Annual Report for AOARD Grant FA2386-10-1-4059 "**Research Title**" Biological information processing in single microtubules

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Name of Principal Investigators: Anirban Bandyopadhyay
- e-mail address : anirban.bandyo@gmail.com
- Institution : National Institute for Materials science
- Mailing Address : 1-2-1 Sengen, Main Building 815, Tsukuba
- Phone : +81-29-859-2167
- Fax : +81-29-859-2801

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Abstract: For the first time in the world we have isolated single brain microtubule reproduced it 200000 times faster than the conventional methods. This radio wave controlled technology to condense proteins adopting protein synchrony. We could take proteins from various samples and could generate synchrony in the water solution; this technology in future would help us in instant testing of inventory drug molecules. Until date it was not known why potential flux moves in the brain at a speed of 400km/hr, when electrons move only a few cm in years, we have found that through microtubule, solitons propagate at the speed of sound which explains mysterious high speed communication in the brain. We have defined what does it meant by information, everywhere we listen to the phrase "brain's information processing" but what does that visualization means? We have experimentally found and suggested it is "soliton".

Introduction: We started working on the brain microtubule way back in 2008, since, I understood that in the brain, neurons separated by 6 inches, synchronize, get phase and frequency locked and that is the source of enormous computability of the brain. However, no experimental evidence existed at that point of time. We became the first group in the world to study single microtubule electronics reliably and identify resonance band of these biological architectures, which was very essential to understand synchrony that would lead to phase and frequency lock conditions in the entire brain. Another problem bothered us for a long time; if we want to compute similar level of complex problems, we need 1000s of megawatts of energy; how brain does that using only 25 watt? We got an answer for that too, we discovered the soliton based information processing in the microtubule, which ensures dissipation less transport of information across the brain. Finally, that I desperately wanted to confirm was the potential of synchrony in brain computing, does brain execute non-linear frequency pulling to introduce simultaneity and drastically reduce the computing time? Yes, brain does that; we could even generate microtubules 200,000 times faster than the conventional methods, only if we triggered synchrony by applying a radio wave to the proteins in a heat bath.

Experiment: We felt three years back in 2008, that it was not sufficient just to try to build the artificial brain, when several fundamental mechanisms for brain operation was not clear—learning and memorizing like fundamental issues of our biological brain is a mystery. We started the microtubule research, extracted from neurons of human brain and several other living species. It was for the first time in the world; the electronics of the material that governed the evolution of living species for 3.5 billion years has been measured in a simple water beaker by exposing radio wave to the solution. The origin of high neurotransmission speed 400km/hr has been a mystery, we unraveled experimentally that soliton delivers the speed, and it defines the unit of information for brain computing. We have also resolved how two contradictory physical phenomena, memory and learning are realized

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14. ABSTRACT
This is the first annual report of a project isolate single brain microtubules reproduced 200,000 times faster than by conventional methods, by using radio wave controlled technology.

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simultaneously in the brain. Finally, we are able to track 3.5 billion years old evolution of living species by ultra-fast protein synchrony.
Porcine's brain, Tree, Algae extracted tubulins (Cytoskeleton, Denver, CO), were preserved at -80o C. To polymerize tubulin into microtubules, Microtubule cushion buffer (60 % v/v glycerol, 80 mM PIPES pH 6.8, 1 mM EGTA, 1 mM MgCl2) was added to General Tubulin Buffer (GTB, 80 mM PIPES pH 7, 1 mM EGTA, 2 mM MgCl2) and/or GTP solution. The mixture is added to 1 mg of tubulin. At 37o C, 10 mM tubulin leads to an uncontrolled growth of microtubule >100μm, we maintain this tubulin concentration for all experiments. GTP, TX (Taxol), VB (Vinblastine), K352 (Pironetin), N-termini, tau, CH (Colchicine) were optionally added to this solution individually or as a combination, produced microtubules were isolated for single nanowire measurement of resonance levels. Ultrasound power varied between pico-watt to femto-watt, if Mg2+ is not added, effect of ultrasound pumping is not observed, so Mg2+ is essential, GTP is not. Phase coherent signals were measured for all combinations of tubulin mixtures when placed in a heat-bath for pure ac pumping. The heat bath of Fig. 2a was placed inside the Raman measurement chamber and growth profile was measured with 532nm laser light from basic Raman spectrum switching between protein and microtubule nanowire shows convergence of Raman vibrations. We have ruled out possibilities of other physical processes detailed arguments in online text B. Rejection arguments: Rejection of DEP, rapid crystallization, ion-induced growth, self-assembly in the collapse of matter. For consistency, all figures in this paper are produced from porcine brain tubulin.

Results and Discussion: Here we describe the experimental results. Chemical route to synthesis microtubule is well established; instead, we develop radiofrequency-induced synchrony to fabricate microtubule-nanowires. Figure 2a shows the design of ac pumping and phase coherent signal capturing devices embedded together. The tubulin solution is added on this chip as a micrometer thick water film, the entire chip is covered with multilayered insulators for operating it as a heat bath. Within a millisecond of ac pumping, microtubule nanowire is produced, and an instantaneous burst of multiple phase coherent signals are captured in the coupled nano-probe circuit (experimental data is shown below Figure 2b scheme). Since protein oscillators are dissolved in water and the water molecules inside the film are continuously polarized before relaxation terminates25 by pumping alternating radiofrequency signal (MHz), proteins respond simultaneously in an orderly manner, which essentially forms the coupled-oscillator network.

Initially, the proteins oscillate with different phases and frequencies, then, due to delocalized energy transfer, attempt to reach equilibrium converge to a single frequency, initiating synchrony.17 Due to ac coupling through water, phase of all oscillators change in a sinusoidal manner, this is called normal modes of vibration, which squeezes to a single phase under strong coupling. If the phase and frequency of all participating proteins were not locked, coherent emission would disrupt immediately. However, the locked criterion sustains when all molecules are arranged in a particular symmetry inside the water film, from which one-step to reach global minima of entropy causes phase transition from liquid to the solid-state structure, which is microtubule. To prove this hypothesis, we pump tubulins without using GTP; then, we find microtubules at the bottom of the heat bath, but broken into small parts (~4μm) along the length, which suggests that even if GTP-hydrolysis that chemically constitutes microtubule14-16 is avoided completely, synchrony is sufficient to assemble proteins into a microtubular form (Fig. 2d). During this phase transition in water, a large number of oscillators switch simultaneously between two fixed energy states, which emit instantaneous burst of phase coherent signal. Unlike conventional assembly process, here reconstitution is extremely fast (105 times the growth rate without ac pumping and with GTP, Fig. 2c) and similar to known coherent collapse we detect ultra-low coherence rich signals, so, instead of assembly, we call it a collapse.

The growth rate of microtubule increases instantaneously as the ac signal is pumped, then

immediately it falls to zero (Fig. 2c inset), in contrast, for natural reconstitution without ac pumping, growth rate increases very slowly and then it remains constant. This suggests that ac pumping does not allow microtubule to grow beyond a certain length as coherent communication disrupts beyond that limit. Moreover, the average length of microtubule remains constant 25μm even if ac pumping is continued after growth rate falls to zero (Fig. 2c). Since average maximum length of microtubule is ~25μm inside a living cell, ultrasound induced non-coherent to coherent signal conversion mechanism and coherent communication among proteins should play a role in the microtubule growth.

Figure 2e,f shows a typical variation of average microtubule length with ac-pumping frequency in presence of GTP, which demands the necessity for several metastable states in the high radio-frequency region that triggers the synchrony of tubulin-assembly. The decomposition of length-frequency variation plot unravels seven such states in Figure S2, which requires laser like actions. The frequency of external ac pumping selects the particular structural symmetry of tubulin to resonate, therefore, six tubulin sites (+C-termini) observed in the microtubules of the living species and seven docking sites identified through molecular dynamics study of tubulin structure have one to one correspondences with these seven metastable states.

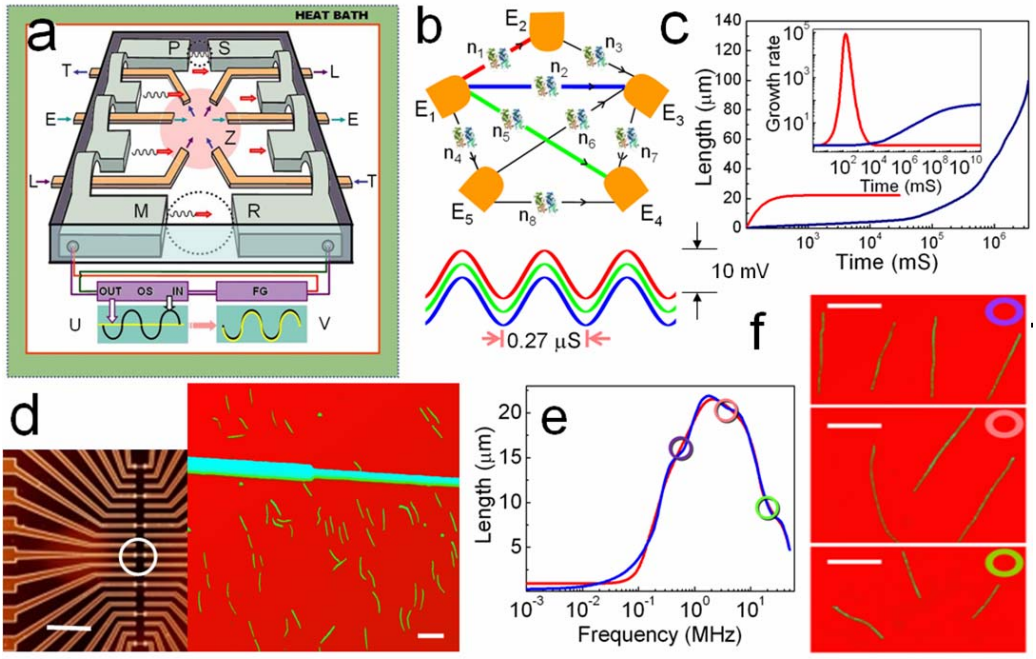

Figure 2. The synthesis of protein condensate: a. The heat bath. As FC forms, PS & MR (200nm gates) close, within ~100ms of Function Generator's (FG) triggering. Instantly, INput/OUTput U at OScilloscope converts to V. The L, E, T electrodes detect coherent signal in the electrically neutral circular region Z. **b**. Three coherent signals measured 1pico-Watt to 10femto-watt (bottom) during condensation (E-electrode (L,E,T), *n*-TD). **c**. Growth rate with GTP for MT when pumped (blue) and not pumped (red). Inset: MT's <L> with time-lapse when FC sets in (blue), in normal in-vitro reconstitution (red). **d**. SEM image of electrode array of heat bath (scale bar 300μm), circular region zoomed, AFM image of MT formed without using GTP (scale bar 4.5μm). **e.** MTs <L> plot, experimental (red) and theoretical (blue) against frequency ω. SEM image of heat-bath electrodes (scale bar 1mm, inset). **f**. AFM images of MTs (top-to-bottom) 500kHz(scale~7μm), 5MHz(scale~5μm) and 15MHz(scale~7μm) on SiO_2/Si (right).

List of Publications:

1. Computational myths and mysteries that have grown around microtubule in the last half a century and their possible verification S. Sahu, S. Ghosh, D. Fujita, A. Bandyopadhyay Journal of Computational and Theoretical Nanoscience (Special Issue) 8, 1-7 (2011), also selected for cover page

Lectures given in the international conferences and in the universities.

1. Remarkable electronic properties of a single Microtubule Google Mountain view campus, workshop on quantum biology 22 October 2010
http://www.youtube.com/watch?v=VQngptkPYE8

2. Practical computing with organic molecules Design and synthesis of a 3D nano brain, International symposium for Young Organic Chemists, Tsukuba, Japan March 1-3, (2011)

3. Quantum aspects of microtubule: Direct experimental evidence for the existence of quantum states in microtubule, Towards a science of consciousness May 2-8 (2011), Sweden

4. Electromagnetic energy of cells and microtubule: how microtubule research will revolutionize the human technologies, Czech Republic 1-3 July 2011

Lecture given in University of Arizona
http://streaming.biocom.arizona.edu/categories/?id=143

DD882: The invention disclosure process is underway. We hope by the end of the next year, we will be able to submit the invention disclosure with significant details.

This document may be as long or as short as needed to give a fair account of the work performed during the period of performance. There will be variations depending on the scope of the work. As such, there is no length or formatting constraints for the final report. Include as many charts and figures as required to explain the work. A final report submission very similar to a full length journal article will be sufficient in most cases.