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### Agent based modeling of viral infections: an investigation across several spatio-temporal scales

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## 2. Modeling HIV-1 Intracellular Replication: two simulation approaches

### 2.1 Introduction

In the field of virology and infectious diseases, mathematical and computational models are developed to provide a better understanding of the underlying biological phenomena and medical processes. In recent years, many computational models have been developed to investigate the complexity of HIV dynamics, immune system response, and drug therapy [1-5]. Simulation of HIV infection can vary from modeling the entire immune response (under virus infection and drug therapy) [30] to simulating the intracellular processes at a molecular level. A concrete example of this wide-range multi-scale modeling is *HIV Decision Support: From Molecule to Man* by Sloot et al. (2009) [6, 7]. Despite the wide range of modeling for HIV dynamics, more and more attention is being paid toward single cell analysis to understand intracellular processes.

At cellular and molecular level different modeling techniques, from deterministic to stochastic [12] and from mathematical to agent-based models [8, 9, 11], have been used to simulate the intracellular process of viral replication. A mathematical model by Reddy et al. (1999) [8] was used to study intracellular kinetics of HIV-1 replication. Reddy's model is based on a system of coupled ordinary differential equations (ODEs) that is solved numerically. Each sub-process of the viral replication cycle is represented by one or more equations. The model provides concentration-based insights on how the overall replication cycle depends on its constituent reactions. Although mathematical models are able to represent the overall behavior of the system, a disadvantage is that they are less helpful in capturing the individual interactions of the system components, ignoring spatial and topological dependencies. On the other hand Agent-Based Simulation (ABS), an emerging field in modeling microbiological phenomena, has been widely used in modeling at cellular and molecular levels [3, 9, 13, 14, 15]. In ABS cells and molecules are modeled as individual agents and the complex behavior of the system emerges from their

interactions. A model using ABS principles was CellAK by Webb et al. (2004) [9]. It includes cellular details such as membrane with lipid bilayers, substrate molecules and enzymes with reaction rules and metabolic pathways. The cellular entities are modeled as agents and the cell behavior emerges from the interaction between agents. Although agent-based modeling has advantages in modeling biological and microbiological systems, such models are computationally more expensive than ODE mathematical models. This problem is becoming less important nowadays with fast supercomputers and distributed computing. In modeling HIV infection, agent-based models are generally large-scale and no intracellular agent-based model exists that specifically concerns HIV.

In this paper, we have modeled the HIV-1 intracellular replication and the virus kinetics inside the cell. The rest of this article is organized as follows: In Section 2 we describe the HIV replication process and present the general model design. Section 3 introduces the two simulation approaches used for implementation. Section 4 presents the simulation results, which are comparable with the experimental data. Section 5 is the conclusions and future work.

## 2.2 Modeling HIV-1 intracellular replication

Despite having a simple structure, HIV has a complex dynamics inside the cell. HIV replication first requires the virus to enter an uninfected host cell such as CD4<sup>+</sup> cells or macrophages. After the virus enters the cell, it utilizes the cell machinery for replication and release of new virions from the cell surface. Figure 1 (a), taken from *Gene Therapy Journal* in ref. [31], illustrates the intra-cellular replication process of HIV-1 from entering the cell to releasing new virus particles from the cell. For simplicity, we have only simulated the major steps that are critical in the viral replication process. Figure 1 (b) shows the general model design and the main replication steps in the model marked in red. The following 7 steps are considered in the model: Reverse transcription, nuclear transport, integration, cell activation, mRNA transcription, transport to the cytoplasm and translation.

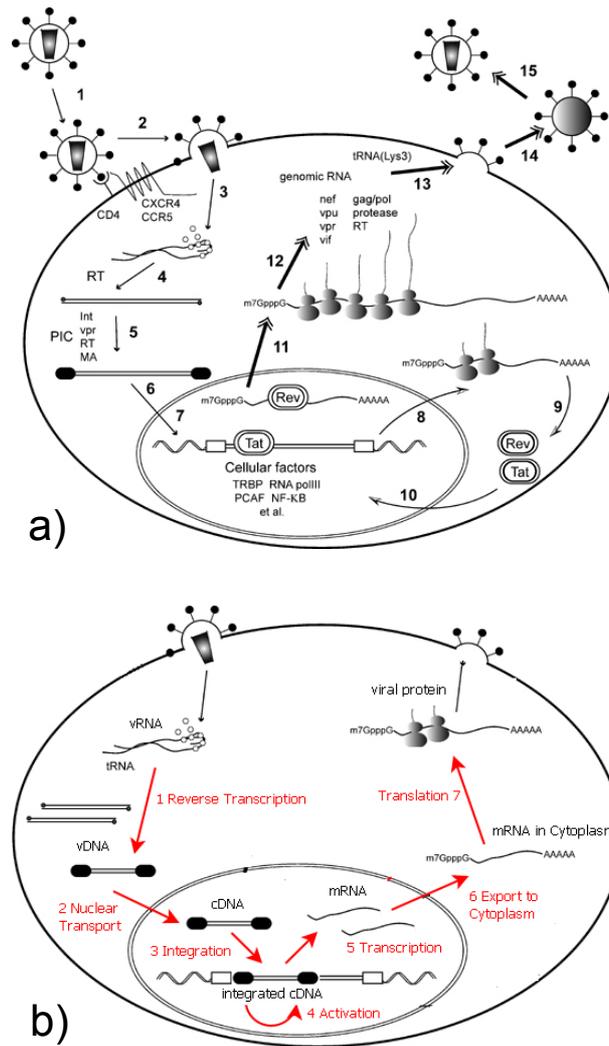


Figure 1. (a) HIV intracellular replication cycle: Entry (Binding to CD4 receptor (1), Co-receptor binding (2), Fusion (3)), Reverse transcription(4,5), Transfer to the nucleus(6), Integration (7), Transcription and regulation(8,9,10), Export to the cytoplasm (11), Translation (12), Assembly, budding and maturation(13,14,15) [31]; (b) 7 main steps of viral replication in the model.

Virus entry to the host cell is not simulated in our model and initiation of infection is modeled as a stochastic process explained in Section 2.1. The cell is modeled as an individual entity with certain states and properties. Over the course of simulation time, we assume the cell to be non-dividing and non-interacting. In what follows we describe the cell infection and different cell states.

### 2.2.1 Cell infection

Cell entry is an important part of the HIV host infection and is target of many drug treatments. In our simulation we decided not to model the entry in detail because a simple description was not enough to represent such a complex mechanism and we preferred to investigate this aspect in a later study [33]. Moreover, the experimental data used to validate the model is based on quantitative methods that use multiplicity of infection (MOI) as a measure of infection. MOI is the ratio of virus particles to cells or the average number of virus particles per cell. In real experiments the MOI is determined by dividing the number of viruses per *ml* by the number of cells per *ml*. Therefore, we used the MOI as a measure of infection, rather than measuring the percentage of infected cells by explicitly modeling the cell entry, until we have the chance to access data with details on the entry process. Cell infection is thus simulated as a stochastic process. For each run the cell may get infected (with 1 or more viruses) or remain uninfected based on the MOI. Although the MOI represents the average number of viruses per cell, the specific number of viruses that infect any given cell follows a Poisson distribution [16]. In a population, the proportion of cells infected by a specific number of viruses is  $P(n)$ ,

$$P(n) = \frac{m^n \cdot e^{-m}}{n!} \quad (1)$$

where  $m$  is the MOI and  $n$  is the specific number of viruses infected the cell. For example if  $m = 1$ , a higher proportion of cells will get infected with one virus or will not get infected at all ( $n = 0$ ), while some cells may get infected with two or more viruses. We have used this approach to substitute the entry process and initiating the cell

infection. The value of MOI is an input to the model and is specified at the start of the simulation.

### 2.2.2 Cell states and transitions

The life cycle of HIV inside the cell is arbitrarily divided into two distinct phases: The early phase refers to the steps of replication from cell entry to integration of viral cDNA into the cell DNA, whereas the late phase refers to the post-integration steps from cell activation and mRNA transcription to budding and release of new viruses from the cell [18]. In this model we have defined 6 states for the cell and each cell has associated a specific state at a time. At each time point the cell can be in one of the following states: Uninfected ( $T_{UI}$ ), early infected ( $T_{EI}$ ), latently infected ( $T_{LI}$ ), and actively infected ( $T_{AI}$ ), productively infected ( $T_{PI}$ ), and dead ( $T_D$ ).  $T_{UI}$  refers to a healthy cell,  $T_{EI}$  is an infected cell before the viral genome is integrated into its DNA,  $T_{LI}$  is the state of a cell which has an integrated provirus in its genome.  $T_{AI}$  is the state of an activated cell which produces new viral mRNAs transcripts, and  $T_{PI}$  is the state of an activated cell that starts to produce new viral particles from its surface. Hence, the state of the cell may change during its lifespan. Figure 2 shows the state transitions of a cell during the viral replication process.

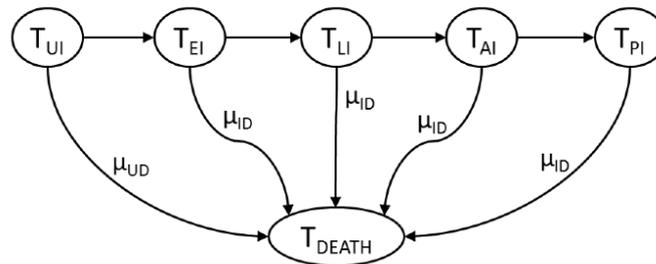


Figure 2. Cell internal states and transitions

The death rate of the cell might change during its lifespan. It is possible that the infected cell death rate is low initially and increases

during cell state transitions. However, the dependency on cell-death rate on the cell age is still not clear [17]. So, we assumed the death rate of the cell to be independent of its age. Hence, the rate of cell dying is stochastic for an individual cell and constant over a population of cells. An Uninfected cell dies with a constant rate  $\mu_{UD} = 0.03$  per day, while an infected cell dies with a higher rate of  $\mu_{ID} = 0.5$  per day [18]. The death rates are rescaled to the time step of the simulation and used as a probability for stochastic cell death.

### 2.3 Model implementation: two different approaches

Two simulation approaches have been used to implement the virus replication model: *Rate-based approach* and *diffusion-based approach*. In the rate-based approach the quantities inside the cell are defined as a set of variables and their rates of change are inferred from literature. On the other hand, the diffusion-based approach defines the internal state of the cell by simulating the molecular quantities inside the cell as agents. The movement of these agents is described by random walk and events occur based on rules defined upon collision between agents. Cell infection and viral replication are stochastic processes and are modeled as such in both approaches. The simulation is time-driven in both approaches and the update scheme is by time advance. The simulation time advances uniformly by a constant time-step at each iteration. We chose a time step of 0.2 minute (12 seconds) in the simulation. This time is small enough to capture the level of detail we are looking at and long enough for completion of occurring chemical reactions such as DNA synthesis and transcription.

#### 2.3.1 Stochastic rate-based approach

In the stochastic rate-based approach the cell is an individual entity and the intracellular quantities are a set of variables known as internal variables. These variables are used to determine the cell state and keep track of the molecular quantities inside the cell. The cell internal variables are: VRNA (viral RNA), VDNA (viral DNA in both cytoplasm and nucleus), PROVIRUS (integrated cDNA), MRNA

(mRNA transcripts), and VP (translated viral protein). At the start of the simulations all variables are set to 0 and if a cell gets infected the number of viral RNAs infecting it is assigned to VRNA. The value of each internal variable changes based on its current value and its rate of change. Figure 3 shows the structure of a single cell. Squares are the internal variables and arrows are their rates of change. An incoming arrow shows an increase in the variable value, while an outgoing arrow shows a decrease.

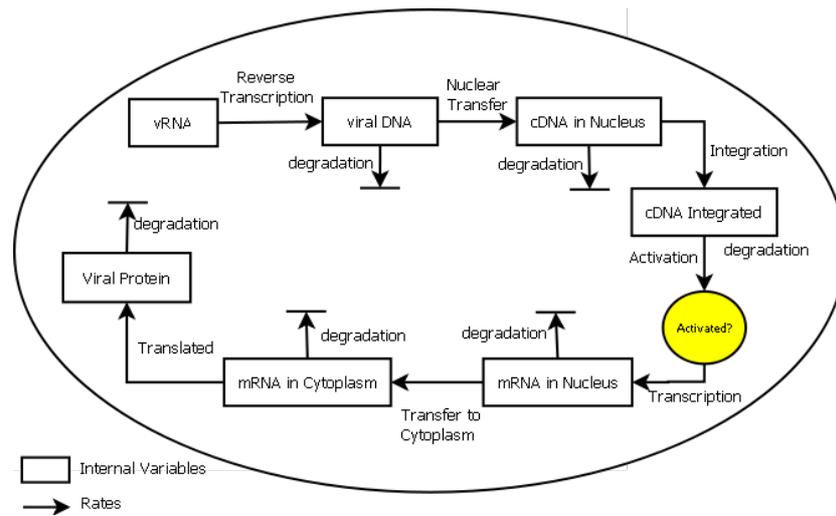


Figure 3. Cell structure in the rate-based approach. Squares are the cell internal variables and arrows are their rates of change. An incoming arrow shows an increase in the variable value, while an outgoing arrow shows a decrease.

Different steps in viral replication occur with different rates. These rates are inferred from literature and listed in Table 1. The rates of major steps in HIV replication are: Reverse transcription, nuclear transport, integration, activation, mRNA transcription, exporting to

cytoplasm and translation rates. Each rate is rescaled to the time step of the simulation and is used as a probability for stochastic occurrence of events. As soon as the cell is infected ( $T_{EI}$ ) the reverse transcription process occurs with the average rate of  $k_{rt}$  nucleotides per minute where the length of HIV RNA component is 9479 nucleotides [19]. The reverse transcription process mainly consists of 3 steps: First synthesis of the first-strand DNA from the viral RNA, next synthesis of the second-strand DNA and finally the full-length double-strand DNA formation which leads to viral RNA degradation. We have considered these 3 steps of reverse transcription by creation of the first and the second-strand DNAs in our model which together form up a full-length viral DNA. Nuclear transfer rate,  $k_{transfer}$ , is the rate by which the viral DNA produced by reverse transcription transfers to the nucleus. The viral DNA in the nucleus is integrated into the host genome, degraded or circulated. DNA circularization is neglected in our model because it is very limited [28]. The viral DNA in the nucleus is integrated into the host cell DNA with rate  $k_{integration}$  and the cell becomes latently infected ( $T_{LI}$ ). A latently infected cell may become activated with an activation rate of  $k_{activation} = 3 \times 10^{-3}$  per day. Activation of the cell is important for a latently infected cell to produce infected mRNA molecules by transcription and marks the passage from early stages of infection to the late stages.

In the previous model of HIV intracellular replication by Reddy et al. [8] all cells were considered to be in an activated state at the time of infection, whereas in our model we make a distinction between activated ( $T_{AI}$ ) and inactivated ( $T_{LI}$ ) cells. At every time step we check for the cell activation. As soon as the cell is activated the transcription process is initiated, otherwise no transcription of viral genes occurs in the infected cell. mRNA transcription is obtained by cellular factors and cell activation and there are three major classes of transcripts: unspliced, singly spliced, and multi-spliced [20]. In our simulation, we are not modeling the splicing event of mRNA transcripts and only consider the full-length mRNAs. Splicing plays regulatory roles and is required for efficient mRNA transcription. It basically adds a delay to the process of transcription, which is balanced in our simulation by the

time delay added for stochastic cell activation. The cell produces mRNA transcripts at every time step with an average transcription rate of  $k_{transcription}$ . The viral mRNA transcripts are exported to the cell cytoplasm by the export rate  $k_{export}$ . In the cytoplasm the mRNA transcripts are translated to viral proteins with translation rate  $k_{translation}$ . The list of rates and parameters used in this approach is summarized in Table 1.

Table 1. Parameters

Parameters	Value	Reference
Reverse transcription rate	165 nucleotides per minute	[21]
Activation rate	$3 \times 10^{-3}$ per day	[22]
Integration rate	4.5 copies per hour	[8]
Transcription rate	1000 copies per hour	[8]
Translation rate	262 proteins per hour	[8]
Export rate to the cytoplasm	2.6 copies per hour	[8]
Transfer rate to the nucleus	0.012 copies per hour	[10]
Half life of mRNA	0.2 per hour	[8]
Elimination rate of proteins	$1.4 \times 10^{-5}$ proteins per second	[23]
Death rate of uninfected T cell ( $\mu_{UD}$ )	0.03 per day	[18]
Death rate of infected T cell ( $\mu_{ID}$ )	0.5 per day	[18]

The modeling flowchart of the virus intracellular replication cycle (in the rate-based approach) is presented in Figure 4. At the start of the simulation cell gets infected randomly based on MOI. The function Replication is executed at each iteration in the simulation. Details of

the replication function are shown in the flowchart with a remark that the function checks the occurrence of an event at each step by taking a random number between 0 and 1. The event occurs if the random number is within the range of the event rate, otherwise it goes to the next step.

The advantage of this approach in comparison to ODE models is first the stochasticity of the model which appears at every step of virus replication. Also cells in the model are considered as individual entities and by looking into these individual cells one can obtain a better understanding of the dynamics of virus replication process at a single cell level.

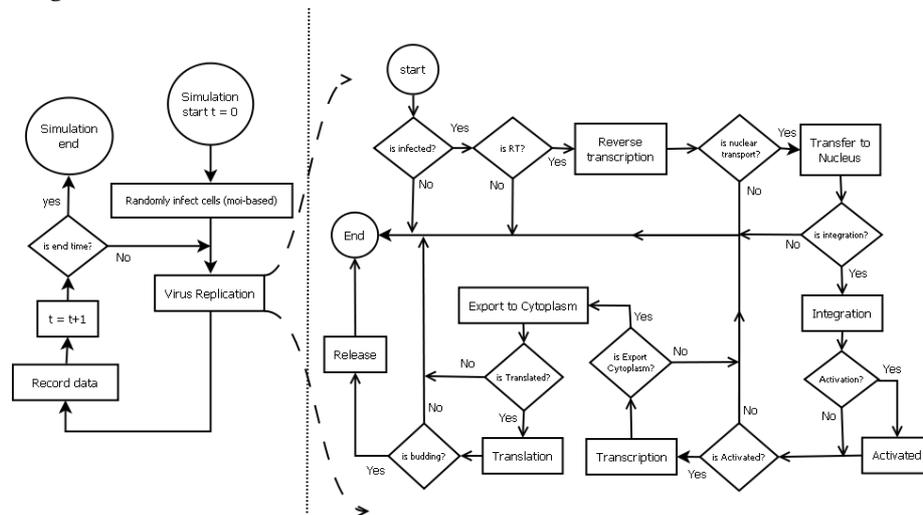


Figure 4. Flowchart of the rate-based model showing the simulation of HIV intracellular replication.

### 2.3.2 Stochastic diffusion-based approach

In the diffusion-based approach we are simulating the HIV intracellular replication based on the movement of particles inside the cell. The cell is an individual entity in the model and includes the cellular and viral compartments, which have a significant role in the viral replication cycle. The cellular compartments are: the cell DNA,

nucleus, cytoplasm (cell area) and tRNA (transfer RNA), while the viral compartments are viral RNA, viral DNA, viral mRNA and viral proteins. We have defined a 2-dimensional grid of 100×100 for placing the cell. The position of the cell is assumed to be constant during the simulation run. The compartments inside the cell are modeled as agents with some properties and behaviors such as position, size and movement. The agents in the model are as follows:

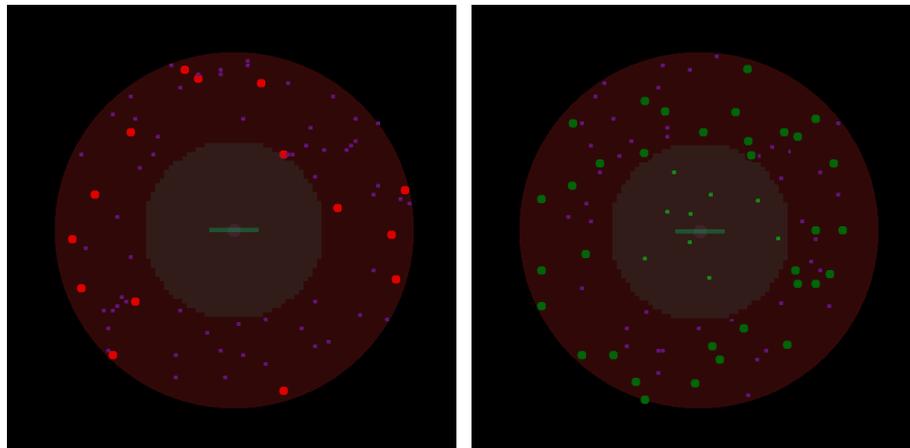
- Cellular nucleus: The cell nucleus has a size and a fixed position in the center of the cell.
- Cellular DNA: The cell DNA has a size and a fixed position in the center of the cellular nucleus.
- Cellular tRNA: The cell tRNA is randomly positioned inside the cell cytoplasm. It binds to the primary binding site (PBS) of the viral RNA and act as a primer for initiation of reverse transcription process.
- Viral RNA: HIV contains 2 viral RNAs which are released inside the cell cytoplasm after infection. Viral RNA randomly moves in the cytoplasm area of the cell.
- Viral DNA: Viral DNA is the product of reverse transcription process. Viral DNA randomly moves inside the cell area (both the cytoplasm and the nucleus). The viral DNAs inside the nucleus are identified as viral cDNA.
- Integrated DNA: The viral DNA which is transferred to the nucleus and is integrated into the host cell DNA.
- Viral mRNA: Viral mRNA is produced in the nucleus and can be exported to the cytoplasm. mRNAs randomly move in both nucleus and cytoplasm areas of the cell.
- Viral protein: Viral proteins are produced in the cytoplasm by translation of viral mRNAs.

At each time step the moving agents can move one grid point, either left, right, up or down, within the cell area or remain in the same grid position (Finite size effects are not studied). The movement of viral particles inside the cell is known to be based on both diffusion and

moving along microtubules [24]. Movement along microtubules leads to a faster transfer of particle toward the cell nucleus. In this simulation we are modeling the movement of particles only based on the diffusion and the diffusion coefficient of particles is tuned to compensate this assumption in the model. The tuned value for the diffusion coefficient is  $0.010415 \mu\text{m}^2/\text{sec}$ , which is approximately 50 times bigger than the largest diffusion coefficient known for HIV particles inside the human 293T cells ( $50\text{-}220\text{nm}^2/\text{sec}$ ) [29]. The advantage of this approach is that we take into account the spatial effects of the cell which cannot be represented in the rate-based model. We chose the cell radius equal to 40 grid points (*gp*). The average T cell radius is between 4 to  $4.5\mu\text{m}$  [25]. Considering the cell size  $4\mu\text{m}$  each grid point would be  $0.1\mu\text{m}$ . In T lymphocytes the diameter of the cell is approximately twice that of its nucleus. Hence, we used the value of 2 for the cell to nucleus size ratio [25, 26], so the nucleus radius would be 20 gp. If the length of T cell DNA is  $0.6\mu\text{m}$ , given that each grid point is  $0.1 \mu\text{m}$ , the DNA length would be 6 gp. The amount of tRNA in the cell is a high value (198000 per cell [25]) however, not all the tRNAs are available to the virus, as a primer for reverse transcription, and a large proportion is used by the cell machinery. Hence, the initial value of the tRNA is used as a free parameter in the model. At the start of simulation, the cell randomly gets infected based on cell infection model (Section 2.1). Then the cell and agents are assigned to the simulation grid. At each time step every moving agent performs a random movement over the grid area and checks for collision. Events in the model occur upon collision between agents and collisions will be processed by the agent-collision rules listed in Table 2.

Table 2. Agent Collision Rules

IF COLLISION (agent1-agent2)	THEN (event)	Rules
vRNA-tRNA	Reverse transcription	Produce vDNA, and vRNA degradation
vDNA-cell membrane	nucleus transfer	vDNA transfers from the cytoplasm to the nucleus (cDNA)
cDNA – cell DNA	Integration	Produce integrated provirus and reduce cDNA
mRNA – cell membrane	nucleus export to cytoplasm	mRNA export from the nucleus to the cytoplasm



(a)

(b)

Figure 5. (a) Simulation visualization of early stages of the simulation viral RNAs (red) are assigned to the cell cytoplasm environment. The purple circles are the cellular tRNA available in the cell (b) Simulation visualization of late stages of simulation: viral mRNA (small green) mainly in the cell nucleus, and translated viral proteins (big green) in the cell cytoplasm. The purple circles are the cellular tRNA available in the cell

Cell activation, transcription and translation occurs with the same rates and conditions explained in the rate-based approach. We have considered these rates constant over time. Figure 5 a shows a snapshot of the model visualization at the early stages of the simulation. The visualized entities are: the cell, the nucleus and the cellular DNA which are all positioned on the center of the grid, while viral RNAs (red) and cellular tRNAs (purple) are randomly positioned in the cell cytoplasmic area. Figure 5 b shows a snapshot of the model at the late stages of the simulation where viral mRNA (small green) is transcribed from the cell DNA inside the nucleus and viral proteins (big green) are translated in the cell cytoplasm.

The model is implemented in Java programming language for both approaches. We have used MASON (A Multi-Agent Simulation library core in Java [27]) libraries and 2D visualization tools in our simulation. The result data of each cell at each run are recorded in a Hash Table at certain time points. These times can be changed at start of the simulation according to the interest of the experiment. The data saved in the history files are used later for analyzing the results in MATLAB.

## 2.4 Simulation results

Although we modeled the intracellular processes at a single cell level, we are analyzing the results over a population of cells. This is closer to what happens in in-vivo and in-vitro experiments, where measurements are an average over all the cells in a volume of blood or on a petri dish. In order to compare our model results with the experimental data we run the simulation for several populations of cells and average over these populations. At each run we compute the first replication cycle up to 72 hours post-infection, corresponding to 3 days, which is approximately 12 hours more than the average life-span of an infected cell. In what follows we will discuss the simulation results of both approaches described in Section 3.1.

The initial number of cells in the simulation is set to  $10^3$  which is the average number of cells in a *ml* of blood [1]. Integration of viral DNA into the host cell DNA is a key step in the virus replication cycle and if

it doesn't occur, the virus is incapable of fully replicating and producing new virions. In experiments conducted by Scott et al. [28] detection of integrated DNA was accomplished using fluorescence-monitored PCR. They used the  $MOI = 0.4$  in their experiments and limited their measurements to a single cycle of HIV replication. We used the same  $MOI$  value in our simulation which indicates that there is on average 0.4 viruses per cell. The simulation results of integrated cDNA are shown in Figure 6. The graph in 6 a is a result of the rate-based approach, while 6 b refers to the diffusion-based approach. The blue (solid lines) line is the average amount of integrated cDNA per cell over time and the red (dashed lines) lines are the standard deviation of the output. Error bars show the experimental data that are measured in 12, 24, 48, and 72 hours post-infection [28]. The concentration of the integrated cDNA first rises, reaches a plateau and then slowly decreases. The decrease in the number of integrated cDNA reflects the death of infected cells. The integrated cDNA is also detected earlier in the simulation results comparing to the experimental data. This difference is explained by the limited sensitivity in detection and accurate quantification of integrated cDNA in PCR experiments [32]. The simulation results of integrated cDNA are consistent with the experimental data in [28]. However, there is an over estimation in the first 36 hours of the diffusion-based approach, which is due to the assumptions made on the diffusion of particles inside the cell. The particles inside an infected cell are in different sizes and have different diffusion coefficients, but we have assumed the same diffusion coefficient for all moving particles. Although this assumption influences the final biological results of the diffusion-based approach, we are fixing it by improving the missing data values. Nonetheless, the modeling approach would still be the same.

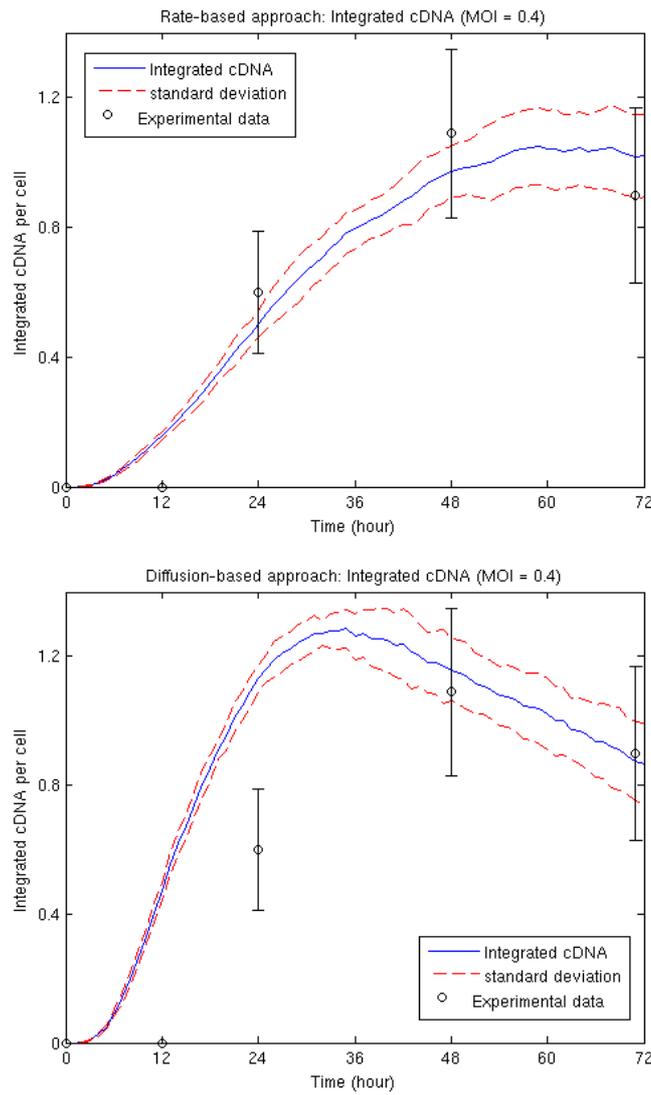


Figure 6. Simulation results of integrated cDNA per cell at MOI = 0.4 (average over 10 simulation runs of 1000 cells); (a) Rate-based approach; (b) Diffusion-based approach

Viral mRNA is the product of transcription process. Once the cell is activated, the transcription process starts. We have measured the

average copies of mRNA transcripts per cell produced over 1000 cells at different MOIs and compared it with experimental data on genomic HIV-1 mRNA molecules. In experiments conducted by Barbosa et al. [10], mRNA of two groups of patients with different viral loads was measured. The patients in the first group had a high viral load and patients in the second group had a low viral load. Accordingly, we choose a range for MOI from 0.01 to 10 and run the simulation for certain values in this range. Then we plot the average amount of mRNA transcripts produced after 72 hours in each run versus the value of MOI in that run (Figure 7 a). The y-axis is the amount of mRNA transcripts produced per cell (72 hours post-infection) and x-axis is the MOI range. Figure 4 a is the results of the rate-based approach and each data point is an average over 1000 cells. The red bars show the range of mRNA produced per cell in the two groups of patients with high (solid bar) and low (dashed bar) viral loads. From these data points we observed that the simulation results of viral mRNA produced per cell is within the same range ( $1.0 \times 10^{-2}$ - $1.0 \times 10^2$  genomes per cell) as the data collected from patients in experiment [10]. Figure 7 b illustrates the trend of viral mRNA production over time for certain MOI values in the rate-based approach. We can see that as the value of MOI increases, the cell activation and transcription processes start earlier and more mRNA transcripts are produced.

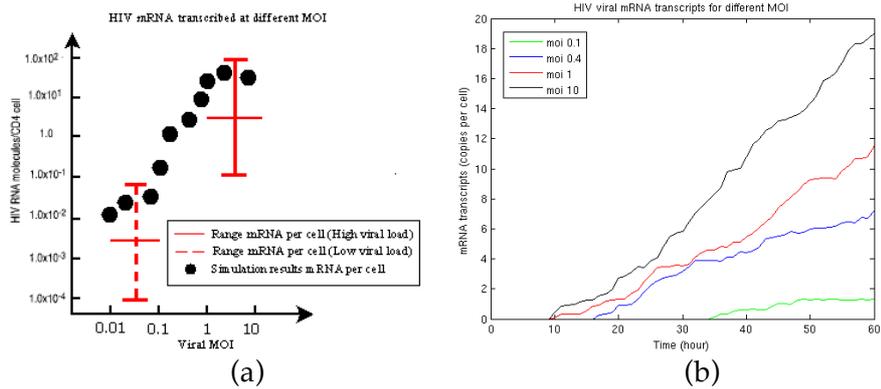


Figure 7. (a) Number of HIV mRNA molecules per cell (y-axis) at different MOIs (x-axis). Each data point is an average over 1000 cells. (b) Trend of mRNA production over time for different MOI values. Simulation results mRNA per cell

## 2.5 Conclusions

We presented a model of HIV-1 intracellular replication, where infected cells undergo a single cycle of virus replication. The cell is an individual entity and cell infection is modelled by a stochastic process. The model keeps track of the main viral proteins and genetic materials inside the cell during the virus replication. Using this model we analyzed each step of the viral replication cycle from reverse transcription to translation. Two simulation approaches were used for implementing the model: rate-based approach and diffusion-based approach. In the rate-based approach the intracellular dynamics of the cell is based on the rates that are inferred from literature, while in the diffusion-based approach the dynamics is based on the modelling intracellular compartments as diffusing agents. Therefore, in the diffusion-based approach the spatial information of the cell is taken into account. The simulation results on the amount of cDNA integrated into the host DNA and the number of mRNA transcripts produced per cell were consistent with the experimental data.

Both simulation approaches are stochastic and by looking at individual cells we get a more realistic description of the dynamics inside the cell. This is a more precise description of the in-vivo and in-vitro experiments compared to the large-scale deterministic models in the literature. For future directions the model can be adapted or extended in both single-cell level and population level. The diffusion-based approach can be extended at the single cell level to focus more on the behavior of the cell under viral replication. A more detailed description of the replication processes such as the movement of particles along the microtubules or the regulatory effects of mRNA splicing will allow a better understanding of the intracellular mechanisms. At a population level the rate-based approach can be extended to simulate re-infection and interaction between cells such as cell-cell transmission of the virus. So far, our model focuses on the first round of replication, while it can be enhanced to have a second, third or multiple rounds of replication. Both approaches in modeling HIV intracellular replication as a whole can be used for investigating the effect of various HIV inhibitors or new drug agents on the replication process. Effect of various drugs can be modeled either in the form of rates or real agents. This will help to identify the efficiency of drugs acting at different stages of virus replication.

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