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The Effectiveness of Using Three-Dimensional Visualization Tools to Improve Students' Understanding of Medicinal Chemistry and Advanced Drug Design Concepts

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Abstract. Computer technology is an integral part of modern research. Most undergraduate pharmacy students are not aware of the use of these techniques in the drug design process. Understanding drug-target interactions plays a vital role in the drug design process, however, teaching the molecular basis of drug action is one of the major challenges we face in medicinal chemistry courses. The increase in the availability of three-dimensional macromolecule crystal structures and computer visualization software have provided better tools to study the drugs effect at the molecular level. This study evaluates the effectiveness of using three-dimensional macromolecule visualization tools in medicinal chemistry lectures on the students understanding of the molecular basis of drug action and drug design concepts. The different examples presented in this work are part of the teaching material that were developed to suite the learning objectives of the course. In addition, the "macromolecular drug targets assignment" was introduced to the course in order to allow the students to have practical experience using the new *in silico* techniques. Two hundred seventy students were surveyed over the past five years, the result showed that the new teaching tools have increased students' interest in medicinal chemistry and allowed them to develop better understanding of the effect of structural modification on compounds' activity and structure activity relationship. In addition, it gave them an insight into the advanced methods used in drug design.

Keywords: three-dimensional visualization; macromolecular crystal structure; medicinal chemistry; drug design; drug-target interactions

1. Introduction

A plethora of three-dimensional (3D) models of biological macromolecules bound to natural substrates, and/or drugs have been elucidated, and are available for download from the RCSB protein data bank (PDB) (Berman et al., 2000). In

addition, the increase in computer processing power has dramatically improved and allowed for the development of many advanced drug discovery software and hence, an increase in the use of computer-aided drug design (CADD) techniques has been observed over the past years (Ferreira, Dos Santos, Oliva, & Andricopulo, 2015). The availability of genomic, proteomic, and structural information of macromolecular drug targets has established the role of rational drug design techniques in the identification, and development of novel compounds (Yu & MacKerell, 2017).

Medicinal chemistry is a core course in the pharmacy degree curricula around the world that focus on teaching a group of therapeutic agents with the focus on their chemistry, chemical structure requirements, mechanism of action, potency, selectivity, and structure activity relationship. Understanding the drug action at the molecular level is essential in deciding the optimum therapeutic approach for any clinical case (Fernandes, 2017). The binding of any drug to its target is highly dependent, not only on the drug's chemistry, but also on its 3D structure. In order for a drug to be active, its functional groups and the 3D shape should complement the site of action (Satyanarayanajois & Hill, 2011). The affinity and the type of interactions between the drug and the target would determine the effect and activity of this drug, any modification of the drug's 3D structure that result in an increase in its affinity and binding is expected to improve activity (Copeland, Pompliano, & Meek, 2006; Satyanarayanajois, 2010). Teaching the molecular aspects of drug-target interactions is not an easy task especially to undergraduate pharmacy students, and is considered one of the major challenges of teaching medicinal chemistry (Tavares et al., 2017). Students' usually do not correlate the importance of the interactions at the molecular level to the therapeutic effect of the drug (Venkataraman, 2009). Visualizing the drug bound to its active site and correlating its specific binding interactions to activity has been previously shown to improve students learning experience (Kurup & Sakharkar, 2019; Tavares et al., 2017).

1.1. 3D Visualizations of Macromolecular Systems

The recent improvements in experimental macromolecular structure determination provided increased information available about structural biology, which resulted in an increment accumulation of information about drug and/or natural substrates binding sites, and protein function (O'Donoghue et al., 2010). Studying the different types of drug-target interactions is necessary for understanding the biological effect of the drugs and is becoming increasingly important in the drug design process. (Günther, Boto, Contreras-Garcia, Piquemal, & Tierny, 2014). 3D Visualization is the essential tool required to study these systems. The visual inspection of different macromolecular systems is now more accessible than ever with the availability of a wide range of advanced software tools (Olson, 2018). The different software platforms and the increased computer power have allowed the scientists to visualize, study, understand complex structures (Chavent et al., 2011).

Drug design is becoming an increasingly important aspect of medicinal chemistry (Anderson, 2012). Taking advantage of the available information and the functionalities imbedded in different software more (CADD) techniques has been

used in the search of new drugs (Ferreira et al., 2015). Many software that exhibit imperative tools are available and used in the different phases of drug design process. A license is required to use the full functionalities of molecular modeling software; however, many software developers have a visualization interface freely available for academic use. Moreover, the new generation of students have increased affinity for technology that should be considered when developing new teaching methods.

2. Methods

3D visualization software was introduced as a teaching tool in the medicinal chemistry course lectures. The main aim of this newly adapted method is to increase students understanding of the molecular basis of drug action, and hence acquiring a better correlation between drugs' chemistry, and the therapeutic outcome.

2.1. 3D Visualization Teaching Tools

3D visualization tools have been shown to improve students' learning in medicinal chemistry (Ferk, Vrtacnik, Blejec, & Gril, 2003; Hayes, 2014). By moving from the traditional methods of teaching the molecular basis of drug action using two-dimensional (2D) structure activity relationship (SAR), students were getting better understanding of the drug effect and the influence of any changes in the chemical structure on activity, and hence, on the therapeutic outcome.

The work described herein focus on the use of 3D visualization in teaching the medicinal chemistry course. The improvement in teaching methods started by the use of the 3D software only to show the students the ligand bound within its binding site. Further improvements in the course has seen an increase in information extracted from the 3D protein structure to include: protein structure and function, binding site features, ligand 3D shape, ligand-target interactions, and functional groups of the ligand and target.

2.2. Active-learning Examples

The following active-learning examples are extracted from medicinal chemistry course teaching tools. The "discovery studio visualizer software"(Dassault Systèmes BIOVIA) was used to prepare structures downloaded from the protein data bank PDB (Berman et al., 2000), to suite the learning objective for each topic. Depending on the type of information available in the macromolecule file, it was used to understand and predict:

- The molecular basis of drug action
- The nature of the ligand-target complex
- Intermolecular interactions, and/or
- SAR principles

β-lactam Antibiotics

Peptidoglycan layer in the bacterial cell wall is essential for cell survival under normal conditions. The enzyme involved in peptidoglycan synthesis is penicillin-binding protein (PBP). This enzyme catalyzes the final stages of bacterial cell wall biosynthesis to preserve cell integrity. β-lactam antibiotics function by inhibiting

PBP that result in the inhibition of peptidoglycan synthesis leading to cell rupture and bacterial death (Nicola, Tomberg, Pratt, Nicholas, & Davies, 2010).

For a compound to bind to any active site, the functional groups and chemistry of this compound should complement those of the active site. Furthermore, the size of the molecule is controlled by the available volume of this binding site. B-lactam antibiotics are structural analogues of the D-Ala-D-Ala moiety present on peptidoglycan precursors. The PBP active site accommodates both substrates, Figure 1 is shown to students and they are asked to find similarities and differences between the two structures, most students would recognize the resemblances in the 3D shape in general, and the presence of carboxylic acid groups, and amide bonds (lactam ring in the antibiotic). This would establish the similarities that should be observed between the natural substrate and drug in general, β-lactam antibiotics in this example.

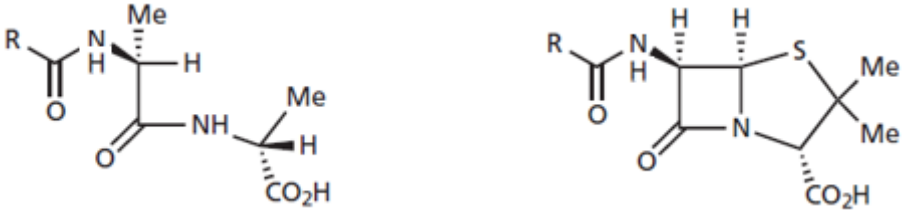

Figure 1: Structural similarity between Acyl-D-Ala-D-Ala and penicillin

A serine residue in the PBP binding site is responsible for the enzyme's activity, this residue undergoes nucleophilic attack on the carbonyl carbon of the β-lactam ring or of the penultimate D-Ala of the pentapeptide substrate to form a covalent acylenzyme complex (Figure 2) (Beadle, Nicholas, & Shoichet, 2001). The bicyclic system ring system is very stained which result in the high susceptibility of the ring's carbonyl group to nucleophilic attack. The relief of this strain after enzymatic β-lactam bond cleavage result in covalent bond formation and the opening of the β-lactam ring (Figure 2) (Lemke & Williams, 2007). The acylenzyme complex formed is structurally different from the hydrolysis intermediate and the enzyme's active site becomes unavailable to react with its peptide substrate, blocking further catalytic activity.

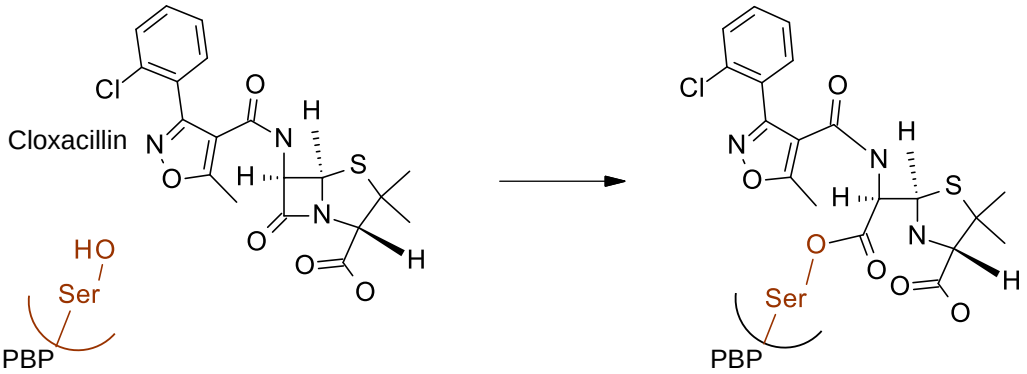

Figure 2: Mechanism of action of β-lactam antibiotics. Cloxacillin binding to the penicillin binding protein (PBP) active site

The 2D mechanism of action shown in Figure 2 is confirmed by studying the 3D structure of cloxacillin bound to PBP from *Escherichia coli* (Figure 3, PDB code: 3MZD (Nicola et al., 2010)). The binding interactions are shown to the students, an electrostatic interaction is observed between the carboxylic acid group and a basic amino acid (Arg248) in the PBP active site (Kishida et al., 2006; Nicola et al., 2010). This interaction will place the β-lactam ring in a position optimum for the nucleophilic attack by the serine residue (Ser44) resulting in covalent bond formation and ring opening. From the interactions formed in the PBP binding site, students can conclude the essential groups for the SAR of β-lactam antibiotics: a free carboxylic acid residue, intact β-lactam ring, optimum distance between the carboxylic group and the ring, and acylamino side chain.

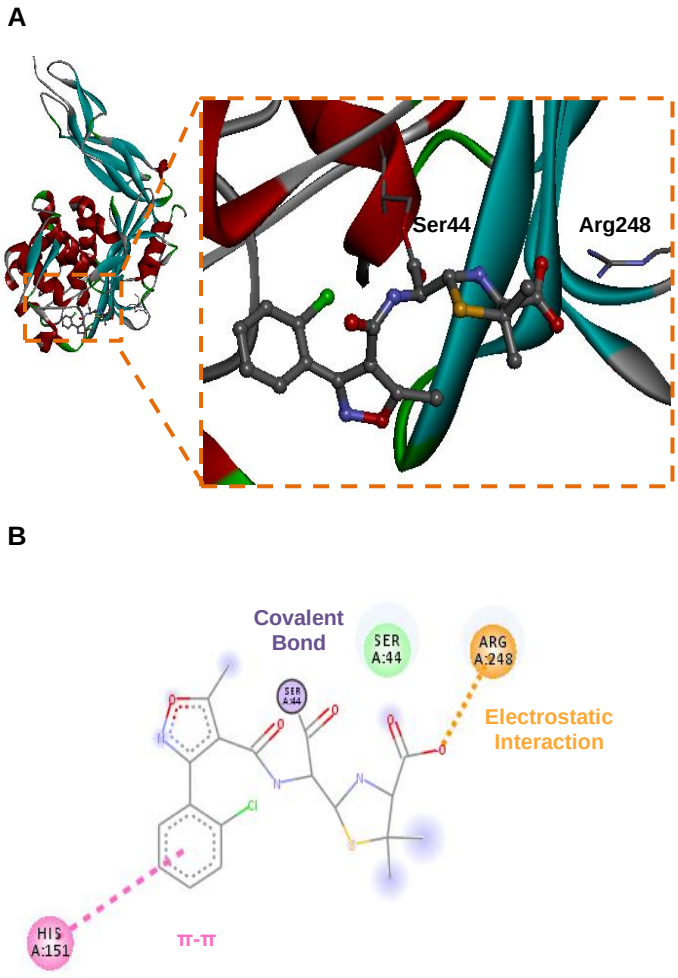

Figure 3. 3D crystal structure of cloxacillin in the PBP binding site (PDB code: 3MZD,(Nicola et al., 2010)). A) Cloxacillin interactions showing the covalent bond with Ser44, and electrostatic interactions with Arg248. B) 2D binding interactions. Not all interactions are shown, and hydrogens were removed for simplicity

Mineralocorticoid Receptor Agonists and Antagonists

Mineralocorticoid receptor (MR) is a steroid hormone-regulated receptor found in the distal nephron of the kidney, colon, and many tissues, including the brain and heart. MR regulates fluid and electrolyte (primarily sodium and potassium

ions) levels in the body and have a large influence on blood pressure.(Hasui et al., 2011) Aldosterone (Aldo, Figure 4), the primary natural ligand for MR, is reported to be synthesized in the heart, and blood vessels. Elevated levels of aldosterone are associated with the development of congestive heart failure, renal dysfunction, and hypertension (Vecchio, Procaccio, Viganò, & Cusi, 2007). MR antagonists, such as spironolactone (Figure 4) show antihypertensive effects in patients with essential hypertension and can be used in heart failure therapy (Pitt et al., 2003; Struthers, Krum, & Williams, 2008).

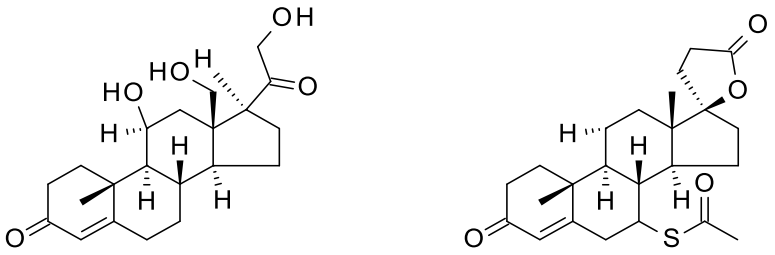

Figure 4: Chemical structures of aldosterone and spironolactone

Upon Aldo binding to the MR structural changes occurs in the receptor leading to its activation, a correct positioning of the agonist within the binding site is essential for activity. Conversely, once MR antagonists bind to the same binding site they would prevent MR from adopting the active conformation thus inhibiting the receptor (Bledsoe et al., 2005). In this lecture students are shown Figure 5 for the superimposition of MR in complex with Aldo (PDB code: 2AA2,(Bledsoe et al., 2005)) and spironolactone (PDB code: 2AB2,(Bledsoe et al., 2005)). The differences between the agonist and antagonist bound receptor (i.e. active and inactive receptor) forms are explained and related to their different therapeutic effects.

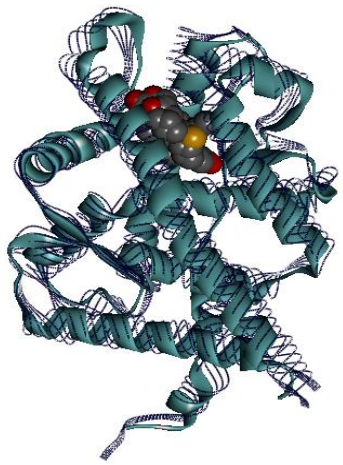

Figure 5: The superimposition of mineralocorticoid receptors in complex with aldosterone in line ribbon (PDB code: 2aa2,(Bledsoe et al., 2005)) and spironolactone in solid ribbon (PDB code: 2ab2,(Bledsoe et al., 2005)). The two ligands are bound in the same binding site; structures of the ligands are shown in CPK

To further explore the difference in the chemistry of the two compounds that would make such difference in the conformation of the receptor, Aldo and spironolactone binding within the MR binding site were studied. The role of the C3 keto group in the binding of Aldo, and other steroids, within the receptor binding site have been established (Kauppi et al., 2003). Spironolactone is characterized by a C17 γ-lactone ring which is characteristic to MR antagonist and, in contrast to Aldo, spironolactone has C7 thioester group. Mutation studies showed that the amino acid methionine (Met852) plays an important in MR activation, interaction of unsubstituted C7 in the MR agonists with Met852 is essential for the receptor to accommodate the agonist, and to acquire its active state. On the other hand, the steric hindrance of the C7 substation in competitive MR antagonists prevents the receptor from reaching the active form thus result in receptor inhibition (Fagart, Seguin, Pinon, & Rafestin-Oblin, 2005). Figure 6 shows the binding positions of Aldo and spironolactone in the MR active site with respect to Met852. Accordingly, students develop better understanding of the chemistry of MR antagonists from the interactions observed at the molecular.

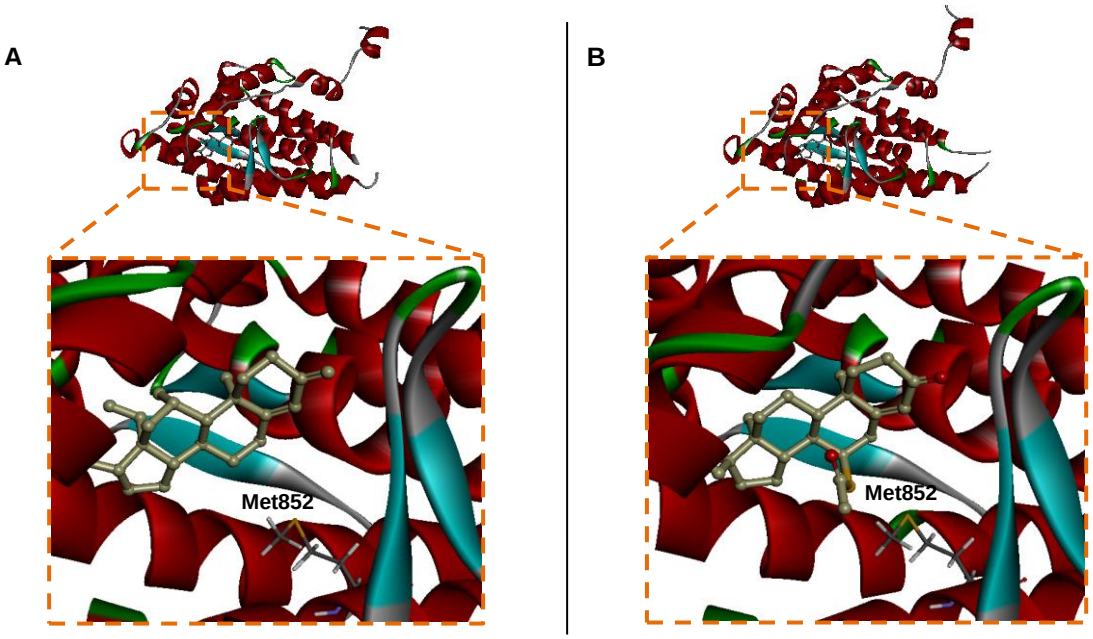

Figure 6: The ligand binding within mineralocorticoid receptor active site showing the amino acid methionine 852 (Met852). A) Aldosterone (PDB code: 2aa2,(Bledsoe et al., 2005)), and B) spironolactone (PDB code: 2ab2,(Bledsoe et al., 2005))

Cisplatin

Cisplatin, *cis*-diamminedichloroplatinum (II), is a chemotherapeutic drug used for the treatment of different human cancers including bladder, head and neck, lung, ovarian, and testicular cancers (Dasari & Tchounwou, 2014). Cisplatin crosslinks with the DNA purine bases, thus interfering with the DNA repair mechanism resulting in DNA damage leading to apoptosis of cancer cells.

Cisplatin, and other organoplatinum anticancer agents on the market, are platinum (PtII) complexes with square planar geometry (Figure 7). The net charge

of the organometallic complex is zero despite the Pt electron deficiency due to the electron-donating effect of chloride. For cisplatin to interact with DNA, the electron-donating chlorides are displaced through nucleophilic attack by cellular water to generate the active hydrated form with a net positive charge that makes it susceptible to nucleophilic attack by DNA bases (Figure 7).

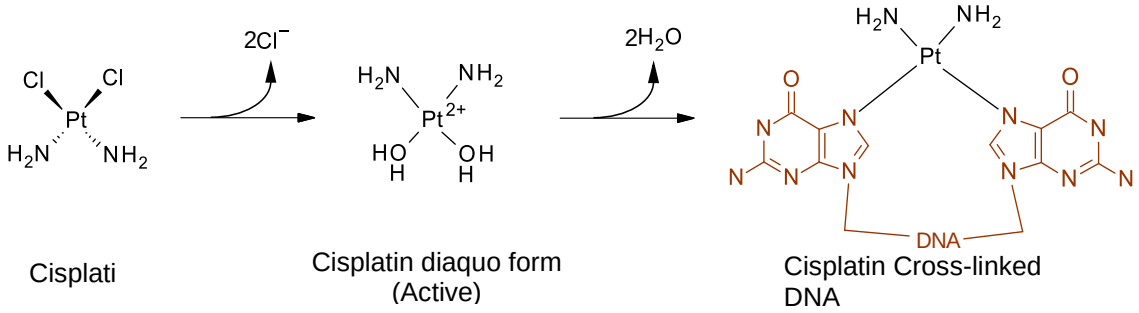

Figure 7: Activation of cisplatin and cross linking of the active form with DNA strand. Nucleophilic attack of N7 of the DNA guanine base on positively charged platinum and the formation of covalent bonds

The two ammine (N7) groups bind irreversibly to the Pt atom and the DNA bases become fixed to the compound in its *cis* configuration (Figure 8). Once bound to the DNA strand it blocks the H-bond interactions between the DNA strands and therefore hinders the repair mechanism resulting in cell death (Ohndorf, Rould, He, Pabo, & Lippard, 1999)

The students gain better understanding of the need for cisplatin activation to the positively charged molecule that can be readily attacked by nucleophilic DNA bases (Figure 7). The orientation of the organometallic compound requires the *cis*-isomer to be able to bind to the DNA strand in the right orientation (Figure 8).

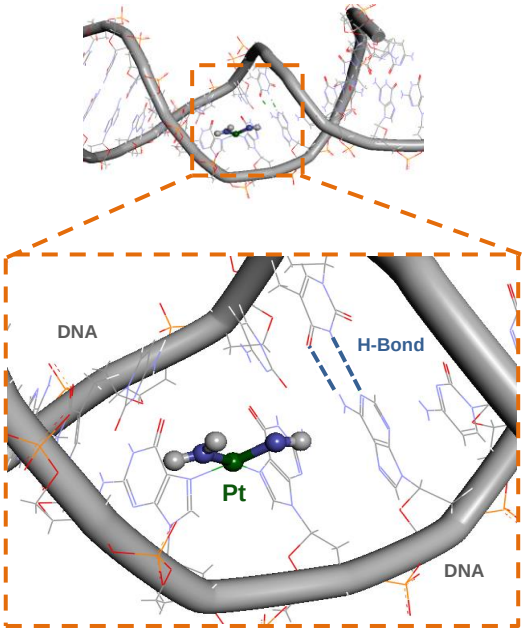

Figure 8: The 3D structure of cisplatin bound to DNA strand preventing H-bond formation between the two DNA strands (PDB code: 1ckt,(Ohndorf et al., 1999))

Topoisomerase Inhibitors

Topoisomerases are enzymes that control the degree of DNA supercoiling. These enzymes can be found in all cell types, and are essential for cell survival (Bates & Maxwell, 2005). Topoisomerases relax the supercoiled DNA strands during DNA replication and transcription to RNA. Due to their important mechanism of action, topoisomerases are key drug targets both for antibacterial and anticancer chemotherapy (Pommier, Leo, Zhang, & Marchand, 2010).

There are two types of topoisomerases: topoisomerase I (TopI) that cuts and religates a single DNA strand, and topoisomerase II (TopII) that catalyze the cleavage of double-stranded DNA (Bates & Maxwell, 2005). Anticancer drugs target different topoisomerase subtypes; for example camptothecins act on the eukaryotic TopIB topoisomerases (Venditto & Simanek, 2010), while human TopIIA are the targets of etoposide, anthracyclines (doxorubicin, daunorubicin), and mitoxantrone (Hevener, Verstak, Lutat, Riggsbee, & Mooney, 2018). On the other hand, TopII, DNA-gyrase, and topoisomerase IV, are found in all bacteria and are the targets of quinolone antibiotics. Humans TopIIA, an analogous enzyme to DNA-gyrase, does not bind quinolones at normal antibiotic concentration and thus maintain the selectivity to bacteria over host cells (Drlica & Zhao, 1997).

All topoisomerase enzyme inhibitors stabilize the topoisomerase-DNA complexes and hinder DNA religation step, leaving damaged DNA strands that are unable to replicate and, thus trigger cell death. The students are shown the interaction of the anticancer drug, etoposide, in human topoisomerase (TopIIB) in complex with DNA (PDB code: 3QX3,(Wu et al., 2011), Figure 9A), and of the antibacterial, ciprofloxacin, in the DNA-gyrase binding site (PDB code: 5BTC,(Blower, Williamson, Kerns, & Berger, 2016), Figure 9B). Students develop better understanding of the molecular mechanism of topoisomerase inhibition as they observe the chemotherapeutic agents interacting with the DNA and the enzyme, and blocking the DNA strand.

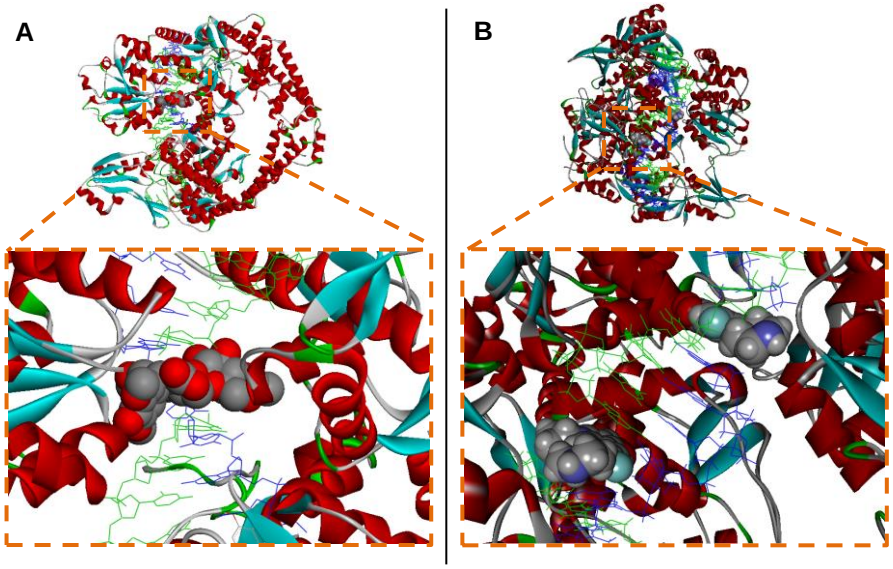

Figure 9: The interaction of: A) etoposide, in human topoisomerase (TopIIB)-DNA complex (PDB code: 3QX3,(Wu et al., 2011)), and B) ciprofloxacin in the DNA-gyrase binding site (PDB code:5BTC,(Blower et al., 2016)). Ligands are shown in CPK

2.3. Macromolecular Drug Targets Assignment

Computer aided drug design (CADD) techniques have played a major role in the development and optimization of novel bioactive compounds over the last three decades. CADD methods includes: ligand docking, molecular modeling, structure-based drug design, virtual screening, quantitative structure activity relationship (QSAR), and computational chemistry.

Although in medicinal chemistry, CADD are now considered routine approaches, there are still deficiency in teaching these fundamental concepts to undergraduate pharmacy students (Carvalho, Borges, & Bernardes, 2005). The dramatic increase in the number of biological macromolecular crystal structures elucidated and deposited in the PDB (Berman et al., 2000) has facilitated the CADD process.

The "macromolecular drug targets assignment" was added to the course in order to allow the students to have practical experience using new *in silico* techniques. The use of advanced software can be troublesome even for postgraduate students and researchers. With the introduction of the assignment to the course, we were faced with the challenge of training large numbers of students on using the 3D interface especially since students were not exposed to the use of modelling software. As a result, a comprehensive step-by-step manual (Appendix 1, can be used after the permission of the author) was made available to students that included an introduction into the PDB, and the 3D visualization software, as well as to figures showing all the steps required for use of the software effectively. Training sessions on the use of PDB and the software were held, students were offered a one to one help on how to deal with any problem in their work. Copies of the "discovery studio visualizer, 2017 R2"(Dassault Systèmes BIOVIA) software were installed in the all computers in the pharmacy building computer laboratory, and the software has been made available for students to download to their personal computers.

Each student group was asked to select a target, of their interest, from the PDB. At the end of the course, students are expected to report a complete description of the bonds formed between the drug and the binding site amino acid residues, the changes on the structure that are expected to affect activity, and to determine SAR from the bound drug conformation.

2.4. Survey

Survey statements were designed to assess the student feedback after the incorporation of the 3D visualization software as part of the teaching methods of medicinal chemistry course, in addition to evaluating their perspective on their experience using the software.

The survey was developed based upon initial positive feedback from many students' in the lectures to measure the level of the effect of using the new tools. In addition, we aimed at measuring students' experience in using the 3D software, it was not surprising that in the training sessions most of the students showed some discomfort and some showed their discontent. This was expected, as it was their first exposure to a very new and even no easy subject.

There were 23 statements, as listed in Table 1, that evaluated students in a three-point Likert scale using 'agree' to 'disagree' where answer '3' was considered

neutral. Since no previous studies were reported on the topic, the questionnaire has been developed based on perceptions of students throughout the course, and the help given to students in their assignments. At the end of the course and after presenting their assignments, students completed the survey anonymously.
All medicinal chemistry students at the end of six semesters after implementing the new tools were asked to fill in the questionnaire. Students had full freedman to refuse to participate in the study, and no incentives were offered to them. However, students were encouraged, and some were inspired, by the fact that finding of this survey will be taken seriously and, in the future, will help improve the medicinal chemistry course.

The average number of students in the medicinal chemistry course is 130 student per semester. From a total of around 780 students, the questionnaire was answered by 270 students. A sample size of 258 was needed to find sense in our study at a confidence level of 95% with a margin of error of 5%. The sample was diverse, as students responding to the survey were with a range of GPAs.

2.5. Student Performance

Students' performance was measured using students' marks in the different assessments, such as quizzes and exams; end of semester course evaluation questioner; and the course intended learning outcomes (ILOs) achievement. Marks for the questions covering topics where 3D examples were used were compared to the marks of questions of the lectures that only depended on conventional 2D SAR explanation.

Table 1: Results of Medicinal Chemistry Course Evaluation Survey

Question	Student Response: N: 270 (%)		
	Agree	Neutral	Disagree
The following questions are about the medicinal chemistry course:			
The layout of the course is suitable	183 (67.7%)	24 (9.0%)	63 (23.3%)
The course increased your interest in medicinal chemistry	92 (34.1%)	130 (48.1%)	48 (17.8%)
The course increases your ability to identify functional groups important for drug activity	260 (96.3%)	3 (1.1%)	7 (2.6%)
The course improved your critical thinking and problem-solving skills	176 (65.2%)	39 (14.4%)	55 (20.4%)
The knowledge gained from this course will be help me relate the chemistry of the drug to its pharmacological effect	263 (97.4%)	6 (2.2%)	1 (0.4%)
The examples covered in the lectures were relevant to other courses in the Pharmacy curriculum	264 (97.8%)	6 (2.2%)	0 (0.0%)
The course helped you understand some concepts covered in other courses in the Pharmacy degree curriculum	201 (74.4%)	8 (3.0%)	61 (22.6%)

The following questions are about the 3D tools used in the lectures:			
Before I took this course, I knew about RCSB PDB protein database	0 (0.0%)	0 (0.0%)	270 (100%)
Before I took this course, I saw a protein 3D structure	3 (1.1%)	0 (0.0%)	267 (98.9%)
Before I took this course, I knew that computer software are used in the drug discovery process	7 (2.6%)	0 (0.0%)	263 (97.4%)
3D visualization should be integrated in other medicinal chemistry courses	255 (94.4%)	10 (3.7%)	5 (1.9%)
3D visualization, where appropriate, should be integrated in other courses in the Pharmacy curriculum	263 (97.4%)	0 (0.0%)	7 (2.6%)
The following questions are about using the 3D visualization software in the assignment:			
It was easy to learn how to use the 3D software	43 (15.9%)	80 (29.6%)	147 (54.4%)
The training on using the software was a good start to using the software	269 (99.6%)	1 (0.4%)	0 (0.0%)
The one to one help from the lecturer was helpful in facing issues of using the software.	167 (61.9%)	103 (38.1%)	0 (0.0%)
Having the software installed in the Pharmacy computer Lab./ home computer improved your 3D software use experience	262 (97.0%)	8 (3.0%)	0 (0.0%)
The use of 3D software has helped you better understand the structural elements of macromolecules	198 (73.3%)	20 (7.4%)	52 (19.2%)
The use of 3D software has helped you better understand the drug-target interactions within the binding site	253 (93.7%)	2 (0.8%)	15 (5.6%)
The use of 3D software has helped you better understand SAR of drugs	240 (88.9%)	8 (3.0%)	22 (8.1%)
You enjoyed using 3D visualization software	97 (35.9%)	122 (45.2%)	51 (18.9%)
You feel that you are now familiar with the developments of drug design techniques	244 (90.3%)	0 (0.0%)	26 (9.6%)
At the end of the assignment your viewpoint about using the 3D software was more positive than at the begging	203 (75.2%)	16 (5.9%)	51 (18.9%)

3. Results

The results of the survey, Table 1, showed that more than half the students were satisfied with the course layout. The new methods appeared to increase the popularity of the medicinal chemistry course for some students, although the majority were neutral in this regard.

Some of the students verbally expressed their increased interest in the topic and some are now considering the topics of medicinal chemistry and drug design for their future postgraduate studies.

One of our best achievements was the unanimous answer that the course has increased the students' ability to identify functional groups important for drug activity. During the lectures, when asked about the importance of any functional group, students answered that it is important for activity and "it should be there". The different examples stressed that any essential functional groups present in the

drug is important for the drug action and any changes in the drug structure would have a great impact on its activity.

According to students' responses, 65% found that the course improved their critical thinking and problem solving skills. However, other more detailed tools may be used in the future to measure the effect of the new changes on students' abilities.

The majority of students found that medicinal chemistry was helpful while studying other pharmacy courses. For a student to be able to relate information of the chemical structure to the drug action was one of the driving forces to improve the course teaching methods.

Most students, prior to this course, had no familiarity with RCSB PDB protein database nor the availability of 3D tools and their utility in the drug design process. For most of them, it was the first time they have seen a protein 3D structure and were able to relate it to the information they studied in the biochemistry courses. Later in the course, they started to relate different concepts they learned in medicinal chemistry to the pharmacology courses. This have influenced them to want to have 3D visualization integrated in other medicinal chemistry and pharmacy courses.

As expected, most students did not find it easy to use the software. However, the training, one to one help offered, and availability of the software on university computer labs and on their personal computers have improved their experience.

The main aim of the macromolecular drug targets assignment" was to allow students to have practical experience in using new *in silico* techniques. The difficulty students faced using the software was expected especially that this was the first time they are using any drug design tool. Although most students did not enjoy using the software, it helped most of them to better understand the drug-target interactions within the binding site and SAR of drugs, and improved their view of the assignment once completed.

Adding to the survey results students' performance was shown to improve progressively in each semester. More correct answers were observed in questions of the 3D explained topics. In general, as more examples were added to the course material, we observed an increase in the overall students' marks in the different assessments. An improvement was also reported in the end of semester course evaluation questioner; and the course intended learning outcomes (ILOs) achievement values.

4. Discussion

One of the major challenges facing medicinal chemistry lecturers is to find a suitable teaching method to make the understanding of molecular aspects of drug-target interactions easier to undergraduate pharmacy students (Tavares et al., 2017). The incorporation of new teaching tools to medicinal chemistry courses have been shown to increase the students understanding, and interest in the course (Kurup & Sakharkar, 2019).

Using computational techniques to visualize 3D structures has been found successful in helping students understand the basics of macromolecules structure

and function (Abreu, Carvalho, Rabelo, & Castro, 2019). Furthermore, the transition to use 3D models and visualization tools to study these structures was fundamental to increase the students understanding of drug-target interactions (Cooper & Oliver-Hoyo, 2017).

The incorporation of laboratory experiments that aimed at teaching students the basic techniques of drug design has been found to increase the students' awareness of the roles of pharmacists in the drug design process.(Szarecka & Dobson, 2019; Tantillo et al., 2019).

The development of the methods used in teaching medicinal chemistry is becoming necessary, now more than ever. In the examples presented in this work, the molecular basis of drug action was introduced to students. The functional groups and the 3D requirements for binding any drug within its target active site were clearly detected by the students while discussing the bound conformation of the drug in the 3D interface. The different types of bonds formed between the drug and the binding amino acids were discussed. The changes of drug affinity, as reflected by the type of bonds formed, was made clearer to them. Students were able to identify the drug's functional group types and their position required for activity. The effect of the differences in strength between reversible and irreversible bonds to drug activity, side effects, and duration of action was experienced firsthand by students. The comparison between the majority of drugs in the market, that are involved in the weaker reversible interactions, to those of drugs with covalent bonds confirmed what students observed in the 3D structure. By comparing the structural features of agonists and antagonists and the effects observed in the overall 3D conformation of the receptor, students were able to differentiate the different SAR parameters required for the agonists and antagonists action at the molecular and effect levels. As we moved from traditional teaching and with the start of using the 3D visualization software, many students were overwhelmed by the experience, and most importantly, they were surprised to know of the availability of such technology.

The survey distributed at the end of the course has helped to obtain the students insight and perspective on the newly used teaching methods and the assignment. The success of using these newer methods had created a more interactive classroom environment. Most of the student, if not all, were interested in visualizing and discussing the 3D structure presented to them. A very positive response was reported, as well as an increase in marks of the students and in different course assessments. As expected most students found it hard to learn to use the software, but most agreed that the training offered was a good start. The one to one help was offered to all students, however, in all six semesters, not all students would seek such help. Students views about the assignment were generally positive once they became familiar with the fundamental tool of drug design process.

Adding to the survey results students' performance was shown to improve progressively in each semester. As expected students performance was better when answering questions covering topics where 3D examples were presented.

In general, as more examples were added to the course material, we observed an increase in the students' marks in the different in class activities, such as quizzes

and exams; end of semester course evaluation questioner; and achievement of the course intended learning outcomes (ILOs). Using 3D protein visualization tools in lectures has improved students understanding of medicinal chemistry and other pharmacy curriculum topics. In addition, many students indicated that they want more advanced tools to be used in different courses in the pharmacy curriculum.

5. Conclusion

The use of 3D visualization tools in teaching undergraduate medicinal chemistry has been shown to be a very valuable tool. A large positive outcome in students' understanding of the subject has been reported. Although the free interface of "Discovery Studio Visualizer" software lacked any advanced modelling tools, it was sufficient to reach our teaching objective. However, many online tools are freely available for use, and can be used for teaching and/or research. It comes as no surprise that most students faced some difficulties when they started using the software, especially that they were not previously exposed to 3D macromolecular structures nor to any visualization tool.

The medicinal chemistry course is usually perceived as difficult, hard to comprehend, and clinically irrelevant to pharmacy students. With the new tools, students gained a better understanding of the molecular basis of drug action and were able to understand, rather than just memorize, the SAR. In addition, the assignment has helped to integrate their chemistry knowledge to the other Pharmacy curriculum topics. In addition, some students showed increased in CADD.

6. Compliances with the Ethical Standards

Conflict of Interest: The authors declare that they have no conflict of interest.
The article is the authors' original work, has not received prior publication and is not under consideration for publication elsewhere.

Research involving Human participants, but the ethical standards are followed.

The Permission Note has been received to use any material in the manuscript such as figures etc. which is not original content.

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Appendix 1

Medicinal Chemistry

Macromolecular Drug Targets Assignment

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Medicinal Chemistry

Macromolecular Drug Targets Assignment

Introduction

The maintenance of life largely depends on the appropriate functioning of a range of macromolecular biopolymers that includes structural proteins, enzymes, receptor proteins and nucleic acids (DNA and RNA). These biomolecules particularly play an important role in the transfer of information between and within cells (signal transduction) that is essential for the coordinated functioning of the whole organism. A general mechanism for signal transduction involves the binding of a small molecule (ligand) to a macromolecular partner (receptor). This binding triggers a change in the 3-dimensional (3D) shape (conformation) of the receptor that leads to a specific biological effect. Examples of this type of system include the interaction of a hormone with its receptor and antigens with T-cell receptors. The binding interaction between a ligand and its receptor is required to form a tight and specific association in order for the induced biological effect to be controlled effectively. As these conditions require the receptor and ligand to be able to specifically recognize each other, the whole process of ligand binding to a receptor is termed "molecular recognition".[1]

Generally, when a ligand binds to its receptor no chemical bonds are formed and the binding is described as being non-covalent in nature. The strength and specificity of the interaction then depends on the accumulation of a large number of weak interactions such as hydrogen bonds, van der Waals and charge-charge attractions. The strength of these interactions depends on the structural complementarity existing between the ligand and its binding site. The original structural binding model for this interaction was proposed by Fischer in the latter part of the 19th century and described the ligand as a key being inserted into the macromolecular lock. This model for the interaction of a ligand and its receptor is still applicable today with the added refinement that both the ligand and the macromolecule are flexible in nature and so have some ability to mold to the structural features of the binding partner. This type of interaction is termed "induced fit" and is the currently most accepted model for the interaction of a ligand and its receptor.[2]

In recent times dramatic advances have been made in the structural characterization of both ligands and macromolecules and the structure of their complex. Experimental techniques such as nuclear magnetic resonance spectroscopy and x-ray crystallography have been developed to the point that determination of structures of complex biomolecules can be determined often in a matter of weeks. Additionally increases in computational power have allowed the development of powerful software capable of both developing model structures of macromolecular receptors and the graphical display of these structures on computer terminals. To facilitate the distribution of structural information regarding biopolymers international data bases have been developed to provide structure coordinate files for free download via the internet. The largest of these is the Protein Data Bank (PDB) that is operated through a number of mirror sites worldwide. The growth in structural information is exemplified by the growth in the number of structures deposited in the PDB with the first structure deposited in 1976 and by July 2019 greater than 154015 biological macromolecular structures are currently available for download.[3]

Macromolecular Biopolymer Structure

Macromolecular biopolymers fall primarily into 3 categories; proteins, nucleic acids or carbohydrates. Like all polymers each of these molecules consists of a long sequence of covalently linked monomeric units. For proteins these monomeric units are amino acids while for DNA and RNA they are nucleotide base units and for carbohydrates the monomers are sugars. For each biopolymer the monomeric units are linked together by specific covalent bonds: in proteins these are the peptide bonds and the full protein is also known as a polypeptide chain. This leads to the first structural characterization of biopolymers known as their primary structure. Primary structure is the linear sequence of monomeric units that make up the biopolymer thus for a protein the primary structure is the specific sequence of amino acids that constitute the polypeptide chain. Generally the primary sequence for the biopolymer is determined by sequential chemical degradation of the molecule followed by chemical analysis to identify the removed monomeric unit. So for a protein this constitutes sequential hydrolysis of the peptide bonds along the protein chain and chemical analysis to identify which of the 20 naturally occurring amino acids has been removed. Biopolymers on the other hand have various energetic forces that result in the molecules adopting an overall specific 3D shape (tertiary structure). These shapes are generally made up from collections of what is known as secondary structural elements including α-helices and β-sheets. A number of ways to depict the secondary structure elements exist when visualizing the protein structure.[1]

Macromolecules Ligand Binding

Ligand (natural substrates or drug molecules) binding by macromolecules are critical both to the healthy functioning of the human body and the therapeutic and toxic effects of drugs. The principles that control ligand binding by biological receptors are known as molecular recognition and depend critically on general complementarity in shape and chemical properties such as charge between the small molecule and the ligand binding site on the macromolecule.[2]

Research Project

This research project will use 3D visualization software to examine the structure of a macromolecular receptor molecule and the interaction of this receptor with specific ligand, which may be a natural compound or drug molecule.
Your results will be submitted in the forms:

1. Figure file sent by email.
2. Poster:
   Poster should be written in a scientific language and includes:
   I. Introduction: the implications of the macromolecule in health and/or disease states and the effect of the ligand binding on the protein.
   II. Discussion should include figures showing all interactions of ligand. Student should not copy material from reference sources including web sites and journal articles without proper referencing.

Software Used:

- Discovery Studio Visualizer, 2017 R2.[4]

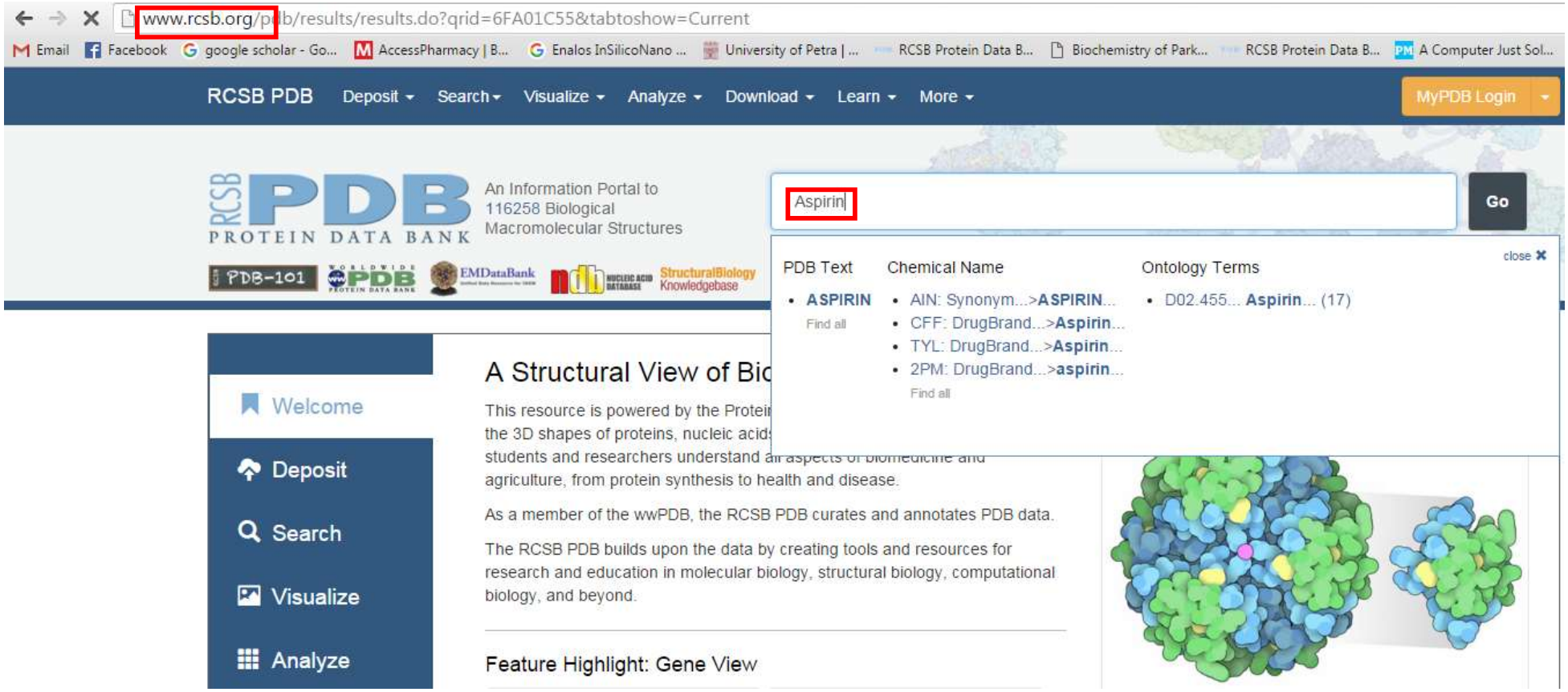
Software is installed in all Faculty of Pharmacy computer lab and a copy is available for students to install to their personal computers.

Assignment Guide

Visualization of receptor alone and in complex with the ligand molecule to examine overall structural features of the receptor, specific structural basis for interaction between the receptor and the bound molecule.

1. Search and select protein structure:

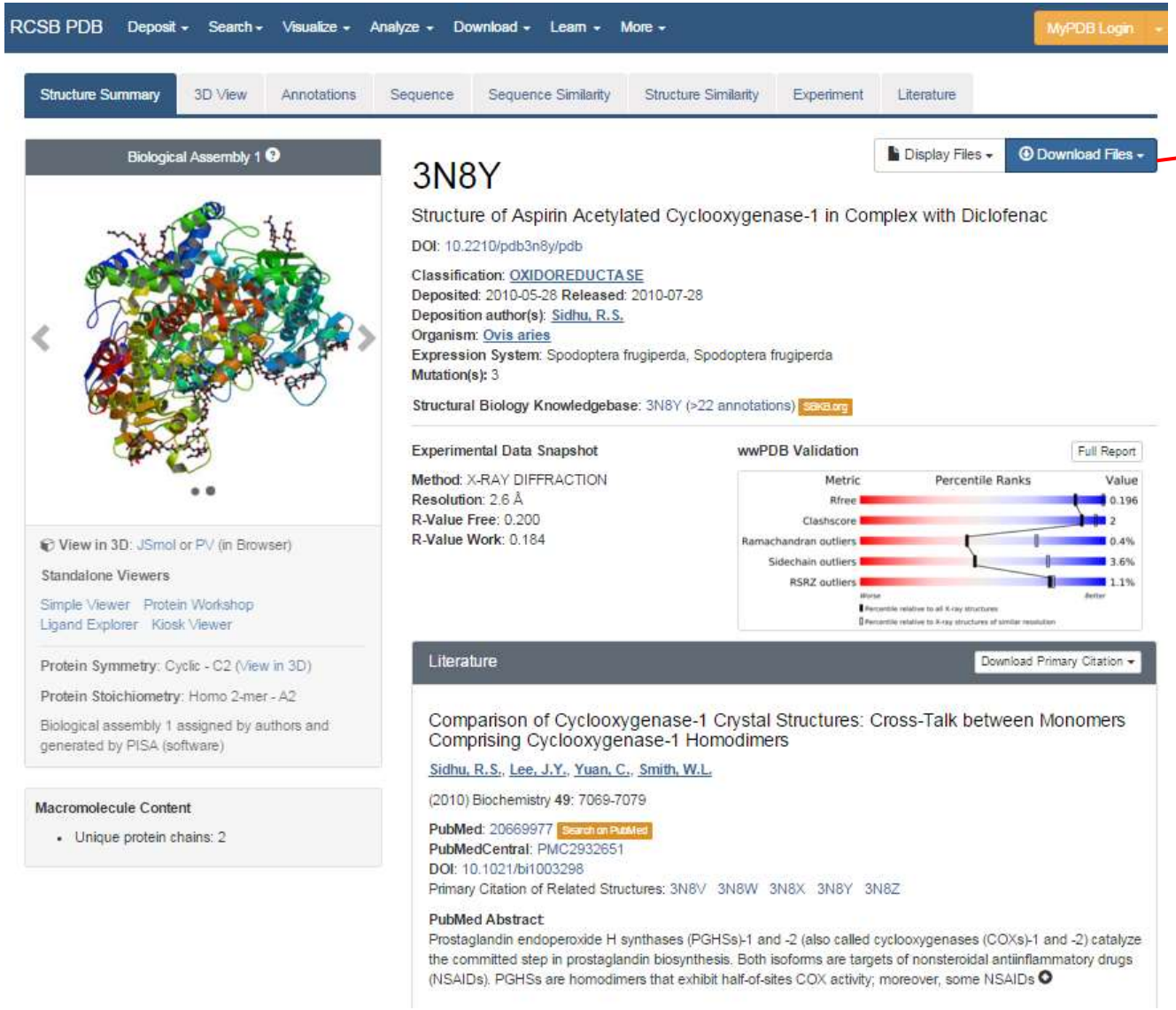
1.1 Visit the webpage: www.rcsb.org
1.2 Search for target.
1.3 Select target and click to open webpage.

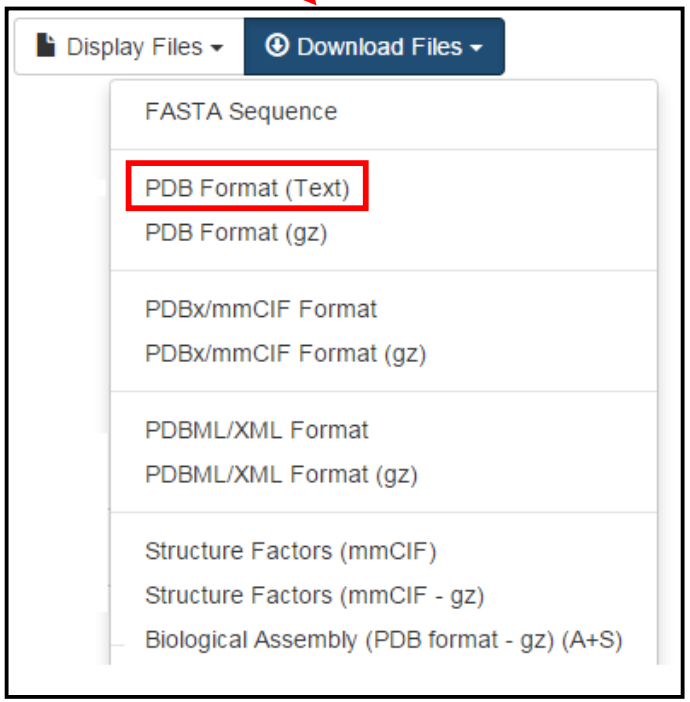


2. Target page:

2.1 Visit the target webpage.

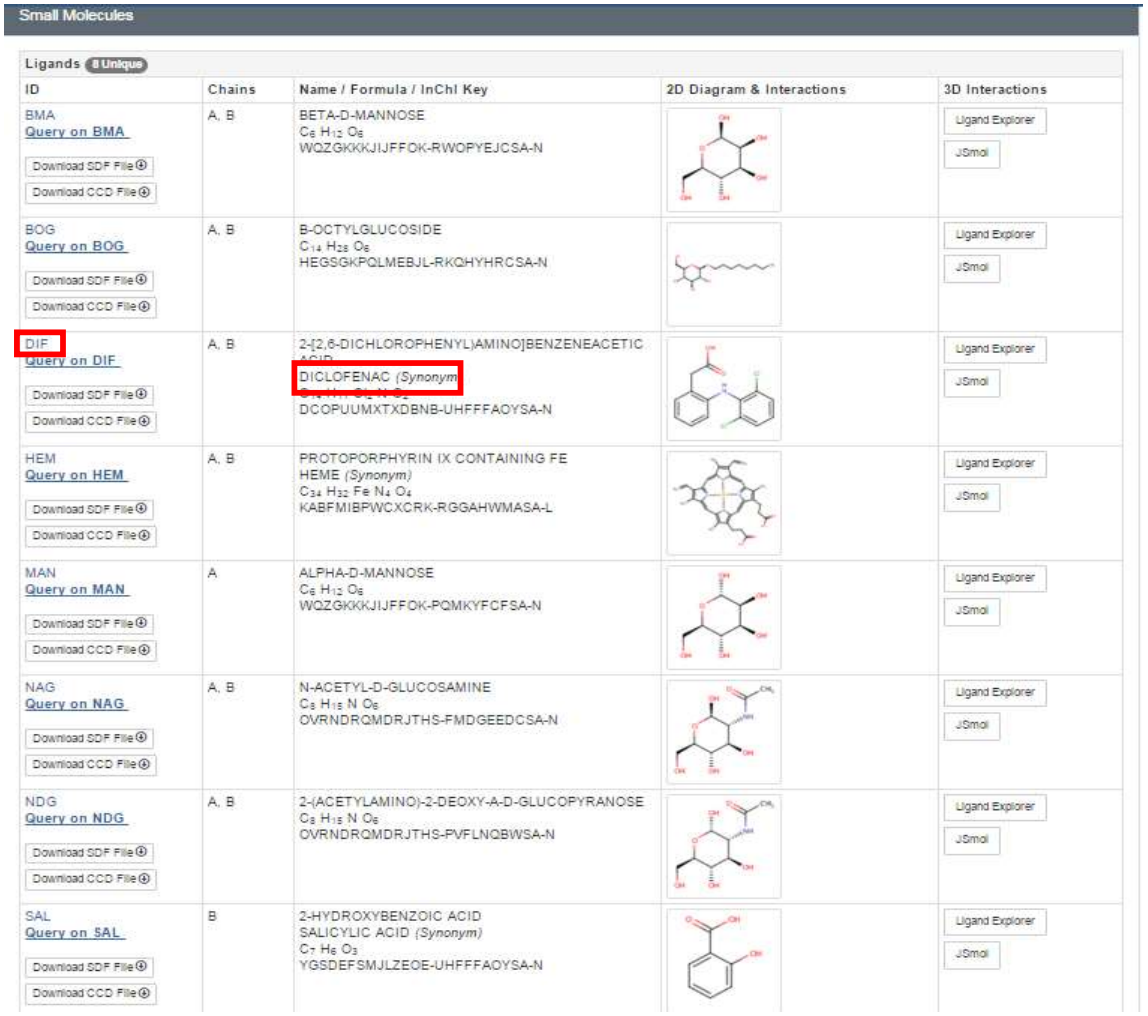
2.2 Download the target file.





3. Small ligands information

Different small molecules and ligands crystalized within the different protein binding sites are listed on the webpage:



Small Molecules

Ligands 8 Unique

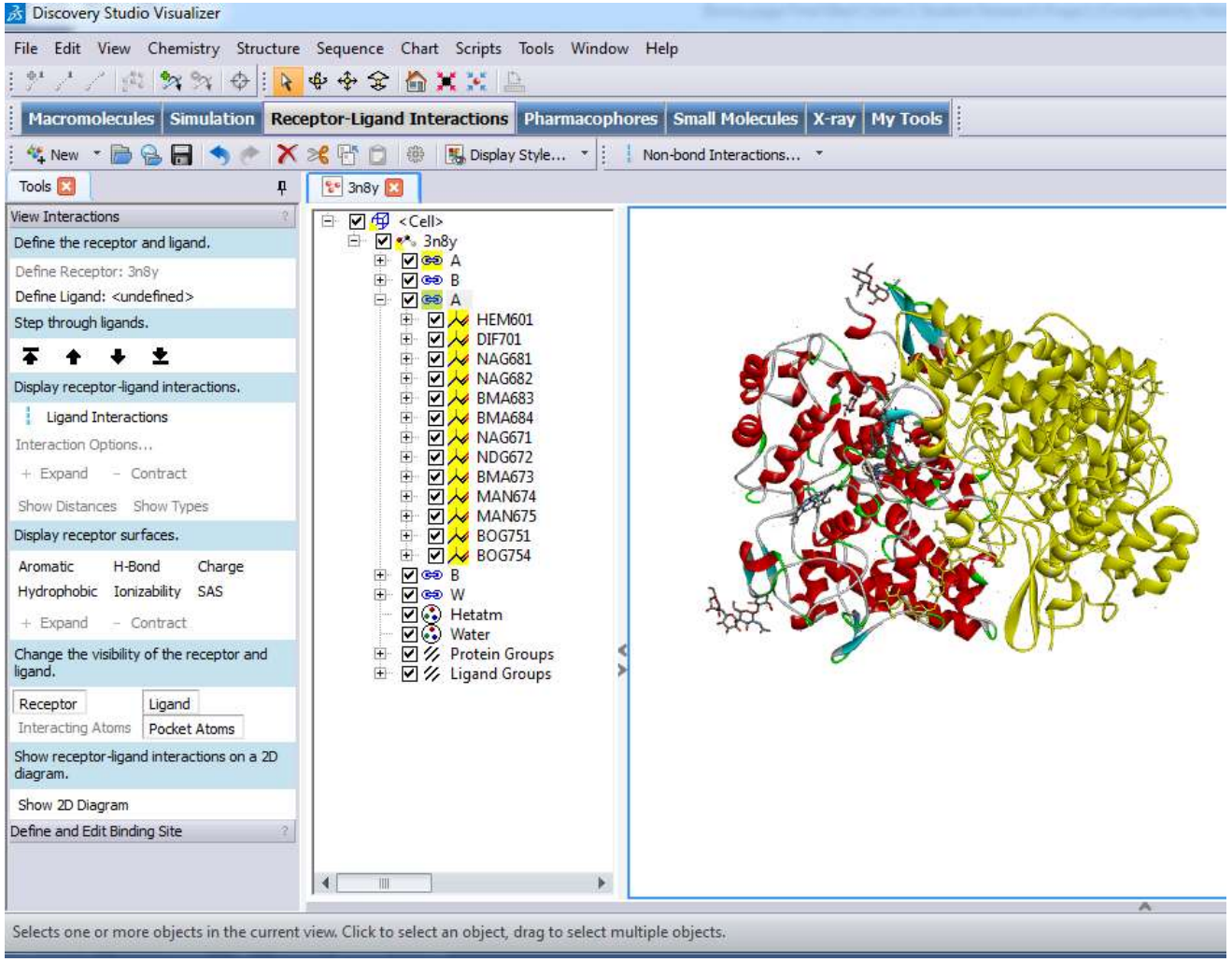
ID	Chains	Name / Formula / InChI Key	2D Diagram & Interactions	3D Interactions
BMA Query on BMA Download SDF File Download CCD File	A, B	BETA-D-MANNOSE $C_6 H_{12} O_6$ WQZGKKKJIJFFOK-RWOPYEJCSA-N		Ligand Explorer JSmol
BOG Query on BOG Download SDF File Download CCD File	A, B	B-OCTYLGLUCOSIDE $C_{14} H_{28} O_6$ HEGSGKPQLMEBJL-RKQHYHRCSA-N		Ligand Explorer JSmol
DIF Query on DIF Download SDF File Download CCD File	A, B	2-[2,6-DICHLOROPHENYL)AMINO]BENZENEACETIC ACID DICLOFENAC *(Synonym)* DCOPUUMXTXDBNB-UHFFFAOYSA-N		Ligand Explorer JSmol
HEM Query on HEM Download SDF File Download CCD File	A, B	PROTOPORPHYRIN IX CONTAINING FE HEME *(Synonym)* $C_{34} H_{32} Fe N_4 O_4$ KABFMIBPWCXCRK-RGGAHWMASA-L		Ligand Explorer JSmol
MAN Query on MAN Download SDF File Download CCD File	A	ALPHA-D-MANNOSE $C_6 H_{12} O_6$ WQZGKKKJIJFFOK-PQMKYFCFSA-N		Ligand Explorer JSmol
NAG Query on NAG Download SDF File Download CCD File	A, B	N-ACETYL-D-GLUCOSAMINE $C_8 H_{15} N O_6$ OVRNDRQMDRJTHS-FMDGEEDCSA-N		Ligand Explorer JSmol
NDG Query on NDG Download SDF File Download CCD File	A, B	2-(ACETYLAMINO)-2-DEOXY-A-D-GLUCOPYRANOSE $C_8 H_{15} N O_6$ OVRNDRQMDRJTHS-PVFLNQBWSA-N		Ligand Explorer JSmol
SAL Query on SAL Download SDF File Download CCD File	B	2-HYDROXYBENZOIC ACID SALICYLIC ACID *(Synonym)* $C_7 H_6 O_3$ YGSDEFSMJLZEOE-UHFFFAOYSA-N		Ligand Explorer JSmol

4. Open the *.pdb file using DS Visualizer 2.0 (Accelrys ®):

4.1 On the left panel you can select amino acids, water or ligand: Use "**Ctrl**" and **H** to show the left panel.

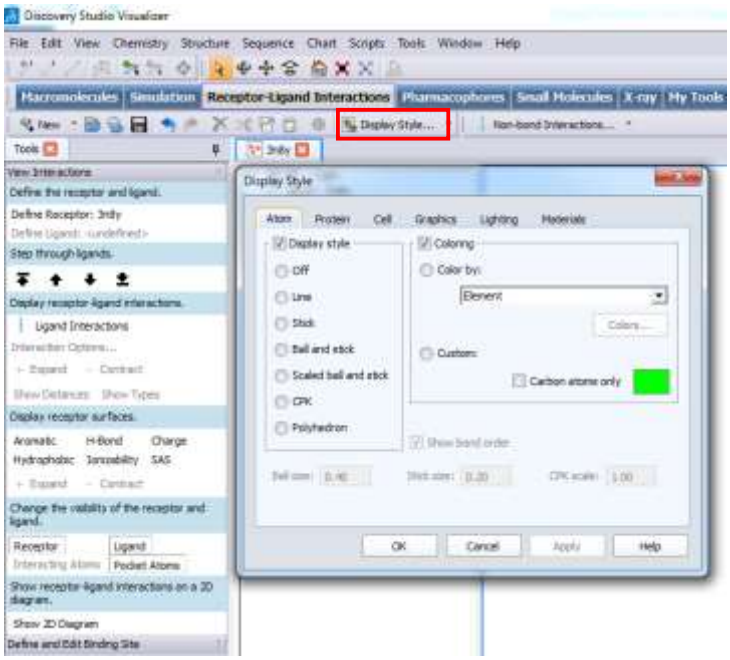
4.2 Similar ligand codes found on this list and on the webpage.

4.3 Protein or ligand can be selected by left mouse click. Use "**Ctrl**" to select more than one entry.
Selected items will be highlighted in yellow.



5. Working with the protein structure:

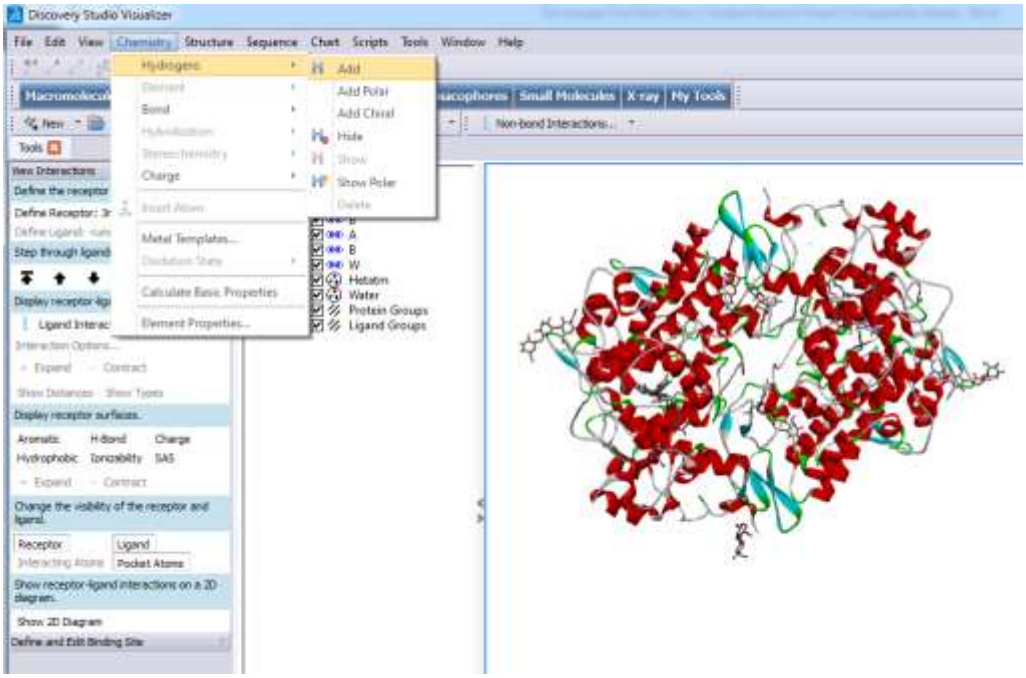
5.1 **Display Style**: change display preferences and background.



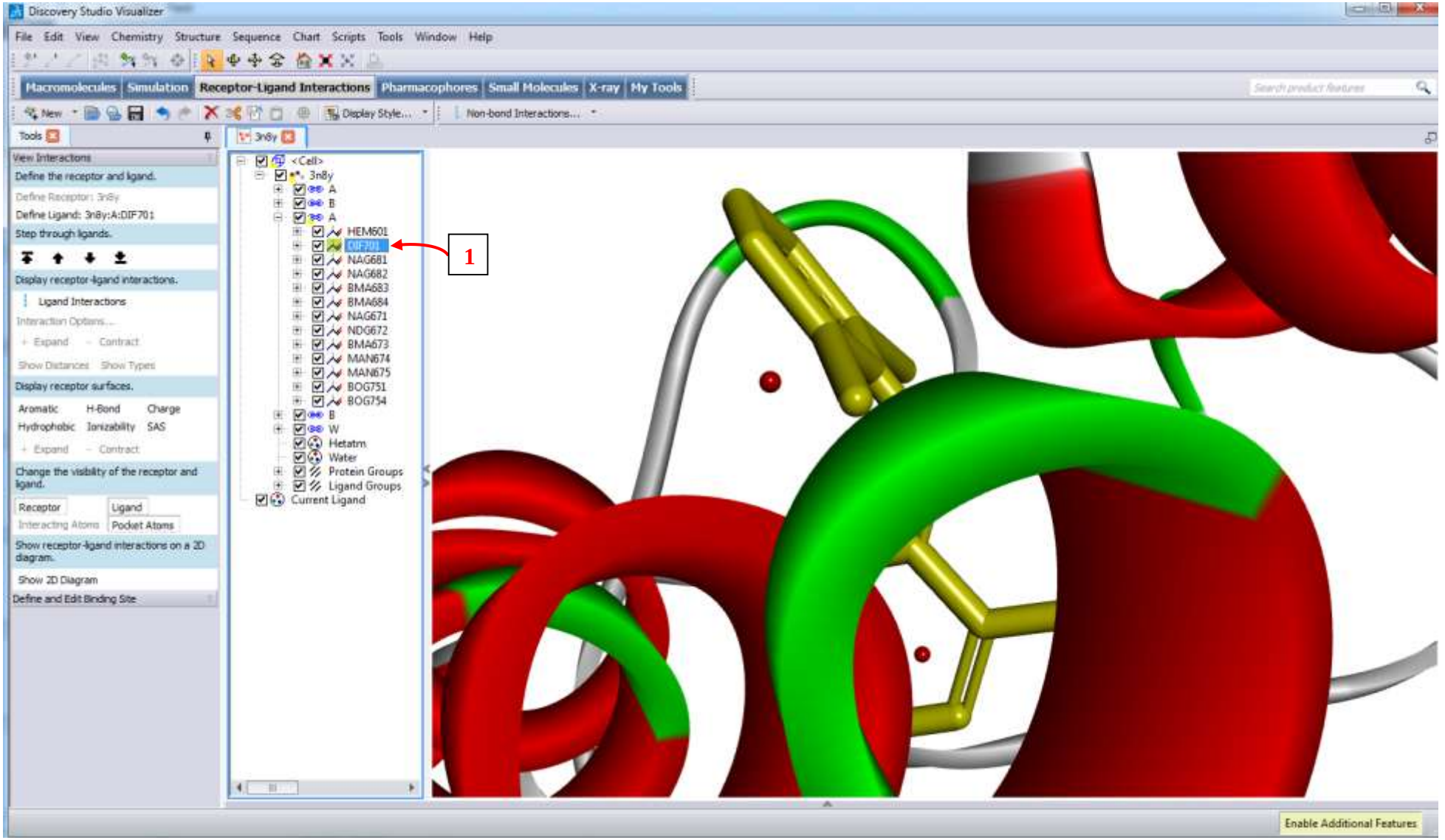
5.2  rotate and move the protein molecule.

6. Receptor - ligand interactions:

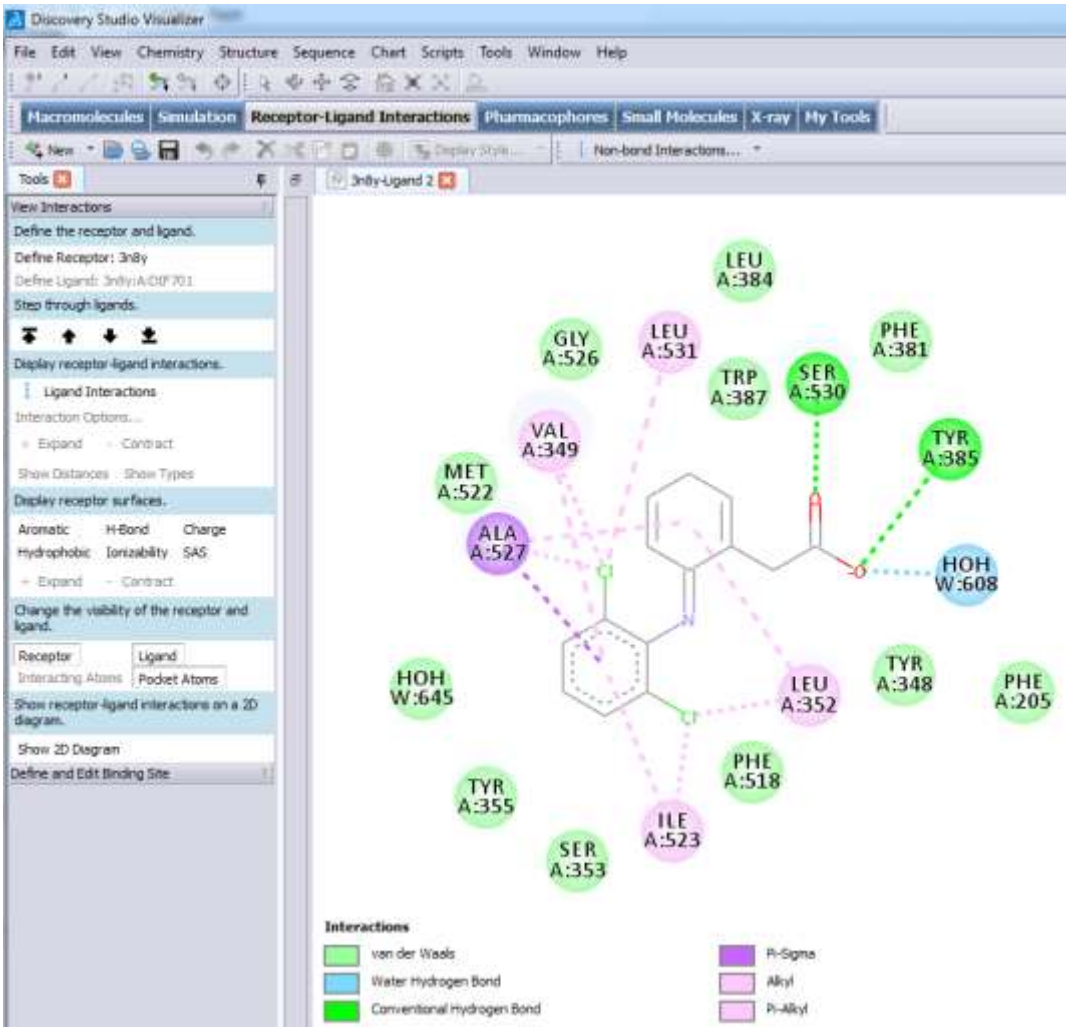
6.1 Add hydrogens to the Protein and Ligand



6.2 Select the ligand form the left panel.
6.3 Define the ligand by clicking **Define Ligand**.
6.4 Show the ligand interactions from **Show 2D Diagram**.



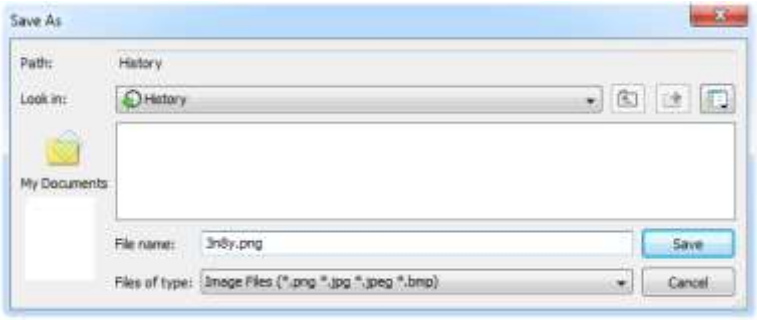
6.5 Record residue names and numbers involved in interactions with the receptor:



7. Reporting the results:

Reported results should include:

a) Amino acids involved in ligand binding
b) Figures for the ligand interactions.
c) Figures can be saved as images:



8. Poster:

8.1 Assignment poster should be:

a) written in a scientific language
b) follow the format of a journal article:
Introduction, Results, Discussion, Conclusion, and References.
c) discussion should include figures for the secondary structural elements of the studied protein, and the different ligand interactions
d) proper referencing of information, diagrams, and figures.

References:

1. Harvey, R. A. P. D., Lippincott Illustrated reviews: Biochemistry. Fifth edition. Philadelphia : Wolters Kluwer Health, : 2011.
2. Metz, G.; Ottleben, H.; Vetter, D., Protein-Ligand Interactions: From Molecular Recognition to Drug Design. 2005; Vol. 19.
3. Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne, P. E., The Protein Data Bank. Nucleic Acids Res 2000**,** 28, 235–242.
4. Dassault Systèmes BIOVIA, BIOVIA Discovery Studio Visualizer. Release 2017 R2, San Diego: Dassault Systèmes, 2017.