Published in final edited form as:

Nat Nanotechnol. 2014 January ; 9(1): . doi:10.1038/nnano.2013.230.

Transport and self-organization across different length scales powered by motor proteins and programmed by DNA

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Abstract

In eukaryotic cells, cargo is transported on self-organised networks of microtubule trackways by kinesin and dynein motor proteins^{1,2}. Synthetic microtubule networks have previously been assembled *in vitro*^{3–5} and microtubules have been used as shuttles to carry cargoes on lithographically-defined tracks consisting of surface-bound kinesin motors^{6,7}. Here we show that molecular signals can be used to program both the architecture and the operation of a self-organized transport system based on kinesin and microtubules and spans three orders of magnitude in length scale. A single motor protein - dimeric kinesin 1⁸ - is conjugated to various DNA nanostructures to accomplish different tasks. Instructions encoded into the DNA sequences are used to direct the assembly of a polar array of microtubules and can be used to control the loading, active concentration and unloading of cargo on this track network or to trigger the disassembly of the network.

In vivo, kinesins carry cargoes including organelles, protein complexes and mRNA¹ on a variety of self-organised microtubule structures including radial arrays in the interphase cytoskeleton, meiotic and mitotic spindles and linear tracks in axons and dendrites². Most synthetic transport systems based on kinesin have used an inverted configuration⁹ in which microtubule shuttles 'glide' on kinesin-coated surfaces. Surface patterning allows the creation of kinesin trackways⁷: directional transport can be achieved by asymmetric patterns that rectify microtubule motion⁶. Microtubule shuttles can be loaded with cargo and unloaded at docking stations¹⁰. Such systems have been used to transport analytes between capture and detection regions in 'smart dust' biosensors¹¹. Networks of microtubules, created using micro-patterned channels, have been used as tracks for single kinesin motors¹². Microtubules can also assemble in self-organized patterns: gliding microtubules modified to adhere to each other form spools⁴ and wires¹³, and microtubules cross-linked by motor proteins form asters³ and polarised bundles^{5,14}.

Kinesin-1 is a homodimer: its two catalytic heads coordinate their chemomechanical cycles to walk processively along a microtubule with 8 nm steps. The energy required for directional motion is provided by ATP hydrolysis¹⁵. We use a fusion between kinesin-1 and

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Author Contributions Experiments were designed by AJMW with input from CSC, HMJC, RAC and AJT. Experiments were performed by AJMW. Data was interpreted and the manuscript written by AJMW and AJT with assistance from all other authors.

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a DNA-binding zinc finger protein¹⁶ to couple kinesin motors to DNA nanostructures¹⁷ (Supplementary Method): the zinc finger binds to a specific 9-base-pair sequence of double stranded DNA with nanomolar affinity.¹⁶ By dynamically controlling the configuration of the nanostructure through hybridization and strand-displacement reactions, we are able to use these DNA-kinesin hybrids to control track assembly, disassembly and cargo transport.

DNA was selected as the material of the nanostructures that control the interactions of motors with microtubules and cargo because of its programmability. The assembly dynamics, structures and conformational changes of DNA nanostructures, including the strand-displacement reactions¹⁸ used to unload cargo and disassemble asters, can be controlled through design of the base sequences of component oligonucleotides. Two types of DNA-kinesin hybrids were used (Supplementary Method; Supplementary Fig. 1): shuttles incorporating one kinesin dimer and assemblers incorporating two. Shuttles consist of a single kinesin dimer bound to a DNA nanostructure which also contains a single-stranded domain which may be used for cargo binding or as a signal to control other shuttles. Cargo modified with the complementary adapter strand of DNA can be loaded by hybridization to a cargo-binding domain. A contiguous domain on the cargo strand that remains single-stranded in this complex (a 'toehold') allows the cargo to be unloaded by a toehold-mediated strand-displacement reaction: the DNA signal for cargo release contains a single-stranded domain that is completely complementary to the cargo adapter and displaces it from its shuttle by strand invasion¹⁸. The DNA template for an assembler includes two zinc finger binding sites in a single DNA duplex. The 5' ends of the binding sites are separated by one and a half turns of DNA (16 base pairs) which means that the two attached kinesin dimers are oriented approximately antiparallel¹⁹. This allows them to crosslink and align microtubules: as the two linked kinesin motors walk towards the 'plus' ends of different microtubules, parallel microtubules are bundled together whilst antiparallel microtubules are slid apart³. The collective action of many such assembler teams creates polarized radial arrays of microtubules²⁰, mimicking natural asters²¹ found in the mitotic spindle. Asters provide a platform for directional transport and form the basis of several proposed devices²². The experiments described here were inspired by the melanophore, a radial track array in the cells of certain fish on which motor proteins concentrate or disperse pigment, making the cell translucent or opaque respectively²³. Here, we use active transport to manipulate the spatial distribution of a fluorescent cargo, the cyanine dye Cy3TM.

Assembly of the synthetic astral track network and a cycle of loading, active concentration and release of the cargo are shown in Figure 1. Microtubules and assemblers are mixed in the presence of ATP to form asters (step 1). ATP is removed and cargo-loaded shuttles are added, binding passively to the microtubules in the rigor²⁴ state (step 2). On addition of ATP (step 3) the shuttles walk towards the centre of the aster, actively concentrating the cargo. Finally, (step 4) shuttles carrying the DNA instruction to unload are added. They bind and walk to the centre of the aster, transporting the DNA signals towards the concentrated cargo shuttles. When a shuttle bearing an unloading instruction encounters a shuttle carrying cargo, unloading is initiated: hybridization between the instruction molecule and the cargo adapter displaces both from their respective shuttles, forming a stable duplex and releasing the cargo back into solution (Figure 1).

Experiments were performed in simple flow chambers using passive pumping²⁵ to allow cargo and signals to be added during imaging (Supplementary Methods). The imaging buffer contained an oxygen scavenging system to prevent photobleaching and an ATP regeneration system to maintain saturating ATP concentration²⁶. DNA nanostructures were prepared by cooling stoichiometric combinations of strands from 95 °C to room temperature over ~30 mins. DNA-kinesin hybrids (assemblers and shuttles) were formed by incubating an excess of the kinesin-zinc finger protein with the required DNA nanostructure for ~30 mins at room

temperature (Supplementary Fig. 2). Asters were assembled from a solution containing taxol-stabilized microtubules, assemblers and ATP: they were immobilized on a streptavidin-coated glass cover slip through biotin attached to the assemblers. Asters were observed after ~10 mins (Supplementary Fig. 3) at a density of approximately $10^{-4} \mu\text{m}^{-2}$. Microtubule and aster size distributions are shown in Supplementary Figs 4 and 5: the mean aster radius of gyration was $4 \mu\text{m}$. No aster formation was observed using control assemblers lacking the half-turn twist between zinc finger binding sites or with one mutated binding site (Supplementary Tables 1 and 2, Supplementary Fig. 2).

The system is shown in operation in Figure 2 and Supplementary Video 1 (results of additional experiments are shown in Supplementary Figs 6-8). Figure 2a shows a sequence of dual-colour fluorescence micrographs of an immobilized aster. Cargo-loaded shuttles were added without ATP: after excess cargo was washed away, cargo decorated the aster uniformly through rigor-state binding of the shuttle kinesins. After addition of ATP the cargo was transported to the centre of the aster within ~1min. Shuttles carrying the release signal were then added: having travelled to the centre of the aster they initiated cargo release and dispersal. Figure 2b shows the intensity of cargo fluorescence along a cross section through the centre of the aster: a central peak formed on addition of ATP and dissipated on addition of the unloading signal, consistent with cargo concentration and release. As another metric of cargo distribution, Figure 2c shows the time-dependent skewness²⁷ (Supplementary Note) of the intensity-weighted pixel intensity histogram corresponding to the cargo channel (Supplementary Fig. 9). Positive skewness indicates the formation of a high-intensity tail in the image intensity distribution corresponding to cargo concentration at the centre of the aster. The data indicate progressive cargo concentration following addition of ATP then dispersion triggered by addition of the release signal.

Supplementary Figures 10 and 11 combine data from 44 experiments in which the cargo was actively concentrated then released by either actively or passively transported signals. Averaged over all observations, the skewness of the intensity-weighted pixel intensity histogram increases after addition of ATP with a time constant of approx. 37s (Supplementary Fig. 10). Supplementary Figure 11 shows the effects of subsequent addition of the release signal, which was actively transported in 33 experiments and passively transported in 11. A range of behaviours is observed in both cases, but actively transported release signals are considerably more effective than signals not attached to shuttles: on average skewness decreases (with a time constant of approx. 32s) after addition of an actively transported release signal but remains approximately constant in the case of passive transport. In both cases, the signal is rapidly delivered to all accessible parts of the aster by flow: the reduced efficiency of passive delivery is consistent with slow transport of the signal, possibly associated with local depletion of its solution concentration, near the centre of the aster where the cargo is concentrated. Single-molecule experiments (Supplementary Fig. 12 and Supplementary Methods) show that the average shuttle velocity on isolated microtubules is $0.6 \pm 0.2 \mu\text{ms}^{-1}$ (shuttles may be slowed down by crowding²⁸ near the centre of an aster). The mean radius of gyration of asters used in these transport experiments was $10 \mu\text{m}$ (Supplementary Fig. 13), so a shuttle can traverse an aster in approx. 20s, consistent with the average timescale of active concentration and release (Supplementary Figs 10 and 11). Diffusive transport of the free signal strand over the same distance in water is significantly faster²⁹, taking of the order of 1s (the crossover between active and passive transport times would occur for structures an order of magnitude larger). We attribute the relative ineffectiveness of passive delivery, and the aster-to-aster variation shown in Supplementary Fig. 11, to a reduction in signal diffusion caused by the dense packing of microtubules and assemblers at the aster centre³. Supplementary Figure 14 shows two successive cycles of cargo concentration and release: the second cycle is less effective than

the first, which may also be a result of the progressive accumulation of kinesin motors near the centre of the aster.

Control experiments were performed to test the release mechanism. In the absence of ATP, shuttles carrying the release strand are immobilized on the aster and have no significant effect on the concentrated cargo (Supplementary Fig. 15). Shuttles carrying ‘dummy’ DNA signals are also ineffective, even in the presence of ATP (Supplementary Fig. 16).

Actively transported DNA signals can also trigger aster disassembly. Figure 3a shows a redesigned assembler in which the link between the two dimeric motors, and hence the cross-links at the centre of the aster that hold it together, can be broken by a second DNA signal (Fig. 3b). Figure 3c shows micrographs of such an aster and its dissolution in response to the disassembly signal, actively transported by shuttle to its centre (Supplementary Video 2; see also Supplementary Fig. 17). The time course of disassembly is also displayed by plotting the radius of gyration of the aster (Supplementary Method) as a function of time (Fig. 3d). In control experiments, actively transported ‘dummy’ signals did not cause disassembly. As in the case of cargo release, passive delivery of the signal was less effective than active delivery (Supplementary Fig. 18).

We have demonstrated an integrated transport system in which a single type of molecular motor is harnessed and controlled by reconfigurable DNA nanostructures to perform a wide range of tasks: creation of an ordered, self-organized track array; capture and concentration of a molecular cargo; transport of signals that lead to cargo release or track disassembly. In each of these, molecular complexes that are a few nanometres in dimension control structure and information flow over tens of micrometres. These experiments demonstrate that, as in eukaryotic cells, active molecular transport can be controlled to perform a wide range of self-organization reactions, spanning many length scales, in synthetic systems. These reactions include the active concentration of components which could be used to create colour changes^{22,23} and to speed up reactions at low concentration or with high activation barriers³⁰. DNA-control of protein motors provides powerful tools for the control of self-organized structure and function.

Supplementary Material

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Acknowledgments

We thank J. Yajima (Department of Life Sciences, Graduate School of Arts and Sciences, University of Tokyo) for protein design and plasmid production and M. Alonso, D. R. Drummond and M. Katsuki (Centre for Mechanochemical Cell Biology, Warwick University Medical School) for assistance with protein production and purification. This research was supported by EPSRC grant EP/G037930/1, BBSRC grant BB/G019118/1 and a Royal Society-Wolfson Research Merit Award (AJT).

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Figure 1. The self-organized transport system

1. DNA-templated kinesin teams organise MTs into radial asters. 2. Shuttles carrying fluorescent cargo decorate the aster uniformly in the absence of ATP. 3. ATP fuels cargo concentration. 4. A DNA-encoded signal is actively transported to the centre of the aster to release the cargo.

Figure 2. Operation of the transport system

a: Fluorescent micrographs showing the transport cycle: **i**, before addition of cargo; **ii**, after cargo + wash with no ATP; **iii**, just after ATP addition; **iv**, ~80s after ATP addition; **v**, just after release signal addition; **vi**, ~180s after release signal. Intensities are scaled consistently in all images. Hylite-labelled microtubules are shown in red; cargo (Cy3) is green. Scale bar 10μm. Times in seconds are indicated **b:** Intensity distributions along a cross section through the centre of the aster (shown as a white line in **a(iii)**) **Left** after ATP addition; **Right** after release strand addition. **c:** Skewness of the intensity-weighted pixel intensity histogram for the cargo fluorescence channel.

Figure 3. Controlled aster disassembly

a: Structures and interactions of the assembler and of the shuttle carrying the disassembly signal. The assembler has two sections, each containing a zinc-finger binding site and a single stranded overhang (blue). These sections are linked by a complementary strand (orange). An additional toe-hold in this strand allows it to be stripped off by the signal strand (orange). The biotin anchorage on the assembler is indicated by a yellow circle. **b:** aster disassembly. **c:** fluorescent micrographs before and after disassembly signal added (red marker). Scale bar $10\mu\text{M}$. Time in seconds indicated. **d.** Time-dependent radius of gyration of the aster (Supplementary Methods) which increases rapidly as the aster is destroyed.