Chemical Feedback in Templated Reaction-Assembly Networks

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ABSTRACT: Chemical feedback between building block synthesis and their subsequent supramolecular self-assembly into nanostructures has profound effects on assembly pathways. Nature harnesses feedback in reaction-assembly networks in a variety of scenarios including virion formation and protein folding. Also in nanomaterial synthesis, reaction-assembly networks have emerged as a promising control strategy to regulate assembly processes. Yet, how chemical feedback affects the fundamental pathways of structure formation remains unclear. Here, we unravel the pathways of a templated reaction-assembly network that couples a covalent polymerization to an electrostatic coassembly process. We show how the supramolecular staging of building blocks at a macromolecular template can accelerate the polymerization reaction and prevent the formation of kinetically trapped structures inherent to the process in the absence of feedback. Finally, we establish a predictive kinetic reaction model that quantitatively describes the pathways underlying these reaction-assembly networks. Our results shed light on the fundamental mechanisms by which chemical feedback can steer self-assembly reactions and can be used to rationally design new nanostructures.

INTRODUCTION

In nature, the biosynthesis of macromolecular building blocks and their assembly often occur simultaneously and within the same cellular compartment.1–4 The spatiotemporal colocalization of covalent and supramolecular reactions, involving the same molecular compounds, results in feedback between the different primary chemical reactions. Chemical feedback in these coupled reaction-assembly networks has profound effects on both the kinetics of the process and the final products that the coupled reactions yield. For example, the structure of virions is different when the translation of viral coat proteins occurs simultaneously with the DNA-templated assembly process as compared to the case when all proteins are already present at the start of the assembly.5–7 Also in protein biosynthesis itself, folding of the polypeptide chain into the desired tertiary structure can be regulated by the rate of its translation.8–10

Supramolecular assembly strategies have become a paradigm in the creation of a plethora of synthetic nanomaterials.11–14 However, while in nature reaction and assembly are invariably coupled, for synthetic materials the synthesis of the building blocks and their subsequent self- or coassembly have traditionally been separated in both space and time. This facilitates experimental control on the purity and characterization of the building blocks and of the details of the assembly process, but it also limits the structures than can be made, and the pathways by which these are reached, as the covalent reaction cannot be used to tune the assembly. In recent years, interest in the use of coupled reaction-assembly processes has emerged as a control mechanism for creating synthetic nanomaterials. A seminal example is polymerization-induced self-assembly (PISA), in which covalent polymerizations of amphiphilic macromolecules are coupled to the formation of micellar and vesicular structures that could not have been created using the classical spatiotemporal separation of the covalent and supramolecular processes.12 Many examples of this strategy have now been reported.13–15 More recently, this approach has been extended to templated assembly reactions. In polymerization-induced electrostatic self-assembly (PIESA), the polymerization of a polyelectrolyte occurs in the presence of oppositely charged macroions that serve as a molecular template for the assembly.16–20 This is of particular interest because templated assembly, in which a macromolecular template acts as a blueprint for the assembly process, is a common approach used by nature to guide multicomponent systems across their complex supramolecular energy landscapes to the desired final structure.21–23 Yet, the control of templated assembly in the synthetic realm remains challenging.24

Despite the promise of these templated reaction-assembly networks, mechanistic insights into the emergence of chemical feedback in these networks remain elusive. For example, it is unclear how the kinetic pathways across underlying elementary reaction steps are affected by the coupling. Moreover, comprehensive and predictive models that capture the essence...
of chemical feedback in these chemical networks are lacking. Yet, these insights are crucial; not only to optimize and rationally design synthetic reaction-assembly networks for nanomaterial fabrication, but also to provide a deeper understanding of how chemical feedback influences structure formation in vivo.

In this paper, we explore chemical feedback in a templated reaction-assembly network that couples covalent polymerizations to supramolecular electrostatic assembly on a macromolecular template. To unveil the kinetic pathways that underlie reaction progress, we perform time-resolved and in situ measurements based on high-resolution NMR spectroscopy and spectrally resolved relaxometry, complemented with light-scattering studies. While classical NMR spectroscopy has been used previously to study reaction-assembly networks,13,16 we extend these NMR spectroscopy measurements with spectrally resolved NMR relaxation measurements to probe the local dynamics of the separate components, which is essential to unravel the underlying kinetic pathways of the network. Our experimental results reveal how the preassembly of monomers onto the oppositely charged template accelerates the covalent reaction and provides feedback to alter the supramolecular assembly process. On the basis of these experimental data, we establish a kinetic reaction-assembly model that quantitatively describes the underlying reaction pathways. These results can help both optimizing the design of synthetic reaction-assembly materials and resolving the role of chemical feedback in structure formation in vivo.

### RESULTS AND DISCUSSION

Our templated reaction-assembly network consists of a neutral macromolecular chain-transfer agent P, positively charged monomers M as reagents and a negatively charged polyelectrolyte that forms the coassembly template, consisting of template binding sites S (Figure 1a). Specifically, we use poly(ethylene glycol methyl) ether 4-cyano-4-(propylsulfanylthiocarbonyl)-sulfanylpentanoate (PEG-CTA) as macromolecular chain-transfer agent, poly(sulfopropylmethacrylate) (PSPMA) with a degree of polymerization of 47 as the negative template, and vinylbenzyltrimethylammonium chloride (VBTC) as the positive monomer. Reversible addition-fragmentation chain transfer (RAFT) polymerization of the positive monomer occurs at the end of the neutral chain, resulting in the growth of the diblock copolymer P–Mn. In the standard case, the targeted degree of polymerization of the positive block n is 50 and the ratio of monomer to template sites is 1:1. The reaction is performed at a potassium chloride (KCl) concentration of 35 mM. Both cationic monomer and the resulting cationic block of the diblock copolymer can bind to the template (Figure 1b), as evidenced by isothermal titration calorimetry measurements (Figure S1). When the cationic block grows sufficiently long, its binding to the anionic template induces micelle formation by complex coacervation. The complex of oppositely charged polyelectrolytes forms the micellar core, and the neutral block forms its corona. These charge-driven micelles are often referred to as complex coacervate core micelles (C3Ms).25

#### Phases in the Reaction-Assembly Process

To unravel the kinetic pathways that underlie this templated reaction-assembly network, we use a combination of NMR spectroscopy, spectrally resolved NMR relaxometry, and light scattering. We use NMR spectroscopy to monitor the monomer conversion and NMR relaxometry to probe the chemical details of the reaction-assembly network (Figure S2). The longitudinal relaxation time T1 and transverse relaxation time T2 both depend on the rotational correlation time τc of (part of) the molecule, with T1 being mostly sensitive to fast molecular dynamics at the NMR measurement frequency and T2 being mostly affected by static sources of restriction in the molecular...
reorientational dynamics. Therefore, the combination of $T_1$ and $T_2$ measurements can be used to probe a wide range of mobility time scales, ranging from the fast dynamics of free monomers to the slow dynamics of polymers. The in situ determination of both $T_1$ and $T_2$ is complicated by the relatively large reaction speed compared to the time needed for quantitative $T_1$ and $T_2$ measurements. To obtain both sufficient time resolution and sufficient information on the local mobility of the components, we perform only quantitative $T_1$ measurements during the reaction while we take the changes in a $T_2$-filtered intensity as a measure for relative changes in $T_2$. We complement the 1H NMR measurements with light-scattering measurements: while NMR relaxation measurements probe the local dynamics of the separate components, light-scattering measurements can probe the overall assembly of the different components together. Based on our experimental observations, we can distinguish four phases in the reaction-assembly process (Figure 2a), which are explained below.

**Phase I: Induction Period.** The reaction network is started by heating the reaction mixture, which leads to thermal decomposition of the radical initiator. First, we observe an induction period (Phase I) during which the monomer conversion is slow as revealed by monitoring the monomer conversion by 1H NMR spectral measurements with a 0.02 s $T_2$ filter (Figure 1c).

Already at the start of this first phase, the situation with the template differs from the one without the template, in which there is no assembly and hence no chemical feedback. We observe that the longitudinal relaxation time $T_1$ of the monomer protons is shorter with the template than without the template (Table S1). For small molecules free in solution, both $T_1$ and $T_2$ decrease with increasing rotational correlation time of the molecule $\tau_c$. A decrease in $T_1$ in the presence of the template thus indicates restricted monomer mobility and can be explained by binding of monomers to the template. On the basis of $T_1$ measurements, we estimate that initially 50% of all monomers present binds to the oppositely charged template (Table S1). We note that differences among the $T_1$ values of the monomer protons are caused by differences in the intramolecular component of the dipole–dipole relaxation process. The latter process is driven by fluctuating fields generated via the interaction between two 1H nuclear magnetic dipoles, and its intramolecular contribution is caused by nuclear vibrations and rotations within the molecule.28

The addition of the template substantially shortens the induction period (Figure 1c). We hypothesize that this is due to the local accumulation of monomers at the macromolecular template, leading to a local increase of monomer concentration. To verify that a higher (local) concentration can shorten the induction phase, we performed different polymerizations without the template at increasing monomer concentration, while keeping the concentration of chain-transfer agent and initiator the same (Figure 3 a). Increasing the monomer concentration from 35 to 105 mM indeed shortens the induction period, while the final pseudo-first-order polymerization rate is the same in both cases (Figure 3b). Further increasing the monomer concentration to 175 mM only has a moderate effect on the induction phase. This reveals that the induction period consists of two contributions, one which is sensitive to the monomer concentration and another which is not. The contribution sensitive to monomer concentration can be explained by the fact that the first monomer added to a growing chain can have a lower effective reactivity as compared to subsequently added monomers, which has also been observed.
increases in Phase II, but because their \( T_3 \) is longer than that of \( M^6 \), the underlying intensity increase that is induced by \( T_2 \) increases becomes negligible compared to the intensity decay because of monomer consumption (Supporting Information Section S3.2). These changes in \( T_1 \) and \( T_2 \) occur only for the polymerization with the template (Figure S3) and are thus the result of chemical feedback.

The onset of faster monomer reorientational dynamics in Phase II is the result of competitive template binding between the monomer and growing diblock copolymer, which binds with a higher affinity because of multivalency. The binding of the polymerizing positive blocks to the template is shown by the broadening of the template peaks in the \(^1\text{H} \) NMR spectrum (Figure 2b). Broader peaks indicate a smaller \( T_2 \) and therefore a decrease in template mobility. The result of the competition between monomers and polymerizing blocks is that the concentration of template-bound monomer decreases, slowing down the polymerization rate at the template, as can be seen from the decrease in the slope of the monomer conversion during Phase II (Figure 1c).\(^{18} \)

**Phase III: Nanostructure Formation.** In Phase III, coassembly of the templates with growing diblock copolymers leads to the nucleation of microphase-separated objects, as revealed by an increase in scattered light intensity (Figure 2e). Nanostructure formation in Phase III results in the net reduction of both \( T_1 \) and \( T_2 \) values of the monomer protons, except for \( M^6 \), suggesting that nanostructure formation somehow hinders the reorientational dynamics of the monomer (Figure 2c,d). Three possible causes, and combinations thereof, can be identified to explain the observed reduction in monomer mobility: (i) the incorporation of bound monomers within the nanostructures leads to a decrease in \( \tau_2 \) (ii) an increase in the bound monomer fraction; (iii) an increase in the viscosity of the sample. The \( T_1 \) of the \( M^6 \) protons stays constant in Phase III, presumably because of that this chemical group is less affected by monomer incorporation in the nanostructure or viscosity increase or because increasing spectral overlap with the adjacent polymer peak prevents accurate evaluation of \( T_1 \) for the \( M^6 \) peak. Another possibility is that the \( \tau_2 \) of the \( M^6 \) protons is around \( 1/(\sqrt{2} \omega_i) \), where \( \omega_i \) is the Larmor frequency. At this point, \( T_1 \) is less sensitive to changes in \( \tau_2 \).\(^{27,28} \)

**Phase IV: Nanostructure Rearrangement.** During Phase IV, continued polymerization and equilibration lead to a decrease in light-scattering intensity and apparent hydrodynamic radius (Figures 2e and S4). A possible explanation for this decrease is the transition from initially elongated objects into smaller spherical micelles. It is known that weakening of the electrostatic interactions can induce a change in shape from spheres to ellipsoids, for example, by the increase in ionic strength.\(^{30} \) On the contrary here, as time progresses, the electrostatic interactions grow in strength as the length of the positive block increases, which could result in the transition from initially elongated objects into smaller spherical micelles. This rearrangement occurs only for shorter template lengths, while for longer templates the light-scattering intensity only increases during polymerization with a lower final intensity and smaller apparent hydrodynamic radius than for the shorter template lengths (Figure S5). This is in accord with literature results where the formation of ellipsoidal micelles was also observed only for shorter homopolymer lengths.\(^{30} \) In fact, also for micelles formed at low concentration from the components of our system, transitions to larger nanostructures at ionic strengths above 10 mM happen only for the short template lengths (Figure S5).
Figure 4. Inverted reaction-assembly network where a negatively charged block is polymerized in the presence of a positively charged template. (a) Different components of the inverted reaction-assembly network. (b) Pseudo-first-order kinetic plots of the monomer conversions for reactions with the template and without the template. Solid lines indicate model predictions for the polymerization with the template, \( k_2 = 1.0 \text{mM}^{-1} \text{h}^{-1} \), \( k = k_2 = 12 \text{mM}^{-1} \text{h}^{-1} \), \( k_{\text{in}} = 500 k_2 n \), \( k_0 = 0.1 \exp(-a E_k \cdot k T) \), with \( a = 3.1 \times 10^8 \text{h}^{-1} \), \( E_k = 6 k_2 T \). \( t = k_2(1, V_{\text{tot}}) = 8 \), \( a = 4.5 \) and \( t_{\text{wh}} = 6.5 \text{h} \), \( [S^0]_0 = 35 \text{mM} \). For the polymerization without the template, the same model parameters are used except for \( k_{\text{in}} = k_2 = k_1 = k_3 = 0 \), \( V_{\text{tot}} / V_0 = 0 \), \( a = 0.9 \), \( t_{\text{wh}} = 5.5 \text{h} \), and \( [S^0]_0 = 0 \). At increasing reaction times of the templated reaction-assembly network, (c) \(^1\text{H NMR spectra; (d) } T_1\)-values for neutral block C and the monomer A protons, with respective error bars obtained from single-exponential fitting; (e) \( T_2\)-filtered NMR spectral intensity (\( T_2\)-filter of 2.4 s) for the C and A protons, normalized to their own signal intensity at the start of the reaction; (f) light-scattering intensity.

Future time-resolved small-angle X-ray scattering (SAXS) measurements could help to verify whether the decrease in light scattering is indeed caused by rearrangements into smaller structures. At the moment, we cannot exclude that other factors than the nanostructure size have a (Figure 2d). This decrease in \( T_2 \) agrees well with the proposed micelle formation: when the neutral block becomes integrated into a dense micellar corona, its mobility is restricted, resulting in a lower \( T_2 \). The neutral block in the final equilibrium nanostructures exhibits three distinct \( T_2 \) values, while at the start of the reaction it has a single \( T_2 \) (Figure S6). The three distinct \( T_2 \) values reveal the occurrence of three main local mobility environments for protons that might be assigned to, in order of increasing local mobility, protons close to micelle core, protons in the middle of the micelle corona, and protons in the outer part of the micelle corona. This might also explain why the \( T_2 \)-filtered signal decreases during Phase IV: the part of the corona close to the core becomes denser when the micelles become spherical. This means that locally the mobility of the protons close to the core gets lower, and thus, the overall average \( T_2 \) decreases even though protons further away from the core can have an increase in mobility upon transition to spherical micelles and their \( T_2 \) might slightly increase. While, as expected, \( T_2 \) is sensitive to slow reorientational dynamic modes, we find that the \( T_1 \) of the neutral block is not much affected by the nanostructure formation (Figure 2c). It might be that the \( r_2 \) of the neutral block is around \( 1 / (\sqrt{2} a_1) \), where \( T_1 \) is less sensitive to changes in \( r_c \). Similar behavior of \( T_1 \) and \( T_2 \) of the neutral block upon C3M formation have been measured earlier at equilibrium conditions.

The occurrence of these four different phases is not restricted to this particular reaction-assembly network: also for a shorter and longer template length, we observe the same phases (Figure S5), with the nanostructure reorganization phase being absent for the longest template length, as discussed above. This demonstrates the relative robustness of our observations. We note that variations between repetitions of the same reaction-assembly experiment (Figures S5 and S7) could be explained by small differences in the trace amount of oxygen present, which results in slightly different radical concentrations. These variations do not affect the general trends: in all these measurements, the polymerization rate in the presence of the template is faster than that in the absence of the template, and at certain point in the reaction, the polymerization rate decreases.

Inverted System. To further verify the robustness of our description of these templated reaction-assembly networks, we invert the system by polymerizing a negatively charged block in the presence of a positively charged template (Figure 4a). In this way, we change the monomer–monomer interactions at the template (Figure S1) and the polymerization reactivity (Figure 3c,d). The inverted system also shows a feedback-enhanced reaction rate as compared to the polymerization without the template (Figure 4b). In addition, \( T_1 \) measurements show that also for the inverted system about 50% of all monomers initially binds to the oppositely charged template (Table S2). Furthermore, the inverted system undergoes the same reaction-assembly network phases: initially the monomer conversion is also slow (Figure 4b). Subsequently, when the main reaction starts, the template peaks also broaden (Figure 4c) and the monomer \( T_1 \) increases (Figure 4d). The monomer \( T_1 \) increases again when the nanostructure formation starts and also the \( T_2 \) of the neutral block decreases upon nanostructure formation (Figure 4e). The inverted system does not show
nanostructure rearrangement: the scattered light intensity only increases during the polymerization reaction (Figure 4f), similar to the reaction-assembly network with the longest negatively charged template. This is because the positively charged template is probably longer than the negatively charged template that we used for Figure 2.

**Effect of Reactant Stoichiometry.** So far, we have focused on reaction networks with a 1:1 ratio of monomer-template sites. The reaction-assembly process and the underlying kinetics can be tuned by various parameters, such as the reactant stoichiometry. If we add an excess of monomer compared to the number of template sites at a 3:1 ratio, first nanostructures form and then disassemble again (Figure 5a). This is similar to what has been observed for another PIESA reaction and agrees with the observations that C3Ms can be formed only around equal charge stoichiometry.

Changing the reactant stoichiometry affects the relative polymerization rate and the evolution of $T_1$ and $T_2$ of the monomers and neutral block during the reaction. The relative polymerization rate is slower when we add an excess of monomer (Figure 5b) because a smaller fraction of the total monomer can preassemble at the template, as it becomes saturated. This is reflected by the larger monomer $T_1$ values at the start of the reaction (Figure 5c). The increase in $T_1$ at the start of the main reaction again indicates expulsion of the monomer from the template because of binding competition and the subsequent $T_1$ decrease again coincides with the start of the nanostructure formation, which reduces the monomer mobility. As the reaction time progresses, the point of equal charge stoichiometry is crossed, leading to an excess of positive monomers that are part of a polycation and polyanion, $([\text{pos}] + [\text{neg}])$, where $[\text{pos}]$ and $[\text{neg}]$ are the concentrations of monomers that are part of a polycation and polyanion, respectively. At equal charge stoichiometry, the intermediate structures at $f_{\text{pol}} = 0.5$ scatter more light, indicating that different structures are formed. This difference can be explained from the effect of stoichiometry on the absolute reaction rate: although the relative reaction rate is lower when the monomer is present in excess, the absolute reaction rate is still higher. This can be seen by comparing the increase of the degree of polymerization of the positive block (Figure 5e). Especially around $f_{\text{pol}} = 0.5$, the polymerization with excess monomer is faster. This higher absolute rate is the result of the larger overall monomer concentration.

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Figure 5. Effect of the monomer-to-template ratio $M_{\text{tot}}/S_{\text{tot}}$. (a) Light-scattering intensity during the reaction-assembly process. (b) Pseudo-first-order kinetic plot of the reaction-assembly process. Solid lines indicate model predictions with the same model parameters as in Figure 1, except for the initial monomer concentration for $M_{\text{tot}}/S_{\text{tot}} = 3:1$, which is $[M_{\text{tot}}]_0 = 105$ nM. (c) $T_1$-values of P and M protons during the reaction-assembly process for $M_{\text{tot}}/S_{\text{tot}} = 3:1$ with respective error bars obtained from single-exponential fitting. (d) Light-scattering intensity as a function of fractional polymer charge ratio $f_{\text{pol}}$. (e) Positive polymer fraction $f_{\text{pol}}$ during the reaction-assembly process. The dashed line indicates the point of equal charge stoichiometry of the oppositely charged polyelectrolytes. (f) Changes in the $T_2$-filtered NMR spectral intensity ($T_2$-filter of 2.4 s) for the P and M protons, normalized to their own signal intensity at the start of the reaction, for the $M_{\text{tot}}/S_{\text{tot}} = 3:1$ reaction-assembly process.
This reveals the important role that the kinetic pathways play in structure formation: the building blocks form different structures when the rate at which they approach the point of equal charge stoichiometry is higher. This is seen by the fact that the scattering intensity is higher when the rate of polymerization around \( f_{\text{poly}} = 0.5 \) is increased. Moreover, direct mixing of presynthesized polymers at \( f_{\text{poly}} = 0.5 \), where equal stoichiometry is achieved almost instantly, results in very turbid samples (Figure 1d). This suggests that in this case kinetically trapped structures are formed when building block synthesis and assembly are decoupled. Indeed, during CSM formation larger nonequilibrium clusters are sometimes observed to be formed, which can either relax to their equilibrium state \(^{33,34}\) or remain trapped if equilibration is too slow. \(^{33,35}\) Gradual transition across the energy landscape for coassembly by coupling synthesis with assembly can avoid the formation of these trapped structures, without necessitating laborious protocols in which salt is added. \(^{33,34}\)

**Kinetic Model.** These data shed light on the fundamental effects that chemical feedback can have on the templated coassembly process explored here. To obtain a quantitative grasp on these effects for a predictive understanding of reaction-assembly networks, we develop a kinetic reaction network model that captures the elementary reaction steps and their couplings. Model predictions are made for the polymerization reaction with different monomer-to-template ratios (a–d) and for the polymerization reaction with template at \( M^\text{tot}/S^\text{tot} = 1:1 \) with decreasing monomer binding strengths by decreasing the value of \( E_a \), which affects both \( k_b \) and \( k_r \). Unless otherwise indicated, the model parameters are the same as in Figure 1. (f) Experimental effects for a predictive understanding of reaction-assembly network on (i) the monomer conversion and on (h) the \( T_P \) of the monomer proton \( M^+ \).

\[ k_{\text{pol}} = \frac{[P]_{\text{tot}}}{k_{\text{pol}}} = \frac{0.5}{0.5} \]
M_j]). The factor by which the local slope at time t deviates from this value gives the correction value ε(t). We note that for the polymerization of the inverted system, deviations from pseudo-first-order kinetics do not happen only at the start of the reaction but also at the end probably because of radical termination reactions (Figure 3c,d). Because we expect the termination reactions to be slightly different for every polymerization reaction, we do not include these effects in ε(t). In order to numerically solve the differential equations of the kinetic model, we need a better time resolution than we can obtain from the NMR reaction experiments. Therefore, we approximate the local overestimation of the rate constant is obtained from additional experiments or the components as a function of the reaction time. Most rate constants are known, this system of differential reaction kinetic equations (Methods). Once the initial concentrations and model constants are known, this system of differential equations can be numerically solved to give the concentrations of the different components as a function of the reaction time. Most rate constants are obtained from additional experiments or the literature; see Supporting Information Section S10 and Figures S10–S17 for parameterization details. We use only the ratio V_ffV_b and the binding rate of the unbound monomers of the bound diblock k_b as adjustable parameters to fit the model to the experimental data. By taking V_b = k_b = 500 k_b, the model prediction of the monomer conversion agrees well with the experimental data for the reaction-assembly network with the negatively charged template (Figures 1c and 5b). In addition, it also correctly describes the time point of free monomer fraction increase (Figures 6b, 2c,d, 5c,f). For the inverted reaction-assembly network, the model overestimates the reaction rate at longer reaction times (Figure 4b) because we did not include the effect of radical termination in the model ε(t) function as explained above. The obtained value for V_b is smaller than that we estimated based on estimations of the ionic bond length and polyelectrolyte blob size (Figure S9). Possible explanations for this difference are that the bound monomers might also be further away than the ionic bond length or that neglecting the prefactors in the estimation resulted in an overestimation of the V_b ratio. In addition, viscosity effects might have slightly slowed down the polymerization, resulting in a lower apparent V_b ratio in the experiments. The fact that k_b has to be much larger than the binding rate constant of a single monomer k_b to accurately describe the data (Figure S11) demonstrates once more that local enrichment can significantly increase the rate at which the process occurs.

The free diblock copolymer concentration decreases before we observe the increase in the fraction of free monomers (Figure 6c). Only when the average degree of polymerization increases sufficiently such that the binding affinity of the diblock becomes larger, the bound monomer fraction decreases. The increased binding affinity of the diblock results in a decrease in free template sites S (Figure 6d). This decrease in S shifts the monomer-template binding equilibrium more toward the free monomers. The binding of the diblock to the template is reflected by the broadening of the template proton peaks in the 1H NMR spectra (Figures 2b, S8).

Within our model, the template plays an essential role by acting as staging area for the chemical reaction. By decreasing the monomer-template interactions, the template effect can be reduced: decreasing the binding energy E_b of a monomer to the template results in less monomer binding and as a result the reaction rate is decreased (Figure 6e,g). In fact, for the lowest binding energies, the decrease might even be larger than shown here because the induction period and thus ε(t) are also affected when the monomer concentration largely decreases (Figures 3 and S12). A way to decrease the interaction strength experimentally is to increase the ionic strength in the solution. A higher ionic strength indeed lowers the monomer binding and the reaction rate (Figures 6f,h and S18). In addition, the induction period seems to lengthen at higher ionic strength, which could indicate that the decrease in local monomer concentration is large enough to affect the induction period. These effects of changing the interaction strength demonstrate that the reaction rate of the templated reaction-assembly network can be tuned, which is essential to regulate the assembly and in some cases can regulate the final structures formed, as discussed above.

CONCLUSIONS

The rational design of synthetic reaction-assembly strategies requires a deep understanding of the chemical feedback between the covalent reaction and the supramolecular assembly. Here, we have unraveled the kinetic pathways of templated reaction-assembly networks and we have shown that chemical feedback fundamentally changes both processes. Supramolecular staging of the monomers on the template strongly accelerates the polymerization reaction. The rate at which the reaction reaches the point of self-assembly in turn affects the structures that are formed.

One of the challenges in the design of self- or coassembling systems is to ensure that they arrive at their equilibrium state and do not become trapped in a local energetic minimum. Our results highlight how the rational design of a reaction-assembly network can achieve exactly this also at relatively high concentrations, where kinetic trapping is often inevitable in conventional assembly processes. These results can be easily extended to other PIESA and PISA systems, where kinetic trapping is avoided or in some cases promoted to arrive at new nanostructures. Also, in these other reaction-assembly networks, spectrally resolved NMR relaxometry can be applied to probe the local dynamics of the separate components. Simultaneously, our kinetic model can serve as a framework to describe also these other systems after implementing some adaptations, for example, including viscosity effects or replacing local enrichment at the template by local enrichment within assembled structures. It would be interesting to complement these future measurements by time-resolved SAXS measurements as the interpretation of SAXS measurements at the relatively high concentrations of the reaction-assembly networks is less ambiguous than for conventional light-scattering experiments. By using this combination of NMR experiments, scattering experiments, and kinetic modeling, the assembly of a wide variety of nanostructures can be linked to the corresponding reaction kinetics, facilitating the rational design of new synthetic supramolecular materials.

METHODS

Sample Preparation. A comprehensive overview of synthetic methods is provided in the Supporting Information (Section S1). For the reaction-assembly experiment at M_b /M_m = 1:1, the positive monomer vinylbenzyldimethylammonium chloride (VBTAC, 35 mM), the macroRAFT chain-transfer agent poly(ethylene glycol methyl)ether 4-cyano-4-(propylsulfanylcarboxyl)-sulfanylpropionate (PEG-CTA, 0.7 mM), the radical initiator 2,2'-azobis[2-(2-
imidazol-2-yl)propane dihydrochloride (VA-044, 0.14 mM), the template poly(sulfopropylmethacrylate) (SPSMA, 35 mM of SPSMA units), and potassium chloride (KCl) (10 mM) were dissolved in 5 mL of D$_2$O. The solution was degassed with N$_2$ for 30 min. Simultaneously, a glass tube for light-scattering experiments and a NMR tube both sealed with a rubber septum were also degassed for 30 min. Subsequently, circa ∼0.8 mL was transferred to the NMR tube and ∼3.5 mL was transferred to the glass tube.

For the polymerization at a [M]/[S] = 3:1 ratio, the monomer concentration was 105 mM instead of 35 mM, for the polymerizations without the template, no PSPMA was added, and for the polymerization at larger ion strength, 120 mM KCl was added instead of 10 mM.

The sample preparation protocol for the inverted reaction-assembly network was similar to the sample preparation protocol for the normal reaction-assembly network with [VBTA] = 35 mM. Only this time, the negatively charged SPSMA template was replaced by the positively charged poly((vinylbenzyl)trimethylammoniumchloride) (PVBTAC) monomer. In addition, the PEG chain-transfer agent was replaced by another PEG chain-transfer agent, poly(ethylene glycol)-4-cyanophenylcarbonothioyl pentanoate. For the polymerizations without the template, no PVBTAC was added.

**Light-Scattering Measurements.** Light-scattering measurements were performed at 44 °C on ALV light-scattering apparatus equipped with an ALV/LSE-5004 light scattering electronic and multiple tau digital correlator and a HeNe-laser operating at 632.8 nm. The scattering angle was set at 90°. The scattered intensities were obtained by averaging the photon count rate over periods of 60 s. The apparent hydrodynamic radius was obtained from second-order cumulant analysis of the intensity autocorrelation function (Figure S4).

**NMR Measurements.** $^1$H NMR reaction experiments were performed at 44 °C on a Bruker 700 MHz AVANCE NMR equipped with a BBI probe. During the reaction, a set of four different NMR measurements was continuously repeated, consisting of (i) single-pulse spectral acquisition; (ii) single-pulse spectral acquisition with a T$_1$-filter based on PROJECT pulse sequence (short filter of 20 ms, Section S3.1 and Figure S2); (iii) same as in (ii) but with long T$_1$-filter of 2.4 s (Section S3.2); (iv) inversion recovery measurement with a PROJECT-based acquisition scheme (Section S3.3 and Figure S2). Here, measurements (ii), (iii), and (iv) were used, respectively, to quantify the monomer concentration, to observe indirect effects of T$_1$ on signal intensity, and to determine T$_1$ relaxation rates. The length of the 90° rf pulse was readjusted at the start of every reaction and was subsequently kept constant within the reaction study. A 14 kHz spectral width was used. In each experiment, the signal from two consecutive acquisitions was added to obtain a sufficient signal-to-noise ratio. The receiver dead time was set to 6.5 μs. The longest T$_1$ was about 3–4 s during most of the reaction, thus the recycle delay was set to 21 s (∼5T$_1$) to obtain quantitative results while achieving the desired time resolution. Only at the end of the reactions with template at $[M]/[S] = 3:1$ and during the reactions without template, the T$_1$ of one M peaks (M$^3$) exceeded 4.2 s (Figure S2); thus, this particular result is not fully quantitative. Yet, this does not affect the data interpretation presented here. The M$^3$ proton peak partly overlaps with the solvent peak and the A$^3$, A$^4$, and A$^6$ proton peaks (partly) overlap with the template proton peaks. Therefore, these protons are not included in the analysis of T$_1$ and of the T$_1$-filtered intensity. After the reaction, the measured free induction decays were Fourier transformed, phased, baseline corrected, and integrated by using the Bruker NMR software TopSpin 4.0.7. Single-exponential data fitting of T$_1$ recovery curves was performed by using a constrained nonlinear least-squares fit algorithm as implemented in the Python package SciPy.

**Kinetic Model.** The kinetic model describes the changes in the concentration of the different components with a set of differential equations. Here, we give a brief overview of these equations. More details are given in the Supporting Information (Section S10).

The change in free monomer concentration is given by

$$\frac{d[M^f]}{dt} = -k_d[M^f][S^f] + k_d[M^d] - k_d(\tau)[M^f] \sum_n \left[ P - M^d_n \right]$$

$$+ \sum_n \left[ P - M^d_n \right]$$

(1)

where the first two terms indicate the change of free monomer concentration because of the exchange of the monomer between the free and the bound state and the last term indicates the change due to the polymerization reaction. In a similar way we get for the bound monomer

$$\frac{d[M^b]}{dt} = k_d[M^b][S^f] - k_d[M^d] - k_d(\tau)[M^b] \frac{V_{\text{tot}}}{V_p} \sum_n \left[ P - M^d_n \right]$$

$$+ \sum_n \left[ P - M^d_n \right]$$

(2)

here, the factor $V_{\text{tot}}/V_p$ accounts for the fact that the monomer concentration at the template is locally increased.

The change in concentration of a free diblock with the degree of polymerization of the positive block $n$ is given by

$$\frac{d[P - M^a_n]}{dt} = -k_d[M^b][P - M^a_n] + k_d(n)[P - M^a_n]$$

$$+ k_d(\tau)([M^b] + [M^d])\left(\left[P - M^a_n\right] - \left[P - M^a_{n-1}\right]\right)$$

(3)

also here, the first two terms correspond to exchange between the free and the bound state and the last term corresponds to the polymerization reaction.

The change in the concentration of a bound diblock with the degree of polymerization of the positive block $n$ follows from

$$\frac{d[P - M^b_n]}{dt} = k_d[M^b][P - M^a_n] - k_d(n)[P - M^a_n]$$

$$+ k_d(\tau)([M^b] + [M^d])\left(\left[P - M^a_n\right] - \left[P - M^a_{n-1}\right]\right)$$

(4)

The neutral block P–M$_0$ cannot bind to the template and therefore always remains in the free state. Its concentration decreases due to the polymerization reaction

$$\frac{d[P - M^b_0]}{dt} = -k_d(\tau)([M^b] + [M^d])\left[P - M^a_0\right]$$

(5)

When a diblock with $n$ positive monomers is bound to the template, part of the monomers can also temporarily unbind from the template and rebind again. The positive monomers of bound diblocks can thus exchange between a free and a bound state. We indicate the concentration of bound positive monomers of bound diblocks with $[n^+]$ and the concentration of free positive monomers of bound diblocks with $[n^+]$. The concentration of bound positive monomers of bound diblocks $[n^+]$ changes according to

$$\frac{d[n^+]}{dt} = -k_d[M^b][n^+] - k_d(1)[n^+] + k_d[S^f] \sum_n \left[ P - M^a_n \right]$$

$$- z \sum_n \left[ P - M^a_n \right] + k_d(\tau)([M^b] + [M^d]) \frac{V_{\text{tot}}}{V_p} [M^d] \sum_n \left[ P - M^d_n \right]$$

(6)

where $z = [n^+]/([n^+] + [n^+])$ is the fraction of bound monomers of the positive block. The first two terms correspond to the exchange between the free and bound state. The third and fourth terms indicate the
changes in $[n^b]$ because of binding and release of the diblock, respectively, and the last term indicates the increase in $[n^b]$ when a bound diblock reacts with a free monomer.

The change in the concentration of free positive monomers of bound diblocks $[n^f]$ is given by

$$\frac{d[n^f]}{dt} = -k_{b}[n^b][S^f] + k_d(1)[n^b] - (1 - z) \sum_k k_d(k)(P - M^b_k)n$$

Finally, the change of the free template site concentration is given by

$$\frac{d[S^t]}{dt} = -k_d(M^t)[S^t] + k_d(M^t) - k_d[S^t]\sum_k [P - M^t\sum_k n + k_d(1)[n^b]]$$

where the first two terms correspond to the binding and release of a single monomer, the third and fourth terms correspond to the binding and release of a diblock, the fifth and sixth terms correspond to the binding and release of the monomers of the bound diblocks, and the last term accounts for changes in $[S^t]$ because of the reactions of a bound monomer with a free diblock or a free monomer with a bound diblock.

We have used the Runge–Kutta fourth-order method to numerically solve this system of differential equations for different sets of model parameters (Figures S10–S17).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://dx.doi.org/10.1021/acs.macromol.0c01915.

Polymer synthesis; isothermal titration calorimetry measurements; overview of the $^1$H NMR measurement methods used to monitor the reaction-assembly network; estimation of the initial bound monomer fraction from NMR $T_1$ measurements; polymerization without template; apparent hydrodynamic radius from light-scattering measurements; effect of the template length; $^1$H NMR $T_2$ measurements of the neutral block; reaction stoichiometry effects; and kinetic model details and the reaction-assembly network at 155 mM KCl (PDF).

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Author Contributions

I.B. and J.S. conceived and designed the project. I.B. synthesized and characterized all compounds. I.B. and C.T. performed experimental work and analysis. I.B. performed the modelling. All authors co-wrote the manuscript.

Notes

The authors declare no competing financial interest.

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