Supplementary Information

1. Materials and Supplementary Methods:

1.1 Materials: PC (polycarbonate) membranes with pore diameters of 3 μm and membrane thickness of 23 μm were obtained from it_4ip Corp. PAH (polyallylamine hydrochloride, Mw 58 kDa), NH_2-NTA (Nα,Nα-Bis(carboxymethyl)-L-lysine hydrate), ALG (sodium alginate, medium molecular weight), isopropyl-b-D-thiogalactopyranoside, ethylenebis(oxyethylenenitrilo)tetraacetic acid, DL-Dithiothreitol and casein were purchased from Sigma. EDC (1-Ethyl-(dimethylaminopropyl) carbodiimide hydrochloride) and NHS (N-hydroxysulfosuccinimide) were purchased from Alfa Aesar. DSS (dextran sulfate sodium) was purchased from MP. DMF (N,N-Dimethylformamide), sodium phosphate, $MgCl_2$, NaCl and $NiCl_2{\cdot}6H_2O$ were purchased from Beijing chemical works. All purchased chemicals have been used without further treatment.

Full length kinesin-1 was expressed and purified as described elsewhere[1]. Briefly, for kinesin expression, the kinesin containing plasmids were expressed in E.coli BL21(DE3)[pLysS]. Cultures grown in LB at 37 °C to an optical density of 1 at 600 nm were induced for 4 h at 20 °C with 0.4 mM isopropyl-b-D-thiogalactopyranoside. The bacterial cells were harvested and resuspended in 30 ml of lysis buffer (50 mM sodium phosphate, 40 mM imidazole, 300 mM NaCl, 5 mM 2-mercaptoethanol, 10 % glycerol, pH 8.0)/liter of culture, lysed in a French press at~19,000 psi, sonicated, and centrifuged (100,000×g) for 30 min at 4 °C. Full-length kinesins were purified using nickel-nitrilotriacetic acid agarose resins filled column. The binding buffer (20 mM sodium phosphate, pH 7.8, 300 mM NaCl, 1 mM $MgCl_2$, 0.5 M ethylenebis(oxyethylenenitrilo)tetraacetic acid, 1 mM ATP, 2 mM DL-Dithiothreitol and 50 mM imidazole) was first injected into the column for kinesin binding. After washing with the washing buffer (20 mM sodium phosphate, pH 7.4, 300 mM NaCl, 1 mM $MgCl_2$, 0.5 M ethylenebis(oxyethylenenitrilo) tetraacetic acid, 1 mM ATP, 2 mM DL-Dithiothreitol and 50 mM imidazole) to remove the unwanted component, the column was eluted with the elution buffer (20 mM sodium phosphate, pH 7.4, 300 mM NaCl, 1 mM $MgCl_2$, 0.5 M ethylenebis(oxyethylenenitrilo) tetraacetic acid, 1 mM ATP, 2 mM DL-Dithiothreitol and 500 mM imidazole) to collect the purified His-tagged kinesin.

Rhodamine-labeled microtubules were copolymerized with biotinylated tubulin, rhodamine modified tubulin and the unlabeled tubulin at the ratio of 1:1:1. These tubulins were copolymerized with a final concentration of 4 mg mL^{-1} (Cytoskeleton, Denver, CO) in BRB80 buffer (80 mM Pipes, pH 6.9, 1 mM EGTA, 1 mM $MgCl_2$) with 4 mM $MgCl_2$, 1 mM MgGTP, and 5% DMSO at 37 ^oC for 30 min. Microtubules were then diluted in BRB80

buffer supplemented with10 μM taxol.

The water used in all experiments was prepared in a three-stage Millipore Milli-QPlus 185 purification system and had a resistivity higher than 18.2 MW cm.

1.2 Methods:

1.2.1 *Synthesis of the assembled tubes:* PC membranes with pore diameters of 3 μm and membrane thickness of 23 μm were used as templates. PAH and DSS were chosen as the scaffold materials of the tubes and then the inner walls were functionalized with NH_2-NTA modified ALG (denoted as ALG-NTA). For the purpose of CLSM observation, PAH was modified with FITC (denoted as PAH). Considering that the inner pores of the PC template are slightly negatively charged, the positively charged PAH (2 mg mL^{-1} in 0.3 M NaCl) was chosen to form the first layer on the wall of the pores.After washing with a solution without polyelectrolyte, the negatively charged DSS (2 mg mL^{-1} in 0.3 M NaCl) was adsorbed onto the surface of PAH as a second layer. The scaffolds of the tubes were obtained after 29.5 times of repetition of this procedure. Then the ALG-NTA (2 mg mL^{-1} in 0.3 M NaCl) was assembled into the inner most of the templates. After Ni^{2+} deposition, the membranes were treated with oxygen plasma to remove the undesired adsorbed film at the top and bottom of the PC membranes. Then they were immersed into DMF to release the tubes. The morphologies of the assembled tubes were examined with a scanning electron microscope (HITACHI S-4800, 10 kV), a transmission electron microscope (JEOL, JEM-1011).Confocal laser scanning (CLSM) micrographs of the tubes were also taken with an Olympus FV500 confocal system (Carl Zeiss) equipped with a 60 × oil-immersion objective.

1.2.2 *Modification of ALG with NH_2-NTA:* For ALG modification, 50 ml of 2 mg ml^{-1} ALG

solution (in 0.1 M pH 6.5 MES buffer) was first mixed with EDC (10 mM) and NHS (20 mM) and was stirred at room temperature for 12 h. Then the mixture was added with NH_2-NTA (10 mM) and stirred at room temperature overnight. The product was purified via dialysis and then lyophilized to get the powder.

1.2.3 *Kinesin modification with Alexa Flour 647-maleimide:* For kinesin modification, 10 μL Alexa Flour 647-maleimide (3.85 mM in DMSO) was first diluted with 80 μL BRB80 buffer. Then 320 μL kinesin solution was dropped with carefully stirring into the dye dilution to avoid protein precipitation. The mixture was allowed to react for 2 h at 4 ^{o}C. Then the modified kinesins were purified through ultrafiltration followed by 3 washing steps with BRB80 buffer.

1.2.4 *Fluorescence experiments:* Tube dispersions with and without inner wall functionalization were both added to the same volume of fluorescently-labeled kinesin solution. The mixtures were incubated under shaking for 1 h in dark and then centrifuged. The supernatants were kept and the precipitates were washed with BRB80 buffer three times. All the washing solution supernatants were added to that kept above to a total volume of 600 μL, respectively. The same volume of purefluorescently-labeled kinesin solution was diluted to 600 μL simultaneously as control.

EDTA was used to further prove the necessity of the Ni-NTA complex for efficient kinesin binding. 60 μL of the functionalized tube dispersion was first mixed with the same volume of 0.2 M EDTA-Na_2 and shaken for 1 h. After centrifugation and washing three times, the precipitate was treated with the same procure mentioned above.

1.2.5 *CLSM observation:* Confocal laser scanning (CLSM) micrographs of the tube

precipitates kept above were taken with an Olympus FV500 confocal system (Carl Zeiss) equipped with a 60 × oil-immersion objective.

1.2.6 *Ultraviolet-Visible spectrosc*opy*:* Same procedure as for the fluorescence measurement was done for UV/Vis measurement to test the tubes' protein binding ability.

1.2.7 *Preparation of the motility solution:* The solution for the motility assay was prepared as follows: The Ni-NTA functionalized tubes were first incubated with casein solution (10 mg mL^{-1} in BRB80 buffer) for 30 min on ice. Then an equal volume of kinesin solution (60 μg mL^{-1} in BRB80 buffer) was mixed with the above tube-casein mixture and incubated on ice for another 30 min. The mixture was centrifuged and washed with BRB80 buffer twice. After centrifugation, the precipitate was mixed with 5 μL rhodamine labeled microtubules and the antifade solution(5 μL 1 mM adenosine 5'-triphosphate (ATP), 20 mM glucose, 0.02 mg mL^{-1} glucose oxidase, 0.008 mg mL^{-1} catalase , respectively , and 0.5 μL 0.5% β-mercaptoethanol). The total volume of the mixture was increased to 100 μL with BRB80 buffer. Tubes with no Ni-NTA functionalization were prepared identically as comparison.

For cargo loading, 5 μL PS particles (in BRB80 buffer) were first mixed with 45 μL rhodamine labeled microtubules dispersion (in BRB80 buffer) for 30 min on ice. The tubes were treated the same as mentioned above. After mixing the cargo and tube solution, antifade solution were added. Additional BRB80 buffer was added to keep thesolution volume to be 100 μL.

1.2.8 *Construction of flow chambers and motility assays:* Motility experiments were performed in the flow chambers with a volume of about 10 μL. The chambers were assembled with a glass slide as the bottom surface, a coverslip to cover the surface, and two

strips of double-sided tapeas spacer. The surfaces of the chambers were first coated with a casein solution (0.5 mg mL^{-1} in BRB80 buffer) on ice for 5 min, and then with a kinesin solution (10 μg mL^{-1} in BRB80 buffer) for another 5 min, finally the chambers were perfused with the motility solution. They were sealed with silicone and observed under the confocal laser scanning microscope (Olympus FV500) with a 60×oil-immersion objective. The field of view was 52.398 μm×52.398 μm, and the scanning speed was 12.5 μs/pixel. The length of the exposure was 640×640×12.5=5,120,000 μs and the effective pixel size of the images was 0.82 μm/pixel.

As comparison, Ni-NTA functionalized tubes with no kinesins were used for motility assay. All the procedures were the same with those mentioned above except that the tubes were not treated kinesin solution.

For experiment where the substrate was modified no kinesins, only the procedure for substrate modification with kineins was omitted, all other procedures were the same with the above experiments.

1.2.9 *Modification of kinesins on the outer tube wall:* The modification of the outer tube walls with kinesins is also realized via the Ni-NTA complexes. As the outer most material of the assembled tubes is PAH which has the primary amine group, we functionalized the outer walls with NH_2-NTA through the conjunction of glutaraldehyde. First, 100 μL 0.025% glutaraldehyde (in water) were added to the tubes precipitate and reacted for 8 h. Then after three times of and washing with water, 100 μL of NH_2-NTA (2mg ml^{-1}) solution was added to the tube precipitate to react for another 8 h. the tube precipitate was added with 100 μL $NiCl_2$ (0.1 M) solution to chelate Ni-(II) for 4 h. The procedures for motility assay with these

outer wall functionalized tubes were the same with those for inner wall functionalized tubes.

2. Supplementary Data:

2.1 *Characterization of the assembled tubes:* As channels, the tube walls must be thick enough to keep the tubular structure. As seen in Figure S1, tubes with only twenty-six bilayers as scaffold collapsed due to the low mechanical strength.

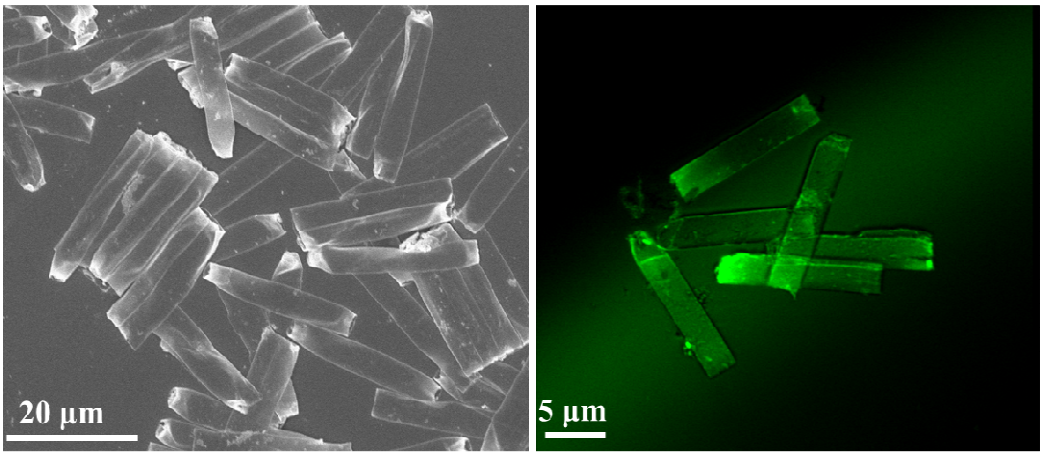
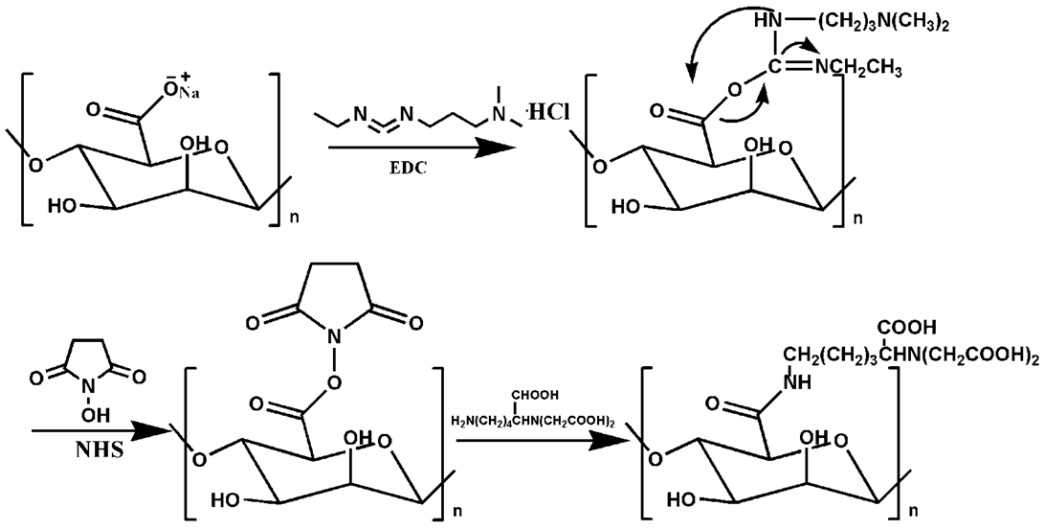


Figure S1 SEM and CLSM images of FITC-PAH /(DSS/PAH)$_{25.5}$

2.2 *Characterization of the NH_2-NTA modified ALG:* The tube inner wall modification with Ni-NTA was achieved by grafting the NH_2-NTA to ALG, assembling the NH_2-NTA modified ALG to the tubes as the inner most-layer, and then immersing these tubes (before removing the template) into Ni^{2+} solution.

The ALG modification was realized by the acylation reaction between the amine group of NH_2-NTA and the carboxyl group of ALG. EDC and NHS were used as cross-linking agents for this reaction (Scheme S1).



Scheme S1 Mechanism for the modification of ALG with NH_2-NTA

The successful modification of ALG with NH_2-NTA was confirmed by FTIR spectroscopy, as shown in Figure S2. The spectrum of NH_2-NTA modified ALG retained the characteristic IR band associated to ALG. However, the peaks at 1734 and 1617 cm^{-1} demonstrate the characteristic IR band of NH_2-NTA. The above analysis indicates that the NH_2-NTA has been successfully grafted to ALG.

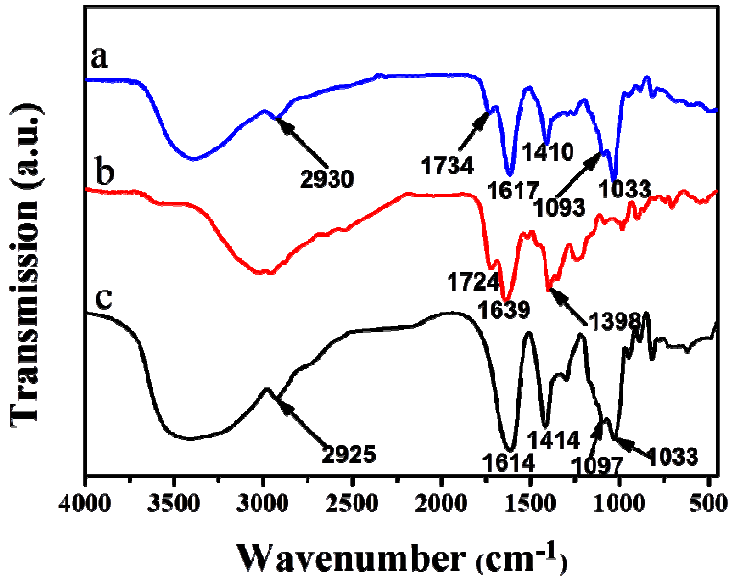
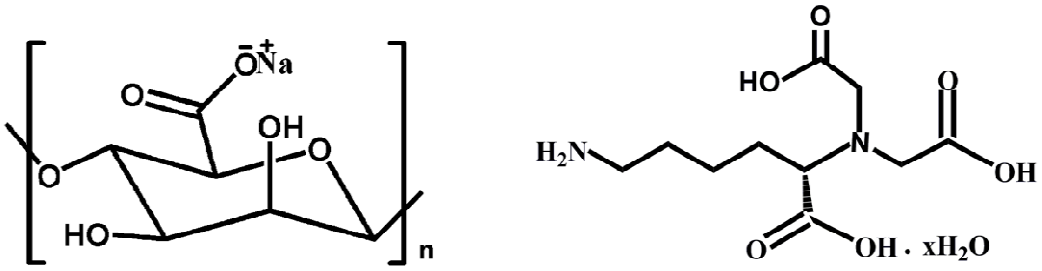


Figure S2 FTIR spectra of NH_2-NTA modified ALG (a), NH_2-NTA (b) and ALG (c)

XPS measurement was also performed to prove the success of the ALG modification. The structural formula of ALG (Scheme S2 left) indicates that there is no N element existing in ALG, which is proven by the XPS peak (Figure S3b). But after reacting with the NH_2-NTA, the N1 peak appears at the location of about 400 eV (Figure S3a). This indicates that NH_2-NTA has been successfully conjugated to ALG, as almost no NH_2-NTA exists as monomer in the system after dialysis. The above results suggest that the ALG had been successfully modified with NH_2-NTA.



Scheme S2 structural formula of ALG (left) and NH_2-NTA (right)

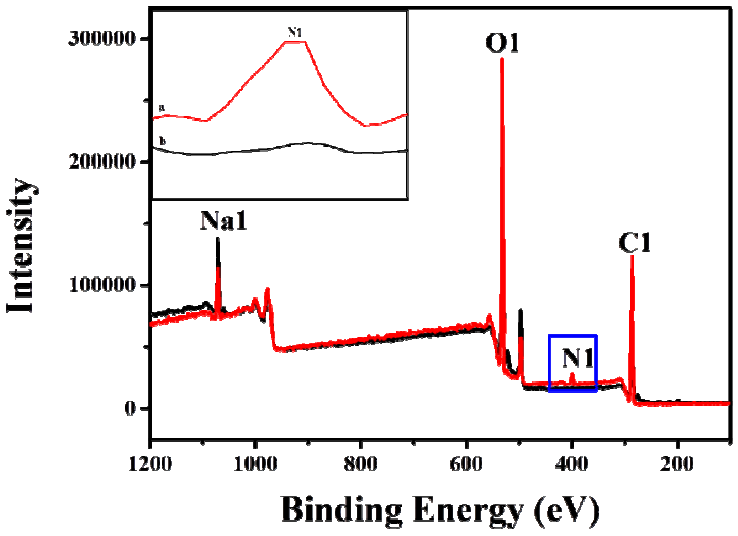


Figure S3 XPS spectra of NH_2-NTA modified ALG (a) and ALG (b)

2.3 *Characterization of the tubes' kinesin-binding ability:* In the Ni-NTA and His-tagged protein binding system, Ni-(II) is the central metal ion. Four of its six ligand binding sites are

occupied by NTA, and the other two are left to interact with the 6 x His tag to provide strong binding with the protein[2]. In order to prove the existing of Ni for protein binding in our tubes, EDX measurement was performed. Figure S4 displays the EDX spectra of the assembled tubes on a silicon substrate. The expected compositions like carbon, oxygen, and nitrogen are detected, which show the main contributions of PAH and DSS. The presence of the Ni component indicates that Ni has been chelated into the tubes. Since there is only one layer of Ni adsorbed, the Ni element takes up a rather small percentage of the total elements.

Element	Weight%	Atomic%
C K	33.87	42.64
O K	58.67	55.44
Ni K	7.46	1.92

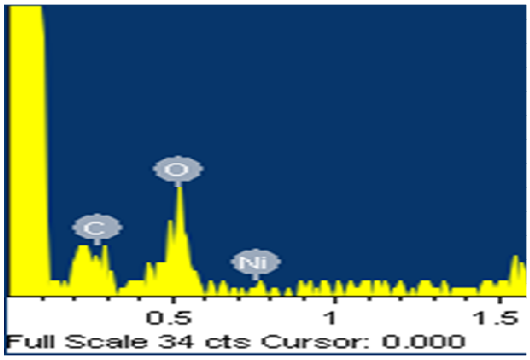


Figure S4 EDX spectra of the Ni-NTA modified tubes

To ensure that the tubes can bind kinesin via the Ni-NTA complex, the fluorescence intensities of the fluorescent labeled motor proteins in solutions before and after mixing with different tubes were investigated (Figure S5 A). The weaker the fluorescence intensity, the less proteins were left in bulk solution. Compared with that of the pure kinesin solution (curve a), the fluorescence intensity of kinesin treated with Ni-NTA functionalized tubes (curve d) and non-functionalized tubes (curve b) both decreased. This suggests that the proteins in bulk solution have been assembled inside the tubes. However the fluorescence intensity of the kinesin supernatant solution treated with Ni-NTA functionalized tubes (curve d) decreased to a much larger degree than that treated with non-functionalized tubes (curve b). This difference results only from the incorporation of Ni-NTA in the inner walls of the tubes.

This indicates that the Ni-NTA complex assisted kinesin binding method is an efficient way for the tubes to immobilize the motor proteins.

To further confirm that the Ni-NTA complex plays a key pole for efficient kinesin binding, EDTA-Na_2 was employed. It could interrupt the interaction of His-tagged protein with the Ni-NTA complex by competing effectively for nickel ions on the NTA sites[3]. Also in Figure S5 A, after the treatment by EDTA, the fluorescence intensity of the kinesin supernatant solution (curve c) decreased only a bit compared to the pure kinesin solution. It suggests that EDTA pretreated tubes adsorb less kinesin than the untreated tubes. This is because that Ni^{2+} interacts stronger with the stronger chelator EDTA rather than the immobilized NTA.

Figure S5 B is the CLSM images of the tubes adsorbing fluorescently labeled kinesin. Pure tubes showed no fluorescence signal at all, while the Ni-NTA functionalized tubes exhibited the strongest fluorescence signal. This result further proves the high protein binding efficiency of the Ni-NTA modified tubes.

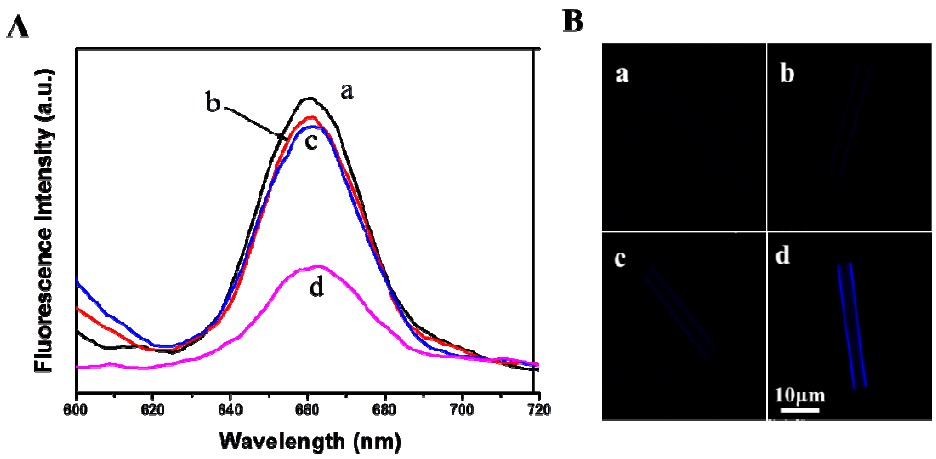


Figure S5 Characterization of the tubes' kinesin-binding ability: A) Fluorescence spectra of the fluorescent labeled kinesin treated with different tubes: a) pure kinesin supernatant solution; b) kineisn supernatant solution after treated with non-functionalized tubes; c) kineisn supernatant solution after treated with Ni-NTA modified tubes that were premixed with EDTA; d) kinesin supernatant solution after treated with Ni-NTA modified tubes; B) CLSM images of the assembled tubes with different treatment: a) pure tube; b) non-functionalized tube treated with fluorescent labeled kinesin; c) EDTA premixed and Ni-NTA modified tubes that was treated with fluorescent labeled kinesin; d) Ni-NTA modified tube that was treated with fluorescent labeled kinesin.

Figure S6 shows the UV/Vis spectra of the tubes with different treatment. Following the same principle as the fluorescence spectra, the weaker the absorption intensity, the more motor proteins that were immobilized to the tubes. Consistent with the result of the fluorescence spectra, tubes with the Ni-NTA modification immobilized the most motor proteins while EDTA disturbs the Ni-NTA mediated adsorption of kinesin to the assembled tubes. All the above results confirm that the Ni-NTA modified tubes can efficiently immobilize the motor proteins.

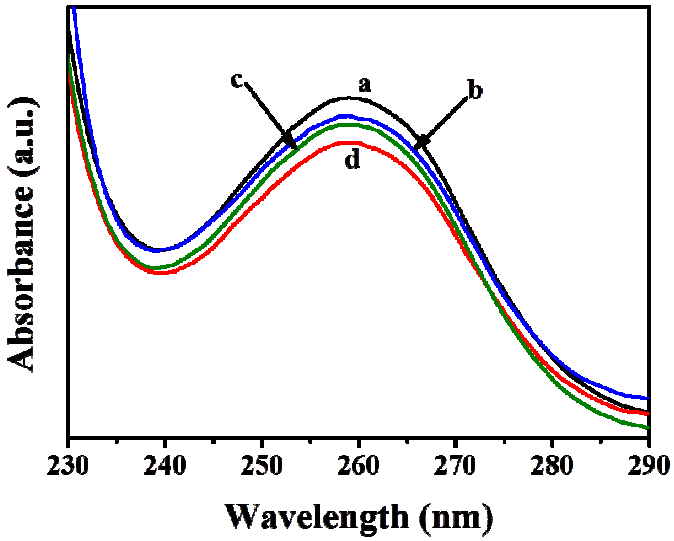


Figure S6 UV/Vis spectra of kinesin treated with different tubes: a) pure kinesin solution; b) kinesin solution after treated with non-modified tubes; c) kineisn solution after treated with Ni-NTA modified tubes that were mixed with EDTA; d) kinesin solution after treated with Ni-NTA modified tubes

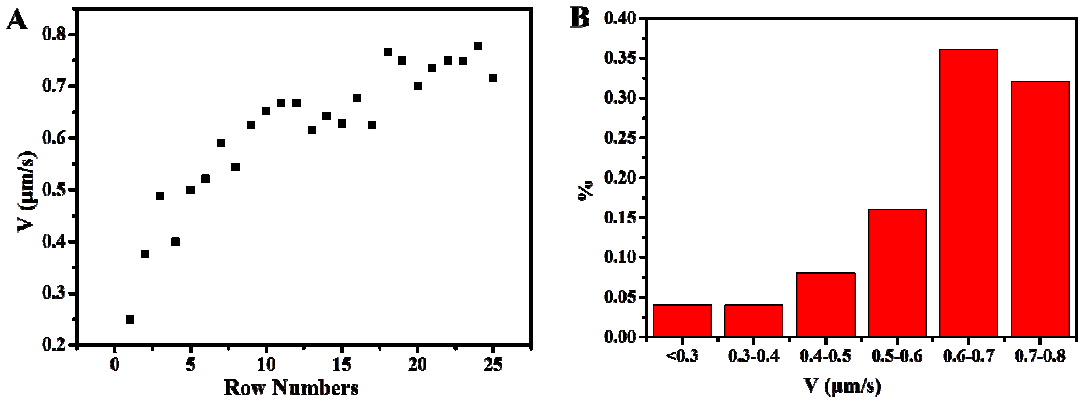


Figure S7 A: The gliding velocities of microtubules in the Ni-NTA modified tubes (n=25. The vertical axis shows the actual mean velocities of every tube transport and the horizontal axis has no physical significance); **B:** velocity distribution of the total 25 times of tube transport.

Table S1: The statistical comparison of the experiments with non-functionalized tubes and Ni-NTA modified tubes.

	N_T	N_E	N_M	N_E/N_T	N_M/N_E	Velocity (μm/s)
Non-functionalized tube	130	60	0	46 %	0 %	0
Ni-NTA functionalized tube	80	51	25	64 %	49 %	0.61±0.15

N_T: Total number of the observation; **N_E:** the number of microtubules entered the tube; **N_M:** The number of microtubules moving unidirectionally along tube wall.

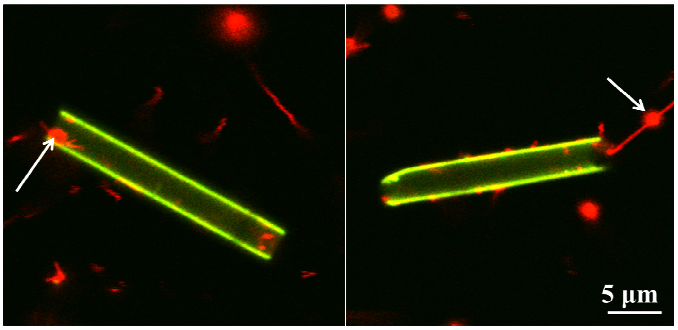


Figure S8 Images of microtubule loading with PS particle interacting with the assembled tubes

As the LbL assembled method allows both the inner and outer surface modification, we further exploited the exterior surfaces of the tubes as tracks for microtubules. Same way with the functionalization of the inner tube wall, we functionalized the exterior surfaces with kinesin via Ni-NTA complex and found they could act as linear tracks for microtubules (movie S5 and Figure S9) as well. The gliding velocity was calculated to be 0.4-0.5 μm/s.

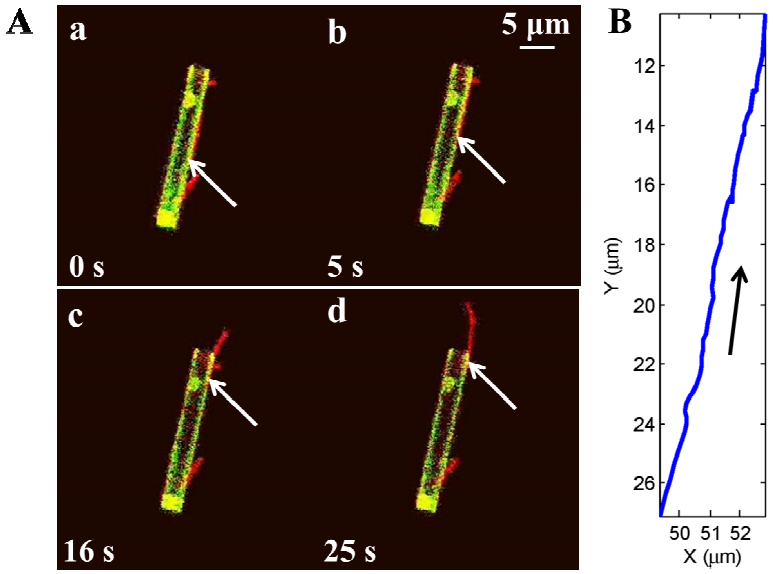


Figure S9 A. Time-lapse images of microtubule moving along the outer wall surface (The white arrows point to the tail of microtubule.). B. Kymograph of the tail of microtubule along the external tube surface (The black arrow points to the moving direction of microtubule).

As comparison, the Ni-NTA modified tubes with no kinesins immobilization were used for motility assay. The modification of tubes with NTA is that NTA can chelate Ni-(II), which specifically binds the His-tagged kinesins to the inner tube walls. NTA itself cannot provide any power to drive the motion of microtubules. This was proved by movie S6 in which microtubule swang in the hollow assembled tube. The entry of microtubule into the tube was probably by Brownian diffusion.

3. Supplementary movies:

Movie S1: Model movie of the experiment;

Movie S2: Movie where the substrate was modified with no kinesins;

Movie S3: Movie of the movement of microtubule in the Ni-NTA functionalized tube;

Movie S4: Movie of the movement of microtubule in the non-functionalized tube;

Movie S5: Movie of microtubule moving along the outer surface of the assembled tube;

Movie S6: Movie of microtubule swinging in the kinesin free but Ni-NTA modified tube

Reference

(1) Coy, D. L.; Hancock, W. O.; Wagenbach, M.; Howard, J. *Nat. Cell Biol.* **1999,** 1, (5), 288-292.

(2) Ho, C.-H.; Limberis, L.; Caldwell, K. D.; Stewart, R. J. *Langmuir.***1998,** 14, (14), 3889-3894.

(3) Yu, T. Y.; Wang, Q.; Johnson, D. S.; Wang, M. D.; Ober, C. K. *Adv. Funct. Mater.* **2005,** 15, (8), 1303-1309.