Emergence of protocellular growth laws

Tristan Rocheleau[1,2], Steen Rasmussen[1,3,4,*], Peter E. Nielsen[4], Martin N. Jacobi[5] and Hans Ziock[1]

[1]*Self-Organizing Systems, EES-6, Los Alamos National Laboratory, MS-D462, Los Alamos, NM 87545, USA*
[2]*University of California Santa Barbara, Santa Barbara, CA 93106, USA*
[3]*Santa Fe Institute, Santa Fe, NM 87501, USA*
[4]*University of Copenhagen, IMBG, Blegdamsvej 3, 2200 Copenhagen, Denmark*
[5]*Chalmers University of Technology, 41296 Gothenburg, Sweden*

Template-directed replication is known to obey a parabolic growth law due to product inhibition (Sievers & Von Kiedrowski 1994 *Nature* **369**, 221; Lee *et al.* 1996 *Nature* **382**, 525; Varga & Szathmáry 1997 *Bull. Math. Biol.* **59**, 1145). We investigate a template-directed replication with a coupled template catalysed lipid aggregate production as a model of a minimal protocell and show analytically that the autocatalytic template–container feedback ensures balanced exponential replication kinetics; both the genes and the container grow exponentially with the same exponent. The parabolic gene replication does not limit the protocellular growth, and a detailed stoichiometric control of the individual protocell components is not necessary to ensure a balanced gene–container growth as conjectured by various authors (Gánti 2004 *Chemoton theory*). Our analysis also suggests that the exponential growth of most modern biological systems emerges from the inherent spatial quality of the container replication process as we show analytically how the internal gene and metabolic kinetics determine the cell population's generation time and not the growth law (Burdett & Kirkwood 1983 *J. Theor. Biol.* **103**, 11–20; Novak *et al.* 1998 *Biophys. Chem.* **72**, 185–200; Tyson *et al.* 2003 *Curr. Opin. Cell Biol.* **15**, 221–231). Previous extensive replication reaction kinetic studies have mainly focused on template replication and have not included a coupling to metabolic container dynamics (Stadler *et al.* 2000 *Bull. Math. Biol.* **62**, 1061–1086; Stadler & Stadler 2003 *Adv. Comp. Syst.* **6**, 47). The reported results extend these investigations. Finally, the coordinated exponential gene–container growth law stemming from catalysis is an encouraging circumstance for the many experimental groups currently engaged in assembling self-replicating minimal artificial cells (Szostak 2001 *et al. Nature* **409**, 387–390; Pohorille & Deamer 2002 *Trends Biotech.* **20** 123–128; Rasmussen *et al.* 2004 *Science* **303**, 963–965; Szathmáry 2005 *Nature* **433**, 469–470; Luisi *et al.* 2006 *Naturwissenschaften* **93**, 1–13).[1]

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1. LIPID-ASSOCIATED TEMPLATE-DIRECTED REPLICATION

Our simple protocell model consists of a lipid container (e.g. a large micelle) with amphiphilic replicating protogene molecules and an associated hydrophobic metabolic system as discussed in Rasmussen *et al.* (2003). However, the details of the coupling between the genes and the container are not important as long as the container defines a localized and restrictive volume or area for the genes (Szostak *et al.* 2001; Pohorille & Deamer 2002; Rasmussen *et al.* 2004; Szathmáry 2005; Luisi *et al.* 2006). In our current minimal representation, only the replicator and container kinetics are modelled explicitly. We can assume that all lipophilic replicators are bound to the exterior surfaces of the lipid aggregates and that both single- and double-stranded templates coexist at these lipid interfaces (figure 1*a*). For the following arguments, it is only necessary to localize the genes. In our model, we

can, for example, assume that the replicator molecules are peptide nucleic acid (Pschl *et al.* 1998; Nielsen 1999; Mattes & Seitz 2001) oligomers with decorated (lipophilic) backbones. We can further assume that the single- and double-stranded templates are at equilibrium with each other as long as the template replication rate is much slower than the hybridization and dehybridization processes.

The local replication reaction can then be written as

$$T_s + \alpha O \rightarrow T_d \rightleftharpoons T_s + T_s, \tag{1.1}$$

where O is a resource replicator substrate molecule and α is the number of such molecules used in each replication. Denoting these local template concentrations by T_s^l and T_d^l for the single- and double-stranded templates, respectively, the local equilibrium condition for the single- and double-stranded templates is then given by

$$K_t = \frac{(T_s^l)^2}{T_d^l}, \tag{1.2}$$

where K_t defines the equilibrium constant. In the following, we assume that $K_t \ll 1$. If T_{tot}^l defines the total local template concentration, single- or double
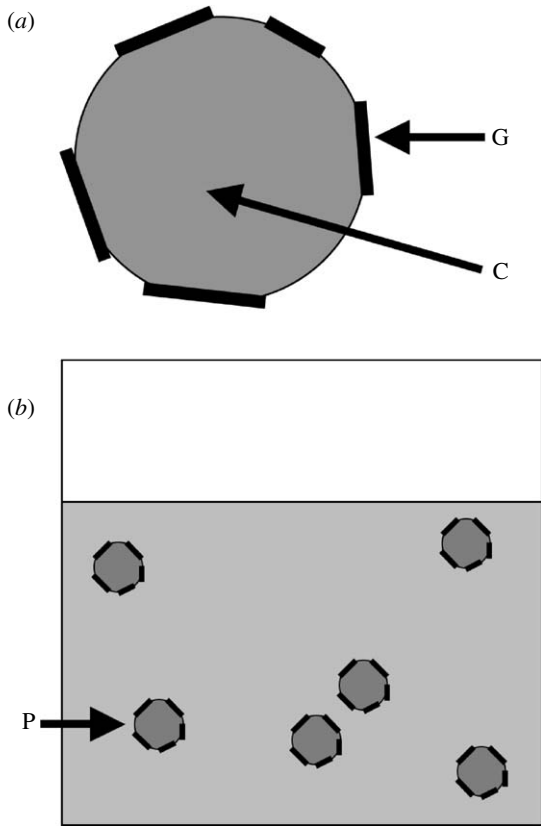
(a)

(b)

Figure 1. (*a*) A protocell consists of a lipid container C (e.g. a large micelle) with amphiphilic gene molecules G at local aggregate concentration $T_{\mathrm{tot}}^{\mathrm{l}}$ attached to the surface (Rasmussen *et al.* 2004). (*b*) The average aggregate of each protocell P is composed of m_0 lipid molecules. The (global) aggregate concentration is A and the global template concentration $T_{\mathrm{tot}}^{\mathrm{g}} = T_{\mathrm{tot}}^{\mathrm{l}} A V_{\mathrm{A}}$, where $T_{\mathrm{tot}}^{\mathrm{l}}$ denotes the local aggregate template concentration (template molecules per aggregate volume).

stranded, we have

$$T_{\mathrm{tot}}^{\mathrm{l}} = T_{\mathrm{s}}^{\mathrm{l}} + 2T_{\mathrm{d}}^{\mathrm{l}}. \tag{1.3}$$

$T_{\mathrm{s}}^{\mathrm{l}}$ and $T_{\mathrm{d}}^{\mathrm{l}}$ can now be expressed as a function of $T_{\mathrm{tot}}^{\mathrm{l}}$ by combining equations (1.2) and (1.3),

$$T_{\mathrm{s}}^{\mathrm{l}} = \frac{-K_t + \sqrt{K_t^2 + 8K_t T_{\mathrm{tot}}^{\mathrm{l}}}}{4}$$

$$= \sqrt{\frac{K_t T_{\mathrm{tot}}^{\mathrm{l}}}{2}} + O_1(K_t), \tag{1.4}$$

$$T_{\mathrm{d}}^{\mathrm{l}} = \frac{T_{\mathrm{tot}}^{\mathrm{l}}}{2} - \frac{T_{\mathrm{s}}^{\mathrm{l}}}{2}$$

$$= \frac{T_{\mathrm{tot}}^{\mathrm{l}}}{2} - \frac{-K_t + \sqrt{K_t^2 + 8K_t T_{\mathrm{tot}}^{\mathrm{l}}}}{8}$$

$$= \frac{T_{\mathrm{tot}}^{\mathrm{l}}}{2} - \sqrt{\frac{K_t T_{\mathrm{tot}}^{\mathrm{l}}}{8}} - \frac{O_2(K_t)}{2}, \tag{1.5}$$

where we have used that $K_t \ll T_{\mathrm{tot}}$ ($K_t \ll 1$) and $O_i(K_t)$, $i = 1, 2$ is small in terms of K_t.

The left side of equation (1.1) defines the template-directed replication of $T_{\mathrm{s}}^{\mathrm{l}}$ using some background precursor template molecules with the local aggregate concentration O^{l}. The template replication rate equation then becomes

$$\frac{\mathrm{d}T_{\mathrm{tot}}^{\mathrm{l}}}{\mathrm{d}t} = k_{\mathrm{T}} f(O^{\mathrm{l}}) T_{\mathrm{s}}^{\mathrm{l}} = \frac{1}{\alpha} k_{\mathrm{T}} O^{\mathrm{l}} T_{\mathrm{s}}^{\mathrm{l}}, \tag{1.6}$$

where $f(\cdot)$ is a reaction mechanism dependent, which is O^{l}/α in the case where α resource oligomers are added sequentially to the template one at the time, while k_{T} is the single oligomer addition rate constant. Substituting equation (1.4) into equation (1.6)

$$\frac{\mathrm{d}T_{\mathrm{tot}}^{\mathrm{l}}}{\mathrm{d}t} = k_{\mathrm{T}} f(O^{\mathrm{l}}) \left(\sqrt{\frac{K_t T_{\mathrm{tot}}^{\mathrm{l}}}{2}} + O_1(K_t) \right), \tag{1.7}$$

where $O_1(K_t)$ is small, which to the leading order results in parabolic growth

$$T_{\mathrm{tot}}^{\mathrm{l}}(t) = \frac{f(O^{\mathrm{l}})^2 K_t k_{\mathrm{T}}^2}{8} t^2 + \frac{\sqrt{T_{\mathrm{tot}}^{\mathrm{l}}(0) K_t} f(O^{\mathrm{l}}) k_{\mathrm{T}}}{\sqrt{2}} t + T_{\mathrm{tot}}^{\mathrm{l}}(0)$$

$$= \left(\frac{f(O^{\mathrm{l}}) \sqrt{K_t} k_{\mathrm{T}}}{2\sqrt{2}} t + \sqrt{T_{\mathrm{tot}}^{\mathrm{l}}(0)} \right)^2, \tag{1.8}$$

where $T_{\mathrm{tot}}^{\mathrm{l}}(0)$ is the concentration at $t = 0$. This result is an example of the well-known growth law for product inhibition in template-directed replication (Sievers & Von Kiedrowski 1994) in an unlimited system.

2. TEMPLATE CATALYSED CONTAINER REPRODUCTION

In our model, the aggregate-associated lipid production L is template catalysed and can be described by

$$pL + T_{\mathrm{d}} \rightarrow T_{\mathrm{d}} + L, \tag{2.1}$$

where pL is the precursor lipid. We assume that the catalysis is dominated by the double-stranded template (Rasmussen *et al.* 2003). The lipid production rate equation is then

$$\frac{\mathrm{d}L^{\mathrm{l}}}{\mathrm{d}t} = k_{\mathrm{L}} pL^{\mathrm{l}} T_{\mathrm{d}}^{\mathrm{l}}, \tag{2.2}$$

where k_{L} is the lipid production rate constant and pL^{l} is the local (background) lipid precursor concentration.

This simple gene–container coupling can be generalized to explicitly account for a variety of details involved in the metabolic processes as discussed in Munteanu *et al.* (2007). In this paper, we focus on a detailed discussion of the above minimal gene–container connection.

We assume the average number of lipid molecules per average aggregate volume V_{A} to be m_0. An average aggregate number can, for example, be maintained through a natural micellar size distribution (Rosen 1988). The relative local (dimensionless) aggregate growth rate can now be expressed as

$$\frac{V_{\mathrm{A}}}{m_0} \frac{\mathrm{d}L^{\mathrm{l}}}{\mathrm{d}t} = k_{\mathrm{L}} pL^{\mathrm{l}} T_{\mathrm{d}}^{\mathrm{l}} \frac{V_{\mathrm{A}}}{m_0}. \tag{2.3}$$

Let A be the global aggregate concentration. Since each aggregate is assumed to divide following growth in order to maintain an average of m_0 lipid molecules per aggregate, the growth in the global aggregate concentration due to the local lipid production in the aggregates can be expressed as

$$\frac{\mathrm{d}A}{\mathrm{d}t} = \frac{A V_{\mathrm{A}}}{m_0} \frac{\mathrm{d}L^{\mathrm{l}}}{\mathrm{d}t} = k_{\mathrm{L}} pL^{\mathrm{l}} T_{\mathrm{d}}^{\mathrm{l}} \frac{A V_{\mathrm{A}}}{m_0}. \tag{2.4}$$

In the above expression, we have implicitly assumed that the volume of the templates and its change are small (constant) compared with the volume of lipid in

an aggregate. We can now transform the local aggregate concentration of templates into the corresponding global concentrations using the simple relation between local and global template concentrations

$$T^{g} = T^{l}AV_{A}, \qquad (2.5)$$

noting that T^{l} is measured in template molecules per lipid aggregate volume V_{A} and A is measured in the number of lipid aggregates (of average size V_{A}) per system volume. Substituting equation (2.5) into equation (2.4) and taking into account that $T_{tot} \approx 2T_{d}$, we obtain

$$\frac{dA}{dt} = \frac{k_{L}pL^{l}}{m_{0}} T_{d}^{g} = \gamma_{A} T_{tot}^{g}, \qquad (2.6)$$

where

$$\gamma_{A} = \frac{k_{L}pL^{l}}{2m_{0}}. \qquad (2.7)$$

It should be noted that we can just as well express the local concentrations in molecules per aggregate, in molecules per aggregate volume using an average aggregate volume of V_{A} or in molecules per aggregate surface area S_{A}.

3. GLOBAL TEMPLATE REPRODUCTION

The growth equation for the local total template concentration T_{tot}^{l} corresponding to equation (1.6) is given by

$$\frac{dT_{tot}^{l}}{dt} = k_{T}f(O^{l})T_{s}^{l} - \frac{dA}{dt}\frac{T_{tot}^{l}}{A}, \qquad (3.1)$$

where the second term stems from the effect the volume change has on the local concentration. Since equation (3.1) is central to the argument, we discuss it in detail. The question that needs to be answered is what happens to the concentrations when one goes from a well-stirred solution to many small containers. This has to do with the origin of the second term in equation (3.1). The volume of the container grows with time and hence the concentration can change, and decreases with increasing container volume as A is measured in aggregates of average size V_{A} per litre. The volume growth of the container must be equal to the growth in the (global) concentration of the aggregates. One also needs a normalization factor to go from the change in container volume to the change in template concentration. All together, this yields a minus sign (the concentration goes down with increasing volume) multiplied by the ratio of the concentration of templates divided by the concentration of aggregates.

Now, since equation (1.2) holds for the local concentration (not the global), we can use the approximate form of equation (1.4) to rewrite the growth equation as

$$\frac{dT_{tot}^{l}}{dt} = k_{T}f(O^{l})\sqrt{\frac{K_{t}T_{tot}^{l}}{2}} - \frac{dA}{dt}\frac{T_{tot}^{l}}{A}. \qquad (3.2)$$

To rewrite the growth in terms of global concentrations, we multiply the growth equation (3.2) by A, and get

$$\frac{d(AT_{tot}^{l})}{dt} - \frac{dA}{dt}T_{tot}^{l} = k_{T}f(O^{l})\sqrt{\frac{K_{t}T_{tot}^{l}}{2}}A - \frac{dA}{dt}T_{tot}^{l}. \qquad (3.3)$$

Note that the volume compensation term vanishes as it should. We then use the relation $T^{g} = T^{l}AV_{A}$ to rewrite the growth in terms of global concentrations

$$\frac{dT_{tot}^{g}}{dt} = \gamma_{T}\sqrt{T_{tot}^{g}A}, \qquad (3.4)$$

where

$$\gamma_{T} = k_{T}f(O^{l})\sqrt{\frac{K_{t}V_{A}}{2}}. \qquad (3.5)$$

4. COUPLED TEMPLATE AND CONTAINER REPRODUCTION

The coupled growth equations (2.6) and (3.4) can be solved by separation of variables, which can be used to rewrite equation (3.4) where we are seeking the solution for large T_{tot}^{g} values. We obtain (Appendix A) the solution,

$$T_{tot}^{g}(t) = \frac{(\exp(\frac{3}{2}at + D_{0}) - b)^{2/3}}{a^{2/3}} \qquad \sim \exp(at), \quad (4.1)$$

where

$$a = \gamma_{T}^{2/3}\gamma_{A}^{1/3}, \qquad (4.2)$$

and

$$b = \frac{\gamma_{T}^{5/3}C_{0}}{3\gamma_{A}^{2/3}}, \quad C_{0} = A(0)^{3/2} - \frac{\gamma_{A}}{\gamma_{T}}T_{tot}^{g}(0)^{3/2}, \qquad (4.3)$$

and $\quad D_{0} = \ln(aT_{tot}^{g}(0)^{3/2} + b).$

Substituting equation (4.1) into equation (2.6) yields

$$\frac{dA}{dt} \sim \gamma_{A}\exp(at), \qquad (4.4)$$

which means that

$$A(t) \sim \exp(at) \sim T_{tot}^{g}(t). \qquad (4.5)$$

Thus, to a leading order, both the coupled template and the aggregate grow exponentially with the same exponent (a). As equations (2.7) and (3.5) are substituted into equation (4.2), we note that the exponent in a direct manner depends on the template and lipid production rate constants, k_{T} and k_{L}, the local background precursor concentration, pL, the background template substrate concentration and the order of the template reaction, $f(O^{l})$, the template duplex equilibrium constant, K_{t} and the average number of lipids per aggregate, m_{0}.

$$a = \left(\frac{1}{4}k_{T}^{2}k_{L}pL^{l}f(O^{l})^{2}\frac{K_{t}V_{A}}{m_{0}}\right)^{1/3}. \qquad (4.6)$$

In figure 2, a simulation of the original kinetic equations (2.6) and (3.4) illustrates these findings. It should be noted that at least one gene duplex must be present in each aggregate for the derived kinetic growth equations to be valid, which is ensured if $\gamma_{A} < \gamma_{T}$.

5. DISCUSSION

A kinetic analysis of a minimal protocell model shows that the local parabolic template growth law is compensated by parallel aggregate division dynamics, which results in an overall exponential growth for the
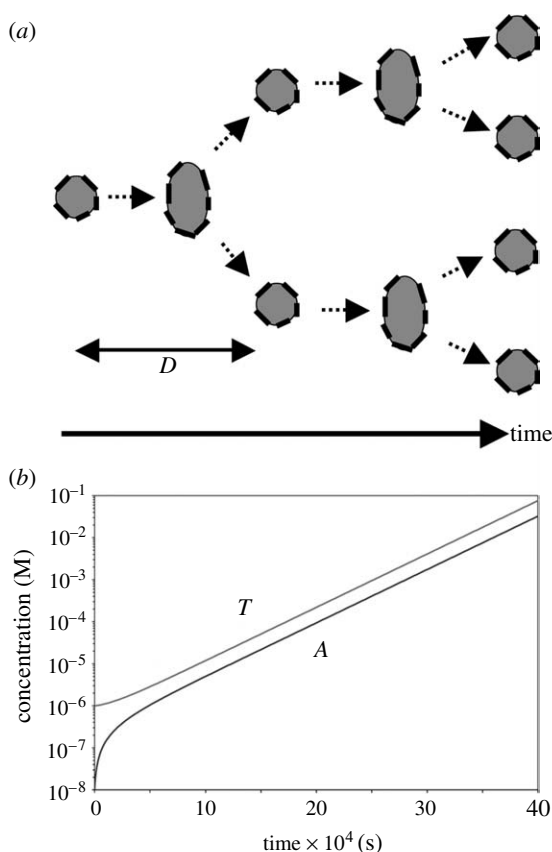
(a)

(b)

Figure 2. (a) The template catalyses the lipid production, while the lipid aggregate makes possible (catalyses) the template replication. Since the local template concentration is kept approximately constant due to the aggregate growth, the template replicates exponentially. The generation (doubling) time is given by $D = \left[(1/4)k_T^2 k_L f(O^l)^2 pL^l(K_t V_A)/m_0\right]^{-(1/3)} \ln 2$. See §5 in the text. (b) The results of a numerical simulation of the original coupled template–container replication system equations (2.6) and (3.4) illustrating the derived coupled asymptotic exponential growth law. Parameter values: $m_0 \sim 1000$, $K_t V_A \sim 10^{-3}$, $pL^l \sim 10^{-3}$ M, $f(O^l) \sim 2 \times 10^{-4}$ M, $k_L \sim 25$ Ms^{-1} and $k_T \sim 10$ Ms^{-1}. Note how the gene and the container concentrations converge to a coordinated growth rate after an initial transient adjustment period, due to an unbalanced set of initial concentrations. The protocell population doubles approximately once a day with these parameter values.

whole system. In simple terms, the coupled replication/container growth through equations (1.1) and (2.1) assures that at steady-state conditions the average local value of the template concentration T_d^l is kept constant as the volume of the system (the number of containers) grow concertedly. Consequently, no shift in the equilibrium of equation (1.1) for the system as a whole is occurring during growth and replication (in contrast to solution replication at constant volume). A second consequence is that the average concentration of single-stranded templates T_s^l and thus the replication rate is kept constant, allowing the system as such (genes + container) to grow exponentially. This result holds for any coupled genetic growth law as well as for an explicit inclusion of the metabolic kinetics or any other detailed intracellular growth dynamics (Munteanu *et al.* 2007). We have expressed analytically how the generation time is affected by the internal gene replication kinetics.

Thus, a detailed stoichiometric growth control of the individual protocell components is not necessary as conjectured, for example, by Gánti (2004). A coupled autocatalytic feedback between the template and the aggregate replication processes generates a coordinated growth of the main components. This result is also an extension of the exponential growth law in the low template concentration limit as discussed by Stadler (Stadler & Stadler 2003) and others. As long as the effective [2] average local replicator concentration is kept constant, in this case by growing the system's total lipid volume, exponential growth is also possible. A discussion of the detailed growth dynamics in modern cells can be found in e.g. Burdett & Kirkwood (1983); Novak *et al.* (1998) and Tyson *et al.* (2003).

ENDNOTES
[1]It should be noted that the main result presented in this paper can be generalized as demonstrated in Munteanu *et al.* (2007).
[2]It should be noted that an exponential amplification is also possible in a naked template-directed replication process if the resulting new template is modified in the ligation process to lower its hybridization energy (Paul & Joyce 2004).

APPENDIX A
The coupled growth equations (2.6) and (3.4) can be solved by separation of variables, i.e. by dividing equation (3.4) by equation (2.6)

$$\frac{dT_{tot}^g}{dA} = \frac{\gamma_T}{\gamma_A} \frac{\sqrt{A}}{\sqrt{T_{tot}^g}}. \qquad (A\,1)$$

Note that equation (A 1) is a special case of a Bernoulli differential equation. From equation (A 1), we obtain

$$(A(t))^{3/2} = \frac{\gamma_A}{\gamma_T}\left(T_{tot}^g(t)\right)^{3/2} + C_0, \qquad (A\,2)$$

where $C_0 = A(0)^{3/2} - \frac{\gamma_A}{\gamma_T} T_{tot}^g(0)^{3/2}$. We use this to rewrite equation (3.4) as

$$\frac{dT_{tot}^g}{dt} = \gamma_T \sqrt{T_{tot}^g}\left(\frac{\gamma_A}{\gamma_T}\left(T_{tot}^g\right)^{3/2} + C_0\right)^{1/3}$$

$$= \gamma_T \sqrt{T_{tot}^g}\left(\frac{\gamma_A^{1/3}}{\gamma_T^{1/3}}\sqrt{T_{tot}^g} + \frac{C_0 \gamma_T^{2/3}}{3\gamma_A^{2/3} T_{tot}^g} + O\left[\left(T_{tot}^g\right)^{-5/2}\right]\right),$$
$$(A\,3)$$

where we have expanded the expression for large T_{tot}^g relative to C_0. Ignoring the last term, which is small in order $T_{tot}^{g-5/2}$, the simplified expression has the solution

$$T_{tot}^g(t) = \frac{\left(\exp\left(\frac{3}{2}at + D_0\right) - b\right)^{2/3}}{a^{2/3}} \quad \sim \exp(at), \qquad (A\,4)$$

to the leading order in $T_{\text{tot}}^{\text{g}}$, where $a = \gamma_A^{1/3}\gamma_T^{2/3}$, $b = (\gamma_T^{5/3}C_0)/3\gamma_A^{2/3}$ and $D_0 = \ln(b + aT_{\text{tot}}^{\text{g}}(0)^{3/2})$. Substituting equation (4.1) into equation (2.6) yields

$$\frac{\mathrm{d}A}{\mathrm{d}t} \sim \gamma_A \exp(at), \tag{A 5}$$

which means that

$$A(t) \sim \exp(at) \sim T_{\text{tot}}^{\text{g}}(t). \tag{A 6}$$

Thus, to leading order, the coupled template and aggregate grow exponentially with the same exponent. Simulation of equations (2.6) and (3.4) verifies these findings (figure 2).

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