Supporting Information

Co- and Distinct Existence of Tris-NTA and Biotin Functionality on Individual and Adjacent Micropatterned Surface Generated by Photo-Destruction

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Experimental Section

Chemicals and Materials: Diamino-polyethylene glycol with MW 2000Da (NH_2-PEG_{2000}-NH_2) and mono Boc amino PEG (NH_2-PEG_{3000}-NH-Boc) were purchased from Rapp Polymere.6-Nitroveratryl chloroformate (NVOC chloride), N,N′-Diisopropylcarbodiimide (DIC) and (3-Glycidoxypropyl)trimethoxysilane (GOPTS) were purchased from Fluka. EZ-Link NHS Biotin was purchased from Thermo Scientific. Trifluoroaceticacid (TFA), Sulfuric acid (about 98% GR), hydrochloric acid (HCl), sodium hydroxide (NaOH), hydrogen peroxide (30% solution), acetone, dichloromethane (DCM) and N,N′-dimethylformamide (DMF) were purchased from Merck. Rhodamine Avidin D dye was purchased from Vector laboratories. Adenosine-5′-triphosphate disodium salt (ATP), imidazole and 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES) were purchased from Himedia. 1,4-Piperazinediethanesulfonic acid (PIPES), Cobalt(II) chloride hexahydrate, nickel(II) chloride hexahydrate, ethylene-bis(oxyethylenenitrilo)tetraacetic acid (EGTA), guanosine-5′-triphosphate sodium salt hydrate (GTP), *β*-casein, catalase, glucose oxidase, *β*-mercaptoethanol, atto-488-biotin and paclitaxel were purchased from Sigma Aldrich. Alexa Fluor 568 carboxylic acid succinimidyl ester was purchased from Invitrogen. O^{t}Bu protected Tris-(nitriloTris-acetic acid) [(O^{t}Bu)Tris-NTA] and plasmids of DK_{612}-His_{10}, V_HH-His_6, Mal3-EGFP and EGFP-His_{10} were received as gift from Dr. Thomas Surrey (London Cancer Research). Masks were commercially procured from available chrome-on-glass (COG) positive photo-masks and negative photo-masks were received as a gift from Dr. Thomas Surrey (London Cancer Research). A 150 W Xenon arc lamp and 280-400 nm dichroic mirror for irradiation were purchased from Laser Spectra Services (a subsidiary of Newport Spectra-Physics, Bangalore, India).

Protein Biochemistry:

Tubulin was isolated from goat brain and it was labelled with Alexa Fluor 568 carboxylic acid succinimidyl ester to obtain the Alexa-568-labelled tubulin. The purification of tubulin from goat brain, Alexa-568 labelling of tubulin, and the polymerization of microtubules were performed as

described in the literature[1]. Deca-histidine tagged EGFP(EGFP-His_{10}), Kinesin612-His_{10}, Mal3-EGFP and V_HH-His_6 proteins were expressed in E-coli and purified through Ni-NTA column in our laboratory.

Methods:

In this paper we have shown two approaches for creating dual functionalised micropatterned surfaces of biotin and Tris-NTA. In case of first approach we have obtained **biotin and Tris-NTA on the same micropattern** (**TBSMP**) and in case of second approach **biotin and Tris-NTA present individually in the adjacent micropattern** (**TBAMP**). Details methods are discussed below.

Preparation of dual functionalised glass surface of biotin and Tris-NTA having both in the same micropattern (TBSMP):

Functionalisation of glass surface with Tris-NTA and biotin separately was already reported[2]. Here, first time, we describe the dual functionalisation. Glass cover slips (50X50 mm) were cleaned with 3M NaOH followed by sonication for 5 minutes. Next, cover slips were cleaned with plenty of water and were treated with piranha (2:3 mixture of hydrogen peroxide and sulphuric acid) followed by ultra-sonication for 30 min under fume hood. Piranha solution was discarded and glass slides were thoroughly cleaned with water and were dried under stream of nitrogen gas. Silanisation of glass surfaces has been achieved by treating with 3-glycidoxypropyltrimethoxysilane (GOPTS) at 75 °C. Next, silanised glass surfaces were treated with 1:1 mixture of diamino-polyethylene glycol and mono-Boc protected polyethylene glycol amine followed by heating at 75 °C for overnight. The polyethylene glycol functionalised surfaces were washed with DMF and plenty of water for complete removal of excess and unreacted polyethylene glycols from surfaces. Next, surface was treated with biotin-NHS for 2 hour at 75 °C followed by washing with DMF and plenty of water. Finally, tert-butyloxycarbonyl protection from polyethylene glycol surface was removed using trifluoroacetic acid, treated for 3 hours and followed by washing with water and dried under N_2 gas stream. Dried glass surface was treated with 2:1 (v/v) mixture of O*t*Bu protected Tris-NTA solution in dry chloroform (20 mg/mL) and DIC at room temperature and was incubated at 75 °C for 4 hours. Finally glass surface was washed with DMF and water and treated with TFA for removal of tertiary butyl esters protection of Tris-NTA. Glass surfaces were washed with plenty of water dried under N_2 gas stream and stored at 4 °C.

Chemical modification of glass surface with 6-Nitroveratryl chloroformate (NVOC chloride) for ″TBAMP″ surface:

We have treated the silanised glass surface (prepared as described before) with diamino-polyethylene glycols (PEG2000) for overnight at 75 °C. The polyethylene glycol functionalised surfaces were washed with DMF for removal of excess and unreacted diamino-polyethylene

glycol and then with plenty of water, dried under N_2 stream. Finally, polyethylene glycol surface was treated with NVOC chloride at 75 °C in dark room[3]. After 1 hour the excess NVOC chloride was washed with chloroform and plenty of water followed by drying under stream of N_2 gas and stored at 4 °C.

UV illumination and immobilization of oligohistidine and biotin tagged biomolecules on the ″TBSMP″ surface and imaging.

The dual functionalised glass surface having biotin and Tris-NTA both in the same micropattern was washed with 100 mM hydrochloric acid, followed by HEPES buffer (20 mM, pH 7.5). For generation of micropatterned surface, glass surface was incubated with 10 mM $CoCl_2$ solution for 7 min and then it was illuminated through microstructured positive photo-mask with UV light produced by 150 W Xenon arc lamp and 280-400 nm dichroic mirror for 2 hours[2b]. Micropatterned glass coverslips were washed by 100 mM hydrochloric acid solution, followed by HEPES buffer and kept in a humid chamber until use. Glass surface was incubated with 10 mM $NiCl_2$ solution for 10 min and it was washed by 200 mM imidazole solution, followed by 20 mM HEPES buffer. A flow chamber was constructed with Ni^{+2}-loaded functionalised glass keeping the functional side down and one poly-L-lysine (PLL)-PEG passivated counter glass, separated by two strips of double sticky tape (Tesa; Hamburg, Germany)[2d]. Flow chamber was equilibrated with *β*-casein solution (1mg/mL) in HEPES buffer for 7 min, placing it on an ice-cold metal block. For visualisation of the pattern, flow chamber was incubated with Solution of EGFP-His_{10} protein (100 nM) and Rhodamin Avidin D dye (100 nM) in HEPES buffer for 7 min. Unbound dyes were washed out by HEPES buffer very well and sealed the flow chamber. Micropatterned surfaces were imaged by 60X objective of NIKON inverted fluoroscence microscope (Eclipse Ti-U) using 488 and 561 nm filter (Figure 1a, b). For relative study experiment (Figure1c, d) we followed the same process and took the images of 20, 30, 45, 60, 90 and 120 min UV light illuminated micropatterned surfaces.

Preparation of side by side biotin and Tris-NTA micropattern as per ″TBAMP″ approach (all the work was performed in dark condition):

For generation of micropatterned surface, NVOC functionalised[3] glass surfaces were illuminated through the negative photo-mask with UV light for 30 min. The position of mask was marked with diamond pen on the glass. The active amino functional ($-NH_2$) group was generated onto the UV light exposed areas (Figure S1). Then Glass surface was washed by HEPES buffer and dried by N_2 gas. Now, newly generated amino functional group was reacted with 2:1 (v/v) mixture of O*t*Bu protected Tris-NTA (15 mg/mL) and DIC in dry chloroform at 75 °C for 4 hours. After that, the unreacted Tris-NTA was removed by washing with plenty of chloroform followed by water and dried with N_2 gas. Then, for biotin functionalisation the position of the negative photo mask was slightly manually changed from the previously marked position and glass surface was illuminated for another 30 min through negative photo-mask. Again amino functional group was generated in different UV light exposed position onto the surface. Further,

newly generated amino functional group was reacted with Biotin-NHS (34 mg/mL) in DMF solution for 2 hour at 75 °C followed by washing with DMF and plenty of water. Next, tertiary butyl esters protection of Tris-NTA was removed by TFA. Finally, the glass surface was washed with water and dried under N_2 gas. Similar method, as described before, was followed for visualization of side by side biotin and Tris-NTA micropatterned surfaces (Figure 3).

Preparation of Alexa Fluor568 labeledTaxol stabilised microtubules (MT)[2b, d]:

Mixture A: 0.5 µL Alexa-568 Tubulin (15 mg/mL) + 2 µL Tubulin (20 mg/mL) + 2.3 µL BRB80 (80 mM PIPES, 1 mM EGTA, 1 mM $MgCl_2$, pH 6.8) + 0.2 µL GTP-Mg (25 mM).

Mixture B: 180 µL of BRB80 and 0.36 µLTaxol (20 µM)

Mixture **A** and **B** were warmed at 37 ° C for 20 minutes and then 45 µL mixture B was added into the mixture **A** and mixed carefully. Then the mixture was centrifuged for 8 min at 12000 rpm at 37 ºC. Then the supernatant was carefully taken out and the red colored pellet was dissolved into the 10 µL mixture **B** solution and stored at room temperature covered with aluminium foil.

Transport of microtubules on ″TBSMP″ surface:

We made flow chamber with Ni^{+2}-loaded dual functionalised glass surface as described before. Flow chamber was equilibrated with *β*-casein solution (1 mg/mL) in HEPES buffer for 7 min, placing it on an ice-cold metal block. To observe the micropattern, we incubated the flow chamber with neutravidin (0.3 µM) solution in HEPES buffer for 10 min. To remove the excess neutravidin after binding with the biotin, flow chamber was washed with HEPES buffer and incubated with 84 nM atto-488-biotin dye solution in HEPES buffer for 7 min. Finally, flow chamber was washed with HEPES buffer to remove the excess dye solution. Now, the flow chamber was exchanged with BRB80 buffer and washed with same buffer containing 200 mM KCl. For microtubule gliding experiment, the flow chamber was incubated with 50 nM $DK_{612}His_{10}$ motor protein in BRB80 buffer containing 200 mM KCl. After 7 min the excess motor protein was washed out by BRB80 buffer containing 200 mM KCl and finally flow chamber was washed with BRB80 buffer to remove excess KCl. Now, motility buffer containing BRB80, taxol (20 µM), *β*-mercaptoethanol (2 mM), ATP (2 mM) was flowed into the flow chamber and it was allowed to warm up to room temperature. Taxol stabilised alexa-568-labelled microtubule solution (stock MT solution, BRB80 buffer, 20 µM taxol, 2 mM Mg-ATP, 1 mM GTP, 1 mM $MgCl_2$) containing oxygen scavenger (20% glucose, 1 mg/mL glucose oxidase and 0.5 mg/mL catalase) was flowed into the chamber. Flow chamber was sealed and observed under TIRF microscope. Time lapse images were taken using 488 and 561 nm laser through an IX-81 fluoroscence microscope (Olympus) with a 60X objective (Olympus; Hamburg, Germany) and an Andor iXon3 897 camera (Figure2a, Movie-S1).

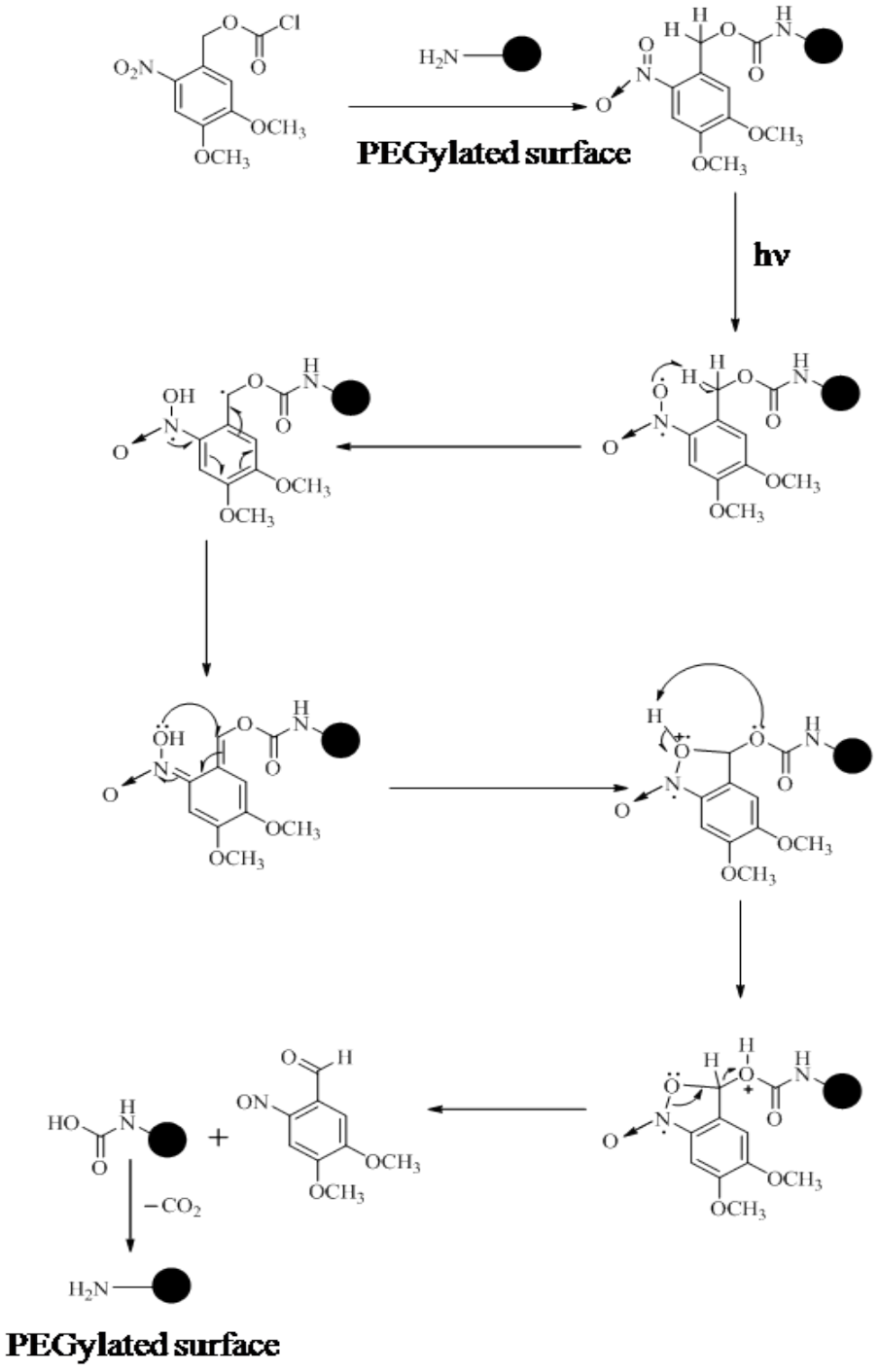
Binding of Mal3-EGFP through V_HH-His_6 antibody and avidin rhodamine red dye on ″TBAMP″ surface:

A flow chamber was constructed with the side by side Tris-NTA and biotin micropatterned glass surface with a counter glass as described before. Flow chamber was equilibrated with β-casein solution (1 mg/mL) in HEPES buffer for 7 min, placing it on an ice-cold metal block. To observe the biotin micropattern 100 nM avidin rhodamin dye in HEPES buffer was flowed into the chamber and incubated for 10 min. Excess avidin rhodamin dye was washed out by repeated washing with HEPES buffer. Next, 500 nM V_HH-His_6 antibody in HEPES buffer was flowed into the flow chamber and incubated for 10 min at 4ºC followed by removal of excess and unbound antibody by repeated washing with HEPES buffer. Next, flow chamber was equilibrated with cold BRB80. 100 nM Mal3-EGFP solution in BRB80 were flowed into the flow chamber and was incubated for 10 min. The flow chamber was washed by BRB80 for removal of excess and unbound protein. Finally, the flow chamber was equilibrated with oxygen scavenger system, containing 20 mM glucose, 1 mg/mL glucose oxidase and 0.5 mg/mL catalase and micropatterns were imaged by 60X objective of NIKON inverted fluoroscence microscope (Eclipse Ti-U) using 488 and 561 filter (Figure 4).

Movie S1: Gliding of microtubule along the dual functionalised line micropatterned surface having Tris-NTA and biotin in the same micropattern:

Data Analysis: All data analysis was performed using Image J software.

A proposed mechanism of photo-destruction step in case of "TBAMP" surface[4]:



Scheme S1.

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