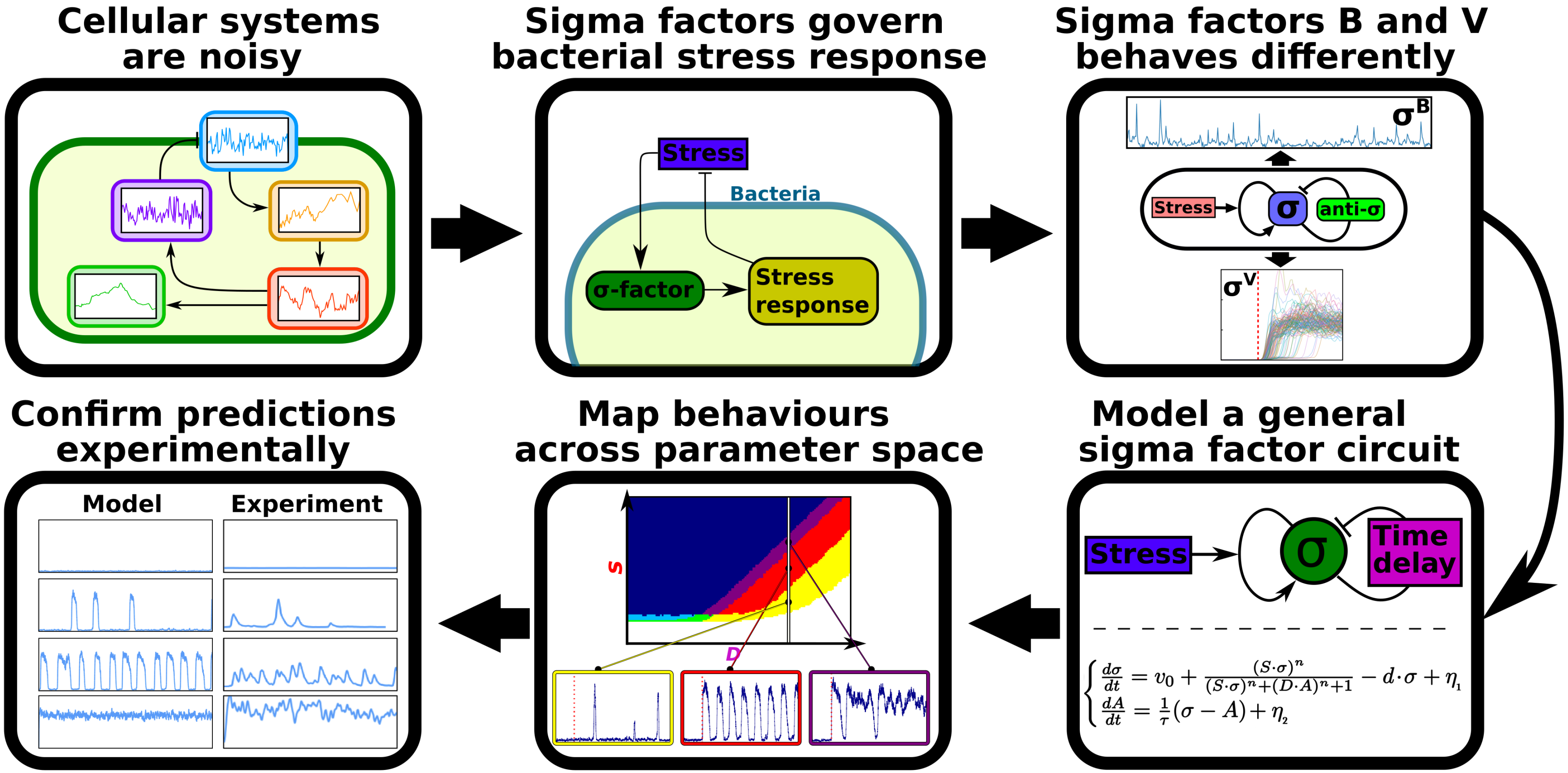
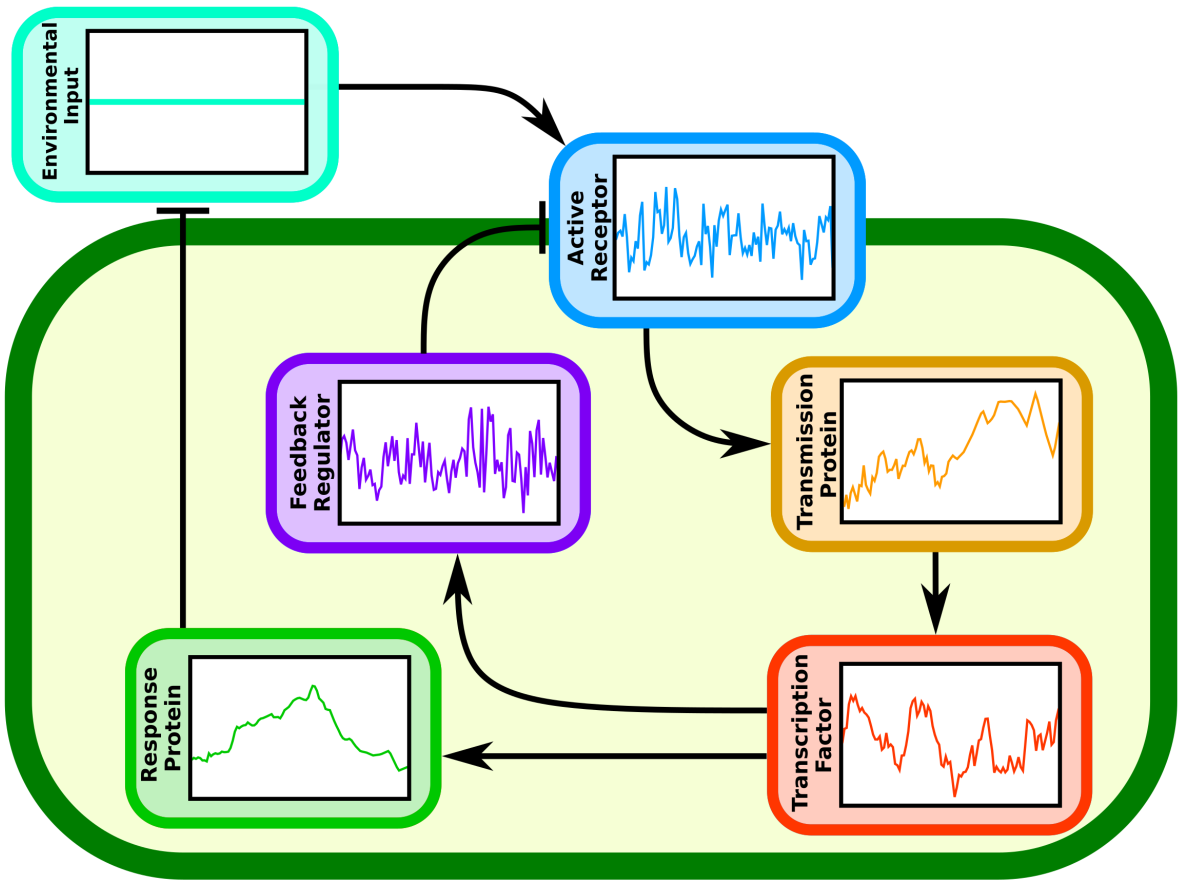
qQ

How bacteria generate beneficial phenotypic heterogeneity through mixed positive/negative feedback loops

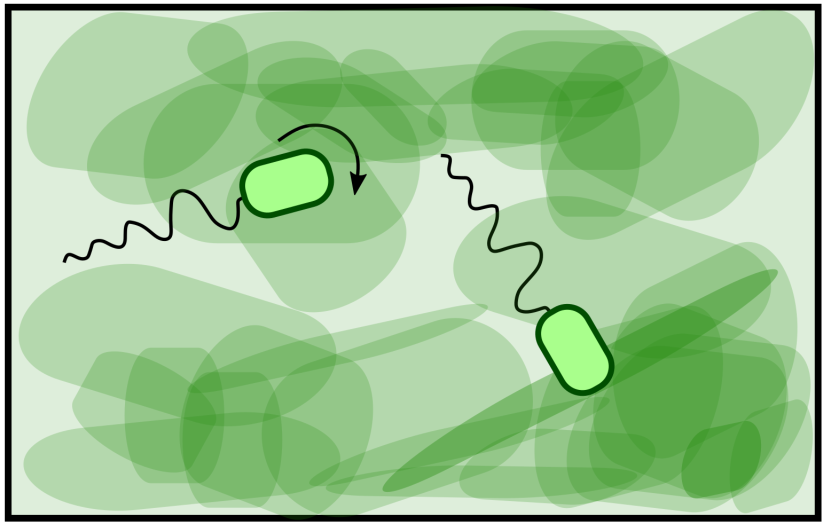


Biological systems are noisy

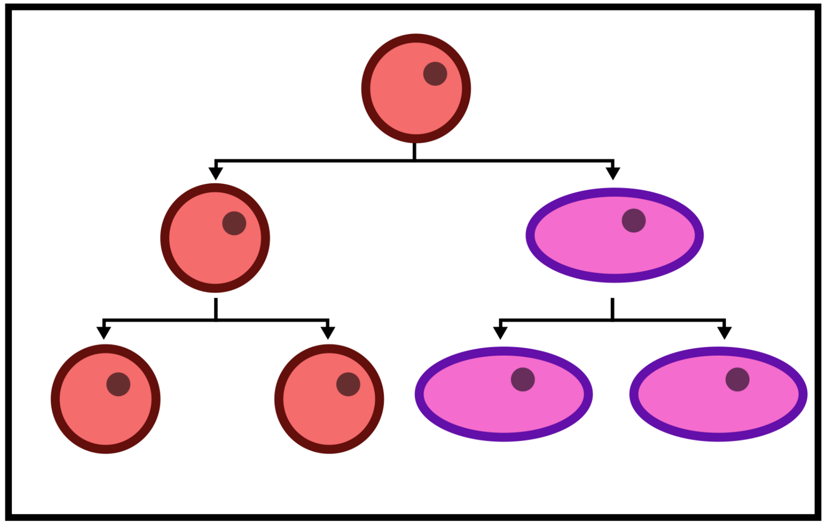


Noise is caused by e.g. randomness in (diffusion based) reactions.

Cellular noise is phenotypically important



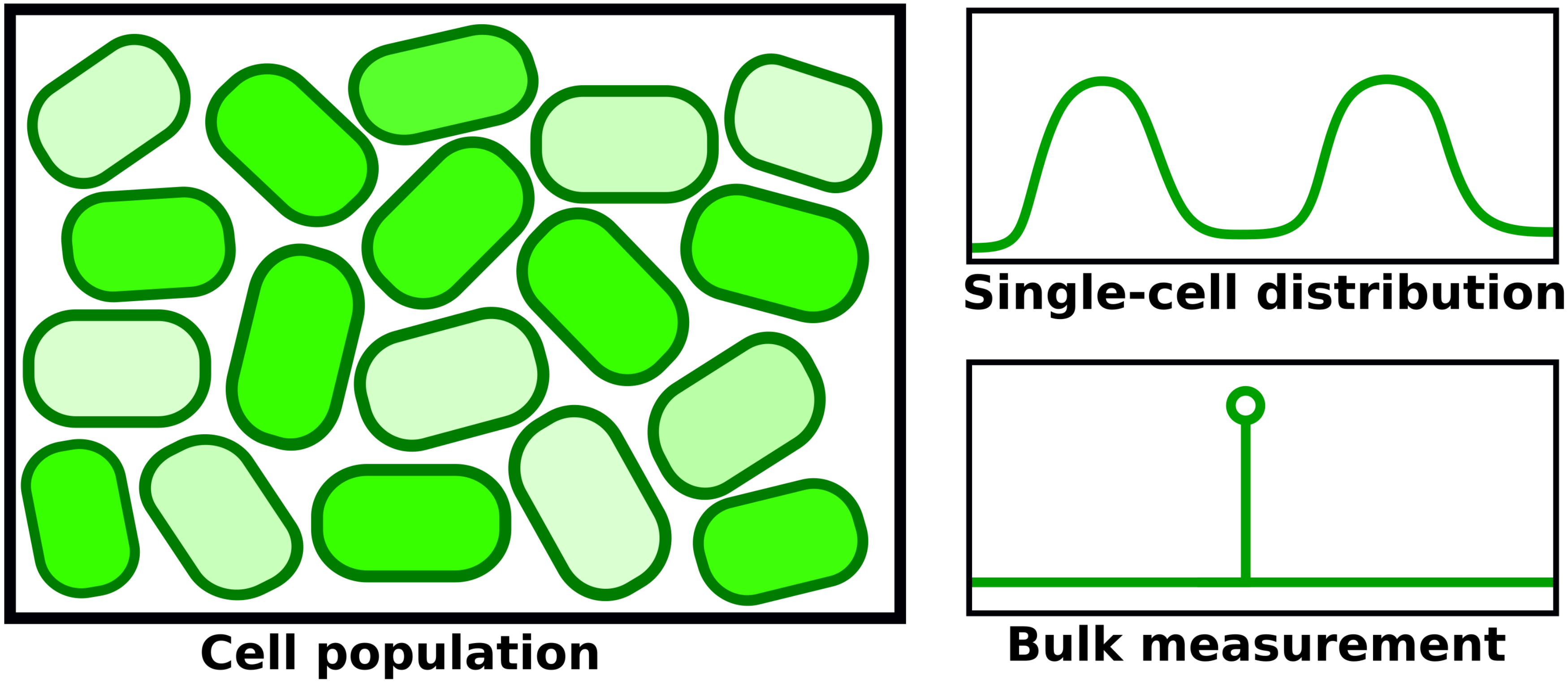
Bacterial Chemotaxis



Cell Differentiation

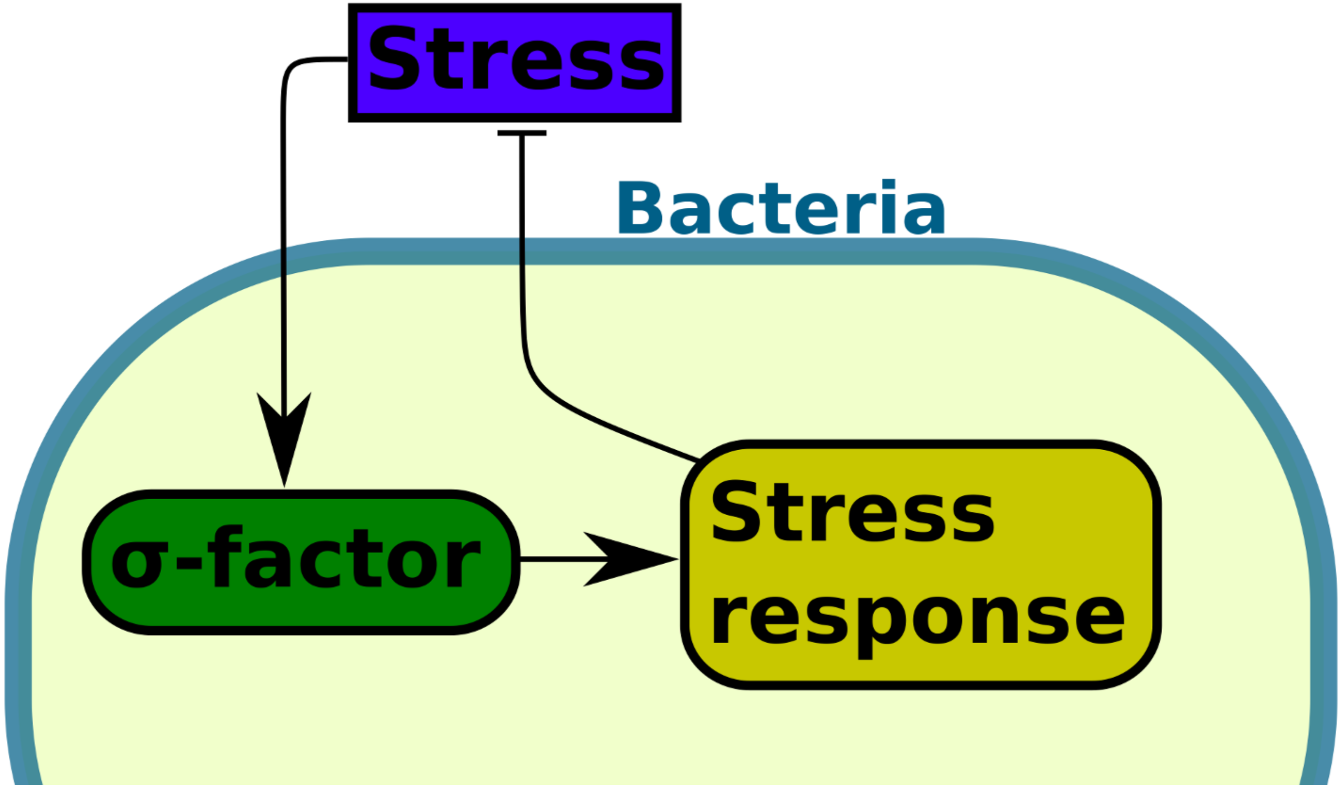
Several decisions are based on noise-induced randomness.

Bulk measurements obscure single-cell dynamics



Single-cell measurements may reveal population heterogeneity.

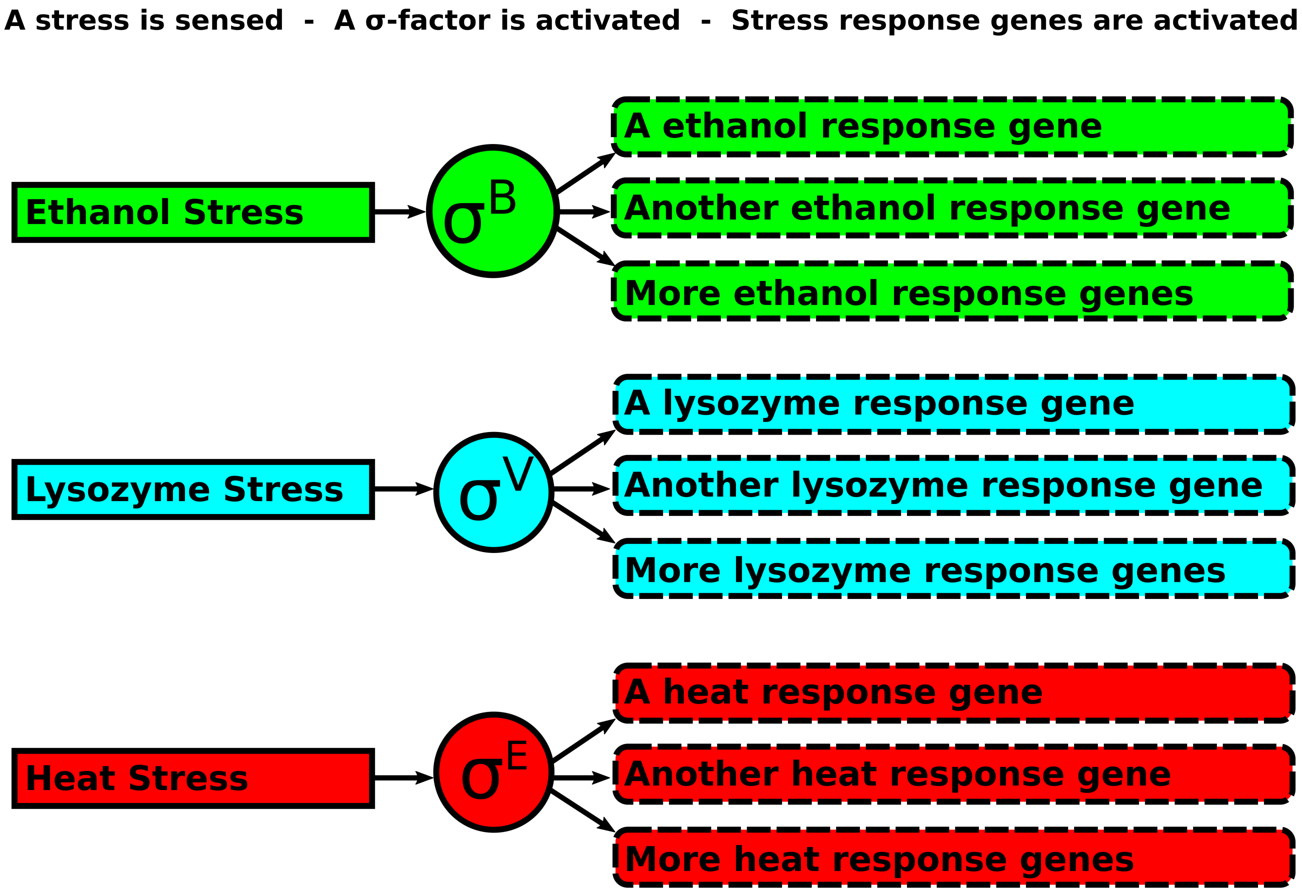
Bacteria activate σ-factors in response to stress



These then activate the stress response genes.

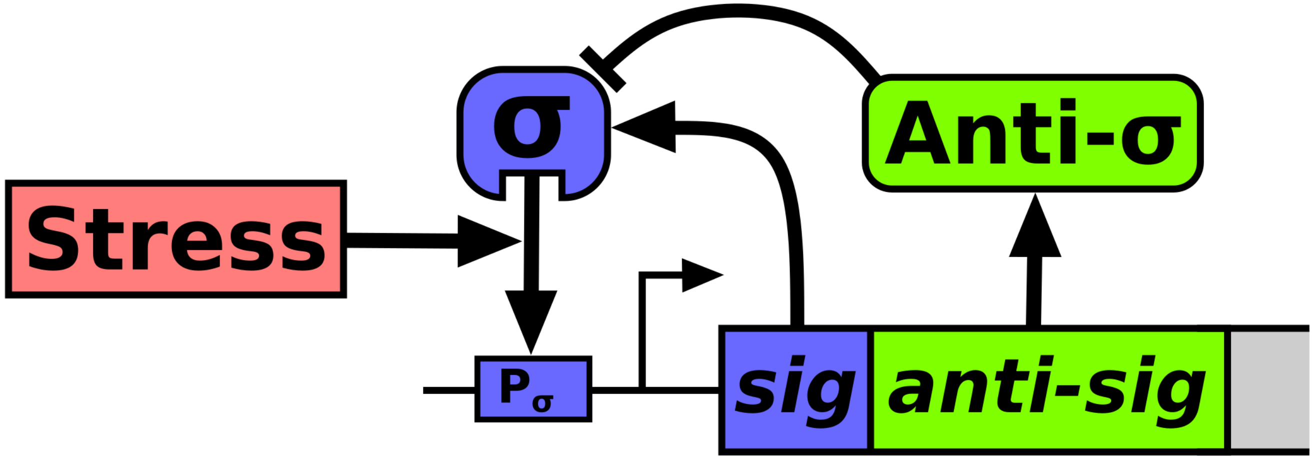
We will use them as a system to study cellular noise.

Bacteria often have several different σ-factors



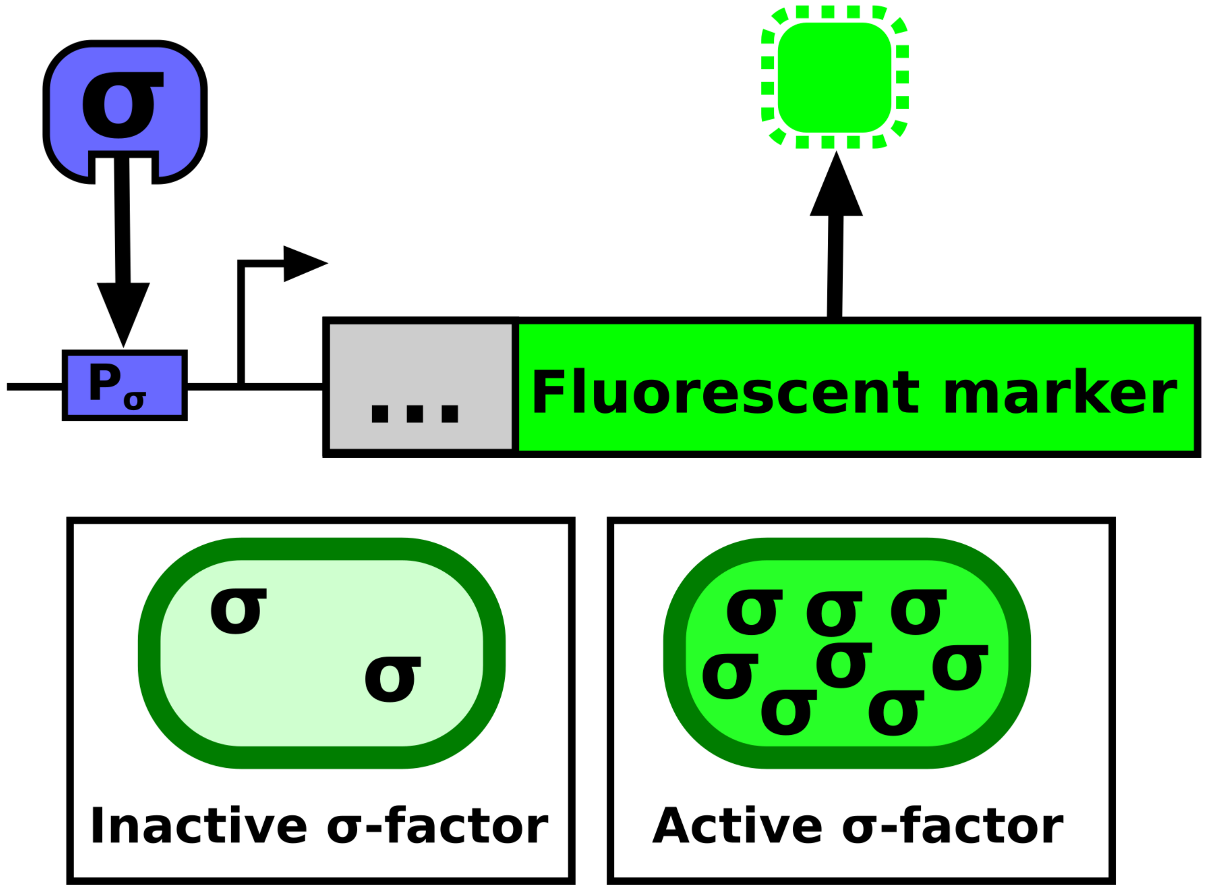
Each activates in response to a specific stress.

Most σ-factors circuits have a similar structure



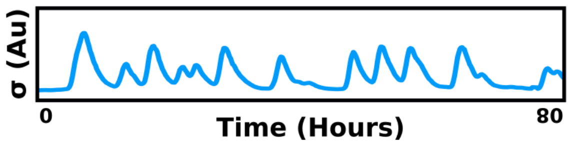
The σ-factor activates both its own production, and that of an anti-σ-factor.
This creates a mixed positive/negative feedback loop.

We measure σ-factors activity through fluorescent markers



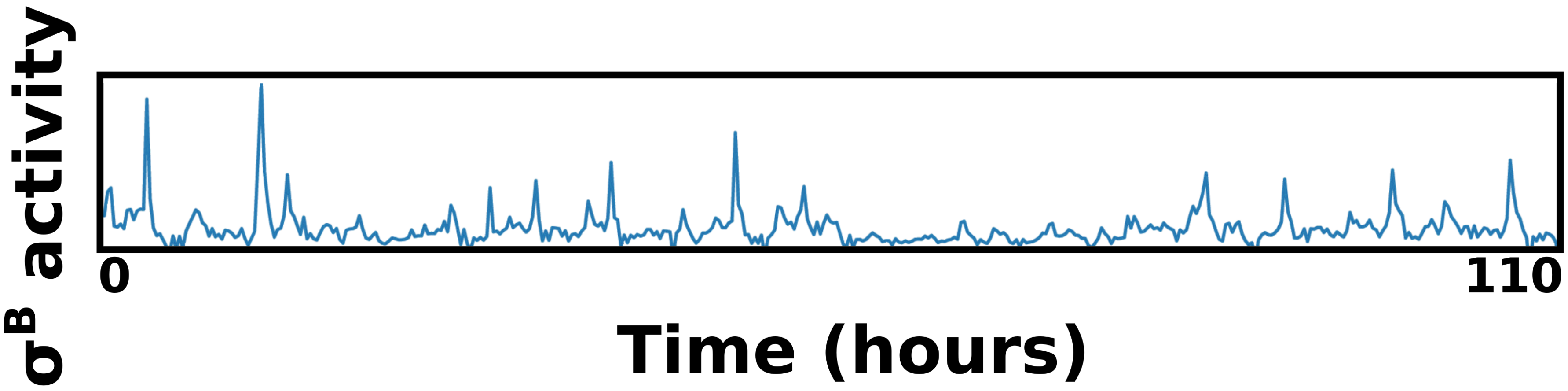
Cellular fluorescence corresponds to σ-factors activity.

We measure σ-factors activity using single-cell fluorescent microscopy



Using image analysis, time trajectories are created.

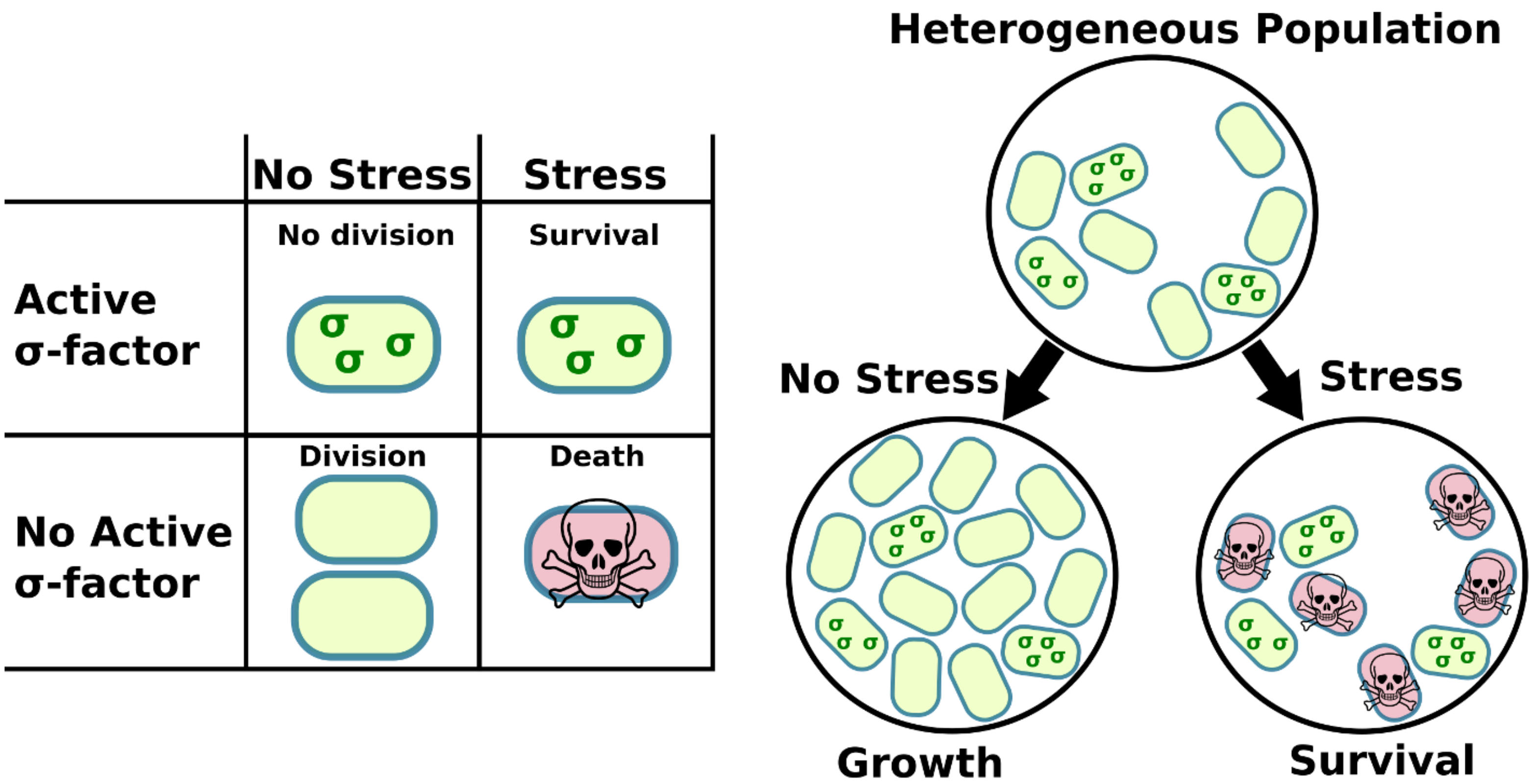
σ-factor B (σ^B) responds to environmental stress



It displays a stochastic pulsing behaviour (in the presence e.g. ethanol).

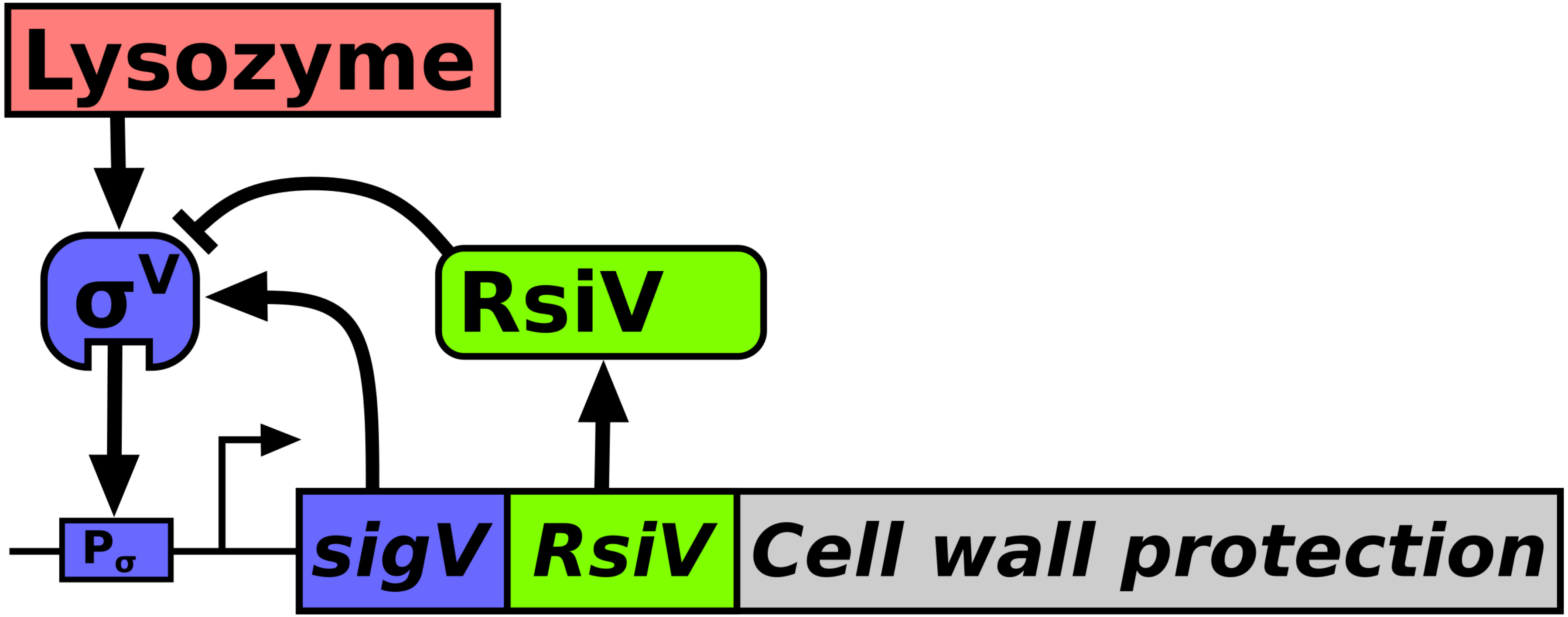
Park et al. *(2018)* *Cell Systems*

This creates population heterogeneity, allowing adaption to an uncertain future



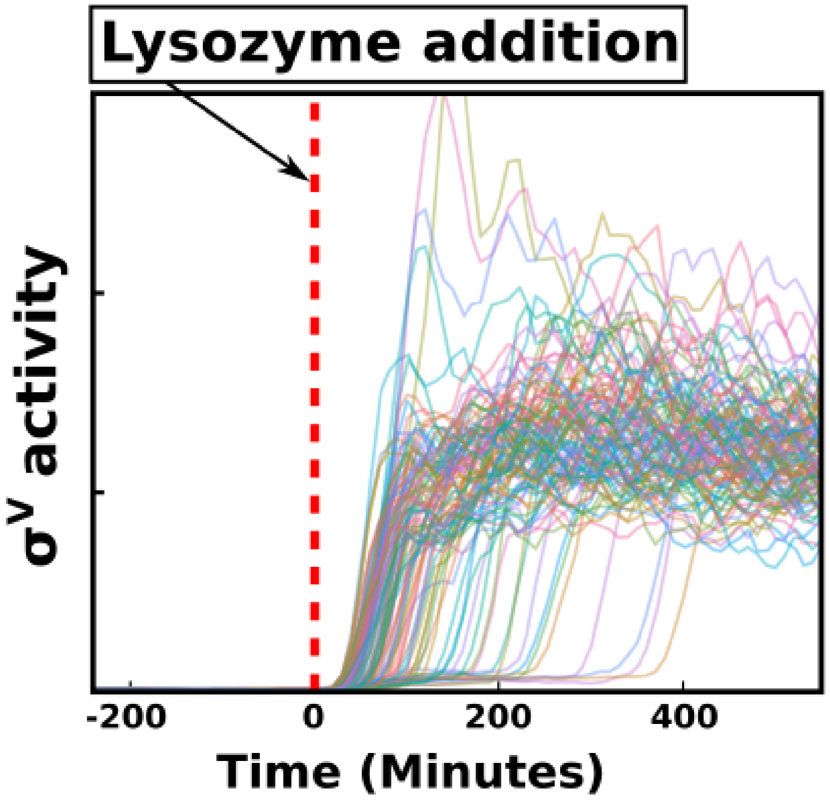
If stress wipes out the non-expressers, the expressers can repopulate the colony.

σ-factor V (σ^V) regulates the lysozyme stress response



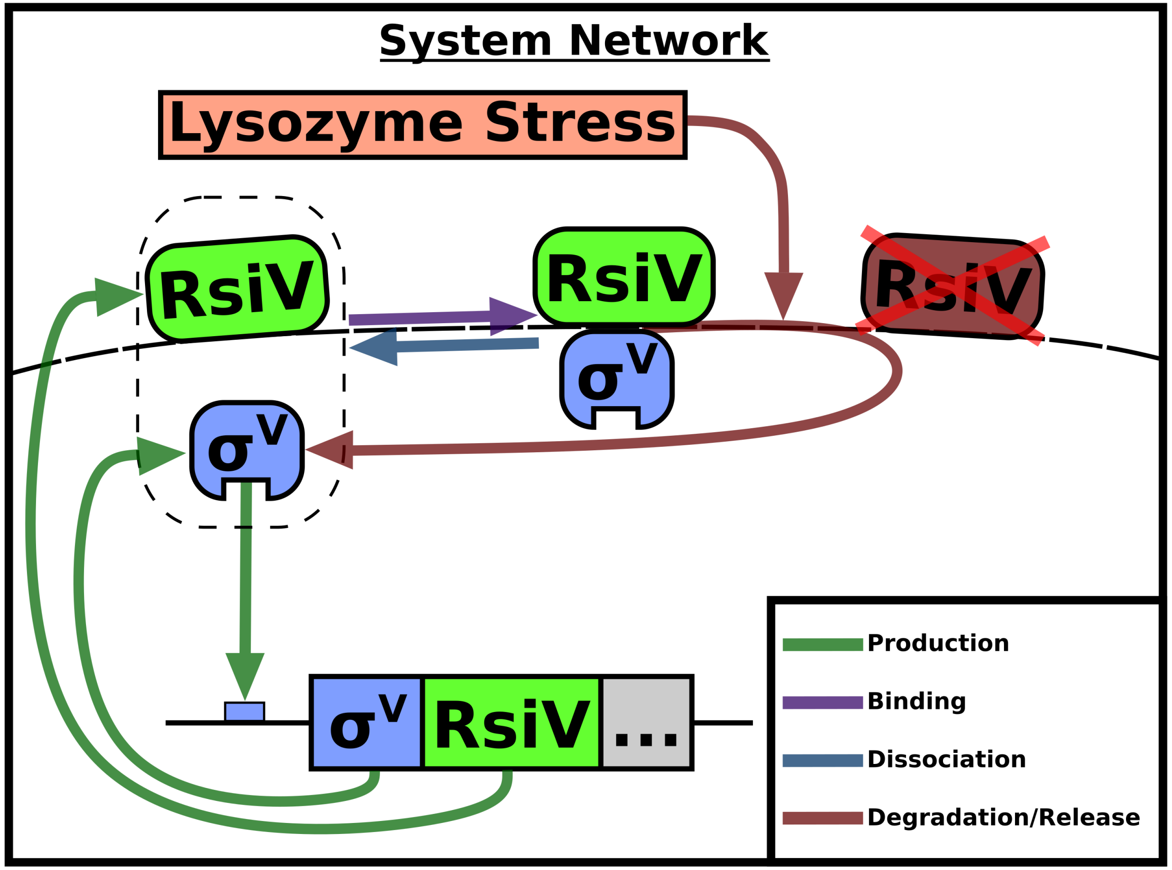
It activates genes for cell wall protection and repair.

σ^V responds through a heterogeneous activation behaviour



The time to activation is heterogeneous across an isogenic population.

We can model the σ^V circuit



Model Reactions

$\varnothing \xrightarrow{\sigma^V} \sigma^V + \mathrm{RsiV}$

$\sigma^V + \mathrm{RsiV} \rightarrow \sigma^V\text{-RsiV}$

$\sigma^V\text{-RsiV} \rightarrow \sigma^V + \mathrm{RsiV}$

$\sigma^V\text{-RsiV} \xrightarrow{L} \sigma V$

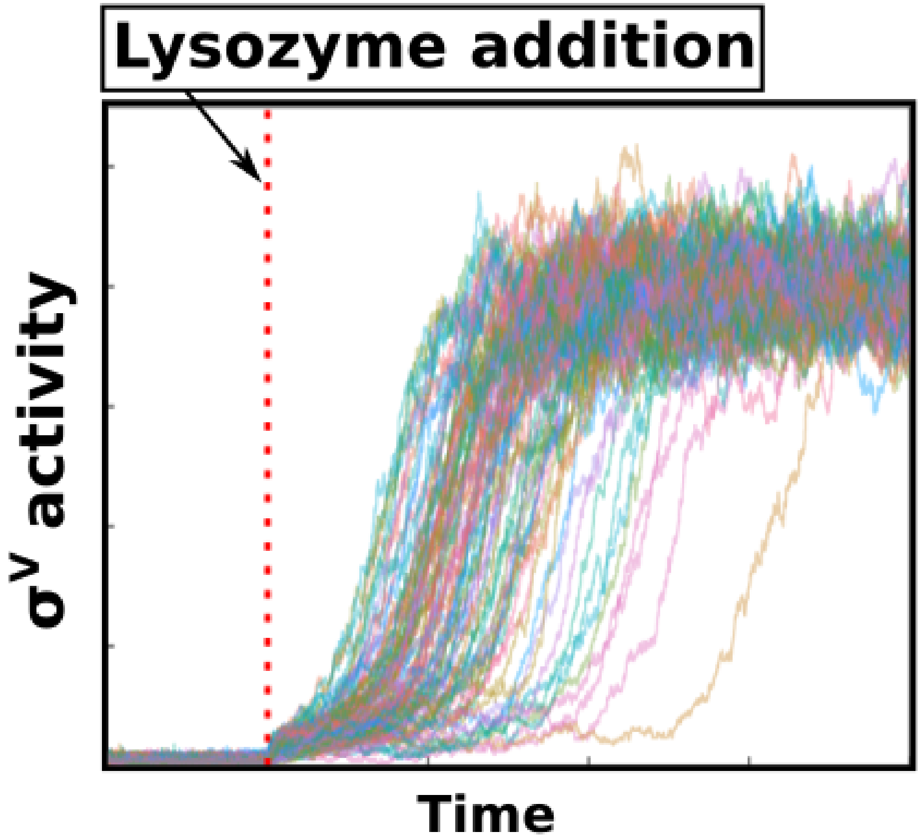
$\sigma^V \rightarrow \varnothing$

$\mathrm{RsiV} \rightarrow \varnothing$

$\sigma^V\text{-RsiV} \rightarrow \varnothing$

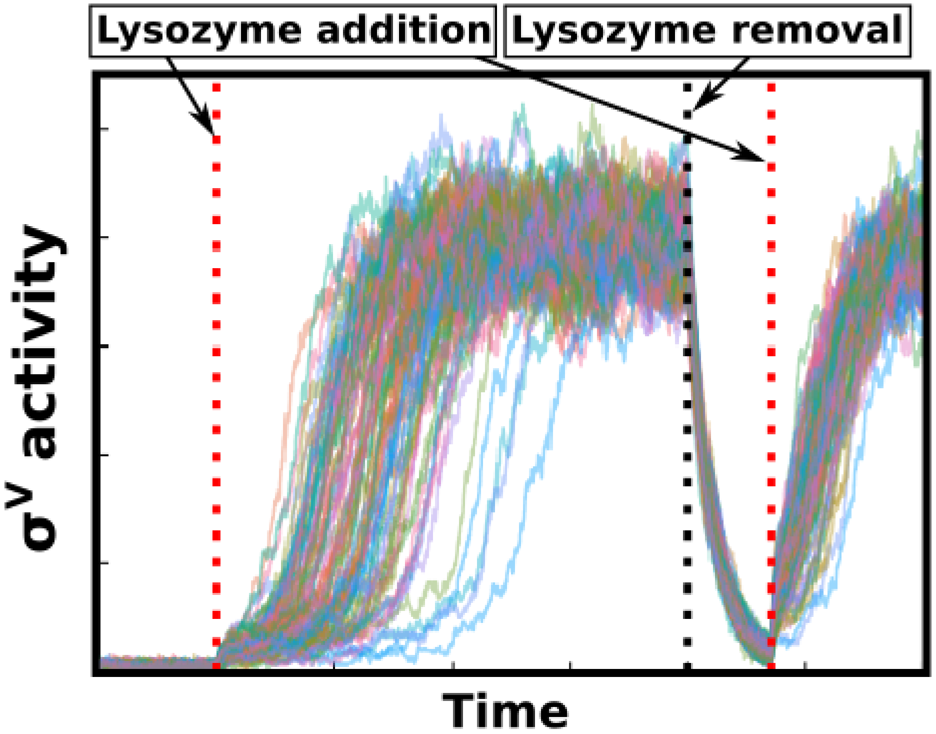
Our *chemical reaction network* model is based on the circuit's reaction events.

The model recreates the heterogeneous response



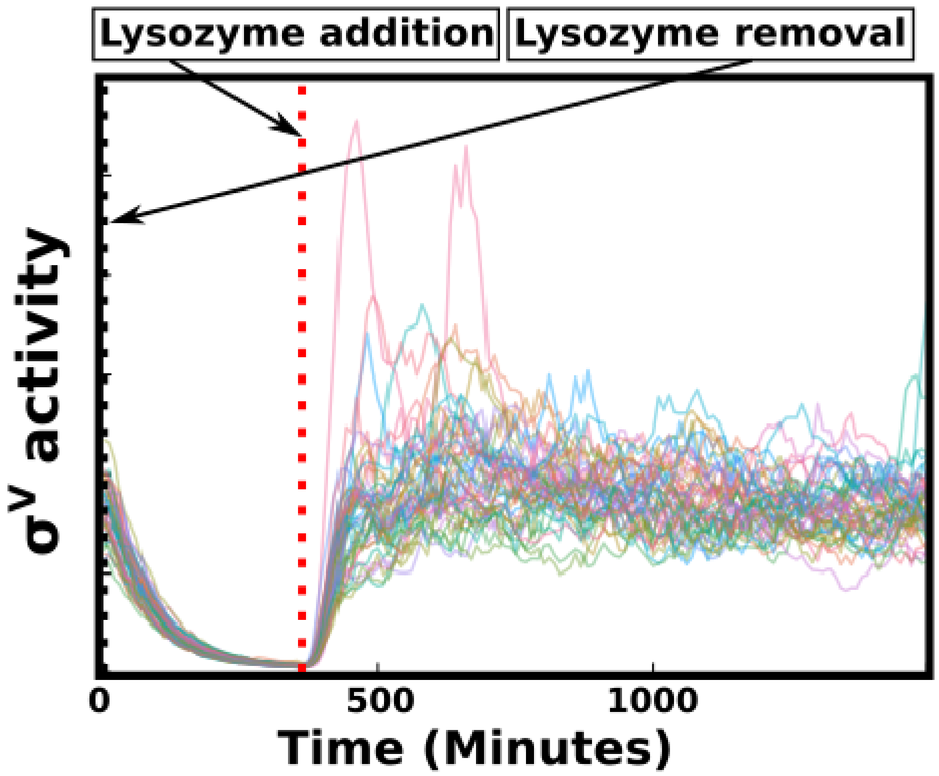
Stochastic reaction network interpretation (Gillespie's algorithm) is used to implement noise.

Our model predicts a memory of previous stresses



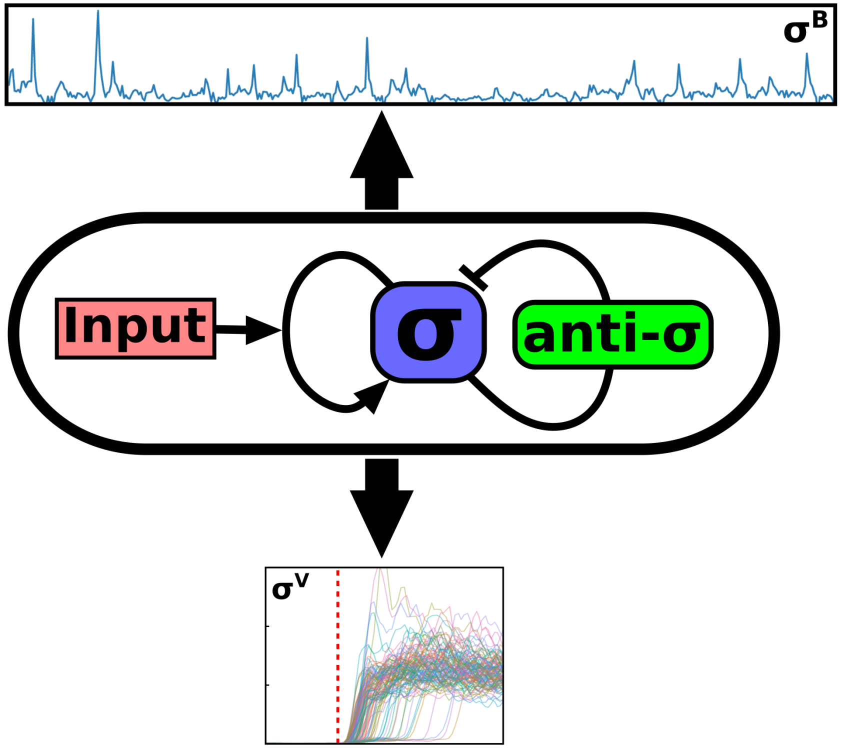
The reactivation (after a stress holiday) is homogenous, not heterogeneous.

We validate the prediction experimentally



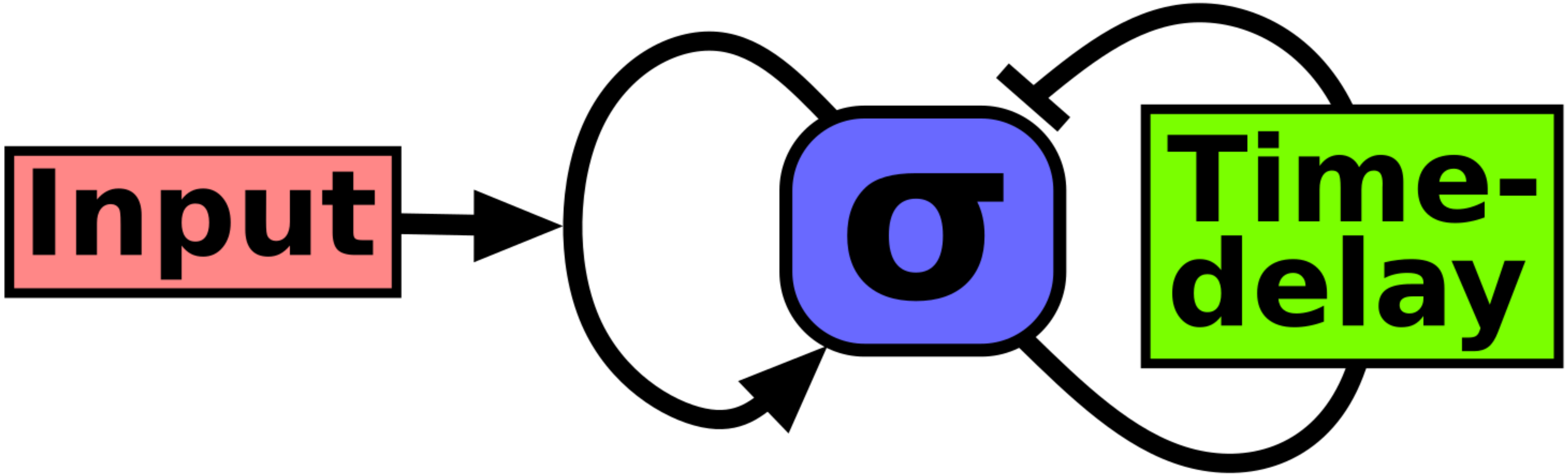
Each validation step increase our confidence in the model.

Both the σ^B and σ^V circuits' contain a mixed positive/negative feedback loop



Can this motif reproduce the two distinct response behaviours?

We model a general σ-factors circuit

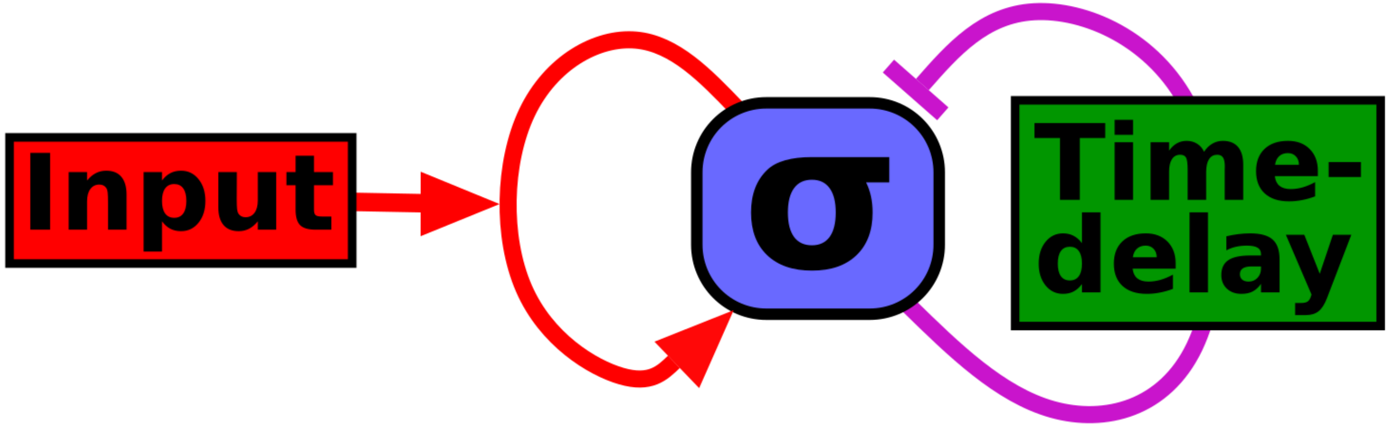


The negative feedback is subject to a *time delay*.

System noise is accounted for by making simulations *stochastic*.

The model depends on only three parameters:

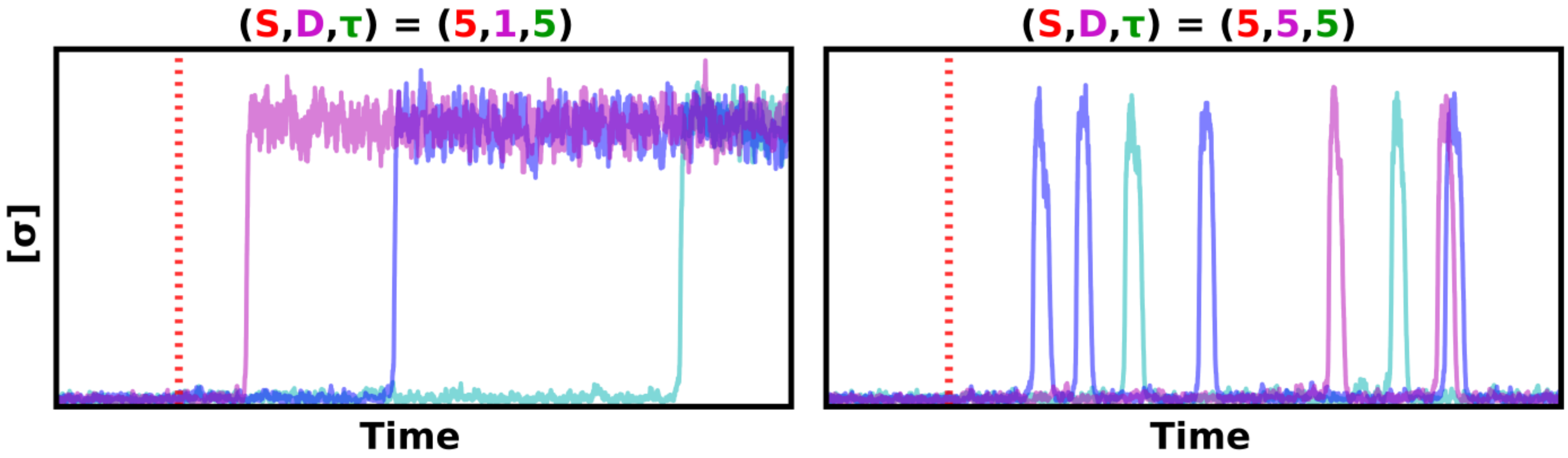
- S: The strength of the self-activation loop.
- D: The strength of the self-deactivation loop.
- τ: The length of the self-deactivation delay.



The system's behaviour is determined by these three properties.

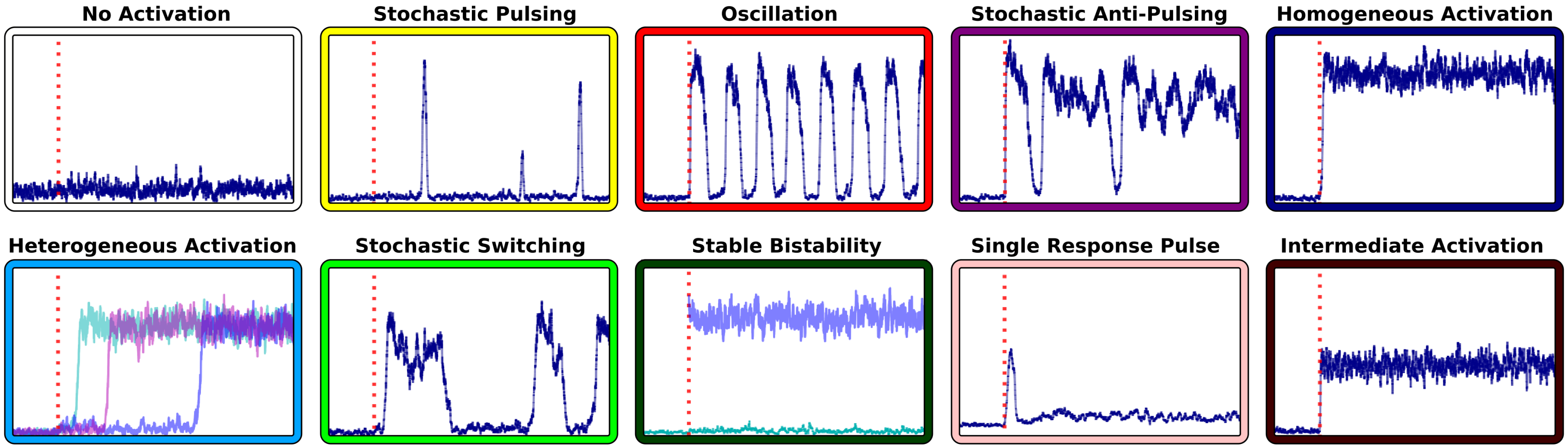
(The model is a one-variable stochastic delay differential equation)

For every parameter set, we get a specific response behaviour



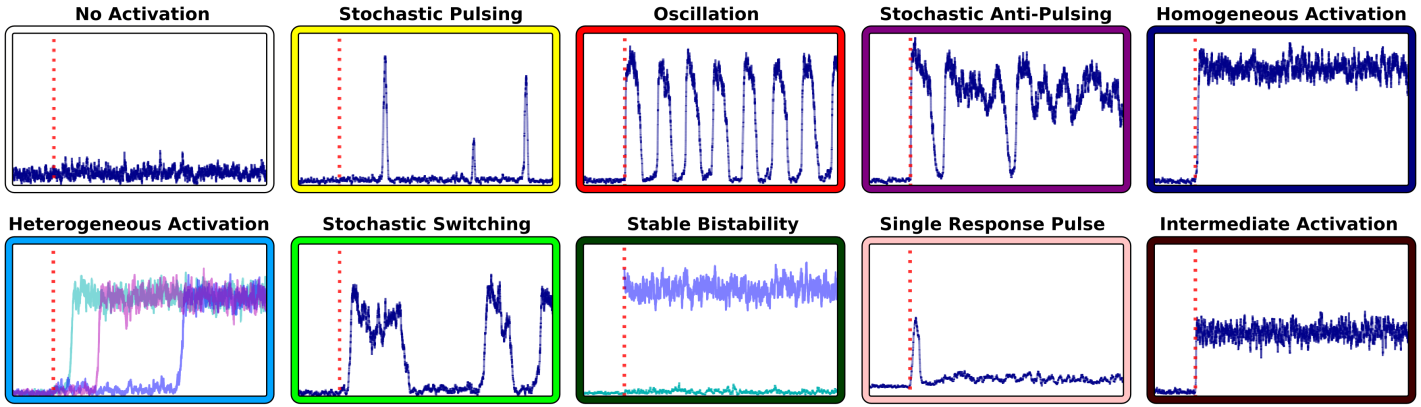
Here we can recreate the behaviours of both the σ^B and σ^V systems.

We have found all the possible response behaviours of the system



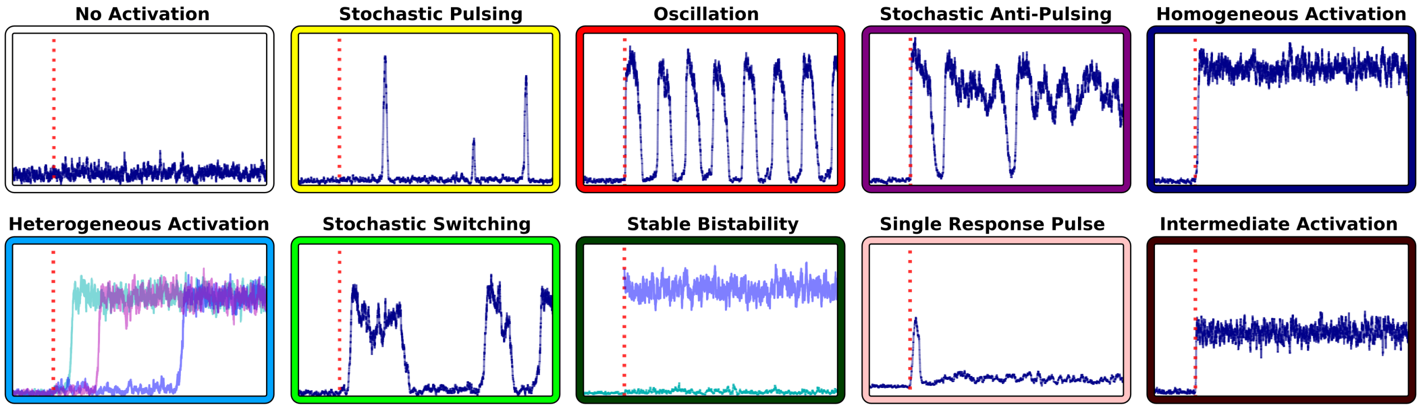
(An automated algorithm is used to classify parameter sets)

We can map these behaviours across parameter space



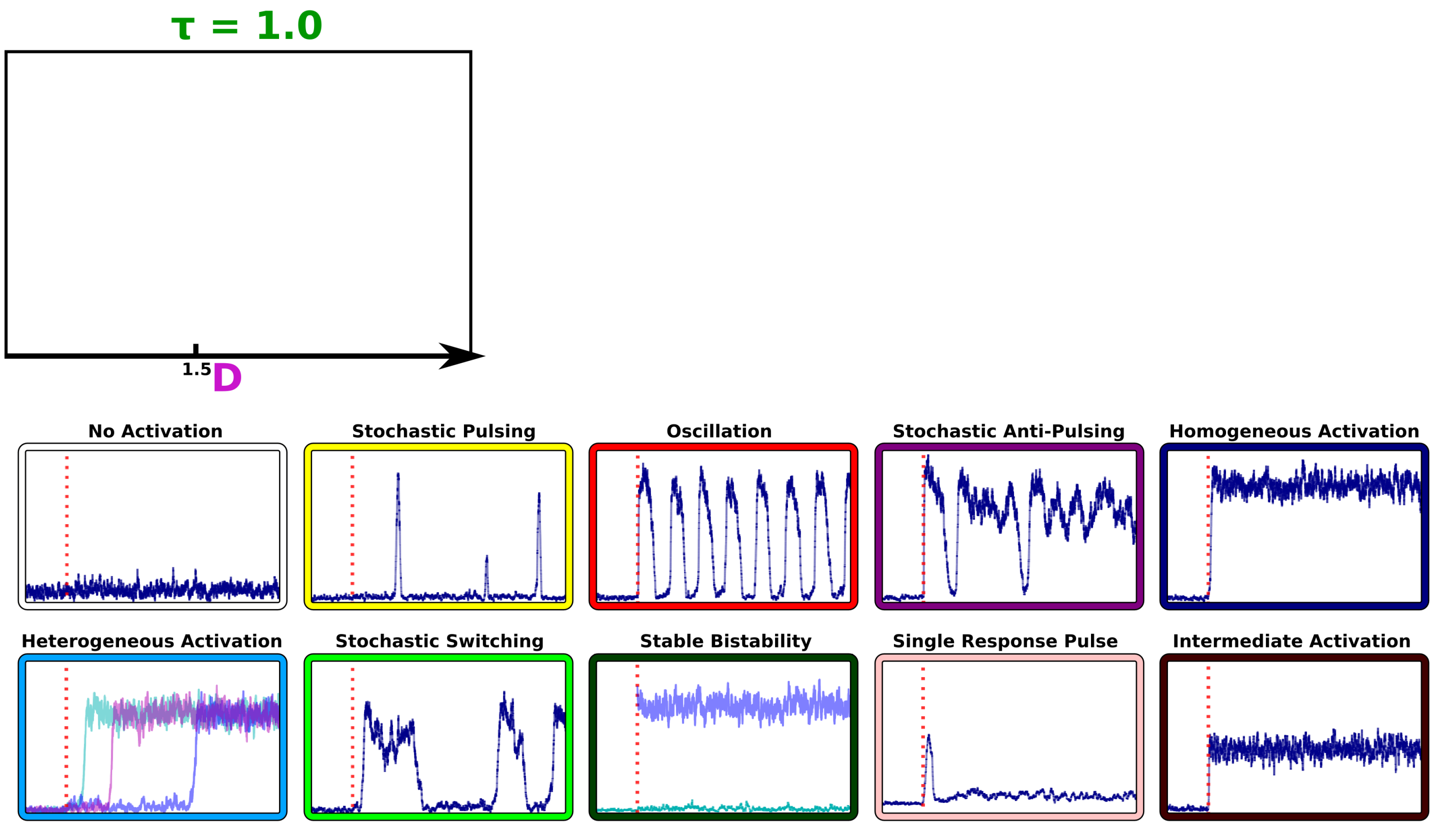
We can map these behaviours across parameter space

τ = 1.0



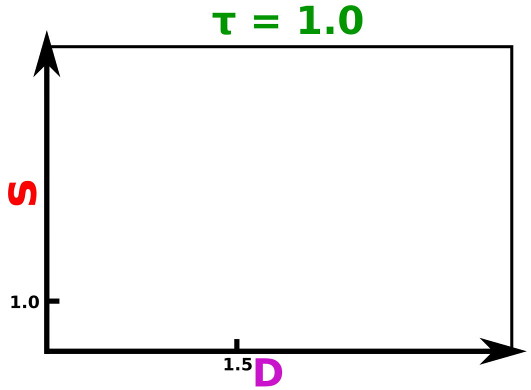
(τ = length of self-deactivation delay)

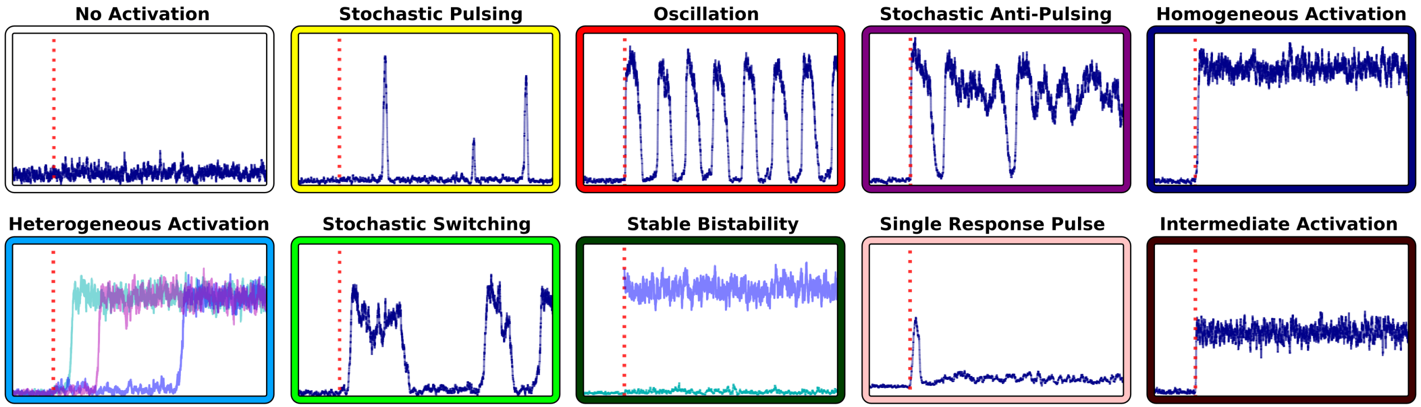
We can map these behaviours across parameter space



(D = strength of self-deactivation, τ = length of self-deactivation delay)

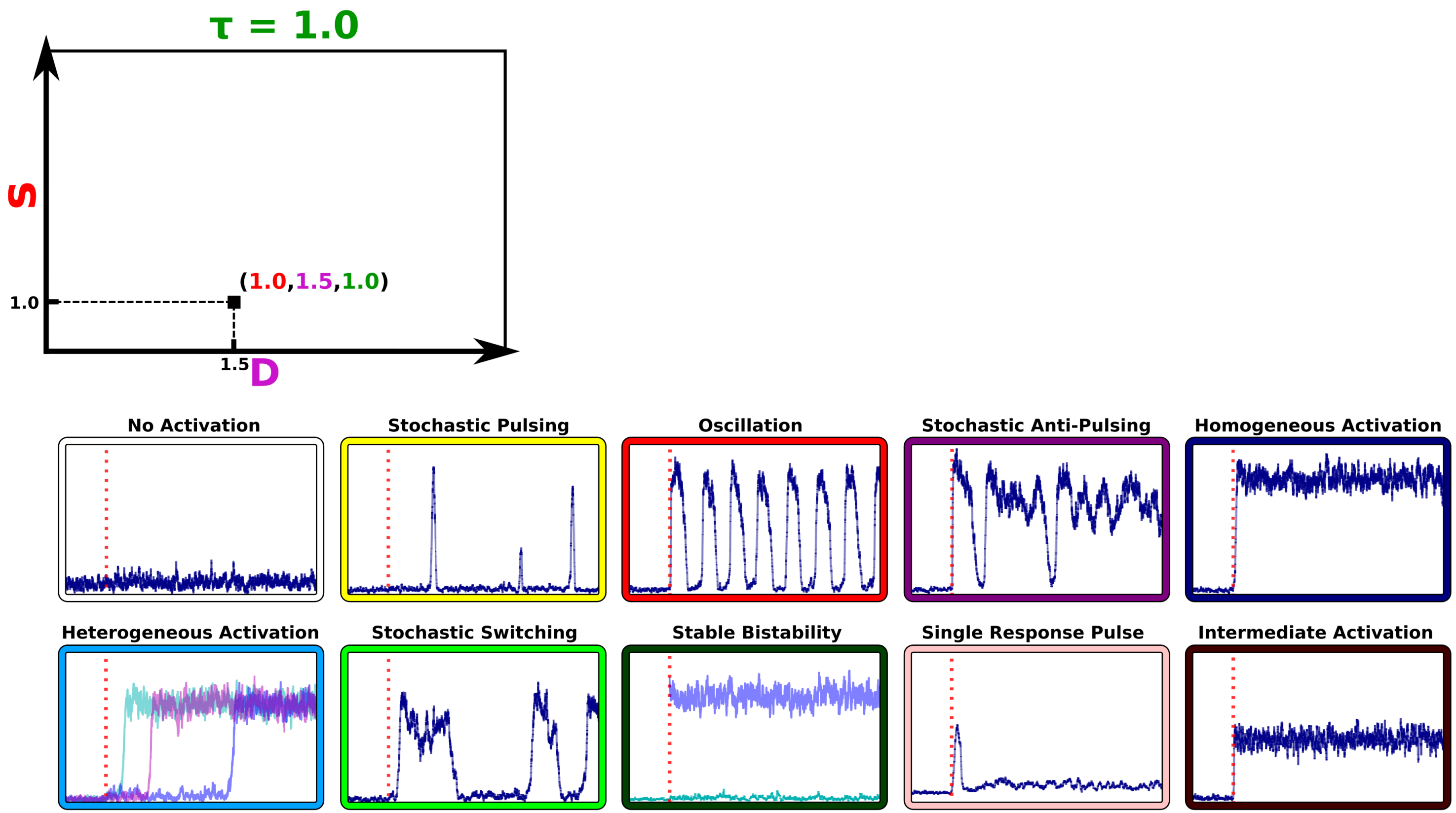
We can map these behaviours across parameter space





(S = strength of self-activation, D = strength of self-deactivation, τ = length of self-deactivation delay)

We can map these behaviours across parameter space



(S = strength of self-activation, D = strength of self-deactivation, τ = length of self-deactivation delay)

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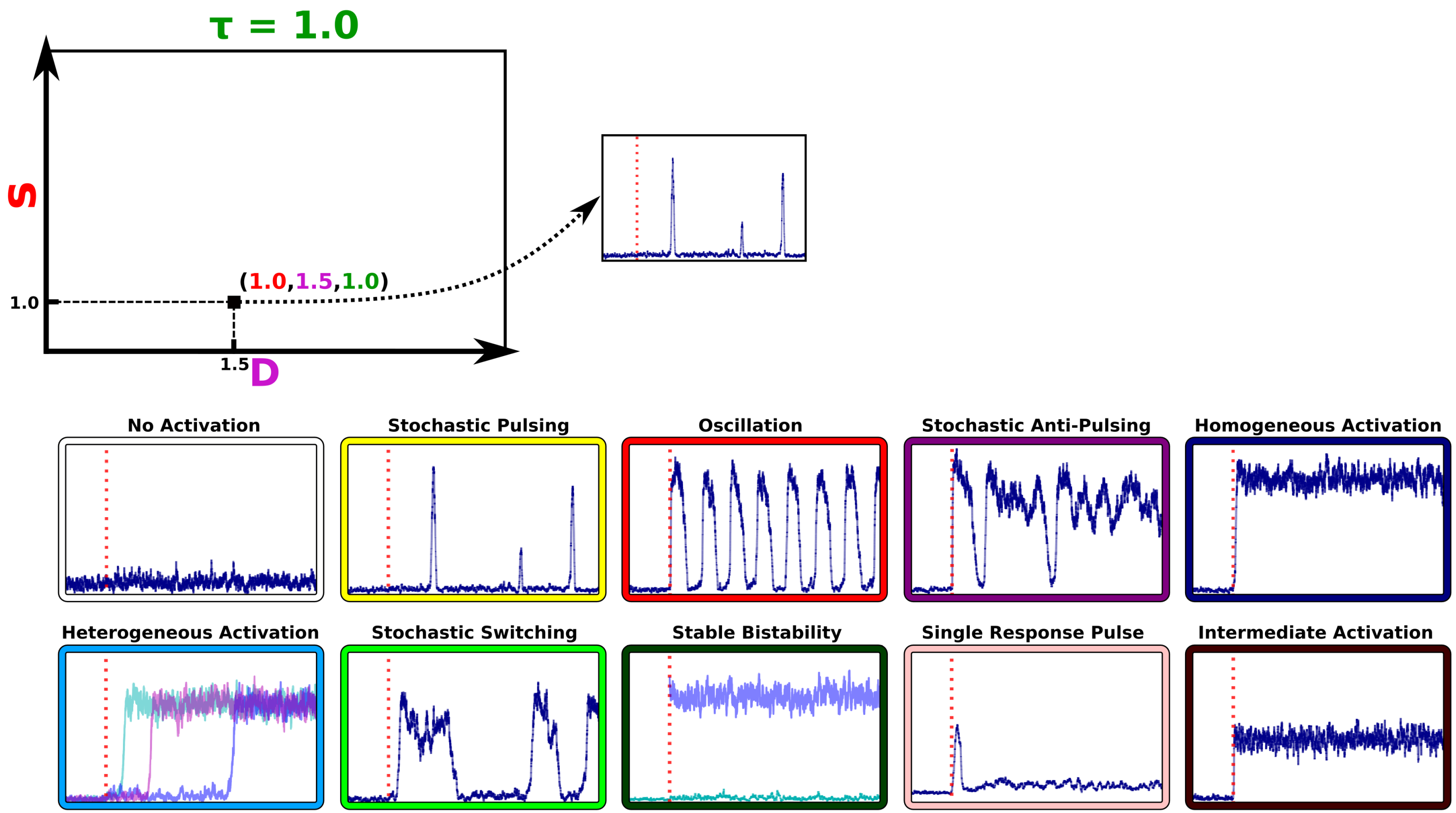

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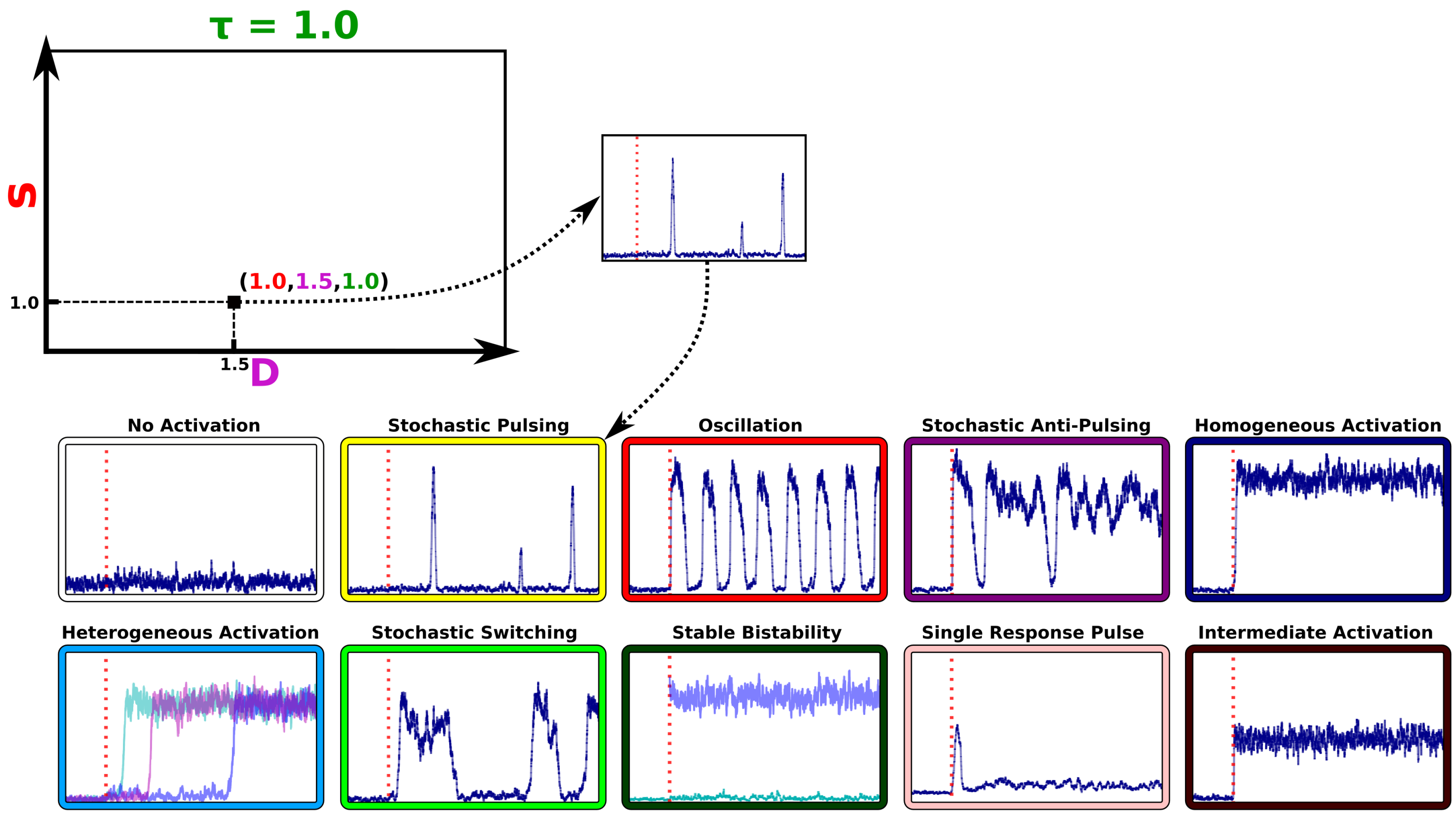
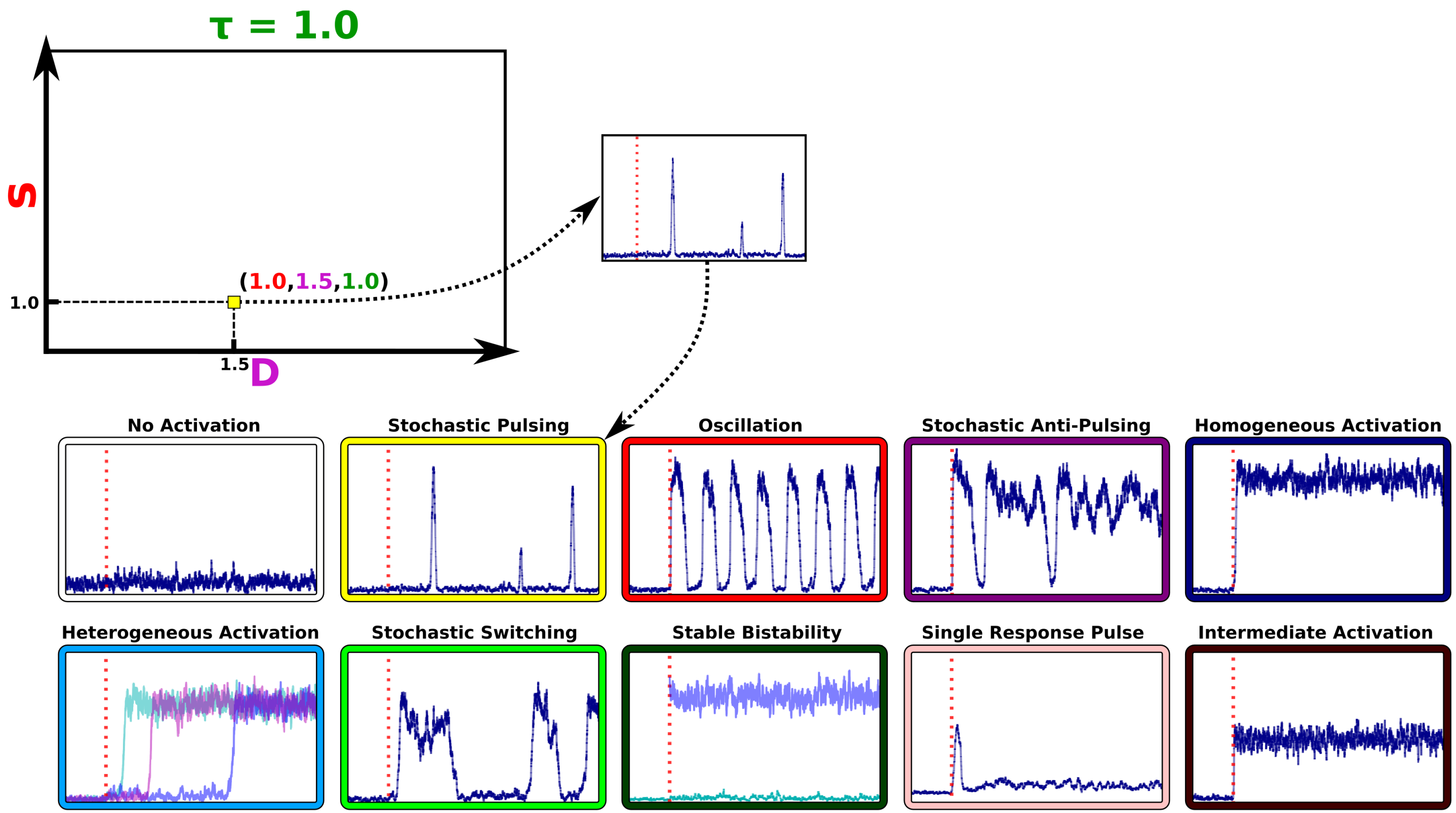
We can map these behaviours across parameter space



(S = strength of self-activation, D = strength of self-deactivation, τ = length of self-deactivation delay)

We can map these behaviours across parameter space



(S = strength of self-activation, D = strength of self-deactivation, τ = length of self-deactivation delay)

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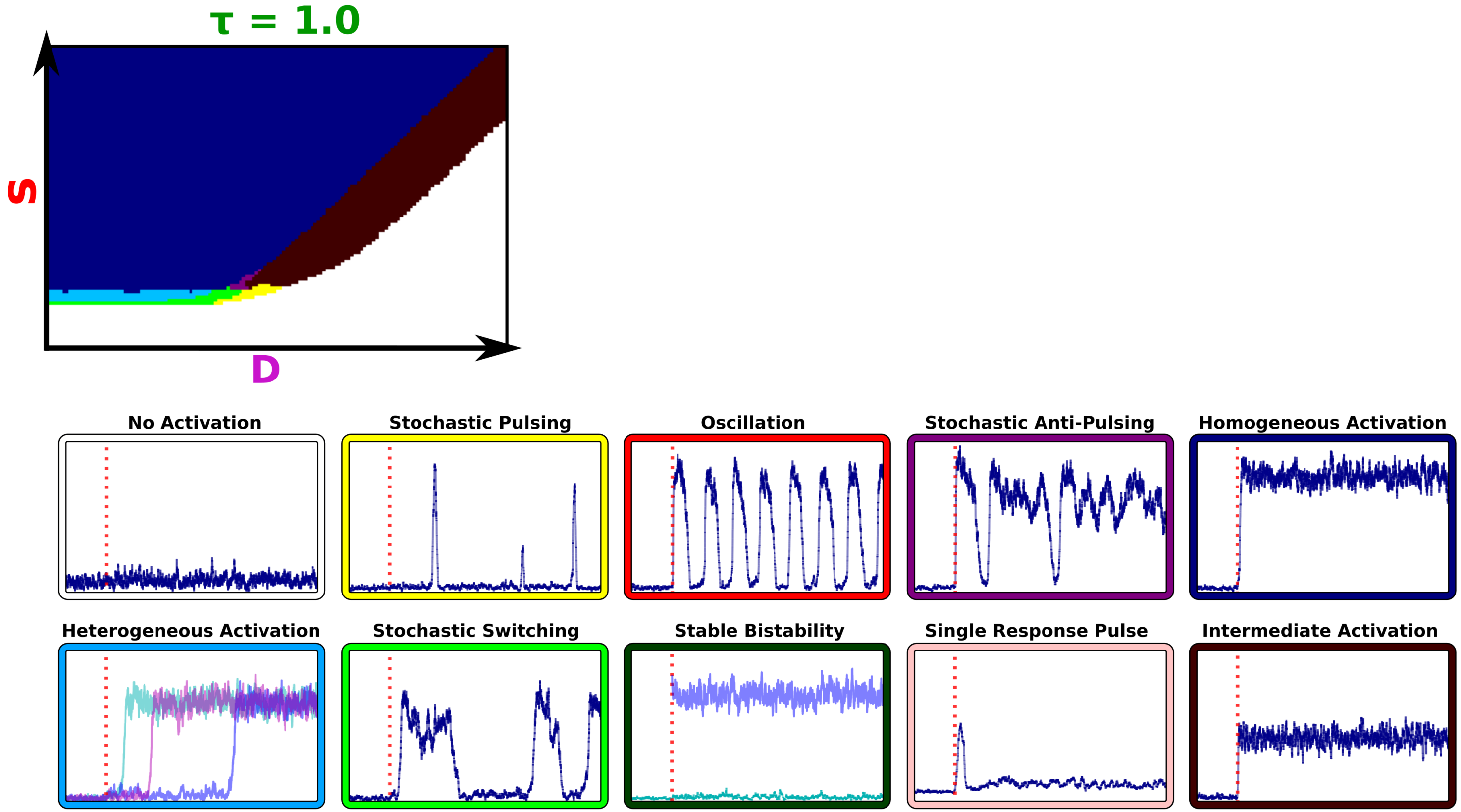
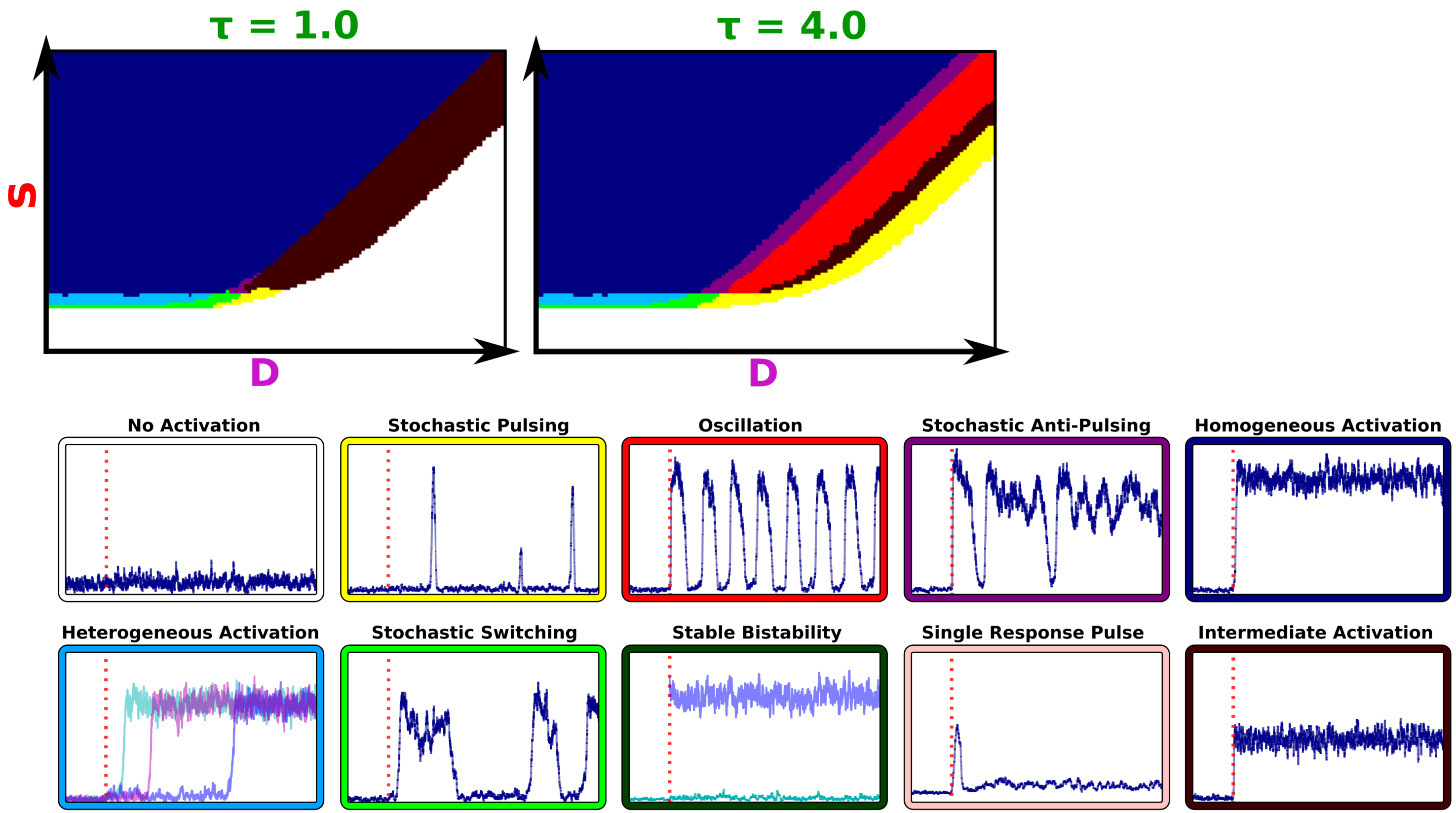


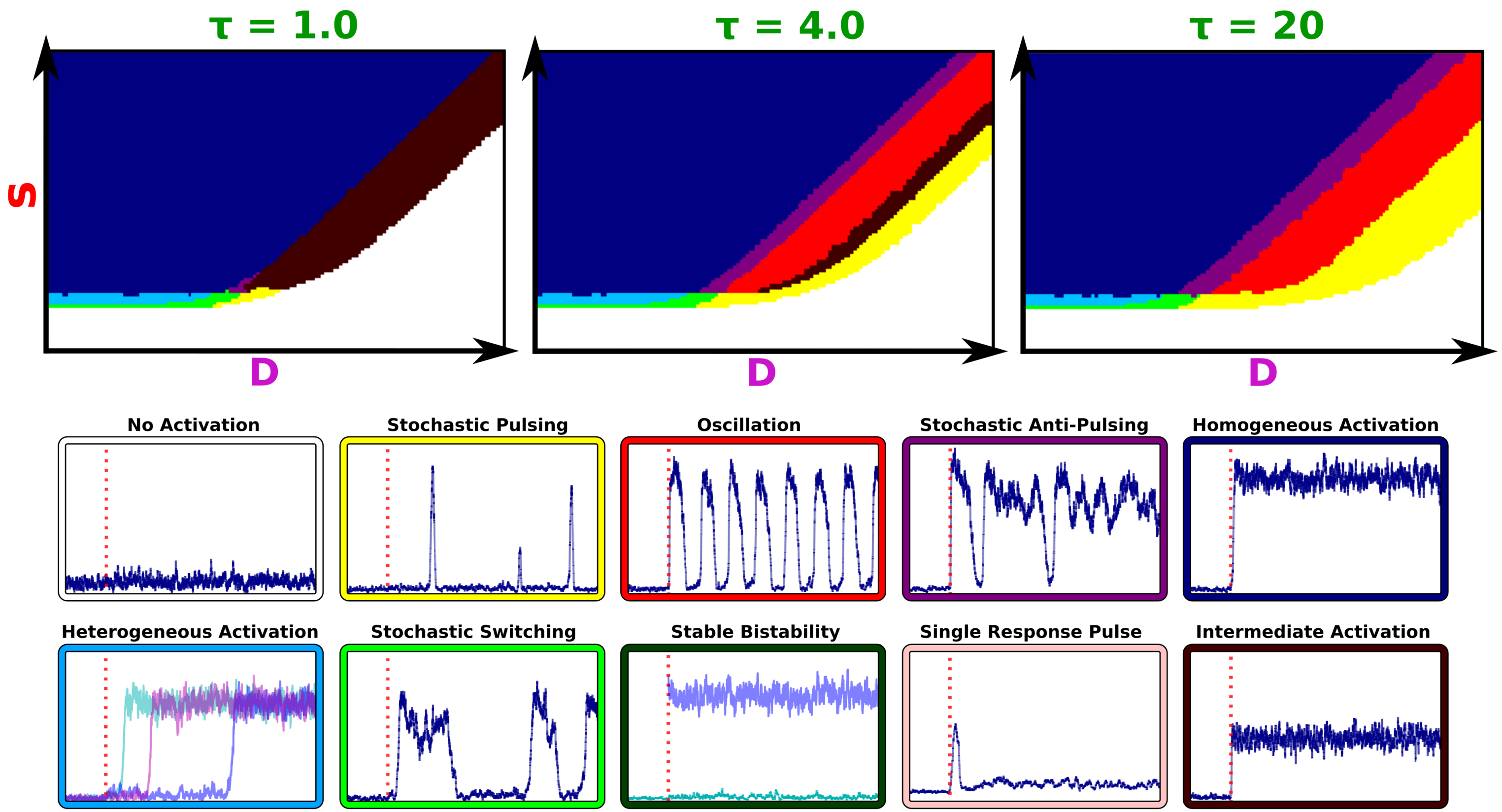
(S = strength of self-activation, D = strength of self-deactivation, τ = length of self-deactivation delay)

We can map these behaviours across parameter space



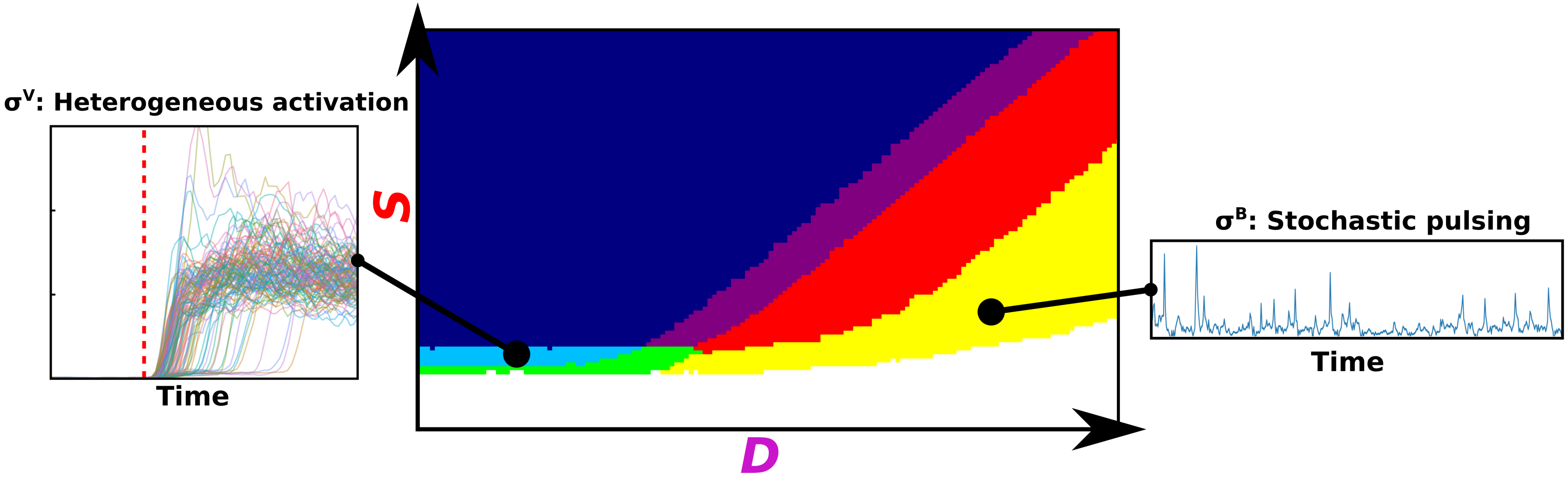
(*S* = strength of self-activation, *D* = strength of self-deactivation, τ = length of self-deactivation delay)

We can map these behaviours across parameter space



(S = strength of self-activation, D = strength of self-deactivation, τ = length of self-deactivation delay)

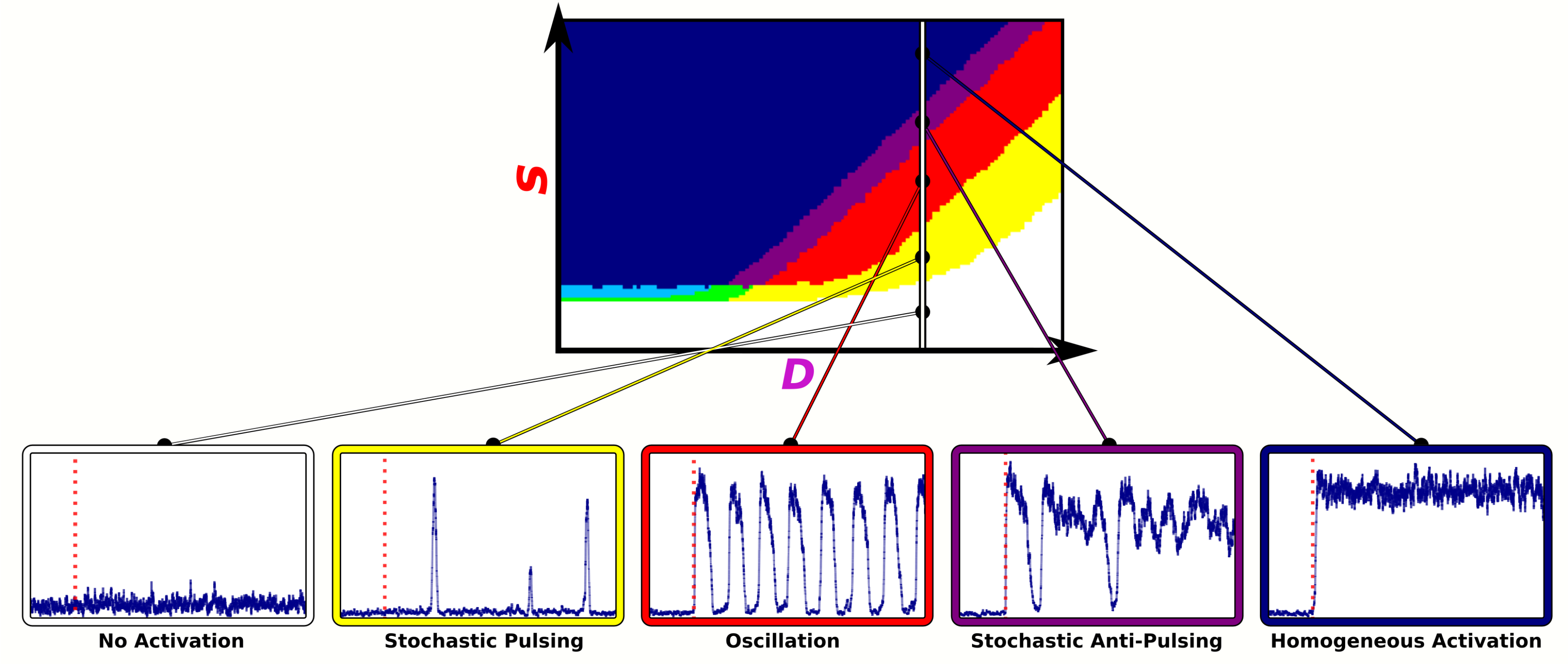
Real systems can be located on our map



This makes predictions on their system-properties.

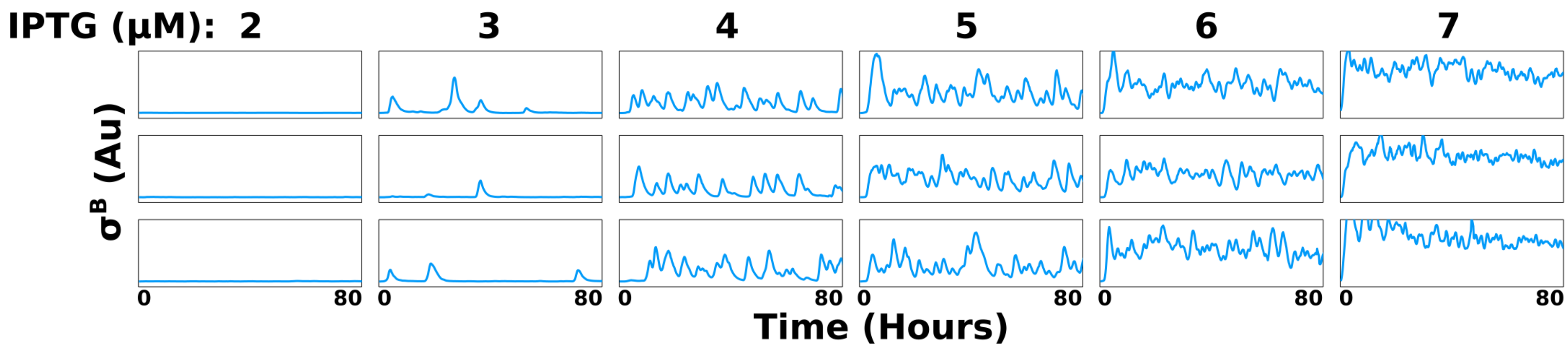
(Here, σ^B should have a higher *D* = strength of self-deactivation than σ^V)

The model predicts a behavioural transition as the parameter *S* is varied



This transition should be observable in the σ^B system (which contains stochastic pulsing).

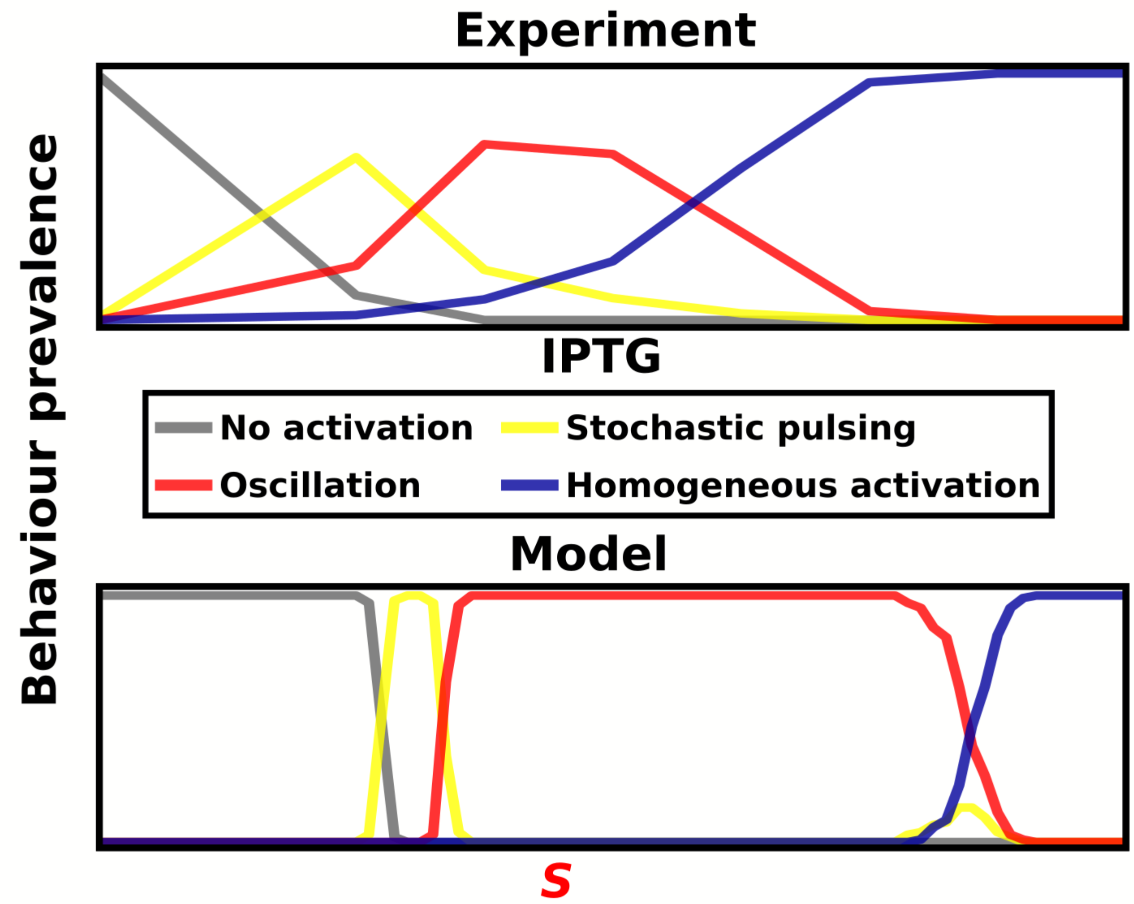
We can modulate this parameter in the real circuit



This confirms that σ^B undergoes the predicted transition.

An increase in IPTG corresponds to an increase in *S*. For each level of IPTG, three repeats are shown.

We can classify model and experiment behaviours as S is varied



A similar transition occurs in both systems.

Goal

- Build system as a tunable synthetic regulator.
- Can be used in synthetic organisms.

Summary

- Biological systems are noisy.
- Cellular noise can generate population heterogeneity.
- Single-cell measurements and models are required to detect this.

Acknowledgements

- Chris Schwall (experiments)
- James Locke (supervisor)

PATH SENSE

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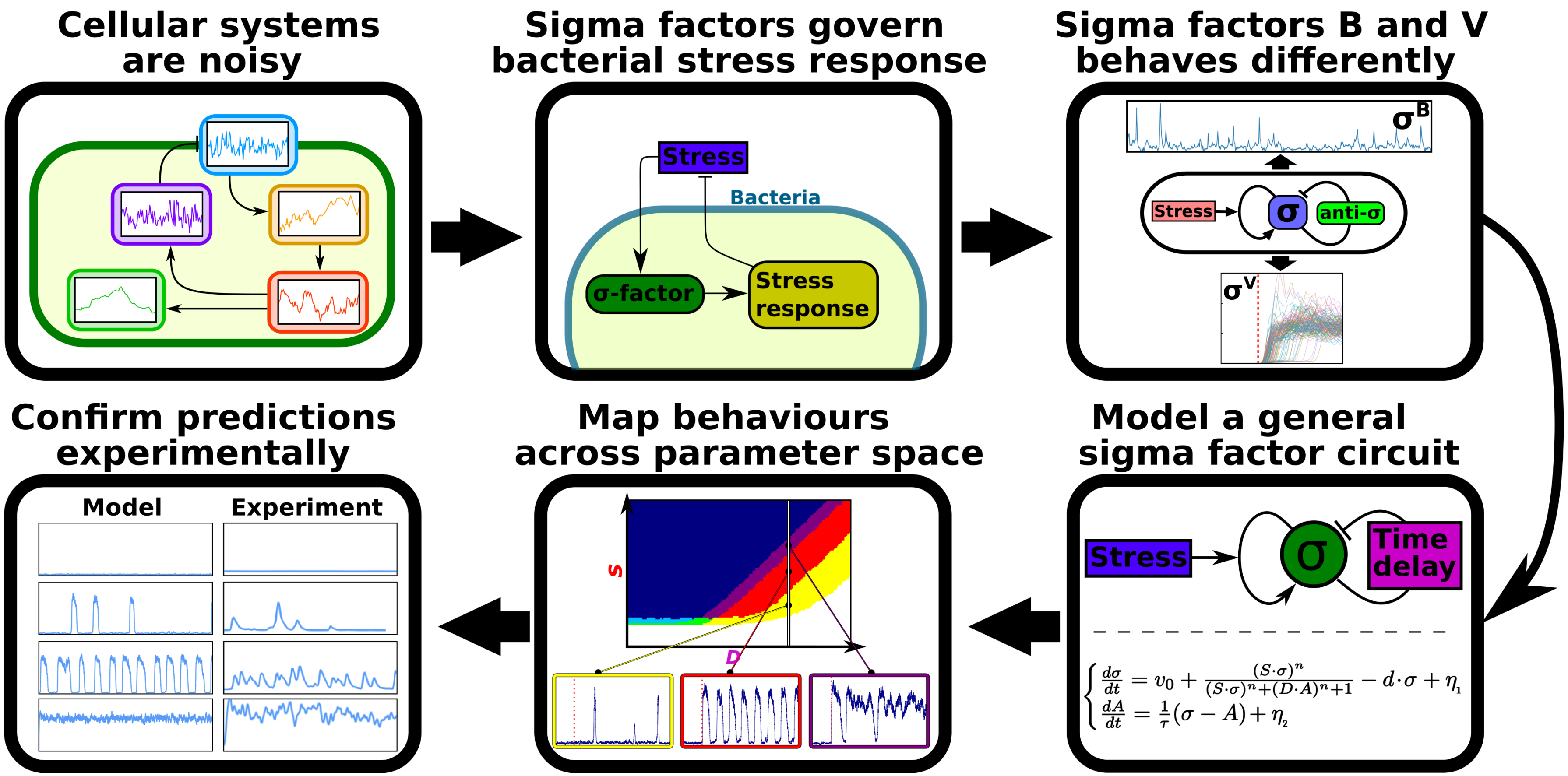
Thank you


Cellular systems are noisy
Sigma factors govern bacterial stress response
Stress
Bacteria
σ-factor
Stress response
Sigma factors B and V behaves differently
σ^B
Stress
σ
anti-σ
σ^V
Model a general sigma factor circuit
Stress
σ
Time delay
$$\begin{cases} \frac{d\sigma}{dt} = v_0 + \frac{(S\cdot\sigma)^n}{(S\cdot\sigma)^n + (D\cdot A)^n + 1} - d\cdot\sigma + \eta_1 \\ \frac{dA}{dt} = \frac{1}{\tau}(\sigma - A) + \eta_2 \end{cases}$$
Map behaviours across parameter space
S
D
Confirm predictions experimentally
Model
Experiment

Our model is a two-variable Stochastic Differential Equation

$$\begin{cases} \frac{d\sigma}{dt} = v_0 + \frac{(S\cdot\sigma)^n}{(S\cdot\sigma)^n+(D\cdot A)^n+1} - \sigma & + \eta\cdot noise_1(\bar{x},\bar{p}) \\ \frac{dA}{dt} = \frac{1}{\tau}(\sigma - A) & + \eta\cdot noise_2(\bar{x},\bar{p}) \end{cases}$$

It depends on only 6 parameters:

- S: The degree of system *self-activation*
- D: The degree of system *self-deactivation*
- τ: The length of the *time delay*
- v_0: The *base production* of the σ-factor
- n: The degree of *system cooperativity*
- η: The *noise amplitude*

(The final terms are functions determining the degree of noise)

(The variable *A* models the time delay)

By simulating the model we can observe its behaviour

Here it exhibits a *Stochastic Pulsing* behaviour.

(A single simulation displayed in *phase space* and over time)
(Nullclines are drawn in red and blue)