STEROID: In Silico Heuristic Target Combination Identification for Disease-Related Signaling Networks

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ABSTRACT

Given a signaling network, the *target combination identification problem* aims to predict efficacious and safe target combinations for treatment of a disease. State-of-the-art *in silico* methods use Monte Carlo simulated annealing (MCSA) to modify a candidate solution stochastically, and use the Metropolis criterion to accept or reject the proposed modifications. However, such stochastic modifications ignore the impact of the choice of targets and their activities on the combination's *therapeutic effect* and *off-target effects* which directly affect the solution quality. In this paper, we present STEROID, a novel method that addresses this limitation by leveraging two additional heuristic criteria to minimize *off-target effects* and achieve *synergy* for candidate modification. Specifically, *off-target effects* measure the unintended response of a signaling network to the target combination and is generally associated with toxicity. *Synergy* occurs when a pair of targets exerts effects that are greater than the sum of their individual effects, and is generally a beneficial strategy for maximizing effect while minimizing toxicity. Our empirical study on the cancer-related `MAPK-PI3K` network demonstrates the superiority of STEROID in comparison to MCSA-based approaches. Specifically, STEROID is an order of magnitude faster and yet yields biologically relevant synergistic target combinations with significantly lower off-target effects.

Categories and Subject Descriptors

J.3 [**Life and Medical Sciences**]: [biology and genetics]

General Terms

Algorithms, Performance

Keywords

drug target combination, Loewe additivity theory, simulated annealing, target prioritization, heuristic rules

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1. INTRODUCTION

Combinatorial control involving redundancy and multifunctionality of biological signaling processes are often implicated in diseases [12] such as cancer. These signaling processes are often modeled as hypergraphs ($G = V, E$) in systems biology, where the nodes V represent molecules (*e.g.*, proteins) and the edges E represent interactions [23]. Fig. 1 depicts the hypergraph representation of the `MAPK-PI3K` signaling cascade [17]. Such graph-based models facilitate our understanding of the underlying disease mechanism, and provide a means to study the effects of targeting different nodes in the network through *in silico* network simulations.

Targeting multiple molecules simultaneously in a signaling network, also known as *combination therapy*, sometimes yields better benefits compared to a single molecule (monotherapy) for complex diseases, for dynamically changing diseases, or for diseases with a heterogeneous population of pathological mechanisms [10]. Even for diseases that are caused entirely by disruption of a single pathway (lacking in dynamics or heterogeneity), combination therapy might still offer benefits over monotherapy by virtue of spreading out the side effects to sub-toxic levels, while concentrating the desired effects on the target pathway. However, not all combination therapies produce better effects than monotherapies. For instance, in a study of combinations of analgesic drugs, some combinations (*e.g.*, `aspirin` and `pentazocine`) were beneficial, while others (*e.g.*, `acetaminophen` and `pentazocine`) were detrimental [26]. Hence, it is important to formulate strategies to develop good drug combinations which maximize the overall *therapeutic effect* while minimizing the *off-target effects*.

The identification of good drug combinations broadly involves two key steps, namely identification of good target combinations and identification of appropriate set of drugs hitting these targets. In this paper, we focus on the first step. That is, we address the *target combination identification problem*, which is complex and non-linear. Informally, this problem involves finding suitable sets of drug targets and the required *target activities* (type and extent of perturbations) for these targets for a given signaling network and a therapeutic goal. The complexity of the biological network (numerous potential drug targets and wide range of *target activities*) makes performing exhaustive search for sets of targets technically challenging, expensive and time consuming since the number of testable combinations increases exponentially with the number of variables associ-

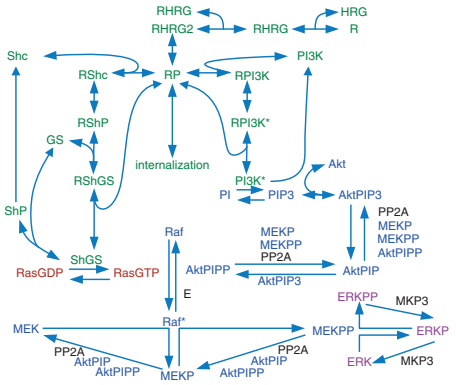

Figure 1: MAPK-PI3K signaling cascade [17].

ated with the network. Tools that can facilitate early detection of inefficacious or toxic target combinations *in silico* can serve as a powerful discovery and prescreening platform when coupled with other complementary technologies such as high-throughput screening.

Informally, the *therapeutic effect* and the *off-target effects* are measures of the intended and the unintended response, respectively, of a biological signaling network to the drug combination. Each drug effect can be simulated *in silico* by modifying appropriate signaling network model parameters. The intended response is the resulting changes to the concentration of the *output node*, while the unintended response is the resulting changes to the sum of the concentration of the rest of the nodes in the network. An *output node* is a molecule that is either involved in some dysregulated biological processes implicated in a disease, or is of interest due to its potential role in the disease. An example of an output node in the MAPK-PI3K network (Fig. 1) implicated in cancer is phosphorylated ERK (ERKPP) [37]. The therapeutic effect of the drug combination, AZD6244 (MEK inhibitor) and sorafenib (Raf inhibitor), can be described as reducing [ERKPP], the node concentration we seek to decrease [11,41].

Few designs of target combinations are automated. These state-of-the-art *in silico* methods are based on sequential decoding (SD) algorithms [4] or Monte Carlo simulated annealing (MCSA) [19,40]. Stochastic search algorithms such as MCSA are expected to perform better than SD for non-linear problems [4]. MCSA modifies a candidate solution stochastically and the proposed modification is accepted or rejected using the Metropolis criterion [28]. Although stochastic candidate modification effectively covers the search space by producing a wide variety of candidates, it has two key limitations when used for identifying target combinations. First, drug targets in real signaling networks influence the therapeutic and off-target effects differently, due to one or more downstream nodes' involvement in other protein-protein interactions [8]. Ignoring this consideration may yield combinations satisfying the user-desired therapeutic effect, but with excessive off-target effects. Second, although the target activity affects the combination effects, it is chosen randomly in MCSA. A judicious selection process can provide us an opportunity to improve efficiency of the overall process.

In this paper, we present a novel and generic approach called STEROID (Heuri**ST**ic-Based Syn**E**rgistic Ta**R**get C**O**mbination **ID**entifier) to address the aforementioned limitations. Instead of only modifying the drug target and target activity stochastically (*e.g.*, [19, 40]), STEROID judiciously modifies candidate solutions by leveraging two additional heuristic criteria for minimizing off-target effects and achieving *synergy* (detailed in Section 4). Off-target effects are generally associated with toxicity. *Synergy* occurs when a pair of targets exerts effects that are greater than the sum of their individual effects, and is generally a beneficial strategy for maximizing therapeutic effect while minimizing toxicity. For instance, medullary thyroid cancer cells treated with AZD6244 and sorafenib had better outcome in terms of cell survival and apoptosis due to drug synergy [24]. STEROID uses heuristics based on *target prioritization* methods (*e.g.*, sensitivity analysis [38, 43] and PANI [8]) which prioritize potential targets in a given disease-related network; and *Loewe additivity isobologram analysis* (LOEWE) [42] which assesses drug interaction in a combination. Specifically, target prioritization-based heuristic is used to select more effective targets to reduce off-target effects. Off-target effects is the main reason why drugs fail, and systems biology offers the hope of improving this trend by avoiding off-target effects throughout the therapy design process. LOEWE-based heuristic is used for pruning the target activity search space to reduce computational cost and to ensure that targets selected are synergistic. As we shall see in Section 5, the above candidate modification strategy leads to efficient identification of superior target combinations compared to MCSA-based techniques [19, 40].

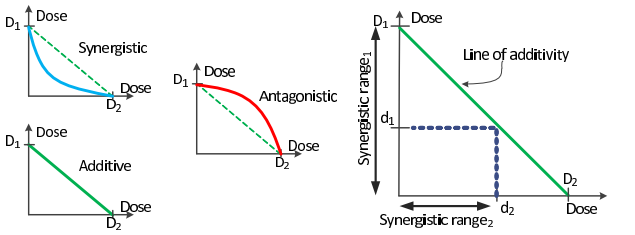

Figure 2: Isobologram. Drug doses D_1 and D_2 achieve the desired therapeutic effect if the drugs are administered alone. d_1 and d_2 together achieves the same effect.

2. BACKGROUND

In this section, we briefly introduce *target prioritization* and *Loewe additivity isobologram analysis* which we shall be exploiting in the sequel. We use the heregulin (HRG)-induced MAPK-PI3K signaling network implicated in ovarian cancer [17] (Fig. 1) as a running example because its nodes are well-studied for the roles they play when targeted with relevant drugs. Details of this ordinary differential equation (ODE) model (BIOMD0000000146) are found in Biomodels.net [25]. We selected ERKPP as the output node due to its role in ovarian cancer [37]. The desired therapeutic effect was set to 50% ERKPP down-regulation for the *in silico* model. Note that in practice, the therapeutic effect is dependent on the stage of the disease and is typically measured as inhibition of certain phenotypic response (*e.g.*, cell growth) which may not be linearly correlated with the inhibition of the output node concentration.

2.1 Target Prioritization

Target prioritization methods assign prioritization rank to individual target nodes based on certain criteria (*e.g.*, the sensitivity of the output node to each target node [38]). Several target prioritization approaches such as sensitivity analysis [38, 43] and PANI [8] have been recently proposed.

Sensitivity analysis assigns node rank according to the sensitivity value which is the ratio of the output node perturbation to parameter (*e.g.*, kinetic rate constant) perturbation. Local sensitivity analysis (LSA) measures sensitivity by varying a single parameter at a time [38] whereas global sensitivity analysis, such as *multi-parametric sensitivity analysis* (MPSA), measures sensitivity by varying multiple parameters simultaneously [43].

PANI [8], in contrast, uses network information and simple empirical scores to prioritize and rank biologically relevant target molecules in signaling networks. First, it prunes the nodes based on a reachability rule to eliminate nodes that are likely to be non-regulators. Then, it ranks the resulting nodes based on the *putative target score* of each node, which is a weighted rank aggregation of a dynamic property (*profile shape similarity distance* (PSSD)) and two structural properties (*target downstream effect* (TDE) and *bridging centrality* (BC)) of the node. PSSD identifies the most relevant upstream regulators of the output node; TDE assesses the potential impact on the network when a node is perturbed; and BC identifies nodes that bridge modular subregions in a network [18]. PANI-prioritized nodes in the `MAPK-PI3K` network (*e.g.*, `AktPIP`) are found to correlate well with known ovarian cancer drug targets [8]. Hence, we reason that they are likely to form safer and more efficacious combinations.

2.2 Loewe Additivity Theory (LOEWE)

The Loewe additivity theory assumes that drugs act without self-interaction and determines drug interaction in a combination using the *combination index* [12]. Given a set of drugs X and therapeutic effect T, let D_x and d_x be the doses of drug $x \in X$ required to achieve effect T when used alone and in combination, respectively. Then, the *combination index* is defined as $CI = \sum_{x \in X} \frac{d_x}{D_x}$. The combination is *synergistic*, *additive* or *antagonistic* if $CI < 1$, $CI = 1$ or $CI > 1$, respectively. The isobologram (Fig. 2) provides a visual interpretation of LOEWE. It is a graph with the individual drug doses (D_1 and D_2) as its axes. The "line of additivity" is used to interpret the drug interaction: synergistic and antagonistic combinations are represented by drug doses that fall below and above the line of additivity, respectively [42]. We adapt this theory by replacing drugs and drug doses with *drug targets* and *target activities*, respectively, for selecting synergistic *target activities*.

3. TARGET COMBINATION IDENTIFICATION PROBLEM

In this section, we formally define the problem of *target combination identification*. Note that the goal of this work is to identify synergistic combinations of targets with reduced off-target effects and excludes the evaluation of drug compounds that bind and regulate the target molecules. We begin by introducing several concepts related to *drug target*.

3.1 Drug Target and Target Activity

A drug asserts its effect on a network through the *target*, while the *target activity* is a variable related to the extent of target perturbation. The perturbation is typically a network parameter (*e.g.*, kinetic rate constant) that controls the concentration of the node associated with the target. The drug effect is typically modeled *in silico* as modulation of the node concentration. The modulation is achieved by modifying either the node's edges (typically represented as ODE reactions) [40] or the node itself (initial concentration) depending on whether the node concentration varies with time. We now formally define these two concepts. We first introduce the notion of *reactant-product edge set* to facilitate exposition. Given a signaling network $G = (V, E)$ and a node $u \in V$, the *reactant-product edge set* of u is defined as $\zeta_u = R_u \bigcup P_u$ where $R_u \subset E$ and $P_u \subset E$ are the edge sets involving u as reactants and products, respectively.

DEFINITION 1. *Given a signaling network $G = (V, E)$, and node $u \in V$ with concentration time-series profile ξ_u and reactant-product edge set ζ_u, the **drug target** of a node u is $c_{fix} = u$ if ξ_u is constant, and it is $c_{var} \in \zeta_u$ otherwise.*

DEFINITION 2. *Given a drug target c perturbed by drug D with dissociation constant K_D, the **target activity** of c is defined as $\Gamma_c = \frac{[D]}{K_D}$ where $[D]$ is the concentration of D.*

The ODE modification varies according to the drug type (*e.g.*, activators or inhibitors) and the mechanism of action. We modeled activation using *nonessential activation* [6], and inhibition using *competitive inhibition* [40]. These reaction modifications make sense only when applied to unidirectional irreversible reactions. Note that reversible reactions can be transformed into equivalent pairs of irreversible reactions using [34].

Formally, let I be an inhibitor, A be an activator, and $c_{fix} = u$ and $c_{var} = r$ be two targets where u is a node with constant concentration time-series profile and r is a reaction in the reactant-product edge set of node v which has a variable concentration time-series profile. Let $r = \frac{V_{max}[S]}{K_m + [S]}$ where V_{max} is the maximum velocity; K_m is the Michaelis-Menten constant; and $[S]$ is the concentration of the substrate S. Then, the *competitive inhibition* of c_{fix} and c_{var} are given by the following equations:

$$\mathcal{I}(c_{fix}) = \frac{[u]_0}{\frac{[I]}{K_I}} \quad (1)$$

$$\mathcal{I}(c_{var}) = \frac{V_{max}[S]}{K_m(1 + \frac{[I]}{K_I}) + [S]} \quad (2)$$

In the above equations, $[u]_0$ is the initial concentration of u and K_I is the dissociation constant of I. Similarly, let K_A be the dissociation constant of A. The *nonessential activation* of c_{fix} and c_{var} are defined as follows.

$$\mathcal{A}(c_{fix}) = \frac{[A]}{K_A}[u]_0 \quad (3)$$

$$\mathcal{A}(c_{var}) = \frac{V_{max}[S](1 + \frac{[A]}{K_A})}{K_m + [S]} \quad (4)$$

3.2 Target Effects

Given a signaling network $G = (V, E)$, a drug target c and the desired *therapeutic effect* t, let $u \in V$ be the node associated with effect t. Let α_u and α'_u be the areas under the concentration-time series profile curves of node u before and after c is perturbed, respectively. Then, the *therapeutic effect* t_c and *off-target effects* ρ_c of c are given by the following equations.

$$t_c = \frac{|\alpha_u - \alpha'_u|}{\alpha_u} \quad (5)$$

$$\rho_c = \sum_{v \in V \setminus u} \left(\frac{|\alpha_v - \alpha'_v|}{\alpha_v}\right) \quad (6)$$

Note that t_c and ρ_c can be determined from *in silico* simulation using *Copasi* [34]. The combination effects are defined similarly and α can be estimated using the linear trapezoidal rule method [5].

3.3 Problem Definition

Intuitively, the goal of the *target combination identification problem* is to identify targets and their activities that achieve a user-specified therapeutic effect (*e.g.*, to achieve 50% inhibition of ERKPP) while minimizing the off-target effects. Hence, the problem can be modeled as the optimization of a *constraint satisfaction problem* (CSP) which is NP-hard [14]. The CSP is represented as a triple (X,D,C), where X, D and C represent the set of variables, the variables' domain and the set of constraints, respectively. The element X represents the set of drug targets and target activities; D represents the set of candidate targets in a given disease-related network and the target activity range; and C represents the condition that the combination therapeutic effect must match the desired therapeutic effect. The objective of the *target combination identification problem* is to minimize the combination off-target effects.

DEFINITION 3. *Given a set of target combination* $C = \{C_1, \cdots, C_N\}$ *and a desired therapeutic effect* t*, let* $C_i = \{c_1, \cdots, c_m\}$ *where* $c_j \in C_i$ *is the* j^{th} *target in the* i^{th} *combination. Let* ρ_{C_i} *and* t_{C_i} *be the off-target effects and therapeutic effect of combination* C_i*, respectively. Then, the* ***target combination identification problem*** *is defined as*

$$C_i = min\{\rho_{C_i} | t_{C_i} = t\}$$

4. IDENTIFYING TARGET COMBINATION

In this section, we describe the heuristic algorithm STEROID for identifying target combinations. We begin by presenting the target prioritization and LOEWE-based heuristics which we shall exploit for modifying the candidate solutions.

4.1 Heuristics

Target prioritization-based heuristic. The goal of using the target prioritization heuristic for target selection is to improve the average solution quality by choosing more effective targets with higher probability, thereby minimizing off-target effects. To achieve this, we first translate the node prioritization rank (Recall from Section 2.1) to an equivalent *target rank*, then convert the rank to a *selection probability* value which is used to decide if the target will be accepted. We now introduce these two concepts.

Given a signaling network $G = (V, E)$ and a target prioritization method P, let $\Psi_{P:u}$ be the rank of node $u \in V$ based on P and $\zeta_v \subset E$ be the reactant-product edge set of node $v \in V$. Let $c_{fix}, c_{var} \in C$ where C is the set of targets in G. Then, the *target ranks* of c_{fix} and c_{var} are denoted as $\Psi_{c_{fix}} = \Psi_{P:u}$ and $\Psi_{c_{var}} = \sum_{w \in W} \Psi_{P:w}$, respectively, where $W = X \bigcup Y$, $X, Y \subset V$, and $c_{var} = (X, Y)$.

The *selection probability* (δ) of a target is the likelihood of selecting the target. We use the rank-based fitness function in [2] to obtain a target's selection probability. The fitness function is based on the individual target ranks and avoids scaling problems associated with using actual objective values. The expected sampling rate of the individual target is controlled by a parameter called *selective pressure* λ^+ [39].

Observe that the aforementioned heuristic is independent of any specific target prioritization method. However, as we shall see in Section 5, PANI-based target combination identification typically generates superior quality results compared to MCSA-based techniques as the former exploits structural and dynamics properties of the signaling network [8] to improve prioritization of targets.

LOEWE-based heuristic. The effects (Section 3.2) resulting from a drug combination can be interpreted as drugs at particular dosages hitting their targets that result in certain target activities causing a particular response of the network. Hence, an interaction of multiple targets in a combination can be assessed the same way as drug interactions (Section 2.2) by replacing the drug doses with target activities. *A target combination is guaranteed to be synergistic if the target activities are chosen from values below the line of additivity.* Based on Section 2.2, we define the *target interaction* as follows.

DEFINITION 4. *Given a therapeutic effect* t *and a target combination* $C = \{c_1, \cdots, c_m\}$*, let* $\Gamma_{0(c_i)}$ *and* $\Gamma_{(c_i)}$ *be the target activities of the* i^{th} *target in* C *that achieve* t *when targeted alone and in combination, respectively. Then, the* ***target combination index*** *of* C *is defined as*

$$\text{TCI}_C = \sum_{c_i \in C} \frac{\Gamma_{(c_i)}}{\Gamma_{0(c_i)}}$$

The combination is ***synergistic***, ***additive*** *or* ***antagonistic*** *if* $\text{TCI}_C < 1$, $\text{TCI}_C = 1$ *or* $\text{TCI}_C > 1$*, respectively. The* ***synergistic ranges*** *of* c_j *and* c_m *are denoted as* $[0\text{–}\Gamma_{0(c_j)})$ *and* $[0\text{–}\Gamma_{(c_m)})$*, respectively, where* $1 \leq j < m$, $\Gamma_{(c_j)} \in [0\text{–}\Gamma_{0(c_j)})$ *and* $\text{TCI}_C < 1$.

Graphically, the synergistic ranges of a 2-target combination can be visualized in Fig. 2 (rightmost isobologram) as "synergistic range$_1$" and "synergistic range$_2$".

4.2 The Algorithm STEROID

Systematic algorithms (*e.g.*, backtracking) proposed for solving CSP [3] assume that partial instantiation of the candidate solutions are possible. However, the target combination identification problem requires full instantiation to find the combination effects. In this section, we present STEROID (outlined in Algorithm 1) that leverages the heuristics in Section 4.1 for modifying the candidate solutions. The signaling network (G) and prioritization rank (Ψ) are used to modify the drug targets and target activities. The signaling network is also used to simulate the target combination effects. Note that the user can specify his preferred target prioritization method for finding Ψ. The input t is used to assess the combination effects while input $\mathcal{S}$ specifies the size of the combination target. Several other parameters (λ^+, θ_t, θ_a, $\mathcal{N}$, τ_0 and i_{max}) that are required by STEROID are set to default values which can be modified if required (Line 2). The parameter λ^+ is used to compute the selection probability of the target. In practice, it is difficult to achieve the therapeutic effect exactly and additive target combinations are generally close to the line of additivity, but seldom "sit" exactly on it. Hence, we specify adjustment factor parameters θ_t and θ_a to relax the condition for therapeutic effect and additive combination into bound conditions, respectively (e.g., 49.5% to 50.5% inhibition of ERKPP and additive if $0.95 \leq \text{TCI} \leq 1.05$). Finally, the parameters $\mathcal{N}$, τ_0 and i_{max} are used to configure the simulated annealing (SA) and they control when the SA terminates: when $\mathcal{N}$ solutions

Algorithm 1 Algorithm STEROID

Input: Signaling network G, prioritization rank set Ψ, therapeutic effect t, combination size $\mathcal{S}$
Output: Solution set $\mathcal{R}$

1: $\mathcal{R} \leftarrow$INITIALIZE($\mathcal{R}$)
2: (λ^+,θ_t,θ_a,$\mathcal{N}$,τ_0,i_{max})$\leftarrow$SETTODEFAULTS(λ^+,θ_t,θ_a,$\mathcal{N}$,τ_0,i_{max})
3: $\tau \leftarrow \tau_0$
4: (G,$\mathcal{C}$,Γ_0)$\leftarrow$PREPROCESSINPUT(G,t,θ_t,τ_0,i_{max})// Phase 1
5: **while** $\tau \geq 0$ and $|\mathcal{R}| \leq \mathcal{N}$ **do**
6: **for** iteration i=1 to i_{max} **do**
7: $\mathcal{X} \leftarrow$GETCOMBI($\mathcal{C}$,λ^+,Ψ,θ_a,Γ_0,$\mathcal{R}$,$\mathcal{S}$)// Phase 2.1
8: ($t_{\mathcal{X}}$,$\rho_{\mathcal{X}}$)$\leftarrow$GETEFFECT(G,t,$\mathcal{X}$)// Phase 2.2
9: $\mathcal{R} \leftarrow$ACCEPTCOMBI($t_{\mathcal{X}}$,$\rho_{\mathcal{X}}$,t,θ_a,θ_t,τ,$\mathcal{R}$)// Phase 2.3
10: **end for**
11: Decrement τ
12: **end while**
13: **return** $\mathcal{R}$

are found or when $\tau_0 \times i_{max}$ iterations are completed where τ_0 is the initial temperature and i_{max} specifies the limit on the number of iteration per temperature cycle.

STEROID consists of two phases, namely, the *preprocessing phase* and the *simulated annealing with heuristics phase*.

Phase 1: Preprocessing. In this phase (Line 4), the reversible reactions in G are converted into pairs of irreversible reactions using [34]. The reactions are then modified (according to Section 3.1) to simulate the effects of the targets when modulated by non-competitive inhibitors or essential activators. The set of drug targets $\mathcal{C}$ is obtained using Definition 1. The individual target activities Γ_0 required to achieve the desired therapeutic effect (50% down-regulation of ERKPP, θ_t=5%) are found using MCSA configured with the parameters τ_0 and i_{max}. Due to space constraints, details for the individual target activities are given in [7].

Phase 2: Simulated Annealing with Heuristics (SAH). The SAH consists of three subphases which are repeated until either the temperature τ reaches zero or the required number of solutions $\mathcal{N}$ is found (Line 5). The subphases consist of target combination generation (the GETCOMBI procedure, Line 7); combination effects calculation (the GETEFFECT procedure, Line 8); and the test for candidate acceptance (the ACCEPTCOMBI procedure, Line 9).

In the GETCOMBI procedure (Algorithm 2), the candidate combination $\mathcal{X}$ consisting of $\mathcal{S}$ targets is generated. Lines 4 to 7 implement the target prioritization-based heuristic and Line 9 LOEWE-based heuristic. The first target $\mathcal{A}$ is randomly selected using SELECTRANDOMTARGET (Line 5, Algorithm 2) and accepted in ACCEPTTARGET (Line 6) if the probability of selecting $\mathcal{A}$ (selection probability) is greater than a random number in the range [0–1] ($\delta_{\mathcal{A}} >$RAND(0,1)). Its activity is then selected within the synergistic range (Definition 4) using SELECTACTIVITY (Line 9). Similar steps are repeated to find subsequent targets and their activities.

Next, the GETEFFECT procedure (Line 8, Algorithm 1) obtains the therapeutic and off-target effects by simulating the candidate solution using Copasi and calculating the effects (Section 3.2). These effects are used to assess the candidate in ACCEPTCOMBI (Line 9). A candidate is accepted if it is synergistic, it achieves the required therapeutic effect, and it has lower off-target effects than the current solution (*curr*); or if it achieves the required therapeutic effect and $e^{-\frac{\rho_{\mathcal{X}}-\rho_{curr}}{\tau}} \geq$RAND(0,1) (Metropolis criterion). Due to space constraints, the formal descriptions of these two procedures are given in [7].

Algorithm 2 The GETCOMBI Procedure (Phase 2.1)

Input: Target candidate set $\mathcal{C}$, selective pressure λ^+, prioritization rank set Ψ, adjustment factor for target interaction θ_a, individual target activity set Γ_0, solution set $\mathcal{R}$, combination size $\mathcal{S}$
Output: Combination candidate $\mathcal{X} = \{(x_1, \Gamma_1), \cdots, (x_S, \Gamma_S)\}$

1: $\mathcal{X} \leftarrow$INITIALIZE($\mathcal{X}$)
2: $\mathcal{Y} \leftarrow$INITIALIZE($\mathcal{Y}$)
3: **for** $(x_i, \Gamma_i) \in \mathcal{X}$ **do**
4: **while** ISNULL(x_i) is TRUE **do**
5: $\mathcal{A} \leftarrow$SELECTRANDOMTARGET($\mathcal{C}/\mathcal{Y}$,$\mathcal{R}$)
6: $x_i \leftarrow$ACCEPTTARGET($\mathcal{A}$,$\mathcal{C}/\mathcal{Y}$,Ψ,λ^+)
7: **end while**
8: $\mathcal{Y} \leftarrow \mathcal{Y} \bigcup x_i$
9: $\Gamma_i \leftarrow$SELECTACTIVITY(x_i,$\mathcal{Y}$,Γ_0,θ_a,$\mathcal{R}$)
10: **end for**
11: **return** $\mathcal{X}$

THEOREM 1. *The time complexity of* STEROID *is* $O(\tau_0 \cdot i_{max} \cdot |E| \cdot |\xi|)$ *where* τ_0 *is the initial temperature;* i_{max} *is the limit on the iterations per cycle;* $|E|$ *is the number of irreversible reactions; and* $|\xi|$ *is the number of time points in the concentration time-series profiles used to estimate the target effects.*

PROOF. The proof is given in [7]. □

5. PERFORMANCE STUDY

STEROID was implemented using Java. In this section, we investigate the performance of STEROID and compare it with state-of-the-art MCSA-based techniques [19, 40]. Since STEROID involves two heuristics and it is orthogonal to any specific target prioritization technique, we use several variants of it for comparative study. We denote variants of STEROID enabled with one and two heuristics as STEROID-X and STEROID-XX, respectively, where X$\in$ {L,P,S,M} and L=LOEWE, P=PANI [8], S=LSA [38] and M=MPSA [43]. Note that modification of the candidate differs depending on the heuristics used. For MCSA, the targets and activities are selected randomly. For STEROID-P, STEROID-S and STEROID-M, the targets are selected using Algorithm 2 (Lines 4–7) while the activities are selected randomly. For STEROID-L, the targets are selected randomly while the activities are selected from within the synergistic range. For STEROID-PL, STEROID-SL and STEROID-ML, the targets and activities are selected using Algorithm 2 (Lines 4–9).

We ran the experiments on an Intel 2.93GHz Xeron processor machine with 12GB RAM running Microsoft Windows 7 to obtain 10 sets of results for each approach. The MAPK-PI3K network [17] was used for analysis and the desired therapeutic condition was chosen as 50% ERKPP down-regulation (Section 2). For all experiments, the combination size was set to 2 and the following default values were used for the rest of the parameters: {τ_0=100; i_{max}=500; $\mathcal{N}$=50; θ_t=θ_a=5%; λ^+=1.8}. We used Copasi for estimating the combination effects and its parameters were set following [8]. Due to space constraints, the effects of these parameters on the results are reported in [7]. In the tables presented, the terms ACT and IN denote activators and inhibitors, respectively. Forward and backward reactions are marked with the superscripts f and b, respectively.

Biological Relevance. First, we examined each approach's ability to find a set of benchmark target combination relevant to ovarian cancer and targeting the MAPK-PI3K network. The benchmark combination set is curated from literature in the PubMed repository using "ovarian", "cancer", "combination" as keywords. Out of 5863 PubMed

PMID	Target 1	Target 2
22180401	Akt In	MEK In
21632463	PI3K In	MEK In
21062259	Akt In	MEK In
14675307	PI3K In	Akt In

Table 1: PubMed results of ovarian cancer drug combinations targeting the MAPK-PI3K network.

Target in Table 1	Inhibition Mechanism	Corresponding target(s) in MAPK-PI3K network
Akt In	Disruption of Akt binding to its membrane localizing factor (PIP3) [30] or dephosphorylation of PIP3 [27]	Reaction 29^b Act, Reaction 30 Act, Reaction 33 Act, Reaction 29^f In, Reaction 31 In, Reaction 32 In
MEK In	MEK dephosphorylation [29] or blockade of MEK phosphorylation [13]. Is also used to achieve ERK inhibition as there is no known ERK inhibitors.	Reaction 16 Act, Reaction 18 Act, Reaction 20 Act Reaction 22 Act, Reaction 15 In, Reaction 17 In, Reaction 19 In, Reaction 21 In
PI3K In	Inhibits PI3K in ATP-competitive manner [27]	Reaction 24^b Act, Reaction 26 Act, Reaction 24^f In

Table 2: Mapping between targets in Table 1 and MAPK-PI3K network.

records (as of 17 March 2012), only 4 (Table 1) fitted the criteria. Table 2 shows the targets in the MAPK-PI3K network that match those in Table 1. We examined our solution set to identify those combinations (Table 3) involving the targets in Table 2 and found that *the majority of the target combinations were found using* Steroid-pl. We noted that the off-target effects of these solutions were relatively low (in the range [4–26]) and were likely to be good combinations, correlating well with literature [1, 21]. Details of the target combinations are reported in [7].

Since the solutions in Table 3 were not the best within our solution set, we went on to examine if the best solutions are biologically relevant and whether one approach is better than another for biological purposes. We pooled solutions from all approaches to identify the top-10 solutions having the least off-target effects involving target activity of less than 100 (*Min10*, Table 4). Note that large target activity (Definition 2) implies either high drug concentration or very small dissociation constant, both of which are likely to cause side effects, especially if the treatment regime requires repeated drug dosing [9]. Steroid-pl *identified a majority of the top-10 solutions (90%).* Most of the targets identified are located downstream of the MAPK-PI3K network. Since none of our solutions corresponded to the curated combinations in PubMed (Table 1), we performed a targeted literature search for each predicted combination (Table 4) to see if it had ever been performed. We found that *60% of the solutions in Min10, all found using* Steroid-pl*, have high biological relevance as potential target combinations.* For instance, from experiments, profound growth inhibition and apoptosis were observed in CI-1040 (MEK1/2 inhibitor) treated ovarian cancer cells with mutations in KRAS or BRAF [32] and these tumors typically overexpress DUSP4 (ERK phosphatase) [35]. This correlates with our computational prediction of combinations 1, 6 and 9 involving ERK phosphatase activator and ERK (or MEK) kinase inhibitor. Note that there is currently no known inhibitor that acts directly on ERK and ERK inhibition is typically achieved through MEK inhibitors. Furthermore, the predicted combination 4 (ERK kinase inhibitor and tyrosine kinase inhibitor) correlates with the fact that the combination of cetuximab (tyrosine kinase inhibitor [22]) and AZD6244 (MEK inhibitor) is currently undergoing a phase 1 clinical trial for solid tumors (NCT01217450). For predicted combinations 7 and 10, we did not find any supporting evidence that they have been performed, successfully or otherwise, in experiments. However, individual components of these combinations have shown efficacy in ovarian cancer [20, 31], and warrant further investigation as potential target combinations. In the remaining predicted combinations, two (combinations 2 and 3) are more akin to monotherapies than combination therapies as they involve the same type of drug (ERK or MEK kinase inhibitor) and we did not find any supporting evidence for the other two (combinations 5 and 8). *We conclude that* Steroid *can identify*

Approach	PubMed Combination (Count)	% Found
Steroid-L	Akt/MEK In (5), MEK/PI3K In (2)	12.73
Steroid-PL	Akt/MEK In (16), MEK/PI3K In (8)	43.64
Steroid-SL	Akt/MEK In (6), MEK/PI3K In (1)	12.73
Steroid-ML	Akt/MEK In (3), MEK/PI3K In (3)	10.91
Steroid-P	Akt/MEK In (5), MEK/PI3K In (3)	14.55
Steroid-S	MEK/PI3K In (1)	1.82
Steroid-M	MEK/PI3K In (1)	1.82
MCSA	Akt/MEK In (1)	1.82

Table 3: Summary of target combinations in $\mathcal{R}$ corresponding to combinations curated from PubMed.

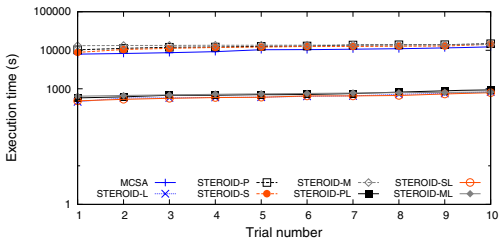

Figure 3: Runtime performance.

biologically relevant combinations with low off-target effects, suggesting that heuristics are useful in improving the solution quality.

We also examined 10 solutions with maximum off-target effects from the pooled solution set (Table 5). All 10 combinations contain tyrosine kinase inhibitors (TKI). Treatments involving TKI tend to lose their effectiveness soon due to resistance, often caused by activating mutations in downstream effectors of the tyrosine kinases [36]. Moreover, they are prone to toxicity caused by the disruption of multiple downstream signaling pathways of the tyrosine kinases, which are involved in normal organ functioning [16]. Hence, designing combinations involving TKI requires knowledge of a patient's tumor's genome to select additional complementary targets that can minimize TKI-resistance. The TKI activity should also be kept low to reduce potential toxicity. However, the predicted combinations involve TKI at high activity level, making them less than ideal. In addition, we noted that several of the targets identified in the predicted combinations (50%) involve promoters of known mediators of cancer (*e.g.*, MEK kinase [33]). The design of such target combinations require extra caution to achieve the required therapeutic effect, since improper management of the balance between the pro- and anti-cancer signal can easily aggravate the cancer. Hence, *large off-target effects may be indicative of less effective or more toxic combinations.*

Runtime Performance. In this set of experiments, we analyzed the execution time needed to complete analysis for the different approaches. Fig. 3 plots the results. We can make two key observations. First, our proposed heuristic-based approach is *an order of magnitude faster than state-of-the-art techniques* (MCSA). Second, approaches incorporating LOEWE are approximately an order of mag-

Target 1 [Activity]	Target 2 [Activity]	ρ	Method
Reaction 21 In [1.858]	Reaction 22 Act [0.173]	1.010	Steroid-pl
Reaction 21 In [2.063]	Reaction 19 In [0.055]	1.010	Steroid-pl
Reaction 21 In [2.248]	Reaction 17 In [2.5×10^{-4}]	1.017	Steroid-pl
Reaction 21 In [2.248]	Reaction 1^f In [3.92×10^{-3}]	1.022	Steroid-pl
Reaction 22 Act [2.227]	Reaction 8^f In [1.2×10^{-4}]	1.023	Steroid-pl
Reaction 22 Act [2.086]	Reaction 21 In [0.064]	1.026	Steroid-pl
Reaction 22 Act [2.143]	Reaction 13 In [0.065]	1.028	Steroid-pl
Reaction 22 Act [2.222]	Reaction 5^b Act [0.011]	1.031	Steroid-l
Reaction 22 Act [2.216]	Reaction 17 In [0.009]	1.032	Steroid-pl
Reaction 21 In [2.163]	Reaction 14 Act [0.010]	1.036	Steroid-pl

Target (Count)	Reaction in [17]	Description
Reaction 21 In (6)	ERKP→ERKPP	ERK kinase inhibitor
Reaction 22 Act (6)	ERKPP→ERKP	ERK phosphatase activator
Reaction 17 In (2)	MEKP→MEKPP	MEK kinase inhibitor
Reaction 1^f In (1)	R+HRG→RHRG	tyrosine kinase inhibitor
Reaction 5^b Act (1)	RShc→RP+Shc	activator of Shc dissociation from RP
Reaction 8^f In (1)	RShgs→Shgs+RP	inhibitor of Shgs dissociation from RP
Reaction 13 In (1)	Raf→Raf*	Raf inhibitor
Reaction 14 Act (1)	Raf* →Raf	Raf inhibitor
Reaction 19 In (1)	ERK→ERKP	ERK kinase inhibitor

Table 4: Top: Target combinations in *Min-10*. Bottom: Details of targets.

Target 1 [Activity]	Target 2 [Activity]	ρ	Method
Reaction 1^b Act [3557]	Reaction 28 In [6870]	3998	mcsa
Reaction 28 In [5050]	Reaction 1^b Act [3602]	3001	mcsa
Reaction 1^b Act [3282]	Reaction 28 In [4397]	2775	Steroid-m
Reaction 32 Act [4298]	Reaction 1^b Act [9192]	361	Steroid-s
Reaction 27 Act [993]	Reaction 3^b In [4717]	361	mcsa
Reaction 1^b Act [9136]	Reaction 31 In [9945]	360	mcsa
Reaction 1^b Act [8992]	Reaction 32 Act [2465]	360	Steroid-s
Reaction 31 In [1215]	Reaction 1^b Act [8406]	353	mcsa
Reaction 32 Act [9963]	Reaction 1^b Act [8086]	351	Steroid-s
Reaction 15 Act [3809]	Reaction 1^f In [1416]	350	mcsa

Target (Count)	Reaction in [17]	Description
Reaction 1^b Act (8)	RHRG→R+HRG	tyrosine kinase inhibitor
Reaction 28 In (3)	PIP3→PI	PIP3 phosphatase inhibitor
Reaction 32 Act (3)	AktPIP→AktPIPP	PI3 kinase activator
Reaction 31 In (2)	AktPIP→AktPIP3	PI3 kinase inhibitor
Reaction 1^f In (1)	R+HRG→RHRG	tyrosine kinase inhibitor
Reaction 3^b Act (1)	RP→RHRG2	tyrosine kinase inhibitor
Reaction 15 Act (1)	MEK→MEKP	MEK kinase activator
Reaction 27 Act (1)	PI→PIP3	PI3 kinase activator

Table 5: Top: Target combinations in *Max-10*. Bottom: Details of targets.

nitude faster than other approaches, likely because LOEWE-heuristic avoids doing full evaluation on non-synergistic combinations which are excluded during candidate generation.

Off-Target Effects. We studied the effects of using different approaches on the off-target effects (Section 3.2) by comparing various descriptive statistics (Fig. 4). We also pooled together the solutions obtained from the 10 trials (pooled trial) and compared the cumulative distribution functions (CDF) using t-test and Kolmogorov-Smirnov (KS) test for further analysis. Observe that Steroid-pl achieved the lowest minimum, maximum, average and median off-target effects. For the pooled trials, using one-tailed t-test, Steroid-pl produced solutions with lower off-target effects compared with other approaches ($p<0.05$); for the CDF, using one-tailed KS-test, Steroid-pl produced CDF which lie further to the left compared with other approaches ($p<0.005$). This implies that Steroid-pl *produces solutions with significantly lower off-target effects when compared to other approaches.* This could be due to Pani and LOEWE seeking solutions with reduced off-target effects in a complementary manner: LOEWE selects for lower target activity by enforcing target synergy while Pani selects for targets with lower off-target effects at higher probability.

Combination Characterization. In this set of experiments, we characterized the solutions based on the target interaction (synergistic, additive or antagonistic) and the

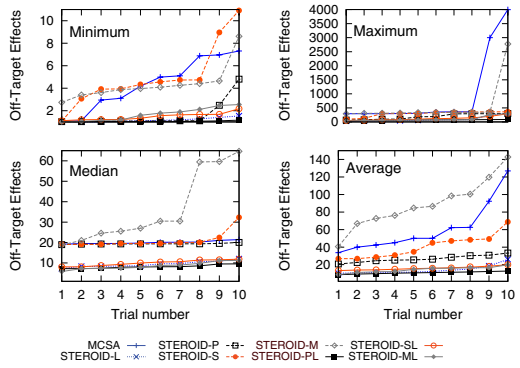

Figure 4: Off-target effects.

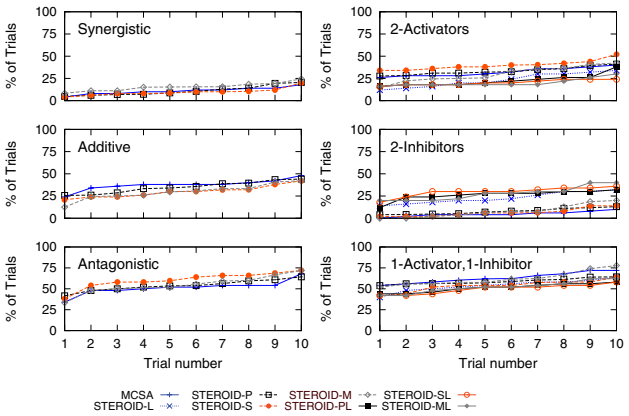

Figure 5: (Left) Target interaction, and (Right) combination types.

combination type (activators, inhibitors, or mixed activator and inhibitor). *Approaches incorporating* LOEWE *produced synergistic combinations by default while other approaches produce mainly antagonistic combinations* (Fig. 5, Left). In terms of the combination type (Fig. 5, Right), we observed that *the bulk of the combinations found are mixed activator and inhibitor.* Development of inhibitors of protein-protein interaction are perceived to be easier than activators which have to achieve binding and be a good replication of the protein interaction to stimulate increase in activity [15]. *Incorporating* LOEWE*-based heuristic improved the fraction of trials with 2-inhibitors by about 1 to 3 folds.*

6. CONCLUSIONS & FUTURE WORK

In this work, we describe Steroid, the first heuristic approach based on LOEWE and target prioritization for modifying target combinations in a signaling network using the simulated annealing framework. Our results highlight the importance of using heuristics to improve the process of generating appropriate candidates during the search for target combinations in terms of both execution time and result quality. Steroid-pl, particularly, produces superior results with high biological relevance and significantly lower off-target results. It also discovers potential combinations (*e.g.*, ERK phosphatase activator with Raf inhibitor) for further exploration. The good performance of Steroid-pl is likely due to the selection of synergistic targets (LOEWE-based heuristic) based on structural and kinetic properties of the network (Pani-based heuristic). Furthermore, we note that LOEWE-based heuristic enriches the search space, improving runtime performance by about an order of magni-

tude, and increasing the fraction of trials with 2-inhibitor combinations by up to 3 folds.

We note that not all target prioritization approaches improve the results and the objectives of the problem (*e.g.*, identifying therapeutic target combinations with low off-target effects) can be used to guide the selection of appropriate approaches. Further extensions of this work include incorporating various "omics" data and drug and disease information as heuristics to find target combinations that exclude combinations akin to monotherapies, and that avoid including activators of pro-disease targets as part of the combinations. In this work, we assume all nodes have equally severe off-target effects. A more flexible strategy is to implement a weighted sum off-target effects to reflect differences in severity of the off-target effects in future work. In summary, our performance comparisons demonstrate the potential value of knowledge-based heuristics for sampling and evaluating targets and STEROID provides a first step in this regard.

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