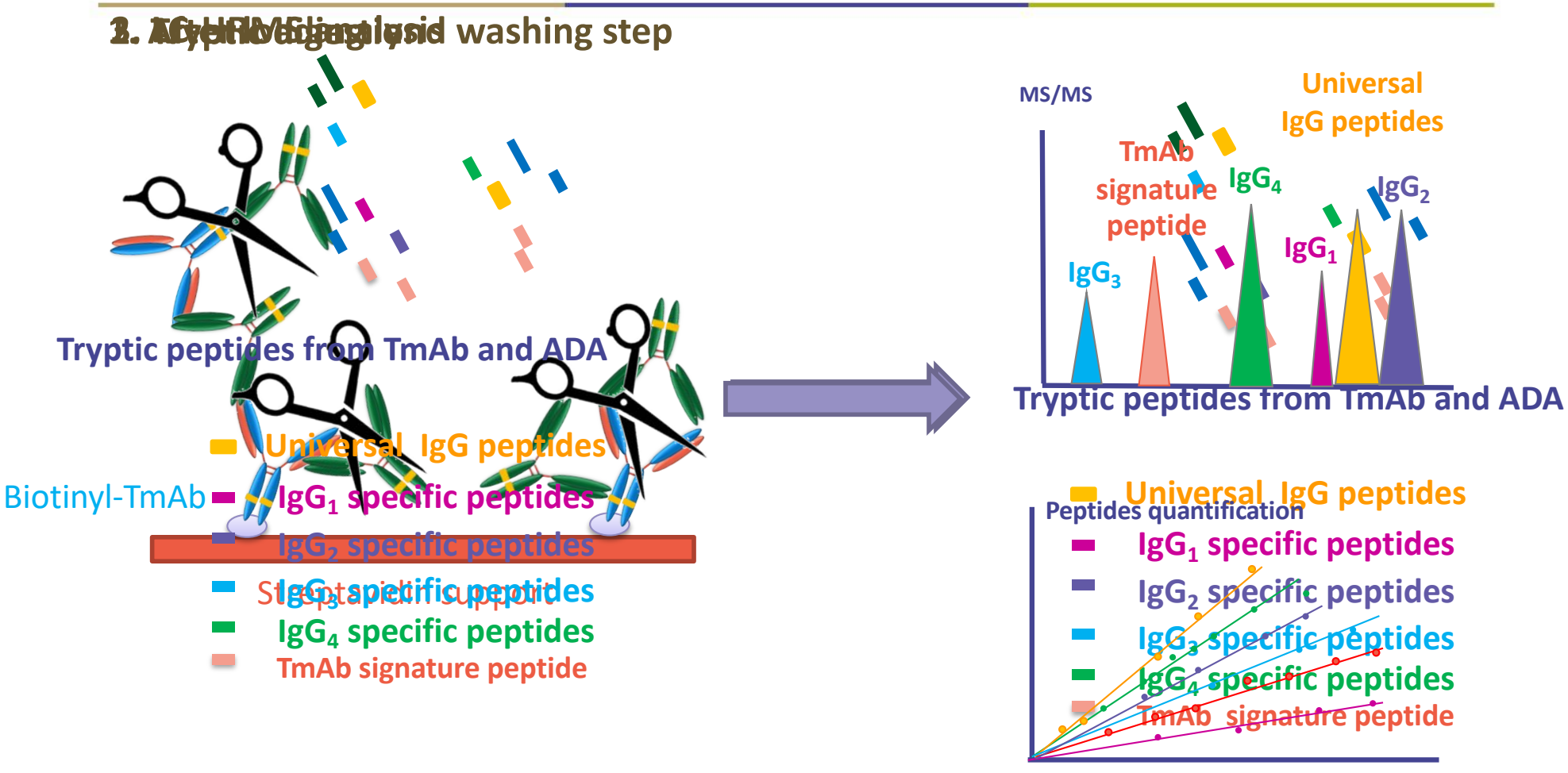
- Universal IgG peptides
- IgG_1 specific peptides
- IgG_2 specific peptides
- IgG_3 specific peptides
- IgG_4 specific peptides
- TmAb signature peptide



Concentration of neutralized TmAb

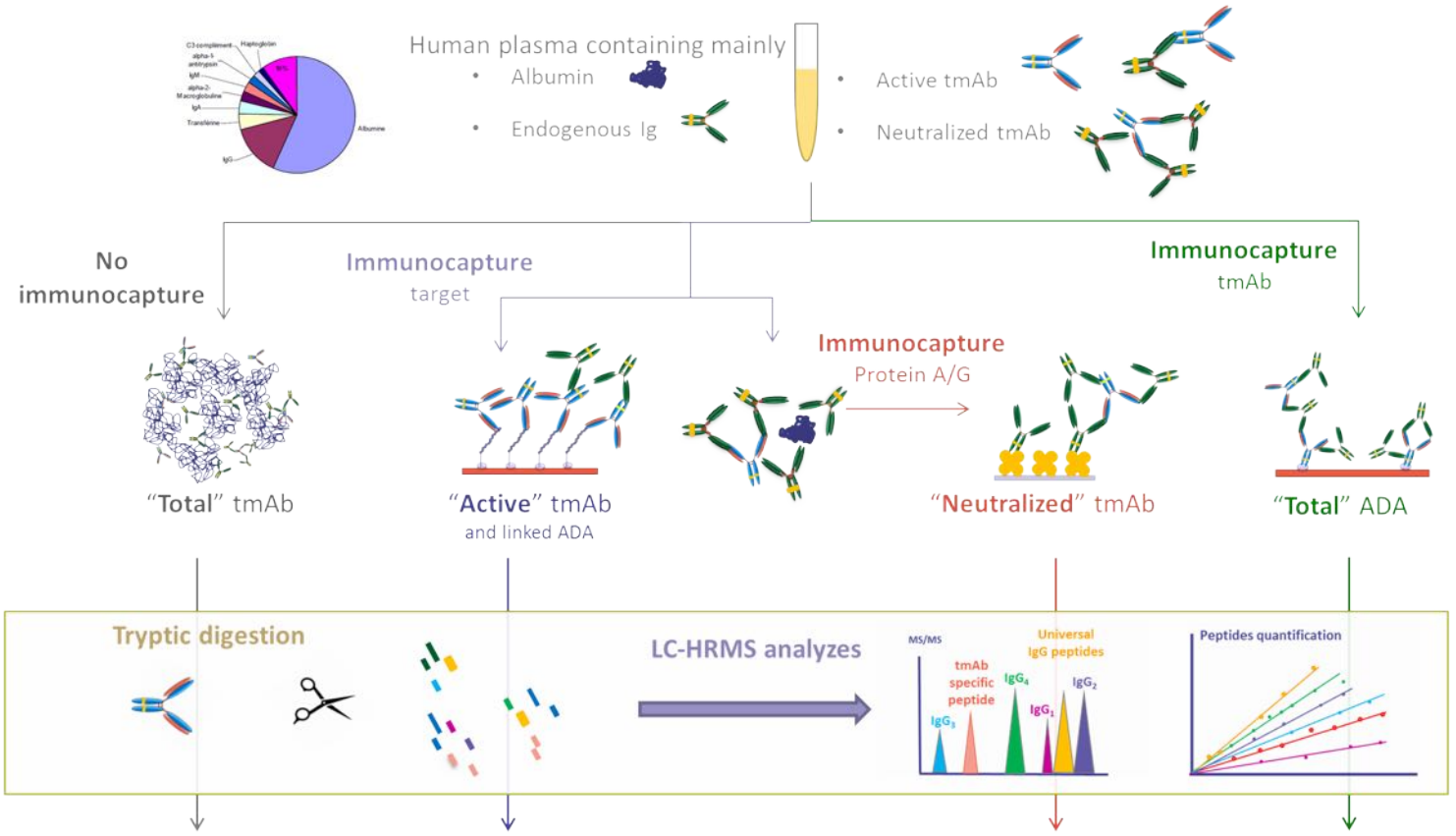
ADA analysis

Total ADA

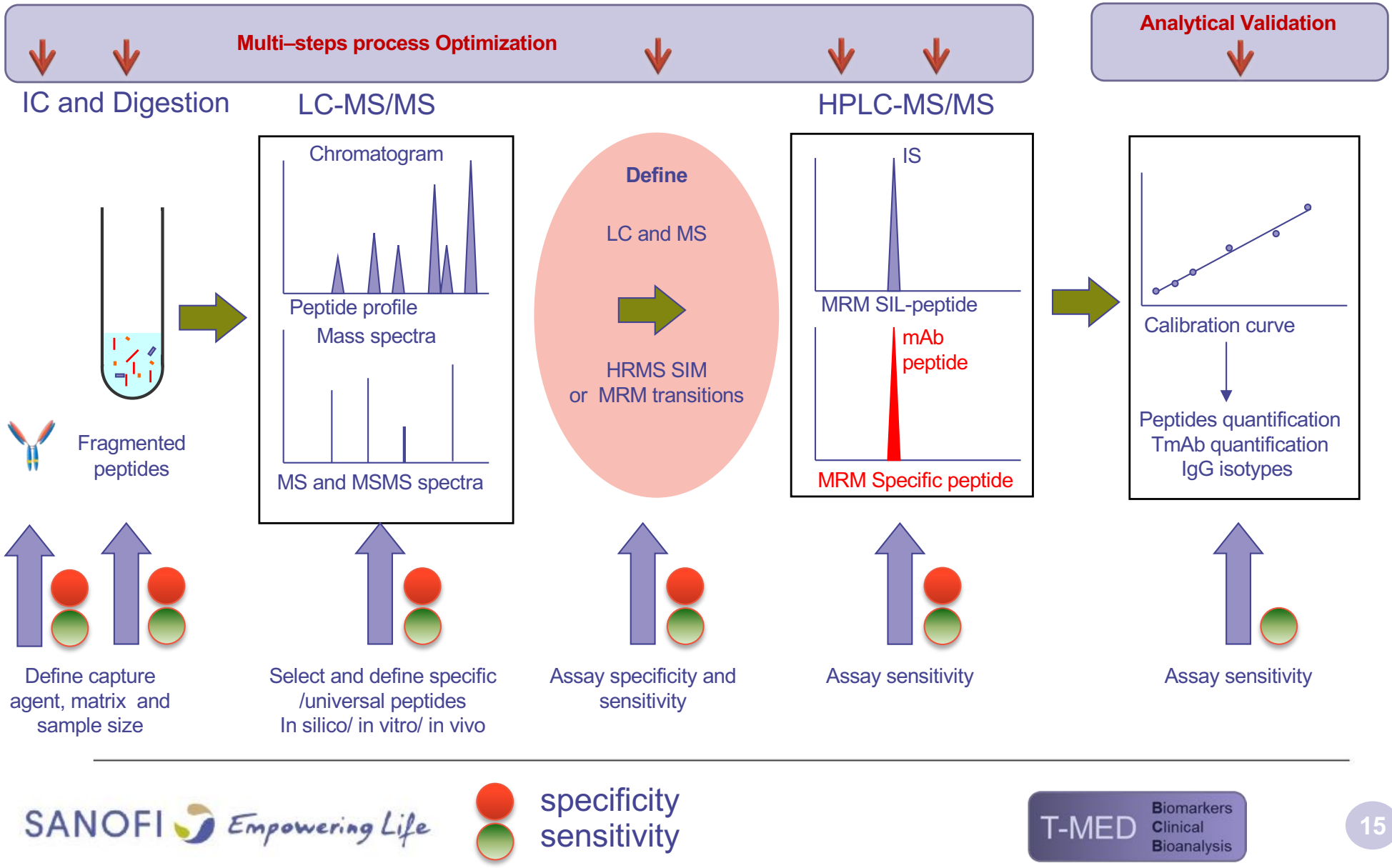


Total ADA + isotype

Summary of IC-LC-MS/MS for PK and Immunogenicity assessment



Optimization of IC-LC-MS/MS analysis: Many steps to explore



Conclusions and perspectives

- Combination of immunocapture and LC-HRMS is not a substitute to ELISA assays but give additional information for both pharmacokinetics and immunogenicity.
- Quantification of total, active and neutralized forms of TmAb is of high interest for PK/PD evaluation.
- Possible and easy ADA characterization and isotype (non neutralizing forms mainly)
- IC-LC-MS/MS is an usable tool to obtain additional information for clinical development of biotherapeutics.

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