Modeling of Hyaluronan-Paclitaxel Targeted Delivery to Cancer Cells

by

Nurliyana Nasarudin

Dissertation submitted in partial fulfillment of
the requirements for the
Bachelor of Engineering (Hons)
(Chemical Engineering)

MAY 2012

Universiti Teknologi PETRONAS
Bandar Seri Iskandar
31750 Tronoh
Perak Darul Ridzuan

CERTIFICATION OF APPROVAL

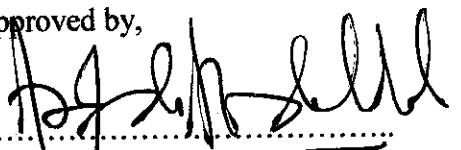
Modeling of Hyaluronan-Paclitaxel
Targeted Delivery to Cancer Cells

by

Nurliyana Nasarudin

A project dissertation submitted to the
Chemical Engineering Programme
Universiti Teknologi PETRONAS
in partial fulfillment of the requirements for the
BACHELOR OF ENGINEERING (Hons)
(CHEMICAL ENGINEERING)

Approved by,



.....

(Assoc. Prof. Dr. Mohd Azmuddin Abdullah)

UNIVERSITI TEKNOLOGI PETRONAS
TRONOH, PERAK
May 2012

CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work is my own except as specified in the references and acknowledgements, and that the original work contained herein have not been undertaken or done by unspecified sources or persons.

NURLIYANA NASARUDIN

ABSTRACT

This project investigates the 3-D molecular modeling of hyaluronan (HA), paclitaxel as anticancer drug, glutaraldehyde as cross-linker and possible crosslinked form of hyaluronic acid paclitaxel molecule along with various physical properties of uni-molecule and multi-molecules. Modeling work had been done using Discovery Studios 2.5 from Accelrys Inc. USA. The study focuses on targeted delivery of hyaluronan crosslink paclitaxel molecule nanoparticle to cancer cells in all-purpose. The generated model will be used in the studies of drug delivery to cancer cells.

ACKNOWLEDGEMENT

Throughout the whole period of conducting the Final Year Project, many have provided immeasurable amount of guidance, ideas, assistance, support and advice. Foremost, I am indebted to my supervisor, Assoc. Prof. Dr. Mohd Azmuddin Abdullah for the continuous support of my final year project, for his patience, motivation, and immense knowledge. His guidance helped me in all the time of research and writing this thesis.

Also to the Final Year Project Coordinator, Norhayati Bt. Mellon for providing me with the necessary information required to conduct the project. I also want to thanks the lectures, staffs of Chemical Engineering Department, Universiti Teknologi PETRONAS for their cooperation, suggestions and guidance in the compilation and preparation this final year project thesis.

Special thanks to Gul-e-Saba, phD student under the same supervisor with me for her support, helps, interest and valuable information and suggestions upon completing this project. Last but not least, deepest thanks to parents, friends and the people who have been contributed by supporting my work directly or indirectly until this project is fully completed. Thank you.

TABLE OF CONTENT

Contents

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: INTRODUCTION

1.1 Background Of Study

Cancer causes 13% of all human deaths in 2007 plays as one of major disease (Gul-e-Saba, et al., 2010). Cancer is a result of uncontrolled growth, invasion and metastatic behavior of group of cells which affects people at all ages and genders with the risk for most type increasing with age (Pal & Nayak, 2010). Chemotherapy cancer drugs are not specific and feared because of a patient's apprehension about toxic consequence. Paclitaxel is an anticancer drug that inhibits angiogenesis and reduces proliferation of tumor cell. However its practical use is limited due to its low solubility in water as well as other pharmaceutical solvents compatible in intravenous administration (Al-Ghananeem, et al., 2009). Effective targeted drug delivery is vital to interfering the cancer cells and tumor growth leaving the normal dividing cells.

Hyaluronan (HA) is a main ligand of CD44 receptor cells and act as the major components of extracellular matrix (ECM) (Gul-e-Saba, et al., 2010). Overexpression of HA-CD44 on cancer cells result in enhanced binding which will play important role in development of targeted control drug delivery to cancer cells (Al-Ghananeem, et al., 2009). Structured-based computational methods boost up the imperative targeted drug delivery with a well characterize target and desired attributes in high speed. To overcome the problem associated with currently available drugs, the need for drug modeling assist better understanding regarding the interactions of the drug with microenvironment in the body at the disease sites (Mandal, et al., 2009). Therefore, the study of hyaluronan crosslink paclitaxel molecule nanoparticle as potential anticancer drug delivery to cancer cells will be modeled and discussed using Discovery Studios 2.5 (Accelrys Inc., USA).

1.2 Problem Statement

When any new type of drugs is to be used in human body, the process of design and development of drug will involve many trial and error experiments based on previous research. This method is time consuming, costly and fraught with uncertainties. Computational modeling to design the molecular structure to see the behavior of an anticancer drug once injected in human cells is a rational approach in drug design which is economical and saves time with limited risk of failures as far as screening for lead compounds is concerned.

1.3 Objectives

1.3.1 To model the molecular structure of Hyaluronan crosslink Paclitaxel molecule nanoparticles targeted drug deliver by using Discovery Studios 2.5.

1.3.2 To estimate the parameters of 3-D molecular modeling structure such as bond length, bond angle, chirality and hybridization.

1.4 Scope Of Study

In this project, the 3-D molecular structure of Hyaluronan crosslink Paclitaxel molecule nanoparticles as potential anticancer drug targeted delivery to cancer cell will be modeled using Discovery Studios 2.5 (Accelrys Inc., USA). Drug design using computational modeling is one of the advanced tools yet that could help drug developers and could play a key role in the pharmaceutical industry (Kumar, et al., 2006).

1.5 Feasibility Study

Discovery Studios 2.5 (Accelrys Inc., USA) is available in Universiti Teknologi PETRONAS computer lab for students to use at all time and together with the project itself involves the compounds that have been known its molecular structure, therefore computational modeling of them is possible to generate and complete within project timeframe.

CHAPTER 2: LITERATURE REVIEW

2.1 The Basic of Cancer and Drug Delivery

Cancer is an intricate and life-threatening syndrome. It is happening as an upshot of progressive growth of chromosomal and epigenetic deviations (Kumar, et al., 2009). There were 1,500,000 new cancer cases were estimated and approximately 560,000 deaths from cancer in 2010 (Jemal, et al., 2010).The hallmarks of cancer cells occur from deregulation of cell proliferation (Shi, et al., 2006). Unlike normal cell which grow and divide and die over some time, cancer cell continues on growing and divide out of control and do not die as shown in figure 1. Rapid growth of abnormal cells is the most important defining feature of cancer which can attack contiguous parts of the body and extents to other organs (Pal, 2003).

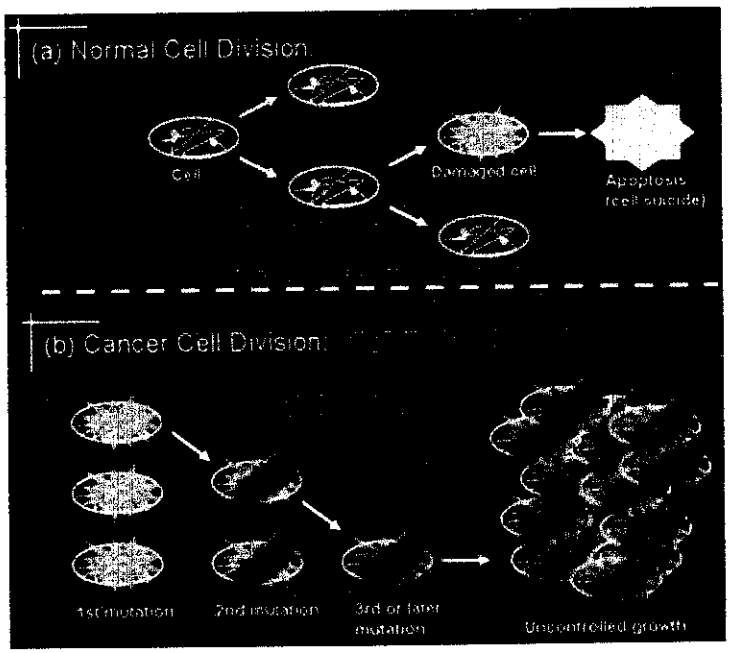


Figure 1: The Differences between Normal Cell and Cancer Cell Division (http://www.chem.ucla.edu/dept/Faculty/gimzewski/nnano2.html)

Although, chemotherapeutic drugs are widely used for the treatment of cancer, but the use of these drugs is often associated with patient toxicity and poor tumor delivery (Rios-Doria, et al., 2012). Cancer cells are sensitive to chemotherapy at early stage. However, it often develops assimilated confrontation upon repeated chemotherapy sequences (Carelle, et al., 2002). The conflict originated by an anticancer drug spreads cross-resistance to a huge range of drugs having diverse chemical structures and cellular targets (O'connor, 2007).

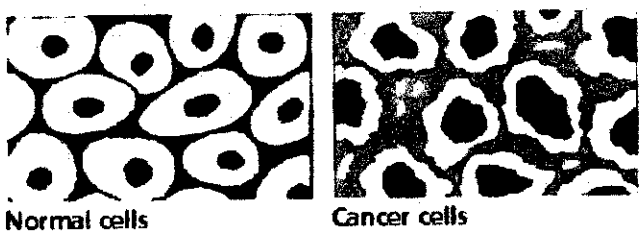


Figure 2: Normal Cells and Cancer Cells Differences
(http://jenneink.blogs.com/jennethink/2007/01/cancer_publicat.html)

Therefore, cancer drug delivery is no longer simply wrapping the drug in new formulations for different routes of delivery but includes the knowledge and experiences from other technologies to provide a vast range of strategies available for drug delivery in cancer (Jain M.D, 2005). The recent focus in development of cancer therapies is on targeted drug delivery to provide therapeutic concentrations of anticancer agents at the site of action and spare the normal tissues. To improve the bio-distribution of cancer drugs, nanoparticles have been designed to solve several limitations of conventional drug delivery systems for optimal size and surface characteristics to increase their circulation time in the bloodstream (Cho, et al., 2008).

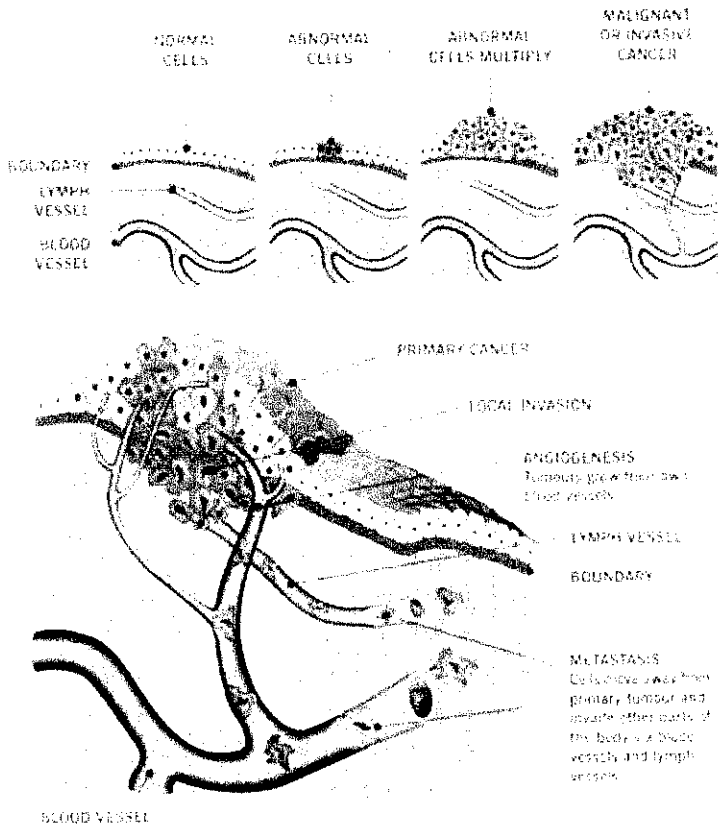


Figure 3: Cancer Diagram
(http://www.asbestos.com/mesothelioma/images/asbestos-cancer-diagram.php)

2.2 Natural Products in Cancer Chemotherapy

Nature is an attractive source of new therapeutic candidate compounds as a tremendous chemical diversity is found in millions of species of plants, animals, marine organisms and microorganisms (B da Rocha, et al., 2001). When it comes to treatment, humans have always turned to nature. The medicinal value of plants has been recognized by almost every society on this planet. Up to the nineteenth century, herbal extracts containing mixtures of natural products provided the main source of folk medicine (Lamari & Cordopatis, 2008). Since the 1940s, 175 anticancer drugs have been developed and are commercially available in the United States, Europe and Japan whereby 65% of these were inspired from natural products, semisynthetic modifications of natural products, natural mimics or synthetic molecules with pharmacophores from natural products (Newman & Cragg, 2007).

Drugs developed from natural sources.

Drug	Medical use	Mechanism of action	Source
Aspirin	Analgesic, anti-inflammatory, antipyretic	Inhibition of COX	Plant
Atropine	Pupil dilator	Antagonist of ACh at muscarinic receptors at post-ganglionic parasympathetic neuroeffector sites	Plant
Caffeine	Stimulant	Adenosine receptor antagonist	Plant
Codeine	Analgesic, antitussive	Opioid receptor agonist	Plant
Digoxin	For atrial fibrillation and CHF	Inhibition of the Na^+/K^+ ATPase membrane pump	Plant
Eugenol	Toothache	Reduces excitability of sensory nerves (increased K^+ efflux and reduced Ca^{2+} influx)	Plant
Morphine	Analgesic	Opioid receptor agonist	Plant
Pilocarpine	Glaucoma	Muscarinic receptor agonist	Plant
Quinine	Malaria prophylaxis	Inhibition of protein synthesis in the malaria parasite	Plant
Taxol	Anticancer agent	Antimitotic agent (binds to and stabilizes microtubules)	Plant
Penicillin	Antibiotic	Inhibition of synthesis of cell wall peptidoglycan	Microbe
Tetracyclin	Antibiotic	Inhibition of protein synthesis by binding to the ribosome 30S subunit.	Microbe
Cyclosporin A	Immunosuppressant	Inhibition of clonal proliferation of T lymphocytes (via inhibition of lymphokine production)	Microbe
Aurantosides	Antifungal	Inhibition of tubulin polymerization	Marine organism
Spongistatin 1	Antifungal	Inhibition of tubulin polymerization	Marine organism
Manoalide	Analgesic, anti-inflammatory	Inhibition of phospholipase A_2	Marine organism

Figure 4: Drugs Developed from Natural Sources (B da Rocha, et al., 2001)

Plant-derived anticancer agents.

Compound	Cancer use	Status
Vincristine	Leukemia, lymphoma, breast, lung, pediatric solid cancers and others	Phase III/IV
Vinblastine	Breast, lymphoma, germ-cell and renal cancer	Phase III/IV
Paclitaxel	Ovary, breast, lung, bladder, and head and neck cancer	Phase III/IV
Docetaxel	Breast and lung cancer	Phase III
Topotecan	Ovarian, lung and pediatric cancer	Phase II/III
Irinotecan	Colorectal and lung cancer	Phase II/III
Flavopiridol	Experimental	Phase I/II
Acronycidine	Experimental	Phase II/III
Bruceantin	Experimental	Preclinical/ phase I
Thalicarpin	Experimental	Preclinical/ phase I

Microbe-derived anticancer agents.

Compound	Cancer use	Status
Actinomycin	Sarcoma and germ-cell tumors	Phase III/IV
Bleomycin	Germ-cell, cervix, and head and neck cancer	Phase III/IV
Daunomycin	Leukemia	Phase III/IV
Doxorubicin	Lymphoma, breast, ovary, lung and sarcomas	Phase III/IV
Epirubicin	Breast cancer	Phase III/IV
Idarubicin	Breast cancer and leukemia	Phase III/IV
Mitomycin C	Gastric, colorectal, anal and lung cancer	Phase III/IV
Streptozocin	Gastric and endocrine tumors	Phase III/IV
Wortmannin	Experimental	Preclinical
Rapamicin[a]	Experimental	Preclinical
Geldanamycin	Experimental	Preclinical

[a]Rapamicin is also a potent immunosupressant.

Figure 5: Nature-derived Anticancer Agents (B da Rocha, et al., 2001)

The development of novel agents from natural sources presents obstacles that are not usually met when one deals with synthetic compounds. Paclitaxel was originally isolated from the bark of the yew tree *Taxus brevifolia*, a finite source of the compound. It took some years to develop a semi-synthetic analog (docetaxel) which is derived from a renewable source, the leaves of *Taxus baccata*. The taxanes paclitaxel and docetaxel show impressive antitumor activity against breast, ovarian and other tumor types in the clinic (B da Rocha, et al., 2001).

Taxol presents as the undoubted star, the best-selling anticancer drug whereby it sales reached US $1.5 billion in the year of 2000 and are still growing in these years. However, it took 20 years from its discovery in 1967 to the first real clinical response observed with ovarian cancer in 1987. The early supply problems were enormous which was about 4000 Pacific yew trees had to be sacrifices for their bark to provide 360 g of Taxol for the early clinical trials, and this rose to 380000 trees for 25 kg of Taxol needed to treat 12000 patients in the early 1990s. The natural product, paclitaxel, has therefore provided not only an effective drug, but also the springboard for further developments (Mann, 2002).

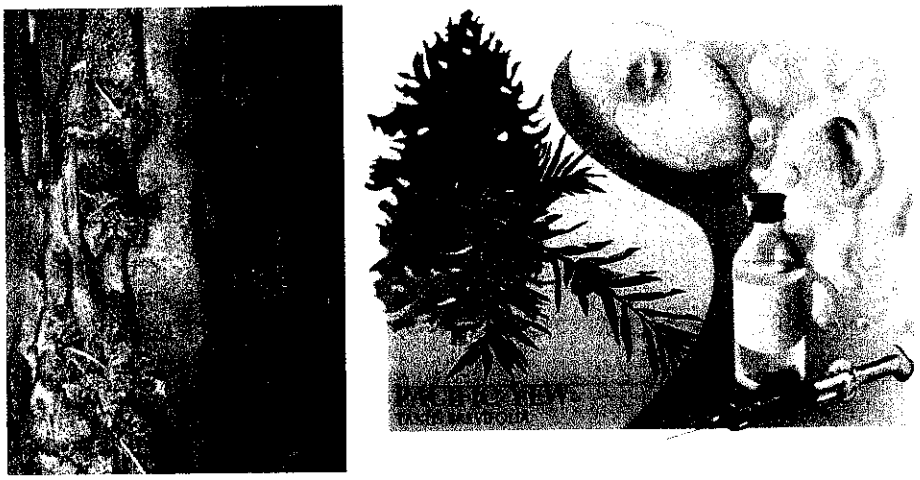


Figure 6: The Bark of Pacific Yew Tree (*Taxus Brevifolia)*
(http://www.art.com/products/p359763516-sa-i4008474/guillermo-gonzalez-pacific-yew-tree-taxus-brevifolia-close_up-of-bark-taxus-brevifolia.htm)

The use of chemotherapy to treat cancer began at the start of the 20th century with attempts to narrow the universe of chemical that might affect the disease by developing methods to screen chemicals using transplantable tumors in rodents. The ability of combination chemotherapy to cure acute childhood leukemia and advance Hodgkin's disease in the 1960s and early 1970s overcame the prevailing pessimism about the ability of drugs to cure advanced cancers, facilitated the study of adjuvant chemotherapy, and helped foster the national cancer program. Chemotherapy has, in fact, transitioned to the age of "targeted therapy" nowadays. (Devita Jr. & Chu, 2008). Thus, the probability of finding one 'magic bullet' drug to cure cancer seems to be nil. Anticancer research has mainly focused on the cancer cells and the development of cytotoxic drugs for efficient and selective chemotherapy. Indeed, in chemotherapy, combinations of chemical compounds are used (Lamari & Cordopatis, 2008).

2.3 Paclitaxel (PTX) As Anti-Cancer Drug and Mechanism of Action

Paclitaxel is an ordinary product with antitumor bustle and a white to off-white crystalline powder with the empirical formula $C_{47}H_{51}NO_{14}$. Paclitaxel is obtained via a Yew tree bark from *Taxus brevifolia* with a molecular weight of 853.9. The Paclitaxel chemical name is 5β, 20-Epoxy-l, 2α,4,7β,10β,13α-hexahydroxytax-l l-en-9-one 4,10-diacetate 2-benzoate 13-ester with (2*R*, 3*S*)-*N*-benzoyl-3-phenylisoserine. Paclitaxel is extremely lipophilic, non-solvable in water and liquefies at around 216-217 °C (Lee, et al., 2008). Besides that, paclitaxel also to the group of cytostatic agents and seems to be suitable due to its tight binding to various cytoskeleton cell constituents thus, resulting in effective local retention at the site of delivery (Patil & Joshi, 2012). The combine therapy of paclitaxel with other anti-tumor agents have been used for countless cancer treatments, but its poor solubility in water and containing certain toxicity (which confines the clinically administrated dose) has limited widespread use in cancer therapy (Leonelli, et al., 2008).

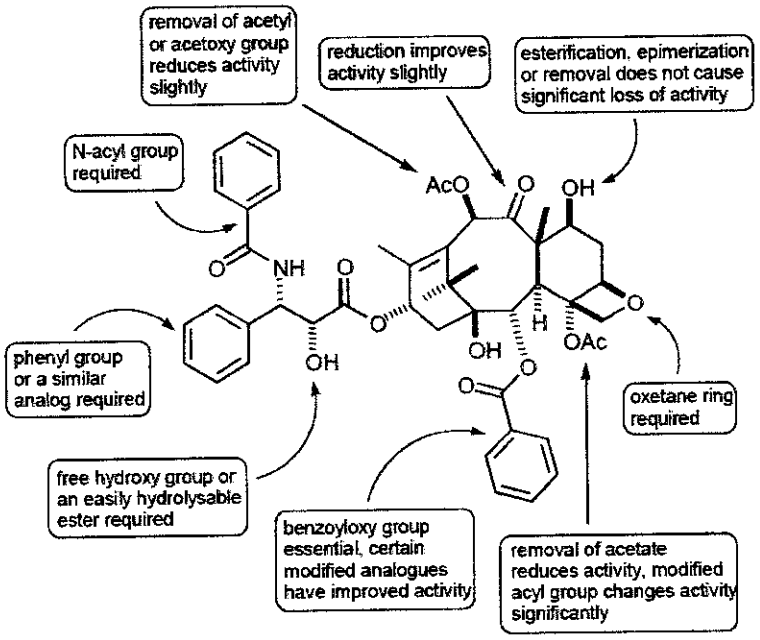


Figure 7: Paclitaxel Skeletal Formula (Haiqing Yuan, 1998)

The cell cycle is divided into several transition periods that are called phase and the cells pass through the G_1 phase during which DNA, and microtubule organizing centers (MTOC) are synthesized. Then, the cells enter the G_2 phase whereby it is a period for preparation for chromosome condensation and mitosis, including the synthesis of tubulin which is the former major component of the mitotic spindle and the latter prerequisite for the interaction of chromosomal fibers into high order structures. Microtubules are essential in cell mitosis and in state of dynamic equilibrium with their subunit tubulins is a major component of the mitotic spindle (Cooper, 2003). The cell cycle stages are shown in figure 8.

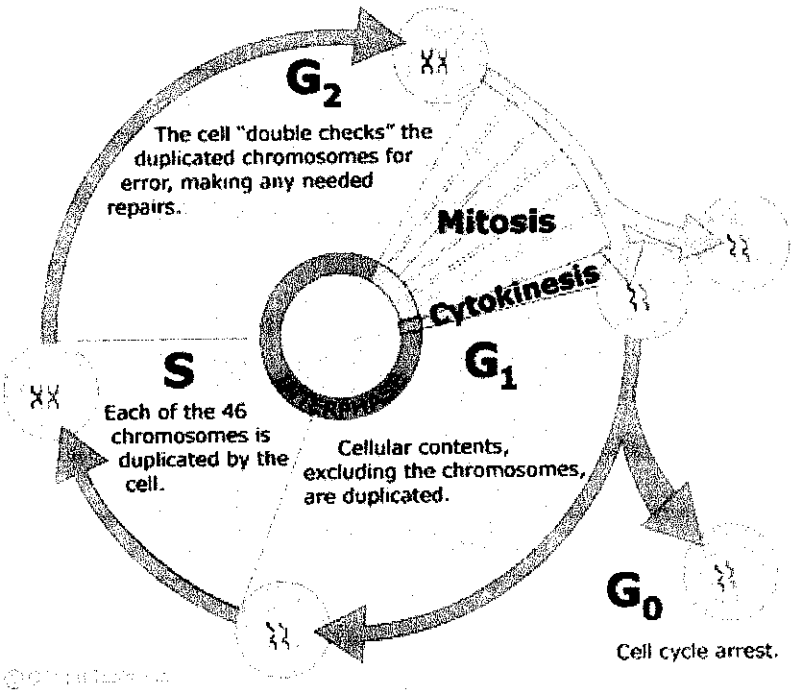


Figure 8: Cell Cycle
(http://scientopia.org/blogs/scicurious/2010/05/31/cell-cycle-p21-depression-and-neurogenesis-and-in-the-hippocampus/)

Microtubules constitute an active role in cell division with their dynamic instability and role in spindle formation during mitosis makes them an interesting target for anticancer drug development. Paclitaxel has been found to bind to microtubules without competing with other microtubule interactive agents, suggesting that paclitaxel binds to a different site on the tubulin. Studies has shown that paclitaxel stabilized microtubules containing microtubule-associated proteins (MAPs) were also found to be stable to both low temperature and to Ca^{2+} in the cell, while under the same conditions, paclitaxel treated microtubules in the absence of MAPs will depolymerize slowly (Mishra, 2011). The stabilization of microtubules results in arrest or delay in normal mitosis in cancer cells and this arrest might result in apoptosis of cancer cells.

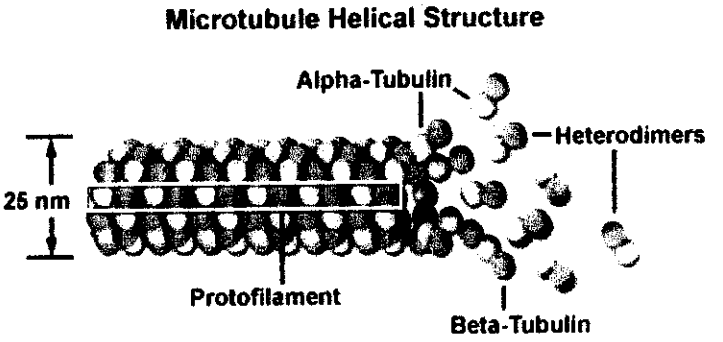


Figure 9: Microtubule Helical Structure
(http://micro.magnet.fsu.edu/cells/microtubules/microtubules.html)

2.4 Hyaluronan (HA)

Hyaluronan (HA) is a linear polysaccharide consisting of alternating units of β1, 3-*N*-acetylglucosaminyl and β1, 4-glucuronic acid. It is occur in the extracellular matrices of numerous tissues and plays a foremost role in cell proliferation, migration and adhesion throughout tissue remodeling, inflammation and tumor invasion (Pure' & Cuff, 2001). Hyaluronan is the bio-macromolecule most directly responsible in the mechanical support of the cell of many tissues for join health which is the major component of synovial fluid and is responsible for the structural and metabolic integrity of articular cartilage (Mohapatra et al., 2008). Evidence has indicated that hyaluronan may be involved in tumor cell metastasis through the interaction between cell receptors with hyaluronan which therefore, it has been used to inhibit the growth of cancer cells (Yu, et al., 1997).

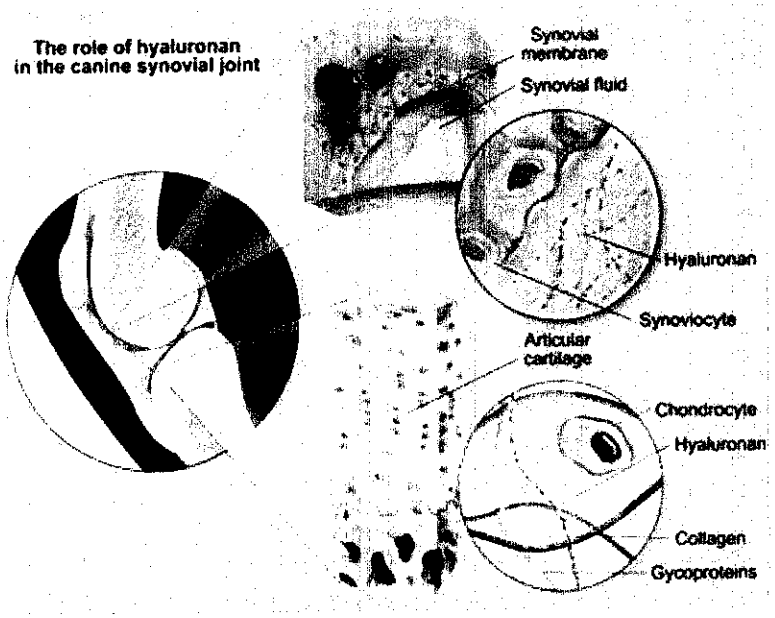


Figure 10: The Role of Hyaluronan (http://www.trixsyn.com/03_hyaluronan.php)

The hyaluronan undergoes constant turnover during the daily maintenance of basement membranes whereby it degraded into small, non-biologically active fragments which is rapidly removed through the liver. Hyaluronan acts as major component of the extracellular matrix (ECM) that can directly regulate inflammatory processes through its interaction with CD44, its cell surface receptor. Furthermore, it is also conceivable that the hyaluronan cable may serve as a temporary scaffold that avoids the loss of (ECM) components during extreme tissue remodeling (Yung & Chan, 2012).

CD44 interaction with HA, its receptor at the cell surface can lead to the targeted drug delivery (Yadav, et al., 2010), which afterward degraded inside the cells (Luo, et al., 2009). CD44 is a trans membrane glycoprotein with a wide tissue distribution and can interact with various cell surface and extracellular ligands but its principal ligand is HA. The binding of HA to CD44 is not constitutive but is activation dependent which must be activated before it can interact with HA. Recognition of HA by CD44 is depending on the degree of post-translational modifications, its phosphorylation status, sulfation pattern and ability to form multivalent aggregates on the cell surface (Yung & Chan, 2012).

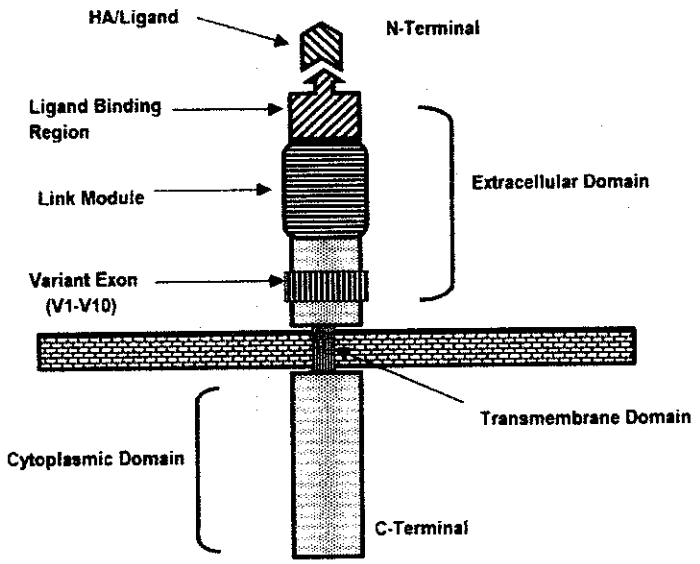


Figure 11: Schematic Representation of HA-CD44 Molecule (Gul-e-Saba, et al., 2010)

Naturally, hyaluronic acid is also present in cross linked form. Studies have shown that HA cross-linking is vital to alleviate the nascent 'cumulus' matrix that forms around the oocyte prior to ovulation which the similar contrivances are likely to transpire at sites in inflammation (Day & de la Motte, 2005). Several methods have been used to cross-link perform dimerization reaction of the styrylpyridinium (SbQ) molecules encouraged by UV light finally produces cross-linked micelles (Tao, et al., 2012).

2.5 Introduction to Cross-linking Methods and Glutaraldehyde as Cross-linkers

Cross-linking is the process of chemically joining two or more molecules by a covalent bond. Cross-linking reagents contain reactive ends to specific functional groups on proteins or other molecule. Cross-linkers are commonly used to modify drugs in which they are selected on the basis of their chemical reactivity and compatibility of the reaction with the application. Cross-linkers are chosen based on the characteristics such as chemical specificity, water solubility and cell membrane permeability, spontaneously reactive or photo-reactive groups, and cleavability. A cross-link introduces a permanent relationship where there may be a need for frequent separation in functioning (Kluger & Alagic, 2004).

Glutaraldehyde possesses inimitable physiognomies that render it one of the supreme operative protein crosslinking reagents and can be exist in at slightest 13 different forms depending on solution conditions as shown in figure 12. It is a linear, 5-carbon dialdehyde, a vibrant, neutral to pale straw-colored, pungent oily liquid that is solvable in all proportions in water and alcohol, as well as in organic solvents which mostly obtainable as acidic aqueous solutions (Migneault, et al., 2004).

In fact, studies of collagen crosslinking reactions with monoaldehyde and dialdehydes having chain lengths of two to six carbon atoms revealed that the reactivity in this series is exploited at five carbons which specify that glutaraldehyde is the maximum operative crosslinking agent (Bowes & Cater, 1968). Glutaraldehyde fixation of bio prosthetic tissue alters the mechanical characteristics of the tissue significantly. Studies showed that the effect of different cross-linking methods on tissue properties whereby cross-linking of bovine pericardium with glutaraldehyde resulted in an increase of the extensibility and a reduction in stress relaxation. Thus, glutaraldehyde has marketable availability and small cost in addition to its great reactivity and chemically steady crosslinks (Nimni, et al., 1987).

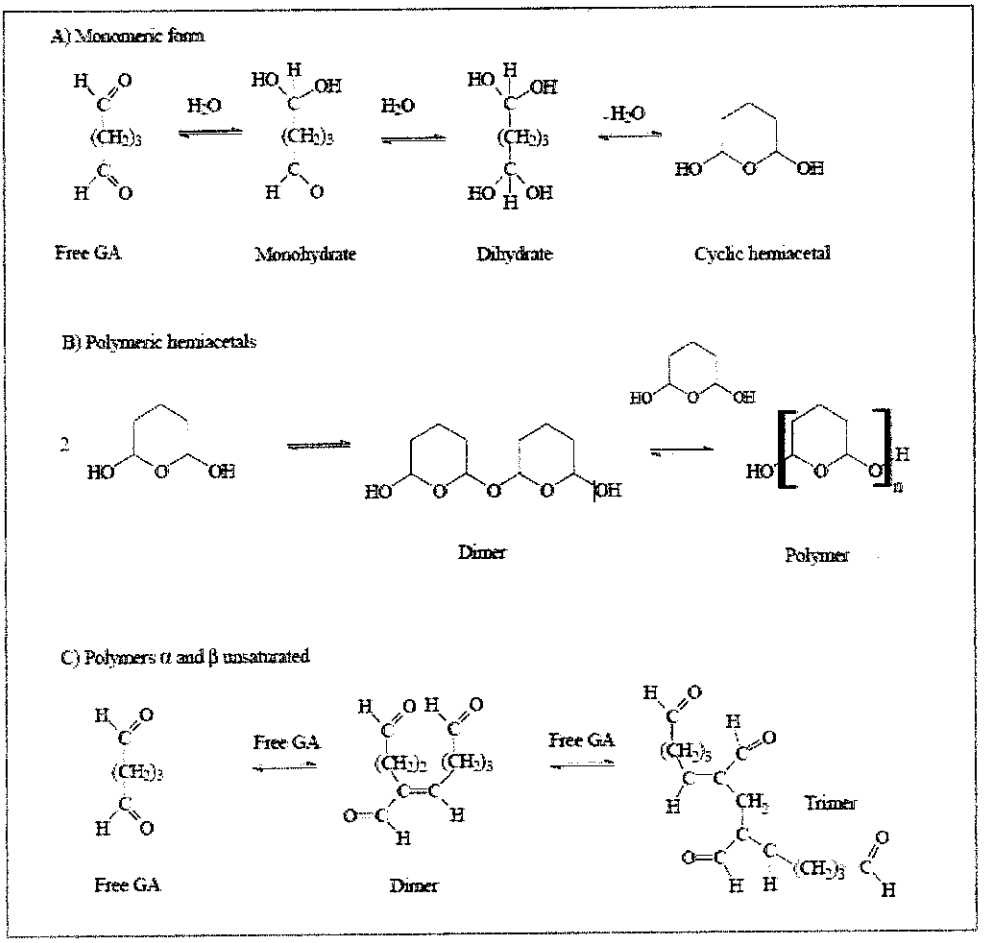


Figure 12: Possible Structure of Glutaraldehyde in Aqueous Solution (Nimni, et al., 1987)

Glutaraldehyde was first applied successfully for bio prosthesis in the late 60s. Previous studies also shown that crosslinking was performed in acetic solution with glutaraldeyhde in order to obtain different degrees of crosslinking. It was observed that for higher crosslinking levels the chitosan membranes become stiffer but their strength decreases for controlling its swelling rate, drug release rate and changing of mechanical properties.

Aqueous solutions of glutaraldehyde contain a mixture of free aldehyde and mono-and dehydrated glutaraldehyde and monomeric and polymeric hemiacetals. Glutaraldehyde appeared as the most widely studied cross-linker of chitosan which was often used as a comparison in the study of novel cross-linkers and its solutions may contain various products resulting from aldol condensation during storage and cyclic glutaraldehyde oligomers having a trioxane structure (Silva, et al., 2004).

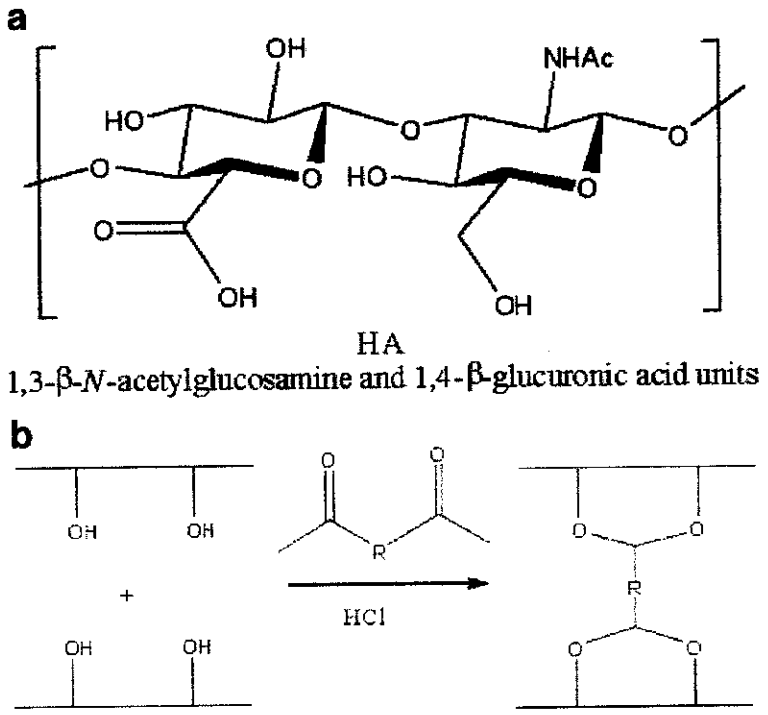


Figure 13: (a) Structure of Hyaluronan (b) Chemical Cross-linking of Hyaluronan using Glutaraldehyde (Al-Ghananeem, et al., 2009)

2.6 Drug Modeling (Structured –based Design Molecular Modeling)

Drug design is a process which involves the identification of a compound that displays a biological profile and ends when the biological profile and chemical synthesis of the new chemical entity are optimized. Drug designing is also known as rational drug design and it is a process of finding new modifications based on the biological receptors and target molecules (Dineshkumar, et al., 2010)

Structure-based computational methods continue to enhance progress in the discovery and refinement of therapeutic agents and drugs. Molecular visualization and molecular modeling are included. The structure-based methods are becoming increasingly important due to the rapid growth in structural data, and the particularly high speed with which structures can be determined as part of a focused drug-discovery effort with a well-characterized target. A drug target is a biomolecule which involved in signaling or metabolic pathways that are specific to a disease process (Marrone, et al., 1997).

Hence, for developing and commercializing drugs within a reasonable amount of time as a potential drawback of the extremely slow natural processes, pharmaceutical companies have devised proactive drug discovery methods, including recent innovation called rational drug design. In this approach, researchers build and test small drug-like molecules based on prior knowledge about the three-dimensional (3-D) structures of known drug molecules (Bandyopadhyay, et al., 2005).

The drug rational model or design is the development of small molecules with desired properties for targets, biomolecules which it is well established and is being applied extensively by the pharmaceutical industries. The development of small molecule such as hyaluronan and paclitaxel require examination of several aspect including bond length, bond angle, chirality and energy. Consequently, all the parameters can be utilized to improve drugs to accomplish desirable attributes such as minimization of toxic side effects, improve bioavailability and overcoming of drug resistance (Mandal, et al., 2009).

2.6.1 Molecular Modeling and Docking

A molecule is characterized by a pair (A: B), in which A represents a collection of atoms, and B represents a collection of bonds between pairs of atoms. Information used for kinematic and energy computations is associated with each of the atoms and bonds. Each atom carries standard information, such as its bond length. Three element of information are associated with each bond are the bond length (the distance between two atom centers), the bond angle (the angle between two consecutive bonds) and whether the bond is rotatable or not (Teodore, et al., 2001). Figure 9 illustrated the rotatable bonds.

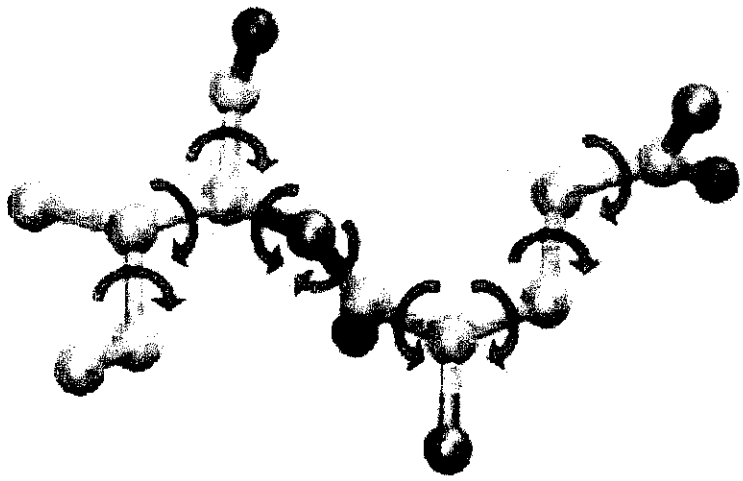


Figure 14: A Drug Molecule. Spheres represent atoms and bonds connecting them are represented by sticks. Curved arrows represent the rotatable degrees of freedom around bonds (Teodore, et al., 2001)

Molecular docking is a computer simulation procedure to predict the conformation of a receptor-ligand complex or as a simulation process where a ligand position is estimated in a pre-defined binding site. Speed and accuracy are key features for obtaining a successful result in docking simulations. In context of docking, energy evaluations are usually carried out with the help of a scoring function (Dias & de Azevedo Jr., 2008). A large number of up-to-date scoring functions are based on force field which is an empirical fit to the potential energy surface. There are many different force field models that can be used to simulate proteins and other organic molecule as AMBER, CHARMM, MM3, MM4 and MMFF94 (Daunay & Micaelli, 2007). This project used CHARMM (Chemistry at HARvard Molecular Mechanics) as the force field.

CHAPTER 3: METHODOLOGY

3.1 Research Methodology

The study has been divided into 2 stages for molecular modeling as follows:

3.1.1 Uni-molecular Modeling

All of the following main molecules will be modeled separately using Discovery Studios 2.5 (Accelrys Inc., USA):

i. An anticancer drug (Paclitaxel)
ii. Hyaluronan (HA)
iii. Glutaraldehyde (Cross-linker)

3.1.2 Multi-molecular Modeling

The following crosslink molecule nanoparticle will be modeled using Discovery Studios 2.5 (Accelrys Inc., USA):

i. Hyaluronan crosslink Paclitaxel molecule nanoparticle

3.2 Project Activities

3.2.1 The Project Work Flow

The project work flow was started by conducting preliminary research which includes the literature review on every aspect of cancer, anticancer drugs, crosslinking and cross-linker agents, targeted drug delivery to cancer cells and drug modeling. Then, the drug modeling was design in Discovery Studio 2.5 software by executing the first stage of designing which is the uni-molecular modeling followed by the second stage of designing the multi-molecular modeling. The results retrieved from the software must be compared with the theory after applying the molecular docking using CHARMM force field.

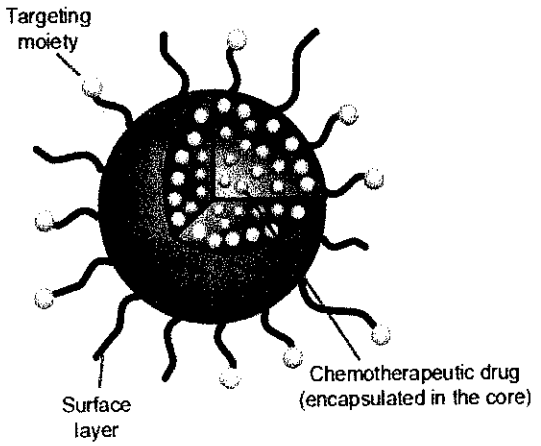


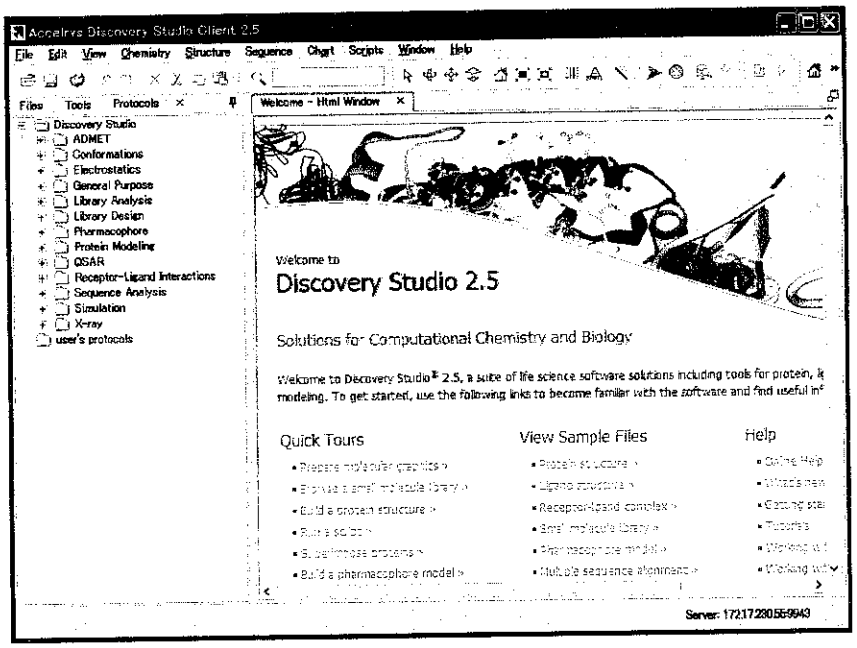
Figure 15: Hyaluronan Crosslink Paclitaxel Molecule Nanoparticle Skeletal View (Dinarvand, et al., 2011)

3.3 Tools Requirement

For this project, the main tool required is the Discovery Studio 2.5 produced by Accelrys. This program is a software suite of life science molecular design solutions for computational chemists and biologist since the modeling help to design the molecular structure in high speed. For all the modeling structure, basic modeling procedure has been applied. Tools requirement as for development of the molecular modeling is shown below.

3.3.1 Software

- Discovery Studios 2.5 (Accelrys Inc., USA)



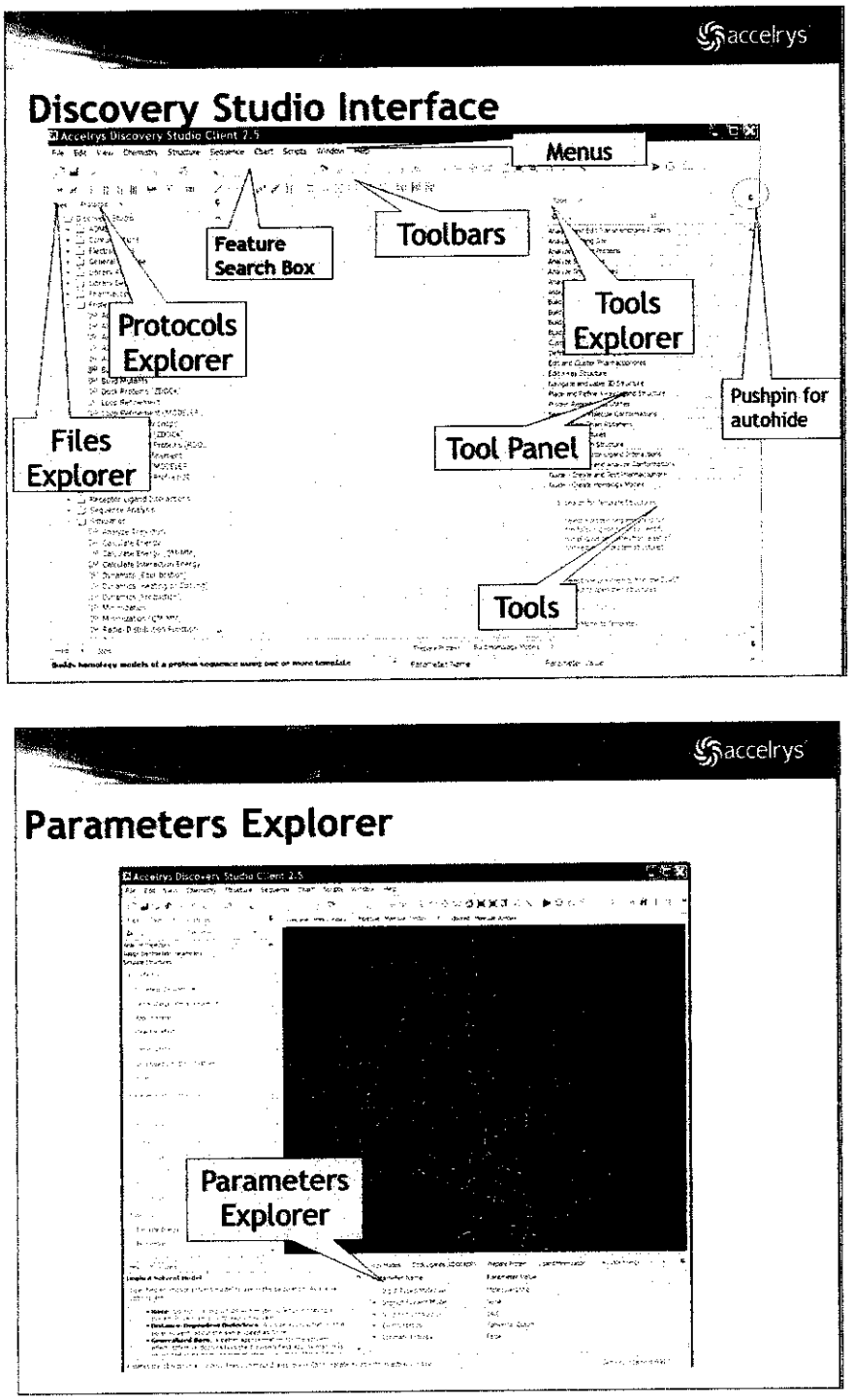


Figure 16: User interface of Discovery Studios 2.5 (Accelrys Inc., USA)

Discovery Studio is a built on Pipeline Pilot platform, allowing a powerful integration with multiple software applications that will guide through template identification, sequence alignments, homology modeling, identification and refinement of CDR loops, as well as subsequent analysis.

3.4 Project Timeline

Table 1: Final Year Project I Project Timeline

Week(s)	1	2	3	4-5	6	7	8-11	12-13	14
Selection Of Project Topic									
Preliminary Research Work On Journal, Books And Previous Thesis									
Project Work Of Review Paper On HA Mediated Targeted Drug Delivery System									
Uni-molecular Modeling - Paclitaxel - Hyaluronic acid - Glutaraldehyde									
Multi-molecular Modeling - HA-Glutaraldehyde - Paclitaxel-HA									
Proposal Defense									
Oral Defense									
Submission Of Interim Report									

Table 2: Final Year Project II Project Timeline

Detail/Week		1-2	3-4	5-6	7	8	9	10	11	12	13	14	15
Continue on modeling	Hyaluronan degrade												
	HA-CD44 Human Binding												
	FERM-CD44 Mouse Binding												
	HA-Paclitaxel (PTX) Cross-linked												
	Glutaraldehyde												
Analysis of Data													
Progress Report Drafting and Pre-presentation to Supervisor													
Submission of Progress Report (hardcopy to coordinator)													
Pre-SEDEX													
Draft Report, Dissertation and Technical Paper (Drafting) to Supervisor													
Submission of Draft Report													
Submission of Dissertation (soft bound – 3 copies)													
Submission of technical paper (softcopy via Turn-it-in, and hardcopy to Coordinator)													
Oral Presentation & Submission of Project Dissertation (Hard Bound – 3 copies)													

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Molecular Modeling

The following content illustrate the molecular modeling developed by using Discovery Studios 2.5 (Accelrys Inc., USA).

4.1.1 Uni-molecular Modeling

4.1.1.1 An anticancer drug (Paclitaxel)

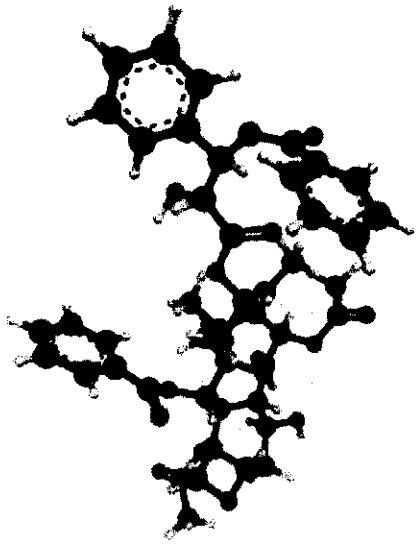
Name Of Compound	2-D Modelling	3-D Modelling
Paclitaxel		

Figure 17: Molecular modeling of Paclitaxel

The modeling of Paclitaxel has been shown in Fig. 17 and 18. The red atom indicates oxygen atom, the black atom indicates carbon atom, the blue atom indicates nitrogen atom and the light grey atom indicates hydrogen atom. The bond length between each atom has also been calculated using function in the software together with its basic properties as shown in Table 3 and 4, respectively.

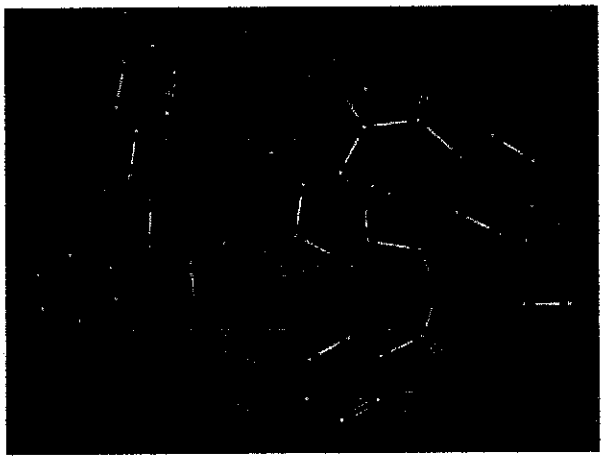


Figure 18: Paclitaxel in 3-D Structure

Table 3: Basic properties and bond length of Paclitaxel

Atoms	Bond	Bond Type	Bond Length
C1 - O2	1	Single	1.4413
O2 - C3	1	Single	1.4490
C3 - C4	1	Single	1.5624
C4 - C5	1	Single	1.5659
C5 - C6	1	Single	1.5599
C6 - C1	1	Single	1.5503
C3 - C7	1	Single	1.5524
C7 - O8	1	Single	1.4253
C6 - N9	1	Single	1.4400
N9 - C10	1	Single	1.3506
C10 - O11	2	Double	1.2537
C10 - C12	1	Single	1.4664
C4 - O13	1	Single	1.4360
C5 - O14	1	Single	1.4497
O14 - C15	1	Single	1.4429
C15 - C16	1	Single	1.5774
C16 - O17	1	Single	1.4376
C16 - C18	1	Single	1.5592
C18 - C19	1	Single	1.5540
C19 - C20	1	Single	1.5748
C20 - C21	1	Single	1.5594
C21 - C15	1	Single	1.5546
C20 - C22	1	Single	1.4856
C22 - O23	2	Double	1.2535
C18 - O24	1	Single	1.4279
C22 - O25	1	Single	1.3553

O25 - C26	1	Single	1.4265
C26 - C27	1	Single	1.5431
C27 - C28	1	Single	1.5499
C28 - C29	1	Single	1.5515
C29 - C30	1	Single	1.5437
C30 - O31	1	Single	1.4303
O31 - C32	1	Single	1.3598
C32 - O33	2	Double	1.2549
C32 - C34	1	Single	1.4921
C34 - C35	1	Single	1.5638
C35 - C36	1	Single	1.5613
C36 - O37	1	Single	1.4355
C36 - C38	1	Single	1.5648
C38 - O39	1	Single	1.4367
C38 - C40	1	Single	1.5527
C40 - O41	1	Single	1.4472
O41 - C34	1	Single	1.4539
C40 - O42	1	Single	1.4405
O42 - C43	1	Single	1.4508
C43 - C44	1	Single	1.5679
C44 - O45	1	Single	1.4355
C44 - C46	1	Single	1.5677
C46 - C47	1	Single	1.5483
C47 - O48	1	Single	1.4317
C46 - O49	1	Single	1.4466
O49 - C50	1	Single	1.4545
C50 - C51	1	Single	1.5668
C51 - C43	1	Single	1.5569
C51 - N52	1	Single	1.4412
N52 - C53	1	Single	1.3503
C53 - O54	2	Double	1.2504
C53 - C55	1	Single	1.4676
C1 - H1	1	Single	1.0920
C1 - H2	1	Single	1.0950
C3 - H3	1	Single	1.0953
C4 - H4	1	Single	1.0945
C5 - H5	1	Single	1.0954
C6 - H6	1	Single	1.0969
C7 - H7	1	Single	1.0928
C7 - H8	1	Single	1.0926
O8 - H9	1	Single	0.9859

N9 - H10	1	Single	0.9668
C12 - H11	1	Single	1.0918
C12 - H12	1	Single	1.0906
C12 - H13	1	Single	1.0902
O13 - H14	1	Single	0.9817
C15 - H15	1	Single	1.0959
C16 - H16	1	Single	1.0945
O17 - H17	1	Single	0.9921
C18 - H18	1	Single	1.0945
C19 - H19	1	Single	1.0944
C19 - H20	1	Single	1.0948
C20 - H21	1	Single	1.0949
C21 - H22	1	Single	1.0950
C21 - H23	1	Single	1.0915
O24 - H24	1	Single	0.9877
C26 - H25	1	Single	1.0946
C26 - H26	1	Single	1.0939
C27 - H27	1	Single	1.0934
C27 - H28	1	Single	1.0936
C28 - H29	1	Single	1.0945
C28 - H30	1	Single	1.0948
C29 - H31	1	Single	1.0937
C29 - H32	1	Single	1.0932
C30 - H33	1	Single	1.0939
C30 - H34	1	Single	1.0946
C34 - H35	1	Single	1.0928
C35 - H36	1	Single	1.0938
C35 - H37	1	Single	1.0952
C36 - H38	1	Single	1.0943
O37 - H39	1	Single	0.9845
C38 - H40	1	Single	1.0934
O39 - H41	1	Single	0.9890
C40 - H42	1	Single	1.0959
C43 - H43	1	Single	1.0974
C44 - H44	1	Single	1.0950
O45 - H45	1	Single	0.9886
C46 - H46	1	Single	1.0967
C47 - H47	1	Single	1.0920
C47 - H48	1	Single	1.0929
O48 - H49	1	Single	0.9893
C50 - H50	1	Single	1.0912

C50 - H51	1	Single	1.0927
C51 - H52	1	Single	1.0948
N52 - H53	1	Single	0.9676
C55 - H54	1	Single	1.0915
C55 - H55	1	Single	1.0908
C55 - H56	1	Single	1.0905

The Paclitaxel structure shows the total measurement of 113 bond length with average bond length of 1.3068 Å.

Table 4: Basic properties of Paclitaxel

Properties	**Paclitaxel**
Number of Atoms	113
Molecular Formula	$C_{47}H_{51}NO_{14}$
Molecular Composition	C: 0.661, H: 0.060, N: 0.016, O: 0.262
Molecular Weight	853.906
Exact Molecular Weight	853.331
Average Bond Length (Å)	1.306825
Number of Rings	7
Number of Aromatic Rings	3
Number of Rotatable Bonds	14

According to Table 4, the exact molecular weight of paclitaxel is slightly lower than its molecular weight due to exact molecular weight is the mass of a molecule calculated with only the most abundant isotopes present which are usually the lightest isotopes (University of Colorado, 2011). The theoretical value of paclitaxel according to material safety data sheet of paclitaxel is 853.92 which is almost near to the value of molecular weight of the uni-molecular paclitaxel 3-D structure, 853.906 retrieved from the software. Paclitaxel shows 7 rings in its molecular structure. However 3 of them are aromatic rings with total 14 rotatable bonds which might be contributes to it various isoforms.

4.1.1.2 Hyaluronic acid (HA)

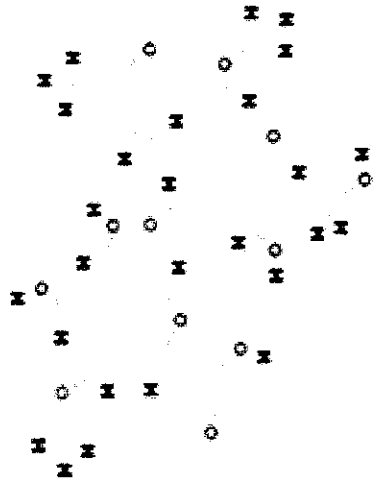
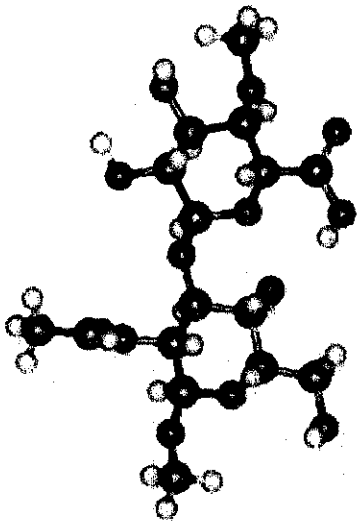
Name Of Compound	2-D Modelling	3-D Modelling
Hyaluronan (HA)		

Figure 19: Molecular modeling of Hyaluronan (HA)

The modeling of Hyaluronan has been shown in Fig. 19. The details of bond length between atoms of HA and its basic properties are shown in Table 5 and 6, respectively.

Table 5: Bond and bond length of HA

Bonds	Bond	Bond Type	Bond Length
O1 - C2	1	Single	1.4010
C2 - C3	1	Single	1.5472
C2 - O4	2	Double	1.2438
C3 - C5	1	Single	1.6925
C5 - O6	1	Single	1.4522
C5 - C7	1	Single	1.4796
C7 - O8	1	Single	1.4295
C7 - C9	1	Single	1.6231
C9 - C10	1	Single	1.6218
C10 - O11	1	Single	1.3701
O11 - C3	1	Single	1.5547
C9 - O12	1	Single	1.4446
C10 - O13	1	Single	1.5333
O13 - C14	1	Single	1.4736

C14 - C15	1	Single	1.6234
C15 - O16	1	Single	1.4351
C15 - C17	1	Single	1.5798
C17 - C18	1	Single	1.5168
C18 - O19	1	Single	1.4327
C17 - O20	1	Single	1.3149
C14 - C21	1	Single	1.4278
C21 - C22	1	Single	1.6633
C22 - O20	1	Single	1.5331
C21 - N23	1	Single	1.4346
N23 - C24	1	Single	1.3390
C24 - O25	2	Double	1.2421
C24 - C26	1	Single	1.4944
O1 - H1	1	Single	0.9863
C3 - H2	1	Single	1.0968
N23 - C24	1	Single	1.3390
C24 - O25	2	Double	1.2421
C24 - C26	1	Single	1.4944
O1 - H1	1	Single	0.9863
C3 - H2	1	Single	1.0968
C5 - H3	1	Single	1.1014
O6 - H4	1	Single	0.9848
C7 - H5	1	Single	1.0986
O8 - H6	1	Single	0.9913
C9 - H7	1	Single	1.0989
C10 - H8	1	Single	1.0996
O12 - H9	1	Single	0.9883
C14 - H10	1	Single	1.0967
C15 - H11	1	Single	1.0973
O16 - H12	1	Single	0.9882
C17 - H13	1	Single	1.1034
C18 - H14	1	Single	1.0991
C18 - H15	1	Single	1.0989
O19 - H16	1	Single	0.9898
C21 - H17	1	Single	1.1023
C22 - H18	1	Single	1.0980
C22 - H19	1	Single	1.0991
N23 - H20	1	Single	0.9936
C26 - H21	1	Single	1.0995

The HA structure shows the total measurement of 47 bond length with average bond length of 1.280556 Å.

Table 6: Basic properties of HA

Properties	**Hyaluronic acid**
Number of Atoms	47
Molecular Formula	$C_{14}H_{21}NO_{11}$
Molecular Composition	C: 0.441, H: 0.061, N: 0.037, O: 0.462
Molecular Weight	381.345
Average Bond Length (Å)	1.280556
Exact Molecular Weight	381.127

Table 6 shows the basic properties of hyaluronic acid. Total number of atom in HA molecules are 47. The molecular weight and exact molecular weight are almost same. Antitumor drug linkage to hyaluronan might improve targeting to cancerous cells and overcome the problem of low drug hydrosolubility due to some specific HA receptors (CD44) are overexpressed in various malignant cell types (Leonelli, et al., 2008). Although various water-soluble synthetic polymers have been exploited, naturally occurring polymers with intrinsic cell specific binding capacity such as HA molecule have tremendous potential as target-specific drug carrier (Manju & Sreenivasan, 2011). Hyaluronan is highly non-antigenic and non-immunogenic, owing to its high structural homology across species and poor interaction with blood components (Amarnath, et al., 2006).

4.1.2 Multi-molecular Modeling

4.1.2.1 Hyaluronan crosslink Paclitaxel molecule nanoparticle

The hyaluronan crosslink were drawn randomly in stick representation as illustrated in Fig. 20. Hyaluronan acts tumor-specific targeting vehicle to interact with CD44 receptor at tumor cell whereas the glutaraldehyde is the cross-linker. The crosslinking occurred by acetalization which occur between hydroxyl groups of HA and the aldehyde group of glutaraldehyde. The hyaluronan crosslink paclitaxel molecule nanoparticle shows the total measurement of average bond length of 1.280745667 Å. The paclitaxel molecules are in particle representation as illustrated in Fig. 20 and the details of the energy of the multi-molecular modeling is shown in Table 7.

Figure 20: Molecular modeling of Hyaluronan crosslink Paclitaxel molecule nanoparticle

Hyaluronan crosslink not only further increases the drug solubility but more importantly it enhances the targeted delivery chances to reach the cancer site. CD44 is overexpressed in human cancer cells and it is cell membrane-localized receptors or HA binding proteins thus HA can bind to the cell surface via interactions with CD44 (Luo, et al., 2009). In similar studies, cross-linked hyaluronan particles are nano-sized and can be attractive delivery candidates for a variety of biomedical applications. Furthermore, the biocompatibility of the HA nanoparticles, the high affinity toward cancer cells, and the suitable physicochemical properties of the HA nanoparticles make it as a nano-drug delivery system being capable of selectivity inhibiting cancer cells of solid tumor and metastatic tumor (Al-Ghananeem, et al., 2009).

Table 7: Energy value retrieved from Discovery Studio 2.5 molecular modeling of Hyaluronan crosslink Paclitaxel molecule nanoparticle

Properties	**Hyaluronan crosslink Paclitaxel molecule nanoparticle**
Improper Energy	0.030
Initial Potential Energy	-61.558
Electrostatic Energy	-131.097
CHARMM Energy/Force field	-61.932
Dihedral Energy/ Torsional energy	72.641
Potential Energy	-61.932
Energy	137.579
Bond Energy	2.012
Initial Energy	191.744
Angle Energy	13.361
Relative Energy	7.530
Van Der Waals energy	-18.879

Molecular docking was applied in this modeling which refers to the computational prediction of the least energy pose between two interacting molecules. The docking allowed to finds the best fit by assuming the molecule to be rigid, and the total potential energy, often referred as energy minimization of the CHARMM potential (or force-fields). The nature of bond is the bond length shrinkage with the assortment of bonds due to attractive forces between electrons and nuclei will rise with the increase in quantity of shared electrons between bonded atoms.

The bond energies and bond length depend on many factors such as electron affinities, size of atom involve in the bond, differences in their electronegativity and the overall structure of the molecule for the covalent bonds. Indeed, there is a general trend such that the bond length is reciprocal with the bond energy. The larger molecule is generally held fixed whereas the side chain atoms of the smaller molecule (the ligand) are free to move during the energy minimization (Sukhwani & Herbodt, 2010).

The overall energy minimization and calculation is showed in Table 6. Initial Potential Energy, electrostatic Energy, CHARMM Energy/Force field for HA cross-linked paclitaxel nanoparticles were negative along with negative Van Der Waals energy might be due to lack of any solvent in modeling and crosslink nanoparticle simulation. However, CHARMM energy usually comes negative. The energy minimization is calculated by the permutation of the total non-bonded and bonded molecule energy. The non-bonded energy is the combination between van der Waals energy and electrostatic energy. Meanwhile, the bonded energy consists of the grouping of 4 different energies which are bond energy, angle energy, torsion energy and improper energy. For stability of molecules the energy matters a lot. High energy states favor the instability in molecules. A real and rational modeled molecule structure should be in state which correspond a model near minimum potential energy and minimum energy arrangement of the atoms corresponds to stable state of the molecule (Ferrara, et al., 2004).

CHAPTER 5: CONCLUSION

5.1 Conclusion

The rational drug design of the hyaluronan crosslink paclitaxel molecule structure using Discovery Studio 2.5 would be multidisciplinary approaches in developing anticancer drugs. The uni-molecular and 3-D structure of hyaluronan crosslink paclitaxel molecule nanoparticle targeted delivery to cancer cells had been successfully modeled. Hyaluronan crosslink have high potential as targeted delivery vehicle to transport the anticancer drug, paclitaxel to cancer cells besides improving the stability, bioavailability of anticancer hydrophobic drug. Hyaluronan crosslink paclitaxel molecule nanoparticle targeted drug deliver to cancer cells molecular modeling is assumed to show that it may be an effective way to inhibit cancerous cells.

REFERENCES

Al-Ghananeem, A. M. et al., 2009. Intratumoral Delivery of Paclitaxel in Solid Tumor from Biodegradable Hyaluronan Nanoparticle Formulations. *AAPS PharmaSciTech,* 10(2).

Amarnath, L. P., Srinivas, A. & Ramamurthi, A., 2006. In Vitro Hemocapability Testing of UV-Modified Hyaluronan Hydrogels. *Biomaterials,* 27(1416-1424).

B da Rocha, A., Lopes, R. M. & Schwartsmann, G., 2001. Natural Products in Anticancer Therapy. *Current Opinion in Pharmacology,* 1(364-369).

Bandyopadhyay, S., Bagchi, A. & Maulik, U., 2005. Active Site Driven Ligand Design: An Evolutionary Approach.

Bowes, J. & Cater, C., 1968. The Interaction of Aldehydes with Collagen. *Biochim.Biophys.Acta,* 168(341-352).

Carelle, N. et al., 2002. Changing Patient Perceptions of Side Effects of Cancer Chemotherapy. *Cancer,* 95(155-63).

Cho, K. et al., 2008. Therapeutic Nanoparticles for Drug Delivery in Cancer. *Clinical Cancer Research,* 14(1310-1316).

Cooper, S., 2003. Reappraisal of Serum Starvation, The Restriction Point G0, and G1 Phase Arrest Points. *The FASEB Journal,* Volume 17.

Daunay, B. & Micaelli, A., 2007. 6 DOF Haptic Feedback for Molecular Docking Using Wave Variables. *IEEE International Conference on Robotic and Automation.*

Day, A. J. & de la Motte, C. A., 2005. Hyaluronan Cross-linking: A Protective Mechanism in Inflammation?. *TRENDS in Immunology,* 26(12).

Devita Jr., V. T. & Chu, E., 2008. A HIstory of Cancer Chemotherapy. *Cancer Res,* 68(21).

Dias, R. & de Azevedo Jr., W. F., 2008. Molecular Docking Algorithms. *Current Drug Targets,* 9(1040-1047).

Dineshkumar, B., Kumar, P. V., Bhuvaneshwaran, S. & Mitra, A., 2010. Advanced Drug Design Softwares and Their Applications in Medical Research. *International Journal of Pharmacy and Pharmaceutical Sciences,* 2(3).

Ferrara, P. et al., 2004. Assesing Scoring Functions For Protein-Ligand Interactions. *J Med Chem,* 41(12)(3032-47).

Gul-e-Saba, Abdah, A. & Abdullah, M., 2010. Hyaluronan-mediated CD44 Receptor Cancer Cells Progression and the Application of Controlled Drug-delivery System. *International Journal of Current Chemistry,* 1(4), pp. 195-215.

Jain M.D, K., 2005. Targeted Drug Delivery for Cancer. *Technology in Cancer Research & Treatment,* 4(4).

Jemal, A., Siegel, R., Xu, J. & Ward, E., 2010. Cancer Statistics, 2010. *CA Cancer Journal for Clinicians,* 60(5), pp. 277-300.

Kluger, R. & Alagic, A., 2004. Chemical Cross-linking and Protein-protein Interactions - A Review With Illustrative Protocols. *ELSEVIER Bioorganic Chemistry,* 32(6 (451-472)).

Kumar, B. et al., 2009. Recent Developments in Cancer Therapy by The Use of Nanotechnoogy. *Digest J Nanometer Biostr,* 4(1)(1-12).

Kumar, N. et al., 2006. Applying Computational Modeling to Drug Discovery and Development. *Drug Discovery Today,* Issue 806-811.

Lamari, F. N. & Cordopatis, P., 2008. Exploring the Potential of Natural Products in Cancer Treatment. *Anticancer Therapeutics.*

Lee, H., Ahn, C. H. & Park, T. G., 2009. Poly[lactic-co-(glycolic acid)]-Grafted Hyaluronic Acid Copolymer Micelle Nanoparticles for Target-Specific Delivery of Doxorubicin. *Macromolecular Bioscience,* p. 336–342.

Lee, H., Lee, K. & Park, T. G., 2008. Hyaluronic Acid-Paclitaxel Conjugate Micelles: Synthesis, Characterization, and Antitumor Activity. *Bioconjugate Chem,* 19(1319-1325).

Leonelli, F., Bella, A. L., Migneco, L. M. & Bettolo, R. M., 2008. Design, Synthesis and Applications of Hyaluronic Acid-PAclitaxel Bioconjugates. *Molecules,* 13(360-378).

Leonelli, F., Bella, A. L., Migneco, L. M. & Bettolo, R. M., 2008. Synthesis and Applications of Hyaluronic Acid-Paclitaxel Bioconjugates. *Molecules,* Issue 360-378.

Luo, Y., Prestwich, G. D., Kopecek, J. & Lu, Z. R., 2009. Hyaluronic Acid Containing Bioconjugates: Targeted Delivery of Anti-cancer Drugs to Cancer Cells. *Patent Application Publication.*

Mandal, S., Moudgil, M. & Mandal, S. K., 2009. Rational Drug Design. *European Journal of Pharmacology 625, 90-100.*

Manju, S. & Sreenivasan, K., 2011. Conjugation of Curcumin onto Hyaluronic Acid Enhances its Aqueous Solubility and Stability. *Journal of Colloid and Interface Science,* Issue 359(1), 318-325.

Mann, J., 2002. Natural Products in Cancer Chemotherapy: Past, Present and Future. *Cancer Nature Reviews,* Volume 2.

Marrone, T. J., Briggs, J. M. & McCammon, J., 1997. Structured-based Drug Design: Computational Advances. *Annu.Rev.Pharmacol.Toxicol.*

Migneault, I., Dartiguenaye, C., Bertrand, M. J. & Waldron, K. C., 2004. Glutaraldehyde: Behavior in Aqueous Solution, Reaction with Proteins, and Application to Enzyme Crosslinking. *BioTechniques,* 37(790-802).

Mishra, R. C., 2011. Microtubule Binding Natural Substance in Cancer Therapy. *Opportunity, Challenge and Scope of Natural Products in Medicinal Chemistry,* Issue 269-282.

Newman, D. & Cragg, G., 2007. Natural Products as Source of New Drugs Over the Last 25 Years. *J Nat Prod,* 70(3)(461-77).

Nimni, M. et al., 1987. Chemically Modified Collagen: A Natural Biomaterial for Tissue Replacement. *J. Biomed Mater. Res,* 21(741-771).

O'connor, R., 2007. The Pharmacology of Cancer Resistance. *Anticancer Res,* 27(1267-72).

Pal, D., 2003. Diet and Cancer. *The J North Orissa Univ,* 2(1)(77-80).

Pal, D. & Nayak, A. K., 2010. Nanotechnology for Targeted Delivery in Cancer Therapeutics. *International Journal of Pharmaceutical Sciences Review and Research,* pp. 1-7.

Patil, S. M. & Joshi, H. P., 2012. Colloidal Drug Delivery System for Tumor Specificity of Paclitaxel in Mice. *Der Pharmacia Lettre,* 4(3)(961-967).

Pure', E. & Cuff, A. C., 2001. A Crucial Role for CD44 in Inflammation. *Trends Mol.Med,* 7(213-221).

Rios-Doria, J. et al., 2012. A Versatile Polymer Micelle Drug Delivery System for Encapsulation and In Vivo Stablization of Hydrophobic Anticacer Drugs. *Journal of Drug Delivery,* Issue ID 951741.

Shi, M. et al., 2006. Antiproliferation and Apoptosis Induced by Curcumin in Human Ovarian Cancer. *Elsevier,* 30(3)(221-6).

Silva, R. M. et al., 2004. Preparation and Characterisation in Simulated Body Conditions of Glutaraldehyde Crosslinked Chitosan Membranes. *Journal of Material Science: Material in Medicine,* 15(1105-1112).

Sukhwani, B. & Herbodt, M. C., 2010. FPGA-based Acceleration of CHARMM-potential Minimization. *Computer Architecture and Automated Design,* Volume MA 00215.

Tao, Y. et al., 2012. Core Cross-linked Hyaluronan-Styrylpyridium Micelles as A Novel Carrier for Paclitaxel. *Carbohydrate Polymers* , 88(118-124).

Teodore, M. L., Phillips Jr., G. N. & Kavraki, L. E., 2001. Molecular Docking: A Problem With Thousands of Degrees of Freedom.

University of Colorado, B. C. a. B. D., 2011. *Exact Molecular Mass versus Molecular Weight.* [Online]

Available at: http://orgchem.colorado.edu/hndbksupport/ms/molmassmw.html [Accessed 16 December 2011].

Yadav, A. K. et al., 2010. Development and Characterization of Hyaluronic Acid Decorated PLGA Nanoparticles for Delivery of 5-fluorouracil. *Drug Delivery,* 17(8), pp. 561-572.

Yung, S. & Chan, T. M., 2012. The Role of Hyaluronan and CD44 in the Pathogenesis of Lupus Nephritis. *Autoimmune Disease,* Issue ID 207190.

Yu, Q., Toole, B. & Stamenkovic, I., 1997. Induction of Apoptosis of Metastatic Mammary Carcinoma Cells In Vivo by Disruption of Tumor Cell Surface CD44 Function. *J. Exp Med,* 186(1985-1996).