

Biophysical principles governing spatiotemporal regulation of compartmentalization in cells

By

ANIRUDDHA N

LIFE10202004001

Institute of Mathematical Sciences, Chennai

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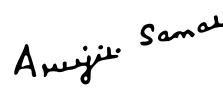
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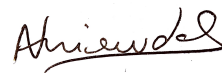
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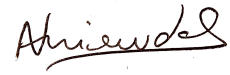
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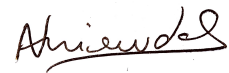
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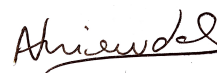
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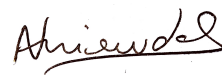


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4. Attended workshop on “[Spins, Games & Networks: Understanding collective coordination in complex systems \(SGN\)](#)” held at the Institute of Mathematical Sciences (IMSc), Chennai, from December 11-24, 2024.
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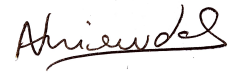
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Aniruddha N

Contents

Abstract	xiv
List of Figures	xxv
List of Tables	xxvi
1 Introduction	1
1.1 Membraneless organelles	2
1.1.1 Tau interaction with microtubules	4
1.2 Membrane-bound organelles	6
1.2.1 Regulation of organelle abundance	7
1.3 Thesis organization	9
2 Liquid-liquid phase separation and wetting phenomena	11
2.1 Theory of phase separation in bulk	11
2.2 Wetting phenomena: Cahn’s graphical theory	14
2.3 Lattice-gas model for phase separation and wetting	18
2.3.1 Phase separation in the bulk	19
2.3.2 Wetting in the presence of a surface	22
2.4 Summary	25
3 Surface-driven condensation of tau on microtubules	26
3.1 Introduction	26

3.1.1	Biology of microtubules and tau proteins	26
3.1.2	Experimental observations	29
3.2	Lattice-gas model for tau condensation on microtubules	31
3.2.1	Model overview	31
3.2.2	Simulation scheme	32
3.3	Results	33
3.3.1	Tau envelopes are surface condensates formed via a prewetting-like transition	33
3.3.2	Microtubule surface heterogeneity dictates spatial pattern of tau condensation	35
3.3.3	Multilayered nature of tau condensates gives rise to two kinetically distinct populations of tau	41
3.3.4	Tubulin partitions into microtubule-condensed tau promoting microtubule growth	44
3.4	Discussion	46
	Appendix	49
3.A	Characterizing phase separation in the bulk and on the surface	49
3.A.1	Identification of the bulk coexistence region	49
3.A.2	Excess density and the fraction of microtubule length covered captures the extent of tau condensation	51
3.B	Parameter selection	52
3.C	An exploration of the model parameters	53
3.C.1	Homogeneous model	53
3.C.2	Heterogeneous model	56
3.D	Previously proposed 1D model captures features of tau condensation on microtubules	58
4	Temporal regulation of organelle biogenesis	61

4.1	Model and methods	61
4.2	Results	65
4.2.1	Mechanisms of organelle biogenesis govern the transient cell-to-cell variability	65
4.2.2	Effect of different mechanisms of organelle biogenesis on the first pas- sage times	73
4.3	Discussion	75
	Appendix	80
4.A	Details of calculation for the moments	80
4.A.1	Numerical solutions using the truncated master equation	80
4.A.2	Exact time-dependent solutions for mean and variance of organelle copy number distribution	82
4.B	Moments of the first passage time distribution	92
4.C	A note on the initial conditions	97
4.D	Supplementary figures	98
5	Conclusions and Outlook	107
5.1	Summary	107
5.2	Directions for future work	109
	Bibliography	112

Abstract

Compartmentalization is a fundamental process for spatiotemporal organization in biology. Compartments provide various advantages, such as creating distinct microenvironments for biochemical reactions, enabling localization of biomolecules, and allowing multiple processes to be carried out simultaneously within the cell. Cells achieve compartmentalization in two ways: i) via membraneless organelles, or biomolecular condensates, and ii) via membrane-bound organelles. In this thesis, we aim to understand the physical principles governing compartmentalization in cells, for both membraneless and membrane-bound organelles.

Proteins can form membraneless compartments called biomolecular condensates that can aid various cellular processes, such as stress response and transcriptional regulation. These proteins also interact with intracellular surfaces like membranes and cytoskeletal filaments, yet the physical principles governing these interactions remain poorly characterized. In this thesis, we study how tau, a microtubule-associated protein, interacts with the microtubule surface. Tau, which is implicated in neurodegenerative disorders, forms condensates in solution at micromolar concentrations. However, recent experiments show that at much lower concentrations, in the nanomolar range, tau forms dense, cohesive patches on microtubules. These patches, previously referred to as envelopes, are distributed non-uniformly along the microtubules and form in a concentration-dependent manner. To shed light on these observations, we employ a lattice-gas framework, incorporating tau-tau and tau-microtubule interactions. We show that tau envelopes are multilayered condensates formed via a prewetting-like transition. The spatial patterns of tau condensates are dictated by inhomogeneities on the microtubule surface, likely arising from post-translational modifications. We demonstrate that the multilayered nature of the condensates can give rise to kinetically distinct populations of tau, with different functional capabilities. In particular, the innermost layer can restrict the access of other proteins, such as severing enzymes, to the microtubule, and the outer layers can recruit tau-interacting molecules like soluble tubulin. Our results thus show that tau forms surface-induced condensates on mi-

crotubules at physiological concentrations, thereby providing additional regulatory control over microtubule-based functional pathways. Our theoretical framework can be generalized to study phase-separating proteins interacting with other surfaces, such as DNA and membranes.

Compartmentalization in cells is also achieved via various membrane-bound organelles, such as nuclei, mitochondria and lysosomes, which carry out specialized functions. The abundance of these organelles in cells is tightly regulated in response to external stimuli, but the underlying mechanisms remain elusive. Time-lapse imaging of fluorescently labelled organelles enables single-cell measurements of organelle copy numbers, revealing the time evolution of their distribution across a cell population. In this thesis, we aim to provide a theoretical framework that can leverage these measurements to discern the regulatory mechanisms. Building on a recently proposed kinetic model of organelle biogenesis, which incorporates de novo synthesis, fission, fusion, and degradation, we explore the time-dependent dynamics of organelle abundance. While previous studies focused on steady-state properties, here we calculate the first two moments of: i) organelle copy numbers over time, and ii) first passage times to reach a specified organelle count. We show that these two moments provide a powerful means to discriminate between different mechanisms of organelle biogenesis. Notably, the time-dependent behaviour of organelle biogenesis reveals richer dynamics compared to the steady-state scenario. Our findings shed light on how cells attain steady-state organelle abundance after cell division and environmental perturbation, which can be used to elucidate the underlying mechanisms regulating organelle abundances.

To summarize, in this thesis, we explored two distinct physical routes by which intracellular organization, via compartmentalization, can emerge. First, we showed that surface-induced condensation of tau can give rise to a functionally diverse microenvironment on microtubules, even below the bulk saturation concentration. Second, we investigated the regulation of membrane-bound organelles, providing a theoretical framework to elucidate mechanisms of organelle abundance control using time-lapse measurements. Together, these studies provide a physical basis for understanding the formation and regulation of diverse intracellular compartments.

List of Figures

1.1	Examples of biomolecular condensates in cells. The figure indicates their positions within cells, including some functions and material properties. Reprinted by permission from Ref.[12]. Copyright © 2019 Springer Nature America, Inc.	3
1.2	Examples of membrane-bound organelles in eukaryotic cells. Image adapted from Wikimedia Commons, licensed under Creative Commons Attribution 4.0 International (CC BY 4.0)	7
2.1	The Flory-Huggins free energy (Eq.2.1) for various values of χ . For the symmetric molecular volume, instability arises for $\chi > 2$.	13
2.2	Phase diagram showing the region of coexistence (coloured area) for a two-component mixture.	14
2.3	Partial wetting	15
2.4	Complete wetting	15
2.5	Wetting transition. Left: The surface free energy can be obtained by the area between the curves described in Eq.2.3. Right: Concentration profiles for complete wetting and partial wetting states.	17
2.6	Prewetting transition. Left: Even in the undersaturated regime, the two curves in Eq.2.4 can intersect at three points, resulting in a prewetting transition. Right: Concentration profiles for a thin, adsorbed layer and a thick, condensed layer.	18
2.7	Phase diagram showing wetting and prewetting transitions. The colored area represents the bulk coexistence region. Note that the prewetting line extends outside of this region, into the undersaturated regime.	18

2.8	A) Schematic for the lattice-gas model. B) Simulation snapshot of droplet formation in the lattice-gas model (parameters: $Jb = 0.3 k_B T$, $\phi = 0.2$)	19
2.9	Representative phase diagram for the lattice-gas system in the NVT ensemble. The region enclosed by the red line indicates the coexistence regime.	20
2.10	A) Phase diagram for a lattice-gas system in the grand-canonical ensemble. The transition between high and low volume fractions becomes sharp for $J_b > J_b^c$ B) The same diagram represented on the $J_b - \mu$ plane shows the criteria for coexistence.)	21
2.11	Change of state variables maps the lattice-gas system phase diagram onto the familiar Ising model phase diagram (for each J_b a different range of μ has been chosen, such that after the transformation $\frac{1}{4}(2\mu + qJ_b)$ they all lie within the same range.)	22
2.12	Lattice-gas model with a solid surface present.	23
2.13	Simulation snapshots showing a dense, finite layer forming on the surface arising from a very small increase in the bulk chemical potential (parameters: $J_b = 1.2 k_B T$, $J_s = 4.2 k_B T$, $h = -5.0 k_B T$)	24
2.14	A-B) The surface excess density and fraction of sites occupied at the surface show a sharp increase as a function of the bulk chemical potential, indicating a prewetting transition. The dotted line indicates the bulk coexistence chemical potential. In the thermodynamic limit, the excess density diverges as this line is approached. (parameters: system size $L = 50$ sites, $J_b = 1.2 k_B T$, $J_s = 4.2 k_B T$, $h = -5.0 k_B T$)	24
3.1	Microtubule structure. Reprinted by permission from Ref.[109]. Copyright © Annual Reviews	27
3.2	Tau sequence showing four repeats in the microtubule-binding domain. Reprinted by permission from Ref.[117] Copyright © 2018, The American Association for the Advancement of Science	28

3.3	Experimental observations showing A) tau interaction with three kinds of microtubules (taxol-stabilized, GDP-tubulin and GMPCPP-tubulin) at various concentrations of tau. B) Kinetics of tau covering various microtubules. C-E) Quantification of tau densities for snapshots is shown in A. (Unpublished results, included with permission from collaborators [96]).	30
3.4	Schematic of lattice-gas model for tau interacting with a microtubule.	31
3.5	Simulation results for a homogeneous surface show distinct states of tau at different concentrations.	33
3.6	The homogeneous model (right) shows a bimodal distribution of tau density, in agreement with experimental observations (left). This implies coexistence between two states, which we identify as adsorbed and condensed states. . . .	34
3.7	The homogeneous model (centre) cannot produce the same positions of the condensates across independent simulations. However, the heterogeneous model (right) can recapitulate this experimental observation (left). The experiments consisted of allowing the tau condensates to form, and then washing away the tau. This was done in a repeated manner.	37
3.8	The heterogeneous model (right) can capture the kinetics of condensate growth along the microtubule in good agreement with the experiment (left). Green stars show nucleation of the condensates, blue arrows show fusion events, and red arrows show fission events.	38
3.9	The heterogeneous model can explain the equilibrium behaviour of tau on taxol-microtubules for a wide range of concentrations. The inset in the left plot is a simple Hill function $\frac{Ac^n}{k^n + c^n}$, with $A = 0.337$, $n = 7$, $k = 1.137nM$	38
3.10	A-C) The heterogeneous model shows good agreement with the experimental observations for different microtubule types over a wide range of concentrations. D-F) shows the quantification for A-C	39
3.11	The kinetics of tau binding to different kinds of microtubules are captured by the heterogeneous model (A-GDP, B-taxol and C-GMPCPP microtubules). . .	40

3.12	Heterogeneities in tau-binding affinity can arise due to PTMs. These, in turn, dictate the condensate positions.	41
3.13	Our model predicts two timescales of tau dissociation (left). This arises because the tau molecules are bound more strongly to the microtubule surface than to each other in the bulk. Experimental observations confirm this prediction (right).	42
3.14	A) The model predicts that with increasing tau concentration in the solution, the number of tau molecules in the slow population remains constant, while the fast population density increases. B) Experimental confirmation of model predictions. C) Schematic representation of model prediction.	42
3.15	Timescales of tau condensate dissociation for GDP and GMPCPP microtubules. A) Our model predicts that the fast timescale should remain unaffected by the surface properties, since it arises only due to tau-tau interactions. B) Experimental results confirm this, showing the same fast timescale for GDP- and GMPCPP microtubules. C) Conversely, the model predicts that the slow timescale should be the same, independent of tau concentration in the solution. D) Experiments show identical slow timescales for GDP-microtubules with condensates formed at different tau concentrations.	44
3.16	Experiments show that tau condensates (cyan) can incorporate tubulin monomers (red) only when they are formed above some threshold concentration (right). . .	45
3.17	Identifying the bulk coexistence curve for the model. A) Shows the representative binodal region obtained by simulation in the absence of a surface, in the NVT ensemble. The critical bulk coupling is at around $0.18k_B T$. B) The peak in specific heat corroborates this (lattice size is $40 \times 40 \times 40$).	51
3.18	A) Kinetics of droplet growth agree with the Sylozov-Lifshitz law. B) The bulk phase diagram in the μVT ensemble.	52

3.19	Homogeneous model. (A) Kymograph showing tau condensation on a homogeneous microtubule. The condensates are not pinned to their location. (horizontal scale bar is $1\mu\text{m}$ and vertical is 5s) (B) The kinetics of the condensate growing on the microtubule show the size increasing linearly with time. (C) Coexistence of the adsorbed state and the condensed state. Intensity distribution, taken from 100 simulations, shows a bimodal shape. (D) The green shows adsorbed tau, red shows condensed tau and purple shows bulk tau droplet. (E) The excess density (orange) and the fraction of microtubule surface covered by condensate (blue) show a sharp jump with increasing bulk chemical potential. $\Delta\mu = 0$ corresponds to bulk phase separation, indicated by a dashed line. (F) Schematic of the phase diagram showing the wetting and prewetting transition, originally proposed by Cahn [64].	54
3.20	An exploration of the behaviour shown by the homogeneous model for various parameter values.	55
3.21	A) The heterogeneous model shows that the tau condensates are spatially pinned. B) Introducing heterogeneity results in a more gradual rise in the excess density and surface coverage, as compared to the homogeneous model (Fig. 3.19). C) Similar to the homogeneous model, the heterogeneous model also exhibits a bimodal distribution of densities.	56
3.22	A) Effect of the heterogeneity parameters ($\bar{h}$ and σ) on tau condensation. B) An exploration of the behaviour shown by the heterogeneous model for various parameter values. The coexistence region gets broader due to the introduction of heterogeneities in the binding affinity.	57

3.23	One-dimensional model for surface condensation. (A) Coarse-graining the 3D model gives us the 1D model of condensation of tau proteins on microtubules. (B) Both homogeneous and heterogeneous models can explain the equilibrium data (homogeneous model, red: $J = 2.3 k_B T$, $\alpha = 0.031 k_B T$, $c_0 = 1.382 nM$; heterogeneous model, black: $J = 4.5 k_B T$, $\alpha = 0.52 k_B T$, $c_0 = 1.43 nM$). (C) The homogeneous model cannot give rise to pinned islands ($A = 100.0$, $\delta = 0.1$). (D) Heterogeneous model gives rise to stable, pinned islands (h_i drawn from $\text{Normal}(0.0, 1.4)$, $A = 500.0$, $\delta = 0.001$).	59
4.1	Model of organelle biogenesis. (A) We consider four processes that together govern organelle abundance in cells; these processes are de novo synthesis (k_d), decay (γ), fission (k_{fis}), and fusion (k_{fus}). (B) Live-cell imaging allows for the monitoring of organelle dynamics within a cell. Here we show a graphical representation of time-lapse snapshots of fluorescently labelled organelle copies in yeast. The bottom panel shows the time evolution of the histogram of organelle abundance across an isogenic cell population.	62
4.2	Mean and Fano factor profiles for various models. (A) De novo synthesis-decay: The mean number of organelles for various values of de novo synthesis and decay rate is shown. (B) De novo synthesis-decay: Here, we plot the time evolution of the Fano factor for various values of k_d , with $\gamma = 1 \text{ min}^{-1}$. (C) Fission-fusion: Dependence of the Fano factor keeping the ratio of parameters constant, $k_{\text{fis}}/k_{\text{fus}} = 20$, and (D) Fano factor profiles for various values of fission rate k_{fis} and the fusion rate k_{fus}	66

4.3	Time evolution of noise profiles for various models, with the initial number of organelles following a binomial distribution. (A) De novo synthesis-fission-decay: In this plot, we vary the fission rate k_{fis} , while keeping the other rates constant; $k_d = 0.4 \text{ min}^{-1}$, $\gamma = 1.0 \text{ min}^{-1}$. (B) De novo synthesis-fission-decay: Fano factor profiles for different values of the de novo synthesis rate k_d , keeping $\gamma = 1 \text{ min}^{-1}$ and $k_{\text{fis}} = 0.8 \text{ min}^{-1}$. (C) De novo synthesis-fusion-decay: Here, we plot the Fano factor as a function of time for various values of the decay rate γ , with $k_d = 5 \text{ min}^{-1}$, $k_{\text{fus}} = 0.1 \text{ min}^{-1}$, and in (D) we vary the fusion rate k_{fus} , with $k_d = 5 \text{ min}^{-1}$ and $\gamma = 0.2 \text{ min}^{-1}$	70
4.4	De novo synthesis-fission-fusion-decay. (A) In this plot, we vary the fission rate k_{fis} and look at the time evolution of the Fano factor, with $k_d = 5.0$, $\gamma = 0.9 \text{ min}^{-1}$, $k_{\text{fus}} = 1.0 \text{ min}^{-1}$. The curve for $k_{\text{fis}} = 0$ (blue) corresponds to the de novo-fusion-decay. (B) Here, we vary the fusion rate k_{fus} , while keeping $k_d = 0.7 \text{ min}^{-1}$, $k_{\text{fis}} = 0.8 \text{ min}^{-1}$, $\gamma = 1 \text{ min}^{-1}$. The other values are the same. The curve for $k_{\text{fus}} = 0$ (blue) is included for comparison with the de novo synthesis-fission-decay model.	72
4.5	Mean first passage times. Here, we plot the mean first passage times as a function of the target number X . In each plot, the parameters are varied while keeping the ratio of rates (hence the mean steady state number) constant. (For B. $k_{\text{fis}}/k_{\text{fus}} = 10.0$)	74
4.6	Coefficient of variation. Here, we plot the CV of the FPT distribution as a function of the target site X . The CV depends only on the ratio of the rates, and hence will be identical for similar processes with the same steady-state levels. (Parameters used for A. $\gamma = 1.0 \text{ min}^{-1}$ and B. $k_d = 10.0 \text{ min}^{-1}$, $\gamma = 1.0 \text{ min}^{-1}$)	75

4.7	Comparison with stochastic simulations. We test our analytical and numerical results by comparing them with stochastic simulation using the Gillespie algorithm. For each parameter set, 10^6 trajectories are simulated and the average quantities computed from them. (A) Mean organelle copy number for various processes as a function of time. (B) Fano factor as a function of time, computed across all the trajectories. (The initial conditions are taken from the binomial distribution (same as in Fig.2-4). C) Mean first passage time for various processes to reach a certain target organelle copy number. (D) CV of the FPT to reach a certain organelle copy number. The initial condition is taken as zero for all processes except fission-fusion, where the initial copy number is taken as one. The parameters not specified are set to zero.	99
4.8	De-Novo Decay: (A) The evolution of the mean with time for different initial distributions, $N = 30, 20, 10, p = 0.5$. (B) The evolution of the mean starting from binomial distributions with mean = 5 and different variances, the trajectories overlap. (C) Evolution of the fano factor with time for different initial distributions as in (A). (D) The evolution of the fano factors with different initial distributions having the same initial mean, mean = 5, initial Fano = 0.5, 0.75, 0.875. $k_d = 7.0 \text{ min}^{-1}, \gamma = 1.0 \text{ min}^{-1}$ for all trajectories	100
4.9	Fission - Fusion: (A) Evolution of mean when initial distributions of different means are chosen (blue = 5, red = 10, green = 15). (B) Evolution of the mean when different initial distributions with the same mean (5) but different Fano factors are taken (blue = 0.5, red = 0.75, green = 0.875). The trajectories overlap. (C) Evolution of the Fano factor when initial distributions with different means are taken as in (A). (D) Evolution of the Fano factor when different initial distributions are taken as in (B). $k_{\text{fis}} = 1.0 \text{ min}^{-1}, k_{\text{fus}} = 0.05 \text{ min}^{-1}$ for all trajectories.	101

4.10 De novo - fission-decay (A) Evolution of mean when initial distributions of different means are chosen (blue = 4, red = 10, green = 15). (B) Evolution of the mean when different initial distributions with the same mean (10) but different Fano factors are taken (blue = 0.5, red = 0.75, green = 0.875). The trajectories overlap. (C) Evolution of the Fano factor when initial distributions with different means are taken as in (A). (D) Evolution of the Fano factor when different initial distributions are taken as in (B). $k_{\text{fis}} = 8 \text{ min}^{-1}$, $\gamma = 10 \text{ min}^{-1}$, $k_d = 8 \text{ min}^{-1}$ for all trajectories.	102
4.11 De novo-fusion-decay: (A) Evolution of the mean when initial distributions with the same mean (5) but different Fano noises are taken. $k_{\text{fus}} = 1.0 \text{ min}^{-1}$, $\gamma = 1.0 \text{ min}^{-1}$, $K_d = 5.0 \text{ min}^{-1}$. (B),(C) Evolution of the mean when initial distributions with different means are taken. (B): $k_{\text{fus}} = 0.01 \text{ min}^{-1}$, $\gamma = 0.2 \text{ min}^{-1}$, $K_d = 5.0 \text{ min}^{-1}$, (C): $k_{\text{fus}} = 1.0 \text{ min}^{-1}$, $\gamma = 1.0 \text{ min}^{-1}$, $K_d = 5.0 \text{ min}^{-1}$. (D) Evolution of the Fano factor when distributions and parameters are taken as in (A). (E), (F) Evolution of the fano factor when the initial distributions and parameters are taken as in (B) and (C), respectively.	103
4.12 De novo - fission - fusion - decay: (A) Evolution of mean when initial distributions of different means are chosen (blue = 2, red = 5, green = 10). (B) Evolution of the mean when different initial distributions with the same mean (5) but different Fano factors are taken (blue = 0.5, red = 0.75, green = 0.875). The trajectories overlap. (C) Evolution of the Fano factor when initial distributions with different means are taken as in (A). (D) Evolution of the Fano factor when different initial distributions are taken as in (B). $k_{\text{fus}} = 1.0 \text{ min}^{-1}$, $k_{\text{fis}} = 10 \text{ min}^{-1}$, $\gamma = 0.9 \text{ min}^{-1}$, $K_d = 5.0 \text{ min}^{-1}$	104

4.13	Asynchronous cell division: we take an initial distribution where f_{ss} fraction of cells follow the associated steady-state distribution and the remaining $(1 - f_{ss})$ fraction of cells have just divided and follow binomial statistics (A) Evolution of mean for de novo synthesis-decay process ($k_d = 10.0 \text{ min}^{-1}$, $\gamma = 1.0 \text{ min}^{-1}$). (B) Evolution of the Fano factor for the same process. The initial Fano factor is higher than one due to the high heterogeneity coming from a combination of two different distributions. (C) Evolution of the mean for fission-fusion process ($k_{fis} = 1.0 \text{ min}^{-1}$, $k_{fus} = 1.0 \text{ min}^{-1}$) (D) Fano factor for the same process. . . .	105
4.14	Asynchronous cell division: we take an initial distribution where f_{ss} fraction of cells follow the Poisson distribution (steady-state) for de novo synthesis-decay and the remaining $(1 - f_{ss})$ fraction of cells have just divided and follow binomial statistics (A) Mean FPT for de novo synthesis-decay process. (B) CV as a function of the target copy number. The CV goes above one here (unlike in the main text due to the difference in initial conditions). ($k_d = 10.0 \text{ min}^{-1}$, $\gamma = 1.0 \text{ min}^{-1}$)	106

List of Tables

3.1	Simulation parameters for the figures in the Results sections. Other common parameters are mentioned in Table 3.3.	34
3.2	Simulation parameters for the figures in the Appendices. Other common parameters are mentioned in Table 4.1.	50
3.3	Parameter values used. (Units are $k_B T$ unless mentioned)	53
4.1	Range of parameters explored. The minimum values indicate the smallest non-zero values used. In processes where a particular mechanism is absent, that rate constant is simply zero.	65

Chapter 1

Introduction

Compartmentalization is ubiquitous in biology. In fact, it can be argued that the ability to form compartments is a prerequisite for life itself to arise. Oparin and Haldane, in their “primordial soup” hypothesis for the emergence of life, proposed that simple organic molecules formed in prebiotic conditions could have spontaneously assembled to form micro-reaction centres [1, 2]. Such assemblies, called coacervates, have been suggested to be the first, primitive forms of cells [3]. Beyond early life, compartmentalization provides indispensable advantages in biology, some of which are listed below:

- They can serve as centres of biochemical reactions by providing a distinct microenvironment from the surrounding medium, for example, by maintaining a different pH or concentration of biomolecules.
- They enable spatiotemporal control of biomolecules, allowing cells to localize molecules to specific locations at required times.
- They allow cells to perform multiple processes simultaneously, especially through specialized compartments having distinct functional capabilities.

Historically, eukaryotic cells were thought to achieve compartmentalization through specialized membrane-bound compartments called organelles [4]. However, in recent years, an alternative paradigm has emerged, in which cells can organize their internal environments through

membraneless compartments, also known as biomolecular condensates [5, 6].

How cells regulate their internal organization is not properly understood. Broadly, in this thesis, we aim to elucidate the physical principles governing the spatiotemporal regulation of compartmentalization inside cells, for both membraneless and membrane-bound organelles.

1.1 Membraneless organelles

Cells can organize the internal environments via membraneless compartments, also known as biomolecular condensates [5, 6]. These are dynamic assemblies of proteins and/or RNA that form in a non-stoichiometric manner and lack a surrounding membrane [7]. They are found in both the cytoplasm and the nucleus, with well-known examples of condensates including P-bodies, nucleoli, stress granules, and Cajal bodies (see Fig.1.1) [5–7]. Since condensates lack a membrane and form via weak, multivalent interactions, they are simpler to assemble than membrane-bound compartments [8, 9]. Therefore, it has been suggested that these membraneless assemblies may also have served as the primitive precursors to cells, acting as microreactor sites [3, 10]. Although such assemblies have been observed for a long time (Santiago Ramón y Cajal first reported observing Cajal bodies in 1903 [11]), their prevalence and significance have come to the forefront only in the past decade or so [5].

Biomolecular condensates can perform a wide range of functions[6], such as: 1) modulating the rates of biochemical reactions by locally increasing the concentration of molecules or sequestering them [13]; 2) spatiotemporally regulating various biochemical processes, for example, transcriptional condensates locally enhance the concentration of transcriptional machinery at the promoter region [14, 15], or P-bodies store untranslated mRNAs to prevent their degradation [16]; 3) buffering stochastic fluctuations in the intracellular concentrations of biomolecules [17], and 4) sensing environmental stresses by switching between one-phase and two-phase states, exemplified by stress granules [18]. A more extensive list of various functions carried out by condensates can be found in the recent literature [5, 6].

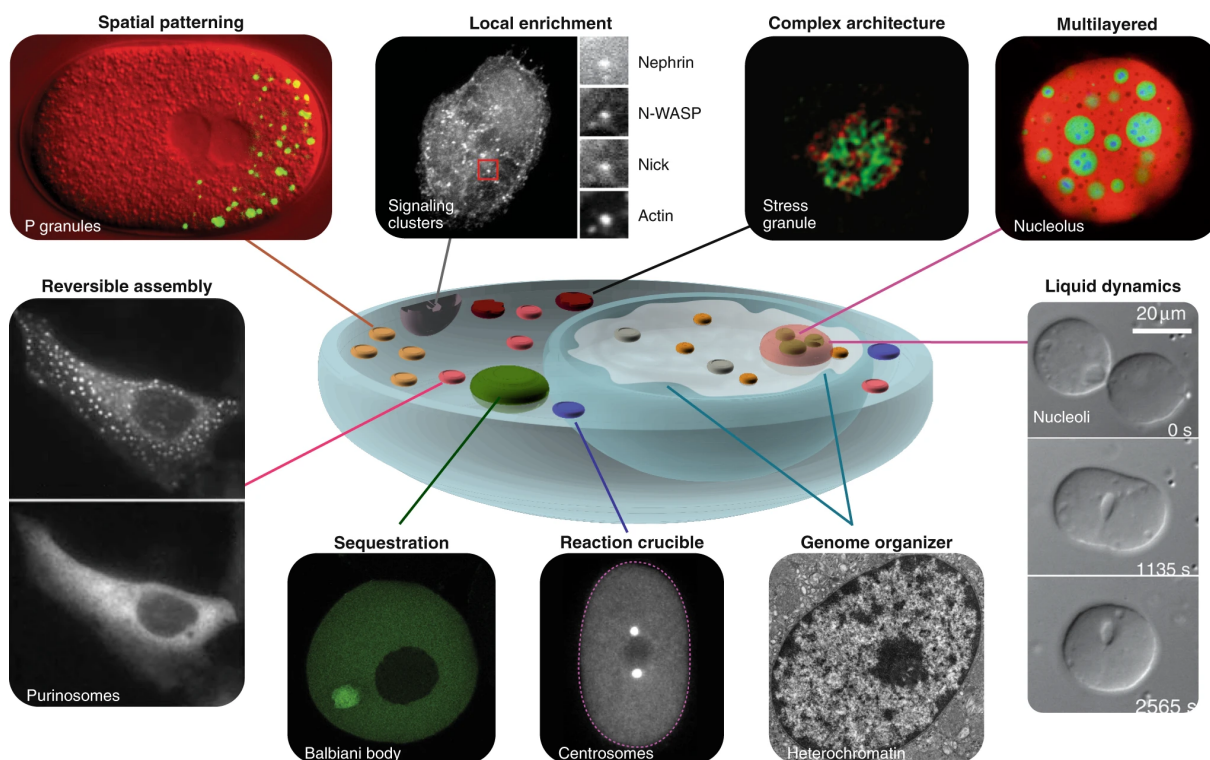


Figure 1.1: Examples of biomolecular condensates in cells. The figure indicates their positions within cells, including some functions and material properties. Reprinted by permission from Ref.[12]. Copyright © 2019 Springer Nature America, Inc.

Condensates often show liquid-like behaviour [19]. These include having spherical, droplet-like morphologies [20], showing properties such as surface tension and viscosity [21, 22], undergoing coalescence and fusion [20], and exchanging molecules with the surrounding medium [23, 24]. Due to these reasons, condensates are often referred to as liquid-like droplets [20], and their formation is primarily attributed to liquid-liquid phase separation (LLPS) [9]. LLPS is a process in which molecules with multivalent interactions overcome entropic mixing to form coexisting dense and dilute phases [25, 26]. Intrinsically disordered proteins (IDPs), which lack well-defined higher-order structures, are more prone to form condensates, driven by non-specific and weak multivalent interactions [27, 28]. We provide a detailed description of phase separation in Chapter 2.

While LLPS acts as a useful introduction to understand the formation of biomolecular condensates, the extent to which it plays a role, especially *in vivo*, remains under discussion [29]. Condensates inside cells differ from simple phase separation in various ways [30, 31]. First, condensates may not necessarily behave like a simple liquid, often showing a wide range of

material states from viscoelastic gel to even solid-like [32, 33]. Second, cellular processes are highly active in nature, meaning that they stay out of equilibrium by constantly consuming energy (for example, by hydrolysing ATP) [31]. Third, the copy number of proteins inside cells may be quite low. Therefore, phase separation, which is a thermodynamic description, may not be best suited. Fourth, intracellular environments are highly multicomponent and crowded [34]. Condensates can interact with many kinds of molecules. Additionally, various intracellular surfaces are present in cells, which can also affect condensates [35].

Biomolecular condensates interact with various intracellular surfaces such as lipid membranes [36–42], DNA [15, 43–46], and cytoskeletal filaments [47–50]. Such an interplay between condensates and intracellular surfaces can result in various functional capabilities for cells, such as increasing dwell times of molecules on membranes [6], nucleating polymerization [50], and organizing the genome [46], to name a few. While several theoretical models have successfully explained specific aspects of surface condensation [48, 49, 51–53], it still remains an emerging field, requiring further comparisons between experiment and theory.

In this thesis, we develop a theoretical understanding of tau proteins interacting with microtubules and provide a general framework for describing phase separation of proteins on intracellular surfaces, such as lipid membranes or DNA.

1.1.1 Tau interaction with microtubules

Microtubules are stiff, cylindrical polymers essential for diverse cellular functions, such as maintaining cell shape, facilitating intracellular transport, and organizing the mitotic spindle during cell division [4]. In neurons, microtubules act as polarized tracks, enabling long-distance organelle and vesicle transport [54]. Their functions are regulated by a variety of microtubule-associated proteins, in particular, neuron-specific protein tau [55]. Dysregulation of tau is involved in severe neurodegenerative pathologies, collectively termed tauopathies [56, 57]. The hallmark of tauopathies is the displacement of tau from microtubules and subsequent formation of insoluble aggregates via tau-tau interactions [56]. Notably, at micromolar concentrations, self-association between tau molecules can induce liquid-liquid phase separation, leading to the

formation of tau droplets, which can also recruit specific proteins [50]. The roles played by these droplets in tau physiology or pathology are still unclear [58].

Recent experiments have shown that at nanomolar concentrations (below those required to form condensates in solution), tau interacts with microtubules as individual molecules, moving diffusively along the microtubule surface [59, 60]. In contrast, at elevated concentrations, tau binds cooperatively, forming cohesive patches on the microtubule surface known as tau envelopes [59, 61]. These envelopes are generally considered restrictive to other proteins. Indeed, they have been suggested to impede the motility of certain molecular motors, such as kinesin-1, while allowing for the movement of others, including dynein, kinesin-3, and kinesin-14 [59]. Moreover, tau envelopes have been shown to limit the access of microtubule-severing enzymes, such as katanin and spastin, to the microtubule lattice [59]. Despite growing interest [62, 63], the physical principles governing tau envelope formation and their functional consequences remain elusive.

Phase-separating proteins can form liquid-like condensates on attractive surfaces, even at concentrations below which droplets are not stable in the bulk solution [51]. This behaviour can arise from a prewetting transition—a surface-driven phenomenon first described by Cahn—in which a dense phase, unstable in the bulk, becomes stabilized at an interface [64]. We provide a more detailed description of this phenomenon in Chapter 2. Recent work has extended this concept to biological systems, suggesting that prewetting may underlie the condensation of proteins on diverse intracellular surfaces, including DNA [44, 65] and lipid membranes [37, 52, 66]. These findings raise the intriguing possibility that tau envelope formation on microtubules may result from a surface-mediated prewetting mechanism.

The work presented in Chapter 3 of this thesis explores this hypothesis and provides a theoretical understanding of the experimental observations. Previous studies have applied the concept of wetting and prewetting in biological contexts using continuum field theories [51, 67]. While this framework has been successful in explaining observed phenomena [42, 67], it is a coarse-grained continuum description that does not account for the discrete nature of these molecules. Biological systems are finite, with regulatory protein copy numbers typically rang-

ing from a few hundred to several thousand [68]. In such mesoscopic systems, particularly when the proteins bind to various surfaces, density fluctuations may become significant. This is especially relevant for prewetting phenomena, where concentrations are far below those required for bulk phase separation. An alternative approach is to employ particle-based methods, such as molecular dynamics or lattice-gas models [52, 69–71]. In this work, we adopt the lattice-gas framework. A more detailed description of this approach is given in Chapter 2.

1.2 Membrane-bound organelles

Cells have an internal organization, an observation which was coincidental with the earliest observations of cells themselves [72]. Improvements in microscopy techniques led to more subcellular structures being discovered, and the term “organelle” was subsequently coined as a diminutive for “little organs” present within cells [73]. These specialized compartments are usually bound by a single or double lipid membrane [4]. Organelles are present only in eukaryotic cells, and not in prokaryotic cells (prokaryote literally meaning “before kernel” or nucleus, indicating their primitivity) [74].

Intracellular organelles carry out specific functions in cells. For example, the nucleus, typically the most prominent organelle in eukaryotic cells, houses the genome and serves as the primary site for DNA replication and RNA synthesis. [4]. Mitochondria are responsible for producing adenosine triphosphate (ATP), the molecular unit of energy, allowing cells to catalyze biochemical reactions [75]. Plant and algal cells additionally contain chloroplasts, which carry out photosynthesis, and also contribute to ATP production and other metabolic processes [76]. Notably, both mitochondria and chloroplasts contain their own genomes [4]. Lysosomes serve as waste-disposal machines for cells, breaking down proteins and other macromolecules. They also process particles external to the cell through endocytosis and can digest defunct organelles of the cell via autophagy [77]. Organelles such as endosomes and peroxisomes are closely related to the lysosome. Some other common examples of intracellular organelles are

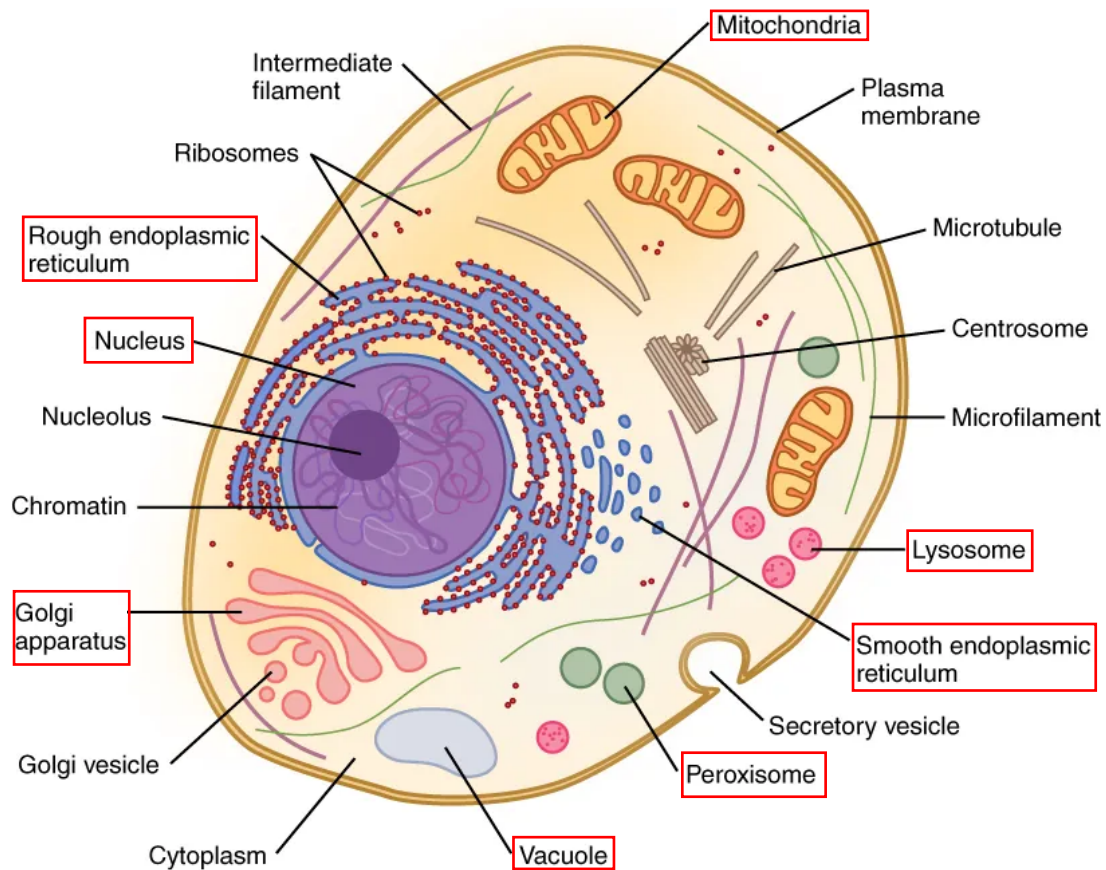


Figure 1.2: Examples of membrane-bound organelles in eukaryotic cells. Image adapted from Wikimedia Commons, licensed under Creative Commons Attribution 4.0 International (CC BY 4.0)

Golgi bodies, vacuoles and the endoplasmic reticulum (ER) [4].

1.2.1 Regulation of organelle abundance

Intracellular organelle abundances are tightly regulated by cells in response to environmental and cellular cues [78]. For example, algal cells tightly regulate the biogenesis of chloroplasts as a function of the existing number of chloroplasts [79]. Similarly, mitochondrial abundance is regulated in response to the metabolic needs of the cell [80]. However, a proper understanding of how a cell regulates its organelle abundance remains elusive.

Live and fixed cell imaging techniques have been employed to uncover mechanisms of organelle biogenesis. These studies have identified various processes that regulate organelle number, namely, de novo synthesis, fission, fusion and decay. Importantly, these processes are inherently stochastic, which leads to cell-to-cell variability in their abundance. One approach to

unravelling the mechanisms of organelle biogenesis has been to count organelle numbers across a population of cells. Counting organelles can be done directly by fluorescence microscopy using antibodies, fluorescent probes, and electron tomography [81–83]. The measured steady-state distribution of organelles is then used to infer the mechanism of organelle biogenesis. In particular, Mukherji et al. [84] constructed a simple model of biogenesis involving the four aforementioned processes. By employing a combination of theory and experiments, they showed that different mechanisms of organelle biogenesis lead to distinct footprints at the level of their cell-to-cell variability, allowing for discrimination between various mechanisms of organelle biogenesis. For instance, Golgi body biogenesis involves de novo synthesis and decay [85, 86], which is characterized by a Poisson distribution. In contrast, vacuole abundance is controlled via fission and fusion [87], which led to a truncated Poisson distribution [88]. Subsequent studies furthered this line of investigation [89].

Previous studies have, however, largely operated under the assumption that organelle abundance distributions have reached a steady state, where the distribution no longer changes with time. While this assumption has been useful for explaining certain experimental observations, it is limited in scope. For example, during cell division, organelles are partitioned between daughter cells [90], altering both the mean organelle number and its cell-to-cell variability. These daughter cells must then readjust to restore steady-state levels for proper function. Similarly, environmental stresses and signalling cues elicit changes in organelle abundance as part of the cellular response. Importantly, these transient phases can reveal regulatory mechanisms and feedback processes that remain hidden at equilibrium. Recent advances in real-time imaging of fluorescently labelled organelles at the single-cell level now allow the direct observation of these temporal dynamics across cell populations [91–94]. However, a theoretical framework that quantitatively links mechanisms of organelle biogenesis to the evolving distribution of organelle abundance, in a manner comparable with live-imaging data, remains lacking.

In the work presented in this thesis, we seek to fill this gap by studying the transient dynamics of organelle abundance, offering new insights into the principles governing organelle number control. To this end, we employ a simple, previously proposed model of organelle biogene-

sis [84] and systematically investigate its transient dynamics using a master-equation approach. First, by tracking how a population-level distribution of organelle abundance evolves with time, computing the time-dependent mean and variance for various model variants. Second, by calculating the mean and variance of the first passage time (FPT) required for a cell to reach a target organelle count from a given starting value. These results are reported in Chapter 4.

1.3 Thesis organization

The thesis is organized as follows:

In **Chapter 2**, we provide an introduction to some key theoretical concepts underlying both liquid-liquid phase separation and surface-induced condensation [25, 26], including concepts from wetting phenomena [64, 95]. We also describe the general lattice-gas framework [71] and Monte Carlo techniques used in the subsequent chapter. We also show results for phase-separation in the bulk and wetting phenomena on a flat surface.

In **Chapter 3**, we introduce results from experiments (from collaboration) on the association of tau proteins with microtubules [59], [96], and describe the lattice-gas model employed to investigate the system. Through a dialogue between experiment and theory, we show that tau envelopes are multilayered condensates formed via a prewetting-like transition. We find that tubulin inhomogeneities along the microtubule lattice govern the spatial condensation patterns of tau. Intriguingly, these condensates can recruit tau-interacting proteins, such as soluble tubulin, thereby enhancing the polymerization of the underlying microtubule. Our results show that surface-dependent condensation of tau results in a local increase of the concentration of tau interactors, forming a distinct local microenvironment on the microtubule surface. The results included in this chapter are from a manuscript submitted for publication and is available as a preprint on bioRxiv [96].

Chapter 4 focuses on membrane-bound organelles. We introduce a previously proposed model used to describe how cells regulate organelle abundances[84]. Previous studies employing this model assume a steady-state condition. We extend the results by obtaining the entire time evolution of the first two moments, the mean and variance, of the number distribution, for different regulatory mechanisms. We also compute the mean and variance of the FPT distribution to reach a target organelle number. We show that these two moments provide a powerful means to discriminate between different mechanisms of organelle biogenesis. The research presented in this chapter is contained in the published manuscript Ref.[97].

We summarise the research presented in this thesis in **Chapter 5**, and provide a discussion on the important results. We also outline potential directions for future work.

Chapter 2

Liquid-liquid phase separation and wetting phenomena

As mentioned in Chapter 1, the formation of biomolecular condensates is primarily attributed to a process called liquid-liquid phase separation [9, 20]. In this chapter, we review the thermodynamics of phase separation, originally developed by Flory and Huggins [25, 26]. We highlight the conditions required for a two-component system to undergo phase separation and form liquid droplets in the bulk. We then introduce the theory of wetting phenomena, developed by Cahn and others [64, 95, 98, 99]. Finally, we employ lattice-gas models to describe the physics of phase separation in the bulk and wetting phenomena on surfaces. The theories and results shown in this chapter serve as an introduction and shape the results of Chapter 3, where we use the principle of wetting transitions in the context of an experimental biological system.

2.1 Theory of phase separation in bulk

In this section, we review the thermodynamic theory of phase separation, originally developed by Flory and Huggins [26, 100]. We consider an incompressible mixture of two kinds of molecules, say A or B , on a lattice with N sites, where either molecule can occupy each site. Suppose N_A represents the number of molecules of species A , we define the volume fraction

of A as $\phi = N_A/N$ (volume fraction of B will simply be $1 - \phi$). Suppose that the molecules interact with their nearest-neighbouring molecules with interaction energy ϵ_{ij} , where $i, j = A$ or B . For such a system, working in the canonical ensemble, we can define the Helmholtz free energy [101] $F(N, V, T) = E - TS$, where the E represents the total internal energy of the system and S the entropy. Under a mean-field approximation for a homogeneous system, which means that we assume that every molecule experiences the same average environment, we can write the free energy density $f = F/V$ as

$$\frac{\nu}{k_B T} f(\phi) = \phi \ln \phi + (1 - \phi) \ln(1 - \phi) + \chi \phi(1 - \phi). \quad (2.1)$$

Here, ν is the molecular volume and $\chi = \frac{z}{k_B T} [\epsilon_{AB} - (\frac{\epsilon_{AA} + \epsilon_{BB}}{2})]$ is the interaction parameter, which captures the difference in affinities between molecules of the same kind and the others. The free energy captures the competition between the entropy of mixing, represented by the first two terms in Eq.2.1, and the interaction energies, represented by the last term. If the interactions between molecules of the same kind are strong enough, the entropic effect can be overcome, thus making the homogeneous state unstable. This results in a phase-separated system, where two phases can coexist. The stability of the homogeneous state requires the free energy density be a convex function of ϕ . We see that above a critical value of χ , the homogeneous state becomes unstable for certain compositions (Fig.2.1).

In Fig. 2.1, we see that above $\chi > \chi_c = 2$ (for symmetric molecular volumes), the region in the middle becomes unstable. For example, if a system is prepared with $\chi = 3$ with composition $\phi_1 > \phi > \phi_2$, the system can partition itself into subsystems of compositions ϕ_1 and ϕ_2 , thereby reducing its overall free energy. The fraction of volumes going to compositions ϕ_1 and ϕ_2 is given by the lever rule, which arises due to incompressibility and particle number conservation. The equilibrium state between the two phases is achieved when the chemical potential $\mu = \left(\frac{\partial F}{\partial N_A} \right)_{V, T}$, and the osmotic pressure $p = - \left(\frac{\partial F}{\partial V} \right)_{N_A, T}$ are equal between the two phases. Graphically, this corresponds to constructing a common tangent at ϕ_1 and ϕ_2 . In the $\chi - \phi$ parameter space, we can see the regions where the homogeneous state is unstable

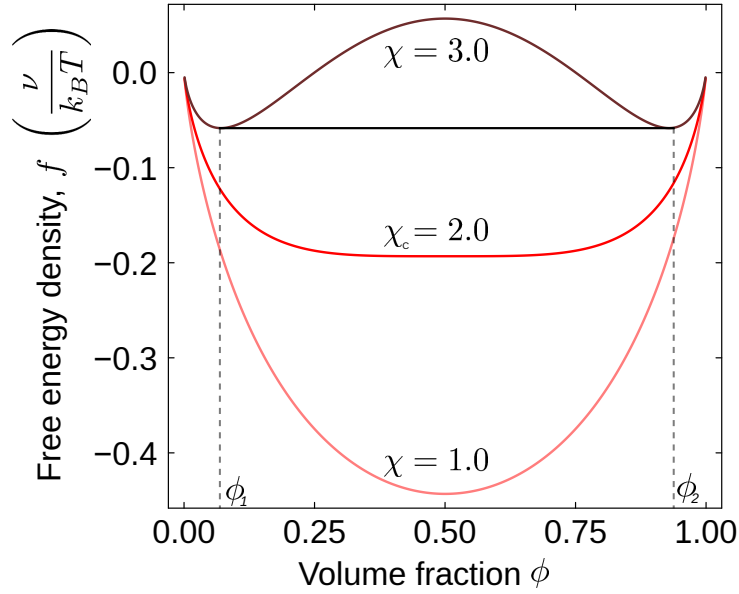


Figure 2.1: The Flory-Huggins free energy (Eq.2.1) for various values of χ . For the symmetric molecular volume, instability arises for $\chi > 2$.

(Fig.2.1). The coexistence curve is called the binodal, and the region enclosed by it corresponds to the coexistence regime.

While the free energy function being a minimum is a requirement for global stability of the homogeneous state, local stability requires $f'' > 0$ at each point on the curve. Within the binodal region, there lies a region of local instability called the spinodal (Fig.2.2). The spinodal curve is defined by $f'' = 0$. The region within this curve (where $f'' < 0$) is unstable. Here, even small perturbations grow, and the system spontaneously undergoes phase separation. Between the spinodal and binodal curves lies the region of metastability. Here, the system can tolerate small perturbations and still remain homogeneous. Only by nucleating droplets above a certain size can the system phase separate. The point where the spinodal and binodal meet is the critical point ($\chi_c=2$ and $\phi_c = 0.5$ for symmetric molecular volumes).

The Flory-Huggins theory shown here provides a useful foundation for understanding phase separation. However, it neglects the interfacial energies between the two phases. This is a valid assumption in the thermodynamic limit, where the volume is infinitely large, and the surface energies become negligible. However, for finite systems or near surfaces, the interfacial energy must be accounted for. A more rigorous approach is to define a free energy functional and

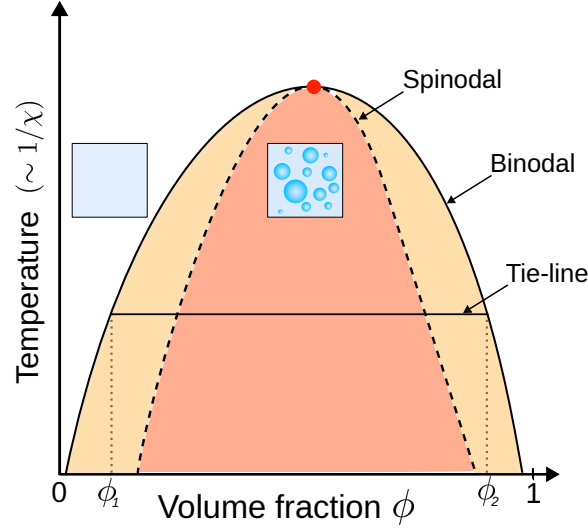


Figure 2.2: Phase diagram showing the region of coexistence (coloured area) for a two-component mixture.

evaluate the spatial profile of the concentration that minimizes it. At this point, one typically switches to a continuous field description of the concentration and approximates the free energy by a Ginzburg-Landau functional [101, 102]. This is the approach used to study wetting phenomena, first described by Cahn [64].

2.2 Wetting phenomena: Cahn's graphical theory

We now introduce concepts from the thermodynamics of wetting phenomena and discuss Cahn's theory of wetting transitions [64, 95], which provides the necessary background for the results shown in Chapter 3.

In the previous section, we described the conditions for liquid droplets to form in the bulk. In the presence of an additional phase, such as a solid surface, new phenomena emerge. Here, we consider a simple, two-phase system (typically considered to be a liquid-vapour system) in contact with a solid surface. With such systems, it is convenient to operate in a grand-canonical ensemble, where the grand potential is defined as $\Omega = F - \mu N$. In the regime where the two phases coexist, we can define the surface tension as the excess free energy per unit area at an interface, $\sigma = \left(\frac{\partial \Omega}{\partial A}\right)_{\mu, V, T}$. Since three different interfaces are present (solid-liquid, solid-vapour and liquid-vapour), there exist three surface tension terms. The relation between them is given

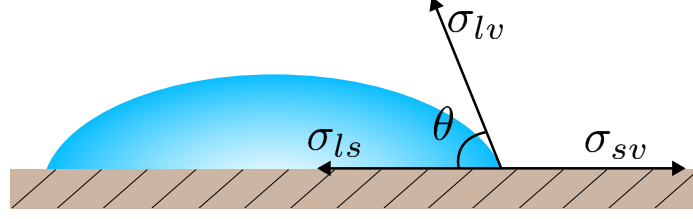


Figure 2.3: Partial wetting

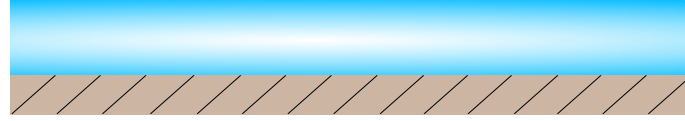


Figure 2.4: Complete wetting

by Young's equation [103]:

$$\sigma_{sv} = \sigma_{sl} + \sigma_{lv} \cos \theta. \quad (2.2)$$

This balance of surface tension is dictated by the thermodynamic variables such as temperature and bulk chemical potential, through their effect on the surface tensions. Changes in these variables would result in changes in the contact angle. When a droplet is present on the surface, with $\theta > 0$, the system is said to be partially wet (Fig.2.3). When the contact angle goes to zero, the entire surface gets covered by a liquid film, and the system is said to be in a state of complete wetting (Fig.2.4).

In his 1977 paper, Cahn gave a thermodynamic theory outlining the conditions under which such a system would show partial and complete wetting [64]. We adopt a continuum field description of a vapour–liquid system in contact with a solid, inert wall. The system is considered to be one-dimensional, perpendicular to the surface, which is at $x = 0$. The concentration of the system is $c(x)$, with the bulk vapour concentration defined as $c = c_0$ as $x \rightarrow \infty$. At the surface, $c = c_s$. Cahn considered a gradient-squared free energy functional given as:

$$\sigma = \Phi(c_s) + \int_0^\infty \left[\Delta f + \kappa \left(\frac{dc}{dx} \right)^2 \right] dx. \quad (2.3)$$

The term $\Phi(c_s)$ denotes the free energy per unit area due to the surface, at composition c_s , assuming a short-range interaction. The first term within the integral is $\Delta f = f(c) - f(c_0) - \mu(c - c_0)$, which is the bulk excess free energy contribution with respect to the vapour phase. The second term within the integral is the energy cost associated with gradients in the concentration field. Subject to the boundary conditions described above, we can minimize the free energy functional with respect to the concentration profile. Using methods of variational calculus and change of variables from x to c one can write for the surface tension [64, 95]:

$$\sigma = \Phi(c_0) + \int_{c_0}^{c_s} \left[c'_s(c) - \sqrt{2\kappa \Delta f(c)} \right] dc. \quad (2.4)$$

The term $\Phi(c_0)$ represents the free energy at the surface if it were at the bulk composition c_0 . The first term within the integral captures the free energy contribution at the surface, and the second term represents the free energy contribution from the bulk. The form of free energy considered by Cahn was the usual double-well form assumed in Ginzburg-Landau theory. Writing the surface tension in this manner allows a graphical treatment of it. The surface tension is simply the area between the two curves within the integral. For certain conditions, the two curves can intersect at three points, as shown in Fig. 2.5.

The middle intersection results in a maximum, and the other two intersections result in minima. Which of the two minima is lower tells us whether the system is in a state of partial wetting or complete wetting. We can split the surface free energy into integrals between the different intersection points as

$$\sigma_{pw} = \Phi(c_0) - A, \quad (2.5)$$

$$\sigma_{cw} = \Phi(c_0) - A + B - C. \quad (2.6)$$

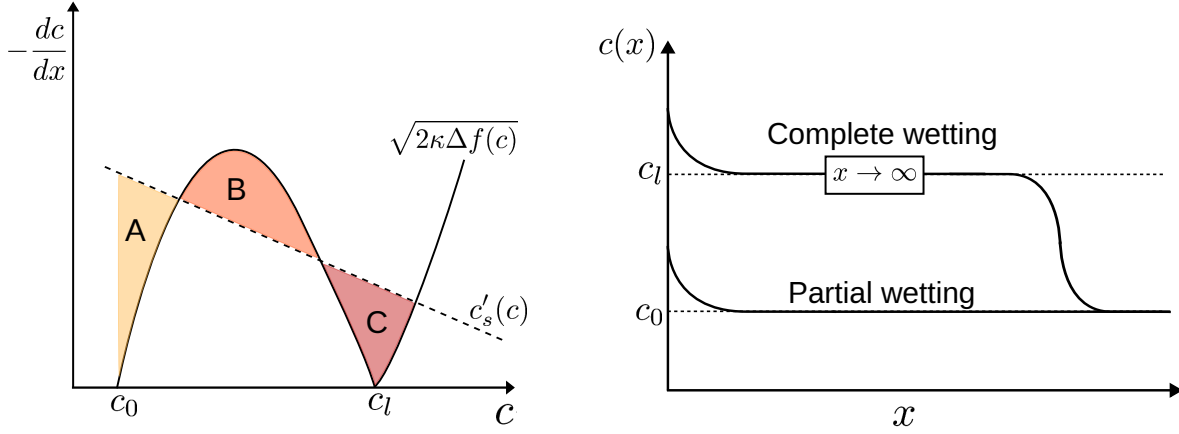


Figure 2.5: Wetting transition. Left: The surface free energy can be obtained by the area between the curves described in Eq.2.3. Right: Concentration profiles for complete wetting and partial wetting states.

This gives us the conditions for the wetting state. The curve indicating the bulk free energy contribution changes with changes in thermodynamic variables such as temperature. For temperatures close to the critical point, the "hump" in the middle is small, making $B < C$. Consequently, $\sigma_{cw} < \sigma_{pw}$, and the system is in a state of complete wetting. However, as the temperature is decreased, the area B increases such that eventually $\sigma_{cw} > \sigma_{pw}$, and the system is in a state of partial wetting. There will be an intermediate temperature at which the two minima will be equal, and therefore the two states can coexist. This is called the wetting transition line. Note that in the regime of complete wetting, the film thickness can be infinitely large. This is because the liquid phase is stable in the bulk as well.

An interesting prediction arises from Cahn's theory. If the system is in the undersaturated regime, only the vapour phase is stable in the bulk. Consequently, the liquid phase does not correspond to a minimum in the phase portrait shown above. However, it is still possible that the two curves intersect at three points, two of which will correspond to free energy minima. These two states correspond to a thin, adsorbed layer and a thick layer at the surface, with the first-order transition line separating them called the prewetting transition [64, 95]. However, in this regime, the complete wetting state corresponds to a layer of finite thickness. This is because the liquid state is not stable in the bulk; it can sustain only near the surface. As μ is increased, and bulk coexistence is approached, the film thickness diverges.

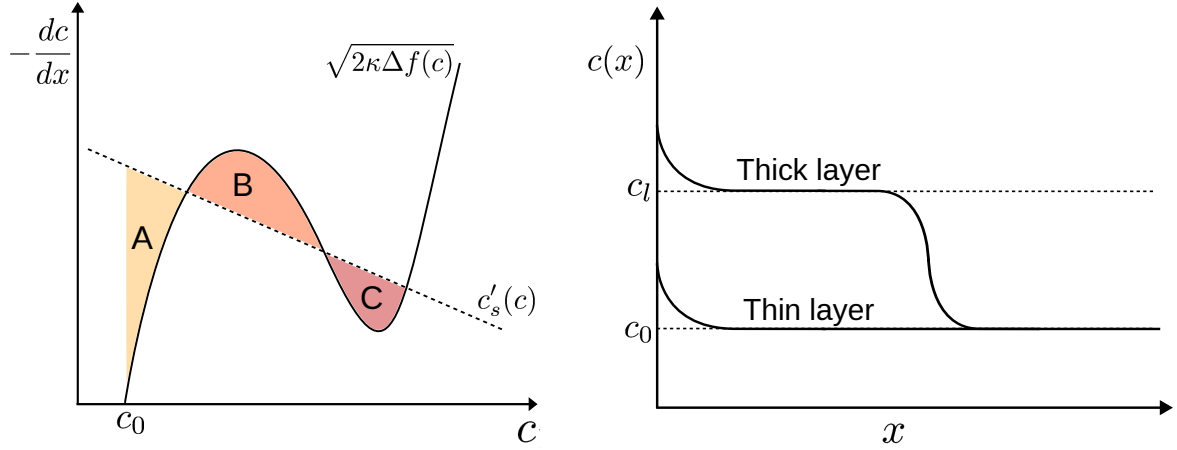


Figure 2.6: Prewetting transition. Left: Even in the undersaturated regime, the two curves in Eq.2.4 can intersect at three points, resulting in a prewetting transition. Right: Concentration profiles for a thin, adsorbed layer and a thick, condensed layer.

In summary, these wetting transitions can be portrayed in the phase diagram shown below:

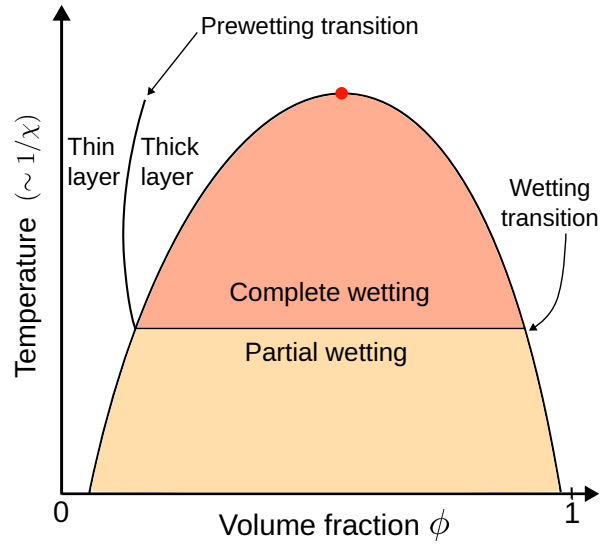


Figure 2.7: Phase diagram showing wetting and prewetting transitions. The colored area represents the bulk coexistence region. Note that the prewetting line extends outside of this region, into the undersaturated regime.

2.3 Lattice-gas model for phase separation and wetting

Previous sections have described the thermodynamic theories of phase separation and wetting transitions. These approaches give a continuous mean-field description. An alternative approach is to adopt particle-based models and simulate via molecular dynamics [104] or Monte

Carlo techniques [105]. In this section, we introduce the lattice-gas framework to study phase separation in bulk and the physics of wetting.

2.3.1 Phase separation in the bulk

For the lattice-gas model, we consider a 3D lattice of $L \times L \times L$ sites, where each site is either occupied by a molecule or empty (Fig. 2.8). The occupancy state at site i is given by $n_i = 1$ if it is occupied, or $n_i = 0$ if it is empty. Molecules interact with their nearest neighbours with interaction energy J_b . We can write the Hamiltonian for this system as:

$$H = -J_b \sum_{\langle i,j \rangle} n_i n_j \quad (2.7)$$

First, we consider the canonical (N, V, T) ensemble. This model is similar to that introduced in the Flory-Huggins theory, in Section 3.17. However, instead of the mean-field free energy density, we find the equilibrium states for the system by carrying out Monte Carlo simulation.

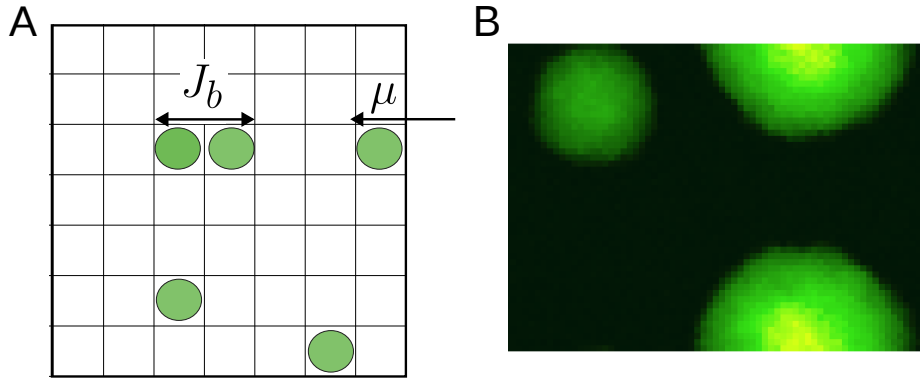


Figure 2.8: A) Schematic for the lattice-gas model. B) Simulation snapshot of droplet formation in the lattice-gas model (parameters: $Jb = 0.3 k_B T$, $\phi = 0.2$)

We initialize the lattice by randomly occupying a fraction $\phi = N/L^3$ of sites, where N is the number of particles in the system and L^3 is the total number of lattice sites. The system is evolved to equilibrium using the Monte Carlo method [105]. In each Monte Carlo step, we randomly choose a particle and propose to move to any empty site anywhere in the lattice (global Kawasaki move). The proposed moves are accepted with a probability given by the Metropolis criterion [105], $p_{acc} = \min[1, \exp(-\beta \Delta H)]$, where ΔH is the change in the energy obtained

from Eq.2.7. This procedure is carried out until the observables reach a steady-state value, within some uncertainty. For the results shown in this section, we carry out 10^4 to 10^5 Monte Carlo sweeps to allow for equilibration. The average quantities are then obtained from the next 10^4 sweeps. Since we are interested only in the equilibrium state here, and not the kinetics of the equilibration, we can allow global displacements of the molecules. In Chapter 3, we study the kinetics of equilibration as well. In that case, the molecules are moved only to their nearest empty neighbouring sites, ensuring that local densities are conserved.

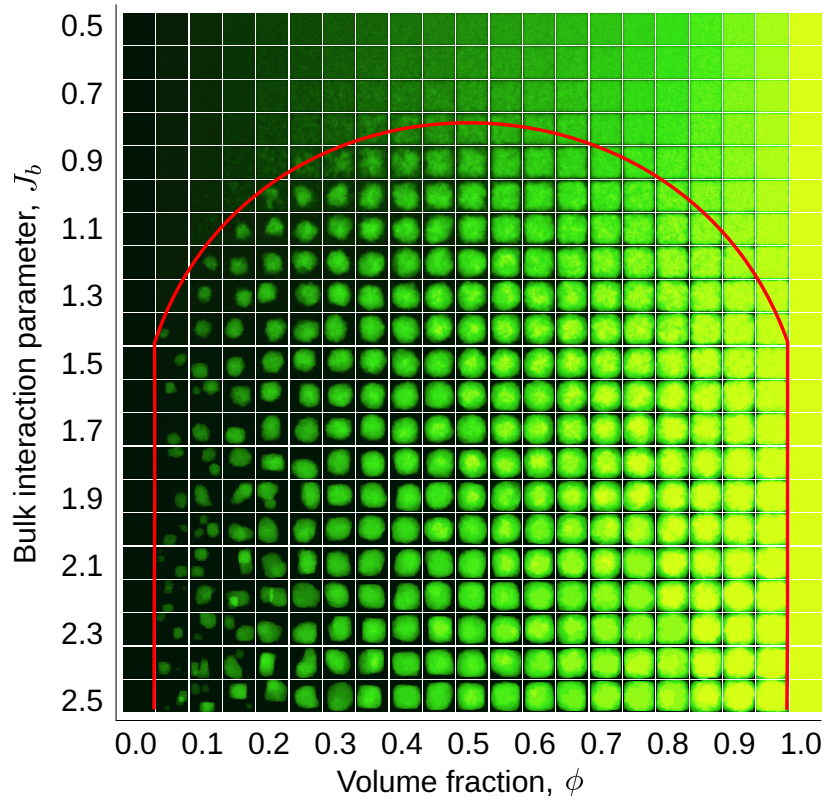


Figure 2.9: Representative phase diagram for the lattice-gas system in the NVT ensemble. The region enclosed by the red line indicates the coexistence regime.

When the interaction strength between molecules is strong enough to overcome thermal fluctuations, the system can form phase-separated droplets, as shown in Fig.2.8B. The range of parameters, J_b and ϕ , for which the system undergoes phase separation is shown in the representative phase diagram in Fig.2.9. We observe the region of coexistence described in Section 3.17. There is a critical value of J_b below which the system cannot phase separate, at any composition. For a simple cubic lattice ($q = 6$), we estimate the critical interaction strength $J_b^c \simeq 0.88 k_B T$.

It must be noted that for finite systems, the coexistence region is generally smaller than that predicted by thermodynamic theories. This is due to fluctuations being significant, which tend to push the system toward a well-mixed state.

Before we introduce surfaces, we must study the bulk system in a grand-canonical ensemble, which is better suited for studying wetting transitions. For this, we introduce an additional term in the Hamiltonian for the bulk chemical potential:

$$H = -J_b \sum_{\langle i,j \rangle} n_i n_j - \mu \sum_i n_i. \quad (2.8)$$

The second term accounts for the change in free energy associated with the addition and removal of molecules from the system. For simulation, particle insertion and deletion moves are accepted with the Metropolis criterion as described earlier. Fig. 2.10 shows the phase diagram in the $J_b - \mu$ parameter space. Here, the bulk volume fraction ϕ is not an input parameter. Instead, the system equilibrates to the volume fraction ϕ which minimizes the free energy.

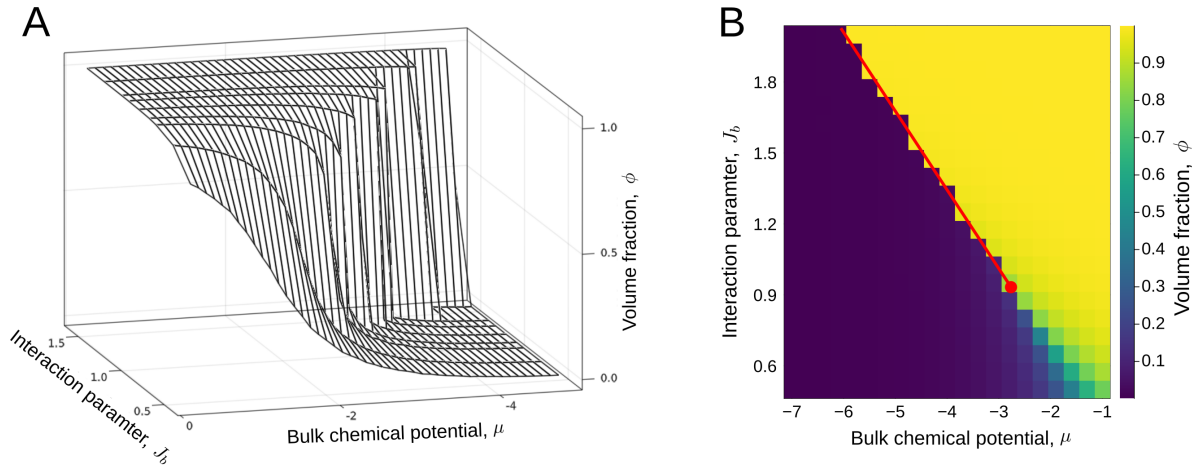


Figure 2.10: A) Phase diagram for a lattice-gas system in the grand-canonical ensemble. The transition between high and low volume fractions becomes sharp for $J_b > J_b^c$ B) The same diagram represented on the $J_b - \mu$ plane shows the criteria for coexistence.)

In Fig. 2.10B, the red line represents the first-order transition between the dense and dilute phases, terminating at the critical point J_b^c . This first-order transition line follows the relation $\mu = -\frac{qJ_b}{2}$, where q is the coordination number and $J_b > J_b^c$. At values of $\mu < -\frac{qJ_b}{2}$, the system will be in the undersaturated regime.

The lattice-gas system described here can be mapped directly to a standard 3D Ising model in the presence of an external field. We see this by replacing n_i in Eq.2.7 by $(s_i + 1)/2$, where $s_i = \pm 1$. This mapping relates the bulk chemical potential to the external field in the Ising model. Transforming the μ axis in Fig. 2.10A, as $\frac{1}{4}(2\mu + qJ_b)$, we get the familiar phase diagram for the Ising model. In the lattice-gas model, setting $\mu = -\frac{qJ_b}{2}$ is equivalent to setting the external field to zero in the Ising model. Furthermore, the critical interaction strength for a 3D Ising model on a simple cubic lattice is $J^c \approx 0.2216 k_B T$ [106]. The corresponding critical interaction strength for the lattice-gas model would be $4 \times J^c \approx 0.8866 k_B T$, which is close to the value we obtain (Fig.2.9).

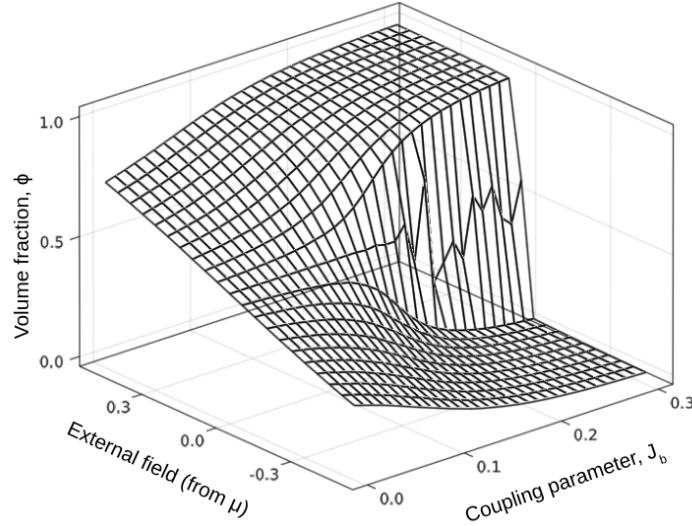


Figure 2.11: Change of state variables maps the lattice-gas system phase diagram onto the familiar Ising model phase diagram (for each J_b a different range of μ has been chosen, such that after the transformation $\frac{1}{4}(2\mu + qJ_b)$ they all lie within the same range.)

2.3.2 Wetting in the presence of a surface

We now introduce a surface at the boundary of our phase-separating system (Fig.2.12). As mentioned in the previous section, the grand-canonical ensemble is better suited for studying wetting phenomena. In the canonical ensemble, the total number of molecules in the system is fixed. Consequently, binding of molecules to the surface depletes the bulk, artificially limiting further binding, even if it is thermodynamically favourable. One approach is to simulate

a very large system, so that the bulk concentration is not depleted significantly. The alternative approach is to adopt the grand-canonical ensemble, which allows the number of particles to fluctuate, keeping the average concentration in the bulk fixed, even as the surface densities form and grow. This is typically the ensemble used to study wetting phenomena [64, 95], and also the approach we adopt here.

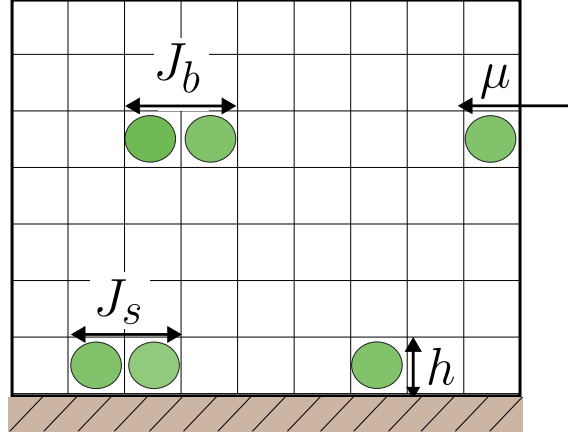


Figure 2.12: Lattice-gas model with a solid surface present.

With a surface present, the Hamiltonian includes additional terms accounting for the molecule-surface interactions:

$$H = -J_b \sum_{\substack{\langle i,j \rangle \\ \in \text{bulk}}} n_i n_j - J_s \sum_{\substack{\langle i,j \rangle \\ \in \text{surf}}} n_i n_j - h \sum_{i \in \text{surf}} n_i - \mu \sum_i n_i. \quad (2.9)$$

The first and last terms represent the bulk interaction and chemical potential contributions as in Eq.2.7. The second term describes interactions between neighbouring molecules when both are at the surface. This captures cooperative effects while binding, governed by the interaction parameter J_s . The third term represents the interaction of individual molecules with the surface, parameterized by the surface field h . This form of Hamiltonian is motivated by earlier Ising model studies of wetting transitions [71, 107, 108]. We determine the equilibrium states of our system by performing Monte Carlo simulations using the Metropolis criterion, as described previously.

In the undersaturated regime, a dense layer of finite thickness can exist at the surface. This

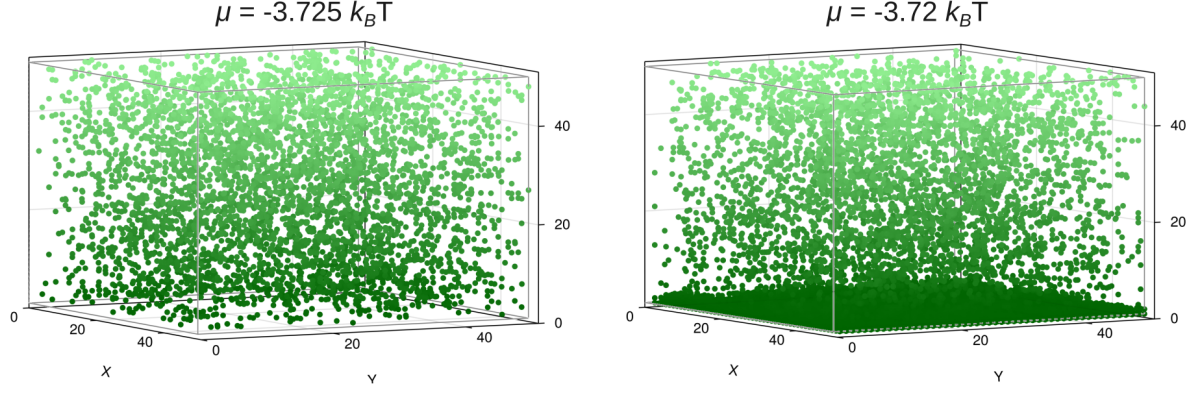


Figure 2.13: Simulation snapshots showing a dense, finite layer forming on the surface arising from a very small increase in the bulk chemical potential (parameters: $J_b = 1.2 k_B T$, $J_s = 4.2 k_B T$, $h = -5.0 k_B T$)

layer forms abruptly upon increasing the bulk chemical potential (Fig. 2.13). This is indicative of a prewetting transition, described earlier in this chapter [64]. To characterize this, we quantify the extent of condensation at the surface using the excess density [107], defined as

$$c_s = \frac{1}{L} \sum_{n=1}^L (\phi_n - \phi_b), \quad (2.10)$$

where ϕ_n is the average occupancy of the n th layer from the surface, and ϕ_b is the density in the bulk far away from the surface. Here, we calculate ϕ_b as the average density of the top 10 layers.

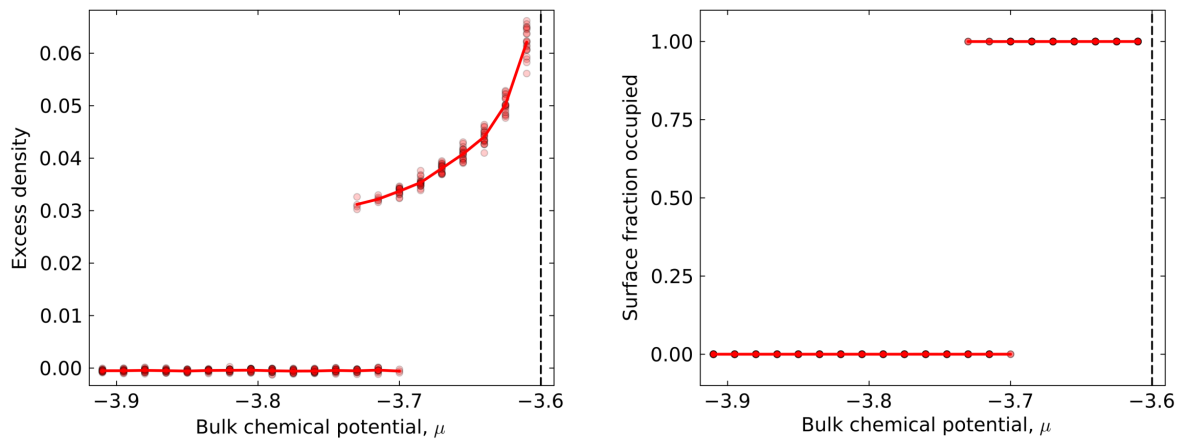


Figure 2.14: A-B) The surface excess density and fraction of sites occupied at the surface show a sharp increase as a function of the bulk chemical potential, indicating a prewetting transition. The dotted line indicates the bulk coexistence chemical potential. In the thermodynamic limit, the excess density diverges as this line is approached. (parameters: system size $L = 50$ sites, $J_b = 1.2 k_B T$, $J_s = 4.2 k_B T$, $h = -5.0 k_B T$)

As shown in Fig. 2.14, the excess density exhibits a sharp jump as the bulk chemical potential is increased, reflecting the sudden formation of a dense surface layer. We also observe a region of coexistence of the thin and thick layer states (around $\mu = -3.72 k_B T$ in Fig. 2.14), arising due to metastability near a first-order transition. This behaviour is characteristic of the prewetting transition. Here, we focus only on demonstrating the prewetting transition within the lattice-gas framework. The full parameter regime is properly explored in Chapter 3 for a similar model.

2.4 Summary

In this chapter, we reviewed the Flory–Huggins theory describing the thermodynamics of bulk phase separation and outlined Cahn’s theory of wetting transitions. We then introduced the lattice-gas framework and used Monte Carlo simulations to study phase separation in the bulk and in the presence of a surface. We also demonstrated the prewetting transition for a flat surface in our model. In Chapter 3, we extend this framework by modifying the surface interactions and coordination number to investigate tau protein binding to microtubule surfaces.

Chapter 3

Surface-driven condensation of tau on microtubules

3.1 Introduction

In this chapter, we present a theoretical model of tau proteins interacting with microtubules. We hypothesize that tau forms surface condensates on microtubules, driven via a prewetting transition. We employ a dialogue between theory and experiments to dissect this hypothesis. The experimental results presented here were carried out by Zdenek Lansky and Marcus Braun's group at the Institute of Biotechnology, Czech Academy of Sciences, Prague. Our role was to develop the theoretical model. **The work presented in this chapter is part of a manuscript submitted for publication and is available as a preprint on bioRxiv [96].**

3.1.1 Biology of microtubules and tau proteins

Microtubules are polymers that form part of the cytoskeletal framework of the cell. They are one of the stiffest polymers in biology, with a persistence length of a few millimetres [4]. A typical microtubule consists of 13 parallel protofilaments stacked together to form a hollow cylinder, with the outer diameter being about 25nm[109]. Each protofilament is composed of $\alpha\beta$ -tubulin heterodimers [110].

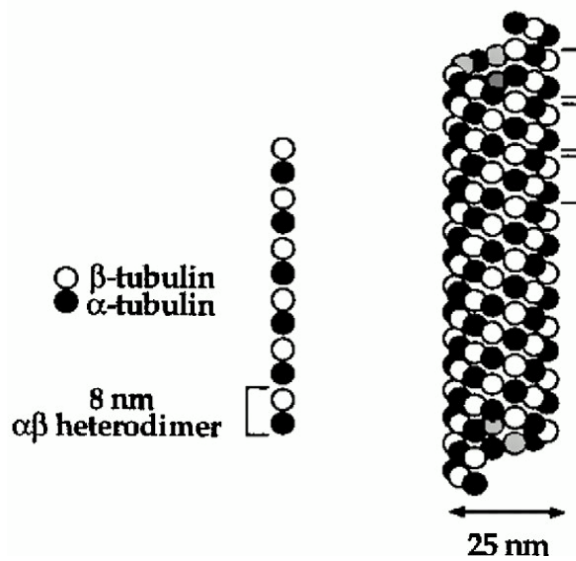


Figure 3.1: Microtubule structure. Reprinted by permission from Ref.[109]. Copyright © Annual Reviews

Tubulin monomers also have nucleotides such as GTP and GDP bound to them [109]. GTP-bound tubulin binds to the polymerizing end of the microtubule, upon which the GTP bound to the β -tubulin gets hydrolysed into GDP. This is accompanied by a 2.5 Å longitudinal compaction of the interdimer interface within the tubulin lattice [109]. The change in lattice conformation makes GDP-tubulin microtubules unstable. If the addition of GTP-tubulin at the polymerizing end outpaces the hydrolysis rate, the growing end remains capped and remains stable. Otherwise, if the hydrolysis is faster than the growth, the microtubule undergoes rapid depolymerization, in a process known as dynamic instability [111]. Additionally, compacted GDP-lattices can be artificially extended by microtubule stabilizing agents, such as the GTP analog GM-PCPP (which extends the lattice reversibly) or taxol (Paclitaxel), which extends the lattice in an irreversible manner[61, 112].

Microtubules perform diverse functions within cells. As a part of the cytoskeleton of the cell, they provide mechanical support to cells [4]. They play an important role in spindle formation during cell division [113]. Microtubules also act as tracks for cargo transport using molecular motors [114]. This function is particularly critical in neurons, whose axons can extend over long distances and rely on motor-driven transport for proper function [54]. In axons, microtubules form polarized bundles, acting as “highways” for cargo transport.

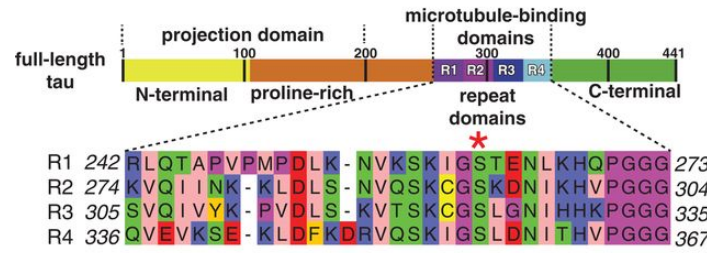


Figure 3.2: Tau sequence showing four repeats in the microtubule-binding domain. Reprinted by permission from Ref.[117] Copyright © 2018, The American Association for the Advancement of Science

Microtubule functioning is regulated by many microtubule-associated proteins (MAPs), which help in maintaining them [55]. A prominent example of this is the protein tau. It is abundant in axons, where it acts to stabilize and bundle microtubules [115], among carrying out other functions [116]. Full-length adult tau is intrinsically disordered and includes a projection domain, an MT-binding region of four imperfect sequence repeats (R1 to R4), and a C-terminal domain (Fig.3.2)[117, 118]. In its pathological state, tau gets hyperphosphorylated and unbinds from microtubules. This leads to the formation of amyloids or tau fibrils, resulting in neurodegenerative diseases collectively called tauopathies [56, 57]. While the structure of tau fibrils is known [119], the physiological state of tau bound to microtubules remains under discussion [117].

Previous studies have demonstrated that under crowded conditions, at tau concentrations in the order of micromolar, self-association between tau molecules can induce liquid-liquid phase separation, leading to the formation of tau droplets [50]. These droplets can also recruit tau-specific proteins, such as tubulin. Nevertheless, the roles played by these droplets in tau physiology or pathology remain a subject of ongoing discussion [58].

Recent experiments have shown that at physiological concentrations, in the nanomolar range, tau interacts with microtubules as individual molecules, moving diffusively along the microtubule surface [59, 60]. In contrast, at elevated concentrations, still far below those required for droplet formation in solution, tau binds cooperatively on the microtubule surface, forming cohesive patches known as tau envelopes [59, 60]. These envelopes are generally considered restrictive to other proteins. Indeed, they have been suggested to impede the motility of certain molecular motors, such as kinesin-1, while allowing for the movement of others, including

dynein, kinesin-3, and kinesin-14 [59]. Moreover, tau envelopes have been shown to limit the access of microtubule-severing enzymes, such as katanin and spastin, to the microtubule lattice. Importantly, the formation of tau envelopes has been shown to strongly depend on the lattice conformation of the microtubules [61]. For instance, tau envelopes form readily on microtubules assembled using GDP-tubulin, whereas no envelopes form on microtubules treated with GMPCPP [61]. These diverse observations highlight the need for a common theoretical framework.

With growing interest in these observations, recent studies have attempted to provide a theoretical description [62, 63, 120]. We discuss a few of these here. A recent perspective [120] remarked that a simple Langmuir binding theory cannot explain these observations. Interestingly, based on stoichiometric arguments, they suggest that tau might be forming multilayered condensates on microtubules. While an alternative theory called the Brunauer-Emmett-Teller (BET) theory is discussed, they highlight that it is unlikely to be useful for condensate biology. Another study proposed a "floor-field" model based on tau-taxol competition to explain the mechanism governing the formation of tau patches [63]. However, the scope of the results shown was limited. Therefore, the physical principles governing tau envelope formation and their functional consequences remain elusive.

3.1.2 Experimental observations

Here, we give a short description of the initial observations made by our collaborators. They attached microtubules to a coverslip surface, added tau-GFP, and followed the kinetics of their interaction using TIRF microscopy (see Methods of [59, 61] for details).

To study tau-microtubule interactions, three microtubule types were considered: (i) GDP-tubulin microtubules, (ii) taxol-stabilized microtubules, and (iii) GMPCPP-treated microtubules. As mentioned earlier, the GDP-microtubule lattice is inherently compacted, whereas taxol- and GMPCPP-microtubule lattices are reversibly and irreversibly extended, respectively [112]. As reported in previous studies [59–61], tau forms cohesive patches on taxol microtubules. These envelopes form by nucleating at distinct spots and undergo a period of elongation, eventually

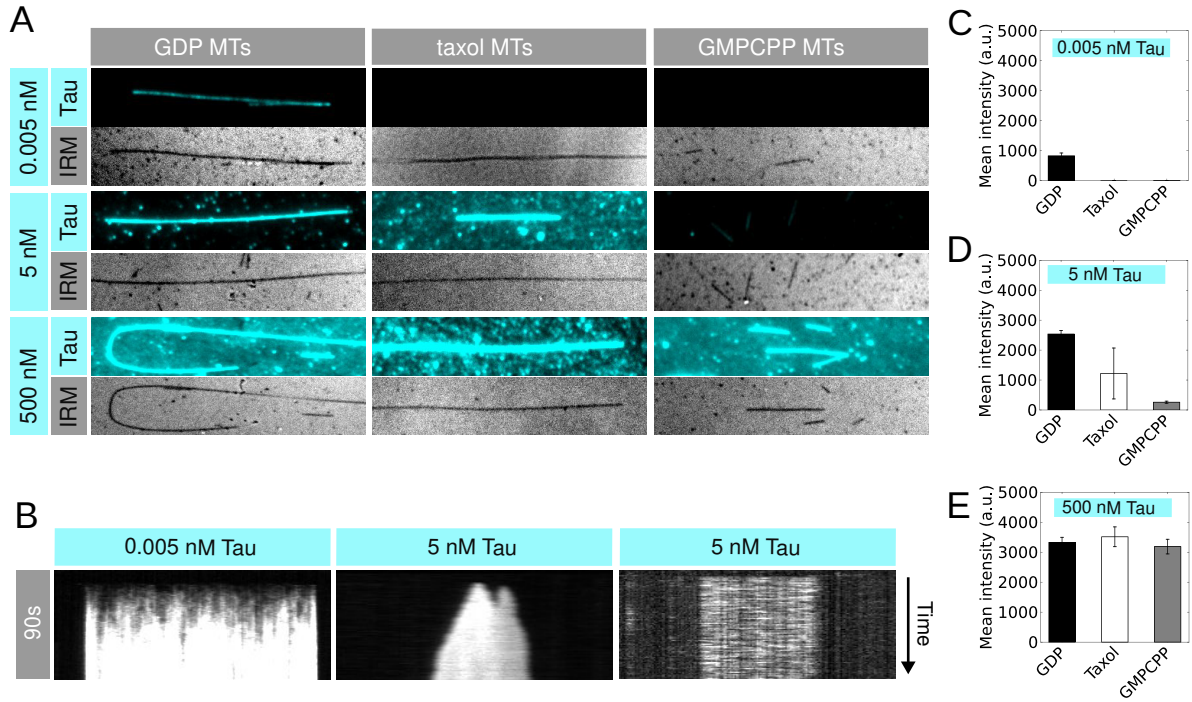


Figure 3.3: Experimental observations showing A) tau interaction with three kinds of microtubules (taxol-stabilized, GDP-tubulin and GMPCPP-tubulin) at various concentrations of tau. B) Kinetics of tau covering various microtubules. C-E) Quantification of tau densities for snapshots is shown in A. (Unpublished results, included with permission from collaborators [96]).

reaching their steady-state lengths (Fig.3.3A, B centre). On GDP-microtubules, the envelopes grow rapidly, covering the entire microtubule at tau concentrations as low as 0.005nM (Fig.3.3A, B left). No envelope formation was observed on GMPCPP-microtubules (Fig.3.3A, right). With taxol-microtubules, increasing the tau concentration leads to increasing lengths of the envelopes, eventually covering the entire microtubule (Fig.3.9, left). Previous studies have further reported that these envelopes can restrict the movement of kinesin-1 motors on the microtubules, and can also shield the lattice from microtubule-severing enzyme katanin[59]. Interestingly, at very high tau concentrations ($> 300\text{nM}$), all three kinds of microtubules get uniformly covered with dense tau, exhibiting similar intensities (Fig.3.3A, E). Notably, even though GMPCPP-microtubules are uniformly coated with tau at these elevated concentrations, previous reports show that the movement of kinesin-1 was not restricted, indicating that tau does not form cohesive envelopes on them[61]. These observations show that even though differences exist, tau exhibits very similar states on different kinds of microtubules, indicating a general underlying mechanism.

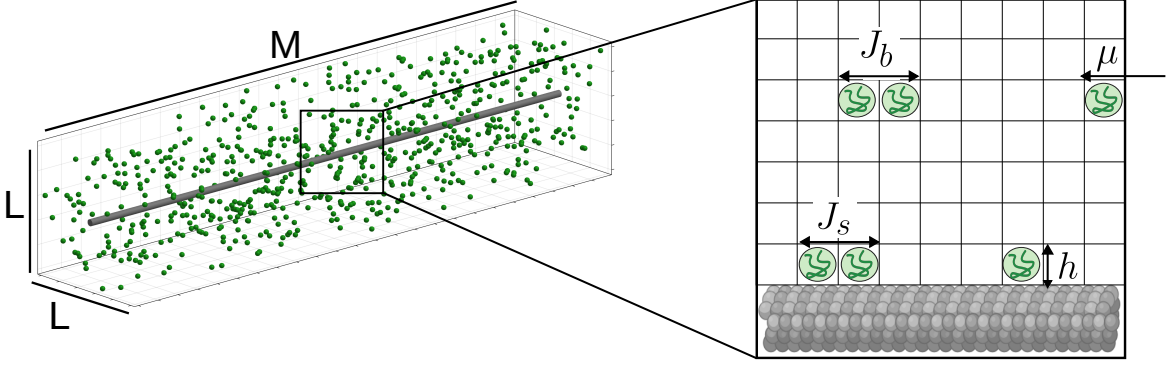


Figure 3.4: Schematic of lattice-gas model for tau interacting with a microtubule.

3.2 Lattice-gas model for tau condensation on microtubules

3.2.1 Model overview

In order to study the interaction of tau molecules with microtubules, we use a simple lattice gas model. In our model, tau molecules are present at the vertices of a three-dimensional cubic lattice with $L \times M \times L$ sites and periodic boundary conditions. The occupancy at site i is given by $n_i = 0$ or 1 , depending on whether it is unoccupied or a tau molecule is present, respectively. The microtubule is represented as a cylindrical surface of length M lattice units, with 12 binding sites radially around it at each position (Fig.3.4). This is motivated by the fact that microtubules have 13 protofilaments on average[112] (see Fig.3.1). Tau molecules interact favourably with each other, forming liquid droplets in the bulk [121], and also interact with microtubules directly [122, 123]. We incorporate these interactions in our framework via the following Hamiltonian:

$$H = -J_b \sum_{\substack{\langle i,j \rangle \\ \in \text{bulk}}} n_i n_j - J_s \sum_{\substack{\langle i,j \rangle \\ \in \text{surf}}} n_i n_j - \sum_{i \in \text{surf}} h_i n_i - \mu \sum_{i \in \text{bulk}} n_i. \quad (3.1)$$

The first term accounts for tau-tau interaction in the bulk, characterized by the bulk coupling parameter J_b . The sum is over all nearest-neighbour pairs in the bulk, where each tau molecule can interact with other molecules at its 26 nearest-neighbouring sites (6 along edges, 12 along face diagonals and 8 along body diagonals). In our model, tau can interact with the microtubule

in two ways: first, individual molecules can bind to the surface at position i with the binding energy h_i , as shown in the third term in the Hamiltonian. Second, tau molecules bound adjacently on the microtubule interact through the surface coupling parameter J_s . This is represented in the second term of the Hamiltonian and accounts for the binding cooperativity of tau. The final term, parametrized by the bulk chemical potential μ , allows the exchange of molecules with an effective reservoir while keeping the average concentration of tau in the bulk fixed [101].

Similar lattice-based models have previously been used to study phase separation, as well as to study wetting phenomena [71, 100, 107].

3.2.2 Simulation scheme

To evolve the system to equilibrium, we simulate it using the kinetic Monte Carlo method [105], described in Chapter 2. Here, each Monte Carlo step is performed as follows: we randomly choose between particle displacement, particle insertion into an empty site, and particle deletion from an occupied site, with probabilities of 0.5, 0.25, and 0.25, respectively. In a displacement step, a randomly chosen particle can be moved to any one of its neighbouring empty sites. For particle insertion, we choose a site at random and add a particle if it is empty. Particle deletion is carried out in a similar way. All particle insertion and deletion moves are done only within a padded region 3 sites from the boundaries parallel to the microtubule, sufficiently far from the surface. This ensures that the local densities are preserved near the surface and that the proper kinetics of condensate growth are obtained. All moves are accepted with a probability given by the Metropolis condition [105], $p_{acc} = \min[1, \exp(-\beta\Delta H)]$, where ΔH is the change in the energy obtained from Eq.3.1. The system is initialized randomly with an initial density $\phi = 0.05$ in the bulk, instead of an empty system ($\phi = 0.0$), since that is a metastable state. We simulate the system for 10^4 to 10^5 Monte Carlo sweeps for equilibration, with each sweep consisting of $L \times M \times L$ moves. For cases where we require only the equilibrium states, we relax the nearest-neighbour constraint on the displacement step and allow the particle to be globally displaced anywhere within the system.

3.3 Results

3.3.1 Tau envelopes are surface condensates formed via a prewetting-like transition

Here, we aim to recapitulate the preliminary observations shown in Fig.3.3. Within our modelling framework, the binding of tau to the three experimentally studied microtubule types can be represented by suitable choices of J_s and h_i . In this section, we show simulation results for taxol-microtubules (see next section for GDP- and GMPCPP-microtubules). First, we consider that the microtubule surface is homogeneous for tau binding, i.e., we assign the same value of h_i to every binding site along the microtubule. We refer to this model as the “homogeneous model”. We set $J_b = 0.2k_B T > J_b^{\text{critical}}$ throughout the main results (other values are explored in the Appendix at the end of the chapter). To explore the equilibrium properties of the homogeneous model, we carry out Monte Carlo simulations, with tau concentration set below the threshold for bulk phase separation (see Appendix).

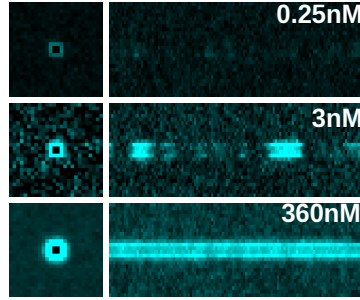


Figure 3.5: Simulation results for a homogeneous surface show distinct states of tau at different concentrations.

At very low concentrations ($<1\text{nM}$), tau molecules get sparsely and individually adsorbed onto the microtubule surface (Fig.3.5, top). As the tau concentration is increased, cooperative binding of tau leads to the formation of cohesive patches or condensates. At these intermediate concentrations (1-10nM), the model predicts the coexistence of high-density regions and low-density regions of tau (Fig.3.5, middle). This is seen in the bimodal distribution of tau densities, consistent with the experimental findings (Fig.3.6).

Figure	$J_b (k_B T)$	$J_s (k_B T)$	$\bar{h} (k_B T)$	$\sigma (k_B T)$	$\mu (k_B T)$
3.5, top	0.2	0.5	0.0	0.0	-3.066
3.5, middle	0.2	0.5	0.0	0.0	-2.929
3.5, bottom	0.2	0.5	0.0	0.0	-2.664
3.6	0.2	0.5	0.0	0.0	-2.905
3.7, middle	0.2	0.5	0.0	0.0	-2.91
3.7, right	0.2	0.5	0.0	0.2	-2.91
3.8, top	0.2	0.5	0.0	0.2	-2.9
3.8, bottom	0.2	0.5	0.0	0.2	-3.1
3.9, A,B	0.2	0.5	0.0	0.2	varied
3.10, A	0.2	0.5	0.3	0.2	[-3.28, -2.9, -2.64]
3.10, B	0.2	0.5	0.0	0.2	[-3.28, -2.9, -2.64]
3.10, C	0.2	0.5	-0.1	0.2	[-3.28, -2.9, -2.64]
3.11, left	0.2	0.5	0.3	0.2	-3.28
3.11, right	0.2	0.5	0.0	0.2	-2.9
3.11, centre	0.2	0.5	-0.1	0.2	-2.9
3.7, right	0.2	0.5	0.0	0.2	-2.91
3.7, right	0.2	0.5	0.0	0.2	-2.91
3.7, right	0.2	0.5	0.0	0.2	-2.91
3.12	0.2	0.5	0.0	[0.01, 0.2]	-2.91
3.13	0.2	0.5	0.0	0.2	[-2.88, -3.2]
3.14	0.2	0.5	0.0	0.2	varied
3.15, A	0.2	0.5	[0.3, -0.1]	0.2	[-2.88, -3.1]
3.15, B	0.2	0.5	0.3	0.2	[-2.86, -3.1]

Table 3.1: Simulation parameters for the figures in the Results sections. Other common parameters are mentioned in Table 3.3.

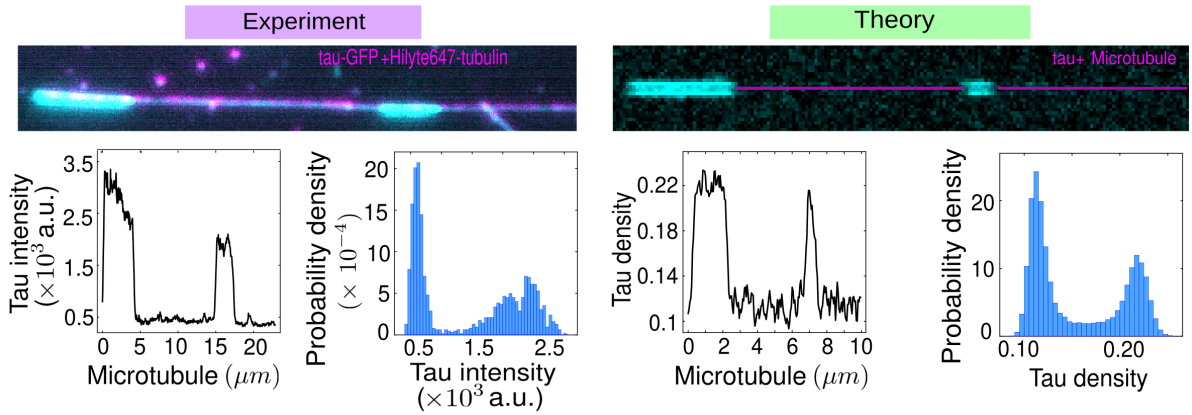


Figure 3.6: The homogeneous model (right) shows a bimodal distribution of tau density, in agreement with experimental observations (left). This implies coexistence between two states, which we identify as adsorbed and condensed states.

Although the tau population on the microtubule predominantly consists of molecules bound to the microtubule surface directly, the presence of tau-tau interactions also leads to the formation of less stable, secondary layers on top of already adsorbed tau. Subsequent increase in tau concentration ($>100\text{nM}$) eventually leads to further tau condensation, resulting in a multilayered structure to emerge (Fig. 3.5, bottom). We use the general term condensate to refer to any surface-bound state of tau beyond simple adsorption. This includes the cohesive patches observed on GDP- and taxol-microtubules (envelopes), and the uniform dense tau coating observed on all three microtubule types at high concentrations. Our model provides a mechanistic explanation for why microtubules with distinct surface properties display comparable tau densities at high protein concentrations (Fig. 3.3, E). The initial adsorption of tau is governed by tau-microtubule affinity, causing low-affinity surfaces, such as GMPCPP-microtubules, to bind less tau at low concentrations. As the concentration increases, tau begins to get adsorbed on the microtubule surface. Once this first layer forms, additional layers of tau assemble through tau-tau interactions in solution, independent of microtubule surface chemistry, resulting in similar overall intensities. The formation of tau condensates on the microtubules is driven by a prewetting-like transition, allowing an unstable liquid phase to get stabilized in the presence of a surface [64]. We elaborate upon this in the Appendix (Fig. 3.19), to show that our model does show a sharp rise in excess density, which is characteristic of a prewetting transition.

Overall, our simple lattice-gas model, which explicitly incorporates tau-microtubule and tau-tau binding, can capture the key experimental observations on different kinds of microtubules.

3.3.2 Microtubule surface heterogeneity dictates spatial pattern of tau condensation

At intermediate concentrations, tau condensates form on taxol-microtubules without covering the entire microtubule length. Although prewetting-like transition can explain the formation of tau condensates, the factors governing their spatial localization on the microtubules remain

unclear. In particular, at lower concentrations, experimental kymographs reveal that the tau condensates remain spatially pinned over time and fully disassemble upon tau withdrawal. Furthermore, experiments with alternating cycles of tau addition and removal on the same set of taxol-microtubules show that the condensates mostly form at the same positions along the microtubule (Fig.3.7, left). Our theoretical model, with a homogeneous tau affinity along the microtubule surface, fails to recapitulate these experimental observations. Our model predicts that tau condensates nucleate and form at arbitrary positions along the microtubule across independent simulations. Additionally, they do not stay pinned to their location but continuously move along the microtubule (Fig.23.7, centre). This behaviour is expected as there is no preferential bias for tau binding on the surface. These discrepancies between the theory and experiments prompted us to hypothesize that the tau affinity along the microtubule is spatially heterogeneous, for example, due to post-translational modifications (PTMs) of tubulin [124], and could be key to explaining the experimental observations. To systematically test this possibility, we introduced heterogeneity into the binding energy profile of tau along the microtubule surface, by sampling h_i randomly from a Normal distribution with mean $0 \text{ } k_B T$ and standard deviation $\sigma = 0.2 \text{ } k_B T$ (see Appendix for the effect of these parameters). We refer to this model as the “heterogeneous model”.

In sharp contrast to the homogeneous model, the heterogeneous model predicts the formation of spatially pinned condensates, in agreement with the experimental findings (Fig.3.8). Additionally, for a given profile of binding affinities, the spatial patterns of condensation are conserved across independent simulation runs, similar to the experiments (Fig.3.7, right).

To further test the equilibrium nature of the condensates, the tau signal was measured experimentally on taxol-microtubules over a wide range of concentrations (Fig.3.9, left). Interestingly, a complex tau-microtubule binding curve was found that could not be fitted to a single Hill equation, suggesting multiple binding regimes at different tau concentrations. In the low concentration regime, the binding exhibited highly cooperative behaviour (Fig. 3.9, left inset), while in the high concentration regime, the binding could be well approximated by a non-cooperative Michaelis-Menten behaviour. A good fit over the entire concentration range was obtained using

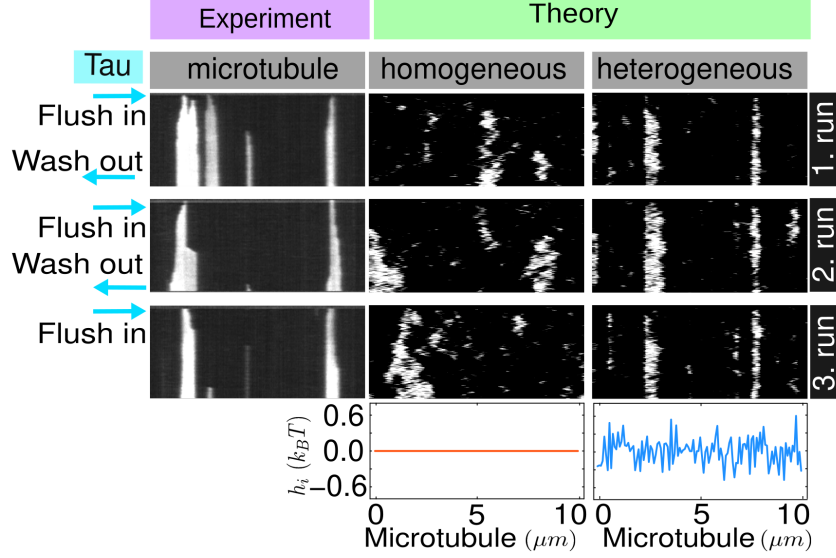


Figure 3.7: The homogeneous model (centre) cannot produce the same positions of the condensates across independent simulations. However, the heterogeneous model (right) can recapitulate this experimental observation (left). The experiments consisted of allowing the tau condensates to form, and then washing away the tau. This was done in a repeated manner.

the heterogeneous model, further substantiating the validity of our theory (Fig. 3.9, left, solid line). Additionally, within the same experiment, the fraction of microtubule length covered by the envelope, with increasing tau concentrations, could also be measured. Our model, with a heterogeneous surface, could quantitatively recapitulate the observed data (Fig. 3.9, right). Additionally, the two curves were used to obtain fits for the conversion parameters (α , c_0 and μ_0 in Eq.3.2) used to relate the concentration of tau in the experiments to the bulk chemical potential in Eq.3.1. The values are reported in Table 3.3.

We further compare the heterogeneous model to the experimental observations with different microtubule types. GDP-microtubules have a more compacted lattice, and GMPCPP-microtubules have an irreversibly extended lattice [112]. This results in different values of tau binding affinities as well as different values of tau cooperativity. We can incorporate this into our model by varying both $\bar{h}$ (mean of tau affinities on the microtubule) and J_s . Here, we take the value of J_s to be the same across all three microtubule types, and consider different values of $\bar{h}$. As mentioned in the previous section, we chose $\bar{h} = 0.0 k_B T$ for taxol-microtubules. For GDP-microtubules, we set $\bar{h} = 0.3 k_B T$, and for GMPCPP-microtubules we set $\bar{h} = -0.1 k_B T$.

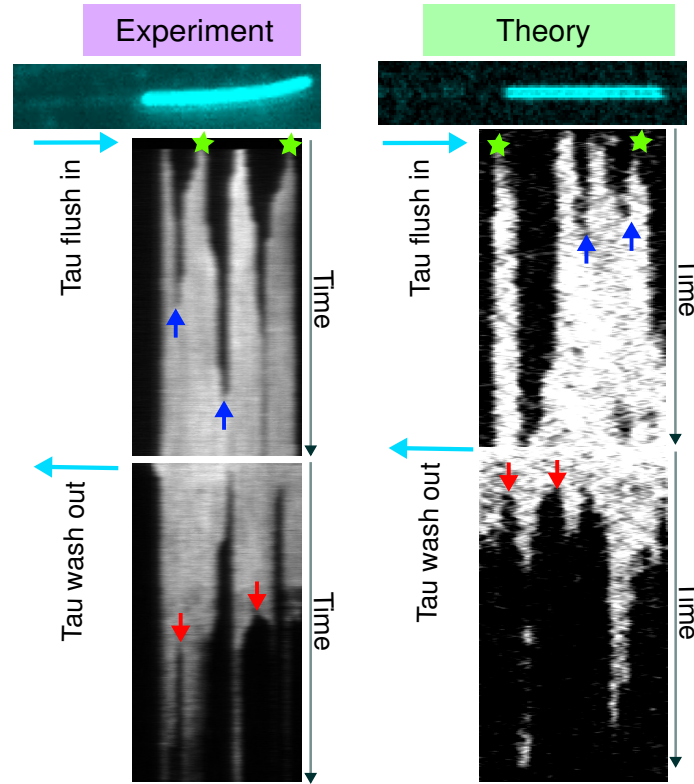


Figure 3.8: The heterogeneous model (right) can capture the kinetics of condensate growth along the microtubule in good agreement with the experiment (left). Green stars show nucleation of the condensates, blue arrows show fusion events, and red arrows show fission events.

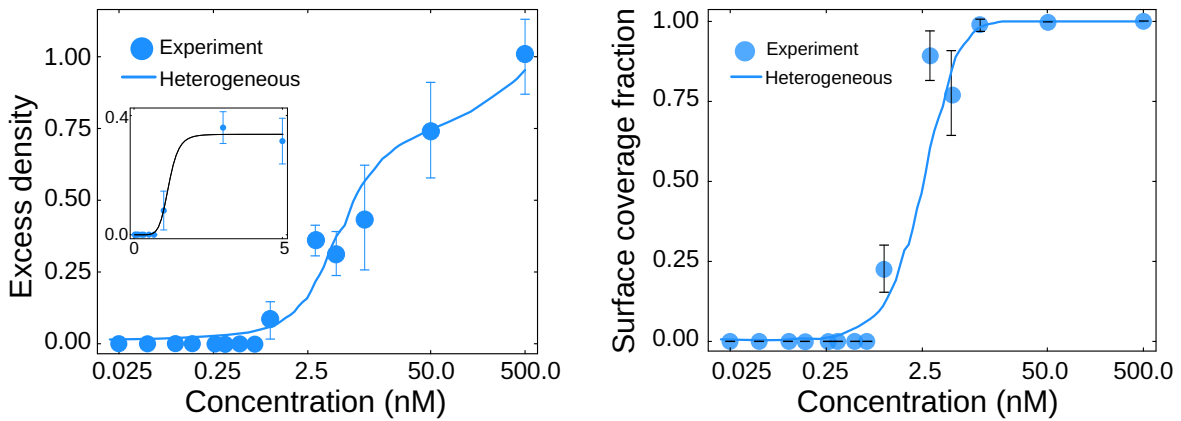


Figure 3.9: The heterogeneous model can explain the equilibrium behaviour of tau on taxol-microtubules for a wide range of concentrations. The inset in the left plot is a simple Hill function $\frac{Ac^n}{k^n + c^n}$, with $A = 0.337$, $n = 7$, $k = 1.137nM$.

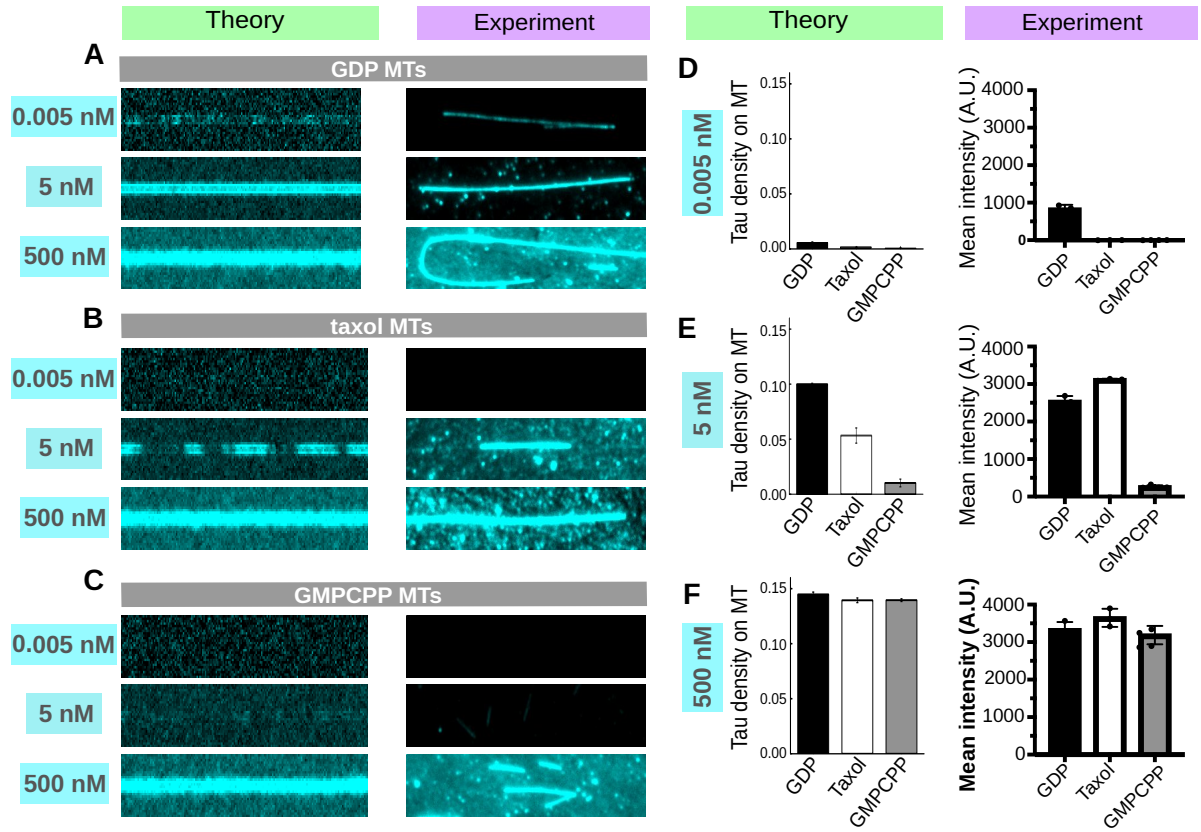


Figure 3.10: A-C) The heterogeneous model shows good agreement with the experimental observations for different microtubule types over a wide range of concentrations. D-F) shows the quantification for A-C

The level of heterogeneity, governed by σ (the standard deviation of tau binding affinities), is taken to be the same ($0.2 k_B T$) for all three microtubules. As mentioned in the previous section, tau covers the entire GDP-microtubule surface even at very low concentrations, whereas GMPCPP-microtubules show a dense, uniform layer only at very high concentrations (Fig.3.3). The heterogeneous model captures the equilibrium and kinetic behaviour across different concentrations for all three microtubule types very well (Fig.3.10, 3.11).

Our results so far suggest that the specificity of tau condensates for certain positions along the microtubule is intrinsic to the microtubule lattice itself and that an uncorrelated random mixture of tubulin isotypes or PTMs is sufficient to explain condensate nucleation and formation at distinct positions on the microtubule surface. To further validate this hypothesis and directly probe the role of microtubule heterogeneity for tau binding, experiments were performed with microtubules containing defined inhomogeneities in the content of tubulin variants in the microtubule

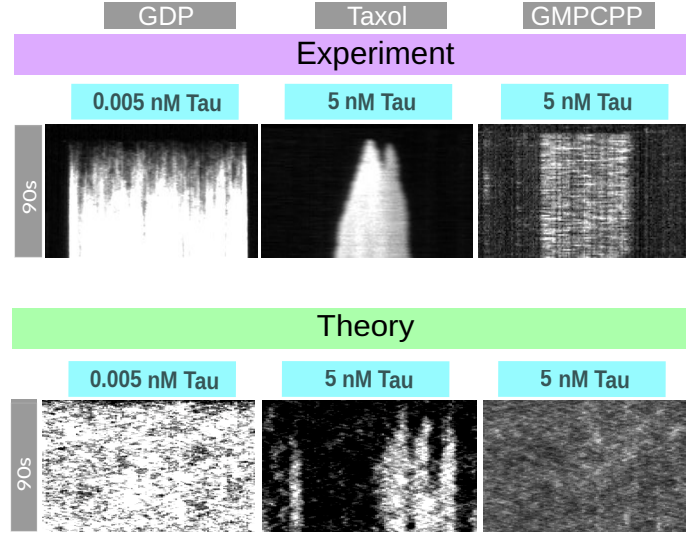


Figure 3.11: The kinetics of tau binding to different kinds of microtubules are captured by the heterogeneous model (A-GDP, B-taxol and C-GMPCPP microtubules).

lattice. To this end, hybrid microtubules were generated by annealing fragments of microtubules polymerized from pig brain tubulin, which have high levels of PTMs, and microtubules polymerized from tubulin isolated from HeLa cells, which have low levels of PTMs [125]. The experiments indicate that tau condensates form predominantly on the brain microtubule sections (Fig.3.12). To compare these results to model predictions, we construct an analogous profile of heterogeneity. Since PTMs contribute to a greater degree of heterogeneity [126], we assign $\sigma = 0.3k_B T$ (the standard deviation in the affinities) to the pig brain microtubules, and $\sigma = 0.01$ to the HeLa microtubules. Simulation results using this construction show good agreement with the experimental findings (Fig.3.12), further highlighting the role of surface heterogeneity.

In summary, a dialogue between theory and experiments indicates that heterogeneities in tau affinities along the microtubule surface, likely mediated by tubulin PTMs and isotypes, dictate the spatial pattern of tau condensate formation. Remarkably, results from our model show that even a random arrangement of these tubulin variants and PTMs is sufficient for the emergence of tau condensates at specific positions along the microtubule.

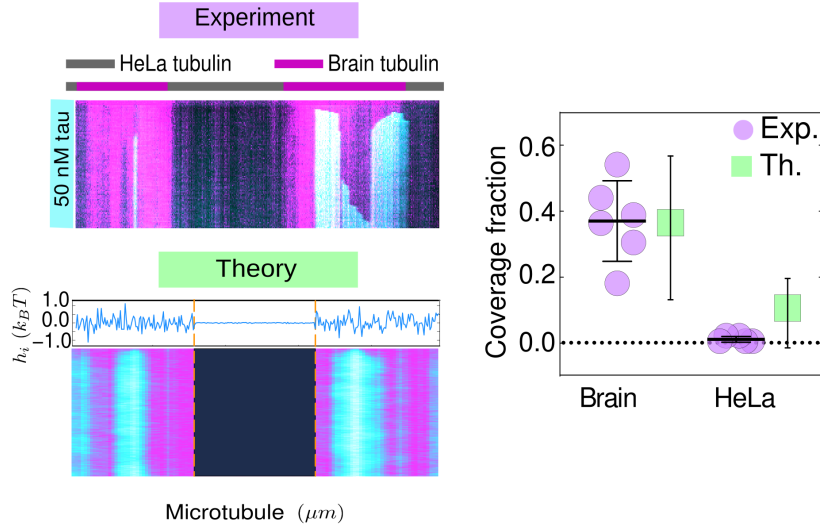


Figure 3.12: Heterogeneities in tau-binding affinity can arise due to PTMs. These, in turn, dictate the condensate positions.

3.3.3 Multilayered nature of tau condensates gives rise to two kinetically distinct populations of tau

Our model predicts the formation of multilayered tau condensates at high concentrations. The first layer of tau adsorbs onto the microtubule through the combined effects of tau-tau cooperativity and tau-microtubule binding affinity, determined by J_s and h_i , respectively. As this adsorbed layer becomes saturated, additional layers of condensed tau are recruited, governed by tau-tau interaction, as characterized by the bulk interaction J_b . The model predicts distinct timescales of dissociation for the adsorbed and condensed tau. More specifically, when the combined effect of J_s and h_i is significantly stronger than J_b , the model predicts that upon depleting tau from the bulk, the condensed layers will disassemble rapidly, followed by a slower decay of the adsorbed layer (Fig. 3.13, left).

To test this prediction, previously published data were reanalyzed [59]. In these experiments, tau condensates were allowed to form on taxol-microtubules. They were then dissolved by washing tau away from the solution, and the change in tau intensity on microtubules was monitored. Strikingly, the analysis shows that tau intensity in condensates decays with two characteristic timescales: within a couple of seconds, the intensity drops significantly, revealing a

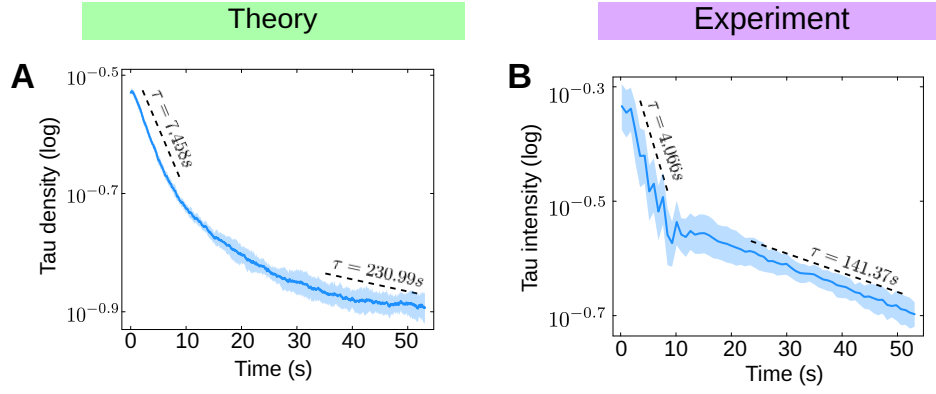


Figure 3.13: Our model predicts two timescales of tau dissociation (left). This arises because the tau molecules are bound more strongly to the microtubule surface than to each other in the bulk. Experimental observations confirm this prediction (right).

fast-turnover population within the condensates. This fast drop was followed by a much slower decay on the order of tens of seconds, during which the condensate disassembled, revealing the second tau population exhibiting slow-turnover (Fig.3.13).

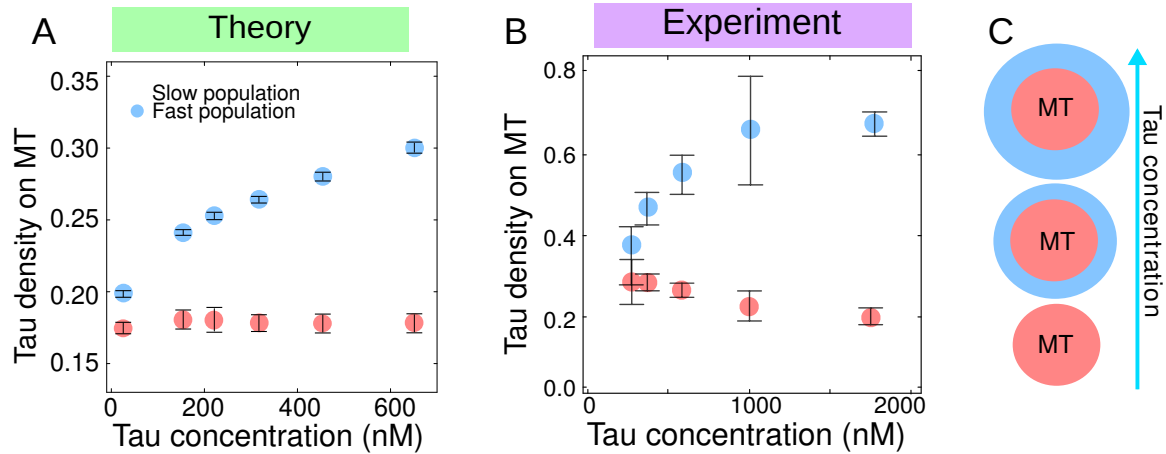


Figure 3.14: A) The model predicts that with increasing tau concentration in the solution, the number of tau molecules in the slow population remains constant, while the fast population density increases. B) Experimental confirmation of model predictions. C) Schematic representation of model prediction.

Moreover, insights from the model reveal how these two populations would behave with increasing tau concentration. Once the adsorbed layer (slow-turnover population) becomes saturated, further increase in tau concentration only leads to the formation of subsequent condensed layers (fast-turnover population). Therefore, the model predicts that the fraction of tau molecules in the slow-turnover population will remain constant, and the fraction of fast-turnover tau will

increase with increasing tau concentrations (Fig.3.14, left and right schematic). To test this model prediction, the wash-out experiment was carried out at different tau concentrations and quantified the relative content of the two populations in tau condensates. The result shows that the slow-turnover population remained approximately constant, whereas the fast-turnover population increased with tau concentration and saturated in the micromolar range (Fig.3.14, right), in close agreement with the model predictions. Monitoring tau turnover in a similar way on taxol-microtubule outside of the condensates showed only one tau population with fast turnover on the order of seconds. This experimental observation is consistent with the theoretical prediction that the regions outside the condensates only consist of a weakly bound layer of tau. The model also predicts that the condensed layer saturates and stops growing in thickness at sufficiently high tau concentration, since the condensate is still stable only near the surface, and not in the bulk.

To further probe the multilayered nature of tau condensates, we again turned to the model. The model predicts that varying the microtubule surface properties (via J_s and h_i) should not affect the fast population of tau, which is governed by J_b and μ . Consistent with this model prediction, experiments performed on GDP- and GMPCPP-stabilized microtubules, with tau condensates formed at 500nM tau, exhibit identical disassembly timescales for the fast-dissociating tau population, despite the markedly different microtubule surface properties (Fig.3.11).

Conversely, the model predicts that varying the tau concentration while keeping the microtubule fixed should not affect the slow population timescale, which corresponds to the first bound layer. Accordingly, the slow disassembly timescale is expected to be independent of tau concentration for a given microtubule (Fig.3.11). This prediction was also corroborated by experiments measuring the disassembly timescales on GDP-microtubules at 10nM and 500nM (Fig.3.11).

In summary, our model predicts, and the experiments confirm, the existence of two kinetically distinct populations of tau in the condensates. The two populations arise as a consequence of the multilayered nature of the tau condensates, with the slow-turnover tau molecules directly adsorbed onto the microtubule surface, and the fast-turnover tau population corresponding to

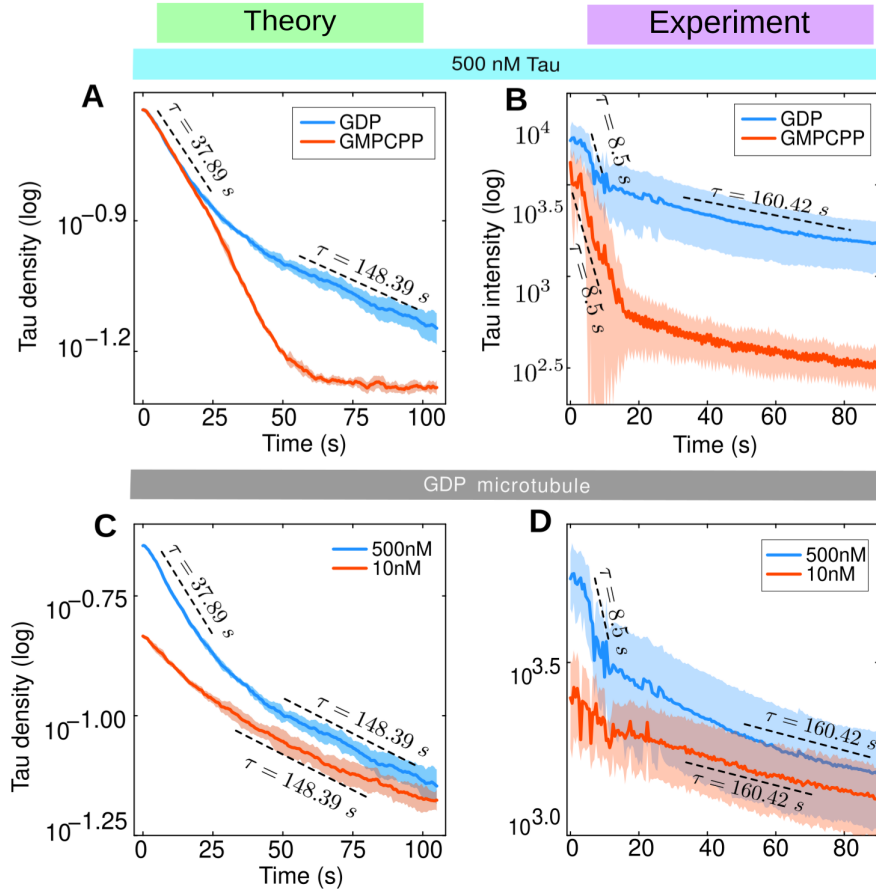


Figure 3.15: Timescales of tau condensate dissociation for GDP and GMPCPP microtubules. A) Our model predicts that the fast timescale should remain unaffected by the surface properties, since it arises only due to tau-tau interactions. B) Experimental results confirm this, showing the same fast timescale for GDP- and GMPCPP microtubules. C) Conversely, the model predicts that the slow timescale should be the same, independent of tau concentration in the solution. D) Experiments show identical slow timescales for GDP-microtubules with condensates formed at different tau concentrations.

molecules condensed onto the adsorbed layer at elevated tau concentrations.

3.3.4 Tubulin partitions into microtubule-condensed tau promoting microtubule growth

A dialogue between our theory and the experiments from collaborators suggests that at nanomolar tau concentrations, which is the physiological range [57], tau can form multilayered condensates on microtubules. We thus wondered whether, analogous to tau droplets formed by liquid-liquid phase separation, these surface-induced tau condensates could recruit tau interac-

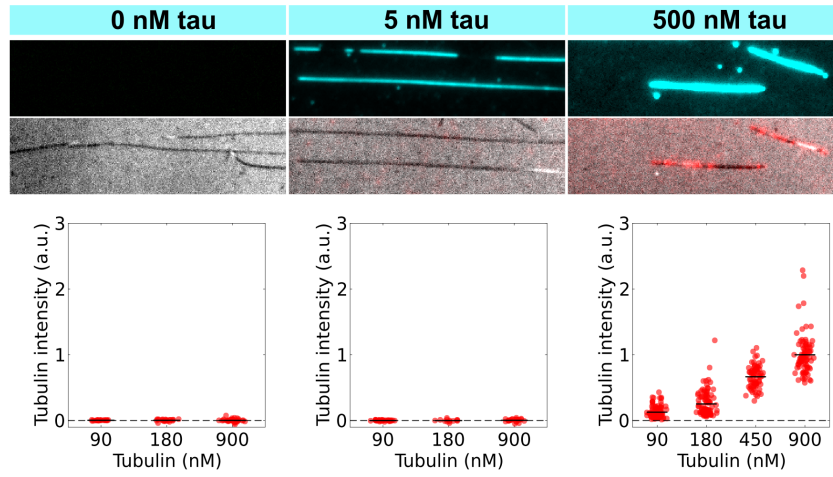


Figure 3.16: Experiments show that tau condensates (cyan) can incorporate tubulin monomers (red) only when they are formed above some threshold concentration (right).

tors, such as tubulin [121].

Experiments using labelled tubulin show that tau condensates can recruit tubulin molecules. However, this effect is seen only when the tau condensates are formed at high tau concentrations (Fig.3.16). We can shed mechanistic light on this observation based on our theory; at low tau concentrations, the condensates, comprising predominantly low-turnover tau, are not sufficiently thick to incorporate tubulin. However, increasing the tau concentration leads to the formation of additional layers, with a significant fraction of high-turnover tau, enabling the recruitment of soluble tubulin. Experiments also show that the recruited tubulin, within tau condensates, can increase the polymerization rate of the microtubules.

Combined with experiments, insights from the model show that the two tau populations comprising the tau condensates have distinct functions. While the low-turnover microtubule adsorbed layer forms the core of the condensates, it is the high-turnover outer layers that can recruit tau interactors, such as soluble tubulin or other microtubules. By recruiting these interactors, condensed tau can form a microenvironment with distinct functional properties, such as locally increased tubulin concentration leading to local enhancement of microtubule growth.

3.4 Discussion

In combination with experimental results from collaborators, we show using a lattice-gas model that tau forms condensates on microtubules. We find that tau–tau interactions drive a prewetting-like transition on the microtubule surface, in which tau binding shifts from a thin, surface-bound layer to a multilayered condensate as the bulk chemical potential is increased. The rapid increase of surface-bound tau density with increasing concentration suggests that a similar principle is at play. This sharp increase illustrates how changes in tau concentration in solution can give rise to distinct surface states: a sparse monolayer at very low concentrations ($< 1\text{nM}$), cooperatively bound tau molecules adsorbed to the microtubule at intermediate concentrations ($\sim 1\text{nM}$), and a multilayered surface-bound tau condensate at high concentrations ($> 100\text{nM}$). These distinct states can be collectively viewed as surface condensates, forming at different concentrations and rates depending upon the microtubule surface properties. Whereas liquid tau droplets form at highly elevated tau concentrations ($\sim 20\mu\text{M}$, in the presence of crowding agents) [50], surface-bound condensates form at sub-micromolar tau concentrations, reported as physiological [127], way below the saturation concentration for bulk phase separation. In this regime, the microtubule surface locally stabilizes the liquid-like condensate.

Here, we have presented a general theoretical framework for studying the condensation of biomolecules on subcellular surfaces. By accordingly modifying the surface, one can explore the interplay of molecules with various surfaces, such as membranes [37] or DNA. Moreover, different parameter regimes of the model can explain a wide range of behaviour. For instance, in the present work, tau first forms a fully saturated, adsorbed layer before subsequent layers assemble. However, this need not always be the case. A recent study [44] on the condensation of transcription factor KIF4 on DNA suggested that multiple layers can form even before the surface is completely saturated. Such behaviour can also be accounted for within our framework, in the regime where the bulk interaction strength is comparable to the surface coupling terms. Thus, our framework provides a basis for gaining mechanistic insights into the interaction of phase-separating proteins with various surfaces.

A dialogue between theory and experiments reveals that the spatial positioning of tau envelopes is governed by local variations in tau-binding affinity. Notably, surface condensates form over extended stretches of the microtubule where the average binding energy is slightly higher than in surrounding regions. These stretches may comprise a mix of weak and strong binding sites, yet collectively present an elevated mean affinity compared to the surrounding regions on the microtubule. This behaviour suggests that surface condensation does not rely on the affinity of single binding sites but instead responds to the average binding energy landscape of tau, enabling it to sense and integrate local heterogeneities to form condensates. A closely related phenomenon has been reported for the nucleoid-associated protein Lsr2, which forms co-condensates along with DNA at AT-rich regions, with condensate position being dictated by the average AT content over extended stretches of the DNA rather than by individual binding sites.[\[45\]](#).

Our results show that the condensed tau layer can recruit other microtubule-associated proteins to the microtubule surface. In the case of soluble tubulin, this process leads to enhanced microtubule growth. Microtubule polymerization is dictated by the binding rate of tubulin dimers to the growing microtubule end [\[109\]](#). This process can be viewed as a target-search problem, whereby free tubulin dimers must locate and bind to the growing microtubule end. Tau condensates, by locally recruiting and trapping tubulin dimers, increase tubulin concentration at the microtubule lattice and enable tubulin dimers to effectively carry out 1D diffusion along the microtubule surface, making the target-search problem more efficient. Thus, in addition to facilitated diffusion, which involves a combination of 3D and 1D search [\[128–130\]](#), surface condensation provides cells with an alternative mechanism for efficient target search on stiffer biopolymers with high persistence lengths. These observations are in line with recent data showing that tau can also enhance microtubule lattice repair by increasing the incorporation of tubulin into the shaft of a pre-existing microtubule [\[131\]](#).

We have employed a minimal model, with a small parameter set, that explains the key experimental observations. However, this also results in the following limitations of the model:

1. Our model is coarse-grained, considering a tau molecule effectively as a particle with no

internal degrees of freedom. Therefore, any analysis that requires consideration of the internal structure or sequence dependence of tau lies outside the scope of our model.

2. Previous experiments have shown that tau binding locally compacts taxol-stabilized microtubules [61]. This compaction, in turn, enables further recruitment of tau. Although this could be the microscopic origin of the cooperative nature of tau binding considered in our model, we do not consider any explicit changes to the microtubule lattice spacing or any consequent feedback mechanisms.
3. Our model does not account for non-equilibrium processes. In particular, effects such as microtubule growth and shrinkage or ATP-dependent activity are not included. This presents a natural direction to extend our framework.

To summarize, in this chapter, we aimed to shed light on the association of tau proteins with microtubules at physiological concentrations. We also sought to develop a general model to study the interaction of phase-separating proteins with intracellular surfaces. Our results, exemplified by tubulin recruitment mediated by surface condensation of tau, suggest a general mechanism for efficient target search on biopolymers in cells. Microtubules in a cell, especially in specific microtubule-rich cell types or cellular compartments, such as neurons and their protrusions, present a vast surface, where cellular factors could be concentrated, locally enabling specific biochemical reactions.

Appendix

3.A Characterizing phase separation in the bulk and on the surface

3.A.1 Identification of the bulk coexistence region

The concentration of tau in all the experiments is below the bulk phase separation threshold (undersaturated regime). Similar to the approach in Chapter 2, we first carry out simulations without the microtubule present to identify the bulk coexistence region. For this, we work in the canonical (NVT) ensemble, where the bulk volume fraction ϕ is specified. The last term in Eq.3.1, with μ , is dropped, and the particles are only allowed to be displaced, not inserted or removed. We identify the bulk coexistence region, as shown in the representative phase diagram in Fig.3.17A. Here, the red line represents the binodal curve described in Chapter 2, outside which only a homogeneous phase is thermodynamically stable [26, 100]. We also identify the critical point for the bulk coupling parameter as $J_b^c \simeq 0.18 k_B T$. This is also corroborated by the peak in the specific heat (Fig.3.17B), which diverges at $J_b = J_b^c$ in the thermodynamic limit [101].

We further verify that our simulations reproduce the expected droplet coarsening dynamics. For the average droplet size, we observe a scaling of $\langle R(t) \rangle \sim t^{1/3}$ (Fig.3.18A), in agreement with the Lifshitz-Slyozov-Wagner scaling, which describes droplet coalescence via Ostwald ripening [132, 133]. Together, these results confirm that our model correctly captures both the equilibrium and kinetic characteristics of classical bulk phase separation.

As mentioned in Chapter 2, the grand-canonical ensemble is better suited for studying wetting phenomena. So, instead of specifying the volume fraction ϕ for the bulk, we specify the bulk chemical potential μ (Eq.3.1). First phase diagram in the $J_b - \mu$ space is shown in Fig.3.18B, where the red line represents the first-order transition line between the dense and dilute phases,

Figure	$J_b (k_B T)$	$J_s (k_B T)$	$\bar{h} (k_B T)$	$\sigma (k_B T)$	$\mu (k_B T)$
3.17, A	varied	-	-	-	ϕ varied
3.17, B	varied	-	-	-	$\phi = 0.5$
3.18, A	0.3	-	-	-	$\phi = 0.2$
3.18	varied	-	-	-	varied
3.19, A	0.2	0.5	0.0	0.0	-2.905
3.19, B	0.2	0.5	0.0	0.0	-2.9
3.19, C	0.2	0.5	0.0	0.0	-2.905
3.19, D green	0.2	0.5	0.0	0.0	-2.92
3.19, D red	0.2	0.5	0.0	0.0	-2.8
3.19, D purple	0.2	0.5	0.0	0.0	-2.6
3.20, A, B	0.2	varied	varied	0.0	-2.92
3.20, C, D	0.2	0.5	varied	0.0	varied
3.21, A	0.2	0.5	0.0	0.2	-2.91
3.21, B	0.2	0.5	0.0	0.2	varied
3.21, C	0.2	0.5	0.0	0.2	-2.91
3.22, A	0.2	0.5	varied	varied	-2.92
3.22, B	0.2	varied	varied	0.2	-2.91
3.21, C	0.2	0.5	varied	0.2	varied

Table 3.2: Simulation parameters for the figures in the Appendices. Other common parameters are mentioned in Table 4.1.

terminating at the critical point J_b^c . This first-order transition line follows the relation $\mu = -\frac{qJ_b}{2}$, where $q = 26$ is the coordination number and $J_b > J_b^c$. At values of $\mu < -\frac{qJ_b}{2}$, the system will be in the undersaturated regime, which is the parameter regime of our interest. Additionally, in the dilute limit, we can relate the bulk chemical potential to concentrations in the experiments via the relation [134]

$$\mu = \mu_0 + \alpha \log \left(\frac{c}{c_0} \right), \quad (3.2)$$

where c_0 is the reference concentration at which $\mu = \mu_0$ and α is a scaling parameter with units $k_B T$.

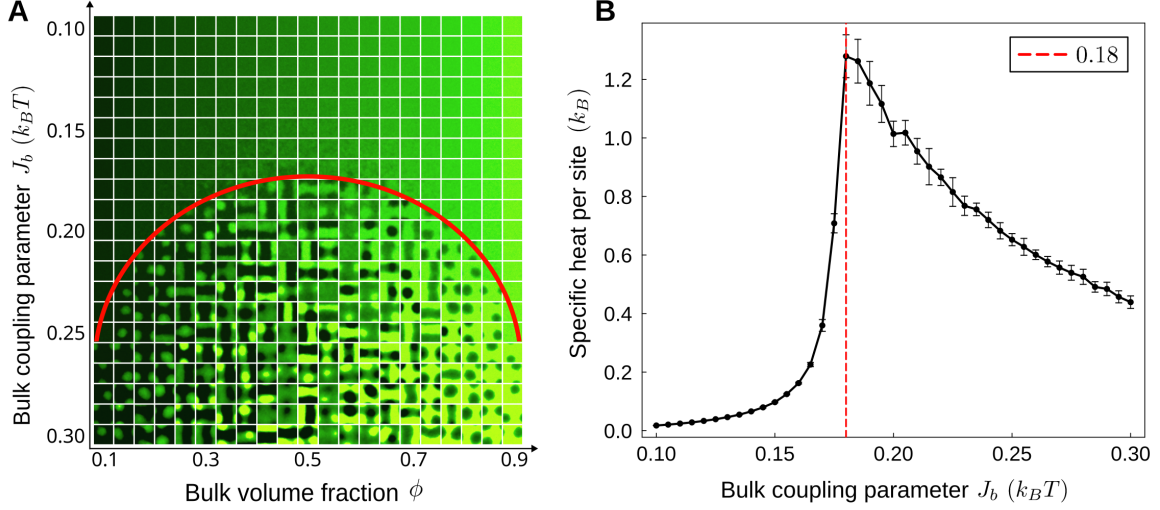


Figure 3.17: Identifying the bulk coexistence curve for the model. A) Shows the representative binodal region obtained by simulation in the absence of a surface, in the NVT ensemble. The critical bulk coupling is at around $0.18k_B T$. B) The peak in specific heat corroborates this (lattice size is $40 \times 40 \times 40$).

3.A.2 Excess density and the fraction of microtubule length covered captures the extent of tau condensation

As shown in the Results section, even in the undersaturated regime, a dense (liquid) phase can form, which is stable only in the vicinity of the surface. The extent of surface condensation of tau can be quantified using 1) the surface excess density[71, 107], and 2) the fraction of the microtubule length covered by the condensates. The excess density is defined as

$$c_s = \frac{1}{N} \sum_{n=1}^N (\phi_n - \phi_b), \quad (3.3)$$

where ϕ_n is the average occupancy of the n th layer and ϕ_b is the density in the bulk far away from the surface. The layers are defined radially outward from the microtubule surface, such that $n = 1$ corresponds to the first layer where the molecules are directly bound to the microtubule, $n = 2$ is the next outward layer and so on.

To obtain the density profile along the microtubule (Fig.3.6), we take the average density in a region of size 10×10 around the microtubule at each position along its length, and subtract the average bulk density from it. This is analogous to measuring the intensity of tau on the

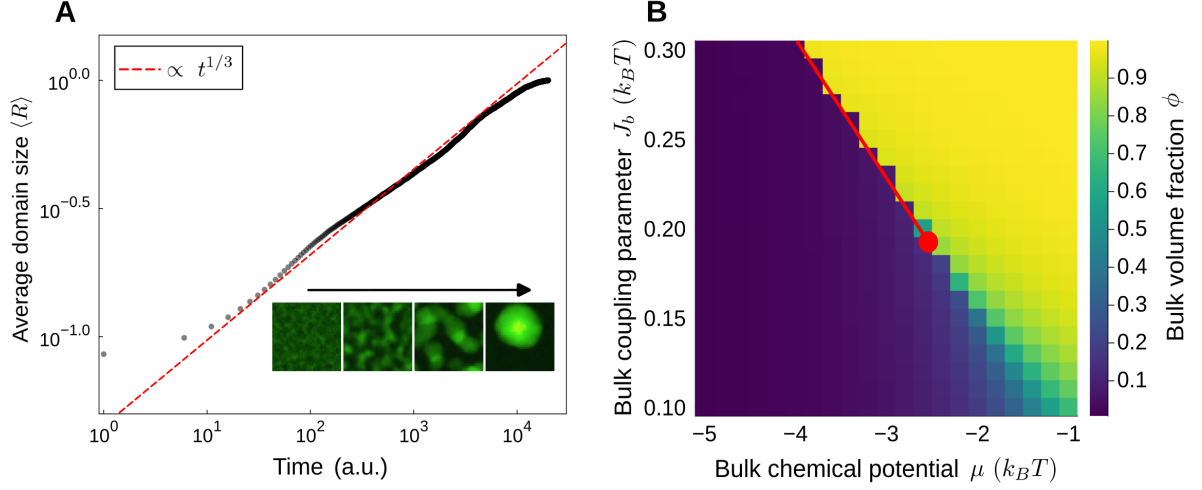


Figure 3.18: A) Kinetics of droplet growth agree with the Sylozov-Lifshitz law. B) The bulk phase diagram in the μVT ensemble.

microtubule surface from the microscopy data. The kymographs from simulation were similarly obtained, measuring the density profile along the length of the microtubule as a function of time (Fig.3.8). To study the disassembly kinetics of the tau condensates (Fig.3.8D), we first let the condensate form and equilibrate with some value of bulk chemical potential μ . Then, using this as our initial configuration, we evolve the system with a much lower value of μ , emulating the wash-out process carried out in the experiments (see respective figure captions for the exact parameter values).

3.B Parameter selection

In this section, we motivate our choice of parameters for the model. The average length of the microtubule in the experiments was about $10\mu\text{m}$. The size of a tubulin dimer is $\sim 8\text{nm}$ [109], with a stoichiometry between 1:2 and 1:5 tau: tubulin [120], giving us around 250-625 binding sites for tau along each protofilament. We take the microtubule to have $M = 300$ binding sites along each protofilament.

Since the tau condensates are localized at the microtubules, we can take a smaller size for the simulation box in the directions perpendicular to the microtubule axis. This is done to minimize the computation in the bulk. We take $L = 26$ sites.

The value of the bulk interaction parameter J_b is taken as $0.2 k_B T$ (above the critical value of $0.18 k_B T$). This sets the range for the bulk chemical potential as $\mu < -qJ_b/2 = -2.6 k_B T$, below which the system is undersaturated. The surface coupling parameter J_s is taken to be higher than J_b , indicating a positive binding cooperativity. Drawing comparisons with the experiments requires us to relate the chemical potential to the concentration of tau used. The parameters for this conversion (μ_0 , α and c_0 in Eq.3.2) were obtained as fitting parameters for the tau density and coverage data in Fig. 3.9. The simulation time step Δt was set to match the timescales of tau envelope assembly and disassembly in the experiments.

Parameter	Range/Value
Bulk coupling parameter, J_b	[0.18, 0.25]
Surface coupling parameter, J_s	[0.3, 1.0]
Surface heterogeneity mean, h	[-0.5, 1.0]
Surface heterogeneity std. deviation, σ	[0.0, 0.5]
Bulk chemical potential, μ	[-4.0, -2.6]
Reference chemical potential, μ_0	-2.904
Chemical potential scaling factor, α	0.05536
Reference concentration, c_0 (nM)	4.715
Number of tau binding sites, M	[120,300]
Simulation box size, L	26
Simulation time step per sweep, Δt (s)	0.035

Table 3.3: Parameter values used. (Units are $k_B T$ unless mentioned)

3.C An exploration of the model parameters

Here, we describe the behaviour shown by the model for different parameter regimes.

3.C.1 Homogeneous model

We first consider a homogeneous microtubule surface, where the binding affinity h_i has the same value at all the sites. As mentioned above, in our model, bulk condensation occurs at $\mu > -2.6 k_B T$. In the undersaturated regime, at low concentrations ($\mu \sim -2.9 k_B T$) dense patches, referred to as condensates, form on the microtubule surface (Fig. 3.5). Following the kinetics of tau condensation on microtubules, we find that the average length of tau condensates

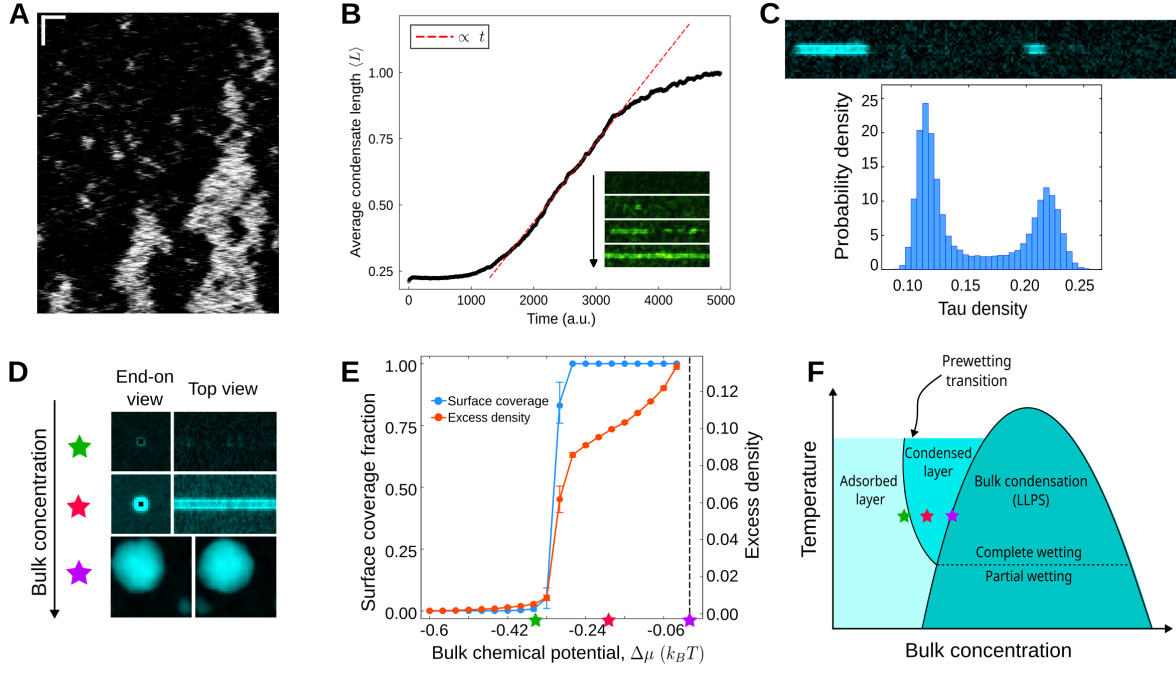


Figure 3.19: Homogeneous model. (A) Kymograph showing tau condensation on a homogeneous microtubule. The condensates are not pinned to their location. (horizontal scale bar is $1\mu\text{m}$ and vertical is 5s) (B) The kinetics of the condensate growing on the microtubule show the size increasing linearly with time. (C) Coexistence of the adsorbed state and the condensed state. Intensity distribution, taken from 100 simulations, shows a bimodal shape. (D) The green shows adsorbed tau, red shows condensed tau and purple shows bulk tau droplet. (E) The excess density (orange) and the fraction of microtubule surface covered by condensate (blue) show a sharp jump with increasing bulk chemical potential. $\Delta\mu = 0$ corresponds to bulk phase separation, indicated by a dashed line. (F) Schematic of the phase diagram showing the wetting and prewetting transition, originally proposed by Cahn [64].

increases linearly with time, $\langle l(t) \rangle \sim t$ (Fig. 3.20B) as expected for condensate growth on a one-dimensional surface [42]. However, the condensates are not spatially pinned, as seen in the experiments.

At equilibrium, tau predominantly exhibits two states: 1) at low concentrations, it is adsorbed directly onto the microtubule as individual molecules, and 2) at higher concentrations, it forms multilayer condensates (Fig. 3.6D). As the tau concentration is increased, the switch from a thin, adsorbed layer to a thick, condensed layer is sharp (Fig. 3.19E). Moreover, the two states can coexist near the transition concentration, as shown in Fig. 3.19C. This rapid switch is akin to a prewetting transition, which was first predicted by Cahn (Chapter 2) [64]. The prewetting transition is a first-order transition, where the surface state goes from a thin adsorbed layer

to a thick layer in a discontinuous manner (Fig. 3.20F). This is captured by a sharp jump in the excess density as we increase the concentration (bulk chemical potential μ) or the surface binding affinity h .

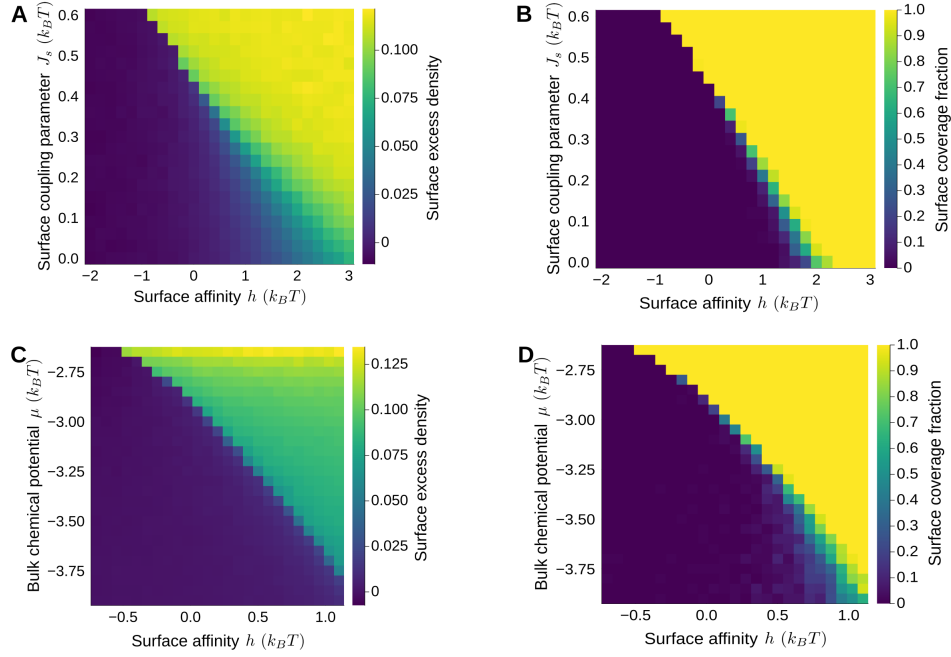


Figure 3.20: An exploration of the behaviour shown by the homogeneous model for various parameter values.

We then systematically vary individual model parameters to explore their effect on the equilibrium behaviour. Varying the surface coupling term J_s and the binding affinity h , we identify the coexistence line along which the thin and thick layers coexist (Fig. 3.20G, H). Similar to the bulk, there exists a critical point at which this first-order transition line terminates, beyond which the transition from an adsorbed layer to a condensed layer is no longer sharp. This can be seen for lower values of J_s (or equivalently for higher temperatures).

Next, we then vary the bulk chemical potential μ and the surface binding affinity h . It can be seen from the Hamiltonian (and from Fig. 3.20C, D) that the coexistence line can be approximated as $\mu + h = \text{constant}$. This means that one can effect an increase in the degree of surface condensation by either making the surface more attractive (increasing h) or by increasing the concentration of molecules in the bulk (increasing μ). For a given condensation profile, the choice of μ and h is not unique. We use this to set $h = 0 \text{ } k_B T$ in our model. Nevertheless,

to describe the entire system uniquely (both bulk and surface states), we must specify both parameters independently.

3.C.2 Heterogeneous model

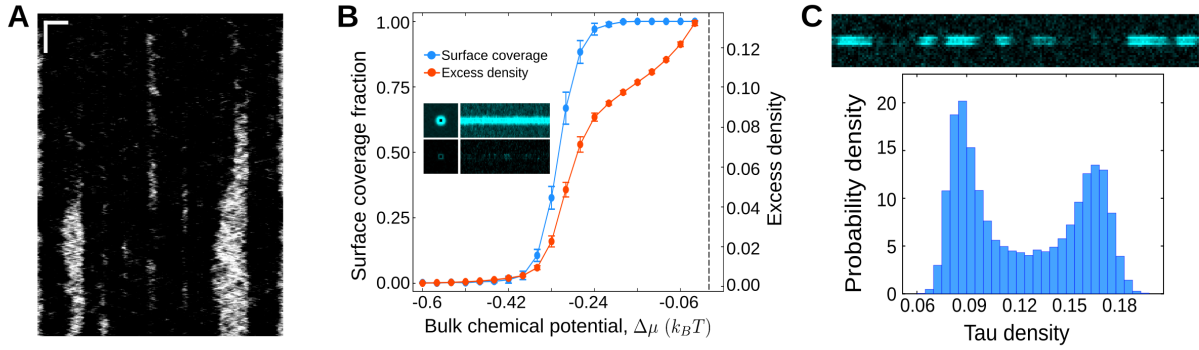


Figure 3.21: A) The heterogeneous model shows that the tau condensates are spatially pinned. B) Introducing heterogeneity results in a more gradual rise in the excess density and surface coverage, as compared to the homogeneous model (Fig. 3.19). C) Similar to the homogeneous model, the heterogeneous model also exhibits a bimodal distribution of densities.

As mentioned in the Results section, the homogeneous model fails to capture the following experimental observations: 1) the condensates form in the same place for a given microtubule, 2) the condensates stay pinned to their location. To explain these observations, we introduce heterogeneity in the binding affinity profile. The affinity at each position along the microtubule i is drawn randomly from a normal distribution $h_i \sim \text{Normal}(\bar{h}, \sigma)$. For simplicity, all 12 sites around the microtubule at position i have the same binding affinity. As described in the previous section, we set $\bar{h} = 0 \text{ } k_B T$, and choose $\sigma = 0.2 \text{ } k_B T$ (these parameters are chosen to capture the behaviour shown by taxol-microtubules; parameters for GDP- and GMPCPP-microtubules are given in the following section).

As in the homogeneous model, tau is adsorbed onto microtubules at lower concentrations and forms multilayer condensates at higher concentrations. However, the transition from thin to thick layer is no longer sharp, and the system exhibits coexistence between the two states for a wider range of concentrations (Fig. 3.22B, C). Importantly, the tau condensates remain pinned to their location on the microtubule, similar to the experiments (Fig. 3.22A)

We next examine how the mean binding affinity $\bar{h}$ and the degree of heterogeneity σ affect the kinetic and the equilibrium behaviour. As expected, increasing $\bar{h}$ leads to an increase in tau condensation on the microtubules. Increasing the heterogeneity σ broadens the region of coexistence between the thin and thick layers. This can be seen in Fig.??D, as the transition is sharper for lower values of σ , with $\sigma = 0 \text{ } k_B T$ (homogeneous surface) showing a very sharp transition. Furthermore, increasing σ results in the shorter condensate lengths on average (Fig. 3.22D).

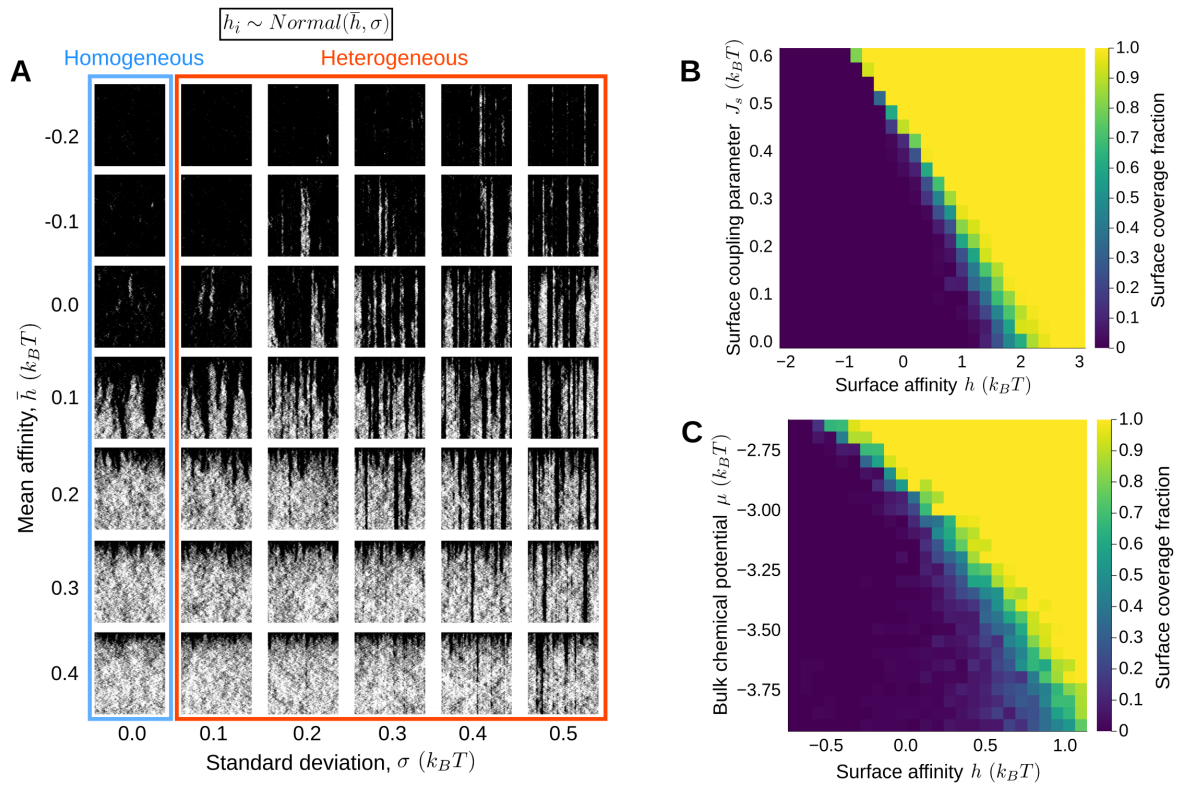


Figure 3.22: A) Effect of the heterogeneity parameters ($\bar{h}$ and σ) on tau condensation. B) An exploration of the behaviour shown by the heterogeneous model for various parameter values. The coexistence region gets broader due to the introduction of heterogeneities in the binding affinity.

3.D Previously proposed 1D model captures features of tau condensation on microtubules

A recent study investigated the interaction between the pioneer transcription factor KIF4 and lambda-DNA [44]. Similar to tau on taxol-microtubules, KIF4 showed a bimodal distribution of intensities on the DNA. The two states of KIF4 were identified as an adsorbed state and a condensed state, with the switch between them attributed to a prewetting transition. The resulting condensation patterns were shown to be correlated to the DNA sequence and were explained using a one-dimensional model. In this model, each site along the 1D lattice is assumed to be in one of two states: adsorbed or condensed. Here, we show that the same model can be used to capture the kinetic and equilibrium properties of tau condensation on microtubules as well.

In this model, the microtubule is considered as a one-dimensional lattice of M sites, where each site corresponds to the local wetting state of tau: -1 for a thin adsorbed layer and +1 for a thick condensed layer (Fig.3.23A). This model is equivalent to a one-dimensional random-field Ising model [108]. The effective free energy of the system is given by

$$E = -J \sum_{\langle i,j \rangle} s_i s_j - \sum_i (h + h_i) s_i \quad , \quad (3.4)$$

where the index $i = 1, 2, \dots, M$ and $\langle i, j \rangle$ indicate the nearest neighbouring pairs. The nearest-neighbour interaction is governed by the parameter J , and it reflects the interfacial tension in the wetted layers (related to J_b and J_s in our 3D model). Each site has some affinity to form a thin or thick layer, represented by the term h_i . The parameter h relates to the bulk chemical potential of the proteins, which in the dilute limit can be given as $h = h_0 + \alpha \log \left(\frac{C}{C_0} \right)$. Here C is the protein concentration in solution, and C_0 is a reference concentration. h_0 is the chemical potential at concentration C_0 and α is a proportionality constant, having the same dimensions as h . For simplification, we set $h_0 = 0$, and C_0 is the concentration at which roughly half of the

microtubule is covered by the condensed region.

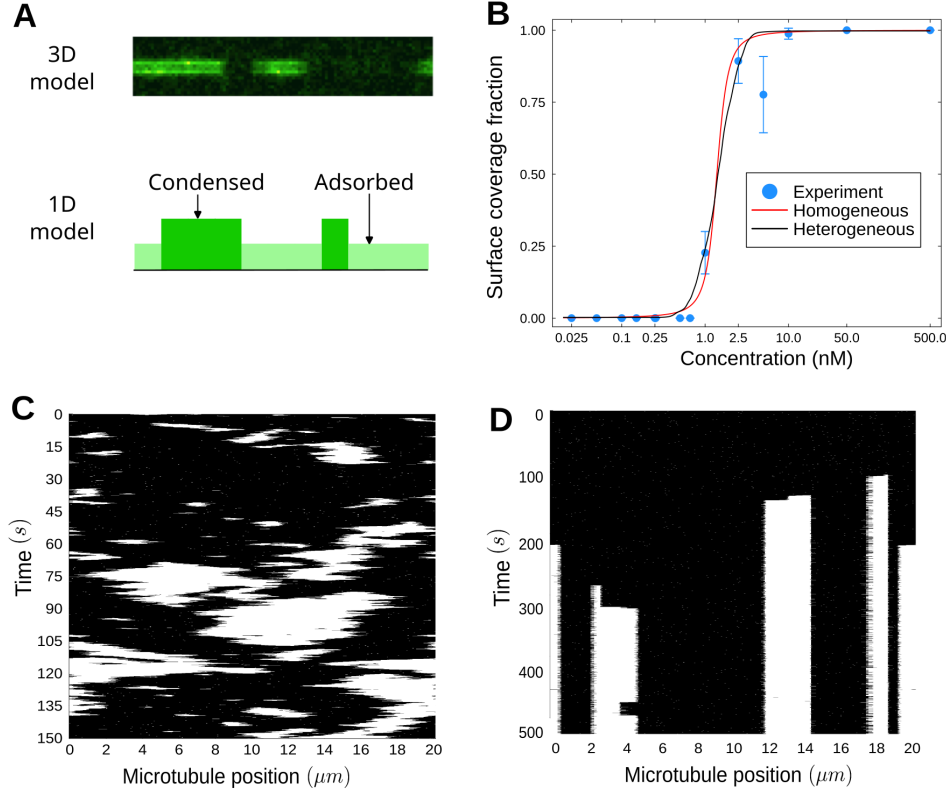


Figure 3.23: One-dimensional model for surface condensation. (A) Coarse-graining the 3D model gives us the 1D model of condensation of tau proteins on microtubules. (B) Both homogeneous and heterogeneous models can explain the equilibrium data (homogeneous model, red: $J = 2.3 k_B T$, $\alpha = 0.031 k_B T$, $c_0 = 1.382 nM$; heterogeneous model, black: $J = 4.5 k_B T$, $\alpha = 0.52 k_B T$, $c_0 = 1.43 nM$). (C) The homogeneous model cannot give rise to pinned islands ($A = 100.0$, $\delta = 0.1$). (D) Heterogeneous model gives rise to stable, pinned islands (h_i drawn from $\text{Normal}(0.0, 1.4)$, $A = 500.0$, $\delta = 0.001$).

As in the 3D lattice gas model, we consider two kinds of surfaces: a homogeneous surface with a constant binding affinity, and a heterogeneous surface with noisy binding affinities. For the case of homogeneous binding affinities, we set $h_i = 0$ and use the transfer matrix method [135] to obtain an expression for the fraction of microtubule covered by the tau condensate [44] as

$$\phi = \frac{1}{2} \left(1 + \frac{1 - \left(\frac{C}{C_0} \right)^{-2\beta\alpha}}{\sqrt{1 + 4e^{(-4\beta J)} + \left(\frac{C}{C_0} \right)^{-4\beta\alpha} - 2 \left(\frac{C}{C_0} \right)^{-2\beta\alpha}}} \right). \quad (3.5)$$

For a heterogeneous surface, we consider the site-dependent affinities h_i to be drawn randomly from a normal distribution with zero mean and standard deviation σ . We numerically compute the microtubule fraction in the condensed state, using J, α and C_0 as fitting parameters (see figure captions for parameter values)[44].

To study the temporal behaviour of the system, we employ the kinetic Monte Carlo method. The rate of switching any given site using the energy given in Eq.3.4 and the detailed balance condition can be defined [44] as. The rate of switching the j th site from $+1$ to -1 is taken as $\omega_j^- = A \exp(2\beta(\delta + 1)H_j)$, and for switching from -1 to $+1$ as $\omega_j^+ = A \exp(2\beta\delta H_j)$, where H_j is the local field at site j , and is given by $H_j = -J(s_{j-1} + s_{j+1}) - (h + h_i)$. The parameters A and δ dictate the timescales of island formation and growth, and are estimated by comparing with the experimental timescales.

We find that a homogeneous surface can explain the equilibrium behaviour for the surface fraction as a function of tau concentration (Fig.3.23B). However, since the binding affinity is the same everywhere on the surface, the homogeneous surface cannot give rise to stable pinned islands (Fig.3.23C). Additionally, across independent simulation trials, the homogeneous surface does not form condensed regions in the same locations, as observed in the experiments (Results section). However, a heterogeneous surface can capture both equilibrium and kinetic behaviour (Fig.3.23D).

While these results are in agreement with those obtained from the three-dimensional lattice-gas model, the latter is more general. The one-dimensional model assumes that there are two states: adsorbed and condensed, and the switch between them is a sharp transition, whereas in our lattice gas model, these states emerge due to tau-tau and tau-microtubule interactions.

Chapter 4

Temporal regulation of organelle biogenesis

In this chapter, we turn to membrane-bound organelles. We extend a previously proposed model of organelle biogenesis [84, 88] and systematically investigate its transient dynamics. First, we track how a population-level distribution of organelle abundance evolves over time (Fig. 4.1.B), computing the time-dependent mean and variance for various model variants. Second, we calculate the mean and variance of the first passage time required for a cell to reach a target organelle count from a given starting value. **The work presented in this chapter is published in Ref.[97].**

4.1 Model and methods

To study the time evolution of organelle abundance, we employ a general model of organelle biogenesis, as proposed by Mukherji et al. [84] and used in subsequent studies[88, 136]. This model consists of four different processes: de novo synthesis, fission, fusion, and decay, as shown in Fig. 4.1. De novo synthesis of an organelle happens from the Endoplasmic Reticulum with a zeroth-order rate constant k_d . An organelle copy can undergo fission with a first-order rate constant k_{fis} , thereby producing two copies. Two organelle copies fuse at a rate k_{fus} . Finally,

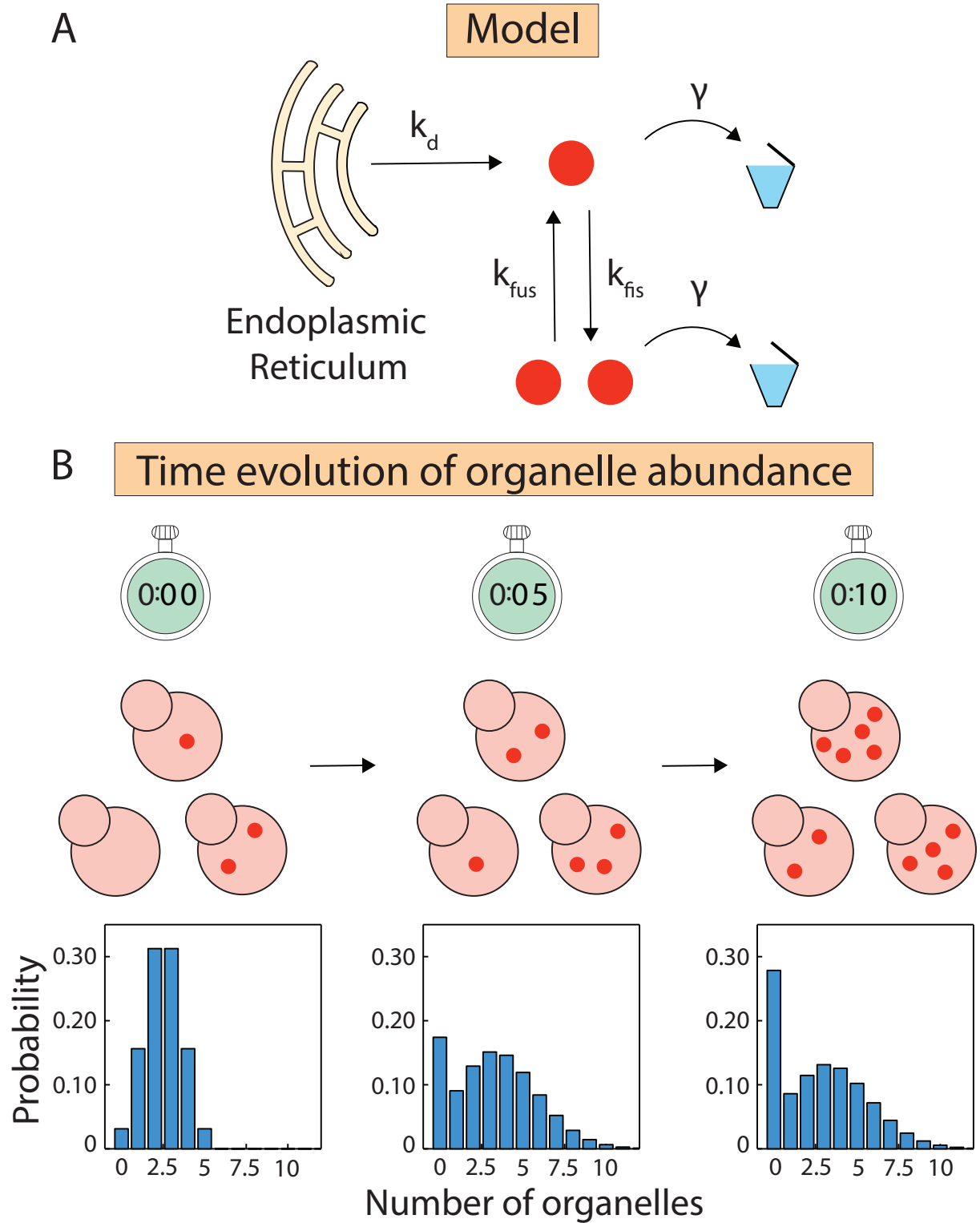


Figure 4.1: Model of organelle biogenesis. (A) We consider four processes that together govern organelle abundance in cells; these processes are de novo synthesis (k_d), decay (γ), fission (k_{fis}), and fusion (k_{fus}). (B) Live-cell imaging allows for the monitoring of organelle dynamics within a cell. Here we show a graphical representation of time-lapse snapshots of fluorescently labelled organelle copies in yeast. The bottom panel shows the time evolution of the histogram of organelle abundance across an isogenic cell population.

an organelle copy can decay with a first-order rate constant γ . While de novo synthesis and fission increase the number of organelles, fusion and decay decrease their number. Through an interplay between these opposing processes, the organelle abundance evolves in time and eventually attains a steady state. Here, we are interested in the transient properties of organelle abundance.

To explore the transient properties of the model, we employ a master equation-based approach and monitor the time evolution of the organelle number. The probability $P_n(t)$ of having n organelles in a cell at a time t is given by the master equation

$$\begin{aligned} \frac{dP_n(t)}{dt} = & [k_d + (n-1)k_{\text{fis}}]P_{n-1}(t) + [(n+1)\gamma + n(n+1)k_{\text{fus}}]P_{n+1}(t) \\ & - [k_d + n(\gamma + k_{\text{fis}}) + n(n-1)k_{\text{fus}}]P_n(t) \end{aligned} \quad (4.1)$$

This equation is a collection of all possible ways that lead to either an increase or a decrease in organelle copy number. Using this equation, we compute the mean and variance of 1) the transient distribution of organelle abundance and 2) the first passage times to reach a specified organelle copy number. These moments allow us to discern between different models of organelle biogenesis.

To unravel the effect of different mechanisms of organelle biogenesis on the transient properties in organelle abundance, we consider different limits of the general model. Following up on previous studies [88], we consider five such limiting scenarios that have a corresponding finite steady state, namely : (i) de novo synthesis-decay, (ii) fission-fusion, (iii) de novo synthesis-fission-decay, (iv) de novo synthesis-fusion-decay, and (v) de novo synthesis-fission-fusion-decay.

First, we focus on the time evolution of organelle copy numbers. The master equation describing the time evolution of organelle numbers is one-dimensional. For such a system, the steady-state solutions for the mean, variance, and its distribution can be computed using different methods [84, 88, 136]. However, the presence of the fusion process renders the transient so-

solutions inaccessible, owing to the corresponding nonlinear terms in the master equation (Eq.4.1). For models without fusion, i.e. (i) de novo synthesis-decay, and (ii) de novo synthesis-fission-decay, we compute the exact mean and variance of organelle distribution as a function of time using the generating function technique [134]. For the rest of the models, we employ a recently developed numerical method by Smith and Shahrezaei [137] to compute the transient organelle distributions. The details of the calculations are provided in the Appendix (4.A). In order to compare the cell-to-cell variability in organelle abundance for various models, we employ a statistical measure of dispersion called the Fano factor. The Fano factor is defined as the ratio of variance to mean.

Additionally, we also seek to compute the first two moments of the distribution of the first passage times (FPT) to reach X copies of the organelle, starting from a given number of copies. The FPT for a random variable is defined as the first time a some specific value is reached [138]. Such first passage times can be extracted from the same data of real-time imaging of fluorescently labelled individual organelles at a single-cell level.

For transient solutions, the initial condition needs to be specified. Here we consider two different scenarios. First, we assume the number of organelle copies to be the same across all the cells in a population. Second, we assume the number of organelle copies across a population to be binomially distributed. This initial condition is motivated by our question of how organelle abundance attains a steady state after cell division. During cell division, the number of organelles is partitioned between the two daughter cells. For a random partitioning of organelles, the number of organelle copies in daughter cells will follow binomial statistics [139]. This can be thought of as a coin toss problem, whereby each organelle copy is assigned to one of the daughter cells with a given probability. Notably, our theoretical framework allows for the use of arbitrary initial conditions, making it well-suited for comparison with experimental data. This flexibility is elaborated upon in the Discussion section of this chapter. Here, we present illustrative examples using binomially distributed initial copy numbers for the mean and Fano factor dynamics, and uniform initial copy numbers (same in all cells) for the first passage time statistics. These choices are intended to demonstrate the working of the theory.

4.2 Results

We consider different limits of the general model and make specific predictions about transient organelle abundance as we tune the relevant model parameters. The parameter values explored lie within the range reported in previous studies employing similar models [84, 88]. A summary of the parameters used is provided in the table below:

Rate constants	Range of values (min^{-1})
De novo synthesis, k_d	0.4-40.0
Decay, γ	0.1-4.0
Fission, k_{fis}	0.1-10.0
Fusion, k_{fus}	0.01 - 10.0

Table 4.1: Range of parameters explored. The minimum values indicate the smallest non-zero values used. In processes where a particular mechanism is absent, that rate constant is simply zero.

4.2.1 Mechanisms of organelle biogenesis govern the transient cell-to-cell variability

We seek to discriminate between the different mechanisms of organelle biogenesis using the temporal properties of organelle numbers. In particular, we focus on i) the transient mean and variance for various subsets of the model, and ii) the first two moments of the first passage time distribution to reach a given organelle count.

For all the mechanisms, the transient mean evolves monotonically to eventually attain steady-state values. In other words, the evolution of mean organelle number as a function of time doesn't allow for distinguishing between different mechanisms. This behaviour persists for a variety of initial conditions (see Appendix, Fig. 4.8 - 4.12). In sharp contrast, the transient Fano factor of organelle numbers, a measure of cell-to-cell variability in organelle abundance, often exhibits qualitatively distinct behaviour corresponding to different mechanisms of organelle

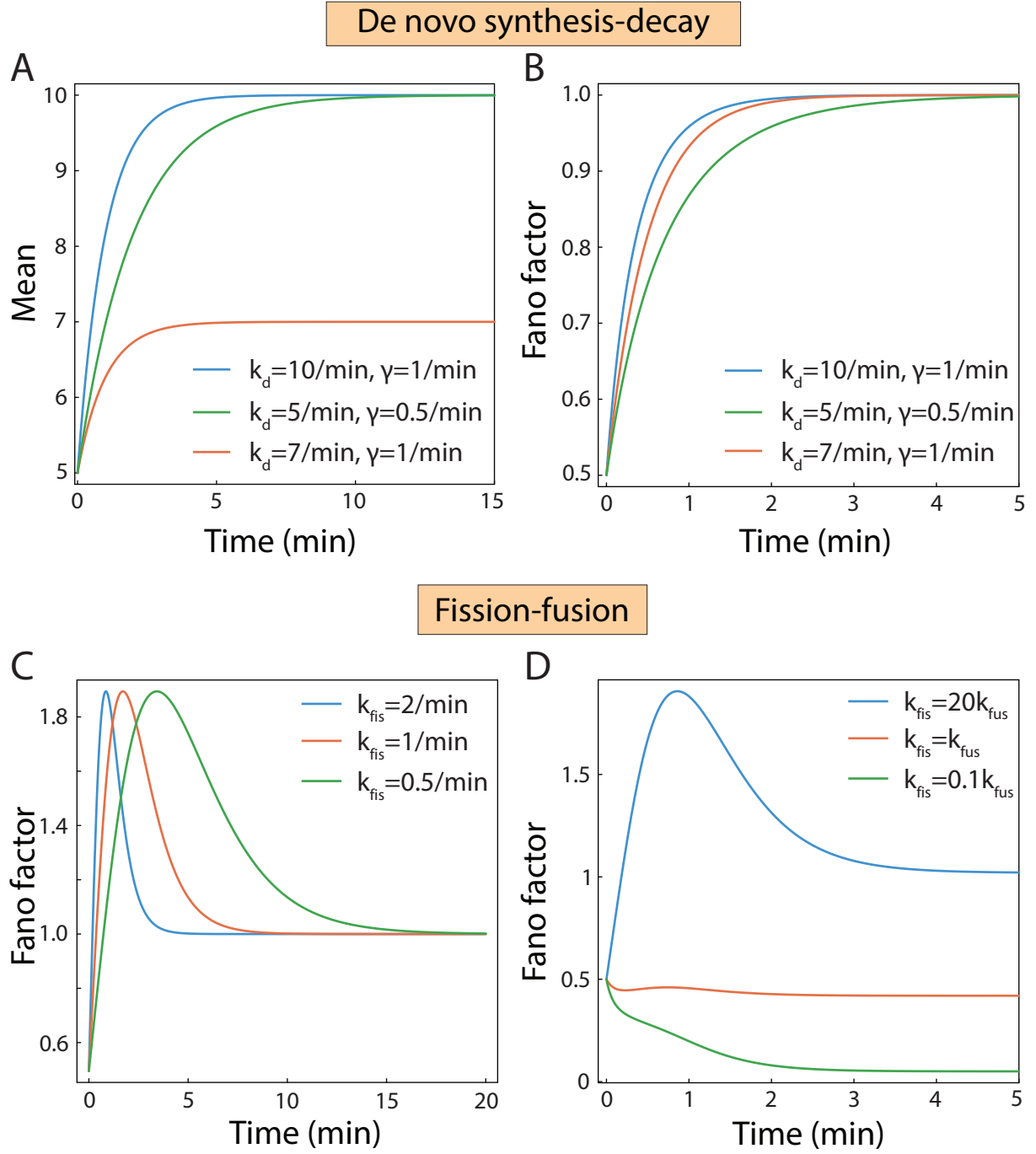


Figure 4.2: Mean and Fano factor profiles for various models. (A) De novo synthesis-decay: The mean number of organelles for various values of de novo synthesis and decay rate is shown. (B) De novo synthesis-decay: Here, we plot the time evolution of the Fano factor for various values of k_d , with $\gamma = 1 \text{ min}^{-1}$. (C) Fission-fusion: Dependence of the Fano factor keeping the ratio of parameters constant, $k_{\text{fis}}/k_{\text{fus}} = 20$, and (D) Fano factor profiles for various values of fission rate k_{fis} and the fusion rate k_{fus} .

biogenesis. In fact, the Fano factor for all mechanisms (except de novo synthesis–decay) shows non-monotonic behaviour. Below, we discuss the results for the individual models in detail.

De novo synthesis – decay

First, we consider the de novo synthesis-decay model. Owing to its simplicity, this model provides a good starting point. This model has been shown to effectively capture the biogenesis of the Golgi body [84, 140–143]. Since the steady-state organelle abundance is Poisson distributed [88], the Fano factor is one. We seek to investigate how the mean and Fano factor of organelle abundance approach the steady state for this model. We compute the mean $\langle n(t) \rangle$ and Fano factor $F(t)$ as a function of time (see Appendix for details).

$$\langle n(t) \rangle = \frac{k_d}{\gamma} - e^{\gamma t} \left(\frac{k_d}{\gamma} - Np \right), \quad (4.2)$$

$$F(t) = \frac{k_d(1 - e^{-\gamma t}) + e^{-2\gamma t}(e^{\gamma t} - p)\gamma Np}{k_d - e^{-\gamma t}(k_d - \gamma Np)}. \quad (4.3)$$

Using these analytical expressions, we find that the mean and Fano factor monotonically approach unity for parameter values and initial distributions (see Appendix, Fig. 4.8), as shown in Fig. 4.2 A, B. These results suggest that the de novo synthesis-decay model can act as a reference to interpret the outcomes of more complex models.

Fission – fusion

Next, we explore the transient properties of the fission-fusion model. Biogenesis of mitochondria in mammalian cells and vacuoles in yeast is controlled through a balance of fission and fusion [84, 87, 144–146]. The steady-state organelle abundance for this model is given by a truncated Poisson distribution, which is characterized by the ratio of the fission and fusion rates [88]. We seek to explore how organelle abundance evolves in time to attain a steady state. To this end, we consider different values of fission and fusion rates. The mean abundance increases monotonically to eventually reach the steady state value (see Appendix, Fig. 4.9, A, B).

In contrast, Fano factor behaves non-monotonically with time, often exhibiting a maximum, see Fig. 4.2 C, D. Interestingly, the transient Fano factor can exceed one when the rate of fission is higher than the fusion rate, that is, the model is fission dominated. This is a key result as, in the steady state, the Fano Factor for this process is bounded by one. This non-monotonic behaviour diminishes as the average organelle number at $t = 0$ grows, even for the same parameter values. To estimate the effect of the spread of the initial distribution at $t = 0$, we choose binomial distributions with a fixed mean but different variances (Appendix, Fig. 4.9 (B, D)). There is a slight increase in the maximum Fano factor for a noisier initial distribution, but the behaviour of the mean has very little dependence on the nature of the initial distribution, and it monotonically reaches the steady state value (see Appendix, Fig. 4.9 B).

A possible reason for this non-monotonicity is due to a competition between fission and fusion. When the fission rate is much higher than fusion, and the initial mean is low, for the first part of the trajectory, fission would dominate and increase the noise, but as the copy number grows, fusion with its n^2 dependence would start to counter fission and lower the noise. In Appendix, Fig. 4.9 (C), we see that a higher initial mean results in a less prominent maximum, as the organelle numbers are high enough for fusion to dominate from early on. This non-monotonic behaviour of the Fano factor may be very useful in discriminating this mechanism of organelle biogenesis from the de novo synthesis–decay case, as the steady state Fano factors of both would be very close to unity, and the steady state distributions would be very similar and difficult to distinguish.

For the aforementioned models, consisting of two competing processes, the Fano factor clearly shows qualitatively distinct behaviour as a function of time. In the ensuing sections, we consider models with three processes.

De novo synthesis – fission – decay

The de novo synthesis-fission-decay model is often invoked to describe the biogenesis of peroxisomes in yeast [84, 136]. While the steady state of this model is characterized by two effective parameters (ratio of de novo synthesis and fission rates, and fission and decay rates,

respectively), the transient behaviour is more complex. Using the generating function technique [134], we compute the mean $\langle n(t) \rangle$ and Fano factor $F(t)$ of organelle abundance as a function of time. The mean and Fano factor are given by

$$\langle n(t) \rangle = \frac{k_d}{\gamma - k_{\text{fis}}} + e^{(k_{\text{fis}} - \gamma)t} \left[Np + \frac{k_d}{k_{\text{fis}} - \gamma} \right], \quad (4.4)$$

$$F(t) = \frac{\left(\gamma k_d + e^{(k_{\text{fis}} - \gamma)t} (\gamma + k_{\text{fis}}) [Np(\gamma - k_{\text{fis}}) - k_d] + e^{2(k_{\text{fis}} - \gamma)t} \{ k_d k_{\text{fis}} - Np(\gamma - k_{\text{fis}}) [k_{\text{fis}}(2 - p) + \gamma p] \} \right)}{(\gamma - k_{\text{fis}}) \left[k_d + e^{(k_{\text{fis}} - \gamma)t} (\gamma - k_{\text{fis}}) \left(Np + \frac{k_d}{k_{\text{fis}} - \gamma} \right) \right]}. \quad (4.5)$$

Using these expressions, we investigate the time evolution of the mean and Fano factor as the different parameters are tuned (see Fig. 4.3 A, B). As earlier, the mean abundance shows a monotonic behaviour for all the parameter values as indicated by the expression for $\langle n(t) \rangle$. The Fano factor, however, shows non-monotonic behaviour and has a maximum for certain parameter values. The steady-state Fano factor is independent of the de novo synthesis rate and the initial distribution, but the maximum Fano factor the system achieves is dependent on these parameters. This maximum is prominent for smaller values of the de novo synthesis rate (which results in a lower steady-state mean) and higher means of the initial distribution of organelle numbers (Appendix, Fig. 4.10 (C)). The behaviour of the Fano factor has very little dependence on the variance of the initial distribution (Appendix, Fig. 4.10 B, D). For higher values of the de novo synthesis rate or a lower mean of the initial distribution, the Fano factor behaves monotonically. The presence of a prominent maximum coupled with a high steady-state value of the Fano factor would be indicative of this model.

De novo synthesis – fusion - decay

The de novo synthesis-fusion model is defined by three parameters, namely, de novo synthesis, decay, and fusion rate constants. Like the previous model, we can expound the behaviour of the mean and Fano factor as a function of time (see Fig. 4.3 C, D). The mean monotonically reaches the steady state value, and its trajectory remains unchanged even if the variance of the

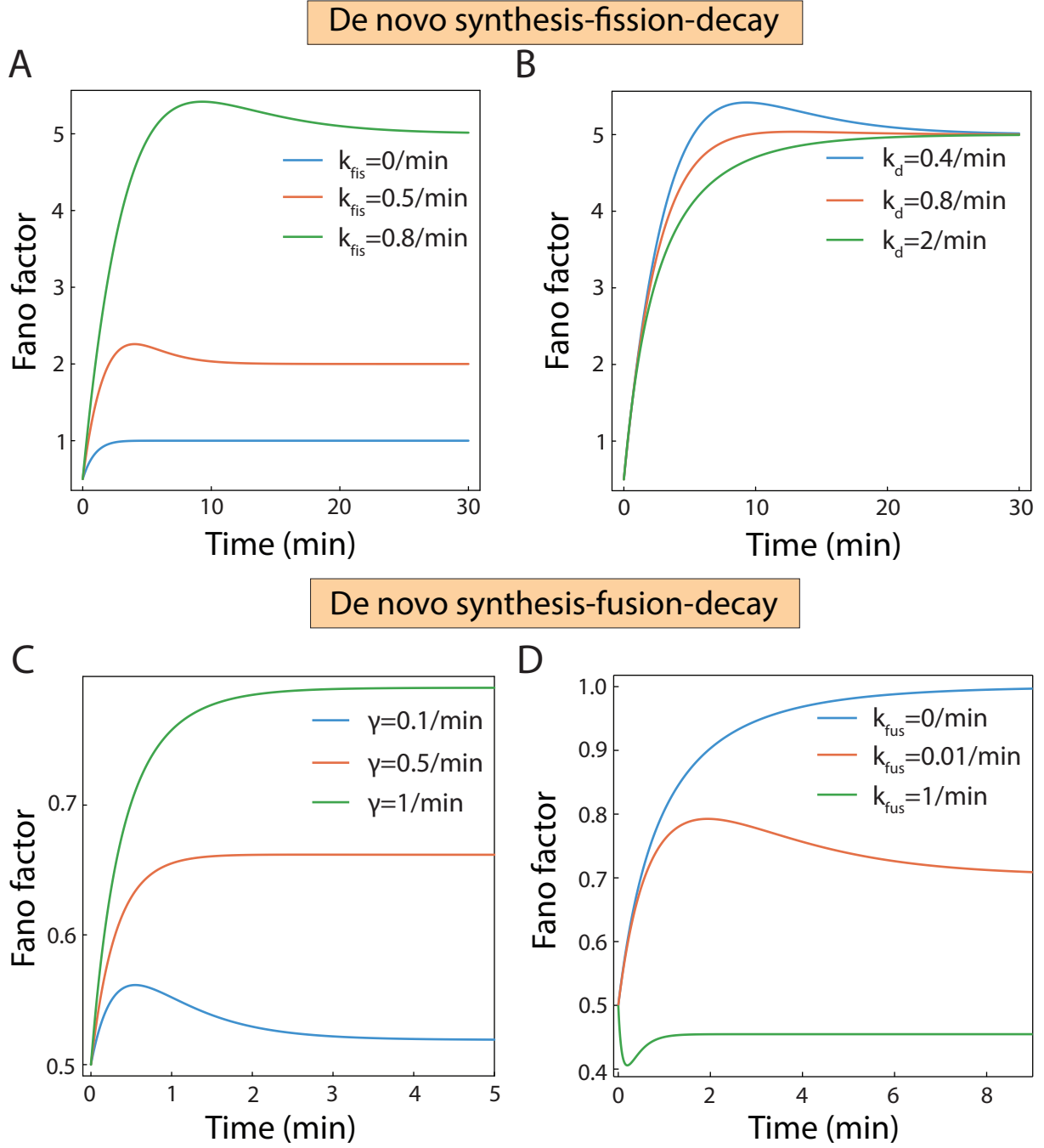


Figure 4.3: Time evolution of noise profiles for various models, with the initial number of organelles following a binomial distribution. (A) De novo synthesis-fission-decay: In this plot, we vary the fission rate k_{fis} , while keeping the other rates constant; $k_d = 0.4 \text{ min}^{-1}$, $\gamma = 1.0 \text{ min}^{-1}$. (B) De novo synthesis-fission-decay: Fano factor profiles for different values of the de novo synthesis rate k_d , keeping $\gamma = 1 \text{ min}^{-1}$ and $k_{\text{fis}} = 0.8 \text{ min}^{-1}$. (C) De novo synthesis-fusion-decay: Here, we plot the Fano factor as a function of time for various values of the decay rate γ , with $k_d = 5 \text{ min}^{-1}$, $k_{\text{fus}} = 0.1 \text{ min}^{-1}$, and in (D) we vary the fusion rate k_{fus} , with $k_d = 5 \text{ min}^{-1}$ and $\gamma = 0.2 \text{ min}^{-1}$.

initial distribution is altered. For certain values of the parameters, again, a non-monotonic behaviour is seen in the behaviour of the Fano factor with time. Whether the Fano factor exhibits a minimum or a maximum depends on the parameters used and the initial distribution. In most of these cases, the extrema are not very prominent compared to the other models. It must be noted that the introduction of fusion to the de novo synthesis-decay model qualitatively alters the behaviour of the Fano factor even for a small fusion rate.

Why do we see the maximum in this model when there is no fission to increase the noise, as in the last two models? The decay rate and the fusion rate are responsible for lowering the organelle number. For low initial values of organelle number, early on in time, decay would dominate as the decay rate is higher than the fusion rate. This would resemble a de novo synthesis-decay process. As the copy numbers increase, fusion with its n^2 dependence would start to dominate over decay and lower the noise. As seen in the figures in Appendix, Fig. 4.11 (E), when the initial mean is higher, both processes would be equally important. Even so, this maximum is weaker compared to the ones seen in models involving fission and is coupled with a low steady-state Fano factor. Conversely, when the fusion rate is higher, or when we take higher initial means, fusion dominates right from the start and the trough becomes more prominent as can be seen in Appendix, Fig. 4.11(F).

De novo synthesis-fission-fusion-decay

De novo synthesis-fission-fusion-decay model captures the biogenesis of endosomes and lysosomes in HeLa cells [147]. The Fano factor of the complete model can show peaks as well as troughs and a range of steady-state values, depending on whether the process is fission-dominated or fusion-dominated. Similar to the fission-fusion model, the Fano factor corresponding to the complete model may exhibit a maximum when it is fission-dominated (Fig. 4.4), but it would have a steady state mean above unity. The mean for this model also evolves monotonically and shows little dependence on the noise in the initial distribution (see Appendix, Fig 4.12).

Overall, our results demonstrate that the mechanisms of organelle biogenesis dictate the

transient cell-to-cell variability in organelle abundance, which allows us to discern between the different mechanisms.

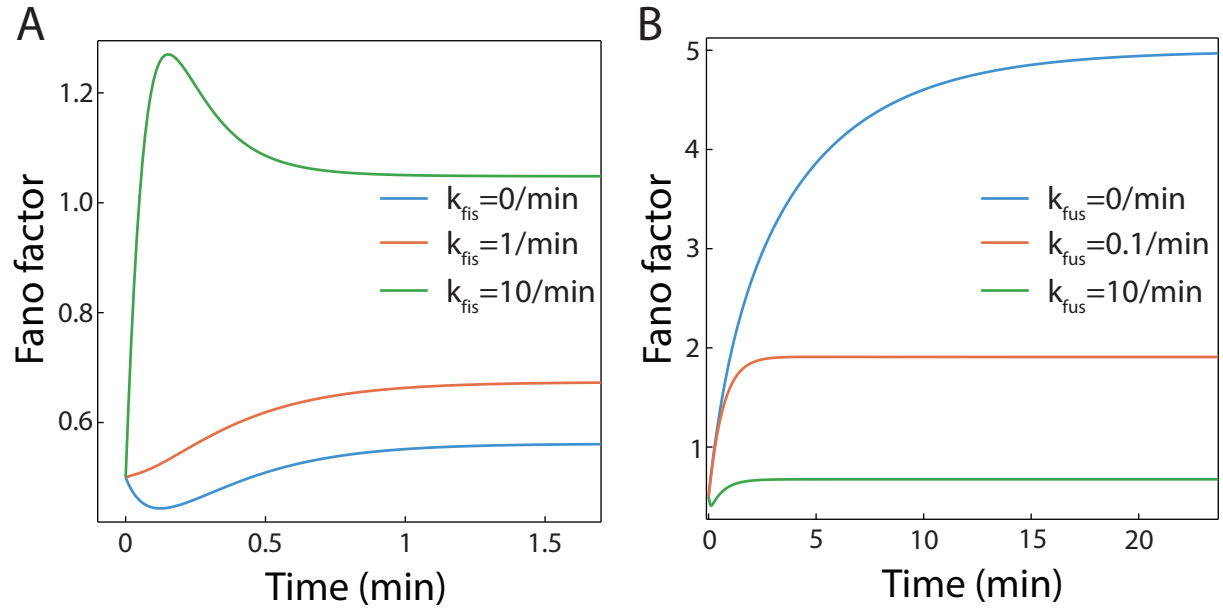


Figure 4.4: De novo synthesis-fission-fusion-decay. (A) In this plot, we vary the fission rate k_{fis} and look at the time evolution of the Fano factor, with $k_d = 5.0$, $\gamma = 0.9 \text{ min}^{-1}$, $k_{\text{fus}} = 1.0 \text{ min}^{-1}$. The curve for $k_{\text{fis}} = 0$ (blue) corresponds to the de novo-fusion-decay. (B) Here, we vary the fusion rate k_{fus} , while keeping $k_d = 0.7 \text{ min}^{-1}$, $k_{\text{fis}} = 0.8 \text{ min}^{-1}$, $\gamma = 1 \text{ min}^{-1}$. The other values are the same. The curve for $k_{\text{fus}} = 0$ (blue) is included for comparison with the de novo synthesis-fission-decay model.

4.2.2 Effect of different mechanisms of organelle biogenesis on the first passage times

We aim to investigate how various mechanisms of organelle biogenesis influence the time required for the organelle copy number to reach a specified value, X , for the first time, starting from zero copies (except the fission-fusion mechanism, which begins with one copy). These times are referred to as first passage times (FPT). Specifically, we compute the first two moments of the FPT distribution for different biogenesis mechanisms, adapting the method described in Ref.[148]. The mean first passage time (MFPT) for the De novo synthesis–fission–fusion–decay model is given by:

$$\langle T \rangle_{0,X} = \sum_{i=1}^X \left[\frac{1}{k_d + (i-1)k_{\text{fis}}} + \sum_{l=i}^{X-1} \frac{\prod_{n=i}^l \beta_n}{k_d + lk_{\text{fis}}} \right], \quad (4.6)$$

where $\beta_n = \frac{n[\gamma + (n-1)k_{\text{fus}}]}{[k_d + (n-1)k_{\text{fis}}]}$.

The second moment of the first passage times distribution is given by

$$\langle T^2 \rangle_{0,X} = 2 \sum_{i=1}^X \left[\frac{\sum_{m=1}^i \eta_m}{k_d + (i-1)k_{\text{fis}}} + \sum_{l=i}^{X-1} \frac{\left(\prod_{n=i}^l \beta_n \right) \left(\sum_{m=1}^{l+1} \eta_m \right)}{k_d + lk_{\text{fis}}} \right], \quad (4.7)$$

where β_n is the same as above, and $\eta_n = \frac{1}{k_d + (n-1)k_{\text{fis}}} + \sum_{l=n}^{X-1} \frac{\prod_{m=n}^l \beta_m}{k_d + lk_{\text{fis}}}$.

The expressions for other models can be obtained by setting the appropriate rate constants to zero. The exception is the fission-fusion process, for which the mean and the second moment of FPT are given by

$$\langle T \rangle_{0,X} = \frac{1}{k_{\text{fis}}} \sum_{i=1}^{X-1} \left[\frac{1}{i} + \sum_{l=i}^{X-2} \frac{\prod_{n=i}^l \beta_n}{l+1} \right], \quad (4.8)$$

and

$$\langle T^2 \rangle_{0,X} = \frac{2}{k_{\text{fis}}^2} \sum_{i=1}^{X-1} \left[\frac{1}{i} \sum_{m=1}^i \eta_m + \sum_{l=i}^{X-2} \frac{\left(\prod_{n=i}^l \beta_n \right) \left(\sum_{m=1}^{l+1} \eta_m \right)}{l+1} \right], \quad (4.9)$$

where $\beta = \frac{(n+1)k_{\text{fus}}}{k_{\text{fis}}}$ and $\eta = \frac{1}{m} + \sum_{l=m}^{X-2} \left(\frac{\prod_{n=m}^l \beta_n}{l+1} \right)$.

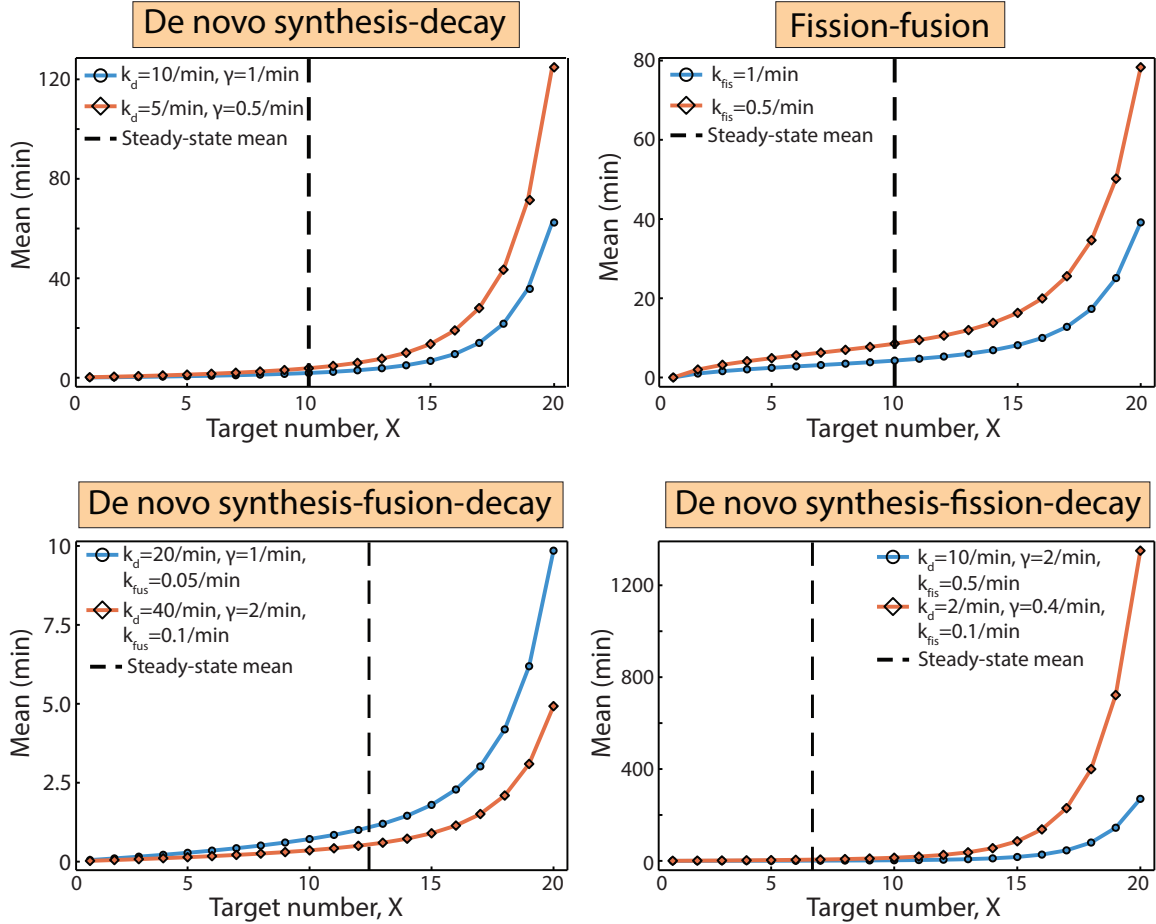


Figure 4.5: Mean first passage times. Here, we plot the mean first passage times as a function of the target number X . In each plot, the parameters are varied while keeping the ratio of rates (hence the mean steady state number) constant. (For B. $k_{\text{fis}}/k_{\text{fus}} = 10.0$)

For all the models considered, the MFPT increases monotonically and rises sharply once X exceeds the mean steady-state levels. Similar to the transient dynamics, the MFPT is dependent on the individual parameter values and not just their ratios. The parameters k_d and k_{fis} promote an increase in organelle number and therefore reduce the MFPT when their values are increased. Similarly, increasing γ and k_{fus} increases the MFPT. However, if multiple parameters are varied keeping the ratios constant, only the effect of changing k_d and k_{fis} is observed, since γ and k_{fus}

occur only in ratio with k_d or k_{fis} (Eq. 4.76). For example, in Fig.4.5(A), keeping the ratio of k_d/γ constant, we see that the MFPT decreases as we increase k_d .

As a measure of noise in the distribution of first passage times, we consider the coefficient of variation (CV). The CV is defined as the ratio of the standard deviation to the mean. Importantly, unlike the MFPT, the CV depends only on the ratio of the parameters, as can be seen from Eq.4.6 and Eq.4.7. At $X = 1$, the CV is one since it is a Poisson process described by a single rate-limiting step [134]. It decreases with increasing X , reaching the minimum value around the steady-state mean level. After this, the CV rises again, eventually reaching 1 for X much larger than the steady-state mean level. This is because at large X the first passage times are governed by a single time scale—the time required to cross the interval $[0, X]$ —which follows an exponential distribution.

Increasing k_{fis} or k_d increases the steady-state mean value, decreases the MFPT (Fig.4.5), and decreases the CV in FPT (Fig.4.6). The opposite is true while increasing γ and k_{fus} .

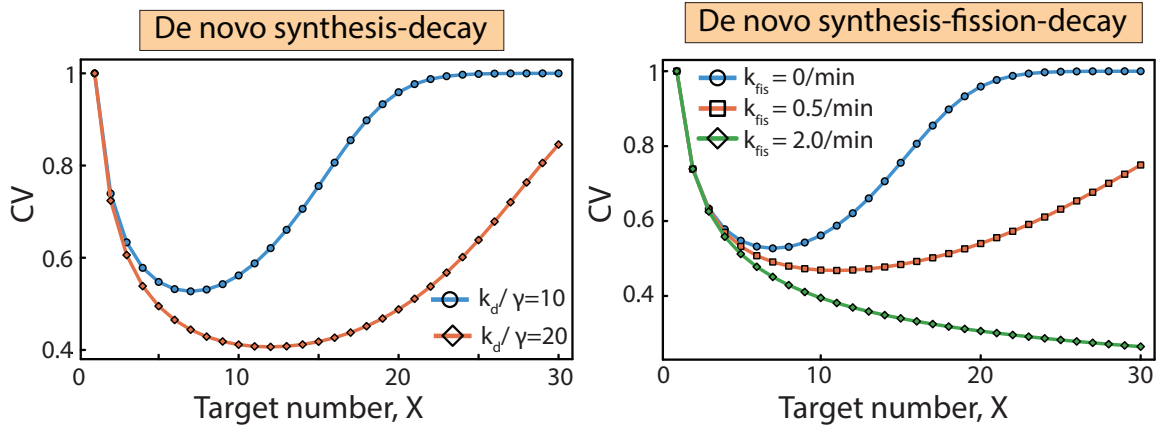


Figure 4.6: Coefficient of variation. Here, we plot the CV of the FPT distribution as a function of the target site X . The CV depends only on the ratio of the rates, and hence will be identical for similar processes with the same steady-state levels. (Parameters used for A. $\gamma = 1.0 \text{ min}^{-1}$ and B. $k_d = 10.0 \text{ min}^{-1}$, $\gamma = 1.0 \text{ min}^{-1}$)

4.3 Discussion

The abundance of organelles is highly regulated in cells. Unravelling the mechanism of organelle biogenesis has been a key area of investigation in cell biology. This area of research

has received a boost in recent years owing to our ability to experimentally observe and count organelle copies at a single-cell level through live and fixed-cell imaging [82, 83, 149, 150]. The advent of these experimental techniques has provided a window of opportunity to undertake a theory-experiment dialogue in order to refine our understanding of the mechanisms of organelle biogenesis. In this chapter, we focus on time-lapse organelle abundance measurements, which remain largely untapped, to decipher how organelle abundance is regulated in response to internal and external cues. In particular, we theoretically investigate how the Fano factor of organelle distribution changes as a function of time for various mechanisms of organelle biogenesis. We find that different mechanisms exhibit qualitatively distinct behaviour, thereby allowing us to discriminate between these mechanisms. Variability in organelle abundance across cells can result from factors such as nutrient levels, environmental conditions [151], and progression through the cell cycle [77]. Our framework accounts for these influences implicitly, as they manifest through changes in the effective rate parameters of organelle biogenesis processes.

Transient solutions are more informative about parameter values than steady-state

Previous studies [84, 88], which tried to falsify models of organelle biogenesis by comparing model predictions to data obtained from fixed cell imaging, were predicated on the assumption that organelle abundance distribution is in a steady state. While this assumption has been useful in interpreting experimental data, it can lead to incorrect conclusions. For instance, the Fano factor for the fission-fusion model in the steady state never exceeds one, which was used as a unique feature of this model to discern it from other models [88]. In sharp contrast, the transient Fano factor for the fission-fusion model can be higher than one, as shown in Fig. 4.2C,D. Hence, it is imperative to be cautious while applying theoretical results to interpret experimentally measured organelle distributions, as obtained from fixed cell imaging. Moreover, the transient behaviour of various models is richer than the steady-state behaviour. For instance, the steady-state distributions of all the models we consider are functions of the ratio of the associated rate constants.

As an example, the steady-state distribution of the fission-fusion model depends on the ratio of fission and fusion rate constants. The average organelle abundance can be increased by decreasing the fusion rate or increasing the fission rate. In a steady state, it is not possible to discriminate between these two possibilities. However, the time it takes to reach the steady state depends on which of the two rates is altered (see Fig. 4.2C, D). Hence, it is possible to discern between these two scenarios by observing the transient Fano factor.

The various processes involved in organelle biogenesis govern the transient as well as the steady-state properties of organelle number distribution. Moments of these distributions can be used to infer the mechanisms of biogenesis. A comparison between transient and steady-state distributions depicts intriguing differences; while the steady-state mean and the Fano factor depend on the ratio of rate parameters, the transient mean and Fano factor often depend on their absolute values. This is evident from the analytical solutions for both the mean and Fano factor of de novo synthesis–decay and de novo synthesis–fission–decay models. For the de novo synthesis–decay model, the time it takes for the mean and Fano factor to attain steady state depends on the degradation rate γ as in Eq. 4.2, whereas the steady-state distribution depends on the ratio of de novo synthesis and decay rates, which is a dimensionless quantity. In Fig. 4.2(A), (B), we show that the rate at which the mean and Fano factor reach steady state depends on γ ; the curves corresponding to $\gamma = 1 \text{ min}^{-1}$ but different k_d values reach the steady states in similar times despite having different steady-state distributions. For the de novo synthesis–fission–decay model, the time taken for the mean and Fano factor to reach steady state (Eq. 4.4) depends on $\gamma - k_{\text{fis}}$ (Fig. 4.3 (A)). In the case of the fission-fusion model (and all other models involving fusion), closed-form analytical solutions are not obtainable. Hence, the dependence of the mean and Fano factor on rate parameters as a function of time is not known. However, it can be seen that the transient mean and Fano factor depend on the absolute values of the rate parameters; in Fig. 4.2 C, we keep the steady state moments fixed by keeping the ratio of k_{fis} and k_{fus} unaltered while changing the value of k_{fis} .

Transient behaviour is often more diverse than steady-state behaviour, depending on the initial conditions. Such diverse behaviour can provide valuable insights into the mechanisms of

organelle biogenesis. By introducing experimental perturbations, it may be possible to observe and analyze transient responses, leading to a deeper mechanistic understanding

Dialogue between theory and experiment

Our work aims to combine theory and experiments to dissect the molecular and biophysical mechanisms driving organelle biogenesis. Such a dialogue entails a careful consideration of the initial distribution of organelle copy numbers within a cell population. In the current work, we have taken the initial distribution for the organelle copy number following binomial statistics for the mean and Fano factor dynamics, and a uniform copy number for the first passage statistics. These are, however, only illustrative examples to demonstrate the theoretical framework. One can, in principle, specify any arbitrary initial condition in Eq.4.16 and Eq.4.67, making the framework well-suited for direct comparisons with the experiments. For example, one can define the initial time point $t=0$ as the moment following a perturbative event, such as cell division, external stresses or signalling cues, and measure the organelle copy number distribution across cells. This empirically determined distribution can then serve as the initial condition for both experimental tracking and theoretical predictions.

The mean and Fano factor dynamics can be obtained from the time-lapse data by tracking the organelle abundance across time in a fairly straightforward manner. The FPT statistics can be extracted from time-lapse data by recording, for each target copy number $X_1, X_2, \dots$, the time it takes for cells to reach the target value. Cells that already exceed the target at $t = 0$ are excluded for that particular X_i . The resulting first passage times can then be used to compute the mean and CV, giving curves directly comparable to those in Fig.4.5, 4.6. Thus, the same time-lapse dataset can be used to compare multiple features of the dynamics to theoretical predictions, and our framework supports this by accommodating arbitrary initial conditions and providing a way for computing both Fano factor and FPT statistics. The behaviour seen in the experiments can then be compared with the theoretical predictions for various models to gain insights into the mechanisms of organelle biogenesis involved. Our framework thus allows the use of experimentally measured initial conditions to generate testable predictions about the

evolution of organelle number distributions under different biogenesis mechanisms.

We make assumptions, mentioned below, for simplification that are also limitations of the model. We highlight them below.

1. During the time taken for the organelle abundance to attain a steady state average level of the underlying mechanism of organelle biogenesis, we assume the corresponding rate constants remain unaltered with time, organelle number, or organelle size, but this may not always be the case [152, 153]).
2. Although the de novo synthesis-fusion and de novo synthesis-fission-fusion models do have finite steady states, the presence of the de novo synthesis process leads to ever-increasing organelle biomass unless there are additional processes or regulatory mechanisms to account for it. Thus, these models have not been explored here.
3. Our current model does not incorporate the spatial dimension. Any dependence of rates on the position of cells, such as gradients of nutrients or signalling molecules, lies outside the scope of this study.

The growing body of work on time-lapse measurements of organelle dynamics provides an alternative to fixed-cell imaging methods. For instance, recent experiments have reported real-time organelle dynamics in *Drosophila* [91], HeLa cells [92, 93], and Neurons [94]. These experiments contain information about organelle abundance; despite the challenges in precisely counting individual organelle numbers from microscopy data [154]. By tapping into these datasets and comparing them with the specific predictions various models make about the transient Fano factor, it should be possible to discriminate between them. The results presented in this chapter should pave the way for furthering the theory-experiment interplay in order to shed light on the process of organelle biogenesis.

Appendix

4.A Details of calculation for the moments

4.A.1 Numerical solutions using the truncated master equation

Eukaryotic cells tightly regulate organelle abundance in response to inter- and intracellular cues. In this manuscript, we seek to obtain the transient properties of organelle abundance distribution. For the model of organelle biogenesis, we consider (see Fig. 4.1 in the main text), we study the time evolution of organelle copy number distribution using a master equation approach. The probability $P_n(t)$ of having n organelle copies in a cell at time t is given by

$$\begin{aligned} \frac{dP_n(t)}{dt} = & [k_d + (n-1)k_{\text{fis}}]P_{n-1}(t) + [(n+1)\gamma + n(n+1)k_{\text{fus}}]P_{n+1}(t) \\ & - [k_d + n(\gamma + k_{\text{fis}}) + n(n-1)k_{\text{fus}}]P_n(t). \end{aligned} \quad (4.10)$$

Owing to the nonlinear nature of Eq. 4.10, an exact closed-form expression for the time-dependent probability distribution doesn't exist. Here we employ an alternative route based on a recent paper that developed a method to obtain time-dependent solutions for a general one-dimensional one-step master equation [137]. Eq. 4.10 is an infinite set of coupled differential equations, but in reality, there will be an upper bound on the number of organelles. Taking this upper bound to be some large integer τ , we can truncate the master equation at $P_{\tau-1}(t)$ and ignore all the higher terms. $P_n(t)$ can be written as a finite vector, $P(t) = (P_0(t), P_1(t), P_2(t), \dots, P_{\tau-1}(t))^T$. Hence, we can rewrite Eq. 4.10 as

$$\dot{P}(t) = AP(t). \quad (4.11)$$

Here A is the transition matrix, given as

$$A = \begin{pmatrix} -a_1 & b_0 & 0 & 0 & \dots \\ a_1 & -a_2 - b_0 & b_1 & 0 & \dots \\ 0 & a_2 & -a_3 - b_1 & b_2 & \dots \\ 0 & 0 & a_3 & -a_4 - b_2 & \dots \\ \vdots & \vdots & \vdots & \vdots & \vdots \end{pmatrix}, \quad (4.12)$$

where $a_n = k_d + (n - 1)k_{\text{fis}}$ and $b_n = (n + 1)\gamma + n(n + 1)k_{\text{fus}}$. The solution to Eq. 4.11 is

$$P(t) = e^{At}P(0). \quad (4.13)$$

Solving this equation requires us to compute the eigenvalues and the eigenvectors of A , which is computationally expensive [155]. However, the matrix is tridiagonal, and the sum of the elements along each column is zero. This method, by reducing the problem to one of finding eigenvalues of a tridiagonal matrix, allows us to numerically compute the transient distributions. The transient probability distribution, evaluated in this manner, is given by

$$P_n(t) = \begin{cases} b_n \dots b_{n_0-1} \sum_{i=1}^{\tau} e^{\lambda_i t} \frac{p_n(\lambda_i) q_{n_0+2}(\lambda_i)}{\prod_{j \neq i} (\lambda_i - \lambda_j)} & n < n_0 \\ \sum_{i=1}^{\tau} e^{\lambda_i t} \frac{p_n(\lambda_i) q_{n_0+2}(\lambda_i)}{\prod_{j \neq i} (\lambda_i - \lambda_j)} & n = n_0 \\ a_{n_0+1} \dots a_n \sum_{i=1}^{\tau} e^{\lambda_i t} \frac{p_{n_0}(\lambda_i) q_{n+2}(\lambda_i)}{\prod_{j \neq i} (\lambda_i - \lambda_j)} & n > n_0 \end{cases}, \quad (4.14)$$

where n_0 is the initial number of organelles, λ_i is the i^{th} eigenvalue, and p and q are orthogonal polynomials defined recursively as

$$\begin{aligned}
p_i(z) &= (z + a_i + b_{i-2})p_{i-1}(z) - b_{i-2}a_{i-1}p_{i-2}(z), \\
p_0(z) &= 1, p_1(z) = z + a_1, \\
q_i(z) &= (z + a_i + b_{i-2})q_{i+1}(z) - b_{i-1}a_iq_{i+2}(z), \\
q_{\tau+1}(z) &= 1, q_{\tau}(z) = z + b_{\tau-2}.
\end{aligned} \tag{4.15}$$

The solution given in Eq. 4.14 assumes that all the cells in the population have the same number of organelles, n_0 , at $t = 0$. This can be generalized to any initial distribution $P_n(0) = Q_n$, with the transient solutions now being given by

$$\tilde{P}_n(t) = \sum_{n_0=0}^{\tau-1} P_n(t) Q_{n_0}. \tag{4.16}$$

We assume two cases for the initial condition; a delta function distribution, i.e., all cells having the same organelle copy number, and a binomial distribution. These are expanded upon in further detail in the ensuing sections.

4.A.2 Exact time-dependent solutions for mean and variance of organelle copy number distribution

In the absence of fusion, Eq.4.10 becomes a linear equation. Two of the models we consider belong to this category, namely i) De novo synthesis-decay and ii) De novo synthesis-fission-decay. We use the generating function technique to solve for the transient mean and variance for these aforementioned models [134].

De novo synthesis-decay

Let us consider the case where only de novo synthesis and decay of organelles occur. The probability $P_n(t)$ of finding n organelle copies at time t is given by the master equation

$$\frac{dP_n}{dt} = k_d P_{n-1} + \gamma(n+1)P_{n+1} - [k_d + n\gamma]P_n. \tag{4.17}$$

The generating function is defined as

$$G(z, t) = \sum_{n=0}^{\infty} z^n P_n(t), \quad (4.18)$$

where $0 < z \leq 1$. We consider the derivatives of the $G(z, t)$, as they will be important for the subsequent calculations.

$$\frac{\partial G(z, t)}{\partial t} = \sum_{n=0}^{\infty} z^n \frac{dP_n(t)}{dt}, \quad (4.19)$$

$$\frac{\partial G}{\partial z} = \sum_{n=0}^{\infty} n z^{n-1} P_n(t). \quad (4.20)$$

We multiply both sides of Eq. 4.17 by z^n and sum over all possible values of n to obtain

$$\sum_{n=0}^{\infty} z^n \frac{dP_n(t)}{dt} = \sum_{n=0}^{\infty} \left[k_d z^n P_{n-1}(t) + \gamma z^n (n+1) P_{n+1}(t) - z^n [k_d + n\gamma] P_n(t) \right]. \quad (4.21)$$

Using Eq. 4.18, 4.19 and 4.20, we can rewrite Eq. 4.21 as

$$\begin{aligned} \frac{\partial G(z, t)}{\partial t} &= z k_d G(z, t) + \gamma \frac{\partial G(z, t)}{\partial z} - k_d G(z, t) - z \gamma \frac{\partial G(z, t)}{\partial z}, \\ &= (z - 1) \left[k_d G(z, t) - \gamma \frac{\partial G(z, t)}{\partial z} \right]. \end{aligned} \quad (4.22)$$

We get the following partial differential equation, which needs to be solved to solve for the generating function,

$$\frac{\partial G(z, t)}{\partial t} + \gamma(z - 1) \frac{\partial G(z, t)}{\partial z} = k_d(z - 1) G(z, t). \quad (4.23)$$

Eq. 4.23 can be solved analytically using the method of characteristics [156]. Using this method, we obtain the auxiliary equations of Eq. 4.23, given by

$$\Rightarrow \frac{dt}{1} = \frac{dz}{\gamma(z-1)} = \frac{dG(z,t)}{k_d(z-1)G(z,t)}. \quad (4.24)$$

Integrating the first two terms leads to

$$\begin{aligned} \int dt &= \int \frac{dz}{\gamma(z-1)}, \\ \Rightarrow \gamma t + \ln A &= \ln(z-1), \\ \Rightarrow A &= e^{-\gamma t}(z-1). \end{aligned} \quad (4.25)$$

Here, A is the constant of integration. Similarly, integrating the second and third terms in Eq. 4.24 gives us

$$\begin{aligned} \int \frac{k_d dz}{\gamma} &= \int \frac{dG}{G}, \\ \Rightarrow \ln \left(\frac{k_d z}{\gamma} \right) + \ln B &= \ln G, \\ \Rightarrow B &= G e^{-z k_d / \gamma}, \end{aligned} \quad (4.26)$$

where B is the constant of integration. Writing $B = f(A)$, where f is some function to be evaluated, we get

$$\begin{aligned} f(A) &= G e^{-z k_d / \gamma}, \\ \Rightarrow f(e^{-\gamma t}(z-1)) &= G e^{-z k_d / \gamma}, \\ \Rightarrow G(z,t) &= e^{z k_d / \gamma} f(e^{-\gamma t}(z-1)). \end{aligned} \quad (4.27)$$

To evaluate the generating function, we need the initial conditions. Below, we consider two

different initial conditions.

Identical organelle copy number in every individual cell Let us assume that at time $t = 0$ every cell in a population contains the same number of organelle copies, N_0 . In other words, the initial distribution has a delta function distribution $P_n(t = 0) = \delta(n - N_0)$, giving us the generating function at $t = 0$, $G(z, 0) = z^{N_0}$. Using this initial condition together with Eq. 4.27 gives us the functional form of $f(z)$,

$$\begin{aligned} G(z, 0) &= e^{zk_d/\gamma} f(z - 1) = z^{N_0}, \\ \Rightarrow f(z) &= e^{-(z+1)k_d/\gamma} (z + 1)^{N_0}. \end{aligned} \quad (4.28)$$

Using this form of $f(z)$ in Eq. 4.27, leads to the following solution for the generating function,

$$G(z, t) = e^{zk_d/\gamma} [e^{-\gamma t}(z - 1) + 1]^{N_0} e^{-\frac{k_d}{\gamma} [1 + e^{-\gamma t}(z - 1)]}. \quad (4.29)$$

The generating function contains all the information about $P_n(t)$; the moments of $P_n(t)$ can be obtained from the generating function using the following relations

$$\langle n(t) \rangle = \left. \frac{\partial G(z, t)}{\partial z} \right|_{z=1}, \quad \langle n^2(t) \rangle = \left. \frac{\partial}{\partial z} \left(z \frac{\partial G}{\partial z} \right) \right|_{z=1}. \quad (4.30)$$

Using Eq. 4.30, we obtain the mean $\langle n(t) \rangle$ and the variance (defined as $\sigma^2 = \langle n^2 \rangle - \langle n \rangle^2$),

$$\langle n(t) \rangle = \frac{k_d}{\gamma} + e^{-\gamma t} \left(N_0 - \frac{k_d}{\gamma} \right), \quad (4.31)$$

$$\sigma^2(t) = \frac{e^{-2\gamma t}(e^{\gamma t} - 1)(\gamma N_0 + k_d e^{\gamma t})}{\gamma}. \quad (4.32)$$

The Fano factor $F(t)$, defined as the ratio of the variance to the mean is given by

$$F(t) = \frac{e^{-2\gamma t}(e^{\gamma t} - 1)(\gamma N_0 + k_d e^{\gamma t})}{k_d + \gamma e^{-\gamma t}(N_0 - k_d/\gamma)}. \quad (4.33)$$

Binomially distributed organelle copy numbers We assume that the organelle copy numbers in individual cells follow a binomial distribution across a cell population, i.e. $P_n(t = 0) = \binom{N}{n} p^n (1 - p)^{N-n}$. Here N is the maximum number of organelle copies a cell can have and p is the corresponding probability for a cell to have a single organelle copy. From the definition of the generating function, at $t = 0$

$$\begin{aligned} G(z, 0) &= \sum_{n=0}^{\infty} z^n P_n(0) = \sum_{n=0}^{\infty} z^n \binom{N}{n} p^n (1 - p)^{N-n}, \\ &= \sum_{n=0}^{\infty} \binom{N}{n} (pz)^n (1 - p)^{N-n}, \\ &= (zp + (1 - p))^N. \end{aligned} \quad (4.34)$$

From Eq. 4.27 and Eq. 4.34, we get

$$\begin{aligned} f(z - 1) &= e^{-zk_d/\gamma} [p(z - 1) + 1]^N, \\ \Rightarrow f(z) &= e^{-(z+1)k_d/\gamma} (pz + 1)^N. \end{aligned} \quad (4.35)$$

By substituting this form of $f(z)$ in Eq. 4.27, we obtain an expression for the generating function

$$G(z, t) = e^{zk_d/\gamma} [pe^{-\gamma t}(z - 1) + 1]^N e^{-\frac{k_d}{\gamma}[1+e^{-\gamma t}(z-1)]}. \quad (4.36)$$

From Eq. 4.30 and 4.36, the mean and the variance are given by

$$\langle n(t) \rangle = \frac{k_d}{\gamma} - e^{\gamma t} \left(\frac{k_d}{\gamma} - Np \right), \quad (4.37)$$

$$\sigma^2(t) = \frac{k_d(1 - e^{-\gamma t}) + e^{-2\gamma t}(e^{\gamma t} - p)\gamma Np}{\gamma} \quad (4.38)$$

The Fano factor $F(t)$ has the following form

$$F(t) = \frac{k_d(1 - e^{-\gamma t}) + e^{-2\gamma t}(e^{\gamma t} - p)\gamma Np}{k_d - e^{-\gamma t}(k_d - \gamma Np)}. \quad (4.39)$$

De novo synthesis-fission-decay

Let us now consider a process in which the organelles undergo de novo synthesis, fission and decay. The probability for n organelles at time t obeys the following master equation,

$$\frac{dP_n}{dt} = [k_d + k_{\text{fis}}(n - 1)] P_{n-1} + \gamma(n + 1)P_{n+1} - [k_d + n(\gamma + k_{\text{fis}})]P_n. \quad (4.40)$$

As in the previous section, we use the generating function, defined in Eq. 4.18. Multiplying both sides of Eq. 4.40 by z^n and summing over all possible values of n gives us

$$\begin{aligned} \sum_{n=0}^{\infty} \frac{dP_n}{dt} z^n &= \sum_{n=0}^{\infty} \left[k_d z^n P_{n-1}(t) + k_{\text{fis}}(n - 1) z^n P_{n-1}(t) + \gamma z^n (n + 1) P_{n+1}(t) \right. \\ &\quad \left. - z^n [k_d + n(\gamma + k_{\text{fis}})] P_n(t) \right]. \end{aligned} \quad (4.41)$$

Using Eq. 4.18, 4.19 and 4.20, we can rewrite Eq. 4.41 as

$$\begin{aligned}
\frac{\partial G(z, t)}{\partial t} &= zk_d G(z, t) + [z^2 k_{\text{fis}} + \gamma] \frac{\partial G(z, t)}{\partial z} - k_d G(z, t) - z[\gamma + k_{\text{fis}} z] \frac{\partial G(z, t)}{\partial z}, \\
&= k_d(z - 1)G(z, t) + (k_{\text{fis}} z - \gamma)(z - 1) \frac{\partial G(z, t)}{\partial z}.
\end{aligned} \tag{4.42}$$

We get the following partial differential equation, which needs to be solved to obtain the generating function

$$\frac{\partial G(z, t)}{\partial t} - (k_{\text{fis}} z - \gamma)(z - 1) \frac{\partial G(z, t)}{\partial z} = k_d(z - 1)G(z, t). \tag{4.43}$$

As before, we use the method of characteristics to solve Eq. 4.43. The auxiliary equations of the partial differential equations are

$$\Rightarrow \frac{dt}{1} = \frac{-dz}{(z - 1)(k_{\text{fis}} z - \gamma)} = \frac{dG(z, t)}{k_d(z - 1)G(z, t)}. \tag{4.44}$$

Integrating the first two terms gives

$$\begin{aligned}
\int dt &= - \int \frac{dz}{(z - 1)(k_{\text{fis}} z - \gamma)}, \\
\Rightarrow (k_{\text{fis}} - \gamma)t + \ln A &= \ln \left(\frac{z - 1}{k_{\text{fis}} z - \gamma} \right), \\
\Rightarrow A &= \left[\frac{z - 1}{k_{\text{fis}} z - \gamma} \right] e^{(k_{\text{fis}} - \gamma)t},
\end{aligned} \tag{4.45}$$

where A is the constant of integration. Similarly, integrating the second and third terms in Eq. 4.44

$$\begin{aligned}
& \frac{-dz}{(z-1)(k_{\text{fis}}z-\gamma)} = \frac{dG}{k_d(z-1)G}, \\
& \Rightarrow k_d \int \frac{-dz}{(k_{\text{fis}}z-\gamma)} = \int \frac{dG}{G}, \\
& \Rightarrow \ln G = \frac{-k_d}{k_{\text{fis}}} \ln(k_{\text{fis}}z-\gamma) + B, \\
& \Rightarrow G(z, t) = [k_{\text{fis}}z-\gamma]^{-k_d/k_{\text{fis}}} B,
\end{aligned} \tag{4.46}$$

where B is the constant of integration. Writing $B = f(A)$, where f is some function to be evaluated,

$$\begin{aligned}
G(z, t) &= [k_{\text{fis}}z-\gamma]^{-k_d/k_{\text{fis}}} f(A), \\
&= [k_{\text{fis}}z-\gamma]^{-k_d/k_{\text{fis}}} f\left(\left[\frac{z-1}{k_{\text{fis}}z-\gamma}\right] e^{(k_{\text{fis}}-\gamma)t}\right),
\end{aligned} \tag{4.47}$$

where we have used the expression for A from Eq. 4.45. Now, we will use the initial condition, and like previously, we consider two cases.

Identical organelle copy number in every individual cell We assume that initially all the cells have the same number of organelles N_0 . Then $P_n(t=0) = \delta(n - N_0)$ and the generating function becomes $G(z, 0) = z^{N_0}$. Using this initial condition with Eq. 4.47, at $t = 0$, gives us

$$G(z, 0) = [k_{\text{fis}}z-\gamma]^{-k_d/k_{\text{fis}}} f\left(\frac{z-1}{k_{\text{fis}}z-\gamma}\right) = z^{N_0}. \tag{4.48}$$

We make the following substitution into Eq. 4.48

$$\frac{z-1}{k_{\text{fis}}z-\gamma} = \omega, \tag{4.49}$$

giving us

$$\begin{aligned} \left[k_{\text{fis}} \left(\frac{1 - \omega\gamma}{1 - k_{\text{fis}}\omega} \right) - \gamma \right]^{-k_d/k_{\text{fis}}} f(\omega) &= \left[\frac{1 - \omega\gamma}{1 - k_{\text{fis}}\omega} \right]^{N_0}, \\ \Rightarrow f(\omega) &= \left[\frac{1 - \omega\gamma}{1 - k_{\text{fis}}\omega} \right]^{N_0} \left(\frac{k_{\text{fis}} - \gamma}{1 - k_{\text{fis}}\omega} \right)^{k_d/k_{\text{fis}}}. \end{aligned} \quad (4.50)$$

Now, we have the functional form of $f(z)$. From Eq. 4.47 and Eq. 4.49, we get the generating function

$$\begin{aligned} G(z, t) &= \left[\frac{1 - \gamma \left(\frac{z-1}{k_{\text{fis}}z - \gamma} \right) e^{(k_{\text{fis}} - \gamma)t}}{1 - k_{\text{fis}} \left(\frac{z-1}{k_{\text{fis}}z - \gamma} \right) e^{(k_{\text{fis}} - \gamma)t}} \right]^{N_0} \\ &\times \left[\frac{k_{\text{fis}} - \gamma}{1 - k_{\text{fis}} \left(\frac{z-1}{k_{\text{fis}}z - \gamma} \right) e^{(k_{\text{fis}} - \gamma)t}} \right]^{k_d/k_{\text{fis}}} (k_{\text{fis}}z - \gamma)^{-k_d/k_{\text{fis}}}. \end{aligned} \quad (4.51)$$

The mean and variance can be obtained using Eq. 4.30 and 4.51,

$$\langle n(t) \rangle = \frac{k_d - e^{(k_{\text{fis}} - \gamma)t} [k_d + N_0(k_{\text{fis}} - \gamma)]}{\gamma - k_{\text{fis}}}, \quad (4.52)$$

$$\sigma^2(t) = \frac{(e^{(k_{\text{fis}} - \gamma)t} - 1) (\gamma k_d - e^{(k_{\text{fis}} - \gamma)t} [k_d k_{\text{fis}} + N_0(k_{\text{fis}}^2 - \gamma^2)])}{(\gamma - k_{\text{fis}})^2} \quad (4.53)$$

The Fano factor, $F(t)$, has the form

$$F(t) = \frac{(e^{(k_{\text{fis}} - \gamma)t} - 1) (\gamma k_d - e^{(k_{\text{fis}} - \gamma)t} [k_d k_{\text{fis}} + (k_{\text{fis}}^2 - \gamma^2) N_0])}{(\gamma - k_{\text{fis}}) [k_d - e^{(k_{\text{fis}} - \gamma)t} (k_d + N_0(k_{\text{fis}} - \gamma))]} \quad (4.54)$$

Binomially distributed organelle copy numbers We consider that the initial organelle abundance is binomially distributed i.e. $P_n(0) = \binom{N}{n} p^n (1 - p)^{N-n}$. We get from the definition of the generating function

$$\begin{aligned}
G(z, 0) &= \sum_{n=0}^{\infty} z^n P_n(0) = \sum_{n=0}^{\infty} z^n \binom{N}{n} p^n (1-p)^{N-n}, \\
&= \sum_{n=0}^{\infty} \binom{N}{n} (pz)^n (1-p)^{N-n}, \\
&= (zp + (1-p))^N.
\end{aligned} \tag{4.55}$$

From Eq. 4.47 and 4.55, we get

$$f(z) = \left(\frac{k_{\text{fis}} - \gamma}{1 - k_{\text{fis}} z} \right)^{k_d/k_{\text{fis}}} \left[\frac{pz(\gamma - k_{\text{fis}})}{zk_{\text{fis}} - 1} + 1 \right]^N. \tag{4.56}$$

Substituting $f(z)$ into Eq. 4.47, we obtain an expression for the generating function

$$\begin{aligned}
G(z, t) &= \left[\frac{p(\gamma - k_{\text{fis}})(z - 1)e^{(k_{\text{fis}} - \gamma)t}}{k_{\text{fis}}(z - 1)e^{(k_{\text{fis}} - \gamma)t} - (k_{\text{fis}}z - \gamma)} + 1 \right]^N \\
&\quad \times \left[\frac{k_{\text{fis}} - \gamma}{1 - k_{\text{fis}} \left(\frac{z-1}{k_{\text{fis}}z - \gamma} \right) e^{(k_{\text{fis}} - \gamma)t}} \right]^{k_d/k_{\text{fis}}} (k_{\text{fis}}z - \gamma)^{-k_d/k_{\text{fis}}}.
\end{aligned} \tag{4.57}$$

Using Eq. 4.30, the mean and variance are given as

$$\langle n(t) \rangle = \frac{k_d}{\gamma - k_{\text{fis}}} + e^{(k_{\text{fis}} - \gamma)t} \left[Np + \frac{k_d}{k_{\text{fis}} - \gamma} \right], \tag{4.58}$$

$$\tag{4.59}$$

$$\sigma^2(t) = \frac{\left(\gamma k_d + e^{(k_{\text{fis}} - \gamma)t} (\gamma + k_{\text{fis}}) [Np(\gamma - k_{\text{fis}}) - k_d] + e^{2(k_{\text{fis}} - \gamma)t} \{ k_d k_{\text{fis}} - Np(\gamma - k_{\text{fis}}) [k_{\text{fis}}(2 - p) + \gamma p] \} \right)}{(\gamma - k_{\text{fis}})^2}. \tag{4.60}$$

The Fano factor $F(t)$ has the following form

$$F(t) = \frac{\left(\gamma k_d + e^{(k_{\text{fis}} - \gamma)t} (\gamma + k_{\text{fis}}) [Np(\gamma - k_{\text{fis}}) - k_d] + e^{2(k_{\text{fis}} - \gamma)t} \{k_d k_{\text{fis}} - Np(\gamma - k_{\text{fis}}) [k_{\text{fis}}(2 - p) + \gamma p]\} \right)}{(\gamma - k_{\text{fis}}) \left[k_d + e^{(k_{\text{fis}} - \gamma)t} (\gamma - k_{\text{fis}}) \left(Np + \frac{k_d}{k_{\text{fis}} - \gamma} \right) \right]}. \quad (4.61)$$

We plot the Fano factor as a function of time for different models as the relevant model parameters are tuned.

4.B Moments of the first passage time distribution

We calculate the first two moments of the distribution of the first passage times to reach X copies of the organelle, starting from a given number of copies. We begin with the master equation, given by

$$\begin{aligned} \frac{dP_n(t)}{dt} = & [k_d + (n - 1)k_{\text{fis}}]P_{n-1}(t) + [(n + 1)\gamma + n(n + 1)k_{\text{fus}}]P_{n+1}(t) \\ & - [k_d + n(\gamma + k_{\text{fis}}) + n(n - 1)k_{\text{fus}}]P_n(t), \end{aligned} \quad (4.62)$$

where $P_n(t)$ is the probability of having n organelles in a cell at a time t and $n = 0, 1, \dots, X - 2$. We treat the site $n = X$ as an absorbing boundary, hence the master equation for the $n = X - 1$ will not have any contribution coming from the site $n = X$, and is given by

$$\begin{aligned} \frac{dP_{X-1}(t)}{dt} = & [k_d + (X - 2)k_{\text{fis}}]P_{X-2}(t) \\ & - [k_d + (X - 1)(\gamma + k_{\text{fis}}) + (X - 1)(X - 2)k_{\text{fus}}]P_{X-1}(t). \end{aligned} \quad (4.63)$$

As before, the set of equations can be written in matrix notation as

$$\dot{\mathbf{P}}(t) = \mathbf{A}\mathbf{P}(t). \quad (4.64)$$

Here $\mathbf{A}$ is the transition matrix, given as

$$A = \begin{pmatrix} -a_1 & b_0 & 0 & 0 & \dots \\ a_1 & -a_2 - b_0 & b_1 & 0 & \dots \\ 0 & a_2 & -a_3 - b_1 & b_2 & \dots \\ 0 & 0 & a_3 & -a_4 - b_2 & \dots \\ \vdots & \vdots & \vdots & \vdots & \vdots \end{pmatrix}, \quad (4.65)$$

where $a_n = k_d + (n-1)k_{\text{fis}}$ and $b_n = (n+1)\gamma + n(n+1)k_{\text{fus}}$. Note that all columns except the last one add up to zero, making the matrix in Eq.4.65 invertible. This is in contrast to the matrix in Eq.4.12, where all the columns add up to zero and the inverse does not exist.

We employ the method from a recent study [148] to obtain analytical expressions for the moments of the distribution of first passage times (FPT) to reach organelle copy number X .

FPT is defined as the random variable $T := \min\{t : x(t) \geq X | x(0) = 0\}$, which is the first time a random walker reaches a target point. The FPT distribution $f_T(t)$ can be written as the product of two vectors as

$$\begin{aligned} f_T(t) &= P(T \in (t, t + dt)) \\ &= \mathbf{U}^T \mathbf{P}(t) \\ &= \mathbf{U}^T \exp(\mathbf{A}t) \mathbf{P}(0), \end{aligned} \quad (4.66)$$

where $\mathbf{P}(t)$ is obtained by solving the master equations in Eq.4.64. $\mathbf{U}^T$ is a column vector where the i^{th} entry is the probability of reaching X number of organelle copies from $n = i$ copy number. $\mathbf{P}(0)$ is the probability at time $t = 0$, where $N = 0$ is given by the column vector

$$\mathbf{P}(0) = \begin{bmatrix} 1 & 0 & 0 & \dots & 0 \end{bmatrix}^T. \quad (4.67)$$

The m^{th} -moment of the FPT, using integration by parts, is given as

$$\langle T^m \rangle = \mathbf{U}^T \left(\int_0^\infty t^m \exp(\mathbf{A}t) dt \right) \mathbf{P}(t) \quad (4.68)$$

$$= (-1)^{m+1} m! \mathbf{U}^T (\mathbf{A}^{-1})^{m+1} \mathbf{P}(0), \quad (4.69)$$

In our problem, since the organelle number can increase in steps of one only the last entry of $\mathbf{U}^T$ will be non-zero, giving us

$$\mathbf{U} = \begin{bmatrix} 0 & 0 & 0 & \dots & k_d + (X-1)k_{\text{fus}} \end{bmatrix}^T. \quad (4.70)$$

To compute the inverse of $\mathbf{A}$, needed for the moments, we use $\mathbf{A}_0$ defined as the $\mathbf{A}$ with $\gamma = 0$ and $k_{\text{fus}} = 0$. $\mathbf{A}_0$ is a bidiagonal matrix whose inverse is a lower triangular matrix, given by

$$\mathbf{A}_0^{-1} = - \begin{bmatrix} \frac{1}{k_d} & 0 & 0 & \dots & 0 \\ \frac{1}{k_d + k_{\text{fus}}} & \frac{1}{k_d + k_{\text{fus}}} & 0 & \dots & 0 \\ \frac{1}{k_d + 2k_{\text{fus}}} & \frac{1}{k_d + 2k_{\text{fus}}} & \frac{1}{k_d + 2k_{\text{fus}}} & \dots & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ \frac{1}{k_d + (X-1)k_{\text{fus}}} & \frac{1}{k_d + (X-1)k_{\text{fus}}} & \dots & \dots & \frac{1}{k_d + (X-1)k_{\text{fus}}} \end{bmatrix} \quad (4.71)$$

We can now write $\mathbf{A}^{-1} = (\mathbf{A}_0 + \mathbf{A}_e)^{-1} = (\mathbf{I} + \mathbf{A}_0^{-1}\mathbf{A}_e)^{-1}\mathbf{A}_0^{-1}$, where

$$\mathbf{A}_e = \begin{bmatrix} 0 & \gamma & 0 & \dots & 0 \\ 0 & -\gamma & 2\gamma + k_{\text{fus}} & \dots & 0 \\ 0 & 0 & -[k_d + 2(\gamma + k_{\text{fus}})] & \dots & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & 0 & \dots & -[(X-1)(\gamma + (X-2)k_{\text{fus}})] \end{bmatrix}. \quad (4.72)$$

One can see that the matrix $\mathbf{E} = (\mathbf{I} + \mathbf{A}_0^{-1}\mathbf{A}_e)$ is a bidiagonal matrix of the form

$$\mathbf{E} = \begin{bmatrix} 1 & -\beta_1 & 0 & \dots & 0 \\ 0 & 1 & -\beta_2 & \dots & 0 \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ 0 & 0 & 0 & 0 & -\beta_{X-1} \\ 0 & 0 & 0 & \dots & 1 \end{bmatrix}, \quad (4.73)$$

where $\beta_n = \frac{n[\gamma+(n-1)k_{\text{fus}}]}{[k_d+(n-1)k_{\text{fis}}]}$. The inverse of this matrix is given by

$$\mathbf{E}^{-1} = \begin{bmatrix} 1 & \beta_1 & \beta_1\beta_2 & \dots & \prod_{n=1}^{X-1} \beta_n \\ 0 & 1 & \beta_2 & \dots & \prod_{n=2}^{X-1} \beta_n \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ 0 & 0 & 0 & 0 & \beta_{X-1} \\ 0 & 0 & 0 & \dots & 1 \end{bmatrix}. \quad (4.74)$$

Mean FPT

From Eq.4.69, the expression for the mean FPT can be given by

$$\langle T \rangle = -\mathbf{U}^T \mathbf{A}^{-2} \mathbf{P}(0) = -\mathbf{U}^T \mathbf{A}_0^{-1} \mathbf{E}^{-1} \mathbf{A}_0^{-1} \mathbf{P}(0). \quad (4.75)$$

One can show that $\mathbf{U}^T \mathbf{A}^{-1} = \mathbf{U}^T \mathbf{A}_0^{-1}$, and for our case $\mathbf{U}^T \mathbf{A}_0^{-1} = -[1 \ 1 \ 1 \ \dots \ 1]$. Using these in Eq.4.75 gives us the expression for the mean as

$$\langle T \rangle_{0,X} = \sum_{i=1}^X \left[\frac{1}{k_d + (i-1)k_{\text{fis}}} + \sum_{l=i}^{X-1} \frac{\prod_{n=i}^l \beta_n}{k_d + lk_{\text{fis}}} \right], \quad (4.76)$$

where $\beta_n = \frac{n[\gamma+(n-1)k_{\text{fus}}]}{[k_d+(n-1)k_{\text{fis}}]}$. This is the mean FPT for the full model, where we begin from 0 organelles and look at the time taken to reach X organelles for the first time.

Second moment

As before, the second moment of the FPT can be calculated as

$$\langle T^2 \rangle_{0,X} = 2\mathbf{U}^T(\mathbf{A}^{-3})\mathbf{P}(0) = -\mathbf{U}^T\mathbf{A}_0^{-1}\mathbf{E}^{-1}\mathbf{A}_0^{-1}\mathbf{E}^{-1}\mathbf{A}_0^{-1}\mathbf{P}(0). \quad (4.77)$$

Evaluating this gives

$$\langle T^2 \rangle_{0,X} = 2 \sum_{i=1}^X \left[\frac{\sum_{m=1}^i \eta_m}{k_d + (i-1)k_{\text{fis}}} + \sum_{l=i}^{X-1} \frac{\left(\prod_{n=i}^l \beta_n \right) \left(\sum_{m=1}^{l+1} \eta_m \right)}{k_d + lk_{\text{fis}}} \right], \quad (4.78)$$

where β_n is the same as above, and $\eta_n = \frac{1}{k_d + (n-1)k_{\text{fis}}} + \sum_{l=n}^{X-1} \frac{\prod_{m=n}^l \beta_m}{k_d + lk_{\text{fis}}}$

Eq.4.76 and Eq.4.78 describe the first two moments for the FPT distribution for the de novo synthesis-fission-fusion-decay model, i.e. containing all four processes. The moments for other models can be obtained by setting the appropriate rate constants to zero.

De novo synthesis-decay

The expressions with $k_{\text{fis}} = 0$ and $k_{\text{fus}} = 0$ simplify to give for the mean

$$\langle T \rangle_{0,X} = \frac{1}{k_d} \left[X + \sum_{i=1}^{X-1} \sum_{j=i+1}^X \frac{(j-1)!}{(i-1)!} \left(\frac{\gamma}{k_d} \right)^{j-i} \right], \quad (4.79)$$

and for the second moment

$$\langle T^2 \rangle_{0,X} = \frac{1}{k_d^2} \left[\sum_{i=1}^X \eta_i + \sum_{l=i+1}^X \left(\frac{(j-1)!}{(i-1)!} \left(\frac{\gamma}{k_d} \right)^{l-i} \left(\sum_{m=1}^l \eta_m \right) \right) \right], \quad (4.80)$$

where

$$\eta_m = 1 + \sum_{l=m+1}^X \frac{(j-1)!}{(m-1)!} \left(\frac{\gamma}{k_d} \right)^{j-m}.$$

Fission-fusion

The expressions for the moments for fission-fusion have to be evaluated separately, since $N = 0$ is an isolated point and the initial condition has to be taken as $N = 1$. The system of equations has one less variable corresponding to $P(0)$. Following the same procedure as above, the mean is given as

$$\langle T \rangle_{0,X} = \frac{1}{k_{\text{fis}}} \sum_{i=1}^{X-1} \left[\frac{1}{i} + \sum_{l=i}^{X-2} \frac{\prod_{n=i}^l \beta_n}{l+1} \right], \quad (4.81)$$

and the second moment is given by

$$\langle T^2 \rangle_{0,X} = \frac{2}{k_{\text{fis}}^2} \sum_{i=1}^{X-1} \left[\frac{1}{i} \sum_{m=1}^i \eta_m + \sum_{l=i}^{X-2} \frac{\left(\prod_{n=i}^l \beta_n \right) \left(\sum_{m=1}^{l+1} \eta_m \right)}{l+1} \right], \quad (4.82)$$

where $\beta = \frac{(n+1)k_{\text{fus}}}{k_{\text{fis}}}$ and $\eta = \frac{1}{m} + \sum_{l=m}^{X-2} \left(\frac{\prod_{n=m}^l \beta_n}{l+1} \right)$

De novo synthesis-fission-decay

Expressions are the same as in Eq.4.76 and Eq.4.78, with $k_{\text{fus}} = 0$ in β .

De novo synthesis-fusion-decay

Setting $k_{\text{fis}} = 0$ in Eq. 4.76 and Eq. 4.78 gives,

$$\langle T \rangle_{0,X} = \frac{1}{k_d} \left[X + \sum_{i=1}^X \sum_{l=i}^{X-1} \prod_{n=i}^l \beta_n \right], \quad (4.83)$$

and

$$\langle T^2 \rangle_{0,X} = \frac{2}{k_d} \sum_{i=1}^X \left[\sum_{m=1}^i \eta_m + \sum_{l=i}^{X-1} \left(\prod_{n=i}^l \beta_n \right) \left(\sum_{m=1}^{l+1} \eta_m \right) \right], \quad (4.84)$$

where $\beta_n = \frac{n[\gamma+(n-1)k_{\text{fus}}]}{k_d}$ and $\eta_n = \frac{1}{k_d} \left[1 + \sum_{l=n}^{X-1} \prod_{m=n}^l \beta_m \right]$.

4.C A note on the initial conditions

The time evolution of the mean and Fano factor of organelle abundance is highly dependent on the initial conditions. We have used two initial conditions in the previous sections: (1) the same organelle copy number in all cells, which acts as a simple starting point. (2) Initial organelle copy number following a binomial distribution, as predicted by stochastic partitioning of organelles between daughter cells without any further regulatory control [157, 158]. However, experimental studies have shown that partitioning may not be random for some organelles, such

as in the case of chromosomes or centrosomes, where partitioning is strictly symmetric, with the daughter cells each inheriting one copy, whereas in endosomes and mitochondria, partitioning may be asymmetric.[159–161]. Such an initial condition can also be incorporated in our model.

As mentioned in the main text, our theoretical framework allows us to choose any arbitrary initial condition. This is helpful as the initial distribution can be empirically obtained and the behaviour predicted under various theoretical models. Obtaining analytical expressions would be possible only for very few simple distributions. However, we can numerically compute the transient properties or any arbitrary initial conditions. As an illustrative example rooted in biological relevance, we consider asynchronous cell division. Suppose we have a cell population which has reached steady state, and now f_{ss} fraction of the cells remain in this state, and the remaining $(1 - f_{ss})$ fraction of cells undergo division. We now have a mixed population, with each subpopulation following different distributions. This can be accounted for by properly specifying the distributions in Eq.4.16. In our example, we study the de novo synthesis-decay case. The steady-state distribution is the Poisson distribution [88], with mean k_d/γ . We can then take, in Eq.4.16 $Q_n = f_{ss} \times \text{Poisson}(k_d/\gamma) + (1 - f_{ss}) \times \text{Binomial}(k_d/\gamma, 0.5)$. We can then vary f_{ss} to see how the results change as we vary f_{ss} . Similarly, the FPT properties can be studied under any arbitrary initial condition by specifying $\mathbf{P}(0)$ in Eq.4.67. The plots are shown in the Supplementary figures for various processes (Fig. 4.13 and Fig. 4.14).

4.D Supplementary figures

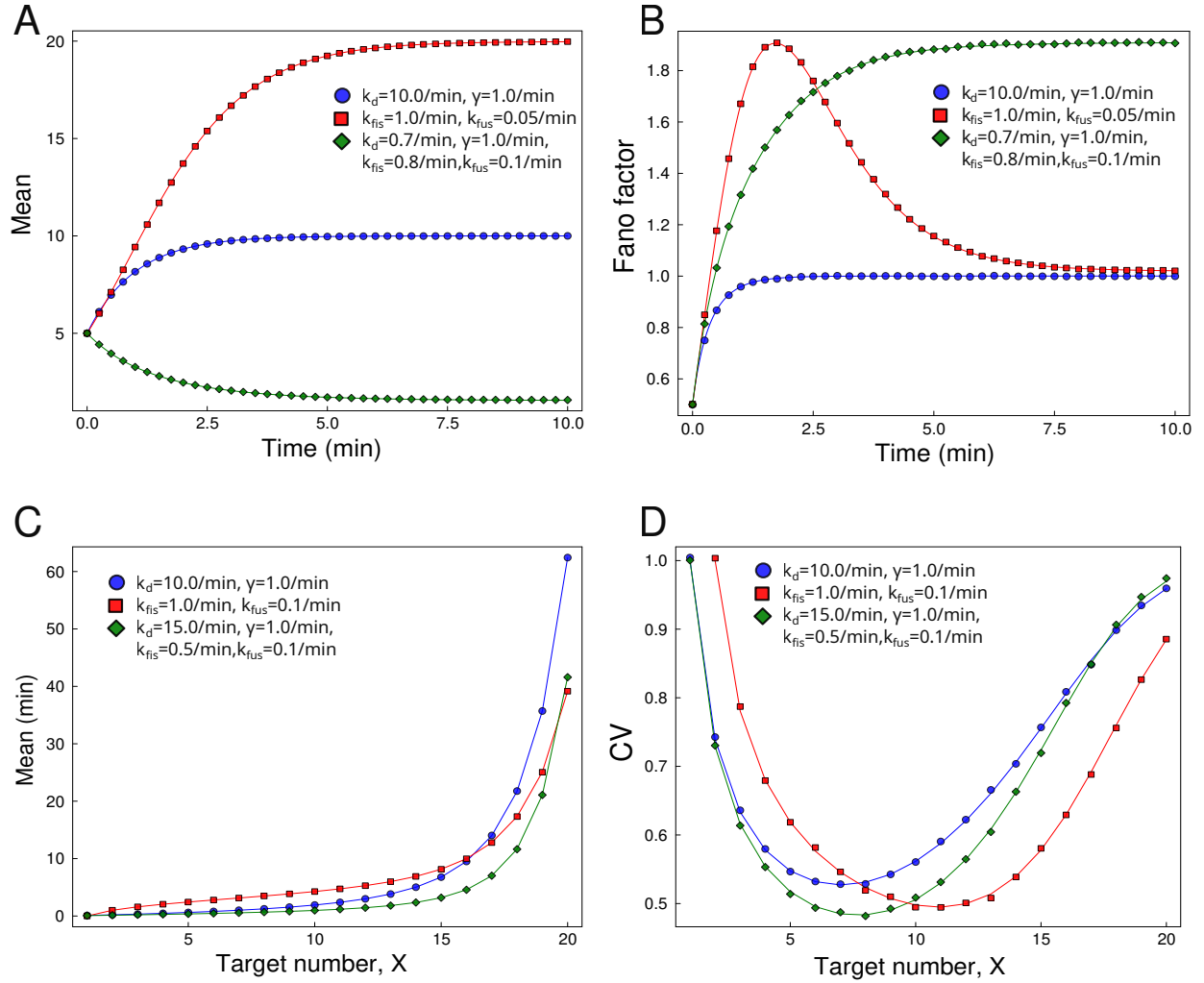


Figure 4.7: Comparison with stochastic simulations. We test our analytical and numerical results by comparing them with stochastic simulation using the Gillespie algorithm. For each parameter set, 10^6 trajectories are simulated and the average quantities computed from them. (A) Mean organelle copy number for various processes as a function of time. (B) Fano factor as a function of time, computed across all the trajectories. (The initial conditions are taken from the binomial distribution (same as in Fig.2-4). C) Mean first passage time for various processes to reach a certain target organelle copy number. (D) CV of the FPT to reach a certain organelle copy number. The initial condition is taken as zero for all processes except fission-fusion, where the initial copy number is taken as one. The parameters not specified are set to zero.

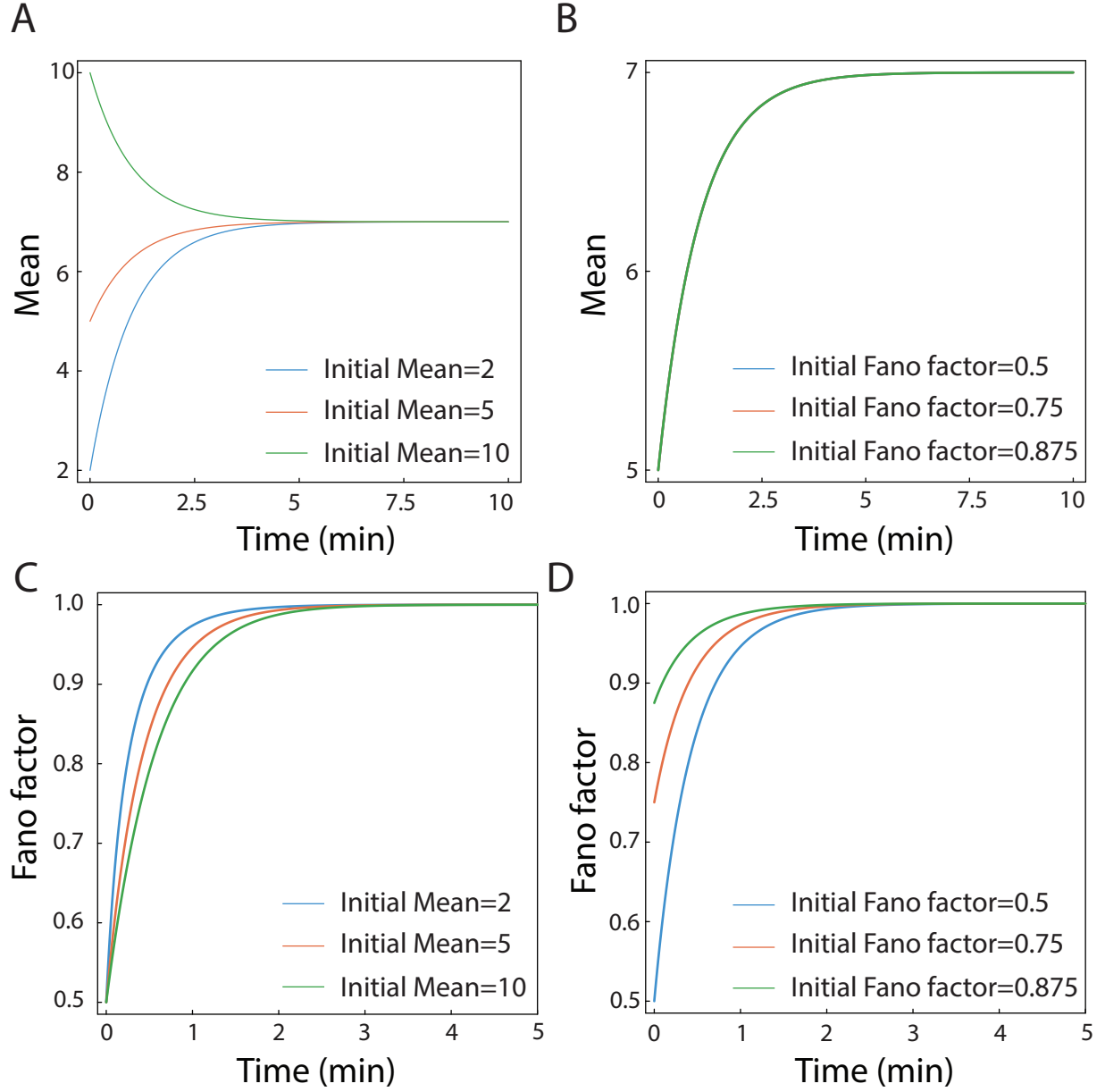


Figure 4.8: De-Novo Decay: (A) The evolution of the mean with time for different initial distributions, $N = 30, 20, 10$, $p = 0.5$. (B) The evolution of the mean starting from binomial distributions with mean = 5 and different variances, the trajectories overlap. (C) Evolution of the fano factor with time for different initial distributions as in (A). (D) The evolution of the fano factors with different initial distributions having the same initial mean, mean = 5, initial Fano = 0.5, 0.75, 0.875. $k_d = 7.0 \text{ min}^{-1}$, $\gamma = 1.0 \text{ min}^{-1}$ for all trajectories

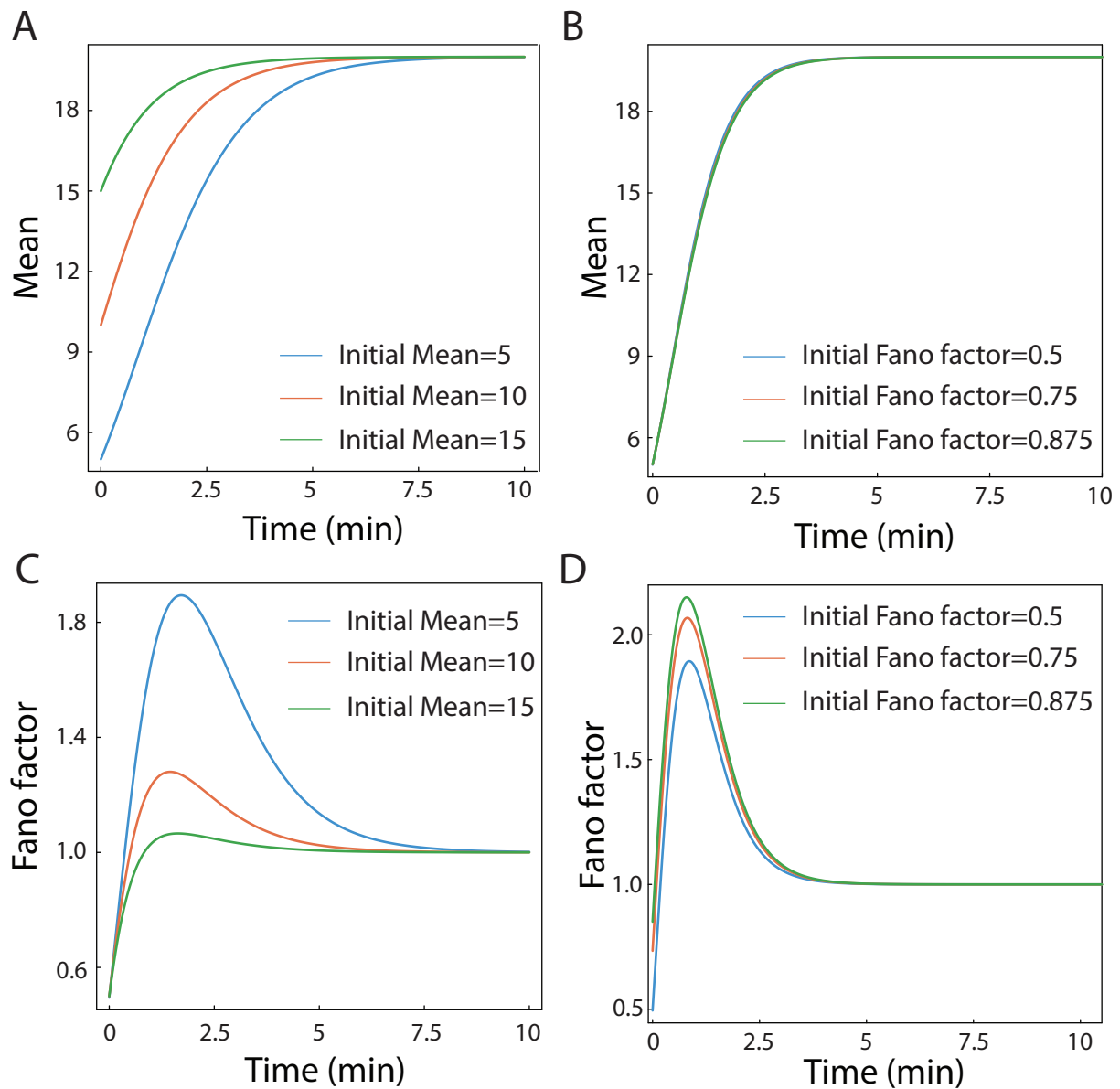


Figure 4.9: Fission - Fusion: (A) Evolution of mean when initial distributions of different means are chosen (blue = 5, red = 10, green = 15). (B) Evolution of the mean when different initial distributions with the same mean (5) but different Fano factors are taken (blue = 0.5, red = 0.75, green = 0.875). The trajectories overlap. (C) Evolution of the Fano factor when initial distributions with different means are taken as in (A). (D) Evolution of the Fano factor when different initial distributions are taken as in (B). $k_{\text{fis}} = 1.0 \text{ min}^{-1}$, $k_{\text{fus}} = 0.05 \text{ min}^{-1}$ for all trajectories.

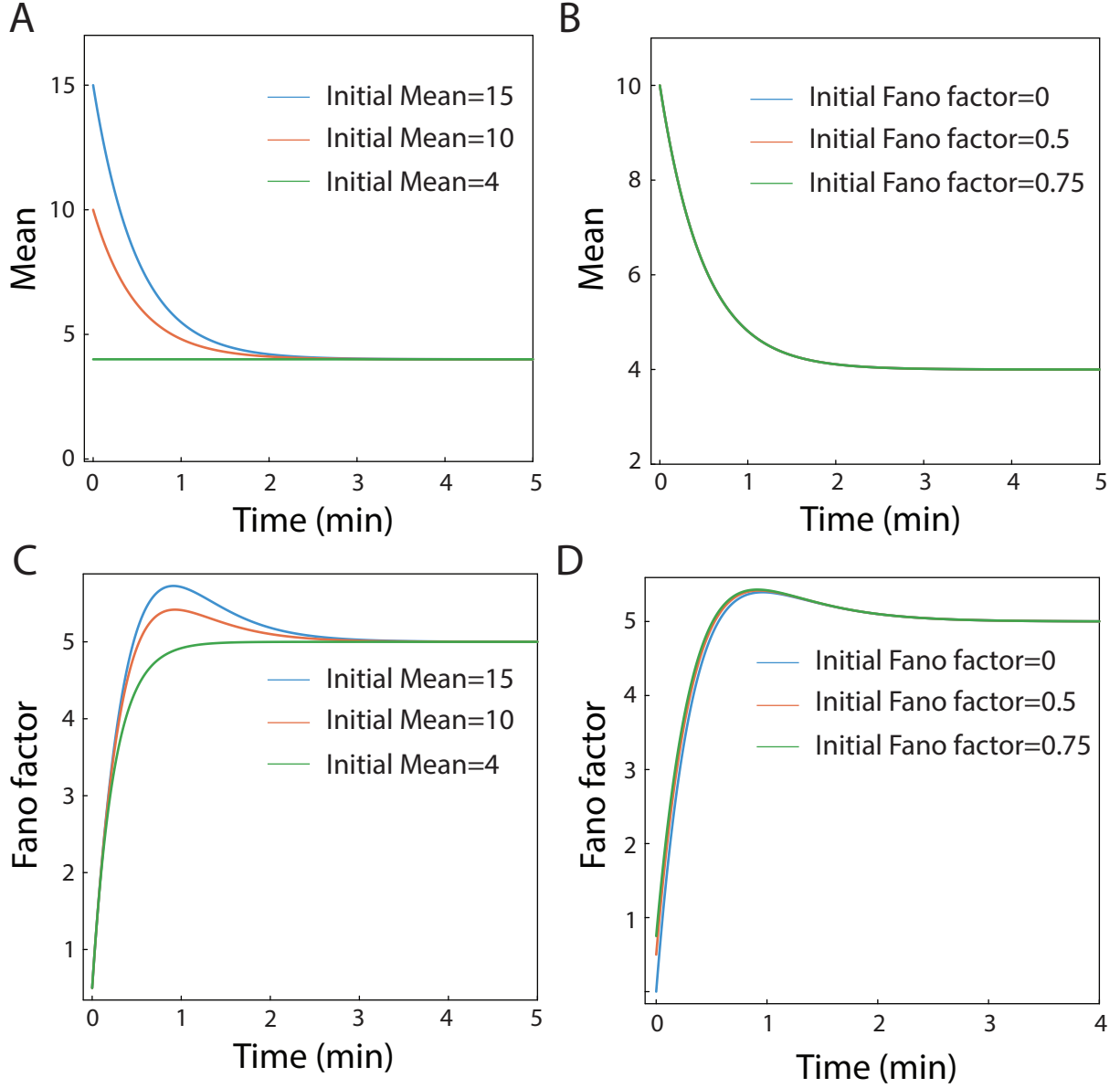


Figure 4.10: De novo - fission-decay (A) Evolution of mean when initial distributions of different means are chosen (blue = 4, red = 10, green = 15). (B) Evolution of the mean when different initial distributions with the same mean (10) but different Fano factors are taken (blue = 0.5, red = 0.75, green = 0.875). The trajectories overlap. (C) Evolution of the Fano factor when initial distributions with different means are taken as in (A). (D) Evolution of the Fano factor when different initial distributions are taken as in (B). $k_{\text{fis}} = 8 \text{ min}^{-1}$, $\gamma = 10 \text{ min}^{-1}$, $k_d = 8 \text{ min}^{-1}$ for all trajectories.

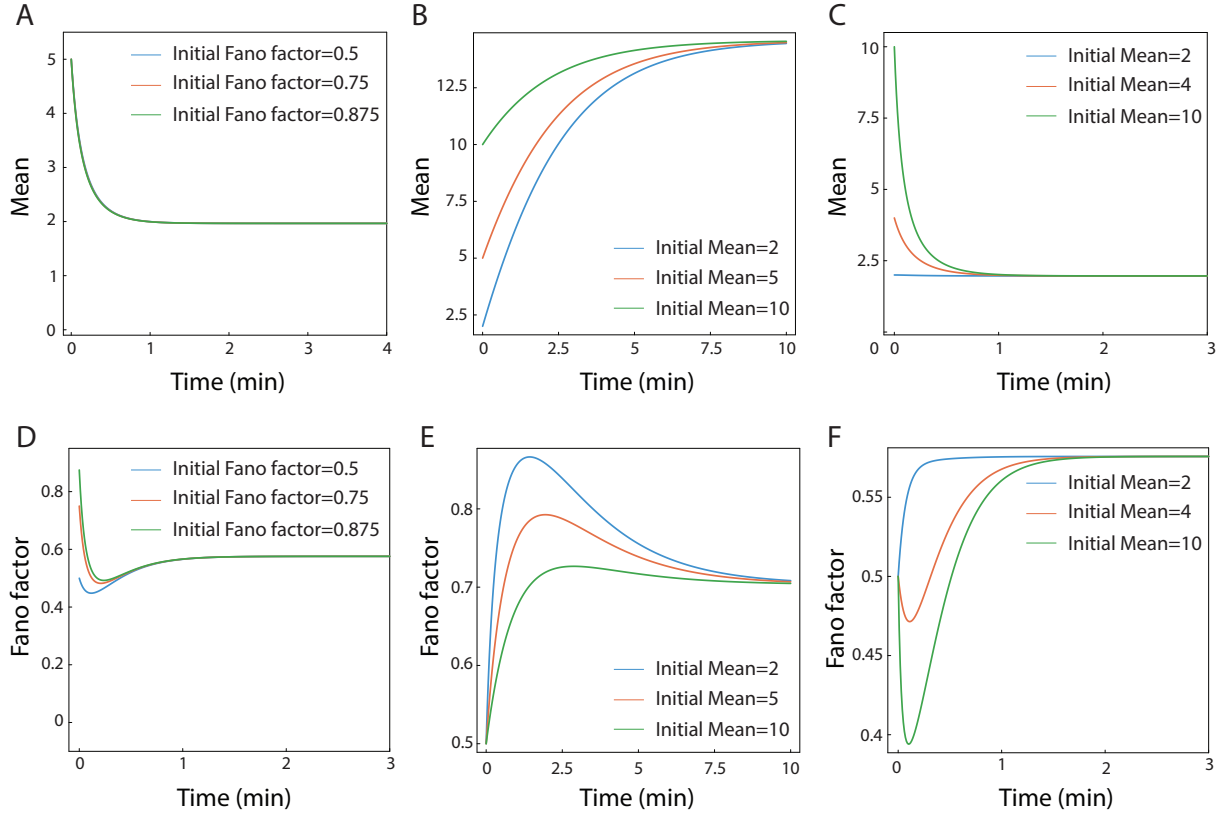


Figure 4.11: De novo-fusion-decay: (A) Evolution of the mean when initial distributions with the same mean (5) but different Fano noises are taken. $k_{\text{fus}} = 1.0 \text{ min}^{-1}$, $\gamma = 1.0 \text{ min}^{-1}$, $K_d = 5.0 \text{ min}^{-1}$. (B),(C) Evolution of the mean when initial distributions with different means are taken. (B): $k_{\text{fus}} = 0.01 \text{ min}^{-1}$, $\gamma = 0.2 \text{ min}^{-1}$, $K_d = 5.0 \text{ min}^{-1}$, (C): $k_{\text{fus}} = 1.0 \text{ min}^{-1}$, $\gamma = 1.0 \text{ min}^{-1}$, $K_d = 5.0 \text{ min}^{-1}$. (D) Evolution of the Fano factor when distributions and parameters are taken as in (A). (E), (F) Evolution of the fano factor when the initial distributions and parameters are taken as in (B) and (C), respectively.

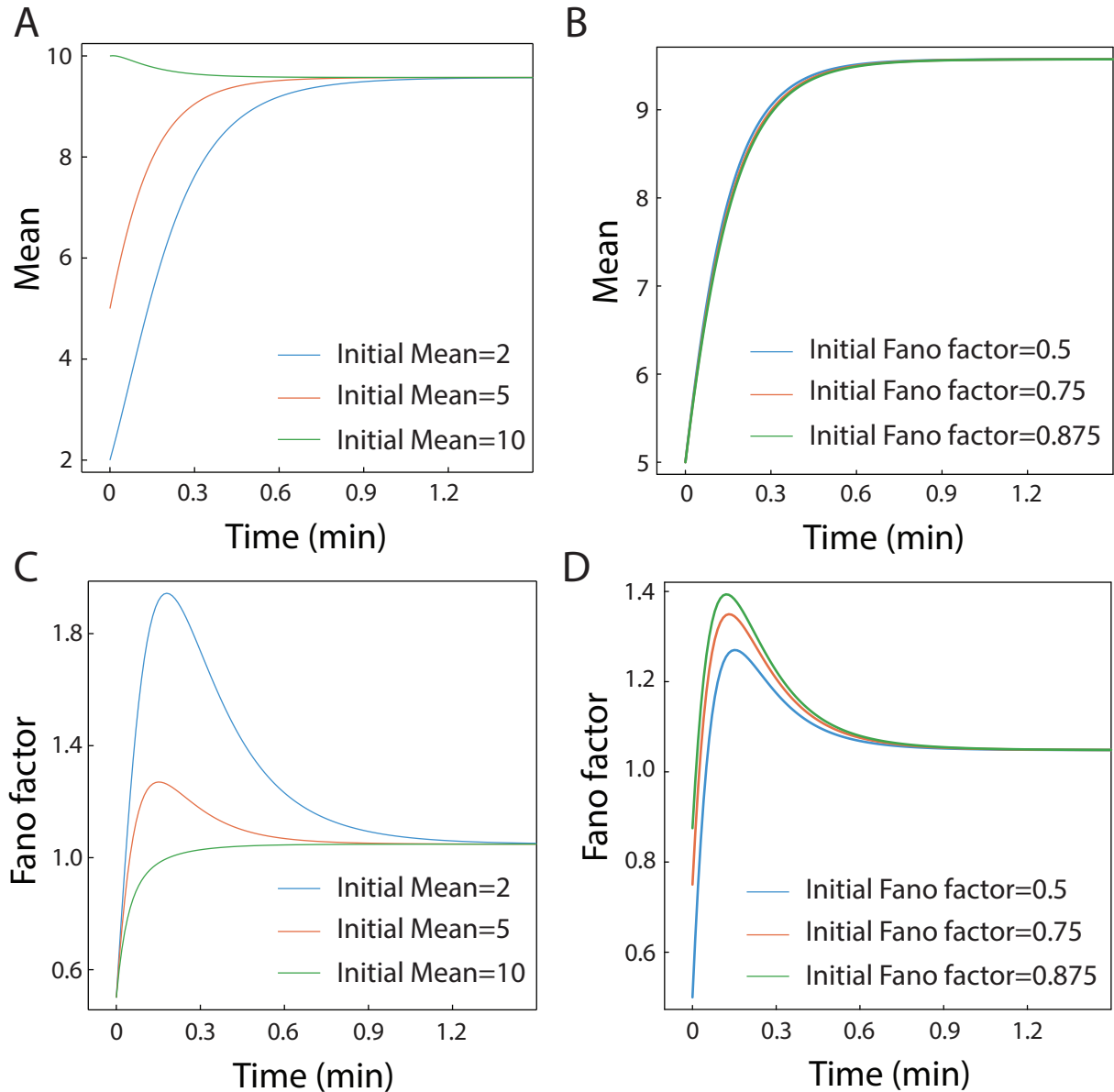


Figure 4.12: De novo - fission - fusion - decay: (A) Evolution of mean when initial distributions of different means are chosen (blue = 2, red = 5, green = 10). (B) Evolution of the mean when different initial distributions with the same mean (5) but different Fano factors are taken (blue = 0.5, red = 0.75, green = 0.875). The trajectories overlap. (C) Evolution of the Fano factor when initial distributions with different means are taken as in (A). (D) Evolution of the Fano factor when different initial distributions are taken as in (B). $k_{\text{fus}} = 1.0 \text{ min}^{-1}$, $k_{\text{fis}} = 10 \text{ min}^{-1}$, $\gamma = 0.9 \text{ min}^{-1}$, $K_d = 5.0 \text{ min}^{-1}$

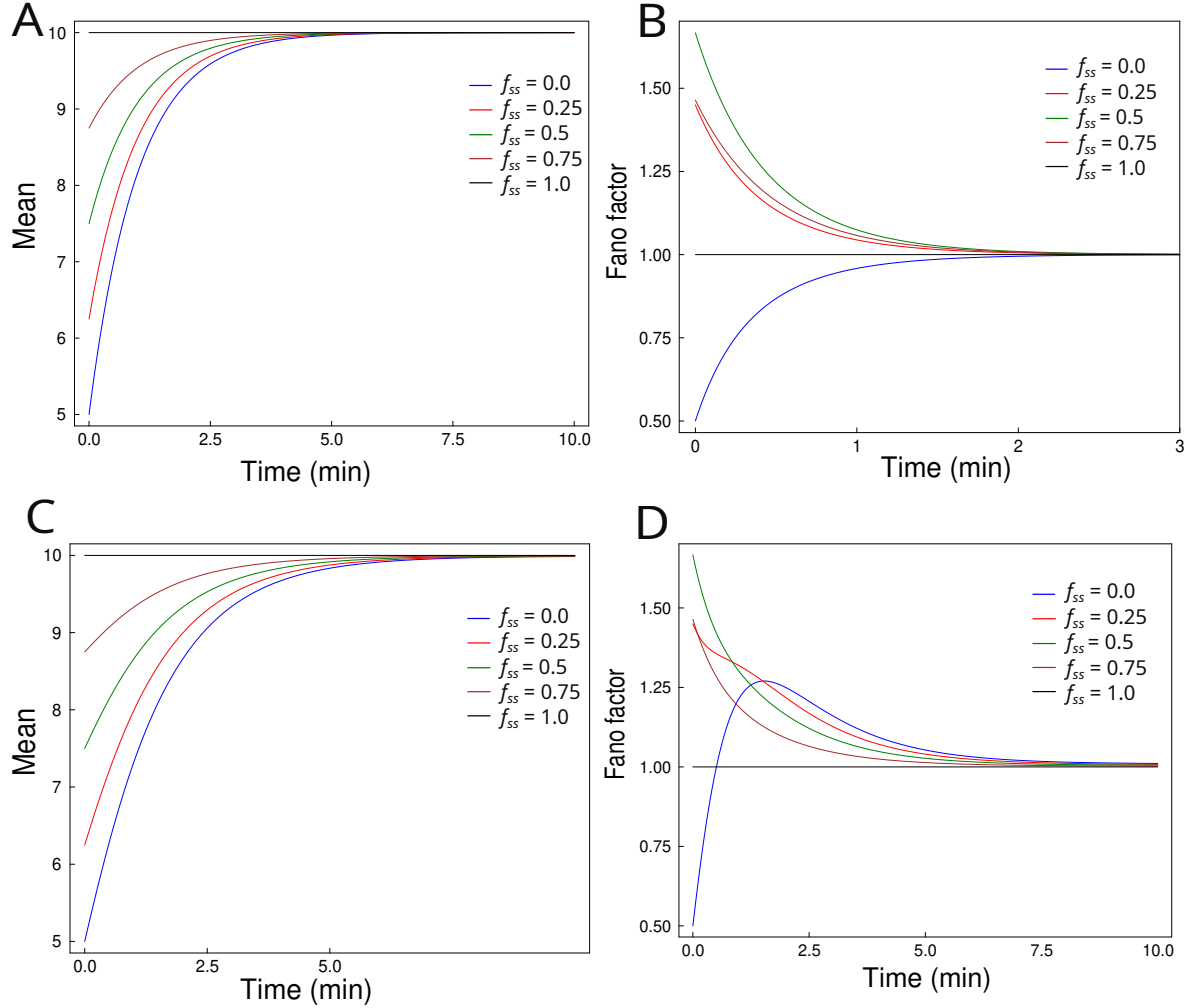


Figure 4.13: Asynchronous cell division: we take an initial distribution where f_{ss} fraction of cells follow the associated steady-state distribution and the remaining $(1 - f_{ss})$ fraction of cells have just divided and follow binomial statistics (A) Evolution of mean for de novo synthesis-decay process ($k_d = 10.0 \text{ min}^{-1}$, $\gamma = 1.0 \text{ min}^{-1}$). (B) Evolution of the Fano factor for the same process. The initial Fano factor is higher than one due to the high heterogeneity coming from a combination of two different distributions. (C) Evolution of the mean for fission-fusion process ($k_{fis} = 1.0 \text{ min}^{-1}$, $k_{fus} = 1.0 \text{ min}^{-1}$) (D) Fano factor for the same process.

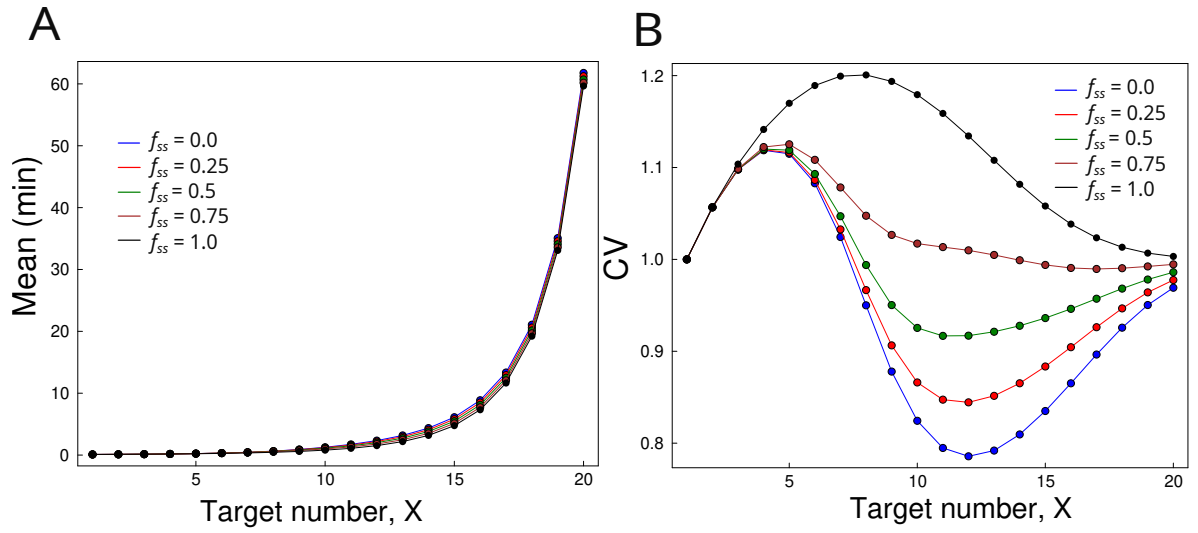


Figure 4.14: Asynchronous cell division: we take an initial distribution where f_{ss} fraction of cells follow the Poisson distribution (steady-state) for de novo synthesis-decay and the remaining $(1 - f_{ss})$ fraction of cells have just divided and follow binomial statistics (A) Mean FPT for de novo synthesis-decay process. (B) CV as a function of the target copy number. The CV goes above one here (unlike in the main text due to the difference in initial conditions). ($k_d = 10.0 \text{ min}^{-1}$, $\gamma = 1.0 \text{ min}^{-1}$)

Chapter 5

Conclusions and Outlook

5.1 Summary

In this thesis, we aimed to elucidate the physical principles underlying the regulation of compartmentalization in cells. We considered both membraneless (biomolecular condensates) and membrane-bound organelles.

In the context of biomolecular condensates, the research presented in this thesis examined how phase-separating proteins interact with intracellular surfaces. In particular, we focused on understanding the association between tau proteins and microtubules in cellular environments. Tau–microtubule interactions are physiologically important because tau dysregulation is implicated in several neurodegenerative diseases. Gaining mechanistic insights into how tau interacts with microtubules in neurons is of considerable interest. Secondly, we aimed to develop a general framework for modelling the interactions between phase-separating proteins and various intracellular surfaces, such as lipid membranes and DNA.

To this end, in collaboration with experimental studies, we found that tau forms surface condensates on microtubules in a concentration-dependent manner. The formation of these condensates is driven by a process similar to a prewetting transition. We further found that the spatial positions of tau condensates are dictated by surface heterogeneities of microtubules, likely

arising from post-translational modifications or various isotypes of tubulin. Guided by model predictions, we demonstrated that tau condensates on microtubules are multilayered in nature. This gives rise to kinetically distinct populations of tau within the condensates. Interestingly, depending on the surface properties of the microtubules, different layers of the tau condensates can perform distinct biological functions. For example, on GDP and taxol-microtubules, the first layer is strongly bound due to cooperative effects. This protects the microtubules from severing enzymes such as katanin. In contrast, outer layers of the condensates, when formed above a certain threshold of tau concentration, can recruit tau interactors such as tubulin. Our minimal model, with a small parameter set, provides a unifying mechanism, explaining observations across different microtubule types, over a wide range of concentrations and biological functionalities.

Cells regulate their organelle abundances in response to external cues and the metabolic needs. Here, we aimed to uncover the principles governing the regulation of membrane-bound organelles within cells. Specifically, we asked if we could decipher the mechanisms governing organelle abundances by counting the copy number of an organelle in a population of cells over time. Such measurements could be obtained from time-lapse microscopy techniques, which are becoming more prevalent. To this end, we employ a previously proposed model describing the regulation of organelle abundances, based on the master equation approach. Previous studies using this model assumed that the organelle copy numbers had reached a steady state. In the work presented in this thesis, we extended the model to provide analytical expressions and numerical results for the first two moments of the organelle number distribution and the first two moments of the first-passage time distribution to reach some target copy number. We found that the transient dynamics show rich behaviour, and depend upon the individual parameters themselves, not just their ratios. We also showed that the characteristic features for various processes shown in previous studies do not hold true if the steady state is not yet reached. Finally, our framework allows the use of experimentally measured initial conditions to generate testable predictions about the evolution of organelle number distributions under different biogenesis mechanisms.

Together, the work presented in this thesis provides a physical basis for understanding the formation and regulation of diverse intracellular compartments.

5.2 Directions for future work

Our work provided a theoretical understanding of the interaction between tau and microtubules [96]. While a minimal lattice-gas model describing wetting was sufficient to explain the experimental observations, other proteins and surfaces may require more complexity, particularly *in vivo*. As discussed in Chapter 1, biomolecular condensates often involve mechanisms that go beyond standard binary phase separation and wetting [30]. Our framework can therefore be extended in several ways to incorporate more realistic biological effects.

Firstly, cellular environments are highly multicomponent [34]. Our framework can be generalized to include molecules of different types by introducing pairwise coupling terms in the Hamiltonian, similar to the multicomponent Flory–Huggins theory [162]. While multicomponent condensates have been studied extensively [163–166], the wetting behaviour of such mixtures in biological contexts remains unexplored. Investigating the effect of molecular compositions on various surface-bound states could provide interesting insights.

Secondly, in this work, we examined the interaction of phase-separating proteins with an inert solid surface. However, biological surfaces are rarely static. For instance, DNA is a flexible polymer that can be mechanically deformed by interacting proteins. Proteins forming condensates on DNA can therefore deform it or even form condensates along with the DNA, a process that has been referred to as co-condensation [45, 65]. Such processes could be studied within our framework by replacing the rigid surface with a flexible polymer [167].

Another important example of dynamic surfaces is lipid membranes, which can be deformed and remodelled by condensates [35, 39]. The lattice-gas framework provides a natural way to incorporate internal degrees of freedom of a membrane. For example, a recent study modelled membranes as a two-dimensional Ising system coupled to a three-dimensional lattice-gas via

mobile tethers [52]. Interestingly, this model demonstrated a relationship between phase separation in the two-dimensional membrane and in the three-dimensional bulk. We applied a similar framework to study the surface condensation of α -synuclein, another intrinsically disordered protein implicated in neurodegenerative disorders. Experiments revealed that α -synuclein forms surface condensates at much lower concentrations than required for bulk phase separation. However, this happens only for specific lipid stoichiometries. Our model could explain this observation by showing that surface condensation depends on membrane properties such as lipid composition [37]. Future work could further explore this line of inquiry.

Finally, cellular environments are inherently far from equilibrium [31, 168]. In the present work, we focused primarily on equilibrium states and relaxation toward equilibrium. However, the framework can be extended to include active processes. Non-equilibrium lattice models are known to exhibit a wide range of rich dynamical behaviours, including periodic or travelling waves, and active phase separation [10, 169, 170]. Incorporating these effects may provide insight into how energy-driven cellular processes regulate biomolecular condensates, especially on surfaces [171].

For our model of membrane-bound organelle abundances [97], the natural step is to systematically compare with experimental data, allowing inference of the kinetic parameters and discriminating between competing biogenesis mechanisms.

Additionally, our framework made some simplifying assumptions that were detailed in Chapter 4. Some natural extensions of our model would be to allow time-dependent rates or consider the spatial organization of the organelles in addition to the temporal evolution. Introducing spatial organization would particularly be relevant since there is a growing body of work studying the effect of cellular perturbations on organelle positioning [172, 173].

Lastly, an intriguing direction would be to allow organelles of different kinds to evolve together under a common constraint. For example, the pool of nutrients in the cell is limited, which means that organelles of different kinds might compete for them [174, 175]. There might be other mutual effects as well [176]. This would also allow us to leverage multiplex microscopy

imaging, which allows tracking more than one species at the same time [[177](#)]. A compelling direction for future work would be to develop such a "multi-species" framework.

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