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Directed Deposition of Single Molecules on Surfaces

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Scanning probe microscopy-based techniques can address and manipulate individual molecules. This makes it possible to use them for building nanostructures by assembling single molecules. Recently the formation of surface structures by positioning single molecules with the Atomic Force Microscope (AFM) was demonstrated on an irreversible delivery process. This inherits the drawback, that the transfer has to occur between differently functionalized surfaces and allows no proofreading of the built structures. Here we demonstrate a procedure for directed deposition of single DNA molecules, which intrinsically allows a reversible positioning. This method uses specific interactions between complementary DNA oligonucleotides for symmetric coupling of the transport molecules to the support and AFM tip, respectively. Thus, it allows for a simple "drag-and-drop" procedure, which relies on the statistical breakage of the molecular interaction under a force load. In addition, the delivery of the transport molecules was observed in real-time by single-molecule fluorescence microscopy.

Keywords: Single Molecule Transport, Atomic Force Fluorescence Microscopy, Molecular Assembly.

The ultimate physical limit in building nanostructures can be reached by assembling one by one molecule.[1,2] Recently, two practical approaches have been demonstrated that make use the atomic force microscope to manipulate single organic molecules and deliver them in a precisely controlled manner to a specific target area.[3–5] Both techniques rely on an irreversible delivery process to guarantee the deposition of the molecule in the target area. This guarantees a high efficiency of the transport process, but has some drawbacks. It allows no proofreading of the delivery, e.g., the removal of molecules that were transported unintentionally by diffusion. More complex delivery processes as the delivery of long DNA molecules that would require a separate positioning of both ends are therefore prohibited. Additionally, separate depots would be required for each type of molecule that has to be delivered.

Here we demonstrate the directed delivery of single DNA molecules using a procedure that intrinsically allows a reversible positioning (see Fig. 1(a)) The DNA to be transported is coupled to the support or the AFM tip using specific molecular interactions between complementary DNA oligonucleotides. This system allows for a simple "drag-and-drop" procedure, which relies on the statistical breakage of the molecular interaction under a force load and does not require any external optical or electrical triggering.[6] Two single stranded DNA oligonucleotides, both with 30 base pairs but different sequences are covalently bound to poly(ethylene glycol) (PEG) coated silicon nitride AFM tips serving as specific anchors for the transport DNA to be transported and to PEG coated glass slides forming a grid for reversible deposition. At the same time the PEG linkers guarantee free accessibility of the DNA strands and passivates the surface against unspecific adsorption.[7,8] The transport DNA itself consists of two sequences, one complementary to the tip anchor, the other to the surface grid DNA. Thus, the molecule can be bound on the surface via one sequence, still leaving the other sequence free to interact with its complementary anchor sequence at the AFM tip and vice versa. Both exhibit equal binding forces of about 45pN (see Fig. 3(a)) due to the same sequence length and A:T/G:C content. The sequences are chosen such, that the hybridization of both sequences with their complementary strands results in a so-called "shear" geometry in which all base pairs of the duplex are loaded in parallel upon forced unbinding (see Fig. 2(a2)).

Thus, contacting a transport DNA which is hybridized to the surface with the functionalized AFM tip results in the formation of a molecular bridge between tip and surface. Upon retraction of the tip one of the two hybridizations will break, either leaving the transport DNA on the

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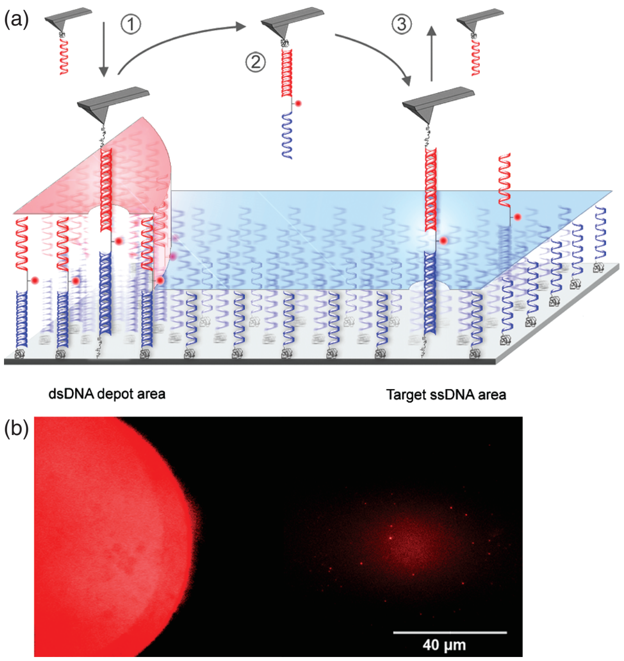

Fig. 1. For demonstration of the reversible deposition process, transport DNA oligonucleotides were taken from an area where they were they were deposited with a high density (b) with a commercial microarray spotter. Functionalized AFM-tip with complementary anchor sequence acquires (a, 1) the transport DNA unit and translocate it (a, 2) to a target area containing only the surface anchor sequence (b). After bringing the DNA transfer-unit in contact with the surface and retraction of the AFM tip (a, 3), the deposition can be observed by single-molecule fluorescence with evanescent excitation (TIRF) of the attached fluorophore (b).

surface or dragging it away. Due to the equal strength of the two anchors, there is an equal probability of 50% for both cases. This inherently guarantees that the process is completely reversible and can be repeated at any number

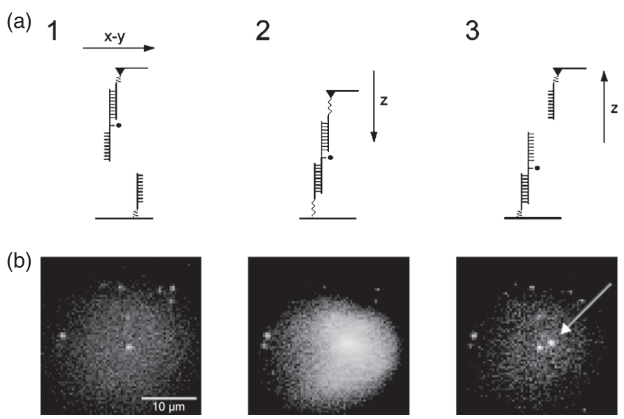

Fig. 2. Scheme and TIR fluorescence images of successful single 30b ssDNA oligonucleotide transport divided in the corresponding single steps. Schematic overview of the incidents during the deposition procedure (a) corresponding to the EMCCD fluorescence images (b). **1**: Target area after moving the functionalized AFM tip with fluorophor labelled ssDNA transport molecule in *x*–*y* position. **2**: Tip approach to the surface for surface ssDNA anchor and ssDNA transport unit. **3**: Fluorescence image of individual deposited DNA unit (white arrow) after AFM tip retraction from surface.

of times until the desired result is achieved. The same situation comes across for the deposition process at the desired target area.

The experiments were performed by a combined Atomic Force Fluorescence Microscopy setup with total internal reflection fluorescence (TIRF) imaging which allows monitoring the deposition process in real-time. For observation of individual transport DNA molecules they were modified with a single Atto647N fluorophore. To minimize photobleaching effects the excitation via the laser diode was triggered together with the camera, being switched on only for fluorescence data acquisition at the deposition process.

For demonstrating the deposition of single DNA oligonucleotides the transport DNA was spotted to the surface with a commercial Microarray-spotter, forming a depot area by hybridisation to the surface bound DNA grid (see Fig. 1(b)) with an optimized average molecular distance of approximately 30 nm.

For taking up a molecule, the functionalized tip was brought in surface contact at the depot area allowing to hybridize with the fluorescently labelled transport DNA, dragging it away upon tip retraction (see Fig. 2). The force peaks measured upon tip retraction verified, that the transport DNA had formed a molecular bridge between surface and tip. After transporting the DNA over a distance of about 80 μm the DNA was dropped in an area where a few molecules are visible, serving as a spatial reference. The process of deposition was monitored simultaneously by an EMCCD camera based fluorescence microscopy showing the fluorescence signal of the single molecule at the predicted position (see Fig. 2(b3)). This ensures that it is the result of directed delivery and not of random diffusion of unbound oligonucleotides (see Fig. 1(b)). The deposition of only one molecule (Fig. 3(b)), was verified by the fluorescence time trace which exhibits a clear indication of a single bleaching step (see Fig. 3(c)). In addition the force peak measured again upon tip retraction verifies that the DNA in fact had been coupled specifically to the surface. One complete transfer cycle takes 4 seconds, containing 1.5 s for taking up and depositing and 0.5 s for changing the lateral position. In each force curve the AFM probe tip was kept in gentle contact with the surface for 0.5 seconds to increase the probability of duplex formation between the anchors and the complementary part of the transport DNA. Figure 3(a) shows force curves measured upon deposition, which are typical for the rupture of a short antiparallel DNA duplex.

Due to the intrinsic 50% probability for taking up a molecule or leaving it on the surface, the maximum efficiency for delivering a molecule with a single transport cycle amounts to 25%. In addition, the probability to form a specific interaction during tip-surface contact is reflected in the frequency of observing a force peak, which appeared approximately at every second surface contact.

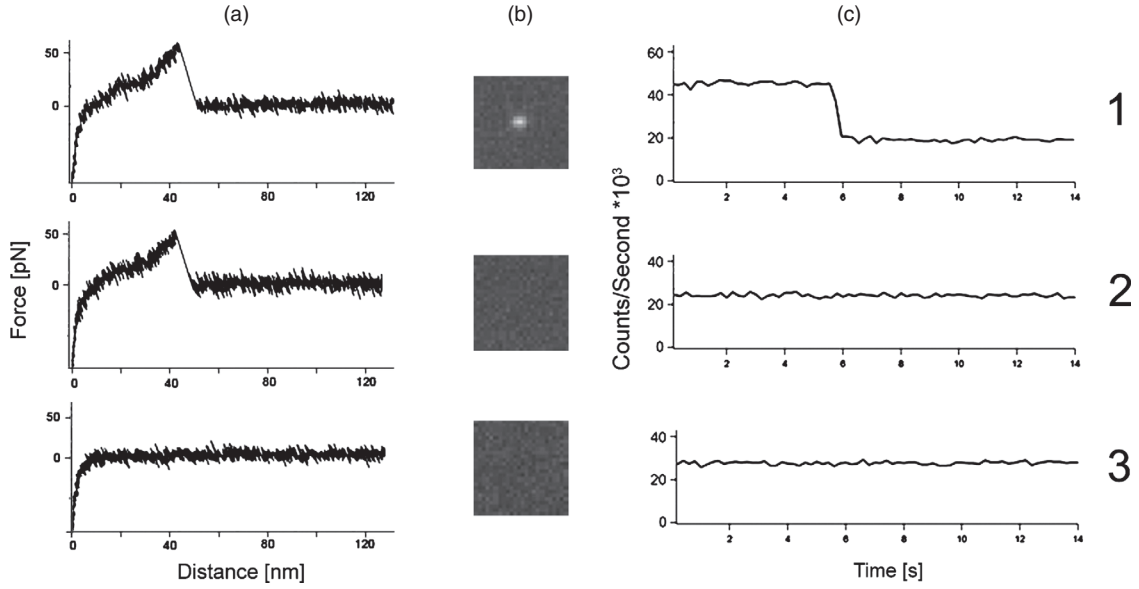

Fig. 3. Experimental data of several transport cycles. Force curves (a) measured by depositing the transfer-DNA unit which corresponds to the single molecule TIR fluorescence images (b) of the target area. The time trace of the Atto647N fluorohor emission for each experimental deposition procedure data is shown in (c) containing the single photobleaching events of deposited transfer units. The first row (**1**) relates to successful transport ssDNA deposition, the second (**2**) and third (**3**) row illustrates the non-deposition state due to surface site rupture or non-transport and the last row the situation without acquired transfer-ssDNA unit at the AFM probe tip.

Bleaching of the fluorophores during DNA labeling and sample preparation and observation as well as incomplete labeling of the DNA oligonucleotides further reduce the probability to observe a directed delivery event. Estimating these bleaching effects to be in the range of 20–50%, the total probability to observe a directed delivery event is given by the product of the single probabilities and results in 4–5% which agrees well with the experimentally observed frequency of 1 in 20 tries.

In the presented procedure the need for optical control of the successful deposition is balanced by the advantage that individual molecules can be reassembled on the same surface not necessarily requiring specific depot areas. The method can be applied to the directed deposition of other molecules and nanoparticles by modification of the transport DNA or by modifying the respective transport unit with the two DNA anchor sequences. In addition the deposited molecules can be used as a template for further self-assembly of other components using specific recognition. This addresses a wide field of applications ranging from ultra sensitive diagnostics in Life Sciences by assembling single molecule based sensors up to applications in nano-electronics concerning the coupling of molecular components to nano-electrodes.

SUPPORTING INFORMATION

DNA sequences, sample preparation, experimental setup and real-time video of a ssDNA deposition are available as supplemental information. This material is available via the Internet.

1. SAMPLE PREPARATION

The borosilicate cover slips and the silicon nitride AFM cantilever tips (MLCT-AUHW, Veeco Probes, California, USA) were prepared as described in Ref. [9]. In summary, the supports were functionalized with ethanol amine as short surface linker and covalently modified with the heterobifunctional polymer NHS-PEG-$COOH_{MW3.400}$ (Laysan-Bio, Alabama, USA). After rinsing with ultra clean H_2O, the different specific 30-bases long anchor DNA oligonucleotides (Fig. S1) were covalently bound with a high density (2 μM) via the peptide binding method to the PEGylated supports. To remove non-specific adsorbed molecules, the supports were subsequently washed 10 minutes in 2× saline sodium citrate buffer (300 mM NaCl, 30 mM sodium citrate, pH 7) and finally shortly twice with ultra clean H_2O. In one depot area (approx. 80 μm of diameter) 2 nM of the 80-bases long Atto647n-modified (Fig. S1) ssDNA transport molecule were spotted to the functionalized borosilicate cover slip via an Microarray-Robot (Genetix, Hempshire, UK) and subsequently hybridized to the surface anchor oligonucleotides in TRIS buffer (20 mM TRIS/HCl, 150 mM NaCl, 1 mM $MgCl_2$, pH 8). After rinsing with ultra clean H_2O after 20 minutes to remove non-hybridized transport DNA, the cover slips were dried in a nitrogen stream. For experimental measurement, the samples were mounted to a custom

Surface anchor sequence AFM-tip anchor sequence

5′ NH_2-(A10) CCGTTGCGATGTCAGAGGTAATGTGGAGTG GTGAGGTGTAATGGAGACTGTAGCGTTGCC(A10)-NH_2 3′

3′ GGCAACGCTACAGTCTCCATTACACCTCAC(A10) -T- (A9)CACTCCACATTACCTCTGACATCGCAACGG 5′

Atto647n ssDNA transport molecuce

Fig. S1. Oligomers used in this transport study. The transfer ssDNA is 80 bases long and is modified with an Atto647n fluorophore at position 41-T (counted from 3′-end). The 30 bases long sequences at the AFM-tipe side and on the surface are used to anchor the transfer ssDNA for both acquiring and deposition due to the partial complementarities to the transfer ssDNA molecule. All oligomers were synthesized and purified (HPLC-grade) form Purimex (Grebenstein, Germany).

made AFM-TIRF sample holder and immersed in previously described TRIS buffer.

2. AFM MEASUREMENTS

All transport experiments were performed with an modified commercial available AFM (MFP3D, Asylum Research, California, USA) at room temperature and optically controlled via an EMCCD camera (IXON, Andor, Belfast, Ireland) in TRIS buffer (see paragraph 1.). The spring constant of the DNA modified cantilever was calibrated in solution using the equipartition theorem.[10,11] The tip was approached and withdrawn from the surface for acquiring and deposition of the ssDNA transport molecule at a speed of 400 nm/s until it was 500 nm above the surface for the translocation over approx. 80 μm. The self-programmed procedures for the ssDNA transport were performed by using Igor Pro 5.03 (Wave Metrics, Oregon, USA). The cantilever positioning for pickup, delivery and deposition was controlled in closed-loop operation.

3. TOTAL INTERNAL REFLECTION FLUORESCENCE (TIRF) MICROSCOPE

Single-molecule fluorescence microscopy was performed via TIRF excitation (Fig. S2). The fluorescence excitation of the Atto647N fluorophor (Atto-Tec, Siegen, Germany) was done by a 636 nm, 25 mW diode laser (Schäfter & Kirchhoff, Hamburg, Deutschland) through a 60×/1.45 oil immersion objective lens (Olympus, Hamburg, Germany), where the collimated laser beam is focused in the back focal plane of the objective lens such that the beam is totally reflected at the cover slip. Fluorescence light was filtered by an emission filter (AHF, Tübingen, Germany)

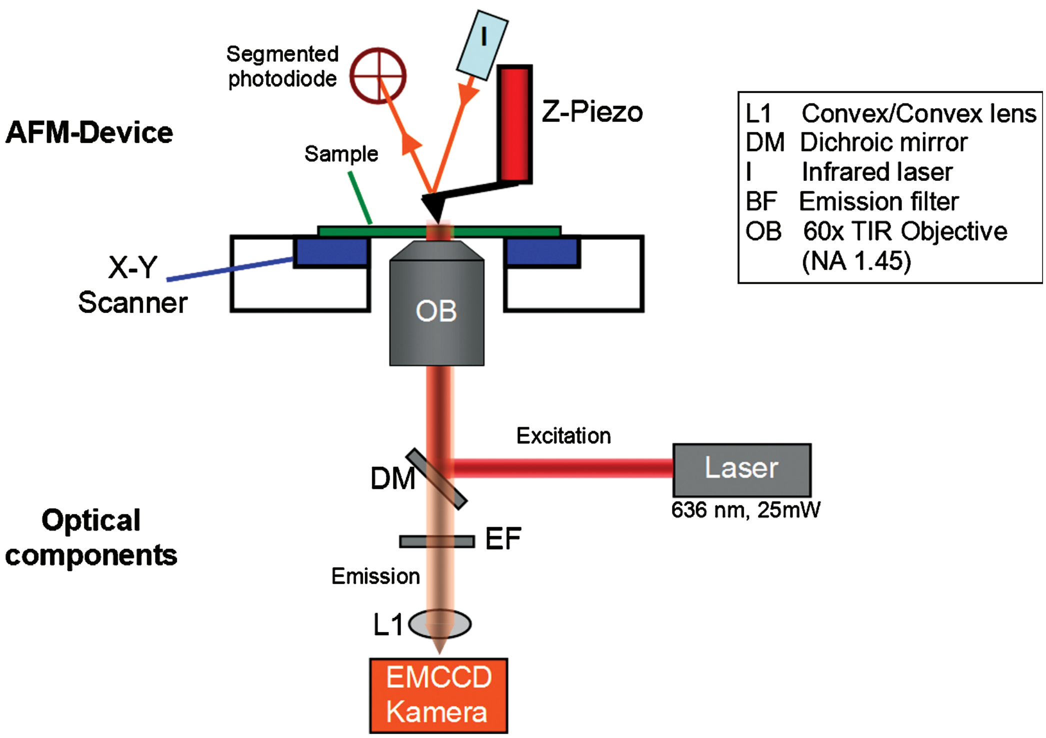

Fig. S2. Experimental combined Atomic Force Fluorescence Microscopy setup with TIRF excitation. The cantilever and the x–y scanner movements are realized via precise piezos in nanometre range. The sample with the depot- and target areas on top is hold via a custom made aluminium support, which is fixed through magnets on the x–y scan stage.

for red (710/50 nm) and a dichroic mirror with a cut-off wavelength of 635 nm (635 RDZ) was used with 636 nm excitation. The emitted light was detected by a peltier-cooled 512×512 pixel back-illuminated EMCCD camera (IXON, Andor, Belfast, Ireland). Time series where recorded in frame-transfer mode with an integration time of 200 ms per frame. The EMCCD chip operated at a temperature of -85 °C with additional water cooling and an electron multiplication gain of EM = 210 was used. One pixel represented in respect to the objective magnification a surface area of approx. 170 nm.

4. REAL-TIME VIDEO SEQUENCE OF DIRECTED ssDNA DEPOSITION

In this real-time video the successful deposition of a ssDNA transfer molecule 5 μm beside another transfer molecule is shown. Separate download via Internet at http://pubs.acs.org.

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