Supplementary Information

Microtubule nucleation from functionalised SiO_2 EM grid

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Chemicals and Materials:

Silicon Dioxide (SiO_2) 40 nm TEM SQ 100μ nine windows grids were purchased from SPI Supplies Division of STRUCTURE PROBE Inc. Sulphuric acid (98% GR) and Trifluoroacetic acid (HPLC grade) were purchased from Merck. N,N-Diisopropylcarbodiimide (DIC) and (3-Glycidoxypropyl)trimethoxysilane (GOPTS) were purchased from Fluka. Acetone (Analytical Reagent grade) was purchased from Rankem. Diamino-polyethylene glycol with MW 2000(H_2N-PEG_{2000}-NH_2) was purchased from Rapp Polymer. Dry N, N′-Dimethylformamide (DMF) and Chloroform ($CHCl_3$)were purchased from Sigma Aldrich and Fisher Scientific respectively. OtBu protected Tris-(nitriloTris-acetic acid) [(OtBu)Tris-NTA]and EGFP-His_{10} protein were received as gift from Dr. Thomas Surrey's laboratory in EMBL, Heidelberg, Germany (Currently London Cancer Research U.K.). Fluorescence Microscopic Imaging was done by Nikon Eclipse Ti Microscope. Confocal dishes (Coverglass Bottom Dish 35 X 10 mm) were purchased from SPL life sciences. Streptavidin Gold Nanoparticle and Uranyl Acetate dihydrate were purchased from Nanocs and Spectrochem respectively. All the commercially purchased chemicals were used without further purification.

Protein biochemistry: XMAP215-His_7 (full-length XMAP215 with a C-terminal hepta-histidine tag was expressed in insect cells and purified as described[1,2]. Deca-histidine tag GFP (EGFP with a C-terminal deca-histidine tag) was expressed in E. coli and purified as described[3]. All proteins were flash-frozen and stored in liquid nitrogen. Concentrations (Bradford) refer to monomers.Tubulin from pig brain was purified and flash-frozen.Then it was stored in liquid nitrogen at a concentration of 200 and 150 μM, respectively. Tubulin concentrations (UV absorbance) refer to tubulin dimers.

Electron Microscope: Imaging was done using Tecnai T12 Spirit TWIN electron microscope operating at 120kV, magnification range 3000x -120000x and in in low dose (< 1000 e/nm²s) mode with single tilt tomography holder.

Surface Chemistry:

Functionalisation onto 40 nm Silicon dioxide (SiO_2) thin film coated TEM window grids.

Preparation of PEGylated Silicon dioxide TEM window grids: SiO_2 thin film coated TEM grids were treated with concentrated Sulfuric acid under fume hood for 10 min. Grids were washed with MilliQ water and dried carefully under the stream of nitrogen gas. Completely dried grids were treated with (3-Glycidoxypropyl)trimethoxysilane (GOPTS) at 75 ^oC for 60 min and washed with dry acetone. After that grids were dried under stream of nitrogen gas quickly to avoid condensation on grid. A solution (40mg/ml) of Diamino-polyethylene glycol with MW 2000 Da (H_2N-PEG_{2000}-NH_2) in dry N,N-Dimethylformamide (DMF) was prepared in PCR tubes. Grids were dipped into the solution and heated at 75 ^oC for overnight. Grids were cooled and washed with plenty of N,N-Dimethylformamide (DMF) and MilliQ water, thoroughly, and dried under the stream of nitrogen gas.

Tris-NTA functionalisation on the PEGylated SiO_2 thin film coated TEM window grids: PEGylated SiO_2 thin film coated TEM window grids were reacted with 2:1 mixture of free carboxylic acid of Tris-NTA (50mg/mL in Chloroform) and coupling reagent N,N'-Diisopropylcarbodiimide (DIC) at 75 ^oC for 4 hours. Tris-NTA functionalised surfaces were washed with plenty of DMF and MilliQ water. Moist grids were dipped into Trifluoroacetic acid and incubated for 4 hours with occasional gentle and careful shaking. Finally, grids were washed with plenty of MilliQ water followed by drying under the stream of nitrogen gas. Grids were stored at 4^oC.

Microscopy:

Immobilisation of EGFP-His_{10} onto the functionalised SiO_2 grid: 5 µL of 10 mM $NiCl_2$ solution was loaded onto the Tris-NTA functionalised SiO_2 grid and incubated for 5 min. Excess $NiCl_2$ was blotted out by filter paper and Ni-loaded grid was washed immediately with 5 µL BRB80 for five times. Next, 5 µL solution of 500 nM EGFP-His_{10} in BRB80 was loaded onto the Ni-loaded functionalised grid and incubated at 4 °C for 10 min. Unbound EGFP-His_{10} onto the grid was washed out with 50 µL BRB80 and placed in a confocal dish with functionalised side down, filled with BRB80 buffer. Grid was visualised under fluorescence microscope (Nikon Plan Fluor 10X DIC objective). Similar procedure was followed using non-functionalised SiO_2 grid for control experiment.

Immobilisation of XMAP215-His_7 onto the functionalised SiO_2 grid: 5 µL of 10 mM $NiCl_2$ solution was loaded onto the Tris-NTA functionalised SiO_2 grid and incubated for 5 min. Excess $NiCl_2$ was blotted out by filter paper and Ni-loaded grid was washed immediately with 5 µL BRB80 for five times. Next, 5 µL solution of 500 nM XMAP215-His_7 in BRB80 was loaded onto the Ni-loaded functionalised grid and incubated at 4 °C for 10 min. Unbound XMAP215-His_7 was washed out with 50 µL BRB80 and 10 µL water and followed by immediate staining with 2% uranyl acetate for 30 sec. Grid was dried in air and imaged under 120 KV electron microscope. Similar procedure was followed using non-functionalised SiO_2 grid for control experiment.

Microtubule nucleation from XMAP215-His_7 immobilised functionalised SiO_2 grid: 5 µL of 10 mM $NiCl_2$ solution was loaded onto the Tris-NTA functionalised SiO_2 grid and incubated for 5 min. Excess $NiCl_2$ was blotted out by filter paper and Ni-loaded grid was immediately washed with 5 µL BRB80 for 5 times. Next, 5 µL solution of 500 nM XMAP215-His_7in BRB80 was loaded onto the Ni-loaded functionalised grid and incubated at 4 °C for 10 min. Unbound XMAP215-His_7 was washed out with 50 µL BRB80 and kept at 4 °C before loading tubulin mix. Finally, 18.5 µM tubulin in nucleation buffer (BRB80 supplemented with 3 mM Mg-GTP) was loaded on top the functionalised grid and incubated at 37 °C humid chamber for 15 min. Next, the grid was washed with warm 50 µL BRB80, 10 µL water and followed by immediate staining with 2% uranyl acetate for 30 sec. Grid was dried in air and imaged under 120 KV electron microscope.

Control Experiment with non-His tagged protein: 18.5 µM tubulin in BRB80 was loaded on top the functionalised grid and incubated at 4 °C in humid chamber for 5 min. Next, the grid was washed with cold 50 µL BRB80, 10 µL water, followed by immediate staining with 2% uranyl acetate for 30 sec. The grid was dried in air and imaged under 120 KV electron microscope.

References:

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2. Reymann, A. C.,Boujemaa-Paterski, R., Martiel, J. L., Guérin, C., Cao, W., Chin, H. F., De La Cruz, E. M., Théry, M., Blanchoin, L.. (2012) Actin network architecture can determine myosin motor activity. *Science336*, 1310-1314.

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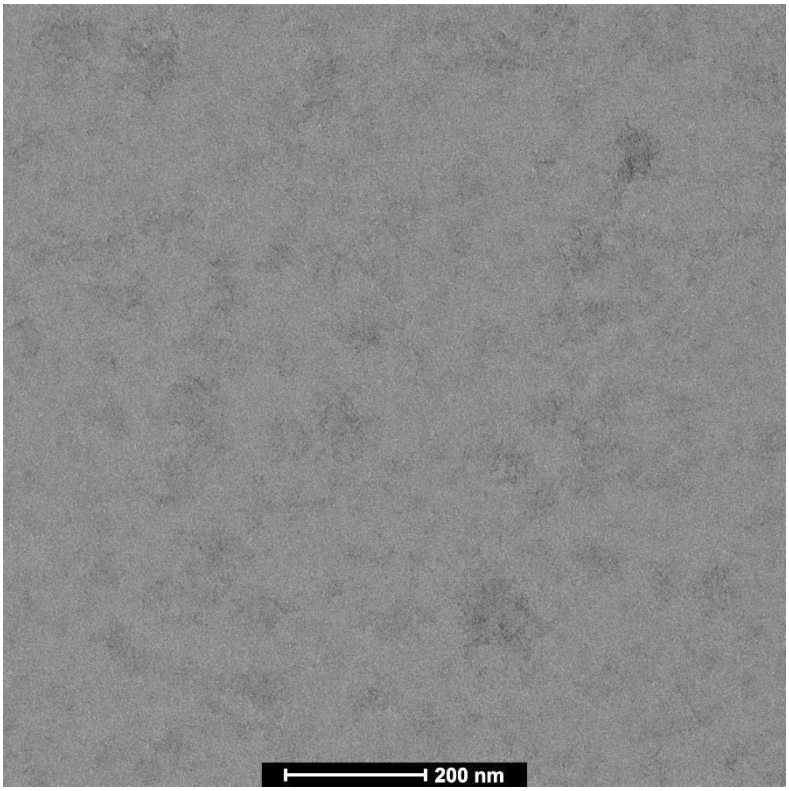

Figure S1. Electron microscopic image of negatively stained functionalised SiO_2 EM grid after loading of tubulin (non His-tagged protein) following similar condition described in XMAP-His_7 immobilisation on functionalised grid. Absences of significant structures indicate that tubulin did not bind with Ni-loaded Tris-NTA functionalised SiO_2 grid.