Transient Colloid Assembly by Fuel-Driven Modulation of Depletion Interactions

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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.

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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,4-13 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.19-21 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 polymers.23-25. 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).
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 (1.15·10^8 # 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 work, 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 colloidal disassembly 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 colloidal 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 colloidal 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 ± 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).
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 particles\textsuperscript{34,35} and silica surfaces\textsuperscript{36}. 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.\textsuperscript{35,36} 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.
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Notes
The authors declare no competing financial interest.

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
“This material is available free of charge via the Internet at http://pubs.acs.org.”

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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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REFERENCES
1. Bialek, W., Biophysics: searching for principles. Princeton University Press: 2012.
2. Branscomb, E.; Biancalani, T.; Goldenfeld, N.; Russell, M., Escapement mechanisms and the conversion of disequilibrium: the engines of creation. Phys. Rep. 2017, 677, 1-60.
3. Gnesotto, F.; Mura, F.; Gladrow, J.; Broedersz, C. P., Broken detailed balance and non-equilibrium dynamics in living systems: a review. Rep. Prog. Phys. 2018, 81 (6), 066601.
4. Alberts, B.; Johnson, A.; Lewis, J.; Morgan, D.; Raff, M.; Roberts, K.; Walter, P., Molecular biology of the cell. Sixth edition ed.; Garland Science: New York, 2014.
5. van Rossum, S. A. P.; Tena-Solsona, M.; van Esch, J. H.; Eelkema, R.; Boekhoven, J., Dissipative out-of-equilibrium assembly of man-made supramolecular materials. Chem. Soc. Rev. 2017, 46 (18), 5519-5535.
6. Singh, N.; Formon, G. J.; De Piccoli, S.; Hermans, T. M., Devising Synthetic Reaction Cycles for Dissipative Nonequilibrium Self-Assembly. Adv. Mater. 2020, 32 (20), 1906834.
7. Boekhoven, J.; Hendriksen, W. E.; Koper, G. J. M.; Eelkema, R.; van Esch, J. H., Transient assembly of active materials fueled by a chemical reaction. Science 2015, 349 (6252), 1075-1079.
8. Leira-Iglesias, J.; Tassoni, A.; Adachi, T.; Stich, M.; Hermans, T. M., Oscillations, travelling fronts and patterns in a supramolecular system. Nat. Nanotechnol. 2018, 13 (11), 1021.
9. Ragazzon, G.; Prins, L., Energy consumption in chemical fuel-driven self-assembly. Nat. Nanotechnol. 2018, 13, 882–889.
10. Heuser, T.; Weyandt, E.; Walther, A., Biocatalytic feedback-driven temporal programming of self-regulating peptide hydrogels. Angew. Chem. Int. Ed. 2015, 54 (45), 13258-13262.
11. Dhiman, S.; Jain, A.; Kumar, M.; George, S., Adenosine-phosphate-fueled, temporally programmed supramolecular polymers with multiple transient states. J. Am. Chem. Soc. 2017, 139 (46), 16568-16575.
12. Debnath, S.; Roy, S.; Ulijn, R. V., Peptide nanofibers with dynamic instability through nonequilibrium biocatalytic assembly. *J. Am. Chem. Soc.* **2013**, *135* (45), 16789-16792.
13. Sorrenti, A.; Leira-Iglesias, J.; Sato, A.; Hermans, T. M., Nonequilibrium steady states in supramolecular polymerization. *Nat. Comm.* **2017**, *8*, 15899.
14. van Ravensteijn, B. G.; Voets, I. K.; Kegel, W. K.; Eelkema, R., Out-of-equilibrium colloidal assembly driven by chemical reaction networks. *Langmuir* **2020**, *36*, 10639-10656.
15. van Ravensteijn, B. G.; Hendriksen, W. E.; Eelkema, R.; van Esch, J. H.; Kegel, W. K., Fuel-mediated transient clustering of colloidal building blocks. *J. Am. Chem. Soc.* **2017**, *139* (29), 9763-9766.
16. Grötsch, R. K.; Angi, A.; Mideksa, Y. G.; Wanzke, C.; Tena-Solsona, M.; Feige, M. J.; Rieger, B.; Boekhoven, J., Dissipative Self-Assembly of Photoluminescent Silicon Nanocrystals. *Angew. Chem. Int. Ed.* **2018**, *57* (44), 14608-14612.
17. Grötsch, R. K.; Wanzke, C.; Speckbacher, M.; Angi, A.; Rieger, B.; Boekhoven, J., Pathway dependence in the fuel-driven dissipative self-assembly of nanoparticles. *J. Am. Chem. Soc.* **2019**, *141* (25), 9872-9878.
18. Heuser, T.; Steppert, A.-K.; Molano Lopez, C.; Zhu, B.; Walther, A., Generic concept to program the time domain of self-assemblies with a self-regulation mechanism. *Nano Lett.* **2015**, *15* (4), 2213-2219.
19. Dehne, H.; Reitenbach, A.; Bausch, A., Transient self-organisation of DNA coated colloids directed by enzymatic reactions. *Sci. Rep.* **2019**, *9* (1), 1-9.
20. Russel, W. B.; Russel, W.; Saville, D. A.; Schowalter, W. R., *Colloidal dispersions*. Cambridge university press: 1991.
21. Evans, D. F.; Wennerström, H., The colloidal domain: where physics, chemistry, biology, and technology meet. 1999.
22. Tuinier, R.; Lekkerkerker, H. N., *Colloids and the Depletion Interaction*. Springer Netherlands: 2011.
23. Asakura, S.; Oosawa, F., Interaction between particles suspended in solutions of macromolecules. *J. Polym. Sci.* **1958**, *33* (126), 183-192.
24. Asakura, S.; Oosawa, F., On interaction between two bodies immersed in a solution of macromolecules. *J. Chem. Phys.* **1954**, *22* (7), 1255-1256.
25. Mao, Y.; Cates, M.; Lekkerkerker, H., Depletion force in colloidal systems. *Physica A* **1995**, *222*, 10-24.
26. Buzzaccaro, S.; Colombo, J.; Parola, A.; Piazza, R., Critical depletion. *Phys. Rev. Lett.* **2010**, *105* (19), 198301.
27. Piazza, R.; Buzzaccaro, S.; Parola, A.; Colombo, J., When depletion goes critical. *J. Condens. Matter Phys.* **2011**, *23* (19), 194114.
28. Liu, Z.; Niu, Z.-w., Temperature responsive 3D structure of rod-like bionanoparticles induced by depletion interaction. *Chinese J. Polym. Sci.* **2014**, *32* (10), 1271-1275.
29. Lele, B. J.; Tilton, R. D., Control of the colloidal depletion force in nonionic polymer solutions by complexation with anionic surfactants. *J. Colloid Interface Sci.* **2019**, *553*, 436-450.
30. Xing, X.; Li, Z.; Ngai, T., pH-controllable depletion attraction induced by microgel particles. *Macromolecules* **2009**, *42* (19), 7271-7274.
31. Gratale, M. D.; Still, T.; Matyas, C.; Davidson, Z. S.; Lobel, S.; Collings, P. J.; Yodh, A., Tunable depletion potentials driven by shape variation of surfactant micelles. *Phys. Rev. E* **2016**, *93* (5), 050601.
32. Feng, L.; Laderman, B.; Sacanna, S.; Chaikin, P., Re-entrant solidification in polymer–colloid mixtures as a consequence of competing entropic and enthalpic attractions. *Nat. Mater.* **2015**, *14* (1), 61-65.
33. van der Helm, M. P.; Wang, C.-L.; Fan, B.; Macchione, M.; Mendes, E.; Eelkema, R., Organocatalytic control over a fuel-driven transient esterification network. *Angew. Chem. Int. Ed.* **2020**, DOI:10.1002/anie.202008921.
34. Burns, J. L.; Jameson, G. J.; Biggs, S., Relationship between interaction forces and the structural compactness of depletion flocculated colloids. *Colloids Surf. A Physicochem. Eng. Asp.* **2000**, *162* (1-3), 265-277.
35. Liang, W.; Tadros, T. F.; Luckham, P., Flocculation of sterically stabilized polystyrene latex particles by adsorbing and nonadsorbing poly (acrylic acid). *Langmuir* **1994**, *10* (2), 441-446.
36. Milling, A. J.; Vincent, B., Depletion forces between silica surfaces insolutions of poly (acrylic acid). *J. Chem. Soc. Faraday Trans.* **1997**, *93* (17), 3179-3183.
Supporting information

Transient colloid assembly by fuel-driven modulation of depletion interactions

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1 Experimental details

1.1 General materials and methods

Unless stated otherwise, all preparations and analyses were performed at room temperature (RT) (~21 °C) and atmospheric pressure. Chemicals were purchased in the highest available purity and used without any further purification unless mentioned otherwise. 3-nitro-L-tyrosine, acetic anhydrous, pyridine anhydrous, imidazole, poly(acrylic acid) (PAA ~130 kDa), N,N-dimethyl formamide (DMF) anhydrous, dimethyl sulfoxide (DMSO), N-(3-Dimethylaminopropyl)-N’-ethylcarbodiimide hydrochloride (EDC) and N,N-diisopropylethylamine (DIPEA) were from Sigma-Aldrich. 3-nitro-L-tyrosine ethyl ester (NY-ethyl ester) was purchased from Chem Impex International and 1-Hydroxybenzotriazole hydrate (HOBt) from Acros Organics. The functionalization of the PAA polymer with 3-nitro-L-tyrosine via EDC/HOBt peptide coupling was performed according to our previous reported procedure. Sodium tetraborate decahydrate (Borax) and boric acid were used as solid salts to prepare borate buffer and were from Sigma Aldrich. Silica microspheres (Si-OH surface functional groups; diameter= 1.21± 0.144 µm; density 2 g/ml; 5.745·10^{10} #colloids/ml; surface area 2.490·10^{12} μm²/g) were purchased from Bangs laboratory. Capillary tubes (rectangle boro tubing: 0.10 x 2.00 mm and 50 mm in length) were purchased from VitroCom. UV-Vis spectroscopic experiments were carried out using Analytik Jena Specord 250 spectrophotometer with a quartz cuvette with a 1 cm path length, volume of 3 mL, at RT. Microscopy images of the capillary tubes were recorded with a Nikon Eclipse E600POL equipped with a U-III Film Camera System. The captured photographs were processed with NIS-elements AR3.0 software.

1.2 Microscopy measurements

Silica microspheres (diameter= 1 µm) suspended in aqueous solution were sonicated and centrifuged (1000 rpm, 1 min, rotor F45-24-11 eppendorf), whereupon the water supernatant was removed and equal amounts of borate buffer (pH 9.0, 200 mM) were added to make the stock solution. The sample with colloid solution (500x dilution; final concentration 1.15·10^8 #colloids/ml) was then transferred to a capillary tube (0.10 x 2.00 mm and 50 mm in length) by dipping the empty capillary vertically into the solution for 5 sec. The capillary tube was placed on a glass slide and sealed with wax on both ends. Subsequently, the silica microspheres were observed through the capillary under the microscope. All stock solutions were filtered with a syringe filter (0.2 µm) before use. Except for the acetic anhydride, all other reagents were mixed beforehand with silica microspheres (500x diluted; final concentration 1.15·10^8 #colloids/ml) in a 4 ml vial. In order to start the fuel cycle acetic anhydride was added to the vial and subsequently the capillary was dipped into this solution. Images of the colloids during the fuel cycle were taken at different magnifications and varying time intervals. Additionally, movies were taken at a speed of 0.8-1.3 fr/sec for 15-25 sec during the fuel cycle. The pH was measured before and after the reaction from the solution left in the 4 ml vial. For the analysis, the aggregation percentage of the colloids was calculated from the recorded microscopy images according to the following equation:

\[ Aggregation (%) = \frac{Particles in aggregate}{Total particles} \times 100\% \]
Where the particles in aggregate represents the sum of particles present as dimers, trimers and larger clusters. The total particles is the sum of the aggregated particles and the single particles. The aggregation percentage was calculated with a Matlab script (see Matlab particle counting).

### 1.3 UV-Vis assay

Stock solutions were prepared in borate buffer (pH 9.0, 200 mM). The fuel cycle was performed with 0.15 mM PAANY, 3 mM acetic anhydride, 0.15 or 0.375 mM of pyridine and 0.375 mM of imidazole in borate buffer (pH 9.0, 200 mM), in quartz cuvettes, path length of 1 cm (total reaction volume of 3 mL) at RT. The stock solutions of the reactants were always added in the following order: PAANY, pyridine, imidazole and acetic anhydride. Teflon caps were used to close the cuvette. The cuvette was turned upside down to mix the solution. The reactant peak was followed using slow time scan, measuring wavelength at 420 nm. The pH was measured before and after the reaction. The conversion was calculated with the extinction coefficient (see calibration line 3-nitro-L-tyrosine in UV-Vis section) and the Lambert-Beer law:

\[
A = \varepsilon l C
\]

Where \(A\) is the absorbance, \(\varepsilon\) the extinction coefficient, \(l\) the path length of the cuvette and \(C\) the concentration.
2 Aggregation calibration

Figure S1: Colloidal aggregation calibration curve of PAANY (depletant) with silica microspheres ($d = 1 \, \mu m$). Different concentrations of PAANY were used, following a sigmoidal trend line (fitted with Origin).
3 UV-VIS spectroscopy

3.1 Calibration line

![Graph showing calibration line with equation and R² value]

**Figure S2:** Extinction coefficient for 3-nitro-L-tyrosine (NY) in borate buffer (pH 9.0, 200 mM) at 420 nm: 5.3 mM\(^{-1}\)cm\(^{-1}\). The absorbance at 420 nm of 3-nitro-L-tyrosine (NY) at different concentrations is shown.

3.2 Conversion plots

![Graphs showing conversion plots](A and B)

**Figure S3:** Conversion of PAANY upon acetylation to PAANY-acyl and subsequent hydrolysis. The absorbance at of the 3-nitro-L-tyrosine moiety at 420 nm is monitored over time during a fuel cycle. (A). PAANY 0.15 mM, pyridine 0.15 mM, imidazole 0.375 mM, acetic anhydride 3 mM in borate buffer (pH 9.0 200 mM). (B). PAANY 0.15 mM, pyridine 0.375 mM, imidazole 0.375 mM, acetic anhydride 3 mM in borate buffer (pH 9.0 200 mM).
4 Aggregation as a function of time

Figure S4: Colloid aggregation as a function time for fuel cycles under different conditions. Upon fuel (acetic anhydride) addition PAANY is acetylated to PAANY-acyl inducing colloidal disassembly (decreased aggregation %), while over time PAANY-acyl is deacetylated again giving rise to the reappearance of colloidal clusters (increased aggregation %). (A). PAANY 0.5 mM, pyridine 0.5 mM, imidazole 1.25 mM, acetic anhydride 10 mM in borate buffer (pH 9.0 200 mM). (B). PAANY 0.6 mM, pyridine 0.6 mM, imidazole 1.5 mM, acetic anhydride 12 mM in borate buffer (pH 9.0 200 mM). (C). PAANY 0.7 mM, pyridine 0.7 mM, imidazole 1.75 mM, acetic anhydride 14 mM in borate buffer (pH 9.0 200 mM). (D). PAANY 0.7 mM, pyridine 1.75 mM, imidazole 1.75 mM, acetic anhydride 14 mM in borate buffer (pH 9.0 200 mM). The standard deviation between two samples is shown (n=2). Silica microspheres (diameter= 1 µm) were used for all samples. The point measured at t=0 min is a different sample without fuel addition, but all other conditions the same.
Figure S5: Representative microscopy images over time for t=0 min (without fuel) with an average aggregation percentage of 54.5 ± 5.78 (Matlab counting) corresponding to the conditions in Figure S4D: PAANY 0.7 mM, pyridine 1.75 mM, imidazole 1.75 mM in borate buffer (pH 9.0 200 mM). The first three pictures are at higher magnification (100/0.7) and the last three at lower magnification (50/0.45). The scale bar in all images is 10 µm.
Figure S6: Representative microscopy images (magnification: 100/0.7) for the fuel cycle over time corresponding to the conditions in Figure S4D: PAANY 0.7 mM, pyridine 1.75 mM, imidazole 1.75 mM, acetic anhydride 14 mM borate buffer (pH 9.0 200 mM). The scale bar is 10 µm in all images.
Figure S7: Representative microscopy images (magnification: 50/0.45) for the fuel cycle over time corresponding to the conditions in Figure S4D: PAANY 0.7 mM, pyridine 1.75 mM, imidazole 1.75 mM, acetic anhydride 14 mM in borate buffer (pH 9.0 200 mM). The scale bar is 10 µm in all figures.
5 Matlab particle counting

The microscopy images were calculated with a Matlab particle counting script. For the latter, the image file obtained with the microscope is loaded into Matlab. Subsequently, the code searches for the single, dimer, trimer and larger clusters. Examples of typical Matlab output files for the fuel cycle microscopy images at 5 min and 3 h are shown below.

![AllParticles](image1.png) ![AggregatedParticles](image2.png) ![SingleParticles](image3.png)

**Figure S8:** Representative Matlab particle counting output. First all particles are identified, whereupon a distinction is made between aggregated and single particles. The aggregated particles are further divided into dimers, trimers and other mers. 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), image recorded at 5 min.
Figure S9: Representative Matlab particle counting output. First all particles are identified, whereupon a distinction is made between aggregated and single particles. The aggregated particles are further divided into dimers, trimers and other mers. 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), image recorded at 3 h.
For a careful examination of the distribution over the various clusters, microscopy video files were also evaluated with the Matlab model. The final calculated distribution is then the average over 20 frames. An example of a Matlab output file for a video frame is shown below.

![Image of Matlab output file](image)

**Figure S10:** Representative Matlab particle processed video frame images. For all videos frames the particles are counted and the clusters identified as dimers, trimers and multimers. 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), image recorded at 5 min (A) and 3 h (B).
6 Mean squared displacement from particle tracking

To examine the mobility of the colloid particles during the course of the fuel cycle, the mean squared displacement (MSD) was calculated based on multiple recorded videos from the microscope as a function of time. The MSD is defined according to the following equation:

$$MSD \equiv \frac{1}{N} \sum_{i=1}^{N} |x^{(i)}(t) - x^{(i)}(0)|^2$$

Where $N$ is the number of particles (i.e. silica colloids), $x^{(i)}(0)$ the reference position and $x^{(i)}(t)$ the position of the $i$-th particle at time $t$. The reference position and the new positions were calculated with a Matlab particle tracking model for every particle for each frame of the video. A typical video consisted of 20 individual image frames with each having about 450 particles in total. The MSD was calculated with single particles and particles in clusters together (Figure S9) and with only single particles (Figure S10).

![Figure S11](image)

**Figure S11:** (A). MSD as a function of time for single particles and particles in clusters together. The standard deviation from two samples is shown (n=2). (B). Example image of particle (single and in clusters) trajectories calculated by the Matlab tracking model. The scale bar is 10 µm. Sample 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).
Figure S12: (A). MSD as a function of time for only single particles. The standard deviation between two samples is shown (n=2). (B). Example image of particle (only single) trajectories calculated by the Matlab tracking model. The scale bar is 10 µm. Sample 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).
References

1. van der Helm, M. P.; Wang, C.-L.; Fan, B.; Macchione, M.; Mendes, E.; Eelkema, R., Organocatalytic control over a fuel-driven transient esterification network, https://doi.org/10.1002/anie.202008921. Angew. Chem. Int. Ed. 2020.
