Motor proteins generate motions in a biological system by converting the chemical energy of adenosine triphosphate (ATP) into mechanical energy. Actin filament/myosin (actomyosin) is a well-studied example, and it performs essential functions in biological systems, such as muscle contraction, organelle transport, and cell motility. A common experimental scheme to study motor proteins is a gliding assay on glass substrates, where the motions of actin filaments are analyzed on randomly distributed myosin on two-dimensional (2D) substrates. In this case, the actin filaments exhibited rather disordered motions close to a 2D random walk, which is somewhat different to that in vivo systems. In muscle cells, myosin goes through one-dimensional (1D) motion on straight actin filaments. Many researchers have tried to build narrow myosin patterns or guiding channels to imitate 1D in vivo motions.

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suspensions were mixed with HMM solution, HMM adhered to the surfaces of the nanowires while maintaining their functionality.[25] We used HMM labeled with primary and fluorescence-conjugated secondary antibody to confirm the assembly of HMM onto the Si-NWs via fluorescence microscopy. We also performed motility experiments using the HMM-functionalized Si-NWs to examine the functionality of the motor protein on the Si-NWs. For the motility experiments, we first deposited the HMM-functionalized Si-NWs, as well as some extra HMM, onto glass substrates in a flow cell. Rhodamine-phalloidin-labeled actin filaments (RhPd-actins) were then infused into the flow cell. The RhPd-actins moved on the Si-NWs, as well as on the glass substrate, after the addition of ATP, and their motility in the flow cell was observed via a fluorescence microscope. In this case, the HMM-functionalized Si-NWs supported the 1D motion of the actin filaments, while we also observed the 2D motion of actin filaments on the glass substrates by some extra HMM deposited onto the glass substrates.

Figure 1B parts (i) and (ii) show optical and fluorescence microscopy images of the functionalized Si-NWs, respectively. It should be noted that the dark regions (representing Si-NWs) in the optical image match well with the brighter regions (representing labeled HMM) in the fluorescence image. The averaged fluorescence intensity of labeled HMM on the Si-NW was $223.22 \pm 24.5$ arbitrary units (au), while that on the glass substrate was $138.9 \pm 10.9$ au. A detailed intensity profile is shown in Figure S1 of the Supporting Information. If we assume that the fluorescence intensity is proportional to the density of HMM, the results indicate that the density of HMM on the Si-NW varied by $\approx 11\%$. The density distribution is quite uniform, although the variation is a bit larger than that on the glass substrate ($\approx 8\%$). However, one should be cautious about this uniformity analysis because the uniformity in the fluorescence image may not accurately represent the uniformity of HMM adsorption due to the spatial resolution of a fluorescence microscope. On the other hand, the averaged values of the fluorescence intensity have often been utilized to estimate the average density of adsorbed proteins on solid substrates.[26] Our results indicate that the density of adsorbed HMM on the Si-NW was about 1.5 times larger than that on glass substrates. This shows the effectiveness of our assembly strategy.

Figure 2A shows an optical image of a HMM-functionalized Si-NW, which was used as a track for the actomyosin motility, and Figure 2B shows a fluorescence image of labeled HMM (blue) and RhPd-actin (red) on the Si-NW. In this experiment, the actin filaments on the nanowires were separated from those on the glass substrates. Thus, when we focused the microscope on the actin filaments on the Si-NWs, those on the glass substrates were out of focus and looked blurred, allowing us to distinguish actin filaments on the Si-NWs from those on the glass substrates. The time-lapse sequence in Figure 2B clearly shows RhPd-actin (red line) moving on the labeled HMM (blue spots) on the Si-NW. The average speed of RhPd-actins on the Si-NWs was $4.953 \pm 1.573$ m/s (averaged value of 48 RhPd-actins). In most cases, individual actin filaments moved at an almost constant speed on the Si-NWs, although the speed of different actin filaments varied even on the same
Si-NW. On the other hand, in less than 2% of our experiments, we observed an actin filament moving at a largely varying speed ($\approx 1$–4 $\mu$m $s^{-1}$) on a single Si-NW with a uniform density of HMM (data not shown). In those instances, we found several other actin filaments fixed on a part of the Si-NW, indicating inactive HMM molecules on the Si-NW. It should be noted that the actomyosin motilities in a conventional gliding assay using APTES- or nitrocellulose-coated glass substrates were 4.740 $\pm$ 1.045 $\mu$m $s^{-1}$ (averaged value of 93 RhPd-actins) and 4.556 $\pm$ 1.863 $\mu$m $s^{-1}$ (averaged value of 65 RhPd-actins), respectively. The measured average speed of the actin filaments on the Si-NWs ($\approx 4.953$ $\mu$m $s^{-1}$) clearly shows that the HMM-functionalized Si-NWs can support actomyosin motility very well, and that they can be an ideal platform for 1D gliding assay. We often observed actin filaments moving over 100 $\mu$m, or longer distances, on the functionalized Si-NWs without any confining physical barrier. This can be partly attributed to the high density of myosin on the Si-NWs. Since this ordered actomyosin system on Si-NWs can generate 1D actomyosin motion without any confining external forces, it can be an ideal mimicking system close to in vivo contractile proteins.

We also found that a single HMM-functionalized Si-NW could support bidirectional motion of actin filaments (Figure 3). At first, we observed that RhPd-actin (red) on a Si-NW (green) moved downward (dotted circle and arrow, Figure 3A). After a while, another RhPd-actin followed the same path in the opposite direction (Figure 3B). Here, those actin filaments moving in opposite directions moved with a similar speed on the Si-NW. The speed of actin filaments moving upward was 3.340 $\pm$ 0.644 $\mu$m $s^{-1}$ (averaged value of five RhPd-actins), and that for downward motion was 3.095 $\pm$ 0.930 $\mu$m $s^{-1}$ (averaged value of three RhPd-actins). This result is consistent with earlier studies, which showed that HMM and kinesin had high torsional flexibility on narrowly patterned motor proteins.\[27,28\]

In previous works, various theoretical models have been introduced to describe motor protein motions in a gliding assay, revealing many interesting biological properties of molecular motors.\[29–36\] However, in most cases, even though actomyosin performed 2D motion on the glass substrates, the actin movement was analyzed as if the actomyosin was performing a 1D motion along their trajectory lines, without considering the 2D nature of their motion. To analyze the qualitative difference between the actomyosin motions on Si-NWs and glass substrates, we performed a more generalized theoretical modeling (Figure 4). The general trajectory of actin filaments can be divided into small trajectory vectors, each denoted $\vec{r}$ (Figure 4A). The $\vec{r}_i$ term represents the $i$th trajectory vector during a short time interval $\Delta t$, and the $\vec{R}$ term, which is the sum of all of the trajectory vectors, represents the final displacement of the trajectory after a certain time period $t$. The nature of actomyosin motion can be analyzed based on the scaling of $\sqrt{\langle \vec{R}^2 \rangle}$ in time $t$ such that

$$\sqrt{\langle \vec{R}^2 \rangle} \approx t^\nu$$

(1)

where the scaling exponent $\nu$ should be $\approx 0.5$ for diffusive motion, $\approx 0.5$–1 for anomalous superdiffusion, and 1 for ballistic motion.\[37\]

Figure 4B shows a log–log plot of $\sqrt{\langle \vec{R}^2 \rangle}$ versus $t$ for the actomyosin motions on Si-NWs (red) and glass substrates (black). We collected positional data of a trajectory with a time interval of 0.3 s between the frames, and calculated the ensemble average, $\langle \vec{R}^2 \rangle$, of the mean square end-to-end distance within a single trajectory of an individual actin filament.\[34\] Previous works have showed that the errors from
the spatial resolution and pixelation of an optical microscope and a charge-coupled device (CCD) camera used for experiments may affect the length measurement, especially when the measured length is shorter than the length of \(\approx 3\) pixels of the CCD camera. However, the measured lengths of actin filaments and individual trajectories for our analysis were much longer than 1 \(\mu\)m (\(\approx 15\) pixels). Thus, such errors from the spatial resolution and pixelation should be small and can be ignored in our analysis. The scaling exponents, \(\nu\), of the trajectories on 1D Si-NWs were close to 1, indicating ballistic motion. This implies that the actin filaments were moving on the Si-NW with an almost constant speed. On the other hand, the scaling exponents of the trajectories on 2D glass substrates were close to 1 at the initial stage, and then decreased to 0.5 after a long time period. This implies that the actin filaments on 2D glass substrates performed superdiffusive motion at the initial stage, and then diffusive motion after later on.

In the case of completely random-walk motion, an object can move along a random direction with a random speed. However, in the gliding assay on 1D Si-NWs or 2D glass substrates, actin filaments were found to move with an almost constant speed (see the Supporting Information for detailed analysis). Thus, the characteristics of actin motions can be explained mainly by the randomness in the direction of actin movements (or directional correlation), which is restricted by the structural polarity and some flexural rigidity of actin filaments. Due to the structural polarity of actin filaments, myosin can only move in a certain direction on actin filaments. In addition, actin filaments have a certain flexural rigidity (often quantified by the “persistence length”), and it is difficult to bend actin filaments with a small radius of curvature. In a 1D assay on 100-nm-diameter Si-NWs, actin filaments can move in only one direction because they cannot change directions in such a narrow path due to their flexural rigidity. Thus, they should exhibit ballistic motion. On the other hand, an actin filament in a 2D assay on glass substrates can bend and change its direction. However, it becomes more difficult for the actin filament to make a sharper turn due to its flexural rigidity. Thus, even on the 2D assay, an actin filament tends to move in a certain direction for a short time period, and the successive motions of actin filaments have directional correlations. The superdiffusive motion of actin filaments on a 2D assay for a short time period can be attributed to such a directional correlation.

For a quantitative analysis of the directional correlation, we introduced Flory’s characteristic ratio, which has been utilized to analyze the conformations of polymer chains (see Supporting Information). If we assume that an actin filament is moving with a constant speed, \(R(t)\) can be expressed as

\[
\sqrt{\langle R^2 \rangle} = \sqrt{C(t) \Delta t^{\nu}} \approx \sqrt{\langle C(t) \rangle} \Delta t^{\nu} \approx t^{\nu+0.5},
\]

where \(\sqrt{\langle C(t) \rangle} \approx t^\nu\), \(C(t)\) is Flory’s characteristic ratio, and \(\Delta t\) is the time interval for the actin filament to move a distance equal to its length \(l\) over a single trajectory vector (see Supporting Information for detailed calculation). Here, Flory’s characteristic ratio, \(C(t)\), shows the directional correlation between trajectory vectors over a time period. For example, if the actin filaments perform 1D motion with a constant speed, \(C(t) \approx t\), and thus \(\sqrt{\langle R^2 \rangle} \approx t\), corresponding to ballistic motion. On the other hand, for the completely random walk without any directional correlations, \(C(t)\) is a constant, resulting in diffusive motion with \(\sqrt{\langle R^2 \rangle} \approx t^{0.5}\). Overall, equation (2) including Flory’s characteristic ratio shows how the directional correlation of the actin trajectory affects the diffusive motion of actin filaments, assuming that the actin filament is moving with a constant speed. We estimated the characteristic ratio \(C(t)\) from the actin trajectories on Si-NWs (red circles) and glass substrates (black squares), as shown in Figure 4C. Since the actin filaments move in only one direction on 1D Si-NWs, Flory’s characteristic ratio increases in time, \(C(t) \approx t\). On glass substrates, since it is difficult for actin filaments to make very sharp turns, \(C(t) \approx t^0\) for a short time period, while \(C(t) \approx t^0\) (i.e., a constant) after a while. This implies that an actin filament tends to move in the forward direction for a short time period, due to its flexural rigidity, while, for a long time period, it loses the directional correlation from its original direction.

For more detailed analysis, we estimated the scaling exponent, \(\nu\), by fitting the log-log plot of \(\sqrt{\langle R^2 \rangle}\) versus \(t\) over different time periods using equation (1). The estimated scaling exponents of the 1D trajectory on Si-NWs are close to 1, indicating ballistic motion. On the other hand, those of 2D motions on the glass substrates tend to decrease in time, which implies that the 2D movement of an actin filament is anomalous superdiffusion at the early stage of movements, and becomes diffusive after a while. The scaling exponents were also calculated using the measured characteristic ratio, \(C(t)\), and Equation (2) assuming that the speed of the actin filament within a single trajectory is constant. It should be noted that, in this case, we are considering only the directional correlations without considering the variation of speeds. Figure 4D shows that both results coincide well. It clearly shows that the characteristics of actin movements on 1D Si-NWs and 2D glass substrates can be explained by the directional correlations of actomyosin motion caused by its structural polarity and flexural rigidity.

In summary, we report a method to functionalize Si-NWs with myosin motor protein. Using HMM-functionalized Si-NWs, we successfully demonstrated in vitro motility assay to study the 1D motion of motor proteins. Significantly, the HMM-functionalized Si-NWs could support actomyosin motility for a long distance (over 100 \(\mu\)m) without any confining physical barriers. It is also interesting that the HMM-functionalized Si-NWs supported the bidirectional transportation of actin filaments. In addition, we developed a simple and versatile model to analyze the motion of actin filaments on 1D and 2D gliding assays. The analysis based on our model revealed the ballistic and superdiffusive nature of actomyosin motions on Si-NWs and glass substrates, respectively. Our analysis also indicates that such characteristics of the actomyosin motion can be attributed to the directional correlations of actin filament movement caused by their structural polarity and a flexural rigidity. Our work can be a significant step toward hybrid nanomechanical systems based
on motor proteins, although there are some remaining challenges, such as positioning nanowires between two points and assembling actin filaments with desired orientation. Furthermore, the development of HMM-functionalized Si-NWs itself should be an important breakthrough in various fundamental researches, such as muscle physiology, since it provides a simple and very efficient tool to study the 1D motion of motor proteins.

### Experimental Section

**Protein Preparation and Labeling:** Actin and myosin were purified from rabbit skeletal muscle, and HMM was prepared from myosin by the method described by Kron et al. Actin filaments were stabilized and labeled with rhodamine phalloidin (excitation wavelength: 542 nm, emission wavelength: 565 nm; Invitrogen). To prepare fluorescence-labeled HMM, we first incubated 1 mL of HMM solution (0.5 mg mL\(^{-1}\)) with 5 μL of primary antibody (mouse monoclonal anti-myosin, purchased from Sigma-Aldrich, part number M&401) at 4°C for 1 h. Subsequently, 5 μL of secondary antibody (anti-mouse IgG labeled with Alexa Fluor 488, purchased from Invitrogen) was added to the solution, which was then incubated at 4°C for 30 min. The antibody-labeled HMM solution was mixed with actin filaments at 4°C for 10 min and centrifuged at 100,000 g for 14 min. In this step, antibody-labeled HMM bound to the actin filaments, and was precipitated during the centrifugation process, while excess antibodies remained in the supernatant. We removed the supernatant and resuspended the pellet in 1 mL BED buffer (0.1 mM NaHCO\(_3\), 0.1 mM ethylene glycol tetraacetic acid (EGTA), 1 mM dithiothreitol (DTT) with 3 mM MgCl\(_2\), 0.1 mM phenylmethylsulfonyl fluoride, and 1 mM ATP so that the labeled HMM was separated from actin filaments by ATP hydrolysis. The solution was then centrifuged at 230,000 g for 14 min to remove actin filaments by precipitation.

**Preparation of HMM-Functionalized Si-NW Suspension:** The Si-NWs grown on the substrates were functionalized with amine groups by dipping the substrates in APTES (Sigma, USA) solution (1:3 and incubated at 4°C for 10 min and centrifuged at 100,000 g for 14 min. To prevent APTES aggregation, NWs grown on the substrates were functionalized with amine groups by dipping the substrates in APTES (Sigma, USA) solution (1:3 and incubated at 4°C for 10 min and centrifuged at 100,000 g for 14 min. Si-NWs grown on the substrates were dried in nitrogen gas flow, and then the 1-cm-by-1-cm substrate was sonicated in 0.5 mL BED buffer with 3 mM MgCl\(_2\) and 0.1 mM phenylmethylsulfonyl fluoride, and 1 mM ATP so that the labeled HMM was separated from actin filaments by ATP hydrolysis. The solution was then centrifuged at 230,000 g for 14 min to remove actin filaments by precipitation.

**Motility Assay:** Flow cells for motility were constructed with vacuum grease and cover glass spacers, and the flow-cell temperature was kept at 30°C throughout all of our experiments. The flow cells were first incubated with HMM-functionalized Si-NWs suspensions for 5 min. Rhodamine-phalloidin-labeled actin filaments were then infused into the flow cells. Note that we did not use any conventional blocking agents such as bovine serum albumin (BSA), which is often utilized to prevent the nonspecific binding of actin filaments on glass substrates. We obtained negligible nonspecific binding of actin filaments on Si-NWs without any blocking agents, presumably due to the small diameter of the Si-NWs and the high density of HMM adsorbed on the Si-NWs. After 1 min, actin buffer (AB) (25 mM imidazole pH 7.4, 25 mM KCl, 4 mM MgCl\(_2\), 1 mM EGTA, 1 mM (DTT)) was infused into the flow cells, followed by the motility buffer (2 mM ATP, 3 mg mL\(^{-1}\) D-glucose, 0.1 mg mL\(^{-1}\) glucose oxidase, 0.02 mg mL\(^{-1}\) catalase, 0.3% methylcellulose in AB).

**Optics System:** Actomyosin motility in the flow cell was observed via a fluorescent microscope (Nikon TE2000U) equipped with an intensified CCD VE-1000 SIT. We used Metamorph analysis software (purchased from Molecular Devices) for the motility analysis of the actin movement.

### Keywords:
- actomyosin
- biomimetics
- molecular motors
- random walks
- silicon nanowires
