Control of enzyme reactions by a reconfigurable DNA nanovault

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Biological systems use compartmentalisation as a general strategy to control enzymatic reactions by precisely regulating enzyme–substrate interactions. With the advent of DNA nanotechnology, it has become possible to rationally design DNA-based nano-containers with programmable structural and dynamic properties. These DNA nanostructures have been used to cage enzymes, but control over enzyme–substrate interactions using a dynamic DNA nanostructure has not been achieved yet. Here we introduce a DNA origami device that functions as a nanoscale vault: an enzyme is loaded in an isolated cavity and the access to free substrate molecules is controlled by a multi-lock mechanism. The DNA vault is characterised for features such as reversible opening/closing, cargo loading and wall porosity, and is shown to control the enzymatic reaction catalysed by an encapsulated protease. The DNA vault represents a general concept to control enzyme–substrate interactions by inducing conformational changes in a rationally designed DNA nanodevice.

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The ability to control single enzymes—defined as the ability to inactivate and activate a specific enzyme in a user-controlled fashion—has great interest in basic research, as well as in application-focused fields, such as biotechnology, synthetic biology, nanomedicine and bio-organic synthesis. DNA nanotechnology allows rational design of nanostructures and devices, and represents a promising approach to organise and control enzymes for several reasons. First, DNA strands can be self-assembled into well-defined DNA nanostructures that can be used to spatially organise several different molecular components. Second, enzymes are easily conjugated to DNA strands through several different methods and can be brought specifically together by base pair complementarity. Third, DNA strand displacement can be used to dynamically assemble and disassemble DNA strands or reconfigure a DNA device between several states. DNA nanostructures have been extensively employed to improve the yield of enzymatic cascades by scaffolding multiple enzymes. On the other hand, only few systems to control single enzymes have been presented so far, mostly relying on the controlled interaction between enzymes of interest and a coenzyme, an inhibitor or a separated subdomain of the enzyme itself. However, these strategies have limited use since they depend on specific properties of the enzymes, and a general method for controlling single enzymatic activities is still missing.

In living organisms compartmentalisation is widely used to gate physical interactions between enzymes and their substrates and results in high local concentrations of specific enzymes that enhance reaction kinetics, channel metabolic substrates through enzymatic cascades, and sequester intermediates, thus reducing potential cell toxicity and competing cross-reactions. Membrane-bound organelles are the most common ways to compartmentalise enzymatic reactions in living organisms, but other varieties of nanoscale compartments delimited by proteins exist such as encapsulin shells, carboxysomes, vault particles and virus capsids. Such protein structures are capable of fuelling the activity of the enclosed enzymes by both passively importing specific substrates through selectively permeable shell proteins, and by dynamically controlling the opening of proteic pores that attract and internalise small substrates molecules within the compartment.

Taking inspiration from nature, scientists have developed artificial enzyme compartmental systems including lipid vesicles, polymersomes, polymer capsules and protein cages. Similarly, rationally designed DNA nano-containers have been constructed characterised by solid walls, with the potential to fully enclose large biomolecules and virus capsids. Such protein structures are capable of fuelling the activity of the enclosed enzymes by both passively importing specific substrates through selectively permeable shell proteins, and by dynamically controlling the opening of proteic pores that attract and internalise small substrates molecules within the compartment.

Inspired by the general method of compartmentalisation, we conceive the idea of a DNA nanostructure that can control reactions catalysed by a single enzyme, by enclosing it within an internal cavity and exposing it to free substrate molecules only in response to specific molecular cues. We name this nanostructure the ‘DNA Vault’ (DV) because it is constructed to have solid walls and a multi-lock system to protect the encapsulated enzyme. Here we present the design of a 3D DNA origami nanodevice, characterise its reversible opening/closing mechanism, and load it with cargos of different chemo-physical properties. Furthermore, we develop an assay to characterise the permeability of DNA nano-containers, and use the DV to control the activity of an encapsulated enzyme. In conclusion, we successfully developed a general approach to control enzyme-substrate interactions by inducing conformational changes in a dynamic DNA nanostructure.

Results
Design of the DNA Vault. The DV was designed using the scaffolded DNA origami method based on the honeycomb lattice (Supplementary Tables 1 and 2). The structure is composed of two halves with dimensions of 26 × 42.5 × 9.5 nm³ and 22 × 42.5 × 9.5 nm³, respectively, that are connected through a hinge, and have an inner cavity of 3.2 zeptoliter (Fig. 1b; Supplementary Fig. 1). Close-packing of helices was used to create a tight interface between the two halves of the DV in the closed state (Fig. 1c, top). To control the opening and closing of the DV, five double-helix locks were placed on the two sides and front, and two hairpin locks were placed close to the hinge (Fig. 1b, c; Supplementary Fig. 2; Supplementary Note 1). The locks function by toehold-mediated DNA strand displacement, and are designed to be reversibly opened and closed by the addition of an opening key (OpK) and a closing key (ClK), respectively (Supplementary Figs. 3 and 4). A single-stranded DNA inside the cavity functions as a cargo-anchoring site (CAS) for enzyme loading (Fig. 1d). Finally, the ends of the helices on the outside of the DV are decorated with 28-nucleotide (nt) poly-T loops (Fig. 1e) to reduce aggregation caused by inter-molecular stacking interactions and to additionally decrease permeability of the side-walls.

The nanostructures were assembled and analysed by gel electrophoresis (Supplementary Fig. 5) showing that ~50% of the self-assembled structures are in the monomeric form while the remaining part forms dimers or higher-order aggregates, which is commonly observed for compact 3D DNA origami structures. Moreover, transmission electron microscopy (TEM) images of the closed DV (Supplementary Fig. 6b) indicate that ~65% of the monomeric structures are correctly folded while 35% show visible deformities that may not allow a correct function of the DV. Thus, we estimate that approximately 30% of all the self-assembled DNA nanostructures are correctly folded monomers. This estimate does not take into consideration minor structural errors such as missing staple strands, that are not distinguishable in TEM images, and could also affect the function of the DV.

The dimensions of closed and open structures were measured by TEM. In the closed sample, monomers were identified with back-to-front length of 26.0 ± 2.4 nm (Supplementary Fig. 7c), matching the theoretical value of 26 nm (Fig. 1b). In the open sample a back-to-front length of 30.5 ± 2.7 nm was measured (Supplementary Fig. 7d), matching the theoretical value of 31 nm which is expected for a structure with a tight hinge designed to open to an angle of 90° (Fig. 1b).

Opening and closing mechanism. In order to encapsulate a native enzyme and control its activity the DV structure must have a flexible hinge to be able to reversibly close and open (Fig. 2a). The functionality of the hinge was investigated by labelling the two halves of the DV with Cy3 and Cy5 fluorophores and measuring Förster resonance energy transfer (FRET) upon addition of both OpK and ClK. It was found that an 8-nt hinge is required to close the structure efficiently (Supplementary Note 2; Supplementary Figs. 7b and 8). The DV with 8-nt hinge was assembled in the open state and investigated by TEM (Fig. 2b): the structures are observed in an open state showing the two halves of the DV
adhering to the TEM grid (Fig. 2b, left). After addition of CIK the TEM imaging reveals closed structures with clear staining and contrast of the inner cavity (Fig. 2b, middle). Finally, after addition of OpK the value increased to 30% indicating device closure (Fig. 2c). Upon addition of a 1.3-fold excess of CIK the value increased to 30% indicating device closure (blue line). After stabilisation of the FRET signal a 5-fold excess of OpK was added, which resulted in a fast decrease in FRET efficiency to 13%. The reason why the initial and final FRET values are not approaching 0% could be explained by the presence of DV aggregates in the sample. The opening and closing mechanism is demonstrated to respond only to specific DNA keys (Fig. 2c, green and red lines) and can be repeated multiple times (Supplementary Fig. 9).

Cargo loading. To test packaging of cargos two different strategies were employed: non-covalent, annealing-driven loading of DNA-modified gold nanoparticles (AuNPs), and covalent bond formation between alkyne-exposing CAS and azide-modified enzyme via copper(I)-catalysed alkyne-azole cycloaddition (CuAAC). Initially, AuNPs of 5 nm in diameter were conjugated with 1:1 molar ratio of thiol-modified DNA strands with complementary sequence to the CAS staple strand, and then incubated together with open DV (Fig. 2d). TEM images of the sample revealed a single AuNP located within the cavity of 10–15% of the DNA nanostructures (Fig. 2e, Supplementary Fig. 10a–c). Next, the endopeptidase alpha-Chymotrypsin (aCt) of ~4.4 × 4.7 × 5.1 nm³ in size (PDB id 1YPH) was conjugated with azide handles. In order to exploit the most generally applicable strategy to modify proteins, we reacted commercially available wild-type aCt with NHS ester-azole heterobifunctional cross-linkers. These cross-linkers attach to exposed lysine residues on the surface of the target protein, and can therefore be used on virtually any soluble protein³¹, ⁴⁵. The resulting azide-modified aCt (Supplementary Fig. 11), was then reacted with open DV assembled with alkyne-modified single-stranded CAS module (DV-ssAlk; Fig. 2f). Enzyme-loaded DNA origami nanostructures were then closed and analysed by TEM, showing a single aCt housed within the cavity of 5–10% of the nanostructures (Fig. 2g, Supplementary Fig. 10d–f). The catalytic activity was partially retained upon chemical modification and packaging (Supplementary Note 3).

Permeability of the DNA Vault. To assess the ability of the DV to shield the internal cavity from free-diffusing molecules, a permeability assay was performed (Fig. 3a). The DV was assembled in the open state with a fluorescent CAS module consisting of the Cy3-modified CAS strand annealed to a Cy5-modified DNA cargo strand exposing a single-stranded toehold. The proximity of the Cy3 and Cy5 fluorophores on the CAS is within FRET distance. In order to measure the molecular permeability of the DNA walls of the nanostructure, open and closed DV were incubated with three invaders of different size: Invader A, a short single-stranded DNA; Invader B, a DNA strand with...
The invaders were designed to displace the Cy5-modified cargo from the Cy3-modified CAS leading to separation of the dyes and loss of FRET. Displacement experiments performed on the CAS/cargo system alone (Supplementary Fig. 12) demonstrate that the difference in size and chemo-physical properties of the invader strands does not affect the cargo displacement process significantly.

The cargo displacement process in the DV was observed by measuring changes in the intensity of the Cy3 and Cy5 peaks and calculating the ratio between them (Fig. 3b). Fast displacement of the cargo is observed when any of the invaders is added to the open structure (from 0.95 ± 0.02 to 0.10 ± 0.01) with Invader A having the faster kinetics. Contrarily, slower kinetics are observed when the closed nanostructure is challenged with the invaders (where slower displacement rates are correlated with bigger invaders), showing that the closed DV is shielding the cargo from the invaders. Initially, a fast decrease in cargo signal is observed indicating that a fraction of the DV sample does not shield the cargo efficiently, which can be explained by the presence of malformed structures in the sample, as indicated by the previously mentioned yield analysis. Nevertheless, well-folded structures with a persistent shielding effect are observed when Invader C is added to the closed DV, where the cargo signal stabilizes at a value of 0.35 ± 0.02. This effect is confirmed by adding Invader A to the reaction (star in Fig. 3b), resulting in further decrease of the cargo signal to baseline levels, which indicates that the closed DV shields the cargo from Invader C, but not from the smaller Invader A.

In order to quantify the shielding efficiency further, we calculated the difference between the cargo displaced from open and closed nanostructures (ΔI). The resulting curves are characterised by two kinetic steps (Fig. 3c). When either Invader A or Invader B are used, ΔI quickly increases at first, indicating that the closed DV is protecting the internal cargo from the invaders. However, after 5 min of incubation, ΔI slowly decreases, suggesting that the invaders eventually penetrate inside the closed DV and displace the cargo from the cavity. However, when Invader C is added, ΔI quickly increases at first, and then stabilizes at around 0.25, suggesting that the closed DV persistently shields its internal cavity from particles of at least 5 nm in diameter.

**Control of enzyme–substrate interaction.** Finally, we tested the effect of opening and closing the aCt-loaded DV on aCt activity.
by using the fluorogenic substrate fluorescein isothiocyanate (FITC)-casein with an estimated size of 4.0–4.1 nm in diameter (the size was calculated based on a molecular weight of 28–30 kDa as estimated by gel electrophoresis) (Fig. 3d). Open DV-ssAlk nanostructures were loaded with azide-exposing aCt, purified from excess unconjugated enzyme (Supplementary Note 4 and Supplementary Fig. 13), and closed. The sample was then split in two tubes: one tube was incubated with correct OpK, the other with unspecific key. Finally, open and closed nanostructures were incubated with FITC-casein and analysed at specific time points by measuring fluorescence. It was observed that the enzymatic activity measured on open aCt-loaded DV was up to 3 times higher than on closed nanostructures and increasing linearly during the time analysed (Fig. 3e, f). Proteolytic activity is also observed for the closed DV sample, which can be explained by the presence of incorrectly folded DV nanostructures identified by the yield analysis.

A double-stranded CAS was also tested in order to investigate the possibility to load DNA-conjugated enzymes into the DV by annealing. However, the measured difference between open and closed DV was less prominent compared to DV-ssAlk, suggesting that a bigger enzyme-anchor can affect the ability of the DV to close and thus decreases its shielding property (Supplementary Note 5 and Supplementary Fig. 14).

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**Fig. 3** Permeability assay of the DNA Vault and enzyme activity control. **a** The DV encloses a fluorescent cargo consisting of a Cy5-modified DNA strand annealed onto the Cy3-modified CAS strand, and exposing a single-stranded toehold region. When an invader is added, it can either penetrate into the nanostructure displacing the cargo, or it is shielded from the cavity, in which case the cargo is unaffected and remains within the DV. Three invader strands of different size are used: simple DNA strand (Invader A), dumbbell DNA strand (Invader B), and Streptavidin-bound DNA strand (Invader C). **b** Fluorescence data showing the decrease of relative peak ratio upon invader strand addition. Invader strands were added at 0 min and the fluorescence immediately measured afterwards. The black arrow indicates addition of invaders; the asterisk indicates addition of Invader A to Closed + Invader C after 30 min to confirm active shielding of the enclosed cargo from free-diffusing Invader C molecules. Bars indicate one standard error from the mean fluorescence signal as recorded in either three (Invader B) or four (Invader A and C) replicates. **c** Quantification of the shielding effect of the DV calculated as the difference of intensity values between closed and open samples, as shown in panel b. The black arrow indicates addition of invaders. **d** The proteolytic enzyme aCt (pink) is encapsulated inside the DV, while the substrate (grey circle) is free in solution. The enzyme catalyses the conversion of substrate to product (yellow circle) in the open structure, while the reaction is inhibited in the closed DV. **e** aCt enzymatic activity measured on open aCt-loaded (blue), closed aCt-loaded (red) and open empty DV (control, grey). Bars indicate one standard error from the mean fluorescence signal as recorded in three replicates. **f**, Normalisation of measured enzymatic activity as in e after control subtraction (closed—control at 30 min = 1).
**Discussion**

The current study provides proof-of-concept for enzyme control by shielding from or exposure to substrate using a dynamic DNA origami device. To achieve this goal, we have combined multiple design principles to construct an unstrained dynamic device with reversible opening/closing mechanism and a cavity with limited accessibility. The ability of the DV to physically hinder enzyme–substrate interactions was demonstrated to inhibit the enzymatic activity of an encapsulated protease. The approach should be generally applicable to several enzyme–substrate systems and we provide a protocol that with slight modification should allow the loading of any enzyme in the DV (Supplementary Note 6).

The DV was purified by polyethylene glycol (PEG) precipitation, which removes excess staple strands without separating well-folded monomers from aggregates. The yield of correctly folded monomeric DNA nanostructures was estimated to be ~30% based on gel electrophoresis and TEM analyses. This is also reflected by the permeability assay against the larger Invader, that shows a shielding effect of ~25%, which clearly indicates that this fraction efficiently shields the enclosed cargo over the timescale assayed. The yield of correctly folded structures can possibly be increased through further purification of monomers, or improved sequence design and self-assembly protocol for the DV.

The enzyme loading yield into well-folded structures was estimated by TEM to be 5–10%. The low yield could be due to unfavourable CuAAC reaction conditions in the constrained cavity of the DV. Another loading strategy that could improve the loading yield could be to conjugate a DNA strand to the enzyme and afterwards anneal it to the CAS of the open DV. However, we showed that a double-stranded CAS partially decreased the shielding function of the DV. Another drawback of the DNA-protein conjugation method used was that it partially inhibited the activity of aCt. The inhibition of enzyme activity may be avoided by using protein engineering or DNA-templated chemistry to produce a site-specific DNA-protein conjugate. However, such approaches are often more time-consuming and less generally applicable.

The enzyme activity assay was performed by using the DV to restrict aCt access to its substrate FITC-casein, and showed that the enzymatic activity of the open sample was 3 times higher compared to the closed sample. This effect was higher than expected since the permeability assay showed that only 25% of the structures efficiently shield the cargo from Invader C, which has similar size as FITC-casein. If every DV contained an aCt cargo, only 1.25 times higher activity would be expected. Thus, we speculate that the loading procedure is biased towards enzyme packaging in well-folded open structures that expose the alkyne-modified CAS, and not into misfolded or aggregated structures that hide the CAS. This procedure differs from the cargo loading protocol used for the permeability assay, where the fluorescent cargo is heat-annealed during the self-assembly process, and is expected to be present on every DV structure. Additionally, the activity of the enzyme could be affected by the microenvironment of the DV particle. In fact, the close proximity of DNA to aCt inside the closed DV will decrease the pH in the proximity of the enzyme and thus lower the catalytic activity of aCt, which has a slightly basic optimum pH.

In conclusion, the DNA Vault integrates the catalytic activity of natural enzymes with rationally designed DNA nanostructures, so that enzymes can be exposed to substrate in response to specific combinations of DNA signals. Moreover, the modular locking system employed here can be modified in order to respond to the presence of proteins, RNA sequences and small molecules, as well as environmental variations in pH, temperature and ion concentration. Modified DNA vaults with higher assembly yields and lower permeability may be needed to control enzymes that act on smaller substrates. The porosity might be modified by addition of more DNA layers or structures such as entropic brushes, chemical compounds, protein shells and lipid membranes to the exterior of the device. Nevertheless, the general approach described here should allow a wide range of enzyme–substrate systems to be controlled. With further development, natural enzymes can be programmed to operate as signal amplifiers for diagnostics applications and as active components of delivery vehicles for advanced applications in medicine.

**Methods**

**Materials.** Chemicals were purchased from Sigma-Aldrich, unless otherwise noted. Standard desalted DNA oligonucleotides were purchased from Integrated DNA Technologies (IDT); oligonucleotides longer than 60 nt and those bearing chemical modifications were ordered as high-performance liquid chromatography-purified, all remaining staple strands were used without further purification.

**Design process.** CaDNAno software was used as primary design tool, with honeycomb lattice. Sequences of the lock module were designed in Nupack. AutoDesk Maya (http://www.autodesk.com) with CaDNAno plugin was used for 3D modelling and rendering of the structure.

**Self-assembly process.** DNA origami self-assembly was performed by mixing scaffold ssDNA (M13mp18, Bayou Biolabs) with 5× molar excess of staple strands in TAE/MgCl2 buffer (40 mM Tris/Acetate, 1 mM EDTA, pH 8.3, 16 mM MgCl2). The assembly reaction was performed by incubating the samples at 75 °C for 15 min, followed by a temperature ramp of −0.1 °C/1.5 min to 60 °C, and afterward a ramp of −0.1 °C every 6 min to 20 °C.

**Structure purification.** When required for experiments the DNA origami structure was purified from excess of staple strands by PEG precipitation, according to the literature, unless noted otherwise.

**Closing and opening.** Open DV is closed by adding MgCl2 to 30 mM final concentration and Cdk at lock: Cdk = 1:1.3 times molar excess, and then incubating the sample at room temperature for 2 h and subsequently at 4 °C for 36 h, unless noted otherwise. Closed DV is opened by adding Opk at Cdk: Opk = 1.5 times molar excess and incubating the sample at room temperature for 1 h, unless noted otherwise.

**Agarose gel analysis.** Annealed DNA structures were directly loaded on 1.5% agarose gel (if not otherwise indicated) and allowed to migrate for 3 h at 4 °C (running buffer: 1X TBE, 10 mM MgCl2; running voltage: 80 V). The gel was stained with SybrGold (Life Technologies) and visualised with Typhoon Trio scanner (GE Healthcare Life Sciences).

**TEM imaging.** Unpurified samples were deposited on glow-discharged 200 mesh carbon-coated copper TEM grids (Ted Pella Inc.) for 1.5 min, and positively stained with 1% uranyl acetate solution for 1 minute. A TEM FEI Tecnai G2 Spirit Bio/Twin was used at either 80 kV or 120 kV acceleration voltage and images were recorded and analysed with either DIAD software (Olympus) or EM-Menuut (TVIPS). Statistical analyses of the DNA origami size (Supplementary Fig. 7c, d) were executed with PAST 2.07 software (http://folk.uio.no/ohammer/past).

**FRET analysis.** The DV was assembled with a 3'-Cy3-modified and a 5’-Cy5-modified staple strand purchased from IDT. Closed and open DV samples were purified from unincorporated staple strands and analysed with FluorMax-3 fluorescence spectrophotometer (Horiba Scientific). Samples were excited at 530 nm (excitation of Cy5) and 600 nm (excitation of Cy5), with entrance slit set to 5 nm, and integration time set to 0.5 s. Emission was recorded at 565 nm and 666 nm for Cy3 and Cy5, respectively. All the experiments were conducted at 25 °C constant temperature. FRET efficiency values were calculated as $E = \frac{I_{DA}(DA + I_{DA})}{I_{DA}}$, where $I_{DA}$ and $I_{DA}$ are the Cy3 and Cy5 emission intensities, respectively, upon donor excitation.

**Cargo displacement experiments.** Biotinylated Invader A was incubated with streptavidin in 1:2 molar ratio for 15 min at room temperature, then used as Invader C without further purification. Native PAGE analysis shows no unreacted biotinylated Invader A (data not shown). Closed purified DV was incubated with either OpK or unspecific key at RT for 30 min. Open or closed cargo-loaded DV were then analysed with FluorMax-4 fluorescence spectrophotometer (Horiba Scientific), and incubated with invader strands in 10 times molar excess at room temperature. Samples were excited at 530 nm (excitation of Cy3) and 600 nm (excitation of Cy5), with
entrance slit set to 5 nm, and integration time set to 0.5 s. Emission was recorded at 565 nm and 666 nm for Cy3 and Cy5, respectively. All the experiments were conducted at room temperature. Relative peak ratio were calculated using the formula \( \text{I}_{\text{Cy3}}/\text{I}_{\text{Cy5}} + \text{I}_{\text{Cy5}} \), where \( \text{I}_{\text{Cy3}} \) and \( \text{I}_{\text{Cy5}} \) are the Cy3 and Cy5 emission intensities, respectively, upon donor excitation. The minimum relative peak ratio was determined on a sample containing a Cy3-modified and a Cy5-modified oligonucleotide ([50]/[121]-[5]Cy3 and [27]/[17]-[5]Cy5) from Supplementary Table 2, respectively) that do not interact with each other. The maximum relative peak ratio was determined as the highest value measured on DV before adding any invader molecule.

**AuNP-oligonucleotide conjugation and AuNP loading.** 5-nm AuNPs were purchased from Ted Pella Inc., thiol-modified oligonucleotide from IDT. Bis(p-sulfonatophenyl)-phenylphosphine-diethyl-dipotassium salt (phosphine, 1 mg) was added to 5 μL of AuNPs, the mix was covered with aluminum foil and stirred slowly for 24 h. NaCl was added until the colour changed from red to bluish, then the mix was centrifuged at 750 g for 15 min. The pellet was resuspended in 100 μL of 2.5 mM phosphine and 250 μL methanol was added. The mix was centrifuged at 750 g for 15 min, and the pellet resuspended in 100 μL of 2.5 mM phosphine (this is the phosphinated AuNP solution). Concentration of the phosphinated AuNPs was calculated with Nanodrop 1000 spectrophotometer (Thermo Scientific), then 1:10 molar ratio of thiol-modified oligonucleotide and phosphinated AuNPs were combined with TBE (0.25x final concentration) and NaCl (30 mM final concentration), and shaken on a horizontal shaker at 900 rpm for 24 h. AuNP-oligonucleotide conjugates were incubated with the open DV in 10:1 molar ratio without further purification for 12 h at room temperature.

**aCt-oligonucleotide conjugation and enzyme loading.** aCt was purchased from Sigma-Aldrich; NHS-C3-azide (BCL-014) and click reaction chemicals were a gift from baceJick GmbH. Enzyme-DNA conjugation was conducted in 2 steps: (i) enzyme conjugation with NHS-azide linker, and (ii) enzyme-azide CuAAC reaction with alkyne-modified oligonucleotide. First, aCt (14 lysine residues/molecule) was resuspended in aCt storage buffer (1 mM Tris/HCl, 2 mM CaCl₂, pH 8.0) at 100 μM final concentration; NHS-azide linkers were resuspended in DMSO at 10 mM final concentration. aCt (2 nmol) was incubated with NHS-C3-azide linker in lysis buffer, n = 1:10 molar ratios, and shaken (400 rpm) at 21 °C for 2 h in 50 μL aCt storage buffer final volume. The sample was then washed 2 times with aCt storage buffer by using Amicon Ultra-0.5 mL centrifugal filters (50 kDa) to remove unconjugated oligonucleotides. aCt-NPs were purified and concentrated using Nanosep centrifugal filters (50 kDa) and then the obtained aCt-NP-DNA conjugates were incubated with the open DV in 101:1 molar ratio without further purification for 12 h at room temperature.

References

1. Seeman, N. C. Nanomaterials based on DNA. Annu. Rev. Biochem. 79, 65–87 (2010).
2. Zhang, F., Nangreave, J., Liu, Y. & Yan, H. Structural DNA nanotechnology: state of the art and future perspective. J. Am. Chem. Soc. 136, 11198–11211 (2014).
3. Saccà, B. & Niemeyer, C. M. Functionalization of DNA nanostructures with enzymes. Chem. Soc. Rev. 40, 5910–5921 (2011).
4. Zhang, D. Y. & Seelig, G. Dynamic DNA nanotechnology using strand-displacement reactions. Nat. Chem. 3, 103–113 (2011).
5. Wilner, O. I. et al. Enzyme cascades activated on topologically programmed DNA scaffolds. Nat. Nanotechnol. 4, 249–254 (2009).
6. Fu, J. et al. Multi-enzyme complexes on DNA scaffolds capable of substrate funnelling with a chemical swinging arm. Nat. Nanotechnol. 9, 531–536 (2014).
7. Linko, V., Eerikainen, M. & Kostiainen, M. A. A modular DNA origami-based enzyme cascade nanoreactor. Chem. Commun. 51, 5351–5354 (2015).
8. Fu, Y. et al. Single-step rapid assembly of DNA origami nanostructures for architecture of nanoscale biocatalysts. J. Am. Chem. Soc. 135, 696–702 (2013).
9. Freeman, R., Sharon, E., Tel-Vered, R. & Willner, I. Supramolecular cocaine–aptamer complexes activate biocatalytic cascades. J. Am. Chem. Soc. 131, 5028–5029 (2009).
10. Liu, M. et al. A DNA tweezer-activated enzyme nanoreactor. Nat. Commun. 4, 2127 (2013).
11. Saphatetian, G. DNA detection and signal amplification via an engineering allostERIC enzyme. J. Am. Chem. Soc. 2003, 344–345 (2003).
12. Gianneschi, N. C. & Ghadiri, M. R. Design of molecular logic devices based on a programmable DNA-regulated semisynthetic enzyme. Angew. Chem. Int. Ed. Engl. 119, 4029–4032 (2007).
13. Frenzel, M., Kuo, C. H. & Niemeyer, C. M. DNA-mediated assembly of cytochrome P450 BM3 subunits. J. Am. Chem. Soc. 133, 16111–16118 (2011).
14. Sancho Olivera, N., Bos, J. & Roelfes, G. Control over enzymatic activity by DNA-directed split enzyme reassembly. ChemBioChem 11, 2253–2258 (2010).
15. Martin, W. Evolutionary origins of metabolic compartmentalization in extant and fossil lineages. Philos. Trans. R. Soc. Lond. B Biol. Sci. 365, 847–855 (2010).
16. Kuchler, A., Yoshimoto, M., Luglinbuhl, S., Mavelli, F. & Walde, P. Enzymatic reactions in confined environments. Nat. Nanotechnol. 11, 409–420 (2016).
17. Chen, A. H. & Silver, P. A. Designing biological compartmentalization. Trends Cell Biol. 22, 662–670 (2012).
18. McHugh, C. A. et al. A virus capsid-like nanocompartment that stores iron and protects bacteria from oxidative stress. EMBO J. 33, 1896–1911 (2014).
19. Yeates, T. O., Kerfeld, C. A., Heinhorst, S., Cannon, G. C. & Shively, J. M. Protein-based organelles in bacteria: carboxysomes and related microcompartments. Nat. Rev. Microbiol. 6, 681–691 (2008).
20. Berger, W., Steiner, E., Grusch, M., Eibleng, L. & Mickaše, M. Vaults and the major vault protein: novel roles in signal pathway regulation and immunity. Cell. Mol. Life Sci. 66, 43–61 (2009).
21. Chowdhury, C. et al. Selective molecular transport through the protein shell of a bacterial microcompartment organelle. Proc. Natl Acad. Sci. USA 112, 2990–2995 (2015).
22. Jacques, D. A. et al. HIV-1 uses dynamic capsid pores to import nucleotides and fuel encapsidated DNA synthesis. Nature 536, 349–353 (2016).
23. Walde, P. & Ichikawa, S. Enzymes inside lipid vesicles: preparation, reactivity and applications. Biomed. Eng. 18, 143–177 (2001).
24. Nardin, C., Widmer, J., Winterhalter, M. & Meier, W. Amphiphilic block copolymer nanocantainers as bioreactors. Eur. Phys. J. E 4, 403–410 (2001).
25. Sakr, O. S. & Borchard, G. Encapsulation of enzymes in Layer-by-Layer (LbL) structures: latest advances and applications. Biomacromolecules 14, 2137–2135 (2013).
26. Glasgow, J. E., Asensio, M. A., Jakobson, C. M., Francis, M. B. & Tullman-Ercek, D. Influence of electrostatics on small molecule flux through a protein nanoreactor. *Nat. Nanotechnol.* **2**, 635–639 (2007).

27. Cornellas-Aragones, M. et al. A virus-based single-enzyme nanoreactor. *Nat. Nanotechnol.* **7**, 57–60 (2012).

28. Andersen, E. S. et al. Self-assembly of a nanoscale DNA box with a controllable lid. *Nature* **459**, 73–76 (2009).

29. Ke, Y. et al. Scaffolded DNA origami of a DNA tetrahedron molecular container. *Nano. Lett.* **9**, 2445–2447 (2009).

30. Kuzuya, A. & Komiyama, M. Design and construction of a box-shaped 3D-DNA origami. *Chem. Commun.* **0**, 4182–4184 (2009).

31. Erben, C. M., Goodman, R. P. & Turberfield, A. J. Single-molecule protein encapsulation in a rigid DNA cage. *Angew. Chem.* **118**, 7574–7577 (2006).

32. Jiao, S. et al. Temperature-controlled encapsulation and release of an active enzyme in the cavity of a self-assembled DNA nanocage. *ACS Nano* **7**, 9724–9734 (2013).

33. Zhao, Z. et al. Nanocaged enzymes with enhanced catalytic activity and increased stability against protease digestion. *Nat. Commun.* **7**, 10619 (2016).

34. Sprengel, A. et al. Tailored protein encapsulation into a DNA host using geometrically organized supramolecular interactions. *Nat. Commun.* **8**, 14472 (2017).

35. Douglas, S. M., Bachelet, I. & Church, G. M. A logic-gated nanorobot for targeted transport of molecular payloads. *Science* **335**, 831–834 (2012).

36. Torelli, E. et al. A DNA origami nanorobot controlled by nucleic acid hybridization. *Small* **10**, 2918–2926 (2014).

37. Rothemund, P. W. & Folding, D. N. A logic-gated nanorobot for targeted transport of molecular payloads. *Science* **335**, 831–834 (2012).

38. Douglas, S. M. et al. Rapid prototyping of 3D DNA-origami shapes with caDNAno. *Nucleic Acids Res.* **37**, 5001–5006 (2009).

39. Sprengel, A. et al. Nanocaged enzymes with enhanced catalytic activity and increased stability against protease digestion. *Nat. Commun.* **7**, 10619 (2016).

40. Zadek, J. N. et al. NUPACK: analysis and design of nucleic acid systems. *J. Comput. Chem.* **32**, 170–173 (2011).

41. Stahl, E., Martin, T. G., Praetorius, F. & Dietz, H. 71 Dynamic DNA devices and assemblies formed by shape-complementary, non-basepairing 3D components. *J. Biomol. Struct. Dyn.* **33** (Suppl 1), 46 (2015).

42. Douglas, S. M. et al. Rapid prototyping of 3D DNA-origami shapes with caDNAno. *Nucleic Acids Res.* **37**, 5001–5006 (2009).

43. Sprengel, A. et al. Tailored protein encapsulation into a DNA host using geometrically organized supramolecular interactions. *Nat. Commun.* **8**, 14472 (2017).

44. Kegler, K. et al. Polyelectrolyte-compression forces between spherical DNA brushes. *Phys. Rev. Lett.* **100**, 118302 (2008).

45. Herrmann, O., Harper, D. A. T. & Ruyan, P. D. STAMP: paleontological statistics software package for education and data analysis. *Palaeontologia Electron.* **4**, 4 (2001).

46. Zhu, Z., Li, Y., Tian, C. & Mao, C. A smart DNA tetrahedron that isothermally assembles or dissociates in response to the solution pH value changes. *Biomacromolecules* **14**, 1711–1714 (2013).

47. van de Waterbeemd, F., Wagenbauer, K. F., Neuner, A. & Dietz, H. 71 Dynamic DNA devices and assemblies formed by shape-complementary, non-basepairing 3D components. *J. Biomol. Struct. Dyn.* **33** (Suppl 1), 46 (2015).

48. Douglas, S. M. et al. Rapid prototyping of 3D DNA-origami shapes with caDNAno. *Nucleic Acids Res.* **37**, 5001–5006 (2009).

49. Zadek, J. et al. NUPACK: analysis and design of nucleic acid systems. *J. Comput. Chem.* **32**, 170–173 (2011).

50. Stahl, E., Martin, T. G., Praetorius, F. & Dietz, H. Facile and scalable preparation of pure and dense DNA origami solutions. *Angew. Chem. Int. Ed.* **53**, 12735–12740 (2014).

51. Hammer, O., Harper, D. A. T. & Ruyan, P. D. PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electron.* **4**, 4 (2001).

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Author contributions

G.G. designed the system under the guidance of E.S.A. G.G. developed and did all experiments. G.G. and M.D.E.J. analysed the FRET data. E.S.A. and J.K. supervised the experiments. G.G. and M.D.E.J. analysed the FRET data. E.S.A. and J.K. supervised the experiments. G.G. would like to thank Jenia Manugurea for support and encouragement. This work was supported by the European Commission under the Seventh Framework Program (FP7/2007–2013), as part of the Marie Curie Initial Training Network, ESSoDNA (No. 317110) and the European Research Council (grant number 683005).

Additional information

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