Native peptide mapping

New method to monitor Higher Order Structure changes in a QC laboratory

Michel Degueldre – ASB PCMD1

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Providence, RI
USA

Inspired by patients.
Driven by science.

Native peptide mapping – Higher order structure (HOS) monitoring

1. Context

2. Development stage

    I. Feasibility assessment
    II. Case study I – IgG4 analysis and bioassay relationship

3. QC applicability

    I. Case study II – IgG1 analysis and bioassay relationship

4. Conclusions and perspective

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Importance of monitoring conformational changes

Sensitive to temperature, pH, Oxidative stress, agitation, light exposure,…

Changes in protein structure - Impact on :

stability, specificity and activity

“Stability indicating” methods

= Pool of physico/chemical & bioassay methods

Regulatory requirement

May be a risk for the patient ?

Analytical and structural characterization package for mAbs

Higher-order structure

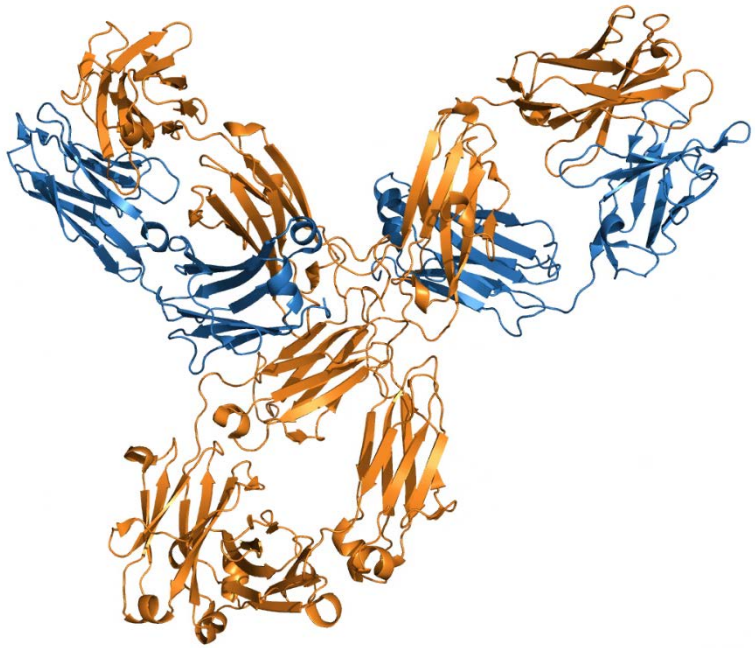
- *Circular Dichroism*
- X-Ray Structure
- *NMR*
- Epitope Detection
- Specific Binding
- *FTIR*

Assay

- OD, HPLC, AAA, Biacore, ELISA, IFMA, Bradford, Lowry, Bioassay

Activity

- Bioassay in vivo and in vitro
- Specific binding assay
- Gene reporter assay (immunogenicity)



Size & Aggregation

- *SE-HPLC* (also identity and assay)
- SDS PAGE / Bioanalyser
- *AUC*
- AF4
- LLS

Purity

- RP-HPLC
- SE-HPLC
- Peptide mapping
- SDS-PAGE
- Field Flow Fractionation
- Elisa (HCP)
- Immunoblot
- DNA assay
- LAL test
- Virus test

Structure / Sequence

- N- and C-terminus
- Amino Acid Analysis
- Peptide Mapping and Sequencing
- Monosaccharide Analysis
- Oligosaccharide Mapping
- Mass Spectrometry
- *Disulphide linkage*

Identity

- N-Terminal Sequence
- Peptide Mapping
- Specific Bioassay
- IEF
- HPLC

Carbohydrate analysis

- ESI-MS (whole molecule)
- MALDI-TOF (released carboh.)
- Separation of labelled released carbohydrates (2-AA, 2-AB)

Surface charge

- IEF
- CZE
- **IEX-HPLC**
- iCE280
- Chromatofocusing

From characterization lab to QC lab…

Current situation…

Characterization

- Circular dichroism
- Ion mobility
- X-Ray structure
- NMR
- H/DX
- SPR
- FTIR

><

QC requirements

- Affordable equipment cost
- Simple data interpretation – Yes or No
- Validated and robust methods designed for long-term use (>10 years)
- Generalist operators
- High throughput and speed essential

These current HOS monitoring methods are not easily amenable to a QC environment.

UCB proposal to monitor HOS in QC environment:
Native peptide mapping

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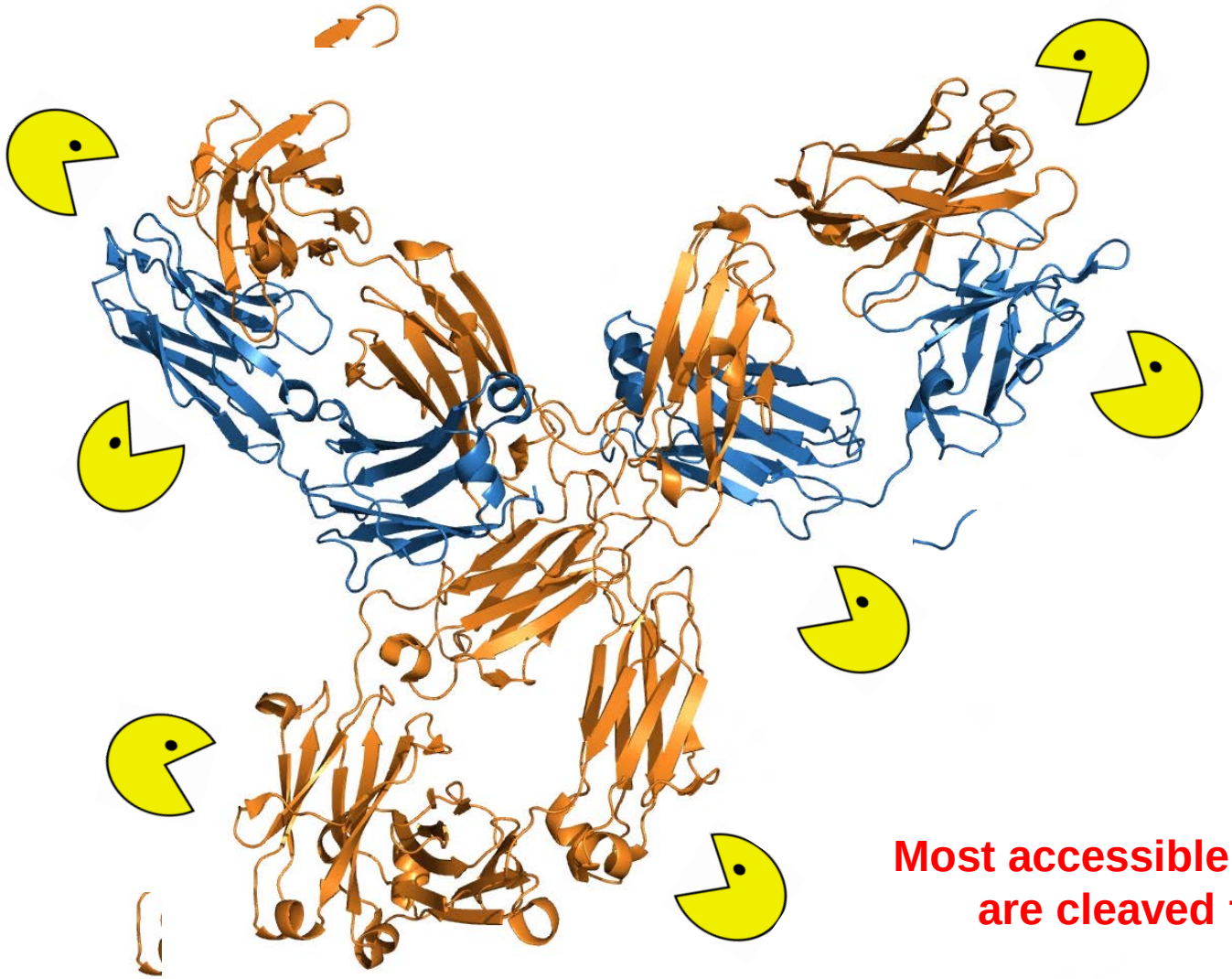
Method development – Native peptide mapping



X min

y min

z min

Most accessible peptides are cleaved first !

Trypsin enzyme – cleaves specifically after K and R residues

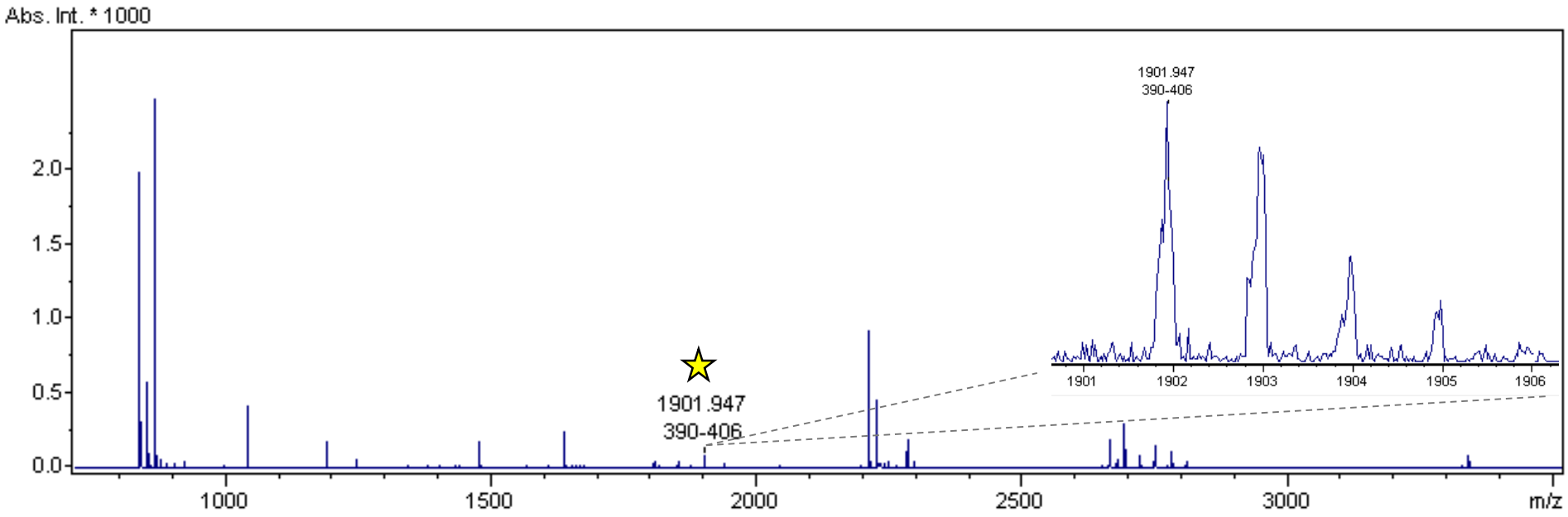
Method development – Native peptide mapping

T1

First development on IgG4 mAb

MALDI-MS identification

Trypsin autolysis peaks but also…



First peptide is observed at first time point

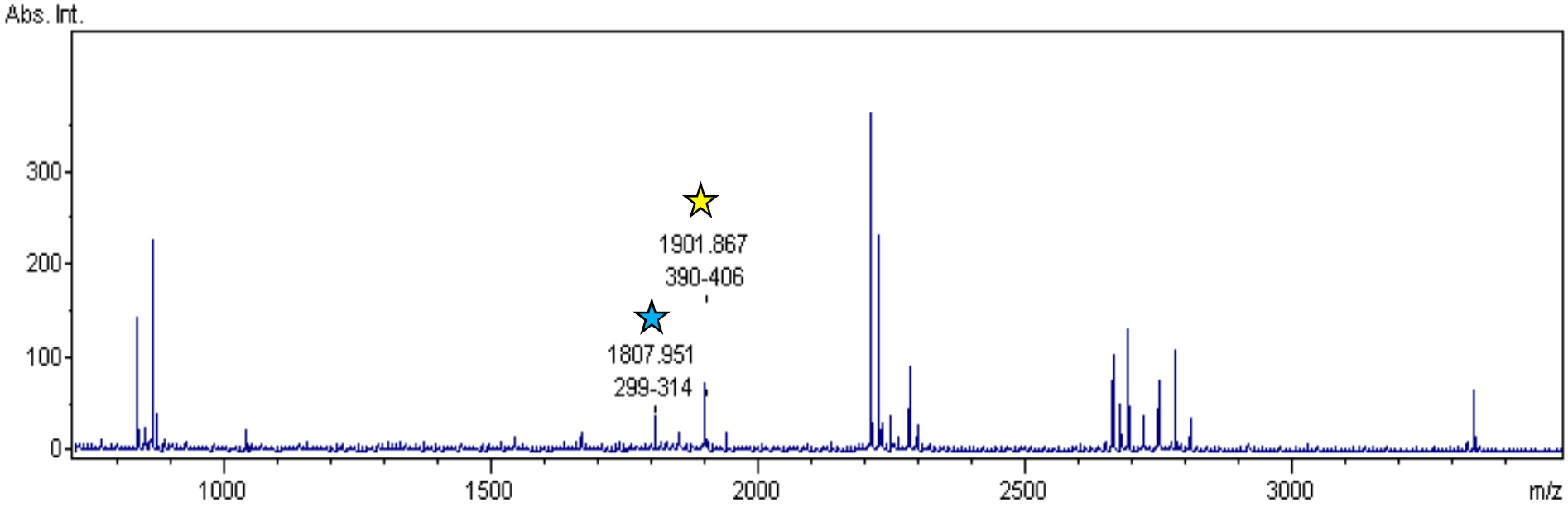
Method development – Native peptide mapping

T2

First development on IgG4 mAb

MALDI-MS identification

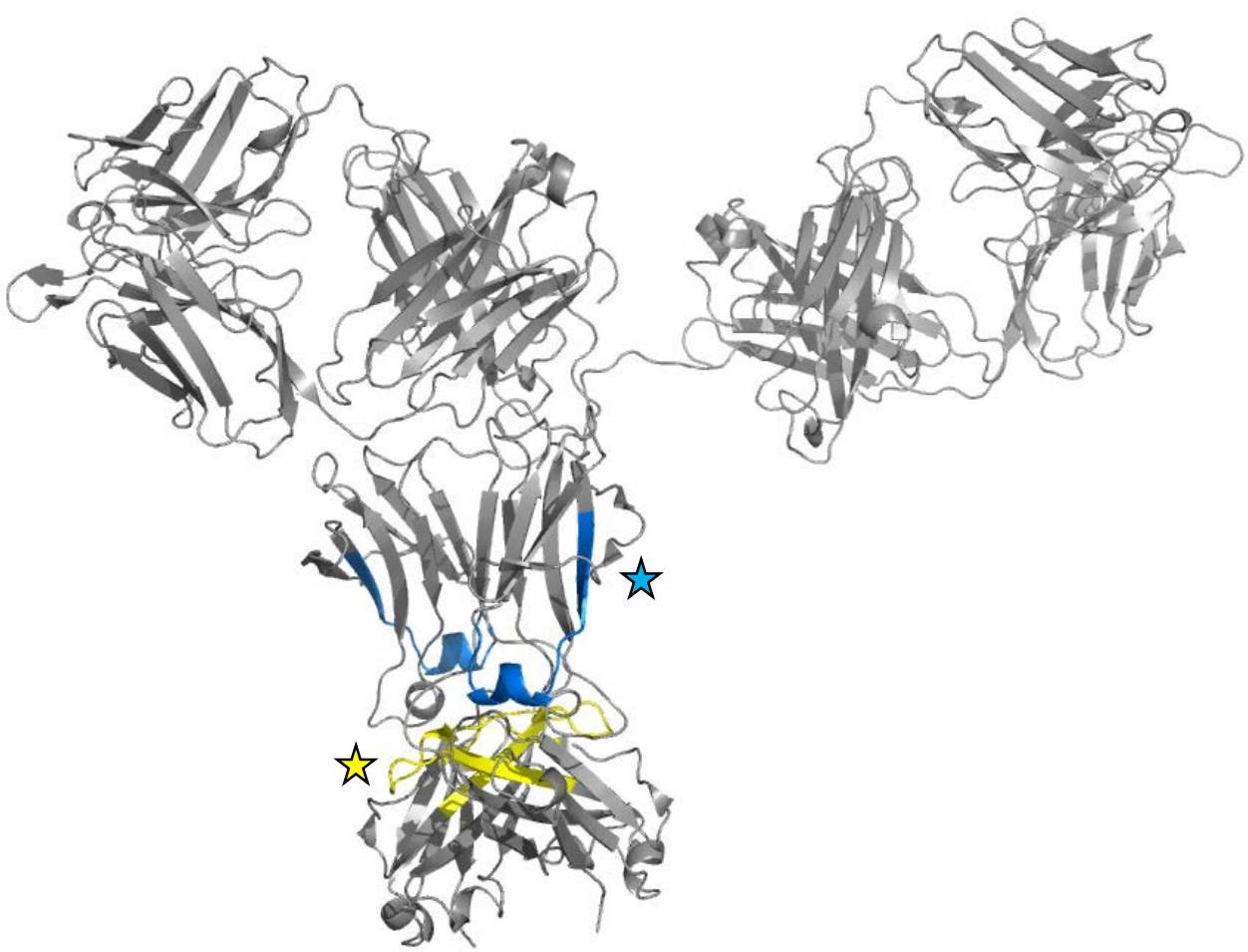
Trypsin autolysis peaks but also…

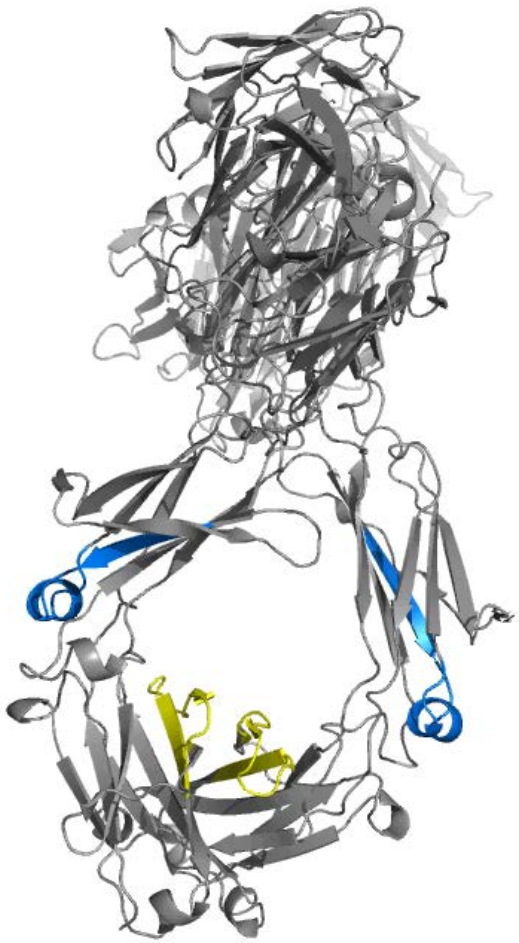


Second peptide is observed after several minutes…

Method development – Native peptide mapping

T2



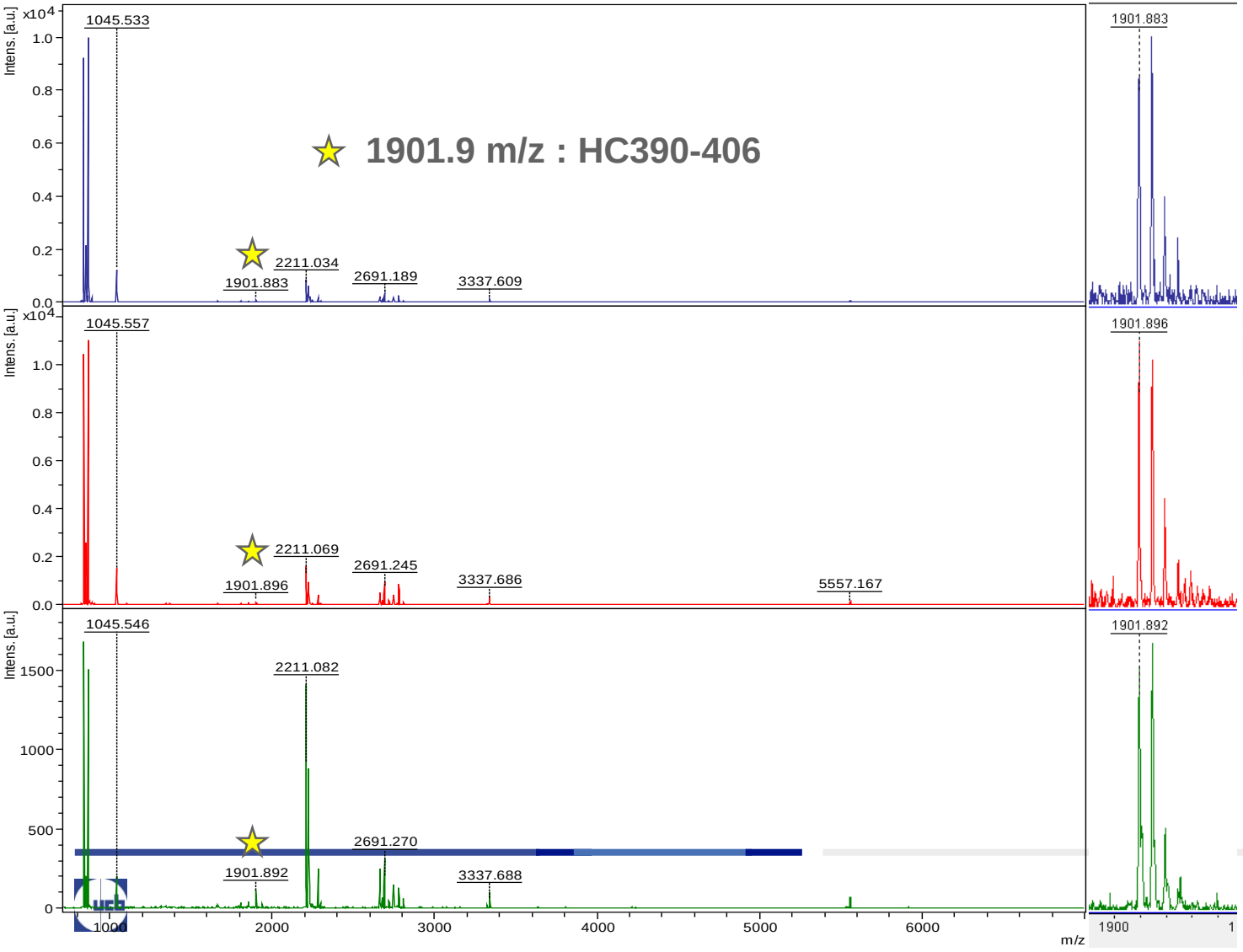


90°

T1

Method development – Native peptide mapping

Repeatability assessment n=3



1st peptide is detected at the same time for all the samples

Same peptides were observed in next time points for triplicates

Native peptide mapping – Higher order structure (HOS) monitoring

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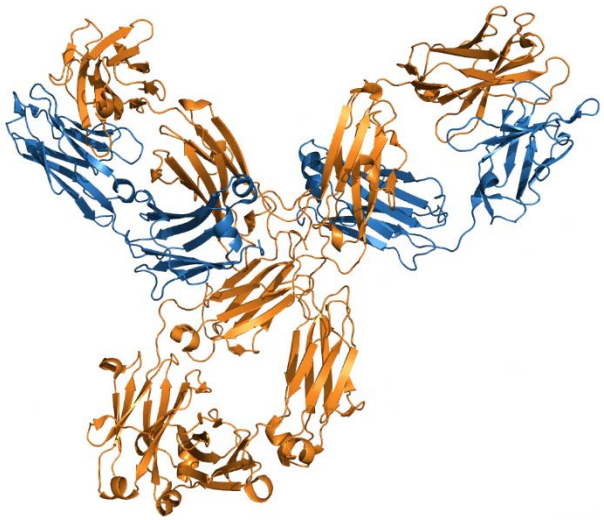
Are we able to observe conformational changes between unstressed and stressed samples ?

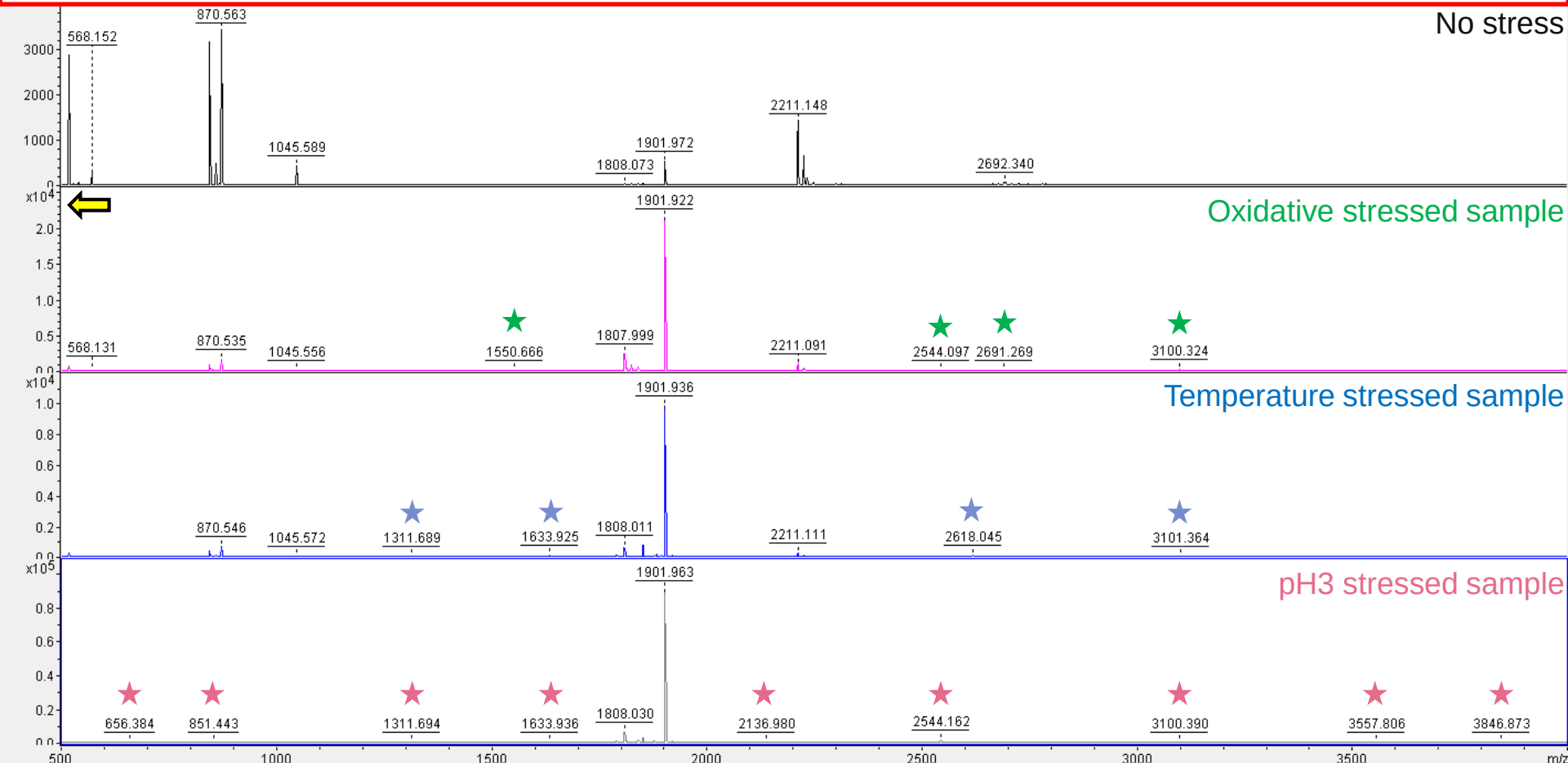


O_2

Unstressed *vs* stressed samples

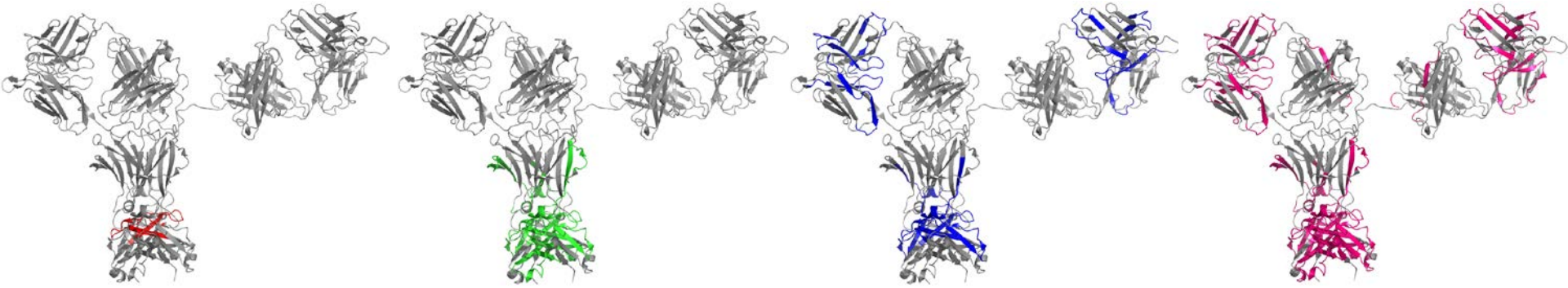
T1

By this method, we are able to distinguish differences between unstressed and stressed samples.
… to distinguish conformational changes.



Which mAb regions have been impacted by the stress according to native peptide map ?

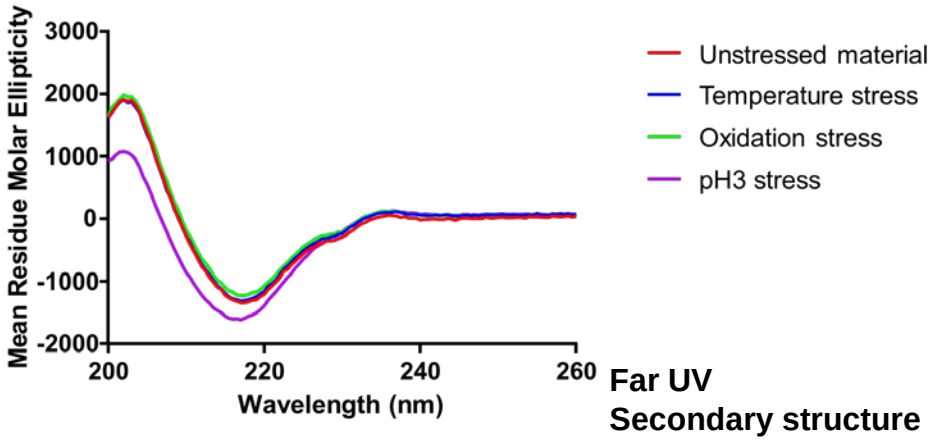
T1



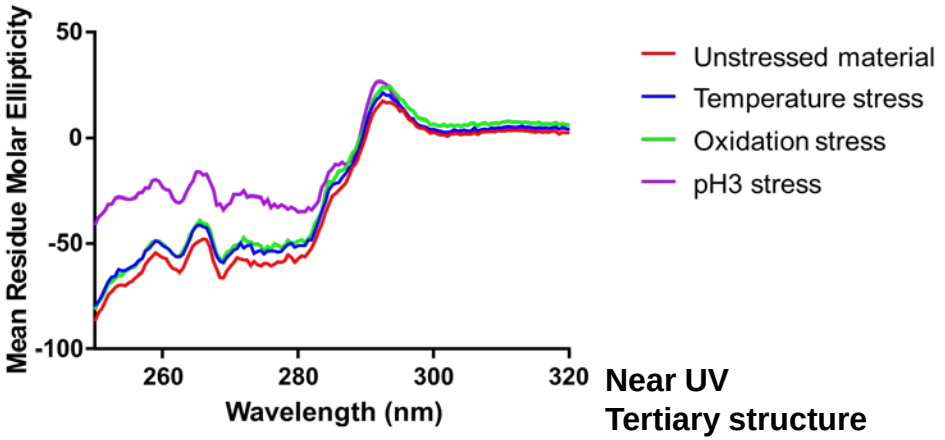
Unstressed sample | **Oxidative stressed sample** | **Temperature stressed sample** | **Acid stressed sample**

Trypsin accessibility

Circular dichroism results confirmation:



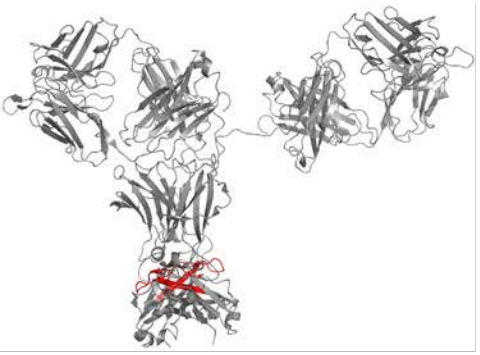
Far UV
Secondary structure



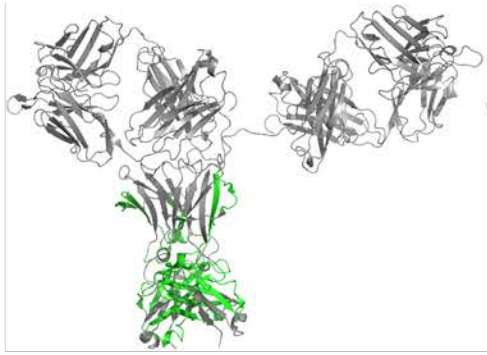
Near UV
Tertiary structure

Is the biological activity impacted by the stress ?

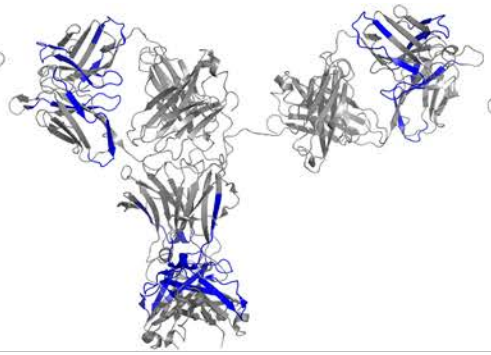
T1



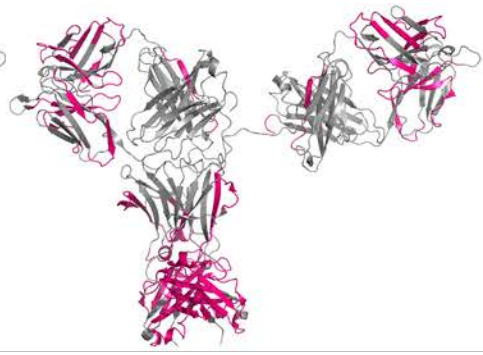
Unstressed sample



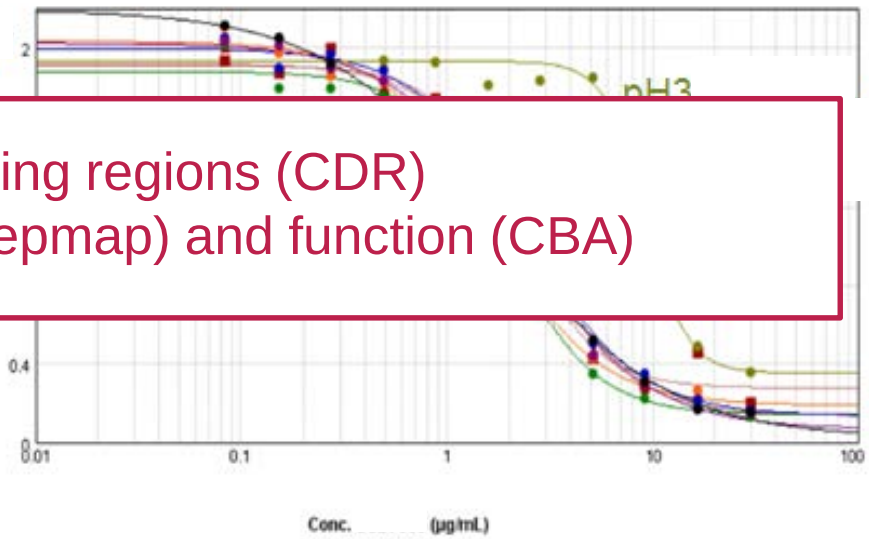
Oxidative stressed sample



Temperature stressed sample



Acid stressed sample

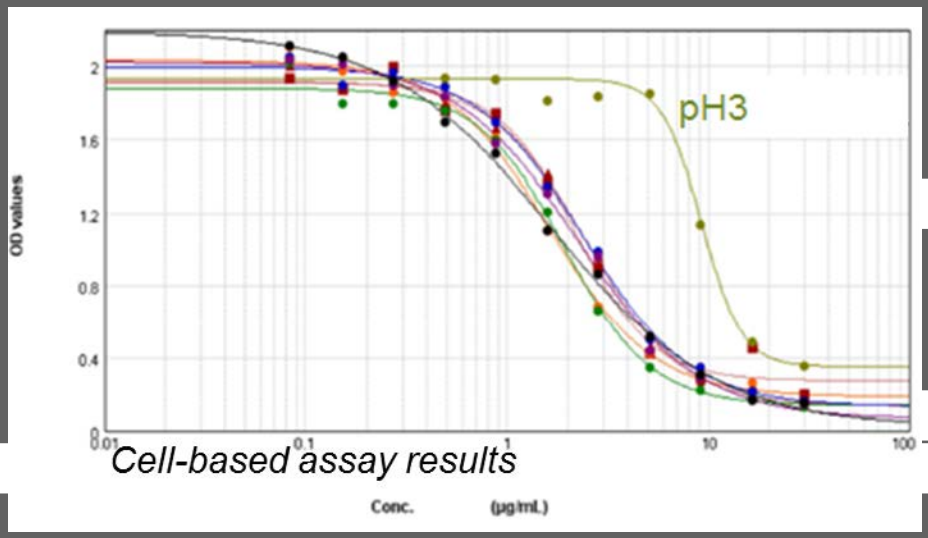


⇨ Complementary determining regions (CDR)
Try to correlate structure (native pepmap) and function (CBA)

Do we observe differences between each stress conditions ?

T1

	Peptide	Unstressed	Ox stress	Temp stress	Acid stress
CDR	HC 1-19			✓	✓
	HC 39-61			✓	✓
	HC 44-61				✓
	HC 44-65				✓
	HC 77-87				✓
	HC 122-133-LC 217-219				✓
	HC 246-252				✓
	HC 286-298				✓
	HC 299-314				✓
	HC 299-317				✓
	HC 342-357				✓
	HC 358-367				✓
	HC 368-389				✓
	HC 390-406				✓
	HC 414-436				✓
	LC 1-18			✓	✓
	LC 36-50			✓	✓
CDR	LC 51-66				✓
	LC 52-66				✓



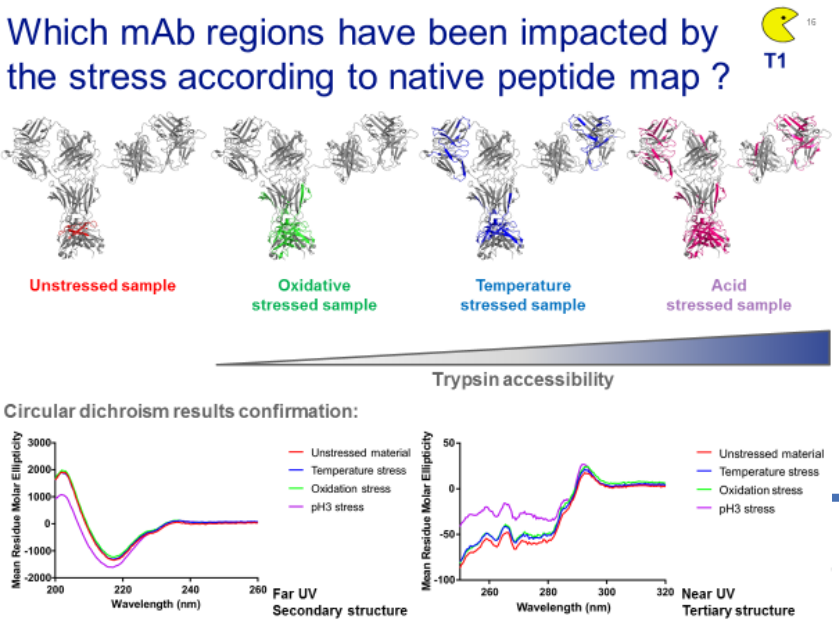
Cell-based assay results

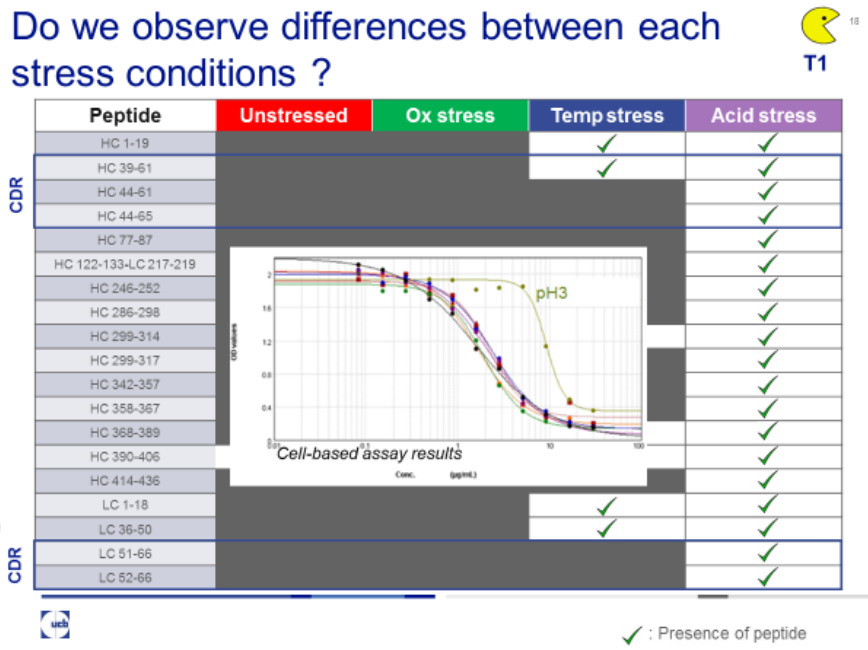
✓ : Presence of peptide

Case study I – IgG4 analysis and bioassay relationship – Conclusions

This new method allows to:

- Detect HOS changes by comparing the samples to an unstressed sample
  - ➢ *Results confirmed by well-established orthogonal technique (CD)*
- Investigate structure – activity relationship





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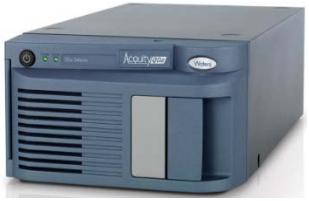
Method development – Native peptide mapping

From development stage to QC applicability
From qualitative results to quantitative results

UPLC
Peptides separation



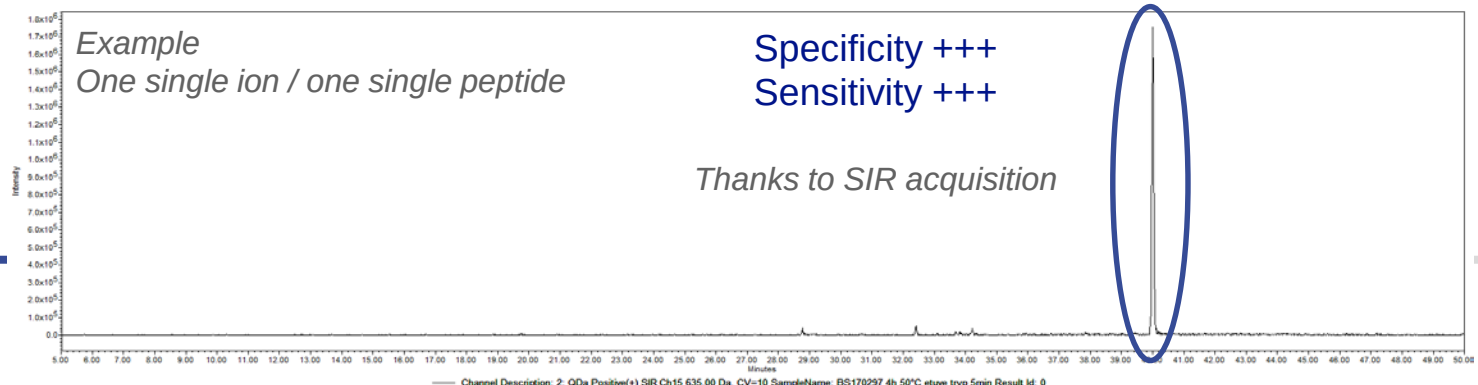
QDa mass detector
Peptides quantitation

- **Follow each peptide thanks to the peptide list determined by MALDI-MS**
  → SIR acquisition

SIR: Single Ion Recording

- **Less expensive than MALDI-MS platform**
- **More user-friendly interface**
- **Need less MS expertise to use it**
- **Managed by CDS - GMP compliance**

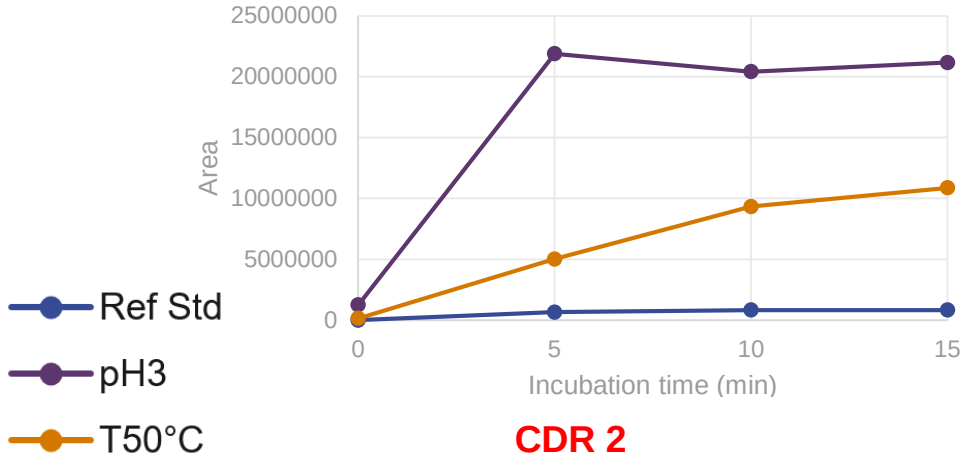
« QC friendly system »



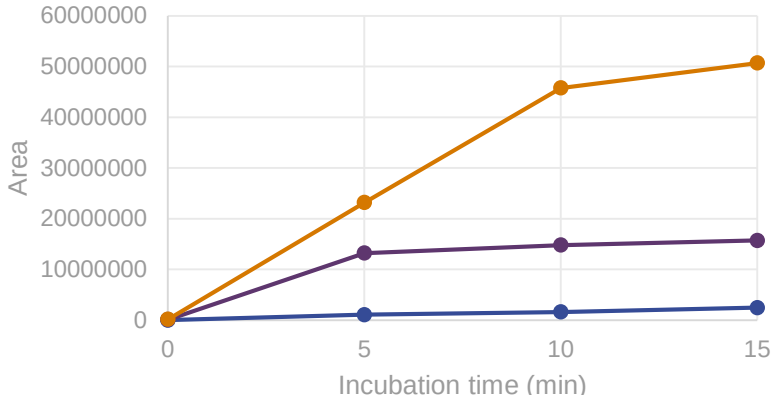
Method development – Native peptide mapping data *vs* Cell-based assay

Quantitative data of IgG1 stressed sample

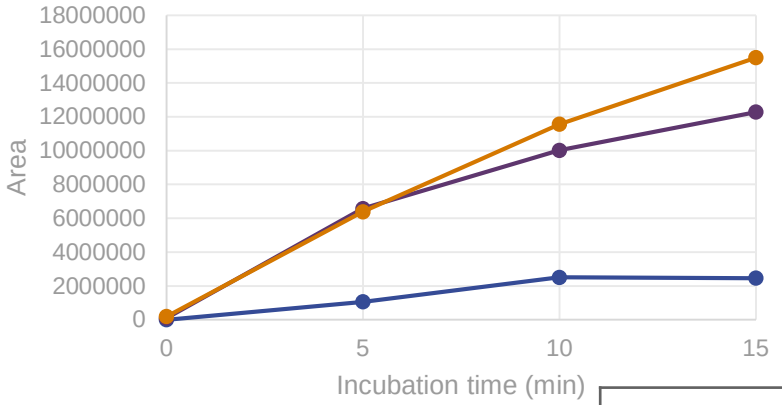
CDR 1

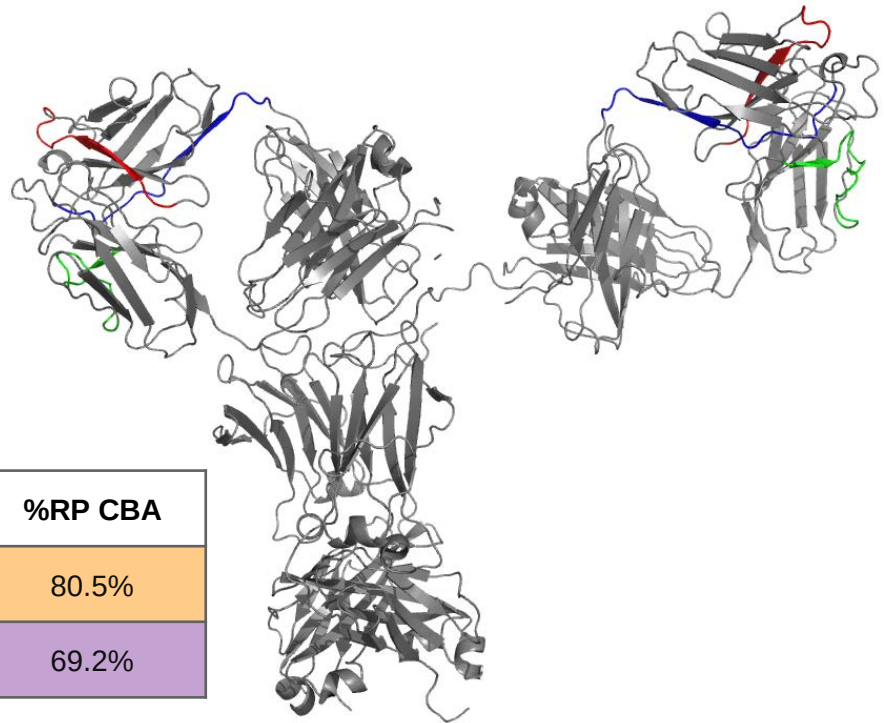


CDR 3



CDR 2

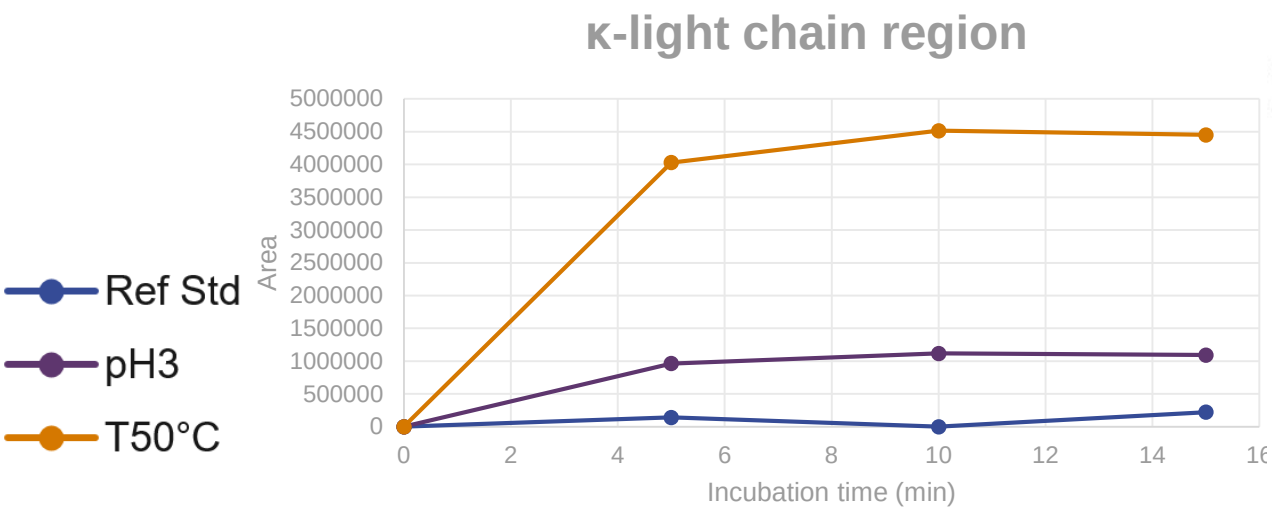


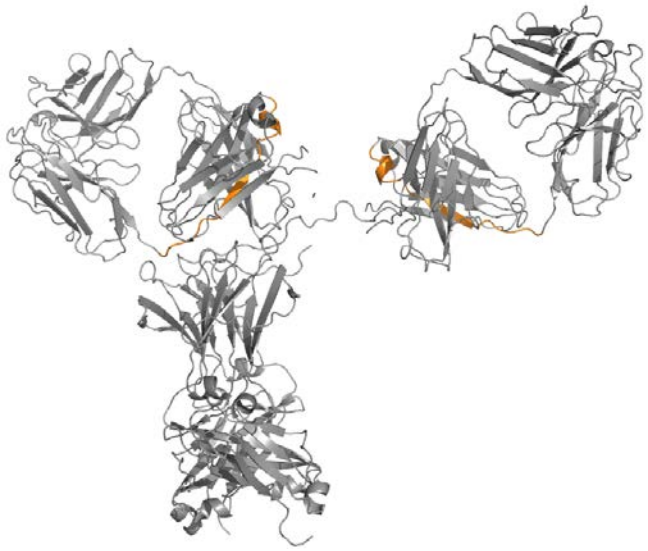


Samples	%RP CBA
50°C	80.5%
pH3	69.2%

Method development – Native peptide mapping data *vs* ELISA binding

Quantitative data of IgG1 stressed sample





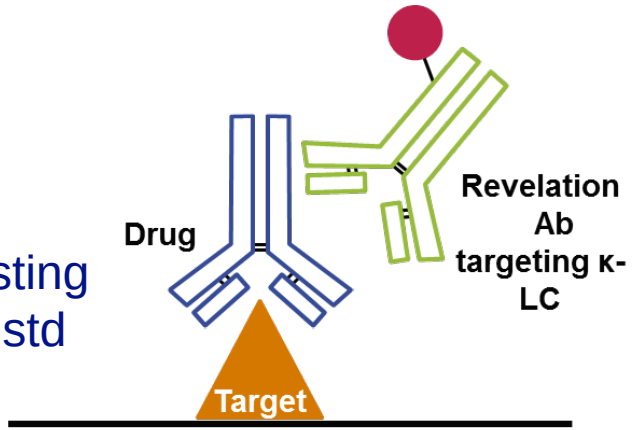
Samples	%RP ELISA
50°C	Not comp
pH3	Not comp

κ-light chain region is impacted by stresses

Hypothesis

➔ « Miss-binding » of the revelation Ab during the ELISA testing

➔ Explain the non-comparability between samples and ref std



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To summarize…

Native peptide mapping:

- Is a **QC-friendly method able to monitor** HOS change for several (4) mAbs.
- May allow **correlation** of protein **conformational changes** with **biological activity**

For the future…

- Generate **more data** to support the use of native peptide mapping to monitor HOS changes and structure-activity relationships
- Evaluate the utility of **native peptide mapping** as a stability indicating method

Special thanks to all our colleagues on this journey:

- **Characterization team – ASB**
- **PCMD1 team - ASB**
- **Bioassay Dev.Team- ASB**

And especially:

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Erica Bortolotto
Sandrine Van Leugenhaeghe
Maria-Teresa Chiusano
Oliver Durrant
Elizabeth Rodriguez
Will Burkitt
Jennifer Halley

Gaël Debauve	Annick Gervais	John O'Hara
Carl Jone		

Thanks!

Questions?