A multi-scale approach for deciphering HIV infection

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Chapter 2

HIV DECISION SUPPORT: FROM MOLECULE TO MAN

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Background

Human Immunodeficiency Virus (HIV) is recognized to be one of the most destructive pandemics in recorded history. Since it was recognized in 1981, acquired immunodeficiency syndrome (AIDS) has claimed more than 25 million lives. The HIV epidemic has become a pandemic and today at least 33 million people are living with HIV [12], [27]. In a report by Mathers and Loncar from the World Health Organization (WHO), projections of global mortality up to 2030 identified HIV/AIDS as one of the three leading causes of death [18]. Each year nearly a billion dollars is invested globally in HIV/AIDS research to change this scenario. Although almost every step of the virus life cycle has been unraveled in the past 25 years, and twenty-four distinct drugs targeted against HIV have been approved, all efforts to achieve an overall eradication of the virus have turned out to be ineffective up to now. On the bright side, effective highly active antiretroviral therapy (HAART) and the availability of genetic screening of patient virus data, has led to sustained viral suppression and higher life expectancy (21.5 years [9]) in patients who have been infected with HIV. Infection by HIV nowadays, is being transformed from “a death sentence” to a controllable disease that needs early diagnosis, life-long patient tailored treatment, continuous follow-up and strict drug adherence.

Several studies have demonstrated that better outcomes are correlated with patients treated by a clinician with greater expertise [15], [5]. This improvement has been described in terms of mortality, hospitalization rate, cost of care, adherence to medications, etc. The definitions of expertise in these studies have varied, but most relied on the number of patients actively managed [6].

Even for the expert clinician, usually a straightforward relational approach is not possible. Given the wealth of data (sequencing and genotypic resistance testing are becoming routine practice) and confounding factors, treatment decisions are highly patient dependent and there is an urgent need for a genotypic resistance interpretation tools that are up-to-date and transparent (provide references for their data sources). The application of artificial intelligence and computational techniques to biomedicine has resulted in the development of computer-based decision support systems (DSSs).

DSSs are designed to enable medical professionals to easily keep up-to-date with the latest clinical expertise and to incorporate this directly in the decision making process.

The Achilles’ heel of the DSSs is the sole dependence on the gathering of information from the published literature by experts, thus overlooking the importance of deductions from other research fields such as molecular dynamics simulations, immune system modeling or Bayesian Network Learning. Our vision is to provide researchers and medical doctors with a virtual laboratory for infectious diseases, which we call ViroLab, to enable easy access to distributed simulations as well as facilitating, processing and analysis of virological, immunological, clinical, experimental and epidemiological data.
The ViroLab Approach to HIV

Figure 2.1: The ViroLab Experimental Platform Framework with its components. From the bottom going left-up: patient specific viral load, mutations and CD4+ information is used by the Molecular Dynamics calculations to estimate the binding affinity of various drugs with Reverse Transcriptase and Protease. The binding affinities as well as the mutations are used to estimate the entry of the virus into the cell. Next the binding affinity, entry process and CD4+ count and viral load are used to estimate the immune response, finally the immune response and the binding affinity are used in modeling the individual nodes in a complex sexual interaction network. From the bottom to right-up: Context sensitive text mining distills relations between drugs and mutations and presents these as ranking rules. The decision support kernel integrates both types of information (simulation and text mining) into integrated decision support for drug ranking.

ViroLab experimental platform consists of complementary, multi-level computational tools as shown in figure 2.1, the details of the links between those will be explained in the following sections. Its components are coordinated to form a framework capable of answering complex questions that clinicians and HIV researchers are facing on a daily basis. Hence, while contributing to fundamental research, ViroLab enhances the conventional DSSs by offering case specific predictions from independent resources.

The ViroLab’s Drug Ranking System estimates the sensitivity of the patient’s virus for available drugs at the genome level. It is a rule based scheme which is based on expert opinion, scientific literature, published data and relating genotype to phenotype experiments.

The HIV population can compensate for the protease inhibitor (PI) resistance mutations by acquiring cleavage site (CS) mutations. To detect the possible associations between PI resistance mutations and CS mutations, co-variation analysis of available HIV sequences has been performed.

At the cellular level, we are modeling the co-receptor use of HIV to understand the virus entry process and how this process evolves in-vivo and its effects on disease progression. Our research in Immune System Response investigates the effects of HIV infection with HAART
treatment and the onset of AIDS on medically measurable parameters such as viral load and CD4 T cell count.

At the epidemiological level, we aim to understand the dynamics of HIV epidemics spreading through sexual contact networks. Therefore we employ a Complex Network (CN), which describes the heterogeneous population of HIV exposure groups. The CN takes as input the results of the simulations on the molecular and cellular level and the demographic and behavioral information available.

The integrated approach to decision support provided by ViroLab experimental platform requires an advanced computational and data management infrastructure. For that we have developed the ViroLab Virtual Laboratory (VL), the backbone of ViroLab, which provides researchers and medical doctors with tools to collaborate, share data resources and services using Grid technology.

VL is a set of integrated components that, used together, form a distributed and collaborative space for science (see figure 2.2). Multiple, geographically dispersed laboratories and institutes use the VL to plan and perform experiments as well as to share their results [11]. Experiment in this context means so-called in-silico experiment that is, a process that combines data and computations in order to obtain new knowledge on the subject of an experiment [1]. Three groups of users have been identified: clinicians using DSSs for drug ranking, experiment developers who plan complex biomedical simulations, and experiment users who apply prepared experiments (scripts). During experiment execution, provenance data is created and stored. The advanced provenance technology of the Virtual Laboratory allows users to trace decisions back to their origin and to share complicated workflows.

![Figure 2.2: Architecture of the ViroLab Virtual Laboratory.](image)

In the remainder of the chapter, we give a brief overview of the various components of ViroLab experimental platform and how they fit together. We also present some new results obtained with this Virtual laboratory for Infectious diseases.

**From Micro to Macro:**

**Molecular Level: Drug-Protein Binding Efficiency Simulations:**
The Molecular Dynamics simulation takes as an input the chemical composition of the drugs and the various mutations of the viral proteins. It calculates the binding affinity of the drug-protein complexes from which we can derive the fitness of the virus and the associated viral load. This will form and input to the tools working on the cellular level as well as the transmission
parameters in the sexual network.

The potential of molecular simulations to enhance our understanding of drug resistance in HIV/AIDS relies ultimately on its capability to achieve an accurate ranking of drug binding affinities, on clinically relevant time scales.

We have investigated the potential of the Poisson-Boltzmann surface area (MM/PBSA) methodology to accurately rank the binding affinities of the inhibitor saquinavir to the WT and resistant variants of HIV-1 protease (PR): L90M, G48V and G48V/L90M. We have also used MM/PBSA to identify the thermodynamic determinants of binding, as a means to understand the mechanisms of emergence of the L90M and G48V mutations under chemotherapeutic pressure.

We identified conformational preferences of the inhibitor and binding site residues and characterize them structurally and energetically [26].

Figure 2.3: a) Comparison of the free energies calculated by MM/PBSA ($\Delta G(\text{calc})$) with experimental data ($\Delta G(\text{exp})$). $\epsilon_1$ is the data from [17], $\epsilon_2$ from [7]. b) Thermodynamic decomposition of MM/PBSA binding energies for saquinavir with the four PRs under study, WT, L90M, G48V and G48V/L90M, based on analysis of the last 4ns of post-equilibration MD.

Detailed studies indicate that we can resolve accurately the binding affinities of saquinavir with four PR mutations (See figure 2.3 and [26])). Obviously these large-scale N-body simulations require a large-scale computational infrastructure. For this we make use of local supercomputers, the European DEISA supercomputer chain and the US TeraGrid. These large-scale computer infrastructures are accessible through the Grid based Virtual Laboratory. For instance a recent DEISA (http://www.deisa.org) allocation as a European Virtual Communities Project allocation (~1.25M CPU hrs) has allowed us to perform part of our work on EU (HPC) grid infrastructure. At Super Computing 2008 we have demonstrated that we can seamlessly link TeraGrid and DEISA resources using Web services via the Application Hosting Environment (GT4 on TG and Unicore 6 on DEISA; [2]).

The binding affinities thus resolved can be used to estimate the drug response of an individual and the individual’s transmission parameter (likelihood of transmission of virus at sexual contact); they provide an indication of the effectiveness of that drug and can then be used
in the subsequent immune response simulations as well as in the individual nodes of the CN where it is reflected in the transmission probability.

**Level of Molecule Populations: Cleavage Site Compensatory Mutation Detection:**
This tool takes as input a set of Gag-Pol sequences including the gene for the viral PR and one or more of its CS, and produces a prediction for potential secondary drug resistance mutations in the latter. The cleavage of the Gag-Pol poly-protein by the PR is essential for the infectivity of HIV virions. PI therapy can give rise to primary resistance mutations in the PR, which are often associated with a decreased activity of the enzyme. Impaired function can be partially restored by compensatory mutations in the CS, probably by providing a better substrate for the mutated proteases. Associated pairs of mutations have been detected from large sets of sequences (‘populations of molecules’) by co-variation analysis [13], and we have extended this approach to identify cleavage site mutations that arise in response to primary resistance mutations in the PR. We analysed HIV-1 subtype B nucleotide sequences containing the protease region from the Los Alamos HIV Sequence Database (www.hiv.lanl.gov). Our final alignment contained 30305 sequences and spanned the entire Gag-Pol region; translation of nucleotide sequences and further analyses were performed using PERL programs and PAUP* (http://paup.csit.fsu.edu). Associations between PR and CS mutations were assessed by chi-square tests of independence. We also implemented a simple, robust ‘phylogenetic jackknifing’ test to exclude non-functional co-variations that arose by ‘common descent’ only. Such apparent co-variations may arise if a pair of mutations (without functional association) is present in an intensively sampled patient or patient group. Briefly, our method tests whether co-variation of a mutation pair can be abolished by excluding any small subset of closely related sequences from the analysis. Failure to do so indicates that the co-variation signal is robust over the whole set of sequences and is therefore likely to bear functional relevance.

We found significant association between several pairs of PR and cleavage site mutations. Most associations were positive, which indicates that the CS mutations may indeed compensate the effect of the PR mutations. Interestingly, some mutation pairs demonstrated a negative correlation, indicating that changes in these cleavage sites are less tolerated by the mutant than by the wild-type protease. Remarkably, a large fraction of the correlated pairs involved a CS position that falls outside the classical definition of a cleavage site, which suggests that positions that lie farther from the cleaved bond may also influence the efficiency of cleavage.

This method generates predictions for CS positions and CS/PR mutation interactions that might be relevant in the complex evolution of PI resistance. The functional relevance of these associations can be corroborated by calculating binding affinities for the mutant PR and CS variants by the computation methods described in Section (a)(i). Confirmed results are applied to refine the rule set for drug resistance prediction and used in the Sexual Contact Network.

**Cellular Level: Modeling the Viral Entry Process through HIV Co-receptor Tropism:**
The Entry Simulation uses the binding affinities as well as the mutations to estimate the entry of the virus into the cell. The result is fed into the Immune System Response Simulator. In 50% of HIV-1 infected individuals a co-receptor switch is observed. Given the transmission with an R5 tropic virus, we aim to evaluate the mutation rate by progressing the virus quasispecies to an optimum fitness by changing co-receptor tropism. The emergence rate of X4 tropic variants, the factors leading to it and the timing of the co-receptor switch are investigated to answer whether accumulation of mutations can be understood from the co-receptor switch.
Viral tropism ranges from R5 tropic to R5X4 tropic and finally to X4 tropic variants and allow for biologically natural trade-off between viral mutation rate and the overall reproduction capacity of the viral quasispecies.

A five-point mutational distance is employed in the model from pure R5 tropic to X4 tropic virus where intermediate steps stand for the R5X4 variant. Three kinds of mutation rates defined as forward (increasing the fitness of the variant), neutral and lethal 0.3, 0.3 and 0.4 respectively [21].

Each simulation is performed for ten person years and the experiment has been conducted for a large range of mutation rates. We have collected the data from the population behavior of T-cells and HIV virions for different substitution rates. For detailed explanation of the model and see chapter 5 and [8].

Figure 2.4: a) T-cell and HIV average population sizes vs mutation rate (per nucleotide site per replication cycle) in log scale. The y-axis scale on the left stands for the number of T-cells and that on the right indicates the HIV-virus population size. b) Co-receptor change rate, Co-receptor change rate to R5X4 (intermediate variants), Co-receptor change rate to X4 tropic variants vs mutation rate in log scale.

In figure 2.4a we observe $10^{-5} – 10^{-4}$ interval as maximum mutation rate for the viral quasispecies. After this interval, the viral population experiences a fitness loss due to emergence of lethal mutations in excess numbers. The upper boundary for the mutation rate obtained from our simulation is in very good agreement with experimental reports [14], [16].

In figure 2.4b, no co-receptor switch is observed under $10^{-5}$. For increased substitution rates ($3.10^{-5} - 10^{-4}$) the viral population experiences co-receptor changes in 50-80 percent of the cases that coincides with the medical data. Larger substitution rates (over $10^{-4}$) produce continuous fluctuations of each tropic variant (Data not shown) indicating that the natural selection halts and all the variants co-exist, although the dominant strain is changing over time.

These results clearly demonstrate that there is an upper boundary for the mutation rate for the selection on co-receptor tropism to work. It is tempting to speculate that there is a lower boundary for the mutation rate that is determined by the negative selection pressure of the humoral immune response. Hence, the efficient and timely production of R5X4 and X4 tropic variants can be achieved only with appropriate mutation rate. This information is being fed into the Immune System Response Simulator.

**Cellular Level: The Immune System Response:**
The Immune Simulator takes as an input the viral load, the CD4+ count and the drug efficacy and then estimates the temporal HIV dynamics. This dynamics is used as an input to the sexual network nodes to model the various stages of HIV infection of the individuals.
We simulate four phases (acute, chronic, drug treatment and onset of AIDS). We investigate the HIV infection dynamics with therapy using microscopic simulations. Non-uniform Cellular Automata (CA) are employed to simulate drug treatment of HIV infection, where each computational domain contains different CA rules, in contrast to normal uniform CA models.

We have defined CD4+ T-cells as the main entities. Each cell is assigned healthy/infected or dead flag depending on the interactions with its neighbors, for more details [22].

Different drug therapies can be modeled by differences in a response distribution function over time. The function is chosen to model the fact that the drug therapy will not immediately influence all of the infected cells, but rather it will affect part of them at each time step. At the same time, the function accounts for the concept of drug resistant virus strains emerging.

The simulation result for treatment naïve individual dynamics is shown in figure 2.5. The result represents qualitatively the HIV infection dynamics. The first phase indicates the fast proliferation of the original HIV strains before the actual immune system response (acute phase). This phase ends when specific immune response occurs for these strains. The next phase, the chronic phase that takes years, is the phase where the viral load increases slowly and CD4 counts decrease slowly. When CD4 T-cell counts drop to a certain level (normal 200 to 500 counts per ml), the drug therapy is started. In this phase, virus replication is blocked and CD4 T-cell counts increase. Once resistant strains against the drugs evolve, the last phase of the disease occurs disrupting the whole immune system.

The main success of the present model is the adequate modeling of the four phases of HIV infection with different time scales in a single model. Our simulation results show a qualitative correspondence to clinical data. The individual HIV dynamics like the ones shown figure 2.5 are given as an input to the individual nodes in the sexual networks and population dynamics simulation.

Population Level: Complex Network Model for HIV Population Dynamics:
The Complex Network Model takes as an input the binding affinities form the MD simulator and the immune response together with epidemiological and demographic information from Centre of Disease Control like databases. It stochastically predicts the evolution of the HIV dynamics as well as the transmission of resistance.
Despite the availability of a large number of mathematical models describing the spreading of HIV, a good understanding of the dynamics through numerical analyses is still a major challenge. The estimate of HIV/AIDS incidence is quite uncertain since many people are still unaware of their infection due to the long asymptotic incubation period. Heterogeneity of the infected population and the various routes of infection among its members exert additional layers of complexity. Therefore it is essential to combine epidemiological processes with sociological models and network sciences. Here we propose a new way to model HIV infection spreading through the use of dynamic CNs.

The heterogeneous population of HIV exposure groups is described through a unique network degree probability distribution. The network model is constructed as a dynamical scale-free network, wherein a node represents each individual and the edges are the links between the individuals. The infection spreads along these edges.

The time evolution of the network nodes is modeled by a Markov process and incorporates HIV disease progression through a configuration model of T-cell CD4 count for each node.

While constructing the model we have taken into account that HIV is primary a Sexually Transmittable Disease (STD). In our model we identify three ways of transmission:

- Men who have sex with men (MSM);
- Heterosexual population;
- Injection drug users (IDU). STD dynamics is not implemented for this group.

Each risk group has its own probability of infection per partner $\lambda$ as well as specific demographic factors and related contact networks.

The results are validated against historical data of AIDS cases in the United States as recorded by the Center of Disease Control. These data include at least three epochs of the epidemic evolution: Pre-ARV, ARV and HAART- treatment and three kinds of HIV spreading.
Figure 2.6: Simulation results and reported data for the AIDS epidemic in USA. MSM exposure groups, power-law distribution. Circles are the annual officially registered number of AIDS cases; the solid line indicates the simulation results. Comparable results were obtained for heterosexuals and IDU users (Data not shown).

As can be seen from figure 2.6 the simulation results provide very close agreement to the officially registered annual AIDS cases. The obvious feature in figure 2.6 is a substantial decline of AIDS cases after introduction of HAART (1996). Our model correctly reproduces this decline and the temporal separation of the peaks of the AIDS epidemic in the heterosexual and MSM populations [23].

In conclusion, the presented parameterized CN model accurately describes the dynamics of HIV spreading taking into account all the existing kinds of HIV spreading. We find a remarkably good correspondence between the number of simulated and registered HIV cases, indicating our approach to modeling the dynamics of HIV spreading through a sexual network is a valid approach that opens up completely new ways of reasoning about various medication scenarios.

This tool, which integrates the clinical information with social behavior, is used by epidemiologists and by decision makers to study scenarios of treatment and prevention measures.

The Virtual Laboratory:
The VL hosts biomedical information related to viruses (proteins and mutations), patients (viral load, CD4 count...) and literature (on drug resistance and etc.), it enables to plan and run experiments transparently on distributed resources in grid systems, clusters and standalone computers.

The VL integrates various tools, modules, protocols and interfaces into a single virtual space, where various types of users are able to (collaboratively) perform tasks. In the upper layer
we have the set of users performing tasks using the tools that are grouped in the interfaces: there are dedicated tools for each group. The centerpiece of the architecture, the unified runtime system, is the part that allows these various users and their tools understand each other. What is more, this runtime layer serves as a bridge to resources dispersed in the virtual laboratory: both data sources and computational services. Finally, these services run on physical equipment that are at their disposal. There are multiple solutions here that are supported by our virtual laboratory; among others it supports also large Grid computing testbeds. Currently the EGEE testbed through the LCG software is supported as well as the DEISA resources.

In this way the virtual laboratory provides an integrated environment to organize computational and ranking experiments in HIV. Details of the ViroLab virtual laboratory are beyond the scope of this manuscript, more information can be found on http://virolab.cyfronet.pl/trac/vlvl.

Discussion

ViroLab experimental platform promotes theoretical understanding of infectious diseases while bridging the gaps not only within each research field but also across different fields. It is integrated in a Grid-based virtual laboratory [1] that facilitates medical knowledge discovery and the interaction and integration of the wide variety of HIV modeling that is available. This offers the potential to provide medical doctors with a DSS to rank drugs targeted at individual patients. Its infrastructure provides virologists with an advanced environment to study trends on molecular, genome, and cellular, individual and epidemiological level.

For instance, to tackle problems such as the development of drug resistance to saquinavir, we envisage being able to simulate the probability of drug resistance with the Binding Affinity Calculator (BAC) and identify compensatory mutations in the cleavage sites. These results could then be integrated with a fitness landscape. This could then be used to interpret patient data in high resolution and provide an improved DSS.

Another potential scenario concerns the transmission of drug resistance at the population level. Viral load and infectivity can estimated on an individual level using the BAC and the Immune System Response model. These parameters are then be supplied to the HIV population dynamics model, eventually resulting in a network with predicted drug resistant nodes. This could help policy makers to answer questions like: “Which drug should be made free and publicly available without creating a universal drug-resistant population?” or “Which combination of HAART would give the best outcome in resource poor areas where adherence is a major problem?”.

In this research, we have already obtained interesting results, showing the viability and the extensibility of the approach taken (e.g. ([23], [25], [1], [3], [4], [26])). The system we have described is still under development with new functionalities added frequently through extensive usability studies in a series of hospitals across Europe.

Note that the majority of the research described in this chapter conducted by collaborators of ViroLab and included in this thesis for explaining the current state of the field from different ends of the spectrum. The author of this thesis
Conclusion

In this chapter we have explained ViroLab, one of the prominent experimental platforms for providing decision support for treating patients with HIV, storing, managing and analyzing patient specific data.

In ViroLab information from different scales are utilized for understanding HIV – human immune system interaction. Molecular dynamics simulations are used for predicting possible drug resistance mutations, immune system modeling in cell level is used to estimate the condition of the immune system in temporal scale and population level complex network simulations are important for determining the epidemiological outcomes of the infection.

In addition to what has been explained in this chapter, there is wide range of protein interaction data available describing the interaction partners as well as the type of interaction. Furthermore, this data is compiled in the HIV-1 Human Protein Interaction Database of National Institute of Allergy and Infectious Diseases [10].

The well annotated characteristics of the data presents a unique opportunity to observe virus-host interaction in protein level. It allows to generate a protein interaction network from all the reports and publications about protein interactions described for HIV proteins over the years. Once curated, the protein interaction network of HIV-1 and Human proteins has the potential to be an indespensible resource for analyzing protein level interactions.

One important advantage is that it gives an overview of what has been described in terms of protein interactions in a single platform. This is a major step forward, after focusing on single gene or protein experiments in the lab and describing findings in terms of pathway diagrams, we are now able to describe all those findings quantitatively.

In the next chapter we will introduce the HIV-1 Human Protein Interaction Network and analyze its network topologically with network centrality measures. Further we are going to provide insight into the network dynamics via network motif analysis.

Chapter References


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