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RA CMC, TRD

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Stability Modeling For Biotherapeutics – a Company Perspective and Case Studies

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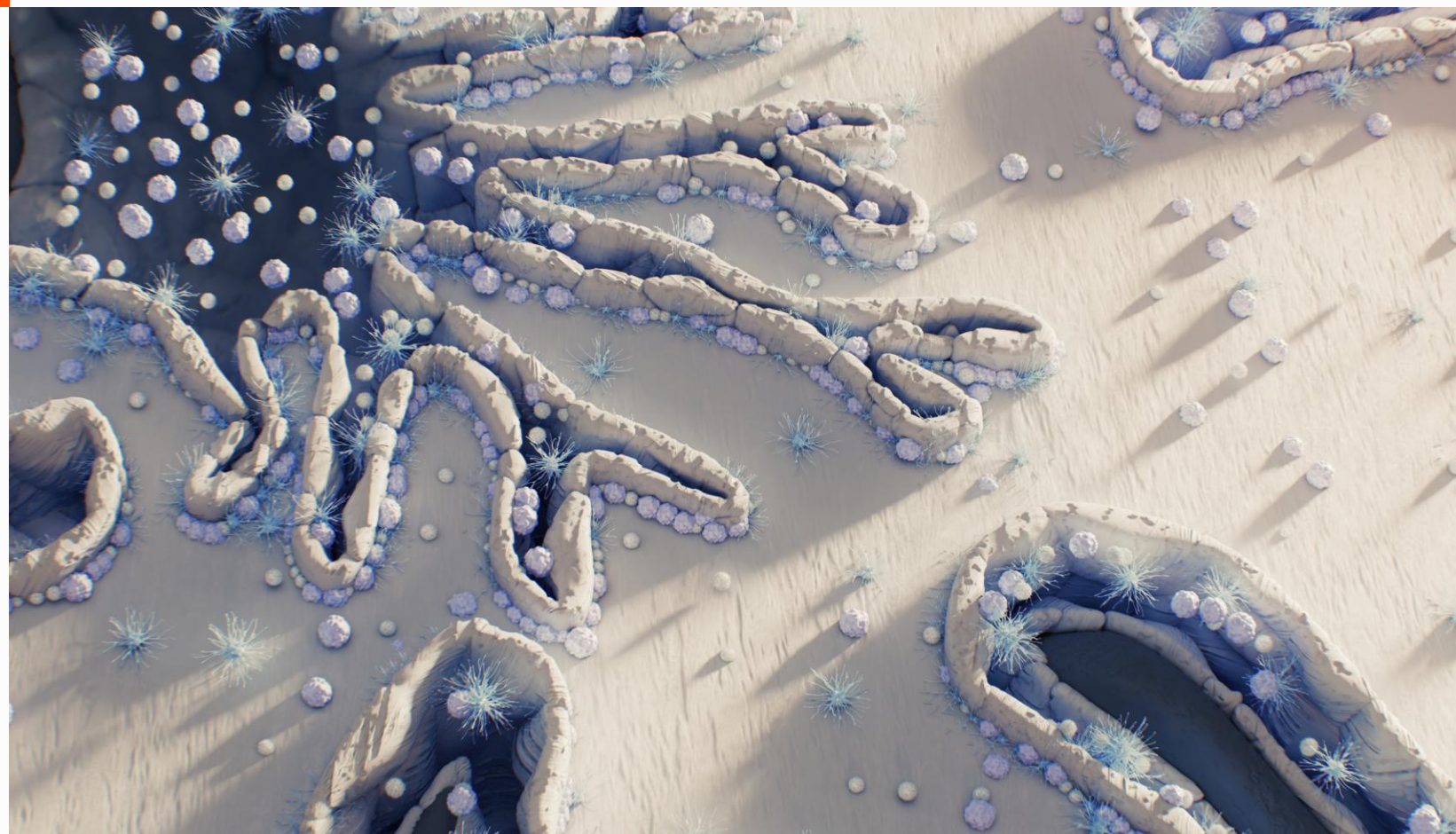

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Agenda

1. **Introduction To Advanced Kinetic Modeling**
2. **Difficult to Model Quality Attributes**
3. **Regulatory applications – with use cases**
4. **Conclusion**

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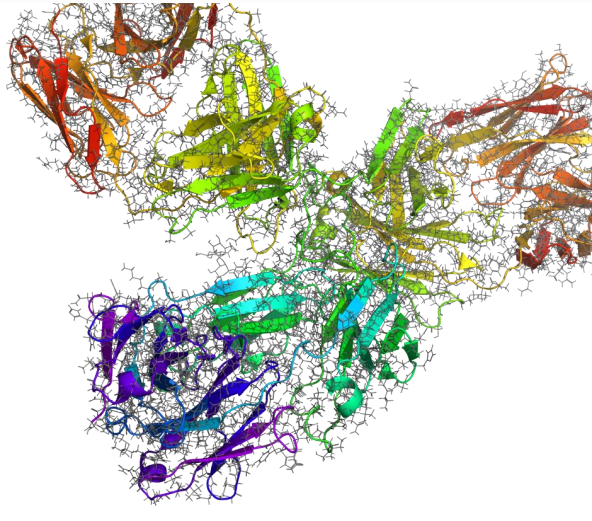
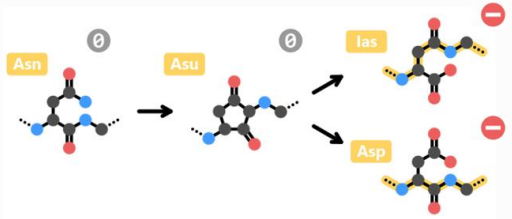
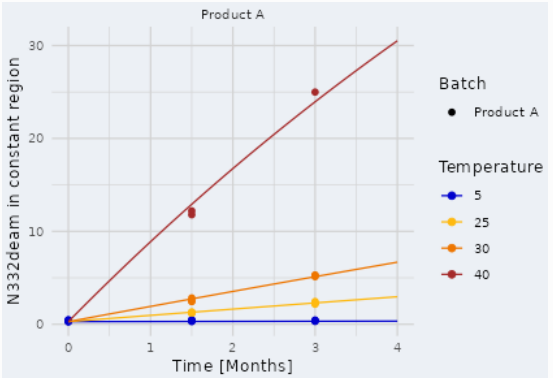
FOCUS : Biotherapeutics - well-characterized Biologics
(e.g. CHO cell expressed mAb-like proteins)



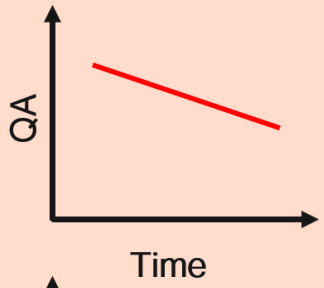
- **Regulatory framework:** The revised ICH Q1 draft guideline, including **Annex 2**, supports alternative modelling approaches for shelf-life extrapolation beyond real-time data for Bx.
- **Advanced kinetic modelling:** Demonstrated as a suitable approach to assess the evolution of multiple Bx CQAs over time, with recognized limitations (e.g. limited change over time)
- **Complementary evidence:** When modelling is not applicable, surrogate or complementary approaches are used, including prior knowledge, platform data, risk assessment, and alternative stability studies.
- **Holistic stability assessment:** Drug stability is evaluated through multiple orthogonal approaches, including molecular modelling of potential liabilities, forced degradation studies, formulation robustness assessments, advanced analytics such as multi-attribute methods to follow degradation of relevant amino acids, and advanced kinetic modelling.





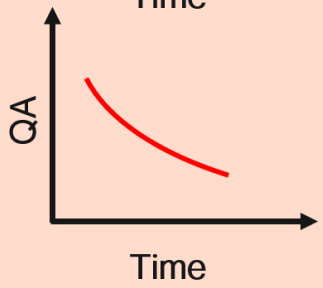
Advanced Kinetic Modeling – basic concepts

Degradation Kinetic Equations



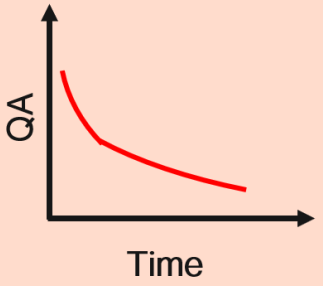
Zero order (linear) model

$$\frac{dQA}{dt} = -k(T)$$



First order (exponential) model

$$\frac{dQA}{dt} = -k(T) \times QA$$



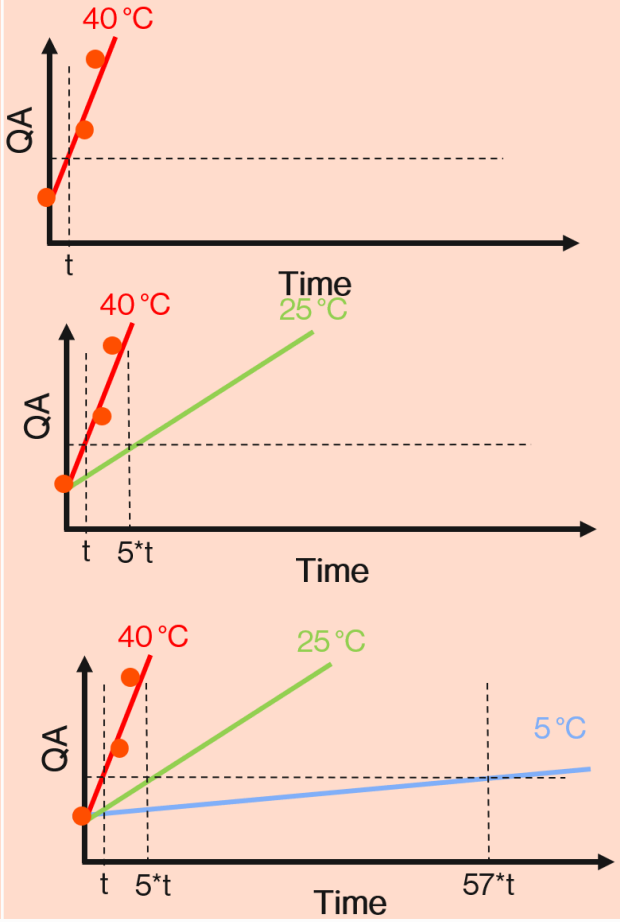
N-order two step model

(Šesták–Berggren equation)

$$\frac{dQA}{dt} = v \times k_1(T) \times (1 - QA_1)^{n1} \times {QA_1}^{m1} \times C^{p1}$$

$$+(1 - v) \times k_1(T) \times (1 - QA_2)^{n2} \times {QA_2}^{m2} \times C^{p2}$$

Extrapolation in Temperature by Arrhenius



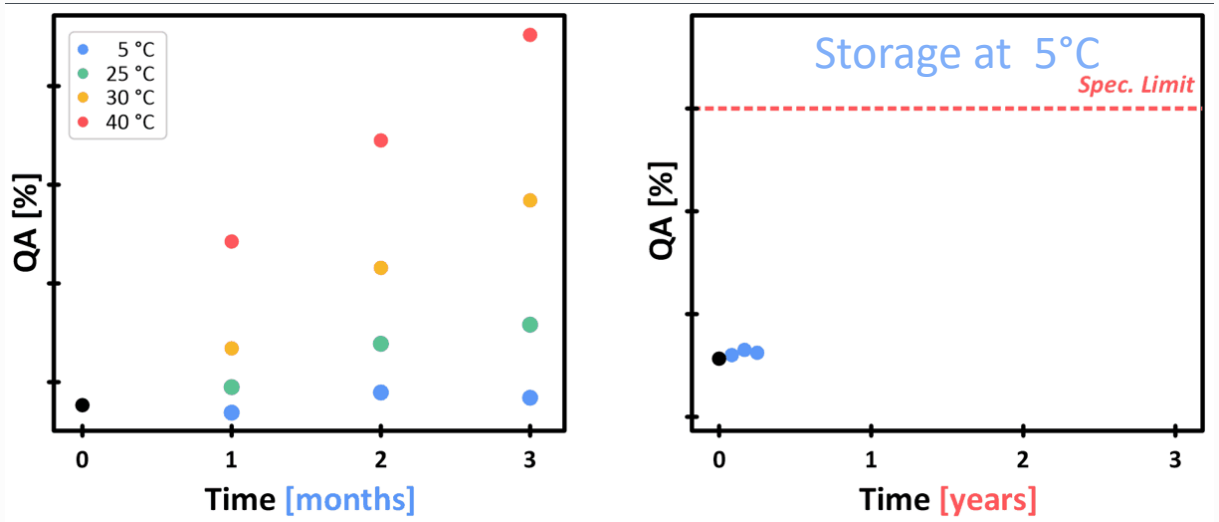
$$k(T) = A \cdot exp^{-\frac{E_a}{R \cdot T}}$$

Temp	Time Factor*
40°C -> 25 °C	5
40 ˚C -> 5 °C	57
25°C -> 5 °C	11

*computed for E_a=20 kcal/mol

Accelerated Predictive stability (APS)

Modellable stability indicating CQAs | **Non-Modellable stability indicating CQAs**



Risk Assessment
based on prior data and knowledge

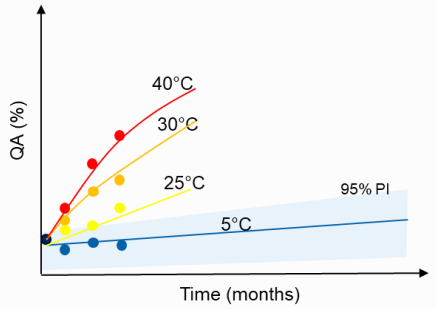
1. **Extrapolation in time** using the **Arrhenius** model is applicable to most degradation processes of biotherapeutics.
2. For monoclonal antibody-like molecules, most degradation profiles can be described using **first-order, exponential kinetic** models

$$k(T) = A \cdot exp^{-\frac{E_a}{R \cdot T}}$$

$$QA(t,T) = (QA_{t\infty} - QA_{t0}) * \left(1 - e^{-k(T)*t}\right) + QA_{t0}$$

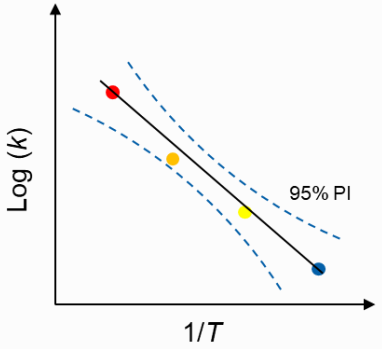
The Toolbox: example for FIH supporting shelf life claim

Model training | Model validation | Shelf life | Shelf life verification

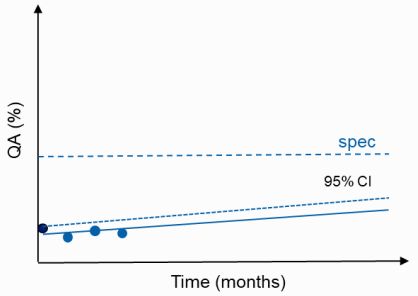


	t0	1 M	2 M	3M
5 °C	X	X	X	X
25 °C		X	X	X
30/35°C		X	X	X
40 °C		X	X	X

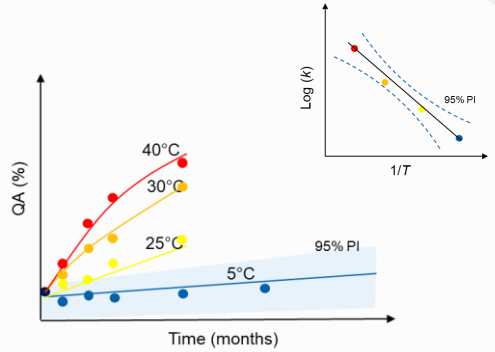
Tox batch



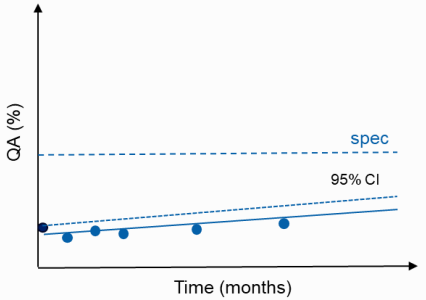
- Goodness of fit
- Arrhenius behavior



Shelf-life assignment for clinical batch based on confidence interval



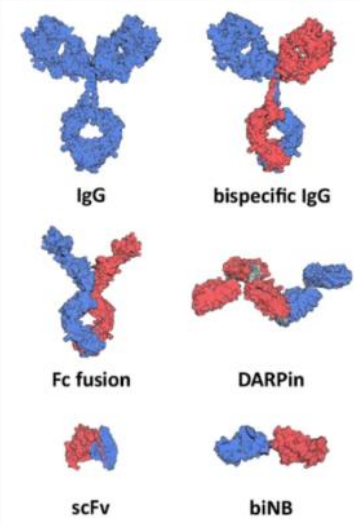
Model re-training by all NEW data
Model re-evaluation



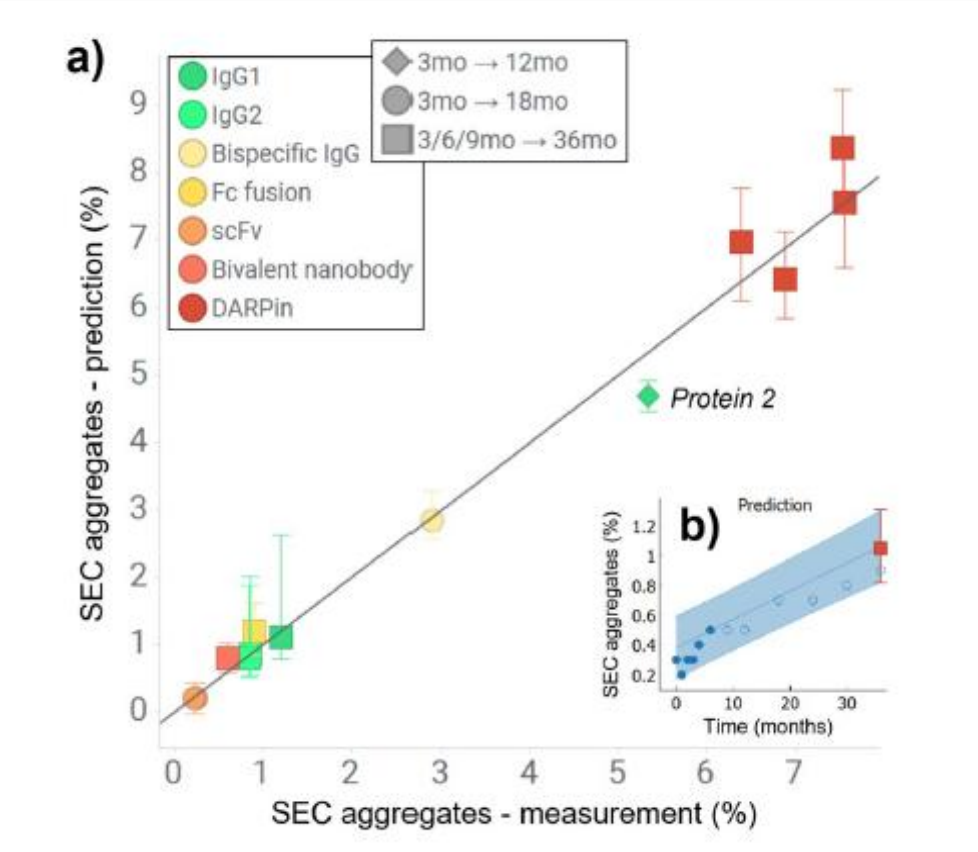
Shelf-life re-evaluation

Risk Assessment for non-modellable CQA

Aggregation Can Be Reliably Modeled



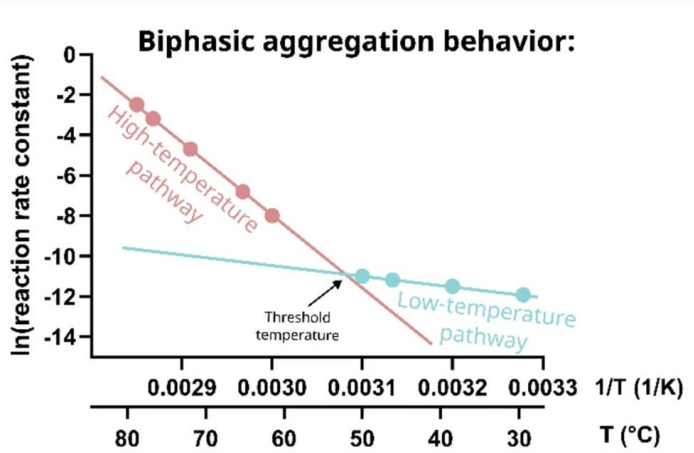
Long-term prediction at 5°C based on short term accelerated stability study



Zidar et at., Scientific Reports 2025, https://doi.org/10.1038/s41598-025-07037-y

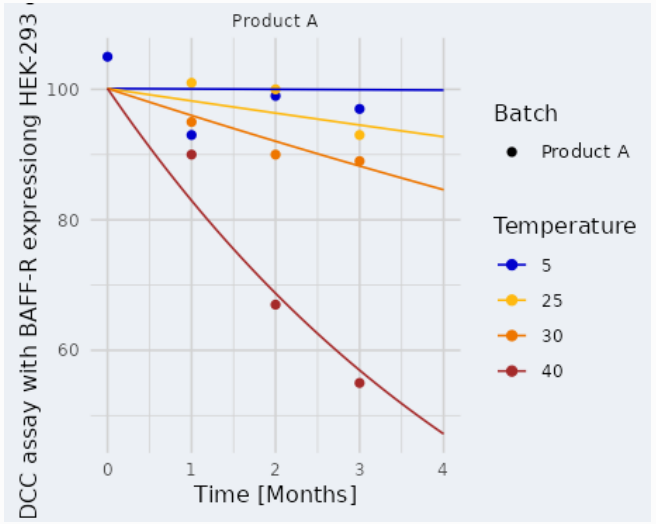
For robust Aggregation modeling it is key to know temperature threshold value allowing you to train the model by using a data from low temperature pathway.

Temperature threshold for mAb like formats is typically >40° C, for bi-valent nano body >35° C and for ScFv and DARpin >30° C.

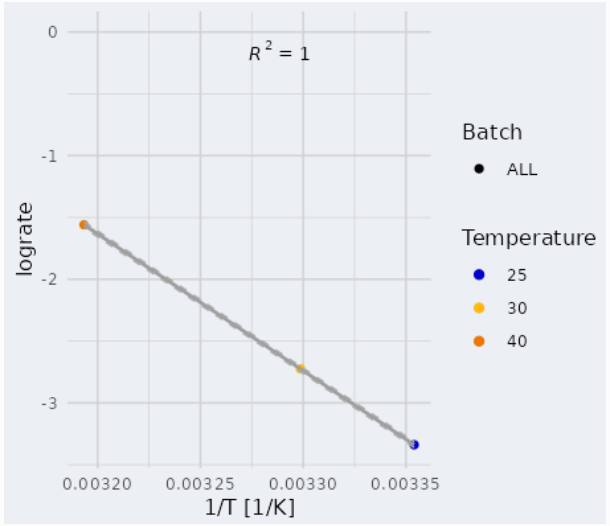


Cell Based Potency Assay- ADCC

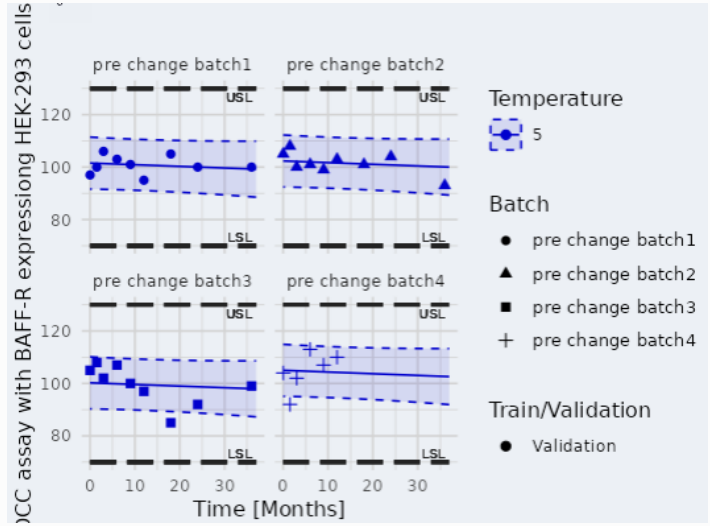
Model training by 3 months data

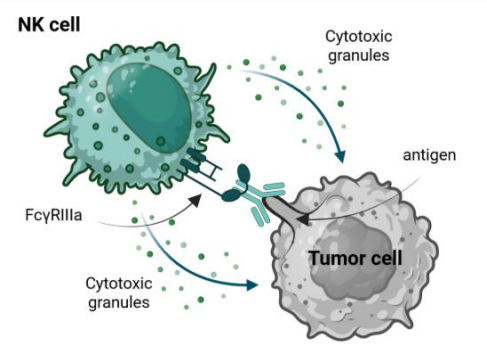


Arrhenius behavior



Prediction Validation





Cell-based assays can often support reliable prediction models despite inherent method variability. In contrast, target-binding assays such as ELISA are often not stability-indicating. In case of "no change" behavior at accelerated conditions data are not suitable for Arrhenius based kinetic modeling.

Risk assessment for stability-indicating Quality Attributes that cannot be modeled

Parameter	Risk assessment
General	All parameters are assessed during formulation robustness studies. Confidence from extensive platform knowledge that these attributes remain within specified limits during storage. Using the APS approach for shelf-life assignment does not increase the risk for out-of-specification events compared to the classical approach.
Appearance (turbidity)	A change of appearance during storage might indicate protein self-association. This risk is assessed already during formulation robustness testing. Formation of non-reversible aggregates would be observed by Size-exclusion chromatography or as sub-visible particles.
Color	A change of the color of the solution during storage might indicate degradation, e.g., linked to a reaction between protein and sugars. This risk is assessed during formulation robustness testing. Relevant degradation would also be observed in purity tests.
pH	The drug product pH is tested during stability studies, but does not change as the protein is kept in a buffered solution.
Protein content	The total protein content is tested during stability studies but does not change during storage in a tightly closed container.
Container Closure integrity	Container Closure Integrity is monitored annually under long-term storage condition. This is supported by He-Leak testing of the empty container and in-process controls. Sterility ensured during manufacturing. Confidence due to platform experience from using the same container closure system for multiple projects.
Sub-visible particles	Increase of sub-visible particles above acceptable limits during storage are rare events, e.g., linked to processing, container or excipient instability.
Visible particles	Visible particles are sporadic events, e.g., linked to processing, container or excipient instability. Mitigation by robust manufacturing experience with 100% visual control and AQL testing.

Regulatory applications: Alternative approach – holistic view

Key considerations when using Accelerated Predictive Stability (APS)

- **Use of front-runner batch(es) data** to establish model
- **Model Suitability** : Model justification, adequate data selection to build model, understanding degradation pathways, goodness of fit, Arrhenius assumption confirmation and model validation
- Conservative predictions (95% statistical intervals) and independent test/validation data sets
- **Addressing CQAs** and attributes that cannot be modelled
- **Leverage prior knowledge** alternative surrogate, increased experimental testing
- **Integrated Control Strategies** : connect to control strategy incl. process perf. indicators and expected oxidation
- **Risk Mitigation** : stage appropriate risk-based scientific assessments as part of a holistic assessment
- **Continuous model Verification** until end of shelf-life and check protocol adherence

Why is it meaningful for regulatory applications ?

Alternative approach – Case 1: approach for phase 1

- **Divergent regulatory expectations for Ph1** for minimal stability data and shelf-life claim (US, JP vs others)
- **Step 1**: systematic science-based driven shelf-life assessment using modelling
  - ➢ **Additional stability conditions** + **MORE timepoints** earlier + **complementary risk assessment** for alternative approach
- **Step 2**: Define regulatory strategy : modelling only informative or supportive of alternative approach?
- **Step 3**: Adapt stability submission to divergent HA requirements (+/- data +/- shelf-life claim)

Ph1	Stability protocol – standard approach	Stability protocol – Novartis alternative approach
CTA content	3 stability conditions	4 stability conditions – **more total timepoints**

Tox	t0	1 M	2 M	3M	6M
5 °C	X	X	X	X	X
25 °C		X	X	X	X
30 or 40°C		X	X	X	X

CB	t0	1.5 M	3M (for some countries)
5 °C	X	X	X
25 °C		X	X
30 or 40°C		X	X

Tox	t0	1 M	2 M	3M	6M (for some countries)
5 °C	X	X	X	X	X
25 °C		X	X	X	X
30/35°C		X	X	X	X
40 °C		X	X	X	X

CB	t0	1 M or 1.5M (for some countries)
5 °C	X	X
25 °C	X	X
30/35°C	X	X
40 °C	X	X

➢ **Benefit: Acceleration of submission and first patient first visit by 4-8 weeks** (clinical supply agility +/- lengthier initial shelf-life)

Some regional regulatory challenges due to inconsistency with regional clinical guidelines : **engage early with HA**

Case 2: Ph3 CTA for new DP strength with future sBLA/LE

Commercial PFS in auto-injector (PFS-AI): new DP strength PFS-AI (new syringe + new AI)

Step 1: new DP strength Ph3 CTA (US IND amendment):

submission with limited stability on Tech Batch + Clinical Batch release + APS + comparability btw DPs

- accelerating CT start with **comparability > prior knowledge > APS**

➢ **Alternative approach submission achieved with clinical trial start accelerated by 6 months**

Step 2: new DP strength future s-BLA / Line Extension (LE)

- US/EU submission with 3 PPQ batches with limited stability data (<<6M)
- Same APS model already verified for clinical batch till EO shelf-life
- No increased risk while anticipating submission with proven comparability
- Subject to prior HA consultation

➢ **Alternative approach could accelerate sBLA/LE submission by 4 months**

Stability modelling in submissions – eCTD Template

- **Developed for all early phase biotherapeutics**

- **Submitted in P8 or P81**: APS chapter included in stability reports
- **Level of detail** sufficient to understand the science :
  - ✓ choice of model
  - ✓ model definition
  - ✓ model validation (goodness of fit, Arrhenius behavior)
  - ✓ conclusion on shelf-life
  - ✓ ongoing verification (e.g. post approval)
- **Risk assessment** included for non-modelled CQAs
- Detailed APS report with full stats available for HA questions

Stability Report in P8

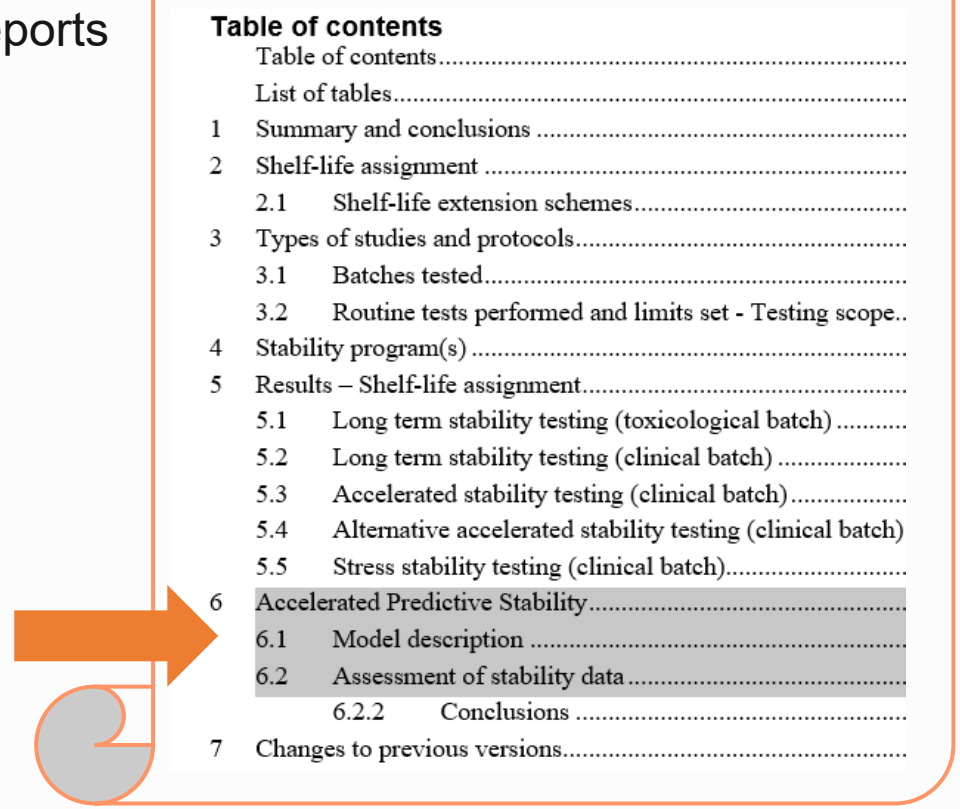


Table of contents

Novartis Overview of modelled CQAs - dev. stage and purpose

	bsAb	tsAb	bsAb	bsAb	IgG1	bsAb	IgG1	IgG1	IgG1	IgG1
Phase **/ APS Regul. submission use**	Ph1 / **CTA**	Ph1 / **CTA**	Ph1 / **CTA**	Ph1 / **CTA**	Ph1 / **CTA**	Ph 2 / **Internal**	Ph3 / **BLA**	Ph3-BLA **Internal**	Ph3 / **BLA**	LCM Ph3 / **Line Ext.**
Purity by rCE-SDS	3	3	6	9	9	12	-	36	18	36
Purity & fragments nrCE-SDS	3	3	6	9	9	12	-	36	18	36
Aggregates by SEC	**3**	3	6	9	9	12	-	36	18	36
Charge variants	3 (bp)	3 (bp)	6 (bp)	9 (bp)	9 (bp)	12 (bp)	-	36	18	36
ELISA	3	-	6	no change	no change	-	-	no change	no change	-
Cell based Assay	-	3	-	-	-	12	-	36	-	36
Multi Attribute Method	-	-	-	-	-	12	-	39	-	-
Candidate selection	x	-	-	x	-	-	-	-	-	-
Formulation & Packaging dev.	x	-	-	x	x	-	x	x	-	x
Comparability	-	-	-	-	-	-	-	x	-	x
Shelf-life with APS	**x**	**x**	**x**	**x**	**x**	**-**	**-**	**-**	**x**	**x**
Out-of-fridge study	-	-	-	-	-	x	-	-	-	x
Control strategy	-	-	-	-	-	-	x	-	x	-

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(bp) - Sum of basic peaks does not follow simple first order kinetic, but can be modeled by Charge variants model

Stability predictions are validated by long-term stability data indicated by numbers in months

5 - Conclusions

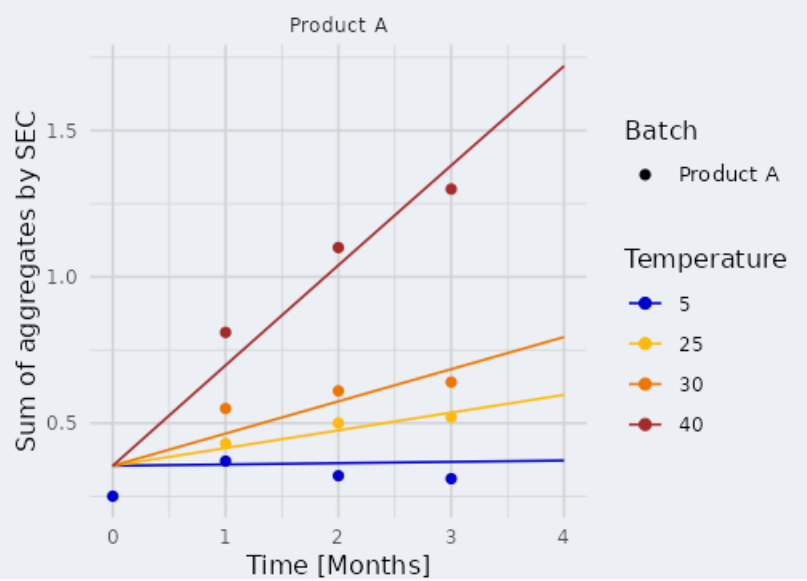
- Stability modelling is a **useful tool** to complement scientific product understanding
  It simulates quality attribute change during long-term storage
- **Combined with molecular understanding** - it enhances risk-based development decisions
- Allows **higher prediction factors for shelf-life** assignments
  more robust assessments of comparative stability studies or investigations of temperature deviations
- High potential to **accelerate patient access** and improve the product control strategy,
  without increasing the risk to patients compared to standard approach
- Stability modelling **informs product understanding**, leads to faster development
  and ultimately to more robust regulatory applications

Thank you

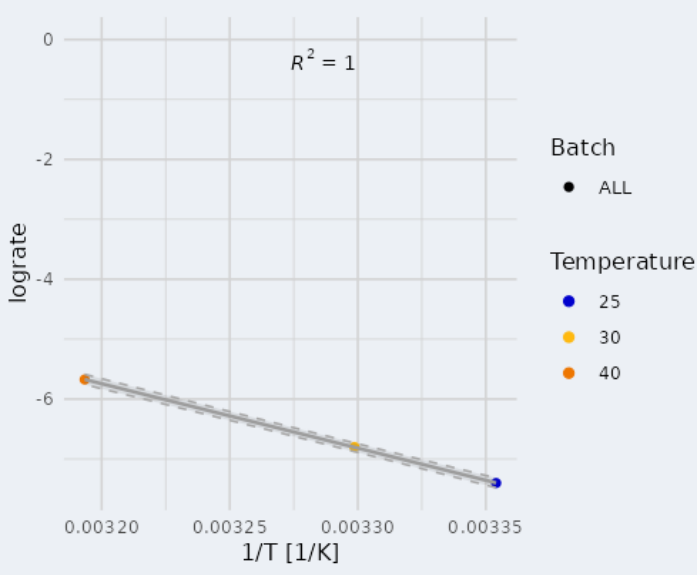
Backup

Aggregation

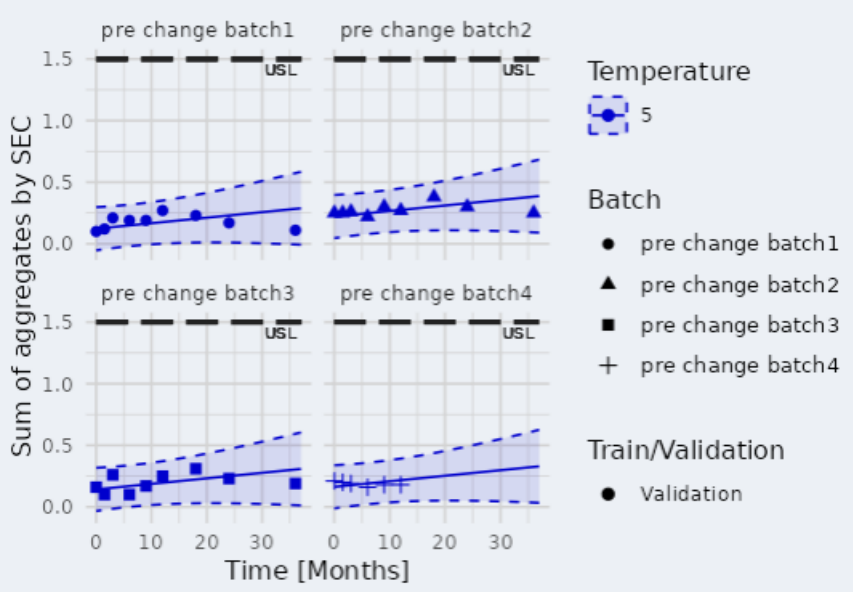
Model training by 3 months data



Arrhenius behavior



Prediction Validation

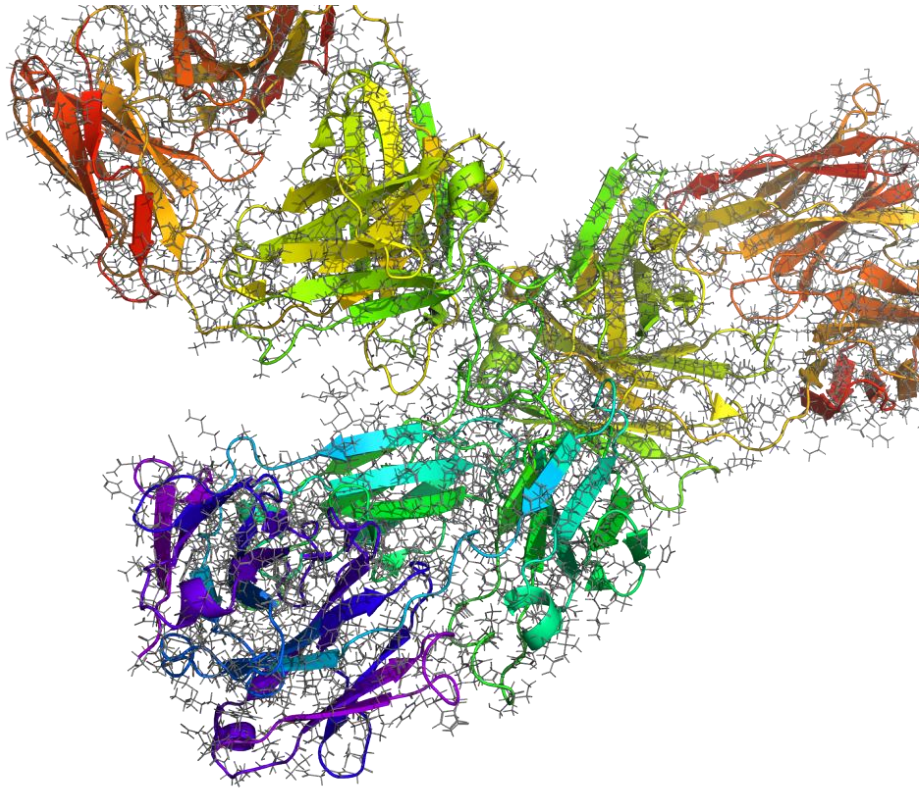


Aggregation can be well described with first order kinetic reaction, when protein is exposed below melting temperature (note that appropriate temperature range is confirmed by linear Arrhenius plot)

Mechanism at 5°C: chemical modifications -> local unfolding of monomer during storage -> aggregation

Zidar et al., High Throughput Prediction Approach for Monoclonal Antibody Aggregation at High Concentration, DOI: 10.1007/s11095-017-2191-6

Heterogeneity of an antibody



Heavy chains:	2 x 448 amino acids
Light chains:	2 x 214 amino acids
Sum of amino acids:	1322 amino acids (145'200 Da)
Glycosylation:	2 x (2.5-3) kDa
Charged groups:	Lys, Arg, His, N-term, Asp, Glu, C-term

Possible modifications at molecular level:

Pyroglutamate (N-term):	2 or 4
Desamidation of Asn:	6
Oxidation of methionine:	4
Glycan variants:	10
Sialylation:	4
O-glycosylation	4
Deletion at C-terminus:	2
Formation of tri-sulfide:	2
Oxydation of Trp:	2
Fragmented backbone:	2
Sequence variants:	it depends

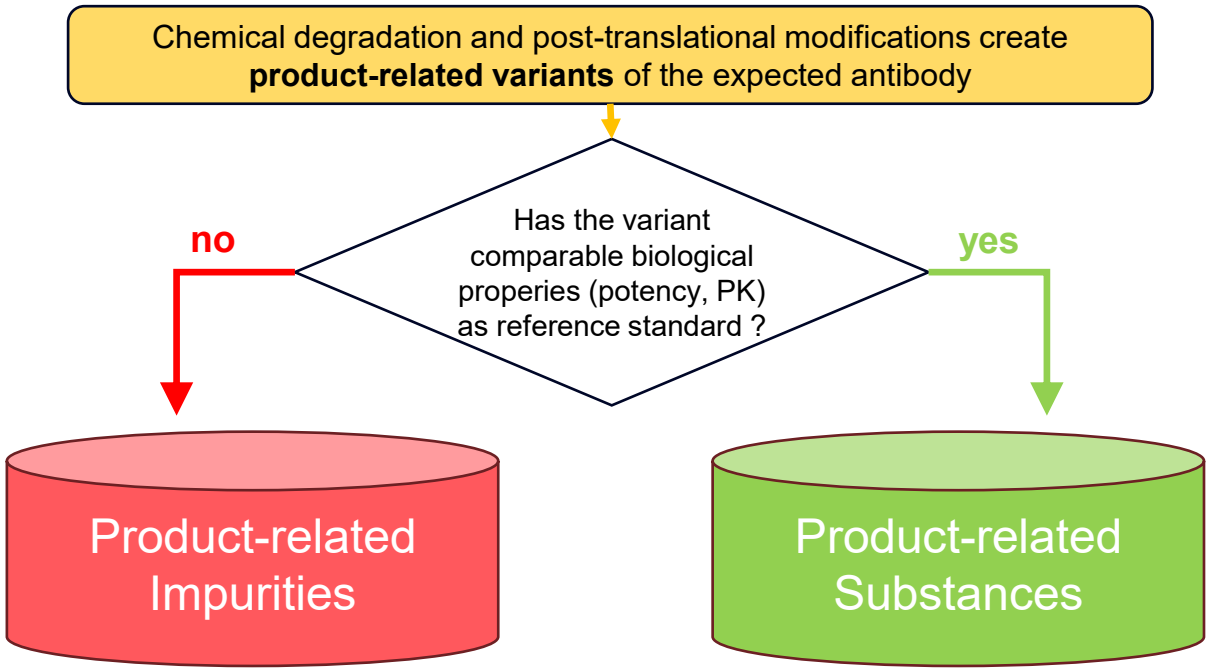
Expected antibody = all amino acids intact

plus:

>40 distinct variants with one chemical modification

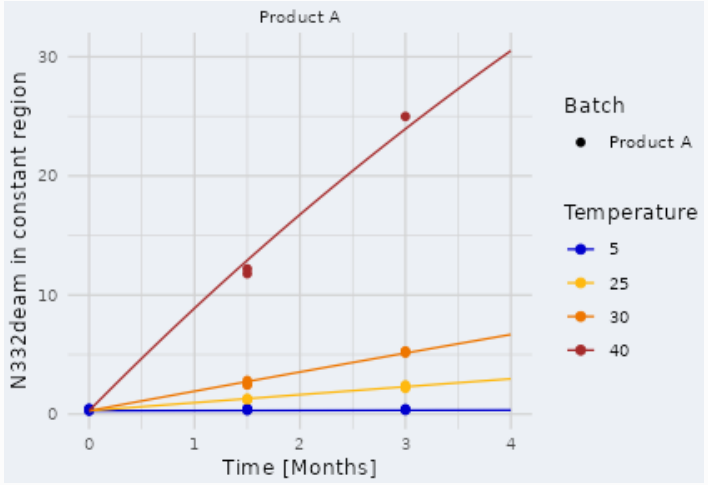
plus:

>1600 variants with two modifications, etc

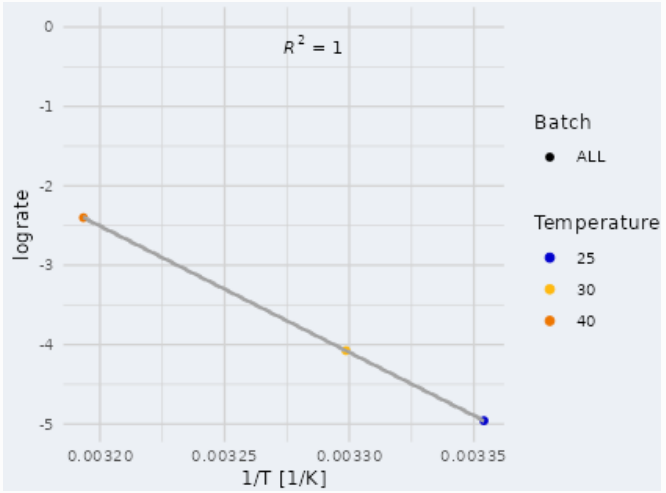


Asparagine deamidation -> Acidic shift

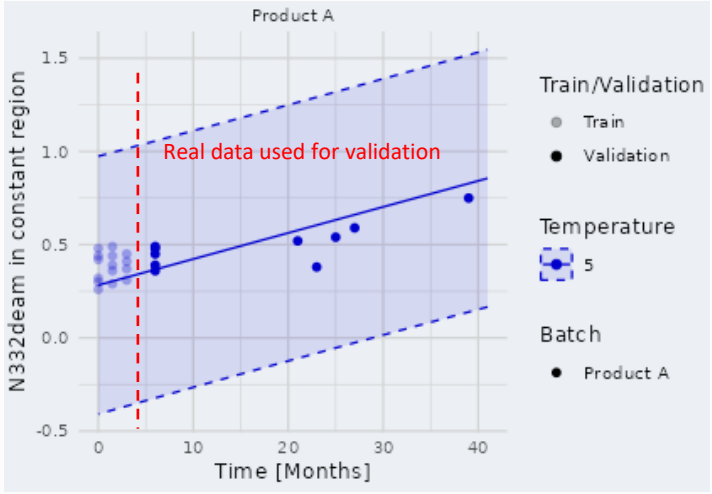
Model training by 3 months data

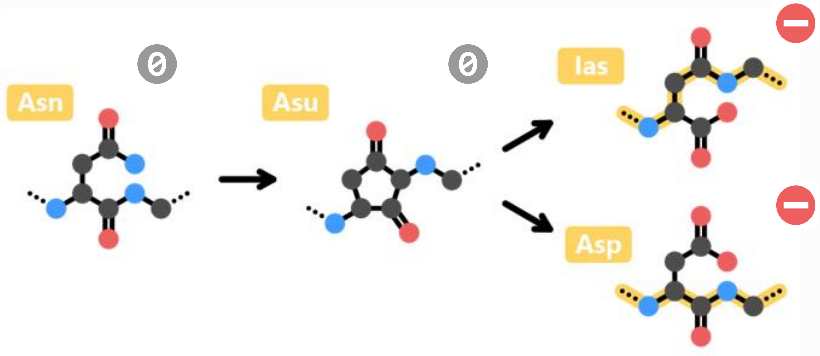


Arrhenius behavior



Prediction Validation

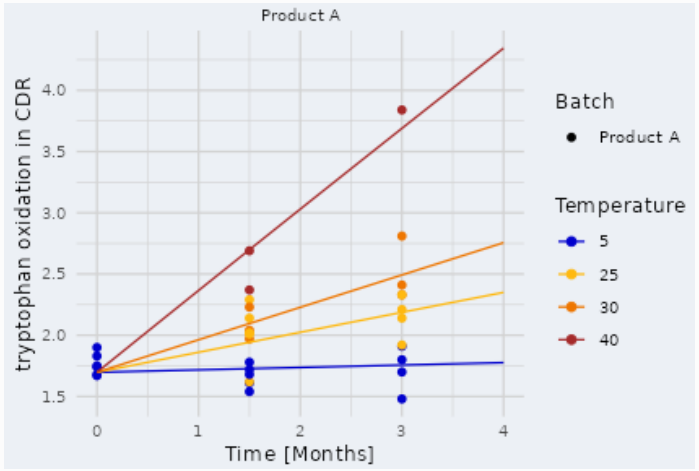




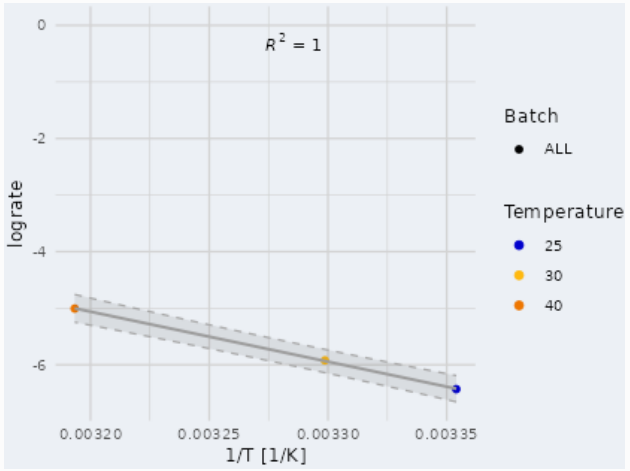
Increase of negative charge (**increase of acidic and decrease of main and basic peaks**), more polar (less hydrophobic) and can affect protein structure or interactions (**aggregation**).

Tryptophane oxidation

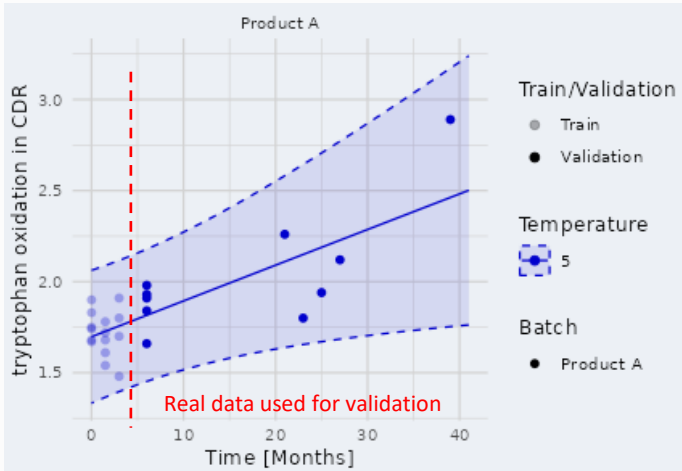
Model training by 3 months data

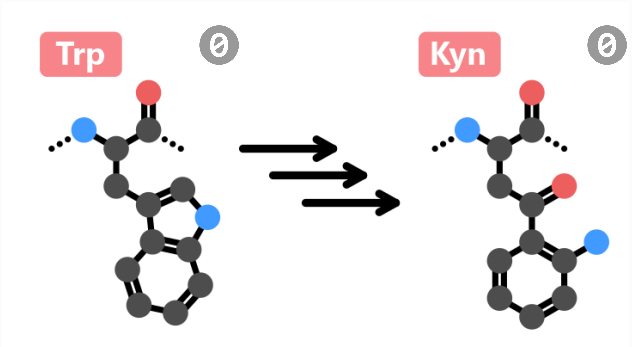


Arrhenius behavior



Prediction Validation





More polar (less hydrophobic) and can affect protein structure or interactions. Converting into new chromophores (for example kynurenine-type products) that absorb light differently, which can appear as **yellowing or browning**.