Magnetic Propulsion of Microswimmers with DNA-Based Flagellar Bundles

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Supporting Information

ABSTRACT: We show that DNA-based self-assembly can serve as a general and flexible tool to construct artificial flagella of several micrometers in length and only tens of nanometers in diameter. By attaching the DNA flagella to biocompatible magnetic microparticles, we provide a proof of concept demonstration of hybrid structures that, when rotated in an external magnetic field, propel by means of a flagellar bundle, similar to self-propelling peritrichous bacteria. Our theoretical analysis predicts that flagellar bundles that possess a length-dependent bending stiffness should exhibit a superior swimming speed compared to swimmers with a single appendage. The DNA self-assembly method permits the realization of these improved flagellar bundles in good agreement with our quantitative model. DNA flagella with well-controlled shape could fundamentally increase the functionality of fully biocompatible nanorobots and extend the scope and complexity of active materials.

KEYWORDS: Propulsion, low-Reynolds-number, slender-body theory, nanorobots, DNA self-assembly, flagella

Many motile micro-organisms possess flagella to propel or swim through viscous fluids. Mimicking these highly evolved microstructures with nanoscale features is challenging but would enable the realization of functional microswimmers. Artificial flagella have been constructed from various materials, such as alloys and glass, with sizes ranging from a few millimeters down to several hundreds of nanometers using top-down fabrication methods. A prominent example of a self-assembled swimmer was demonstrated by linking magnetic particles while an applied magnetic field induces the formation of particle chains. For the actuation of such artificial flagella, externally applied magnetic fields have proven to be advantageous as they are fully biocompatible and can be applied in aqueous environments from afar, avoiding the need for potentially toxic chemical reactions commonly used to propel phoretic swimmers. Within these approaches, miniaturization to the nanoscale, complex maneuvering, functionalization for targeted drug delivery, large scale production, and a certain degree of biocompatibility were shown. However, for future applications it is desirable to develop more versatile and naturally biocompatible means of realizing artificial flagella with the potential to mimic the complex hierarchically assembled protein structure of real flagella while ideally combining all of the above-mentioned features in one single system. DNA-based self-assembly can meet these requirements as it allows for systematic design at the nanoscale, large-scale production and straightforward functionalization, while being fully biocompatible. We show that even isotropically covered spherical particles of 1 μm in diameter can be propelled by virtue of hydrodynamically formed DNA flagellar bundles. Interestingly, compared to swimmers with a single appendage, our bundles exhibit a stiffness profile that is shown to underlie favorable swimming shapes that can also be found in flagellar bundles of bacteria.

To realize artificial flagella, we employed DNA tile-tube assembly schematically depicted in Figure 1. In this technique, short single-stranded DNA fragments assemble into n-helix tubes (nHT) (Figure 1a), where each tube consists of repeating structural units of n adjacent DNA duplexes, which can polymerize along the axis of the tube. From a large variety of possible tube types (Supporting Information Figure S1), three designs with varying degree of twist and stiffness are shown in Figure 1b: straight 8-helix-tubes (st8HT), twisted 8-helix tubes (tw8HT), and super-twisted 13-helix tubes (stw13HT). The tubes were attached to 1 μm iron oxide particles via the hybridization of complementary DNA strands.
For imaging and tracking, a subset of the DNA strands forming the tubes were modified with Cy3 dyes on their 5′ ends. All three types of the tubes (Figure 1d−f) and their respective hybrid structures (Figure 1g−i) were imaged in a liquid cell of a few micrometers in height. Remarkably, the hybrids visually resembled flagellated bacteria, were similar in size, and possessed equal amounts of twist as natural flagella. For example, the stw13HT (Figure 1f,i) had a pitch of 2.51 ± 0.51 μm and a diameter of 0.57 ± 0.19 μm, which compares well to an E. coli filament in its normal form, whereas the tw8HT (Figure 1e,h) had a pitch of 0.98 ± 0.22 μm and a diameter of 0.30 ± 0.17 μm, which resembles the curly state of the E. coli.21

To test the ability of the DNA-flagellated magnetic particle hybrids to propel at low Reynolds number, we externally applied a rotating homogeneous magnetic field. Magnetic beads can also be pulled by a magnetic gradient field, however, it is generally not viable to achieve significant gradients at a distance, and so homogeneous (nongradient) fields are much more practical for potential applications. The samples were placed in the center of a three-axis Helmholtz coil system that was integrated into an inverted fluorescence microscope (Supporting Information Note S2). The hybrids were exposed to rotating homogeneous magnetic fields of up to 100 G, and their motion was captured on video. Video samples containing hybrid structures (displayed in Figure 1g−i) revealed that all the particles rotated in phase and approximately 10% of the stw8HTs and tw8HTs decorated structures formed a bundle of DNA flagella on only one side of the bead (see Figure 2a and Supporting Information Video 1 for a well-assembled structure and Supporting Information Figure S7 for non-functional side
products). Because we did not observe a qualitative difference in the bundle structure of st8HTs, tw8HTs, and stw13HT-bundles (see Supporting Information Figure S8 and S9), and since stw13HT formed with a yield of only \( \sim 1\% \), we limited our study to the tw8HT-bundle swimmers. Figure 2a shows eight snapshots of a tw8HTs decorated 1 μm magnetic particle during one full rotation. Similar to real bacterial flagella, upon rotation the DNA flagella formed a hydrodynamically assembled corkscrew-like bundle with a length of several micrometers. The rotated tube bundle propelled the DNA–magnetic bead hybrids (see Figure 2b and Supporting Information Video 2) head first along the rotation axis. The propulsion speed of up to 1/10 of a body length per turn (along the rotation axis) is in good agreement with the propulsion speed of up to 1/10 of a body length per turn for additional bundle analysis). The fluorescence intensity of the bundles in supporting the theoretical model, swimmers with decreasing stiffness of the bundle (solid lines) achieve a higher speed than swimmers with constant stiffness of the bundle (dashed lines) for a broad range of bundle lengths and stiffnesses.

**Figure 3.** Analysis of a DNA tile-tube bundle. (a) The fluorescence intensity of an artificial flagellar bundle (inset) drops exponentially (red dashed line) when moving away from the bead due to a decreasing number of tubes in the bundle. Data was gained by averaging over several movie frames to rule out errors emerging from a change in the fluorescence distribution along the particle during rotation (see Supporting Information Figure S7 for additional bundle analysis). The fluorescence decay length of the demonstrated swimmer is 3 μm. (b) Numerical solutions of the shape of one stiff (top) and floppy (middle) bundle with constant bending stiffness in comparison to a bundle with decreasing stiffness (bottom) at eight positions during one full rotation. (c) Within the theoretical model, swimmers with decreasing stiffness of the bundle (solid lines) achieve a higher speed than swimmers with constant stiffness of the bundle (dashed lines) for a broad range of bundle lengths and stiffnesses.

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\Gamma \ddot{\mathbf{r}} = -\sigma (A \ddot{\mathbf{r}}^2) + \frac{\partial \sigma}{\partial \dot{\mathbf{r}}} 
\]

Here, \( \dot{\mathbf{r}} \) is the local velocity of the bundle, \( A \) is the local bending stiffness, \( \sigma \) is the bundle position and \( \Gamma \) is a Lagrange multiplier ensuring inextensibility. All variables depend on the arc length position \( s \) along the bundle. We solved this equation by generalizing previous work on actuated swimmers \(^{17}\) using boundary conditions taking into account that the bundle is attached to a rotating bead (Supporting Information Note S3). The crucial difference to earlier works, \(^{17}\) however, is that in our case the bending stiffness \( A(s) \) changes along the bundle, which extends the scope of our model to bundles of variable stiffness.

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At low Reynolds numbers, swimming requires a swimming stroke that is not time-reversible. Therefore, a rotating straight passive bundle needs to bend into a chiral shape to generate propulsion. The propulsion efficiency then depends on the curvature and the beat amplitude of this induced shape. In order to ascertain how well our bundle of variable stiffness meets these requirements, we compared it to the cases of constant high and low bending stiffness. Figure 3b shows that constant high stiffness (top) achieves high amplitudes but lacks bending especially toward the end of the bundle. In contrast, a constant low stiffness (middle) permits bending but lacks beat amplitude. A bundle with variable stiffness (bottom), however, can achieve both large amplitudes and bending along the entire length of the bundle (see also Supporting Information Video 6). Figure 3c shows a comparison of the swimming speed as a function of bundle length for bundles of decreasing stiffness (solid), and bundles of constant stiffness (dashed) comprising 1, 6, 20, and 50 tubes. Remarkably, the swimming speed of the variable stiffness swimmer is faster for nearly all parameters. In other words, a bead with a bundle of multiple tubes of different lengths surpasses one with the same number of equal-length tubes, which is a feature that arises naturally in our method of construction.

Finally, we tested our theoretical model by comparing the predicted and the observed swimming speeds of 10 individual tw8HT-bundle swimmers (Figure 4). The solutions from the model allowed us to predict the swimming speed of a swimmer from its geometric parameters, which we determined for each swimmer from the experimental data. The so predicted swimming speeds differed by less than 40% from the observed speed. Differences between the model and the observations are attributed to errors in determining some of the parameters that enter the model (see Supporting Information Note S3) as well as to neglecting the additional drag, which arises in the vicinity of the glass surface. Despite these differences, our observations agree remarkably well with the quantitative predictions from the model.

Our general and flexible self-assembly scheme allows constructing biocompatible artificial flagella of comparable size to small bacteria and in large numbers. Coupling of DNA-tubes to magnetic beads results in hybrid structures that swim by means of a flagellar bundle when actuated by an external magnetic field, which also allows for complex maneuvering. Our quantitative model reveals that our artificial and bacterial flagellar bundles whose stiffness decreases toward their ends achieve an improved speed compared to bundles with homogeneous rigidity. Design rules derived from the theoretical model can be applied in future experiments to optimize the swimmers to reach even higher speeds. To better understand and improve the assembly process of our artificial flagella, real-time bundle formation dynamics could be studied using magnetic tweezers, which can trap and rotate the swimmers in solution with no interfering glass surface nearby. If unfolding of the bundle can be achieved, further insights into run-and-tumble and swimming strategies used by microorganisms can be gained. Our method demonstrates the feasibility and advantages of DNA self-assembly techniques for the construction of new microswimmer and nanorobot designs, including hierarchically assembled fully biocompatible soft materials that are difficult to realize with other fabrication methods.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.nanolett.5b03716.

Methods and materials, DNA sequences, additional fluorescence microscopy and TEM images, gels, statistical analysis, and model for swimmers with length-dependent bending modulus. (PDF)
Rotation of swimmer from Figure 2a. (AVI)
Directed motion of swimmer from Figure 2b. (AVI)
Structural change of the artificial bundle upon changing the rotation frequency. (AVI)
Swimmer from Figure 2c steered to follow a curved path. (AVI)
Swimmer exposed to a rotating magnetic field that repeatedly switches from clockwise- to counterclockwise. (AVI)
Analytic solution for the shape of a swimmer with decreasing tail stiffness. (AVI)

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A.M.M. and C.W. contributed equally to this work. A.M.M., C.W., E.F., and T.L. conceived the experiments. A.M.M. produced the structures. A.M.M., P.O., and P.F. performed and analyzed the swimming measurements. C.W. provided the analytic and numeric calculations. A.M.M., C.W., E.F., P.F., and T.L. interpreted the results and wrote the manuscript.

Notes
The authors declare no competing financial interest.
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