Reversible and Directional Self-Assembly of Bio-Molecular Templates for Nanotechnology Interconnects

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In recent years, the exponential growth in semiconductor technology has been sustained by extending the capabilities of top-down manufacturing processes based on lithography to shorter and shorter wavelengths. Unfortunately, the costs of these top-down approaches are projected to be prohibitive at sizes and tolerances in the nanometer size range. In response, a new paradigm has arisen based on the bottom-up or molecular engineering approach to the mass replication of nanoscale electronic circuits that promises to be cheaper, more flexible, and efficient. Control of interconnections emerges as one of the major challenges in the development of these bottom-up approaches. Microtubules (MT) are self-assembled subcellular proteinaceous filaments with nanometer scale diameters and micrometer scale lengths. MTs are biopolymers assembled from two, related protein monomers; α and β tubulin. The aspect ratio of MT, the reversibility of their assembly and their ability to be metallized by electroless plating make them excellent candidates to serve as templates for the fabrication of nanowires. Within the cell, the slower-growing microtubules minus ends are tethered to microtubule-organizing centers, and the faster growing plus ends extend into the cytoplasm. γ-tubulin, a tubulin isoform, is believed to nucleate MTs by forming nucleation complexes (NC). Our work focuses on developing technology for bottom-up approaches to nano-electronics manufacturing inspired by biological processes. The ability to interface biological structures, such as MT to an inorganic substrate is a prerequisite to the development of such an approach toward the controlled fabrication of nanoscale interconnects between microelectronic devices on silicon wafer.

Our "in situ" approach to manufacturing a MT interconnection on a silicon wafer consists of (see Fig. 1)(a) a starting electrode functionalized with a derivatized MT nucleating complex (cap) via specific ligands, (b) controlled growth of MTs from the starting electrodes toward a target electrode, (c) binding of the MT plus end to capping agent bound to the target electrode via specific ligands, and (d) disassembly of uncapped MTs and subsequent metallization of interconnecting protein template. The project has several tasks that overlap in time: (1) Synthesis of end specific capping proteins, (2) Development of linkage and inorganic surface functionalization, (3) Control of MT growth and stability, (4) Metallization of MTs and (5) Protection, encapsulation and testing of nanointerconnects.

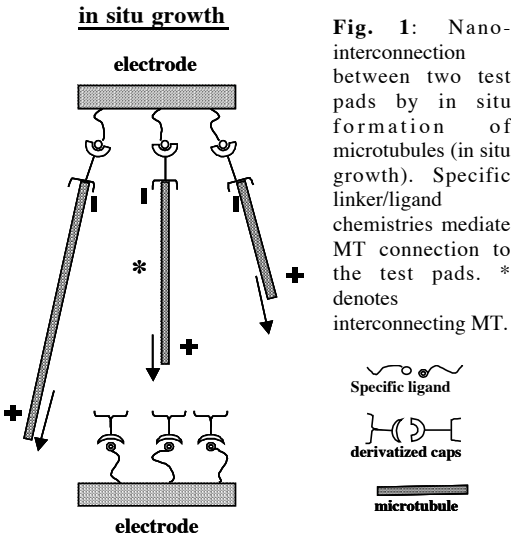

Fig. 1: Nano-interconnection between two test pads by in situ formation of microtubules (in situ growth). Specific linker/ligand chemistries mediate MT connection to the test pads. * denotes interconnecting MT.

(1) Synthesis of a Gamma-tubulin fusion protein for the nucleation of microtubules from functionalized electrodes

We have chosen the fusion protein, GST(Glutathione s-transferase) and γ-tubulin, as nucleating agent for the initiation of microtubule growth.. The GST-γ-tubulin was developed by extracting RNA from human cells, and amplifying the human γ-tubulin using RT-PCR. That clone was then sequenced

to verify that it was the appropriate gamma tubulin, and the entire gene was present. The sequence was cloned into a plasmid containing Glutathione S-transferase (GST), which resulted in the creating of a recombinant GST-gamma tubulin protein. The recombinant plasmid was then inserted into *Escherichia coli*. The *E. coli* were then grown in large flasks of growth media containing ampicillin to select for those *E. coli* containing the plasmid of interest. When the culture reached an optical density of 0.9, 1mM Isopropyl-_-D-thiogalactopyranoside (IPTG) was added to induce the expression of the GST-gamma tubulin. *E. coli* were collected via centrifugation and then lysed using lysozyme. The supernatant was loaded onto a column that binds to the GST protein. Unbound proteins were washed from the column and the GST-gamma tubulin was eluted using free glutathione. The protein was verified using SDS-PAGE electrophoresis and found to be the correct size, 85kD. The protein was also verified using ELISA, and antibodies to both the GST and γ-tubulin were highly specific. A similar approach is currently used to synthesize a GST-_-tubulin MT plus end cap for functionalizing target electrodes.

(2) Functionalization of gold electrodes with self-assembled monolayers for attachment of fusion protein

We have investigated the application of self-assembly of reactive alkanethiols together with genetically engineered fusion protein to develop a protein assembling method for incorporation of MTs as bio-interconnects. Reactive Au surfaces have been functionalized using functionalized self assembled monolayers (FSAM's). FSAM of carboxylic acid terminated alkanethiol followed by coupling of specific ligands for selective binding and attachment of a derivatized microtubule-nucleating protein. The nucleating protein, GST-γ-tubulin, is the fusion protein of GST(Glutathione s-transferase) and γ-tubulin, described in Activity 1. GST serves as a linking tail to γ-tubulin, which can bind very specifically to Glutathione (anti-GST). Anti-GST is bound to the FSAM through the carboxylic acid group at the end of the alkyl chains (Fig. 2(a)). A specific immunoglobulin for the anti-_-tubulin bearing a fluorescent moiety (IgG-Cy3) is used as evidence and to quantify the extent of the formation of the protein assembly. Strong fluorescence from a functionalized gold electrode on a SiO_2 substrate coated with the fusion protein indicates that the approach we have developed gives a very good coverage of the electrode (Fig. 2(b)).

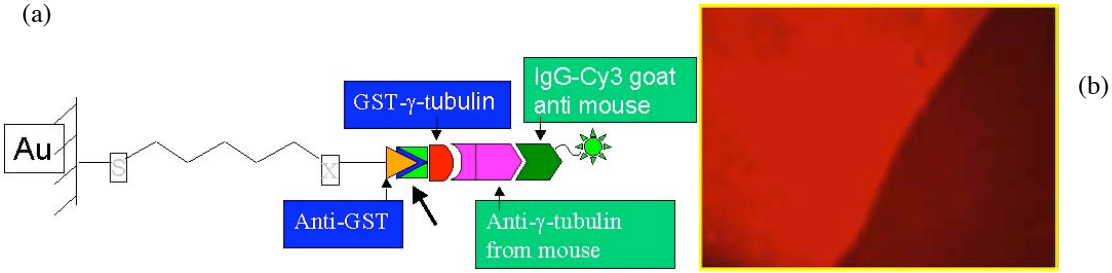

Fig. 2: (a) Schematic of functionalized gold surface. S stands for sulfur, X for carboxylic acid. The fusion protein GST- _-tubulin binds to the anti-_-tubulin. A specific immunoglobulin for the anti-_-tubulin bearing a fluorescent moiety (IgG-Cy3) is incorporated as evidence and extent of the formation of the protein assembly. (b) The strong actual fluorescence of the gold electrode on a SiO_2 substrate in the fluorescence microscopy image on the right, indicates good coverage of the electrode.

In addition to experimental development of ligand chemistries, an ab-initio simulation approach has been developed towards building a database of ligands targeting proteins to self-assembled monolayers of alkanethiols on gold. The approach is based on interactions between chelating agents and aminoacid moieties. The results suggest that high-level quantum mechanical methods

applied to relatively small systems can be used to develop adequately a database of self-assembled monolayers-ligand-protein interactions.

(3) Controlled nucleation and growth of MT from _-tubulin functionalized gold electrode

In-vitro MT assembly is performed in PEM 80 buffer at PH~6.9 with a final concentration of tubulin of 1.5 mg/ml. Polymerization is performed by the addition of GTP. Taxol is added as a stabilizing agent during polymerization. Immersion of functionalized electrodes in the solution containing _ and _ tubulin leads to the nucleation and assembly of MTs from the electrode as shown in Fig. 3(a). This process results in MTs attached and growing from the electrodes that can be subsequently aligned and directed by flow over the surface (Fig. 3(b)).

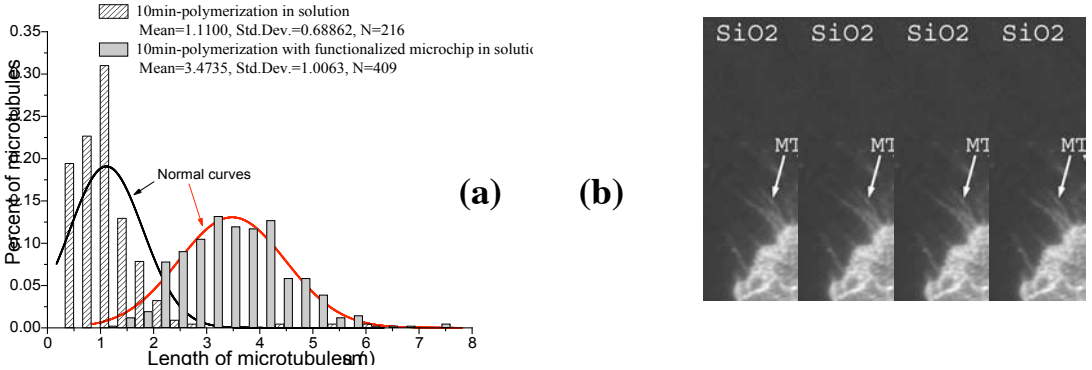

Fig. 3: (a) Length distribution of MT polymerized in solution and from a _-tubulin functionalized gold electrode showing that _-tubulin nucleates and promotes MT growth (temperature is 37ºC); (b) Fluorescent microscope image of MT nucleated and grown for 30min from a functionalized gold substrate. The alignment of the MTs by flow demonstrates that MTs are bound by one end to the substrate via the _-tubulin.

A multiscale kinetic Monte Carlo simulation model of MT growth has also been developed to understanding the effect of tubulin concentration and tubulin diffusion on the dynamics of MT growth from nucleation centers. The model predicts strong diffusion-controlled competition between growing MTs for tubulin in solution leading to growth morphologies comparable to those observed in experiments.

(4) Metallization of MTs

Copper has emerged as the metal of choice for interconnect structures in the fabrication of modern high-speed integrated circuits. Copper metallization of microtubules has been accomplished using biologically compatible benign chemistries and through the method of electroless deposition. Copper reduction was initiated from a copper sulfate solution using ascorbic acid. Acetic acid was incorporated into the chemistry to alter the kinetics of reduction of copper and control copper particle size. Deposition of copper onto the microtubules was obtained under a pH vicinity of 4 for roughly 4 minutes (see Fig. 4).

Fig. 4: Microtubules metallized with Cu imaged using scanning electron microscopy (SEM). The presence of copper particles on the microtubule surface was confirmed using energy-dispersive spectroscopy (EDS). The thickness of the Cu coating ranges from 5 to 45nm.

References

[1] For further information about this project email:
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