Supplementary material for

Evaluating the effect of two-dimensional molecular layout on DNA origami-based transporter

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Supplementary Discussion

We evaluated the 2D layout effect on two key parameters of the transporter (velocity and run length) using the DNA origami platform with a defined number and layout of motor molecules. We found that the layout had a moderate effect on the velocity, while the densely packed layout decreased the run length (when comparing transporters with the same motor number).

For the velocity, please see the main text.

In the present study, in contrast to velocity, molecular layout affected the run length under these conditions, and we observed short run lengths with densely packed layouts (when only comparing transporters with the same motor number. We note that the run length was also affected by the ionic strength (NaCl concentration, Fig. 3 and Supplementary Fig. 21), suggesting that the electrostatic interaction is also important for transporter activity [1, 2]). Regarding the motile mechanism, there are two key parameters (on- and off-rates of motor, [3, 4]). Currently, the reason is unknown, but we speculate that steric hindrance might affect both on- and off-rates. For on-rate, the searching area of a tethered motor increases with an increase in the intermolecular distance (D) between motors. Meanwhile, the effective tubulin concentration decreases with increasing D, as volume scales with D^3 while the number of tubulin subunits scales with D ([4], Supplementary Fig. 27). Although the local tubulin concentration is higher for a layout with short intermolecular distance, we hypothesize that steric hindrance and a rigid/huge scaffold (DNA origami) might cancel the high local tubulin concentration, resulting in a short run length. For example, the rotational degree of freedom in the DNA origami transporter along the longitudinal axis would be much lower than that of a single dsDNA-based transporter in the literature, reducing the freedom of kinesin

molecules in a three-dimensional (3D) search (from the literature using a similar structure (gold nano-particles) to estimate the diffusion constants of DNA origami, translational and rotational diffusion constants for DNA origami would be 6 $\mu m^2/s$ and 2/ms, while these of 20–60bp dsDNA are 20-100 $\mu m^2/s$ and 10^2-10^4 /ms, respectively) [5–7]. In this scenario, the steric hindrance effect is much greater in the DNA origami-based transporter than in the dsDNA-based previous system in the literature, decreasing the effective on-rate of tethered kinesin molecules for a densely packed layout. Furthermore, we speculate that the load-independent off-rate for densely packed layouts may be high, as the presence of nearby kinesin may affect the rebinding of the tethered head at a single-head bound state, resulting in the higher off-rate for densely packed layouts.

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