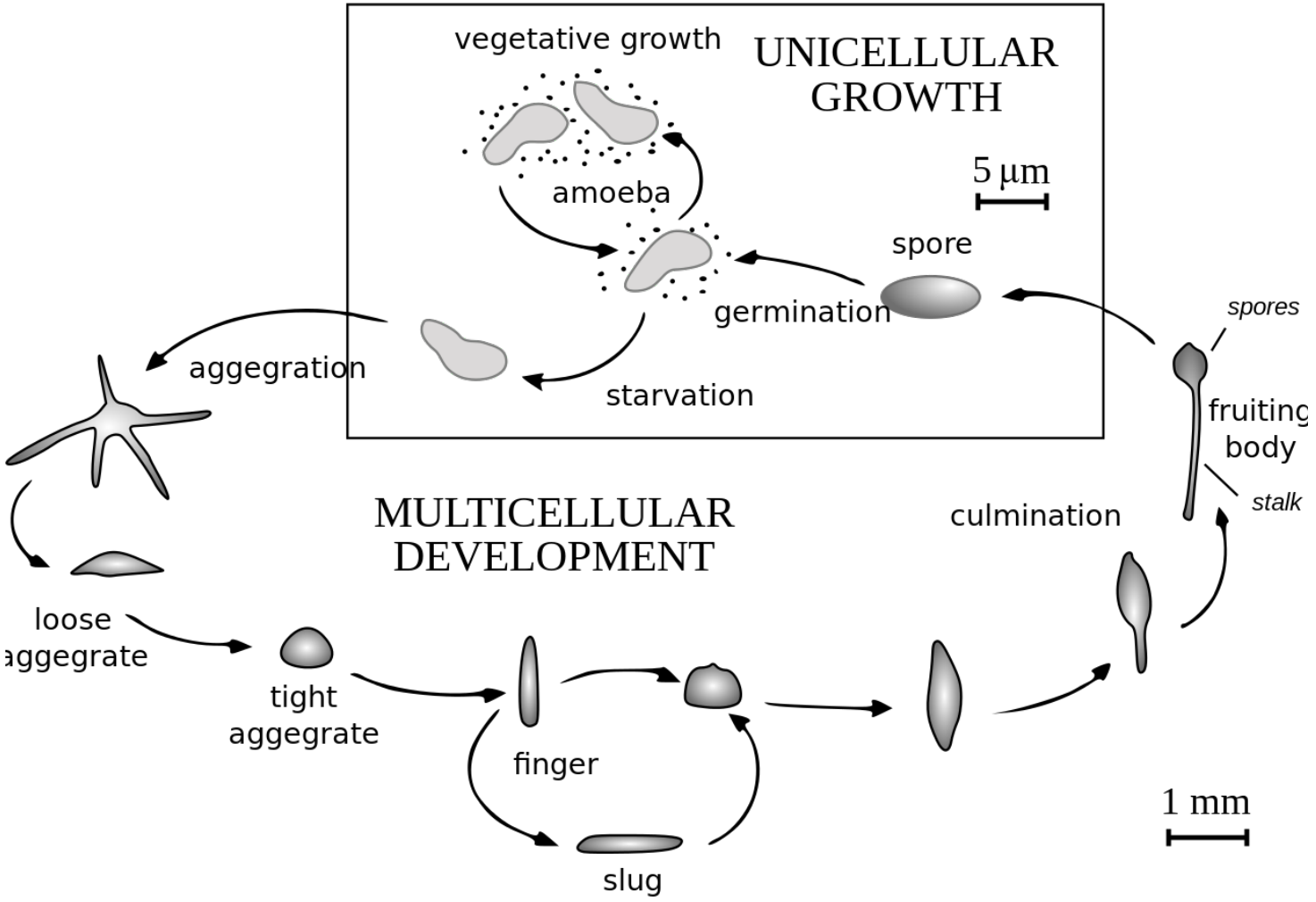
# Coupling positive feedback loops with cell-cell signalling to overcome noise during developmental initiation in *Dictyostelium*

**Daniel Kornai**

# Overview

- **Introduction to *Dictyostelium***
- **Noise and synchronization: Biological motivations of the model**
- **Growth-Differentiation pathway: Overview and Boolean network model**
- **Behaviour of the single cell model**
- **Overview: 1D spatial model of the Dictyostelium colony**
- **Behaviour of the 1D spatial model across a range of noise levels**

# *Dictyostelium* lifecycle



# Noise and synchronization: Biological motivations of the model

- **A Dictyostelium colony can only aggregate successfully if the cell fate decisions of individual cells are synchronized**

- **Synchronization is difficult due to two compounding factors:**

  - **Variability in the environment cells are exposed to**
    **→ cells receive different signals**

  - **Intrinsic noise from the stochastic dynamics of gene expression**
    **→ cells have different responses to identical signals**

***How can Dictyostelium guarantee that cells choose to enter the social-developmental cycle synchronously?***

# *Dictyostelium* phylogeny and the biological motivations of the model

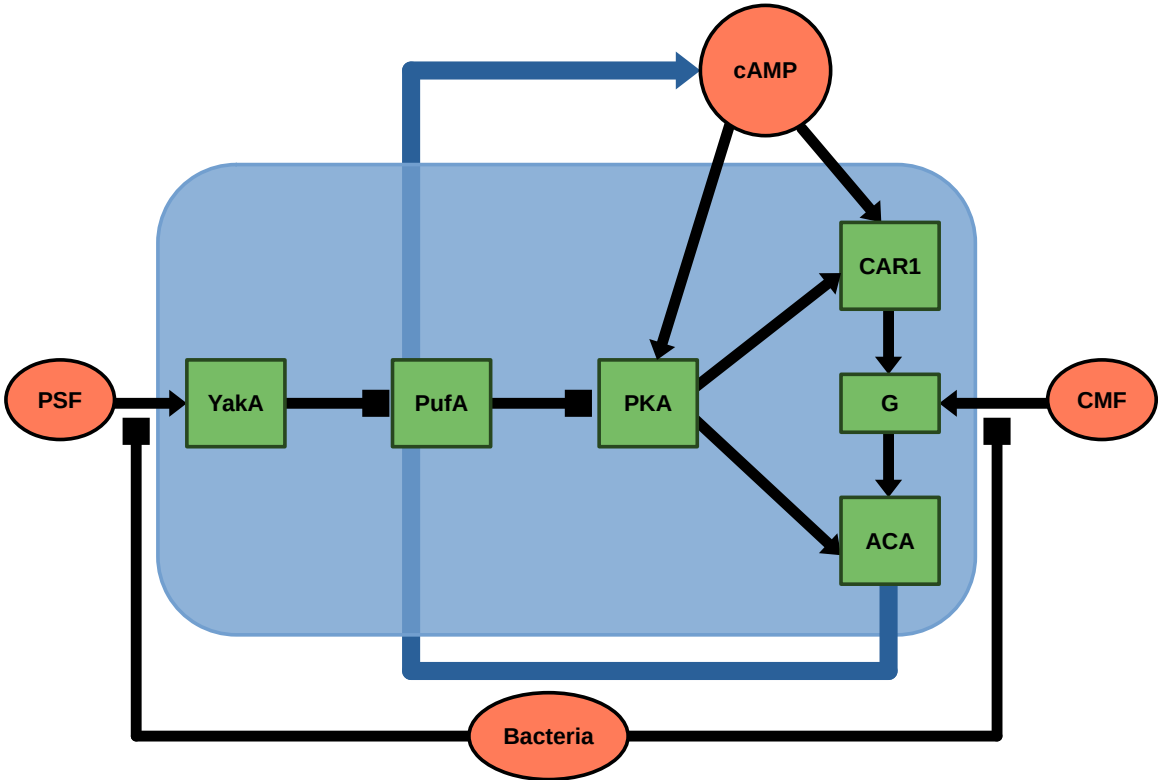
- **Dictyostelid phylogeny can be subdivided into two branches each containing two major groups.**
- **Colony sizes vary across groups, with Group 4 species having much larger colony sizes than groups 1-3**
- **Larger colonies are more difficult to synchronize:**
  - **Larger colonies inhabit larger areas, which implies that cells are subjected to a wider range of microenvironments.**
  - **A higher number of cells are distributed across a larger area, meaning that any cell-cell communication that relies on the diffusion of signaling molecules is less efficient at transferring information between the most distant cells**
- **This suggests that the synchronization mechanisms utilized by group 4 species are more robust. Why?**
- **Group 4 species export a signalling molecule (cAMP) which is involved in a positive feedback loop to coordinate aggregation, while groups 1-3 only utilize cAMP as an intracellular signal during early development**

**How did the coupling of cell-cell signalling to a positive feedback loop enable group 4 *Dictyostelia* to lower the amount of stochastic variation in the timing of the onset of aggregation, thereby allowing larger groups to collaborate successfully?**

# The growth-differentiation pathway: controlling the transition from unicellular growth to aggregation

- **Well-fed cells secrete the PSF (Pre-starvation Factor)**
  - PSF receptors are only produced by starving cells. Thus, well fed cells will not respond to PSF
  - PSF concentrations increase with cell density, allowing cells to estimate the density of kin
- **Starving cells secrete CMF (Conditioned Medium Factor)**
  - Starving cells make CMF receptors, and secrete CMF.
  - CMF concentrations indicate the density of starving kin.
- **PSF indirectly activates PKA**
- **PKA activates CAR1 (a cAMP receptor) and ACA (the cAMP synthase). Most cAMP is exported outside the cell**
- **CMF activates G proteins which enable the activation of ACA by CAR1**
- **This completes a positive feedback loop, as cAMP exposure activates PKA and CAR1, leading to further cAMP production**

***Exposure to cAMP induces the expression of aggregation competence genes (such as the cell adhesion genes csA, tgrB1, and tgrC1), allowing these cells to start multicellular development***



# Boolean network model of the growth-differentiation pathway

- Scalar input in the form of signal concentrations
- Boolean circuit representing internal elements
- Signals are secreted according to the internal state

**Gene**
(can be *OFF* or *ON* )

**internal mRNA concentration**
(scalar value)

**Concentration of external signal**
(scalar value)

**Wire**

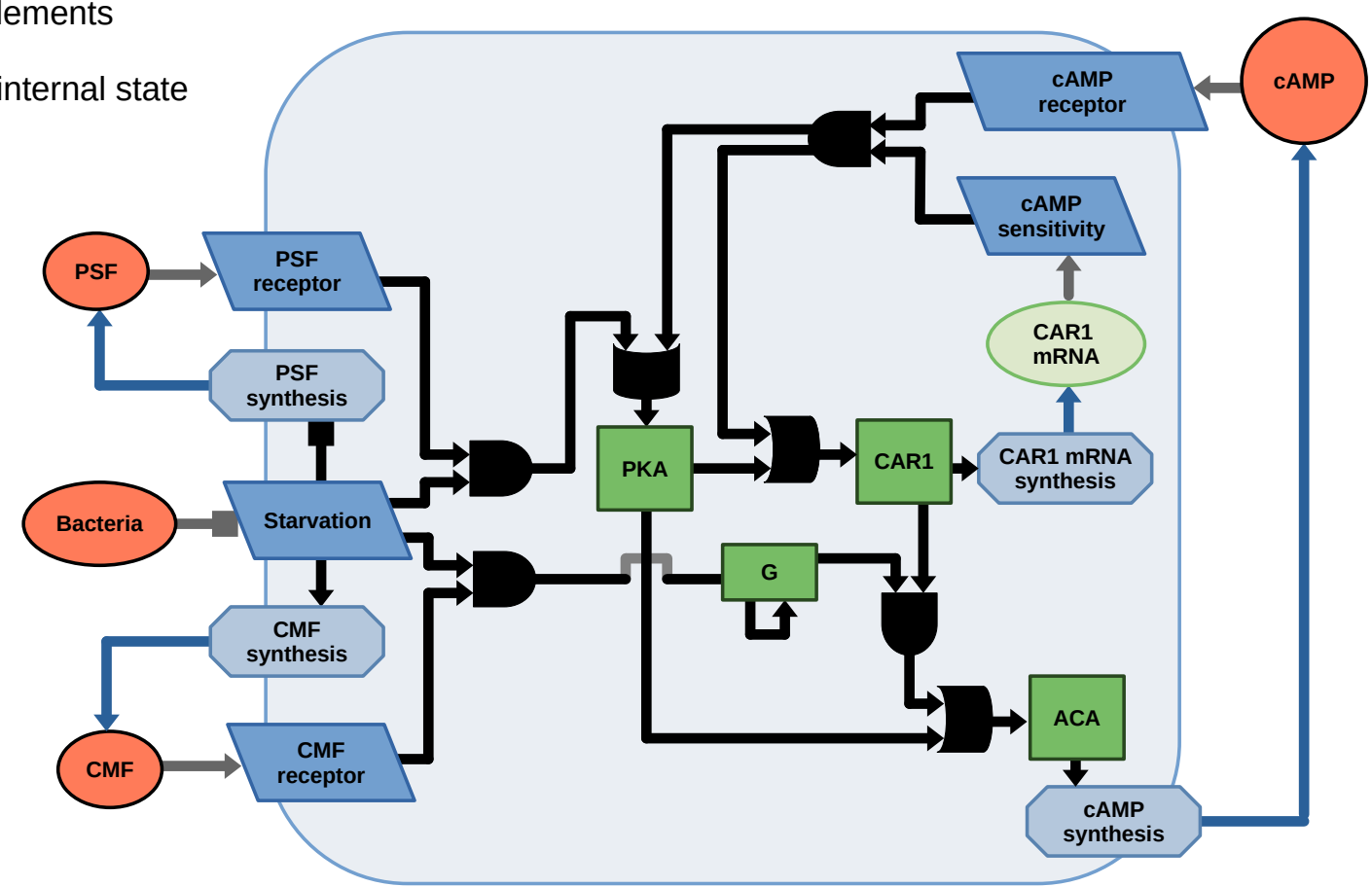
**Boolean NOT**

**AND gate**

**Probabilistic OR gate**
Returns *TRUE* with probability of 0.75 if only 1 input is *TRUE*

**Signal molecule secretor**
If boolean input is *TRUE* , increases the concentration of a signal by predefined amount

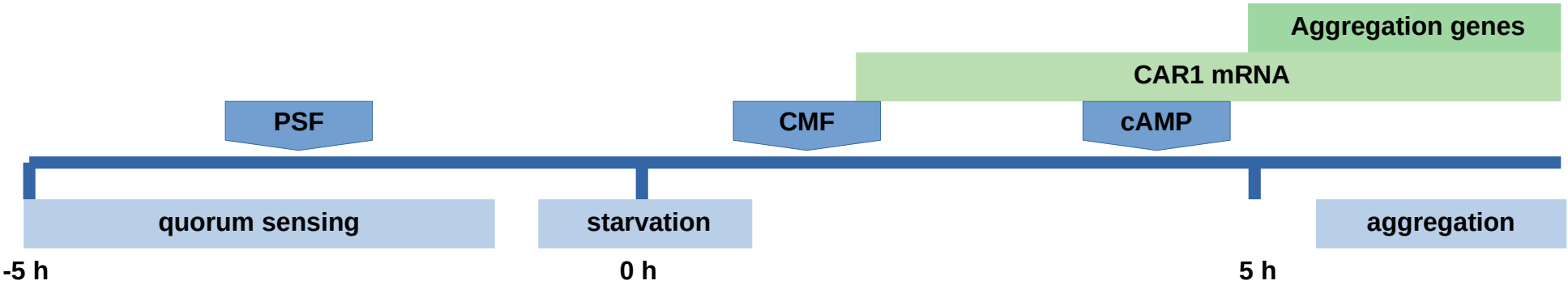
**Signal molecule sensor**
Takes scalar input concentration, returns *TRUE* with probability input/100



# Single cell model

- **Given the boolean network, the production (and consumption) rates of signals impact the time domain behaviour of the model.**

- **Production rate parameters set to match timescales established in the literature**

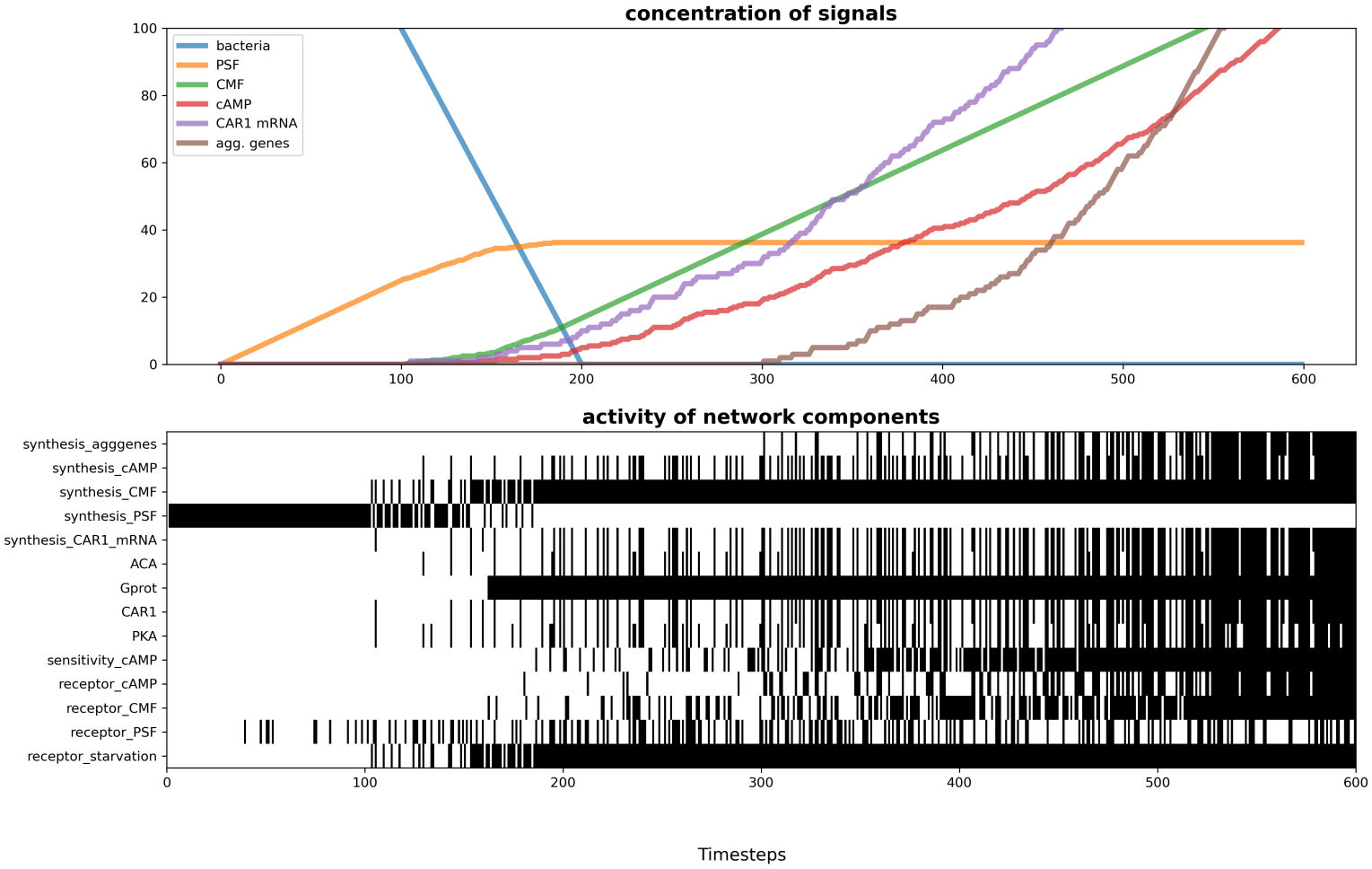



  - **PSF production starts 2-3 hours before starvation**
  - **CMF production is significant after 1-2 hours of starvation**
  - **cAMP production following 4-5 hours of starvation**

  - **CAR1 mRNA accumulates 2 hours following starvation**
  - **Aggregation competence genes are expressed at 5-6 hours following starvation**

- **Internal cell state, the level of extracellular signals (Bacteria, PSF, CMF, cAMP) and internal signals (CAR1 mRNA) are tracked over time**

# Network component activity and signal levels in a single cell over time

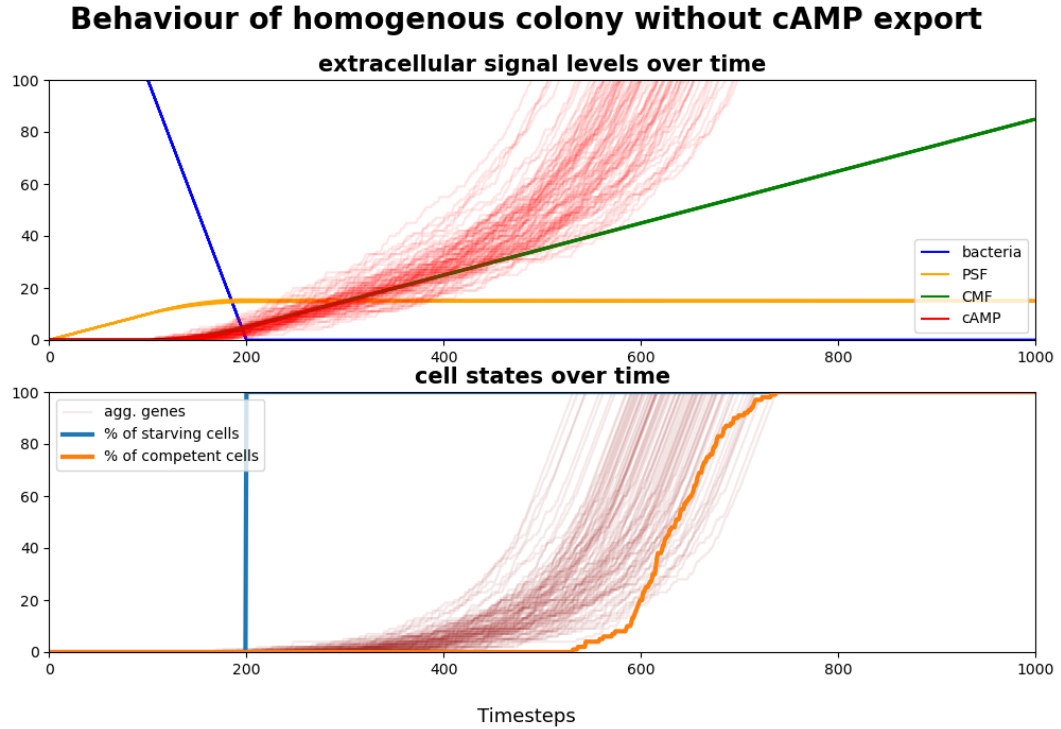



- **Single cell model reproduces expected temporal behaviour, and the inherent stochasticity of transcription**

# 1D spatial model

- **Single cell model is extended by allowing extracellular signals (PSF, CMF, cAMP) to diffuse between neighbouring grid cells arranged in a line**
  - **Diffusion rates for the signals depend on the size of the molecule**
- **The collective behaviour of cells in a grid unit is modelled via a single cell. However, grid units now have a cell density parameter, which multiplies the rate at which signals are produced**
- **The starting cell density, and starting bacterial concentrations are drawn from truncated normal distributions with variable standard deviation**
  - **Truncation is to avoid ≈0 cell densities or bacterial concentrations**
- **Internal cell state, the level of extracellular signals (Bacteria, PSF, CMF, cAMP) and internal signals (CAR1 mRNA) are tracked over time**
- **The lack of cAMP export by Dictyostelia in groups 1-3 is modelled by setting the diffusion rate of cAMP to 0, thereby making it an intracellular signal**

# 1D model: Colony behaviour in groups 1-3 without cAMP export




- **When starvation is synchronous, the moderate de-synchronization in the timing of aggregation competence is due to intracellular stochasticity alone.**

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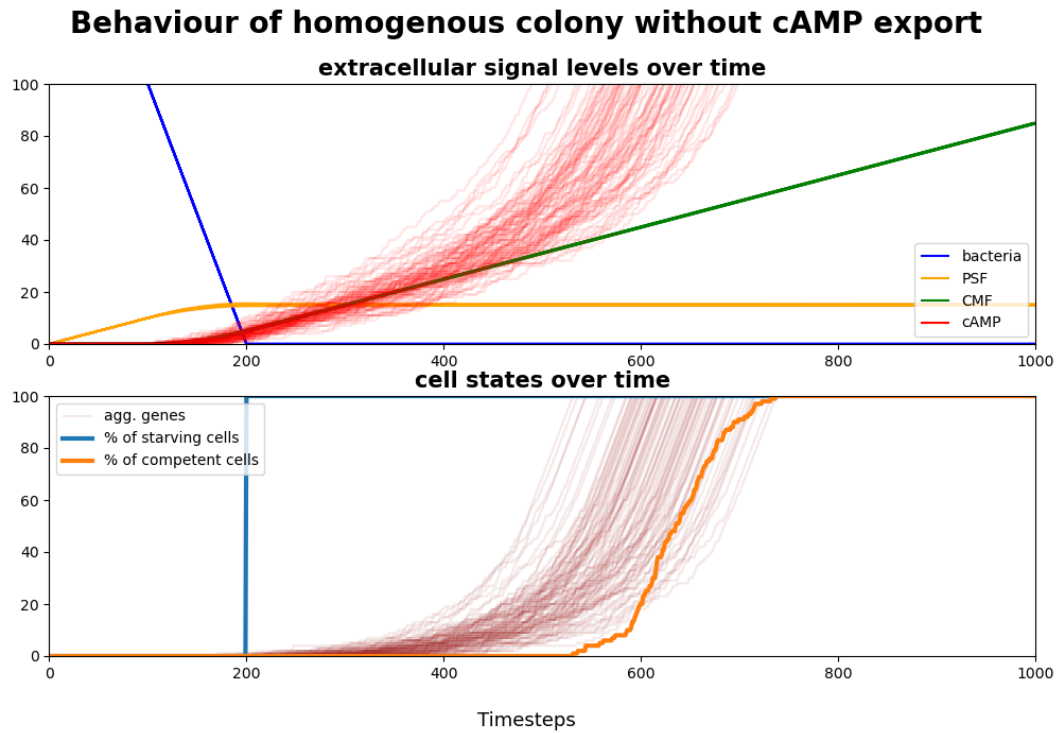
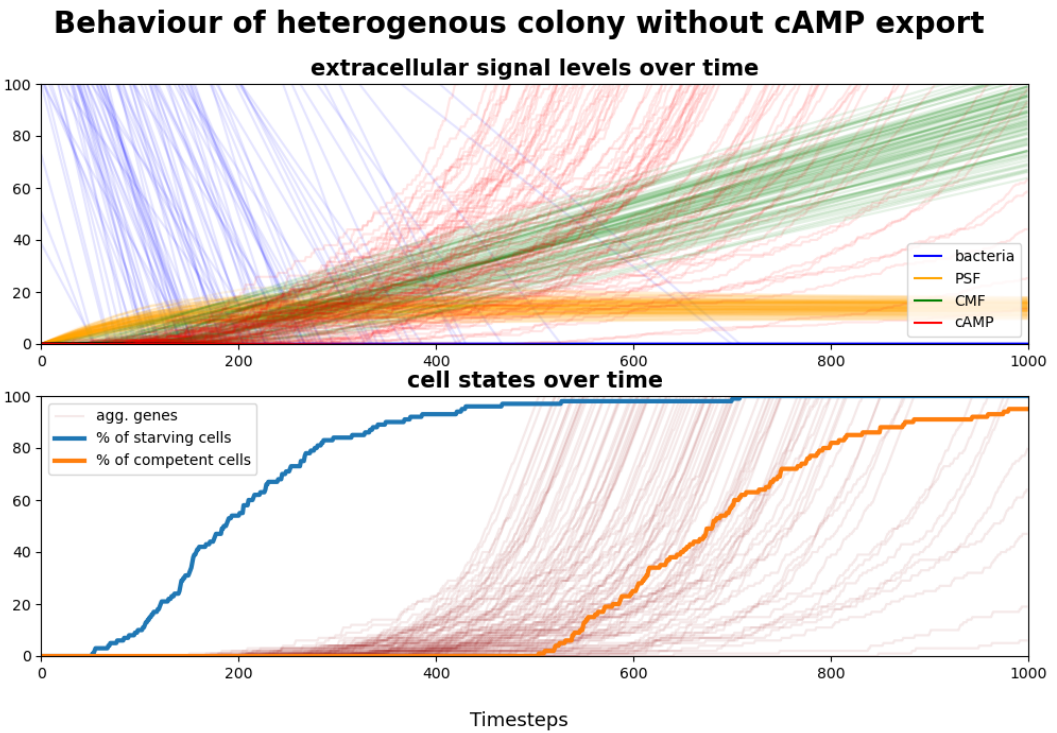
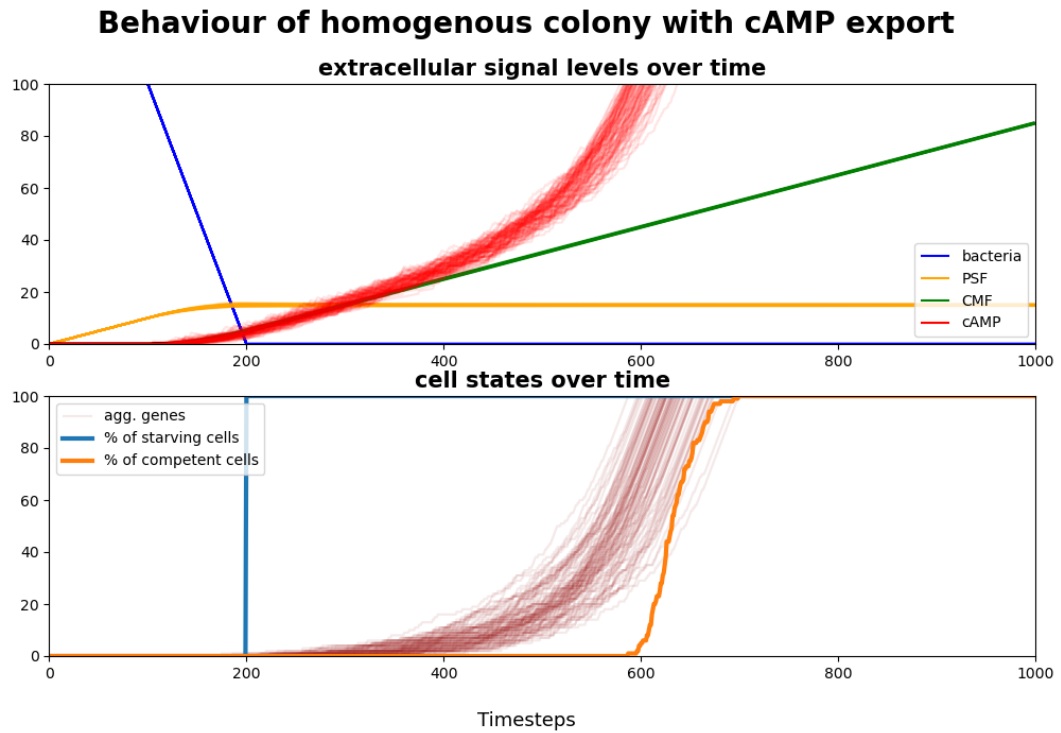



- **When starvation is synchronous, the moderate de-synchronization in the timing of aggregation competence is due to intracellular stochasticity alone.**
- **When starvation is asynchronous due to variability in cell densities and starting bacterial concentrations, intracellular stochasticity and environmental heterogeneity compound to increase the spread in the timing of aggregation competence**
- **Despite PSF and CMF signals diffusing across the colony, cell-cell communication alone is not able to synchronize the population**

# 1D model: Colony behaviour in group 4 with cAMP export




- **When starvation is synchronous, diffusing cAMP signals can overcome intracellular stochasticity to synchronize aggregation**

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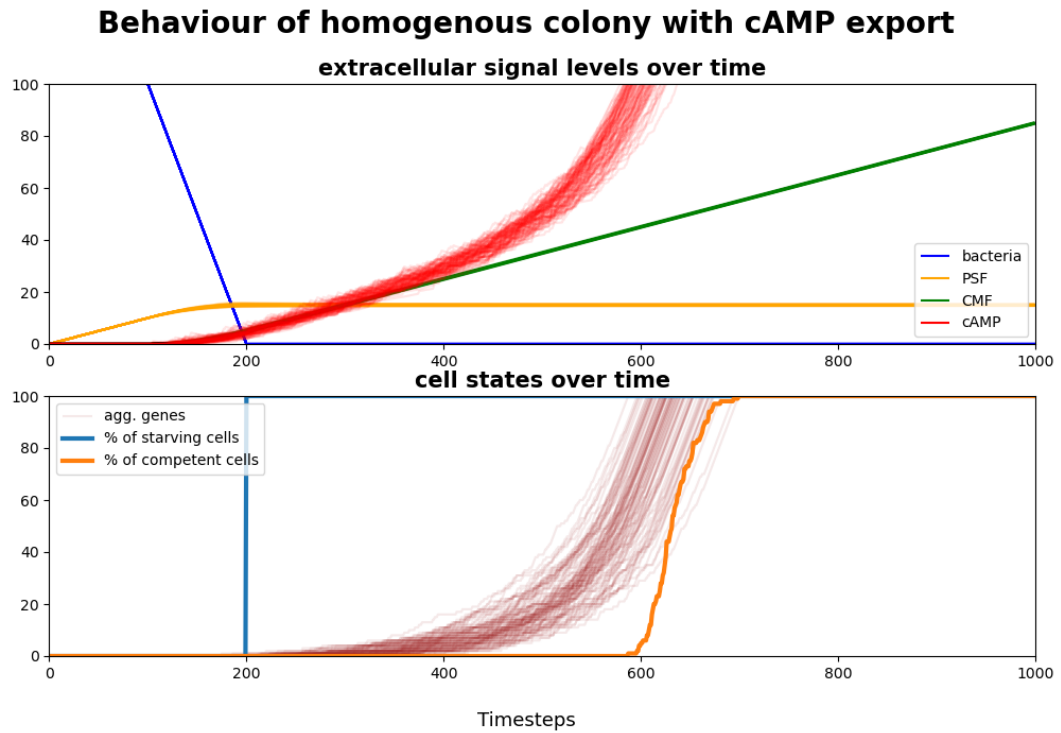
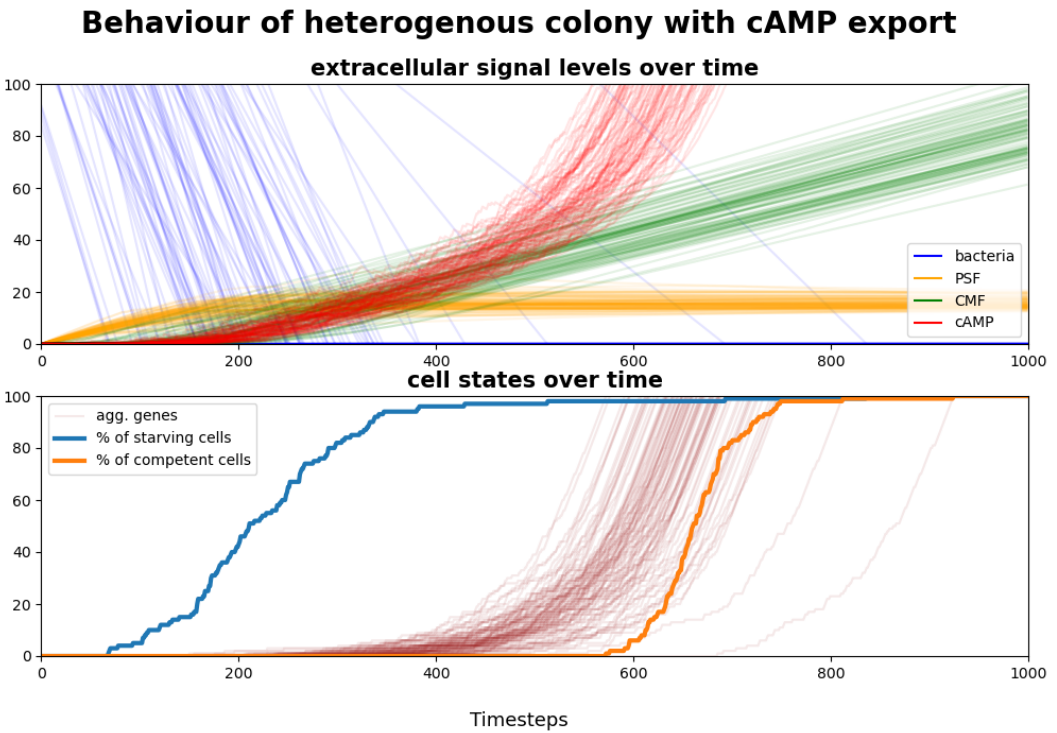
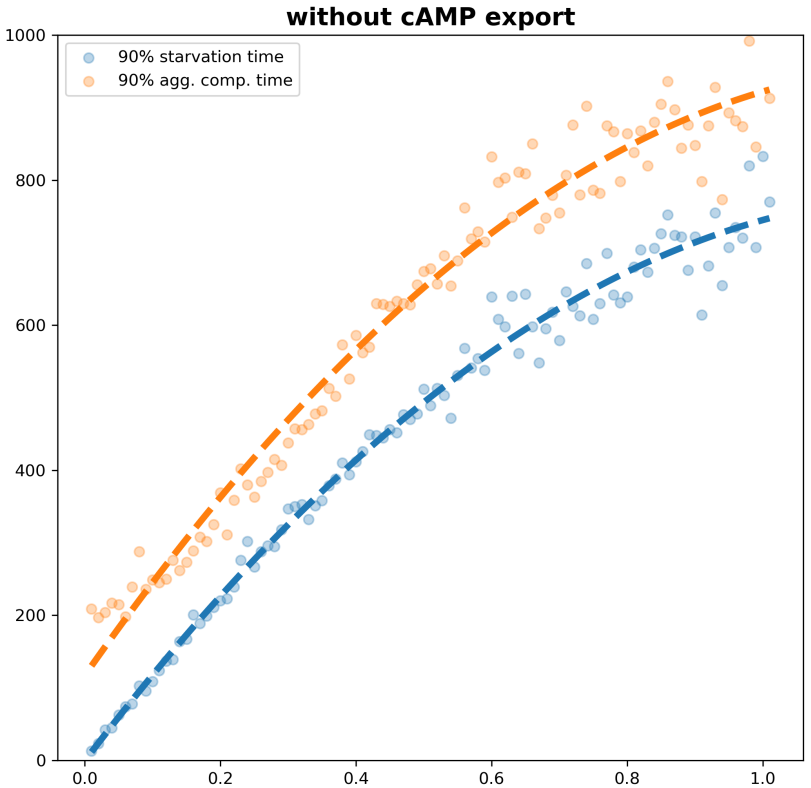



- **When starvation is synchronous, diffusing cAMP signals can overcome intracellular stochasticity to synchronize aggregation**
- **When starvation is asynchronous due to variability in cell densities and starting bacterial concentrations, the positive feedback loop induced by cAMP signals synchronize aggregation competence, despite varying PSF and CMF levels**
- **cell-cell communication coupled with positive feedback loops can maintain colony synchronicity, even in the face of significant noise**

# Comparison of predicted colony behaviour in groups 1-3 vs group 4 across a range of heterogeneity levels using the 1D model



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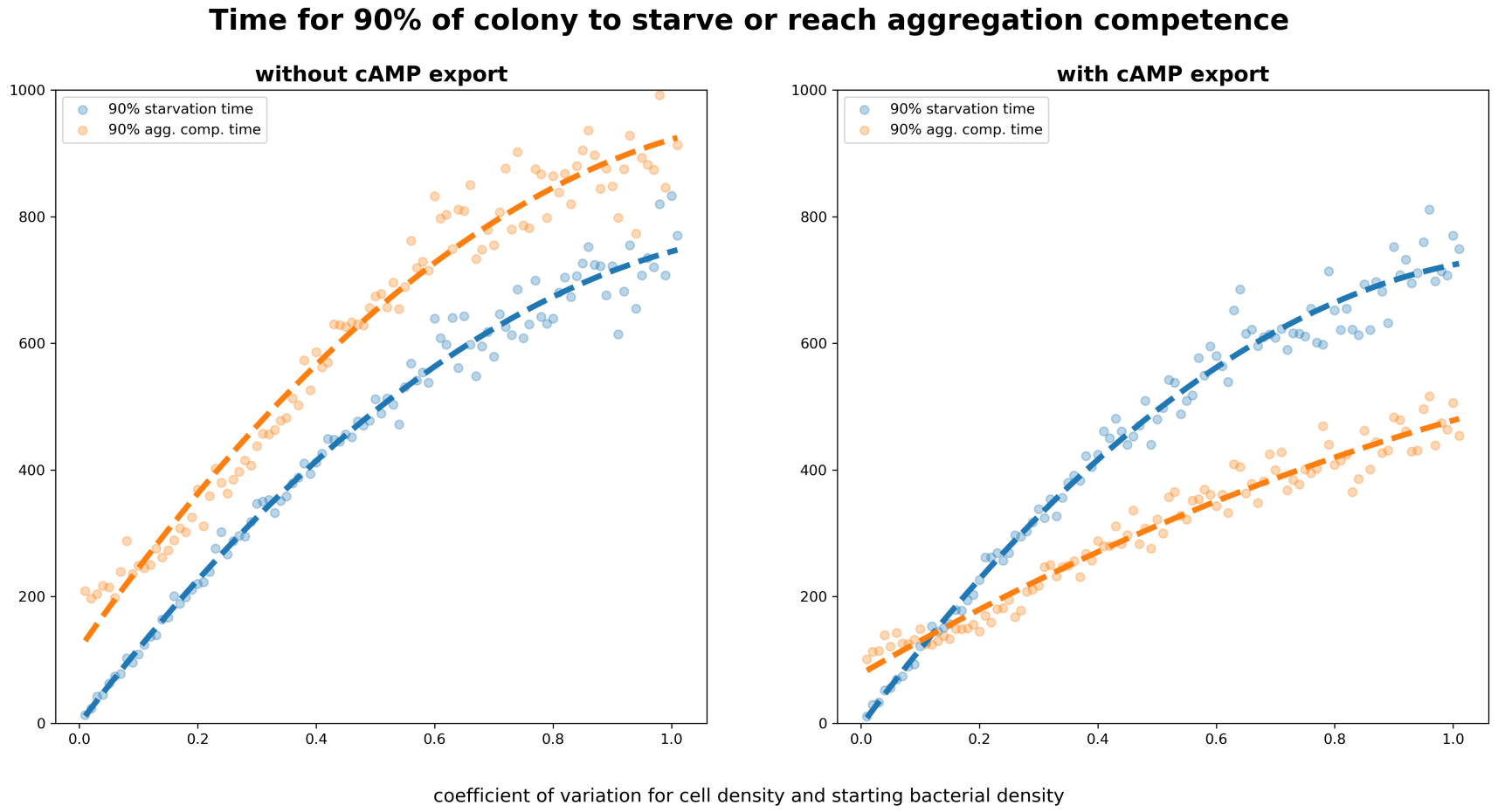

# Conclusions

- **Dictyostelium groups must synchronize cell fate decisions to succeed in their multicellular development**
- **The synchronization of autonomous cell fate decisions is made difficult by the intrinsic stochasticity of cells, and variability in their environment**
- **Phylogenetic analysis suggests that the increased colony sizes of group 4 Dictyostelia are due to the export of cAMP, a signalling molecule involved in a positive feedback loop.**
- **The 1D spatial model suggests that when cell-cell signalling is coupled with a positive feedback loop, noise arising from environmental heterogeneity and intracellular stochasticity can be effectively buffered, allowing the population to make collective deterministic cell fate choices**

# Limitations

- **A boolean circuit model represents a significant simplification of the intracellular processes driving decision-making**
- **Precise measurements for the level of extracellular signals over time are not available in the literature**
- **Time domain RNAseq data used to calibrate transcription levels only has a resolution of 1-2 h**
- **Other signalling molecules are known to be involved in the initiation of aggregation, but their regulatory networks are not currently known**

# Thank you for your attention!

## Any questions?