

Chapter 2

Molecular Robots Guided by Prescriptive Landscapes⁰

Traditional robots [Siegwart, 2004] rely for their function on computing to store internal representations of their goals and environment and to coordinate sensing and any actuating of components required in response. Moving robotics to the single-molecule level is possible in principle, but faces the limited ability of individual molecules to store complex information and programs. One strategy to overcome this problem is to use systems that can obtain complex behavior from the interaction of simple robots with their environment [Braitenberg, 1984, Brooks, 1991, Turing, 1936]. First steps in this direction were the development of DNA walkers [Bath and Turberfield, 2007], which have transitioned from being non-autonomous [Sherman and Seeman, 2004, Shin and Pierce, 2004], to being capable of directed but brief motion on one-dimensional tracks [Bath et al., 2005, Tian et al., 2005, Yin et al., 2008, Omabegho et al., 2009]. Here, we demonstrate that previously developed random walkers [Pei et al., 2006], so-called molecular spiders that comprise a streptavidin molecule as inert body and three deoxyribozymes as catalytic legs, exhibit elementary robotic behavior when interacting with a precisely defined environment. Single-molecule

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microscopy observations confirm that such walkers achieve directional movement by sensing and modifying tracks of substrate molecules laid out, much like bread crumbs, on a two-dimensional DNA origami landscape [Rothemund, 2006]. When using appropriately designed DNA origami, the molecular spider robots autonomously carry out sequences of actions such as “start”, “follow”, “turn”, and “stop”. We anticipate that this strategy can result in more complex robotic behaviour at the molecular level, by incorporating additional control mechanisms. One example might be interactions between multiple molecular robots leading to collective behaviour [Bonabeau et al., 1999, Rus et al., 2002], another the ability to read and transform secondary cues on the DNA origami landscape as a means for implementing Turing-universal algorithmic behaviour [Turing, 1936, Von Neumann and Burks, 1966, Bennett, 1982].

We previously described polycatalytic assemblies, comprised of streptavidin molecules and several attached nucleic acid catalysts, that function as walkers and are referred to as molecular spiders [Pei et al., 2006]. The molecular spiders used in this study comprise one streptavidin molecule as inert body and three catalytic “legs”. Legs are adapted from DNA enzyme 8-17 that binds and cleaves oligodeoxynucleotide (henceforth “oligonucleotide”) substrates with a single ribose moiety (Fig. 2.1) into two shorter products that have lower affinities for the enzyme [Santoro and Joyce, 1997]. The different substrate and product affinities ensure that a spider’s interactions with a layer of immobilized substrate and/or product sites can be modeled using a simple memory principle [Antal, 2007]: each leg moves independently from sites to accessible neighboring sites, but if a leg is on a site not visited before, it will stay longer on average. Put biochemically: a deoxyribozyme attached to a site that was previously converted to a product will dissociate faster, whereas it will stick longer to substrates and eventually cleave them. Because spiders have multiple legs that prevent complete dissociation, a single dissociated leg will quickly reattach to a nearby product or substrate site. After cleaving, each leg will thus explore neighbouring sites until it finds another substrate to bind to for longer. This ensures that the body of a spider positioned at the interface between products and substrates will move toward the substrate region, and that it will move directionally along a linear track as the substrates are cleaved. Previously engineered walkers using “burnt bridge” mechanisms [Sherman and Seeman, 2004, Bath et al., 2005, Tian et al., 2005, Omabegho et al., 2009] and Brownian ratchets found in nature [Saffarian et al., 2004] ren-

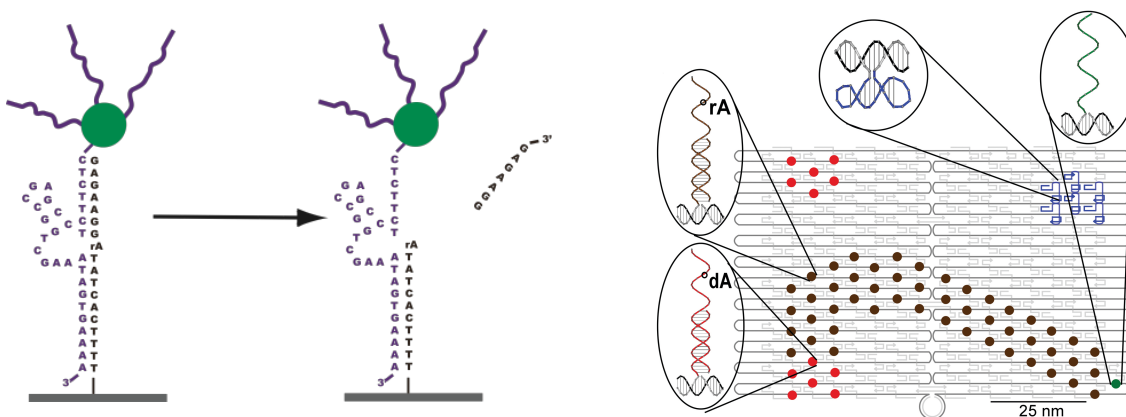


Figure 2.1: Deoxyribozyme based molecular walker and origami prescriptive landscape schematics. The $\text{NICK}_{3.4A3+1}$ spider consists of a streptavidin core that displays a 20 base ssDNA that positions the spider at the start (green), and three deoxyribozyme legs. The 8-17 deoxyribozyme cleaves its substrate at an RNA base creating two shorter products (seven and eleven bases). Dissociation from these products allows legs to associate with the next substrate. Spider actions: after release by a 27-base ssDNA trigger, the spider follows the substrate track, turns, and continues to a stop site (red). Schematic of the DNA origami landscape with positions A-E labeled; track EABD is shown. A representative origami landscape shows the start position (green), the substrate track (brown), stop and control sites (red), and a topographical marker (blue).

der revisiting the same path impossible, but our spiders will perform random Brownian walks on product sites until they again encounter a substrate track.

In analogy to the reactive planning used in simple robots [Brooks, 1991], the sensor-actuator feedback afforded when legs sense and modify nearby oligonucleotides allows us to design prescriptive landscapes that direct the spider's motion along a predefined path (Fig. 2.1). Prescriptive landscapes were constructed using the DNA origami scaffolding technique [Rothemund, 2006]. The scaffold consists of a 7249-nucleotide single-stranded DNA folded by 202 distinct staple strands into a rectangular shape roughly $65 \times 90 \times 2$ nm in size and with 6-nm feature resolution (Fig. 2.1). Each staple can be extended on its 5' end with probes that recruit substrates, products, goal and control DNA strands [Ke et al., 2008]. We designed pseudo-one dimensional tracks on origami of about spider width (three adjacent rows of substrates, Fig. 2.1). Tracks are coded by a sequence of points (A, B, C, D, E; i.e., on an ABD landscape the spider starts at A, and passes through B before ending at D). Staples were modified to position: (1) A START

oligonucleotide, used to position a spider at the start of the experiment, that is complementary to a TRIGGER oligonucleotide used to release the spider [Yurke et al., 2000] (the “start” action); (2) Substrate TRACK probes to capture the 5' extension on substrates forming the TRACK (directing the “follow” and “turn” actions); (3) STOP probes complementary to the 5' extension on STOP strands (non-chimeric and uncleavable analogs of the substrate) that do not influence directional movement but trap spiders to prevent them from walking backwards after completing the track (the “stop” action); (4) CONTROL probes (identical to the STOP, but disconnected from the track), used to assess the extent to which free-floating spiders are captured directly from solution; and (5) MARKER oligonucleotides based on inert dumbbell hairpins, aiding in origami classification within atomic force microscopy (AFM) images (Fig. 2.1). To position spiders at START sites, we replaced one of the four catalytic legs of the NICK_{4.4A12} spider with a tethering oligonucleotide (Supplementary Information) partially complementary to the START oligonucleotide.

To estimate the efficiency of spider motion directed by the TRACK, we defined and tested four paths with no (EAC), one (ABD), or two (EABD, EABC) turns (Fig. 2.2). The basic experimental procedure involves: (1) Assembling the origami; (2) attaching spiders to START sites; (3) adding TRACK, STOP, and CONTROL strands to complete the landscape; and (4) initiating an experiment by releasing spiders through addition of TRIGGER and 1 mM Zn⁺⁺ cofactor [Li et al., 2000]. We sampled the origami solution before and after spider release, and imaged individual samples by AFM to determine the locations of spiders. We scored only “face-up” origami (substrates projected away from mica) to avoid artifacts, using procedures that minimize readout bias (see Supplementary Information for details). In all samples imaged before spider release, 30-40% of the assembled origami carry at least one spider, 80-95% of which are singly occupied, and of these 80-90% bound their spider at the START position. Upon adding trigger, all four landscapes with substrate tracks showed that the fraction of spiders at the START diminishes with a concomitant increase in spiders observed on the STOP sites (Fig. 2.2 c,g). A spider’s ability to reach the STOP sites decreased with increased TRACK length and with decreased time of incubation in solution. In time-lapse experiments on a long path (EABD, spanning 90 nm) we observed a gradual increase of up to 70% of spiders on STOP sites within 60 minutes (Fig. 2.2 c,g). A short path (ABD, 48 nm) was completed to the same extent within 30 minutes. We captured one series of AFM images

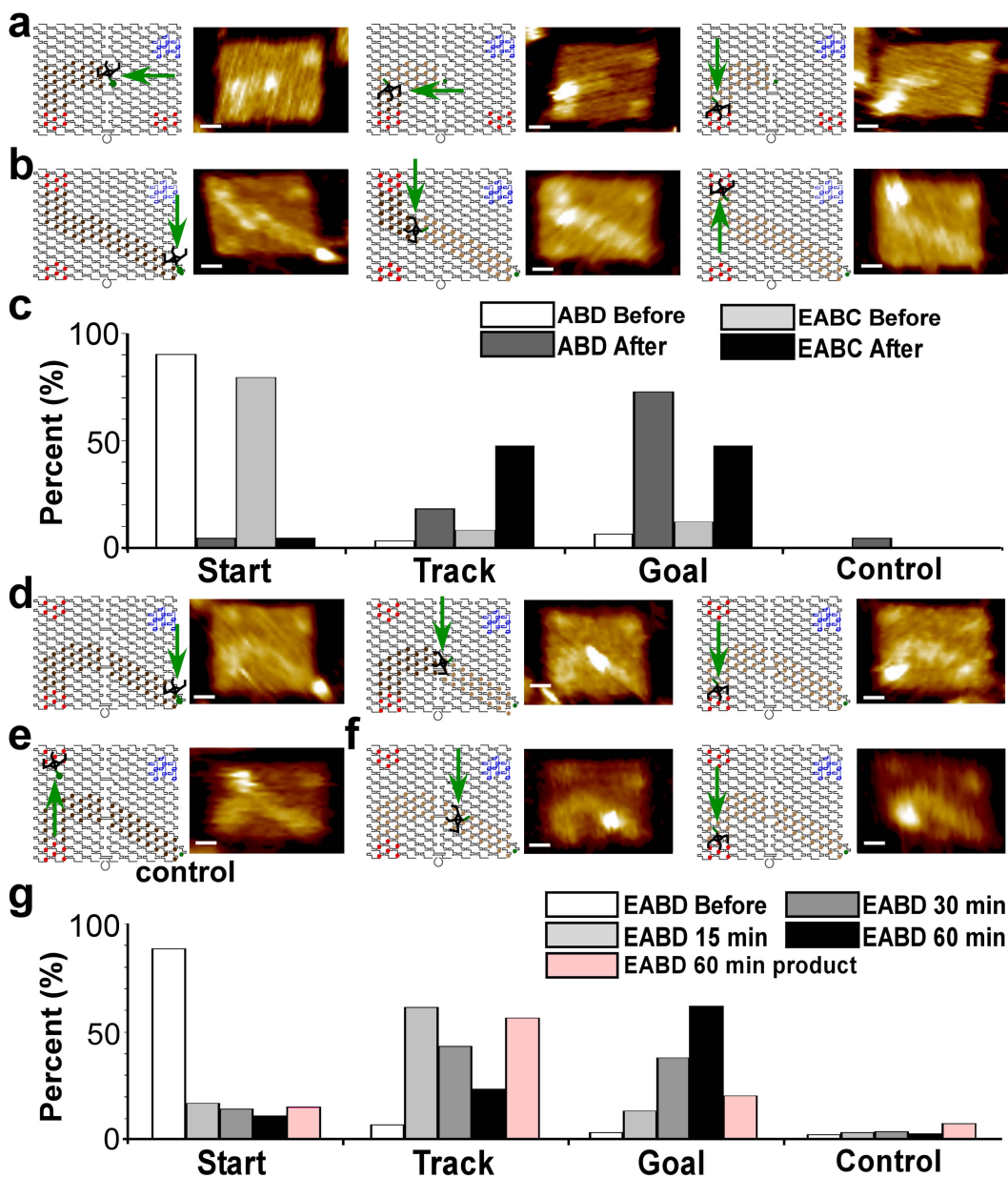


Figure 2.2: Results of spider movement along three tracks with schematics and AFM images of the spider at the start, on the track, and at the stop site. a, ABD track. b, EABC track. c, Graph of ABD and EABC spider statistics before and 30 minutes after release. d, EABD track. e, EABD track with spider on control. f, EABD product-only track. g, Graph of the EABD spider statistics before, and 15, 30 and 60 minutes after release, and 60 minutes after release on the EABD product-only track. All AFM images are 144×99.7 nm, the scale bar is 20 nm. Legend text indicates the number of origami with a single spider that were counted for the given sample.

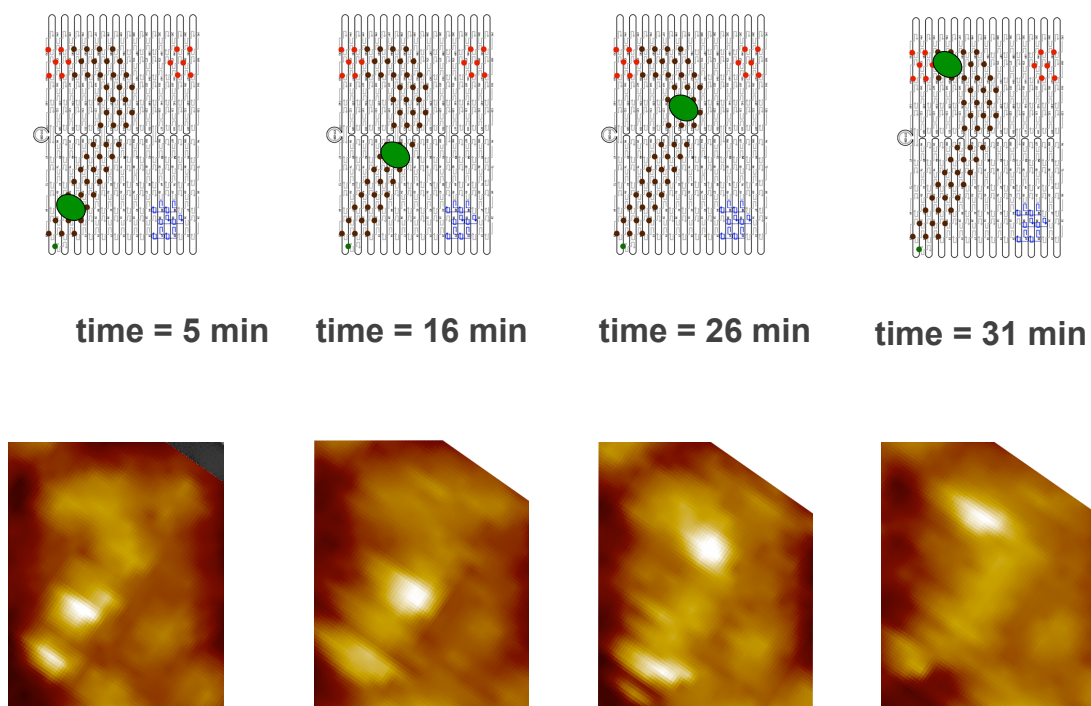


Figure 2.3: Schematics and AFM images of the spider moving along a track at 5 minutes, 16 minutes, 26 minutes, and 31 minutes after trigger was added. AFM images are 300×300 nm and the scale bar is 100 nm.

of a spider moving along an origami track (Fig. 2.3). The rate of spider movement (90 nm over 30 minutes, with approximately 6 nm per three parallel cleavage events) was consistent with the processive cleavage rates (1 min^{-1}) of spiders on a 2D surface as obtained by SPR.

More systematic sequential imaging proved difficult due to micas inhibitory effects on the spider. To test that spiders can indeed traverse product tracks by means of unbiased random walks, we challenged spiders with EABD origami in which the substrate was replaced by product on the TRACK. Spiders still reached the STOP sites albeit more slowly (Fig. 2.2 f,g), as expected from purely Brownian spider movement even if individual steps are somewhat faster [Antal, 2007]. If all

three legs simultaneously dissociate before any leg reattaches, a spider could ‘jump’ by completely dissociating from the origami and subsequently reattaching elsewhere at random. Evidence against frequent jumping (or an excess of spiders in solution during the initial assembly stage) comes from the low level of spider occupancy at CONTROL sites in both substrate and product track experiments (Fig. 2.2 c,e,g) and the stable proportions of unoccupied and multiply-occupied origami (both before and after the addition of trigger, 5-10% of origami displayed more than one spider on its track). In contrast, when spiders were released on ABD landscapes with no TRACK strands, after 30 minutes we observed an equal distribution between STOP and CONTROL sites, as expected for a process that involves spider dissociation from and random rebinding to the origami. In independent ensemble experiments using surface plasmon resonance to monitor spider attachment and with a constant flow passing over the surface, up to 15% of spiders dissociate from a non-origami 2D product-covered surface within 60 minutes. When using similar surfaces but covered with substrate, spiders show an average processivity of 200 substrate sites before being removed by flow. Together, the results of our control experiments rule out the possibility that spiders move predominantly by jumping; there is insufficient jumping even on product tracks to explain the 50 – 70% occupation of the STOP sites after walks on ABD, EABC, and EABD substrate tracks.

To observe the movement of individual spiders in real-time, we applied particle tracking by super-resolution total internal reflection fluorescence (TIRF) video microscopy [Walter et al., 2008]. Four biotin molecules were attached to the underside of the origami for immobilization on the avidin-coated quartz slide. Spiders were covalently labeled with on average 2.3 Cy3 fluorophores, and STOP sites were labeled with 6 Cy5 fluorophores. The labeling allowed us to monitor changes in spider position relative to the STOP site by two-color fluorescent particle tracking [Churchman et al., 2005, Yildiz and Selvin, 2005]. In a typical experiment, spider-loaded tracks were incubated with TRIGGER and immobilized on the slide, then Zn^{++} was added to promote spider movement via substrate cleavage. Recognizing that the 8-17 activity depends on buffer conditions [Li et al., 2000], we obtained the best results from SSC or HEPES with increased Zn^{++} concentrations but without Mg^{++} .

Our resolution was not sufficient to reliably detect turns, so we focused on EAC landscapes. Individual particle traces showed a distribution of behaviors that may result from variations across

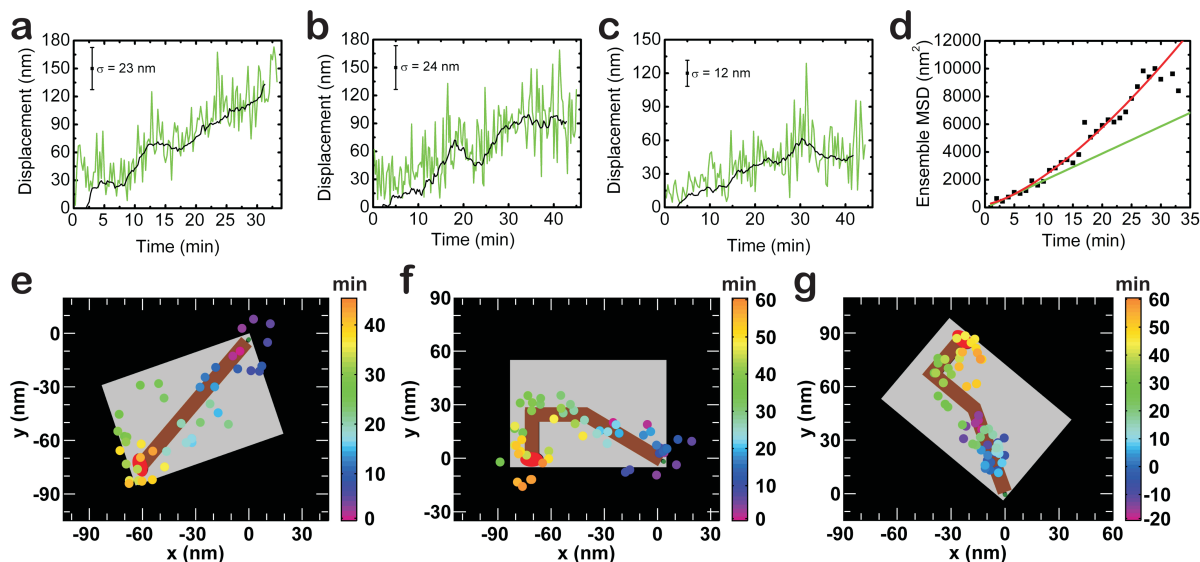


Figure 2.4: Spiders imaged on origami tracks in real-time using super-resolution TIRF microscopy. a, Position-time trajectory of a selected spider (EAC 2, Cy3-labeled) on the EAC substrate track. The position as a function of time is represented by color-coded dots. A small green dot represents the START and a large red oval represents the Cy5-labeled STOP site. ZnSO_4 was added at time zero. b, Displacement of the spider trajectory in panel a from its initial position as a function of time. The green line represents displacement calculated using averaged position measurements at 1 minute intervals, and the black line represents the displacement from a rolling 4-minute average. c, Ensemble root mean square displacement (RMSD) of exemplary spiders on the EAC substrate track in the presence (red, 15 spiders) and absence (black, 7 spiders) of Zn^{++} , with the corresponding displacements used to calculate each ensemble RMSD for each buffer condition (similarly colored line graphs). d, Ensemble RMSD for spiders on EAC tracks satisfying simple filtering criteria. Curves are shown for spiders on EAC substrate track (red, 85 spiders), EAC product track with TRIGGER introduced to the sample 10-15 minutes before imaging (blue, 18 spiders), and EAC product track with TRIGGER introduced 30-60 minutes before imaging (black, 29 spiders). EAC substrate and 10-15 minute trigger product RMSD plots are fit to a power law function, and the EAC 30-60 minute trigger product RMSD is fit to a straight line. Individual displacements are shown with colors corresponding to the respective ensemble RMSD plots.

molecules, idiosyncrasies of the sample preparation, the stochastic nature of the observed process, photobleaching, and/or instrument measurement error (Fig. 2.4 a,b). Despite this variability, traces of moving particles commonly exhibited net displacements between 60 and 140 nm and mean velocities between 1 and 6 nm/min; within error, these values are consistent with track length (90 nm) and deoxyribozyme cleavage rate ($1 \text{ min}^{-1} \text{ leg}^{-1}$), respectively. Tests with and without Zn^{++} and/or TRIGGER, both on substrate and product tracks, yielded RMSD plots of the particle traces that in each case varied as expected based on the behavior of spiders on origami tracks, despite the inherent noise associated with single particle tracking over tens-of-nanometer length scales and tens-of-minute time scales (Fig. 2.4 c,d). For instance, RMSD plots indicated substantially more movement on substrate tracks in the presence of Zn^{++} and TRIGGER than in their individual absence (Fig. 2.4 c). On product tracks, results were consistent with an unbiased random walk independent of Zn^{++} . When product tracks were pre-incubated with TRIGGER 30-60 minutes prior to addition of Zn^{++} and onset of imaging (as were substrate tracks), little or no movement was observed (Fig. 2.4 d), consistent with spiders having been released and moved toward or to the STOP sites prior to imaging. In contrast, when TRIGGER and Zn^{++} were both added shortly prior to imaging, substantial movement was observed (Fig. 2.4 d), consistent with our AFM results for spiders on product tracks (Fig. 2.2 f,g) and with Monte Carlo simulations of spider movement.

The results of our single-molecule experiments are consistent with DNA-based random walkers guided by their landscapes over distances as far as 100 nm, for up to 50 cleavage steps, at speeds of roughly 3 nm/min. We note, however, that the distance over which a spider can move is limited by dissociation and backtracking, so any increase in processivity comes at the cost of a slower velocity [Pei et al., 2006]. Other limitations arise from the mechanism-consuming substrate that must be recharged to sustain directed movement, and from spiders being subject to the stochastic uncertainty as to whether each one can accomplish its task (cf., “faulty” behavior in robotics and “yield” in chemistry). And compared to protein-based walkers using solution phase fuels [Hess, 2006b], our walkers are not as fast, efficient, or powerful. But as candidates for molecular robots, they offer the advantages of programmability [Bath and Turberfield, 2007, Yin et al., 2008, Adleman, 1994, Stojanovic et al., 2003, Seelig et al., 2006], predictable biophysical behaviour [Bath and Turberfield, 2007], and interaction with designable landscapes [Rothemund, 2006].

The ability to obtain programmed behavior from the interaction of simple molecular robots with a complex and adjustable environment suggests that by exploiting stochastic local rules and programming the environment, we can effectively circumvent the limitations that molecular construction places on the complexity of robotic behaviour at the nanoscale.