Modeling of hyaluronan-cisplatin targeted delivery in human colorectal cancer cells.

by

Adibah Bazilah binti Abdul Rani

Supervisor: AP Dr. Azmuddin Abdullah

Dissertation submitted in partial fulfillment of

the requirements for the

Bachelor of Engineering (Hons)

(Chemical Engineering)

MAY 2011

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CERTIFICATION OF APPROVAL

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Approved by,

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(AP Dr. Azmuddin Abdullah)

UNIVERSITI TEKNOLOGI PETRONAS

TRONOH, PERAK

May 2011

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.

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ADIBAH BAZILAH BINTI ABDUL RANI

ABSTARCT

A modeling study on effective delivery of hyaluronan-cisplatin (HA-Pt) in human colorectal cancer was carried out. The main objective was to investigate how the HA-Pt conjugate improves drug delivery to colorectal cancer cells. The software, Discovery Studio 2.5 generates a three-dimensional simulation of the conjugated drug. The drug cisplatin is classified as an alkylating agent, the first platinum based anticancer drug that is effective against cancer. It inhibits DNA actual utilization by base pairing and causes a miscoding of DNA. Hyaluronan is a naturally occurring linear polymer that is a major component of extracellular matrix (ECM). The hyaluronan receptor, CD44 is overexpressed in cancer cells compared to normal cells. As a result, HA binding to CD44 can be explored for targeted anticancer drug delivery in human cancer cells.

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CHAPTER 1

INTRODUCTION

1.1 Background of study

Cancer evolution is a complex process that involves many different phenomena that occurs at different scales. The phenomenological description strongly depends on the enlargement used in real or ideal microscopic scale such as the nanoscale simulation. From the modeling point of view, a connection can be drawn between the cellular level and the microscopic, mesoscopic and macroscopic scales.

As the factors that affect drug delivery are linked to biochemistry and metabolism taking place at the nanoscale in the body, nanotechnology holds better potential in overcoming problems related to drug delivery. It involves the intersection of variety of disciplines including engineering, material science, chemistry and physics with cancer biology (Mansoor, 2007). Nanotechnology enables the maintaining of drug level in therapeutic range, increasing specificity, sustained drug delivery and decreasing toxicity side effects. Knowledge in polymer chemistry is combined together in order to develop novel methods for drug delivery.

1.2 Problem Statement

Abnormal cell proliferation is what defines tumorigenesis. It is the increase in tumor cell number, and thus tumor burden that ultimately accounts for the adverse effects on the host. The objective of current cancer therapy is to reduce the number of tumor cells to prevent further accumulation. For this reason, the understandings of nanoscale drug delivery system to cancer cells play a significant role in improving cancer treatment. The interaction between anti-cancer drug towards the cancer cells should first be understood.

Up to date, there are numerous available anticancer drugs with distinct mechanism of action. The effects they pose to the normal and cancer cells may vary from one drug to another. Besides that, the chosen drug may also face problem such as poor solubility, unsuitable retention time, poor bio compatibility or some other problems. As the drugs used to fight cancer affect normal cells as well as cancerous cell, treatment with these drugs must be balanced between maximum killing of cancer cells and minimum effect on normal cells. Therefore, improvement in drug delivery system is important to ensure high selectivity on cancer cells and decreasing negative side effects.

Molecular modeling combines the theoretical method and computational to mimic the behavior of the molecules in three dimensional manner. Some features of molecular modeling is that it allows structure optimization, energy minimization and molecular docking. In molecular docking, the software regonizes interaction between ligands and targets at atomic level.

In the past, the anti-cancer drugs development does not focus on macromolecular targeting. Currently, the development search for specific target that subsist in cellular compartments to assist treatment of cancer. Therefore, molecular modeling is used to explore such target to support experimental effort. Besides that, this method can also be used to design new and improve drug with minimum toxicity.

1.3 Objectives

The objectives of the project are:

- To model the nanoscale drug delivery system to cancer cells using Discovery Studio 2.5.
- To investigate the cisplatin mechanism of action towards cancer cells.
- To simulate the delivery of hyaluronan-cisplatin conjugate to cancer cells by CD44 signaling pathway.

1.4 Scope of Study

The project comprises a study of molecular modeling to increase the solubility of anticancer drugs and CD44 mediated drug delivery. Assumptions are made and defined in order for the project objectives to be achieved in the time frame given.

In the modeling framework, the assumptions were:

- Hyaluronan-CD44 interaction follows the lock and key analogy
- Cisplatin automatically released into cells after hyaluronan-CD44 interacts
- Van de Waals forces can be ignored

The modeling of this project focuses on three dimensional molecular modeling to produce hyaluronan-cisplatin conjugate. Prior to that, the properties and the reaction between hyaluronan-cisplatin, hyaluronan to CD44 and cisplatin to cancer cells must be thoroughly studied.

CHAPTER 2

LITERATURE REVIEW

2.1 Cancer cells

Disregulation of cell proliferation and apoptosis are the hallmarks of cancer cells (Shi et al., 2006). Normal body cells grow and divide and know to stop growing. Over time, they also die. Unlike these normal cells, cancer cells just continue to grow and divide out of control and do not die when they are supposed to. In addition for regulation of cell proliferation, cell numbers are also controlled by regulated apoptosis (Shi et al., 2006).However, cancer cells have developed many kinds of mechanisms to be refractory to this regulatory signal and thus do not undergo apoptosis under appropriate conditions (Elliott & Blobe, 2005; Hanahan & Weinberg, 2000). The differences between a normal and cancer cells are shown in Figure 2.1.

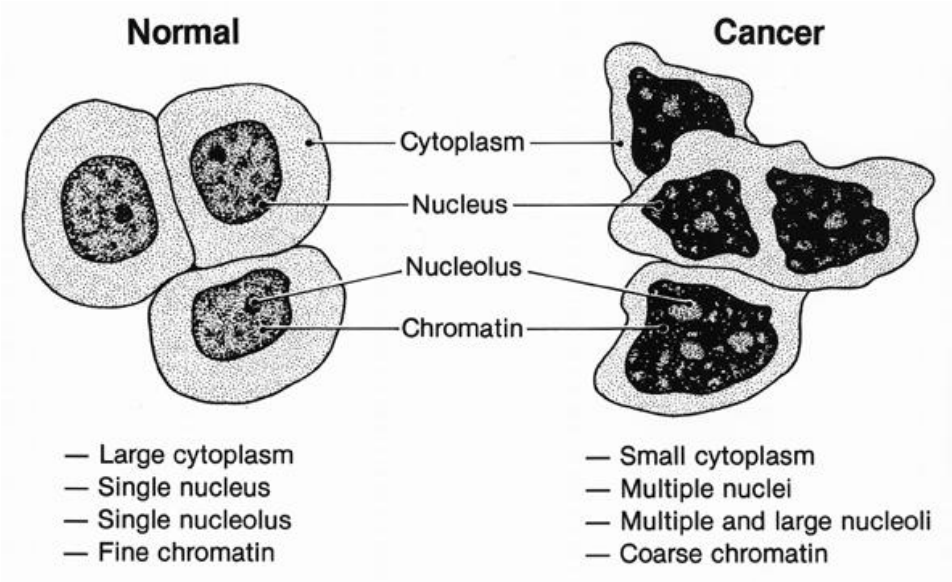

Figure 2.1: Differences of cell structure of normal and cancer cells.
(Source: http://breastcancer.about.com/od/diagnosis/tp/tumor_grade.htm)

The changes in physical properties of the cells may be caused due to the reasons summarized as follows:

Table 2.1: The changes in physical properties of cancer cells. (Changes in Physical Properties of Cancer Cells, 2010)

Physical changes	Explanation
Cytoskeletal changes	The distribution and activity of the microfilaments and microtubules may change and this allows the cell to interact with neighboring cells and alter the appearance of the cells.
Cell adhesion/motility	The reduction of cell to cell and cell to extracellular matrix adhesion allows large masses of cells to form.
Nuclear changes	The shape and organization of the nuclei of cancer cells may be markedly different from that of the nuclei of normal cells of the same origin.
Enzyme production	Cancer cells often secrete enzymes that enable them to invade neighboring tissues. These enzymes digest away the barriers to migration and spread of the tumor cells.

2.2 Cancer cell metabolism and proliferation

Cancer cells show clear differences in energy metabolism when compared to normal cells. The metabolism of cancer is approximately 8 times greater than the metabolism of normal cells. The first tumor-specific alteration of altered metabolism was discovered by the Nobel Prize winner Otto Warburg in the 1920s (Kroemer & Pouyssegur, 2008). The ‘‘Warburg phenomenon’’ consists of an increase in glycolysis that is maintained in conditions of high oxygen tension (‘‘aerobic glycolysis’’) and gives rise to enhanced lactate production (Brahimi-Horn, Chiche, & Pouysségur, 2007; Warburg, Posener, & Negelein, 1930)

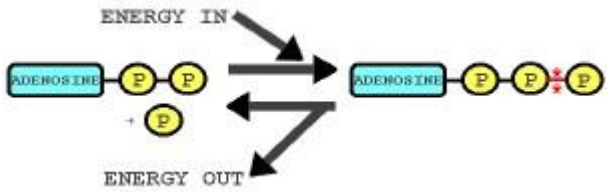

Figure 2.2: Energy production through the breaking down of ATP molecule.
(Source: http://www.atpdepletion.com/)

All cells need energy to survive and divide. The energy is produced from the production on Adenosine Triphosphate (ATP) through glycolysis. Compared to normal cells, cancer cells need to generate ATP at a much higher rate which is required to synthesize new molecules and rapidly divide. For that reason, the energy metabolism of cancer cells are significantly altered and they generate an excessive amount of ATP.

A normal cell is surrounded by a membrane which selectively allows materials to flow in and out. Oxygen and nutrients, such as glucose, flow in and the waste products of cellular chemistry flow out. The cells are protected by the immune system whereby a well functioning immune system is the best defence against the formation of cancer cells.

However, when environmental toxins or carcinogens overpower the immune system, the entire program is altered. The cell membrane is affected first, losing its ability to exchange oxygen causing the cell to revert to a primitive survival mechanism which is fermentation. The newly formed anaerobic cancer cell cannot be repaired as fermentation is not reversible and the cell is now out of control and must be destroyed as rapidly as possible.

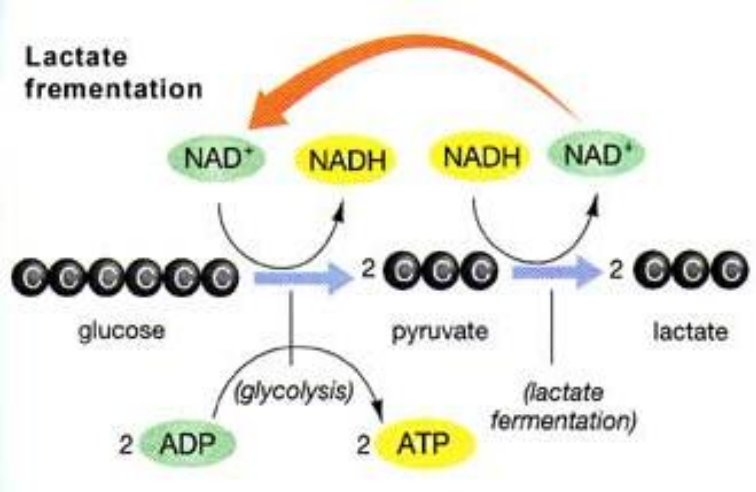

Figure 2.3: Glycolysis and fermenatation.
(Source: http://forums.studentdoctor.net/showthread.php?t=207239&page=4)

The lactic acid produced by fermentation lowers the cell pH and destroys the ability of DNA and RNA to control cell division causing uncontrolled cancer cell multiplication. Unlike normal cell whose pH is 7.3 to 7.4, cancer cells have pH as low as 4.0 or 5.5 in the case for terminal cancer. Besides that, the charge of cancer cell drops from 90 milivolts to less than 40 milivolts causing only 5 substances that can pass in or out of the cell which are water, sugar, potassium, cesium and rubidium. Oxygen cannot enter the cell despite the large amount of oxygen in the blood. Cesium, because of its electrical properties can still enter the cancerous cell. Therefore, it is usually used in high pH therapy of cancer. Besides cesium, other strong alkaline that is mainly used to counter cancer are rubidium and potassium.

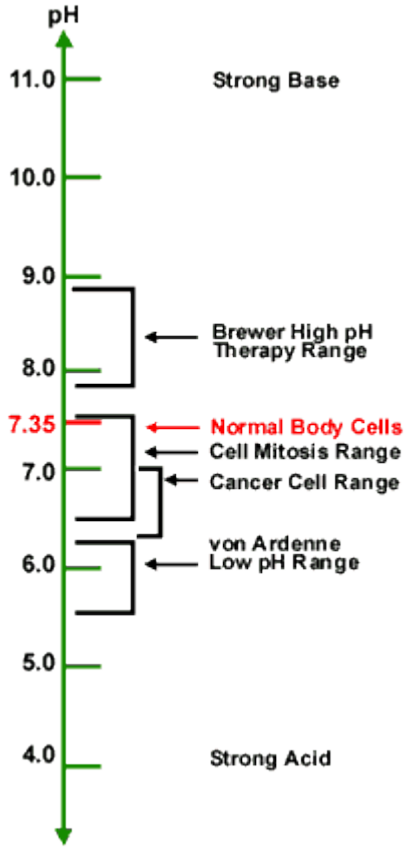

Figure 2.4: The relationship between pH of cancer cells and cancer progression. (Source: http://www.mindfully.org/Health/High-pH-Therapy-Brewer1984.htm)

Cancer cells contain the full complement of biomolecules that are necessary for survival, proliferation, differentiation, cell death, and expression of many cell-type-specific functions. Cancer happens when these functions are not properly regulated. Hence, to understand cancer, it is important to understand proliferation in normal cells and how it becomes uncontrolled in cancer cells.

Fundamentally, accumulation of clonal cells causes cancer. Cell proliferation is an increase in the number of cells as a result of cell division. The rate of cell proliferation depends on three parameters:

(*a*) Rate of cell division (*Tc*) referring to the time needed to complete cell division cycle

(*b*) Fraction of cells within the population undergoing cell division

(*c*) Rate of cell loss from the population due to terminal differentiation or cell death

In normal cell development, each organ maintains control over *Tc*, growth fraction and cell loss. Physiological stimuli can change these parameters leading to increased growth in tissues. However, a new steady state is achieved once the stimulus is removed.

The cell division cycle must be comprehended to know the defects that may have happen that cause abnormal proliferation process in cancer cells. Cancer cells tend to continuously grow and divide out of control while normal cell follows the cell division cycle orderly and die over time.

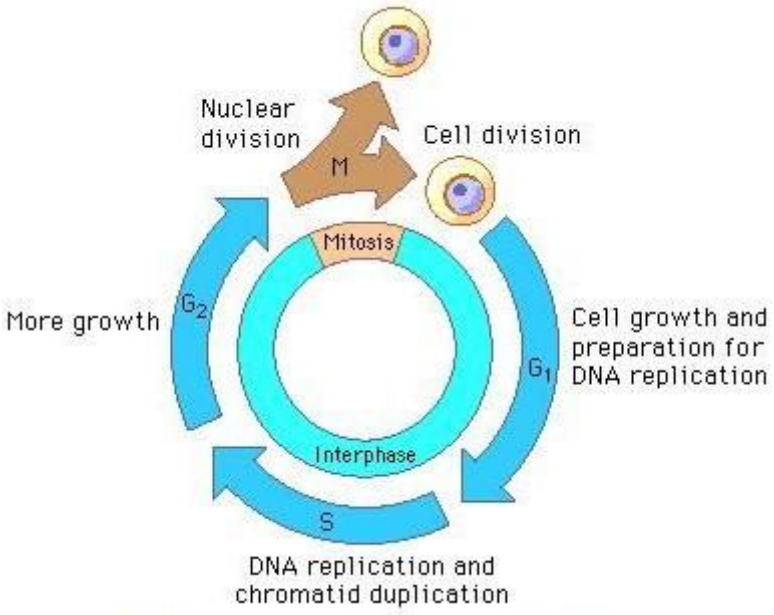

Figure 2.5: The cell division cycle.
(Source: http://www.phschool.com/science/biology_place/biocoach/mitosisisg/cellcyc.html)

The cell division cycle consist of two major phase which is the interphase and mitosis phase. The interphase is the phase where the cell grows and replicates its DNA. It consists of 3 stages known as Gap 1 (G1), DNA synthesis (S) and Gap 2 (G2). The mitosis phase (M) is where cells divide into two cells that are identical to the mother cell. The functions of each stage are shown in Table 2.2.

Table 2.2: The cell cycle functions.

Phase	Stage	Description
Interphase	G1	Growth and preparation of the chromosomes for replication
	S	DNA replication
	G2	Ensure cell is ready to enter M phase and divide
Mitosis	M	Cell growth stops and cellular energy is focused on the division into two daughter cells

Tumor cells can proliferate without proliferation signals. Despite that, the tumor cells do not necessarily proliferate faster than normal cells. Biopsy samples from normal, inflammatory, and neoplastic lesions of the lung, cervix, vocal cord, or pharynx have been analyzed for the rate of cell proliferation. These studies show that benign inflammatory lesions can grow over 20 times faster than cancer in a discrete time and place (Teodori et al., 1990). Hence, not only rapid growth of cell distinguishes neoplasia but also uncontrolled growth over time.

The growth rate of cell decreases as the tumor mass increases. The change in growth rate with larger tumor may be caused by (Andreeff, Goodrich, & Pardee, 2000):

(a) Decrease in the growth fraction

(b) Increase in cell loss

(c) Nutritional depletion of tumor cells resulting from outgrowth of available blood supply

(d) Lengthening of *Tc*

However, the biochemistry of growth appears to be very similar qualitatively in tumor and normal cells (Weber, 1983). The fundamental difference probably lies in a relaxation of the regulation of cell growth (Fingert, Campisi, & Pardee, 1991). For instance, proliferation of cancer cells still occurs in physiological level of growth factor whereby normal cells will remain inactive. Besides that, normal cell lies on secreted extracellular matrix (ECM) where various proteins stimulating cell growth present. Tumor cells however often are partly or completely independent of ECM for optimal growth, and they may secrete little matrix material (Liotta, 1986).

2.3 Colorectal Cancer

Colorectal cancer is currently one of the most common cancers in Malaysia (Balraj & Ruhana, 2007). Based on the Malaysia Cancer Statistics 2006, in Peninsular Malaysia only, the colorectal cancer is the most common cancer among men and second most common among women. All in all, it is the second most common cancer after breast cancer in Peninsular Malaysia. A total of 2,866 cases were registered with National Cancer Registry in 2006, representing 13.2% of all cases registered.

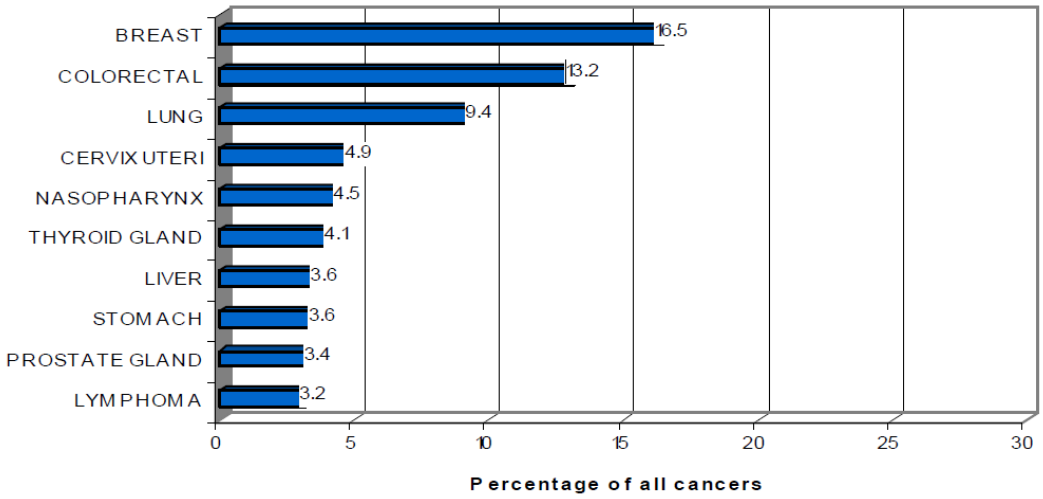

Figure 2.6: Ten most frequent cancer in Penisular Malaysia in 2006.
(Sourcs: http://www.makna.org.my/PDF/MalaysiaCancerStatistics.pdf)

Colorectal cancer is cancer that develops in the colon or the rectum. The colon and rectum are parts of the digestive system, also called the gastrointestinal system. The digestive system processes food for energy and rids the body of solid waste (fecal matter or stool) (Alteri et al., 2011). Colorectal cancer is also known as bowel cancer or colon cancer.

The colon consists of four sections which are the ascending colon, transverse colon, descending colon and sigmoid colon. Colorectal cancer usually develops slowly over a period of 10 to 15 years (Kelloff et al., 2004). Most of the colon cancers develop out of colon polyps, which grow like a mushroom out of the lining of the large bowel with a stalk and a fleshy polyp head. Certain kinds of polyps, called adenomatous polyps or adenomas, are the most likely to become cancers, though fewer than 10% of adenomas progress to cancer (Levine & Ahnen, 2006).

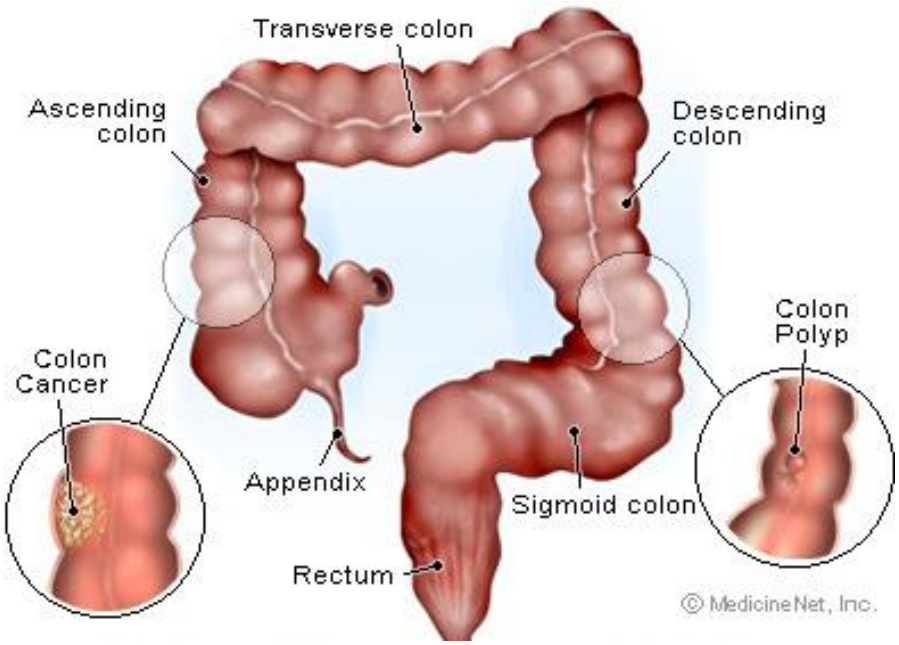

Figure 2.7: Colon sections and colon polyp.
(Source: http://www.medicinenet.com/colon_cancer/article.htm)

The extent to which colorectal cancer has spread is described by its stages. Staging is essential in determining the choice of treatment and in assessing prognosis.

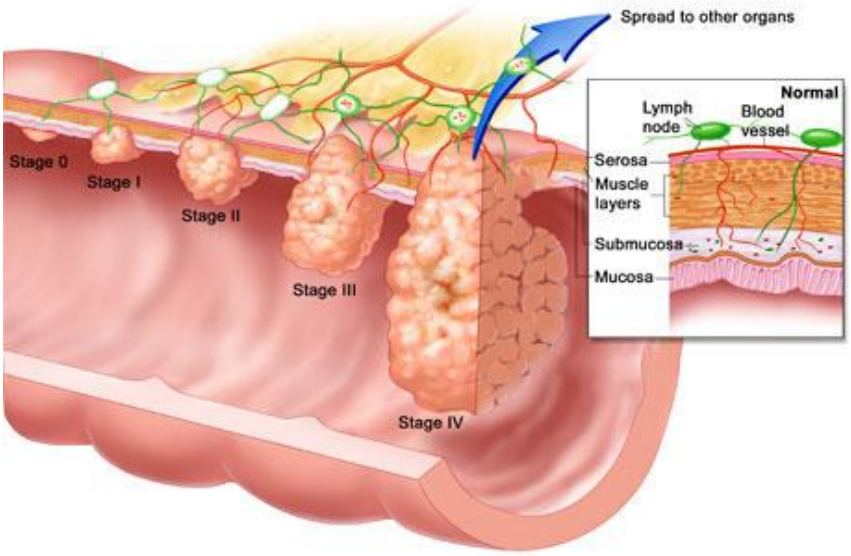

Figure 2.8: Stages of colorectal cancer.
(Source: http://www.ipsas.upm.edu.my/caed/download/colon-ca.pdf)

Table 2.3: Stages of colorectal cancer.

Stage 0	The cancer is very early. It is found only in the innermost lining of the colon or rectum
Stage I	The cancer involves more of the inner wall of the colon or rectum.
Stage II	The cancer has spread outside the colon or rectum to nearby tissue but not to the lymph nodes.
Stage III	The cancer has spread to nearby lymph nodes but not to other parts of the body.
Stage IV	The cancer has spread to other parts of the body. Colorectal cancer tends to spread to the liver and/or lungs.

2.4 Targeted Cancer Treatement

For decades, the hallmark of medical treatment for cancer has been intravenous cytotoxic chemotherapy (Gerber, 2008). This conventional chemotherapy typically works by killing rapidly dividing cells, which could be the tumor but also certain normal cells, causing side effects like hair loss, nausea, diarrhea and anemia (Pollack, 2010). Recent progress in understanding the molecular changes in cancer development offer the prospect of specifically targeting malfunctioning molecules and pathways to achieve more effective and rational cancer therapy (Sawyers, 2004). Targeted therapy may include monoclonal antibodies, small molecule inhibitors and targeted drug delivery.

Monoclonal antibodies are antibodies that are identical derived from one type of immune cell and each a clone of a single parent cell. In targeted cancer therapy, they are directed against molecules unique to, overexpressed in, or mutated in cancer cells. Monoclonal antibodies exert their anticancer effects through variety of mechanisms: by recruiting host immune functions (including natural killer cells and the complement cascade) to attack the target cell; by binding to ligands or receptors, thereby interrupting essential cancer cell processes; or by carrying a lethal payload, such as a radioisotope or toxin, to the target cell (i.e., conjugated monoclonal antibodies) (Gerber, 2008).

Small molecule inhibitors typically interrupt cellular processes by interfering with the intracellular signaling of tyrosine kinases (Gerber, 2008). Tyrosine kinase signaling can lead to cell growth, proliferation, migration, and angiogenesis in normal and malignant tissues. Unlike monoclonal antibodies, small molecule inhibitors are usually administered orally rather than intravenously.

Some therapies take advantage of the features in cancer in order to develop targeted therapy. Most solid tumors possess unique features and defects of their associated vasculature, such as extensive angiogenesis, defective vascular architecture, and increased vascular permeability, all of which can be used for delivering therapeutics (Juillerat-Jeanneret, 2008). Recognizing these features allows the usage of functionalized drug colloidal carrier for targeted delivery of anticancer drugs to tissues.

This can be done passively by increasing vascular permeability in defined location or actively by chemically modified drugs or nanoparticle. Alternatively, direct modification and/or functionalization of drugs, involving the chemical conjugation of drugs to disease-targeting biological markers, can be used to achieve direct active targeting of drugs (Juillerat-Jeanneret, 2008).

2.5 Cisplatin

In 1893, Alfred Werner suggests that $Pt(NH_3)_2Cl_2$ actually consists of two isomers of square planar which is the cis-isomer namely cis-diaminedichloroplatinum (II) and trans-isomer also known as transplatin. In 1969, Barnett Rosenberg has shown that only the cis-isomer has antitumor activity and is now known as cisplatin in short. In contrast, even though transplatin produce the same complex with DNA, it does not have useful anticancer activity. The different geometries of these two isomers result in different binding modes with DNA (Trzaska, 2005).

Cisplatin **Transplatin**

Figure 2.9: Molecular structure of cisplatin and transplatin.
(Source: http://clipartist.net/2011/04/page/138/)

Cisplatin, or cis-diaminedichloroplatinum(II) (cis-DDP), is an anticancer drug widely used to treat a variety of tumors, especially those of the testes, ovaries, head and neck (Comess & Lippard, 1993). At normal condition, cisplatin is found as a clear liquid. It is given to patient intravenously. For example, it may be introduced via drip infusion, central line or peripherally inserted central catheter (PICC) line. Central lines are typically inserted into a vein near the collar bone whereas PICC lines are inserted into a vein in the arm.

The exact dosage of cisplatin given to a patient is different from one to another. It depends on many factors such as the person height, weight, general health, other health problems and also the type of cancer of the patient.

Cisplatin is the first platinum-based drug that is developed for cancer treatment. Since the introduction, other platinum based drug such as carboplatin and oxaliplatin are introduced. Cisplatin is classified as an alkylating agent. It works by preventing the

production of genetic material by the cell. This is done by forming links with the strands of DNA and by doing so it binds them together. This prevents the cell from reproducing.

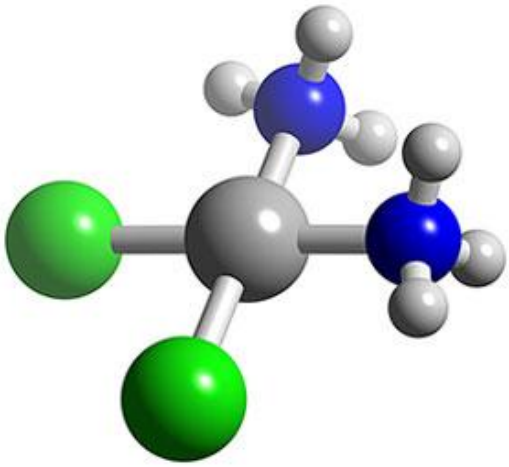

Figure 2.10: 3D structure of cisplatin.
(Source: http://www.3dchem.com/molecules.asp?ID=214)

Platinum atom = dark grey, Chlorine = green,
Nitrogen = dark blue, Hydrogen =light grey

2.6 Cisplatin Mechanism of Action

Although the mechanism has not yet been fully elucidated, cisplatin is generally believed to kill cancer cells by binding to DNA and interfering with the cell's repair mechanism, which eventually leads to cell death (Trzaska, 2005). In studies of the mechanism of action of cisplatin, it has been known that the cytotoxicity of cisplatin arises from its capacity to damage DNA, resulting in cisplatin-DNA adducts (A. M. J. Fichtinger-Schepman, 1985; Takahara, Rosenzweig, Frederick, & Lippard, 1995; Huang, Zhu, Reid, Drobny, & Hopkins, 1995). The formation of these adducts cause the purines to become destacked and the DNA helix to become kinked.

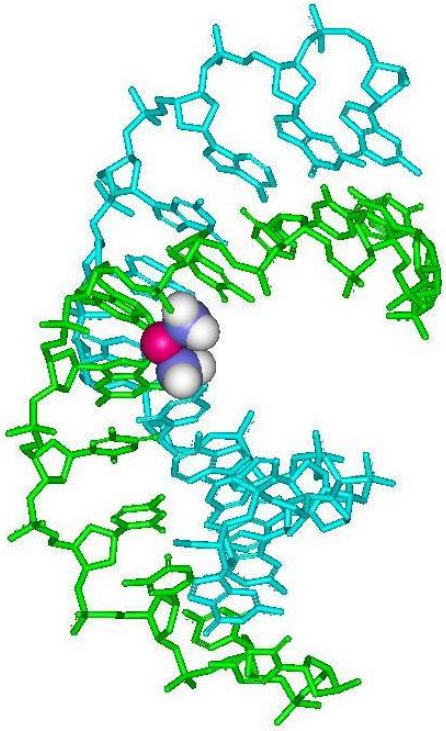

Figure 2.11: Cisplatin causing DNA to be kinked.
(Source: http://pharm1.pharmazie.uni-greifswald.de/bednarski_web/web/data/personen/Lectures/Alkylans/)

The mechanism of action of platinum-based anticancer drugs such as cis-diamminedichloroplatinum(II), or cisplatin, involves three steps: cell entry, drug activation, and target binding. The drug first enters the cell by active uptake or passive

diffusion. Inside the cell, cisplatin undergoes hydrolysis producing the highly reactive charged platinum complex $[Pt(NH_3)_2ClH_2O]^+$. Further hydrolysis displaces the remaining chloride ligand, and the platinum can bind to a second nucleotide base. The hydrolysis reaction occurs as the following equation:

$$Pt(NH_3)_2Cl_2 + H_2O \rightarrow [Pt(NH_3)_2Cl(H_2O)]^+ + Cl^-$$

$$[Pt(NH_3)_2Cl(H_2O)]^+ + H_2O \rightarrow [Pt(NH_3)_2(H_2O)_2]^{2+}$$

This complex coordinates to DNA through the N7 atom of either a guanine or adenine base (Trzaska, 2005). There are many types of adducts that can be formed by cisplatin and DNA. The major intrastrand cross-links are formed at guanine and adenine sites and represent ~90% of total platinum adducts (Eastman, 1987; Reedijk, 1992; Bruhn, Toney, & Lippard, 2007; Sip & Leng, 1993). The adducts formed are 1,2-intrastrand cross-links, where platinum coordinates to two adjacent guanine residues or an adjacent adenine and guanine (Takahara, Frederick, & Lippard, 1996). The most common adduct formed by reaction of activated cisplatin with DNA is the 1,2-d(GpG) cis-diammineplatinum(II) intrastrand cross-link, with 1,2-d(ApG) and 1,3-d(GpNpG) intrastrand cross-links being formed to a lesser extent (Ober & Lippard, 2008).

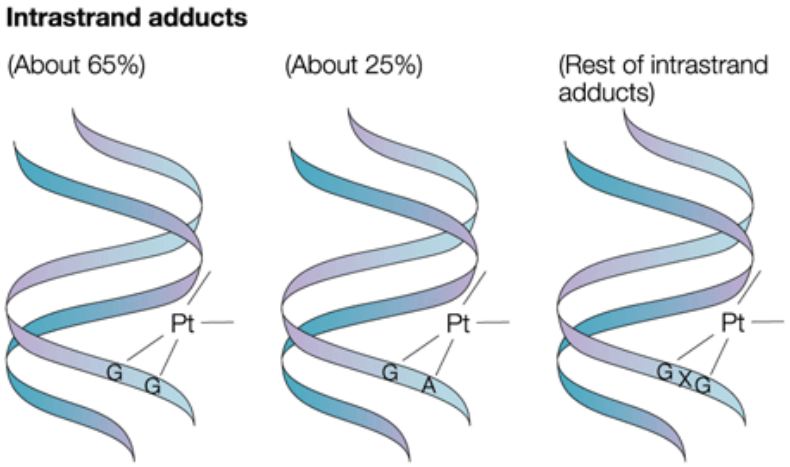

Figure 2.12: Types of intrastrand adducts.
(Source: http://www.narainsamy.com/cisplatin-molecule&page=3)

Interstrand cross-links are formed between two guanine residues on opposite strands at d(GpC)·d(GpC) sites and represent 5–10% of total platinum adducts

(Hopkins et al., 1991). The remaining are other intrastrand cross-links, interstrand cross-links, monofunctional adducts, or protein-DNA cross-links.

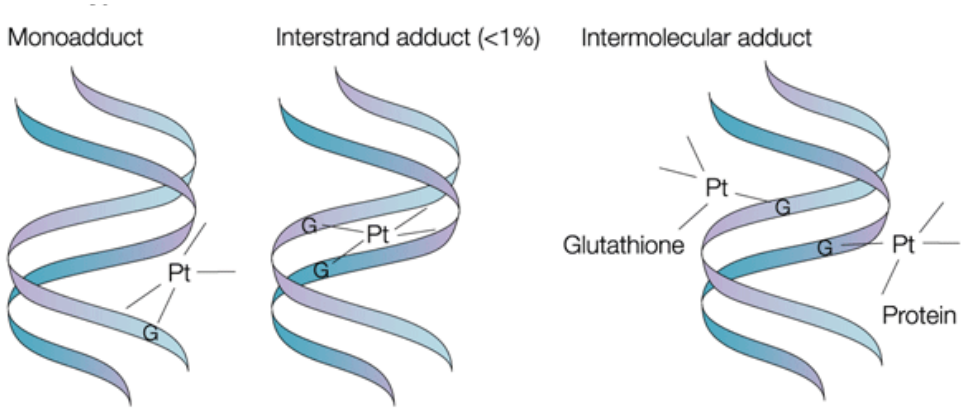

Figure 2.13: Monoadduct, interstrand adduct and intermolecular adduct.
(Source: http://www.narainsamy.com/cisplatin-molecule&page=3)

All in all, the drugs enter the cell by diffusion or with the aid of a transporter, become activated by forming aqua complexes, and bind to nuclear DNA, which in eukaryotes is packaged in highly condensed chromatin structures comprising nucleosome building blocks (Ober & Lippard, 2008). The figure below summarized cisplatin mechanism of action.

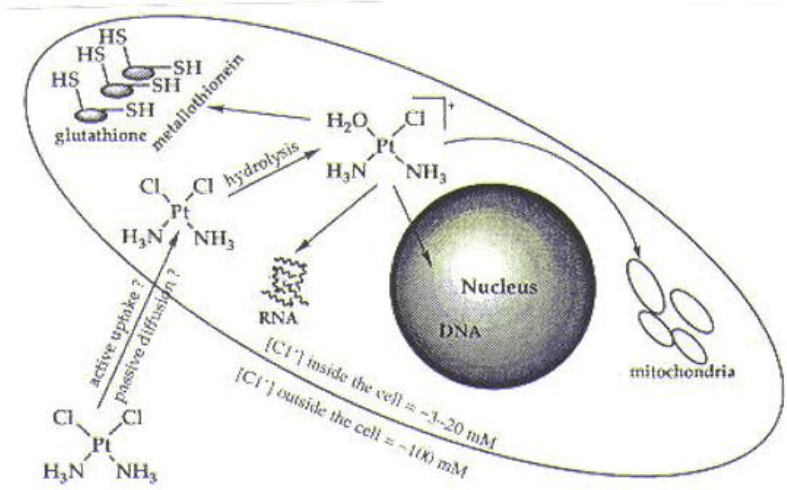

Figure 2.14: Steps involved in cisplatin mechanism of action.
(Source: http://www.chemcases.com/cisplat/cisplat12.htm)

2.7 Hyaluronan

Hyaluronan or hyaluronic acid was discovered in bovine vitreous humour by Meyer and Palmer in 1934. Hyaluronic acid (HA) is a naturally occurring polyanionic, polysaccharide that consists of N-acetyl-glucosamine and β-glucoronic acid (Brown & Jones, 2005). Hyaluronan is found in all tissues and body fluids of vertebrates as well as in some bacteria (Fraser & Laurent, 2003). Hyaluronan is a high-molecular-mass linear polymer found in the extracellular matrix, especially of soft connective tissues (Fraser & Laurent, 1992). It is most frequently referred to as HA due to the fact that it exists in vivo as a polyanion and not in the protonated acid form (Brown & Jones, 2005).

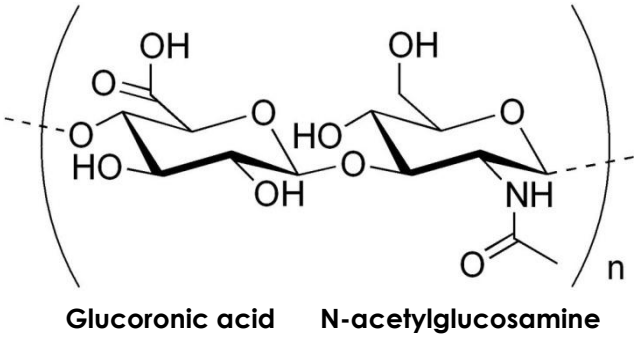

Figure 2.15: Structure of hyaluronic acid.
(Source: http://www.skinrejuvenex.com/educational-news/beauty-product-research-101-palacia-hydroptimal-gel/)

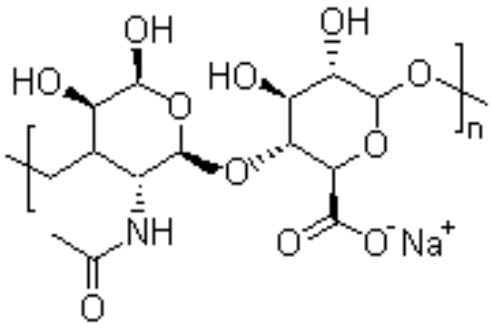

Figure 2.16: Structure of hyaluronan sodium salt.
(Source: http://www.buyersguidechem.de/AliefAus.php?pnumm=780708239236)

HA is made by enzymes called hyaluronan synthases (HAS). HA is synthesized at the plasma membrane with the polymer being extended from the reducing end, which results in its extrusion from the cell surface. This is typical for glycosaminoglycans (Tolg, Hamilton, & Turley, 2004). HA dissolves in water to form a viscoelastic solution, with increasing concentration raising the solution viscosity (10 mg/ml has a viscosity 5000x that of water) (Varti et al., 1999). The unique viscoelastic nature of HA along with its biocompatibility and non-immunogenicity has led to its use in a number of clinical applications, which include: the supplementation of joint fluid in arthritis, as a surgical aid in eye surgery, and to facilitate the healing and regeneration of surgical wounds (Fraser & Laurent, 2003). As a result of its inherent biocompatibility, biodegradability, and bioactivity, HA has proven to be a versatile molecule in both drug delivery and tissue engineering applications.

2.8 Hyaluronan-Cisplatin Conjugate

A conjugation of a drug with a polymer is called a 'polymeric pro-drug'. The polymeric conjugates of drug possess some advantages compared to their low molecular weight precursors. The main advantages include: (1) an increase in water solubility of low soluble or insoluble drugs, and therefore, enhancement of drug bioavailability; (2) protection of drug from deactivation and preservation of its activity during circulation, transport to targeted organ or tissue and intracellular trafficking; (3) an improvement in pharmacokinetics; (4) a reduction in antigenic activity of the drug leading to a less pronounced immunological body response; (5) the ability to provide passive or active targeting of the drug specifically to the site of its action; (6) the possibility to form an advanced complex drug delivery system, which, in addition to drug and polymer carrier, may include several other active components that enhance the specific activity of the main drug (Khandare & Minko, 2006).

Cisplatin (CDDP) administered intravenously can cause severe side effects including increased risks of leucopenia, nausea, anemia, acute nephrotoxicity, and chronic neurotoxicity (both hearing loss and nerve damage) (Carrick, Ghersi, Wilcken, & Simes, 2004). These undesirable side effects could cause the patient reluctant to complete their chemotherapy sessions or their decision to use less effective chemotherapeutic agents. In addition, untargeted chemotherapeutics will result in low drug concentration of drug that reaches the cancer cells. By the linkage of an anticancer drug on the HA chain, a more selective target and release of the chemotherapeutic agent to tumor cells can be achieved (Meo, 2008; Platt & Szoka, 2008).

Cisplatin conjugated to hyaluronan (HA) which is a highly biocompatible and nonimmunogenic polymer (Cai, Xie, Bagby, Cohen, & Forrest, 2008). A hyaluronan-cisplatin (HA-Pt) conjugate may increase CDDP concentrations and decrease systemic toxicity. HA-Pt conjugates had high anti-tumor activity *in vitro* similar to the free drug. Pathology studies demonstrated that animals with HA-Pt treatment showed milder degenerative changes in livers, less congestion as well as necrosis in kidneys and 60% of the animal were completely cured of head and neck cancers (Cai et al., 2008). In

another study, the area-under-the-curve of cisplatin in the axially lymph nodes shows 74% increase when injected with HA-Pt compared with normal cisplatin.

Hyaluronan sodium salt is used as it is more water soluble compared to actual hyaluronan. HA sodium salt also reacts readily with chlorine in CDDP. One or more chlorides on CDDP can be hydrolyzed and then replaced by carboxylate(s) on HA. This reaction releases NaCI which is not harmful for the body system and produces HA-Pt conjugate. The resulting conjugate is referred to as HA–Pt for clarity, although the conjugate is $Pt(NH_3)_2(H_2O)OOC^-HA$ (mixture of mono- and di-conjugates) (Xie et al., 2010).

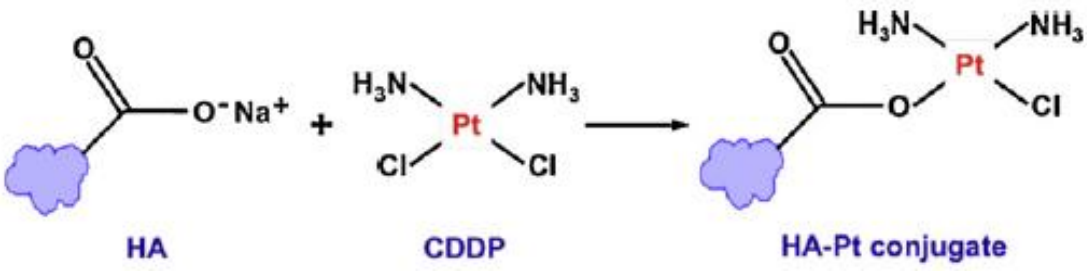

Figure 2.17: Reaction to produce HA-Pt conjugate (Xie et al., 2010).

2.9 CD44

CD44 is a family of integral cell membrane glycoproteins, with multiple functions (Nair, 2006). CD44 is encoded by a single gene which is composed of at least 20 exons (Man-Sun, 1997). The protein has many different forms, generated by alternative mRNA splicing and by post-translational modification, which may mediate different functions (Kennel, Lankford, Foote, Shinpock, & Stringer, 1993). The most abundant form of CD44, standard CD44 (CD44s), consists of an N-terminal signal sequence (exon 1), a Link-homology hyaluronan-binding module (exons 2 and 3), a stem region (exons 4, 5, 16 and 17), a single-pass transmembrane domain (exon 18) and a cytoplasmic domain (exon 20) (Thorne, Legg, & Isacke, 2004).

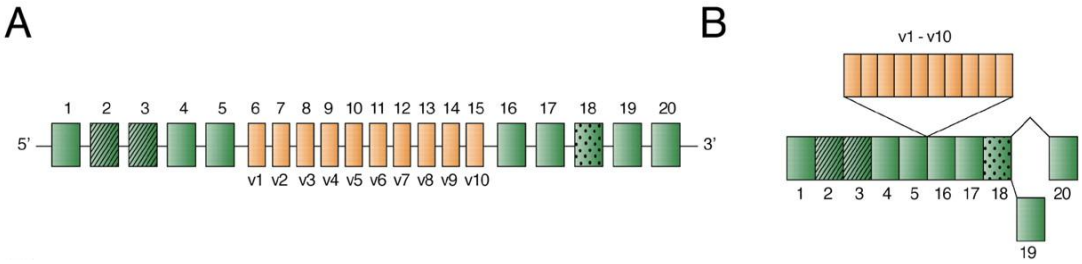

Figure 2.18: (A) Genomic organization of CD44. The exons encoding the hyaluronan-binding domain and transmembrane domain are cross-hatched and stippled, respectively. (B) mRNA splicing patterns in CD44. The standard form of CD44, CD44s, comprises exons 1-5, 16-18 and 20 (green). Most variant forms of CD44, CD44v, contain the standard exons with combinations of exons 6-15 (v1-v10) (orange). The inclusion of exon 19, normally absent in most CD44 transcripts, results in a CD44 short-tail variant owing to use of an alternative translation stop codon.
(Source: http://jcs.biologists.org/content/117/3/373.full)

These proteins have been implicated in many biological processes, such as cell adhesion, cell substrate, cell to cell interactions, including lymphocyte homing haemopoiesis, cell migration and metastasis (Makrydimas, Zagorianakou, Zagorianakou, & Agnantis, 2003). In immunobiology, CD44 is a valuable marker for memory cells, since B-cell and T-cell activation in the immune response leads to high expression of CD44. When an antigen triggers the immune response and activates the T-helper cells, CD44 activity is increased by upregulation. CD44 variant isoforms are

expressed on different types of normal cells. In addition some isoforms are overexpressed on tumor cells including breast, cervical, endometrial and ovarian cancer (Makrydimas et al., 2003).

CD44 is a single pass transmembrane protein which has four functional domains. One of the extracellular domains interacts with the glycosaminoglycan hyaluronan of the matrix. This interaction is modulated by other lipids and accessory proteins that interact with the transmembrane domain of CD44. The cytoplasmic domain interacts with the cytoskeletal proteins for transmission of signal.

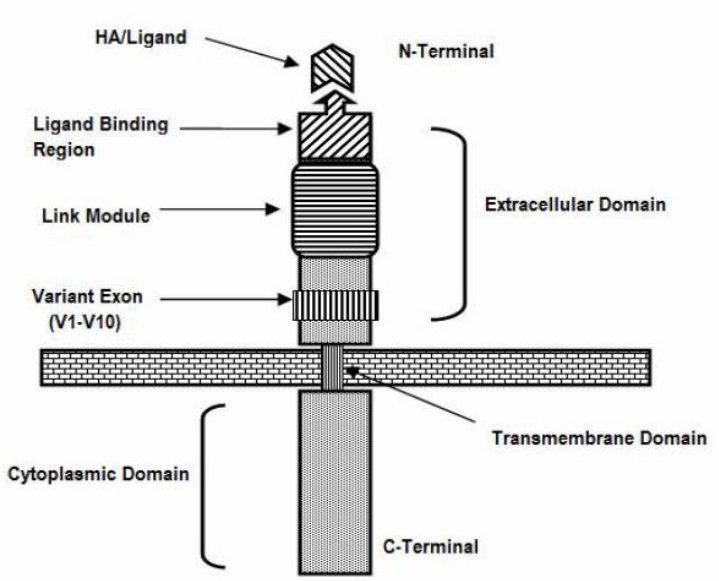

Figure 2.19: Schematic of CD44 molecule. (Gul-e-Saba, Abdah, & Abdullah, 2010)

2.10 HA-CD44 Interaction

Hyaluronan has six characterized surface receptors namely CD44, RHAMM, LYVE-1, HARE, layilin and Toll-4 (Tolg, Hamilton, & Turley, 2004; Ingber, 2006). Many physiological functions of HA are thought to relate to its molecular characteristics, including its physiochemical properties, its specific interactions with hyaladhereins, such as CD44 and RHAMM (receptor for HA-mediated motility), and its mediating effect on cell signalling and behavior (Toole, 2001).

All CD44 isoforms exhibit HA-binding site in their extracellular domain, and thereby serve as a major cell surface receptor for HA (Underhill, 1992). Hyaluronan interactions with CD44 mediate at least three important physiological processes which are signal transduction, assembly of pericellular matrices, and receptor-mediated internalization (Knudson, Chow, & Knudson, 2002; Toole, 2001).

CD44 is a single pass transmembrane protein which has four functional domains. One of the extracellular domains interacts with the glycosaminoglycan hyaluronan of the matrix. This interaction is modulated by other lipids and accessory proteins that interact with the transmembrane domain of CD44. The cytoplasmic domain interacts with the cytoskeletal proteins for transmission of signal (Nair, 2006).

CD44 receptor is expressed in both normal and tumor stem cells (displaying unique ability to initiate normal and/or tumor cell-specific properties) and CD44 has been suggested as one of the important surface markers for both normal stem cells and cancer stem cells (Al-Hajj, Wicha, Benito-Hernandez, Morrison, & Clarke, 2003).

HA conjugates, nanoparticles, and microspheres have been widely used in the delivery of therapeutics to metastatic tissues that overexpress CD44. This drug delivery strategy may allow a controlled release of the drug and a high targeting selectivity on tumor cells, increasing drug cytotoxicity and decreasing its undesirable side effects (Serafino et al., 2011).

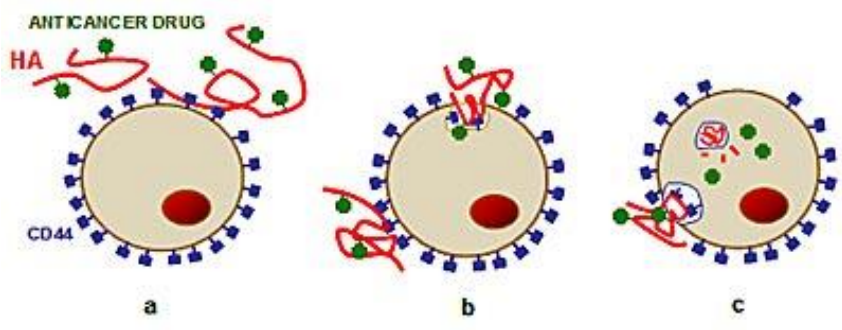

Figure 2.20: (a) Interaction of HA-drug with CD44 receptors on tumor cell, (b) cell absorbs molecule by engulfing it through the CD44 "door", (c) HA-drug degrades and drug is released directly into the key areas of the cell causing it to die (Source: http://derstandard.at/1308680364519/Carrying-drugs-to-target)

2.11 Case Studies of Molecular Modeling

Molecular modeling is a computer based science of deriving, representing and manipulating the structure and reactions of molecules. Numerous software has been developed and different type of software may produce different output. Examples of molecular modeling softwares are COSMOS, CHARMM, Discovery Studio and AutoDock. Some of the techniques applied in molecular modeling includes molecular visualization and molecular mechanics methods.

An example of a work based on molecular modeling is the application of molecular dynamics simulation to predict the compatibility between water-insoluble drugs and self-associating poly(ethylenapplication of molecular dynamics simulation to predict the compatability between water-insoluble drugs and self-associating poly(ethylene oxide)-b-poly(ε-caprolactone) block copolymers. All the molecular modeling simulations were performed using the Material Studio software package running on Silicon Graphics (SGI) workstation cluster. Molecular dynamics (MD) simulation was applied to study the solubility of two water-insoluble drugs, fenofibrate and nimodipine, in a series of micelle-forming PEO-b-PCL block copolymers with combinations of blocks having different molecular weights (Patel, Lavasanifar, & Choi, 2008). By implementing the Flory-Huggins interaction parameter in the simulation, the final results are proven to be consistent with the solubility data of the drug/PEO-b-PCL systems.

Material Studio is also used in a case study of molecular modeling of gel nanoparticles with cyclosporine A for oral drug delivery. The Flory-Huggins theory as implemented in Material Studio modeling environment was used to study the interaction between cyclosporine A with different surfactants (Tokarsky, Andrysek, & Capkova, 2011). Finally, structural parameters and energy characteristics of all systems have been compared and one composition was selected as a very promising for further experimental study.

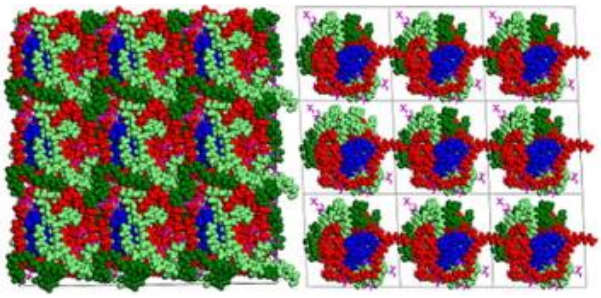

Figure 2.21: Example of result using Material Studio.
(Source: http://www.sciencedirect.com/science/article/pii/S0378517311002432)

Another type of molecular modeling is using QSAR which could also stand for an approach of quantitative structure-activity relationship. It correlate the chemical structure with a defined process such as activity or reactivity. The QSAR also support 3D QSAR for molecular modeling. In University of Helsinki, the researcher in drug delivery and pharmaceutical nanotechnology group uses QSAR for structure based models for oral drug absorption. The study focuses on models for efflux transport and volume of drug distribution (University of Helsinki, 2010).

The next case study of molecular modeling is on ligand docking and binding site analysis. In structure-based drug design process, the docking and binding affinity complex of a small molecule to the binding site of receptor is very important. For a thorough understanding of the structural principles that determine the strength of a protein/ligand complex both, an accurate and fast docking protocol and the ability to visualize binding geometries and interactions are mandatory (Seeliger & Groot, 2010). Hence, Autodock Vina is used to demonstrate the how docking and visualization can help structure based designs. It also produces atomic affinity grids of the complex instantly.

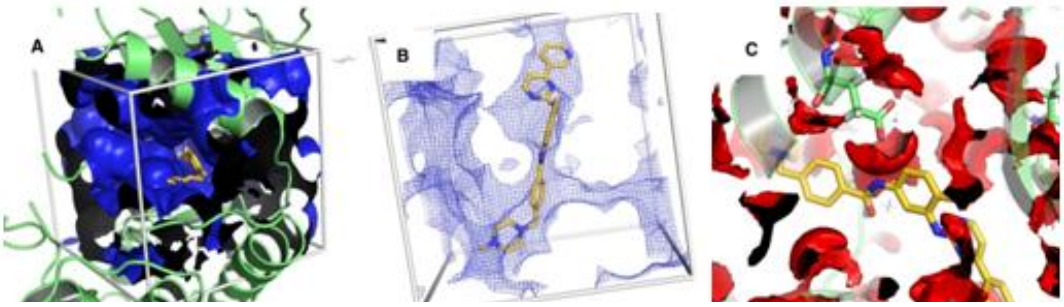

Figure 2.22: Autodock grid maps displayed with different contour levels. **a** Map for interactions of aliphatic carbon atoms at contour level 5kcal/mol. **b** Same map at contour level -0.3 kcal/mol. **c** Hydrogen bond donor map at contour level -0.5 kcal/mol.
(Source: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2881210/)

In a study of computational approach for the identification of anti-HIV phytocompoun with respect to T-cell receptor through molecular docking, a combination of software were used including Discovery Studio. Discovery Studio is used for docking and ligand preparation. Molecular mechanics like scoring function which includes terms of hydrogen bonds is employed by DS to rank the docked posses (Daisy, Rajalakshmi, Lilly, & Anusta, 2011). In ligand preparation, hydrogen bonds were added to the three dimensional phytocompound and energy was minimized using CHARMm force field. Lipinski's properties like molecular weight, log P and number of Hydrogen-bond donors and acceptors for the active principles were also noted.

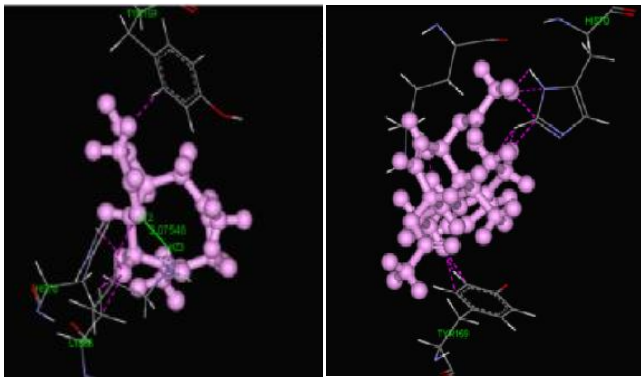

Figure 2.23: Different interaction of drug-receptor complex. The complexes are a) costunolide b) gymnemic diacetate with T-cell receptor.
(Source : http://derpharmachemica.com/vol3-iss3/DPC-2011-3-3-18-31.html)

CHAPTER 3

METHODOLOGY / PROJECT WORK

3.1 Project Flow Chart

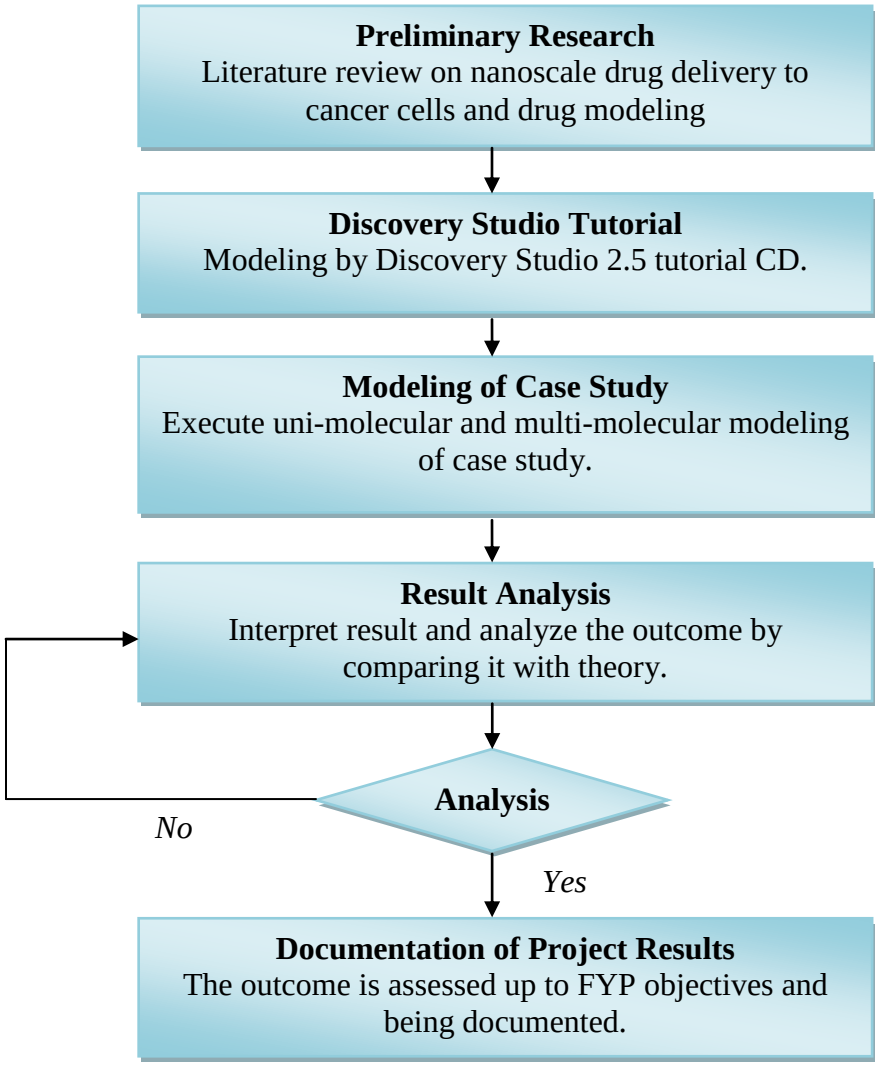

Figure 3.1: Project flow chart.

Preliminary research involved a thorough literature review drawn from all aspects of the project. This enables complete understanding of the nature of the project and what can be manipulated to get the end results. Next, as Discovery Studio 2.5 is a new program purchased, the program must be studied and familiarized. In accomplishing this, a tutorial for the program is installed and studied. After understanding the project and the program, the three dimensional molecular modeling can be carried out. The modeling phase consists of two phase namely uni-molecular modeling and multi-molecular modeling.

In uni-molecular modeling, the molecules of hyaluronan, cisplatin and CD44 are prepared based on their molecular structure identified through literature. After that, the interaction between the molecules are identified and studied in order to proceed to the multi-molecular modeling phase.

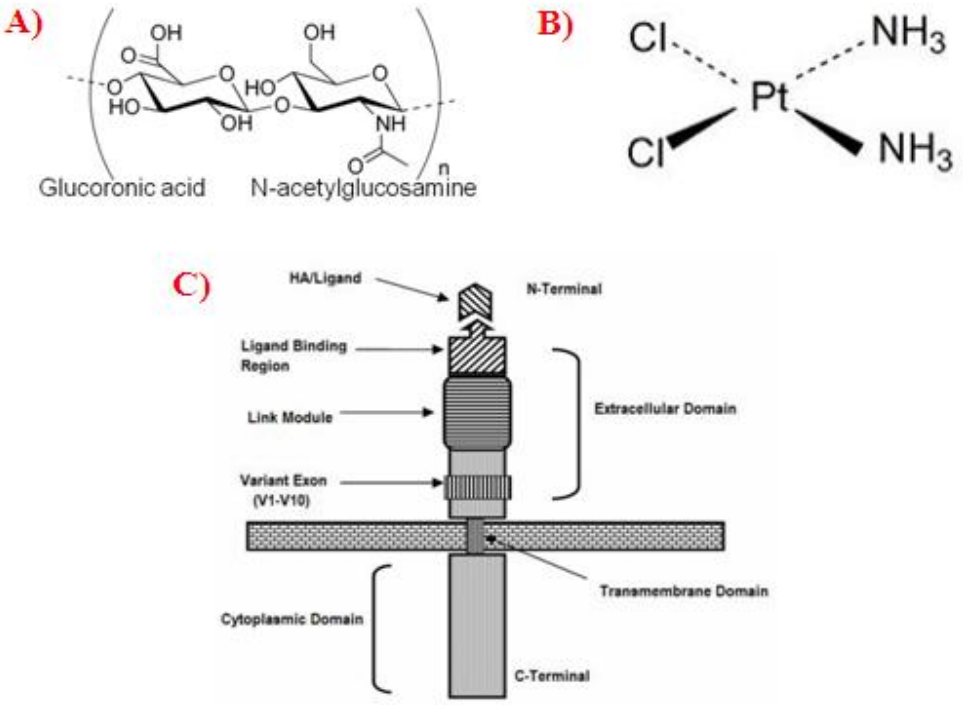

Figure 3.2: Structure involved in uni-molecular modeling:
A) Hyaluronan, B) Cisplatin and C) Schemetic of CD44

After identifying the active side of hyaluronan and cisplatin, the modeling for hyaluronan-cisplatin was prepared using Discovery Studio 2.5. The interaction between hyaluronan and CD44 are also modeled out.

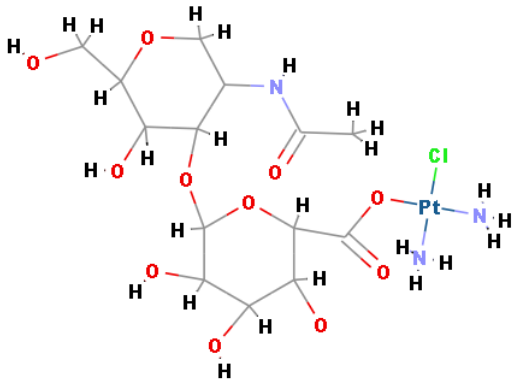

Figure 3.3: Hyaluronan-cisplatin 2D structure.

The result from the molecular modeling was then compared with the theory drawn out through literature review in the result analysis step. If the result contradicted the theory, it must be analyzed again and the problems must be identified so that corrective actions can be made.

3.2 Project Gantt Chart

No	Detail/Week	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1.	Project work continues	■	■	■	■	■	■	■								
2.	Submission of progress report								●							
3.	Project work continues								■	■	■	■	■			
4.	Pre-EDX											●				
5.	Submission of draft report												●			
6.	Submission of dissertation (soft bound)													●		
7.	Submission of technical paper													●		
8.	Oral presentation														●	
9.	Submission of project dissertation (hard bound)															●

Figure 3.4: Gantt chart of FYP 2.

Process	■
Suggested Milestone	●

3.3 Tools and Equipment

For this project, the main tool required is Discovery Studio produced by Accelrys. This program is a software suite of life science molecular design solutions for computational chemists and computational biologists. Some of the main modeling that will help in this project is molecular and protein modeling. Besides that, the Discovery Studio Integrated QASR and Library Design tools will also be very helpful as it could quickly uncover compounds that have the greatest potential of becoming of new drugs.

For all modeling software, a basic procedure will be applied. During the earlier stage, the geometry and parameters of the problem must first be identified. Next, the physically modeling will take place. The modeling result is to correspond to the case study and is analyzed for its validity.

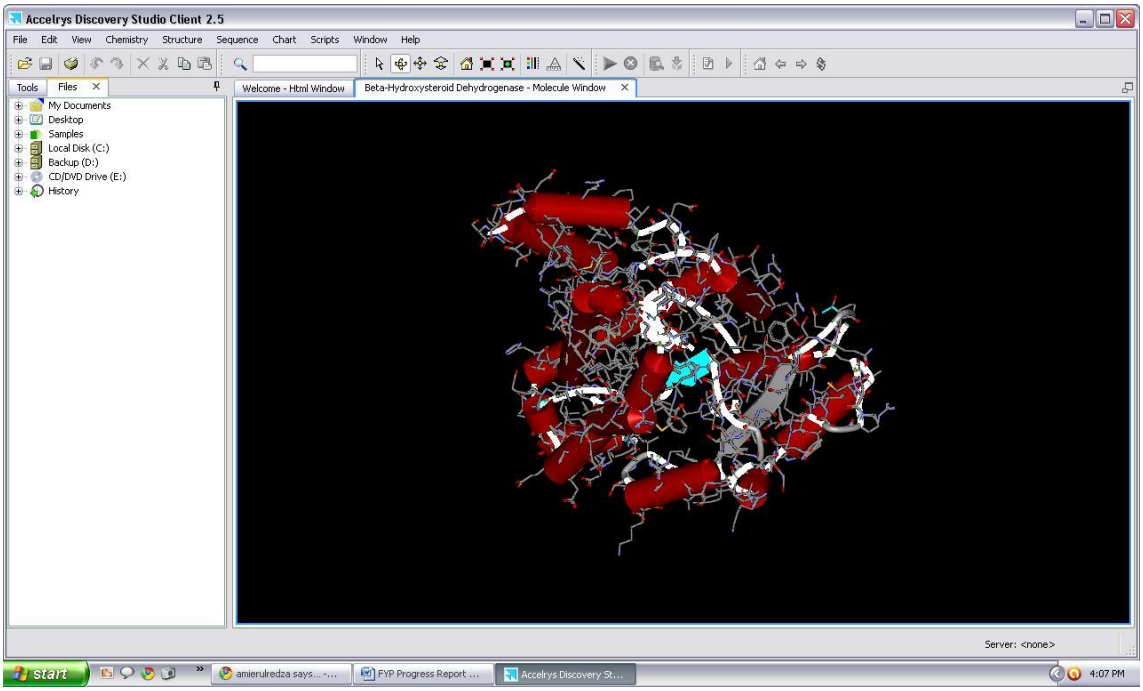

Figure 3.5: Screenshot of Discovery Studio.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Modeling of Cisplatin

CDDP is a square planar molecule that exists in cis and trans form. Unlike cisplatin, transplatin does not function for anticancer drugs. Some of the reasons are because the cis form of $(NH^3)_2Pt^{2+}$ with N-Pt-N angle of 97 has lower enegery than trans form with N-Pt-N angle of 180 (Li & Jena, 2011). The rotation barrier to change N-Pt-N from 180 to 97 is about 1.0 eV (Li & Jena, 2011). The cis form of $(NH^3)_2Pt^{2+}$ reacts with two Guanines in DNA, the two N atoms in Guanines can readily bind to the Pt atom in cisplatin. However, transplatin does not provide this opportunity due to steric reasons (Li & Jena, 2011). As a result, it has faint cytotoxicity effect because of its geometry allowing less adduct formation with DNA if compared to the cis form.

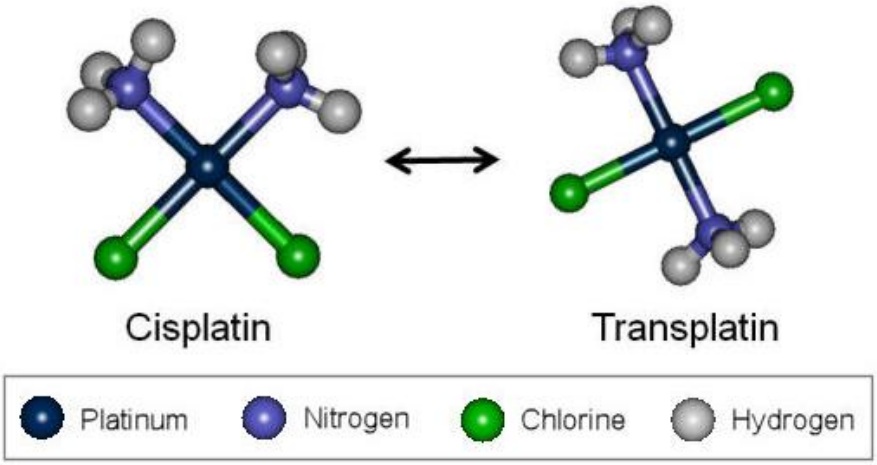

Figure 4.1: Cisplatin and transplatin.

Cisplatin is classified as an alkylation agent defined as a compound capable of covalently binding an alkyl group to a biomolecule under phsyiological conditions (aqueous solution, 37°C, pH 7.4) (Avendano & Menendez, 2008). DNA alkylating agents interact with resting and proliferating cells in any phase of cell, but they are more

cytotoxic during the late G1 and S phases (as mentioned). Before cisplatin reacts with DNA, two chlorine atoms is eliminated when attacked by water molecules. Therefore, the active piece in the cell is not cisplatin but $(NH^3)_2Pt^{2+}$. The modeling data of cisplatin can be referred below.

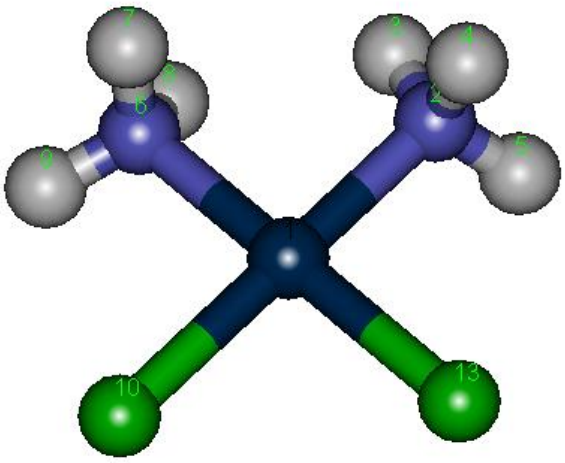

Figure 4.2: Cisplatin with each atom numbered.

Table 4.1: Bond and length of cisplatin.

No	Bond	Length
1	Pt1- N2	2.0036
2	N2- H3	1.0299
3	N2- H4	1.02943
4	N2- H5	1.02965
5	Pt1- N6	2.00446
6	N6- H7	1.02941
7	N6- H8	1.02974
8	N6- H9	1.02945
9	Pt1- Cl10	2.30145
10	Pt1- Cl13	2.3007

Average bond length = 1.478779

In plasma, the high concentration of chloride prevents its hydrolysis, but when it enters the cell the much lower chloride concentration prompts its reaction with water to

give the positively charged species. The active complexes enter the nucleus and become attracted by the negatively charged DNA. The antitumor properties of cisplatin are attributed to the kinetics of its chloride ligand displacement reactions leading to DNA crosslinking activities causing DNA bending and interfering with DNA replication, transcription and other nuclear functions and arresting cancer cell proliferation and tumor growth. Other mechanisms of cisplatin cytotoxicity include mitochondrial damage, decreased ATP activity, and altered cellular transport mechanisms. It was early checked that the cisplatin-DNA adducts were stable for at least three days at 37°C after their formation.

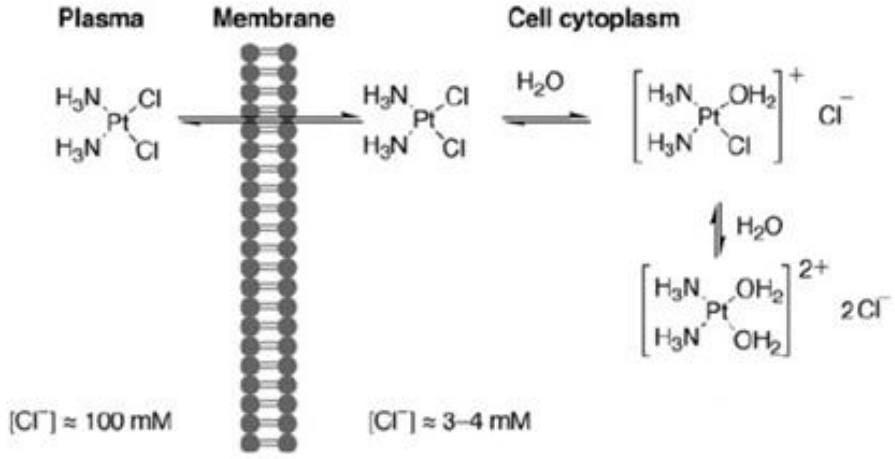

Figure 4.3: Intracellular bioactivation of cisplatin.
(Soure: http://www.scribd.com/doc/11639473/Medicinal-Chemistry-of-Anticancer-Drugs)

In cisplatin, there are two types of ligands which are the leaving ligands and the non-leaving ligands. It was established that amine ligands are the non-leaving ligands. Leaving ligands are usually anions of medium stability, and their replacement can take place on physiologically and therapeutically useful scale. A number of best leaving ligand are halogenides, carboxylates, sulphates, water and hydroxo ligand. Hydroxo ligand is important as in can increase the low solubility of platinum complexes in aqueous solution (Ašperger, 2003). It is a poorly soluble compound, maximum solubility in aqueous solution being approximately 1 mg/mL (Allwood, Stanley, & Wright, 2002).

The chemical stability of cisplatin is adversely affected by pH and light. Cisplatin is unstable in aqueous solution unless chloride ions are present in sufficient concentration. The optimum pH for stability is within the range 3.5–5.5 (Williams, 1990). Cisplatin is also relatively sensitive to light (Zieske et al., 1991). Degradation occurred during exposure to short-wavelength (350-490-nm) visible light (Zieske et al., 1991).

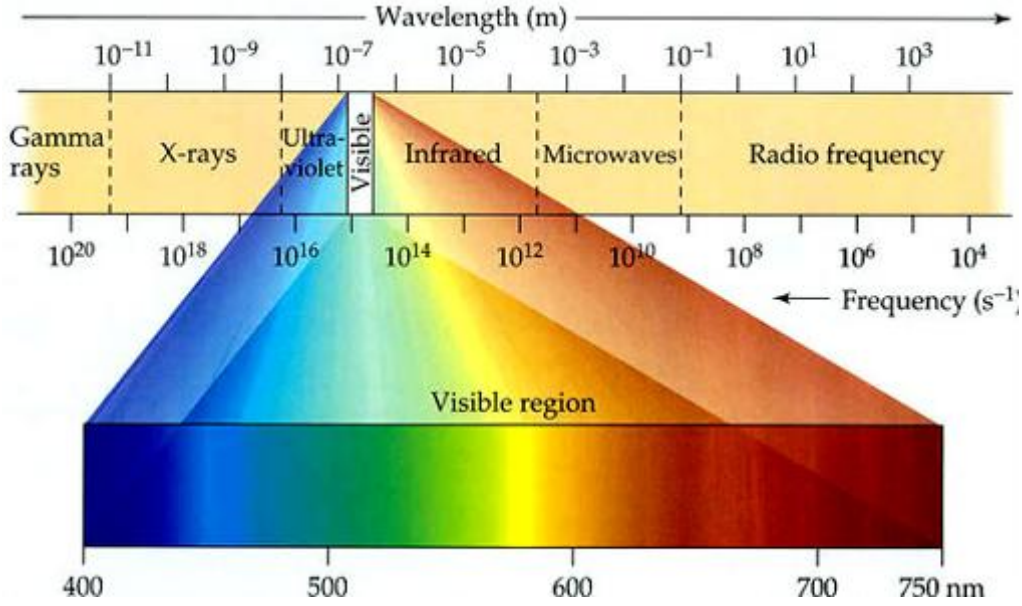

Figure 4.4: Electromagnetic spectrum.
(Source: http://infraredtv.com/IR_Explained.html)

4.2 Modeling of Hyaluronan

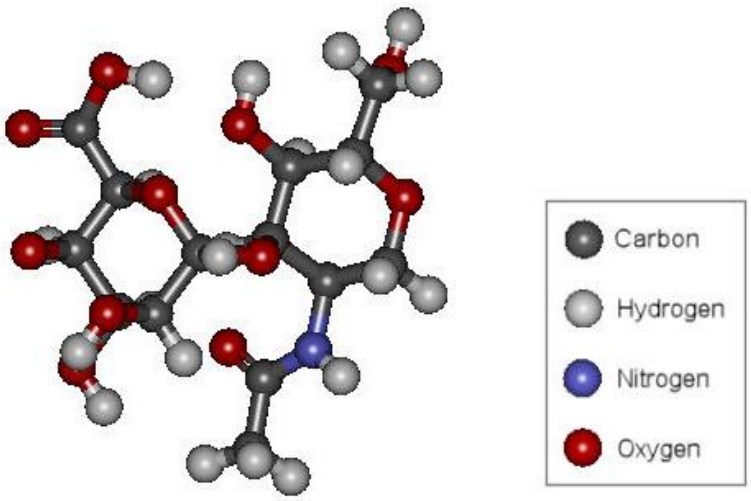

Figure 4.5: Hyaluronan.

Hyaluronan (HA) is a linear polysaccharide of high-molecular by alternating addition of glucoronic acid and N-acetylglucosamine to the growing chain using their activated nucleotide sugars as subtrates. The number of repeat disaccharides in a completed hyaluronan molecule can reach 10 000 or more, a molecular mass of 4 million Daltons (Necas, Bartosikova, Brauner, & Kolar, 2008). An average disaccharide has a length of 1nm. Hence, a hyaluronan molecule of 10 000 repeats could extend 10μm if stretched from end to end, a length approximately equal to diameter o a human erythrocyte (Cowman & Matsuoka, 2005). The modeling data of hyalutonan can be referred in Table 4.2.

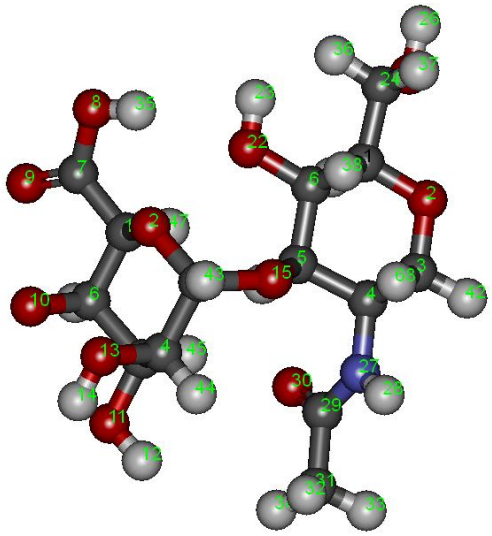

Figure 4.6: Hyaluronan with each atom numbered.

Table 4.2: Bond and length of hyaluronan.

No	Bond	Length
1	C1 - O2	1.43753
2	O2 - C3	1.43433
3	C3 - C4	1.5338
4	C4 - C5	1.54146
5	C5 - C6	1.56386
6	C6 - C1	1.56423
7	C1 - O2	1.48894
8	O2 - C3	1.48694
9	C3 - C4	1.57292
10	C4 - C5	1.54096
11	C5 - C6	1.55293
12	C6 - C1	1.52777
13	C1 - C7	1.49645
14	C7 - O8	1.39092
15	C7 - O9	1.23592
16	C6 - O10	1.42946
17	C5 - O11	1.42497
18	O11 - H12	0.989177
19	C4 - O13	1.42844
20	O13 - H14	0.989262
21	C3 - O15	1.53745
23	O15 - C5	1.46808
24	C6 - O22	1.43051
25	O22 - H23	0.982349

No	Bond	Length
26	C1 - C24	1.53903
27	C24 - O25	1.42748
28	O25 - H26	0.988526
29	C4 - N27	1.44567
30	N27 - H28	0.993596
31	N27 - C29	1.33488
32	C29 - O30	1.23673
33	C29 - C31	1.49486
34	C31 - H32	1.09977
35	C31 - H33	1.09927
36	C31 - H34	1.09947
37	O8 - H35	0.989564
38	C24 - H36	1.09841
39	C24 - H37	1.09886
40	C1 - H38	1.09931
41	C6 - H39	1.09844
42	C5 - H40	1.09099
43	C3 - H42	1.09833
44	C3 - H68	1.09909
45	C3 - H43	1.09787
46	C4 - H44	1.099
47	C5 - H45	1.09076
48	C6 - H46	1.09841
49	C1 - H47	1.07837

Average bond length = 1.290528

Hyaluronan is degraded by a family of enzymes called hyaluronidases. Some of hyaluronidases are hyase, β -D-glucuronidase and β-N-acetyl-hexosaminidase. In general, hyase cleaves high molecular weight HA into smaller oligosaccharides while β -D-glucuronidase and β-N-acetyl-hexosaminidase further degrade the oligosaccharide fragments by removing nonreducing terminal sugars (Leach & Schmidt, 2004). The degradation products of HA, oligosaccharides and very low molecular weight hyaluronan exhibit pro-angiogenic properties (Mio & Stern, 2002).

Jansen et al. (2004) investigated the possible cytotoxic effects, biocompatibility and degradation of hyaluronan based conduit for peripheral nerve repair. Based on this study, it was found that hyaluronan conduit is not cytotoxic and shows good biocompatibility. HA is highly non-antigenic and non-immunogenic, owing to its high structural homology across species and poor interaction with blood components (Amarnath, Srinivas, & Ramamurthi, 2006). In the body, HA occurs in salt form, hyaluronate and is found in high concentrations in several soft connective tissues.

4.3 Modeling of Hyaluronan-cisplatin

Hyaluronan-cisplatin is a drug conjugate to improve drug delivery system by implementing the CD44 receptor signaling. Besides that, the conjugate also is used to improve the properties of the free drug. The reaction to produce hyaluronan-cisplatin is described as the figure below.

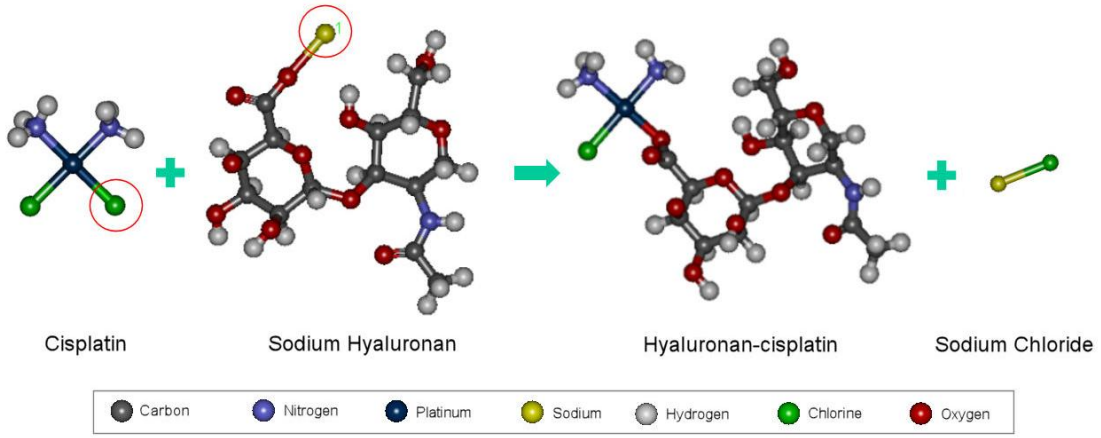

Figure 4.7: Reaction to produce hyaluronan-cisplatin.

From the reaction above, it can be seen that cisplatin reacts with hyaluronan salt to produce hyaluronan-cisplatin (HA-Pt) by releasing sodium chloride. Sodium hyaluronan is used instead of hyaluronan because it is more water soluble and reacts readily with cisplatin. From Figure 4, the red circle shows the reacted atom which is the chlorine from cisplatin and sodium from sodium hyaluronan. The platinum forms bond with the oxygen in the glucoronic side of sodium hyaluronan to produce hyaluronan-cisplatin conjugate.

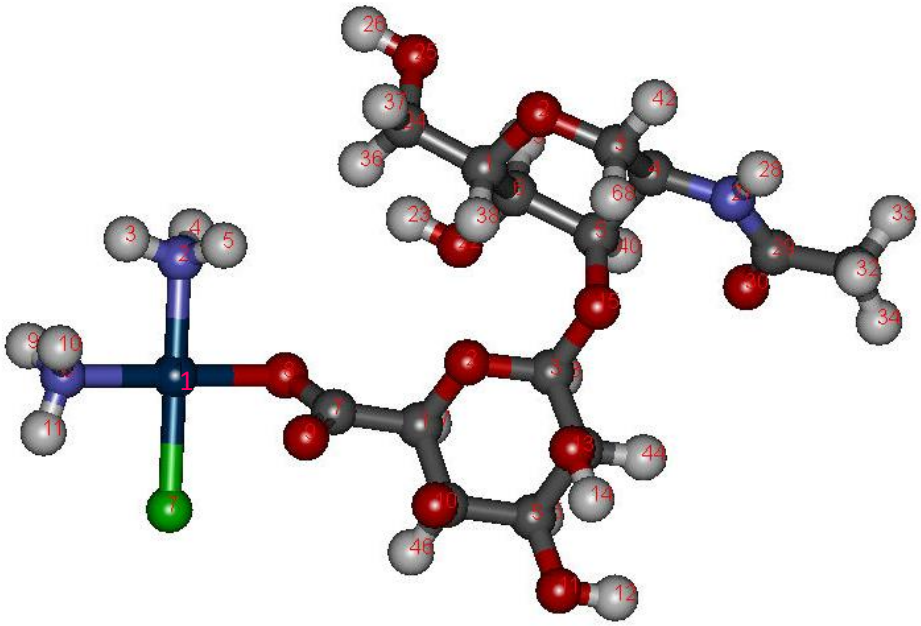

Figure 4.8: Hyaluronan-cisplatin conjugate with each atom numbered.

Table 4.3: Bond and length of hyaluronan-cisplatin.

No	Bond	Length
1	Pt1-N2	2.00372
2	N2-N3	1.0319
3	N2-H4	1.02902
4	N2-H5	1.02899
5	Pt1-C17	2.30474
6	Pt1-N8	2.00929
7	N8-H9	1.03079
8	N8-H10	1.02879
9	N8-H11	1.03025
10	C1-O2	1.4384
11	O2-C3	1.43576
12	C3-C4	1.5323
13	C4-C5	1.55843
14	C5-C6	1.56874
15	C6-C1	1.5618
16	C1-O2	1.45542
17	O2-C3	1.46922
18	C3-C4	1.5362
19	C4-C5	1.5367
20	C5-C6	1.52808
21	C6-C1	1.54337

No	Bond	Length
22	C1-C7	1.51446
23	C7-O8	1.39882
24	C7-O9	1.23623
25	C6-O10	1.43006
26	C5-O11	1.42505
27	O11-H12	0.989215
28	C4-O13	1.42579
29	O13-H14	0.99098
30	C3-O15	1.46198
31	O15-C5	1.4863
32	C6-O22	1.44277
33	O22-H23	0.982757
34	C1-C24	1.53603
35	C24-O25	1.4292
36	O25-H26	0.987727
37	C4-N27	1.47688
38	N27-H28	0.994418
39	N27-C29	1.34271
40	C29-O30	1.23554
41	C29-C31	1.50135
42	C31-H32	1.10101

No	Bond	Length
43	C31-H33	1.10088
44	C31-H34	1.09807
45	C24-H36	1.09851
46	C24-H37	1.0986
47	C1-H38	1.09917
48	C6-H39	1.09796
49	C5-H40	1.09742
50	C3-H42	1.09976
51	C3-H68	1.09726
52	C3-H43	1.09815
53	C4-H44	1.09898
54	C5-H45	1.10002
55	C6-H46	1.09933
56	C1-H47	1.0979
57	O8-Pt1	1.97837

Average bond length = 1.32301

Cisplatin as a free drug has low solubility in water. Poor water solubility has always been one of the most fundamental problems in drug delivery (Patel, 2011). It is estimated that around 40% of drugs in the pipeline cannot be delivered through the preferred route or in some cases, at all owing to poor water solubility (Salata, 2004). Bioconjugates of low molecular weight hyaluronic acid with cytotoxic agents is designed to improve solubility of the cytotoxic agent and facilitate its intravenous administration (Brekke & Gubbe, 2008). As hyaluronan soluble in water, it indirectly increases the cisplatin solubility in water. This is important so that it can be passed in the bloodstream to deliver its function.

Although various water-soluble synthetic polymers have been exploited for conjugation of hydrophobic drugs, naturally occurring polymers with intrinsic cell specific binding capacity have tremendous potential as a target-specific drug carrier (Manju & Sreenivasan, 2011). For example, hyaluronic acid (HA), a naturally occurring polysaccharide composed of N-acetyl-d-glucosamine and d-glucuronic acid has a strong affinity with cell-specific surface markers such as CD44 (Maeda, Seymour, & Miyamoto, 1992). As CD44 is overexpressed cancer cells including colorectal cancer, malignant cells with high metastatic activities often exhibit enhanced binding and uptake of HA.

CD44 signaling improves the drug delivery system through targeted delivery to cancer cells. By this method, higher accumulation of cisplatin on cancer cells can be achieved. As a result, cisplatin cytotoxicity to the cancer cell increases while the healthy cells that surround them remain untouched and unaffected by the therapy. Consequently, the side effects of cisplatin such as nausea, kidney toxicity, low white blood cells and anemia is reduced.

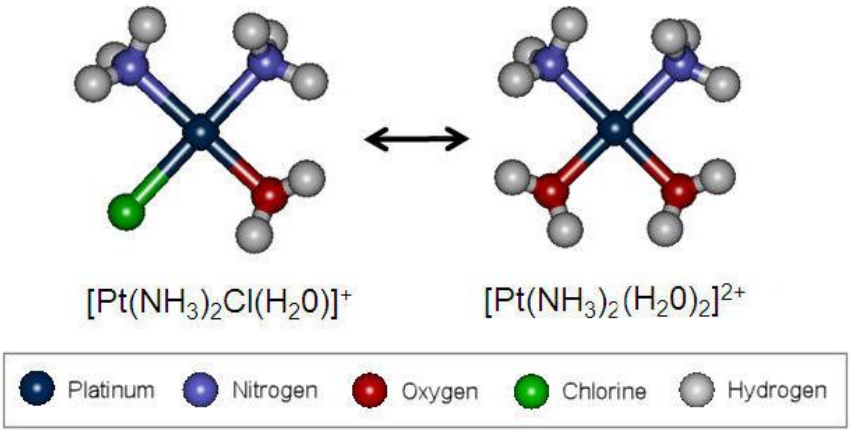

Figure 4.9: Mono and di-hydrated cisplatin.

When HA is attached to the CD44 on its ligand, it will release a highly reactive cisplatin as shown in Figure. The resulting cisplatin allows platinum to bind with one or two nucleotide base depending on the extend of hydrolysis it went through. The attraction between DNA and activated cisplatin is also contributed by the opposite charges they own, positive for activated cisplatin and negative for DNA.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

The hyaluronan-cisplatin conjugation could be the answer to improve drug delivery system through targeted delivery by CD44 signaling. Based on the outcomes of this project, three dimensional molecular modeling of hyaluronan-cisplatin was successfully generated using Discovery Studio 2.5. In addition, the hyaluronan-cisplatin conjugate also improve water solubility of cisplatin. Through targeted delivery, cisplatin toxic effect on cancer cell increases while its effect on normal cells is minimized. It was also identified that cisplatin cytotoxicity is delivered by binding itself on DNA causing the DNA to be kinked and loses its ability to proliferate as well as promoting cell death.

Recommendation and future work to improve the efficiency and to gain better insight of the project are stated as follow;

- Correlates the simulation with lab results.
- Preparation of CD44 and DNA structure if possible.
- Further discussion of the approach in molecular level by applying organic chemistry and coordination chemistry.

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