



Chemically Fueled Autonomous Sol→Gel→Sol→Gel→Sol Transitions

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Abstract: Complex non-equilibrium phase behaviors are a hallmark of natural self-assembling systems. Here we show how intricate phase transitions can be achieved through a chemically fueled reaction cycle to yield autonomous sol→gel→sol→gel→sol transitions. A relay of chemical transformations based on thiazinane metathesis leads to two consecutive transient gelations in a closed system. Within seconds of fuel addition to deactivated thiazinane monomers, an imine-based hydrogel forms that consists of fibrillar microspheres. This gel quickly loses its mechanical strength and forms a solution, from which a second aldehyde-based gel nucleates and remains stable for over one day. Overall, our reaction cycle gives rise to two consecutive re-entrant phase transitions without any experimental intervention.

Chemically fueled reaction cycles can activate and deactivate building blocks, leading to the assembly and disassembly of complex soft materials, respectively. Transient assembly of colloids,^[1] nanoparticles,^[2] coacervates,^[3] cages/macrocycles^[4] and gels^[5] has been achieved using cleverly designed reaction cycles. The life-time of such transient structures depends on the balance of activation and deactivation rates versus the total amount of activating fuel that is available in the system.^[6] Often, spontaneous deactivation by hydrolysis is used, but other deactivating fuels have been explored.^[7] Self-dividing coacervates,^[8] supramolecular oscillations,^[9] transient catalysis,^[10] an autonomous information ratchet and molecular pump,^[11] fueled drug delivery^[12] are some of the bio-inspired functions that have been recently developed using fueled reaction cycles.

In supramolecular systems chemistry, one of the goals has been to control autonomous sol→gel→sol, gel→sol→gel, or gel→gel→gel transitions using reaction cycles.^[5–7] However, more complex sequences of chemically fueled phase transitions have yet to be reported.

Here we present a fully autonomous sol→gel→sol→gel→sol transition that leverages the multitude of chemical reactions possible with an aldehyde moiety (Figure 1a). Specifically, a single small molecule (SachThiaz) can transition through different chemical states in one pot, namely: thiazinane (starting solution), imine (**gel1**), or aldehyde (**gel2**). The first **gel1** (SachImine) is composed of fibrous spherical microgels and forms within a minute after the addition of the chemical fuels. Within 10 minutes the SachImine **gel1** disassembles and is converted to aldehyde solution **sol2** that forms a second hydrogel **gel2** consisting of long rigid fibers. After 72 hours the SachCHO gel reverts back to a solution state **sol3** to partially recover the non-assembling thiazinane (SachThiaz), which eventually degrades to chemical waste PdT (phenyl dithiazinane). We will first break down the individual chemical steps, before combining everything to achieve the sol→gel→sol→gel→sol transitions (see also Supporting Information Section 3 for additional exploration of the parameter space).

SachThiaz is the deactivated (i.e., non-assembling) derivative of the SachCHO hydrogelator that was previously described.^[13] SachThiaz forms by condensation of D,L-homocysteine with the aldehyde group of SachCHO forming a 1,3-thiazinane and is water soluble due to its free carboxylic acid. When treated with formaldehyde (HCHO) it can undergo thiazinane metathesis to form 1,3-thiazinane-4-carboxylic acid^[14] (waste) and release the SachCHO hydrogelator. However, irrespective of the concentrations, when SachThiaz is treated with HCHO the released SachCHO never yields a hydrogel but only suspended aggregates over several hours. This could be due to incomplete and slow formation of SachCHO. The thiazinane→aldehyde conversion can, however, be accelerated using anhydrous piperazine (PIP) as a base, leading to 75 % conversion and full gelation within 3 minutes (SachThiaz 48.2 mM, PIP 124.8 mM, HCHO 398 mM; see Supporting Information Figure S1). Thus, the combination of PIP + HCHO as chemical fuels can carry out SachThiaz to SachCHO conversion for rapid sol→gel transformation.

For deactivation and disassembly of the SachCHO hydrogel we use homocysteine thiolactone.HCl (HCT) as deactivating fuel. HCT can react with aldehydes to form imines and then through a concerted mechanism convert into the thiazinane.^[14] Previous studies report much slower

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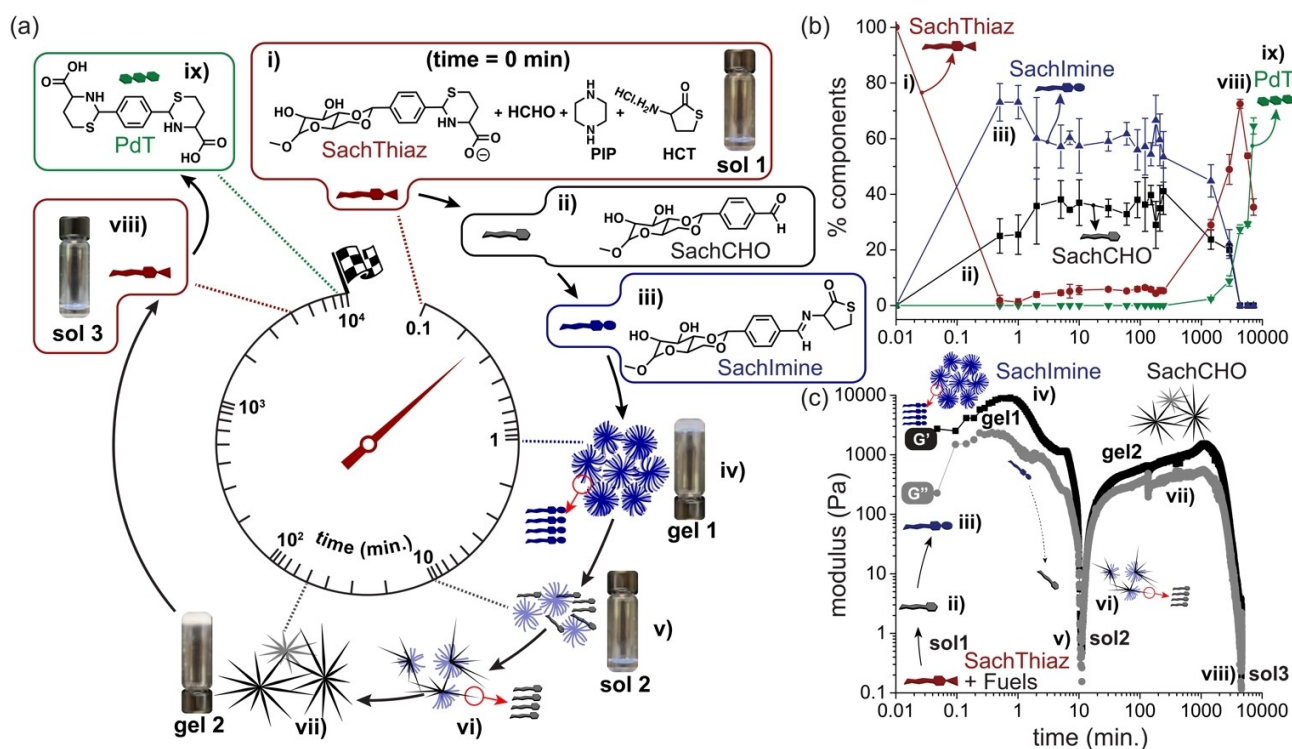


Figure 1. a) Scheme of chemical species and phases during an autonomous sol→gel→sol→gel→sol transition. At $t=0$, formaldehyde (HCHO), anhydrous piperazine (PIP) and homocysteine thiolactone.HCl (HCT) are added to a SachThiaz solution, starting sequential chemical transformations that result in two different transient hydrogels (sachImine-based **gel1**, and **gel2** with characteristic SachCHO fibers). Indices i–ix) correspond to all Figure panels and are described in the main text. b) Composition of SachThiaz, sachImine, SachCHO and phenyl dithiazinane (PdT) calculated from ^1H NMR experiments after addition of fuels to SachThiaz solution (at $t=0$ min: SachThiaz = 48.2 mM PIP = 124.8 mM, HCT = 1.75 M, HCHO = 398 mM). Error bars show standard deviations over triplicate experiments. c) Rheology: three solutions states **sol1–3** and two gel states **gel1–2** can be distinguished (see additional experiments in Supporting Information Figure S2).

kinetics for reaction of HCT and aldehydes (acetaldehyde and formaldehyde),^[14] compared to optimized conditions for SachThiaz→SachCHO conversion and hydrogelation. This should permit HCHO to first react with SachThiaz and form SachCHO assemblies without getting irreversibly consumed by HCT forming 1,3-thiazinane-4-carboxylic acid. However, when we introduce HCT with PIP + HCHO at $t=0$ min to the previously optimized sol→gel transformation (SachThiaz 48.2 mM, PIP 124.8 mM, HCHO 398 mM; see Supporting Information Figure S1), we observe very interesting phase changes in the system. Within seconds of adding the fuels to the clear SachThiaz solution, it turns turbid and forms **gel1** within one minute (Figure 1a, i–iv). This cloudy gel starts disappearing and yielding clear **sol2** at $t \approx 10$ min (Figure 1a, v). Several minutes later at $t=11$ –13 min we start seeing the formation of white assemblies that quickly grow to form a white **gel2** that is fully formed at ≈ 30 min (Figure 1a, vi–vii). Over the next two days **gel2** turns slightly pale and starts breaking down. After three days ($t \approx 4000$ min, Figure 1a, viii), the assemblies disappear completely and finally a yellowish solution remains ($t \approx 7000$ min, Figure 1a, ix). Overall, the system undergoes two back-to-back re-entrant phase transitions that together yield an autonomous sol→gel→sol→gel→sol transition (Supporting Information Movie 1).

The chemical transformations responsible for these dynamic changes were followed by NMR (Nuclear Magnetic Resonance) and LCMS (Liquid Chromatography Mass Spectrometry) techniques. We observe that SachThiaz is consumed almost quantitatively ($\approx 97\%$) within seconds of fuels addition, and is converted into SachCHO which in turn keeps reacting with HCT to form SachImine (Figure 1b, and characteristic NMR peaks in Supporting Information Figure S3 and S4). SachImine-based **gel1** is formed when the composition of the total system is $\approx 75\%$ SachImine and $\approx 22\%$ SachCHO (Figure 1b). Over the next 7–10 minutes, the SachImine drops to $\approx 55\%$ and SachCHO increases to $\approx 40\%$ in overall composition (Figure 1b). This change correlates with the disappearance of **gel1** which turns into **sol2** followed by the formation of the second weaker hydrogel **gel2** which that is characteristic for SachCHO^[13] (Figure 1a,b and Supporting Information Movie 1). Additional NMR and microscopy experiments at lower HCT concentration confirmed that SachImine is indeed responsible for **gel1** and SachCHO for **gel2** (Supporting Information Figure S3). After 24 hours ($t=1440$ min) we observe reformation of SachThiaz in the system (from ≈ 5 to 27%), while both SachCHO (42% to 27%) and SachImine (55% to 45%) concentrations decrease. After 48 hours ($t=2880$ min), SachImine further reduces to 22% forming more SachThiaz (50%) while the SachCHO concentration re-

mains constant. After 3 days ($t=4320$ min), we observe 75 % SachThiaz while both SachCHO and SachImine have completely disappeared (Figure 1b). By this point the SachCHO gel disappears in the vial forming **sol3** (Figure 1a). Interestingly, we see presence of a dithiazinane containing phenyl ring (phenyl dithiazine, PdT in Figure 1a,b) from day 1 onwards which gradually increases from 5 % on day 1 to 25 % on day 3 and 65 % by day 5 ($t=7200$ min, see also Supporting Information Figure S4, S5). The pH in the system drops to 2 within a day (cf. Supporting Information Figure S6), which can cause acetal cleavage^[13] to release the saccharide moiety of SachThiaz and allow another 1,3-thiazinane ring to form in the para-position giving PdT. Upon further addition of activating fuels (HCHO, PIP, and HCT) the sol→gel→sol→gel→sol transition could not be repeated, likely due to the low pH combined with a significant amount of waste products that are present at that time.

The sol→gel→sol→gel→sol transition was also followed by rheology to observe the mechanical properties of the system (Figure 1c and Supporting Information Figure S2). SachImine forms a strong hydrogel **gel1** within a minute of fuel addition and reaches a G'_{\max} of around 5500 kPa. Both G' and G'' start to decrease quickly over the next 7–10 minutes to give **sol2** which turns visibly into a solution (see Supporting Information Movie 2, $t=4:30$). The cross-over of G' and G'' happens at ≈ 1 Pa close to the noise level of

the rheometer and therefore cannot be determined reliably. Shortly after, SachCHO **gel2** forms with a G'_{\max} of ≈ 500 Pa. This gel is stable for 24 hours after which it starts losing its mechanical properties and dissociates over the next 2 days.

The different sol→gel→sol→gel→sol transitions can be clearly seen using confocal microscopy (Figure 2 and Figure 3, Supporting Information Movie 3). SachImine **gel1** is composed of fibrillar microspheres with diameters in the range of 10 to 50 μm that start to appear within seconds of fuel addition (Figure 2b,c and Figure 3a). The microspheres consist of smaller SachImine nanofibers with a width of 50–150 nm as seen by transmission electron microscopy (see inset ii in Figure 3a). Interestingly, when these “hairy” spheres start to disappear we see that the rigid fibers of SachCHO exclusively start forming on top of these disappearing structures through heterogeneous nucleation (Figure 3a–f and Supporting Information Movie 4). It is likely that conversion of SachImine in the microspheres creates a much higher local concentration of SachCHO in their vicinity than elsewhere. Thus, as soon as the critical concentration for SachCHO assembly is around the disassembling microgels, the monomers start to heterogeneously nucleate from their points of release where the concentration would be the highest. Eventually, the microspheres disappear and we observe long fibers typical of the SachCHO hydrogel (and confirmed by NMR & microscopy in Supporting Information Figure S3).^[5a,13] These fibers

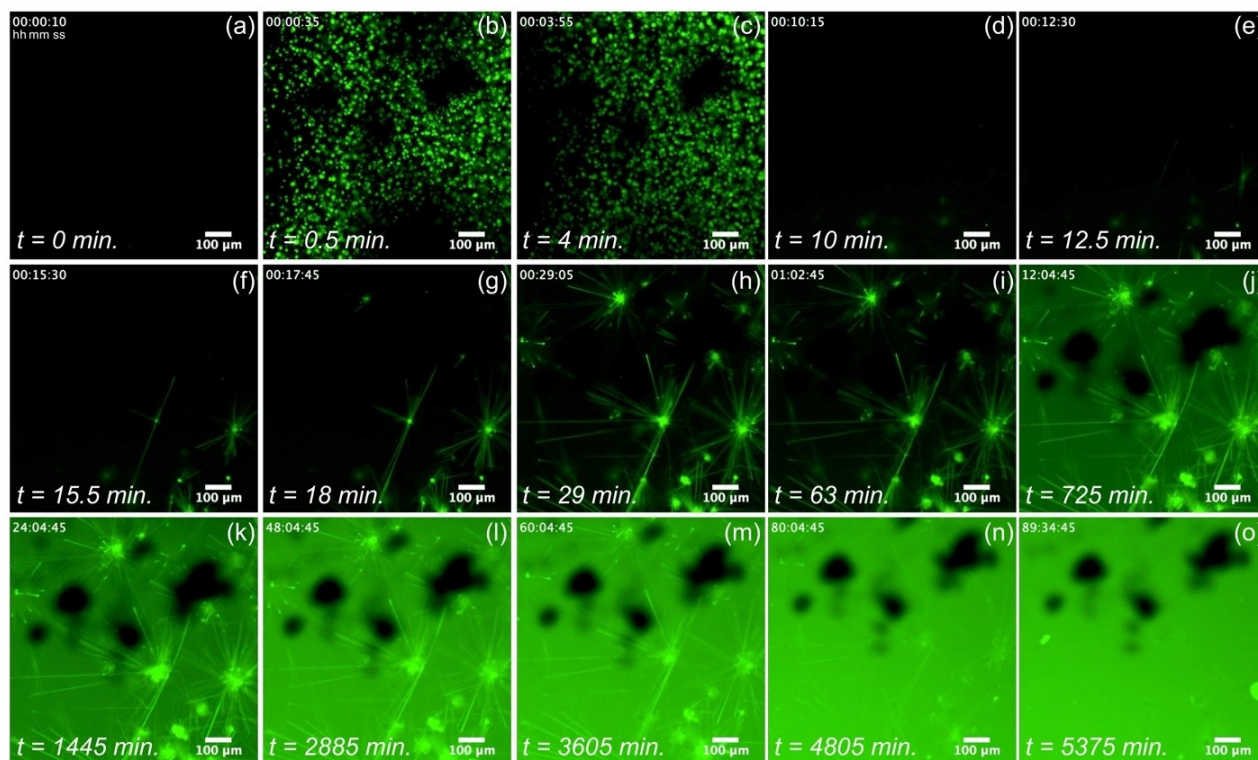


Figure 2. Confocal microscopy images showing the assembly and disassembly of SachImine microsphere **gel1** (a–e) followed by the formation and disappearance of SachCHO fibers of **gel2** (f–o) over time after the addition of fuels to the SachThiaz solution. Thioflavin-T 10 μM is used as a fluorescence marker. Scale bar is 100 μm . The brightness of the images has been increased after 7 min to better see the fibers (note: this results in a high background signal of thioflavin-T for panels k–o). See Supporting Information Movie 3.

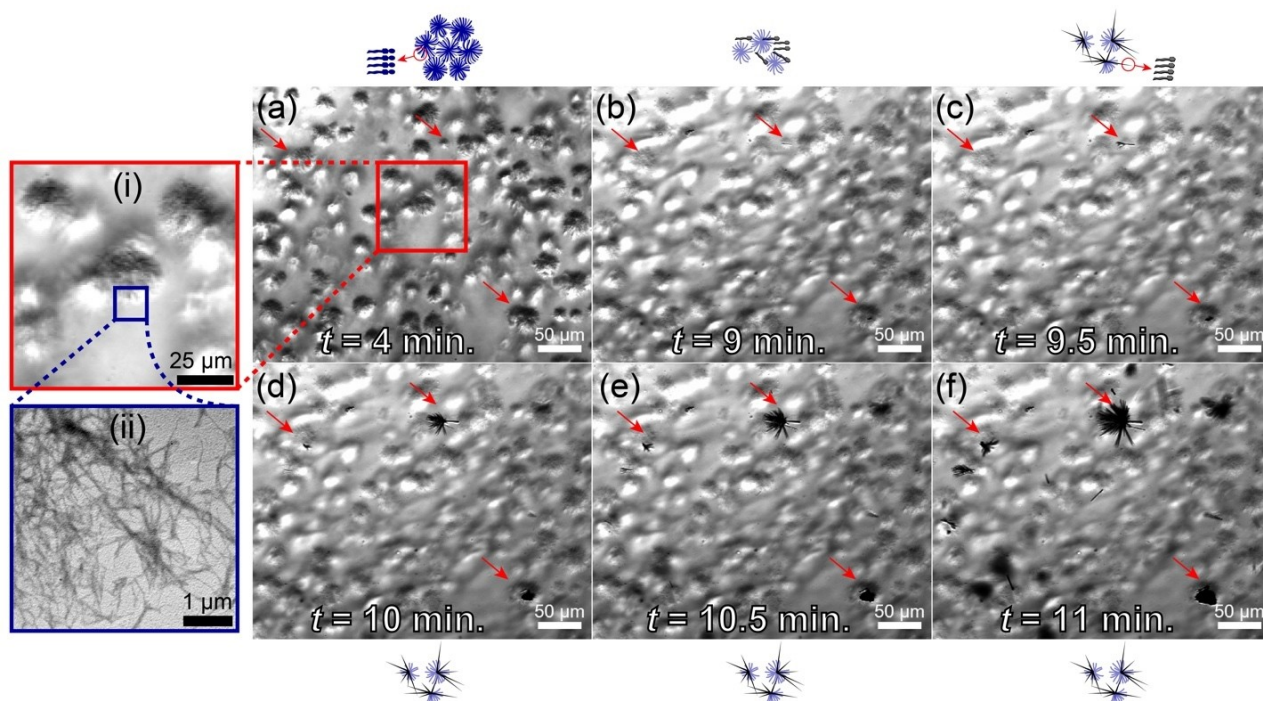


Figure 3. Transmitted light images from confocal microscopy showing the nucleation of SachCHO from fibrous microgels of sachlmine. Scale bar is 50 μm unless otherwise indicated. See Supporting Information Movie 4 for several examples and longer times after addition of fuels. Inset (i) shows an enlarged portion of the image in panel a, and inset (ii) shows transmitted electron microscopy of a comparable sample (i.e. not exactly in the region of the blue box).

disappear over the next 3 days forming solution again (Figure 2m–o).

Recently, Meijer and co-workers have shown how multi-component systems combining surfactants and hydrogelators can undergo multiple reentrant phase transitions (gel→sol→gel→sol) upon stepwise dilution under thermodynamic control.^[15] Here we have shown that intricate phase transitions (sol→gel→sol→gel→sol) can be achieved autonomously—without needing experimental intervention—using a chemically fueled approach. The use of both an activating (HCHO) and deactivating fuel (HCT), combined with the multiple reactivity of the aldehyde moiety—capable of forming either an imine or thiazinane—is what allows these phase transitions. The discovery of solution-based supramolecular^[6,9] and micellar^[16] oscillators, combined with multiple phase transitions similar to our current work, should allow for autonomous sol-gel oscillators to be developed.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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