CATÓLICA DE VITÓRIA CENTRO UNIVERSITÁRIO

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HALYSSON FREITAS ALVES DA SILVA

A PROPOSAL FOR FREE TOOL-BASED PROCESSES TO CONDUCT *IN SILICO* DOCKING EXPERIMENT FOR BUILD THREE-DIMENSION LEARNING OBJECT

Vitória

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Course Conclusion Paper presented to Católica de Vitória Centro Universitário, as a mandatory requirement to obtain the title of Bachelor of Pharmacy.

Adivisor: MSc. Danilo Soares Costa

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Approved on _____ of _____ of _____, by:

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I dedicate this work to my father Jarbas Alves da Silva and my Mother Clemilda de Freitas e Silva by your unconditional love given to me in all moments of my life. They were essential to my success until this moment, I'm very grateful for their love and I praise God for their lives. I love them.

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ABSTRACT

The needed about improvements on education tools for better and more effective learning is a constant reality one time when the world evolution is a constant. This can be seen a portion of this in pharmacy industries and specificity drugs because difference in expression of the genes evolution. It throws a new area called pharmacogenomics, but for this is necessary very especial capable people. One requisite to enter in pharmacogenomics is to know about *in silico* tools and many other subjects into pharmacy course. But, *in silico* tools use in class looks not be a reality in it. To alter this, this work proposed an approximation of this reality by aux the teacher to introduce *in silico* tools into your class, primarily for Pharmacology and Pharmaceutical Chemistry subjects to see ligands into proteins activity site. To allow this, a scrip to make news complex ligand into receptor activity site by the docking process was established. The methodology to develop this scrip was a qualitative, exploratory and descriptive research to do a specification of the solution. For the three firstly was described the theorical concepts and the *in silico* tools. To the specification four phases was established: the theorical solution idea, the implementation, the usage case, and the use of molecular docking result as a Learning Object. The theorical solution has five modules – data source, viewer, editor, translator and docking. The implementation was made with Autodock Vina, Autodock Tools, Babel, and Discovery Studio. The usage case has as input molecules the Caffeine and the CYP450-3A4. Finally, the result of the docking process as showed and discussed in the context of education to power the learning, in the way to understanding the ligand and receptor interaction as Learning Object in context of facilitations provided by *in silico* tools.

Key words: Molecular Docking. *In Silico*. AutoDock Vina. Learning Object. Learning And Education.

FIGURES LIST

ACRONYMS LIST

ADT - Auto Dock Tools

CFF - Federal Council of Pharmacy

G6PD - Glucose-6-phosphate dehydrogenase

MEC - Ministry of Education and Culture

MGL - Molecular Graphics Laboratory

NAD - Nicotinamide adenine dinucleotide

NADP - Nicotinamide adenine dinucleotide phosphate

NCBI - US National Library of Medicine National Institutes of Health

SUMÁRIO

1   INTRODUCTION

The drugs are in human life present all the time, since birth up until the moment before death. Prescription drug sales in the world increase every year. As presented in statistical analysis by a top-ranked company for pharmaceutical analysis named Evaluate Group. This statistical displays a $119 billion growth between 2008 up to 2016, and a projected a growth of $286 billion between 2017 until 2022, starting from $774 billion in 2017 up to outlook $1,060 billion in 2022 (LERVOLINO; URQUHART, 2017, p. 8).

A rapidly search in US National Library of Medicine National Institutes of Health – NCBI – with the sentence "adverse drug events" and filter "to 1980/12/31" provides the count of 2960 articles, for filter "to 1990/12/31" - 5537, for filter "to 2000/12/31" - 12435, for filter "to 2010/12/31" – 53205, and prospects for 2020 is a number superior to 240 thousands articles. These demonstrate that it is a very important research field for humanity, and so on. Many articles titles demonstrate innumerous diseases and genetics challenges on therapeutic drug and adverse drug events.

Some of these adverse drug events are studied by pharmacogenetics and pharmacogenomics, because they derivate on variability in drug response (ROBERT, 2004). Pharmacogenetics is a term created by Vogel of Heidelberg in 1959 (PATIL, 2015) and according to (ROBERT, 2004) the difference with pharmacogenomics is only the name, consequently a fashion to adding the suffix '... omics'.

Drugs available on the market are test in a rigorous way in a big group of people. In this case, adverse drug events are derivate on gene unknown variations and no by the drug itself. Thus, on one hand, this research and clinical field generally acts on people that's do not fit the general safety profile studied and guaranteed by the steps of launching a new drug. On other hand, in drug development phase, pharmacogenomics is important to predict the best configuration of a molecule.

Drugs are thinking to patient benefit. In contrast, the same drugs that can obtain good results maybe do badly, depending on numerous factors. Paracelsus (1493-1541), a renaissance physician, postulated: "What is there that is no poison? All things are poison and nothing is without poison. Solely the dose determines that a thing is not a poison" (BRUNTON; CHABNER; KNOLLMAN, 2011, p. 73).

In known human history, we often find reports of medicines and poisons as in the case of Socrates, on book "Phaedo: The Immortality of the Soul" (PLATO, 2019), where Socrates was subjected to drink poison because of his death sentence. The poison used is a cocktail of extract from Conium maculatum, also known as water hemlock, mixed with opium (ALI KEMAL, 2016, p. 5).

Using plant Cicuta dry matter parts to test the potential poisoning in mice, the extracts of tuber and green seeds were respectively 17 mg/kg and 1320 mg/kg to obtain of LD50 (median lethal dose). Other a bit minors doses didn't achieve death but took to intoxication, much smaller doses didn't manifest toxicity effect (ORLANDO-GOULART et al., 2018, p. 8). Clinical signs and symptoms can be rhabdomyolysis, renal failure, ataxia, respiratory paralysis, headache and dermatitis, as were show in three cases reported, two children and one adult (ALI KEMAL, 2016, p. 4).

In the above case, for all people the poison works. Other history dated 510 B.C., where fava beans for some people behave like poison, and for others no ill effects were observed in investigation performed by Pythagoras (ROBERT, 2004).

The first case is an example of full population toxicity once was used to kill everyone with a death sentence. In second case, it is able to see a case to be study with pharmacogenomics concepts and tools. These difference between peoples that have toxicity, and others no, is due the first haven a deficiency of G6PD because no expression of this gene. This is a problem were DNA with the code of G6PD don't generate RNAm to generate protein.

More one case is related about patients of African ancestry and establish a question over why they don't have a relative less response to other population ACE inhibitors and β-adrenergic blockers in article (BREWSTER; SEEDAT, 2013). This has conclusions that patients of African ancestry have therapy resistance but the pharmacogenomics yield heterogeneous and little data, what complicates the study.

In the pharmacogenomics field, DNA can express RNAm and then generate protein, but DNA possesses a polymorphism, in the other words, a DNA has a mutation that changes your nucleic acids sequence. These polymorphisms can do protein to increase or decrease your activity.

Metabolisms are a way that the animal body, include human body, encounter to eliminate xenobiotic. Xenobiotic (drug, poison, food) is anything external to the body that in some way goes into to the body by enteral and parenteral administration routes.

Metabolisms are divided in phase 1 - oxidation and phase 2 - conjugation. The two phases objective is turning the xenobiotic more hydrophilic to be more easily eliminated on the bile or in kidney on urine (BRUNTON; CHABNER; KNOLLMAN, 2011, p. 123–124). The oxidation and conjugation process are driven and executed by enzymes. Polymorphisms in these enzymes can be cause an increase or decrease in these processes.

Cytochrome P450 has the heme group, like the heme group in red blood cells hemoglobin. It has an iron atom that capture oxygen – O2 – in triplet state (state stable) and transform to oxygen singlet state (state reactive). Then, this is one step to xenobiotic oxidation. Next is necessary that the enzyme site of metabolism be compatible with xenobiotic, to xenobiotic realizing fit into enzyme and be oxidize (YANG et al., 2015).

Variations on site metabolism in CYP450 can result in an augment or deduct in effect on drug or prodrug depends whether a polymorphisms augment or deduct the enzyme activity. If has polymorphisms in the metabolism enzyme then drug effect is deducted and prodrug effect is augment and vice versa.

Given such characteristics provided by polymorphisms, the study of the impact in enzymes metabolism is important for current health mainly to pharmacists and physicians.

Learn experiments can be the same way that researches do. They are three mainly types of experiments: in vivo, in vitro, and in silico. The two firsts depend a high cost and specialize laboratory. The third can be executed in a personal computer, accessible to more people like graduated students.

In this way, this work is pursuing a compatible way with average computational hardware graduated students' resources and free and computationally treatable software for experience and learning about docking drugs in a metabolite site on CYP450.

The tools that executing docking process over ligand and receptor can show results about these relations and thus correlation with relations founded in in vitro and in vivo experiments. These in silico tools to do docking process can be a good thing to aux educations process and in this direction this work run.

The education need for national growth is an axiom for all nations of the world. Health is other, equals to education, concern problem to be more and more developed in a way to improve citizen's life. Gathering the two requirements for a better nation life quality, the government, private initiative and universities seem a big challenge to train professionals for the news technologies.

Countries like Brazil, USA, Portugal, and Spain have a good regulation in aim to educate new professionals to practice pharmacist activities.

At Brazil, the MEC (Ministério da Educação e Cultura - Ministry of Education and Culture) jointly with CFF (Conselho Federal de Farmácia - Federal Council of Pharmacy), private initiative and numerous universities professors make the documentation that guides all universities in Brazil about the minimum curriculum. This is called Diretrizes Curriculares Nacionais do Curso de Graduação em Farmácia (Pharmacy Undergraduate National Curriculum Guidelines) (BRASILEIRO, 2017).

Focusing attention on new drug development, *in silico* methods about drug metabolism and docking technologies, the Brazilian pharmacist curriculum legislation has some topics that lead these themes. Item II in Art. 5 explicit an axis in technology and health innovation to be worked joint pharmacist's students. The term technology appears fourteen times in the whole document, and also terms such as biopharmaceuticals, biomedicines, biotechnology, biochemistry, genetics, and molecular biology, obviously, appear in Portuguese.

And more important two terms, already cited in introduction, are pharmacogenetics and pharmacogenomics. One time they are showed in Brazilian pharmacist curriculum legislation, more specific in when, among all other skills necessary to a pharmacist, talk about exclusively pharmacist sciences and ability to innovation search and development (BRASILEIRO, 2017, p. 6).

Searching the string 'farmacogenômica graduação Brasil' (pharmacogenomics graduation Brazil) in NCBI nothing specific was found, and the string in English too.

The intuit is to discover if these skills ruled in Brazilian pharmacist curriculum legislation are fulfill for undergraduate students.

The same string searched in Google Scholar Search Tool no return most satisfactory results. Only one low impact article that has one information about pharmacogenomics curriculum. This relate that exist a college subject called "Farmacocinética e Toxicocinética e Farmacogenética" (Pharmacokinetics and Toxicokinetics and Pharmacogenetics) in two universities only, and de subject isn't just pharmacogenetics (REGINA; MORAES, 2016). This gap shows the necessity to improve and growth this subject in Brazil for undergraduate students.

With the previous string we found a subchapter in a master dissertation from Portugal about pharmacogenomics education (BESSA, 2018, p. 15;23-24). This text explains that most pharmacists know about existence of pharmacogenomics, but they don't know what to do and how to do. These conclusions are made with bibliography resources and field research with pharmacists.

No one of these texts found give a manner and concern to discuss methods and materials to teacher an undergraduate about pharmacogenetics, pharmacogenomics, and more less, this two in context of drug docking. The article (SOUZA, 2013) among others preoccupations talk about the importance of these concepts in a context of health professional education to a personalized medicine.

It is possible to observe that the insertion of Bioinformatics in Brazil is very rare, and even so, it is usually done in post-graduate courses, with only a few universities introducing the knowledge of Bioinformatics during graduation (RIBEIRO JUNIOR; OLIVEIRA; CECCATTO, 2012).

Approach this theme go meet the news skills necessary to now undergraduate and future graduate pharmacist. Drug docking, not only to research, but also a way to develop pharmacogenomics and pharmacology ability.

The main goal of this work is discovering applications for three-dimensional in silico representation of the pharmacological ligand and receptor interaction to build a script for preparing didactic material.

In direction to dismember the main goal and plus more understanding about, it is intentions of this work to: superficially understand the existing software and to delve in a practical way in the chosen ones with focus and delimitation in solving the

problem; understand the necessary files and extensions that represent the molecules; establish, given the literature, the existing forms of molecular interaction and find out how to explain them in the chosen software; and perform a proof of concept of the built script.

2 METHODOLOGY

Using the theoretical framework of (GERHARDT; SILVEIRA, 2009), the methodology to be used in this work is established here. The terms and their respective semantics, to transcribe the methodology, which are described here will be in a broader description in the source established above.

Scientific knowledge depends on scientific research and carried out by scientific methods, above all, the acquisition of empirical knowledge can and is in many cases disassociated from clear and objective methods. In this way, the realization of this research touches both approaches, especially with the continuous and absolute care to only record knowledge when it is validated as scientific knowledge.

In this work the scientific research is characterized by being: as for the approach, a qualitative research, because it is concerned with joining solutions of *in silico* applications to improve a subjective task, which is the preparation of didactic material; as for nature, it is applied research given the use of various tools that are already aimed at solving practical problems, thus expanding its field of action; as for the objectives, it is an exploratory and descriptive research when the analysis of tutorials and documentation of the tools and description of these are carried out to reach the established objectives; as for the procedures, a part, not being the most important, is a bibliographic search to gather concepts and other information in order to propose the script described in the objectives and proceed with a specification of solution architecture. The most important part, involving one of the cytochrome enzymes P450 is a drug that inhibits this enzyme, thus explaining the validity of the script.

This work will be conduct with three stages that are the theorical reference, the collection of *in silico* tools and source of data, and third, by the development of the solution with the orchestration over the two first to do a solution.

To the theorical reference many repositories of articles will be consulted for obtained the necessary information to base with conceptual framework as PubMed, NCBI - National Center for Biotechnology Information, Google Scholar, and so on. The focus of this chapter is to obtain information about the CYP450 - the receptor, ligand as

inhibitor on receptor and the molecular interactions, the basis for these topics, and about the process involved in learning.

The collection of *in silico* tools and source of data will be done starting with information obtained from articles reads for theorical reference, by information derived from web forums and official tools sites. This nature of this research part is different because the technical information is poor described in scientific articles.

The development will be a structuration to accomplish the goals in a way to describe each detail that was study in the two first steps and coordinate that into a solution. It is a desire to cut the development in four phases.

The first phase is to think the architecture of solution, where it will be a conceptual solution that can be implemented in a variety of ways with a variety of tools. The second phase is one possible implementation of architecture of solution, and will has a defined set of tools and details about the process to use it. The third phase is thinking to describe a use of this implementation of architecture of solution, and some organization definitions to better use of it, to generate a product. Finally, this product will be thinking to be used into a learning and education process.

3   THEORICAL REFERENCE

At first glance, every business wants success, and that goes for the assertion of risk and cost reduction when dealing with research and development, or the fact of time also comes into the calculation. According to the European Federation of Pharmaceutical Industries and Associations, a new drug available to the market has taken 12-13 years since its first synthesis, the cost in 2016 was close to € 1,926 million (at the time the Euro ranged from approximately R$ 3.50 to R$ 4.5, Brazilian coin), and of the tested substances only one or two out of 10,000 (EFPIA, 2018).

Therefore, means to reduce risks, costs and time are developed. Computing has allowed the development of so-called in silico technologies where simulations of in vitro and in vivo experiments can be performed. They argue (TYZACK; KIRCHMAIR, 2019) it is important to understand the metabolism of new molecules to mitigate the possibility of failures in the research and development process due to ADMET problems (absorption, distribution, metabolism, excretion and toxicity), so it is important to have reliable in silico methods.

Taking into consideration such tools, their importance for the development of new drugs and the objective of the present work, which is to elaborate new educational possibilities to explain the pharmacological ligand and receptor interaction, below follows a conceptual and technological arsenal describing in silico tools designed for research of new molecules and focusing on the understanding of metabolism regarding the oxidation performed by the family of enzymes called CYP450, more specifically CYP450-3A4 and its inhibition by some drugs.

3.1   CYTOCHROME P450

Cytochrome P450, which is briefly called CYP450, is a metabolizing enzyme family of the endogenous substances and xenobiotics, which are exogenous substances such as drugs. They are part of first phase metabolizing enzymes, whose main and major role is to oxidize substances with lipophilic characteristics, thus giving more hydrophilic characteristics, an important fact for the elimination of these substances from the body mainly through the kidneys, with drug metabolism being the focus of

the study of CYPs450 in pharmacology (BRUNTON; CHABNER; KNOLLMAN, 2011, p. 27–28, 124–125).

Thissuper family called CYP450 or Cytochrome P450 because are a cellular prontein ('cyto'), colorful ('cromo'), and is a pigment that your light absorption is on 450nm wave-length (SOUZA, 2013).

Figure 1 - Scheme of the P450 nomenclature
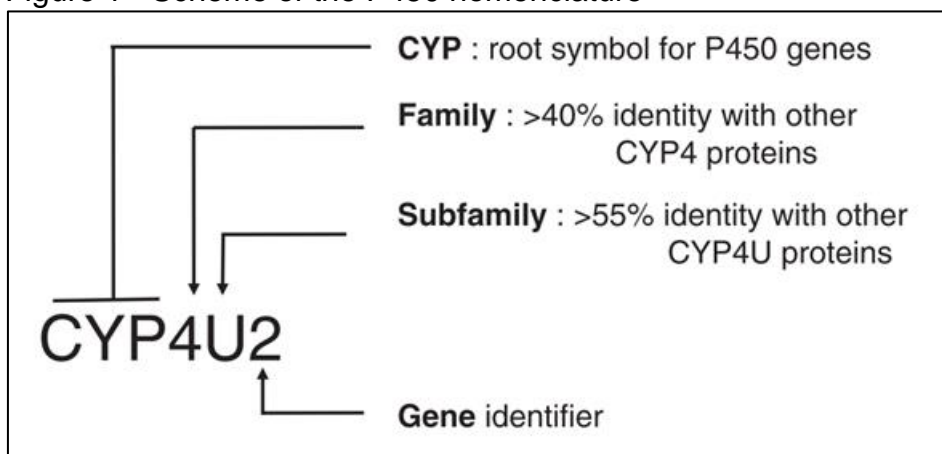
Source: Adapted from(FEYEREISEN, 2012)

The structure of naming the families and sub families and gene was given by (FEYEREISEN, 2012; NELSON et al., 1993) where by example CYP450-3A4 the CP450 is the family, 3 is the sub family, A4 the gene, or by example in   Figure 1. There are many families of CYP like: CYP1, CYP2, CYP3, CYP4, CYP5, CYP7, CYP21, and so on. Some are most important for xenobiotics metabolization like CYP2D6, CYP2C19, CYP2C9, CYP3A4 and others (BRAZ et al., 2018; GONG et al., 2018; SOUZA, 2013; THOMFORD et al., 2016).

This Heme group at the active site of the CYP450 acts primarily in catalyzing the reaction. As can be seen in   Figure 2 (YANG et al., 2015), the Fe^{3+} of the Heme group forms a complex with the RH carbon and by donating electrons from the NADPH via the enzyme NADPH-P450 Oxyreductase, iron is reduced to Fe^{2+} and an equilibrium reaction between the conjugate $Fe^{2+}RH$ and Fe^{2+} + RH products.

In performing xenobiotic oxidation, the CYP450 family of enzymes performs a stoichiometric operation as reported in (YANG et al., 2015) where: $NAD(P)H + O_2 + R \rightarrow NAD(P)+ + RO + H_2O$. Nicotinamide adenine dinucleotide (NAD) or nicotinamide adenine dinucleotide phosphate (NADP) in their reduced forms NADH

and NADPH participate as electron donating substrates, so the products are their oxidized forms NAP+ and NADP+. The portion R of the equation is the substrate carbon and RO is the oxidized product.

Figure 2 - CYP450 oxidative cycle
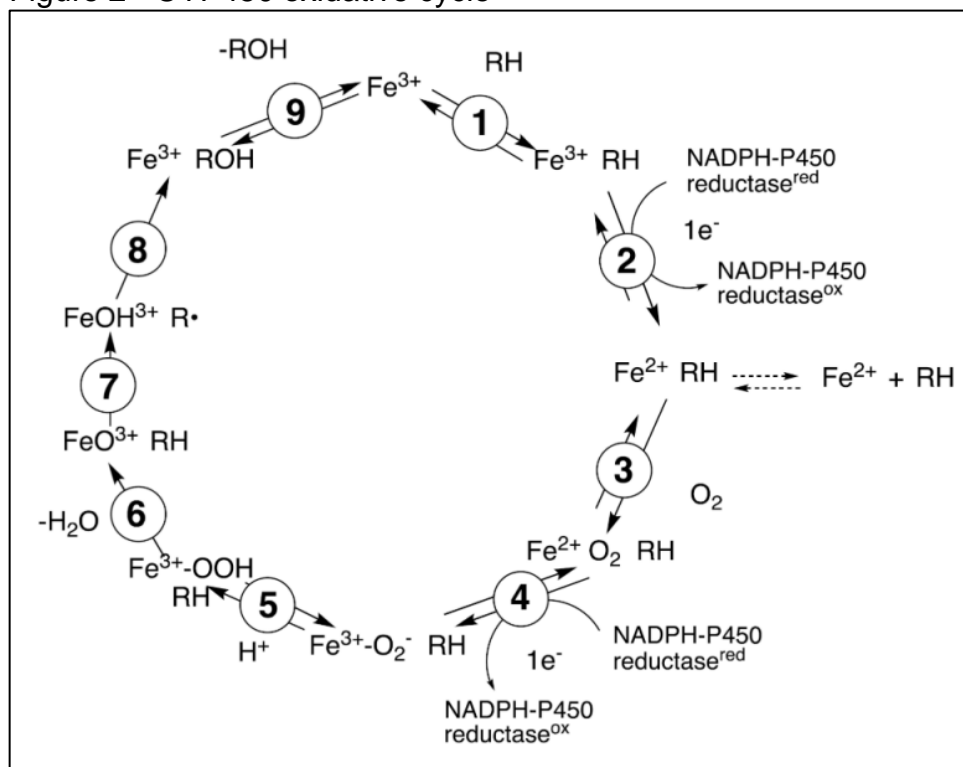
Source: Adapted from (YANG et al., 2015)

The CYP450 family is considered a hemoprotein because it contains in its active site an invariable chemical component called heme (ferric porphyrin) which is also contained in hemoglobin (TYZACK; KIRCHMAIR, 2019; USSELMAN, 2017). Proteins that contain only amino acids are called simple, and in the case of CYP450 are called conjugates, the Heme being called prosthetic group (NELSON; COX, 2014, p. 89).

The enzyme NADPH-P450 Oxyreductase is a mandatory flavoprotein for CYP450 activity, according to (WASKELL; KIM, 2015). Its activity is by removing electrons from the donated NADPH to the heme acceptor of CYP450.

In the third phase, advancing in Figure 2, one molecular oxygen enters the reaction forming a conjugate $Fe^{2+}O_2RH$ and later $Fe^{3+}-O_2-$ RH, in which the iron is once again oxidized. Because it has iron in the Heme group, CYP450 is also considered a metalloprotein. This feature is fundamental because oxygen arrives in the triplet state

while organic molecules are in the singleton state, which is a ban on spin, especially iron metal breaks this barrier (YANG et al., 2015).

3.2 CYP450-3A4 ENZYME AND YOURS INHIBITORS

Given a simple mathematical relationship, given that a drug with potential toxicity and a low therapeutic index, and its metabolizing enzyme is inhibited, an adverse effect can easily occur in the drug treatment process as there will be no systemic clearing. Such substances that inhibit their enzyme are called inhibitors of this enzyme. (DERUNGS et al., 2016; USSELMAN, 2017; YANG et al., 2015).

Going in the direction of providing statistical data on the use of CYP450-active drugs, the drugs were classified as inducers, inhibitors or substrates according to specialized literature. Totaling 94 (59.1%) of the drugs acting on CYP450 enzymes and 172 patients (90.5%) using any of these drugs, many with polypharmacy because they are all elderly. Another very important fact is that 47 (29.6%) of the drugs are inhibitors of CYP450-3A4 enzymes and may cause toxicity (BRAZ et al., 2018). Both CYP450-3A4 inducers and inhibitors were tabulated, and here we adapt only inhibitors in Table 1.

Table1 – CYP450-3A4 enzyme inhibitor drugs

Weaks	Valproic Acid, Carbamazepine, Cyclophosphamide, Clonazepam, Dexamethasone, Diazepam, Entacapone, Glibenclamide, Hydralazine, Losartan, Mirtazapine, Omeprazole, Paracetamol, Paroxetine, Pravastatin, Prednisone, Propranolol, Risperidone, Sildenafil, Theophylline, Ticlopidine, Trazodone, Venlafaxine
Moderates	Cyclosporine
Strongs	Amiodarone, Amitriptyline, Amlodipine, Atazanavir, Atorvastatin, Caffeine, Cimetidine, Diclofenac, Diltiazem, Efavirenz, Erythromycin, Fluconazole, Fluoxetine, Haloperidol, Isoniazid, Midazolam, Nifedipine, Norfloxacinin, Norfloxacinin, Norfloxacinin, Noravoxacinin

Source: Adapted from (BRAZ et al., 2018)

A very interesting situation was observed in the study (HO et al., 2010) which started from the suggestions given in some studies and tested whether garlic supplementation, a very common spice, in the diet was a factor inhibiting drug metabolism such as warfarin - anticoagulant - and saquinavir - antiretroviral, which are metabolized by CYP450-2C9 and CYP450-3A4. In this case it was concluded that garlic supplementation not only inhibited CYP450-2C9 activity but also reduced its expression. For CYP450-3A4 no change was detected. Although it did not affect CYP450-3A4, it demonstrates that foods can also generate inhibitions on cytochrome P450, and that it can target CYP450-3A4 enzyme.

From the same point of view and now applied to CYP450-3A4, grain fungi in temperate climates can produce a class of organic compounds called Enniatins, a secondary metabolite. The Enniatins B1 subtype substantially reduces hydroxylation levels of midazolam, a benzodiazepine anxiolytic that has been specially controlled in clinical practice, as a substrate for CYP450-3A4 (IVANOVA et al., 2019). Also demonstrating interaction with midazolam as a substrate for CYP450-3A4, plant *G. inflata* has moderate inhibition (LI et al., 2017).

3.3   MOLECULAR DOCKING

In silico tools exist for many applications ranging from single ribbon DNA analysis, gene expression in RNA transcription, to mRNA translation into a protein. From protein in its primary structure to a tertiary or quaternary structure many in silico processes need to take place and validations of the three-dimensional structures to build a closer representation of the real.

In counterpart addition to all of these previous steps there are in silico tools aimed at analyzing various aspects of the tertiary and/or quaternary structures of enzymatic action proteins.

The work entitled Computational Methods And Tools To Predict Cytochrome P450 Metabolism For Drug Discovery (TYZACK; KIRCHMAIR, 2019) brings a collection of many concepts and types and examples of in silico applications. It is divided into: methods for predicting the active site of metabolism; methods for predicting metabolite structure; methods for predicting the specificity of CYP450, and this is

very important for the present work because it deals with how a drug interaction and enzyme inhibition happens and the molecular docking method is responsible for showing the substrate binding event in the enzyme.

Molecular docking is a way to predict how one molecule can fit in other molecule, normally a micro molecule to a macromolecule, by generating a variety of conformations over these molecules and calculate forces like Van der Walls between they to evaluate the best position by lowest free energy one (SCHLEINKOFER; WANG; WADE, 2006). To do this task, many tools exists like AutoDock, DOCK, FlexX, GOLD, ICM, and so on.

To person that know about chemistry and biochemistry in a travel begin over Molecular Docking knowledge maybe think about a big amount of information that a computer should calculate and what information is necessary to do that. In general, Molecular Docking uses a model of reality that ignores or simplifies data to gain in the low-cost computer process. In alternativity, Molecular Dynamics uses a big quantity of data, but has a high-cost computer process.

The (SALMASO; MORO, 2018) argue that the Molecular Dynamics is the direction to the future, however, for this work where we must think in low-cost computing process the Molecular Docking satisfies this requisite and give to us a good solution.

Figure 3 - A typical docking workflow
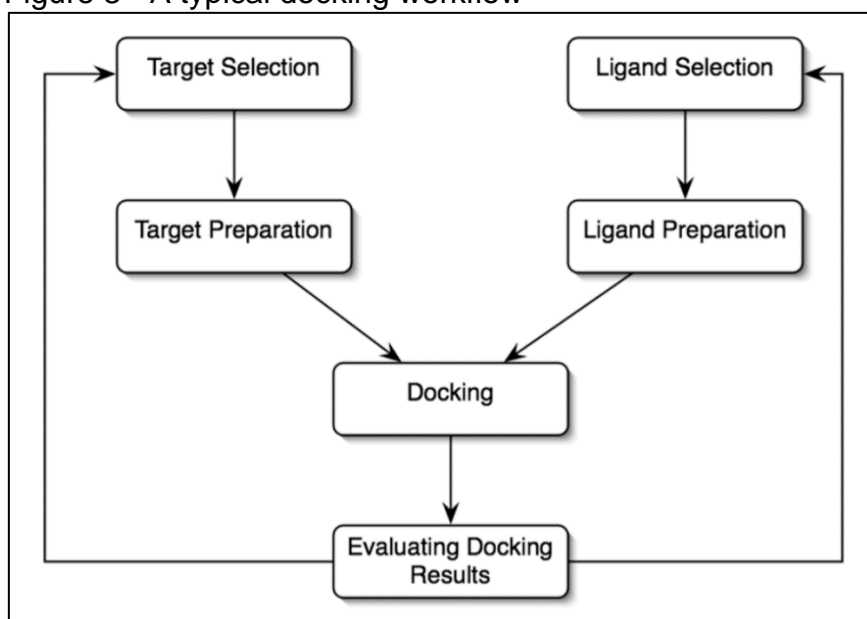
Source: Adapted from(SCHLEINKOFER; WANG; WADE, 2006)

The Molecular Docking process commonly is do with a micro molecule, the ligand, and a macromolecule with the active site, the target. In the   Figure 3, a typical Molecular Docking process is drawn with simples' steps: select the ligand and target, prepper these whatever necessary, execute the docking with some in silico software for this, and evaluating or analysis the results.

It is easier to realize a docking process with a drug and your protein target, in fact, this is a commonly use to this technique. We can think that molecules have flexibilities properties and movement in a variety of freedoms like translational androtational. In this way, the (SALMASO; MORO, 2018) classifies docking process by theses flexibilities and methods to calculate the process, see Figure 4. In this work, we won't describe all details about this, for this, see the reference.

Figure 4 - Molecular docking techniques organized according to ligand-protein flexibility and conformational searching engines.
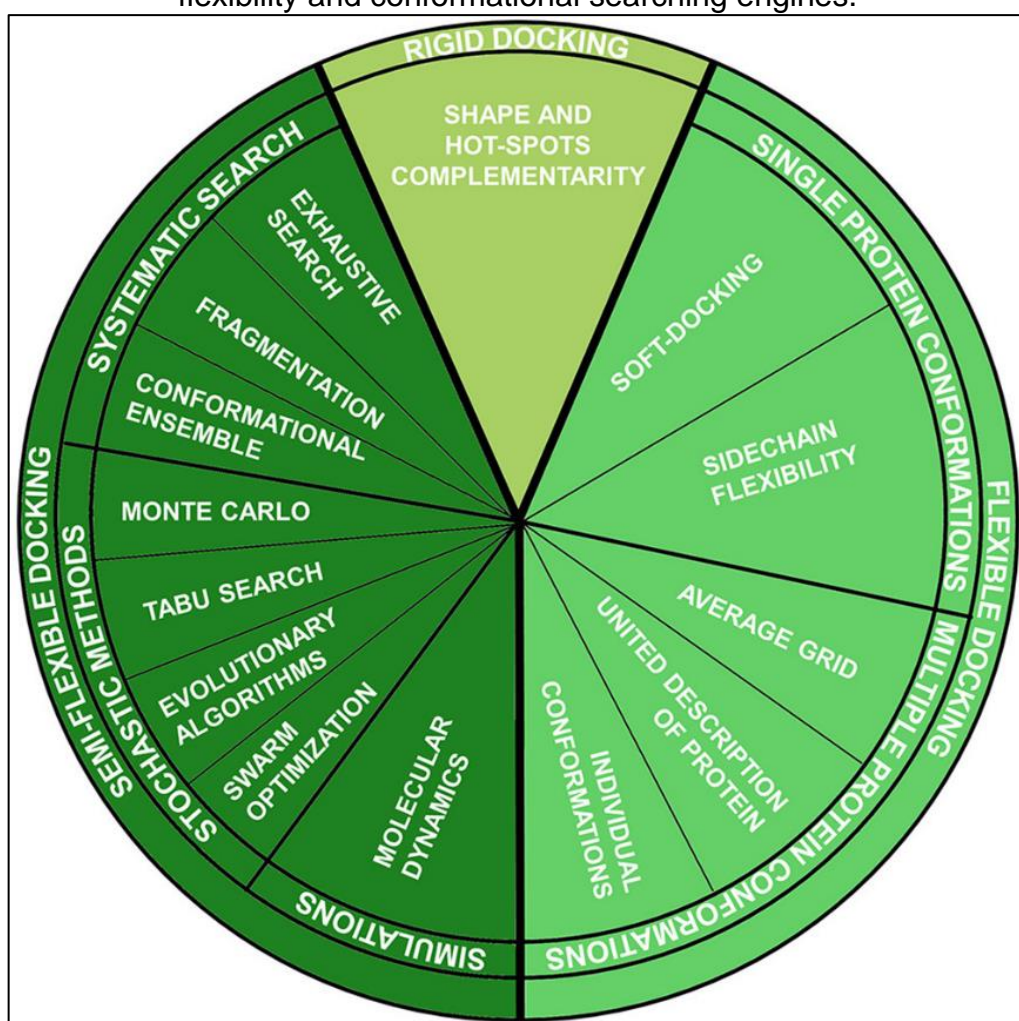
Source: Adapted from (SALMASO; MORO, 2018)

Assuming the computation cost problem this work will focus on a semi-flexible docking where the ligand can be flexible and the target can't. The ligand, for drug cases, is a small molecule in proportion compared to protein target. The flexibility of ligand augments the computation cost relatively with non-flexibility, however, this bring to result more details with a plausible cost.

3.4 CHEMICAL STRUCTURES

For understand about molecules is necessary to know about many things. One of this knowledge is about functional groups that would behave yourself like radical in a molecule, ex.: methyl, ethyl, phenyl, carbonyl (aldehyde or ketone), carboxyl, hydroxyl (alcohol), ether, ester, anhydride (tow carboxylic acids), amino, amido, guanidino, imidazole, sulfhydryl, disulfide, thioester, phosphoryl, phosphoanhydride, and others no cited (COX, 2013, p. 14).

From that, many based hydrocarbon compounds should be interpreted by having one or more radical or functional groups. Amino acids forming a group of several based hydrocarbon compounds that are the basis of proteins. All amino acids have obligatory two functional groups and a third radical that depends on your name. In the same manner, deoxyribonucleic and ribonucleic acids have functional groups, and innumerous other molecules in a cell.

The three-dimensional structure of give molecule is very important because one same molecule can be different configuration and conformation and this impact on interactions between biomolecules (COX, 2013, p. 16).

3.5 ORGANIC CHEMISTRY INTERACTIONS

In the organism, water is very important to guide many organic chemistry interactions. This chapter, mostly, is a resume of (COX, 2013) chapter two. Water is a universal solvent and it is capable to dissolve polar molecules and organize the nonpolar molecules in micelles.

Thus, water molecule breaks down your interaction with other water molecule in favor to do new interaction with an ion. Salts have two parts that dissolves in water, one anion and one cation, both interact with dipolar water. This is called ionic dissociation, because salts have ionic interact.

Water interaction occurs in two fronts, one hand by oxygen with two negative charges and by other hand by hydrogens, that each one has a positivity charge. In the case of NaCl, a salt, the Na is the metal and has a positive charge that interact with water oxygen. For Cl, a halogen highly electronegativity, it interacts with one of water hydrogen.

Not only attraction interaction that is provided by water. Repulsion is too an interaction supplied by water, and it is must important to life. This repulsion take place when water and some organic molecule interacts. This force happens because organic molecules are nonpolar and water are polar.

The lipidic bilayer that forms cell membranes is an example of molecules that have two parts, a polar head and a nonpolar tail. Cells exist in an aqueous place and have water inside your body. Water act repulsing from inside and out the nonpolar part, and attract polar part of this molecules that form lipidic bilayer.

Water too helps to form proteins three dimensional structures because have amino acids that can be a side chain polar or nonpolar. The nonpolar amino acids are pushed into protein body because repulsion interaction with water and polar amino acids are pulled out to protein surface to attraction interaction with water. When protein interact with water because your polar amino acids and stay in the solution this procedure is called solvation.

The van der Wallsinteractions are forces that can classifies some those cited above or more. For (COX, 2013, p. 53), the van de Wall interactions are synonymy of London interaction, but for (SOLOMONS; FRYHLE, 2012, p. 76)the synonymy isn't true, but the London interaction are a subpart of the it. Here, we will use this last concept.

The van de Walls interactions are dipole-dipole, hydrogen bonds and dispersion forces. The dipole-dipole occurs when a molecule has an atom with strong electronegativity and pulls the electrons towards itself, therefore, it has more

electrons on its side, forming a high-density cloud of them. The side with this cloud is more negative.

Hydrogen bonds are like a subtype of dipole-dipole that occur only when hydrogen is in a covalent ligation with oxygen, nitrogen or flour atom. These three atoms have a big electronegativity that pulls the electrons towards itself in a strong way and the hydrogen pushes the electrons because of your negligible electronegativity.

Dispersion forces occur when an apolar molecule has a moment when the uniformity distribution of your electrons has a disturb. At this moment, some side is more negativity and other side is more positive. This effect impacts another neighbor molecule that moves your cloud of electrons, by example, whether this neighbor molecule stays in the positive side, then your cloud of electrons is attracted to near of the first.

3.6   BIOCHEMISTRY INTERACTIONS AND PROPERTIES

A range of interactions occurs in vivo into the body of some animal, include de human body. An important set of interactions happen on proteins. These proteins and yours correctly conformations are very important to do many vital activities. These conformations depend on the combination of forces derived from interactions.

Proteins could have four levels of structures. The first level is unidimensional and is only a tape of amino acids. Among other properties, we shall remember that after a peptide ligation a reside of amino acid has two charges, a negativity with carbonyl and a positivity with amino group.

Those two charges are very important to build the second level, where the protein can assume two major structures the alpha helix or beta sheet, and exist some variations of both. These structures are mainly formed by hydrogen bonds between oxygen derived of the carbonyl and a proton derived of the amino group.

The proteins tertiary structures are formed when amino acid residues far from each other interacts. These interactions former the completely folded structure of a protein, and are molded by different kinds of weak bonding interactions. Other important

bonds are disulfide bridge, a type of covalent bond. The quaternary structures are a junction of tertiary like subunits of a protein.

3.7   LEARNING AND EDUCATION

Technology and informatic have become a reality in life today. Since more than a decade, this is a reality observed and this is called digital culture (CASTELLS, 2008).

The bioinformatic is a discipline necessary to insertion in modern scientific research, to develop scientific community with skills and knowledge in the life science and to prepare the educational community to lesson for informed citizen. More than, it has powerful tools to do a more solid learning for students improve yours concepts in curricular matters and informatic abilities (MACHLUF et al., 2017).

It is argue in (PINTO; BOTELHO, 2012) that frontiers between work, entertainment and learning are decreasing, in this way, it is necessary to rethink how to teach and what are the tools that can be significant in learning. Thus, software that most look-like electronic games where the ambience is more rich, interactive and stimulating are better.

In a society that technology is ubiquitous, the learn is more effective whether the content is near to the student cognitive structure, then it is nearby to significative learning.

Courses with a gamification proposal is one solution to ensure that students don't give up from it. This is the case of (NEVES et al., 2016) that found in others works and implements a gamification process to solve the problem of evasion in courses on the web. Gamification has goal to motivate the student in a process more attractive to learn.

The trouble with ways to visualize cells is one problem in education where students have difficulties with identification concepts because abstract and can't visualize that. To solve this problem (AUGUSTO et al., 2016) propose diminish distance between theorical and practical printing a three dimensional model with a 3D printing.

We can consider this cell printed object as an Learning Object as define in (BRAGA, 2015). Learning Objects has a diversity of conceptualization, overall, this can be

understood as any object thinking and planning to auxiliary in learning something in education process.

As the same way that (AUGUSTO et al., 2016) make to transform information and resources to a an physical object as an Learning Object is possible to transform or use digital resources *in silico* as too.

A model of learning is stated in Figure 5 where are input and outputs (skill, will, thrill), three phases of the learning process (surface, deep, transfer), and success criteria for the task. More others details are present in that work, but for here only the input, output and learning process will be used instead (HATTIE; DONOGHUE, 2016).

Surface learning process refers more to decorate facts without understand the whys about that fact. Deep learning process involve create your own idea fruit of your effort to interact with the matter and the correlation with concepts of it. Transfer learning process is relating to the ability to use the learned for reasoning over a context and apply this to another context.

Figure 5 - A model of learning
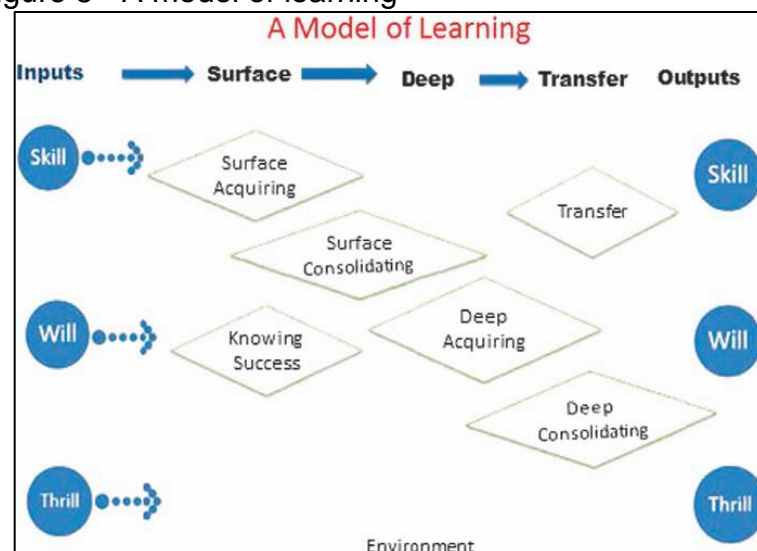
Source: Adapted from (HATTIE; DONOGHUE, 2016)

The North American psychologist D. P. Ausubel formulated one theory about learning that is called Significative Learning. In this theory is necessarily has the desire to learn, or in other words motivation,previous concepts or subsumption concept, and the content to be learning must be potentially significant (PELIZZARI et al., 2002).

Continuing the argumentation, (PELIZZARI et al., 2002) say:

> The more the new content is substantially and non-arbitrarily related to some aspect of the previous cognitive structure that is relevant to it, the closer it is to meaningful learning. The less this type of relationship is established, the closer it is to mechanical or repetitive learning.

Both (HATTIE; DONOGHUE, 2016) and Ausubel are correlated about the skills (as input) or previous concepts, the will and thrill that link with motivation. The content potentially significant is just one think to motivation, however, this is very important to be think and planning to augment the likelihood of attract and motivate the student to pass over all learning process. The content can be in the form of a Learning Object and in this work, we believe that would be fun and technological.

In direction to fun and motivational content is necessary conceptualize the playful in multiple ways in a wealth of details as being an essential feature of the psychophysiology of human behavior, a basic need for personality, body and mind, thus being an experience of fullness that we fully engage. The play becomes activity in the action itself and in the movement experienced, requiring an internal predisposition to go a little further. A learning process involving playfulness is not supported by the traditional liturgy of expository classes because it lives with randomness where the individual experiences his autonomy in a different time than the other, generating the possibility of self-knowledge. The playful facilitates learning at any age and exemplifies a child with a new object to explore, so it is in playing that cognition develops, thus being the education style for the third millennium for many educators (ALMEIDA, 2009).

With all discuss in this subchapter, the learn pass into some process, need some inputs, and delivery some results depending on the way that is approached. If this learning process is being support by a good experience and tool to facilitate the interaction with content, the likelihood of gain in learn is potentiated.

4   SOFTWARES, TYPE FILES AND DATA BANKS

On course to develop our solution for solve the problem of the gap in visualization the theorical to factual model, some tools are select and reported bellow.

4.1   AUTODOCK, AUTODOCK VINA AND AUTODOCK TOOLS

According to ("AutoDock", 2020) site, the first AutoDock version was written in Prof. Arthur J. Olson's Laboratory in 1989-1990 by Dr. David S. Goodsell with other name, AutoDoq. Thus, it was the first docking program to model the ligand with full conformational flexibility. After, the fourth version brought a capability to model flexibility both in ligand and macromolecule.

AutoDock Vina, that is only called Vina henceforward, is a new version of AutoDock, in truth, Vina is other software completely different to AutoDock. Analyzing AutoDock and Vina to the final user usage, Vina is more easy because has less steps (VINA, 2020). Vina has improved the accuracy and speed of docking relatively to AutoDock with your new score function, that is a calc to select the best ligand position and conformation (TROTT; OLSON, 2010).

Auto Dock Tools, that is only called ADT from now on, is a software developed by Molecular Graphics Laboratory (MGL) of The Scripps Research Institute, the same institute that developed AutoDock and Vina (MGLTOOLS, 2020). ADT are part of MGLTools and is a graphical powerful tool to visualize and configuration files to be use in AutoDock and Vina. Beyond this, ADT is too a possible way to visualize results from AutoDock and Vina.

AutoDock, AutoDock Vina and ADT has some official material to study like your own web site and documents like AutoDock3, AutoDock4.2.5, AutoDock4.2.6 users guide, AutoDock Vina Manual, and MGT Tools Documentation ("AutoDock Vina Manual", 2020; "Documentation — MGLTools", 2020; FORLI et al., 2012; MORRIS et al., 2014).

Like some a thousand other software of innumerous purposes, theses softwares too have many information in a vastly communities in the internet. Some in forum,

personal and institutional web sites, videos, and etc. The learn in the manipulation of the AutoDock, AutoDock Vina and ADT to this work was made both in official information and nonofficial, because the nonofficial has some approaches that is not achieved in official.

4.2 SMINA AND VINARDO

Smina is a fork of Vina that has implement a way to allow different score functions that can be selected by user when run a docking process. This project hasn't a nice website and is hosted in a count in sourceforge, where your poor documentation is too found ("Smina", 2020).

Vinardo, in its turn, is a score function that has a simplified calculation that improve your speed. Second (QUIROGA; VILLARREAL, 2016), "a combination of scoring, minimization, and re-docking on carefully curated training datasets allowed us to develop a simplified scoring function with optimum docking performance". Vinardo outperforms Vina in all tests.

4.3 DISCOVERY STUDIO 2020 CLIENT

Discovery Studio 2020 Client – DSC – is a software product belonging to Biovia. This company has many software solutions among these products to Collaborative Science, Unified Lab Management, Process Production Operations, and Quality and Regulatory Management as they classified. Beyond this, they have provided services like consulting, training and support.

Biovia has a mission to "enhance the scientific excellence and build the prestige of BIOVIA's scientists, software and services by guiding the direction of company-wide applied science innovation, in alignment with the company strategy" (BIOVIA, 2020).

For this work, only Discovery Studio 2020 Client was select because this tool is very well to use as viewer and manipulator ligand and receptor. In many cases, it found a crystalized protein with a ligand that can be a simple ligand, an inducer or an inhibitor, however we need to separated them for three reasons: more used to get

the protein, to get the ligand, or to get both to executing re-docking. DSC is the easiest tool to perform these tasks found so far by our search.

4.4 PROTEIN DATA BANK AND PDB DATA FILES

Protein Data Bank was the first to provide free access to digital files about biomacromolecules both in biology and medicine. This work began in 1971 with digital files arrived from nuclear magnetic resonance (NMR) methods. Today, digital files are provided by innumerous techniques like X-ray crystal structure determination, NMR, cryoelectron microscopy and theoretical modeling (BERMAN et al., 2002; BIOINFORMATICS, 2020).

All file in PDB has an PDB ID that is unique for each. Some protein can be having many representations and conformations depending on the method to obtain its, the ligand that come together in your active site, and so on. These characters can be show in each PDB file page.

In a PDB file page firstly us will encounter a PDB ID, some images about this protein, resources to visualizer and download this file, the method, resolution, citations, the history of version, and so on.

4.5 DRUG BANK

Drug Bank is more than an inventory of digital files on drugs but an encyclopedia that contains primally digital drugs files and more other information about these. Some information that we can found is: drug indications, drug binding data as well as drug-drug and drug-food inter-actions, the influence of hundreds of drugs on metabolite levels (pharmacometabolomics), gene expression levels (pharmacotranscriptomics) and protein expression levels (pharmacoprotoemics) (WISHART et al., 2018).

4.6   BABEL

In addition, to manipulate digital files about proteins, drugs or other molecule we can found these files in a vastly types and configurations. For our need, a PDBQT file is necessary to be convert to PDB. PDB is a filetype that can be found at Protein Data Bank and Drug Bank among others and this will be primarily used here. PDBQT is a extended PDB filetype that is create by ADT and has some information and properties necessary to do the docking process. PDBQT is made from a PDB file to be an input file, however after docking process an output file is generate in PDBQT that is not accepted in DSC. For this case, it will need the Babel to do this translation.

According to (BABEL, 2020) "Open Babel is a chemical toolbox designed to speak the many languages of chemical data. It's an open, collaborative project allowing anyone to search, convert, analyze, or store data from molecular modeling, chemistry, solid-state materials, biochemistry, or related areas."

Many information about a vastly supported filetypes, installation, details about conversions and filters, how to write software using the Open Babel library, and so on can be found in Open Babel Documentation Release 2.3.1 (HUTCHISON et al., 2011).

5 DEVELOPMENT

The development of this work will be giving in three times to do the theorical idea, implementation with your current's limitations and usage case with CYP450-3A4. Theorical idea remits to think an ideal solution that not always is possible to implement at all. Implementation will give life to the theorical idea and those technical features that was not possible to implement by any reason are the limitations. The last is the usage case with CYP450-3A4 where the implementation will be run with the receptor CYP450-3A4 and one or more ligand as inhibitor.

5.1 THE THEORICAL IDEA

In the direction of design the theoretical idea is necessary to get together all classes of tools and your relationship. These tools are data source, dockers, viewers, editors, and translators.

Figure 6 - Solution Architecture
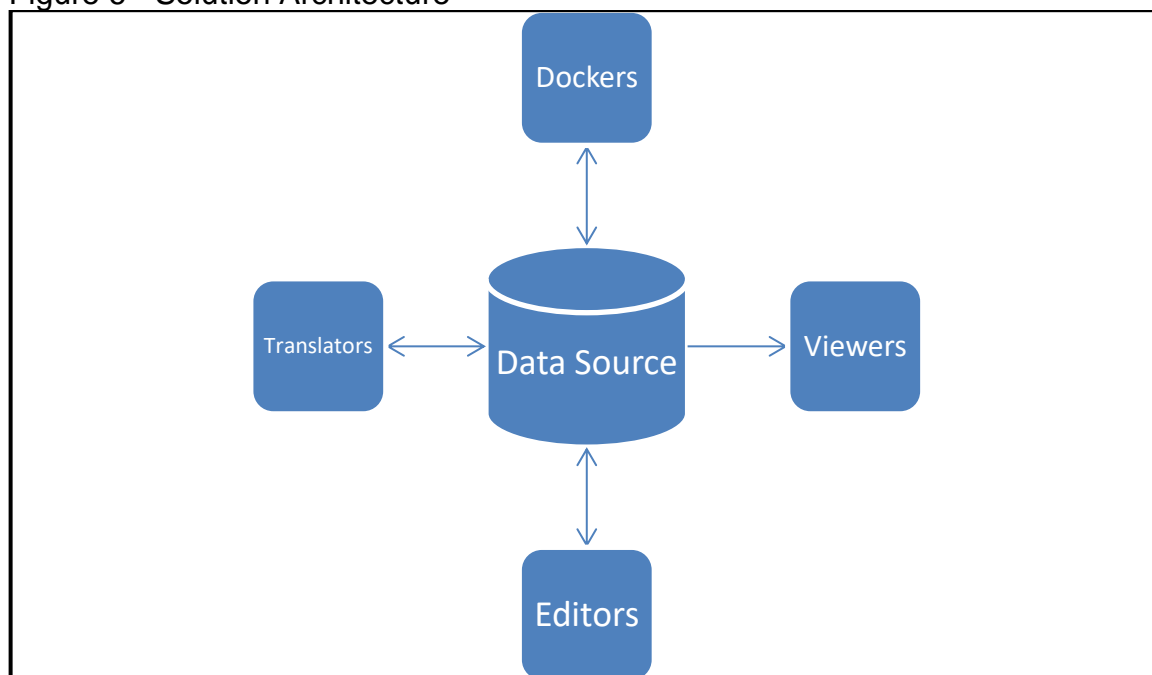
Source: self-elaboration

These tools class and your relationship constitute the solution architecture as can see in   Figure 6. The center module is the Data Source where all information is

concentrated and all others modules communicate with, in a bidirectional way, except viewers. From data source to other module it will be called a read process and going to the data source it will be called a write process.

The Data Source is a conceptual way to relate to the files of interest. These files can be accessed by many ways depending on the company web site, internet protocol, internet URL protocol, software facilities, local files, and so on.

Represented by arrows, the read process and write process are simple proceeding for computer user for many popular software solutions like a text, plan or slide editor because actual software for these generally hides all complexity behind the scenes. This facility isn't usually a truth for biological files.

The Viewers Module is a solution to transform the structured text from data source in a range of formats to show and manipulate vision in many perspectives. These perspectives can be linked one to other by example a three-dimensional view linked to amino acid letters sequence view that when select some molecule part in one view the selection will be show on another view.

For this work, the more important and mandatory perspective in Viewers Module is a three-dimensional to show the molecule with two basics manipulate functionalities: zoom and rotation. Furthermore, ways to visualize the three-dimensional are interesting like that in the Protein Data Bank 3D View tool called Style. This Style is a property of any visualizer tool, where we can view molecule structure in deferent presentation styles like: backbone, surface, cartoon, spacefill, licorice, and line.

The Editors Module is necessary to prepare molecules to docking. A range of preparations can be needed. The two most important are: cut off unwanted ligands from desire proteins obtained from one web Data Source and adding hydrogens which would be necessary for some docking tools.

Some others features can be interesting in the Editors Module like a possibility to adding or removing functional groups of the drug to execute docking process with a drug variant and compare these results. We come to think a way adding, removing or alter parts of proteins to analyses the results in docking process, but this is more complex than a simple edition. If in the future we found in literature some indication that it come be a simple task with some new method or tool, it will be automatic a part of this solution architecture.

The Translator Module becomes necessary when one data file in Data Source Module is incompatibly with some other module that is needed for a moment. A variety of filetypes exist for describe molecules like: pdf files, smiles, vmol, pdbqt, and so on. Each filetype can be differences in data that is stored. Because of that when execute a translation where the destination filetype need some information that isn't in the origin filetype is necessary be careful and study about this specific translation.

The Dockers Module is the principal and more complex process in our solution architecture. Exists in literacy a range of algorithms to solve this problem and each of that use some different theory, however, discuss this algorithms and theories aren't the goal here. The goal is to understand the use of the tools that will compose this module and how up to maximum the quality of the results when more than one result is given.

The solution architecture was think to be more flexibility in your implementation and cover a range other approaches in education like study the files by translation process, protein mutation edition, concepts related to lock and key analogy or induced fit concept by algorithms in dockers software, however it will funnel to study with a focus on visualization after docking, so let's establish a procedure on solution architecture. The data will be given in Data Source, treated by Edition and/or Translation if necessary, after the Dockers module will process inputs and output the results, again the Translation if necessary, and at long last the Viewers. This procedure is not rigid on solution architecture, still it is a way to maintain concentration on goal.

5.2   IMPLEMENTATION

The implementation will cover the established procedure on solution architecture. Firstly, for it, the Data Source module implementation, followed by a discussion of some needs of Dockers module implementation that be satisfied by Editors and Translators implementation, the Dockers module implementation then is present and yours results passed by Translator module and discussed on the Viewers implementation. As mentioned previously, the procedure is not rigid, one example of

this is that Viewers will be present in many situations disorderly in relation to the theoretical procedure.

The Data Source module has a folder in computer that will storage files accrue from Protein Data Bank and Drug Bank. Protein Data Bank is accessed by https://www.rcsb.org/ link and the search field provide a way to look up by PDB ID, author, macromolecule, sequence, or ligand. In most cases the search is by macromolecule name where the result show structures of it, citations that can be helpful for an academic work, some filters like: organism, taxonomy (eukaryote or bacteria), experimental method that this protein was obtained, and so on. Some sorting like the most important match score where it shows the quality of the assembly protein.

After the choice of one structure related to the desire protein by a click on the link in list result, a vastly information about this protein is show like a way to view 3D structure and a button to download in some formats, but for this implementation of Data Source module only PDB filetype will be downloaded to a local folder.

The Drug Bank is accessed by https://www.drugbank.ca/ link and the search field provide a way to look up firstly by name and after that a drug was found is possible to search by similar structures. In the similar structures screen appear some drugs with approximately structures and a way to drawing a new molecule to search from this.

It is pleasing to search in the Drug Bank, in contrast with Protein Data Bank, because here when one drug is search one result is given. This has a logical, drugs don't have more than one montage, on the other hand, proteins given its complexity can be more than one montage. After found a desire molecule, we can view it in 2D and 3D, this is what we consider too Viewers implementations, simple but powerful to the moment. In parallel, the download can be done and in spite of has many filetypes, the choice is always PDB format for this work. Again, the download will be executed in the same folder where the protein is.

Generally, proteins come with a ligand. In this case, it is necessary to edit the protein with aim to remove this ligand. This is one implementation of functionalities from Editors module. Thus, the Discorery Studio 2020 Client – DSC – will implement with an easy click selector on your implementation of Viewers module.

By a classic navigation to open some document in software to edit text, in DSC is the same File>Open and a popup window appear and is possible to navigate into folder that contains our desire protein. Here, it is an access to Data Source module by Editors and Viewers modules. Once that open the file one view will show the protein in 3D, where is allowed to select some molecule part with mouse click over or by hold press down in left mouse click and draw an area over the desire part. With hold press down right mouse click and move, the molecule will rotate in all three Cartesian planes.

Other way to do selection is by other Viewers module implementation by a right click over molecule in 3D view and in popup menu click in view>hierarchy. When the file come with divisions over molecule is fine to use that resource at hierarchy view, where sometimes is more ease to select some parts of interested.

Once one part is not interest for, it is possible to select that and delete by button 'Del' in computer keyboard. Other way is by selecting the part of interest and copy with classical Ctrl+c and past that in another new file, the cut with Ctrl+x also works. New file can be created by menu File>New>Molecule Window and after with the classical Ctrl+v to past into. To finish, save that by menu File>Save that will bring up a popup window. Write the desire name in field name, in the type field select the Protein Data Bank Files option, then click on button save.

Since we have a protein and ligand molecule to do the docking process, we will open Auto Dock Tools – ADT – to do some others editions and now translations. ADT implements solutions direct or indirect for all parts of our Solution Architecture, but ADT is not complete or the best for all. To explain ADT in your interest, each part will be relating over the process to prepare for the docking process.

Firstly, ADT implements Viewers module with a 3D and Dashboard views, where these are similar to 3D and hierarchy viewers respective in DSC, but the facilities to manipulate in DSC is better and easier for these views.

By clicking in menu File>Read Molecule, navigate to the desire folder, select and open the molecule that was prepared in before steps. It will be show in a 3D and Dashboard views. Here, the exploration in 3D has some differences in comparison with DSC. To rotate the molecule, use to hold press down left mouse click and move,

but to selection in ADT is not possible like in DSC, it is very limited and not interactively.

It is necessary to do some editions in the protein to prepper it for docking. One of this is remove waters and add hydrogens. Remove waters when they exist diminish the computer cost to realize this task. The Vina need the hydrogens that normally doesn't come in protein file. To realize these jobs is very easy by click in menu Edit>Delete Water and Edit>Hydrogens>Add>OK.

One time that we have prepared the protein is necessary now to translate this for the format required by Vina that is the PDBQT filetype. For this moment, the ADT implements one function of the Translators module. By click in menu Grid>Macromolecule>Choose a popup window with list appear to select the desire molecule. In this case, only one option is listed because one molecule was loaded in ADT then select that and click on button Select Molecule. A popup window with some information will appear and that can be closed, after, a window to save in PDBQT format is show and then save that in the same work folder.

The same translation is necessary to drug that will be the ligand in docking process, but the manner to do that is a little bit different. Firstly, is necessary to open the PDB file that contains this drug by menu Ligand>Input>Open and chosen that is computers files. The molecule will be show in a 3D and Dashboard views.

The Vina can be works with a flexibly ligand but this has a computational cost when more flexibly is the molecule. For this, the Vina accept a configuration to augment or diminish points of flexibility and this can be set by ATD by click in menu Ligand>Torsion Tree>Set Numbers of Torsions. A popup appear where is possible to choose the number of torsions and for this quantity if we want so be in points that impact more or less atoms.

The flexibility can be configured to turn not-rotatable some specific ligations as: all peptide backbone, amide bonds, guanidinium bonds, by selected atoms, or all rotatable bonds. To select one or more of these options is by click in menu Ligand> Torsion Tree>Choose Torsions. All these configurations tools are ADT implements of Editors module.

Now, it is important to translate too the drug from PDB to PDBQT filetype. To do that is very simple by click in menu Ligand>Output>Save as PDBQT. After that is only click in Save on new popup window of course choosing the work folder.

The next step is preparing the grid to docking process. The grid is a cartesian coordinates and size in Angstroms to a cube or parallelepiped that specifies a search space in macromolecule. This space is referent to that who we believe be an activity site where the drug will be to fit in.

ADT has a way to facilitate in a visual way to delimited this space for a grid. By click in menu Grid>Grid Box a popup window with title Grid Options appear and a parallelepiped is show in 3D view that whether rotate the grid show yours face in blue, red and green. On Grid Options color on controls help to better identifier the coordinates and size, one more control is the property called Space that for Vina is necessary to set for one Angstrom.

The easier and most use way to select an activity site is by use that come from indicated by ligand on original macromolecule. To facilitate this process is possible to load an original macromolecule with the ligand in 3D view in ADT. How are they the same one overlaps the other visually and the original ligand is show. Thus, it is possible to configure the grid on this visual information.

One time that we have the receptor, the ligand, the grid coordinates and sizes is time to make the configure file that will be the collection of parameters to be passed to Vina. This configure file need, as can be seen in   Figure 7, the receptor, the ligand, the out to output file, the center each cartesian axis and the size for each cartesian axis.

The configuration file must be saved with name conf.txt. The name is usage pattern, the extension is the basic text in Windows operation system, and on other like Unix based system the file has no extension.

In this point all preparation for docking process is made. As mentioned before the Vina is the principal part of Dockers module implementation. To run Vina firstly is necessary to use the CMD on Windows, also know how MS-DOS, however, on Unix based system like Linux or Mac-OS the same functionality is called Shell or Bash, and for all we should call Line Command.

Figure 7 - The conf.txt file

```
receptor = protein.pdbqt

ligand = ligand.pdbqt

out = out.pdbqt

center_x = -19.637

center_y = -19.851

center_z = -10.672

size_x = 22

size_y = 22

size_z = 22
```

Source: self-elaboration

One time on Line Command is recommended to navigate into the work folder to facilitate the construction of the command to call Vina with all your parameters. One time into work folder we can put the command Vina in Line Command and press enter on keyboard, this will run Vina without parameters and show to us all possible parameters, a specie of help or instruction manual.

By having all in hands, now is necessary to create the command to run Vina that is compose by name "vina" plus "--config" parameter plus the name of file that contains the configuration. The final line is "vina --config conf.txt". If you don't want to use the config parameter is achievable to pass all information by specify all parameters like this line example "vina --receptor protein.pdbqt --ligand ligand.pdbqt --center_x -15.01 --center_y -19.0 --center_z 10.0 --size_x 22 --size_y 22 --size_z 22 –out out.pdbqt". This last way is more complex to plan and execute because is all in one line which leads to a greater likelihood of errors.

One time that the command is written on Line Command is enough press enter on keyboard and expect the progress bar finish. When itfinishes, a small table with nine results sorted by scoring function will be briefly shown. The lower is affinity (kcal/mol) better is the result.

Navigating into work folder by way that wants is sure that a file "out.pdbqt" is there. This file encloses the nine molecules results that we will use with the protein to see the fit in activity site. However, this file is in PDBQT filetype that is not possible to be open in DSC then we need to convert it to PDB filetype.

The Open Babel implements a big range of functionalities in Translators module, but for us the only interest is in transform PDBQT in PDB. For this execute de Open Babel select the input format to PDBQT and the output format to PDB. After, select the file to be the input and the name to the output, finish, run by button with caption "convert". This process accesses the Data Source module in read and write process.

To finish the processes, we will return to DSC to join the macromolecule with the results ligand positions and conformations in the same view. By click in menu File>Open a popup window appear to select files, navigate to work folder and select the file with macromolecules and the file with results then click in open. It will create two news tabs with each one having at first a 3D view.

To joint these are necessary copy from results to macromolecule view and for this go to the view with the results. Choice a point with a considerable radius from the center of the molecules, press and hold left mouse button and draw a circle around the molecules, after, release the left mouse button. The molecules will be with gold color then they are selected. In the menu Edit>Copy you can copy this selection and navigating to another tab where macromolecule is found click in menu Edit>Paste.

Now, to analyses one by one ligand result is necessary to turn visible the hierarchy view by Ctrl+h. Clicking in checklist box is possible to turn on or off visible each result and so on work with it.

5.3   USAGE CASE WITH CYP450-3A4 AND INHIBITORS

With all things considered before, now is appealing to show this in practice. Because of this, in this subchapter we will discuss a docking process with a structure of CYP450-3A4 human protein obtained by Protein Data Bank with PDB ID 4D75 and the caffeine, Drug Banc Accession Number - DB00201, as strong inhibitor.

At Protein Data Bank site, the search was executed with string "p450 3a4" and the choice based on the higher match score that means select the first file listed on found list, because this list is organized by default higher to lower in match score. The match score higher means that the structure has more relevance. The caffeine choice to inhibitor is arbitrary because your popularity in Brazil both in the form of the coffee drink or pure nutraceutical pill.

On the computer is good practice create a folder called "WorkSpace". This is a classical good practice in programmer life to host yours projects. Inside this folder, each docker project is standardized by this work with macromolecule name and PDB ID followed by ligand and Drug Banc Accession Number, all this information separated by underscore character, prohibited to use any especial character or accent, and all letter caps. By example for this work case, the name to folder would be a formation like "CYP4503A4_4D75_CAFFEINE_DB00201". From now on, this folder will be cited as project folder and that is the local repository where the molecules will be downloaded as the way discuss in before subchapter.

Figure 8 - CYP4503A4_4D75, in the left with inhibitor and the right without inhibitor
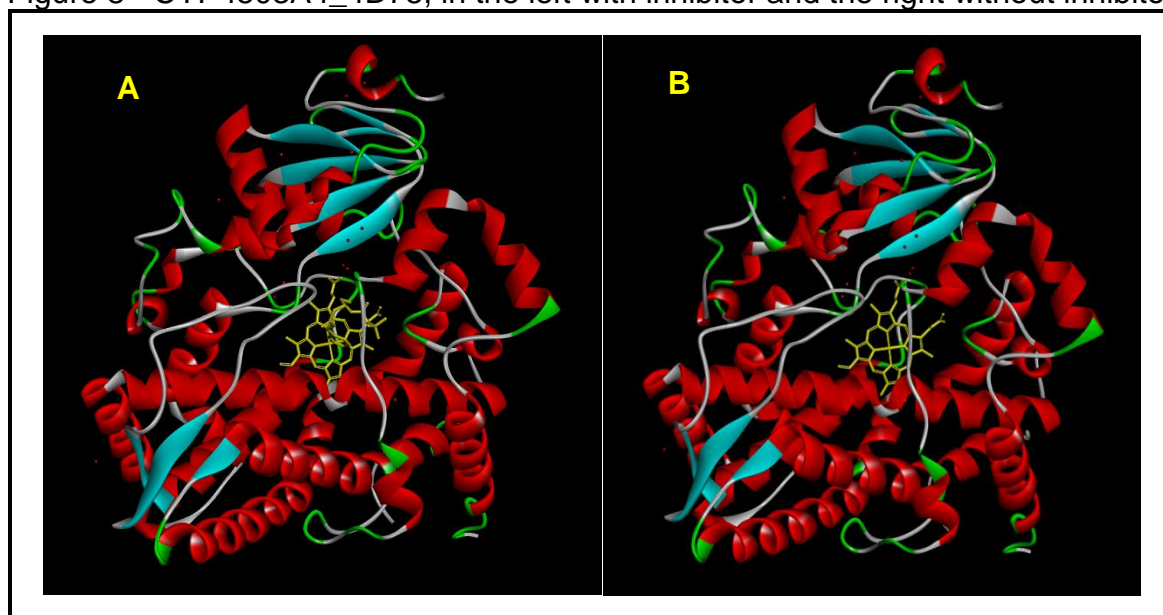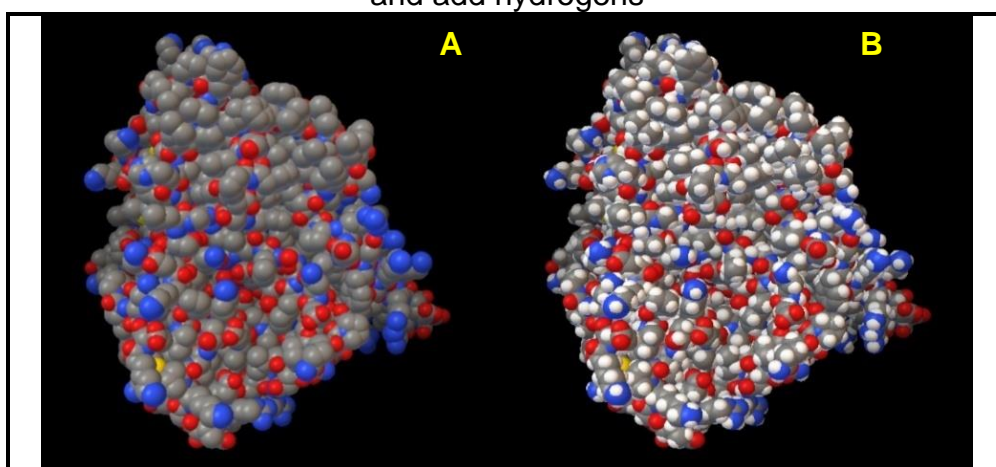

Source:self-elaboration

The 4D75 molecule comes with an inhibitor together and is necessary to remove it. For this, use the DSC to remove it like described before how to do this process. The before and after about 4D75 is show in Figure 8. The part A is with original inhibitor comes in 4D75 and part B is without removed by DSC. The part B must be saved, we

use to name only the name of molecule in human body like "CYP4503A4.pdbqt" into project folder.

Now is necessary to prepare the molecule to docking process adding some hydrogens and remove waters molecules from macromolecule, and next, translate both macromolecule and ligand to PDBQT filetype. For this use the ADT as was described before. The adding of hydrogens is very clear to see in Figure 9 by white balls in subpart B in contrast with subpart A.

Figure 9 - CYP4503A4_4D75, A: With waters and no hydrogens; B: Without waters and add hydrogens

Source: self-elaboration

Figure 10 - The Grid Box configuration.
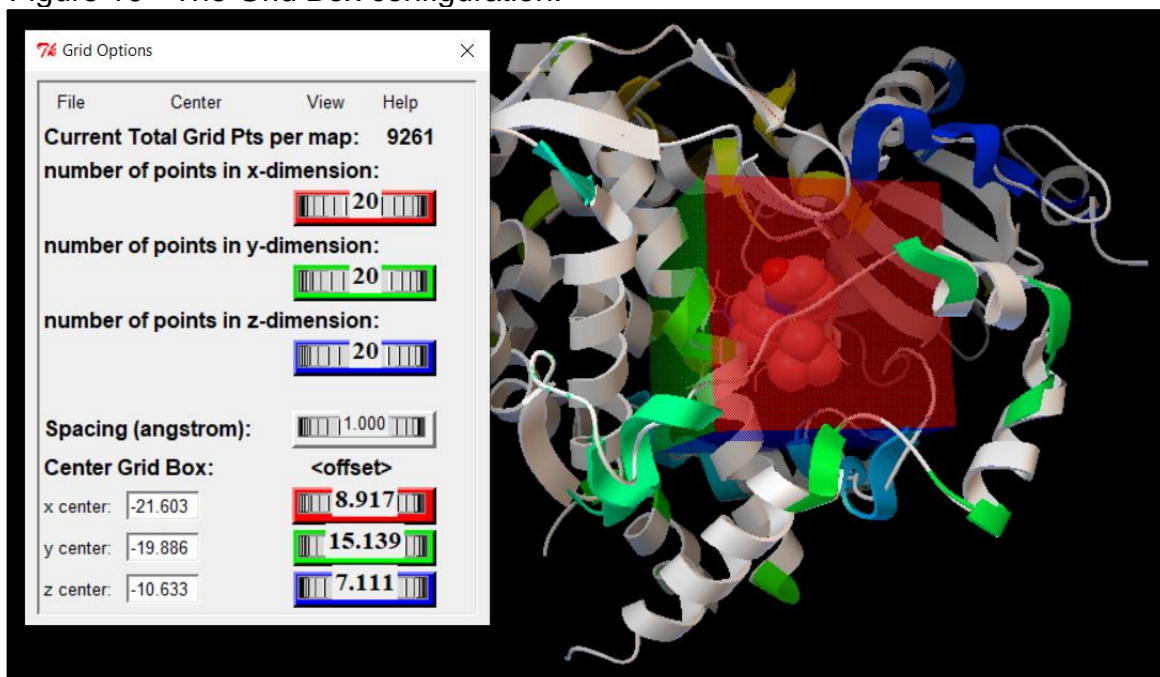
Source: self-elaboration

During the save of macromolecule in PDBQT filetype a messenger will pop up to talk that de Fe (iron) atom is without a charge. It is because Vina accept Fe atoms but can't assign a charge to this. Many messages in official e-mail list and forums were consulted and many people has this problem but no one know how to solve it, at least, in the searches performed nothing was found. In this work, we will ignore this problem.

The drug needs to be set about your flexibility and translate from PDB to PDBQT filetype. In the specific case no one set about flexibility of the Caffeine will be done because your structure is all rigid. Caffeine is a xanthine molecule with three methyls add to it and has an official name as 1,3,7-Trimethylxanthine. Xanthine base is rigid and these methyls don't add a chance to flexibility. To finish, save the molecule as PDBQT as specified before with name "caffeine.pdbqt".

Figure 11 - The conf.txt file.

```
receptor = CYP4503A4.pdbqt

ligand = caffeine.pdbqt

out = out.pdbqt

center_x = -21.603

center_y = -19.886

center_z = -10.633

size_x = 20

size_y = 20

size_z = 20
```

Source: self-elaboration

The configuration file is show in   Figure 11 where all needed information to run docking process in Vina is show. The information about coordinate over the Grid Box was perform with original macromolecule to view the ligand that comes with, as show in   Figure 10. Now, it run Line Command, navigate to the project folder and run Vina.

Figure 12 - Part A: Begin of docking process; Part B: End of docking process.

Source: self-elaboration

In the   Figure 12, the A part begins in navigation into the user folder to project folder, after executing Vina by command "vina –config conf.txt". The B part show the execution with a progress bar and a table result. The next step is convert result file "out.pdbqt" to "out.pdb" by program Babel as show in   Figure 13.

Figure 13 - Babel convert from PDBQT to PDB

Source: self-elaboration

By finish, open the DSC and into it open the "CYP4503A4.pdb" and "out.pdb" files. Copy from the "out.pdb" all content and past into "CYP4503A4.pdb". The Figure 14 show this result, but only one result is visible because was select in hierarchy view only "out_model_1" to not tangle the view. The caffeine is the molecule in gold color.

Figure 14 - Enzyme CYP450-3A4 with caffeine as inhibitor ligand show in DSC.
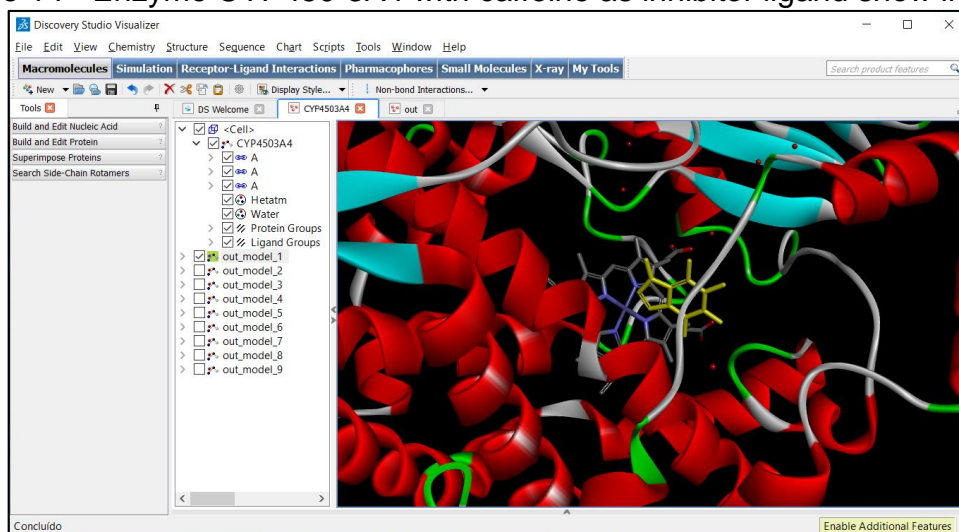
Source: self-elaboration

By the way, this subchapter shows the usage case with CY450-3A4 and the caffeine as inhibitor ligand in a docking process. This is an important and complex step to achieved a way to analyze in 3D the ligand-receptor interaction in classroom.

5.4   DISCUSSION ABOUT MOLECULAR DOCKING AND LEARNING

Originally, the idea was to do a script in which the teacher would be capable to do more attractive material for your class. This material is a way to view molecules and your interactions nearer to real, to the three-dimensional immersion in contrast with the actual two-dimensional resources like blackboard and 2D figures in books. More than this, a way to allow interaction with like zoom or hide some parts, rotate the molecules, highlight parts or molecular interaction, show thorough atom information about each part, and so on.

This work can be seen much more beyond than a script to teacher make better your act to teaching. However, this work can be dismembering in a range of pieces like Learning Objects. These possibilities are the use of those tools to innumerous planning tasks to growth significance of content to potentially the motivation sensation.

Become with Data Source module is possible to use that by example to teacher about functional groups or enantiomers in Organic Chemistry subject, peptide

ligations or the proteins structure levels in Biochemistry subject, the drug molecules in Pharmacology or Pharmaceutical Chemistry subject, DNA and RNA details in Genetic subject. In three first cases are possible to think a Learning Object with the Protein Data Bank or Drug Bank as a source of information and too as an easier, free, simply and low computer cost Viewers module implementation to see the three-dimensional of molecules. To DNA and RNA exist other platforms tolls and techniques, not cited in this work, however this can be related with the proteins in the level of *in silico* data.

It is feasible to argument about many others uses over these *in silico* tools like a Learning Object. Above all, more important to this work is discuss the result of docking process with the needs of visualizer details about the interaction macromolecule with ligand in activity site.

A new Learning Object is defined here with some resources, desired previous concepts that the student should have to possess to interact with it, and the expected learned concepts that the student should know after the free time of interaction.

The desired previous concepts will be the first to describe here because a logical reasoner. The student needs to know, at left and reasonably, the information present in the subchapters 3.4 up until 3.6. These is important to be capable to think about interactions of molecules. Others interesting knowledge are about Pharmaceutical Chemistry and Pharmacology.

The resources are a docked result, the macromolecule and the visualizer. The tools of visualizer have to show the interactions like hydrogens bonds, Van Der Waals forces, and so on, and show molecules in a variety of ways, highlight parts like the atoms of lateral chain of an amino acid, contrast the limits of macromolecule with the ligand, and so on. The more resources that the visualization tool has is cool to the exploration and student can be learn it by yourself curiosity, however is much important that the teacher instruct the student about those minimum necessary.

This minimum necessary is just like in Figure 15, where has some colors margins edging areas of interesting to discuss. Into yellow margin in gold color a caffeine molecule as ligand near to the heme prosthetic group with some dashed lines from caffeine parts to macromolecule parts indicating the interactions.

The red margin shows the hierarchy of the data that is showing in visual three-dimensional molecule. There has too here the description of ligations showed into yellow margin. These ligations are sub items of "Non-bond Monitor 1" where is possible to see the amino acids and yours numbers involved in.

The orange margin another way to show data from molecules. Many tabs are there to aux in search the specified information. In the tab "AminoAcidChain" is possible to find the amino acids involved in ligations that we see into the red margin.

The blue margin, continuing in Figure 15, is where all interaction is configured to be show. In Interaction Types box is possible to select what favorable or unfavorable interaction that is desired to create, and by create we understanding as a visual object listed in data. The box Molecular Scope let to choose the coverage that will be applied a search over interactions, and finally, the box Selection Scope that is very important to not pollute it with more information that is necessary by select only interactions that involves the caffeine molecule.

Figure 15 - DSC showing some interactions after configured to.
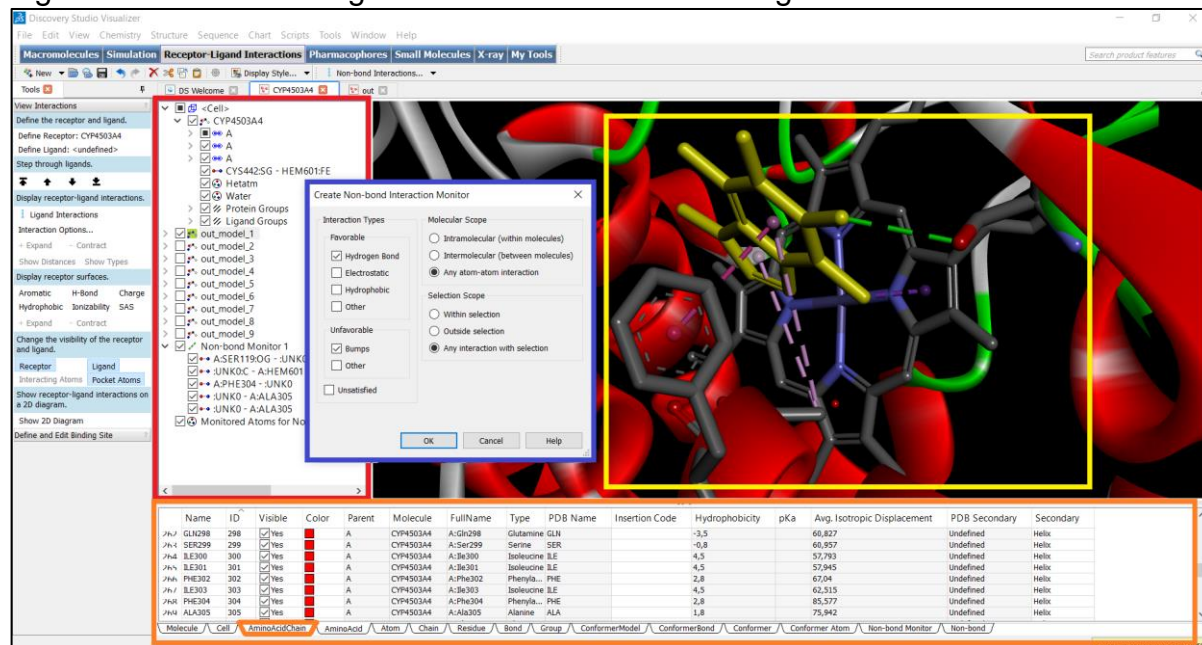
Source: self-elaboration

After this point, the student is able to navigate over the information, manipulate this tool and would explore this information in a goal to understanding the existing interactions between macromolecule and ligand.

The expected learned skills that the student should know after the free time of interaction are the capacity to recognize the attraction or repulsion parts between macromolecule and ligand, and the possible types of these relations. Plus, it is important in a future moment as a new interaction cycle with the Learning Object that the student make a correlation between your experience and observations on manipulating the complex macromolecule/ligand with the literacy about this.

6 CONCLUSIONS

After all discussed in theorical reference over a variety of subjects in the field of molecular docking and some about learning for the characteristics of new students that brings with technology in the education/learning process, and base on the build in this work, a list of important conclusions points is here put from now on.

Recovering the goal of this work to create a script to facilitate the molecules visualization and receptor-ligand interaction class planning for teachers in college subjects at the pharmacy course, it was thinking about what to use for a better class experience.

In this way, the motivation was conceptualized in context to approximate to the technological approach bringing with student reality and given possible more realistic interaction with content.

This basis to think a solution that involves tools that approximate to the student's reality and their entertainment preferences. It concludes an obvious fact that the blackboard is not a better way and today students have more familiarity with digital against pen and paper.

Another fact, the nature of molecules is in three dimensions and teachers attempt to draw it on blackboard or books to express this in two dimensions, by far it isn't the full experience in interaction with molecules.

Adding *in silico* software tools to this equation was the solution. It brought new concepts and problems. Concepts surrounding the idea lock and key analogy and more actually induced fit concept. Problems arising from the modeling of molecules and your interactions like a variety of forms to model and ways to calculate them.

We encounter a class of software called molecular docking that own an *in silico* superclass. These applications are capable to simulate, with limitations, a receptor-ligand interaction by a big amount of calcs derived of one algorithm and output in a file the result. All of that, receptor, ligand and output are files that has information about molecules that describe the same in a three-dimensions, but in text body.

To show receptor, ligand and output with a result of interaction, it is necessary to have programs specifically to visualize in three-dimensions. Theses software read

the text files with coordinates information about each atom in molecule and your ligations to show it in the screen. The free rotation is possible to see molecules in all angle combination of rotation in 360º degrees in each axis for three-dimensions.

In possession of these resources, this work proposed, in a light way, a manner to prepared a class plan with these softwares and how to use this for education/learning process, in fashion to improving the molecule visualization and decreasing the gap between theoretical description text and two-dimensions pictures to real things.

Some limitations in this step is that these *in silico* tools have a computational problem, if how much is detailed the model of representation like in a model that comprise induced fit concept then more computational cost. Because of that problem, we choose a representation model that covers a model more like lock and key analogy, and thus, these propose is more compatible with personal computers, because this model has less details to be computed in calculations.

The script to prepare a class was executed and tested with a protein called CYP450-3A4, an important enzyme to oxidize drugs, but some drugs are your inhibitor and this can carry a drug-drug interaction with the patient use two or more drugs, and this may cause an intoxication. Besides that, many strong CYP450-3A4 inhibitors was used to execute and test the script.

The results must be compared with literacy by the teacher and student to check with molecular docking process results. Even so, with a variety of limitation in each one docking result because computational scarce resource, we believe that this proposed solution has a good quality to use for education process in reference of exemplification better than traditional two-dimensional ligand-receptor interaction.

For the future works, the application of these knowledges gained here by the construction of this proposed scrip to make Learning Objects to aux the process education/learning will validate e enable to do improvements.

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