Title
Hidden suppressive interactions are common in higher-order drug combinations

Author
Zhou, April

Publication Date
2021

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA

Los Angeles

Hidden suppressive interactions are common in higher-order drug combinations

A thesis submitted in partial satisfaction of the requirements for the degree of Master of Science in Bioinformatics

by

April Chengxin Zhou

2021

ABSTRACT OF THE THESIS

Hidden suppressive interactions are common

in higher-order drug combinations

by

April Chengxin Zhou

Master of Science in Bioinformatics

University of California, Los Angeles, 2021

Professor Van M. Savage, Chair

Combinational therapy has been one method used to combat the growing concern of multi-antibiotic resistant bacteria. In identifying the interaction type of a drug combination, the focus is often on the overall effect of the combination derived from comparison to each drug alone. These identifications tend away from suppression, where bacteria grow better in combination than when treated with a single drug component. They also miss “hidden” cases of suppression, where the highest-order can be suppressive to a lower-order but not to a single drug. We examined an extensive dataset of 5-drug and all lower-order combinations using computational methods and regression analysis and found that over half of combinations contain hidden suppression. My specific focus was on examining possible structures of hidden suppression at these higher orders. Overall, understanding this is important because of how it can affect our predictions of antibiotic resistance evolution in combinational treatments.

The thesis of April Chengxin Zhou is approved.

Pamela Yeh

Nandita Garud

Van M. Savage, Committee Chair

University of California, Los Angeles

2021

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF BOXES

LIST OF EQUATIONS

ACKNOWLEDGEMENTS

This thesis is a version of Natalie Ann Lozano-Huntelman, April Zhou, Elif Tekin, Mauricio Cruz-Loya, Bjørn Østman, Sada Boyd, Van M. Savage, Pamela Yeh, "Hidden suppressive interactions are common in higher-order drug combinations." It is published in *iScience* volume 24, issue 4, 2021, https://doi.org/10.1016/j.isci.2021.102355. Natalie Ann Lozano-Huntelman was instrumental in the analysis and co-authoring of this work. Elif Tekin was central to identifying the types of interactions of the combinations present. Mauricio Cruz-Loya aided with the statistical analysis; Bjørn Østman aided with the fitness landscape; Sada Boyd aided with the writing process. Pamela Yeh and Van M. Savage were critical in both guiding the research and editing this work. I would specifically like to thank Pamela Yeh for allowing me to work on this following her conceptualization, and for the time she has allowed me to spend with her lab during my time at UCLA.

We are grateful for funding from the Hellman Foundation (P.J.Y.), a KL2 Fellowship (P.J.Y.) through the NIH/National Center for Advancing Translational Sciences (NCATS) UCLA CTSI Grant Number UL1TR001881.

CHAPTER 1. INTRODUCTION

Drugs are prevalent in our daily lives, and over the years, drug resistance—and especially antibiotic resistance—has become a leading public health concern (Bloom et al., 2018, Chokshi et al., 2019, Povolo and Ackermann, 2019). Though a natural occurrence in that resistance can evolve, its sheer prevalence has been expedited by inappropriate prescriptions as well as antibiotic overuse in agriculture and the community (Ventola, 2015, Aslam et al., 2018). To compound this problem, the discovery of new antibiotics is low, due to lack of financial lucrativity, regulatory barriers, and limited mechanisms of actions that can be targeted with traditional therapeutic methods (Cooper and Shlaes, 2011, Aslam et al., 2018). Currently, all major infectious diseases face the issue of antibiotic resistance and even multi-antibiotic resistance. This has all come at a great cost in multiple ways. Most notably, resistance hampers treatment against diseases, leading to higher risk of mortality for patients—especially in clinical settings, where vulnerable patients are clustered in one setting (Dadgostar, 2019). Hence, efforts have been made to combat the spread of antibiotic resistance.

One strategy for working against antibiotic resistance is use of combinational antibiotic therapy, exploiting therapeutic effects to combat pathogens and drug-resistant strains more effectively and to reduce resistance evolution (Fischbach, 2011, Rieg et al., 2018, Liu et al., 2020). The key therapeutic effect typically desired is that of synergy when it comes to the drug-drug interaction within the combination (Yin et al., 2018). Overall, there are three categorizations for the way two or more drugs interact: synergistically, antagonistically, or additively. In synergistic interactions, the effect of the drugs together is better than the expected effect based of each drugs' individual effects. Conversely, in antagonistic interactions, the

combined effect is lesser than the expected effect; in additive interactions, the combined and expected effects are matched (Bliss, 1939) (Box 1).

In the most extreme form of antagonism, now termed suppression, drugs work more ineffectively together than at least one of the drugs alone (Yeh et al., 2006, Chait et al., 2007). This was first described by Fraser (1870), who showed that individually administering two toxins to a rabbit would kill it, but that administering jointly would keep it alive. Now, over a century later, interest in this phenomenon has been renewed (Yeh et al., 2006, Chait et al., 2007, Cokol et al., 2014, de Vos and Bollenbach, 2014, Bollenbach, 2015, Singh and Yeh, 2017, Lukačišin and Bollenbach, 2019, Tyers and Wright, 2019, Dean et al., 2020). Regarding antibiotic interactions, the term suppression was first brought to attention in a systematic study of two-drug interactions in 21 antibiotics, which found that approximately 10% of all interactions could be classified as such (Yeh et al., 2006). In the past decade, more studies have been published elucidating the effects of suppression on resistance evolution, its mechanisms, and its prevalence as well. For instance, studies have pointed to suppressive drug combinations possibly decreasing the rate of bacterial adaptation to and evolution of drug resistance (Hegreness et al., 2008). They have also indicated suppressive combinations could decrease the likelihood of resistance evolution resulting from spontaneous mutations, as well as decrease such likelihood by lowering evolutionary fitness of high-resistant strains and favoring wildtype, drug-sensitive strains in direct competition *in vitro* as a result (Chait et al., 2007). Relating to mechanisms, it has been found that nonoptimal ribosomal gene regulation drives the suppressive nature of drugs that act on protein and DNA synthesis (Bollenbach et al., 2009). Relating to prevalence, amount of suppression found has varied. Cokol et al. (2014), in screening a dataset of 175 two-drug combinations to identify synergistic combinations, found approximately 17% to be suppressive.

Yeh et al. (2006) reported 8% of 2-drug combinations examined to be suppressive and Beppler et al. (2017) reported 5% to be suppressive. In higher-order combinations of more than two drugs, Beppler et al. (2017) reported 3% of combinations examined and Tekin et al. (2018) reported 8%.

At the two-drug level, suppressive interactions are easy to identify, as bacterial growth under either single drug is lesser than that of the growth under both drugs (Figure 1). However, suppressive interactions can also occur in high orders of drug combinations (Figure 1). Notably, this is the direction combinational drug therapy is heading, with some prominent combinational treatments already involving three-drug combinations (Mokhtari et al., 2017, Guerrero-Garcia and Rubio-Guerra, 2018). For instance, in a 5-drug combination, the drugs together can have a lesser effect than one of the single drugs alone. Alternatively, the 5 drugs together could have a lesser effect than any of the 3 or 2 drugs together. Within this, a 4-drug subset of the 5 drugs can be suppressive to a 3- or 2-drug combination or a single drug; a 3-drug subset can be suppressive to a 2-drug combination or a single drug as well. Usually, studies examine interaction type based on the effects of single drug components—thus, if the suppressive interaction occurs between the highest-order and a lower-order combination within it, the interaction is termed "hidden" (Beppler et al., 2017, Tekin et al., 2018) (Figure 1). In other words, identification of suppressive effects can be missed if lower-order, non-single drug effects are not examined. Suppressive effects that can be identified using just the highest-order combination at hand in addition to the singular components, meanwhile, are "net" suppressive (Cokol et al., 2011, Stergiopoulou et al., 2011, Otto-Hanson et al., 2013, Tekin et al., 2017, Katzir et al., 2019) (Box 1). For instance, given a 3-drug combination, it is possible for suppression to not appear between the 3 drugs and any of the single drugs, yet appear between the 3 drugs and one of the 2-drug subsets within it.

Given this possibility of hidden interactions, examination of lower-order combinations is of importance in determining the interaction type of higher order drug combinations.

Hidden interactions play a role both clinically and scientifically. Clinically, if combinations are screened without considerations towards interactions with lower-order sub-combinations, then screening outcome and expected efficacy of the treatment may be different because of hidden suppressive interactions. Scientifically, hidden suppressive interactions can significantly alter the topography of a fitness landscape, which visualizes the relationships between stressors or mutations and their effects on fitness (Wright, 1932, Wright, 1988). Specifically, they can alter the peaks and valleys, with peaks being environments beneficial to bacterial growth and valleys resulting in areas difficult for populations to cross, owing to intermediate traits or environments that face an overall decrease in fitness (Figure 2). Hence, a better understanding of hidden interactions is useful from both an evolutionary and clinical standpoint.

Traditionally, interaction within a combination of drugs is determined solely based off comparison to effect of all single drug components; this is reflective of the difficulty of studying hidden interactions. First, studying these interactions in higher orders requires obtaining the growth measurements of all single and combinational drug subsets under a select higher-order combination. For a 5-drug combination, this includes the one 5-drug combination, five 4-drug combinations, ten 3-drug combinations, ten 2-drug combinations, five single drugs alone, and a no-drug control. Together, these measurements make up one full-factorial of growth measurements. Second, classifying higher-order interactions requires calculations with contributions from all lower levels in a combination, which amounts to a high number. Applying the scope of emergent interactions to this makes for more intricate calculations for each

combinatorial component of a full-factorial. This is because calculations then account for how single drugs may affect the interactions of another included subset—as an example, a third drug can alter the interaction between two other drugs, rather than just how the third drug affects the two drugs on an individual level (Beppler et al., 2016, Tekin et al., 2016, Tekin et al., 2017, Tekin et al., 2018). For net interactions where only single drugs and their effects on the highest-order combination are concerned, the calculation is simpler but can still be computationally time consuming. Overall, there are logistical hurdles to examining interactions.

Net and emergent interactions aside, the identification of hidden interactions is a challenge because after identifying interactions at all order levels, these values must then be compared to one another. At the 5-drug level, this makes for 120 comparisons per combination studied. On another note, understanding how to theoretically and conceptually quantify higher-order drug combination interactions is difficult. However, advances in hardware and automated robotics have alleviated the logistical hurdle because of a better ability to handle large quantities of computation and measurements. Regarding the conceptual side, framework for the categorization of combinations and their interactions, including emergent properties, have become more elucidated.

Here, we perform a systematic examination of the structure of suppressive interactions in higher-order drug combinations. Specifically, we ask: 1) how prevalent are hidden suppressive interactions? 2) What is the structure of a suppressive interaction: are they likely to be suppressive to the next lower-order combination? For example, do we primarily see a 5-drug combination that is suppressive relative to a 4-drug combination, or are there larger jumps in suppression, for example, a 5-drug combination that is suppressive relative to a 2-drug combination? Or is suppression likely to be nested—that is, if a 5-drug combination is

suppressive, is it likely to be suppressive to 4-drug and 3-drug subsets within the 5-drug combination? 3) Lastly, are some antibiotics or main mechanism of actions more likely to be involved in general suppressive interactions? The focus of my thesis was question 2; however, I contributed to questions 1 and 3 as well.

CHAPTER 2. METHODS

We re-examined the data set collected and published in Tekin et al. (2018) to examine the presence and patterns of suppressive interactions (both hidden and net) within these combinations.

A. Experimental set-up of Tekin et al. (2018)

The data set examined was originally collected and published in Tekin et al. (2018). A pathogenic *E. coli* strain CFT073 was isolated from human clinical specimens and obtained from ATCC (700928). A culture of CFT073 was streak-purified on Luria Broth (LB) (10 g/l tryptone, 5 g/l yeast extract, and 10 g/l NaCl) agar and a single colony was selected to make individual aliquots of bacteria stored in 25% glycerol and frozen at -80°C. For each day of experiments, a new aliquot was used, which was thawed and diluted by a factor of 10^2 in LB and a culture was grown for approximately 4 hours at 37°C.

Eight different antibiotics that span a range of mechanisms of action was used (Table 1): Ampicillin (A9518), Cefoxitin Sodium Salt (C4786), Ciprofloxacin Hydrochloride (MP Biomedicals 199020), Doxycycline Hyclate (D9891), Erythromycin (E6376), Fusidic Acid Sodium Salt (F0881), Streptomycin (S6501), and Trimethoprim (T7883) (Table 1). All drugs were obtained from Sigma Aldrich unless otherwise noted. Each antibiotic was prepared in solution in 100% DMSO, except for streptomycin which was dissolved in 50% DMSO.

Dose-response curves were generated using GraphPad Prism 7 (http://www.graphpad.com/quickcalcs/Ecanything1/) to estimate IC10, IC5, and IC1 for each antibiotic, using 20-step 2-fold dilutions beginning at 0.1mM. For fusidic acid, the concentration used to begin the 2-fold dilutions was 1mM, since using 0.1mM to begin the dilutions resulted in the inability to determine an IC50 using Graphpad Prism 7. Three concentrations at the sub-inhibitory level were used so that growth still occurred but was slowed in comparison to no-growth bacteria (Table 1). Once usable concentrations were determined, source plates (one plate with one antibiotic and two plates with two antibiotics combined in DMSO) were made using 100% DMSO except in the case of streptomycin where 50% DMSO was used.

All possible 2-, 3-, 4-, and 5-drug combinations of the antibiotics listed in Table 1 at each of the three possible drug concentrations were tested. This resulted in 13,608 5-drug-dose combinations, 5,670 4-drug-dose combinations, 1,512 3-drug-dose combinations, 251 2-drug-dose combinations, and 24 single drug treatments. Each well was filled on each experimental plate to a total volume of 50μL. 25μL of LB was pinned with 250nL of antibiotics from the appropriate source plates and 25μL of the inoculum (a 10^{-4} dilution of the over day culture). Plates were incubated at 37°C and read at OD_{590} every 4hr for 16hr. Each combination had a minimum of three replicates.

B. Calculation of growth measurements performed by co-author

Growth measurements for each well were approximated from the maximum linear slope of the log transformed optical density (OD) readings that occurred over each time step (0hr to 4hr, 4hr to 8hr, 8hr to 12hr, and 12hr to 16hr) as a relative proxy to an exponential growth rate. These growth measurements were then normalized to the positive no-drug control wells to

determine relative fitness values. Fitness values below 5% were considered to be lethal and fitness values that were +100% were set back to be 100%. These fitness values were then used to evaluate drug interactions based on the methods used in Tekin et al. (2018).

C. Measurement of interactions by Tekin et al. (2018)

To measure the deviation from additivity, known as "net interactions," Bliss Independence methods (Bliss, 1939) were followed. The Bliss independence method is widely used to categorize interactions (Sühnel, 1998, Meletiadis et al., 2005, Yeh et al., 2006, Petraitis et al., 2009, Zhao et al., 2014, Baeder et al., 2016, Koch et al., 2016, Liu et al., 2018). Bliss independence assumes that at a set concentration of an antibiotic the relative effect is completely independent of each other. A deviation from this expectation results in either a synergistic interaction (positive deviation, Figure 1) or antagonistic interaction (negative deviation, Figure 1).

To measure net interactions, methods outlined in Beppler et al. (2016), Tekin et al. (2016), and Tekin et al. (2018) were used. This framework is used to examine 2-, 3-, 4-, and 5-drug combinations but can also be expanded to *N* number of drugs (Tekin et al., 2018). To find the net interaction, or the deviation from additivity for N drugs (DA_N) the fitness effects (w) contributed by each drug alone are removed from the overall fitness effect ($w_{D_1,D_2,D_3 \ldots D_N}$) assuming Bliss independence (Equation 1).

$$\textbf{Equation 1: } [\boldsymbol{DA_N}]_{\boldsymbol{D_1,D_2,D_3 \ldots D_N}} = \boldsymbol{w_{D_1,D_2,D_3 \ldots D_N} - w_{D_1} w_{D_2} w_{D_3} \ldots w_{D_N}}$$

After the initial interaction value is determined, a rescaling process is used to better distinguish between interaction types (Tekin et al., 2016). For rescaling, when the DA is synergistic one

rescales to the lethal case. This is because when measuring growth, it is not possible to be deader than dead. If the interaction was not synergistic then it was normalized to the minimum fitness of an individual drug within the deviation from additivity formulas. Equation 2 shows the example for a 3-drug combination.

$$\textbf{Equation 2: } DA_{rescaled} = \frac{[DA_N]_{D_1,D_2,D_3 \ldots D_N}}{\left|min(w_{D_1}, w_{D_2}, w_{D_3}, \ldots w_{D_N}) - w_{D_1} w_{D_2} w_{D_3} \ldots w_{D_N}\right|}$$

Emergent interactions were also examined. An emergent interaction is the interaction that is unique to either the three, four, or five drugs being present within a combination. For example, when considering all possible drug effects that can be occurring within a single 3-drug combination there are a total of seven effects. First, all three individual drugs have their own effect. These effects are accounted for when we are determining the deviation from additivity. Next, there are three pairwise interactions that can also interact with the individual drug effects of the third drug. And finally, there is the emergent effect, which is the interaction that is strictly because of the three drugs being in combination. Similar to the DA calculations the emergent calculations (*E3*) removes the effects of the single drugs but then also removes the effects of the pairwise interaction only leaving the effects uniquely due to the 3-drug combination (Equation 3). This can then be rewritten only in fitness effects. (Equation 4).

$$\textbf{Equation 3: } E3 = DA_{X,Y,Z} - w_X DA_{Y,Z} - w_Y DA_{X,Z} - w_Z DA_{X,Y}$$

$$\textbf{Equation 4: } E3 = w_{XYZ} - w_X w_{YZ} - w_Y w_{ZX} - w_Z w_{YZ} + 2 w_X w_Y w_Z$$

The same principals can be expanded out to accommodate *N* number of drugs within a combination Tekin et al. (2018). These emergent interaction were then rescaled in a similar way as the DA values as described in Tekin et al. (2018).

D. Analysis of Prevalence and Patterns of Suppression and Hidden Suppression

Following the calculations for the measurements of interactions, I focused on examining the drug-dose replicates and developing a framework to determine the prevalence of hidden suppression in the drug combinations. I also determined cases to classify the types of structures of hidden suppression that can be found within a combination and evaluated the abundance of combinations that fell into each specified case. Cases are defined in Table 2.

The median DA_N of drug-dose replicate experiments was used to determine patterns of suppression in three, four, and five drug-dose combinations. A cutoff value of $DA_N \geq 1.3$ to classify combinations as net suppressive was used. This cutoff value is based on the framework used by Beppler et al. (2017), which only examined 2-drug and 3-drug combinations. All combinations, regardless of net interaction, were screened for hidden suppression.

Following this identification of net interactions, "paths" were generated for each of the drug-dose combinations. A "path" is a unique heterarchical grouping containing one representative of each of all the lower-order combinations within the highest-order combination. These paths facilitate comparisons of nested fitness values within *N*-order combinations, which are used to determine cases of suppression and hidden suppression. For instance, when evaluating possible hidden suppression in a 4 drug-dose combination, pairwise drug-dose combination values can only be compared to those of 3 drug-dose combinations that they are a part of, rather than those of all possible 3 drug-dose combinations (Figure 3A). Fitness values of all combinations and single drugs were included in these paths, resulting in six paths for each 3-drug-dose combination, 24 paths for each 4-drug-dose combination (Figure 3A), and 120 paths for each 5-drug-dose combination (Figure 3B).

To identify the presence of hidden suppression, the fitness of the highest-order combination $\left(w_{\boldsymbol{D_1,D_2,D_3\ldots D_N}}\right)$ was divided by the fitness of the lower-order combination with the smallest fitness $\left(\min\left(w_{\boldsymbol{D_1,D_2,D_3\ldots D_{N-1}}} \ldots\ w_{\boldsymbol{D_1,D_2}}\right)\right)$ (Equation 5).

Equation 5: $\boldsymbol{Hidden\ suppresion} \Leftrightarrow \frac{w_{\boldsymbol{D_1,D_2,D_3\ldots D_N}}}{min\left(w_{\boldsymbol{D_1,D_2,D_3\ldots D_{N-1}},\ldots w_{\boldsymbol{D_1,D_2}}}\right)} \geq \mathbf{1}$

A value greater than or equal to 1.3 indicates the presence of hidden suppression. Once the presence of hidden suppression was determined within a combination, each path was examined in-depth for all possible hidden suppression relationships. The net interaction, representative fitness values of inclusive combinations, and single drugs were compared and used to assess if the combination could be considered a special case (Table 2).

Data for combinations with any suppressive interactions, net or hidden, was analyzed in through the use of logistic regression in R using the glm function. The variables were first changed to binary, with 1 indicating presence and 0 indicating the absence of drug or the main mechanism of action creating the initial sets of predictors. Because hidden suppressive interactions require at least three drugs to be present to be defined, this makes it necessary for the logistic regression model to not have an intercept term. This is because the case where all dummy variables are zero corresponds to no drug being present, in which case any suppressive interaction is not possible by definition. Single drugs and 2-drug combinations were evaluated separately for a clearer interpretation of the data and to ensure model identifiability without removing variables. Coefficients, confidence intervals, p-values, odds ratios, and the probability from the logistic regressions are available in Tables 3-14.

E. Program Language and Code Availability

The data analysis is performed in MATLAB version 2015a, Python version 3.7.0, and R 4.0.2. PRISM was used by Tekin et al. (2018) for their study but was not needed in the reanalysis performed by this study. Measurement of interactions and interaction type determination was performed in MATLAB. Generation of paths and the identification of hidden suppression and special cases were performed in Python. Code for these parts can be found in the appendix. The determination of the growth measurements and logistic regressions were performed in R.

CHAPTER 3. RESULTS

I compared the fitness of the highest-order interaction to all lower-order interactions, to determine if hidden suppression was present within the combination. This information was then examined through the use of paths. A path, to refresh, is a unique heterarchical grouping containing one representative of each of all the lower-order combinations within the highest-order combination (Figure 3). I use these paths to identify what suppressive interactions occur within a combination and to detect nesting of hidden suppression. That is, for example, "full" nesting occurs in a 5-drug combination when the 5-drug combination (A+B+C+D+E) is suppressive to a 4-drug combination (A+B+D+E) and that 4-drug combination is suppressive to a 3-drug combination (A+D+E) which is then suppressive to a 2-drug combination (A+D). Analyzing paths will enable us to understand the structure of the interactions—determining which comparisons between a specific lower-order combination and the highest-order combination are suppressive (Box 1). More specifically, hidden suppression was identified by comparing the fitness of the highest-order combination with that of the lowest fitness from its lower-order combinations. Paths of hidden suppressive combinations were also analyzed to see what types of suppression could be identified. Please note that when referring to a single drug the

full name of the antibiotic is written out, when referring to a combination as a single entity the abbreviations of the drugs (Table 1) within the combination are used. For example, a combination containing the drugs ampicillin, fusidic acid, and streptomycin is listed as AMP+FUS+STR.

A. The Prevalence of Hidden Suppression

Nearly all higher-order combinations of unique drugs had at least one dose that produced a hidden suppressive interaction. Out of all the possible 182 higher order drug combinations (56 3-drug combinations + 70 4-drug combinations + 56 5-drug combinations) only five (four 3-drug combinations and one 5-drug combination) had no unique dose that had hidden suppression: AMP+FUS+ERY, AMP+FOX+FUS, FOX+CRP+FUS, STR+FOX+FUS, and TMP+STR+FUS+DOX. Among all 20,790 of unique drug-dose combinations studied, suppressive interactions are observed in 54% (11,302) of combinations. With only 17% (3,534) of the total combinations identified as net suppressive (Tekin et al., 2018) the remaining 7,768 combinations with suppressive interactions only contain hidden suppressive interactions. By solely considering the highest-order combination and the single drug effects, 69% of the combinations with suppressive interactions would not be identified (i.e., 7,768 out of 11,302). As the number of drugs in a unique drug dosage combination increases so does the percentage of combinations with hidden suppression: 33% of the 3-drug combinations, 48% of the 4-drug combinations, and 59% of the 5-drugs combinations had hidden suppression (Figure 4).

In cases where the net interaction is synergistic or additive, hidden suppression can still occur when the highest-order combination is compared to a lower-order combination (Figure 1, Figure 5-6). Importantly, for a combination to contain hidden suppression, it is not dependent on

the interaction type based on comparing the fitness values to the single drugs alone. For instance, a synergistic 4-drug combination that results in 20% relative fitness compared with no-drug environments can have a lower-order synergistic 2-drug combination that results in 10% relative fitness. This example then also has hidden suppression because the 4-drug combination results in more bacterial growth than the lower-order 2-drug combination but is still below the additive effects of the single drugs (Figure 1). Net additive combinations had hidden suppression in 27% of 3-drug combinations, 40% in 4-drug combinations, and 67% in 5-drug combinations (Figure 7). In net synergistic combinations, hidden suppression was found in 0% of 3-drug combinations, 7% of 4-drug combinations, and 23% in 5-drug combinations (Figure 7). Hidden suppression in net antagonistic combinations also increased as the number of drugs increased: 52% of 3-drug combinations, 71% of 4-drug combinations, and 72% in 5-drug combinations. In contrast, combinations that are net suppressive showed a decrease in the amount of hidden suppression as the number of drugs increased; 96% of 3-drug combinations, 92% of 4-drug combinations, and 88% in 5-drug combinations. These trends—the increase in the amounts of hidden suppression in synergistic, additive, and antagonistic with the increase in the number of drugs, and the decrease in hidden suppression with the increase in the number of drugs among the suppressive interactions—are also observed when examining emergent interactions (Figure 7).

B. The Structure of Hidden Suppression

When addressing the structure of hidden suppression, it is important to recognize that in each drug combination multiple lower-order interactions are occurring. For example, in a 3-drug combination, there are three unique 2-drug combinations within it. Using the same framework, in a 5-drug combination there are ten unique 2-drug combinations, ten unique 3-drug combinations,

and five unique 4-drug combinations. This results in a total of 25 possible hidden interactions. Combinations that contain hidden suppressive interactions can have suppressive interactions with one of the 25 possibilities, all of them, or any amount in between.

The highest-order combination has *N* drugs and is compared to all of the lower-order combinations to see where hidden suppression took place (Table 15). When comparing net suppressive combinations and those that only have hidden suppression, there are more instances of hidden suppression in combinations that are net suppressive no matter the number of drugs in the lower-order combination (Table 15, Figure 8). For example, in a 4-drug combination, there is suppression to the 3-drug combinations in 71% in net suppressive combinations while in combinations with only hidden suppression it was only observed 60% of the time. Combinations that are net suppressive also have the highest amounts of hidden suppression occurring between all possible lower-order combinations (Figure 8). For example, in a 5-drug combination, there are a total of ten possible 2-drug combinations. In net suppressive 5-drug combinations, hidden suppression occurs between the highest-order combination and all possible 2-drug combinations roughly 60% of the time. This occurs in less than 20% of 5-drug combinations that only have hidden suppression.

In breaking this down from a paths perspective, 78% of paths in net suppressive 3-drug combinations were suppressive to a possible 2-drug combination. 14% of paths in net suppressive 4-drug combinations were suppressive to a possible 2-drug combination only; 10% were suppressive to a 3-drug combination only, and 61% were suppressive to both a 2- and 3-drug combination. In net suppressive 5-drug combinations, 4% of paths were suppressive to a 2-drug combination only; 2% to a 3-drug combination only; 5% to a 4-drug combination only; 6% to both a 2- and 3-drug combination; 4% to both 2- and 4-drug combination; 5% to both a 3- and

4-drug combination; and 66% to all 2-, 3-, and 4-drug combinations in a path (Table 16). In combinations with hidden suppression only, 77% of paths in 3-drug combinations were suppressive to a possible 2-drug combination. 17% of paths in hidden suppression only 4-drug combinations were suppressive to a possible 2-drug combination only; 16% were suppressive to a 3-drug combination only, and 45% were suppressive to both a 2- and 3-drug combination. In hidden suppression only 5-drug combinations, 6% of paths were suppressive to a 2-drug combination only; 4% to a 3-drug combination only; 18% to a 4-drug combination only; 7% to both a 2- and 3-drug combination; 5% to both 2- and 4-drug combination; 7% to both a 3- and 4-drug combination; and 23% to all 2-, 3-, and 4-drug combinations in a path (Table 17).

This trend, of hidden suppression being more common in net suppressive combinations than only hidden suppression combinations, can be observed no matter how many drugs are in the highest-order combination or the number of drugs in the lower-order combination it is being compared to. It also strengthens as the number of drugs in the highest-order combination increases. Figure 8 compares the amounts of hidden suppression in net suppressive combinations and only hidden suppression combinations. Overall, the difference between net suppressive combinations and only hidden suppression combinations is smaller in 3-drug combinations than in 5-drug combinations. This is especially true when observing if there is hidden suppression for all possible options of *N*-drugs in a lower-order combination.

For net suppressive combinations, full nesting—when fitness at any order is greater than the fitness of the next lower-order combination in all paths including single drug effects—was only observed in the 3-drug and 4-drug combinations. A majority of net suppressive combinations were considered to have partially nested suppression, wherein at least one path, the fitness at any order must be greater than the fitness of all lower-orders (defined in Table 2)

(Figure 9). When examining the potential nesting of non-net suppressive combination, single drug effects do not need to be considered because by definition there would be no suppression to the single drugs. All net synergistic combinations only contain hidden suppression that does not fall into any special case.

Disregarding highest-order interaction type, 52.4% of net suppressive 3-drug combinations had fully nested suppression; 44.0% had partially nested suppression and 3.6% had no hidden suppression. In net suppressive 4-drug combinations, 0.5% had fully nested suppression, 87.7% had partially nested suppression, 3.3% were partially suppressed, and 8.5% had no hidden suppression. In net suppressive 5-drug combinations, 81.2% had partially nested suppression, 6.7% were partially suppressed, and 12.1% had no hidden suppression (Table 18). When classifying interaction using emergent interactions, fully nested suppression only appeared in 3-drug combinations. Based on emergent interactions, 36.8% of suppressive 3-drug combinations had fully nested suppression, 46.9% had partially nested suppression, and 16.3% had no hidden suppression. In suppressive 4-drug combinations, 49.6% had partially nested suppression, 5.9% were partially suppressed, 0.4% were suppressive with some form of hidden suppression, and 44.1% had no hidden suppression. In suppressive 5-drug combinations, 33.8% had partially nested suppression, 7.1% were partially suppressed, and 59.1% had no hidden suppression (Table 19). Overall, whether net suppressive or suppressive based on emergent interactions, higher-order drug combinations that fit into a defined case mostly fit the definition for partially nested suppression.

In regard to 3-drug combinations that have hidden suppression only and were neither net suppressive nor deemed suppressive using emergent interactions, all such combinations did not fit definitions for the fully hidden suppression or partially hidden suppression cases. This was

also the case for all 4- and 5-drug combinations that only had hidden suppression. Using net interactions, 0.3% of 4-drug combinations with hidden suppression only experienced fully nested hidden suppression; 78% experienced partially nested hidden suppression, and 21.8% just had a hidden suppressive interaction of some kind. In 5-drug combinations, 39.2% had partially nested hidden suppression, and 60.8% had a hidden suppressive interaction of some kind (Table 20). Using emergent interactions, 0.4% of 4-drug combinations with hidden suppression only experienced fully nested hidden suppression; 81.8% experienced partially nested hidden suppression, and 17.8% only had hidden suppressive interaction of some kind. In 5-drug combinations, 55.9% of combinations had partially nested hidden suppression and 44.1% had hidden suppressive interactions of some kind (Table 21). In general, the trend of 4- and 5-drug combinations with hidden suppressive interactions only mostly falling into the partially nested hidden suppression case is shared between net and emergent-identified combinations. Across all combinations determined to be suppressive or have hidden suppression using both net and emergent interactions, the number of combinations that fit the case of partially nested suppression is most abundant.

C. Likelihood of Specific Drugs or Mechanisms of Action Involved in Suppressive Interactions

We used logistic regressions to determine if any drug or the main mechanism of action may have a positive association with general suppressive interactions (hidden and net). The presence of trimethoprim alone was found to be significantly positively associated with suppressive interactions for 3-drug, 4-drug, and 5-drug combinations (Table 3, Table 7, Table 11). Ciprofloxacin, doxycycline, and erythromycin only had a significant positive association with suppressive interactions in 4-drug and 5-drug combinations (Table 7 and Table 11). The

presence of trimethoprim increased the odds of a 3-drug, 4-drug, and 5-drug combination being suppressive by roughly two-fold ($p < 0.001$). The combined presence of ciprofloxacin and trimethoprim (CPR+TMP), and cefoxitin and trimethoprim (FOX+TMP) were also found to significantly increase the probability of finding suppressive interactions in 3-drug, 4-drug, and 5-drug combinations ($p < 0.001$) (Table 4, Table 8, Table 12). The combined presence of ampicillin and ciprofloxacin, ciprofloxacin and erythromycin, doxycycline and cefoxitin, and erythromycin and trimethoprim, had a positive association with suppressive interactions for 4-drug, and 5-drug combinations ($p < 0.001$) (Table 8 and Table 12).

When considering the main mechanism of action rather than individual antibiotics, the presence of the antibiotic acting on folic acid biosynthesis (trimethoprim) was found to be significantly positively associated with suppressive interactions ($p < 0.01$) in 3-drug, 4-drug, and 5-drug combinations (Table 5, Table 9, Table 13). There were only two positive associations that occur across all levels of higher-order drug combinations (i.e. 3-drugs, 4-drug, and 5-drug combinations): they are with the antibiotic acting on folic acid biosynthesis, trimethoprim, alone ($p < 0.0001$) and the combination of two main mechanism of actions—folic acid biosynthesis and the DNA gyrase ($p < 0.001$) (Table 6, Table 10, Table 14). The probability of a combination having suppressive interactions decreases with the presence of an antibiotic acting on the 30S ribosomal subunit alone in the 3-drug, 4-drug, and 5-drug combinations ($p < 0.0001$) (Table 5, Table 9, Table 13).

CHAPTER 4. DISCUSSION

While it was previously reported that higher-order drug combinations had a substantial amount of suppressive interactions (14% in Beppler et al. (2017) and 8% in Tekin et al. (2018)),

there has been no further work on understanding the patterns and prevalence of higher-order suppressive interactions, particularly hidden interactions. The idea of hidden suppressive interactions was first introduced by Beppler and colleagues several years ago (Beppler et al., 2017). New technologies are now allowing rapid detection of suppressive interactions using both very small volumes of bacterial culture and antibiotic combinations (<1uL) and very short time frames of several hours (Cokol et al., 2011, Churski et al., 2012, Cokol et al., 2014). New conceptual advances allow us to examine higher-order interactions and emergent properties of drug combinations (Beppler et al., 2016, Tekin et al., 2016, Katzir et al., 2019, Lukačišin and Bollenbach, 2019). Because of this, suppressive interactions have received more focus recently (see review Singh and Yeh (2017)). We have shown that even with recent advancements and interest in suppression, one can severely underestimate the number of suppressive interactions by not considering hidden suppression.

When examining hidden suppression, increasing the number of drugs in a combination also increases the number of possible lower-order combinations thus possibly increasing the total number of combinations with hidden suppression interaction. When we look at the overall percentage of combinations with hidden suppression this value steadily increases from 33% to 48% to 59% as the number of drugs increases (Figure 4). This would explain the trends we see in Figure 5 for synergistic, additive, and antagonistic combinations. However, this does not offer a viable explanation for the negative correlation between the amount of hidden suppression and the number of drugs in a combination of net and emergent suppressive combinations.

In 2-drug combinations, it has been shown that a combination of DNA synthesis inhibitors and protein synthesis inhibitors have higher amounts of suppression (Yeh et al., 2006, Chait et al., 2007, Bollenbach et al., 2009). Thus, we expected that we might find some drugs or

main mechanisms of actions more consistently involved in suppressive interactions, and this was indeed the case. We have shown that there is a significant positive association with suppressive interactions and interference with the 50S ribosomal subunit in combination with a DNA gyrase in 4-drug combinations and a significant positive association with suppressive interactions and interference with the 30S ribosomal subunit in combination with a DNA gyrase in 5-drug combinations. These findings are supported by the one suppressive mechanism that is very well understood (Bollenbach et al., 2009).

The main mechanism of action is one way that antibiotics are commonly grouped. We expected to see similar patterns of association between the logistic regressions based on specific drugs and based on the main mechanism of actions. We observe this similarity with the main mechanism of actions affecting folic acid biosynthesis trimethoprim, affecting the 50S ribosomal subunit—doxycycline and erythromycin, and affecting DNA gyrase—ciprofloxacin. As previously described the identification of DNA gyrases and protein syntheses can be expected to be positively associated with suppressive interactions. However, folic acid biosynthesis interference is positively associated with suppressive interactions in all levels of drug combinations (3-drug, 4-drug, and 5-drug combinations). We suggest that this cellular mechanism may also be a mechanism for suppression and could be a fruitful avenue for future studies.

Further, though use of net and emergent interactions resulted in different values of combinations fitting the cases defined (Table 2), the partially nested suppression case was identified the most. This may give insight into a prevalent structure of hidden suppression in higher-order combinations when present. Admittedly, though, the combinations screened as having hidden suppression with a different highest-order interaction are of the most interest.

Combinations deemed synergistic but with hidden suppression would be of further interest, but no synergistic combinations had hidden suppression that fell into any special case. This is because such combinations could possibly reap the benefits of both the highest-order interaction and the hidden suppressive interactions to lower-orders.

Hidden suppressive interactions can affect fitness landscapes, which means they ultimately could affect the evolutionary trajectory of populations. For example, if we use a drug combination with a corresponding fitness landscape based only on information from the single drugs and the 5-drug combination, we could end up with a landscape topography that looks very different from a fitness landscape where we had information from all lower-order drugs (Figure 10). This is not surprising because we have more information in the latter than the former. Qualitatively, the fitness landscapes are similar, but there are quantitative differences (Sanchez-Gorostiaga et al., 2019). In contrast, in cases where hidden suppression is present, a landscape without the lower-order interaction information would look very different from a landscape with all the lower-order interactions (Figure 10-11). Qualitatively, there are important differences between the fitness landscape because there are local valleys and peaks that are present in the latter and not present in the former. These valleys and peaks can affect how a population evolves, and where it ends up (Østman et al., 2011, Palmer et al., 2015, Bendixsen et al., 2017).

Within a specific drug pair, recent work has shown that the concentrations at which two drugs veer into suppressive territory (from, for example, additivity) could be understood via a cost-benefit analysis. There is a trade-off between a drug inducing resistance (good for the bacterial cell) and increasing toxicity (bad for the bacterial cell), and this trade-off could explain why certain concentrations in one drug pair are suppressive, whereas other concentrations exhibit different interaction types (Wood and Cluzel, 2012). Furthermore, with some exceptions,

suppressive interactions, as with most interactions, are typically robust to genetic mutations (Chevereau and Bollenbach, 2015).

Clinicians traditionally favor treatments with synergistic combinations, because it limits the number of antibiotics prescribed to the patient limiting any potential adverse effects (Lepper and Dowling, 1951, French et al., 1985, Sun et al., 2013, Arya et al., 2019), rather than treatment with suppressive combinations. This is because by definition, using suppressive interactions means using higher drug concentrations to achieve the same bacterial killing effect as drugs that are additive or synergistic. Thus, hidden suppressive interactions are ones that could be confounding in the clinic. As more treatments move to higher-order combinations of drugs (Mbuagbaw et al., 2016, Sun et al., 2016, Morimoto et al., 2018, Tsigelny, 2019), it becomes critical to understand where suppressive interactions may be hidden, to avoid surprising and unwelcome clinical outcomes. For example, as shown in Figure 10, if one were to use a combination of CPR+ERY+STR+FUS+TMP, if we only compared the results of the five drugs together with all the single drugs alone, we would think this was a potentially useful combination, in that killing efficiency seems to increase relative to the five single drugs by themselves. But once we examine these in light of emergent properties, what we see is that CPR+ERY+STR+FUS+TMP has a lower killing efficiency than CPR+STR+FUS+TMP.

In conclusion, we show here that higher-order drug combinations exhibit a large number of suppressive interactions, and these interactions are primarily hidden. That is, we would never know there was a suppressive interaction if we only looked at the effects of the highest-order combinations and compared that to all the single-drug effects. Uncovering hidden suppressive interactions could decrease surprises regarding how populations evolve to drug combinations. At

the same time, identifying hidden suppression can yield valuable information about underlying reasons regarding which drug combinations could be useful and which ones should be avoided.

CHAPTER 4. LIMITATIONS AND RESOURCE AVAILABILITY

Limitations of the Study

Here we exemplify the need to consider hidden interactions and the possible implications of hidden suppression. To do this we examined an extensive data set and found intriguing results. However, ideally, additional data could be analyzed with an even larger group of drugs examined, allowing for multiple representatives from each antibiotic class and the main mechanism of actions. The data set from Tekin et al. (2018) used low levels of inhibition for each individual drug in an attempt to have detectable growth when antibiotics are used in 5-drug combinations. The low inhibition of each individual drug can affect the fraction of net-suppressive interactions by narrowing the range of a suppressive interaction. But ultimately these concentrations were chosen to avoid killing off the entire bacterial populations before a 5-drug combination could be examined.

Additionally, given that we used the same cutoff for hidden suppression as is used for net suppression, identifying a more formal cutoff value for hidden suppression would be ideal. Our value is somewhat arbitrary given its determination came from the same dataset used. It resulted from scaling the interaction values and identifying peaks in the distribution of drug combinations against the interaction metric that best-informed cutoffs for the different interaction types (Tekin et al., 2018). The same process could be used again across multiple datasets to see if a more fitting cutoff can be generalized.

Resource Availability

Materials Availability

This study did not generate new unique reagents.

Data and Code Availability

All data and code has been made freely available via Mendeley Data (https://data.mendeley.com/datasets/ts2hnd72yf/draft?a=4fec844a-e75b-402b-9883-e34bfeff5c2a).

CHAPTER 5. FIGURES AND TABLES

A. FIGURES

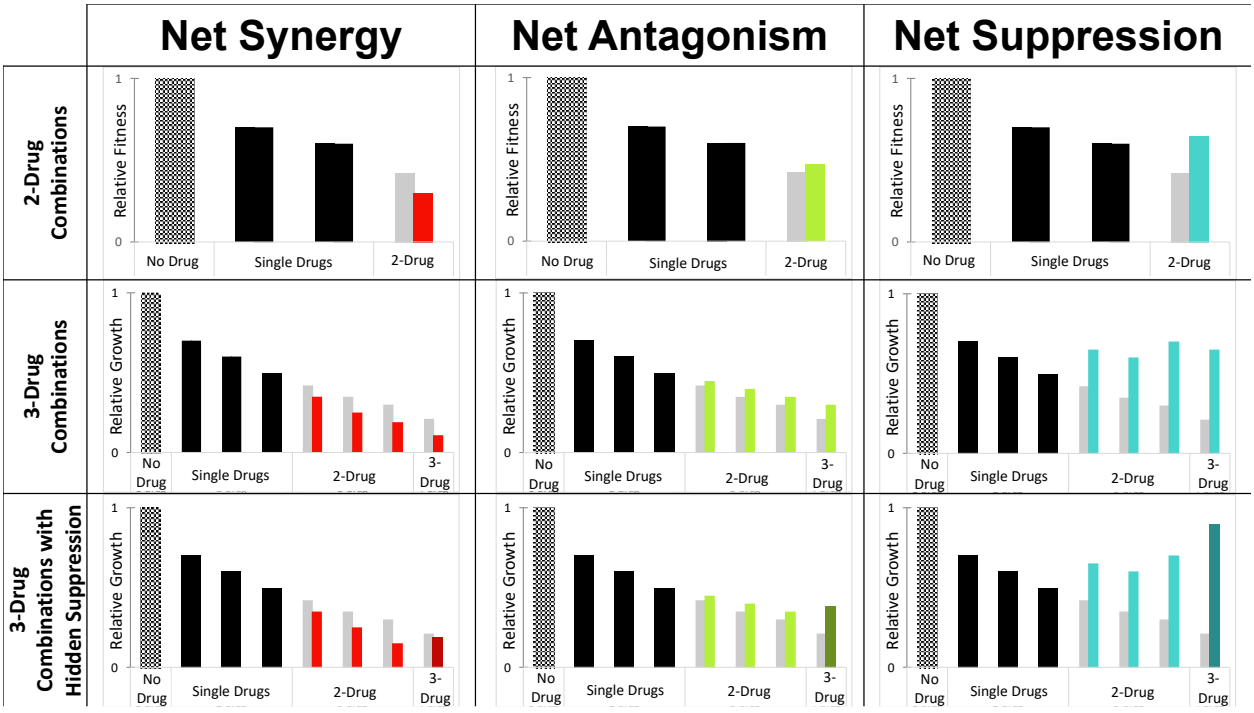

Figure 1. Antibiotic interactions in 2-drug and 3-drug combinations. Hatched bars represent growth in a no-drug environment, black bars represent the fitness of bacteria treated with a single antibiotic. Light gray bars represent the fitness of additive drug interactions, synergistic interactions are in red, antagonistic interactions are in green and suppressive interactions are in teal. Note that the 2-drug combinations do not need to have the same net interaction type for a 3-drug combination to have a particular net interaction. Suppressive interactions are an extreme form of antagonism: notice that the bacteria treated with the suppressive drug combination has a higher fitness then the single drugs.

Importantly, suppressive interactions can be hidden: this occurs when the highest-order combination has higher fitness than a lower-order combination but it does not have higher fitness than any of the single drugs. Thus, hidden suppression can only occur in a combination of 3 or more drugs. Also note, that bacteria treated with the 3-drug combination with hidden suppression has a higher fitness compared to any of the 2-drug combinations but not one of the single drugs.

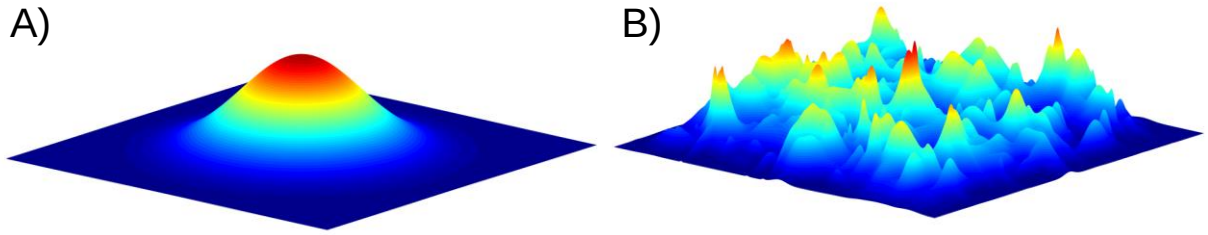

Figure 2. An illustration of the fitness landscapes and the importance of ruggedness in evolutionary trajectories. A) A smooth landscape only has one peak. As a population evolves to an environment there is always a path that leads to the optimum set of traits resulting in the highest possible fitness. **B)** In a rugged landscape, multiple peaks and valleys make evolving to the highest fitness not as straightforward as in a smooth landscape. Populations may have to cross a valley which means (1) a loss of fitness must first occur before a net increase in fitness, (2) the population can become stuck at a local peak rather than evolve and ascend to the global peak, or (3) the population must make a jump from one peak to the next. Without the lower-order interactions, we may miss key details of intermediate peaks and valleys in the fitness landscape.

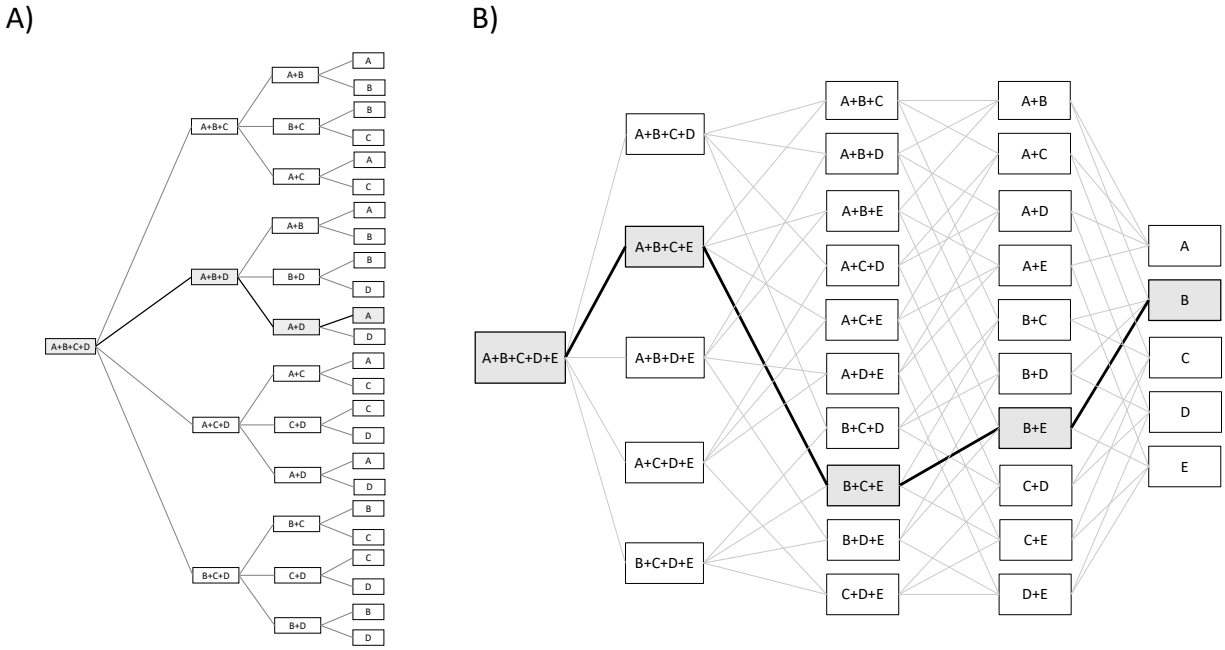

Figure 3. The paths for a 4-drug and a 5-drug combination consisting of drugs A, B, C, D, and E. A) All 24 possible paths are shown for a 4-drug combination. **B)** All 120 possible paths are shown for a 5-drug combination. For both the 4-drug (A) and 5-drug (B) combinations**,** a single path is shown in a bold

line with the highest-order combination and each lower-order combination highlighted in gray. This single path represents a unique set of drugs, one at each level of combinations (4-drug, 3-drug, 2-drug, and a single drug), allowing for an assessment of any nesting. For this example, nested hidden suppression occurs when the 5-drug combination is suppressive to the 4-drug, the 4-drug combination is suppressive to a 3-drug combination, and the 3-drug combination is then suppressive to a 2-drug combination. And, if appropriate, the 2-drug combination is suppressive to the single drug effects (this is only considered if the combination is net suppressive). If this is true for all paths the combination is considered to be fully nested. If this is only observed in some paths the combination is considered to be partially nested.

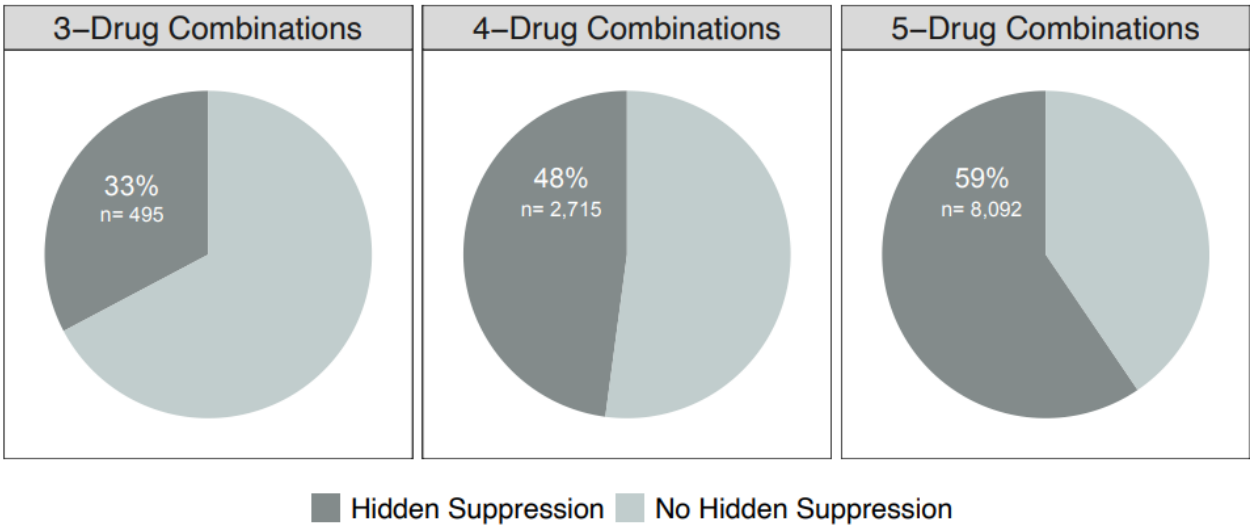

Figure 4. Hidden suppression is present in a majority of higher-order combinations. Hidden Suppression was found in all levels examined—3-drug, 4-drug, and 5-drug combinations. The amount of hidden suppression increases as the number of drug increase.

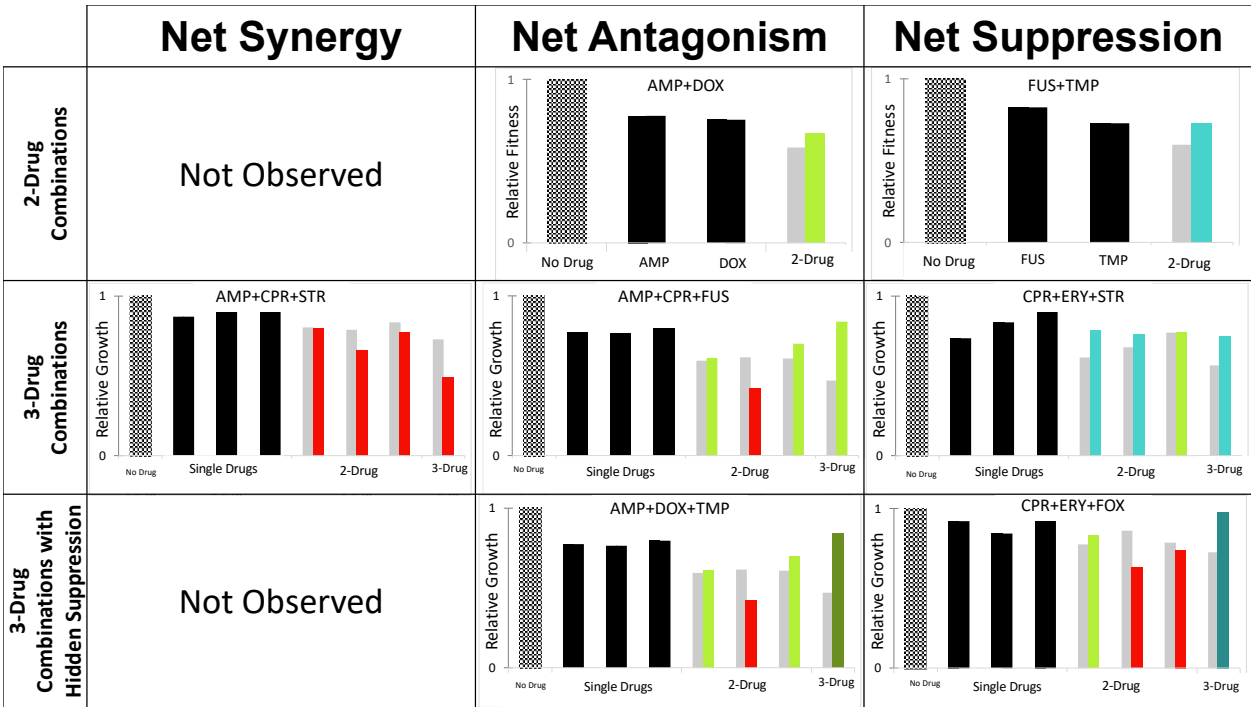

Figure 5. Examples from the data of antibiotic interactions in 2-drug and 3-drug combinations. Combinations are listed above bar graphs for each example (for abbreviations see Table 1). Hatched bars represent growth in a no-drug environment, black bars represent the fitness of bacteria treated with a single antibiotic. Light gray bars represent the fitness of additive drug interactions, synergistic interactions are in red, antagonistic interactions are in green and suppressive interactions are in teal. Note that the 2-drug combinations do not need to have the same net interaction type for a 3-drug combination to have a particular net interaction. Suppressive interactions are an extreme form of antagonism: notice that the bacteria treated with the suppressive drug combination has a higher fitness then the single drugs. Importantly, suppressive interactions can also be hidden when the highest-order combination has higher fitness than a lower-order combination and not the single drugs. Thus, hidden suppression can only occur in a combination of 3 or more drugs. Also note, that bacteria treated with the 3-drug combination with hidden suppression has a higher fitness compared to any of the 2-drug combinations but not one of the single drugs.

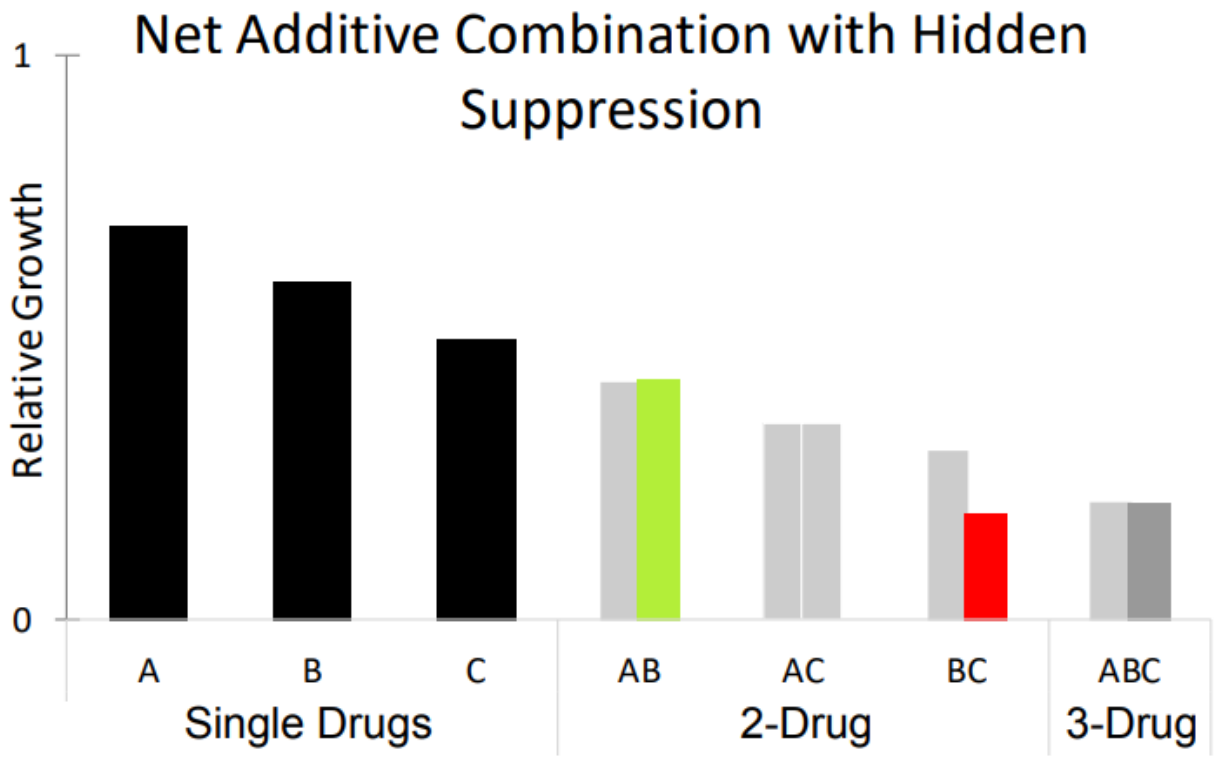

Figure 6. Hidden suppression can be within a net additive combination. The bars in black show the effects of the single drugs. The grey bars on the left show the additive expectations given the single drug effects while the bar on the right shows the actual relative growth when exposed to the combination. The 2-drug combinations have varying interactions, combination AB is an antagonistic interaction (green bar), combination AC is an additive interaction so the expected grey bar is the same as the relative growth that is observed, and BC is a synergistic combination (red bar). Due to the nature of a hidden suppressive interaction, a net additive combination can have hidden suppressive interactions (3-drug combination in dark gray) as long as at least one of the lower-order interactions is synergistic (2-drug combination BC in red). Note that although the three-drug combination (dark gray) has the same value as the strictly additive case (light gray) it is considered to have hidden suppression because one of the lower-order 2-drug combinations is synergistic (red). This makes the 3-drug combination have higher fitness than the 2-drug lower-order combination.

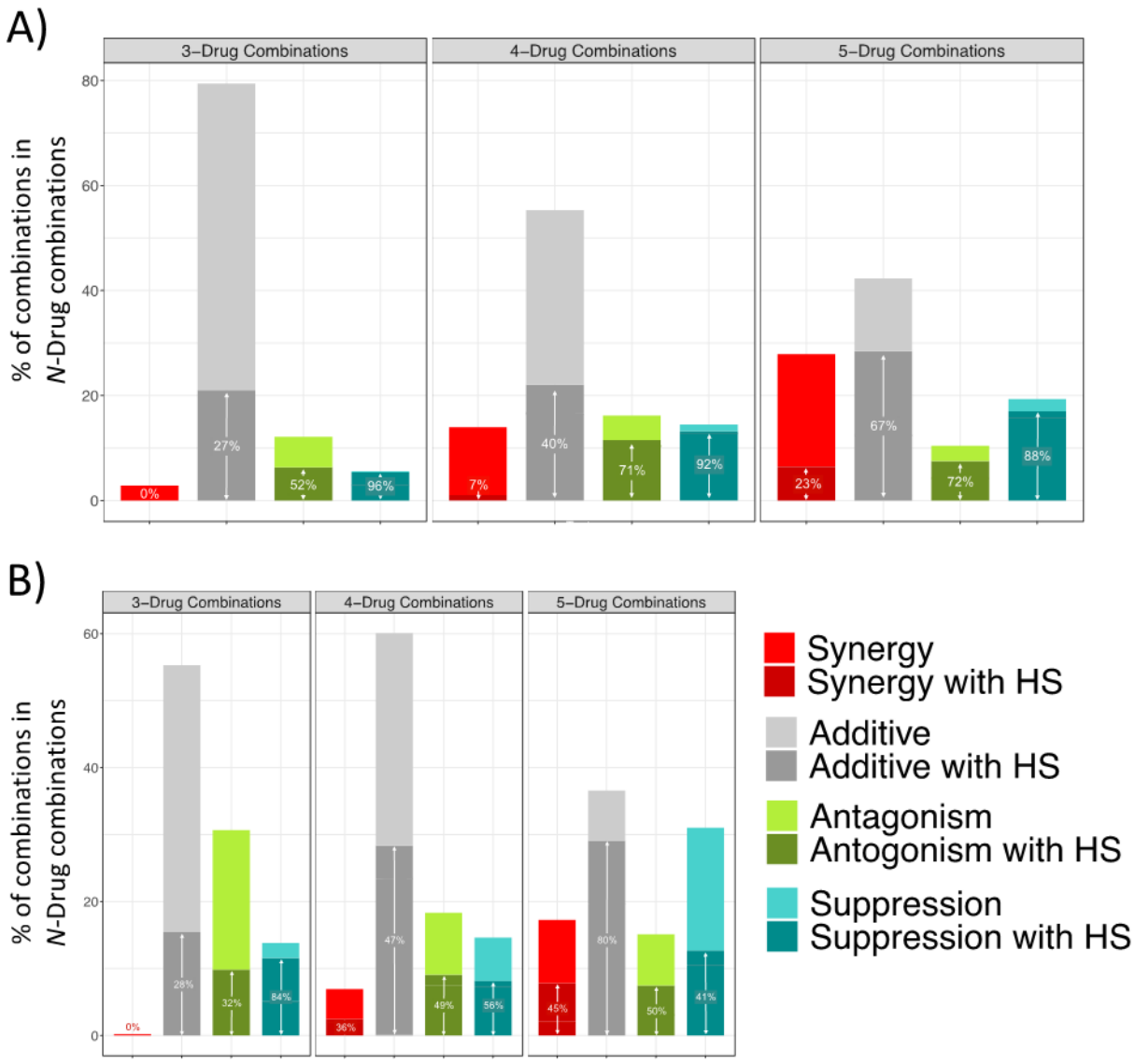

Figure 7. The distributions and relative proportion of hidden suppression for each interaction type for net (A) and emergent (B) interactions for 3-, 4-, and 5- drug combinations. The proportion of combinations with hidden suppression (HS) of suppressive interactions (teal) decreases as the number of drugs in a combination increases. The percentage written inside the darker shades of the bars represents the proportion of combinations with hidden suppression present in that specific interaction type. The y-axis is the percentage of each interaction type within the designated level of the drug combination, showing the overall distribution of net or emergent interactions. For example, in A) the net suppressive 4-drug combinations, 92% of the combinations have hidden suppression within them. As the number of drugs increases, the amount of hidden suppression within additive, synergistic, and antagonistic combinations also increase.

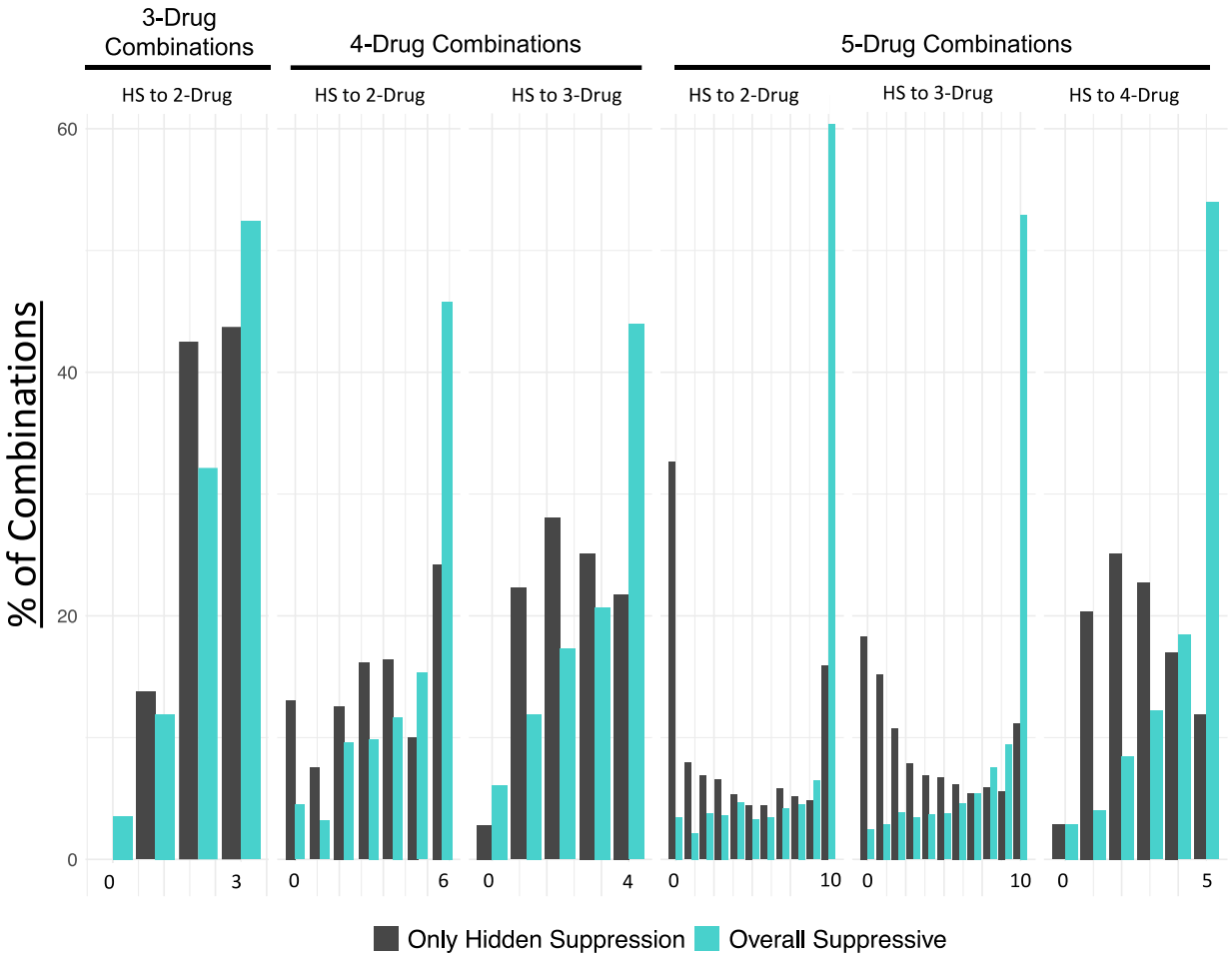

Figure 8. Hidden suppressive interactions occur more frequently within net suppressive combinations rather than within non-net suppressive combinations. The amounts of hidden suppression are shown out of the total number of lower-ordered combinations within a single higher-order combination that is either net suppressive (teal) or have some instances of hidden suppression (gray).

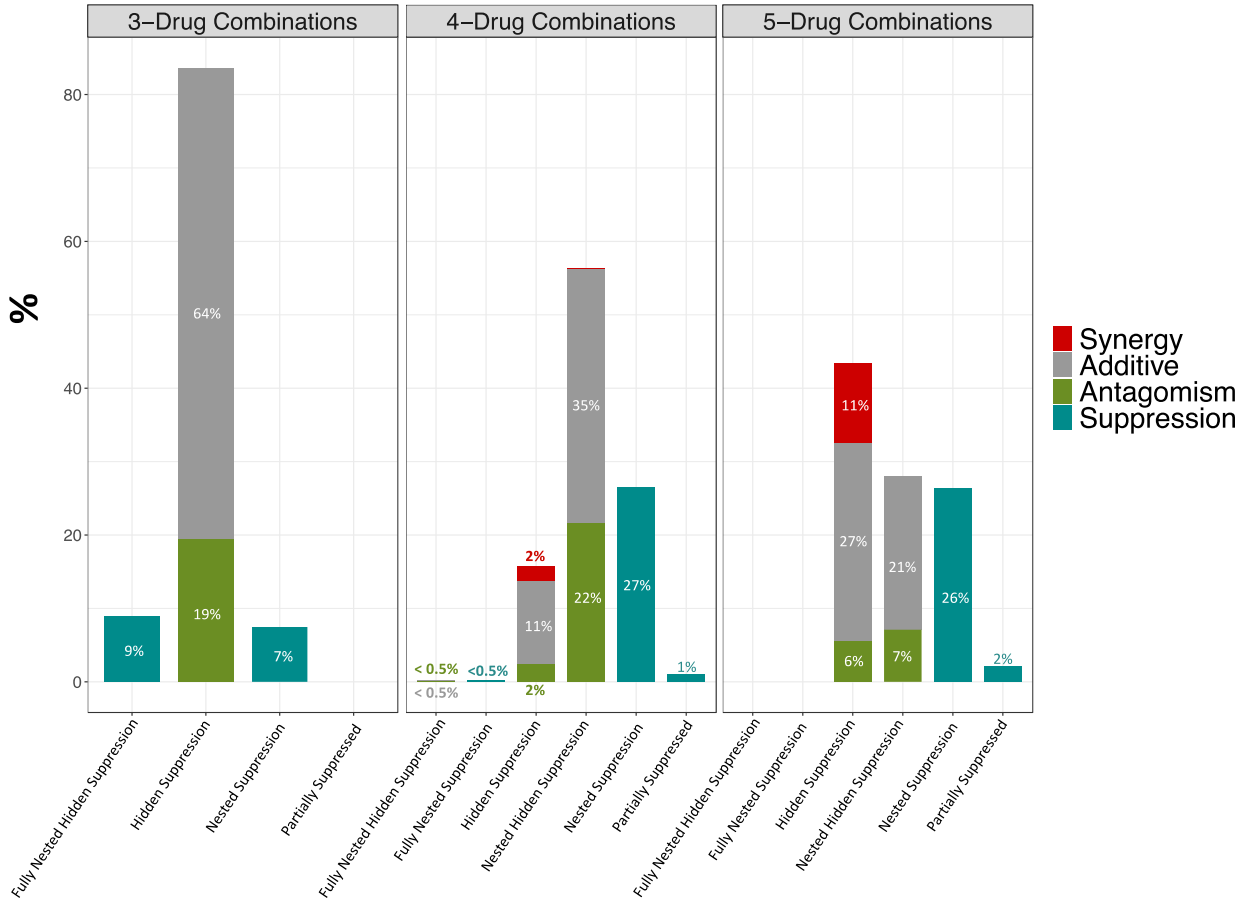

Figure 9. Distribution of special cases of hidden suppression structure. All net synergistic combinations only have hidden suppression that does not adhere to any special case. 3-drug combinations were only tested for fully hidden suppression, hidden suppression, nested suppression, and partially suppressed, because all other special cases are trivial in a 3-drug combination.

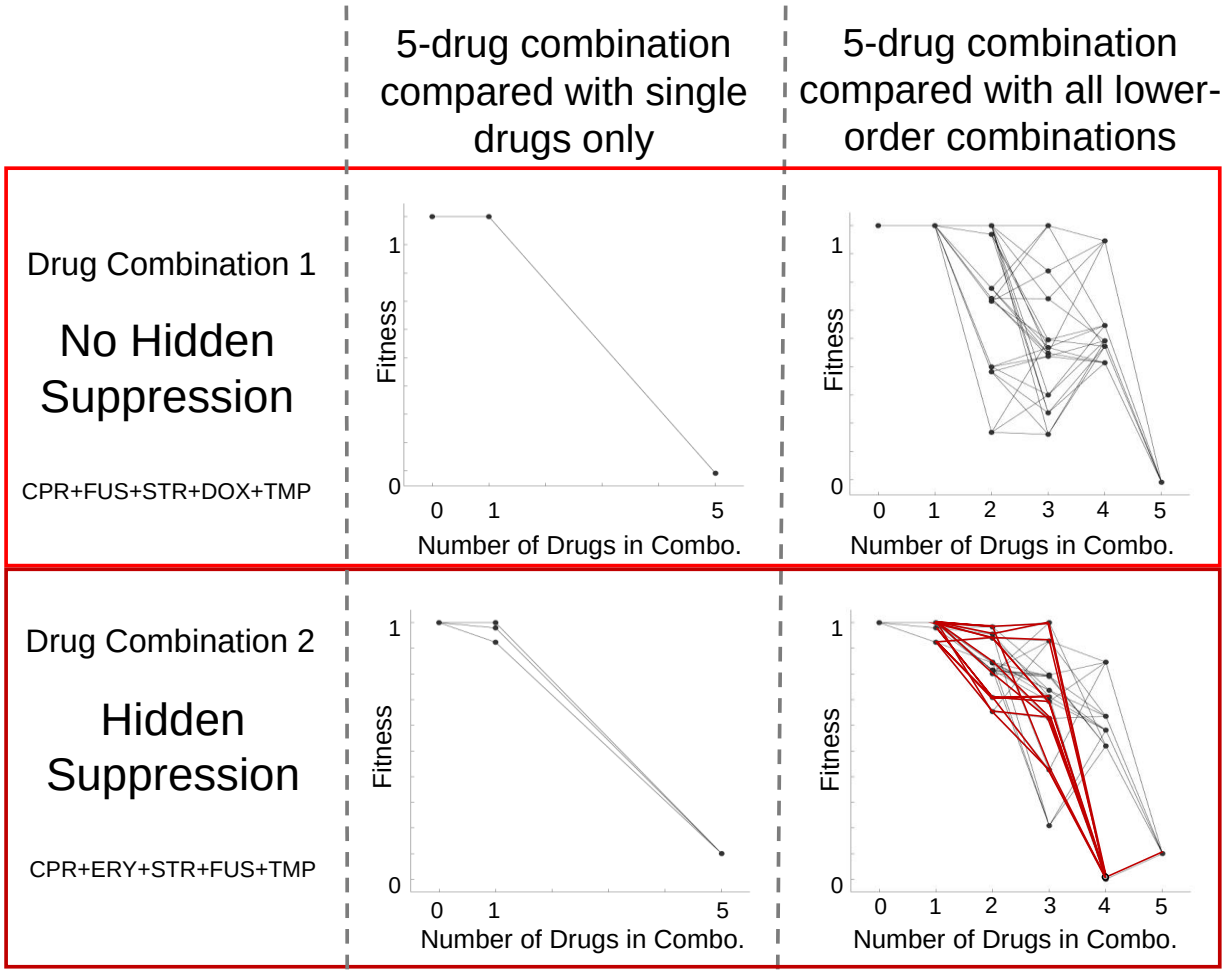

Figure 10. Fitness graphs show the importance of considering hidden interactions. Fitness graphs show similar information as a fitness landscape, they both help to visualize the relationships between stressors or genetic mutations and their effects on fitness. However, fitness graphs can be more appropriate for discrete data. Here we show fitness graphs of two synergistic 5-drug combinations (for abbreviations see Table 1). Drug combination 1 has no hidden suppression (top) and drug combination 2 has hidden suppression (bottom). The left-hand side shows the fitness graphs not considering the hidden suppression notice how similar these two appear to be. While the figures on the right-hand side show the fitness graphs including the lower-order combinations, notice the increase in ruggedness is due to the hidden suppressive interactions (the decrease in fitness at one of the 4-drug combinations) in the bottom right. The edges in red highlight the paths involved in hidden suppression. For more detailed information about these paths please see Figure 11.

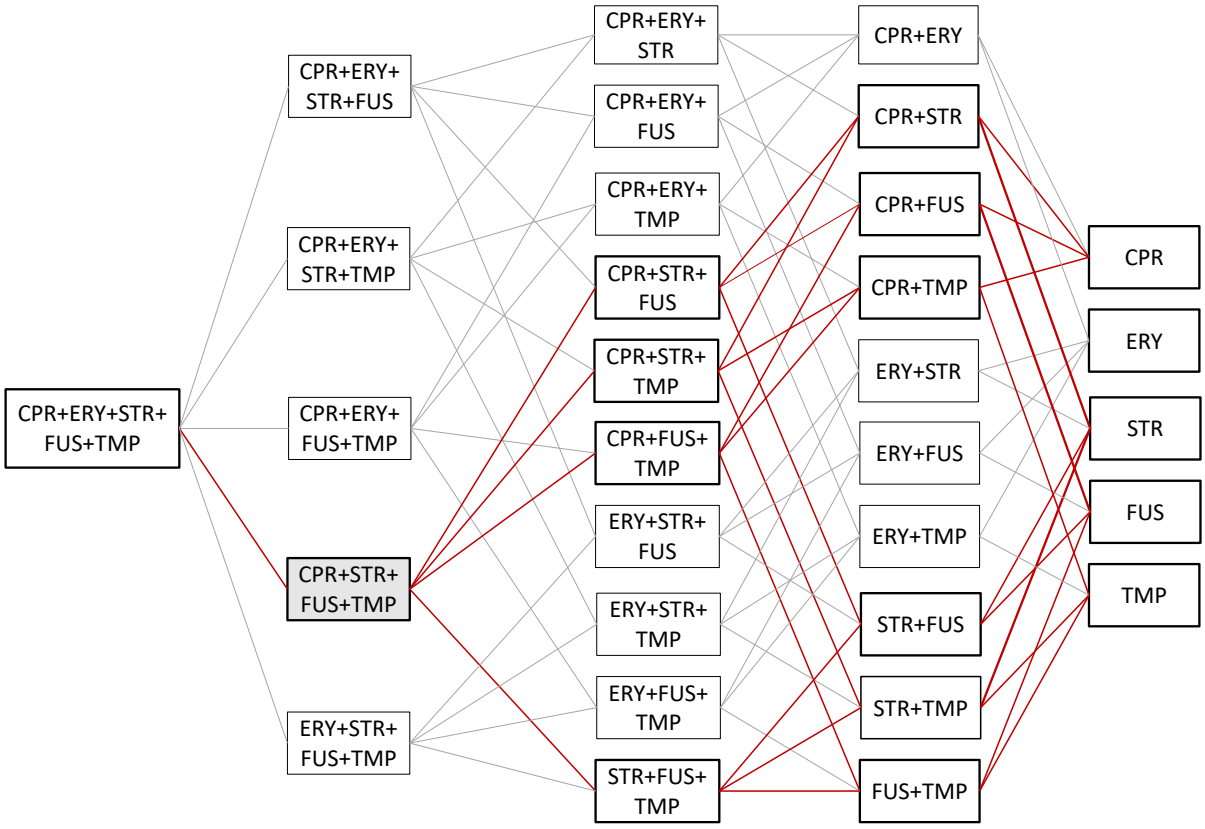

Figure 11. Paths of example synergistic drug combination with hidden suppression. All 120 paths for the combination CPR+ERY+STR+FUS+TMP (for abbreviations see Table 1). Paths highlighted in red with bold edges contain hidden suppression between the 5-drug combination and the 4-drug combination CPR+STR+FUS+TMP (shaded in grey). These highlighted paths are the same paths shown in Figure 10.

B. TABLES

Table 1. A list of the names, concentrations, main mechanism of action, mean relative growth compared to a no-drug control, and the abbreviation of the antibiotics used in this study.

Name (Abbreviation)	Main Mechanism of Action	Concentration (μM)	Relative Growth (%)	Standard Error (%)
Ampicillin (AMP)	Cell Wall	1- 2.89 2- 2.52 3- 1.87	1- 77.43% 2- 86.01% 3- 87.06%	1- 3.05% 2- 1.74% 3- 2.42%
Cefoxitin sodium salt (FOX)	Cell Wall	1- 1.78 2- 1.37 3- 0.78	1- 83.46% 2- 92.13% 3- 93.33%	1- 4.73% 2- 2.58% 3- 1.81%
Trimethoprim (TMP)	Folic Acid Biosynthesis	1- 0.22 2- 0.15 3- 0.07	1- 79.59% 2- 74.63% 3- 68.20%	1- 3.89% 2- 4.26% 3- 3.93%
Ciprofloxacin hydrochloride (CPR)	DNA gyrase	1- 0.03 2- 0.02 3- 0.01	1- 92.14% 2- 92.14% 3- 91.06%	1- 1.69% 2- 2.40% 3- 2.17%
Streptomycin (STR)	Aminoglycoside Ribosome, 30S	1- 19.04 2- 16.6 3- 12.25	1- 81.10% 2- 90.77% 3- 83.53%	1- 6.50% 2- 1.37% 3- 4.30%
Doxycycline hyclate (DOX)	Ribosome, 50S	1- 0.35 2- 0.27 3- 0.15	1- 75.15% 2- 76.53% 3- 70.01%	1- 5.51% 2- 5.13% 3- 4.73%
Erythromycin (ERY)	Ribosome, 50S	1- 16.62 2- 8.29 3- 1.78	1- 84.25% 2- 84.29% 3- 79.63%	1- 5.77% 2- 5.60% 3- 5.91%
Fusidic acid sodium salt (FUS)	Ribosome, 30S	1- 94.42 2- 71.01 3- 37.85	1- 82.31% 2- 78.82% 3- 82.62%	1- 2.51% 2- 2.83% 3- 2.47%

Table 2. Special Case Definitions. A description of each special case definition for both net suppressive interactions and not net suppressive interactions.

Net Suppression Classification $(\boldsymbol{DA_N > 1.3})$		**Hidden Suppression Classification** $\left(\boldsymbol{{w_N}/{w_{\text{min of lower orders}}} > 1.3}\right)$	
Special Case	Definition	Special Case	Definition
Fully Nested Suppression	In all paths, fitness at any order must be greater than the fitness of all lower-orders.	Fully Nested Hidden Suppression	In all paths, fitness at any order must be greater than the fitness of all lower-orders, **excluding** the single drugs.

Partially Nested Suppression	In at least one path, fitness at any order must be greater than the fitness of all lower-orders.	Partially Nested Hidden Suppression	In at least one path, fitness at any order must be greater than the fitness of all lower-orders, **excluding** the single drugs.
Fully Suppressed	In all paths, fitness at the highest-order(w_N) is greater than the fitness of all lower-orders.	Fully Hidden Suppression	In all paths, fitness at the highest-order(w_N) is greater than the fitness of all lower-orders, **excluding** the single drugs.
Partially Suppressed	Only some paths have the highest-order(w_N) fitness greater than all lower-order fitness.	Partially Hidden Suppression	Only some paths have the highest-order(w_N) fitness greater than all lower-order fitness, **excluding** the single drugs.
Suppressive Interaction with Hidden Suppression	The highest-order combination does not fulfill any other conditions but is still has at least one hidden suppressive interaction.	Hidden Suppressive Interaction	The highest-order combination does not fulfill any above conditions, but still has an element of hidden suppression.
No Hidden Suppression	No paths have the highest-order(w_N) fitness greater than lower-order fitness, excluding first-order(w_1).		

Table 3. Logistic regression of a single drug with 3-drug combinations with some levels of suppressive interactions (hidden and net). Terms in **bold** have a significant positive association with suppressive interactions.

Term	Coefficient	Confidence Interval		p-value	Odds Ratio	Probability
		0.30%	99.70%			
AMP	-0.416	-0.720	-0.117	1.58E-04	0.660	40%
CPR	0.019	-0.277	0.311	0.863	1.019	50%
DOX	0.096	-0.198	0.388	0.371	1.100	52%
ERY	-0.112	-0.409	0.183	0.302	0.894	47%
FOX	-0.085	-0.382	0.208	0.429	0.918	48%
FUS	-0.868	-1.185	-0.560	2.96E-14	0.420	30%
STR	-1.684	-2.053	-1.337	5.02E-38	0.186	16%

TMP	**0.729**	**0.443**	**1.018**	**3.75E-12**	**2.074**	**67%**
AIC: 1678.2	Bonferroni- corrected α: 0.00625				Degrees of Freedom: 1512	

Table 4. Logistic regression of pairwise drugs with 3-drug combinations with some levels of suppressive interactions (hidden and net). Terms in **bold** have a significant positive association with suppressive interactions.

Term	Coefficient	Confidence Interval		p-value	Odds Ratio	Probability
		0.10%	99.90%			
AMP+CPR	0.126	-0.576	0.817	0.571	1.134	53%
AMP+DOX	0.252	-0.379	0.877	0.208	1.287	56%
AMP+ERY	-0.778	-1.498	-0.097	4.95E-04	0.460	31%
AMP+FOX	-0.524	-1.291	0.212	0.029	0.592	37%
AMP+FUS	-1.671	-2.593	-0.861	1.27E-09	0.188	16%
AMP+STR	-1.432	-2.477	-0.559	2.23E-06	0.239	19%
AMP+TMP	**0.953**	**0.305**	**1.627**	**6.08E-06**	**2.594**	**72%**
CPR+DOX	0.159	-0.439	0.753	0.403	1.172	54%
CPR+ERY	-0.208	-0.836	0.406	0.294	0.812	45%
CPR+FOX	-0.857	-1.565	-0.182	1.01E-04	0.425	30%
CPR+FUS	-0.307	-1.008	0.359	0.159	0.736	42%
CPR+STR	-0.755	-1.561	-0.027	1.94E-03	0.470	32%
CPR+TMP	**0.739**	**0.122**	**1.379**	**2.25E-04**	**2.094**	**68%**
DOX+ERY	-0.189	-0.798	0.407	0.326	0.828	45%
DOX+FOX	0.570	-0.039	1.185	3.51E-03	1.768	64%
DOX+FUS	0.388	-0.238	1.002	0.050	1.474	60%
DOX+STR	-0.939	-1.783	-0.186	2.10E-04	0.391	28%
DOX+TMP	-1.044	-1.685	-0.420	2.31E-07	0.352	26%
ERY+FOX	0.182	-0.485	0.846	0.392	1.199	55%
ERY+FUS	-0.775	-1.498	-0.101	4.93E-04	0.461	32%
ERY+STR	0.030	-0.682	0.699	0.890	1.031	51%
ERY+TMP	0.464	-0.155	1.094	0.020	1.590	61%
FOX+FUS	-0.848	-1.632	-0.122	4.23E-04	0.428	30%
FOX+STR	-1.607	-2.635	-0.740	8.27E-08	0.201	17%
FOX+TMP	**1.026**	**0.387**	**1.698**	**9.05E-07**	**2.790**	**74%**
FUS+STR	-0.942	-1.924	-0.104	1.06E-03	0.390	28%
FUS+TMP	-0.058	-0.688	0.559	0.769	0.943	49%
STR+TMP	-0.978	-1.756	-0.259	4.08E-05	0.376	27%
AIC: 1579.7	Bonferroni- corrected α: 0.00179				Degrees of Freedom: 1512	

Table 5. Logistic regression of the main mechanism of actions with 3-drug combinations with some levels of suppressive interactions (hidden and net). Terms in **bold** have a significant positive association with suppressive interactions.

Term	Coefficient	Confidence Interval		p-value	Odds Ratio	Probability
		0.50%	99.50%			
Cell Wall	-0.267	-0.517	-0.018	5.76E-03	0.765	43%
Folic Acid Biosynthesis	**0.742**	**0.470**	**1.017**	**2.74E-12**	**2.100**	**68%**
DNA gyrase	0.035	-0.246	0.314	0.747	1.036	51%
Ribosome, 30S	-1.462	-1.719	-1.212	5.36E-50	0.232	19%
Ribosome, 50S	0.062	-0.188	0.312	0.524	1.064	52%
AIC: 1716	Bonferroni- corrected α: 0.01			Degrees of Freedom: 1512		

Table 6. Logistic regression of the pairwise main mechanism of actions with 3-drug combinations with some levels of suppressive interactions (hidden and net). Terms in **bold** have a significant positive association with suppressive interactions.

Term	Coefficient	Confidence Interval		p-value	Odds Ratio	Probability
		0.20%	99.80%			
Cell Wall+Folic Acid Biosynthesis	**1.016**	**0.548**	**1.495**	**5.52E-10**	**2.762**	**73%**
Cell Wall+DNA gyrase	-0.417	-0.949	0.096	0.021	0.659	40%
Cell Wall+Ribosome, 30S	-1.565	-2.050	-1.109	6.46E-22	0.209	17%
Cell Wall+Ribosome, 50S	0.265	-0.114	0.645	0.044	1.303	57%
Folic Acid Biosynthesis+DNA gyrase	**0.742**	**0.175**	**1.326**	**1.90E-04**	**2.100**	**68%**
Folic Acid Biosynthesis+Ribosome, 30S	-0.304	-0.790	0.172	0.068	0.738	42%
Folic Acid Biosynthesis+Ribosome, 50S	-0.522	-1.022	-0.038	2.10E-03	0.593	37%
DNA gyrase+Ribosome, Ribosome, 30S	-0.529	-1.082	-0.009	4.25E-03	0.589	37%
DNA gyrase+Ribosome, Ribosome, 50S	-0.039	-0.507	0.428	0.808	0.961	49%
Ribosome, 30S+Ribosome, 50S	-0.160	-0.572	0.252	0.262	0.852	46%
Cell Wall+Cell Wall	-0.584	-1.148	-0.044	2.18E-03	0.558	36%
Ribosome, 30S+Ribosome, 30S	-1.565	-2.411	-0.857	3.93E-09	0.209	17%
Ribosome, 50S+Ribosome, 50S	-0.402	-0.905	0.085	0.019	0.669	40%
AIC: 1670.9	Bonferroni- corrected α: 0.0039			Degrees of Freedom: 1512		

Table 7. Logistic regression of single drug with 4-drug combinations with some levels of suppressive interactions (hidden and net). Terms in **bold** have a significant positive association with suppressive interactions.

Term	Coefficient	95% CI		p-value	Odds Ratio	Probability
		2.5%	97.5%			
AMP	-0.270	-0.373	-0.168	2.50E-07	0.763	43%
CPR	**0.430**	**0.327**	**0.533**	**2.37E-16**	**1.537**	**61%**
DOX	**0.214**	**0.112**	**0.317**	**4.22E-05**	**1.239**	**55%**
ERY	**0.517**	**0.414**	**0.620**	**7.30E-23**	**1.677**	**63%**
FOX	**0.385**	**0.282**	**0.488**	**2.09E-13**	**1.469**	**60%**
FUS	-0.829	-0.934	-0.724	3.75E-54	0.437	30%
STR	-1.333	-1.439	-1.228	2.61E-135	0.264	21%
TMP	**0.799**	**0.696**	**0.903**	**1.11E-51**	**2.223**	**69%**

AIC: 6693.7 Bonferroni-corrected α: 0.00625 Degrees of Freedom: 5670

Table 8. Logistic regression of pairwise drugs with 4-drug combinations with some levels of suppressive interactions (hidden and net). Terms in **bold** have a significant positive association with suppressive interactions.

Term	Coefficient	95% CI		p-value	Odds Ratio	Probability
		2.5%	97.5%			
AMP+CPR	**0.329**	**0.145**	**0.513**	**4.65E-04**	**1.389**	**58%**
AMP+DOX	0.039	-0.143	0.220	0.677	1.039	51%
AMP+ERY	-0.548	-0.730	-0.366	3.69E-09	0.578	37%
AMP+FOX	-0.185	-0.368	-0.003	0.047	0.831	45%
AMP+FUS	-0.356	-0.542	-0.171	1.71E-04	0.700	41%
AMP+STR	-0.416	-0.604	-0.229	1.36E-05	0.660	40%
AMP+TMP	**0.694**	**0.506**	**0.882**	**5.26E-13**	**2.001**	**67%**
CPR+DOX	**0.622**	**0.436**	**0.809**	**5.87E-11**	**1.863**	**65%**
CPR+ERY	**0.860**	**0.671**	**1.051**	**7.30E-19**	**2.363**	**70%**
CPR+FOX	-0.519	-0.705	-0.334	4.02E-08	0.595	37%
CPR+FUS	-0.520	-0.705	-0.335	3.69E-08	0.595	37%
CPR+STR	-0.652	-0.838	-0.467	5.72E-12	0.521	34%
CPR+TMP	**0.958**	**0.758**	**1.160**	**8.83E-21**	**2.606**	**72%**
DOX+ERY	0.191	0.008	0.375	0.042	1.211	55%
DOX+FOX	**0.517**	**0.335**	**0.699**	**2.48E-08**	**1.677**	**63%**
DOX+FUS	-0.132	-0.313	0.049	0.153	0.877	47%
DOX+STR	-0.574	-0.759	-0.391	8.63E-10	0.563	36%
DOX+TMP	-0.159	-0.350	0.033	0.104	0.853	46%
ERY+FOX	0.122	-0.063	0.307	0.198	1.129	53%
ERY+FUS	-0.026	-0.207	0.154	0.774	0.974	49%
ERY+STR	-0.098	-0.279	0.082	0.286	0.906	48%
ERY+TMP	**0.724**	**0.528**	**0.922**	**5.59E-13**	**2.063**	**67%**
FOX+FUS	-0.250	-0.432	-0.069	6.77E-03	0.779	44%

FOX+STR	-0.012	-0.194	0.169	0.893	0.988	50%
FOX+TMP	**1.204**	**1.011**	**1.400**	**7.50E-34**	**3.334**	**77%**
FUS+STR	-0.054	-0.246	0.138	0.584	0.948	49%
FUS+TMP	-0.497	-0.686	-0.310	2.11E-07	0.608	38%
STR+TMP	-1.087	-1.278	-0.898	2.92E-29	0.337	25%
AIC: 6308.8	Bonferroni-corrected α: 0.00179				Degrees of Freedom: 5670	

Table 9. Logistic regression of the main mechanism of actions with 4-drug combinations with some levels of suppressive interactions (hidden and net). Terms in **bold** have a significant positive association with suppressive interactions.

Term	Coefficient	95% CI		p-value	Odds Ratio	Probability
		2.5%	97.5%			
Cell Wall	**0.280**	**0.171**	**0.390**	**5.69E-07**	**1.324**	**57%**
Folic Acid Biosynthesis	**0.848**	**0.743**	**0.954**	**1.64E-55**	**2.335**	**70%**
DNA gyrase	**0.493**	**0.388**	**0.598**	**3.68E-20**	**1.637**	**62%**
Ribosome, 30S	-1.706	-1.836	-1.579	1.47E-149	0.182	15%
Ribosome, 50S	**0.613**	**0.503**	**0.725**	**3.23E-27**	**1.847**	**65%**
AIC: 6871.4	Bonferroni-corrected α: 0.01				Degrees of Freedom: 5670	

Table 10. Logistic regression of the pairwise main mechanism of actions with 4-drug combinations with some levels of suppressive interactions (hidden and net). Terms in **bold** have a significant positive association with suppressive interactions.

Term	Coefficient	95% CI		p-value	Odds Ratio	Probability
		2.5%	97.5%			
Cell Wall+Folic Acid Biosynthesis	**1.112**	**0.897**	**1.329**	**6.62E-24**	**3.040**	**75%**
Cell Wall+DNA gyrase	-0.219	-0.437	-0.001	0.049	0.803	45%
Cell Wall+Ribosome, 30S	-0.488	-0.728	-0.246	7.11E-05	0.614	38%
Cell Wall+Ribosome, 50S	0.263	0.037	0.487	0.022	1.301	57%
Folic Acid Biosynthesis+DNA gyrase	**0.870**	**0.662**	**1.083**	**5.15E-16**	**2.388**	**70%**
Folic Acid Biosynthesis+Ribosome, 30S	-0.737	-0.971	-0.506	5.13E-10	0.479	32%
Folic Acid Biosynthesis+Ribosome, 50S	0.183	-0.050	0.417	0.124	1.201	55%
DNA gyrase+Ribosome, Ribosome, 30S	**-0.418**	**-0.656**	**-0.182**	**5.34E-04**	**0.658**	**40%**

DNA gyrase+Ribosome, Ribosome, 50S	**0.782**	**0.562**	**1.004**	**3.76E-12**	**2.185**	**69%**
Ribosome, 30S+Ribosome, 50S	-0.300	-0.532	-0.066	0.012	0.741	43%
Cell Wall+Cell Wall	-0.081	-0.222	0.060	0.263	0.923	48%
Ribosome, 30S+Ribosome, 30S	-0.749	-0.899	-0.602	4.70E-23	0.473	32%
Ribosome, 50S+Ribosome, 50S	**0.346**	**0.204**	**0.489**	**1.82E-06**	**1.413**	**59%**

AIC: 6695.6 Bonferroni-corrected α: 0.0039 Degrees of Freedom: 5670

Table 11. Logistic regression of single drug with 5-drug combinations with some levels of suppressive interactions (hidden and net). Terms in **bold** have a significant positive association with suppressive interactions.

Term	Coefficient	95% CI		p-value	Odds Ratio	Probability
		2.5%	97.5%			
AMP	0.033	-0.032	0.097	0.317	1.033	51%
CPR	**0.351**	**0.287**	**0.415**	**9.27E-27**	**1.420**	**59%**
DOX	**0.148**	**0.083**	**0.212**	**7.02E-06**	**1.159**	**54%**
ERY	**0.261**	**0.197**	**0.326**	**1.65E-15**	**1.299**	**56%**
FOX	**0.205**	**0.140**	**0.269**	**4.60E-10**	**1.227**	**55%**
FUS	-0.465	-0.530	-0.399	5.03E-44	0.628	39%
STR	-0.292	-0.357	-0.227	1.36E-18	0.747	43%
TMP	**0.572**	**0.508**	**0.636**	**2.25E-68**	**1.771**	**64%**

AIC: 17458 Bonferroni-corrected α: 0.00625 Degrees of Freedom: 13602

Table 12. Logistic regression of pairwise drugs with 5-drug combinations with some levels of suppressive interactions (hidden and net). Terms in **bold** have a significant positive association with suppressive interactions.

Term	Coefficient	95% CI		p-value	Odds Ratio	Probability
		2.5%	97.5%			
AMP+CPR	**0.738**	**0.618**	**0.857**	**1.41E-33**	**2.091**	**68%**
AMP+DOX	-0.008	-0.127	0.111	0.891	0.992	50%
AMP+ERY	-0.480	-0.599	-0.362	1.78E-15	0.619	38%
AMP+FOX	-0.065	-0.183	0.054	0.286	0.937	48%
AMP+FUS	**0.298**	**0.172**	**0.425**	**3.75E-06**	**1.347**	**57%**
AMP+STR	-0.131	-0.254	-0.008	0.037	0.877	47%
AMP+TMP	-0.041	-0.161	0.078	0.499	0.960	49%
CPR+DOX	0.128	0.009	0.247	0.035	1.136	53%
CPR+ERY	**0.513**	**0.394**	**0.632**	**3.81E-17**	**1.670**	**63%**
CPR+FOX	-0.337	-0.456	-0.219	2.29E-08	0.714	42%
CPR+FUS	-0.238	-0.361	-0.114	1.60E-04	0.788	44%
CPR+STR	-0.570	-0.693	-0.448	7.58E-20	0.565	36%

CPR+TMP	**0.704**	**0.584**	**0.826**	**4.02E-30**	**2.023**	**67%**
DOX+ERY	**0.266**	**0.147**	**0.386**	**1.31E-05**	**1.305**	**57%**
DOX+FOX	**0.496**	**0.377**	**0.616**	**3.98E-16**	**1.642**	**62%**
DOX+FUS	-0.502	-0.624	-0.379	9.13E-16	0.606	38%
DOX+STR	-0.461	-0.584	-0.339	1.73E-13	0.631	39%
DOX+TMP	**0.740**	**0.619**	**0.861**	**3.89E-33**	**2.095**	**68%**
ERY+FOX	**0.369**	**0.250**	**0.489**	**1.28E-09**	**1.447**	**59%**
ERY+FUS	-0.483	-0.605	-0.361	7.90E-15	0.617	38%
ERY+STR	-0.183	-0.305	-0.060	3.45E-03	0.833	45%
ERY+TMP	**0.821**	**0.700**	**0.943**	**2.54E-40**	**2.274**	**69%**
FOX+FUS	-0.609	-0.731	-0.487	1.19E-22	0.544	35%
FOX+STR	**0.466**	**0.341**	**0.591**	**2.80E-13**	**1.594**	**61%**
FOX+TMP	**0.315**	**0.195**	**0.435**	**2.59E-07**	**1.371**	**58%**
FUS+STR	**1.174**	**1.022**	**1.331**	**3.24E-50**	**3.236**	**76%**
FUS+TMP	-0.388	-0.511	-0.265	6.09E-10	0.678	40%
STR+TMP	-0.802	-0.926	-0.679	2.60E-37	0.448	31%
AIC: 17458	Bonferroni-corrected α: 0.00179				Degrees of Freedom: 13602	

Table 13. Logistic regression of the main mechanism of actions with 5-drug combinations with some levels of suppressive interactions (hidden and net). Terms in **bold** have a significant positive association with suppressive interactions.

Term	**Coefficient**	**95% CI**		**p-value**	**Odds Ratio**	**Probability**
		2.5%	97.5%			
Cell Wall	**0.498**	**0.404**	**0.592**	**3.289E-25**	**1.645**	**62%**
Folic Acid Biosynthesis	**0.677**	**0.608**	**0.746**	**1.343E-82**	**1.967**	**66%**
DNA gyrase	**0.457**	**0.388**	**0.526**	**1.416E-38**	**1.579**	**61%**
Ribosome, 30S	-1.296	-1.416	-1.178	8.45E-102	0.274	21%
Ribosome, 50S	**0.585**	**0.491**	**0.679**	**2.201E-34**	**1.795**	**64%**
AIC: 17234	Bonferroni-corrected α: 0.01				Degrees of Freedom: 13602	

Table 14. Logistic regression of the pairwise main mechanism of actions with 5-drug combinations with some levels of suppressive interactions (hidden and net). Terms in **bold** have a significant positive association with suppressive interactions.

Term	**Coefficient**	**95% CI**		**p-value**	**Odds Ratio**	**Probability**
		2.5%	97.5%			
Cell Wall+Folic Acid Biosynthesis	-1.609	-1.925	-1.305	2.14E-24	0.200	17%
Cell Wall+DNA gyrase	-0.984	-1.263	-0.709	3.45E-12	0.374	27%
Cell Wall+Ribosome, 30S	-0.983	-1.411	-0.564	5.34E-06	0.374	27%
Cell Wall+Ribosome, 50S	**3.715**	**3.160**	**4.299**	**1.67E-37**	**41.06**	**98%**

Folic Acid Biosynthesis+DNA gyrase	**0.431**	**0.279**	**0.583**	**2.47E-08**	**1.540**	**61%**
Folic Acid Biosynthesis+Ribosome, 30S	**1.932**	**1.518**	**2.365**	**3.81E-19**	**6.900**	**87%**
Folic Acid Biosynthesis+Ribosome, 50S	0.193	-0.087	0.472	0.174	1.213	55%
DNA gyrase+Ribosome, Ribosome, 30S	**1.300**	**0.921**	**1.696**	**4.53E-11**	**3.669**	**79%**
DNA gyrase+Ribosome, Ribosome, 50S	0.030	-0.243	0.301	0.827	1.031	51%
Ribosome, 30S+Ribosome, 50S	-3.349	-3.799	-2.913	1.04E-49	0.035	3%
Cell Wall+Cell Wall	**0.322**	**0.237**	**0.407**	**1.44E-13**	**1.379**	**58%**
Ribosome, 30S+Ribosome, 30S	**0.395**	**0.311**	**0.480**	**4.36E-20**	**1.485**	**60%**
Ribosome, 50S+Ribosome, 50S	**0.521**	**0.435**	**0.608**	**4.36E-32**	**1.683**	**63%**

AIC: 16981 Bonferroni-corrected α: 0.0039 Degrees of Freedom: 13602

Table 15. Net suppressive combinations have more hidden suppression than combinations that are not net suppressive

Hidden suppression found between	Hidden Suppression Only	Net Suppression
5-Drugs v. 4-Drugs	53%	80%
5-Drugs v. 3-Drugs	41%	79%
5-Drugs v. 2-Drugs	40%	80%
4-Drugs v. 3-Drugs	60%	71%
4-Drugs v. 2-Drugs	61%	75%
3-Drugs v. 2-Drugs	76%	77%

Table 16. Path breakdown of hidden suppression between highest-order and lower-order sub-combinations in net suppressive combinations. There are a total of 504 paths across net-suppressive 3-drug combinations, 19704 paths across net-suppressive 4-drug combinations, and 315600 paths across net-suppressive 5-drug combinations.

Combination	Order of hidden suppression found against							
	2 only	3 only	4 only	2 and 3	2 and 4	3 and 4	2, 3, and 4	None

3 Drug	78%							22%
4 Drug	14%	10%		61%				15%
5 Drug	4%	2%	5%	6%	4%	5%	66%	8%

Table 17. Path breakdown of hidden suppression between highest-order and lower-order sub-combinations in non-net suppressive combinations. There are a total of 2484 paths across non-net suppressive 3-drug combinations, 47136 paths across non-net suppressive 4-drug combinations, and 693720 paths across non-net suppressive 5-drug combinations.

Combination	Order of hidden suppression found against							
	2 only	3 only	4 only	2 and 3	2 and 4	3 and 4	2, 3, and 4	None
3 Drug	77%							23%
4 Drug	17%	16%		45%				23%
5 Drug	6%	4%	18%	7%	5%	7%	23%	31%

Table 18. Case categorization of net-suppressive drug combinations. There are a total of 84 net-suppressive 3-drug combinations, 821 4-drug combinations, and 2629 5-drug combinations.

Case	Drug Combination		
	3	4	5
Fully Nested Suppression	52.4%	0.5%	0%
Partially Nested Suppression	44.0%	87.7%	81.2%
Fully Suppressed	0%	0%	0%
Partially Suppressed	0%	3.3%	6.7%
Suppressive Interaction with Hidden Suppression	0%	0%	0%
No Hidden Suppression	3.6%	8.5%	12.1%

Table 19. Case categorization of suppressive drug combinations determined through emergent interactions. There are a total of 209 suppressive 3-drug combinations, 827 4-drug combinations, and 3871 5-drug combinations determined through emergent interactions.

Case	Drug Combination		
	3	4	5
Fully Nested Suppression	36.8%	0%	0%
Partially Nested Suppression	46.9%	49.6%	33.8%
Fully Suppressed	0%	0%	0%
Partially Suppressed	0%	5.9%	7.1%
Suppressive Interaction with Hidden Suppression	0%	0.4%	0%
No Hidden Suppression	16.3%	44.1%	59.1%

Table 20. Case categorization of net-suppressive drug combinations with hidden suppression only. There are a total of 414 non-net suppressive 3-drug combinations, 1964 4-drug combinations, and 5781 5-drug combinations with hidden suppressive interactions only.

Case	Drug Combination		
	3	4	5
Fully Nested Hidden Suppression		0.3%	0%
Partially Nested Hidden Suppression		78.0%	39.2%
Fully Hidden Suppression	0%	0%	0%
Partially Hidden Suppression	0%	0%	0%
Hidden Suppressive Interaction	100.0%	21.8%	60.8%

Table 21. Case categorization of drug combinations with hidden suppression only determined through emergent interactions. There are a total of 382 3-drug combinations, 2258 4-drug combinations, and 5544 5-drug combinations with hidden suppressive interactions only as determined through emergent interactions.

Case	Drug Combination		
	3	4	5
Fully Nested Hidden Suppression		0.4%	0.0%
Partially Nested Hidden Suppression		81.8%	55.9%
Fully Hidden Suppression	0%	0%	0%
Partially Hidden Suppression	0%	0%	0%
Hidden Suppressive Interaction	100.0%	17.8%	44.1%

C. BOX

Box 1. Definitions of important terms used

Combination Types

Higher-Order Combination: a drug combination of three or more drugs

Lower-Order Combination: a drug combination consisting of a smaller number of drugs that are included within a higher-order combination; in a 5-drug combination all combinations with four of those drugs, all combinations with three of those drugs, and all combination of two of those drugs within the 5-drug combination are considered to be a lower-order combination to that specific 5-drug combination.

Drug Interactions

Additive Interaction: no interaction between drugs; under Bliss independence, the combined effect is as expected assuming each drug is acting independently (Bliss, 1939)

Synergistic Interaction: interaction between drugs is stronger than expected; drugs in combination are more effective at inhibiting growth than expected under the additive model

Antagonistic Interaction: interaction between drugs is weaker than expected; drugs in combination are less effective at inhibiting growth than expected under the additive model

Suppressive Interaction: interaction between drugs results in increased bacterial growth rate compared to the effects of fewer numbers of drugs; drugs in combination are not only less effective at inhibiting growth than expected under the additive model but increases growth compared to lower-order combinations or single drugs

Net Suppression: a suppressive interaction that occurs between the combination of drugs and the single drug effects; there is greater bacterial growth when exposed to a drug combination than when exposed to a single drug

Emergent Suppression: a suppressive interaction that occurs solely because all drugs are present in the combination

Hidden Suppression: a suppressive interaction that occurs between the combination of drugs and a lower-order combination

Other Useful Terms

Full-Factorial: a dataset that examines higher-order combinations with all their possible lower-order combinations, single drug effects, along with positive and negative controls. For example,

the full-factorial dataset for a single 5-drug combination includes the effects of the 5-drug combination as well as all possible 4-, 3-, and 2-drug combinations of those five drugs, all single drugs, positive controls, and negative controls.

Structure: the way to describe where interactions (net and hidden) occur within a combination

Path: a unique heterarchical grouping containing one representative of each of all the lower-order combinations within the highest-order combination

Nesting: a special type of structure where suppressive interactions occur when an N-drug combination is suppressive to an (N-1)-drug combination and that (N-1)-drug combination is suppressive to an (N-2)-drug combination which is suppressive to an (N-3)-drug combination, this nesting can continue until you compare a 2-drug combination with a single drug.

CHAPTER 6. APPENDIX

This appendix contains code snippets to exemplify what was used to create the paths, as well as evaluate hidden suppression and any particular cases as defined in Table 2. Again, all data and code has been made freely available via Mendeley Data (https://data.mendeley.com/datasets/ts2hnd72yf/draft?a=4fec844a-e75b-402b-9883-e34bfeff5c2a).

A. Path creation

For each combination order, the median DA_N of drug-dose replicate experiments was used and rearranged to create paths. Starting with a highest-order combination of interest, the names of subsequent inclusive lower-order combinations were generated, with median DA_N values of those combinations then searched for. For instance, in the code below, the 2-drug combinations under a 3-drug combination are identified and the relevant value attached to each

2-drug combination is then searched for. Next, the process is done for the single drugs under each 2-drug combination. The final output is a .csv for each specific highest-order combination, with each row being a path that contains fitness values. Net-suppressive and non-net suppressive combination outputs were split in different destinations.

```
import pandas as pd # use “pd” to call for pandas
import itertools # for combinations
import os
for index, row in DAF3.iterrows():
      SORTING3 = pd.DataFrame(columns =
['C3','F3','C2','F2','C1','F1','INTR']) # format for .csv output

      if row["DA"] < 1.30: ##### SEARCH FOR HIDDEN SUPPRESSION #### >= 1.30
USED FOR SUPPRESSIVE COMBINATIONS #####
            d1 = row["Drug1"] # NOTE: uses title of columns
            d2 = row["Drug2"]
            d3 = row["Drug3"]
            f3 = row["fitness"] # goes into PD
            c3 = frozenset((d1,d2,d3))
            print3 = d1+d2+d3 # goes into PD
            intr3 = row["interaction"]

            f2 = 0
            print2 = 0

            combo_gen2 = [d1,d2,d3]
            combo2 = list(itertools.combinations(combo_gen2,2)) # combos
within higher level combo
            for i in combo2:
                  dr1, dr2 = i
                  c2 = frozenset((dr1,dr2)) # for comparison
                  print2 = dr1+dr2 # rewrites above; goes into PD

                  for a,b in DAF2.iterrows():
                        r1 = b["Drug1"]
                        r2 = b["Drug2"]
                        comp2 = frozenset((r1,r2)) # for comparison (current
combo w/ what's generated from above)
                        if comp2 == c2:
                              f2 = b["fitness"] # rewrites above; goes into
PD
                              break

                  for ind, rw in E1.iterrows():
                        drug1 = rw["DRUG"]
                        if drug1 == dr1:
                              f1 = rw["MEDIAN"]
                              SORTING3 =
SORTING3.append({'C3':print3,'F3':f3,'C2':print2,'F2':f2,'C1':drug1,'F1':f1,'
INTR':intr3}, ignore_index=True) # a single path
```

```
                        if drug1 == dr2:
                              f1 = rw["MEDIAN"]
                              SORTING3 =
SORTING3.append({'C3':print3,'F3':f3,'C2':print2,'F2':f2,'C1':drug1,'F1':f1,'
INTR':intr3}, ignore_index=True)

            f_name = "/u/.../HD/C3/%s%s.csv"%(COMB3,str(number3))
            SORTING3.to_csv(f_name) # export csv

            number3 = number3 + 1
```

B. Evaluating for hidden suppression

In evaluating for hidden suppression, files containing the paths for both net-suppressive and non-net suppressive combinations were iterated through. The value of the highest-order combination at hand was divided by that of the lowest among all the lower-order combinations within. This new value was then compared against a cutoff as detailed in Methods; the final output is a .csv similar to that of the input, but with an additional column containing the new value. The code below illustrates this for a 3-drug combination.

```
COMB3 = "nC3_" # naming purposes
number3 = 0
for num in range (0,1428): ##### CHANGE VALUE DEPENDING ON AMOUNT OF COMBOS
IN QUESTION AT THE FILE FOLDER DESTINATION (by drug and whether net-
suppressive or not) #####
      look = storage[num] # looping through dictionary of paths for these
files
      lowFit = look.iloc[0]["F2"] # temporary value

      for index, rows in look.iterrows(): # looking for smallest fitness at
lower orders
            nf2 = rows["F2"]
            if nf2 < lowFit:
                  lowFit = nf2

      EDIT3 = pd.DataFrame(columns =
['C3','F3','C2','F2','C1','F1','newDA','INTR'])
      for indx, rws in look.iterrows(): # adding adjusted DA row
            c3 = rws["C3"]
            f3 = rws["F3"] #
            c2 = rws["C2"]
            f2 = rws["F2"]
            c1 = rws["C1"]
            f1 = rws["F1"] # don't need to check bc already gone over via
DA3 >= 1.3 check
            i3 = rws["INTR"]
```

```
            if lowFit == 0:
                  lowFit = 0.000000000001 # so no division by 0
            nd = f3/lowFit

            EDIT3 =
EDIT3.append({'C3':c3,'F3':f3,'C2':c2,'F2':f2,'C1':c1,'F1':f1,'newDA':nd,'INT
R':i3}, ignore_index=True)

      f_name = "/u/.../HD/nC3/%s%s.csv"%(COMB3,str(number3))
      EDIT3.to_csv(f_name, index = False)

      number3 = number3 + 1
```

C. Defined cases

To evaluate for defined cases of hidden suppression, the values within each path of a select higher-order combination were compared against each other. Counts of how comparisons could be categorized were then evaluated to see if any special cases, defined in Table 2, were met. The final outputs are .csvs containing the counts of cases met by combination order. The code below is a snippet for the 3-drug combination scenario. Note that a dictionary containing paths to the files created in the last step, which includes the new values used to identify hidden suppression, was iterated through.

```
x3 = 0
#----------------- # category counts
types3 = pd.DataFrame(columns = ['FNS','NS','FS','PH','NHS','NA'])
FNS3 = 0 # fully nested suppression
NS3 = 0 # nested suppression
FS3 = 0 # fully suppressed
PH3 = 0 # partially hidden suppression
NHS3 = 0 # no hidden suppression
NA3 = 0 # no "case"
#----------------- # combo lists
fns3 = pd.DataFrame(columns = ['combos','INTR']) # intr is "original"
interaction
ns3 = pd.DataFrame(columns = ['combos','INTR'])
fs3 = pd.DataFrame(columns = ['combos','INTR'])
ph3 = pd.DataFrame(columns = ['combos','2 only','INTR'])
nhs3 = pd.DataFrame(columns = ['combos','INTR'])
na3 = pd.DataFrame(columns = ['combos','INTR'])

for num in range (0,84): ##### CHANGE VALUE DEPENDING ON CSVS APPLICABLE
#####
      look3 = YN3[num] # looping through dictionary - 1 combo per dictionary
key
```

```
      name = look3.iloc[0]["C3"]
      i3 = look3.iloc[0]["INTR"]
      #----------------- overall counts
      nested_count = 0
      LRsml_count = 0
      f32_count = 0
      #----------------- PH counts
      h2 = 0

      for index, rows in look3.iterrows(): # counts
            C3 = rows["C3"]
            F3 = rows["F3"]
            C2 = rows["C2"]
            F2 = rows["F2"]
            C1 = rows["C1"]
            F1 = rows["F1"]

            # -------------- overall counts
            if F3 > F2 and F3 > F1 and F2 > F1:
                  nested_count = nested_count + 1
            if F3 > F1:
                  LRsml_count = LRsml_count + 1
            if F3 > F2:
                  f32_count = f32_count + 1

            # -------------- PH counts
            if F3 > F2:
                  h2 = h2 + 1

      # classification
      if nested_count == 6: # fully nested suppression
            FNS3 = FNS3 + 1
            fns3 = fns3.append({'combos':name,'INTR':i3}, ignore_index=True)
      elif nested_count > 0: # nested suppression
            NS3 = NS3 + 1
            ns3 = ns3.append({'combos':name,'INTR':i3}, ignore_index=True)
      elif nested_count == 0:
            if LRsml_count > 0 and f32_count == 6: # fully suppressed
                  FS3 = FS3 + 1
                  fs3 = fs3.append({'combos':name,'INTR':i3},
ignore_index=True)
            elif LRsml_count > 0 and f32_count > 0: # partially hidden
                  PH3 = PH3 + 1
                  ph3 = ph3.append({'combos':name,'2 only':h2,'INTR':i3},
ignore_index=True)
            elif LRsml_count > 0 and f32_count == 0: # no hidden
                  NHS3 = NHS3 + 1
                  nhs3 = nhs3.append({'combos':name,'INTR':i3},
ignore_index=True)
            else:
                  NA3 = NA3 + 1
                  na3 = na3.append({'combos':name,'INTR':i3},
ignore_index=True)
```

```
types3 =
types3.append({'FNS':FNS3,'NS':NS3,'FS':FS3,'PH':PH3,'NHS':NHS3,'NA':NA3},
ignore_index=True)
types3.to_csv(r"/u/.../SUPR/suprSORT/Supr3_Counts.csv",index=False)
# all other dataframes .to_csv as well
```

CHAPTER 7. REFERENCES

Arya, D., Chowdhury, S., Chawla, R., Das, A., Ganie, M. A., Kumar, K. P., Nadkar, M. Y. & Rajput, R. 2019. Clinical Benefits of Fixed Dose Combinations Translated to Improved Patient Compliance. *Journal of The Association of Physicians of India*, 67, 58.

Aslam, B., Wang, W., Arshad, M. I., Khurshid, M., Muzammil, S., Rasool, M. H., Nisar, M. A., Alvi, R. F., Aslam, M. A., Qamar, M. U., Salamat, M., & Baloch, Z. 2018. Antibiotic resistance: a rundown of a global crisis. *Infection and drug resistance*, 11, 1645–1658.

Baeder, D. Y., Yu, G., Hozé, N., Rolff, J. & Regoes, R. R. 2016. Antimicrobial combinations: Bliss independence and Loewe additivity derived from mechanistic multi-hit models. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 371, 20150294.

Bendixsen, D. P., Østman, B. & Hayden, E. J. 2017. Negative epistasis in experimental RNA fitness landscapes. *Journal of molecular evolution*, 85, 159-168.

Beppler, C., Tekin, E., Mao, Z., White, C., Mcdiarmid, C., Vargas, E., Miller, J. H., Savage, V. M. & Yeh, P. J. 2016. Uncovering emergent interactions in three-way combinations of stressors. *Journal of The Royal Society Interface*, 13, 20160800.

Beppler, C., Tekin, E., White, C., Mao, Z., Miller, J. H., Damoiseaux, R., Savage, V. M. & Yeh, P. J. 2017. When more is less: Emergent suppressive interactions in three-drug combinations. *BMC microbiology*, 17, 107.

Bliss, C. 1939. The toxicity of poisons applied jointly. *Annals of Applied Biology*, 26, 585-615.

Bloom, D. E., Black, S., Salisbury, D. & Rappuoli, R. 2018. Antimicrobial resistance and the

role of vaccines. *Proceedings of the National Academy of Sciences,* 115, 12868-12871.

Bollenbach, T. 2015. Antimicrobial interactions: mechanisms and implications for drug discovery and resistance evolution. *Current opinion in microbiology,* 27, 1-9.

Bollenbach, T., Quan, S., Chait, R. & Kishony, R. 2009. Nonoptimal microbial response to antibiotics underlies suppressive drug interactions. *Cell,* 139, 707-718.

Chait, R., Craney, A. & Kishony, R. 2007. Antibiotic interactions that select against resistance. *Nature*, 446, 668-671.

Chevereau, G. & Bollenbach, T. 2015. Systematic discovery of drug interaction mechanisms. *Molecular systems biology,* 11.

Chokshi, A., Sifri, Z., Cennimo, D. & Horng, H. 2019. Global contributors to antibiotic resistance. *Journal of global infectious diseases,* 11, 36.

Churski, K., Kaminski, T. S., Jakiela, S., Kamysz, W., Baranska-Rybak, W., Weibel, D. B. & Garstecki, P. 2012. Rapid screening of antibiotic toxicity in an automated microdroplet system. *Lab on a Chip*, 12, 1629-1637.

Cokol, M., Chua, H. N., Tasan, M., Mutlu, B., Weinstein, Z. B., Suzuki, Y., Nergiz, M. E., Costanzo, M., Baryshnikova, A. & Giaever, G. 2011. Systematic exploration of synergistic drug pairs. *Molecular systems biology*, 7.

Cokol, M., Weinstein, Z. B., Yilancioglu, K., Tasan, M., Doak, A., Cansever, D., Mutlu, B., Li, S., Rodriguez-Esteban, R. & Akhmedov, M. 2014. Large-scale identification and analysis of suppressive drug interactions. *Chemistry & biology*, 21, 541-551.

Cooper, M.A. and Shlaes, D., 2011. Fix the antibiotics pipeline. *Nature*, 472, 7341, 32.

Dadgostar, P., 2019. Antimicrobial resistance: implications and costs. *Infection and drug resistance*, 12, 3903.

De Vos, M. G. & Bollenbach, T. 2014. Suppressive drug interactions between antifungals. *Chemistry & biology*, 21, 439-440.

Dean, Z., Maltas, J. & Wood, K. 2020. Antibiotic interactions shape short-term evolution of resistance in E. faecalis. *PLoS pathogens*, 16, e1008278.

Fischbach, M. A. 2011. Combination therapies for combating antimicrobial resistance. *Current opinion in microbiology*, 14, 519-523.

Fraser, T. 1870. On atropia as a physiological antidote to the poisonous effects of physostigma. *Practitioner*, 4, 65-72.

French, G., Ling, T., Davies, D. & Leung, D. 1985. Antagonism of ceftazidime by chloramphenicol in vitro and in vivo during treatment of gram negative meningitis. *British medical journal (Clinical research ed.),* 291, 636.

Guerrero-García, C. and Rubio-Guerra, A.F., 2018. Combination therapy in the treatment of hypertension. *Drugs in context*, 7.

Hegreness, M., Shoresh, N., Damian, D., Hartl, D. & Kishony, R. 2008. Accelerated evolution of resistance in multidrug environments. *Proceedings of the National Academy of Sciences,* 105, 13977-13981.

Katzir, I., Cokol, M., Aldridge, B. B. & Alon, U. 2019. Prediction of ultra-high-order antibiotic combinations based on pairwise interactions. *PLoS computational biology,* 15, e1006774.

Koch, G., Schropp, J. & Jusko, W. J. 2016. Assessment of non-linear combination effect terms for drug–drug interactions. *Journal of pharmacokinetics and pharmacodynamics,* 43, 461-479.

Lepper, M. H. & Dowling, H. F. 1951. Treatment of pneumococcic meningitis with penicillin compared with penicillin plus aureomycin: studies including observations on an apparent

antagonism between penicillin and aureomycin. *AMA archives of internal medicine*, 88, 489-494.

Liu, J., Gefen, O., Ronin, I., Bar-Meir, M. & Balaban, N. Q. 2020. Effect of tolerance on the evolution of antibiotic resistance under drug combinations. *Science*, 367, 200-204.

Liu, Q., Yin, X., Languino, L. R. & Altieri, D. C. 2018. Evaluation of Drug Combination Effect Using a Bliss Independence Dose–Response Surface Model. *Statistics in biopharmaceutical research,* 10, 112-122.

Lukačišin, M. & Bollenbach, T. 2019. Emergent gene expression responses to drug combinations predict higher-order drug interactions. *Cell systems,* 9, 423-433. e3.

Mbuagbaw, L., Mursleen, S., Irlam, J. H., Spaulding, A. B., Rutherford, G. W. & Siegfried, N. 2016. Efavirenz or nevirapine in three‐drug combination therapy with two nucleoside or nucleotide‐reverse transcriptase inhibitors for initial treatment of HIV infection in antiretroviral‐naïve individuals. *Cochrane Database of Systematic Reviews*.

Meletiadis, J., Verweij, P. E., Te Dorsthorst, D. T., Meis, J. F. & Mouton, J. W. 2005. Assessing in vitro combinations of antifungal drugs against yeasts and filamentous fungi: comparison of different drug interaction models. *Medical mycology,* 43, 133-152.

Mokhtari, R.B., Homayouni, T.S., Baluch, N., Morgatskaya, E., Kumar, S., Das, B. and Yeger, H., 2017. Combination therapy in combating cancer. *Oncotarget*, 8, 23, 38022.

Morimoto, M., Shimakawa, S., Hashimoto, T., Kitaoka, T. & Kyotani, S. 2018. Marked efficacy of combined three-drug therapy (Sodium Valproate, Topiramate and Stiripentol) in a patient with Dravet syndrome. *Journal of clinical pharmacy and therapeutics*, 43, 571-573.

Østman, B., Hintze, A. & Adami, C. 2011. Impact of epistasis and pleiotropy on evolutionary

adaptation. *Proceedings of the Royal Society B: Biological Sciences*, 279, 247-256.

Otto-Hanson, L., Grabau, Z., Rosen, C., Salomon, C. & Kinkel, L. L. 2013. Pathogen variation and urea influence selection and success of Streptomyces mixtures in biological control. *Phytopathology*, 103, 34-42.

Palmer, A. C., Toprak, E., Baym, M., Kim, S., Veres, A., Bershtein, S. & Kishony, R. 2015. Delayed commitment to evolutionary fate in antibiotic resistance fitness landscapes. *Nature communications*, 6, 1-8.

Petraitis, V., Petraitiene, R., Hope, W. W., Meletiadis, J., Mickiene, D., Hughes, J. E., Cotton, M. P., Stergiopoulou, T., Kasai, M. & Francesconi, A. 2009. Combination therapy in treatment of experimental pulmonary aspergillosis: in vitro and in vivo correlations of the concentration-and dose-dependent interactions between anidulafungin and voriconazole by Bliss independence drug interaction analysis. *Antimicrobial agents and chemotherapy*, 53, 2382-2391.

Povolo, V. R. & Ackermann, M. 2019. Disseminating antibiotic resistance during treatment. *Science*, 364, 737-738.

Rieg, S., Kern, W. V. & Soriano, A. 2018. Rifampicin in treating S aureus bacteraemia. *The Lancet*, 392, 554-555.

Sanchez-Gorostiaga, A., Bajić, D., Osborne, M. L., Poyatos, J. F. & Sanchez, A. 2019. High-order interactions distort the functional landscape of microbial consortia. *PLoS Biology*, 17.

Singh, N. & Yeh, P. J. 2017. Suppressive drug combinations and their potential to combat antibiotic resistance. *The Journal of antibiotics*, 70, 1033.

Stergiopoulou, T., Meletiadis, J., Sein, T., Papaioannidou, P., Walsh, T. J. & Roilides, E. 2011.

Synergistic interaction of the triple combination of amphotericin B, ciprofloxacin, and polymorphonuclear neutrophils against Aspergillus fumigatus. *Antimicrobial agents and chemotherapy,* 55, 5923-5929.

Sühnel, J. 1998. Parallel dose-response curves in combination experiments. *Bulletin of mathematical biology*, 60, 197-213.

Sun, W., Sanderson, P. E. & Zheng, W. 2016. Drug combination therapy increases successful drug repositioning. *Drug discovery today*, 21, 1189-1195.

Sun, X., Vilar, S. & Tatonetti, N. P. 2013. High-throughput methods for combinatorial drug discovery. *Science translational medicine*, 5, 205rv1-205rv1.

Tekin, E., Beppler, C., White, C., Mao, Z., Savage, V. M. & Yeh, P. J. 2016. Enhanced identification of synergistic and antagonistic emergent interactions among three or more drugs. *Journal of The Royal Society Interface,* 13, 20160332.

Tekin, E., Savage, V. M. & Yeh, P. J. 2017. Measuring higher-order drug interactions: a review of recent approaches. *Current Opinion in Systems Biology*, 4, 16-23.

Tekin, E., White, C., Kang, T. M., Singh, N., Cruz-Loya, M., Damoiseaux, R., Savage, V. M. & Yeh, P. J. 2018. Prevalence and patterns of higher-order drug interactions in Escherichia coli. *NPJ systems biology and applications*, 4, 31.

Tsigelny, I. F. 2019. Artificial intelligence in drug combination therapy. *Briefings in bioinformatics*, 20, 1434-1448.

Tyers, M. & Wright, G. D. 2019. Drug combinations: a strategy to extend the life of antibiotics in the 21st century. *Nature Reviews Microbiology,* 17, 141-155.

Ventola, C.L., 2015. The antibiotic resistance crisis: part 1: causes and threats. *Pharmacy and therapeutics*, 40, 4, 277.

Wood, K. B. & Cluzel, P. 2012. Trade-offs between drug toxicity and benefit in the multi-antibiotic resistance system underlie optimal growth of E. coli. *BMC systems biology,* 6, 48.

Wright, S. 1932. *The roles of mutation, inbreeding, crossbreeding, and selection in evolution*, na.

Wright, S. 1988. Surfaces of selective value revisited. *The American Naturalist,* 131, 115-123.

Yeh, P., Tschumi, A. I. & Kishony, R. 2006. Functional classification of drugs by properties of their pairwise interactions. *Nature Genetics,* 38, 489.

Yin, Z., Deng, Z., Zhao, W. and Cao, Z., 2018. Searching synergistic dose combinations for anticancer drugs. *Frontiers in pharmacology*, 9, 535.

Zhao, W., Sachsenmeier, K., Zhang, L., Sult, E., Hollingsworth, R. E. & Yang, H. 2014. A new bliss independence model to analyze drug combination data. *Journal of biomolecular screening,* 19, 817-821.