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A model system to study transport of self-assembled cargos

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Intracellular transport is the process by which cellular cargos, such as organelles and proteins, are moved throughout the cell. Motor proteins bind these cargos and walk along microtubule tracks to deliver them to specific regions of the cell. In axons, cargos are transported by either fast or slow axonal transport. Fast axonal transport is performed by fixed teams of motors bound to membranous cargos, whereas slow axonal transport is thought to be performed by motors that transiently self-assemble with cargos, assembling and disassembling throughout transport. While recent studies have begun to shed light on the nature of slow axonal transport, there are many open questions about the mechanism of action for transient motor association, and how they could result in effective, yet slow, long-range transport. Here, we describe an in vitro system to study self-assembled cargos using quantum dots (Qdots) as artificial cargos. In this system, kinesin motors are able to form transient interactions with Qdot cargos, allowing for the study of self-assembled cargos that assemble and disassemble during transport. Using this system, we can begin to probe the effects of self-assembly on cargo transport properties.

Keywords: microtubule, kinesin-1, intracellular transport, slow axonal transport, quantum dots

Abbreviations: Qdot, quantum dot; TIRF, total internal reflection fluorescence

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Intracellular transport is imperative for the survival of all cell types. This process allows organelles, proteins and other cellular cargos to be transported within the cell in a more efficient manner compared with diffusion-based transport.[1] This efficient transport process is especially important in the case of neurons.[2] These cells are comprised of a cell body, where the majority of all new cell material is synthesized, and an extended axon that functions to transmit signals to neighboring cells. In order to properly maintain these long processes, newly synthesized material must be transported from the cell body to the end of the axon and material must also be recycled back to the cell body.[3]

Intracellular transport relies on microtubules and motor proteins. Microtubules are cytoskeletal filaments that possess an inherent structural polarity, having a plus and minus end. In the case of axons, microtubule plus ends are oriented away from the cell body and point toward the axon terminus. Kinesin-1 is a motor protein that walks along microtubule tracks toward the plus end and is important in transporting newly synthesized material from the cell body into the axon.

In axons, there are two types of intracellular transport: fast and slow axonal transport. Fast axonal transport involves the transport of membranous organelles at approximately 100–400 mm/day.[4] Cytosolic proteins are transported by slow axonal transport, which is characterized by periods of fast transport interrupted by extended pauses that slow the overall rate of transport to approximately 0.2–8 mm/day.[4] Unlike fast axonal transport, which is achieved by fixed teams of motors continuously bound to membranous cargos, cytosolic proteins are thought to form transient complexes with microtubule motors that self-assemble and disassemble throughout transport.[5] While fast axonal transport has been well studied, further research is necessary to fully elucidate the mechanism that underlies slow axonal transport.

We have developed a novel in vitro system to study the transport of

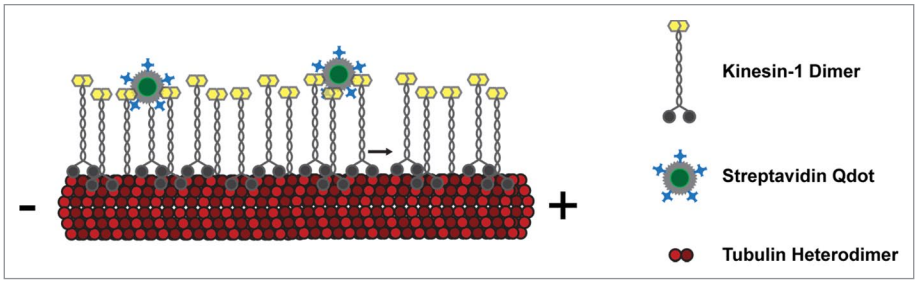

Figure 1. Representation of assay set-up with Qdot-kinesin added onto microtubules with high densities of excess non-biotinylated motors. Arrow indicates the direction in which kinesin motors walk along the microtubule. Plus and minus signs denote the polarity of the microtubule filament.

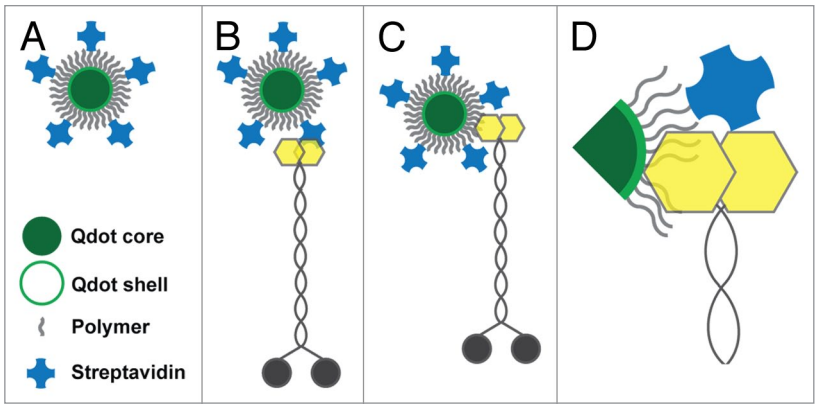

Figure 2. Potential mechanisms by which non-biotinylated kinesin-1 could non-specifically associate with Qdots. (**A**) Representation of streptavidin conjugated Qdots used in this study. (**B**) Kinesin-1 could bind Qdot via streptavidin molecule. (**C**) Kinesin-1 could bind Qdot via polymer coating. (**D**) Kinesin-1 could bind Qdot by getting lodged into splayed polymer coating.

self-assembled cargos, similar to those that could be utilized in slow axonal transport. We create quantum dot (Qdot) labeled kinesin motors by specifically linking a single Halo tagged kinesin motor to a streptavidin-conjugated Qdot via a biotin ligand. These Qdots act as artificial cargos in our assay and are also used to track cargo motion during transport using total internal reflection fluorescence (TIRF) microscopy. Qdot-tagged kinesins are added onto microtubules with different densities of non-biotinylated kinesin motors that are able to transiently bind Qdot cargos, allowing for the study of self-assembled cargos (**Fig. 1**).

The binding of non-biotinylated motors to Qdot cargos is through a non-specific interaction. Using two-color imaging to observe Qdot motility and individual GFP-tagged kinesin motors in these assays, we were able to visualize events where: (1) GFP-kinesin associates with a Qdot already moving along the microtubule; (2) GFP-kinesin dissociates from a Qdot moving along the microtubule; (3) GFP-kinesin bound to a Qdot dissociates simultaneously with the Qdot; (4) Qdot and GFP-kinesin bind the microtubule simultaneously; and (5) Qdot binds a GFP-kinesin already bound to the microtubule.[6] Thus, the experimental system we developed can be used to gain further insight into the effects of transient assemblies of motors, which may be closer to the reality with cells and could inform on the mechanisms of axonal transport.

How non-biotinylated motors non-specifically associate with Qdot cargos is unclear. The Qdot streptavidin conjugates used in this study are comprised of a semiconductor core and shell that is coated with a polymer to attach streptavidin molecules. Three potential mechanisms by which motors could bind non-specifically to Qdots are: (1) motors could bind non-specifically to streptavidin molecules, but this would be limited to the small number of streptavidin molecules on the Qdots; (2) motors could bind non-specifically to exposed polymer; or (3) motors could get caught in the polymer coating if the brush is splayed because of the high radius of curvature of the Qdot (**Fig. 2**).

Interestingly, bulk assays where only Qdots and non-biotinylated motors are mixed in solution do not show detectable binding events between Qdots and motors, suggesting that binding in solution, in the absence of microtubules, is rare. This is in contrast to our in vitro system, where binding events are observed frequently. This discrepancy can be explained when both the energy and local concentration of the species involved are considered.

Free in solution, kinesin motors and Qdots move purely by diffusion and have energies that are on the same order of magnitude, ~1 k_BT. Since we observe little binding between Qdots and non-biotinylated motors in solution, we conclude that such low energies are smaller than the energy barrier required for kinesin-Qdot binding. The result is that the probability that an interaction event between a kinesin motor and a Qdot would result in a non-specific binding event is low in free solution. However, in our assay, there are two parameters that lower the energy barrier required for Qdot-kinesin binding. First, in our assay, kinesin motors have a higher energy than in solution, as they generate force as they walk along microtubules. Kinesin motors produce a maximum of ~5 pN of force for every 8 nm step they take along the microtubule.[7,8] Thus, we can estimate that in our assay, kinesin motors can do maximal work on the order of ~10 k_BT. Given that we observe collisions between a non-biotinylated motor walking behind or in front of a Qdot to

result in a high probability of binding, we conclude that the energy barrier for such binding is below 10 k_BT. The energy barrier of insertion of a particle into a polymer brush is on the order of 10 k_BT,[9] so the kinesin motor is likely to have enough energy to penetrate the brush. After intercalating into the brush, the motors might be kept there through van der Waals forces, but we were still able to observe motors dissociating from the Qdots,[6] implying that the 10 k_BT of energy is also enough to escape the brush.

Another factor in our system that could lower the energy barrier required for Qdot-kinesin binding is the local concentration of each species. An increased concentration of either species will drive the binding reaction forward, resulting in the production of more Qdot-kinesin complexes. In our system, microtubules are attached to a glass surface and kinesin motors bind these microtubules with high affinity (K_d = 60 nM). This generates a high local concentration of motors and Qdots at the microtubule surface, favoring their interaction.

We show that in solution, the energy barrier for non-specific binding of kinesin motors to artificial Qdot cargos is high, resulting in few non-specific interactions. In our assay, this energy barrier is lowered due to the high local concentration of kinesin at the microtubule surface and the ability of kinesin to do work. The possibility for kinesin to be able to penetrate the brush is supported by the ability of kinesin to do more than enough work to overcome the energy barrier to non-specifically bind Qdots as it walks along microtubules. Although we do not know for such if this is the mechanism, it is an interesting possibility. The non-specific interactions with artificial Qdot cargos allow the formation of self-assembled cargos. This novel system can be used to model the transport of self-assembled cargos that are thought to be the basis of slow axonal transport. Using this system, we show that these self-assembled cargos move at slow velocities, but are transported over long distances compared with single motors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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