

Self-organisation and other *emergent* properties in a simple biological system of microtubules.

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Abstract.

In biological systems, *emergent* properties develop when numerous individual molecular elements in a population are coupled in a non-linear manner. Under suitable conditions, the formation *in vitro* of a population of microtubules, a major component of the cellular skeleton (cytoskeleton), behaves as a *complex* system and develops a number of *emergent* phenomena. These preparations, which initially contain just two molecular species, a nucleotide and a protein, self-organise by reaction and diffusion and the morphology that develops is determined at a critical moment early in the process by weak external factors such as gravity and magnetic fields. Other *emergent* phenomena; namely, replication of form, generation of positional information, and collective transport and organisation of colloidal sized particles, develop. Microtubules are responsible both for cellular organisation and the transport of sub-cellular particles from one part of the cell to another. Frequently, this behaviour is triggered by some weak internal or external factor. The *in vitro* observations outlined illustrate how in a simple biological system, a *complex* behaviour may give rise to *emergent* phenomena outwardly resembling major biological functions.

Over the last hundred years, enormous advances in biology have been made based upon the general concept of molecular reductionism. This has found its apogee in the molecular basis of DNA and RNA function and more generally with the association of molecular structure with function. The implication is that knowledge of the exact disposition of atoms in a molecule will eventually (if established for a sufficiently large number of proteins and other macromolecules) lead to a description of living systems. Many biologists make substantial efforts to identify individual molecular agents involved with a specific biological function. Molecular reductionism is, however, subject to limitations and most biologists realise that some biological properties or functions somehow arise simply because a large number of interacting molecular species are present. To anyone familiar with non-linear dynamics, this suggests that populations of biological molecules might behave as *complex* systems and develop *emergent* phenomena. Some scientists are asking whether some of the global properties of biological systems can be accounted for in terms of *emergent* properties and even whether life itself should be considered as such.

Until recently, biologists have played little attention as to the manner by which populations of specific biological molecules might behave as *complex* systems. A major *emergent* phenomenon in many *complex* systems is self-organisation. Here, I would like to outline a very simple biological system of a population of microtubules in a test-tube, comprised initially of just two molecular species (a nucleotide and a protein) that behaves as a *complex* system and shows a number of *emergent* phenomena including self-organisation.

1. Self-organisation by reactive processes

The 2nd law of thermodynamics teaches us that at equilibrium order will be progressively and ineluctably lost with time. Two miscible liquids, initially separated from one another, will slowly mix by way of diffusion and convection and the existing order progressively lost. One of the characteristic properties of living systems is order and self-organisation. This naturally raises the question as to the nature of the physical-chemical processes by which order and form spontaneously develop in an initially largely unstructured biological object such as an egg or seed. Biological processes are based upon biochemical and chemical reactions. However, normally solutions of reacting chemicals in a test-tube do not self-organise. Because of this, for very many years, it was not believed possible that solutions of reacting chemicals or biochemicals could self-organise by reactive processes.

Nevertheless, very slowly over the last hundred years, both theoreticians and experimentalists have progressively shown that this is not necessarily the case. Since the late 1930's, some theoreticians (Kolmogorov, Rashevsky, Turing, and Prigogine and co-workers) [1-5] have proposed that some particular types of chemical reaction might, due to being sufficiently far-from-equilibrium, show strongly non-linear reaction dynamics. They predicted that these non-linear dynamics could, in some cases, result in a macroscopic self-organisation of the sample. Some chemical systems originally discovered in the 1920's [6] and 1950's [7] have been shown to self-organise this way [8, 9]. At a molecular level, self-organisation results from a coupling of reaction and diffusion and the patterns that arise are comprised of periodic variations in the concentration of some of the reactants. Such structures are often called reaction-diffusion or Turing structures; the latter after the British mathematician who was one of the first persons to propose such a mechanism in 1952 [3]. Prigogine and co-workers called them 'dissipative' structures [5, 10] 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. Rashevsky, Turing, Prigogine et al, and others, all proposed that biochemical mechanisms of this type might provide an underlying physical chemical explanation for biological pattern formation and morphogenesis. Although such terms were not used at the time, what these theoreticians predicted was that biological self-organisation could arise as an *emergent* phenomenon in a *complex* system by molecular processes of reaction and diffusion.

A different aspect of these systems is the manner by which some reaction-diffusion systems may show bifurcation properties and can hence be sensitive to weak external factors. In the early 1970's, Kondepudi and Prigogine explicitly calculated that the presence of an external factor, such as

gravity, or an electric or magnetic field, at a critical moment early in the process, might determine the self-organised morphology which subsequently develops [11].

The pioneer workers in this field were fully aware of the possible implications that their approach might have towards some problems in biology, and the concepts outlined above have aroused interest and debate at various times over the last 50 years. However, for a variety of reasons, the majority of biologists and chemists have not adopted this approach. Although the main reason is conceptual, another reason is the scarcity of simple experimental systems proven to self-organise this way. For example, in chemistry, it was not until 1990 that a chemical reaction, similar to those first discovered long ago by Bray (1921) [6] and Belousov (1951) [7] was finally accepted as the first example of a Turing-like structure [8, 9].

The same situation has prevailed in biology. Since the work of Turing and Prigogine and co-workers, many authors have compared the morphologies that occur in biological organisms with the mathematical predictions of reaction-diffusion theories. There is a whole body of literature in this area [12-14]. More recently, other workers [15, 16] have demonstrated that the patterns of calcium waves observed *in vivo* in the cytosol arise from reaction-diffusion processes. In spite of these advances, one of the elements lacking has been an example of a simple biochemical system in a test-tube that self-organises this way.

Under suitable conditions, we have found that the *in vitro* formation of microtubules, a major component of the cellular skeleton (cytoskeleton), does behave this way. These preparations, which initially contain just two molecular species (a nucleotide and a protein) self-organise by reaction and diffusion and the morphology that develops is determined at a critical moment early in the process, by weak external factors such as gravity and magnetic fields. This behaviour is not a result of the sum of the properties of individual microtubules and cannot be understood in terms of molecular reductionism. On the contrary, it arises from the collective action of the entire microtubule population in which individual microtubules interact and communicate with one another by way of the chemical trails that they themselves form. These observations illustrate how in a simple biological system, reactive processes give rise to a population of interacting elements that behaves as a *complex* system and shows a number of *emergent* phenomena as a consequence. In addition to self-organisation, and its triggering by weak external factors, a number of other collective phenomena develop; namely, replication of form, generation of positional information, and collective transport and organisation of colloidal sized particles. These *emergent* phenomena, outwardly at least, resemble the major biological properties of microtubules and they may turn out to be of considerable biological significance.

2. Self-organisation in colonies of living organisms

Colonies of living organisms provide many examples of self-organisation [17]. In many cases, structure and organisations develop, not by action at the level of the individual, but by way of the dynamics in which individuals, strongly coupled to one another in a non-linear manner, behave as a collective ensemble. Similar types of morphology often develop in spite of large differences in the nature and size of the individual element. Striped arrangements frequently 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. For example, over a distance scale of several centimetres some bacteria colonies form a stationary pattern. Observations at higher magnification show that the pattern is comprised of regions containing differing bacterial densities. At even higher magnification, the individual bacteria are seen to be undergoing a rapid, seemingly random movement. Individual bacteria interact indirectly with one another via trails in the concentration of chemical attractants and repellents that they themselves produce. It is by way of dynamic processes involving the collective movement of many bacteria that the stationary pattern arises. The energy source driving this process is the chemicals consumed by the bacteria. When it runs out, the bacteria stop moving and the pattern disappears.

A similar mechanism is the basis for the self-organisation of ant colonies and other social insects. The behaviour of the population results essentially from the actions of individuals strongly coupled to one another by a form of chemical communication [17, 18]. 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, will in its turn, deposit more pheromone on the trail thus reinforcing it. The progressive reinforcement of these chemical trails leads to the self-organisation of the ant population. Although the rules governing the behaviour of individual ants are relatively simple, the overall behaviour is extremely sophisticated.

One of the advantages of this type of process is that ants rapidly establish the shortest route between a food source and the nest. Consider a situation where there are two food sources close to a population of ants; but where one of the food sources is closer than the other. As ants return to the nest with food, they leave behind themselves chemical trails. These trails are then followed by other ants who in their turn, deposit chemicals that reinforce the original trails. In such a way, progressively more and more ants follow the paths to the food sources. However, because the trail from the closer of the two sources is shorter, it takes less time for an ant to return to the colony. This results in a slightly larger number of ants taking the path to this food source, thus reinforcing the strength of the chemical trail of the shorter path at the expense of the longer path. Hence, progressively more and more ants take the shorter path to the closer food supply until they nearly all follow this route. This illustrates how self-organisation results from the progressive reinforcement of chemical trails by moving objects which themselves produce these trails. If, the two food sources are at approximately equal distance from the nest, then the ants still mostly accumulate on the path to one of the food sources. This comes about because any small factor which early in the process favours the reinforcement of one of the chemical trails over the other, will progressively lead to nearly all the ants using this pathway. Once the reinforcement of one pathway has gone sufficiently far, then the determining factor may be removed without affecting the subsequent behaviour. This is a simple example of a bifurcation due to a weak external factor in a self-organising *complex* system.

3. Microtubules

Microtubules [19, 20] are a major filamentary component of the internal skeleton of cells (cytoskeleton). They have two major cellular roles; they organise the cell interior, and they permit and control the directional movement of intracellular particles and organelles from one part of the cell to another. Microtubules participate in many fundamental cellular functions including the maintenance of shape, motility, and signal transduction. They frequently organise or reorganise in response to weak internal and external stimuli of either physical or biochemical nature. Microtubules are a significant component of brain neurone cells and they make up the mitotic spindles that separate chromosomes during cell division. They play a determining role in the organisational changes that occur during the early stages of embryogenesis. Microtubule organisation is a fundamental cellular property affecting numerous biological functions and the viability of a cell is compromised when it does not occur correctly.

Microtubules are long tubular shaped supra-molecular assemblies with inner and outer diameters of about 16 nm and 24 nm respectively. Although their length is variable, they are often several microns long. The walls of the tube are comprised of a protein, tubulin, and microtubules arise from the self-assembly of this protein by way of reactions involving the hydrolysis of a nucleotide, guanosine triphosphate (GTP), to guanosine diphosphate (GDP). Once microtubules form in this way, they continually grow and shrink by processes in which additional tubulin molecules are added to one end of a microtubule whilst other tubulin molecules are lost from the opposite shrinking end. This process is likewise associated with the hydrolysis of GTP to GDP. The system is hence chemically irreversible and there is a continual consumption and dissipation of chemical energy. Biologists have established in living cells that microtubule organisation and reorganisation results from the chemical dynamics of the reactive processes associated with their formation and maintenance.

Microtubules can be readily formed and studied *in vitro*. A solution of purified tubulin, in the presence of an excess of GTP, when warmed from about 4°C to 36°C, assembles within a few minutes into microtubules. After the microtubules have formed, this reaction continues by processes in which the complex, tubulin-GTP, is added to the growing (+) end of a microtubule and tubulin-GDP is lost from the opposite shrinking (-) end. An unusual and important feature of microtubules is that they possess a reactive polarity, and the reaction dynamics at opposite ends of the microtubule are different. Due to this difference in reactivity, microtubules often grow from one end (+) whilst shrinking from

the other end (-). 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 termed '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 very large range of microtubule reaction dynamics.

A shrinking microtubule is capable of forming a trail of free tubulin. This tubulin is initially liberated in the form of the complex, tubulin-GDP. This progressively diffuses out into the solution. Simultaneously, excess GTP present reconverts the tubulin-GDP to tubulin-GTP. At this point, the tubulin-GTP can be incorporated into the growing ends of neighbouring microtubules. Because the incorporation of tubulin into the growing ends of microtubules increases strongly with tubulin-GTP concentration, neighbouring microtubules will preferentially grow into regions of higher tubulin-GTP concentration whilst avoiding those of lower concentration. Hence, for some types of microtubule reaction dynamics (and rates of tubulin diffusion) neighbouring microtubules can communicate with one another, and modify their rate and direction of growth, by way of the chemical trails that they themselves produce. In this way, a population of microtubules is capable of behaving as a *complex* system. It can self-organise and generate other *emergent* phenomena in a manner that shows many analogies with the way that ants and other social insects self-organise.

4. Microtubule self-organisation and other emergent phenomena

4.a. Self-organisation

Under many conditions, microtubule solutions show neither temporal nor spatial self-organisation. However, in 1987 it was reported that they could show regular damped oscillations of assembly and disassembly [21]. In 1990, we reported experiments under different buffer conditions in which macroscopic self-ordering occurred [22]. When assembled in glass containers, measuring 40 mm by 10 mm by 1 mm, the microtubule solution progressively self-organises over approximately 5 hours to form a series of periodic horizontal stripes of about 0.5 mm separation. Once formed, the striped pattern remains stationary and it is stable for between 48 to 72 hours, after which the system progressively runs out of reactants. In each striped band, all the microtubules are very highly oriented with respect to one another. The direction of orientation is at about either 45° or 135° to the direction of the stripe, but adjacent stripes differ from one another in having a different orientation from their neighbours. Hence, the microtubule orientation flips from left to right periodically up the length of the sample container. In addition to this orientational pattern, a pattern of variations of microtubule concentration is also present that coincides with the changes in orientation [23]. The microtubule concentration drops by about 30% and then rises again each time the microtubule orientation flips from acute to obtuse or vice versa (Figure 2). Experiments, that will not be described here, show that self-organisation contains both reactive and diffusive contributions and arises from processes involving the continual growth and shrinking of individual microtubules [24-26].

4.b. Replication of form

The structure is complicated, for each 0.5-mm stripe also contains within it another series of stripes of about 100 μm separation. These, in their turn, contain other sets of stripes of about 20 μm , 5 μm and 1 μm separation [24, 27]. In samples made up in a 15 mm diameter test tube, an additional level of ordering of several mm arises. These large stripes contain the lower levels of organisation already mentioned. Hence, similar types of pattern spontaneously arise over distances ranging from a few microns up to several centimetres. So here, we already see two emergent phenomena; self-organisation and replication of form.

The range of dimension over which these microtubule structures occur is typical of those found in many types of higher organisms. Cells are about 10 μm in size, eggs are often about 1 mm, and a developing mammalian embryo is several centimetres long. Self-organisation also arises when

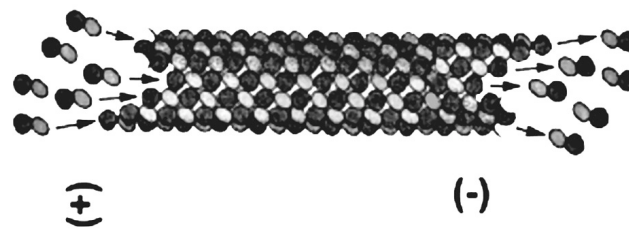


Figure 1 Schematic illustration of a microtubule growth and shrinking". Tubulin-GTP is added to the growing end of a microtubule (+) and tubulin-GDP is lost from the other shrinking end (-). During this process, GTP is hydrolysed to GDP.

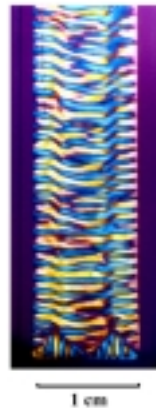


Figure 2. Self-organised microtubule structures as formed in optical cells, 40 mm by 10 mm by 1 mm, positioned vertical. Microtubules were formed by warming a solution containing 10mg/ml of tubulin from 4°C to 36°C in the presence of an excess of GTP. Microtubules form within 2-3 minutes after warming the solution, and the structure shown progressively develops over the next 5-6 hours. The structure once formed is stationary and the solution is stable for about 3 days. The strong optical birefringence indicates that the microtubules are highly aligned. The structure is photographed through crossed polars (0° and 90°) with a wavelength retardation plate at 45°. The retardation plate produces a uniform mauve background. Microtubule orientations of about 45°, such that their birefringence adds to the birefringence of the wavelength plate, produce a blue wavelength shift whereas orientations at about 135° subtract from the birefringence and result in a yellow interference colour. The alternating blue and yellow stripes arise from periodic variations in microtubule orientation from obtuse to acute.

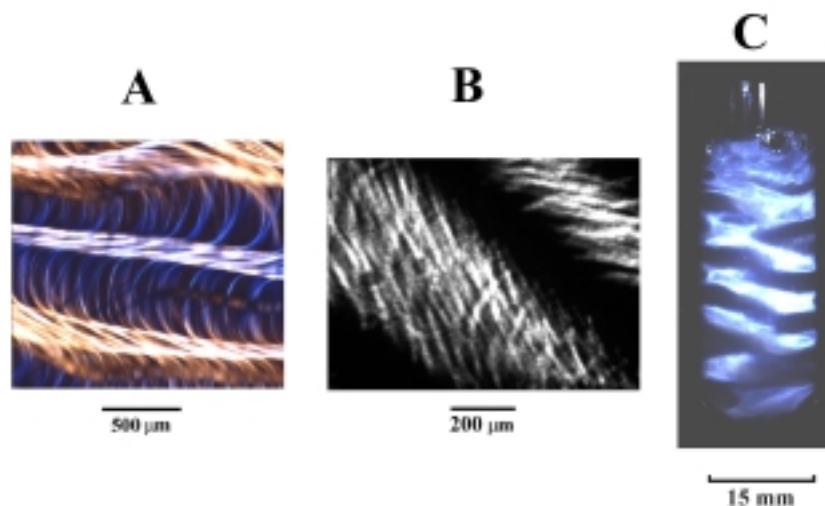


Figure 3. Replication of form. The striped structure, as shown in figure 2, is itself comprised of stripes of smaller periodicity. Photographs A) and B) show one of the individual stripes at higher magnification. Separations of approximately 100 μm and 20 μm are clearly visible. C) is a photograph of the structure that forms in a 15 mm diameter test tube.

samples are prepared in small containers (50-200 μm) of dimensions comparable to those of cells and embryos.

4.c. Positional information

Another feature of these structures and organisations is that they contain a considerable amount of positional information. This is clearly seen in the self-organised morphology, shown in Figure 4, in which the pattern has a clearly defined centre. Moreover the centre of the pattern is positioned in the centre of the sample. Thus, in some way or other, the microtubules have worked out where the centre of the sample is. 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.

4.d Dependence of self-organisation on gravity.

The difference in conditions leading to the two different morphologies shown (figures 2 and 4) is merely the orientation of the sample with respect to gravity during self-organisation. Striped morphologies occur when the microtubules are prepared rectangular sample cells that are upright, but concentric circles arise when they are prepared in the same containers lying horizontal, flat down [28]. This fact indicates that gravity in some way intervenes in the self-organising process. Once formed, the structures are stationary and independent of their orientation with respect to gravity. To establish at what moment during self-organisation the sample morphology depends on the gravity direction, we carried out the following simple experiment [24]. Twenty samples of purified tubulin in the presence of GTP (at 4°C) were placed vertical. Samples were simultaneously warmed to 36°C to instigate microtubule formation. Consecutive cells were then turned from vertical to horizontal at intervals of one minute and left in this position for the rest of the self-organising process and examined 12 hours later after the structures had formed. Twenty minutes after instigating microtubule formation, when the last sample was rotated from vertical to horizontal, there are no obvious signs of any striped structure. Since the structures form while the cells are flat, one might expect that they would all form the horizontal pattern. This is the case for samples turned during the first few minutes. However, samples which were upright for six minutes or more all formed striped morphologies similar to preparations that remained vertical all the time. The final morphology depends upon whether the sample container was horizontal or vertical, at a critical time six minutes after instigating assembly, early in the self-organising process. This can be described as a bifurcation between pathways leading to two different morphological states, and in which the direction of the sample with respect to gravity determines the morphology that subsequently forms.

An obvious question is; what would happen if gravity was not present at the bifurcation time? To answer this we carried out an experiment under conditions of weightlessness produced in a free-falling rocket of the European Space Agency. This produced conditions of weightlessness for the first 13 minutes of the self-organising process. We found that, contrary to reference samples assembled on an on-board 1g centrifuge, samples assembled under conditions of weightlessness did not self-organise [27]. This result shows that under the conditions used in this experiment, the presence of gravity at the bifurcation time actually triggers self-organisation. To study the effects of weightlessness, it is not necessary to go to the trouble, expense, and risk-to-life, of carrying out experiments in space. Gravity effects may be substantially reduced in ground-based laboratories using simple inexpensive methods such as clinorotation and magnetic levitation. We have also carried out experiments using these methods and observed behaviour very close to that obtained in space-flight [29]

4.e. Proposed molecular mechanism

In far-from-equilibrium systems that self-organise, bifurcations are associated with an instability in the initially homogenous state. When self-organisation arises from a chemical processes, as in the present case, then this instability will involve reactive elements. For the microtubule case, we would hence expect a chemical instability, involving the relative concentrations of microtubules and free tubulin, to occur close to the bifurcation time. This is the case. Frequently, the kinetics of microtubule self-assembly, after an initial increase due to the formation of microtubules from the tubulin solution, remains at a stationary level. In general, microtubule solutions showing this type of

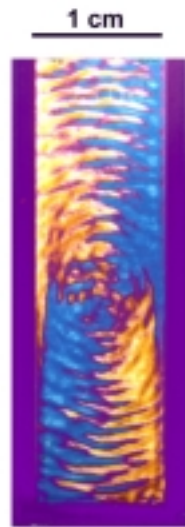


Figure 4. The morphology that forms is dependent on the gravity direction. A different stationary morphology forms when the sample container is positioned horizontal during self-organisation. For this morphology, the centre of the sample is determined by the centre of the pattern. This illustrates the generation of positional information.

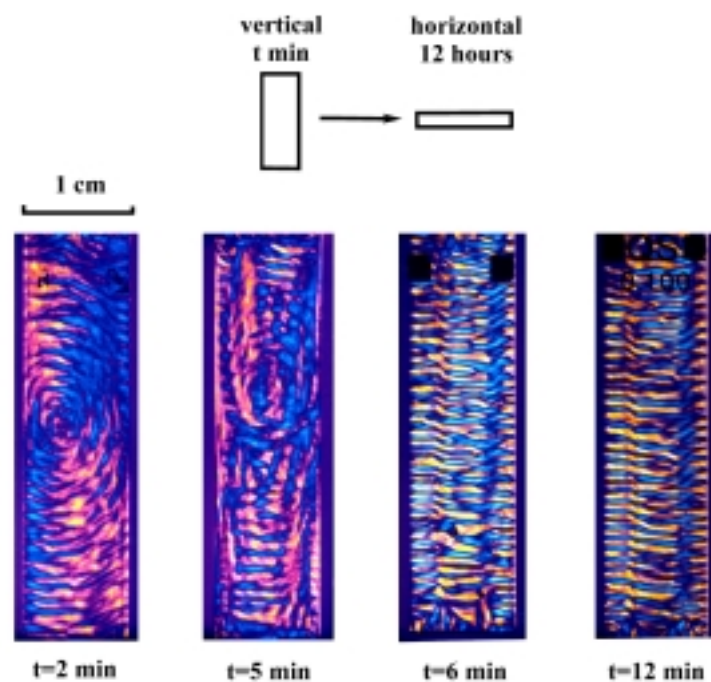


Figure 5. Bifurcation behaviour of self-organised microtubule preparations. The morphology that forms is determined by the gravity direction at a critical time early in the process.. The photographs show the final stationary morphologies for samples rotated from upright to horizontal at different times, t , during the first twenty minutes of self-organisation. 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

behaviour do not self-organise. However, microtubule preparations that do self-organise do not show this type of assembly kinetics. Instead, after an initial rapid increase, corresponding to the formation of microtubules from tubulin, the microtubule concentration shows an overshoot and progressively decreases over the next 30 minutes to a value about 20% lower than at the maximum [24, 30]. The maximum in the microtubule concentration occurs approximately six minutes after instigating microtubule assembly, and coincides with the bifurcation time when self-organisation is determined by gravity.

Microtubule self-organisation depends not only upon the presence of gravity at an early critical moment. It also depends on other weak external factors, such as magnetic fields, sheering and weak vibrations and geometrical factors [30, 31]. These experiments strongly suggest that any factor, which at the bifurcation time, leads to a privileged direction of microtubule orientation, will trigger self-organisation. This conclusion provides an important clue to the molecular mechanism by which self-organisation occurs. Microtubules are continually growing from one end and shrinking from the other. For appropriate values of reaction dynamics, the shrinking end of a microtubule will leave behind itself a chemical trail of high tubulin-GDP concentration. Excess GTP in the reaction mixture then converts tubulin-GDP back to tubulin-GTP. At this point, the tubulin-GTP is again available either to be incorporated in the growing end of a neighbouring microtubule, or to nucleate with other tubulin-GTP molecules to form a new microtubule. During this time, the tubulin freely diffuses into the surrounding solution. Likewise, growing microtubule ends produce regions depleted in tubulin-GTP. Because reaction rates increase with increasing concentration, neighbouring microtubules will preferentially grow into regions of high tubulin-GTP concentration whilst avoiding those of low concentration. We postulated that for appropriate reaction dynamics, the chemical trails produced by individual microtubules, can modify and determine the direction of growth of their neighbours [27]. Thus neighbouring microtubules will "talk to each other" by depleting and accentuating the local concentration of active chemical. Under such circumstances, the coupling of reaction with diffusion will progressively lead to macroscopic variations in microtubule orientation and concentration.

When the microtubules first form from the tubulin solution, they are in a phase of growth and are distributed uniformly through the solution in an isotropic manner. At this stage, there is almost no disassembly from their shrinking ends. However, the rapid initial growth of the microtubules depletes the concentration of free tubulin in solution and this in turn provokes the partial disassembly of the microtubules. This partial disassembly manifests itself as the 'overshoot' in the assembly kinetics. When partial disassembly starts to occur, just prior to the bifurcation time, it leads to the formation of the chemical trails outlined above. The isotropic arrangement of microtubules is now unstable, for at this time, orienting just a few microtubules will induce their neighbours to grow along the same orientation. Once some microtubules have taken up a specific orientation, then neighbouring microtubules will also grow into the same direction. Orientational order will then spread from neighbour to neighbour, and so on. The process mutually reinforces itself with time and leads to self-organisation. Hence, in agreement with experiments, any small factor that at the instability (bifurcation time) directly orients microtubules, or leads to a privileged direction of microtubule growth, will trigger self-organisation.

4.f. Numerical simulations

To investigate whether such an explanation is realistic we carried out computer simulations of a population of growing and shrinking microtubules, incorporating microtubule reaction dynamics consistent with experimental values, [32, 33]. Simulations involving just a few microtubules, demonstrated both the formation of the tubulin trails outlined above and the growth of neighbouring microtubules into these trails, along their direction. When the simulations were extended to a population of about 10^4 microtubules on a two-dimensional reaction space, $100\ \mu\text{m}$ by $100\ \mu\text{m}$, then after 2-3 hours of reaction time, a self-organised structure comprised of regular bands of about $5\ \mu\text{m}$ separation developed [32, 33]. This structure is comparable with the experimental self-organised structure that arises over a similar distance scale. In addition, the simulations also predict an 'overshoot' in the microtubule assembly kinetics. At the calculated 'overshoot', the simulations predict that the strongly shrinking microtubules result in strong fluctuations of concentration and density (3%). For self-organisation to occur, the algorithm also requires the presence, at this critical moment, of a small asymmetry in the reaction-diffusion process. The asymmetry acts either by directly

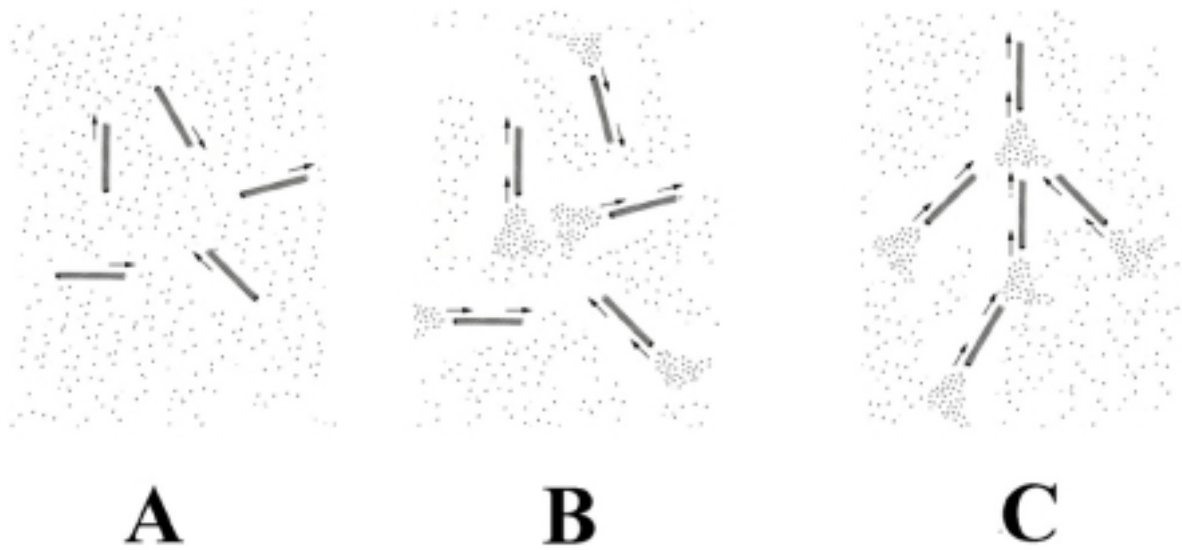


Figure 7 Proposed mechanism for the formation of the self-organized structure. Microtubules are chemically anisotropic, growing and shrinking along the direction of their long axis. This leads to the formation of chemical trails, comprised of regions of high and low local tubulin concentration from their shrinking and growing ends respectively. These concentration trails (density fluctuations) are oriented along the direction of the microtubule. Neighbouring microtubules will preferentially grow into regions where the local concentration of tubulin is highest. 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 bifurcation time. This produces trails of high tubulin concentration from the shrinking ends of the microtubules. In C) microtubules are growing and forming preferentially into these tubulin trails. The isotropic arrangement shown in B) is unstable. Once a few microtubules start to take up a preferred orientation then neighbouring microtubules will also grow into the same orientation. Once started, the process mutually reinforces itself with time and leads to self-organisation. At the instability, any small effect that leads to a slight directional bias will trigger self-organisation. Gravity acts by way of its directional interaction with the macroscopic density fluctuations present in the solution.

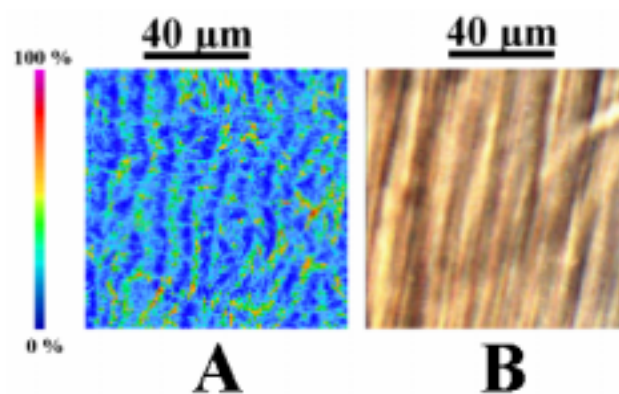


Figure 8. Numerical simulations (A) containing only reactive and diffusive terms predict macroscopic self-organisation comparable with experiment (B).

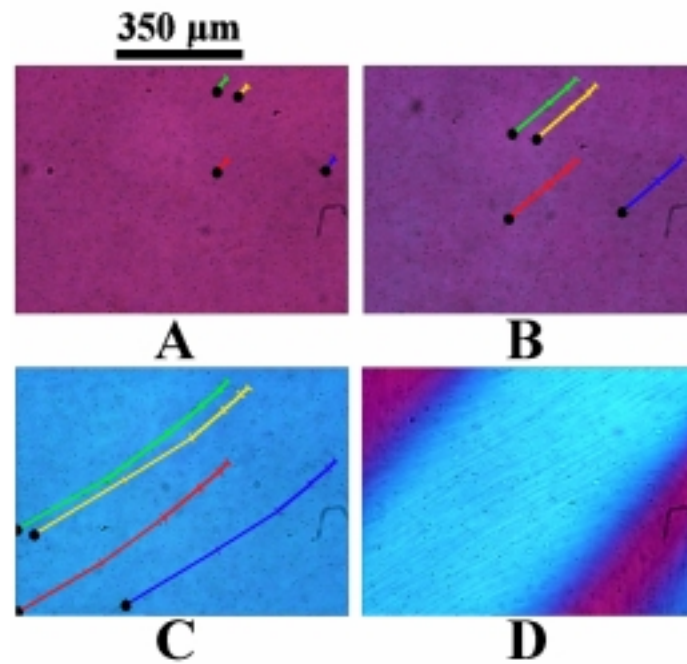


Figure 9. Transport of colloidal polystyrene particles during microtubule self-organisation. Images of the preparation at different times during self-organisation; A), 20 min; B), 40 min; C), 60 min; D), 5 hours. The numerous small dots are polystyrene beads of 1.1 μm diameter. Several have been highlighted 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.

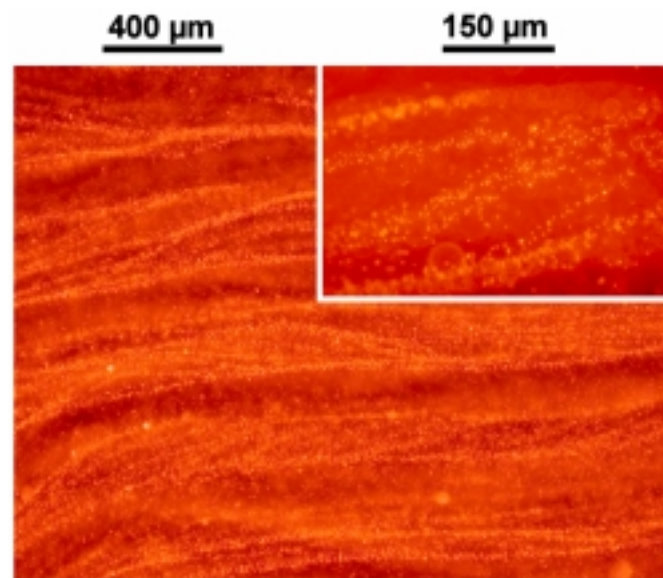


Figure 10 Microtubule self-organisation also results in the organisation of colloidal particles. The photograph shows the distribution of 1.0 μm diameter fluorescent polystyrene particles in a self-organised preparation. This pattern coincides with the microtubule pattern. The particle distribution was homogenous prior to self-organisation.

orienting some microtubules, or by making tubulin diffusion faster along one direction than the others. The latter favours the growth of microtubules along this direction and triggers self-organisation by the orientational effect thus produced. Gravity, by interacting with the density fluctuations produced by strongly shrinking microtubules at the 'overshoot' (bifurcation time), gives rise to increased molecular transport along the vertical direction and so triggers self-organisation. Magnetic fields and sheering, on the other hand, act by directly orienting microtubules at the bifurcation time. Hence, gravity and magnetic fields may break the symmetry of the initially homogenous state and thus lead to the emergence of form and pattern. Gravity and magnetic fields can thus intervene in a fundamental cellular process and will indirectly affect other cellular processes that are in their turn dependent upon microtubule self-organisation. Other external factors, such as vibrations, have the same effect. Processes of this type could form a general type of mechanism by which outside environmental factors are transduced into living systems. Such processes may have played a role in the development of life on earth.

4.g .Collective particle transport and organisation

The computer simulations outlined above also suggest an explanation for another emergent property of this system; namely the directional transport and organisation of transport of colloidal sized particles [34]. 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. For a variety of reasons, we suspected that the self-organising process could also result in collective particle transport. This turned out to be the case. We observed the following behaviour. When 1 μm diameter colloidal polystyrene particles were added to the initial preparation of tubulin and GTP [34] then about 15 minutes into the self-organising process, all the beads start to move in the same direction at speeds of several μm per minute. The direction of movement corresponds to the direction of microtubule orientation that develops at this time. When self-organisation is complete after about 5 hours, then there is no further particle transport. Particle transport does not occur when self-organisation is not triggered by gravity, or if microtubules are assembled under different reactive conditions such that self-organisation does not occur. Numerical simulations of the self-organised arrangement at different times during the process, show that the parallel fronts of oriented microtubules shown in figure 8A, cross the reaction space at speeds of several μm per minute [32]. These travelling fronts correspond to variations in microtubule concentration of at least 30%. As the microtubule preparation is extremely viscous, they also correspond to waves of differences in viscosity of several thousand poises. Such travelling waves, comprised of variations in concentration and viscosity, would be quite capable of transporting colloidal sized particles along with them.

Moreover, the distribution of particles, which was initially homogenous, takes on a pattern coincident with that of the microtubules. So, in addition to being transported, the colloidal beads are also themselves organised by the self-organising process [34]. We believe that this 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 concentration develop in the sample. As these develop, the rate of particle movement will not be the same everywhere. Particles will hence tend to accumulate into different regions of space in a manner analogous to that which cars travelling at different speed aggregate into clusters or form traffic jams.

5. Conclusions

Under appropriate conditions, *in vitro* microtubules preparations behave as a *complex* system. They self-organise and show a number of other *emergent* phenomena by way of a reaction-diffusion process, which shows analogies with the way ants and other social insects self-organise. The principal *emergent* properties that develop; self-organisation, collective particle transport, and their triggering by a weak factor, outwardly resemble the major features of microtubule behaviour in living systems. It may turn out that the mechanism and *emergent* properties outlined above are of major biological significance. Although biologists have long known that the self-organising behaviour of microtubules in living systems arises from their reaction dynamics, as yet they do not view this in terms of *emergent* properties in a *complex* system.

The question thus naturally arises as to whether the processes outlined above might also occur *in vivo*; and in particular whether or not they might arise during the cell cycle and the early stages of embryogenesis. One of the characteristic properties of microtubule self-organisation by reaction and diffusion is its dependence on various external factors such as gravity. It is known that cellular functions are modified when cells are cultured under conditions of weightlessness [35, 36]. Recent experiments on cell lines cultured under conditions of weightlessness show a disorganised microtubule network compared to control experiments under normal gravity conditions [37-40]. This latter behaviour is consistent with the *in vitro* observations reported here and raises the possibility that the processes outlined above might occur in living cells.

Rashevsky, Turing, Prigogine and co-workers, first developed their theories as a possible underlying physical-chemical explanation for biological self-organisation during embryogenesis. They predicted a way by which macroscopic chemical patterns could spontaneously develop from an initially unstructured egg. Although there is evidence that microtubule self-organisation by reaction and diffusion occurs during *drosophila* embryogenesis [23, 41, 42], it is too early to affirm whether or not this process plays a role in determining the body plan of the resulting organism. What we can say is that non-linear reaction dynamics can in principle account for biological self-organisation and pattern formation, and that an important cellular component, microtubules, behaves this way in a test-tube.

The overall phenomenological behaviour of the microtubule preparations shows a qualitative resemblance to some aspects of living organisms in the following ways. Firstly, macroscopic ordering appears spontaneously from an initially homogenous starting point. Secondly, the final state depends upon small differences in conditions at a critical moment at an early stage 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, a non-linear system could be described in biological vocabulary as being ‘determined but not yet differentiated’.

The mechanism of self-organisation outlined above shows significant differences from the type of reaction-diffusion scheme originally proposed by Turing. In the Turing system, the molecules communicate with one another by diffusion (fast diffusion of the inhibitor and slow diffusion of the activator). In the microtubule system, on the other hand, as for ants, communication occurs essentially by way of the chemical trails that the microtubules produce by their own reactivity. It is a reaction-diffusion system, since without tubulin diffusion at the appropriate rate, self-organisation would not occur. Another difference with the Turing scheme is the reactive anisotropy and heterogeneity of the microtubule system. In a normal reaction-diffusion scheme there is no inherent anisotropy in the reactive process. This is not the case for an individual microtubule. Here, reactive growing and shrinking can lead to chemical trails along only one specific direction. The system has an in-built propensity for symmetry breaking under the effect of a weak external factor. In addition, in a microtubule preparation, chemical reactions can only occur at the ends of individual microtubules, and these ends are often several microns apart. The solution, once microtubules have assembled, is hence chemically heterogeneous and this factor likewise favours self-organisation. It may be that the specific type of mechanism encountered here, based on reactive growth and shortening of tubes or rods, is particularly suited to self-organisation. At present, it is not yet clear whether these processes are widespread in biology or if they are limited to microtubules.

The results outlined above demonstrate how a very simple biological system comprised initially of just a protein and GTP, and without DNA, can show a *complex* behaviour and develop *emergent* phenomena that outwardly resemble certain biological functions. These phenomena, which may be of considerable biological importance, are not the sum of properties of individual molecules, but come about spontaneously as a consequence of non-linear reaction dynamics in a population of strongly interacting elements.

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