Evaluation of chemical stability during developability assessment of therapeutic antibodies

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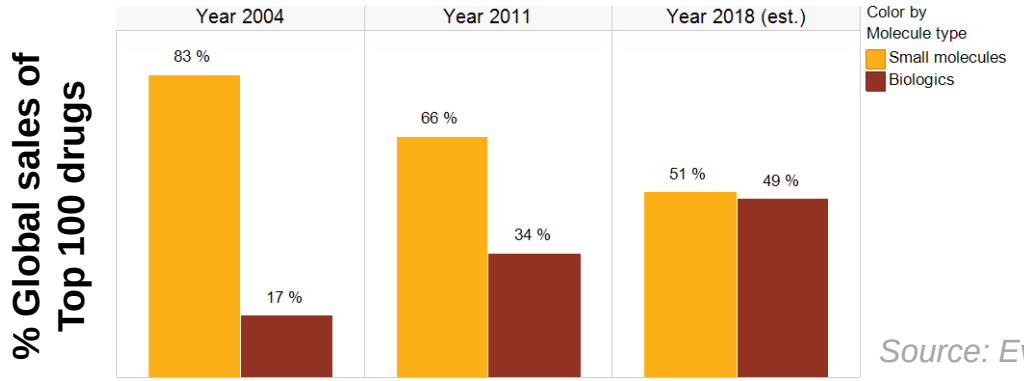
The future of Healthcare

Challenges and Opportunities

- **Controlling costs** will be an important social and economical challenge for healthcare industry to still deliver affordable drugs in the future
- **Innovative Drugs and Therapies** required to meet currently unmet medical needs
- Major contributors will be **Biological Drugs**, which will make up 50% of the drug sales of the top 100 drugs by 2018



Source: EvaluatePharma, 2013

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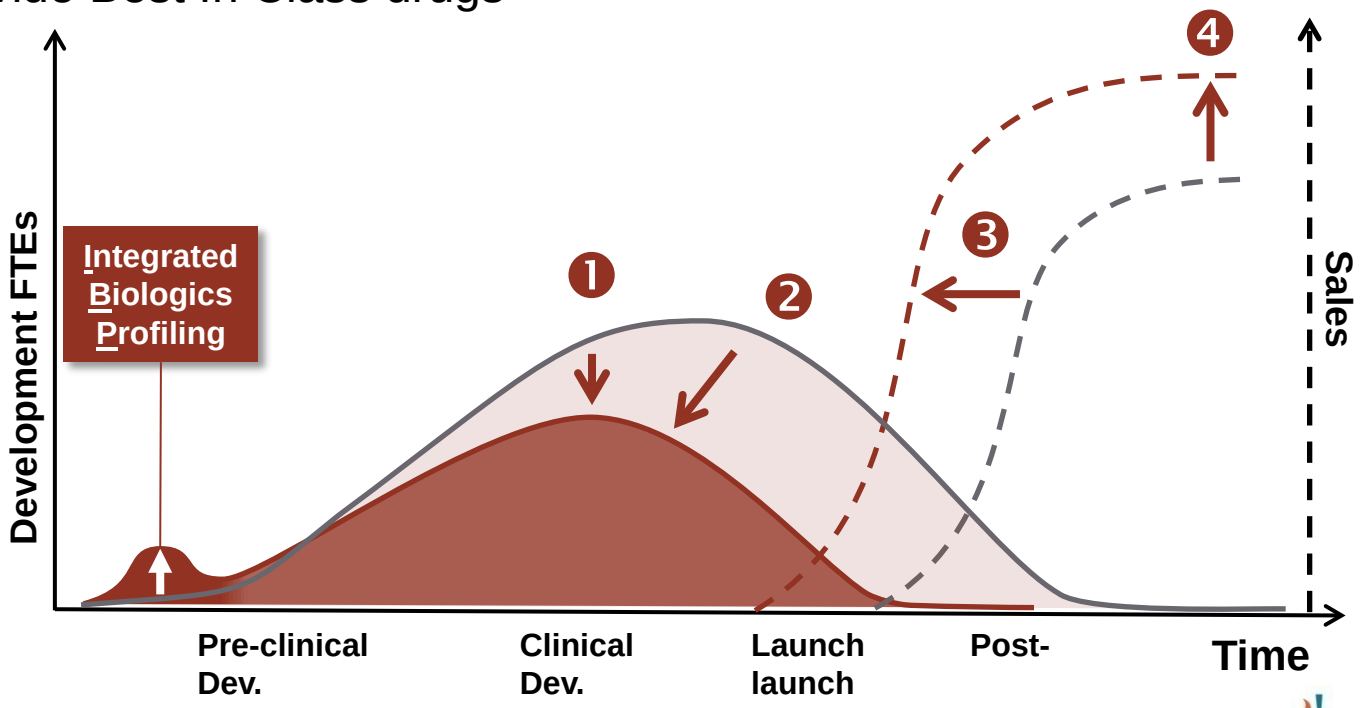
Value Proposition

Early Dev Resource Investment for High-Quality Biologics Pipeline

➔ Invest in **Quality** of early Biologics Pipeline to

1. De-risk Development process and avoid excessive effort
2. Improve productivity (e.g. platform approaches)
3. Accelerate the development process and subsequently time to reach the patients
4. Provide Best In Class drugs



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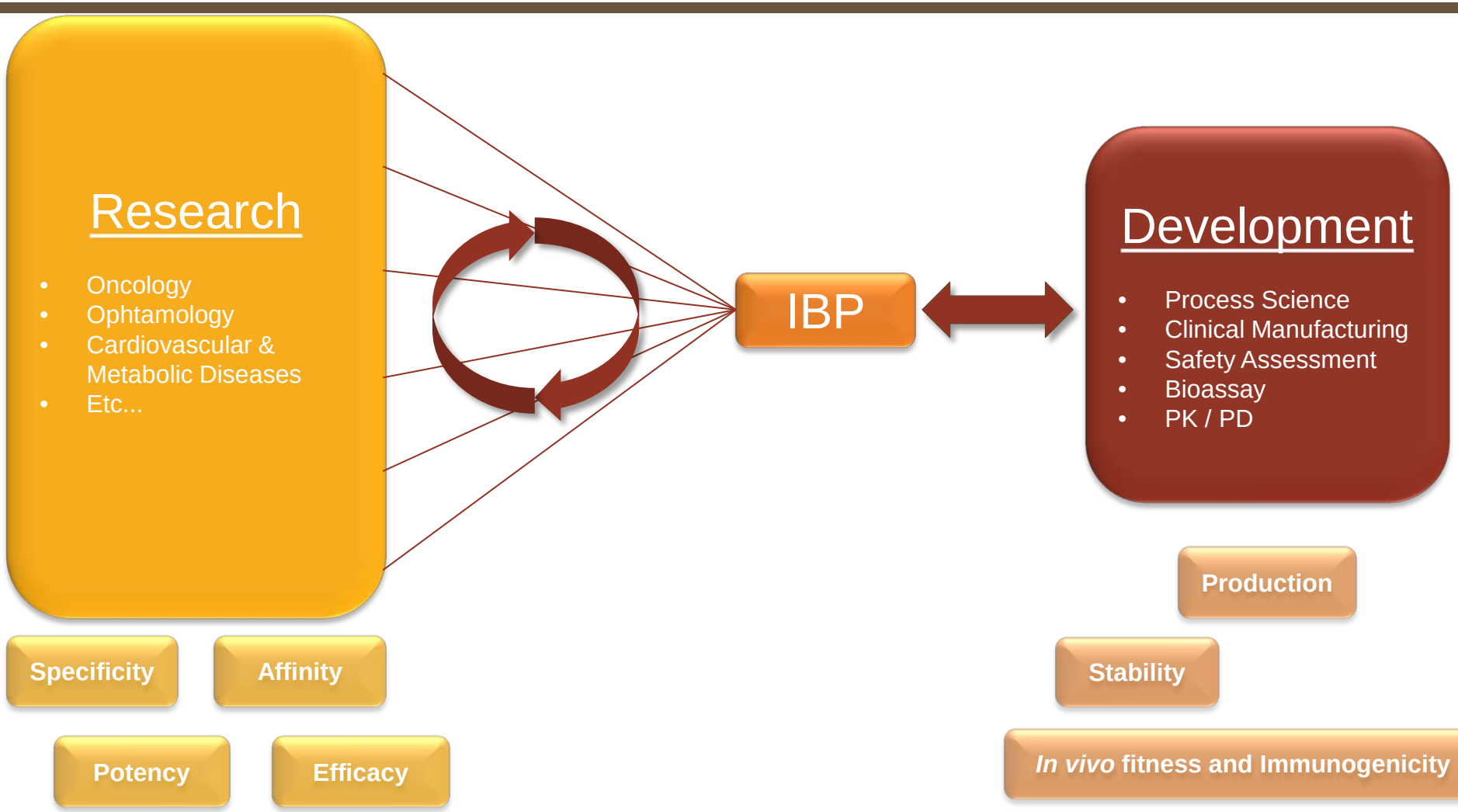
Role of IBP: Major Interfaces and Deliverables



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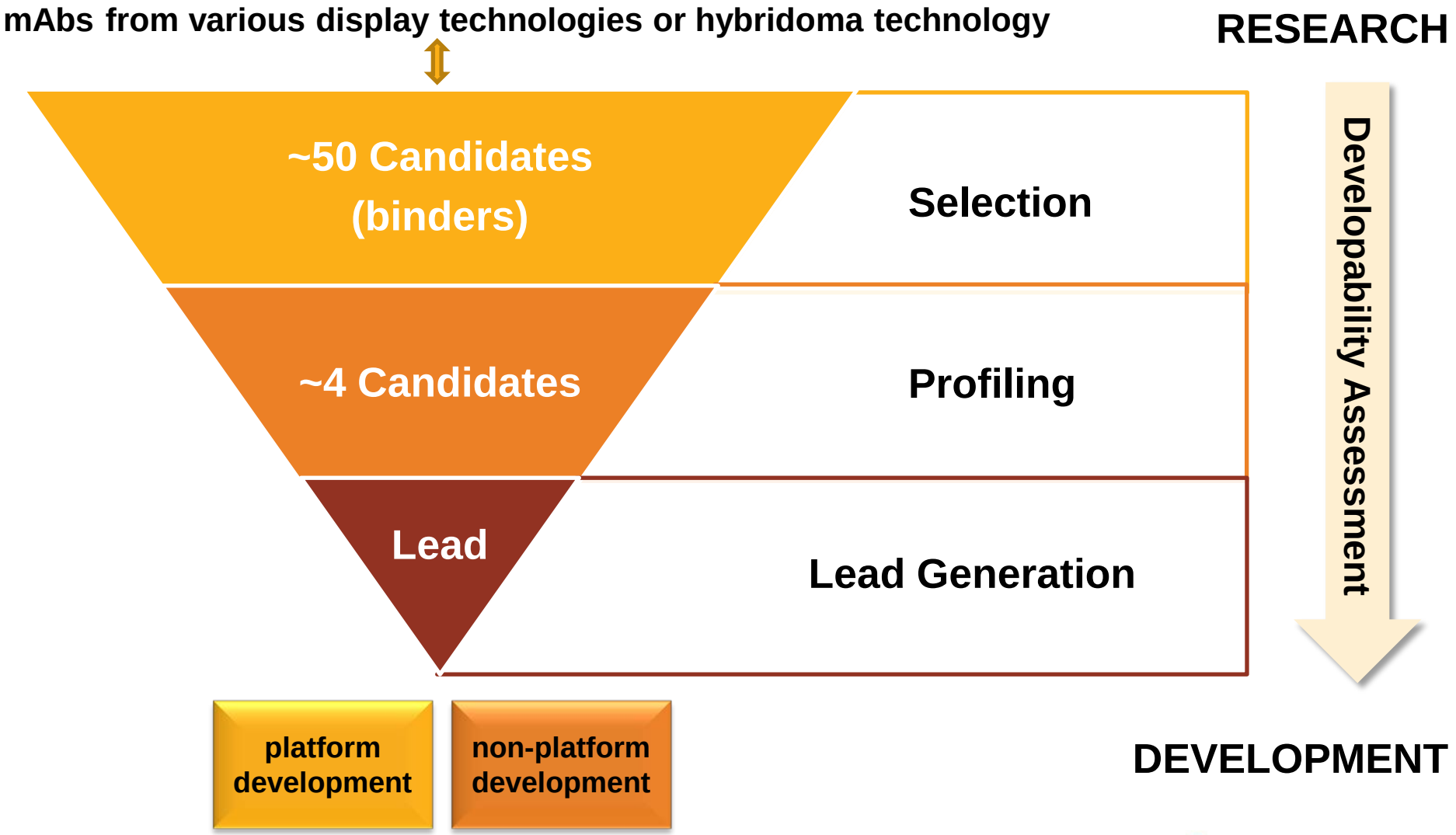
Developability Assessment of Antibodies

A concept for Early Lead Selection



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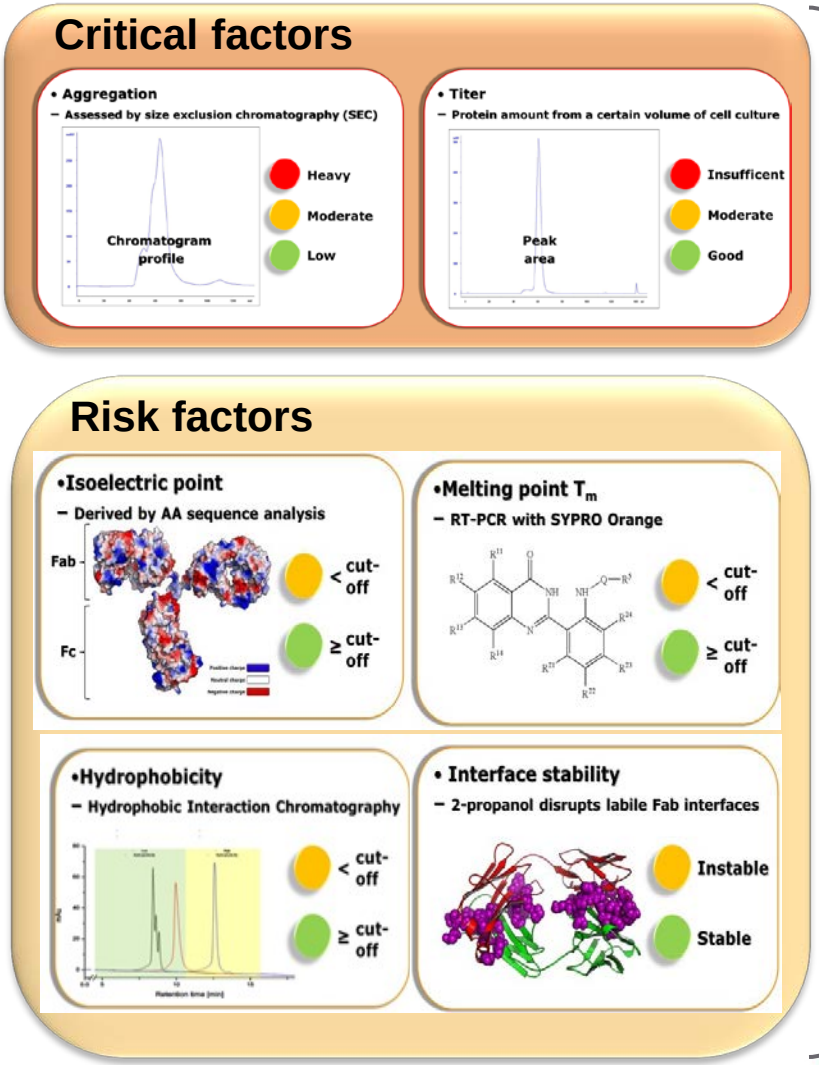
HT Purification and Biophysical Screening

- HT purification of large number of candidates from HEK
- HT Biophysical screening and «traffic light» rating
  - Critical parameters directly affect candidate rating
  - Risk factors affect rating in combination with each other



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Key aspects for Biologics Development and major impact factors

Key Aspects for Development

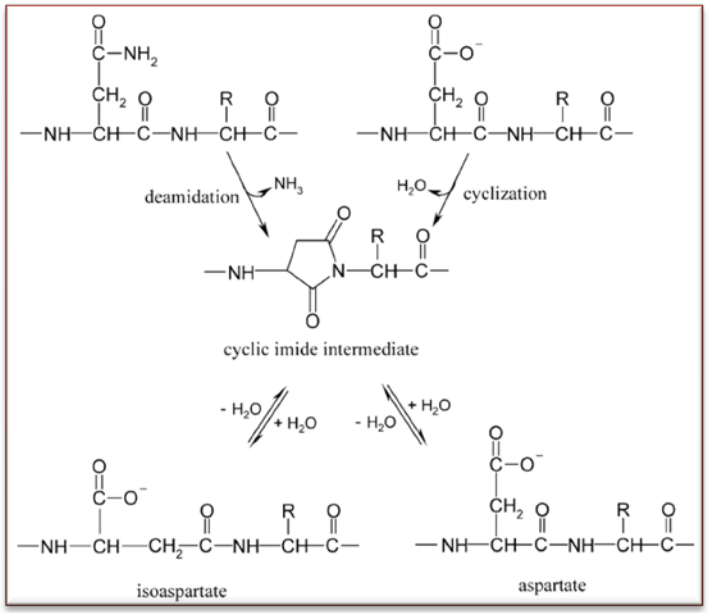
- Manufacturability
  - Acceptable cost of goods for production
- Comparability
  - Process scale up should not alter the product
- Stability
  - Product stable within the shelf-life
- Compatibility
  - Product properties are compatible to the planned route of administration

Major Impact Factors

- Aggregation / Precipitation
  - Irreversible or reversible oligomer formation
- Degradation / Clipping
  - Chemical / Protease dependent decomposition of proteins
- Post-translational modifications
  - Deamidation / Iso-Asp formation / etc.
- Viscosity
  - Reversible formation of higher order protein interactions

Deamidation / iso-Asp isomerization as a stability risk in biologics development

- Major degradation pathway in antibodies
- Potential effect on potency, stability, in-vivo stability, immunogenicity
- Deamidation:
  - Sequence motifs reported in IgGs (Fab, Fc): NG, NS, NN, NT, NA
  - pH dependent : highest deamidation rates at pH >7
- Iso-Asp isomerization
  - Known isomerization motifs in mAbs: DG, DS, DD, DH, DK (DP)
  - Increased kinetics at elevated temperatures and low pH (>5)



W. Zhang, M.J. Czupryn / J. Pharm. Biomed. Anal. 30 (2003) 1479/1490

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Kettenberger et al, Plos One 2014

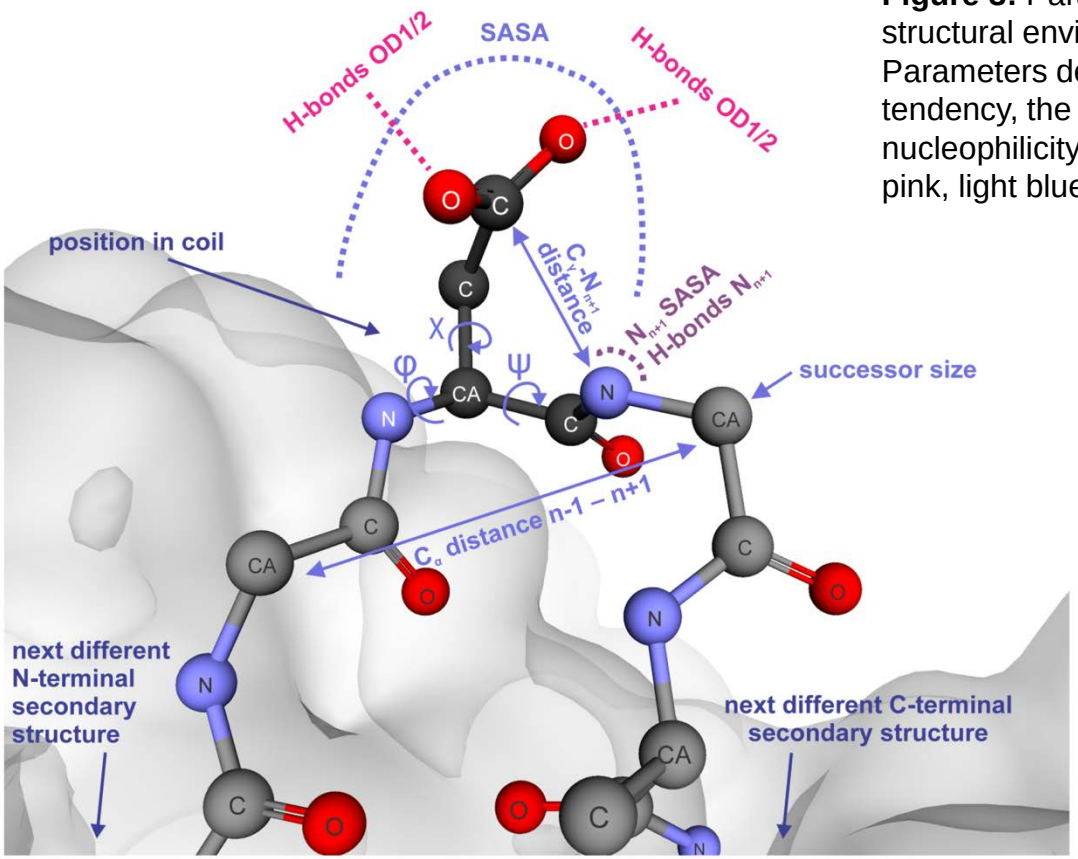

Figure 3: Parameters characterizing Asn and Asp residues in a structural environment outlined at an exemplary Asp residue. Parameters describing the carboxyl/amino group leaving tendency, the transition state accessibility, the N_{n+1} nucleophilicity, and the structural environment are depicted in pink, light blue, purple, and dark blue, respectively

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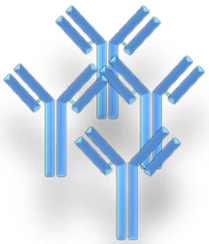
PTM profiling strategy during candidate selection



best binders

PTM site removal engineering

in silico sequence analysis

potential critical PTM sites in CDR?

Selection Phase

no critical PTM sites in CDR

PTM check

- **LC-MS**
- SEC
- CEX
- CE-SDS
- bioactivity

unusual PTM finding

PTM stress testing

- forced deamidation
- forced Asp-isomerization
- forced glycation
- forced oxidation

Profiling Phase

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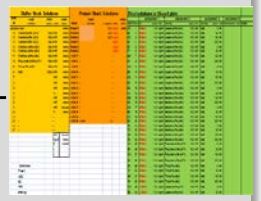
High-throughput Pre-formulation assessment

Setup and Readout

Sample preparation

Liquid handler

Buffer & excipient matrix



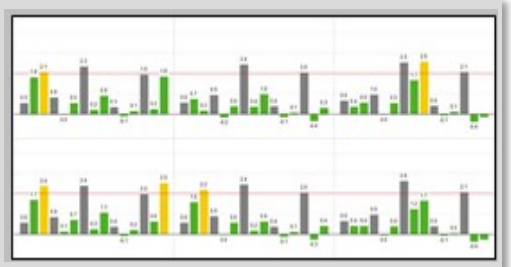
formulations in 96-well format

Stress conditions

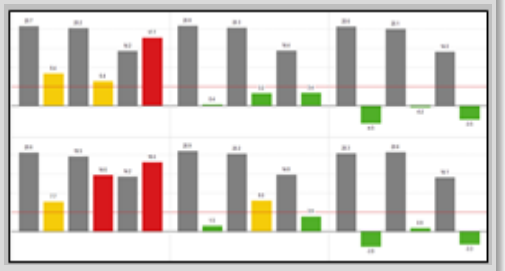
- Thermal
  - Stability assessment under accelerated temperature conditions
- Mechanical stress
  - Freeze/Thaw and Shaking
  - Robustness during handling, shipping, etc.
- Photostress
  - Sensitivity to photo degradation
- Silicon-oil-compatibility
  - Suitability for prefilled syringe development
- ...

Analytics & Candidate Ranking

- SEC
- DLS
- CEX
- Labchip
- MS
- MFI
- ...



Change in AP levels



Change in Charge variants

➢ **Assessment of stability under representative formulation conditions to highlight potential challenges for Drug Product development**

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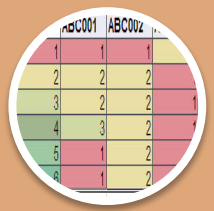
Mass Spectrometry for early biologics development

Candidate Screening

Candidate Characterization



Developability Assessment

HR Mass Spectrometry

- ID check / confirmation
- cell line development

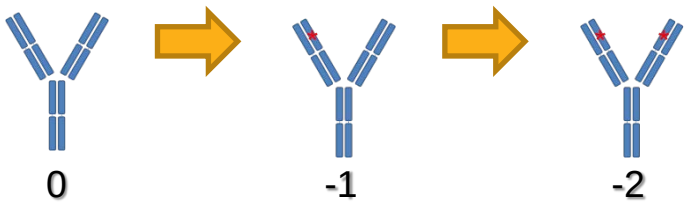
- sequence confirmation
- chain assembly
- clipping / degradation products
- **PTMs / deamidation**
- drug-antibody-ratio (DAR)
- in-process control

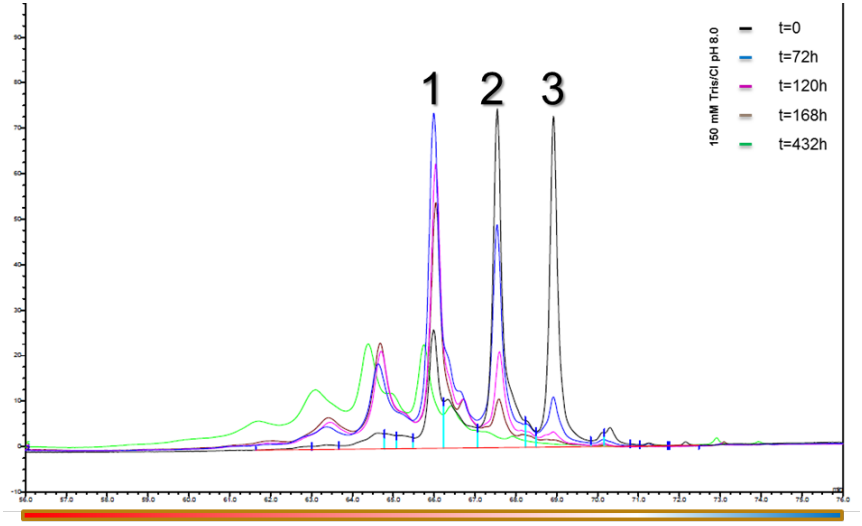
- **pre-formulation / stability testing**
- PTM stress tests
- serum stability (PK assays)
- stability in cell culture (clipping studies)

Deliver rapid qualitative and semi-quantitative information about a large set of different biologics for candidate selection

Case study 1: Analytical CEC time-course profile reveals presence of deamidation

- Peak 3: Main peak
- Peak 2: Single HC deamidated
- Peak 1: Both HC's deamidated





Time dependent deamidation stress test analysis by CEC / salt gradient

- Strong tendency to accumulate acidic variants → Indication for deamidation
- Confirmed by LC/MS-Pepmap
- Strong impact on potency assay of deamidated variants

Sample	Potency (%)
Bioassay reference (cell pool)	100
Peak 3 - Main peak	~ 120
Peak 2 - (single HC deamidated)	~ 100
Peak 1 - (both HCs deamidated)	~ 60

Rel. potency of isolated peaks (prep. CEC)

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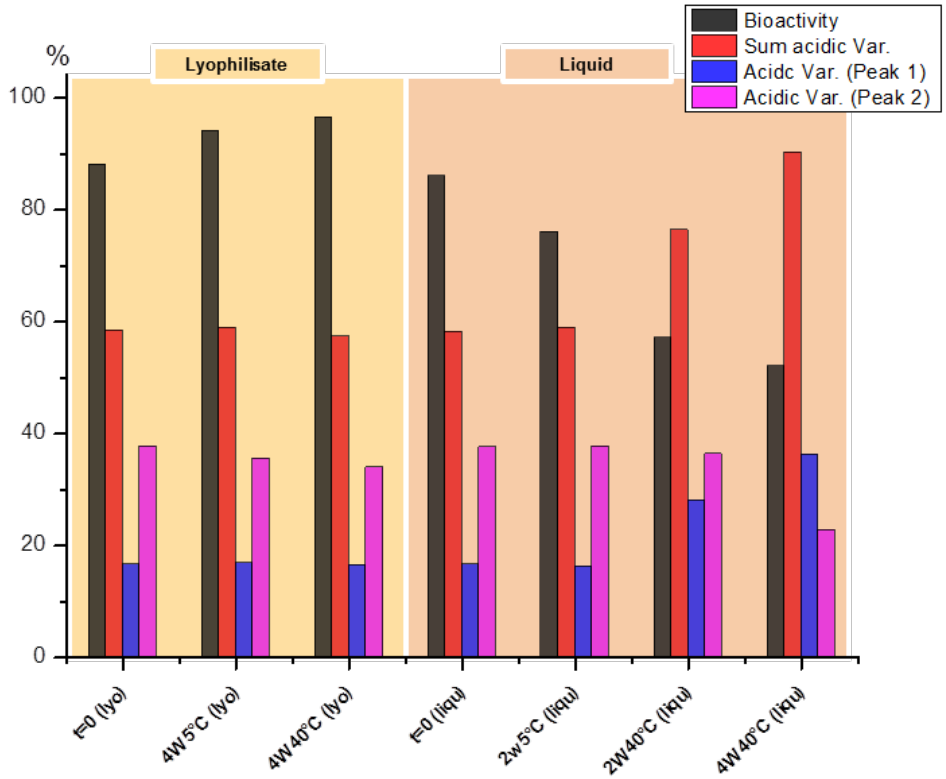
Formulation stability data

- Effect of deamidation on relative potency
- Deamidation susceptibility impacts liquid formulation development significantly

➔ Recommendation to the team:

Removal of deamidation site from primary sequence



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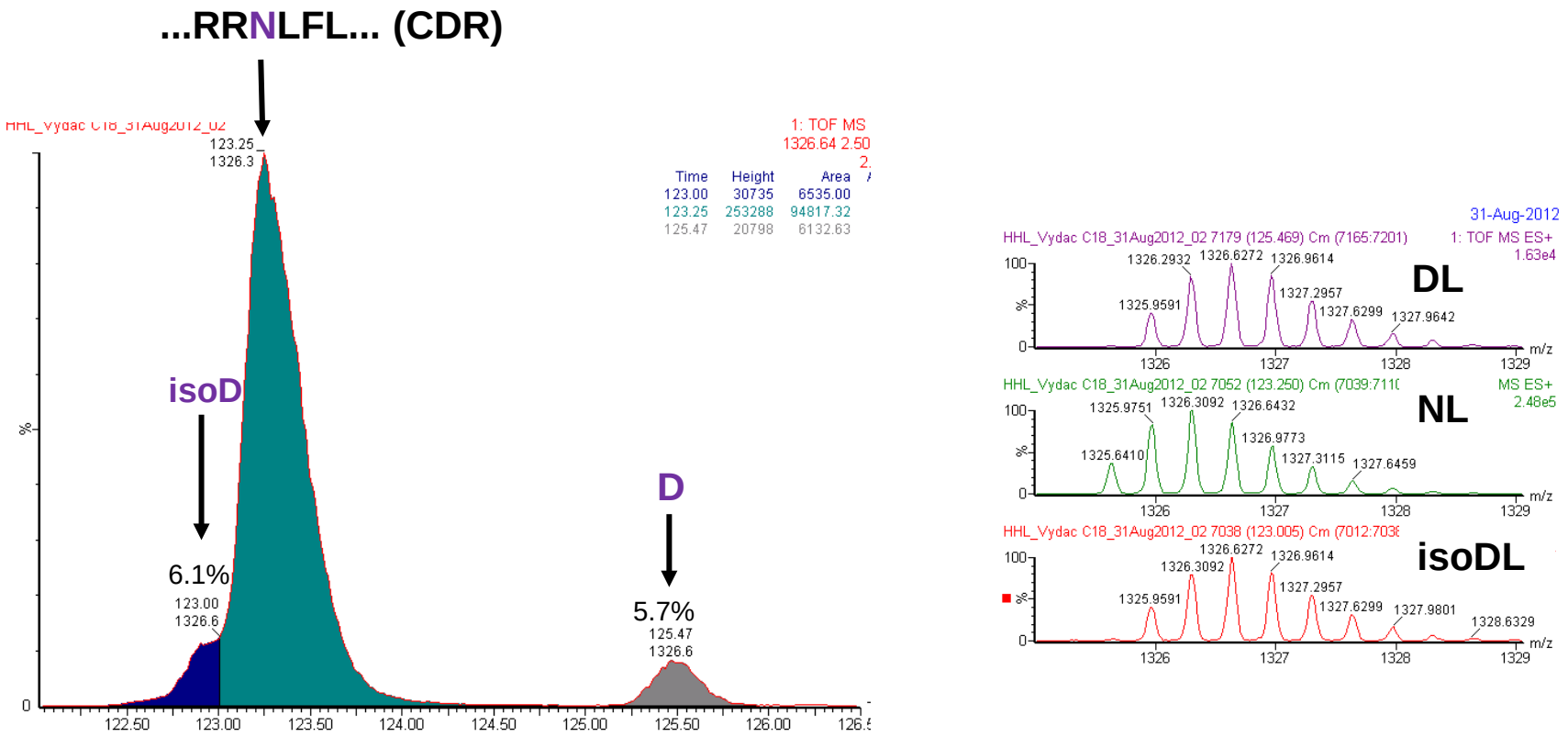
Case study 2: Observation of unusual deamidation site in CDR

Extracted ion chromatogram



Deamidation susceptibility to be evaluated in stability samples from formulation assessment

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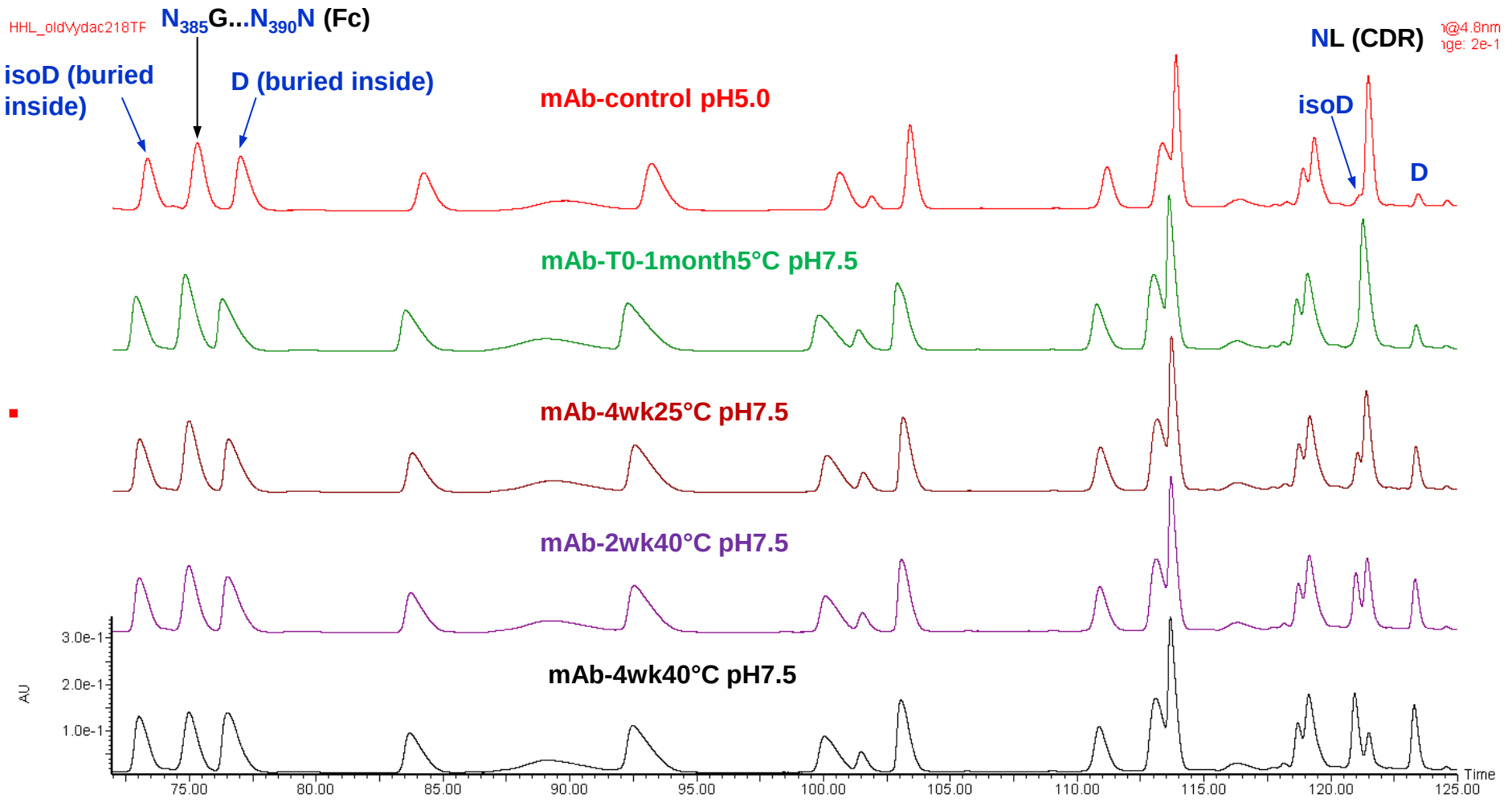
LysC LC-MS peptide mapping UV trace overlay



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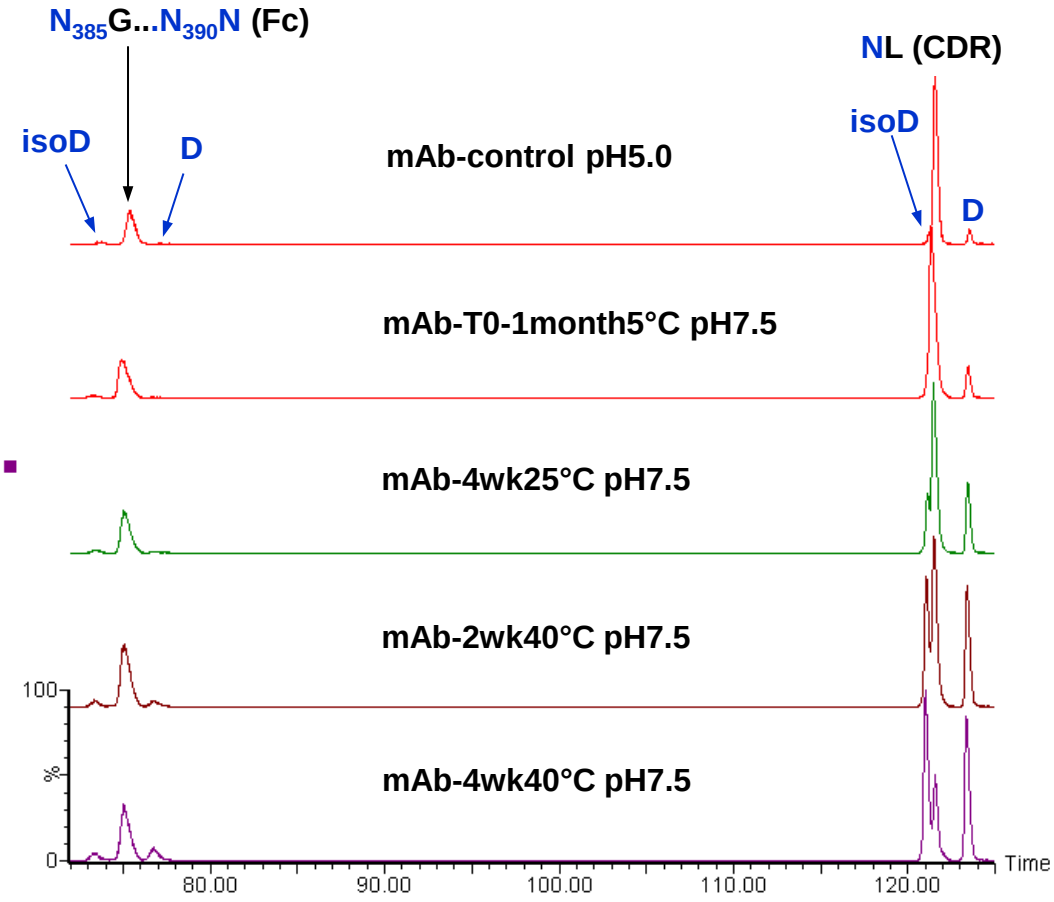
CDR deamidation after thermal stress @ pH 7.5

LysC peptide mapping – Extracted ion chromatogram



Samples	%deamidation at NL (CDR) via MS EIC	%deamidation at $N_{385}G...N_{390}N$ (Fc) via MS EIC
mAb-control pH5.0	**11.4**	**8.1**
mAb-T0-1month5°C pH7.5	**16.2**	**8.1**
mAb-4wk25°C pH7.5	**37.9**	**10.7**
mAb-2wk40°C pH7.5	**55.6**	**15.7**
mAb-4wk40°C pH7.5	**77.9**	**25.9**

Deamidation at NL site significantly increased under thermal stress

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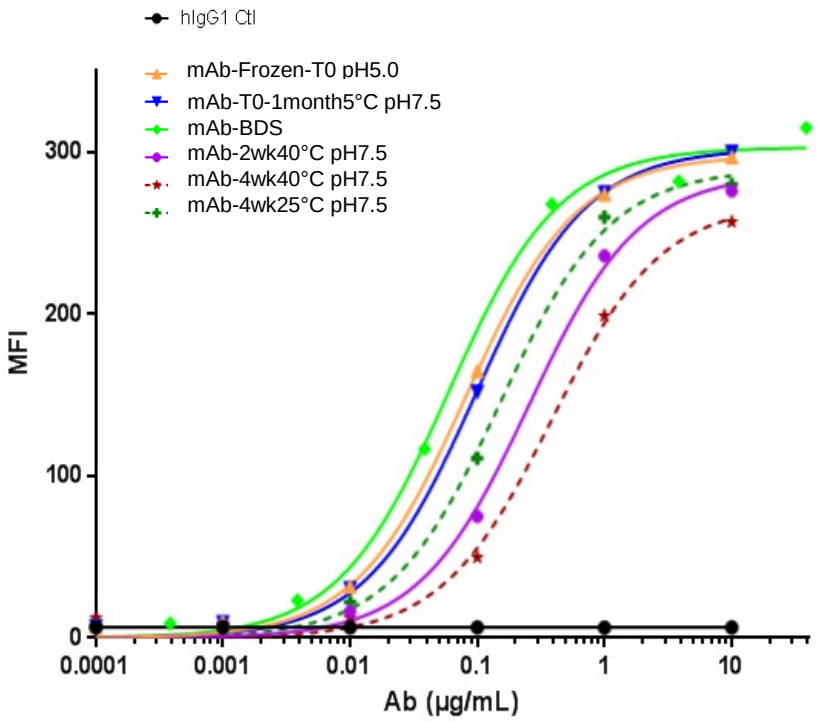
Risk assessment mAb NL (CDR) deamidation

Effect on potency and serum stability

Cell-based binding assay

Sample	Deamidation at NL (CDR)	Cellular Kd (nM)
mAb-BDS	ND	0.059
mAb-control pH5.0	11.4%	0.081
mAb-T0-1month5°C pH7.5	16.2%	0.098
mAb-4wk25°C pH7.5	37.9%	0.150
mAb-2wk40°C pH7.5	55.6%	0.254
mAb-4wk40°C pH7.5	77.9%	0.382



Large drop in binding affinity

Stability in Cyno serum 37°C

Decreased stability

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Case study 3: Forced deamidation testing after in-silico analysis of critical PTM sites

- *In-silico* analysis of CDRs of a favored antibody candidate reveals a Asn-Ser (NS) deamidation motif in the heavy chain CDR2 region

- Asn cannot be removed during antibody engineering without loss of function

- **risk mitigation:** forced deamidation stress test

➡ thermal stress at 40 °C and pH 8.0 for 0-2 weeks

➡ LysC peptide mapping UPLC-MS

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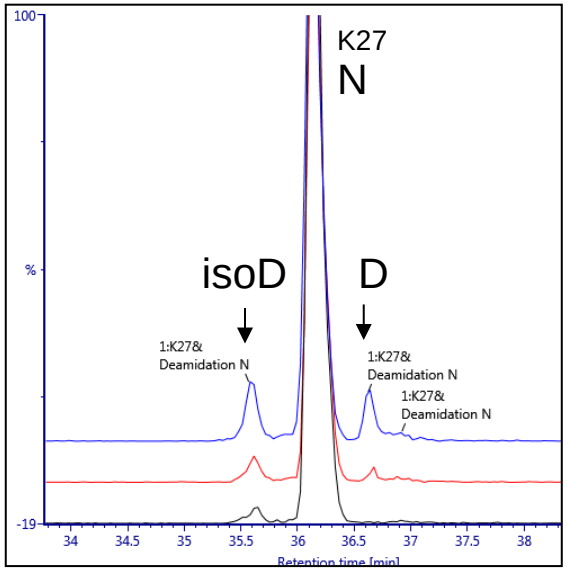
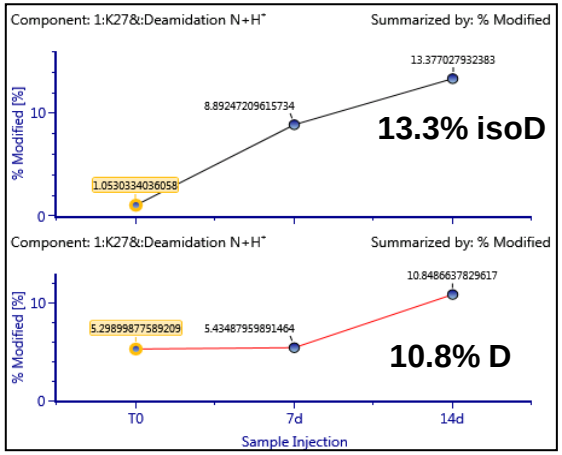
Deamidation levels after thermal stress at pH 8.0

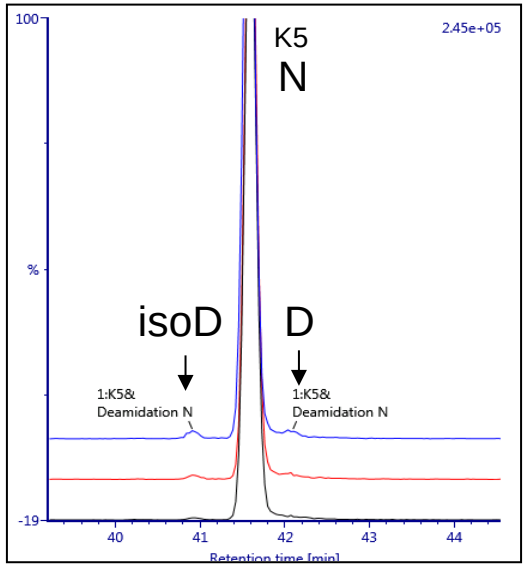
LysC Peptide mapping

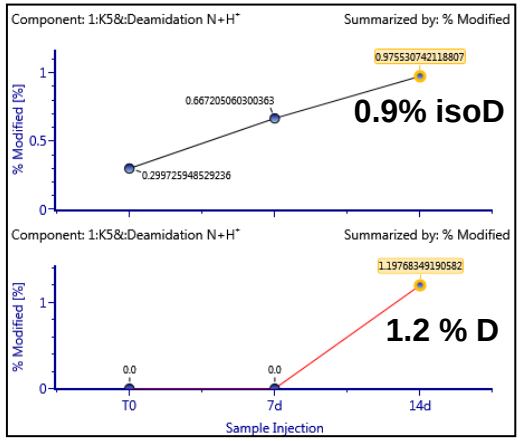
control Fc peptide MS chromatogram





HCDR2 peptide MS chromatogram





Waters UNIFI 1.6 software

14d, 40°C, pH 8

7d,40°C, pH 8

t0

low deamidation level in HCDR2 after stress:

no developability risk (PTM)

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Summary

- Developability assessment is a concept that can help to optimize timelines and costs during biologics development
- Deamidation and iso-Asp formation can have significant impact on the potential shelf-life of a biologics and needs special focus in formulation and analytics
- Stress testing or high throughput formulation stability testing helps to understand the susceptibility of potential post-translational modification motifs

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Stefan Wildt
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Thorsten Lorenz
Torsten Kuiper
Tugce Nur Tas
Yu Tang
... and many more!!