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Functional localization of kinesin/microtubule-based motility system along metallic glass microwires

K. Kim,¹ A. Sikora,¹ K. S. Nakayama,¹ H. Nakazawa,² M. Umetsu,¹,² W. Hwang,³,⁴,⁵ and W. Teizer¹,⁴,⁶,a)

¹WPI-Advanced Institute for Materials Research (WPI-AIMR), Tohoku University, Sendai, Japan
²Department of Biomedical Engineering, Graduate School of Engineering, Tohoku University, Sendai, Japan
³Department of Biomedical Engineering, Texas A&M University, College Station, Texas 77845, USA
⁴Materials Science and Engineering, Texas A&M University, College Station, Texas 77843, USA
⁵School of Computational Sciences, Korea Institute for Advanced Study, Seoul, South Korea
⁶Department of Physics and Astronomy, Texas A&M University, College Station, Texas 77843, USA

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We report an approach using metallic glass microwires for functional organization of kinesin/microtubule-based molecular motility systems along a quasi-one-dimensional track. The molecular motility system assembled along a metallic glass microwire exhibits the typical kinesin-powered gliding motion of microtubules, while the variance of the gliding direction depends on the wire diameter. As a result of the geometrical boundary condition given by the wire tracks, the angle within which the orientations of gliding microtubules fall becomes narrower for smaller wire diameter. Such behavior supports the feasibility of using microwires as a simple and flexible means of spatial regulation of the molecule-based in-vitro motion. Furthermore, the metallic glass wires interact with microtubules, the negatively charged polyelectrolytes, along the wire. The existence of the glassy phase of metallic glass materials allows the formation of long conductive micro/nano-wires of varying composition.¹²,¹³ These characteristics are advantageous for designs of flexible functional elements directly bridging the gap between macro-scale devices and micro/nano-systems. However, for practical applications of the metallic glass wire as a functional track of the molecular motion, it is crucial to prove the compatibility between the wire material and the motility system. Here, we describe an experimental study that demonstrates the motional activity of the kinesin/microtubule-based motility system organized along Pd₄₂.₅Cu₃₀Ni₇.₅P₂₀ metallic glass (Pd-MG) microwires.

Truncated Drosophila kinesin proteins (400 residues, starting from MSAEREIPAEGEDLMEASTPN) with an additional ending domain including the biotin attachment site and the hexa histidine-tag were expressed in Escherichia coli (E. coli) and purified by following a standard protocol.¹⁴ Microtubules were polymerized from a 7:3 mixture of non-labeled/rhodamine-labeled commercial tubulin (Cytoskeleton, Inc.) at 5 mg/ml by following a standard protocol.¹⁵ Detailed procedures for the preparation of proteins are described elsewhere.¹¹ Pd-MG microwires were produced by a customized gas atomization process.¹³ Flow

Cells consist of a variety of active proteins whose spatio-temporal regulatory associations are responsible for the dynamic cellular differentiation. Such rigorous regulations may be one of the major mechanisms on how nature has sorted out a vast number of species while maintaining the diversity of organisms. The cytoskeletal filaments, such as microtubules and actin filaments, and the filament-associated linear motor proteins, such as kinesin and myosin, comprise the key intracellular machinery essential for driving systematic cellular cycles. Successful reconstruction of the proteins of this machinery in-vitro has led to a new paradigm of cellular mimetic demonstrations based on the dynamic behavior of active proteins.¹–⁴ Their inherent molecular length scales and their well established motion, even in noisy environments, readily meet the prerequisites for micro/nano-fluidic devices. However, these proteins are, in general, randomly dispersed upon reconstruction in-vitro. Therefore various novel strategies achieving artificial organization of the motility systems have been emphasized for practical applications.⁵–⁷ In recent studies, the general approach to achieve this goal, which has traditionally relied on surface patterning, has become more flexible by including pre-structured templates, such as nanowires, carbon nanotubes, and glass microwires, as organizers for the proteins and their motion.⁸–¹¹ Their potential secondary functionalities (e.g., optical, electrical, magnetic, etc.) are beneficial for constructing advanced molecular motility-based devices. Moreover, three-dimensional manipulation is also viable as these templates are individual entities. Particularly, using a glass microwire is relatively straightforward because it has the length scale suitable for interfacing typical macro-scale fluidic devices with molecular-level transport, and the native surface condition compatible with the motility systems. Substitution of the non-metallic glass wire with a metallic wire can create an additional functionality. Upon substitution, the microwire plays the role of an anodic electrode producing an electric field to organize microtubules, negatively charged polyelectrolytes, along the wire. The existence of the glassy phase of metallic glass materials allows the formation of long conductive micro/nano-wires of varying composition.¹²,¹³ These characteristics are advantageous for designs of flexible functional elements directly bridging the gap between macro-scale devices and micro/nano-systems. However, for practical applications of the metallic glass wire as a functional track of the molecular motion, it is crucial to prove the compatibility between the wire material and the motility system. Here, we describe an experimental study that demonstrates the motional activity of the kinesin/microtubule-based motility system organized along Pd₄₂.₅Cu₃₀Ni₇.₅P₂₀ metallic glass (Pd-MG) microwires.

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¹E-mail: teizer@physics.tamu.edu. Tel.: 1-979-845-7730. Fax: 1-979-845-2590.
cells were assembled by a typical method using a slide glass, a glass coverslip, and double-sided scotch tapes. A tiny skein of the Pd-MG microfibers was fixed by scotch tape near the center of the flow channels. For motility assays, a flow cell was first filled with the prepared kinesin solution without further dilution (∼40 μM) by using a micropipette. After 10 min incubation at room temperature, the kinesin solution was flushed out by filling the cell with microtubule solution using a micropipette. For the microtubule solution, microtubules polymerized for 15 min at 37 °C were diluted in the stabilizing solution (PEM buffer with Taxol 10 μM and the oxygen scavenging system: see Ref. 11) including 10 mM of MgATP. The final concentration of tubulin in the motility assays was 2.5 μg/ml. The flow cell was then mounted on a fluorescence microscope (Olympus IX71, Objective lens: UPlanFL 40×, and Filter set: XF204 (Omega Optical)). Movies were recorded using Metamorph (streaming parameters: 180 ms exposure per frame, total 3000 frames) through a CCD camera (ImagEM, Hamamatsu, Adapter: U-PMTVC4XIR) on regions including a Pd-MG microwire along which microtubules are bound. A total of five flow cells were observed for 18 different strands of the Pd-MG microwire skins.

Figures 1(a)–1(c) (see multimedia view of Figures 1(a)–1(c)) are fluorescence (bright-field-assisted) images showing microtubules on a kinesin-treated Pd-MG microwire (diameter is 1.2 μm) with time lapse (bottom-left in min:sec). The leading end of a microtubule moving progressively is marked with white arrows as an indication of the typical kinesin-powered gliding motion of the microtubule (triangles are fixed at the initial location of the end as references). Microtubules fully bound to the wire show nearly one-dimensional motion propelled along the wire axis. Helical motion of the microtubules with respect to the wire axis is also observed since the wire and the kinesin motor distribution are three-dimensional. On the other hand, microtubules partially bound to the wire point in arbitrary directions. Their fluctuating movements end up with two distinctive events, either detachment from the wire or re-binding. In the case of re-binding, the microtubules resume the gliding motion along the wire. Such gliding motion of microtubules guided by a wire is clearly distinguished from the motion of microtubules gliding on a two-dimensional kinesin-treated substrate. Figure 1(d) is an overlap of 600 sequential fluorescence images of microtubules gliding on a kinesin-treated glass coverslip for ∼18 min (image size ∼200 μm × 200 μm). The motion directions are irregular as expected. The guided motion of microtubules on a wire track becomes more dispersive in direction as the wire widens. Figure 1(e) (see multimedia view of Figure 1(e)) is an overlap of 300 sequential fluorescence images of microtubules gliding on a thicker Pd-MG wire for ∼9 min (the diameter is 8.8 μm, the image size ∼50 μm × 50 μm). Microtubules gliding on the track surface are seen as definite contours. On the contrary, microtubules either free in solution or partly bound to the track show up fuzzy as a consequence of thermal fluctuation. The traces of microtubules gliding near the ends of the track are also seen a bit hazily. This is because these regions are out of the focal plane. In order to exclude such thermal motion-activated trajectories in the motion direction analysis, the area outside of the wire track was cropped out. The wire region was then rotated in a way that the wire axis pointing to the right is defined as the orientational reference (see the definition in the middle of the panel (f) in Figure 1).

The adjusted wire track, which is marked with the white-dotted rectangle in Figure 1(e), appears at the top in Figure 1(f). The image in the second row is the top image filtered by using “Tubeness” (ImageJ plug-in) for enhancement of the curvilinear traces. We then utilized “OrientationJ” (ImageJ plug-in) to build a histogram of the local orientations of the traces. (e) Traces of microtubules gliding on a kinesin-treated Pd-MG microwire with diameter of 8.8 μm. (f) Top row: The orientation-adjusted area of the wire defined in the image (e) by a dotted rectangle. Second row: The top image after filtering. Third row: Color-coded local orientations of the traces. Rightward wire axis defines zero degree. Angle increases counter-clock-wise. Bottom row: Histogram of the orientations. (Multimedia view) [URL: http://dx.doi.org/10.1063/1.4896964.1] [URL: http://dx.doi.org/10.1063/1.4896964.2]
clearly distinguished from the case of the motion on a flat surface (inset in Figure 1(d)).

In order to look at the dispersion in the motion direction dependent on the wire diameter, orientation histograms were built in Figure S1 in the supplementary material. For the production of the histograms, the same procedure as described above was applied in each case. The histograms show the gliding angle generally broadening with the wire diameter. We interpret this as a result of the kinesin motor proteins being bound on a cylindrical surface. Microtubules have millimeter-long persistence length, much greater than the size of typical cells. Such a high rigidity of microtubules predicts the existence of a limiting angle in orientation that allows microtubules to bind entirely on the surface of a wire with diameter of several micrometers. This is because of the mechanical energy building on the microtubule which must be bent for full binding along the cylindrical surface of a wire. The bending energy depends on the wire diameter and the microtubule orientation, competing against the binding energy of the microtubule to the kinesin layer. The latter depends on the kinesin surface density and the binding affinity between a tubulin dimer and a kinesin motor head. In the infinite-diameter limit, the wire is identical to that of a flat surface where no bending-induced restriction in the gliding orientation arises. On the other hand, in the zero-diameter limit, the microtubule gliding motion will be one-dimensional with the possible freedom of helical motion depending on the kinesin motor configuration. Thus, a gradual decrease of the angle confining the fully-bound microtubule motion is expected as the wire diameter decreases.

We regard the curvilinear traces stamped on the wires after the image processing mentioned earlier as consequences of gliding motion of fully bound microtubules or, at least, fully bound sections of microtubules. Assuming that the asymmetric populations \( A(\theta) \) of the histograms are purely due to statistical accident, we symmetrized the histograms to define the deviation of the microtubule orientation. The symmetric histogram, \( S(\theta) = \{ A(\theta) + A(-\theta) \}/2 \), is plotted in Figure 2(a) for the wire diameter of 8.8 µm as an example. The maximum angles allowed for full binding of microtubules are then defined as the widths of the Gaussian distribution fitting to the symmetric histograms. The widths are plotted in Figure 2(b). These values, gradually increasing with the wire diameter, define a boundary dividing the space into the two domains favorable and unfavorable for microtubule gliding. Figure 2(c) shows the relationship between the limiting angle and the wire diameter in a polar plot. In the above interpretation, we assumed that the asymmetric angular distribution solely arises from stochastic binding. While our data does not provide any evidence for this scenario, it may be interesting for future studies to investigate the effect of the twisted tubulin lattice. Due to the intrinsic helicity, it may be possible for microtubules to exhibit asymmetry in their torsional activities. Such fine-structure in the binding sites may affect the helical motion direction, particularly in the case of a nano-wire whose diameter is comparable to that of the microtubule.

The above analysis is based on a static picture. Thus, the conclusion, that the particular geometrical condition given by a wire template favors microtubules to be aligned along the wire upon full binding, is perhaps valid only for emphasizing a passive term of its functionality as a guiding track. However, the cylindrical surface can also introduce an additional interesting scenario in a dynamic picture previously.

FIG. 2. (a) Symmetrized histogram in Figure 1(e). Dotted line is the Gaussian fitting curve with a width of 55.32°. (b) Widths plotted as a function of wire diameter. The plotted values differentiate between the two domains favorable and unfavorable for microtubule gliding. (c) Polar-plotted graph (b). The schematic on the graph indicates the wire axis.
studied for microtubules gliding on a kinesin-coated flat surface. An important concept in this study was the diffusive motion of the free leading head (leading section not pinned by successive motor proteins) of a gliding microtubule to search for the next motor to bind. The diffusive motion is basically swiveling of the leading head within a solid angle centered at the point pinned by the last kinesin motor. The degree of swiveling, namely, the solid angle, primarily depends on the microtubule’s flexibility. For a flat surface, assuming that the motor proteins are homogeneously covering the surface, the likelihood for the leading head to meet motors on the left hand side from its propelled direction (defined by the pinned part of the microtubule) is equal to those to meet motors on the right. Therefore, there is no directional preference in such a case, regardless of the initial gliding direction. This is not entirely true for the case of a wire template. If the free leading head of a microtubule which is partly bound to motors on the wire and oriented perpendicular to the wire axis, then we will encounter a situation exactly identical to the case of a flat surface. However, if the propelling direction leans towards the wire axis with an arbitrary angle, then the leading head will face to higher probability of being coupled with kinesin motors sitting on the side towards which the leading head inclines. This directional preference is due to the cylindrical geometry of the wire and probabilistically enhanced with a decrease in the angle between the propelling direction and the wire axis. Therefore, the gliding motion of microtubules on a wire becomes more and more likely to be oriented parallel to the wire axis as it progresses. This scenario elucidates that the wire geometry is also coupled with the gliding dynamics, supporting the capability of the wire-type templates as guiding tracks.

In addition to the structural boundary condition discussed above, non-structural boundary condition set by a local electric field can also be considered. This is achievable because now the wire track is metallic. Electrical controls of microtubules have been performed based on the electrophoretic movements of microtubules, which are triggered by the electrostatic interactions between the local electric field produced by the electrode and the effective charges defined along the microtubules. The electrostatic potential energy (U) for a microtubule whose center region is touching a wire electrode with an arbitrary angle (θ) is given below as a function of θ

\[ U(\theta) = x \left\{ \frac{a}{\sin \theta} \tan^{-1} \left( \frac{L}{2a} \sin \theta \right) - \frac{L}{2} \right. \\
\left. + \frac{L}{4} \ln \left( \frac{L^2}{4a^2} \sin^2 \theta + 1 \right) \right\} \]

Here, L and a are the length of the microtubule and the radius of the wire electrode, respectively, θ is defined as an angle between the wire axis and the microtubule axis, and x is a constant depending on applied potential, linear charge density of the microtubule, and electrode configuration. We consider the electric field cylindrically symmetric and the microtubule as a one-dimensional uniformly charged straight rod. This potential energy profile is a double-well with two minima of the same depth at θ = 0 and 180°. The energy barrier (U at θ = 90°) increases with an increase of L and with a decrease of a. The energetically most favorable orientation is either parallel or antiparallel to the wire axis. An experiment was performed to investigate the effect of electric fields on the microtubule orientation distribution. Detailed information about the experiment is described in the supplementary material. Figure 3(a) shows fluorescence images of microtubules accumulated around a Pd-MG microwire (8 μm in diameter) 55 s after electric fields formed around the wire. (See multimedia view of Figure 3(a): Numbers on top of the images are applied voltages (left) and time in min:sec (right). Image size is ∼50 μm × 50 μm.) Applied DC-voltages (in Volt) are marked in each image. A higher density of microtubules is recognized at higher voltages as the electrostatic attractive force acting on the microtubules increases with electric field strength. The histograms of microtubule orientation distribution in Figure 3(b) indicate that the electric fields become clearly effective for orienting microtubules along the wire axis from an applied voltage of 2.0 V. Full temporal sequences of the histograms are in the supplementary material. The width of the distribution generally decreases with the applied voltage (Figure 3(c), see also Figure S4 in the supplementary material). The increase in width at the highest voltage could be the result of microtubules largely losing their freedom of rotation upon binding to the wire surface as the interaction with the surface becomes significant. Also the interaction between microtubules, whose mechanism is not clear, may not be ignored particularly in a highly crowded situation.

In summary, we performed template-assisted kinesin-powered microtubule gliding assays using Pd-MG microwires with diameter ranging from 1 μm to 12 μm. Fluorescence microscopy indicated the typical gliding motion of rhodamine-labeled microtubules from the motility system assembled...
along the wire templates. Orientation analysis revealed the influence of the wire diameter on the gliding directions. The directions are less deviating from the direction of the wire axis as the wire diameter becomes thinner. Our observation proves the compatibility between the Pd-MG microwire and the motility system, and also emphasizes the role of geometrical factors in the characteristics of the gliding motion. Together with the discussed secondary functionality of the Pd-MG microwire track, this study supports the effectiveness of utilizing metallic microwires for artificial regulation of molecular motility systems. Also, with further minimization of the wire dimension, this approach may be appropriate for electrical monitoring of molecular motilities.

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18See supplementary material at http://dx.doi.org/10.1063/1.4896964 for orientation histograms for microtubules gliding on several Pd-MG microwires with diameter ranging from 1 μm to 12 μm and for a detailed description of the experiment about the effect of electric fields on the microtubule orientation distribution.