GDD/TRD/Bx


# Stability Predictions for mAbs Using Arrhenius-Based Kinetics

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**CMC Strategy Forum Europe – CASSS**
**October 18th, 2023**

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# Agenda

- From two to one step Arrhenius based kinetic models
- Does aggregation of proteins follow Arrhenius relation?
- Example of application
- Conclusions

# Excellent Agreement With the Experimental Real-time Data

Evaluation of Arrhenius-based kinetic models through a cross-company perspective on stability modeling:

- key stability indicating attributes
- different types of biotherapeutics, vaccines and biomolecules combined in in vitro diagnostic kits
- five biopharma companies
- stability predictions up to 3 years for products at storage conditions
- temperature excursions outside the cold-chain

| Type of product | Company | Stability attributes |
|---|---|---|
| **Biotherapeutics** | Abbvie | Acidic isoforms |
| **Biotherapeutics** | Novartis | Acidic variants, aggregates |
| **Biotherapeutics** | Sanofi | Aspartate isomerization |
| **Biotherapeutics** | Sanofi | Charged isoforms |
| **Biotherapeutics** | Sanofi | Glass transition temperature (Tg), RMC |
| **Biotherapeutics** | Abbvie | Monomer content (SEC) |
| **Biotherapeutics** | Sanofi | HMW % (SEC) |
| **Biotherapeutics** | MSD | The emergence of impurity % |
| **Biotherapeutics** | Novartis | purity (rCE-SDS) |
| **Biotherapeutics** | Novartis | Aggregates HMW(SEC) |
| **Biotherapeutics** | Novartis | Aggregates HMW (SEC) |
| **Bulk vaccine** | Sanofi | Cell viability |
| **Multivalent vaccine** | Sanofi | Depolymerization (%) |
| **Flu vaccine** | Sanofi | Antigen content |
| **Polysaccharide-Protein conjugate vaccine** | Sanofi | Free polysaccharide (%) |
| **Live-attenuated virus** | Sanofi | Infectious titer (CCID50) |
| **Quadrivalent vaccine (Tetravac)** | Sanofi | Antigenicity of diphteria and FHA (ELISA) |
| **Live-attenuated virus (Stamaril)** | Sanofi | Infectious titer (CCID50) |
| **Live-attenuated virus (vYF)** | Sanofi | Infectious titer (CCID50) |
| **In vitro diagnostic - VIDAS ® PTH (1-84)** | bioMérieux | VIDAS ® relative fluorescent value on Ctr C1 vial |
| **In vitro diagnostic - VIDAS ® Cortisol S** | bioMérieux | VIDAS ® relative fluorescent value on Std S1 vial |
| **In vitro diagnostic - VIDAS ® NEPHROCHECK ®** | bioMérieux | VIDAS ® relative fluorescent value on Ctr C1 vial |
| **In vitro diagnostic - VIDAS ® NEPHROCHECK ®** | bioMérieux | VIDAS ® relative fluorescent value on Ctr C1 vial |

Kinetic rate $d\alpha/dt$ was phenomenological described as the sum of two individual one-step reactions:

$$\frac{d\alpha}{dt} = v \times A_1 \times exp\left(-\frac{E_{a1}}{RT}\right) \times (1-\alpha_1)^{n1} \times {\alpha_1}^{m1} \times C^{p1} + (1-v) \times A_2 \times exp\left(-\frac{E_{a2}}{RT}\right) \times (1-\alpha_2)^{n2} \times {\alpha_2}^{m2} \times C^{p2}$$

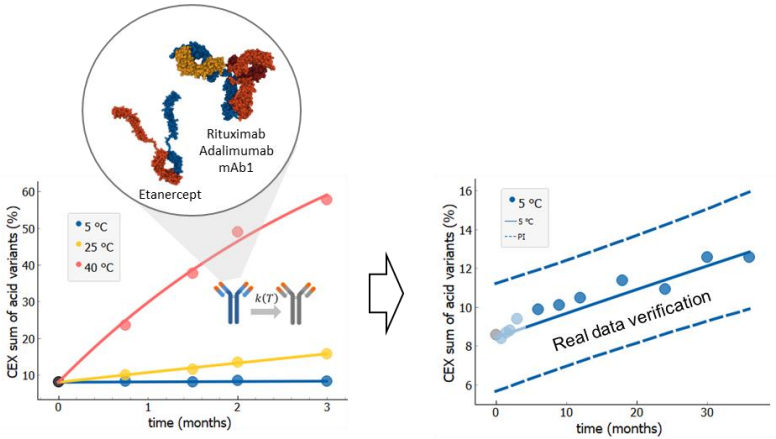
Sci Rep. 2023 Jun 21;13(1):10077. doi: 10.1038/s41598-023-35870-6.

# Simplified Biotherapeutic Degradation Models: One Step First-Order Kinetics

$$\frac{d\alpha}{dt} = v \times A_1 \times exp\left(-\frac{E_{a1}}{RT}\right) \times (1-\alpha_1)^{n1} \times {\alpha_1}^{m1} \times C^{p1} + (1-v) \times A_2 \times exp\left(-\frac{E_{a2}}{RT}\right) \times (1-\alpha_2)^{n2} \times {\alpha_2}^{m2} \times C^{p2}$$

$$\frac{d\alpha}{dt} = A \times exp\left(-\frac{E_a}{RT}\right) \times (1-\alpha)$$

Stability profiles of Charge profiles, aggregation, fragmentation, potency at standard storage conditions.




Accelerated stability data

Three years prediction

Sci Rep. 2021 Oct 15;11(1):20534. doi: 10.1038/s41598-021-99875-9.



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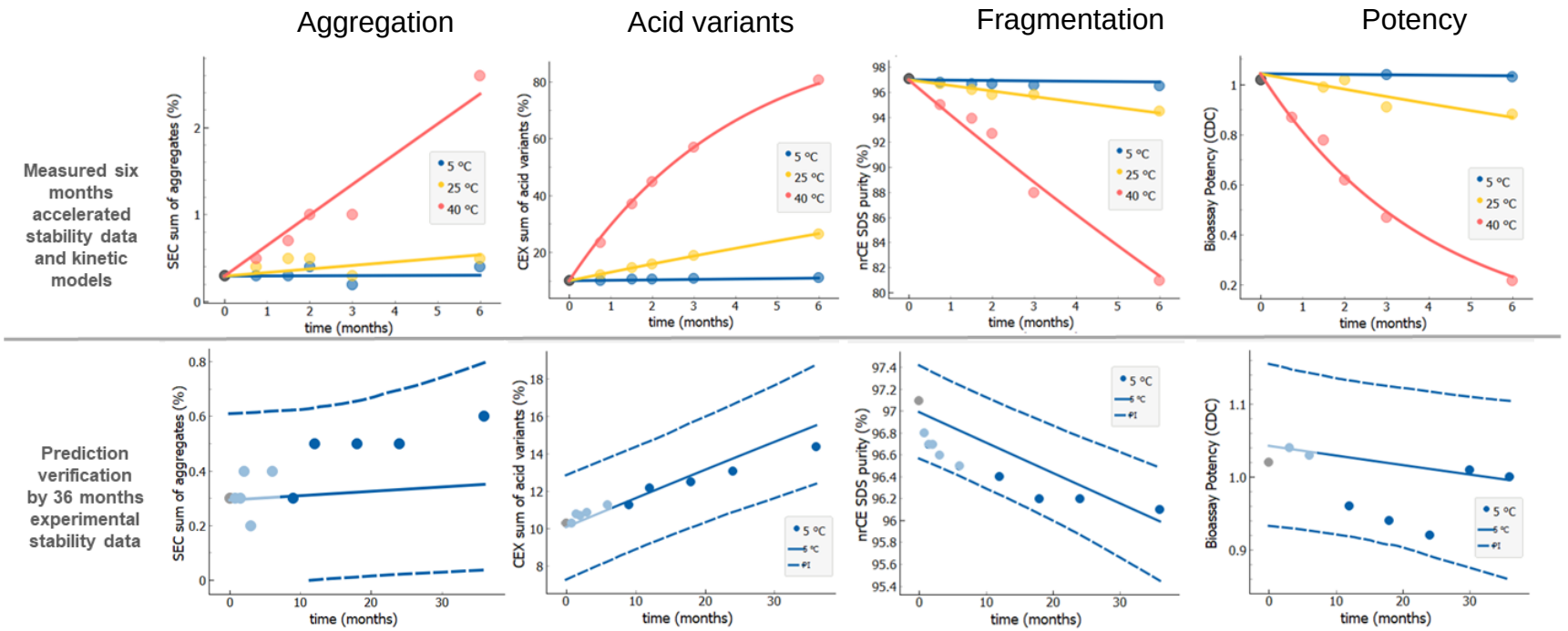
# Example Models for a Rituximab batch




Sci Rep. 2021 Oct 15;11(1):20534. doi: 10.1038/s41598-021-99875-9.



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# Long-Term Stability Predictions Verifications with Experimental Data

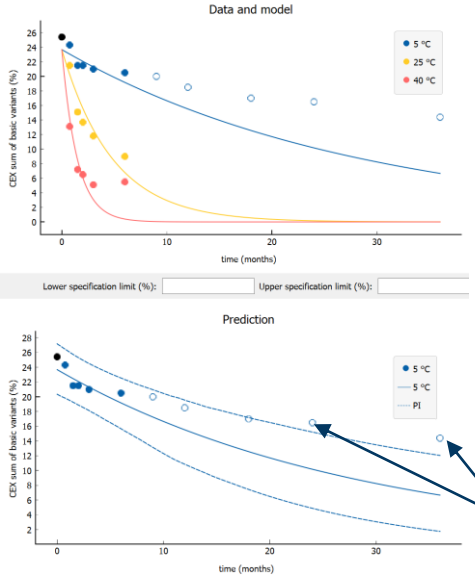
| | mAb1 | Rituximab | Etanercept | Adalimumab |
|---|---|---|---|---|
| CEX amount of main variants (%) | 100% | 91% | 100% | 99% |
| CEX sum of acid variants (%) | 100% | 97% | 100% | 99% |
| nrCE SDS purity (%) | 100% | 95% | 100% | 98% |
| nrCE SDS sum of fragments (%) | 100% | no data | no data | no data |
| Relative potency | n.a. | 97% | 100% | 100% |
| SEC purity (%) | 100% | 98% | 90% | 100% |
| SEC sum of aggregates (%) | 100% | 94% | 90% | 97% |
| CEX amount of basic variants | 85% | 90% | 74% | 99% |
| **Number of batches** | 8 | 36 | 11 | 19 |

Ratio of experimental long-term data points between 6 and 36 months at 5 °C that fall within the 95 % prediction interval relative to the total number of all measured values during that time (e.g. 100 % - all longterm data fall within prediction interval)

Sci Rep. 2021 Oct 15;11(1):20534. doi: 10.1038/s41598-021-99875-9.

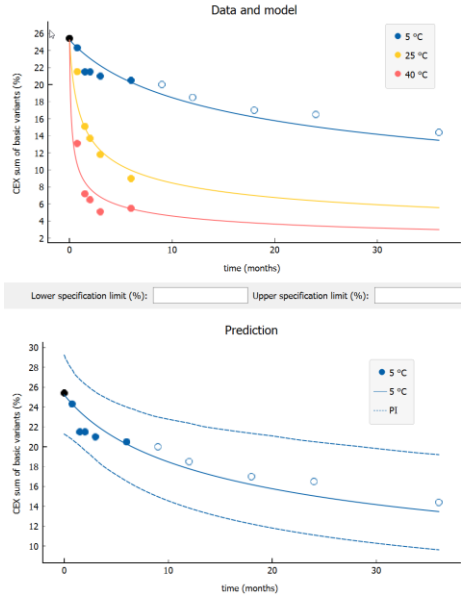
# Degradation of „Sum of basic variants“ Described by 2-Step Model

One step – first order model




Above the Prediction Interval

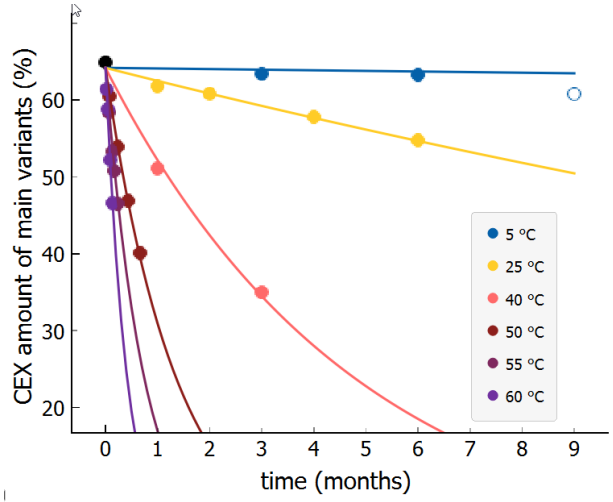
Two steps model



# (Non) Arrhenius Degradation

Arrhenius relation for temperature dependent kinetic rate of degradation proces:

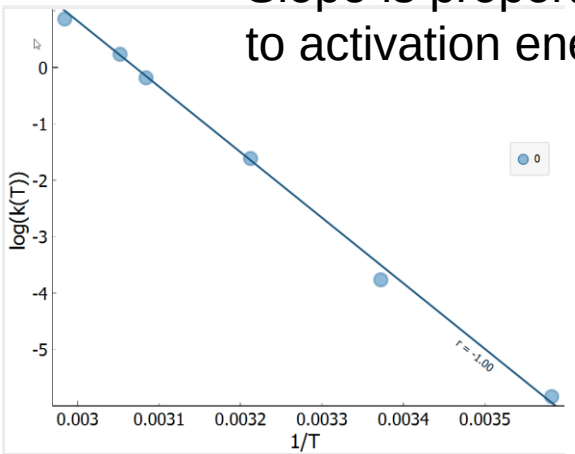
$$k = A \times e^{-\frac{E_a}{RT}}$$




Arrhenius plot:

$$ln(k) = ln\,(A) - \frac{E_a}{R} \times \frac{1}{T}$$

Slope is proportional to activation energy



# Aggregation: Non-Arrhenius Plots from Literature

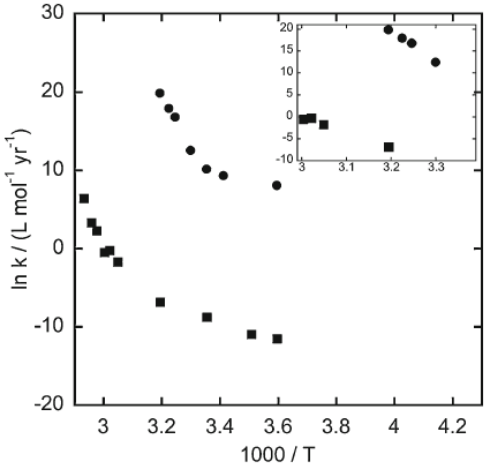



**Fig. 4.** Arrhenius plot of observed rate coefficient values for aggregation of bG-CSF (*circles*) and a MAb (*squares*). See main text for additional details

Wang & Roberts: AAPS Journal, Vol. 15, No. 3, July 2013

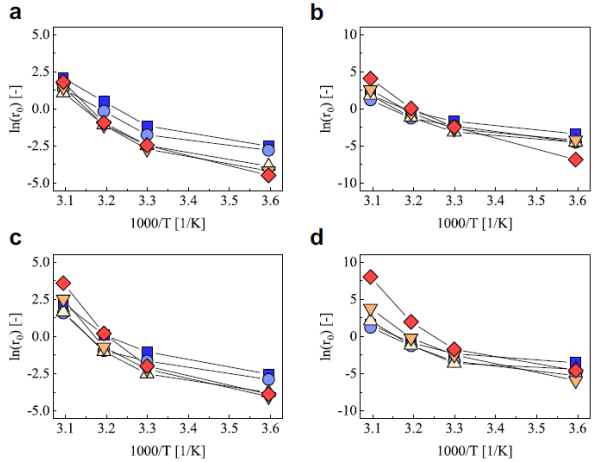
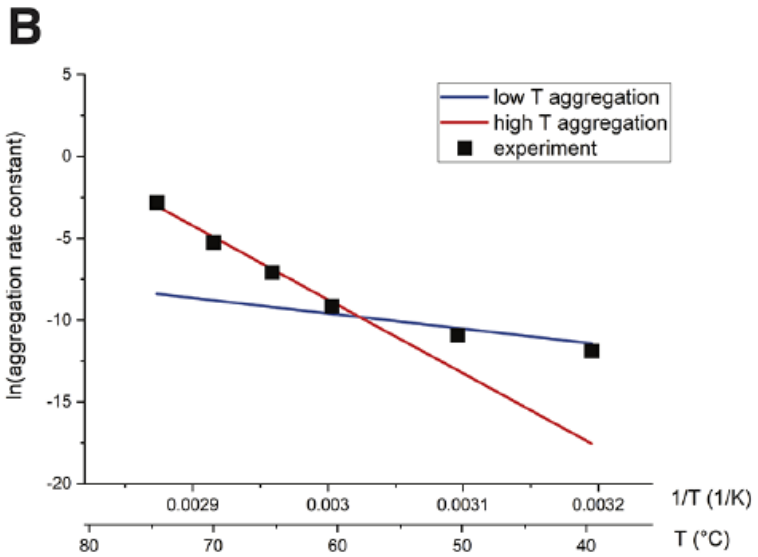
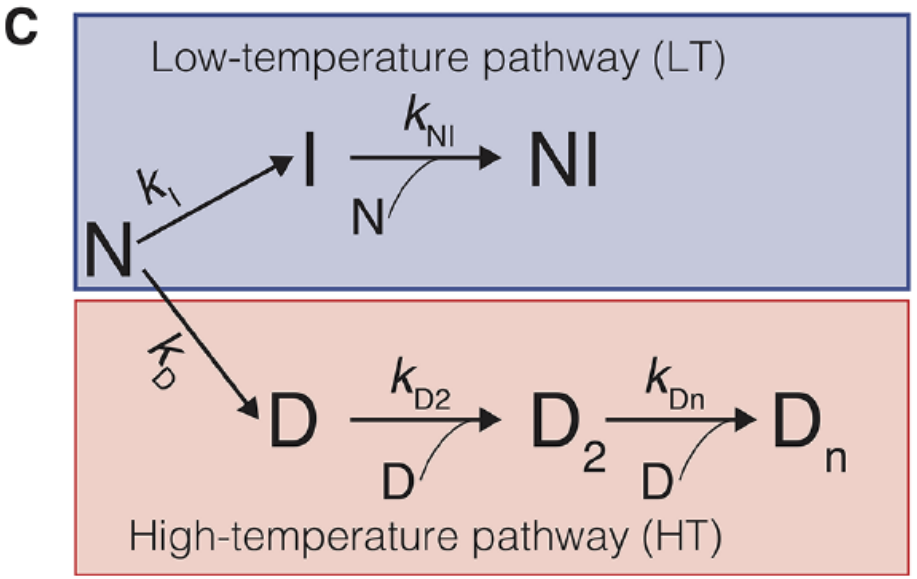



**Figure 2.** Arrhenius plot of the initial aggregation rate at 100 mg/mL mAb concentration and pH = pI (blue squares), pI − pH = 1 (light blue circles), pI − pH = 2 (yellow upward triangles), pI − pH = 3 (orange downward triangles), and pI − pH = 4 (red diamonds). (a) mAb-1 without excipient, (b) mAb-2 without excipient, (c) mAb-1 with 150 mM sodium chloride, (d) mAb-2 with 150 mM sodium chloride.

Wälchli: Journal of Pharmaceutical Sciences 109 (2020) 595-602

# Temperature is Promoting Different Aggregation Pathways

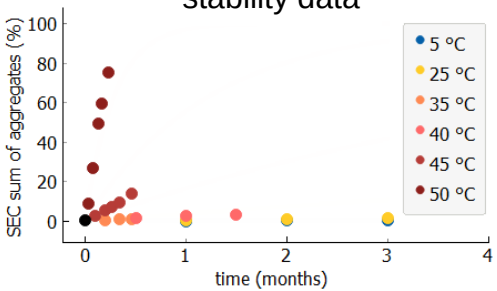


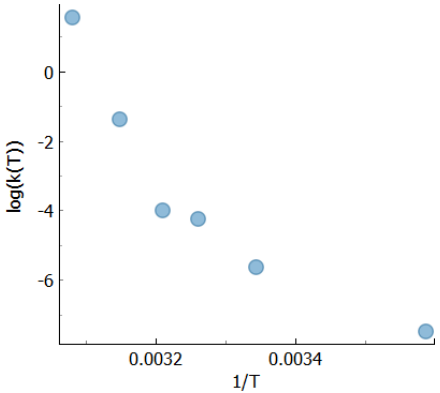

Journal of Medicinal Chemistry 2022 65 (3), 2623-2632,
DOI: 10.1021/acs.jmedchem.1c02010

# Aggregation Prediction for a Fusion Protein

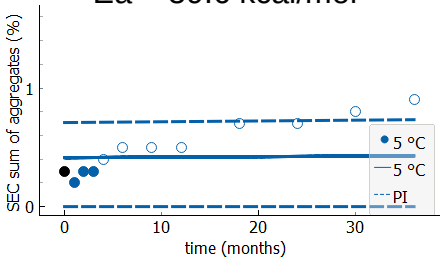
Accelerated stability data




Arrhenius plot




T [5 - 50 °C]
Ea = 59.6 kcal/mol



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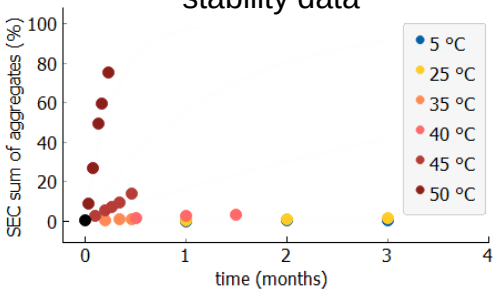
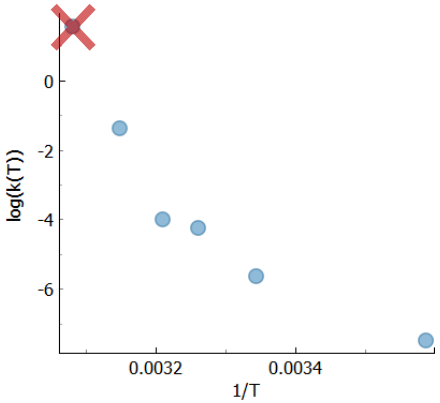
# Aggregation Prediction for a Fusion Protein

Accelerated stability data




Arrhenius plot

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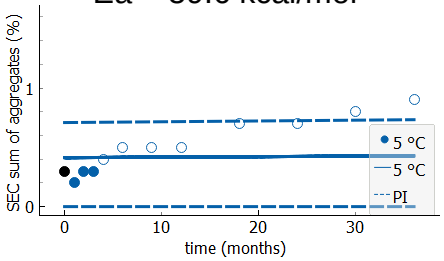
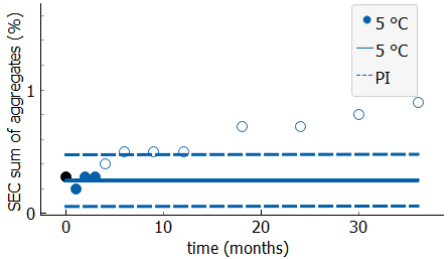
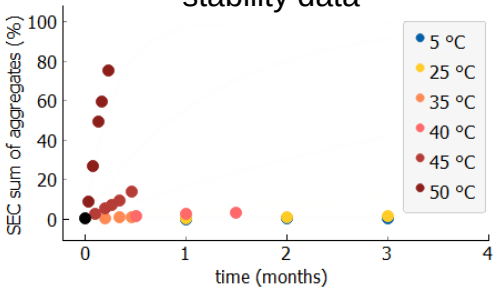



T [5 - 45 °C]
Ea = 54.0 kcal/mol

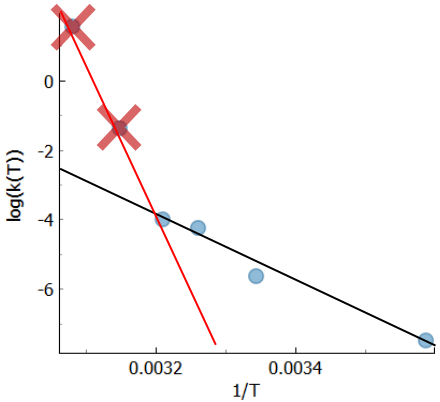


# Aggregation Prediction for a Fusion Protein

Accelerated stability data




Arrhenius plot

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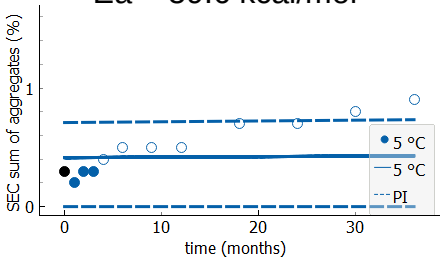


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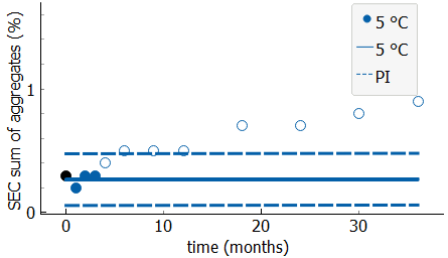
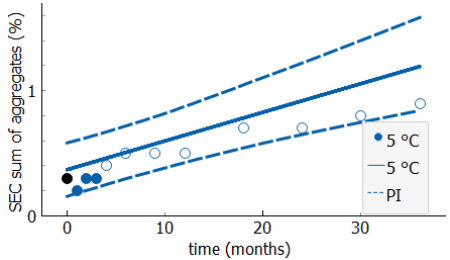



T [5 - 40 °C]
Ea = 21.4 kcal/mol



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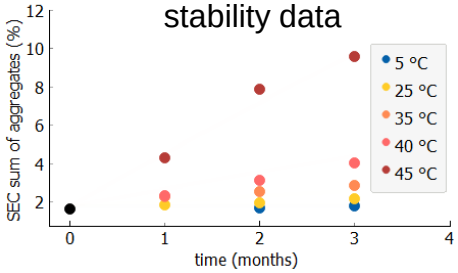
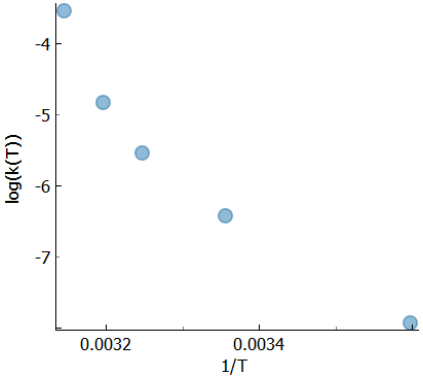
# Aggregation Prediction for a mAb

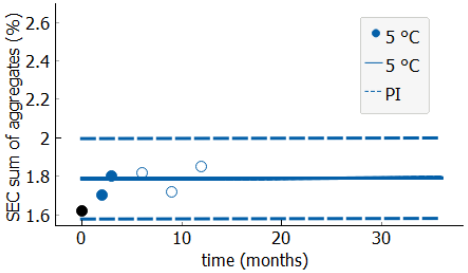
Accelerated stability data




Arrhenius plot




T [5 - 45 °C]
Ea = 44.9 kcal/mol



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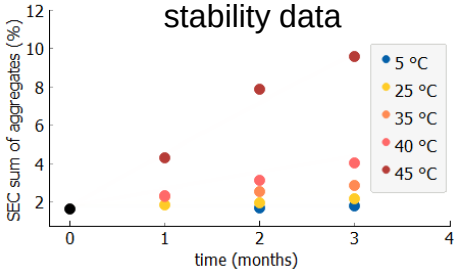
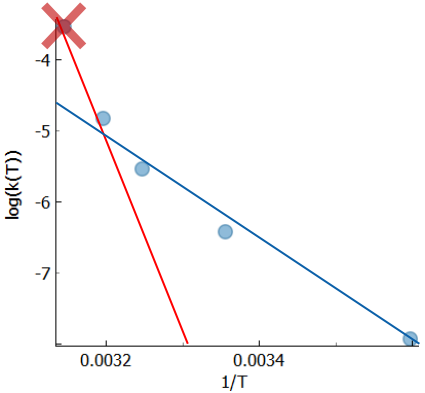
# Aggregation Prediction for a mAb

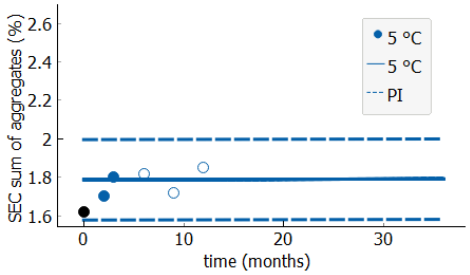
Accelerated stability data



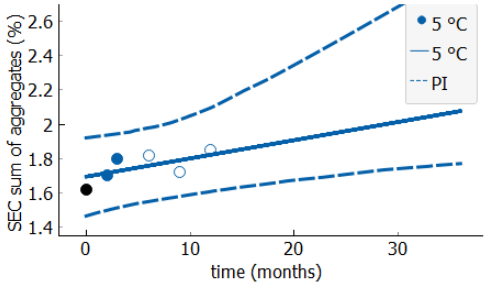

Arrhenius plot




T [5 - 45 °C]
Ea = 44.9 kcal/mol

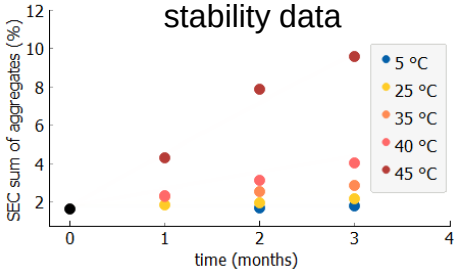



T [5 - 40 °C]
Ea = 21.0 kcal/mol

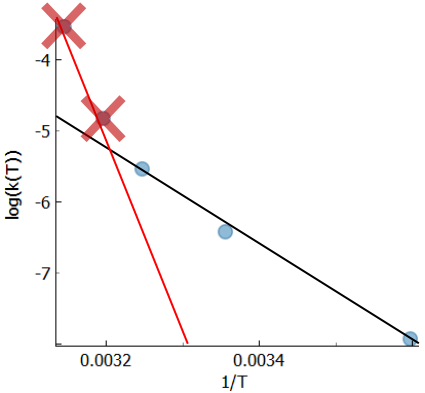


# Aggregation Prediction for a mAb

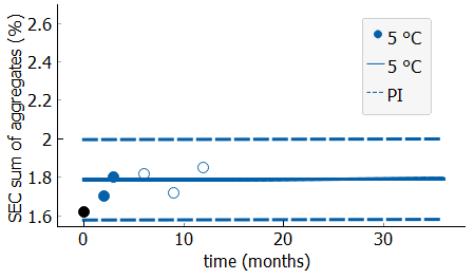
Accelerated stability data



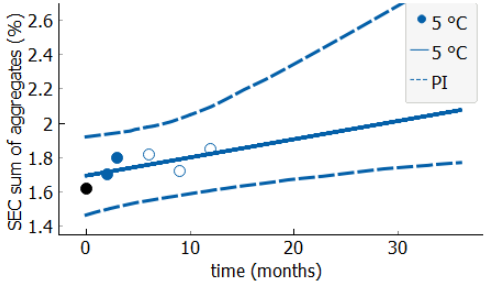

Arrhenius plot



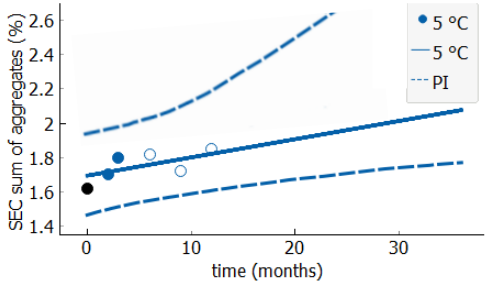

T [5 - 45 °C]
Ea = 44.9 kcal/mol



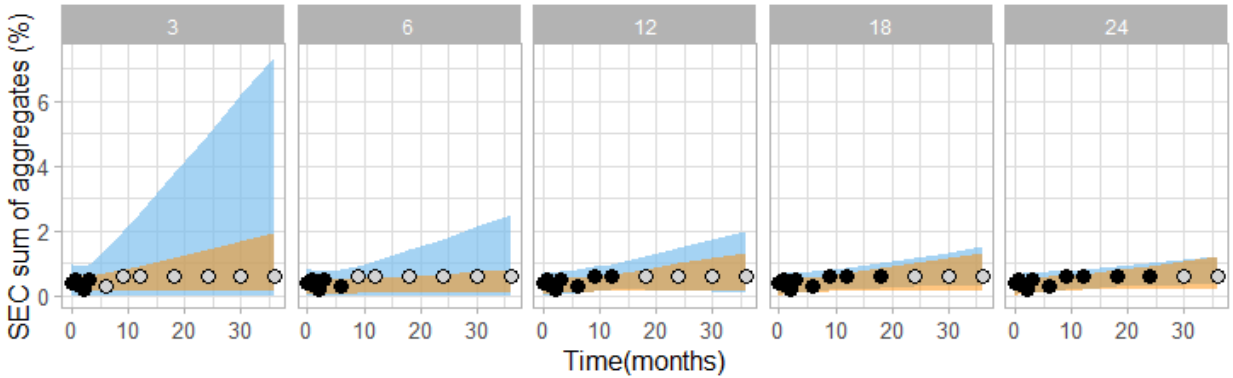

T [5 - 40 °C]
Ea = 21.0 kcal/mol




T [5 - 35 °C]
Ea = 18.3 kcal/mol



# Improved robustness, speed and accuracy of stability predictions




Aggregation of Rituximab: comparison of Arrhenius kinetic model (orange) and linear extrapolation (blue) prediction intervals by using 3, 6, 12, 18 or 24 month experimental stability data (black data points) respectively. In grey, remaining measured data points at intended storage are shown for visual verification of the prediction intervals.

Sci Rep. 2021 Oct 15;11(1):20534. doi: 10.1038/s41598-021-99875-9.

# Conclusions

- Simplified one-step Arrhenius-based kinetics for mAbs longterm stability predictions (6 months accelerated stability data -> 3 years prediction verified by real data)
- Arrhenius relation applies to protein aggregation, with attention to temperature range
- Practical applications: formulation development, temperature excursions
- Valuable insights for assessing shelf life and ensuring product quality and safety

Check-out publications:
2021: D. Kuzman *et al.*, *Long-term stability predictions of therapeutic monoclonal antibodies in solution using Arrhenius-based kinetics* (Sci. Reports)
2022: M. Bunc *et al.*, *Aggregation Time Machine: A Platform for the Prediction and Optimization of Long-Term Antibody Stability Using Short-Term Kinetic Analysis* (J. Med. Chem.)
2023: M. Huelsmeyer *et al.*, A universal tool for stability predictions of biotherapeutics, vaccines and in vitro diagnostic products (Sci. Reports)

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Miha Ravnik

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Blaž Zupan

Revelo d.o.o.

Bruna Pezzi Zupan

Lan Žagar




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# Thank you

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