A SWARM ENGINEERING FRAMEWORK FOR MICROTUBULE SELF-ORGANIZATION

by

SANJAY SARMA ORUGANTI VENKATA

(Under the Direction of Ramana Pidaparti)

ABSTRACT

Microtubules are highly dynamic polymers distributed in the cytoplasm of a biological cell. Alpha and beta tubulins combine to form these tubules through polymerization, controlled by the concentrations of GTPs and MAPs. These play a crucial role in many intra cellular processes, predominantly in mitosis, organelle transport and cell locomotion. Current research in this area is primarily focused on understanding these exclusive behaviors of organization of tubules and their association with different MAPs through organized laboratory experiments. However, the intriguing intelligence behind these tiny machines resulting in complex self-organizing structures is largely unexplored. Understanding this can support researchers in validating many hypotheses in quicker and cost-effective ways. On these lines, we propose a novel swarm engineering framework in modeling rules for these systems, by convolving the principles of design with swarm intelligence. The proposed rules were simulated on a game engine and this approach demonstrated self-organization of rings and protofilaments.

INDEX WORDS: Microtubules, Microtubule Associated Proteins, Self-Organization, Swarm Engineering, Swarm Intelligence, Game Engine, Protofilaments.

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DEDICATION

Amma, Appa, Gurus and Swami

ANYATHAA SHARANAM NAASTI TWAMEVA SHARANAM MAMA |

TASMAT KARUNYA BHAAVENA RAKSHA RAKSHA MAHESWARA ||

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This work is a continuation of my pursuit in building smart and simple swarm agents addressing real world problems. Even after many years of research trying to develop novel algorithms and applications in this field, the fervor to explore newer areas has never diminished. And I am fortunate to have found a great mentor supporting my ideas through Dr. Ramana Pidaparti. I would like to express my deepest sense of gratitude to him for guiding me through my research during my masters program and for giving me space and time in learning through my own mistakes.

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CHAPTER – 1

INTRODUCTION

1.1 Motivation

Human beings perceive themselves as autonomous thinkers trying to figure out things on their own in solving problems. While also being autonomous, sometimes we accept and borrow thoughts from others. In fact, many great ideas have evolved through populations and at some point, we tend to attribute our survival and growth in all the fronts to our togetherness in the form of 'societies'. This powerful social coordination combined with our great cognitive abilities made us the lone dominant species on this planet, which is beyond any other specie's evolutionary scope. However, it is not a wonder that a colony of ants is more intelligent than a human being in many ways.

Though species other than humans lack a high level of cognition, their intelligence amplification is through collective thinking and coordination. Their low-level interactions lead to highly complex accomplishments, which otherwise are beyond the scope of individual ones. Some examples of these intelligent phenomenon include foraging in ants [1], stigmergy [2], hiving [3], flocking of birds [4] and schooling in fish [5]. Observation of these biological systems demonstrating complex social behaviors through emergence, lead to the development of the new field of Swarm Intelligence (SI) [6].

Given its unique nature of problem solving, the field of swarm intelligence has attracted a vast number of researchers over the past two decades [7]. Today, its application areas include

robotics [8, 9], communication [10], optimization [1, 5], crowd simulations [11] and self-assembly [8]. Many current day design principles in the field of SI are inspired from the field of swarm robotics, which focuses on the design of rules for coordination of large number of robots as swarm agents [9].

Self-assembly on the other hand is demonstrated by nearly every biological process in our body, starting from replication of DNAs to organization of cells in an organelle. These structures are expected to behave like swarms at some level in their organization [12]. Of them, microtubules are one such intracellular structures whose self-organizing behavior is under wide study at different levels [13].

In these lines, in our current research we propose a frame work integrating the design principles of swarm engineering [8], with the observed behavior of microtubules sub-structures, in simulating self-organization.

1.2 Challenge

Microtubules (MT), are highly dynamic polymers undergoing continuous assembly and disassembly in the cell structure. These are hollow cylinders with the heterodimers arranging on their walls in chains, growing up to a length of 50μm inside biological cells [14]. Heterodimers, are made of globular proteins called 'Tubulins'. Each dimer is identified to have one α-tubulin and one β-tubulin and these alternate to form strings called protofilaments [15]. Microtubules demonstrate many complex organizations like separation of chromosomes during mitosis, organelle transport, cell locomotion through cilia and flagella [16], by associating themselves with various Microtubule Association Proteins (MAPs).

Many hypotheses on self-organization and functionality of microtubules have been proposed over the past two decades, and the area still remains very active in molecular cell biology

research [17]. Though these investigations were focused on understanding the complex processes, not much of effort has been made in understanding the intelligence in these complex intra cellular organizations. Understanding this intelligence through simulations can support researchers in hypothesizing many unknown processes like the effect of various cancer drugs at cellular level [18]. However, the primary challenge lies in designing an intelligent framework supporting their real-world behavior. Also, an additional challenge lies in demonstrating the framework's effectiveness through dynamic graphical simulations.

1.3 Proposed Approach

We propose a behavior-based design method as formulated by Brambilla et al.,[8] in designing rules for our agents behaving as tubulins and heterodimers. These swarm agents are expected to interact spatially and connect to each other through morphogenesis. We propose a strategy for demonstrating self-organization of sub structures like rings and protofilaments by introducing GTPs and analyze their behavior through a simulation framework run on a game engine. These swarm agents are expected to be autonomous, with an ability to sense and interact with the environment without any centralized control, while also maintaining coordination for task accomplishment [6, 8, 9]. At agent level, we combine the general rules of self-assembly with swarm engineering, where the geometry, interactions and environment parameters at each level are precisely defined [19, 20]. Along with it we ensured our adherence to the fundamental rules of swarms i.e., robustness, scalability and flexibility [9].

1.4 Contributions

The current study is one of the few using a swarm engineering approach in addressing a real-world problem. At first, we investigate the general behavior of microtubules in self-organizing

tubulins through lower level structures and the interaction of MAPs with tubulins as proposed in the literature. These behaviors are further formulated into rules based on the guidelines provided for the design of swarm robots [8, 9] in combination with self-organization principles [19, 20], where agent's geometry, environment and interactions are defined. Geometry rules are with respect to shape and bond locations in globular proteins at monomer level and bonding rules are defined based on three types of interactions, i.e., agent-agent, agent-environment and agent-MAP. The proposed rules are for forming protofilaments and ring structures in a constrained 3D space. Further, we extend these rules to probabilistic behaviors of dimers as agents, involving a bond breakage strategy and thus introducing stochasticity into the system.

Our final contribution is in the form of developing a 3D simulator for testing the proposed rules. The 3D simulator was developed in Unity game engine, which provided an ideal environment for real-time simulations through its physics engine. Performance parameters for ring and protofilaments were logged for quantitative analysis. We also draw some experience from a similar system for foraging developed in our previous work [21], where a swarm searches for a potential food source through communication protocols.

1.5 Outline

This thesis is organized into 5 chapters, starting with the outline and introduction. In chapter 2 we introduce microtubule's structural and functional properties, and also introduce related works proposed in literature on self-assembly. We introduce our framework in chapter 3 followed by the results in chapter 4 for varying parameters. We, end the chapter with a discussion on the obtained results. Finally, our conclusions and future works are presented in chapter 5.

CHAPTER – 2

SELF-ORGANIZATION IN MICROTUBULES

2.1 Background and Related Works

Microtubules are complex networked protein structures responsible for many crucial intra cellular processes in eukaryotic cells. These are highly dynamic polymers undergoing continuous assembly and disassembly. Structurally, these are hollow cylinders with dimers arranged on the cell walls and have approximately, 25nm and 112nm of outer and inner walls respectively and grow up to a length of 50μm inside the cells. The building blocks of the tubules are 'Heterodimers', and each heterodimer is identified to have a pair of α and β Tubulins. These dimers attach in the cytoplasm to form long strings called protofilaments, with alternating α, β monomers [14]. Also, theyare understood to undergo polymerization forming strings, rings and sheets as intermittent structures leading to the formation of microtubules.

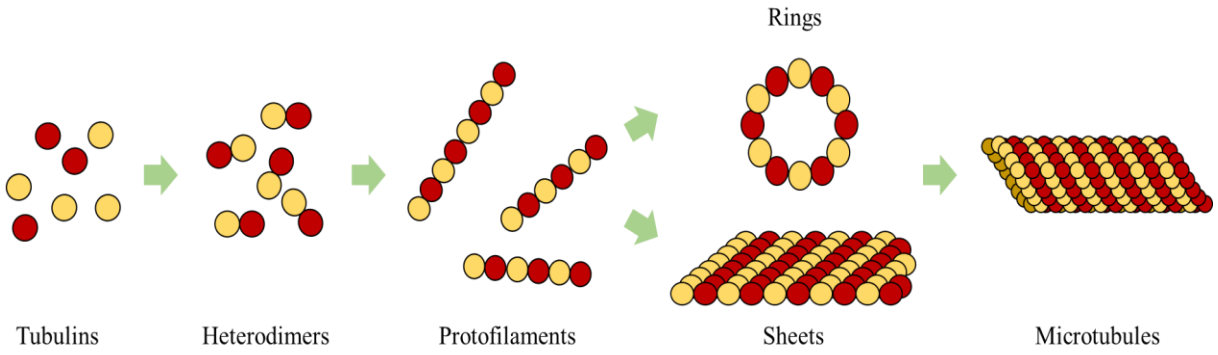


Figure 2.1 Self-organization of Tubulins, Heterodimers, Protofilaments, Sheets, Rings, leading to Microtubules.

Microtubules are known to demonstrate complex organizations and intracellular functions by associating themselves with various Microtubule Association Proteins (MAPs). Some of these functions include chromosome separation during mitosis [16], organelle transport [22] and cell locomotion through cilia and flagella [14, 23].

2.1.1 Microtubule Synthesis

Synthesis of Microtubules starts from Microtubule Organizing Centers (MTOC) in animal cells which are otherwise called Centrosomes [24]. Centrosomes contain Centrioles with cart wheel structures, where a pair of them are arranged perpendicular to each other. These centrioles also control cilia, flagella and basal bodies through microtubules.

Centrosomes hold the negative ends of the MTs and the growth of the MTs is from the positive ends through the association of GTP bound dimers suspended in the cytoplasm. A γ-tubulin is generally involved in initiating the tubule formation, which is only localized to the centrosomes.

The growth or shrinkage of a tubulin is dependent on the ratio of the rate of polymerization to the rate of hydrolysis. A ratio >1 favors growth and also, is dependent on the GTP cap at the positive end [14]. So, controlling this GTP cap will alter the growth of MTs and will have a significant effect on mitosis. Hence, selective targeting of MTs through drugs can reduce malignancy in cancers [18], by hindering mitosis.

2.1.2 Dynamic Instability

Microtubules display a unique property of ‘dynamic instability’ [13-15] where they undergo continuous assembly on the positive ends and disassembly on the negative ends simultaneously. Desai et al., [14] reviewed various hypothesis and configurations proposed in the

formation of tubules. They also summarized the works on dynamic instability, where the tubulin is expected to undergo polymerization and de-polymerization altering through altering catastrophe and rescue phases supported by a thermodynamic basis for its instability.

Dynamic Instability is controlled by the hydrolysis of GTP to GDP associated with the tubulins. This weakens the bonds between the dimers and hence disassembly takes place from the negative end, as shown in figure 2.2. A single MT can sometimes alternate between cycles of assembly and disassembly depending on the Microtubule Associated Proteins (MAPs) it is associated with, a process called thread milling [14].

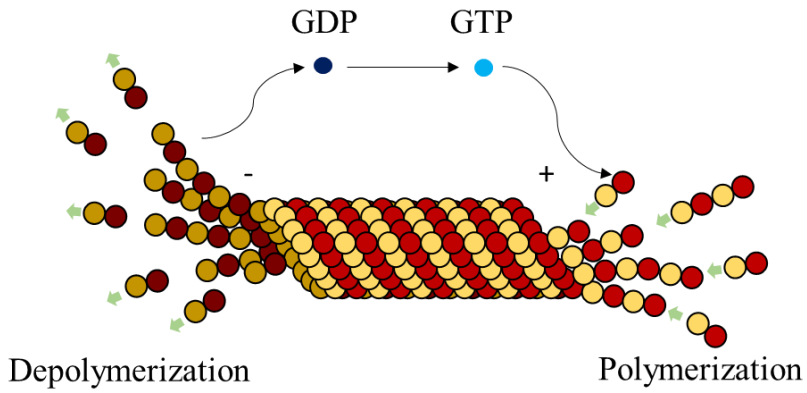


Figure 2.2 Dynamic instability in microtubules

2.1.3 Microtubule Associated Proteins (Maps)

Microtubule's dynamic instability controlled by Microtubule Associated Proteins (MAPs) was investigated by Drewes et al., [23]. They also investigated another set of proteins called MT-affinity-regulating kinases (MARKs) that are responsible for the detachment of MTs increasing dynamics through phosphorylation. Many MAPs have been discovered by the researchers since the discovery of microtubules, with each discovery leading to a new hypothesis on their functions. Some of these interesting MAP functions are in guiding MTs towards selective targets like

chromosomes in cytoplasm during mitosis, bundling multiple MTs or maintaining gaps between the fibers. Apart from these, some MAPs also vary the strength of bonds between dimers. The intelligence at molecular level organizes these associations depending on the required functionalities. Figure 2.3 shows different MAPs acting on microtubules. CLASP- De-polymerizes microtubules at a rapid rate, STOP holds the tubulins from depolymerizing, PRC1 bundles with other microtubules, Kinesin and dynein are motor proteins which walk towards positive and negative polarities respectively and XMAP215 increases the rate of polymerization[14, 23]. Also, Tau proteins [25] are found to be the dominant players in causing Alzheimer's due to the formation of abnormal aggregates.

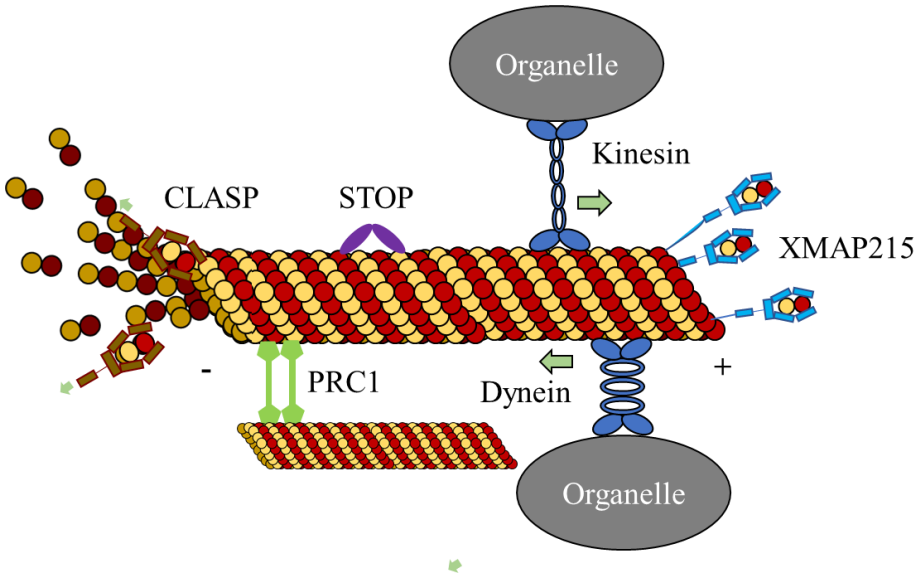


Figure 2.3 Different MAPs acting on a microtubule.

2.1.4 Motor Proteins

Motor proteins a type of MAP plays a pivotal role in intra cellular movements in vesicles, organelle transport, beating of cilia and flagella. Tom Hays et al.,[22] investigate intracellular organelle transport by kinesins in neurons. These proteins are highly polar in nature and utilize the

polarity in microtubules for their directional walking. Other motor proteins involved in organelle transport in cytoplasm include dyneins and mysonins. Given the polarity of MTs, these proteins walk towards opposite sides, i.e., kinesins towards +ve and Dyneins towards – ve respectively. These proteins attach themselves at active locations on organelles and while moving across MTs.

2.1.5 Microtubules in Mitosis

Microtubules play very crucial roles in the mitosis process [16], especially in the separation of chromosomes, distribution of organelles for the daughter cells and in cell locomotion during interphase. Three varieties of MTs have been identified with each associated to a functionality in the cell division process. Kinetochore microtubules separate daughter chromosomes, while polar MTs form the mitotic spindles and Astral MTs attach themselves to the cell periphery, working on cell wall separations.

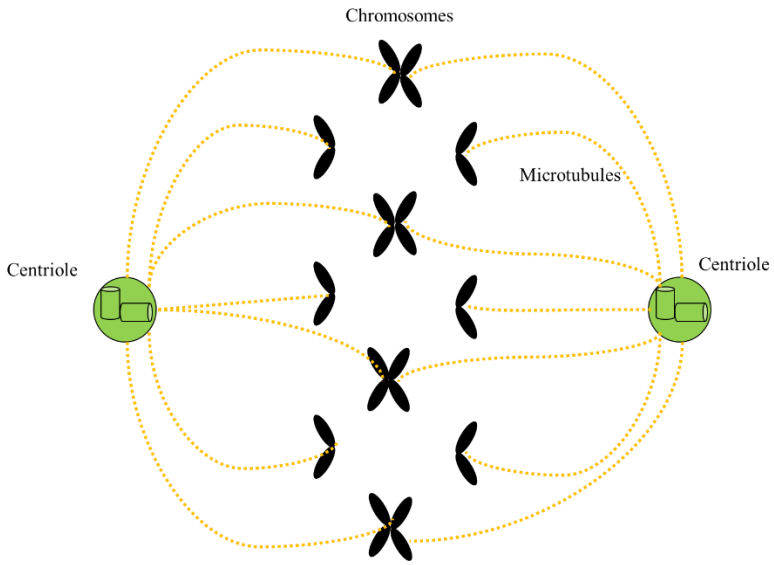


Figure 2.4 Microtubules separating chromosomes during mitosis.

Centrosomes duplicate themselves prior to mitosis and move to the opposite side of the cell nucleus, after which the nuclear envelope breaks in prophase freeing the chromosomes into

cytoplasm. The condensed chromosomes are attached to kinetochore microtubules originating from the centrosomes on the opposite ends. These chromosomes are broken into two halves and carried towards the negative polarity of MTs by motor proteins as shown in figure 2.4. This is followed by the ripping of the cell at the center by polar and astral MTs.

2.2 A Survey on Self-Organization Simulations

Many of these self-organization models and the interactions of MAPs and GTPs proposed so far were purely through experimental observations. However, they have not been practically observed in many cases because of the limits in microcopy. This necessitates the requirement of an alternative approach to understand these mechanisms, which was realized through computational modeling and simulations. Some of the works, followed for our current research are presented here.

Bassetti et al., [26] proposed a 2D model for stabilized microtubule organization with a combination of rotational DOF and MT polarity dynamics. This resulted in a driving field which was configuration dependent. A simplistic numerical simulation of self-organization of MTs was modelled by Nicolas et al., [27], reproducing the molecular diffusions in a chemical process. This goes with the idea of varying concentrations of tubulins on growing and shrinking ends, i.e., with higher concentration on shrinking end a directionality is obtained. They also simulated the effect of gravity on this organization process. Jun et al.,[28] focused on the self-organization of cortical microtubules, in which MTs in 2D cortex of plant cells were simulated demonstrating the effect of inter MT collisions on de-polymerization and their self-organization into parallel arrays due to their polarity. Also, the effect of several mutants destroying the self-organization was analyzed in their work.

Our current work draws some inspiration from the work of Gutmann et al., [29] in simulating gliding behavior in microtubules in a real time 3D environment. Gliding driven by motor proteins forms dynamic rings and bundle structures and this involves millions of computations, posing a challenge to its real-time simulations. This was overcome by using general-purpose computing on graphic processing units (GPGPU) for their programs.

CHAPTER – 3

FRAMEWORK

3.1 Background

In our current work, we aim to achieve self-organization in tubulins leading to protofilaments and ring structures. Microtubule behavior discussed in the previous chapter is modelled as per the swarm engineering principles discussed in the following sections.

3.1.1 Swarm Intelligence

Swarm Intelligence (SI) is the collective behavior of simple agents working together, in accomplishing tasks which are beyond their scope through coordinated effort and interactions called emergence. The term first coined by Gerardo Beni [30], was based on intelligent behavior observed in natural swarms of fishes, ants, bees, sheep etc., Though the cognitive abilities at individual level are insignificant, their simple low level interactions gives them the ability to accomplish highly complex tasks. The tasks at individual level are performed by the swarm agents, without the knowledge of the global outcome and without any centralized control.

Initial attempts in developing computer simulations for these behaviors were made by Craig Reynolds in 1986 [4]. He proposed three simple rules of separation, alignment and cohesion for boids (bird-oid objects), demonstrated flocking like behavior observed in birds. In his strategy, a boid tries to keep away from colliding with the swarm through ‘Separation’, while heading in the mean direction of the swarm through ‘Alignment’ and ‘Cohesion’ by steering towards the mean

position of the surrounding neighbors. These rules though sound complementary, are weighted vectors whose resultant values generate a flocking pattern. An illustration of these rules in presented in figure 3.1.

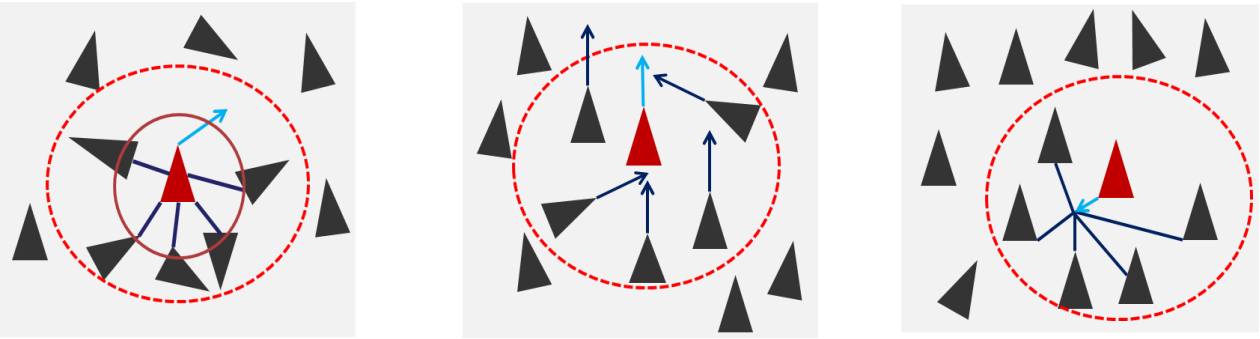


Figure 3.1 Separation, Alignment and Separation demonstrated by Reynold's boids

More complex behavior of fishes and insect swarms were first modelled by Kennedy and Eberhart through their famous Particle Swarm Optimization (PSO) [5]. PSO is a simple two step update for position and velocity of the swarm agents. The velocity computations preceding position updates are obtained from the cognitive and social affinity factors multiplied with the agent's relative personal best position and its relative position with swarm's best (leader), respectively. The position of the swarm is updated iteratively leading to a convergence either at local or global optima. PSO, gained reputation for its ability to optimize functions in a non-linear fashion and hence is applied on problems in which finding an optimal value turns impossible through conventional techniques. However, these heuristic based algorithms are not guaranteed to find a global optimum and hence in improving their performance, many variants were proposed over the last two decades. Also, we proposed a variant called Graded Particle Swarm Optimizer [31], a hierarchical gradation based technique. Other algorithms belonging to this class of population based search include, Ants Colony Optimization [1], Artificial bee colony algorithm [32], Fish school search [33] etc.,

3.1.2 Swarm Engineering

Swarm engineering is the application of knowledge, science and engineering design principles in modelling and developing swarm intelligent systems. Kazadi et al., [34] defined swarm engineering as “The design of predictable, controllable swarms with well-defined global goals and provable minimal conditions.” And a swarm engineer as the one who models these systems which can reliably achieve the targets on time. This area still being in its infancy, has seen major developments in the recent times through its applications in the field of robotics.

Swarm Robotics though directly does not define the engineering principles, it is the nearest approach we can find in addressing real-world problems through swarm intelligence. Hence, in the current project, we formulated our design principles from a swarm robotic perspective.

3.1.3 Self-Organization

Self-organization is an order arising in a system spontaneously from local interactions of the parts of a disordered system. It is a spontaneous decentralized process and hence no central agent controls it [35]. This phenomenon is observed in many physical, chemical and natural processes like swarming, which is often described as self-assembly.

In a review on swarm robotics by Brambilla et al., [8], self-assembly was classified under the sub class of spatially organized behaviors in collective behaviors alongside aggregation, pattern formation, object clustering and assembling. Spatially organized behavior is the distribution, organization and collective movement of robots in space in forming chains, patterns, aggregations and complex structures. These are similar to the self-organizing behaviors in natural systems, except for the difference in the rates with which they occur.

3.2 Proposed Framework

In our frame work, we segregated the agents into multiple levels based on their level of association. Any agent which on interaction with other agents does not disintegrate into smaller agents was considered as a primary agent in that level. In which case, tubulins become the primary agents in level 1. A spherical shape was chosen, in order to avoid artifacts raising due to irregular geometries during simulations and also, globular proteins are spherical in shape in general (hence called globular). We modelled our agents by a behavior based design approach [8], where the design of their behavior was by understanding the processes occurring at tubulin level. This is a trial and error process, in which the behavior of the agents was altered till the desired global configuration was attained. Also, our models were designed at microscopic level, in which the agents are individually analyzed. This contrasts with a macroscopic model, where the whole system is analyzed through a set of differential and rate equations.

For our current simulations, we considered three proteins, α, β tubulins and GTP/GDPs as swarm agents. These agents are spherical in shape and are colored in black, gray and cyan colors for clear distinction. They were restricted to move only in a 2D plane, and the collisions, interactions with the environment and other agents was monitored through a physics engine.

3.2.1 Tubulins as Agents

In our current framework, we segregated all the proteins (tubulins and MAPs) into three types based on their functionalities. These were termed as primary, secondary or control, and functional agents, based on assembly levels.

Primary agents at any level are the basic building block proteins and any assembled primary agent at a lower level becomes a primary agent at a higher level. To demonstrate this, let

us consider the general hypothesis of MT formation. At the basic level, both α and β tubulins act as primary agents. After the formation of heterodimers from tubulins, the definition of agent is moved to the next level where the heterodimers are treated as primary agents. Similarly, this progresses through protofilaments, sheets and tubules, where each of these act as primary agents in that level.

Secondary or control agents are the agents which influence the behavior of primary agents directly or indirectly. Each control agent is associated with a unique functionality and can also vary its state based on its association with other agents. For example, GTPs and MAPs acting on dimer level primary agents, vary bonding strengths or increase the probability of bond breakage, by which they demonstrate the dynamic instability behavior. These agents have specific effects on the primary agents and a group of different MAPs are responsible for achieving a target, say chromosome separation with kinetochore configuration [14].

The third type are the functional agents which can work on the primary agents irrespective of their level, organization and association with other agents. Examples of these include searchers and breakers. A summary of these types of agents is presented in figure 3.2. This level upgradation strategy was planned in order to improve validation and support retraction of a model due to failures occurring while introducing new MAPs in to the framework.

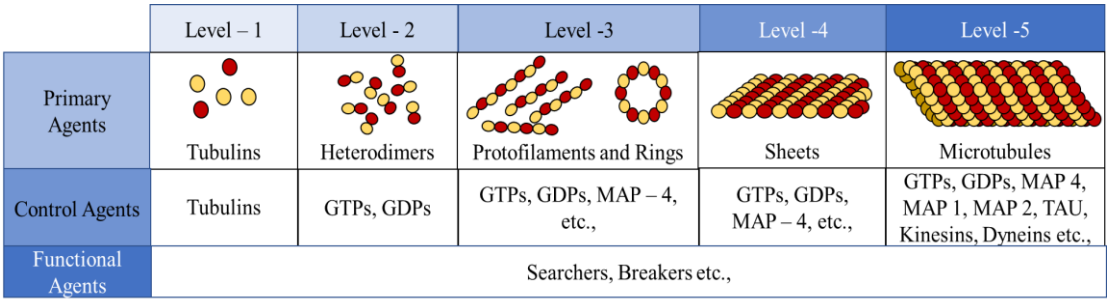


Figure 3.2 Agents segregated into different levels and groups

In summary, at each level, a primary agent is a combination of primary agents from the lower levels and hence has a different geometric configuration. Also, the interaction of control agents is limited to the level of their corresponding primary agents i.e., control agents can interact with the primary agents of only certain levels. However, functional agents can interact with the primary agents irrespective of their level.

For our current problem, we limit ourselves to the formation of dimers, rings and protofilaments at level 3 and the interaction with GTPs as control agents.

3.2.2 Tubulin Bonding Sites

Geometry, Environment and Interactions are the necessary parameters to be defined before starting with any self-organization simulations [19]. As mentioned earlier, the shape of all the agents was limited to spheres. In addition to that, we add additional tiny cube like structures on the sphere surfaces for creating bonding sites as shown in figure 3.3. Bonding sites are the locations where, one agent attaches itself with another agent after meeting all the criterion.

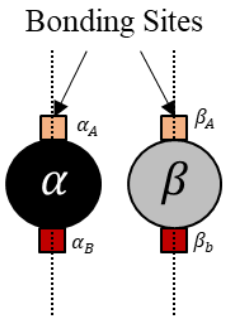


Figure 3.3 Bonding sites (exaggerated) on tubulin agents

Each tubulin has 2 bonding sites and a primary axis passing through the bond and sphere centers as shown in figure 3.3. This axis is chosen as a reference for bond angles or azimuth angles, with respect to which variations in bond positions are made depending on the kind of interactions

the tubulins has with different control agents. Initially, these bond angles are set to zero. Also, these bonds come in pairs and follow the thumb rule, like sites never bond. That means, all A bonding sites try to bond only with the B bonding sites. A summary of agent properties is presented in table 3.1.

	Tag	Alpha	Beta	GTP
Geometry	**Shape**	Sphere	Sphere	Sphere
	No. of Bonding Sites	2	2	0
	Color	Black	Gray	Cyan
	Initial Bond Angles	(0,0)	(0,0)	-
Motion	**DOF**	2	2	2
	Forces	Random	Random	Random
	Boundaries	Yes	Yes	Yes
	Collision	Yes	Yes	Yes
Interacting Agents	**Primary Agents**	α - Tubulin	β - Tubulin	α, β -Tubulin
	MAPs	GTP	GTP	-

Table 3.1 Agent Properties

3.2.3 Rules for Assembly

Having defined the geometric parameters of the agents, to get organized structures, it is important to define rules of interactions between different agents and the environment. In this section, we explicitly define these rules for motion and interactions.

Rule 1: Move tubulins randomly in a 2D space with a random force updating at equal time intervals, given by equation 3.1.

$$(F_x, F_y) = \begin{cases} 0 & \text{if } \Delta t \neq T \\ \text{random}(-F_{max.}, F_{max.}) & \text{if } \Delta t = T \end{cases} \quad (3.1)$$

Where, (F_x, F_y) is a 2D force vector. The values of these are chosen randomly between $[-F_{max.}, F_{max.}]$ once in every T seconds. T and $F_{max.}$ are force update interval and

maximum directional force respectively. These are given as user inputs prior to starting the simulations. This gives a Brownian motion in the particles and hence the value of F_{max}. should be moderately selected. Also, this force rule is applied on all the agent types in our framework, however, the values of F_{max} change depending on the agent.

Each bonding site acts as a communication center for the other agents. An agent detects its neighbors through these bonding sites before deciding its interactions with them. Hence, we set a trigger zone around the bond in detecting its neighbors as shown in figure 3.4. Also, each agent is assigned a group of tags for the ease of detection and response.

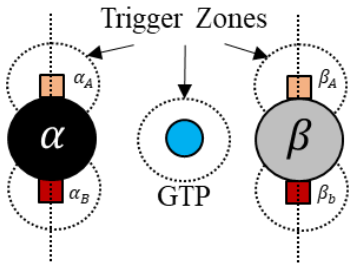


Figure 3.4 Trigger zones on tubulins and GTPs

Every bonding site upon entering the trigger zone of other agent's bonding site is checked for its tag and the following rule 2 is applied. It must be noted that, a set of flags were maintained to get the state of the agent prior to making a bond. These flags are updated after any changes in the bonds and after satisfying the rules. At this point, it can be noted that there are no specific bonding sites specified for GTPs and hence they are free to associate themselves with the tubulins.

Rule 2: On collision with any one of the bonds with other agent's unlike bonds, a joint is created upon meeting the conditions in 3.2.

$$\text{Joint}_{\text{dimer}} = \begin{cases} \textbf{false} & \text{if} \quad \text{Tag}_{\text{thisAgent}} = \text{Tag}_{\text{collider}} \\ & \quad \text{or} \ \text{Tag}_{\text{thisParent}} = \text{Tag}_{\text{colliderParent}} \\ & \quad \text{or} \ \ \text{Flag}_{\text{bond}} = \text{true} \\ & \quad \text{or} \ \ \text{Flag}_{\text{dimer}} = \text{true} \\ \textbf{true} & \text{if} \quad \text{Tag}_{\text{thisAgent}} \neq \text{Tag}_{\text{collider}} \\ & \quad \text{and} \ \text{Tag}_{\text{thisParent}} \neq \text{Tag}_{\text{colliderParent}} \\ & \quad \text{and} \ \ \text{Flag}_{\text{bond}} \neq \text{true} \\ & \quad \text{and} \ \ \text{Flag}_{\text{dimer}} \neq \text{true} \end{cases} \tag{3.2}$$

A joint is created when $\text{Joint}_{\text{dimer}}$ is true. Consider the bonding site α_A which is a bonding site linked to α tubulin. All bonding A bonding sites are tagged with the same names and similarly for the bonding sites B and each parent is also given a tag separately. When $\alpha_A$detects β_B falling in its vicinity, its tag is compared to the collider's tag, which in this case are unlike bonds, 'Bond A' and 'Bond B' respectively. Then the tag of the parents they are linked to are checked, i.e., the tubulin they are attached to. In this case the parents are 'Alpha' and 'Beta' respectively. Hence as the tags don't match and it is checked if these bonding sites are already bonded or if the bonding agent is already a dimer through $\text{Flag}_{\text{bond}}$ and $\text{Flag}_{\text{dimer}}$ statuses. If false, a joint is created between α_A and β_B and upon forming a joint, the status of the flags, $\text{Flag}_{\text{bond}}$and $\text{Flag}_{\text{dimer}}$are set to true.

Rule 2 was designed based on the formation of heterodimers from tubulins as explained in chapter 2. These heterodimers are inactive and cannot bond with other dimers unless they are activated by GTPs.

For rule 3, we defined two different approaches based on the type of final shape we desire. Each of these rules is based on the change of bond locations due to interactions with GTPs as proposed by Ravelli et al., [36]. This idea of relocating the bonds on the spheres draws inspiration from the doctoral dissertation of Teich-McGoldrick [37], in which they propose a model through

bonding patches on spheres, which self-organize to form honeycomb structures . The details of these rules are presented in two parts. In addition to the rules of association with GTPs, we added a probability function to GTPs, as their bonding is not limited to a specific location on a tubulin.

Rule 3 (for protofilaments): For an inactive dimer on collision with GTP, the following flags are checked

$$\mathrm{GTP_{bond}} = \begin{cases} \textbf{false} & \text{if } \mathrm{Flag_{dimer}} = \text{false} \\ & \text{or } \mathrm{Flag_{gtp}} = \text{true} \\ & \text{or } \mathrm{P_{random}} \leq \mathrm{P_{GTP}} \\ \textbf{true} & \text{if } \mathrm{Flag_{dimer}} = \text{true} \\ & \text{and } \mathrm{Flag_{gtp}} = \text{false} \\ & \text{and } \mathrm{P_{random}} \leq \mathrm{P_{GTP}} \end{cases} \tag{3.3}$$

In 3.3, $\mathrm{P_{GTP}}$ is probability of association for GTPs with tubulins. This is a constant, given as a user input. A random number $\mathrm{P_{random}}$ is picked between 0 and 1 and checked against $\mathrm{P_{GTP}}$. Upon, satisfying 3.3, the colliding GTP is destroyed assuming its association with the colliding tubulin. Also, the color of the tubulin is changed for our identification. Now, the tubulin is active and can participate in polymerization by joining with other active tubulins.

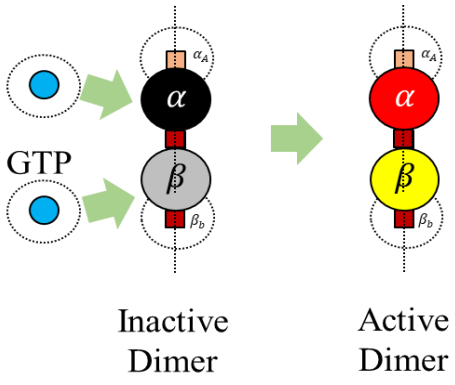
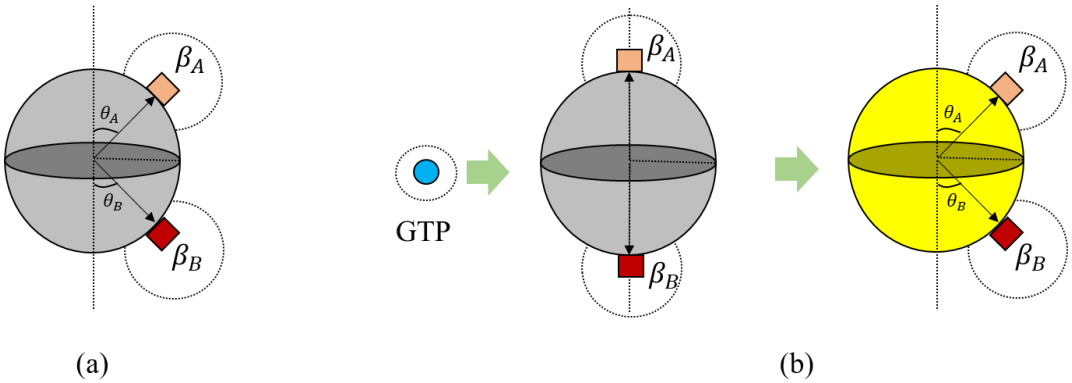


Figure 3.5 Transformation of inactive dimer to an active dimer through GTP interactions.

To identify the status and level of the tubulins, the $Flag_{dimer}$ is set to false as the tubulin now belongs to a polymer than a dimer and hence the other flag $Flag_{gtp}$ is set to true. An illustration of the process is shown in figure 3.5. It must be noted that, the bonds don't change their angle with respect to their axis for protofilaments.

The bonds in 3D placed on a spherical surface can be rotated with respect to their azimuth and elevation angles as shown in figure 3.6 for rings. But for our current problem, the elevation angle was not considered as the motion of the agents is limited only to two dimensions. Now these angles are computed with respect to the axis as shown in the figure 3.6.



(a) (b)

Figure 3.6 a) The azimuth angles of bonds β_A and β_B are θ_A and θ_B respectively. b) Relocation of the bonds in β tubulin on interaction with GTP

Rule 4 (for Rings): The condition in equation 3.3 is applicable for the ring formation as well. However, the bond angles θ_A and θ_B change as shown in figure 3.6. These values are chosen prior to the simulations, based on which the ring size and the number of members required. Hence in a dimer, the bonds tilt after collision with GTPs as shown in figure 3.6 and thus forming ring structures in our current problem. The transformation in bond angles in dimers is shown in figure 3.7.

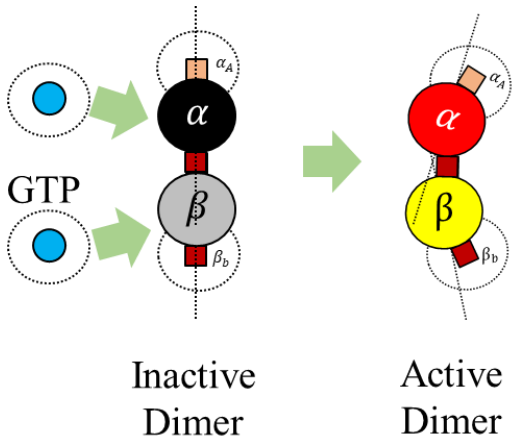


Figure 3.7 Transformation of inactive dimer to an active dimer through GTP interaction and change in bond angles.

The next and final rule is assembling dimers in forming protofilaments and rings. Which are through direct collisions like rule 1

Rule 5: An active dimer bond, on collision with another active dimer bond the following condition is checked for making a joint.

$$\text{Joint}_{\text{polymer}} = \begin{cases} \textbf{false} & \text{if} \quad \text{Tag}_{\text{thisAgent}} = \text{Tag}_{\text{collider}} \\ & \quad \text{or} \ \text{Tag}_{\text{thisParent}} = \text{Tag}_{\text{colliderParent}} \\ & \quad \text{or} \ \text{Flag}_{\text{bond}} = \text{true} \\ & \quad \text{or} \ \text{Flag}_{\text{dimer}} = \text{false} \\ & \quad \text{or} \ \text{Flag}_{\text{GTP}} = \text{false} \\ \textbf{true} & \text{if} \quad \text{Tag}_{\text{thisAgent}} \neq \text{Tag}_{\text{collider}} \\ & \quad \text{and} \ \text{Tag}_{\text{thisParent}} \neq \text{Tag}_{\text{colliderParent}} \\ & \quad \text{and} \ \text{Flag}_{\text{bond}} \neq \text{true} \\ & \quad \text{and} \ \text{Flag}_{\text{dimer}} = \text{true} \\ & \quad \text{and} \ \text{Flag}_{\text{GTP}} = \text{true} \end{cases} \tag{3.4}$$

A joint is created between two hetero dimers as shown in figure 3.8. When $\text{Joint}_{\text{polymer}}$ is true. We monitor the length of the strings and rings forming during this phase by logging data into a text file.

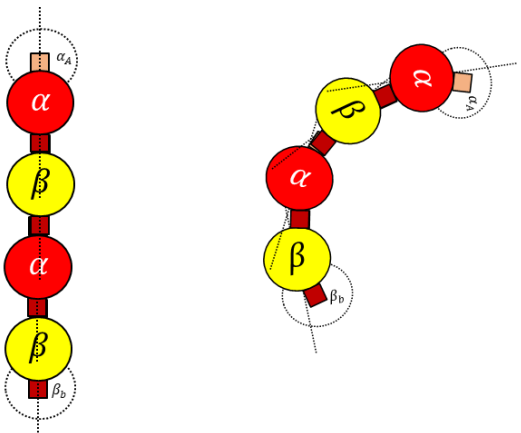


Figure 3.8 Polymerization in dimers forming (a) Protofilaments and (b) Rings

3.2.4 Extended Rules

Apart from the regular rules for assembly discussed in the previous section, we also propose two additional rules in line with the bond breakage in protofilaments Vis a vis, hydrolysis of GTP to GDP and vice versa. These rules are currently only for the protofilament formation and breakage; however, they can also be extended to the formation of rings.

Extended Rule 1: A GTP is formed from a GDP as per the following condition

$$\text{State}_{\text{GTP}} = \begin{cases} \textbf{true} & \text{if} \quad \text{State}_{\text{GDP}} = \text{true} \\ & \text{and} \quad \text{Duration}_{\text{GDP}} \geq \text{Limit}_{\text{GDP}} \\ \textbf{false} & \text{if} \quad \text{State}_{\text{GDP}} = \text{false} \\ & \text{or} \quad \text{Duration}_{\text{GDP}} \leq \text{Limit}_{\text{GDP}} \end{cases} \tag{3.5}$$

A GTP is formed from a GDP which is associated with a water molecule. However, the water molecules are replaced with a duration, $\text{Limit}_{\text{GDP}}$. A duration value, $\text{Duration}_{\text{GDP}}$ is

maintained for a GDP since it is spawned and upon crossing $\mathrm{Limit}_{\mathrm{GDP}}$, the flag $\mathrm{State}_{\mathrm{GTP}}$ is set true and the tag of the agent is changed to "GTP" from "GDP", allowing its collisions with other dimers.

Extended Rule 2: A terminal dimer breaks from the protofilament as per the following condition

$$\mathrm{Joint}_{\mathrm{Break}} = \begin{cases} \textbf{true} & \text{if} \quad \mathrm{P}_{\mathrm{random}} < \mathrm{P}_{\mathrm{computed}} \\ & \text{and} \quad \mathrm{Flag}_{\mathrm{terminal}} = \mathrm{true} \\ & \text{and} \quad \mathrm{Joint}_{\mathrm{polymer}} = \mathrm{true} \\ \textbf{false} & \text{if} \quad \mathrm{P}_{\mathrm{random}} \geq \mathrm{P}_{\mathrm{computed}} \\ & \text{or} \quad \mathrm{Flag}_{\mathrm{terminal}} = \mathrm{false} \\ & \text{or} \quad \mathrm{Joint}_{\mathrm{polymer}} = \mathrm{false} \end{cases} \tag{3.6}$$

A joint is broken when the random number picked in $\mathrm{P}_{\mathrm{random}}$is less than the computed probability as shown in equation 3.7, and when the bond belongs to the terminal dimer

$$\mathrm{P}_{\mathrm{computed}} = 1 - (1 - \mathrm{P}_{\mathrm{break}})^{\mathrm{Length}_{\mathrm{polymer}}} \tag{3.7}$$

In equation 3.7, $\mathrm{P}_{\mathrm{break}}$is a constant, given as user input and $\mathrm{Length}_{\mathrm{polymer}}$is the length of the polymer, the dimer is present in. From the equation 3.7, the probability of breakage increases with the increase in length and hence longer lengths tend to break dimers in the terminal ends. This rule was designed considering the future possibility of adding MAPs which alter the growth of protofilaments or tubulins, these can be directly linked to $\mathrm{P}_{\mathrm{break}}$ values. After a bond breakage, GDPs are released by the dimers mimicking the hydrolysis process and turn inactive getting ready to associate themselves with GTPs. GDPs progress

through to turn into GTPs as per the extended rule 1. An illustration of the process in presented in figure 3.9.

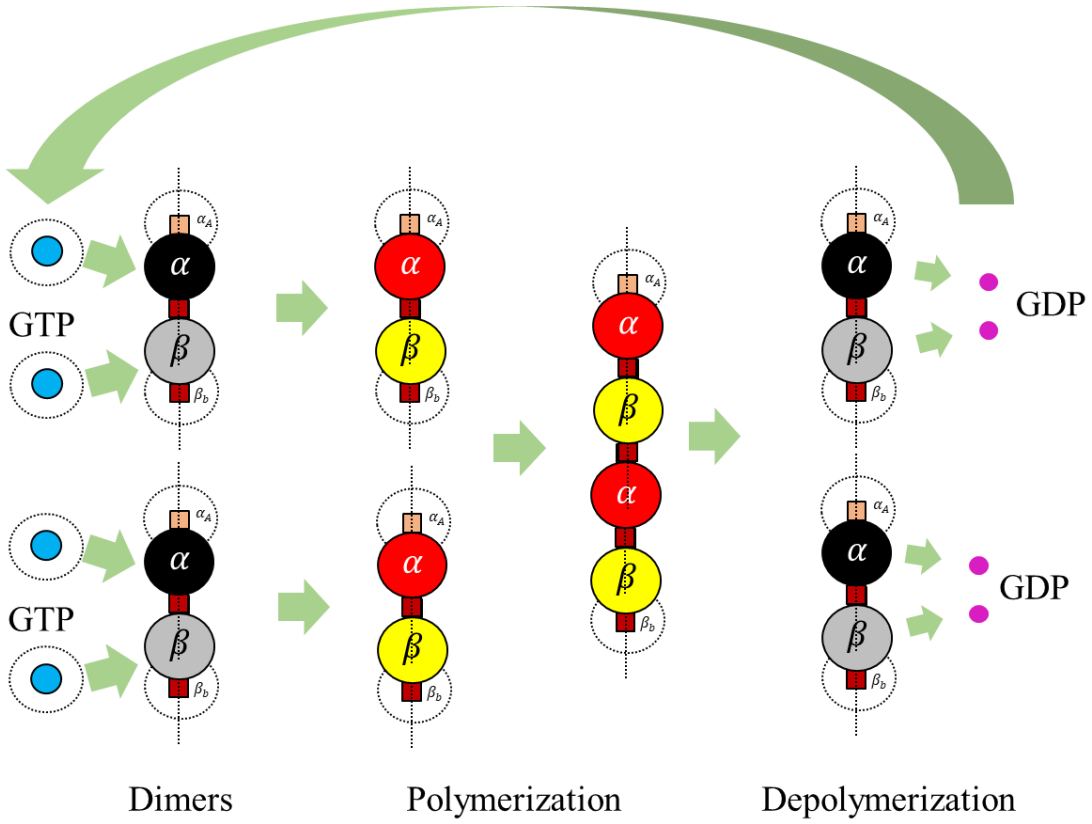


Figure 3.9 Complete life cycle of a GTP

In the current chapter, we introduced the frame work and designed rules for microtubule self-organization for forming protofilaments and ring structures applying the swarm engineering principles. We further proposed two extension rules where the transition between GTPs and GDPs was defined. Also an additional probabilistic bond breakage rule was proposed, where the length of the protofilament has a direct influence on the bond breakage. We summarize all these rules in the form of a flow diagram in figure 3.10. We implement these interaction rules on Unity game engine discussed in the next chapter. We also analyze their behavior on both the basic and extended

rules. We begin with details about the simulation environment, parameters and followed by analysis. A combination of parameters were tested on GTPs and bond breakage probabilities.

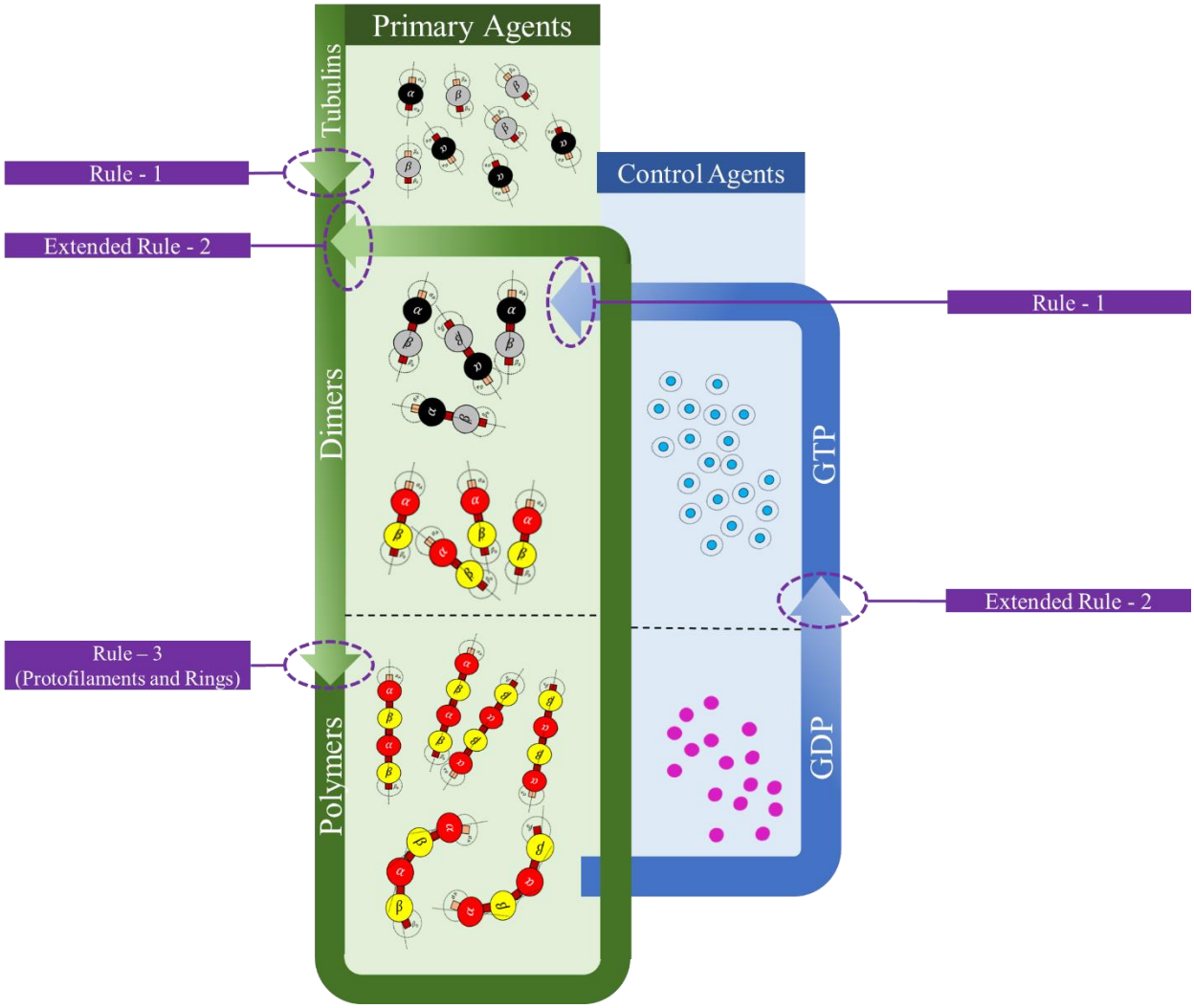


Figure 3.10 Process and rule diagram for Microtubule self-organization.

CHAPTER – 4

EVALUATION

4.1 Simulation Environment

We simulated our proposed frame work in Unity 5 game engine. Unity 5 developed by Unity technologies is a cross platform game engine widely used for game development and simulations. It has a strong physics engine and executes programs in parallel in a real time like environment. This was our best choice as it supports our multi agent strategy by allowing a single code to run on all the game agents, instead of time based allotment of processor for each agent, as done in other sequential ways. Another advantage of running swarms in unity was scalability. The number of swarm agents can be increased by large numbers without compromising the real-time performances. This software also supports integration with Virtual Reality. This gives a very different perspective to the researchers in understanding the swarm systems and molecular dynamics alike. Developing interaction tools for molecular dynamics in virtual reality is one of our future agendas. Most important of all it is freely available for non-commercial purposes.

4.2 Configuration Space

We designed a 3D configuration space in unity, but limited most of the movements of the swarms agents on to a 2D plane. The configuration space is a transparent tray, designed to constrain the motion of agents along one axis using colliders. Also, through this setup, we made sure that the agents don’t fly away during simulations by adding colliders on the walls. The dimensions of

the tray were maintained at 3 X 3 units and all the agents were initialized at random locations. A sample picture of the configuration space and agents is shown in figure 4.1.

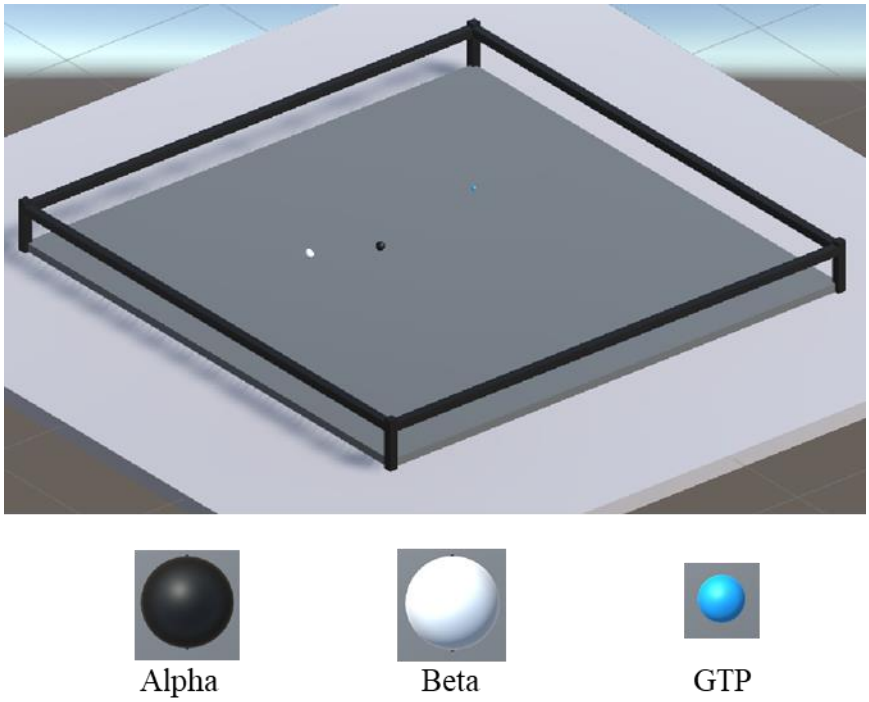


Figure 4.1 Configuration space and different agent types

4.3 Simulation Parameters

In all our simulations, the population of tubulins was kept constant at 250 each, for both α and β. We chose the frame count instead of time as termination criterion to avoid issues related to processing speeds on different computers. Hence, we got the same amount of data irrespective of system speeds. We ran all our simulations on an Alienware PC with a configuration of 3.40 GHz Clock Speed (i7 6700), 32 GB RAM, NVIDIA 1080 GTX Founder's edition GPU and high speed solid-state drive. The simulations ran very smooth without any noticeable lags. We logged all the data into a text file and was later processed in MATLAB 2017b in obtaining graphs. For our analysis, we varied the relative population of GTPs to understand their effect on the self-assembly

process and, we varied the parameter P_{GTP} to see the effect of the GTP association probability on the assembly. A summary of the simulation parameters is presented in table 4.1.

Parameter	**Value**
Configuration space size	3 units x 3 units (l x b)
Tubulin population	250 - α, 250 - β
GTP association probability (P_{GTP})	0, 0.2, 0.4, 0.6, 0.8, 1
GTP Population	200, 300, 400, 500, 600, 700, 800
Bond Breakage Probability P_{break}	0, 0.1, 0.3,0.5
Max. frames	100,000

Table 4.1 Simulation parameters

4.4 Simulation Results

We ran the simulations and recorded videos for all the parameter combinations for analysis. We also captured photos at different intervals as shown in figures 4.2 and 4.3. We present two sample simulations for protofilament and ring formations. The first simulation was with 500 tubulins and 500 GTPs with P_{GTP}= 1. This was followed by the simulations for rings, with the same parameter settings and the results are presented in figures 4.2 and 4.3 respectively. The analysis charts are presented in figure 4.4. In our current simulations, we started with a distribution of inactive tubulins and GTPs in the configuration space. Inactive tubulins are black and gray in colors and these tubulins combine to form heterodimers. A hetero dimer after bonding with a GTP, changes its color to red and yellow and gains the ability to polymerize, thus forming protofilaments and rings.

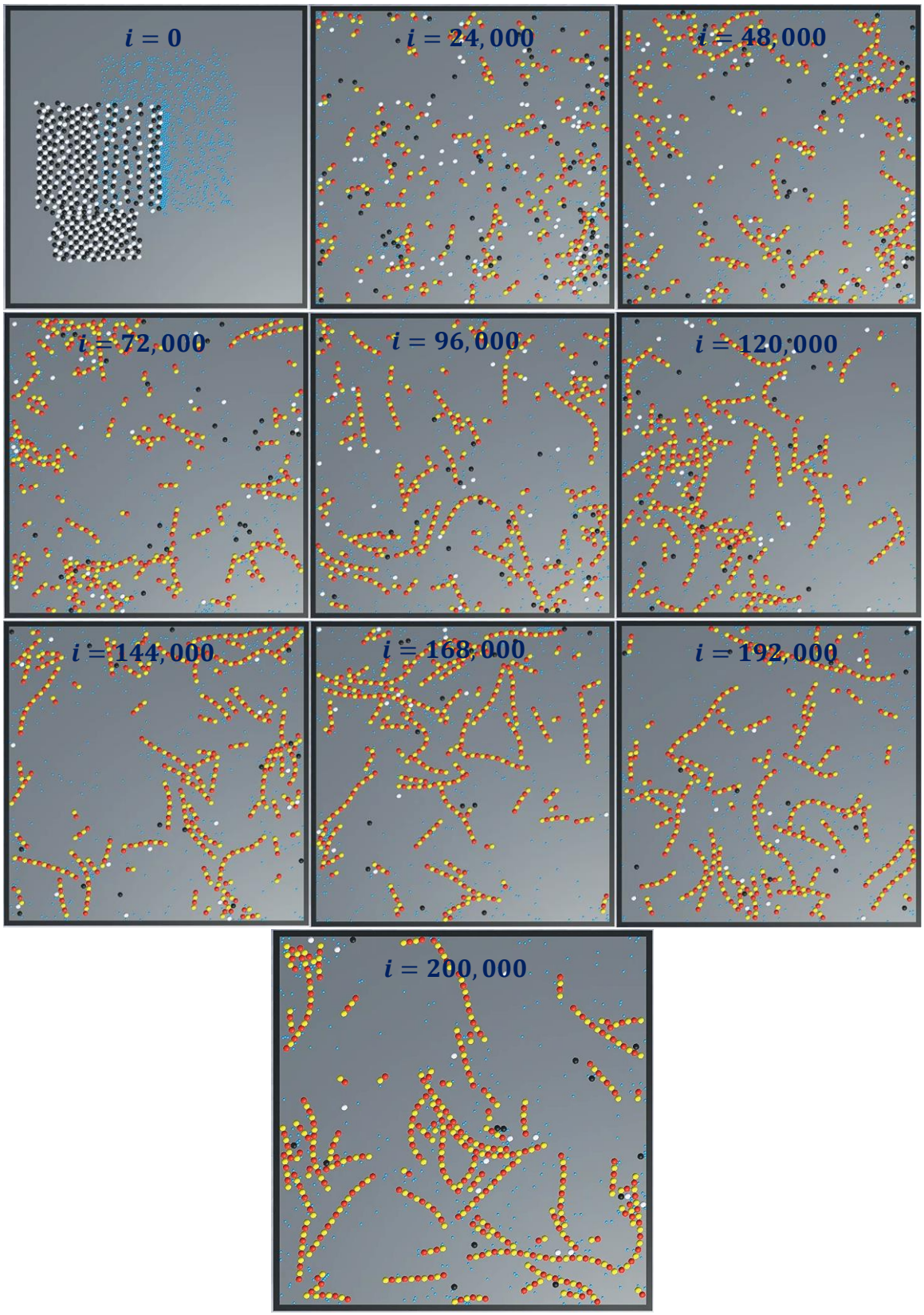


Figure 4.2 Self-organization of protofilaments from tubulins and GTPs

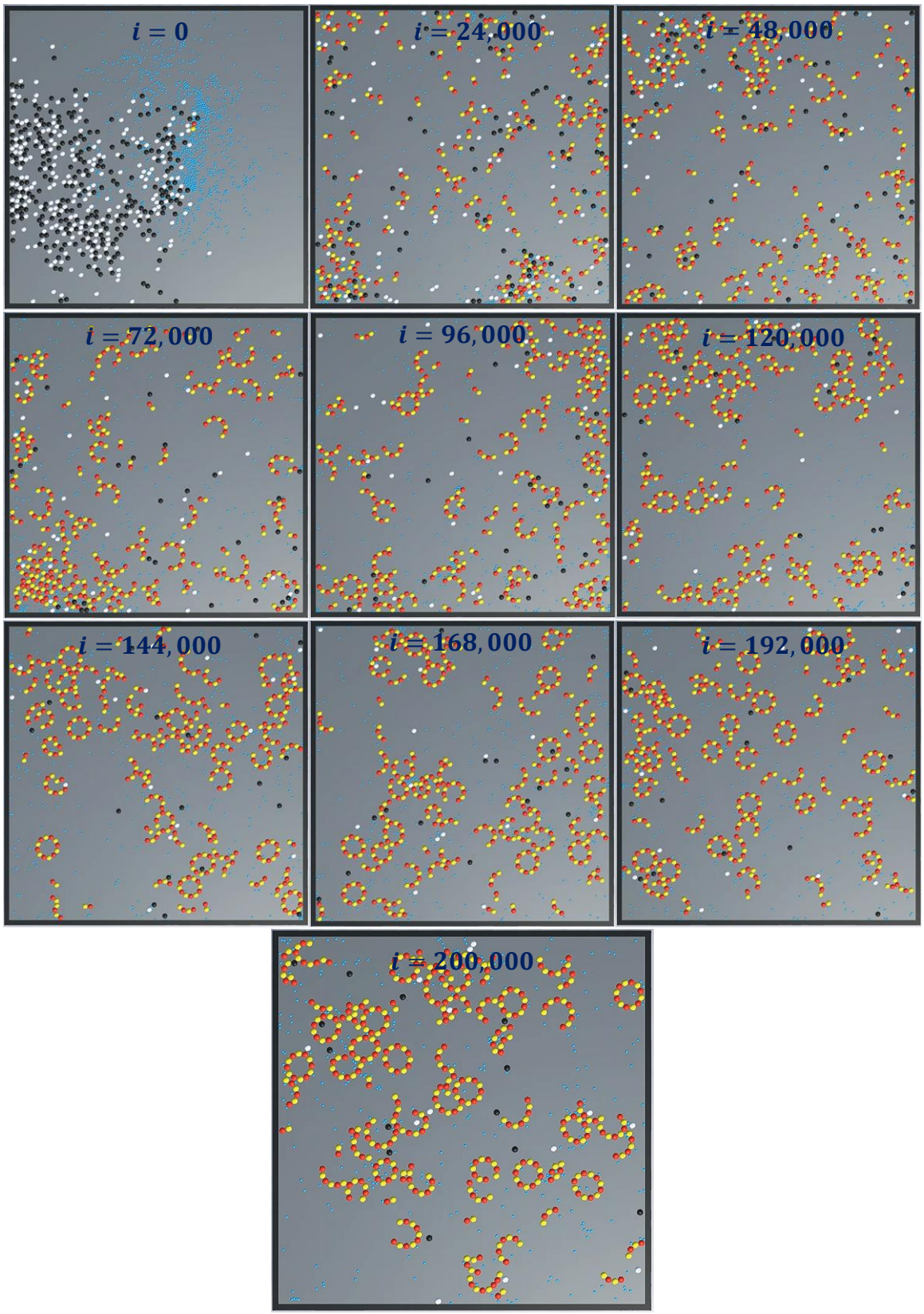


Figure 4.3 Self-organization of ring structures from tubulins and GTPs

From the simulations run, we obtained total number of dimers, number of inactive dimers, active dimers, GTPs and the maximum length of the protofilaments or rings forming. Their numbers in each frame and the rate change between frames is shown in the graphs in figure 4.4 for both protofilaments and rings.

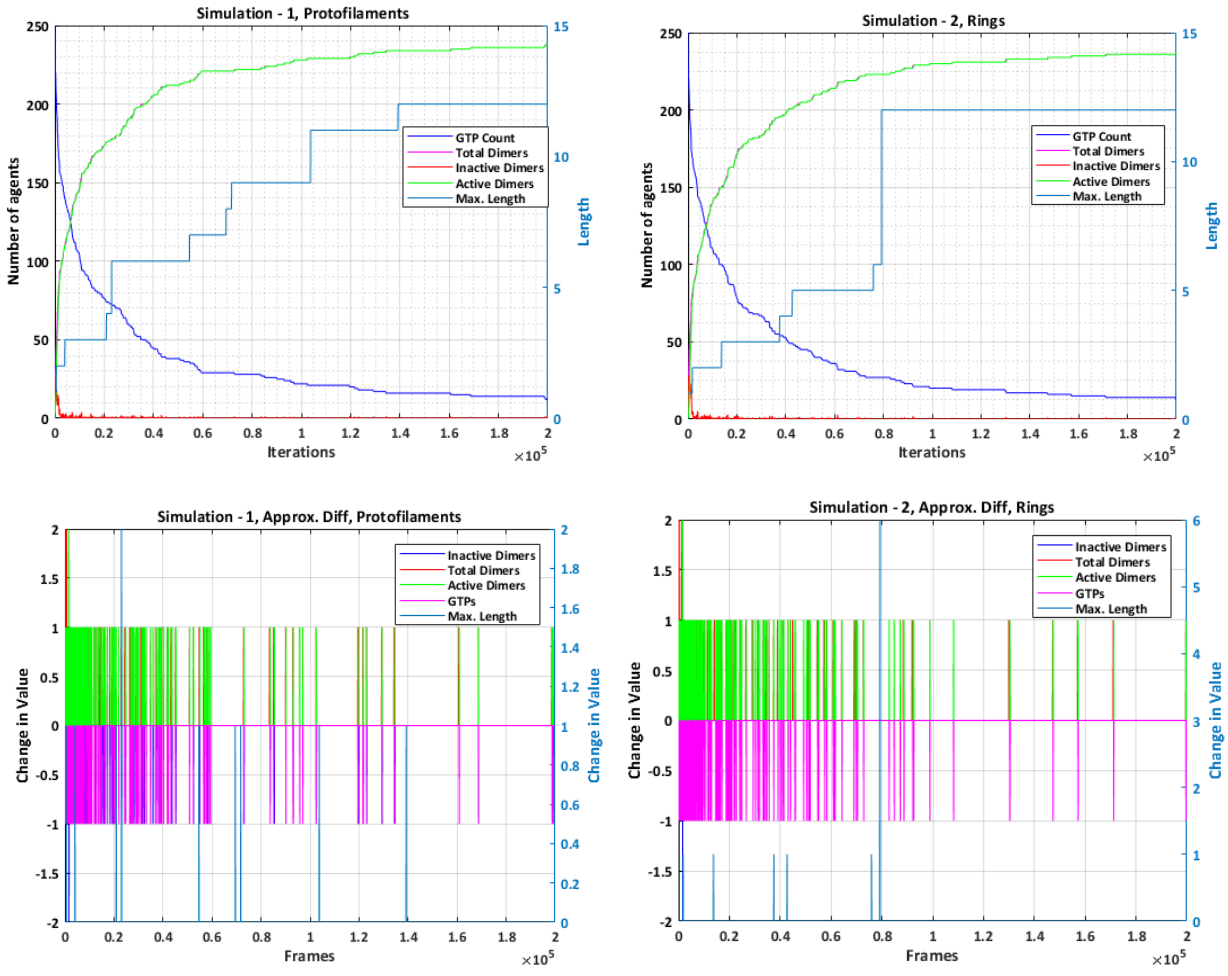


Figure 4.4 Plots tracking number of agents in each frame, rates and frequency of change

The first set of graphs in figure, shows variation in numbers of different agents, while the second set tells about the rate and the frequency of change in numbers. In the agent number graph, it can be noted that the sudden shifts in the max. length values are seen whenever a smaller or larger sub unit combine and thus increasing the length by a large value. Also, the number of

inactive dimers is low most of the time, as any inactive dimer on its formation is immediately targeted by a GTP.

4.5 Influence of GTPs

We went on to vary the parameters associated with GTPs to understand the factors influencing the assembly process. In the first set of analysis, we started with a constant population of 500 in GTPs and 250 in tubulin, but varied their P_{GTP} values as shown in the table 4.1 for both protofilaments and rings. And during the simulations, we obtained agent numbers as explained in the previous section and their corresponding graphs are shown in figures 4.5 and 4.6.

It can be noticed that there was no significant difference in the number of agents changing levels, with change in GTP association probabilities. However, in the inactive dimer count graph, for a probability of zero, the inactive dimer count took a reverse trend as there are not ready GTPs to make them active. This is equivalent to having no GTPs in the process. This is an indirect validation for the understanding the importance of presence of GTPs.

Most of the parameter graphs trends were close to each other demonstrating no significant changes with change in probability values, however, a low probability signified relatively mild shoot-ups in inactive dimer counts as sheen their corresponding graphs. This was more significant in the case of rings which might be because of entrapments of GTPs in partially formed rings.

The next step in our analysis was to vary the population of GTPs with constant probability. For to avoid complexities, we maintained a hundred percent chance of association for the GTPs with inactive dimers but varied the GTP population between 200 and 800 units, which is relatively between -80% and 80% , in the intervals of 20% for a fixed total tubulin count at 500. The results of these simulations are presented in figures 4.7 and 4.8.

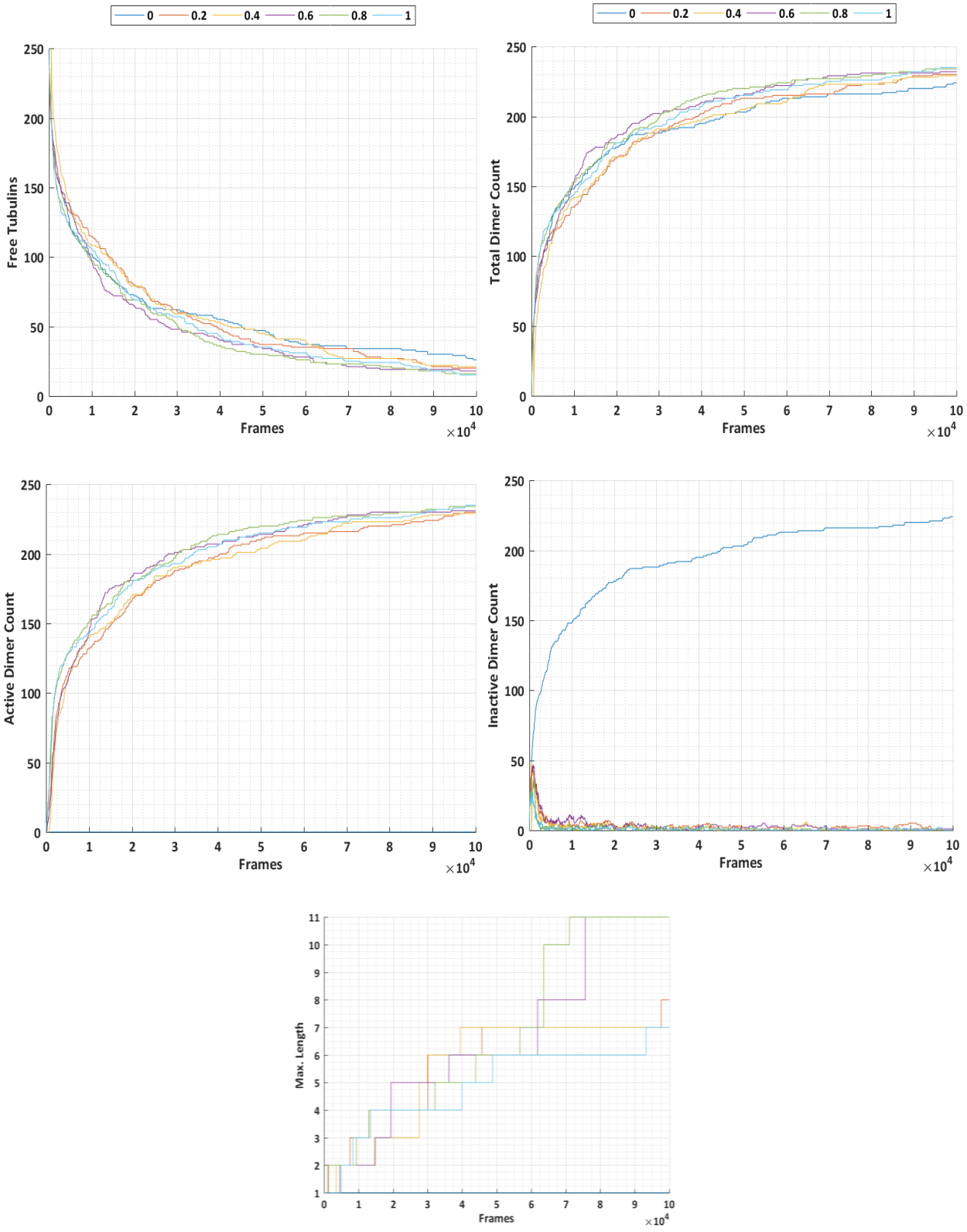


Figure 4.5 Graphs showing variation in agent nos. and length for varying P_{GTP} values in protofilaments

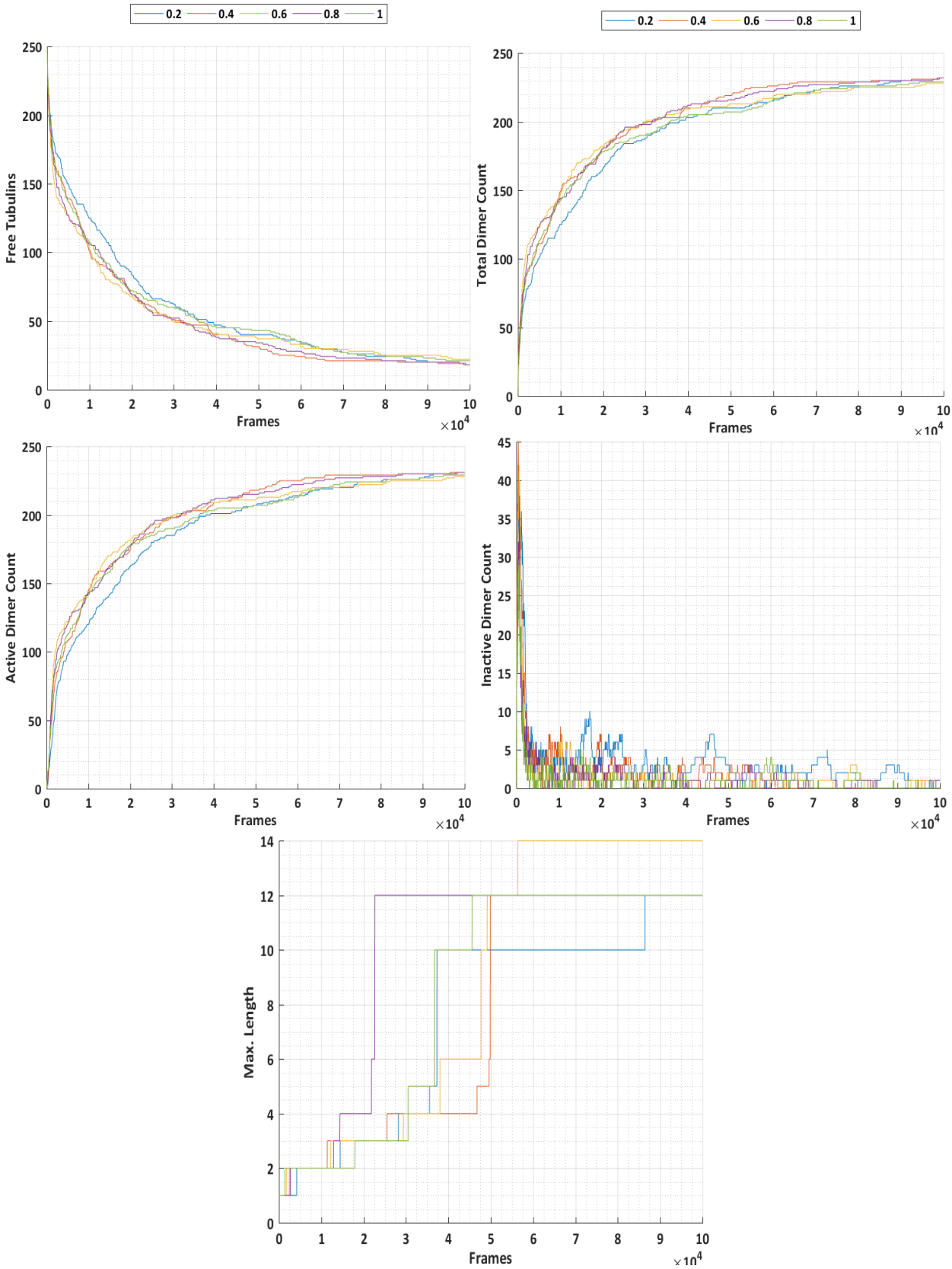


Figure 4.6 Graphs showing variation in agent nos. and length for varying P_{GTP} values in rings

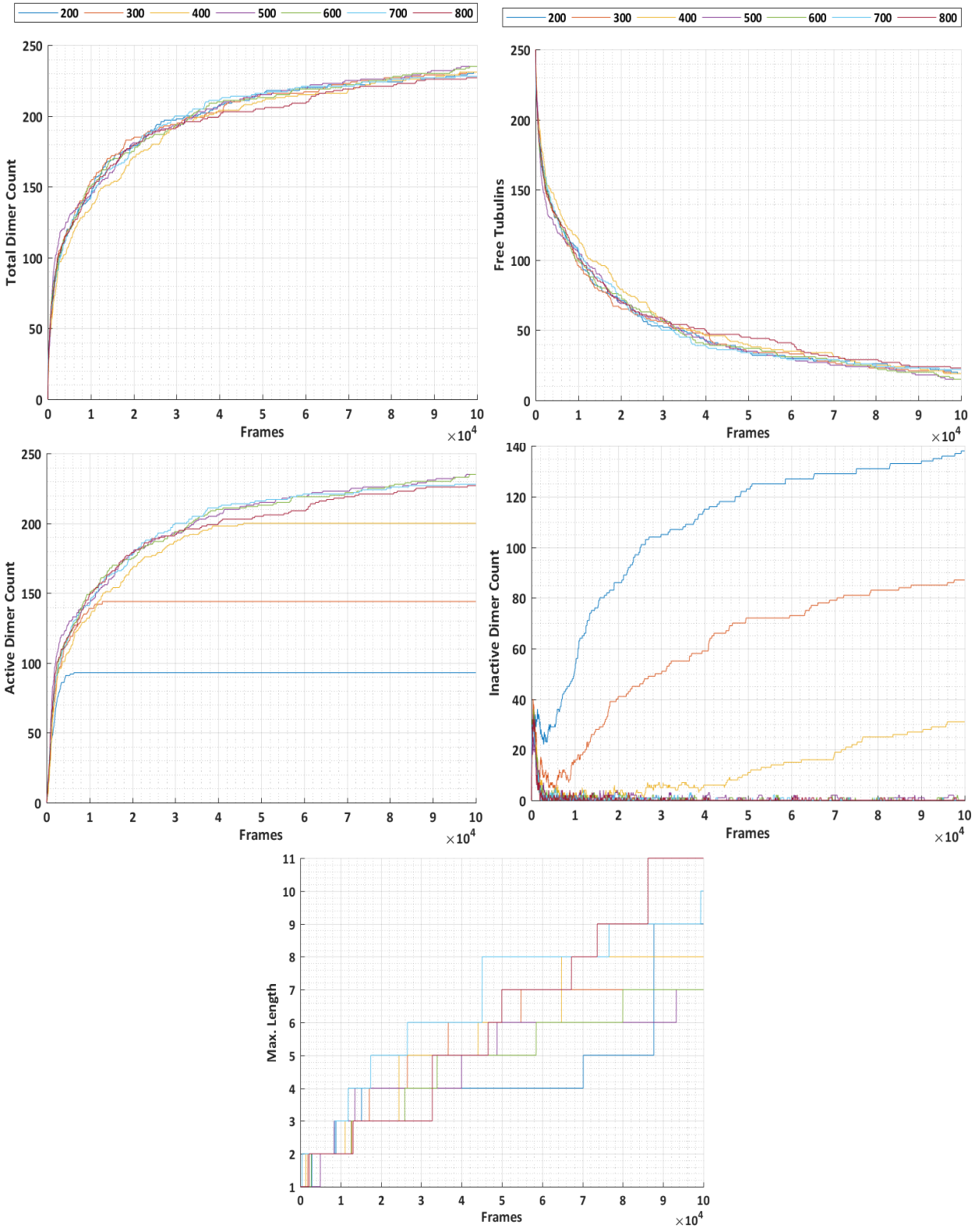


Figure 4.7 Graphs showing variation in agent nos. and length for varying GTP population in protofilaments

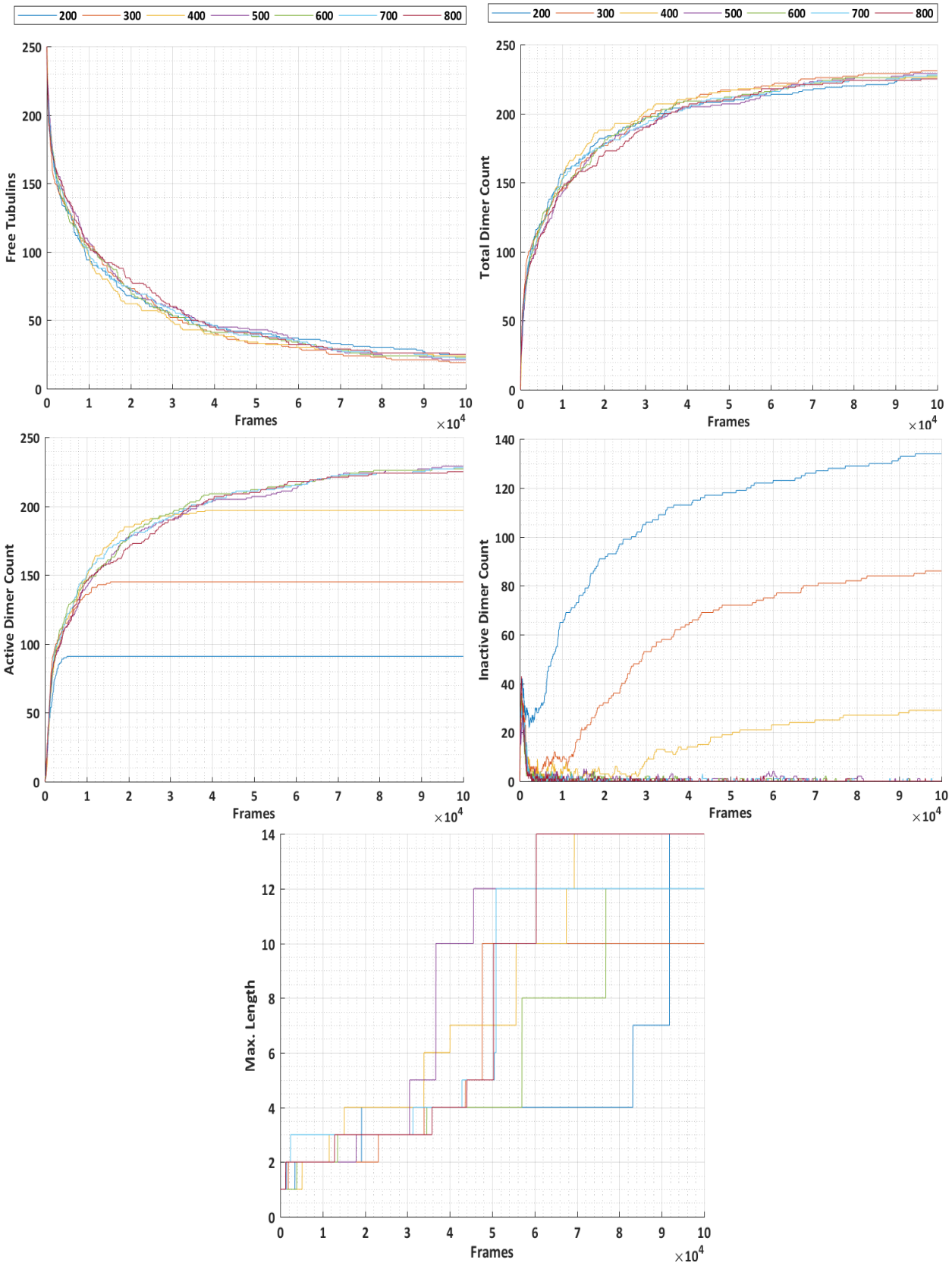


Figure 4.8 Graphs showing variation in agent nos. and tubulin length for varying GTP population in rings

4.6 Simulation of extended rules

We simulated the extended rules for varying bond breakage probabilities as discussed in the previous chapter. The probabilities were chosen to be as 0, 0.1, 0.3 and 0.5. A probability of 0 means no breakage, which was discussed previously. The simulation frames are presented in 4.9 and its corresponding quantitative results in figures 4.10 and 4.11.

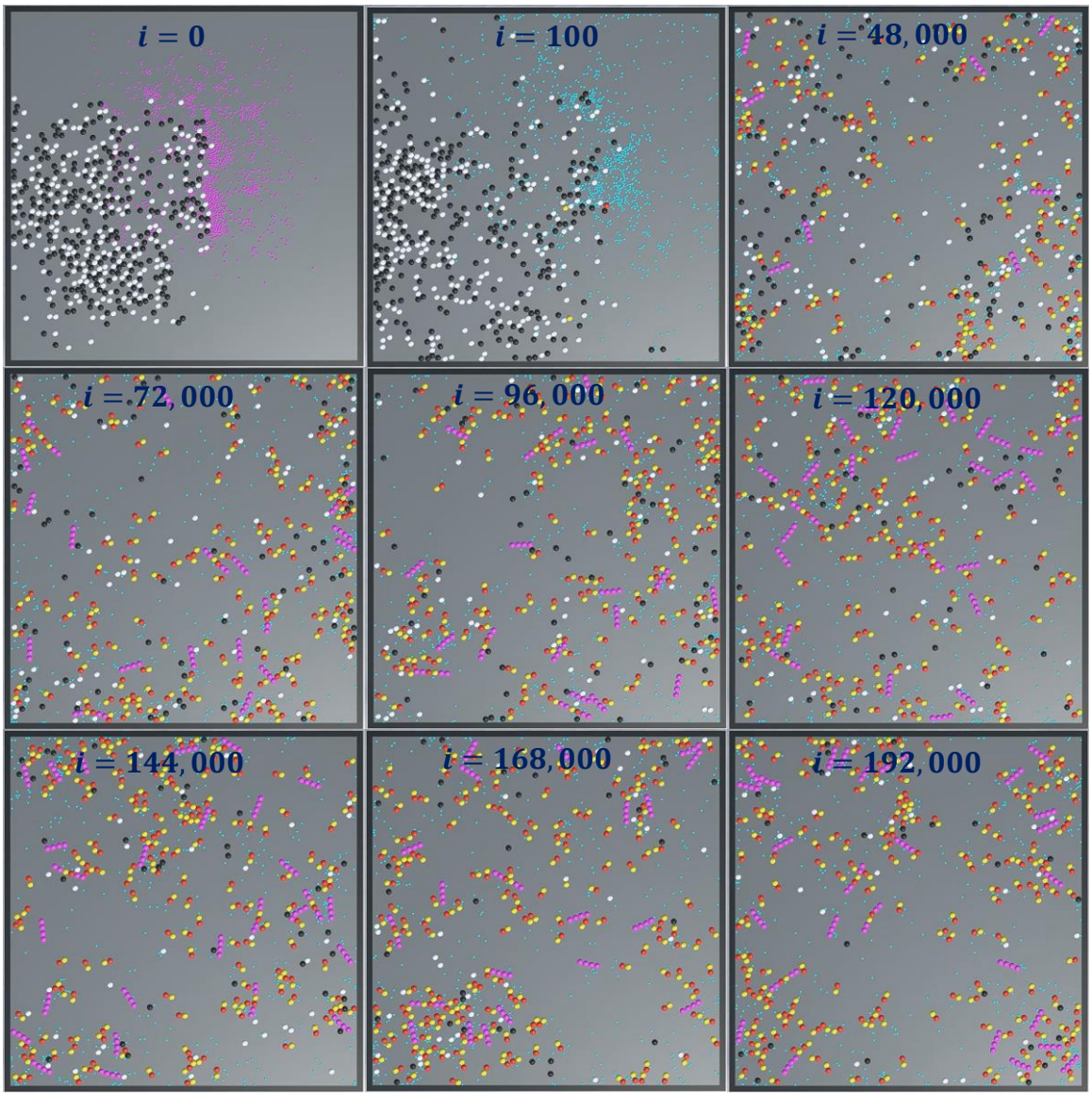


Figure 4.9 Sample simulations with extended rules for bond breakage

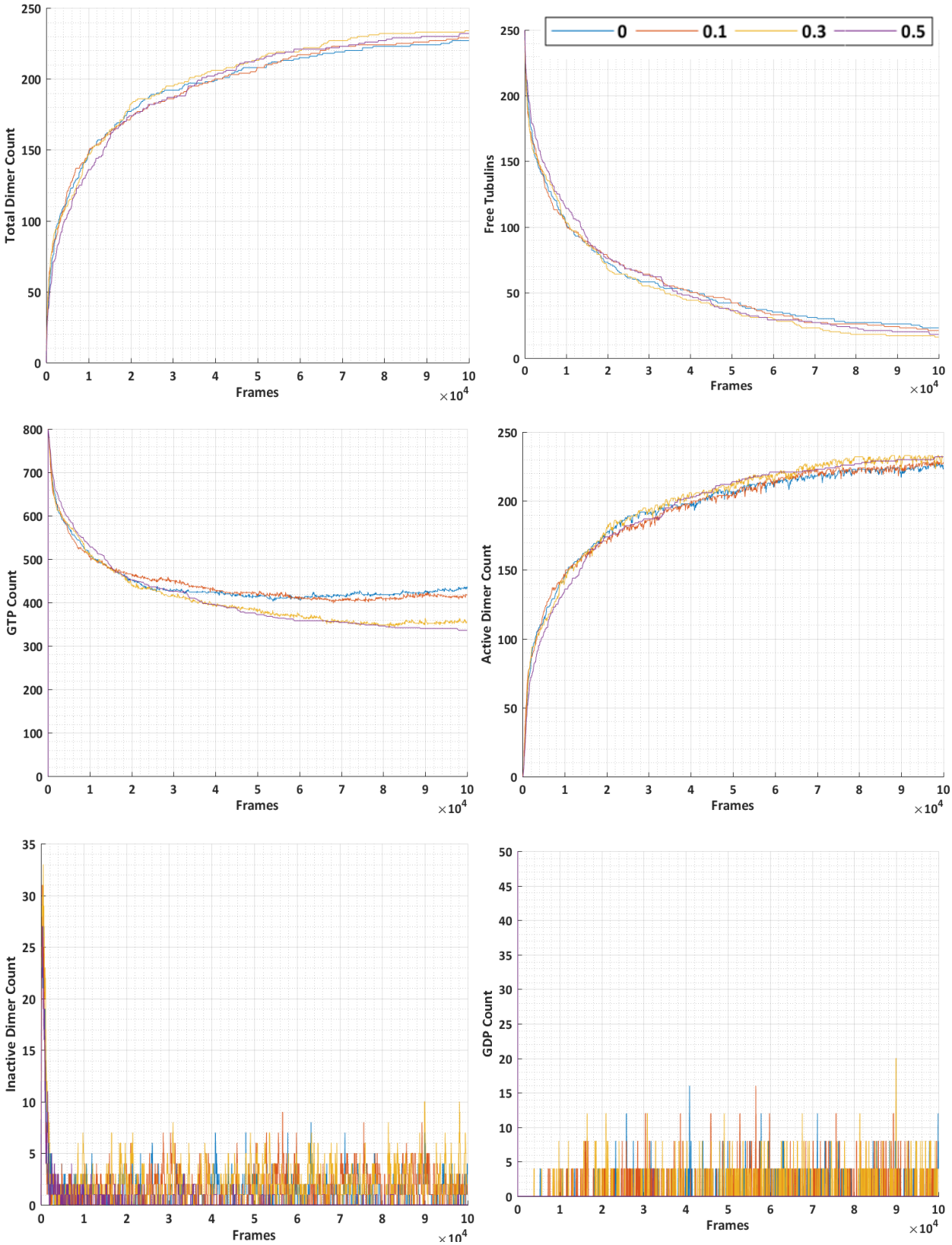


Figure 4.10 Graphs showing variation in agent nos. and tubulin length for varying breakage probability

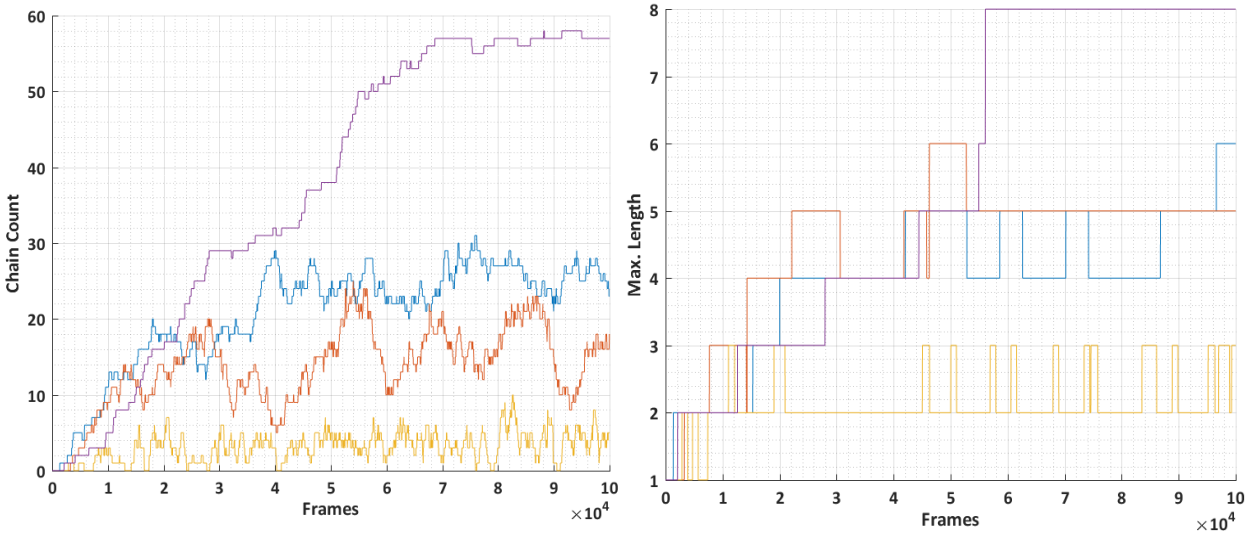


Figure 4.11 Graphs showing variation in number of chains and chain lengths for varying breakage probability

4.7 Discussion

From the above analysis graphs, it was found that the trends in free tubulin and total dimer count are not influenced either by the GTP association probability or the GTP count itself. It is also logical that, their levels of association are not same as the level in which GTP interacts. Most changes are in the numbers of active dimers and inactive dimers which are in level 2 as per our segregation model in which the control agents are GTPs.

The change in active dimer counts and inactive dimer counts are also not significant in the case of a constant population of GTPs irrespective of the change in probabilities of association. This is also evident from the second set of simulations where the populations were varied. In both rings and protofilament formations, the active dimer count reaches a saturation after certain number of frames when the population of GTPs was lesser than the population of tubulins. Also, the corresponding inactive tubulin count keeps raising after an initial dip.

An important observation in these graphs is that the active dimer count does not show any change in trend when the GTP population is greater than or equal to the tubulin populations. Hence, we observe that excessive concentrations of MAPs do not affect an MT function, however low concentrations do significantly affect. The threshold to this value can be determined by the tubulin concentrations.

Also, no conclusive observation could be made on the lengths of the protofilaments and rings as the data relating to their formation is more randomly driven.

In our extended rules simulations, we observed that the chain numbers largely vary with the probability values with the number of chains dropping with the increase in breakage probability. However, a reverse trend was observed with a 0.5 probability and also the chain lengths were maximum for high breakage probability.

CHAPTER – 5

CONCLUSIONS AND FUTURE WORKS

5.1 Conclusions

In the current work, a swarm intelligence framework for simulating sub structures of microtubules was presented. The simulations show the formation of protofilaments and rings, in an environment with almost real-time settings. Different shape formations like rings were observed just by relocation or rotation of bonds

It was seen that GTP as a control agent influenced these rotations leading to rings and string formations. The graphs presented explain the variation in the state of agents with time.

Though the GTP association probabilities had no major effect on the self-organization process, their concentrations significantly affected it. And it can be concluded that the relative concentration of tubulins and GTPs plays a significant role in influencing rates of polymerization.

5.2 Future Works

For our future works, we plan to extend our simulations to 3D with bond displacements along all the three axes. We expect that the current frame work would contribute towards this in forming complete microtubule structures starting from monomer units and GTPs.

After, the formation of microtubules, we plan to extend this Swarm Engineering framework in simulating dynamic instability in microtubules and the effect of different MAPs. Also, we plan to investigate the effect of cancer drugs like Colchicine, which are growth inhibiting proteins.

5.2.1 Agents as blocks strategy

We also plan to introduce machine learning and computational intelligence methods in automatic design of rules in comparison to the behavior based strategy followed currently in our work [8]. In this strategy, the agents are directly picked by the computational intelligence algorithms and tried out to match with the desired results defined in terms of a complex fitness function. These agents at different levels as discussed in the previous chapters have a set of predefined geometric properties and behavior rules and cannot be altered. However, a different agent can be picked and tried out till the targets are met.

The CI algorithms are expected to come up with the right combinations of agents and process sequences at each level and hypothesize the observed phenomenon. For example, let us consider the polymerization problem. The presence of GTP dimers extends the chains but immediate hydrolysis weakens them and eventually de-polymerize. With the primary blocks given to the algorithm, it tries out all the control agents and selects those which are extending the lengths. Further, the complexity increases when dynamic instability is observed and the algorithm is expected to use the right control agents (MAPs) to sustain good lengths. In the end the agent generates multiple hypothesis and process steps in achieving its goal.

However, this is constrained by the design of the agents itself. Absence of agents which stabilize the chains can lead to solutions which are non-convergent. In order to achieve this, we also propose a free agent strategy

5.2.2 Free Agent strategy

Free agents are in short agents whose rules can by dynamically selected by a learning algorithm. This is preceded by agents as block strategy, as the system is expected to learn the

behavior of the rules through supervised and reinforcement learning techniques before it tries designing its own agents. This is very helpful in the scenarios where the available agent's properties or rules are insufficient to answer a new problem.

As most of the agents in the prior strategy are de-signed based on the findings in literature, designing a hypothetical agent for a naturally observed process opens up new scopes of research in identifying their true existence. A summary of these strategies is presented in figure 5.1.

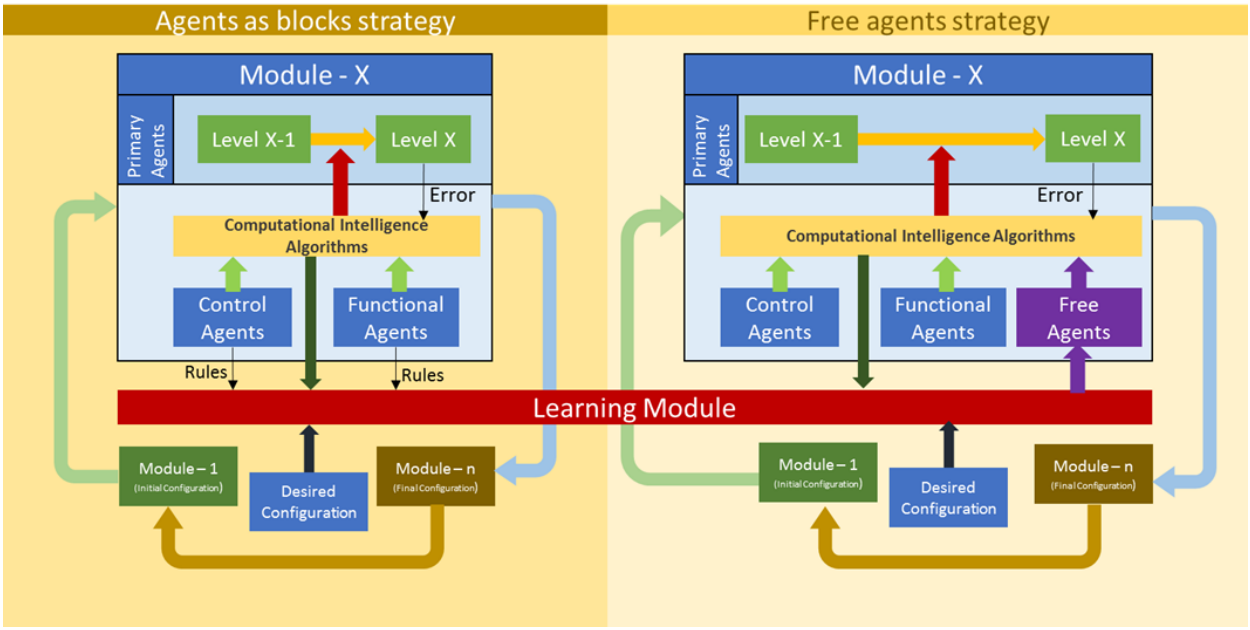


Figure 5.1 Layout for the proposed strategy for future works

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