Optimizing Combinatory Drugs using Markov Chain-Based Models

Shuang Ma
State Key Laboratory of Robotics, Shenyang Institute of Automation, Chinese Academy of Sciences
Dan Dang
Faculty of Medical Devices, Shenyang Pharmaceutical University
Wenxue Wang (wangwenxue@sia.cn)
State Key Laboratory of Robotics, Shenyang Institute of Automation, Chinese Academy of Sciences
Yuechao Wang
State Key Laboratory of Robotics, Shenyang Institute of Automation, Chinese Academy of Sciences
Lianqing Liu
State Key Laboratory of Robotics, Shenyang Institute of Automation, Chinese Academy of Sciences

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Optimizing Combinatory Drugs using Markov Chain-Based Models

Shuang Ma[1,2,3], Dan Dang[4], Wenxue Wang[1,2,*], Yuechao Wang[1,2], Lianqing Liu[1,2,*]

1. State Key Laboratory of Robotics, Shenyang Institute of Automation, Chinese Academy of Sciences, Shenyang, China;

2. Institutes for robotics and Intelligent Manufacturing, Chinese Academy of Sciences, Shenyang, China

3. University of Chinese Academy of Sciences, Beijing, China

4. Faculty of Medical Devices, Shenyang Pharmaceutical University, Shenyang, China

Email: mashuang@sia.cn (S.M.), dangdan@syphu.edu.cn (D.D.), wangwenxue@sia.cn(W.W.), ycwang@sia.cn(Y.W.), lqliu@sia.cn(L.L.)

Tel: +86-24-2397-0215(W.W.); +86-24-2397-0181(L.L.)

* To whom correspondence should be addressed.

Abstract

Background: Combinatory drug therapy for complex diseases, such as HSV infection and cancers, has a more significant efficacy than single-drug treatment. However, one key challenge is how to effectively and efficiently determine the optimal concentrations of combinatory drugs because the number of drug combinations increases exponentially with the types of drugs.

Results: In this study, a searching method based on Markov chain is presented to optimize the

combinatory drug concentrations. Its performance is compared with four stochastic optimization algorithms as benchmark methods by simulation and biological experiements. Both simulation results and experimental data demonstrate that the Markov Chain-based approach is more reliable and efficient than the benchmark algorithms.

Conclusion: This article provides a versatile method for combinatory drug screening, which is of great significance for clinical drug combination therapy.

Keywords: Combinatory Drug Optimizations; Markov Chain; Transition Probability; Stationary Balance Distribution; Combinatory Therapy

Background

In the practice of clinical treatment, a single drug often fails to achieve the desired efficacy because the single drug in general aims at a single target of diseased cells and cannot remedy all aberrantly functioning pathways because of the robustness of organisms. The drug may also have poor safety profiles owing to various factors [1], including compensatory changes in cellular networks upon drug stimulation [2], redundancy [3], crosstalk [4], and off-target activities [5]. In contrast, drug mixtures are generally more effective than single effectors because multiple drugs simultaneously act on different pathways and cell targets, potentially leading to higher efficacy and lower toxicity because of drug synergy [6]. Therefore, in the clinical treatment of complex diseases, such as parasitic nematode infections or herpes simplex virus (HSV), a variety of drugs have been used in combination for treatment improvement [7]. The infection of parasitic nematodes (or roundworms) poses a serious safety hazard to humans and livestock [8], and the anthelmintics (or antinematode drugs) are highly susceptible to drug

resistance. It has been proved that a variety of combinations of multiple anthelmintic drugs, rather than a single medicine, can enhance the deworming effect [9]. In the case of the eradication of wild-type *Caenorhabditis elegans* worms, it is more effective to use four combinatory drugs (levamisole, pyrantel, tribendimidine, and methyridine) than single drugs [10]. Traditional treatments of HSV-I, one of the most common sexually transmitted infections, often include virus-specific drugs, which are effective at the beginning but exhibit limited long-term efficacy as drug-resistant strains develop. However, a combination of six drugs (IFN-α, acyclovir, IFN-γ,ribavirin, IFN-β and TNF-α) was demonstrated to be the most promising therapy for the reason that the drugs in the combinatory treatment can act simultaneously on the multiple pathways and cellular protein complexes, and, therefore, regulate all relevant pathways, potentially blocking HSV-I replication [11]. Combined use of multiple drugs is also a common practice in the treatment of cancers to achieve higher efficacy and potency. For example, in the treatment of non-Hodgkin's lymphoma, the drugs, pirarubicin, velet, cytarabine and prednisone, are usually used in combination, which the chemotherapy effect is remarkablely enhanced [12].

However, owing to the inherent complexity of biological systems and internal structure of cells and, particularly, to the huge searching space, it is extremely challenging to effectively and efficiently to determine the optimal drug mixture from all possible drug combinations through trial and error. For example, there are n drugs and each drug has m concentration candidates, it is necessary to find the optimal drug mixture in the space of m^n combinations. Obviously, as the types of drug increase, the number of combinations increases exponentially, and it is impossible to test all cases of drug combinations because it takes a considerable amount

of time to perform the testing experiments. Therefore, it is important to explore how to reduce the number of experiments and predict the optimal combinatory drug concentration accurately and quickly.

For these reasons, the optimization of drug combination has attracted considerable attention in recent years, and several methods for predicting the optimal combinatory drug concentrations have been proposed [13-20]. A feedback system control (FSC) method was developed to search for optimal synergistic combinatory drugs for the treatment of diseases [16]. The FSC method starts with a set of initial concentrations of combinatory drugs with defined drug doses, and the efficacies of the combinatory drugs on the cells at the given concentrations are evaluated according to the phenotypic output response of the cells. Then, the next predictions of the concentrations of drug mixtures are conducted based on the previous drug testing results with a certain searching algorithm, such as the Gur game (GG) algorithm, modified Gur game (MGG) algorithm, differential evolution (DE) algorithm, and continuous adaptive population reduction (CAPR) method, and the FSC method iteratively approaches a globally optimal combinatory drug mixture [19]. However, in some cases, these algorithms may degrade the overall performance of FSC owing to the inherent shortcomings of these algorithmic frameworks [17-20]. FSC with the GG and MGG algorithms often falls in oscillatory curves instead of giving a convergent output. It converges too early to a local extremum with the DE algorithm, thereby forming a premature convergence phenomenon, and it lacks a unified parameter controlling strategy with the CAPR algorithm to satisfy various applications. Furthermore, the FSC iterates its searching process, in which the next iteration requires biological experiments with the predicted combinatory drug doses for further

85 evaluation and prediction. Thus, the optimization of combinatory drugs with FSC is quite
86 inefficient because a significant amount of time is spent on the testing experiments.

87 In this paper, an optimization method based on Markov chain models is proposed to search
88 for optimal combinatory drug concentrations with excellent performance. In this method, the
89 searching process of the optimal drug concentration is converted into a Markov chain with
90 $N = m^n$ state variables representing all possible drug combinations, where n refers to the
91 number of drugs, and m is the number of discretized concentrations for each drug. This
92 Markov chain can be depicted by a network of N nodes in the space of R^n, where the nodes
93 refer to the state variables. Assuming that all the possible drug combinations have equal
94 probability to be the optimal mixture without having prior knowledge about the efficacy of the
95 drug mixtures, a matrix of transition probability can be initialized so that the stationary
96 distribution vector of the Markov chain has an equal value of $1/N$ for all its states. Then the
97 searching process for the optimal combinatory drug concentrations is equivalent to updating
98 the transition probability matrix and seeking the the state with the maximum value in the
99 stationary distribution vector.

100 The proposed method was validated by both simulation and biological experiments. In the
101 simulation experiments, the proposed Markov-chain-based method was compared with the four
102 benchmark algorithms (GG, MGG, DE, and CAPR) in the FSC framework. In biological
103 experiments, the survival rate of cells under two combinatory drugs is regarded as the response
104 function, and the Markov-chain-based method was compared with GG and MGG in FSC. The
105 results of the simulation and biological experiments prove that the algorithm based on the

Markov chain outperforms the selected benchmark algorithms in terms of accuracy and efficiency. In summary, this study provides a versatile, novel method for efficiently optimizing combinational drug concentrations, and the work is of great significance for clinical drug combination therapy.

The remainder of this article is organized as follows. First, the preliminary theories of the Markov chain are discussed briefly, and the Markov-chain-based method is presented. Then the simulation and biological experiments are described, and the experimental results are discussed to compare the performance of the proposed Markov chain-based method with other benchmark algorithms. In the last, we conclude this article.

Methods

In this section, first, some basic theories of the discrete-time Markov chain are briefly reviewed. The optimization problem of combinational drug therapy is formulated with assumptions, and the general idea of the Markov-chain-based approach to the optimization of drug combinations are described. The detailed algorithms in the cases of one drug and two drugs are given in Supplementary Materials.

Markov Chain Theory

A Markov chain is a special kind of Markov stochastic process with a set of discrete states. It starts in one of these states and moves successively from one state to another, satisfying the Markov property. Markov chains are a mathematical model to describe a process in which the next state of the system depends only on the present state, and not on the preceding states. In other words, the process loses its memory of the past over time.

Definition of a Markov Chain: When $\{X_n, n \geq 0\}$ is a random sequence taking values in a finite or countable discrete set, where $\Phi = \{1,2,\dots,N\}$ or $\Phi = N$ typically, the process $X(n) = X_n$ for $n = 1,2,\dots$ is a Markov chain if

$$P(X_{n+1} = s_{n+1} | X_n = s_n, X_{n-1} = s_{n-1}, \dots, X_0 = s_0) = P(X_{n+1} = s_{n+1} | X_n = s_n) \quad (1)$$

where $n \geq 0$ and $s_{n+1}, s_n, s_{n-1}, \dots, s_0 \in \Phi$, and the values taken by the random variables X_n are called the states of the chain. Moreover, if the transition probability $P(X_{n+1} = s_{n+1} | X_n = s_n) = p_{ij}$ for $s_{n+1} = j$ and $s_n = i$ is independent of n, then $P = \{p_{ij}\}$ is called the transition probability matrix.

Stationary Distribution: For a Markov chain with the transition probability matrix $P = \{p_{ij}\}$, a probability distribution vector π is called a "stationary distribution" if π has entries $\{\pi_j \geq 0, j \in \Phi\}$ such that the following conditions hold.

$$\begin{cases} \pi = \pi P \\ \sum_{j \in \Phi} \pi_j = 1 \end{cases} \quad (2)$$

where $\pi = \pi P$ is called the "balance equation."

Assumptions

Before introducing the combinatorial drug optimization method based on the Markov chain, it is assumed that there are two assumptions:

- With the slow change in the concentration of the combination drugs, the effect of the drug on the experimental subject also changes smoothly.
- The number of drug combinations is limited.

The above two assumptions are reasonable for the optimization of combined drug concentrations. The organism does not change dramatically under smooth input from the outside world. In the experiment, the concentration of the drug combination is a few discrete points. Under the above two assumptions, the combinatorial drug optimization problem can be expressed using a finite-state Markov chain .

Method Description

First, a general example is depicted to illustrate the main idea of the method. Suppose that there are n kinds of drugs and each drug has m possible concentrations, then the state space $\Phi = \{1,2,\dots,m^n\}$ represents the set of m^n combinatory drug concentrations in an ascending order. Our goal is to find the optimal concentration from the state space. Here, the drug response function or death rate of cells can be represented by a normalized function $f(x) \in [0,1]$ for $x \in \Phi$. The higher value of the $f(x)$, the better effect of the drug combination at the corresponding concentration, leading to higher cell death rate. $f(x)$=0 means that the drug combination at the concentration level x is completely ineffective while $f(x)$=1 indicates that the drug combination achieves its best treatment efficacy. Our aim is to find the best concentration x^* for drug combination with the maximum value of the objective function $f(x)$ as follows:

$$x^* = argmax\ f(x), x \in \Phi \quad (3)$$

As shown in Fig. 1, in the case of three drugs, it is necessary to construct a three-dimensional network structure of Markov chain. Each drug has *m* concentration levels and a

total of m^3 concentration combinations constitutes the state space $\Phi = \{1,2,\ldots,m^3\}$. The states in Φ represent the drug combination of different concentrations. For any $i,j \in \Phi$; if $f(i) > f(j)$, it is implied that the efficacy of the drug combination at the concentration level i greater than that at concentration level j. Likely, in the general case of n drugs, an n-dimensional network of Markov chain can be constructed, and the state space Φ consists of m^n states if each drug has m concentration levels.

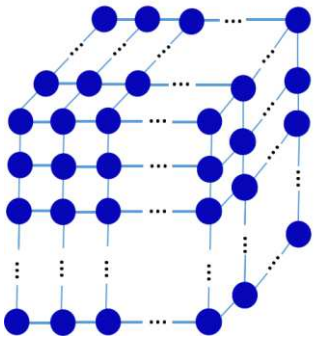


Fig. 1. State-transition diagram of an irreducible homogeneous aperiodic and positive recurrent Markov chain with m^3 states

In order to search for the optimal drug combination, a key assumption with the Markov chain model is that, for any state $x(t)$, the state $x(t+1)$ at the next moment always comes from the current state $x(t)$, and the states have a larger probability shifting to the direction with a larger objective function. In other words, at the step t, if the objective function for the state $x(t)$ is $f(x(t))$, then the state $x(t)$ can select to transfer to its adjacent states to obtain the next state by comparing its function value with those at the adjacent states and choosing the state with a relatively higher objective fucntion value for the next step. The benefits of this approach are obvious. As t approaches infinity, the state transfers to the optimal state x^*, which means that the probability at the global maximum of the objective function is the greatest.

Reconsidering the Markov chain model described above, from the state transition diagram shown in Fig. 1, it is obvious that it is a random walk with any two adjacent states. Searching for the optimal drug concentration is equivalent to seeking the state with the largest steady-state probability in the stationary distribution. Therefore, a transition probability matrix P of $m^n \times m^n$ is initialized and then updated iteratively for searching for the optimal drug combination with the Markov chain model. Firstly, according to the initial state, the corresponding matrix P is constructed, and two suitable experimental points are selected from $m^n \times m^n$. Secondly, the state transition probability matrix is updated and then the balance equation is solved to achieve the stationary state distribution. After multiple iterations, the algorithm converges with a predefined criteria, the maximum value in its stationary distribution is the corresponding optimal state sought, that is, the optimal combination of drug concentration levels.

It is noteworthy that the initialization of the transition probability matrix is not unique. Without having prior knowledge about the efficacy of the drug mixtures, it is reasonable to assume that all the possible drug combinations have equal probability to be the optimal mixture and a matrix of transition probability can be initialized so that the stationary distribution vector of the Markov chain has an equal value of $1/N$ for all its states. In this study, the transition matrix is initialized such that, on the network, every pair of adjacent states has an equal transition probability to move back and forth between each other, and every state has the same transition probability to move to all its adjacent states. In particular, the state on the edge of the network has a certain probability to go back to itself. Then, the Markov-chain-based approach to optimizing the combinatory drugs turns into a process of repeatedly updating the transition

matrix by comparing the efficacies of pairs of adjacent drug combinations and then computing the corresponding stationary distribution vector until a certain convergent criterion is satisfied. The steady state that has the maximal distribution probability is referred to as the optimal drug combination.

The general procedure of the optimization algorithm for combinatory drugs based on the Markov chain model is described as follows.

Step 1: The Markov chain and the corresponding transition probability matrix are initialized according to the numbers of drugs and concentration levels

Step 2: Suitable adjacent combinations of experimental points are selected.

Step 3: The transition probability matrix of the Markov chain is updated according to the difference in the drug response functions at the corresponding suitable experimental points.

Step 4: The corresponding stationary distribution is solved according to the updated transition probability matrix using the balance equation.

Step 5: It is determined whether the stationary distribution converges. If it converges, the algorithm stops; otherwise, it returns to the second step, or, when the predetermined number of iterations is reached, the algorithm stops.

A single drug and two kinds of drugs are taken as examples to introduce the searching algorithm we proposed in Supplementary Materials.

Simulation Experiments and Discussion

A simulation was used to compare the performances of the Markov-chain-based algorithm we proposed and four other algorithms: the GG algorithm, MGG algorithm, DE algorithm and CAPR method. The principle of these four algorithms are introduced briefly in Supplementary Materials.

Predicting the Optimal Concentration of Single-Drug

As shown in Figs. 2(a)~(o), three drug response functions are used to compare the performance using the Markov-chain-based algorithm, GG algorithm and the DE algorithm. As shown in Fig. 2, the GG-based algorithm oscillates around some states (Fig. 2(d)) or stays in a suboptimal state (Fig. 2(e) and Fig. 2(f)). The DE algorithm converges too early to the local extremum (Fig. 2(i)). The CAPR algorithm oscillates in a relatively small range but does not converge to a final state (Figs. 2(g)~(i)). The proposed Markov chain algorithm can find the optimal state in a few steps (Figs. 2(j)~(l)).

Unlike the limitations of GG- and DE-based algorithms we have mentioned in Supplementary Materials, the Markov-chain-based algorithm can avoid the disadvantages we mentioned above and predict the state at which the optimal drug combination concentration should have excellent performance (Figs. 2(j) ~(l)). The experimental points in the state space are selected evenly. The state with the largest steady-state probability in the balance distribution is the output we found by the algorithm based on the Markov chain and the output is usually unique. Moreover, using the GG- and DE-based algorithms, the prediction and the experiment results of the drug concentration combination are serial. Thence, our proposed algorithm using parallel experiments is more efficient than the GG- and DE-based algorithms

using serial experiments.

As shown in Figs. 2(m)~(o), the stationary distributions $\pi = (\pi_1, \pi_2, \ldots, \pi_N)$ change gradually with the update of transition probability matrices. Finally, the shape of the stationary distribution resembles the shape of the drug response function, which explains why the algorithm we proposed is effective for searching for the optimal combinatory drugs.

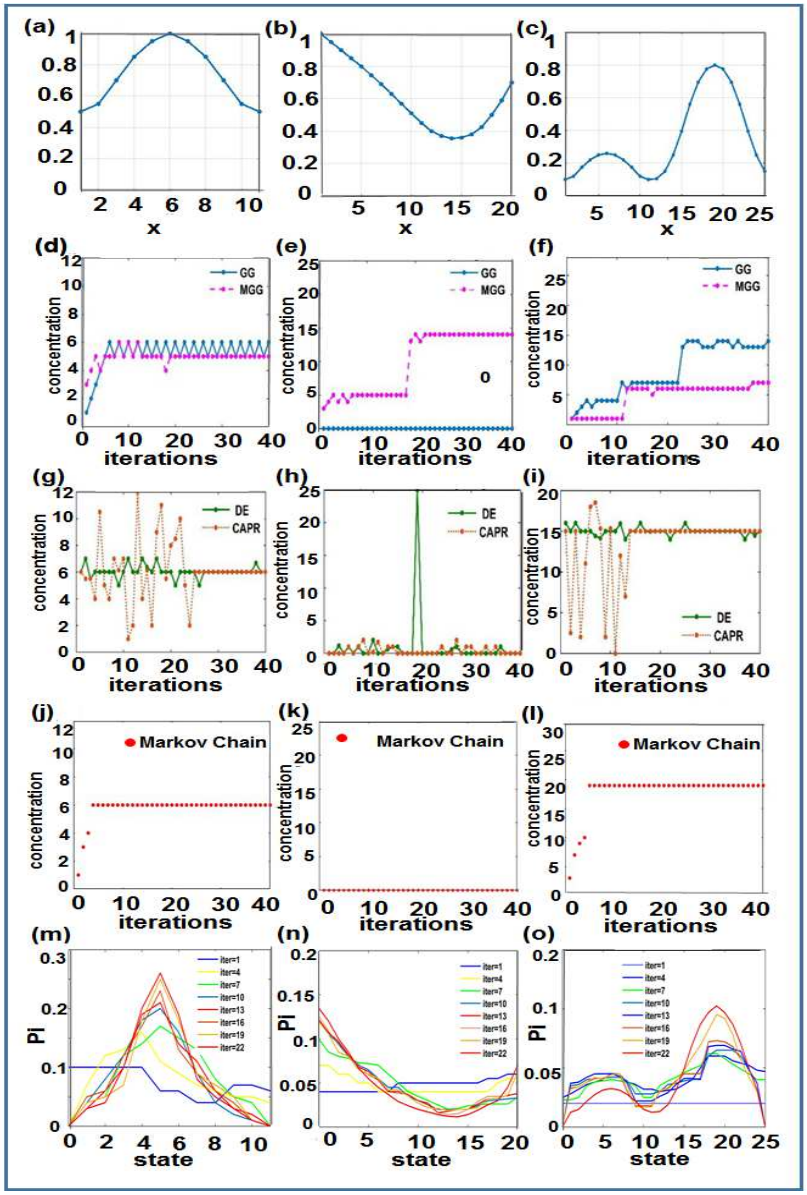


Fig. 2 Three drug response functions and numerical simulations using five different algorithms. (a)~(c): drug response functions; (d)~(f): GG algorithm and MGG algorithm;

(g)~(i):DE algorithm and CAPR algorithm; (j)~(l): Markov-chain-based algorithm,(m)~(o) the stationary distributions.

Predicting the Optimal Combination of Multiple Drugs

As shown in Fig. 3A-(a) Fig. 3B-(a), two drug response functions were used to evaluate the performance of the Markov-chain-based algorithm. As shown in Figs. 3A-(b)~(f) and Figs. 3B-(b)~(f), according to the smooth distribution of the peak function using Markov-chain-based optimization algorithm, the number of interval iterations between each graph is 10 steps. As the number of iterations increases, the smooth distribution surface gradually converges to the response function of the two drug combinations.

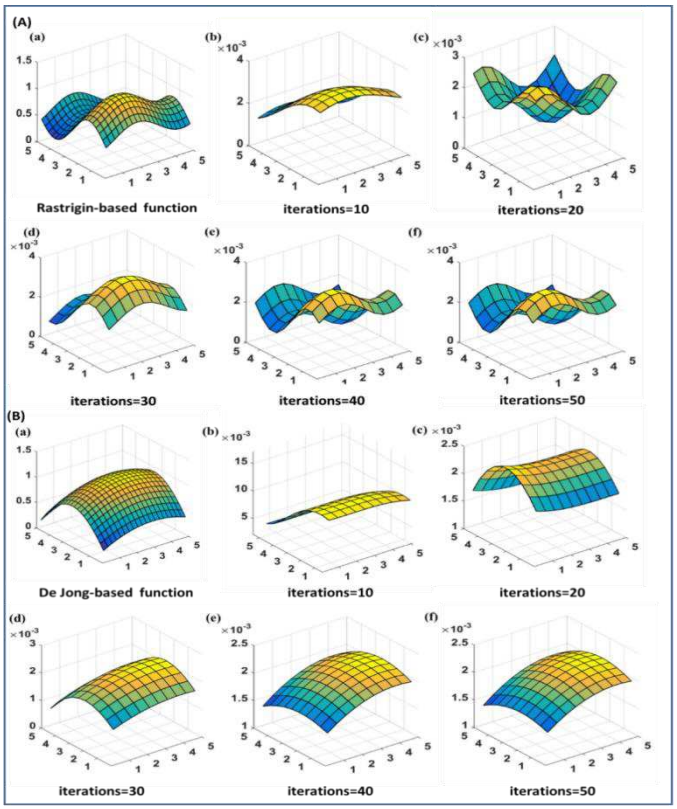


Fig. 3 Two drug response functions and corresponding stationary distributions based on Markov chain: as the number of iteration steps increases, the steady distribution surface

converges to the drug response surface: (A) Rastrigin-based function and (B) De Jong-based function

Table 1 lists the comparisons of the performance between the algorithm we proposed and the other four algorithms. The algorithm is regarded as effective if the optimized output $f(x, y)$ is larger than the threshold (λ) or the output we predicted is among the top P%(even if the results we predicted is far from the real maximum value). It can be concluded that the reliability and efficiency of the algorithm we proposed are better than that of the other algorithms.

Table 1. Performance comparison of five algorithms

		GG		MGG		DE		EDE		Markov chain	
		Success rate	# of iters	Success rate	# of iters	Success rate	# of iters	Success rate	# of ites	Success rate	# of iters
A	$\lambda = 0.95$	0.82	85.6	0.92	60.2	0.78	88.4	0.85	78.2	1.00	43.6
	$P = 5\%$	0.95	35.2	0.99	25.3	0.62	43.3	0.72	44.5	1.00	22.3
B	$\lambda = 0.95$	0.13	45.1	1.00	53.4	0.26	43.4	0.33	38.4	1.00	15.2
	$P = 5\%$	0.15	32.3	1.00	45.7	0.09	47.7	0.21	45.2	1.00	19.9

Biological Experiments and Discussion

Cell Culture

The cell lines used in this study were obtained from the School of Medical Device, Shenyang Pharmaceutical University (Shenyang, China). MCF-7 cells (human breast cancer cell line) and BXPC-3 cells (human pancreatic cancer cell line) were cultured in RPMI-1640 (Thermo Scientific HyClone, Logan, UT, USA) containing 10% fetal bovine serum and 1% penicillin–streptomycin solution at 37°C (5% CO2).

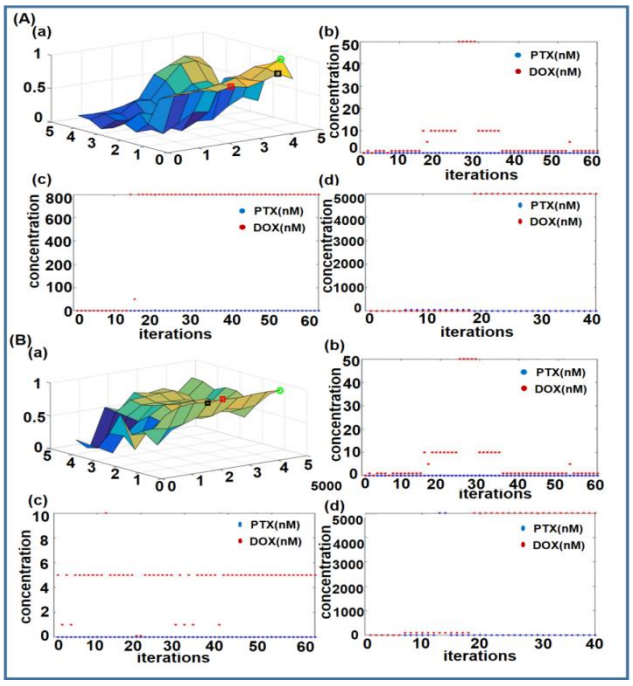
Cell Proliferation Assay

Cells were plated onto 96-well plates (8 × 103 cells/well for MCF-7 and 8 × 103 cells/well for BXPC-3) and allowed to attach for 24 h. Cells were incubated with free drugs dissolved in an appropriate cell culture medium at serial concentrations for 72 h. For treatments containing DOX and PTX, each contained nine concentrations ranging from 0 to 5000 nM according to DOX-equivalent concentration with a total of 81 concentration combinations with six complex holes per concentration. Following incubation, 10 μL of cell counting kit-8 (CCK8) (Dojindo) was added to each well in the dark and incubated at 37°C (5% CO_2) for 2 h. After incubation, a microplate reader (Thermo, Multiskan FC) was used to measure the number of viable cells in each well of a 96-well plate at a wavelength of 450 nm.

Performance Comparison

The response functions of two combined drugs, paclitaxel (PTX) and doxorubicin hydrochloride (DOX), on two kinds of cell, MCF-7 and BXPC-3, were selected to compare the performance of the algorithm we proposed and the other two GG-based algorithms. Fig. 4A-(a) and Fig. 4B-(a) are the two combined drug response functions. (The green circle drawn in

299 the fig is the maximum point of the drug response function, the red square is the maximum
300 point found using the GG algorithm, and the black square is the maximum point found using
301 the MGG algorithm.) Fig. 4A-(b) and Fig. 4B-(b) are the performance of the original GG
302 algorithm, Fig. 4A-(c) and Fig. 4B-(c) are the performance of the MGG algorithm, and Fig.
303 4A-(d) and Fig. 4B-(d) are the performance of the Markov-chain-based algorithm to find the
304 optimal combination of PTX and DOX. Figs. 4A-(b)~(c) and Figs. 4B-(b)~(c) show the
305 nonrobustness of the GG and MGG algorithms. From Fig. 4, we can draw conclusions similar
306 to those in the simulation. The original GG algorithm can easily lead to falling into the local
307 optimal value (Fig. 4A-(b) and Fig. 4B-(b)). As shown in Fig. 4A-(c) and Fig. 4B-(c)，the
308 MGG algorithm may take many iterations if the starting point and the optimal state are far away.
309 It is obvious that the point found by MGG algorithm is the local optimal value as shown in the
310 black square in Fig. 4A-(a) and Fig. 4B-(a).



311

312 Fig. 4 Two drug response functions and numerical simulations using three different algorithms:

(A) drug response function of PTX and DOX on MCF-7 cells. (a): drug response function; (b): using GG algorithm; (c): using MGG algorithms; (d): using Markov-chain-based algorithm; (B) drug response function of PTX and DOX on BXPC-3 cells: (a): drug response function; (b): using GG algorithm; (c): using MGG algorithms (d): using Markov-chain-based algorithm

In the Fig. 4A-(d) and Fig. 4B-(d), when the Markov-chain-based algorithm is used, the global optimal combination can be found within only a few iterations. As the experiment and calculation are parallel, the proposed algorithm is much more efficient.

Shown in Table 2 are performance comparisons of the two GG-based algorithms and the Markov-chain-based algorithm. Similar to the results of simulation, the efficiency and accuracy of the Markov-chain-based algorithm we proposed are much better than that of the other two GG-based stochastic algorithms.

Table 2. Performance comparison of three algorithms

		GG Algorithm		**MGG Algorithm**		**Markov Chain Algorithm**	
		Success rate	**# of iterations**	**Success rate**	**# of iterations**	**Success rate**	**# of iterations**
MCF-7	**$\lambda = 0.7$**	**0.20**	**10.8**	**0.39**	**5.4**	**1.00**	**19.0**
	$\lambda = 0.8$	**0**	**NaN**	**0**	**NaN**	**1.00**	**19.0**
	$\lambda = 0.9$	**0**	**NaN**	**0**	**NaN**	**1.00**	**19.2**
	P = 5%	**0**	**NaN**	**0**	**NaN**	**1.00**	**19.2**

BXPC-3	**λ = 0.7**	**0.48**	**11.0**	**0.20**	**1.0**	**1.00**	**7.0**
	λ = 0.8	**0.05**	**8.2**	**0**	**NaN**	**1.00**	**7.0**
	λ = 0.9	**0**	**NaN**	**0**	**NaN**	**1.00**	**19.0**
	P = 5%	**0**	**NaN**	**0**	**NaN**	**1.00**	**19.0**

Stationary Distribution Evolution and Find the Optimal Value

As shown in Fig. 5, two drug response functions of the two combinatory drugs were used. Fig. 5A-(a) and Fig. 5B-(a) are the two combinatory drugs acting on MCF-7 and BXPC-3 cell lines respectively (the green circle is the maximum value of the drug response function). Figs. 5A-(b)~(f) and Figs. 5B-(b)~(f) are the stationary distribution at different iterations. (The red circle is the maximum point found at the current iteration.) The number of interval iterations between each graph is 10 steps. It can be concluded that the stationary distributions of the response functions are going to vary as the updating of the transition probability matrices. Finally, the shape of the stationary distribution is similar to the that of drug response functions. At approximately 20 iterations, the optimal drug concentration combination can be found, which explains why the Markov-chain-based algorithm performs very well for optimizing the combinatory drugs.

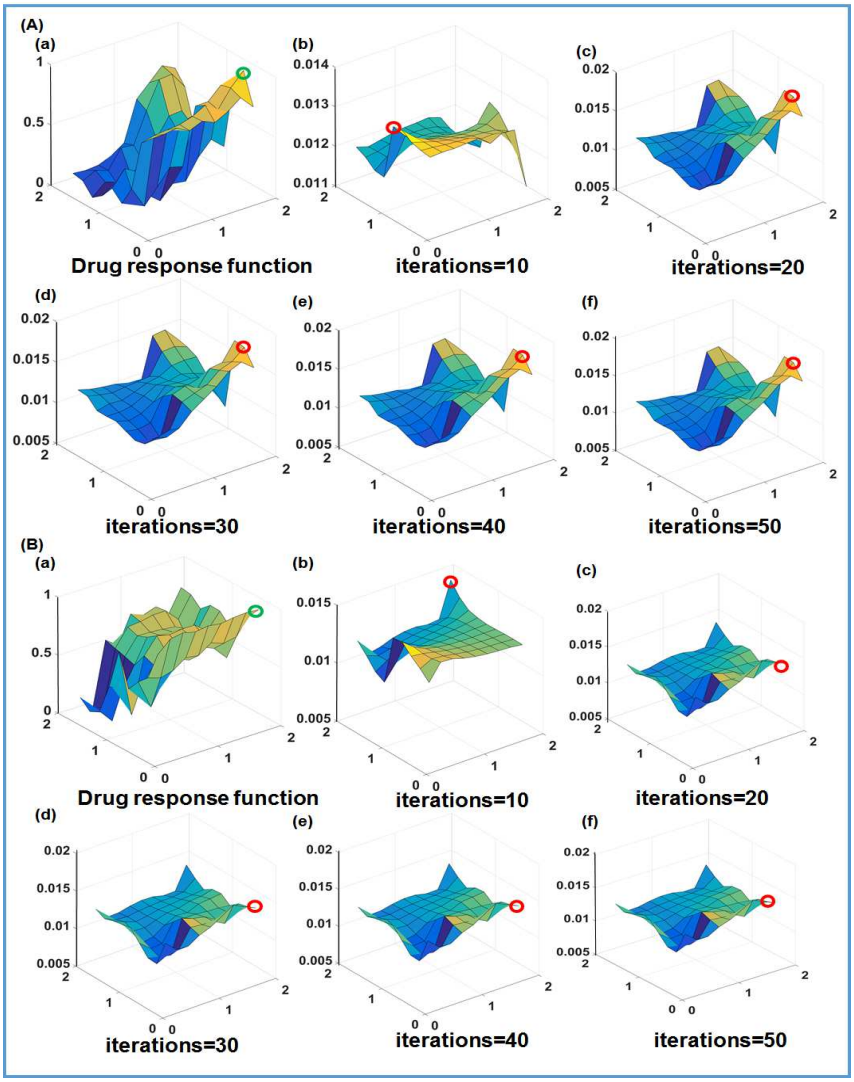


Fig. 5 Stationary distributions of two drug response functions converging to the shapes similar to the drug response function and finding the optimal drug concentration combination as the transition probability matrices are updated.

Conclusion

In this study, a novel Markov-chain-based approach was proposed to solve the problem of the optimization of combinatory drugs. The basic principle of the proposed method was introduced, and the steps of the algorithm used in the general case were illustrated in detail. Furthermore, the algorithm was promoted to cases of one-dimensional and two-dimensional

346 situations. In the simulation part, three one-dimensional functions and two two-dimensional
347 functions with different characteristics were introduced. The performances of two GG-based
348 algorithms, two DE-based algorithms, and the proposed Markov chain method were compared,
349 and the shortcomings of the other four algorithms were shown and analyzed. Based on the
350 results of the cell inhibitory rate experiments, the response functions of two combined drugs
351 were used to compare the performances of the Markov-chain-based method and two GG-based
352 algorithms. The simulation and experiment results show that the Markov-chain-based
353 algorithm performs much better than that of the other two algorithms in terms of efficiency
354 as well as quality. The stationary distributions converged to a similar shape to the response
355 functions of two combinatory drugs, which is consistent with the results of the simulations.
356 This proves the Markov-chain-based algorithm we proposed has an excellent performance.

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359 **Author's contributions**

360 Shuang Ma performed experiments, coding and manuscript writing. Dan Dang provided early
361 code for the algorithm. Wenxue Wang, Yuechao Wang, Lianqing Liu provided crucial guidance
362 and ideas throughout the project. All authors approved the final manuscript.

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Availability of data and materials

The data and algorithm mentioned in this paper are available in the [data and algorithm] package.

Ethics approval and consent to participate

Not Applicable

Consent for publication

Not Applicable

Competing interests

The authors declare that they have no competing interests.

Author details

[1.] State Key Laboratory of Robotics, Shenyang Institute of Automation, Chinese Academy of Sciences, Shenyang, China;

[2.] Institutes for robotics and Intelligent Manufacturing, Chinese Academy of Sciences, Shenyang, China

[3.] University of Chinese Academy of Sciences, Beijing, China

[4.] Faculty of Medical Devices, Shenyang Pharmaceutical University, Shenyang, China

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Figures

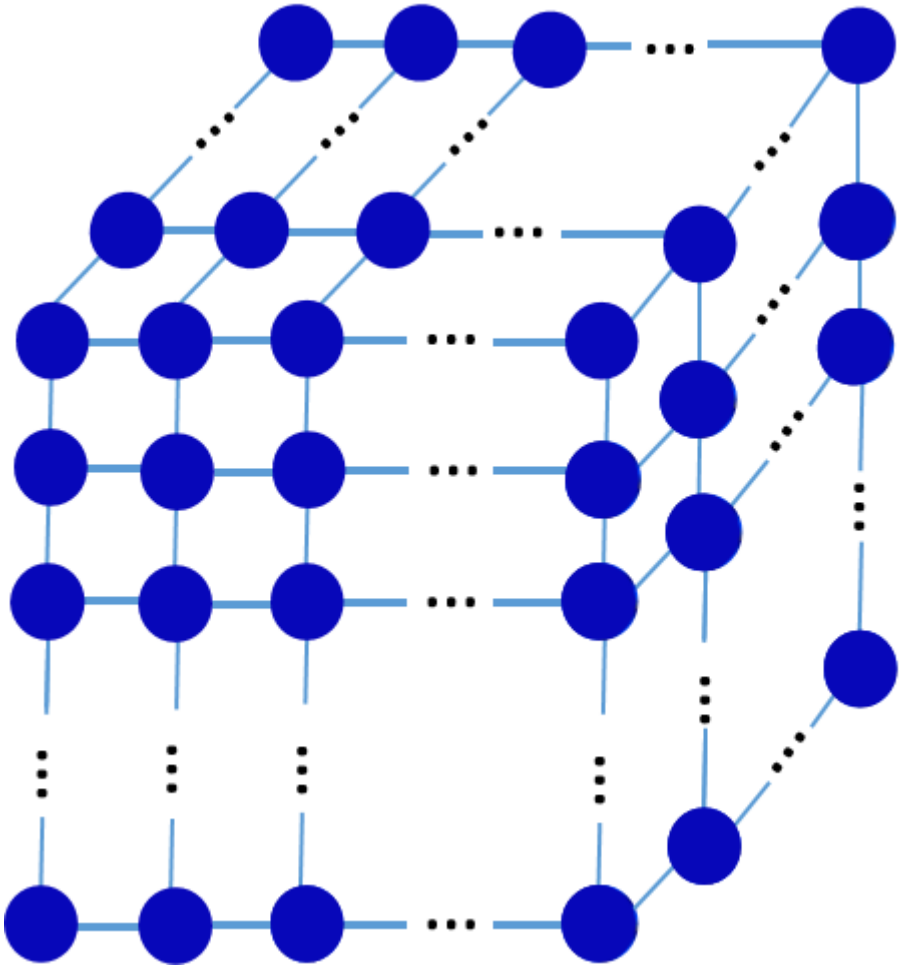

Figure 1

State-transition diagram of an irreducible homogeneous aperiodic and positive recurrent Markov chain with m3 states

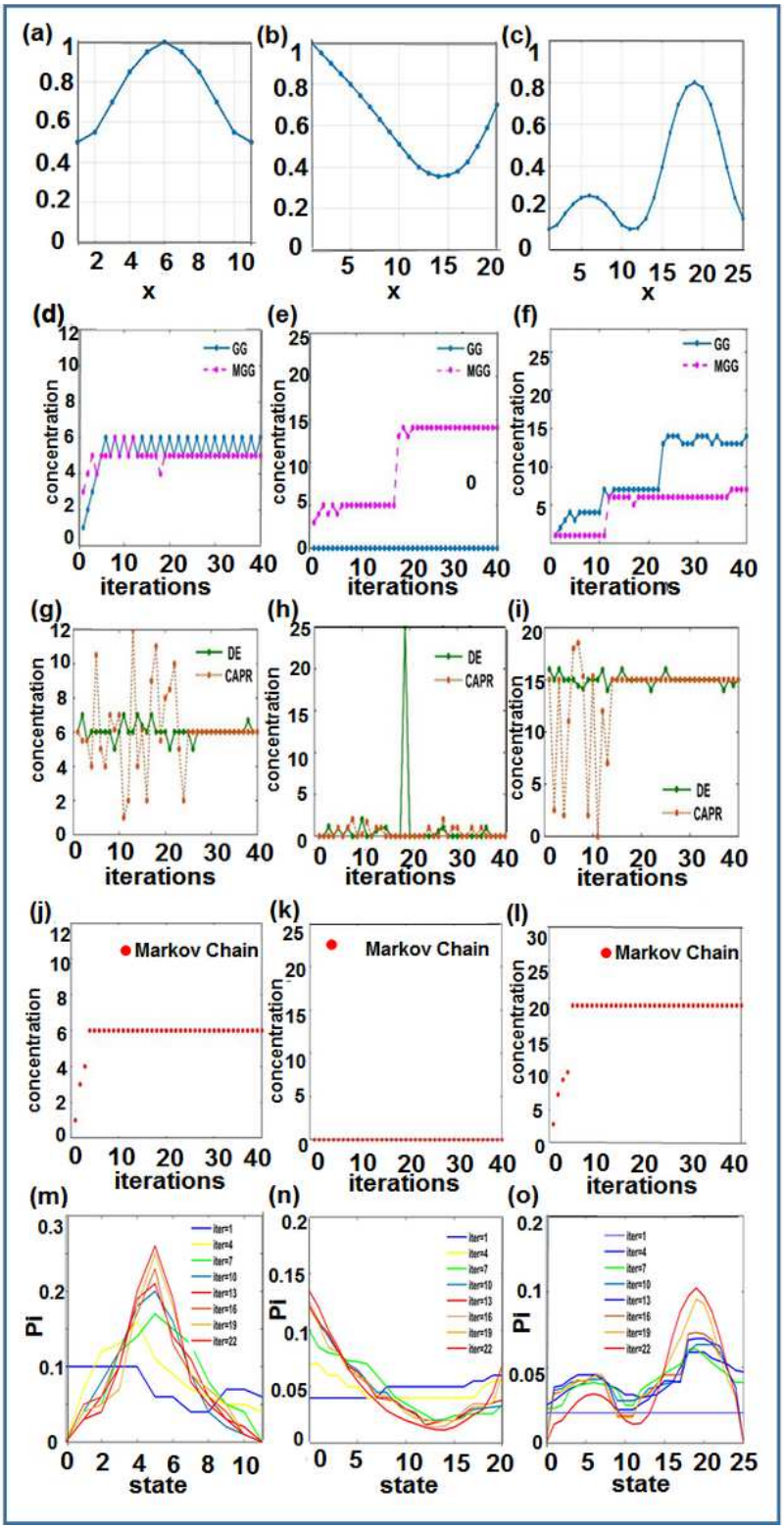

Figure 2

Three drug response functions and numerical simulations using five different algorithms. (a)~(c): drug response functions; (d)~(f): GG algorithm and MGG algorithm; (g)~(i):DE algorithm and CAPR algorithm; (j)~(l): Markov-chain-based algorithm,(m)~(o) the stationary distributions.

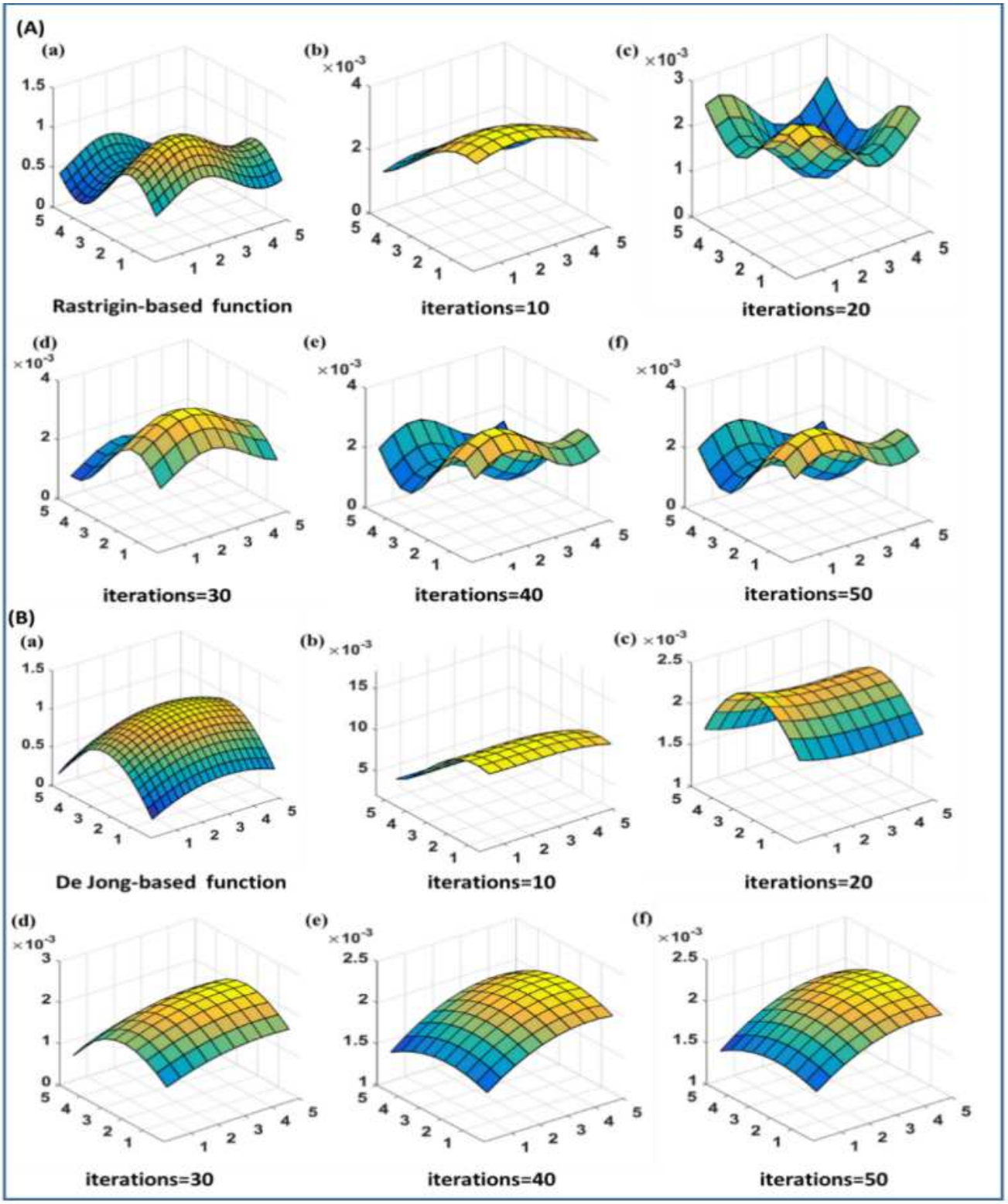

Figure 3

Two drug response functions and corresponding stationary distributions based on Markov chain: as the number of iteration steps increases, the steady distribution surface converges to the drug response surface: (A) Rastrigin-based function and (B) De Jong-based function

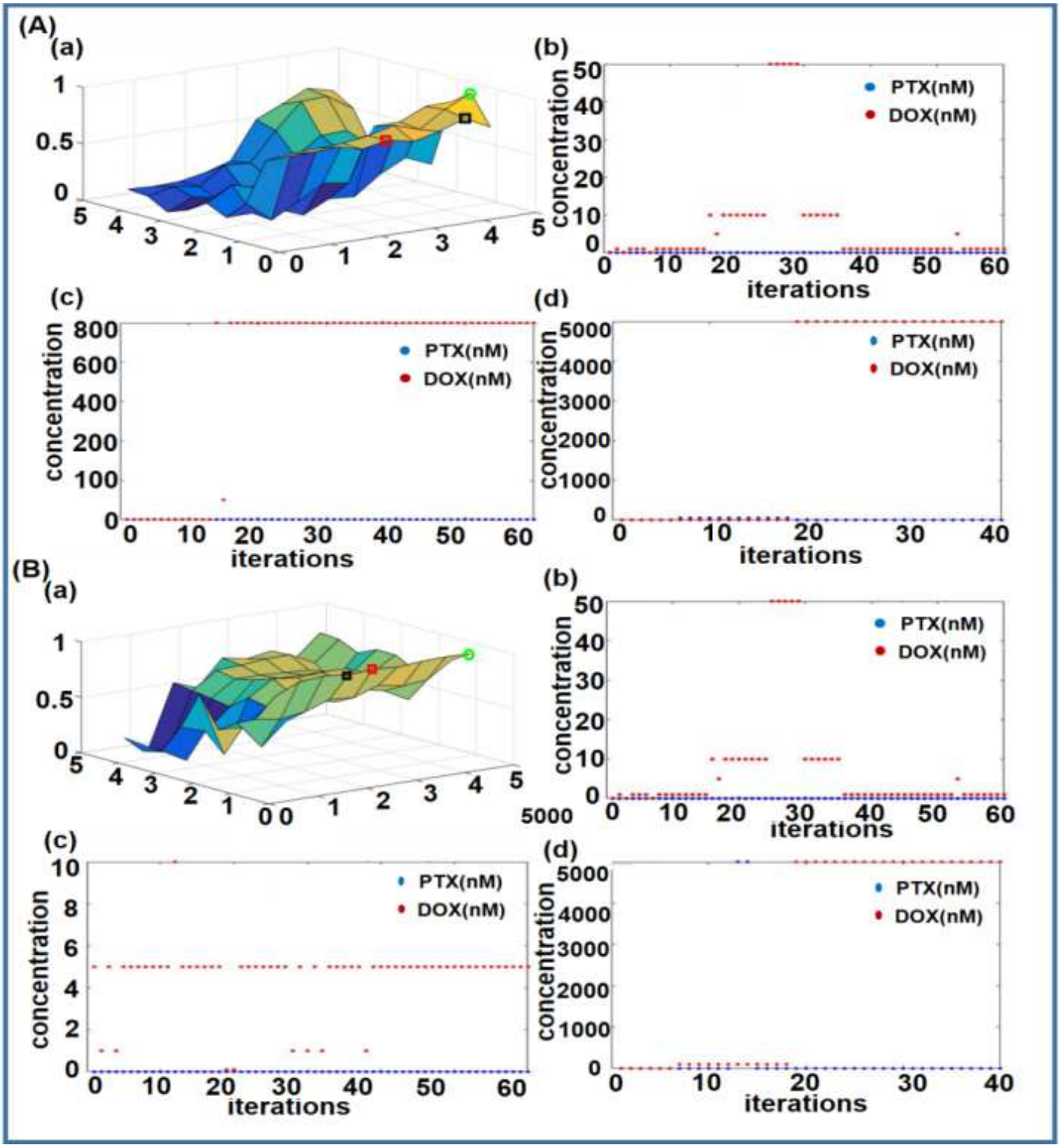

Figure 4

Two drug response functions and numerical simulations using three different algorithms: (A) drug response function of PTX and DOX on MCF-7 cells. (a): drug response function; (b): using GG algorithm; (c): using MGG algorithms; (d): using Markov-chain-based algorithm; (B) drug response function of PTX and DOX on BXPC-3 cells: (a): drug response function; (b): using GG algorithm; (c): using MGG algorithms (d): using Markov-chain-based algorithm

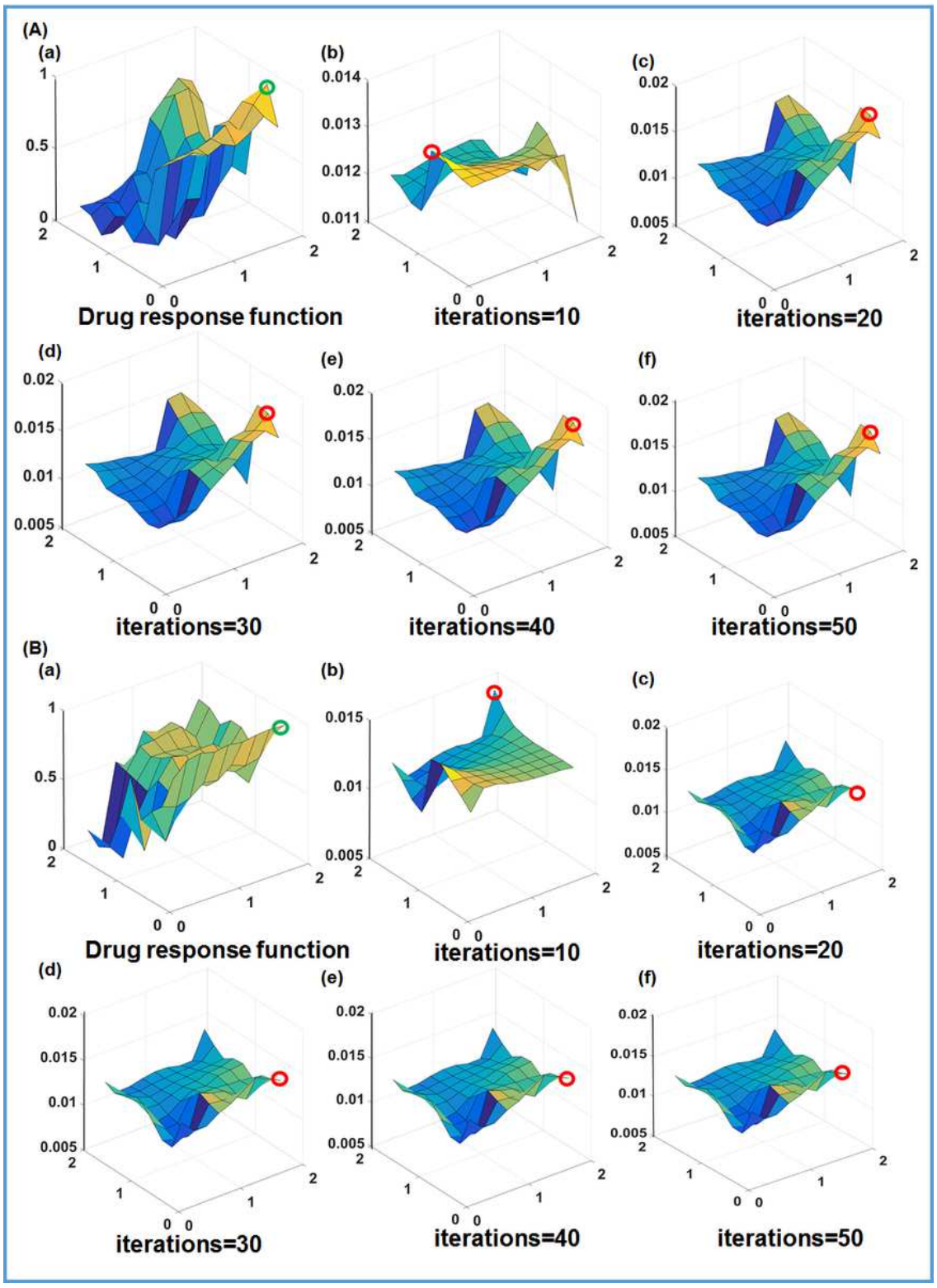

Figure 5

Stationary distributions of two drug response functions converging to the shapes similar to the drug response function and finding the optimal drug concentration combination as the transition probability matrices are updated.