Mathematical Cell Biology Graduate Summer Course

University of British Columbia, May 1-31, 2012

Leah Edelstein-Keshet

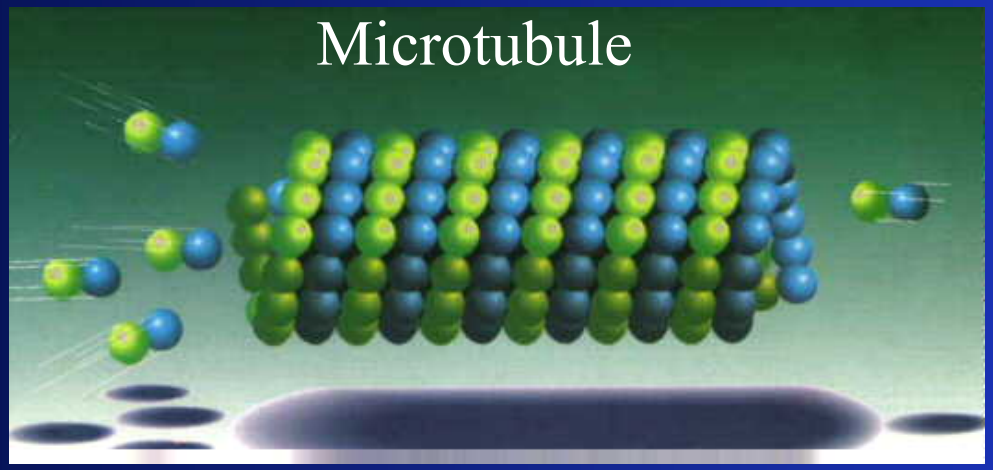
Reverse engineering a polymerization reaction

Deciphering polymerization steps from biochemical data

Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 5975–5979, June 1996
Applied Mathematics

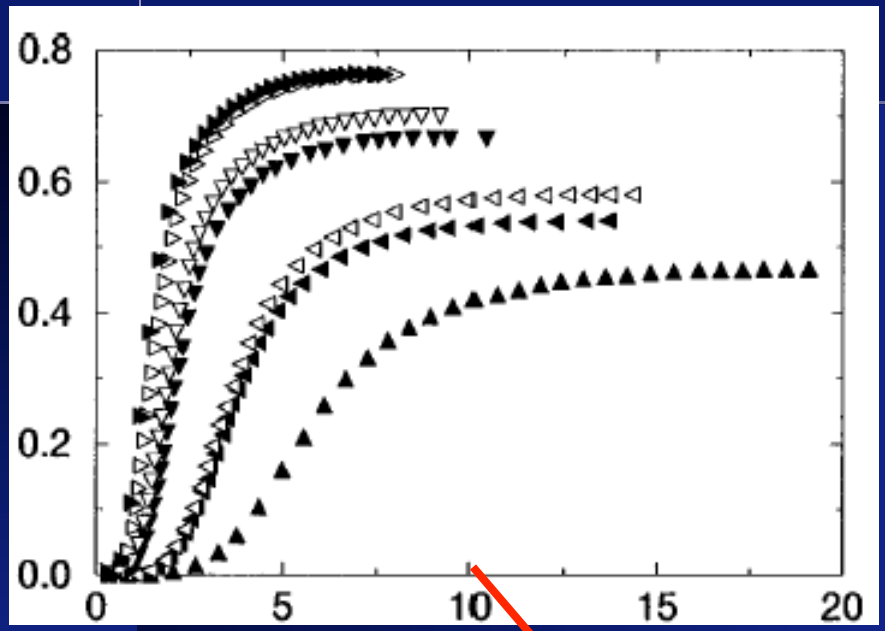
Kinetics of self-assembling microtubules: An "inverse problem" in biochemistry

HENRIK FLYVBJERG*†‡, ELMAR JOBS*, AND STANISLAS LEIBLER†



Henrik Flyvbjerg

Flyvbjerg (1996) showed how to "reverse engineer" polymer assembly based on scaling laws.



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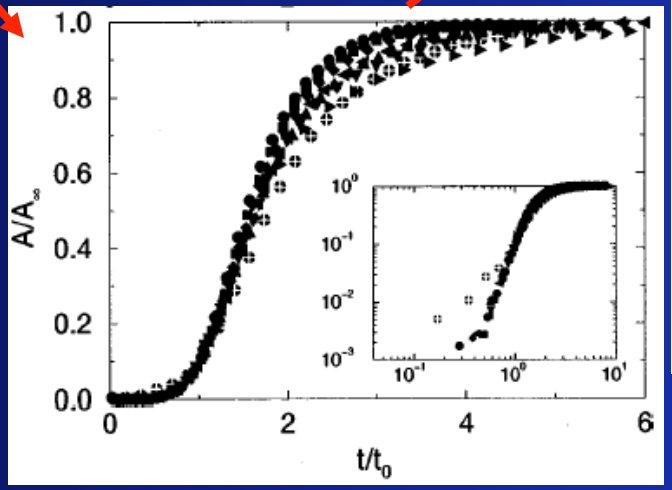
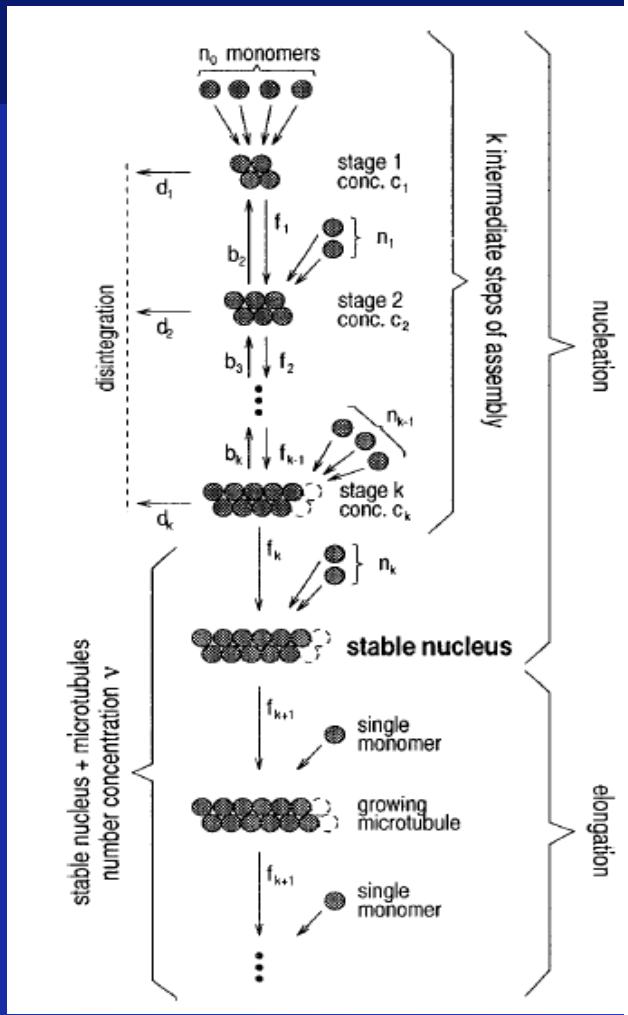




The same idea was applied to understanding the assembly of

Islet Amyloid Poly-Peptide (IAPP)

a protein that forms toxic fibrils in the pancreatic beta cells.

(These cells secret insulin, and their disfunction leads to Type 2 Diabetes)

Amyloid in Type 2 Diabetes:

J Bailey[1], K J Potter[2], C B Verchere[2], L Edelstein-Keshet[1] and D Coombs[1]

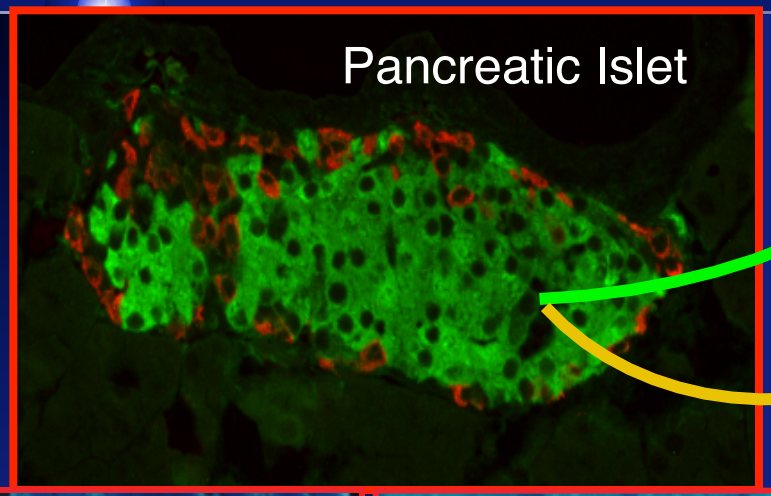
Reverse engineering an amyloid aggregation pathway with dimensional analysis and scaling

Phys. Biol. **8** (2011) 066009 (9pp)

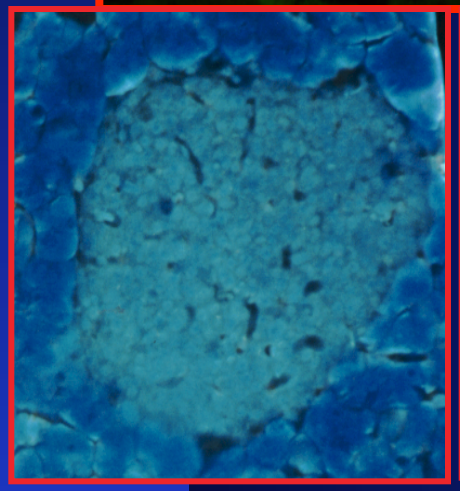
With James Bailey (UBC MSc)
Daniel Coombs
Bruce Verchere

The Islet Beta Cell Peptide IAPP

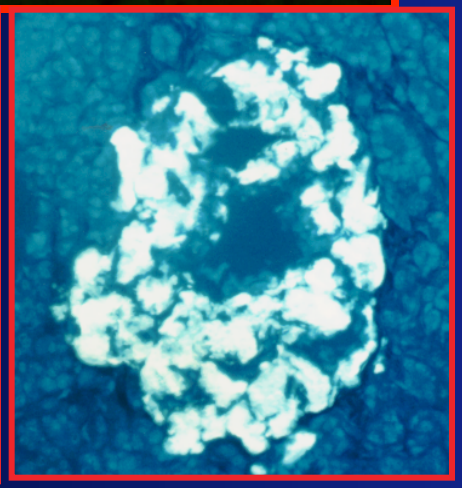
Insulin



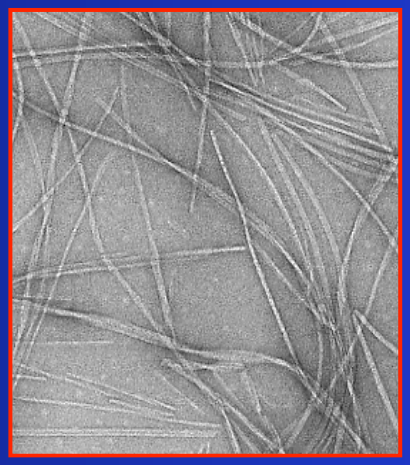
Islet Amyloid Polypeptide
(IAPP; amylin)



Normal



Type 2 Diabetes



IAPP-derived amyloid fibrils

Islet Amyloid Polypeptide Assembly

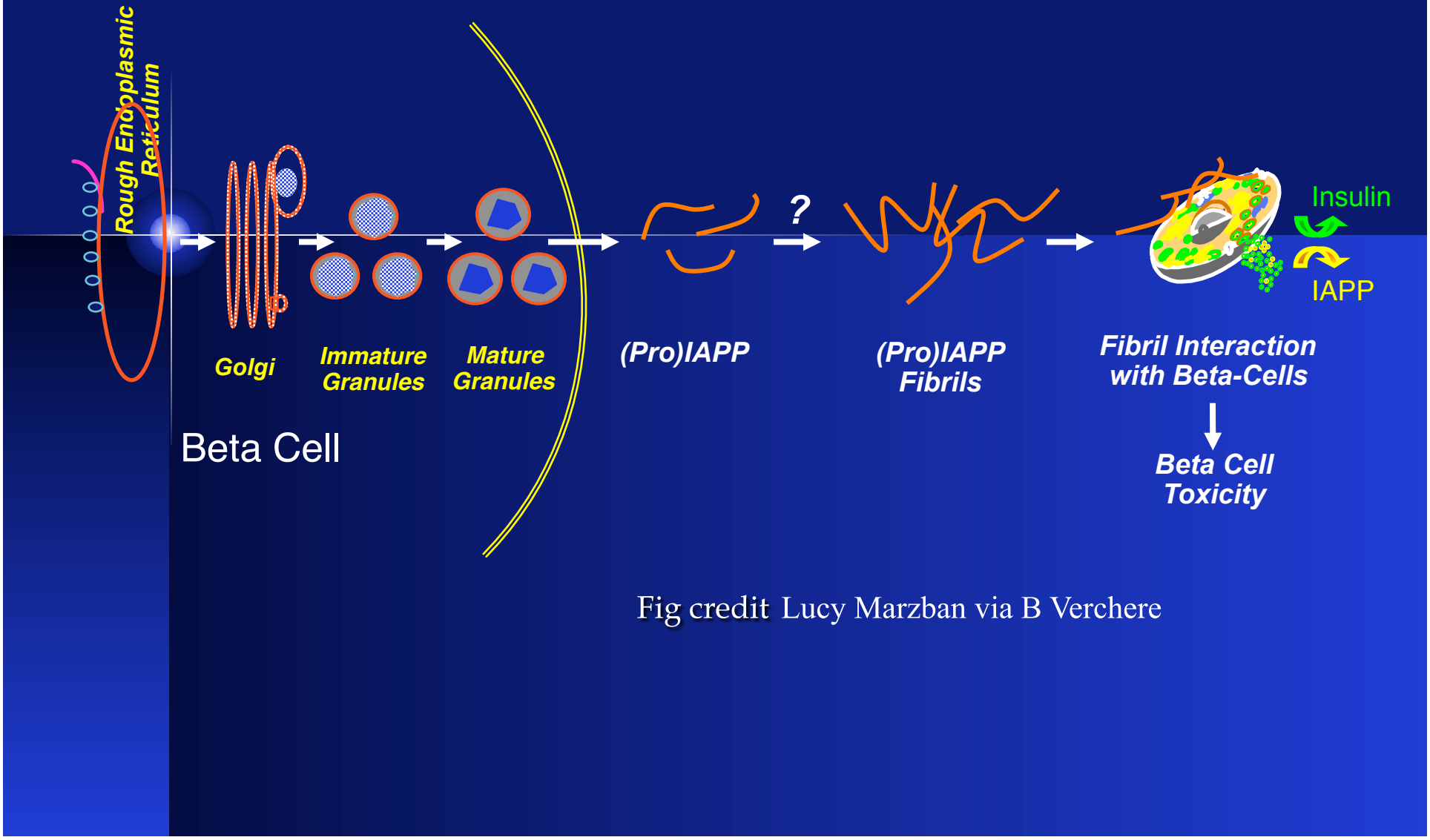
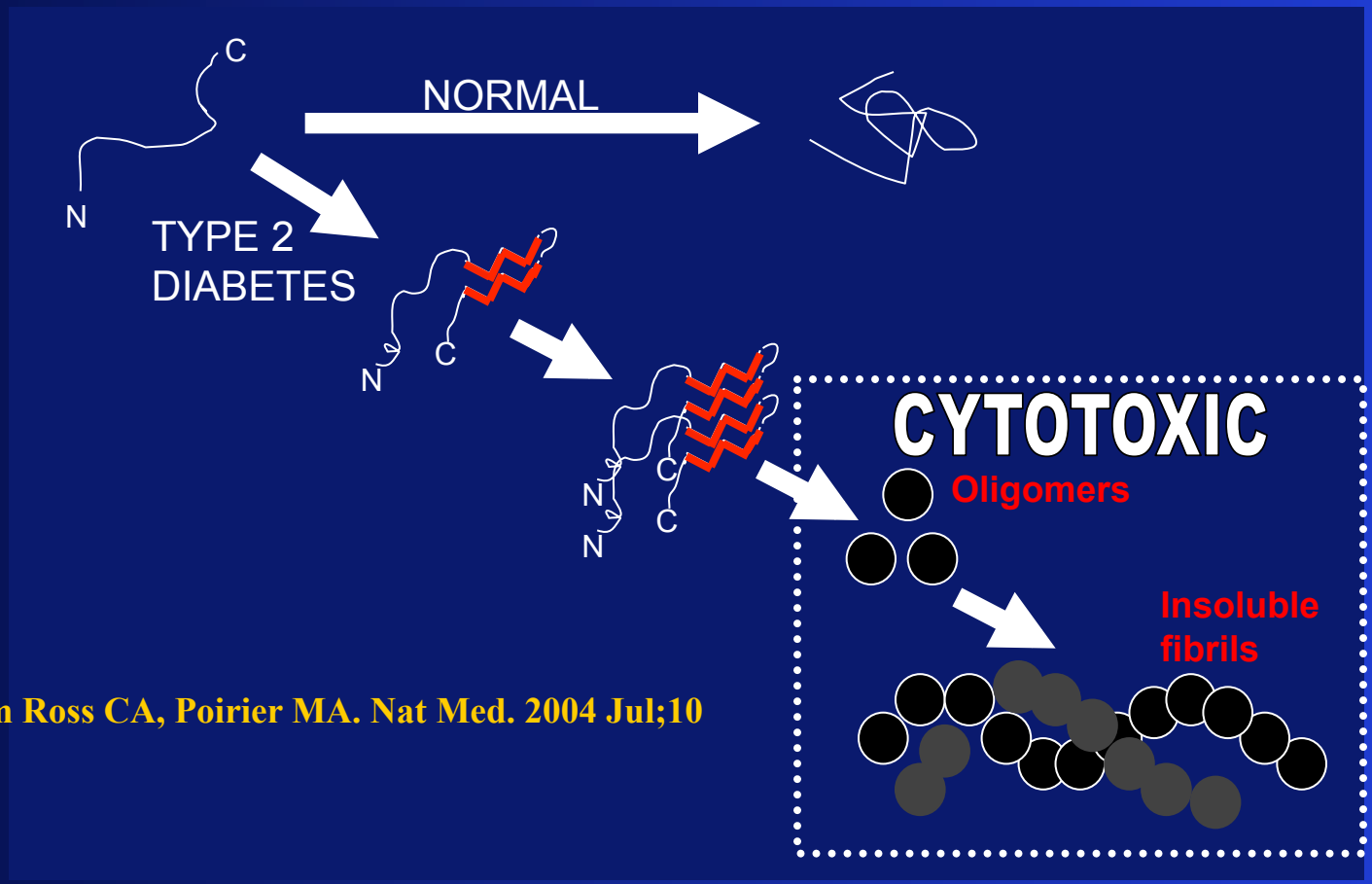


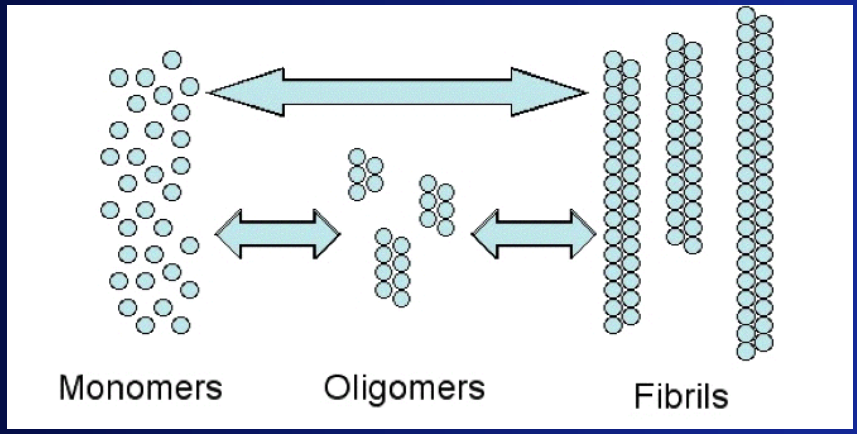
Fig credit Lucy Marzban via B Verchere

IAPP



Adapted from Ross CA, Poirier MA. Nat Med. 2004 Jul;10 Suppl:S10-7.

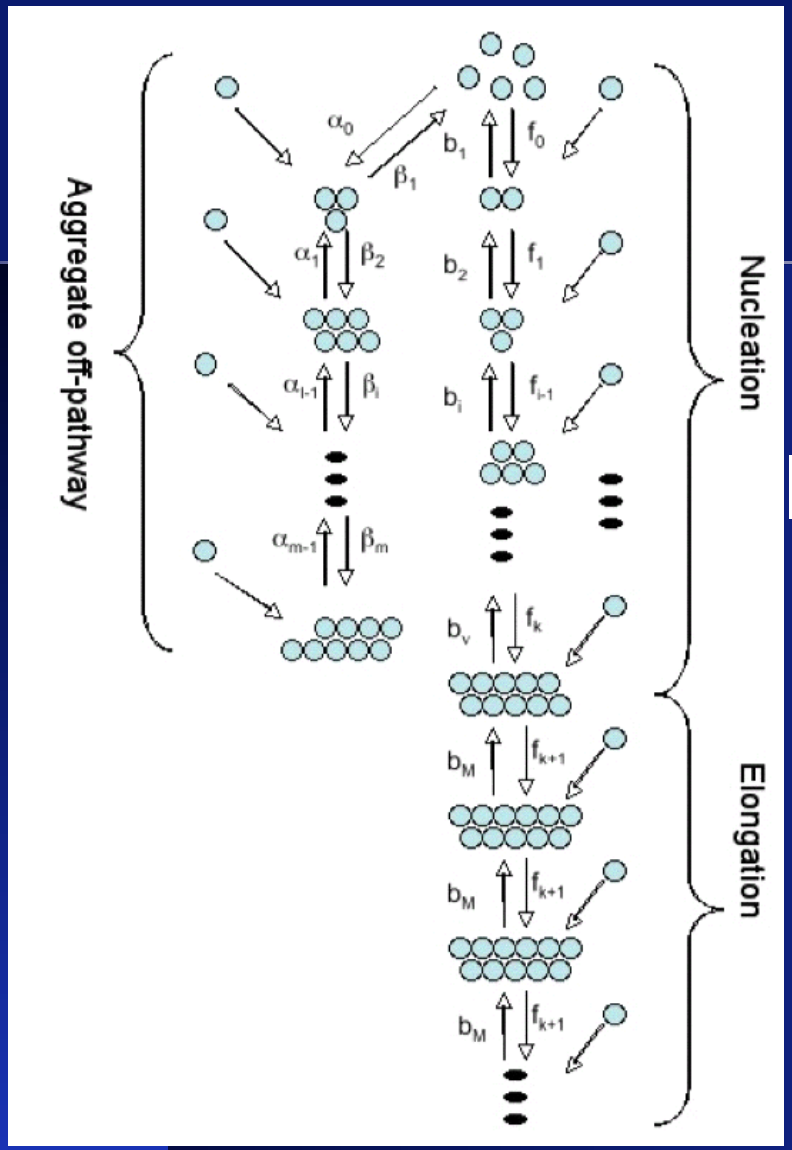
Previous models



Lee et al

Assume nuclei form as individual monomers and join a growing nucleus

Lee C C, Nayak A, Sethuraman A, Belfort G and McRae G J 2007 *Biophys. J.* **92** 3448–58

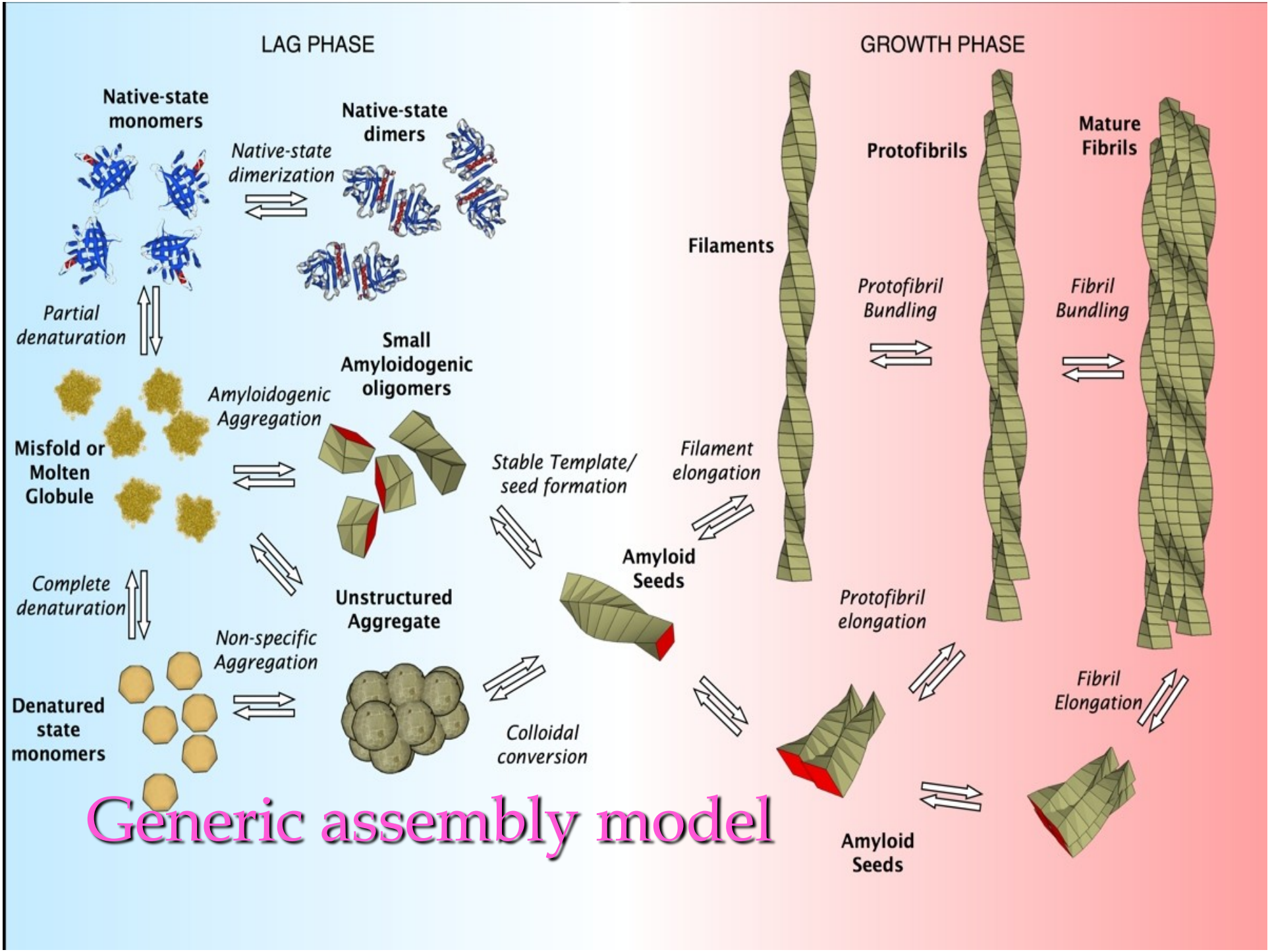


Previous models

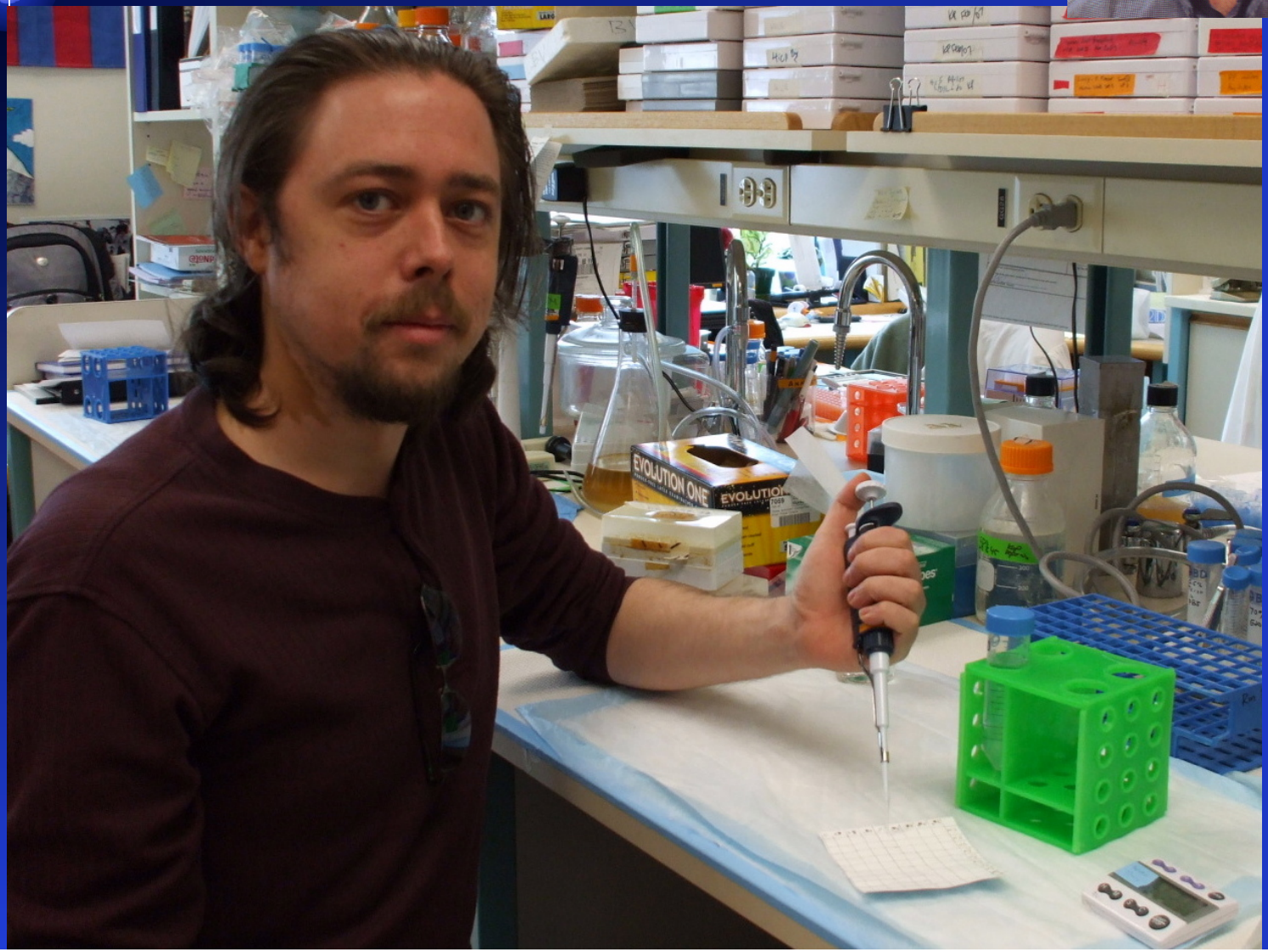
Powers & Powers

Powers E T and Powers D L 2008 *Biophys. J.* **94** 379–91

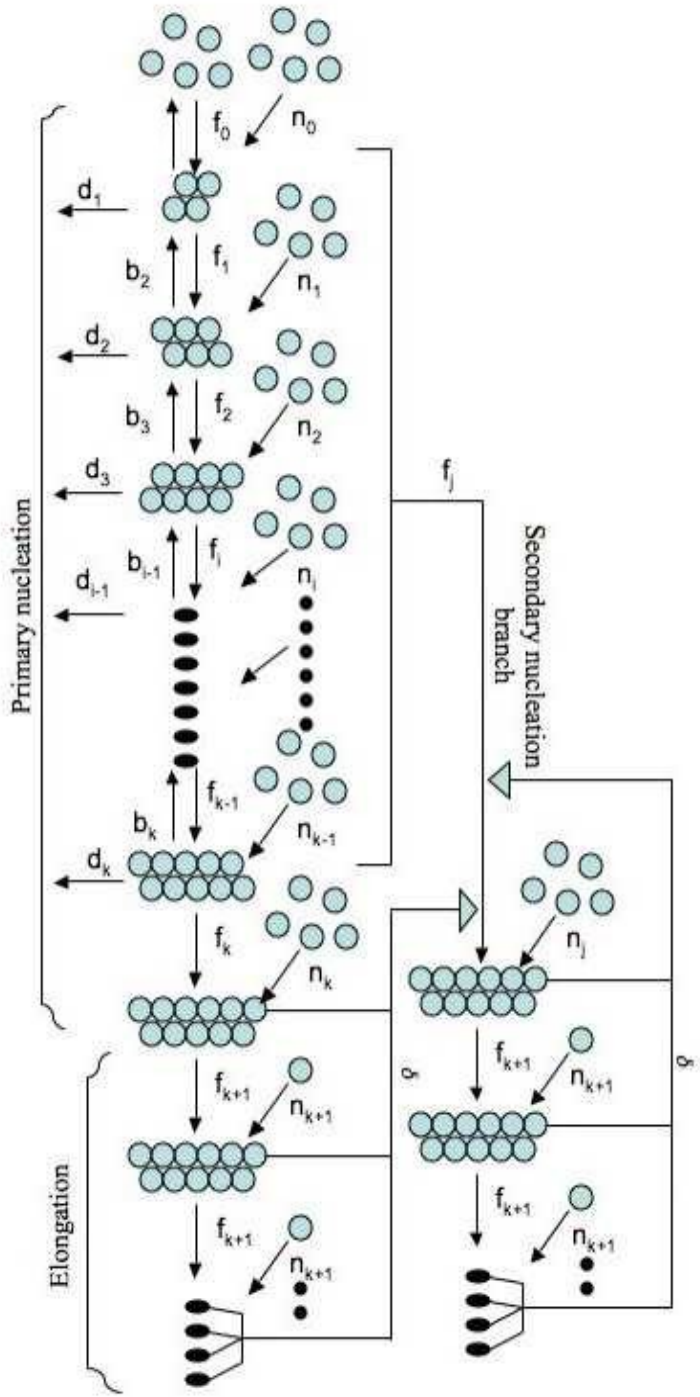
nucleated polymerization with a competing off-pathway aggregation sequestering monomers away from main pathway


LAG PHASE
GROWTH PHASE
Native-state monomers
Native-state dimers
Native-state dimerization
Partial denaturation
Misfold or Molten Globule
Small Amyloidogenic oligomers
Amyloidogenic Aggregation
Stable Template/ seed formation
Filament elongation
Filaments
Protofibrils
Mature Fibrils
Protofibril Bundling
Fibril Bundling
Amyloid Seeds
Complete denaturation
Unstructured Aggregate
Non-specific Aggregation
Protofibril elongation
Fibril Elongation
Denatured state monomers
Colloidal conversion
Amyloid Seeds
Generic assembly model

James Bailey in the Verchere lab

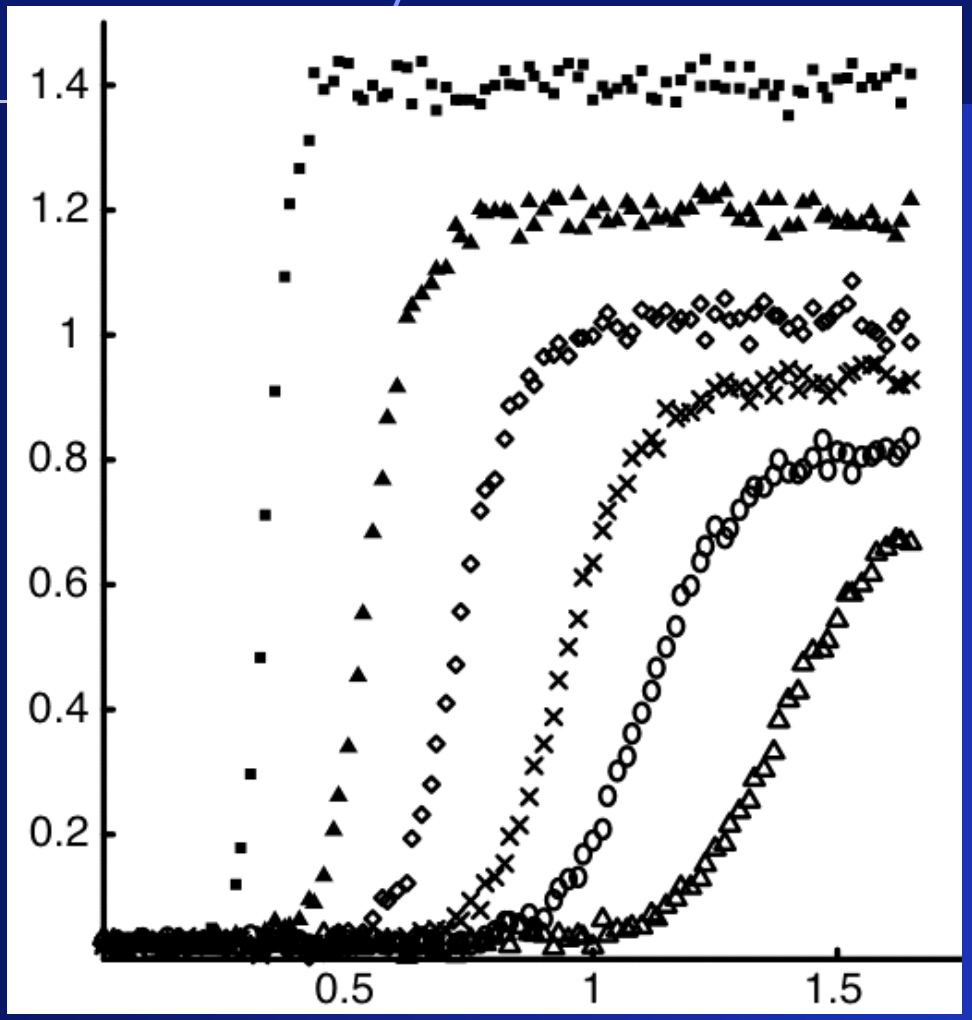


Default polymerization model

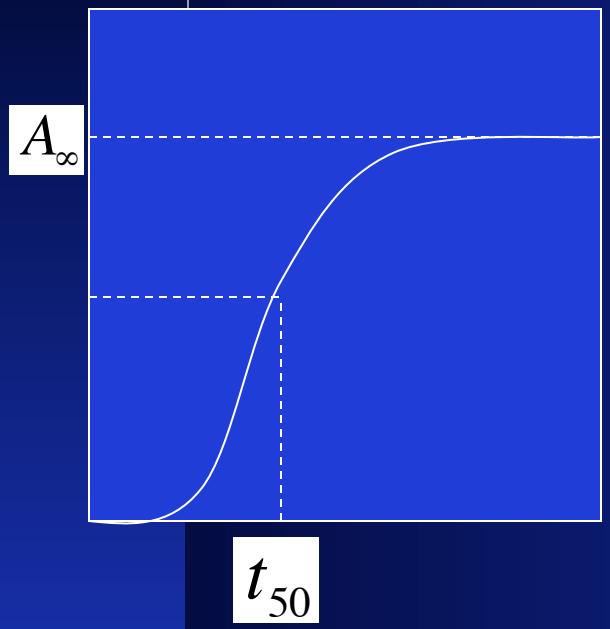


We do not know ahead of time how the polymer assembles, what are the nuclei, and how many monomers add at each step. We also do not know the rates of the forward and reverse reactions of these steps. The data will enable us to determine many of these based on the scaling property.

hIAPP Polymerization



Scale the data:



Scale time: t/t_{50}

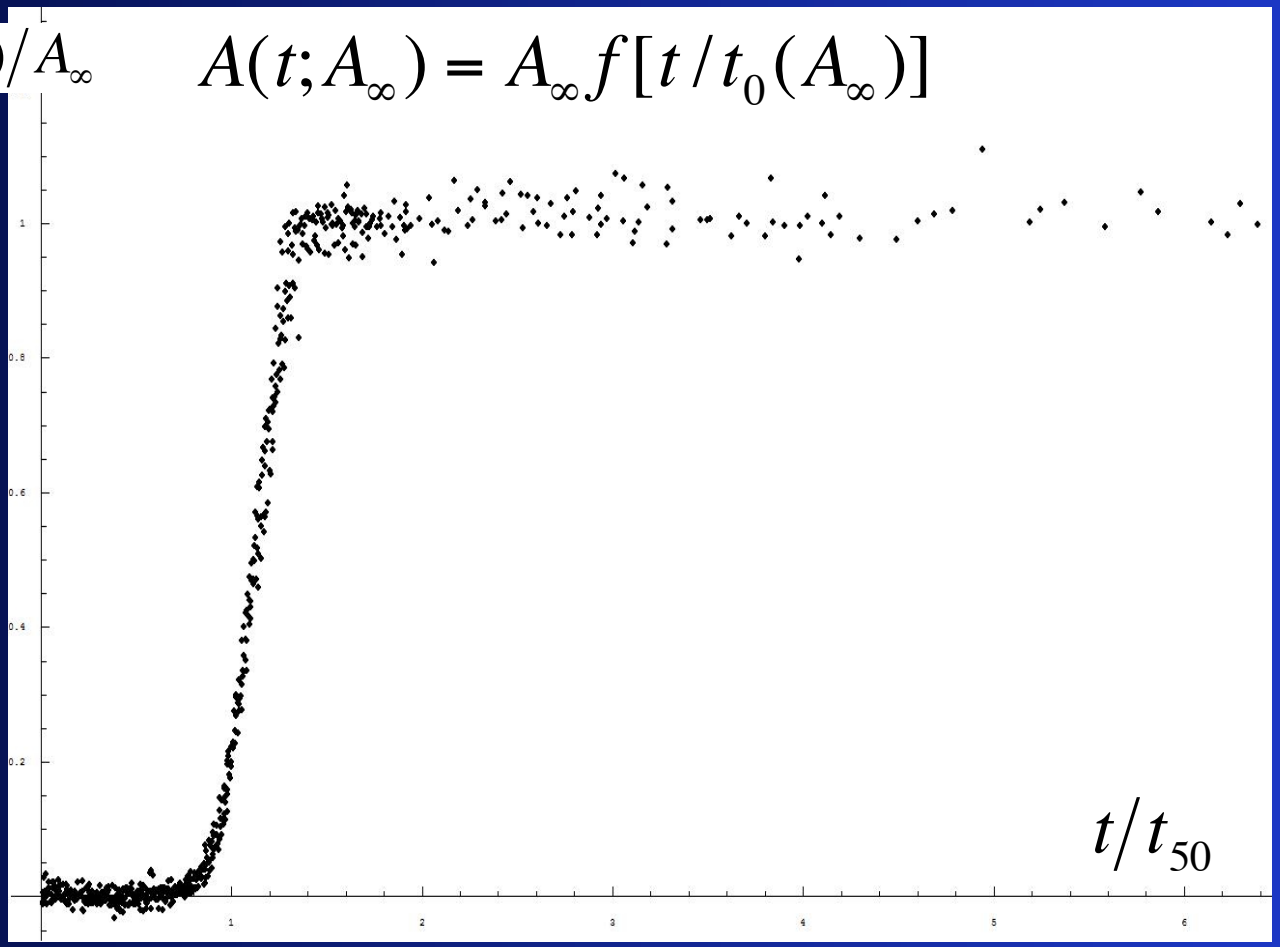
Scale fluorescence: $A(t)/A_\infty$

$$A(t;A_\infty) = A_\infty f[t/t_{50}(A_\infty)]$$

Scaling collapses the data

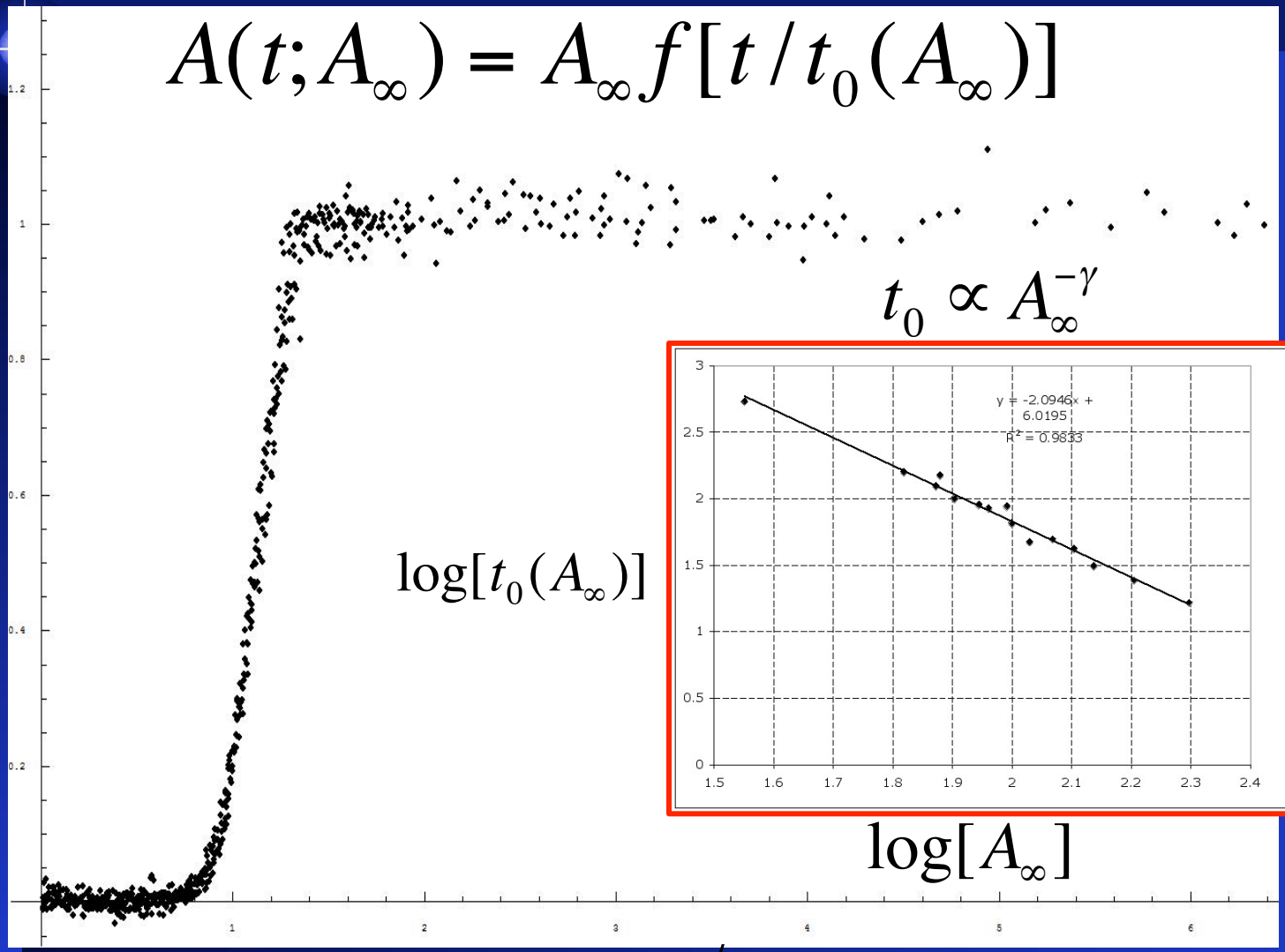
$A(t)/A_\infty$

$$A(t;A_\infty) = A_\infty f[t/t_0(A_\infty)]$$



Scaled data

$$A(t;A_\infty) = A_\infty f[t/t_0(A_\infty)]$$



$A(t)/A_\infty$

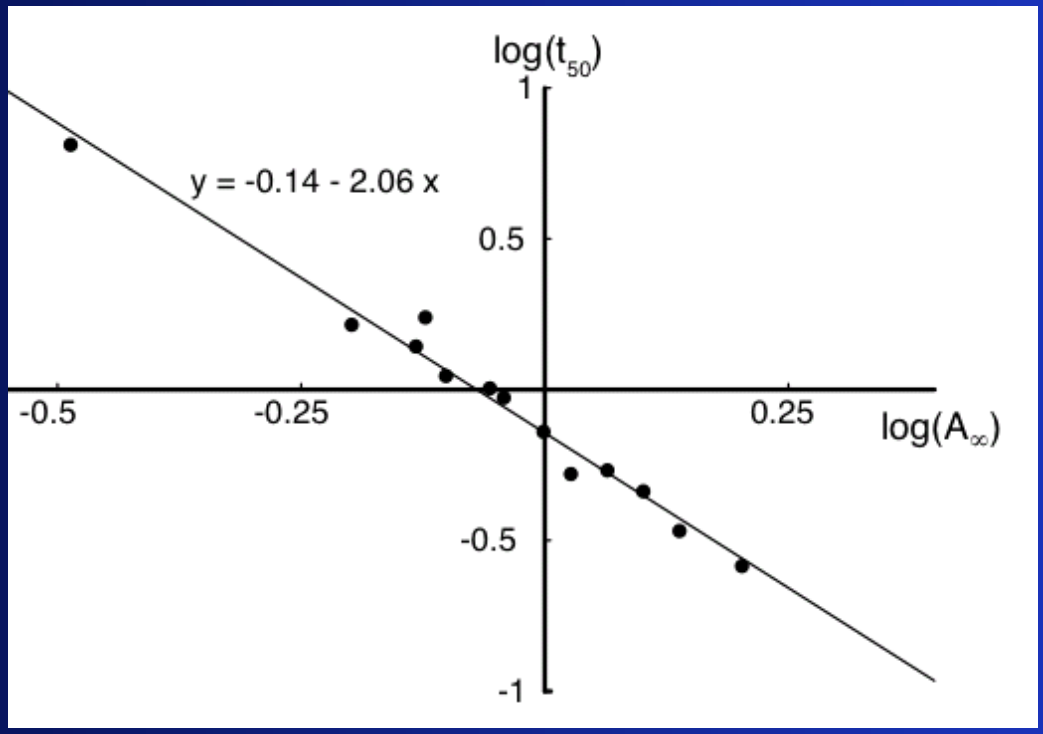
$t_0 \propto A_\infty^{-\gamma}$

$\log[t_0(A_\infty)]$

$\log[A_\infty]$

t/t_0

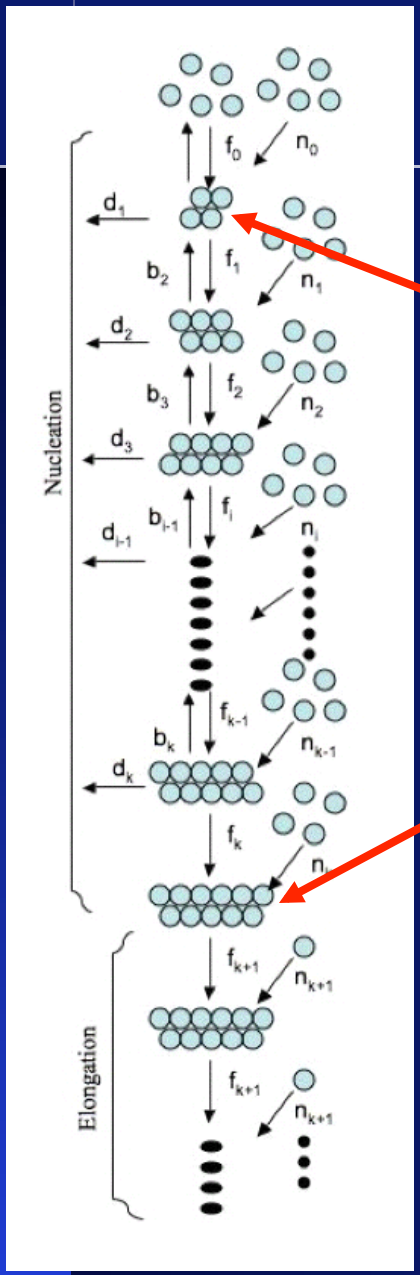
Find a scaling law



$$t_{50} \propto A_{\infty}^{-\gamma}$$

$\gamma \approx 2$

Nucleation-dependent polymerization



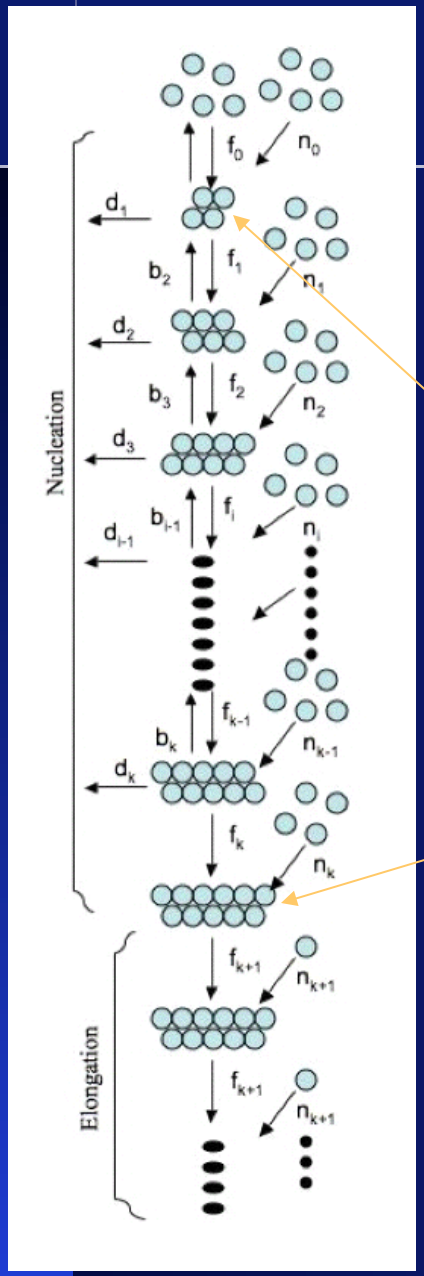
First stable oligomer (mass p_1)

ith stable oligomer (mass p_i) $i=2..k$

First stable nucleus (mass ν)

Fibrils (mass M)

Mass action kinetics (odes)



$$\frac{dp_1}{dt} = f_0 c^{n_0} - f_1 c^{n_1} p_1 + b_2 p_2 - d_1 p_1, \qquad (1)$$

$$\frac{dp_i}{dt} = f_{i-1} c^{n_{i-1}} p_{i-1} - f_i c^{n_i} p_i - b_i p_i + b_{i+1} p_{i+1} - d_i p_i \quad (2 \leqslant i \leqslant k), \qquad (2)$$

$$\frac{dv}{dt} = f_k c^{n_k} p_k,$$

$$\frac{dM}{dt} = f_{k+1} c^{n_{k+1}} v.$$

We define dimensionless variables as follows:

$$\hat{t} = \frac{t}{t_0}, \qquad \hat{c} = \frac{c}{c_0}, \qquad \hat{p}_i = \frac{p_i}{X}, \qquad \hat{\upsilon} = \frac{\upsilon}{X},$$

The dynamics described by the rescaled equations must be independent of c_0. This is the same as saying that all the scaled curves in the polymerization data are the same when superimposed.

Scaling the model

$$\hat{t} = \frac{t}{t_{50}} = \frac{t}{\lambda c_0^{-\gamma}},$$

$$\hat{c} = \frac{c}{c_0},$$

$$\hat{c}_i = \frac{c_i}{X},$$

$$\hat{\nu} = \frac{\nu}{\mu},$$

$$\hat{M} = \frac{M}{c_0}$$

Require that c_0 not appear in scaled eqns

$$\mu = c_0^{\gamma}$$

$$X = \mu$$

$$n_i = \gamma$$

$$n_0 = 2\gamma$$

$$- b_i p_i + b_{i+1} p_{i+1} - d_i p_i = 0$$

Dimensionless equations

After some careful work, obtain:

$$\frac{dp_1}{dt} = f_0 c^{2\gamma} - f_1 c^{\gamma} p_1,$$

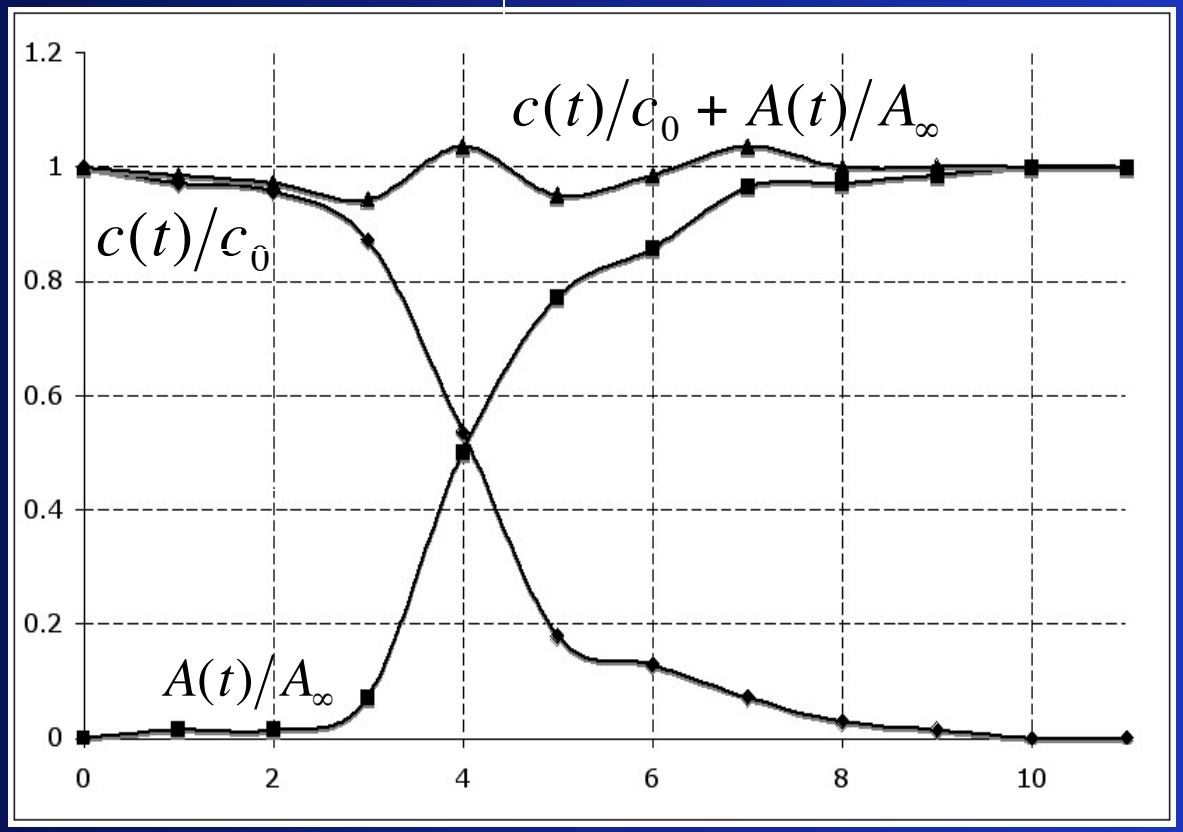
$$\frac{dp_i}{dt} = f_{i-1} c^{\gamma} p_{i-1} - f_i c^{\gamma} p_i$$

$$\frac{dv}{dt} = f_k c^{\gamma} p_k,$$

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

The fact that the data scales imposes strong constraints on the possible underlying kinetics

Mass mostly in monomer and fibrils



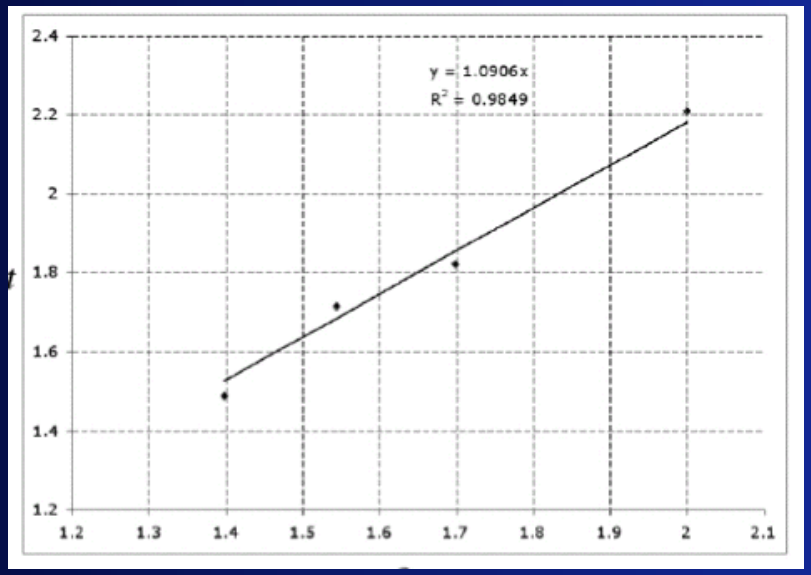
$M(t) + c_0(t) \approx \text{constant}$

Fiber elongation

Add monomers to a "mature reaction" and watch fiber growth. Nuclei are roughly constant, and

$$\frac{dM}{dt} \approx -\frac{dc_0}{dt} = f_e F c_0^{n_{k+1}} \quad \Rightarrow \quad \frac{dA}{dt} \propto c_0^{n_{k+1}}$$

Log(*dA/dt*)

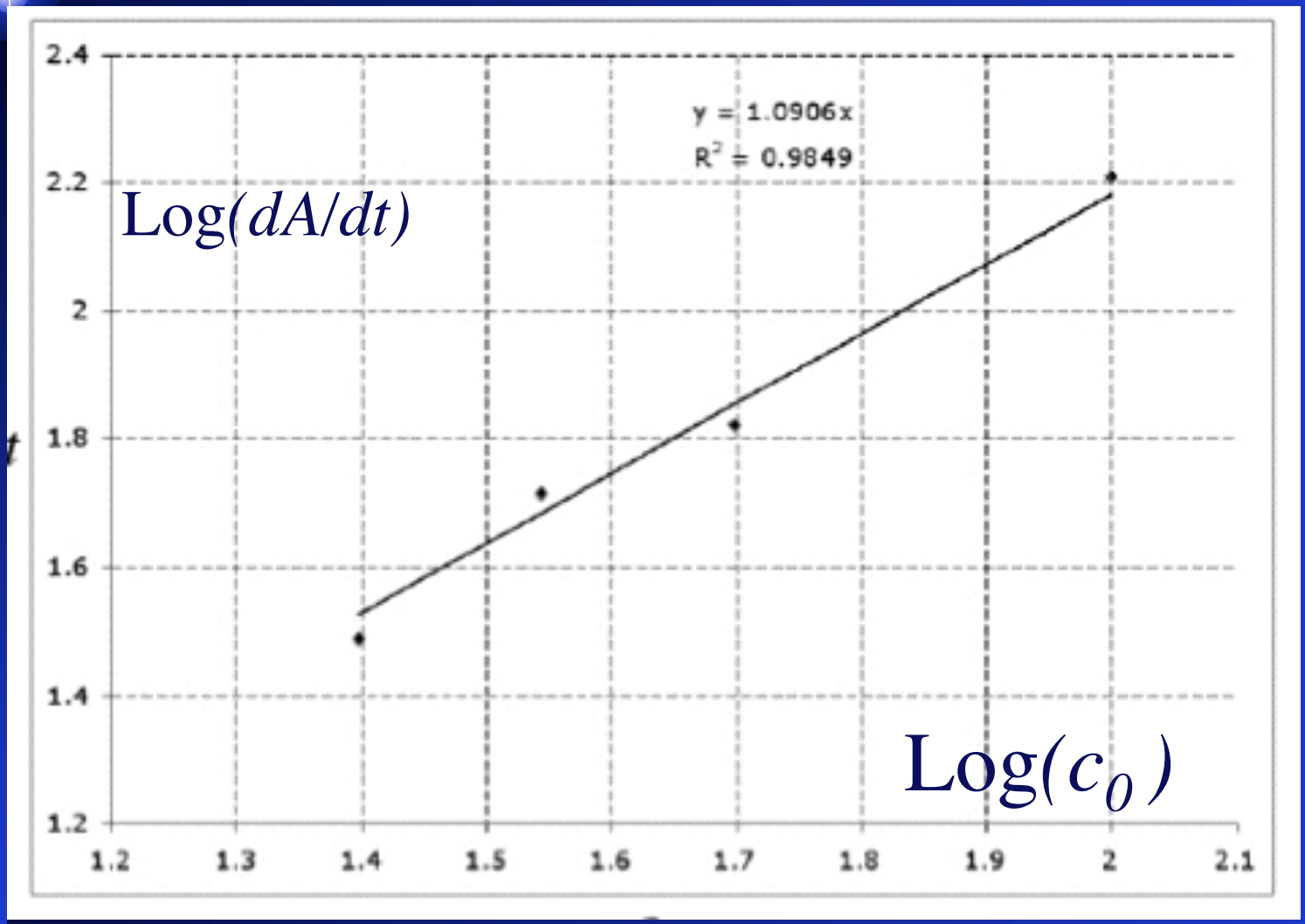


Log(c_0)

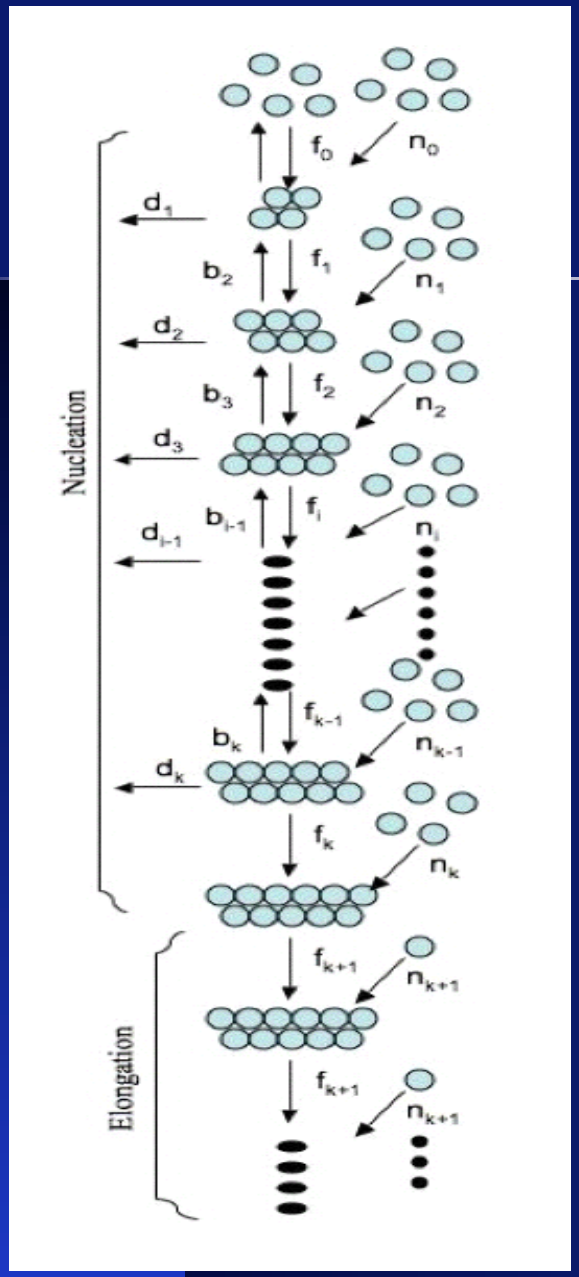
Find that $n_{k+1}=1$

Fibers grow by adding single monomers

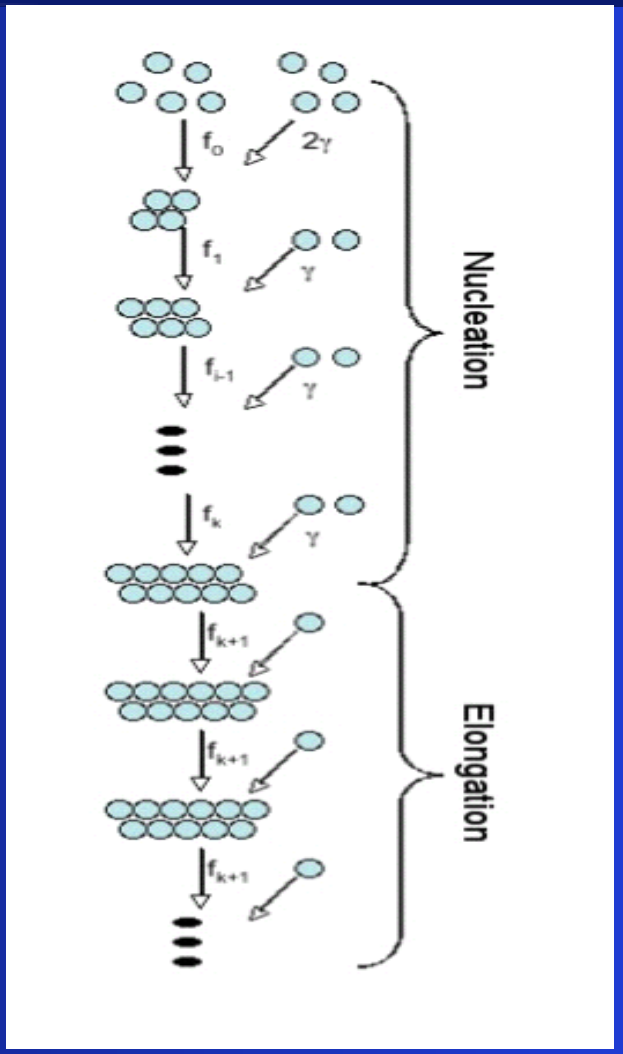
$$\frac{dA}{dt} \propto c_0^{n_{k+1}}$$



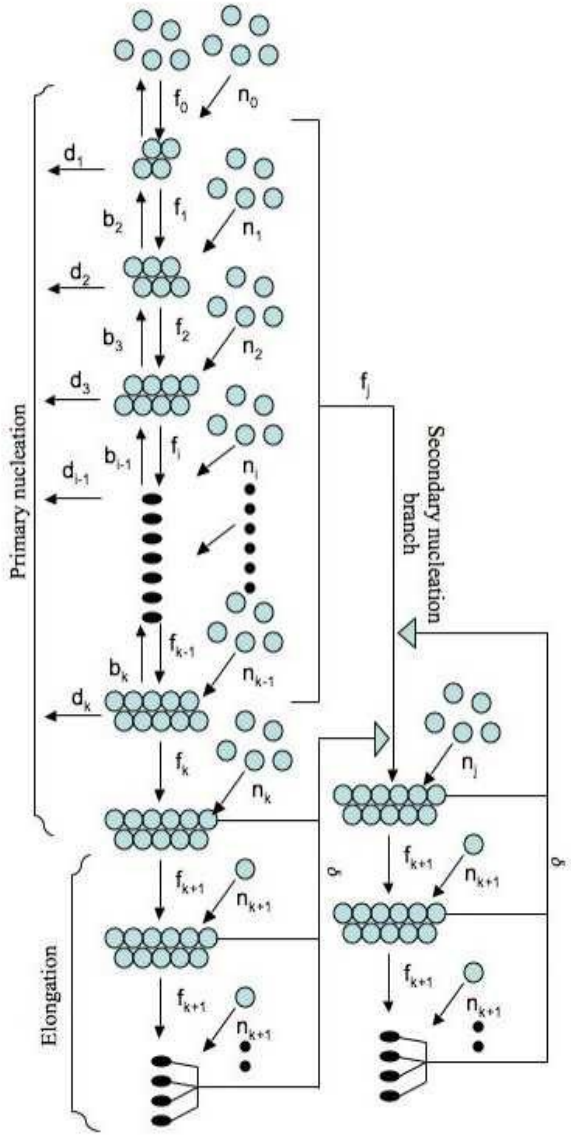
Summary: what the scaling showed:

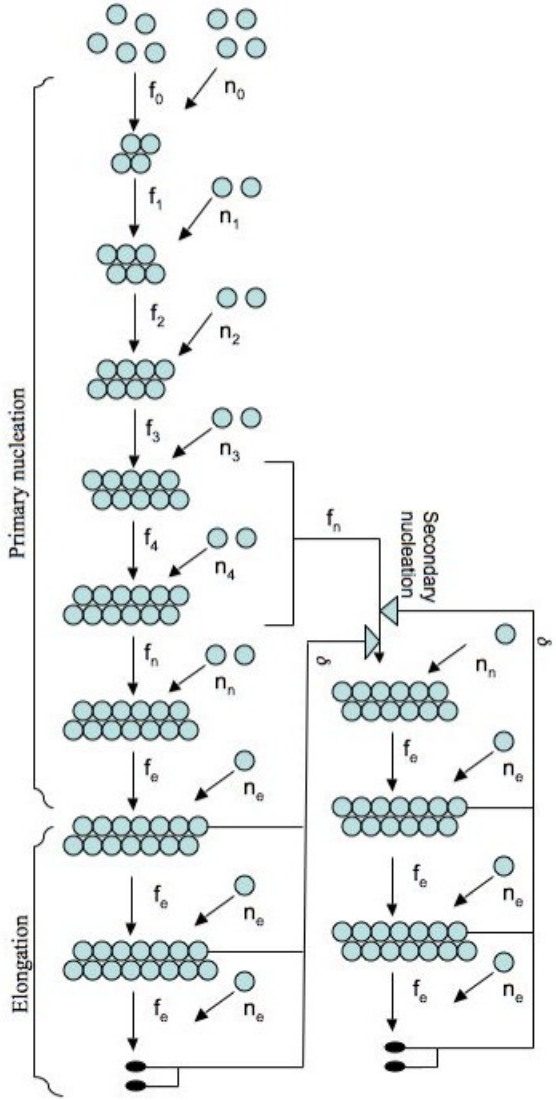


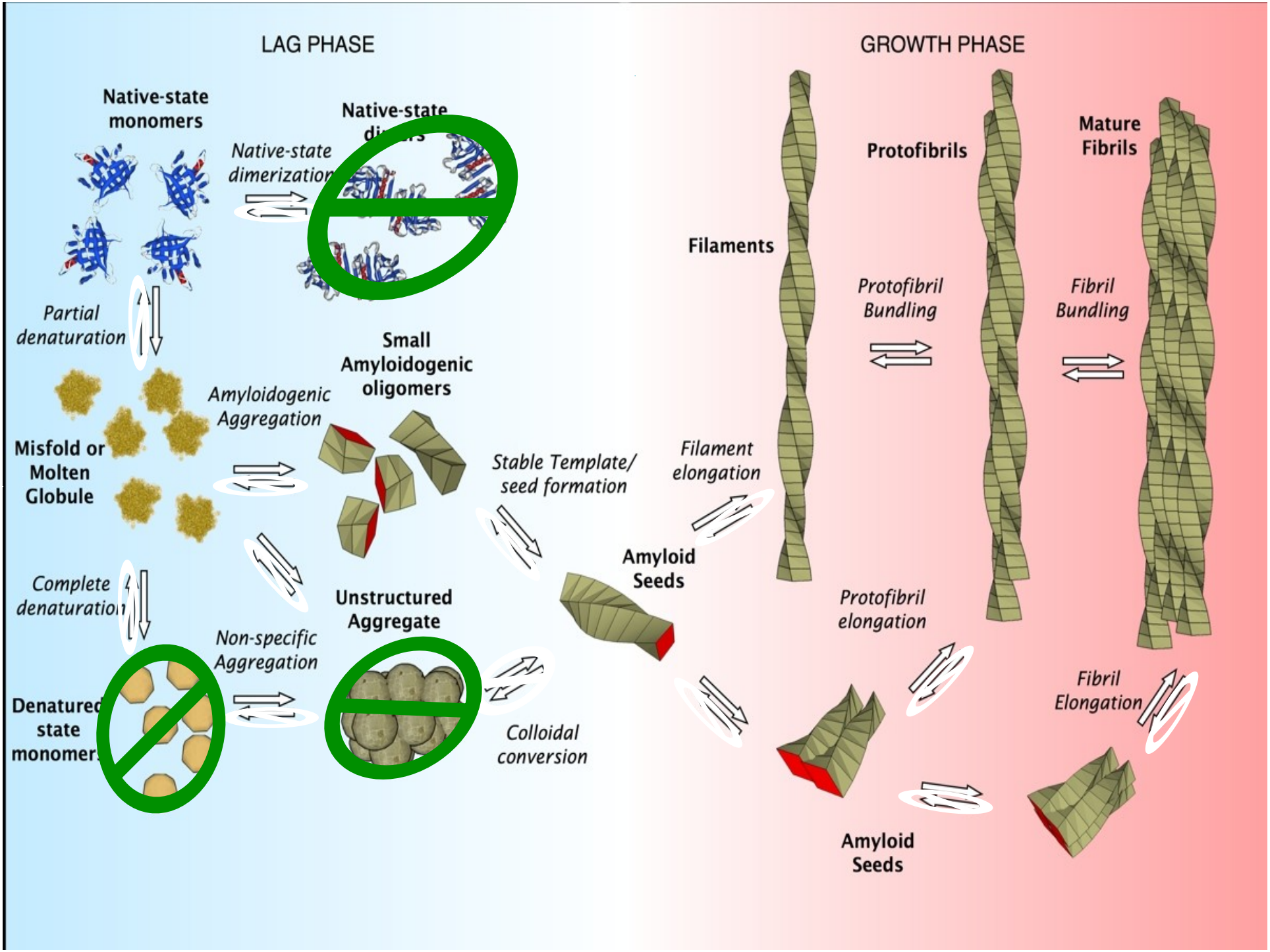
Scaling and model



Secondary nucleation






LAG PHASE
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Native-state monomers
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Amyloid Seeds
Filament elongation
Filaments
Protofibril Bundling
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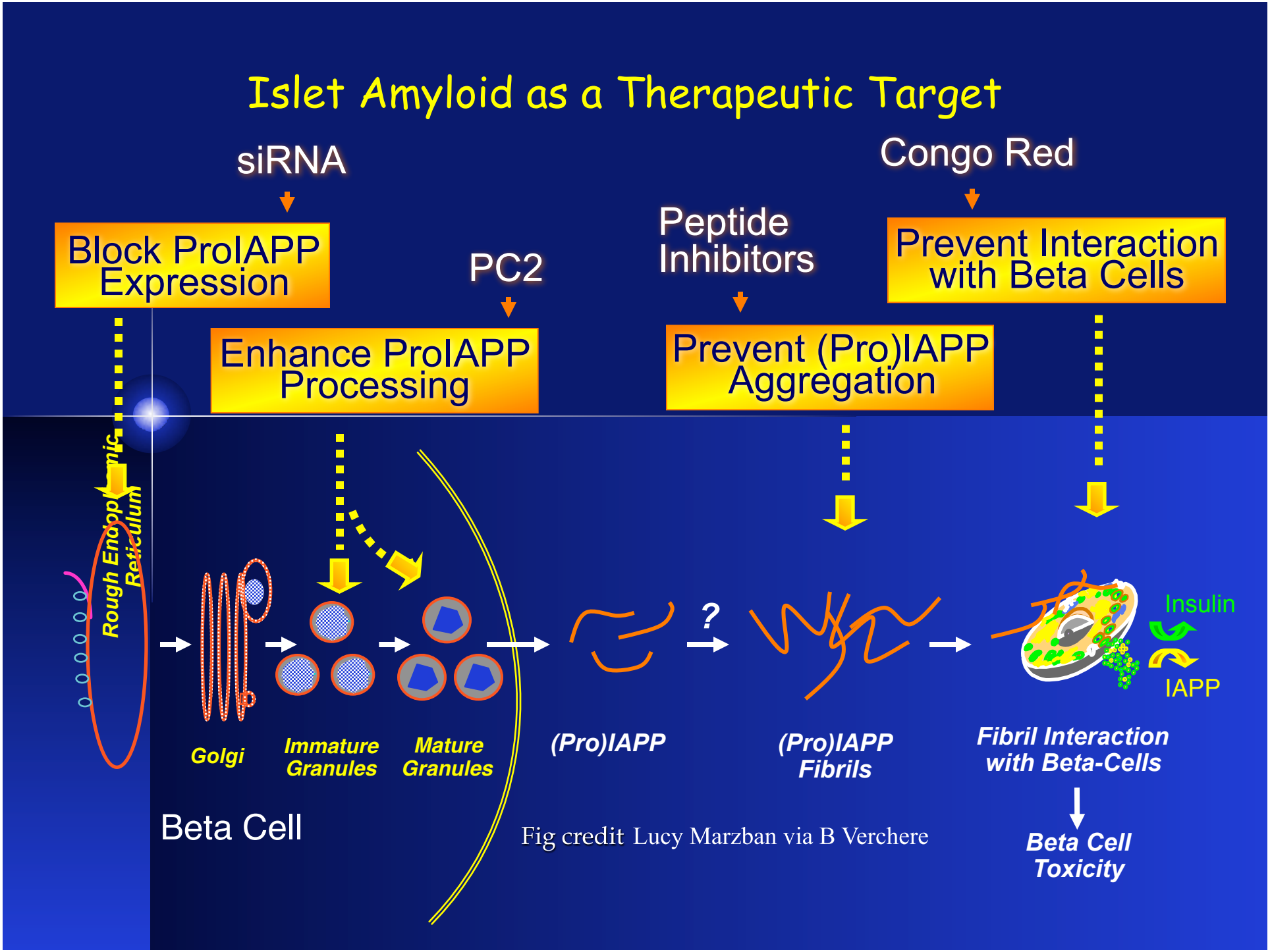
Islet Amyloid as a Therapeutic Target
siRNA
Block ProIAPP Expression
PC2
Enhance ProIAPP Processing
Peptide Inhibitors
Prevent (Pro)IAPP Aggregation
Congo Red
Prevent Interaction with Beta Cells
Rough Endoplasmic Reticulum
Golgi
Immature Granules
Mature Granules
Beta Cell
(Pro)IAPP
?
(Pro)IAPP Fibrils
Insulin
IAPP
Fibril Interaction with Beta-Cells
Beta Cell Toxicity
Fig credit Lucy Marzban via B Verchere

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NSERC support (Leah Keshet)

MITACS support and Accelerate BC internship (James Bailey)