

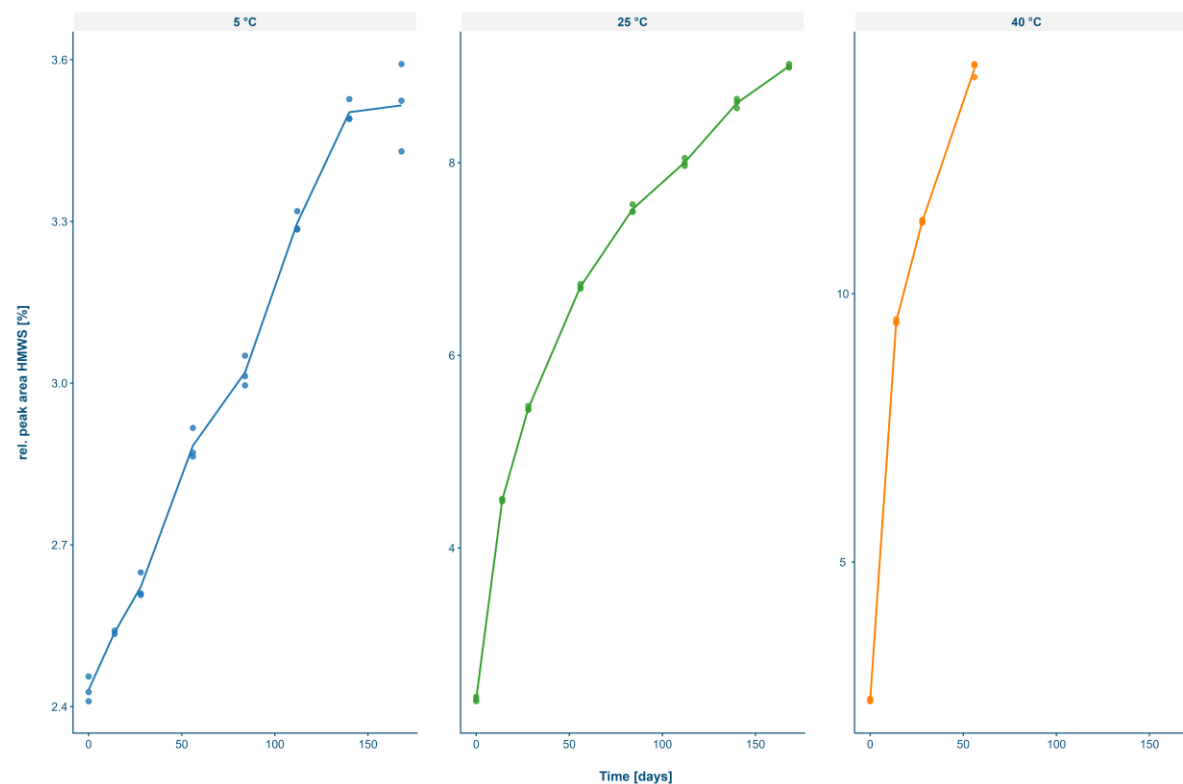
## A two-stage bootstrap framework for long-term stability modelling of biologics

September 23, 2026  
Kshitij Singh, Leukocare



## Scientific Question

- *How should accelerated stability data inform predictions at storage conditions?*
- *Temperature-specific rates first, or kinetic and temperature parameters jointly?*
- *Can one kinetic equation describe a biologic's stability?*

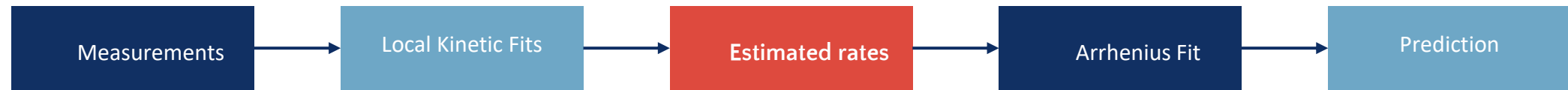


### Different Temperatures <- different Story

- At 25°C and 40°C, HMWS rises fast, then slows
- Zero-order is linear

## Global or two-stage?

- Two-stage + bootstrap: fit rates per temperature, then Arrhenius across temperatures, then bootstrap



- Arrhenius needs one consistently defined kinetic quantity

| Selected model | Equation                | Units of k                      |
|----------------|-------------------------|---------------------------------|
| Zero-order     | $C = C_0 + kt$          | AU/day                          |
| First-order    | $C = C_0 \cdot e^{-kt}$ | $\text{day}^{-1}$               |
| Second-order   | $C = C_0 / (1 - kC_0t)$ | $\text{AU}^{-1}\text{day}^{-1}$ |



*Incompatible units — not comparable on one axis*

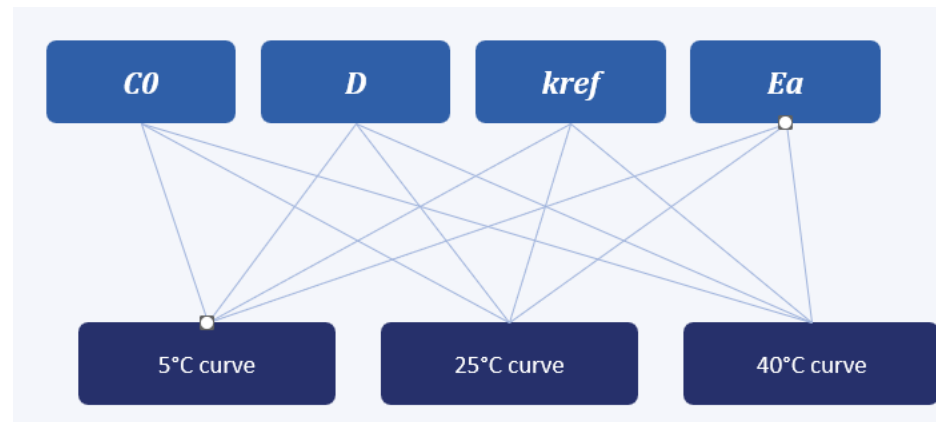
- Different models per temperature give rate constants with different units and meanings.

## Global or two-stage?

- Global kinetic modelling: one kinetic equation and constant  $E_a$  across all temperatures



- Shared parameters feed all 3 curves ( $C_0$ ,  $D$ ,  $k_{ref}/E_a$ )

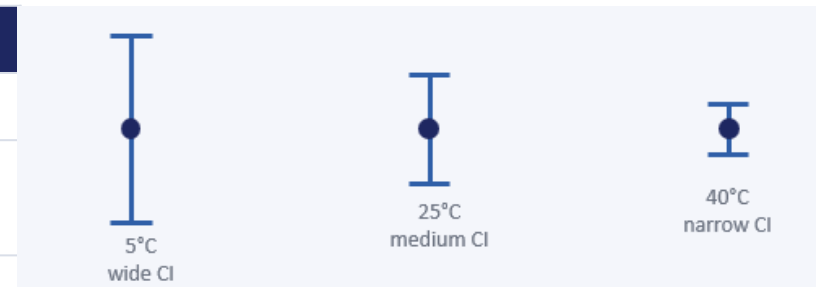


## Global or two-stage?

- Global kinetic modelling: one kinetic equation and constant  $E_a$  across all temperatures



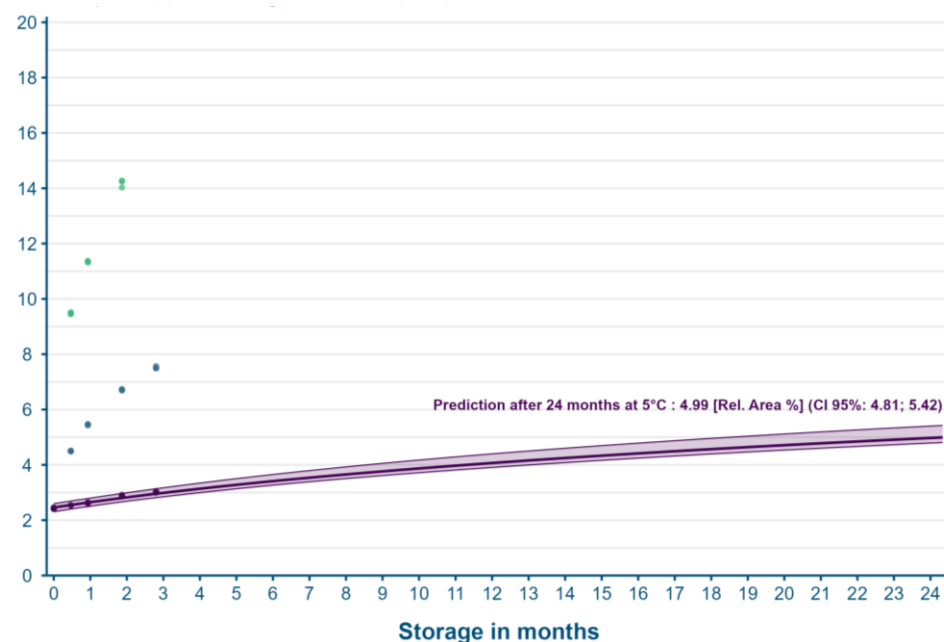
| Parameter     | Best informed by                      | Risk if that's wrong                      |
|---------------|---------------------------------------|-------------------------------------------|
| $C_0$         | All 3 temps, if independent           | Biases every curve if truly shared        |
| $D$           | Mostly 25°C/40°C (visible curvature)  | 5°C curve inherits an unconfirmed plateau |
| $K_{ref}/E_a$ | Joint curvature + spread, all 3 temps | An unresolved temperature pulls on both   |



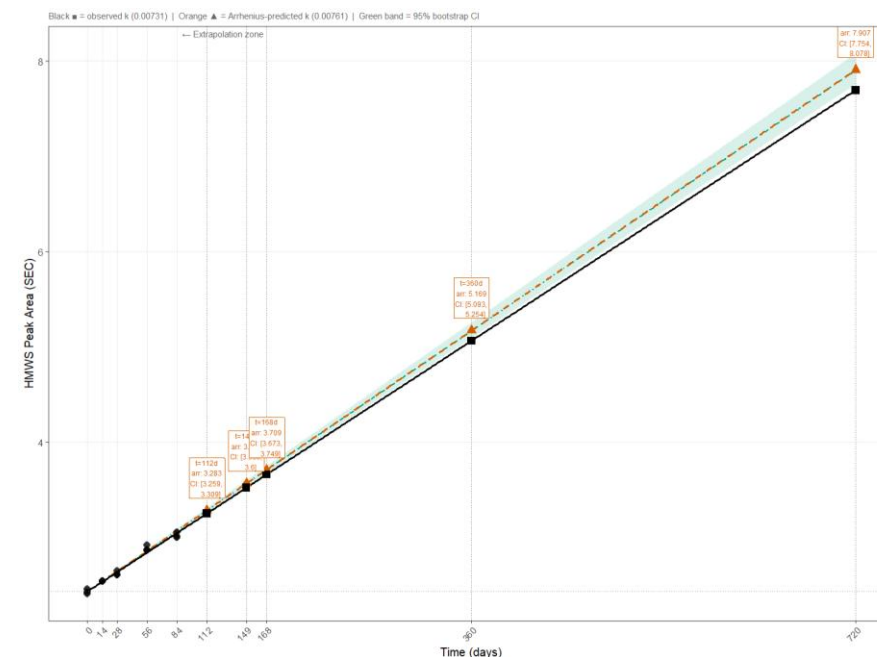
Two-stage regression treats all three as equally informative – discarding real information.

## Case Study I: HMWS [%]

| Time [days] | Tested [%] | Global Kinetic Modelling [%] | Two-stage + Bootstrap [%] |
|-------------|------------|------------------------------|---------------------------|
| 84          | 3.02       | 2.96 [2.81, 3.14]            | 3.07 [3.05, 3.09]         |
| 112         | 3.30       | 3.10 [2.96, 3.30]            | 3.28 [3.26, 3.31]         |
| 140         | 3.50       | 3.23 [3.09, 3.45]            | 3.50 [3.47, 3.53]         |
| 168         | 3.52       | 3.36 [3.21, 3.59]            | 3.71 [3.67, 3.75]         |
| 720         |            | 4.99 [4.81, 5.42]            | 7.97 [7.75, 8.08]         |



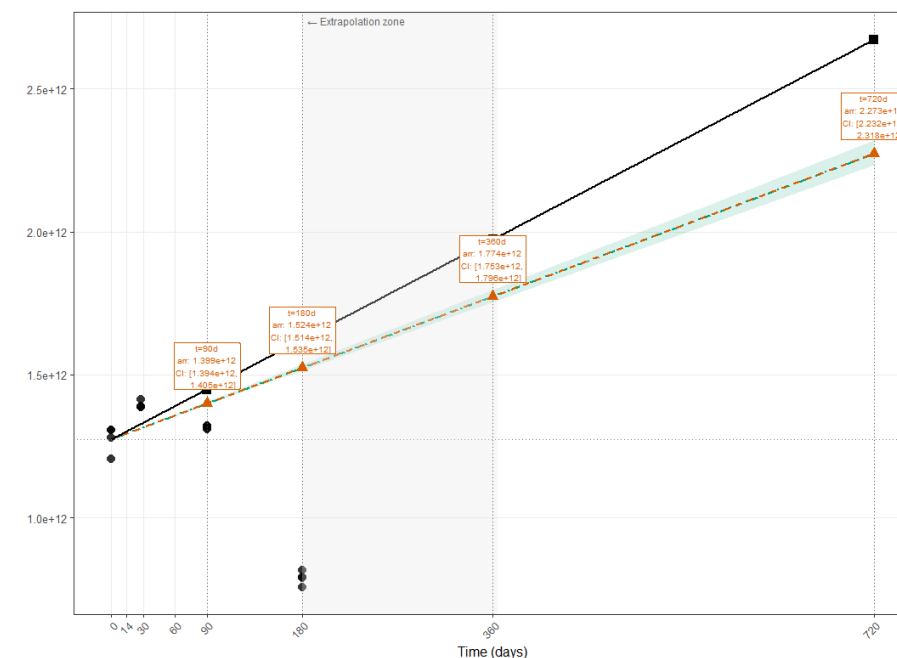
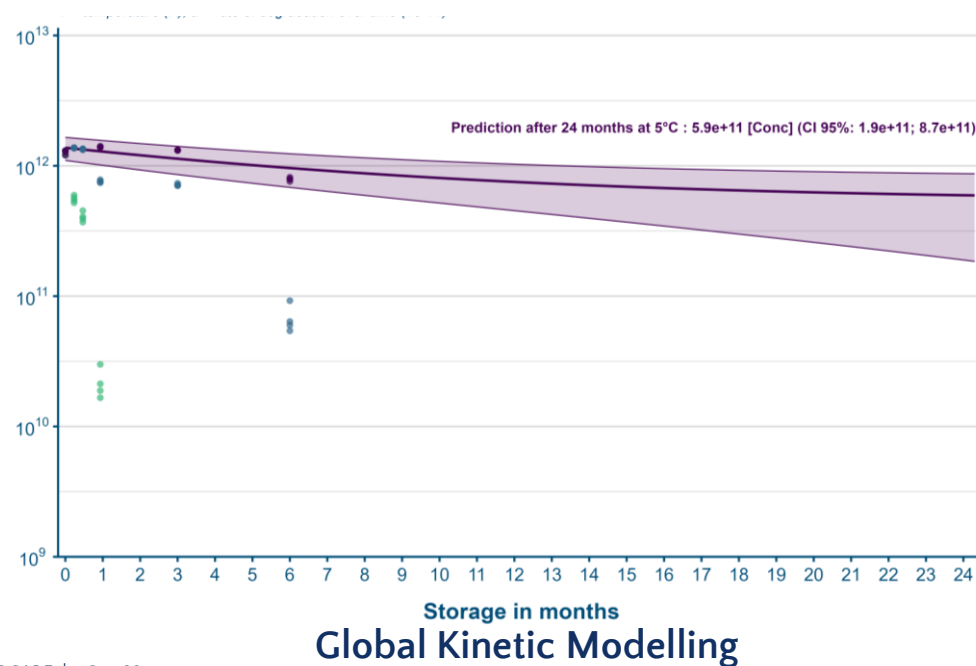
Global Kinetic Modelling



Two Step

## Case Study II: Viral Titer

| Time [days] | Tested [%] | Global Kinetic Modelling [%]  | Two-stage + Bootstrap [%]     |
|-------------|------------|-------------------------------|-------------------------------|
| 90          | 1.32e+12   | 1.13e+12 [8.57e+11, 1.41e+12] | 1.40e+12 [1.39e+12, 1.41e+12] |
| 180         | 7.90e+11   | 9.61e+11 [6.83e+11, 1.24e+12] | 1.52e+12 [1.51e+12, 1.54e+12] |
| 360         |            | 7.52e+11 [4.53e+11, 1.03e+12] | 1.77e+12 [1.75e+12, 1.80e+12] |
| 720         |            | 5.94e+11 [1.89e+11, 8.71e+11] | 2.27e+12 [2.23e+12, 2.32e+12] |



## Objection: Why?

- Different pathways may dominate at different temperatures
- Two-stage replaces raw data with 3+ rates ( $k_5$ ,  $k_{25}$ ,  $k_{37/40}$ )
- Degrees of freedom drop to 1 (3 points, 1 line) vs  $n-3$ ; raw AIC/BIC not comparable across temperatures
- Three temperatures say little about temperature-law curvature or heterogeneity
- The global joint likelihood is the Maximum Likelihood Estimator — asymptotically the best any regular estimator can do.

## Solution

| Method                         | Sensitive to                                            | Blind to                               |
|--------------------------------|---------------------------------------------------------|----------------------------------------|
| SEC (%HMWS/%LMWS)              | Aggregation, fragmentation                              | Charge and hydrophobicity changes      |
| CEX / icIEF                    | Deamidation, Asp isomerization, C-terminal Lys clipping | Size-only changes with no charge shift |
| RP-HPLC                        | Oxidation (Met, Trp), some clipped variants             | Non-covalent aggregation               |
| CE-SDS (reduced / non-reduced) | Fragmentation; covalent aggregates SEC can miss         | Native, non-covalent size changes      |

- Look beyond one attribute — compare activation energies across the stability profile
- Cross-attribute correlations require independently modeled profiles
- Compare equations scientifically, not only by goodness of fit.