

Direct Single-Molecule Observation of Mode and Geometry of RecA-Mediated Homology Search

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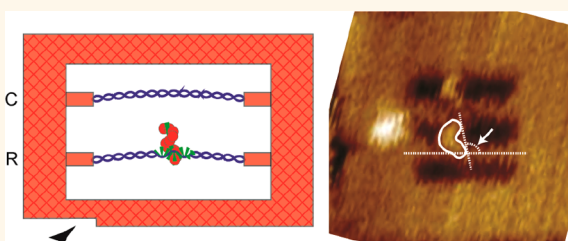
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Supporting Information

ABSTRACT: Genomic integrity, when compromised by accrued DNA lesions, is maintained through efficient repair via homologous recombination. For this process the ubiquitous recombinase A (RecA), and its homologues such as the human Rad51, are of central importance, able to align and exchange homologous sequences within single-stranded and double-stranded DNA in order to swap out defective regions. Here, we directly observe the widely debated mechanism of RecA homology searching at a single-molecule level using high-speed atomic force microscopy (HS-AFM) in combination with tailored DNA origami frames to present the reaction targets in a way suitable for AFM-imaging. We show that RecA nucleoprotein filaments move along DNA substrates via short-distance facilitated diffusions, or slides, interspersed with longer-distance random moves, or hops. Importantly, from the specific interaction geometry, we find that the double-stranded substrate DNA resides in the secondary DNA binding-site within the RecA nucleoprotein filament helical groove during the homology search. This work demonstrates that tailored DNA origami, in conjunction with HS-AFM, can be employed to reveal directly conformational and geometrical information on dynamic protein–DNA interactions which was previously inaccessible at an individual single-molecule level.



KEYWORDS: homologous recombination, DNA repair, RecA, recombinase A, high-speed AFM, DNA origami

The repair of DNA damage, in particular double stranded breakages, is critical to the maintenance of genomic integrity in all forms of life. Where lesions occur, the DNA sequence is normally recovered by homologous recombination, which exchanges identical or very similar DNA strands, in order to overcome deleterious cell activity or carcinogenesis. Homologous recombination is mediated by the ubiquitous recombinase A (RecA) family of recombinases and involves three steps: formation of a nucleoprotein filament; location of sequence homology; and finally strand exchange. An overview of this process is given in Figure 1a. In the first step, RecA monomers polymerize non-sequence specifically on single-stranded DNA (ssDNA)—at a stoichiometry of three nucleotides (nt) to one RecA monomer—in the presence of Mg^{2+} and ATP (or ATP analogues such as ATP γ S) to form a right-handed helical nucleoprotein filament, 10 nm wide, and with a helical pitch of 10 nm.^{1,2} The encapsulated ssDNA is held in the core of the filament bound through hydrogen bonds formed between the nucleic acid phosphate groups and residues in the L1 and L2 loops which collectively make up DNA-binding-site I of the

RecA protein. The nucleobases are orthogonal to, and spiral around, the helical axis of the filament with 6.2 RecA monomers and 18.5 nt per turn.

Once formed, the nucleoprotein complex searches available and accessible double-stranded DNA (dsDNA) for sequence homology between the encapsulated ssDNA and the dsDNA substrate.² Molecular dynamics (MD) simulations suggest that the incoming dsDNA enters the filament via the filament's helical groove,³ giving rise to a characteristic interaction geometry which is as yet unverified experimentally. Access to the helical groove is gated by the predominately negatively charged C-terminal domain which protrudes radially from the complex.^{3,4}

The incoming dsDNA is thought to bind transiently along a strongly electronegative cleft located at the C-terminal side of the L2 loop, commonly referred to as DNA-binding-site II, with three nucleotides roughly in B-form bound by each RecA

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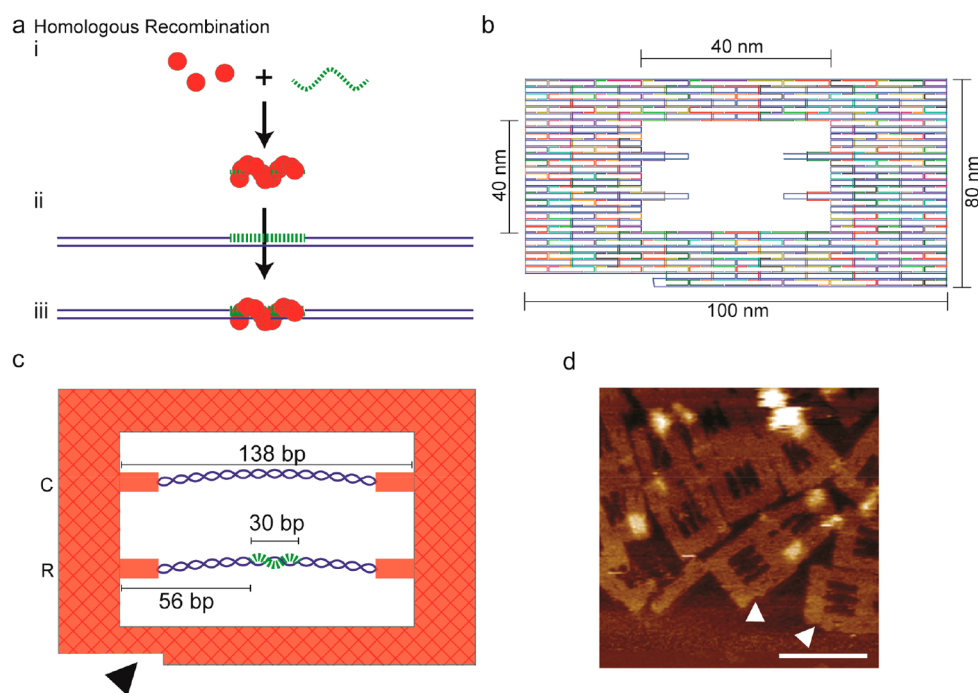


Figure 1. Experimental setup for conducting RecA homology searching within a DNA origami frame. (a) Schematic diagram depicting RecA-mediated homologous recombination. (i) RecA monomers (red circles) polymerize upon ssDNA (green line) in the presence of Mg^{2+} and ATP forming a nucleoprotein filament. (ii) The nucleoprotein filament searches for sequence homology (green dashed line) between the encapsulated ssDNA and a dsDNA substrate (blue lines). (iii) A stable complex is formed where sequence homology is located. (b) Schematic diagram of the DNA origami frame containing two sets of anchor points where dsDNA molecules can be suspended. (c) The arrangement of two dsDNA strands within the DNA frame, a heterologous control (C) and a reaction (R) strand containing a 30 bp region of sequence homology (green dashed line). The central strands are 138 bp long (including the anchors) and interact only weakly with the surface and thus are easily accessible by the nucleoprotein filament. A polarity marker (black triangle) is included for geometrical reference. (d) AFM micrograph showing the folded DNA origami frames with polarity markers indicated by white triangles. Scale bar = 125 nm. Z-scale = 5 nm.

monomer and with large stretches between neighboring triplets.⁵ Binding occurs *via* salt bridges formed with the backbone of the strand that is not probed for homology, the outgoing strand, leaving the other complementary strand of the dsDNA free to interact with the encapsulated ssDNA.³

It has been proposed that a number of L2 loop residues intercalate in the stretches between the nucleotide triplets of the incoming dsDNA supporting the extension of the dsDNA from the B-form and causing a kink which disrupts the base stacking. This allows the complementary strand of the incoming dsDNA to be presented in registration with the encapsulated ssDNA in binding-site I such that new base pairs can be formed.^{3,6} Where sequence homology is located, further base pairing occurs, continually stabilizing the pairing as the incoming strand processively winds into the nucleoprotein filament. The new duplex is finalized and released from the nucleoprotein complex as RecA dissociates upon hydrolysis of the ATP which is bound at the monomer–monomer interface.^{7–9}

To date, the mechanism and interaction geometry by which RecA orchestrates its homology search remains widely debated, particularly given that no motor activity has been identified despite the inclusion of ATP in the nucleoprotein filament. The current understanding is derived from a multitude of biochemical,^{10,11} average reconstructed structural information,^{12–18} and MD simulations.^{3,4,19} Typically, single-molecule experiments on the RecA homology searching mechanism have relied on indirect reporting,²⁰ for example, FRET-based studies,^{21,22} which are limited in their ability to resolve unambiguously specific events of individual RecA filament–

DNA encounters where contributions of other simultaneous interactions may contribute to the signal. This is a particular problem where RecA has been shown to interact cooperatively during homology searching under these conditions.²³

In contrast, atomic force microscopy (AFM) offers the spatial and temporal resolutions to interrogate directly nucleoprotein interactions unambiguously at the single-molecule level. Here, we utilize surface-immobilized DNA origami nanostructures to present localized but only weakly bound dsDNA on which individual RecA nucleoprotein filaments can undertake homology searches. This system enables the interrogation of individual searching events through high-speed atomic force microscopy (HS-AFM), which provides detailed information regarding the geometry and mode of interaction between the nucleoprotein filament and the DNA substrate (Figure 1b).

RESULTS AND DISCUSSION

The DNA origami nanostructure was designed as a frame and is based on our previous work,²⁴ featuring two dsDNA strands at its core, a control (fully heterologous) and a reaction (with a 30 bp homologous region) strand (Figure 1b,c and Supporting Information Figure S4). This enables nonsequence-specific searching activity across heterologous sequences to be discerned from specific interactions resulting from the location of homology. Furthermore, searching activity across non-contiguous dsDNA can be observed.

RecA nucleoprotein filaments were imaged *in situ* with HS-AFM while searching for homology on the dsDNA presented by the DNA origami nanostructures. Once individual filaments

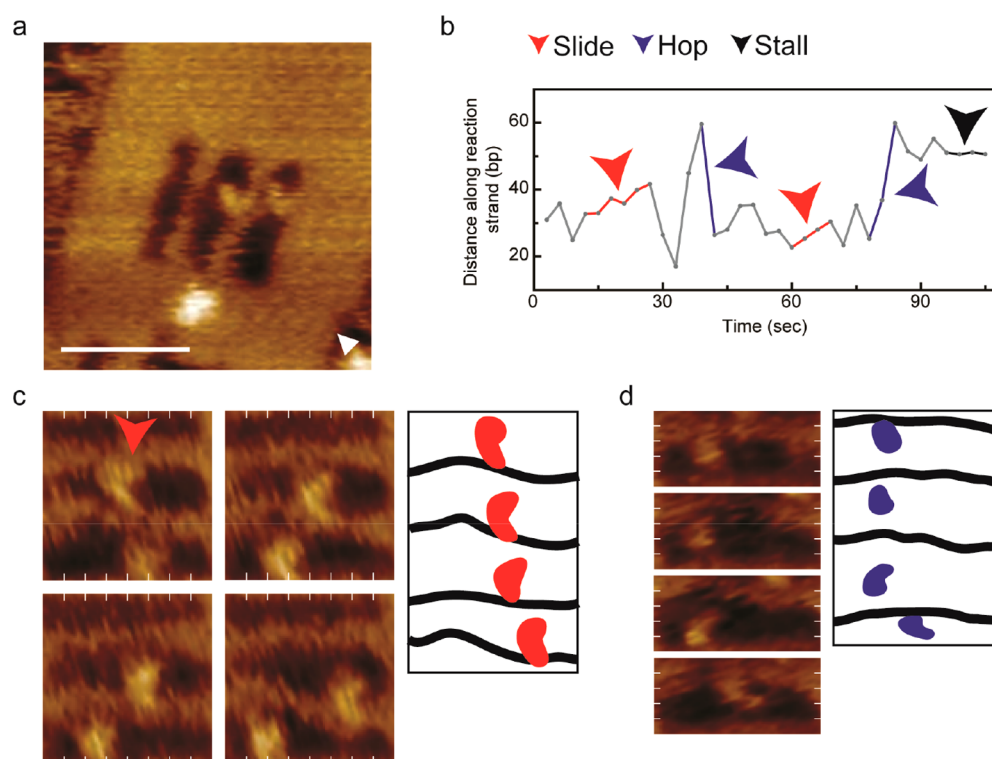


Figure 2. Direct observation of RecA nucleoprotein filament homology search. (a) Snapshot of HS-AFM movie showing nucleoprotein filaments searching for homology on a dsDNA suspended within a DNA origami. The orientation of the origami is determined from the polarity marker (white triangle). (b) Motion of a representative filament, plotted as the distance of the filament from the end of the reaction strand closest to the polarity marker *vs* time. Examples of distinct behaviors are highlighted: facilitated diffusion, “slides” along the DNA (red arrows); large distance movements along the dsDNA or dissociation from the DNA, “hopping” (blue arrows); and “stalling” at the region of homology (black arrow). Representative examples of these events can clearly be seen in the HS-AFM images and associated schematics, showing typical sliding (c) and hopping (d) movements. Scale bar (A) = 40 nm; Z-scale = 6 nm.

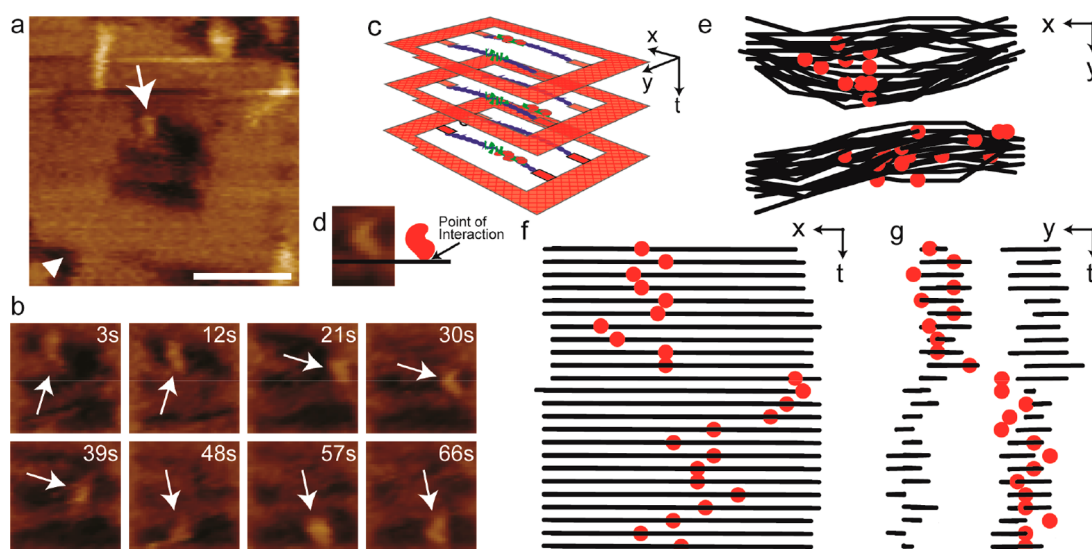


Figure 3. Homology searching on noncontiguous DNA. (a) AFM micrograph showing a 30 nt nucleoprotein filament (white arrow) interacting with one of the suspended DNA strands in the origami frame (polarity marker indicated by the white triangle). (b) HS-AFM image series showing a nucleoprotein filament sliding along and swapping from one suspended DNA strand to the other. The position of the filament relative to the position of the dsDNA is shown in (e–g) using the coordinate system shown in (c). The red circles represent the point of interaction of the filament with the dsDNA as shown in (d), and the black lines represent the dsDNA strands. Scale bar = 40 nm. Z-scale = 6 nm.

associate with the dsDNA, they can be seen to move freely along the partially constrained dsDNA despite being confined to the central cavity of the DNA origami nanostructure,

tracking back and forth along the DNA strands as well as jumping from one strand to the other (Figure 2 and Movie S1). The searching activity is shown to be independent of the AFM

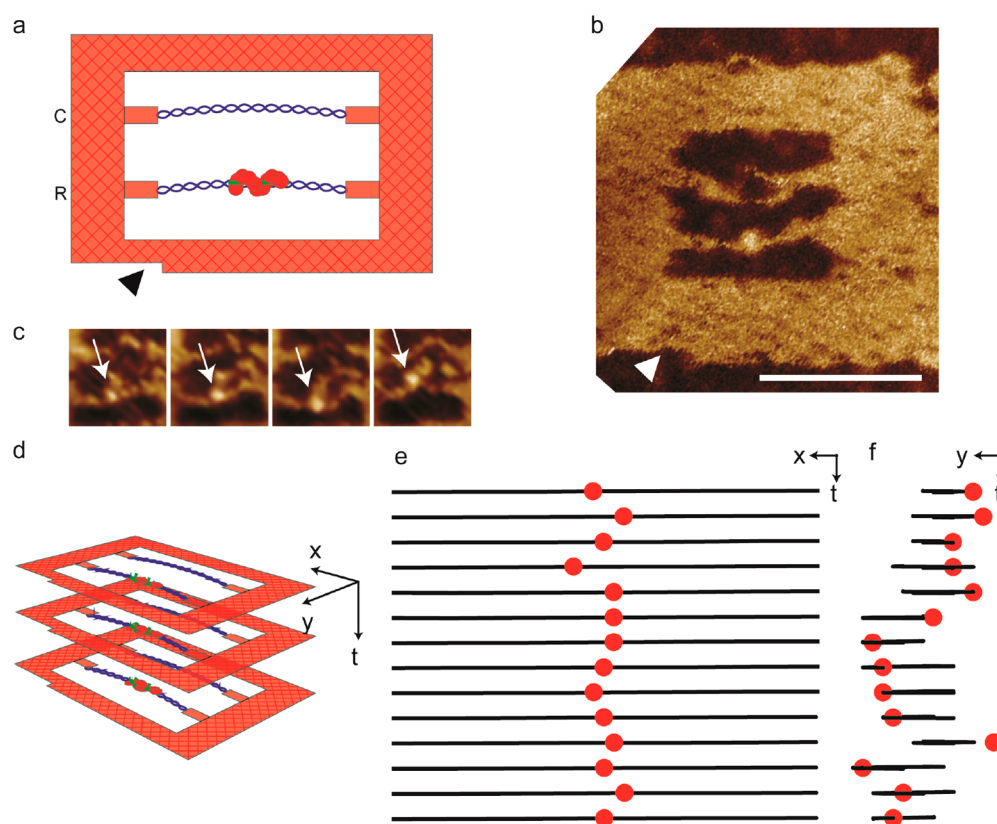


Figure 4. Observation of homology location with the HS-AFM. (a) Schematic and (b) AFM micrograph depicting a RecA filament bound to the region of sequence homology on the reaction strand (indicated by “R”). The white triangle indicates the polarity marker of the DNA frame. (c) A time-series of AFM micrographs demonstrating the stability of the synaptic joint, which moves in concert with the dsDNA, at the region of sequence homology. (e and f) The positions of the center of mass of the nucleoprotein filament (red circles) and the dsDNA (black lines) according to the coordinate system shown in (d). Scale bar in (b) and size of images in (c) is 40 nm. Z-scales are (b) 6 nm and (c) 5 nm.

scanning direction, demonstrating the minimal influence exerted by the scanning AFM probe (Figure S1).

The discrete motions of the nucleoprotein filaments were determined with respect to the internal dsDNA strands using the DNA origami frame as a reference. The frame presents the dsDNA in a predictable position, and hence the center of mass of individual filaments can be plotted along its length *vs* time (Figure 2b), where two distinct types of motion can be discerned. Short regions of consistent unidirectional movement, referred to as slides (Figure 2b, red arrows and Figure S3, black arrows) are observed, separated by large random jumping motions between a variety of positions along the DNA strand, referred to as “hops” (random sampling) (Figure 2b, blue arrows). The procedure for identifying these motions is detailed in Figure S2. The direct observation of persistent dsDNA-associated motion further validates the previously reported mechanism of 1D facilitated diffusion or sliding along a DNA substrate during RecA-facilitated homology search.²¹ Examples of the two distinct motions can be seen in Figure 2c,d for slides and hops, respectively (see also Movie S1).

From the analysis of more than 30 motion plots similar to the one reported in Figure 2b, we observe sliding motions over an average distance of 6.0 ± 1.0 bp, with up to a maximum distance of 25 bp (Figure S3), yielding an average search rate of 0.9 ± 0.3 bp s^{-1} . We note that despite the biological reaction being confined to the surface, and thus a solid–liquid interface (as required for AFM studies), the slides observed here are only three times slower than those suggested by recent FRET

experiments.²¹ The short distances and the low occurrence frequency when compared to random sampling suggest that sliding facilitates checking for homology around the current location and is not a primary sampling method. The search rate for hops is considerably faster, at 6.2 ± 3.7 bp s^{-1} , than that observed for slides and with an average movement distance of 19.5 ± 2 bp. The overall combined search rate is 3.5 ± 2.0 bp s^{-1} under the conditions used in this study. The nucleoprotein filaments search both the reaction and control strands with no discernible differences, demonstrating that all of the available sequence space is sampled.

The average dwell-time of a filament on the dsDNA is of the order of a few seconds—unless it encounters a sequence of homology—after which the filament detaches from the dsDNA and can reattach to either the same dsDNA at a different position, or in fact attach to another dsDNA. This predominately random sampling approach enables the filament to search noncontiguous DNA, here the control and reaction strands, in concert as shown in Figure 3 (and also Movie S2). *In vivo* this is likely to be accompanied by intersegmental transfer, where disparate regions of the same filament are able to interact with separate dsDNA sequences independently.²⁰ However, the filaments in this work are too short, with only 30 nt representing 1.5 turns of the nucleoprotein helix, for this to be observed.

Although filaments searching for homology were frequently observed throughout this study, successful location of homology was rare. This is likely a result of the reduced degrees of freedom of the dsDNA substrate given its tethered

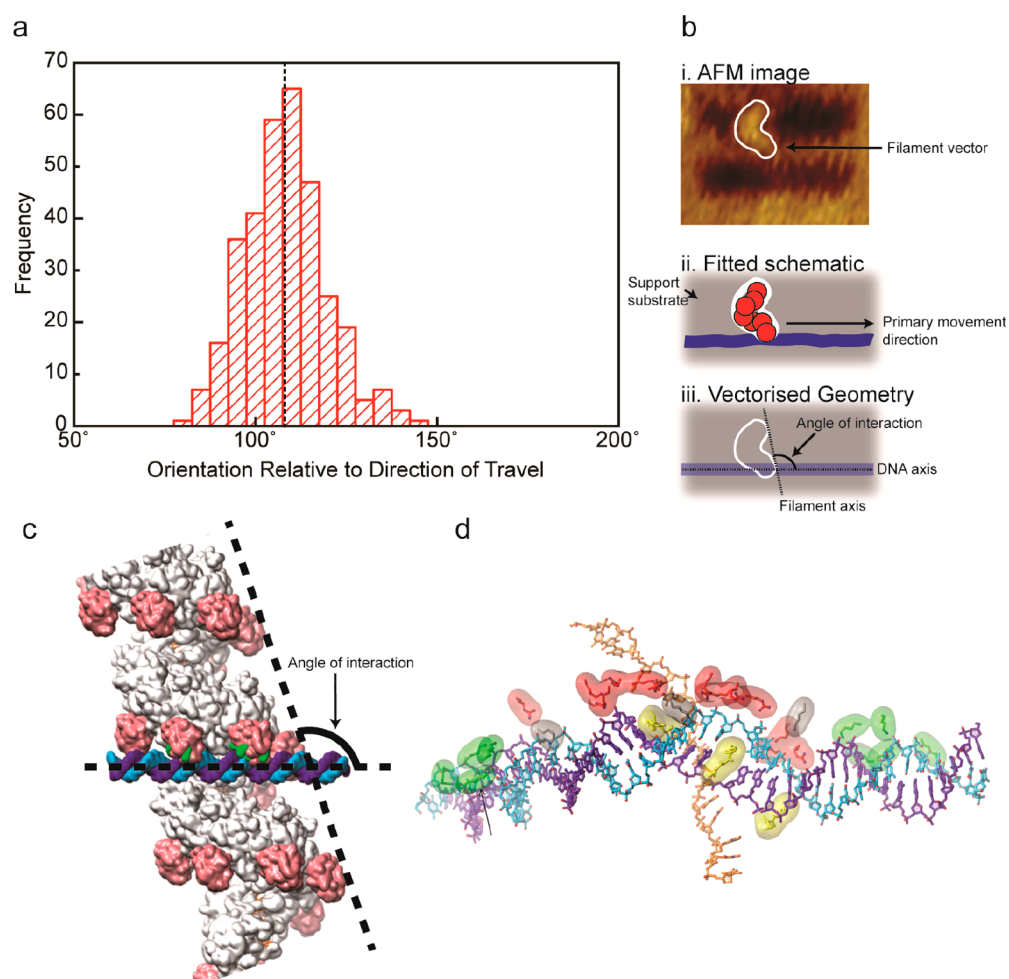


Figure 5. Geometry of the nucleoprotein filament–DNA interaction. (a) Distribution of the angle of interaction between the nucleoprotein filament and dsDNA, measured as shown in (b). The angle of 108° , as predicted by the MD simulations of Yang *et al.*³ is indicated (black dashed line). (b) A vector is fitted around the nucleoprotein filament in the AFM micrograph (i) which can be directly related to the predicted interaction geometry, showing the curvature of the helical groove (ii). The angle between the filament axis and dsDNA axis is measured from the vector forms (iii). The gray area indicates the surface onto which the DNA origami frame is immobilized, and the blue line indicates the dsDNA. (c and d) The observed interaction angle is in-line with the prediction from molecular dynamic simulations of Yang *et al.*³ (c) The incoming dsDNA is bound into the helical groove of the nucleoprotein filament through interactions with the C-terminal-side of L2 (red), thereby aligned into binding site II. (d) The encapsulated ssDNA is shown in orange, with the incoming dsDNA shown in purple and blue. The residues responsible for DNA interactions are highlighted for the C-terminal domain (green), binding site II (red), and L2 binding loop (yellow). Panels (c) and (d) are adapted from Yang *et al.*³

termini, which may restrict the ability of additional dsDNA to enter the helical groove of the filament to probe for further sequence homology. Nonetheless, stable nucleoprotein complexes can be observed at the region of homology due to the inclusion of ATP γ S which prevents the RecA from dissociating (Figure 4). The bound complexes remain located at the homologous sequence through successive images and in fact move in concert with the fluctuations of the dsDNA, suggesting substantial stability of the nucleoprotein–dsDNA complex, as well as confirming that they are not only transiently bound (Figure 4c,e,f and Movie S3).

The geometry of the interaction of the RecA-filament with an incoming dsDNA has been predicted through MD simulations,³ where it was estimated that the angle between the filament and the dsDNA is 108° during the interaction. Here, we are able to verify experimentally and directly the proposed interaction geometry throughout the dynamic homology searching process, which was enabled by the use of the DNA origami frame as a reference (Figure 5). Given that the

nucleoprotein filament has a helical symmetry and making the reasonable assumption that it lies within the surface plane, the orientation of the interaction can be determined between the dsDNA and the filament during its search. Figure 5a shows the distribution of interaction angles, measured as indicated in Figure 5b, revealing a characteristic interaction angle of $106 \pm 11^\circ$ between the nucleoprotein filament and the dsDNA with reference to the direction of travel.

The observed interaction geometry fits well with that proposed by the simulations of Yang *et al.*³ (108° ; Figure 5c,d), suggesting that the incoming dsDNA indeed aligns with binding site II within the helical groove of the filament. From Figure 5d, the encapsulated ssDNA running the length of the filament (orange) can be seen to be orientated with respect to the incoming dsDNA (purple and blue) at an angle very similar to that observed in this study.

Although HS-AFM does not yet have sufficient spatial resolution to infer the exact geometry of the section of the dsDNA where it enters the filament or to confirm the existence

of a kink in the incoming dsDNA owing to the localized melting, it provides direct experimental evidence of the transient alignment of dsDNA within the nucleoprotein filament's helical groove during homology searching.

CONCLUSIONS

We have demonstrated that HS-AFM combined with the DNA origami frame system, where a biological interaction can take place within a structurally robust reference, can provide conformational and geometrical information on dynamic nucleoprotein interactions at a single-molecule level, revealing details unobtainable by any other method.

Exploiting this system, we directly show that RecA nucleoprotein filaments undergo facilitated diffusion along the incoming dsDNA during homology searching. In this mode only short distances are explored—approximately 6 bp at a time—and hence it is likely to relate to the checking of sequence registration surrounding locations of microhomology, while the overall sequence space is randomly sampled.

Furthermore, we demonstrated that the nucleoprotein filament interaction with the incoming dsDNA occurs with a distinct and defined geometry. The observed interaction geometry suggests that the dsDNA enters the nucleoprotein filament aligned with the DNA-binding-site II of RecA which follows the right-handed helical groove, experimentally corroborating the findings of the MD simulations of Yang *et al.*³ The ability to extract critical geometrical information from dynamic single-molecule experiments is thereby demonstrated as a key strength of this approach and will aid in the further understanding of other nucleoprotein interactions and systems.

MATERIALS AND METHODS

Materials. All DNA oligonucleotides that formed the central strands, DNA frame staples, and the nucleoprotein filament substrate used in this study were synthesized by Integrated DNA Technologies (IDT; Coralville, USA). The M13mp18 ssDNA was purchased from New England Biolabs (NEB; Ipswich, USA). The sequences for all DNA species used in this study are provided in [Supporting Information](#). RecA protein (*E. coli*) was purchased from NEB at a concentration of 2 mg/mL and was used without further purification. Adenosine 5-(γ -thio)triphosphate (ATP γ S) tetralithium salt, made up to 5 mM, Mg(OAc)₂, made up to 100 mM, Tris acetate, made up to 100 mM, pH 7.4, and NiCl₂, made up to 10 mM in deionized water, were all purchased from Sigma-Aldrich (St. Louis, USA).

Formation of Origami Nanostructure. The DNA frame was formed as from the design published by Yamamoto *et al.*,²⁴ using the caDNAno software.^{25,26} The DNA origami structure was formed from the collective self-assembly of 224 oligonucleotide staples (sequence list, [Table S1](#)) and M13mp18 ssDNA scaffold. Structures were folded in 10 mM Tris acetate (pH 7.4), 10 mM Mg(OAc)₂, and 1 mM EDTA following a cooling gradient of $-1\text{ }^{\circ}\text{C min}^{-1}$ from 95 to 15 $^{\circ}\text{C}$. The central reaction and control DNA strands were hybridized from their constituent oligonucleotides (sequence list, [Table S2](#)) as above and were incorporated into the DNA origami following a gradient of $-0.5\text{ }^{\circ}\text{C min}^{-1}$ from 45 to 15 $^{\circ}\text{C}$. Completed structures were purified using a Sephacryl S400 (GE Healthcare, Buckinghamshire, UK) size exclusion matrix in a buffer containing 10 mM Tris acetate (pH 7.4), 10 mM Mg(OAc)₂ and 1 mM EDTA to remove excess staples and unincorporated central strands.

RecA Nucleoprotein Filament Formation. RecA nucleoprotein filaments were formed as previously described by Sharma *et al.*²⁷ Briefly, RecA protein was introduced to the 30 nt DNA oligonucleotide at a ratio of 1 RecA monomer to 3 nucleotides in the presence of 500 μM ATP γ S, 10 mM Tris acetate (pH 7.4), and 2 mM Mg(OAc)₂. The reaction was incubated at 37 $^{\circ}\text{C}$ for 15 min.

In Situ Homology Searching. Complete DNA frames were deposited upon freshly cleaved mica, incubated for 15 min, and rinsed off with deionized water before being immersed in a buffer containing 10 mM Tris acetate (pH 7.4) and 10 mM Mg(OAc)₂.

Nucleoprotein filaments were introduced to the surface-bound DNA frames at a ratio of 1:10 (nucleoprotein filament: DNA frame) and incubated on the surface for 5 min at 22 $^{\circ}\text{C}$. The subsequent homology searching interactions were followed by continually scanning with the HS-AFM in the same buffer at 22 $^{\circ}\text{C}$.

HS-AFM Imaging. All samples were imaged in tapping mode (amplitude modulation), in aqueous buffer, with a Bruker Dimension Fastscan AFM (Bruker Nanosurfaces, Santa Barbara, USA), using Fastscan D etched Si₃N₄ cantilevers (nominal spring constant = 0.25 N/m, resonant frequency approximately 110 kHz in liquid) containing a Si tip (nominal radius of curvature = 5 nm). Cantilevers were driven close to resonance under liquid, and images were typically acquired with a pixel density of 512 \times 512, a scan speed of 38 Hz and a tapping amplitude of 4–6 nm. Images were flattened by plane-fitting using the associated Nanoscope analysis software (Bruker Nanosurfaces, Santa Barbara, USA). ImageJ software (<http://rsbweb.nih.gov/ij/>) was subsequently used to determine the nucleoprotein filament coordinates throughout sequential images in a series. The conditions for the classification of the different nucleoprotein filament motions are defined in [Figures S2 and S3](#).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsnano.7b06208. Data supporting this work can be accessed via the University of Leeds repository: <https://doi.org/10.5518/296>.

Additional figures demonstrating the minimal influence of the scanning probe, definitions of the sliding motion boundaries, further motion plots, design details of the DNA origami frame, supporting movies depicting the observed searching behaviors and all sequences used in this study ([PDF](#))

Movie S1: RecA nucleoprotein filament searching for homology within a DNA origami frame ([AVI](#))

Movie S2: Homology searching on non-contiguous DNA strands ([AVI](#))

Movie S3: A stable synaptic joint formed at the region of homology ([AVI](#))

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Notes

The authors declare no competing financial interest.

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REFERENCES

- (1) Galletto, R.; Amitani, I.; Baskin, R. J.; Kowalczykowski, S. C. Direct Observation of Individual RecA Filaments Assembling on Single DNA Molecules. *Nature* **2006**, *443*, 875–878.
- (2) Cox, M. M. Regulation of Bacterial RecA Protein Function. *Crit. Rev. Biochem. Mol. Biol.* **2007**, *42*, 41–63.
- (3) Yang, D.; Boyer, B.; Prevost, C.; Danilowicz, C.; Prentiss, M. Integrating Multi-Scale Data on Homologous Recombination into a New Recognition Mechanism Based on Simulations of the RecA-ssDNA/dsDNA Structure. *Nucleic Acids Res.* **2015**, *43*, 10251–10263.
- (4) Saladin, A.; Amourda, C.; Poulain, P.; Ferey, N.; Baaden, M.; Zacharias, M.; Delalande, O.; Prevost, C. Modeling the Early Stage of DNA Sequence Recognition Within RecA Nucleoprotein Filaments. *Nucleic Acids Res.* **2010**, *38*, 6313–6323.
- (5) Chen, Z.; Yang, H.; Pavletich, N. P. Mechanism of Homologous Recombination from the RecA-ssDNA/dsDNA Structures. *Nature* **2008**, *453*, 489–494.
- (6) De Vlaminck, I.; van Loenhout, M. T. J.; Zweifel, L.; den Blanken, J.; Hoening, K.; Hage, S.; Kerssemakers, J.; Dekker, C. Mechanism of Homology Recognition in DNA Recombination from Dual-Molecule Experiments. *Mol. Cell* **2012**, *46*, 616–624.
- (7) Kowalczykowski, S. C.; Krupp, R. A. DNA-Strand Exchange Promoted by RecA Protein in the Absence of ATP: Implications for the Mechanism of Energy Transduction in Protein-Promoted Nucleic Acid Transactions. *Proc. Natl. Acad. Sci. U. S. A.* **1995**, *92*, 3478–3482.
- (8) Reymer, A.; Babik, S.; Takahashi, M.; Norden, B.; Beke-Somfai, T. ATP Hydrolysis in the RecA-DNA Filament Promotes Structural Changes at the Protein-DNA Interface. *Biochemistry* **2015**, *54*, 4579–4582.
- (9) Menetski, J. P.; Bear, D. G.; Kowalczykowski, S. C. Stable DNA Heteroduplex Formation Catalyzed by the Escherichia coli RecA Protein in the Absence of ATP Hydrolysis. *Proc. Natl. Acad. Sci. U. S. A.* **1990**, *87*, 21–25.
- (10) Müller, B.; Koller, T.; Stasiak, A. Characterization of the DNA Binding Activity of Stable RecA-DNA Complexes. Interaction Between the Two DNA Binding Sites within RecA Helical Filaments. *J. Mol. Biol.* **1990**, *212*, 97–112.
- (11) Adzuma, K. No Sliding During Homology Search by RecA Protein. *J. Biol. Chem.* **1998**, *273*, 31565–31573.
- (12) Egelman, E. H.; Stasiak, A. Structure of Helical RecA-DNA Complexes. Complexes Formed in the Presence of ATP-gamma-S or ATP. *J. Mol. Biol.* **1986**, *191*, 677–697.
- (13) Egelman, E. H.; Stasiak, A. Structure of Helical RecA-DNA Complexes. II. Local Conformational Changes Visualized in Bundles of RecA-ATP-gamma-S Filaments. *J. Mol. Biol.* **1988**, *200*, 329–349.
- (14) Ellouze, C.; Takahashi, M.; Wittung, P.; Mortensen, K.; Schnarr, M.; Norden, B. Evidence for Elongation of the Helical Pitch of the RecA Filament Upon ATP and ADP Binding Using Small-Angle Neutron Scattering. *Eur. J. Biochem.* **1995**, *233*, 579–583.
- (15) Nordén, B.; Wittung-Stafshede, P.; Ellouze, C.; Kim, H.-K.; Mortensen, K.; Takahashi, M. Base Orientation of Second DNA in RecA DNA Filaments Analysis by a Combination of Linear Dichroism and Small Angle Neutron Scattering. *J. Biol. Chem.* **1998**, *273*, 15682–15686.
- (16) Timmins, P. A.; Ruigrok, R. W.; DiCapua, E. The Solution Structure of RecA Filaments by Small Angle Neutron Scattering. *Biochimie* **1991**, *73*, 227–230.
- (17) Datta, S.; Ganesh, N.; Chandra, N. R.; Muniyappa, K.; Vijayan, M. Structural Studies on MtRecA-Nucleotide Complexes: Insights into DNA and Nucleotide Binding and the Structural Signature of NTP Recognition. *Proteins: Struct., Funct., Genet.* **2003**, *50*, 474–485.
- (18) Story, R. M.; Weber, I. T.; Steitz, T. A. The Structure of the E. coli RecA Protein Monomer and Polymer. *Nature* **1992**, *355*, 318–325.
- (19) Klapstein, K.; Chou, T.; Bruinsma, R. Physics of RecA-Mediated Homologous Recognition. *Biophys. J.* **2004**, *87*, 1466–1477.
- (20) Forget, A. L.; Kowalczykowski, S. C. Single-Molecule Imaging of DNA Pairing by RecA Reveals a Three-Dimensional Homology Search. *Nature* **2012**, *482*, 423–427.
- (21) Ragunathan, K.; Liu, C.; Ha, T. RecA Filament Sliding on DNA Facilitates Homology Search. *eLife* **2012**, *67*, e00067.
- (22) Ragunathan, K.; Joo, C.; Ha, T. Real-Time Observation of Strand Exchange Reaction with High Spatio-temporal Resolution. *Structure* **2011**, *19*, 1064–1073.
- (23) Lee, A. J.; Sharma, R.; Hobbs, J. K.; Wälti, C. Cooperative RecA Clustering: the Key to Efficient Homology Searching. *Nucleic Acids Res.* **2017**, *45*, 11743–11751.
- (24) Yamamoto, S.; De, D.; Hidaka, K.; Kim, K. K.; Endo, M.; Sugiyama, H. Single Molecule Visualization and Characterization of Sox2-Pax6 Complex Formation on a Regulatory DNA Element using a DNA Origami Frame. *Nano Lett.* **2014**, *14*, 2286–2292.
- (25) Douglas, S. M.; Dietz, H.; Liedl, T.; Högberg, B.; Graf, F.; Shih, W. M. Self-Assembly of DNA into Nanoscale Three-Dimensional Shapes. *Nature* **2009**, *459*, 414–418.
- (26) Douglas, S. M.; Marblestone, A. H.; Teerapittayanon, S.; Vazquez, A.; Church, G. M.; Shih, W. M. Rapid Prototyping of 3D DNA-Origami Shapes with caDNAno. *Nucleic Acids Res.* **2009**, *37*, 5001–5006.
- (27) Sharma, R.; Davies, A. G.; Wälti, C. Directed Assembly of 3-nm-Long RecA Nucleoprotein Filaments on Double-Stranded DNA with Nanometer Resolution. *ACS Nano* **2014**, *8*, 3322–3330.