Invited Article

Self-Organization and Higher Level Emergent Phenomena in a Population of Microtubules

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Abstract

This article summarizes the self-organising behaviour of *in vitro* microtubule preparations and the manner that it is triggered and affected by weak external factors, in particular, gravity. In these preparations, self-organisation also leads to the development of other, higher level, phenomena such as the collective transport and positioning of any colloidal or sub-cellular particles present. Self-organisation results not from static interactions but occurs by way of the chemical reactions involved in the formation and maintenance of microtubules from tubulin and guanosine triphosphate (GTP). An essential feature of these experiments is that the system is extremely simple; being initially comprised of only two reacting species, purified tubulin and GTP. No other biological agents, such as molecular motors, nucleating centres or associated proteins, are present. Both experiments and numerical simulations indicate that self-organisation arises from the reactive growth and shrinking of microtubules. We postulate that individual microtubules are strongly coupled to their neighbours via the chemical trails they produce by their reactive growing and shrinking and which causes the whole microtubule population to behave as a *complex* system. Self-organisation and its related phenomena then develop as *emergent* properties in a manner showing analogies with the way that ant colonies self-organise. The fact that the latter develop highly sophisticated behaviour extending up to what is termed 'swarm intelligence' raises the question as to what extent microtubules are likewise capable of 'swarm intelligence'; and if so, whether similar processes also occur *in vivo*.

Key words: bio-complexity, swarm intelligence, reaction-diffusion, microtubules weightlessness

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1. Introduction

Microtubules (Alberts, Bray *et al.*, 2002) are tubular (16 nm and 24 nm diameter) shaped super-molecular assemblies, several μm long, whose walls are comprised of a protein, tubulin. Together with actin and intermediate filaments, they form the cellular cytoskeleton. Within the cell, microtubules play two major roles; they organise the cell's internal structure and they permit and control the directional movement and localisation of sub-cellular particles and organelles from one part of the cell to another. Microtubule organisation is frequently strongly modified in many of the cellular processes they are involved in. Often, this occurs not by the rearrangement of an existing structure but by disassembly followed by reassembly to form a different arrangement. For example, at cell division, microtubules disassemble then reassemble to form the oriented arrays which make up the mitotic spindle and along which the chromosomes are transported. Another characteristic aspect of their behaviour is that their organisation is often triggered by weak internal or external factors, either biochemical or physical in nature.

A feature distinguishing microtubule assembly from many other super-molecular assemblies, such as phospholipid mesophases, is that it is associated with chemical reactions. Microtubule assembly requires the presence of a nucleotide, guanosine triphosphate (GTP) that is hydrolysed to guanosine diphosphate (GDP) during their assembly. Moreover, these reactive processes continue once microtubules have formed. Biologists have for a long time realised that much of the intriguing behaviour of microtubules arises from the fact that they are transient dynamic entities; and that within cells their organisation and reorganisation results from the reactive processes associated with their formation and maintenance. Since solutions of reacting chemicals do not normally self-organise, nor are they strongly dependent on weak external physical stimuli, this raises the question of the underlying physical chemical processes by which this type of behaviour might come about.

One possible manner by which solutions of reacting chemicals or biochemicals might behave in such a way is through certain types of reaction-diffusion processes. Since the late 1930's, theoreticians have predicted that a coupling of reactive processes with molecular diffusion can, under some conditions, result in self-organisation (Kolmogorov, Petrovsky *et al.*, 1937; Rashevsky, 1938; Turing, 1952; Glansdorff and Prigogine, 1971; Nicolis and Prigogine, 1977). Some chemical systems have been established as behaving this way (Bray, 1921; Belousov, 1958; Castets, Dulos *et al.*, 1990). In addition, it has been predicted that self-organisation can depend on the presence at a critical moment early in the process of weak

external fields such as gravity (Kondepudi and Prigogine, 1981). When self-organisation occurs in this way, it is because the individual molecules (or groups of molecules) in a population, strongly coupled to one another by a mechanism of reaction and diffusion, behave as a *complex* system (Nicolis and Prigogine, 1977; Coveney and Highfield, 1995). In *complex* systems, comprised of strongly coupled elements, new so-called *emergent* phenomena develop that are collective properties of the overall population. Self-organisation is a major *emergent* phenomenon, frequently determined by weak external factors that break they symmetry of the self-organising process.

Little attention has been devoted to the way that populations of specific bio-molecules might develop *emergent* properties by reactive processes. Below, I summarise our studies of some *in vitro* microtubule preparations that under appropriate conditions develop a number of *emergent* properties by a reaction-diffusion process. The principal phenomena which arise are self-organisation and its triggering by weak external factors, such as gravity and magnetic fields. In addition, other more sophisticated collective phenomena develop; namely, replication of form, generation of positional information, and the collective transport, organisation and positioning of any colloidal or sub cellular particles present. The behaviour arises from the collective action of the entire microtubule population in which individual microtubules are strongly coupled to one another by processes involving the chemical trails they form by their own reactive growing and shrinking. The way we believe this comes about shows many analogies with the way ant colonies self-organise (Tabony, 2006a). Knowing that ant, and other social insects, colonies can spontaneously develop very high-level behaviour extending up to what is called 'swarm intelligence' (Bonabeau, Dorigo *et al.*, 1999; Bonabeau and Theraulaz, 2000), raises the question as to what extent microtubules might likewise be capable of 'swarm intelligence'; and if so, whether, and to what degree, similar processes might also occur *in vivo*.

2. Self-Organisation By Chemical Reaction

Under equilibrium conditions, the 2nd law of thermodynamics tells us that over time, order will be progressively and ineluctably lost. Two miscible liquids, initially separated from one another, slowly mix by way of diffusion and convection, and the existing order gradually disappear. Because of this, for very many years, it was not believed possible that solutions of chemicals could self-organise by reactive processes. Nevertheless, progressively over the last hundred years, often against much opposition, researchers have shown that this is not always the case.

Theoreticians (Kolmogorov,

Petrovsky *et al.*, 1937; Rashevsky, 1938; Turing, 1952; Nicolis and Prigogine, 1977) have proposed, due to being sufficiently far-from-equilibrium, that some types of chemical reaction might show strongly non-linear reaction dynamics which in some cases could result in macroscopic self-organisation. At a molecular level, self-organisation results from a coupling of reaction and diffusion and the patterns that develop are comprised of periodic variations in the concentration of some of the reactants. Such structures are often called reaction-diffusion or Turing-like structures; the latter after the mathematician who was one of the first persons to propose such a mechanism in 1952 (Turing, 1952). Prigogine and co-workers called them 'dissipative' structures (Nicolis and Prigogine, 1977) because a dissipation of chemical energy is required to drive and maintain the system sufficiently far-from-equilibrium such that self-organisation occurs. It is this flux or dissipation of chemical energy that provides the thermodynamic driving force for self-organisation.

A further aspect of these systems is the manner by which some of them might show bifurcation properties and hence, at a critical moment early in the process, be sensitive to weak external factors (Nicolis and Prigogine, 1989). Systems at, or close to, equilibrium may be described by linear equations. The unique solution to these equations leads to the equilibrium state as the only stable state. When however, a system is progressively moved away from equilibrium, a point is reached where its dynamics are no longer approximated by linear relationships but become strongly non-linear. A non-linear equation such as the quadratic, $y=ax^2+bx+c$, may, depending on the parameter values, have two real solutions in x for a given value in y. Hence, under given experimental conditions, a system showing non-linear dynamics can show more than one stationary state. When such a system is progressively displaced from equilibrium, at the point where the linear behaviour becomes unstable, the system adopts from the different non-linear dynamic pathways open to it, the pathway leading to the non-linear state that subsequently develops. At this critical instant, or bifurcation point, there is little to choose between the different pathways open to the system. Hence, the presence of a weak external factor, otherwise too weak to effect an equilibrium state, can favour one of them and so determine the system's future development. Furthermore, this factor need only be present at the critical moment when the initial state is unstable. Once the system has bifurcated, it follows the selected pathway and forms the pre-determined state. After the bifurcation has occurred, the critical determining factor may be removed without any further effect on the system and which behaves as though it retained a memory of the conditions prevailing at the bifurcation.

In the early 1980's, Kondepudi and Prigogine (Kondepudi and Prigogine, 1981)

explicitly calculated for certain reaction-diffusion systems, that the presence of weak external fields (such as gravity, or an electric or magnetic field) at a critical moment early in the process might determine the self-organised morphology that subsequently developed.

The pioneer workers in this field were fully aware of the implications that this approach might have towards some problems in biology, and at various times over the last 50 years the concepts outlined above have aroused interest and debate. Nevertheless, for a variety of reasons, they have not been adopted by the majority of chemists and biologists. Although the main reason is conceptual, another reason is the scarcity of experimental systems proven to self-organise this way. For example, in chemistry, it was not until 1990 that a chemical system, similar to those first discovered long ago by Bray (Bray, 1921) and Belousov (Belousov, 1958) was finally accepted as the first example of a Turing-like structure (Castets, Dulos *et al.*, 1990). Likewise, in biology, one of the elements lacking has been the example of a simple *in vitro* system that self-organises (and is dependent upon weak external factors) by reactive processes. Microtubules are obviously good candidates for this type of behaviour. The *in vitro* preparations described below self-organise by a reaction-diffusion process and this self-organisation is triggered by external factors such as gravity and magnetic fields.

3. Self-Organisation in *Complex* Systems

In parallel with this approach on self-organising chemical systems, researchers over a wide range of disciplines have progressively discovered that new global phenomena may develop in populations of strongly coupled elements. These phenomena are not sum of the properties of the individual elements, but on the contrary arise collectively by way of the non-linear dynamics by which the individual elements are coupled to one another. In recent years, systems of this type have been called *complex* and the global phenomena that arise are known as *emergent* (Nicolis and Prigogine, 1989; Coveney and Highfield, 1995). Self-organisation frequently occurs as a major *emergent* property. Striped arrangements often arise; when they do, they are nearly always the result of an outside external perturbation that induces a directional bias on the actions of the individual element in the population. Self-organising chemical systems make up a particular type of *complex* system.

Colonies of living organisms provide many examples of self-organising *complex* behaviour. A well-studied example is that of ant colonies, and in which the behaviour of the population results essentially from the actions of individuals strongly coupled to one another by a

form of chemical communication (Camazine, Deneubourg *et al.*, 2001). Although the rules governing the behaviour of individual ants are relatively simple, the overall behaviour is extremely sophisticated. A moving ant leaves behind itself trails of chemicals, known as pheromones, that attract or repel other ants. An ant encountering a trail of attractive pheromone will change its direction to follow this trail. This ant, in its turn, deposits more pheromone on the trail thus reinforcing it. In this way ants form the long columns with which we are all familiar. The progressive reinforcement of these chemical trails leads to the self-organisation of the ant colony. Based on such a mechanism, ants rapidly establish the shortest route between a food source and their nest (Camazine, Deneubourg *et al.*, 2001). This behaviour, which is not trivial, is the equivalent in computing of resolving the 'travelling salesman' problem (Bonabeau and Theraulaz, 2000; Dorigo, Bonabeau *et al.*, 2000). Algorithms using virtual ants are now used in the internet and telecommunications industry to find the shortest pathway between sites and efficiently route data from one point to another.

Consider a situation where two food sources are close to an ant population; one closer than the other (Camazine, Deneubourg *et al.*, 2001). As ants return to the nest with food, they leave behind themselves chemical trails. These are then followed by other ants; who in turn deposit chemicals that reinforce the original trails. Thus, progressively more and more ants accumulate onto the paths to the two food sources. However, for the shorter trail, it takes less time for an ant to return to the colony. This results in a slightly larger number of ants taking this path, thus reinforcing it at the expense of the longer path. Progressively more and more ants take the shorter path to the closer food supply until they nearly all follow this route. If the two food sources are at an equal distance, a weak external factor will suffice to favour one pathway over the other and hence determine on to which of the pathways the ants accumulate. It is easy to see that this choice is determined early on, before pathway reinforcement has gone very far. As pathway reinforcement progresses, it takes the application of an increasingly stronger external factor to induce a change whereby the alternate pathway develops. In the absence of such an effect, the deciding external factor need only be present for a critical period early in the process. Once pathway reinforcement has started, it will continue until the food source is consumed. This behaviour is a simple example of a bifurcation in a *complex* system.

A further aspect of some types of *complex* system in that an *emergent* phenomenon may itself modify the subsequent collective behaviour of the population and so lead to the development of higher-level *emergent* properties. This type of behaviour, often

known as stigmergy (Grassé, 1959), is defined as "the production of certain behaviour in agents as a consequence of the effects produced in the local environment by previous behaviour" (Beckers, Holland *et al.*, 1994). Based on it, in populations of coupled elements, a hierarchy of collective processes can spontaneously generate advanced behaviour extending up to what is sometimes termed "swarm intelligence" (Bonabeau, Dorigo *et al.*, 1999). For example, ants in a colony build when the pheromone concentration produced by the queen is within a certain range. The construction by the ants of a wall or gallery, then, in its turn, modifies the pheromone diffusion profile, and this affects future construction. In such a way, intricate architectures and structures spontaneously develop (Camazine, Deneubourg *et al.*, 2001). An even higher level of behaviour, also based on the stigmergy, is the capability of some species of blind ants to form armies that penetrate along a swarm front, and raid and attack other organisms (Camazine, Deneubourg *et al.*, 2001). This illustrates how stigmergy involving indirect chemical communication following simple rules can spontaneously give rise to extremely sophisticated behaviour. As I hope to demonstrate below, there are many analogies between the way ants self-organise and develop high-level *emergent* phenomena and the way that microtubules self-organise.

4. Microtubule Assembly and Reaction Dynamics

Microtubules are readily assembled *in vitro* by warming a solution of purified tubulin, in the presence of excess of GTP, from about 4°C to 36°C. Within a few minutes microtubules form; whilst at the same time GTP is hydrolysed to GDP. After the microtubules have formed, this reaction continues by processes in which molecules of tubulin are added to end of a microtubule whilst other tubulin molecules are liberated from the opposite end. There is hence a continual consumption or dissipation of chemical energy through the system. Microtubules grow by the addition of the complex, tubulin-GTP, and shrink by the loss of the complex, tubulin-GDP. The hydrolysis of tubulin-GTP to tubulin-GDP occurring during the time tubulin molecules are incorporated into the microtubule. The tubulin-GDP liberated at the shrinking end of a microtubule progressively diffuses out into the solution. Simultaneously, excess GTP present converts it back to tubulin-GTP; at which point, it is once again available for incorporation into microtubules; either into the growing end of a neighbouring microtubule, or by nucleation to form a new one.

An unusual and important feature of microtubules is that the reaction dynamics at opposite ends are different. Due to this, microtubules often grow from one end whilst shrinking from the other. When the rates of growth and shrinking are comparable, individual microtubules

retain the same approximate length but change position at speeds of several µm per minute. This type of behaviour is termed 'treadmilling'. Another type of behaviour called 'dynamic instability' occurs when individual microtubules either shrink or grow very abruptly. By modifying experimental conditions, such as buffer composition, it is possible to observe *in vitro* a wide range of microtubule reaction dynamics.

An aspect of microtubule reaction dynamics that appears to have been overlooked is that a shrinking microtubule is capable of forming a trail of free tubulin. Likewise, the growing end of a microtubule can cause zones that are depleted in tubulin-GTP. We postulate that because the addition of tubulin into the growing ends of microtubules increases in a strongly non-linear manner with tubulin-GTP concentration, then for suitable microtubule reaction dynamics, and rates of tubulin diffusion, neighbouring microtubules preferentially grow into regions of higher tubulin-GTP concentration whilst avoiding those of lower concentration. Hence, under appropriate conditions, neighbouring microtubules may communicate indirectly with one another by a stigmergic process involving the chemical trails that they themselves produce. In this way, a population of microtubules is capable of behaving as a *complex* system that self-organises and generates other *emergent* phenomena.

5. Experimental Behaviour of In Vitro Microtubule Preparations

5.1 Self-Organisation

In these experiments, tubulin solutions at concentrations of between 4 to 25 mg ml^{-1} (many of them at 10 mg ml^{-1}) are assembled into microtubules by warming the cold solution to 36°C in the presence of excess GTP. Microtubules form rapidly within 2-3 minutes. Progressively over a period of about 5 hours, the initially homogenous solution spontaneously self-organises to form a macroscopic structure (Tabony and Job, 1990). Once formed, the structure is stationary. The solution remains stable for about 3 days, after which time it runs out of reactants; the microtubules progressively disassemble, and the tubulin denatures. Figure 1, shows the striped arrangement of about 0.5 mm separation that develops when microtubules are assembled in spectrophotometer cells, 40 mm by 10 mm by 1 mm, placed upright.

The preparations are of high optical birefringence (Figure 1B, 1C) thus indicating that the microtubules are highly aligned with respect to one another. An electron micrograph showing some of these oriented microtubules is shown in Figure 2. Small angle neutron scattering measurements show that the microtubules in each striped band are highly oriented at either 45° or 135° to the stripe direction; but that adjacent stripes differ in having alternating orientations. Hence, the microtubule orientation flips regularly from 45° to

135° every 0.5 mm up the length of the spectrophotometer cell. The neutron scattering measurements also show that the majority of microtubules are present within these oriented arrays. The pattern of variations in microtubule orientation may also be observed by placing the sample between crossed linear polars with a wavelength retardation plate placed between them at 45° to their axis (Figure 1C). The retardation plate produces a uniform mauve background interference colour. Microtubule orientations, such that their birefringence add to the birefringence of the wavelength plate produce a blue wavelength shift, whereas orientations that subtract cause a yellow shift. Sample regions made up of microtubule orientations that are either acute or obtuse differ by producing yellow or blue interference colours. Hence the regular changes in microtubule orientation manifest themselves as a series of alternating yellow and blue stripes.

As mentioned above, reaction-diffusion processes predict the formation of chemical patterns comprised of variations in reactant concentration. The observations described above, however, show a pattern made up of variations in the microtubule orientation. It was hence necessary to ascertain if variations in microtubule concentration are also present in these structures. Both small angle neutron scattering experiments and imaging a fluorescent microtubule marker show that this is the case (Papaseit, Vuillard *et al.*, 1999). In fact, the microtubule concentration pattern superimposes on the orientational pattern (Figure 3) with the microtubule concentration dropping by about 30% and then rising again every time the direction of orientation flips from 45° to 135°.

The patterns are somewhat more complicated than they appear at first sight. The 0.5 mm stripes contain within them, another series of stripes of about 100 µm separation, and these in turn contain other striped arrangement of about 20 µm separation (Tabony, 1994; Papaseit, Pochon *et al.*, 2000; Tabony, Glade *et al.*, 2002a). At distances below this there are additional levels of organisation of about 5 µm and 1 µm.

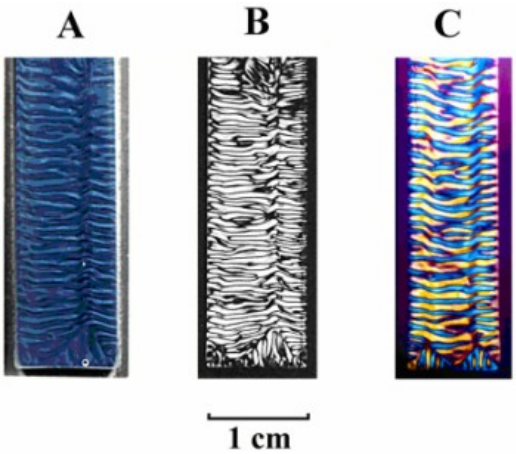

Figure 1. Self-organised microtubule structures as formed in optical cells, 40 mm by 10 mm by 1 mm (Tabony and Job, 1990; Tabony, 1994; Tabony, Glade *et al.*, 2002a). Microtubules were formed by warming a solution containing tubulin (10 mg ml^{-1}) from 4°C to 36°C in the presence of excess GTP. The structure is photographed; A) in reflected light; B) through crossed linear polars (0° and 90°); C) through crossed polars (0° and 90°) with a wavelength retardation plate (550 nm) at 45°. The strong optical birefringence shown in B) and C) indicate that the microtubules are highly aligned.

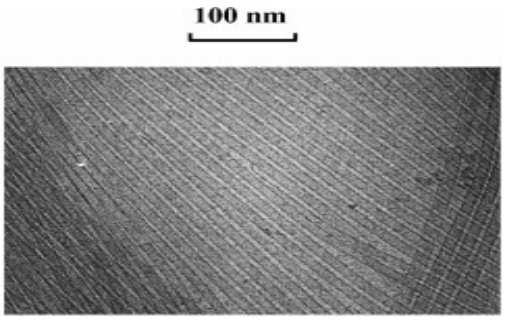

Figure 2. Electron microscope image of part of the self-organised microtubule preparation showing arrays of highly aligned microtubules (Tabony, Glade *et al.*, 2002a).

Some of these structures are shown in Figure 4. An additional level of ordering of several mm periodicity is observed when samples are made up in larger sample containers. These large stripes in turn contain the lower levels of organisation already mentioned. Hence, similar types of pattern spontaneously arise over distances ranging from a few µm up to several cm. The length of the microtubules in these preparations, as estimated from both biochemical assays and numerical simulations, is approximately 10 µm. It should be borne in mind that the optical images, even at high magnification, do not correspond to individual microtubules as such, but arises from the variations in the preparation's optical properties that their arrangement causes.

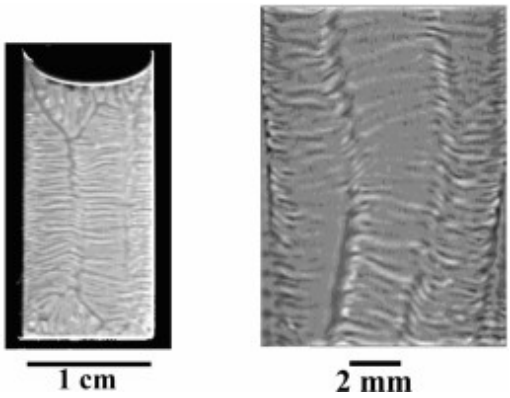

Figure 3. Microtubule concentration patterns as shown by fluorescent imaging (Papaseit, Vuillard *et al.*, 1999).

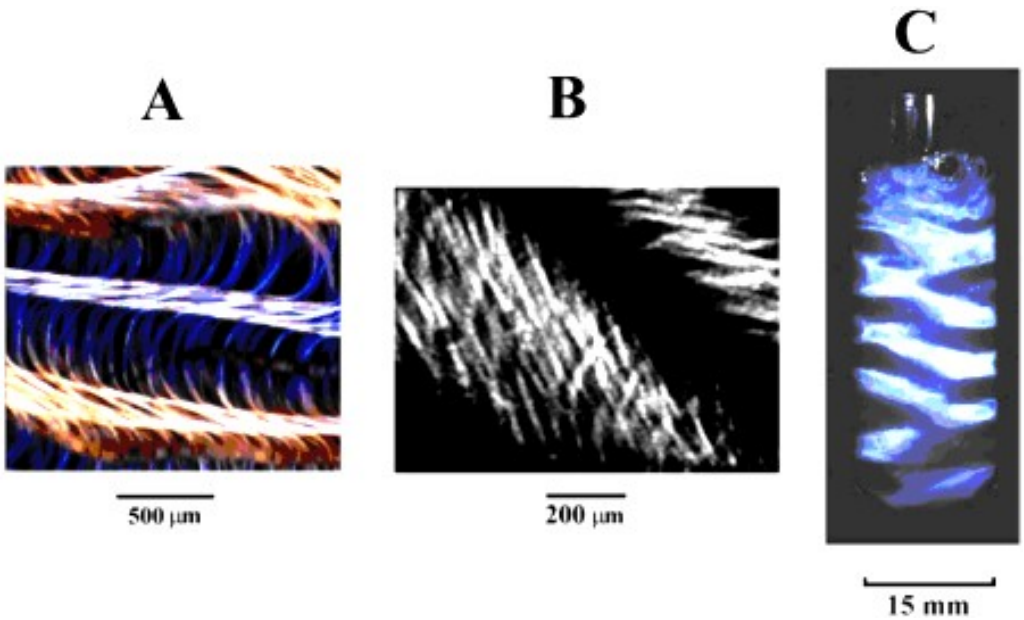

Figure 4. Replication of form. The striped structure shown in figure 1 is itself comprised of stripes of smaller periodicity. Photographs A) and B) show one of the individual stripes at higher magnification (Tabony, 1994). C) is a photograph of a larger scale structure formed in a 15 mm diameter test-tube (Tabony, Glade *et al.*, 2002a).

A feature of these patterns is that they contain a considerable amount of positional information. This is clearly seen in the self-organised morphology shown in Figure 5, where the pattern has a clearly defined centre, itself positioned in the middle of the sample. In addition, the positional information thus produced is expressed and manifested in a clear-cut manner. The generation of positional information is a basic phenomenon underlying embryogenesis and biological pattern formation. Its creation by reactive processes in a simple *in vitro* preparation, initially devoid of it, is an important feature of the observed behaviour.

5.2 Effects of Gravity

5.2.I. Gravity direction

Striped morphologies occur when the microtubules are prepared in upright sample containers, but a different pattern arises when they are prepared in the same containers lying flat (Figure 5) (Tabony and Job, 1992; Tabony, 1994). This observation indicates that gravity intervenes in the self-organising process. A simple way of testing this hypothesis is to form the structures with the sample cells lying flat down, but this time placed on the turntable of a record player (rotating at 33 rpm) with the sample's long axis along the direction of the centrifugal field (0.14 g). A striped morphology once again forms; the direction of the stripes being perpendicular to that of the applied centrifugal field (Tabony and Job, 1992).

Once formed after 5-6 hours, the structures are independent of their orientation with respect to gravity. To establish at what moment the sample morphology depends upon the gravity direction, microtubule formation was simultaneously instigated in twenty different samples placed upright (Tabony, 1994). Consecutive cells were turned from vertical to horizontal at intervals of one minute, and the samples examined 12 hours later after the structures had formed (Figure 6). Twenty minutes after instigating microtubule formation, when the last sample is turned from vertical to horizontal, there are no obvious signs of a striped structure. Since the structures forms while the sample cells are flat, one might expect that they would all form the horizontal pattern. This is the case for samples turned from vertical to horizontal during the first 3-4 minutes. However, samples upright for 6 minutes or more all formed striped morphologies identical to preparations that were vertical all the time. The final sample morphology depends upon whether the sample is horizontal or vertical at a critical time, 6 minutes after instigating assembly, and at an early stage in the formation of the self-organised structure before any striped pattern is visible. The process can be described as a bifurcation between pathways leading to two different morphological states in which the direction of the sample with respect to

gravity determines the morphology that subsequently forms.

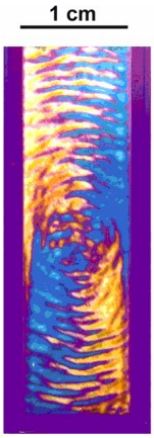

Figure 5 (right). The morphology that forms is dependent on the gravity direction. A different stationary morphology forms when the sample container is positioned horizontal during self-organisation (Tabony and Job, 1992; Tabony, 1994). For this morphology, the centre of the sample is determined by the centre of the pattern. This illustrates the generation of positional information.

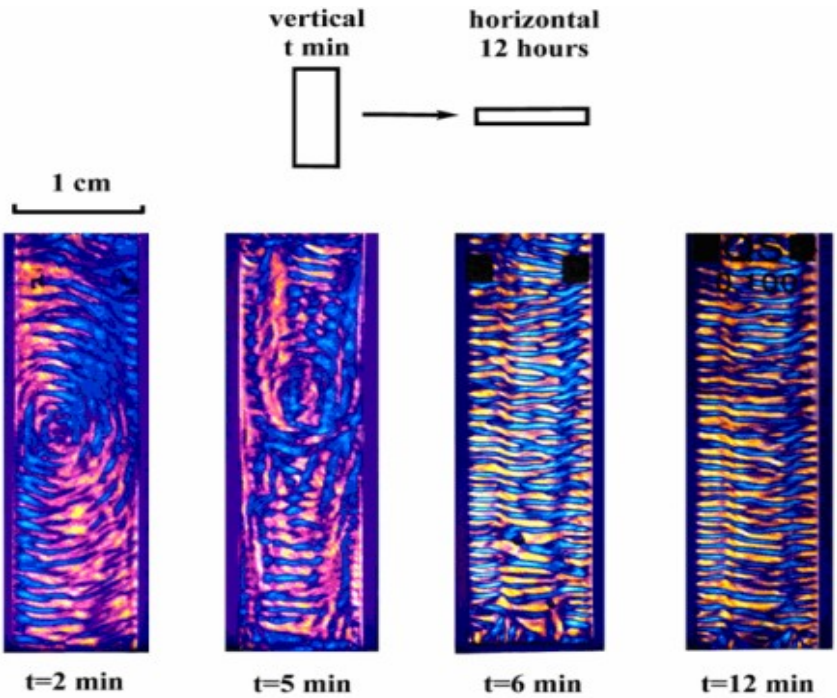

Figure 6. Bifurcation behaviour of self-organised microtubule preparations (Tabony, 1994). The morphology that forms is determined by the gravity direction at a critical time early in the process. The photographs show the final stationary morphologies for samples rotated from upright to horizontal at different times, t, during the first twenty minutes following microtubule assembly. Samples that remained vertical for 6 minutes or more formed striped structures as though they had remained vertical throughout the entire period of structure formation.

5.2.II. Effect of weightlessness

An obvious question that arises is; what would happen if the experiment were carried out under conditions of weightlessness? There are several methods by which the effects of gravity can be removed or attenuated. One method is space-flight. Close to the earth, terrestrial gravity is still present, but in a free falling object its effects are nullified. In sounding rockets, or orbiting craft such as the space-shuttle, conditions of weightlessness of between 10^{-2} to 10^{-4}g are readily obtained (Cogoli and Gmunder, 1991).

However, to study the effects of weightlessness it is not always necessary to go the trouble and expense of carrying

out experiments in space. Two quite different ground-based methods which strongly attenuate either, the gravity force, or the effects it causes, are magnetic levitation (Beaugnon and Tournier, 1991) and clinorotation (Briegleb, 1992; Cogoli, 1992). In magnetic levitation, a high magnetic field gradient interacts with matter to produce a force, repulsive for diamagnetic substances such as water or an organic compound, whose value and direction can be adjusted to counterbalance gravity. Because magnetic fields act on diamagnetic matter at a level of the electrons in individual molecules, magnetic levitation causes a substantial reduction in weight. Gravity may act upon matter in several manners. One possible effect is to cause mechanical deformations due to 'weight'. Another is that it will interact with any density differences present in the sample to cause a buoyancy force that may lead to transport in the vertical direction. In principle, magnetic levitation will counterbalance both of these effects.

A different, even simpler method, capable of substantially reducing one of the major effects of gravity, is rotation of the sample about the horizontal axis (clinorotation) (Briegleb, 1992; Cogoli, 1992). In these experiments, gravity is still present, but clinorotation continually changes the 'direction of fall', so that the net directional transport which gravity causes is cancelled out. In practice, speeds of rotation about 60 rpm are often effective.

None of these methods are perfect. Space-flight suffers from high costs, limited access, limited experimental observations and frequent technical failures. Interpretation of results can be complicated by the possible effects of launch and return-to-earth conditions (vibrations and high centrifugal fields). As discussed below, in magnetic levitation experiments, the fact that a high magnetic field is also present can induce effects of its own that complicate interpretation. In clinorotation, sample rotation acts mainly by annulling the transport effects that gravity causes. In addition, the attenuation of gravity effects is limited by the centrifugal force that rotation itself causes. For example, at 60 rpm, 1 and 10 mm off the axis of rotation, the centrifugal forces are $4x10^{-3}g$ and $4x10^{-2}g$ respectively (Briegleb, 1992).

We carried out experiments using all three methods and observed a similar behaviour (Papaseit, Pochon *et al.*, 2000; Glade, Beaugnon *et al.*, 2006). Namely, at a critical period early in the process, conditions of weightlessness strongly inhibit self-organisation. By combining these experiments with others, such as those carried out in a uniform magnetic field and in miniature containers, we came to the conclusion that self-organisation is induced by a number of different external factors capable of either directly orienting some of the

microtubules or leading to a favoured direction of microtubule growth.

A space flight experiment (Papaseit, Pochon *et al.*, 2000) was carried out during the free-fall period of a European Space Agency sounding rocket that provided approximately 13 minutes of weightlessness. Since on the ground, the sample morphology is determined by the sample orientation with respect to gravity 6 minutes after instigating microtubule assembly, 13 minutes of weightlessness should suffice to investigate the effect of weightlessness on self-organisation.

Approximately thirty samples were contained in an experimental module divided into two compartments; a 'weightlessness' compartment and a '1g on-board centrifuge' compartment. As soon as conditions of weightlessness were achieved in the sounding rocket approximately 3 minutes after launch, the microtubules were assembled by warming the cold solution of tubulin and GTP to 36°C. Simultaneously, the 1g centrifuge was switched on. The payload then remained under conditions of weightlessness for the next thirteen minutes before falling back to earth. As soon as re-entry started, the on-board 1 g centrifuge was switched off. After returning to earth, the payload was recovered and returned to the launch-site laboratory within the next two hours. During this time the samples were maintained at 36°C. The samples were then removed from the experimental module and examined. We found that the samples placed in the 1g centrifuge part of the module self-organised in a manner similar to normal laboratory 1g conditions; namely, stripes when the centrifugal field was parallel to the long axis of the cell (Figure 7A), and a circular morphology when it was perpendicular (Figure 7B). These observations showed that self-organisation was unaffected by payload re-entry and recovery. However, in contrast to this behaviour, samples subject to weightlessness showed practically no self-organisation (Figure 7C). Hence, under these conditions, the presence of gravity for the first 13 minutes actually triggers self-organisation.

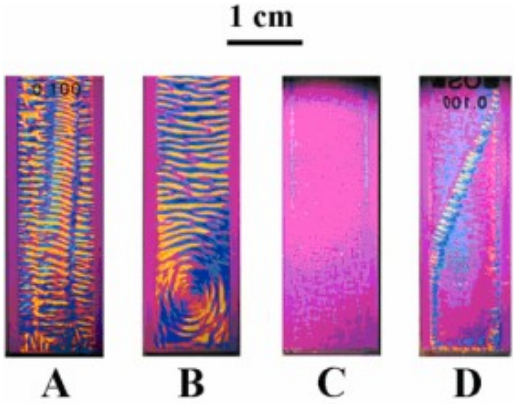

Figure 7. Microtubule structures as formed during space-flight. Microtubules were assembled when conditions of weightlessness were obtained in the payload. Photographs A) and B) show the self-organised morphologies that arise for samples assembled on a 1g on-board centrifuge with the centrifugal field parallel (A) and perpendicular (B) to the long axis of the sample cell. The centrifuge was stopped after 13 minutes, immediately prior to re-entry, and the samples left under 1g conditions for a further 5 hours while the structures developed. The photograph C), also taken after 5 hours, shows that almost no self-organisation occurs when samples are subject to weightlessness during the first 13 minutes of the experiment. D) is a photograph, taken after 5 hours, of a sample in

which an air bubble in the neck of the sample cell traversed the sample during re-entry. Microtubules were oriented along the bubble trajectory, thus triggering partial self-organisation perpendicular to the path of the bubble.

Self-organisation should not be confused with self-assembly. Both in these experiments and those described below using clinorotation and magnetic fields, microtubules self-assemble from tubulin to the same extent, and with the same reaction kinetics, as under normal 1g gravity conditions. It is the self-organisation of the assembled microtubules into the macroscopic arrangement of orientation and concentration described above that depends on gravity.

One of the practical difficulties that can occur in space-flight experiments is problems due to air bubbles. In the sounding rocket experiment mentioned above, although care was taken to prevent it, in some of the samples small air bubbles formed in the neck of the sample container. For these samples, during re-entry, when the sample was subject to centrifugal forces of about 30g, the air bubble was pushed through the sample. In one sample, this process was filmed. A strongly birefringent lined formed along the trajectory of the air bubble showing that the bubble had oriented the microtubules along its path. Subsequently, striped regions, limited in extent, developed perpendicular to this trajectory (Tabony, Glade *et al.*, 2002a; Tabony, Glade *et al.*, 2002b) (Figure 7D). This observation provides a clue to the mechanism by which self-organisation occurs; for it tells us that orienting the microtubules at an early stage in the process induces self-organisation.

We also carried out experiments under conditions of clinorotation. The commercial apparatus we used permitted simultaneous investigation of both rotating and stationary samples. To keep the centrifugal field which rotation causes at a value low enough that it does not itself trigger self-organisation, samples were contained in 4 mm internal diameter tubes (8 cm high). As discussed below, self-organisation can also depend on sample shape. For the long cylindrical geometry used in these experiments, striped morphologies form for both vertical and horizontal dispositions. Experiments were carried out in the following way. Samples containing tubulin and GTP at 4°C were warmed to 36°C by putting them in the clinostat placed in a hot room, and the clinostat started. Some samples were rotated about the horizontal axis at 60 rpm, whereas others (1g reference) remained stationary. The microtubule preparations have a high viscosity of about 10^4 poise and sample rotation does not cause mixing. After 15 minutes, the clinostat was stopped and the samples left for the next 5 hours. The reference samples that had not undergone clinorotation self-organised in the normal manner (Figure 8B). However, samples subject to clinorotation for the

first 15 minutes did not self-organise (Glade, Beaugnon *et al.*, 2006) (Figure 8A). This behaviour strongly resembles that observed in the space-flight experiment. As clinorotation acts mainly by suppressing any transport effects that gravity may induce, the concordance of the results from both methods tells us that gravity acts on the system via a directional transport term. This can only come about by an interaction of gravity with density differences present in the sample at the critical time when it acts on it. It likewise suggests that the directional transport term gravity induces leads to a preferred direction of microtubule growth and that this orientational effect triggers self-organisation.

By increasing the centrifugal force at the edge of the sample, by for example increasing the radius of sample rotation, it is possible progressively instigate self-organisation. We estimate that the value of the centrifugal force at which self-organisation is triggered as approximately 10^{-2}g (Tabony, Rigotti *et al.*, 2007). We also observed that both the extent and rate at which self-organisation occurs increases with the magnitude of the centrifugal force.

This simple experimental set-up can now used to remove or strongly attenuate the triggering of self-organisation by gravity, and so investigate how the system responds to other external factors whose effects would otherwise be masked or complicated by gravity induced self-organisation. Terrestrial gravity produces a constant force of acceleration. A different, oscillating, type of acceleration may be produced by simply vibrating the sample back and forth. To see what effect this might have, we attached a small vibrator to the outside of the sample tube that produced a displacement of approximately 10 μm at a rate of 125 Hz (maximum acceleration of 0.13g). The vibrator rotated with the sample tube. Hence, unlike gravity, any effect it might have will not be averaged out by clinorotation. We found, under conditions of clinorotation, that applying these weak low frequency vibrations for the first fifteen minutes of the process also triggered sample self-organisation (Glade, Beaugnon *et al.*, 2006).

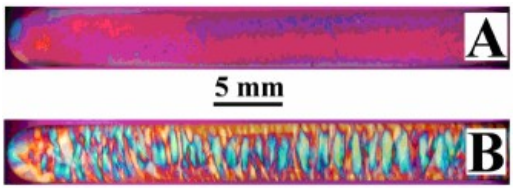

Figure 8. Effect of near weightlessness produced by clinorotation on microtubule self-organisation (Glade, Beaugnon *et al.*, 2006). In A) microtubules were assembled in a 4 mm diameter tube rotating (60 rpm) around the horizontal for the first 15 minutes, then photographed 5 hours later. Self-organisation does not occur. This contrasts with the 1g reference sample shown in B) assembled simultaneously at another position on the clinostat, not undergoing rotation, then photographed at the same time as A).

5.3 Experiments in Magnetic Fields

Microtubules have a negative diamagnetic susceptibility which is strongly anisotropic. When placed in a magnetic field, they hence experience a

torque, capable of orienting them under appropriate conditions. Bras et al (Bras, Diakun *et al.*, 1998) carried out experiments demonstrating, in the first few minutes after instigating their assembly (when both the sample viscosity and the microtubule length are low), that microtubules can be oriented by magnetic fields of between 4-11 T. As discussed below, this orienting effect can trigger or modify self-organisation. When placed in a high magnetic field gradient, microtubules also experience a repulsive force. Under these conditions, three different forces act on the sample that may mutually affect self-organisation; the orienting torque arising from the magnetic field, the force produced by the magnetic field gradient, and gravity. In addition, the magnitude of the magnetic field and the magnetic field gradient may be varied, and they can be disposed to have different relative directions compared to gravity.

5.3.I. Uniform magnetic fields.

Before considering the situation where a high magnetic field gradient is present, it is more convenient to first consider the action of a uniform magnetic field. We carried out experiments where the magnetic field was either vertical or horizontal. In both cases, when samples were assembled directly in the magnet, we found on removal from the field after 15 minutes, that they showed a very strong uniform optical birefringence indicating the microtubules were almost perfectly aligned along the field direction. When placed vertical in a vertical magnetic field for 15 minutes, samples rapidly self-organised to form a striped arrangement. The stripes are however more regular, and form far more rapidly, than those for reference samples formed outside of the field. Hence, in this case the orienting effect of the magnetic field during the first 15 minutes reinforces the self-organisation induced by gravity.

When samples were left in a vertical magnetic field for several hours (rather than just the first 15 minutes) we observed that practically no self-organisation occurred. On the contrary, the microtubules retained the uniform orientation which develops after just a few minutes in the magnet. Hence, in this case, the continued presence of the orienting torque produced by the magnetic field is sufficient to retain the microtubules in their orientation, and thus by preventing them adopting other orientations inhibits the self-organisation that gravity would otherwise have induced.

To determine at what early stage of the process magnetic fields are effective in orienting the microtubules we carried out the following experiments. Microtubule formation was initially instigated with samples vertical, at time t=0, outside of a vertical magnet field. Samples were then placed in the magnetic at time, t min, before being

removed at t=15 min. For samples placed in the magnetic field after the first 5 minutes, we found that the microtubules were not oriented by the magnetic field and there was no noticeable effect on self-organisation.

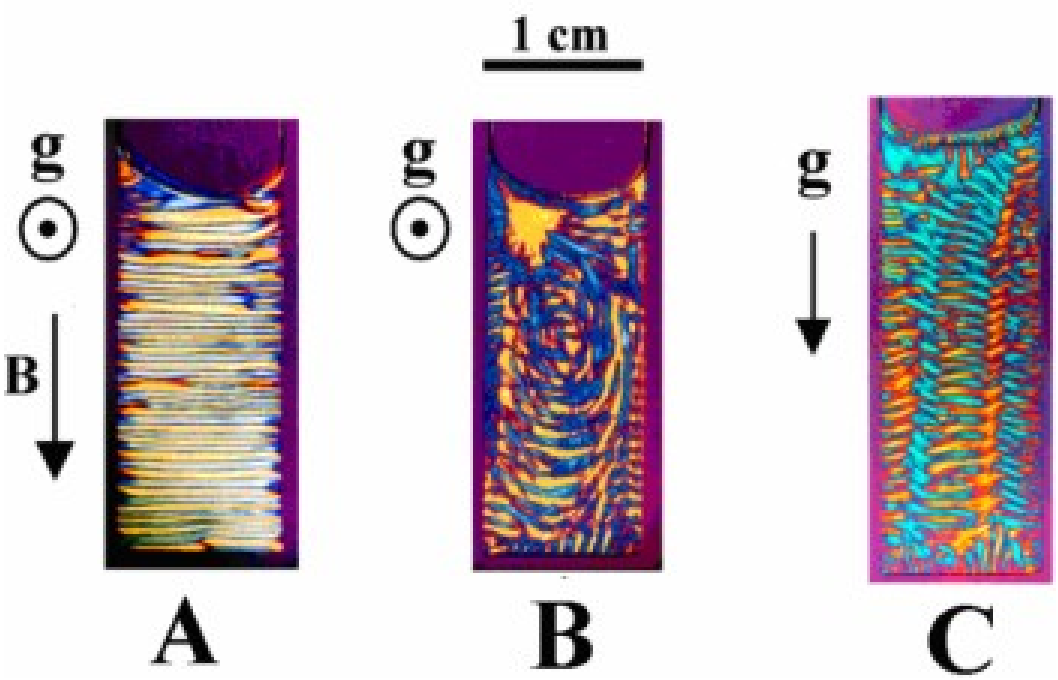

Figure 9. Effect of exposure to a 13 T horizontal magnetic field for the first 15 minutes after instigating microtubule assembly, on the final self-organised morphology (Glade and Tabony, 2005). The sample shown in A) was placed in the magnetic field whereas sample B) was outside of it. Both samples were simultaneously positioned horizontal. For comparison, C) shows a sample prepared outside the magnet in the vertical position.

The determining role of orienting microtubules by way of a magnetic field is illustrated even more clearly by carrying out experiments in a horizontal rather than a vertical magnet (Glade and Tabony, 2005). In this case, the sample was also placed horizontal. We found that when the microtubules were assembled in the magnet for the first 15 minutes, then instead of the circular 'horizontal' morphology, a striped morphology similar to that for vertical samples rapidly developed (Figure 9). Hence, in this case, the orienting effect of the magnetic field is sufficient to modify the self-organised arrangement that would otherwise have arisen. This effect first becomes noticeable for magnetic field values greater then 4T. It should also be noted that self-organisation occurs progressively faster in the presence of orienting fields of increasing intensity. For example, self-organisation is complete within 90 min in a horizontal field of 10 T, whereas in its absence it takes about 5 hours.

5.3.II Magnetic field gradients: magnetic levitation

In the central region of a superconducting magnet, the magnetic field is relatively uniform and magnetic field gradients are low. Samples paced here are subject to the orienting torque discussed above, but will not experience any significant repulsive forces compared to gravity. However, at the periphery of the magnet, high field gradients are also present. For a 15 T magnet, these field gradients are large enough to produce a force capable of levitating diamagnetic materials (Beaugnon and Tournier, 1991). To do this, the direction of the magnetic field needs to be vertical and the sample has to be placed in the upper region of the magnet where the repulsive force acts upwards against gravity. The value of the field gradient where levitation exactly counterbalances gravity depends on sample composition. For microtubule preparations we determined this value by levitating a drop of microtubule preparation with a magnetic field gradient greater than the minimum required for levitation, and then progressively reducing the field intensity until the drop fell through the magnet. At this field value, the position of maximum field gradient in the magnet corresponds to conditions where magnetic levitation forces exactly counterbalance gravity.

We then carried out experiments similar to those carried out in space-flight or under clinorotation. As the sample volume for which the magnetic levitating force is homogenous to within a few percent is limited to a region about 2 cm high, samples were contained in glass cells measuring 20 mm by 10 mm by 1 mm. When microtubule formation was instigated under conditions of magnetic levitation, but starting from time, t=0 min, we found as expected, due to the orienting effect of the magnetic field, that self-organisation still occurred. We then, to minimise this effect, assembled the microtubules outside of the magnet for the first 3 minutes. The sample was then quickly lowered into the magnetic field, placing it under conditions of magnetic levitation, 2-3 minutes before the critical time (6 min) when gravity acts on it; then removed from the magnet 12 minutes later. As shown in Figure 10, self-organisation is strongly inhibited (Glade, Beaugnon *et al.*, 2006). This behaviour should be contrasted with that of the reference '1g' sample, positioned outside the magnet and which self-organised in the normal manner. In this experiment self-organisation is strongly inhibited, rather than being completely suppressed. We believe the remaining self-organisation arises from a residual orienting effect of the magnetic field.

In these experiments, three different forces are operating on the sample; gravity, the levitating force due to the magnetic field gradient, and the orienting torque due to the magnetic field. By

adjusting the strength of the magnetic field, we found that the self-organising effects of these forces can also be made to counterbalance one other. For example, when samples were assembled from time t=0 min to time, t=15 minutes, in a magnetic field of 10 Tesla, producing a magnetic field gradient force equivalent to about 0.8g, then self-organisation was almost completely suppressed.

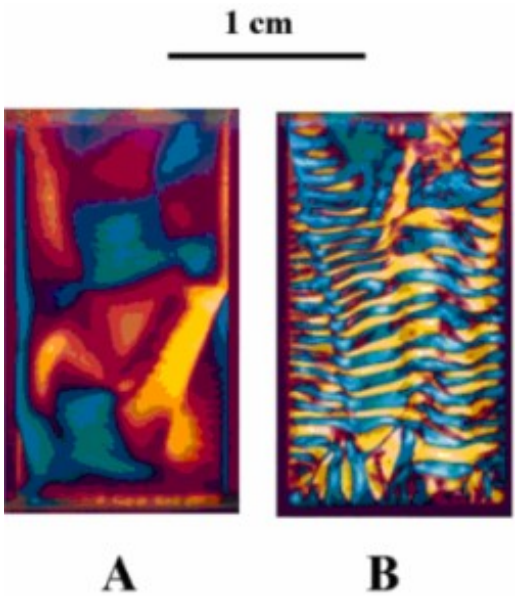

Figure 10. Effect of magnetic levitation on microtubule self-organisation (Glade, Beaugnon *et al.*, 2006). Microtubules were assembled by warming the sample to 36°C for 3 minutes and then placing it in the super-conducting magnet (also at 36°C) under conditions of magnetic levitation. The sample was removed from the magnet, 12 minutes later and photographed after 5 hours. As shown in A), self- organisation is strongly inhibited. This observation contrasts with the sample shown in B) assembled simultaneously, at another position on the sample holder, under normal 1g gravity conditions outside the magnetic field, and photographed at the same time as A).

5.4 Self-Organisation in Miniature Cell-Sized Containers

The experiments outlined above indicate that various factors which at a critical moment early in the process, either orient, or induce a preferred direction of microtubule growth, strongly affect self-organisation. In miniature containers, a somewhat different method of orienting microtubules is also present. It comes about because the growing end of a microtubule has to stop growing when it arrives at an impenetrable rigid boundary. For the type of reaction dynamics thought to be present here, microtubules that no longer grow, shrink. Hence, close to a boundary, microtubules growing perpendicular to the boundary are at a disadvantage compared with those growing parallel to it. The presence of a boundary thus favours the growth of parallel microtubules and this orienting factor can strongly affect self-organisation. Its effect increases both with decreasing sample dimension and with increasing geometrical anisotropy.

One of the limitations in comparing the *in vitro* behaviour outlined above with the results of *in vivo* experiments, is the large size of the sample container (several cm) compared with the dimensions of many biological systems. Mammalian cells are often about 50 μm in size; plant cells are often somewhat larger (200 μm) and many embryos and seeds are approximately a millimetre in dimension. Both, to make a better comparison with microtubule organisation *in vivo* and to investigate how factors such as small size and shape might affect self-organisation, we assembled microtubules in miniature

(50-500 µm) containers of various shapes (Cortes, Glade *et al.*, 2006).

The containers were made by moulding poly-dimethyl siloxane (PDMS) elastomer onto a template comprised of islands of resin standing out from a flat surface. The cured PDMS sheet, when peeled away from the mould, contains numerous small wells about 70 µm deep of the desired shape and size. This sheet can then be cut to size, the wells filled with cold tubulin solution, and microtubules assembled in them. We found that conditions which lead to self-organisation in centimetre-sized containers also resulted in self-organisation in these miniature wells. The morphology that forms depends strongly on sample shape and dimensions. As might be expected, elongated forms particularly favour self-organisation.

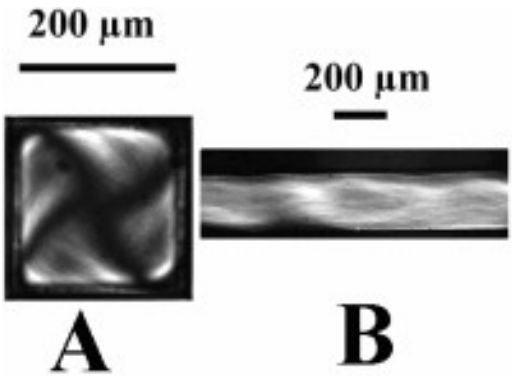

Figure 11. Self-organisation in miniature wells as viewed through cross polars (Cortes, Glade *et al.*, 2006).

In canal shaped wells, a self-organised morphology rapidly develops in which many microtubules are oriented along the general direction of the long axis (Figure 11) (Cortes, Glade *et al.*, 2006). The microtubule orientation alternates periodically between acute to obtuse whilst transiting through a parallel orientation close to the sample boundaries. The microtubule concentration shows a similar pattern, with regions of high and low concentration alternating along the canal. This pattern shows many common features with the striped pattern which develops in centimetre-sized containers. In this case, the geometrical anisotropy of the well furnishes an orienting factor capable of strongly intervening in the self-organising process. For example, in this case, when the experiment is carried out under conditions of weightlessness for the first 15 minutes of the process, then self-organisation occurs in the same manner as under normal gravity conditions (Tabony, Rigotti *et al.*, 2007).

This behaviour changes when the geometrical anisotropy is reduced. In square shaped containers, patterns of microtubule orientation and concentration form that describe circles around the perimeter of the well (Figure 11). Microtubule concentration and orientation are often lower in the corners than elsewhere and the patterns frequently show a swastika or spiral like appearance. In this case, when the experiment is carried out under conditions of weightlessness for the first 15 minutes, then as for centimetre sized containers self-organisation does not occur.

Additional effects arise when geometrical units are connected together. Depending on the exact geometry, different parts of the sample may either inhibit or reinforce self-organisation. For example, when a canal is connected to one of the sides of a square, then the strongly self-organised microtubules coming from the canal inhibit the weaker self-organisation that would have otherwise developed within the square. When two canals are joined orthogonal to one another, then in the region away from the intersection self-organisation occurs as for isolated canals. However, at the junction, where oriented microtubules arriving from orthogonal directions meet up, there is little or no self-organisation. On the contrary, when the geometry is comprised of four squares connected at the corners of another central square, the effect of the connections on the four adjoining boundaries is to strongly reinforce self-organisation. Oriented microtubule arrays develop at the connections which penetrate well into the individual squares, and in appearance resemble mitotic spindles (Cortes, Glade *et al.*, 2006).

5.5. Self-Organisation Induces Collective Particle Transport and Organisation

One of the major biological properties of microtubules is the transport of sub-cellular particles, such as chromosomes and vesicles, from one part of a cell to another. Growing microtubules are known to be able to exert a force on objects in their path. For a number of reasons, we suspected that the self-organising process might also result in collective particle transport; and this turns out to be the case. When 1 μm diameter colloidal polystyrene particles are added to the initial preparation of tubulin and GTP, then about 15 minutes into the self-organising process, the beads all start to move in the same direction at speeds of several μm per minute (Glade, Demongeot *et al.*, 2004). The direction of movement corresponds to the direction of microtubule orientation as it develops during self-organisation (Figure 12). The rate of particle transport increases with increasing reaction rates and ceases when self-organisation is complete after about 5 hours. When self-organisation does not occur; either because it not triggered by gravity, or because the microtubules are assembled under different buffer conditions not permitting it, particle transport does not occur either. Transport behaviour is independent of whether the added particles are polystyrene beads, phospholipid vesicles, purified chromosomes, or isolated nuclei. Molecular motors are not present in these preparations.

In addition to this collective transport the distribution of particles which was initially homogenous takes on a pattern coincident with the microtubule pattern (Figure 13). So as well as being

transported, the colloidal beads are also themselves organised by the self-organising process (Glade, Demongeot *et al.*, 2004). We believe this behaviour comes about in the following way. The speed of particle transport depends on the reaction rate and is strongly dependent on the initial tubulin concentration. During self-organisation, regions of different microtubule and tubulin concentration develop in the sample. As these concentrations differences develop, the reaction rate, and hence the particle speed, is no longer everywhere the same. Hence, in a manner analogous to that by which cars travelling at different speed aggregate into clusters and form traffic jams, particles will tend to accumulate into different regions of space.

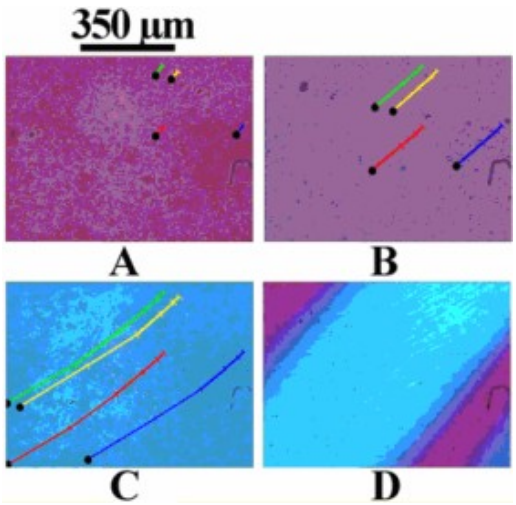

Figure 12. Transport of colloidal polystyrene particles during microtubule self-organisation (Glade, Demongeot *et al.*, 2004). Images of the preparation at different times during self-organisation; A), 20 min; B), 40 min; C), 60 min; D), 5 hours. The numerous small dots are polystyrene beads of 1 µm diameter. Several have been highlighted and the coloured lines indicate their trajectories. During the first hour of self-organisation the microtubules orient along the direction indicated by the bead trajectories.

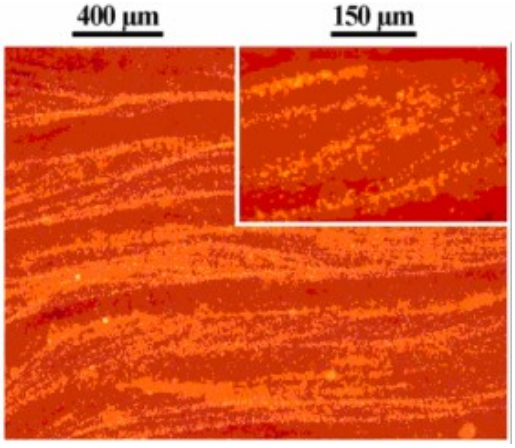

Figure 13. Microtubule self-organisation also results in the organisation of colloidal particles (Glade, Demongeot *et al.*, 2004). The photograph shows the distribution of 1 µm diameter fluorescent polystyrene particles in a self-organised preparation. This pattern coincides with the microtubule pattern. The particle distribution was homogenous prior to self-organisation.

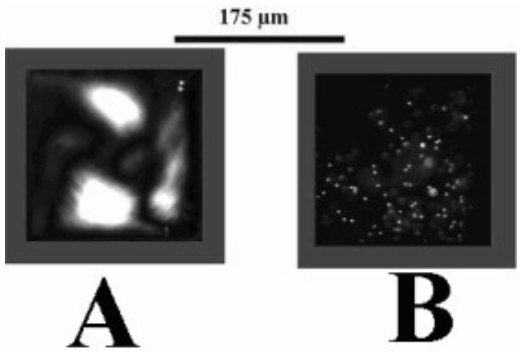

Figure 14. Self-organisation and particle positioning in miniature square-shaped wells. Images were recorded; A) through crossed polars, indicating variations in microtubule orientation; B) from 1 µm diameter fluorescent polystyrene beads present; initially the particles were uniformly distributed (Tabony, Rigotti *et al.*, 2007).

This effect is strongly enhanced in miniature containers. For example, in square wells we found when self-organisation was complete that the sample had spontaneously separated into regions of high and low particle density

with a large proportion of the particles concentrated into one part of the sample. In this case, the self-organising process has transported and positioned, the initially uniformly distributed particles, to a specific region; namely where high microtubule concentrations develop.

5.6. Self-Organisation Results from Reactive Processs and Not Static Interactions

Although in cells, biologists have established that changes in microtubule organisation occur by their disassembly in an existing arrangement followed by reassembly to form a different structure, nevertheless, in the present case, it is important to establish whether self-organisation (and its gravity dependence) result from reactive processes or if they might arise from static interactions related to the liquid crystalline properties of the solution. Here, I summarise some of the observations and arguments indicating that self-organisation arises from reactive processes involving microtubule disassembly and reassembly.

An experiment readily carried out is to first form the self-organised structure as described, and then destroy it by mixing (Tabony and Papaseit, 1998). After mixing the solution contains microtubules at the same concentration and temperature as before; however, the reaction dynamics and chemical energy consumption are significantly less than when the microtubules were initially formed. If the self-organised structure arises from static interactions, such as occur in some liquid crystals, then the structure will reform after mixing. The fact that this is not the case argues against self-organisation arising from static interactions.

A further argument against static interactions being the cause of self-organisation is its triggering by gravity. Static interactions, such as may occur in liquid crystals, are equally present under conditions of weightlessness as under normal gravity conditions. If they are the cause of self-organisation, then self-organisation would also occur under conditions of weightlessness. The fact that this is not the case indicates that self-organisation does not arise from them. Likewise, if the structure involved the separation of phases of different density under the action of gravity (for example a liquid crystalline and an isotropic phase), then even if the structure did not form under conditions of weightlessness, it would form if a brief period of weightlessness were followed by normal '1g' conditions. The fact that this is not the case argues against this possibility.

Microtubules may also be assembled, either under different buffer conditions, or in the presence of stabilising agents such as taxol, such that their reaction dynamics are very different from those in the self-organising preparations described here. In these cases, even though

microtubules are present at the same concentration, self-organisation does not occur (Tabony and Papaseit, 1998; Tabony, Vuillard *et al.*, 2000).

Microtubules disassemble when the solution is cooled to 4°C. When it is re-warmed to 36°C, then providing it contains sufficient GTP, microtubules reform (and GTP is hydrolysed to GDP). When we carried out this experiment under self-organising conditions, the striped self-organised structure also reformed (Tabony and Papaseit, 1998; Tabony, Vuillard *et al.*, 2000).

Static interactions in liquid crystals, although they can give rise to macroscopic variations in orientational order, do not lead to macroscopic variations in concentration. On the contrary, the central prediction of reaction-diffusion theories is the formation of macroscopic variations in the concentration of reacting species. Both neutron small angle scattering and fluorescence imaging observations demonstrate that substantial macroscopic microtubule concentration variations are present in the self-organised preparations (Papaseit, Vuillard *et al.*, 1999).

Another possibility is that the pattern might involve a coupling of reactive processes with flow due to thermal gradients during warming. The high viscosity of microtubule preparations renders thermal convection difficult. Samples prepared under conditions when there was no significant thermal convection gave the same self-organised patterns as those where the bottom of the sample was 5°C warmer than the top (Tabony and Job, 1992). Moreover, it is not necessary to form microtubules by warming a pre-mixed solution of cold tubulin and GTP. They may also be formed by mixing together separate pre-warmed solutions of tubulin and GTP. We found that the self-organised structures which develop are the same as that obtained by warming a cold solution of tubulin and GTP (Tabony, Vuillard *et al.*, 2000). Hence thermal convection appears to play no significant part in the self-organising process.

An important parameter in any reactive process is the overall reaction rate. This will be strongly dependent upon experimental parameters such as concentration and temperature. We determined the rate of hydrolysis of GTP to GDP in self-organising microtubule preparations (Tabony, 1994; Tabony and Papaseit, 1998; Glade, Demongeot *et al.*, 2004) for different initial tubulin concentrations and at different assembly temperatures using P^{31}NMR spectroscopy. We found (Tabony, 1994) that the periodicity of the striped structure decreased, and the structure formed increasing rapidly, both with increasing initial tubulin concentration and temperature. An increase in the reaction rate by a factor, x, resulted in a decrease in the spacing by $x^{1/2}$.

These experiments indicate that the

striped structure arises via chemical processes associated with microtubule formation and maintenance and not from static interactions between the microtubules. However, the most convincing argument that self-organisation is a consequence of reactive processes involving the assembly and disassembly of microtubules comes from neutron small angle scattering measurements (Tabony, 1994). During the initial stages of self-organisation, the left and right-hand halves of the sample, when observed through crossed polars with a wavelength interference plate, rapidly develop either yellow or blue interference colours. These interference colours correspond to regions in which the microtubules within them have taken up either an obtuse (blue) or acute (yellow) orientation. The striped structure subsequently develops by blue zones forming in some parts of the yellow part of the sample, and yellow zones forming in some parts of the blue region. In the zones where there is no colour change, the microtubules retain their orientation, whereas in the regions where there is a colour change the microtubule orientation flips from acute to obtuse, or vice versa. We carried out a series of neutron small angle scattering measurements where the incident neutron beam was limited to a horizontal band of the approximate dimensions of a stripe (Tabony, 1994). In these measurements, the orientation of the microtubules is manifested by the azimuthal angular direction of the scattering onto a two dimensional detector, whilst their concentration is proportional to the overall intensity of the scattered neutrons. In agreement with the optical observations outlined above, we observed that stripe formation corresponded to a flipping over of the scattering pattern on the detector from an acute to an obtuse arc (or vice versa). Simultaneous with this orientational re-ordering, the intensity of the microtubule scattering, proportional to their concentration, decreased, then rose, before declining again. Hence, orientational re-ordering, which is itself the stripe forming process, is concurrent with a process in which oriented microtubules (in specific regions) partially disassemble and then reassemble into an orthogonal orientation. In other words the stationary pattern arises because microtubules disassemble and reassemble with different orientations and concentrations in alternating parts of the sample. This neutron scattering experiment clearly shows that self-organisation is associated with the reaction dynamics of microtubule dis-assembly and re-assembly.

5.7. Microtubule Assembly Kinetics in Self-Organising Preparations

Due to their length, microtubules strongly scatter light. Because of this, once assembled from tubulin, the initially

limpid solution often appears milky or turbid. Hence, as they form, the optical density of the solution at wavelengths of around 350 nm, increases from a value close to zero to a value proportional to the microtubule mass. The kinetics of microtubule assembly is routinely measured this way.

By varying various parameters such as buffer conditions, it is possible to observe a large range of microtubule reaction dynamics. These are often accompanied by different assembly kinetics. For example, under appropriate conditions, microtubule solutions show a series of damped oscillations of assembly and disassembly that can be monitored as oscillations in the solution's optical density. Under other conditions, the microtubule mass monotonically rises to a stationary value. The self-organising preparations discussed in this article do not show this type of behaviour. Instead, approximately 6 minutes after instigating their formation, the microtubule mass attains a maximum value, after which it progressively declines over the following hour to a stationary level approximately 20% lower. When the reaction dynamics are modified, by for example increasing the concentration of magnesium ions in the buffer from 1 mM to 10 mM, then microtubules assemble to the same extent as at 1 mM Mg, but the assembly kinetics show a monotonic increase to a stationary value instead of an 'overshoot'. Under these conditions, the solution remains homogenous and self-organisation does not occur. We monitored microtubule assembly kinetics for various different buffer conditions where self-organisation either occurred or was absent. For monotonic assembly kinetics, we have not observed self-organisation. On the contrary, an 'overshoot' in the assembly kinetics is present whenever self-organisation does arise (Figure 15). These observations suggest that the reaction dynamics which give rise to an 'overshoot' in the assembly kinetics are intimately associated with self-organisation.

In any out-of-equilibrium system, the bifurcation point, when the system is sensitive to weak external factors, coincides with a condition of instability in the homogenous state. Hence, in microtubule preparations, where self-organisation is thought to result from reactive processes associated with the formation and maintenance of microtubules from tubulin, we might expect to find a chemical instability involving different relative proportions of tubulin and microtubules, close to, or just prior to, the critical time when self-organisation is triggered by gravity. The ‘overshoot’ in the assembly kinetics, where the proportion of microtubules is at a maximum compared with the mass of free tubulin, describes such a chemical instability (Tabony, 1994). Moreover, it occurs after approximately 6 min; the moment when the system is gravity

dependent. By modifying various conditions it is possible to displace the assembly 'overshoot' to longer times. We found that the critical time at which the self-organised morphology was dependent on the gravity direction was also displaced to the same time as the 'overshoot' (Tabony, 1994).

The molecular basis for the assembly 'overshoot' is the following. Within the first three to four minutes, when the microtubules initially form from the tubulin solutions, free tubulin is rapidly consumed, thus depleting its concentration and reducing its availability. This affects the reaction dynamics in such a way, that not only the microtubules can no longer sustain further growth and assembly but on the contrary they progressively disassemble by about 20%. This partial dis-assembly then results in a progressive increase in free tubulin, which, in its turn, slows the rate of dis-assembly until a stationary level of microtubule mass is attained.

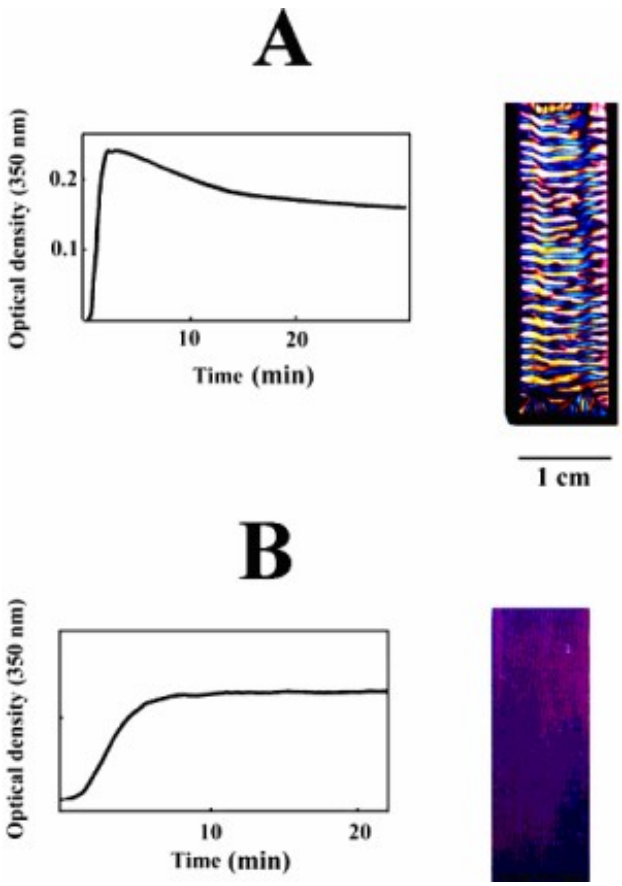

Figure 15. An 'overshoot' in microtubule assembly kinetics is associated with self-organisation (Tabony, 1994; Papaseit, Pochon *et al.*, 2000). The kinetics of microtubule assembly as measured by the optical density at 350 nm for preparations that A), self-organise; B), do not self-organise. The overshoot corresponds to an instability in the chemical composition (relative proportions of microtubules and tubulin) of the sample and coincides approximately with the gravity induced bifurcation time (6 min) in the initially homogenous solution. Preparations that do not show this 'overshoot' do not self-organise.

6. Proposed Molecular Basis of Self-Organisation

Microtubules are continually growing from one end and shrinking from the other. For suitable microtubule reaction dynamics and tubulin diffusion, we hypothesize that the shrinking end of a microtubule will leave behind itself a trail of raised tubulin concentration. Likewise, the growing ends can cause zones depleted in tubulin-GTP. In this scenario, it is immaterial whether a specific microtubule shrinks (or grows) by a given amount, or if it disassembles completely (or a new one forms by nucleation). In either case, trails of free tubulin are liberated from the shrinking ends, and regions depleted in tubulin are formed close to the growing ends. The tubulin liberated from shrinking ends is initially in the form of the complex, tubulin-GDP. This progressively diffuses out into the solution. Simultaneously, excess GTP present reconverts this tubulin-GDP to tubulin-GTP; at which point it is once again available for incorporation either into the growing ends of neighbouring microtubules, or to nucleate and form a new microtubule.

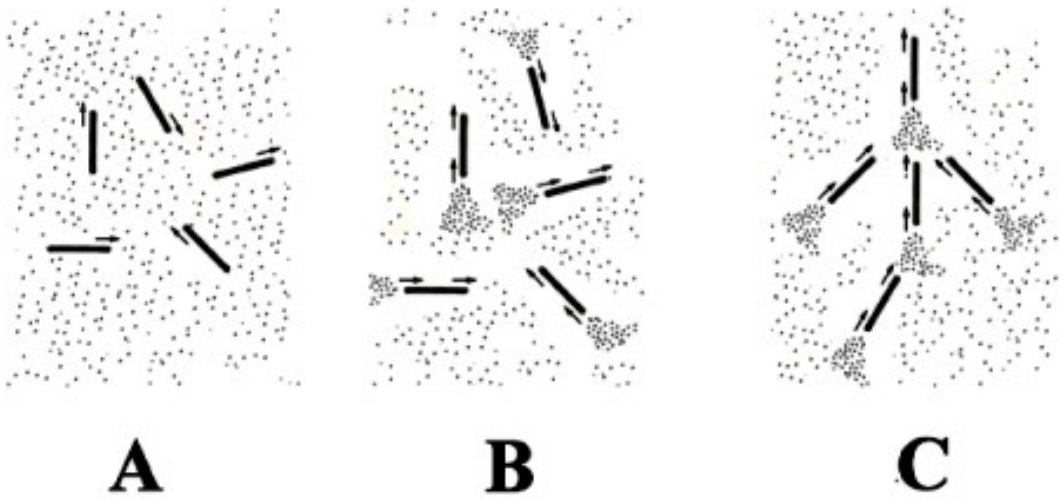

Figure 16. Proposed mechanism for the formation of the self-organised structure. In A), microtubules have just formed from the tubulin solution: they are still in a growing phase and have an isotropic arrangement. In B), microtubule disassembly has started to occur at the 'overshoot' and produces trails of high tubulin concentration from the shrinking ends. In C), neighbouring microtubules grow preferentially into these trails. The isotropic arrangement shown in B) is unstable. Once some microtubules take up a preferred orientation, then neighbouring microtubules will progressively grow into the same direction. Once started, the process mutually reinforces itself with time and leads to self-organisation. Thus, at the 'overshoot', any small effect that leads to a slight oreintational bias will trigger self–organisation.

Because the addition of tubulin into the growing ends of microtubules (or the nucleation and growth of new microtubules) increases in a strongly non-linear manner with tubulin-GTP concentration, we postulate that neighbouring microtubules will preferentially grow into regions of higher

tubulin-GTP concentration whilst avoiding those of lower concentration. Hence, under appropriate conditions, neighbouring microtubules may communicate indirectly with one another by a process involving the chemical trails they produce. In this way, a microtubule population is capable of behaving as a *complex* system that self-organises and generates other *emergent* phenomena in a manner showing analogies with the way in which ant colonies self-organise (Tabony, 2006b).

When the microtubules first assemble from the tubulin solution, experimental observations (neutron small angle scattering, optical birefringence) within the first three to four minutes show that the preparation is both isotropic and homogeneous, i.e. the microtubules are not oriented and they are uniformly distributed throughout the solution. It is only after 6 minutes, when overall dis-assembly starts to occur, that optical birefringence, synonymous with microtubule orientation, starts to develop. This marks the beginning of the self-organising process. At this point, the solution will no longer be chemically homogeneous, but will instead show substantial heterogeneities in chemical composition. The overall disassembly that starts to occur at this time will strongly favour the formation of the chemical trails outlined above. We propose that the isotropic arrangement of microtubules at this time is unstable to weak orienting factors. Orienting just a few microtubules will induce their neighbours to grow along the same direction and so on. Hence, once started, the process will mutually reinforce itself and lead to self-organisation. Thus, in agreement with experiments, the 'overshoot' in the assembly kinetics is intimately associated with self-organisation, and any factor which at that time partially orients microtubules, or leads to a privileged direction of microtubule growth, will trigger self-organisation (Figure 16).

To investigate whether the above explanation is realistic, we carried out computer simulations of a population of growing and shrinking microtubules (Glade, Demongeot *et al.*, 2002). These simulations incorporated as parameters experimentally realistic microtubule reaction dynamics and the experimentally determined tubulin diffusion constant. Where simulations were limited to just a few microtubules on a two dimensional reaction space, they demonstrated both the formation of the tubulin trails outlined above and the growth of neighbouring microtubules into them (Figure 17).

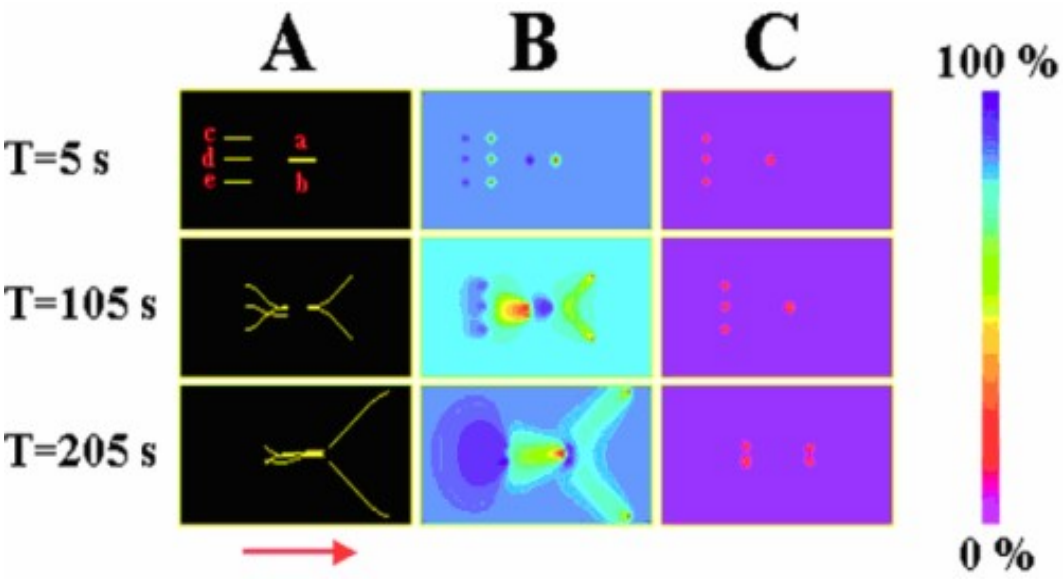

Figure 17. Numerical simulations illustrate the formation of chemical trails (Glade, Demongeot *et al.*, 2002). A) shows microtubules, and B), C) the concentration profiles of tubulin-GTP and tubulin-GDP respectively. Initially five microtubules were positioned as shown and the simulation started. The growing ends of the microtubules form regions depleted in tubulin-GTP whereas the shrinking ends form trails rich in tubulin-GTP. Microtubules a) and b) move apart, away from the region of low tubulin-GTP concentration produced by their neighbour. Conversely, microtubules c), d), and e) grow into the path of the chemical trails of tubulin-GTP produced by the shrinking ends of microtubules a) and b).

When simulations were extended to a population of about 10^4 microtubules on a two dimensional reaction space, 100 µm by 100 µm, then after 2-3 hours of reaction time, a self-organised structure comprised of regular bands of about 5 µm separation developed. This arrangement compares well with the experimental self-organised structure over a similar distance scale (Figure 18). The simulations likewise predict an 'overshoot' in the microtubule assembly kinetics that also compares favourably with the experimental assembly kinetics (Figure 18). At the 'overshoot', when substantial microtubule dis-assembly occurs, the simulations forecast the development of strong fluctuations of concentration and hence density (3%) (Glade, Demongeot *et al.*, 2002).

In these simulations, microtubules initially nucleate and grow from free tubulin. Subsequently, depending on the local reaction conditions, individual microtubules shrink and grow spontaneously in a manner that encompasses extremes varying from "treadmilling" to "dynamic instability". In addition, where regions of high tubulin concentration form via microtubule disassembly, new microtubules are permitted to nucleate and grow. The main parameters affecting self-organisation are the initial tubulin concentration, microtubule growth and shrinking rates, the rate of regeneration of tubulin-GTP from tubulin-GDP, nucleation of new microtubules, and the

tubulin diffusion constant. Self-organisation is favoured by high initial tubulin concentrations and the rate of generation of tubulin-GDP to tubulin-GDP needs to be fast. For the mechanism to operate individual microtubules must be within the range of the diffusive trails produced by their neighbours. This depends on the diffusion constant of free tubulin. If diffusion is very fast, then the trails will rapidly disappear and there will be no self-organisation. Neither will self organisation occur when diffusion is very slow, for in this case microtubules will grow or reform into exactly the same positions as the disassembling ones. As the solution is initially unorganised, it will stay so.

When we first carried out these simulations we noticed that the direction of the stripes was always along the diagonal of the reaction space and this suggested that a directional bias had unwittingly been built into the algorithm. This turned out to be the case. It came from a small asymmetry in the way tubulin diffusion had been digitised. When this asymmetry was removed, then although clumps of oriented microtubules formed, macroscopic self-organisation into stripes did not develop. We then reintroduced a directional bias into the algorithm in two different ways. One way was to simply impose an orientation on some of the microtubules at an early stage in the process before the assembly 'overshoot' (bifurcation time). This resulted in the development of a striped morphology and would resemble the manner uniform magnetic fields would act on the system. Of course, the other way of breaking the symmetry is to make tubulin diffusion anisotropic. As expected, by favouring the growth of microtubules along one direction more than others, this asymmetry also resulted in the development of a striped morphology.

According to this scenario, gravity triggers self-organisation in the following way. At the 'bifurcation' time, it interacts with strong density fluctuations produced by the partial overall disassembly of the microtubules. This interaction causes a 'drift' term which breaks the symmetry of the transport processes. By promoting microtubule growth along a specific direction over the entire sample, self-organisation is triggered. Thus in agreement with experiments, gravity acts on the system via the directional transport it induces from its interaction with the density fluctuations caused by microtubule disassembly at the assembly 'overshoot'.

These simulations also furnish a possible explanation as to how collective particle transport might arise. At different time intervals during self-organisation, they predict the formation of parallel fronts of oriented microtubules which cross the reaction space at speeds of several μm per minute (Glade, Demongeot *et al.*, 2004). Growing

microtubules are known to be able to exert a force on objects in their path. Hence, the observed particle transport could arise by the collective force thus generated in each travelling front. However, the travelling fronts also correspond to variations of at least 30% in microtubule concentration. Since, the microtubule preparation is extremely viscous, they likewise correspond to viscous waves of several thousand Poise. Such travelling waves of concentration and viscosity would be quite capable of transporting colloidal sized particles along with them. At present, we cannot say whether the particles are pushed by growing microtubules in the fronts and/or transported by effects of viscosity and concentration associated with them. However, the fact that the speed and direction of the travelling fronts compares favourably with the experimental speed and direction of colloidal particle transport is consistent with the travelling fronts being responsible for the observed behaviour.

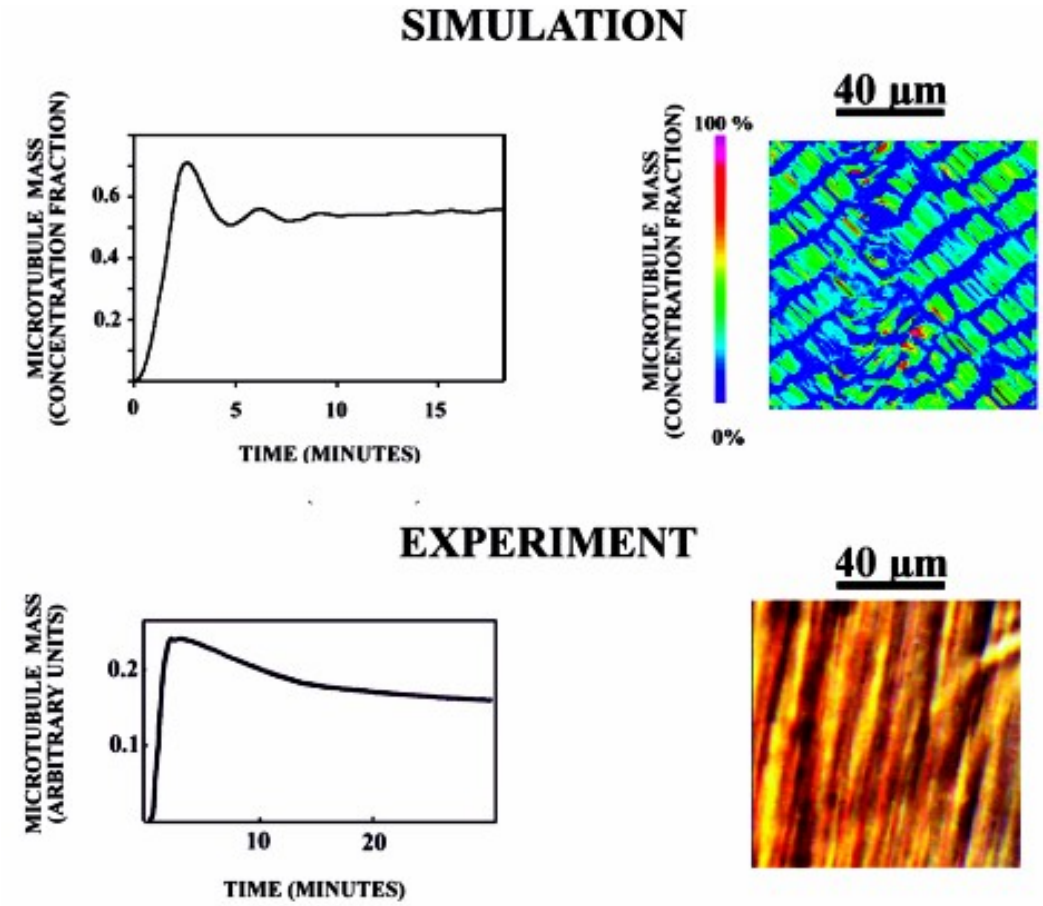

Figure 18. Numerical simulations predict microtubule assembly kinetics and self-organisation comparable with experiment (Glade, Demongeot *et al.*, 2002). The left hand part of the figure compares the kinetics of microtubule assembly as measured experimentally with that calculated by the simulation. The right hand side compares the simulated self-organised structure with the experimentally observed structure over the same distance scale. The colour scale is proportional to microtubule concentration.

The numerical simulations, based upon experimental reaction dynamics, thus predict the major features of the observed behaviour; namely, self-organisation; it's triggering by external orienting factors; and its association with assembly kinetics showing an 'overshoot'. They are likewise consistent with the observed collective directional transport of colloidal particles. In these simulations, self-organisation and other *emergent* properties arise by processes involving chemical trials of raised and lowered tubulin concentration produced by the reactive growing and shrinking of individual microtubules combined with tubulin diffusion. The fact that they do successfully predict experiment encourages us to believe that the same underlying mechanism underlies the experimental behaviour.

Using an analytical theoretical approach, likewise based on a reaction-diffusion-asymmetric transport process involving the reactive growing, shrinking, and nucleation of individual microtubules, Portet et al (Portet, Tuszynski *et al.*, 2003) came to similar conclusions. More recently, Baulin et al (Baulin, Marques *et al.*, 2007) consider theoretically a rather more simplified situation in which the reactive shrinking and growing of individual microtubules is limited to the complete disassembly of individual microtubules accompanied by the nucleation and formation of new ones. They also predicted the formation of aligned arrays of microtubules - although not patterns of microtubule concentration - that would self-organise under the effect of a weak external orienting factor.

7.Do These Processes Also Occur in Vivo?

Microtubules have two major biological functions; they organise the cell interior and they are responsible for the directional transport from one part of the cell to another of sub-cellular particles such as vesicles and chromosomes. Major cellular activities are perturbed when these processes do not occur normally. The data outlined above demonstrate that under suitable conditions microtubules can, by behaving as a *complex* system, develop *emergent* phenomena that, outwardly at least, resemble these major microtubule functions. The question thus arises as to whether such processes might also occur *in vivo*. When a fundamental physical-chemical process arises *in vitro* in a test-tube there is a high likelihood that under some circumstances it will also occur *in vivo*. However, to establish whether or not this is the case requires experiments capable of distinguishing this particular process from other mechanisms. One of its characteristic properties, not shown by other physical chemical mechanisms, is that self-organisation, together with particle transport and segregation, is triggered, or modified, by a number of external factors

such as gravity. Hence, experiments in cells or embryos showing that microtubule organisation and particle transport are simultaneously affected by these factors, in a manner resembling the *in vitro* behaviour outlined above, is *prima facie* evidence in favour of the same type of process also occurring *in vivo*. Here, I summarise some data suggesting that this might be the case.

In certain types of egg, it has long been known that gravity is involved in early development (Morgan, 1904). For example, in *xenopus* eggs, gravity is an essential factor in determining the body plan of the organism that develops. Substantial malformations result when these eggs are rotated through 90° at a critical time early in development, when the so called 'grey crescent' forms (Cook, 1986). The 'grey crescent' consists of a macroscopic array of aligned microtubules (Ziskind and Elinson, 1990; Houliston and Elinson, 1991; Houliston, 1994). During its formation, the microtubules of which it is comprised transport along their direction of orientation yolk particles containing mRNA at speeds of several μm per min. This has the effect of transporting the mRNA contained in them to a specified location in the embryo and this genetic localisation then participates in determining future development. The global behaviour; microtubule self organisation into aligned arrays, dependent upon the gravity direction at a critical instant, and associated with the collective transport and positioning of colloidal particles, strongly resembles the behaviour observed *in vitro* (c.f. experiments carried out in miniature containers). Figures 19 compares the microtubule structure of the 'grey crescent' in *xenopus* eggs with the *in vitro* self-organised structure. It likewise compares the rate of yolk particle transport, due to the oriented microtubules in the 'grey crescent', with the *in vitro* rate of transport. These favourable comparisons, combined with the absence of other physical-chemical mechanisms capable of giving rise to such behaviour, strongly suggests that the *in vivo* behaviour outlined above arises from the same type of process which occur *in vitro*.

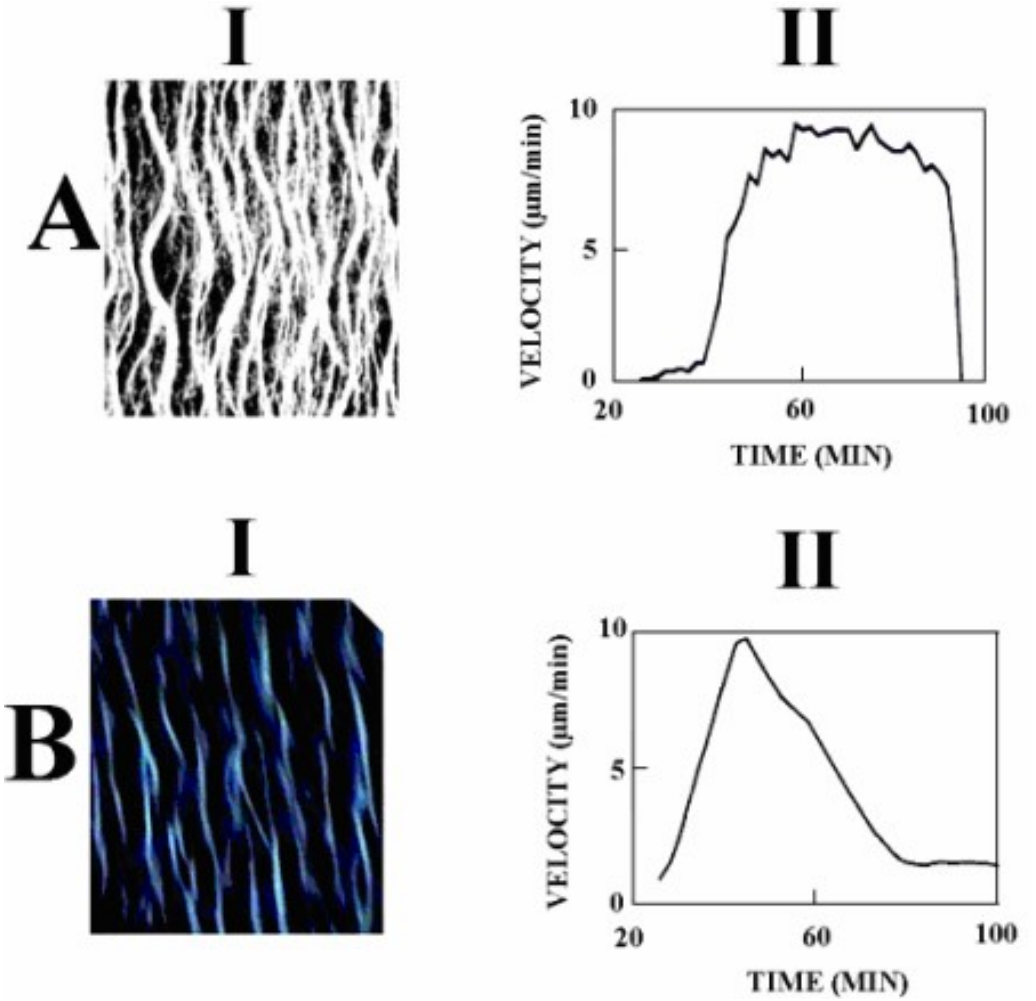

Figure 19. Comparison (Tabony, 2006a) of microtubule organisation (I) and particle transport (II) during early *xenopus* embryogenesis (A) with those observed *in vitro* by the reaction-diffusion process described in the text (B). Both processes are dependent on the gravity direction. A(I) is taken from Houliston and Elinson (Houliston and Elinson, 1991); A(II) is taken from Larabell *et al* (Larabell, Rowning *et al.*, 1996): by taking the time for the first cleavage as 90 minutes, the scale in A(II) has been changed from the time normalised with respect to the first cleavage to the time in minutes. In B), the initial tubulin concentration was 16 mg ml^{-1}.

A further example where such processes may occur is the striped patterns that develop during *drosophila* fruit fly embryogenesis. Organisation of the cytoplasm by microtubules, associated with microtubule driven particle transport, is known to play a major role in the morphogenetic processes that take place. The first stages of development take place by consecutive nuclear divisions in the non-compartmentalised egg (Alberts and Foe, 1983). Microtubules then transport the nuclei, initially uniformly distributed within the egg, to the surface region where between nuclear divisions 10 to 14 cellularisation progressively occurs. The cells are open towards the inside as this occurs, and they remain open until the end of the 14th nuclear division. Just prior to this (for around 5 minutes), when the ventral and cephalic furrows appear at

gastrulation, the distribution of microtubules in the egg displays a striped arrangement (Figure 20A) (Calliani, 1989; Papaseit, Vuillard *et al.*, 1999; Tabony, Glade *et al.*, 2002a). The stripes occur in the central part of the egg only; the end regions are not striped.

As outlined above, one of the factors on which the *in vitro* self-organised morphology depends is sample geometry. Since this is a parameter that can be modified *in vivo* as well as *in vitro,* it provides an external variable by which the behaviour of the two systems can be compared. For example, when we prepared *in vitro* microtubule self-organised structures in cylindrical containers whose shape mimic that of a *drosophila* egg, we found that the morphology which formed was also comprised of a striped central zone with stripe-free regions at each end (Figure 21). The exact morphology of the *in vitro* pattern depended on the length of the sample. Below a certain critical length, the striped central region did not form. For longer samples, the end stripe-free zones remained of the same length and the number of stripes in the central region increased with sample length. For samples of appropriate length, the pattern strongly resembled the microtubule pattern observed in *drosophila* eggs.

Drosophila eggs can be shortened, shortly after they are laid, by ligation. This consists of using a blunt razor blade, positioned in a guillotine, to pinch the egg in two compartments. If the egg does not burst, then within ten minutes a membrane forms which separates it into two un-connected fragments. Development can then occur in either one, or sometimes both, fragments. As the dependence of the microtubule pattern on sample length is a feature of the *in vitro* pattern, we examined microtubule patterns in such ligated eggs as a function of egg fragment length. We found that the length of the end stripe-free zones was independent of fragment length and approximately the same as for un-ligated eggs. The number of stripes decreased with fragment length and stripes did not form when the fragment was less than a critical length. The length dependence of the microtubule patterns in the *drosophila* eggs is hence similar to that observed *in vitro;* thus suggesting that in both cases similar mechanisms might be operating.

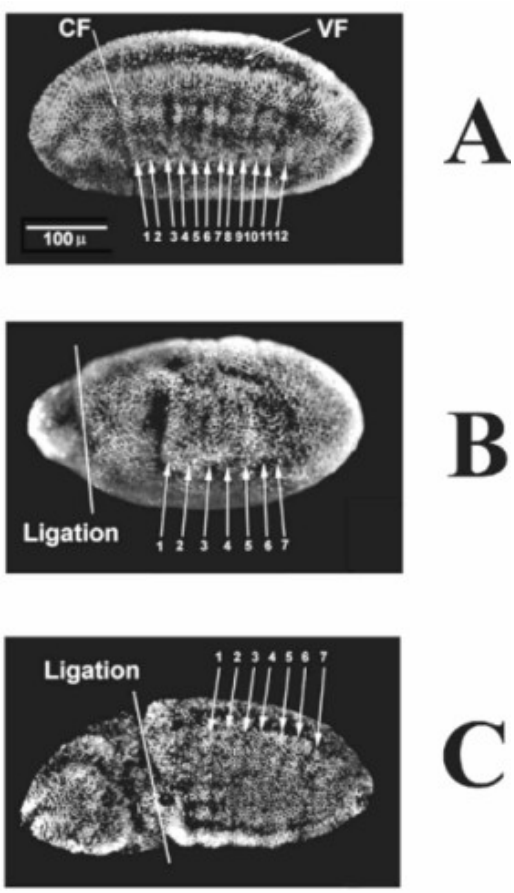

Figure 20. Microtubules patterns observed by immunofluorescence, in A) whole, and B, C) ligated *drosophila* eggs. The position of the ventral and cephalic furrows are shown in A). Ligation divides the egg into two unconnected fragments, and development continues in one, or both, of the fragments (Papaseit, Vuillard *et al.*, 1999).

For several decades, experiments in space have shown that weightlessness in humans depresses the immune system and reduces bone formation. These, and other, effects are thought to arise at a cellular level. Over the years, a substantial body of evidence has accumulated demonstrating that various cellular processes, such as growth rates, signalling pathways and gene expression are substantially modified when cells -in particular cells of the immune and bone systems - are placed under conditions of weightlessness. A number of experiments point to an involvement of the cytoskeleton (Hughes-Fulford, 2002; Lewis, 2002). Since, terrestrial gravity is not normally considered as being capable of intervening in chemical or biochemical processes, the question arises as to how it is that gravity affects cellular function? Of course, one possibility is that it does so by modifying microtubule self-organisation and sub-cellular particle transport and localisation as described above. In which case, then the processes I have been discussing here must also occur in cells under normal gravity conditions.

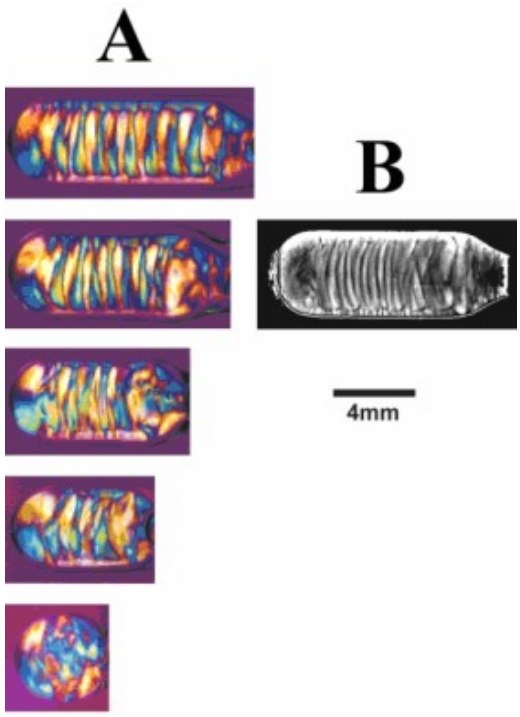

Figure 21. Effect of sample length on microtubule patterns (Papaseit, Vuillard *et al.*, 1999). A) Birefringent patterns formed by microtubules assembled in cylindrical 'egg' shaped containers of different length. B) Microtubule concentration variations as detected by fluorescence imaging: here, the overall morphology resembles the microtubule pattern in *drosophila* eggs.

Recent experiments on human epithelial (MCF-7), human lymphocyte (Jurkat), glial, rat utricular hair cells, and thyroid carcinoma cells, cultured under conditions of weightlessness, show substantial reductions in microtubule organisation compared with those at 1g (Lewis, Reynolds *et al.*, 1998; Vassy, Portet *et al.*, 2001; Gaboyard, Blanchard *et al.*, 2002; Lewis, 2002; Uva, Masini *et al.*, 2002; Hughes-Fulford, 2003) (Figure 22). These observations, consistent with the effect of weightlessness on the self-organisation of microtubules *in vitro*, thus raise the possibility that they arise from similar physical chemical processes. However, in addition to self-organisation, weightlessness will also have a substantial effect on the transport and organisation of colloidal and sub-cellular particles that self-organisation causes. Hence, if the type of reaction-diffusion process considered here also occurs in cells then one might expect that microtubule driven particle transport and localisation will be similarly affected by exposure to brief periods of weightlessness. Some important biological processes dependent on these phenomena are cell-division, signalling pathways, exo and endo-cytosis, and transport and localisation of mRNA containing vesicles. Based upon this argument, for the cellular systems where microtubule organisation is inhibited by weightlessness, we predict that weightlessness will lead to the following changes in cell function:- retarded cellular division and reduced growth rates, inhibited signal transduction, and impaired protein localisation and synthesis (Tabony, Rigotti *et al.*, 2007).

Whether or not this is the case in general will also depend upon a variety of symmetry-breaking factors - such as the shape of a cell or embryo - which may, or may not, also be present. Depending on circumstances, such factors might either oppose or reinforce the action of gravity. Hence, in some cases, the effect of weightlessness may be to strongly inhibit microtubule organisation and particle transport; in other cases it may perturb and slow them down; in other cases the effect may be negligible. Considerations of this type can account for why different cell types show differing sensitivities towards weightlessness. Based upon this argument, one might expect that circular or spherical shaped cells and embryos would show stronger gravity dependence than strongly elongated cells such as neurons. Cells of the immune system, many of which are spheroidal, are often reported as showing a gravity dependence (Lewis, 2002; Hughes-Fulford, 2003). As mentioned, the early developmental stages of *xenopus*, zebra fish and chicken embryos show a gravity dependence (Cook, 1986; Ziskind and Elinson, 1990; Wacker, Herrmann *et al.*, 1994) and they are likewise close to spherical. For the *drosophila* embryo, on the other hand, its elongated shape will probably on its own suffice to trigger

self-organisation, and hence these embryos would at best be only weakly gravity dependent.

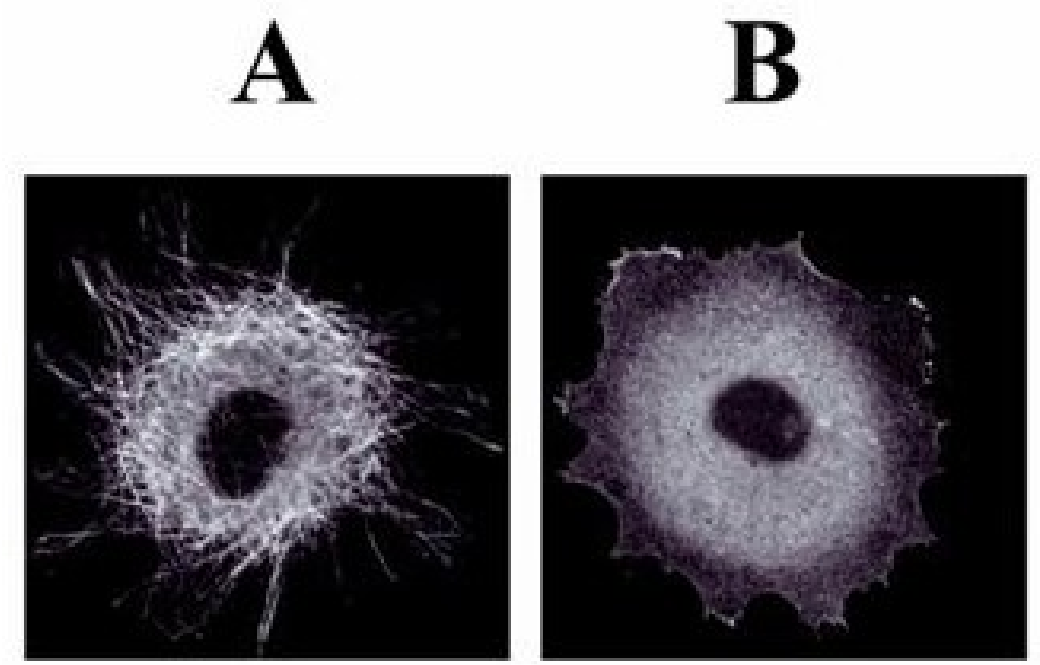

Figure 22. Microtubule organisation in human breast cancer (MCF 7) cells. A) at 1g; B) under weightlessness. The microtubules, as in the case of the *in vitro* preparations described above, do not self-organise under conditions of weightlessness. Reproduced from Vassy et al (Vassy, Portet *et al.*, 2001)

8. Summary and Outlook

The results outlined above show that under suitable conditions a population of microtubules behaves as a *complex* system that self-organises by reactive processes. Self-organisation is triggered, and affected, by a number of weak external factors such as gravity, vibrations, magnetic fields, and sample geometry, that induce an orienting effect (or preferred direction of microtubule growth) at a critical moment early in the reaction-diffusion process. When self-organisation occurs, it gives rise to, and is associated with, other higher-level *emergent* phenomena, such as the replication of form, generation of positional information, and the collective transport, organisation and positioning of colloidal and sub-cellular particles. It must be emphasised that the observed *in vitro* behaviour is quite unusual; normally solutions of reacting chemicals do not behave this way. An important feature of these experiments is the extreme simplicity of the system; being initially comprised of only two reacting species, tubulin and GTP, and without any other chemical or biological agent.

Self-organisation is believed to occur by a process in which individual microtubules in the population are

coupled via chemical trails of raised and lowered tubulin concentration caused by their reactive growing and shrinking. Simulations of a population of such shrinking and growing microtubules, based on experimentally realistic values of microtubule reaction dynamics, predict both the formation of these trails and the major overall features of the experimental behaviour; namely, self-organisation and its triggering at a critical early moment (corresponding to an 'overshoot' in the microtubule assembly kinetics) by microtubule orienting factors.

At this stage, it is not clear whether these processes are widespread in biology, or if they are limited to microtubules. It may be that the specific type of mechanism encountered here, based on reactive growth and shortening of tubes or rods, is a mechanism that is particularly suited to self-organisation. Since other elements of the cytoskeleton, such as actin and intermediate filaments show reaction dynamics of the same type, it is plausible that they may behave in a similar manner.

The overall phenomenological behaviour of these microtubule preparations shows a qualitative resemblance to some aspects of living organisms in the following ways. Firstly, macroscopic ordering appears spontaneously from an initially disorganised and unstructured starting point. Secondly, the final state depends upon small differences in conditions at a critical moment early in the process. This is reminiscent of what occurs during biological development, when after a certain stage, cells of identical genetic content take different developmental pathways to form different cell types. Just after bifurcating, the microtubule preparation could be described in biological vocabulary as being 'determined but not yet differentiated'.

Self-organisation is both dependent upon, and triggered by, weak external factors that break the symmetry of the process and lead to the emergence of form and pattern. Processes of this type could form a general class of mechanism by which weak environmental factors are transduced into biological systems. At some time during the development of life, an initially homogeneous biochemical 'soup' must have spontaneously self-organised and developed other high-level properties. The type of mechanism outlined here, in which self-organisation and other *emergent* properties are triggered into development by a variety of external factors, may have played a role in this process.

The major properties observed in this *in vitro* system, microtubule self-organisation associated with the directional transport and positioning of sub cellular particles, triggered by weak external factors shortly after microtubule assembly, correspond, outwardly at least,

to the major *in vivo* properties of microtubule. This raises the question as to whether such processes also arise *in vivo*. There is a growing body of evidence, in particular observations of the effect of the gravity vector on microtubule organisation in different cell lines and embryos, which suggests that this may indeed be the case.

Microtubule organisation and particle transport are involved in a number cell functions, including cell division. If they do not occur in the normal manner, then these cellular functions may be perturbed. The observation that weak external fields triggers microtubule self-organisation and microtubule driven particle transport thus raises the question as to whether effects on living organisms, both therapeutic and harmful, can result from exposure to brief periods of external physical stimuli. For example, the long-term survival of certain organisms in space may be compromised without a corrective action to replace terrestrial gravity.

The title of this special edition is "Microtubules, information processing and consciousness". In this respect, it is worth commenting on some particular aspects of this system. Firstly, the fact that it is extremely simple; being comprised of just two reacting species. Secondly, when self-organisation occurs, the structures that spontaneously develop generate a significant amount of 'information'. For example, in the 'horizontal' morphology shown in Figure 5, the centre of the sample is well defined; the corners are readily distinguished from the centre. This contrasts with the initial homogenous state in which there is no positional information; all parts of the sample are indistinguishable from one another. Thirdly, the structure which forms is, in this case, positioned at the centre of the sample container. Hence, in some way or another, the microtubules have 'felt' the sample container and worked out or 'calculated' where the centre is. Fourthly, the organisation contains within itself other, self-similar, structures. Thus, the process has, in some way, replicated the information contained in the structure that it generates.

In the bifurcation behaviour, where the morphology that develops is dependent upon conditions at a critical time early in the process, the sample behaves as though it retains a memory of the conditions prevailing at the bifurcation time. Just after the bifurcation, the new self-organised morphology that will subsequently develop is determined, even though at the time no organisation is visible. The gravity dependence in particular illustrates to what extent the system is sensitive to (responds to, interacts with, feels) and adapts to its external environment.

When colloidal particles are present in the preparation, the system develops other properties. The self-organising process now behaves in a way where the

overall microtubule population is itself capable of carrying out collective actions; namely, the directional transport, organisation, and positioning of colloidal particles. In a certain sense, the microtubule population spontaneously acts as a nano-machine, carrying out work and fulfilling a function. In this case, transporting and positioning particles to a specified location. This is an illustration of how a hierarchy of *emergent* properties can spontaneously generate sophisticated behaviour. The microtubule population, like ants colonies, self-organise and develop new high-level phenomena by a mechanism where individual elements lay down chemical trails that affect the subsequent behaviour of their neighbours. Knowing that ants and social insects spontaneously develop very high-level behaviour extending up to what is called 'swarm intelligence' (Bonabeau, Dorigo *et al.*, 1999) raises the intriguing question as to what extent microtubules might also be capable of 'swarm intelligence'.

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