A Model of Cellular Decision Making in Photodynamic Therapy of Cancer

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***Abstract*— The aim of this report is to provide a mathematical model of the mechanism for making binary fate decisions about cell death or survival, during and after type II photodynamic therapy (PDT) treatment, and to supply the logical design for this decision mechanism as an application of rate distortion theory to the biochemical processing of information by the physical system of a cell. Based on system biology models of the molecular interactions involved in the PDT processes previously established, and regarding a cellular decision-making system as a noisy communication channel, we use rate distortion theory to design a time dependent three dimensional Blahut-Arimoto algorithm where the input is a stimulus vector composed of the time dependent concentrations of three PDT related cell death signaling molecules and a cell fate decision as output. The molecular concentrations are determined by a group of rate equations. The output is the cell decision with a probability of cell survival or death. The optimality of the cell decision strategy is assessed by the cell survival probability, which might be modified to account for heterogeneous cell resistance to therapy.**

Keywords-component;; cell killing model; rate equations;photodynamic therapy.

I. INTRODUCTION

It is accepted that in vivo type II PDT treatment of cancer cells and subsequent cell killing may involve all three main cell death pathways as described by the terms of apoptotic, necrotic and autophagic cell death. Based on existing system biology models [1], [2], [3], we have developed a detailed molecular PDT model that includes 70 types of molecules and their corresponding interactions, pathways and biochemical events induced by PDT treatment. The molecular interaction diagram and major features have been summarized in [18] and the system of all molecular interactions, rate equations, reaction constants and initial conditions has been described analytically in [1-3, 18]]. We briefly discuss here some major aspects of the cell biochemistry related to type II PDT since our focus is the study of the cell decision mechanism of a single cell model in response to PDT treatment.

Singlet oxygen $^1O_2$ is a cytotoxic agent generated during PDT treatment. First, a photosensitizer, a light-absorbing molecule that is activated by light is delivered to the cultured cells under study. During treatment molecular oxygen is consumed as a result of the photochemical reaction to produce cytotoxic agents, thus leading to cell killing with appropriate doses of photosensitizer and light. Besides singlet oxygen and other reactive oxygen species (ROS), activation of caspase cascades known as "executioner caspases" such as caspase-3, -6 and -7 is the next step of the apoptosis/necrosis process [4]. The active executioner of caspases cleave cellular substrates, which leads to characteristic biochemical and morphological changes observed in dying cells. A very early step upon illumination is cytochrome c release from the mitochondria into the cytosol of treated cells [5, 6]. The cytochrome c release might be correlated to the loss of the mitochondrial membrane potential that has been observed in experiments, and might be related to MOMP (mitochondrial permeability transition pore). Ca2+ release through MOMP is correlated to cytochrome c loss. PDT has a very subtle effect on mitochondrial membrane. Cells could die from ATP depletion (necrosis) or follow the apoptosis activation of the caspase-pathway. Caspase 3 is the caspase that cleaves a large number of proteins that are involved in cell structure and maintenance, such as. PARP has been used as the marker of the apoptotic extent. PDT treatment with Pc 4, BPD, or aluminum phthalocyanine (AlPc) has been shown to lead to cleavage of PARP in different cell lines [7].

II. RATE DISTORTION THEORY

Whenever energy is transferred information is transferred. In PDT, light energy is absorbed by the photosensitizers and then transferred to oxygen and other molecules, through a cascade of reactions in the environment of a cell. The PDT treatment acts as a "source" generating the input information that the system of molecular network and interactions within a cell must communicate to the "receiver" or the cell. The information is encoded by the parameters of the light and the photosensitizer doses as the source "words" or "code" (death signals) is transformed into a form through activated photosensitizers that can be

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transmitted through the "channel" of molecular interactions. When decoded by molecular "thresholds", the input information can be converted to a channel output that has the form of a cell's state in terms of necrosis, apoptosis, autophagy or survival. In order to determine quantitatively whether or not the performance-efficiency of this bio-communication system is useful for modeling the experimental data it is necessary to assign numerical values to the various statistical variations and errors that the system may produce. The statistical mechanism that governs the generation of the source and the distortion measure that penalizes the bio-coding errors and determines the fidelity of the reproduction of the cell killing signal need to be quantified specifically in order to have a complete description. Eventually, we want to design an optimal treatment strategy that leads, through intracellular biochemical reactions, to reproduction of the PDT death signal output by the cell, with an average distortion that does not exceed a specified upper level D, for a single tumor cell model or a tumor cell population. To quantitatively answer this question, we use the rate distortion function R(D) which has been initially defined as the effective rate of the source producing information and passing it to the user, subject to the constraint that the user can tolerate an average distortion D[8]. In this model, it will be the effective rate at which the signaling molecular concentrations start the cell death pathways when the cell can faithfully reproduce this signal only if the distortion does not exceed D. The treatment pattern of the a priori setting parameters (lght density, photosensitizer concentration, etc.), is related to the data bio-compression of the death signal through molecular interactions, and the classification of the signal as to cell death or cell survival is done with a possible statistical error that is assigned a numerical penalty (distortion function d).

III. MATHEMATICAL BACKGROUND

An input vector signal will represent the combined stimulus vector of different death inducing molecular concentrations by PDT. We solve an ODE group that describes the major molecular interactions underlying the PDT treatment in time domain and obtain the stimulus vector of normalized molecular concentrations of x(t) = (x_1(t), x_2(t), x_3(t)) where $x_1(t)=[^1O_2](t)/[^1O_2]_{max}$; $x_2(t)$ = [cPARP](t)/$[cPARP]_{max}$; $x_3(t)$ = [Casp3](t)/$[Casp3]_{max}$. The PDT treatment starts at t=0, and ends at a later time $t=t_d$ (this can be 10 to 30 minutes and determines the fluence or optical dose for a given photon density). Observation of the treated cells ends at $t = t_{max} = 30$ hours. We define $p_X(x_1, x_2, x_3)$ as the probability by which the source produces the "word" (normalized molecular concentration levels) (x_1(t), x_2(t), x_3(t)) [1] at time *t*. For a continuous distribution, starting with ε = 0.001 or ε <0.001 we define the bump function approximation [2] to the delta function, in the phase space of the normalized concentrations:

$$p(x_1, x_2, x_3) = \frac{1}{\varepsilon^2}\eta\left(\frac{x_1, x_2, x_3}{\varepsilon}\right) \tag{1}$$

$$\eta(x_1, x_2, x_3) = \begin{cases} \exp\left(-\frac{1}{1-x^2}\right) & if \quad |x| < 1 \\ 0 & if \quad |x| \geq 1 \end{cases} \tag{2}$$

Then the marginal probability distribution of the cell decision are defined as the binary values for the variable y as equal to:

$$q(y) = \begin{cases} q_0 & for \quad y = 0(death) \\ 1-q_0 & if \quad y = 1(survival) \end{cases} \tag{3}$$

A distortion measure is then defined as $d(x_1, x_2, x_3|y)$, which is a measure of the penalty charged for reproducing the strength of the cell death signal described by the vector stimulus x=(x_1, x_2, x_3), by the decision $Y = y$ and thus quantifies how disadvantageous a given decision *Y* is in response to a given stimulus *X*. The mechanism by which data is gathered, stored, and utilized are poorly understood, and rate distortion theory may provide some insight into this function[3]. The "decompressed" data strength (cell decision) may be different from the original data (level of cell death stimulation). Typically, there is some distortion between the original and reproduced signal. This distortion measure, may be cell dependent, or time dependent, describing essential features of a cell, such as how does a cell estimate the state of its environment, how does it quantify alternative decisions, and how does it relate these decisions to the maximization of the fitness of the population [3]. For the purposes of information theory a discrete channel is described by a probability transition matrix Q(y/ x_1,x_2,x_3) where Q is the conditional probability of receiving the y output - signal letter given that the x_1,x_2,x_3 input letter signals were transmitted. As a conditional probability it is related to the probability distributions of the random vector x(t) and the random variable y, by the equation:

$$q(y) = \sum_{x_1 x_2 x_3} p_X(x_1, x_2, x_3) Q(y|x_1, x_2, x_3) \tag{4}$$

In the minimization of the mutual information (equation (6) below), conditional probability matrix Q is calculated through a condition that is defined by: [1][3]

$$Q(y|x_1, x_2, x_3) = \frac{q(y) \cdot e^{s \cdot d(x_1, x_2, x_3|y)}}{\sum_{y'} q(y') \cdot e^{s \cdot d(x_1, x_2, x_3|y')}} \tag{5}$$

where s is taken to be a negative number. This is the Lagrange multiplier for the method of calculus of variations, which is used to find the optimal cell decision probability q and conditional probability *Q* by minimizing the average mutual information between source (stimulus vector) and

receiver (cell/cell decision) [4]. We obtain the strategy as defined by equation (4) and (5) that minimizes the average mutual information between the input x (death stimuli, normalized concentrations) and the output (decision y), and the decision probability for cell survival or cell death by implementing a time dependent optimization Blahut Arimoto algorithm that is shown below (Fig.1). The average mutual information is defined as [1]:

$$I(p_X, Q) = \sum_{x_1 x_2 x_3 y} p_X(x_1, x_2, x_3) Q(y|x_1, x_2, x_3) \times \times \log \frac{Q(y|x_1, x_2, x_3)}{q(y)} \quad (6)$$

The rate distortion function $R(D)$ is defined as

$$R(D) = \min_{Q \in Q_D} I(p, Q) \quad (7)$$

where, Q_D is defined as the collection of all conditional probabilities-strategies $Q(y|x_1, x_2, x_3)$ such that $d(Q) \le D$ where the expected distortion is given by:

$$D = \sum_{x_1 x_2 x_3, y} p_X(x_1, x_2, x_3) Q(y|x_1, x_2, x_3) \times \times d(x_1, x_2, x_3|y) \quad (8)$$

The function R(D) describes the amount of information needed to be preserved by this biochemical data compression scheme of the source output which is given in the form of levels of molecular concentrations, so that reproduction of the death/survival signal can be subsequently generated from the compressed data with average distortion less than or equal to some specified value *D*. According to [3] the complexity and (metabolic) cost of a channel generally varies directly with its capacity, and a less complex strategy is more likely to be followed to be realized by a biological system. With minimal information I and a cell dependent distortion measure d, the optimal strategy incorporates randomness to generate biological variation.

IV. Mathematical Modeling and Simulation

The molecular reactions, rate equations, definitions of constants and their values and initial conditions that were used to mathematically model the molecular network that generates the concentrations of the stimuli as functions of time for the input of the Blahut Arimoto algorithm for the cell decision mechanism, have been published in [5], [6], [7] and a complete reference can be found in [38]. A system of 70 ODE (ordinary differential equations) was solved numerically to characterize the main molecular interactions involved in Type-II PDT. The output of this equation group is the time dependent levels of molecular concentrations for the stimulus vector of $x_1(t), x_2(t), x_3(t)$ corresponding to singlet oxygen $^1O_2$, cPARP and Caspase 3. The concentrations were normalized with respect to their maximum values and their range is [0, 1]. The total time for the simulations was up to 30,000s to monitor post- treatment cell killing. We used the stiff solver (ode15s) by MATLAB (The Math Works, Natick, MA) to obtain the solution vector as a function of illumination and observation times, t = 0 to 1800 (s) (end of illumination time) and from 1800 to 30,000 (s). Experimental verification of these quantities that describe the levels of all these molecular concentrations can be very difficult if not impossible and they relate indirectly to the ultimate consequence of PDT for cell killing. The output of the time dependent Blahut Arimoto algorithm is the cell survival probability. The distortion measure *d* that quantifies how disadvantageous a decision y is, in response to the stimulus vector x = (x_1, x_2, x_3) is defined by the equation $d(x_1, x_2, x_3|y = survival) = 10$ if $x_i \ge x_i^{th}$ and $d(x_1, x_2, x_3|y = survival) = 10^{-1}$ if $x_i < x_i^{th}$ for i=1,2,3 and a small number otherwise. The thresholds x_i^{th} for the normalized concentrations were all set to 0.5. This distortion measure penalizes a cell survival error more than cell death error for given stimuli, by one order of magnitude.

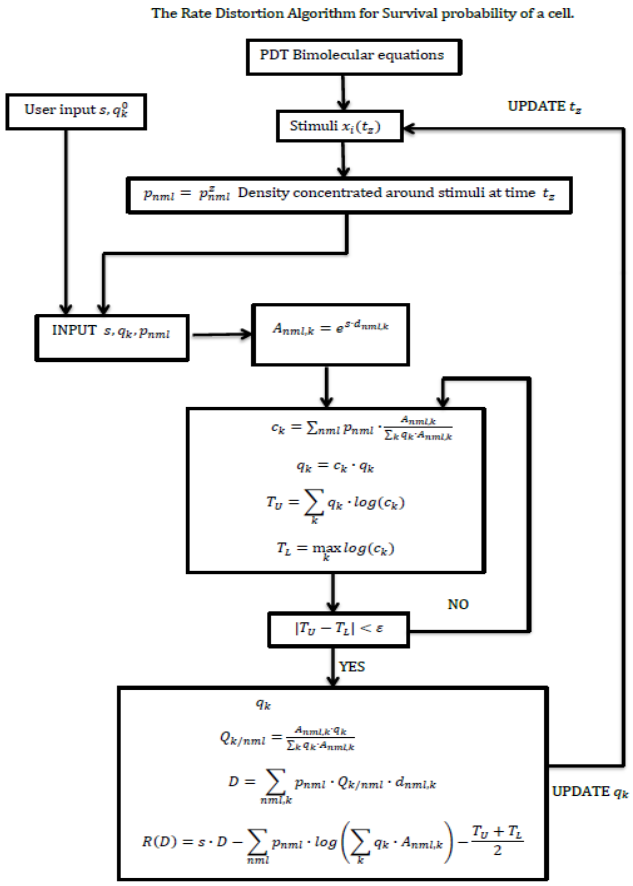

Figure 1. The Blahut Arimoto algorithm for cell decision mechanism in PDT.

For the range of the Lagrange multipliers the equation $s = -10^{-n}$ was used and in the simulations n varied over a finite set of integers (a sample of n values from 1 to 20 was taken for the simulations. The initial survival probability $q^0(y = surv) = 1 - q_0$ was set equal to 0.9. The treatment parameters for the PDT model that was introduced in our previous work [5], and was linked to the input of this algorithm were set to: Photon density $\rho=10^6$ cm^{-3}. Photo sensitizer (Photofrin) concentration in a cell $[S_0]$ = $5x10^{13}cm^{-3}$. Single cell oxygen concentration $[^3O_2]$ = $6.06x10^{17}cm^{-3}$ [1]. Figure 1 presents the flow chart of the algorithm and Figure 2 show an example of the simulation results about the cell survival probability as a function of observation time. Note in the latter figure, only apoptosis is considered for modeling of cell death which usually requires much longer time to complete its processes after the PDT treatment.

V. CELL REPAIR AND RESISTANCE

Cells have special machinery that rapidly recognizes damage and repairs it, therefore allowing the cell to retain its structure and survive [8]. The cell repair mechanisms that are related to the development of drug resistance in cancer cells are complex and of various kinds and are associated with many factors, such as cell type and intracellular and extracellular environment. For example, superoxide dismutase, which is present in both the mitochondria and cytoplasm of eukaryotic cells, is an enzyme that restrains the toxicity of reactive oxygen species such as $^1O_2$, one of

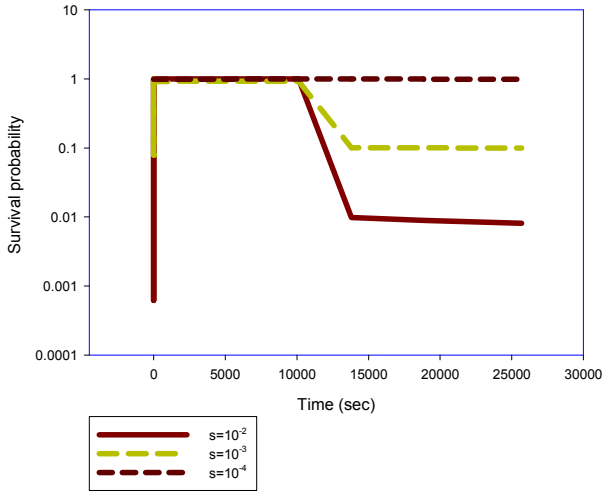

Figure 2. A sample of a survival probability curves as predicted by the Blahut Arimoto algorithm for the cell model. Value of the parameter s = -10^{-2},-10^{-3},-10^{-4} Photon density $\rho=10^6cm^{-3}$. Photo sensitizer (Photofrin) concentration in a cell $[S_0]$ = $5x10^{13}cm^{-3}$. Single cell oxygen concentration $[^3O_2]$ = 6.06x1017cm^{-3}.

the PDT agents. Cell killing through PDT is a unique case of study for system biology in which cell repair and death in response to combined stimulations of photosensitizer and light can be quantitatively investigated and modeled [1][5].In this approach of a cell decision mechanism of our report, the cell survival/death probability $q(y)$ can be modified and assisted by a term proportional to its dynamic survival state (a "restoring term"), to account for repair, resistance and increase in survivability that does not follow from the communication channel model and the distortion of the death signal induced by the three stimulating molecular concentrations, but rather represents the collection of processes by which a cell identifies and corrects mitochondrial and/or nuclear damage or any other form of cellular damage. We replace $q(y)$ by $\tilde{q}(y)$ where

$$\tilde{q}^0(y = surv) = q^0(y = surv) \quad (9)$$

$$\tilde{q}^{t+\Delta t}(y = surv) = \\ = b_1 \cdot q^{t+\Delta t}(y = surv) + b_2 \cdot \tilde{q}^t(y = surv) \quad (10)$$

In the equation (10), the survival probability $\tilde{q}(y)$ is updated as the sum of two weighted contributions: The survival probability predicted by the rate distortion function and calculated by the Blahut Arimoto algorithm , and a term that represents self-renewal capabilities of the cell and its ability to generate drug resistance [9], which is linearly proportional the survival state of the cell at the given time. The more photo-pharmacological damage is induced, the less the potency of the cell is, to resist this damage that corrupts its integrity. This modeling method reported here, of using a restoring term, has been studied in [18].

VI. CONCLUSIONS

In this study a model of cell death as a decision mechanism on the basis of rate distortion theory is proposed, which captures certain observed characteristics of a cell behavior after PDT treatment. The model obtains the cell survival probability as a consequence of a binary decision process for cell to survive or die. The main components of the model consist of the time dependent distribution of molecular stimuli vector as the input, the distortion function or measure, the conditional probability of the cell decision strategy, the expected distortion and the rate distortion function which quantifies stochastically the fate of a cell given the stimulation. The results are independent of the biological mechanism by which the cell strategy is implemented and the Blahut Arimoto algorithm is used to derive the optimal pathway. According to [17] cellular decision-making has the following main features: a cell must (1) estimate the state of its environment by sensing stimuli; (2) make a decision informed by the consequences of the alternatives; and (3) perform these functions in a way that maximizes the fitness of the population. These characteristics are obtained numerically by solving the rate

euqations for concentrations of signaling molecules and the rate distortion theory for cell survival prbability. Further study is underway to apply this model to a population of cells with different model parameters to reflect the diverse response of cells to PDT and other treatment and obtain simulation results that can be compared to experimental data on cell survival.

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