

# A Thermostable Kinesin Motor Protein for Hybrid Nanoscale Systems

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**Motivation**—Living organisms assemble, organize, and disassemble nanoscale materials through the active consumption of chemical energy sources. Such energy-driven processes enable these materials to change composition, structure, and morphology in response to environmental conditions and stimuli. Our overall goal is to understand and to exploit key strategies used by living systems to develop new nanocomposite materials whose assembly and configuration can be programmed or “self-regulated” in artificial environments. To this end, we have isolated and characterized a thermostable kinesin motor protein that plays a central role in trafficking nano- and molecular scale materials within cells.

**Accomplishment**—A key hurdle to integrating active biomolecules into hybrid nanoscale systems centers on the ability to interface intrinsically diverse components. Fortunately, Nature provides an extraordinary array of organisms and molecular machines that have evolved to function under a wide range of conditions. The thermophilic, deuteromycetous fungus *Thermomyces lanuginosus* was selected for our work based on its high temperature growth optimum, and anticipated robustness in artificial environments. A gene encoding a kinesin motor protein was isolated using reverse transcription and the polymerase chain reaction to amplify the sequence from purified mRNA. The *Thermomyces* kinesin (*TKIN*) gene was then cloned into a bacterial expression plasmid, and engineered to introduce a 10x Histidine tag into the motor protein. The recombinant *TKIN* was expressed in the Tuner (DE3) pLacI strain of *Escherichia coli*, and purified from lysates using Ni-NTA chelate chromatography.

The temperature-dependent biochemical and biophysical properties of the recombinant *TKIN*

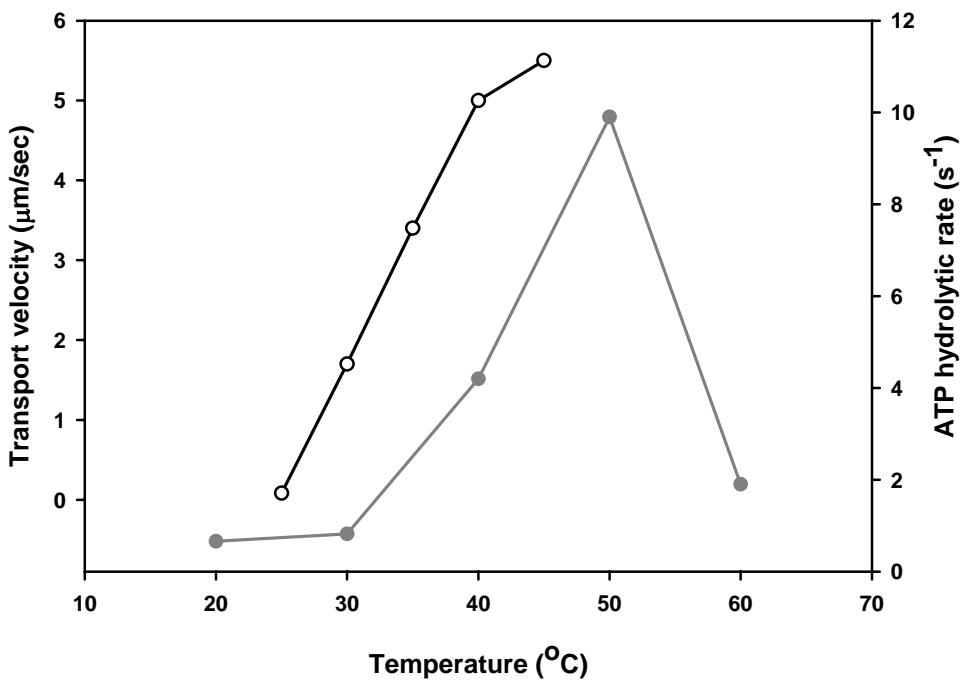
were characterized with respect to use in synthetic architectures. DNA analysis of the coding sequence indicated that the *TKIN* is a member of the Unc104/KIF1A subfamily of kinesins. Size exclusion chromatography and gel electrophoresis confirmed that the *TKIN* exists as a functional monomer, compared to the conventional dimeric kinesin motor protein. The rate of ATP hydrolysis displayed a clear temperature dependency, with an apparent maximum at 50°C followed by a rapid decline due to microtubule depolymerization (Fig. 1). The rate of microtubule transport in gliding motility assays displayed a maximum at ~45°C (Fig. 1). The transport rate increased from 1.5 μm/sec at 25°C, to a maximum of 5.5 μm /sec at 45°C, which is substantially greater than any other characterized kinesin. Directional gliding motility assays indicated that *TKIN* is a plus-end directed motor protein, which is consistent with other members of this kinesin subfamily. We then evaluated the long-term performance of *TKIN* by monitoring changes in ATP hydrolytic rates over an extended period of time. No change in the hydrolytic rates was observed over this period, suggesting that this kinesin is capable of functioning at maximum performance for more than 11 days at room temperature (Fig. 2), which has not been previously demonstrated for any kinesin.

**Significance**—The ability to actively transport nanoscale cargo is a requisite first step for developing dynamic nanocomposite materials. We have isolated, engineered, and characterized a thermostable kinesin motor protein that possesses the necessary characteristics for integration at synthetic interfaces. Our detailed analysis and understanding provides a critical framework for defining the operational range in which *TKIN* can be used to assemble and organize nanocomposite materials.

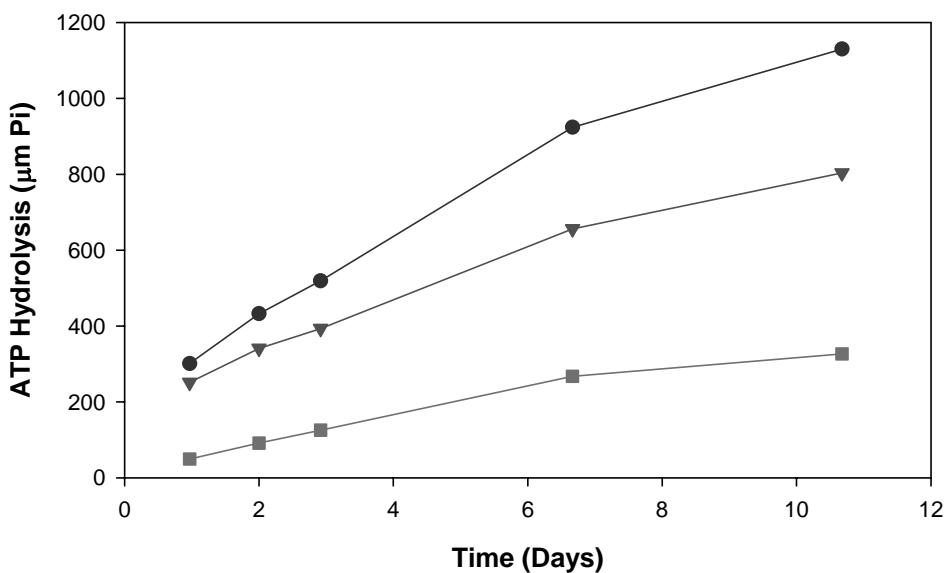
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**Figure 1.** Temperature-dependent effects on the transport rate (-○-) and ATP hydrolysis (-●-) observed for *Thermomyces* kinesin. Both the transport and hydrolytic rates display an apparent peak at  $45 - 50^{\circ}\text{C}$ , which is consistent to the temperature optimum for this fungus.



**Figure 2.** Rate of ATP hydrolysis of *Thermomyces* kinesin over 11 days at room temperature. The corrected rate of kinesin hydrolysis (-●-) was determined by subtracting the background rate of ATP hydrolysis (-▼-) from the total rate of ATP hydrolysis (-■-).