The time complexity of self-assembly

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Time efficiency of self-assembly is crucial for many biological processes. Moreover, with the advances of nanotechnology, time efficiency in artificial self-assembly becomes ever more important. While structural determinants and the final assembly yield are increasingly well understood, kinetic aspects concerning the time efficiency, however, remain much more elusive. In computer science, the concept of time complexity is used to characterize the efficiency of an algorithm and describes how the algorithm’s run-time depends on the size of the input data. Here we characterize the time complexity of nonequilibrium self-assembly processes by exploring how the time required to realize a certain, substantial yield of a given target structure scales with its size. We identify distinct classes of assembly scenarios, i.e., “algorithms” to accomplish this task, and show that they exhibit drastically different degrees of complexity. Our analysis enables us to identify optimal control scenarios for nonequilibrium self-assembly processes. Furthermore, we suggest an efficient irreversible scheme for the artificial self-assembly of nanostructures, which complements the state-of-the-art approach using reversible binding reactions and requires no fine-tuning of binding energies.

Significance

An important limiting factor for self-assembly processes is the time it takes to assemble large structures with high yield. While equilibrium self-assembly systems slowly relax toward a state of minimal free energy, nonequilibrium systems offer various ways to control assembly processes and to optimize their time efficiency. We show that these different control scenarios can informatively be characterized by their time complexity, i.e., their scaling of the assembly time with the structure size, analogous to algorithms for computational problems. Especially for large structures, differences in the time complexity of the scenarios lead to strongly diverging time efficiencies. Most significantly, we show that by effectively regulating the supply of constituents, high resource and time efficiency can be achieved for self-assembly processes.

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robust against model modifications. We mainly consider irreversible processes, in which structures can only grow. To assess the relevance of reversible binding, we also discuss a scenario in which individual monomers may detach from the edges of incomplete structures at a finite detachment rate \( \delta_0 \) that decreases exponentially with the number \( n \) of bonds that need to be broken: \( \delta_n = Ae^{-n \delta} \) (Arrhenius’ law). Here \( E_B \) is the binding energy per contact (bond) in units of \( k_B T \), and the constant \( A \) can typically be assumed to be large relative to the rate of reactions (19, 20). Note that we consider only the detachment of single monomers at the edges of one- (1D), two- (2D), or three-dimensional (3D) heterogeneous structures of edge length \( L \) (only the 2D case is illustrated explicitly). A constant influx of monomers of species \( i \) takes place during the time interval \( [T_i, T_i + \tau] \) with net influx rate \( N \alpha \).

Once added to the system (activated), monomers start to self-assemble. A monomer of a bulk species has two (1D), four (2D), or six (3D) possible binding partners as shown. In the 1D case, we assume periodic boundary conditions, i.e., species 1 and 5 can bind as well as the final structures form closed rings. In the higher-dimensional cases, we assume open boundaries, implying that the species located at the boundary have a reduced number of binding partners. Any two fitting monomers can dimerize with rate \( \mu \). Subsequent to dimerization, structures grow by attachment of single monomers with rate \( \nu \) per binding site. Furthermore, monomers can detach from a cluster with rate \( \delta_n = Ae^{-n \delta} \), where \( n \) is the number of bonds that need to be broken and \( E_B \) the binding energy per bond. We set \( A = 10^{18} \text{C}/\text{m} \), with \( C = N/V \) denoting the concentration of monomers per species. Our aim is to minimize the assembly time \( T_90 \) when 90% of all resources are assembled into complete structures. To this end, we control particular elements of the assembly process (control parameters) and distinguish four scenarios that are defined through the respective control parameter(s). The other parameters are fixed from the following set of “default” values: \( T_i = 0, \alpha = \infty, \mu = \nu, E_B = \infty (\delta_0 = 0) \). Each scenario can be used to elucidate kinetic traps and achieve a high assembly yield but how much time do these different strategies require?

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**Fig. 1.** Schematic description of the model. \( N \) identical copies of \( S \) different species of monomers assemble into one- (1D), two- (2D), or three-dimensional (3D) heterogeneous structures of edge length \( L \) (only the 2D case is illustrated explicitly). A constant influx of monomers of species \( i \) takes place during the time interval \( [T_i, T_i + \tau] \) with net influx rate \( N \alpha \). Once added to the system (activated), monomers start to self-assemble. A monomer of a bulk species has two (1D), four (2D), or six (3D) possible binding partners as shown. In the 1D case, we assume periodic boundary conditions, i.e., species 1 and 5 can bind as well as the final structures form closed rings. In the higher-dimensional cases, we assume open boundaries, implying that the species located at the boundary have a reduced number of binding partners. Any two fitting monomers can dimerize with rate \( \mu \). Subsequent to dimerization, structures grow by attachment of single monomers with rate \( \nu \) per binding site. Furthermore, monomers can detach from a cluster with rate \( \delta_n = Ae^{-n \delta} \), where \( n \) is the number of bonds that need to be broken and \( E_B \) the binding energy per bond. We set \( A = 10^{18} \text{C}/\text{m} \), with \( C = N/V \) denoting the concentration of monomers per species. Our aim is to minimize the assembly time \( T_90 \) when 90% of all resources are assembled into complete structures. To this end, we control particular elements of the assembly process (control parameters) and distinguish four scenarios that are defined through the respective control parameter(s). The other parameters are fixed from the following set of “default” values: \( T_i = 0, \alpha = \infty, \mu = \nu, E_B = \infty (\delta_0 = 0) \). Each scenario can be used to elucidate kinetic traps and achieve a high assembly yield but how much time do these different strategies require?

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**Time Complexity Analysis**

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**Appendix A: Time Complexity Analysis**

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**Table 1:**

| Scenario | Control Parameter | Time Complexity |
|----------|-------------------|-----------------|
| Reversible binding | \( E_B \) | \( T_90 \) |
| Dimerization | \( \mu \) | \( T_90 \) |
| Activation | \( \alpha \) | \( T_90 \) |
| Just-in-sequence | \( \{ T_i \} \) | \( T_90 \) |
parameter that maximizes the time efficiency. We are interested in the asymptotic dependence of $T_{\text{min}}^{\text{opt}}$ on the structure size $S$ for $S, N \gg 1$. In particular, while we have shown previously (31) that for small copy numbers $N$, the activation scenario is strongly influenced by stochastic effects, we here assume $N$ to be large enough so that stochastic effects can be considered irrelevant.

Maximal time efficiency can then be obtained by a proper choice of the relative frequency of nucleation and growth events: Initiation of new structures must be sufficiently retarded relative to the growth of existing structures to avoid kinetic traps ("slow nucleation principle") (31–33). The larger the target structure is, the smaller the ratio between the effective nucleation and growth rate has to be to achieve a yield of 90%. However, too small a nucleation rate severely limits the required assembly time on the other hand. The various scenarios (with the exception of the one-dimensional reversible-binding scenario, which constitutes a special case) represent different mechanisms to control the ratio between the nucleation and growth rate and therefore allow one to tune it to an optimal value.

However, the effectiveness with which the ratio is controlled, and thus the minimum assembly time that can be achieved, varies greatly between the different strategies. In all cases, we find numerically that both the optimal control parameter and the minimal assembly time exhibit power-law dependencies on the size of the target structure (Fig. 2). The corresponding exponents are referred to as the control parameter exponent $\phi$ and the (time) complexity exponent $\theta$, respectively. Both exponents are scenario specific and, moreover, depend on the dimensionality of the assembled structure, as is discussed in detail below for each scenario.

To derive analytical estimates for the exponents, we use that the optimal ratio between nucleation and growth rate should approximately scale inversely with the structure size,

$$\frac{\text{number of nucleation events per time}}{\text{number of attached monomers per time}} \sim S^{-1}.$$

A detailed mathematical evaluation of the scaling of the terms on the left-hand side with the system parameters can be found in SI Appendix, section 3. In the main text, we restrict ourselves to a discussion of the phenomenology of our numerical results and use heuristic scaling arguments.

Reversible-Binding Scenario. In the reversible-binding scenario, the time complexity strongly depends on the dimensionality of the structure. For one-dimensional structures, the rate of monomer detachment is the same for all unfinished structures. Hence, it is not possible to selectively disfavor nucleation of new structures relative to the growth of existing structures by varying the binding energy $E_B$. In this respect, the one-dimensional reversible-binding scenario constitutes a special case among all scenarios, since it realizes a profoundly different self-assembly mechanism. We find that structures are initially formed in such an amount that the overall attachment and detachment processes of the monomers balance out and the concentration of monomers becomes stationary. Growth and shrinkage of a structure then become approximately equally likely and the cluster sizes evolve (approximately) diffusively, with diffusion constant given by $D = \nu m + \delta$, where $m$ denotes the stationary monomer concentration. Hence, varying the detachment rate $\delta$ allows one to maximize the diffusive flux. We show in SI Appendix, section 3 that the optimal detachment rate and the resulting effective diffusion constant scale like $\delta_{\text{opt}} \sim D \sim S^2/CvS^{-2}$. This implies that the assembly time for one-dimensional structures scales like the diffusive timescale (to diffusively transverse a distance $S$) $T_{\text{diff}} \sim S^2/D \sim \nu^{-2}S^4$ with time complexity exponent $\theta = 4$, which agrees very well with the results obtained from stochastic simulations (Fig. 2A).

In higher dimensions, large clusters are typically bound more tightly and hence become energetically favored over clusters of small size, as illustrated in Fig. 3A. This creates an effective nucleation barrier, which allows one to strongly enhance the time efficiency compared to the one-dimensional case. Essentially, the monomer concentration is thereby much larger than in the one-dimensional case, which enables nucleated structures to grow quickly. However, to guarantee both high resource efficiency (high yield) and time efficiency, the binding energy must be fine-tuned to within few percent of its optimal value (Fig. 3B). Larger binding energies imply a lower nucleation barrier and lead to kinetic trapping, whereas lower binding energies progressively reduce the effective nucleation rate. By fine-tuning of the binding energy $E_B$, we obtain the time complexity exponent $\theta_{\text{2D}} \sim 1.19$ and $\theta_{\text{3D}} \sim 0.75$, respectively, for the two-dimensional (2D) and three-dimensional (3D) cases (Fig. 2B and C). Both exponents can also be estimated analytically from
Eq. 1 by deriving effective rates for nucleation and attachment reactions (SI Appendix, section 3 and tables in Fig. 2 B and C). Note that the binding energy $E_B$ is measured in units of $k_B T$ and the detachment rate relative to the reaction rate ($C_\nu$). Hence, the most feasible way to fine-tune the control parameter in experiments will be to adapt either the temperature or the monomer concentration $C$.

**Dimerization Scenario.** We then analyzed the remaining irreversible assembly scenarios, setting $\delta_1 = 0$. In the dimerization scenario, decreasing the dimerization rate $\mu$ disfavors initiation of new structures relative to the growth of existing structures. Fig. 4 A shows the corresponding transition from zero to perfect final yield, with $\mu_{\text{Dopt}}$ indicating the rate at which a final yield of 90% is achieved. We find that the optimal dimerization rate $\mu_{\text{Dopt}}$ that minimizes the time required to achieve 90% yield is only slightly lower than $\mu_{\text{opt}}$ and, for linear structures, scales as $\mu_{\text{opt}} \sim \nu S^{-2}$ (Fig. 4 A). This dependence of $\mu_{\text{Dopt}}$ on $S$ for linear structures can be explained as follows: According to Eq. 1, when increasing the structure size $S$, the ratio between nucleation ($=\text{dimerization}$) and growth rate must be reduced to allow the structures to grow to the larger size. However, to achieve the desired scaling, $\mu_{\text{Dopt}}$ must scale quadratically with $S$, because the number of dimerization events per time increases with the number of possible dimerization partners ($\sim S$) leading to an additional factor of $1/S$.

Since dimerization is the rate-limiting step, we expect that the assembly time will predominantly be determined by the total dimerization rate $T_{90}^{\text{min}} \sim (C_{\mu\text{Dopt}} S)^{-1} \sim (C_\nu)^{-1} S$. This estimate correctly predicts the time complexity exponent $\theta = 1$ for linear structures (Fig. 2A). For target structures of higher dimension, the effective growth rate of clusters is increased compared to the one-dimensional case because structures grow radially. This allows for a simple possibility to relate the exponents for target structures of higher dimension to the one-dimensional case by rescaling the binding rate $\nu$. Note that the number of possible binding partners of a globular structure with $s$ particles is proportional to its surface area and thus scales approximately as $s^{(d-1)/d}$, where $d$ is the dimensionality of the structure. Thus, defining an effective average binding rate $\nu_S \sim \nu S^{(d-1)/d}$ for a target structure size $S$ allows one to map higher-dimensional growth processes to an effective one-dimensional process along the radial coordinate. Replacing $\nu \rightarrow \nu_S$ therefore translates the scaling laws for linear objects into approximate scaling laws for higher-dimensional structures. This scaling idea for the dimerization scenario accurately yields the control parameter exponents for higher-dimensional structures (table in Fig. 4 A) and only slightly overestimates the time complexity exponents in higher dimensions (Fig. 2 B and C). These deviations may be attributed to the subleading contribution of the growth process to the total assembly time, which becomes more pronounced in higher dimensions. The dimerization scenario is the most time-efficient scenario because reducing the dimerization rate allows one to specifically control the effective nucleation speed without simultaneously affecting the attachment rate. In contrast, changing the binding energy in the reversible-binding scenario at the same time reduces the effective attachment speed and therefore renders this strategy less efficient.

**Activation Scenario.** In the activation scenario, nucleation is inhibited by controlling the concentration of available (or active) monomers. Decreasing the influx rate $\alpha$ reduces the momentary concentration of active monomers and therefore reduces the effective dimerization rate. As in the dimerization scenario, this leads to a transition from zero to perfect final yield (Fig. 4 B). The transition is not strictly monotonic but exhibits some small-scale peaks, whose origin is not entirely clear. From the stochastic simulations and scaling analysis (SI Appendix, section 3) we infer for linear structures an optimum influx rate scaling as $C_\alpha \sim \nu S^{-3}$ (Fig. 4 B, Inset). The dependence of $C_\alpha_{\text{opt}}$ on $S$ can be explained similarly to that for the dimerization scenario: When increasing $S$, according to Eq. 1, the ratio between effective nucleation and growth rate must be reduced, while the increase of the total nucleation rate with increasing number of species must be balanced. Together, this accounts for a factor of $1/S$ in $C_\alpha_{\text{opt}}$, analogous to the dimerization scenario. Additionally, however, increasing $S$ would enhance the total influx of particles and thus the momentary concentration of monomers. This again would increase the nucleation rate and needs to be balanced, thus explaining the third factor of $1/S$. The control parameter exponents $\phi$ for higher-dimensional structures can again be derived with our rescaling argument, $\nu \rightarrow \nu S^{(d-1)/d}$, and are found to be only slightly larger than those obtained from simulations (table in Fig. 4 B). As the monomers are activated over a time span $1/\alpha$, the time complexity exponents are the reciprocals of the parameter exponents (Fig. 2).

We expect that the exponents for the activation scenario remain the same if other forms of monomer input are considered. For example, monomers could (reversibly or irreversibly) switch between an assembly inactive and active state. In
**Discussion**

Fig. 2 shows the dependence of the minimal assembly time on target structure size, together with the resulting time complexity exponents for the different scenarios and dimensionalities. All exponents decrease with increasing dimensionality of the target structure and can even change their relative order. For the dimerization, activation, and reversible-binding scenario, one can show that the analysis is independent of the heterogeneity of the building blocks (SI Appendix, section 2). Remarkably, the exponents are furthermore robust to various modifications of the model such as heterogeneous binding rates, modified boundary conditions, or altered definitions of the assembly time (SI Appendix, section 4). Similarly, advanced protocols like annealing or different forms of monomer input in the activation scenario leave the exponents invariant. This invariance shows that the time complexity analysis yields a reliable and robust characterization of self-assembly processes. Furthermore, the invariance of the parameter exponents allows for an optimal control strategy to be identified in dependence of the size of the target structure in each of the four scenarios.

The dimerization scenario turns out to be the most time-efficient scenario in all dimensions. Controlling the dimerization rate is efficient as it allows one to initiate just as many structures as are needed, followed by a rapid growth phase if all particles are readily available. For linear structures, the supply-control strategies rank second and third, with coordinated supply in the JIS scenario being more efficient than uncoordinated supply in the activation scenario. Reversible binding is the least efficient approach to assembling large linear structures, but it is efficient for the assembly of higher-dimensional structures and then becomes competitive with the JIS scenario, slightly outperforming it for large structure sizes.

The reason why reversible binding is inefficient for one-dimensional structures is that for linear objects—in contrast to higher-dimensional objects—nucleation cannot be slowed down by the sequential addition of particles.

**SI Appendix, section 4**, we simulate the reversible case explicitly, assuming that the switch is fast so that active and inactive monomers are at equilibrium. This case might indeed be relevant in virus capsid assembly (26) and it exhibits the same scaling as the constant influx scenario (SI Appendix, Fig. 5C). Controlling the switching rate between particle configurations (for example with light) (34) could also be a feasible way to implement the activation scenario experimentally.

**Just-in-Sequence Scenario.** In the irreversible assembly scenarios discussed so far, all species are made available simultaneously. Consequently, excess nucleation of structures can only be suppressed by using a low dimerization or activation rate. In contrast, the JIS scenario favors specific assembly paths by regulating the order in which building blocks are supplied. The species supplied first in this temporal sequence define the nuclei for subsequent growth. Formation of other competing nuclei (dimers) during the assembly process is suppressed by the sequential delivery of building blocks, which ensures that mutual binding partners are supplied successively. Binding of newly added monomers to existing structures is therefore more likely than formation of new dimers. The frequency of competing nucleation events can be controlled by adjusting the interval \( \Delta T \) between the equidistant time points \( T_i \) at which subsequent “batches” of monomers are provided. Longer time intervals increase the yield at the cost of a lower time efficiency.

To minimize the total number of batches, we chose an “onion” supply protocol, which allows structures to grow radially from the inside out, like the skins of an onion (Fig. 5C). Furthermore, the time efficiency can be enhanced by using increasing, nonstoichiometric concentrations for the monomers in successive batches (Fig. 5B). Nonstoichiometric concentrations in a properly chosen ratio (SI Appendix, section 1, Just-in-sequence scenario) reduce competition for resources between growing structures (Fig. 5A) and thereby greatly enhance the time efficiency, as well as robustness to extrinsic noise in the particle numbers supplied, especially for higher-dimensional structures (Fig. 5D and E). Therefore, nonstoichiometric concentrations are the key to successful implementation of the JIS strategy for higher-dimensional structures. Since we assume equidistant time intervals \( \Delta T \) between subsequent batches, the total assembly time is the product of \( \Delta T \) (\( \sim S \)) and the total number of batches (\( \sim L \sim S^{1/4} \)), yielding the complexity exponents \( \theta = 1 + 1/d \), as shown in Fig. 2. To demonstrate the broad experimental applicability of the JIS supply strategy with a concrete example, we discuss in detail in SI Appendix, section 5 how the JIS strategy could efficiently be used to assemble artificial \( T = 1 \) capsids. Artificial capsids have important potential technological and medical applications (35–37) and the simulations show that the JIS strategy might indeed be a feasible and efficient way to assemble these structures.
down relative to growth by increasing the detachment rate. This strong dependence of the efficiency on the dimensionality implies that, generally, the morphology of the assembled structures plays an important role for the reversible-binding scenario. For example, assembling quasi-linear objects with two (or more) layers of subunits instead of a one-layered linear object might significantly increase the assembly efficiency. Identifying and designing those morphologies that are particularly favorable and assemble efficiently could therefore be an interesting direction for future research.

In conclusion, our time-complexity analysis of self-assembly describes lower bounds for the required assembly time as a function of the target structure size. Furthermore, it provides a robust description of how the parameters of the system must be controlled to achieve optimal time and resource efficiency. The analysis enables us to compare the efficiency of different self-assembly scenarios. In computer science, the complexity of a computational problem is defined as the complexity of the fastest algorithm available to solve it (38). Among the assembly scenarios discussed here, limiting the dimerization rate defines the fastest assembly process and might thereby determine the time complexity of self-assembly (of course, we cannot exclude the possibility of even faster assembly strategies). Experimentally, however, controlling the dimerization rate is difficult, as it effectively requires building blocks that exhibit allosteric binding effects. So far, experiments have typically resorted to rendering binding reactions reversible (21–23, 25, 39, 40). Our analysis shows that this common approach is time efficient for the assembly of higher-dimensional structures. However, to be truly competitive, fairly precise tuning of bond strengths, temperature, and the concentration is required. Our analysis suggests that a supply-control strategy like the JIS scenario is a promising alternative that offers similar or better time efficiency using irreversible self-assembly. As a significant advantage, this strategy does not rely on sophisticated properties of the building blocks (like allosteric effects or fine-tuned bond strengths) but only on temporal supply control and hence on parameters that might be more amenable to regulation and adaptation in experiments: In its simplest implementation, the different species could just be added manually to the system in the designated temporal sequence.

Compared to the current state-of-the-art approach via reversible reactions, irreversible assembly schemes might thus provide a complementary and more versatile strategy for assembling complex structures, requiring control over relative concentrations, rather than fine-tuning of the molecular details. Importantly, the idea underlying the JIS scenario entails a rather specific design principle for efficient irreversible assembly protocols of complex nanostructures (“batches without mutual binding partners”); we demonstrate in SI Appendix, section 5 how this principle is applied exemplarily for the assembly of artificial $T = 1$ capsids. This design principle thereby provides a clear path toward the experimental realization of the JIS scenario, suggesting that the strategy will be broadly applicable to the assembly of artificial structures.

An interesting question for future research concerns the prospects for spatiotemporal supply control, i.e., controlling not only the time interval but also the site at which monomers are injected into a spatial system, for further enhancement of the time efficiency. Moreover, it would be interesting to consider the time complexity of assembly schemes like hierarchical self-assembly (41–44), which include polymer–polymer interactions, or assembly schemes in which interactions among the particles allow for multiple self-assembly states. Finally, other potentially important aspects of self-assembly include susceptibility to errors in the case of reduced binding specificities or defective particles, as well as robustness to stochastic effects for small copy numbers. If particle numbers are large and nonspecific bonds are sufficiently weak and reversible, we expect that these factors will not considerably affect the assembly dynamics. Otherwise, it might be instructive to test how the different assembly scenarios are influenced by these factors and compare the robustness of the various strategies in this respect.

**Materials and Methods**

This paper is accompanied by a detailed SI Appendix file, which discusses the numerical and analytical methods that were used to simulate the four scenarios and to determine their time complexity exponents. Specifically, SI Appendix, section 1 shows the details of the numerical simulation and, in particular, explains how the concentrations for the various species in the just-in-sequence scenario were determined. SI Appendix, section 2 analyzes the master equation and shows mathematically that the heterogeneity...
(distinguishability) of the building blocks is irrelevant for the dynamics in the limit of large particle numbers. SI Appendix, section 3 is dedicated to the mathematical scaling analysis and explains how the analytic estimates for the time complexity and control parameter exponents are derived. Furthermore, SI Appendix, section 4 demonstrates the robustness of the time complexity exponents to various modifications of the model and variations in the parameters. Finally, SI Appendix, section 5 illustrates how the just-in-sequence supply strategy can be used in practice for the concrete example of artificial T = 1 capsid assembly and thereby demonstrates the broad applicability of the just-in-sequence scenario.

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Data Availability. C++ code for simulations and data have been deposited in GitHub (https://github.com/FloGat88/Self_Assembly.git) (45).

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