

Transient colloid assembly by fuel-driven modulation of depletion interactions

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ABSTRACT: In biology, energy stored in chemical fuels is used to drive processes energetically uphill, enabling the highly dynamic behavior of living organisms. The out-of-equilibrium behavior can propagate from molecular reaction networks to the micro- and macroscopic scale. These natural phenomena have sparked the design of man-made out of equilibrium chemical reaction networks (CRNs) and dissipative assembly systems with hydrogels, (supra)polymers, vesicles/micelles and colloids. In colloidal systems, the assembly process is typically controlled by balancing the interaction forces. Here, we use a polymeric depletant integrated in a fuel driven esterification CRN to induce transient colloidal assembly. The polymer undergoes a temporal coil-globule transition upon acetylation by the chemical fuel. In the random coil conformation it acts as depletant agent for the silica colloids, promoting colloidal aggregation. As compact globule, the polymer loses its depletant characteristics. During the fuel cycle the polymer cyclically transitions from one form to the other, directly influencing colloidal aggregation and redispersion. Thus, a fuel-driven CRN on the molecular scale results in a microscopic response with a transient colloidal depletion cycle. Overall, the time-dependent propagation of out-of-equilibrium activity across length scales presented here, offers opportunities to design responsive materials with life like properties.

Living systems use energy in the form of chemical fuels to drive thermodynamically unfavourable processes, which gives them the ability to sense, replicate, proliferate, self-heal and amplify signals. These biological out-of-equilibrium systems have a molecular origin, yet can give rise to micro- or even macroscopic responses.¹⁻³ Prime examples from biology are microtubules and actin filaments that play key roles in cellular motility. Their underlying CRNs are based on the consumption of a chemical fuel (i.e. GTP or ATP) to drive the assembly of transiently stable supramolecular polymers from individual activated tubulin or actin building blocks.⁴ Inspired by these natural systems, many dissipative assembly processes have been designed,⁵⁻¹³ including fuel-driven colloidal assembly¹⁴⁻¹⁹. The majority of these colloidal systems decorate the colloids' surface with functional groups that can then participate in a specific fuel-driven CRN. That way the interaction potential of the colloids can be altered during the fuel cycle, leading to a transient assembly response. In general, colloidal stability and assembly are governed by an interplay between different interaction forces.²⁰⁻²¹ On top of that, nanoscale polymeric particles and surfactant assemblies can also promote colloidal assembly via depletion.²² Depletion is an attractive force of entropic origin which arises when colloids are dispersed in a solution containing non-adsorbing polymers²³⁻²⁵. In this scenario colloids are surrounded by a so-called exclusion zone, a volume depleted of polymers which thickness corresponds to the radius of gyration of the polymers. When two colloids approach each other so

that the corresponding exclusion zones overlap (i.e. polymers cannot fit between the colloids anymore), colloids become attractive due to an osmotic pressure imbalance. The size, orientation and hydrophilicity of the depletants can be controlled by external factors, such as pH, temperature and ionic interactions for reversible regulation of depletion interactions.²⁶⁻³² In the current work, however, we aim to control colloidal interactions with a polymeric depletant that is integrated in a fuel-driven CRN. The fuel cycle integration provides autonomous control over the time domain and does not require any external temperature or pH changes. Previously, we developed a fuel-driven transient polymer acetylation CRN with acetic anhydride as chemical fuel to regulate product yield and lifetime.³³ We were able to control polymer conformation and aggregation with two organocatalysts (pyridine and imidazole) and by varying fuel levels. The polymer we developed consisted of a poly(acrylic acid) (PAA) backbone with 25% coverage of 3-nitro-L-tyrosine (NY), which could undergo transient acetylation causing a temporal coil to globule transition. Now, we exploit this fuel-driven polymer coil-globule transition to dynamically control its depletion ability, leading to transient colloidal assembly (Figure 1).

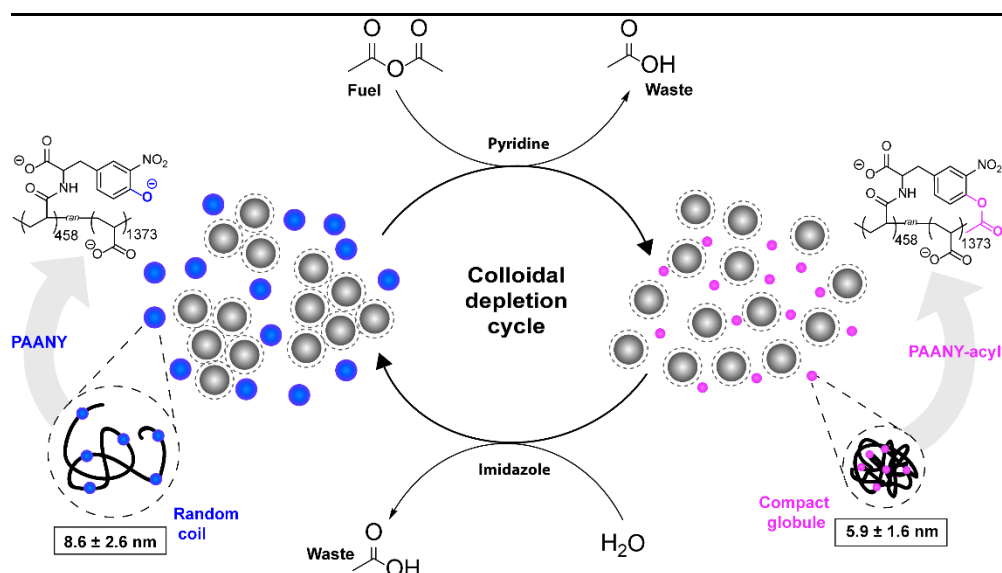


Figure 1. Colloidal depletion cycle regulated by a fuel-driven esterification network with a polymer random coil (large size - depletant) to compact globule transition (small size - no depletant) due to transient acetylation of 3-nitro-L-tyrosine functionalized (poly)acrylic acid (PAANY).

PAANY with negatively charged NY groups has a random coil conformation (diameter 8.6 nm). Upon addition of the chemical fuel (acetic anhydride) the NY moieties get acetylated, generating PAANY-acyl, which adopts a compact globule conformation (diameter ~5.9 nm) due to a decreased charged density and increased hydrophobic surface. We hypothesize that native PAANY in the random coil conformation is a polymeric depletant for silica colloids (diameter = 1 μm), making the colloids assemble. PAANY-acyl, as a smaller compact globule, loses the depletion capacity, leading to disassembly of the colloids. Over time, PAANY-acyl will revert back to native PAANY through hydrolysis, leading to a conformational change and a concomitant increase in depletion ability. Hence, a full fuel cycle initiated by the addition of chemical fuel on the molecular scale causes a transient colloidal assembly-disassembly-assembly cycle on the microscopic level.

At first, the depletion capability of PAANY was investigated. To this end, different concentrations of PAANY in borate buffer (pH 9.0, 200 mM) were added to a solution of silica microspheres ($1.15 \cdot 10^8$ # colloids/mL) to construct an aggregation calibration curve (Figure S1). From this curve, it becomes clear that at least 0.5 mM PAANY should be used to have a significant depletion effect. In contrast to our previous work³³, for these colloidal experiments borate buffer of pH 9.0 was used as colloidal silica was found to stick to the glass wall of the capillary at lower pH. In the absence of the anhydride fuel the colloids form a stable dispersion with PAANY acting as a depletant. Subsequently, different concentrations of PAANY (0.5/0.6/0.7 mM) were used in fuel cycles with acetic anhydride (chemical fuel), pyridine (catalyst forward reaction) and imidazole

(catalyst backward reaction) (Figure 2 and Figure S3-4). With lower pyridine concentration (0.7 mM) only 50% of PAANY is acetylated at its maximum (Figure S3A - UV-VIS conversion) and the colloid (dis)assembly response is less strong (Figure S4A,B,C) compared to the optimized conditions (Figure 2A and Figure S4D). With more pyridine present (1.75 mM) almost 90% of the NY groups on the polymer are acetylated (Figure S3B - UV-VIS conversion) and the colloidal assembly cycle is more pronounced (Figure 2A and Figure S4D). In Figure 2A the colloid aggregation (%) as a function of time is shown for the optimized conditions (0.7 mM PAANY with 2.5 equivalents of pyridine and imidazole). Initially, the colloids are 55% aggregated due to effective depletion interactions with PAANY. After addition of 20 equivalents of the anhydride fuel, the colloid clusters immediately disassemble and an aggregation of only 30% can be observed with mostly dimer structures present (Figure 2C - microscopy image 5 min). Hence, the polymer in its globule state with is not able to deplete the colloids anymore at this concentration and the large clusters disassemble. During the fuel cycle the colloidal aggregation increases again and microscopy images after 90 min and 180 min show the reappearance of the larger colloidal clusters (Figure 2D,E). This can be attributed to an increase in the depletion interactions as the polymer is deacetylated back and in its random coil conformation again. The colloidal clusters from the reference microscopy images of the starting sample can be viewed in Figure S5 (high and low magnification), overall corresponding to an average aggregation percentage of $54.5 \pm 5.78\%$. Microscopy images of colloidal assembly over the course of a full fuel cycle are presented in Figures S6,7 (high and low magnification).

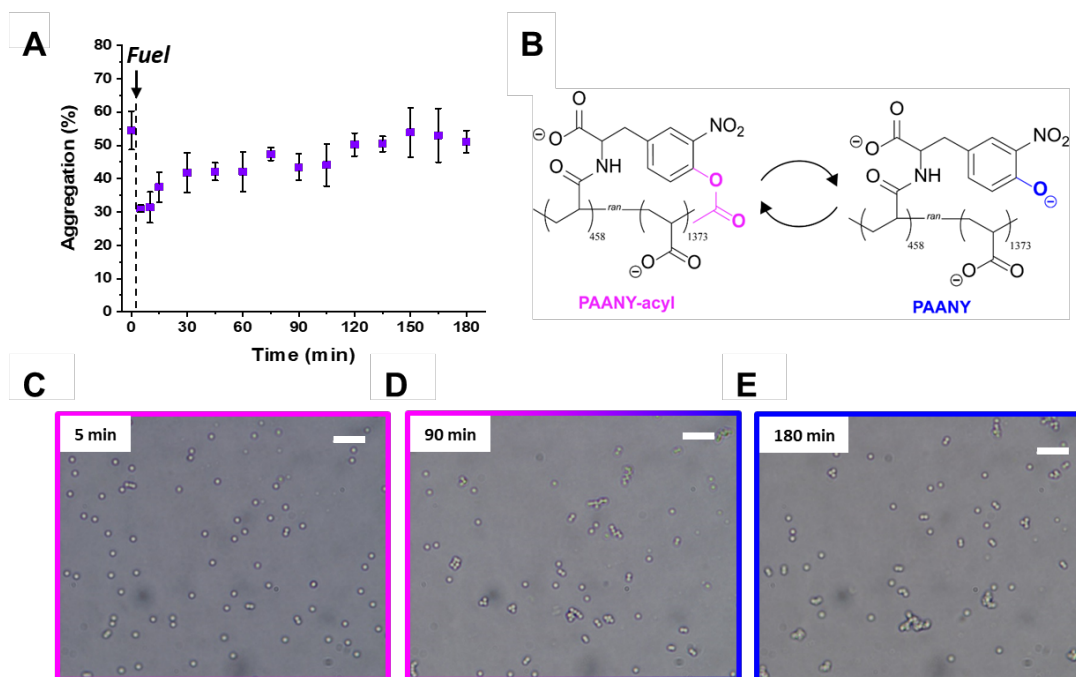


Figure 2. (A). Colloid aggregation as a function time for a fuel cycle. (B). Upon fuel (acetic anhydride) addition PAANY is acetylated to PAANY-acyl. (C). The acetylation induces colloidal disassembly (30% aggregation at 5 min). (D-E). Over time PAANY-acyl is deacetylated giving rise to the reappearance of colloidal clusters (40% aggregation at 90 min and 50% aggregation at 180 min). Conditions: PAANY 0.7 mM, pyridine 1.75 mM, imidazole 1.75 mM, acetic anhydride 14 mM in borate buffer (pH 9.0 200 mM) with silica microspheres (diameter= 1 μm). The standard deviation between two samples is shown ($n=2$). The point measured at $t=0$ min is a different sample without fuel addition, but all other conditions the same. The scale bar in all images is 10 μm . The aggregation (%) was determined by using Matlab.

Next to the overall aggregation over time, the distribution over the various cluster sizes was evaluated (Figure 3). As apparent from Figure 3, the percentage of dimer structures (Figure 3A) remains fairly constant over the course of the fuel cycle. On the contrary, the contribution of trimers (Figure 3B) and especially larger clusters (> 3 particles) (Figure 3C) changes over time and follows the trend of the overall aggregation. Larger clusters are present from the start and disappear completely upon fuel addition, while over time they slowly reassemble. The cluster structures that we observe for all samples are not entirely crystalline, but show a fractal-like open structure (Figure 3D). We hypothesize that this is due to the existence of a secondary minimum in the interaction potential. The formation of the fractal structures is most likely caused by an increased depletion attraction force and hence reduced probability of escape for a particle in a cluster. This phenomenon has also been observed in other research with PAA polymers used for depletion-flocculation studies of polystyrene latex particles³⁴⁻³⁵ and silica surfaces³⁶. The secondary minimum is also quite shallow, as corroborated by the presence of single particles alongside the larger clusters (Figure 3D). Another reason for the presence of open structures can be polymer adsorption onto the colloid surface. However, adsorption here can be ruled out since it usually happens for PAA polymers having protonated carboxylates at low pH.^{34, 36} Furthermore, the mobility of the particles is not influenced by the fuel cycle. We confirmed this by calculating the mean

squared displacement (MSD) as a function of time (see SI section Mean squared displacement from particle tracking).

In this work, we have shown how polymer conformational changes regulated by a fuel-driven esterification CRN can induce transient colloidal depletion aggregation. Upon acetylation by a chemical fuel, the polymer undergoes a temporal transition from a random coil to a compact globule. In the random coil conformation it acts as a polymeric depletant for silica colloids, while in its globular form it is too small to act as depletant for this system. Later in the fuel cycle the polymer is deacetylated again, reobtaining its depletion capacity. The concept was visualized using optical microscopy. The colloidal clusters are fractal-like structures due to the existence of a secondary minimum and the colloids remain dynamic over the course of the fuel cycle. Overall, a fuel cycle initiated by the addition of chemical fuel on the molecular scale results in a microscopic response with a transient colloidal depletion cycle and thus provides a temporary way out of thermodynamic equilibrium on large length scales. Understanding the propagation from a molecular reaction network to the microscopic or even macroscopic world is important for further research in this field and can support the design of responsive materials with life-like properties.

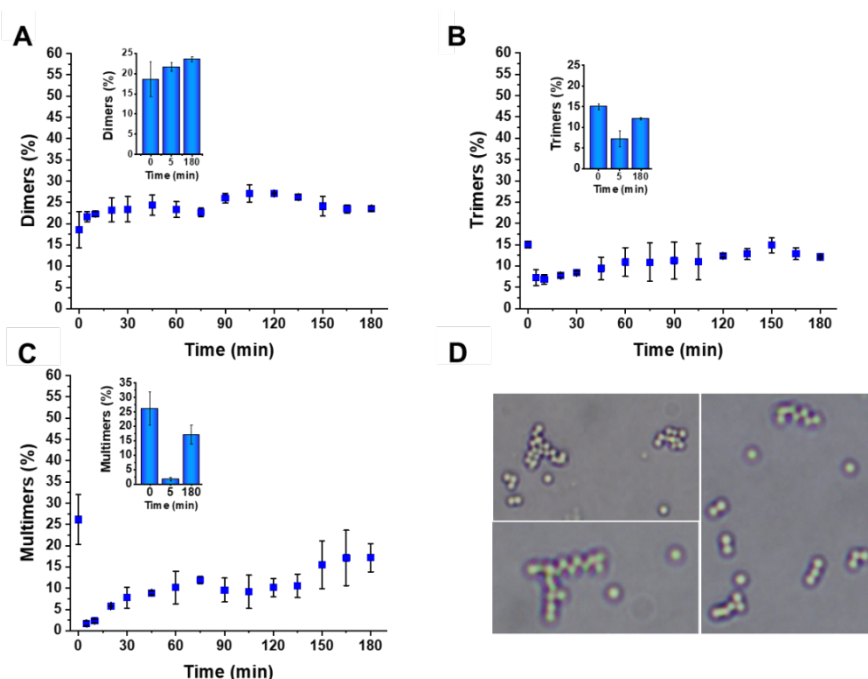


Figure 3. Cluster distribution over time: (A). Dimers contribution. (B). Trimers contribution. (C). Multimer (>3 particles) contribution. (D). Representative image of multimer fractal structures. Conditions: PAANY 0.7 mM, pyridine 1.75 mM, imidazole 1.75 mM, acetic anhydride 14 mM in borate buffer (pH 9.0 200 mM) with silica microspheres (diameter= 1 μ m). The standard deviation between two samples is shown (n=2). The point measured at t=0 min is a different sample without fuel addition, but all other conditions the same. The bar charts inside the plots highlight the starting point (0 min), the start of fuel addition (5 min) and the endpoint (180 min).

ASSOCIATED CONTENT

Supporting Information.

Experimental details, supplemental figures referred to in the main text obtained with different techniques (PDF).

Video of colloidal particles at reference t=0 min, both at high and low magnification

Video of colloidal particles in fuel cycle at t=5 min, both at high and low magnification

Video of colloidal particles in fuel cycle at t=180 min, both at high and low magnification

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Author Contributions

M.P.v.d.H and C-L.W. carried out the experiments and analyzed the data. R.L. and S.S. developed the Matlab particle counting and tracking scripts. L.R. provided suggestions on experiments, analysis and improvements. M.P.v.d.H, C-L.W, L.R. and R.E. designed the experiments. M.P.v.d.H. wrote the manuscript. R.E. and L.R. conceived and directed the overall research project and revised the manuscript. All authors commented on the work and the manuscript.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS

CRN, Chemical reaction network; PAANY, (poly)acrylic acid 3-nitro-L-tyrosine; ATP, Adenosine triphosphate; GTP, Guanosine triphosphate.

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