

Annual Report for AOARD Grant FA2386-11-1-0001 "**Research Title**" Study of opto-electronic properties of a single microtubule in the microwave regime,

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Abstract: We establish the supremacy of synchrony by proving that though apparently microtubule grows continuously from tubulins, in that growth randomly triggered rapid growth and long silence modes are superimposed by synchrony and de-synchrony glues. When such synchrony is introduced in an artificial cell like environment, microtubule does not grow freely, similar to living cell a lower and upper limit appears which was impossible to observe outside the living cells. We test synchrony protocol in plants, animals and in fungi tubulins to find that the limits for length and speed are valid therein; however, they do not synchronize at a common signal frequency, rather, select isolated & distinct frequency domains. Such a sharing of frequency space cannot occur right now, since, plants, fungi and animal cells were separated more than three billion years before. Thus, a hitherto unknown parameter synchrony actively defines fundamental parameters for the cellular operations.

1. Introduction:

A change in the continuous growth, decay and catastrophe of microtubule namely dynamic instability is a vital cellular expression used to estimate the effect of artificially designed drugs, language of cell-defense under foreign invasions etc¹⁻³. All these interpretations would change if one finds that multiple events are occurring invisible to our measurements. The rate of growth is surprisingly distinct across the species to regulate the vital cell processes—disrupted, if there is a modest change in this rate⁴⁻⁶(Figure 1A). Therefore, one can tune delicate cellular features like chromosomal instability⁷, nuclear transport⁸ simply by targeting the dynamic instability. The tubulins from plant, animal and fungi retains 90-95% genetic similarity⁹, yet plant-microtubules rapidly recognize to re-organize with the environmental changes, animal or fungal cannot, in contrast animal-microtubules are extremely sensitive to regulate the growth precisely¹⁰. The universal growth-rate control mechanism cutting across the species is unresolved—several basic questions of this primary activity of living cells have remained unanswered. For example, no *in vitro* synthesis could reproduce lower (200 nm) and upper length limits (24 μ m), as observed *in vivo* wherein limits follow unique relationships with the cell-shape. How microtubule defines its limits? Unlimited growth and random speeds of growth are always observed *in vitro*, even, the dynamic instability is modeled as a stochastic & random process¹¹. Yet, major contemporary discoveries on dynamic instability ignore the limitations of *in-vitro* growth scenarios¹²⁻¹⁴.

The missing common minimum protocol in microtubules of different species:

Plenty of mathematical models within the chemical kinetics framework fit the conventional *in vitro* growth process¹⁵. Though advanced tools allow observing dynamic instability with a nano-seconds resolution, the existing models do not explore beyond the random fluctuations of growth, recovery and catastrophe protocol¹⁶. Literatures have argued for the concerted

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actions of tubulins with the stabilizers and de-stabilizers to regulate the microtubule dynamics¹⁴; however, no state-of-the-art study is done to underpin the origin and mechanism of concerted action. Thus, studies have neglected the possibility that all isoforms & homologs of plant, animal and fungal tubulin, drug molecules and ions inside a single cell¹⁷ could still follow a single communication protocol to decide collectively the speed and the growth limits.

Fusion of "synchrony" and "dynamic instability":

Here, as we bring in the additional experimental criterion—triggered-synchrony of proteins¹⁸—to the conventional in vitro route, the missing speed-control and limiting-length-control return from in-vivo to in-vitro (Figure 1B). In synchrony, the applied field expands and contracts two or more tubulin dimers together at the same time, (same phase and frequency). Here, an artificial cell-like environment (Figure 2A) works as cavity so that the pumped ac electromagnetic signal reflects within the geometrical boundary, repeatedly, to induce synchrony such that the spontaneous decay stops, and the effect that is only visible to us is the effect of pure growth. In conventional non-synchronized in vitro synthesis, we do not observe a rapid mode or a silent mode, though the possibility for a “stop” mode¹⁹ and rapid-growth²⁰ were suggested earlier. Dynamic instability is not a sequential growth process as believed earlier instead, parallel wireless communication¹⁴ among proteins govern the process, as it the foundation of synchrony. By introducing and disrupting synchrony, we could activate or deactivate the limiting length control and speed control in vitro (Figure 2B).

Protein-synchrony is so generic that even pristine tubulin forms cylindrical shape without GTP. This finding would seek modifications in the models to answer one of the hallmark questions of microtubule research (Why does microtubule grow and shrink?^{21, 22}), and supports particularly controversial findings^{23, 24}. Our direct experimental evidence is consistent with the previous proposals that have challenged the role of GTP²⁵. The GTP, like other neuro-drugs, controls dynamic instability following particular synchrony rules. The rule is that the positive catalyst to synchrony (stabilizer) grows microtubule to a longer limit and at faster speed, the negative catalyst does the reverse (de-stabilizer, Figures, 4 A B).

By collecting tubulin samples from species that radically differs in genetic signature [porcine (brain neuron), human (MCF 7 active breast cancer cell), fungi²⁶ (Agaricus bisporus mushroom,) and plant (six days old soybean germ lings)] we have experimentally proved that synchrony activates at particular frequency domain for a particular species, the remarkable frequency-space sharing is unprecedented (Figure 3B). However, tubulins extracted from breast cancer cells are so sensitive to synchrony that any ac frequency from 1kHz to 50MHz they grow limit-less, thus, one could detect cancer tubulin directly using the synchrony-test²⁷.

2. Results and discussions:

Creation of an artificial cell-like environment:

A living cell has nano-gaps in the cell membrane to exchange molecular & ionic components; we have kept such provision in the artificial cell designed by us (see PC and MS 200nm wide nano-gaps in Figure 2A). The cell shape is square, geometry of local boundaries selected as argued earlier mathematically¹¹ so that microtubules are mostly found in the homogeneous electric field region with diameter ~ 100μm in the central part of the cell. By changing the cell geometry, we tuned the homogeneous electric field region, only Soybean adjusted its limiting lengths and growth rate with the changing environments. We have used common cell-geometry-parameters for all the species and for all drugs. We grow micrometer thick film of tubulin solution on the chip, kept inside a chamber at 37°C, the

electrode-array remains dipped inside the water-film similar to a living cell. Electromagnetic signal is pumped through the cell boundary electrodes, ions in the buffer solutions are dragged into the ion channels PC and MS (200 nm gap between +ive and -ive electrodes) resembling ion-membrane transport in a living cell. The ion-migration continues until ions block PC and MS gates. This chip is created by e-beam lithography and the electrodes are

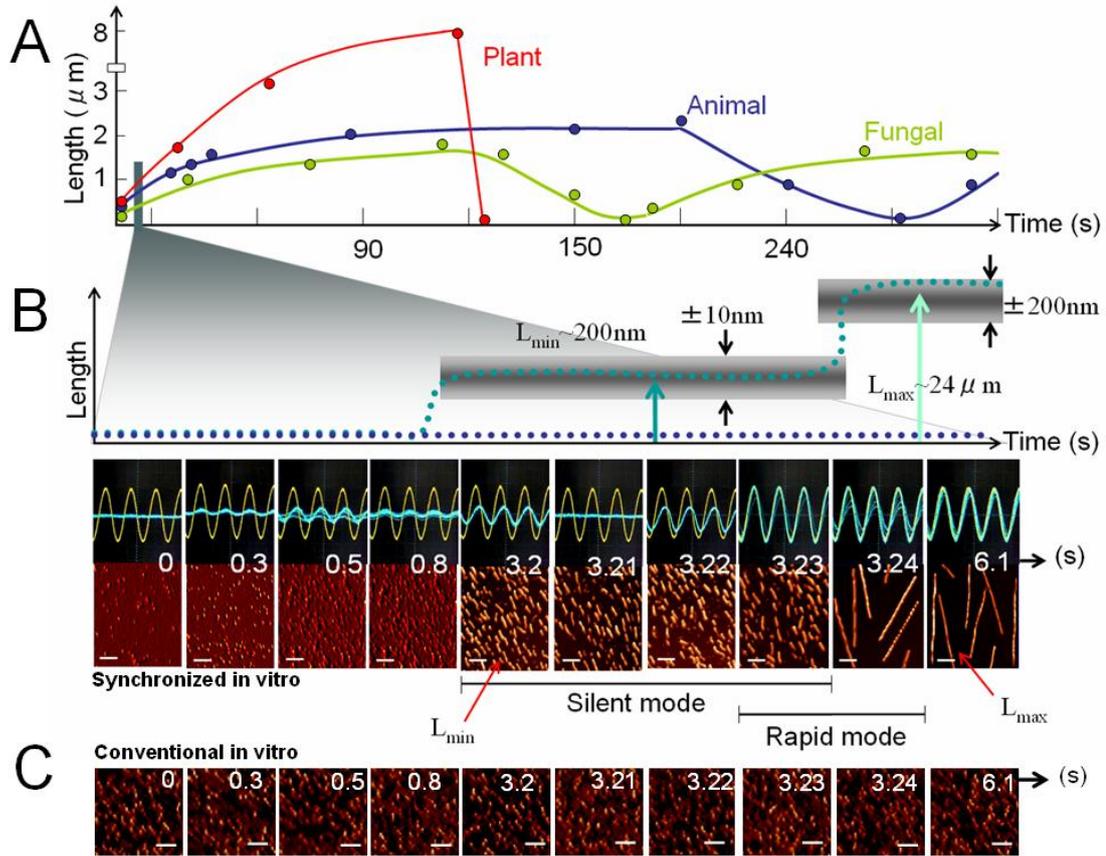


Figure 1: Comparison between conventional in vitro and synchronized in vitro processes: A. Dynamic instability for an animal, plant and fungi samples; shows growth, catastrophe and recovery, as observed in conventional in vitro process. **B.** Length variation for 6.1 seconds of growth for Porcine brain neuron-extracted tubulins, when we pump an ac signal (yellow trace in oscilloscope-captured image), emission from solution is measured as an ac signal output (blue). Growth stopped by rapid drying the micrometer thick film after ac pumping at 2.001 seconds (data of 100 chips each with 10 cells and 20,000 events for 6.1 seconds). We pump with $\sim 1\mu\text{s}$ pulse, and dry; then $\sim 2\mu\text{s}$ pulse, and dry; this way up to 20ms. Then measure length of every single microtubule on the entire chip to get $\langle L \rangle$, so we see variation $1\mu\text{s}$ to 20ms, with constant drying time. AFM image of the chip, scale bar from left to right are, 30nm (first four), 300nm (next four), $5\mu\text{m}$ (next two). For AFM, Average maximum microtubule length $\langle L \rangle$, for ~ 100 samples, synthetic conditions optimized to standard deviation $SD \sim \pm 10\text{nm}$ for $L_{\text{min}} \sim 200\text{nm}$ and $SD \sim \pm 200\text{nm}$ for $L_{\text{max}} \sim 24\mu\text{m}$. AFM images for entire 6.1 seconds in conventional in vitro process. **C.** AFM images of chip for the conventional in vitro process capture only tubulins, time-lapse kept identical, the scale bar is $\sim 20\text{nm}$.

made of gold, the heights of the electrodes were $\sim 500\text{nm}$, hence, all geometrical parameters replicate a typical cell environment as described in the literature²⁸. Chemical composition & biological activities of a cell dramatically differs than our artificial analogue, since we study the effect of synchrony, a clean artificial cell environment provides the artifact-free data.

Experimental procedure & synchrony detection measures:

Since earlier experiments detected MHz ac signal emission from living cells and assigned it

to microtubule²⁹, we tune protein-synchrony source inside a cell-like environment by pumping 1 pico-watt to 1 femtowatt 3.7 MHz ac signal into the micrometer thick tubulin-water film created on a Si/SiO₂ chip for 1 μ s to 200 ms (T). Pump-time control enables us to see evolution of growth with a few microseconds resolution (Figure 2A). We

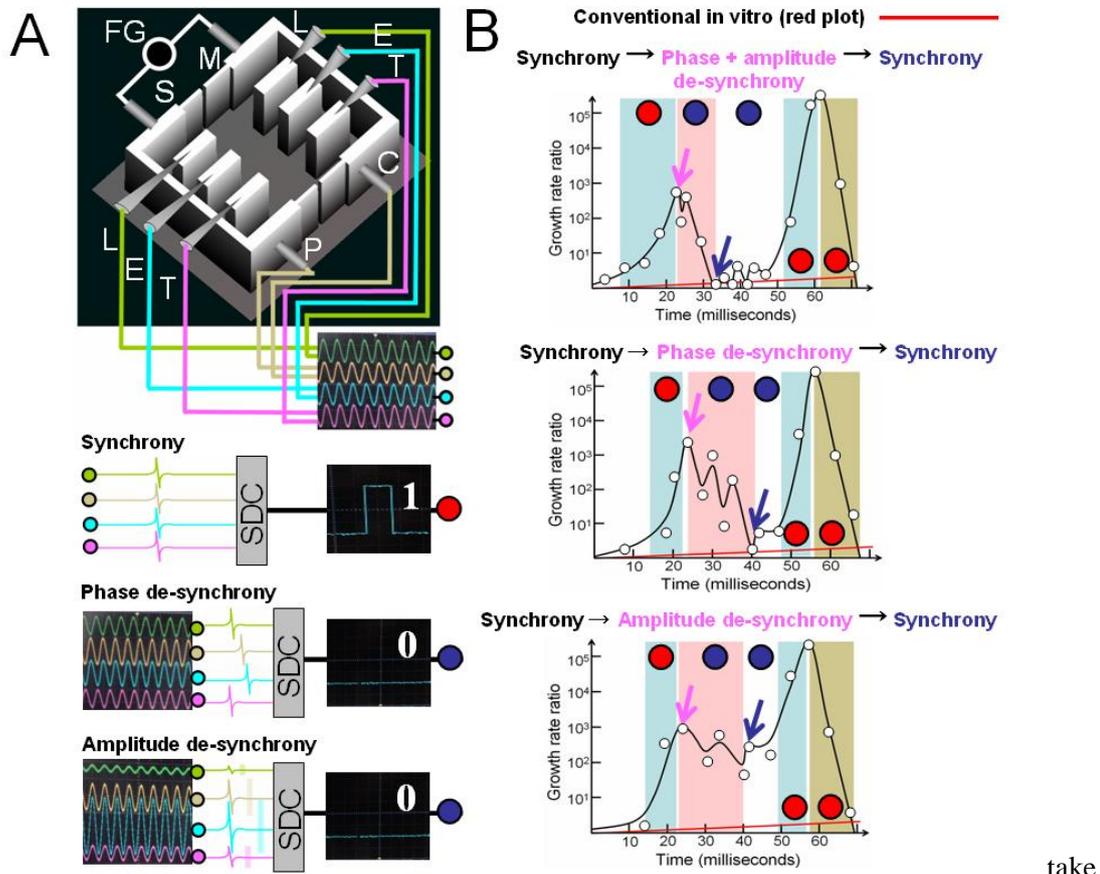


Figure 2: **Switching between synchrony and de-synchrony to switch between two kinds of in vitro processes:** **A.** Replica of living cell, with PCMS ion gates and heat bath at 37⁰C, cell dimension of Figure 2A fixed for all species (200 μ m), two 200nm ion-channels. FG is function generator, and connected circuit is the ac pumping circuit. L, E, T signals and two cables from PCMS is connected to oscilloscope. Signals of synchrony is demonstrated on top, phase de-synchrony in the middle and amplitude de-synchrony is at the bottom. An AND gate inside SDC checks whether signals arrive at same time, or same amplitude if “yes” we see “1” in the oscilloscope, and if “no” then “0”. **B.** Growth rate is switched off by disrupting synchrony in three ways, de-synchronizing both phase and amplitude, (top), de-synchronizing only phase (middle), and desynchronizing only amplitude (bottom) by sending noise into the solution. Pink arrow shows when noise is applied to de-synchronize. Blue arrow shows when noise is off and synchrony recovers slowly. Once microtubule nucleates from tubulin, silent mode is not distinctly visible, hence recovery to synchrony is fast. Growth rate ratio means Growth rate for tubulin density at higher than natural synchrony to growth rate at lower tubulin density.

out chip, dry, and using Raman confirm that the nanowires are truly microtubule, using AFM measure the length of the microtubules produced to get maximum average length $\langle L \rangle$, and $\langle L \rangle / T$ is the growth rate. We prepare protein solution exactly as described in the literature for spontaneous microtubule growth and use it without incubation; drugs are added whenever required (*see experimental section*). The chip embeds two independent hardwares (Figure 2A). First, PCMS electrodes pump ac signal to the micrometer thick tubulin-water film created in the middle of this chip and second, electronically independent L, E, T electrode systems connected to synchrony-detection-circuits. The L, E and T do not cross

talk, collect signals from various parts of the tubulin-film, feed them to SDC and whenever they get signal at a time (between 0.1 ns) with the same phase and amplitude, its logic output is one, if not it is zero. Thus, the existence of synchrony is validated more extensively advancing earlier studies³⁰.

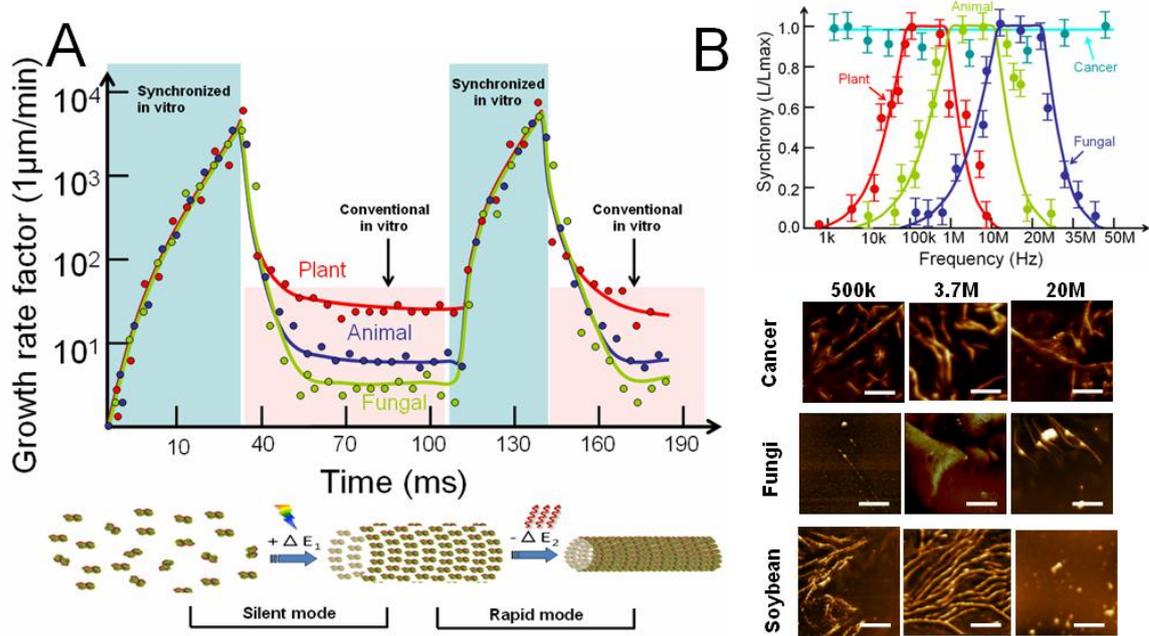


Figure 3: **Entire living kingdom shares electromagnetic frequency band in a complementary manner:** **A.** We have plotted growth factor (how many times the $1\mu\text{m}/\text{min}$) during synchronized in vitro for three species. Sudden fall is the point where several drugs were added, growth returns the specific features observed in conventional in vitro process. Then, more tubulin is added to supersede the drug effect and remove noise, as soon as synchrony is established, high growth returns, the difference between three kingdoms disappears. Below the plot, we schematically present our model, during silent mode, due to synchrony the cylindrical form is produced, then it absorbs energy, and afterwards rapid assembly starts which releases energy, this is the rapid mode. **B.** We plot ratio of the maximum average length $\langle L \rangle$ to the maximum length $\langle L_{\text{max}} \rangle$ measured from AFM chip as a function of frequency for all drugs and for all species put together. For three samples, MCF7 breast cancer cells, Fungi, and Soybean tubulins, we show AFM images when chips were pumped with 500kHz, 3.7MHz, and 20MHz ac signals respectively. Scale bar is $10\mu\text{m}$.

Detection of “silent mode” and a “rapid mode”: re-defining dynamic instability

As soon as the ac pumping starts, initially, L, E & T signal transmission tries to reach coherence repeatedly as we see L, E & T output jumps in the oscilloscope, within 3-4 seconds, in-phase coherent output is visible, but output is 30% of the input (Figure 1B). If pumping is stopped during "silent mode", we found only $\sim 200\text{nm}$ long microtubules for all four species we studied. We tried to pump smaller and shorter pulses, changed the tubulin-density to grow shorter than 200 nm, but failed, thus, it is the lower length limit. The "silence" is thus, essential to nucleate the microtubule to 200 nm up to the lower limit, from which "rapid mode" takes over as L, E & T signal shows logic “one” and output signal is 100% of the input (Figure 2A bottom). Then microtubule grows at around $\sim 10\mu\text{s}$ to the maximum length $\langle L \rangle \sim 24\mu\text{m}$. For porcine, breast cancer, soybean & mushroom, the duration T is $10\mu\text{s}$, hence, growth rate is $24\mu\text{m}/10\mu\text{s} \sim 1.44 \times 10^7 \mu\text{m}/\text{min}$, at tubulin concentration $200\mu\text{M}$ — orders faster the expected rate of $\sim 1\text{-}10\mu\text{m}/\text{min}$. Even if we pump solution for a long time, or increase density, the limiting length does not change, which is again independent of tubulin density and constant across the four species we studied, we call it "rapid mode" (Figure 1B). At high tubulin & Mg^{2+} concentration ($>60\mu\text{M}$), microtubule

grows $\sim 10^2$ - 10^3 times faster due to spontaneous protein-synchrony¹⁸, here, induced synchrony increases the speed even further by $\sim 10^3$ times. This is consistent with the recent single microtubule growth-rate-study, which has established that since tubulins bind laterally and longitudinally together to form microtubule, any protocol that ensures pre-assembly of tubulins into a 2D sheet prior to chemical reaction would speed up the growth rate dramatically²³.

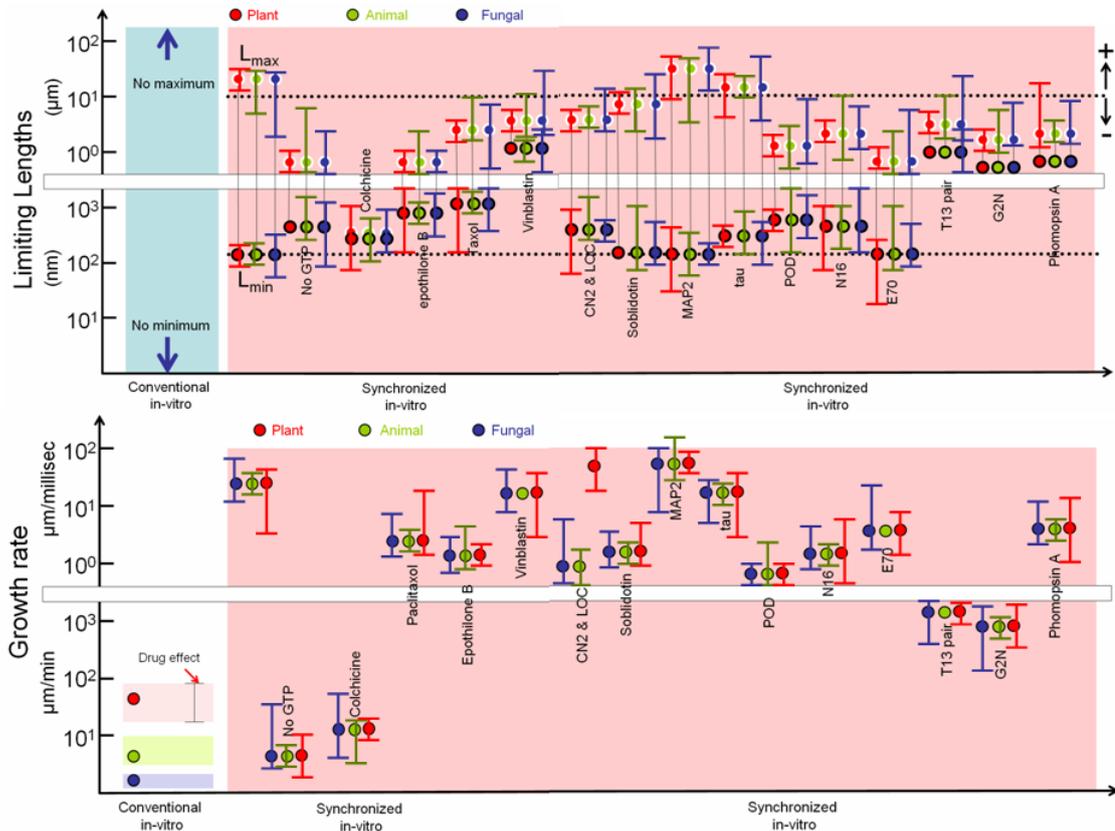


Figure 4: Two tables showing universal limiting lengths and growth rate control in the entire living kingdom (we study 4 species, 16 drugs; thus, 64 sub-species): The Limiting length (above) and growth rate tables for synchronized in vitro starts with a case where GTP + Taxol + Mg^{2+} is added with tubulin as common apart from drugs. Notably, GTP and Etoposide A (*Sorangium Cellulosum*) have same effect on limiting length and speed. Taxol decreases the difference between upper and lower limits, Vinblastin (*Catharanthus roseus*) CN2 & LOC does not help in the polymerization of microtubule in animal cells and in contrast it's presence in plant cell speeds up dynamic instability. For several drugs, the effect is independent of the species.

If cell-like environment and ac pumping is removed, unlimited growth and random growth rate is observed again and the two unique modes disappear. We have also found that if both phase and amplitude synchrony disappears, slow modes become essential for generating synchrony once they are brought back. Consequently, in our artificial cell, when noise is poured in continuously, new materials are brought into the domain of synchrony and some materials are discarded via de-synchrony by the system itself, then continuous transitions between slow and rapid modes give rise to a slow growth exactly as observed in a living cell. In earlier version of dynamic instability, microtubule growth was continuous, now we introduce a “stop” between two rapid growths, together it is very slow but strictly quantized, such possibilities were noted earlier when remarkable metastable states of microtubule growth was detected¹⁹. Even the possibilities of extremely rapid modes were argued earlier²⁰. It has also been argued that dynamic instability might consist of two distinct phases and

phase transition occurs frequently during growth itself³¹.

Switching between synchrony and de-synchrony and observe microtubule growth:

Since L, E & T captured electromagnetic emission from solution just at the onset of microtubule formation (10 pW), and the input power to the solution are same—microtubule growth does not consume any external energy, pumping only triggers synchrony. We induce de-synchrony artificially by sending de-phased/random amplitude signal via L, E and T, which decreases the speed, and if we break synchrony using different amplitude inputs, limiting lengths change, thus, we return to conventional in-vitro growth process. In the Figure 2B we have shown a sequence of synchrony-de-synchrony-synchrony to prove that the process of synchrony is generic and it has one to one correspondence with the growth rate of microtubule. In de-synchronized state, we triggered synchrony again and re-start the silent & the rapid modes (Figure 2B). By comparing three cases of synchrony-de-synchrony switching, we find that phase synchrony is the key to maintain coupling among tubulins during growth, if it survives, during de-synchrony, as in Figure 2B middle, then rapid mode is triggered faster. However, if phase synchrony is not there but amplitude synchrony survives as in the Figure 2B bottom, faster growth falls rapidly and it takes time to recover. If both kinds of synchrony disappear then we do not observe polymerization for a certain time, even if noise is removed and ac signal to activate synchrony is started once again.

Therefore, Figure 2B suggests that synchrony controls the rapid modes and the silent modes, which are not observed in the milli-seconds resolution, random onsets make it a very slow process. Synchrony induced growth is similar for all the four species and 14 drug molecules to the one demonstrated in Figure 2B. In the living species, tubulin is doped with Taxol, GTP, GDP and other drugs especially for the animal and fungal, therefore in the living cells we observe a compound effect. One interesting aspect of our findings is that some tubulins accept the drug molecules inside, some molecules affect the microtubule growth from outside keeping synchrony-rule intact. The combination of drug molecules have dramatic effect on determining the limiting lengths and the eventual speed of growth, and it most cases the speed decreases more as we add more number of drug molecules.

Switching between synchronized in vitro and conventional in vitro protocol: Difference between plant, animal and fungi appears and disappears

Video microscopy techniques capture dynamic instability in vitro³², we add drug molecules to modify the growth rate significantly and to match the observed in vivo activity of the microtubule dynamics³³. In Figure 3A we have switched back and forth between "conventional in vitro" process where the growth rate is very slow, and "synchronized in vitro" where the growth rate is very high to find that initial chaotic fluctuation prior to synchrony activation makes the process slower. We get similar growth plots for all tubulin species even when we do not add noise but simply add drug molecules. Since, we get cylindrical form with synchrony and without GTP or any molecular drug, we propose a model below Figure 3A, where we suggest as argued before²³, that if cylindrical shape is constructed before the reaction begins, the polymerization speed would be remarkably faster since hydrolysis-induced bonding takes place at multiple points simultaneously.

The common minimum protocol valid across the four species:

We have studied how frequency variation affects the coupling among tubulins and determined the maximum length of microtubule obtained from the chip as described before for 64 cases (4 species and 16 drugs makes 64 test samples). The frequency vs. maximum length plot normalized in Figure 4 shows that in the lower frequency domain (~100kHz) plant tubulins (Soyabean tubulin and its mixture with drugs) synchronize, as a result they fail to absorb the drug molecules as efficiently as animals or fungi—drug molecules require a higher frequency for co-synchrony. This is consistent with the evolution argument that

animal and fungi share similar protistan origin^{34,35}. Plant microtubules tend to form unique pattern in the artificial cell under synchrony, as seen here in the AFM images—tuned by ac noise. Around ~1MHz, animal tubulins (Porcine neuron) synchronize but fungal tubulins synchronize at much higher frequency domain (~20MHz). Thus, plant animal and fungal tubulins synchronize at specific frequency domains. A billion years before, when plants, animal, fungal were separated; the basic synchrony protocol was conserved so that addition of specialization is consistent over the years. Why synchrony-activation-frequency regions were separated would remain an open question (Figure 4). However, active breast cancer cell extracted tubulins reach synchrony at any frequency between 1 kHz-50 MHz, we see a straight line in the Figure 3B for cancer, which means AFM images of microtubules produced for any triggering frequency were identical statistically and no significant changes were observed. Thus, the global synchrony-activation rule that is valid uniquely for plant animal and fungal kingdom is not valid for the cancer tubulin. However, this observation suggests that simple synchrony test could reveal if tubulin has mutated into activate cancer in the cells²⁷.

A database of growth rate and limiting length controls for all four species:

Any parameter, positively or negatively interfering with synchrony would increase or decrease the limiting lengths and the growth speed respectively. In vivo, soybean tubulin grows faster than porcine or human tubulins^{16,36}, which are even faster than the mushroom tubulins. However, all the limiting speeds and lengths appear identical under ac pumping—tubulin acts as the basic synchronizer. Administered drugs to the tubulin solution prior pumping modulates the synchronization and changes the growth rate and the limiting lengths (Figure 4A). For example, Colchicin stops the rapid growth unlike a non-synchrony case³⁷—taking us closer to in vivo; Taxol keeps it unchanged. Changing density does not change the limiting lengths, this is a significant conceptual shift observed here from previous studies. When synchrony is active, instead of density, the coupling factor becomes the ultimate control parameter. Thus, change in tubulin density in the cells cannot affect the fundamental life processes. Theoretically, if tubulin proteins act as fundamental oscillator unit, the microtubule would act as an elastic tuning fork generating MHz oscillations, as we observe here³⁸; such MHz emission is already detected outside a living cell. By applying oscillator's synchronous communication models, we have theoretically generated the limiting lengths exactly as observed experimentally. Thus, the drug molecules determine the domain in which the synchronous MHz communication of tubulin protein-oscillators is active (40000 tubulins for 24 μ m), the upper limit of this domain sets the maximum length, and the minimum oscillators required to activate a synchrony-network of tubulins determine the minimum length.

Growth rate of microtubule is primarily the folding-time of 2D tubulin sheet into a cylindrical shape. We find that resonant oscillations of tubulins are alone sufficient to generate microtubular structure. The ac signal induced synchrony forms microtubules even in absence of GTP, taxol and buffer solutions, but it breaks apart beyond a certain length ~4 μ m. Thus, synchronization brings tubulins into a cylindrical shape even without hydrolysis, which provides the stability to grow longer, hydrolysis does not define the cylindrical shape. However, we could not grow microtubule at all, in absence of Mg²⁺, since it controls the polymerization, not GTP. It has always been a great mystery in the microtubule growth that one GTP molecule is hydrolyzed but not the other one, then, what that GTP does sitting idle in every single tubulin dimer? This is clear now; one GTP hydrolyzes to strengthen the bonding between small tubular units of microtubule, and the other one plays vital role in global synchronization, which helps microtubule to grow much longer than 4 μ m (~24 μ m in animals). Since without GTP, the rapid growth rate turns a few thousand times faster, not 2 million times, as we observe in the GTP-case, therefore, GTP is essential for synchrony that directly controls the speed. For all four species, only the inclusion of GTP & similar drugs

tune the speed (Figure 4B). All drug-tubulin-data produced in Figure 3B are in presence of GTP, therein, it is evident that the remarkably faster growth of plant has matched limiting parameters with that of animal and fungi; this is in sharp contrast to the conventional belief³⁹. This similarity between plant and animal under certain condition shades significant light on the chaos or self-order debate in the green kingdom⁴⁰.

The global scaling law does not rule out plethora of molecular mechanisms proposed thus far, it suggests that when all positive catalyst to synchrony acts as stabilizer and negative catalyst to synchrony acts as a de-stabilizer, then, to molecular mechanisms are locally driven to satisfy the global cause demonstrated in Figure 4A and 4B. In a sharp contrast, the existing models, while attempting to generate a common mechanism for stabilizers and de-stabilizers, considered that all molecular mechanisms modulates the very basic mechanism of GTP hydrolysis, which bonds tubulins along the ring and longitudinally⁴¹.

3. Conclusion:

A critical review of the unique phenomenon observed here:

We have redefined dynamic instability. The apparent slow growth rate is made of a sudden rapid growth followed by a long pause and then a rapid growth once again, the interval is undefined, and the sequence of rapid and slow modes is undefined. We verified the videos published in the web to find that the growth is not continuous; the phenomenon remained unnoticed and it is visible distinctly here as synchrony multiplies the effect manyfold.

Secondly, we observed a contradictory behavior. In one hand we see that in Figure 3B, plant, animal and fungi tubulins share the frequency space in a mutually exclusive manner, if that was so essential to follow a billion years back, then why in Figure 4A,B show that in most of the cases all three species behave in an identical manner? It leads us to the conclusion that reaching synchrony defines a species; the protocol to deal with its environment makes them distinct. However, after reaching synchrony, it is essential to communicate with the drugs or foreign materials in an universal language, so, they are designed to respond in an identical manner¹⁸. If species at all were separated from single cell based on their environmental response, then, an unknown factor made sure that tubulins/microtubules do not merge, had nature decided it otherwise, drugs in the plant kingdom (Figure 4) would never have suited animals.

Thirdly, synchrony bound results are density independent for critical lengths and growth rate, a closer observation reveals that for a reliable transport and information processing in the living cell, it is a must that the critical parameters remain unchanged. The supremacy of synchrony is so profound that two particular observations (i) rolling of 2D tubulin sheet into a cylindrical shape without GTP and (ii) remarkable growth of cancer cell extracted tubulins, firmly establish that synchrony can mastermind extreme events.

Recently the practice of explaining dynamic instability in terms of chemical kinetics model has been challenged, and it is argued that non-equilibrium phase transitions cause microtubule growth and decay in vivo⁴³. Conventional protocols failed to explain how 40000 tubulins assemble in less than a microsecond. The possible answer is in the theoretically rich yet ignored literatures for decades, where proteins act as resonant oscillators and engage in wireless communication⁴⁴. Thus, synchrony of microtubules is well known⁴⁵ but its origin lies in its fundamental structural unit tubulin that brings eukaryotes under a single umbrella, and time has come to unearth this complex oscillator as an unprecedented composition of tuning forks.

4. Experimental section:

Different solutions of tubulins:

Porcine (brain neuron), human (MCF 7 active breast cancer cell), fungi (Agaricus bisporus mushroom) and plant (six days old soybean germ lings), samples were obtained from Cytoskeleton Inc kept at -70°C . Ingredients are lyophilized tubulin, PIPES, General tubulin Buffer, GTP Stock, DMSO, PEG, Taxol, Glycerol, Colchicin etc. Porcine's brain extracted tubulins were received from Cytoskeleton (Denver, CO), preserved at -80°C . To polymerize tubulin into microtubules, Microtubule cushion buffer (60 % v/v glycerol, 80 mM PIPES pH 6.8, 1 mM EGTA, 1 mM MgCl_2) was added to general tubulin buffer (80 mM PIPES pH 7, 1 mM EGTA, 2 mM MgCl_2) and/or GTP solution. Solution Pr combinations were: Pure tubulin+ H_2O (pH~7.0), Pure tubulin+ H_2O + MgCl_2 , Pure tubulin+ H_2O ± MgCl_2 ±GTP stock. Solution Cl, we kept identical procedure for all four species, exactly as described in the cytoskeleton website for all species; except that for fungi GTP stock had 10% PEG. Stock solutions were rotated in 15000 rpm, 0°C , 30 minutes to de-polymerize prior use. For breast cancer cells, 10% Glycerol, for fungi 10% PEG, for Soybean 5% DMSO was used prior pumping. We fitted limiting lengths using the oscillator models.⁴⁶⁻⁵⁰

List of Publications:

Memory switching properties of a single brain microtubule, S. Sahu, S. Ghosh, K. Hirata, D. Fujita, A. Bandyopadhyay Appl. Phys. Lett. (accepted).

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.

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