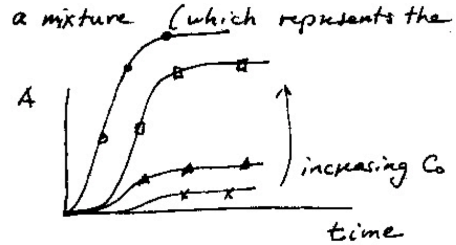
Kinetics of Self assembly of fibrils - Intro

Given some observations about a chemical reaction's product formation, can we deduce something about the underlying mechanism?

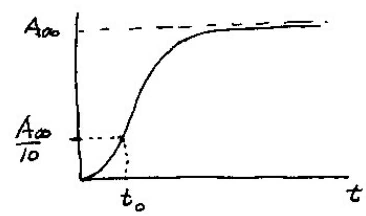
"Reverse Engineering"

Flyvbjerg et al (1996) analyzed microtubule polymerization kinetics, by following the turbidity $A(t)$ of a mixture (which represents the total mass $M(t)$ in polymer form).



They found curves of the form for various initial monomer conc

$$C(0) = C_0$$

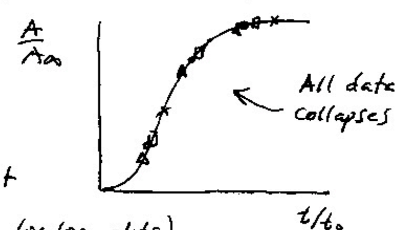


Each curve has a sigmoidal shape, and is assigned two parameters, A_∞ (its asympt.) and t_0 (which they arbitrarily chose ~~as t~~ such that

$$A(t_0) = A_\infty/10 \quad \text{(see Figure.)}$$

The data showed a particularly convenient SCALING PROPER·

A plot of $\frac{A(t)}{A_\infty}$ vs $\frac{t}{t_0}$ led to one MASTER CURVE



i.e. data collapses onto one curve.

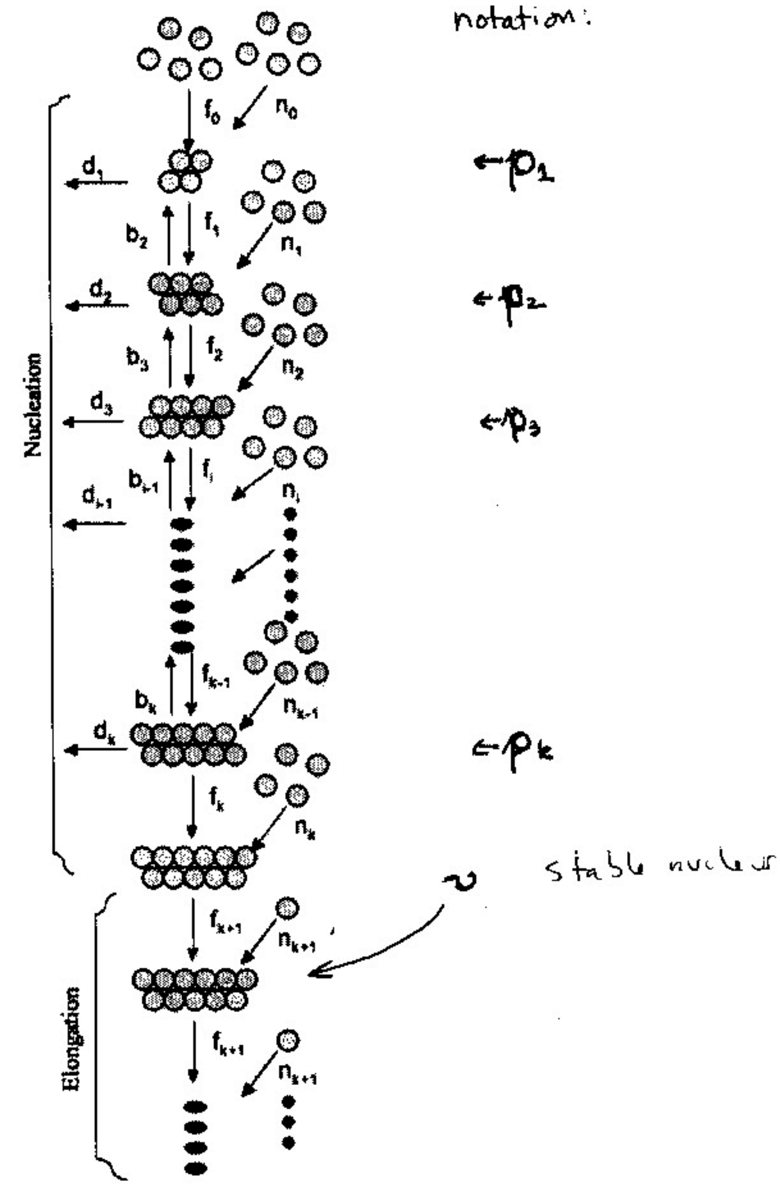
$\leftarrow$ slope γ / $\log 1/A_\infty$ — $\log t_0 = \gamma \log 1/A_\infty$

Using this fact, and the property that

$$t_0 \propto A_\infty^{-\gamma} \quad \text{(seen by log log plots)}$$

they were able to figure out DETAILS of the underlying mechanism

A great example of the utility of scaling and dimensional arguments)



'igure 4: Generic nucleation dependent model,the basic model that describes the nucleat
›olymerization of a self assembling polymer. Monomers are assumed to quickly associa
.nd dissociate to form short lived oligomeric species, we will denote as oligomers. The
›ligomers are assumed to quickly come into steady state with the monomer populatic
›table nuclei are then formed on a slower time scale and quickly elongate to form polyme

Kinetics of self-assembly of polymer fibrils.

Flyvbjerg, Jobs, Leibler (1996) PNAS 93: 5975-5979.
Janas Bailey

Scaling law:

$$A(t, A_\infty) = A_\infty\, f\left(\frac{t}{t_0(A_\infty)}\right)$$

$$t_0 \propto A_\infty^{-\gamma}$$

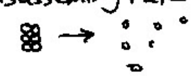
$C_0 = C(0) =$ monomer conc at $t=0$

$p_i(t) =$ conc of i-mer " time t

n_i = number of monomers needed for $C_i \xrightarrow{n_i \text{ mon.}} C_{i+1}$

$C(t) =$ monomer conc at time t

f_i } forward reaction
b_i } backward kinetics
d_i = disassembly rate



Basic events:

$$p_{i-1} + n_{i-1}\,\text{mon} \underset{b_i}{\overset{f_{i-1}}{\rightleftharpoons}} p_i + n_i\ \text{Monomers} \underset{b_{i+1}}{\overset{f_i}{\rightleftharpoons}} p_{i+1} + \cdots$$

(p_{i-1} ↓, p_i ↓ d_i, p_{i+1} ↓ d_{i+1})

Assumption: (1) Basic Mass-action kinetics apply. There is successive addition / loss of unit but no breakage into 2 or more large subunits.

$\frac{dp_1}{dt} =$

$\frac{dp_2}{dt} =$

⋮

$\frac{dp_i}{dt}$

Put your equations here

We refer to all complexes up to size k as "oligomers".

Stable nuclei:

$\nu(t) =$ conc of nuclei (in terms of monomer equivalents)

Assumption: (2) There are k "nucleation steps" in which oligomers form and grow the first stable nucleus forms by growth of the k'th oligomer.

(3) A stable nucleus has negligible disassembly rate.

$$\Rightarrow \quad \frac{d\nu}{dt} = f_k\, C^{n_k} p_k$$

-ssumption (4): Let M = mass in fibrilar form (monomer equivalents)

After a nucleus forms, it elongates by single monomer addition, and does not disassemble nor break

$$\Rightarrow \quad \frac{dM}{dt} = f_{k+1}\, c\, v$$

$\Rightarrow$ M keeps growing as long as monomer is available.

Assumption (5): Oligomers / nuclei form slowly, but fibrils grow rapidly so the amt of mass in oligomer form is small compared to mass in polymer form

$$\Rightarrow \quad c + M \approx c(0) = \text{monomer conc at time } 0$$

$$M \approx C(0) - C$$

By (4 and 5) as $t \to \infty$ $M \to C(0)$, $c \to 0$

i.e. let $M_\infty = C(0) = C_0$ (*)

$$\frac{M}{M_\infty} = \frac{C(0) - C}{M_\infty} = \frac{C(0)}{C(0)} - \frac{C}{C(0)} = 1 - \frac{C}{C(0)}$$

Assumption (6) : The expt'l measured turbidity $A(t)$ of the polymer solution is proportional to the polymer mass i.e.

$$\frac{A(t)}{A_\infty} = \frac{M(t)}{M_\infty}$$

(this assumes that the oligomers and nuclei have negligible contribution to turbidity)

$\Rightarrow$ By (6) and previous result (*)

$$\frac{A(t)}{A_\infty} = 1 - \frac{c}{C(0)}$$

Exercises for Unit on kinetics of self-assembly of polymer fibrils.

(1) Use the assumptions to formulate a set of ODE's for the concentrations of oligomers $p_2, p_3, \cdots p_k$, nuclei ν, and mass in polymer form. Assume at each step that $n_0, n_1, n_2 \ldots n_{k-1}$ monomers add to form the next (oligomer) complex.

(2) Given that mass in oligomers and nuclei is much smaller than mass in monomers and fibrils, i.e. that $c(t) + M(t) \approx c(0)$, argue that $\frac{A(t)}{A_\infty} \simeq 1 - \frac{c(t)}{c(0)}$ where A is the experimentally measured variable that represents fibril level. (Assume $\frac{A(t)}{A_\infty} = \frac{M(t)}{M_\infty}$)

(3) ~~A~~ Rewrite your eqns from (1) in dimensionless form using the rescalings : $t = t_0 \hat{t}$, $c = c_0 \hat{c}$, $p_i = X \hat{p}_i$, $\nu = \mu \hat{\nu}$, $M = c_0 \hat{M}$ (where $\wedge$ denotes dimensionless variables). ~~and to~~

(4) Now use the fact that data scales as $t_0 \propto c_0^{-\gamma}$, i.e.

$$t_0 = \frac{\lambda}{c_0^\gamma}$$

(and that this scaling makes the behaviour independent of c_0) to identify appropriate choices for X, μ, $n_0, n_1, \ldots$ etc. Explain ~~the~~ what happens to the rates $b_1, b_2, \ldots$ $d_1, d_2, \ldots$ and what this implies.

(5) Write down the (scaled) equations for $\hat{p}_1, \hat{p}_2, \ldots \hat{p}_k$, $\hat{\nu}$, $\hat{M}$

(6) Based on (4), how many monomers n_0 are needed to make the first oligomer? How many (n_j) to then make the 2nd, 3rd oligomer? How many monomers does it take to make the first stable nucleus?

7) Close to the start of the experiment, $c(t) \approx c(0) = C_0 \Rightarrow$

$$\hat{c}(t) \equiv \frac{c(t)}{C_0} \approx 1$$

Use this to simplify the eqns and solve them one by one.

show that
$$c_1(t) \sim f_0 t$$
$$c_2(t) \sim \tfrac{1}{2} f_1 f_0 t^2$$
$$\vdots$$
$$c_k(t) \sim \quad t^k$$

And that
$$M(t) \sim \quad t^{k+2}$$

Thereby establish that $\log\left(\frac{A(t)}{A_\infty}\right) = (k+2)\log t + \text{constant}$.

8) An attached excel file contains simulated 'turbidity data' for self-assembly of amyloid fibrils from three different monomer concentrations $c(0) = 1.0$, $c(0) = 2.0$, and $c(0) = 3.0$. Use the data as follows:

(a) Identify A_∞ and t_0 for each set.

(b) Plot $A(t)$ vs t for the 3 series on one plot

(c) Plot $\log(A/A_\infty)$ vs $\log(t)$ " " "

Use the early few data points to determine the number of distinct oligomeric species

(d) Plot $\log(A_\infty)$ vs $\log(t_0)$. What ~~does~~ do the slopes of these lines indicate?

(e) Plot A/A_∞ vs t/t_0 to show that the data collapses.

(f) Use the data to determine the full assembly scheme and sketch a diagram