

# Stochastic Model of Prion's Disease: Nucleation-Polymerisation Model Part II

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April, 22nd, 2010

## Experimental heterogeneity

- Heterogeneity of the structure

- Heterogeneity of the dynamics

- Seeding experiments

- Model proposed

## Modelling each reaction

- Precise reactions step and parameters

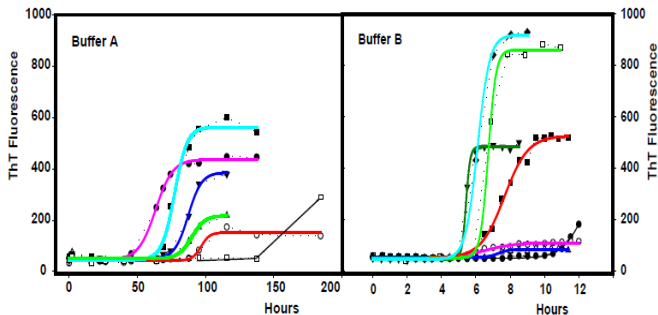
- Deterministic rate equations

- Stochastic algorithm

- Reproducing the experimental variability

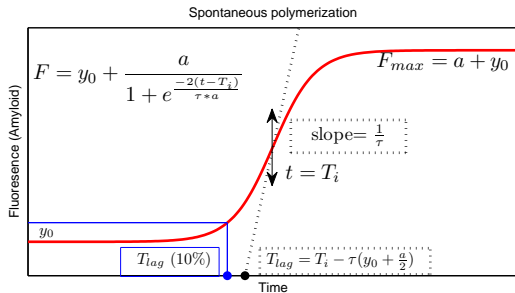
## Further...

# Experiments



Nucleation-Polymerisation were performed by an initially population of recombinant Prion protein  $PrP^C$  upon different conditions (pH, temperature, concentration, solution) Here are represented several experiment performed upon two different conditions. The *ThT* fluorescence is used as a measurable quantity which is correlated to the global mass of the polymer during experiments were performed.

# Simoid shape

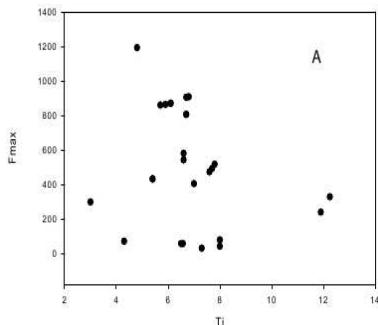


Four quantities appear to be characteristic of the dynamics.

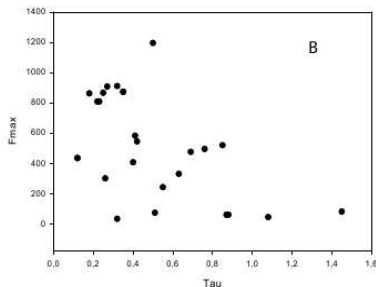
- ▶  $F_{max}$  is the maximal fluorescent value reached.
- ▶  $\frac{1}{\tau}$  is the maximal growth rate during the polymerization, which is achieved at  $t = T_i$ .
- ▶  $T_{lag}$  is the waiting time before the polymerization starts truly

All of these quantities can be measured on each experimental curves.

## Relation between $F_{max}$ and $T_i$ and $\tau$



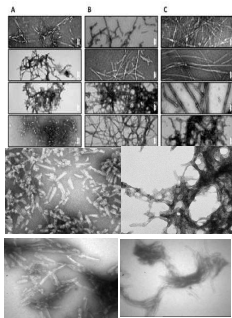
(a) Relation between  $F_{max}$  and  $T_i$



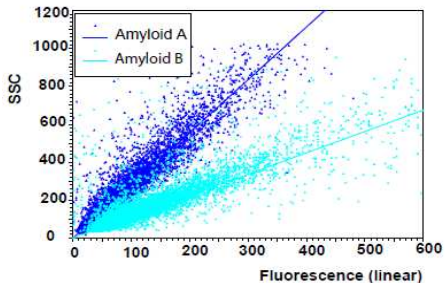
(b) Relation between  $F_{max}$  and  $\tau$

The maximum value does not correlate with the remain quantity of monomers at the end of the experiment, and does not correlate neither with the lag time and the maximum growth rate This proves that no "off-pathway" occurs.

# Observed heterogeneity of the structure



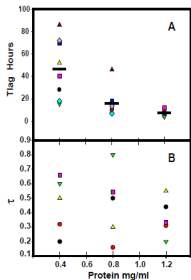
(c) microscopy photos



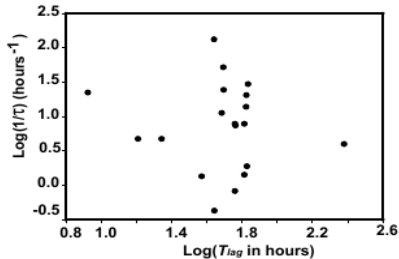
(d) Fluorescence and Polymer size

This lead to conjecture to an heterogeneity of the final structure itself of the polymer. The electron microscopy analysis give some clue to this heterogeneity and the relation between the size of polymer with its fluorescence response to ThT confirm it.

# Variability in Lag Time and Growth rate



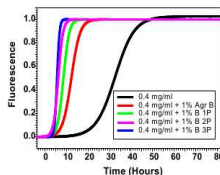
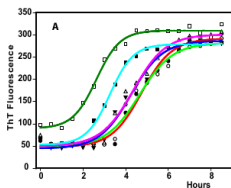
(e) Relation between  $M(0)$  and  $T_{lag}$  and  $\tau$



(f) Relation between  $T_{lag}$  and  $\tau$

These result can also be interpreted as heterogeneity of nucleation and subsequent polymer formation. It can be suggested that different nucleus generated structurally different polymers each exhibiting specific polymerisation dynamics.

# Seeding



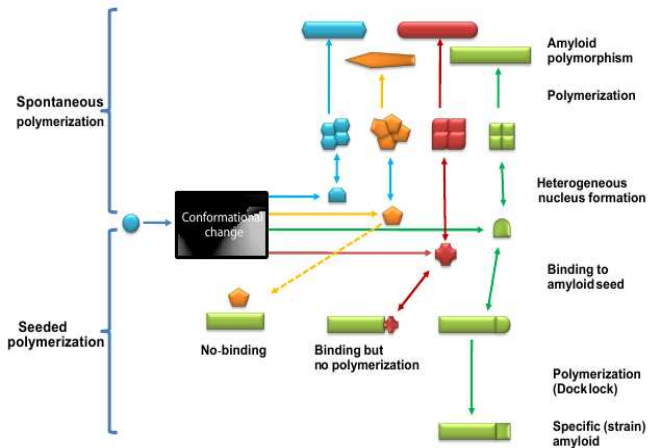
(g) Seeded experiment with increased initial quantity of seed

(h) Successive seeding experiment

Seeding experiments explain the infectiousness of the prion disease. **But the nucleation is predominant in the formation of prion amyloids.** The conformational change has to take place during the nucleation process more than during the polymerisation process.



# Model



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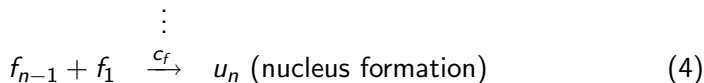
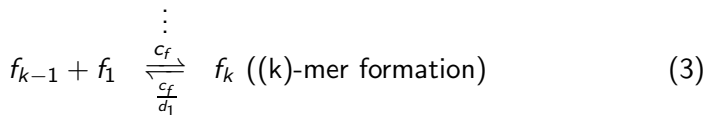
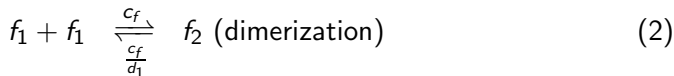
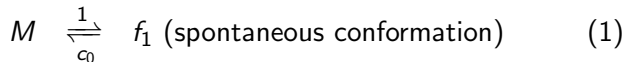
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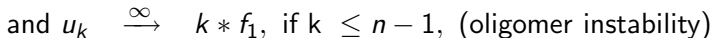
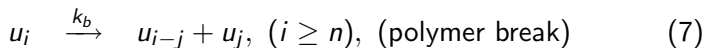
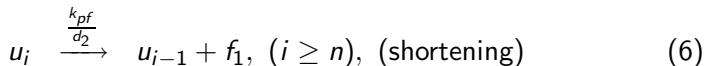
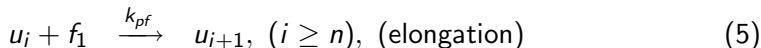
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- ▶  $n$ : is the size of the nucleus.

# Nucleation



# Polymerization





# Parameters

- ▶  $c_0$ : equilibrium constant between inactive monomers  $M$  and active monomers  $f_1$ . We suppose (fast variable)

$$M = c_0 * f_1$$

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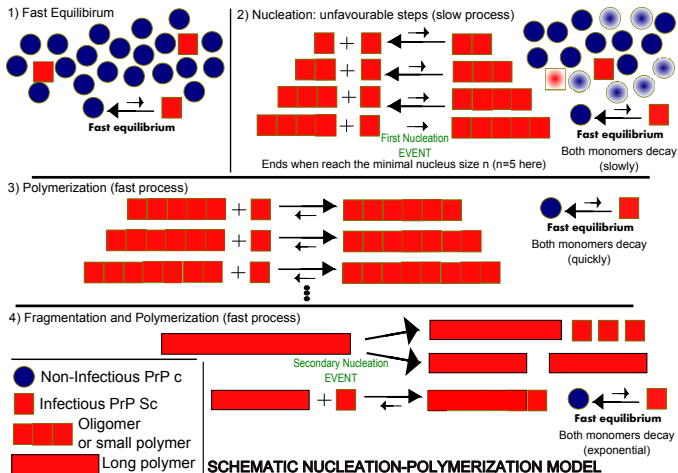
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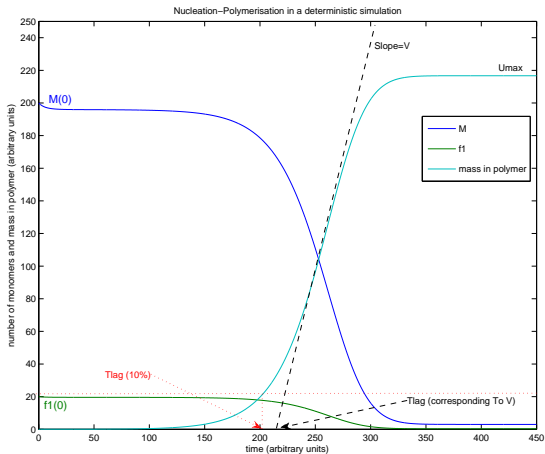
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- ▶  $n$ : nucleus size.

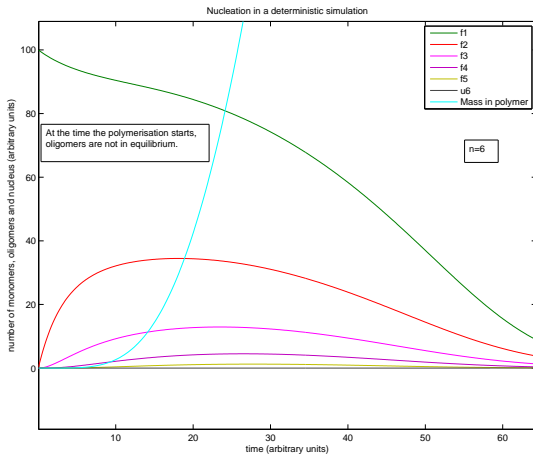




# General Behavior



# General Behavior: nucleation dynamics



## Influences of parameters

- ▶  $c_0$ : Changes drastically the Lag time and the Growth rate. If  $c_0$  increases, less active monomers will be available, resulting in increasing the time of nucleation and slowing down also the polymerization. It has also by the same way an influence on the final equilibrium.

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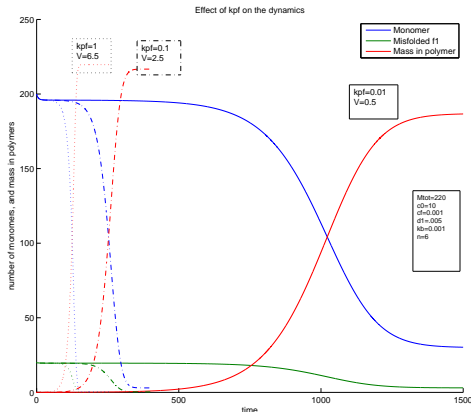
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- ▶  $c_f$ : Only changes the Lag time.
- ▶  $d_1$ : Influences the nucleation time. Can also limit the polymerisation if the oligomers don't disappears quickly enough (ie if  $d_1$  high, which is unlikely to be).

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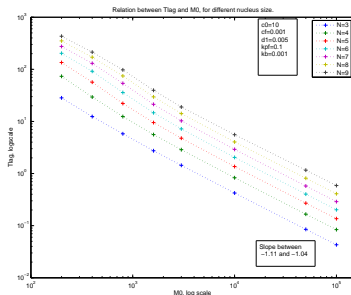
- ▶  $k_{pf}$  and  $k_b$ : Influence both polymerization and nucleation in the same way. Change also the final equilibrium.



## Influences of parameters

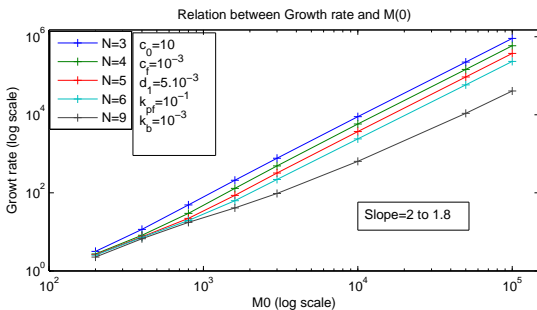
- ▶  $M(0)$  and  $n$ :  $M(0)$  reduces the lag time, while  $n$  increases it. Up to a constant,  $T_{lag}$  follows the same relation with  $M(0)$ , whatever the nucleus size is. In agreement with experimental date. We have

$$\log(T_{lag}) \simeq -1.07 * \log(M(0)) \quad (8)$$



# Influences of parameters

- ▶  $M(0)$  and  $n$ :  $M(0)$  speeds up the polymerization, while  $n$  slightly decreases it. This contradicts experiments.



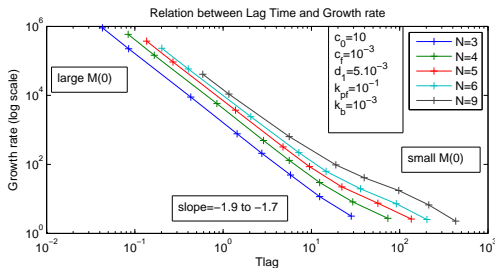


## Some key factor: general scaling laws

- ▶  $M_{tot} * k_{pf} \gg k_b$  guarantee an initially growing polymer.

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- ▶  $M_{tot} * k_{pf} \gg k_b$  guarantee an initially growing polymer.
- ▶  $Tlag$  versus  $V$ : For large  $M(0)$ , they are clearly inversely correlated. This contradicts experiments.



## experimental variability

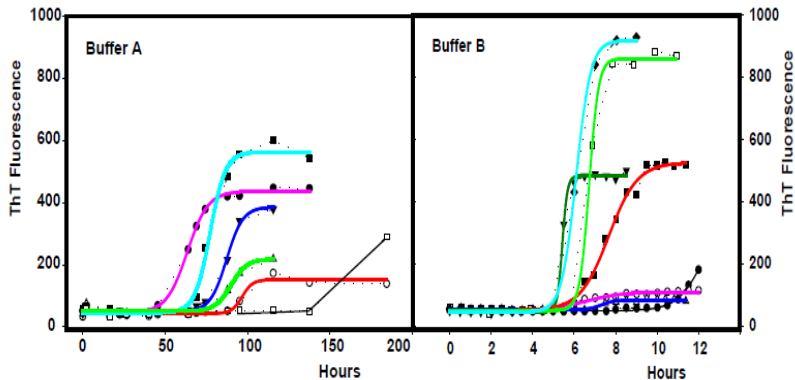
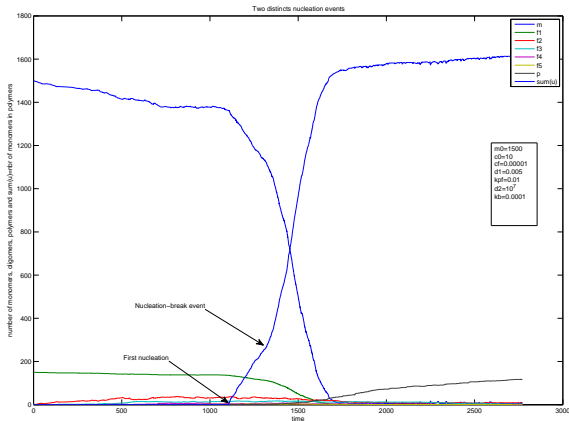


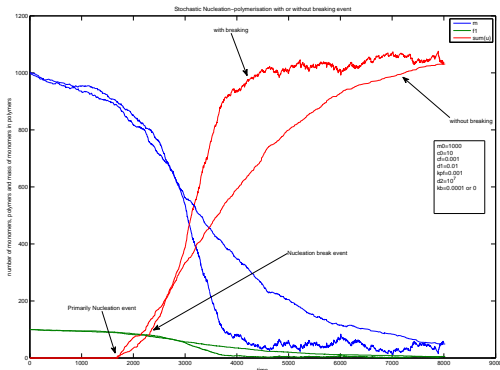
Figure: Several polymerisation experiments

# Stochastic algorithm - global dynamic



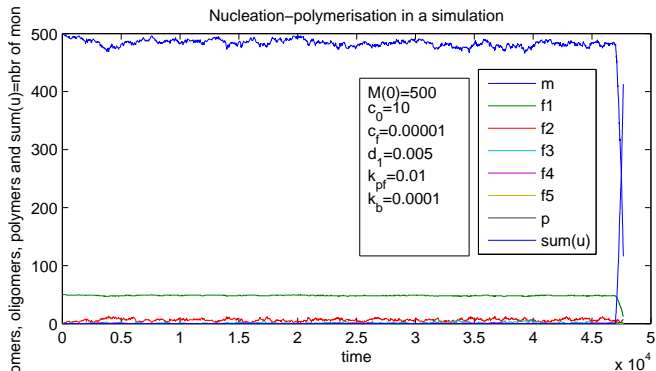
## Break events or Secondary nucleation pathway

- ▶ One single nucleation event then elongation and break events can explain the sigmoid form



## Stochasticity in the oligomer

- ▶ One can have many (small) fluctuations in the oligomer species, before reaching nucleation



## Influences of parameters - stochastic algorithm

- ▶  $c_0$ : if it increases, the relative proportion of normal and abnormal monomer will **increase variability of the Lag Time**. Indeed, larger  $c_0$  will result in less active monomer available (while keeping the total value of monomer constant) making the nucleation event a rare event with high variability.

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- ▶  $d_1$ : The equilibrium constant of the nucleation step has no evident effect on variability.

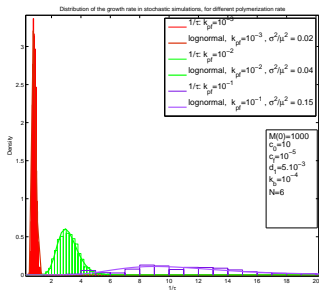
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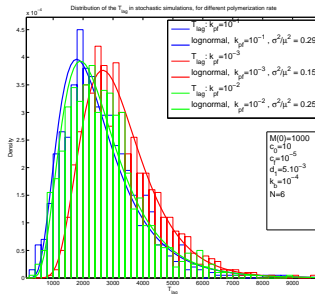
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- ▶  $k_b$ : the fragmentation rate influences on the respective numbers of nucleation events/ break events. **Small  $k_b$  appears to give higher variability on the growth rate**. Almost no influence on the lag time.

# Influences of parameters - example of $k_{pf}$

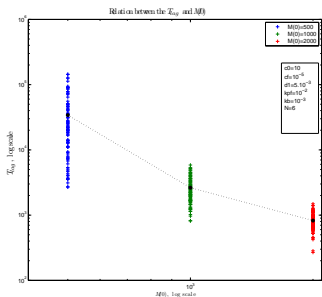


(a) Influence of  $k_{pf}$  on  $1/\tau$

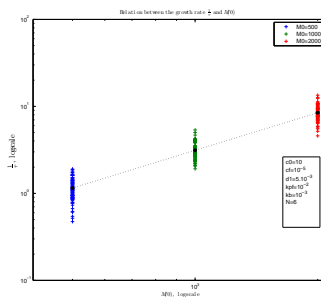


(b) Influence of  $k_{pf}$  on  $T_{lag}$

# Evolution of the Lag Time and the Growth rate on the stochastic algorithm, for different population size



(c)  $T_{lag}$



(d)  $1/\tau$

For both, large population decrease the variability, but it much more obvious for the lag time than the growth rate.

# Relation between the Lag Time and the Growth rate on the stochastic algorithm

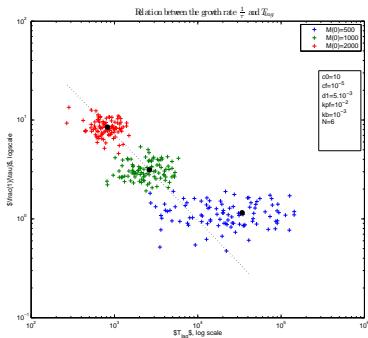
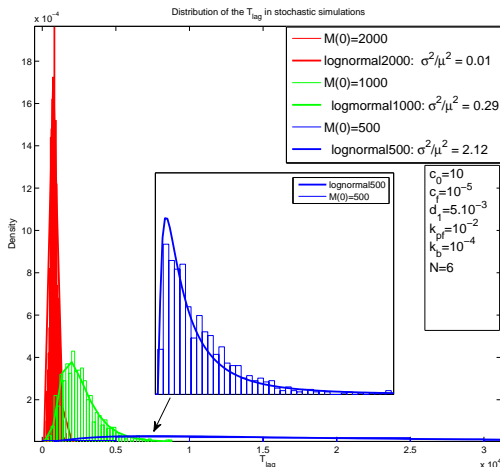
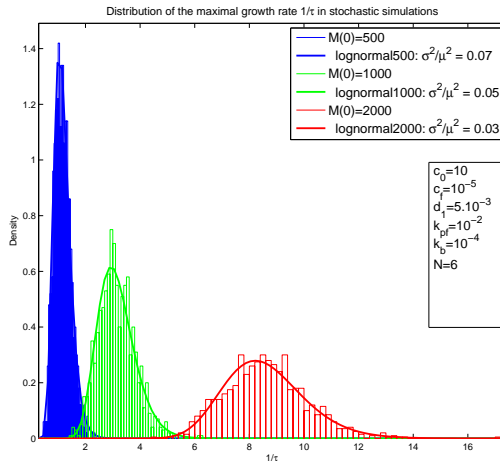


Figure: Relation between lag time and growth rate (log scale)

# Distribution of the Lag time on the stochastic algorithm



# Distribution of the Growth rate on the stochastic algorithm





## Coexistence of two structure

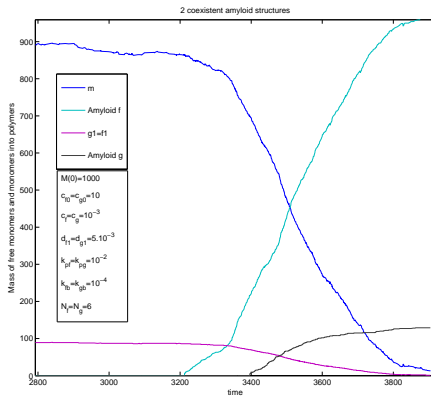


Figure: One example of coexistence of two structures

## Coexistence of two structure

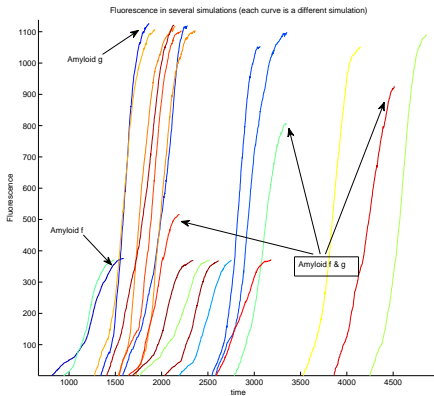


Figure: Reproducing experimental variability

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- ▶ Interaction between structures.