Precise Coating of a Wide Range of DNA Templates by a Protein Polymer with a DNA Binding Domain

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* Supporting Information

ABSTRACT: Emerging DNA-based nanotechnologies would benefit from the ability to modulate the properties (e.g., solubility, melting temperature, chemical stability) of diverse DNA templates (single molecules or origami nanostructures) through controlled, self-assembling coatings. We here introduce a DNA coating agent, called C8−BSso7d, which binds to and coats with high specificity and affinity, individual DNA molecules as well as folded origami nanostructures. C8−BSso7d coats and protects without condensing, collapsing or destroying the spatial structure of the underlying DNA template. C8−BSso7d combines the specific nonelectrostatic DNA binding affinity of an archael-derived DNA binding domain (Sso7d, 7 kDa) with a long hydrophilic random coil polypeptide (C8, 73 kDa), which provides colloidal stability (solubility) through formation of polymer brushes around the DNA templates. C8−BSso7d is produced recombinantly in yeast and has a precise (but engineerable) amino acid sequence of precise length. Using electrophoresis, AFM, and fluorescence microscopy we demonstrate protein coat formation with stiffening of one-dimensional templates (linear dsDNA, supercoiled dsDNA and circular ssDNA), as well as coat formation without any structural distortion or disruption of two-dimensional DNA origami template. Combining the programmability of DNA with the nonperturbing precise coating capability of the engineered protein C8−BSso7d holds promise for future applications such as the creation of DNA–protein hybrid networks, or the efficient transfection of individual DNA nanostructures into cells.

KEYWORDS: directed self-assembly, protein engineering, DNA nanotechnology, single molecule, protein polymer, nanomaterials

New DNA nanotechnologies such as those based on DNA origami,1−4 single molecule DNA imaging,5 single molecule sequencing strategies such as optical mapping,6−9 and nanopore sequencing10,11 increasingly rely on precise control of physical-chemical properties of individual DNA molecules: mechanical properties, interactions with nanoscale environments,12 etc. While there is some opportunity to exert control via tuning of solution conditions, many cases exist in which incompatible solution conditions greatly hinder the ability to achieve high assembly yields for products containing diverse building materials. We expect that much higher levels of control and greater assembly yields can be obtained by developing dedicated nonelectrostatic DNA binders capable of modulating specific DNA properties. A general toolbox of DNA binders that modulate physical properties of individual DNA molecules may therefore be expected to be useful for a wide range of DNA-based technologies.

With this in mind, we have recently designed, produced and characterized a recombinant, protein-based polymer that coats individual double-stranded DNA (dsDNA) molecules and significantly increases its persistence length.13 The polymer coating protects against enzymatic degradation without making the DNA completely inaccessible to strong (sequence specific) binders. It is composed of two polypeptide domains and its
sequence is abbreviated as \( \text{C}_4-\text{B}^{\text{Sso7d}} \). The \( \text{C}_4 \) domain is a tetramer of a previously published collagen-inspired sequence \( \text{C}_4 \), a 99 amino acid polypeptide that is highly hydrophilic and forms soluble random coils over a wide range of solution conditions. As a DNA binding domain, a simple stretch of 12 lysines was used (K12). The enhanced DNA stiffness provided by the \( \text{C}_4-\text{B}^{\text{K12}} \) polymer coating has already been shown to be useful for nanochannel-based optical mapping of DNA, where it allows for full stretching of DNA in rather wide nanochannels (250 × 250 nm). While highly effective in coating linear dsDNA, we show here that the \( \text{C}_4-\text{B}^{\text{K12}} \) polymer causes undesirable distortion of DNA origami structures.

As a DNA binding domain, the K12 oligolysine domain is nonspecific. Virtually all anticipated applications of DNA binding domains that modulate specific DNA properties involve complicated background solutions composed of biopolymers other than DNA, and the oligolysine domain may also bind to those molecules. The same of course holds for most synthetic polycationic blocks currently used for nonviral gene transfer; those molecules. The same of course holds for most synthetic polycationic binding blocks other than DNA, and the oligolysine domain may also bind to other large engineered protein-based polymers is also attainable. Bulk purification using ammonium sulfate precipitation was sufficient to separate the secreted protein polymers from most other proteins secreted in the extracellular medium, as shown by SDS-PAGE in Figure 1b. Multimers of the hydrophilic \( \text{C}_4 \) domain are known to poorly bind SDS and hence move with anomalously low speeds in SDS-PAGE. Multimers of the hydrophilic \( \text{C}_4 \) domain are known to poorly bind SDS and hence move with anomalously low speeds in SDS-PAGE.

**RESULTS AND DISCUSSION**

**Protein Design and Production.** The amino acid sequence of the \( \text{C}_4-\text{B}^{\text{Sso7d}} \) protein-based polymer and a cartoon of it bound to dsDNA is shown in Figure 1a, where we have used the published X-ray crystallographic structure of Sso7d bound to a short DNA double helix. Note the large asymmetry of the polymer in terms of the domain lengths (63 amino acids for the \( \text{B}^{\text{Sso7d}} \) binding domain and 797 amino acids for the hydrophilic \( \text{C}_4 \) domain). The addition of a larger C-block to Sso7d than K12 aims to increase the solubility and rigidity of resultant protein–DNA complexes without affecting the nature of the interaction between protein and DNA, which is driven solely by the binding block. The \( \text{C}_4-\text{B}^{\text{Sso7d}} \) protein-based polymer, with a predicted molar mass of 80 372 Da, was successfully produced by Pichia pastoris as a secreted protein at an approximate yield (purity of the secreted protein to volume of cell-free medium) of 0.72 g/L. Secretion of the DNA binding domain linked to the unstructured polypeptide region was well tolerated by the \( P. \) pastoris cells suggesting that production of other large engineered protein-based polymers is also attainable. Bulk purification using ammonium sulfate precipitation was sufficient to separate the secreted protein polymers from most other proteins secreted in the extracellular medium, as shown by SDS-PAGE in Figure 1b. Multimers of the hydrophilic \( \text{C}_4 \) domain are known to poorly bind SDS and hence move with anomalously low speeds in SDS-PAGE. Indeed, the apparent molar mass of the purified polypeptides as deduced from electrophoretic mobility would be around 190 kDa. The molar mass of the purified protein was analyzed by MALDI-TOF mass spectrometry (Figure 1c), which shows three peaks that correspond to the \( \text{M}^+ \) (80 210 Da), \( \text{M}^{2+} \) (40 190 Da) and \( \text{M}^{3+} \) (26 645 Da) ions. This agrees with the predicted molar mass of 80 372 Da within experimental accuracy. Furthermore, the existence of the protein in a monomer state in solutions of the purified biosynthesized protein was demonstrated by Dynamic Light Scattering by...
detection of a single molecule population with a hydrodynamic radius of 7.9 nm, as expected for a hydrophilic polypeptide coil of approximately 860 amino acids.

**Protein Polymer Binding Isotherms for Different Types of DNA Templates.** In order to probe the DNA binding properties of C8−Bso7d for different types of DNA templates we characterize the mobility shift with agarose gel electrophoresis (Figure 2) after the addition of C8−Bso7d to 2.0 kbp linear DNA, 2.6 kbp supercoiled pDNA and 7249 nt circular ssDNA from M13mp18 virus (the scaffold typically used in the production of DNA origami) since Sso7d is reported to also bind to ssDNA.24 We find that the addition of C8−Bso7d reduces the electrophoretic mobility of all DNA templates, confirming the interaction of the protein with these DNA templates. The shift in mobility follows a similar trend for each sample. The mobility begins to decrease at a low protein/DNA ratio, 0.017 protein molecules per base pair (ptn/bp) for linear dsDNA and pDNA or per nucleotide (ptn/nt) for ssDNA. The mobility shift saturates at around 0.067 ptn/bp or ptn/nt. Higher protein concentrations do not lead to further changes in the observed mobility (see Supporting Information Figure S1) but do appear to cause a reduction in staining efficiency due to competition with the binding of the DNA stain. In contrast, using the C4−BK12 protein polymer that binds to DNA purely via nonspecific electrostatic interactions, required the addition of a much larger excess amount of protein polymers (>0.667 ptn/bp) to achieve a complete saturation of binding as deduced from the electrophoretic mobility, as we also reported previously.13 Structural and crystallographic studies report that Bso7d binds to dsDNA every 4 bp, equivalent to 0.25 ptn/bp.17,25 We find that binding to dsDNA saturates at around 0.084 ptn/bp or nt, which is equivalent of one protein every 12 bp or nt. Presumably, steric interactions of the large C8 domains prevent the coating from achieving higher densities.

Since the Sso7d domain has a tryptophan residue in the binding site, we probed the interaction between tryptophan with the dsDNA through its fluorescence quenching. In Figure 3 can be appreciated that, when excited at 285 nm, the fluorescence intensity emitted at 340 nm by tryptophan is reduced linearly upon addition of nucleic acid. The fluorescence intensity reached a minimum at protein/DNA bp <0.09, which is in good agreement with the observed results by agarose gel electrophoresis for dsDNA (Figure 2a). This confirms the interaction between C8−Bso7d and the DNA.

**Secondary Structure of Sso7d Domain in the Context of C8−Bso7d Protein Polymer.** In order assess whether Sso7d folding is influenced by the attachment of the C8-block, we carried out circular dichroism spectroscopy (see Supporting Information Figure S2). We use a C4 protein polymer to obtain a reference spectrum and subtracted twice the C4 spectrum from the spectrum of the full length C8−Bso7d (on a molar basis), to obtain the spectrum of the Sso7d block in the context of the C8−Bso7d protein polymer. Despite the large noise due to the large mass of the C8-block in comparison to that of the Sso7d block, we find a difference spectrum that is very similar to those previously reported for free Sso7d.18 Additionally, considering that the tryptophan of the binding domain Sso7d is actually interacting with those of dsDNA (Figure 3), we can conclude that the folding and interaction with DNA of the domain Sso7d is not significantly undermined by the fusion to the C8-block.

![Figure 2. C8−Bso7d binding of one-dimension DNA templates. Electrophoretic Mobility Shift Assay for (a) 2.0 kbp linear dsDNA, (b) circular M13 ssDNA and (c) 2.6 kbp pDNA complexed with C8−Bso7d. Protein/DNA bp or nt ratio is shown at the top. (d) Plot of bound DNA as a function of protein/DNA bp ratio for dsDNA and Protein/DNA nt ratio for ssDNA.](image-url)
Figure 4. Comparison of structures of complexes with C₈−B_Sso7d and C₈−B_K12 protein polymers for different types of DNA templates. AFM images of dried complexes. (a,b) 8.0 kbp linear dsDNA coated with 0.125 ptn/bp of (a) C₈−B_Sso7d and (b) 0.125 ptn/bp C₈−B_K12. Scale bars are 500 nm. (c,d) 4.0 kbp supercoiled dsDNA coated with (c) 0.375 ptn/bp of C₈−B_Sso7d and (d) 0.833 ptn/bp of C₈−B_K12. Scale bars are 400 nm. (e,f) Circular ssDNA from M13mp18 virus coated with (e) 1.45 ptn/bp of C₈−B_Sso7d and (f) 0.792 ptn/bp of C₈−B_K12. Scale bars are 500 nm. (g) Zoom of (a) for linear dsDNA+ C₈−B_Sso7d, showing a protein polymer bottle-brush structure around a DNA core. Scale bar is 150 nm. (h) Zoom of the square section of the C₈−B_Sso7d + ssDNA complex shown in (e), showing a short stretch of complex (arrow) for which the C₈−B_Sso7d protein polymer coating did not prevent intramolecular basepairing. Scale bar is 50 nm.

Table 1. Height and Contour Lengths of Protein Polymer Coated Linear 8 kbp dsDNA

<table>
<thead>
<tr>
<th>ptn/bp</th>
<th>C₈−B_Sso7d</th>
<th>C₈−B_K12</th>
</tr>
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<tbody>
<tr>
<td>height (nm)</td>
<td>0.06</td>
<td>0.13</td>
</tr>
<tr>
<td>contour length (nm)</td>
<td>2720±65</td>
<td>2382±83</td>
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*Theoretical contour length for bare linear 8 kbp dsDNA, ND: not determined.

Structures of Protein Polymer-Coated 1D DNA Templates from AFM Imaging. We used AFM imaging to investigate the nanoscale structure of complexes of the C₈−B_Sso7d protein polymers with a range of DNA templates: linear 8.0 kbp dsDNA, 4.0 kbp supercoiled dsDNA, and 7249 nt circular ssDNA (Figure 4a, 4c and 4e). For all templates, we compare the structures obtained with C₈−B_Sso7d with C₈−B_K12 diblock polymer, for which DNA binding is purely electrostatic and not specific to DNA (Figure 4b, 4d and 4f). AFM images of complexes of C₈−B_Sso7d with 8.0 kbp linear double stranded DNA are qualitatively similar to those formed with the C₈−B_K12 diblock protein polymer (compare Figure 4a and b): the coating is homogeneous and preserves the contour length of the template. Upon increasing the protein to DNA ratio, the DNA molecules become progressively coated (Height in Table 1), without any indication of intramolecular aggregation. The bottle-brush architecture of the complexes can be observed in high resolution AFM images for complexes with C₈−B_Sso7d (Figure 4g). The C₈−B_Sso7d complexes have a somewhat larger height than the C₈−B_K12 complexes. This most likely reflects both the larger and globular binding domain B_Sso7d and the larger length of the stabilizing C-block for C₈−B_Sso7d. A quantitative analysis (as described in the Materials and Methods section) also reveals that contour lengths of C₈−B_Sso7d complexes are significantly shorter than those of the bare DNA (Table 1). Indeed, it has been reported that Sso7d causes kinking that may reduce the effective contour length of dsDNA by 10−20%.²⁰

For 4 kb supercoiled pDNA, complexation with C₈−B_Sso7d seems to lead to global opening up of plectonemic supercoils (Figure 4c) exposing its circular topology, possibly due to stiffening effects from the protein coating and due to induced twist of the topologically constrained DNA caused by protein binding. The complexes have a contour length of 1395±29 nm, which is about the theoretical contour length of the naked pDNA (1360 nm, assuming a length of 0.34 nm per nt). B_Sso7d binding has been reported to lead to DNA unwinding through placement of its triple beta-sheet domain across the minor groove.²⁰,²⁷ At a fixed linking number this will be compensated by the introduction of positive supercoiling that balances part or all of the original negative supercoiling. The marked shortening of the apparent contour length of complexes that was observed for linear DNA is absent in the case of supercoiled pDNA. In clear contrast to complexation with C₈−B_Sso7d, the C₈−B_K12 diblock leads to a tightening of plectonemic supercoils (Figure 4d), possibly via bridging of the opposing sides of a plectonemic supercoil by the binding domain. As a consequence, single thick contours are observed for C₈−B_K12 pDNA complexes, that appear as flexible rod-like structures with a contour length that is close to half that of the contour length of bare pDNA, as previously reported.²³ For more images of the complexes of pDNA with both proteins, see the Supporting Information (Figure S3).

Complexes with single-stranded DNA are again markedly different for the two proteins (Figure 4e and f). It appears that the C₈−B_Sso7d protein polymer can almost completely prevent the formation of secondary structures due to intramolecular base pairing of the ssDNA. Therefore, it disentangles and stretches out the structure (Figure 4e and Supporting Information Figure S4). In most of the images a single short piece of an apparent duplex segment remains visible (marked segment with an arrow in Figure 4h) that could correspond to a local (nearly) palindromic sequence with particularly strong base pairing. The complexes with C₈−B_Sso7d have a contour length of 1326±95 nm, which is 53.8% of the calculated contour length of naked ssDNA (2465 nm, assuming a
DNA appears more compact (Figure 5a), rotates more rapidly, and di
stresses faster than T4 DNA coated with C₄—B¹²¹² (Figure 5b) or C₈—B⁷⁰⁷⁰ protein polymers (Figure 5c). Especially when T4-DNA is coated with C₈—B⁷⁰⁷⁰, the DNA molecules are quite extended and a coarse linear contour can typically be distinguished. In the videos it appears to diffuse more slowly (Supporting Videos S1–S3). C₄—B¹²¹² has a similar effect but the decrease in mobility and the stretching is less pronounced. Hydrodynamic radii deduced from the estimated diffusion constants are roughly twice larger for C₈—B⁷⁰⁷⁰ coated than for bare T4 DNA. In no case aggregation was detected. While the hydrodynamic radius of the (coated) T4 DNA mainly reflects the stiffness of the coated DNA, it is also sensitive (to a lesser extent) to the thickness of the bottle-brush coating, and to excluded volume effects between the bottle-brush coated DNA segments. Therefore, while it is clear that, like the C₄—B¹²¹² coating, the C₈—B⁷⁰⁷⁰ coating leads to a significant stiffening of the DNA, more detailed studies will have to be performed to precisely quantify the induced stiffening, and to determine whether it is larger or smaller than what we have previously found for C₄—B¹²¹².

**Impact of Protein Polymer Coating on Hydrodynamic Radius of T4 DNA.** For the C₄—B¹²¹² diblock protein polymer, stiffening of linear dsDNA induced by the bottle-brush coating was previously studied using both AFM and nanochannel stretching experiments.⁸ Here we qualitatively address stiffening of linear DNA by measuring changes in the translational diffusion constant (and hence the hydrodynamic radius) of linear double stranded T4 DNA (169 kbp) when it is coated with C₈—B⁷⁰⁷⁰ and C₄—B¹²¹². Diffusion constants are deduced from the mean square displacements as a function of time (see Materials and Methods), by tracking the centers-of-mass of YOYO-1-stained T4 DNA using fluorescence microscopy. Representative images of bare T4 DNA, and T4 DNA coated with either C₄—B¹²¹² or C₈—B⁷⁰⁷⁰ are shown in Figure 5. Estimated diffusion coefficients D and the corresponding hydrodynamic radii RH of bare T4 DNA, and T4 DNA coated with C₄—B¹²¹² and C₈—B⁷⁰⁷⁰ are given in Table 2. Bare T4 DNA appears more compact (Figure 5a), rotates more rapidly, and di
stresses faster than T4 DNA coated with C₄—B¹²¹² (Figure 5b) or C₈—B⁷⁰⁷⁰ protein polymers (Figure 5c). Especially when T4-DNA is coated with C₈—B⁷⁰⁷⁰, the DNA molecules are quite extended and a coarse linear contour can typically be distinguished. In the videos it appears to diffuse more slowly (Supporting Videos S1–S3). C₄—B¹²¹² has a similar effect but the decrease in mobility and the stretching is less pronounced. Hydrodynamic radii deduced from the estimated diffusion constants are roughly twice larger for C₈—B⁷⁰⁷⁰ coated than for bare T4 DNA. In no case aggregation was detected. While the hydrodynamic radius of the (coated) T4 DNA mainly reflects the stiffness of the coated DNA, it is also sensitive (to a lesser extent) to the thickness of the bottle-brush coating, and to excluded volume effects between the bottle-brush coated DNA segments. Therefore, while it is clear that, like the C₄—B¹²¹² coating, the C₈—B⁷⁰⁷⁰ coating leads to a significant stiffening of the DNA, more detailed studies will have to be performed to precisely quantify the induced stiffening, and to determine whether it is larger or smaller than what we have previously found for C₄—B¹²¹².

**Protein Polymer Coating of DNA Origami Nanostructures.** Next we move on to the coating of more complicated DNA templates, viz. self-assembled DNA nanostructures. Specifically, we will use DNA origami, which is based on the programmed formation of Holliday junctions between M13mp18 viral ssDNA scaffold and small synthetic ssDNA staple strands. The specific structure that we will work with is the "tall rectangle" structure designed by Paul Rothemund.³¹ We first characterize the binding isotherms of the protein polymers when binding to the two-dimensional origami DNA templates using an electrophoretic mobility shift assay (EMSA) using agarose gel electrophoresis.

Results are shown in Figure 6. We find that the addition of the protein polymers reduces the electrophoretic mobility of the DNA origami, much like the behavior of the other DNA templates, again confirming the generic affinity of the protein polymers for DNA templates. For complexes of DNA origami with the C₄—B¹²¹² protein polymer, mobility begins to decrease at around 0.054 pt)/(bp, and saturates at around 0.861 pt)/(bp (Figure 6a). For complexes of DNA origami with the C₈—B⁷⁰⁷⁰ protein polymer (Figure 6b), the mobility is already reduced at much lower protein polymer concentrations, namely, at 0.008 pt)/(bp. The mobility shift also saturates at a much lower protein polymer concentration, namely at around 0.094 pt)/(bp. Approximate binding curves deduced from the EMSA data is shown in Figure 6c. The different binding behavior for the two protein polymers most likely arises from the fact that the Ssö7d binding domain is DNA specific, whereas, as discussed above, the K₁₁ domain binds through electrostatic interactions alone, and is not DNA-specific.

Next, using AFM we investigated the spatial structures of complexes of the protein polymers with the DNA origami. Results are shown in Figure 7. Coating DNA origami with the C₈—B⁷⁰⁷⁰ preserves the designed structure of the DNA.

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**Table 2. Diffusion Constants D and Hydrodynamic Radii RH of T4 DNA, Coated with C₄—B¹²¹² and C₈—B⁷⁰⁷⁰**

<table>
<thead>
<tr>
<th></th>
<th>bare C₄—B¹²¹²</th>
<th>C₈—B⁷⁰⁷⁰</th>
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<tbody>
<tr>
<td>D [10⁻¹⁵ m²/s]</td>
<td>3.50 ± 0.09</td>
<td>2.42 ± 0.04</td>
</tr>
<tr>
<td>RH [μm]</td>
<td>0.70 ± 0.02</td>
<td>1.007 ± 0.02</td>
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origami, as can be seen in Figure 7a–d. In contrast, coating DNA origami with C₄−BK₁₂ produces distorted origami structures at high ptn/bp ratios (Figures 7e–i). Note that lower protein polymer concentrations were used for C₈−BSso7d than for C₄−BK₁₂, in view of the much lower concentration needed to saturate binding for the C₈−BSso7d protein polymer. This may also have helped in preventing structural distortion of the coated DNA origami for the case of coating with C₈−BSso7d. It is also interesting to notice that the height of the coated origami seems not to increase when more protein is added. This effect can be because the hydrophilic domain is quite flexible and could flatten out on and around the DNA origami and remain sufficiently dynamic that it is difficult to observe on top of the origami.

Enzymatic Accessibility of Coated DNA. Accessibility of macromolecular agents to DNA coated by molecules such as proteins or other polymers is relevant for a wide range of possible applications. As a way to estimate the DNA accessibility, we evaluated the ability of the C₈−BSso7d protein to protect the DNA from degradation by nuclease enzymes (Figure 8, electrophoresis gel images given in Supporting Information Figure S5). Solutions containing pDNA or DNA origami complexed with C₈−BSso7d or C₄−BK₁₂ were incubated with a high concentration of DNase I, a nonsequence specific endonuclease enzyme. At different incubation times aliquots of the sample were taken and the reaction quenched with EDTA. The samples were then run in an agarose gel. The intensity of the band corresponding to the protein–DNA complex was quantified with the ImageJ software and plotted against time.
The experiment shows that the protein polymer coating offers a moderate degree of protection against nucleases for both DNA origami and pDNA. Coated templates are degraded in about 10 min, which is about 5 times slower than the bare DNA templates (see the curve for bare origami DNA in Figure 8). Protection by C₈−B₈so7d is somewhat stronger than by C₄−B₁₂, possibly due to the longer hydrophilic brush and the DNA-specific binding domain.

CONCLUSIONS

We have shown that we can harness the high specificity and affinity of naturally occurring DNA binding domains in combination with a long unstructured domain for controlled binding and coating of a wide range of different types of DNA templates, including DNA origami nanostructures. Using the binding properties of the designed protein C₈−B₈so7d, we can modulate physical-chemical properties of DNA templates such as their stiffness, surface chemistry and accessibility to enzymes. In comparison to the C₄−B₁₂ protein polymer that only binds through nonspecific electrostatics, the So7d DNA-binding domain of the C₈−B₈so7d protein polymer has advantages of resulting in stable coatings at lower protein concentrations, prevention of intramolecular bridging in ssDNA and supercoiled pDNA templates, and preservation of shape of DNA templates. The BSso7d domain will be less sensitive to undesired molecular cross-talk with other negatively charged surfaces or polyelectrolytes. Additionally, the B₈so7d is insensitive to intramolecular bridging in ssDNA and supercoiled pDNA templates, and preservation of shape of DNA templates (see the curve for bare origami DNA in Figure 8).

PSYCHOSIS OF PROTEIN.

The fermentation was similar to the previously described method.† Fed-batch fermentations using minimal basal salts medium were performed in 2.5-L Bioflo 3000 fermentors (New Brunswick Scientific, Edison, NJ). The methanol fed-batch phase for protein production lasted 2–3 days. A homemade semiconductor gas sensor—controller was used to monitor the methanol level in the off-gas and to maintain a constant level of 0.2% (v/v) methanol in the broth. The pH was maintained at 3.0 throughout the fermentation by addition of ammonium hydroxide. At the end of the fermentation, the cells were separated from the broth by centrifugation for 15 min at 10 000g (room temperature or 4 °C) in an SLA-3000 rotor (Thermo Scientific, Waltham, MA), and the supernatant was microfiltered ( Pall Corporation, Port Washington, NY) and stored at 4 °C for subsequent purification.

Protein Purification.

All centrifugation was done for 30 min at 20 000g at 4 °C, interchangeably in a Sorvall SLA-1500 or SLA-3000 rotor (Thermo Scientific, Waltham, MA). First, medium salts were removed from the cell-free broth by adjustment of the pH to 8.0 with NaOH, followed by centrifugation. Subsequently, the protein-based polymer was selectively precipitated from the solution by adding ammonium sulfate to a saturation of 45%, incubating overnight at 4 °C, and subsequent centrifugation. The pellet was resuspended in an equal volume (relative to the cell-free broth) of Milli-Q water and precipitation was repeated once at 4 °C, using an overnight incubation. The pellet was resuspended in 0.2 volumes (relative to the cell-free broth) of Milli-Q water and stored at 4 °C for subsequent purification.

MATERIALS AND METHODS

Materials. Linear 8.0, 2.5, and 2.0 kbp dsDNA were purchased from Thermo Scientific (Waltham, MA, USA). M13mp18 single-stranded DNA 7249 nt (ssDNA) was purchased from New England Biolabs (Ipswich, MA, USA) and T4-DNA were purchased from Nippon Gene (Tokyo, Japan) and used without further purification, supercooled 4.0 and 2.7 kbp pDNA were recovered from recombinant E. coli by using the GeneJet plasmid Miniprep kit from Thermo Scientific. All short ssDNA staples used for DNA origami formation were purchased from IDT (Integrated DNA Technologies, Inc.). The precise sequence of staples and scaffold/staple layout for the “Tall Rectangle” design can be found in ref 31. YOYO-1 was purchased from Invitrogen (Life Technologies, Carlsbad, CA, USA). Restriction enzymes were purchased from New England Biolabs or from Thermo Scientific. The C₄−B₈so7d and C₁₂ protein polymers were produced and purified following previously published methods.†‡

Molecular Cloning. A double-stranded adapter encoding the So7d binding domain (B₈so7d) was prepared by annealing of overlapping codon-optimized oligonucleotides (Eurogentec, Belgium; Supporting Information Table S1). The vector containing the DNA coding for the hydrophilic random coil protein “C₈” was prepared in the following way: a fragment C₈ from plasmid pMTL23-C₈ (see ref 14) by double digestion with the restriction enzymes DraIII/Van91I was ligated into the plasmid pMTL23-C₄ previously digested with Van91I to obtain pMTL23-C₈. The plasmid pMTL23-C₈ was obtained by ligating the double-stranded B₈so7d adapter into the vector pMTL23-C₈ previously digested with restriction enzymes Van91I and EcoRI. The fragment encoding the C₈−B₈so7d protein-based polymer was released through digestion of plasmid pMTL23-C₈−B₈so7d with Xhol/EcoRI and ligated into the correspondingly digested P. pastoris expression vector pPIC9 (Invitrogen). The resulting plasmid pPIC9-C₈−B₈so7d was linearized with Sall and electroporated into P. pastoris strain GS115 (Invitrogen). The plasmid integrates into the genome through homologous recombination at the his4 locus providing normal growth on methanol. The presence of the gene was verified by polymerase chain reaction.

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All centrifugation was done for 30 min at 20 000g at 4 °C, interchangeably in a Sorvall SLA-1500 or SLA-3000 rotor (Thermo Scientific, Waltham, MA). First, medium salts were removed from the cell-free broth by adjustment of the pH to 8.0 with NaOH, followed by centrifugation. Subsequently, the protein-based polymer was selectively precipitated from the solution by adding ammonium sulfate to a saturation of 45%, incubating overnight at 4 °C, and subsequent centrifugation. The pellet was resuspended in an equal volume (relative to the cell-free broth) of Milli-Q water and precipitation was repeated once at 4 °C, using an overnight incubation. The pellet was resuspended in 0.2 volumes (relative to the cell-free broth) of Milli-Q water and stored at 4 °C for subsequent purification.

MATERIALS AND METHODS

Materials. Linear 8.0, 2.5, and 2.0 kbp dsDNA were purchased from Thermo Scientific (Waltham, MA, USA). M13mp18 single-stranded DNA 7249 nt (ssDNA) was purchased from New England Biolabs (Ipswich, MA, USA) and T4-DNA were purchased from Nippon Gene (Tokyo, Japan) and used without further purification, supercooled 4.0 and 2.7 kbp pDNA were recovered from recombinant E. coli by using the GeneJet plasmid Miniprep kit from Thermo Scientific. All short ssDNA staples used for DNA origami formation were purchased from IDT (Integrated DNA Technologies, Inc.). The precise sequence of staples and scaffold/staple layout for the “Tall Rectangle” design can be found in ref 31. YOYO-1 was purchased from Invitrogen (Life Technologies, Carlsbad, CA, USA). Restriction enzymes were purchased from New England Biolabs or from Thermo Scientific. The C₄−B₈so7d and C₁₂ protein polymers were produced and purified following previously published methods.†‡
calibration was done based on Protein Calibration Standard II (Bruker).

**Preparation of Protein–DNA Complexes.** Protein–DNA complexes dissolved in 10 mM sodium phosphate buffer, pH 7.4, were prepared by mixing of pipetted portions of DNA stock solution, protein stock solution and 100 mM phosphate buffer, pH 7.4, in Milli-Q water. Mixtures were vortexed for 1 min. Volumes of the mixed portions of stock DNA and protein solutions were varied according to their initial concentration and the desired final molar protein/DNA-bp (ptn/bp) ratio. Protein stock solutions were prepared just before use by dissolving a weighted amount of lyophilized protein in Milli-Q water.

**Preparation of DNA Origami.** To assemble the tall rectangle DNA origami designed by Rothemund,\(^5\) 5 nM single-stranded M13mp18 DNA (NEB, 7249 nt long) was mixed in 1× TAE buffer (40 mM Tris, 20 mM Acetic acid, 2 mM EDTA and 12.5 mM Magnesium acetate, pH 8.0) with 50 mM n-tape strands. The solution was heated to 80 °C and then cooled to 20 °C over 2 h and subsequently kept at 4 °C.

**Preparation of Protein–DNA Origami Complexes.** Protein–DNA origami complexes were prepared by mixing of pipetted portions of DNA origami stock solution in 1× TAE buffer (40 mM Tris, 20 mM Acetic acid, 2 mM EDTA and 12.5 mM Magnesium acetate, pH 8.0) and protein stock solution in 10 mM acetate buffer, pH 4.85. Mixtures were vortexed for 10 s. Volumes of the mixed portions of stock DNA and protein solutions were varied according to their initial concentration and the desired final protein/DNA-bp (ptn/bp) ratio.

**Electrophoretic Mobility Shift Assay (EMSA).** Aliquots of DNA (50 ng/μL for pDNA and 2.0 kb linear dsDNA and 30 ng/μL for circular M13 ssDNA) were mixed with different volumes of a CsCl–B sötväger solution (0.035 or 0.35 g L\(^{-1}\)) and with 10× Tris–acetate–EDTA (TAE) buffer (pH 8) for a final volume of 10 μL. After incubation for at least 60 min at room temperature, the mixtures were mixed with 6× loading buffer and 10–12 μL of the final mixture were subjected to electrophoresis in an agarose gel (1%) for at least 60 min at 95 V using 1× TAE buffer (pH 8). Bands were visualized using Sybr gold. In the case of DNA origami, aliquots of 5 nM origami (40 ng/μL) in 1× TAE buffer (40 mM Tris, 20 mM Acetic acid, 2 mM EDTA and 12.5 mM Magnesium acetate, pH 8.0) were mixed with different volumes of a CsCl–B sötväger solution (0.05 to 0.2 g L\(^{-1}\)) in 10 mM Acetic acid buffer, pH 4.85. Mixture was vortexed for 10 s and then incubated for 60 min at room temperature. The mixtures were mixed with 6× loading buffer and subjected to electrophoresis in an agarose gel (1%) for 30 min at 90 V using 1× TAE buffer. Bands were visualized using ethidium bromide.

**Fluorescence Quenching.** Protein + dsDNA sample were incubated between 1 and 4 h at room temperature before measuring the fluorescence intensity in a Cytation 3 imaging reader (Biotek). For fluorescence measurements, 150 μL of sample were deposited in a 96-well Greiner 96 Black Flat Bottom Fluorotrac. Using top optics, samples were excited at 285 nm and fluorescence emission at 340 nm was collected with an electron multiplying charge-coupled device (EMCCD) camera (Andor iXon X3). The diffusion coefficient and hydrodynamic radius of bare T4 DNA and T4 DNA and coated with C8–B\(^{12}\) (0.834 ptn/bp) and C8–B\(^{20}\) (0.5 ptn/bp) were calculated from the fluorescence intensity decay. 10 μL complexes dissolved in 10 mM sodium phosphate buffer (pH 8). Bands were visualized using Sybr gold. In the case of DNA origami, 1 μL of 5 nM DNA origami-protein-based polymer complex solution was mixed with 9 μL of filtered Milli-Q water and immediately incubated at 4 °C for 1 h in AC Molecule tapping mode. Images were recorded at 1.95 Hz and 1024 samples per line. Height profile measurements were performed with Igor software.

**Fluorescence Microscopy Imaging.** Protein-coated single T4-DNA molecules were stained at room temperature with the intercalating fluorescent dye YOYO-1 in 10 mM Tris-HCl, pH 8.0 (intercalation ratio of one every 25 bp). The samples were incubated at least 30 min and the final T4-DNA concentration was ∼5 μg/mL. The fluorescent protein–DNA was imaged with a Nikon Eclipse Ti inverted fluorescence microscope equipped with a 200 W metal halide lamp, a filter set and a 100× oil immersion objective. The exposure time was controlled using an UV light shutter and the images were collected with an electron multiplying charge-coupled device (EMCCD) camera (Andor iXon X3). The fluorescence signal of bare T4-DNA and T4-DNA molecules were stained at room temperature with the intercalating fluorescent dye YOYO-1 in 10 mM Tris-HCl, pH 8.0 (intercalation ratio of one every 25 bp). The samples were incubated at least 30 min and the final T4-DNA concentration was ∼5 μg/mL.

**DNA Protection Test.** pDNA 2.6 kbp (concentration of 19.7 ng/μL) was complexed with C8–B\(^{20}\) (concentration 0.44 mg/mL) in TAE buffer (pH 8) for 1 h at room temperature (0.188 ptn/bp). Then 1 μL of the enzyme DNase I (RNase free, Thermo Scientific) 0.055 U was added to 35.5 μL complexes dissolved in reaction DNase I buffer (100 mM Tris-HCl, pH 7.5, 25 mM MgCl\(_2\), 1 mM CaCl\(_2\)) for a final [DNA] = 17.2 g/mL and immediately incubated at 25 °C using a thermo block. Aliquots of 3.5 μL were taken at different times and mixed with 3.5 μL of EDTA 50 mM. After addition of loading buffer (6×) the sample was electrophoresed in agarose gel 1% at 100 V for 45 min. DNA bands were visualized using ethidium bromide. The same procedure was repeated for DNA Origami 7.2 kbp (concentration 20 ng/μL). To estimate fractions of intact DNA as a function of time, gel images were analyzed using the ImageJ software.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsnano.6b05938.

Extra methods and Supporting Figures S1–S5 (PDF)

Video S1 (AVI)

Video S2 (AVI)

Video S3 (AVI)

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**Notes**

The authors declare no competing financial interest.

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REFERENCES


