Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Systems Biology

Measuring higher-order drug interactions: A review of recent approaches

Elif Tekin[1,2], Van M. Savage[1,2,3] and Pamela J. Yeh[2]

Abstract

Using drugs in combination can be important in therapeutic strategies, both to decrease the risk of toxic side effects as well as to decrease the likelihood of resistance evolving. These properties are largely affected by interactions among drugs. Given the increased use of three or more drugs in clinics, we provide a review of challenges researchers face when studying higher-order drug interactions together with a review of current theoretical and methodological advances in resolving these issues. The challenges include deriving theoretical measures for interaction effects compared with single drug efficacies, disentangling higher-order emergent effects from lower-order interactions, enhancing the resolution of data to better classify interaction types, and considering practical difficulties such as measuring responses to drug combinations across a range of concentrations. The systems biology and interaction modeling approaches reviewed here offer ways to go beyond-pairwise interactions and to quantify and better understand higher-order drug interactions. However, there are many research directions yet to pursue to provide mechanistic insights and to determine the consequences of these interactions.

Addresses

[1] Department of Biomathematics, University of California, David Geffen School of Medicine, Los Angeles, CA 90095, United States
[2] Department of Ecology and Evolutionary Biology, University of California, Los Angeles, CA 90095, United States
[3] Santa Fe Institute, Santa Fe, NM 87501, United States

Corresponding author: Yeh, Pamela J (pamelayeh@ucla.edu)

Current Opinion in Systems Biology 2017, **4**:16–23

This review comes from a themed issue on **Pharmacology and drug discovery (2017)**

Edited by **Lars Kuepfer** and **Tobias Bollenbach**

For a complete overview see the Issue and the Editorial

Available online 22 May 2017

http://dx.doi.org/10.1016/j.coisb.2017.05.015

Keywords

Synergy, Antagonism, Drug combinations, Higher-order interactions, Emergent interactions.

Introduction

Clinical therapeutics for combatting complex diseases such as HIV, cancer, and tuberculosis [1–5] are increasingly moving towards combinations of three or more drugs. The benefit of such therapies may result from higher-order (more than two-drug) interactions that can only emerge and be observed when three or more drugs are present. The complexity of treating many diseases typically arises because pathogens use different metabolic pathways to fuel growth to proliferate and also because of the accumulation of mutations in the course of the pathogen proliferation. As a result of these processes, fitness landscapes for the pathogen can possess many peaks (maxima) and valleys (minima) that determine the potential set of trajectories and strategies for evolutionary adaptation by the invasive pathogen population. Traditional treatment strategies that only consider effects of single or even pairs of drugs will often result in simple fitness landscapes that can be easily evolutionarily navigated by the pathogen. Consequently, traditional strategies often do not eliminate the pathogen and may increase the rate of drug-resistant cases, creating daunting challenges for scientists and clinicians. Focusing on higher-order interactions may help create a path forward.

Combinations of drugs have been shown to provide major advantages in dealing with complex disease dynamics [6–8]. First, use of drugs that work by different mechanisms of action to target different metabolic, genetic, cell wall, or other pathways have been shown to disrupt the progression of certain diseases and increase treatment efficacies [9]. Drugs are referred to as *synergistic* when they enhance the treatment outcome compared to the expectation based on single-drug effects [10,11]. Synergistically interacting drugs are used in the clinic as they help to reduce the toxicity to the patient by requiring less dosages of each drugs in the combination. On the other hand, some drugs interact *antagonistically*, meaning effects of the drugs counteract each other and result in diminished combined effect. Antagonistic combinations are mostly avoided in clinical settings [12,13].

Intriguingly, several studies have shown that more antagonistic drug combinations slow the rate of evolution of drug-resistance [14,15] by creating fitness landscapes that are more difficult to traverse through evolution. These effects are amplified for the rare case of hyper-antagonistic (suppressive) drug combinations in which one drug reverses some of the effects of the other drug. This case is especially effective in terms of the fitness landscape because suppression decreases selection forces that push towards resistant strains because the wild-type (drug-sensitive) strains can outcompete (out-proliferate) the resistant strains [16]. Hence, synergy and antagonism may both be useful in

clinical and scientific settings, and a more nuanced view that incorporates both at multiple levels between multiple drugs may lead to advances. For instance, a desirable fitness landscape might be created by a three-drug combination in which each drug pair interacts antagonistically but the full emergent interaction between all three drugs is synergistic. Such scenarios may help negotiate trade-offs between the treatment efficiency and the evolution of resistance.

For these reasons, it is of paramount importance to map the space of the fitness landscapes that are determined by genetic and drug interactions, and a major component of this is to accurately assess and quantify drug interactions. This mapping will likely be a key part of developing novel treatments. To accomplish this necessitates methods to quantify drug interactions at all levels (2-way, 3-way, 4-way, etc.) and of all types (net and emergent). The first step is to determine what experimental measurements need to be taken. The effectiveness of drug therapies are typically determined by a drug's ability to inhibit the pathogen's growth. This is commonly referred to as relative fitness and denoted as w_D for drug combination D and typically takes values between 0 and 1, corresponding respectively to no-growth (complete death of pathogen and best treatment) and maximum growth (highly proliferating pathogen and the worst treatment) (Figure 1a). Notably, a more sophisticated method in the future might rely on predictive models like Zimmer et al. [17] for higher-order interactions that could enable a great reduction or compression of the measurements and information needed to map the fitness landscape.

The next step is to define what it means to "interact", usually defined based on its converse—what do we expect or predict when there is no interaction. Given a definition of no interactions ("additive"), the classification of an interaction is based on the assessment of how strongly and in what direction the effects of a drug combination deviate from this null hypothesis that drugs do not interact. A broad range of examples would include Bliss Independence and Loewe Additivity defined below as well as covariance, mutual information, and ANOVA. The choice of interaction metric depends both on the data type (fixed concentrations versus a range) and implicit assumptions about how fitness (growth rate) depends on the drug concentration. Note that apart from utilizing a fitness measure as in most drug-drug interaction studies, one can also look at other essential dynamical properties of pathogen population in the presence of multi-drug combinations, such as toxicity [18,19], protein-expression levels [20], or promoter activity dynamics [21].

Classification of pairwise interactions

There are two commonly used and well-established methods for identifying pairwise drug interactions, namely Bliss Independence [10] and Loewe Additivity [11]. Bliss Independence characterizes interactions when drugs are combined at fixed concentrations, whereas Loewe Additivity classifies interactions based on the drugs combined over a concentration range (Figure 1). Predicting the values of these metrics may be possible by also using dose-response relations—defined by Hill functions relative to certain pathogen inhibition level—for each single drug alone as in [6,8,22] and excitingly expanded and utilized in a recent paper by Zimmer et al. [17]. Moreover, for detecting pairwise interactions based on Bliss Independence, it has been shown that the identification of interactions is greatly enhanced when a rescaling method is applied to the interaction metric [23,24] (Figure 2). Quantifying the deviation relative to the special cases of lethal synergy—two-drug combination completely kills the pathogen—and antagonistic buffering—two-drug combination effect is equal to the effect of the strongest individual drug—leads to a much more directly interpretable interaction strength in terms of its magnitude and leads to an explicit separation of interaction classes [23,24].

Measuring higher-order interactions—beyond pairwise parts

Even though higher-order drug combinations are becoming more prevalent in the clinic [1–5], a deep understanding of how to quantify and to characterize higher-order drug interactions has not yet been fully established. This is mainly because complexities arise when the number of drugs in the system increases, such as going from two-drug to three-drug combinations. The first essential step is to extend the pairwise classification methods—Bliss Independence and Loewe Additivity—to higher-order drug combinations (Figure 1) in order to develop new measures of higher-order interactions. This approach has been effectively accomplished by several studies that have either focused on fixed or varied concentrations of drugs. In particular, Otto-Hanson et al. [25] and Stergiopoulou et al. [26] characterized three-way interactions based on a Bliss Independence expectation that individual drugs act independently (Figure 1a). They used this expectation to study combination treatment strategies against *Streptomyces scabies*—a common and highly invasive soil bacteria—and *Aspergillus fumigatus*—causing life-threatening diseases to immune deficient individuals.

On the other hand, Berenbaum et al. [27] introduced a concentration-based mathematical framework of quantifying higher-order drug interactions by adapting the Loewe method to any number of drugs (Figure 1b). Due to the laborious nature of data collection for three- or more drug combination responses across concentration ranges, Berenbaum's model (or Loewe with N drugs) has just started to garner attention in empirical studies with the increased data availability. For instance, a study by