Switchable Supracolloidal 3D DNA Origami Nanotubes Mediated through Fuel/Antifuel Reactions

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3D DNA origami provide access to the *de novo* design of monodisperse and functional bio(organic) nanoparticles, and complement structural protein engineering and inorganic and organic nanoparticle synthesis approaches for the design of self-assembling colloidal systems. We show small 3D DNA origami nanoparticles, which polymerize and depolymerize reversibly to nanotubes of micrometer lengths by applying fuel/antifuel switches. 3D DNA nanocylinders are engineered as basic building block with different numbers of overhang strands at the open sides to allow for their assembly via fuel strands that bridge both overhangs, resulting in the supracolloidal polymerization. The influence of the multivalent interaction patterns and the length of the bridging fuel strand on efficient polymerization and nanotube length distribution is investigated. The polymerized multivalent nanotubes disassemble through toehold-

mediated rehybridization by adding equimolar amounts of antifuel strands. Finally, Förster Resonance Energy Transfer yields *in situ* insights into the kinetics and reversibility of the nanotube polymerization and depolymerization.

Introduction

Nature and man-made technologies display abundant examples for hierarchically self-assembled structures and functional materials, which react to external stimuli and reconfigure on demand.^{1, 2} Particularly intriguing is for instance the cytoskeleton, which employs multiple assemblies for transport, cell movement and cell reorganization: actin filaments, intermediate filaments and microtubules.^{3, 4} In nature, many of the sophisticated structures – in particular filaments and nanotubes – are built up by monodisperse proteins that organize in a highly specific manner. Using protein engineering concepts, there has been relevant progress to obtain protein-based nanotubes or filaments *ex vivo*.⁵⁻¹¹ Even though structural engineering of such proteins has advanced considerably, it remains a challenge to *de novo* design protein nanoparticle building blocks capable of fibrillar assembly and with elaborate control of switching interactions. In contrast, tailor-made, synthetic building blocks with defined dimensions and controlled interaction patterns could provide an alternative. This way, rational design strategies can be applied for *de novo* design of self-assembling nanoparticle systems and new types of switching mechanisms can be implemented.¹²⁻¹⁶ One particular challenge is to go beyond simple fibrillar assemblies, that are for instance attainable by block copolymer systems¹⁷ or gold nanorods¹⁸, and find pathways for the rational design of filamentous nanotube structures that can contain a spatially segregated compartment in the interior.

DNA is a promising material to design building blocks for hierarchical assembly due to its precise programmability. For instance, nanotubes¹⁹⁻²² can be self-assembled with DNA tiles composed of a few strands. Franco and coworkers demonstrated reversible growth by using pH²³ and toehold-mediated strand

displacement,²⁴, using a sequence overhang to allow strand displacement by an invading DNA strand,²⁵ a technique which becomes of growing importance in DNA strand displacement cascades and DNA computing.²⁶⁻²⁹ While such strategies deliver nanotubes, they do not proceed via an intermediate defined nanoparticle/colloid level - similar to engineered proteins - which could provide advantages for *e.g.* functionalization with enzymes or inorganic nanoparticles. A potential strategy towards precision design of supracolloidal self-assembling systems is the use of 3D DNA origami, where a long DNA scaffold is folded into a pre-designed and distinct nanoparticle shape by staple strands.³⁰ DNA origami have been successfully employed as building blocks for hierarchical self-assembly, yielding finite-size superstructures^{31, 32} as well as periodic assemblies like 2D lattices or fibrils.^{15, 33-35} Hollow 3D DNA origami were used to harbor enzymes for cascade reactions and enhanced catalytic activity could be shown by dimerization of them.³⁶ We previously demonstrated supracolloidal fibrils of solid divalent 3D DNA origami cuboids and showed how classical dsDNA hybridization as well as non-DNA host/guest interactions can be used for their organization, and how multivalency and cooperativity effects can be unraveled using such monodisperse building blocks.^{34, 37} Saccà and coworkers showed 3D DNA origami fibrils formed by base stacking and reconfigured their stiffness using DNA hybridization.³⁸ However, reports on DNA origami-based nanotubes are still scarce,³⁹⁻⁴² and triggers giving additional external control for reversible growth have not been employed for DNA-origami based fibrils so far.⁴³ Growing interest in DNA strand displacement has shown the extreme potential of using DNA itself as trigger. Toehold-mediated strand displacement uses the thermodynamic gain offered by the DNA toehold to Isolated nanocylinders and their polymerized nanotubes would be of general interest for controlled drug delivery, templated material growth, as membrane channels or even as artificial filaments for biomaterials.^{19, 22, 39, 44}

Here, we present a first approach towards switchable supracolloidal nanotube assemblies based on distinct 3D DNA nanocylinders (3D-DNA-NC), that are monodisperse in their size and cavity. We report how

external fuel strands bridging the ssDNA overhangs emanating from the two faces of these 3D-DNA-NC guide the supracolloidal self-assembly as a function of overhang connector density, salt content and hybridization length. Building on this, we implement fuel/antifuel switching mechanisms using a strand displacement reaction. Moreover, we use Förster Resonance Energy Transfer (FRET) of appropriately functionalized interaction patterns to *in situ* read out details of the assembly/disassembly as a function of changes in the interaction strength.

Results and Discussion

Design of 3D DNA Origami Building Blocks

Our building block for nanotube formation consists of a 3D DNA origami hollow nanocylinder, that is abbreviated as 3D-DNA-NC. The DNA strands exiting at both open sides are in general passivated with ssDNA overhangs of 15 thymine nucleobases (nb) to prevent unspecific interactions. However, up to 24 ssDNA strands with a specific sequence protrude from each end, termed “connector overhangs”, shown in blue in Figure 1. These connectors are not complimentary, but need an additional bridging strand (fuel strand $A^* = A_1^*A_2^*$, shown in black) to hybridize both connector ends A_1 and A_2 (Figure 1b). This allows to trigger the supracolloidal polymerization and nanotube formation. The bridging strand can be modified with a toehold sequence B (Figure 1c). This introduces the possibility to add an external antifuel trigger strand, B^*A , to remove the bridging strand, BA^* , via toehold-mediated strand displacement, allowing for a depolymerization of the nanotubes to single colloidal 3D-DNA-NC. Moreover, up to six connector strands per side can be end-modified with fluorophores Alexa Fluor 568 (AF568) and Alexa Fluor 647 (AF647) to enable FRET measurements (Figure 1d). The sequences of all DNA strands used are in Figure S1, Table S1 to S10 and the positions of all connector strands are shown in Figure S2.

Figure 1. Design and characterization of 3D-DNA-NC and their switchable self-assembly into supracolloidal DNA nanotubes. (a) Left: 3D-DNA-NC are folded from the M13mp18 scaffold and 5x excess of staple strands. The design shows core staples in green, T₁₅ poly-thymine overhangs in pink and connector strands in light blue. Center: Front view of the 3D-DNA-NC. Positions of the connector strands are marked in light blue. Right: The 3D-DNA-NC can be switched reversibly using fuel/antifuel strands (b) Self-assembly of 3D-DNA-NC into nanotubes. The fuel strand (black) hybridizes with connector strands A₁ and A₂ from two different DNA origami. (c) Toehold-mediated strand displacement with an antifuel reversibly depolymerizes the supracolloidal nanotubes to single building blocks. (d) End-modification of connector strands with AF568 (green) and AF647 (red) and their positions in the 3D-DNA-NC. (e) Negatively stained TEM image of 3D-DNA-NC. (f) AGE of 3D-DNA-NC with different connector density, each containing six AF568 modified connectors and six AF647 modified connectors. The green channel corresponds to the AF568 filter and the red channel corresponds to the AF647 filter. The AGE image is a merger of green and red channel. DNA ladder not fluorescently labelled, thus not shown.

The 3D-DNA-NC was folded from a M13mp18 scaffold in the presence of a 5x excess of staple strands using a temperature ramp. Transmission electron microscopy (TEM) confirms the correct folding into the 3D-DNA-NC with a length of 30 nm, a diameter of 25 nm and an inner cavity diameter of 15 nm (wall thickness = 5 nm; Figure 1e). Purification by spin filtration was used to remove all excess staple strands. Lanes 1 to 3 of the agarose gel electrophoresis (AGE, Figure 1f) indeed only display one sharp fluorescent band for the 3D-DNA-NC with different connector strand density and modified with AF568 and AF647. Comparison with reference lane 4 confirms that no free fluorophore-labeled connector strands remain. Some 3D-DNA-NC dimers are visible in the AGE. We however attribute their observation to non-specific interactions in the AGE, as TEM displays well separated objects due to the T₁₅ passivation.

Supracolloidal Nanotube Polymerization

The driving force for the self-assembly into supracolloidal nanotubes should in principal depend on the length of the fuel strand (overlap) and on the number of connectors (*i.e.* the multivalency) at the patches. Additionally, different procedures for assembly can be considered. Indeed, we first investigated two different procedures for the growth into the nanotube structures: (1) *in situ* fibrillation and (2) post-folding fibrillation. For both we use a fixed connector density of 16 with a bridging fuel strand of 22 nb, hence with a hybridization overlap of 11 nb to both sides (T_m of 38 °C). Firstly, for the *in situ* fibrillation (Figure 2a), the bridging fuel strand is directly added to the origami folding mixture with a 1:1 equivalence to the connector strands, which again are in 5x excess to the scaffold. Even though this means fuel strands are in excess with respect to the fully formed origami (20 nM), TEM images (Figure 2b) prepared at room temperature show that nanotubes of up to 1 μm (~30 origami) and a number average degree of polymerization of $\bar{X}_n = 5.7$ form. This is due to the multivalent design, where cooperative binding and entropic effects ensure that nanotube polymerization is favored over passivation of origami ends by dangling non-bridging fuel strands.³⁷ After removal of excess staple and fuel strands by spin filtration and further incubation at 37 °C for 2 days the nanotubes grow to over 2 μm (~60 origami) and $\bar{X}_n = 8.7$. Further incubation allows for re-shuffling of fuel strands due to the proximity of the incubation temperature to the T_m of 38 °C. This triggers some dynamic self-correction mechanisms and further nanotube growth as the fuel strand is able to dehybridize and rehybridize easily at this temperature, leading to a thermodynamically preferred polymerization.

Figure 2. Two strategies for nanotube assembly of 3D-DNA-NCs using 16 connectors and fuel strands with 11 nb per side. (a) Scheme for *in situ* fibrillation: Addition of fuel strand (black) to folding mixture of scaffold and staple strands. Formation of short nanotubes during cooling ramp and long nanotubes after removal of excess strands by spin filtration and further incubation. (b) TEM images of 3D-DNA-NC nanotubes for *in situ* fibrillation. Left: nanotubes before purification. Right: nanotubes after purification and incubation at 37 °C for 2 days. (c) Scheme for post-folding fibrillation: Addition of fuel strand (black) after folding and purification of single 3D-DNA-NCs. (d) TEM images for post-folding fibrillation. Left: purified, isolated 3D-DNA-NCs before addition of fuel. Right: nanotubes after addition of equimolar amounts of fuel and incubation at 37 °C for 2 days. (e) Statistical TEM image analysis of nanotube length for both procedures. Lines are calculated as Gaussian distributions and added as guide for the eye. Conditions: Concentration of 3D-DNA-NC = 20 nM, 5 mM Mg^{2+} , pH 7.2.

Secondly, in post-folding fibrillation, the bridging fuel strands are added after the 3D-DNA-NC are folded and purified (Figure 2c). After incubation at 20 nM and 37 °C for 2 days to ensure completed polymerization, the nanotube lengths are generally a bit shorter and reach up to 700 nm (~20 origami) with $\bar{X}_n = 4.9$. Figure 2e compares the statistical TEM image analysis of the nanotube length distribution for both procedures. *In situ* fibrillation leads to longer nanotubes, most likely due to the higher dynamics in the system during the annealing procedure as nanotube assembly and building block formation takes place simultaneously.

Variation of the Connector Strand Density and Mg^{2+} Concentration

Next, we analyze the influence of changing the connector density and the salt concentration on the length distribution of the growing nanotubes for the *in situ* fibrillation method. All evaluations were done after purification to remove excess strands and incubation for 2 days at 37 °C. Interestingly, when reducing the

connector density from 16 (above Figure 2) to only 8, nanotubes hardly form and mostly dimers are visible (5 mM Mg^{2+} ; Figure 3a, c). An increase to 24 connectors leads to a slight increase in nanotube length with $\bar{X}_n = 8.8$ (Figure 3e), compared to the previously used 16 connectors. Hence, increasing the multivalency by increasing the connector density intensifies the binding strength between the origami. The cooperativity arises when after the first binding event the other connectors are brought into vicinity, favoring further hybridization with bridging fuel strands.⁴⁵⁻⁴⁷

Figure 3. Supracolloidal nanotube polymerization as function of the connector density and salt concentration. (a) TEM images of nanotubes with 8, 16 and 24 connectors (top to bottom). (b) TEM images of nanotubes with 16 connectors and 10, 15 and 20 mM Mg^{2+} (top to bottom). (c) Statistical TEM image analysis of the nanotube length distribution for 8 (black), 16 (red) or 24 (blue) connectors. (d) Statistical TEM image analysis of nanotube length distribution for increasing salt content for nanotubes with 16 connectors. (e) Degree of polymerization for increasing connector density at 5 mM Mg^{2+} . (f) Degree of

polymerization depends on salt content for all connector densities. Scale bars 500 nm. All nanotubes were formed *in situ* at a total concentration of 20 nM, purified, and were further annealed for 2 days at 37 °C.

An increase of the Mg^{2+} concentration after purification supports nanotube growth by shielding the negative charge of the 3D-DNA-NC (Figure 3b, d). By elevating the Mg^{2+} concentration from 5 mM to 20 mM the $\bar{X}_n$ increases considerably from 8.7 to 13.3 at 16 connectors (Figure 3f). A particular change in the distribution occurs, as monomers, dimers and short oligomers are less abundant. Since the electrostatic repulsion of the 3D-DNA-NCs is reduced at higher ionic strength, the overall binding affinity between the building blocks increases. A similar trend is consistently observed for all connector strand densities, yet for the 8 connectors, the increase in nanotube growth levels off at ca. 10 mM Mg^{2+} . This may relate to the general challenge in imaging such fibrils, which is that rupture of nanotubes during droplet deposition cannot be fully excluded and because largest fibrils tend to aggregate (and hence cannot be considered in the statistical evaluation). Therefore, some even longer DNA nanotubes cannot be incorporated into the statistics. Shear-induced rupture during sample preparation is more likely for this low connector density (8) with a mechanically weaker connection and may contribute to an apparent restriction of the nanotube length. TEM images and statistical distributions of the nanotube length for 8 and 24 connectors at higher Mg^{2+} concentrations are shown in Figure S3.

Influence of Hybridization Length of the Bridging Fuel Strand

Moreover, the choice of the fuel length is decisive for nanotube growth with respect to the system temperature. We tested this effect for overlap lengths (hybridization lengths) of 5, 8, 11 and 13 nb on each side of the bridging fuel strands, which leads to T_{ms} of ~ 0, 22, 38 and 50 °C as measured by UV-Vis. When performing the *in situ* fibrillation at a connector density of 16, the shortest bridging strand with 10 nb (5 nb on each side) does not lead to any assembly during incubation at 37 °C (Figure 4a, b). This demonstrates that the multivalency effects are not pronounced enough and lead to a too low binding affinity. This behavior is different to DNA mediated colloid assembly, where large colloids can be

efficiently linked using overhangs as short as 4 nb due to strong multivalency effects at comparably flat and large surfaces in such larger systems.⁴⁸⁻⁵⁰ Short nanotubes form for a hybridization length of 8 nb at each overhang (total fuel strand length 16 nb) with a T_m of 22 °C. The longest nanotubes with a $\bar{X}_n$ of 8.7 are observed when the T_m matches the incubation temperature of 37 °C, which is the case for 11 nb per side with a T_m of 38 °C. Interestingly, a further increase of the hybridization length to 13 nb with a T_m of 50 °C leads to a significant decrease in nanotube length with $\bar{X}_n$ dropping down to 4.2 (Figure 4c). This behavior can be explained by the loss of dynamics for the fuel strand exchange at a T_m substantially higher than the incubation temperature, where rehybridization of fuel strands and corrections of oversaturated origami faces are less efficient. Additionally, TEM indicates a higher amount of ill-formed 3D-DNA-NC for such samples, which is likely due to the fact that the early hybridization with the bridging fuel strands during the folding impedes proper 3D-DNA-NC folding. Hence, we conclude that the inter-origami recognition forces need to be strong enough to ensure stable nanotubes at room temperature, but weak enough to allow reversible binding for self-correction mechanisms.¹ Due to the multivalent design using 16 connectors, small changes in the length of the bridging fuel strand drastically change the interaction strength on the colloidal level (multivalency) and, therefore, the overall behavior of the system as a whole.⁵¹

Figure 4: Influence of the hybridization length of the fuel. (a) TEM images of nanotubes formed with fuel having 5 to 13 nb hybridization overlap with each connector, respectively. T_m by UV-Vis, except for 5 nb, as predicted by the IDT OligoAnalyzer. (b) Statistical TEM image analysis of nanotubes polymerized with fuel of different hybridization lengths. (c) Degree of polymerization for increasing hybridization length. All nanotubes were formed *in situ* at a total concentration of 20 nM, purified and further annealed for 2 days at 37°C .

Reversible Switching of Nanotubes

Building on this understanding, we will next turn to realizing a switching of the 3D-DNA-NC nanotubes by adding a 15 nb toehold to the original fuel strand containing 11 nb on both sides (Figure 5a). We now use the post-folding fibrillation method and start from individual 3D-DNA-NC (20 nM) and first polymerize them at 37°C by addition of an equimolar quantity of fuel strand (320 nM) for the 16 connector strands at the 3D-DNA-NC (2 days). Afterwards, the addition of an antifuel strand leads to toehold-mediated strand displacement that we hypothesized to be strong enough for DNA nanotube breakage.

Indeed, once the antifuel strand is added (1 eq, 1 h incubation at 37 °C), TEM clearly depicts breakage into individual units. This whole process is highly reversible and further addition of fuel repolymerizes the nanotubes, as confirmed by TEM images shown for three consecutive switches in Figure 5b. Although waste in the form of stable duplexes accumulates with each switch, the statistical distribution of nanotubes shows a very similar length distribution for each cycle. Hence, the switch is highly reversible. This strategy underscores that toehold-mediated strand displacement reactions can be applied in highly multivalent 3D DNA fibrillating systems with strong confinement of the interacting strands at the two sides.

In the previous approaches trying to break multivalent 3D DNA origami superstructures a heavy excess of binding partners (at least 10 eq) was needed to break such fibrillar assemblies, because the multivalent binding gain needed to be overcome.^{34, 37} Here the multivalency can be overcome by the gain in free energy provided by the toehold-mediated hybridization of the full fuel/antifuel strand pair involving the additional toehold area.²⁵ The susceptibility of the switch to operate with equimolar quantities limits waste accumulation, which may provide higher levels of robustness for reversible switching and reduces crosstalk in more complex systems. Our switching of the nanotubes therefore extends the toehold-mediated fuel/antifuel switching mechanism from previous switching of DNA tile assembly^{24, 29} and 2D DNA origami⁵² to 3D DNA origami filamentous superstructures.

Figure 5: Reversible switching of supracolloidal 3D-DNA-NC-based nanotubes by toehold-mediated fuel/antifuel rehybridization using equimolar quantities. (a) Scheme of the fuel/antifuel switching mechanism. (b) TEM images of 3D-DNA-NC reversibly polymerizing to nanotubes and depolymerizing back to single building blocks. Insets show the statistical distribution of nanotube length. Nanotubes were formed after fuel addition for 2 days at 37 °C. Samples were incubated for 1 h at 37 °C after antifuel addition.

***In Situ* Analysis of Reversible Nanotube Assembly by FRET**

Next, we turn to the particular challenge of developing a strategy to measure this switching mechanism with an ensemble average *in situ* technique, which to the best of our knowledge has not been realized for such periodic 3D DNA origami assemblies. To this end, we modified six of the connector strands on the respective sides of the 3D-DNA-NC with fluorophores amenable to FRET (Figure 6a). One side bears AF568, while the other side is modified with AF647. Upon nanotube assembly these fluorophores are

brought into close vicinity, allowing a FRET from the donor dye AF568 to the acceptor dye AF647. The whole fluorescence spectrum is shown in Figure S4.

We first focus on a 3D-DNA-NC with 16 connector strands (Figure 6b). Indeed, the FRET ratio as measured by the ratio of the two emission maxima ($I_{AF647}/I_{AF568} = I_{670nm}/I_{590nm}$) increases as soon as 1 eq fuel is added to the fluorophore-modified 3D-DNA-NCs (Figure 6b). The FRET ratio reaches a plateau after ca. 2 h, which is indicative of the majority of the assembly being completed in this time frame. A negative control without any fuel does not show any FRET, as expected.

FRET measurements allow to quickly access the *in situ* behavior at different hybridization lengths of the bridging fuel strand. For post-folding fibrillation at 37 °C, the FRET ratio is highest upon addition of 1 eq fuel with a hybridization length of 13 nb, where the bond is stable at this elevated temperature ($T_m = 50$ °C, Figure 6b). On the contrary, for 11 nb the bridging fuel is partly hybridized ($T_m = 38$ °C), giving a reduced FRET. The bridging fuel strand with only 8 nb is almost fully dehybridized at this temperature ($T_m = 22$ °C), and, consequently, shows no FRET increase at all. This FRET behavior is confirmed by statistical TEM image analysis (Figure 6c), in which an increase of nanotube lengths is achieved by using longer hybridization lengths of the fuel strand. This behavior is slightly different to the *in situ* assembly method (Figure 4), where the longest fuel strand with a hybridization length of 13 nb yields considerably shorter nanotubes compared to 11 nb due to some crosstalk in the folding process. Here we use pre-folded 3D-DNA-NC and post-folding assembly, which prevents this unwanted crosstalk.

Interestingly, despite the large size of the origami units, polymerization proceeds quite fast with the maximum FRET ratio reached in only 0.5 h. The slope of the time-resolved FRET ratio curves correlates with the polymerization rate. By evaluating the initial slope of the FRET increase, it can be concluded that polymerization rate qualitatively increases with the hybridization length and connector density (Figure S5) as the inter-origami binding forces are strengthened.

Figure 6. *In situ* monitoring of reversible nanotube polymerization by FRET. (a) Scheme illustrating the decoration of 3D-DNA-NC with fluorophores and reversible FRET during (de)polymerization. (b) Time-resolved FRET ratio at 37 °C for 3D-DNA-NC with 16 connectors and different hybridization lengths. 3D-DNA-NC with no fuel added as reference (green). The measurements are an average of three, the shaded area is the standard deviation. (c) Statistical analysis of TEM images of nanotube length distribution for post-folding fibrillation after 2 h at 37 °C for different hybridization lengths. (d) Time-resolved FRET ratio showing repeated addition of fuel and antifuel at 25 °C for different connector densities. The measurements are an average of two, the shaded area is the standard deviation. Origami concentrations are 10 nM at 5 mM Mg²⁺.

Most importantly, FRET enables to measure the reversible (dis)assembly of nanotubes *in situ*. We investigated this behavior for the 22 nb fuel strand (11 nb per overhang) with a toehold in conjunction with 3D-DNA-NC bearing different connectors (8, 16 and 24; Figure 6d). Since the post-folding fibrillation at 37 °C does not lead to strong assembly for 8 connectors due to reduced inter-origami interaction (Figure 3), we investigated the reversible switching of nanotubes at 25 °C (for switching at 37 °C, see Figure S6). At this temperature, an increase in FRET ratio can be observed for all connector densities, and a scaling of the FRET ratio with the connector density occurs. This in turn correlates very well with the previously observed increase in nanotube lengths for higher connector densities in the *in situ* fibrillation (Figure 3). After 2 h of incubation, 1 eq of antifuel was added and the FRET ratio decreases as the nanotubes disassemble. Using this concept, Figure 6d displays a successful and repeated switching for three times. Some shifts in the respective FRET ratios after multiple switching appear, but we suggest that this may be caused by scattering effects as the volume increases with each fuel/antifuel

addition and some photobleaching effects. Nonetheless, these shifts are minor thanks to a stable switching of the system and low standard deviations confirm its reproducibility.

Conclusion

In summary, we demonstrated the regulation of the supracolloidal polymerization of 3D DNA origami-based nanotubes using fuel/antifuel strand principles and exploiting toehold-mediated strand displacement reactions. We first explored in detail how multivalency and fuel length overlap provide sufficient driving force for the polymerization and how increased salinity and the correct assembly protocols can assist the formation of long nanotubes. We found that the energetic gain by addition of a short toehold to the bridging fuel strand provides sufficient thermodynamic driving force for disassembly using equimolar amounts of antifuel even for multivalent assembled systems. Additionally, we introduced *in situ* FRET measurements to monitor supracolloidal self-assembly of 3D DNA origami structures and could give first insights into the kinetics and dynamic behavior of the nanotube polymerization. We believe this work is a promising approach to merge the fields of colloidal self-assembly and DNA nanotechnology while advancing the implementation of biological self-assembly principles into the supracolloidal world. Our approach is versatile and the antifuel approach could be extended to other switches, such as the introduction of a DNA catalytic circuit²⁹, an enzymatic reaction network⁵³ or application of sensors using for example pH²³. We believe that the FRET approach will allow to study the self-assembly trajectories and energy landscapes of such systems in greater detail in future, as it grants higher temporal resolution and presents a non-invasive form of monitoring the system compared to classical *ex situ* imaging techniques.

Experimental

Materials. M13mp18 scaffold and folding buffer was purchased from tilibit, DNA strands were purchased from IDT and IBA Lifesciences. Agarose gel was received from AppliChem. MgCl₂ (1 M) and

NaCl (5 M) were ordered from Fisher Scientific. Boric acid, hexadecane, magnesium acetate and TRIZMA were obtained from Sigma Aldrich. EDTA was purchased from Carl Roth. Carbon film 300 mesh copper grids and uranyl acetate (>98%) were bought at EMS.

Devices. TEM images were taken with a FEI L120 operating at 120 kV. Agarose gel electrophoresis was executed in a water-cooled CBS Scientific HSU-020 gel electrophoresis chamber using an Enduro 300 V power source. Gel imaging was done with an INTAS ECL Chemostar. DNA origami were folded in a Biometra TPersonal Thermocycler. Nanotubes were incubated in an Eppendorf ThermoMixer C. UV-Vis measurements were conducted on an AnalytikJena ScanDrop 250 using a Tray cell cuvette from Hellma with a path length of 1 mm or a Hellma microcuvette with a path length of 3 mm. Fluorescence spectroscopy was done with the Tecan Spark plate reader in top mode.

Folding of 3D-DNA-NC. The 3D-DNA-NC were designed with the program cadnano⁵⁴ and their design confirmed with the software cando.^{55, 56} Three master mixes of staple strands were prepared. Master mix 1 (MM1) contained all staple strands to form the core of the origami. Master mix 2 (MM2) contained the poly-T passivated strands and connector strands while master mix 3 (MM3) contained ssDNA overhangs (not used here) for additional functionalization. The concentrations of each staple strand in the master mixes were 666.7 nM, 666.7 nM and 1000 nM, respectively.

Typical folding mixtures contained 15 μ L MM1, 15 μ L MM2, 10 μ L MM3, 20 μ L scaffold (100 nM), 10 μ L of 50 mM NaCl, 11 μ L of 200 mM MgCl₂ to give 100 μ L of folding mixture with 20 nM scaffold, a 5x excess of staple strands (100 nM) and 22 mM MgCl₂. The volume of milliQ water varied depending on whether fluorophore-modified connector strands were added (1.25 μ L, 100 nM) and/or fuel strand.

Temperature ramp for annealing: 80 °C: 15 min, 80 to 60 °C: 5 min per 1 °C, 60 to 40 °C: 3 h per 1 °C, 40 to 25 °C: 1 h per 1 °C, Stay at 4 °C, Lid temp: 80 °C; Folded DNA origami were stored at 4 °C until further use.

Purification of 3D-DNA-NC. The folded 3D-DNA-NC mixtures were purified by spin filtration with Amicon 100 kDa spin filters at 10,000 g and 15 °C for 5 min. The samples were washed 6x with FoB5 buffer (5 mM TRIS, 1mM EDTA, 5 mM NaCl, 5 mM MgCl₂, pH 7.2)³⁰ and recovered by turning them upside down into a fresh tube and centrifuging at 5000 g for 3 min.

Polymerization of 3D-DNA-NC. *In situ* fibrillation: Fuel strands were added to the origami folding mixture (800 nM for 8 connectors, 1600 nM for 16 connectors, 2400 nM for 24 connectors) and the origami were folded and purified as described above. After purification, the Mg²⁺ concentration was increased if needed and samples were incubated for another 2 days at 37 °C at 300 rpm in a thermoshaker. Post-folding fibrillation: 3D-DNA-NCs were purified by spin filtration and the Mg²⁺ concentration was adjusted. The concentration was evaluated by UV-Vis at 260 nm and the fuel strand was added in a 1:1 ratio, in respect to the ssDNA connector strands. The samples were incubated for 2 days at 37 °C and 300 rpm in a thermoshaker unless otherwise stated.

Reversible switching of 3D-DNA-NC nanotubes. 3D-DNA-NCs were purified and diluted to 20 nM. 1 eq of 22 nb fuel with toehold was added and the mixture was incubated at 37 °C, 300 rpm for 2 days. For depolymerization, 1 eq of antifuel was added and the sample was incubated for 1h. Next, 2 eq of fuel was added to assure sufficient quantity and the sample was again incubated for 2 days. For the next depolymerization, 2 eq of antifuel was added, followed by incubation for 1 h. For repolymerization, 3 eq of fuel was added, followed by incubation for 2 days. For imaging, nanotubes containing waste of fuel/antifuel from previous switches were washed twice with 200 µL Tris-buffer to improve imaging.

TEM sample preparation. 3 µL of sample were incubated for 60 s on plasma-cleaned copper grid, then blotted away using filter paper. 3 µL of milliQ water was dropped on the grid and blotted away immediately afterwards. For negative staining, 3 µL of 1 wt% uranyl acetate solution was incubated on the grid for 20 s before being blotted away.

Agarose gel electrophoresis. Gels were prepared with 1.5 wt% agarose in TBE buffer (22.25 mM Tris base, 22.25 mM boric acid, 0.5 mM EDTA, 6 mM magnesium acetate) and cast without stain. Gels were run at 3 V/cm in a cooled chamber set to 15 °C for 2.5 h. A fluorescent DNA ladder was used and the gels were imaged without staining using the fluorescence of the fluorescent connector strands.

FRET measurements. For fluorescence intensity measurements, a black 384 well plate from Costar Corning was used. Each well contained 20 μ L solution and 10 nM origami. Evaporation was reduced by adding 4 μ L of hexadecane on top. The excitation wavelength was set to 495 nm using an excitation filter. Emission wavelengths were measured using filters at 590 nm and 670 nm. Well plates were pre-incubated for 30 min in the plate reader before the fuel was added. If run at 37 °C, the plate was kept on a thermoshaker at 37 °C during pipetting to prevent cool-down. Each measurement was done at least in duplicate and the average and standard error calculated.

Quantification of TEM images. Nanotubes were counted using ImageJ. For each sample, an average of 300 species was counted.

Measurement of melting curves for fuel. One connector strand was mixed with the fuel in equimolar amounts in FoB5 buffer (480 nM). A UV-Vis spectrum was measured every 180 s while the temperature was cooled down to 2 °C, then heated up to 90 °C and cooled back down over a span of 255 min. An average of at least three separate measurements was used for each fuel strand. The T_m of the 10 nb fuel is too low to be measured and was therefore calculated with the OligoAnalyzer from IDT.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgments

The authors thank Katja Schumann for assistance in laboratory work. We acknowledge support by the European Research Council starting Grant (TimeProSAMat) Agreement 677960. This work made use of

the microscopy facilities provided through the Core Facility “Imaging of Materials Systems” at the Freiburg Center for Interactive Materials and Bioinspired Technologies (FIT).

Conflict of Interest

There are no conflicts to declare.

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Table Of Contents Entry:

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