Electronic Supplementary Material (ESI) for ChemComm.

Covalent, Sequence-Specific Attachment of Long DNA Molecules to a Surface Using DNA-Templated Click Chemistry-- Supplementary Information

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Materials and methods

The following oligonucleotides were used in this study:

Name	DNA Sequence & Modifications	Purpose
SF-17	gctacctcgtgagcagtcagtacgttttt/(C11-SS)	Anchor strand control (no alkyne)
SF-47	gctacctcgtgagcagtcagtacg/(Oct-dU)/ttttt/(Am)	precursor to anchor strand
SF-57	ccagacatccgacacatactgaac/(Oct-dU)/ttttt/(Am)	precursor to anchor strand control (sequence mismatch)
SF-77	gctacctcgtgagcagtcagtacgttttt/(Am)	precursor to anchor strand control (no alkyne)
SF-27	gctacctcgtgagcagtcagtacg/Oct-dU/ttttt/(TEG)/(C3-SS)	anchor strand with alternative spacer
SF-88	(Az)/cgtactgactgctcacgaggtagc	anchor strand complement (+ azide)
Sf-18	Cgtactgactgctcacgaggtagc	anchor strand complement control (no azide)
SF-28	(Az)/cgtactgactgctcacgaggtagc/(C3)/tctgaactgtttaaagcatttgaggg	anchor complement (+ azide) + target strand primer
SF-78	cgtactgactgctcacgaggtagc/C3/tctgaactgtttaaagcatttgaggg	control anchor compliment + primer (no azide)
SF-58	(Am)/tctgaactgtttaaagcatttgaggg	target primer (+ amine)
SF-396	Accttatgcgattttaagaactgg	reverse primer for 396 bp target strand
SF-3679	Tccttgaaaacatagcgatagcttag	reverse primer for 3.6 kbp target strand

Abbreviations for DNA modifications: C11-SS = undecyl disulfide; Oct-dU = octadiynyl deoxyuracil; Am = amine; TEG = triethylene glycol spacer; C3-SS = propyl disulfide; Az = azide; C3 = propyl spacer

The first sequence was purchased from Biosearch Technologies, Inc. (Petaluma, CA, USA), and all others were purchased from Integrated DNA Technologies (Coralville, IA, USA). The purity of the custom ordered oligonucleotides was verified by the manufacturers using mass spectrometry, and the DNA was used without further purification. DNA was stored long-term at -20°C and short-term at 4°C in either TAE buffer (1X TAE = 40 mM Tris(hydroxymethyl)aminomethane acetate, 1 mM EDTA, pH 8.3) or phosphate buffer (PB, 20 mM sodium phosphate, pH 7).

Preparation of DNA anchor strands

The DNA anchor strands were prepared by attaching the 3'-amino-modified precursor oligonucleotides (SF-47, -57, -77) to a carboxyl-terminated alkyl disulfide via amide coupling chemistry. The precursor strand (20 μM) was mixed with a 50-fold molar excess of bis(10-carboxydecyl)disulfide along with 10 mM each of 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-Hydroxysuccinimide (NHS) activators and 20 mM sodium phosphate buffer (pH 6.5) in 1:1 aqueous isopropanol, in a total volume of 250 μL. The reaction tubes were backfilled with nitrogen gas and kept in the dark to react for 3 hours. The reaction mixtures were then purified using Illustra NAP-5 columns from GE Healthcare Life Sciences (Pennsylvania, USA), using 0.025X TAE (0.1 mM TrisAc, 2.5μM EDTA) as the elution buffer (low-salt buffer was necessary for a downstream purification step). After purification, the disulfide-modified DNA was stored at 4°C for up to two weeks. In order to minimize the possibility of an unwanted thiol-yne reaction between the strands, the disulfide was not reduced to a thiol until immediately prior to use, as described in a later section. The undecyl disulfide-modified anchor strands were characterized with electrospray ionization mass spectrometry by the Campus Mass Spectrometry Facilities at the University of California, Davis (see Fig. S1).

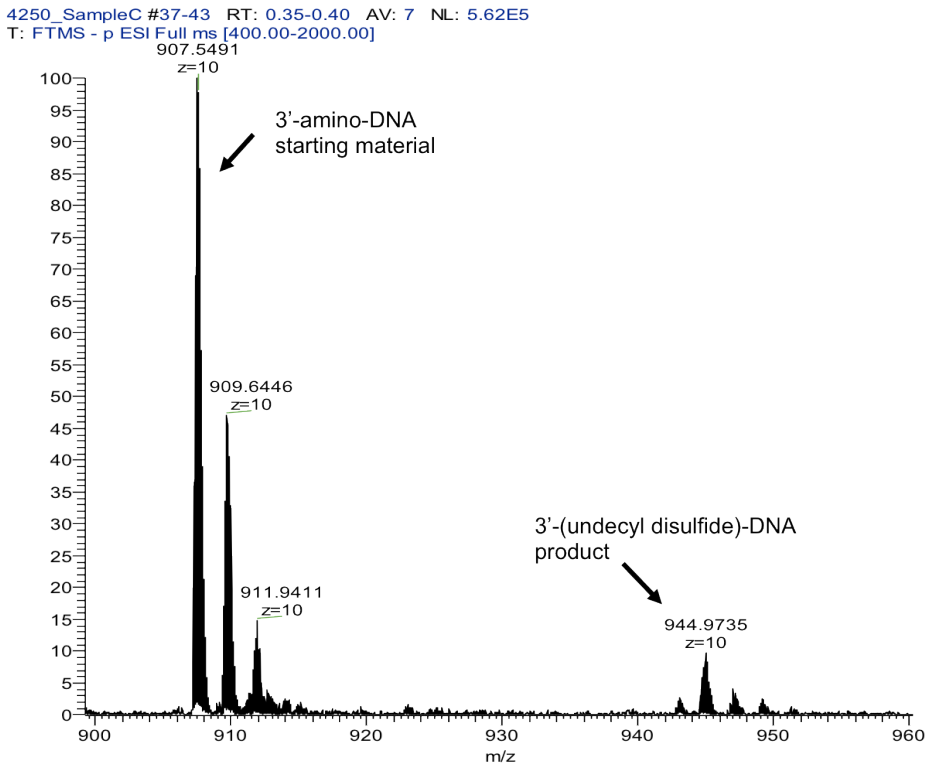


Fig. S1: Electrospray ionization mass spectrum of the undecyl disulfide-modified DNA anchor strands.

Preparation of DNA target strands

In order to hybridize with the surface anchor strands, the double-stranded DNA targets contain a terminal 24-base single-stranded DNA tail segment that is complementary to the anchor strand sequence. The target strands are generated via polymerase chain reaction (PCR) with a forward primer that is connected to the 24-base tail by a propyl spacer group, which prevents the polymerase enzyme from copying over the tail region. Therefore, PCR can produce a double-stranded product with a short, single-stranded tail (see Fig. S2). To serve as a template for PCR, M13mp18 RF I DNA (New England Biolabs Inc, Massachusetts,USA) was linearized using the EcoRI restriction enzyme (New England Biolabs). For the PCR reaction, 50 pg of linearized M13 DNA was combined with Taq DNA polymerase master mix (Bioexpress, Utah, USA), along with 200nM each of the appropriate forward (SF-28, -58 or -78) and reverse (SF-396 or -3679) primers in a 250 μL PCR tube. (The numerals at the end of the reverse primer name indicate the total base length of the target strand produced by PCR with that primer, including the 24-base tail). Ultrapure water produced by a Barnstead Nanopure Diamond water purification system (Thermo Scientific, North Carolina, USA) was used to bring the solution up to a volume of 50 μL. Solutions were incubated through the following program on an thermal cycler (Eppendorf, Hamburg, Germany): an initial melting step of 94°C for 2 min, followed by 35 cycles of

melting at 94°C for 30 sec, annealing at 49°C for 45 sec, and extension at 70°C for 30 sec or 200 sec (for SF-396 or -3679, respectively), followed by a final cycle with an extension of 5 minutes. Remaining primers and enzymes were removed using the QIAquick PCR Purification Kit (QIAGEN, Limburg, Netherlands). Purified DNA targets were kept in TAE buffer at 4°C for short-term, and -20°C for long-term storage.

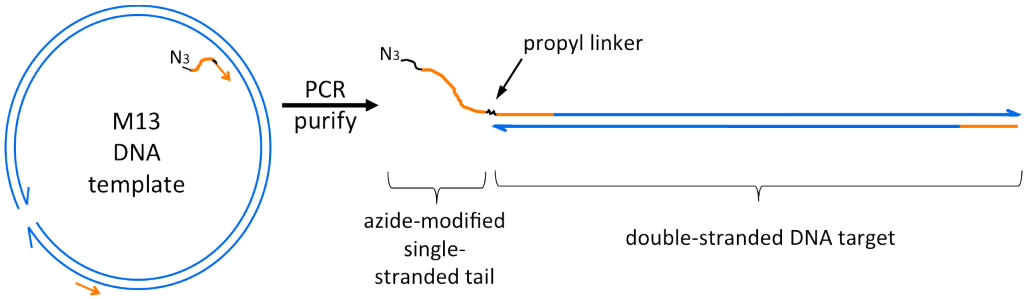


Fig. S2: Generation of the double-stranded DNA targets via PCR with modified primers (primers in orange).

Preparation of the Cu(I)-binding ligands

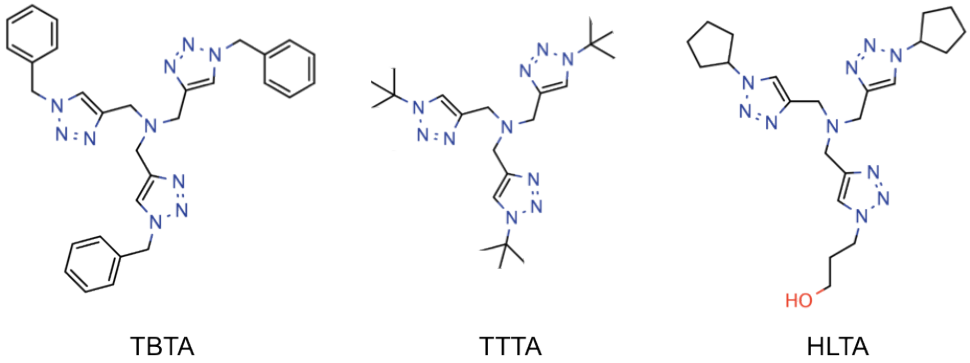
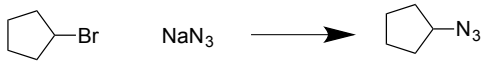


Fig. S3: Molecular structures of the Cu(I)-binding ligands used in this study.

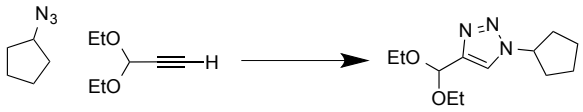
TBTA: The tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine ligand was purchased as a 1:1 Cu(II) complex in 55% aqueous DMSO from Lumiprobe Corporation (Florida, USA).

TTTA: The tris(1-t- butyl) ris(t-butyltriazolylmethyl)amine ligand was synthesized following an established protocol. [1]

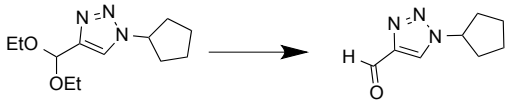
HLTA: The 3-(4-((bis((1-cyclopentyl-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-1H-1,2,3-triazol-1-yl)propan-1-ol ligand was synthesized with the following protocol.



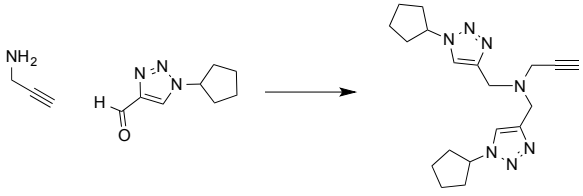
Sodium azide (7.2 g, 111 mmol) was dissolved in DMSO (200 mL). The solution was then treated with bromocyclopentane (15 g, 101 mmol), and stirred overnight. The reaction was quenched with water (1 L) and extracted with diethyl ether (3 × 100 mL). The organic layers were combined and washed with brine solution (200 mL). This was then dried with $MgSO_4$ filtered and concentrated under reduced pressure to give azidocyclopentane (10.5g, 94%) as a clear oil.



Azidocyclopentane (2.9 g, 26.1 mmol) was dissolved in acetonitrile (52 ml), treated with $Cu(II)(OAc)_2$ (0.237 g, 1.305 mmol) and the mixture was stirred until homogeneous. 3,3-diethoxyprop-1-yne (3.34 g, 26.1 mmol) was subsequently added. The solution was treated with DIPEA (0.456 ml, 2.61 mmol) and allowed to stir overnight. Solvent was evaporated under vacuum to give crude material which was purified by flash chromatography (4: 1 - Hexanes: Ethyl Acetate) to yield 1-cyclopentyl-4-(diethoxymethyl)-1H-1,2,3-triazole (5.69g, 91%).

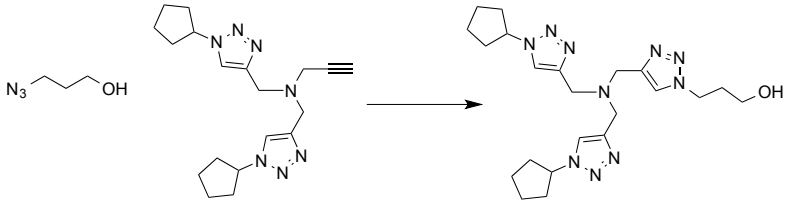


1-cyclopentyl-4-(diethoxymethyl)-1H-1,2,3-triazole (1 g, 4.18 mmol) was dissolved in a mixture of DCM (6 ml) water (3 ml) and TFA (0.4 ml). The mixture was allowed to stir overnight and then EtOAc (100 ml) was added. The mixture was washed with $NaHCO_3$ solution (3 × 40 mL) and finally with brine (40 ml). The organic phase was dried with $MgSO_4$ filtered and then concentrated under reduced pressure to yield 1-cyclopentyl-1H-1,2,3-triazole-4-carbaldehyde (0.566g, 82%) as a low melting solid.



1-cyclopentyl-1H-1,2,3-triazole-4-carbaldehyde (0.6616 g, 4.01 mmol) was dissolved in DCM (16.02 ml)

and treated with prop-2-yn-1-amine (0.096 ml, 1.602 mmol). Sodium triacetoxyborohydride (0.747 g, 3.52 mmol) is then added to the mixture, and the reaction was stirred overnight. The completion of the reaction was monitored by LCMS. Sample was washed with 2M NaOH solution (~5 ml) and extracted with DCM (3 × 4 mL). The combined organic layers were dried with Na_2SO_4 and then concentrated under vacuum to give N,N-bis((1-cyclopentyl-1H-1,2,3-triazol-4-yl)methyl)prop-2-yn-1-amine (0.5g, 88%)



N,N-bis((1-cyclopentyl-1H-1,2,3-triazol-4-yl)methyl)prop-2-yn-1-amine (1.35 g, 3.82 mmol) was dissolved in MeCN (20 ml). 3-azidopropan-1-ol (0.463 g, 4.58 mmol) was added dropwise to the reaction followed by $Cu(II)(OAc)_2$ (0.035 g, 0.191 mmol). The reaction was stirred for 24 h, then the solvent was evaporated under vacuum. The residue was taken up in MeCN and passed through a small plug of activated alumina. Fractions from the column were combined and then evaporated under vacuum. The residue was suspended in MTBE (~15mL) and a minimum of MeCN was added dropwise while vigorously stirring. This produced a white powder, which was triturated with MTBE to yield 3-(4-((bis((1-cyclopentyl-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-1H-1,2,3-triazol-1-yl)propan-1-ol as a white powder (1.2g, 2.64 mmol, 69%).

The final product was characterized by nuclear magnetic resonance spectroscopy (NMR). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.00 (d, *J* = 16.8 Hz, 3H), 4.91 (t, *J* = 7.0 Hz, 2H), 4.53 (t, *J* = 6.5 Hz, 2H), 3.95 (s, 6H), 3.55 (t, *J* = 4.8 Hz, 2H), 2.52 (s, 1H), 2.28 – 2.19 (m, 4H), 2.14 – 1.98 (m, 6H), 1.95 – 1.66 (m, 7H).

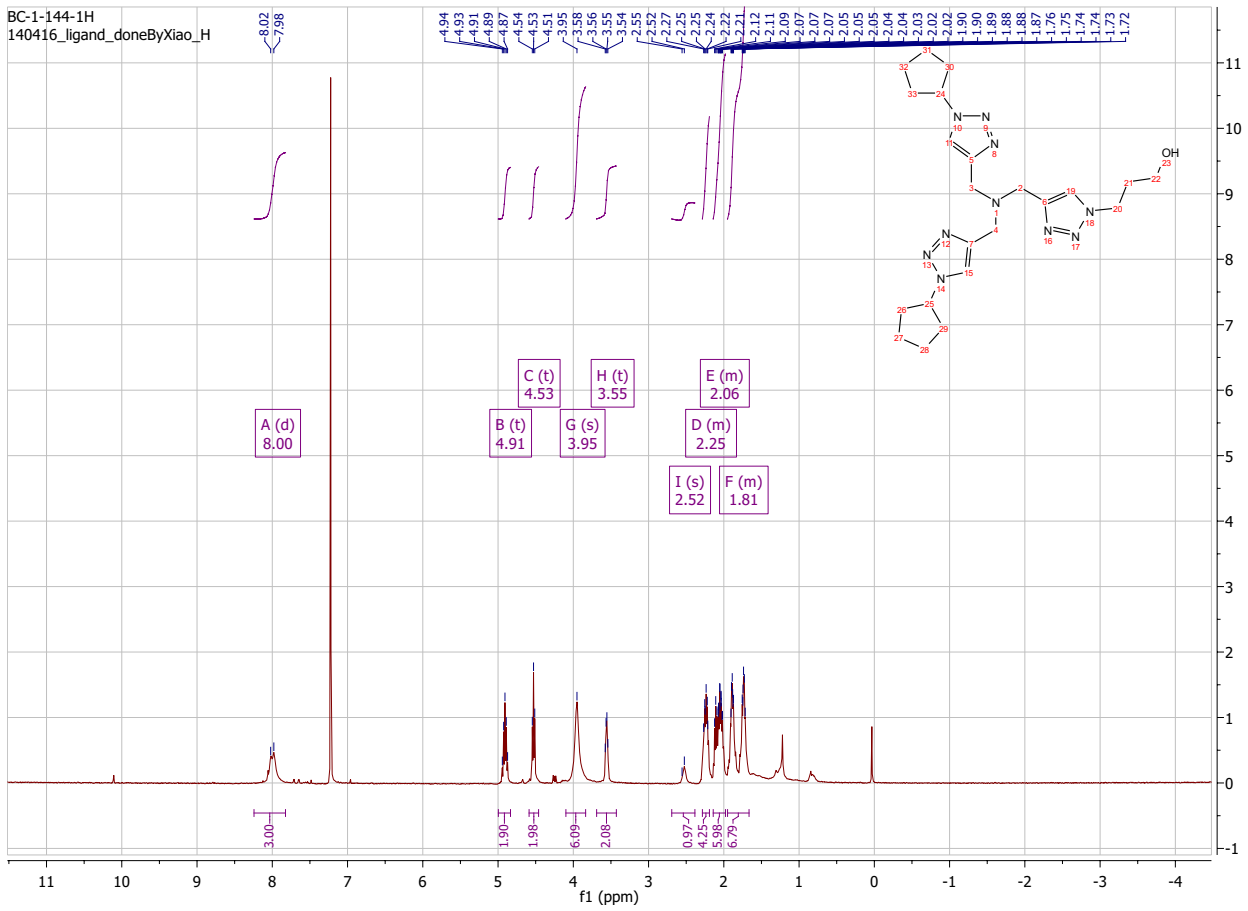


Fig. S4: ^{1}H NMR spectrum of the HLTA ligand.

Testing the DNA-templated coupling reaction in solution

Prior to the surface-coupling experiments, the reaction conditions were optimized by performing the coupling reaction in solution and measuring the yield with denaturing polyacrylamide gel electrophoresis (D-PAGE).

Hybridization and reaction with copper: First the single-stranded DNA anchor and target strands (SF-47 and SF-88, respectively) were hybridized by mixing in a 1:1 molar ratio in 1.0M NaCl, 20 mM PB, pH7, heating briefly to 70°C, and cooling from 65°C to 22°C at a rate of 0.75°C/min. The copper solutions were prepared by dissolving Cu(II) sulfate in water and mixing with the appropriate ligand (TBTA, TTTA, or HLTA) in an aqueous organic solvent mixture containing phosphate buffer (pH 7) and 200 mM NaCl. Nitrogen gas was bubbled through the copper solutions for 10 min prior to use to remove dissolved oxygen. Then the sodium ascorbate reducing agent was added to the copper solution, which was mixed. After a 30 sec delay, the hybridized DNA was added to the copper solution

in a 0.2 mL PCR tube, and the tube was backfilled with nitrogen gas and capped. The final DNA concentration was 1.25μM, and the Cu(II), ligand, and ascorbate concentrations were all systematically varied. At predetermined time points, a 4μL aliquot of the reaction mixture was removed and added to 16μL of 1.25X quenching buffer (1X QB = 3:1 formamide:water, 20mM Tris acetate, 2.5 mM EDTA, 0.15X gel loading dye). The quenched reaction aliquot was immediately placed on ice and transferred to the freezer (-20°C) until loading into the gel.

Denaturing polyacrylamide gel electrophoresis: Polyacrylamide gels were prepared with a polyacrylamide/bisacrylamide (29:1) concentration of 9-12%, and contained 8.3M urea in order to denature the DNA while running the gel. After casting the gel and setting for at least 30 min, the wells were rinsed with 0.5X TBE running buffer (1X = 89mM Tris base, 89mM boric acid, 2mM EDTA, pH 8.3). The gel was then pre-run in 0.5X TBE for at least 45 min at 150V in an Enduro vertical gel electrophoresis system (Labnet International, Inc, New Jersey, USA). Prior to loading the gel, the samples were removed from the freezer and heated to 80°C in QB for at least 4 min to completely denature the DNA. They were then immediately loaded into the gel and run for 60-90 minutes at a voltage of 150V. Gels were then removed and stained by soaking in 0.5X TBE containing a 1X concentration of Sybr Green II dye (Life Technologies, California, USA) for 10 min. The gels were then visualized and imaged on an Enduro Gel Documentation System (Labnet) or on a UV lamp with a handheld camera.

Determination of reaction yield from gel images: It has been demonstrated previously that the efficiency of DNA crosslinking reactions can be estimated by comparing band intensity from denaturing PAGE.[2] Due to the urea content of the gel and the elevated temperatures during running, the DNA remains denatured as it runs through the gel.[3] Any anchor/target hybrid DNA that has successfully reacted will be crosslinked by the triazole product, and thus will migrate as a single, slower-moving product band, while any DNA that has not reacted will be separated into two different faster-moving bands (see Fig. S5). To estimate the yield of the reaction at different time points, we cropped out the product band from the gel image and compared the average pixel intensity of the band to that of a standard reference band using MATLAB (The Mathworks, Inc., Massachusetts, USA). This normalized intensity was then plotted as the estimated yield (in arbitrary units) as a function of time, as shown in Fig. 2 (main text). Note that any of the product DNA that has been degraded during the reaction is expected to run faster than the product band due to a smaller size, and thus degradation over time also leads to a reduction in the product band intensity.

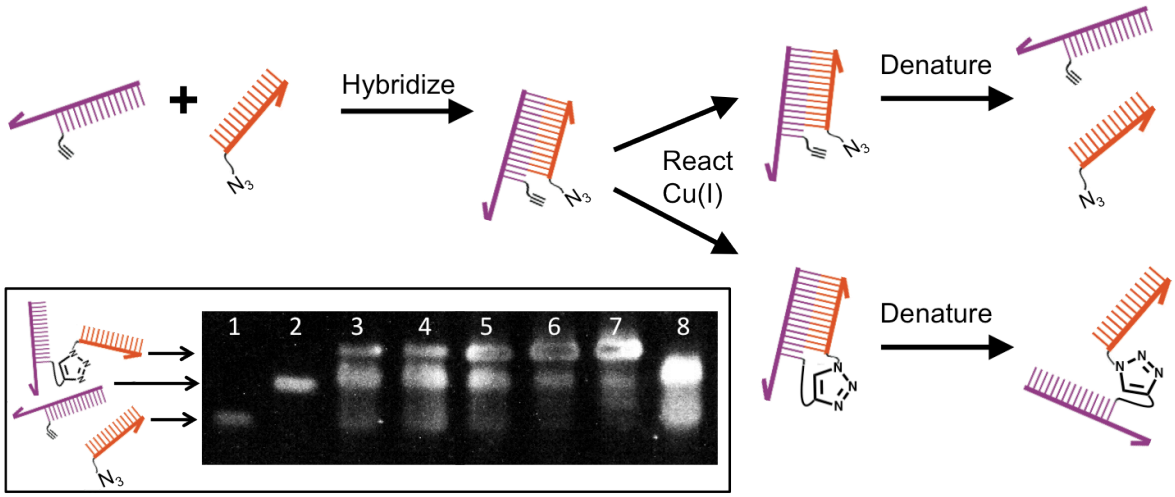


Fig. S5: Reaction scheme for the DNA-templated coupling reaction in solution. DNA that has not successfully reacted is separated into two different bands in the denaturing gel, while DNA that has reacted is crosslinked by the triazole product and migrates as a single, slower-moving band. (Bottom) Sample image of results from D-PAGE. Lanes 1 and 2 are the target and anchor strands, respectively. Lanes 3-7 are a series of time points from a mixture of both strands after hybridization and reaction with the Cu(I) catalyst under the same conditions used for the surface coupling reaction. As time in the presence of Cu(I) increases, the intensity of the product band increases, while the reactant band intensities decrease. Lane 8 is a control where the Cu(I) catalyst was omitted; in this case, only the reactant bands appear.

Surface-coupling reaction and denaturation

Preparation of switchable self-assembled monolayer surface: Single-crystal gold bead substrates were prepared in house following an established protocol,[4] and were used for all AFM experiments. The gold bead substrates were cleaned by sonication in organic solvents and thorough rinsing with ultrapure water, followed by immersion in hot nitric acid for 20 min. Substrates were then rinsed with pure water, dried with compressed air. After being briefly annealed under a hydrogen flame, the substrates were cooled under a stream of nitrogen gas and immediately placed into an ethanolic solution of 11-mercaptoundecanoic acid (MUDA) containing 10% acetic acid by volume. Thiol solutions were backfilled with nitrogen gas, sealed and kept in the dark at room temperature to minimize thiol oxidation during monolayer assembly. Assembly times of approximately one hour were found to result in ordered self-assembled monolayers (SAMs) that still contain enough monolayer defects to accommodate the thiolated oligonucleotides during the insertion step (see below).

Insertion of thiolated anchor strands into the monolayer:

After monolayer assembly, the substrates were removed from the solution and immediately rinsed and sonicated for 10 s in a 9:1 ethanol:acetic acid solution, then rinsed with pure ethanol and gently blow-dried with filtered air. They were then placed in a custom-built PTFE fluid cell and rinsed several times with a TAE buffer. Immediately prior to use, the alkyl disulfide-modified anchor DNA strands were incubated with 2mM Tris(2-carboxyethyl)phosphine (TCEP) to reduce the disulfide groups to thiols. The reaction tube was backfilled with nitrogen gas and kept in the dark to minimize unwanted thiol-yne addition between two different strands.[5] After 10 minutes, the thiolated DNA was purified using a QIAquick Nucleotide Removal Kit (QIAGEN). The thiolated DNA anchors were then inserted into defects in the monolayer by immersing the MUDA/gold substrate in an aqueous TAE buffer solution containing approximately 1.0 μM thiolated DNA, 2mM TCEP, and 50 mM NaCl for 20-40 minutes in the dark. Following the insertion step, the surface was repeatedly rinsed with TAE buffer to remove any unbound DNA.

Hybridization with the target DNA: The purified target DNA PCR product was diluted 10 times in hybridization buffer (HB, 200mM NaCl, 40mM Tris acetate, 1mM EDTA, 1.0mM sodium dodecyl sulfate, pH 8.3), and was incubated with the anchor DNA-functionalized surface for 30-60 minutes. After hybridization, the surface was rinsed repeatedly with HB.

Reaction with Cu(I): The Cu(I) catalyst solution was prepared as follows. Cu(II) sulfate was dissolved in water, and was mixed with the HLTA ligand in a 4:1 ligand:Cu(II) molar ratio in an aqueous phosphate-buffered saline solution (PBS, 200mM NaCl, 100mM sodium phosphate, pH 7) containing 5% dimethyl sulfoxide (DMSO) by volume. The final Cu(II) concentration was 200μM. Nitrogen gas was bubbled through the solution for 10 min prior to use to remove dissolved oxygen. Immediately prior to the surface reaction, the surface was rinsed three times with PBS to thoroughly remove any Tris or EDTA, which both inhibit the reaction.[6] Then the sodium ascorbate reducing agent was added to the Cu(II) solution to a final concentration of 2.0mM. After a 30 sec delay, the surface was exposed to the Cu(I) solution and was placed in a sealed chamber that was purged with nitrogen gas to help minimize the presence of oxygen. After a reaction time of 25 min, the surface was rinsed repeatedly with saline TAE buffer (STAE, 200mM NaCl, 40mM Tris acetate, 5mM EDTA, pH 8.3). We found that although rigorous oxygen exclusion was not necessary, running the reaction in air for extended periods resulted in noticeable degradation of the DNA (data not shown).

Denaturation of unreacted DNA: The surface-bound DNA was denatured using alkaline conditions, which disrupt the hydrogen bonds in the base pairs and remove any DNA that is not covalently tethered to the surface. The surface was rinsed and left under an aqueous solution of 10mM NaOH and 330μM EDTA (pH 12) for 5 minutes. It was then rinsed with the same solution to remove any free DNA.

AFM imaging

All imaging was carried out using an NTEGRA Vita Atomic Force Microscope, manufactured by NT-MDT (Moscow, Russia). Images were acquired while operating in semi-contact (tapping) mode under an aqueous Ni(II) imaging buffer (NB, 5mM Ni(II) acetate, 0.1X TAE), using silicon tips mounted on silicon nitride cantilevers with a nominal spring constant of 0.3 N/m and a resonant frequency of approximately 16 kHz in liquid (model SNL-10, manufactured by Bruker, California, USA).
As described in a previous publication,[7] the Ni(II) ions are coordinated by the surface carboxylate groups and function as salt bridges that immobilize the anionic DNA molecules. Prior to the hybridization, reaction, or denaturation steps, the surface was rinsed repeatedly with STAE to remove any Ni(II) ions that were bound to the surface.
A note on cleanliness: All glassware, teflon fluid cells, and ceramic tweezers were cleaned in piranha solution and rinsed thoroughly with water before use. (Piranha is 3:1 sulfuric acid: hydrogen peroxide. CAUTION— piranha is highly corrosive and reacts violently with organics).

AFM image analysis

Determination of reaction yield from AFM images: To estimate a yield for the surface coupling reaction, the surface was imaged both before and after carrying out the denaturation step. An average surface density of target DNA molecules was determined by counting the number of corresponding features in images over an area of at least 10 μm^2 (>200 molecules). Any molecules that were not at least halfway inside the boundary of the image were excluded. The yield was then defined as the average density of molecules after the denaturation step divided by the average density before denaturation.
Semi-automated digital tracing of DNA contours: In order to extract conformational statistics from the AFM images, the contour of each molecule on the surface was digitized in a semi-automated fashion using a published algorithm.[8] The result was a set of coordinates representing the digitized paths of the DNA molecules within each image (Fig. S6). These coordinates were then used to determine

contour lengths, end-to-end distances, and bending angle distributions for the molecules. For this purpose, 1.0 μm² images at 256 x 256 pixel resolution were used, and only molecules that were fully within the image boundary were measured.

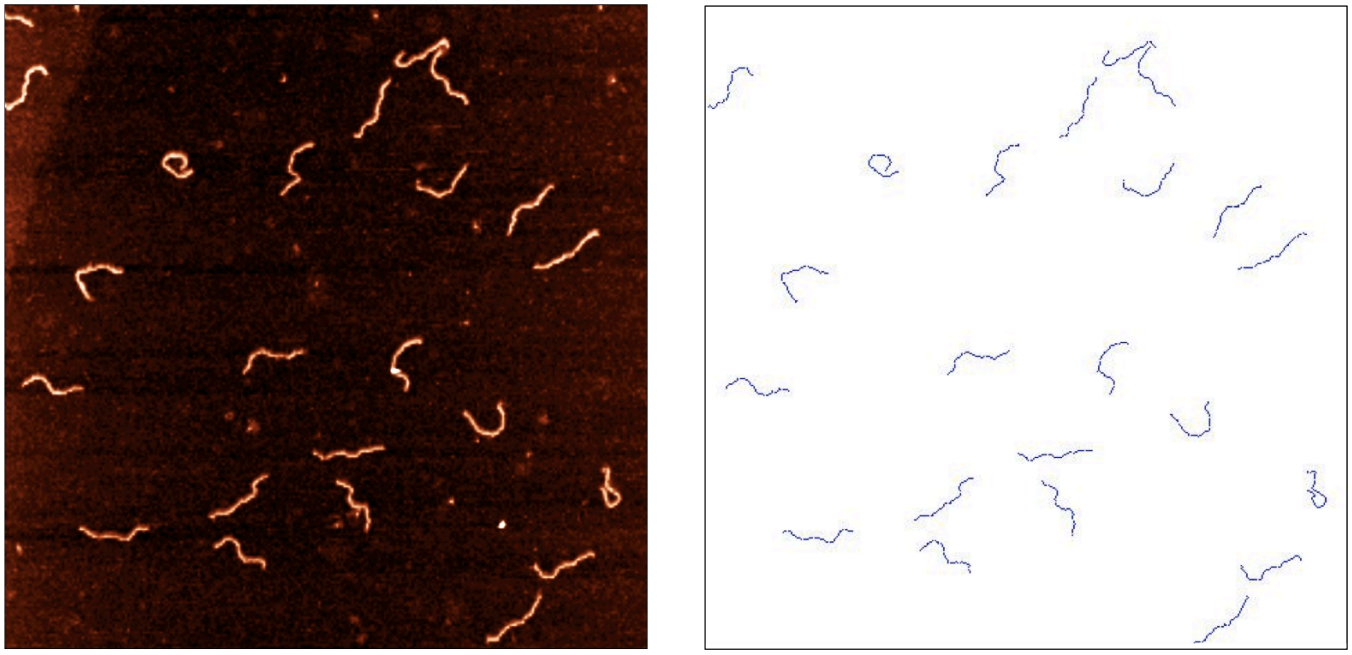


Fig. S6: Semi-automated digital tracing of DNA conformations. Shown on the left is a sample 1.0 μm^2 image, and on the right is a plot of the digitized coordinates of the DNA molecules obtained using the tracing algorithm described in reference 8.

Assessment of DNA degradation by statistical chain conformation analysis: In order to estimate the extent of oxidative damage to the DNA backbone caused by reactive oxygen species[9] during the reaction step, we carried out statistical analysis on the chain conformations in the images before and after exposure to the Cu(II) catalyst. In our case, a double-stranded break in the surface-tethered DNA would result in cleavage and release of part of the molecule from the surface; this would result in a shortening of the contour length L_c of the molecule. We analyzed the coordinates of a total of 243 molecules and found no significant change in the average measured contour length $\langle L_c \rangle$ after the reaction step, indicating that the occurrence of double-stranded breaks is negligible (Fig. S7). On the other hand, single-stranded breaks (or 'nicks') in the backbone are more difficult to directly identify due to the limited resolution of the AFM images. Such nicking is expected to occur at random sites along the DNA backbone, also making it difficult to detect by analyzing the bend angles along the molecule. In contrast, introduction of a nick has a pronounced effect on the distribution of end-to-end distances, causing both a shift in the peak to shorter distances as well as a broadening of the

distribution.[10] Given that we observed no significant change in the mean or the standard deviation of end-to-end distances after the reaction step, we conclude that any single-stranded breaks are limited to a small minority of molecules (Fig. S7).

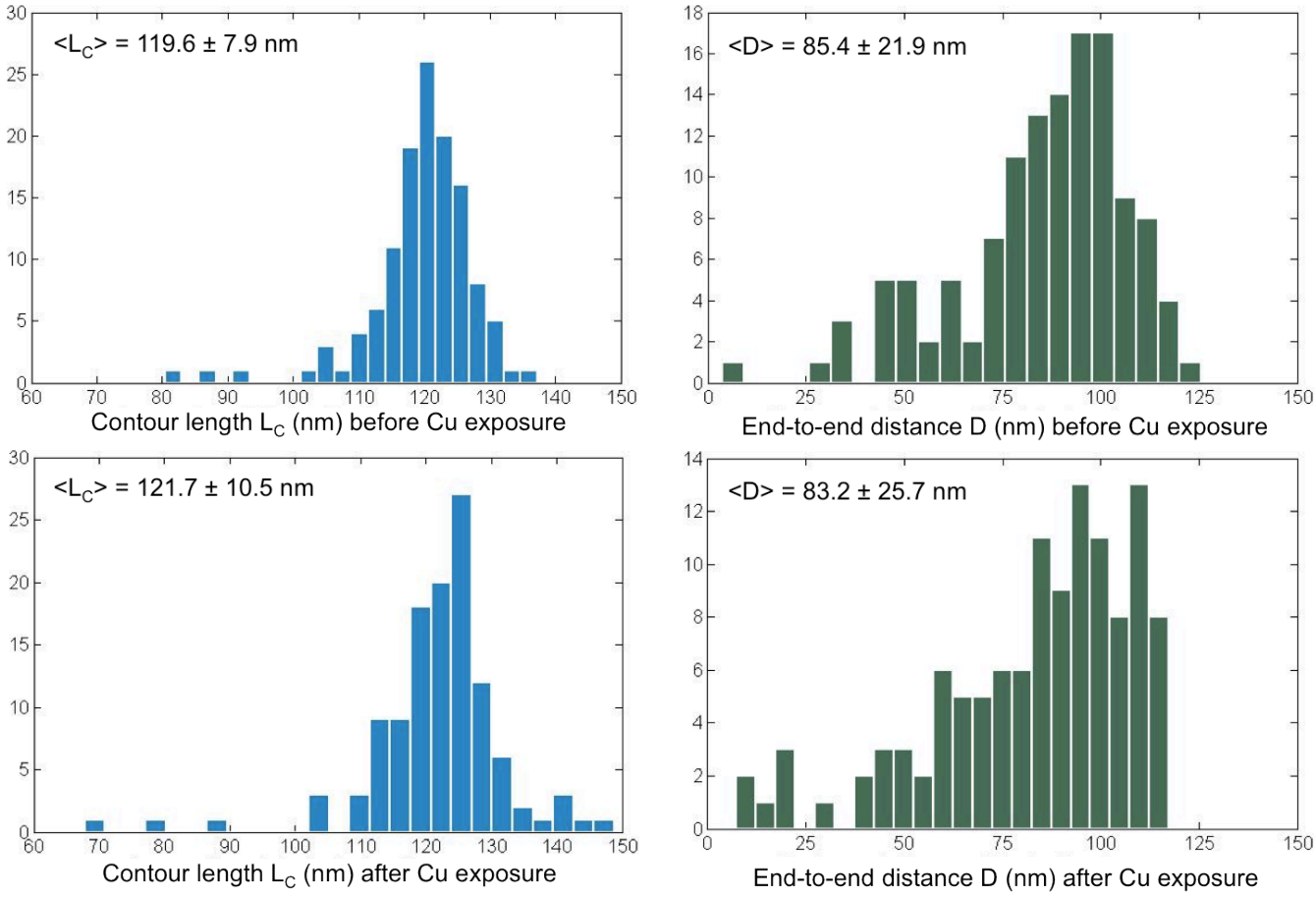


Fig. S7: Chain conformation statistics extracted from AFM images. Histograms of the measured contour length L_C (left) and end-to-end distance D (right) are shown for molecules before (top) and after (bottom) exposure to the Cu(I) catalyst. The mean value ± one standard deviation is displayed on each graph. The vertical axes show the count.

Additional AFM data:

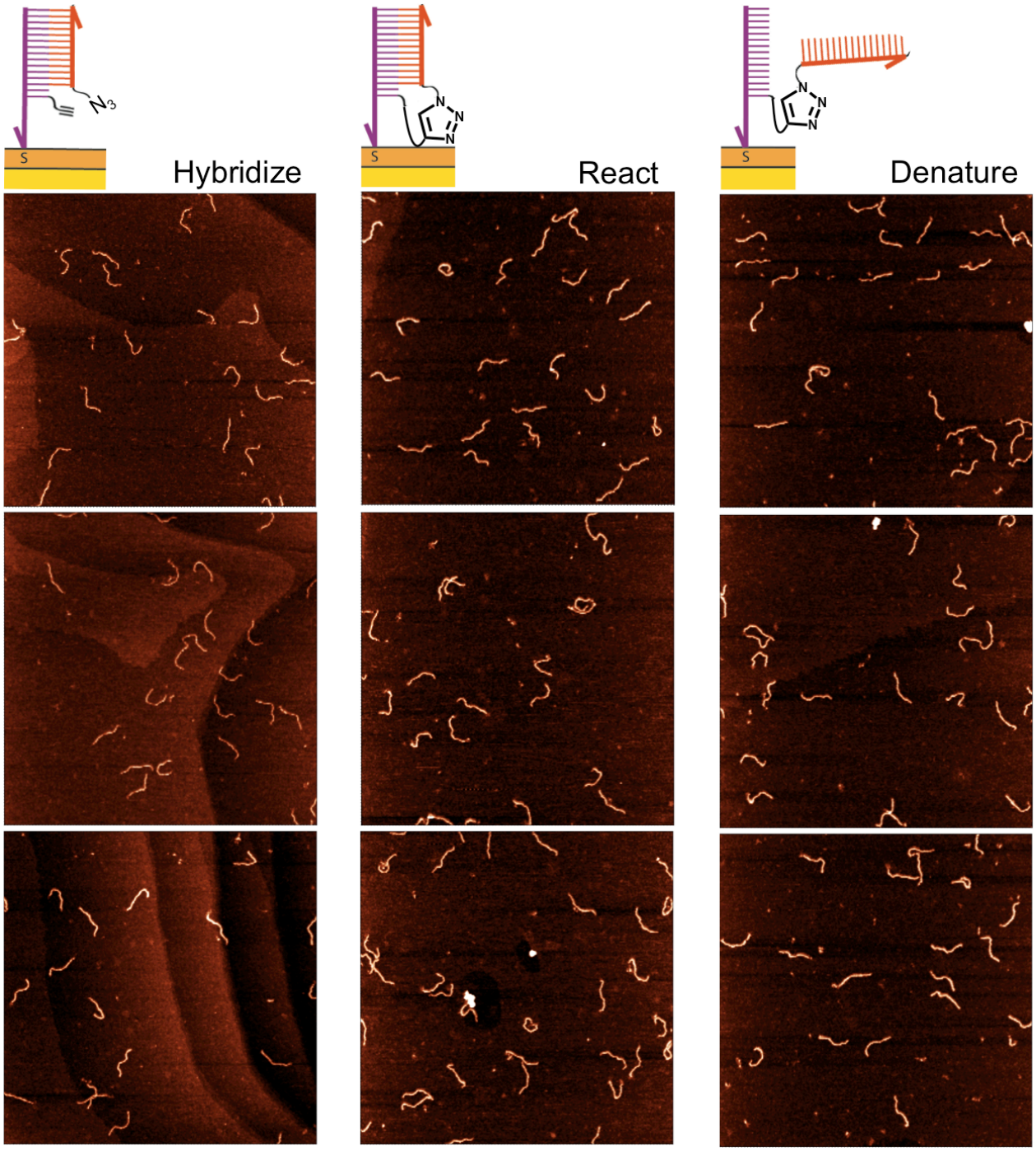


Fig. S8: AFM images taken after each step of the DNA-templated surface coupling reaction. In this experiment, the 396 bp azide-modified DNA targets were hybridized with alkyne-modified anchor DNA strands (left). The surface was then exposed to the Cu(I) catalyst solution for 25 min, and imaged again after the reaction (middle). Finally, the surface was exposed to a denaturing alkaline solution for 5 min, and then imaged (right). The measured yield in this experiment was 80%, as determined from a total area of 14 μm^2. The height scale is the same as in Fig. 3, and the image size is 1.0 μm^2 for all images.

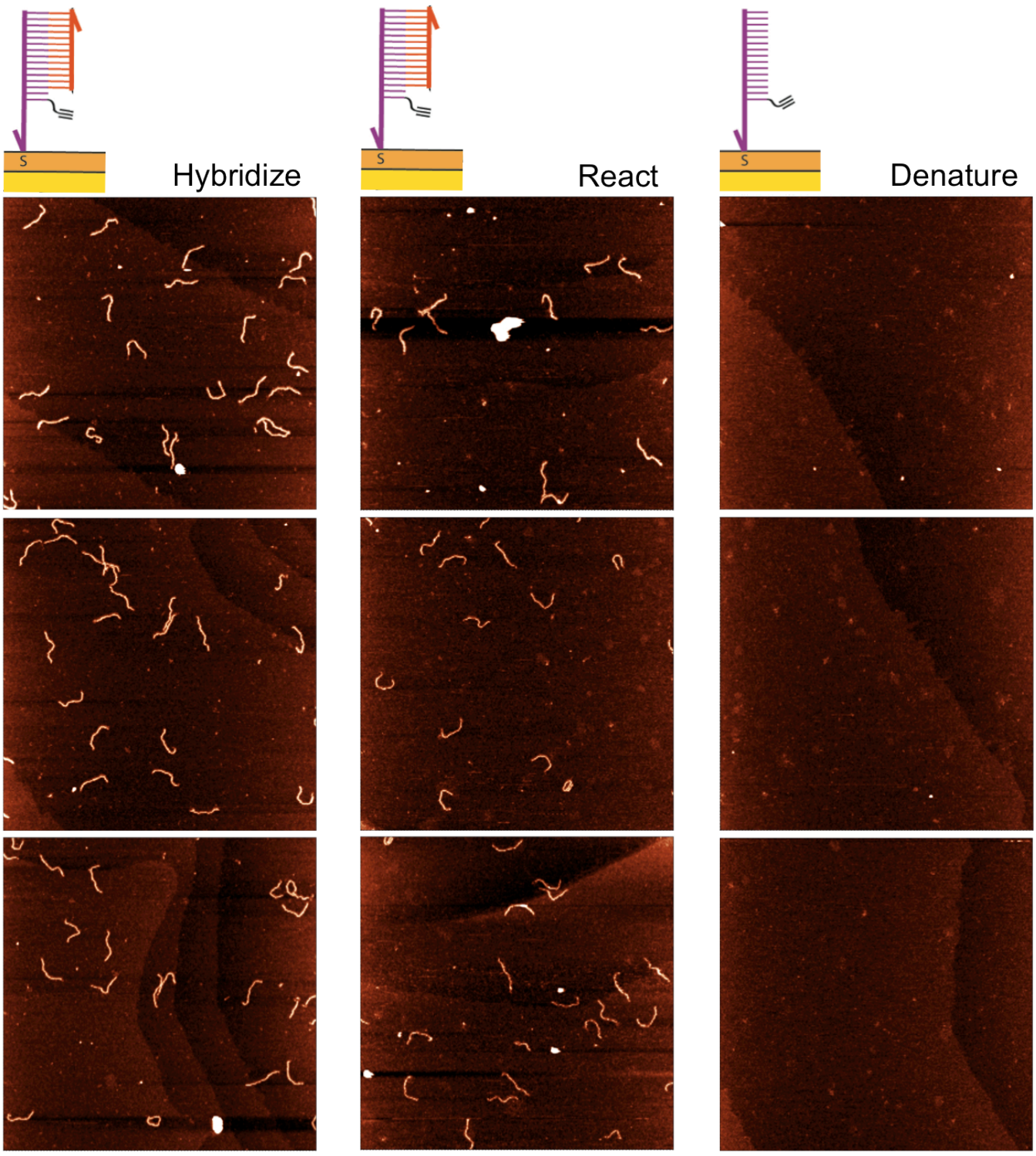


Fig. S9: In this control experiment, the 396 bp DNA targets did not contain azide groups. The targets were hybridized with alkyne-modified anchor DNA strands and then imaged (left). The surface was then exposed to the Cu(I) catalyst solution for 25 min, and imaged again after the reaction (middle). Finally, the surface was exposed to a denaturing alkaline solution for 5 min, and then imaged (right). The measured yield in this control experiment was < 1%. The height scale is the same as in Fig. 3, and the image size is 1.0 μm^2 for all images.

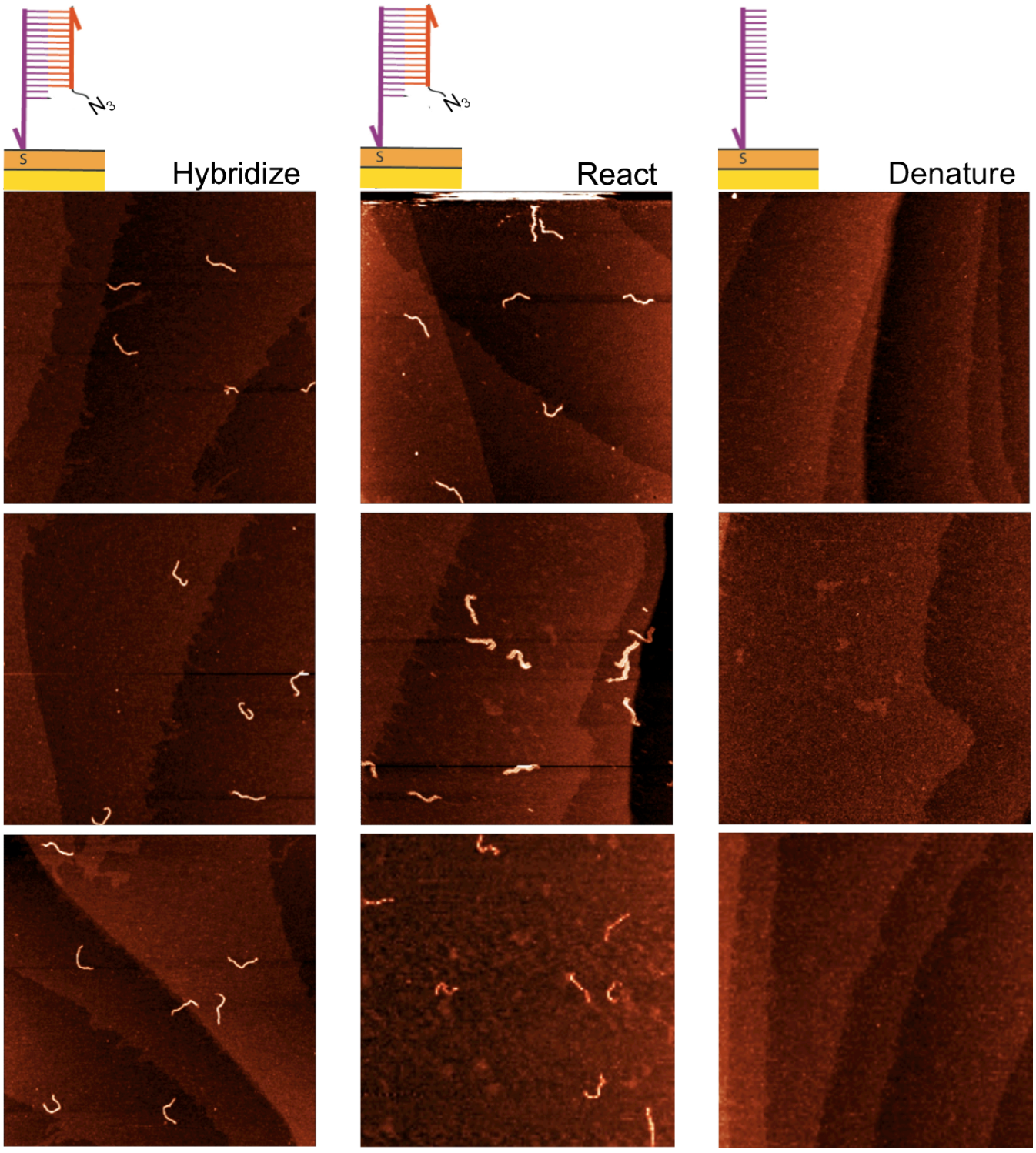


Fig. S10: In this control experiment, the 396 bp DNA targets contained azide groups, but the anchor strands did not contain alkyne groups. The targets were hybridized with the anchor DNA strands and then imaged (left). The surface was then exposed to the Cu(I) catalyst solution for 25 min, and imaged again after the reaction (middle). Finally, the surface was exposed to a denaturing alkaline solution for 5 min, and then imaged (right). The measured yield in this control experiment was < 1%. The height scale is the same as in Fig. 3, and the image size is 1.0 μm^2 for all images.

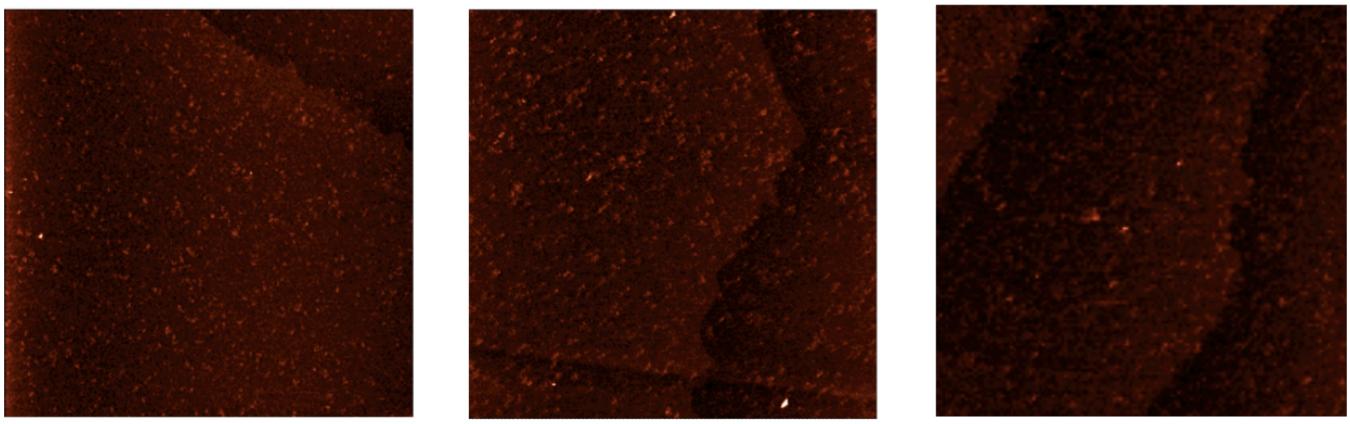


Fig. S11: In this control experiment, the sequence was mismatched between the alkyne-modified anchor strands and the azide-modified 396 bp DNA targets. After the hybridization step, no target DNA was observed on the surface for a total area of >10 μm^2 that was surveyed. The height scale is the same as in Fig. 3, and the image size is 1.0 μm^2 for all images.

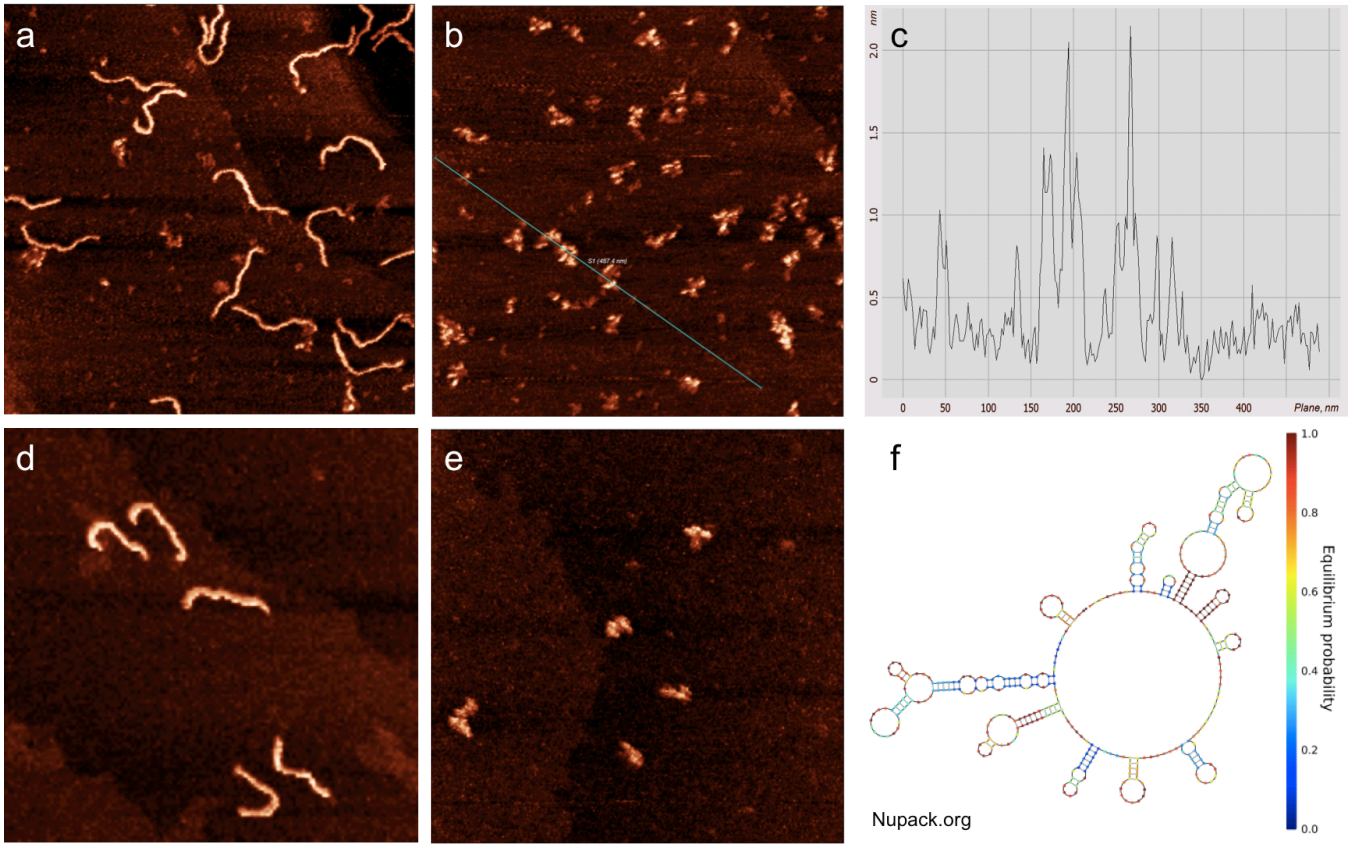


Fig. S7: AFM images showing selective denaturation of the target DNA to produce surface-tethered, single-stranded DNA. (a) The 396 bp azide-modified target DNA was hybridized and coupled to the surface, as before. (b) For this experiment, the NaOH concentration was increased to 30mM (pH 12.5) for the denaturation step. This was sufficient to fully denature the entire 396 bp DNA, as observed in the AFM images. (c) Height profile from line drawn across the image in (b). After denaturation, the 2.0nm-tall worm-like features were replaced by small, globular features containing regions with heights varying from 0.75nm to 2.0nm. These heights are consistent with features containing regions of both single- and double-stranded DNA, as compared with heights measured

previously.[7] (d) and (e) show images after the reaction and denaturation steps, respectively, from a repeated trial of the same experiment. The surface density of the globular features observed after denaturation was found to correlate with the surface density of target DNA before denaturation, which is interpreted as further support that the features correspond to the same DNA that has been fully denatured. (f) Results of a thermodynamic analysis of the minimum free energy secondary structure of the 396-base DNA strand, as determined using the online Nucliec Acid Package tool (www.nupack.org). The color scale gives the equilibrium probability of forming intramolecular base-pairs at 23°C. Notice that under these conditions, the DNA strand is expected to contain both single-stranded and double-stranded regions, consistent with what is observed in the AFM images. The height scale is the same as in Fig. 3, and the image size is 0.25 μm^2 for all images.

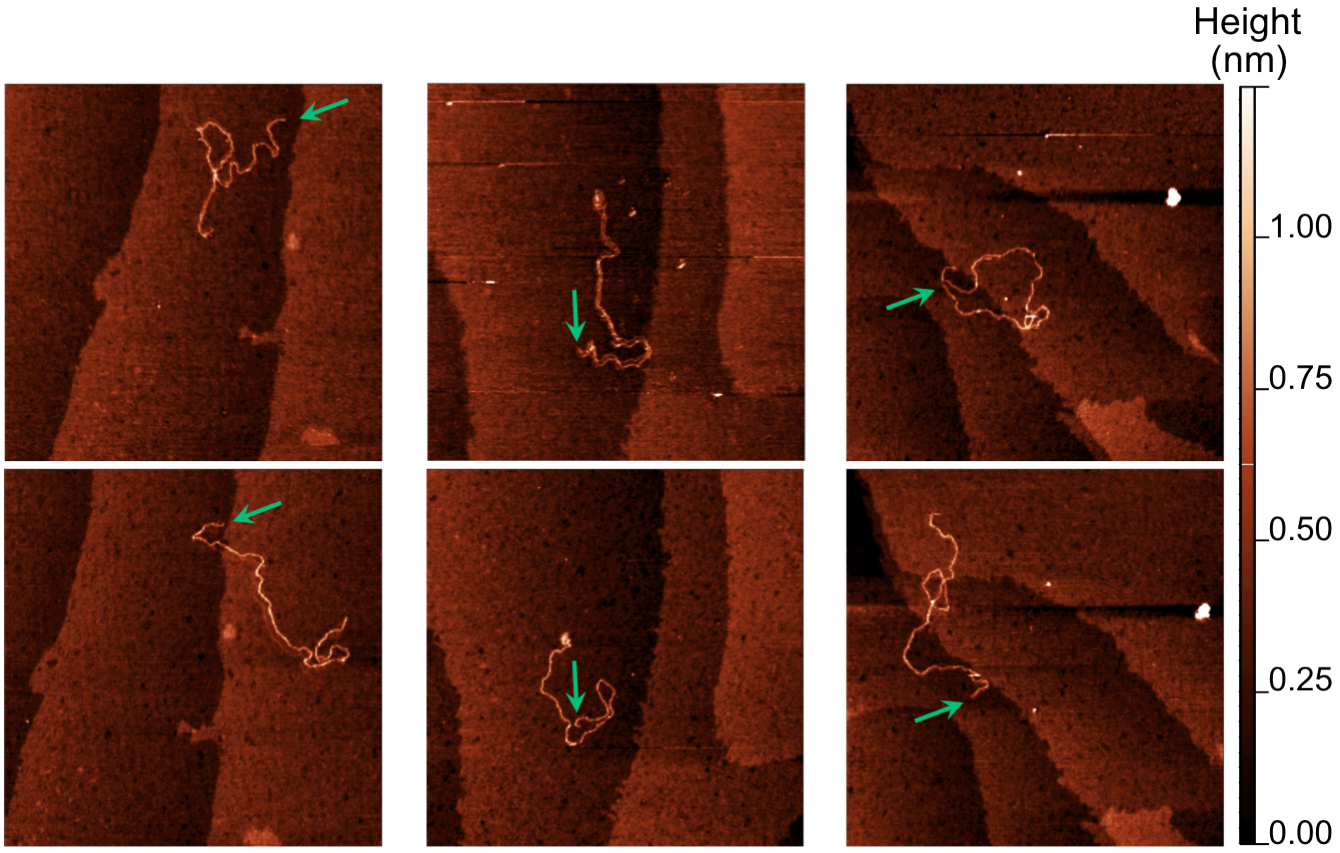


Fig. S8: AFM images showing long DNA that is tethered to the surface at one end. The top row shows three different 3679bp DNA molecules after hybridization and coupling to the surface. The bottom row shows the same three molecules after the surface was rinsed. Because the molecules are only tethered to the surface at one end, they are free to partially desorb from the surface and change conformation during rinsing, and only the tethered end remains in the same place. Using the atomic steps in the background as a reference, the attachment point of each molecule can be inferred, and is indicated with a green arrow. The height scale is shown on the right, and the image size is 1.0 μm^2 for all images.

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