Experimental Design to Identify Antibiotic Synergy

Jennifer Brennan JRB@CS.WASHINGTON.EDU
Paul G. Allen School of Computer Science & Engineering
University of Washington
Seattle, WA 98195, USA

Lalit Jain LALITJ@UW.EDU
Foster School of Business
University of Washington
Seattle, WA 98195, USA

Sofia Garman, Ann E. Donnelly and Erik S. Wright {SCG63,ESWRIGHT}@PITT.EDU
Department of Biomedical Informatics
University of Pittsburgh
Pittsburgh, PA 15260, USA

Kevin Jamieson JAMIESON@CS.WASHINGTON.EDU
Paul G. Allen School of Computer Science & Engineering
University of Washington
Seattle, WA 98195, USA

Abstract

Antibiotic resistance is an important public health problem. One potential solution is the development of synergistic antibiotic combinations, in which the combination is more effective than the component drugs. However, experimental progress in this direction is severely limited by the number of samples required to exhaustively test for synergy, which grows exponentially with the number of drugs combined. In this extended abstract we introduce the normalized diagonal sampling (NDS) design, an experimental design that samples along all appropriately normalized diagonals in concentration space. Under a benign assumption on antibiotic behavior, we prove that the NDS design identifies all synergies according to a novel, clinically motivated definition of synergy. We applied our method to screen two- through eight-way combinations of eight antibiotics at 10 concentrations each. Our method showed that there are no clinically relevant synergies among these eight antibiotics, accomplishing in two weeks what previously would have taken years.

Keywords: Antibiotic Combination Therapy, Drug Synergy, Experimental Design

1. Introduction

Antibiotic resistance poses a clinical problem for which there are few available solutions. One promising strategy is the use of synergistic antibiotic pairings whose collective potency is greater than expected (Tyers and Wright, 2019). Commercially available examples include the antibiotics trimethoprim and sulfamethoxazole, which inhibit separate steps in the folate biosynthesis pathway (Bushby and Hitchings, 1968), and quinupristin and dalfopristin, which both inhibit the ribosome (Noeske et al., 2014). Very few examples of synergistic combinations exceed two antibiotics (Booth et al., 1994), partly because the number

of measurements required to detect multi-antibiotic synergy increases exponentially with the number of antibiotics tested. Exhaustively testing 10 concentrations of five antibiotics would require on the order of 10^5 experiments, which limits the search space even when employing robotics to facilitate experimentation (Tekin et al., 2018). Scaling exhaustive testing beyond five antibiotics is therefore impractical, and another approach is needed to explore the space of possible synergies.

In this extended abstract we present an experimental design that provably identifies all synergies among d drugs at m concentrations using just $m \cdot 2^d$ measurements, an improvement upon the m^d measurements of the exhaustive design that makes high-dimensional synergy screening practical for the first time. Our contributions are fourfold: (1) we define a new metric of synergy motivated by the clinical use of combination therapy; (2) we use domain-specific knowledge about the behavior of antibiotics to define a class of high-dimensional dose-response curves; (3) we introduce the Normalized Diagonal Sampling design, a novel experimental design, and show that sampling according to this design identifies all synergies under our metric when the dose-response belongs to aforementioned function class; and (4) we perform experiments using our design, and show that a set of eight common antibiotics has no clinically relevant synergies against a wild-type strain of *E. coli*. We conclude with a discussion of future work, in which we plan to apply adaptive experimental design to identify drug combinations with high coverage over a variety of *E. coli* strains with varying degrees of antibiotic resistance. Such a drug combination could be used in the clinic to provide initial treatment even before the bacterial strain is identified.

Related work. Scientists are interested in understanding the behavior of combinations of biological agents in fields as diverse as immunosuppressants (Berenbaum, 1977), environmental toxins (Cedergreen, 2014), anesthetics (Hendrickx et al., 2008), and anticancer drugs (Scripture and Figg, 2006). Understanding this behavior requires both a metric by which to measure interactions and an experimental design for collecting measurements. Our proposed metric, the Minimax Effective Concentration Index, combines elements of the popular Highest Single Agent (Berenbaum, 1989) and Loewe (Loewe, 1953) models to reflect how antibiotics are combined in the clinic. Our experimental design is similar to past work that reduces the sample complexity via subsampling the combination space (Cokol et al., 2017), but unlike past work, we provide formal guarantees on the recovery of synergistic responses.

2. Provably identifying synergy with sample-efficient experimental design

In this section we describe our theoretical and methodological contributions. We begin by introducing a novel metric for synergy, the Minimax Effective Concentration Index. Next, we describe a biologically sound assumption on the function class of high-dimensional dose-response curves. Finally, we introduce our experimental design, the normalized diagonal sampling design, which provably identifies all synergies using a small subset of all possible measurements, as long as the dose-response function does in fact lie within our assumed function class. Figure 1 summarizes these contributions.

2.1 The Minimax Effective Concentration Index

In clinical practice the goal is to administer antibiotic combinations that are effective while avoiding high doses, which may cause adverse effects. We define the *Minimax Effective*

Concentration Index (MECI), which captures the idea of avoiding high doses by minimizing the highest single antibiotic's concentration (appropriately normalized) among antibiotic combinations that are effective at inhibiting growth.

Let $\Omega = \{1, 2, 3, \ldots, d\}$ index a set of d drugs. An experimental measurement consists of a vector $\mathbf{x} \in \mathbb{R}^d_{\geq 0}$ encoding a concentration for each drug; an experiment determines whether this vector is *effective* or *ineffective* against the bacteria. Let N_i be the normalization constant for drug i, which captures biologically or clinically relevant notions of scale between drugs, with $\mathbf{N}$ the vector of normalization terms. The set of possible experimental measurements, denoted $\mathcal{X}(\Omega)$, is all combinations of drugs at a given set of m ratios relative to the normalization, $\frac{x_i}{N_i} \in \{0, c_1, c_2, \ldots, c_{m-1}\}$. These ratios are commonly taken to be powers of two, $c_j = 2^{-j}$. Finally, let $\frac{\mathbf{x}}{\mathbf{N}}$ denote elementwise division, resulting in a normalized concentration vector. The MECI is defined as:

$$MECI(\Omega) = \min_{\mathbf{x} \in \mathcal{X}(\Omega)} \left|\left| \frac{\mathbf{x}}{\mathbf{N}} \right|\right|_\infty \quad \text{such that} \quad \mathbf{x} \text{ is effective.} \tag{1}$$

We define the Total Synergy Score (TSS(Ω)) as the improvement of the best combination in Ω upon the best single drug in Ω. The Emergent Synergy Score (ESS(Ω)) is similarly defined as the improvement over the best (strict) subset of drugs in Ω, and captures the marginal benefit of combining the drugs Ω. We say a synergy is *clinically relevant* whenever $\log_2(ESS(\Omega)) \leq -2$.

$$TSS(\Omega) = \frac{MECI(\Omega)}{\min_{i \in \Omega} MECI(\{i\})}; \qquad ESS(\Omega) = \frac{MECI(\Omega)}{\min_{\Omega' \subset \Omega} MECI(\Omega')}.$$

2.2 The non-paradoxical growth assumption

Our goal is to identify synergies among all subsets of drugs in Ω, which means we must solve the constrained optimization problem (1) for each subset. Observe that we only have sample access to the constraint function; we must experimentally measure bacterial growth under dose combination $\mathbf{x}$ to determine if $\mathbf{x}$ is effective.

Without any restrictions on the constraint function, we cannot identify the MECI using any method other than exhaustive search. To overcome this problem, we identify a biologically meaningful function class of unimodal dose-response functions, which we say do not exhibit *paradoxical growth*. The function class is defined formally in Definition 1, with an example of paradoxical growth illustrated in Figure 1B.

Definition 1 (Absence of paradoxical growth) *Let Ω be a set of antibiotics. Let the vector $\mathbf{x_0} \in \mathbb{R}^{|\Omega|}_{\geq 0}$ represent a fixed background concentration of antibiotics to which we add increasing amounts of another antibiotic combination $\mathbf{x} \in \mathbb{R}^{|\Omega|}_{\geq 0}$. We say the set of drugs Ω* ***does not exhibit paradoxical growth*** *if, for all $c_3 > c_2 > c_1 \geq 0$, the response $r : \mathbb{R}^{|\Omega|}_{\geq 0} \to \mathbb{R}$ satisfies $r(\mathbf{x_0} + c_2\mathbf{x}) < r(\mathbf{x_0} + c_1\mathbf{x}) \implies r(\mathbf{x_0} + c_3\mathbf{x}) \leq r(\mathbf{x_0} + c_2\mathbf{x})$.*

2.3 The normalized diagonal sampling design

We introduce the *normalized diagonal sampling (NDS) design*, an experimental design that samples all combinations of drugs in Ω at equal concentrations relative to the normalizations N_i. The NDS design evaluates each combination $\Omega' \subseteq \Omega$ at all $m - 1$ normalized concentrations $\{c_1\mathbf{1}, c_2\mathbf{1}, \ldots, c_{m-1}\mathbf{1}\}$, where $\mathbf{1}$ represents a vector of all ones of size $|\Omega'|$.

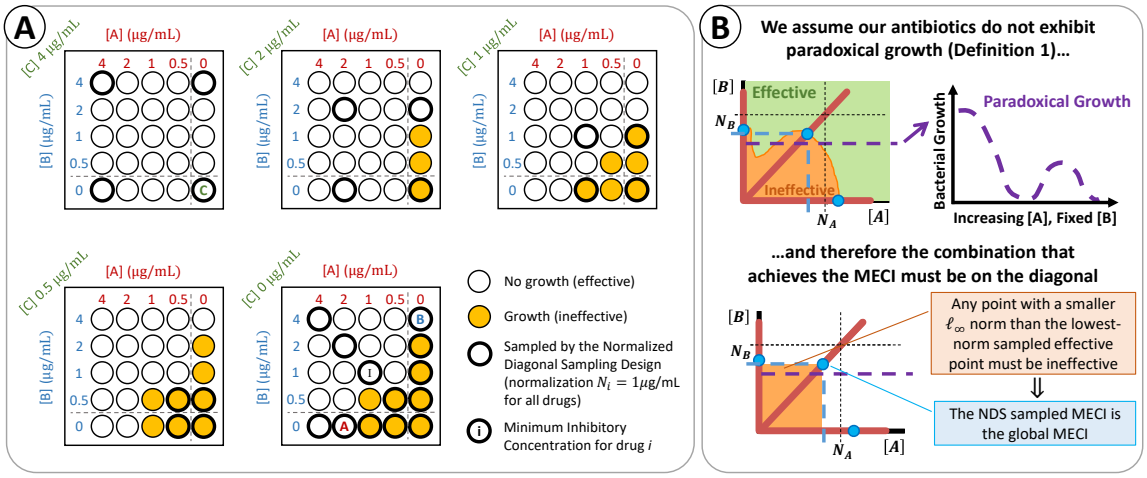

Figure 1: (A) Testing three drugs at four concentrations each could be performed exhaustively using a checkerboard assay (all circles), or via the NDS design (bold circles). (B) "Proof by picture" of NDS correctness. Blue circles show the minimum-norm effective concentration along each diagonal (solid red line) sampled by the NDS design. Points with a smaller norm than the NDS-identified MECI *must* be ineffective, otherwise the drugs would exhibit paradoxical growth.

Exhaustive tests for synergy are conducted with checkerboard assays (see Figure 1A) requiring m^d wells to screen d drugs at m concentrations each. The NDS design significantly reduces the required number of wells by testing along the "diagonal" – testing each combination with all drugs present at the highest concentration, then at the second-highest concentration, and so on (bold measurements in Figure 1A). Under the NDS design, each of the 2^d drug combinations requires only m wells, for a total requirement of $m \cdot 2^d$ wells. For eight drugs and 10 concentrations per drug, this requires $m \cdot 2^d = 10 \cdot 2^8 = 2,560$ wells, or about twenty six 96-well plates. This is experimentally feasible, whereas $m^d = 10^8$ wells (requiring approximately 10^6 plates) is not.

Suppose we define a concentration vector $\mathbf{x}$ as *effective* whenever the measured response falls below some threshold ($r(\mathbf{x}) \leq t$). Then, as long as the response behaves according to Definition 1, we can identify entire regions of the antibiotic combination space as *ineffective* using only measurements on the boundary of the space. This leads to our main result: the correctness of the NDS design in the absence of paradoxical growth.

Theorem 2 *Assume the set of drugs Ω does not exhibit paradoxical growth (Definition 1). Then, the normalized diagonal sampling design applied to Ω identifies $MECI(\Omega')$ for all $\Omega' \subseteq \Omega$. In other words, of all the concentration vectors $\mathbf{x}$ sampled by the NDS design, the one with the minimum N-normalized ℓ_∞ norm among effective vectors is also the minimum norm effective point among the full set of possible combinations $\mathcal{X}(\Omega')$, for each $\Omega' \subseteq \Omega$.*

Since the NDS design provably finds the MECI, high-dimensional antibiotic combination screens can be run with the confidence that if no synergies are identified, then none exist.

Table 1: Experimental results show no strong synergy among eight tested drugs

	Breakpoint Normalized							MIC Normalized						
Number of Drugs	2	3	4	5	6	7	8	2	3	4	5	6	7	8
Weak Synergy	5	4	6	1	0	1	0	18	14	11	1	0	0	0
No Synergy	23	52	64	55	28	7	1	10	42	59	55	28	8	1

2.4 Experimental results: no clinically relevant synergies among eight drugs

We applied our method to identify synergies among a set of eight common antibiotics: ampicillin, aztreonam, ceftazidime, chloramphenicol, ciprofloxacin, gentamicin, trimethoprim and tobramycin. Experiments were fully randomized with the use of robotics, and drug effectiveness was measured by computing the area under the curve of optical density (600nm) over time.

We performed two separate experiments according to two different normalizations N_i, EUCAST breakpoint and minimum inhibitory concentration (MIC), to illustrate the flexibility of our synergy metric and experimental design. The breakpoint normalized experiment is motivated by clinical applications, where the breakpoint serves as a proxy for a standard dose of antibiotics, while the MIC normalized experiment more closely aligns with the methods biologists use to understand the mechanisms of synergy. Table 1 shows the number of drug combinations with weak synergy ($\log_2 ESS = -1$) and no synergy ($\log_2 ESS = 0$), stratified by the number of drugs in the combination. We found no combination of any cardinality that exhibited clinically relevant levels of synergy ($\log_2 ESS \leq -2$).

Our findings are consistent with previous studies that found synergy to be rare (Cokol-Cakmak et al., 2020; Tekin et al., 2018; Chandrasekaran et al., 2016; Yilancioglu and Cokol, 2019). Our method provides an advantage over these previous methods; under the assumption that the drugs do not exhibit paradoxical growth, we guarantee that no clinically relevant synergies exist among these eight drugs in the experimental conditions tested, even though our design required only a fraction of the samples of an exhaustive screen.

3. Future directions: optimizing the empiric therapy

We have so far motivated antibiotic combination therapy with the promise of synergy, with implications for reduced side effects or decreased acquisition of resistance when treating a single bacterial strain (genetic variant). A different motivation for combination therapy comes from the clinical goal of *coverage*, in which we seek a combination that is effective against the largest number of bacterial strains possible.

Definition 3 (Coverage) *Let bacterial strains s be drawn from a population S, as in a hospital setting in which patients arrive with infections of unknown origin. Let Ω be the set of drugs available. We define the* ***coverage*** *of the combination $\mathbf{x} \in \mathbb{R}_{\geq 0}^{|\Omega|}$ as $C(\mathbf{x}) := \mathbb{P}_{s\sim S}(\mathbf{x}$ is effective against $s)$.*

Coverage is a property of the population S of infectious strains, and varies across category of infection (e.g. sepsis, pneumonia) and geographical region. Finding a combination with

high coverage is complicated by the existence of antibiotic resistance, with many strains exhibiting multiple antibiotic resistance. In addition, resistance to one drug can confer resistance to an entire class or mechanism of antibiotics, so that resistance patterns are typically correlated between strains.

Identifying an antibiotic combination with high coverage is important in the administration of the so-called *empiric therapy*, the combination prescribed to all patients with a given set of symptoms before doctors know specific information about the infectious strain. This problem can be formalized as a constrained maximization of the coverage:

Problem Statement 1 (Optimal Empiric Therapy.) *Identify the combination of K antibiotics that maximizes coverage while applying no antibiotic at a dose greater than its normalization N_i.*

$$\arg\max_{\mathbf{x}} C(\mathbf{x}) \qquad s.t.\ ||\mathbf{x}||_0 \leq K, \quad \left|\left|\tfrac{\mathbf{x}}{\mathbf{N}}\right|\right|_\infty \leq 1$$

We present several ideas for future directions to address this problem statement.

Subset selection. One constraint of the empiric therapy is that it should not prescribe any drug above its normalization constant N_i. By choosing N_i to be the EUCAST breakpoint, which is a proxy for the concentration the drug achieves in the human body when administered in a clinical setting, this constraint translates to prescribing each drug at no more than its "standard" dose. Our non-paradoxical growth assumption tells us that the highest-coverage dosing will always occur when each drug in the combination is prescribed at $x_i = N_i$, so the problem becomes one of selecting the best K of d drugs.

Best-of-K Bandits. When the problem is cast as one of subset selection, it bears some resemblance to the Best-of-K bandits problem. Each round, a strain s is chosen by nature, the player chooses a set of K antibiotics, and the player receives reward 1 if the strain is sensitive to any drug in the player's set, or a reward of 0 if the strain is resistant to all drugs in the player's set. Simchowitz et al. (2016) show that the Best-of-K bandits problem is very challenging when individual drugs have low average effectiveness but drug sensitivities are correlated among strains. Our setting may additionally involve interactions between the drugs, which is not precisely captured by the Best-of-K framework.

Matrix completion. If there were no interactions between drugs, or those interactions were of a small magnitude, then the behavior of high-dimensional combinations could be predicted using just single-drug response data for each strain. If we take S to be an infinite population of strains, then an important experimental design goal is to identify which single drug responses to collect from which strains, where at each time step we may choose to measure the susceptibility of one new or previously-measured strain to a single antibiotic. Since resistance patterns among strains are correlated, it is natural to suspect that the matrix describing the resistance of each strain to each drug has a low-dimensional factorization. We could leverage this idea to take fewer measurements from each strain, allowing us to sample more strains from S. If we believed the set of drugs exhibited interactions up to some order M, then we could extend this to $M+1$-dimensional tensor factorization.

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