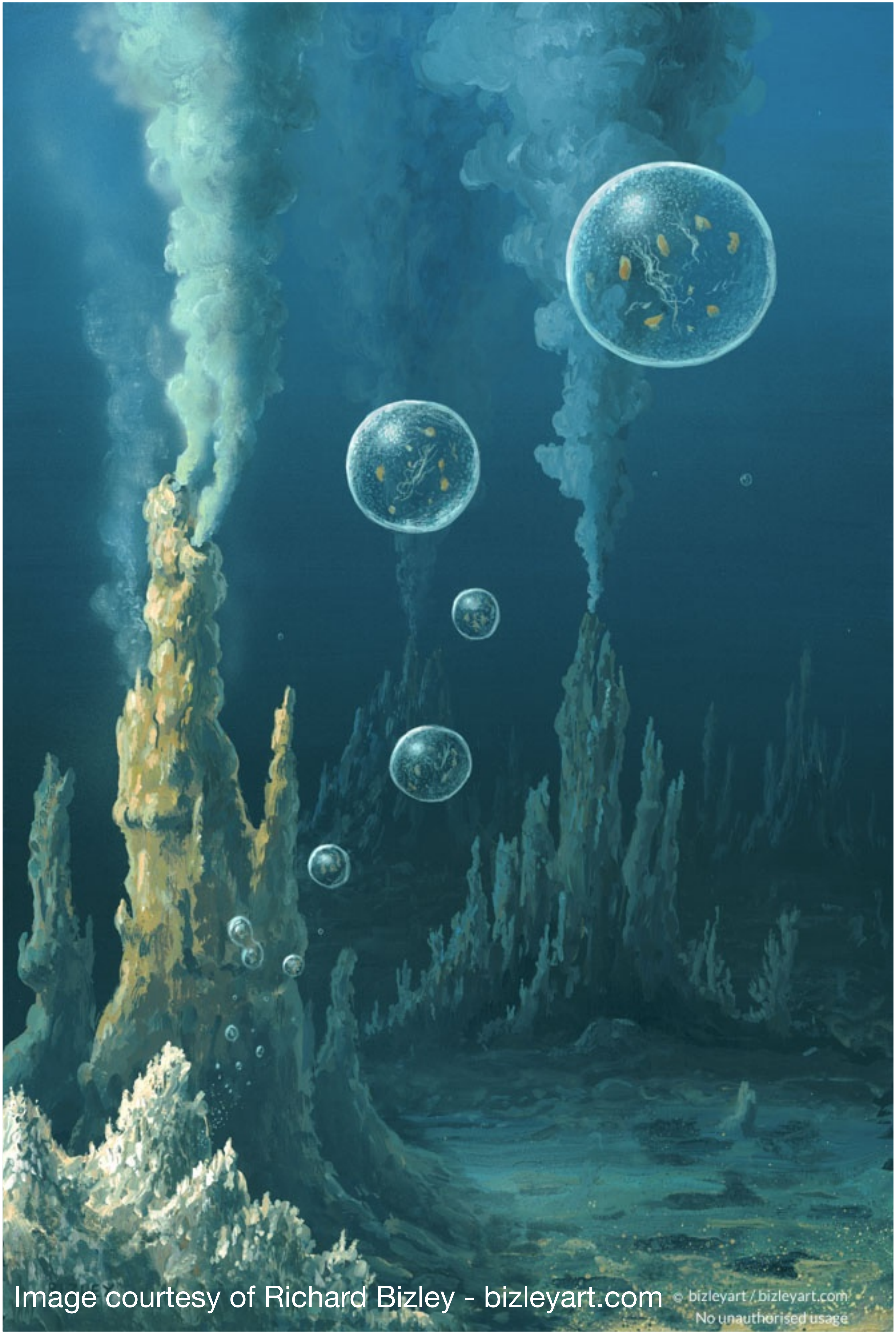


Image courtesy of Richard Bizley - bizleyart.com

Modelling the prebiotic origins of regulation & agency in evolving protocell ecologies

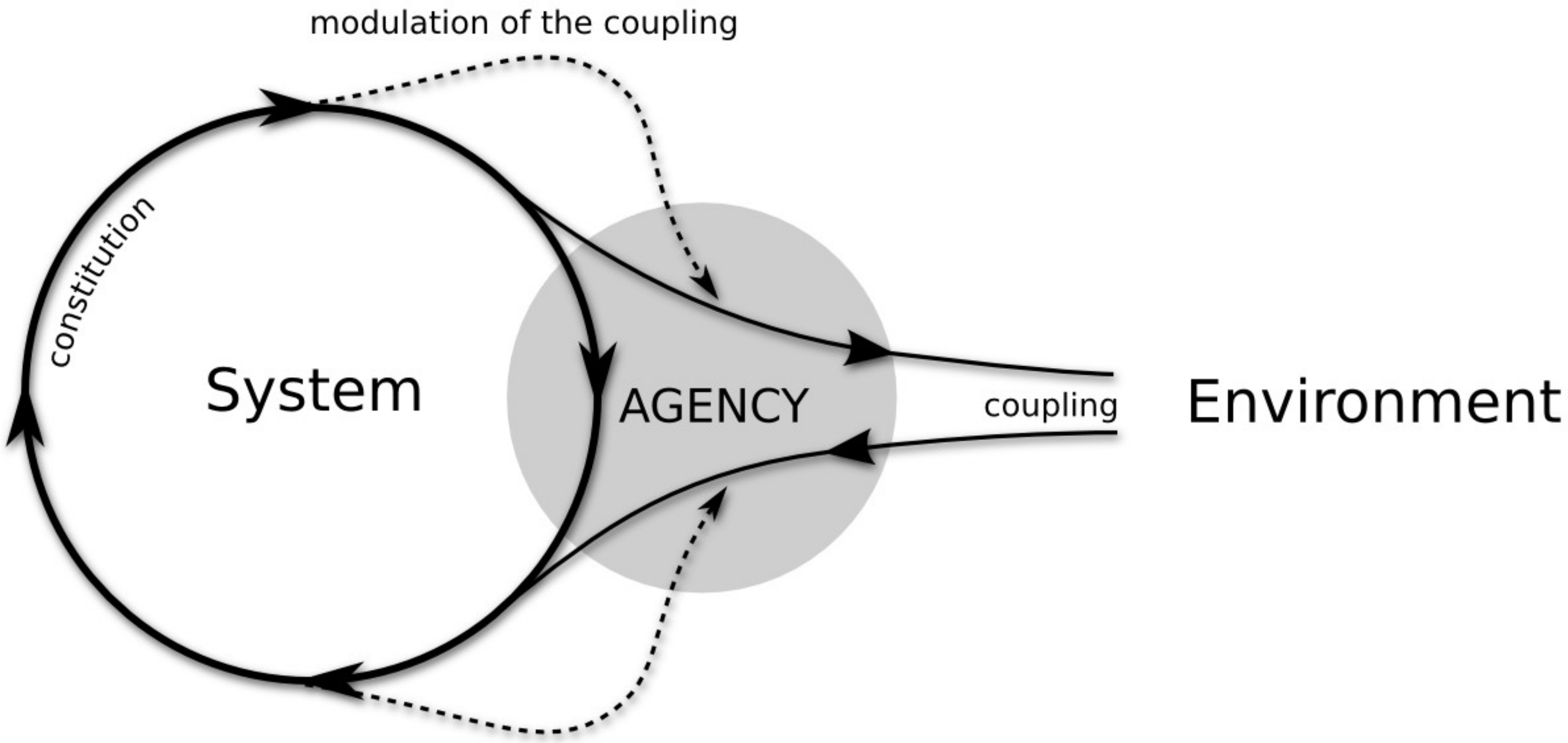
Ben Shirt-Ediss

Donostia International Physics Center

IAS Research Talk | November 2024

Broad Aim

How/why did the first minimal chemical **agents** emerge at the origins of life?



Biological agent: "An individuated autonomous organisation that adaptively regulates its coupling with its environment and contributes to sustaining itself as a consequence".

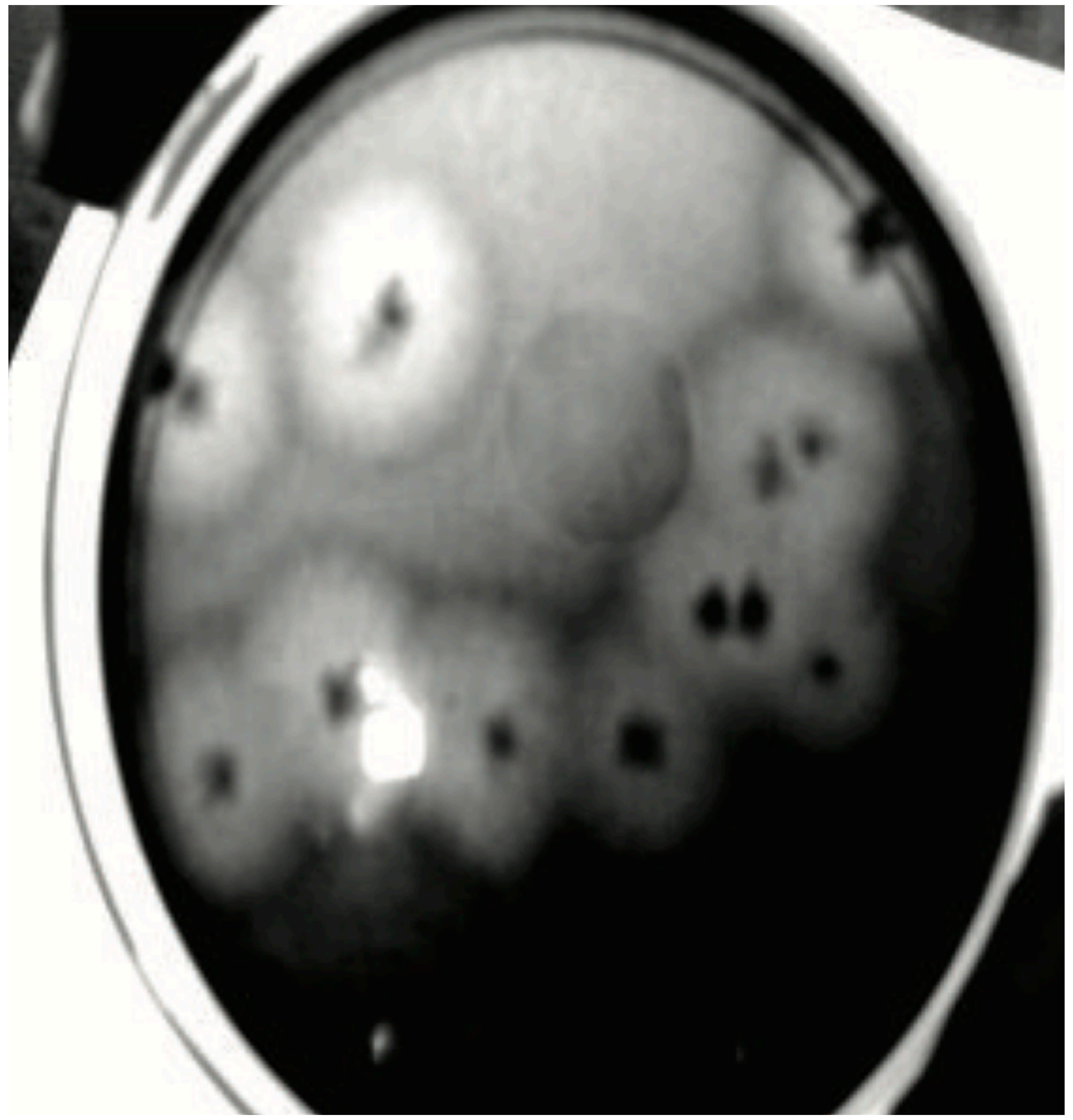
Defining Agency. Barandiaran et al. Journal of Adaptive Behaviour (2009)

Regulation Mechanisms in Cells

Cellulolytic bacteria secretion

IF sufficient cellulose in environment **THEN** secrete cellulase enzymes to break it down into useable glucose

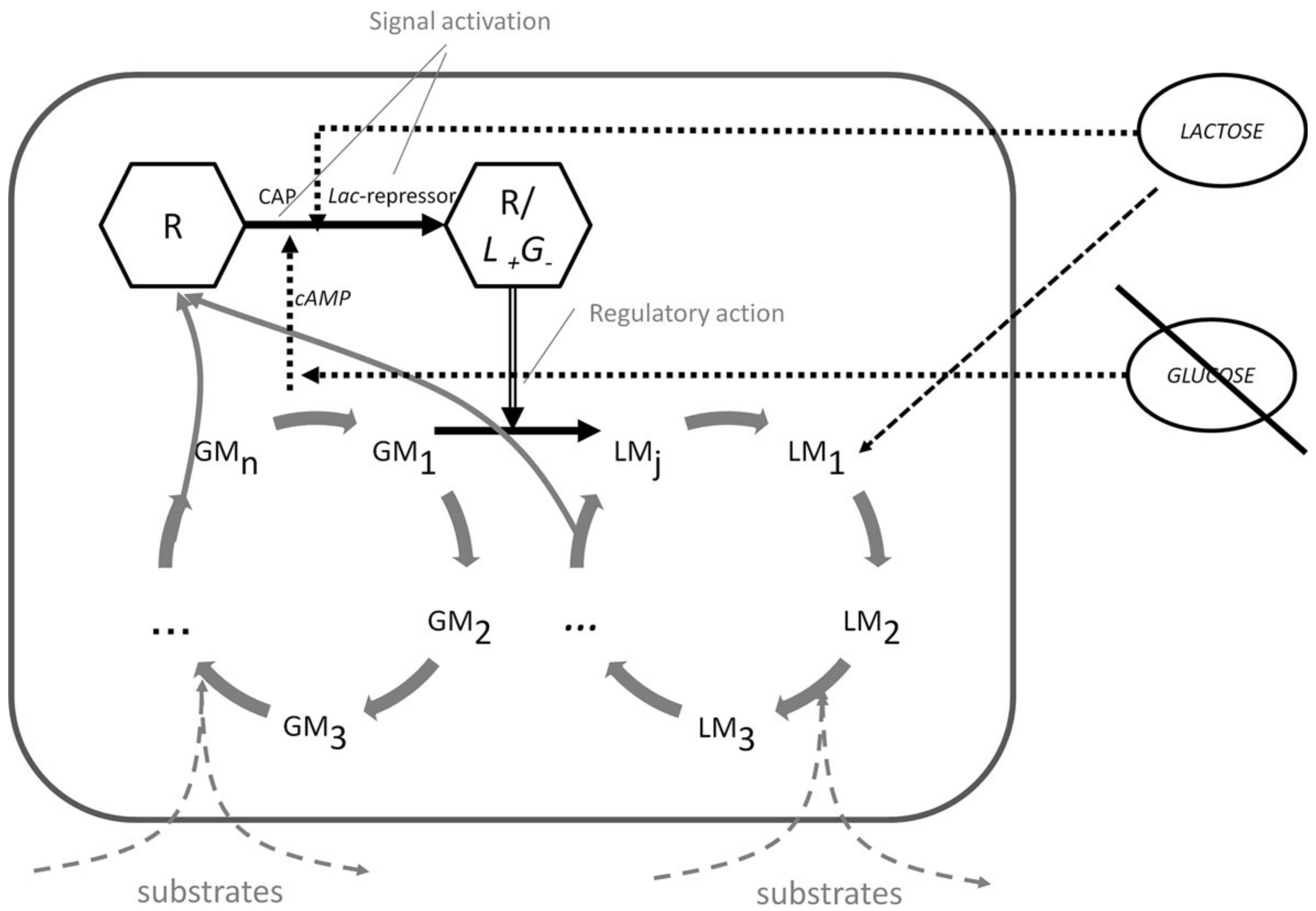
Changes local environment



E.coli nutrient source switching (lac Operon)

IF env glucose conc insufficient **AND** env lactose present **THEN** begin importing and metabolising lactose

Changes pathways of energy/ matter flow through metabolism itself



B.subtilis sporulation

IF env conditions too harsh **THEN** sporulate

Shuts down metabolism

Prebiotic Origins of Regulation

How did protocells develop mechanisms to:

1. Regulate **internal dynamics**
2. Regulate **behaviour towards environment** (including agent-agent interactions)

...that enabled them to survive in **variable** and **challenging** environmental conditions?

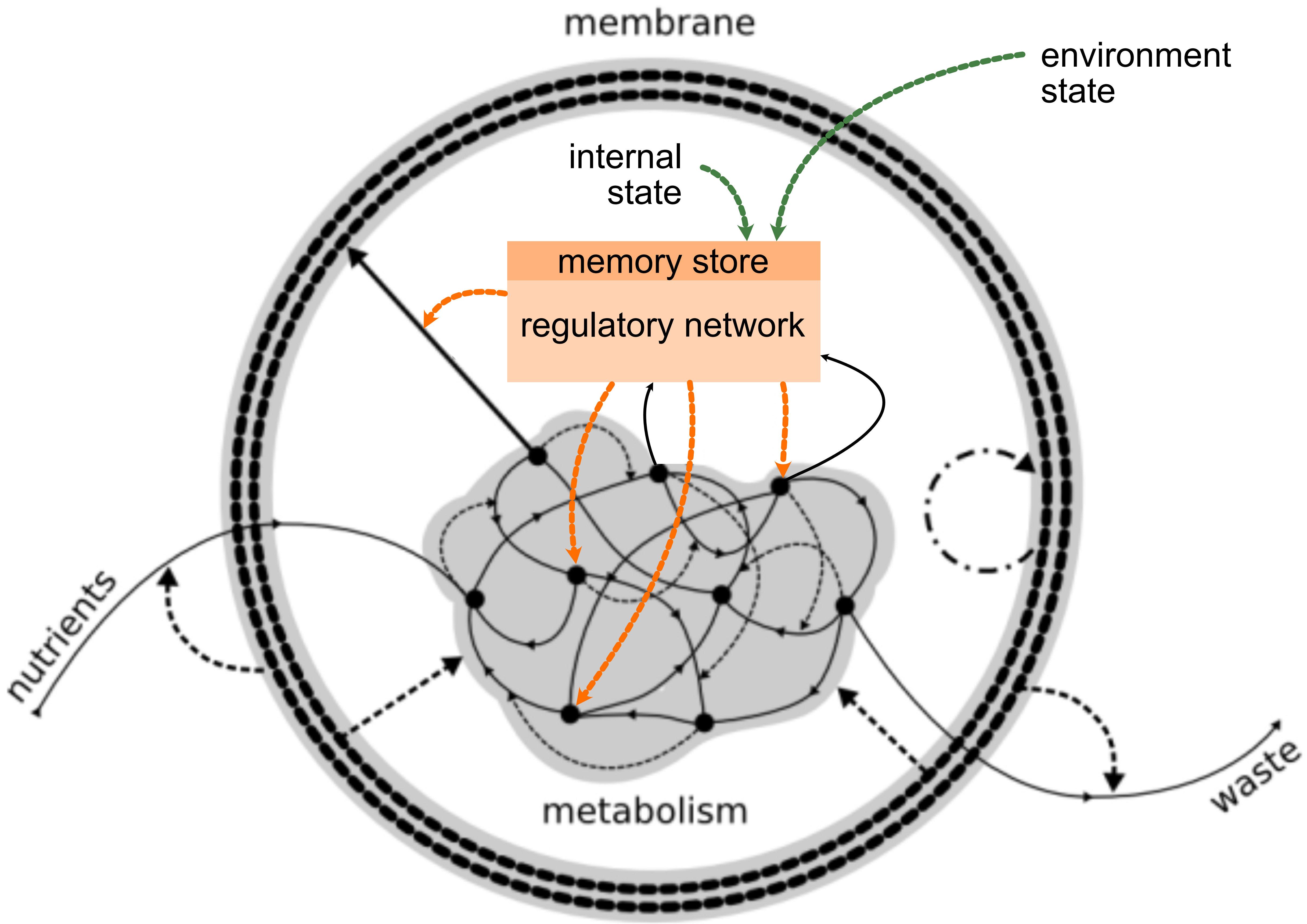


Figure adapted from: xabier.barandiaran.net

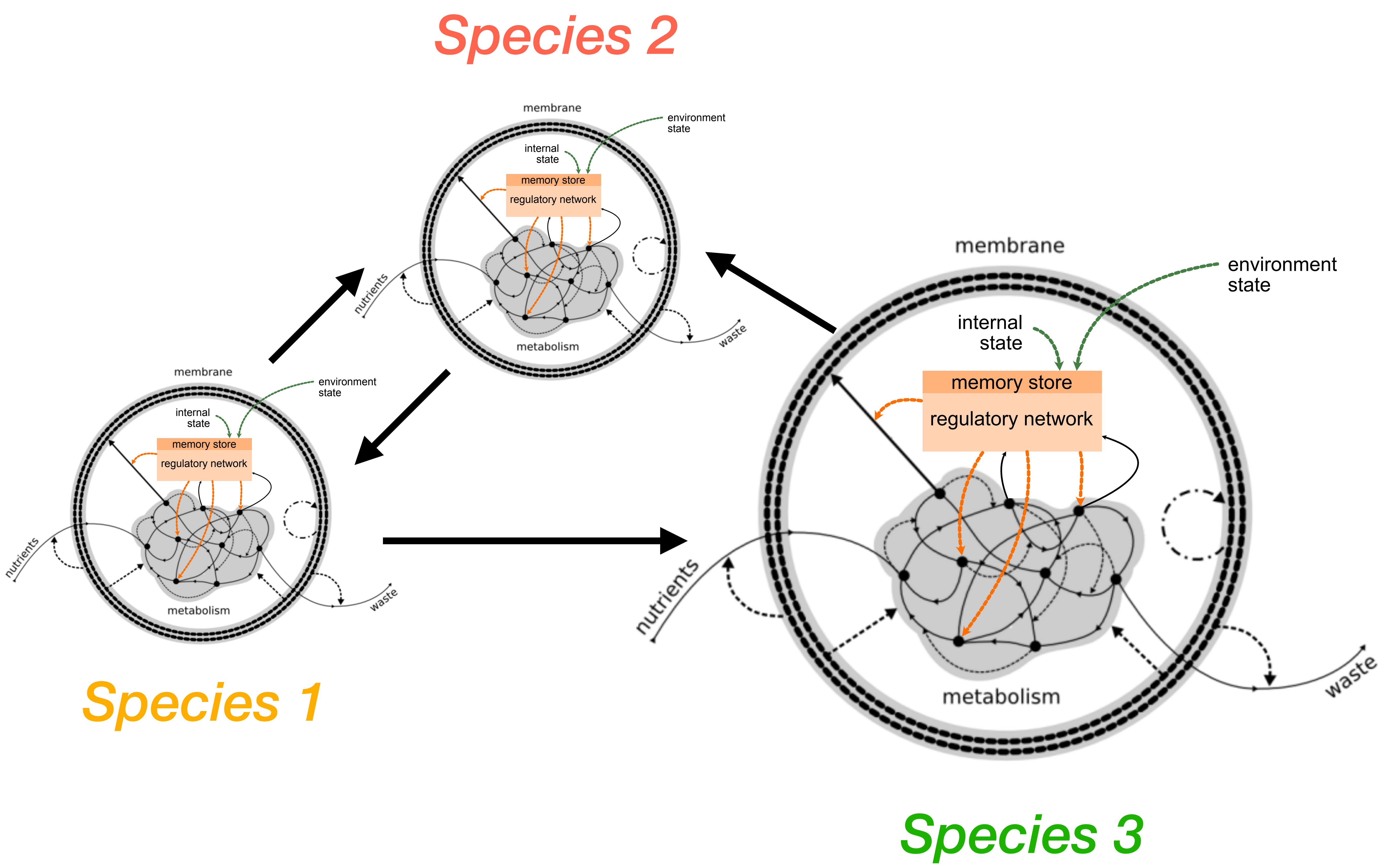
Origins of Regulation

How did protocells develop mechanisms to:

1. Regulate **internal dynamics**
2. Regulate **behaviour towards environment** (including agent-agent interactions)

...that enabled them to survive in **variable** and **challenging** environmental conditions?

Protocell Ecology



The Model

Araudia

…Basque for

'Formal set of directives'

'Procedural rules'

'Code of conduct'

Chemostat

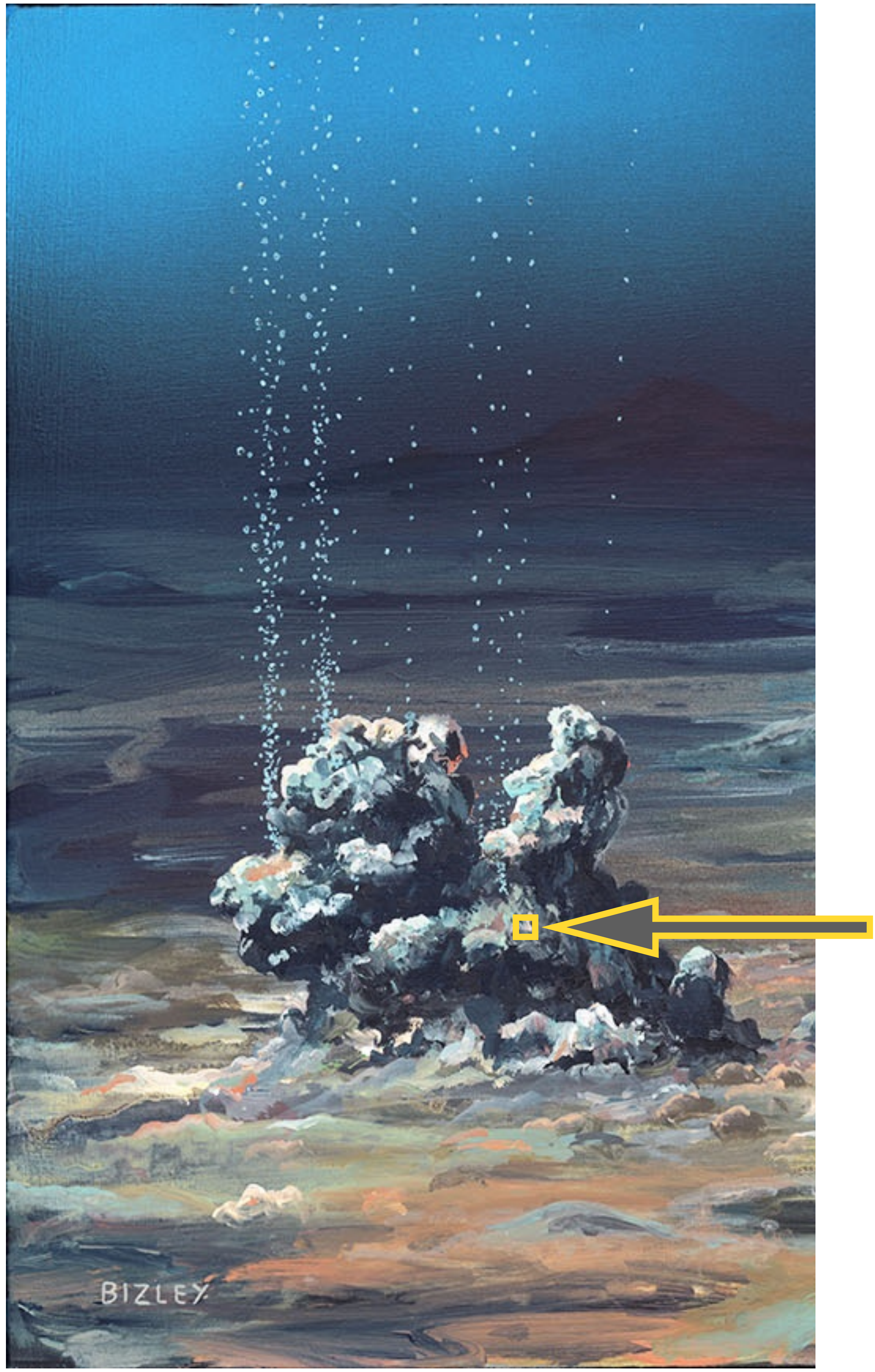
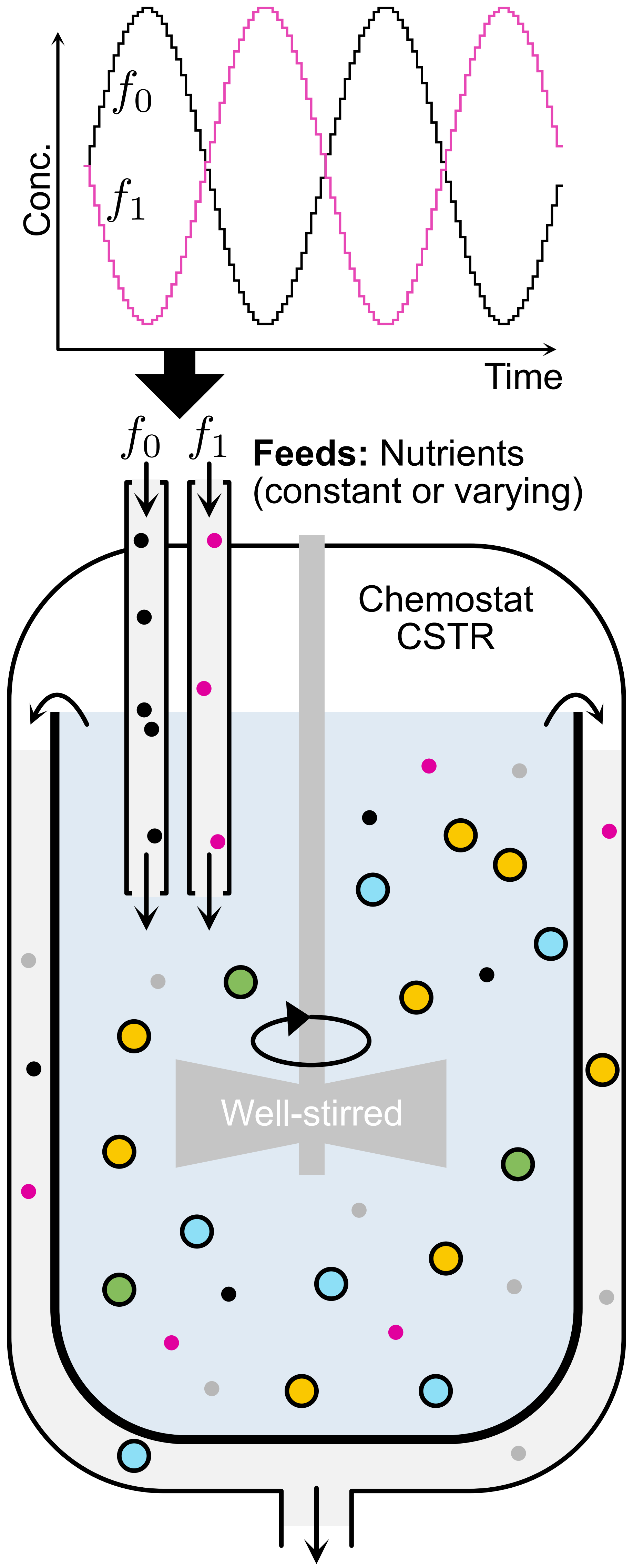


Image courtesy of Richard Bizley - bizleyart.com



Feeds: Nutrients
(constant or varying)

Chemostat
CSTR

Outflow: Surplus nutrients,
metabolic by-products,
protocells

Protocell Ecology in Chemostat

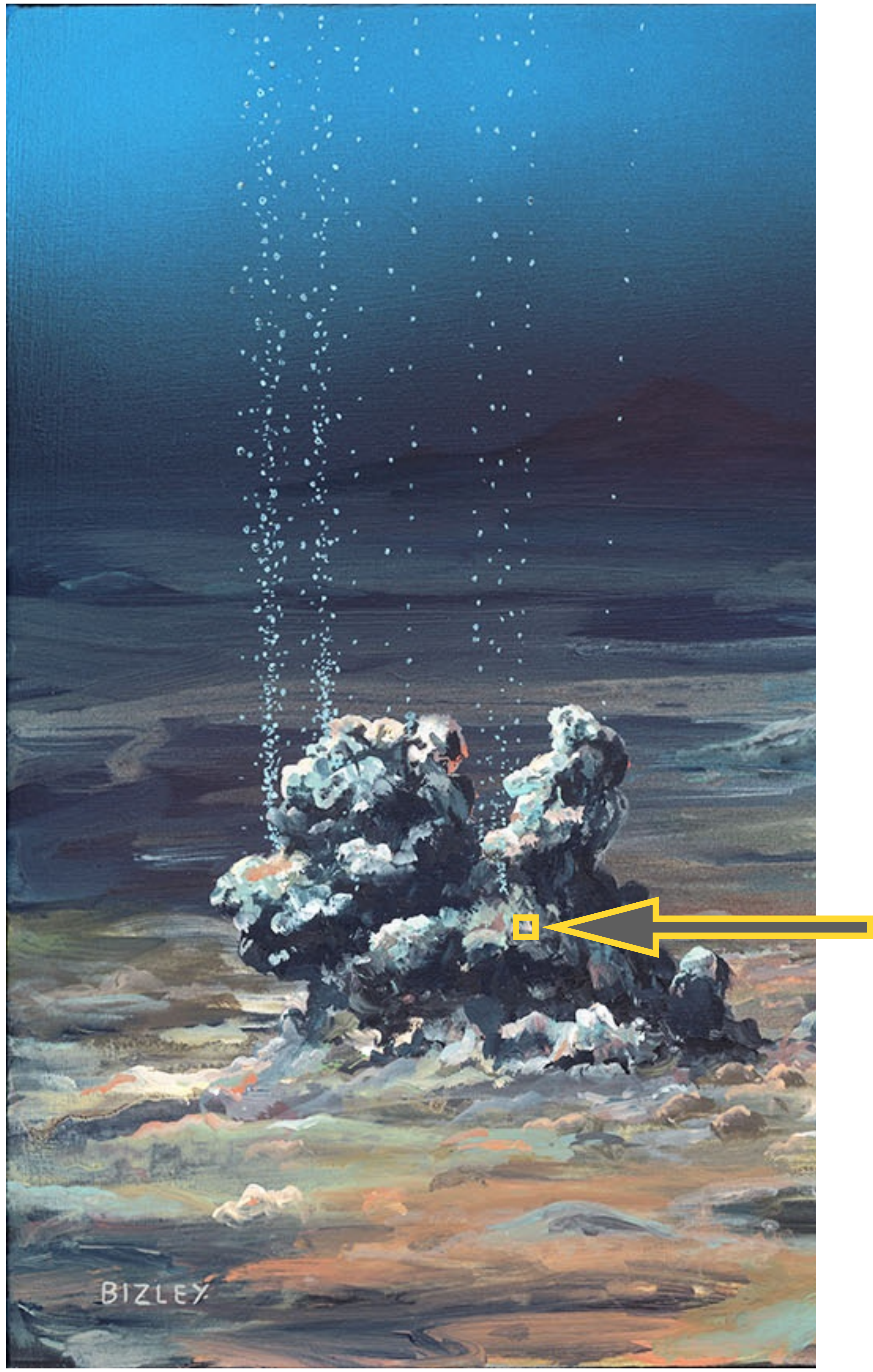
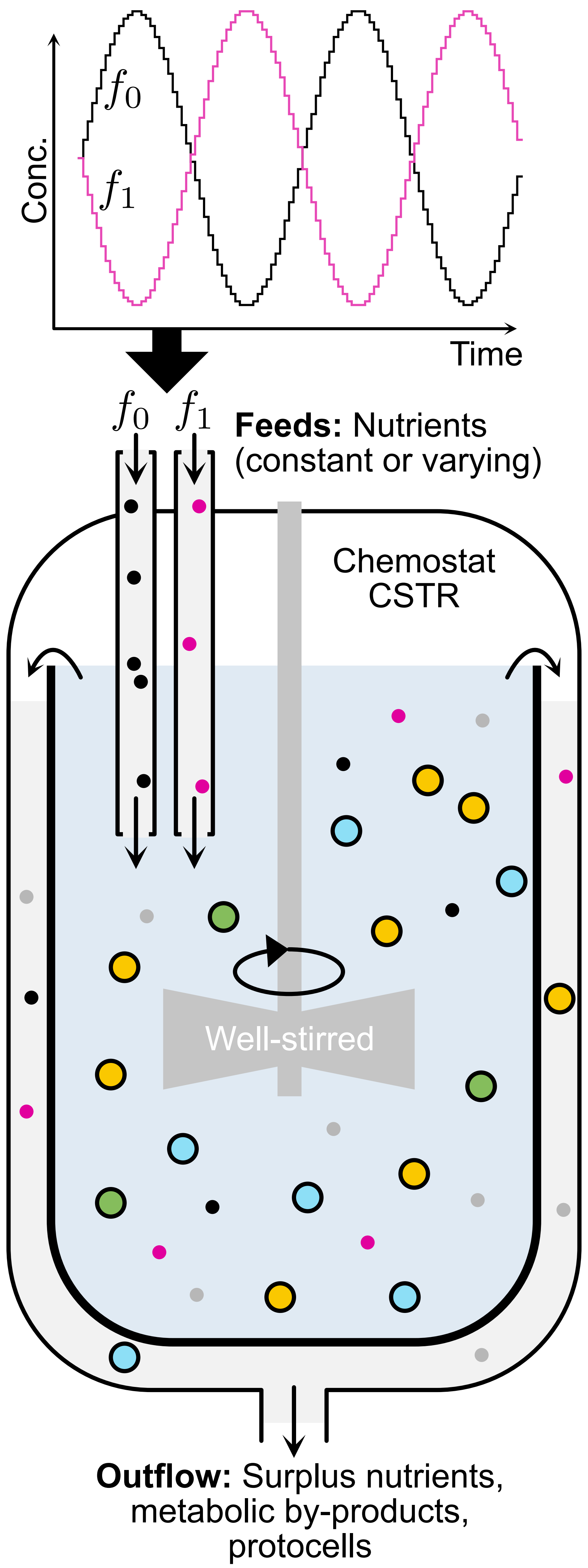
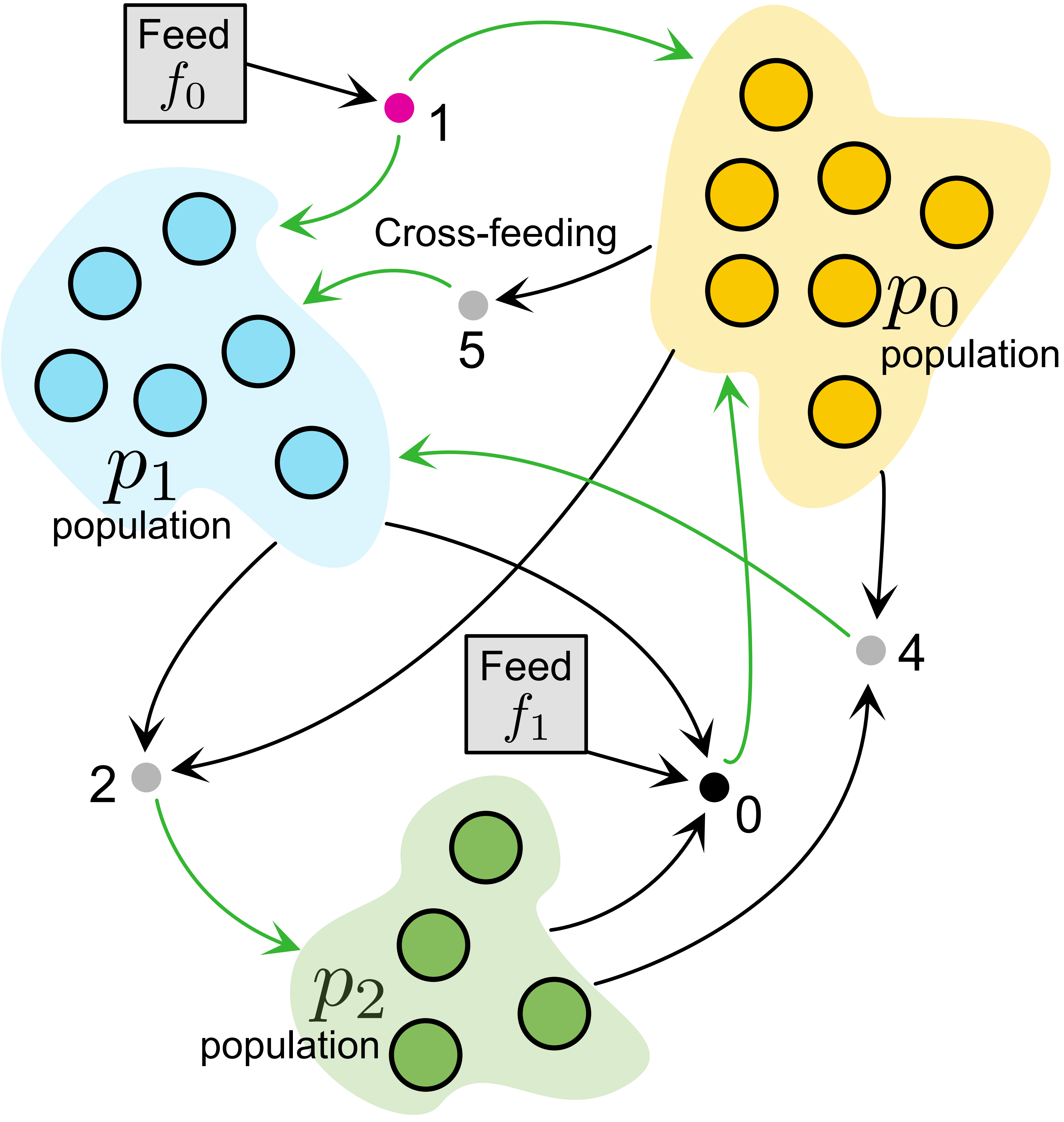


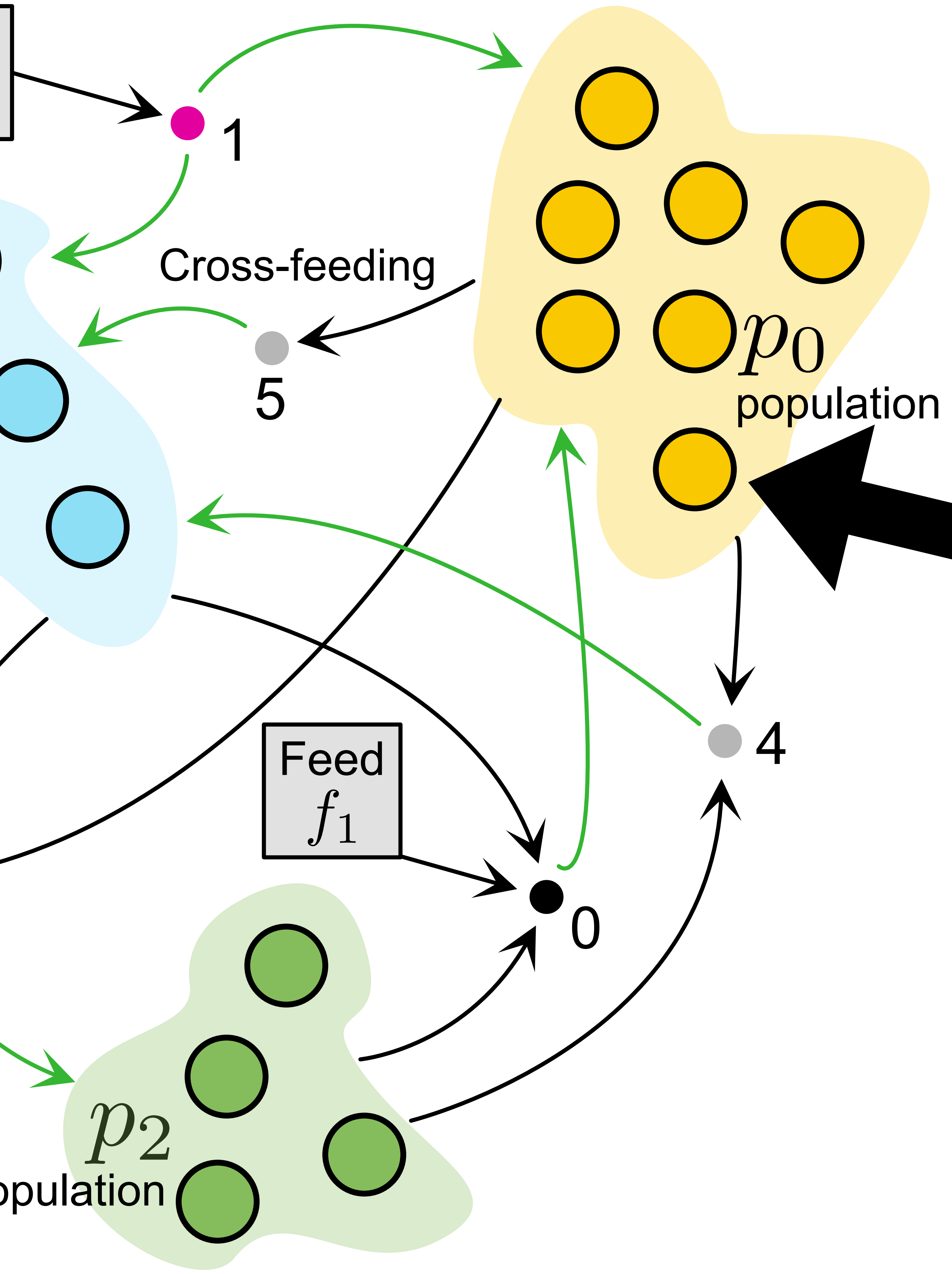
Image courtesy of Richard Bizley - bizleyart.com

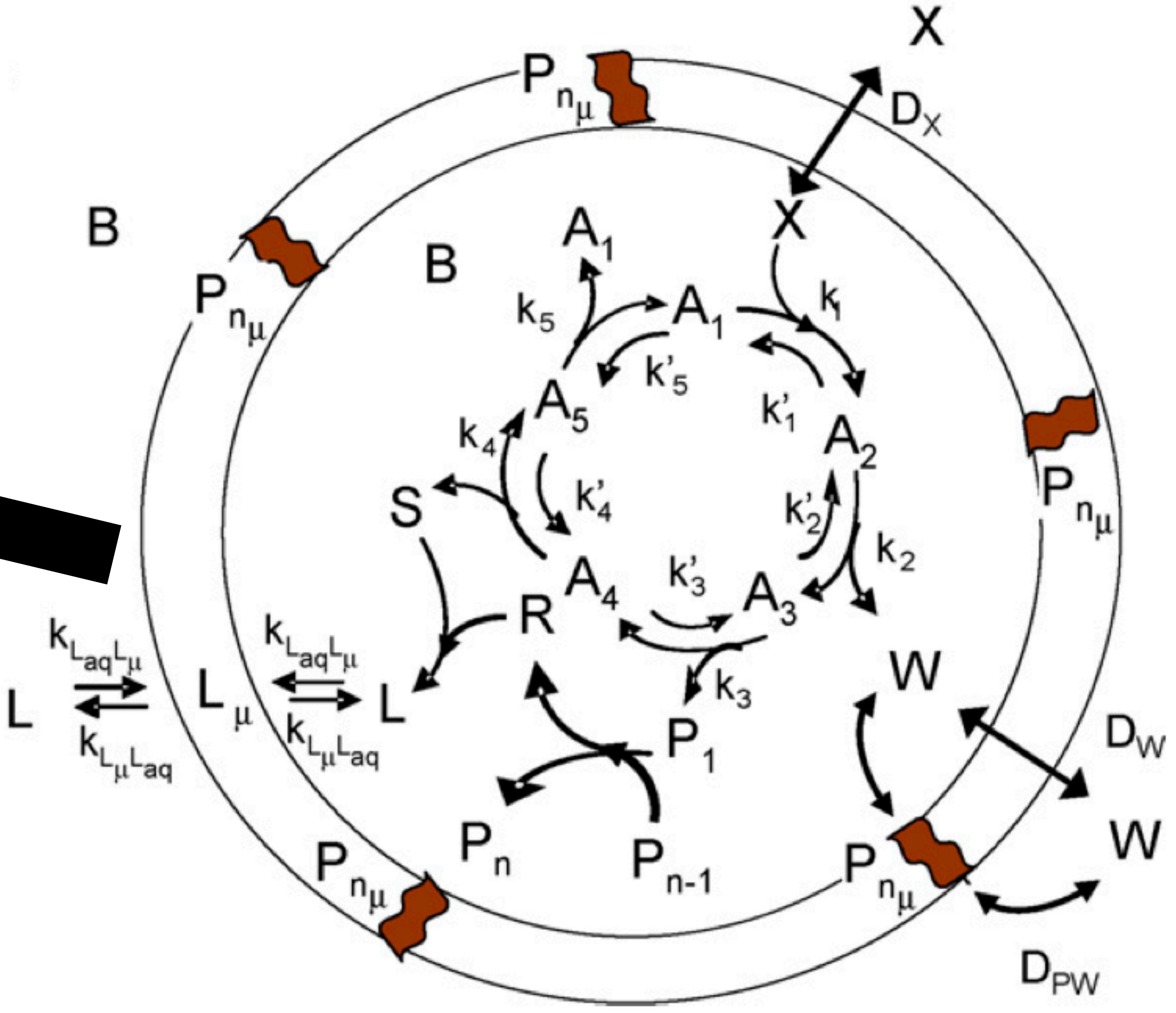


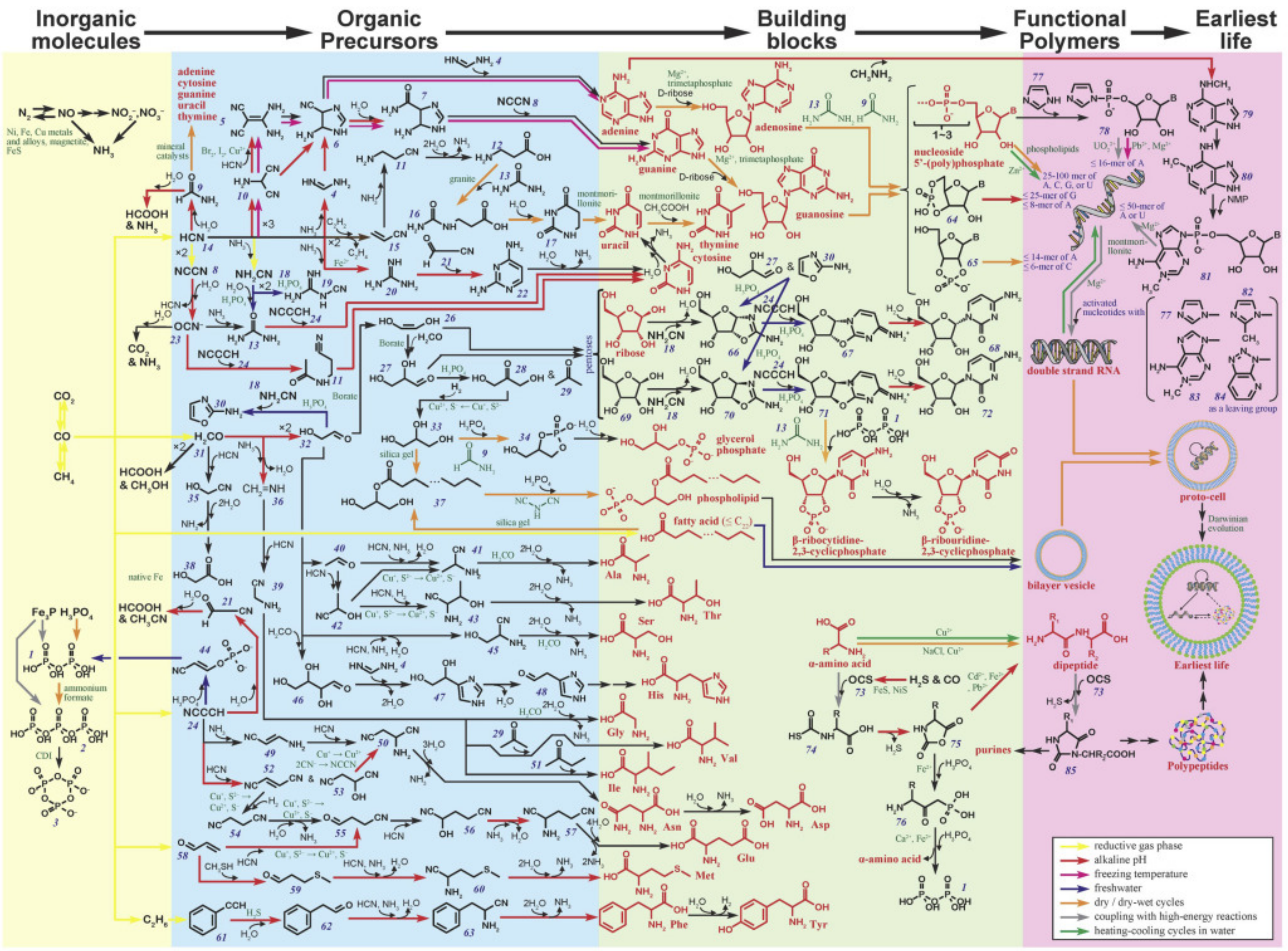


Secretory Agency

Protocell Abstraction





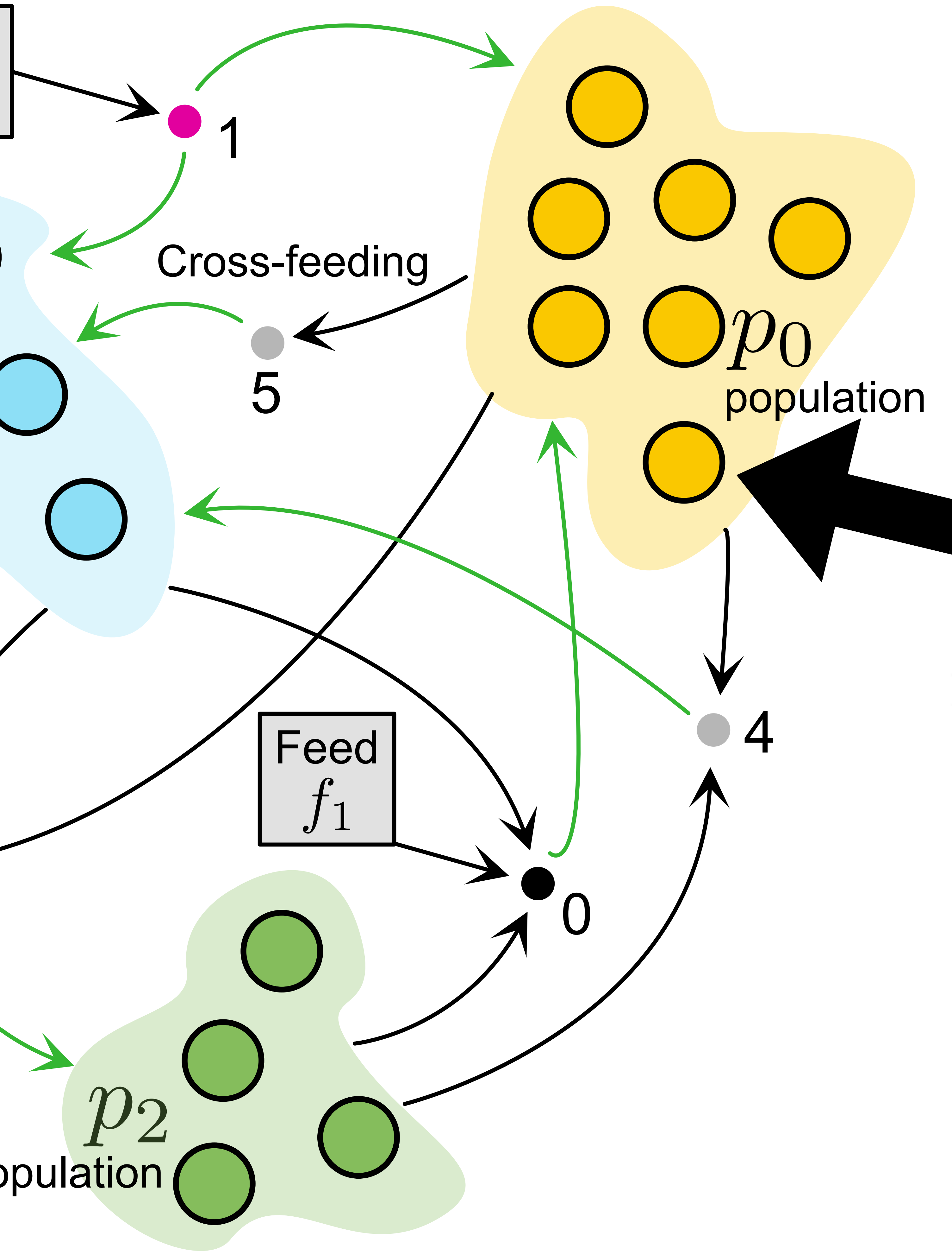


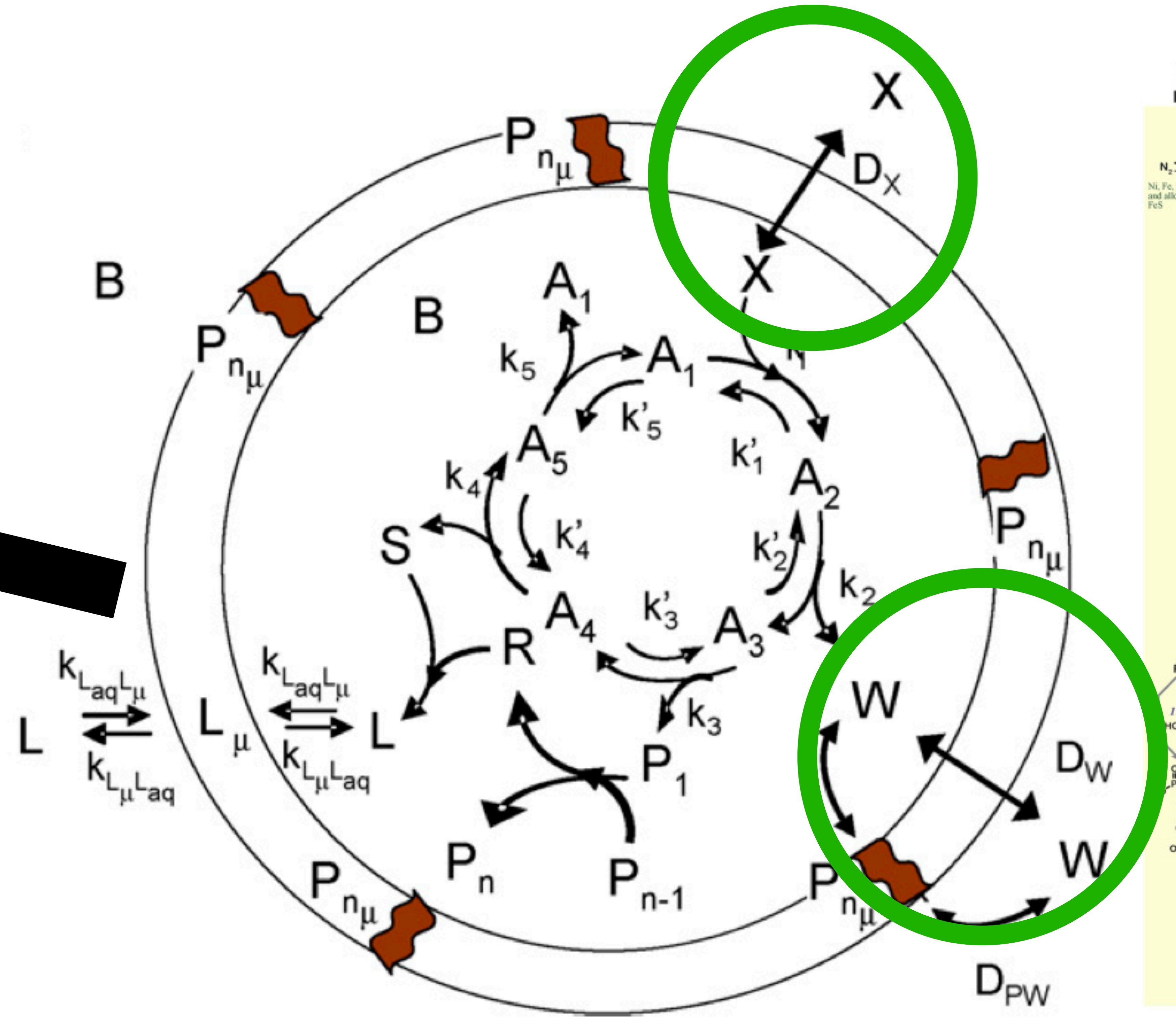
- We don't capture detailed protocell biophysics!
- We assume reliable division

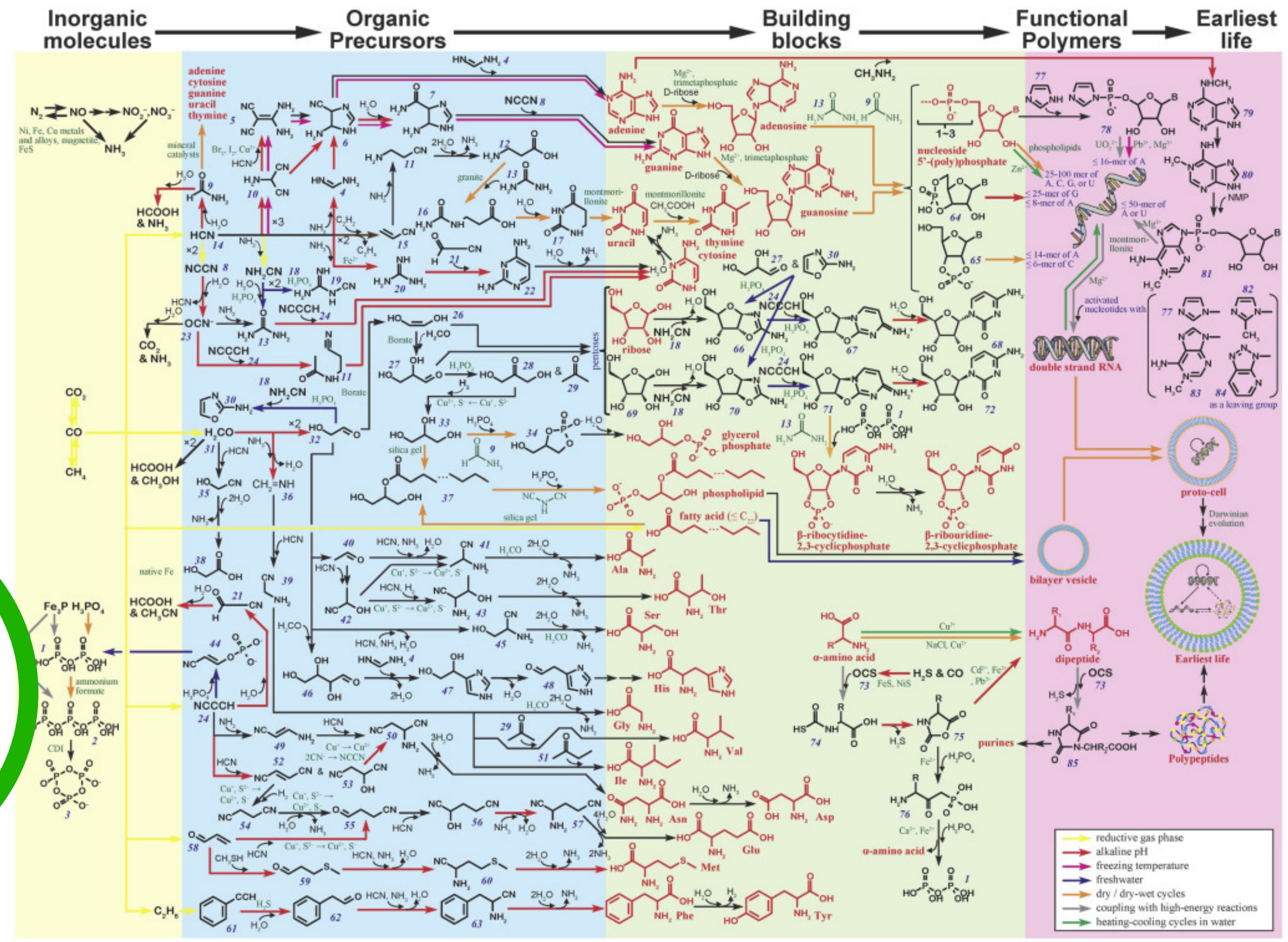
- We don't explicitly model pre-biotically plausible chemical pathways, or catalysts

Origins of building blocks of life: A review. Kitadai et al. Geoscience Frontiers (2018)

Protocell Abstraction

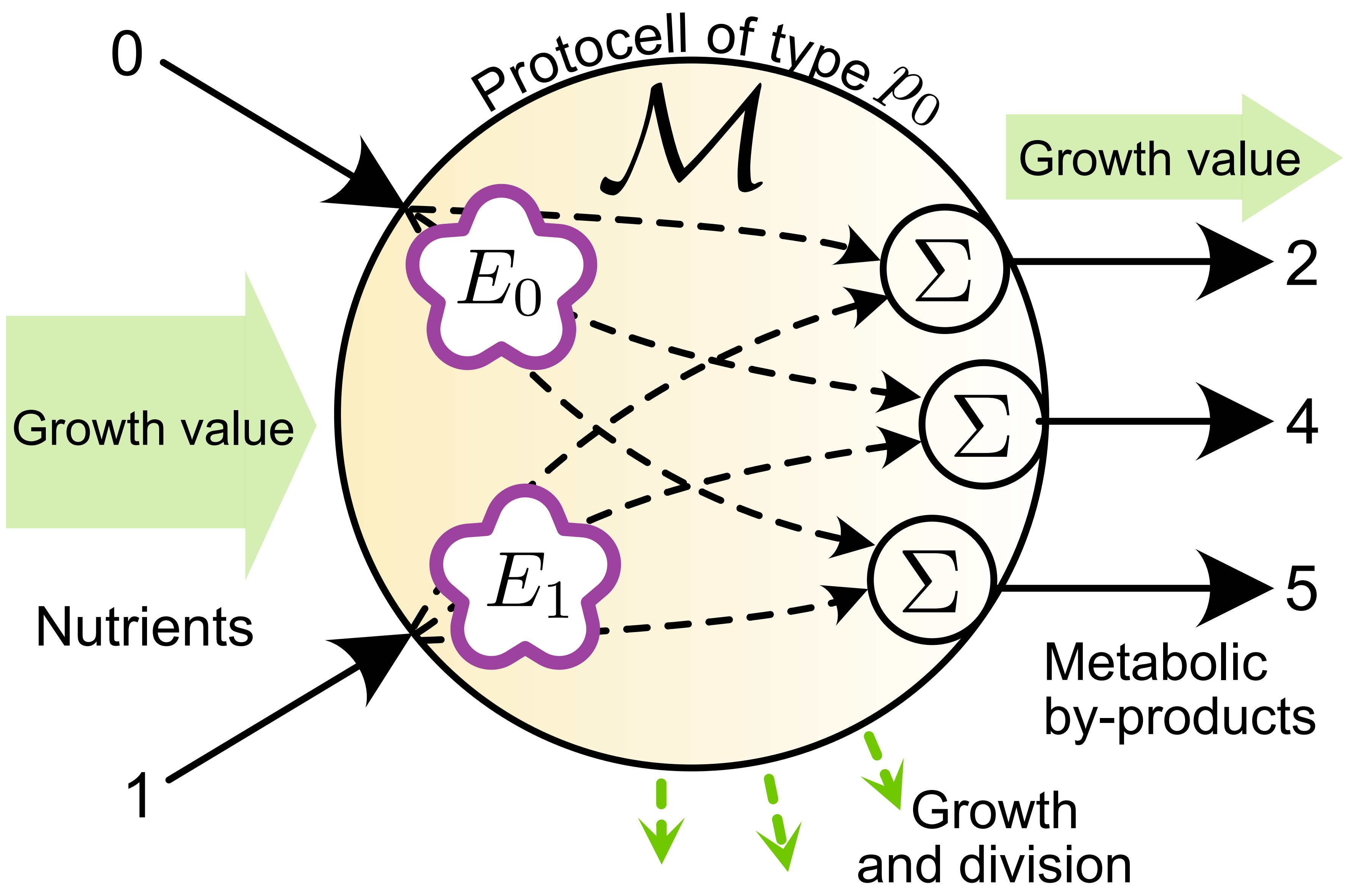






Origins of building blocks of life: A review. Kitadai et al. Geoscience Frontiers (2018)

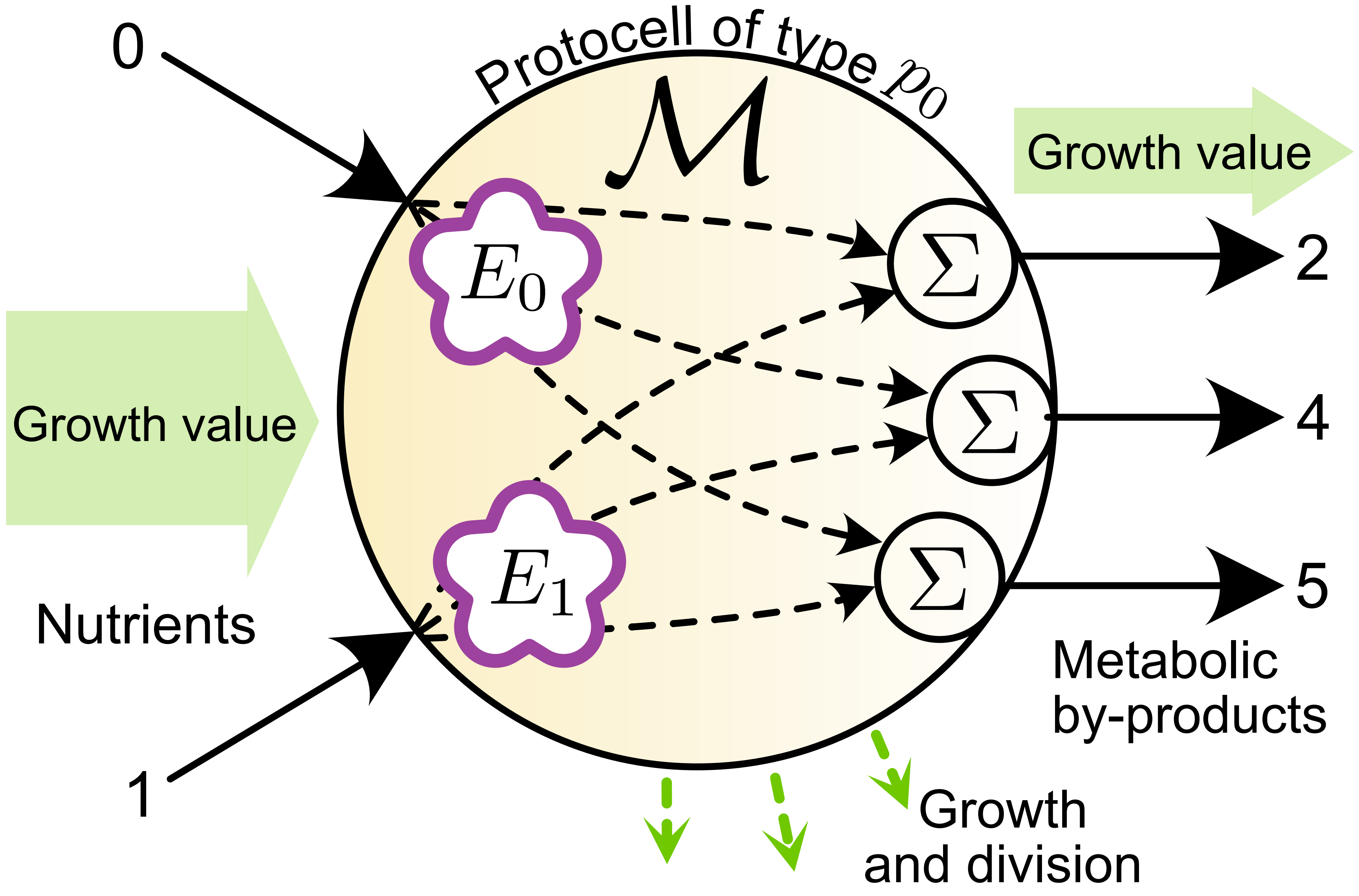
Metabolic Network



Metabolic Network

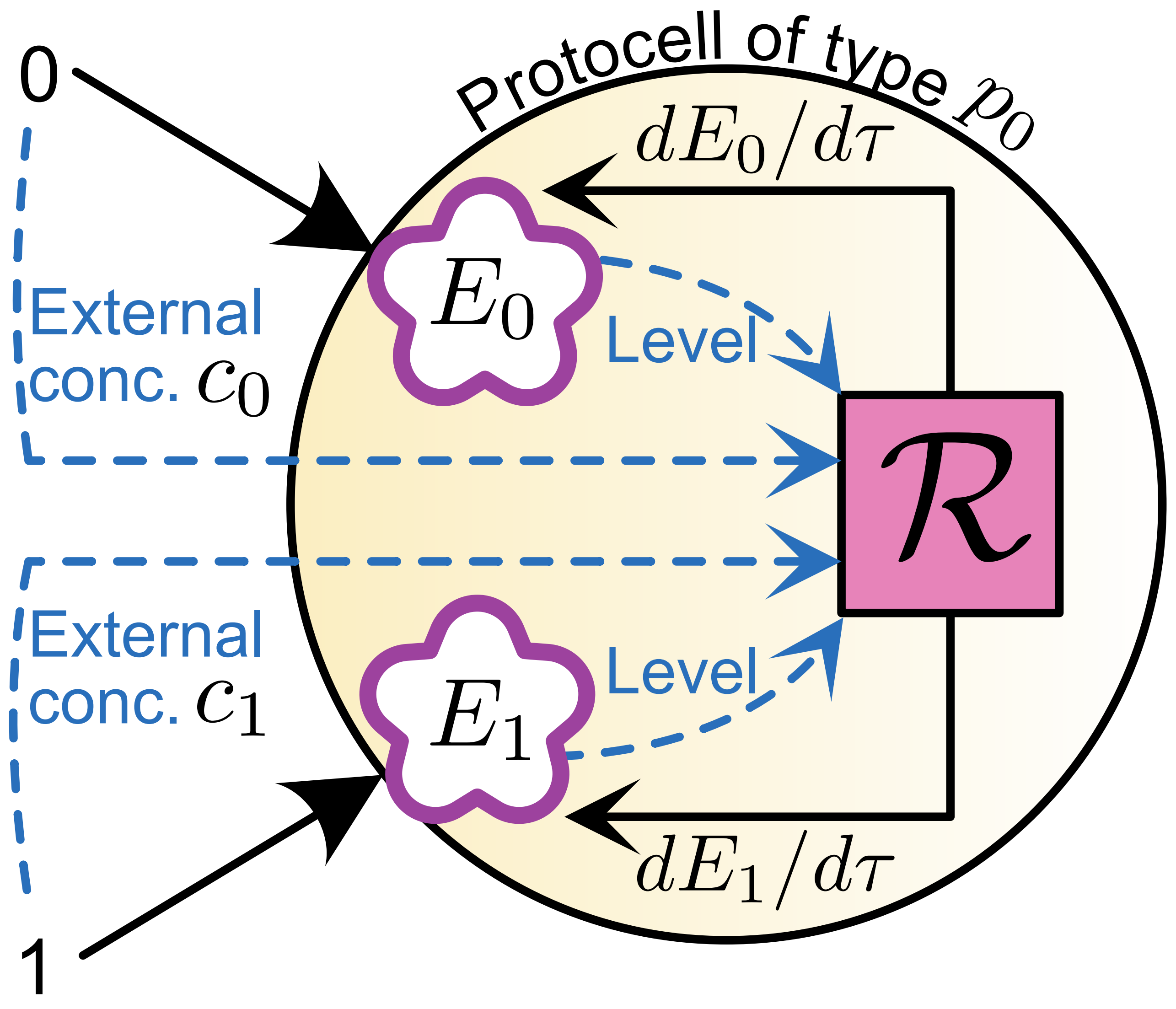
- Consuming nutrients
- Secreting by-products back to medium
- Growing and dividing (into equal daughters)

Regulatory Network



Metabolic Network

- Consuming nutrients
- Secreting by-products back to medium
- Growing and dividing

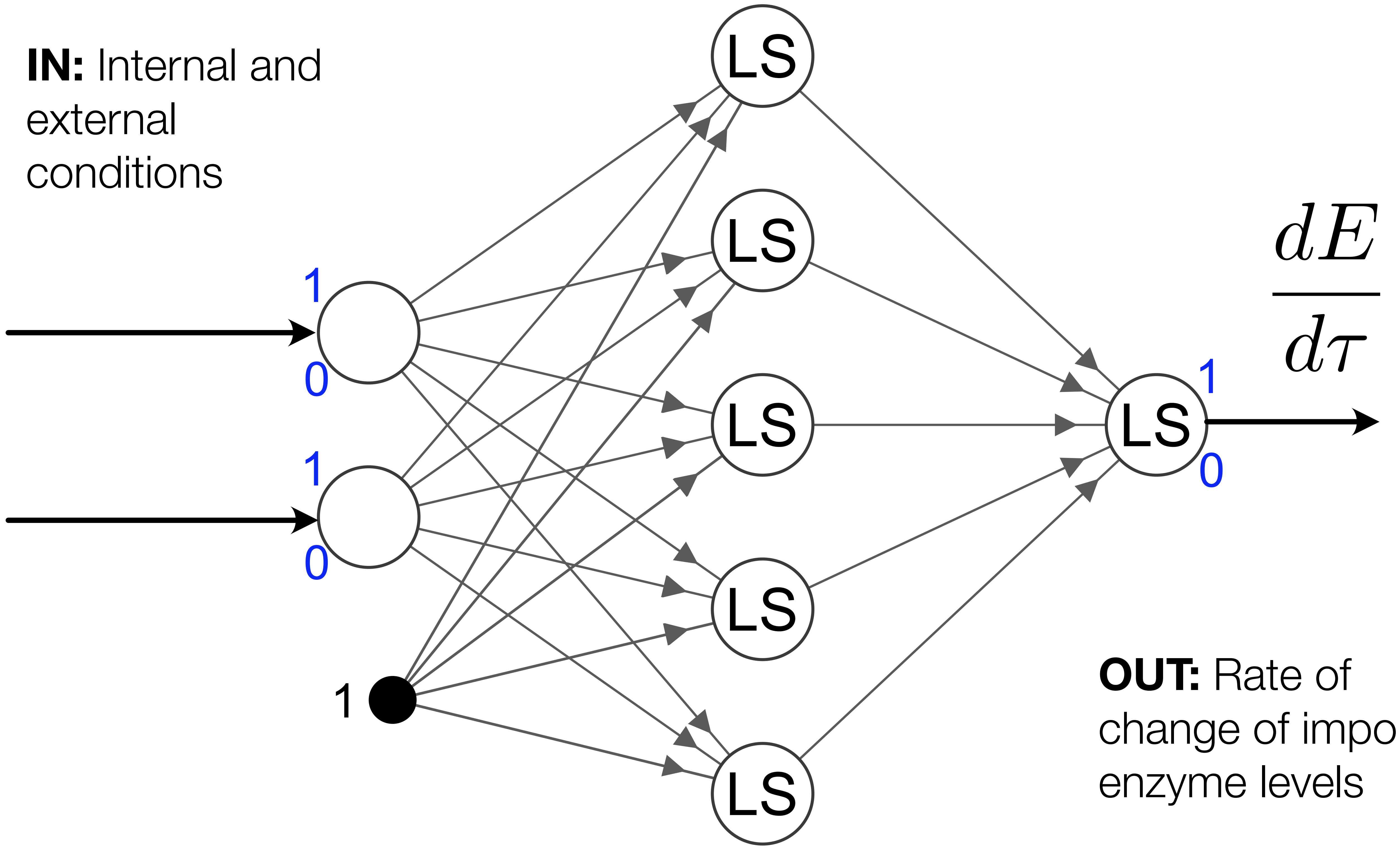


Regulatory Network

- Dynamically changes import enzyme levels
- ...in response to internal state and environmental state

Regulatory Network

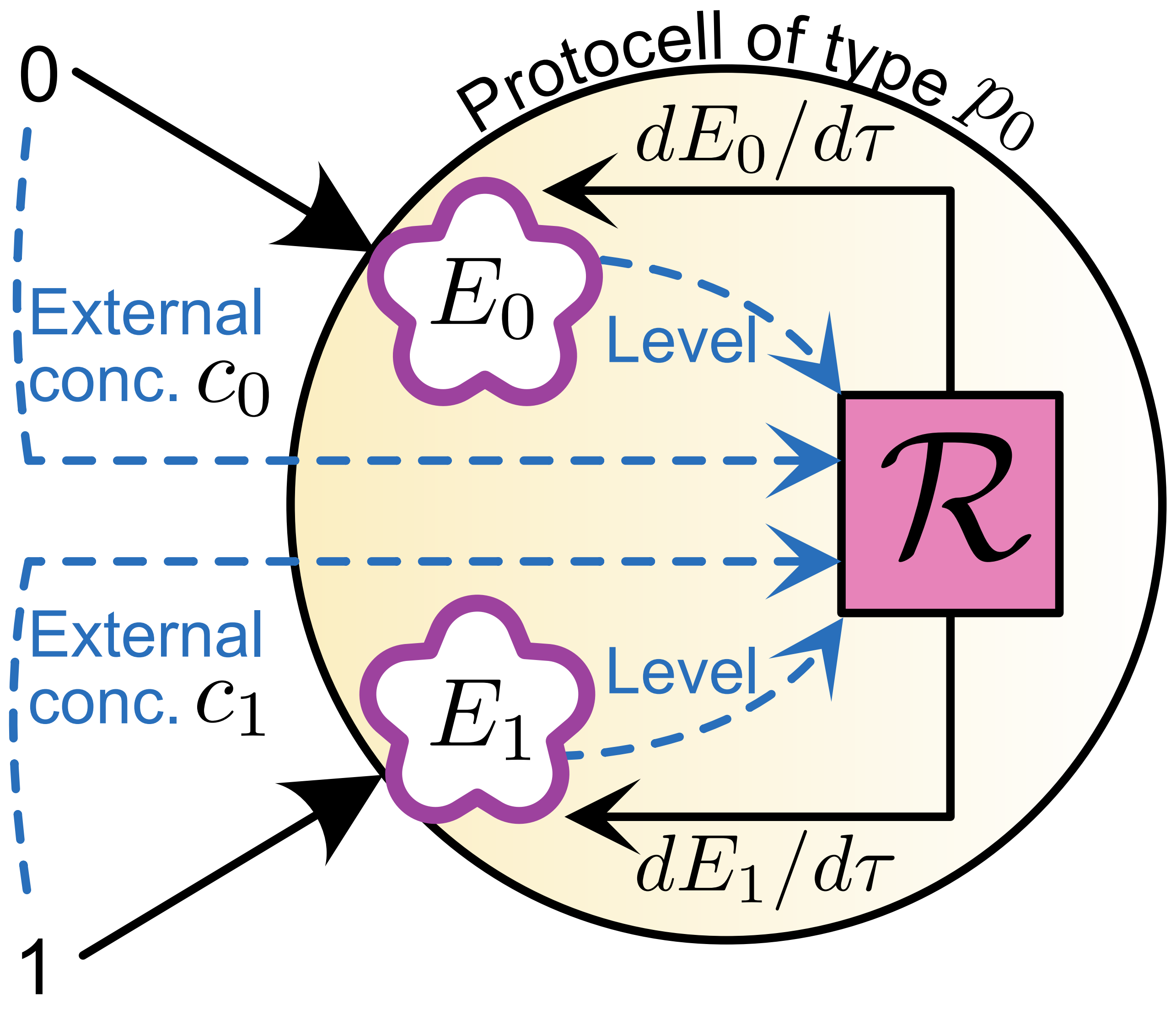
Neural ODE Dynamical System



IN: Internal and external conditions

OUT: Rate of change of import enzyme levels

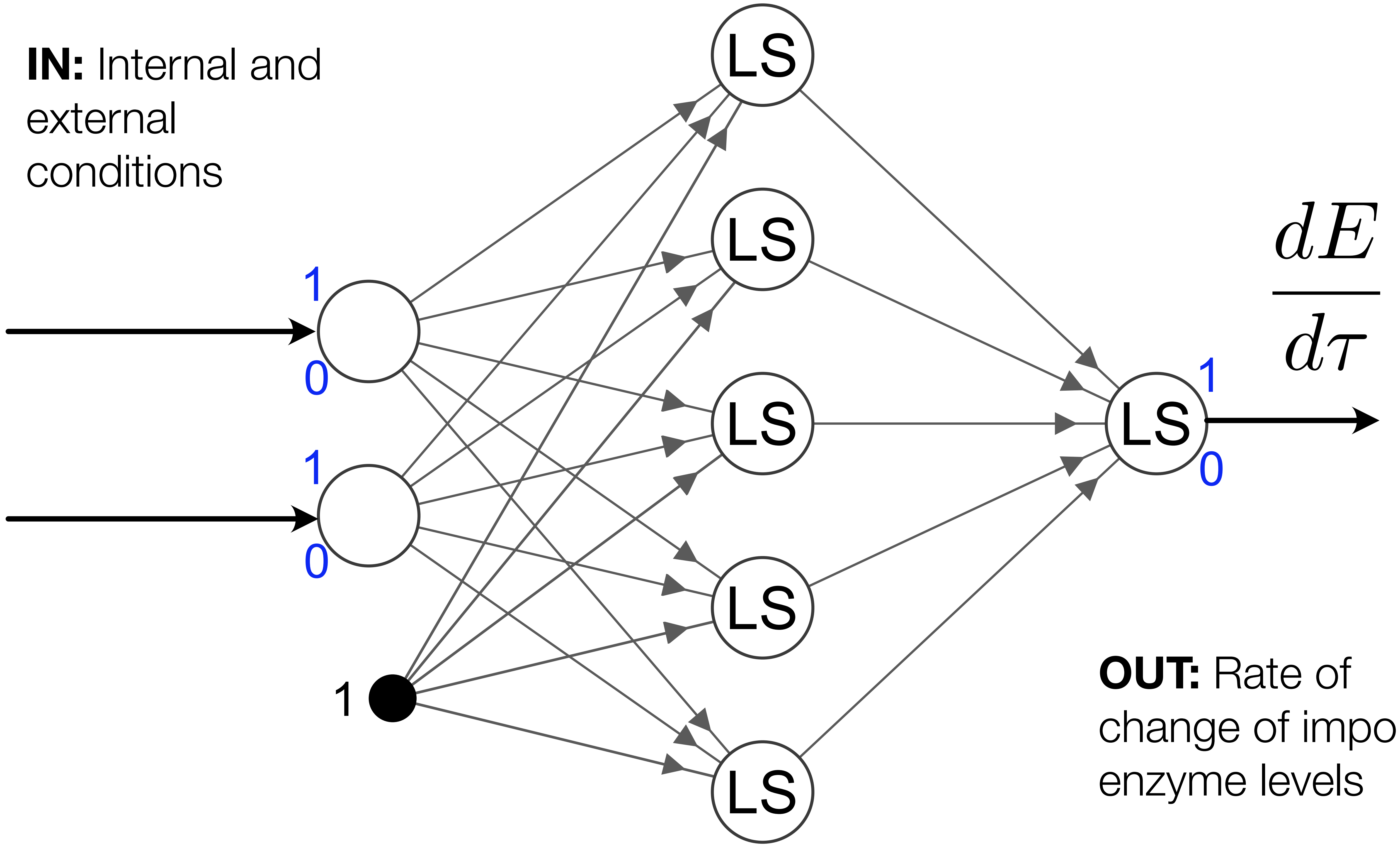
Initially regulatory network is disabled: all weights are 0



Nothing to do with machine learning!

Regulatory Network

Neural ODE Dynamical System



IN: Internal and external conditions

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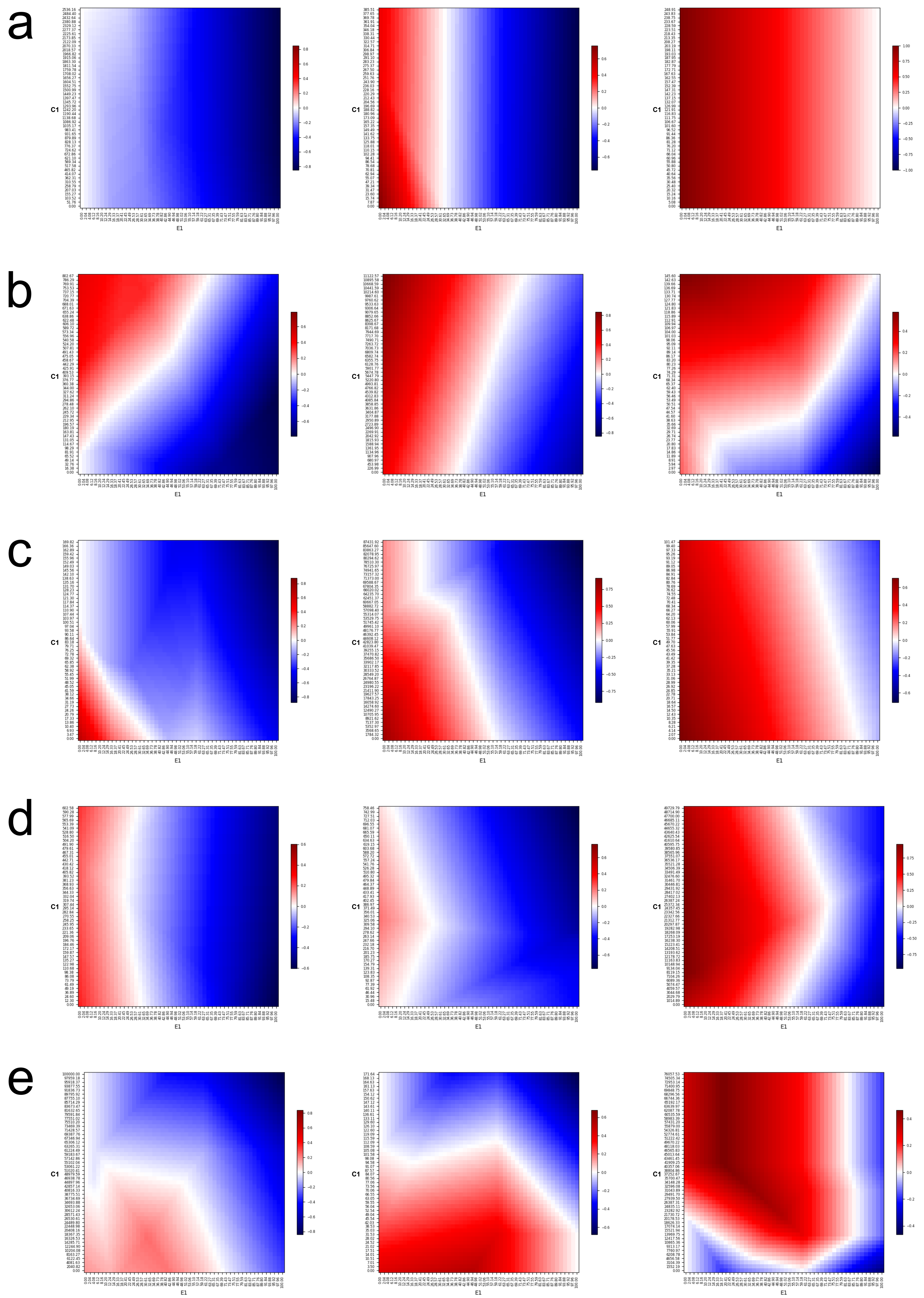
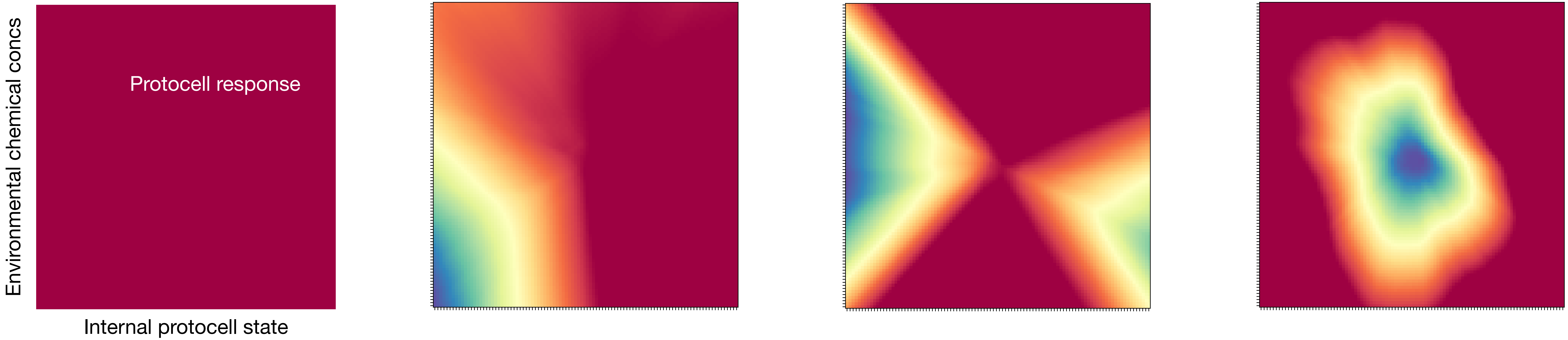


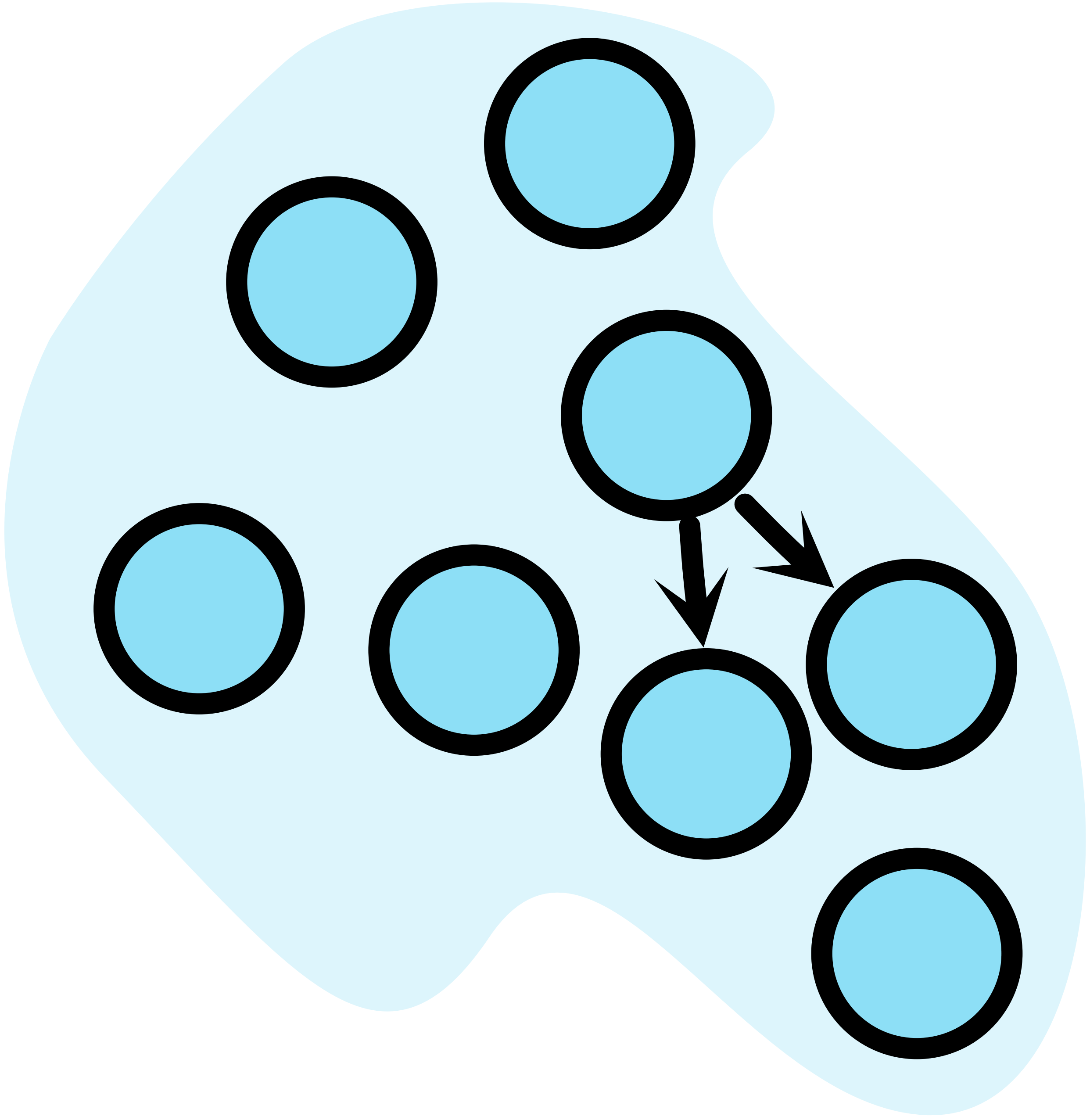
Regulatory Network Tinkered into Functionality by Evolution



'Knowledge' about how to best respond to potential environmental conditions, over evolutionary time becomes "sedimented" in regulatory network function

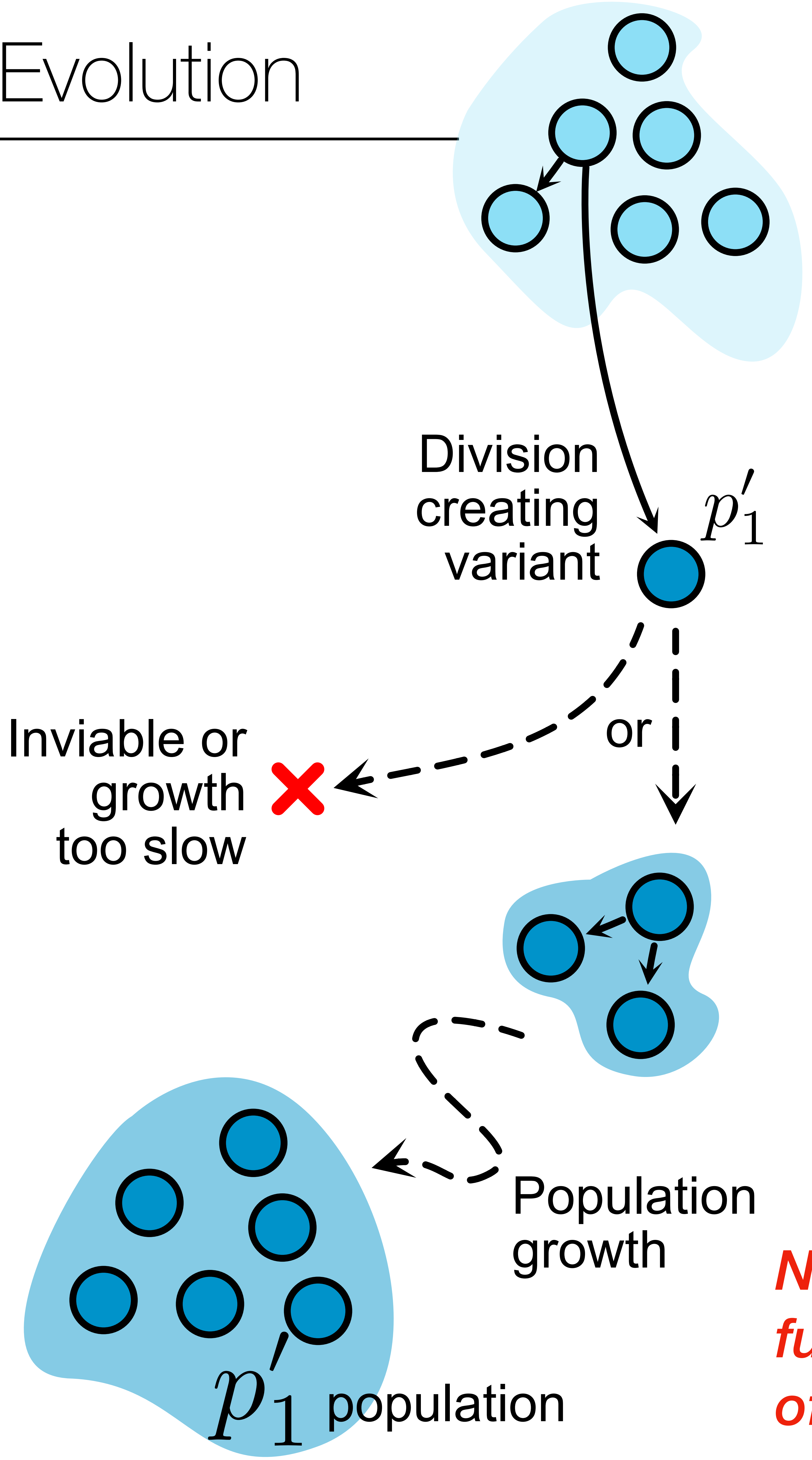
(Micro) Evolution

Frequent



Normal division

Occasional



Division creating variant

p'_1

Inviable or growth too slow

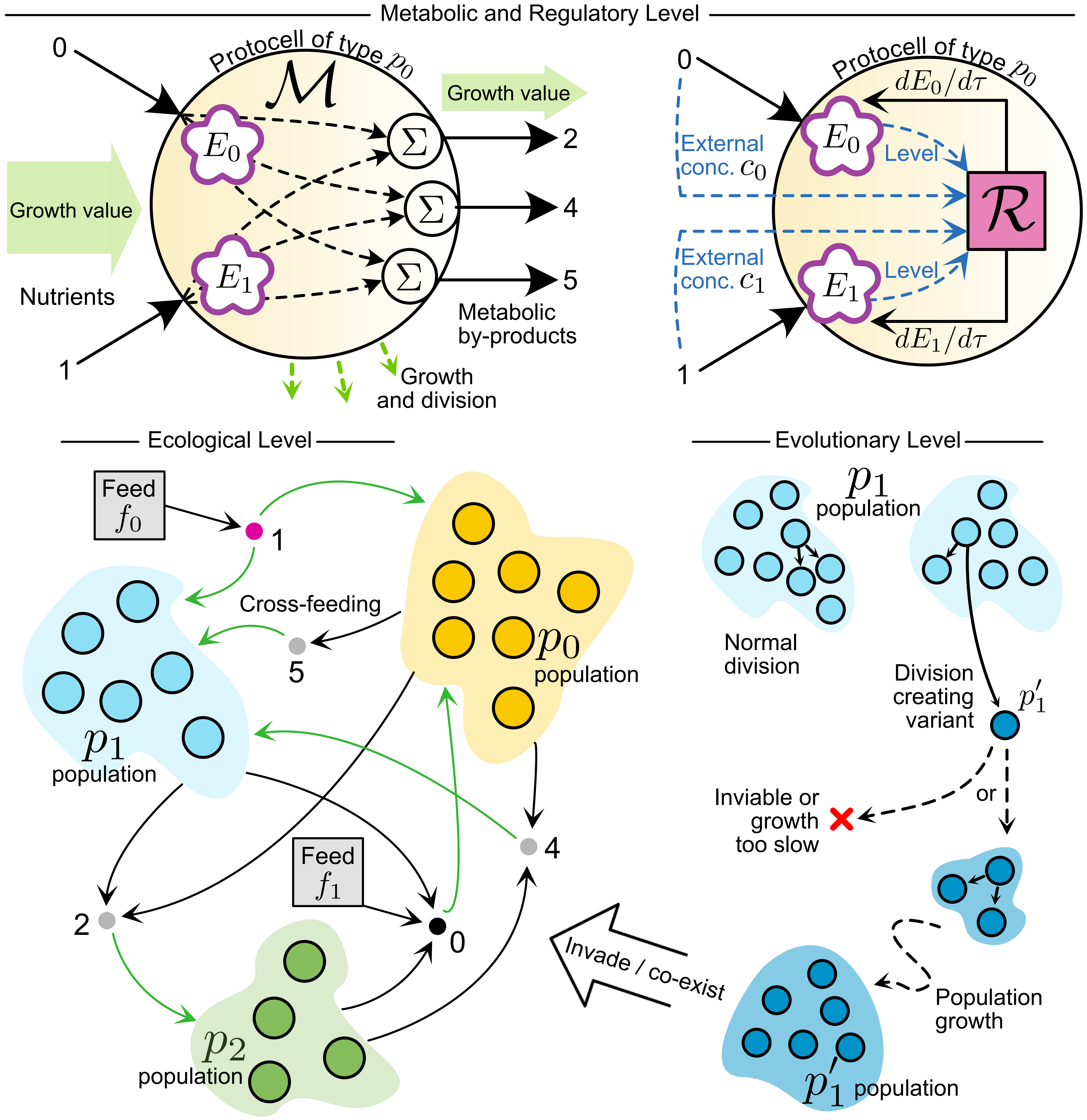
or

Population growth

p'_1 population

No explicit fitness function! only survival of reactor wash out!

Three Levels of Abstraction



Metabolism

Ecology

Evolution

Mathematical Basis: Microbial Consumer-Resource Model

Robert MacArthur
Consumer-Resource Model (1970)

b **Generalized consumer–resource model**

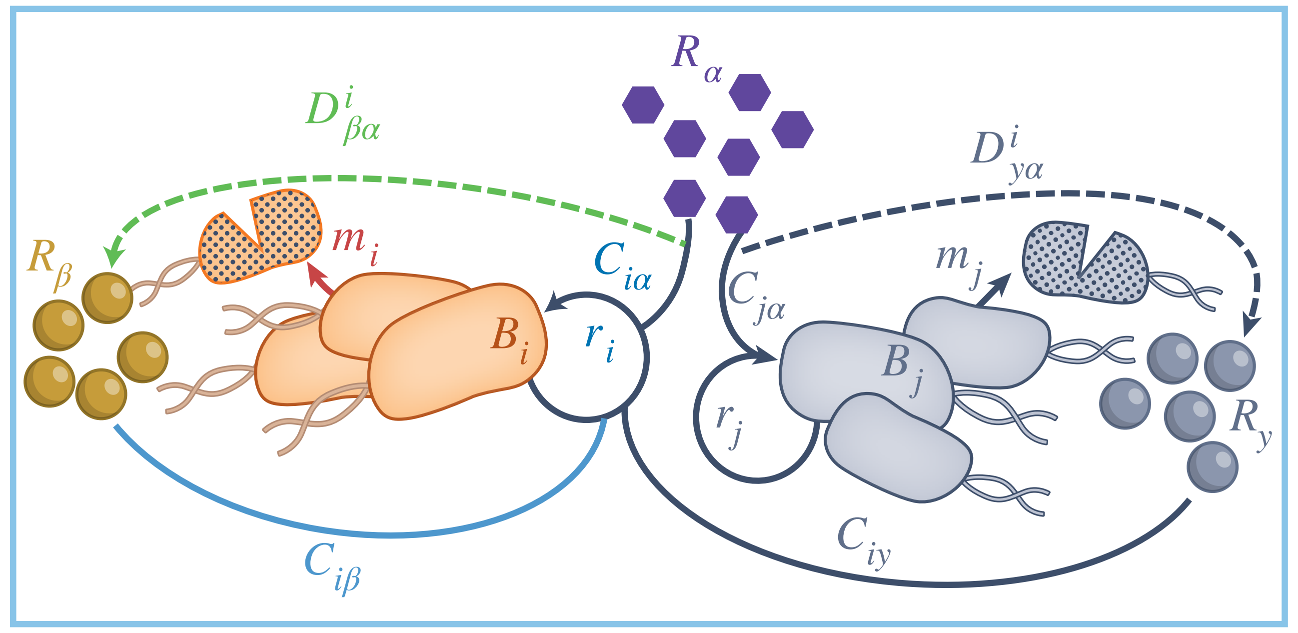
Growth per population

$$\frac{dB_i}{dt} = r_i B_i \left(\sum_\alpha \Delta w_{i\alpha} C_{i\alpha} R_\alpha\right) - m_i B_i$$

$$\Delta w_{i\alpha} = w_\alpha - \sum_\beta D^i_{\beta\alpha} w_\beta$$

Resource dynamics and metabolism

$$\frac{dR_\beta}{dt} = -\sum_i C_{i\beta} R_\beta B_i + \sum_{\alpha,i} D^i_{\beta\alpha} C_{i\alpha} R_\alpha B_i$$



Goldfold 2018

RESEARCH

RESEARCH ARTICLE

MICROBIOLOGY

Emergent simplicity in microbial community assembly

Joshua E. Goldford[1,2*], Nanxi Lu[3*], Djordje Bajić[3], Sylvie Estrela[3], Mikhail Tikhonov[4,5], Alicia Sanchez-Gorostiaga[3], Daniel Segrè[1,6,7], Pankaj Mehta[1,7]†, Alvaro Sanchez[2,3]†

A major unresolved question in microbiome research is whether the complex taxonomic architectures observed in surveys of natural communities can be explained and predicted by fundamental, quantitative principles. Bridging theory and experiment is hampered by the multiplicity of ecological processes that simultaneously affect community assembly in natural ecosystems. We addressed this challenge by monitoring the assembly of hundreds of soil- and plant-derived microbiomes in well-controlled minimal synthetic media. Both the community-level function and the coarse-grained taxonomy of the resulting communities are highly predictable and governed by nutrient availability, despite substantial species variability. By generalizing classical ecological models to include widespread nonspecific cross-feeding, we show that these features are all emergent properties of the assembly of large microbial communities, explaining their ubiquity in natural microbiomes.

Microbial communities play critical roles in a wide range of natural processes, from animal development and host health to biogeochemical cycles (*1*–*3*). Recent advances in DNA sequencing have allowed us to map the composition of these communities with high resolution. This has motivated a surge of interest in understanding the ecological mechanisms that govern microbial community assembly and function (*4*). A quantitative, predictive understanding of microbiome ecology is required to design effective strategies to rationally manipulate microbial communities toward beneficial states.

Surveys of microbiome composition across a wide range of ecological settings, from the ocean to the human body (*2*, *3*), have revealed intriguing empirical patterns in microbiome organization. These widely observed properties include high microbial diversity, the coexistence of multiple closely related species within the same functional group, functional stability despite large species turnover, and different degrees of determinism in the association between nutrient availability and taxonomic composition at different phylogenetic levels (*3*, *5*–*10*). These observations have led to the proposal that common organizational principles exist in microbial community assembly (*6*, *7*). However, the lack of a theory of microbiome assembly is hindering progress toward explaining and interpreting these empirical findings, and it remains unknown which of the functional and structural features exhibited by microbiomes reflect specific local adaptations at the host or microbiome level (*10*) and which are generic properties of complex, self-assembled microbial communities.

Efforts to connect theory and experiments to understand microbiome assembly have typically relied on manipulative bottom-up experiments with a few species (*11*–*13*). Although this approach is useful for providing insights into specific mechanisms of interactions, it is unclear to what extent findings from these studies scale up to predict the generic properties of large microbial communities or the interactions therein. Of note is the ongoing debate about the relative contributions of competition and facilitation (*14*, *15*) and the poorly understood role that high-order interactions play in microbial community assembly (*11*, *16*, *17*). To move beyond empirical observations and connect statistical patterns of microbiome assembly with ecological theory, we need to study the assembly of large numbers of large multispecies microbiomes under highly controlled and well-understood conditions that allow proper comparison between theory and experiment.

Assembly of large microbial communities on a single limiting resource

To meet this challenge, we followed a high-throughput ex situ cultivation protocol to monitor the spontaneous assembly of ecologically stable microbial communities derived from natural habitats in well-controlled environments; we used synthetic (M9) minimal media containing a single externally supplied source of carbon, as well as sources of all of the necessary salts and chemical elements required for microbial life (Fig. 1A). Intact microbiota suspensions were extracted from diverse natural ecosystems, such as various soils and plant leaf surfaces (methods). Suspensions of microbiota from these environments were highly diverse and taxonomically rich (fig. S1), ranging between 110 and 1290 exact sequence variants (ESVs). We first inoculated 12 of these suspensions of microbiota into fresh minimal media with glucose as the only added carbon source and allowed the cultures to grow at 30°C in static broth. We then passaged the mixed cultures in fresh media every 48 hours with a fixed dilution factor of $D = 8 \times 10^{-3}$ for a total of 12 transfers (~84 generations). At the end of each growth cycle, we used 16*S* ribosomal RNA (rRNA) amplicon sequencing to assay the community composition (Fig. 1A and methods). High-resolution sequence denoising allowed us to identify ESVs, which revealed community structure at single-nucleotide resolution (*18*).

Most communities stabilized after ~60 generations, reaching stable population equilibria in nearly all cases (Fig. 1B and fig. S2). For all of the 12 initial ecosystems, we observed large multispecies communities after stabilization that ranged from 4 to 17 ESVs at a sequencing depth of 10,000 reads; further analysis indicated that this is a conservative estimate of the total richness in our communities (figs. S3 and S4 and methods). We confirmed the taxonomic assignments generated from amplicon sequencing by culture-dependent methods, including the isolation and phenotypic characterization of all dominant genera within a representative community (fig. S5).

Convergence of bacterial community structure at the family taxonomic level

High-throughput isolation and stabilization of microbial consortia allowed us to explore the rules governing the assembly of bacterial communities in well-controlled synthetic environments. At the species (ESV) level of taxonomic resolution, the 12 natural communities assembled into highly variable compositions (Fig. 1C). However, when we grouped ESVs by higher taxonomic ranks, we found that all 12 stabilized communities—with very diverse environmental origins—converged into similar family-level community structures dominated by Enterobacteriaceae and Pseudomonadaceae (Fig. 1D). In other words, a similar family-level composition arose in all communities despite their very different starting points. This is further illustrated in fig. S6, where we show that the temporal variability (quantified by the β diversity) in family-level composition is comparable to the variability across independent replicates. The same is not true when we compare taxonomic structure at the subfamily (genus) level.

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[1]Graduate Program in Bioinformatics and Biological Design Center, Boston University, Boston, MA 02215, USA. [2]The Rowland Institute at Harvard University, Cambridge, MA 02142, USA. [3]Department of Ecology and Evolutionary Biology, Microbial Sciences Institute, Yale University, New Haven, CT 06511, USA. [4]John A. Paulson School of Engineering and Applied Sciences, Harvard University, Cambridge, MA 02138, USA. [5]Department of Applied Physics, Stanford University, Stanford, CA 94305, USA. [6]Departments of Biology and Biomedical Engineering, Boston University, Boston, MA 02215, USA. [7]Department of Physics, Boston University, Boston, MA 02215, USA.
*These authors contributed equally to this work.
†Corresponding author. Email: alvaro.sanchez@yale.edu (A.S.); pankajm@bu.edu (P.M.)

Goldford *et al*., *Science* **361**, 469–474 (2018) 3 August 2018

1 of 6

Postfai 2017

PRL **118,** 028103 (2017) PHYSICAL REVIEW LETTERS week ending 13 JANUARY 2017

Metabolic Trade-Offs Promote Diversity in a Model Ecosystem

Anna Posfai,[1] Thibaud Taillefumier,[2] and Ned S. Wingreen[1,3]
[1]*Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, New Jersey 08544, USA*
[2]*Department of Mathematics and Department of Neuroscience, The University of Texas at Austin, Austin, Texas 78712, USA*
[3]*Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544, USA*

(Received 20 September 2016; published 12 January 2017)

In nature, a large number of species can coexist on a small number of shared resources; however, resource-competition models predict that the number of species in steady coexistence cannot exceed the number of resources. Motivated by recent studies of phytoplankton, we introduce trade-offs into a resource-competition model and find that an unlimited number of species can coexist. Our model spontaneously reproduces several notable features of natural ecosystems, including keystone species and population dynamics and abundances characteristic of neutral theory, despite an underlying non-neutral competition for resources.

DOI: 10.1103/PhysRevLett.118.028103

An astonishing characteristic of life on Earth is its great variety. In tropical rainforests, more than 300 tree species may be found on a single hectare [1], while in one gram of soil, the number of distinct microbial genomes has been estimated at ~2000–18, 000 [2]. Explaining this great biodiversity has been a main focus of research in ecology. One major conceptual challenge is embodied by the so-called "paradox of the plankton" [3]: in the framework of simple resource-competition models, it has been argued that the number of species indefinitely coexisting cannot exceed the number of resources [4–7]. Yet, in apparent contradiction to this theory, which is known as the competitive exclusion principle [8], some marine ecosystems host a hundred or more coexisting species of phytoplankton [9], competing for only a handful of abiotic nutrients [10].

The limit on diversity set by the competitive exclusion principle could be overcome in many possible ways. Even within simple resource-competition models, diverse populations may emerge from intrinsically oscillatory or chaotic dynamics [11,12], though the stability of such solutions in the face of long-term evolution has been challenged [13]. Looking beyond resource competition, there are many proposed mechanisms for diversity, generally falling into three (nonexclusive) categories: (1) systems never approach steady state due to temporal variation of the environment, e.g., weather changes [3,14] or seasonal cycles [15]; (2) real environments are heterogeneous in space, e.g., due to environmental gradients such as temperature, salinity, or exposure to light [16]; (3) ecosystems are limited by factors other than resources, e.g., predation [17,18] or self-limiting toxin production [19]. (For reviews see [20,21].)

While the above mechanisms are likely all broadly relevant, in the context of phytoplankton, it was recently suggested that diversity may also persist due to trade-offs between different traits or abilities [22]. With this in mind, we present a simple resource-competition model in which species are constrained by a trade-off between their different resource utilization abilities. In this model, organisms collectively shape the resource concentrations around them to produce a state equally favorable for all, and hence, an unlimited number of species can coexist. While the model is highly simplified, it highlights how both trade-offs and environmental shaping can contribute to ecological diversity.

We employ a classical resource-competition model [23] to investigate the population dynamics of m species competing for p types of nutrients. A "species" σ is specified by its metabolic strategy, namely the coefficients of its rate of utilization of each nutrient: $\vec{\alpha}_\sigma = (\alpha_{\sigma 1}, \ldots, \alpha_{\sigma p})$. Conceptually, $\alpha_{\sigma i}$ is proportional to the number of enzyme molecules allocated by the organism to importing and processing nutrient i. We assume that enzymes for different nutrients may have different costs w_i, but to reflect "trade-offs," all organisms have the same fixed enzyme budget: $\sum_{i=1}^{p} w_i \alpha_{\sigma i} = E$.

We further assume a well-mixed system such that the concentration of nutrients is homogeneous and is determined by the nutrient supply rates $\vec{s} = (s_1, \ldots, s_p)$, by the uptake of nutrients by organisms, and by a degradation or loss rate μ_i. We denote the per-enzyme rate of consumption of nutrient i by r_i. A relevant choice for r_i is the Monod function $c_i/(K_i + c_i)$, but it can be any monotone increasing, continuously differentiable function of c_i with $r_i(0) = 0$. The kinetics of nutrient concentration c_i is therefore given by

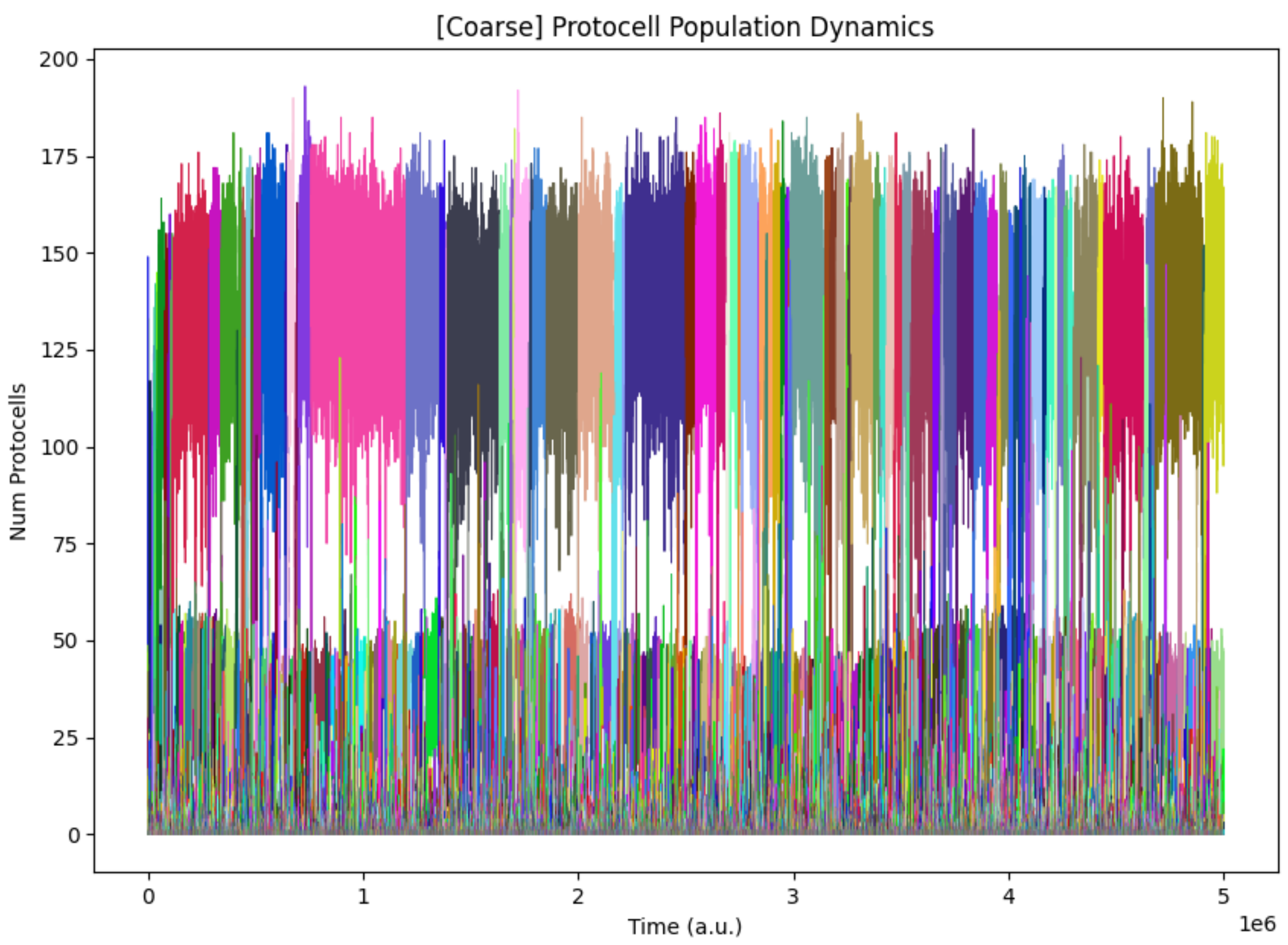
$$\frac{dc_i}{dt} = s_i - \left(\sum_\sigma n_\sigma(t)\alpha_{\sigma i}\right) r_i(c_i) - \mu_i c_i(t), \qquad (1)$$

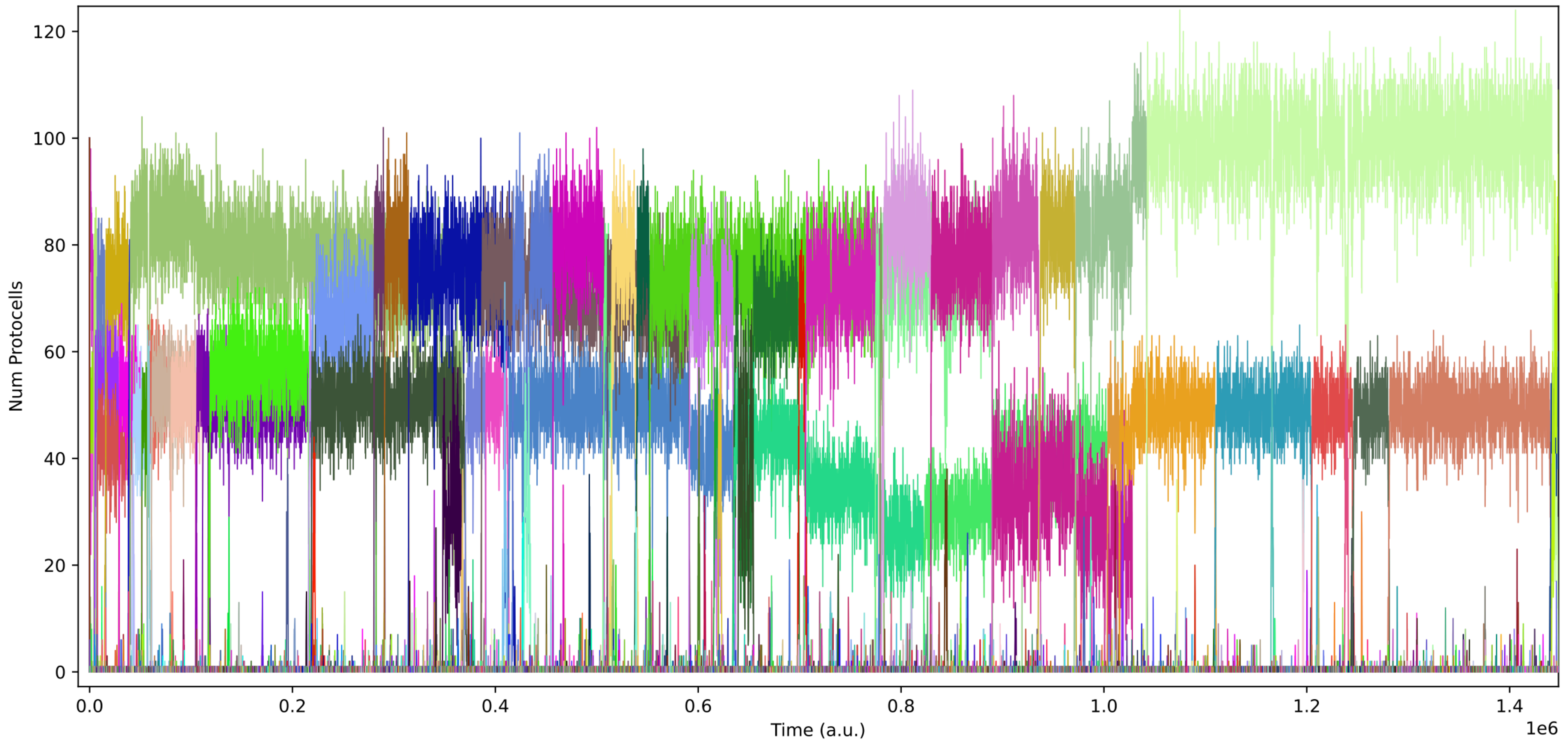
where n_σ is the population of species σ. Since metabolic reactions typically occur on a faster time scale than cell division, we assume a separation of these time scales. It

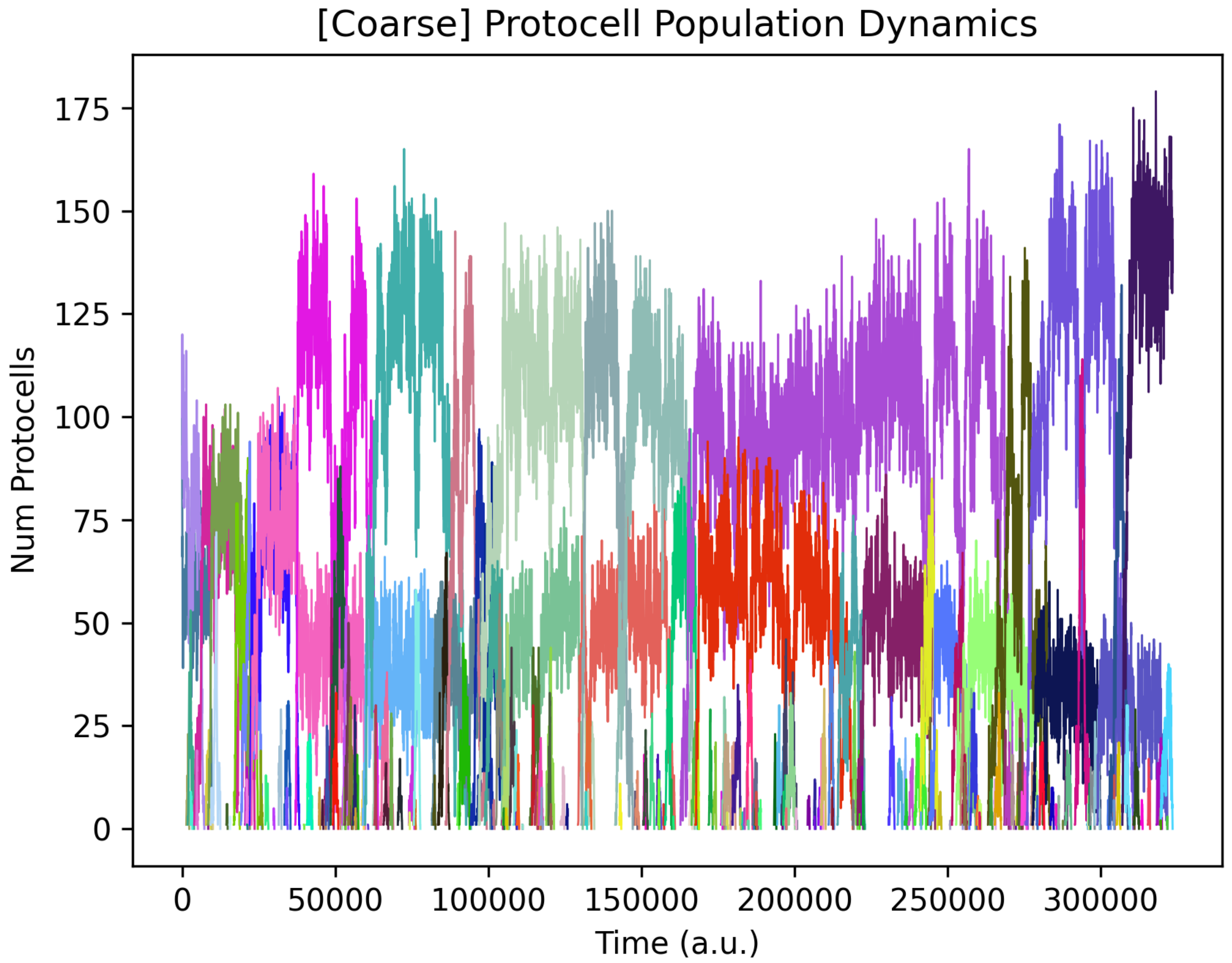
0031-9007/17/118(2)/028103(5)

+ Evolution + Regulation + Stochasticity!

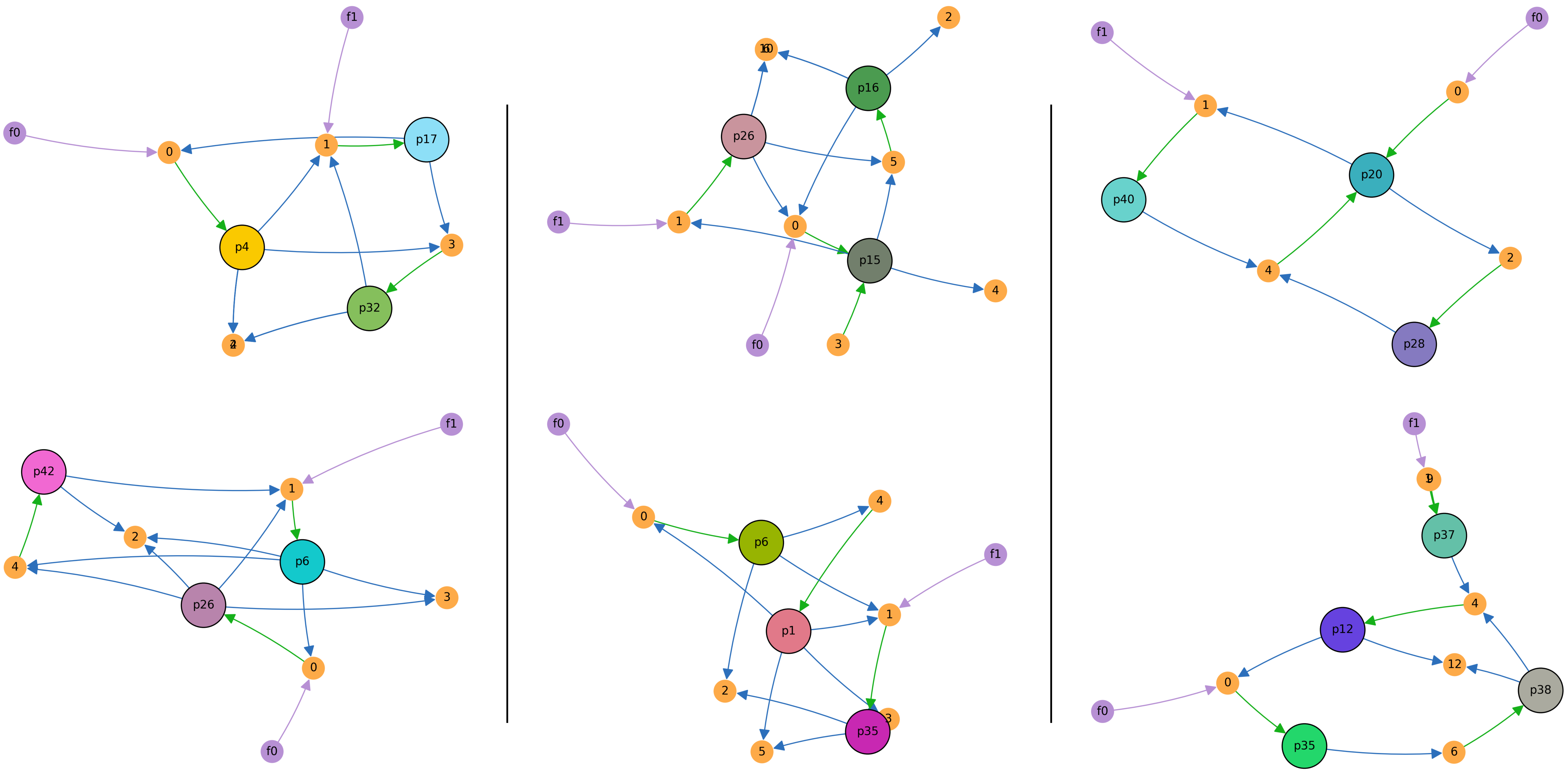
Protocell Population Dynamics and Evolutionary Lineage



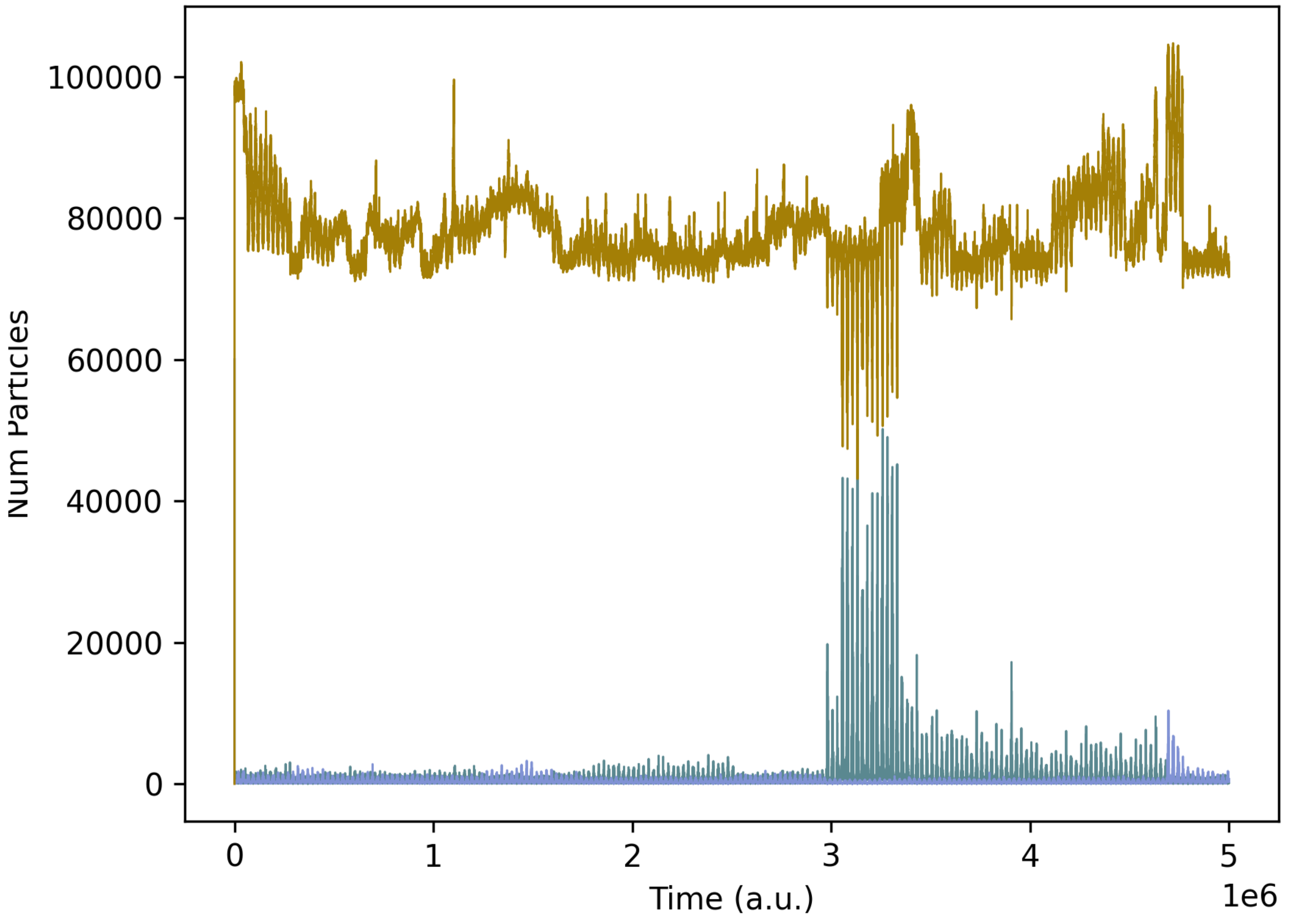


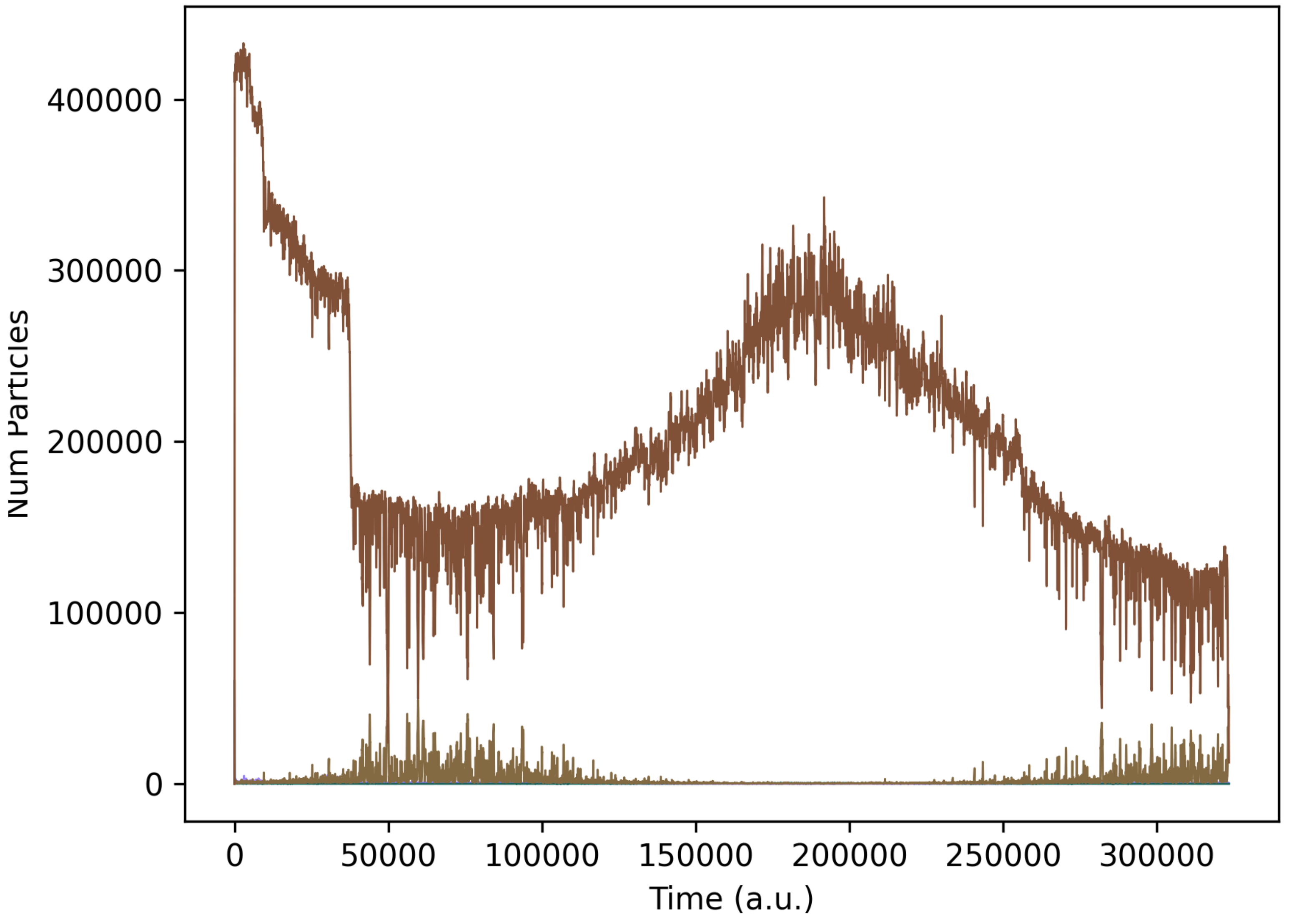


Example Cross-Feeding Ecological Arrangements



Chemical Dynamics in Chemostat


[Coarse] Reactor Chemical Dynamics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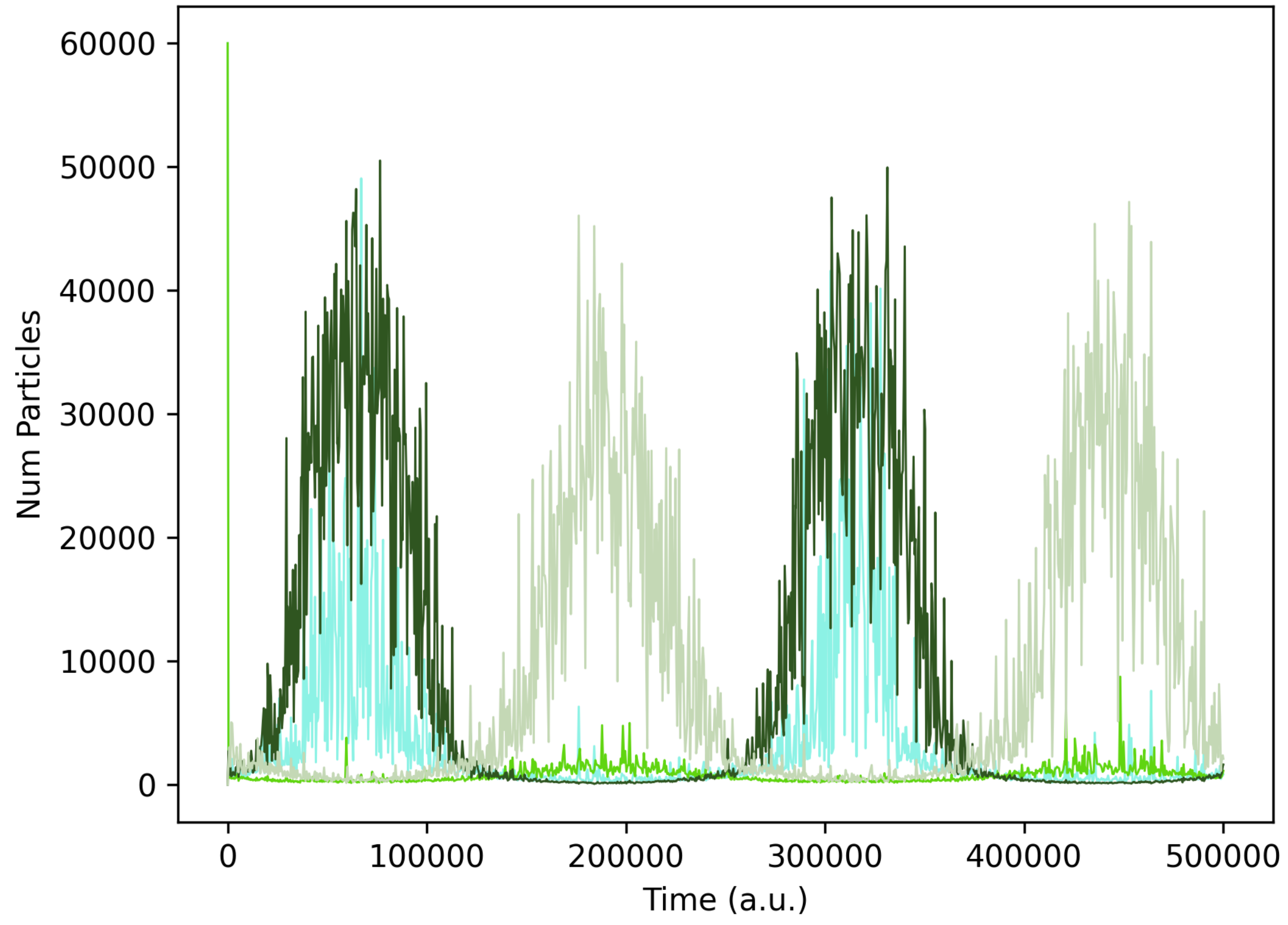
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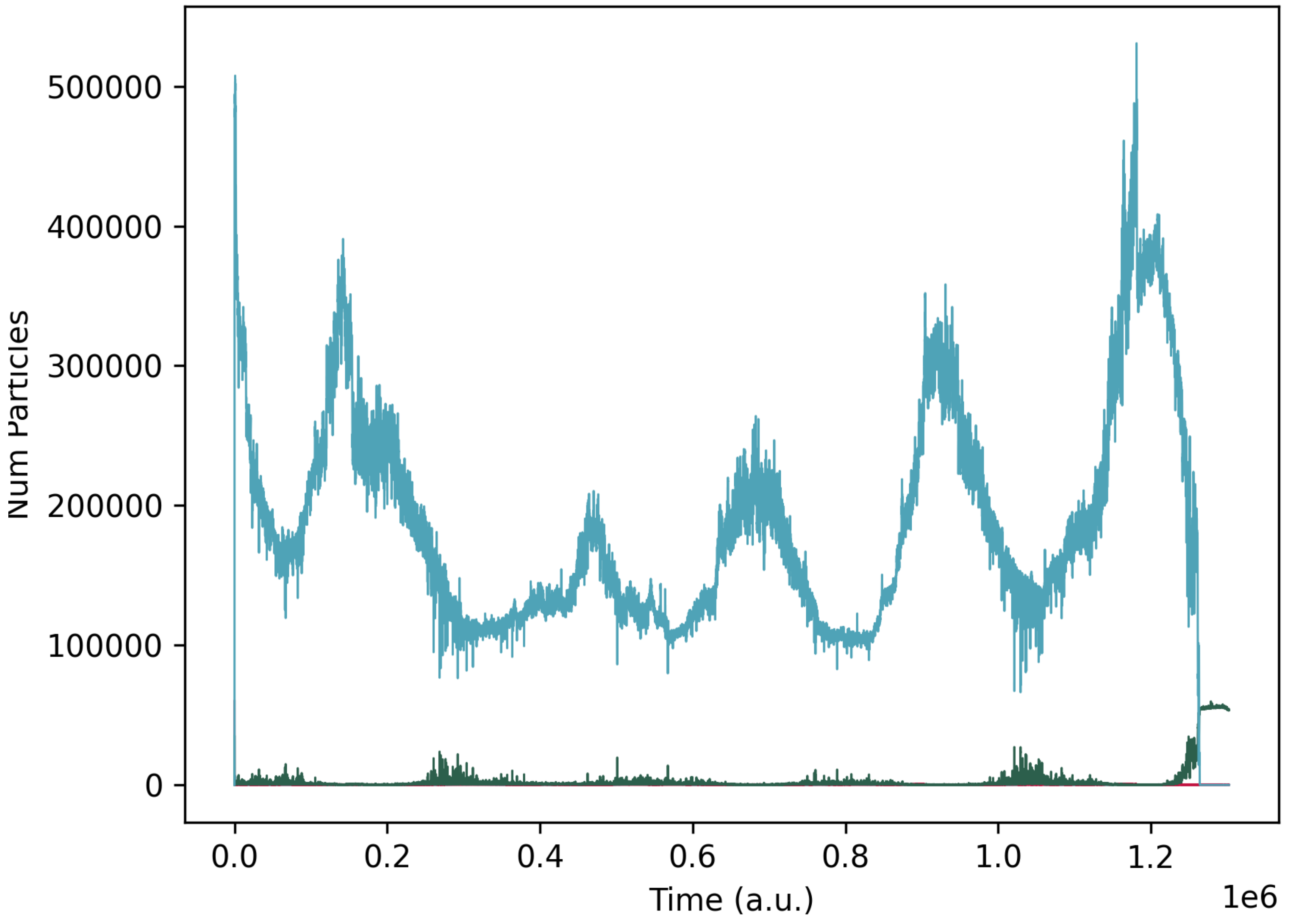
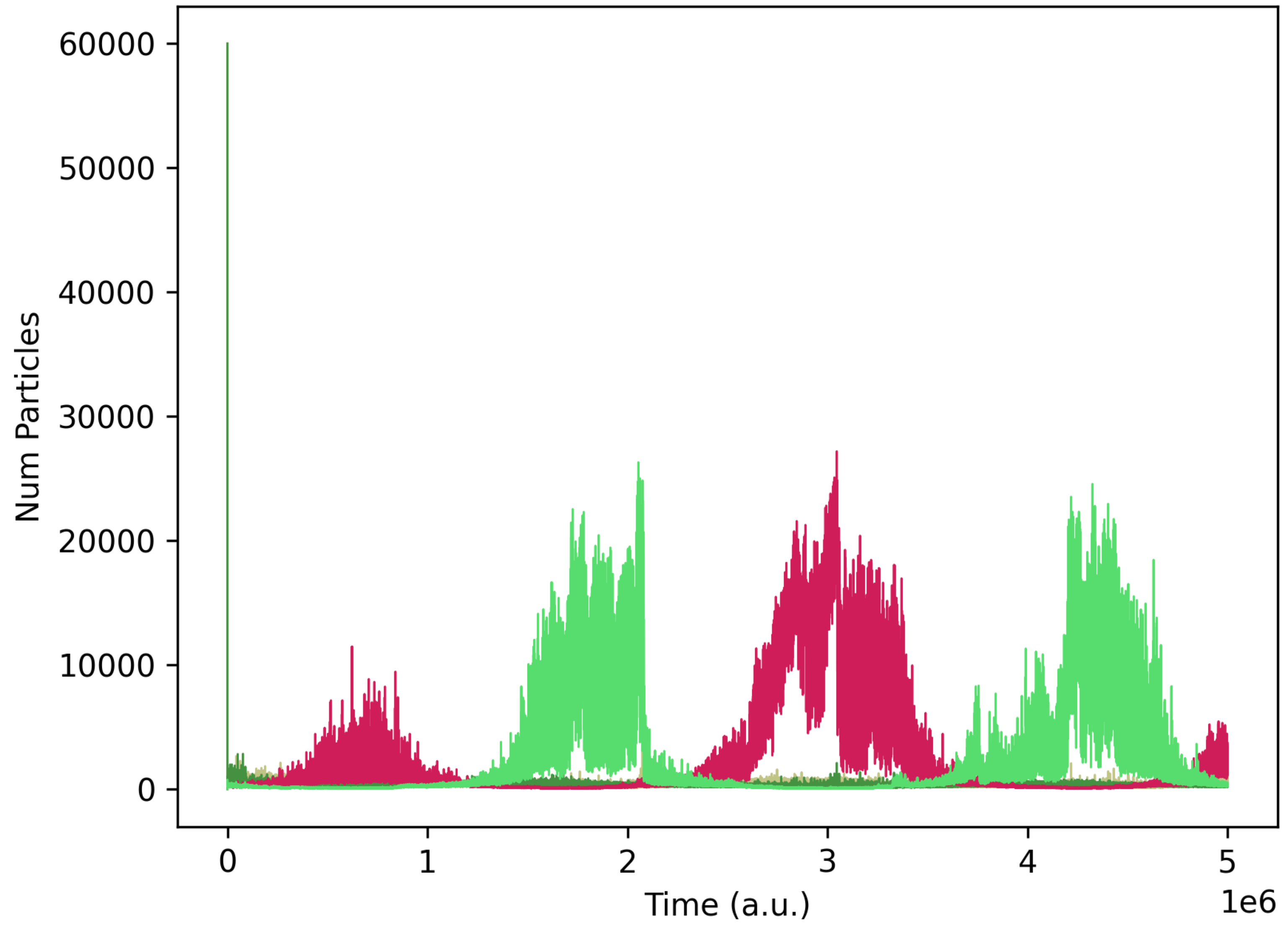
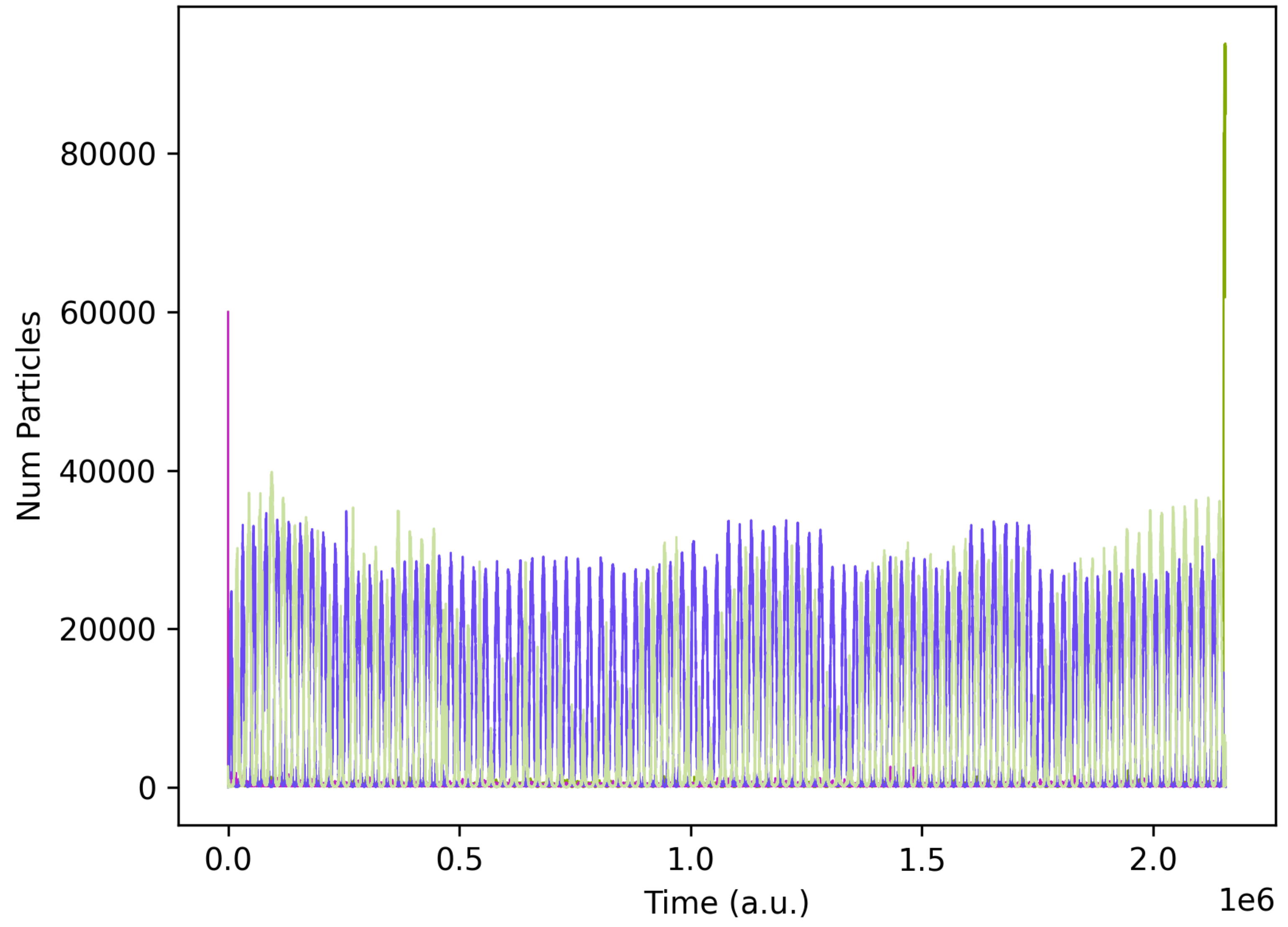
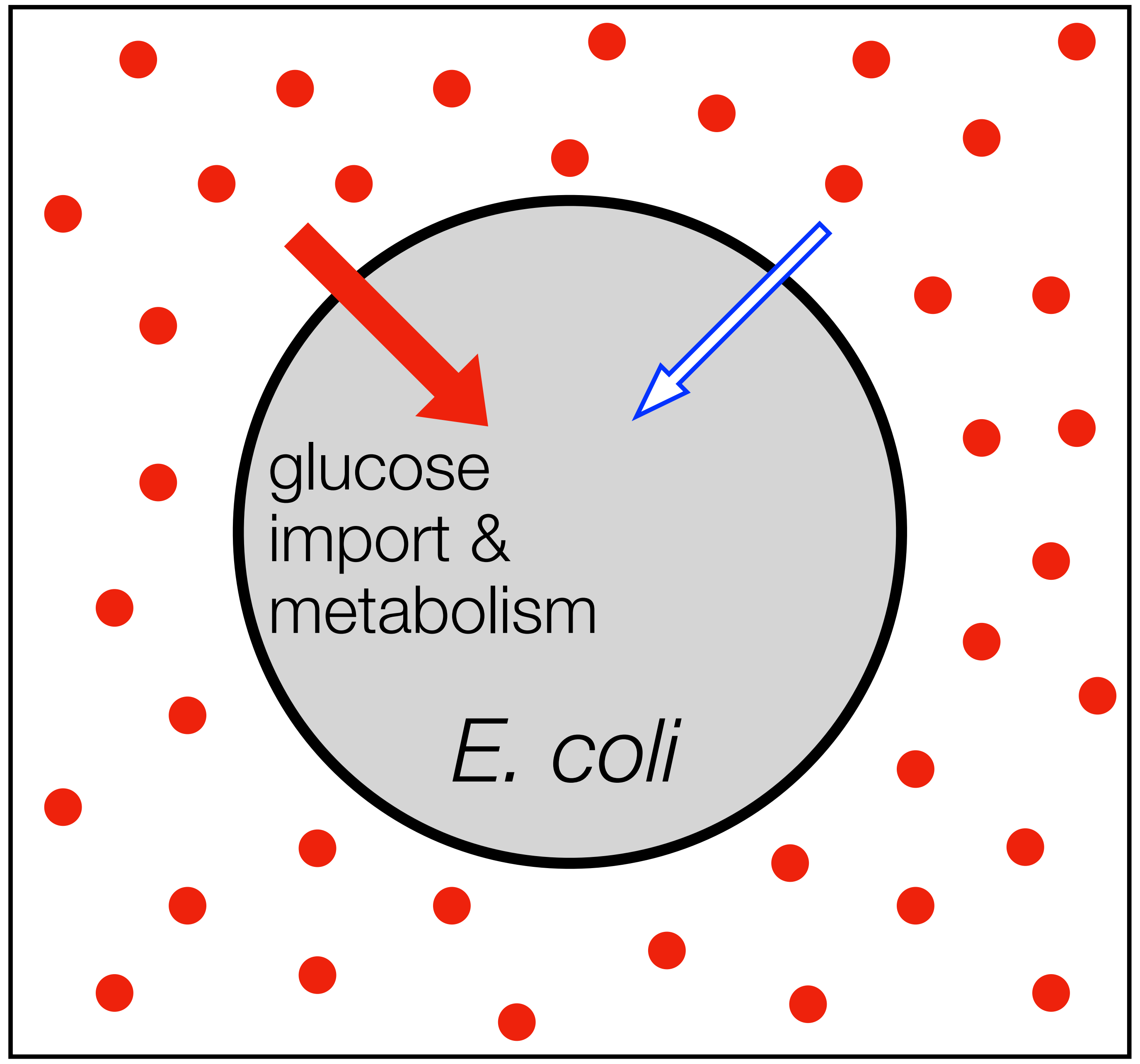

[Coarse] Reactor Chemical Dynamics


[Coarse] Reactor Chemical Dynamics


[Coarse] Reactor Chemical Dynamics

Results

Can Rudimentary lac Operon Behaviour Evolve?



- **Glucose** present
- lac operon **off** (not expressed)
- E. coli metabolises **glucose** (preferred energy source)

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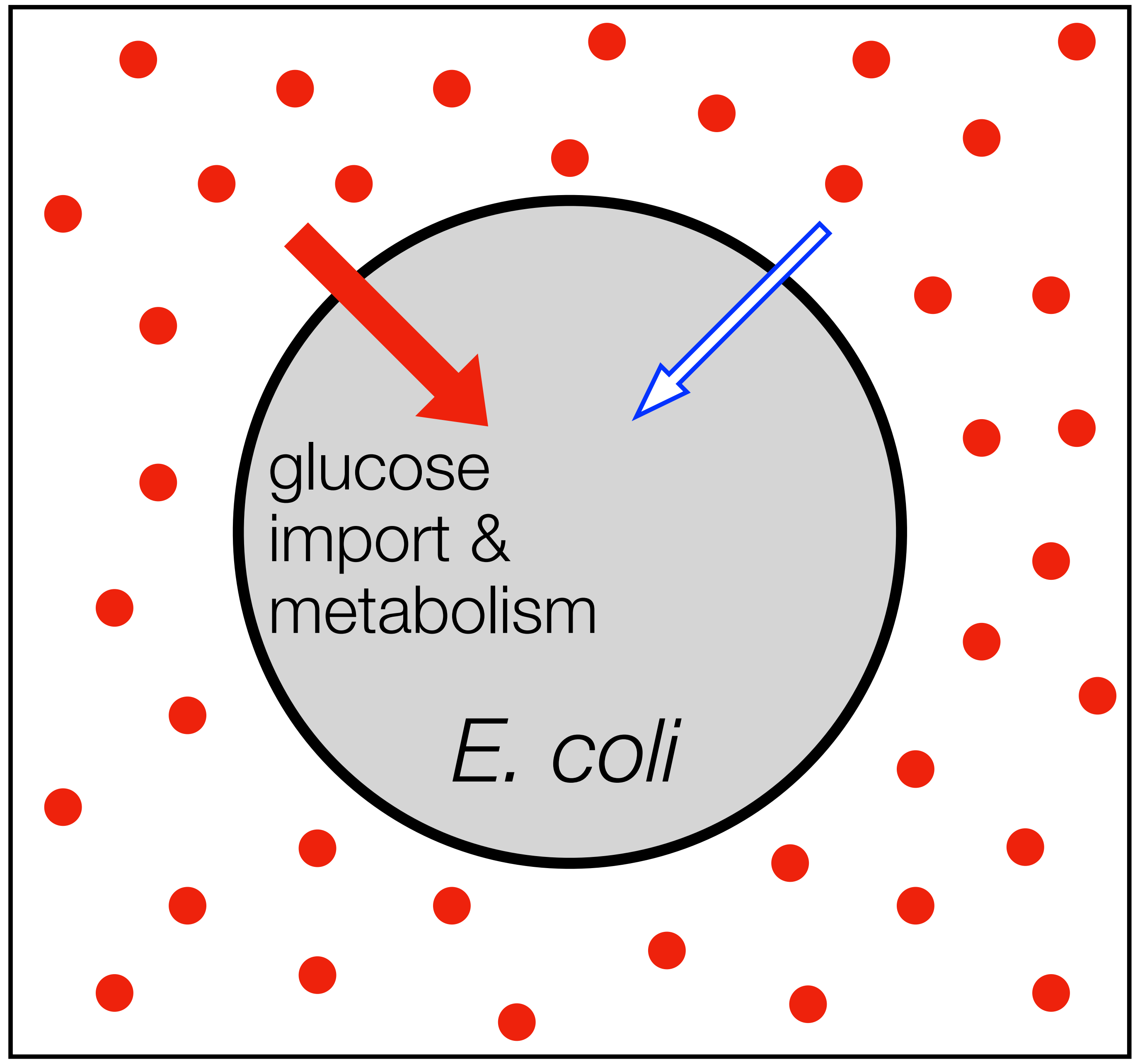

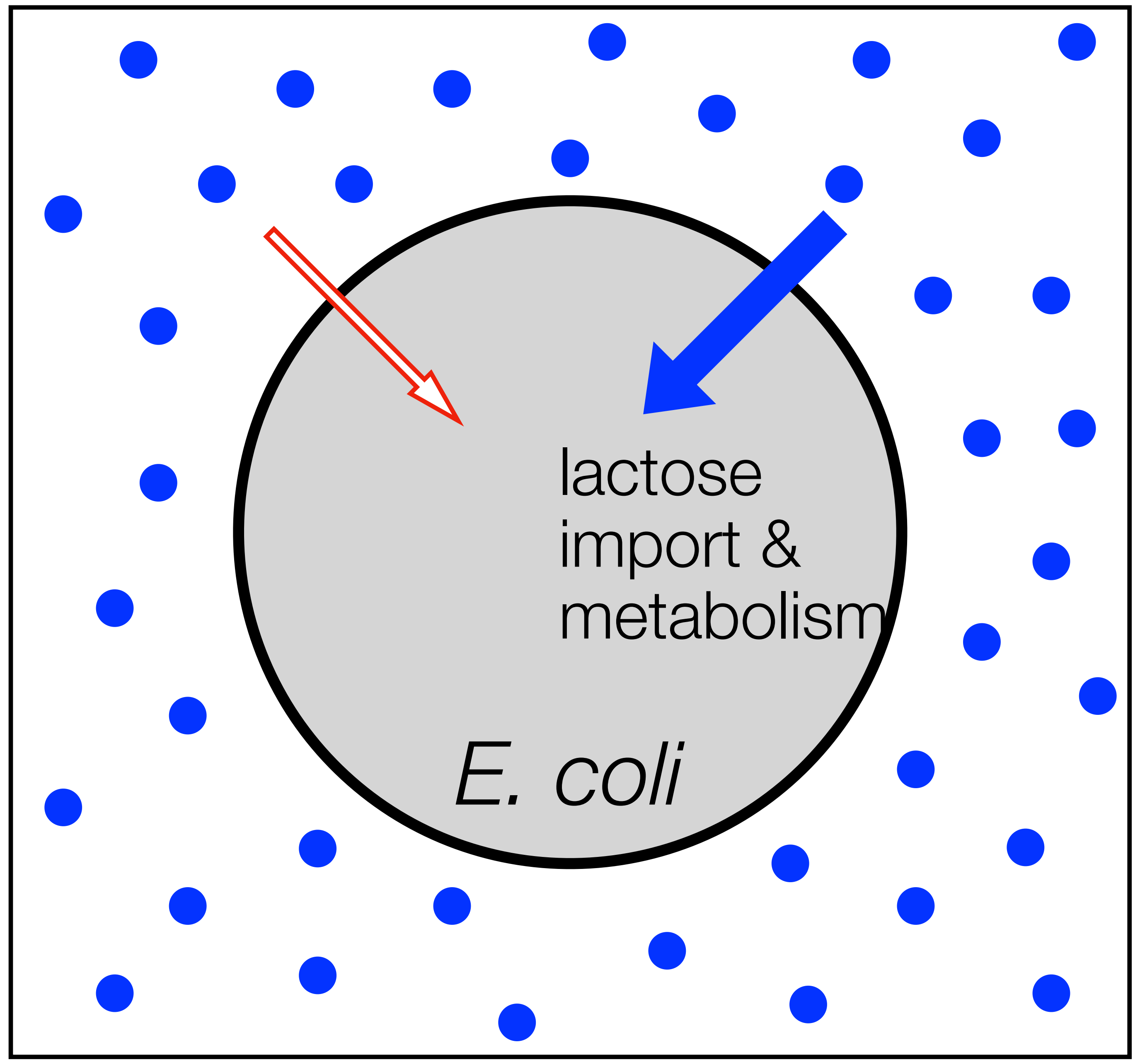

- **Glucose** present
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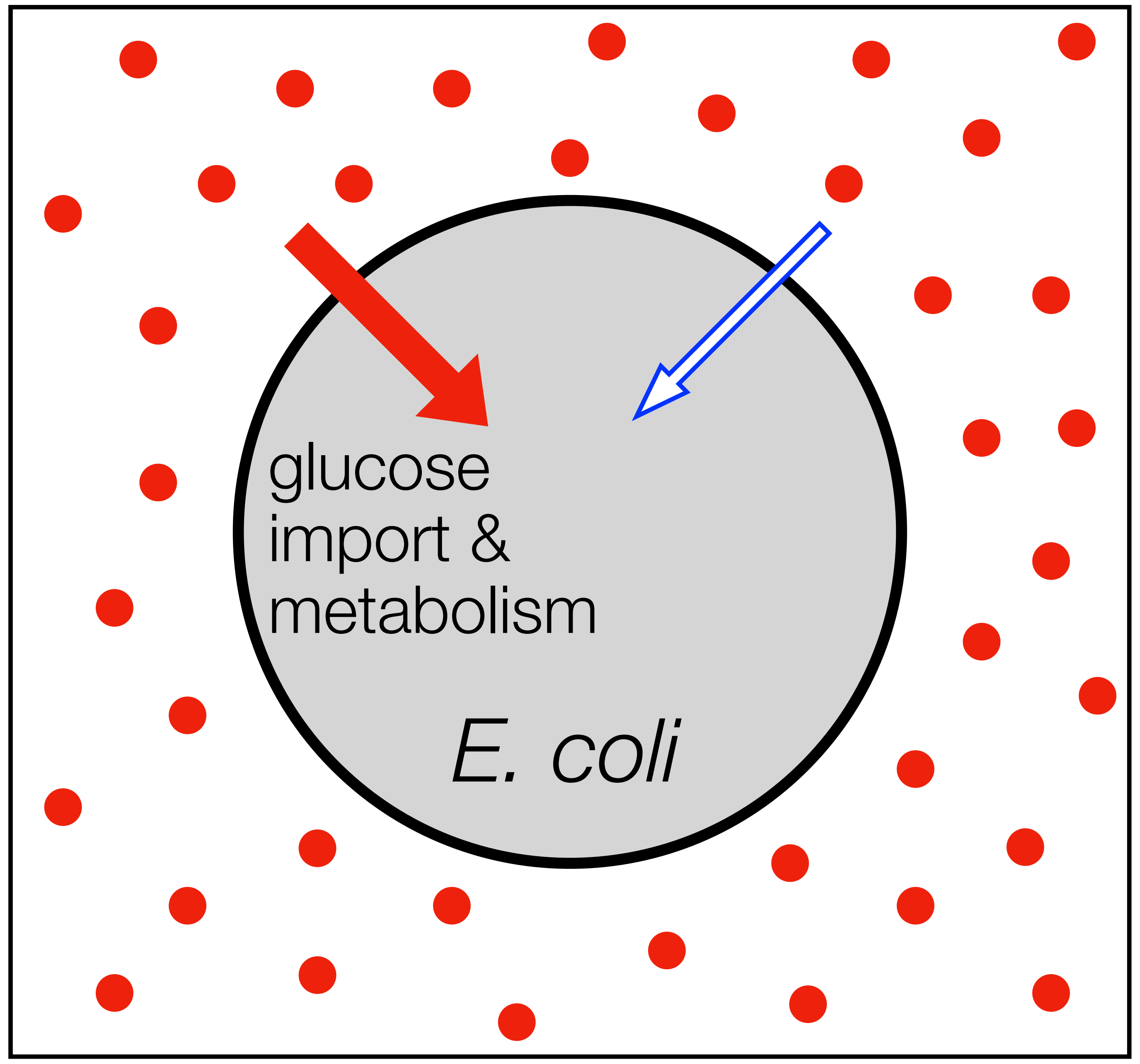
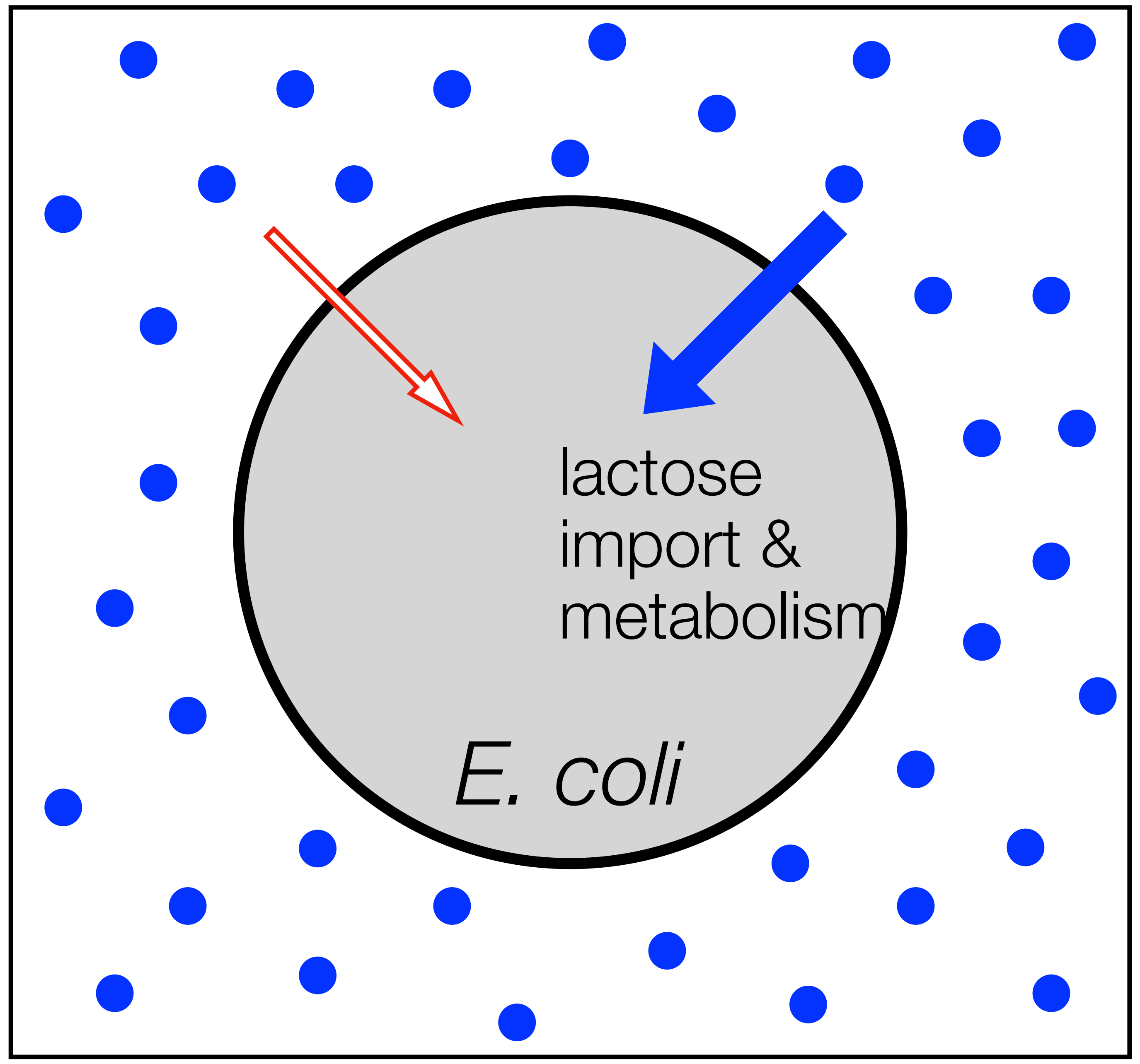
- E. coli metabolises **glucose** (preferred energy source)

- **Lactose** present, glucose absent
- lac operon **on** (expressed)
- E. coli metabolises **lactose** instead

Can Rudimentary lac Operon Behaviour Evolve?





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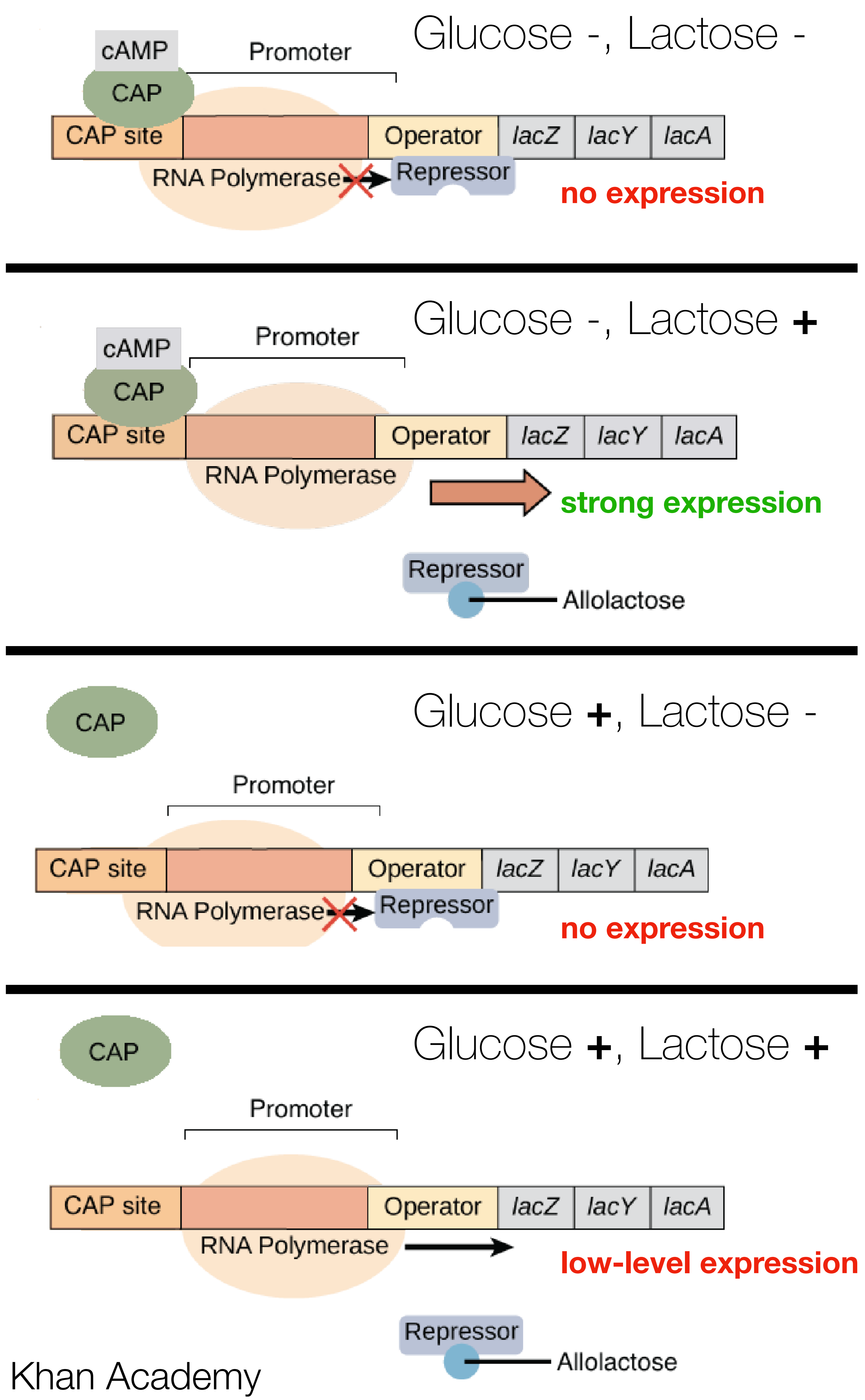
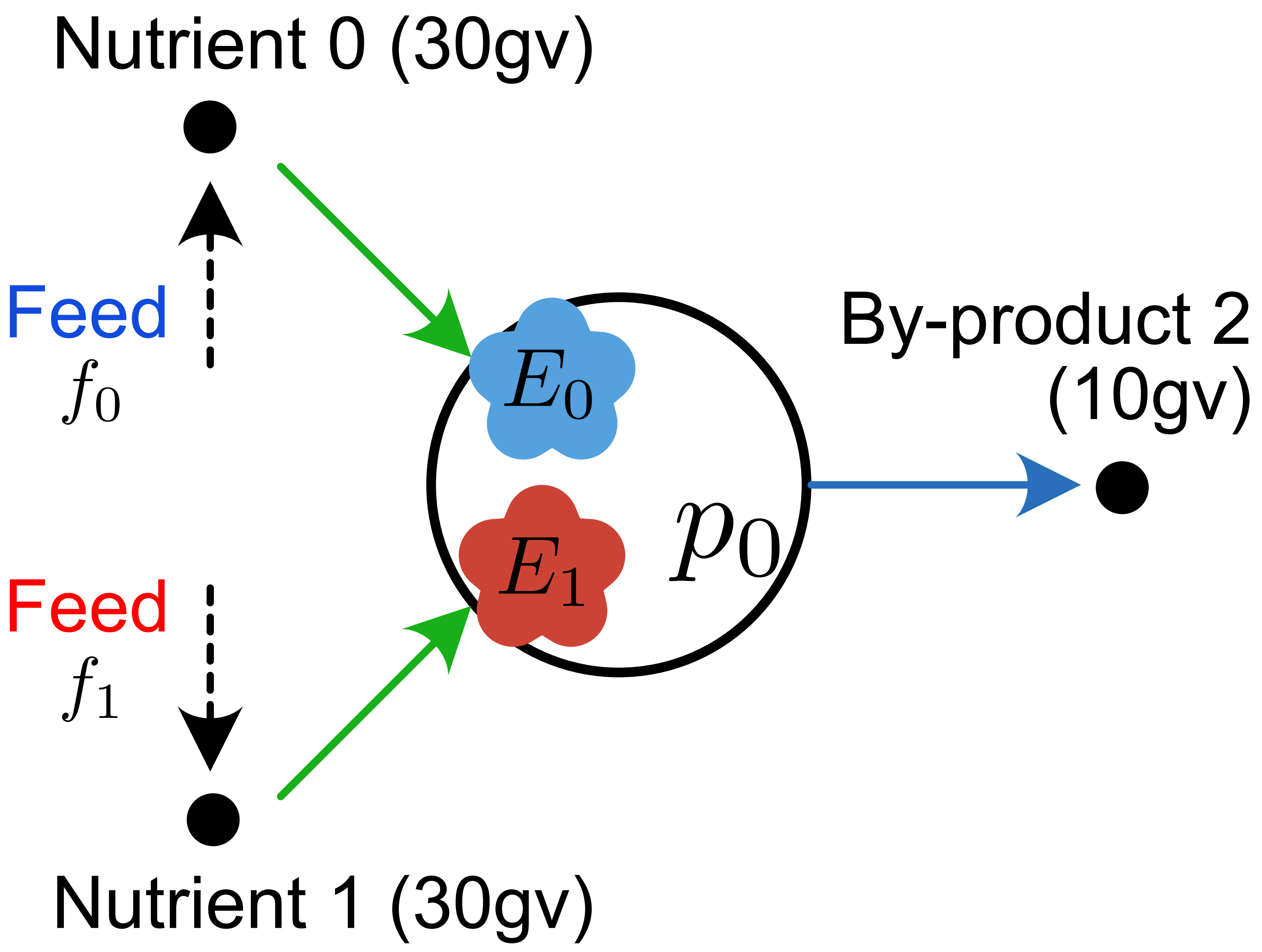


Khan Academy

Case Study 1: Single Protocell Species



Nutrient 0 (30gv)

Feed f_0

E_0

E_1

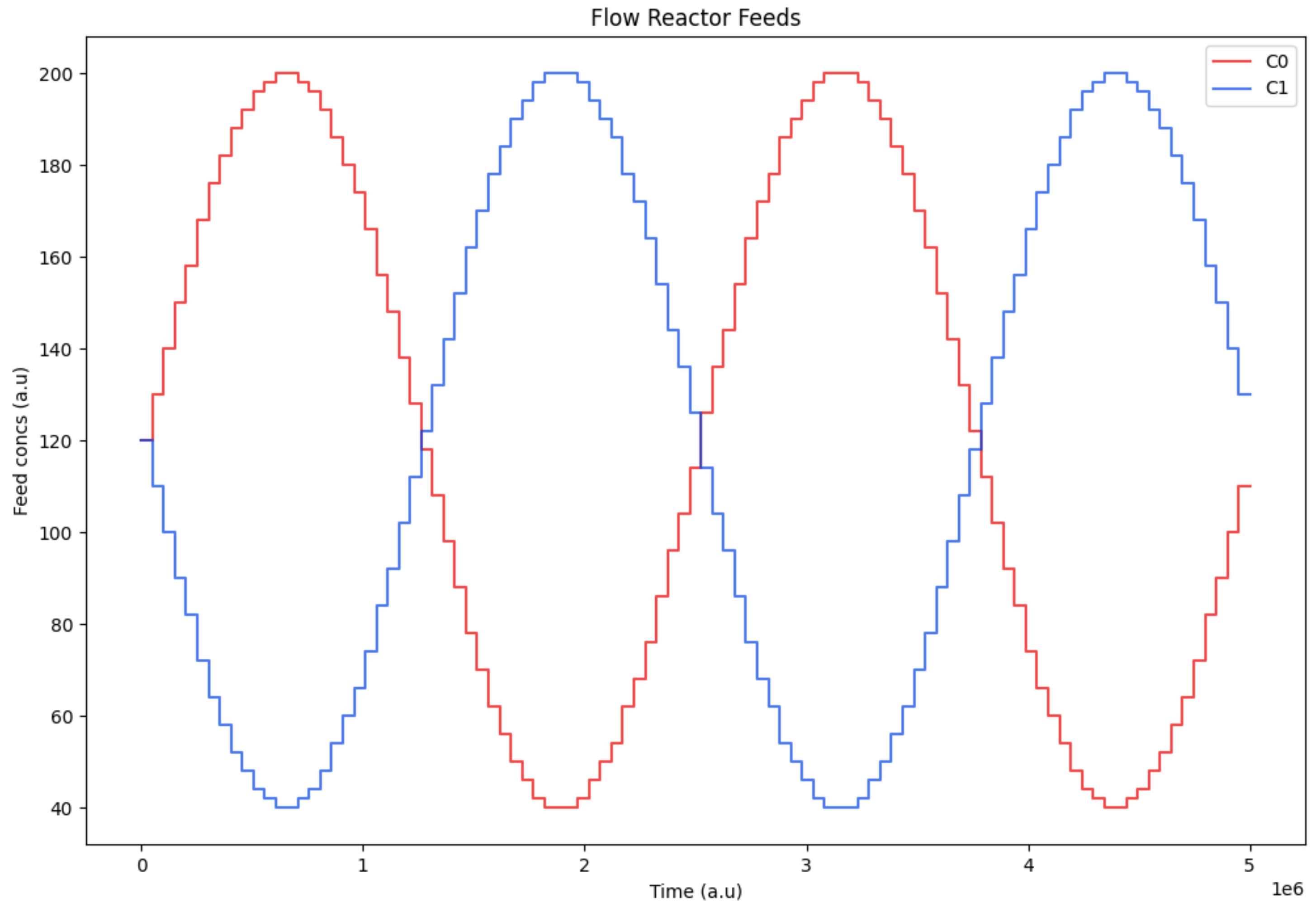
p_0

By-product 2 (10gv)

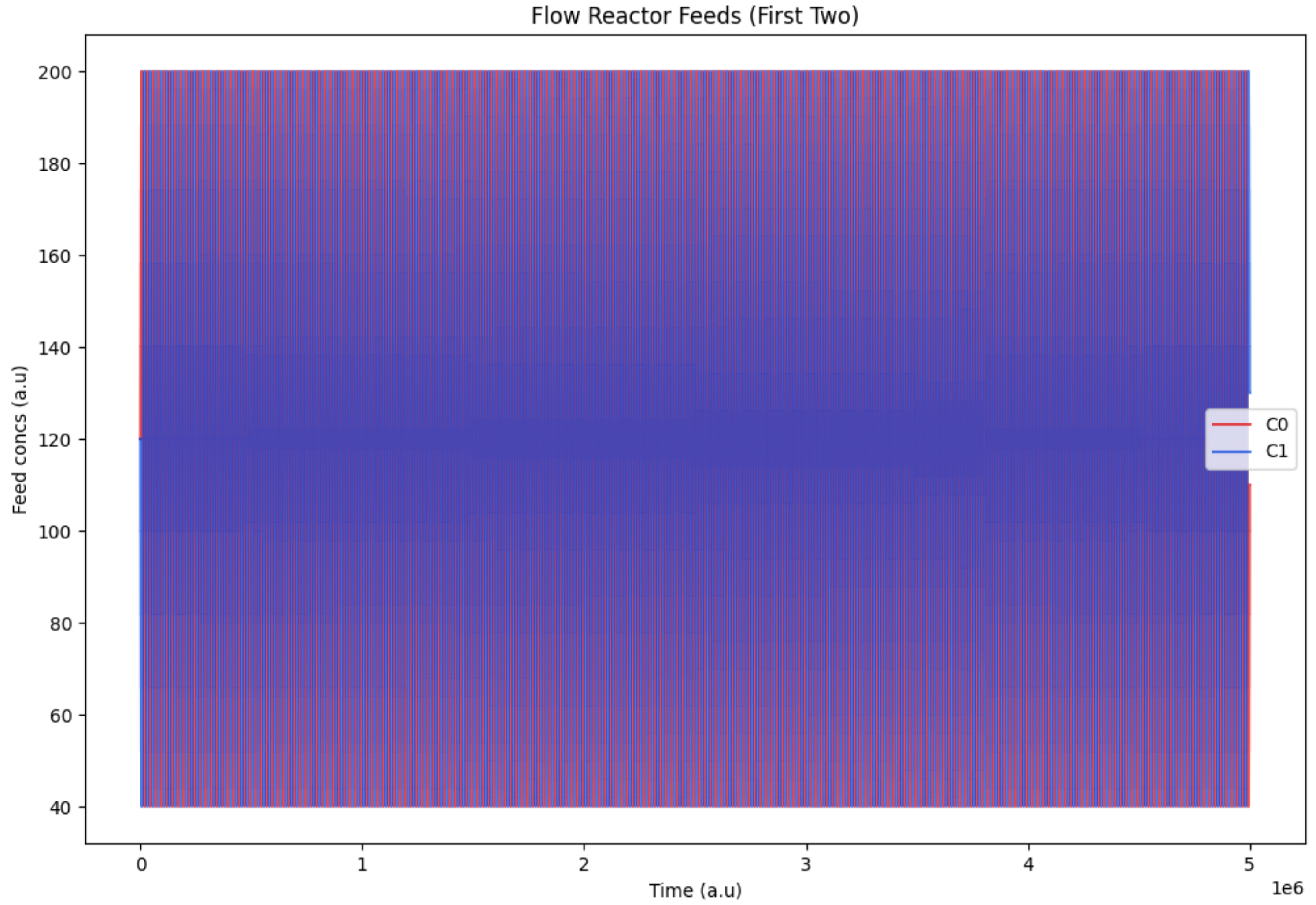
Feed f_1

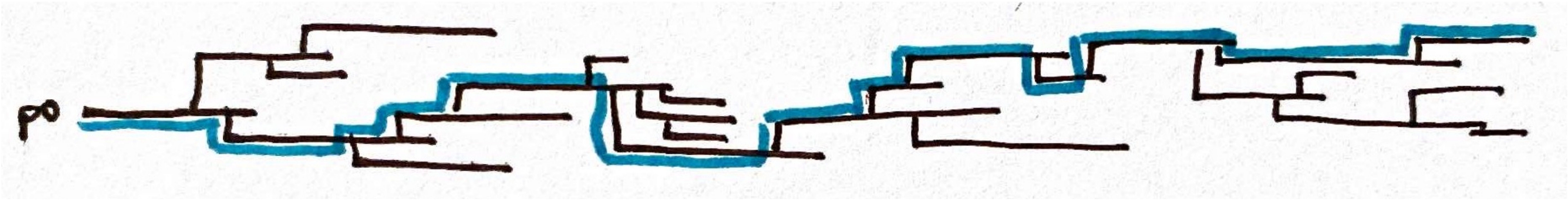
Nutrient 1 (30gv)

Slow Nutrient Changes



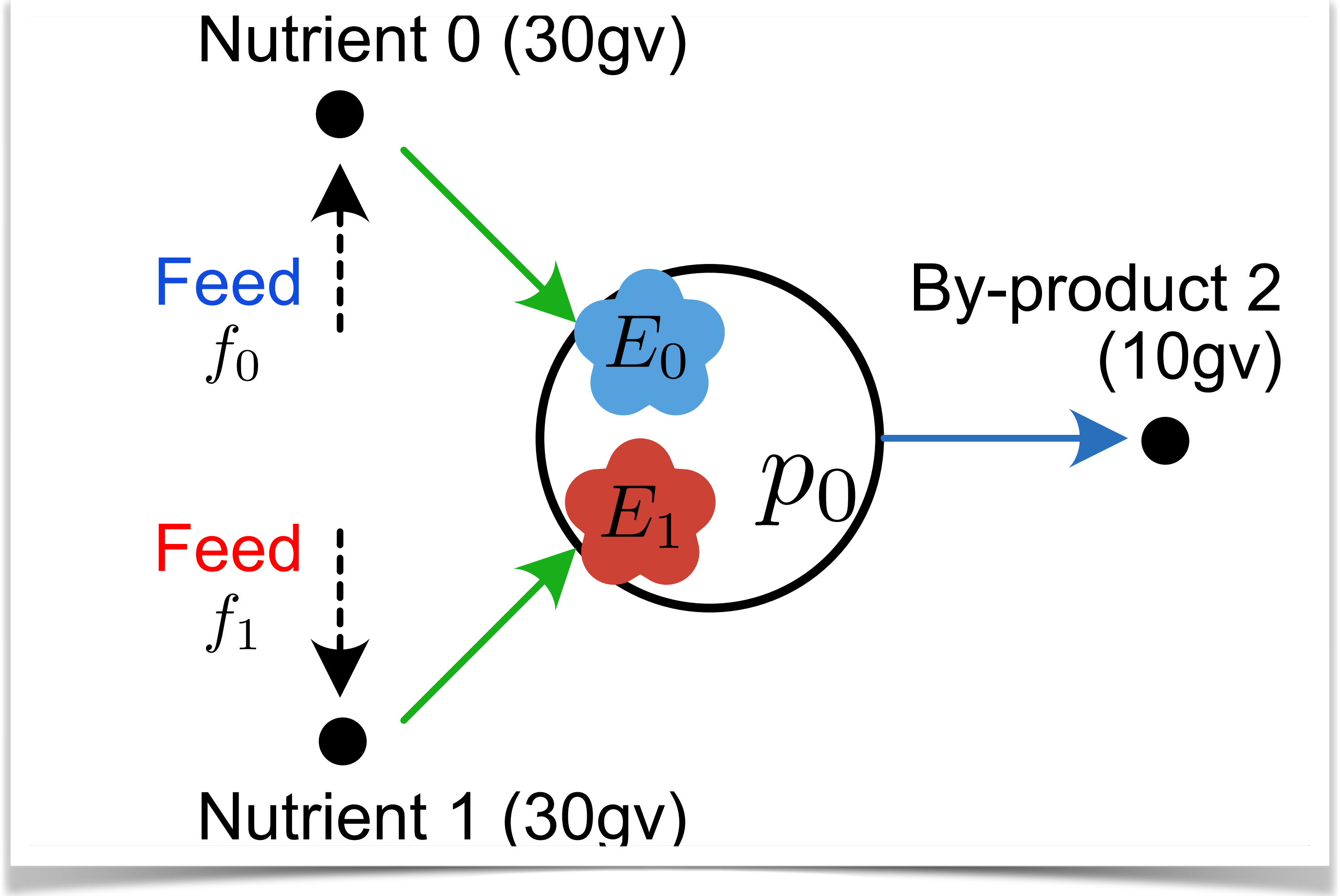
Fast Nutrient Changes





- We track enzyme dynamics across the "trunk" of the evolutionary lineage

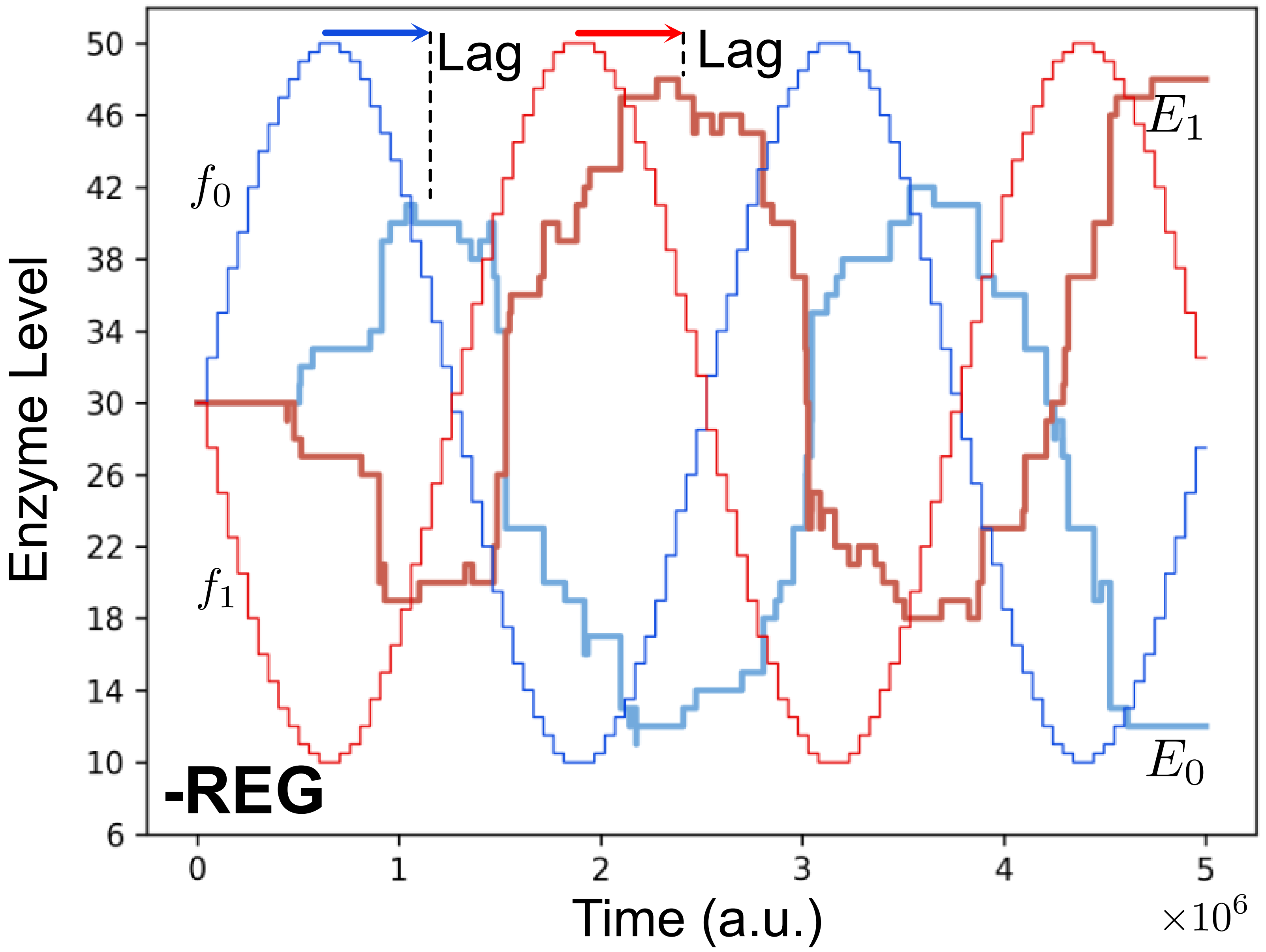
Case Study 1: Single Protocell Species



Slow Nutrient Forcing

Phylogenetic Adaptation Only

-REG



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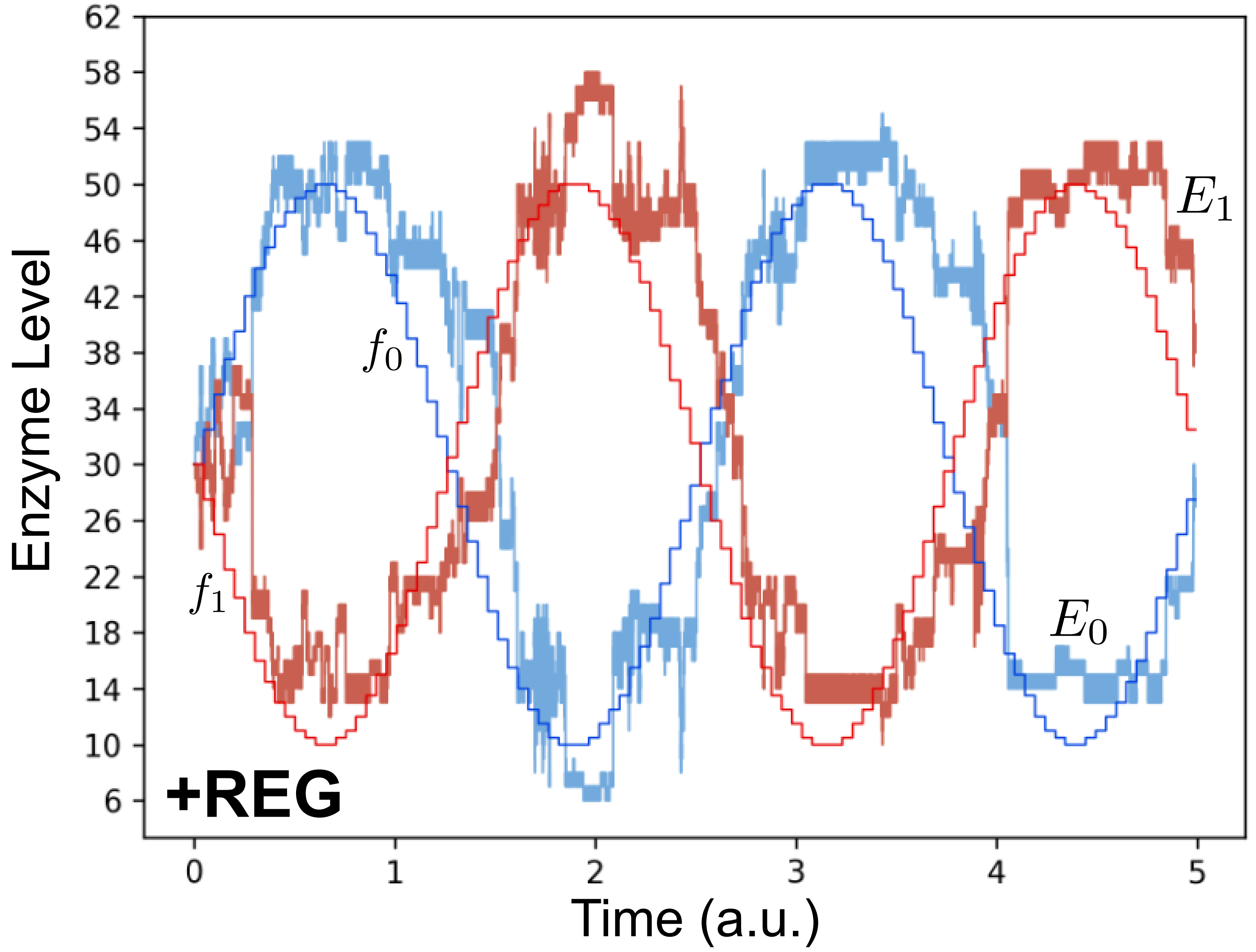
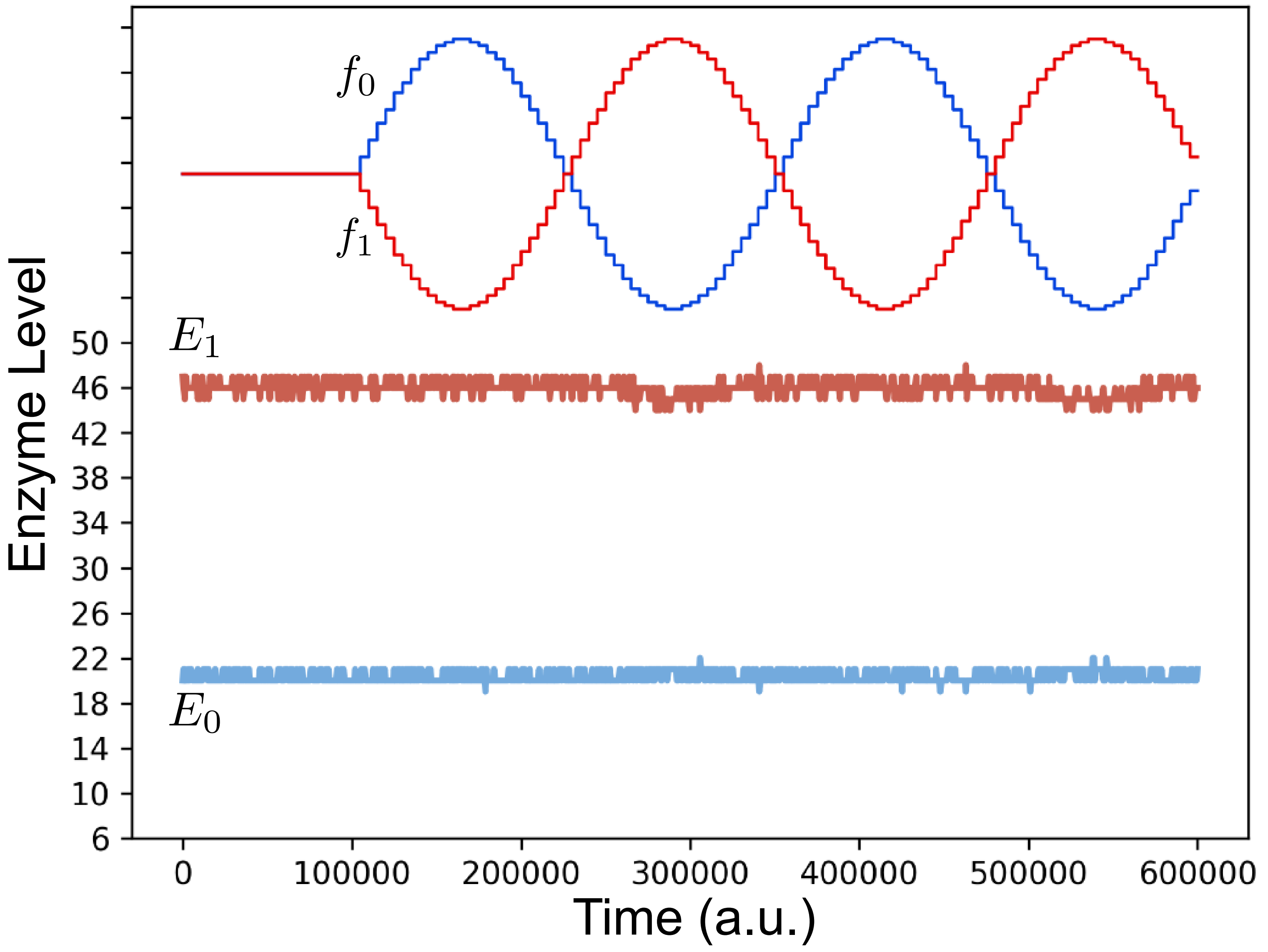
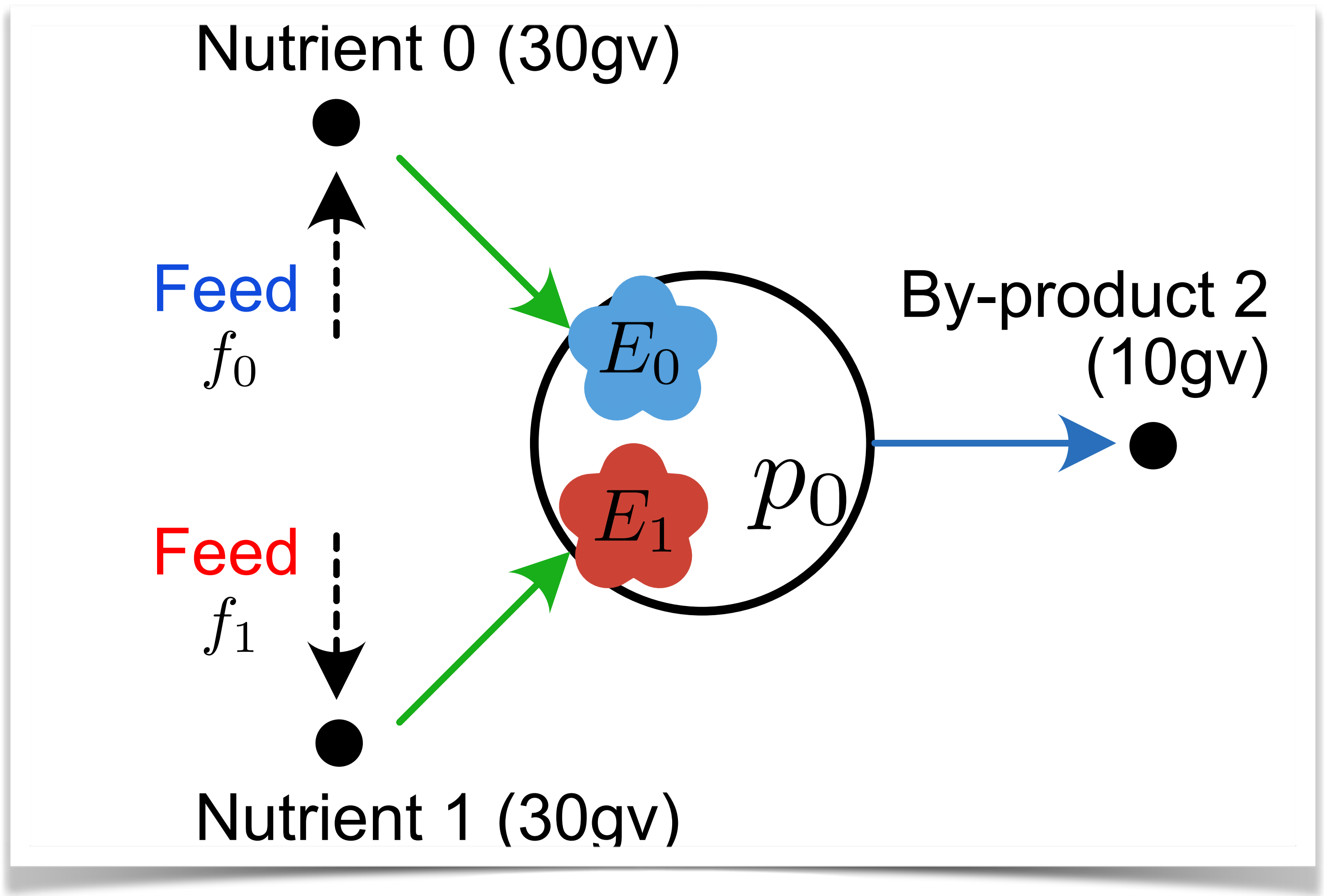


Reg Net Functional? (No)

Protocell variant @ t = 4.9$\times 10^6$ under slow forcing



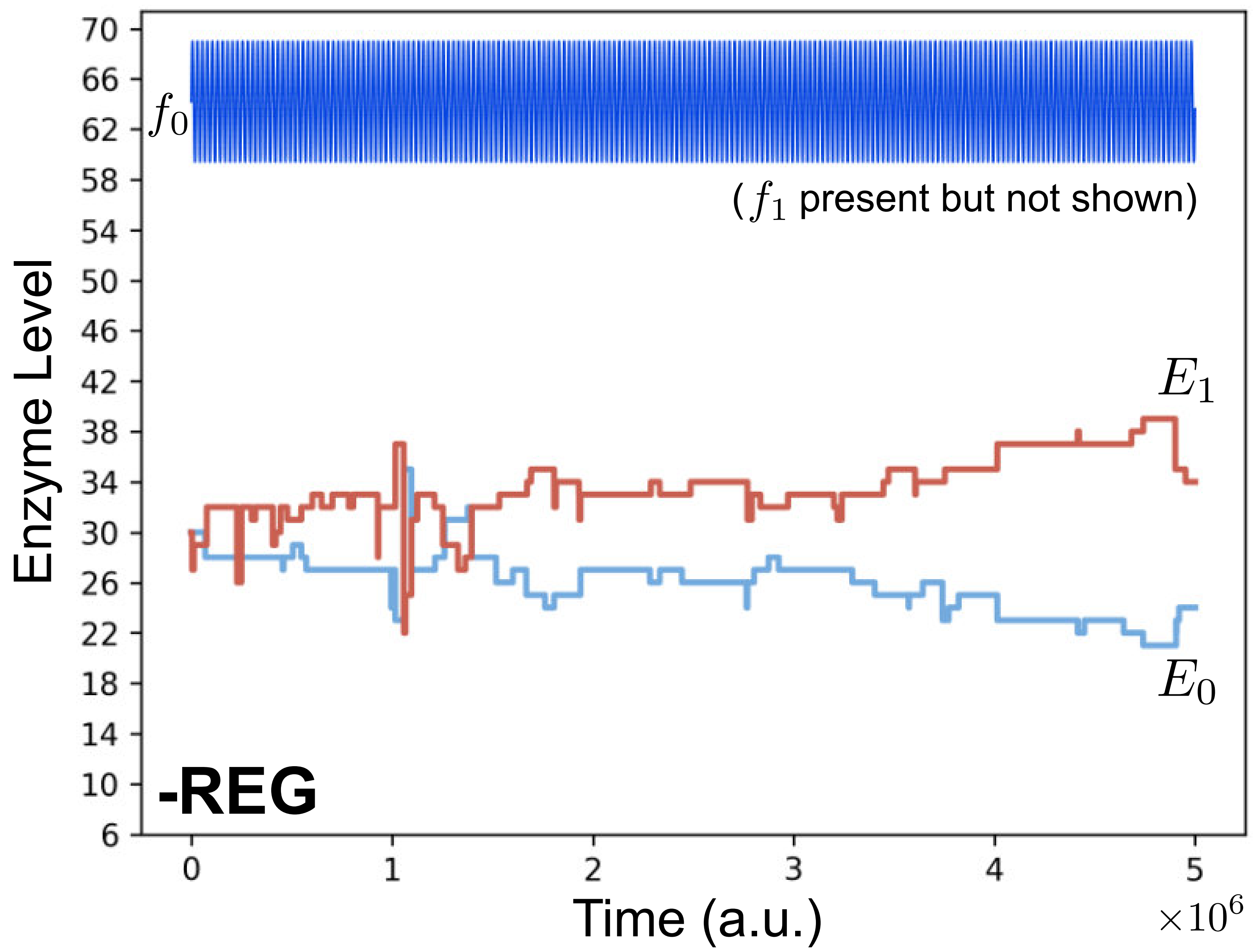
Case Study 1: Single Protocell Species



Fast Nutrient Forcing

Emergence of Bursts of Functional Regulation

-REG



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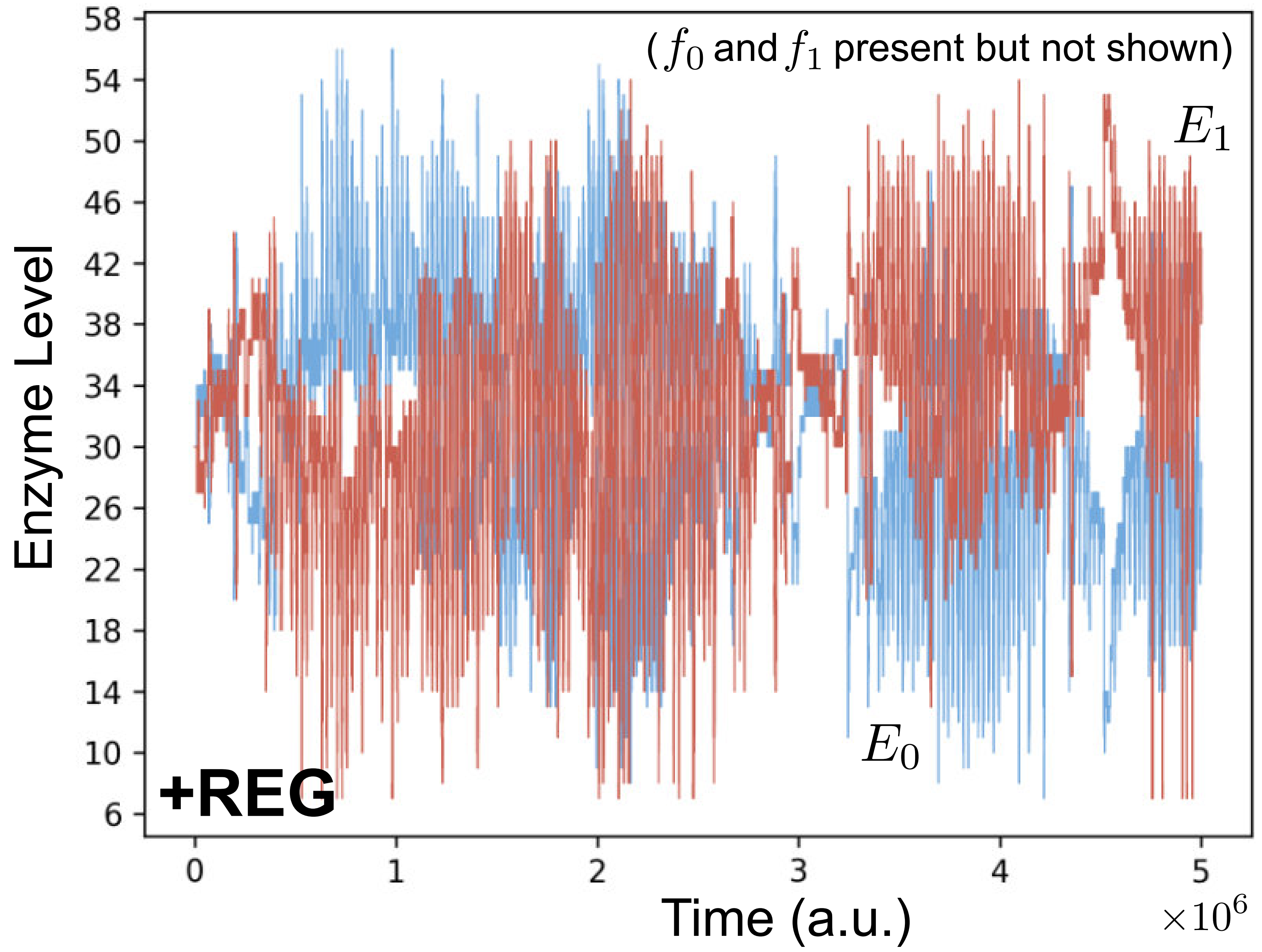
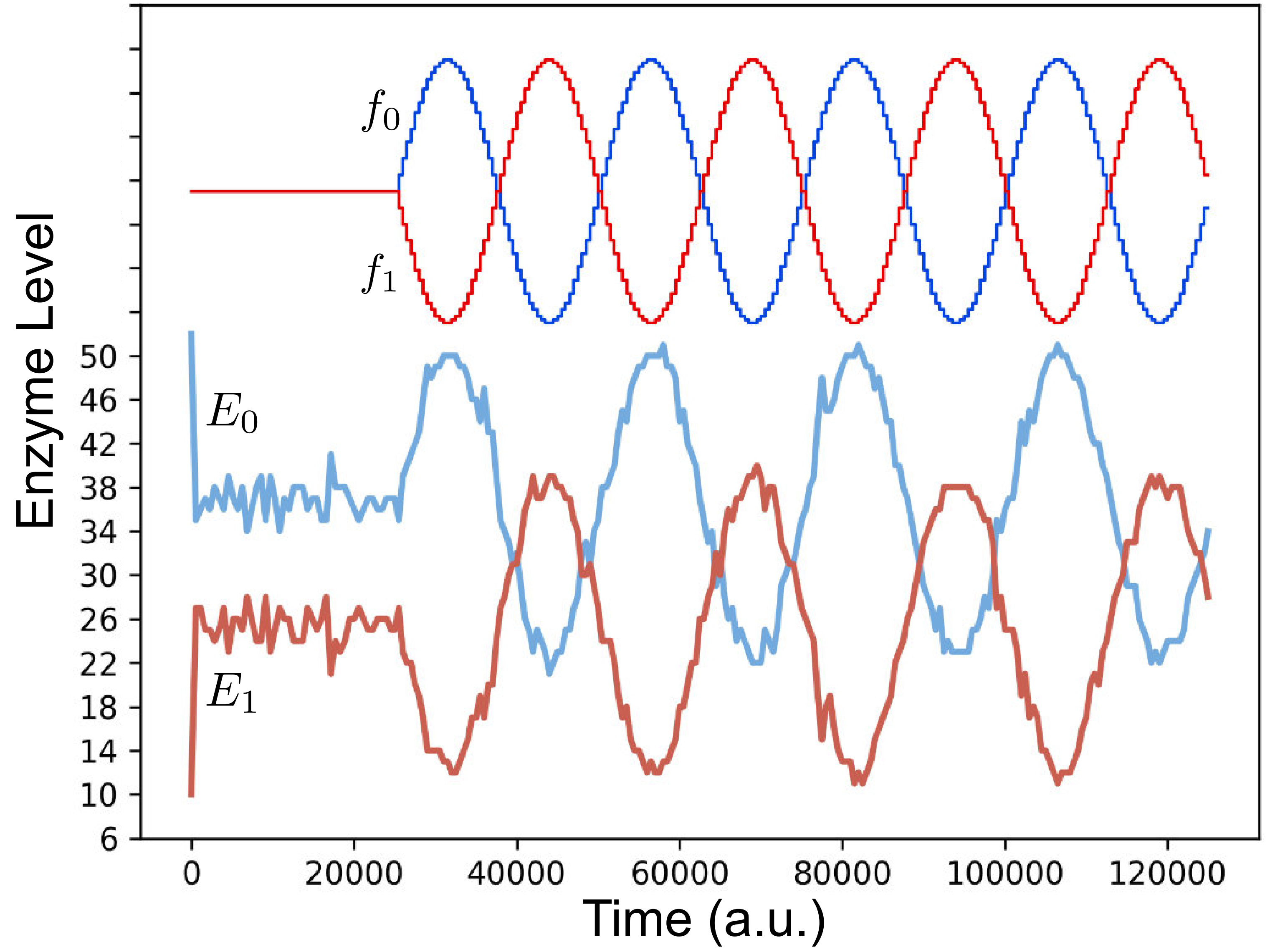
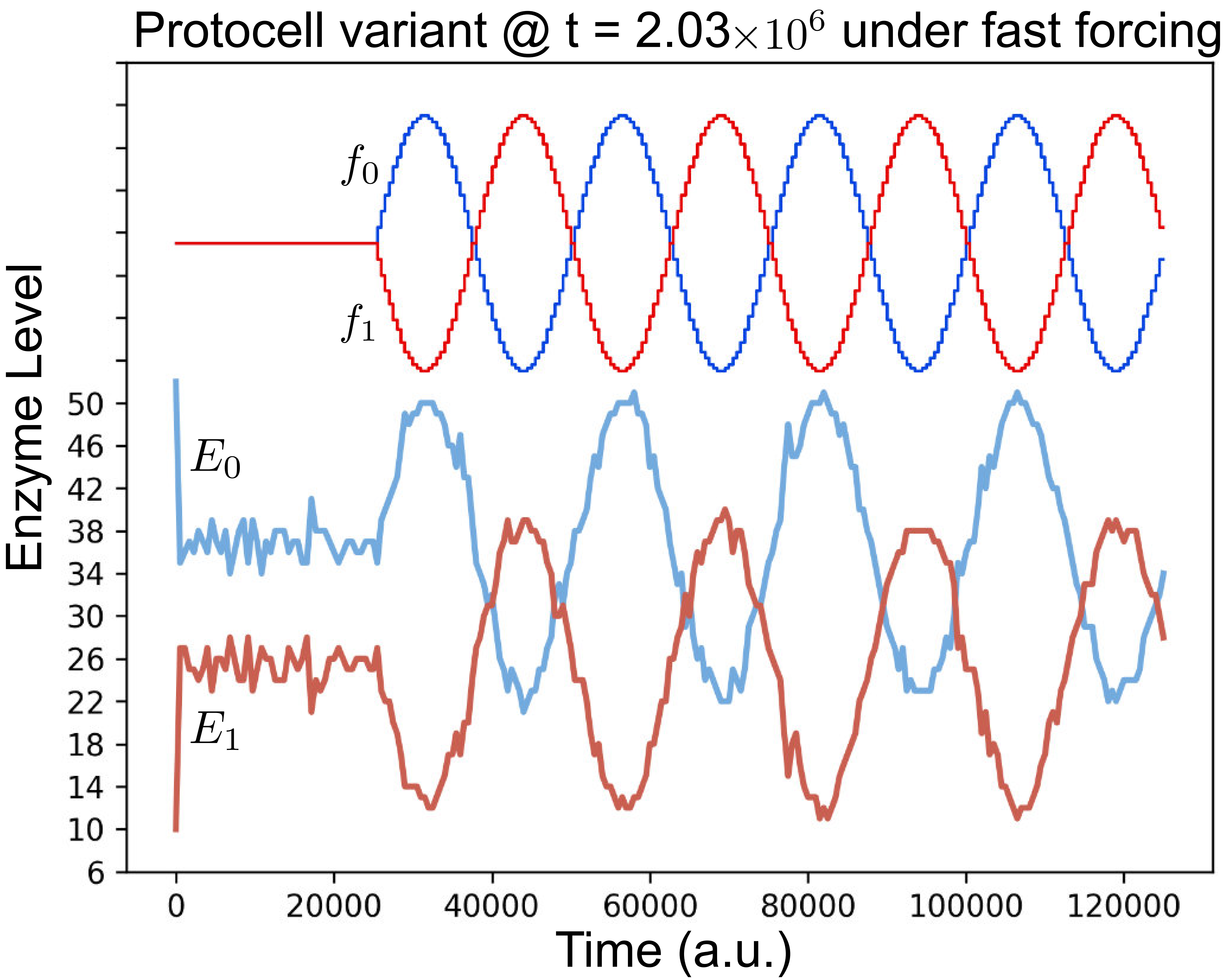


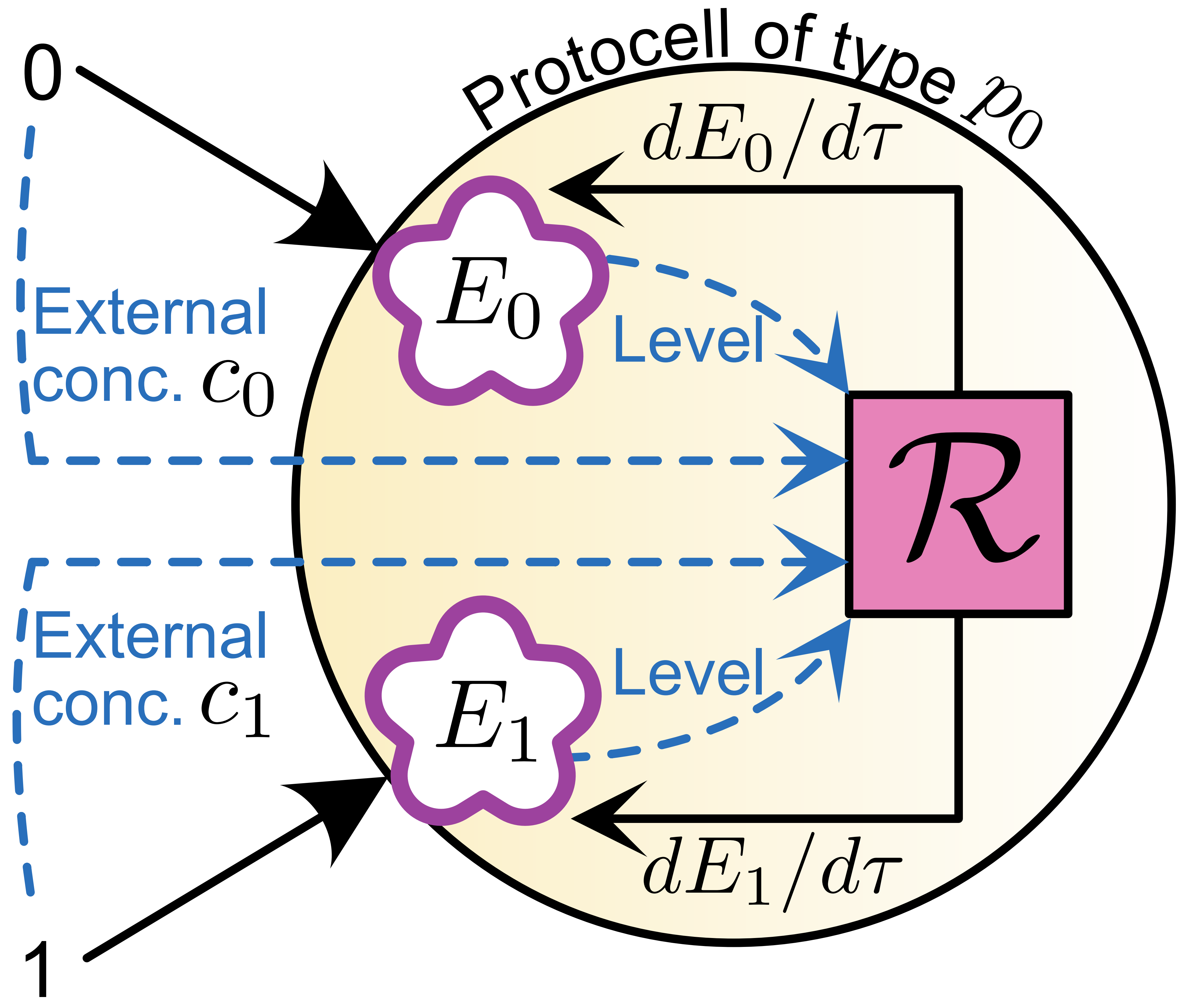
Reg Net Functional? (Yes)

Protocell variant @ t = 2.03$\times 10^6$ under fast forcing



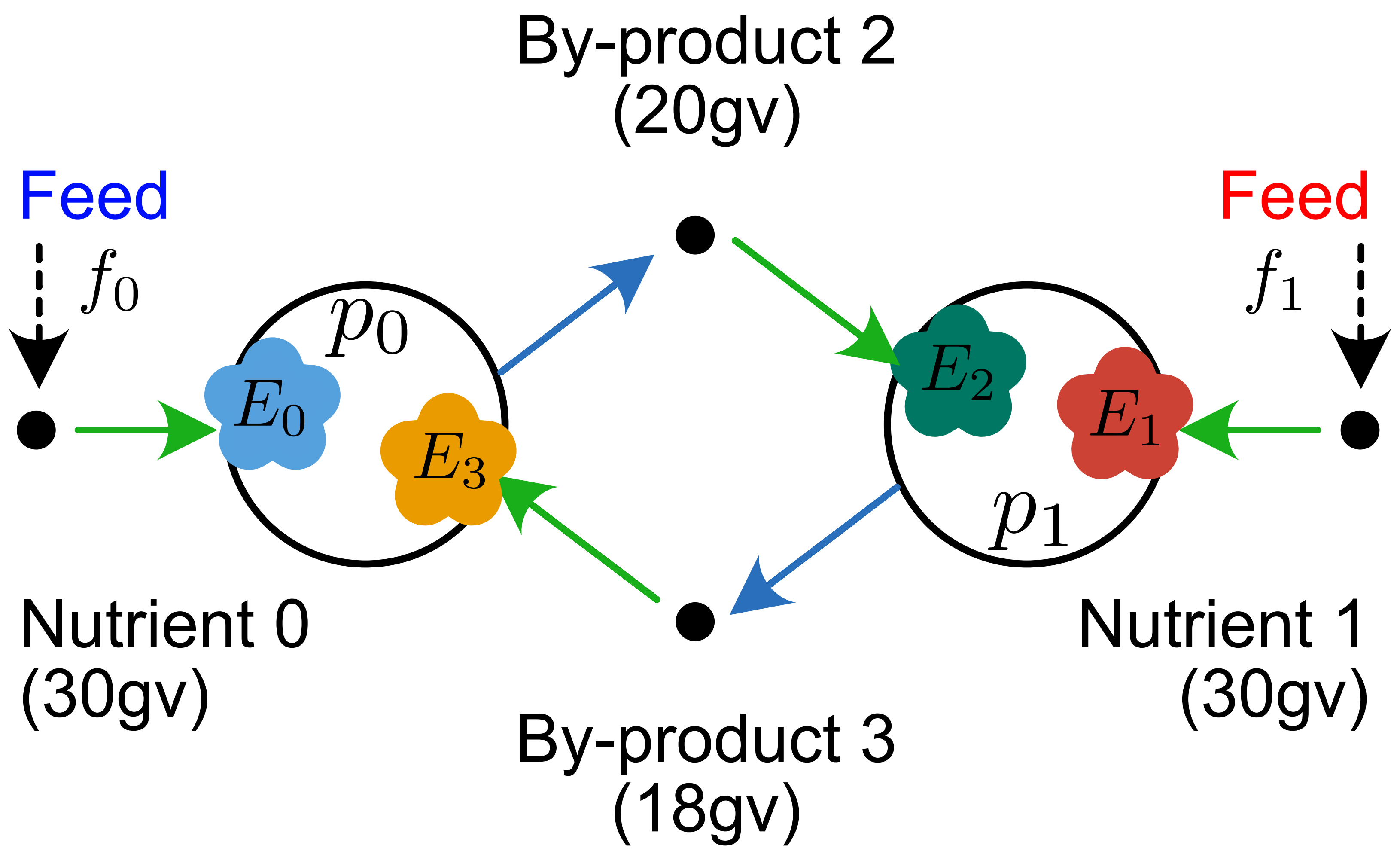
Case Study 1: Single Protocell Species



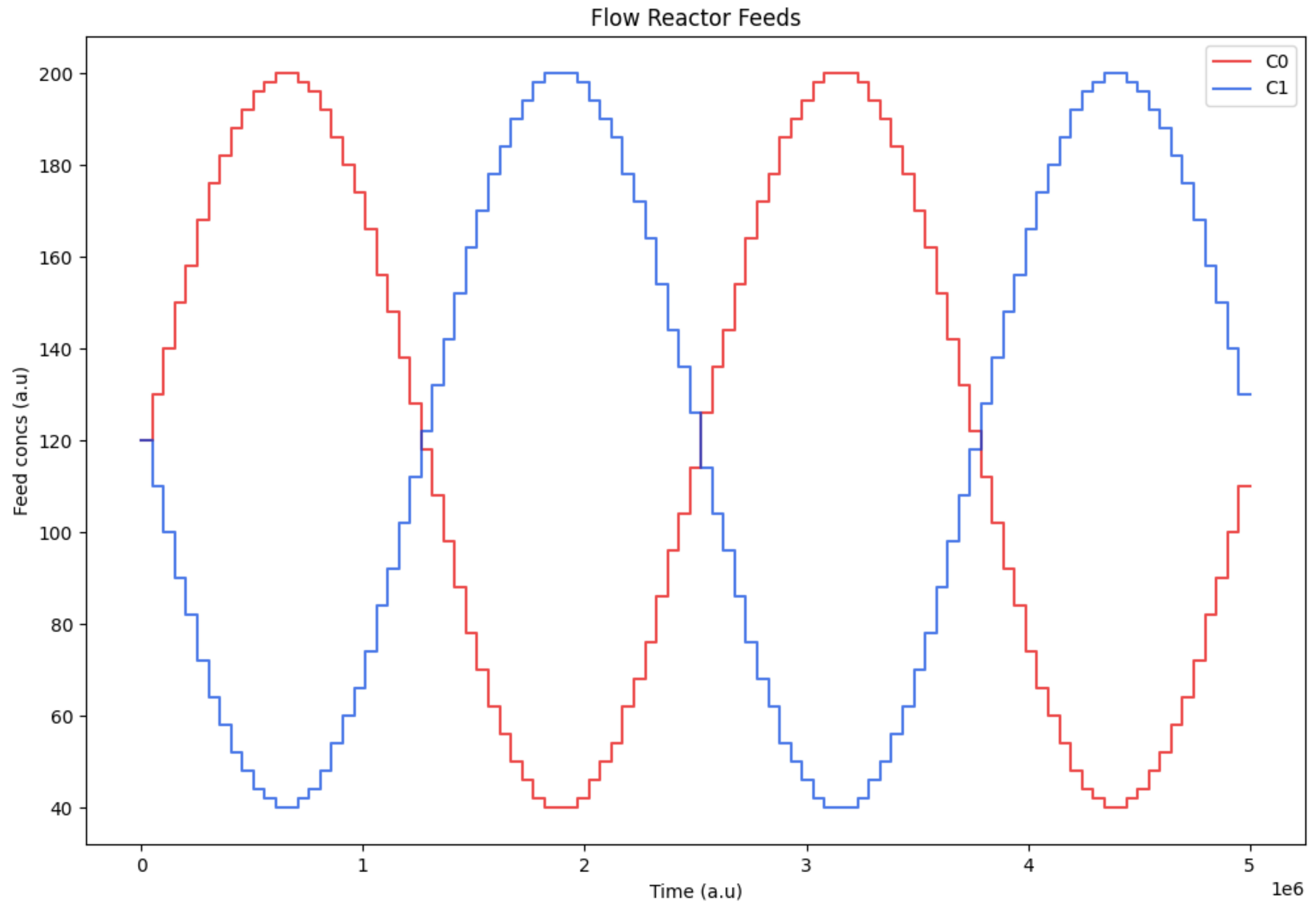


Evolution of "lac Operon" like regulatory behaviour!

Case Study 2: Minimal Mutualism



Slow Nutrient Changes



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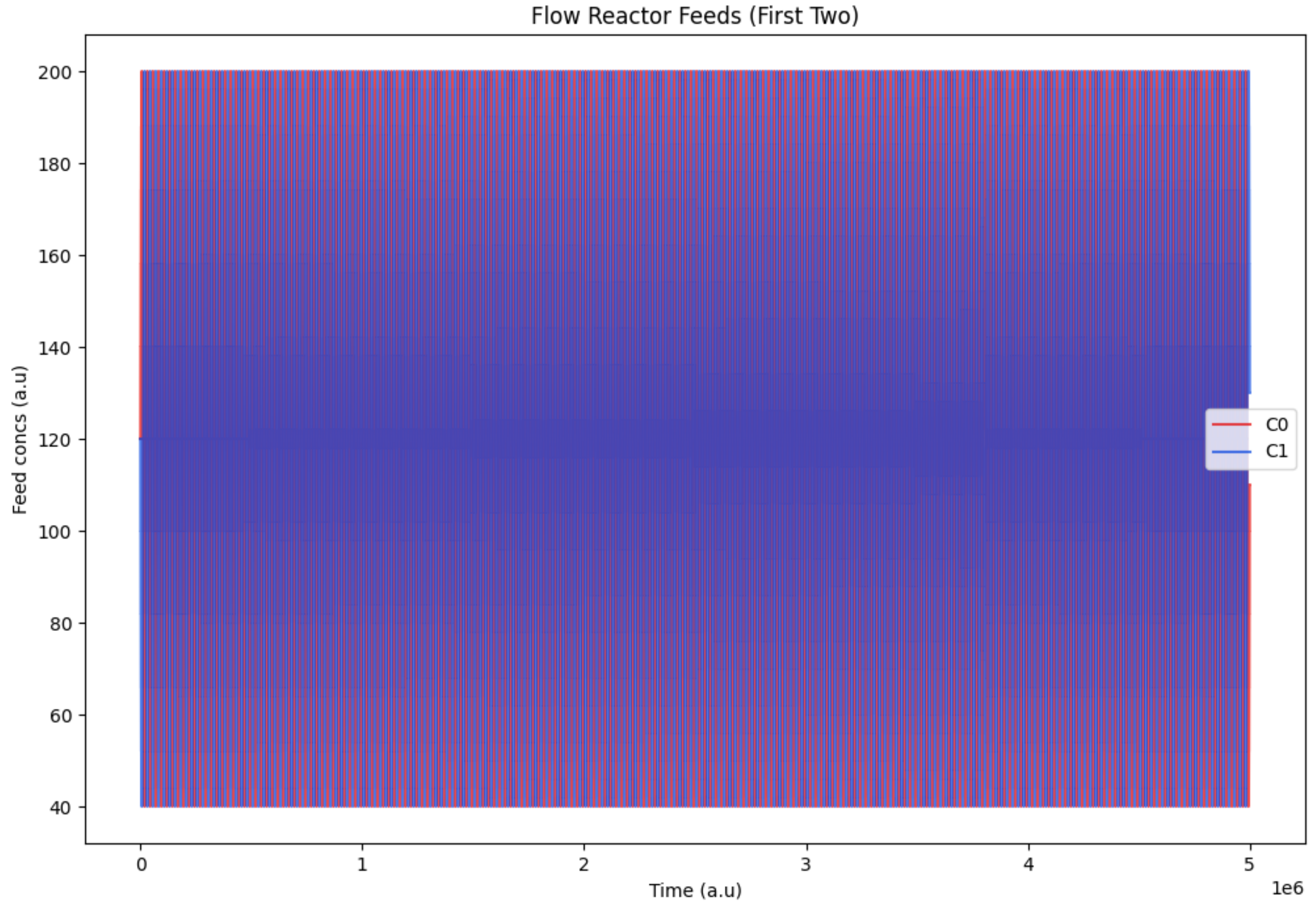
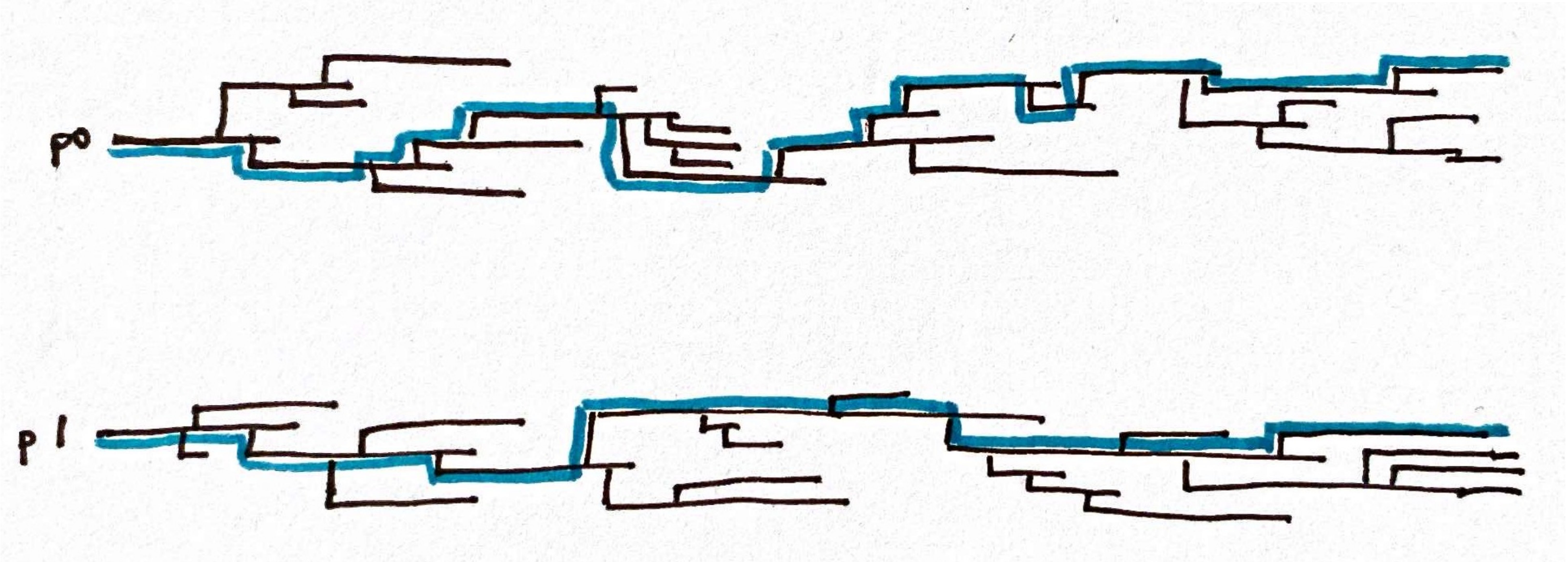
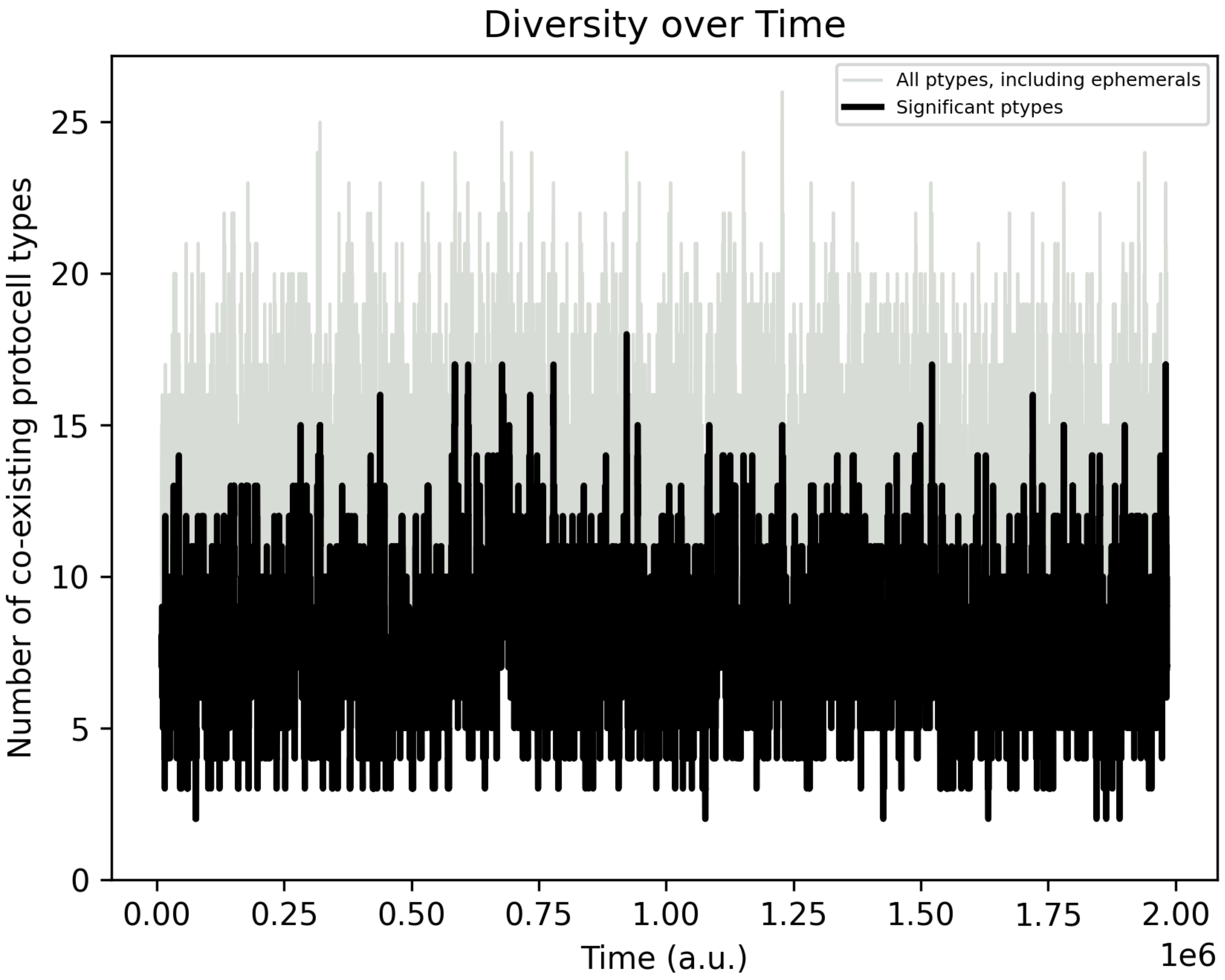
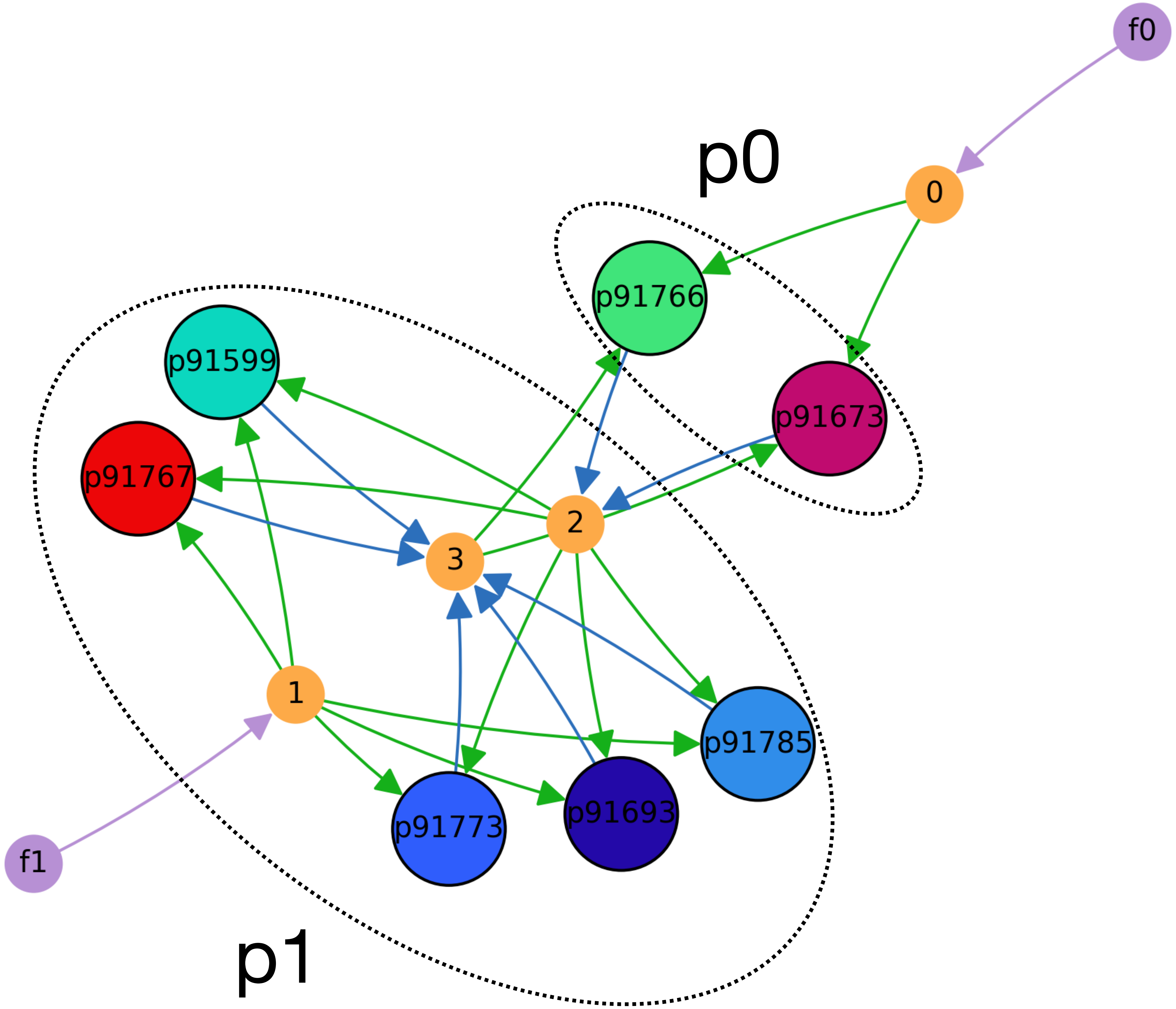


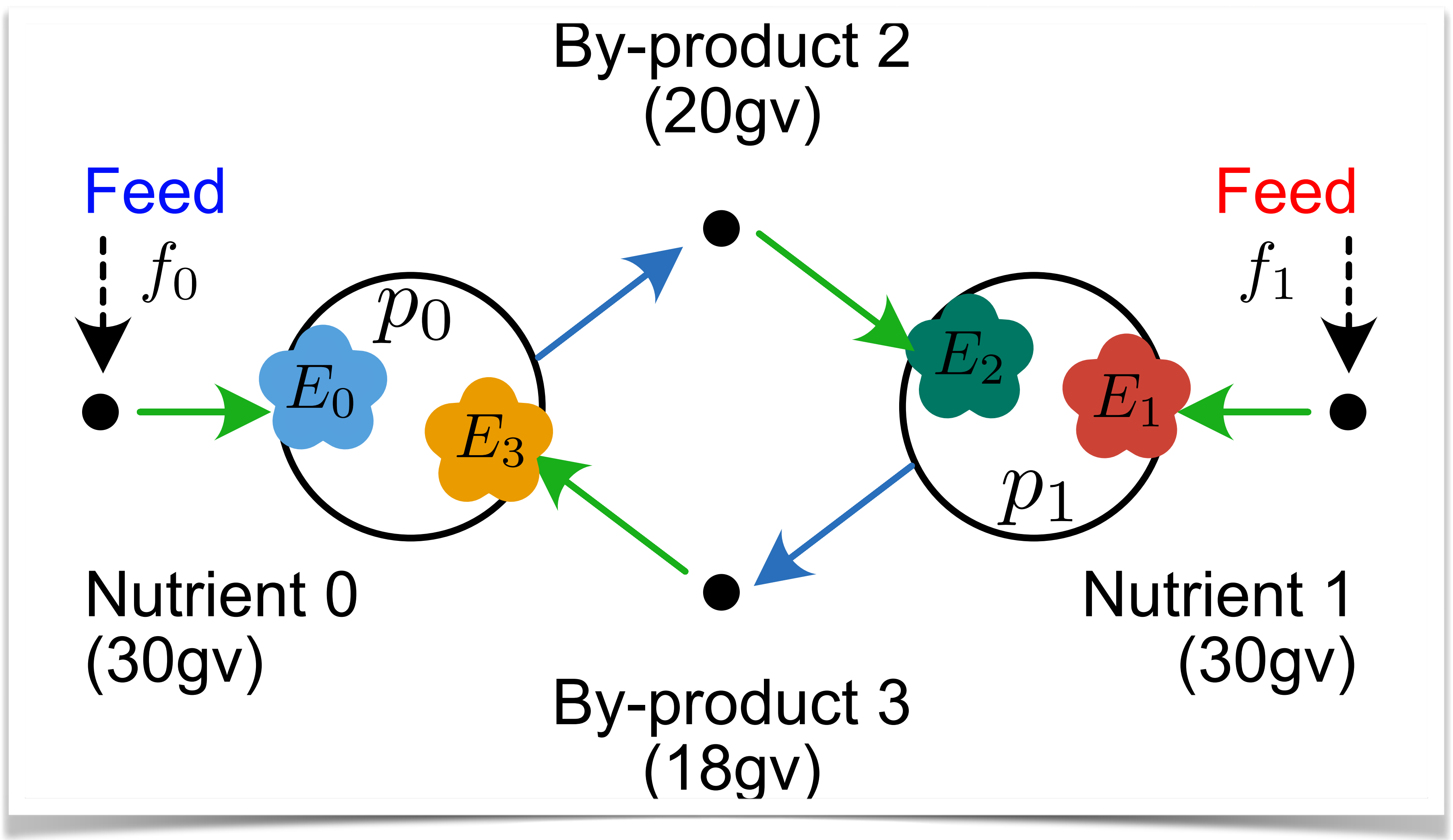


A Note: Temporary "Quasi-Species" Often Develop





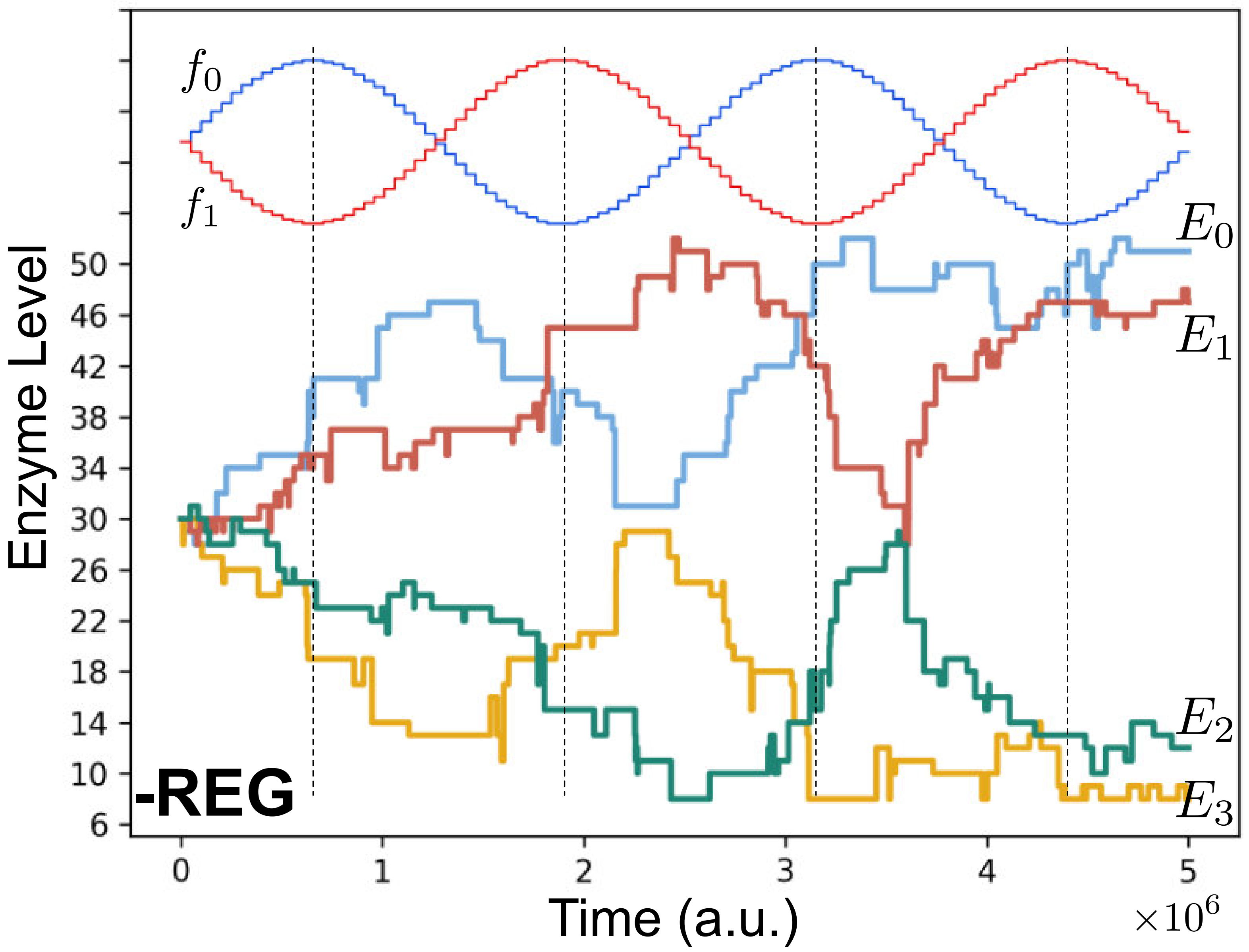
Case Study 2: Minimal Mutualism



Slow Nutrient Forcing

Phylogenetic Adaptation Only

-REG



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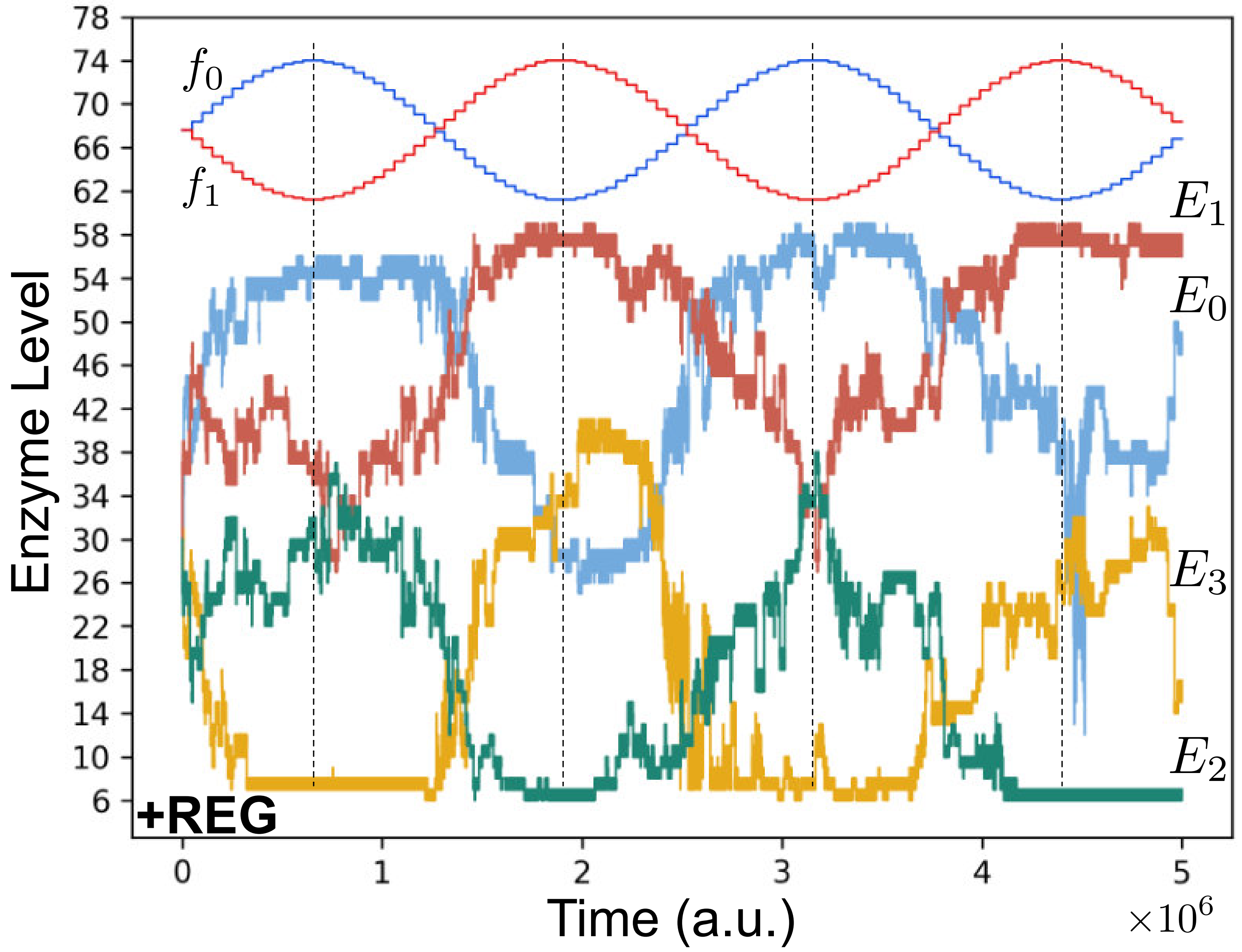

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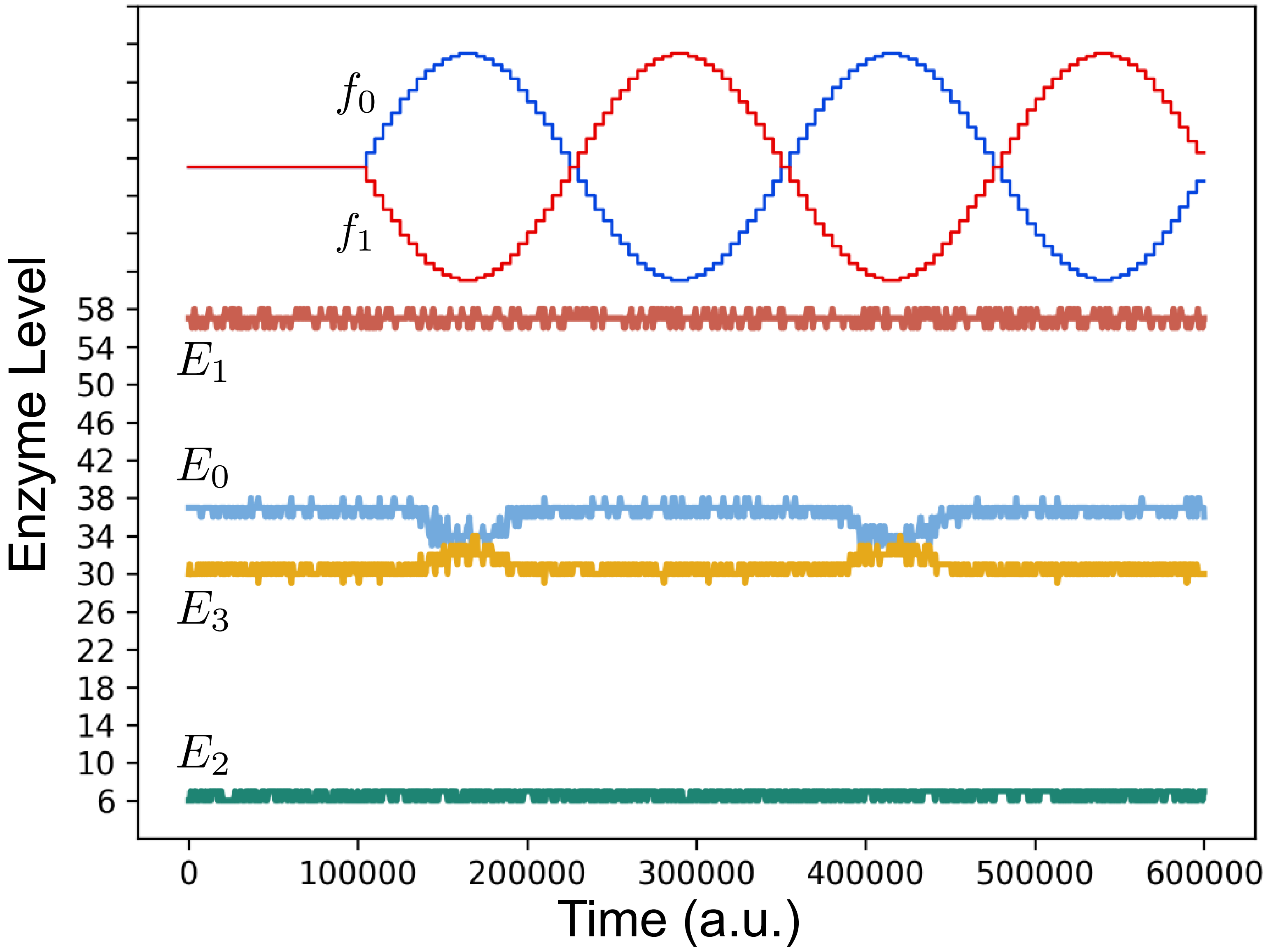

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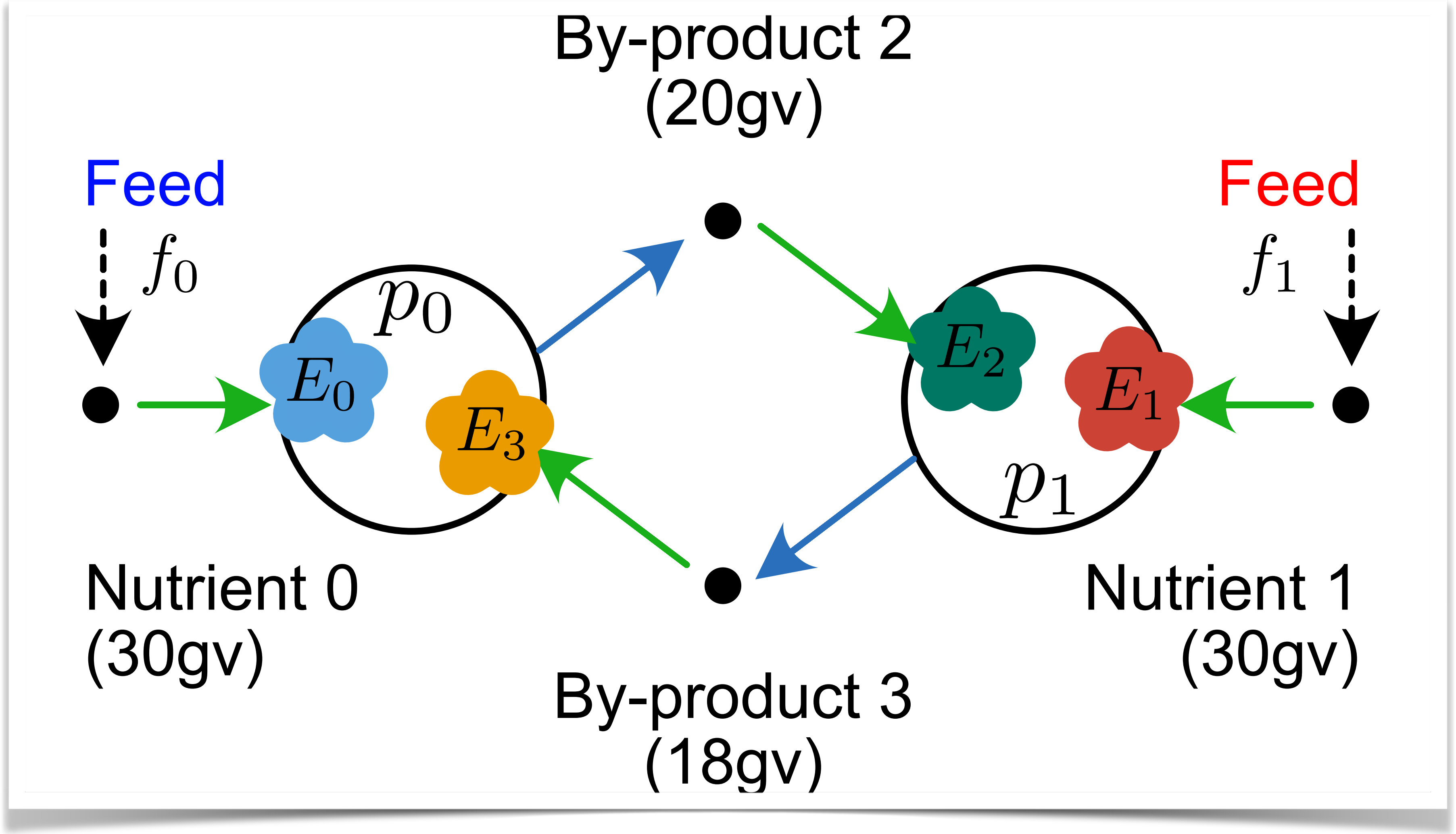
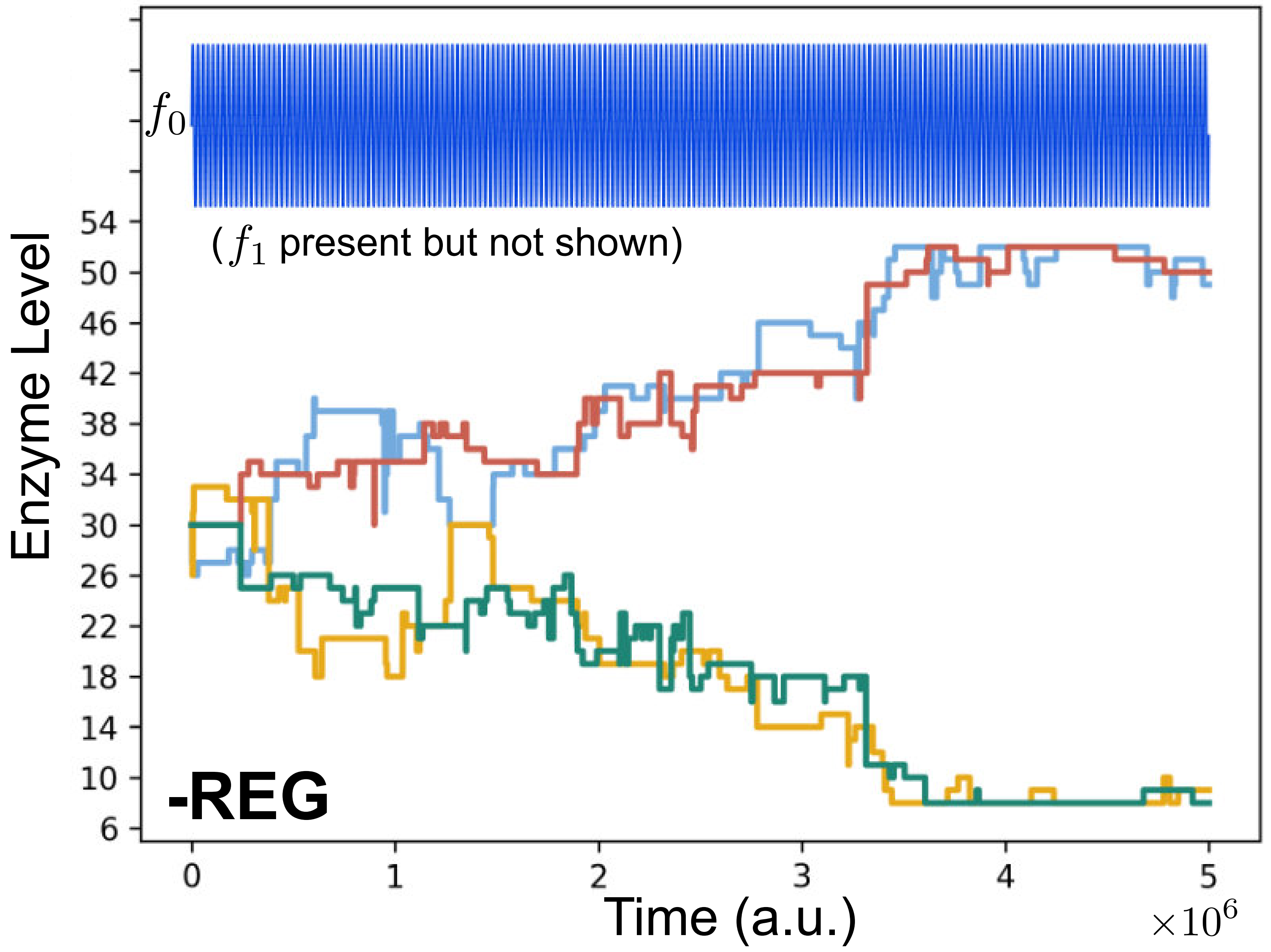


Fast Nutrient Forcing

Emergence of Bursts of Functional Regulation

-REG



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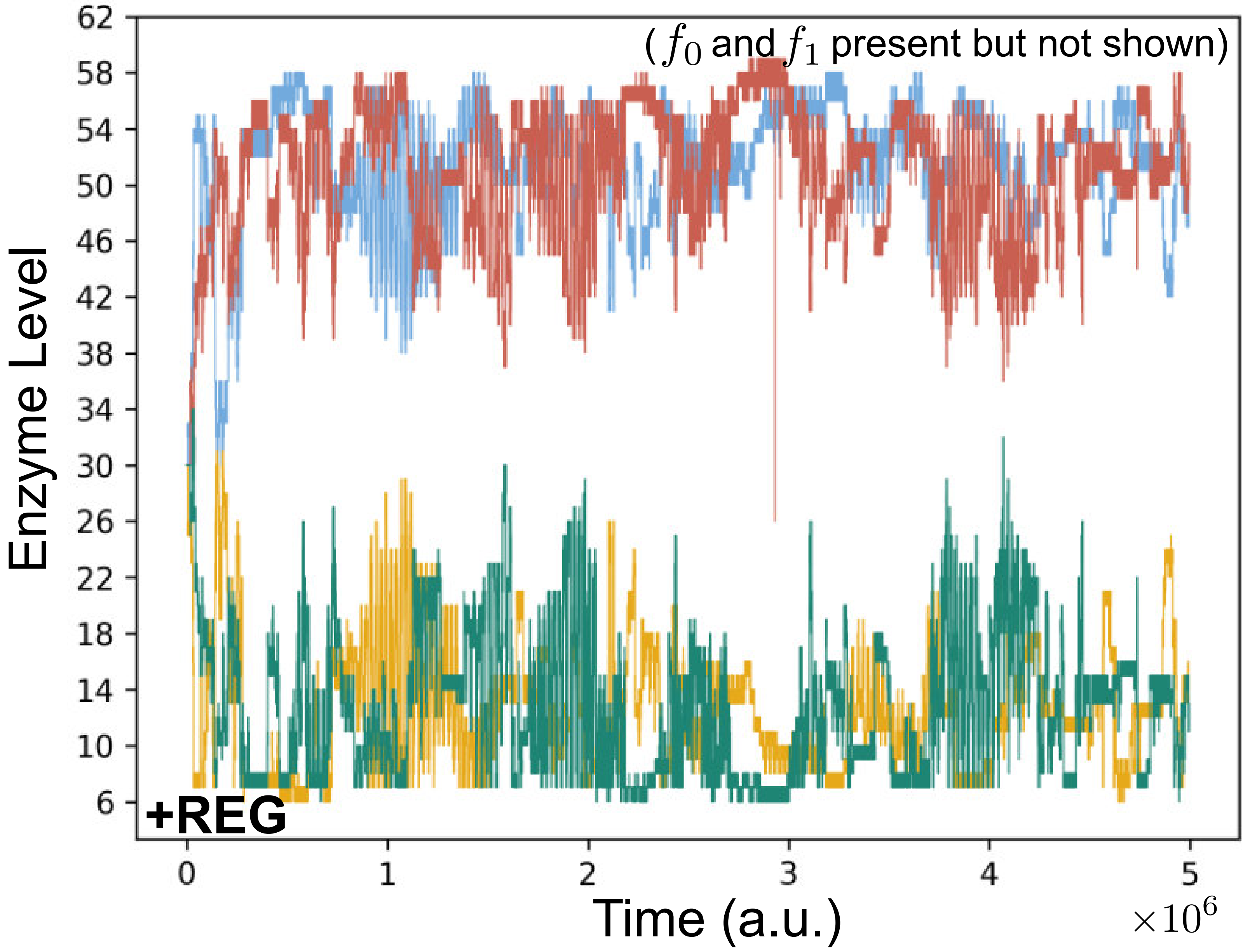

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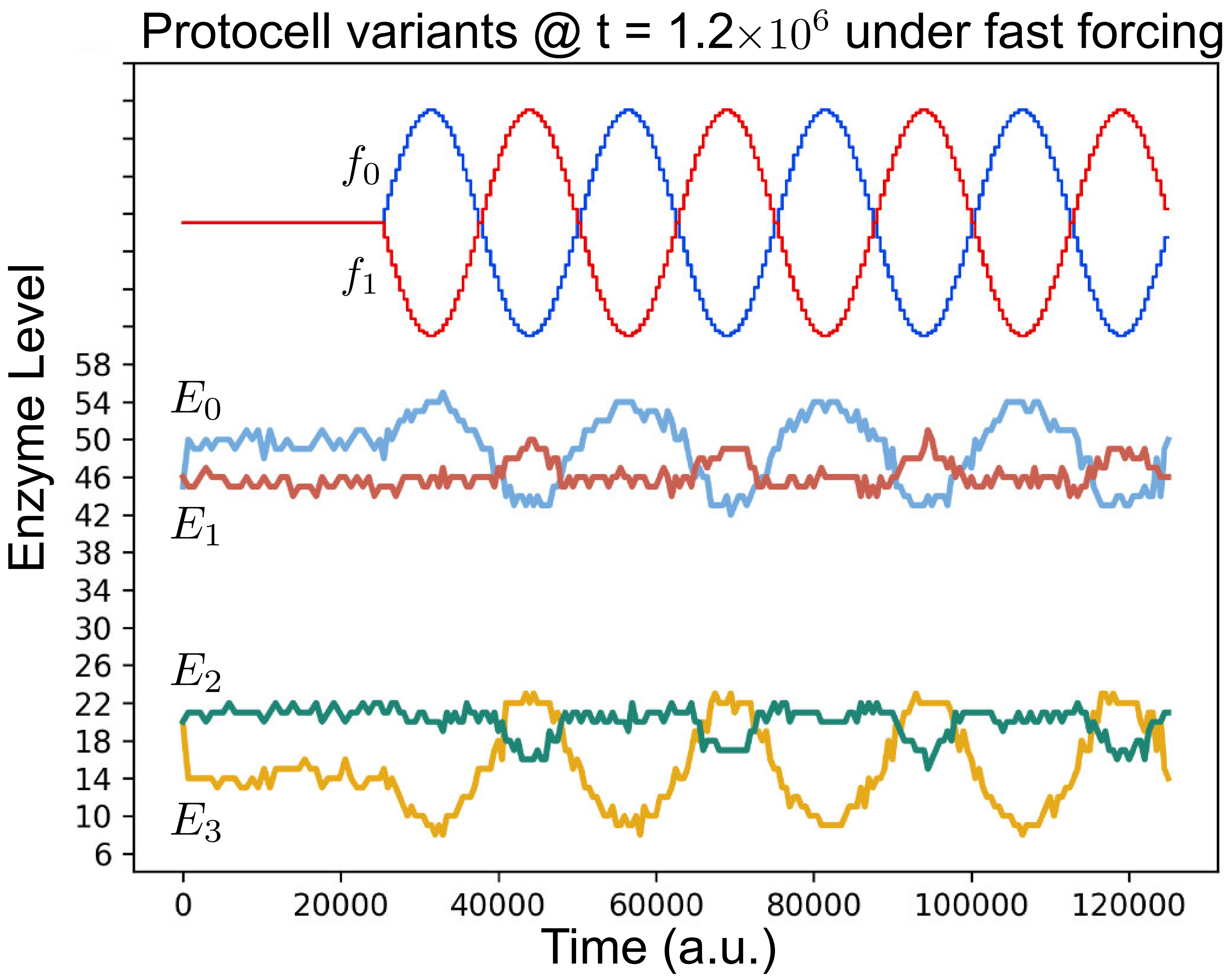

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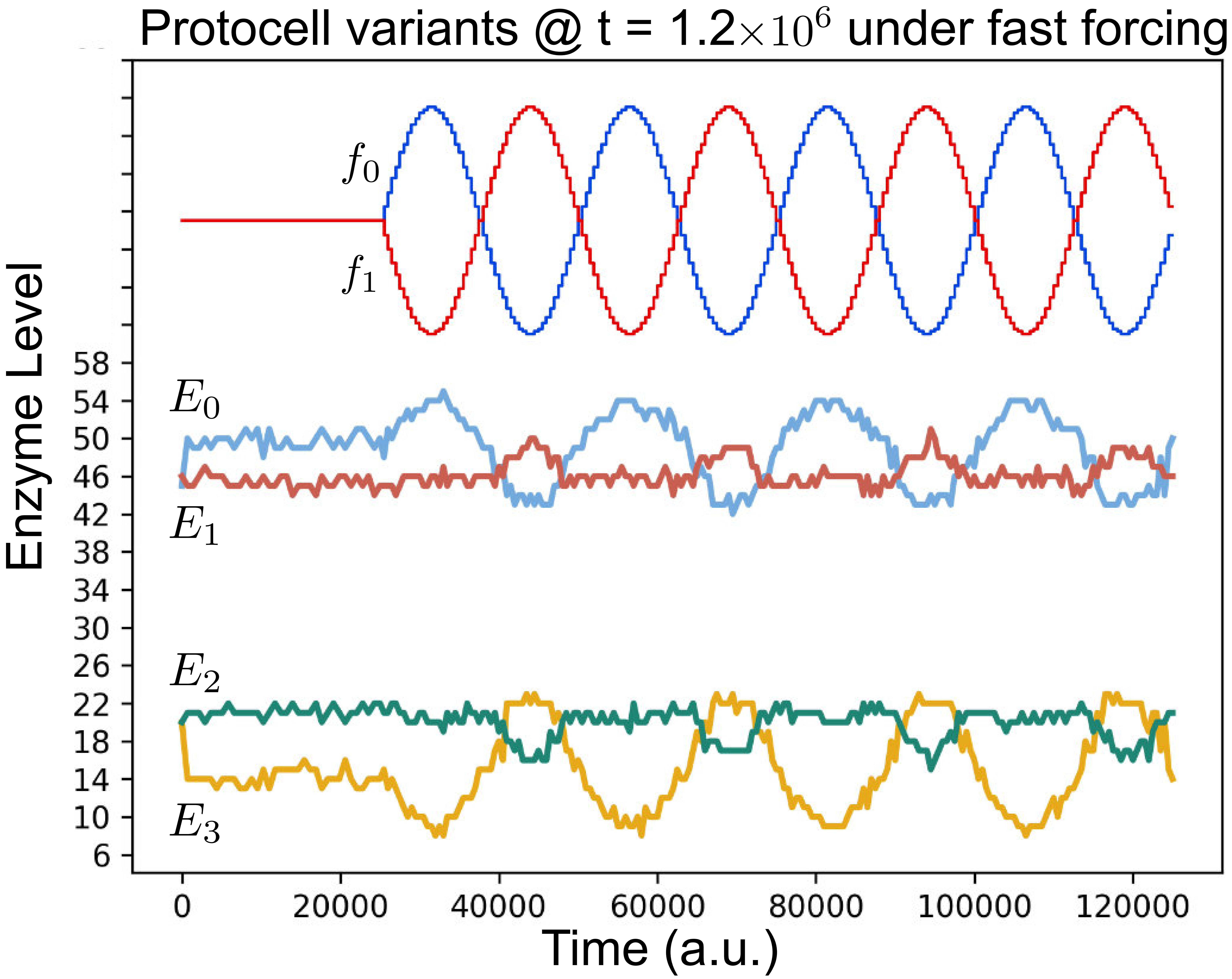
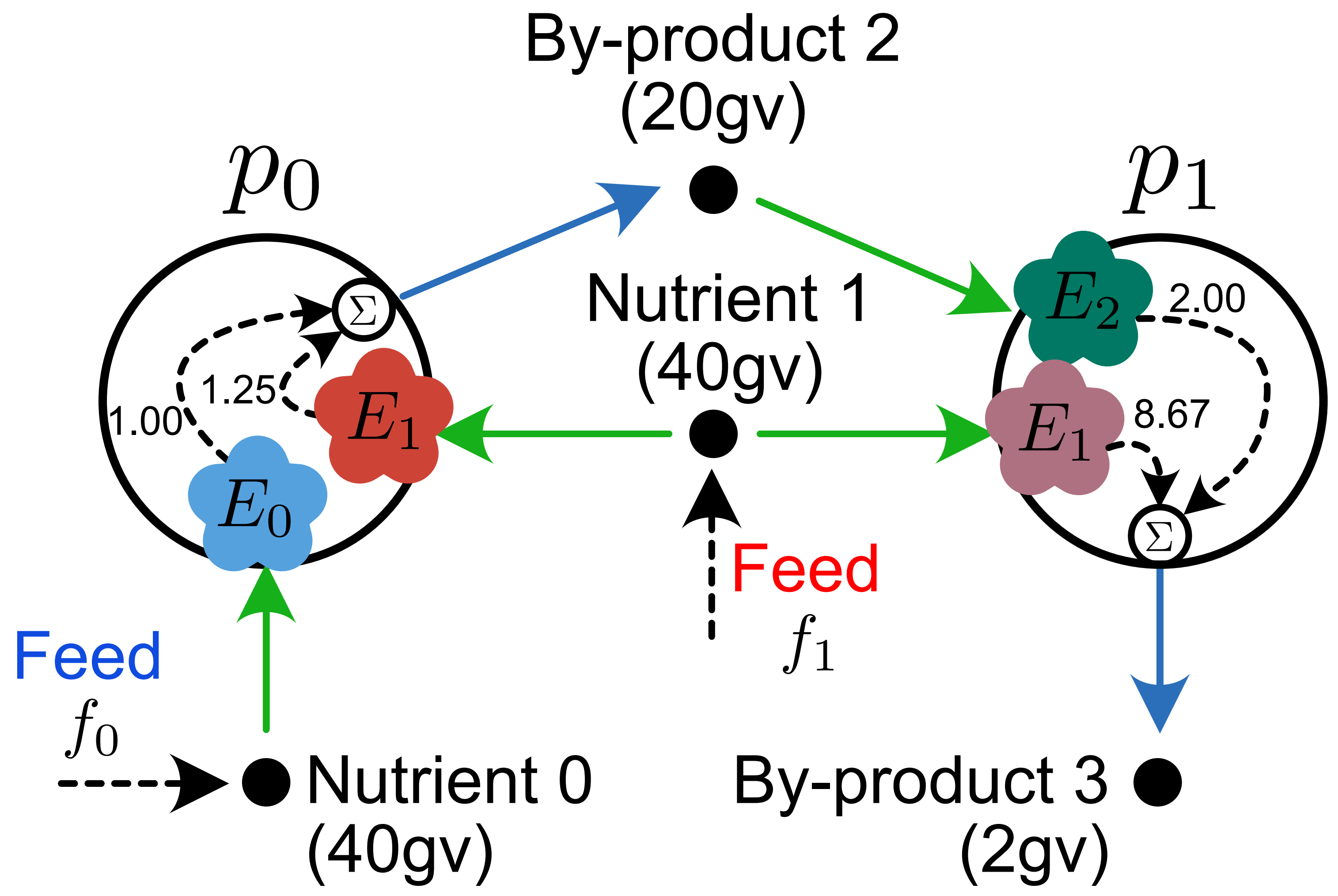
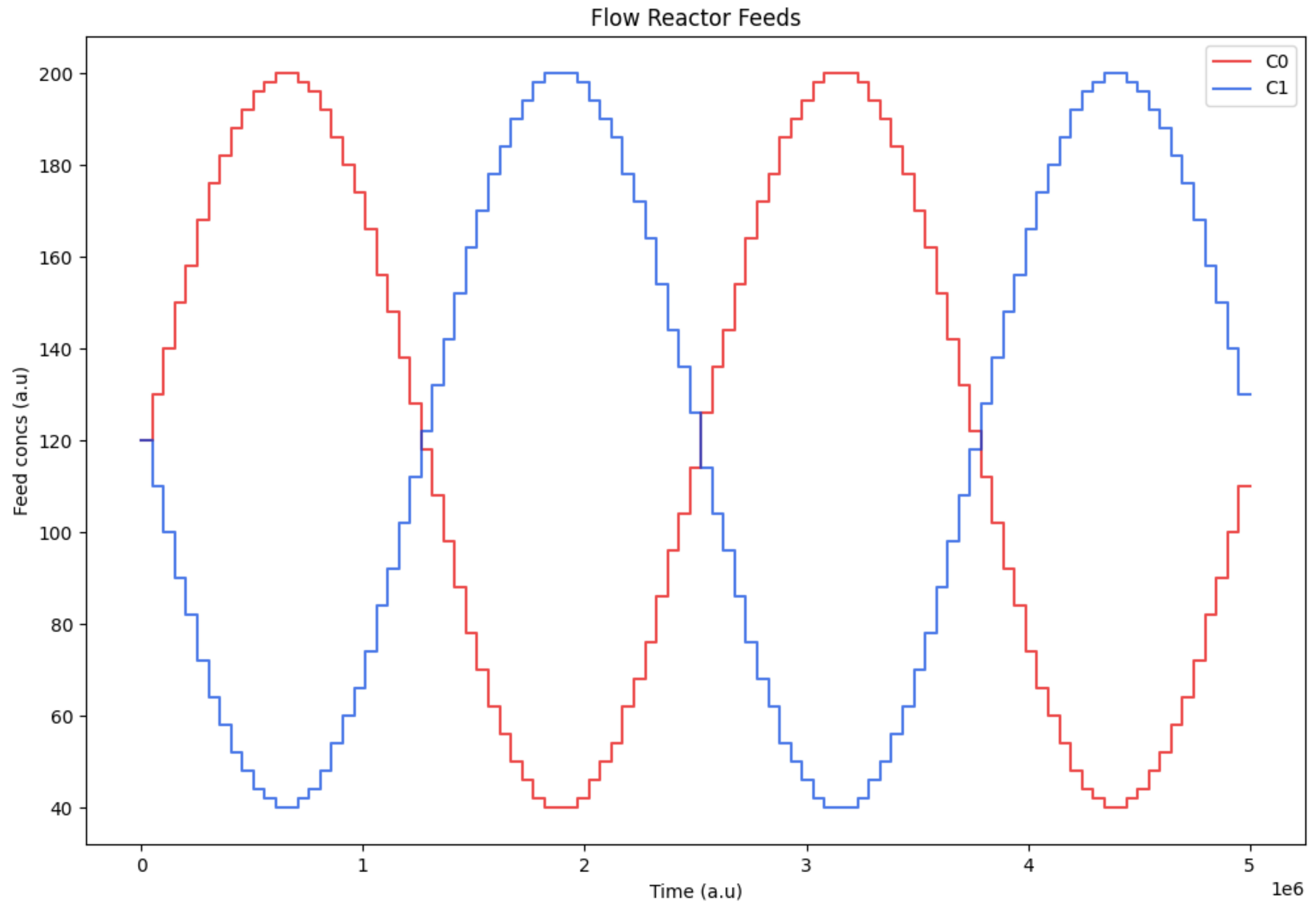


Case Study 3: Competition



Slow Nutrient Changes



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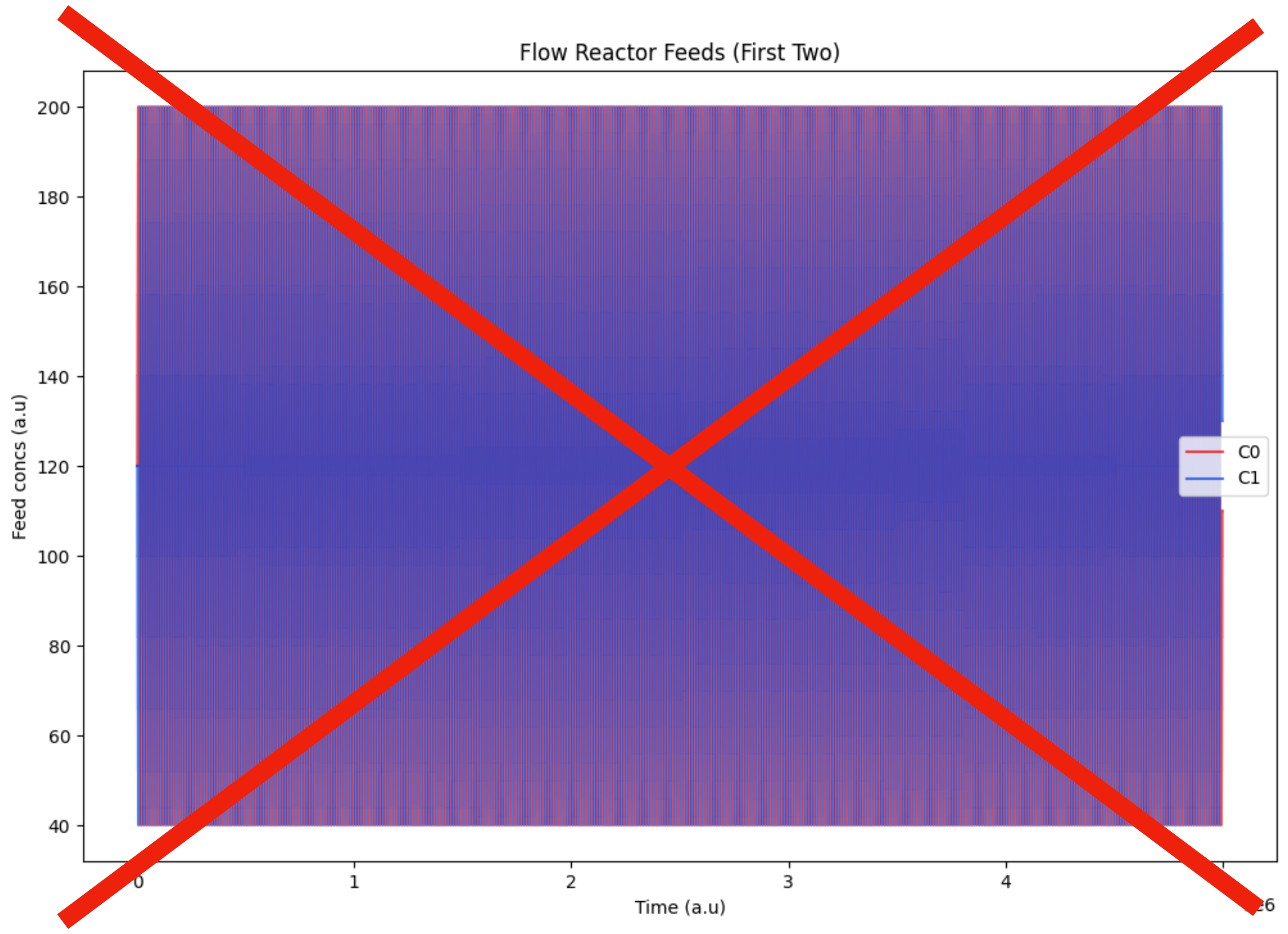

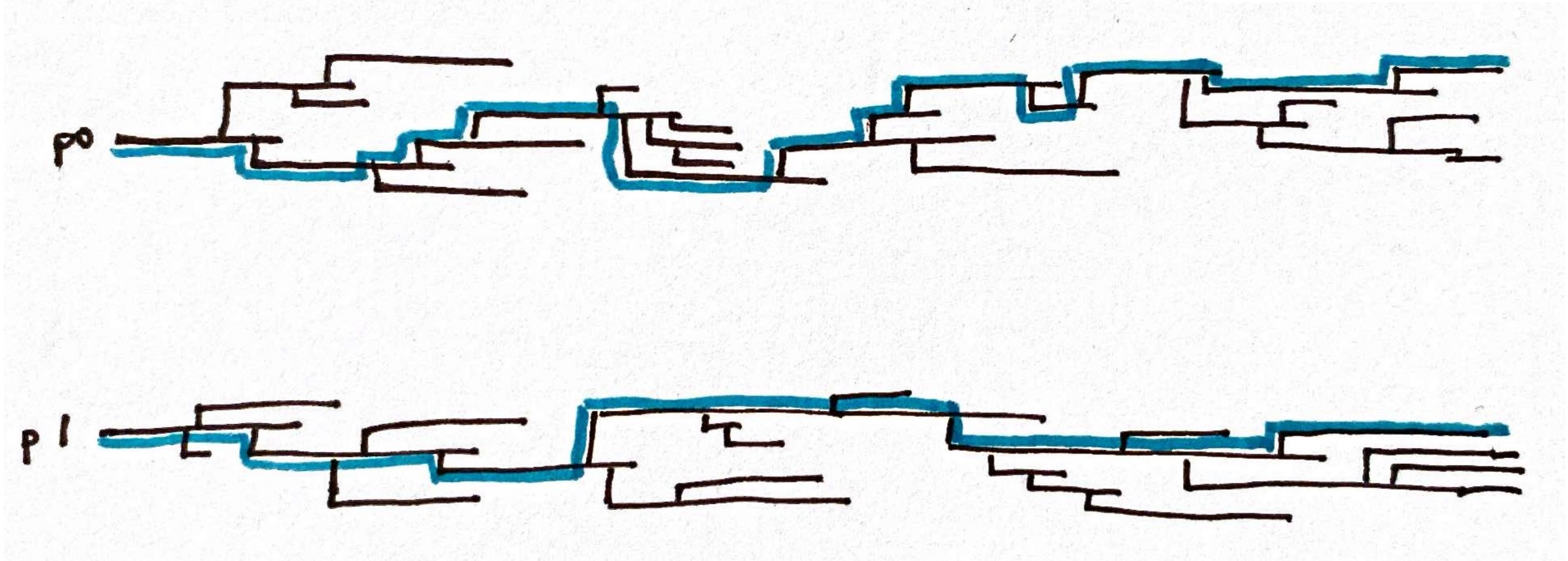

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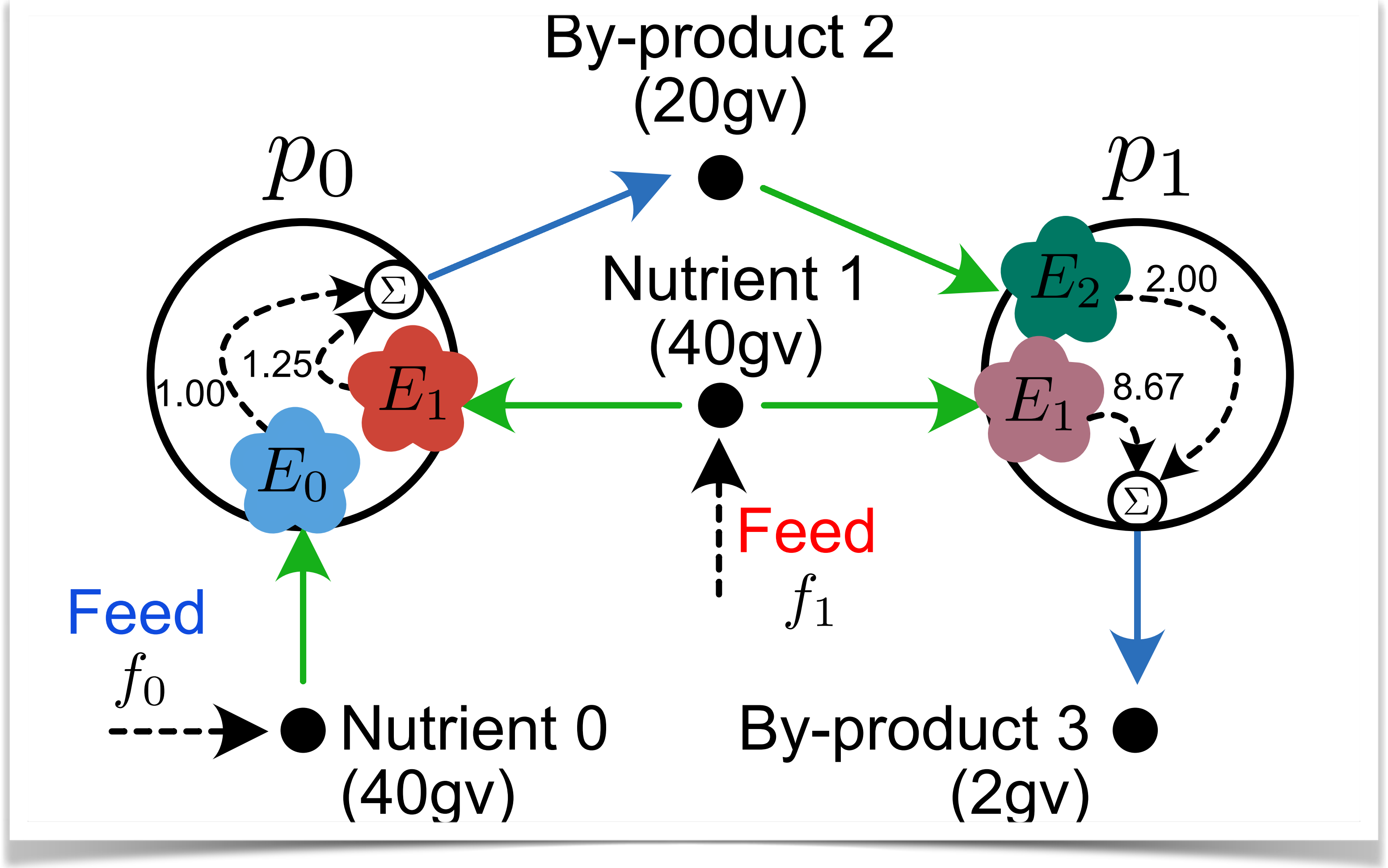
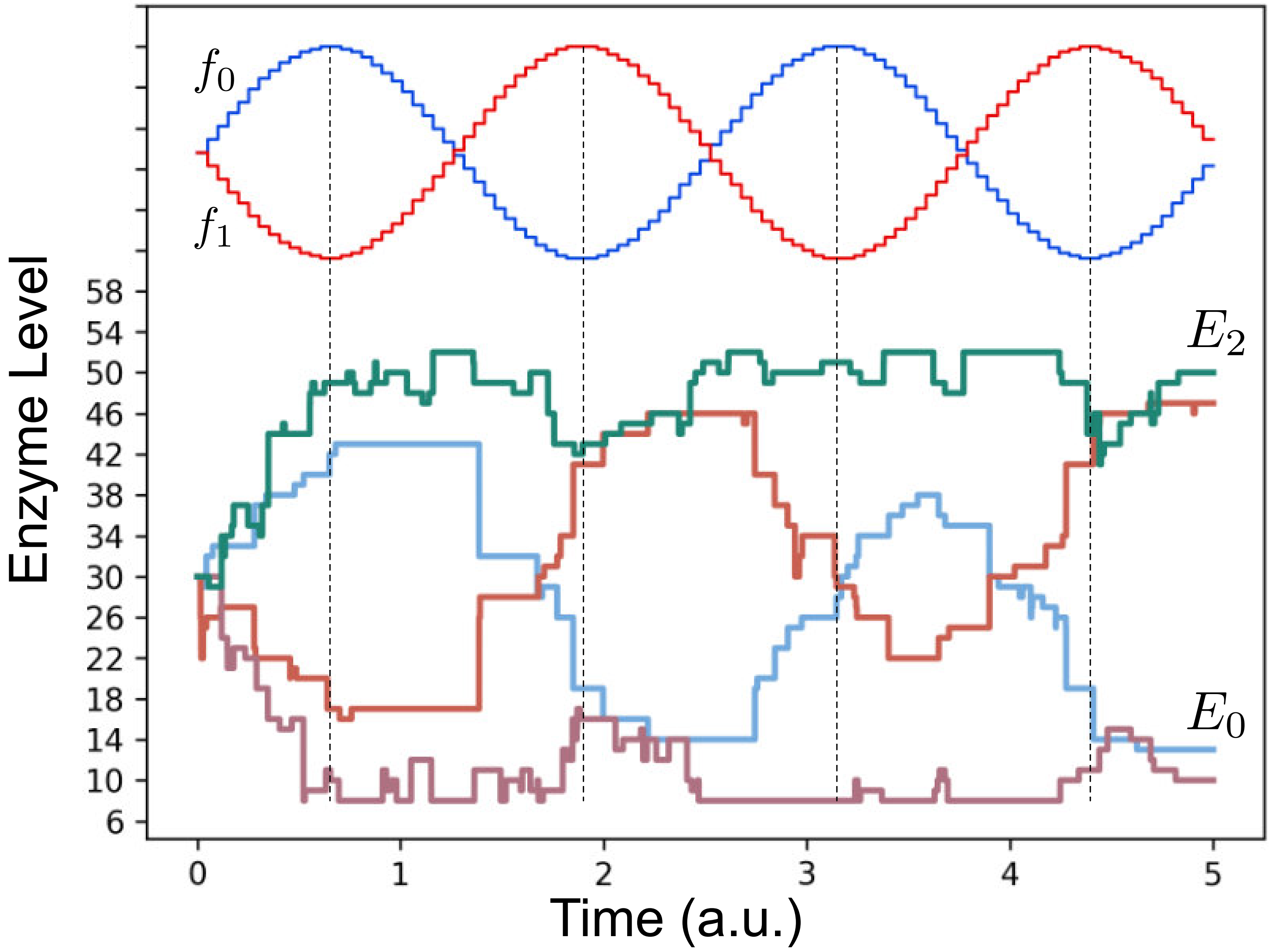


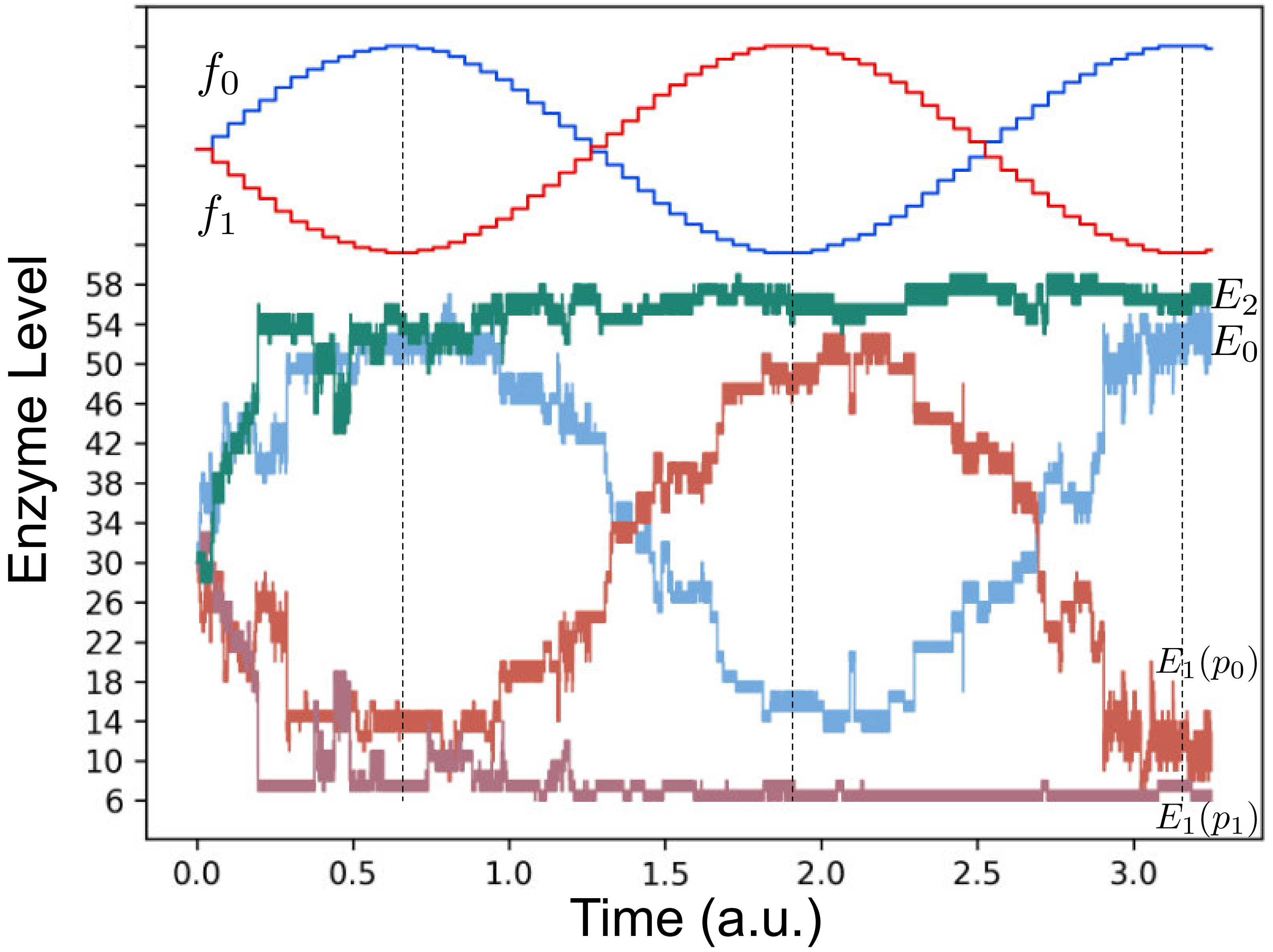
Slow Nutrient Forcing

Different phylogenetic adaptations can happen but regulation does not emerge (we cannot get past slow forcing)

-REG



+REG (1)



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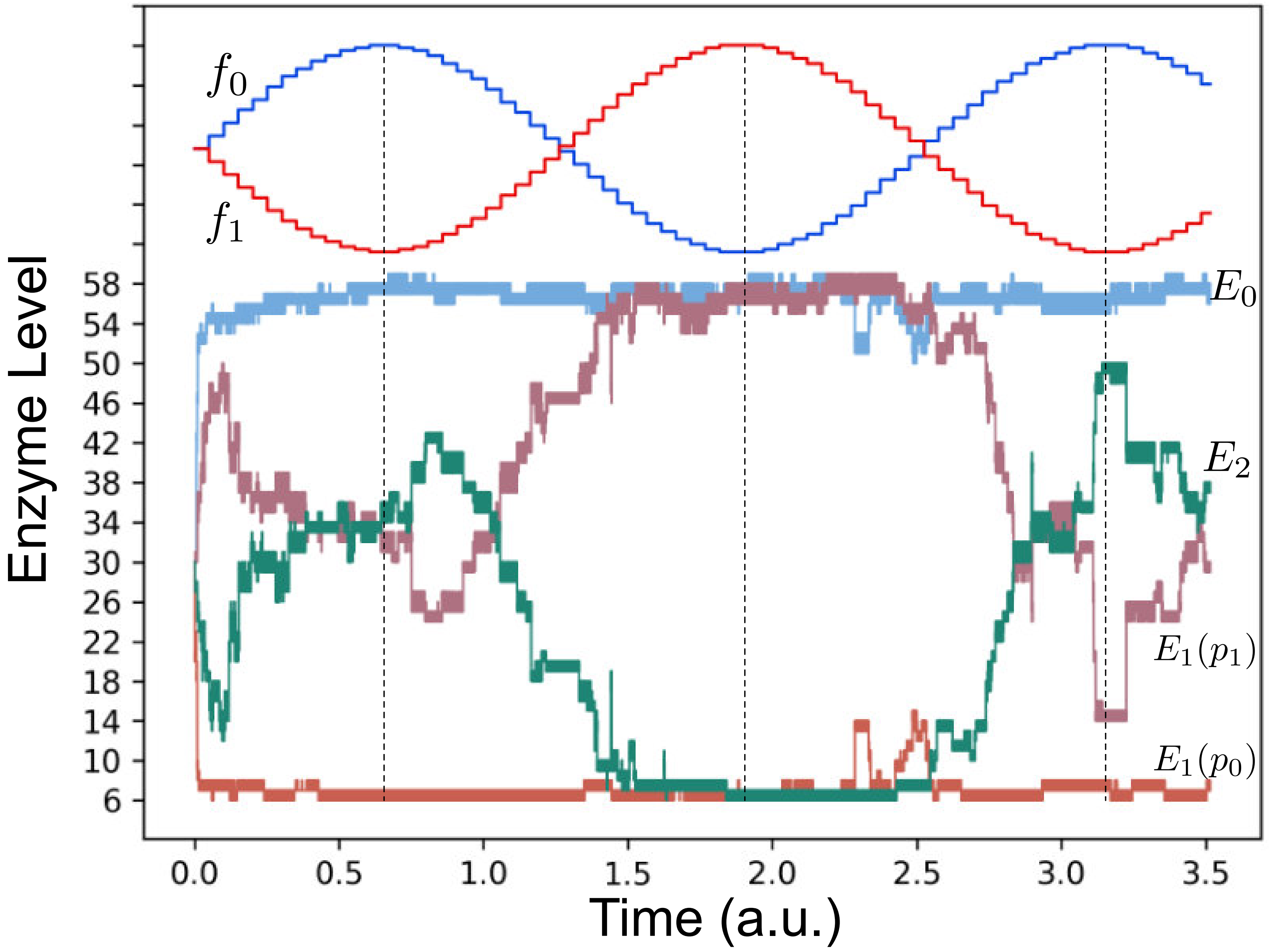
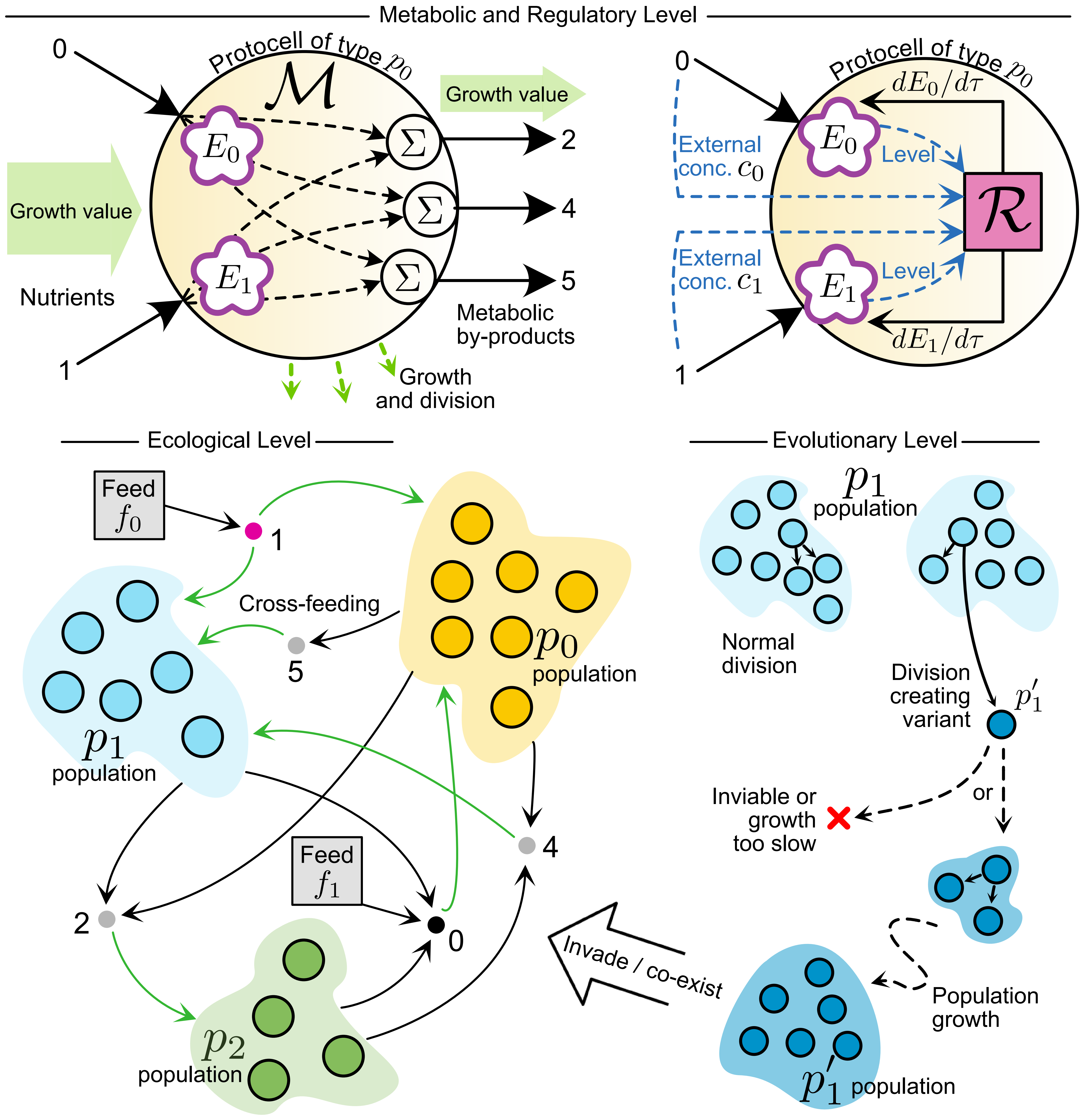


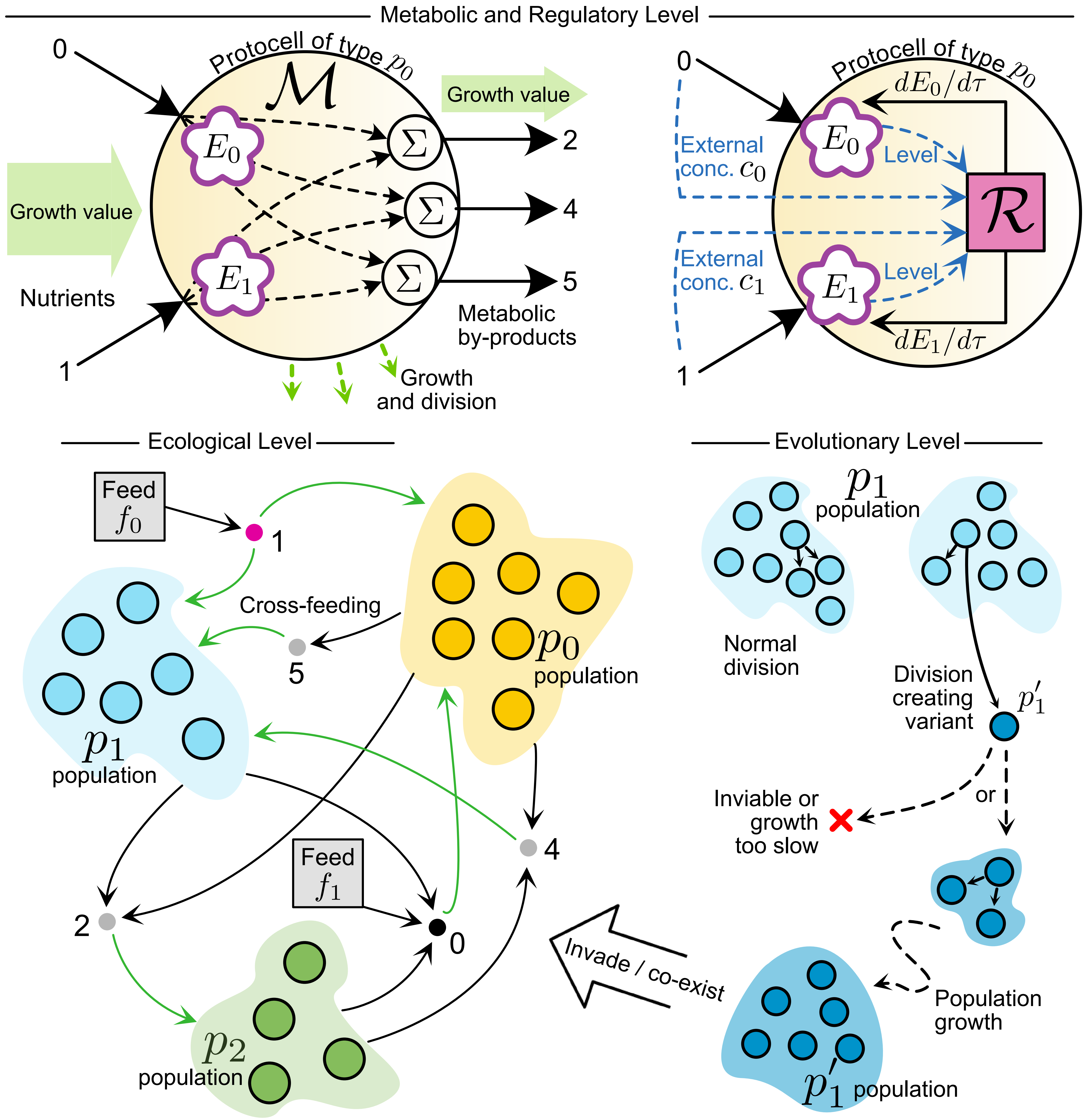
Summary

Our Approach



- Metabolism / Ecology / Evolution multi-scale model, exploring if regulatory mechanisms emerge in an evolving protocell ecology under forcing input of a flow reactor

Our Approach



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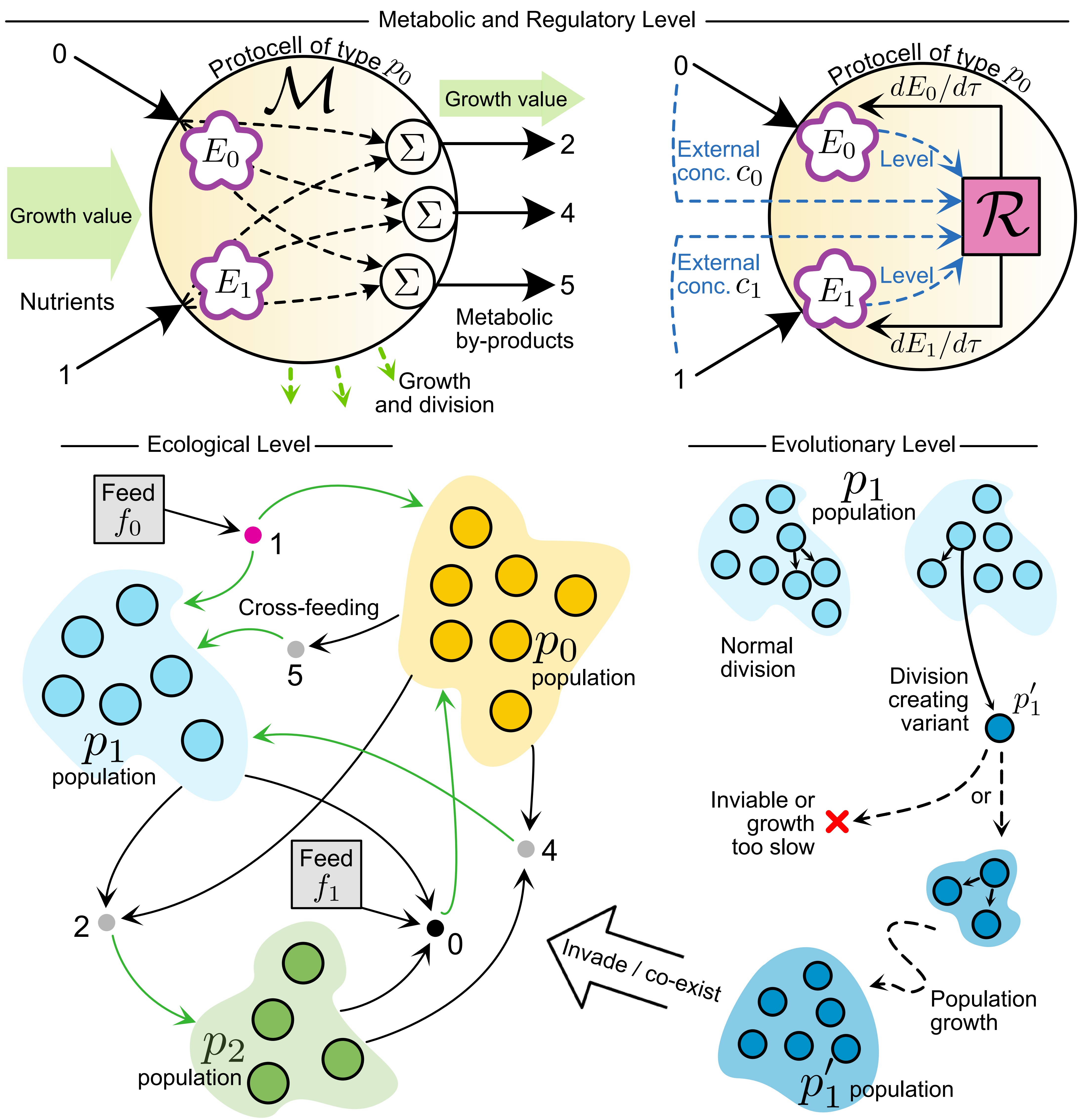
- Model is abstract, but is intended as a tool to help us think about why minimal agency evolved in protocell populations

Our Approach



- Metabolism / Ecology / Evolution multi-scale model, exploring if regulatory mechanisms emerge in an evolving protocell ecology under forcing input of a flow reactor
- Model is abstract, but is intended as a tool to help us think about why minimal agency evolved in protocell populations
- Does not give mechanistic detail about how regulation emerged, but rather sees under what conditions regulatory networks develop in response to environmental challenges, if there is the potential to develop (abstract) regulatory networks

We Wanted to Answer Questions Like These

Do protocell regulatory networks:

✓ • Get selected for? (over static enzyme levels)

✓ • Increase ecological robustness to varying reactor inputs?

✓ • Allow protocell species to learn long-term trends?

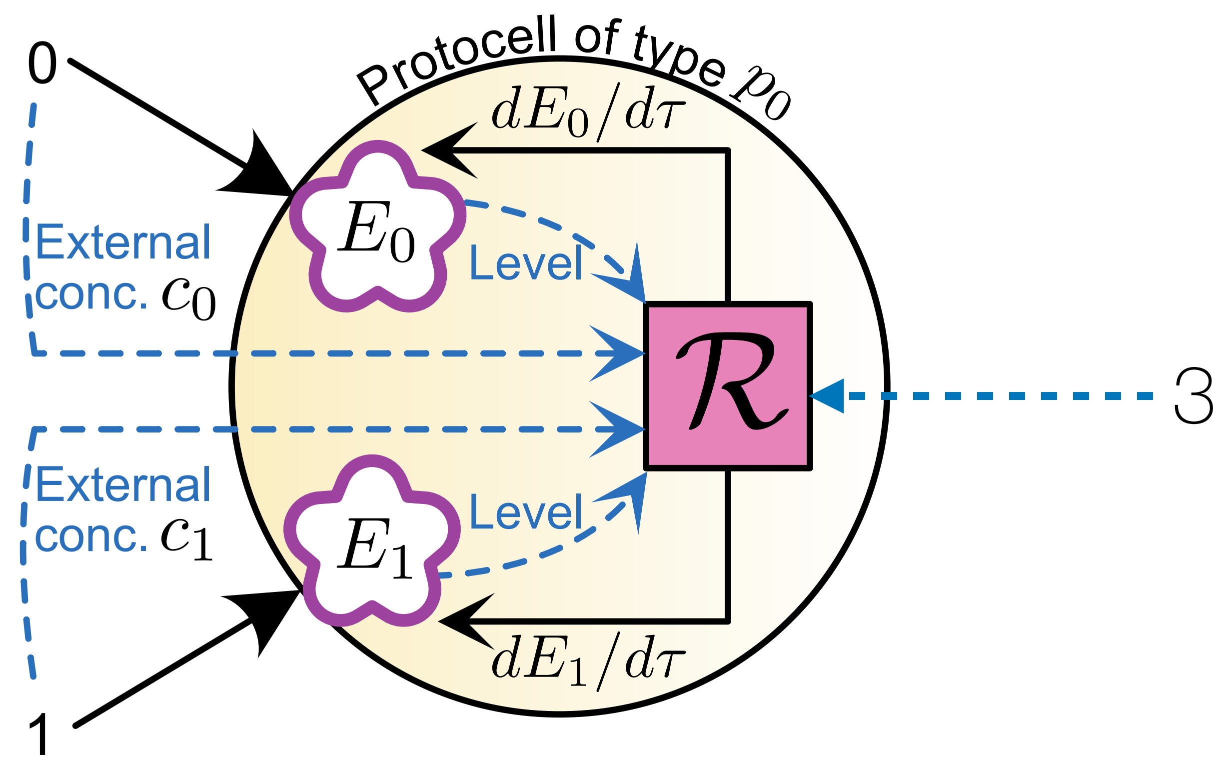
? • Increase ecological diversity / dynamic complexity?

More experimentation needed

✓ • How is phylogenetic adaptation related to ontogenetic adaptation?

Interesting Research Avenues

- More complex protocell ecologies



- Toxins

Protocells unfold novel behaviours in terms of competition/cooperation strategies with their peers

- Reg. networks also sensitive to non-imported nutrients in environment

- Major evolutionary changes enabled (protocells can change diet)

- AND requirement for nutrient uptake

Phil. Trans. R. Soc. B. – manuscript for submission

PHILOSOPHICAL
TRANSACTIONS B

Phil. Trans. R. Soc. B.
doi:10.1098/not yet assigned

Modelling the prebiotic origins of regulation & agency in evolving protocell ecologies

Ben Shirt-Ediss[1,2], Arián Ferrero-Fernández[2,3], Daniele De Martino[3,4], Leonardo Bich[2], Alvaro Moreno[1] and Kepa Ruiz-Mirazo[2,3]*

[1]*Donostia International Physics Centre (DIPC – CSIC, UPV/EHU), San Sebastián, Spain*
[2]*Department of Philosophy, IAS-Research Centre for Life, Mind and Society, University of the Basque Country (UPV/EHU), Donostia-San Sebastián, Spain*
[3]*Biofisika Institute (CSIC, UPV/EHU), Leioa, Spain*
[4]*Ikerbasque Foundation, Bilbao, Spain*

Keywords: Prebiotic systems evolution, minimal metabolism, ecopoiesis, regulation, adaptive agency

With thanks to co-authors:

Kepa Ruiz-Mirazo

Alvaro Moreno

Leonardo Bich

Daniele De Martino

Arián Ferrero

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