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Chemically Fuelled Dissipative Self-Assembly exploiting Cooperative Catalysis

Subhajit Bal, Krishnendu Das, Sahnawaz Ahmed and Dibyendu Das*

Abstract: In living systems, dissipative processes are driven by the endergonic hydrolysis of chemical fuels such as nucleotide triphosphates. Here we demonstrate through a simple model system, the realization of a transient self-assembled state by utilizing the catalytic effect of histidine on the formation and breaking of ester bonds. Firstly, histidine facilitates the ester bond formation, which then rapidly co-assembles to form a self-supporting gel. Out of equilibrium state is realized due to the cooperative catalysis by the proximal histidines in the assembled state, driving the second pathway and resulting in disassembly to sol. Cooperative effects seen in natural and synthetic systems which use dual role of imidazoles as nucleophile and as proton donor, is utilized here to achieve transient assemblies. This simple system mimics the structural journey seen in microtubule formation, where the substrate GTP facilitates the non-covalent assembly and triggers a cooperative catalytic process leading to hydrolysis of substrate and subsequent disassembly.

Nature elegantly utilizes transient self-assembled structures that are capable of exhibiting functions through energy consuming processes.[1-3] These transient self-assembly processes rely on the consumption of chemical fuels to perform reactions that are thermodynamically unfavourable.[4-9] Chemical fuels are high energy molecules which are kinetically stable and the stored energy is utilized to drive biological processes. [10-19] One of the classic examples of transient assemblies seen in Nature is in the form of cytoskeleton proteins such as tubulins that self-assemble to form microtubules.[18,19] Structurally, the individual tubulin dimers assemble non-covalently using weak forces of hydrophobic and electrostatic interactions in presence of guanosine triphosphate (GTP) and the polymerization moves towards equilibrium.[20, 21] Interestingly, in the assembled state the catalytic potential of these individual tubulin dimers gets significantly amplified resulting in hydrolysis of GTP to GDP which causes conformation change and diassembly.[21,22] Intrigued by this, we pursued to devise a simple system (Figure 1) which can undergo fuel driven self-assembly and utilize the accelerated catalytic potential at assembled state to consume the fuel. [21,22] To achieve this, we propose the use of cooperative catalysis from proximal imidazole groups of histidine based amphiphiles in assembled state (Figure 1). In this context, works of Miravet/Escuder/Ulijn, Scrimin Lab and recent works of Prins and co-workers have utilized cooperative catalysis at assembled state in artificial systems.[22-27]

The amphiphile (**C18H**) was synthesized by simply stearoylation (**C18**) of histidine (Figure 2a).[28] **C18H** (12 mM) was dissolved in aqueous solvent containing 20% v/v DMSO (See SI for details) resulting in a free flowing solution. For the creation of the high energy ester bond, 4-nitrophenol (NP) as the nucleophile was used. Notably, when we added a water soluble coupling agent, 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 4 mM) to the mixture of NP and **C18H**, the solution rapidly became more viscous and turned into a self-supporting gel within 2 min. EDC has previously been used by Boekhoven and coworkers as the high energy fuels for generation of transient supramolecular materials.[29,30] Interestingly, after 30 min the gel showed transition to a solution (Figure 2c) suggesting the formation of a transient assembled state. Addition of EDC or NP separately did not result in gelation. Varying the concentration of the coupling agent and nucleophile resulted in different lifetimes of the transient gels, reaching up to ca. 12 h (Figure 2b).

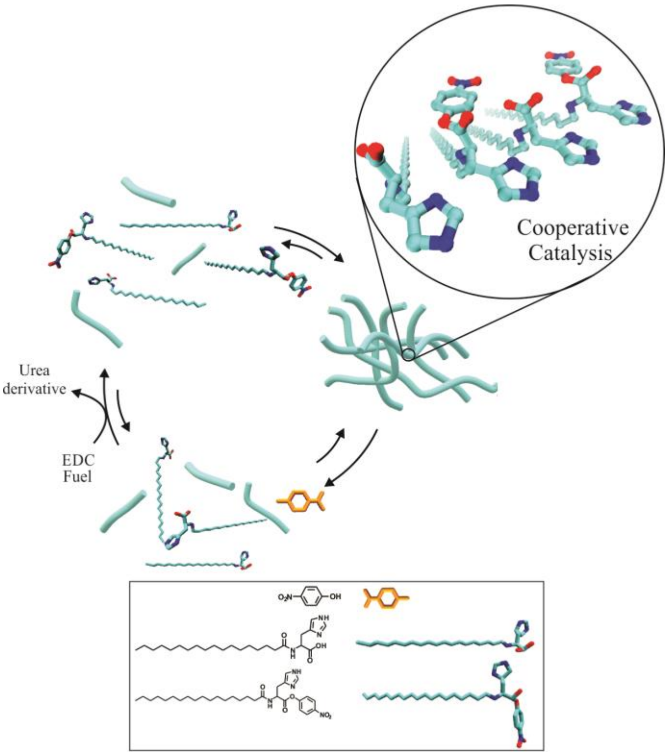

Figure 1. Schematic overview of fuel driven self-assembly and dissipation by cooperative catalysis

To investigate this transient behavior further, UV Vis spectroscopy was used (Figure 3a). In case of **C18H** (12 mM) with NP (4 mM), within two minutes of addition of EDC (4 mM), we could see the generation of a low intensity peak at 268 nm arising from the n to π^* transition of the generated ester **C18H-NP**.[31] Notably, after 30 minutes, this peak gradually disappeared indicating the transient existence of the high energy ester **C18H-NP**. To investigate in a control system, we synthesized phenylalanine (**F**) analogue of compound **C18H**, which was made following a similar process of reacting the corresponding amino acid **F** with stearoyl chloride (**C18F**, Figure

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2a). However, treatment with NP and EDC did not result in generation of ester peak as presence of exogenous base is required for the condensation of acid and alcohol. Addition of 4 mM N,N-diisopropylethylamine (DIPEA) resulted in the generation of the peak at 268 nm suggesting formation of the ester **C18F-NP** (Figure 3b). This peak plateaued after ca. 45 min and no decrease of intensity of the peak could be seen even after 10 h, suggesting higher stability of the kinetically stable ester. Also, the fact that absence of exogenous base does not result in any ester conversion, underpins the importance of the built-in base in form of histidine's imidazole of **C18H**. To investigate further, we checked this dissipative system with nuclear magnetic resonance (NMR) spectroscopy. Time dependent ^{1}H NMR of the sample containing the **C18H** in presence of NP and EDC indeed showed the generation of the NP protons (yellow bands, protons marked H$_a$ and H$_b$, Figure 3c) of the ester**.** Notably, after 45 min, the signals of the NP aromatic protons gradually decreased and eventually disappeared completely showing the transient existence of the ester (**C18H-NP**). In contrast, **C18F** showed presence of the aromatic ester protons without any signs of decrease of peak area with time till 10 h (Figure S1, Supporting Information). The morphological transformations of the sample were checked at different time periods by transmission electron microscopy (TEM, Figure 4). Samples imaged immediately after mixing EDC, NP with **C18H**, showed no network like morphologies with only some aggregated structures (Figure 4a). Notably, after aging of 10 min, the formed transient gel showed dense networks with extensive entanglements (Figure 4b). Interestingly, the same sample which turned to solution after 30 minutes, showed substantial decrease of fibrillar network structure (Figure 4c). Control samples of **C18H** with or without addition of NP or EDC separately, did not show any nanofibrillar network like morphology. Powder XRD was done with the dried powder and xerogel of **C18H** solution and the transient gel respectively. The xerogel samples of the transient gel showed crystalline character with sharper peaks compared to only **C18H** (Figure 4d). Spacings of 4.38 Å and 3.9 Å suggest the distance between two lipid layers of the amphiphiles and their sharper profiles indicate closer packing distance.[32] Furthermore, rheological measurements done with the transient gels showed superior mechanical strength compared to the precursor acid **C18H** due to the better packing in the transient coassembled gels (Figure S2). The reason for the temporal existence of networks and gel was likely due to cooperative catalysis in the assembled state. This arises due to the proximity of the histidines in the closely packed assembled nanostructures formed from the coassembly of the precursor acid **C18H** with the transiently generated **C18H-NP** ester. In extant biocatalysis, proximal localization of active site residues remarkably facilitate the catalytic efficiencies. Also, histidine dyads perform catalytic roles in hydrolases by shuttling protons and influencing the local pH.[33] To investigate this cooperative effect further, **C2H** was synthesized by simply acetylating histidine amino acid (Figure 2a). Due to drastic decrease of the tail hydrophobicity it was expected that **C2H** will not access the self-assembled nanostructures and hence will not show cooperative catalysis which is critical for dissipation in these systems. Addition of NP and EDC to **C2H** also resulted in the conversion to the corresponding ester which was followed at 268 nm (Figure 3b). The peak intensity did not show any decrease over time, observed till 10 h. Also, from ^{1}H NMR spectroscopy, the peak intensity of marked protons (Ha, Hb, yellow band in Figure 3d) of NP in the corresponding ester remained unchanged with time with no indication of transient nature. This mixture containing the **C2H-NP** ester and **C2H** acid was also monitored by circular dichroism (CD) spectroscopy which showed weak signals suggesting the absence of assembled structures (Figure S3). In contrast, CD spectroscopy done with transient ester of **C18H** analogue at ca. 10 min showed stronger signals supporting the presence of

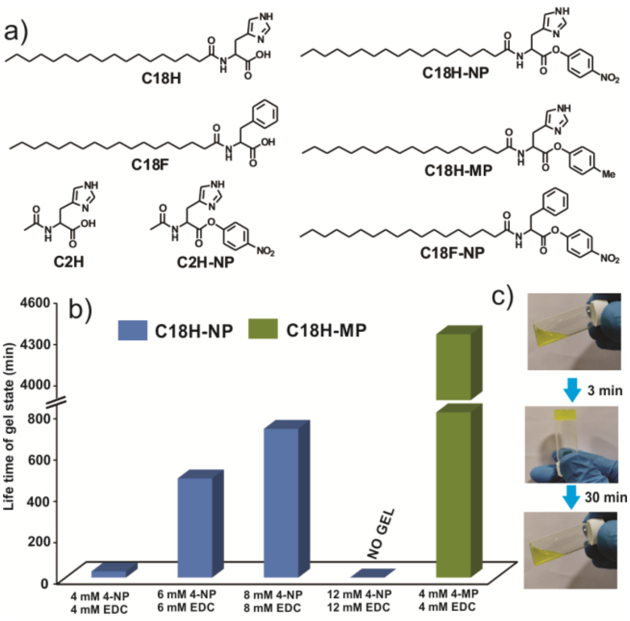

Figure 2. a) Chemical structures of molecules involved. b) Life time of transient gels with varying concentration of EDC and NP or 4-methyl phenol c) Vial images of **C18H** (12 mM**)** in presence of NP (4 mM) and EDC (4 mM) in different time periods.

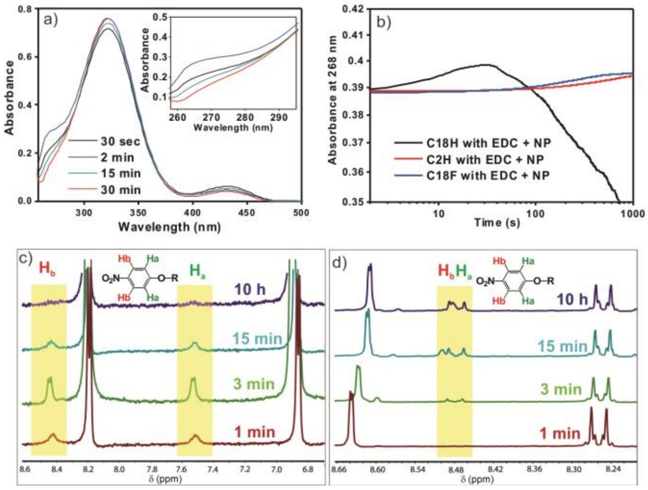

Figure 3. a) Time dependent absorption spectra of **C18H** with NP and EDC. Inset shows the enlarged view of the absorption peaks at 268 nm. b) Change of absorption at 268 nm with time. Time dependent ^{1}H NMR spectra of c) **C18H** and d) **C2H** with NP and EDC at 27 °C (R in the chemical structures denote the rest of the molecular structure).

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supramolecular networks as observed from TEM (Figure 4). TEM also revealed no network like morphologies for **C2H-NP** containing sample (Figure S4). These results point that in the coassembled state of **C18H-NP** and **C18H**, the proximal histidines could be exhibiting cooperative effect. In this context, if a less hydrolysable ester can be accessed, this out of equilibrium state may be realized for longer time, a phenomenon experimentally observed in case of microtubules.[20] Hence, 4-methylphenol (MP) was used as the nucleophile to access the kinetically more stable ester (Figure 2a, **C18H-MP**). Due to replacement of electron withdrawing group of -NO_2 with positive inductive effect promoting group of methyl, the ester bond will be less prone to hydrolysis in the assembled state. Indeed, addition of 4-methylphenol along with EDC resulted in the partial conversion to **C18H-MP** which formed a stable gel with life time of ca. 3 days (Figure 2b). For support of cooperative catalysis, rates of hydrolysis of ester were measured as a function of pH, from 5.5 to 7.9 in buffer controlled conditions (Figure 4e). Notably, the rate of the hydrolysis of the C18H-NP showed a bell shaped dependence with highest activity at pH=6.5 which is in the proximity of the p*K*a of the imidazoles (Figure 4e). This suggests the dual cooperative role of the proximal imidazoles as nucleophile and as proton-donor.[34] At pH=7.9, base catalysed rate of formation of the ester could be observed (V*i*=1.11 µM min^{-1}).

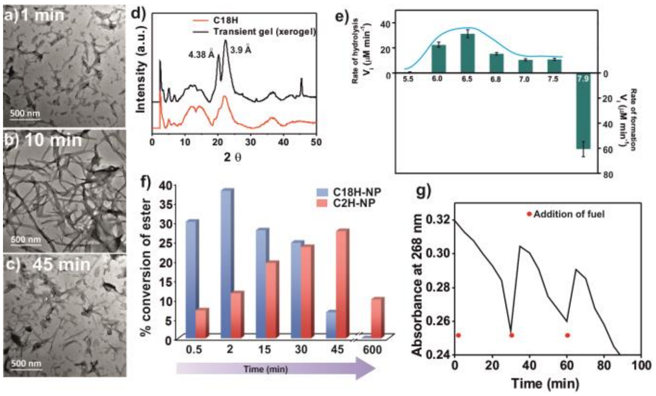

Figure 4. a-c) Time resolved TEM images of **C18H** in presence of NP and EDC. d) PXRD patterns of **C18H** and transient gel (xerogel). e) Rate of hydrolysis of ester as a function pH 5.5 to 7 and the rate of formation of ester at pH 7.9. f) Time resolved conversion of **C2H**-NP and **C18H**-NP. g) Reversible cycles of dissipative assembly-disassembly process fuelled by repeated addition of EDC measured by monitoring absorbance at 268 nm.

To measure the extent of conversion of acid to ester, UV Vis spectroscopy was used. From a standard plot created with synthesized **C2H-NP** (Figure S5), the concentration of the transient ester **C18H-NP** was calculated. Rapid conversion from acid to ester with conversion of ca. 1.5 mM occurred in the first 2 minutes (Figure 4f). This corresponded to ca. 38 % of the NP present (4 mM). The concentration of the ester gradually decreased with time (Figure 4f). This result suggested that coassembly of **C18H** and **C18H-NP** is responsible for the creation of the transient gel. This finding from UV Vis spectroscopy is supported by the observation from ^{1}H NMR spectroscopy (Figure 3c). Strikingly, the gel remains stable even when only a fraction of transient ester (ca. 0.79 mM) is present in the system after 30 min. It will be important to note here that **C18H** on its own does not form gel till 30 mM concentration. To investigate whether the two components are involved in co-assembly or self-sorting, CD spectra were recorded (Figure S6, SI).[35] Since **C18H-NP** is prone to hydrolysis, we chose the more stable ester **C18H-MP** (Figure 1). The CD spectrum of **C18H** showed a positive Cotton peak at 265 nm and was clearly distinguishable from the spectrum of **C18H-MP**, which showed two low intensity broad peaks at 290 nm and 322 nm (Figure S6a, S6b). Notably, CD spectrum of 1:1 concentration mixture of **C18H** and **C18H-MP** did not match with the spectrum obtained from the simple sum (theoretical) of the CD traces of these components (Figure S6c) suggesting co-assembled structures. Further, the plot of CD intensity at λ=265 nm versus concentration ratios of the components did not yield a linear relationship suggesting the absence of self-sorted assembled structures (Figure S7, SI).[35, 36] To check whether the system can be made reversible by addition of fuel again, EDC was mixed after the gel to sol transition occurred. Addition of 4 mM of the coupling agent resulted in the reformation of the self-supporting gel which showed similar dissipative nature and showed transition to sol in ca. 30 min. The cycle could be repeated for 3 times in total. Refueling on the fourth occasion did not result in gelation presumably due to the accumulation of waste in form of urea in the closed system. By monitoring the kinetics at wavelength 268 nm, the generation and subsequent disassembly was demonstrated through spectroscopy (Figure 4g).

In summary, we demonstrate a chemically fuelled transient self-assembled system which dissipates energy from the assembled state by utilizing cooperative catalysis. Lipid functionalized histidine is used to facilitate both the reverse hydrolysis step in disassembled form and augmented hydrolysis step due to cooperative effects in the assembled state. This simple chemical system mirrors the microtubule filament formation, where fuel drives assembly and the accelerated catalytic activity at assembled state results in degradation of fuel and disassembly.[21,27]

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Conflict of interest

The authors declare no conflict of interest.

Keywords: Catalysis • Cooperative effects • Dissipative self-assembly • Gels • Systems chemistry

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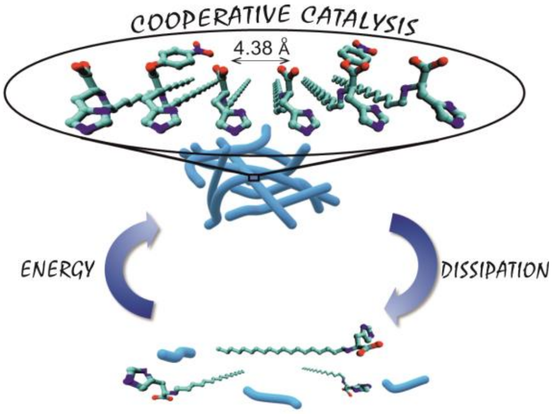
Self-Driving: A simple fuel driven self-assembled system is demonstrated which achieves out of equilibrium state by dissipating energy from the assembled structures. This is done utilizing cooperative effects of the proximally located histidines which catalyse the hydrolysis of ester bonds of the lipid tailed amphiphile. This simple system mirrors the dissassembly observed in case of microtubule filaments where augmented catalysis at assembled state results in energy dissipation and subsequent disassembly.



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