Delft, May 31st 2021

Dear KIND directors,

Hereby we, Jos Zwanikken and Liedewij Laan, assistant and associate professor at BN are re-applying for Kavli seed money with our proposal:

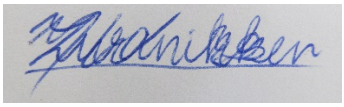
"Optimizing noisy protein concentration via non-genetic feedback"

In this proposal we have addressed (in red) the questions you raised about the previous version of this proposal. In the current draft we describe how we will integrate the statistical physics expertise of Zwanikken with an experimental project aimed at dissecting the role of protein expression noise in evolution, so far executed in the Laan lab, and extended on which theoretical modelling will be used and how the experimental data will be fed into the model. We will use the seed money to obtain experimental data and to interpret this data in microscopic terms by fitting a statistical physics model. We will use the seed money (50k) to hire Caspar Schmeits as a research assistant for a year. Caspar is currently a master student is the Laan lab, finishing his master in biology and he already has a master in statistics. His background makes him a perfect fit for the project we propose here. He will perform the experimental work in collaboration with Jos who will focus on the theoretical work. Caspar has indicated that he would like to perform a PhD project at the interphase of physics and biology and this project will provide him with relevant experience in this direction. Our goal is to submit a paper by the end of the project, which will allow us to apply for further external funds to deepen our collaboration in the future.

Yours sincerely,



Liedewij Laan



Jos Zwanikken

1. Applicant
Dr. ir. Liedewij Laan and Dr. Jos Zwanikken

2. Title of the project
Optimizing noisy protein concentration via non-genetic feedback

4. Abstract Provide a brief abstract of the project.
Noise is a factor in systems from biology to physics. Rather than a nuisance, noise such as in gene expression can be beneficial by enabling fortuitous evolutionary adaptation. We aim to investigate a system where this luck is presumably heritable and even reinforced. Based on detailed knowledge on budding yeast polarity, we aim to experimentally assess whether lucky feedback makes yeast more adaptable. Interpretation of the data necessitates careful theoretical analysis, after which current models can be extended to non-idealized systems to estimate the evolutionary relevance of feedback across organisms.

5. Benefit to the synergy of KIND
We propose to start a new collaboration with the help of KIND, combining the biological knowledge and experimental work from the Laan group, with the modelling and statistics expertise of the Zwanikken group. Our collaboration will allow us to experimentally characterize whether gene expression noise indeed can lead to evolutionary feedback in cells, and to use our observations to further improve the theory. Moreover this collaboration will help the Zwanikken group to enter the field of physics of life. And as both groups bring in complementary expertise on this problem, the collaboration initiated here has the potential to be the start of a long term collaboration. With the results of this collaboration we will apply to the ENW-M2 research scheme.

6. Description of the proposed research
Organisms continuously face the challenge to adapt to their surroundings. The most stable solutions are genetically encoded, and on the cellular level often involve tuning the concentration of expressed proteins (1). However, for the short-term, faster non-genetic mechanisms are required. Well studied examples include chemical marks on DNA (2) and self-sequestering proteins (3).

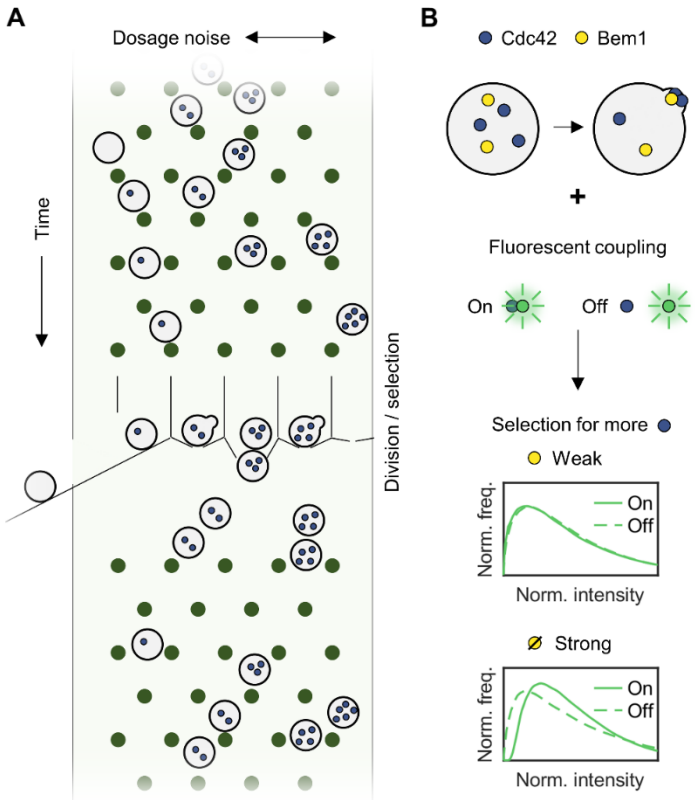


Figure 1 Non-genetic, adaptive feedback on protein dosage variation created by noise, conceptually and in practice. (A) Dosage feedback depicted as cells falling down an infinite bean machine. Expression noise is represented through random bounces on the junctions determining protein dosage (increasing from left to right) across time. As selection filters out certain outcomes, in this case purging low dosages, the starting point for the population is biased each round in a favorable manner.(B) In budding yeast, the vital process polarity requires concentrating Cdc42 to one point, aided by Bem1. Without Bem1, a higher Cdc42 dosage is required. This selection affects the distribution of Cdc42 across the population and is made visible by fluorescently labelling of Cdc42.

By contrast, a simple mechanism via protein inheritance after cell division has so far been unexplored. As noise in gene expression is ubiquitous, genetically identical cells randomly differ in protein concentration. After every division, selection purges unlucky cells and promptly biases the starting concentration of those that multiply (as conceptually in Figure 1A). The lucky state thereby propagates

across generations. This constitutes a non-genetic feedback across generations (4) towards the optimal concentration, whose simplicity suggests universality across many organisms. Our aim is to demonstrate this feedback and quantitatively estimate its importance for evolution.

For this purpose, we investigate the effect of expression noise in budding yeast polarity, a process essential for survival where yeast chooses the direction for a new daughter cell using the key protein Cdc42 (see Figure 1B). A sufficient abundance of this protein is necessary to start daughter cell formation, particularly when the protein Bem1 is not there to assist (5). When the Cdc42 concentration in the population of cells is on average suboptimal, the lucky part of the population will drive the concentration towards optimality. Our recently constructed polarity model and simulations (5,6) allows us to distill the feedback effect from noisy population distributions of Cdc42.

Concretely, we detect Cdc42 concentrations in the population by measuring cells with fluorescently labeled Cdc42 proteins (5) using (imaging) flow cytometry. When Cdc42 concentration becomes more important by removing Bem1 through chemical induction (6), a concentration feedback occurs. This induces a shift in the fluorescent distribution compared to our control experiment where we do not attach the fluorescent label to Cdc42. The significance of the shift can be shown by measuring multiple control populations with unattached labels, to quantify the unrelated distribution variations. Since fluorescence intensities from flow cytometry are measured in arbitrary units, whereas our current simulation model predicts protein abundances, we will initially focus on linking fluorescence intensity to protein abundance and/or concentration. We will start by refining the experimental procedure. For example, with imaging flow cytometry (IFC), it becomes possible to obtain microscopy images of every passing cell, thereby greatly increasing the measurement resolution. Our next aim is to validate a relationship between intensity and abundance by (statistical) modelling.

We will build on a recently devised analytical model that was devised in the Laan Lab for exploring the effects of gene expression noise and epigenetic feedback on population fitness. In this model, cells can occupy two states of Cdc42 abundance (above or below the required threshold for cell polarization) (7). By extending the model to allow for multiple Cdc42 abundance states, applying an analytical approach informed by stochastic simulations, we will be able to directly compare the simulated Cdc42 distributions to the experimental results, and interrogate the relation between epigenetic feedback and the population distribution of Cdc42. The comparisons will allow us to identify a minimal model and form new hypotheses about the nature of Cdc42 expression, and potentially quantify time scales for epigenetic feedback.From our molecular models and experiments our goal is to distil a theoretical understanding of the role of noise in evolution that is applicable to many different biological systems. Practically, our goal is to submit a research paper before the end of the project.

Requested budget:
Experiments and data analysis will be performed by Caspar Schmeits, a MSc Student in the Laan Lab, who will submit his thesis in June. The theoretical work will be conducted by Dr. Jos Zwanikken and assisted by Schmeits. The seed money will be used to hire Schmeits for the period of one year as a research assistant. Laan will pay lab materials and other lab work related costs from other resources.

References:

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