Data Analysis of Accelerated Stability Data from Proteins for Shelf-Life Prediction

> NCS2024, Wiesbaden <
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Life forward

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Shelf-Life Prediction for Proteins from Accelerated Stability Data

Slides: my own personal, subjective opinion; results are preliminary

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Using the **Arrhenius-Equation** to predict shelf-life …

1ˢᵗ : obtain decay constant k,

$$A = -k \cdot t + b$$

$$A = A_0 \cdot \exp(k \cdot t)$$

$$\frac{dA}{dt} \propto A^n$$

2ⁿᵈ : fit ln(k) against time and get E_a
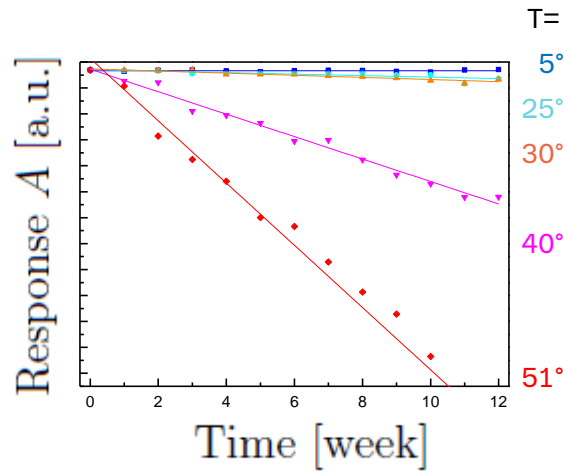
$$k = K \cdot \exp\left(-\frac{E_a}{R \cdot T}\right)$$

$$\ln(k) = -\frac{E_a}{R} \cdot \frac{1}{T} + \ln(K)$$
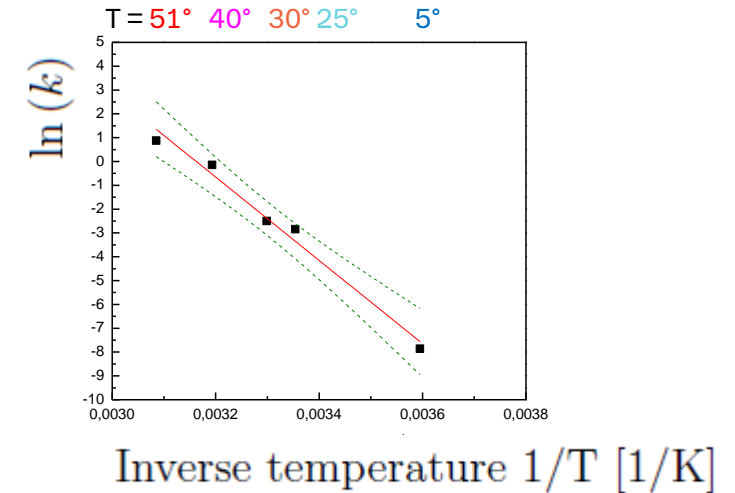
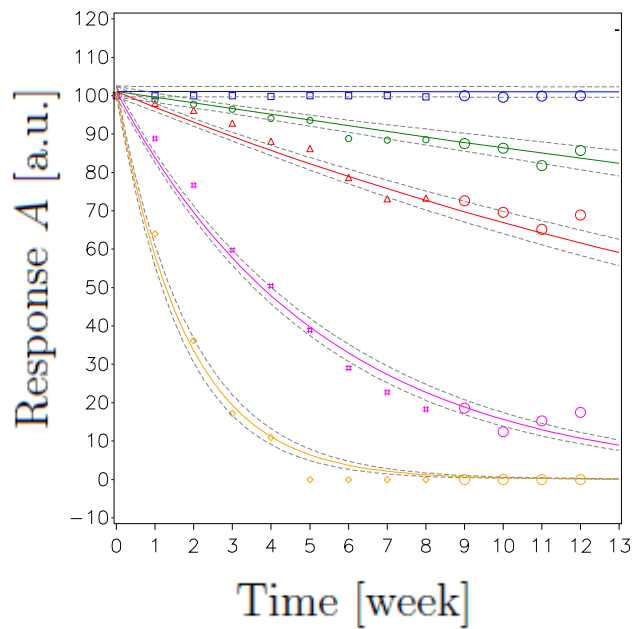**Alternative: fit decay constant k, and activation energy E_a simultaneously:** ⟶

⟶ Predicting response *A* for 1, 2, … years of shelf-life

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Predictions in time and temperature can be done

by a simultaneous fit in time/k $_{(0^{th}, 1^{st}, AKM ...)}$ and T/ E$_a$ $_{(Arrhenius, mod. Arrh. ...)}$:

Predicting response *A*
for 1, 2, ... years
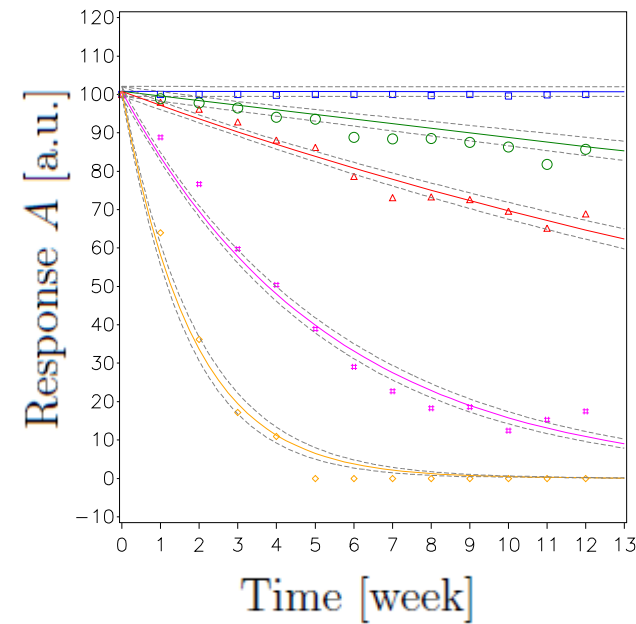
Predicting response *A*
for new temperatures

Advanced kinematic modelling (AKM)

$$\alpha = 1 - \frac{A(t)}{A_0}$$

AKM with AKTS software:

one-step kinetics

$$\frac{d\alpha}{dt} = A \cdot \exp\left(-\frac{E_a}{R \cdot T}\right) \cdot (1-\alpha)^n \cdot \alpha^m$$

Dimer (Peptide of 5 kDa,
as compared to protein/anti-body)

two-step kinetics

$$\frac{d\alpha}{dt} = A_1 \cdot \exp\left(-\frac{E_{a1}}{R \cdot T}\right) \cdot (1-\alpha)^{n1} \cdot \alpha^{m1} + A_2 \cdot \exp\left(-\frac{E_{a2}}{R \cdot T}\right) \cdot (1-\alpha)^{n2} \cdot \alpha^{m2}$$

- 5 temperatures:
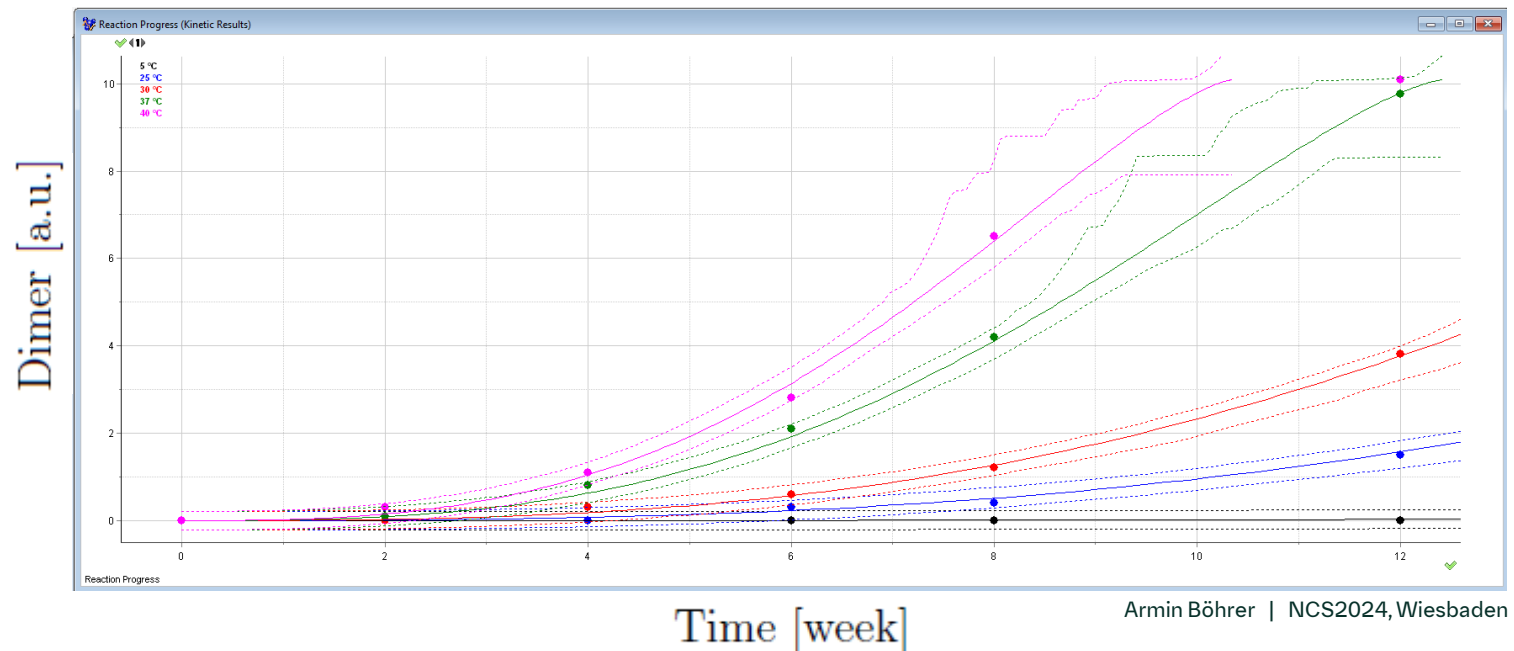 5°, 25°, 30°, 37°, 40°

- 6 time points:
  0, 2, 4, 6, 8, 12 weeks

- Model

  ● 1 step : A -> ...

  ○ 2 steps : A -> ... ; A -> ...

  ○ 2 steps : A_1 -> ... ; A_2 -> ... with A = $A_1 + A_2$

- Result ➜

  n = 0.278
  m = 0.647

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Possible solutions using the Arrhenius-Equation

Strategies/Alternatives:

- Advanced kinematic modelling (AKM),
  Fit with the AKTS software [1]

- Parabola for the time dependence

- Solve numerically using standard software to fit the differential equation

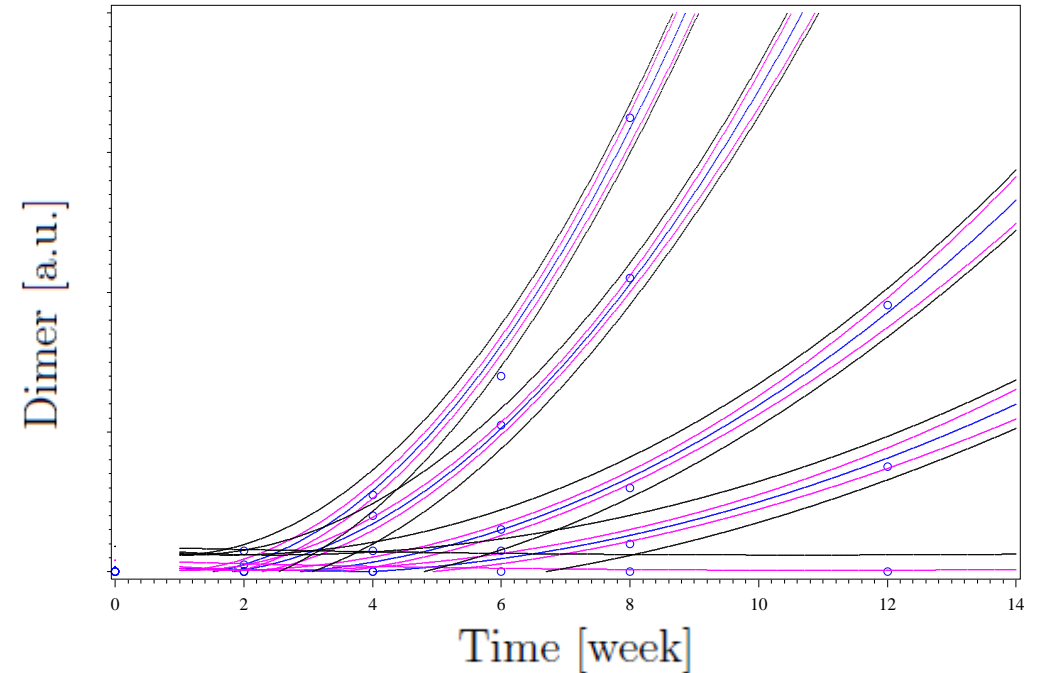- Solve differential equation and fit within standard software

[1] AKTS Home Page, Advanced Kinetics and Technology Solutions, https:// www. akts. com/

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Choose simple solution

Parabola for time dependence

Selection:

- Use Dimer < 70% of maximum
- Avoids sigmoid part at "high" T
- Simple model
- solves $dA/dt \sim A^n = A^{(1/2)}$
- result ➜ extrapolation 5°
  ➜ agreement with AKM

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R-package "deSolve": ODE [2]

Accuracy and precision:

~~Messages, warnings?~~

- **Result** $\min(RSS(\alpha(t=0))$ ➜

  n = 0.28
  m = 0.65

- **Bias?**

- **Simulated data
  from IBeta.inv**

  (predicted +rnorm) data
  n = 0.28
  m = 0.65

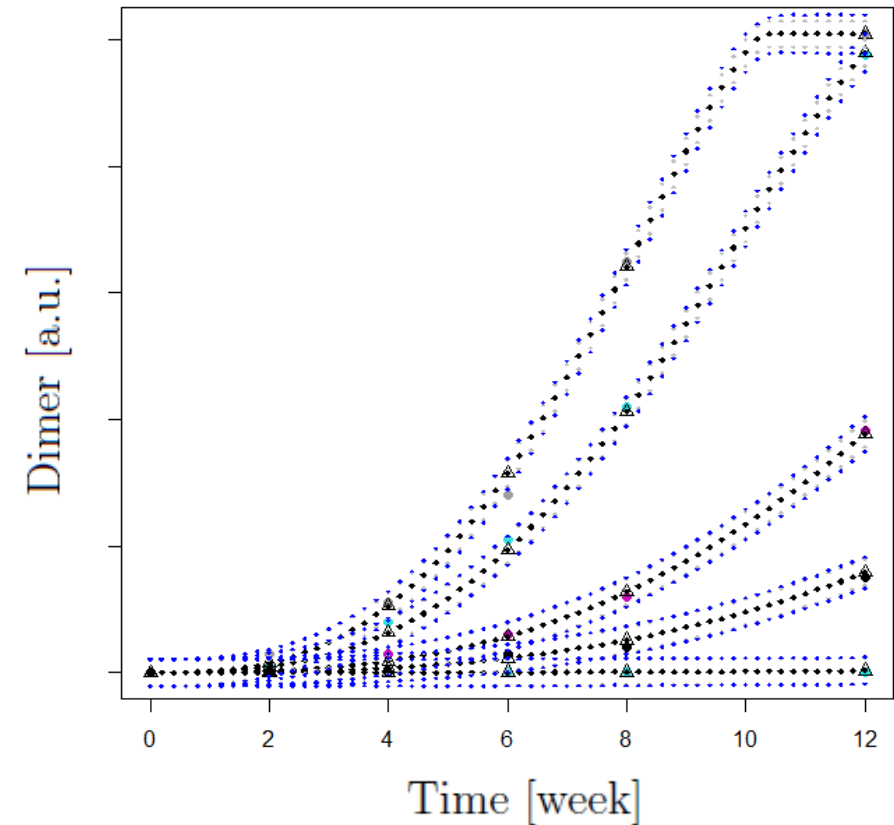[2] Karline Soetaert, Thomas Petzoldt, R. Woodrow Setzer (2010)...
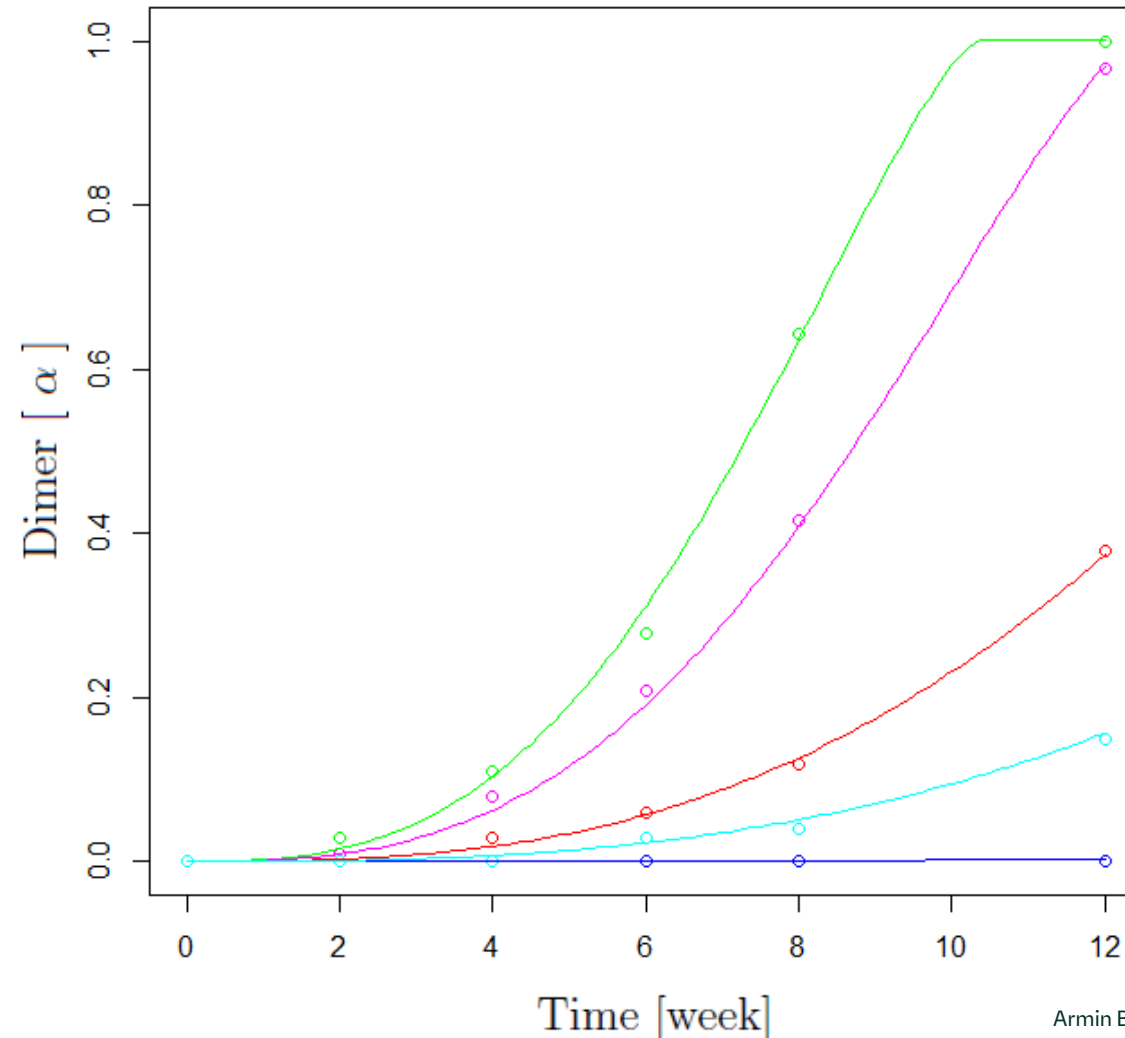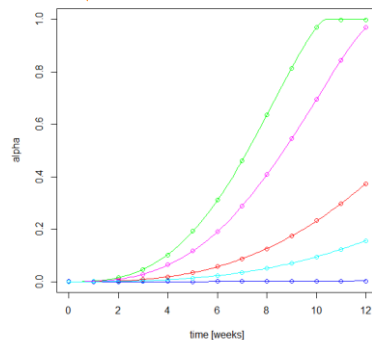
Solve differential equ. $\sim(1-\alpha)^m\alpha^n$ (Šesták–Berggren equation)

For m, n < 1 analytical solution exists
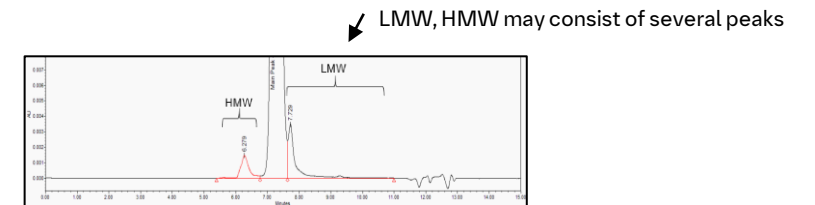
$$I(x;\ a,\ b) = \int_0^x t^{a-1} \cdot (1-t)^{b-1}\, dt$$
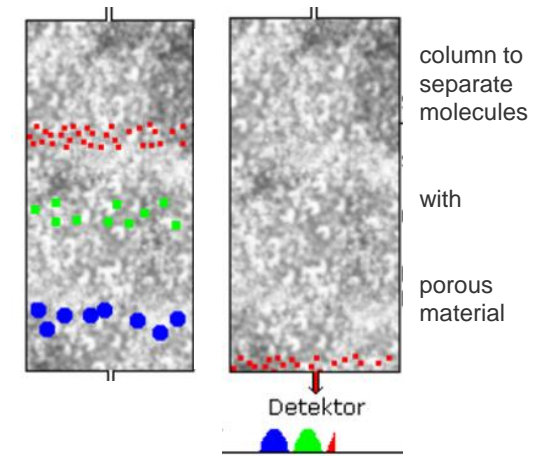
- Solved with Incomplete beta function: [3]

- Inverse needed: R-package "zipfR": Ibeta.inv [4]

- Add analytic continuation

- result ➜ all temperatures, parameters etc.
  ➜ agreement with AKM
  n + b = 1.00…
  m + a = 1.00…

- Limited support = maximum reached in finite time

3) Stroh: Abramowitz, Stegun; 4) S. Evert (2020). Inside zipfR. Technical report…

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Back to proteins: "measure change of fraction(%) of 3 components"

Analytical methods:

column to separate molecules

with

porous material

Detektor

- **IEC:** separate **3 components** by charges
  (Main Peak, acid peaks, basic peaks)

  ➔ data analysis see next slides

LMW, HMW may consist of several peaks

HMW

LMW

Main Peak

- **SEC:** separate **3 components** by size
  (Main Peak, acid peaks, basic peaks)

  ➔ data analysis still in progress

Ion exchange chromatography (**IEC**) uses electrostatic or ionic interactions between polar and ionic analytes to separate molecules.

Size exclusion chromatography (**SEC**) separates molecules based on their size by filtration through a gel.
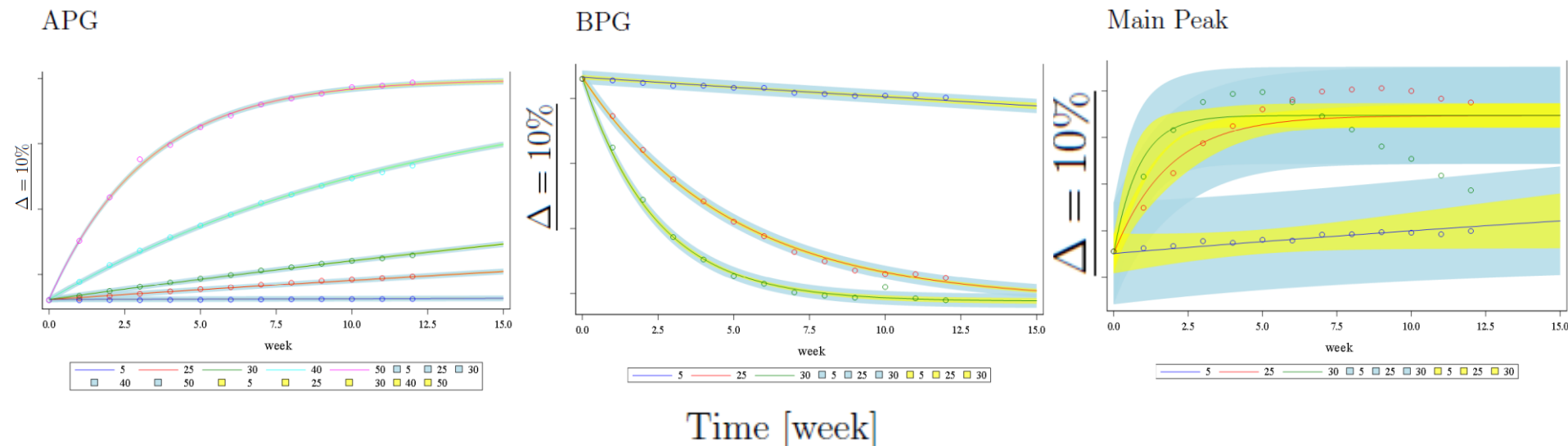
IEC: 0th or 1st order good, SEC: 0th or 1st order fair description, maybe 2-step (~ for AKM)

Example IEC: 1st order is best, but for 3rd component <u>no good model</u> found

This is also seen by others

- Data from Ion Exchange Chromatography (IEC) with 1st order fit

- 5 temperatures: 5°, 25°, 30°, 40°, 50°
- 13 time points: 0, 1, 2, 3, … 12 weeks
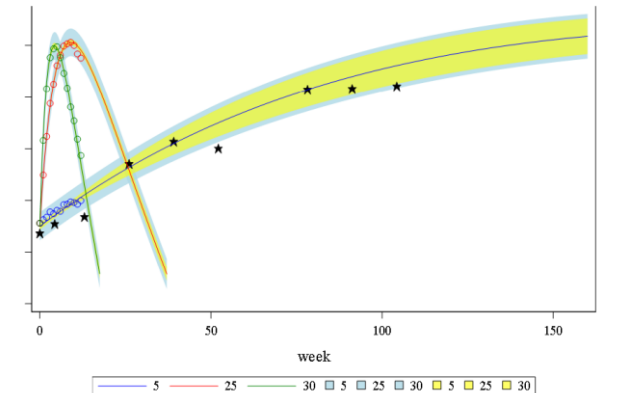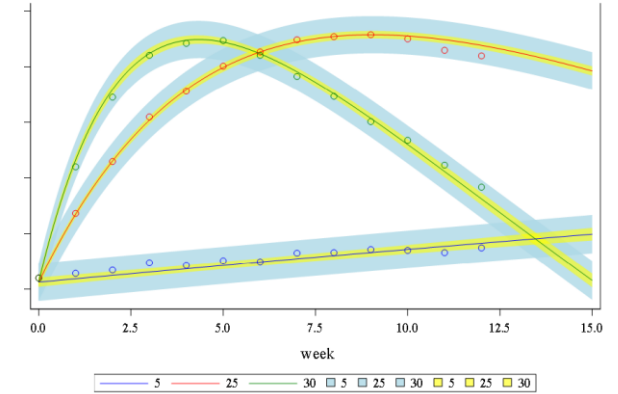
solution for 3rd component problem

The 3rd component

Fit 3rd components as sum of 2 simple functions
- No stable fit

Perform simultaneous fit it all 3 components

- MainPeak= 100% - APG - BPG

AKM studied,
Šesták–Berggren approach can be solved analytically for m, n < 1
➔ Solved by inverse of incomplete Beta function

Selection important, which T to use, check relevance of data points
➔ simple models (e.g., parabola) and
    analytic solutions help for process understanding

IEC: 0th or 1st order does it, AKM similar
➔ 3rd component by constraint/simultaneous fit

Dependence on molecule and analytic method

What next?
(temperature-) modified Arrhenius equation

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Thank you for your attention!