Stochastic π simulations of several RNA-interference models and its efficacy to impede HIV-1 virus

Muskan Kukreja[1,2]

Biodesign Institute
Arizona State University,
Tempe, AZ , USA 85287

Manish K Gupta[3]

Laboratory of Natural Information Processing
Dhirubhai Ambani Institute of Information and Communication Technology (DA-IICT),
Gandhinagar, India 382007

Abstract

RNA-interference has been considered as a promising anti-viral treatment. Many mathematical models have been proposed for RNA-i mechanism and HIV-1 separately, however, little work is known from systems biology approach. On the other hand recently stochastic π calculus has been widely used to model complex biological process to understand them at systems level but not much has been done to successfully inhibit HIV-1 using RNA-i. In this work, using stochastic π calculus, we have modelled pathway of si-RNA transfection at several stages of HIV-1 replication cycle in order to prevent HIV-1 escape from RNA-i. In particular, we have considered transfection of CD4, pre-sliced mRNA virus, viral genes such as TAT, NEF and GAG for more effective inhibition and also p24 si-RNA for efficient prevention of integration and reconstruction of HIV-1 progeny.

Keywords: Anti-viral, RNA-i, HIV-1, Systems Biology, AIDS, si-RNA, π-calculus, stochastic, virus

1 Introduction

Acquired Immune Deficiency Syndrome (AIDS) has been widely known as killer epidemics caused by the HIV-1 virus since 1981. Many approaches have been considered in the literature to fight this virus including techniques such as machine learning, mathematical modelling and RNA-interference (RNA-i) [10] [14] [20] [9].

[1] The authors would like to thank Luca Cardelli (Microsoft Research, UK) for useful suggestions in stochastic π calculus modelling. The work of the first author was done while he was at the Laboratory of Natural Information Processing, Dhirubhai Ambani Institute of Information and Communication Technology (DA-IICT), Gandhinagar, India 382007

[2] Email: mkukreja@asu.edu

[3] Email: mankg@computer.org

This paper is electronically published in
Electronic Notes in Theoretical Computer Science
URL: www.elsevier.nl/locate/entcs

Though RNA interference techniques is quite successful for gene deactivation it was found later that virus can escape the RNA-i [3] [22]. Many mathematical models have been proposed in understanding the complex pathway of RNA-interference [8] [9]. In this paper, we consider a systems biology approach towards this problem. Systems biology can be used to understand the biological components and their interactions in biological process [11] [6] [13]. This includes developing models that create understanding of the functions and behavior of biological systems. One of the popular candidate for modelling is stochastic π calculus which has been widely used to model complex biological process to understand them at systems level. In particular, HIV-1 population dynamics have been modelled by Kukreja and Gupta [12] and Agarwal, Mishra and Gupta [1] in stochastic π calculus. In this work, we consider inhibition of HIV-1 virus by RNA-interference mechanism through stochastic π calculus. We show that this process can inhibit HIV-1 replication and potentially act as powerful anti-retroviral therapy. The first step in modelling this pathway is to understand the RNA silencing mechanism at systems level. The modelling is done in stochastic π calculus and simulations are done in stochastic π machine. The rates of reactions of these models are taken from the literature [2] [9].

To achieve this, we have first considered different models of RNA-interference mechanism through stochastic π calculus and verified important results. We have also developed viral replication model and anti-viral silencing model to show that RNA-interference can inhibit the viral response. Using this, we finally modelled the complex pathway of si-RNA transfection at various stages of HIV-1 replication cycle to show that continuous transfection can downregulate the HIV-1 viral response.

The paper is organized as follows. Section 2 gives a short introduction to stochastic π calculus. The section 3 gives a brief introduction to RNA-interference technology and also discusses the modelling of the RNA silencing process. Section 4 is about modelling the viral replication to show that virus replicates in a silencing system and Section 5 is about anti-viral silencing which can inhibit the viral response. Section 6 of the paper gives introduction to HIV-1 virion structure and its genome and section 7 deals with our final model of the mechanism of RNA-interference inhibition of HIV-1 virus. The paper concludes with section 8.

2 Stochastic π Calculus

Process calculi are widely used in computer science for describing interactions between simultaneously running processes. In 1992, Robin Milner [15] developed a formal language for concurrent computational processes, such as mobile telephone systems, known as the π-calculus. It is an expressive formal language for describing systems of concurrent actors and their interactions. Many variants of π -calculus have been developed since then. In 1995, Corrado Priami [4] developed a stochastic extension of π-calculus, known as the stochastic π-calculus. This added a rate r to every activity in the system modelled. The stochastic rate and the associated exponential distribution which are attached to every reaction describes the stochastic behavior of that activity. These rates are used by the stochastic simulation algorithm which calculates the probability of all possible reactions at each step and

stochastically choose the next step reaction based on this probabilities. One special feature of π calculus is, it allows dynamic communication topology which can be used to model mobility. It is this feature which makes it a perfect representation of biological transaction [19] [13]. Here molecules are modelled as process, interaction capability as channels, interaction as communication, modification as state change [19] [13]. The advantage of using stochastic π-calculus for modelling of these or any other biological system is that biological systems are very complex and probabilistic in nature and thus highly unpredictable. It helps us in taking into account the complexity, concurrency and probabilistic nature of these systems. For further details on this calculus the reader is referred to the excellent references such as [17] [15] [6].

3 RNA-Interference

In this section, first we describe the basics of RNA-interference followed by a basic stochastic model. Finally we extend it to many segment model. Ever since the discovery of RNA interference, it is considered as a common denominator for several post transcriptional gene silencing process induced by long double stranded RNA molecule (ds-RNA). It is based on the defence mechanism developed within the species to fight against foreign invaders [7]. RNA interference works inside every species by generating a specific response against the foreign elements. They amplify these responses to fight against this threat. RNA silencing mechanism also has a risk of mounting certain undesirable responses which work against itself. It also has specific immune response against viruses. It does it by identifying the non self elements or genes and by generating the target immune response against invaders and finally amplifying this response.

For the proper functioning of the RNA-interference, the major challenge occurs in recognizing the self and non self genes. Some of the induced responses can be proved boom to us but in parallel there can also be response which might deter our goal. In the amplification process, there is an accidental production of antisense transcript which can lead to the formation of double strand RNA (ds-RNA) which can in turn trigger the silencing mechanism [2].

3.1 Basic Biological Model

In this basic model, we have considered the simple RNA silencing pathway developed earlier [2]. The long double strand RNA molecule is cleaved by enzyme dicer. These small pieces generally of $22 - 30$ nucleotides are known as short interfering RNA's (si-RNA). These are actually the key mediators. This si-RNA act as a template for RNA-induced silencing complex (RISC). These RISC target to m-RNA to form the m-RNA RISC complex in short we call them **complex**. There is an amplification of ds-RNA molecules as some part of this complex forms into ds-RNA molecule and some part gets degraded. See Figure 1.

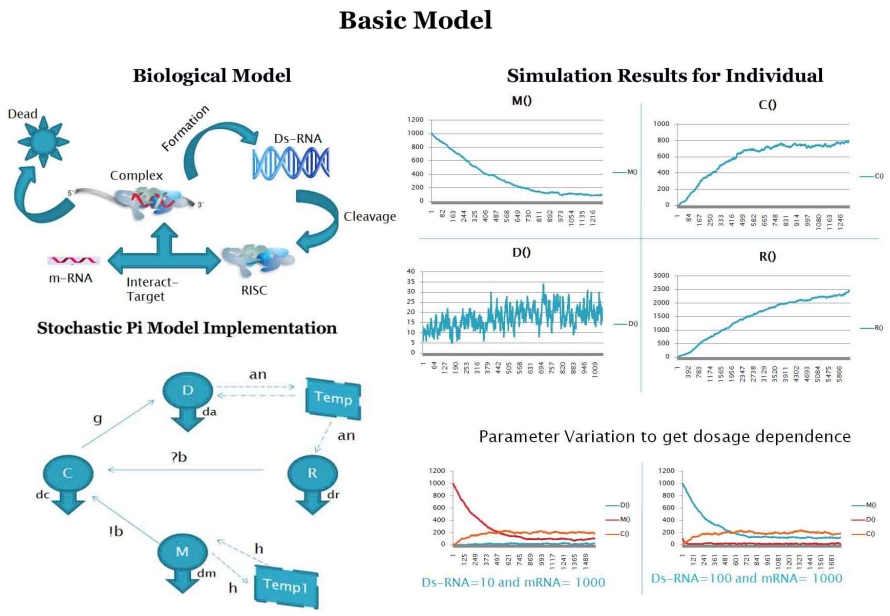

Fig. 1. Stochastic π modelling and simulation of RNA silencing process [2]

3.2 Stochastic π Calculus Modelling

In order to verify the issue discussed above regarding the RNA silencing process, there is a need to model this biological phenomenon. For the modelling purpose we have chosen stochastic π-calculus, to check the capability of the model to amplify its response and identity non self and self genes thereby limiting the responses against itself. The D, R, M and C (ds-RNA molecule; RISC, m-RNA and complex) are considered as process. Here process are represented as nodes and down arrows as decay rates of process. Edges represent the interaction and transformation of one process into another. ? represent input from the channel and ! represent output of the channel. The ds-RNA molecule cleaves with rate an to form RISC complex where n the number of segments of small interfering molecules formed or decays with rate da. Temp is imaginary process used to pump the values of D into R constantly while maintaining the values of D as constant. Similarly, R takes the input from channel b and M gives the output at the same channel to give C. Finally m-RNA molecule M increases with rate of h and decay at rate dm. The new complex formed C either forms D at rate g or decays with rate dc. The rates of the reactions have been taken from the literature [2].

3.3 Simulation Results

In this modelling, we have considered that silencing occurs starting with some initial inoculum of ds-RNA. After performing simulations for small and large amount of ds-RNA(10-100) we have seen the following observations

- There is sudden degradation of ds-RNA concentration
- Rapid increase in RISC complex concentration
- There is a amplification of the response, after initial decay of ds-RNA. It reaches to a steady state but still managed to continue the very high RISC concentration.

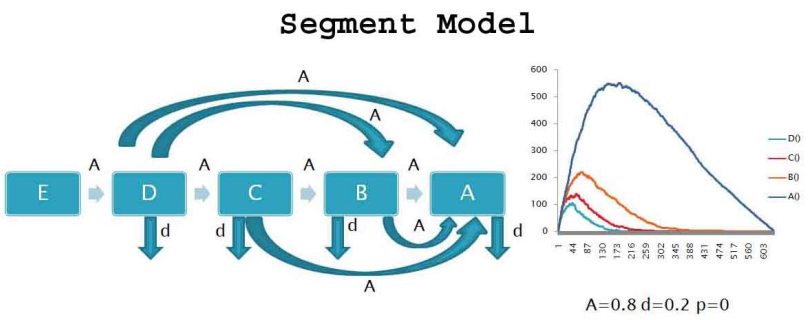

Fig. 2. In this segment model there is an amplification of target m-RNA to many folds. The segment A being in upstream shows the highest number. Due to imbalance silencing reaction dies

- There is decrease of m-RNA concentration because of the silencing process.

3.4 Drawbacks and Conclusion of Basic Model

There is no dosage dependence seen in the simulation, any amount of ds-RNA would lead to equal level of silencing process and steady state. This dosage dependence is seen experimentally as large amount of ds-RNA is quite a good indicator of the presence of non and self genes. For detail analysis and simulation of basic model see Figure 1.

This model can generate specific immune response rapidly but fails to shut down any responses that occur by accident. The main drawback of the model is that accidental responses are also amplified and are not shut until the self transcripts are also removed. Secondly the system is not working to recognize between self and non self genes. A small mistake would be amplified and cause a permanent silencing mechanism. Since this model fails to recognize the self genes, there is a need to develop a new model. We have tried to build a stochastic π calculus model for the number of si-RNA molecules and tried to show that upstream molecule leads its follower in terms of concentration during the amplification. As described earlier [2] there is a unidirectional amplification of ds-RNA molecule from 3' to 5' prime direction. We have built the model for 5 segments $A, \ldots, E$. For the purpose of modelling we would assume that amplification process is starting from segment D. Every segment can amplify into its next segment with a rate A and do not amplify with a rate d or do nothing with rate p.

Using stochastic π calculus we have verified that number of segments from downstream to upstream are sub exponential and not exponential. The stochastic π segment model and parameter variation for the values of A, d and p can be found in supplement material available at http://www.guptalab.org/mainweb/publication.html. The model and simulation results are given in Figure 2. Our final motive is to observe how the change in distribution of si-RNA population affects the overall dynamics of silencing. Unidirectional amplification has shown the path to down regulate the self responses that are generated accidently.

Here, we have developed a model that takes into account for various segments of ds-RNA molecules and their corresponding RISC complex (R) and complex (C). We have built stochastic π calculus model for 5 segments but since the model is

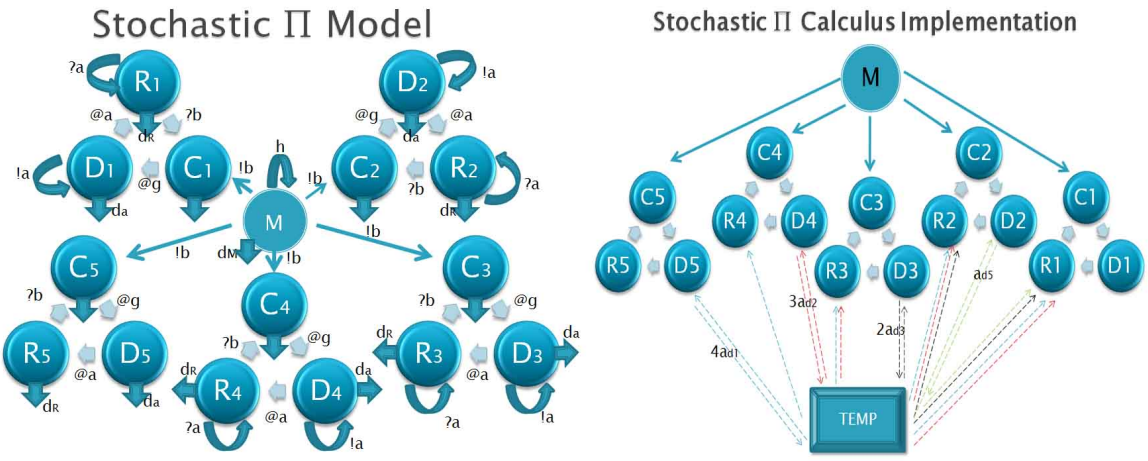

Fig. 3. Stochastic π-calculus model for 5 segments

symmetric, around M(), it can be further extended for n segments easily by adding corresponding D_{n+1}, C_{n+1} and R_{n+1} . For sake of simplicity and analysis we have shown the results for $2, 4, 5$ and 10 segments (see supplement material for $2, 4$ and 10 segments). The model and simulation for 5 segments is shown in Figure 3 and Figure 4.

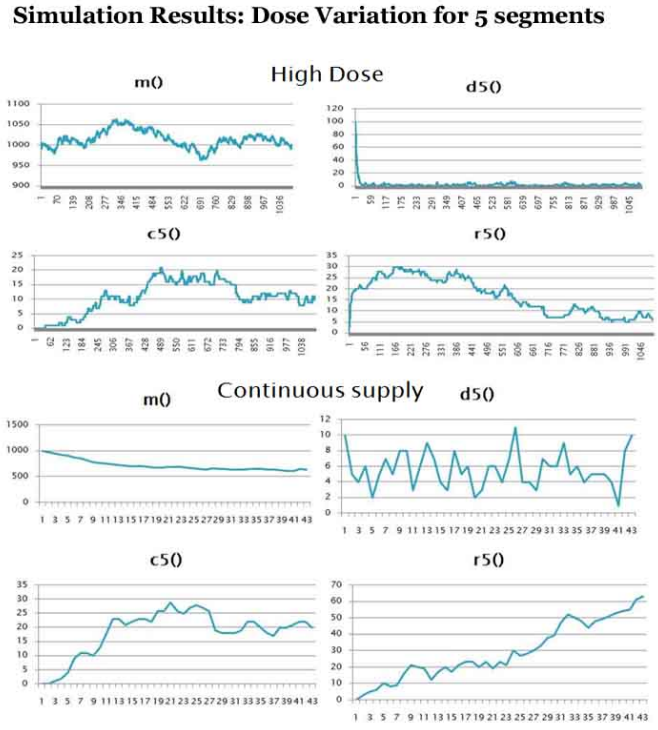

Fig. 4. Simulation results for 5 segments for high value of ds-RNA(100) and continuous supply of ds-RNA

The model we developed for 5 segments is an extension of our basic model,

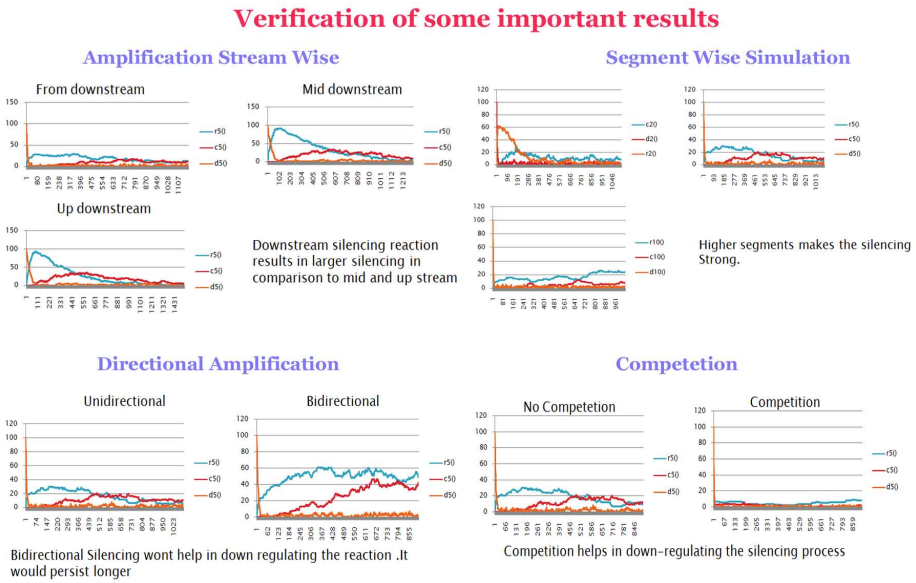

Fig. 5. Simulation results involving bidirectional, stream wise amplification, segment variation and competition

difference lies in the fact that for each si-RNA segment corresponding to $A, \ldots, E$, we have $D_1, \ldots, D_5$; $R_1, \ldots, R_5$ and $C_1, \ldots, C_5$. Since there is a unidirectional amplification the concentration of upstream segment A is contributed by all $n-1$ D_i's. For this case all D_1 to D_5 contribute in formation of R_1 and D_2 to D_5 in formation of R_2 and so on. Each D_i can die at rate of da or transform into corresponding R_i with rate of a. Each R_i can take input from channel b and M can give the corresponding output at channel b to give complex C_i. Each complex C_i can either form D_i with rate g or die at rate of dc. The Temp is imaginary process for pumping the values of corresponding D_i's that are responsible for formation of R_i's.

3.5 Analysis

Taking the unidirectional amplification into account, we observe the change in our results compared to our basic model. The ds-RNA initiates the reaction but even the higher amount is unable to continue the silencing reaction. This means for continuous silencing response there should be continuous input supply of ds-RNA. The reaction is persistent as long as there is continuous input. This system amplifies the response but also limit the self accidental response. We can control the silencing process with the external supply of ds-RNA input. The rates of the reaction have been taken from literature [2].

3.6 Verification of some important results

Some trivial manipulations on this model, we found that bidirectional nature of amplification process does not down regulate self response, increasing the number of segments, competition among the si-RNA and RISC molecules would end the silenc-

ing process more quickly and downstream amplification results in higher silencing reaction. Simulation results are given in Figure 5.

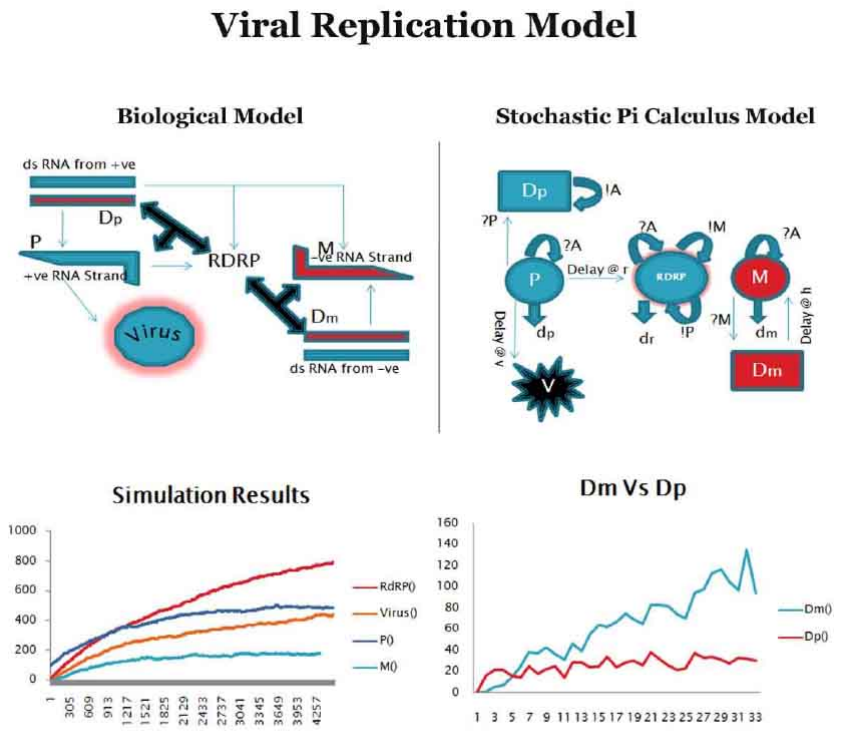

Fig. 6. The RdRP(), P(), M() and virus first increases and then becomes stable, this is how virus replicates and ds-RNA strand formed by minus strand(Dm) always dominates over ds-RNA formed by plus strand(Dp)

4 Viral Replication in RNA-interference Models

In the earlier section, we have modelled the RNA-interference and find its ability to silence ds-RNA and endogenous genes. In this section, we have developed the model that incorporate RNA-interference technique with viral replication. First we would consider the mechanism of viral replication when the RNA strand enters into our body.

Retroviruse like HIV-1 first translates its RNA genome to DNA and then inserts it into the human genome. As discussed earlier, the *ds*-RNA is considered as a signal of non self genes. In our earlier model we have seen that the *m*-RNA RISC complex amplifies the response by forming ds-RNA, but recent studies have shown that virus encoded *RdRP* (RNA Directed RNA Polymerase) helps in formation of ds-RNAs through a secondary pathway hence replicating the virus indirectly [20].

Among the two strands of HIV, the RNA plus strand (denoted by P) generates the virus. Initially *RdRP* is generated by P. Then two strands +ve(P) and -ve(M) interact with *RdRP* to form the complementary strands respectively resulting in *ds*-RNAs i.e., *Dp* (by +ve strand) and *Dm* (by -ve stand). The *Dp* has a complex which again forms *RdRP*, RNA plus and a minus strand. *Dm* forms only a minus

strand. Finally a virus is generated which is of plus strand. The biological model and stochastic π-calculus model is shown in Figure 6. As seen from the simulation results we observe that constant replication of virus in the system and also the concentration of Dm dominates over Dp (see Figure 6).

5 Anti-viral RNA Silencing

In this section, we show that RNA-silencing helps in downregulating the viral response, depending upon the silencing strength and other factors like rate of formation of si-RNA's would effect the viral patterns in the RNA-interference systems. We have extended the viral replication model to develop the anti-viral silencing model. Here we consider the role of dicer that form siRNA's from ds-RNA's. Sip and Sim denote the siRNA's formed by cutting Dp and Dm respectively by dicer at rate of Gd. SiRNA's are formed by the both Dp and Dm and also by Plus and Minus strand at the rate of Gs. These si-RNA's are hence combine with free $RISC$ to form Rp and Rm respectively. Free $RiSC$ replicates itself with rate i and all these protein complexes have their death rates as shown. The biological and stochastic π model is shown in the Figure 7.

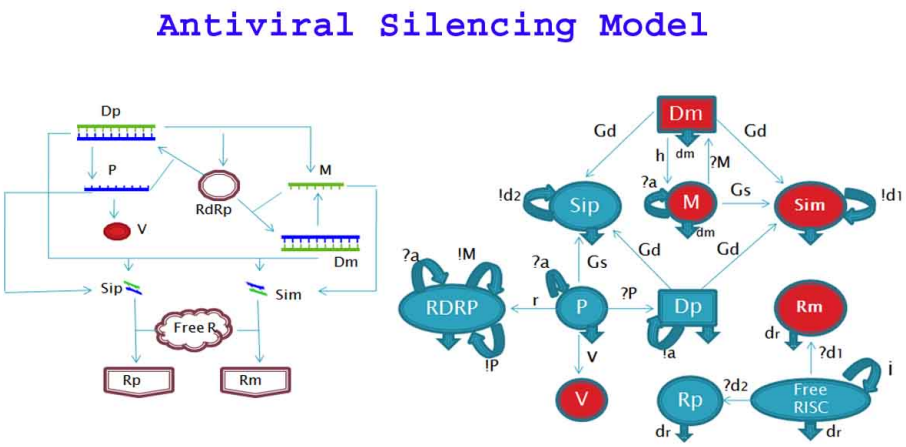

Fig. 7. Anti-viral replication model

Earlier in viral replication model, we observe the viral replication in the system. Due to RNA-interference, we conclude from this model that there is a change in viral patterns. From the simulation results (see Figure 8), we observe that RNA-silencing helps in down regulation of virus to a considerable amount. Silencing strength which depends upon Gs, Gd and the number of si-RNA's formed play a vital role in determining the downregulation of viral patterns. We see that as there is an increase in silencing strength, viral patterns tends to lower its concentration.

6 HIV-1 Virion Structure and its Genome

HIV-1 has a viral core which has two copies of viral RNA copies. The surrounding environment comprised of cellular lipids. Gp120 binds have high affinity towards

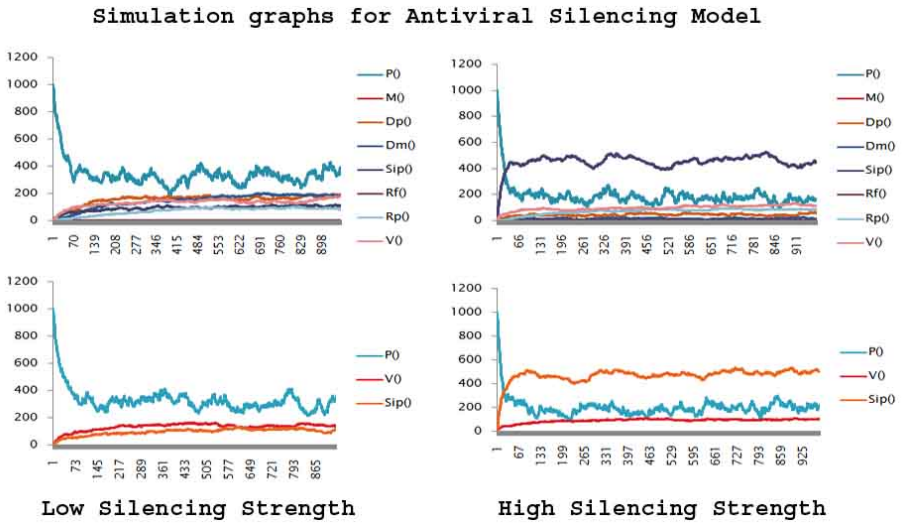

Fig. 8. Simulation graphs of anti-viral replication model

CD_4 protein and cellular chemokine receptor (CCR5 and CXCR4). These two co-factors are necessary for HIV-1 replication thereby helping in infecting CD_4 and macrophages during early and later period of infection respectively.

The virus has nine genes. These are classified into 3 categories viz. structural, transactivation and accessory genes. **Gag, Pol and Env** are basically the **structural genes** responsible for encoding the viral core, enzymes and virus envelope respectively. The next category is of **transactivation genes** that are responsible for replication. **Tat and Rev** regulates the viral transcription and RNA transport respectively. The final category is of **accessory genes**. **Nef** is one of the important of this category which down regulates CD_4 and essential for viral disease induction. Apart from **Nef**, **Env** gene is also responsible for it. CD_4 downregulation is vital property for virion release from infected cells. Another important key feature of T-cell activation initiated by **Tat** and **Env** by interacting with cellular kinase to promote the virus replication [14] [20].

7 RNA-i Attacks HIV-1

Studies have shown that si-RNA directed against the HIV-1 genome can inhibit viral infection [18] [21]. Silencing activity towards CD_4 major protein decreased HIV-1 entry. Targeting viral and cellular RNA's by transfecting it with si-RNA and then exposed to HIV lead to many folds reduction in HIV-1. si-RNA can inhibit viral replication at several stages of infection, including early stages when the viruses are most vulnerable, more infection can also be blocked by targeting viral genes and host genes that are involve in HIV-1 replication cycle [5]. Major challenges in this silencing process is that all viral si-RNA are not equally accessible to si-RNA's. There are also issues in non recognition of progeny molecules towards si-RNA. As described earlier, RNA-i mechanism is potent for HIV-1 inhibition, in this work we have considered pathway involving Magi-CCR5, CD_4 and chemokine receptor CCR5 and CXCR4, the pre-sliced viral m-RNA, viral-DNA, viral-protein and viral genes such as NEF, REV and GAG that are transfected with si-RNA [16]. Initially, HIV-1

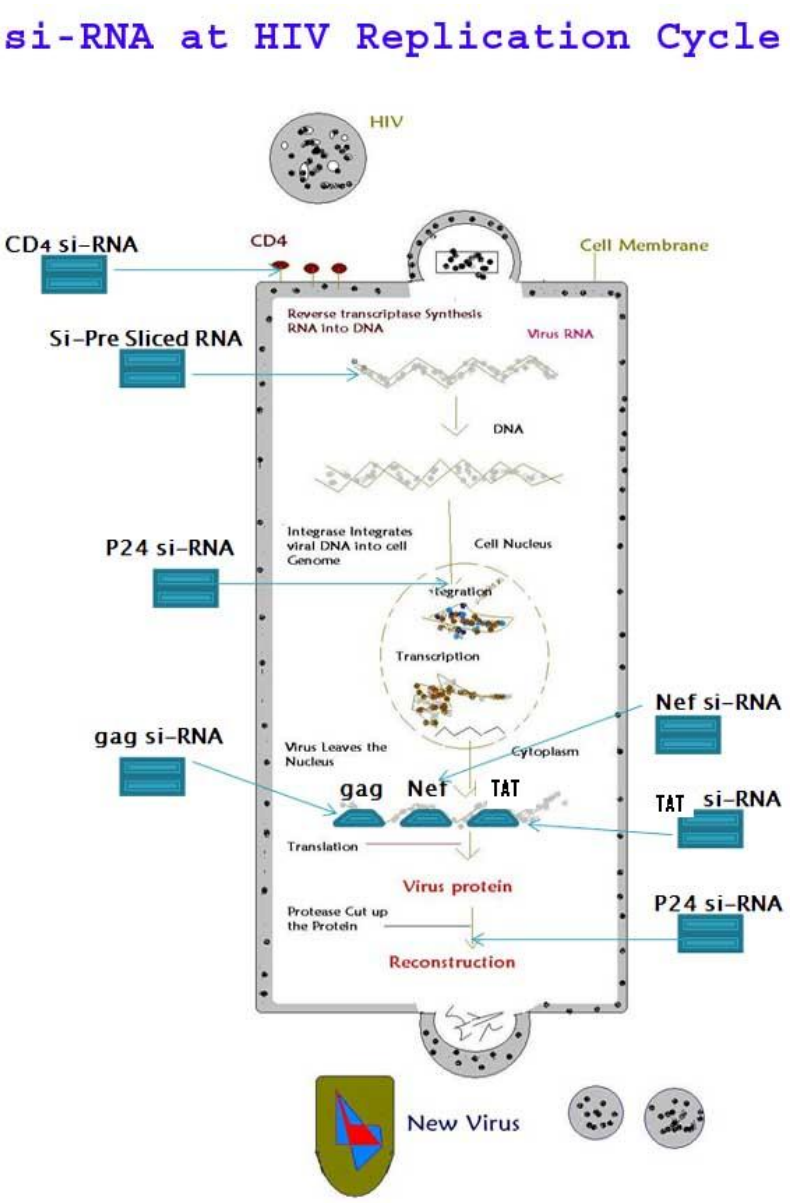

Fig. 9. si-RNA targeted at various levels of HIV-1 replication cycle

virus attacks CD_4T Cell which are source of CD_4 protein and CXCR4 chemokine receptor respectively. After infection with HIV-1 they become infected and then die due to infection. As soon as the virus inserts its RNA and useful enzymes for replication in the nucleus. We transfect the m-RNA of virus at several stages with corresponding si-RNA's which forms a complex. This si-RNA m-RNA Complex is different for every transfection but for the sake of simplicity for modelling purpose we have chosen this to be m-RNA RISC complex (we have called them complex as discussed in section 3.4). We first tried to transfect the pre-sliced m-RNA of virus with its corresponding si-RNA. Then with p-24 si-RNA we have tried to prevent integration and reconstruction. After this, we have try to target three genes GAG, NEF and REV. The biological model is shown in Figure 9 and stochastic π modelling

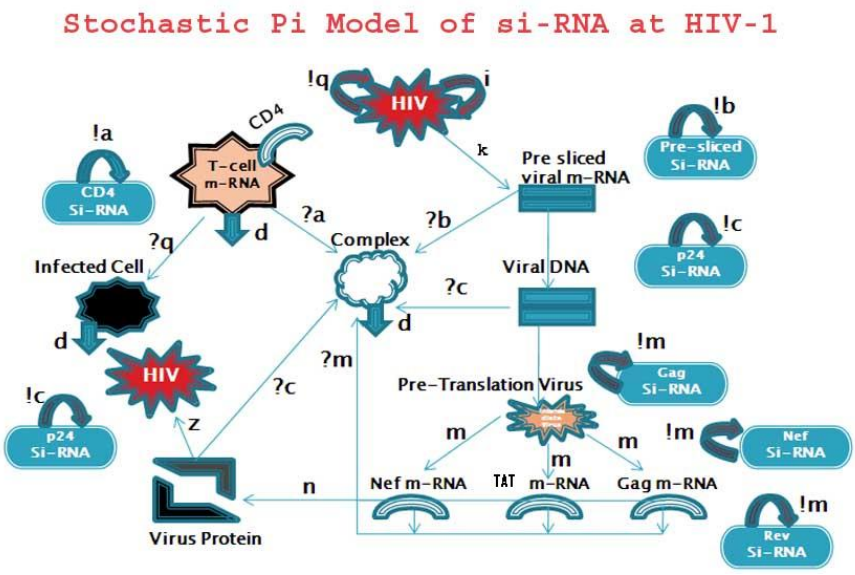

Fig. 10. Stochastic π modelling of the complex pathway of si-RNA directed against various stages of HIV-1 replication cycle

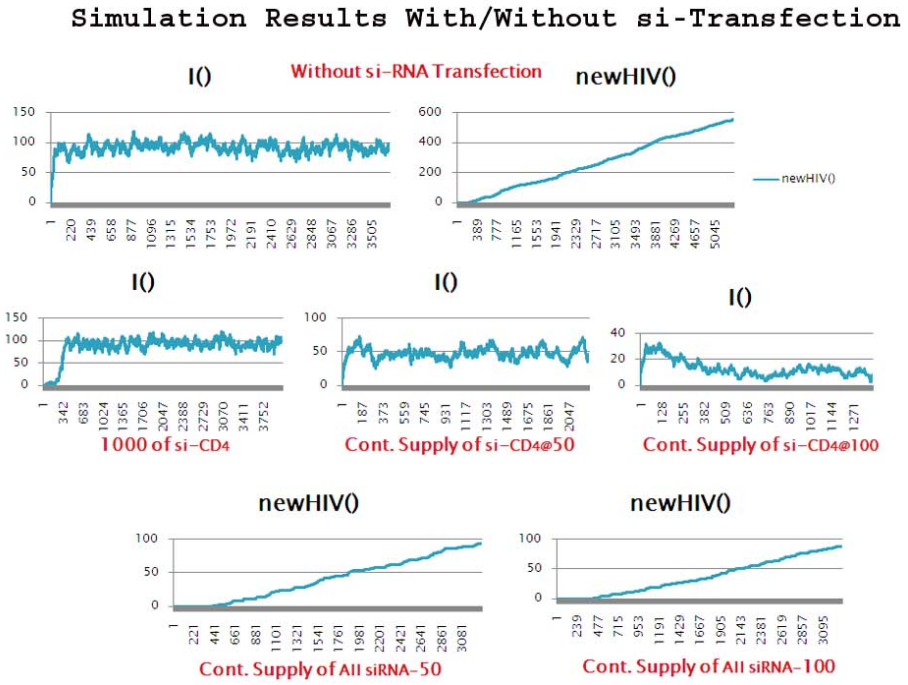

Fig. 11. Simulation Results for final model

is shown in Figure 10. Here channels a, b, c and m are used for transfecting. The pre-translation virus delay at rate m into three components REV, NEF and GAG which is reformed at rate n. These genes also from complex when transfected with their corresponding si-RNA's.

The simulation results shown in Figure 11 shows infected cells and new HIV-1 virus generated before and after transfection with si-RNA's. If we transfect 1000 units si-CD_4 which can target the CD_4 which can suppress the HIV-1 entry, there

is a delay in HIV-1 entry but the amount of new HIV-1 virus produced aren't changing. But if we supply continuous amount of si-RNA at rate of 50 then there is 2 fold reduction in the infected cells. The amount tend to decrease more as we increase the rate of transfection with CD_4.

After transfection continuously with all si-RNA's at rate of 50 and 100, we observe that there is a 6 fold reduction in formation of new virus. Continuous supply of si-RNA hence inhibit the HIV-1 replication process to a large extent, hence shown the way to inhibit HIV-1 virus.

There is an intricate interplay relationship between virus and RNA- interference mechanism. Viruses take advantage of silencing systems and use them to regulate their response. In addition they can also encode protein which can suppress RNA interference mechanism of inhibiting the viral response [3] [22]. The intermediate of the Interference mechanism si-RNA's can arise from RNA secondary structures and also from ds-RNA from virus. In addition to si-RNA, mi-RNA can also inhibit as well as promote viral replication. In particular, mi-RNA is encoded by NEF gene and on the contrary, TAT gene act as suppressor of RNA-interference by blocking the Dicer. It has been seen that when sh-RNA anti-viral treatment is given, HIV-1 escape from RNA-i. The HIV-1 escape is quite prevented by Tar RNA binding protein (TRBP) which is a vital component to increases HIV-1 interference with RNA-i.

7.1 How our model takes care of HIV-1 escape

Studies show that using a single si-RNA can result in HIV-1 escape [3] [22]. For this we have used continuous supply of different si-RNA's which are target at multiple points in HIV-1 replication cycle which minimizes the HIV-1 escape. For the purpose of gene silencing, we have chosen TAT and NEF so to minimize the RNA-i suppressor activity as discussed above.

8 Conclusion

In this work, we exploited natural phenomenon of RNA-interference to inhibit HIV-1 virus and its viral response. Stochastic π model of RNA-interference in section 3.4 is well versed in predicting various hidden phenomenon that can carry out inside the RNA-i process. Stochastic π simulations of this model substantiated it by being coherent to various observations proposed/done in the laboratory (section 3.6)

Viral replication model simulations results reveals virus response is a fast process and it takes advantage of RNA-i machinery for its replication. To counter this phenomenon our anti-viral silencing model simulation results in down regulating viral response. The rates of reactions of these models are taken from the literature [2] [9]. These model are flexible to incorporate other factors in future compare to model developed earlier based on system of differential equation [2] [9]. Finally we exploit this process in inhibiting HIV-1 virus by transfecting it at various stages. Simulation results reveal continuous and multiple si-RNA transfection at CD_4, previral mRNA, viral genes (**GAG**, **REV** and **NEF**) and p24 si-RNA at integration and reconstruction inhibits HIV-1 viral response and prevents HIV-1 escape from RNA-i. We hope this systems biology approach would add a new

dimension in finding solutions and ongoing research towards HIV-1.The modelling of this complex pathway would give us the real insight towards HIV-1 inhibition process by RNA-i and contribute greatly to ongoing research in finding solutions to HIV-1. The supplementary material of the paper such as SPiM codes of the models and high resolution images of the paper are available at author's home page at http://www.guptalab.org/mainweb/publication.html.

References

[1] Agarwal, A., R. Mishra and M. K. Gupta, *Modelling of HIV-1 infection of $CD4^+$ T cells and induced NF-κB activation and regulation using stochastic π -calculus*, preprint, Gupta Lab, DA-IICT (2008).

[2] Bergstrom, C., E. McKittrick and R. Antia, *Mathematical models of RNA silencing : unidirectional amplification limits accidental self-directed reactions*, Proc. Natl. Acad. Sci. U.S.A **100** (2003), pp. 11511–11516.

[3] Berkhout, B. and J. Haasnoot, *The interplay between virus infection and the cellular RNA interference*, FEBS Letters (2006), pp. 2896–2902.

[4] C. Priami, A., *Stochastic π-calculus*, Computer Journal **6** (1995), pp. 578–589.

[5] Capodici, J., K. Kariko and D. Weissman, *Inhibition of HIV-1 infection by small interfering RNA-mediated RNA interference*, Journal of Immunology (2002).

[6] Cardelli, L., *Abstract machines of systems biology*, Transactions on Computational Systems Biology **3** (2005), pp. 145–168.

[7] Diallo, M. and U. Schepers, *RNA Interference: Everything, what one ever wanted to know about silencing!*, Bioforum Europe (2003), pp. 143–145.

[8] Groenenboom, M., A. Maree and P. Hogeweg, *The RNA silencing pathway: the bits and pieces that matter*, PLoS. Comput. Biol (2005).

[9] Groenenboom, M. A. and P. Hogeweg, *The dynamics and efficacy of antiviral RNA silencing: a model study*, BMC Systems Biology (2008).

[10] Jojic, N., V. Jojic, B. J. Frey, C. Meek and D. Heckerman, *Using epitomes to model genetic diversity: Rational design of HIV vaccines*, in: *NIPS*, 2005.

[11] Kitano, H., *A graphical notation for biochemical networks*, Biosilico **1** (2003), pp. 169–176.

[12] Kukreja, M. and M. K. Gupta, *Stochastic π simulations of few HIV-1 models*, Poster Number 18 at The Microsoft Research- Computational and Systems Biology Course-08, Italy (2008).

[13] Kuttler, C., "Modeling Bacterial Gene Expression in a Stochastic π-Calculus with Concurrent Objects," Ph.D. thesis, University of Lille 1 (2006).

[14] Levy, J. A., "HIV and the pathogenesis of AIDS," ASM Press, Washington, DC, 1994.

[15] Milner, R., J. Parrow and D. Walker, "A calculus of mobile processes," Cambridge University Press, 1992.

[16] Novina, C. D. and M. F. Murray, *si-RNA directed inhibition of HIV-1 infection*, Nature Medicine **8** (2002), pp. 681–686.

[17] Phillips, A. and L. Cardelli, *A correct abstract machine for the stochastic π-calculus*, Transactions on Computational Systems Biology VII **4230** (2006).

[18] Pomerantz, R. J., *RNA interference meets HIV-1: Will silence be golden?*, Nature Medicine **8** (July), pp. 659–660.

[19] Regev, A., W. Silverman and E. Shapiro, *Representation and simulation of biochemical processes using the π-calculus process algebra*, Pacific Symposium on Biocomputing (2001), pp. 459–470.

[20] Tan, W. and W. Yuan, "Stochastic Modeling of AIDS Epidemiology and HIV Pathogenesis," World Scientific, 2000.

[21] Wang, Q.-C., Q.-H. Nie and Z.-H. Feng, *RNA interference: Antiviral weapon and beyond*, World J Gastroenterol **9** (2003), pp. 1657–1661.

[22] Westerhout, E., M. Ooms, M. Vink, A. Das and B. Berkhout, *HIV-1 can escape from RNA interference by evolving an alternative structure in its RNA genome*, Nucleic Acids Research **33** (2005), pp. 796–804.