

## Fuel-Mediated Transient Clustering of Colloidal Building Blocks

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### Supporting Information

**ABSTRACT:** Fuel-driven assembly operates under the continuous influx of energy and results in superstructures that exist out of equilibrium. Such dissipative processes provide a route toward structures and transient behavior unreachable by conventional equilibrium self-assembly. Although perfected in biological systems like microtubules, this class of assembly is only sparsely used in synthetic or colloidal analogues. Here, we present a novel colloidal system that shows transient clustering driven by a chemical fuel. Addition of fuel causes an increase in hydrophobicity of the building blocks by actively removing surface charges, thereby driving their aggregation. Depletion of fuel causes reappearance of the charged moieties and leads to disassembly of the formed clusters. This reassures that the system returns to its initial, equilibrium state. By taking advantage of the cyclic nature of our system, we show that clustering can be induced several times by simple injection of new fuel. The fuel-mediated assembly of colloidal building blocks presented here opens new avenues to the complex landscape of nonequilibrium colloidal structures, guided by biological design principles.

In this paper, we present a colloidal system capable of undergoing transient, reversible assembly. Driven by molecular fuel-mediated surface reactions, a cyclic alternation between hydrophobic attraction and electrostatic repulsion as dominating contribution to the interparticle potential was established. Optimization of the reaction conditions revealed an experimental window where transient molecular attractions could be maintained sufficiently long to allow for time-dependent, mesoscale assembly.

Using molecular strategies to control intercolloidal interactions has been frequently exploited and recently led to particles that rely on, e.g., DNA-hybridization or supramolecular motifs, to guide their assembly.<sup>1</sup> Regardless of the chemical details of the molecules that drive assembly, the building blocks are typically reverse-engineered to form structures that exist in or near equilibrium. By definition, the formed assemblies are rather static and may only respond to environmental changes that constitute a drastic change in the system's thermodynamic ground state.

This lack of structural dynamics is in sharp contrast to a variety of biological assembly processes that rely on continuous

energy input, typically in the form of energy-rich molecules, e.g., adenosine triphosphate (ATP) and guanosine triphosphate (GTP).<sup>2</sup> These fuel consuming assembly processes yield structures that exclusively exist and operate out of equilibrium. Their properties depend on mass and energy flux densities, rather than on thermodynamic equilibrium states. Consequently, the assemblies will spontaneously disintegrate as fuel supply is ceased, and the system returns to equilibrium. Striking examples are microtubules and actin filaments, where fuel controlled structure formation leads to assemblies capable of full-filling dynamic functions, e.g., cellular transport, cell motility and proliferation.<sup>2,3</sup>

Being able to imprint similar transient behavior into colloidal systems paves the way to create inherently dynamic superstructures capable of self-healing and adaptability via continuous regeneration. Additionally, dynamic interparticle potentials yield materials with mesoscopic architectures unreachable by conventional equilibrium assembly and allows for the faster fabrication of defect-free superstructures.<sup>4</sup> Lastly, these dynamic systems can serve as models for exploring nonequilibrium statistical mechanics.

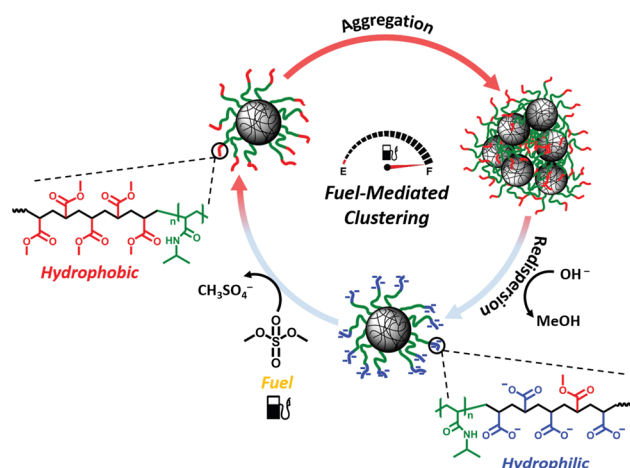
Recently, dynamic assemblies of metallic nanoparticles were developed that show reversible aggregation upon application of external light triggers or dynamic pH oscillations.<sup>5</sup> Here, we extend the class of dynamic structure-formers by developing significantly larger colloidal particles that can assemble, driven by chemical fuel conversion, in close analogy to biological systems. The use of chemical fuels enables control over assembly behavior through reaction kinetics and catalysis, while also storing energy in the system instead of using external energy sources.

The envisioned colloidal fuel-mediated clustering cycle is depicted in Scheme 1, and its design is inspired by a molecular, dissipative system we previously published.<sup>6</sup> The cycle starts with particles that carry charged carboxylic acids causing mutual electrostatic repulsion, safeguarding colloidal stability. Upon addition of chemical fuel, in this case a strong methylating agent (dimethyl sulfate, (CH<sub>3</sub>)<sub>2</sub>SO<sub>4</sub>), the charged carboxylic acids are converted into hydrophobic methyl esters. By removing both the charges and increasing the hydrophobic character of the building blocks, aggregation should be induced. By using a basic, buffered reaction environment, the formed

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Scheme 1. Fuel-Mediated Clustering of Colloidal Building Blocks<sup>a</sup>

<sup>a</sup>In the initial situation, colloids are dispersed in a high pH reaction medium. This causes the outer p(MMA) segments of the grafted polymers to be negatively charged due to deprotonation of carboxylic acids (bottom). The negative charges provide colloidal stability through electrostatic repulsion. Addition of chemical fuel ( $(\text{CH}_3)_2\text{SO}_4$ ) removes these charges by methylation, thereby increasing the hydrophobicity of the polymer brush's outer corona (left). The increased hydrophobicity triggers clustering between the colloidal particles (right). After fuel depletion, hydrolysis of the formed methyl esters becomes dominant. Over time, the charges are reintroduced on the grafted polymers, leading to disintegration of the formed clusters and returning to the initial, well-dispersed state (bottom). For sake of clarity, the methyl groups along the backbone of the p(MMA) segments are discarded.

methyl esters are not stable and slowly hydrolyze back to the charged state. Naturally, reintroduction of the charges reinstates electrostatic repulsion between the building blocks leading to disassembly of the formed clusters.

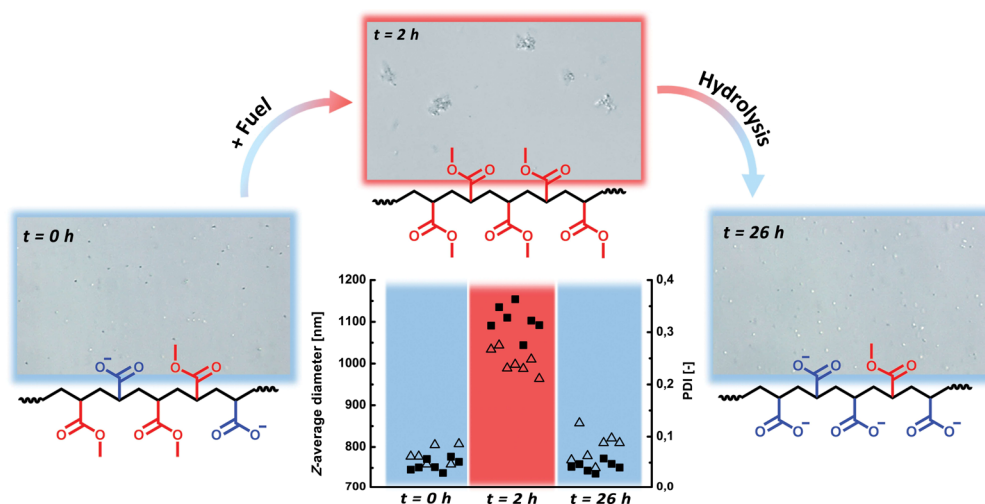
The building blocks used throughout this study comprise a polystyrene core particle grafted with a block copolymer brush. Surface-Initiated Atom Transfer Radical Polymerization (SI-ATRP) was selected as grafting method of choice, since its controlled features allow for the growth of uniform and chemically well-defined brushes.<sup>7</sup> The outer segments of the surface-immobilized polymer brush contain a mixture of hydrophobic methyl esters and charged carboxylic acid moieties along the backbone formed by polymerization of methyl methacrylate (MMA) and *in situ* hydrolysis of a small fraction of the incorporated pending ester moieties. These moieties participate in the fuel-mediated transesterification reactions and will therefore be exploited to control the interparticle potential. The inner segment (poly(*N*-isopropylacrylamide), p(NIPAM)) provides hydrophilic character to the brush ensuring steric stabilization. The buffered reaction environments required to promote base-catalyzed hydrolysis of the formed methyl esters and therefore ensure reversibility of the clustering process, are unavoidably associated with high ionic strengths. By introducing steric stabilization to the particles, we could maintain colloidal stability in the absence of fuel.<sup>8</sup> Furthermore, the inner p(NIPAM) segments act as spacers guaranteeing that the fuel-responsive outer segments are well-separated from the colloidal surface. Consequently, fuel-induced attractions will only act at the outer rim of the polymer brush. This assures that during the aggregation process the polystyrene cores of neighboring

particles cannot approach each other closely, preventing formation of strong irreversible Van der Waals mediated bonds.<sup>8</sup> Naturally, impeding strong attractions between building blocks promotes cluster disassembly after fuel depletion.

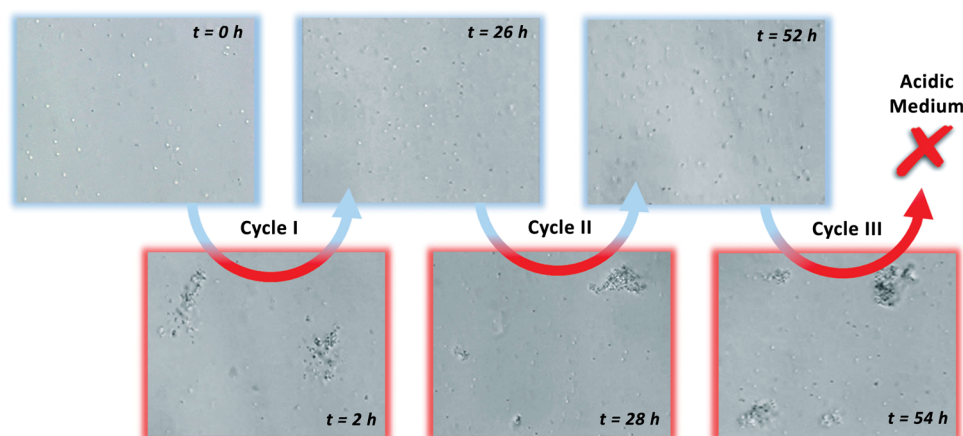
The colloidal stability of the particles was probed using dynamic light scattering (DLS), in both pure water and high ionic strength media. Grafting the polymer brush did not affect colloidal stability as was apparent from the low polydispersity indices (PDIs) measured regardless of the ionic strength of the dispersing medium. As mentioned before, this colloidal stability is attributed to the hydrophilic segments and the charged carboxylic acids in the outer segments of the grafted polymers. The presence of charges was reflected in a slightly negative zeta ( $\zeta$ ) potential. The importance of these charges was underlined by a strong pH-dependence on the colloidal stability. At pH values below the typical  $\text{pK}_a$  of carboxylic acids, protonation generates neutral, mainly hydrophobic outer brush surfaces, promoting particle aggregation. The pH sensitivity of the colloidal stability provided us with a strong indication that fuel-induced clustering might be feasible as well. Fuel addition does not only remove charges from the polymer end-segments, it also boosts hydrophobicity by methyl ester formation, further enhancing clustering.

To investigate the fuel dependent aggregation behavior of the prepared particles, we dispersed them in a borate buffer ( $\text{Na}_2\text{B}_4\text{O}_7$ , 75 mM based on boron) setting the pH of the dispersion to 9.<sup>9</sup> The colloidal stability of the buffered dispersion was verified with DLS. No increase in polydispersity and hydrodynamic size was measured 6 days after dispersing the particles in the buffer, making this situation an ideal reference point to probe the effect of added fuel on the colloidal stability. Increasing the buffer concentration to  $\geq 100$  mM led to irreversible salt-induced aggregation. The upper limit for the buffer concentration of 75 mM has implications for the maximum allowable fuel concentration. Upon reaction of the fuel with the pending carboxylic acids of the particles or via hydrolysis, highly acidic protons are released into the reaction medium.<sup>10</sup> Despite the fact that we use a buffer, generating too many acidic species will eventually saturate the buffer and lead to an acidic environment. Under acidic conditions, the colloids are unstable, making it impossible to differentiate between fuel and pH-mediated clustering. Addition of fuel in different concentrations to buffered reaction media in the absence of particles revealed allowable fuel concentrations of 1–25 mM. Within this concentration range, the pH could be maintained between 8 and 9.

After fuel addition to dispersions containing the polymer grafted colloids, the mixtures were stirred for 2 h. On the basis of the reaction kinetics of  $(\text{CH}_3)_2\text{SO}_4$  at the given initial pH, the 2 h time window is sufficient to convert all fuel to methyl sulfate ( $\text{CH}_3\text{SO}_4^-$ ), either by methylation of carboxylic acids or direct hydrolysis.<sup>10</sup> The effect of fuel on colloidal stability was probed using DLS. The data presented here were obtained using a fuel concentration of 5 mM and is illustrative for all fuel concentrations within the allowable experimental window. As anticipated, fuel injection led to a significant increase in the measured Z-average diameter (Figure 1, squares). This strong indication for aggregation is supported by an increase in PDI (Figure 1, triangles), which reflects the formation of objects of many different hydrodynamic dimensions. High PDIs were expected since the aggregation process is governed by random particle collisions. The obtainable cluster size is limited by the



**Figure 1.** Representative optical microscopy images of colloidal block copolymer grafted particles (p(St)-*g*-p(NIPAM-*co*-MMA)) during a fuel-mediated clustering cycle. From left to right: The colloidal dispersion before fuel addition, 2 h after fuel injection (5 mM), after an additional 24 h period. The chemical details of the p(MMA) outer segments of the grafted brush are shown below each image. Bottom middle panel: Hydrodynamic diameters (squares) and corresponding PDIs (triangles) of the colloids measured with DLS before fuel addition ( $t = 0$  h; blue), 2 h after injection of fuel ( $t = 2$  h; red), and after an additional period of 24 h ( $t = 26$  h; blue). Each symbol represents a single DLS measurement underlining the reproducibility of our results.



**Figure 2.** Optical microscopy images obtained from multiple sequential fuel-mediated aggregation cycles with block copolymer grafted colloidal particles (p(St)-*g*-p(NIPAM-*co*-MMA)). Bottom (red): Images obtained 2 h after each fuel injection. Fuel concentrations of 7.5 and 10 mM were used for Cycle I and Cycle II/III, respectively. Top (blue): Pictures recorded 24 h after fuel injection.

low particle collision probabilities under the applied dilute conditions and the relatively short period in which the particles are mutually attractive. The DLS data are consistent with optical microscopy images. Before fuel injection, well-dispersed colloids were observed (Figure 1, left and Figure S4a), whereas aggregates were present 2 h after  $(\text{CH}_3)_2\text{SO}_4$  addition (Figure 1, middle and Figure S4b).

On the basis of the DLS and optical microscopy results, we concluded that the first half, i.e., fuel-induced aggregation, of the proposed reversible fuel-mediated clustering strategy was successful. To check if colloidal stability was indeed regained after fuel depletion, the samples were stirred for an additional 24 h and the previously described DLS and microscopy analyses were repeated. DLS revealed a significant decrease in both the hydrodynamic diameter (Figure 1 squares) and PDI (Figure 1, triangles) compared to values measured after 2 h. The obtained values are comparable to those measured for the blank, which contained no fuel. Optical microscopy analysis further accentuates the transient behavior by revealing a stable

dispersion without any colloidal clusters after the additional stirring period. Unfortunately, we were not able to probe directly the reintroduction of the charged moieties at the outer particle surface via, e.g., electrophoretic measurements. These measurements were hindered due to the high ionic strength of the dispersing medium. Nevertheless, the strong dependence of colloidal stability on surface charge and the fact that methyl esters are marginally stable at elevated pH makes the proposed mechanism for cluster disintegration highly plausible. Furthermore, we confirmed that particles grafted with p(NIPAM) homopolymers did not respond to the presence of fuel, underlining the indispensability of the methyl esters and related carboxylic acids to steer the interparticle potentials in these fuel-mediated clustering studies.

Because this fuel-driven assembly is in principle a reversible process, we should be able to move through more cycles by consecutive fuel addition. The stable dispersion shown in Figure 1 (right) was used for this stagewise fuel addition experiment. Colloidal stability was monitored using optical

microscopy throughout the experiment (Figure 2). Evidently, two additional consecutive cycles could be performed without any problems, emphasizing the transient switching between attraction and repulsion dominated interparticle potentials even further. Aggregation was induced after fuel addition (Figure 2, bottom row), whereas stable dispersions were obtained after fuel depletion and hydrolysis of the formed methyl esters (Figure 2, top row). We must, however, note that for the consecutive cycles higher fuel concentrations were required (7.5–10 mM) compared to the first cycle (5 mM). Applying higher fuel concentration leads to a progressively faster decreasing pH of the dispersing medium due to loss of buffering capacity. Eventually, an acidic environment was obtained in which the particles were not stable and formed irreversible clusters (Figure 2, bottom right panel, pH  $\approx$  4). We hypothesize that the need for more fuel in the consecutive cycles is caused by a mismatch in the number of carboxylic acids that are methylated versus the number that is formed by hydrolysis. The applied hydrolysis period might be too long, causing demethylation of more methyl esters than were initially formed. To methylate this increasing number of acids, more fuel is required to trigger colloidal clustering. To increase the number of cycles, the kinetics of cluster disintegration should be followed in more detail to minimize the hydrolysis period and therefore keep the number of carboxylic acids to a minimum. Additionally, design parameters, e.g., grafting density, hydrophilic/hydrophobic balance and molecular weight of the grafted polymers provide additional variables that could be tuned to elucidate the role of brush characteristics on the observed transient behavior and to reveal the minimal fraction of fuel-responsive moieties per particle required to induce clustering.

Concluding, in this work we provide proof of principle of fuel-mediated clustering of colloidal building blocks. This is achieved by controlling interparticle potentials in a transient fashion through conversion of a chemical fuel. The fuel concentration provides a new degree of freedom to tune interactions between colloids, enabling assembly into out-of-equilibrium structures. Spatially controlled formation of assemblies is in principle within reach by applying fuel gradients or patterns. Evidently, the basic design rules presented here are not limited to spherical polystyrene particles and extension to more elaborate particle designs, e.g., patchy particles paves the way to directional, dynamic colloidal structures.<sup>11</sup>

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.7b03263.

Synthetic and characterization details (PDF)

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## Notes

The authors declare no competing financial interest.

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