Supplementary Material

for

Uptake and transfection efficiency of PEGylated cationic liposome–DNA complexes with and without RGD-tagging

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Intracellular Motion of CL–DNA NPs

For single particle tracking studies of intracellular motion, live cells were incubated with fluorescently labeled RGD-tagged CL–DNA NPs at high membrane charge density and imaged 2 h after incubation (Fig. S1). For analysis of active transport, cells were transfected with GFP-tubulin before incubation with the CL–DNA NPs, allowing visualization of their cytoskeletal microtubule network. Particle tracking was performed with a custom routine in Matlab that was written using algorithms from [1].

Imaging and analysis of particle trajectories revealed two types of non-Brownian motion, both of which are indicative of endosomal entrapment of particles.

Evidence of active transport

Fig. S1 A,B shows still frames and the trajectory for an example of an intracellular particle undergoing active transport. The particle moved rapidly along a trajectory which is colocalized with GFP-labeled microtubules. Additional evidence for active transport comes from the double-logarithmic plot of mean square displacement against time (Fig. S1E): the observed slope (the exponent α in $<\Delta R^2>=(D\ \delta t)^{\alpha}$) is much larger than 1, the value expected for Brownian motion. Motor protein-mediated transport along microtubules is facilitated through specific interactions, suggesting that CL–DNA NPs undergoing active transport are enclosed within intact endosomes.

Evidence of confined diffusion

Fig. S1 C,D shows an example of an intracellular particle undergoing confined diffusion. The still frames in Fig. S1D show a small bright particle that is moving within a diffusely fluorescent circular area with R≈1 μm. The bright fluorescent particle's trajectory remains within this diffuse fluorescent spot for the entirety of its motion (Fig. S1C). The low value for the exponent α (Fig. S1E; see above for definition of α) determined for the particle's motion is consistent with this observation, implying that the particle is confined within a finite volume. The boundary of the volume is labeled with fluorescent lipid (thus the diffuse fluorescence), suggesting that the particle is enclosed within a membrane-bound organelle and not the molecularly crowded cytoplasm or mesh-like cytoskeletal network. It is likely that collision of the CL–DNA complex with the confining endosomal membrane results in lipid exchange and thus fluorescent labeling of the endosomal membrane.

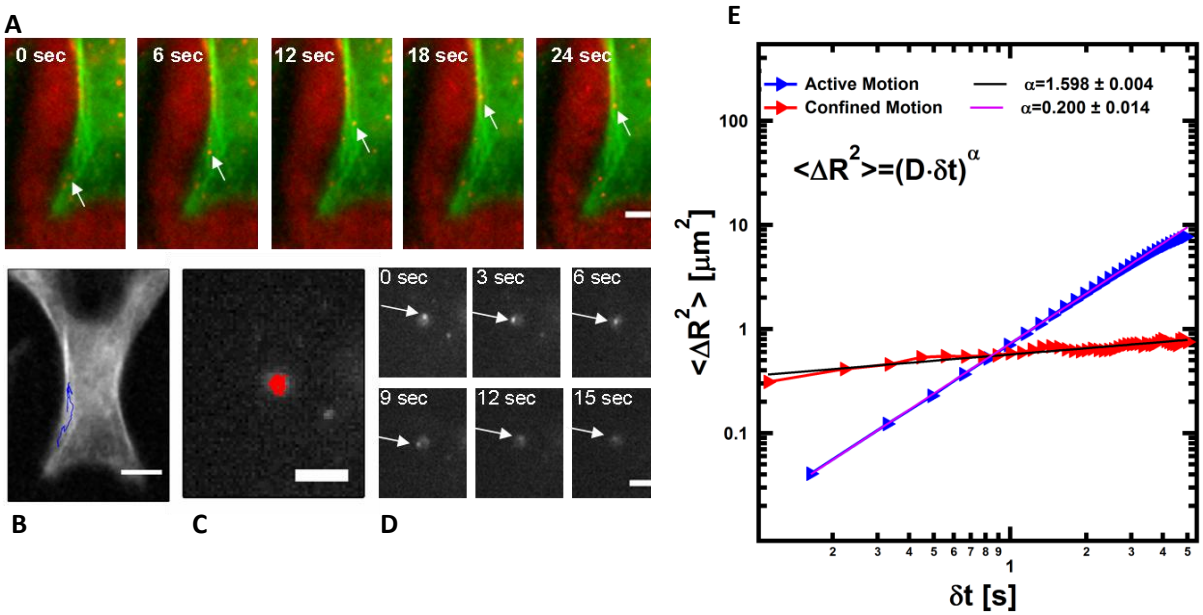

Fig. S1. Intracellular motion of RGD-tagged CL–DNA nanoparticles (DOTAP/DOPC/RGD-PEG2K-lipid = 80/10/10 mol/mol/mol). (A) Still frames showing fluorescently labeled CL–DNA nanoparticles within a GFP–tubulin expressing fibroblast. The white arrow marks a nanoparticle undergoing active transport. (B) The trajectory of the particle, as determined by particle tracking, shows colocalization with GFP-labeled microtubules. (C, D) Trajectory and still frames for a fluorescently labeled nanoparticle undergoing confined Brownian motion. The particle is confined within a diffusely fluorescent spot as evident from the colocalization of the particle's trajectory and the diffuse spot (C). (E) Log-log plots of mean square displacement against time for the particles from parts (A) and (C). In both cases, the slope (α) deviates strongly from α=1, the value expected for Brownian motion.

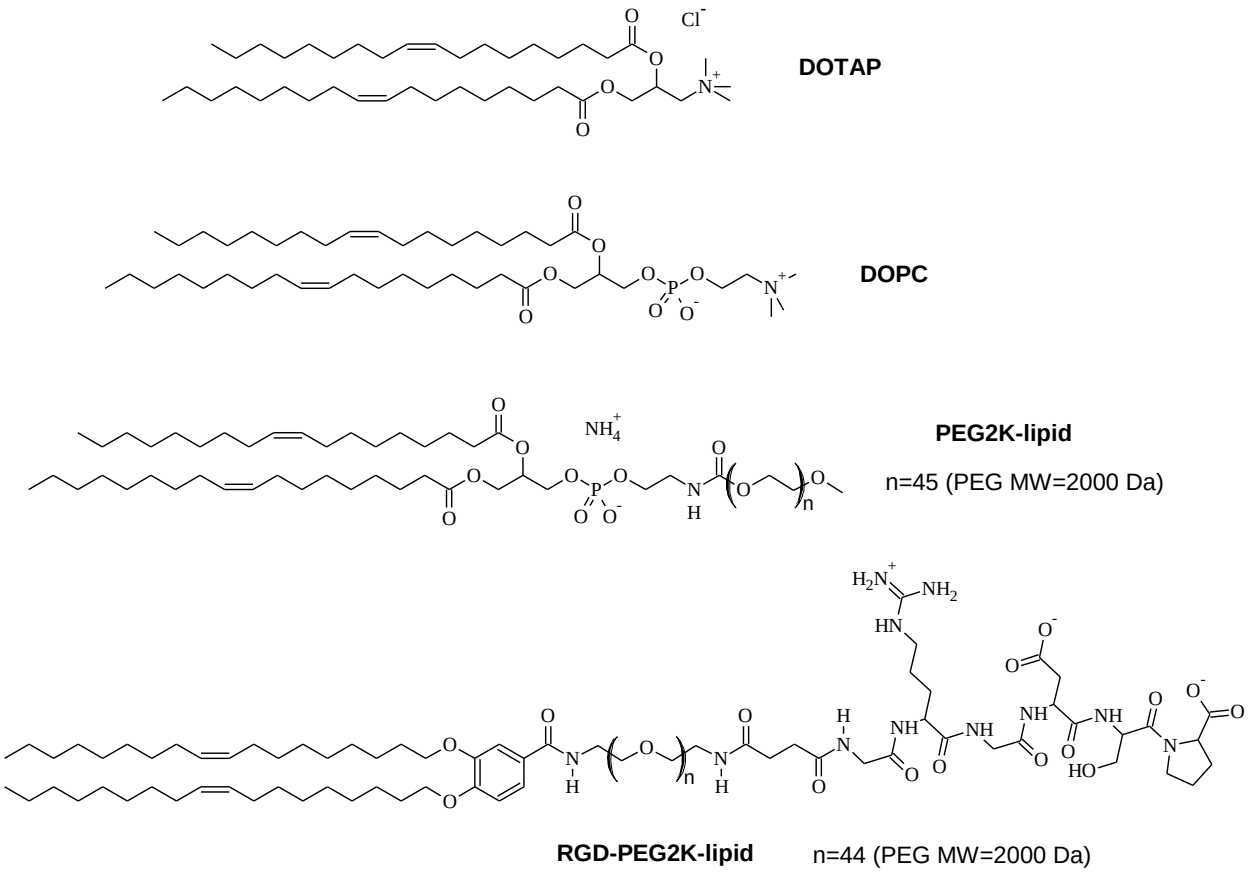

Fig. S2. Chemical structures of the lipids used in this work.

References

1 Crocker JC, Grier DG. Methods of Digital Video Microscopy for Colloidal Studies. J Colloid Interface Sci 1996;179:298–310.