Existence and Persistence of Microtubule Chemical Trails - A Step Toward Microtubule Collision-Based Computing

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Abstract. Microtubules and actin fibres are dynamic fibres constituting the cell skeleton. They show seemingly computational behaviours. Indeed, depending on conditions of reaction, they self-organise into temporal and/or spatial morphologies *in vitro* and act as micro-machine processors and actuators in living cells. I discuss here about their possible reuse to construct microtubule-based computational systems. I analyze particularly the possible existence of computational events based on 'chemical collision' between microtubules. A molecular model of microtubule disassembly has been developed to verify that heterogeneities of composition and/or concentration of the chemical medium can form from shrinking microtubules and persist. They could serve as an efficient communication channel between microtubules. Numerical simulations show that diffusing tubulin molecules can explore large distances (0.5 μm) during the disassembly of only one molecule. This leads to the formation of only very weak heterogeneities of composition. However, the model predicts that they could be more important due to microtubule arrays.

1 Introduction

Dynamic reacting fibres are numerous in nature. In living systems, they are, for instance, microtubules and actin fibres, both forming the living cells cytoskeleton, or helical tubular structures of some viral capsids such as those of T4 phages or tobacco mosaic viruses. Carbon nanotubes or DNA-designed nanotubes [1] are examples in artificially produced systems.

Some of these systems, in particular microtubules, are able to self-organise from homogeneous 'soups of molecules' to well structured morphologies made of ordered populations of dynamic reacting fibres. Little is known about their self-organisation. It is thought to be out-of-equilibrium and to imply reaction and diffusion processes coming from the chemical or physical interactions of large collections of reacting fibres. Static interactions are also present and may reinforce self-organisation.

Such systems are studied for the consequences of their self-organisation on the function of living systems. Self-organisation of microtubules is proposed to be one way by which living cells are sensible to gravity or other external fields [2–4].

Actin fibres were also shown to self-organise. Liquid-crystalline processes were proposed to explain the phenomenon [5]. Associated with other proteins, they show different forms of self-organisation, in particular the formation of 'actin comets' propelling bacteria in infected cells [6, 7]. Moreover, an artificial system constituted of carbon nanotubes, in particular conditions of reaction, is also able to self-organise [8, 9]. They form coral like 'macroscopic' structures made of self-organised carbon nanotubes. These structures form dynamically and imply permanent processes of creation, growth (catalyzed by cobalt particles) and shrinkage (due to atomic hydrogen) of carbon nanotubes. The manner this system self-organises has similarities with that of microtubule solutions.

In all these systems, self-organisation results from the numerous individual interactions that occur between 'molecular agents'. They look to be computed. Information and its 'quasi algorithmic' processing are primordial principles of the biological function and organisation. Indeed, living matter acts since more than 3.5 billion years to develop the most efficient forms of information processing and nano-machines that could exist. It includes the exploitation of the informative DNA molecule, all organisation, transport and communication processes, and extends to most complex levels of information processing and nano-machinery as the function and organisation of the organisms, their reproduction, or the 'calculi' realised in the nervous system, bringing to the emergence of consciousness.

Such sources of inspiration abound in Nature. They motivate new research approaches and begin to be exploited in computational sciences. Their use has been thought since many years. For instance, cellular automata models were directly inspired from living systems. They were used in particular to answer the question of self-replication and were also shown to be able to realise computational tasks. However, molecular computation is relatively new and a consensus upon it was initiated by M. Conrad that furnishes its principal concepts [10]. Yet, he mentioned the importance of self-organisation in biological systems and cites Hameroff ideas about microtubules [11]. Because of their structure, their kinetic functioning – their ability to self-assemble - and their self-organisation properties, they are of great interest in computational science. Theoreticians proposed that computational events could occur inside microtubules or between them [11–18]. From this, it has been proposed that they could constitute a kind of 'cellular nano brain' able to intervene in particular in neuronal brain functions.

This article focuses on microtubule self-organisation and on the molecular processes that generate it. In particular I develop the aspect of the possible communication between microtubules by the way of the formation of tubulin trails. I emphasize that self-organisation could be viewed as the result of numerous computational events coupled to various communication channels. I also propose several manners to control this phenomenon. Notions such as programmability (structural control), adaptability, robustness of a computation with microtubule solutions, are discussed.

Microtubule solutions are particularly interesting because they are easy to produce, to use and to manipulate. They can be considered as a toy-model system usable to develop and test the concepts of computation with dynamic fibres. Moreover, they don't only exist in the form of a simulated program. They are real ! So they could be used to conceive real processing units. Nature has beaten us: cells and organisms are yet equipped with such chemical processors [17].

2 Microtubule Self-Organisation

Microtubules are tubular shaped supra molecular dynamic assemblies of about 25 μm diameter and several microns length. They are present in all eukaryotic cells, constituting, with actin fibres, the major part of their cytoskeleton. Cell shape, cell moves, intracellular transport and cell division are biological processes in which they play a substantive role. Moreover, they are important consumers of chemical energy (GTPases) and should probably participate greatly to energy regulation in living cells.

It's known since more than 15 years that *in vitro* solutions containing only purified microtubules and chemical energy, the GTP (guanosine triphosphate), can self-organise into macroscopic stationary morphologies [19, 20]. The forming patterns present periodic variations of concentration and orientation of microtubules [21]. They are also known to contain imbricated self-similar substructures on several scale levels [22]. These patterns form progressively, in several dozen minutes to hours, permanently consuming chemical energy to maintain the reaction out of the thermodynamic equilibrium [20]. The reaction implies only two molecules, the protein tubulin (the constituting brick of microtubules) and a nucleotide, the GTP (a chemical energy source allowing microtubules to disassemble). The reaction is initiated by warming the solution from 4 $^\circ$ C to 35 $^\circ$ C. Microtubules form within one or two minutes and, during hours, they reorganise progressively into these macroscopic well structured morphologies. Moreover, this phenomenon is sensible to weak external fields such as gravity [2, 3, 23], vibrations [26], magnetic [24–26] or electric fields [24, 27]. The intensity or the orientation of these fields can trigger and modify the manner that microtubules will organise.

A liquid-crystalline self-organisation mode like in actin birefringent solutions [5] can't be excluded [28–30]. Nevertheless, microtubules are highly dynamic assemblies. Reaction and diffusion processes should intervene in these processes. Tabony's team proposed that they could form from reaction-diffusion processes [3, 31]. One of the principal assumptions of the proposed model was that microtubules could communicate between each other via a chemical way. Indeed, microtubules, while growing or shrinking, are locally modifying the concentrations and the chemical nature of the free molecules present in the medium such as, at least, tubulin and energetic nucleotides. For example, while shrinking, a microtubule is liberating a chemical trail constituted by free tubulin molecules (they are inactive, associated with GDP (guanosine diphosphate), but they are rapidly regenerated into tubulin active molecules, associated with GTP). An-

other – more debatable – hypothesis has been made concerning the chemotactic ability of microtubules. They used this subtlety to explain why new microtubules preferentially 'choose' to grow in these chemical trails. Of course, microtubules are not equipped with sensors that guide them. Nevertheless, this can be understood in terms of probability of presence and of preferentially growing direction. The probability that another microtubule nucleates or develops into this recumbent region is higher than in other parts of the solution. Moreover, the probability that the new microtubule grow in the direction of the trail is higher than in other directions. Microtubules are highly reactive. If they form and grow in these trails, they are stable and continue to exist; if not – if they nucleate in the chemical trail and grow in another direction, going out of the trail – they could penetrate in an area of the solution with unfavourable conditions of reaction. Then, they would probably disassemble rapidly. Once formed, the tubulin trails extend in all directions by molecular diffusion and, with time, decrease in intensity until the solution is homogenous. Then, microtubules just have to be in the neighbourhood of a trail to perceive its influence. Population of social insects, like ant colonies, are self-organising due to a communication between individuals by way of chemical trails called pheromone trails. The idea was that populations of chemically interacting microtubules could behave in same manner. Self-organised patterns of concentration and orientation of microtubules were obtained with this model. Populations of microtubules were self-organising by reaction-diffusion processes, at a microscopic level only [32] or at a macroscopic level also. The model was taking into account the triggering effect of the weak external fields [3,14,33]. Further, other teams made experimental observations showing evidences of such processes: new microtubules form and grow in the pathways let by other disassembling microtubules [19,34,35].

Other forms of microtubule self-organisation were observed. In other conditions of reaction, for example when the rate energy regeneration is low, one can observe periodic temporal oscillations of the bulk solution [36,37], or periodic microtubule concentration waves travelling at high speed rates [19,38]. When adding microtubules associated molecules (e.g. molecular motors like dynein or kinesin) more complex dynamic structures can appear (e.g. vortices [39]).

To resume, microtubule self-organisation seems to be an underlying mechanism linked to microtubule collective dynamics. The process is highly sensitive to molecular factors acting directly on the dynamics of microtubular ends – such as ion concentrations (calcium, magnesium ...), or other molecules like taxol, colchicine, nocodazole, used particularly in tumour therapeutics –, or acting on populations of microtubules such as microtubule associated proteins (MAPs). They are also sensitive to the presence or absence of external fields – gravity, vibrations, magnetic or electric fields – or geometric factors [40,41], acting globally on the whole solution. Using light is also a good manner to act globally or locally (e.g. with the punctual UV beams sources of microscopes or with UV lasers) on these solutions by breaking microtubules into small microtubules, producing more reacting microtubular ends and changing their dynamics [42], or by acting locally via other molecules, for example by using caged GTP molecules [43]. All

these factors could be used to guide microtubule self-organisation to an expected result.

3 Principles of a Microtubule Chemically-Colliding Computing System

Although no experimental prototypes of microtubule-based computers have been realised yet, number of experiments and numerical simulations have respectively shown or predicted macroscopic and microscopic behaviours compatible with a possible use in computational sciences. This section contains a review of theoretical ideas (and original ideas) on how microtubules could naturally compute. I also propose techniques to control them so as to obtain expected results. All these ideas about computation are only propositions and different manners to look at the microtubular system. However, each of them are reinforced by true data and experimental observations.

3.1 Microtubules as 'Turing Machine'-Type Supramolecular Assemblies

Turing machines are the formal representation of any computing process. They don't exist as physical objects but can be rewritten in any algorithmic programming language and implemented into von Neumann computers to realise calculations. The classic Turing machine is represented as a unique reading and writing head, moving and acting on a data tape (e.g. magnetic or optical data storage). The data tape contains a series of discreet finite states. The head also contains its own finite state. The program of a Turing machine can be expressed with 4 symbols: one for the initial state of the head, one for the output action – writing to the tape or moving on it –, one for the data value in front of the head (reading), one for the final state of the head after processing. Depending on these values and related actions, the head reads and writes the tape, and moves on it until the machine can not move and change. During that, a process is realised by successive individual steps. The result is the new data of the tape.

In comparison (fig. 1), microtubules are existing physical objects that can also be represented by an algorithmic programming language in a numerical model [10]. Their two reacting ends can be assimilated to two 'independent' Turing machines – they however can be linked internally by soliton waves [13,16,17,44] and transport of ions inside the tube [45] – and the body of the microtubule to an internal memory.

Microtubules form spontaneously from tubulin proteins associated with a nucleotide triphosphate, the GTP. When forming, the GTP is hydrolysed and a phosphate is liberated for each molecule of Tubulin-GTP assembled. Note that the hydrolysis of GTP is due to a structural change of tubulin after assembly. It is a consequence of the assembly. However, once formed, the tubulin-GDP is allowed to disassemble. GTP hydrolysis, during the microtubule assembly, is a

necessary condition for the microtubule disassembly. Then, the main body of the microtubule is principally constituted of tubulin associated with GDP. Nevertheless, it's a little more complicated at the microtubular ends. The assembly or the disassembly of microtubules is not linear with the concentration of free tubulin because of the presence of 'microtubule caps'. They are small zones of variable length (the length of, at least, only one molecule of tubulin to several nanometres long) having chemical and structural differences with the body of the microtubule. Their precise nature – chemical (cap made of a buffering layer of tubulin-GTP or tubulin-GDP-P, with the phosphate not yet hydrolysed or liberated) and/or structural (open sheet shape for growing microtubules, inside-out curled filaments for shrinking microtubules) – is still debated [46–48] but all agree to say they are protective zones against microtubule disassembly at the growing ends – and, respectively, against assembly at the shrinking ends. They act as buffer zones, permitting a microtubule to survive to an unfavourable reactive event and can introduce delays in the changes of reactivity of the microtubular ends. Further, considering the computational approach, they introduce internal states at the tips of the microtubules.

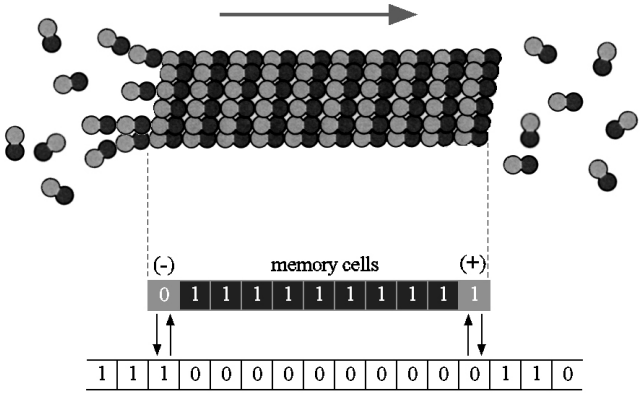

Fig. 1. Shows the scheme of a microtubule (up) growing at the right end and shrinking from the left end. At the ends, free tubulin heterodimers are diffusing. In parallel (down) is shown a microtubular Turing machine evolving on a 1D Boolean world (white cells). The concentrations of free tubulin heterodimers are idealised by Boolean values, 0 meaning 'no tubulin', 1 meaning 'presence of tubulin. The microtubule Turing machine is schematized by a non-reactive body, a series of 1 (black cells), acting as an internal memory of the previously encountered values in the world, and 2 writing and reading heads (gray cells). The processes at the heads depend on their internal state (0 for the shrinking end, 1 for the growing end) and on the local state encountered in the medium in front of the head. When moving (growing or shrinking), they modify locally the medium, writing a 1 (when liberating a 1) or a 0 (when consuming a 1).

Then, as would do Turing machines, microtubular ends 'read' and 'write' in the chemical informative support – the medium –, modifying locally the concentrations – and the compositions – of tubulin and nucleotides. The reacting states at their ends and the manner they will react are dependent to their own state – the chemical composition and the structure of the caps – and to the local concentration of their constituting bricks – tubulin-nucleotide molecular complexes – in the medium. If the end of a microtubule is in a stable state and if it is surrounded by a sufficient concentration of tubulin-GTP molecules, it can grow. In this case, it consumes the local molecules of tubulin-GTP, then creating a local depletion of the concentration of tubulin-GTP. On the contrary, if the end of the microtubule is in an unstable configuration and the chemical environment unfavourable, it disassembles, liberating locally tubulin-GDP molecules.

Moreover, when assembling from free tubulin-GTP, the microtubule stores in its body the information that was locally present in the medium – in the form of free molecules – and can restore it locally when disassembling. Indeed, when a microtubule disassembles, it leaves along its trajectory a concentrated trail of tubulin-GDP molecules. Before diffusion acts, homogenising the medium, information is locally restored. This information is available for other neighbouring microtubules. Note that the storage of information into microtubule bodies has a cost: it consumes GTP to assemble. Information is completely restored when tubulin-GTP molecules are regenerated by exchange of the tubulin-associated GDP with free GTP nucleotides present in the medium.

3.2 Microtubular Turing Machines Compute Self-Organised Morphologies

If microtubules are 'Turing machines'-type supramolecular assemblies, do they realise computational processes in microtubule solutions and how ?

Computational processes occurring inside microtubules have been already proposed and theoretically studied. In particular, S. Hameroff and J. Tuszinsky initiated and have developed a framework for a possible quantum computation in microtubules and its eventual role in biological processes, in particular brain processes such as memory, signal processing and consciousness [13, 16–18, 44].

Here, I focus on more macroscopic phenomena, easiest to observe and to control, that could also realise computational tasks. I consider the manner by which collections of microtubule Turing machines are interconnected, producing local and global ordering. I propose that this could be understood as the result of numerous computational events. In time, solutions of microtubules reach asymptotic behaviours – temporal and/or spatial – maintained by a permanent consumption of chemical energy. The outcoming morphological or temporal attractors are, in themselves, results.

In general, in complex systems, when some individuals are close together, they can interact – directly or via their surrounding environment – and this affects their internal states and their behaviours. These simple events are elementary computation tasks, also called 'collision-based computing' events [15].

They also could be associated to single instructions of a computer program. Correctly ordered and interconnected, these collision computing elements can realise more complex tasks. Ordering them to realise a particular task corresponds to a programming of the system.

From the concerted specific action of such computational elements can emerge a result in the form of a shape, a construction (social insects nests), a morphology (body patterning by pigmented cells [49]), or other emerging tasks (sorting out of objects and optimization tasks [50–53]). This may also be the case for microtubule populations. From their dynamics coupled to communications between them, a temporal [36,37] or spatio-temporal [2,19,22,31,54] self-organising behaviour emerges.

Saying that self-organised asymptotic behaviours are 'results' of a computation brings up the question of the stopping problem. Indeed, in these systems, by definition, as long as they are self-organising, equilibrium is never reached, so the systems never stop on a particular result (the state of the system can always change). It's easier to look at them as real time adaptable processors. The morphologies that form correspond to the spatial stationary states of the chemical processor. They are maintained because of a permanent consumption of energy and reactants, and are waiting for external stimulations to change. In case of an n-states periodic temporal behaviour, the associated possible states of the chemical processor would be each of the n-states. When external stimuli are applied to (load in) the solution, its behaviour adapts itself to the new conditions producing new asymptotic states. When all energy or reactants is dissipated, all out-of equilibrium emergent behaviour disappears. The system then stops to compute but this is not equivalent to the stopping of a program.

Such behaviours can be controlled or biased to realise programmed tasks. Examples are known where populations of interconnected Turing machine type individuals, well ordered and sharing a common informational support, produce computational tasks. This is the case in cellular automata simulations such as the well known Conway's 'game of life', where individuals self-organise and compute, depending on their internal state and on local neighbouring states [15, 55, 56]. Experiments concerning the effect of external factors on microtubule spatially self-organising solutions have shown that the self-organising behaviour can be influenced to produce one type of morphology or another [19,36,37]. It's reasonable to think that the same could be done with only temporal or spatio-temporal microtubule self-organising solutions.

In both cases – self-organising system or pre-ordered (programmed) system – computation can occur because information is shared and exchanged between individual agents. In addition, any self-organising system that can be controlled is susceptible to be programmed.

At a collective level, microtubules are controllable. We 'only' have to understand how microtubules interact and how to control them finely. Let's look at the list of possible communication ways that could exist in microtubule solutions, allowing self-organisation to occur.

3.3 Inter-Connexions Between Microtubules - Communications in the Solution

They are numerous manners by which microtubules can 'discuss' in a solution. These ways of communication are different in terms of rapidity, distance range, directionality and efficiency. Some are well established experimentally or are predicted by simulations; others are more hypothetic and hard to be observed.

- *Microtubules treadmilling* (and moves in general). *In vitro* or in living cells, microtubules are always-moving objects due to their dynamic nature. They have been observed to behave frequently in dynamic instability (stochastic-like behaviour of their reactive ends) or in treadmilling motion. During the former, they grow at one end and shrink at the other. A moving object is observed although the assembled tubulin does not move. While travelling, they behave as long range carriers of information in the solution, on the understanding that they can deliver this information locally after a long travel throughout the solution. This way of communication exists during all the life of the microtubule, is directional, but slow. It can't exceed the maximum growing rate of about 1 to 10 μm.min^{-1}.

- *Microtubular chemical trails or depletion areas - diffusion around the ends of the microtubule.* They will be more discussed in chapter 4. By growing or shrinking, microtubules modify locally, around their reacting tips, the composition and/or the concentration of reactants. A possible formation of chemical trails behind microtubule shrinking ends and of depletion areas around growing ends have been stressed by several teams. They suggested and calculated that the activity of a microtubule could influence locally, or at a more important distance, the reactivity of neighbouring ones [3,32,57,58]. Let in the pathway of microtubules, they should be directional. Their propagation by molecular diffusion is quick at the microtubule scale. If they exist, they would constitute one of the most fundamental mechanisms for the local microtubular inter-connexion. This can be called a chemical colliding computational event [15].

- *Molecular diffusion inside microtubules* [45]. Odde shown in 1998 that small molecules (ions, taxol, antibodies, nucleotides...) and tubulin heterodimers can diffuse inside the tube of microtubules. This was suspected because of the exclusive localization of interaction sites of molecules like taxol inside microtubules. Odde evaluated the diffusion for small molecules and for tubulin heterodimers. He showed that tubulin diffuses rapidly inside the microtubule, reaching the equilibrium inside of a 20 μm length microtubule in about 1 minute. What is important here is that this transport is purely directional, driven by the shape of the microtubule. When a molecule of tubulin explores a sphere of about 6 μm radius in 1 second outside microtubules, the same molecule would travel at the same rate but in only one direction inside the microtubule. This transport is directional, can be long range, depending on

the size of the microtubule, and fast. Other molecules, such as ions or energetic nucleotides that modify the reactivity of microtubules, can also travel inside microtubules.

- *Avalanches of disassembly in solutions near to instability.* Molecular diffusion is fast at microtubule scale but is limited in range. Tubulin-GDP emitted by a microtubule and travelling far from the tip will be rapidly diluted in the population of GDP-tubulin molecules emitted by other microtubules. In return, locally, an inhibitive modification of the composition or the concentration of reactants (increase of the tubulin-GDP concentration or depletion of tubulin-GTP) would affect the nearest microtubules, causing their backhanded disassembly. If, at one moment of the reaction, numerous microtubules in the solution are in a close-to-disassembly state and their density number sufficient, the locally-emitted information of disassembly could propagate very quickly as in excitable media. Microtubules will function as amplifiers and relays of the signal. In self-organised solutions, microtubules are spaced by only one or two microtubule diameters [59]. It's a very short distance for the molecular diffusion. The propagation rate will only be limited by the reactive rates of each microtubule and their levels of instability. It will propagate very quickly at long distances if all the solution is in an instable state. The phenomenon will start from instable nodes (groups of microtubular ends) and will propagate all around. In spite of the global instability state, stability nodes, as to say small areas where microtubules groups are more stable than all around, can survive in the solution. The avalanche of disassembly encounters these regions of hindrance, is locally stopped by them, and progressively decreases in intensity. At a macroscopic level, this could produce very quickly microtubule concentration gradients over millimetres or centimetres. The macroscopic manifestation of this plausible process is observed in microtubule solutions *in vitro* where microtubule concentration waves can form and propagate at rates of about 1 $\mathrm{mm.min}^{-1}$ [19]. It implies that microtubule reactivity is synchronised by any process over long distances. This avalanche phenomenon can explain that as well as the formation, in only few minutes, of the 'longitudinal bar' of microtubules observed in spatially self-organised solutions [40]. At least two observations reinforce this suggested mechanism. First, moments of instability exist in microtubule self-organising solutions: in Tabony's solutions, a chemical instability occurs 6 minutes after the beginning of the reaction, a moment where microtubules are particularly concentrated and instable (the instability is followed by a phase of rapid disassembly) [60]. In Mandelkow's solutions, growing phases alternate locally with massive disassembling phases, producing microtubule concentration waves. Moreover, the fact that the concentration waves or the spatial self-organised stripes depart from – or are influenced by – the sample boundaries [3, 19, 38, 40, 41] is consistent with this suggested mechanism.

- *Quasi-particle (conformational solitons) waves inside microtubules* [16, 17, 44]. Although not yet proved experimentally, theoreticians suggested that

tubulin heterodimers, as 'electrets' (electric dipoles), behave as bits of information, the microtubule lattice (the regular arrangement of tubulin in the body of the microtubule) becoming the place of internal computational events. Changes of the states of one or some heterodimers at one site of the microtubule propagate rapidly all around. Coherent states of neighbouring tubulin heterodimers can appear and propagate as soliton waves on the surface of the tube. Collisions of soliton waves can occur, giving rise to computational events directly at the surface of the microtubule. Ingoing and outgoing information comes from exchanges with the outer medium and with the neighbouring microtubules by way of local external fields (electromagnetic fields). Indeed, the formation of zones of coherent states on microtubules generates local oriented fields (perpendicular to the surface of the microtubules) that can be perceived by other microtubules. Moreover, it has been predicted that incoming soliton waves could affect the reactivity of microtubular ends. Reciprocally they could be generated by changes at microtubular ends (converting the energy of GTP into soliton waves). In this model, microtubule associated proteins would have an important role maintaining microtubules close together. Such a mechanism would allow the information to travel very rapidly on an individual microtubule from one end to the other (linking them by a propagation of internal states in the microtubule). Individual microtubules would then behave as nerves, receiving and integrating a signal at one end and/or along the body, propagating it, and emitting changes at the other end by the way of proportional changes of reactivity.

- *Mechanical interactions mediated by MAPs.* Microtubule associated proteins play an important role in living cells, controlling the precise structure of the microtubule cytoskeleton. They are of different types, producing various effects. Most of the time, they are molecular motors using energy of triphosphated nucleotides to travel along microtubules to one direction or to the other. Recent works shown that adding MAPs to microtubule solutions produces well ordered patterns [39]. As additive instructions to a programming language, they increase the diversity of behaviours of the microtubular system.

3.4 Control of the Process

The self-organisation phenomenon is autonomous and adaptable to external stimuli. It could then be used directly as an enslaved system, controlled by an interaction loop with the environment. Nevertheless, in regard to the huge combinations of elementary computational events in microtubule solutions, it would be interesting to consider their programmability.

The von Neumann computer opts for structural programmability [10]. That means that two programs having exactly the same structure, initiated with the same set of data, will produce the same result. On the contrary, in a molecular

computer, structural programmability is difficult to implement. Programming the system consists in the initiation of its configuration by setting exactly all states, positions and orientations of the molecules in the system. For the microtubules, that means to organise them precisely. Giving that, excepted in numerical simulations, the exact programming of the real molecular computer will not ensure to obtain the same result each time. Genuinely, due to thermal agitation and to the stochastic nature of chemical reactions, two identically-programmed processes will rapidly diverge. Two microtubules, in the same conditions, will not assemble or disassemble in the same way. So, controlling them is probably not interesting because the result of their interaction is weakly deterministic.

However, when considering a population – even small – of individuals, it may not be completely non-deterministic. If it was the case, we would not obtain reproducible self-organised behaviours. Two self-organising processes, initiated with similar conditions of reaction, will not give identical but very similar behaviours (morphologies, waves ...).

Solutions exist to consider structural programmability of microtubule solutions. They will be more discussed in the concluding remarks. In brief, they could consist in the use of the advantages of self-organisation, in its control, and in the isolation of small quantities of self-organising populations of microtubules. Micro-volumes of solution can be isolated physically – using physical boundaries – or virtually – using local actions (e.g. local electric fields delivered by electrode arrays). In fact, they self-organise in very small samples of cellular dimensions, as PDMS designed micro test tubes, micro-capillaries, phospholipidic vesicles and solution droplets [41, 61].

For instance, a structural programming of the system would be to use these micro-samples as micro-chips, controlling locally their individual self-organisation and configuring the connections of a population of these chips as could be done in cellular automata. Each chip will behave independently, reaching progressively its own morphological or temporal attractor, and will be influenced by information received from the neighbouring connected chips. Self-organisation of microtubules, in each chip, is controllable by external fields such as gravity or vibrations, magnetic, electric and probably electromagnetic fields, by temperature variations applied on each micro-sample, by UV-light ... Controlling the shape and the dimensions of each sample – and the channels connecting them – is also a good manner to control the computation. In living tissues, similar situations occur. For example, in the cardiac tissue, excitation waves are initiated tissue by changing the shape of one of the cells by local application of a pressure [62].

4 Simulation of the Formation of Tubulin-GDP 'Trails': Chemical Communication Between Microtubules is Possible

The chemical activity of microtubules causes the formation of local variations of concentration and composition of the chemical medium around their react-

ing tips. This assumption is intuitive. It has been proposed repeatedly that the formation of such variations could influence microtubule dynamics and self-organisation. In 1990, Robert et al published a simple chemotactic model of microtubule self-organisation where individual microtubules coordinate each other and self-organise, following the gradients of tubulin concentration, themselves modified by the activity of the microtubules [32]. The general idea was there but the influence of the activities of the microtubular ends on other microtubules, by the intermediate of free tubulin concentration variations, remained hypothetic.

A quantitative model of formation of a depleted area of free GTP-tubulin around the growing tip of a microtubule was proposed by Odde [58]. The motivation of this study was to estimate if the formation of this zone and its homogenisation by diffusion could be a limitation for the growth reaction. The result – an analytical solution of a reaction-diffusion equation – estimated that for a microtubule growing at 7 μm.min^{-1}, the concentration at the tip was 89% of the concentration far from the tip and that the concentration gradient was extending to less than 50 μm from the tip (about 1-2 microtubule diameters). The model proposed by Glade et al [3] also predicted the formation of similar depleted areas at the growing ends of microtubules with the formation of tubulin concentrated trails at the shrinking ends (fig. 2). This local phenomenon was hypothesised to be the most fundamental manner by which microtubules communicate. It is reinforced by observations *in vitro* [19] and *in vivo* [34] showing that new microtubules preferentially grow in the trajectory of shrinking ones.

Nevertheless, these models were simulating continuous quantities of tubulin molecules, expressed in μM, that way approximating the reality. In real solutions only few molecules diffuse from the reacting ends of microtubules and are diluted in large amounts of free tubulin. In consequence, the differences of tubulin amounts surrounding a shrinking end are very small. In these conditions, it could call into question the molecular explanation – the communication between microtubules mediated by free tubulin molecules – of microtubule self-organisation [3, 32].

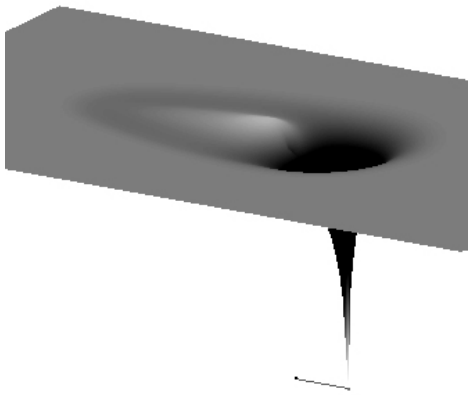

Fig. 2. A tubulin trail as simulated by the spatiotemporal differential equation system of a reaction-diffusion 2d-model similar to that proposed by Glade et al [3]. The figure shows the tubulin-GTP concentration map (represented in false 3D and grey scales), in superposition of a microtubule (above). In this simulation, an isolated microtubule is in treadmilling motion, growing at the right end and shrinking at the left one. At the growing end, an 'intense' depletion of tubulin forms (the black hole). At the shrinking end, tubulin-GDP is liberated and rapidly converted into tubulin-GTP producing a concentrated trail (white trail at the left of the depletion). The microtubule measures 10 μm.

I wanted to verify the possible existence of such tubulin 'trails' and their survival inversely depending on diffusion. I also wanted to answer the question of whether spatial variations of tubulin amounts were detectable by other microtubules and if it was the case, how ?

I conceived a molecular model where all tubulin molecules were represented. Tubulin heterodimers are approximated by ellipsoids of 8 x 4 x 4 nm diameters. Their translational and rotational diffusion rates along the 3 axis where calculated from their shape – the 3 radii of the ellipsoid – or from the macroscopic diffusion constant of tubulin in the cytoplasm [45, 58, 63]. Using the Stoke's relationship, corrected for a prolate ellipsoid (the approximated shape of tubulin heterodimers), I obtained the following. In water, for the X (great axis) and Y (short axis Y=Z) axis of the heterodimers, the translational rates are respectively 5.7 nm.μs^{-1} and 4.0 nm.μs^{-1}, and the rotational rates 11.1 $^\circ$.ns^{-1} and 2.0 $^\circ$.ns^{-1}. In the cytoplasm, the translational rate values are respectively 2.0 nm.μs^{-1} and 1.4 nm.μs^{-1}, and 1.4 $^\circ$.ns^{-1} and 0.24 $^\circ$.ns^{-1} for the rotational rates. The time step was fixed at 2.5 ns for the most precise simulations (to quantify the macroscopic diffusion) and at 5 μs for the others. At each time step, all molecules diffused randomly according to these values, with the constraint that 2 molecules couldn't exist in the same place. From these simulations, I verified the measured macroscopic diffusion of the population of tubulin heterodimers. It was perfectly consistent with the value measured experimentally (5.9 10^{-12} m^2.s^{-1}

in the cytoplasm and 4.9 10^{-11} $m^2.s^{-1}$ in water). The boundary conditions were toric for tubulin-GTP molecules, to maintain their density in the sample, and permeable for tubulin-GDP molecules, to obtain their correct density profiles.

The objective was to observe the diffusion of the liberated molecules of GDP-tubulin from the tip of a shrinking microtubule. Preformed microtubules – with a space step between tubulin heterodimers of 0.57 nm along the X axis, and an angle step of 27.69 ° between two successive heterodimers – were designed.

To test the formation of a tubulin trail, I placed an isolated microtubule of 0.5 μm long at the centre of a simulated cubic sample of 1.4 μm side, oriented along the X axis, in a medium containing 1 μM tubulin-GTP (5400 molecules.μm^{-3}).[1] The microtubule was allowed to disassemble constantly. I simplified the microtubule disassembly considering the liberation of GDP-tubulin molecule by molecule and not the liberation of tubulin coiled oligomers from proto-filaments. Assembly was not permitted. The conversion of tubulin-GDP into tubulin-GTP was not either permitted.

In this simulation, the microtubule was disassembling 100 x faster than a real microtubule (normally shrinking at a maximum of about 20 $\mu m.min^{-1}$, as to say at a rate equivalent to 2000 $\mu m.min^{-1}$. In this case, an eye-observable concentrated area formed around the shrinking end (fig. 3).

Fig. 3. Concentrated area of tubulin-GDP (black) in formation at the shrinking end of a microtubule. Tubulin-GTP heterodimers are displayed in grey. In this simulation, the microtubule disassembles 100 x faster (at 2000 $\mu m.min^{-1}$) than a normal microtubule.

[1] Note that tubulin concentrations of 100 μM or the presence of numerous microtubules distributed in the sample don't really affect the diffusion rate of free tubulin (results not shown).

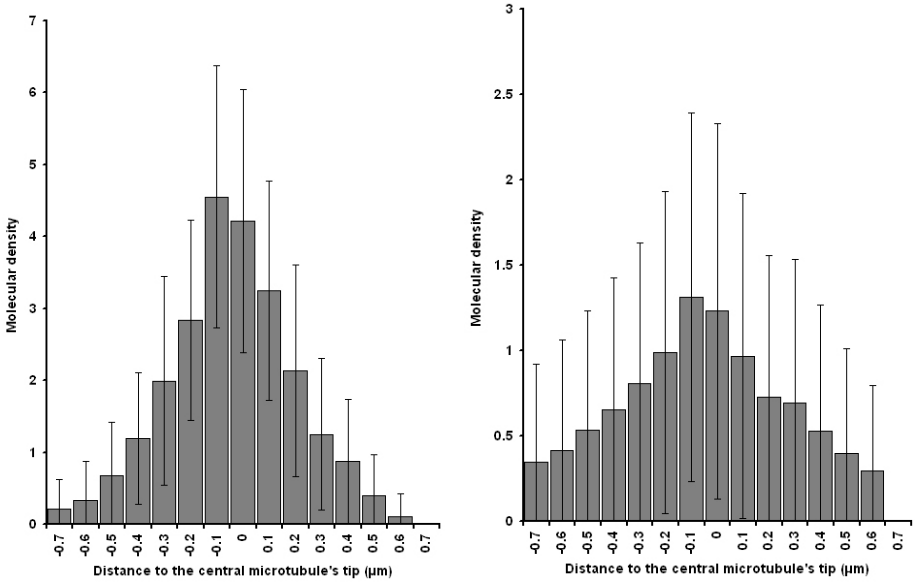

Fig. 4. (**Left**) Profile Tubulin-GDP density around the tips of disassembling microtubules, measured from the centre of an array of 5 disassembling microtubules, each of them respectively separated by 30 nm (one microtubular diameter). All microtubules disassemble simultaneously at 20 μm.min^{-1} (0.54 heterodimer.ms^{-1}). The translational diffusion rate of individual molecules has been determined from the measured value of tubulin diffusion in the cytoplasm (5.9 10^{-12} m^2.s^{-1}) [63]. The quantity of tubulin-GDP molecules liberated is very low. In consequence integration in time is necessary to obtain its density profile. It has been reconstructed by integration of the density maps of 6 independent simulations, during 1.8 ms, between the simulation times 9.2 ms and 11 ms, along the 3 axis (a total of 6642 profiles). (**Right**) The same with a different diffusion rate. Here, translational diffusion rate of individual molecules has been determined from the shape of tubulin, using the viscosity of water at 37 $^\circ$C. The average value of the global diffusion constant obtained (4.9 10^{-11} m^2.s^{-1}) is 8x larger than in the cytoplasm, as measured in water [64]. The resulting density profile of tubulin is weaker but detectable.

At realistic rates of disassembly (20 μm.min^{-1}) however, the variation of molecular density around the tip was hard to detect, in particular in water. Indeed, molecular diffusion is a very fast process compared to microtubule disassembly. During the disassembly of one molecule in the cytoplasm, the previously liberated molecule has a sufficient time (about 1.8 ms) to explore an average sphere of 150 nm of radius (0.5 μm in water). In consequence, during microtubule disassembly - or assembly [58] - the solution is rapidly homogenous at the micron scale (largely greater than microtubules scales). Nevertheless, although it's not intense, the very weak gradient of GDP-tubulin molecules can be measured (fig. 4). Globally, the GDP-tubulin heterodimers are nearest to the tip than far away.

This can be better observed by allowing several group microtubules to disassemble. I simulated 5 parallel aligned microtubules disassembling at 20 μm.min^{-1}. This time, the formation of a gradient of GDP-tubulin was clearly eye-observable and measurable. After 11 ms of reaction in cytoplasmic conditions, the gradient extends to about 0.7 μm from the tips of the microtubules and has an average maximum value of 4.5$\pm$1.8 molecules at the tips of the microtubules (fig. 4 - Left).

In water, the intensity of the gradient is weaker. After 11 ms, the gradient of tubulin-GDP extends more rapidly to a distance of about 1.5 μm from the tips of the microtubules. It has an average maximum value of 1.31$\pm$1.08 molecules at the tips of the microtubules (fig. 4 - Right).

In conclusion, microtubules, by their assembling or disassembling activity can produce local chemical heterogeneities. Unfortunately, even if dozens of tubulin-GDP-producing microtubules are located in the same place, the increase of total tubulin concentration is undetectable: the variation of density of tubulin-GDP is largely lower than the natural fluctuations of total tubulin density (there are about 1000 molecules of tubulin-GTP per molecule of liberated tubulin-GDP). These heterogeneities are not concentrated areas, but composition modified areas.

For individual microtubules, they are very weak and extended in space, so, particularly in water-based solutions, it is improbable that an individual microtubule can influence another one. The effect is probably sensible when produced by a group of synchronously reacting microtubules. To produce an intense composition or concentration heterogeneity, there are two possible scenarios: (1) in a solution of randomly distributed microtubules, nodes of microtubular ends naturally exist that can form initial composition (or concentration) heterogeneity nodes; (2) microtubule arrays form locally by another mechanism (for example by electrostatic interactions). Then, the formation of heterogeneity nodes can provoke the recruitment or inhibition of microtubules as proposed in [3, 32]. Moreover, because of the rapid diffusion of tubulin molecules in comparison to the reactivity of the microtubules, tubulin 'trails' are not directional as proposed in the reaction-diffusion model of Glade et al [3].

Other effects could reinforce a little the intensity of tubulin-GDP concentrated areas. Indeed, microtubules can liberate individual heterodimers of tubulin-GDP but also oligomers of several assembled tubulin-GDP heterodimers. Such oligomers are N times longer than N separated tubulin-GDP heterodimers. Their diffusion rate is then approximately N times lower than that of free tubulin. This could help to maintain the free tubulin-GDP more concentrated in the neighbourhood of the tip. Further, during disassembly, short assembly events can occur sometimes and convert locally tubulin-GTP into tubulin-GDP immediately liberated and added to the concentrated pool at the tip.

5 Concluding Remarks

Is it utopia to think microtubule solutions in terms of computing systems ?

Writing this article was something like revisiting the works concerning microtubule dynamics and self-organisation. Nevertheless, the reader will have noted down that I don't give any plans to construct a microtubule-based computer. Indeed, reconsidering microtubule solutions and placing them in the context of molecular computation was similar to take to pieces an electronic computer and, looking at each piece, wondering what is its function and how it works.

Several elements of a possible computation are present in microtubule self-organising solution – and more generally in dynamic fibre populations. Now it's an inverse problem: with these pieces and tools, we have to reconstruct – and first, figure out – the computer. Theoretical basis exist that describe what could be a reaction-diffusion computer [65] or a chemical collision based computer with reacting polymers [15]. However, a real implementation is not trivial.

A chosen approach could be to consider only the macroscopic level of self-organisation – generated morphologies or global behaviours – as done in other works to control external systems by an environment interaction loop [66,67]. As in [67] it could be easy to use Mandelkow's microtubule solutions – with travelling waves of microtubule concentration –, instead of a Belousov-Zhabotinski (BZ) reaction, with a similar environment interaction loop, to control the behaviour of any *Animats*. It would probably not provide any additional advantage in comparison to BZ processors. Indeed, they react faster and are easier to realise.

I think there's something more powerful that can be extracted from microtubule systems and used, due to their particular dynamics and to the numerous communication ways that coexist in these solutions. The best would be to be able to program these systems at the molecular level, as to say, to initiate the microtubule processing system by placing microtubules (and other molecules) in such an arrangement (and state) that they compute in accordance with an known algorithm. The problems of structurally programmed molecular computers were underlined by Conrad [10]. Imagining that it's possible to set the initial states and connections at the beginning of the reaction, due not only to non-deterministic molecular agitation but also to an uncertainty of the control of this initialization, the local computing events would rapidly diverge and produce a result far from the expected one at the macroscopic level.

The two major problems are the precision of the final result and the manner to control the system with the maximum of accuracy and at the smallest scale.

Microtubules are not as sophisticated as neurons. They don't self-organise as precisely as neurons do to form a functional tissue realising a computation. The structural programming of a microtubule brain will then be delicate. Nevertheless microtubule solutions could learn more or less as would do neural networks, if the reactive conditions allow them to self-organise and to reorganise after an external stimulation (the asymptotic behaviour must not be too much stable). Tabony's conditions of self-organisation are too much stable: the pro-

duced stationary morphologies are difficult to modify after the 6^{th} minute of the process that corresponds to the chemical instability, when the solution is sensible to external factors. Moreover, this process is very slow. Something closer to Mandelkow's conditions (more reactive) would be more appropriated. Once controlled, self-organisation would be directly used to initiate a 'more or less well programmed' processor that would realise quite well an expected calculus. The asymptotic behaviour would be refined by a learning feedback loop adjusting self-organisation, and so on.

Moreover, the feasibility of a fine control of a reaction-diffusion system to create computing elements has been shown on other systems, in particular with single chemical waves of the BZ reaction [65, 68]. A proposition would be to create small elements of computation, well localised in containers like vesicles, droplets or PDMS micro-tubs, containing the minimal amount of microtubules. Thereafter, they could be inter-connected like small electronic chips. The computation would emerge from the coupled dynamics of the individual chips.

Most of the methods presented before to control microtubule self-organisation (magnetic fields, vibrations, gravity and temperature ...) act on the entire sample without any discrimination in space. A fine control is possible using functionalized elements added to the system. A combination of different microtubules associated proteins is the most simple. Depending of the composition of the MAPs mix (type and concentration of the MAPs used), one can obtain a variety of self-organised morphologies [39]. MAPs can be added as free molecules in the solution, or be part of a functionalized surface (for example a surface patterned with MAPs). More powerful, microtubules functionalized with magnetic nanoparticles [69] allow controlling the positioning of individual microtubules with local weak magnetic field. Small populations of microtubules could be localised in vesicles or micro-samples elaborated in silicon polymers [41] and interconnected by other channels of communication like the gap junctions in the cells [16]. Finally, if microtubules are not the best fibres to do computation (their lifetime is short, about 1 or 2 days, and they react slowly), one could imagine to use other dynamic nano-fibres such as dynamic behaving carbon nanotubes [8,9] or specially designed fibres [1].

In this article, I presented the beginning of a study concerning the existence of elementary computational events in microtubule solutions, in particular a communication between microtubules via 'chemical composition heterogeneities'. The logical continuation of this study is to verify by similar numerical simulations whether or not microtubular ends are sufficiently sensible to these variations of composition in the medium and that this mechanism can drive a certain self-organisation. The next step is to simulate and quantify the variation of the chemical states of two ore more microtubules during the chemical collisions that certainly occur in microtubule solutions *in vitro* or in living cells. This will depend on angle and distance parametres between microtubules. Moreover, due to thermal agitation, microtubular ends are always moving. This introduces a fuzzy term in the chemical interaction between microtubules.

Seeing natural processes as computing processes gives to biologists and physicists a different point of view and sometimes helps to break accepted paradigms. Microtubules serve as simple mechanical elements in the cell structure (tensegrity models of the cytoskeleton). In addition, the cytoskeleton is acting as an autonomous system sensible to external stimuli, conferring very complex behaviours to the cell. Comparing behavioural phenomena at the cellular scale and those that exist at the level of entire organisms is revealing. The motion of a migratory cell looks similar to that of an octopus, an organism more complicated. Flagella organelles are primitive caudal fins of swimming cells (e.g. spermatozoids). The bacteria *Listeria* reuse the actin cytoskeleton as a propelling motor in infected cells [6, 7]. These simple organisms developed many techniques to reuse the cytoskeleton, creating that way micro-machines. In more simple – non living – systems, microtubule activity and/or its self-organisation is intrinsically capable to act as a micro-machine, directly in a solution [54] or in phospholipidic vesicles [41, 61].

Over the aspect of micro-machinery, it's a temptation to imagine the role of primitive brain it could have had in simple organisms (e.g. ciliate organisms) during millions years of evolution. To end the article, I can't refrain citing these words of C. S. Sherrington – yet mentioned by Hameroff and Tuszynski in [17] – about the 'seemingly intelligent' behaviours of single cell protozoa: "*of nerve there is no trace, but the cytoskeleton might serve*".

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