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Enhanced stability of kinesin-1 as a function of temperature

K. Chase [a], F. Doval [b], M. Vershinin [b, c, *]

[a] Department of Physics, Oregon State University, 301 Weniger Hall, Corvallis, OR 97331-6507, United States
[b] Department of Physics and Astronomy, University of Utah, 115 South 1400 East, Salt Lake City, UT 84112-0830, United States
[c] Center for Cell and Genome Science, University of Utah, 257 South 1400 East, Rm. 201, Salt Lake City, UT 84112-0840, United States

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ABSTRACT

Kinesin-1 is a mechanochemical enzyme which mediates long distance intracellular cargo transport along microtubules in a wide variety of eukaryotic cells. Kinesin is also relatively easy to purify and shows robust function in vitro, leading to numerous proposals for using the kinesin-1/microtubule system for nanoscale transport in engineered devices. However, kinesin in vitro shows signs of degradation at ~30 °C which severely limits its usability in biomimetic engineering. Notably, kinesin-1 functions robustly in animal cells at body temperatures as high as 40 °C which suggests that kinesin functioning can be stabilized beyond what is observed in vitro. The present study investigated the effect of trimethylamine N-oxide (TMAO) as a potential heat-protecting agent for kinesin function and microtubule stability. We show that at a concentration of 200 mM, TMAO can indeed stabilize kinesin-based motility up to a little over 50 °C and that such motility can be sustained for extended periods of time. Our results suggest that intracellular crowding (mimicked in vitro by TMAO) can indeed stabilize kinesin-1 at high temperatures and helps resolve a long standing discrepancy between thermal stability of kinesin-1 observed in vivo and in vitro. Moreover, when considered together with our previous report that kinesin-1 can function well down to near-freezing conditions, this study establishes kinesin-1/microtubule motility as a thermally viable engineering platform.

Kinesin-1 motors are processive mechanochemical enzymes whose prototypical intracellular function consists of transporting cargos along the microtubule cytoskeleton. Individual motors significant but limited processivity [1–3], while teams of kinesin-1 motors attached to the same cargo can jointly achieve far longer travel [2]. Moreover, wild-type full-length motors cooperate well enough that unloaded velocity for small motor teams shows no significant decline from single motor value [1,4]. Such robust nanoscale transport has strong potential for biomimetic engineering (e.g. [5–7]). Some of the key design issues facing such applications include cargo packaging, routing, storage and disposal/recycling which are all ripe for biomimetic and de novo solutions.

An important issue that any engineered system has to address is stability with respect to the expected operating temperature extremes. Kinesin-1 motility is currently very limited in this respect: it shows signs of degradation at approximately 30 °C [8,9]. However, there are several a priori reasons to expect that this is not the inherent limit of the system. Kinesin-1 has been shown via temperature-pulse microscopy to be fully functional up to 50 °C for very short time intervals [10]. Non-processive kinesin TKIN has been shown to function up to at least 45 °C in vitro [11]. TKIN is a non-processive motor which limits its use to microtubule gliding and its engineering applications are further limited due to motor aggregation. In summary, even though there are practical limitations which make kinesin-based transport difficult to adapt for practical uses, it is clear that the microtubule/kinesin system can be functional in a wide temperature range in vitro.

Kinesin-based transport is remarkably more stable in vivo

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* Corresponding author. Department of Physics and Astronomy, University of Utah, 115 South 1400 East, Salt Lake City, UT 84112-0830, United States.
E-mail address: Vershinin@physics.utah.edu (M. Vershinin).

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than in vitro. Thermophilic eukaryotes can maintain microtubule-based transport up to at least 60 °C [12]. However, even mammalian kinesin-1, which is so often used to study in vitro motility, maintains stable motile function at animal body temperature (up to at least 40 °C). Our study was therefore motivated by two questions: Why does the thermal range of mammalian kinesin-1 extend 10 °C higher in vivo than in vitro? And whatever this mechanism is, could it be used in vitro to enhance engineered nanoscale transport?

We hypothesized that kinesin function in cells is stabilized at elevated temperatures by molecular crowding. To test this hypothesis, we have measured kinesin-1 motility in the presence of trimethylamine-N-oxide (TMAO). TMAO is an osmolyte found in many eukaryotes, most notably in marine species [13,14]. It has been shown to stabilize secondary structure of several proteins [15,16]. There are also a numerous published examples of TMAO stabilization of protein enzymatic activity, especially at extremely high TMAO concentrations not found in living systems (e.g. [17, 18]). At the same time, the stabilizing effect of TMAO is not universal to all proteins [19] and must be tested in each case. Highly concentrated TMAO has a range of effects on the many types of bonds responsible for protein folding (e.g. Ref. [20]) but the biggest effect appears to be to decrease solubility of peptide backbones [21,22]. It has been suggested that TMAO activity mimics the effects of molecular crowding in cells [22]. Despite these promising reports, the effect of TMAO on cytoskeletal motility has never been tested. Our work fills this gap and shows that TMAO is an excellent stabilizer of kinesin-microtubule transport.

We first measured the velocities of identically prepared beads decorated with kinesin-1 (full length recombinant KIF5A heavy chain dimers) at varying concentrations of TMAO (Fig. 1). We found that buffers containing 200 mM TMAO allowed for healthy kinesin-1 motility. Velocity was slightly elevated relative to the no TMAO condition ($p < 1e-6$). On the other hand, 500 mM assays showed significantly slower velocity distribution ($p < 2e-6$). We observed reduced bead-microtubule binding at even higher TMAO concentrations. In addition, we found that buffers containing in excess of ~1 M TMAO showed cloudiness under 100× magnification (even though saturating concentration of TMAO exceeds 4 M [23]) and we did not explore this regime.

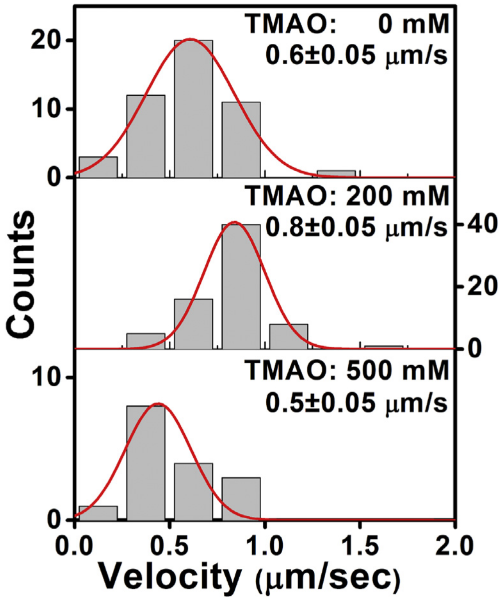

Fig. 1. Cargo velocities at room temperature as a function of TMAO concentration. Gaussian fit is shown in all cases (solid red). TMAO concentration and characteristic velocity (mean ± SEM) is shown for each panel. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

We then incremented temperature of the assays in the range from 22 °C to 46 °C and observed robust motility of the kinesin-driven cargos at all temperatures (Fig. 2). Generally, microtubule imaging was harder at elevated temperatures, however microtubules were consistently observed even after 10 min incubation at 70 °C and kinesin motility was steady suggesting that partial or local degradation of microtubules was negligible. The data represents sustained kinesin performance and microtubule stability for at least 10 min of measurements at a given temperature. The fastest velocity observed at 46 °C was 4.86 μm/s. This may be the absolute record holder for processive kinesin-1 motility reported to date although Ishiwata lab has only reported aggregate numbers for their 50 °C assay (10) for their temperature pulsed assays.

The histograms for all velocities (Fig. 3) show a well-defined peak at all temperatures and the peak mode consistently increases with temperature. The data fits well to an Arrhenius trend although a fit to only the lower temperature data points exceeds the measured value at 46 °C which may be interpreted as the very onset of thermal degradation. Our observations in the presence of TMAO are numerically comparable to previously reported [9,10] values (Fig. 3B, black). The difference is that our observations represent sustained motor activity over extended periods of time. The activation energy in the presence of TMAO was 49 ± 2 kJ/mol (fit value ± standard error of the fit). This is comparable to previous reports of 50–60 kJ/mol without TMAO [9,24]. These results show that kinesin activity can be stabilized at high temperatures and that this can happen without drastic alteration of kinesin enzymatic activity.

We have attempted to raise the temperature beyond 46 °C. At 53 °C, we observed inconsistent kinesin performance, resulting in a very broad velocity distribution (not shown). None or only sporadic motility was seen above 53 °C although it is conceivable that consistent activity can be attained at higher temperatures via temperature pulsing [10]. In particular, reducing the temperature down to 53 °C quickly restored bead motility, which argues against substantial unfolding of kinesin at these temperatures.

In conclusion, our work also bears on the long standing question of why kinesin-1 shows degrading motility above 30 °C in vitro but persists up to at least 40 °C in cells. We show that molecular crowding in cells, which is mimicked by TMAO, is likely sufficient to stabilize kinesin mechanochemical function. Our data does not rule out the possibility that other factors, such as heat shock proteins (e.g. [25]) contribute to motor and microtubule stability in cells. We also demonstrate that kinesin movement along microtubules can be stabilized in vitro via a simple addiction of a readily available and cheap compound (TMAO). This makes using microtubule-based transport in a wide range of nano-devices far more practical than before.

1. Materials and methods

Bead motility assays were set up as previously described [24,26].

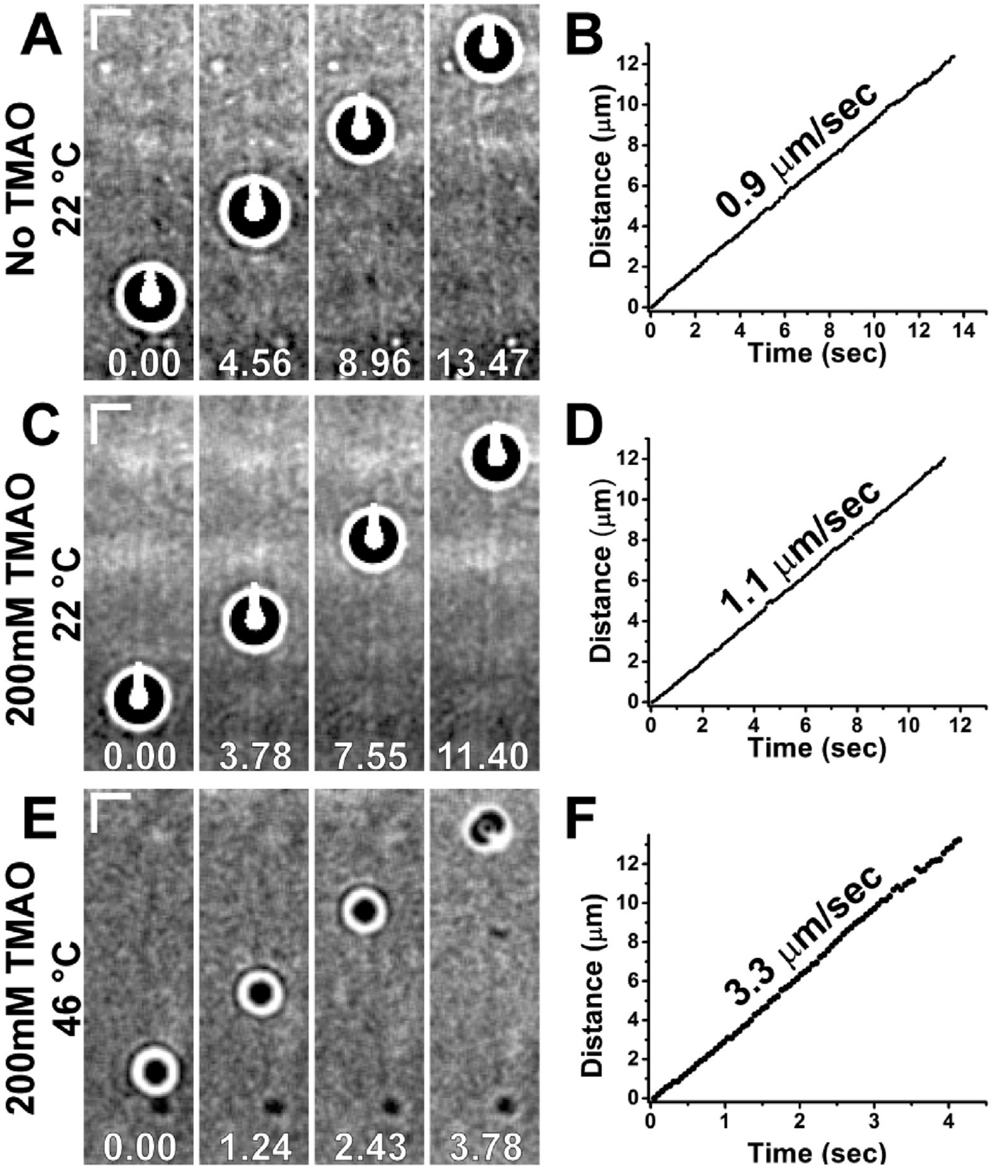

Fig. 2. Examples of bead motility as a function of temperature. TMAO concentration and measurement temperature are shown on the left of each row. Four frames cropped from bead motility recordings are shown for each condition (left panel) along with the track of bead motion and the corresponding overall velocity (right panel). Relative recording time for each frame is indicated at the bottom of each frame in seconds. Vertical and horizontal scale bars are 2 μm.

TMAO was received in powder form (Sigma-Aldrich, St. Louis, MO) and diluted with PMEE buffer (35 mM Pipes, 5 mM MgSO4, 1 mM EGTA, 1 mM EDTA, pH 7.0). TMAO was then mixed with beads to a desired final concentration after they were incubated with kinesin (right before admission into the flow cell).

At all temperatures, unless otherwise indicated, the stage was allowed to settle at a desired temperature for 10 min, followed by motility measurements for an additional 10–15 min.

Velocity data for different assay conditions were approximately Gaussian-distributed and were thus compared via two-sample Student's t-test. Non-parametric testing yielded equivalent results (not shown).

Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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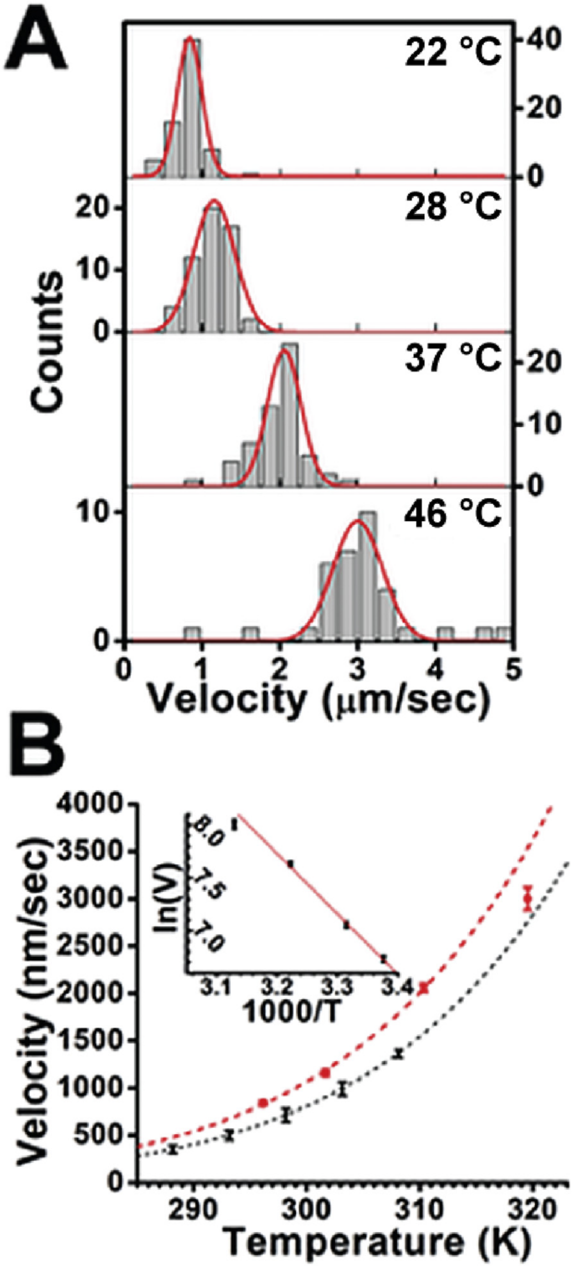

Fig. 3. Velocity vs temperature in the presence of 200 mM TMAO. (A) Bead velocity distributions for 22 °C, 28 °C, 37 °C, 46 °C (as indicated in each panel). Characteristic velocities were 0.84 ± 0.02, 1.16 ± 0.03, 2.06 ± 0.04, 3.02 ± 0.12 respectively (mean ± SEM). Gaussian fit for each case is shown in solid red. (B) Velocities measured in this work (red) along with Arrhenius fit (red dashed line) to the lowest three data points. Data from Ref. [9] is replotted in black (Arrhenius fit - dashed black line) for comparison. (inset) Arrhenius plot for our data and a linear fit for the lowest three data points (solid red line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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