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Two-stage polyelectrolyte assembly orchestrated by a clock reaction

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Supporting information for this article is given via a link at the end of the document.

Abstract

Controlling the transient self-assembly of (macro)molecular building blocks is of fundamental interest, both to understand the dynamic processes occurring in living systems and to develop new generations of functional materials. The subtle interplay between different types of physicochemical interactions, as well as the possible reaction pathways, are crucial when both thermodynamic and kinetic factors play substantial roles, as in the case of transient supramolecular assemblies. Clock reactions are a promising tool to achieve temporal control over self-assembly in non-living materials. Here, we report on the tunable association of poly(allylamine hydrochloride) (PAH) fueled by the formaldehyde-sulfite clock reaction. The electrostatic interaction between the large macromolecules and the small, oppositely charged sulfite ions gives rise to micron-sized coacervate-like complexes. As the clock proceeds, sulfite is completely depleted and the complexes dissociate. However, under suitable conditions, a subsequent reaction between the polyelectrolyte and formaldehyde can lock-in the preformed supramolecular structure, giving rise to covalently crosslinked colloidal particles.

Introduction

In nature, the building blocks of living materials can interact in a myriad of ways, by means of both non-covalent and covalent bonds, and on very different time- and length scales. The subtle interplay between the variety of interactions is critical to determine the dimensions and properties of the final products.[1,2] At the same time, their specific assembly pathway further impacts the outcome. The structural complexity and associated functions accessible in nature (*e.g.* signaling, sensing and reproduction) have become an inspiration for exploring assembly under out-of-equilibrium conditions. Such an approach allows to generate a huge variety of structures from relatively simple chemical building blocks. An important point in this process is that the building blocks can be primed to associate in a tunable manner. One such example are polyelectrolytes, intrinsically multi-responsive macromolecules, bearing a large number of electrostatically charged groups. Their dimensions and physicochemical properties are highly dependent on the pH and ionic strength of their direct environment. This makes polyelectrolytes particularly appealing as stimuli-responsive building blocks for the transient assembly of functional materials.[3–6]

Notably, control over self-assembly in the time domain is a highly promising route in materials science to achieve the design of autonomously behaving systems.[7,8] In this direction, temporal changes in pH have been used to induce swelling and shrinking of polymersomes,[9] (dis)assembly of cargo-loaded micelles with a complex coacervate core[10] and for the regulation of polymerization and gelation.[11] Many of such examples make use of enzymatic systems to obtain temporal control over pH.[11,12] These enzyme-based approaches have some advantages, such as the ability to generate cycles by repeated addition of fuel. Nevertheless, enzymatic systems suffer from several drawbacks, including limited scalability and compatibility with additional components. Clock reactions, molecular reaction networks characterized by an abrupt increase in the product(s) concentration after a well-defined lag time,[13] are promising alternatives for the programming of pH changes.[14–17] Clock reactions have been applied to temporally modify the viscosity of polyelectrolyte solutions,[18] to regulate the (dis)assembly of micelles and polymeric networks[19], to control the rate of polymerization[20] or the formation of nanoparticles[21] and supramolecular assemblies[22,23] in time. In this regard, the formaldehyde-sulfite (F-S) clock reaction is of special interest.[24,25] The mechanism of this well-known acid-to-alkali clock reaction can be summarized by two main equations. First, formaldehyde reacts with sulfite in water, producing hydroxide ions (Equation 1). If bisulfite is also present, and is in excess compared to sulfite, the produced hydroxide ions are quickly scavenged by the bisulfite-sulfite equilibrium reaction (Equation 2), so that there is no pH increase. Only after the full consumption of the bisulfite, this buffering effect vanishes, and the pH increases abruptly, from acidic (around pH 5.5) to alkaline (above pH 10).

$$\mathrm{CH_2O + SO_3^{2-} + H_2O \rightarrow HOCH_2SO_3^- + OH^-} \quad (1)$$

$$\mathrm{HSO_3^- \rightleftharpoons SO_3^{2-} + H^+} \quad (2)$$

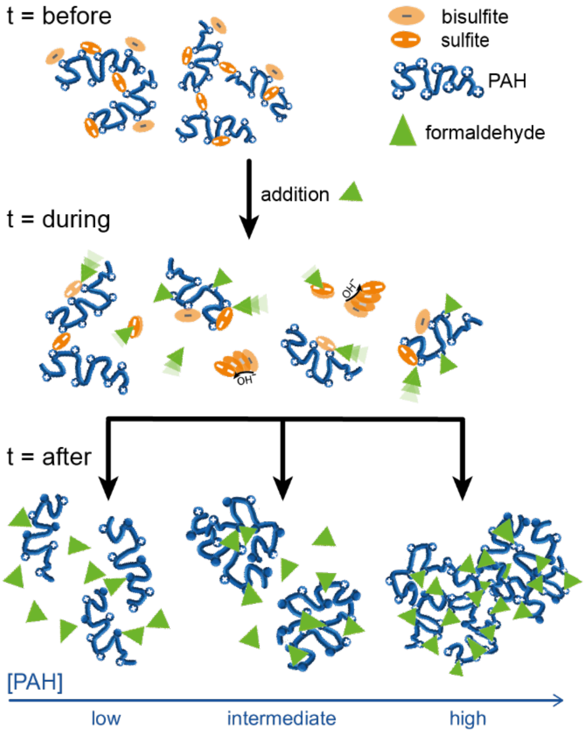
The lag time after which the jump in pH occurs can be tuned by changing the formaldehyde concentration at a given ratio of sulfite to bisulfite in the buffer.[26] Moreover, the gap between the initial (acidic) and final (alkaline) pH is determined by the net amount of hydroxide produced during the reaction, which is directly dependent on the sulfite concentration.

Here, we discuss the use of the F-S clock reaction to control the temporal assembly of poly(allylamine hydrochloride) (PAH), a cationic polyelectrolyte. We show that the generation of coacervate-like droplets, resulting from electrostatic interactions between PAH and sulfite, can act as a transient template for the generation of covalently crosslinked particles. The interplay between these different interactions leads to a variety of end states for a relatively narrow range of polyelectrolyte concentrations. The initial electrostatic crosslinking of the polyelectrolyte chains by the sulfite anions results in turbid solutions, containing micrometer-sized charged complexes. Upon addition of formaldehyde, we first observe the disappearance of the complexes, followed by the formation of polymeric nanoparticles and, for the highest PAH concentration, even of precipitating aggregates.

We propose that the electrostatically crosslinked complexes act as a template, bringing the amine-functional monomers closer, thereby increasing the local density of reactive sites that are covalently crosslinked by their subsequent reaction with formaldehyde. These results show how transient compartmentalization, due to non-covalent interactions, provides a template wherein covalent crosslinks lock-in the preformed structure. In addition, we demonstrate that the formation of nanoparticles or large aggregates can be decided by tuning the initial polymer concentration, as well as pH of the solution.

Results and discussion

The formaldehyde-sulfite (F-S) clock reaction produces a surge in pH after a lag time, which, for a fixed bisulfite-sulfite ratio, can be controlled by changing the formaldehyde concentration. For our experiments, we choose a set of initial conditions yielding a pH change from around 5.7 to almost 11 after a delay of 50 seconds. By introducing poly(allylamine hydrochloride) (PAH) to the F-S clock reaction, different interactions between the components play a role at various time domains. As the components react with each other and are consumed over the course of the clock reaction, we anticipate a pronounced temporal evolution of different solution structures (**Scheme 1**). Upon mixing the polymer with the sulfite-bisulfite buffer, complexation occurs between the positively charged PAH and the negatively charged ions, via a Coulombic interaction (t = before). The subsequent addition of formaldehyde initiates the F-S clock (t = during). Sulfite and bisulfite are then consumed by reactions (1) and (2), which disrupts the electrostatic interactions. At the same time, crosslinking reactions take place between the polyelectrolyte and the formaldehyde, which is always present in excess. Covalent chemical modifications can subsequently alter the solubility of PAH, induce aggregation or even precipitation of the polymer (t = after).



Scheme 1. Graphic representation of the temporal evolution of structures during the F-S clock reaction. Initially, PAH-sulfite electrostatic complexes form initially (t = before).The addition of formaldehyde starts a series of reactions (t = during), that lead to different structures depending on the PAH concentration (t = after).

Electrostatic crosslinking of PAH

We study the influence of PAH concentration ($c_{\mathrm{NH_2}}$, from 1 to 10×10^{-3} M) on the initial electrostatic interactions with fixed concentrations of F-S buffer components (5×10^{-3} and 5×10^{-2} M of sodium sulfite and sodium bisulfite, respectively). Upon mixing these components, turbidity appears for all PAH concentrations (**Table S**1 shows the associated transmission values), which is attributed to complex formation between the protonated amines of PAH and the divalent sulfite anions. This electrostatic crosslinking of the polyelectrolyte leads to the formation of coacervate-like complexes. The bisulfite ions can also complex with PAH, but are unable to form electrostatic crosslinks like the sulfite. Indeed, when the polymer is mixed only with sulfite we obtain a highly turbid solution, while mixing it only with bisulfite results in a clear solution (Table S2). To visualize the complexes at various concentrations, we use a mix of unmodified PAH and Rhodamine B-labelled PAH (1:1 ratio, details in SI) and the sulfite-bisulfite buffer, and we image the samples using confocal laser scanning microscopy (CLSM, **Figure 1**).

Since the turbidity increases with higher concentration of PAH, the complexes should increase, either in size or in number. We find from CLSM analysis that the complexes are around 1 µm in diameter (Figure 1), regardless of the PAH concentration. The complexes must therefore increase in number with higher concentrations of polyelectrolyte. Moreover, a large amount of adsorption to the glass cover slide is observed during the imaging time, which increases with PAH concentration (**Figure S1**). We then measure the electrophoretic mobility of the PAH-sulfite complexes, from which we compute the zeta potential. Increasing the polymer concentration from 1 to 10×10^{-3} M results in positive zeta potential values going from 11.5 up to 17.9 mV (Figure 1b), suggesting the presence of excess polycation in the complexes. This result is reasonable since the concentrations of sulfite and bisulfite remain constant for increasing PAH concentrations. The net positive charge of the PAH-sulfite complexes contributes to their adsorption to the cover slide, as glass is known to have a slightly negative surface charge.[27]

Evolution of PAH-sulfite complexes during the clock reaction

Having established the size and zeta potential of the initial PAH-sulfite complexes, we add formaldehyde to induce the clock reaction. Upon its addition, we start measuring pH (**Figure 2**) as well as transmission (**Figure 3**) in real time. As previously mentioned, the lag time of the F-S clock reaction, after which the pH suddenly increases, directly depends on the initial formaldehyde concentration.[26] In particular, with a constant sulfite-bisulfite buffer concentration, changing the formaldehyde concentration between 6×10^{-2} M and 1.4×10^{-1} M leads to a decrease in lag time from 140 to 30 seconds (**Figure S2**). For all the experiments presented here, we choose a constant formaldehyde concentration of 10^{-1} M, which results in a lag time of 50 ± 8 s and a pH gap (ΔpH), between the initial and final pH, of 5.2 ± 0.2.

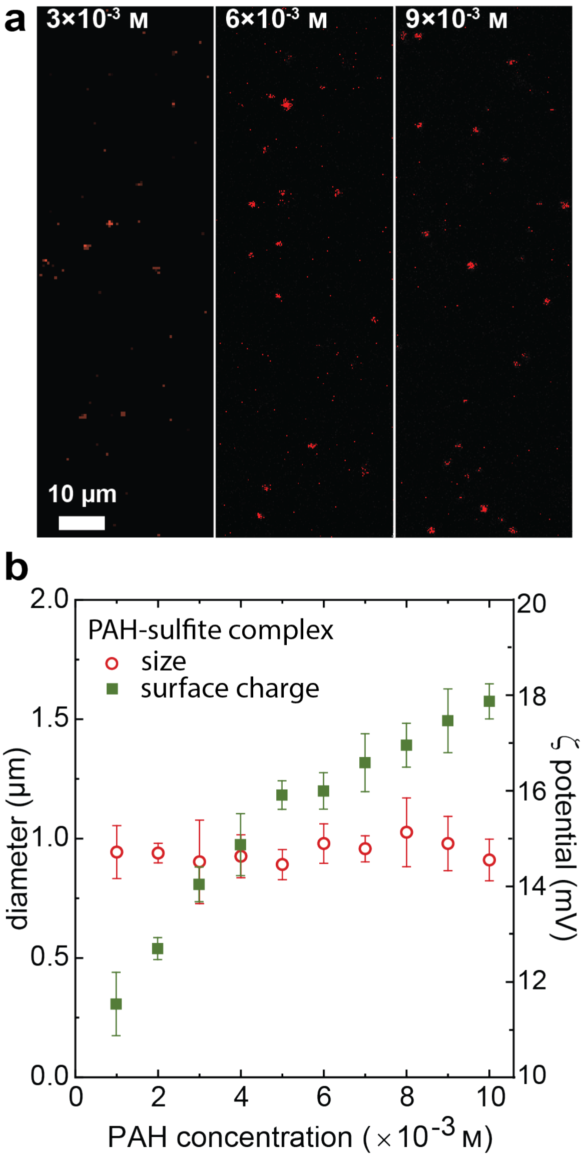


Figure 1. Characterization of PAH-sulfite complexes obtained in sulfite-bisulfite buffer (5×10^{-3} M sodium sulfite, 5×10^{-2} M sodium bisulfite) with increasing polymer concentration (1 to 10×10^{-3} M). a) Confocal micrographs show micron-sized complexes. b) Diameter (red circles) of the complexes is constant, while zeta potential (green squares) increases for increasing PAH concentration.

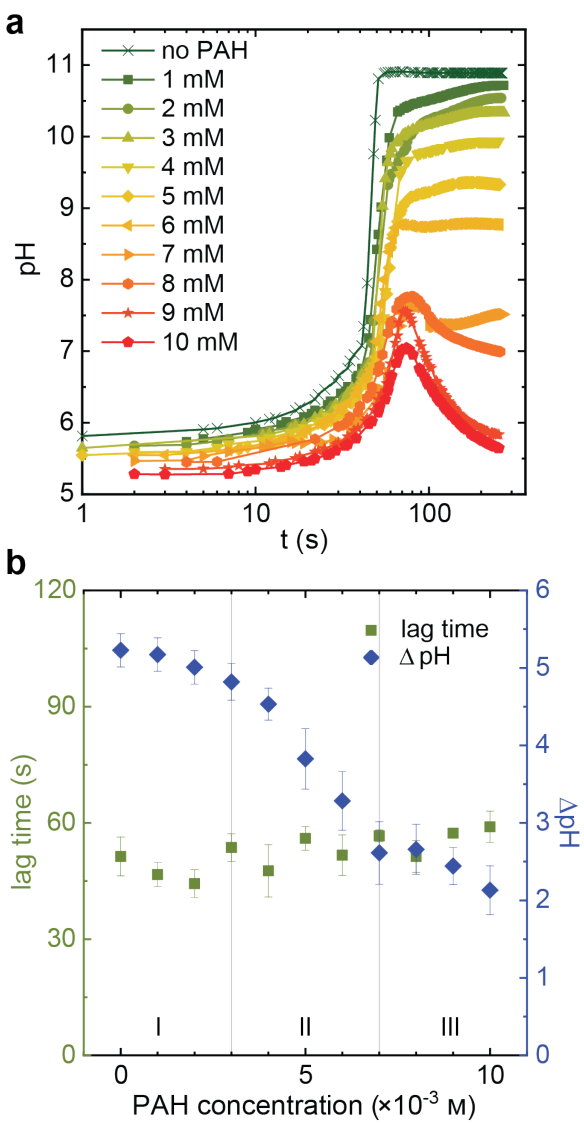


Figure 2. Influence of PAH concentration, from 1 to 10×10^{-3} M, on the F-S clock reaction (5×10^{-3} M sodium sulfite, 5×10^{-2} M sodium bisulfite and 10^{-1} M formaldehyde). a) Temporal pH evolution with increasing polymer concentration. b) Average lag time of the clock reaction (green squares) and the gap between the initial and final pH (ΔpH, blue diamonds). Error bars indicate standard deviation of three replicate samples.

The addition of PAH at low concentration ($c_{\mathrm{NH_2}} \leq 3\times10^{-3}$ M, regime I) to the F-S clock does not significantly impact the pH evolution of the clock reaction (Figures 2a and **S3**). A slight decrease in both the initial and final pH of the solution is observed, while the lag time remains fairly constant (Figure 2b). As (bi)sulfite ions are consumed, the electrostatic crosslinks disappear and the complexes dissociate, leaving a clear solution (transmission of nearly 100 %, **Figure 3**a). Evidence of this transition at the microscale is given in Supporting video 1, showing the course of the F-S clock reaction with 3×10^{-3} M of PAH acquired *in situ* by CLSM.

By increasing the polyelectrolyte concentration (4×10^{-3} M $\leq c_{\mathrm{NH_2}} \leq 6\times10^{-3}$ M, regime II), there is a further decrease of the starting pH, along with an even more pronounced decrease of the final pH. As a result, ΔpH is smaller (Figure 2b). In this regime, the turbidity decreases as the pH increases as a result of the F-S clock reaction, marked by a steep rise of transmission to about 75 %. Nevertheless, the final solution is never completely transparent (Figure 3b).

For the highest PAH concentrations ($c_{\mathrm{NH_2}} \geq 7\times10^{-3}$ M, regime III), the pH only increases to about 7.5, and then decreases over time. The threshold concentration of PAH above which a subsequent decrease in pH is observed, lies at 7×10^{-3} M. In regime III, the lag time remains still similar to that in regimes I and II, while the pH gap decreases from 5.2 ± 0.2 (at $c_{\mathrm{NH_2}} = 0$) to below 3 (Figure 2b). In this regime, the measured transmission shows a similar temporal profile as the pH, first going up after the lag time, leaving an almost clear solution, and then decreasing due to the formation of white flakes in suspension (Figure 3c). These small precipitates keep growing after the completion of the clock reaction and ultimately sink to the bottom, forming a sediment.

With increasing polymer concentration, the rate of pH increase, d(pH)/dt, is somewhat reduced (**Figure S4**). This observation may be attributed to the combined effects of a lower starting pH and the deprotonation of the polymer's primary amine groups, both hampering the increase in pH caused by the clock reaction.

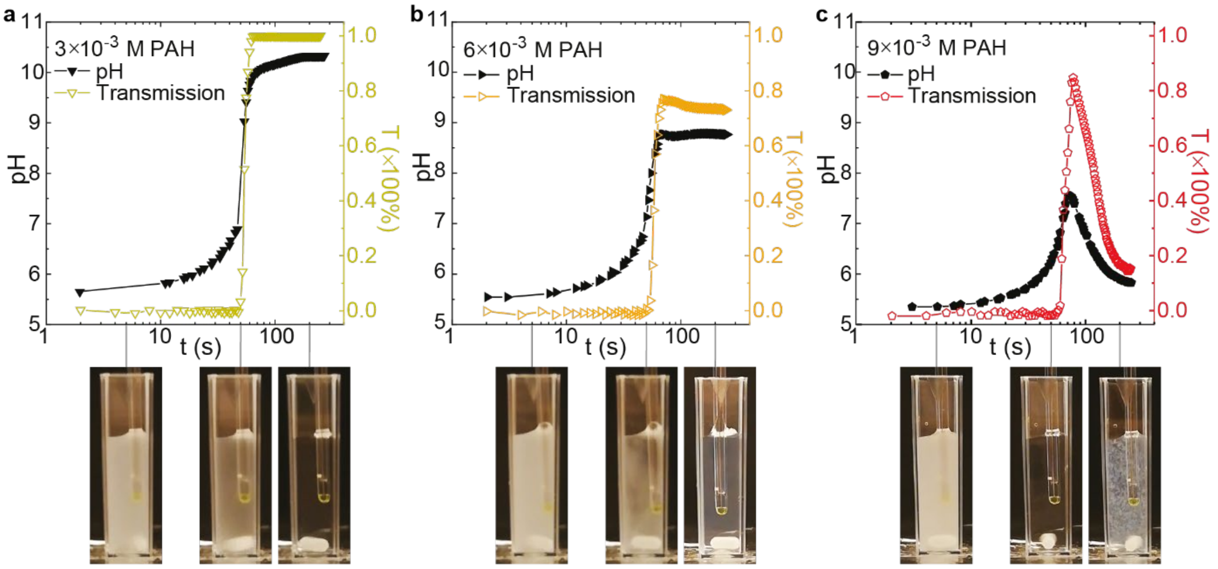
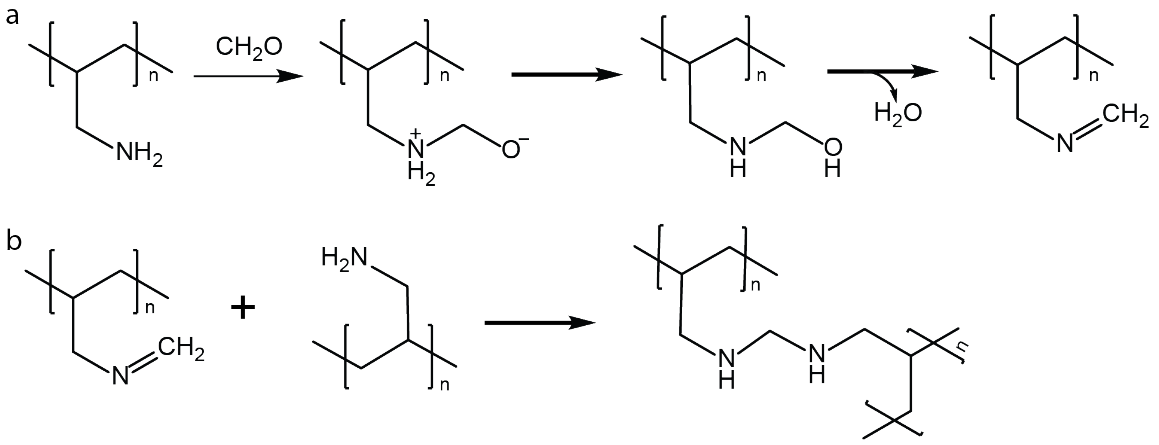


Figure 3. Overlay of the temporal evolution of pH and transmission, measured at the same time for representative experiments with: a) low PAH concentration (regime I, 3×10^{-3} M), b) intermediate PAH concentration (regime II, 6×10^{-3} M) and c) high PAH concentration (regime III, 9×10^{-3} M). PAH is added to the F-S clock reaction (5×10^{-3} M sodium sulfite, 5×10^{-2} M sodium bisulfite and 10^{-1} M formaldehyde). Below each graph, photographs taken at the start, during and after the reaction show the evolution of turbidity for each concentration regimes.

Covalent crosslinking mechanism

The ΔpH decrease observed for regimes I and II could be reasonably attributed to a buffering effect exerted by PAH. The apparent pK_a of PAH has been reported to be around 8.8.[28] Release of protons from the polyelectrolyte compensates the hydroxide ions generated by the F-S clock, reducing the final pH. However, this effect alone cannot explain the drop in pH which is observed at higher PAH concentrations, *i.e.* regime III. We hypothesize that this phenomenon might originate from a direct reaction between formaldehyde and PAH. This hypothesis is further supported by the concomitant solubility decrease observed for PAH in regime III, which suggests a chemical modification of the polymer.

Under certain conditions, the amine groups of PAH can perform a nucleophilic attack on the carbonyl group of formaldehyde. An ammoniomethanolate intermediate is generated, which is rapidly converted to a hemiaminal by proton transfer. Subsequent dehydration produces an imine, but its formation is dependent on the solution pH (**Scheme 2**a). If it is too acidic, nearly all amines will be protonated and therefore not enough nucleophilic. On the contrary, if the solution is too basic, dehydration of the hemiaminal is unfavorable.[29] After dehydration, the resulting imine can react with a nearby primary amine, producing a methylene bridge (**Scheme 2**b). Either intrachain or interchain crosslinks can form, depending on the polymer concentration. Given that the highest polymer concentration we use is lower than 1 mg·mL^{-1}, that is, below the polyelectrolyte overlap concentration,[30] interchain crosslinking is unlikely. However, the complexation by sulfite (and bisulfite) significantly affects the interaction between polymer chains. A templating effect can result in multiple chains being crosslinked together by formaldehyde, as they are brought in closer proximity by the electrostatic complex formation. Evidence for such transient supramolecular templating effect is given by an additional experiment (**Figure S5**). Namely, in the absence of sulfite, the reaction between formaldehyde and PAH still occurs, as is apparent from a similar pH decrease over time, but does not lead to the formation of an insoluble precipitate.



Scheme 2. Proposed reaction pathway between an amine-functional polyelectrolyte (PAH) and formaldehyde. a) Imine formation occurs in three steps: nucleophilic attack, proton transfer and dehydration. b) Reaction between imine-modified PAH and an amine group, also from PAH, resulting in the formation of methylene bridges.

Analysis of the polymers obtained after the clock reaction

To differentiate between the final states of the clock reaction in the three respective concentration regimes, we start by characterizing the samples containing $c_{\mathrm{NH_2}} = 3\times10^{-3}$ M and 6×10^{-3} M by dynamic light scattering (DLS) at 90° scattering angle. The diffusion coefficients, obtained from the exponential decay of the normalized intensity autocorrelation function, are used to calculate hydrodynamic radii (R_{H}) of the particles in the samples. We find two different particle size distributions for the respective samples (**Figure 4**). In the 3×10^{-3} M sample, a major population with a mean $R_{\mathrm{H}} = 33.1$ nm and a minor population with $R_{\mathrm{H}} = 4.4$ nm are observed. Doubling the polymer concentration (6×10^{-3} M) results in a single peak with a mean size $R_{\mathrm{H}} = 113.7$ nm. By repeating the DLS measurement on the same samples after two weeks, we find that the size distributions have not changed, indicating good stability of the particles once the reaction is finished (**Figure S6**). The increase in particle size with increasing PAH concentration elucidates the difference in turbidity between the samples after the clock reaction. It is likely that the degree of crosslinking goes up with increasing polymer concentration, resulting in the formation of larger polymer particles. By choosing the right concentration, we can thus tune the size of the nanoparticles formed as a result of the clock reaction system. In the highest concentration regime (III), the degree of crosslinking is possibly so high, that the particles grow, aggregate and finally sediment. Because these samples cannot be characterized with DLS, we turn to spectroscopic analysis to get insight about the nature of bonds that are formed during the reaction.

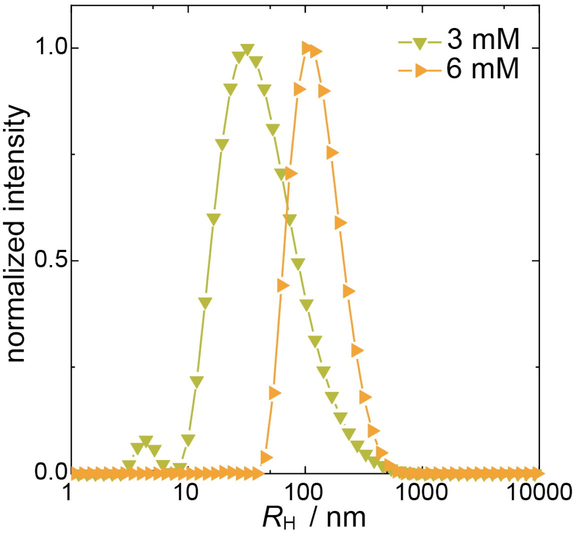


Figure 4. Normalized intensity-weighted particle size distributions showing the hydrodynamic radii of samples containing 3×10^{-3} M and 6×10^{-3} M PAH after the F-S clock reaction, centered at 33.1 and 113.7 nm, respectively. A smaller second distribution, centered at 4.4 nm, is observed in the 3×10^{-3} M sample.

To elucidate the mechanism of PAH crosslinking through its reaction with formaldehyde, we first purify the precipitate obtained in regime III conditions, separating it from other reaction products and residual formaldehyde by centrifugation and several washing steps with water. After lyophilization, we obtain a white product which is insoluble in water, as well as in alcohols and other solvents such as acetone, dimethyl formamide, tetrahydrofuran and acetonitrile. This insolubility in a wide range of solvents is indicative of a crosslinked structure.

We then acquire the Fourier transform infrared (FTIR) spectra of both the purified precipitate and of as-supplied PAH, (**Figure 5**a). Comparing the two spectra, we find several distinct differences. The broad band centered around 2900 cm^{-1}, which originates from the merging of the NH_3^+ and CH_2 stretching signals, is significantly reduced in the precipitate compared to the original polymer. This drastic reduction is attributed to the conversion of primary amines into secondary or tertiary amines. More evidence for secondary amine formation, and thus crosslinking, comes from the shift to higher wavenumbers of the peaks in the 1450 to 1650 cm^{-1} range (highlighted in orange in **Figure 5**). Moreover, a striking change between the polymer before and after the clock reaction is the appearance of two strong bands at 1150 and 1022 cm^{-1}, which we associate with the C–N stretching of hexahydrotriazine groups (highlighted in blue in Figure 4).[31] The intensity of these bands indicate that a significant amount of these crosslink moieties is present in the precipitate (**Figure 5**b).[32] These results support our hypothesis that the products obtained with the highest concentrations of PAH are crosslinked.

To check whether the reaction between formaldehyde and PAH can lead to formation of nanoparticles in the absence of (bi)sulfite, we perform a manual titration to mimic a temporal evolution of pH (**Figure S7**). We titrate small amounts of 1 M sodium hydroxide solution into samples containing 3, 6 and 9×10^{-3} M PAH, at constant formaldehyde concentration (10^{-1} M). Upon a pH increase, the first signs of precipitate formation appear, for all samples. These results show that hydroxide ions are necessary for the crosslinking reaction to take place, but manual addition is not controlled enough to result in well-defined nanoparticles. In the clock reaction conditions, we can tune the final product simply by changing PAH concentration. In contrast, the manual addition of sodium hydroxide solution results in the formation of large aggregates for the whole PAH concentration range.

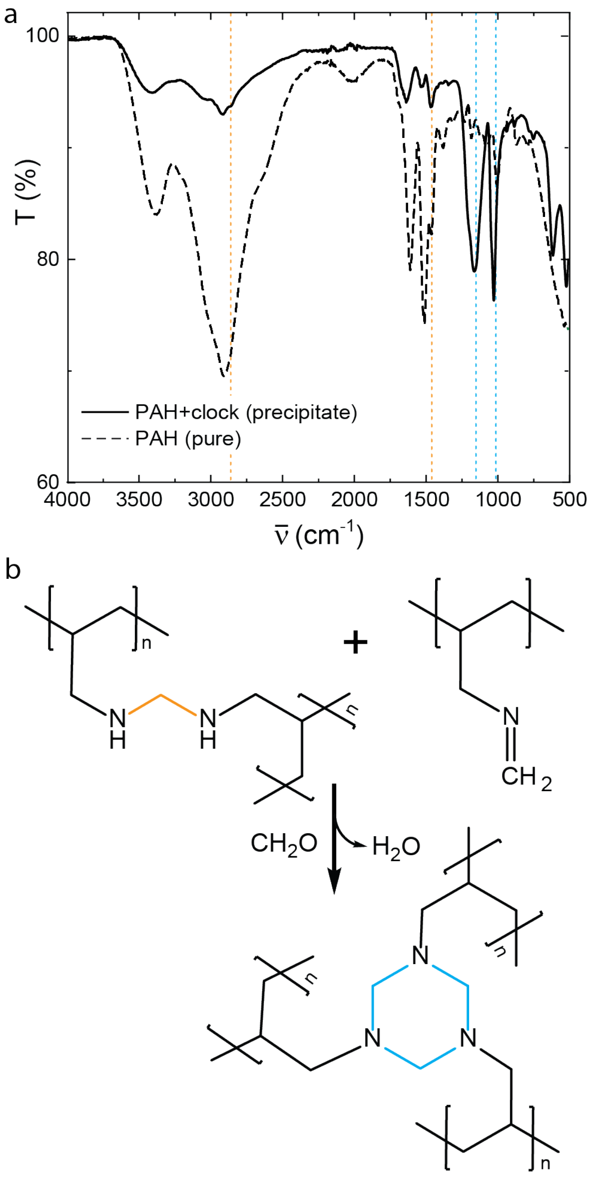


Figure 5. Analysis of the precipitate formed by mixing PAH (concentration regime III) with the F-S clock reaction. a) FTIR spectra of PAH as supplied (dark grey dashes) and of the washed and freeze-dried precipitate (black solid line). Distinct differences between the PAH before and after the clock indicate chemical modification in the form of crosslinking by reaction with formaldehyde. b) Pathway for the formation of hexahydrotriazine crosslinks. Evidence for the formation of C-N bonds is indicated in (a) with vertical lines in matching colors.

Tuning the temporal evolution by initial pH control

As the PAH concentration is increased towards 10×10^{-3} M, the initial pH of the sulfite-bisulfite buffer decreases from 5.7 to 5.2, as shown in Figure 2a. This decrease in pH causes a shift in the sulfite-bisulfite equilibrium in favor of the bisulfite, slightly delaying the pH jump. To study the influence of such slight variations in pH on the evolution of the system, we conduct the following experiment. We manually adjust the pH of the sulfite-bisulfite solution containing 9×10^{-3} M PAH by adding small amounts of a 1 M aqueous sodium hydroxide solution. Next, we trigger the clock reaction by the addition of formaldehyde. We observe that the lag time shortens as increasing amounts of base are added (**Figure 6a-b**). Furthermore, the pH decrease following the clock reaction (see **Figure 2**) becomes less pronounced, and even absent at 4×10^{-3} M NaOH. In addition, flaky precipitates are no longer formed when more than 1×10^{-3} M NaOH is added. Instead, a turbid suspension remains after the clock reaction, similar to what is observed in regime II (without addition of base). Further increasing the concentration of added base leads to reduction in turbidity. This observation supports the proposed crosslinking mechanism, in which imine formation can occur only in presence of a certain amount of protons. The additional NaOH promotes a faster increase in pH during the clock reaction and therefore, the crosslinking reaction becomes less favorable, averting precipitation. The expected decrease in aggregate and particle size upon addition of more NaOH is confirmed by DLS analysis of the obtained samples (**Figure S8**).

As mentioned before, the equilibrium between sulfite and bisulfite is highly pH-dependent. Adjusting the initial pH to be constant for all concentrations of PAH results in a fixed ratio between sulfite and bisulfite, so that the clock behavior should remain unchanged. We therefore adjust the pH also for samples over the full range of PAH concentration, to check whether the temporal progressions of pH overlap. In this case, the initial pH is changed to 5.7, which is the initial condition for the (bi)sulfite buffer without polyelectrolyte (**Figure S9**). Instead of the curves overlapping with the negative control (no PAH present), we find again a decrease of the lag time and the absence of precipitation. The observed deviation in behavior suggests that the polymer perturbs the otherwise predictable operation of the clock. Thus, we can drive the formation of crosslinking products from nanoparticles to large aggregates, not only by changing the PAH concentration, but also by the addition of small amounts of base to the clock reaction components.

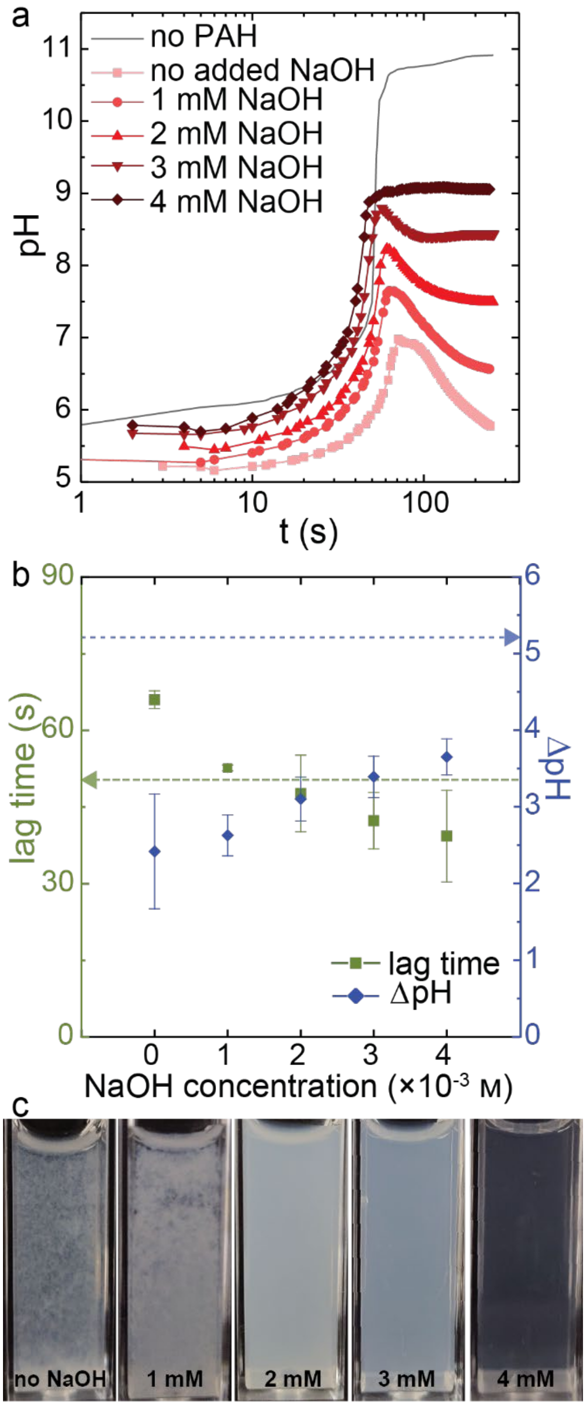


Figure 6. Effects of NaOH addition before the start of the clock reaction. a) pH *vs* time for stepwise addition of NaOH at fixed PAH concentration ($c_{\mathrm{NH_2}}= 9\times10^{-3}$ M). b) Lag time and pH gap at $c_{\mathrm{NH_2}}= 9\times10^{-3}$ M, averaged over three measurements with standard deviation indicated by error bars. The dashed arrows indicate the average lag time and pH gap in absence of PAH. c) Images taken after the clock reaction ($c_{\mathrm{NH_2}}= 9\times10^{-3}$ M) showing precipitation and turbidity, which decrease with increasing NaOH concentration.

Conclusion

We have shown how a variety of structures can be obtained from a system of relatively simple components, thanks to temporally-controlled changes in their interactions. Combining a sulfite-bisulfite buffer with poly(allylamine hydrochloride), we generate reversible electrostatic complexes about one micrometer in size. Upon addition of formaldehyde, we start a clock reaction, producing an abrupt pH increase as (bi)sulfite is consumed, after a tunable lag time. To our delight, the compartmentalization of polyelectrolyte in the transient complexes enhances a crosslinking reaction between formaldehyde and the polymer which generates permanent hydrocolloids. Simply by changing the polymer concentration, we can obtain final structures of different sizes. At the lowest concentrations, a low degree of crosslinking produces small nanoparticles and the solution becomes clear. In the intermediate concentration range, crosslinked polymer chains form nanoparticles, around 200 nm in diameter, which scatter more light and produce a turbid suspension. Finally, using the highest polymer concentration gives rise to highly crosslinked polymer aggregates that precipitate from the solution. Aside from tuning the polymer concentration, by adjusting the initial pH of the solution at a constant polymer concentration, we can control the final state, ranging from a clear solution to precipitation, giving us a second handle to control the size of the crosslinked polymer particles. Our results give useful insight on the formation of nanostructures in dynamic systems, especially about how the balance between different types of interactions in time can be used to generate a variety of new materials.

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Keywords

Clock reaction • electrostatic interactions • polyelectrolytes • self-assembly • temporal control

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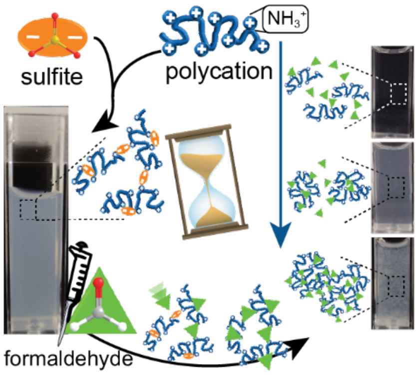
Table of contents entry for “Two-stage polyelectrolyte assembly orchestrated by a clock reaction”.



A cationic polyelectrolyte is assembled with temporal control by means of the formaldehyde-sulfite clock reaction. Electrostatic interactions template microscopic complexes, which are then crosslinked covalently to lock-in a preformed structure. The final particle size can be controlled simply by tuning either the concentrations of components, or the initial pH.