ERC Starting Grant 2020
Research proposal [Part B1]
(Part B1 is evaluated both in Step 1 and Step 2,
Part B2 is evaluated in Step 2 only)

[redacted]

EnBioSys

Cover Page:

Principal Investigator: [redacted]
Host Institution: **Physics of Life Excellence Cluster, TU Dresden**
Proposal Duration: **60 months**

Proposal Summary

All living systems function out of thermodynamic equilibrium and require a continuous supply of energy. To understand how cells and organisms function, we need to determine how metabolic energy is partitioned among the complex array of cellular processes that are necessary for life at any scale, from isolated biochemical networks to quiescent and highly proliferative cells to organismal growth and development. To investigate the energetics of living systems, I established calorimetry to measure the energy exchanged in form of heat between biological systems and their environment. By combining these measurements with specific perturbations, I have shown that the energetic costs associated with a given biological process can be calculated, and thus, provides a means towards understanding the energetics of biological systems. This proposal aims to understand the energetic costs of accurate cell signaling, and of homeostasis, proliferation and growth of cells and organisms. It will further investigate how these biological systems are governed by energetic trade-offs. First, the trade-off between energy dissipation and accuracy of biochemical signaling pathways. Second, the trade-off between power and yield during cell growth and organismal development. Specifically, I will:

1) Develop approaches to quantify the overall energetics of biological systems
2) Elucidate the role of energy dissipation on the accuracy and reproducibility of cell cycle signaling
3) Determine how energetics drive embryonic development and cell growth

This work will overcome the current lack of non-invasive techniques to quantitatively measure metabolic rates, especially rates of energy conversion and dissipation in biological systems. The results will yield quantitative thermodynamic data needed to determine the energetics of biological systems and will be essential for kinetic growth studies of normal and diseased systems.

Significance and proposal overview

Living biological systems are metabolically active, open systems that constantly exchange matter and energy with their environment. They function out of thermodynamic equilibrium and continuously use metabolic pathways to obtain energy from chemical bonds derived from nutrients to fulfil the systems energetic requirements to stay alive, grow, and develop. Although the major biochemical networks in metabolism were identified in the 1950s, understanding how and why biological systems organize their metabolism in a particular manner remains a major challenge that has led to the re-emergence of metabolism as a quantitative field of study [1].

Work over the last decades shows that change in the metabolic state of the cell is important for cell differentiation and proliferation. For example, the shift from oxidative metabolism to aerobic glycolysis promotes growth in many cancers [2-4], and has also been implicated in normal tissue growth [5,6]. How and why many cells of various organisms often use this specific metabolic strategy to grow is currently debated [7-12]. These studies have highlighted the principle(s) of how metabolism allows cells to proliferate at the level of biochemical networks within cells. However, they lack quantitative thermodynamic data and a general framework for the energetic principles that maintain and govern cellular states. How much energy does a cell require to stay alive and maintain a non-equilibrium state? An organism? How much more energy does that organism need to grow and develop? How efficiently used is the energy required for various cellular processes? These crucial aspects of biological systems are not understood, in part due to the difficulties of measuring thermodynamic quantities such as rates of energy uptake, conversion and dissipation *in vivo.*

I aim to identify the energetic principles of organismal development, cell growth and cellular signaling pathways on the molecular level. To do so, Objective 1 will overcome obstacles in measuring energetics by establishing novel, non-invasive biophysical methods using calorimetry. The non-invasive nature of this approach enables adaptation to biological systems on different scales, from reconstituted biochemical networks to different cell types, organoids, *ex vivo* cultured tissues to small aquatic and terrestrial organisms. Objective 2 will measure the energetic cost of accurate and reproducible signaling necessary to allow for robust cell cycle progression. It will shed light on how biological signaling systems use energy dissipation to function robustly against variations in their underlying biochemical parameters and environment. Objective 3 will determine the energetic costs of maintaining a non-equilibrium steady-state and contribute new knowledge to understand the energetic costs of building a new cell and organisms. It will generate quantitative thermodynamic data of the energetic driving force of growth as well as energy conversion in form of newly grown biomass which is essential for kinetic growth studies of normal and diseased systems.

Background and approach

Living systems can be represented as dissipative structures, which are open, non-equilibrium thermodynamic systems that use metabolism to obtain energy and matter from nutrients [13,14]. Metabolism, a set of life-sustaining, enzyme-catalyzed chemical reactions ultimately drive the systems energetics. Here, the system energetics is defined as: the energy flux into new biomass and into usable energy that continuously drives various essential cellular processes that keep the system away from chemical equilibrium and facilitate growth. The energy which is not converted into new biomass is ultimately dissipated to the environment giving rise to the system net metabolic rate.

From a thermodynamic perspective, metabolism can be regarded as an energy converter [26-28], which, conceptually, can be divided into two parts. One is "energy yielding" *catabolic* reactions, i.e. the combustion/oxidation of substrates to CO_2 and H_2O. And the other is "energy converting" *anabolic* reactions, the biosynthetic reactions required for the production of new biomass (Fig. 1). Most living systems are growing non-steady state systems that convert energy in newly formed biomass over time. In order for growth to occur at a finite rate, a thermodynamic driving force must exist in the form of free energy dissipation by the system. This reduction in free energy produces heat at a certain rate, which equals the net change in enthalpy of the all reactions taking place in the system. It represents the enthalpic rate of energy dissipation due to the net balance of catabolic and anabolic reactions and can be measured using calorimetry.

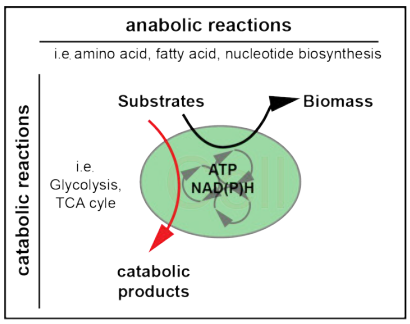

Figure 1. Schematic representation of metabolism of biological systems, conceptually divided in catabolic (i.e. reactions glycolysis and TCA cycle) and anabolic reactions (i.e. amino acid, fatty acid and nucleotide biosynthesis).

To investigate the energetic principles of biological systems two approaches can be chosen. First, a molecular approach, which aims to

determine the flux of matter and energy through each of the possible metabolic reactions. This approach involved measurement of steady state metabolite levels and their flux through metabolic pathways to constrain computational models to ultimately infer the flux of matter within biological systems [15-19]. Because the number of reactions is large, knowing all of the details either through measurement or inferred metrics is cumbersome [20]. Thus, studying the energetics of biological systems requires a certain degree of abstraction to reduce the complexity to a level where key parameters that sufficiently describe the system energetics can be estimated. Alternatively, a second, coarse-grained approach, assumes the system is a "black box" where its energetics can be inferred by the input and output fluxes of energy and matter [14,21-25]. Varieties of this approach originate in ecology and bioengineering on different scales, but have not been applied to fundamental questions in cell and developmental biology.

Previous work in bacteria, yeast, plants and Chinese hamster ovary cells has shown that combined measurements of heat dissipation by calorimetry, O_2 consumption, CO_2 production and the construction of mass, enthalpy and redox balances is a powerful tool to infer rates of energy dissipation, and the catabolic and anabolic reactions of growing systems [33-36]. This approach can provide quantitative thermodynamic information on rates of catabolic and anabolic reactions as well as biomass yield and efficiencies of growth. The yield of a growing system may be increased by increasing the amount of synthesized biomass relative to the amount energy consumed by catabolism. But, as the net energy dissipation, and thus the combined driving force decreases, the whole process is slowed as predicted by non-equilibrium thermodynamics [28,37-39]. Thus, the rate of growth should increase with its energy dissipation at the cost of yield, an energetic trade-off between power and yield. In other words, growth can, from an energetic perspective, be "fast and wasteful" or "efficient and slow".

In [redacted] laboratory at [redacted] University, I have focused my postdoctoral research on establishing approaches to the quantitatively measure the energy exchanged between biological systems and their surroundings, and to investigate the energy costs the of building a new organism. I established isothermal calorimetry to quantitatively measure the flow of energy in form of heat between developing vertebrate embryos and their surroundings with high sensitivity and temporal resolution.

I discovered that the overall heat dissipation increased by ~40% during cleavage stage development suggesting an increase in energetic costs associated with the expansion in the number of cells in the absence of volumetric growth (Fig. 2). My work demonstrated that heat increase scaled with total cell surface area rather than total cell number and the calculated costs of maintaining and assembling plasma membranes and associated proteins probably accounts for a significant proportion of the heat increase [40]. This work showed that the cell's membrane is likely to contribute significantly to the total energy budget of the early embryo.

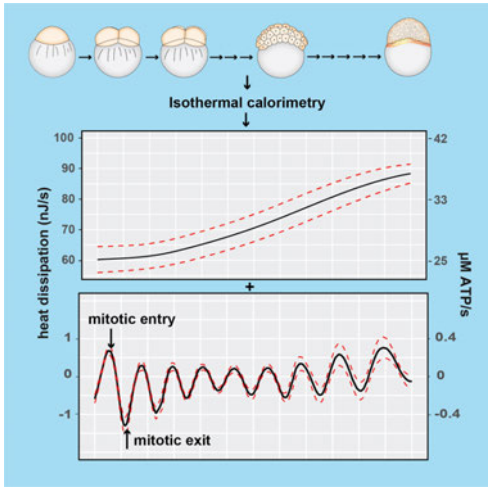

Figure 2. Isothermal calorimetry reveals increasing and oscillatory heat dissipation in conjunction with synchronous cleavage divisions during early embryogenesis.

By further investigating the heat dissipation of early zebrafish development, I discovered prominent heat flow oscillations, with periods matching the synchronous early reductive cleavage divisions, that persisted even when DNA synthesis and mitosis were blocked by inhibitors (Fig. 2). Instead, the heat flow oscillations were driven by the phosphorylation and dephosphorylation reactions catalyzed by the cell cycle oscillator, the biochemical network controlling mitotic entry and exit [41]. Thus, this work revealed the energetic costs of cell cycle signaling and was one of the first to measure the energy dissipated in form of heat by biochemical signaling networks. The energetic cost of cell cycle signaling is many orders of magnitude higher than predicted by theoretical approaches, indicating that coordinating the cell cycle spatially as well as temporally over large embryonic volumes with precision reflects a high energetic burden on cell proliferation during development.

I have since begun to expand the heat dissipation measurements to other aquatic species and mammalian tissue culture systems. Preliminary data from this work shows that the heat dissipation by a single xenopus embryo undergoing development can be measured (Fig. 3A). The single embryo measurements are a necessary advance for the work proposed Objective 2. In addition, I have tested a commercially available calorimeter that is modular and allows for media perfusion, atmosphere control and the insertion of different measurement probes. This work revealed that tissue culture cells can be maintained within the calorimeter vessel and preliminary data in Fig. 3B shows that the heat dissipated by synchronized HeLa cells is consistent with dynamic changes in the cell's energetics during the cell cycle.

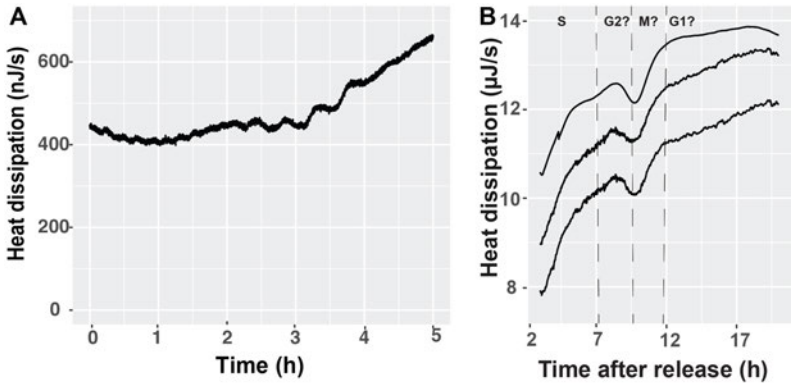

Figure 3 (A) Heat dissipation of a single *X. laevis* embryo undergoing embryonic development plotted against time. **(B)** Heat dissipation of 3 HeLa cell populations seeded at different densities after release from G1/S arrest. Dotted lines indicate possible cell cycle phase transitions based on time after release

Together, my postdoctoral findings reveal that quantitative heat dissipation measurements combined with perturbation experiments and theoretical modeling is a powerful approach to dissect the energetic costs of various cellular processes in biological systems. This work forms the basis of this proposal, which aims to expand this approach to investigate fundamental questions about the energetics of biological systems. My long-term research goal is to understand the energetic costs of building a new cell and of building a new organism, and determine the energetic costs of a non-equilibrium state. I am particular interested in understanding how cell growth, organismal development and cellular signaling pathways on the molecular level are governed by energetic trade-offs. First, the trade-off between energy dissipation and accuracy of biochemical signaling pathways. Second, the trade-off between power and yield during cell growth and organismal development. I aim to develop a quantitative approach to measure the energetic driving force of growth as well as energy conservation in form of newly grown biomass and thus growth of the system. Specifically, I propose the following Objectives:

Objective 1: Develop approaches to quantify the overall energetics of biological systems

I have shown that quantitative heat dissipation measurements combined with perturbation experiments and theoretical modeling can be used to infer the energetic costs of various cellular processes [41]. However, heat dissipation measurements alone are insufficient to determine the overall energetics of biological systems in terms of rates of energy conversion by catabolism and rates of energy conservation in newly formed biomass by anabolism. Thus, I will use an engineering approach to establish a bioreactor-like system within a commercially available calorimeter or in parallel to measure additional parameters sufficient to determine the energetics of biological systems. I aim to measure byproducts of metabolism, namely heat, CO_2, NH_3, and lactate production, as well as O_2, glucose and glutamine consumption by calorimetry, microrespirometry, and biochemistry (Fig. 4).

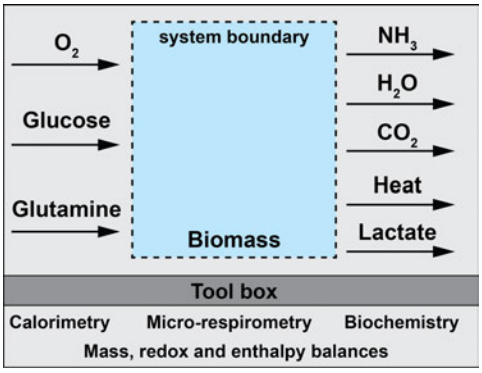

Figure 4 Conceptual schematic open system exchanging energy and matter with the environment. The input and output parameters will be measured and analyzed by methodologies displayed in the toolbox to infer the systems energetics.

These measurements can be used by two complimentary enthalpy balance models with different levels of abstractions to study the energetics of biological systems. First, measurements of CO_2 and O_2 can be used to describe the enthalpy changes for anabolism and catabolism based on a redox balance of the systems. Three important ratios can be calculated from these measurements: the respiratory quotient r_{CO2}/r_{O2}, and the calorespirometric ratios: r_Q/r_{O2} and r_Q/r_{CO2} [30]. The respiratory quotient can indicate the oxidation state of the substrates consumed [29]. At steady state in non-growing systems, the calorespirometric ratios, r_Q/r_{O2} and r_Q/r_{CO2} are related to Thornton's constant (ΔH_{O2}, the enthalpy of organic compound combustion is approximately constant when expressed per mole O_2). release). Importantly, deviation of the measured calorespirometric ratios from steady state conditions indicates a non-steady state, growing system and provides quantitative thermodynamic information on the rate of anabolic reactions and the overall yield of the growth

(Table 1) [30,31,42,43]. Measurement of pH changes and lactate production can be used to expand this approach to systems with mixed catabolic metabolism, comprised of oxidative phosphorylation and aerobic glycolysis.

Table 1: Measurement and analysis of energetic parameters

Measure	Calculate	No growth	Growth	Infer
Heat dissipation rate (R_Q)	r_{CO_2}/r_{O_2}	= 0.7-1	= <0.7-1>	oxidation state of the substrate
O_2 consumption rate (R_{O_2})	r_Q/r_{O_2}	$= \Delta H_{O_2}$	$\neq -\Delta H_{O_2}$ $r_Q = -\Delta H_{O_2} * r_{O_2} - r_a * \Delta H_{an}$	Catabolic & anabolic reactions, metabolic paths
CO_2 production rate (R_{CO_2})	r_Q/r_{CO_2}	$= \Delta H_{CO_2}$ $= (1-y_s/4)\Delta H_{O_2}$	$\neq -\Delta H_{CO_2}$ $r_Q = -\Delta H_{CO_2} * r_{CO_2} - r_a * \Delta H_{an}$	Catabolic & anabolic reactions, metabolic paths, carbon yield

Where R_{CO_2}/R_{O_2} is the respiratory quotient, R_Q/R_{O_2} & R_Q/R_{CO_2} are the calorespirometric ratios, ΔH is enthalpy, ΔH_{O_2} is Thornton's constant (-455kJ mol^{-1} O_2), ΔH_{CO_2} is enthalpy of combustion mol^{-1} CO_2, and y_s is the chemical oxidation state of the combusted substrate, ΔH_{an} is the enthalpy of combustion of biomass, r_a is the rate of anabolic reactions, "No growth" refers to the system at steady state. "Growth" refers to the system at non-steady state.

Second, this redox balance model can be expanded by measured rates of non-gaseous substrates and products. This approach describes the formation of biomass by anabolic reactions relative to the extent of catabolic reactions by stoichiometry models based on mass and enthalpy balances [24,36,44,45]. It assumes that a biological system can be sufficiently determined by a macrochemical equation with stoichiometric coefficients for the individual components. The stoichiometry of this equation, for any particular system, can be derived by defining the system input and output using the C, H, O, N and charge balances. This reaction can be formulated to include heat dissipation which is directly related to the stoichiometric coefficients. This allows analysis of the system's thermodynamics and provides a means to test the validity of the chemical reaction by an enthalpy balance approach [44,45]. *Together, these approaches will overcome the current lack of non-invasive techniques to quantitatively measure metabolic rates, especially rates of energy conversion to biomass and energy dissipation in growing system and, thus, determine the energetic driving force, power and yield of the system. The non-invasive nature of this approach enables adaptation to biological systems on different scales, from different cell types, organoids, ex vivo cultured tissues and embryos to small aquatic and terrestrial organisms. It has the potential to generate quantitative thermodynamic data essential for kinetic growth studies of normal and diseased systems.*

Objective 2: Understanding the role of energy dissipation on the accuracy and reproducibility of cell cycle signaling.

Theoretical studies have estimated the amount of energy dissipation needed to drive biological oscillators accurately and reproducibly, such that the period and phase remain relatively constant over time. The embryonic heat flow measurements show that many orders of magnitude more energy is dissipated than predicted by theoretical approaches and likely reflect the thermodynamic burden of imposing accurate and robust timing on cell proliferation during development [46]. This raises the question of how much energy do biochemical signaling systems cost *in vivo* and what is the role of energy dissipation by these systems? Objective 2 investigates this phenomenon by using the embryonic cell cycle oscillator as a model system. To do so, I work with biophysicist Jonathon Howard (Yale University) and started to collaborate with theorist Yuhai Tu (IBM, T.J Watson Research Center). We aim to develop a theoretical model to predict the energetic costs of coordinating the cell cycle over large embryonic volumes in time and space, and to identify targets to manipulate the amplitude, phase and period of the heat dissipation oscillations. This approach will be complemented by experimental approaches in my lab. I will measure heat dissipation oscillations by a single embryo frog undergoing development (Fig. 3A). This is necessary to quantify the variances in oscillatory heat dissipation amplitude, phase and period. Furthermore, I aim to establish a frog cycling egg extracts as a manipulative biochemical system. I will implement perturbations predicted by the theoretical model to manipulate the heat oscillation amplitude, phase and period *in vitro* (in embryo extracts) and *in vivo* (in embryos) and to test their impact on the embryonic cell cycle. *I aim to understand the energetic cost of accurate and reproducible signaling necessary to allow for robust cell cycle progression. Thus, this work will shed light on how biological signaling systems use energy dissipation to function robustly against variations in their underlying biochemical parameters and environment. This will allow future work to investigate how energy dissipation by the cell cycle oscillator affects downstream mitotic processes such as DNA damage or spindle assembly in wild-type conditions and where the cell cycle oscillator is energetically compromised. Furthermore, it will enable studies on how mitochondrial energetics influence the function and accuracy of cell cycle signaling. This can be achieved by heat dissipation measurements of embryonic extracts with added and purified wild-type and energetically compromised mitochondria e.g. mitochondria harboring known disease-associated DNA mutations.*

Objective 3: Determine how energetics drive embryonic development and cell growth

The goal of this objective is to determine the energetic costs of maintaining a non-equilibrium steady-state for quiescent non-growing cells and not developing unfertilized oocytes, and the costs of building a new cell and organism in growing and developing systems. Furthermore, I hypothesize that cell growth and organismal development is governed by an energetic trade-off between power and yield so that these processes are sufficiently fast and efficient to support life in a given environment. To investigate this, my lab will use two model systems. **1)** Zebrafish embryos and unfertilized oocytes as a model for organismal development. **2)** Quiescent and heterogeneously or synchronously growing tissue culture cells as a model system for cell maintenance, growth and proliferation. Preliminary data in Fig. 3B indicates that heat dissipation measurements from synchronized HeLa cells is consistent with dynamic changes in the cell's energetics during the cell cycle. For both systems and conditions, my lab will define the systems energetics by the following classes of measurable parameters: *1)* ***thermodynamic****, i.e. rates of energy dissipation, energy conversion, and energy conservation, power, yield (See objective 1)*: These results will be augmented by 2) ***morphometric*** *i.e. cell number, growth rate, mass, volume, and surface area*; 3) ***biosynthetic*** *i.e. biomass composition, total organic carbon yield of growth*. The result of these experiments will determine a basal energetic state for unfertilized oocytes and quiescent cells and how this basal state changes in growing and developing systems. We will relate these measurements to overall rates of the systems, such as organismal development, cell proliferation, the time period of different cell cycle phases. We further aim to combine this approach with two types of perturbation experiments and theoretical modeling. The first is phenomenological in nature and aims to determine how the energetics of non-growing and growing systems change in response to environmental perturbations such as temperature, pressure, oxygen levels, pH, energy availability in nutrients, and growth factor concentrations. The second aims to expand the systems energetics to the molecular scale by allocating the measured energy dissipation to the energetic costs of various cellular processes by chemical and genetic perturbations. *This work will determine the energetic costs of building a new cell and of building a new organism from the bottom up, and determine the energetic costs of a non-equilibrium state. Long-term, my lab will learn whether an energetic trade-off between power and yield explains the different rates of development, stem cell differentiation, and cell cycle times between different cell types, between normal versus diseased cells, and within and between species. For example, I predict that fast proliferating cells such as cancer cells are thermodynamically optimized for power at the cost of yield. Similarly, different thermodynamic optima might be able to explain why the evolutionary conserved process of stem cell differentiation occurs at different rates in related species. Fundamental knowledge of embryonic energetics may be used in future to predict in-vitro fertilization outcome, improve culture conditions, and provide therapeutic approaches to fertility.*

Risks and innovation

I have intellectual expertise in developmental biology, metabolism, signaling and quantitative biology and technical proficiency in calorimetry, imaging, respirometry, metabolite quantification and genetic manipulations, which will ensure successful execution of the proposed work. I have long standing collaborations with theorist which is vital for the theoretical physics aspect of this work. This proposal can be characterized as a high risk, high reward and part of the achievements in Objective 3 rely on a bioengineering approach to expand the calorimetry in Objective 1. However, this effect is mitigated by the fact the already established heat dissipation measurements alone combined with perturbation and theoretical estimates is a powerful tool to dissect the energetic costs of cellular processes during cell growth and organismal development. A detailed risk analysis follows in Part B2.

This proposal is innovative in multiple ways. Through an interdisciplinary approach, this work will establish a direct, non-invasive measurements to investigate energetics of biological systems. The innovation results from the combination of traditional metabolic rate measurements with heat dissipation measurements, which can quantify the amount of energy conserved in newly grown biomass per unit time. Current state of the art techniques to measure metabolic rates which are non-invasive are lacking this information. Second, this work will derive a thermodynamic description of cell growth and organismal development generating fundamental information about the how biological systems are driven by their energetics. This approach and the derived energetic parameters have applicability in physics, computational and systems biology and can complement current research in metabolomics, which centers on interconnected biochemical reactions and signaling pathways.