Ben-Gurion University of the Negev

מכון אילזה כ"ץ למדע וטכנולוגיה בתחום הננומטרי
Ilse Katz Institute for Nanoscale Science and Technology

ISRAEL SCIENCE FOUNDATION

Computer Controlled Fast and Processive Bipedal Motor that Travels Long Distances

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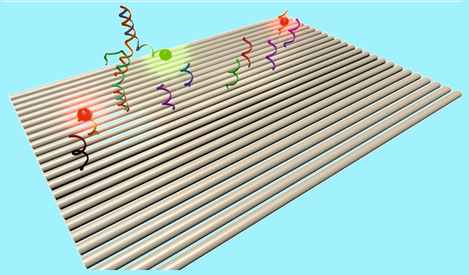
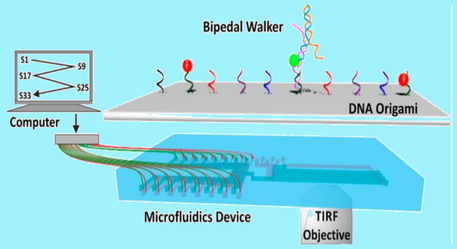
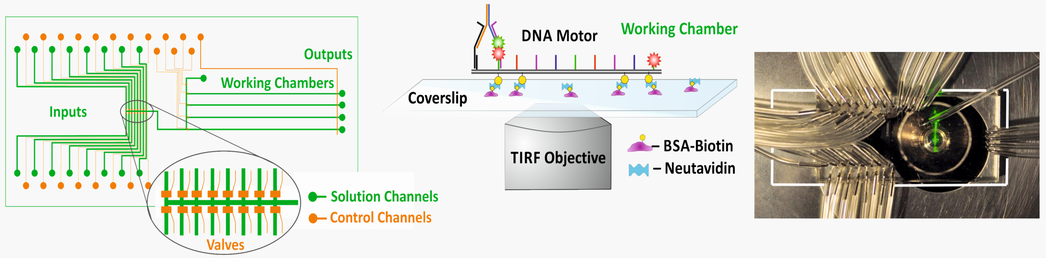




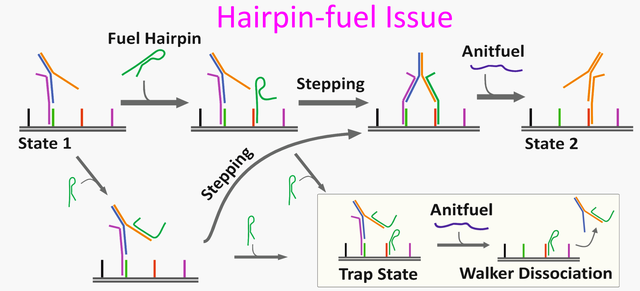
ABSTRACT: Biological molecular machines can perform hundreds of serial operations with remarkably high speed. In comparison, synthetic molecular machines, including those made of DNA, perform orders of magnitude fewer operations and are orders of magnitude slower. In response, a major effort in our group is to develop effective and fast DNA based molecular machines. In recent years we have demonstrated a DNA bipedal motor that strides on a DNA origami and operates by responding to 'fuel' and 'anti-fuel' DNA strands. The strands are provided to the motor by a computer-controlled microfluidics device and motor progress is observed by single-molecule fluorescence. Importantly, the microfluidics allows the removal of excess strands and motors waste, crucial for proper operation of the motor. With this setup, we demonstrate the performance of 32 walking steps (64 consecutive chemical reactions with ~98.9% yield per reaction) which amount to 370 nanometers traveled by the walker. Furthermore, a redesign of the walking mechanisms (such that higher fuel concentration does not result in decreased yield, as was the case for earlier motor versions) resulted in two orders of magnitude increase in motor speed for the same motor yield. However, motor dissociation rate-per-step remains above ~1%, limiting the number of steps to about 50. Here we aim to identify the factors that lead to the ~1% motor dissociation per step, and by rational design of solutions, decreasing motor dissociation and increasing motor processivity.

To enable unidirectional walking of a bipedal DNA motor capable of striding over micrometer distances, it is necessary to understand how to reliably and controllably connect several unique origami tiles to create stable micrometer-long tracks. In the current study, we combine a purification approach suggested in our previous work with a sticky end-based (SE) bridging of six rectangle origami tiles to assemble a 950 nm track for a DNA walker. The observations are made by immobilizing the hexamer track in microfluidics and fluorescent microscopy technique is used to visualize the track in which the first and the last tiles are labeled by ATTO 647N, showing two clear peak pairs with ~750 nm distance between them. On the contrary, attempts to assemble the hexamer track from the same origami tiles using weaving-welding techniques failed to gain a pure product. To summarize, using our method we have tailored and fabricated a 950 nm long track ready for a DNA motor.

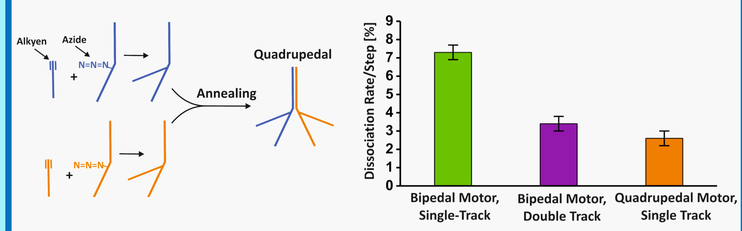
Microfluidic Chip on TIRF Microscope



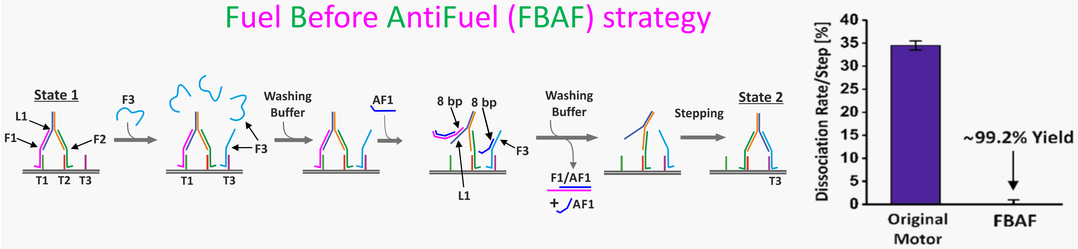
Hairpin-fuel Issue



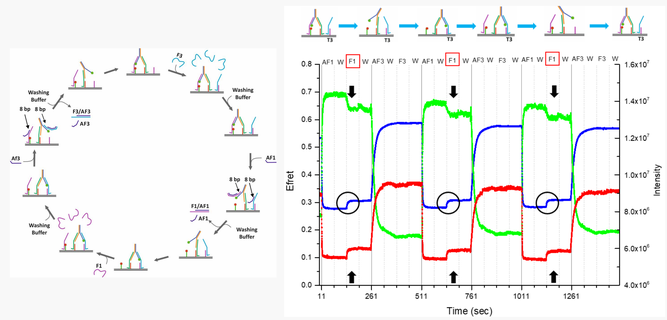
Multi-legged Walker and Double Track



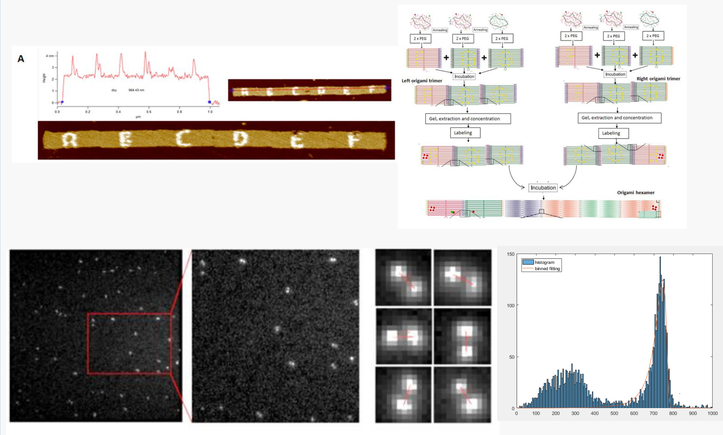
Fuel Before AntiFuel (FBAF) strategy



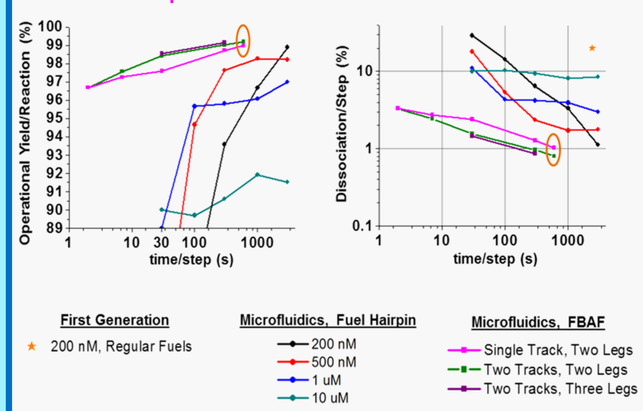
Kinetic Measurement of Leg Lifting and Leg Placing



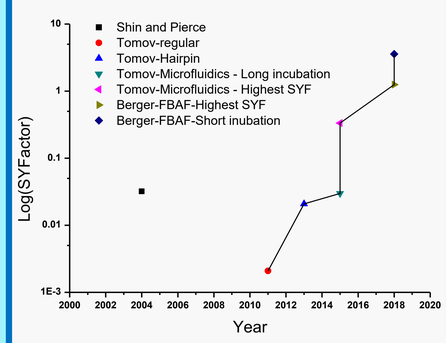
Sticky-end based fabrication of long track



Operational Yield Per Reaction



SY Factor



$$\text{SYFactor} = \frac{Speed}{1 - Yield/Reaction}$$

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(2) Liber, M., Tomov, T. E., Tsukanov, T., Berger, Y., Nir, E., "A Bipedal DNA Motor that Travels Back and Forth between Two DNA Origami Tiles", *Small*, 2014

(3) Tomov, T., Tsukanov, R., Glick, Y., Berger, Y., Liber, M., Avrahami, D., Gerber, D., Nir, E., "A DNA Bipedal Motor Achieves a Large Number of Steps Due to Operation Using Microfluidics-Based Interface", *ACS Nano,* 2017

(4) Khara, D. C., Schreck, J. S., Tomov, T. E., Berger, Y., Ouldridge, T. E., Doye, J. P. K., Nir, E., DNA bipedal motor walking dynamics: an experimental and theoretical study of the dependency on step size", *Nucleic Acids Research*, 2017

(5) Berger, Y., Tomov, T., Tsukanov, R., Khara, D. C., Liber, M., Nir, E., "Fast and Processive Computer Controlled DNA Bipedal Walker", *in preparation*

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