Picking-up product degradation by bioassay.

Function and Structure, pieces of the puzzle !

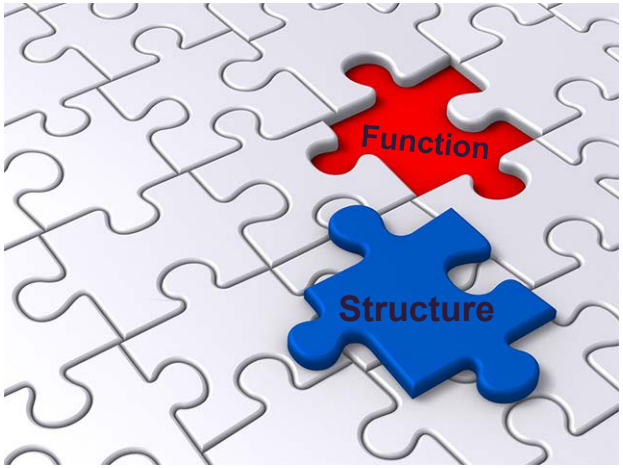
UCB Pharma – Annemie Wielant

AT Europe CASSS - March 2019

Inspired by patients.
Driven by science.

Agenda

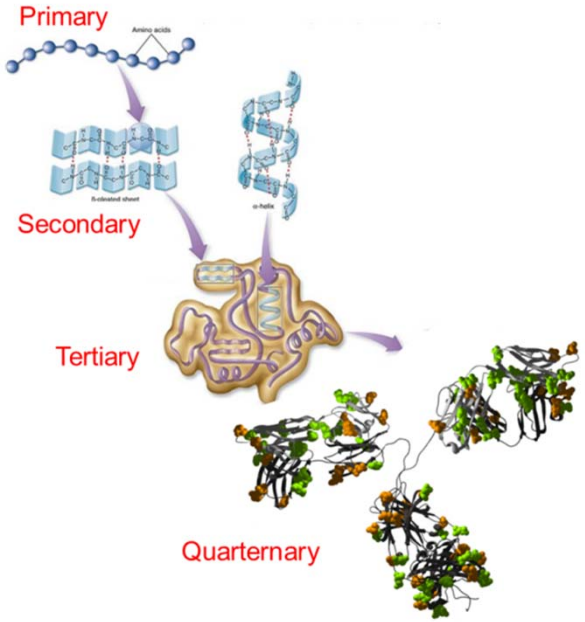
- Risks of changes in Higher Order Structure ?
- **Function** : Measurement of binding activity by Bioassay.
- **Structure** : Higher order structure analysis by native peptide mapping.
- Potential link between Structure and Function (case studies).
- Take home messages.



Higher Order Structure (HOS) of bio-molecules

- Includes the secondary, tertiary and quaternary structures of a protein.
- Mandatory for structure and function (specificity).
- Complex structures with many potential sites of heterogeneity and degradation !

- Regulatory agencies (ICH Q5E*) require assessment of HOS to see how protein structure is affected during the lifecycle of the drug.
- Analytical methods should be able to pick-up pertinent changes in HOS.
- Key component in defining the critical quality attributes (CQAs)
- Gain in knowledge on the molecular structure to ensure the quality of the drug !



* International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use – Comparability of biotechnological/biological products subject to changes in their manufacturing process Q5E

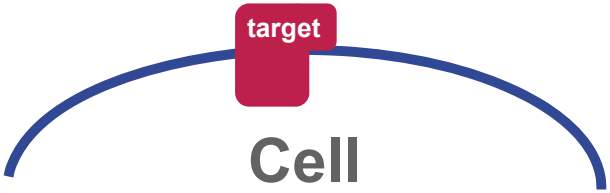
Risks of HOS confirmational change

Degradation by temperature, pH, Oxidative stress, agitation, light exposure,…

Changes in protein structure can result in changes to :

efficacy, stability, specificity and affinity.

No drug efficacy



Risk for the patient ?

Could potentially affect drug safety – trigger disease progression
(increased potential for immunogenicity and loss of biological function)

“Stability indicating” methods

= Pool of physico/chemical & bioassay methods

Inducing change in HOS by forced degradation studies

= intentional degradation at conditions more severe than accelerated conditions.

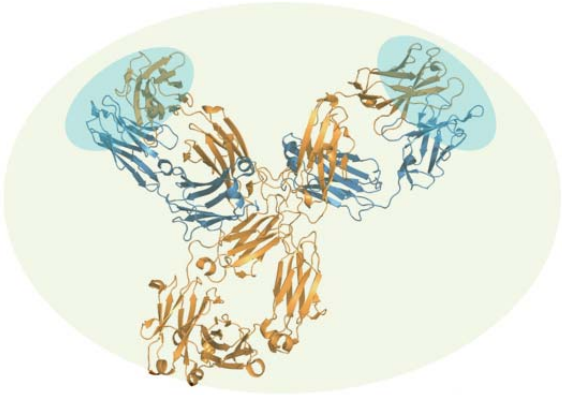
➔ forced degradation study ≠ shelf-life stability study

Concept Paper « Forced Degradation Studies for Therapeutic Proteins », EBE, March 2015

The analysis of FDS samples will help us to reveal the relationship between a change in higher order structure and biological activity

⇩ **Native peptide mapping**

⇩ **Bioassay**



« At which point will a change in HOS form a real risk for the patient ? »

Why do we need a Bioassay ?

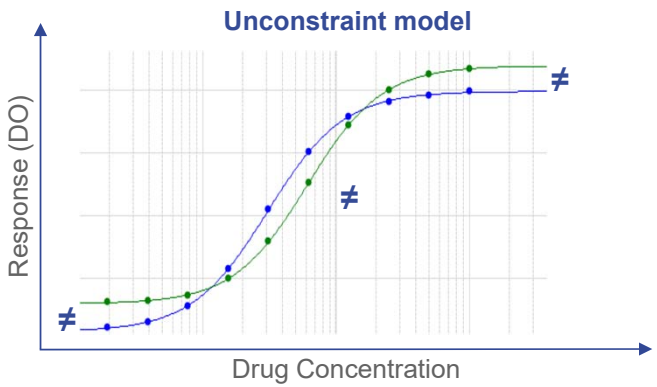
- To evaluate the **potency/activity** of a pharmaceutically active substance.
- Method should reflect **Mode of Action** of the drug.
- Potency (%RP) is a **product specific measurement.**
- Part of the analytical package to effectively control the **quality/stability** of the drug during it's lifecycle.
- **Two main categories** : binding assays and cell-based assays.
- **A fully validated bioassay** has to be in place before a product is released **for commercial use**.

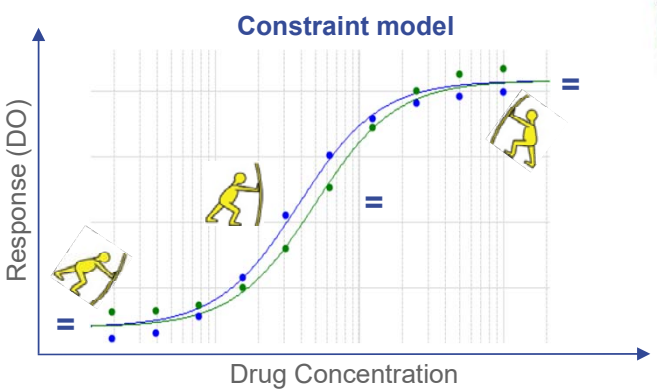
Bioassay - Evaluating similarity

- **Nonlinear relationship** between the response and the analyte concentration - 4PL curves*
- **RP = horizontal distance** between Sample (S) and Standard (RS) dose-response curve…**if and only if they are… similar** !!
- Need of a **similarity assessment <u>metric</u>** and **similarity <u>limit</u> (equivalence limit)**

➔ use of a composite measure : considers all curve parameters together in one single measure**: RSSEnonPar***

RSSEnonPar = (RSSE constraint model – RSSE unconstraint model)



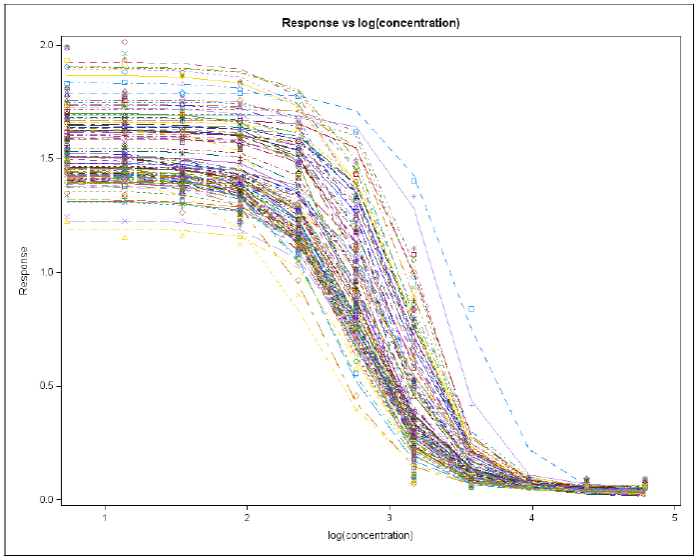


Article « Assessing similarity with Parallel-Line and Parallel-Curve Models », Bortolotto and al., BioProcess International, June 2015

* 4-parameter logistic regression
* Residual Sum of Square Error

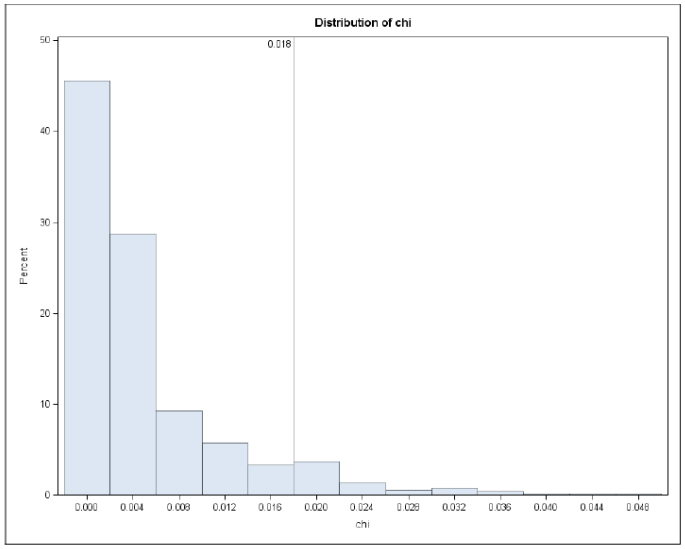
Evaluating similarity – Equivalence limit determination

➔ need to define an acceptable **limit** to this (non)similarity value (Approach A – USP1032)



STATISTICAL SUPPORT !!

Figure 5–3: The distribution of the 518 chi-square statistics used to update the equivalence margin

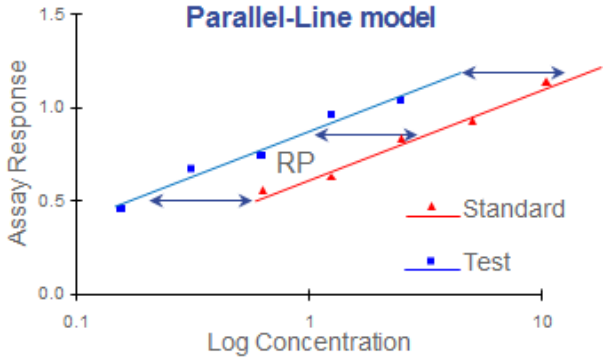


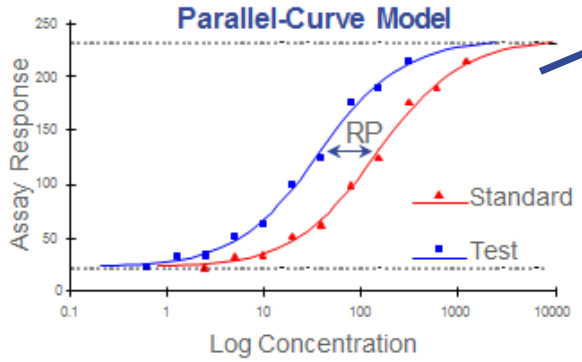
➔Tolerance limit derived from historical values (comparison of Standard to itself – as much as possible) of non similarity values

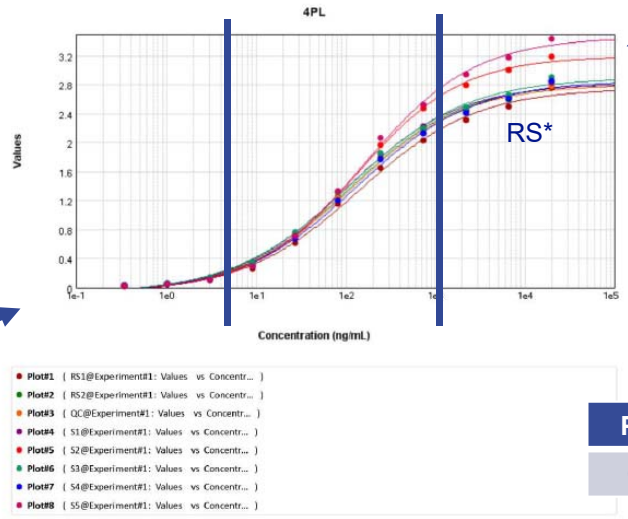
➔ Non similarity value defined to conclude similarity – If in the future, RSSE value is above, the pair of reference standard/sample will be rejected

Model selection - Why our preference for the parallel curve model ?

➔ 2 major models to fit the data over the range of concentration of interest (**parallel-line and parallel-curve model**)







Sample degraded at 50°C/14d

Parallelism	Linear part	Full 4PL
Operator 1	Pass	Fail

The full dose-response relationship is the most appropriate if you use the assay for lot release and for characterization/stability testing.

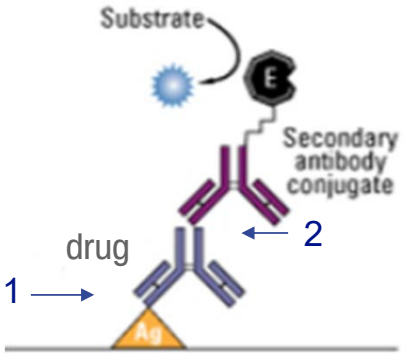
RS : Reference standard

Is a Bioassay really « stability indicating » ?

Case study 1: measurement of IgG binding with ELISA and cell-based assay

Different assays: different assay principals, equivalence margins,…
➔ can respond differently towards degradation !

ELISA : both part 1 and 2 play a role.



CBA : Assay in which only the active part of the molecule plays a role in the setup.

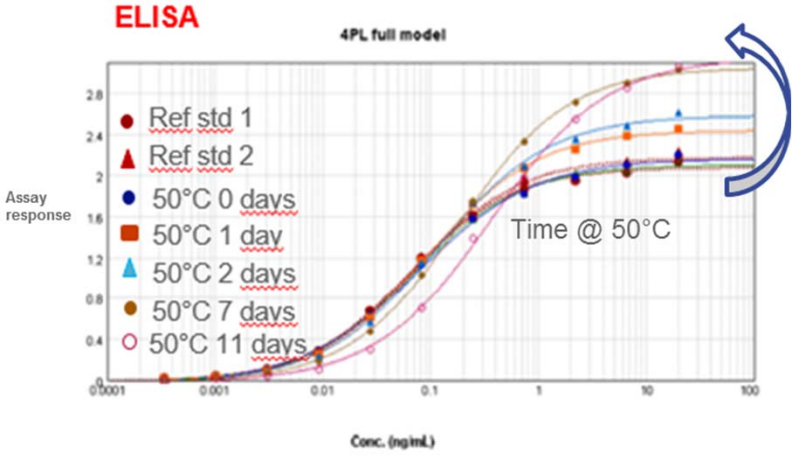
1 : active part of antibody
2 : epitope recognized secondary detection antibody

Is a Bioassay really « stability indicating » ?

Case study 1: stressed IgG

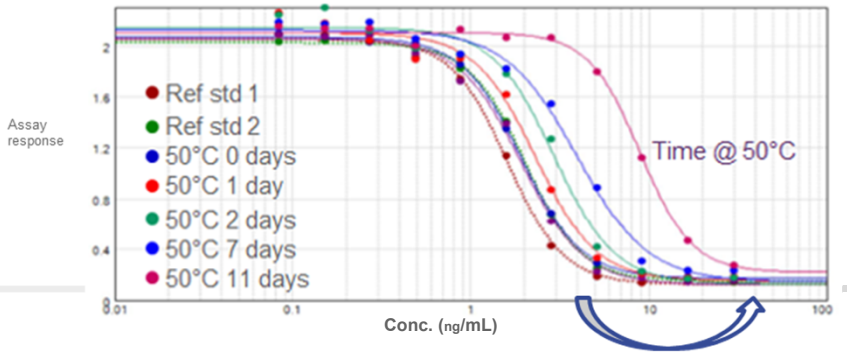
Summary Table	FDS samples - degradation at 50°C	
Sample	Mean RP ELISA	Mean RP CBA
0 days	93,00%	86,50%
1 day	Failed parallelism – no %RP calculated	72,70%
2 days	Failed parallelism – no %RP calculated	69,40%
7 days	Failed parallelism – no %RP calculated	41,60%
11 days	Failed parallelism – no %RP calculated	24,70%

ELISA



➔ **Fail in similarity does NOT mean that there is no activity anymore !**

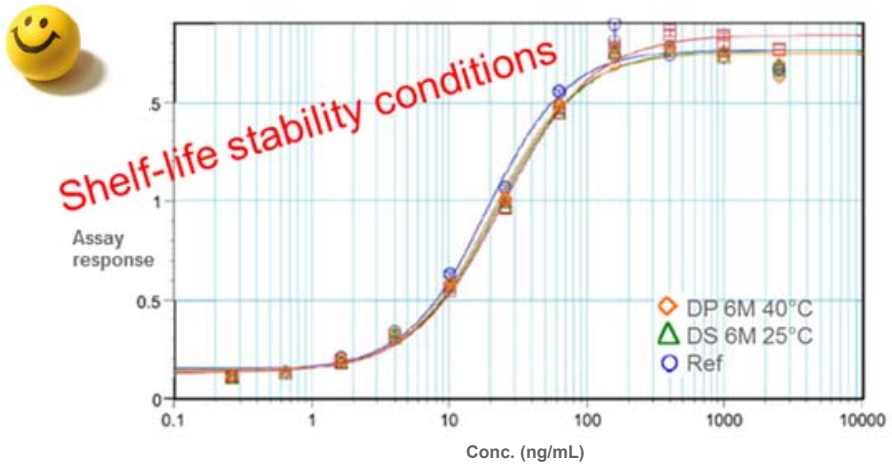
CBA



No exact answer to the question « Which part of the molecule is impacted by the degradation ? »

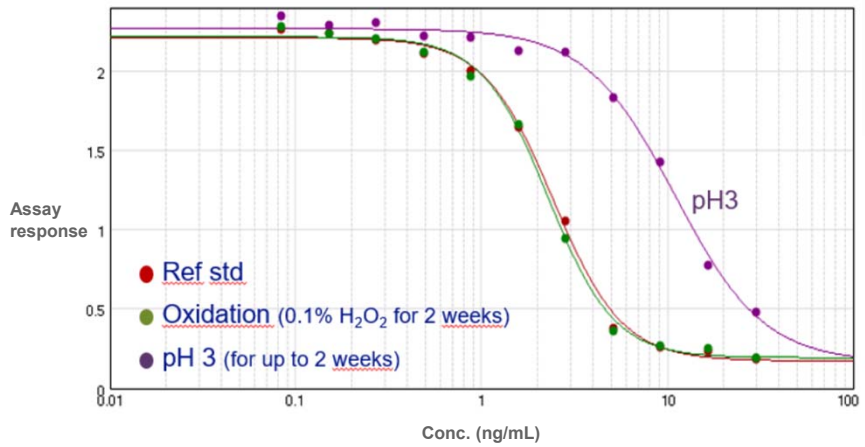
Stability versus forced degradation

Drug exposed to ICH stability storage conditions



Here, in this particular case, bioactivity is not affected by (small) degradations resulting from ICH stability storage conditions (even not at most extreme conditions e.g. 40°C during 6 month).

Bioactivity is only affected by harsh degradations

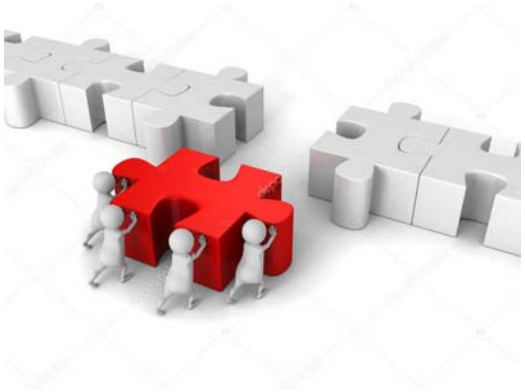


Here stress conditions as used in the Forced Degradation Study.

➔Even if the bioassay is not the most sensitive stability indicating method, testing in **stability program** still required (ICH Q1A (R2) and ICH Q5C).

Is a Bioassay really « stability indicating » ? Conclusions…

- **Not all methods can pick-up all forms of degradation.**
- **Degradation product may be found similar to original.**
- **Method may not reflect the full impact of the degradation.**

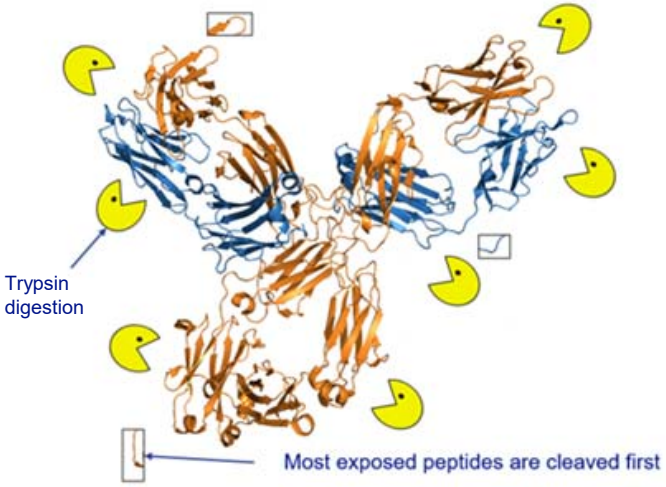


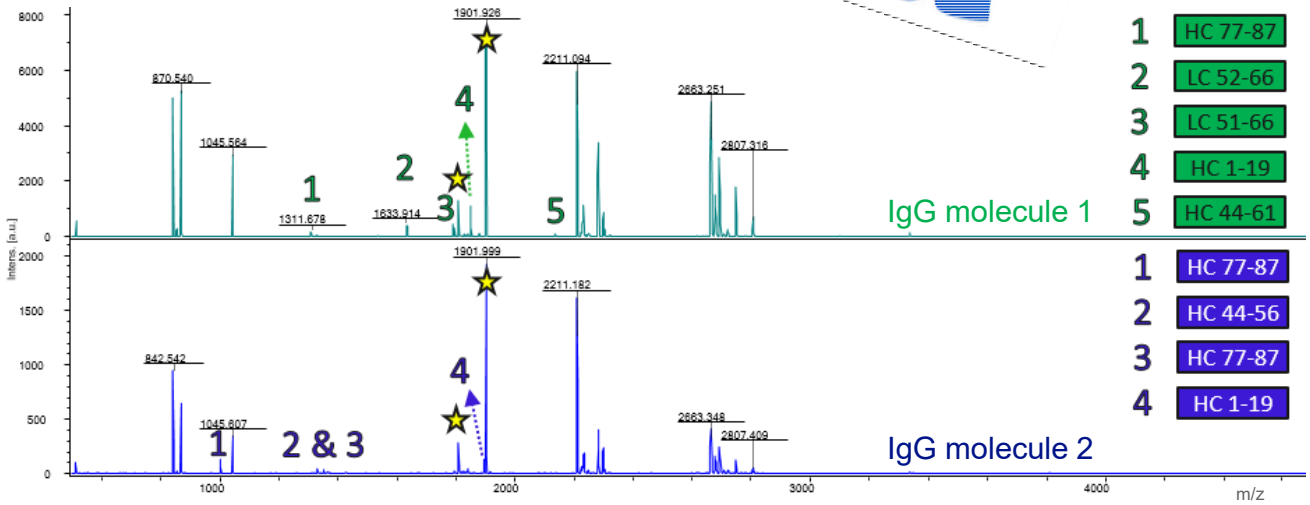
- **Method variability** can have an impact on the sensitive to pick-up degradation.
- **Regulatory expectation**: to have a bioassay representative of the mode of action of the drug: therefore cell- based bioassay is preferred to ELISA!
- Need **of Phys/Chem methods to support bioassay results**!

Native peptide mapping - Higher order structure analysis

Analysis by Mass Spectrometry (MS) =
Mapping of protein - peptide ID

Exposed peptides reflect the state of the HOS!





2 different non-stressed IgG molecules – 10 minutes of trypsin incubation

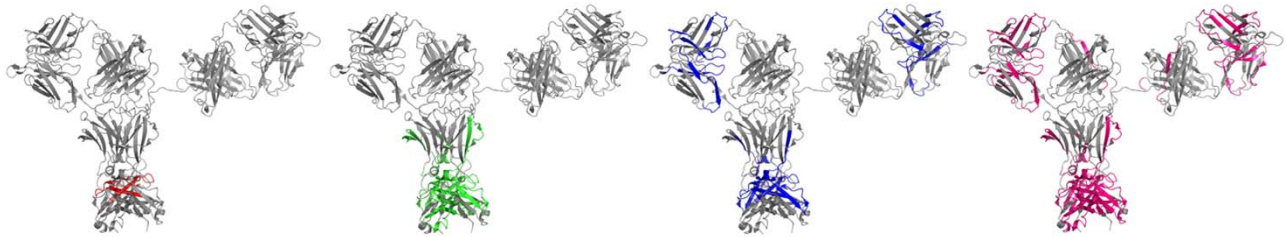
☆Peptides from the constant region.
Other peptides come from the variable region.

➔ After 5 min of trypsin : only peptides from constant region detected
➔ peptides from constant region: consistent for a subclass of mAb
➔ peptides from variable region: specific for each molecule

Structure-Function (case study)

Case study 2: Native peptide mapping of a stressed IgG (5 min trypsin digestion)

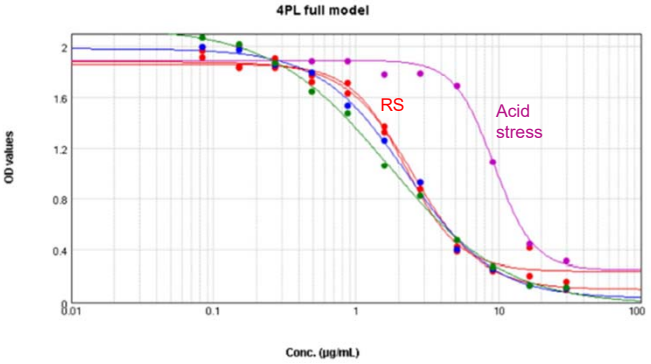
Degradation type



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

	MAb Region	RS	Oxydation	Temp	Acid pH
	HC[390-406] 1902,0 m/z	1	1	1	1
	HC[1-19] 1851,0 m/z	0	0	1	1
CDR	HC[44-65] 2566,1 m/z	0	0	0	1
	HC[77-87] 1311,7 m/z ox 1327,7 m/z	0	0	1	1
	HC[299-314] 1808,0 m/z	1	1	1	1
	HC[299-317] 2228,3 m/z	0	1	1	1
	HC[342-357] 1876,9m/z	0	0	0	1
	HC[358-367]-HC[414-436] 3846,7 m/z	0	0	0	1
CDR	LC[51-66] 1790,0 m/z	0	0	1	1
CDR	LC[52-66] 1633,9 m/z	0	0	1	1

Cell-based assay



- ➔ Degradations/structural changes will impact at 3 levels:
  - Number and localization of exposed peptides (trypsin accessibility)
  - Release rate of exposed peptides
- ➔ Here active part (CDR) peptides affected by temperature and pH (very harsh conditions)
- ➔ Link with biological activity !!

CDR= Complementarity-determining regions

Structure-Function (case study)

Back to case study 1: Native peptide mapping of gradual temperature stressed IgG - Liquid Chromatography-MS results

Summary Table	FDS samples - degradation at 50°C	
Sample	Mean RP ELISA	Mean RP CBA
0 days	93,00%	86,50%
1 day	Failed parallelism – no %RP calculated	72,70%
2 days	Failed parallelism – no %RP calculated	69,40%
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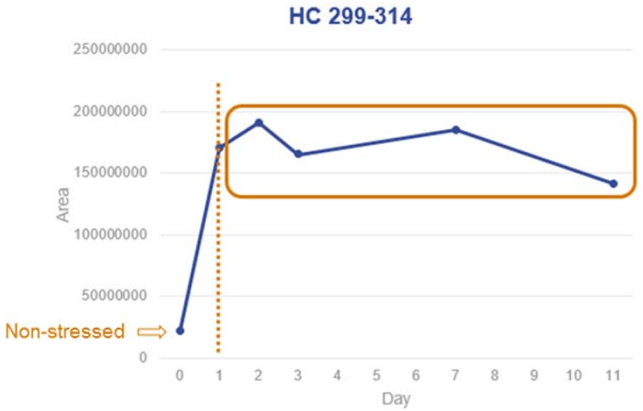
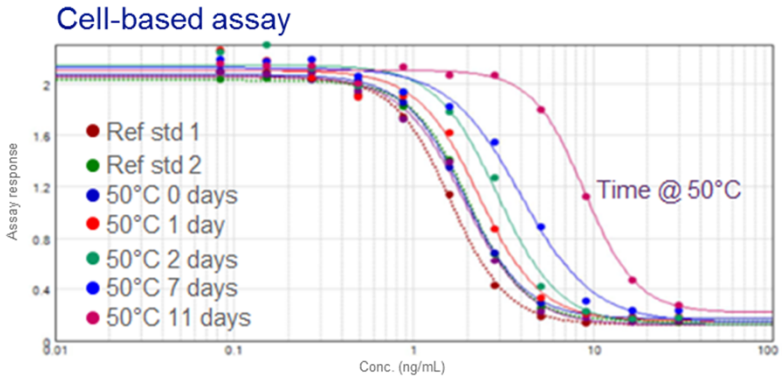
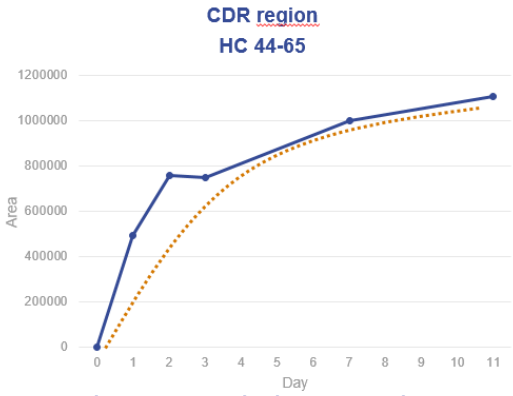


Plateau reached after 1 day - stress/50°C





CDR – here continuous evolution over time - stress/50°C

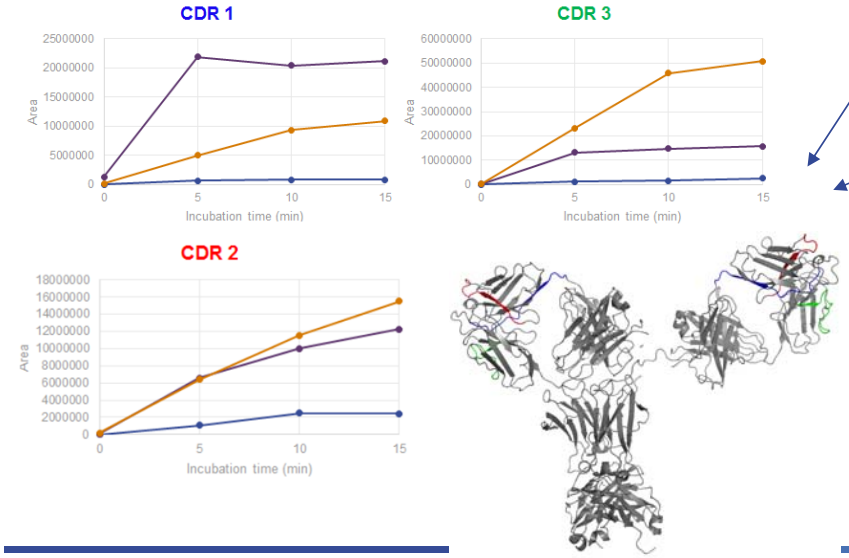
Outcome :

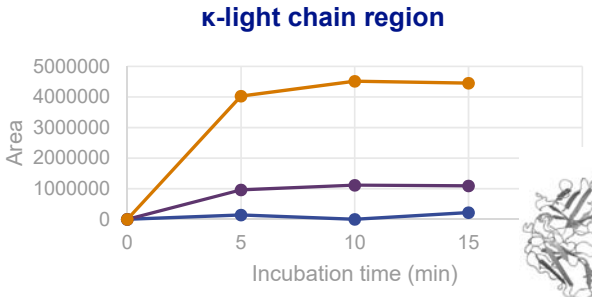
- Here HOS change occurs gradually during the stress duration.
- Bioassay response is gradually affected when the active part (CDRs) of the drug is impacted – active part not first impacted.

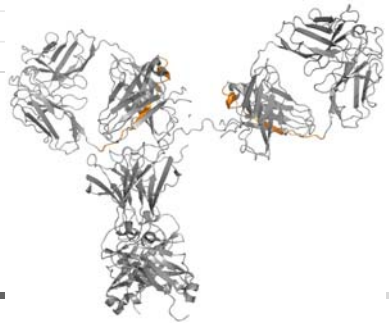
Structure-Function (case study)

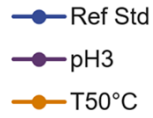
Case study 3: Native peptide mapping results versus bioassay results of a IgG stressed sample

Stress conditions	Cell-based assay	ELISA
50°C/14days	80,5%	Not parallel – no %RP
pH3/14days	69,2%	Not parallel – no %RP





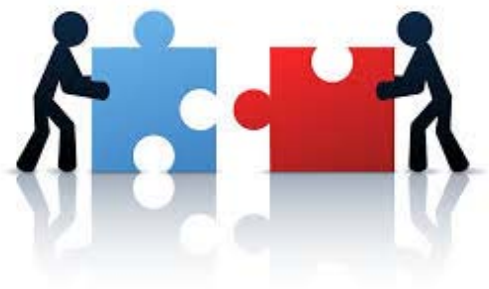




More details should shortly be available in a paper submitted in MAbs: Degueldre *et al.*, "Native peptide mapping – A simple method to routinely monitor higher order structure changes and its relation to functional activity".

In conclusion… take home messages.

- The sensitivity of the available bioassay formats should be evaluated as early as possible and should be correlated in a structure/function analysis.
- ICH stability storage conditions ≠ forced degradation conditions !
- Even if the ICH stability storage conditions might not be sufficient to affect the biological activity…keep the bioassay in the stability indicating method package as it is a regulatory expectation.
- Understanding which and how degradations impact the active part of the drug is crucial.



Towards the future…

- Generate more data to support the link between bioassay and the native peptide mapping (HOS) results.
- Know-how on molecule degradation is built from the projects (past and present).
- Predictive tool for drug candidate engineering/screening.
- Evaluate implementation of the native peptide mapping in a QC environment (as part of stability package) -> anticipate regulators expectations in term of product knowledge.
- Tool to further characterize the correlation between structure – function and… immunogenicity (patient safety) ?

Special thanks to my colleagues on this journey …

Driving force behind this project:
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Questions?

Thanks!