# Evolution of Antibiotic Resistance under Treatment: from *in-vitro* Insight to the Clinic and back

Irit Levin Reisman, Jiafeng Liu and Nathalie Q. Balaban

The evolution of antibiotic resistance in microorganisms is a major heath issue. Understanding the evolutionary trajectories from susceptibility to resistance and the factors that affect it is crucial. Our results show that tolerance, a form of survival under antibiotics that is distinct from resistance, plays a major role in promoting the evolution of resistance *in vitro*. In order to determine the relevance of the *in vitro* experimental evolution results for the clinic, we followed the course of infections in patients. A striking similarity between the *in vitro* and in host evolution is observed. However, further dissection of the response of the clinical strains to combination of antibiotics reveals a new way by which resistance is strongly promoted by the tolerance phenotype.

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# Dynamics of confined cell migration

David Brückner[1], Alexandra Fink[2], Christoph Schreiber, Joachim Rädler, Chase broedersz[3]

**In many biological phenomena, cells migrate through confining structured environments. We study how migrating cells overcome physical obstacles in the form of a thin constriction. Specifically, we ask whether such confined migration exhibits emergent stochastic dynamical laws. To this end, we develop two-state micropatterns, consisting of two adhesive sites connected by a thin constriction, allowing the cells to perform repeated stochastic transitions between the sites. For this minimal system, we obtain a large data set of single cell trajectories, enabling us to infer an equation of cell motion, which decomposes the dynamics into deterministic and stochastic contributions. Our data-driven approach reveals that these cells exhibit intricate non-linear migratory dynamics, with qualitatively similar features for cancerous (MDA-MB-231) and non-cancerous (MCF10A) cells. In both cases, the cells drive themselves deterministically into the thin constriction, a process that is sped up by noise. Interestingly, the deterministic dynamics of the cancerous cells exhibits a limit cycle, while the non-cancerous cells show excitable bistable dynamics.**

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# Dynamics, Noise, and Antibiotic Resistance in Single Cells

Mary J. Dunlop

The majority of our understanding on antimicrobial drug resistance comes from studies on the genetic changes that cause it, however bacteria can also transiently survive antibiotic exposure even without permanent genetic changes. Using a combination of time-lapse microscopy experiments and stochastic modeling I will show how *E. coli* bacteria use feedback to generate dynamics and noise in expression of a key regulatory protein, providing transient antibiotic resistance at the single-cell level. In addition, I will discuss how expression of resistance genes can predispose cells towards mutation. Our findings show that even in the absence of antibiotic exposure, cells that are transiently resistant are more mutation prone, suggesting that these transient mechanisms may act as a stepping stone towards higher levels of drug resistance. These results are significant because they reveal important dynamic information about the period over which transient resistance develops and ultimately how it can lead to permanent genetic changes encoding multidrug resistance.

# Mutation dynamics and fitness effects followed in single cells

Lydia Robert[1], Jean Ollion[2], and Marina Elez[3]

**Mutations have been investigated for more than a century but never witnessed in action in single cells, thus preventing direct characterization of their dynamics and reliable estimation of the distribution of their fitness effects. Such estimates have wide application and are critical for most evolutionary models/studies. We accomplished this in *Escherichia coli* by employing microfluidics, time-lapse imaging, and by visualizing mutations in single cells by using a fluorescent tag of the Mismatch Repair System. I will present our results obtained for strains having a wide range of mutation rates and growing under controlled condition in the absence of exogenous stress.**

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# Multiscale model predicts dynamics of metabolic reprogramming in tumor spheroids

Mahua Roy[1] and Stacey D. Finley[2]

Mathematical modeling provides the predictive ability to understand the metabolic reprogramming and complex pathways that mediate cancer cells' proliferation. We present a computational model [1] to predict the impact of intracellular metabolism on tumor growth. The model spans multiple time and length scales to recapitulate tumor growth: a detailed kinetic model of intracellular metabolite dynamics [2] on the order of minutes, reaction diffusion equations describing extracellular nutrient concentrations that change on the order of seconds, and cellular behavior including adhesion, proliferation, viability and cell state transitions, occurring on the timescale of hours. Through our model simulations, we investigate the responses of individual intracellular species under metabolic perturbations and quantify how those responses contribute to the response of the tumor as a whole. Excitingly, the model provides quantitative insight into the dynamic reprogramming of tumor cells at the intracellular level in response to specific metabolic perturbations. Overall, the model is a useful framework to study targeted metabolic strategies for inhibiting tumor growth.

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Acknowledgements: This work was funded by The Rose Hills Foundation and the USC Provost's Office (research grant to SF).

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# Building Tissues to Understand how Tissue Build Themselves

Zev J. Gartner[1]

**The capacity of cells to self-organize into tissues is critical to their normal developmental and their ability to self-repair. Thus, a better understanding of how tissues self-organize will improve our ability to build tissues and organs in the lab, and suggest new strategies to slow the breakdown of tissue structure that contributes to the initiation and progression of disease. We are working to understand the mechanisms used by cells to self-organize robustly by measuring the properties of individual cells, then linking these properties to collective behaviors in tissues. We focus on self-organizing programs in the breast and gut, and how these programs are susceptible to the perturbations that underlie diseases such as cancer.**

# What can single-cell profiling tell us about blood cancers?

Sahand Hormoz[1,2]

**In some types of cancer, the same genetic alteration can result in drastically different disease phenotypes in different patients. For example, myeloproliferative neoplasm (MPN), arises when a hematopoietic stem cell (HSC) acquires a somatic driver mutation. The mutated HSC thereby gains a proliferative advantage over the other HSCs and gives rise to a disproportionately large population of differentiated blood cells that carry the same mutation. Intriguingly, the same somatic point mutation in the *JAK2* gene can give rise to an increase in the number of red blood cells, an increase in the number of platelets, or scarring of the bone marrow tissue, in different patients. This disconnect between genotype and phenotype is partly because the same mutation can have different consequences depending on the identity of the stem cell in which the mutation first occurs and the extent to which the population of mutated stem cells subsequently expands in each patient. Different HSCs –even within the same patient– can exhibit very different proliferation and differentiation dynamics. In addition, the dynamics of the expansion of the cancer stem cell population is potentially strongly stochastic; the population grows and shrinks from random fluctuations. I will discuss our recent efforts to understand this complex disease using emerging single-cell technologies and quantitative modeling.**

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# Together is better than alone: Modulating transcription elongation efficiency through RNA polymerase group dynamics

Sangjin Kim[1, 2] and Christine Jacobs-Wagner[1]

**Transcription by RNA polymerases (RNAPs) is essential for cellular life. Here, we provide in vitro and in vivo evidence that co-transcribing RNAPs display either collaborative or antagonistic group dynamics over long distances through transcription-induced DNA supercoiling. Our findings suggest a model in which RNAPs assist each other's motion by relieving torsional stress, largely independently of promoter strength and thus RNAP density, as long as cancellation between positive and negative DNA supercoils can be achieved. Promoter repression reduces the apparent speed and processivity of RNAPs over two kilobases downstream of the promoter due to accumulated negative DNA supercoils, quickly arresting the synthesis of proteins that are no longer needed. Antagonistic RNAP dynamics can also be intergenic such that transcription of a highly-expressed gene can dampen the transcription elongation rate of a divergently-transcribed gene. Our findings may be broadly applicable given that transcription on topologically-constrained DNA is the norm across genes and organisms.**

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# Single-cell measurement and control to unravel yeast gene expression heterogeneity

Kieran Sweeney[1] and Megan N. McClean[1]

**From microbes to cancer, variability in gene expression can lead to nongenetic phenotypic heterogeneity. This heterogeneity is important in determining how populations of cells grow, survive fluctuating environments, and develop drug resistance. For example, individual yeast cells within isogenic populations show striking heterogeneity in stress tolerance. Though genetic forces (*e.g.* mutation) determining population heterogeneity are well appreciated, non-genetic forces (*e.g.* stochastic gene expression) have been less thoroughly elucidated. Recently, we used single-cell RNA sequencing to quantify transcript heterogeneity in *Saccharomyces cerevisiae* cells treated with and without salt stress to explore population variation and identify cellular covariates that influence the stress-responsive transcriptome [1]. There is significant regulatory variation in individual yeast cells, both before and after stress. Heterogeneity in the expression of transcription factor targets implicated regulatory variability in establishing population-level heterogeneity. Live-cell imaging of cells expressing pairs of fluorescent regulators, including the transcription factor Msn2 with Dot6, Sfp1, or MAP kinase Hog1 revealed coordinated and decoupled nucleocytoplasmic shuttling. The live cell imaging coupled with analysis of the single-cell expression data suggests that cells may filter decoupled bursts of transcription factor activation but mount a stress response upon coordinated regulation, even in a subset of unstressed cells. We have developed an optogenetic toolkit that allows us to construct light-activated transcription factors. Using these transcription factors, we are working to resolve the relationship between bursts of transcription factor activity, burst coordination, and gene expression leading to population-level heterogeneity**

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# Stochastic nature of bacterial eradication using antibiotics

Jessica Coates[1], Bo Ryoung Park[2], Dai Le[2], Emrah Simsek[2], and Minsu Kim[1,2,3]

**Frequent antibiotic failure is a serious threat to public health. To cope with this threat, it is critical that we betterunderstand population dynamics of bacteria exposed to antibiotics. In this talk, I will present our recent laboratory studies showing stochastic nature of bacterial eradication using antibiotics. Bactericidal drugs induce population fluctuations, leading to stochastic population dynamics. Consequently, bacterial clearance does not follow a deterministic course but is highly probabilistic. These population fluctuations may be manipulated to facilitate bacterial eradication.**

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# Transport network in living systems by current-reinforcement rule

Toshiyuki Nakagaki[1],

**A kind of huge amoeboid organism named Physarum plasmodium constructs an intricate network of veins for circulating nutrients and signals over the entire body. The network shape (topology of connectivity, and sequence of branching in vein network, for instance) is drastically re-organized within hours in response to external conditions. The past studies showed that the network shape was optimized to maximize possibility of survival, in some senses. So we may extract an algorithm for optimal design of functional network from the primitive organism. The key thing is adaptive dynamics of current-reinforcement rule: each vein of network becomes thicker when current is large enough through the vein itself, while it becomes thinner and dies out otherwise. We propose the equations of motion for this simple rule, and functions and formation of transport network is analyzed. We will show that the rule is applicable to the other bio-systems: (1) social dynamics of public transportation, (2) formation of network structure in sponge bone (bone remodeling in other words). A tractable perspective to think similarly of a variety of bio-network is given from the viewpoint of current-reinforcement rule.**

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# Automated, predictive, and interpretable inference of phenomenological models of biological dynamics

Ilya Nemenman[1]

**The cost of an empirical bit in biophysics has fallen dramatically, and high-precision data are now abundant. However, biological systems are notoriously complex, multiscale, and inhomogeneous, so that we often lack intuition for transforming such measurements into theoretical frameworks. Modern machine learning can be used as an aid. I will discuss our Sir Isaac platform for automatic inference of phenomenological models of complex dynamics from noisy time series, even if the dynamics are nonlinear, and only a few of the relevant variables are measured. I will illustrate the method on applications to toy problems, including inferring the iconic Newton's law of universal gravitation, as well as a few synthetic biochemical reaction networks. Finally I will illustrate how the approach has been used for automatic construction of a model of *C. elegans* escape dynamics, which is more accurate than that curated manually, is biophysically interpretable, and makes nontrivial predictions about the system.**

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# Model-guided evolution of microbial species and communities

Paula Jouhten[1,2], Dimitrios Konstantinidis[1], Natalia Gabrielli[1], Filipa Pereira[1], Ramon Gonzalez[3], Pilar Morales[3] and Kiran R. Patil[1]

**Directed evolution of metabolic networks in microbial species and communities is much desired in several biotechnological and environmental applications. This, however, is difficult in the case of complex traits that are not directly growth/fitness associated. Towards addressing this challenge, I will present two approaches that my lab is presently working on: engineered exaptation and synthetic mutualism. Both approaches make use of genome-scale metabolic models to identify optimal nutritional environment for adaptive laboratory evolution experiment so as to exert a strong selection pressure on the desired metabolic traits. I will discuss the underlying theory, algorithmic implementation, and our first experimental results based on case studies involving wine yeasts and lactic acid bacteria.**

Acknowledgements: This work was partly sponsored by the German Ministry of Education and Research (grant 031A601B and 031A605)
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# Single-cell bacterial electrophysiology

Ekaterina Krasnopeeva[1], Leonardo Mancini[1], Uriel Perez Barbosa[1] and Teuta Pilizota[1]

**Electrochemical gradient of protons, or proton motive force (PMF), is at the basis of bacterial energetics. It powers vital cellular processes and defines the physiological state of the cell. Here we use an electric circuit analogy of an *Escherichia coli* cell to mathematically describe the relationship between bacterial PMF, electric properties of the cell membrane and catabolism. We combine the analogy with the use of bacterial flagellar motor as a single-cell "voltmeter" to measure cellular PMF in varied and dynamic external environments. For example, we apply several different stresses and find that butanol acts as an ionophore, and we functionally characterise membrane damage caused by the light of different wavelengths. We next investigate, from the point of view of energetics, differences between dormancies of *E. coli*, all thought to be low-energy states, and suggest energy maintenance strategies that are emerging from our results. Our approach coalesces non-invasive and fast single-cell voltmeter with a well-defined mathematical framework to enable quantitative bacterial electrophysiology.**

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Acknowledgements: This work was funded by HFSP grant RGP0041/2015.

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# Single cell analysis in cancer

Benjamin L. Emert, Eduardo Torre, and Arjun Raj

***Short Abstract* — Cancer treatment often involves the use of drug that kill most, but not all cancer cells. These remaining cells often contribute to therapy resistance, leading to relapse. We use quantitative experimental methods to reveal the ways in which single cells survive therapy. These mechanisms may be exploited to make new treatment regimens designed to prevent therapy resistance.**

***Keywords* — Single cell biology, cancer.**

Cancer is a disease that originates from single cells, and the treatment of cancer also is a problem of single cells: anti-cancer therapies can often kill the vast majority of tumor cells but a few rare cells remain and grow despite treatment. Often, it is thought that the underlying basis for the behavior of these rare cells is a genetic difference. However, we and others have shown that non-genetic differences may be a key driver of rare, drug resistant cells, yet the precise molecular nature of these differences often remains mysterious. We here describe the development of a cellular "time machine" that allows us to link the ultimate cellular fate to the initial cellular state on a single cell basis, thus revealing markers for pre-resistant cells in the population. Further, we use genetic screening technologies to elucidate the pathways that control the formation of these rare cells and discuss their therapeutic implications.

# Real-time visualization of the inception of drug tolerance in single melanoma cells

Chen Yang[1,2], Chengzhe Tian[1], Nicole Jacobsen[1],and Sabrina L. Spencer[2]

***Short Abstract* — Drug resistance is a major problem in cancer therapy. However, little is known about how an initially drug-sensitive population persists in the presence of drug to eventually re-enter a proliferative state in which cells can acquire *bona fide* drug resistance mutations. Here we use long-term single-cell time-lapse microscopy and cell tracking to uncover cell-to-cell variability in signal transduction that enables some cancer cells to evade drug treatment. Our results implicate rapid signal rewiring events in the incomplete response of cancer cells to drug, and identify means to reduce heterogeneity in drug response.**

## I. PURPOSE

SPONTANEOUS genetic mutations allow an initially drug-sensitive population of cancer cells to acquire a drug-resistant phenotype. However, little is known about how drug-sensitive cells first evade drug action and survive in the presence of drug, referred to as 'drug tolerance', a crucial step on the road to resistance. To better understand the timescale of the inception of drug tolerance and the heterogeneity within the drug-tolerant population, we combined single-cell time-lapse microscopy and MATLAB-based automated cell tracking to monitor Dabrafenib-treated melanoma cells harboring a BRAFV600E mutation.

## II. RESULTS

By monitoring single melanoma cells expressing a live-cell CDK2 activity sensor that marks the proliferation-quiescence decision over the first five days of treatment, we discovered that the majority of the cells stop proliferating and remain quiescent for the duration of the drug treatment while a subset of cells escape drug action and occasionally divide in drug. These 'escapees' rapidly revert to the drug-sensitive state upon drug withdrawal, clearly implicating a non-genetic mechanism enabling proliferation in drug.

We further find that Erk is reactivated specifically in escapees several hours prior to cell-cycle re-entry, whereas Erk activity remains low in non-escapees. Consistently, co-treatment with a Mek or Erk inhibitor further inhibits Erk activity and nearly eliminates the escapee population, indicating that MAPK pathway reactivation is required for re-proliferation and that Erk activity is not fully suppressed by Dabrafenib treatment alone. This finding reveals the molecular underpinnings of the benefit of using combination therapy over the mono-therapy in melanoma patients. Profiling the escapee population by single-cell RNA-seq reveals that escapees rely on multiple mechanisms to re-proliferate in Dabrafenib, and that blocking each mechanism reduces the escapee population.

## III. CONCLUSIONS

Together, our findings suggest that rapid signaling pathway rewiring in the first few days of drug treatment drives occasional cell cycling in drug, which enables the acquisition of genetic mutations and permanent drug resistance.

Acknowledgements: This work was funded by a Searle Scholar Award, a Kimmel Scholar Award, and a Beckman Young Investigator Award.

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# Identifying the key control parameters driving collective multicellular signaling and pattern formation

Allyson E. Sgro[1-3]

**One of the key outstanding challenges in understanding coordinated multicellular pattern formation is identifying what single cells tune within themselves to change population-wide patterns. A major driver of multicellular patterns is oscillations in single-cell signaling networks, but it is unknown what features single cells naturally modulate in these oscillations to change global patterns. An ideal system for addressing this challenge exists in the social amoeba, *Dictyostelium discoideum*. *Dictyostelium* uses travelling waves of cyclic AMP as a chemoattractant between cells to drive aggregation into a multicellular state when starving. These waves originate within single cells that release cyclic AMP to the environment, and the single-cell signaling network phenomena that drive the creation of these waves are well-characterized[1]. Using new experimental data in conjunction with an existing phenomenological model[1,2], I explore what parameters single cells can modulate to control the properties of these signaling oscillations and the patterns they coordinate.**

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Acknowledgements: This work was funded by a Burroughs Wellcome Fund Career Award at the Scientific Interface to A.E.S. and NSF Award #1838341

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# Engineering bacterial two-component systems

Jeffrey J. Tabor

**Two-component histidine kinase signal transduction systems (TCSs) are the largest family of multi-step signal transduction pathways and a treasure trove of genetically-encoded sensors for synthetic biology. Bacteria utilize TCSs to sense a remarkable range of chemical and physical stimuli in the environment, and respond by activating appropriate gene expression programs including virulence and antibiotic-resistance pathways. We have computationally identified over 25,000 non-redundant TCSs in bacterial genomes, with a typical organism containing several dozen. Despite the diversity and importance of these pathways, the vast majority remain uncharacterized. Major challenges are that most bacteria cannot be cultured in the laboratory, and that TCS output promoters are typically unknown or highly cross-regulated. We have developed a suite of technologies to overcome these challenges and dramatically improve TCS performance. For example, we have shown that pathway leakiness and dynamic range can be dramatically increased by optimizing the expression levels of the sensor kinase and response regulator proteins [1]. In addition, we have developed a general method for rewiring TCSs to synthetic output promoters, enabling these pathways to be ported between distantly-related host bacteria, converted into one-input/one-output sensors, and subjected to high throughput screens for input discovery [2]. Once functional, we have demonstrated that the input detection thresholds of TCSs can be tuned over more than two orders of magnitude by introducing mutations that specifically alter the secondary phosphatase activity of the sensor histidine kinase, enabling these sensors to be tailored to the application at hand [3]. We have deployed our methods to engineer bacteria that sense nitrate levels in soil, contaminants in seawater, and diagnose colon inflammation [4], among other applications.**

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