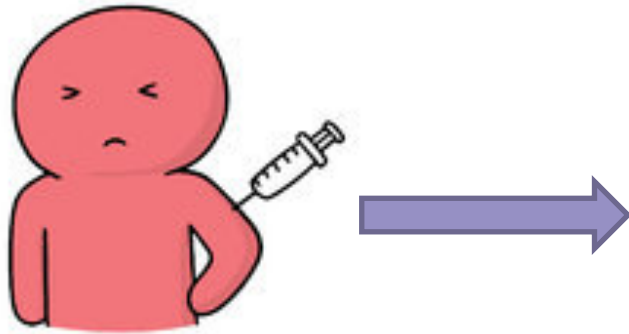


Innovative combination of ImmunoCapture (IC),
Liquid Chromatography and High Resolution Mass Spectrometry
(IC-LC-HRMS) approaches for the quantitative analysis of
therapeutic monoclonal antibody (tmAb), and simultaneous
characterization of Anti-Drug Antibodies (ADA) populations,
in clinical trials.

Pauline Bros, PhD – *Montpellier, France*



Injection of exogenous protein (i.e., therapeutic monoclonal antibody, protein, peptides...)

Immunogenicity

- Property of an exogenous substance to provoke a natural immune response when introduced into an organism, leading to the formation of anti-substance antibodies.
- Response is dependent of the individuals and the injected product.
 - Wanted → Vaccine
 - Unwanted → **Anti-Drug Antibodies**

Immunogenicity observed in human is of major safety concern



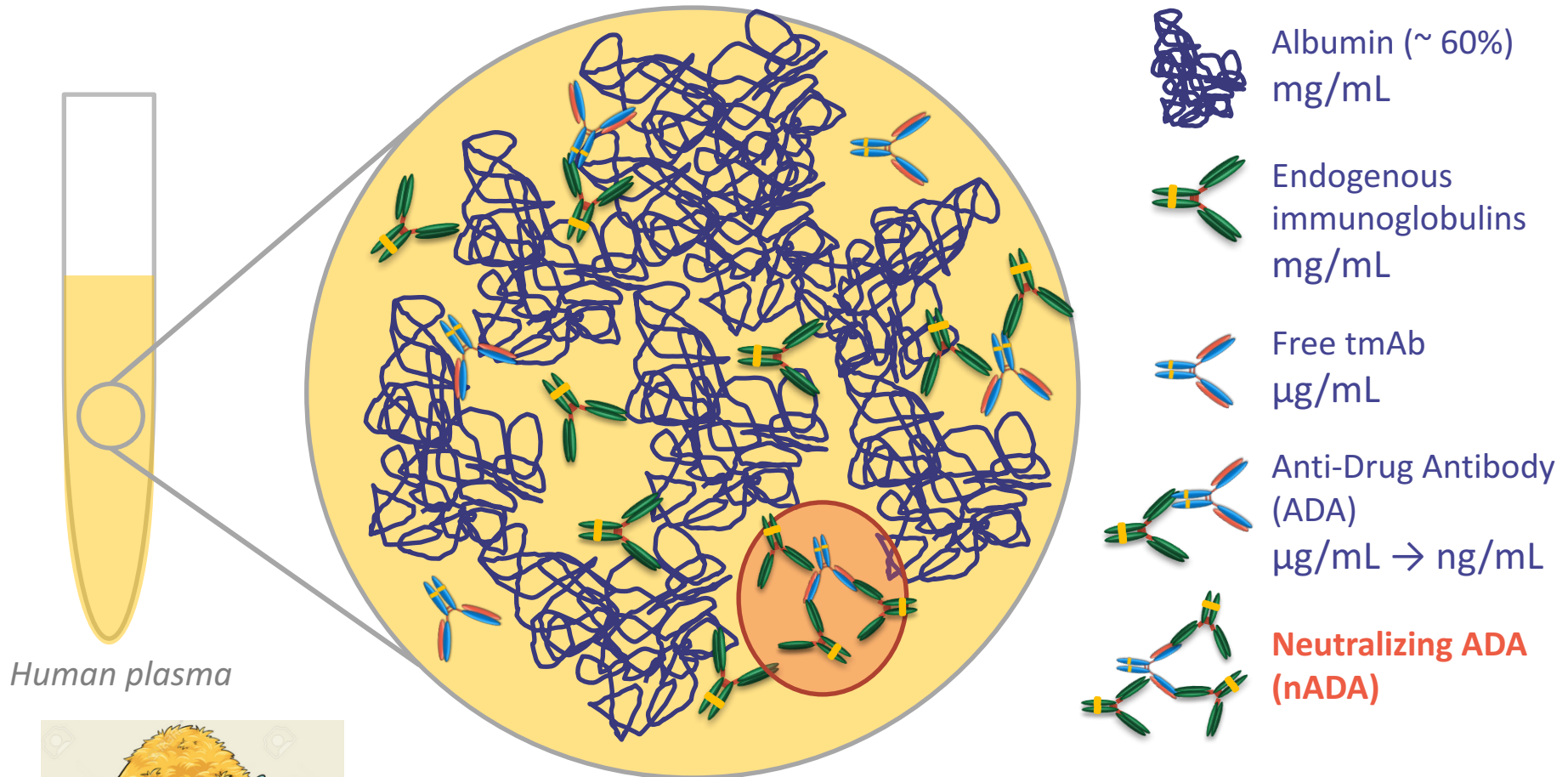
Efficacy

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

Safety



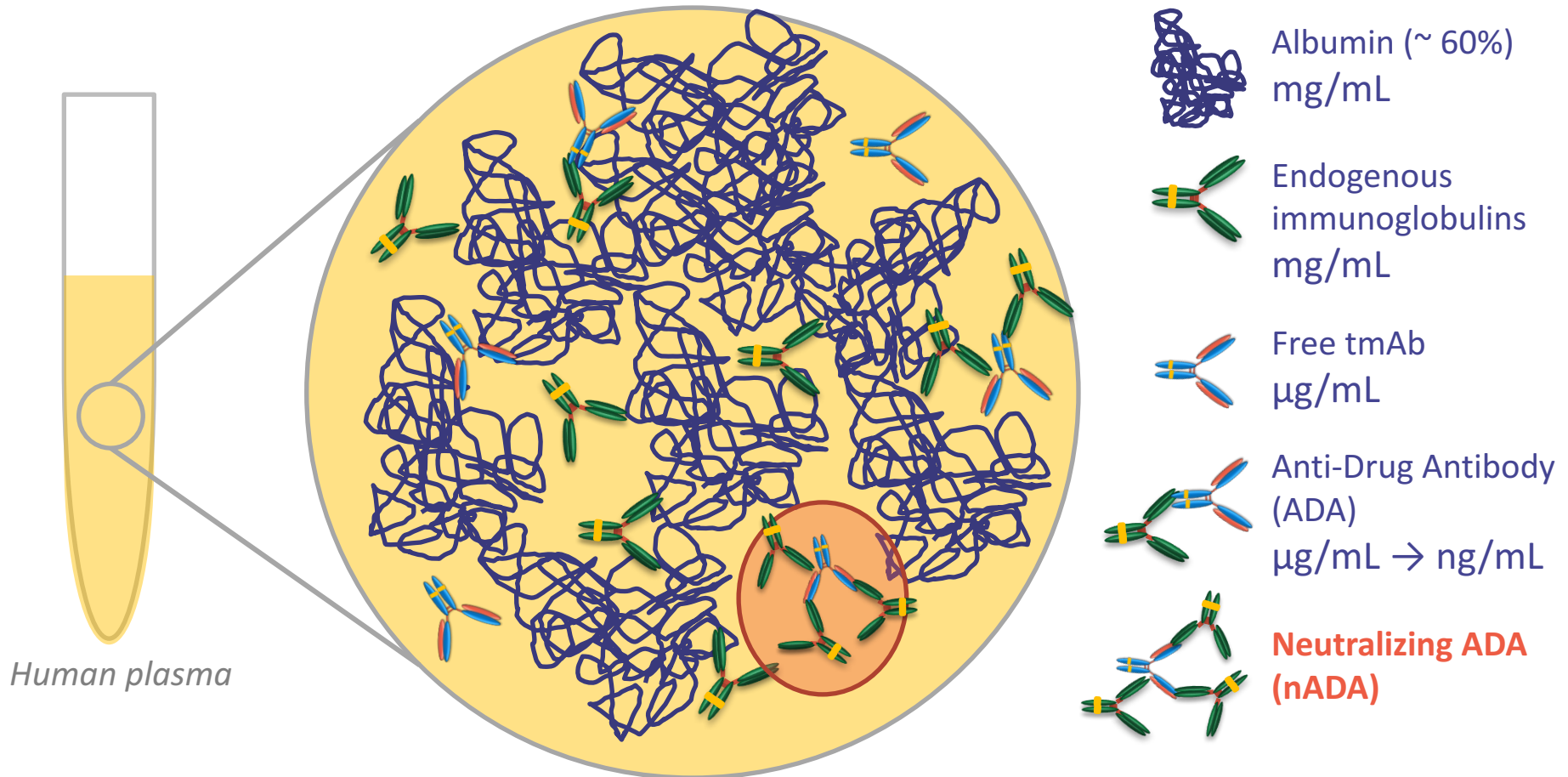
- Immunologic reaction
- Severe loss of treatment efficacy
- Cross reactivity with endogenous compound
- Induced autoimmune reaction



Human plasma



nADA = a needle in a haystack
Ratio ~ 1/1 000 000
LoQ to reach for nADA ~ 100 ng/mL

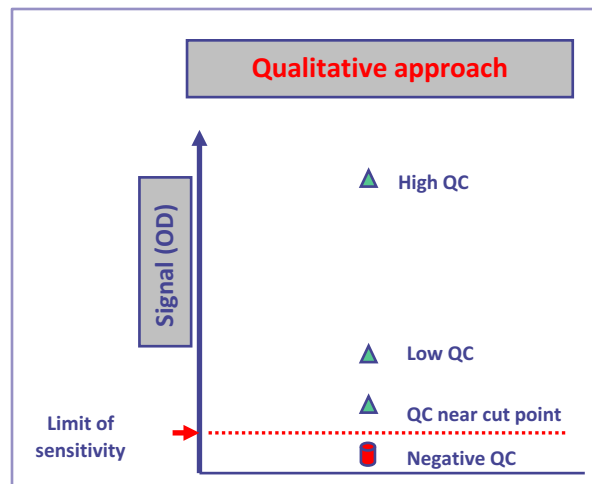
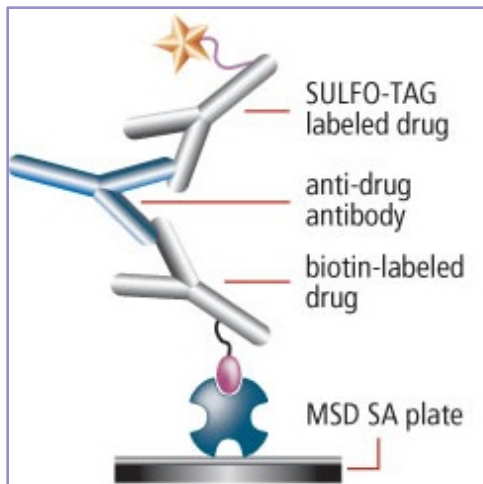


Efficient sample preparation and sensitive/specific analysis method are crucial!

Monitoring immunogenicity by ELISA

Mainly Bridging ELISA (with or without acid dissociation)

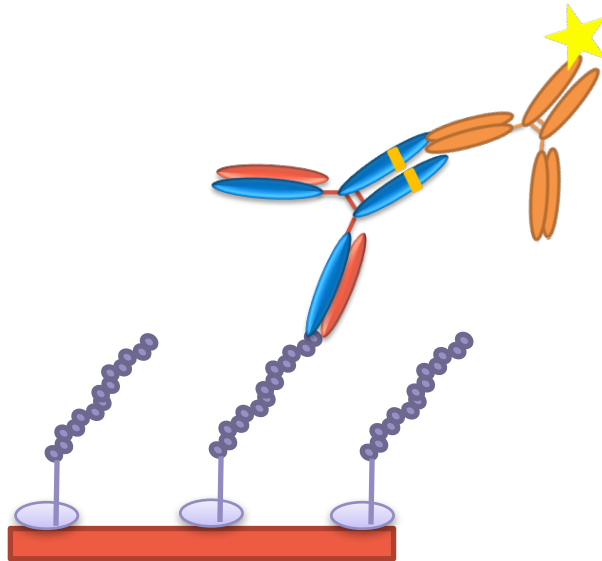
- *Qualitative detection of ADA based on Cut-point approach*
 - 1st step : Screening test = detect positive/negative ADA*
 - 2nd step : Confirmatory test = competition approach with excess of drug*
 - 3rd step : Titration = serial dilution of sample to reach cut-point*
 - 4th step : Neutralizing ADA (CBA or LBA)*
- *Qualitative approach – No “gold” standard (Rabbit polyclonal or monoclonal ADA is only “representative”)*
- *Possible interferences in ADA assay*



ADA interferences in ELISA

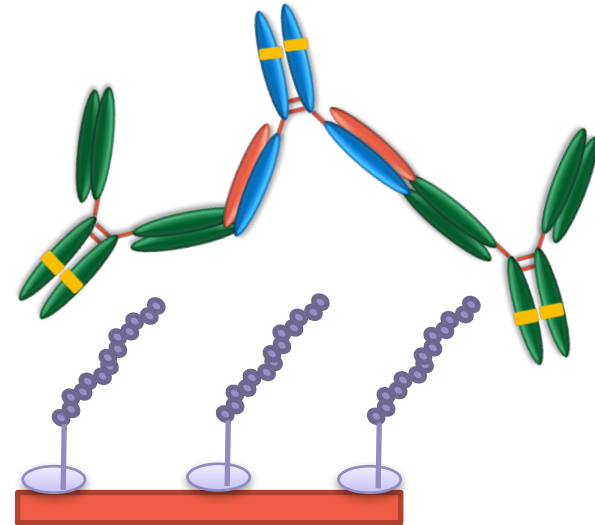
1/2

1st case: ideally one!



Real active tmAb concentration

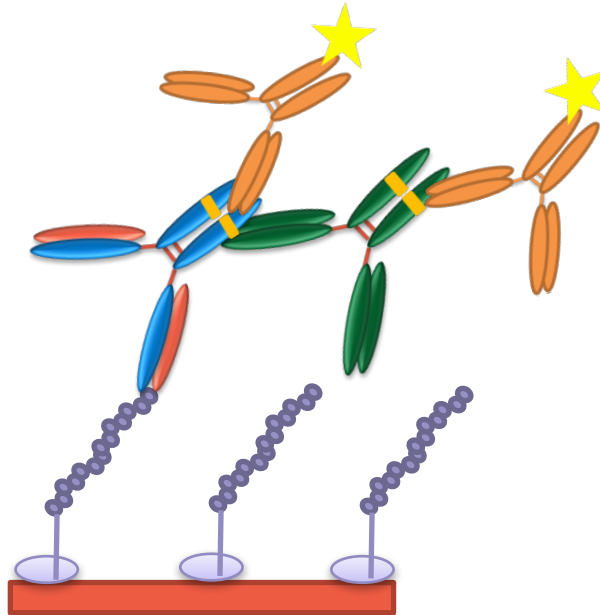
2nd case: presence of nADA



Underestimation of tmAb concentration

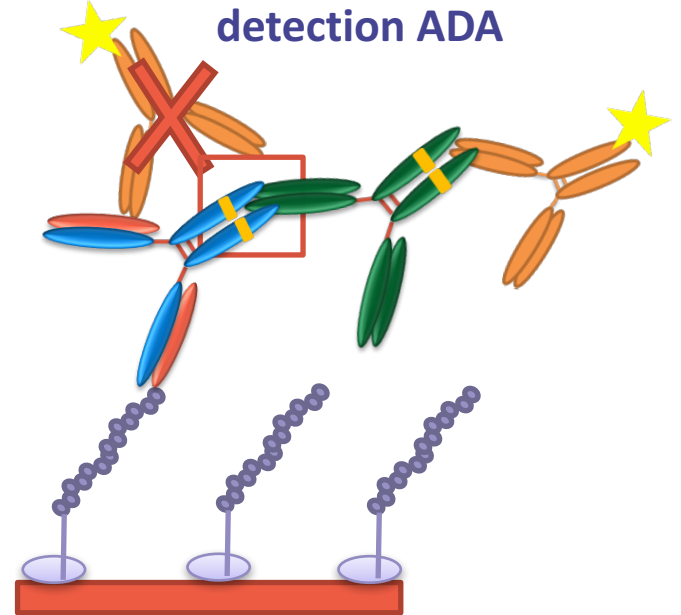
tmAb: therapeutic monoclonal antibody

3rd case: presence of ADA



Possible overestimation of tmAb concentration

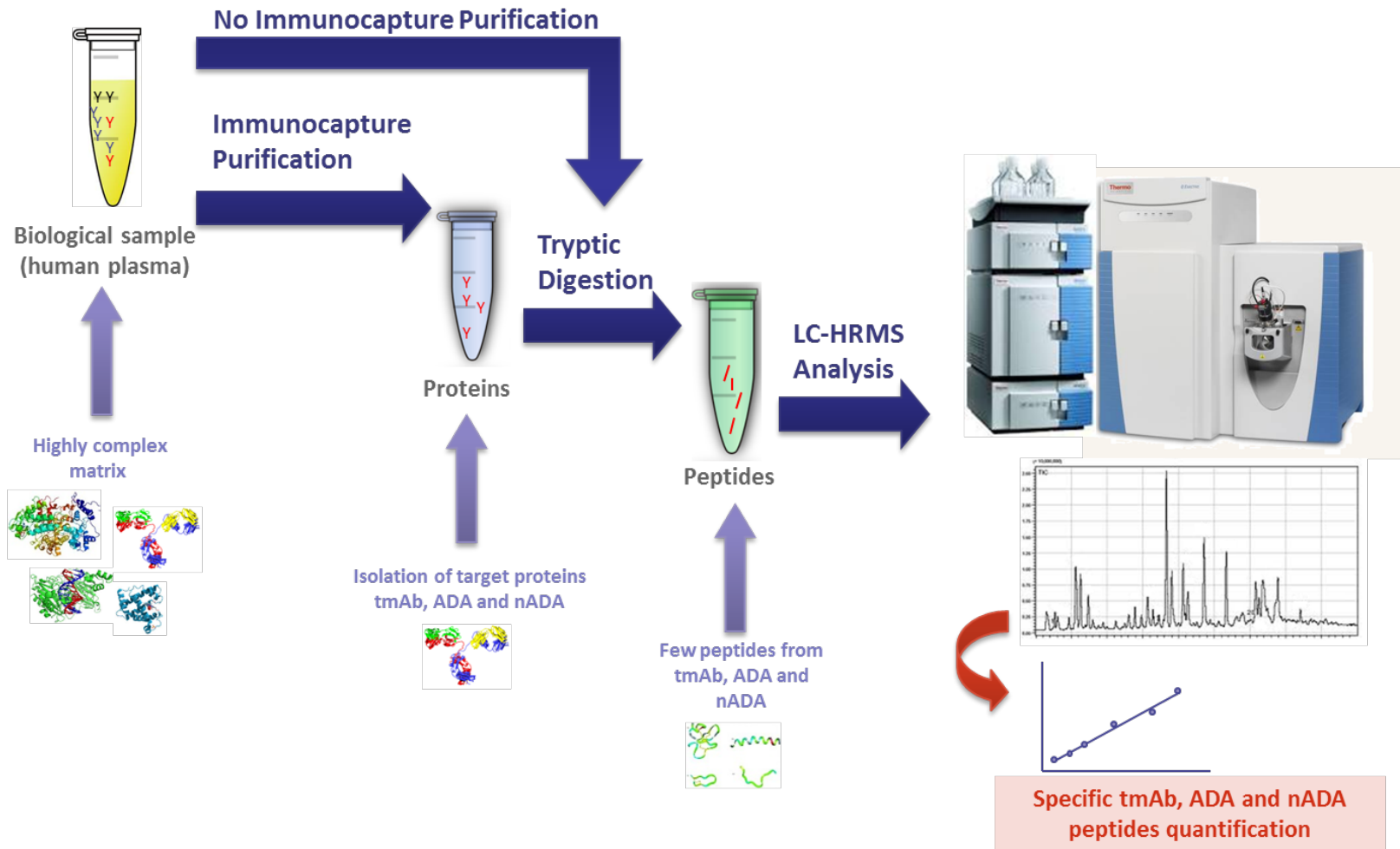
4th case: presence of masking detection ADA



Jeopardized/Hazardous estimation of tmAb concentration

tmAb: therapeutic monoclonal antibody

LC-HRMS analytical process strategy



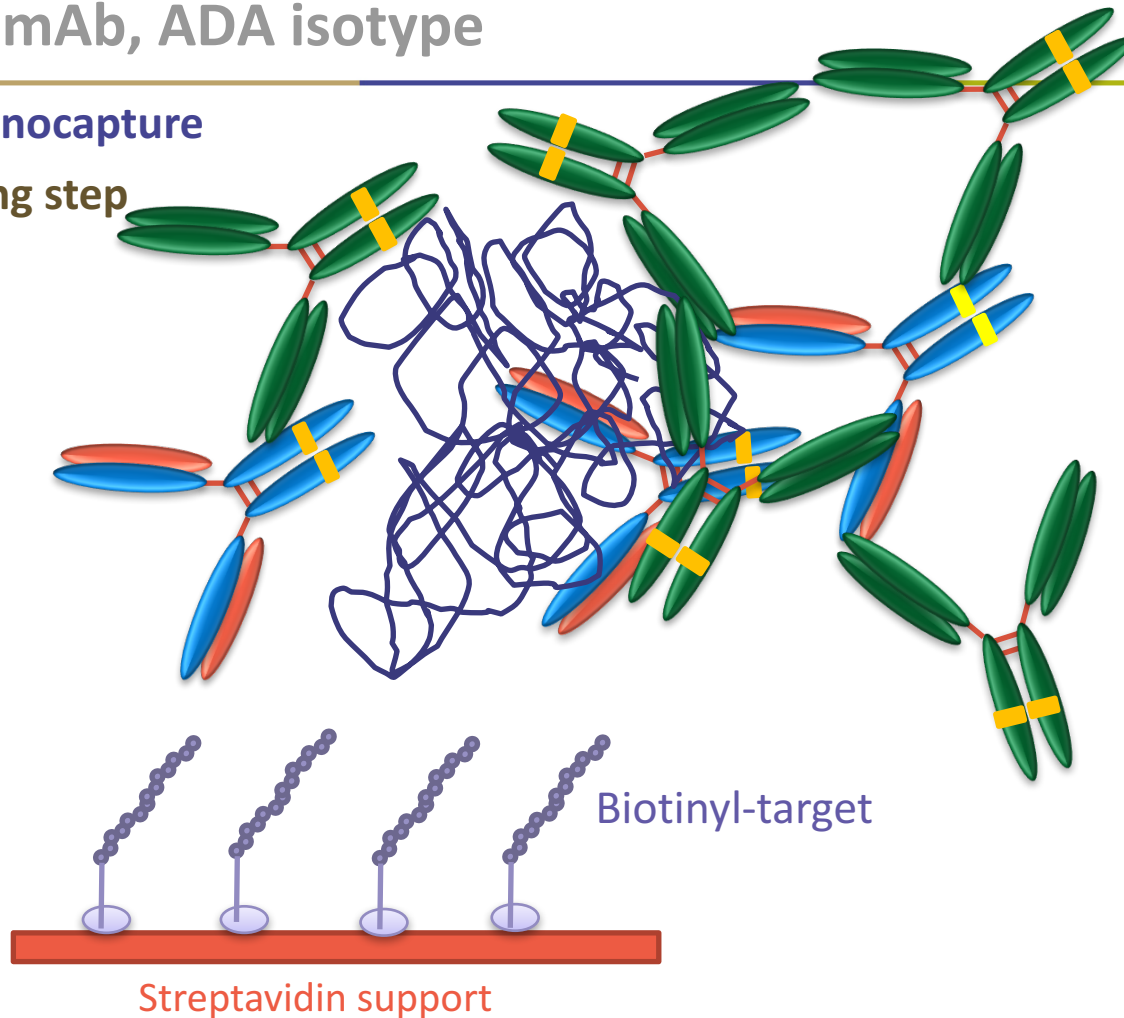
Efficient sample preparation

1/4

Active tmAb, ADA isotype

1st immunocapture

1. Loading step



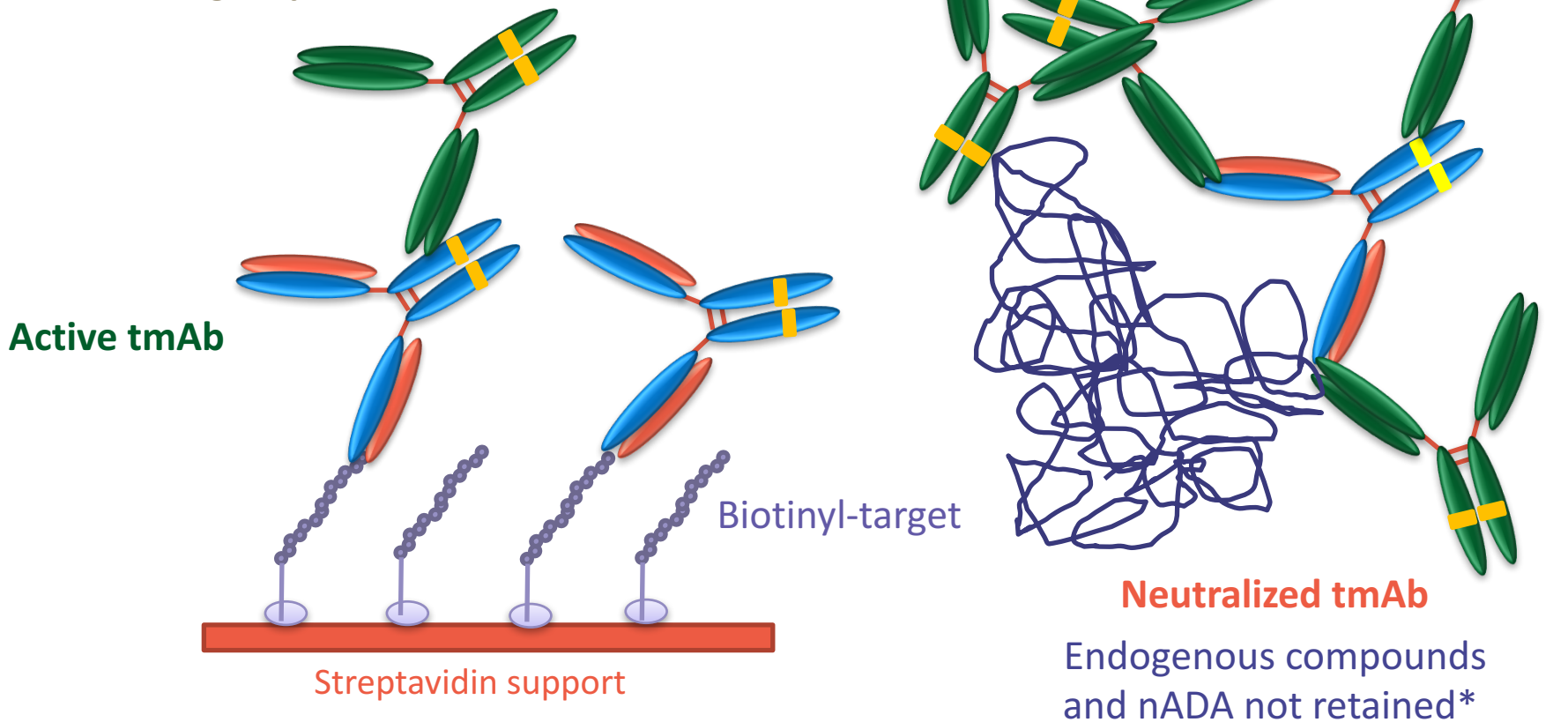
Efficient sample preparation

1/4

Active tmAb, ADA isotype

1st immunocapture

2. Washing step



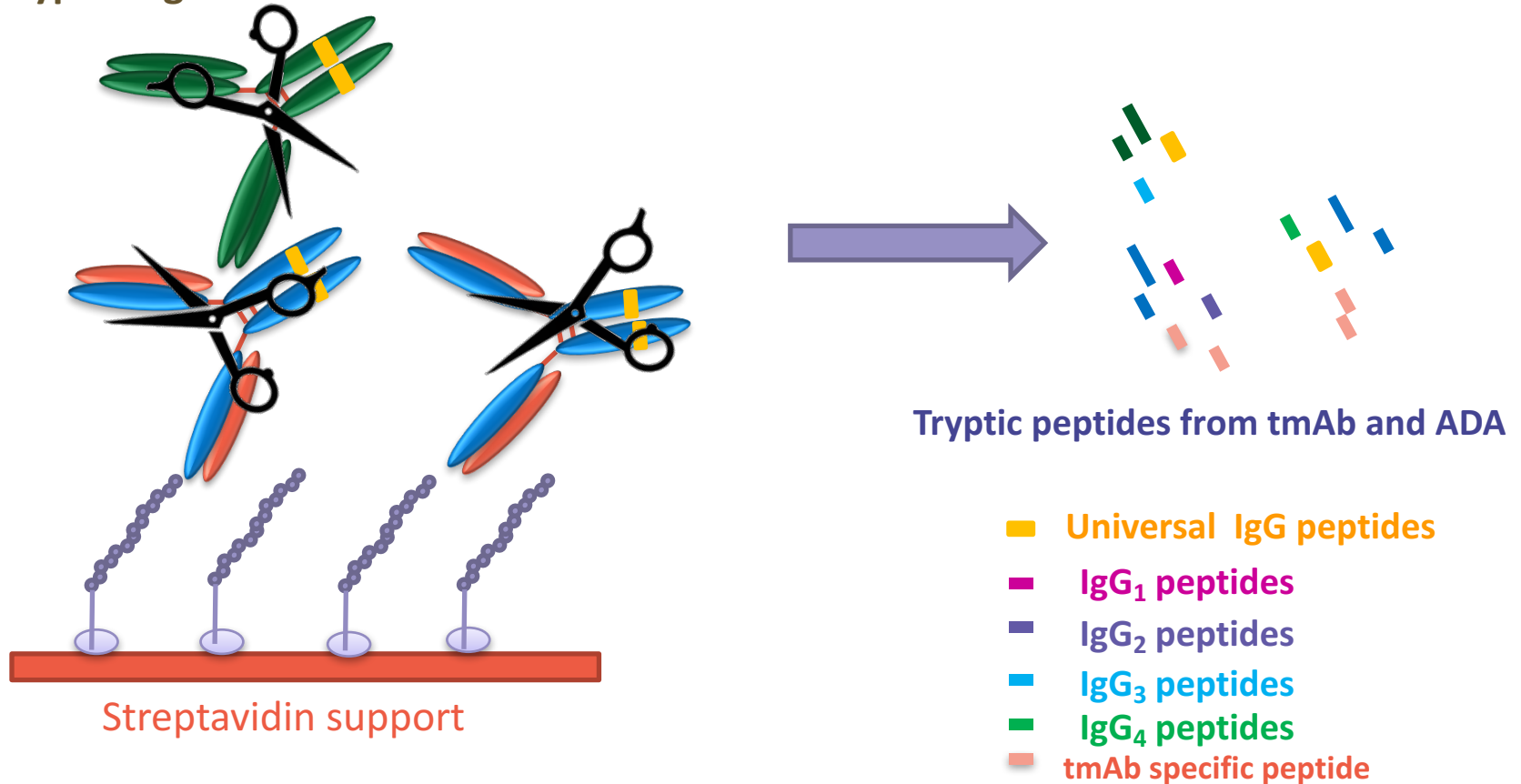
Efficient sample preparation

Active tmAb, ADA isotype

2/4

1st immunocapture

3. Tryptic digestion



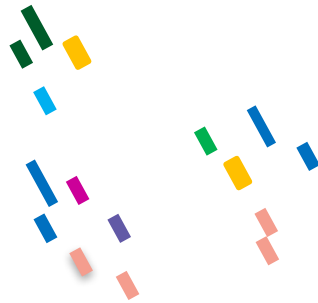
Efficient sample preparation

Active tmAb, ADA isotype

2/4

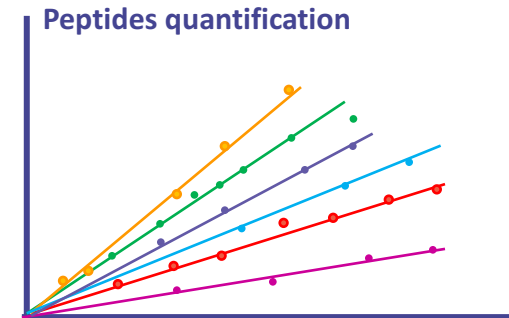
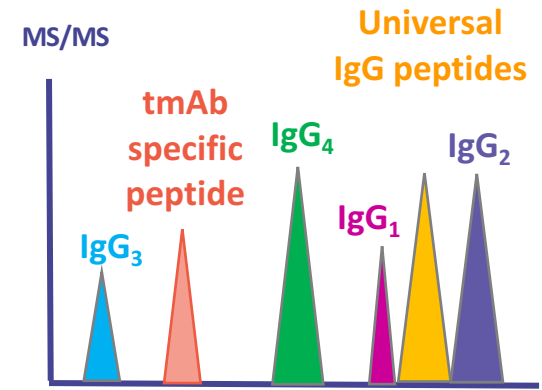
1st immunocapture

4. LC-HRMS analyse



Tryptic peptides from tmAb and ADA

- Universal IgG peptides
- IgG₁ peptides
- IgG₂ peptides
- IgG₃ peptides
- IgG₄ peptides
- tmAb specific peptide



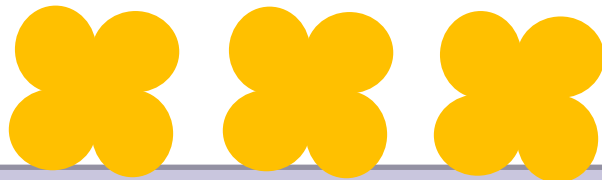
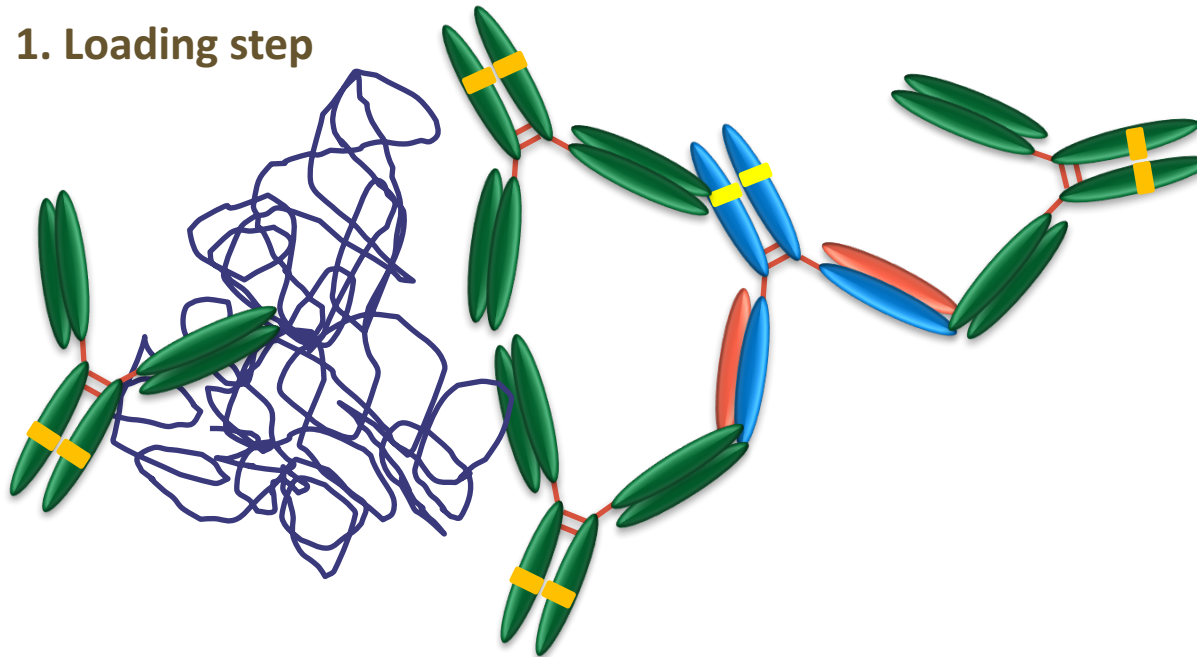
Efficient sample preparation

Neutralized tmAb

3/4

2nd immunocapture* to decomplex matrix
(on unrecovered fraction**)

1. Loading step



Protein A/G

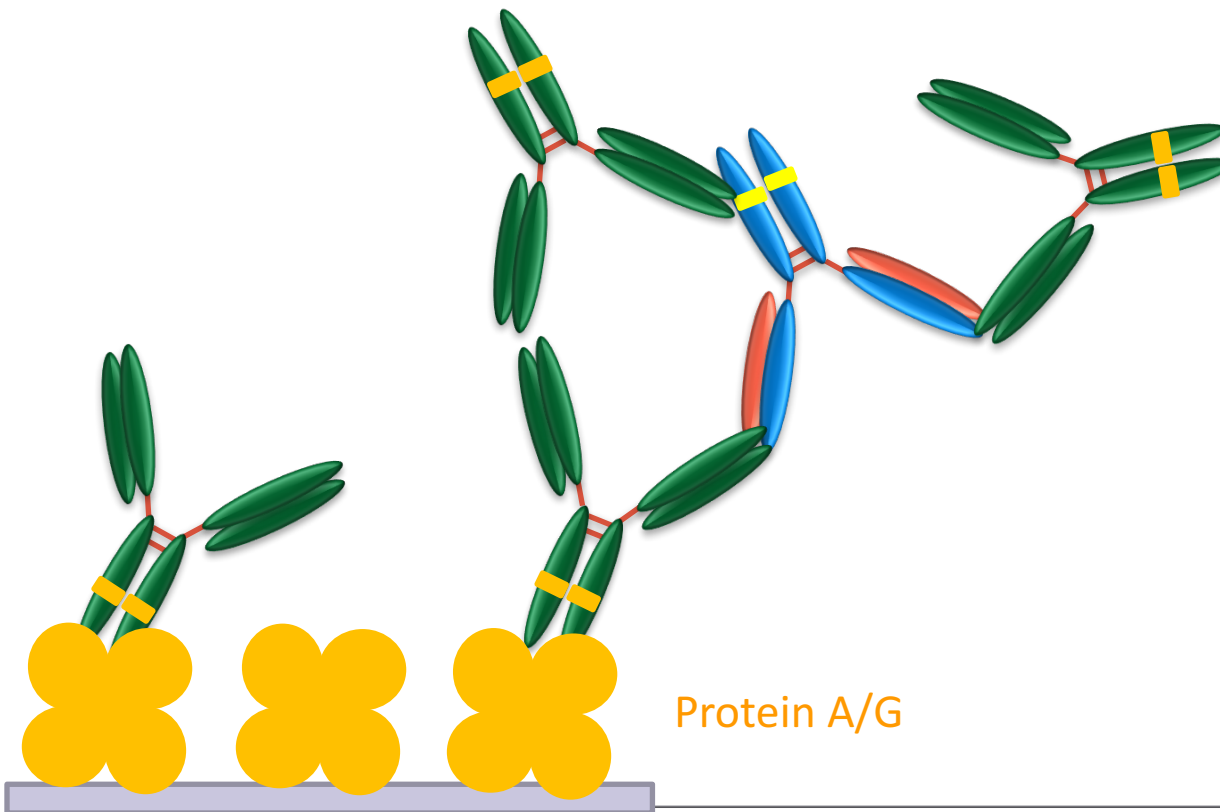
Efficient sample preparation

Neutralized tmAb

3/4

2nd immunocapture* to decomplex matrix
(on unretained fraction**)

2. Washing step



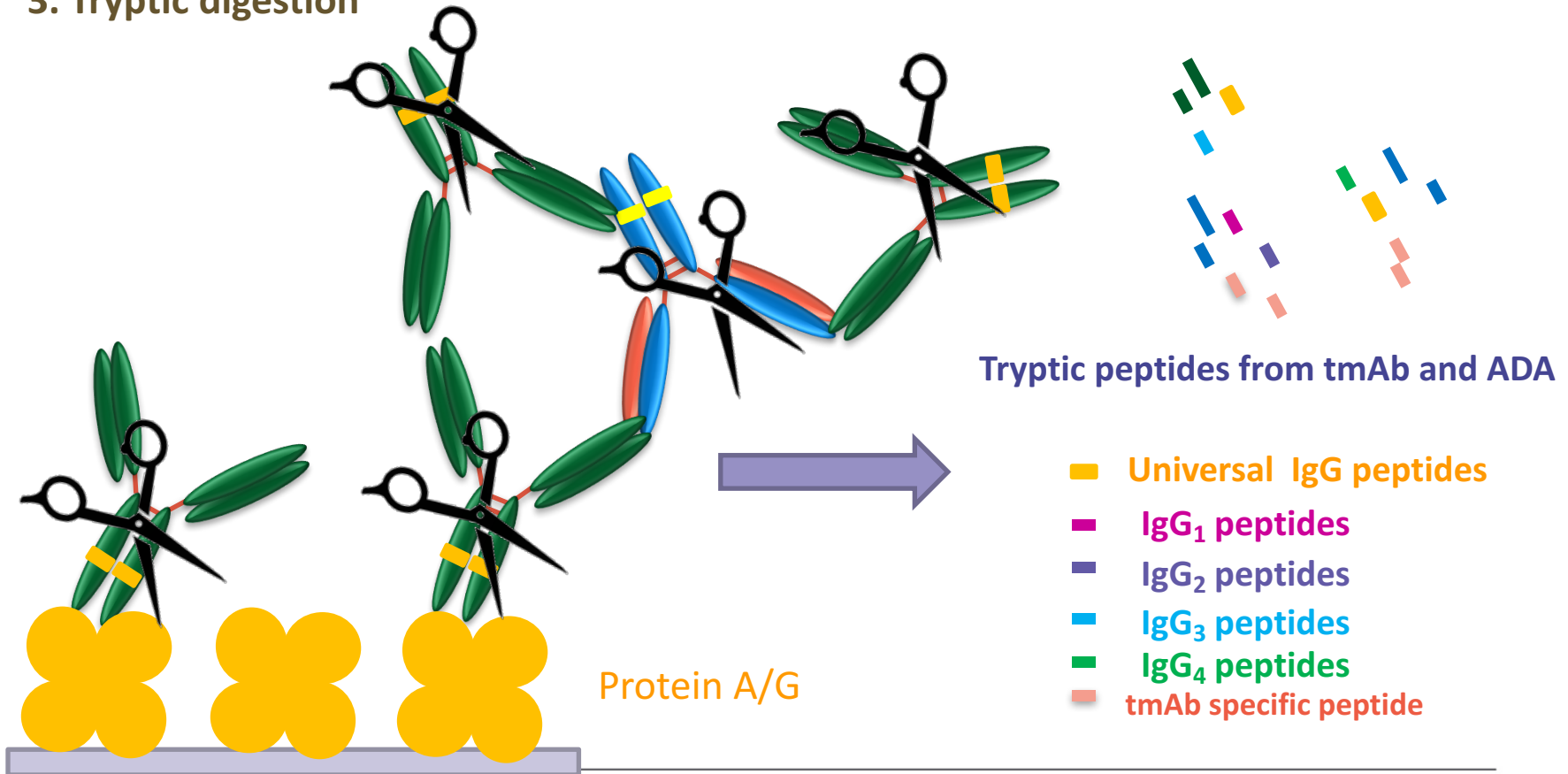
Efficient sample preparation

Neutralized tmAb

3/4

2nd immunocapture* to decomplex matrix
(on unretained fraction**)

3. Tryptic digestion



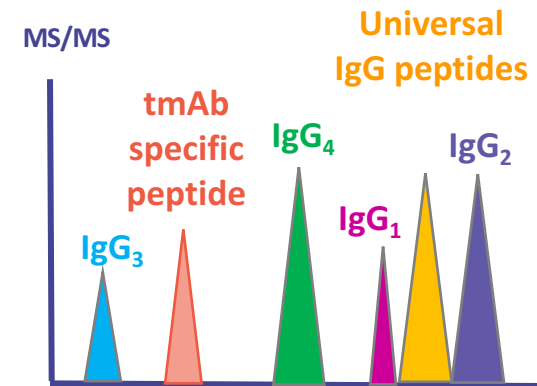
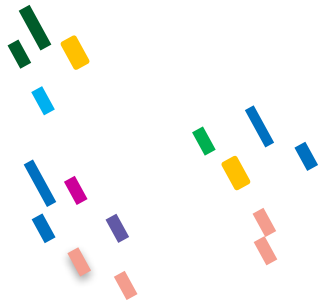
Efficient sample preparation

Neutralized tmAb

4/4

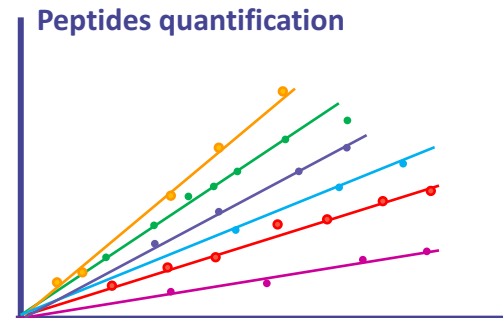
2nd immunocapture
(on unretained fraction)

4. LC-HRMS analyse



Tryptic peptides from tmAb and ADA

- Universal IgG peptides
- IgG₁ peptides
- IgG₂ peptides
- IgG₃ peptides
- IgG₄ peptides
- tmAb specific peptide



Concentration of neutralized tmAb

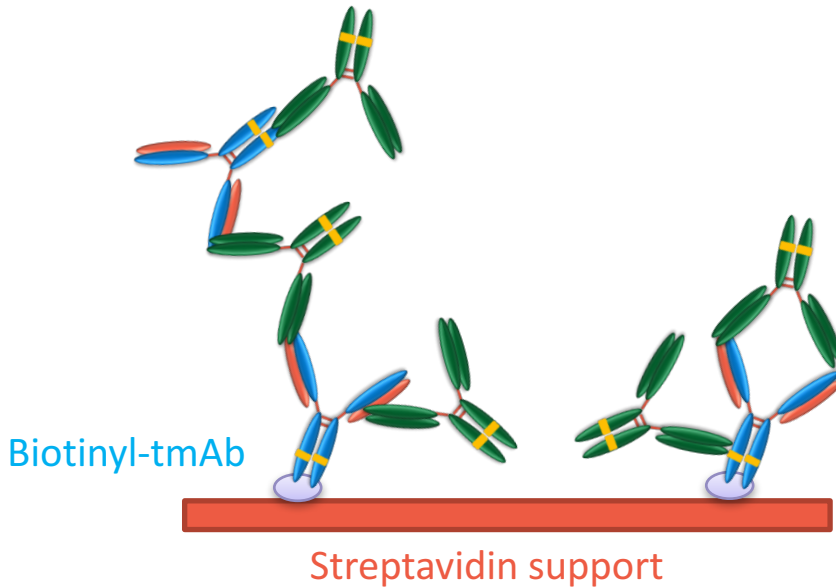
ADA isotype

Total ADA

1. After loading and washing step

For samples :

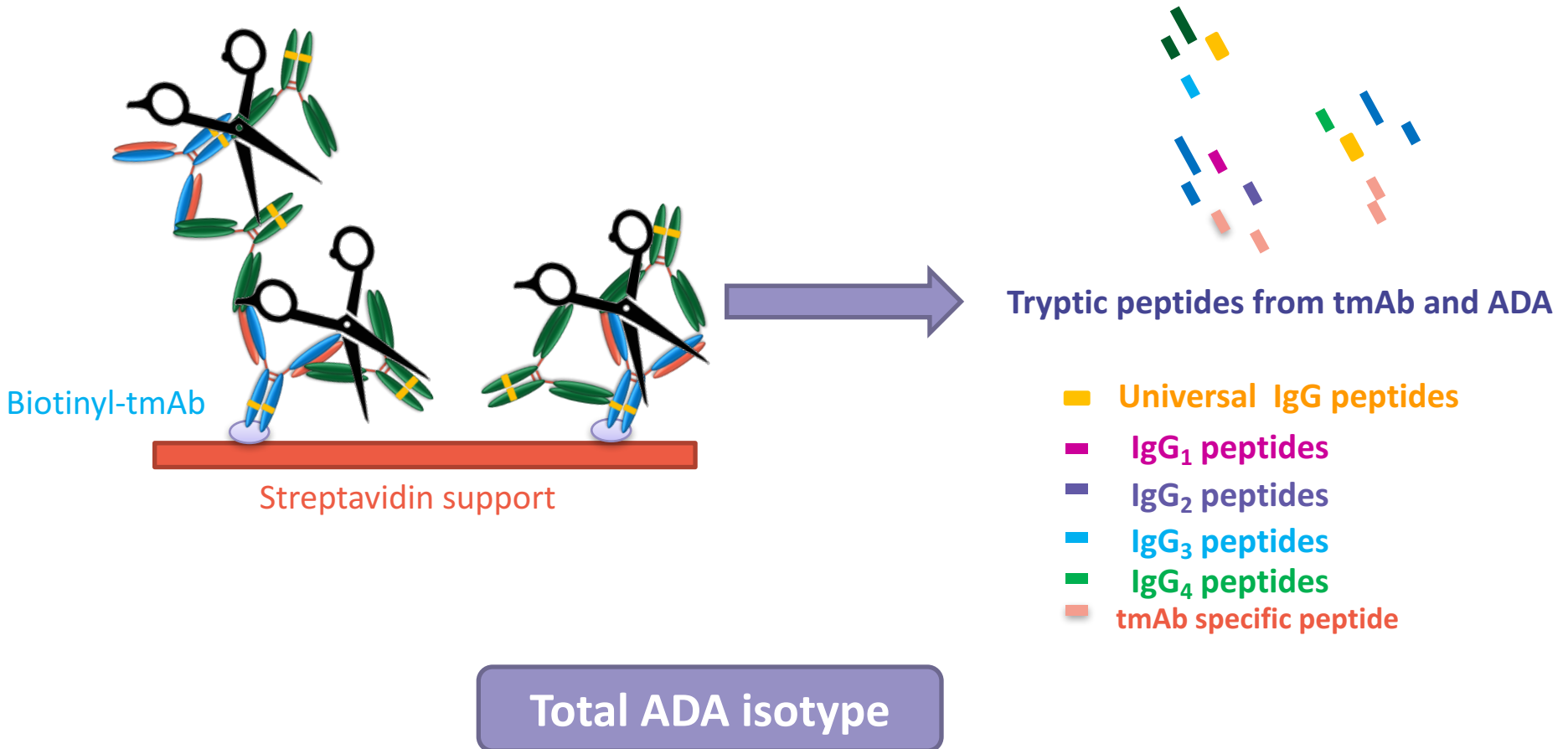
- With tmAb
- Without tmAb in the case of late collected sample with total tmAb < LLOQ



ADA isotype

Total ADA

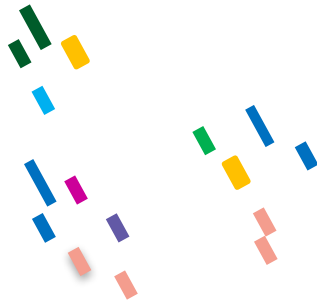
2. Tryptic digestion



ADA isotype

Total ADA

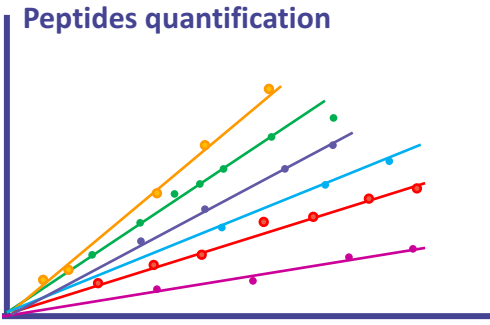
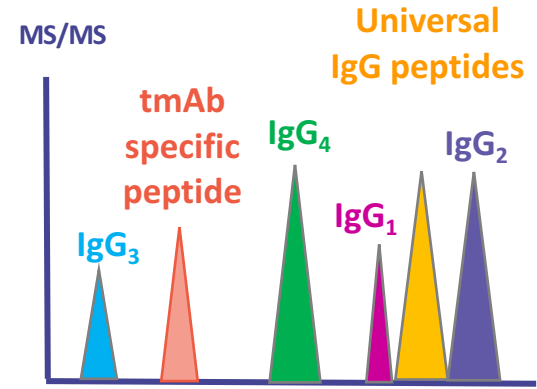
3. LC-HRMS analyse



Tryptic peptides from tmAb and ADA

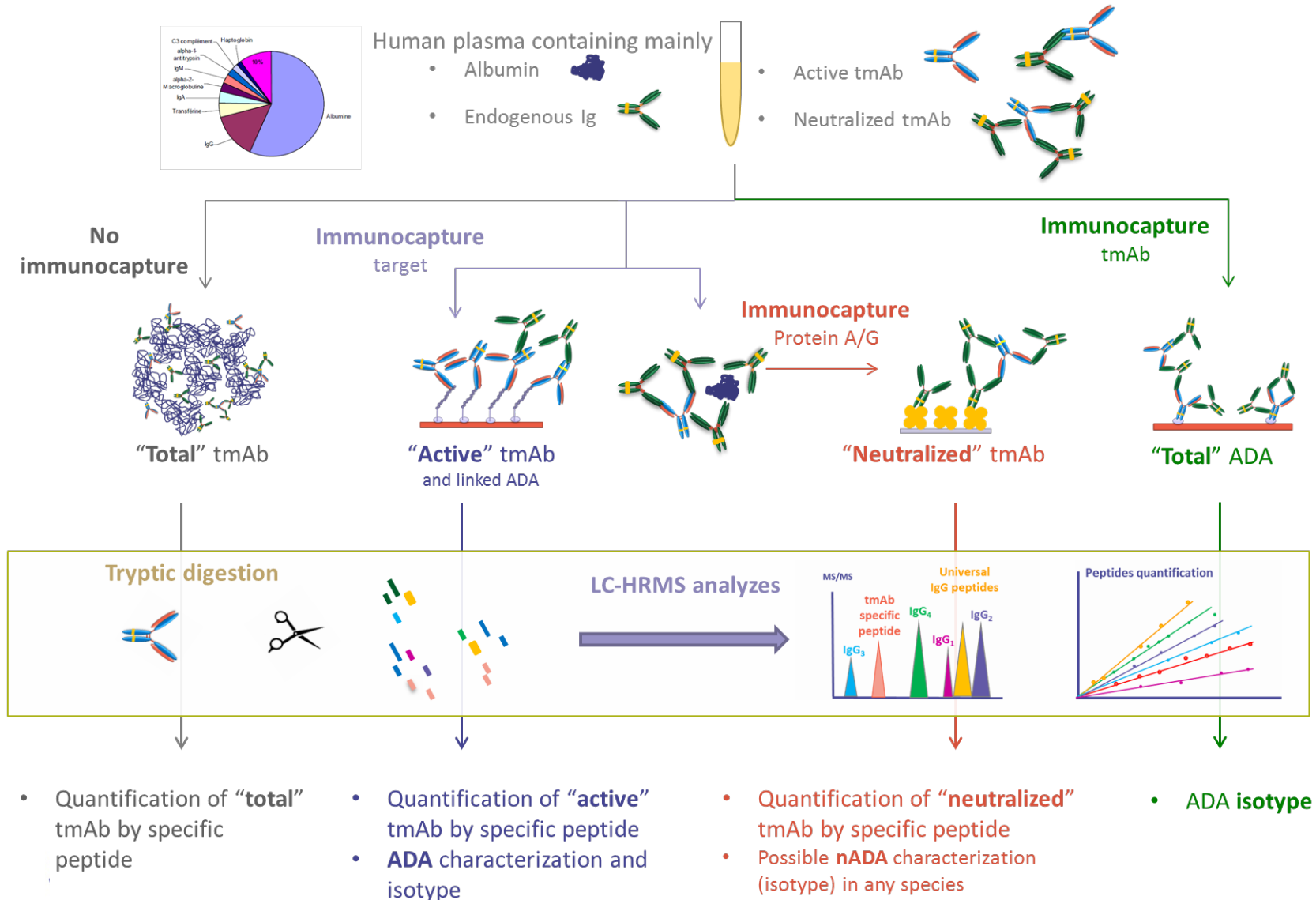
- Universal IgG peptides
- IgG₁ peptides
- IgG₂ peptides
- IgG₃ peptides
- IgG₄ peptides
- tmAb specific peptide

Total ADA isotype



Conclusions and perspectives

1/2



Innovative points of this strategy:

- Combination of immunocapture and LC-HRMS for immunogenicity concern
- Quantification of active and neutralized tmAb
- ADA characterization and isotype

Acknowledgements

Aline Cournut
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