A Unidirectional DNA Walker Moving Autonomously Along a Track

Peng Yin*, Hao Yan*, Xiaoju G. Daniell*, Andrew J. Turberfield†, John H. Reif*
*Department of Computer Science, Duke University, Durham, NC 27708, USA
†University of Oxford, Department of Physics, Clarendon Laboratory Parks Road, Oxford OX1 3PU, UK

A nanoscale object moving autonomously over a self-assembled microscopic structure has important nano-robotics applications, e.g. serving as a nano-particle and/or information carrier. Recent successes in self-assembly of DNA nanostructures provide a solid structural basis to meet this challenge. However, existing nanoscale synthetic DNA devices are unsuitable for the above purpose: they only exhibit localized non-extensible motions (open/close, extension/contraction, and reversible rotation), mediated by external environmental changes. Here we report an experimental construction of unidirectional DNA walker that moves autonomously along a linear DNA track. The self-assembled track contains three anchorages at which the walker, a six-nucleotide DNA fragment, can be attached. At each step the walker is ligated to the next anchorage, then cut from the previous one by a restriction endonuclease. Each cut destroys the previous restriction site and each ligation creates a new site in such a way that the walker cannot move backwards. The device is powered by the hydrolysis of ATP by T4 ligase. The prototype device can be embedded in other self-assembled DNA structures and in principle be extended beyond 3-step operation.

The structural design of the device is shown in Figure. The track consists of three evenly spaced DNA double helical 'anchorages' (A, B, and C), each tethered to another DNA duplex segment which forms part of the backbone of the track by means of a 4-nucleotide 'hinge'. A 6-nucleotide DNA 'walker', labeled * and coloured red, moves sequentially along the track from anchorage A to B, then to C. The motion of the walker depends on alternate enzymatic ligation and restriction (cleavage). Before the motion starts the walker, whose position is indicated by *, resides at anchorage A. In this state anchorages A* and B have complementary sticky ends which can hybridize with each other. T4 ligase can then heal the nicks at either end of the newly-hybridized section, covalently joining the two anchorages (A* + B $\rightarrow$ A*B); this is an irreversible step that consumes energy provided by the hydrolysis of ATP. The ligation of A*B creates a recognition site for endonuclease PflM I. PflM I then cleaves A*B in such a way that the walker moves to anchorage B: A*B $\rightarrow$ A + B*. The sticky end of anchorage B* can then hybridize with the complementary sticky end of anchorage C, and the two anchorages are ligated to form B*C. Ligation product B*C contains a recognition site for the second endonuclease BstAP I and hence is cleaved by BstAP I to regenerate anchorage B and create C*. Thus the walker moves from anchorage B to C, completing the autonomous, programmed motion of the walker.

The operation of the device was verified by tracking the radioactively labeled walker using gel electrophoresis.