Supporting Information for:

Herding nanotransporters: Localized activation via release and sequestration of control molecules

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Analytical solution: The analytical solution for the steady state ATP concentration was obtained under the assumptions that ATP concentration inside the illumination zone is constant, and the consumption of ATP by kinesin is negligible compared to that by hexokinase. For steady state diffusion and sequestration of ATP outside the illumination zone we can write (assuming cylindrical symmetry):

$$D(\frac{\partial^2 C}{\partial r^2}+\frac{1}{r}\frac{\partial C}{\partial r})-\frac{A}{K_m}C=0 \qquad (1)$$

where D is the diffusion constant of ATP, A is the activity of hexokinase and K_m is the hexokinase Michaelis constant for ATP. This expression can be rewritten as:

$$r^2\frac{\partial^2 C}{\partial r^2}+r\frac{\partial C}{\partial r}-\frac{r^2}{r*^2}C=0 \qquad (2)$$

where $r*^2 = K_m/DA$. This is the modified Bessel equation for which one of the solutions is given by [17]:

$$C=aK_0(\frac{r}{r*}) \qquad (3)$$

where K_0 is the modified Bessel function of the second order and a is an integration constant. At the boundary of the illumination zone r_i, the concentration $C(r_i)$ is equal to concentration C_0 inside the illumination zone. Thus:

$$C=\frac{C_0}{K_0(\frac{r_i}{r*})}K_0(\frac{r}{r*}) \qquad (4)$$

$K_0(x)$ can be approximated as (1/x)*exp(-x). Accordingly, eq. (4) reduces to:

$$C = C_0\sqrt{\frac{r_i}{r}}\exp[\frac{(r_i - r)}{r*}] \qquad (5)$$

For the calculation of C_0, we equate the ATP generation in he illumination zone to the sequestration in the zone and the ATP diffusion out of the zone at steady state:

$$J_{ATP}\big|_{r_i} \cdot 2\pi r_i + \frac{A}{K_m} C \cdot \pi r_i^2 = G \cdot \pi r_i^2 \qquad (6)$$

From eq. (5), we can calculate the flux J_{ATP} going out of the illumination zone:

$$J_{ATP} = -D\frac{\partial C}{\partial r}$$

which gives

$$J_{ATP} = DC(\frac{1}{r*} + \frac{1}{2r}) \qquad (7)$$

Inserting eq. (7) into eq. (6):

$$DC_0(\frac{1}{r*} + \frac{1}{2r_i}) \cdot 2\pi r_i + \frac{b}{K_m} C_0 \cdot \pi r_i^2 = G \cdot \pi r_i^2$$

Rearranging, substituting r* and solving for C_0,

$$C_0 = \frac{G}{D(\frac{1}{r*} + \frac{1}{r_i})^2} \qquad (8)$$

Defining quality of control Q as the ratio of the maximum ATP concentration (C_0) to the characteristic ATP diffusion outside the illumination zone ($r_i + r*$), control for a given radius of illumination zone r_i can be found to be maximized for $r* = 2r_i$ by differentiating

$$Q = \frac{G}{D(\frac{1}{r_i} + \frac{1}{r*})^2 (r_i + r*)} \qquad (9)$$

with respect to r* and equating it to 0.

Fuel efficiency: The fuel efficiency is the ratio of the ATP used by kinesin motors (given by $N_{motor}v_{avg}/d$ with N_{motors} – number of microtubule-bound motors in the activation volume, s – step length of kinesin) to the total amount of ATP released (given by $C_{cATP}kI_{hv}V_{illum}$). If the activation zone is defined as extending to $r_{10\%}$, under optimum conditions for control ($r^* \sim r_1$, $r_{10\%} \sim 3r_1$) the average velocity of the microtubules in this activation region is found to be $v_{avg} \approx v_0/2$. Utilizing eq. (8) and $v_0 = v_{max}^{kin} C_0 / K_m^{kin}$, $C_{motors} = N_{motors}/V_{activation}$,, $G = C_{cATP} \times k \times I_{hv}$ and $V_{activation} = 9V_{illum}$ gives the expression of fuel efficiency in terms of the illumination radius for optimum quality conditions as

$$\eta = C_{motors} \frac{v_{\max}^{kin}}{s \times K_m^{kin}} \cdot \frac{2r_i^2}{D_{ATP}} \quad (10)$$

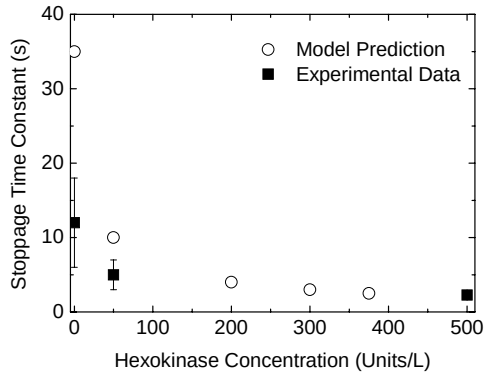
Thus, fuel efficiency decreases with the size of the illumination region.

Diffusion-limited uncaging of ATP at high light intensities. The diffusion-limited generation term G is given by $G \approx 2D_{cATP}C_{cATP}/(r_i^2 \ln(R/r_i))$, where R is the distance from the center of illumination to the edge of the flow cell[47]. Assuming that the diffusion constants of caged-ATP and ATP are similar, calculations for the diffusion-limited case (high light intensity) yield:

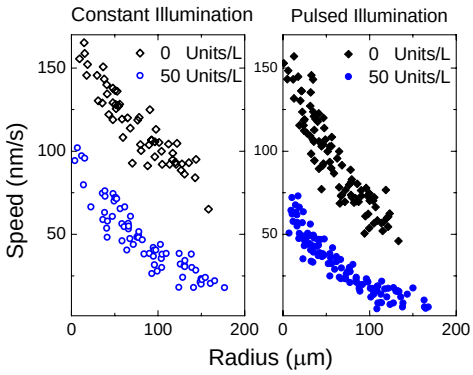
$$C_0 = \frac{2C_{cATP}}{(\frac{r_i}{r^*}+1)^2 \ln(\frac{R}{r_i})} \quad (11)$$

Under these conditions, the gradient $C_0/r_{10\%}$ is still maximized when r* is approximately twice r_i but increases in proportion to $1/(r_i * \ln(R/r_i))$. In order to photolyze nearly every caged ATP molecule entering the illumination zone, the illumination intensity has to be increased to more than $I = 4D/kr_i^2$ (3 W/cm^2 for r_i=15 μm). However, since we observe UV photodamage of microtubules on the timescale of

hours for the currently employed intensity of 2 mW/cm^2, a thousand-fold increased intensity may lead to an unacceptable level of UV-related degradation.



Supplementary Figure 1: Exponential decay of modeled and experimental stoppage time. After the UV source was turned off the velocity of microtubules drops exponentially. Here, the time constant of the drop-off from numerical modeling and experiments are plotted as function of the hexokinase concentration for an illumination zone with a 15 μm radius. As the hexokinase concentration increases, the time to stop the shuttles decreases (measured by the time to reach v_0 x 1/ e). All illumination zone sizes showed an exponential decrease of velocity with almost identical time constants.



Supplementary Figure 2: Comparison of constant and pulsed illumination. Shown are speed vs. radius (measured from center of pinhole) plots for constant (left) and pulsed (right) illumination. Both illumination methods resulted in similar maximum speeds and speed distributions.

Supplementary Movie 1: Localized illumination without sequestration. This movie shows microtubules gliding over a kinesin coated glass surface, fueled by they hydrolysis of ATP. The cylindrical zone of ultraviolet light releases the caged-ATP which rapidly diffuses throughout the flowcell. Microtubules closer to the illumination zone move at a higher velocity than those at the periphery of the field of view, as seen in Figure 3. This movie is accelerated 250 times.

Supplementary Movie 2: Localized illumination with sequestration. This movie shows microtubules gliding over a kinesin coated glass surface in a solution also containing hexokinase, an ATP hydrolyzing enzyme. Due to the sequestration of ATP by hexokinase the activation zone (the area in which microtubules move) is restricted to the area immediately surrounding the illumination zone. This movie is accelerated 8,000 times.