DNA Origami Design of Dolphin-Shaped Structures with Flexible Tails

Ebbe S. Andersen,[†,‡] Mingdong Dong,[†,§] Morten M. Nielsen,[†,‡] Kasper Jahn,[†,‡] Allan Lind-Thomsen,[∥] Wael Mamdouh,[†,§] Kurt V. Gothelf,[†,⊥] Flemming Besenbacher,[†,§] and Jørgen Kjems[†,‡,*]

[†]Danish National Research Foundation, Centre for DNA Nanotechnology (CDNA) at the Interdisciplinary Nanoscience Center (iNANO), University of Aarhus, DK-8000 Aarhus, Denmark, [‡]Department of Molecular Biology, University of Aarhus, DK-8000 Aarhus, Denmark, [§]Department of Physics and Astronomy, University of Aarhus, DK-8000 Aarhus, Denmark, [⊥]Department of Chemistry, University of Aarhus, DK-8000 Aarhus, Denmark, and [∥]Wilhelm Johannsen Centre for Functional Genome Research, Department of Cellular and Molecular Medicine, University of Copenhagen, Denmark

ABSTRACT The DNA origami method allows the folding of long, single-stranded DNA sequences into arbitrary two-dimensional structures by a set of designed oligonucleotides. The method has revealed an unexpected strength and efficiency for programmed self-assembly of molecular nanostructures and makes it possible to produce fully addressable nanostructures with wide-reaching application potential within the emerging area of nanoscience. Here we present a user-friendly software package for designing DNA origami structures (http://www.cdna.dk/origami) and demonstrate its use by the design of a dolphin-like DNA origami structure that was imaged by high-resolution AFM in liquid. The software package provides automatic generation of DNA origami structures, manual editing, interactive overviews, atomic models, tracks the design history, and has a fully extendable toolbox. From the AFM images, it was demonstrated that different designs of the dolphin tail region provided various levels of flexibility in a predictable fashion. Finally, we show that the addition of specific attachment sites promotes dimerization between two independently self-assembled dolphin structures, and that these interactions stabilize the flexible tail.

KEYWORDS: DNA origami · self-assembly · dynamics · design software · AFM (atomic force microscopy)

A central challenge of nanotechnology is to efficiently design and construct well-defined functional structures and devices at the nanoscale. Self-assembly is a promising route toward this goal, but it is often hampered by the lack of fundamental understanding, control, and reproducibility of the stochastic processes involved. Nucleic acids are suitable materials for self-assembly since they form well-defined structures and are programmable for predefined designs and interface well within biological systems.[1] The use of DNA for the precise structuring and addressability at the nanoscale has promising applications in chemistry, molecular computing, and potentially within the emerging area of nanomedicine.[2,3]

A first step in the development of the field of structural DNA nanotechnology was the movement from initial theoretical considerations[4] to the design of small geometric structures such as the DNA cube.[5] A second step was the design of DNA "tiles" that forms larger complexes by unique recognition and that can perform algorithmic calculations by this process.[6,7] A third step was the realization that an efficient route for self-assembly was to use nucleation on a larger scaffold to facilitate higher-order folding.[8] Recently, a novel approach was introduced by Rothemund, named the "DNA origami" method,[9] where the 7.2 kb single-stranded DNA genome of the M13 bacteriophage is folded with the help of 200−250 short synthetic oligonucleotides to create arbitrary planar structures with dimensions in the area of 100 × 100 nm^2. The DNA origami method provides a platform with hitherto unseen possibilities for directed self-assembly since the 250 short staple strands can, in principle, be extended from the origami surface and serve as 250 individually addressable pixels located exactly 6 nm apart on the origami surface. Since the pioneering work of Rothemund, the DNA origami structures have been used for constructing nanoarrays of proteins, gold nanoparticles, and oligos,[10−12] and as a scaffold for algorithmic calculations.[13] However, there is currently a lack of advanced scientific tools for computer-aided designs of complex DNA structures. Only a few dedicated programs have been developed so far,[14,15] and programs for DNA origami design are not yet available.[9,16] Here we present a highly interactive software package for designing DNA origami structures of arbitrary shape, and we have used this software to construct a dolphin-like DNA origami structure with a flexible tail region. The study uses an interdisciplinary approach where software development is aimed at designing the DNA origami structures that in turn are

*Address correspondence to jk@mb.au.dk.

Received for review April 8, 2008 and accepted May 23, 2008.

Published online June 10, 2008.
10.1021/nn800215j CCC: $40.75

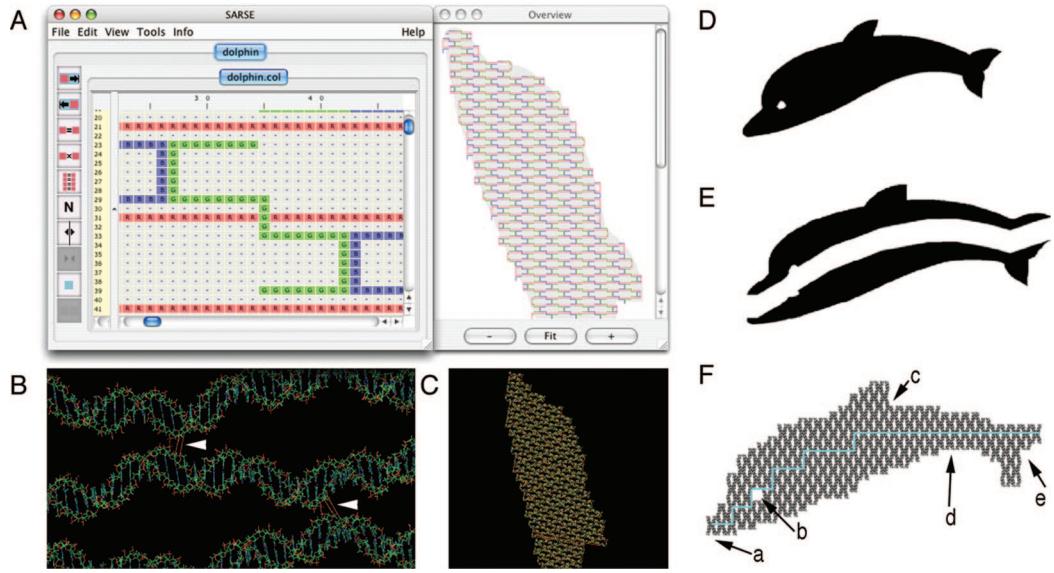

Figure 1. Design process of a DNA origami dolphin. (A) SARSE editor window showing a small part of the DNA origami design with backbone strand in red and staple strands in green and blue. The overview window, shown to the right, can be used to navigate the editor window. (B) An atomic model can be generated during the design process and visualized in a 3D viewer such as PyMol.[18] White arrows indicate crossover positions. (C) The same atomic model as in B at a lower zoom level. (D) The dolphin shape used as input. (E) The shape was stretched 1.125×, divided down the middle to insert a seam, and modified on back and tail fins. (F) Atomic model of the DNA origami design with indication of special features: a, nose; b, eye; c, 90° angle at back fin; d, narrow tail region; e, branching tail fins. Blue line in E indicates the position of a seam.

prepared in the molecular biology laboratory and characterized by atomic force microscopy.

RESULTS AND DISCUSSION

Construction of a DNA Origami Dolphin. A user-friendly software package for making DNA origami structures was developed based on a semiautomated scientific data editor, called SARSE.[17] The program uses an editor and an overview window to facilitate the editing and navigation of the large DNA origami structures (Figure 1A). The program also has a history window that allows the user to undo unwanted changes and logs files to help reproducing complex designs. A toolbox is used to activate programs that can both analyze and modify the design or generate an atomic model to be inspected in a 3D viewer (Figure 1B, 1C).

As an example of designing DNA origami structures of arbitrary shape, we have reconstructed the shape of a common dolphin, *Delphinus delphis*, which makes up a central part of the logo from the University of Aarhus[19] with the motto "*solidum petit in profundis*"[20] (Figure 1D). A graphical editor was used to adjust the size of the dolphin bitmap file to match the length of the M13 sequence (the size of the black area of the bitmap should be approximately 10 × 7249 pixels since each pixel on every tenth line will be assigned to a single base of the M13 sequence which is 7249 bases long). In order to get the exact desired dimensions of the final dolphin origami structure, the bitmap has to be stretched by 1.125 times in the direction perpendicular to the helices (*e.g.*, the horizontal strands in Figure 1A) taking into account that the length of 10 bases of a DNA helix is 3.375 nm and the distance between two helices of a DNA origami structure with 1.5 turns between crossovers was previously reported to be ~3 nm.[9] The bitmap was modified on the back and tail fins to allow simple folding (Figure 1E). The SARSE program allows an easy way of inserting a seam into an origami design simply by dividing the bitmap into two halves, which was used to create an eye on the dolphin (Figure 1E). Currently, the SARSE origami design software only allows 1.5 helical turns between staple strand crossovers and one central seam to be added. However, it can be extended to a different spacing between crossovers and multiple seams by modifying certain parameters in the design program.

The bitmap files of the upper and lower part of the dolphin were imported in the SARSE editor; the upper part was folded from the nose in the 5′ to 3′ direction, and the lower part was folded from the nose in the 3′ to 5′ direction to represent one continuous strand. The SARSE editor was used to modify the design and add T-loops and T-extensions on the edges to inhibit intermolecular helical stacking interactions.[9] The design was inspected by the automatic generation of an atomic model (Figure 1B, 1C). The two dolphins were designed to fit on top of each other with the approximate orientation of the two dolphins in the University of Aarhus logo,[19] and intermolecular crossover strands were made to connect them. A thermodynamically stable DNA hairpin structure that appears naturally in the M13 viral DNA[9] was placed at the tip of the dolphin tail. This defines two parts of the M13 sequence that was inserted in the two parts of the dolphin design to gener-

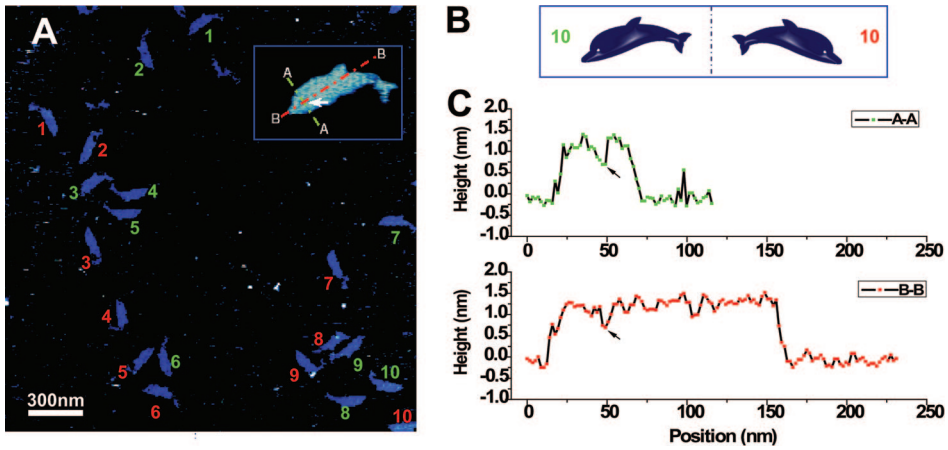

Figure 2. AFM imaging of single dolphin shapes. (A) AFM image of DNA origami dolphins. Inset shows a high-resolution image of a single dolphin shape with an arrow pointing to the eye and section profile lines A−A and B−B. Green and red numbers count each of the two alternative configurations of the dolphin shape. (B) The two types of dolphin shapes with the mirror plane indicated with a dotted line. Green and red numbers correspond to the count numbers in panel A. (C) Section profile of the dolphin shape in the inset of panel A showing a dimension of 50 nm along the A−A axis, and a dimension of 150 nm along the B−B axis. An arrow indicates the eye feature in both profiles.

ate a list of oligonucleotide sequences. Seam oligos and intermolecular crossover strands were designed by hand (oligos used in the design are listed in the Supporting Information).

The dolphin shape was chosen for this study because it possesses several interesting and challenging features. It is asymmetric and has easily identifiable features such as the nose, the 6 nm eye, the 90° angle at the back fin, and the branching tail (a−c,e in Figure 1F). Also it has a narrow tail region and a staggered seam that defines the interaction between the upper and lower part of the dolphin (d and blue line in Figure 1F). A tutorial of the design process and the final design files are available at http://www.cdna.dk/origami.

AFM Imaging of DNA Dolphins with Flexible Tails. In the initial experiments, dolphin origami structures were self-assembled and deposited on a freshly cleaved flat mica surface and then visualized using tapping mode atomic force microscopy (AFM) in liquid. In Figure 2A, a high-resolution AFM image of the resulting dolphin structures adsorbed on the mica surface is shown, which clearly reveals DNA origami structures with the expected features of the dolphin such as the nose, fin, tail, and eye.

In Figure 2B, a schematic illustration with two mirror image dolphin structures (only valid for the dolphin overall shape and not at the molecular level) is presented.[21] The dolphin structure can land on the surface in one of two different orientations, which allows one to distinguish which surface of the dolphin is facing up or down, respectively. The dolphin structure lands in one of two mirror symmetric orientations, one that is left-handed and one is right-handed (Figure 2B), a phenomenon that is referred to as "2D chirality".[21] In all recorded AFM images, we observe an approximately equal distribution of dolphin origami structures with face-up and face-down orientations as seen from the red and green numbers in Figure 2A,B, respectively. This suggests that the DNA origami dolphin structure in solution has no distinct structural conformation that would bias the deposition on the surface; that is, in solution, the dolphins are achiral, whereas when they land on the surface they become 2D chiral. If one could control the attachment of DNA origami structures in only one of the possible orientations, this will be an important step for creating well-defined larger DNA origami assemblies at the surface.

On the basis of section profiles along the A−A and B−B axes (Figure 2A, inset, and 2C), the eye is revealed as a concave feature of approximately 6 nm in width (as indicated by the arrows in Figure 2C), and its depth depends in most cases on the AFM tip convolution. A similar concave feature is observed in the red cross section in Figure 2C, which is most likely caused by the absence of a DNA oligo at that location.

In the original design, the dolphin tail did not have crossovers in the seam to allow for sliding of the helices and tail flipping. Indeed, this specific tail design resulted in the observation of many coexisting alternative

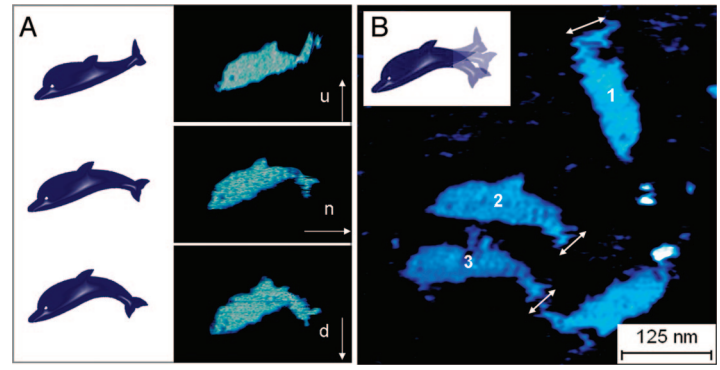

Figure 3. Observation of tail flexibility. (A) AFM images of dolphin DNA structures in several different conformational states. Arrows indicate orientation of tail as up (u), normal (n), and down (d). (B) In high-resolution AFM images, distortion patterns were observed for structures that were aligned perpendicular to the direction of scanning. Dolphins are labeled as 1, 2, and 3.

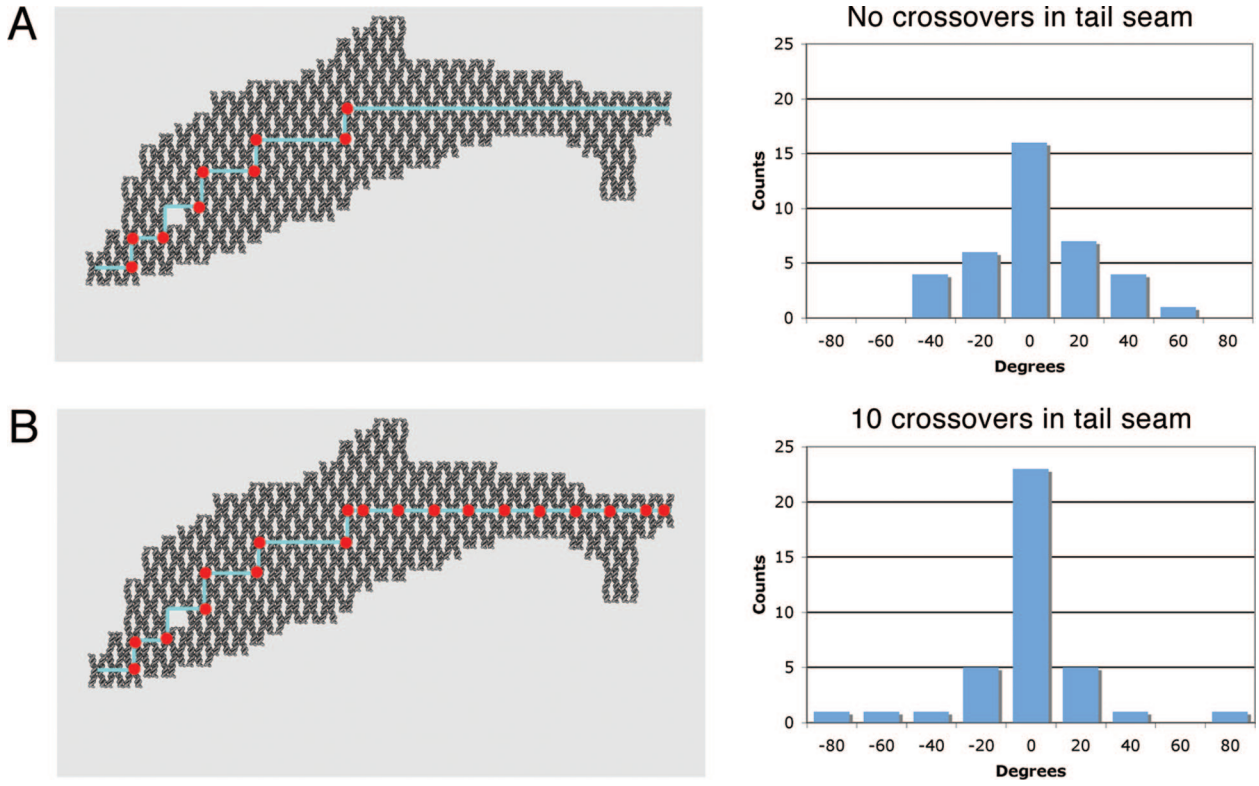

Figure 4. Characterization of tail flexibility. (A) The molecular model of the DNA origami dolphin is shown with the seam in blue and with indication of the positions of crossover points in red. The histogram to the right shows the distribution of tail angles measured in AFM images. (B) Model of DNA origami dolphin with 10 seam crossovers inserted in the tail region and the corresponding histogram to the right.

conformations of the tail in all recorded AFM images. The tail could either be flipped up (u, Figure 3A top), with respect to the normal position (n, Figure 3A middle) or down (d, Figure 3A bottom).

Surprisingly, upon applying a higher force by the AFM tip, the tails appeared to be slightly distorted in the high-resolution AFM images, as seen by comparing Figure 3B with Figure 2A. Thus, this tail distortion can clearly be assigned as an influence of the force exerted by the AFM tip. In that context, the tail distortion is most significant when the dolphin origami is oriented perpendicular to the scanning direction of the AFM tip (Figure 3B, dolphin 1) as compared to the other dolphins aligned along the scanning direction (Figure 3B, dolphins 2 and 3).

Next we investigated the effect of inserting crossover points in the tail region (Figure 4A, 4B, models) on the overall assembled structure. The tail angle was determined by using three reference points on the dolphin DNA structure: the nose, the middle of the body below the right angle of the back fin, and the middle of the tail fin. The experimentally determined angle was in good agreement with the theoretical angle of 145°. For the dolphin origami without crossovers in the tail, a broad distribution of tail angles was observed with only 42% of the structures being within ±10° of the theoretical value (Figure 4A, histogram). Analyzing the dolphin structure with 10 crossovers in the tail showed that 61% of the dolphins had a tail angle within ±10° of the theoretical value (Figure 4B, histogram). By fitting a Gaussian curve to the two histograms, we found a Gaussian variance of ±15° for the unstable tail and a Gaussian variance of ±11° for the stabilized tail. The same level of stability and variance was observed when adding 18 crossovers in the tail, suggesting that the data are statistically significant (data not shown). The observed difference might be explained by the potential sliding of helix ends when no crossovers are present. We conclude that the tail region can be stabilized (while maintaining some flexibility) by the amount of seam crossovers.

Dimer Interaction Stabilizes Tail Conformation. One of the visions within the emerging area of nanotechnology is to form self-assembled structures with incorporated macroscopic function, and to fulfill this goal, the DNA origami structures must be placed by specific interconnections at well-defined and known positions. The higher-order assembly of DNA origami structures is a key for future DNA nanotechnology applications. We therefore tried to stick two dolphin structures together by intermolecular base pairing to create a dolphin dimer. This was accomplished by the independent assembly of two structurally distinct dolphins—one with sticky ends and receptor sites on the abdomen and another with sticky ends and receptor sites on the back (Figure 5A, 2C) that will stick together by proper annealing to form a dolphin dimer (Figure 5C).

After the assembly process, the two dolphin origami structures were purified from excess oligos that may compete with the interaction. The dolphins were mixed with 1:1 molar ratio and allowed to dimerize in the assembly buffer for 30 min at 37 °C.

AFM imaging revealed that we predominantly observe the formation of the dolphin dimers. Nevertheless, trimers could also be formed as seen in Figure 5D, where a monomer unintentionally sticks to a predesigned dolphin dimer. A more statistical investigation of the yield, number, and strength of contacts of dolphin dimers *versus* monomers and/or other larger assemblies is currently in progress. Most of the paired dolphins were interacting at the three designed sites (Figure 5C) and maintained their orientation on the surface as observed by an equal amount of face-up and face-down species (Figure 5E, 5F). The tail interaction of the two dolphins constrains both tails in the correct conformation with an angle close to the theoretical value of 145° (Figure 5E, 5F). In some cases, the dolphin dimers do not interact at the tail site, and as a result, the tails are observed to be flexible (Figure 5G). The specific interaction also allows the annotation of otherwise identical dolphin structures in the AFM images (Figure 5E–G).

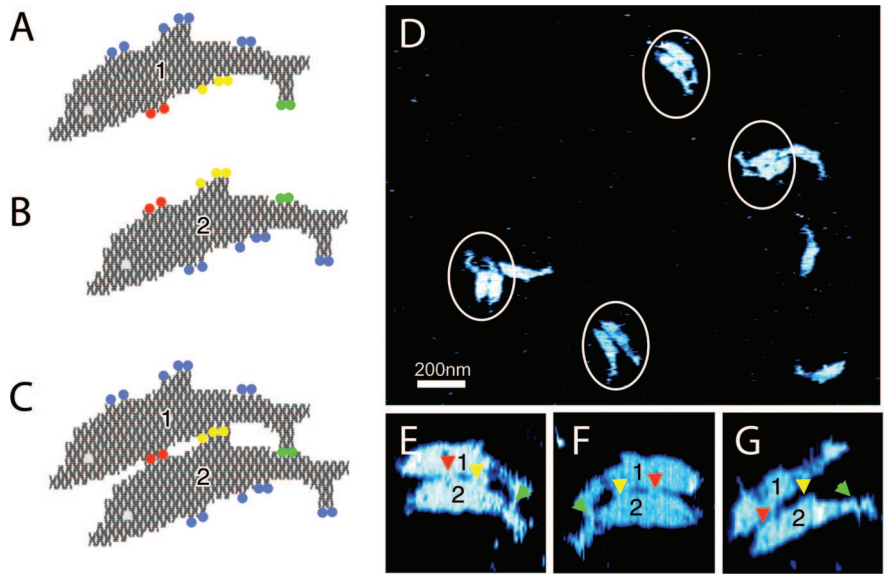

Figure 5. Specific recognition between two dolphin structures. (A) Dolphin 1 with back protected with T-extensions (blue) and the abdomen with sticky ends (red, yellow, green). (B) Dolphin 2 with sticky ends on the back (red, yellow, green) and T-extensions on the abdomen (blue). (C) Dolphins 1 and 2 interacting *via* sticky-end interactions at three interaction points (red, yellow, green). (D) AFM image showing the sample where dolphins 1 and 2 were allowed to dimerize for 30 min at 37 °C prior to imaging. Single dolphins are also observed. (E–G) Three different dimeric dolphins are shown with red, yellow, and green arrows indicating the three interaction sites. Dolphins 1 and 2 are annotated.

The DNA dolphin dimer structure presented here is the first DNA origami to be assembled from nonidentical parts, and more interestingly, it presents a unique structural recognition motif that stabilizes the flexible tail feature. These results reveal a new and important route for the construction of more sophisticated nanodevices composed of DNA origami structures.

CONCLUSION

A new software package for the design and construction of DNA origami structures is presented, and it is applied to the construction of DNA dolphin-like origami structures. This dolphin shape has specific features that made it interesting for a thorough investigation of the flexibility of the narrow tail region. The flexibility was shown to be dependent on the amount of seam crossover strands within the tail region, and its movement could be controlled with the flexible design and AFM probe tip. Furthermore, we have demonstrated that specific docking of DNA origami structures can constrain flexible regions and provide a functional tool for the assembly of larger, higher-order structures. The flexibility is an interesting property that might be exploited in future DNA origami designs to form structures with small flexible extensions that can be used as nanorobotic arms or nanocantilevers. As shown here, the nanorobotic arms can be moved top-down by the AFM cantilever or be designed to have intrinsic flexibility. Alternative ways to move nanorobotic arms by hybridization topology have been designed by Ding *et al.*[22] The nanorobotic arms could potentially be used to execute particular operations at the nanoscale and be moved by molecular motors. Flexible DNA origami structures may thus provide the basis for creating new controllable dynamic nanostructures for future interesting applications.

METHODS

Software Development. Several programs were developed to facilitate the DNA origami design process and made available as a program package for the semiautomated scientific data editor called SARSE.[17] The design process proceeds by the following steps: (1) a bitmap file is imported, and the shape can be further modified in the SARSE editor; (2) a program is activated that automatically finds a folding path through the shape and adds staple strands with crossovers in the plane of the figure; (3) the SARSE editor is used to make changes to the origami design by the editing of different symbols for the scaffold strand and the staple strands; (4) a program is triggered that generates the 3D atomic model which subsequently can be inspected in molecular viewers such as PyMol[18] or QuteMol;[23] (5) finally, a program is started that inserts a given sequence in the design, annotates the staple strands, and creates as an output a list of oligos for ordering (programs are described in more detail in the Supporting Information). The SARSE editor facilitates the design process by its ability to iteratively ex-

ecute programs to modify the data. The process is overviewed in a window that allows interactive navigation of the large DNA origami design. By tracking the history of the design process, it is possible to jump back if undesired results are obtained. The SARSE editor was extended to allow new modes for data processing to be defined by the user with specific menus and tool icons. The software for DNA origami design is available at http://www.cdna.dk/origami. The Java editor and origami program package are free software distributed under the GNU General Public License version 3 (GPLv3).

Preparation of M13 DNA. The M13mp18 DNA (from New England Biolabs) was introduced into the bacterial strain XL-1 Blue (Stratagene) by heat shock transformation. The transformed bacteria were plated in soft topagar on LB plates and incubated at 37 °C overnight. M13 plaques are visible as clear spots on the otherwise turbid bacteria lawn. One of the plaques was used to infect a large 250 mL culture of XL-1 Blue bacteria and allowed to grow overnight. The culture was centrifuged at 4000*g* for 15 min at 4 °C. The supernatant containing the M13 phages was transferred to a beaker glass, and 10 g of polyethylene glycol (PEG-8000) and 7.5 g NaCl were added. The solution was stirred for 30 min followed by centrifugation for 20 min at 10 000*g* at 4 °C. The pellet was suspended in 10 mL of 10 mM Tris-Cl, pH 8.0. Then 0.1% SDS was added to denature proteins, and the solution was subjected to several rounds of phenol extraction to remove all traces of protein.

Self-Assembly Reactions. The oligos were retrieved in 96-well plates at 100 μM from DNA technology A/S. Several different dolphin structures were assembled: (1) unstable tail, (2) semistable tail, (3) stable tail, (4) "up" dolphin with sticky end abdomen and stable tail, and (5) "bottom" dolphin with sticky end back and stable tail (see Supporting Information for the oligo sets used in each structure). The assembly reactions were performed in Tris-acetate-EDTA buffer with 12.5 mM MgAc (TAEM) and 1.6 nM M13 and 100-fold excess of each oligo. The samples were heated to 95 °C and cooled to 20 °C in steps of 0.1 °C/6 s. Dolphins 1 and 2 were self-assembled with a 4-fold excess of primer to M13 ssDNA. The assembly reactions were purified on columns (Microcon) with repeated washing to remove excess primers, and the concentration was calculated as devised by Ke *et al.*[11] The two dolphins were mixed 1:1 in TAEM buffer and allowed to dimerize for 30 min at 37 °C.

AFM Imaging. After the self-assembly reaction was completed, the dolphin-like origami structure was incubated at room temperature for 1 h prior to AFM imaging. The sample (5 μL) was deposited onto a freshly cleaved mica surface (Ted Pella, Inc.) and left to adsorb for 5 min. Buffer (TAEM, 200 μL) was added to the liquid cell, and the sample was scanned in a tapping mode using either an Agilent AFM series 5500 (Agilent Technologies, USA) or Nanoscope IV MultiMode SPM (Veeco Instruments, Santa Barbara, CA) with 0.4 nN/nm force constant cantilever of an oxide-sharpened (triangular, NP-S, Veeco Instruments, Santa Barbara, CA) and a vertical engage E-scanner. After engagement, the tapping amplitude set point was typically 0.5 V and the scan rates ranged from 1 to 2 Hz. During the liquid AFM imaging, the highest resolution was in most cases obtained with minimal loading forces applied and optimized feedback parameters. Several AFM images, all 512 × 512 pixels, were obtained from separate locations across the mica surfaces to ensure reproducibility of the results. All the images were analyzed by using the commercial Scanning Probe Image Processor (SPIP) software (Image Metrology ApS, version 4.2, Lyngby, Denmark).[24]

AFM images of 2 × 2 μm recorded with low force were used for measuring the tail angle. The tail angles were measured in the SPIP program by using three reference points on the dolphin DNA structure: the nose, the middle of the body below the right angle of the back fin, and the middle of the tail fin. On the theoretical model, this corresponds to an angle of 145°. The measured angles were used to calculate the deviation from the theoretical value.

Acknowledgment. We gratefully acknowledge the support from the Danish National Research Foundation to the CDNA center and the Danish Research Agency for support to the iNANO center. M.M.N. was supported by the Carlsberg Foundation. We thank Dr. Mads Bruun Hovgaard and Dr. Jacob Ask Hansen for the valuable discussions and suggestions concerning graphics and website, respectively.

Supporting Information Available: Description of the programs in the DNA origami program package and the sequences of the oligonucleotides used in the dolphin origami structures used in this study. This material is available free of charge *via* the Internet at http://pubs.acs.org.

REFERENCES AND NOTES

1. Seeman, N. An Overview of Structural DNA Nanotechnology. *Mol. Biotechnol.* **2007**, *37*, 246–257.
2. Gothelf, K. V.; LaBean, T. H. DNA-Programmed Assembly of Nanostructures. *Org. Biomol. Chem.* **2005**, *3*, 4023–4037.
3. Simmel, F. C. Towards Biomedical Applications for Nucleic Acid Nanodevices. *Nanomed.* **2007**, *2*, 817–830.
4. Seeman, N. C. Nucleic Acid Junctions and Lattices. *J. Theor. Biol.* **1982**, *99*, 237–247.
5. Chen, J. H.; Seeman, N. C. Synthesis from DNA of a Molecule with the Connectivity of a Cube. *Nature* **1991**, *350*, 631–633.
6. Rothemund, P. W.; Papadakis, N.; Winfree, E. Murata, S. Algorithmic Self-Assembly of DNA Sierpinski Triangles. *PLoS Biol.* **2004**, *2*, e424.
7. Yan, H.; LaBean, T. H.; Feng, L.; Reif, J. H. Directed Nucleation Assembly of DNA Tile Complexes for Barcode-Patterned Lattices. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 8103–8108.
8. Yan, H.; Park, S. H.; Finkelstein, G.; Reif, J. H.; LaBean, T. H. DNA-Templated Self-Assembly of Protein Arrays and Highly Conductive Nanowires. *Science* **2003**, *301*, 1882–1884.
9. Rothemund, P. W. Folding DNA to Create Nanoscale Shapes and Patterns. *Nature* **2006**, *440*, 297–302.
10. Högberg, B.; Olin, H. DNA-Scaffolded Nanoparticle Structures. *J. Phys.: Conf. Ser.* **2007**, *61*, 458–462.
11. Ke, Y.; Lindsay, S.; Chang, Y.; Liu, Y.; Yan, H. Self-Assembled Water-Soluble Nucleic Acid Probe Tiles for Label-Free RNA Hybridization Assays. *Science* **2008**, *319*, 180–183.
12. Williams, B. A.; Lund, K.; Liu, Y.; Yan, H.; Chaput, J. C. Self-Assembled Peptide Nanoarrays: An Approach to Studying Protein−Protein Interactions. *Angew. Chem., Int. Ed.* **2007**, *46*, 3051–3054.
13. Fujibayashi, K.; Hariadi, R.; Park, S. H.; Winfree, E.; Murata, S. Toward Reliable Algorithmic Self-Assembly of DNA Tiles: A Fixed-Width Cellular Automaton Pattern. *Nano Lett.* **2007,** 10.1021/nl0722830.
14. Pierce, N. A. NuPack http://www.nupack.org/ 2007.
15. Birac, J. J.; Sherman, W. B.; Kopatsch, J.; Constantinou, P. E.; Seeman, N. C. Architecture with GIDEON, a Program for Design in Structural DNA Nanotechnology. *J. Mol. Graph. Model.* **2006**, *25*, 470–480.
16. Sims, M. *NanoEngineer-1 (*http://www.nanoengineer-1.com/), 2008.
17. Andersen, E. S.; Lind-Thomsen, A.; Knudsen, B.; Kristensen, S. E.; Havgaard, J. H.; Torarinsson, E.; Larsen, N.; Zwieb, C.; Sestoft, P.; Kjems, J.; Gorodkin, J. Semiautomated Improvement of RNA Alignments. *RNA* **2007**, *13*, 1850–1859.
18. DeLano, W. L. *The PyMOL Molecular Graphics System* http://www.pymol.org/ 2002.
19. The logo of the University of Aarhus can be found at http://www.au.dk and http://info.au.dk/au-design/segl/.
20. Latin for "Seek a firm footing in the depths.
21. Ernst, K. Supramolecular Surface Chirality. *Top. Curr. Chem.* **2006**, *265*, 209–252, and references therein.
22. Ding, B.; Seeman, N. C. Operation of a DNA Robot Arm Inserted into a 2D DNA Crystalline Substrate. *Science* **2006**, *314*, 1583–1585.
23. Tarini, M.; Cignoni, P.; Montani, C. Ambient Occlusion and Edge Cueing to Enhance Real Time Molecular Visualization. *IEEE Trans. Vis. Comput. Graph.* **2006**, *12*, 1237–1244.
24. Dong, M. D.; Howard, K. A.; Oupicky, D.; Bisht, H.; Kjems, J.; Besenbacher, F. Visualization of Thermally Activated Nanocarriers Using *In Situ* Atomic Force Microscopy. *Nanotechnology* **2007**, *18*, 185501.