

Research Statement

Khalid Boushaba

Starting in 2003, when I joined Iowa State I became interested in the application of mathematics to medicine (Mathematical Biology) and began working with biologists and mathematicians, in particular with Dr. Marit Nilsen-Hamilton and Dr. Howard Levine. Part I highlights some aspects of my past and present research. Part II is devoted to future research.

I. Past and present Research.

- **Mathematical feasibility for the use of aptamers in chemotherapy and imaging:**

A central challenge for drug design is to create molecules with optimal function that also partition efficiently into the appropriate in vivo compartment(s). This is particularly true in cancer treatments because cancer cells up regulate their expression of multidrug resistant trans- porters, which necessitates application of higher concentrations of extracellular drugs to enable cell killing. Here we give proof in principle with a mathematical model based on chemical kinetic considerations that intracellular RNA aptamers can increase the effective intracellular concentration of a drug is by "pulling" the drug in [7]. We evaluate the use of cell-expressed aptamers with affinity for the drug to increase the efficiency of drug transport across the cell membrane and to increase the intracellular concentration of drug. Using the maximum principle we showed that this outcome will occur if the aptamer diffuses throughout the cytoplasm. The ability of the aptamer to increase the intracellular concentration of its target molecule could also be used for imaging cells. We showed by using simulation that an intracellular aptamer can be enlisted for an integrated approach to both increase drug effectiveness and image aptamer-expressing cells. An important finding from this study is the identification of the role of receptor diffusion in moving a drug from the membrane into the cell interior. The study predicts that the efficiency of drug action will be higher if the drug target molecule diffuses rather than being sequestered in an intracellular location such as is true for many enzymes.

- **Mathematical model for tumor dormancy:**

We developed a two compartment model for tumor dormancy [6], which is based on the suggestion by Zetter [10] that vascularization of a secondary (daughter) tumor can be suppressed by inhibitor originating from a larger primary (mother) tumor. We applied this idea at the avascular level to develop a model for the remote suppression of secondary avascular tumors via the secretion of primary avascular tumor inhibitors. The model gives good agreement with observations made in a clinical report that excision of a primary polyploid melanoma resulted in no recurrence at the site of surgery. Instead, by one month after the excision secondary tumors at sites arose five to seven centimeters from the primary site [8]. We provided a reasonable biochemical/cell biological model for this phenomenon to show that, when the tumors are sufficiently remote, the primary tumor will not influence the secondary tumor. However, if the primary and secondary tumors are within close enough range, the primary tumor can prevent the growth of the secondary tumor even after the primary tumor has been removed.

The overall idea for the two-compartment model for tumor dormancy involves the interaction between two tumor masses by way of secreted proteins and can be described by four chemical equations (Fig. 5). Suppose Ra is a receptor on a tumor cell capable of being activated by a growth factor. Let G be a molecule of growth factor, for example FGF2, expressed by the tumor cell. The signal transduction pathway by which G induces the tumor cell to express a plasminogen degrading protease, C can be modeled in terms of enzyme kinetics: $(1) G + Ra \leftrightarrow \{Ra G\} \rightarrow C + Ra$. The enzyme C (uPA, urokinase plasminogen activator) cleaves the matrix-associated plasminogen (Pg_g) by hydrolysis of certain peptide

bonds: (2) $C + P_g \leftrightarrow \{C:P_g\} \rightarrow C + P_g' + P_m$. This creates the protease plasmin (P_m) and other products of proteolysis (P_g'). Tumor cells also secrete the latent form of $TGF\beta$ (I_i), which is cleaved by plasmin to produce active form of $TGF\beta$ (I_a) via: (3) $I_i + P_m \leftrightarrow \{I_i:P_m\} \rightarrow I_a + L + P_m$. Here L denotes LAP, the latency associated peptide. $TGF\beta$ inhibits the production of uPA by blocking growth factor receptor signals in (4) $R_a + I_a \leftrightarrow R_i$, where R_i represents the inactive receptor complexed with I_a ($\{R_a:I_a\}$).

Overview of the biochemical pathway.

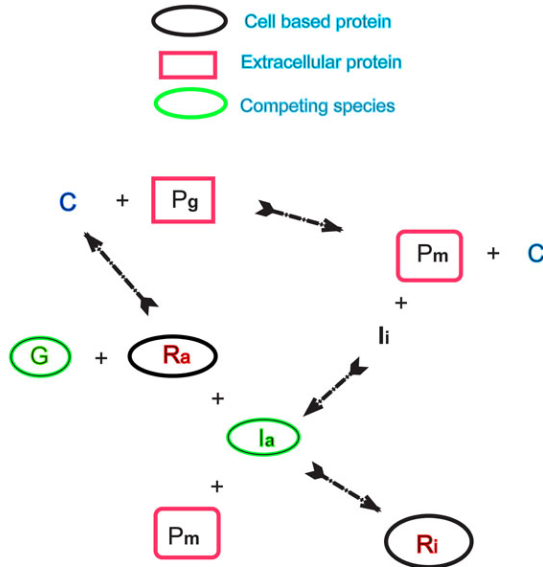


Figure 1. Overview of the biochemical pathway to regulate tumor dormancy. This wiring diagram shows that the active and inactive receptors (R_a and R_i) on tumor cells are mediated by active $TGF\beta$ (I_a) and how latent $TGF\beta$ (I_i) is activated by plasmin (P_m). Plasmin is released from plasminogen (P_g) by uPA from tumor cells. uPA is expressed by tumor cells in response to FGF (G). This is an example of positive-negative feedback.

species	notation	concentration
receptor	R	$[R]$
fibroblast growth factor, FGF	G	$[G]$
urokinase plasminogen activator, uPA	C	$[C]$
tissue growth factor beta $TGF\beta$	I_a	$[I_a]$
latent $TGF\beta$	I_i	$[I_i]$
plasminogen	P_g	$[P_g]$
plasmin	P_m	$[P_m]$

These four chemical equations constitute a positive-negative feedback loop (Fig. 1). More plasmin is created as more enzyme is produced from the matrix-associated plasminogen in response to growth factor. Plasmin cleaves more latent $TGF\beta$ (I_i) to produce active $TGF\beta$, which in turn inactivates cell receptors. As cell receptors are inactivated, there are fewer available to catalyze the first reaction, hence the production of enzyme falls and cleavage of the plasminogen to plasmin decreases. The lower plasmin concentration produces less active $TGF\beta$, the equilibrium in the fourth equation is driven further to the left and more receptors are returned from the inactive to the activated state.

$$\begin{aligned}
\partial_\tau G &= \mathcal{D}_G \Delta G - \mu_G G + \frac{\sigma_G - \Lambda_1 G}{1 + I_a + G} \mathcal{N}, \\
\partial_\tau C &= \mathcal{D}_c \Delta C - \mu_C C + \frac{\Lambda_1 G}{1 + I_a + G} \mathcal{N}, \\
\partial_\tau P_m &= \mathcal{D}_P \Delta P_m - \mu_P P_m + \Lambda_2 C, \\
\partial_\tau I_a &= \mathcal{D}_A \Delta I_a - \mu_A I_a + \Lambda_3 I_i P_m, \\
\partial_\tau I_i &= \mathcal{D}_i \Delta I_i - \mu_I I_i - \Lambda_3 I_i P_m + \frac{\sigma_I}{1 + I_a + G} \mathcal{N}, \\
\frac{\partial \mathcal{N}}{\partial \tau} &= \nabla \cdot \left\{ \mathcal{D}_\eta \nabla \left[\mathcal{N} \ln \left(\frac{\mathcal{N}}{\tau_1(G) \tau_2(I_i)} \right) \right] \right\} + \frac{\Lambda G}{\mathcal{K} + G} \mathcal{N} (1 - \mathcal{N}) - \mu_N \mathcal{N}.
\end{aligned}$$

Figure 2. Nondimensionalized partial differential equations for the tumor dormancy problem from which follow the system of 12 nonlinear ordinary differential equations for the compartment model (not shown). The resulting odes have no cell movement terms (unlike the last pde below). Notice that the activity coefficient in this pde is a function of both FGF and TGF β concentrations.

All these models require input of kinetic, diffusion and cell movement constants that describe the biological systems. This continues to be one of the greatest stumbling blocks to this type of modeling. Never the less, by using sensitivity experiments, as was done in [6,9], we were able to identify, within ranges, values for the various parameters for the models for which literature numbers are either not available or only available as in vitro values.

- **Mathematical modeling of phytoplankton:**

We introduced a mathematical model for the dynamics of the phytoplankton in the sea [1]. We then performed the analysis of the model [2, 3, and 5]. The work was part of a collaborative effort of mathematicians, biologists and experts of fisheries aiming at describing the growth and survival of the larvae of the anchovy of the Bay of Biscay, whose phytoplankton is the main food during the passive larval stage. The model focuses on the effect of the current (3D) and vertical mixing. Our method consists of describing these effects as the product of the horizontal current, which is solved along the characteristic lines, and the coupled action of vertical current and vertical diffusion, restricted on each characteristic line of the horizontal current. We show that the trivial steady state loses its stability and nontrivial (non-constant in space) steady state is created. In [5], we develop a multi layer method to solve a generalized case of a phytoplankton model introduced in [1]. It is treated by means of a sequence of approximations: the mixed layer is subdivided into a finite number of thin layers within each of which horizontal velocity can be considered constant with respect to depth. Existence, uniqueness and non negative solutions are investigated.

Consideration of nitrogen fixation adds a positive nonlinear feedback to plankton ecosystem models. We investigate the consequences of this feedback for secondary phytoplankton blooms and the response of phytoplankton dynamics to physical forcing [4]. The dynamics of phytoplankton, Trichodesmium (the nitrogen fixer), and nutrients is modeled with a system of three differential equations. The model includes two types of nonlinear interactions: the competition of phytoplankton and Trichodesmium for light, and the positive feedback resulting from Trichodesmium recycling. A typical simulation of the model in time, with forcing by a varying mixed-layer depth, reveals a clear successional sequence including a secondary or 'echo' bloom of the phytoplankton. We explain this sequence of events through the stability analysis of three different steady states of the model. Our analysis showed the existence of a critical biological parameter, the ratio of normalized growth rates, determining the occurrence of 'echo' blooms and the specific sequence of events following a physical perturbation. The interplay of positive and negative feedbacks appears essential to the timing and the type of events following such a perturbation.

II. Future Research.

- **Mathematical modeling of angiogenesis in living zebrafish embryos**

(collaborators: H.A Levine and J. Essner)

Angiogenesis is the process by which the body produces new blood vessels from an

existing vasculature. Angiogenesis is an extremely complex biological mechanism, occurring in various contexts, such as during placental growth, fetal development and wound healing. Angiogenesis is also the heart's first repair response to coronary occlusion and is an important factor for tumor growth and arthritis, as well as in many other pathological circumstances. The purpose of this proposal is to study angiogenesis in living zebrafish embryos from a biochemical/cell biological perspective. We start from the assumption that all biological processes are fundamentally biochemical in nature. Therefore we seek meaningful models that reflect this assumption to as great a degree as possible. While the proposal focuses on a relatively narrowly defined problem from development biology, *the broader impact* of the work is to develop modeling techniques and algorithms which will have a wider impact on the modeling and theoretical understanding of various aspects of development biology.

- **Mathematical modeling morphogenesis of the mammary gland**

(collaborator: M. Nilsen-Hamilton)

After birth, there are three main stages of morphogenesis of the mammary gland in the female. The first is during puberty when the epithelium grows into the mammary fat pad to form the familiar branched epithelial mammary tree. The second stage of morphogenesis is during pregnancy when the epithelial cells differentiate to form alveolar buds, which then become mature alveoli prior to lactation. The third stage of mammary gland morphogenesis in the adult is when the female has terminated lactation and the gland regresses during involution to regain a structure similar to the virgin mammary gland. These stages of mammary morphogenesis involve the biological and biochemical processes that drive differentiation and pattern formation are inherently kinetic in nature. These processes include the interaction of growth factors with their receptors that activate signaling pathways to regulate gene expression, receptors and protein degradation, the interactions between epithelial and mesenchymal cell types that are driven by receptor-ligand interactions, production and activity of proteases and protease interactions with their inhibitors. The result is a complex nonlinear system involving feedback and synergistic interactions that can be best understood through new theoretical approaches and numerical simulation.

- **Modeling Membrane Transporter Mechanisms**

(Collaborators: Hans Weinberger and Ed Yu)

The overall goal is to elucidate the fundamental mechanisms of membrane transporters, in a project that integrates mathematical modeling, biochemical and cell biology technique. The target of the work is a bacterial multidrug transporter that recognizes scores of structurally dissimilar toxic compounds and actively extrudes them from cells. The experimental data will be employed to model the concentration of drug accumulation in bacterial cells. This information will be directly applied to the use of antibiotics with enhanced efficiency for treating clinical infectious diseases.

References:

1. O. Arino, K. Boushaba and A. Boussouar, **Modelization of the role of currents and turbulence on the growth and dispersion of the marine phytoplankton**, Comptes Rendus de l'Académie des Sciences Life Sciences 323 (2000), 113-118.
2. O. Arino, K. Boushaba and A. Boussouar, **A mathematical model of the dynamics of the phytoplankton-nutrient system**, Journal of Non linear Analysis and Application, Real World Applications 1 (2000), 69-87.
3. O. Arino, K. Boushaba and A. Boussouar, **A mathematical model of phytoplankton**, Mathematical Models and Methods in Applied Science (M3AS) 12 (2002), no. 6, 871-901.
4. K. Boushaba and M. Pascual, **Dynamics of the 'echo' effect in a phytoplankton system with nitrogen fixation**, Bulletin of Mathematical Biology 67 (2005), no. 3, 487-507.
5. K. Boushaba, **A multi layer method applied to a model of phytoplankton**, Networks and Heterogeneous Media 2 (2007), no. 1, 37-54.
6. K. Boushaba, H. A. Levine, M. Nilsen-Hamilton, **A mathematical model for the regulation of tumor dormancy based on enzyme kinetics**, Bulletin of Mathematical Biology 68 (2006), no. 7, 1495-1526.
7. K. Boushaba H. A. Levine and M. Nilsen-Hamilton, **A Mathematical feasibility for the use of aptamers in chemotherapy and imaging**, Proceedings of the National Academy of Sciences, under review (2008)
8. V. DeGiorgi, D. Massai, G. Gerlini, F. Mannone, E. Quercioli, and P. Carli, **Immediate local and regional recurrence after the excision of a polypoid melanoma: tumor dormancy or tumor activation?** Derm. Surgery, 29 (2003), pp. 664--667.
9. J. P. Peters, K. Boushaba and M. Nilsen-Hamilton, **A mathematical model for fibroblast growth factor competition based on enzyme kinetics**, Mathematical Biosciences and Engineering 2 (2005), no. 4, 789-810.
10. B. Zetter, **Angiogenesis and tumor metastasis**, review Ann. Rev. Me., (1998), 49 pp. 407--22.

