MSB1004 Modelling Biosystems;

Assignment 2

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**SECTION 3 – 7 marks**

**a) Let us assume there is a genetic mutation that causes chemoresistance in the tumor cells (the drug does not kill them anymore). Describe how you would capture the variety in genetic mutation status in different tumor cells in an ABM model. Write some pseudo-code for your implementation of the genetic mutation status and its effect on tumor growth and chemotherapy. (4 marks)**

Environment

* 3D space /2D (if looking at different slices)
* Nutrients (Gradient; most on the edge and no nutrients in the core of tumor)
* Chemotherapeutic drug

Agents

* Tumor cells

States

* Proliferation rate
  + proliferation rate is higher further outward
* Non-chemoresistant
* Chemoresistant (due to mutation)
* Energy level

Rules

* Becoming chemoresistant due to chemoresistant mutation
* Non-resistant cells can be killed by drug
* Tumor cells gain energy when uptaking nutrients
* Both resistant and sensitive tumor cells can die when energy is 0
* Proliferate when energy > 10 → decrease energy by 5

Actions

* Die
* Proliferate
* Become resistant
* Absorb nutrients / increase energy level

The initial condition could be a random distribution of mutations in cancer cells due to the intratumor heterogeneity. These cancer cells initially have different proliferation rates and energy levels, and these values are also dependent on the amount of nutrients that can reach cancer cells (which affects the carrying capacity of tumor growth). When a normal tumor cell mutates, it will adopt the behavior of the mutated tumor cells. But only when it gains the chemoresistance mutation, it will develop the properties of resistant cells and can not be killed by the drug anymore. This mutation can be acquired during cell proliferation.

When the tumor is treated with chemotherapeutic drugs, incorporating stochasticity in the mutation process would result in a probability for the chemoresistance mutation. If this probability is higher than the threshold, this tumor cell will accumulate the chemoresistance mutation and become a resistant cell. If not, it will stay a sensitive cell and can be killed by drugs. Moreover, the selective pressure would favor the tumor cells which have accumulated the chemoresistance mutation. These resistant cells will not be killed by the chemotherapeutic drug and then proliferate rapidly to expand the resistant population. The ABM model will capture the heterogeneity in realistic tumor behavior, it is the difference in the number of resistant and sensitive cells over time. The imbalance between resistant population and sensitive population can affect the tumor growth and outcome of chemotherapy.

**Pseudo code for mutation:**

to gain-energy

if nutrient in radius

set energy energy + energy\_from\_nutrients

remove nutrient

to mutate

if resistance? false

If random number (0 to 1) > 0.95 ; *5% chance of mutation each tick is required to gain the chemoresistant mutation*

set resistance? true

to killed-by-chemo

if resistance? false

if drug in radius

if random number (0 to 1) > 0.5; *% if the death rate is higher than the threshold, this cell will be killed by drug*

tumor cell dies

remove drug

to proliferate

if cells < carrying\_capacity

if energy > 10

cell divides

set energy energy - 5

if resistance? true

new cell resistance? true

**More pseudo-code for model outline:**

%Set up

Set random mutation state of cancer cells from 0 to 1

Set random energy level of cancer cells from 0 to 20

Set random death state caused by drugs from 0 to 1

Set-default-shape turtles "circle 3"

%All initial cancer cells are sensitive cells

%Function of identifying resistant cells and sensitive cells

Check all sensitive cells

+/ If mutation state > 0.95 then this sensitive cell gains the chemoresistance mutation, the number of resistant cells is increased by 1 while the number of sensitive cells is decreased by 1

%Function of identifying cell proliferation and cell death

Check all cells

+/ If energy level > 10 and then this cell is divided into 2 cells and the energy level of new cells will be: previous energy level - 5.

If the previous cell is the sensitive cell, the number of sensitive cells is increased by 1. Otherwise, the number of resistant cells is increased by 1.

+/ If death state > 0.5 then this sensitive cell is killed by drugs, and the number of sensitive cells is decreased by 1. Or if energy level = 0, both resistant cells and sensitive cells can die due to the lack of nutrients.

%New death state

Check all cells

+/ If resistant cells, death state = 0

+/ If sensitive cells, death state = previous death state + random number (-1 to +1)\*0.01

If new death state < 0, set to 0

If new death state > 1, set to 1

%New mutation state

Check all sensitive cells

New mutation state = previous mutation state + random number (-1 to +1)\*0.01

If new mutation state < 0, set to 0

If new mutation state > 1, set to 1

%New energy level

Check all cells

New energy level = previous energy level + random number (0 to 1)\*5

If new energy level > 20, set to 20

**b) Describe a research question that you could answer with the above ABM that you**

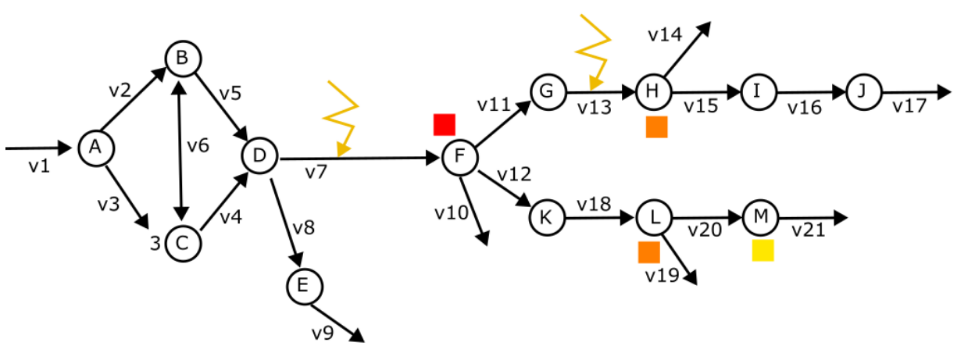
**could not answer with an ODE or PDE model. (3 marks)**

Research question: “How do chemotherapeutic drugs affect the heterogeneity in the spatial distribution of cancer cells and the treatment response?”

Description: When treating cancer cells with chemotherapeutic drugs, it can result in selective pressure to favor cancer cells that acquire the mutation that causes chemoresistance during proliferation, these cells are referred to as resistant cells. The tumor cells that cannot resist chemotherapeutic drugs are referred to as sensitive cells. The competition between the resistant population and sensitive population would determine the outcome of chemotherapy. For example, if the sensitive population gains favors in survival and proliferation, the tumor growth can be suppressed by drugs. Otherwise, the tumor still progresses due to the dominance of drug-resistance cells. One of the resources that cancer cells compete for is space, as it influences cellular survival, growth, and proliferation. Another resource that the cells will compete for is nutrients. Therefore, we hypothesize that chemotherapeutic drugs can lead to the appearance of resistant cells and sensitive cells which are different in spatial distribution and this heterogeneity of the tumor can be used to predict the treatment outcome.

**Why ODE and PDE models could not answer this question?**

The ODE model cannot answer this question because it cannot capture the spatial heterogeneity of cancer cell distribution. Therefore, an agent-based model with spatial information is more suitable for modeling the treatment response of cancer than a non-spatial model like ODE. Although PDE takes space into account when modeling the tumor dynamics and treatment response, it assumes that this system is homogeneous and cancer cell behaviors are deterministic. However, the chemotherapeutic-resistance mutation in resistant cells could be a stochastic element because it is randomly accumulated during cell division. Plus, even cancer cells with the same mutation status can respond differently to the therapy due to other stochastic factors, such as differences in the proliferation rate, drug uptake ability, metabolism, or epigenomes. Therefore, a deterministic model like PDE cannot capture the tumor behavior as realistically as the stochastic ABM. Finally, we need a multi-scale model that considers both space (cell distribution) and time (treatment response over time), and both cellular levels (mutation acquisition) and population levels (treatment response or drug resistance), thus making ABM more suitable to answer our research question, rather than ODE and PDE models.



**SECTION 4 – 20 marks**

**a) Take a look at the network. What happens under dark conditions? Which reactions will carry a flux to maximize the production of E? (3 marks)**

Under dark conditions, D can not be converted into F. Thus, all D is eventually converted into E and optimizes the production of E. The reactions v1, v3, v4, v8 will carry a flux to maximize the production of E. v9, v2, v6, v5 would have no flux if the goal is to maximize E. The bottom pathway producing 3C will yield more D so this is the preferred pathway for the objective of maximization of E.

**b) Fill in the stoichiometric matrix for this network. For simplicity, you can just enter the nonzero entries. (5 marks)**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
| A | +1 | -1 | -1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| B |  | +1 |  |  | -1 | -1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| C |  |  | +3 | -1 |  | +1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| D |  |  |  | +1 | +1 |  | -1 | -1 |  |  |  |  |  |  |  |  |  |  |  |  |  |
| E |  |  |  |  |  |  |  | +1 | -1 |  |  |  |  |  |  |  |  |  |  |  |  |
| **F** |  |  |  |  |  |  | +1 |  |  | -1 | -1 | -1 |  |  |  |  |  |  |  |  |  |
| G |  |  |  |  |  |  |  |  |  |  | +1 |  | -1 |  |  |  |  |  |  |  |  |
| **H** |  |  |  |  |  |  |  |  |  |  |  |  | +1 | -1 | -1 |  |  |  |  |  |  |
| I |  |  |  |  |  |  |  |  |  |  |  |  |  |  | +1 | -1 |  |  |  |  |  |
| J |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | +1 | -1 |  |  |  |  |
| K |  |  |  |  |  |  |  |  |  |  |  | +1 |  |  |  |  |  | -1 |  |  |  |
| **L** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | +1 | -1 | -1 |  |
| M |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | +1 | -1 |

**c) Consider the following constraints listed below. What is the maximal production of**

**carotenoids that you can achieve using these constraints? (7 marks)**

**Given:**

**upper bound: v1 = 10 mmol/(gDW\*h)**

**flux: v10 = 5 mmol/(gDW\*h)**

**v11 = v12**

**flux: v17 = 3 mmol/(gDW\*h)**

carotenoids (metabolites F (red), H (orange), L(orange), M(yellow)).

+/ Maximum production of F = 30 mmol/(gDW\*h)

To maximize the production of F, we should maximize the v1 to the upper bound = 10 mmol/(gDW\*h) to get as much material into the system as possible. The outflux of A should go to B or C and then go to D and F; however, to produce maximum of F, the flux of v3 would be maximized while the flux of v2 would be set as 0 because the bottom pathway has higher outflux of C and is thus the favorable pathway. This will all result in D being A\*3, which then all goes to F if v8 is 0; thus, the maximal influx of F would be 3\*10 = 30 mmol/(gDW\*h).

+/ Maximum production of H = 12.5 mmol/(gDW\*h)

Because the outflux for v10 (or the storage rate of F) is set at 5 mmol/(gDW\*h), a total flux of 25 mmol/(gDW\*h) stays in the system. Ultimately, the set flux of v10 at 5 means that no more than 5 mmol/(gDW\*h) can be stored. In this case, the total number of v11 and v12 is 25 mmol/(gDW\*h), v11 is equal to v12 so they can be 12.5 mmol/(gDW\*h).

F should be maximal for all cases because it is required for maxima of the other carotenoids. Given that 25 flux from F will stay in the system, v11 and v12 have to be 12.5 mmol/(gDW\*h), because they have to be equal. To maximize the production of H, v13 has to then be 12.5 mmol/(gDW\*h).

+/ Max production of L = 12.5 mmol/(gDW\*h)

The maximum of L production would be achieved by maximizing v18. v12 and v11 are equal and to maximize these pathways these fluxes have to both be 12.5 mmol/(gDW\*h). v18 should then also be 12.5 mmol/(gDW\*h).

+/ Max production of M = 12.5 mmol/(gDW\*h)

Because v18 is optimized to 12.5 mmol/(gDW\*h) to produce as highest L as possible, the total outflux of L is 12.5 mmol/(gDW\*h) and could be the storage of L or the production of M. To maximize the production of M, v20 should be maximized to 12.5 mmol/(gDW\*h) and v19 should be 0.

Taken together, after maximizing the production of all carotenoids, we acquire these following fluxes :

Production rate of F = v7 = 30 mmol/(gDW\*h).

Production rate of H = v13 = 12.5 mmol/(gDW\*h).

Production rate of L = v18 = 12.5 mmol/(gDW\*h).

Production rate of M = v20 = 12.5 mmol/(gDW\*h).

Thus, the maximal production rate of carotenoids should be: 30 + 12.5 + 12.5 + 12.5 = 67.5 mmol/(gDW\*h). It has to be noted that the products (carotenoids) are consumed in further reactions.

**d) Market research has indicated that the consumers would be really interested in a yellow variety of tomatoes. Without affecting any of the storage reactions (v10, v14, v19 and v21), suggest a reaction knockout (= target for genome editing) in the network that would maximize production of yellow pigments. How does this change the flux distribution throughout the network? Is this change sufficient to produce yellow tomatoes? (5 marks)**

**Under the constraints of C:**

If v11 is knocked out, L can maximally be 25 now instead of 12.5, since nothing goes to the top reaction anymore. The storage of red is however set at 5, so there will always be storage of red. This results in a storage of 25 for yellow as well, but because it is mixed with red it will result in orange.

Therefore it is impossible to generate a yellow color with a single knockout using the constraints from C. The storage of F (v10) should be set to 0, otherwise there will always be red interfering. This is however prevented by the constraint.

**Ignoring all constraints;**

Ignoring the constraints set in C, a yellow color could be produced if the objective would be maximizing v20 and knocking out v11. This would result in the only carotenoid that could be stored to be M (yellow). With the objective of maximizing v20, everything would stay in the system and be stored as M.

To achieve the yellow color, the only stored color should be yellow (M). This would mean that the products should either leave the system through v9, v17 or v21. Since the actual reaction rates are unknown, no definite conclusion can be made, but it seems very unlikely that there would be no storage of other carotenoids when only knocking out one reaction, in this case v11. Since the combination of orange and yellow carotenoids will result in orange tomatoes, it is not possible to achieve yellow tomatoes under these conditions.

The color is mostly dependent on the storage reactions, knocking out a single other reaction could only result in elimination of M (yellow) carotenoid storage if reaction rates are unknown.

**External discussion:**

If we only base on the given model and color combinations, the knockout of v11 reaction is not sufficient to produce yellow tomatoes. However, numerous studies have shown that the mutant tomatoes have yellow color due to the absence of carotenoids [1-3]. This recessive mutation of yellow-flesh in tomatoes leads to the elimination of fruit carotenoids by disrupting the activity of an enzyme called phytoene synthase, the first step in the carotenoids synthesis signaling pathway [2,3]. Thus, we assume that the knock-out of reaction v7 results in no carotenoids being produced through this pathway, which will confer yellow-color flesh in tomatoes.

**References**

1. Liu, L., Shao, Z., Zhang, M., & Wang, Q. (2015). Regulation of carotenoid metabolism in tomato. *Molecular plant*, *8*(1), 28-39.
2. Kachanovsky, D. E., Filler, S., Isaacson, T., & Hirschberg, J. (2012). Epistasis in tomato color mutations involves regulation of phytoene synthase 1 expression by cis-carotenoids. Proceedings of the National Academy of Sciences, 109(46), 19021-19026.
3. Gady, A. L., Vriezen, W. H., Van de Wal, M. H., Huang, P., Bovy, A. G., Visser, R. G., & Bachem, C. W. (2012). Induced point mutations in the phytoene synthase 1 gene cause differences in carotenoid content during tomato fruit ripening. *Molecular Breeding*, *29*, 801-812.

**SECTION 5 – 10 marks**

**Assume that a multi-scale model, coupling a constraint-based module with an agent-based module, is able to accurately determine which (combination) therapy is best based on patient-specific tumour characteristics that can easily be determined by routine clinical measurements. You want to bring this technology to the clinic. Write a short essay on which steps you would undertake to ensure the quality and reproducibility of the in silico models to get regulatory approval. (max 250 words) (10 marks)**

Using an *in silico* model to predict patient responses can be of great benefit to the medical field. However, given the high impact and the involvement of patients, it is crucial to ensure the model’s quality and reproducibility. To ensure that the model is credible and receive regulatory approval, it should adhere to various standards, such as those outlined in the ASME VV-40-2018 [1].

The ASME VV-40-2018 introduces the risk-informed credibility assessment framework, which has a multi step approach. Firstly, the context of use must be defined, which explains how the model should be used. This model could be used in clinical settings to predict the best therapy based on patient-specific tumour characteristics. Secondly, a risk analysis evaluates the model’s impact on clinical decisions. This step would help to identify incorrect predictions by the model, as this can lead to adverse outcomes for the patient. Thirdly, the model should be credible, this can be evaluated through verification and validation. Model verification checks whether the model is free from any errors and makes mathematical sense. Validation ensures that the outcomes predicted by the model align with real data. Here, clinical applicability should also be taken into account. To achieve this, a pilot study can be performed to make sure the model’s predictions are in line with the situations in the real world. Lastly, an uncertainty quantification should evaluate how the model deals with uncertainties and how these impact the model’s predictions. A sensitivity analysis can be used to evaluate this.

**References**

1. Viceconti, M. *e.a.* In silico trials: Verification, validation and uncertainty quantification of predictive models used in the regulatory evaluation of biomedical products. *Methods* **185**, 120–127 (2020).