Synergy, Toxicity, Efficacy
In Vitro Drug Interaction Inference

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UW Madison - Biostatistics

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Introduction

> **Question**
>
> How do we quantify the effectiveness of drug combinations in a drug discovery context?

Drug combinations serve myriad purposes

- Overcoming resistance

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Drug combinations serve myriad purposes

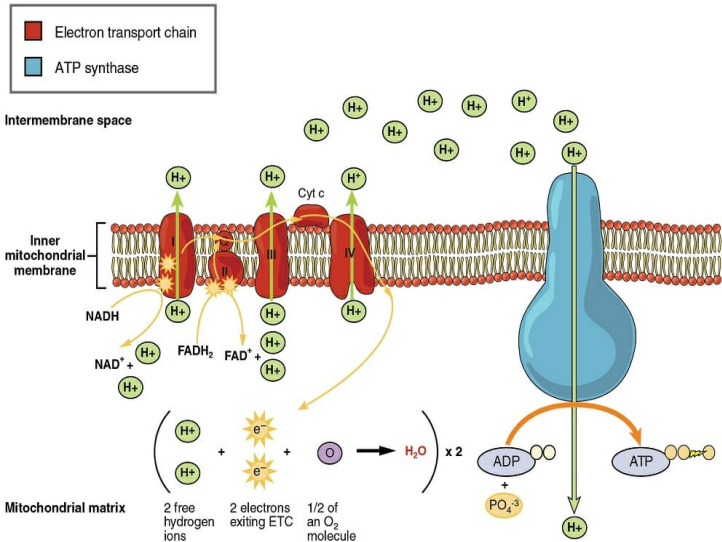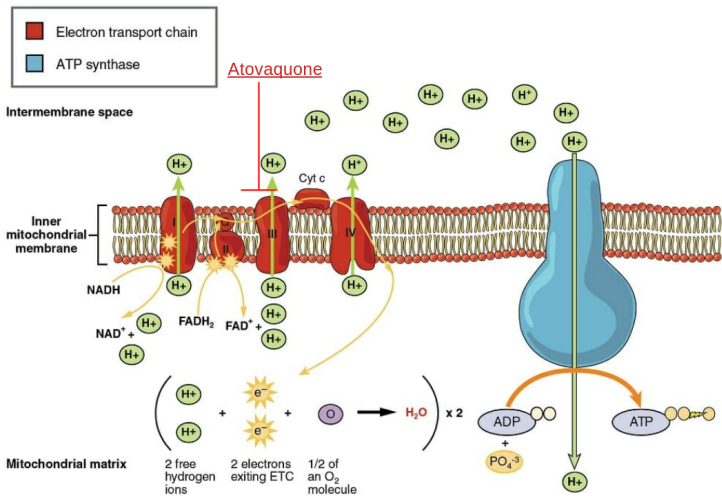- Overcoming resistance
- Increasing effectiveness
- Reducing toxicity
- Repurposing existing drugs
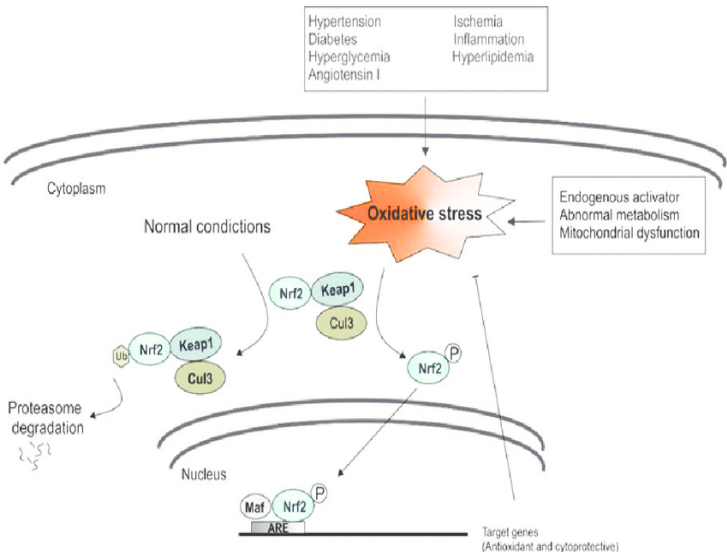
Example: Atovaquone-Brusatol

Credit: *biologydictionary.net/electron-transport-chain-and-oxidative-phosphorylation/*
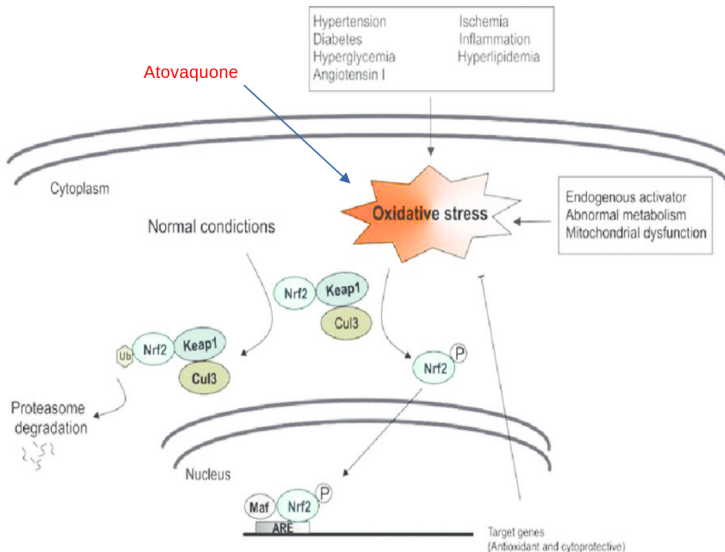
Example: Atovaquone-Brusatol

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Atovaquone-Brusatol (cont'd.) - Nrf2

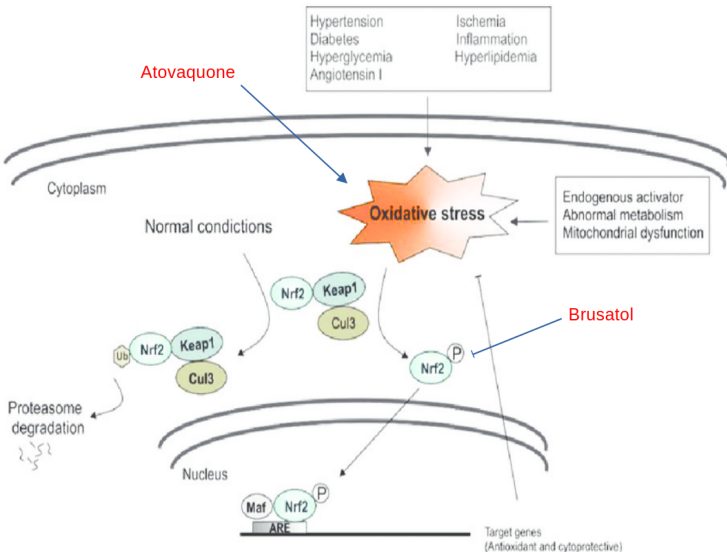Credit: *The Effect of Nrf2 on Diabetic Complications, Reis et. al., 2016*

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Atovaquone-Brusatol (cont'd.) - Nrf2

Credit: *The Effect of Nrf2 on Diabetic Complications, Reis et. al., 2016*

Atovaquone-Brusatol (cont'd.)

Hypothesis

Atovaquone and Brusatol, in combination, will exhibit a synergistic effect in cell mortatility in cancer cell lines.

Test - Nicha Boonpattrawong, Patankar Lab

- A 96-well plate was populated with the cancer cell line OVCAR3.
- Then, the wells were treated with various combinations of Atovaquone, Brusatol, and control (DMSO).
- After 72 hours, the plates were fed through a plate-reader that determines the Optical Density (OD) in each well.

Atovaquone-Brusatol Experimental Design

Set 1:

ATO/BRU	1	2	3	4	5	6	7	8	9	10	11	12
A												
B			ATO 16uM	ATO 32uM	ATO 64uM	ATO 128uM	ATO 256uM	128/100	64/50	32/25	16/12.5	
C												
D		DMSO										
E			BRU 6.25nM	BRU 12.5nM	BRU 25nM	BRU 50nM	BRU 100nM	256/50	128/25	64/12.5	32/6.25	
F												
G												
H												

Set 2:

ATO/BRU	1	2	3	4	5	6	7	8	9	10	11	12
A												
B			256/25	128/12.5	64/6.25	256/100	128/50	64/25	32/12.5	16/6.25	256/6.25	
C												
D		DMSO										
E			256/12.5	128/6.25	64/100	32/50	16/25	32/100	16/50	16/100		
F												
G												
H												

Atovaquone-Brusatol Results

Ato (μM)/Bru (nM)	0.0	6.25	12.5	25.0	50.0	100.0
0.0	2.10	1.73	1.78	1.44	0.95	0.59
16.0	1.83	1.67	1.72	1.50	0.98	0.59
32.0	0.76	0.75	0.90	0.98	0.84	0.60
64.0	0.75	0.74	0.72	0.65	0.60	0.58
128.0	0.95	0.91	0.70	0.74	0.69	0.79
256.0	1.12	0.89	0.99	0.76	0.94	0.90

Table: Mean OD for each Atovaquone/Brusatol dose combination in the OVCAR3 cell line. Blue and yellow highlighted squares are dose combinations found exclusively in Set 1 and 2, respectively.
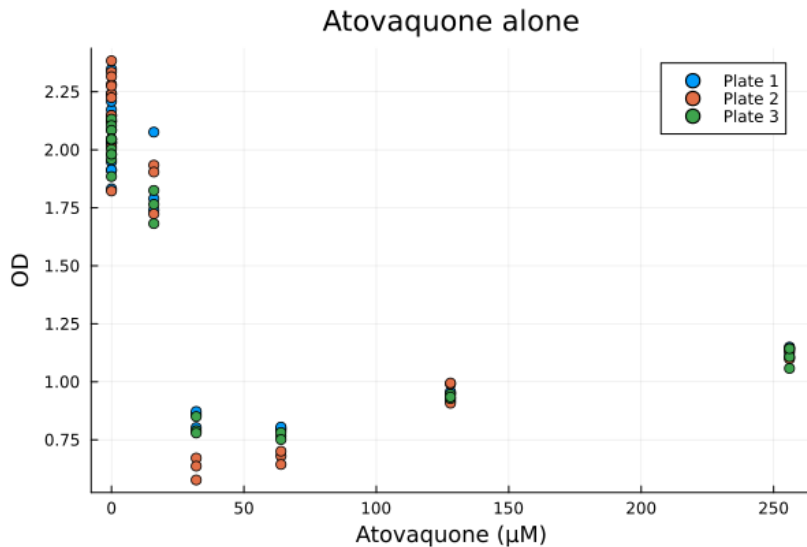
Atovaquone-Brusatol Results (cont'd.)

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Synergy

Two (or more) drugs can either be

- Synergistic
- Additive
- Antagonistic

- What it means for two drugs to be synergistic or antagonistic seems intuitive.
- However, the meaning of drug *additivity* is much less clear, and there are several dozen different definitions.
- This makes actually quantifying the notion of synergy and antagonism much more

Example: Atovaquone and Brusatol Inference

Atovaquone and Brusatol

- had very small Loewe synergy scores for small doses of Atovaquone, but ultimately, was not overall statistically significant

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- was statistically significantly antagonistic via Bliss/ZIP

Example: Atovaquone and Brusatol Inference

Atovaquone and Brusatol

- had very small Loewe synergy scores for small doses of Atovaquone, but ultimately, was not overall statistically significant
- was statistically significantly antagonistic via Bliss/ZIP
- had several dose-combinations which were statistically significantly HSA antagonistic

This is weird

Credit: *The Effect of Nrf2 on Diabetic Complications, Reis et. al., 2016*
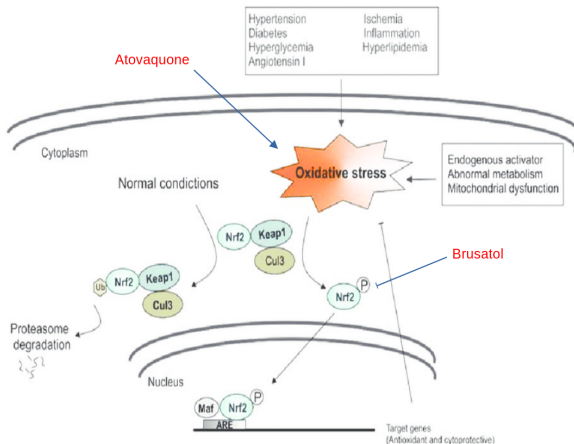
Possible explanations:

- Brusatol might need to be introduced first
- Brusatol has other effects
- Second pathway
- Error

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Goals for Drug Combinations

Recall drug combinations goals/promises:

- Overcoming resistance
- Increasing effectiveness
- Decreasing toxicity
- Repurposing existing drugs

Goals for Drug Combinations

Recall drug combinations goals/promises:

- Overcoming resistance ✓
- Increasing effectiveness ✓
- Decreasing toxicity
- Repurposing existing drugs ✓

Relevant XKCD

Credit: *XKCD - Cells: https://xkcd.com/1217/*

Synergy Can Be Undesirable

Synergy tells us we can achieve a similar inhibitory effect using "less drug"

- Not, inherently, a beneficial result

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- Beaten resistances may be necessary for healthy cells to survive

The ideal drug combination would be synergistic(?), and more potent in cancerous tissue than in healthy.

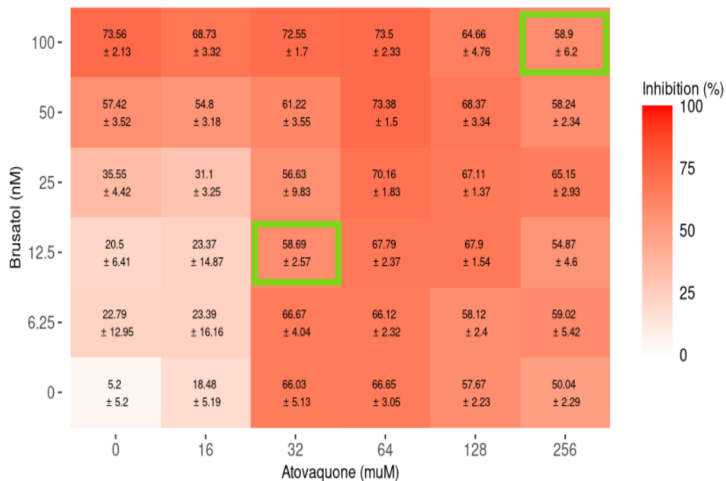A more general metric would consider synergy, while comparing efficacy and toxicity.

U-shaped Dose Response Curves

Heatmap of % inhibition for Atovaquone and Brusatol
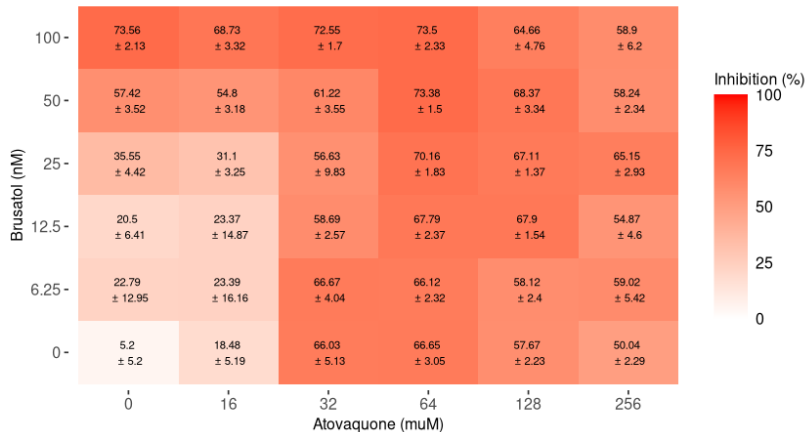
U-shaped Dose Response Curves

Heatmap of % inhibition for Atovaquone and Brusatol

DrugCombDB - Cancer Cell Lines

DrugCombDB, and similar databases, contain no data on healthy tissue.

> Specifically, DrugCombDB includes 448 555 drug combinations derived from HTS assays, covering 2887 unique drugs and 124 human cancer cell lines. In particular, DrugCombDB has more than 6000 000 quantitative dose responses from which we computed multiple synergy scores to determine the overall synergistic or antagonistic effects of drug combinations. In addition to the
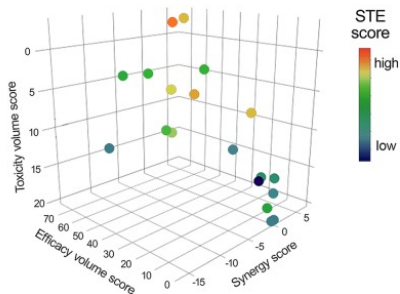
SynToxProfiler
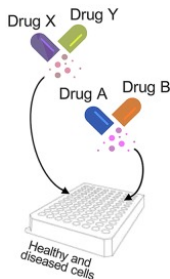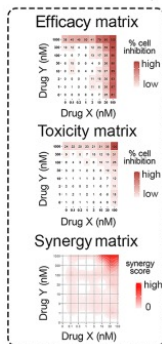
Credit: *Ianevski et. al., 2020*

SynToxProfiler Methods

Calculate (normalized) volume under

- E_{AB}: Efficacy curve (cancer tissue)
- T_{AB}: Toxicity curve (healthy tissue)
- S_{AB}: Synergy curve (cancer tissue)

For each combination, calculate and rank by STE score

$$\mathrm{STE}_{AB} = \frac{\mathrm{rank}(S_{AB}) + \mathrm{rank}(E_{AB} - T_{AB})}{2N}$$

SynToxProfiler Test

TODO: Discuss the course project I did for Christina's course.

Moral: SynToxProfiler screened out at least one drug combination that had a very high synergy but very poor selective efficacy, and that combination was found to be overly toxic in clinical trials.

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Synergy Score Aggregation

Many of the most popular methods of determining synergy are *non-parametric*, which means we estimate a different synergy score for each dose-combination.

To make a decision of additive vs synergistic vs antagonistic, we need to aggregate this 3D data into a single value.

1. Pick your favorite (common in non-methods literature)
2. Sum all the synergy scores
3. TODO: others?

These have problems:

1. Ignores opposite effects elsewhere, allows for biased selection of "favorite".
2. Removes relevant features of the 3D curve
   - For example, do we see synergy in high dose of A and low dose of B, but antagonism when switched?

Synergy Score Aggregation

Is there a better method of aggregating synergy scores?

1. Generate both a synergy ($\max\{f, 0\}$) and an antagonism ($\min\{f, 0\}$) score separately.
   - Doesn't let antagonistic regions drag down synergistic regions
   - May allow a combination to be all three of additive, synergistic, and antagonistic
2. Look for *large, connected* dose-combination regions with high synergy.
   - Hopes to make claims such as "high doses of A react synergistically with low doses of B".

Example: Loewe Synergy for Atovaquone-Brusatol
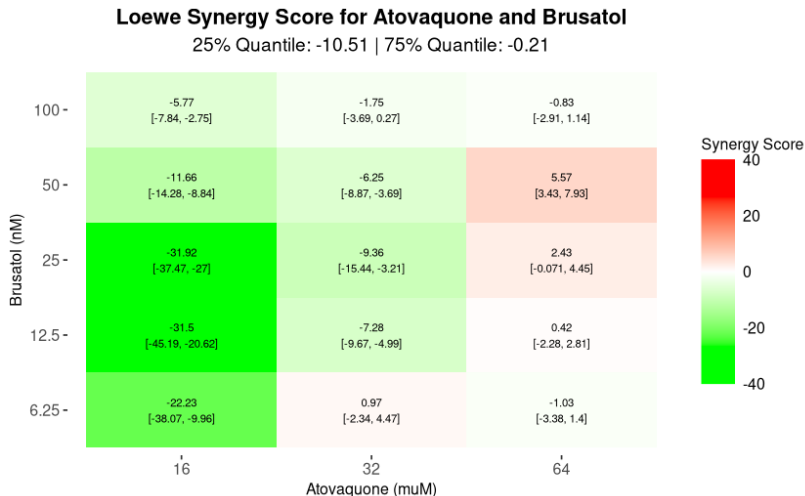
Network Analysis of Synergy

Many modern methods (TODO: citations) incorporate gene or protein interaction networks to supplement synergy analysis through, e.g.

- Prediction, especially in HTS contexts
- Explaining synergy outcomes

However, to the best of my knowledge, nobody has ever defined synergy *within* a network.

Synergy within Networks

Such a method would seek to find subnetworks of, say, a GRN that are "especially (in)active" in the presence of the drug combination than would be expected if there drugs didn't interact.

Potential benefits:

1. Networks can inherently convey information on relationships between drugs
2. May answer more pertinent questions — synergism within a subnetwork similar to a gene set can enable qualitative analysis of the potential therapeutic benefit of the combination
3. Networks are cool and flashy

Cons:

1. More expensive and time-consuming analyses would be needed, such as RNASeq.
2. TODO: Others?

Non-monotonic Dose-Response Curves

Heatmap of % inhibition for Atovaquone and Brusatol
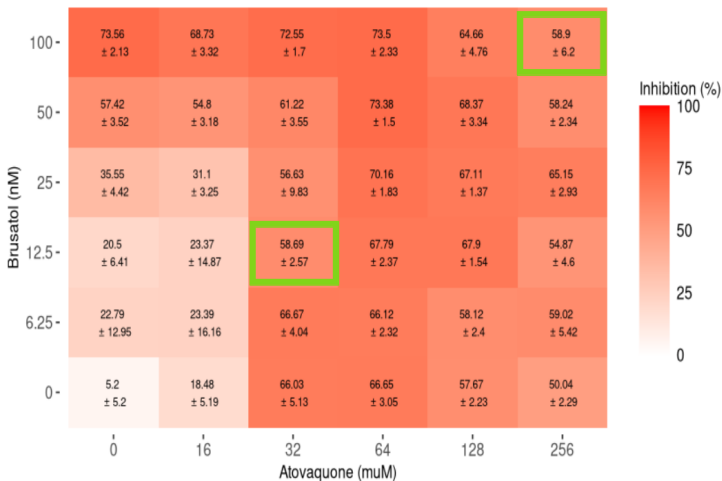
References I

📄 Ting-Chao Chou, *Derivation and properties of michaelis-menten type and hill type equations for reference ligands*, Journal of Theoretical Biology **59** (1976), no. 2, 253–276.

📄 Cong Cheng, Fang Yuan, Xiao-Ping Chen, Wei Zhang, Xie-Lan Zhao, Zhi-Ping Jiang, Hong-Hao Zhou, Gan Zhou, and Shan Cao, *Inhibition of nrf2-mediated glucose metabolism by brusatol synergistically sensitizes acute myeloid leukemia to ara-c*, Biomedicine and Pharmacotherapy **142** (2021), 111652.

📄 Aleksandr Ianevski, Anil K Giri, and Tero Aittokallio, *SynergyFinder 3.0: an interactive analysis and consensus interpretation of multi-drug synergies across multiple samples*, Nucleic Acids Research **50** (2022), no. W1, W739–W743.

References II

📄 Aleksandr Ianevski, Sanna Timonen, Alexander Kononov, Tero Aittokallio, and Anil K Giri, *SynToxProfiler: An interactive analysis of drug combination synergy, toxicity and efficacy*, PLoS Comput Biol **16** (2020), no. 2, e1007604 (en).

📄 Hui Liu, Wenhao Zhang, Bo Zou, Jinxian Wang, Yuanyuan Deng, and Lei Deng, *DrugCombDB: a comprehensive database of drug combinations toward the discovery of combinatorial therapy*, Nucleic Acids Research **48** (2019), no. D1, D871–D881.

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Median-Effect Equation

Definition (Median-Effect Equation (MEE))

$$\log \frac{f_a}{1 - f_a} = n \log D - n \log D_m,$$

where

- f_a is the *fraction effected* of a receptor
- D is the dose of ligand
- D_m is the *median effect dose*
- n is the *Hill coefficient*

- Governs how concentration of ligand influences the proportion of receptors effected
- Derived by Ting-Chao Chou in 1976 from the mass effect law
- Provides a nice justification for linear models

Atovaquone/Brusatol MEE Curves

Define

$$Survival_{ijk} = OD_{ijk} / \max_{i'}\{OD_{i'jk}\}$$

for the ith well in the jth plate and kth set.
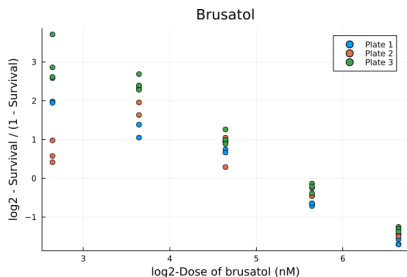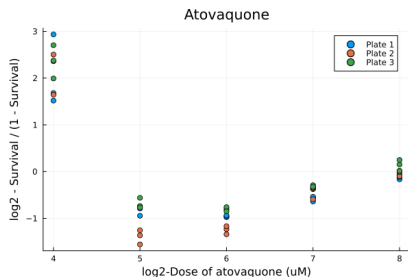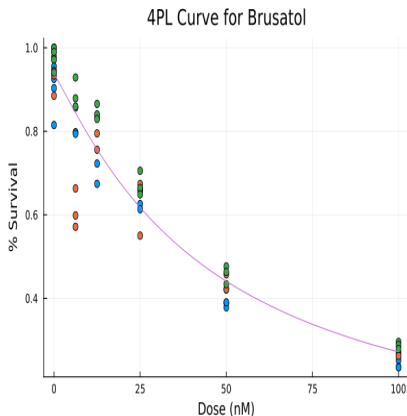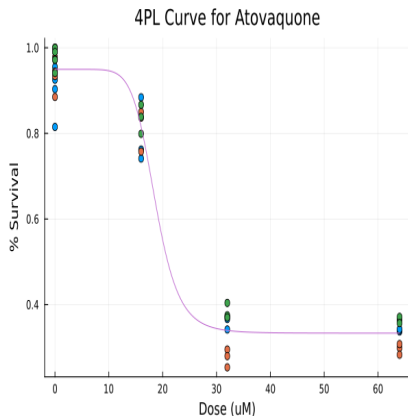
4 Parameter Logistic Regression

> ## Definition (4 Parameter Logistic (4PL) Curve)
>
> $$f(D) = \eta_0 + \frac{\eta_f - \eta_0}{1 + \left(\frac{D}{D_m}\right)^{-n}},$$
>
> where
>
> - η_0 is the *minimal effect*
> - η_f is the *maximal effect*

- Generalizes the Hill equation
- Enables minimal and maximal effects
- No longer has a nice linear form
- Still asserts monotonicity

Atovaquone/Brusatol 4PL Curves

Loewe Additivity

Definition (Loewe Additivity)

We say that two drugs, A and B are Loewe Additive if

$$\frac{\lambda D}{f_A^{-1}(f_{AB,\lambda}(D))} + \frac{(1-\lambda)D}{f_B^{-1}(f_{AB,\lambda}(D))} = 1.$$

- Most common method of defining synergy
- Passes the Sham-experiment test - a drug acts additively with itself
- The LHS is referred to as the *Combination Index*
- Can be undefined for some D
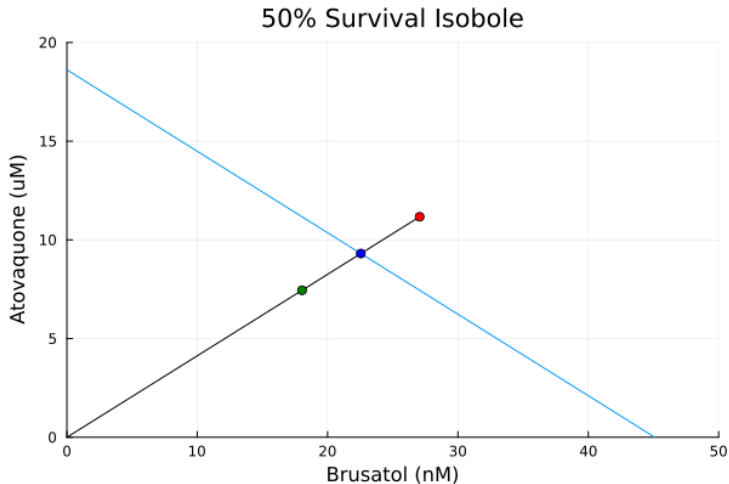
Isoboles

Loewe additivity results in linear *Isoboles*.

- Atovaquone $D_m = 18.62\ \mu M$
- Brusatol $D_m = 45.10$ nM

50% Survival Isobole

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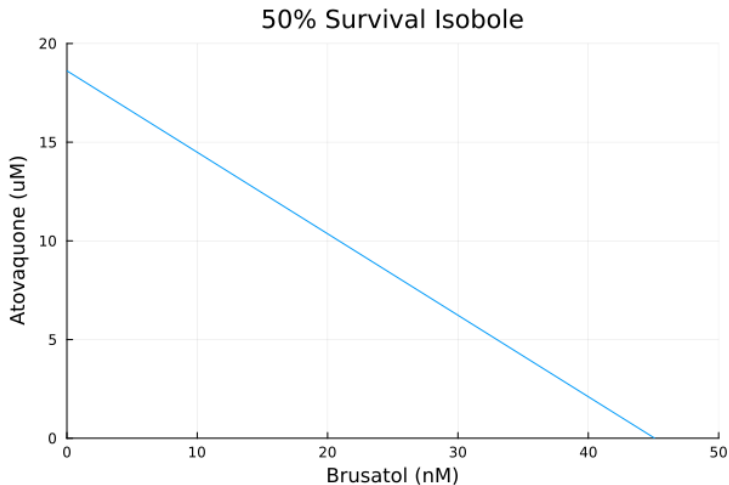
Chou-Tulalay Combination Index Method

Definition (Combination Index)

$$CombInd = \frac{\lambda D}{f_A^{-1}(f_{AB,\lambda}(D))} + \frac{(1-\lambda)D}{f_B^{-1}(f_{AB,\lambda}(D))}$$

Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors

TC **Chou**, P Talalay - Advances in enzyme regulation, 1984 - Elsevier

A generalized method for analyzing the effects of multiple drugs and for determining summation, synergism and antagonism has been proposed. The derived, generalized equations …

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- Assumes AB acts as a third drug, thus, $f_{AB,\lambda}(D)$ should follow a MEE
- Generates a value for CombInd at each effect level in $(0, 1)$
- Produces biased results

Bias in Chou-Tulalay Combination Index

<u>Toy Example</u>

Suppose we have two drugs, A, B that follow MEE curves, are truly Loewe additive, and we know that

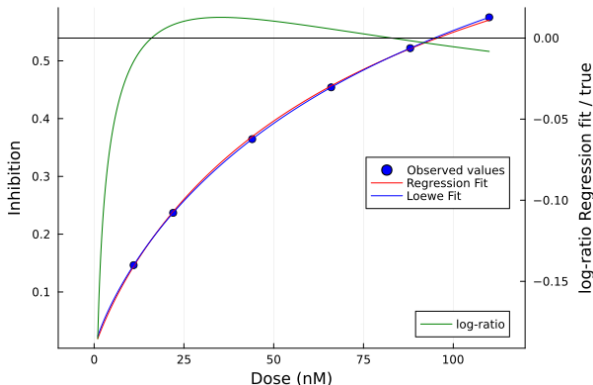- $n_A = 1.9$, $n_B = 0.7$
- $D_{mA} = 27.0$ nM, $D_{mB} = 100.0$ nM

and, further, suppose we observe the true inhibition, $f_{AB,\lambda}(D)$ for

- $\lambda = 1/11$
- $D \in \{11, 22, 44, 66, 88, 110\}$ nM

Bias in Chou-Tulalay Combination Index
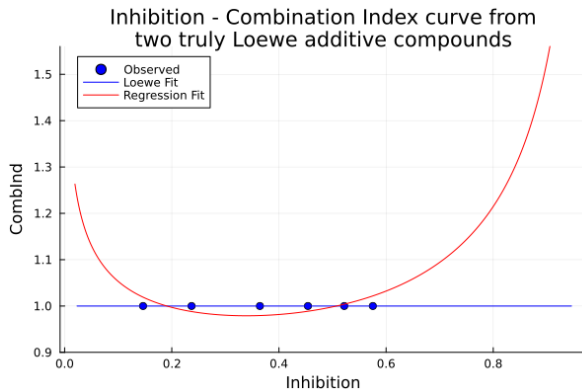
Toy Example

We would observe the following Dose-Response curve:

"Sometimes the [CombInd] values are > 3 or much greater, especially at low effect levels..."

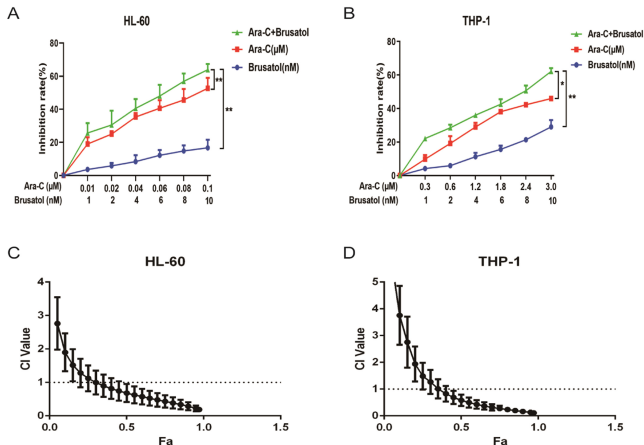# Bias in Chou-Tulalay Combination Index

Toy Example

Then we would generate the following Inhibition-Combination Index plot:

"For anticancer or antiviral agents, synergy at high effect levels... is more relevant to therapy than at low effect levels..."
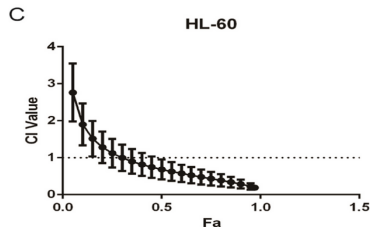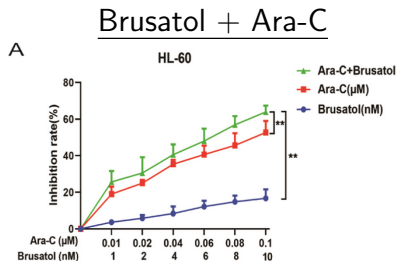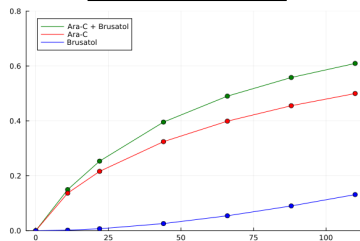
Chou-Tulalay Combination Index Example

Credit: *Cheng et. al., 2012*

Conclusion: Synergy is most highly expressed at 0.1μM Ara-C, 10nM Brusatol, so that dose was used in further experiments.
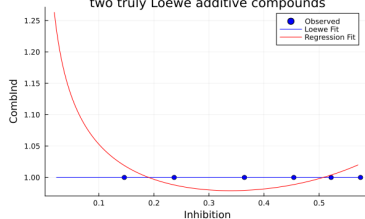
Chou-Tulalay Combination Index Example (cont'd.)

Using Loewe Additivity

Only fit the curve once!

Eliminates double-fitting bias

- Values are compared to the additive curve
- Results are not dependent on range of doses tested

Can only get CombInd values at observed effects

- Can't use, say, $CombInd_{50}$
- How do we compare different combinations?

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- How do we compare different combinations?

Aggregate results over doses tested

Synergy Finder

R package

- Calculates Loewe synergy scores without fitting a second multi-compound dose-response curve
- Performs bootstrapping to get dose-level confidence intervals
- Aggregates values to get confidence interval for mean score over all dose combinations

Loewe Synergy Score

> **Definition**
>
> Let $y_{\lambda,D}$ be an observation of $f_{AB,\lambda}(D)$, and let $y_{Loewe,\lambda,D}$ be the fitted value of $f_{AB,\lambda}(D)$ given some monotherapeutic dose-response data and model. Then the Loewe synergy score is
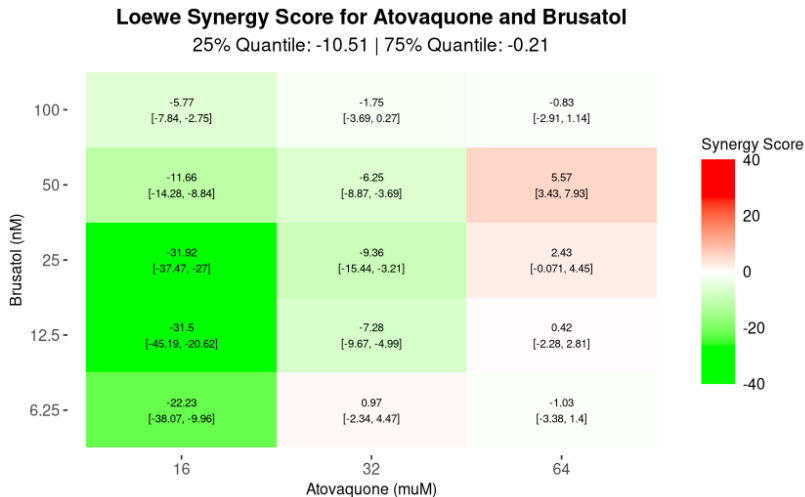>
> $$S_{Loewe,\lambda,D} = y_{\lambda,D} - y_{Loewe,\lambda,D}.$$

Loewe Synergy for Atovaquone-Brusatol

Heatmap of % inhibition for Atovaquone and Brusatol

Loewe Synergy for Atovaquone-Brusatol

Loewe Synergy Score for Atovaquone and Brusatol
25% Quantile: -10.51 | 75% Quantile: -0.21
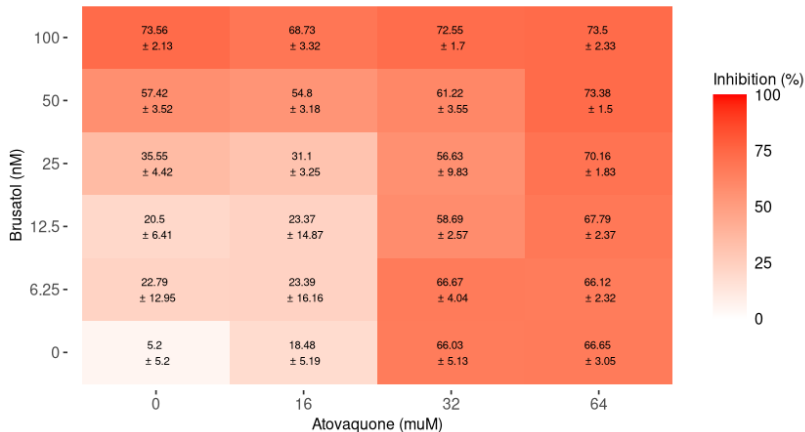
Other Synergy Scores

Synergy Finder also includes the three other synergy scores used by DrubCombDB

- Bliss additivity: Cell death as a result of one drug is statistically independent of cell death as a result of the other
- ZIP additivity: After some initial effect, the presence of one drug has no effect on the dose-response curve of the other
- HSA additivity: The drug combination will perform as well as the highest performing of its constituent parts
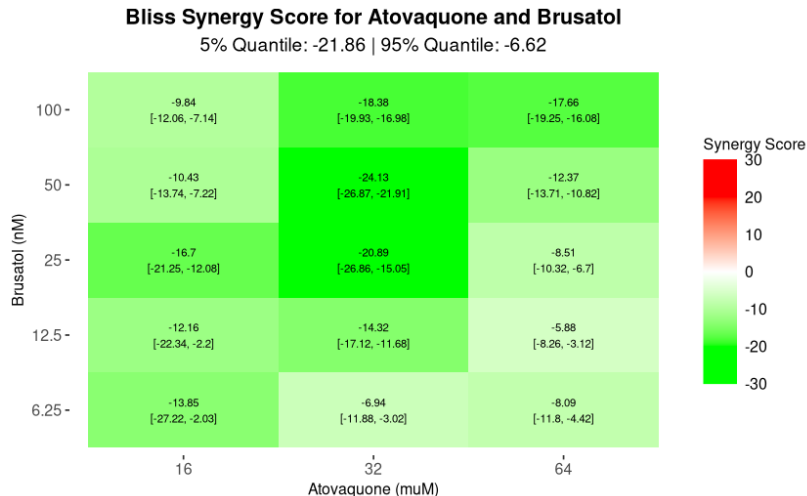
Bliss Additivity

Definition (Bliss Additivity)

Drugs A and B, in ratio $\lambda : (1 - \lambda)$ are Bliss additive at dose D if

$$f_{AB,\lambda}(D) = f_A(\lambda D) + f_B((1 - \lambda)D) - f_A(\lambda D) \times f_B((1 - \lambda)D).$$

- Fails Sham-combination experiment
- Measurements are not actual observations of death/no death
- Does not even require monotherapeutic dose-response fitting

Bliss Additivity for Atovaquone and Brusatol

ZIP Additivity

> **Definition (ZIP additivity)**
>
> Let A and B be compound, where A has hill coefficient n_A and median effect dose D_{mA}. For simplicity, assume drugs A, B, both follow MEE's. Let
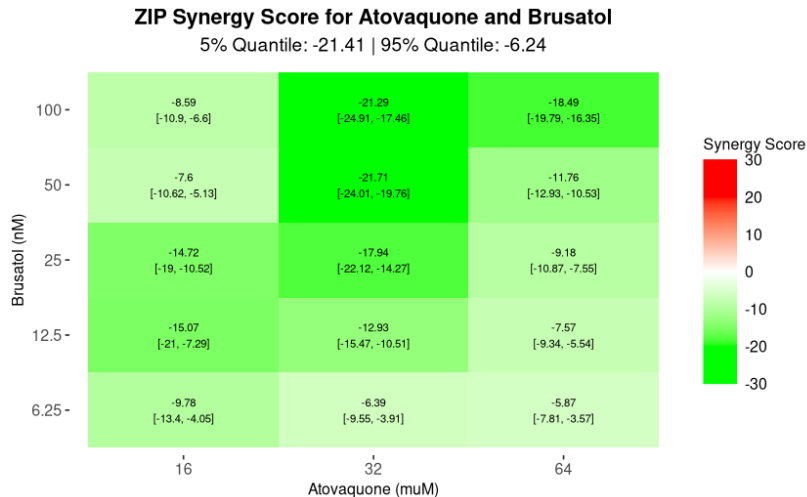>
> $$f_A(D|B = d_b) = f_{AB}(D, d_b).$$
>
> A and B are ZIP additive if
>
> $$f_A(D|B = d_b) = f_B(d_b) + \frac{1 - f_B(d_b)}{1 + \left(\frac{D}{D_{mA}}\right)^{-n_A}}.$$

- "Combines" Loewe and Bliss additivity
- ZIP synergy score passes sham-combination experiment
- $f_{AB}(d_a, d_b) = f_A(d_a) + f_B(d_b) - f_A(d_a)f_B(d_b)$

ZIP Additivity for Atovaquone and Brusatol

ZIP Synergy Score for Atovaquone and Brusatol

5% Quantile: -21.41 | 95% Quantile: -6.24

HSA Additivity

Definition (HSA additivity)

We say that compounds A and B are HSA additive if

$$f_{AB}(d_a, d_b) = \max\{f_A(d_a), f_B(d_b)\}.$$

- Provides a lower-bound for additivity definitions
- Not very useful for determining synergy
- Very handy for determining antagonism
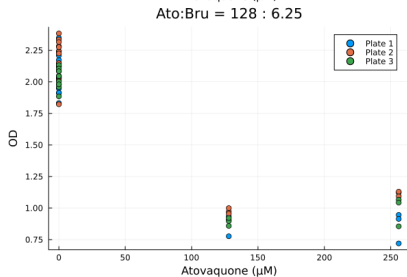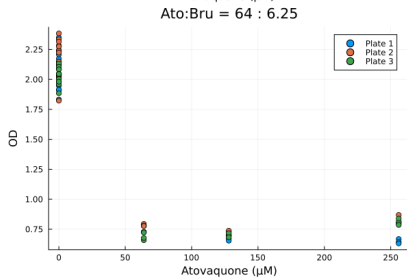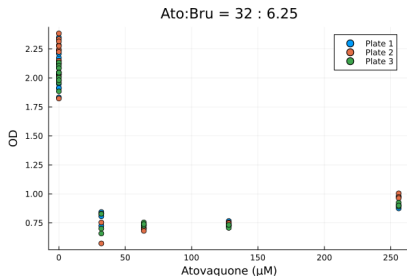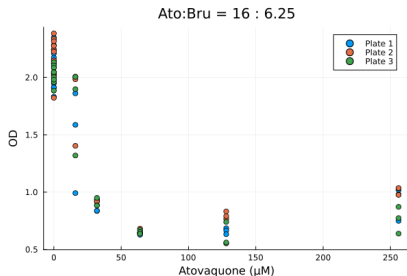- May be preferred when one drug has little effect

HSA Additivity for Atovaquone and Brusatol
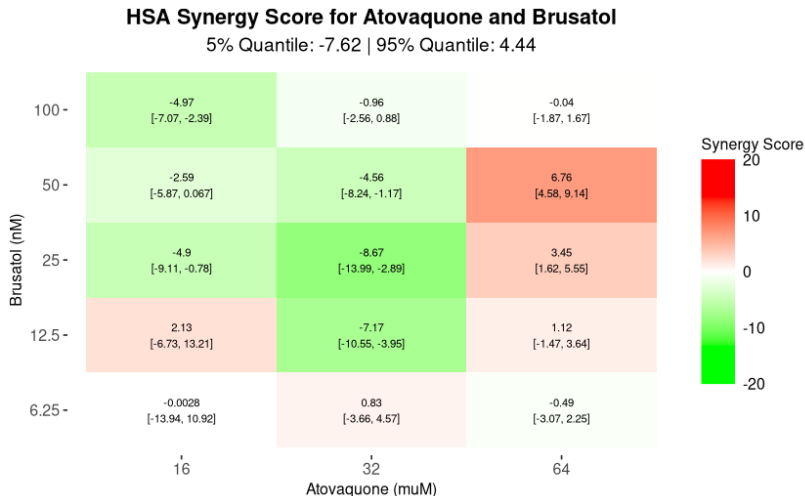
Holistic Synergy Inference

- Many more definitions of synergy exist
    - Non-parametric, e.g., Hand
    - Parametric, e.g., BRAID / Linear models
- No clear "winner", people have their favorites
- Best to report multiple, and make inferences holistically

Constant Ratio Dose-Response Curves

Synergy Finder (cont'd.)

For each observed λ, D, assume replicates $y^k \sim N(\bar{y}, \hat{\sigma}^2)$.

- Draw B bootstrap samples $y^{k\prime}$
- Re-estimate monotherapeutic dose-response curves
- Calculate Loewe synergy scores $(s_{\lambda,D}^b)_{b=1}^B$
- Calculate average synergy score as $s_b' = \mathrm{mean}\{s_{\lambda,D}^b \mid \lambda \in (0,1)\}$

Obtain confidence intervals / p-values for each dose, and the average over all doses