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by

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A Multiscale Model for Self-Assembly with Secondary Nucleation-like Properties

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Most of the biological polymers that make up our cells and tissues are hierarchically structured. For biopolymers ranging from collagen, to actin, to fibrin, this hierarchy provides vitally important versatility, allowing a multitude of structurally and functionally distinct structures to be constructed from a limited set of biomolecular constituents. This structural hierarchy must be encoded in the self-assembly process, from the earliest stages onward, in order to produce the appropriate substructures in the correct sequence. In this Letter, we explore the kinetics of such multi-stage self-assembly processes in a model system which is formulated as a set of discrete master equations capturing the underlying hierarchical molecular-scale process, but which may be homogenized to yield a practical, continuum description in terms of PDEs to compare to bulk experiments such as light scattering or turbidity measurements. We present the general framework, and apply it to recent turbidimetry data on the self-assembly of collagen fibrils. Furthermore, our analysis suggests a connection between diffusion-limited aggregation kinetics and fibril growth, supported by slow, power-law growth at very long timescales observed in both systems.

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Biological matter is hierarchically structured. The folding of individual proteins is a standard and powerful example, but the hierarchy does not stop at the level of tertiary structure. Indeed, quaternary aggregates of proteins form the basis of all structural biological materials: Collagen networks [1–6] may be cast into into at least five stages of hierarchical organization, starting from the tropocollagen monomers. These monomers assemble into microfibrils, which in turn assemble into fibrils. The fibrils become the basic building blocks for the larger fibers, which eventually assemble into the collagen network. Nature abounds with many more examples (*e.g.*, actin and tubulin) of such hierarchical biopolymers which, together, are responsible for the mechanical functioning of cells and organisms [7–11]. Even more generally, hierarchical organization is one of the most pervasive motifs in biology. From the smallest scales of the primary structure of a protein, to the largest scales of herds or packs of animals - at every level units assemble or cooperate to shape larger structures. Why is hierarchical organization so advantageous? Why does it make sense to invest energy in a two or multi-step assembly procedure? Is a hierarchical polymer, in general, better suited than a single-chain polymer to structural purposes? Do hierarchical self-assembly processes permit a description in terms of intuitive continuum models such as those describing simple linear polymerization? This Letter considers these questions partially in terms of the generic, two-step assembly process of a fibrous polymer. Higher levels of hierarchical organization may be treated by repeatedly applying the model to adjacent rungs in the hierarchical ladder provided, of course, that the time scales at which assembly steps takes place are sufficiently well separated. Under these assumptions, we set up a set

of master equations, to which we apply a homogenization scheme to yield a continuum, PDE description that describes ensemble/bulk measurements. We use these to produce and interpret recent, anomalous turbidity measurements reported in [6], see Fig. 1.

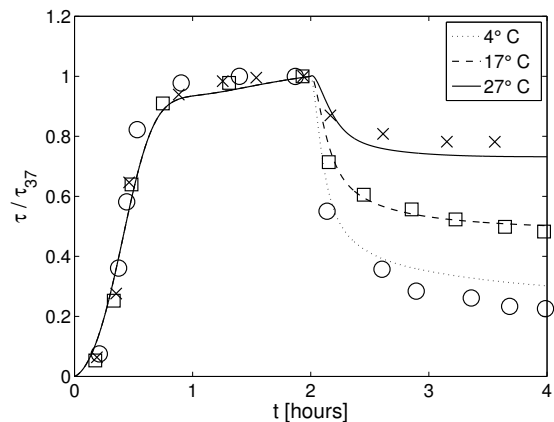


FIG. 1. Turbidity τ measured for three different quenching temperatures versus time in hours. Experiments indicated with points and theory indicated with lines. The first 2 hours of each quenching experiment is assembly at 37°C , followed by 2 hours of disassembly at different temperatures. Each experimental curve is independently normalized with its maximum turbidity after 2 hours of assembly at 37°C .

Multiscale model of self-assembly. Consider N monomers in a fixed volume, leading to N different possible species, the largest of which is simply the N monomers stuck together. For any aggregation number n , we only consider one configuration of the monomers. Alternatively, we could say that we consider an average

of configurations for each aggregation number. For this reason, we say that our model is quasi two-dimensional. We assume that only monomer addition and dissociation takes place. There are no collisions between larger aggregates and we assume aggregates are strong enough not to break into smaller pieces. The breaking apart of aggregates can only occur by dissociating one monomer at a time.

The association rates from species n to $n + 1$ are given by a_n , while the dissociation rates from n to $n - 1$ are given by b_n . In a one-dimensional end-growth and evaporation model, all the association and dissociation rates would be equal [12]. These two assumptions are sketched in Figure 2.

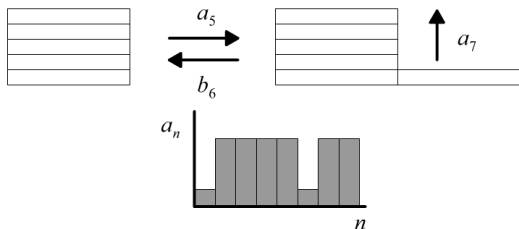


FIG. 2. Sketch of the quasi two-dimensional model, indicated is the forward reaction rate as a function of the aggregation number.

The first monomer in each column will have an energy barrier to cross while the rest of the monomers in the column can latch on fairly easy. This mechanism resembles secondary nucleation in crystals and leads to periodic association and dissociation rates. The period of the rates will be H , the thickness of the polymer.

We consider the mass fractions P_n , where n is fixed, for which we can write down the evolution equations, given by

$$\frac{dP_1}{dt} = -a_1 P_1^2 + \sum_{n=2}^N \left[\frac{1}{n} b_n P_n - \frac{1}{n} a_n P_1 P_n \right], \quad (1)$$

$$\frac{dP_2}{dt} = a_1 P_1^2 + \frac{2}{3} b_3 P_3 - \left(a_2 P_1 + \frac{1}{2} b_2 \right) P_2, \quad (2)$$

⋮

$$\frac{dP_n}{dt} = \frac{n}{n-1} a_{n-1} P_1 P_{n-1} + \frac{n}{n+1} b_{n+1} P_{n+1} - (a_n P_1 + b_n) P_n, \quad (3)$$

where $3 \leq n \leq N$ indexes the different equations, these are known as the master equations. Given suitable initial conditions, (1) - (3) yield a unique solution.

Now that we have established the discrete system of equations, we will pass to a continuum system. We use a continuum system since it is easier to work with analytically. We introduce $u = P_1$, the monomer mass fraction, and the polymer distribution v , which is a continuous

version of $(P_n)_{n=2}^N$. To find the polymer distribution, we introduce the domain $\Omega := [0, 1]$ and $x_n := \frac{n-\frac{3}{2}}{N-1}$, where $n = 2, \dots, N$. We shall refer to Ω as the mass space. We define v such that

$$P_n(t) = \int_{x_{n-\frac{1}{2}}}^{x_{n+\frac{1}{2}}} v(x, t) dx. \quad (4)$$

This leads to a continuum system given by

$$(P) \begin{cases} \frac{du}{dt} + a_1 u^2 - b(0) v(0, t) = - \int_{\Omega} s v dx, \\ \frac{\partial v}{\partial t} + \frac{\partial}{\partial x} \left(D \left(\frac{x}{\varepsilon}, u \right) \frac{\partial v}{\partial x} - q \left(\frac{x}{\varepsilon}, u \right) v \right) = s v, \\ D(0, u) \frac{\partial v}{\partial x}(0, t) - q(0, u) v(0, t) = a_1 u^2 - b(0) v(0, t), \\ D(1, u) \frac{\partial v}{\partial x}(1, t) - q(1, u) v(1, t) = 0, \\ u(0) = \phi_m, \\ v(x, 0) = \phi_p(x), \end{cases} \quad (5)$$

where ϕ_m is the monomer initial condition and $\phi_p(x)$ is the polymer distribution initial condition. In most cases we choose $\phi_m = 1$ and $\phi_p = 0$, corresponding to tropocollagen freshly dissolved in water. The various coefficients are given by

$$D \left(\frac{x}{\varepsilon}, u \right) = \frac{1}{(N-1)^2} \frac{1}{2} \left(a \left(\frac{x}{\varepsilon} \right) u + b \left(\frac{x}{\varepsilon} \right) \right), \quad (6)$$

$$q \left(\frac{x}{\varepsilon}, u \right) = \frac{1}{N-1} \left(a \left(\frac{x}{\varepsilon} \right) u - b \left(\frac{x}{\varepsilon} \right) \right), \quad (7)$$

$$s \left(\frac{x}{\varepsilon}, u \right) = \frac{1}{x + \frac{1}{2}(N-1)} \left(a \left(\frac{x}{\varepsilon} \right) u - b \left(\frac{x}{\varepsilon} \right) \right), \quad (8)$$

where $a(x)$ and $b(x)$ are the continuous versions (e.g. linear interpolations) of a_n and b_n and $\varepsilon = \frac{H}{N-1}$, the period of the constants in Ω . Note that $a(x)$ and $b(x)$ have a period of 1 such that $a(\frac{x}{\varepsilon})$ has period ε . We see that from a set of difference equations (1) - (3), we obtain a convection-diffusion partial differential equation coupled to a non-linear ODE (5).

We can introduce the *microscale* variable $y := \frac{x}{\varepsilon}$, which can be interpreted as the radial growth direction in mass space. Using the new microscale variable, we have converted our model to a multiscale description of self-assembly.

Homogenization results. We can redefine our system now not in Ω but in a new space $\Omega \times Y$. This step is necessary for the periodic homogenization procedure [13, 14]. This procedure leaves the ODE in (5) unaltered but obtains a new PDE given by

$$\frac{\partial v_0}{\partial t} = \frac{\partial}{\partial x} \left(\bar{D}(u) \frac{\partial v_0}{\partial x} - \bar{q}(u) v_0 \right) + \bar{s}(x, u) v_0, \quad (9)$$

where the effective coefficients are

$$\bar{D}(u) := \left(\int_Y \frac{1}{D(y, u)} dy \right)^{-1}, \quad (10)$$

$$\bar{q}(u) := \bar{D}(u) \int_Y \frac{q(y, u)}{D(y, u)} dy, \quad (11)$$

$$\bar{s}(x, u) := \int_Y s(x, y, u) dy. \quad (12)$$

The *homogenized limit* v_0 in (9) is valid in the limit of $\varepsilon \downarrow 0$ and is a very good approximation in the case that $\varepsilon \ll 1$, corresponding to polymers with a high aspect ratio. We see that by fine-tuning the rate coefficients as a function of y , we can have much more control over the polymer distribution.

Rate coefficients. The system (5) has the necessary number of initial and boundary conditions and hence has a well-defined solution. By specifying the rate constants, we can model several self-assembling systems. The association and disassociation rate constants are of course related by a modified Arrhenius' equation [15], by

$$\frac{a(y)}{b(y)} = F(T) e^{-\frac{\Delta E(y)}{k_B T}}, \quad (13)$$

where $\Delta E(y)$ is the energy barrier as function of y , k_B is Boltzmann's constant and T is temperature. Choosing $b(y)$ constant in Y , in which case we find that

$$a(y) := k_+ e^{-\frac{\Delta E(y)}{k_B T}}, \quad b(y) := k_-, \quad (14)$$

where k_+ and k_- are the trial frequencies. After this identification we rescale time k_- , resulting in a dimensionless system. In case nothing is known about the trial frequencies, we use $F(T) = 1$.

Comparison to experimental data. A common experimental method for monitoring assembly is measuring the turbidity τ of a solution. The turbidity, or cloudiness, arises due to light scattering in solution. Here we assume that the scattering of light is due to the size of particles alone. We therefore find that $\tau \sim \langle M \rangle$, where $\langle M \rangle$ is the average polymeric mass, defined by

$$\langle M \rangle := \int_{\Omega} v x dx. \quad (15)$$

The reasoning here is that increasingly larger aggregates contribute more and more to light scattering.

Application to collagen. Collagen is one of the main components in animal tissues. In fact, collagen typically makes up 25% of an animal's proteome [5]. Collagen *in vitro* spontaneously self-assembles into large fibrils,

consisting of smaller microfibrils again. At the smallest scales, we find the monomeric unit of collagen structure, called tropocollagen [1]. It is a rod-shaped macromolecule of about 300 nm length and 1.5 nm in diameter. Large numbers of tropocollagen monomers spontaneously self-assemble into micrometer-long bundles called microfibrils [2]. These in turn assemble *in vivo* to form thicker fibrils, which become the building blocks for collagen fibers. The fibers are used in network-like structures in fibrous tissue. However, *in vitro* fibrils often form into network gels.

We aim to apply our multiscale self-assembly model to fibrous collagen in order to find corresponding growth curves. We will first find the correct function $F(T)$ in (13). We assume that collagen association is diffusion-limited, which is justified by the entropic nature of collagen self-assembly [16]. Hence, we find for the association trial frequency $k_+ = 8\pi RCD$, where R is a typical polymer dimension, C is the monomer concentration and D is the monomer diffusion constant. The collagen fibrils are long semi-flexible polymers, however, the persistence length l_p is not very well known. Still we can estimate the typical radius of a collagen fibril using the freely jointed chain model [17], $R_g^2 = ml_0^2$, where m is the number of links and l_0 is the Kuhn length. Furthermore we can estimate the number of links using $m \approx \frac{L}{l_p}$, where L is the total length of the collagen fibril. Using this approximation we use Kuhn segments the length of one persistence length and hence we find $R^2 \approx \frac{L}{l_p} l_p^2 = Ll_p \sim T^{-1}$. Using the Stokes-Einstein relation [18] for the diffusion constant for monomers, we find

$$k_+ = 8\pi RCD \sim T^{-\frac{1}{2}} T = T^{\frac{1}{2}}. \quad (16)$$

By comparing the Stokes-Einstein relation modified for rod-shaped particles with the relation for spherical particles, we see that the diffusion constant of monomers is approximately linear in temperature [19].

Using (15), we compute turbidity curves Figure 1 and Figure 3. We see some very typical growth characteristics like a nucleation phase and a rapid growth phase. Contrary to older models [20], we do not see a real plateau phase, but rather a slow growing phase starting off after the rapid growth phase.

These curves were produced using the energy barrier given by,

$$\frac{\Delta E(y)}{k_B T} = 2 \frac{310.15}{T} - 3.06 \sin(\pi y), \quad (17)$$

where y is the radial growth or microscale coordinate. The energy barrier in (17) is energetic in nature while the energy gain is entropic in nature, resulting in faster assembly at higher temperatures.

Power-law behaviour. The large-time slow growth behaviour of the model seems to be also present in experimental systems [6, 7]. The lack of a real plateau phase in

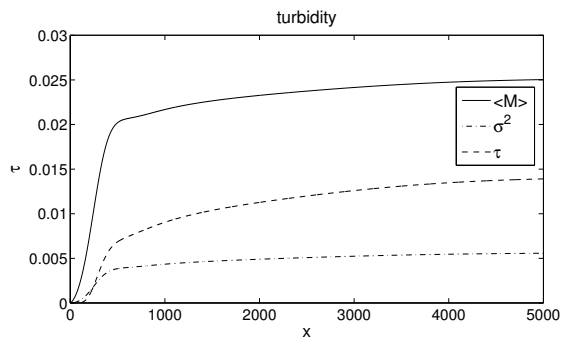


FIG. 3. Turbidity measured for three different quenching temperatures. Experiments indicated with points and theory indicated with lines. One hour corresponds to 550 time units.

experiments is what sparked the interest in a new model for collagen self-assembly. By performing a long computing run and cutting off the initial nucleation and rapid growth phase, we obtain the large-time behaviour. Plotting this tail end on a double logarithmic scale, see Figure 4, we find a power law given by

$$\tau(t) = 1.15 \cdot 10^{-2} t^{0.09}, \quad (18)$$

which is valid between 400 and 5000 time units, approximately 8 hours, with a relative error of 1.5 %. In long-run experiments, however, the slow growth behaviour was observed for far longer times. Hence, we conjecture that this power-law behaviour is valid for much larger time intervals.

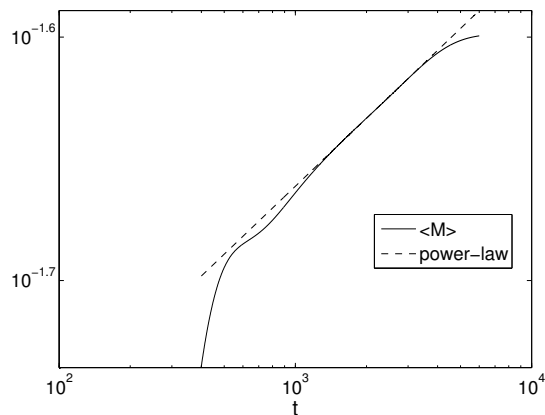


FIG. 4. Power-law behaviour after the initial nucleation and rapid growth phases.

The power-law growth behaviour stems from the diffusion-limited growth process [21, 22]. A regular diffusion-limited aggregate grows from any point on its surface, leading to Brownian tree shapes. For a constant addition rate, one can derive that [23]

$$R \sim t^{\frac{1}{d_f}}, \quad (19)$$

where R is the radius of gyration and d_f is the fractal dimension of the Brownian tree. However, in our case we only allow monomers to attach in very small regions, which might lead to a relation resembling (19).

A similar power-law behaviour was found by Botet and Jullien [24] for the steady-state radius of gyration of a diffusion-limited aggregate with disaggregation. They found the power-law

$$R \sim N_s^{0.07}, \quad (20)$$

where N_s is the number of monomers. Supposing the typical time between monomer addition is larger than the equilibration time, we have here again a very similar power-law: if we assume that $\tau \sim R$ then our result is indeed strikingly similar.

Conclusion. Our coarse grained evolution equations reproduce both growth and temperature-quench depolymerization curves very similar to those reported in experiments. Secondary nucleation, or indeed any type of growth process involving multiple energy scales, result in a distribution of time scales in the associated kinetic process. This manifests itself, even at the level of a single hierarchical fibril, as a superposition of ordinary growth kinetics involving lag, growth and saturation phases with very slow transients. Interestingly, this behaviour is already seen for single fibrils and does not require assumptions on the behaviour of the networks into which these fibrils assemble themselves. We may, however, obtain information on the microscopic kinetic properties of assembly processes using our model. By fitting coefficients to bulk experimental turbidity measurements, we obtain microscopic rates and we are able to estimate energy barriers and gains. Finally, we have seen that our model predicts a power law behaviour for long times with a very small exponent. This power law is strikingly similar to the power law found by Botet and Jullien [24] under the added assumption of slow monomer addition. A direct test of our basic assumptions would be to establish whether or not the slow transient is in fact a power law in time. In summary, we have derived a very general microscopically motivated, macroscopically applicable evolution equation for hierarchical self-assembly processes involving distinct energy scales. It reproduces experimental observations, and permits their interpretation in terms of underlying processes.

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