Modeling Self-organization of Microtubules from Tubulins

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ABSTRACT
Self-assembly is a ubiquitous, naturally occurring, robust process in many living organisms. Microtubules are a well-studied type of natural self-organization systems that assemble into functional units by attaching to cellular structures. Modeling microtubule self-organization is of broad interest as they form a network of protein filaments that is critical to many processes in eukaryotic cells. In this paper, we propose a modeling algorithm starting from alpha and beta tubulins as basic building blocks in the self-organization of microtubules. The preliminary results obtained from our algorithm demonstrate that such self-organization stems from the properties of the basic building blocks that can be used to form the complex microtubule structures.

Categories and Subject Descriptors
J.3 [**Medical information systems**]

General Terms
Algorithms, Measurement, Performance Design, Experimentation.

Keywords
Self-organization, microtubules.

1. INTRODUCTION
Molecular self-assembly [1] is defined as the spontaneous organization of molecules, typically repeated protein subunits, under thermodynamic equilibrium into structurally well-defined and rather stable arrangements. Biological self-assembly systems include actin and tubulin filaments, which form a network of protein filaments called the cytoskeleton that is critical to many processes in eukaryotic cells. One such network formed from tubulin filaments is the microtubule. Microtubules (MT) form a framework for structures such as the spindle apparatus that appears during cell division, and are responsible for various kinds of movements, cell division, intracellular structure organization and transport. The basic building blocks of MTs are "tubulin monomers" (α and β tubulins) with slightly different properties. Both α and β tubulins spontaneously bind one another through polymerization to form a functional subunit named "heterodimer". These heterodimers assemble themselves into linear "protofilaments" under controlled intracellular conditions that in turn assemble into MTs [1]. Among other related works, [6, 7] describe a self-assembly model guided by dynamic assembly of mitotic spindle and justify the apparent stability in the final formation of an MT as "out-of-equilibrium stochastic interactions" between tubulins at the molecular level. Another related work [8] describes a mechanochemical stochastic model to describe gradual microtubule formation from an open sheet to a closed tube. This model was shown to be regulated by energy distributions within the MT.

In order to investigate MT self-assembly, we propose a modeling algorithm starting from alpha and beta tubulins. Our model considers tubulins as basic building block in the self-organization of a microtubule instead of dimers. In this paper, the modeling algorithm and the resulting patterns of self-organization obtained are presented and discussed in terms of the initial and final affinity factor (mentioned in the paper as "stickiness factors") distribution within the microtubule.

2. SELF-ORGANIZATION APPROACH
A microtubule consists of basic building units called the alpha and beta tubulins. Alpha and beta tubulins combine to form a structure referred to as dimers. Such dimers were considered as the basic building block in the formation of microtubules in [1, 2]. However in this paper, tubulins instead of dimers are considered as basic building blocks that avoid unnecessary complications and also make more realistic biological assumptions. This consideration adds flexibility to the proposed algorithm as we can impose finer self-organization attributes in the model at the molecular level. Moreover, the alternating occurrences of alpha and beta tubulins are maintained in the final microtubule to make the structure biologically valid. In this approach focus has been given to generate a grid data structure that would contain all the physical and geometric parameters that are sufficient to render the corresponding 3-D structure for the microtubule. We observe two types of geometric parameters that control the 3-D shape of the microtubule. In a microtubule protofilament, the dimers can have relative angular displacement from each other, whereas the protofilaments in a microtubule themselves can have relative angular displacement that gives the microtubule a helix like pattern. Fig. 1 explains these two scenarios.

Fig. 1(a) shows two cases: one of a straight protofilament with no vertical angular displacement relative to the dimers, whereas the other protofilament have non-zero vertical angular displacement. If protofilaments with non-zero vertical angular displacement are arranged to form a MT it would give the microtubule an appearance shown in Fig. 1(c). The complementary case of non-zero horizontal angular displacement between protofilaments is shown in Fig. 1(b) whereas the corresponding microtubule appearance is shown in Fig. 1(d).

3. MODELING SELF-ORGANIZATION
The final microtubule structure in [1] suggests that an alpha or beta tubulin of a microtubule has four sites where it can attach

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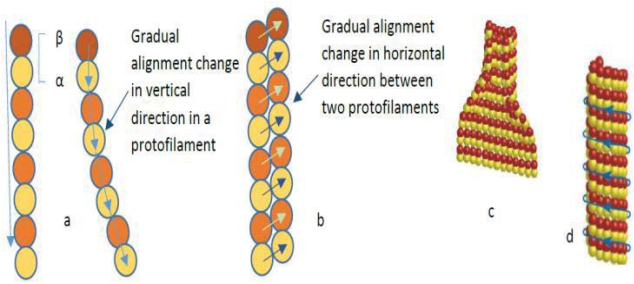

Figure 1: (a) Vertical and (b) horizontal angular displacement in microtubules; (c) and (d) represents the corresponding structure in different microtubule formation phases [1].

itself to its neighboring tubulins. These four sites of one tubulin would be characterized by "stickiness factors" shared by its four neighbors. In the proposed algorithm these factors denoted as X are generated using a normal distribution. Specifications of this distribution are as follows:

$$X \sim N(\mu, \sigma);\ \mu = 25;\ \sigma = 18.75; \qquad (1)$$

The algorithm considers resolution of 0.1 in stickiness factors i.e. the normal distribution sample size is ≈ 1510. From (1) above it is evident that the distribution allows negative "stickiness" factors. Such negative stickiness given to a tubulin would imply repulsion rather than affinity between tubulins. Therefore, the corresponding units sharing negative "stickiness" would be further away from each other. This negative factor would therefore add flexibility to the model and would justify intermediate formation stage of a MT shown in Fig. 1(c) where the dimers within the terminal protofilaments are not connected to each other and the separation varies by different degrees along the microtubule length.

The final microtubule is represented as a 2-D array $M_{a\times b}$ where each element $M_{ij} \in M$ is an array of dimension 1×6. An arbitrary element M_{ij} in i^{th} row and j^{th} column is described as follows:

$$M_{ij}[k] = \begin{cases} \text{randomly generated stickiness factor, when } 1 \le k \le 4 \\ \text{vertical angular displacement } \theta_v \text{, when k} = 5 \\ \text{horizontal angular displacement } \theta_h \text{, when k} = 6 \end{cases}$$

Apart from the "stickiness" factors stored in the first four places of the element, the fifth and sixth components of each element i.e. θ_v and θ_h represent two externally supplied parameters which are used to find longitudinal and lateral angular displacement. In addition to this we have considered two additional parameters (d_v and d_h) to account for the gradual change of angular displacement longitudinally and laterally respectively. The parameters θ_v and θ_h acts as an initial value for calculating angular displacement. In Fig.1, we describe the visual justification for these parameters. In Fig. 1(a) two protofilaments are shown. The right one shows gradual longitudinal angular displacement (i.e. $\theta_v \neq 0$ and $d_v \neq 0$) whereas for the left one $\theta_v = 0$ (and $d_v = 0$). Fig. 1(b) shows constant lateral angular displacement (with $d_h = 0$) between two consecutive protofilaments. The corresponding microtubule formation by arranging such protofilaments are shown in Fig. 1(c) and (d) respectively. Based on the initial distribution of "stickiness" factors, the algorithm computes a global compactness factor (CF) that determines the termination condition of the algorithm. Compactness factor, CF, signifies the global variance of the "stickiness" factors throughout the MT. On termination of the algorithm, CF should be within the range $(\mu - \sigma, \pi + \sigma)$ signifying a relatively uniform distribution of "stickiness" factors across MT and thus a more stable structure.

3.1 Microtubule Self-assembly Algorithm

We now formally represent the following algorithm, **SAM** (Self-Assembly of Microtubule), which takes into account the aforementioned parameters.

SAM:

Input: Length of final microtubule L; Width of final microtubule W; Vertical angular displacement θ_v; Horizontal angular displacement θ_h; Horizontal gradual angular variation factor $d_h \in \{1,0\}$; Vertical gradual angular variation factor $d_v \in \{1,0\}$.

Output: The final microtubule represented in array *M*.

Steps:

1. Calculate number of rows a of M as $a \leftarrow \left\lceil \frac{L}{d} \right\rceil$; Here d is the diameter of one tubulin.
2. Calculate number rows b of M as $b \leftarrow \left\lceil \frac{W}{d} \right\rceil$;
3. For $i = 1: a$
   For $j = 1: b$
   For $k = 1:4$
   $M_{ij}[k] \leftarrow r \in X$ from distribution (1);
   End
   $M_{ij}[5] \leftarrow (\theta_v + \theta_v(i-1)/a)(d_v)$;
   $M_{ij}[6] \leftarrow \theta_h + (\theta_h(j-1)/b)(d_h)$;
   End
   End
4. Calculate compactness factor
   $CF \leftarrow \sqrt{\sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{4}\left(M_{ij}[k] - \mu\right)^2}/(ab)$;
5. Post-processing step:
   $\delta \leftarrow 0.1$; // Factor controlling rate of convergence.
   1. Repeat until both
      $(CF < \mu + \sigma)$ and $(CF > \mu - \sigma)$
      a. For $i = 1: a$
         For $j = 1: b$
         //Calculate the average "stickiness" for
         // local tubulin
         sum $\leftarrow \sum_{k=1}^{4} M_{ij}[k]/4$;
         If $((\mu - \sigma) \le \text{sum} \le (\mu + \sigma))$
         Continue to next <*i,j*> tubulin;
         Else
         If$((\mu - \sigma) \ge \text{sum})$
         For $k = 1 : 4$
         $M_{ij}[k] \leftarrow M_{ij}[k] + \delta(\mu - \sigma - sum)$;
         End
         If$((\mu + \sigma) \le \text{sum})$
         For $k = 1 : 4$
         $M_{ij}[k] \leftarrow M_{ij}[k] + \delta(sum - \mu - \sigma)$;
         End
         End Else
         b. Re-calculate compactness factor
         $CFN \leftarrow \sqrt{\sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{4}\left(M_{ij}[k] - \mu\right)^2}/(ab)$;

c. If $((CFN < \mu + \sigma)$ and $(CFN\mu - \sigma))$
Exit;

Else
// Assign new values to δ so that the compactness factor in the next round of computation would lead towards convergence.//
If $(CFN > \mu + \sigma)$
$\delta \leftarrow \delta - 0.1$;
If $((CFN < \mu - \sigma))$
$\delta \leftarrow \delta + 0.1$;
$CF \leftarrow CFN$;

The algorithm starts by calculating the number of tubulins required in one protofilament that also signifies the number of rows in *M*. Subsequently the number of such protofilaments in the final microtubule, i.e. the number of columns in *M* is also calculated. Based on these numbers of tubulins required in each protofilament, equal numbers of alpha and beta tubulins are homogeneously distributed along one protofilament. All other protofilaments are initiated in a similar manner. Once a microtubule is initialized, SAM starts simulating the self-assembly. The user inputs also include the horizontal and vertical angular displacements as θ_v and θ_h. Another parameter d_h determines whether neighboring protofilaments would maintain uniform angular displacement relative to each other; one such structure created with constant horizontal angular displacement θ_h is shown in Fig.1(d).

Subsequently in step 3, *self-organization of the microtubule* assigns random stickiness factors to all the tubulins in the array. Note that in each element of the array the fifth and sixth elements are reserved for θ_v and θ_h and does not play an important part in the post-processing step of the algorithm. Based on the initial assignment of the random stickiness factor, a global compactness factor *CF* is calculated in step 4. The goal of the subsequent post-processing step (step 5) is to force the global *CF* to be in the range $(\mu - \sigma, \pi + \sigma)$ at termination of the algorithm to ensure global numerical proximity of stickiness factors of all tubulins in the final microtubule. This would most likely enforce stability to the final microtubule structure. In step 5. (a) all the tubulins are re-assigned new stickiness factors if compactness factor CF does not belong to the aforementioned range. The momentum factor δ determines the rate at which the factors will be updated and therefore in a way prevents the algorithm from following a greedy approach. After all assignment of stickiness factors in step 5. (b), compactness factor is re-calculated and stored in a variable called *CFN*. Based on the region *CFN* belongs to, the momentum factor δ is changed accordingly to force the subsequent compactness factors towards the target range during subsequent iterations. These steps are reiterated (from step 5.1) until **SAM** converges towards the specified range.

4. RESULTS AND DISCUSSION

The developed algorithm was programmed in MATLAB and/or Eclipse platform to generate the visualization from matrix *M*. Some preliminary results are presented in Figs. 2 and 3. The initial and final distribution of "stickiness" factor across the microtubule is shown using color codes in an array. The four-element array in each cell corresponds to the four factors of one tubulin. It can be seen from Fig. 2 that at the final distribution, overall stickiness factors are similar and represented by relatively uniform color distributions. We have used lighter to darker shades to represent higher to lower numerical "stickiness" factors. These shades are computed automatically in the program and span a very wide range corresponding to the entire range of random "stickiness" factors. This color coding scheme was chosen as darker to lighter shades to represent lower to higher factors is intuitively appealing.

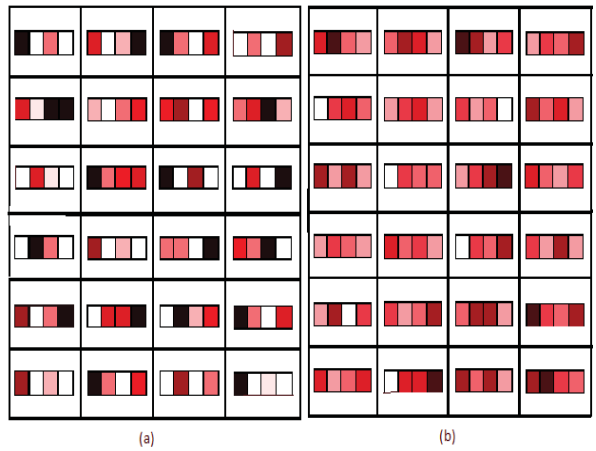

Figure 2: Initial and final distribution of "stickiness" factor across microtubules shown using color codes.

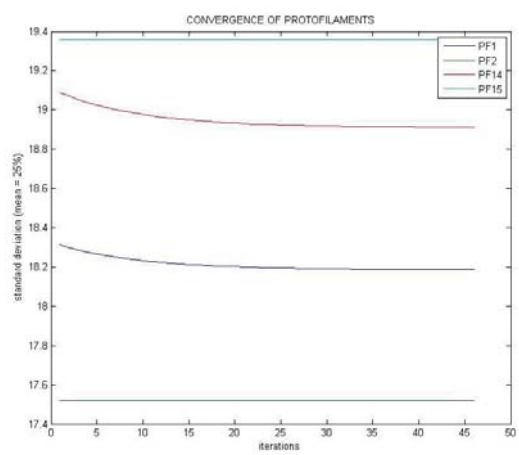

Figure 3: Convergence of "stickiness" factors for protofilaments 1, 2, 14 and 15 for a microtubule having 15 protofilaments and 20 tubulins per filament.

The convergence of "stickiness" factors for protofilaments 1, 2, 14 and 15 for a microtubule having 15 protofilaments and 20 tubulins per filament are shown in Fig. 3. It can be seen from Fig. 3 that after 30 iterations, the stickiness factor remains constant.

We carried out 16 different simulations for self-assembly and found the results given by each simulation to follow a similar trend as shown above.

5. CONCLUSION

In this paper, an algorithmic framework is developed to model the self-organization of the microtubule from tubulins. The proposed algorithm and the necessary steps were described in detail. The preliminary results obtained from the model demonstrate that self-organization from basic building blocks can indeed form the final microtubule structure. However, further investigation is necessary to evaluate the parameters considered here and their sensitivity in

the model for realistically simulating the microtubule self-organization process. Some scopes of future work are mentioned in the following.

Microtubules are not static struts. They display a high degree of dynamicity which enable them to grow or shrink very rapidly. Our proposed algorithm has considered parameters such as θ_v and θ_h to incorporate structural dynamicity during their growth phases as described in [1]. Subsequently, we optimize the distribution of "stickiness" factors across the microtubule to enforce the assumption that these factors with less deviation between them would display a more compact microtubule structure. However, these considerations for incorporating dynamicity or compactness are by no means exhaustive. There are ample scopes for further sophisticated modelling of inherent dynamic instability within a microtubule. In addition, accurate modelling of interaction between neighboring tubulins involving M-loop and H1-S2 loop are necessary to properly simulate molecular level assembly of microtubules. Another aspect of microtubule formation is protofilament twisting where protofilaments slides with respect to each other under influence of thermal forces on the microtubule. As a result, the inter-protofilament bonds are stretched. This effect can be mitigated by applying a twisting force along the microtubule axis. Therefore, effective modelling of microtubule formation needs to consider such issues.

Many previous works considers microtubules as isotropic tubes which, recent research shows, is an inadequate assumption. Under influence of osmotic pressure above 600 Pa [4], microtubule walls forms into an ellipse from a circle. Such deformation can be modelled by further accurate modelling of inter-protofilament interactions [5]. In a more recent work [9] the authors propose a nonlocal transport model that justifies the evolution of MT when they interact with stationary distributions of motor proteins. Further investigation and simulation of these interactions at molecular and sub-molecular levels could lead to better understanding of MT assembly and organization influenced by heterogeneous molecular interactions. Another direction for future work could be to model self-assembly of microtubule bundles as microtubules are often found together within a bundle. The challenge for this assembly is to successfully model the cross-linkers between such microtubules.

6. ACKNOWLEDGMENTS

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