MODELING MOTION OF CONTAMINANT BAP IN CYTOPLASM

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ABSTRACT

The response of cells to contaminant stressors like nicotine is of great importance for human health. The focus of the project is to model the way of the contaminant until the entrance of the nucleus. Therefore, in the first step the cell culture surrounded by the fluorescent contaminant is imaged by a laser microscope. Filters and contour extracting algorithms are used to extract the cell geometry. Finally the movement of the contaminant is modeled using reaction-diffusion-equations and random-walk-processes. The long term goal of the project is to understand the influence of contaminant molecules on biological cell functions.

1. INTRODUCTION

Our first step is to model the motion of the contaminant Benzo[a]pyrene BaP within the cytoplasm.
It is well known that a fraction of contaminants is able to react with receptors (AhR) and the motion of larger particles like this complexes is slowed up compared to unbound contaminants. [1] Further, the flow of a receptor-bounded complex is more directed towards the nucleus of the cell than the molecules which are unbounded. [2] So we model two kinds of motion: the normal diffusion via Random-Walk-Process and the directed diffusion via Random-Walk-Process with drift. [3]
It is assumed that there is an equilibrium of the bounded and the unbounded fraction. This means that the association and dissociation rates have to be modeled as well.
The reason of modeling the motion of contaminants is that we need all parameters which describe the behaviour of our substance BaP. In practice, you have the possibility to accomplish FRAP experiments to get all these parameters. [4, 5] At a later date we want to compare our model-parameters with the calculated ones out of the FRAP data. At that time we don't have such data, so we simulate FRAP experiments with different input values and analyse the results we get.

2. MATERIALS AND METHODS

We describe now the steps of modeling: motion of unbounded and bounded particles, association and dissociation rates at equilibrium and FRAP experiments.

2.1. Motion of unbounded Contaminants

The motion of a free particle is modeled by a Random-Walk-Process.
Let the position of the particle at a given time t be (x_t, y_t). The particle jumps within 1 timestep 1 or -1 unit in x-direction and in y-direction. This yields 4 possible positions of the particle after 1 timestep:

$$(x_{t+1}, y_{t+1}) = \begin{cases} (x_t - 1, y_t - 1) \\ (x_t - 1, y_t + 1) \\ (x_t + 1, y_t - 1) \\ (x_t + 1, y_t + 1) \end{cases}$$

The probability of the incidence is the same for every point.

$$P\left[(x_{t+1}, y_{t+1}) = (x_t \pm 1, y_t \pm 1)\right] = \frac{1}{4} \qquad (1)$$

2.2. Motion of bounded Contaminants

The motion of a bounded particle is modeled by a Random-Walk-Process with drift. This means that the compound have a preferred direction.
Let the preferred direction be a point (x_{Dir}, y_{Dir}) and the position of the particle at a given time t be (x_t, y_t). The possible positions of the compound are the same as in section 2.1, the probabilities on the other hand are different. A step in the preferred direction is more probable than a step in the opposite direction. Let the probability of a jump in preferred direction be $p, p \geq \frac{1}{2}$. This yields the probabilities of the 2 possible positions in x-direction:

$$P[x_{t+1} = x_t + s_t] = p \qquad (2a)$$
$$P[x_{t+1} = x_t - s_t] = 1 - p \qquad (2b)$$

whereas:

$$s_t = \text{Sign}[x_{Dir} - x_t] = \begin{cases} 1 & , x_{Dir} > x_t \\ 0 & , x_{Dir} = x_t \\ -1 & , x_{Dir} < x_t \end{cases}$$

The same equations apply to y-direction as well.

2.3. Association and Dissociation

Note, that compounds can unbind and free particles can be bind.

Let B_t the fraction of bounded particles at time t and F_t the fraction of free particles at time t.
The rate of unbinding molecules k_{off} per timestep describes this process of dissociation. On the other hand the parameter k_{on}, which specifies the rate of new bounded molecules per timestep, characterises the process of association.
Now, we can calculate the fractions of the different particles at time $t+1$ out of the fractions at the time t:

$$B_{t+1} = B_t + k_{on} \cdot F_t - k_{off} \cdot B_t \quad (3a)$$
$$F_{t+1} = F_t - k_{on} \cdot F_t + k_{off} \cdot B_t \quad (3b)$$

We assume an equilibrium of free and bounded particles at initial time $t = 0$. The equilibrium situation yields:

$$B_t = const. \quad \forall t \geq 0 \quad (4a)$$
$$F_t = const. \quad \forall t \geq 0 \quad (4b)$$

Making use of the equations (4) equations (3) are simplified:

$$\frac{k_{on}}{k_{off}} = \frac{B_t}{F_t} \quad (5)$$

Futher, we assume that the sum of bounded and unbounded fraction is 1. This yields:

$$B_t = \frac{k_{on}}{k_{on} + k_{off}} \quad (6a)$$
$$F_t = \frac{k_{off}}{k_{on} + k_{off}} \quad (6b)$$

The relationship between dissociation rate and mean binding time BT is well known [4]:

$$k_{off} = \frac{1}{BT} \quad (7)$$

Equation (7) and equation (5) yields :

$$k_{on} = \frac{B_t}{BT \cdot (1 - B_t)} \quad (8)$$

2.4. Simulation of FRAP experiments

As we show in the sections above, we only need a few input parameters to simulate the motion of the contaminant molecules.
First, to guarantee the equilibrium situation during the whole simulation, we need

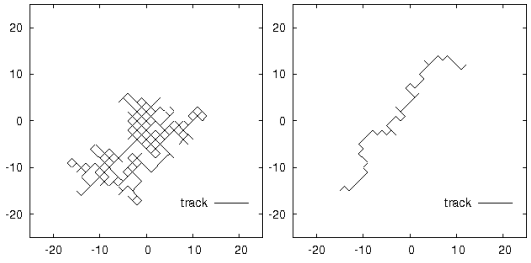
- the mean binding time BT and the fraction of bounded particles B_t or
- the association rate k_{on} and the dissociation rate k_{off}

We choose the first possibility.
Second, we need a direction (x_{Dir}, y_{Dir}) and a probability p for the Random-Walk motion with drift. In the case of modeling contaminants the preferred direction of bounded particles is the position of nucleus. So we modeled the nucleus as a circle with centre (x_{Dir}, y_{Dir}) and radius r_{Dir}.
In case of a particle enters the nucleus we modeled two different kinds of behavior. We assume on the one hand that only bounded particles can be captured by the nucleus and on the other hand that all (bounded and free) particles are captured by the nucleus.
As an application of the motion-model we simulate Fluorescence Recovery After Photobleaching (FRAP) experiments. FRAP is a method of the confocal laser scanning microscopy (cLSM). You are able to assign parameters of diffusion and binding by these experiments. The proceeding of FRAP is to bleach fluorescent particles irreversible within a bleaching spot. Afterwards you monitor the recovery of fluorescent molecules from the outer part of the bleaching spot. They enter the bleaching spot by their motion.
So, we define the radius of a circular bleaching spot r_{Spot} and the centre of the spot $(x_{Spot}, y_{Spot}) = (0, 0)$ as well as the size of the monitored square area a.
For simulation we initialize the model with the number of tracked particles $Samples$, the time steps of simulation $TimeSteps$ and the number of simulations $SimSteps$ we used to create an average recovery.

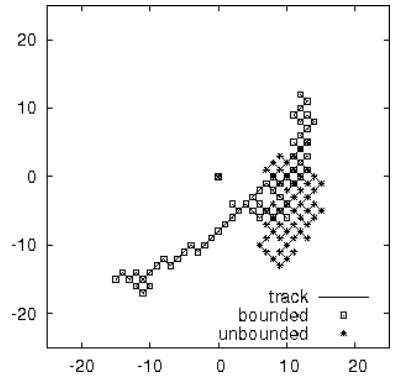
3. RESULTS

3.1. Motion of unbounded und bounded Contaminants

The motion of unbounded contaminants is a diffusion process. A track of on particle is shown in Figure 1(a). On the other hand the motion of contaminants which are bounded by another particle modeled as a diffusion with a drift as you can see in Figure 1(b).



(a) free particle (b) bounded particle



(c) free and bounded particle

Figure 1. track of single particles

3.2. Association and Dissociation

The next step is to integrate the fact that unbounded contaminants can be bounded and the other way around. Now the track is a combination of directed and undirected walk as you can see in Figure 1(c).

3.3. Simulation of a FRAP experiment

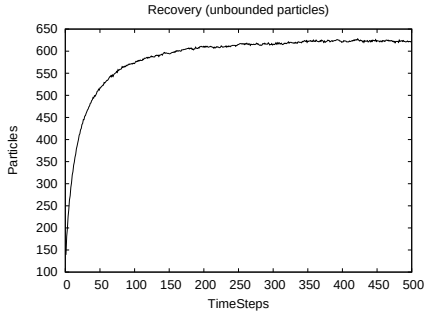
The following simulations are set up with the same parameters unless otherwise noted (see Table 1).

Samples	# of particles	20000
TimeSteps	# of simulated time steps	2000
SimSteps	# of Simulations for averaging	100
a	length of square monitored area	50
r_{Spot}	radius of bleaching spot	5
x_{Dir}	x-coord. of nucleus	15
y_{Dir}	y-coord. of nucleus	15
r_{Dir}	radius of nucleus	5
p	prob. of jump to nucleus	0.55

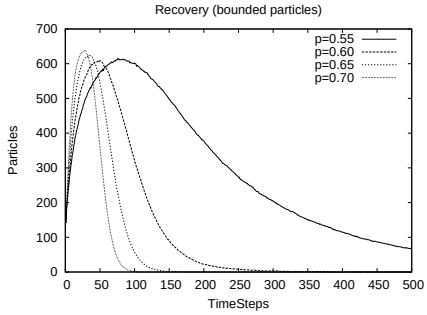
Table 1. simulation parameters

First, we simulate a FRAP experiment with particles which are unbounded, walk undirected and can not enter the nucleus (see Figure 2(a)).

Second, we simulate several FRAP experiments of particles which are bounded and walk with a drift (see Figure 2(b)). We vary the probabilities of a jump into the direction of the nucleus. Note, particles that enter the nucleus are captured in this case.



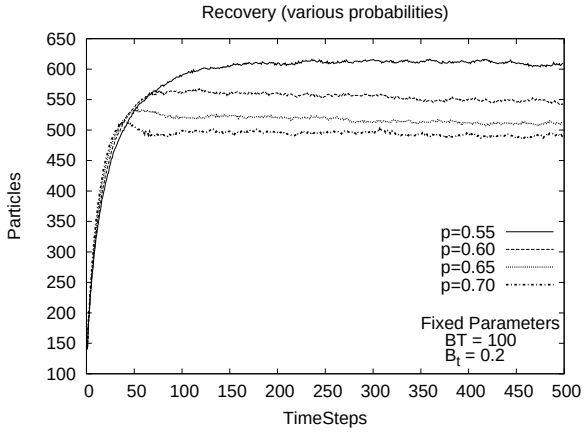
(a) only unbounded particles



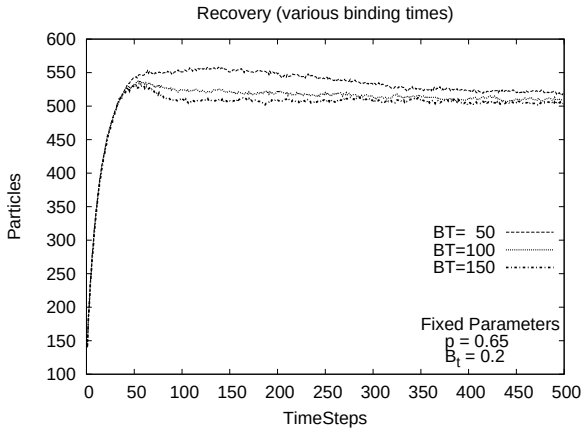
(b) only bounded particles

Figure 2. FRAP simulations

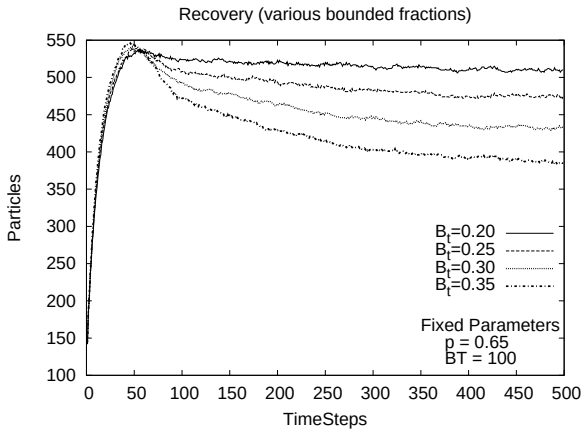
Third, we simulate three different types of FRAP experiments by varying the parameters of the probability of a jump towards the preferred direction p, the mean time of binding BT and the bounded fraction B_t. On the one hand we assumed that only the bounded fraction can be captured by the nucleus (Figure 3) and on the other hand all particles can be capured by the nucleus (Figure 4).



(a) different probabilities

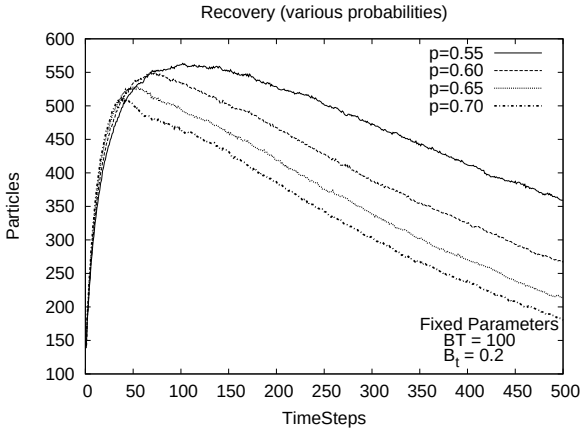


(b) different binding times

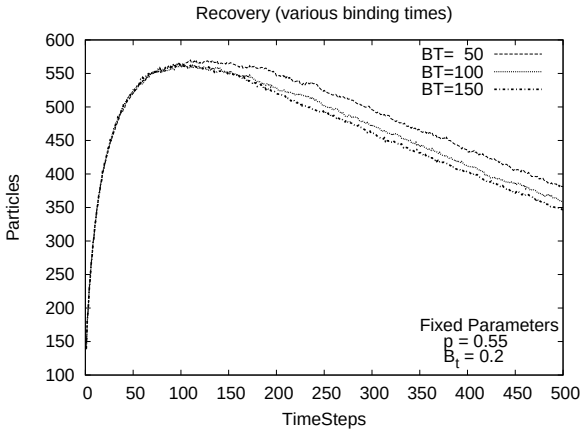


(c) different bounded fractions

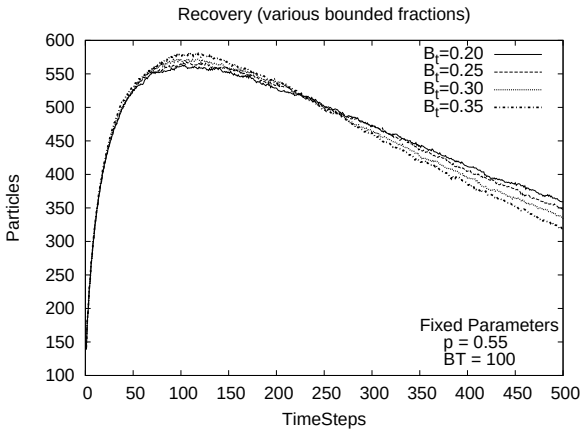
Figure 3. FRAP simulations (capture of bounded particles)



(a) different probabilities



(b) different binding times



(c) different bounded fractions

Figure 4. FRAP simulations (capture of all particles)

4. DISCUSSION AND FUTURE WORK

The influences on the recovery of FRAP experiments of directed particle movement and the possibility of particle capture in cell membranes are rarely described in the literature. A standard figure found in the literature is Figure 2(a) which correspondends in our simulation to unbounded particle movement. The recovery converges towards a non-zero value because no particle sink like capturing by the nucleus is modeled. In contrast, particle capture by the cell nucleus causes a zero limit in the recovery as displayed in Figure 2(b).
Further, the recovery is fastened and shifted to smaller values

1. by definition of a higher probability value for steps towards the sink (Figure 3(a), 4(a))
2. by definition of a higher mean binding time (Figure 3(b), 4(b))
3. by definition of a higher fraction of bounded particles (Figure 3(c), 4(c))

In the future we plan to derive an analytical solution for the FRAP recovery to change this qualitive conclusions into quantitive. This solution will allow to infer all parameters which describe the diffusion and binding processes from real FRAP data. Therefor different diffusion coefficients have to be modeled in a next step.

5. REFERENCES

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[4] B. L. Sprague, R. L. Pego, D. A. Stavreva, and J. G. McNally, "Analysis of binding reaction by fluorescence recovery after photobleaching," *Biophysical Journal*, vol. 86, pp. 3473–3495, June 2004.

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