A Landau-Ginzburg Model of the Co-existence of Free Tubulin and Assembled Microtubules in Nucleation and Oscillations Phenomena

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Abstract. A link is shown between reaction-diffusion kinetics for microtubule assembly and time-dependent Landau-Ginzburg phenomenology. In the latter, microtubule assembly is treated as a first-order phase transition using a postulated Landau-Ginzburg free energy expansion. The results establish a connection between the oscillations observed in experiment and the phase diagram for microtubule assembly. The model also predicts a specific heat behavior which could be verified experimentally.

Key words: Microtubule assembly, phase diagram, tubulin concentration

1. Introduction

Microtubules are key components of the eukaryotic cytoskeleton. Despite being relatively rigid cylindrical protein polymers, microtubules are constantly rearranging themselves to participate in and perform various functions within the cell. One of the most interesting observations is what has been termed *dynamic instability* where individual microtubules have been seen to continuously switch between growing and shrinking states [1, 2]. A different type of assembly behavior is also found above a threshold of tubulin concentration where ensembles of microtubules undergo smooth, damped oscillations [3–6]. These patterns are oscillations in the total amount of assembled tubulin within an *in vitro* buffer. The dynamics of a single microtubule have been extensively modeled using a variety of techniques such as Monte Carlo simulations, chemical kinetics equations and the master equation formalism [7–12]. In this paper we will be interested in the behavior of the bulk system. Previous models of microtubule oscillations have used Monte Carlo methods or coupled reaction schemes [13–16]. As we mentioned earlier, when an ensemble of microtubules has the correct buffer conditions, the total amount of assembled tubulin undergoes damped oscillations [3–6]. These oscillations are typically observed turbidimetrically. This method measures the amount of light scattering from the sample which is proportional to the amount of assembled tu-

bulin. Since this is a bulk measurement, all of the spatial information about where assembly is taking place is eliminated, thus leaving the impression that these are simply oscillations in time, homogeneous throughout the sample. This may be the case in some situations, but other possibilities have been seen to exist. Mandelkow et al. [17] performed an experiment where they could actually view microtubule oscillations by eye and they observed waves in the sample. These waves of assembly were nucleated at the boundary of the vessel containing the sample and propagated inwards through the buffer.

Previously [18], a model involving chemical kinetics was shown to reproduce many of the observed microtubule oscillations phenomena in the bulk. The chemical kinetics model developed earlier did not aspire to account for any spatial patterns. It was based on five basic reactions involving microtubule and tubulin dimers bound with either *GTP* or *GDP*. The set of reactions included in this description was:

where MT_i , is a microtubule containing i dimers, $Tu - GTP/Tu - GDP$ is an unassembled tubulin dimer with *GTP*/*GDP* bound at the exchangeable site (E-site) in the β -monomer (see [18] for more details). It was assumed that all the variables depended only on time, but we will now assume space dependence as well in order to gain insight into the emergence of patterns. We also want to extend our model to include diffusion, however not all quantities will diffuse to the same extent. Assembled microtubules are much larger than their constituent dimers and would thus diffuse much more slowly, so their diffusion constant D_1 , is much smaller than that of free tubulin, i.e. $D_2 \gg D_1$. With these added terms, we can derive the set of reaction-diffusion equations for the above processes:

$$\dot{N} = -k_c N + k_n T_t - k_i N T_d \quad (1.6)$$

$$\dot{T}_a = k_+ N T_t - k_c T_a + k_n T_t - k_i T_a T_d + D_1 \nabla^2 T_a \quad (1.7)$$

$$\dot{T}_d = k_c T_a - k_r T_d + k_i T_a T_d + D_2 \nabla^2 T_d \quad (1.8)$$

$$\dot{T}_t = -k_+ N T_t - k_n T_t + k_r T_d + D_2 \nabla^2 T_t, \quad (1.9)$$

where the diffusion constant D_2 is taken to be the same for all free tubulin dimers, independent of their bound nucleotide. The four dependent variables in these equations are: N , the microtubule number density, T_a , the density of assembled tubulin and T_f/T_d , free tubulin dimers with GTP/GDP bound at the E-site. The rate constants for these reactions can be determined from experimental data [18], likewise the diffusion of tubulin within the cytoplasm has been measured. Salmon et al. [19] found an *in vivo* value of $D_2 = 5.9 \times 10^{-8} \text{ cm}^2 \text{ s}^{-1}$. From this they also estimated a value of $D_2 = 56 \times 10^{-8} \text{ cm}^2 \text{ s}^{-1}$ for tubulin in an *in vitro* buffer [19]. Since we are modeling *in vitro* assembly, we will adopt the second value for our choice of D_2 . To the best of our knowledge, the diffusion constant D_1 , for the ensemble of microtubules has not been directly measured but we expect it to be at least an order of magnitude less than free tubulin.

We will simplify the description even further and introduce just the concentration fields ψ for the unassembled tubulin (including both GTP - and GDP -rich tubulin), and ϕ for the combined concentration of microtubules of all lengths. Under these conditions the four Equations in (1.6)–(1.9) can be replaced by just two equations below

$$\dot{\phi} = D_1 \nabla^2 \phi - k_1 \phi \psi - k_2 \phi + k_3 \psi \quad (1.10)$$

and

$$\dot{\psi} = D_2 \nabla^2 \psi + k_1 \phi \psi - k_3 \psi + k_2 \phi. \quad (1.11)$$

We now take into account the fact that the diffusion constant D_2 of free tubulin is much greater than D_1 leading to conditions of a virtually uniform distribution of free tubulin ψ under steady-state conditions with $\dot{\psi} = 0 = \nabla^2 \psi$ and any spatial patterns formed by the system will be those exhibited by microtubules via ϕ . Thus solving, (1.11) for ψ yields

$$\begin{aligned} \psi &= \frac{k_2 \phi}{k_3 - k_1 \phi} \\ &\cong \frac{k_2}{k_3} \phi \left(1 + \frac{k_1}{k_3} \phi + \dots \right), \end{aligned} \quad (1.12)$$

where we have expanded the denominator in a series assuming a sufficiently small concentration of microtubules to warrant such a procedure. Finally, we substitute (1.12) into (1.10) to obtain

$$\dot{\phi} = D_1 \nabla^2 \phi + k_2 \phi - \frac{k_1^2 k_2}{k_3^2} \phi^3 + \dots \quad (1.13)$$

The above equation is in the form of the so-called time-dependent Landau-Ginzburg equation ($TDLG$) which has played a prominent role in the development of the theory of phase transitions [20–22]. We will demonstrate in the remainder of

Figure 1. Plots of Equations (2.3) and (2.4) along with the experimental data points from Fygenon et al. (1994).

the paper that there is another connection to the *TDLG* equations given by both the phase diagram and the oscillations observed in the ensembles of microtubules.

It is appropriate to comment on the results of the mathematical analysis done for the *TDLG* in $(3 + 1)$ dimensions [23] which showed the existence of both concentric spherical waves and plane wave propagations with velocity v such that the *TDLG* in the latter case reduces to:

$$D_1\phi'' + v\phi' + k_2\phi - k_4\phi^3 = 0 \quad (1.14)$$

where

$$\xi = x - vt; \quad \phi' = \frac{d\phi}{d\xi} \quad \text{and} \quad k_4 = \frac{k_1^2 k_2}{k_3^2}. \quad (1.15)$$

We will obtain a similar equation for ϕ in a later section and show its relevance to spatial oscillations.

2. Landau-Ginzburg Free Energy Expansion

In this section we follow an entirely different course (but as we shall see later, ultimately equivalent mathematically) and treat the process of microtubule assembly as a first-order phase transition with the objective of shedding new light on this as yet incompletely understood non-equilibrium phenomenon. The parallels between inhomogeneous nucleation in a first order phase transition and that of microtubule nucleation and growth are sufficient for us to explore this possibility. We can directly equate the liquid phase with that of free tubulin. This is the regime where the solid phase (i.e. microtubules) is unstable. Similarly, the solid phase

Figure 2. Plots of the free energy potential as a function of the order parameter reflecting the three phase regions in our theory.

would correspond to free tubulin being the unstable solution of the equation of state and microtubules being stable. In this regime, we would see self-nucleation of microtubules just as in standard nucleation phenomena. Between these two phases is the co-existence region where both phases can exist under the same set of experimental conditions. In experimental situations, we can find ourselves in any of these three regions. When the buffer conditions are not correct, the temperature too low or perhaps the tubulin concentration insufficient, microtubules are unstable. This would correspond to the liquid phase. As conditions are altered so that microtubules can form, the system would enter the co-existence region. Once the tubulin concentration is raised beyond a certain threshold level, microtubules would begin to self-nucleate within the solution. This is the solid phase. Figure 1 shows a phase diagram constructed using data points from Fygenon et al. [24] which depicts these three regions of the phase diagram.

One preferred and time tested method of dealing with such a phase transition is using a phenomenological Landau-Ginzburg approach. In this case we would expand the free energy of the system undergoing a phase transition in terms of a suitable order parameter. Temperature is a natural choice for a control parameter in our system, but the concentration of tubulin will also control the phase dynamics. This parallels once again liquid-solid transitions where temperature and pressure (or alternatively density) control the phase of the system. From the phase diagram, we see that upon raising the temperature and/or the concentration of tubulin, we move from the state of free tubulin to the coexistence region. As for the order parameter, experiments tend to measure the turbidity which is proportional to the

amount of assembled tubulin, i.e. the total mass of protein contained in all microtubules formed. This will be our choice of an order parameter since it is zero in the disordered state (free tubulin) and non-zero in the ordered state (assembled tubulin). Since we have no symmetry requirements for the order parameter, we choose the expansion for the free energy as:

$$F(\phi) = A\phi^2 + B\phi^3 + C\phi^4, \quad (2.1)$$

where $A = A([Tu], T)$, $B < 0$, $C > 0$ and ϕ is the order parameter whose meaning is the local density of the protein such that assembled tubulin corresponds to a higher value of local density while free tubulin in solution corresponds to a lower density. For the parameter A in our expansion, we choose it to be a function of the tubulin concentration $c = [Tu]$ and the temperature T , both of which can be viewed as control parameters. The requirement that $B < 0$ is chosen arbitrarily simply so that the ordered phase corresponds to $\phi > 0$. What makes this formulation different from standard Landau phenomenology is that instead of approaching the ordered phase as we lower the control parameter (T for example), in our case we approach the ordered phase by raising either the temperature or the tubulin concentration. This point must be kept in mind when we choose a particular form for A . Taking the expression for the free energy given in Equation (2.1), we first solve the equilibrium condition $\frac{\partial F}{\partial \phi} = 0$ which gives us the non-zero solutions in terms of free energy coefficients as

$$\phi_{\pm} = \frac{-3B \pm \sqrt{9B^2 - 32AC}}{8C}. \quad (2.2)$$

In order for $\phi_{\pm}$ to be real roots, we require that $9B^2 - 32AC \geq 0$. The point at which this is equal to zero is exactly the point at which the solid phase (i.e. a stable population of microtubules) begins to appear. If we continue to raise the control parameter(s) (which in our formulation causes A to become smaller), we eventually reach the point $A = 0$ which defines the liquid-solid transition point. Beyond this point, the liquid (free tubulin) state is unstable (see Figure 2 for clarification).

As is customary for phenomenological approaches, we have several adjustable parameters in our free energy expansion. We can gain some information about the value of the expansion coefficients by looking at the experimental phase diagram in Figure 1. We first perform a fit to the data for the top curve (solid transition line). This line is characterized by the temperature-concentration scaling law given by $(T - T_c) \propto (c - c_c)^{-0.5}$ where we choose $T_c = 4$ °C and $c_c = 1.5$ μ M based on experimental data. This curve corresponds to the point where $A = 0$, therefore we postulate that

$$A = a[105 - (T - 4)(c - 1.5)^{0.5}] \quad a > 0, \quad (2.3)$$

where we have added a constant of proportionality a . Recall the requirement that we approach the ordered phase as we *raise* the control parameter(s). Thus we must choose the particular form given above for A . For small temperatures and/or

concentrations, $A > 0$. As we raise one or both of the control parameters, A approaches zero which is what we require. The bottom line in Figure 1 is the point where the coexistence region begins. As shown before, at this point $A = \frac{9B^2}{32C}$ which directly leads to

$$(T - 4) = \frac{105 - \frac{9B^2}{32aDC}}{(c - 1.5)^{0.5}}. \quad (2.4)$$

If we assume, as required in a Landau approach, that a , B and C be constants, we can best fit the data by choosing $\frac{9B^2}{32aC} \simeq 48$. Plots of both Equation (2.3) and Equation (2.4) are shown to approximate the experimental results reasonably well as is shown in Figure 1. It is possible that a better fit may be obtained by assuming that one or more of the coefficients depend on one or both of the control parameters, but this would needlessly complicate our calculations. Our model fits the data points from experiment sufficiently well and is based on a very modest set of adjustable parameters. Aside from the phase diagram, Landau-Ginzburg theory can make predictions for order parameter solutions which is our next step.

3. Mean Field Dynamics

We wish to extend our model free energy to include surface energy, due to the interface between the two phases (liquid-free tubulin and solid-assembled tubulin). This energy contribution is proportional to an interfacial energy density parameter D and basically represents the GTP energy stored in a lateral cap. We will treat our system as one-dimensional (along the protofilament axis) and thus our free energy expansion is now a functional of the form

$$F = \int dx \left[\frac{D}{2} (\nabla \phi)^2 + A\phi^2 + B\phi^3 + C\phi^4 \right]. \quad (3.1)$$

We minimize the above free energy using standard procedures [25] which gives rise to the equation of motion for the solid-liquid boundary in the form of the time-dependent Landau-Ginzburg equation

$$\gamma \frac{\partial \phi}{\partial t} = D(\nabla^2 \phi) - 2A\phi - 3B\phi^2 - 4C\phi^3, \quad (3.2)$$

where the ‘relaxation’ term $\gamma(\partial \phi / \partial t)$ accounts for the presence of energy dissipation in a microtubule which is well documented [26] and γ is a phenomenological relaxation coefficient. As mentioned earlier assembly-competent tubulin dimers must contain *GTP* molecules whose energy is higher than the hydrolyzed *GDP* product molecule when tubulin dimers disassemble from a microtubule. Therefore, quasi-periodic oscillations of microtubule growth and shrinkage can be seen as dissipative processes. The equation above represents the motion of the phase front, i.e. the leading edge of the microtubule growth region. The friction term is representative of the fact that the microtubule can only grow when there is both *GTP* and

tubulin around. The less tubulin that there is, the lower the microtubule growth rate. Also, as the tubulin becomes used up, the rate of growth will lower. Therefore, we assume that γ will depend inversely on the tubulin concentration. Although we are not directly considering the differences in the two nucleotides, *GDP* and *GTP*, as the assembly competent (*GTP* rich) tubulin becomes used up, there will be more *GDP*-tubulin in the solution and hence more effective ‘friction’ to the solid phase growth.

If we assume a traveling wave type solution to Equation (3.2), so that our order parameter has the functional dependence $\phi = \phi(\xi)$ where $\xi = x - vt$ is a moving coordinate, our equation of motion can be written as an ordinary differential equation

$$D_1\phi'' + v\phi' + k_2\phi + k_3\phi^2 - k_4\phi^3 = 0, \quad (3.3)$$

and the new coefficients are: $D_1 = D/\gamma$, $k_2 = -2A/\gamma$, $k_3 = -3B/\gamma$, $k_4 = 4C/\gamma$ where $' = \partial/\partial\xi$. The solutions to this nonlinear ordinary differential equation will give us our order parameter patterns in terms of the moving coordinate ξ . We have chosen the above parameter in such a way as to obtain direct correspondence with the reaction-diffusion result of Equation (1.14). The only difference here is the presence of a quadratic nonlinearity. However, it is possible to obtain such terms in the reaction-diffusion scheme by assuming, for example, that two tubulin dimers may form a microtubule nucleus.

Solving the equation of motion (3.3) is quite straightforward. The choice of initial conditions is really the key to the behavior. We know that the critical parameters are the concentration of seeds and the concentration of *GTP* [6]. Our order parameter represents the amount of assembled tubulin. The concentration of seeds for microtubule growth is essentially an amount of pre-assembled tubulin, therefore it is natural to choose $\phi(0)$ according to this criterion. The amount of available *GTP* is indicative of the potential growth and hence could be thought of as defining the initial derivative $\phi'(0)$. These choices are certainly open to question and revision, but as we shall see, they produce acceptable results. The coefficient of the first order derivative, i.e. the so-called ‘friction’ term, should depend on the tubulin concentration $c = [Tu]$. This is because the more tubulin there is, the higher the number of tubulin dimers that may encounter the end of the microtubule. With this in mind, we will assume that γ depends on c as $\gamma \propto c^{-1}$ so that low concentration means high ‘friction’ and vice versa.

In order to compare our results to those from experiments, we need to have similar conditions. Thus we will fix our temperature T at 37 °C and only work with one control parameter, $c = [Tu]$ the tubulin concentration. Comparing this with the phase diagram, we see that this gives us a critical concentration for microtubule growth of $c = 4.7 \mu\text{M}$ and a critical concentration of $c_c = 13 \mu\text{M}$ for microtubule self-nucleation. Using the initial conditions stated above, the equation of motion was solved numerically. The results are shown in Figure 3 for four different tubulin concentrations. The solutions that we acquire reveal a transition that occurs as we

Figure 3. The amount of assembled tubulin versus time for (a) $3 \mu\text{M}$, (b) $6 \mu\text{M}$, (c) $15 \mu\text{M}$ and (d) $25 \mu\text{M}$ tubulin concentration. All simulations performed at 37°C .

raise the tubulin concentration, a condition clearly seen in experiment [14]. As the tubulin concentration is increased, the system moves from a region of no assembly to monotonic growth and then from growth to oscillations.

4. Discussion

The first new feature we see in this model is that the region of self-nucleation corresponds to a very high degree to the region where we see oscillations. This should not be too surprising since self-nucleation is a required condition for microtubule oscillations to occur [6]. In comparing these solutions with the oscillations seen in experiments we find several similarities. First, the tubulin concentrations at which growth begins and oscillations occur are in agreement with experiment. The exact points at which these transitions occur depends strongly on additional factors such as Mg^{2+} concentration, but the basic behavior is correct. In this sense, we can only provide meaningful comparisons for the order of magnitude since there are many additional factors that we have not included in this phenomenological theory. Second, as we raise the tubulin concentration, the steady-state value that

Figure 4. Specific heat predicted by our model as a function of temperature and tubulin concentration.

the system works towards increases. Also, as we increase either T or $c = [Tu]$, the size and extent of oscillations increases. Lastly, we have treated the system using a very simple approach and have been able to reproduce both a phase diagram for assembly and nucleation as well as microtubule oscillations. Due to the simplicity of our free energy expansion in Equation (3.1), we can make a prediction for the specific heat behavior in this system. We would expect to see thermal hysteresis, and the extent of hysteresis should decrease with increasing tubulin concentration (see Figure 4). This effect should be observable experimentally and could further validate our treatment as a first-order phase transition.

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References

1. Mitchison, T. and Kirshner, M.: *Nature* **312** (1984), 232.
2. Mitchison, T. and Kirshner, M.: *Nature* **312** (1984), 237.
3. Pirollet, F., Job, D., Margolis, R. and Garel, J.: *EMBO J.* **6** (1987), 3247.
4. Melki, R., Carlier, M.-F. and Pantaloni, D.: *EMBO J.* **7** (1988), 2653.
5. Carlier, M., et al.: *Proc. Natl. Acad. Sci. USA* **84** (1987), 5257.

6. Obermann, H., Mandelkow, E.-M., Lange, G. and Mandelkow, E.: *J. Biol. Chem.* **265** (1990), 4382.
7. Hill, T. and Chen, Y.: *Proc. Natl. Acad. Sci. USA* **81** (1984), 5772.
8. Chen, Y. and Hill, T.: *Proc. Natl. Acad. Sci. USA* **82** (1985), 1131.
9. Martin, S., Schilstra, M. and Bayley, P.: *Biochim. et Biophys. Acta* **1073** (1991), 555.
10. Martin, S., Schilstra, M. and Bayley, P.: *Biophys. J.* **65** (1993), 578.
11. Gliksman, N., Skibbens, R. and Salmon, E.: *Mol. Biol. Cell* **4** (1993), 1035.
12. Flyvbjerg, H., Holy, T. and Leibler, S.: *Phys. Rev. E* **54** (1996), 5538.
13. Chen, Y. and Hill, T.: *Proc. Natl. Acad. Sci. USA* **84** (1987), 8419.
14. Marx, A. and Mandelkow, E.: *Eur. Biophys. J.* **22** (1994), 405.
15. Houchmandzadeh, B. and Vallade, M.: *Phys. Rev. E* **53** (1996), 6320.
16. Jobs, E., Wolf, D. and Flyvbjerg, H.: *Phys. Rev. E* **79** (1997), 519.
17. Mandelkow, E., et al.: *Science* **246** (1989), 1291.
18. Sept, D., Limbach, H., Bolterauer, H. and Tuszynski, J.A.: *J. Theor. Biol.* **197** (1999), 77.
19. Salmon, E., et al.: *J. Cell Biol.* **99** (1984), 2157.
20. Landau, L.D. and Lifshitz, E.M.: Statistical physics, Pergamon, London, 1980.
21. Gunton, J.D. and Droz, M.: Introduction to the theory of metastable and unstable states, Springer, Berlin, 1983.
22. Metiu, H., Kitahara, K. and Ross, J.: *J. Chem. Phys.* **64** (1976), 292.
23. Skierski, M., Grundland, A.M. and Tuszynski, J.A.: *J. Phys. A* **22** (1989), 3789.
24. Fyngenson, D., Braun, E. and Libchaber, A.: *Phys. Rev. D* **50** (1994), 1579.
25. Ma, S.: Modern theory of critical phenomena, Benjamin, New York, 1976.
26. Erickson, H. and O'Brien, E.: *Ann. Rev. Biophys. Biomol. Struct.* **21** (1992), 145.