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Department of Applied Mathematics & Physic Sciences

Mathematical Models of Double Strand Breaks Repair in DNA

Dissertation

of

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Approved by 3 members of commitee 1st October 2017.

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Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less.

Marie Curie

Summary

The theme of dissertation is the reproduction of Reza Tallei and Hooshang Nikjoo biochemical kinetic models for the repair damages of DNA induced by ionizing radiation.

The study of ionizing radiation included in the branch of Radiobiology. In the first chapter we will describe the definitions of radiobiology, which could help us understand the base of our problem,as well as the biology description of our model. In the second chapter we will quote the principles of mathematical modelling, the differential equations, the biochemical processes and how these equations solved by the computer. The damages creation are a chemical process. Chemical reactions with reactants and products expressed by the mass kinetics. Also, chemical process which is defined in our mathematical model express the binding of enzyme in the site of damage. The above system described by non-linear differential equations and scalling methods, and the computational lanquage that we utilize for the solution of the mathematical system is C++ used in Cygwin platform. In the third chapter we will analyze the results and the graphs of models and how they differ from the expected results. In the last chapter we will say our results, and there will be a discussion about future plans and goals that we want to face.

Περίληψη

Το θέμα της διπλωματικής εργασίας είναι η αναπαραγωγή βιοχημικών κινητικών μοντέλων του Reza Taleei και Hooshang Nikjoo, που αναφαίρονται στην επιδιόρθωση βλαβών του DNA, οι οποίες προκαλούνται από ιονίζουσα ακτινοβολία.

Η μελέτη της ιονίζουσας ακτινοβολίας υπάγεται στο πεδίο μελέτης της Ραδιοβιολογίας. Στο πρώτο κεφάλαιο θα περιγραφούν οι ορισμοί ραδιοβιολογίας, που μπορούν να θέσουν τις βάσεις για την κατανόηση και την περιγραφή του προβλήματός μας. Στο δεύτερο κεφάλαιο θα παραθέσουμε τις αρχές της μαθηματικής μοντελοποίησης, τις διαφορικές εξισώσεις, τις βιολογικές διαδικασίες και πως αυτές οι εξισώσεις επιλύονται από τον υπολογιστή. Η δημιουργία βλαβών είναι μια χημική διαδικασία. Οι χημικές αντιδράσεις μαζί με τα αντιδρώντα και τα προϊόντα εκφράζονται μέσα από τη κινητική μαζών(mass kinetics).Η χημική διεργασία του μαθηματικού μας μοντέλου στηρίζετα στο τρόπο που το ένζυμο προσδένεται στη θραύση. Το σύστημα των χημικών μας αντιδράσεων εκφράζεται από μη γραμμικές συνήθεις διαφορικές εξισώσεις, οι οποίες επιλύονται από τη γλώσσα προγραμματισμού C++ στο παραθυρικό περιβάλλον του Cygwin. Η επιδιόρθωση των θράυσεων θα μελετηθεί στο βιοχημικό μονοπάτι για τη μη ομόλογη ένωση άκρων, για τρεις περιπτώσεις απλές θραύσεις, σύνθετες και τις θραύσεις στην ετεροχρωματίνη. Στο τρίτο κεφάλαιο θα αναλύσουμε τα αποτελέσματα και τις γραφικές παραστάσεις των 3 διαφορετικών μοντέλων και θα δούμε ομοιότητες και διαφορές των αναμενόμενων αποτελεσμάτων. Επίσης θα παραθέσουμε στα ήδη υπάρχοντα μοντέλα δικές μας σταθερές και θα συζητήσουμε τα συμπεράσματα που λαμβάνουμε, με μια διάθεση αναζητήσεως προσδοκιών για το μέλλον και στόχους που θέλουμε να αντιμετωπίσουμε.

Contents

Chapter 1

Introduction

1.1 Introduction

The recent years, one dangerous phenomenon is the exposure of ionizing radiation. Ionizing Radiation characterized by excess amount of energy that ends up in the free atoms and molecules and ionized them. Radiobiology, is a branch of science that deals with the action of ionizing radiation on biological tissues and living organisms, is a combination of two disciplines; radiation physics and biology. All living things are made up of protoplasm that consists of 7 inorganic and organic compounds dissolved or suspended in water. The smallest unit of protoplasm capable of independent existence is the cell.

Exposure to ionizing radiation causes damage to living tissue, and can result in mutation, radiation sickness, cancer, and death although,ionizing radiation is generally harmful and potentially lethal to living things but can have health benefits in radiation therapy for the treatment of cancer and thyrotoxicosis. [**2**]

Although,irradiation could lead cells to death, they can protect themselves using Repair Mechanisms. These Repair Mechanisms, are some biochemical processes that combines specific enzymes and proteins from the detecting of cell's damages to the error-free or error-prone repair. For the whole understanding of biochemical process of DNA Repair we reproduced already known mathematical models such as, Cuccinota's, Reza Taleei's.

1.2 History

Although radiation was discovered in late 19th century, the dangers of radioactivity and of radiation were not immediately recognized. In contrast, the first observation happened in 1895 by Wihelm Rontgen. He puplished his observations concerning the burns that developed in his body through the use of X-rays.As a field of medical sciences, radiobiology originated from Leopold Freund's 1896 demonstration of the therapeutic treatment of a hairy mole using a new type of electromagnetic radiation called x-rays, which was discovered 1 year previously by the German physicist, Wilhelm Röntgen. After irradiating frogs and insects with X-rays in early 1896, Ivan Romanovich Tarkhanov concluded that these newly discovered rays not only photograph, but also ”affect the living function”. At the same time, Pierre

and Marie Curie discovered the radioactive polonium and radium later used to treat cancer.The genetic effects of radiation, including the effects on cancer risk, were finding much later, and especially in 1927 by Hermann Joseph Muller, thats way in 1946 we awarded the Nobel prize.

Before the biological effects of radiation were known, many physicians and corporations had begun marketing radioactive substances as patent medicine and radioactive quackery. Examples were radium enema treatments, and radium-containing waters to be drunk as tonics. Marie Curie spoke out against this sort of treatment, warning that the effects of radiation on the human body were not well understood. Curie later died of aplastic anemia caused by radiation poisoning. Eben Byers, a famous American socialite, died of multiple cancers (but not acute radiation syndrome) in 1932 after consuming large quantities of radium over several years; his death drew public attention to dangers of radiation. By the 1930s, after a number of cases of bone necrosis and death in enthusiasts, radium-containing medical products had nearly vanished from the market.

In the United States, the experience of the so-called Radium Girls, where thousands of radium-dial painters contracted oral cancers (but no cases of acute radiation syndrome), popularized the warnings of occupational health associated with radiation hazards. Robley D. Evans, at MIT, developed the first standard for permissible body burden of radium, a key step in the establishment of nuclear medicine as a field of study. With the development of nuclear reactors and nuclear weapons in the 1940s, heightened scientific attention was given to the study of all manner of radiation effects.

Last but not least, the explosings of atomic bombings of Hiroshima and Nagasaki resulted in a large number of incidents of radiation poisoning, allowing for greater insight into its symptoms and dangers. Red Cross Hospital Surgeon, Dr. Terufumi Sasaki led intensive research into the Syndrome in the weeks and months following the Hiroshima bombings. Dr. Sasaki and his team were able to monitor the effects of radiation in patients of varying proximities to the blast itself, leading to the establishment of three recorded stages of the syndrome. Within 25–30 days of the explosion, the Red Cross surgeon noticed a sharp drop in white blood cell count and established this drop, along with symptoms of fever, as prognostic standards for Acute Radiation Syndrome. Actress Midori Naka, who was present during the atomic bombing of Hiroshima, was the first incident of radiation poisoning to be extensively studied. Her death on August 24, 1945 was the first death ever to be officially certified as a result of radiation poisoning (or ”Atomic bomb disease”). [**1**]

1.3 Symbolisms and Units

Symbols Definitions

SYMBOLS	DEFINITIONS
LET	*Linear energy transfer*
D	*Dose of radiation*
bp	*Base Pairs*
DB's	*Double Breaks*
SSB's	*Single Strand Breaks*
DSB's	*Double Strand Breaks*
WR	*Radiation weighting Factors*
HT	*Equivalent Dose*
QF	*Radiation Quality Factors*

1.4 Principle Definitions of Radiobiology

In this subunit will be referred to the definitions of Radiobiology, as well as general definitions that we will use in the explanation of our mathematical problems. The Radiation that causes damages is actually photons. A photon is an elementary particle, the quantum of the electromagnetic field including electromagnetic radiation such as light, and the force carrier for the electromagnetic force. The photon has zero rest mass and always moves at the speed of light within a vacuum.[**17**]

Photon Interactions with matter

Recall that photons are individual units of energy. As an x-ray beam or gamma radiation passes through an object, three possible fates await each photon:[**18**]:

- It can penetrate the section of matter without interacting.
- It can interact with the matter and be completely absorbed by depositing its energy.
- It can interact and be scattered or deflected from its original direction and deposit part of its energy.

There are two kinds of interactions through which photons deposit their energy; both are with electrons. In the first one type of interaction the photon loses all its energy; in the second, it loses a portion of its energy, and the remaining energy is scattered. These two interactions are shown below.

In the photoelectric (photon-electron) interaction, as shown above, a photon transfers all its energy to an electron located in one of the atomic shells. The electron is ejected from the atom by this energy and begins to pass through the surrounding matter. The electron rapidly loses its energy and moves only a relatively short distance from its original location. The photon's energy is, therefore, deposited in the matter close to the site of the photoelectric interaction. The energy transfer is a two-step process. The photoelectric interaction in which the photon transfers its energy to the electron is the first step. The depositing of the energy in the surrounding matter by the electron is the second step.

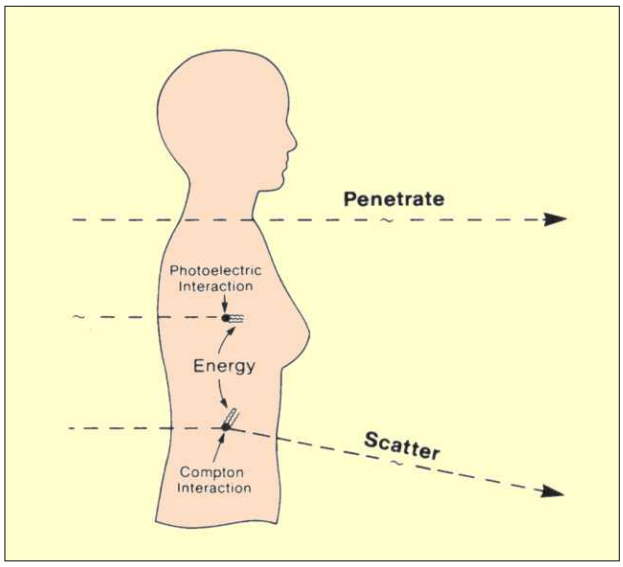

Figure 1.1: *The image adopted by "Interaction of Radiation with Matter- Perry Sprawls- The Physical Principles of Medical Imaging, 2nd Ed."*

Photoelectric interactions usually occur with electrons that are firmly bound to the

atom, that is, those with a relatively high binding energy. Photoelectric interactions are most probable when the electron binding energy is only slightly less than the energy of the photon. If the binding energy is more than the energy of the photon, a photoelectric interaction cannot occur. This interaction is possible only when the photon has sufficient energy to overcome the binding energy and remove the electron from the atom.

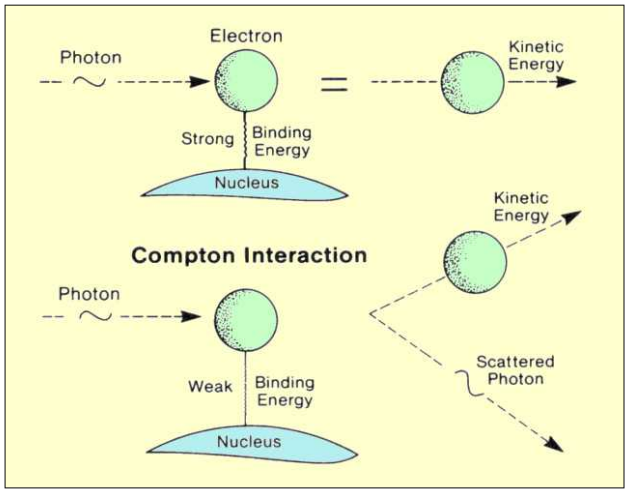

Figure 1.2: *The image adopted by "Interaction of Radiation with Matter- Perry Sprawls-The Physical Principles of Medical Imaging, 2nd Ed."*

A Compton interaction is one in which only a portion of the energy is absorbed and a photon is produced with reduced energy. This photon leaves the site of the interaction in a direction different from that of the original photon, as shown in the previous figure. Because of the change in photon direction, this type of interaction is classified as a scattering process. In effect, a portion of the incident radiation "bounces off' or is scattered by the material. This is significant in some situations because the material within the primary x-ray beam becomes a secondary radiation source. The most significant object producing scattered radiation in an x-ray procedure is the patient's body. The portion of the patient's body that is within the primary x-ray beam becomes the actual source of scattered radiation. This has two undesirable consequences. The scattered radiation that continues in the forward . direction and reaches the image receptor decreases the quality (contrast) of the image; the radiation that is scattered from the patient is the predominant source of radiation exposure to the personnel conducting the examination.

Probability of Compton Interactions

- directly proportional to number of outer shell electrons, i.e. the electron density, physical density of the material
- inversely proportional to photon energy
- does not depend on atomic number (unlike photoelectric effect and pair production)

In other words, the probability of a Compton effect is dependent on the number of electrons in the absorbing material which for almost all elements is approximately the same per unit mass. Thus, the Compton effect is independent of the atomic number (Z) of the absorber.

Linear Energy Transfer(LET)

The energy transferred to the tissue by ionizing radiation per unit track length and have the above characteristcs:

- Is a function of the charge and the velocity of the ionizing radiation.
- Increases as the charge on the ionizing radiation increases and its velocity decreases.
- Alpha particles are slow and positively charged. Beta particles, on the other hand, are fast and negatively charged. Therefore, the LET of an alpha particle is higher than that of a beta particle.
- Lethal effects increase as the LET increases.
- The unit of the LET is keV/μm.

Dose, Absorbed Dose, Equivalent Dose: Three main definitions that we must quote for the understanding of the procedure are Dose, Absorbed Dose, and Equivalent Dose. [**3**]

Figure 1.3: *Relationship between exposure and equivalent dose.The image adopted by "Comprises three sections covering the essential aspects of radiation physics, radiobiology, and clinical radiation oncology-Murat Beyzadeoglu,Gokham Ozyigit,Cuneyt Ebruli-Basic Radiation Oncology, Springer-Verlag Berlin Heidelberg, (2010)"[**3**]*

DOSE: This is the dose delivered per unit of time. If a radiation dose that causes irreparable damage when delivered over a short time period, is delivered over longer periods the cell or organism may survive.

ABSORBED DOSE :The basic quantity of radiation measurement in radiotherapy is the "absorbed dose." This term defines the amount of energy absorbed from a radiation beam per unit mass of absorbent material. The unit of absorbed dose is the Gray (Gy). It changes continuously along the path of the radiation because the radiation slows down. In addition, secondary radiation energies occur due to secondary scattering from the particle's path in tissue. The type and effects of each form of radiation type should be known exactly in order to define the total effect of the radiation.

EQUIVALENT DOSE: Different radiations cause different damages in human tissues. The absorbed dose is not adequate for studies of radiation protection. Thus, the absorbed dose in tissue should be multiplied by the radiation weighting factor for this radiation type. The calculated result is defined as the equivalent dose. If the mean absorbed radiation dose (Gy) in a tissue or organ is multiplied by the appropriate radiation weighting factor (WR), the equivalent dose (HT) is found. Radiation weighting factors (WR) are determined in order to compare the biological effects of different radiation types. These weighting factors are also called radiation quality factors(QF)

Description of Biological Effect Proccess

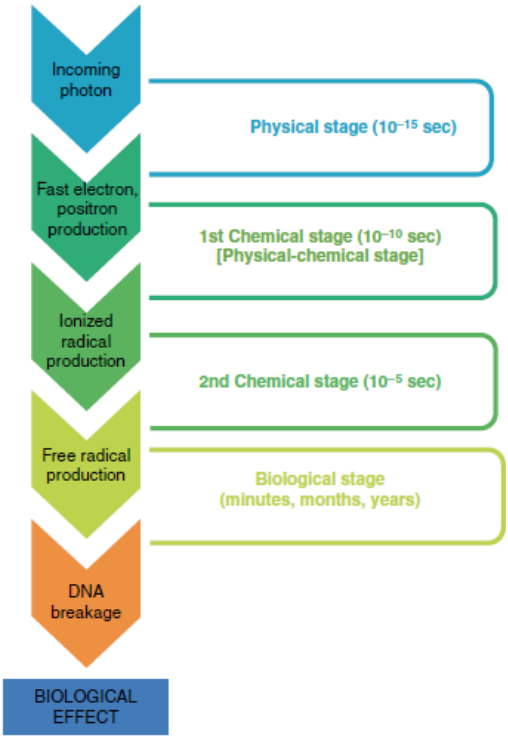

Figure 1.4: *Duration of all stages until the biological effect. The image adopted by "Comprises three sections covering the essential aspects of radiation physics, radiobiology, and clinical radiation oncology-Murat Beyzadeoglu,Gokham Ozyigit,Cuneyt Ebruli-Basic Radiation Oncology, Springer-Verlag Berlin Heidelberg, (2010) "***[3]***

The irridiated cells may have the below outcomes [**2**]

- No effect
- Division delay: the cell is delayed from going through division.
- Apoptosis: the cell dies before it can divide or afterwards by fragmentation into smaller bodies which are taken up by neighbouring cells.
- Reproductive failure: the cell dies when attempting the first or subsequent mitosis.

1.5 Chemical Base of DNA Damage

The branch of Radiobiology

The Radiation Biology Branch research activities are focused on pre-clinical basic science research aimed at identifying and incorporating novel approaches to cancer treatment, evaluation, and prevention. One sector of investigation is whether Ionizing Radiation induction is beneficial or devastating. Its most common impact is the induction of cancer with a latent period of years or decades after exposure. High doses can cause visually dramatic radiation burns, and/or rapid fatality through acute radiation syndrome. Controlled doses are used for medical imaging and radiotherapy. Some scientists suspect that low doses may have a mild hermetic effect that can improve health. Some effects of ionizing radiation on human health are stochastic, meaning that their probability of occurrence increases with dose, while the severity is independent of dose. Radiation-induced cancer, teratogenesis, cognitive decline, and heart disease are all examples of stochastic effects. Other conditions such as radiation burns, acute radiation syndrome, chronic radiation syndrome, and radiation-induced thyroiditis are deterministic, meaning they reliably occur above a threshold dose, and their severity increases with dose. Deterministic effects are not necessarily more or less serious than stochastic effects; either can ultimately lead to a temporary nuisance or a fatality. [**1**]

Direct or Indirect Radiation Induction

There are two types of actions in irradiation the direct and indirect: The former type of Radiation affects DNA molecules in the target tissue. The direct ionization of atoms in DNA molecules is the result of energy absorption via the photoelectric effect and Compton interactions. If this absorbed energy is sufficient to remove electrons from the molecule, bonds are broken, which can break one DNA strand or both (Figure 5). A single broken strand can usually be repaired by the cell, while two broken strands commonly result in cell death. [**3**]

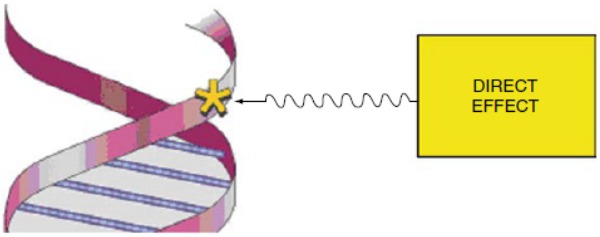

Figure 1.5: *Direct radiation. The image adopted by Comprises three sections covering the essential aspects of radiation physics, radiobiology, and clinical radiation oncology-Murat Beyzadeoglu,Gokham Ozyigit,Cuneyt Ebruli-Basic Radiation Oncology, Springer-Verlag Berlin Heidelberg, (2010)*

The latter type of Radiation on molecules includes the formation of free radicals by energy transfer from radiation, and the resulting molecular damage caused by the interactions of these free radicals with DNA (Figure 6). This phenomenon is most probably due to the interaction of radiation with water molecules, since the human body is approximately 70 percent of water. Free radicals are electrically neutral atoms that contain “free” (i.e., unbound) electrons. They are highly electrophilic and reactive. Free radicals formed by the hydrolysis of water affect DNA. [**3**]

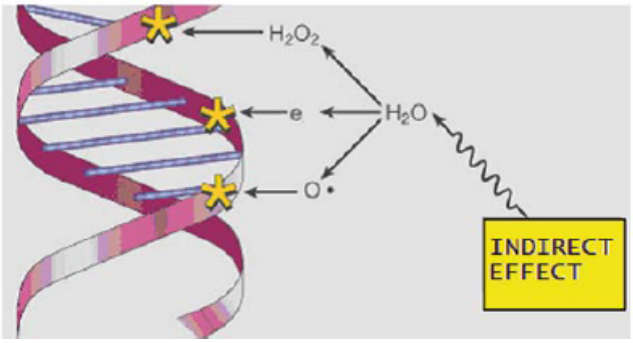

Figure 1.6: *Indirect radiation. The image adopted by" Comprises three sections covering the essential aspects of radiation physics, radiobiology, and clinical radiation oncology-Murat Beyzadeoglu,Gokham Ozyigit,Cuneyt Ebruli-Basic Radiation Oncology, Springer-Verlag Berlin Heidelberg, (2010)"*

Water exposed to ionizing radiation forms free radicals of hydrogen and hydroxyl, which can recombine to form gaseous hydrogen, oxygen, hydrogen peroxide, hydroxyl radicals, and peroxide radicals. In living organisms, which are composed mostly of water, majority of the damage is caused by the reactive oxygen species, free radicals produced from water. The free radicals attack the biomolecules forming structures within the cells, causing oxidative stress.

In cooling systems of nuclear reactors, the formation of free oxygen would promote corrosion and is counteracted by addition of hydrogen to the cooling water. The hydrogen is not consumed as equal for each molecule, so it is reacting with oxygen one molecule is liberated by radiolysis of water; the excess hydrogen just serves to shift the reaction equilibriums by providing the initial hydrogen radicals. The reducing environment in pressurized water reactors is less prone to buildup of oxidative species. The chemistry of boiling water reactor coolant is more complex, as the environment can be oxidizing. Most of the radiolytic activity occurs in the core of the reactor where the neutron flux is highest; the bulk of energy is deposited in water from fast neutrons and gamma radiation, the contribution of thermal neutrons is much lower. In air-free water, the concentration of hydrogen, oxygen, and hydrogen peroxide reaches steady state at about 200 Gy of radiation. In presence of dissolved oxygen, the reactions continue until the oxygen is consumed and the equilibrium is shifted. Neutron activation of water leads to buildup of low concentrations of nitrogen species; due to the oxidizing effects of the reactive oxygen species, these tend to be present in the form of nitrate anions. In reducing environments, ammonia may be formed. Ammonia ions may be however also subsequently oxidized to nitrates. Other species present in the coolant water are the oxidized corrosion products (e.g. chromates) and fission products (e.g. pertechnetate and periodate anions, uranyl and neptunyl cations).[16] Absorption of neutrons in hydrogen nuclei leads to buildup of deuterium and tritium in the water. Behavior of supercritical water, important for the supercritical water reactors, differs from the radiochemical behavior of liquid water and steam and is currently under investigation. [**16**]

The magnitude of the effects of radiation on water is dependent on the type and energy of the radiation, namely its linear energy transfer. A gas-free water subjected to low-LET gamma rays yields almost no radiolysis products and sustains an equilibrium with their low concentration. High-LET alpha radiation produces larger amounts of radiolysis products. In presence of dissolved oxygen, radiolysis always occurs. Dissolved hydrogen completely suppresses radiolysis by low-LET radiation while radiolysis still occurs with

The presence of reactive oxygen species has strongly disruptive effect on dissolved or-

ganic chemicals. This is exploited in groundwater remediation by electron beam treatment.

When normal cell DNA is damaged by radiation provided in the kinds of doses normally used in radiotherapy, the cell cycle is stopped by the protein $p53$. The DNA is repaired; the cell then re-enters the cell cycle and continues to proliferate. If the DNA cannot be repaired, the cell enters apoptosis- the programmed cell death pathway. At high radiation doses, the molecules utilized by the DNA repair mechanisms are damaged, so repair is not possible, the cell loses its ability to divide, and it subsequently dies. Water H_2O is ionized when exposed to radiation, and as we know from physic, a positively charged water molecule and a free electron are formed $H_2O \rightarrow H_2O_+ + e_-$ This free electron e_- interacts with another water molecule in the reaction resulting in the formation of a negatively charged water molecule. These charged water molecules undergo the reactions, yielding H_+ and OH_- ions. These H and OH free radicals may combine with other free radicals or with other molecules. If the LET of the radiation is high the free OH_- radicals do not recombine with $H+$radicals, and so they do not form H_2O. They combine with each other in the reactions $OH_- + OH_- \rightarrow H_2O_2$and $H_+ + H_+ \rightarrow H_2$, forming hydrogen peroxide and hydrogen gas molecules .
There are 3 types of damages by irradiation in mammalian cells, with the highest fatality: $DSB's$ (Double strand breaks), $BD's$ (base damages), $SSB's$ (Single strand damages). More specific, while $DSB's$ considered to be the most lethal damages $DB's$ ans $SSB's$ have severe effects icluding mutagenesis, high incidents of various pathologies, such as cancer, neurological disorders and ageing [**7**].

1.6 Phases of Cell Cycle

In cells with a nucleus, as in eukaryotes, the cell cycle is also divided into three periods: interphase, the mitotic (M) phase, and cytokinesis. During interphase, the cell grows, accumulating nutrients needed for mitosis, preparing it for cell division and duplicating its DNA. During the mitotic phase, the chromosomes separate. During the final stage, cytokinesis, the chromosomes and cytoplasm separate into two new daughter cells. To ensure the proper division of the cell, there are control mechanisms known as cell cycle checkpoints. During its life, a cell generally exhibits a long period or phase (interphase) during which no division occurs, and a division phase (mitosis). This is called the cell cycle. The cell cycle is repeated at each cellular stage, and the length of time corresponding to a cell cycle varies with cell type. In some cells the interphase is very long period for instance, the neuron does not divide during the life period of organism. Generally, cells grow until they reach a certain size, then they divide. [**6**]

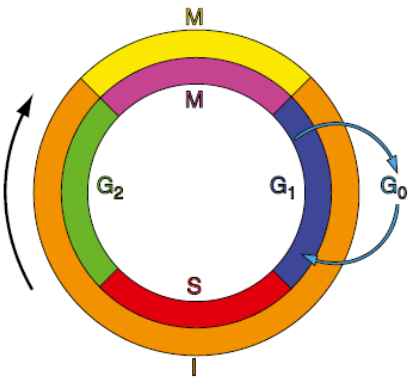

Figure 1.7: *Cell Cycle divided to an outer and an inner circle. The outer cirlce referred to the Mitotic phase and the Interphase and the inner cirlce to the subunits of Interphase and the Mitosis.The image adopted by "Comprises three sections covering the essential aspects of radiation physics, radiobiology, and clinical radiation oncology-Murat Beyzadeoglu,Gokham Ozyigit,Cuneyt Ebruli-Basic Radiation Oncology, Springer-Verlag Berlin Heidelberg, (2010)."*[**3**]

The Stages of Mitosis

- Mitosis is the division of a cell into two cells through the mating of its genome. Mitosis is only observed in eukaryotic cells. Somatic cells are formed via mitosis, whereas germ cells are formed by meiotic division.[**2**]

The Stages of Interphase

- Prophase: The nuclear membrane and endoplasmic reticulum disappear. The chromosomes shorten and thicken. Centrosomes move towards opposite poles. The nucleolus disappears. Spindle cells form from the poles to the center.

- Metaphase: The chromosomes shorten and thicken further. Sister chromatids are kept together using centromeres. The chromosomes are arranged side-by-side in a row in the equatorial plane. The chromosomes hold on to spindle cells with their centromeres.
- Anaphase: The contraction and relaxation movements of spindle cells break the centromeres that lock the chromatids together. The sister chromatids are separated from each other and are moved to opposite poles.
- Telophase : The chromosomes stop moving. The chromosomes unwind their helices and become chromatins. The nucleolus reappears. RNA and protein syntheses start. Spindle cells disappear. The nuclear membrane forms, and the endoplasmic reticulum takes on a shape again. Vital events restart in the cell. Cytogenesis occurs, and division finishes.

Another significant part of the cell cycle, is the cell proliferation, defined by two well-defined time periods:

- mitosis (M) where division takes place
- the period of DNA synthesis (S).

The S and M portions of the cell cycle are separated by two periods (gaps) $G1$ and $G2$. The $G1$ or $Gap1$ Phase is the first of the for phases of interphase. In this part of interphase, the cell synthesizes mRNA, proteins in the preparation for subsequent steps leading to mitosis, it ends when the cell moves into the S phase of interphase. The $Sphase$ of interphase is the second ''step" between $G1, G2$ phases. The $G2$ phase or $Gap2$ phase is the third and final subsequence of interphase, in which the DNA of cells is completed replicated.The $G2$ ends and the Mitosis starts, in which the cells chromatin condenses into chromosomes. Also,the time between successive divisions (mitoses) is called cell cycle time. For mammalian cells growing in culture the S-phase is usually in the range of 6-8 hours, M less than an hour, $G2$ in the range of 2-4 hours, and $G1$ from1-8 hours, making the total cell cycle in the order of 10-20 hours. In contrast, the cell cycle for stem cells in certain tissues is up to about 10 days. [**3**] The phase consting of $G0, G1, G2, M$. The $G1, G2, M$ phase compose a big period of cell evolution, which names interphase and takes place in the cell division, mitosis. The size of each phase is different and depend on the type of each type of cell. More specific:

Phase G0: The Phase G0 or zero Phase or relax is a period in cell cycle, which cells exist quiescent, also the G0 phase is a extension of G1, which cells are diviated or they brace themselves for deviation. We can say without hesitation, that is a distinct calm phase within cell cycle.

Phase G1: It is the first of four phases of cell cycle. At the duration of G1 phase the cells are growing in size and they compose the mRNA and proteins(histones), which they are necessary components for DNA composition. By the time that the necessary proteins have already grown up , they enter in S Phase.

Phase G2: The G2 phase is the third and last subphase of Interphase at the exact time before mitoses and subsequently the division of cell. In this way, the S phase completed, where DNA is doublicate. Finally, the G2 phase reach at the end, when the prophase begins, the first phase of mitoses which cell's cromatin composed in chromosomes. This phase is characterized as the period of cells and proteins fast evolution and composition. In many types of cells the G2 phase is not obligatory, so the transition of the DNA doublicate to mitoses happens immediately.

Phase M: The M Phase or at the duration of mitoses the chromosomes which have been created in G2 phase divided. In the final step cytocine, chromosomes and cytoplasm divided into two new sisters cells. For the confirmation of right process division and regulation, there are mechanisms which control some ponits of cell cycle (cell cycle checkpoints).

- In general, cells are most radiosensitive in the M and G2 phases, and most resistant in the late S phase.
- The cell cycle time of malignant cells is shorter than that ofsome normal tissue cells, but during regeneration after injury normal cells can proliferate faster.
- Cell death for non-proliferating (static) cells is defined as the loss of a specific function, while for stem cells it is defined as the loss of reproductive integrity (reproductive death). A surviving cell that maintains its reproductive integrity and proliferates indefinitely is said to be clonogenic. [**3**]

1.7 Processing (repair) of DNA damage

Cellular metabolism is a fundamental biological system consisting of myriads of enzymatic reactions that together fulfill the basic requirements of life.[**15**] The characteristic of the above systems are the ability of conservativeness, and organism achieve it under lethal and crucial circumstances. Such circumstances are the creation of DNA damages. As also described above, there is the possibility of occurence of single (isolated) DNA damage and complex (DSBs and non-DSB lesions) as shown here in figure 8:

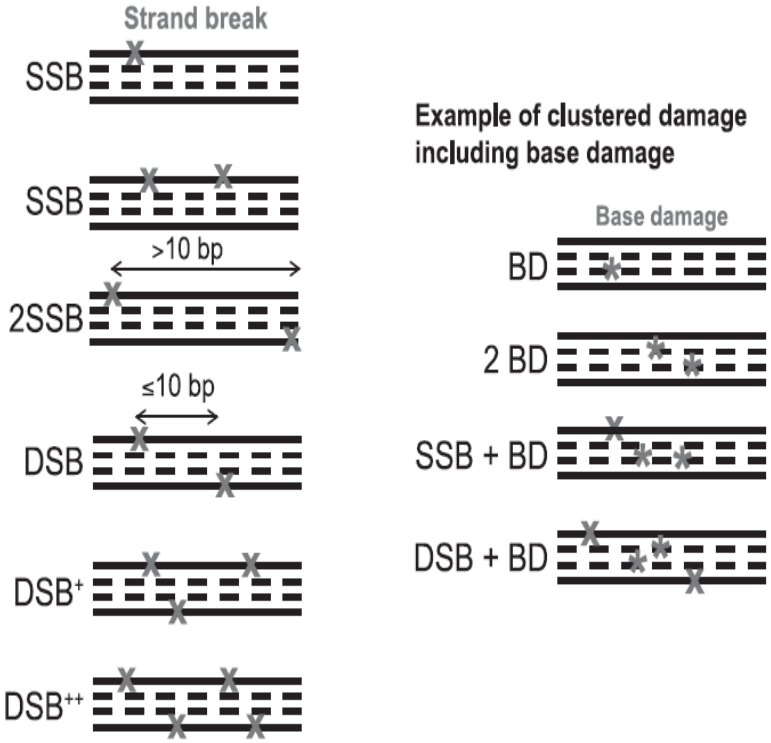

Figure 1.8: *Type of damages simple,complex SSB,DSB,lessions. The SP are the sugar-phosphate backbones and the B the base pairs. The image adopted by '"Spectrum of Radiation-Induced Clustered Non-DSB Damage/A Monte Carlo Track Structure Modelling and Calculations, Ritsuko Watanable, Shirin Rahmanianand Hooshang Nikjoo- MEDLINE, (2015)." [**13**]*

This dissertation will be referred on the size of strand breaks as bp (base pairs). So, the DNA damages of a cell characterized as crucial as the amount of bps have been damaged or altered or even if the breaking bps are too close to each other. The figure 8 shows the cells types of Damages, and analytically they are:

$SSB's$: Only one base is beeing damaged. If there are two damages in different strands in a distance bigger than 10bp we call it 2SSB, because it is two different SSBs, not a complex system.

$DSB's$: Two or more damages occured in a distance below or equal to 10bp.

DSB_{+} : A DSB on one strand and a SSB on a another strand.

$DSSB_{++}$: Two base damages on two different strands. If the system consisting of a

SSB and a DSB within10 bp is defined as one kind of complex DSB(DSBc)

Ionizing radiation produces a variety of DNA lesions, including single- and double-strand breaks $(SSB's, DSB's)$ and various types of base damage $(DB's)$. Although, such lesions also arise as a result of endogenous processes in the cell, radiation-induced lesions differ from endogenous lesions in their spatial distribution along the DNA molecule. It has been well established that DSBs are the most harmful type of damage to the cell. However, most DNA lesions, including DSBs, are repaired effectively by the cell, and only a small proportion of radiation-induced lesions are repaired with very slow repair kinetics. Among different types of DNA damage, clustered damage or ‘‘multiple damaged sites’’ have been postulated to be more difficult to repair. These findings have now been extended to include a slow component in base excision repair (BER) , a result of closely spaced base lesions and $SSBs$. This finding is important, since non-DSB lesions can also contribute to deleterious effects, such as mutagenesis and cytotoxicity. Also, each type of damage has a variety of paths. For example,damages characterized as $SSB's$, regulated by BER(Base excision Repair), NER(Nucleid excision Repair), MMR(Mismatch Repair) pathways, as well as damages described as $DSB's$ regulated by $NHEJ$, $MMEJ$ and HR paths.The mainly repair pathway of SSB’s and DSB’s is extremely conserved amongst higher eukaryotes and are reffered as the base excision repair (BER) .

There are three paths in DSB’s repair damages, the non-homologous end joining (NHEJ), the homologous recombination (HR), and the microhomology-mediated end joining (MMEJ). In each types of damage there are both slow and fast paths. As it is obvious, the slow paths are most difficult to repair than the fast and for this reason the slow ones are the most compex ones. Complex damages are the damages that are very close to each other and for this reason is more difficult for them to be repaired correctly. Among the three major repair pathways, the $NHEJ$ is a simplerand faster repair process in comparison to the $MMEJ$ and the HR repair. Biochemical end processing of DSB could lead to short deletions or insertions that are common throughout NHEJ and MMEJ.In addition, the processes involving rejoining or mis-rejoining ofthe original broken ends could lead to chromosome translocations.NHEJ is active through the whole cell cycle in mammaliancell lines. It has been proposed that HR is active during late S andG2 phases of the cell cycle, where a sister chromatid is available as a template for repair. The latter suggestion is supported by furthermolecular experiments that show post-translational activation of HR proteins such as CtIP, and Rad51 in G2 and S phases and not in G1 and G0 phases. In the G1 phase and in early S phase of cell cycle the NHEJ and MMEJ repair paths depending on the type of double strand breaks. The simple DSB are substrate for NHEJ and MMEJ, while complex DSB and DSB heterochromatin require furthermore process in the end rejoing.The repair of all DSB begins with NHEJ presynaptic process and according to the type of DSB pursue simple ligation, furthermore process end joining after ligase or resection. In G1 phase and in early S phase the model must act in a farsighted way of kinetic equations of DSB repair for DNA damages induced by hight $LET\, radiation$. The kinetic repair of DSB’s damages has at least two main repair components, the fast and the slow component. Between three paths of repair the NHEJ is the more simple and fast process compared to MMEJ and HR. The added

end by biochemical processes in double breaks, could be lead to small deletions or inductions which solved towards NHEJ and MMEJ paths. Furthermore, the repair processof the union of initial end or even the wrong union of initial damaged end could be leadin chromosome translocations. The NHEJ path is active in the duration of cell cycle; the HR path is active in the $S, G2$ phases and it is inactive in $G1$ and $G0$ phases.

Figure 1.9: *Repair paths depend on Cell cycle. NHEJ and MMEJ paths are regulating in G1, early S and Mitotic phases and NHEJ and HR are regulating in late S and G2 phases. As we notice the NHEJ path existing active in all cell cycle phases. The image adopted by "The Non-homologous End-Joining(NHEJ) Pathway for the Repair of DNA Double-Strand Breaks: I.A Mathematical Model-Reza Taleei, Hooshang Nikjoo-Radiation Research 179,000-000,(2013)."[**14**]*

Chapter 2

Models-Methods

2.1 Assumptions

The models will be discussed represent pathways of DSB Repair Models, specifically NHEJ,HR, MMEJ. In this chapter we will quote the equation's mathematical systems, simulation process and the model's code. The initial model described was the principal pathway Non Homologous End Joining. A mechanistic description of the processing of DNA double-strand breaks (DSBs) is important for the understanding of ionizing radiation effects leading to cell death, mutation, genomic instability, and carcinogenesis. Mathematical models of DSB repair are important for the description of radiation modalities. Because the damages of DNA form with the interacting equations we can use the mass kinetics[**12**]:modalities not accessible by experimental means and for their possible predictive capabilities. The below model is referred to Cuccinota's DSB's Repair Model (NHEJ) [**4**]

One of the most important part of modelling process is the correct assumptions. Assumptions do not work everytime, so it is important to choose the most appropiate.

- Firstly, we assume that the quantity of proteins are abudant and still stable in the system. If the total proteins moving or even are fewer the model does not work.
- Secondly, we assume that the process of each enzyme or protein is happening one after other, with the right sequence, all enzymes finishing their repair regulation and then the process continues with the next repair enzyme. If damages finishes earlier, the model can not go to the next step.
- Also, our scaling approach results in a significant reduction in parameter space since it avoids the need to estimate values for the total cellular concentrations of enzymes,which are effectively replaced by a single constant, H1.
- Moreover, we assume that at the time zero we have the maximum size of damages. Something that it is not surely right in the real experiments, since new repair damages can be created by the repair of the old and overcome the number of initial damages
- Lastly, we assume that every Gy induce 35-45 DSB/cel.

2.2 Models of Simple and Complex Paths

The tackle of simulation problem diverses whether the system is simple or complex. To put it in other words when the system has both paths fast and slow, or one only path, the methods differ. The DSB rejoining with single processing pathway has this interacting equations [**12**]:

$$IR + DNA \rightarrow DSB$$

The biochemical kinetic model, mass-action equations describe the interaction of an enzyme, E with a substrate, S , in a biomolecular reaction to form an enzyme-substrate complex, C . This step is followed by a secpond-step: the formation of a reaction product, P , and the release of the enzyme. This is an approach that describes the exposure of cells DNA to ionizing radiation (IR) resulting DSB (double strand breaks)[**12**]. It is assumed that a fast damage recognition step (presynapsis) is followed when the DSB's are formed. In the step of presynapsis a damage-processing enzyme is binding to the DSB and formed an enzyme-substrate complex.

$$[DSB] + [E] \rightarrow [C] \rightarrow [P] + [E]$$

where quantities in brackets represent time-dependent concentrations of biochemical species and the reaction rate-constants are labelled as k with an appropriate subscript used. The products of the reaction are correctly or incorrectly rejoining DNA. And an important assumption is that $k1rev$ must not be much smaller than k_2 . The mass-action equations corresponding to the reaction of above equation is :

$$\frac{dDSB}{dt} = a\frac{dD}{dt} - k_1[E][DSB]$$

where $a\frac{dD}{dt}$ is the rate of Dose in time, another assumption that the initial quantity of DSB $[DNA]_0$ is proportional to quantity of Dose (D) . The quantities of enzyme, E evaluated by the conservation law $E = E_0 - C$, where E_0 is the concentration of E at $t = 0$. The solutions of mass-action equations are depend on quasi-steady-state approximation, where every points of the system are equal to zero except from the first ones(reacted species) and the last ones(product species). So if the quantity of substrates is in great excess of the quantity of enzymes the time-change of complex repair C is very small, and we use some theorems like pertubation theorems to check the system out or correct it. In our problem we will not use this assumption in the fitting process.The excision for the repair complex is:

$$\frac{dC}{dt} = k_1[E][DSB] - k_2[C]$$

When the initial double strand breaks $[DSB]_0$ do not fixed by rejoining the excision is :

$$\frac{DSB}{dt} = -k_1[E][DSB]$$

Then we insert a function that will explain the efficiency of DSB repair for this reason we divide the concentration of $[DSB]$ with the number of initial protein. Do not forget we have assumed that the initial number of $DSB's$ have the higher concentration. $f(t) = [DSB]/E_0$, $c(t) = [C]/E_0$, $a_1 = k_1 E_0$ are the scalling variables sfter the irradiated time, so we have the below system :

$$\begin{aligned} \frac{df(t)}{dt} &= -a_1[1 - c(t)]f(t)] \\ \frac{dc(t)}{dt} &= a_1[1 - c(t)]f(t) - k_2 c(t) \end{aligned}$$

The equations are valid when the $k_2 >> k_1[E_0]$, also the concentration of free enzymes approaches $[E0]$

Following a fast damage recognition process, competition between the two pathways with rates k1 and k3 occurs for a single. The model of competing DSB's pathways as described by

$$DSB + E_1 \rightarrow C_1 \rightarrow P_1 + E_1$$

$$DSB + E_2 \rightarrow C_2 \rightarrow P_2 + E_2$$

and assuming the back reactions $k1rev$ and $k3rev$ may be neglected. Recombination repair involving movement of damaged DNA to a homologous undamaged DNA then occurs followed by DNA syntesis and ligation. The products of these reactions are rejoined DNA or exchange aberrations.It is assumed that some fraction of the DSB produced by ionizing radiation is repaired by such recombination events.

$$\frac{dDSB}{dt} = a_j \frac{dD}{dt} - k_1[E_1][DSB] - k_3[E_2][DSB]$$

The repair complexes of the competing system:

$$\begin{aligned} \frac{dC_1}{dt} &= k_1[E_1][DSB] - k_2[C_1] \\ \frac{dC_2}{dt} &= k_3[E_2][DSB] - k_4[C_2] \end{aligned}$$

The solutions for the enzyme concentration laws $E01 = [E1] + [C1]$ and $E02 = [E2] + [C2]$. For simplicity the case of saturation of $[C2]$ pathway and the resulting effects on the kinetics of formation abberations from $[C1]$ are considered. The formation of an exchange abberation of type m is described by the differential equation(assuming $k4r >> k4m$),where $k2m$ is the rate of formation of exchange abberation of type m from

complex $[C1]$ respectively.

$$\frac{d[A_m]}{dt} = k2m[C_1]$$

The rate constants for product formation obey the auxilarity condition:

$$k_2 = k_{2r} + \sum_m k_{2m}$$

, where k2r is the rate of corrected rejoing from [C1], and the summation represents the type of exchange abberations could occur. We solve the equations with numerical methods and we use scalling methods to simplify them. For instance, $f(t) = [DSB]/E_{01}$, $c_1(t) = [C_1]/E_{01}$, $c_2(t) = [C_2]/E_{02}$, $a_1 = k_1 E_{01}$, $a_3 = k_3 E_{02}$, $r = E_{01}/E_{02}$ are the scalling variables after the irradiated time, so we have the below system :

$$\begin{aligned}
\frac{df(t)}{dt} &= -a_1[1 - c_1(t)]f(t) - a_3[1 - c_2(t)]f(t)] \\
\frac{dc_1(t)}{dt} &= a_1[1 - c(t)]f(t) - k_2 c_1(t) \\
\frac{dc_2(t)}{dt} &= ra_3[1 - c_2(t)]f(t) - k_4 c_2(t)
\end{aligned}$$

The form of models will be analysed in the dissertation are based on the below schematic process [**12**]

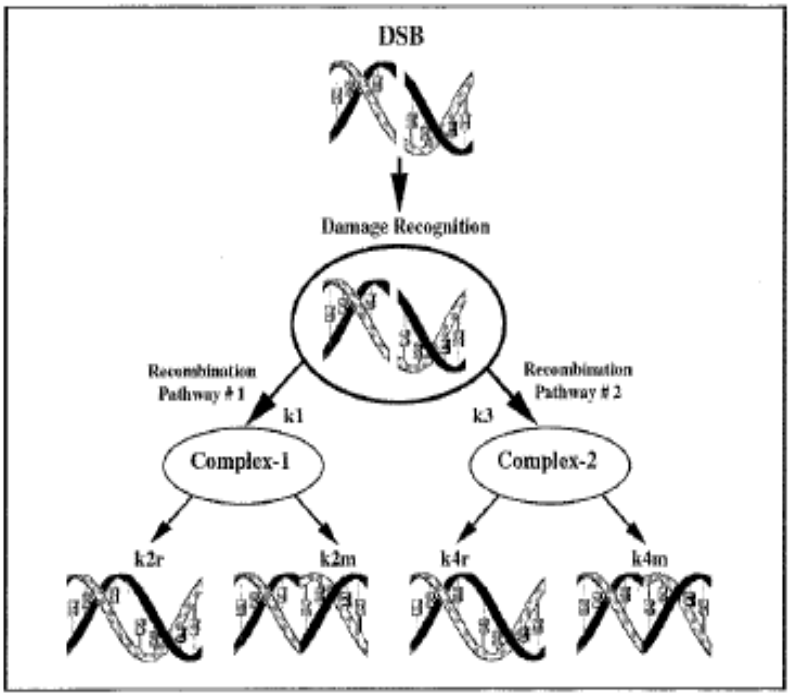

Figure 2.1: *A general model for competing DSB's pathways. The image adopted by "Kinetics of DSB rejoining and formation of simple chromosome echange aberrations-F.A Cucinotta, H.Nikjoo, P.O'Neil, D.T. Goodhead- INT.J. RADIAT.BIOL VOL.76,NO 11, 1463-1474 , (2000)."*

2.3 The NHEJ pathway in each phase of cell cycle

The repair process has three options the NHEJ,HR,MMEJ repair pathways nevertheless, will be focused on the NHEJ processes. The NHEJ is divided again to three pathways regarding to the type of damage induced. For example, if the damage induce in Heterochromatin,the cell will follow different repair process instead of a clustered damage(complex damage). These biochemical paths have the same start (prosynaptic process), but the proteins competing. In 1.3 will quote altogether the processes and the mathematical equation, nonetheless in 1.4-1.5-1.6 will give more detail to each process seperately.

Modeling Approach

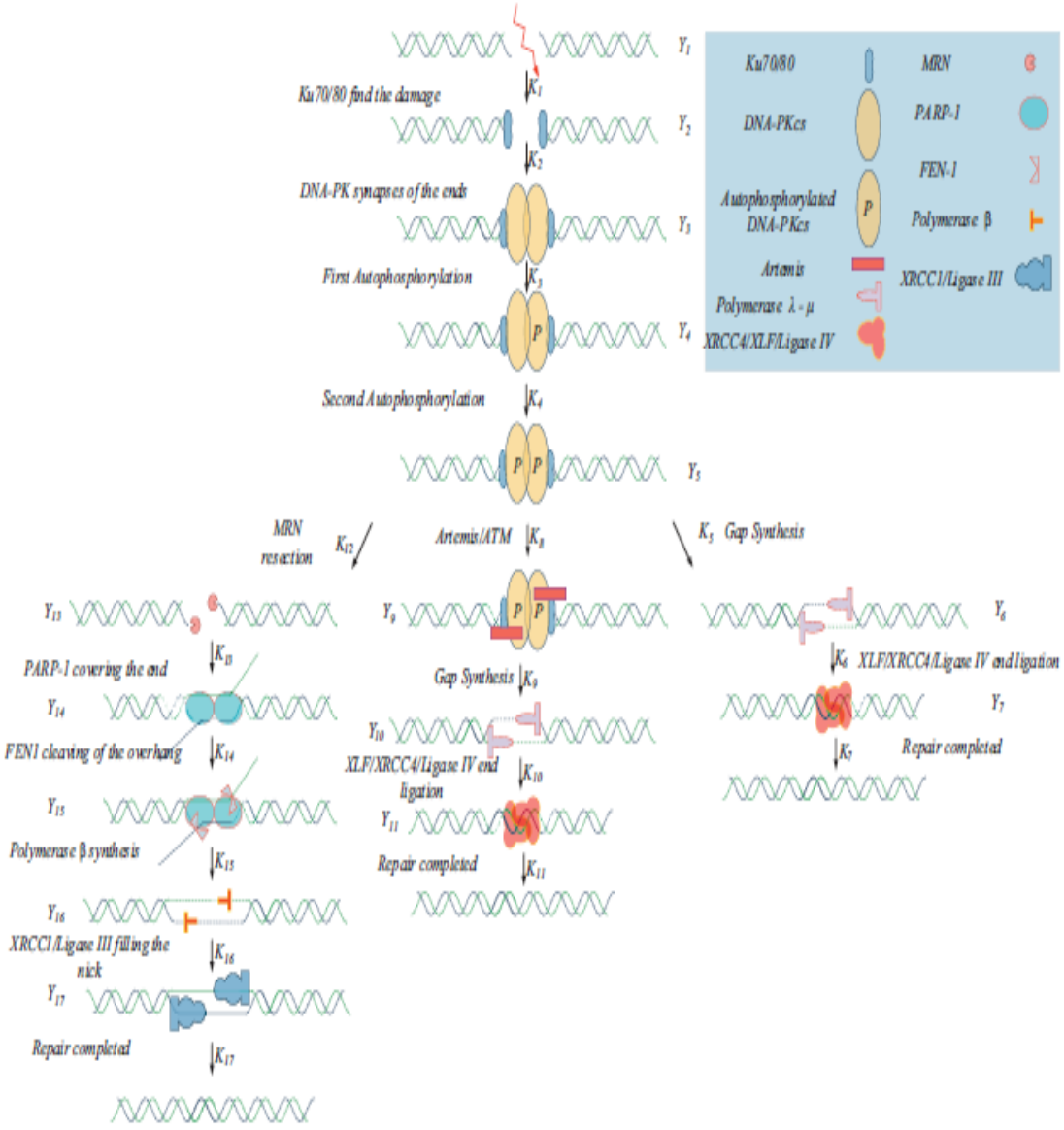

Figure 2.2: *The Graph illustrates the repair proteins-enzymes taking part in the three compeeting pathways. The equations 1.1-1.4 is the presynaptic process of NHEJ, 1.5-1.7 describes the simple DSB repair, the 1.8-1.10 describes the Heterechromating remodelling and the 1.11-1.15 the complex DSB repair.Fiqure adopted from[**14**].*

$$\frac{dY_1}{dt} = a\frac{dD}{dt} - V_1 \qquad V_1 = K_1[Ku70/80]Y_1 \tag{2.1}$$

$$\frac{dY_2}{dt} = V_1 - V_2 \qquad V_2 = K_2[DNAPKcs]Y_2 \tag{2.2}$$

$$\frac{dY_3}{dt} = V_2 - V_3 \qquad V_3 = K_3Y_3 \tag{2.3}$$

$$\frac{dY_4}{dt} = V_3 - V_4 \qquad V_4 = K_4Y_4 \tag{2.4}$$

$$\frac{dY_5}{dt} = V_4 - V_5 - V_8 - V_{12} \qquad V_5 = K_5[Polymerase\lambda - \mu]Y_5, \qquad V_8 = K_8[Artemis/ATM]Y_5 \tag{2.5}$$

$$\frac{dY_6}{dt} = V_5 - V_6 \qquad V_6 = K_6[XLF/XRCC4/LIGIV]Y_6 \tag{2.6}$$

$$\frac{dY_7}{dt} = V_6 - V_7 \qquad V_7 = K_7Y_7 \tag{2.7}$$

$$\frac{dY_9}{dt} = V_8 - V_9 \qquad V_9 = K_9[Polymerase\lambda - \mu]Y_9 \tag{2.8}$$

$$\frac{dY_{10}}{dt} = V_8 - V_9 \qquad V_{10} = K_{10}[XLF/XRCC4/LIGIV]Y_{10} \tag{2.9}$$

$$\frac{dY_{11}}{dt} = V_{10} - V_{11} \qquad V_{11} = K_{11}Y_{11} \tag{2.10}$$

$$\frac{dY_{13}}{dt} = V_{12} - V_{13} \qquad V_{13} = K_{13}[PARP - 1]Y_{13} \tag{2.11}$$

$$\frac{dY_{14}}{dt} = V_{13} - V_{14} \qquad V_{14} = K_{14}[FEN1]Y_{13} \tag{2.12}$$

$$\frac{dY_{15}}{dt} = V_{14} - V_{15} \qquad V_{15} = K_{15}[Polymerase\beta]Y_{15} \tag{2.13}$$

$$\frac{dY_{16}}{dt} = V_{15} - V_{16} \qquad V_{16} = K_{16}[XRCC1\ LIGIII]Y_{16} \tag{2.14}$$

$$\frac{dY_{17}}{dt} = V_{16} - V_{17} \qquad V_{17} = K_{17}Y_{17} \tag{2.15}$$

These concentrations characterized by the action of some enzymes-proteins taking part in the repair process.

$$[C0] \rightarrow Ku70/80$$

$$[C1] \rightarrow DNA - PKcs$$

$$[C2] \rightarrow ABCDE\ sites$$

$$[C3] \rightarrow PQR\ sites$$

$$[C4] \rightarrow Artemis$$

$$[C5] \rightarrow Polymerase\lambda - \mu$$

$$[C6] \rightarrow MRN$$

$$[C7] \rightarrow PARP - 1$$

$$[C8] \rightarrow FEN-1\ sites$$

$$[C9] \rightarrow Polymerase\beta\ sites$$

$$[C10] \rightarrow XRCCIV/LigaseIII$$

Scalling Variables

$$H_i = [E_i] + \sum_{n=1}^{n} [C_j] = constant$$

$$h_i = \frac{\sum_{n=1}^{n} [C_i]}{H_i}$$

$$c_i(t) = \frac{[C_i]}{H_i}$$

$$K_i = \frac{k_i}{H_i}$$

$$[Ku70/Ku80] = \frac{E_1}{H_i} = 1 - h_1(t)$$

$$[DNAPKcs] = \frac{E_2}{H_i} = 1 - h_2(t)$$

$$[XLF]/[LIGIV]/[XRCC4] = \frac{E_4}{H_i} = 1 - h_4(t)$$

$$[Artemis] = \frac{E_7}{H_i} = 1 - h_7(t)$$

$$[Polymerase\lambda - \mu] = \frac{E_8}{H_i} = 1 - h_8(t)$$

$$[XLF]/[LIGIV]/[XRCC4] = \frac{E_9}{H_i} = 1 - h_9(t)$$

$$[MRN] = \frac{E_{11}}{H_i} = 1 - h_{11}(t)$$

$$[PARP-1] = \frac{E_{12}}{H_i} = 1 - h_{12}(t)$$

$$[FEN-1] = \frac{E_{13}}{H_i} = 1 - h_{13}(t)$$

$$[Polymerase\beta] = \frac{E_{14}}{H_i} = 1 - h_{14}(t)$$

$$[XRCCI]/[LigaseIII] = \frac{E_{15}}{H_i} = 1 - h_{15}(t)$$

The above equations are actually the probability of each protein to bind to the site of damage.Moreover, substituting the above equations in mass kinetics of the system we lead to the below parametrical equations, with dependent variable t . Also, we substitute the enzymes as we have said in the asumptions with the scalling variables avoiding the specific evaluation of concentrations. More specific:

$$\frac{dc_0}{dt} = a\frac{dD}{Hidt} - k_1 c_0(t)[1 - h_1(t)] \tag{2.16}$$
$$\frac{dc_1}{dt} = k_1 c_0(t)[1 - h_1(t)] - k_2 c_1(t)[1 - h_2(t)] \tag{2.17}$$
$$\frac{dc_2}{dt} = k_2 c_1(t)[1 - h_2(t)] - K_3 c_2(t) \tag{2.18}$$
$$\frac{dc_3}{dt} = K_3 c_2(t) - K_4 c_3(t) \tag{2.19}$$
$$\frac{dc_4}{dt} = K_4 c_3(t) - k_5[1 - h_4(t)]c_4(t) - k_8[1 - h_7(t)]c_4(t) - k_{12}[1 - h_{11}(t)]c_4(t) \tag{2.20}$$
$$\frac{dc_5}{dt} = k_5 c_4(t)[1 - h_4(t)] - k_6 c_5(t)[1 - h_5(t)] \tag{2.21}$$
$$\frac{dc_6}{dt} = k_6 c_5(t)[1 - h_5(t)] - K_7 c_6(t) \tag{2.22}$$
$$\frac{dc_7}{dt} = K_7 c_6(t) - k_8 c_4(t)[1 - h_7(t)] \tag{2.23}$$
$$\frac{dc_8}{dt} = k_8 c_4(t)[1 - h_7(t)] - k_9 c_8(t)[1 - h_8(t)] \tag{2.24}$$
$$\frac{dc_9}{dt} = k_9 c_8(t)[1 - h_8(t)] - k_{10}[1 - h_9(t)]c_9(t) \tag{2.25}$$
$$\frac{dc_{10}}{dt} = k_{10}[1 - h_9(t)]c_9(t) - K_{11} c_{10}(t) \tag{2.26}$$
$$\frac{dc_{11}}{dt} = K_{11} c_{10}(t) - k_{12}[1 - h_{11}(t)]c_4(t) \tag{2.27}$$
$$\frac{dc_{12}}{dt} = k_{12}[1 - h_{11}(t)]c_4(t) - k_{13}[1 - h_{12}(t)]c_{12}(t) \tag{2.28}$$
$$\frac{dc_{13}}{dt} = k_{13}[1 - h_{12}(t)]c_{12}(t) - k_{14}[1 - h_{13}(t)]c_{12}(t) \tag{2.29}$$
$$\frac{dc_{14}}{dt} = k_{14}[1 - h_{13}(t)]c_{12}(t) - k_{15}[1 - h_{14}(t)]c_{14}(t) \tag{2.30}$$
$$\frac{dc_{15}}{dt} = k_{15}[1 - h_{14}(t)]c_{14}(t) - k_{16}[1 - h_{15}(t)]c_{15}(t) \tag{2.31}$$
$$\frac{dc_{16}}{dt} = k_{16}[1 - h_{15}(t)]c_{15}(t) - K_{17}]c_{16}(t) \tag{2.32}$$

Then we substitute the scaling variables and we have the below equations that we will use to computational calculations

$$\frac{dc_0}{dt} = \frac{adD}{H_1 dt} - k_1 c_0(1 - c_0 - c_1 - ..... - c_{15} - c_{16}) \quad (2.33)$$

$$\frac{dc_1}{dt} = k_1 c_0(1 - c_0 - c_1..... - c_{15} - c16) - k_2 c_1(1 - c_1 - ..... - c_{15} - c_{16}) \quad (2.34)$$

$$\frac{dc_2}{dt} = k_2 c_1(1 - c_1 - ..... - c_{15} - c_{16}) - K_3 c_2 \quad (2.35)$$

$$\frac{dc_3}{dt} = K_3 c_2 - K_4 c_3 \quad (2.36)$$

$$\frac{dc_4}{dt} = K_4 c_3 - k_5 c_4(1 - c_4... - c_1 6) - k_8 c_4(1 - c_7.... - c_{16})c_4 - k_{12} c_4(1 - c_{11}... - c_{16}) \quad (2.37)$$

$$\frac{dc_5}{dt} = k_5 c_4(1 - c_4.. - c_{16}) - k_6 c_5(1 - c5... - c_{16}) \quad (2.38)$$

$$\frac{dc_6}{dt} = k_6 c_5(1 - c_5.. - c_{16}) - K_7 c_6 \quad (2.39)$$

$$\frac{dc_7}{dt} = K_7 c_6 - k_8 c_4(1 - c_7.... - c_{16}) \quad (2.40)$$

$$\frac{dc_8}{dt} = k_8 c_4(1 - c_7.... - c_{16}) - k_9 c_8(1 - c_8.... - c_{16}) \quad (2.41)$$

$$\frac{dc_9}{dt} = k_9 c_8(1 - c_8... - c_{16}) - k_{10} c_9(1 - c_9.... - c_{16}) \quad (2.42)$$

$$\frac{dc_{10}}{dt} = k_{10} c_9(1 - c_9... - c_{16}) - K_{11} c_{10} \quad (2.43)$$

$$\frac{dc_{11}}{dt} = -K_{11} c_{10} - k_{12} c_4(1 - c_{11}.... - c_{16}) \quad (2.44)$$

$$\frac{dc_{12}}{dt} = k_{12} c_4(1 - c_{11}.... - c_{16}) - k_{13} c_{12}(1 - c_{12}.... - c_{16}) \quad (2.45)$$

$$\frac{dc_{13}}{dt} = k_{13} c_{12}(1 - c_{12}.... - k_{14} c_{12}(1 - c_{13}.... - c_{16}) \quad (2.46)$$

$$\frac{dc_{14}}{dt} = k_{14} c_{13}(1 - c_{13}.... - c_{16}) - k_{15} c_{14}(1 - c_{14}.... - c_{16}) \quad (2.47)$$

$$\frac{dc_{15}}{dt} = k_{15} c_{14}(1 - c_{14}.... - c_{16}) - k_{16} c_{15}(1 - c_{15} - c_{16}) \quad (2.48)$$

$$\frac{dc_{16}}{dt} = k_{16} c_{15}(1 - c_{15} - c_{16}) - K_{17} c_{16} \quad (2.49)$$

rate constants	Complex Path	rate constants	Complex Path
a	16	k9	2
k1	350	k10	0.8
k2	500	k11	0.5
K3	50	k12	3
K4	20	k13	1
k5	25	k14	0.7
k6	18	k15	0.75
k7	3	k16	0.5
k8	9	k17	0.15

Table 2.1: *The coeficient table has actualy two column, the first column indicates* $constant_i$ *of NHEJ simulation model and the second column indicates the rate constant(number) of NHEJ simulation model. The coefficients adopted by [***14***]*

2.4 Presynaptic Process and Simple DSB repair

Biological Description

Non-homologous end joining (NHEJ) is the primary pathway for DSB repair in eukaryotic cells, also it defects in NHEJ increase radiation sensitivity and the risk of carcinogenesis. Many of the steps involved in NHEJ have been characterized experimentally, including the initial recognition of DSBs by the Ku70/80 heterodimer(presynapsis), subsequent recruitment of the DNA-dependent protein kinase catalytic subunit (DNA-PKcs), and formation of the DNA dependent protein kinase (DNA-PK).

The repair process starts when the Ku70/80 (presynaptic process) detect the cell and finds the damages, then it recruits suitable repair proteins and enzymes(inhibit MRN, PARP-1 proteins) for each damage. The duty of Ku70/80 is to be unified with the next enzyme (DNA-PKcs) and form DNA-PK complex. DNA-PKcs includes several serine-threonine residues that are autophosphorylated. This step of autophosphorylation of various subsets of these sites is thought to be important in regulating the choice between NHEJ or homologous recombination repair(HR). DNA-PKcs is a gatekeeper for prematyre ligation of damage, and the subsequently the wrong repair of damage site. The autophosporylation of serine(PQR) and threonine(ABCDE) residues is required for the ligation. If the ABCDE autophosphorylation do not achieved, the cell will not leave the end termini repaired by the pathway. In contrast, if the PQR autophosphorylation do not complete the cell will interupt the process ad will find an alternative pathway. After that step LigIV comes linking all this repairs together and give an end to the procedure.

Modeling Approach

We use the mass-action kinetics approach to describe the binding of repair enzymes to DSBs with several intermediate repair complexes leading to DNA rejoining:

- an initial complex bound by the Ku70/Ku80 heterodimer,
- Ku-mediated DNA-PKcs binding,
- the regulation of the DSB-DNA-PKcs complex through autophosphorylation by DNA-PK,
- a final repair complex involving the Ligase IV/XRCC4 heterodimer, denoted LiIV.

The model illustrates the sequential repair processes step-by-step. Law of mass action is used to translate the model to mathematical formalism. Yi, Vi, and Ki represent the repair–protein complex concentration, repair rate, and repair rate constant at step of repair, respectively. The number of DSB is linearly proportional to radiation dose with DSB induction-rate per unit dose constant. The law of mass action is employed to derive that explains Y1 increases with the initial dose and decreases with Ku70/Ku80 heterodimer recruitment at the site of damage. Our kinetics model of NHEJ consists of a system of eight coupled nonlinear ordinary differential equations for each class of DSBs (simple and complex). This system of equations describe major components in the NHEJ repair pathway and the phosphorylation

of H2AX by DNA-PKcs. The value of H1 can be denoted as the total number of copies of Ku70/Ku80. However, in the model, other constants, Hj, could be used as the scaling variable, and we prefer to interpret the value of H1 as the total number of DNA repair complexes that could occur in a cell. We have fixed this value at a large number $(H1 = 3000)$ to ensure that the shape of the DSB rejoining[**14**]. Actually the amount of proteins to be larger than the quantity of $a * Dose$. The series of repair complexes are denoted Cj and the repair in the autophosphorylation complex denoted as Cp or Cpp. The first complex Cj (C1) is formed by Ku70/80 binding to the DSB, the second through binding by DNA-PKcs to the first complex forming C2, the third is the autophosphorylation of ABCDE site or PQR site then a part convert to H2AX, the final step is the ligation(C3). Because these proteins are post-transcriptionally regulated, the total number of enzymes in free form or complex form is assumed to be conserved.

$$[C0] \rightarrow Ku70/80$$
$$[C1] \rightarrow DNA - PKcs$$
$$[C2] \rightarrow DNA - PK$$
$$[C2p] \rightarrow ABCDE\ sites$$
$$[C2pp] \rightarrow PQR\ sites$$
$$[C3] \rightarrow LigaseIV$$

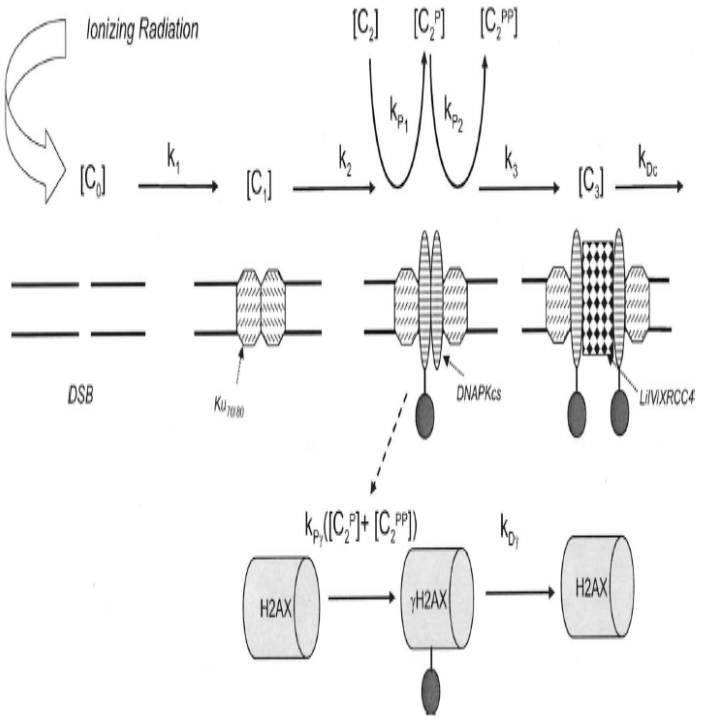

Figure 2.3: *Schematic represetation of Non Homologous End Joining (NHEJ) presynaptic process, with the regulative enzymes taking part in the repair. Such as Ku70/80, DNA-PCks, LiIV/XRCC4 and the two activation steps considered; phosphorylation of DNA-PKcs and H2AX.The fiqure adopted by[**4**]*

Equations DSB's repair Model (NHEJ)

$$\frac{dC_0}{dt} = a\frac{dD}{dt} - K_1[Ku70/80][C0] \tag{2.50}$$
$$\frac{dC_1}{dt} = K_1[C_0][Ku70/80] - K_2[DNAPKcs][C_1] \tag{2.51}$$
$$\frac{dC_2}{dt} = K_2[DNAPKcs][C1] - K_{p1}[C_2] \tag{2.52}$$
$$\frac{dC_2p}{dt} = K_{p1}[C_2] - K_{p2}[C_2p] - K_{res}[C_2p] \tag{2.53}$$
$$\frac{dC_2pp}{dt} = K_{p2} - K_3[LiIV][C_2pp] \tag{2.54}$$
$$\frac{dC_3}{dt} = K_3[LiIV][C_2pp] - K_{Dc}[C_3] \tag{2.55}$$
$$\tag{2.56}$$

Scalling Variables

$$H_i = [E_i] + \sum_{n=1}^{n}[C_j] = constant$$

$$h_i = \frac{\sum_{n=1}^{n}[C_i]}{H_i}$$

$$c_i(t) = \frac{[C_i]}{H_i}$$

$$K_i = \frac{k_i}{H_i}$$

Substituting the above equations in mass kinetics of the system we lead to the below parametrical equations, with dependent variable t . Also, we substitute the enzymes as we have said in the asumptions with the scalling variables avoiding the specific evaluation of concentrations. More specific,

$$[Ku70/Ku80] = \frac{E_1}{H_i} = 1 - h_1(t)$$

$$[DNAPKcs] = \frac{E_2}{H_i} = 1 - h_2(t)$$

$$[LIGIV]/[XRCC4] = \frac{E_3}{H_i} = 1 - h_3(t)$$

$$\frac{dc_0}{dt} = a\frac{dD}{Hidt} - k_1 c_0(t)[1 - h1(t)] \quad (2.57)$$
$$\frac{dc_1}{dt} = k_1 c_0(t)[1 - h_1(t)] - k_2 c_1(t)[1 - h_2(t)] \quad (2.58)$$
$$\frac{dc_2}{dt} = k_2 c_1(t)[1 - h_2(t)] - K_{p1} c_2(t) \quad (2.59)$$
$$\frac{dc_2p}{dt} = K_{p1} c_2(t) - (K_{p2} + K_{res}) c_{2p}(t) \quad (2.60)$$
$$\frac{dc_2pp}{dt} = K_{p2} c_{2p}(t) - k_3 c_{2pp}(t)[1 - h_3(t)] \quad (2.61)$$
$$\frac{dc_3}{dt} = k_3 c_{2pp}(t)[1 - h_3(t)] - K_{Dc} c_3(t) \quad (2.62)$$
$$\frac{dDSBs}{dt} = K_{res} c_{2p}(t) \quad (2.63)$$
$$(2.64)$$

If we reput the scalling variables to the above parametrical equations we have the follow system.

$$\frac{dc_0}{dt} = \frac{adD}{H_1 dt} - k_1 c_0(1 - c_0 - c_1 - c_2 - c_{2p} - c_{2pp} - c_3 - c_{res}) \quad (2.65)$$
$$\frac{dc_1}{dt} = k_1 c_0(1 - c_0 - c_1 - c_2 - c_{2p} - c_{2pp} - c_3 - c_{res}) - k_2 c_1(1 - c_1 - c_2 - c_{2p} - c_{2pp} - c_3 - c_{res}) \quad (2.66)$$
$$\frac{dc_2}{dt} = k_2 c_1(1 - c_1 - c_2 - c_{2p} - c_{2pp} - c_3 - c_{res}) - K_{p1} c_2 \quad (2.67)$$
$$\frac{dc_2p}{dt} = K_{p1} c_2 - (K_{p2} + K_{res}) c_2 p \quad (2.68)$$
$$\frac{dc_2pp}{dt} = K_{p2} c2p - k_3 c2pp(1 - c_3) \quad (2.69)$$
$$\frac{dc_3}{dt} = k_3 c2pp(1 - c_3) - K_{Dc} c_3 \quad (2.70)$$
$$\frac{dcres}{dt} = K_{res} c2p \quad (2.71)$$
$$(2.72)$$

We define H_i the number of proteins that remains stable(according the first assumption) in the system: $H_1 = H_2 = H_3 = 3000$ **[4][12]** ,as well as that the initial quantity of DSBs is the biggest quantity $a\frac{dD}{dt} >> k_1[E][DSB]$ **[12]**

rate constants	Complex Paths	Simple Paths
a	25	25
k3	0.5	8
Kρ1	10	10
Kρ2	0.5	10
KDc	4	4
kDγ	2	2
kM	0.5	0.5
kres	0.05	0

Table 2.2: *The coeficient table has three columns, the first column indicates the* $constant_i$ *of the model, the second column indicates the rate of constant(number) in complex paths and the third in simple paths.**[4]***

Finally a significant information for the repair problem is how many damages remain at the duration of process (DSB remaining) :

$$DSBremain(t) = H_1[\sum_{j=0}^{3} c_j(t) + cres(t)]$$

The method used was Runge-Kuta and the platform for simulations and graphs was Cygwin in a Linux enviroment and ulitilized the programming language C++. [**22**]

```
/=========================================================
//Program to solve an  ODE system using Runge-Kutta Method
//=========================================================
// Compile with the commands:
// gfortran -O2 -c rksuite/rksuite.f;
// g++ -O2 dna.cpp rksuite.o -o dna -lgfortran
#include <iostream>
#include <fstream>
#include <cstdlib>
#include <string>
#include <cmath>
using namespace std;
//---------------------------------------------------------
// Parameteral  conditions:
const double H1      =  3000.0;
const double dDdt    =     1.0;
const double kappa1 =   100.0;
const double kappa2 =   100.0;
const double kappa3 =    8 ;
const double kP1     =    10.0;
//-------------- V79 cells
//const double alpha  =    16;
//const double kP2    =    10;
//const double kDc    =     4.0;
//const double kres   =     0;
//------------- HF19 cell
 const double alpha  =    25.0;
const double kP2    =    10.0;
 const double kDc    =     4.0;
 const double kres   =     0.0;
const double adDdt  = alpha*dDdt/H1; //first term in c0 equation
//-----------------------------
double c0            = 0.0; //alpha*dDdt/H1;
double c1=0.0,c2=0.0,c3=0.0,c2P=0.0,c2PP=0.0,cres=0.0, c7=0.0;
//-----------------------------
const int NEQ    = 8;
```

```
const int LENWRK = 32*NEQ;
const int METHOD = 2;
//---------------------------------------------------------
extern "C" {
  void setup_(const int& NEQ,
              double& TSTART,double* YSTART, double& TEND,
              double& TOL   ,double* THRES ,
              const int& METHOD, const char& TASK,
              bool  & ERRASS,double& HSTART, double* WORK,
              const int& LENWRK,       bool& MESSAGE);
  void ut_   (void f(double& t,double* Y   , double* YP ),
              double& TWANT,   double& TGOT, double* YGOT,
              double* YPGOT,   double* YMAX, double* WORK,
              int& UFLAG);
}
//---------------------------------------------------------
//Function that computes derivatives
void f(double& t,double* Y, double* YP){
  double x1,x2,x3,v1,v2,v3;
  c0 = Y[0]; c3   = Y[3];
  c1 = Y[1]; c2P  = Y[4];
  c2 = Y[2]; c2PP = Y[5]; cres = Y[6]; c7=Y[7];
  //derivatives:
  YP[0] = alpha*dDdt/3000-kappa1*c0*(1.0-c0-c1-c2-c3-c2P-c2PP-cres);                //c0
  YP[1] = kappa1*c0*(1.0-c0-c1-c2-c3-c2P-c2PP-cres)-kappa2*c1*(1.0-c1-c2-c3-c2P-c2PP-cres);  //c1
  YP[2] = kappa2*c1*(1.0-c1-c2-c3-c2PP-c2P-cres)-kP1*c2;                   //c2
  YP[3] = kappa3*c2PP*(1.0-c3-cres-c2PP-c2P)-kDc*c3;                    //c3
  YP[4] = kP1*c2-(kP2+kres)*c2P;                          //c2P
  YP[5] = kP2*c2P-kappa3*c2PP*(1.0-c3-c2PP-cres);                     //c2PP
  YP[6] = kres*c2P;                                       //cres
  YP[7]=(c2P+c2PP+c3)/(0.5+c2P+c2PP+c3)-c7 ;          //foci
}
```

```
int main(){
  string buf;
  double T0,TF,X10,X20,X30,V10,V20,V30;
  double t,dt,tstep;
  int    STEPS, i;
  // rksuite variables:
  double TOL,THRES[NEQ],WORK[LENWRK],HSTART;
  double Y[NEQ],YMAX[NEQ],YP[NEQ],YSTART[NEQ];
  bool   ERRASS, MESSAGE;
  int    UFLAG;
  const char TASK = 'U';
  //Input:
  cout << "# Enter c0, STEPS,T0,TF:\n";
  cin  >> c0 >> STEPS >> T0  >> TF;getline(cin,buf);
  cout << "# c0 = "        << c0    << endl;
  cout << "# No. Steps= " << STEPS << endl;
  cout << "# Time: Initial T0 =" << T0
       << "  Final TF="           << TF  << endl;
  //Initial Conditions:
  dt         = (TF-T0)/STEPS;
  YSTART[0] = c0;
  YSTART[1] = c1;
  YSTART[2] = c2;
  YSTART[3] = c3;
  YSTART[4] = c2P;
  YSTART[5] = c2PP;
  YSTART[6] = cres;
  YSTART[7]=c7;
  //Set control parameters:
  TOL = 5.0e-6;
  for( i = 0; i < NEQ; i++)
    THRES[i] = 1.0e-10;
  MESSAGE = true;
  ERRASS  = false;
  HSTART  = 0.0;
```

```
  //Initialization:
  setup_(NEQ,T0,YSTART,TF,TOL,THRES,METHOD,TASK,
         ERRASS,HSTART,WORK,LENWRK,MESSAGE);
  ofstream  myfile("dna.dat");
  myfile.precision(16);
  myfile << T0<< " "
         << YSTART[0] << " " << YSTART[1] << " "
         << YSTART[2] << " " << YSTART[3] << " "
         << YSTART[4] << " " << YSTART[5] << " "
         <<YSTART[6]<< "  "  << YSTART[7] << "  "
         <<  '\n';
  //The calculation:
  for(i=1;i<=STEPS;i++){
    t = T0 + i*dt;
    ut_(f,t,tstep,Y,YP,YMAX,WORK,UFLAG);
    if(UFLAG > 2) break; //error: break the loop and exit
    myfile << tstep << " "
           << Y[0]  << " " << Y[1] << " "
           << Y[2]  << " " << Y[3] << " "
           << Y[4]  << " " << Y[5] << " "
           << Y[6]  <<"  " <<Y[7]<< "  "
           << '\n';
  }
  myfile.close();
}
```

```
file = "dna.dat"

H1   = 3000


set xlabel "t"


plot file using 1:(H1*$2) with lines title "[C_0]",\
    file u 1:(H1*$3) w l t "[C_1]",\
    file u 1:(H1*$4) w l t "[C_2]",\
    file u 1:(H1*$5) w l t "[C_3]",\
    file u 1:(H1*$6) w l t "[C_2^P]",\
    file u 1:(H1*$7) w l t "[C_2^{PP}]",\
    file u 1:(H1*$8) w l t "[C_{res}]",\
    file u 1:(H1*($2+$3+$4+$5+$8)) w l t "DSB_{remaining}"
```

2.5 Heterochromatin DSB Repair

The below model is reffered to heterochromatin DSB repair and it is an extented form of NHEJ presynaptic process with alternative enzymes and repair complexes added. For this reason, it is reproduced from both biochemical paths;single and competing. The previous section explained the biological evidence on which the model was constructed. The assumptions still the same, thus the rate of DSB induction is linearly related to the dose rate(dD/dt), with the DSB induction-rate per unit dose constant (a).We assume that the total dose is delivered at time zero (no repair during irradiation) and the initial number of DSB is the initial condition of the equations.

Biological Description

The repair process is starting with the NHEJ proteins Ku70/Ku80 finding the crucial damages, the DNA-PKcs phosphorylation. In late S and G2 phases, there might be competition between NHEJ and HR repair pathways or between Ku70 and the MRN complex. Ku is capable of translocating inward even when it binds to different damage configurations . Ku also has a higher affinity for doublestranded than single-stranded DNA. When the Ku70/Ku80 heterodimer binds to the DSB, it changes configuration and translocates inward to provide space for other proteins like DNA-PKcs to bind to the Ku-DNA complex. Proteins involved in NHEJ bind to the Ku-DNA complex with higher affinity than to the DSB ends, i.e., DNA-PKcs can bind independent of Ku to DSB, but the affinity increases ;100-fold when it binds to the Ku- DNA complex.

DNA-PK regulates access to the damage ends by autophosphorylation at threonine and serine sites (ABCDE and PQR clusters) of the DNA-PKcs protein. The ABCDE autophosphorylation is required for efficient ligation by the XRCC4-like factor (XLF)/XRCC4/LIG IV complex. Surprisingly, cells that are deficient in DNA-PKcs autophosphorylation of the ABCDE site are more radiosensitive than cells that lack DNA-PKcs, probably because the nonphosphorylated DNA-PKcs remains bound to the termini rendering them inaccessible to alternative repair pathways. In contrast, inhibition of PQR autophosphorylation renders the ends more accessible for repair by the HR pathway; therefore cells deficient in PQR autophosphorylation are more radioresistant than cells that lack DNA-PKcs.

XRCC4 plays a key role in the recruitment and activation of the end processing enzyme polynucleotide kinase/phosphatase (PNKP) and DNA ligase IV. XLF mediates the activity of XRCC4. According to definition simple DSB are not accompanied by further strand breaks in close proximity (within 10 bp). We assume that simple DSB are easily ligatable by the XLF/XRCC4/LIG IV complex. Repair proteins crystallography images illustrate that proteins such as Ku70/80 heterodimer bind to 2–3 helical turn of the DNA, therefore it is hypothesized that close proximity of strand breaks to the DSB could inhibit proper binding of DSB repair proteins. We define a complex DSB as a DSB in close proximity to another SSB (named DSBþ), or in close proximity to another DSB (named DSBþþ). Artemis is an exo/ endonuclease that plays a key role in dealing with the fraction of DSB that is repaired slowly . Phosphorylation of Artemis by DNA-PKcs is essential for its endonuclease activity. It has been shown that preligation proteins like Artemis (polymerases that function as end processing protein) work

together with core NHEJ repair enzymes and are required for DSB in close proximity of abasic sites. It has also been shown that Artemis deficient cells show the same repair kinetics for fast components of overall repair while the slow component becomes impaired in comparison to nondeficient cells. In the NHEJ model presented here, Artemis is involved in the repair of the complex DSBs, which require further end-processing before ligation. The DNA ligase complex composed of XLF/XRCC4/LIG IV could be sufficient for some end ligation, however some end configurations(complex form of damage) may require additional nucleotide addition by Polymerase l or k before the ligation process can seal the nick. The XLF/XRCC4/LIG IV complex then seals the nick for the complex damages after Artemis end-processing. For the difficult ligatable ends, the gaps are first filled by the synthesis activity of Polymerase l or k with C8 repair rate and then ligated with by XLF/XRCC4/LIG IV.

Modeling Approach

We use the mass-action kinetics approach to describe the binding of repair enzymes to DSBs with several intermediate repair complexes leading to DNA rejoining: The rate of induction of the first complex (dC1/dt) increases linearly with the rate of the DSB induction and decreases with the Ku70/Ku80 repair rate at the site of damage.[**14**]The rate of the second complex (C2) increases with the Ku70/Ku80 repair activity and decreases with the DNA-PKcs repair activity. In our model, V3 and V4 represent the rates of DNA-PKcs autophosphorylation of the two sites(ABCDE,PQR).The Artemis complex (C5) increases with V4 and decreases with Polymerase
$lambda - \mu$ repair activity.Then the rate of ligation and filling the gap is shown by V5 and V6 for the simple ligatable damages in the model, finaly the ligase activity increases with K7 rate constant.

$$[C1] \rightarrow Ku70/Ku80$$

$$[C2] \rightarrow [DNA - PKcs]$$

$$[C3] \rightarrow [DNA - PK]$$

$$[C4] \rightarrow ABCDE\ sites$$

$$[C5] \rightarrow PQR\ sites$$

$$[C9] \rightarrow [Artemis]$$

$$[C10] \rightarrow [Polymerase\lambda - \mu]$$

$$[C11] \rightarrow [XLF]/[XRCC4]/[lLIGIV]$$

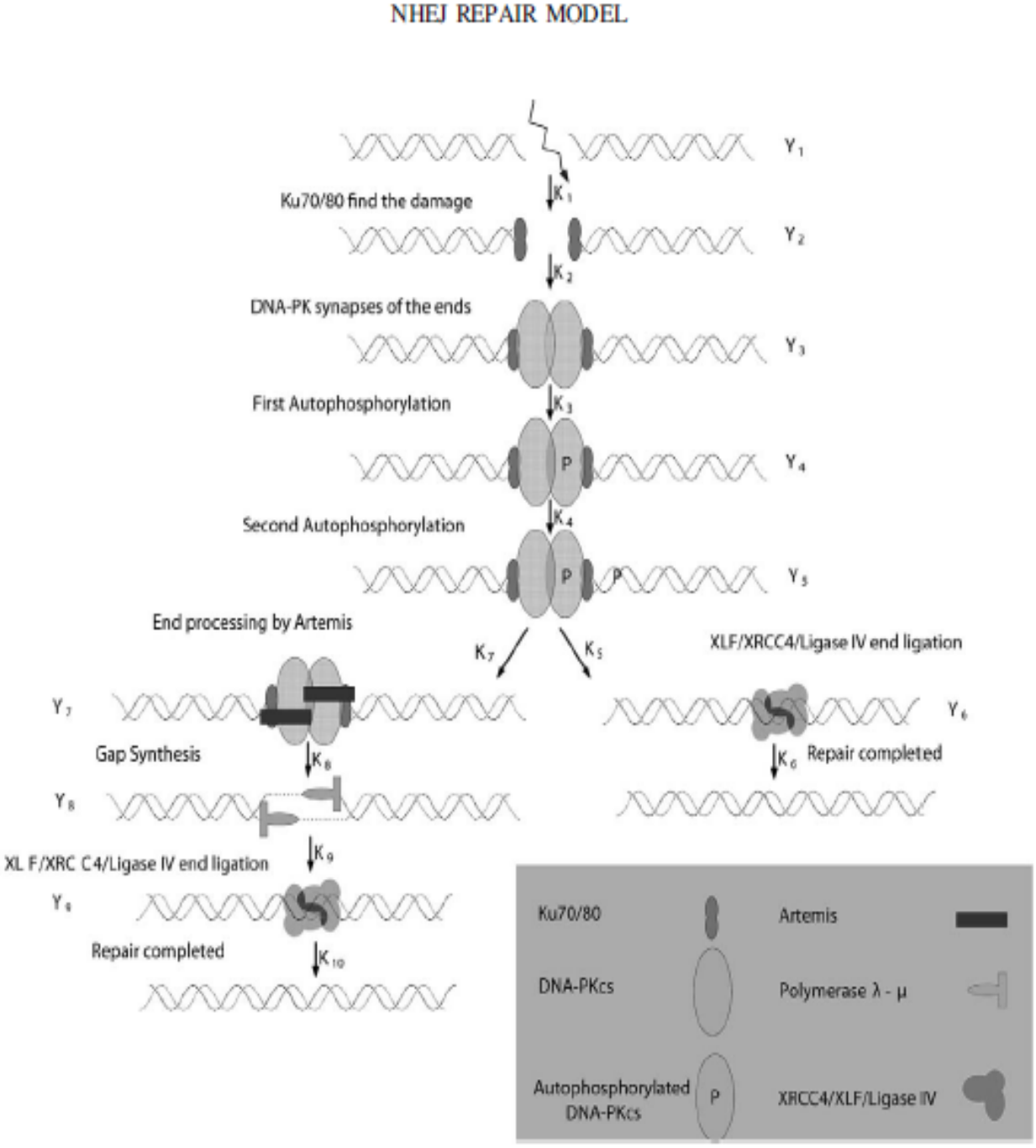

Figure 2.4: *The NHEJ repair between simple damages and damages induced in Heterochromatin. This process happens in early S, G2 phase. The proteins-enzymes taking part are the above:Ku70/80,DNA-PKcs, Autophosphorylated DNA-PKcs, Artemis, Polymerase* $\lambda - \mu$ *, XRCC4/XLF/Ligase IV. The image adopted by [***14***]*

$$\frac{dC_1}{dt} = a\frac{dD}{dt} - V_1 \qquad V_1 = K_1[Ku70/80][C_1] \tag{2.73}$$
$$\frac{dC_2}{dt} = V_1 - V_2 \qquad V_2 = K_2[DNAPKcs][C_2] \tag{2.74}$$
$$\frac{dC_3}{dt} = V_2 - V_3 \qquad V_3 = K_3[C_3] \tag{2.75}$$
$$\frac{dC_4}{dt} = V_3 - V_4 \qquad V_4 = K_4[C_4] \tag{2.76}$$
$$\frac{dC_5}{dt} = V_4 - V_5 - V_7 \qquad V_5 = K_5[XRCC1, LigIII][C_5], \quad V_7 = K_7[Artemis][C_5] \tag{2.77}$$
$$\frac{dC_6}{dt} = V_5 - V_6 \qquad V_6 = K_6[C_6] \tag{2.78}$$
$$\frac{dC_7}{dt} = V_7 - V_8 \qquad V_8 = K_8[Polymerase\lambda - \mu][C_7] \tag{2.79}$$
$$\frac{dC_8}{dt} = V_8 - V_9 \qquad V_9 = K_9[LigI][C_8] \tag{2.80}$$
$$\frac{dC_9}{dt} = V_9 - V_{10} \qquad V_{10} = K_{10}[C_9] \tag{2.81}$$
$$\tag{2.82}$$

We substitute each concentration as well as we scalling the variables.

$$[C1] \rightarrow [C0]$$

$$[C2] \rightarrow [C1]$$

$$[C3] \rightarrow [C2]$$

$$[C4] \rightarrow [C3]$$

$$[C5] \rightarrow [C4]$$

$$[C9] \rightarrow [C5]$$

$$[C10] \rightarrow [C6]$$

$$[C11] \rightarrow [C7]$$

Scalling Variables

$$H_i = [E_i] + \sum_{n=1}^{n}[C_j] = constant$$

$$h_i = \frac{\sum_{n=1}^{n}[C_i]}{H_i}$$

$$c_i(t) = \frac{[C_i]}{H_i}$$

$$K_i = \frac{k_i}{H_i}$$

Substituting the above equations in mass kinetics of the system we are leading to parametrical equations, with dependent variable t . Also, we substitute the enzymes as we have

said in the asumptions with the scalling variables avoiding the specific evaluation of concentrations. More specific,

$$[Ku70/Ku80] = \frac{E_1}{H_i} = 1 - h_1(t)$$

$$[DNAPKcs] = \frac{E_2}{H_i} = 1 - h_2(t)$$

$$[XLF]/[LIGIV]/[XRCC4] = \frac{E_4}{H_i} = 1 - h_4(t)$$

$$[Artemis] = \frac{E_6}{H_i} = 1 - h_6(t)$$

$$[Polymerase\lambda - \mu] = \frac{E_7}{H_i} = 1 - h_7(t)$$

$$[XLF]/[LIGIV]/[XRCC4] = \frac{E_8}{H_i} = 1 - h_8(t)$$

$$\frac{dc_0}{dt} = a\frac{dD}{Hidt} - k_1 c_0(t)[1 - h1(t)] \tag{2.83}$$
$$\frac{dc_1}{dt} = k_1 c_0(t)[1 - h_1(t)] - k_2 c_1(t)[1 - h_1(t)] \tag{2.84}$$
$$\frac{dc_2}{dt} = k_2 c_1(t)[1 - h_2(t)] - K_3 c_2(t) \tag{2.85}$$
$$\frac{dc_3}{dt} = K_3 c_2(t) - K_4 c_3(t) \tag{2.86}$$
$$\frac{dc_4}{dt} = K_4 c_3(t) - k_5[1 - h_4(t)]c_4(t) - k_7[1 - h_6(t)]c_4(t) \tag{2.87}$$
$$\frac{dc_5}{dt} = k_5[1 - h_4(t)]c_4(t) - K_6 c_5(t) \tag{2.88}$$
$$\frac{dc_6}{dt} = k_7[1 - h_6(t)]c_4(t)[1 - h_7(t)] - k_8[1 - h_7(t)]c_6(t) \tag{2.89}$$
$$\frac{dc_7}{dt} = k_8[1 - h_7(t)]c_6(t) - k_9[1 - h_8(t)]c_7(t) \tag{2.90}$$
$$\frac{dc_8}{dt} = k_9[1 - h_8(t)]c_7(t) - k_{10} c_8(t) \tag{2.91}$$
$$\tag{2.92}$$

Then we substitute the scaling variables and we have the below equations that we will use to computational calculations

$$\frac{dc_0}{dt} = \frac{adD}{H_1 dt} - k_1(1 - c_0 - c_1 - c_2 - c_3 - c_4 - c_5 - c_6 - c_7 - c_8)c_0 \quad (2.93)$$

$$\frac{dc_1}{dt} = k_1(1 - c_0 - c_1 - c_2 - c_3 - ..... - c_8)c_0 - k_2(1 - c_1 - c_2 - c_3 - ..... - c_8)c_1 \quad (2.94)$$

$$\frac{dc_2}{dt} = k_2 c_1(1 - c_1 - c_2 - c_3 - c_4 - c_5 - c_6 - c_7 - c_8)c_1 - K_3 c_2 \quad (2.95)$$

$$\frac{dc_3}{dt} = K_3 c_2 - K_4 c_4 \quad (2.96)$$

$$\frac{dc_4}{dt} = K_4 c_3 - k_5(1 - c_4 - c_5 - c_6 - c_7 - c_8)c_4 - k_7(1 - c_6 - c_7 - c_8)c_4 \quad (2.97)$$

$$\frac{dc_5}{dt} = k_5(1 - c_4 - c_5 - c_6 - c_7 - c_8)c_4 - K_6 c_5 \quad (2.98)$$

$$\frac{dc_6}{dt} = k_7(1 - c_6 - c_7 - c_8)c_4 - k_8(1 - c_7 - c_8)c_6 \quad (2.99)$$

$$\frac{dc_7}{dt} = k_8(1 - c_7 - c_8)c_6 - k_9(1 - c_8)c_7 \quad (2.100)$$

$$\frac{dc_8}{dt} = k_9(1 - c_8)c_7 - K_{10} c_8 \quad (2.101)$$

(2.102)

rate constants	Complex Paths
a	25
k1	350
k2	500
k3	50
k4	20
k5	15
k6	5
k7	3.6
k8	8
k9	0.25
k10	0.55

Table 2.3: *The coeficient table consists of two columns, the first column indicates the* $constant_i$ *, the second column indicates the rate constants(number) [**14**]*

```
//=========================================================
//Program to solve an  ODE system using Runge-Kutta Method
//=========================================================
// Compile with the commands:
// gfortran -O2 -c rksuite/rksuite.f;
// g++ -O2 dna.cpp rksuite.o -o dna -lgfortran
#include <iostream>
#include <fstream>
#include <cstdlib>
#include <string>
#include <cmath>
using namespace std;
//---------------------------------------------------------
// Parameteral  conditions:
const double H1      =  3000.0;
const double dDdt    =    80.00;
const double kappa1 =   350.0;
const double kappa2 =   500.0;
const double kappa3 =  50.0;
const double kappa4 =   20.0;
const double kappa5 =   15.0;
const double kappa6 =   5.0;
const double kappa7 =   3.6;
const double kappa8 =    8.0 ;
const double kappa9 =   0.25;
const double kappa10 =  0.55;
const double alpha =    25.0;
const double adDdt  = alpha*dDdt/H1; //first term in c0 equation
//-----------------------------
double c0=0.93; //alpha*dDdt/H1;
double c1=0.0,c2=0.0,c3=0.0,c4=0.0,c5=0.0,c6=0.0, c7=0.0, c8=0.0;
//-----------------------------
const int NEQ    = 9 ;
const int LENWRK = 32*NEQ;
const int METHOD = 2;
//---------------------------------------------------------
```

```
//---------------------------------------------------
extern "C" {
  void setup_(const int& NEQ,
              double& TSTART,double* YSTART, double& TEND,
              double& TOL   ,double* THRES ,
              const int& METHOD, const char& TASK,
              bool  & ERRASS,double& HSTART, double* WORK,
              const int& LENWRK,       bool& MESSAGE);
  void ut_   (void f(double& t,double* Y   , double* YP ),
              double& TWANT,   double& TGOT, double* YGOT,
              double* YPGOT,   double* YMAX, double* WORK,
              int& UFLAG);
}
//---------------------------------------------------
//Function that computes derivatives
void f(double& t,double* Y, double* YP){
  double x1,x2,x3,v1,v2,v3;
  c0 = Y[0]; c3   = Y[3];
  c1 = Y[1]; c4  = Y[4];
  c2 = Y[2]; c5= Y[5]; c6 = Y[6]; c8=Y[8];   c7=Y[7];
  //derivatives:
  YP[0] = (alpha*dDdt)/H1-kappa1*c0*(1.0-c0-c1-c2-c3-c4-c5-c6-c7-c8);                //c0
  YP[1] = kappa1*c0*(1.0-c0-c1-c2-c3-c4-c5-c6-c7-c8)-kappa2*c1*(1.0-c1-c2-c3-c4-c5-c6-c7-c8);  //c1
  YP[2] = kappa2*c1*(1.0-c1-c2-c3-c4-c5-c6-c7-c8)-kappa3*c2;    //c2
  YP[3] = kappa3*c2-kappa4*c3;          //c3
  YP[4] = kappa4*c3-kappa5*(1.0-c4-c5-c6-c7-c8)*c4-kappa7*c4*(1.0-c6-c7-c8);   //c4
  YP[5] = kappa5*c4*(1.0-c4-c5-c6-c7-c8)-kappa6*c5;                  //c5
  YP[6] = kappa7*c4*(1.0-c6-c7-c8)-kappa8*(1.0-c7-c8)*c6;          //c6
  YP[7] =   kappa8*c6*(1.0-c7-c8)-kappa9*(1-c8)*c7;     //c7
  YP[8] = kappa9*c7*(1-c8)-kappa10*c8 ; //c8


}
```

```
int main(){
  string buf;
  double T0,TF,X10,X20,X30,V10,V20,V30;
  double t,dt,tstep;
  int    STEPS, i;
  // rksuite variables:
  double TOL,THRES[NEQ],WORK[LENWRK],HSTART;
  double Y[NEQ],YMAX[NEQ],YP[NEQ],YSTART[NEQ];
  bool   ERRASS, MESSAGE;
  int    UFLAG;
  const char TASK = 'U';
  //Input:
  cout << "# Enter c0, STEPS,T0,TF:\n";
  cin  >> c0 >> STEPS >> T0  >> TF;getline(cin,buf);
  cout << "# c0 = "       << c0    << endl;
  cout << "# No. Steps= " << STEPS << endl;
  cout << "# Time: Initial T0 =" << T0
       << "  Final TF="          << TF  << endl;
  cout << "# kappa1= " << kappa1 << " kappa2=  " << kappa2  << endl;
  cout << "# kappa3= " << kappa3 << " kappa4=  " << kappa4  << endl;
  cout << "# kappa5= " << kappa5 << " kappa6=  " << kappa6  << endl;
  cout << "# kappa7= " << kappa7 << " kappa8=  " << kappa8  << endl;
  cout << "# kappa9= " << kappa9 << " kappa10= " << kappa10 << endl;
  //Initial Conditions:
  dt        = (TF-T0)/STEPS;
  YSTART[0] = c0;
  YSTART[1] = c1;
  YSTART[2] = c2;
  YSTART[3] = c3;
  YSTART[4] = c4;
  YSTART[5] = c5;
  YSTART[6] = c6;
  YSTART[7] = c7;
  YSTART[8] = c8;


     //Set control parameters:
     TOL = 5.0e-6;
     for( i = 0; i < NEQ; i++)
       THRES[i] = 1.0e-10;
     MESSAGE = true;
     ERRASS  = false;
     HSTART  = 0.0;
     //Initialization:
     setup_(NEQ,T0,YSTART,TF,TOL,THRES,METHOD,TASK,
            ERRASS,HSTART,WORK,LENWRK,MESSAGE);
     ofstream  myfile("dna1.dat");
     myfile.precision(16);
     myfile << T0<< " "
            << YSTART[0] << " " << YSTART[1] << " "
            << YSTART[2] << " " << YSTART[3] << " "
            << YSTART[4] << " " << YSTART[5] << " "
            << YSTART[6] << " " << YSTART[7] << " "
            << YSTART[8] << " " <<'\n';
     //The calculation:
     for(i=1;i<=STEPS;i++){
       t = T0 + i*dt;
       ut_(f,t,tstep,Y,YP,YMAX,WORK,UFLAG);
       if(UFLAG > 2) break; //error: break the loop and exit
       myfile << tstep << " "
              << Y[0]  << " " << Y[1] << " "
              << Y[2]  << " " << Y[3] << " "
              << Y[4]  << " " << Y[5] << " "
              << Y[6]  << " "<<  Y[7] << " "
              <<Y[8]   << " "<< '\n';
     }
     myfile.close();
   }
```

2.6 MMEJ

The below model is an extented form of presynaptic process of NHEJ model with more enzymes and repair complexes added. For this reason, it is reproduced from both biochemical paths;single and competing. The previous section explained the biological evidence on which the model was constructed. The assumptions still the same, thus the rate of DSB induction is linearly related to the dose rate(dD/dt), with the DSB induction-rate per unit dose constant (a).We assume that the total dose is delivered at time zero (no repair during irradiation) and the initial number of DSB is the initial condition of the equations.

Biological Description

As MMEJ is masked by NHEJ, the proteins involved in DSB repair and their molecular mechanisms are therefore not fully known yet. The repair process is starting with the NHEJ proteins Ku70/Ku80 finding the crucial damages, the DNA-PKcs phosphorylation. In late S and G2 phases, there might be competition between NHEJ and HR repair pathways or between Ku70 and the MRN complex. Ku is capable of translocating inward even when it binds to different damage configurations . Ku also has a higher affinity for doublestranded than single-stranded DNA. When the Ku70/Ku80 heterodimer binds to the DSB, it changes configuration and translocates inward to provide space for other proteins like DNA-PKcs to bind to the Ku-DNA complex.

$$[C1] \rightarrow Ku70/Ku80$$

$$[C2] \rightarrow [DNA - PKcs]$$

$$[C3] \rightarrow [DNA - PK]$$

$$[C4] \rightarrow ABCDE\ sites$$

$$[C5] \rightarrow PQR\ sites$$

$$[C13] \rightarrow [MRN]$$

$$[C14] \rightarrow [PARP - 1]$$

$$[C15] \rightarrow [FEN1]$$

$$[C16] \rightarrow [Polymerase\beta]$$

$$[C17] \rightarrow [XRCC11]$$

$$\frac{dC_1}{dt} = a\frac{dD}{dt} - V_1 \qquad V_1 = K_1[Ku70/80][C_1] \tag{2.103}$$
$$\frac{dC_2}{dt} = V_1 - V_2 \qquad V_2 = K_2[DNAPKcs][C_2] \tag{2.104}$$
$$\frac{dC_3}{dt} = V_2 - V_3 \qquad V_3 = K_3[C_3] \tag{2.105}$$
$$\frac{dC_4}{dt} = V_3 - V_4 \qquad V_4 = K_4[C_4] \tag{2.106}$$
$$\frac{dC_5}{dt} = V_4 - V_{12} \qquad V_{12} = K_5[XRCC1, LigIII][C_5] \tag{2.107}$$
$$\frac{dC_{13}}{dt} = V_{12} - V_{13} \qquad V_{13} = K_6[C_6] \tag{2.108}$$
$$\frac{dC_{14}}{dt} = V_{13} - V_{14} \qquad V_{14} = K_8[Polymerase\lambda - \mu][C_7] \tag{2.109}$$
$$\frac{dC_{15}}{dt} = V_{14} - V_{15} \qquad V_{15} = K_9[LigI][C_8] \tag{2.110}$$
$$\frac{dC_{16}}{dt} = V_{15} - V_{16} \qquad V_{16} = K_{10}[C_9] \tag{2.111}$$
$$\frac{dC_{17}}{dt} = V_{16} - V_{17} \qquad V_{17} = K_{17}[C_{17}] \tag{2.112}$$
$$\tag{2.113}$$

We substitute each concentration as well as we scalling the variables.

$$[C1] \rightarrow [C0]$$

$$[C2] \rightarrow [C1]$$

$$[C3] \rightarrow [C2]$$

$$[C4] \rightarrow [C3]$$

$$[C5] \rightarrow [C4]$$

$$[C13] \rightarrow [C5]$$

$$[C14] \rightarrow [C6]$$

$$[C15] \rightarrow [C7]$$

$$[C16] \rightarrow [C8]$$

$$[C17] \rightarrow [C9]$$

Scalling Variables

$$H_i = [E_i] + \sum_{n=1}^{n} [C_j] = constant$$

$$h_i = \frac{\sum_{n=1}^{n} [C_i]}{H_i}$$

$$c_i(t) = \frac{[C_i]}{H_i}$$

$$K_i = \frac{k_i}{H_i}$$

$$[Ku70/Ku80] = \frac{E_1}{H_i} = 1 - h_1(t)$$

$$[DNAPKcs] = \frac{E_2}{H_i} = 1 - h_2(t)$$

$$[PARP - 1] = \frac{E_5}{H_i} = 1 - h_5(t)$$

$$[FEN1] = \frac{E_6}{H_i} = 1 - h_6(t)$$

$$[Polymerase\beta] = \frac{E_7}{H_i} = 1 - h_7(t)$$

$$[XRXCC1] = \frac{E_8}{H_i} = 1 - h_8(t)$$

Substituting the above equations in mass kinetics of the system we are leading to parametrical equations, with dependent variable t . Also, we substitute the enzymes as we have said in the asumptions with the scalling variables avoiding the specific evaluation of concentrations. More specific,

$$\frac{dc_0}{dt} = a\frac{dD}{Hidt} - k_1 c_0(t)[1 - h1(t)] \tag{2.114}$$
$$\frac{dc_1}{dt} = k_1 c_0(t)[1 - h_1(t)] - k_2 c_1(t)[1 - h_2(t)] \tag{2.115}$$
$$\frac{dc_2}{dt} = k_2 c_1(t)[1 - h_2(t)] - K_3 c_2(t) \tag{2.116}$$
$$\frac{dc_3}{dt} = K_3 c_2(t) - K_4 c_3(t) \tag{2.117}$$
$$\frac{dc_4}{dt} = K_4 c_3(t) - k_{12}[1 - h_5(t)]c_4(t) \tag{2.118}$$
$$\frac{dc_5}{dt} = k_{12}[1 - h_5(t)]c_4(t) - k_{13}[1 - h_5(t)]c_5(t) \tag{2.119}$$
$$\frac{dc_6}{dt} = k_{13}[1 - h_5(t)]c_5(t)[1 - h_7(t)] - k_{14}[1 - h_6(t)]c_6(t) \tag{2.120}$$
$$\frac{dc_7}{dt} = k_{14}[1 - h_6(t)]c_6(t) - k_{15}[1 - h_7(t)]c_7(t) \tag{2.121}$$
$$\frac{dc_8}{dt} = k_{15}[1 - h_7(t)]c_7(t) - k_{16}[1 - h_8(t)]c_8(t) \tag{2.122}$$
$$\frac{dc_9}{dt} = k_{16}[1 - h_8(t)]c_8(t) - K_{17}c_9(t) \tag{2.123}$$
$$\tag{2.124}$$

Then we substitute the scaling variables and we have the below equations that we will use to computational calculations

$$\frac{dc_0}{dt} = \frac{adD}{H_1 dt} - k_1(1 - c_0 - c_1 - c_2 - c_3 - c_4 - c_5 - c_6 - c_7 - c_8)c_0 \quad (2.125)$$

$$\frac{dc_1}{dt} = k_1(1 - c_0 - c_1 - c_2 - c_3 - ..... - c_8)c_0 - k_2(1 - c_1 - c_2 - c_3 - ..... - c_8)c_1 \quad (2.126)$$

$$\frac{dc_2}{dt} = k_2 c_1(1 - c_1 - c_2 - c_3 - c_4 - c_5 - c_6 - c_7 - c_8)c_1 - K_3 c_2 \quad (2.127)$$

$$\frac{dc_3}{dt} = K_3 c_2 - K_4 c_3 \quad (2.128)$$

$$\frac{dc_4}{dt} = K_4 c_3 - k_{12}(1 - c_5 - c_6 - c_7 - c_8 - c9)c_4 \quad (2.129)$$

$$\frac{dc_5}{dt} = k_{12}(1 - c_5 - c_6 - c_7 - c_8 - c_9)c_4 - k_{13}(1 - c_5 - c6 - c7 - c8 - c9)c5 \quad (2.130)$$

$$\frac{dc_6}{dt} = k_{13}(1 - c5 - c_6 - c_7 - c_8 - c9)c_5 - k_{14}(1 - c_6 - c_7 - c_8 - c_9)c_6 \quad (2.131)$$

$$\frac{dc_7}{dt} = k_{14}(1 - c_6 - c_7 - c_8 - c_9)c_6 - k_{15}(1 - c7 - c_8 - c_9)c_7 \quad (2.132)$$

$$\frac{dc_8}{dt} = k_{15}(1 - c7 - c_8 - c_9)c_7 - k_{16}(1 - c_8 - c_9)c_8 \quad (2.133)$$

$$\frac{dc_9}{dt} = k_{16}(1 - c_8 - c_9)c_8 - K_{17}c_9 \quad (2.134)$$

(2.135)

rate constants	Complex Paths
a	25
k1	350
k2	500
K3	50
K4	20
k12	3
k13	1
K14	0.7
k15	0.75
k16	0.5
k17	0.15

Table 2.4: *The coeficient table consists of two columns, the first column indicates the* $constant_i$ *, the second column indicates the rate constants(number) [8]*

```
//=========================================================
//Program to solve an  ODE system using Runge-Kutta Method
//=========================================================
// Compile with the commands:
// gfortran -O2 -c rksuite/rksuite.f;
// g++ -O2 dna.cpp rksuite.o -o dna -lgfortran
#include <iostream>
#include <fstream>
#include <cstdlib>
#include <string>
#include <cmath>
using namespace std;
//---------------------------------------------------------
// Parameteral  conditions:
const double H1      =  3000.0;
const double dDdt    =     1.0;
const double kappa1 =   350.0;
const double kappa2 =   500.0;
const double kappa3 =    50.0;
const double k4      =    20.0;
const double k12     =    3.0 ;
const double k13     =    1.0 ;
const double k14     =    0.7 ;
const double k15     =    0.75;
const double k16     =    0.5 ;
const double k17     =    0.15;
const double alpha   =    40;
const double adDdt   = alpha*dDdt/H1; //first term in c0 equation
//-----------------------------
double c0            =0.08; //alpha*dDdt/H1;
double c1=0.0,c2=0.0,c3=0.0,c4=0.0,c5=0.0,c6=0.0,c7=0.0,c8=0.0,c9=0.0;
//-----------------------------
const int NEQ    = 10;
const int LENWRK = 32*NEQ;
const int METHOD = 2;
//---------------------------------------------------------
```

```
extern "C" {
  void setup_(const int& NEQ,
              double& TSTART,double* YSTART, double& TEND,
              double& TOL   ,double* THRES ,
              const int& METHOD, const char& TASK,
              bool  & ERRASS,double& HSTART, double* WORK,
              const int& LENWRK,       bool& MESSAGE);
  void ut_   (void f(double& t,double* Y   , double* YP ),
              double& TWANT,   double& TGOT, double* YGOT,
              double* YPGOT,   double* YMAX, double* WORK,
              int& UFLAG);
}
//---------------------------------------------------------
//Function that computes derivatives
void f(double& t,double* Y, double* YP){
  double x1,x2,x3,v1,v2,v3;
  c0 = Y[0]; c3  = Y[3];  c6=Y[6];   c9=Y[9];
  c1 =Y[1] ; c4  = Y[4];  c7=Y[7];
  c2 = Y[2]; c5  = Y[5];  c8=Y[8];
  //derivatives:
  YP[0] = adDdt-kappa1*c0*(1.0-c1-c2-c3-c4-c5-c6-c7-c8-c9);                 //c0
  YP[1] = kappa1*c0*(1.0-c1-c2-c3-c4-c5-c6-c7-c8-c9)-kappa2*c1*(1.0-c2-c3-c4-c5-c6-c7-c8-c9);//c1
  YP[2] = kappa2*c1*(1.0-c2-c3-c4-c5-c6-c7-c8-c9)-kappa3*c2;                   //c2
  YP[3] = kappa3*c2*(1.0-c3-c4-c5-c6-c7-c8-c9)-k4*c3;                     //c3
  YP[4] = k4*c3-k12*(1-c5-c6-c7-c8-c9)*c4;                          //c4
  YP[5] = k12*(1-c5-c6-c7-c8-c9)*c4-k13*c5*(1.0-c5-c6-c7-c8-c9);            //c5
  YP[6] = k13*c5*(1.0-c5-c6-c7-c8-c9)-k14*c6*(1.0-c6-c7-c8-c9)  ;         //c6
  YP[7] =   k14*c6*(1.0-c6-c7-c8-c9)-k15*c7*(1.0-c7-c8-c9) ;              //c7
  YP[8]=  k15*c7*(1.0-c7-c8-c9)-k16*c8*(1.0-c8-c9)    ;                  //c8
  YP[9]= k16*c8*(1.0-c8-c9)-k17*c9    ;                                //c9

}
//--------------------------------
int main(){
  string buf;
```

```
double T0,TF,X10,X20,X30,V10,V20,V30;
double t,dt,tstep;
int    STEPS, i;
// rksuite variables:
double TOL,THRES[NEQ],WORK[LENWRK],HSTART;
double Y[NEQ],YMAX[NEQ],YP[NEQ],YSTART[NEQ];
bool   ERRASS, MESSAGE;
int    UFLAG;
const char TASK = 'U';
//Input:
cout << "# Enter c0, STEPS,T0,TF:\n";
cin  >> c0 >> STEPS >> T0  >> TF;getline(cin,buf);
cout << "# c0 = "        << c0    << endl;
cout << "# No. Steps= " << STEPS << endl;
cout << "# Time: Initial T0 =" << T0
     << "  Final TF="           << TF  << endl;
//Initial Conditions:
dt         = (TF-T0)/STEPS;
YSTART[0] = c0;
YSTART[1] = c1;
YSTART[2] = c2;
YSTART[3] = c3;
YSTART[4] = c4;
YSTART[5] = c5;
YSTART[6] = c6;
YSTART[7] = c7;
YSTART[8] = c8;
YSTART[9] = c9;
//Set control parameters:
TOL = 5.0e-6;
for( i = 0; i < NEQ; i++)
  THRES[i] = 1.0e-10;
MESSAGE = true;
ERRASS  = false;
HSTART  = 0.0;
//Initialization:
```

```
  setup_(NEQ,T0,YSTART,TF,TOL,THRES,METHOD,TASK,
         ERRASS,HSTART,WORK,LENWRK,MESSAGE);
  ofstream  myfile("dna3.dat");
  myfile.precision(16);
  myfile << T0<< " "
         << YSTART[0] << " " << YSTART[1] << " "
         << YSTART[2] << " " << YSTART[3] << " "
         << YSTART[4] << " " << YSTART[5] << " "
         << YSTART[6] << " " << YSTART[7] << " "
         << YSTART[8] << " " << YSTART[9] <<" "
         << '\n';
  //The calculation:
  for(i=1;i<=STEPS;i++){
    t = T0 + i*dt;
    ut_(f,t,tstep,Y,YP,YMAX,WORK,UFLAG);
    if(UFLAG > 2) break; //error: break the loop and exit
    myfile << tstep << " "
           << Y[0]  << " " << Y[1] << " "
           << Y[2]  << " " << Y[3] << " "
           << Y[4]  << " " << Y[5] << " "
           << Y[6]  << " " << Y[7] << " "
           <<Y[8]   << " " << Y[9] << " "
           <<'\n';
  }
  myfile.close();
}
```

Chapter 3

Results

3.1 Results of First Model

The problem of DNA Repair has been executed with the help of computational language $C++$. Must be added that the method used was Runge-Kuta. As it refferred in our reference's papers as well as we notice in the compiling system, the form of equations are slightly stiff, nonetheless they are in a way that it does not bother us. If the system was actually stiff that means that exists a range of numbers in the specific system of equations that they have very sensitive solutions, and system tends to unstability.First of all for the computational part we set up the MATE desktop on Cygwin and we use the graph tool of Gnuplot. As we said before, we want to reproduce the Non-homologous end joining pathway, the primary pathway of DSB's damages; for this reason, we reproduce the model of Cuccinota's paper. In each model reproduced graph of proteins-enzymes capacities, DSB rejoining and fraction unrejoined, as well as comparisons between computational and experimerimental results .The outcomes we received are a little bit different from the paper's results[**4**], such as the number of the initial damages. Nevertheless, the model rescalled in the same initial numbers of damages, so could be compared with ours.

Another significant graph is the Fraction Unrejoined that describes the percentage of damages have not repaired yet. In other words this is the propability of unrepaired damages through the time or else a frequency that counts the unrepaired damages in every step. Analyticaly, the unit of total damages is consisting of two subunits, the repaired one and the unrepaired:

$$N_{tot} = N_{rep} + N_{unrep}$$

So the probability of non repaired damages is:

$$P_{unrep} = \frac{N_{unrep}}{N_{tot}} \Leftrightarrow$$
$$P_{unrep} = \frac{N_{tot} - N_{rep}}{N_{tot}} \approx fraction\ unrejoined$$

Another measurement for the unrepaired damages give us the DSB's Unrejoing, instead of this measurement counts the percentage unrepaired damages $(\approx fraction\ unrejoined * 100\%)$

As we can observe from the graph of the enzyme's capacities contributing in NHEJ repair, the Ku70/80 is the only enzyme which do not leaves any residue in the repair process. To the contrary, finishes the proteins recruitment in the 36 initial seconds. The other enzymes are leaving breaks residues. The Ligase IV leaves the bigger amount of residues and unified with the DSB remaining approximately in 3 hours, so the residues of Ligase IV are the residuals of whole DNA repair process.The unrepaired strand breaks reach the percentage of $4,8\%$. The code, the constants and the process of Model1 have been discussed before (Chapter1.3) for the below outcomes.

The peak quantities of each enzyme process of our model(Fiqure 2.1) gathered and compared with the paper's model [**4**]. The realized outcomes confirm adequately our numerical results(Fiqure 2.2). Especially, in the begining, the two different graphs overlapp each other. The deviation is approximately two units.

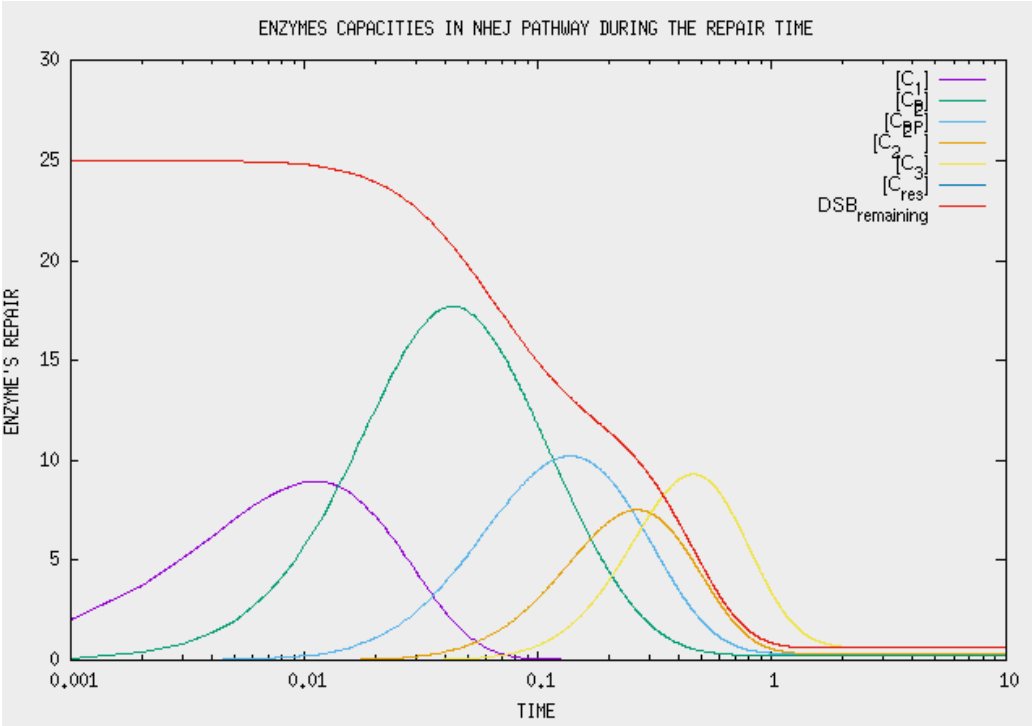

Figure 3.1: *The graph illustarates the quantities of 6 repair enzyme-proteins that are used in NHEJ pathway, and the Remaining Double Strand Breaks in each step of Repair, D=1Gy, a=25. Denoted C1 as DNA-PKcs, C2 as DNA-PK, C2p as ABCDE cites, C2pp as PQR cites, C3 as LigaseIV*

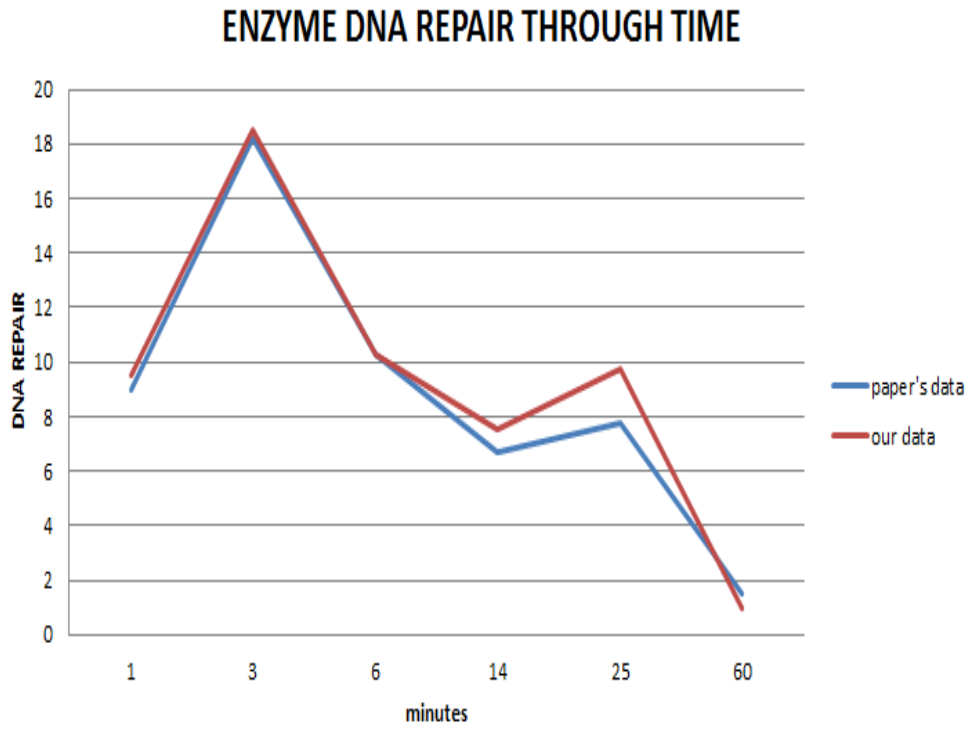

Figure 3.2: *The graph compares the results of simple NHEJ pathway between our mathematical model and paper's mathematical model[**4**]. For the graph it is utilized the peak of each enzyme's capacity.*

time(minutes)	Paper's Results	Our Results
1	9	9.5
3	18.2	18.5
6	10.3	10.3
14	6.7	7.5
25	7.8	9.75
60	1,5	1

Table 3.1: The coeficient table has three columns, the first column indicates the time, second column indicates the paper's results[**4**] and the third column indicates our outcomes coming out Runge-Kuta method.

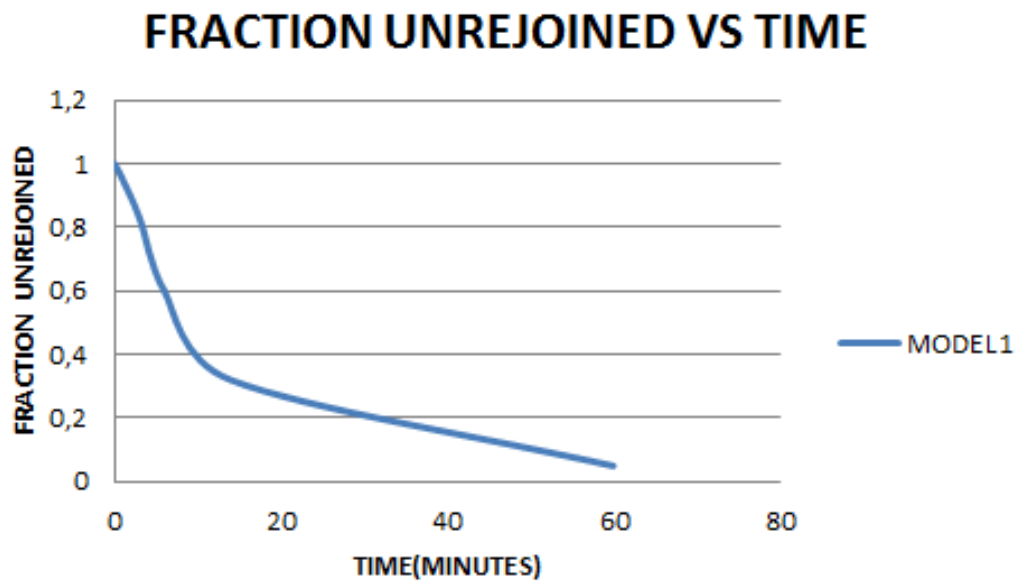

Figure 3.3: *Fraction Unrejoined in simple path of Non-homologous End Joining (NHEJ), and the Remaining Double Strand Breaks in each step of Repair process. The model1 describes our computational simulation of NHEJ simple path,our data indicated below*

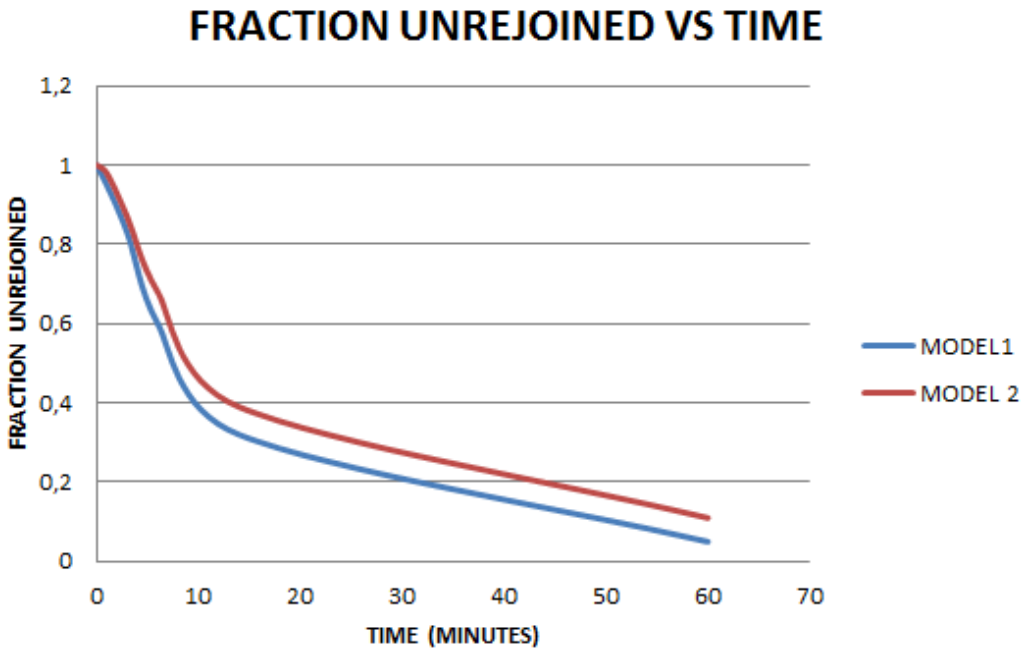

Figure 3.4: *Fraction Unrejoined in simple path of Non-homologous End Joining (NHEJ) , and the Remaining Double Strand Breaks in each step of Repair process. The model1 describes our simulation of simple path of NHEJ and the model2 the model of paper's results [**4**]*

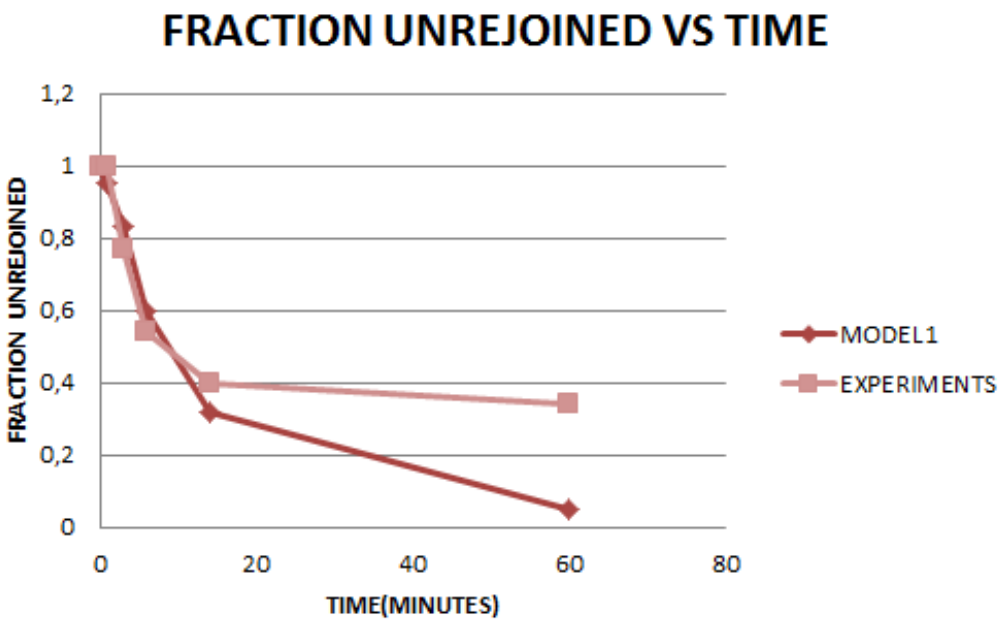

Figure 3.5: *Fraction Unrejoined in simple path of Non-homologous End Joining (NHEJ) pathway, and the Remaining Double Strand Breaks in each step of Repair process. The model1 describes our simulation of simple path of NHEJ and experiments the experimental data of model2 [**21**]*

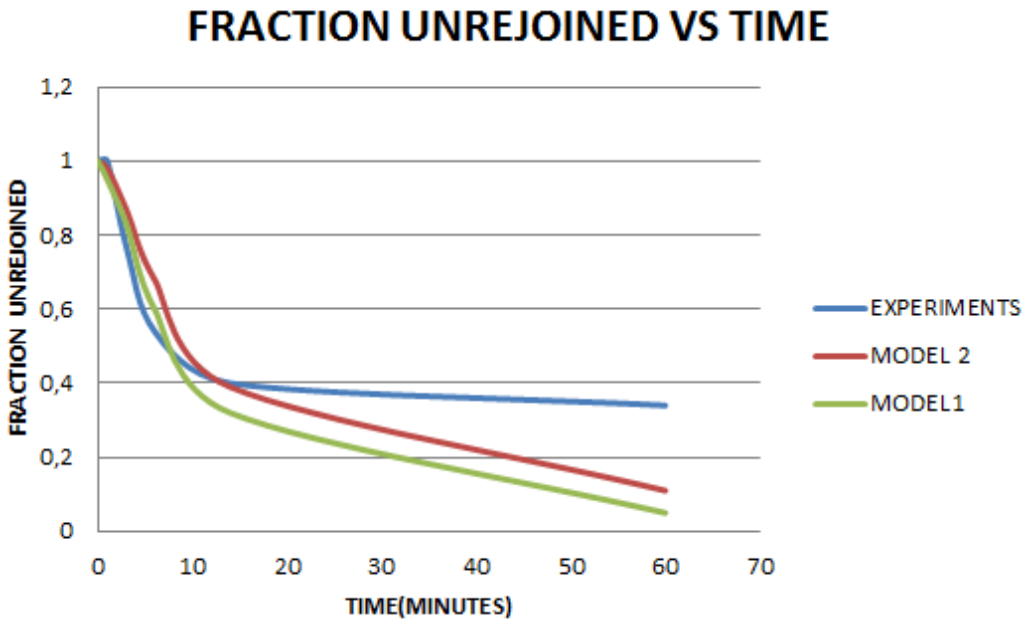

Figure 3.6: *Fraction Unrejoined in Non-homologous End Joining (NHEJ) pathway, and the Remaining Double Strand Breaks in each step of Repair process. The model1 describes our simulation of simple path of NHEJ the model2 the model of paper's results and experiments the experimental data of model2 [**4**]*

time(minutes)	Our Results	Experimental Results
1	1	1
3	0.95	1
6	0.83	0.77
14	0.6	0,54
25	0.32	0.4
60	0.05	0.34

Table 3.2: *The coeficient table has three columns, the first column indicates the time, second column indicates the paper's results and the third column indicates our outcomes coming out Runge-Kuta method.[**22**]*

For the Complex Path of Non-Homologous End Joining (NHEJ) we use the constants of the 1.3 [**4**] and the results for Dose=1Gy and a=25 is the below graph. The concentrations of specific biochemical processes are bigger in amount than in simple path. Such processes are first autophosphorylation, second autophosporylation. Nevertheless, the LigIV/XRCC4 decreased dramatically according to simple path. The breaks residuals has an increasement approximately to 21% , 16% deviation from simple's path residuals. Because the complex path is more slow the processes lasting more. Thus, in the duration of 1 hour the repaired percentage is 20% and the unrejoined 80% ,as we observe in the graph of Unrejoined fraction.

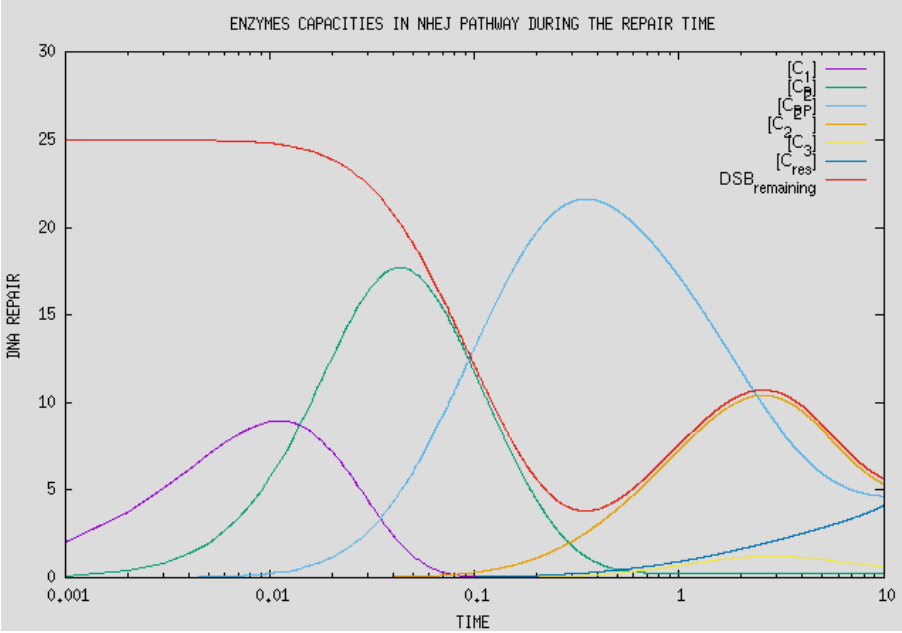

Figure 3.7: *The graph illustarates the quantities of 6 repair enzyme-proteins that are used in the complex path of simple NHEJ , and the Remaining Double Strand Breaks in each step of Repair, D=1Gy, a=25. Denoted as C1 as DNA-PKcs, C2 as DNA-PK, C2p as ABCDE cites, C2pp as PQR cites, C3 as LigaseIV.*

time(minutes)	Our Results	Experimental Results
1	25	23
3	49	45
6	70	75
10	100	102

Table 3.3: *The coeficient table has three columns, the first column indicates the time, second column indicates the paper's results and the third column indicates our outcomes coming out Runge-Kuta method.*

3.2 Results of Second Model

The 9 non-differential equations were solved numerically with the rate constants that reffered in Unit 1.4. The results of NHEJ pathway in Heterochromatin is given below: We use Ei=3000 D=1 and a=25 and we compare them with the results of [**20**]. Actually, the two models have different initial damages because we do not know the exact values of a and D nevertheless we can scale and fit the results so we can compare the general form of mechanism in each step of repair.

As we can observe from the second model, if we want having less break residues, we would have regulate the below quantity approximately equal to unit.

$$a * \frac{Dose}{Hi} \approx 1$$

For this reason, (Fiqure 3.12) having $6,6\%$ of breaks residues in contary of (Figure 2.13) having $4,8\%$ of break residues. The deviation is above $1,8\%$

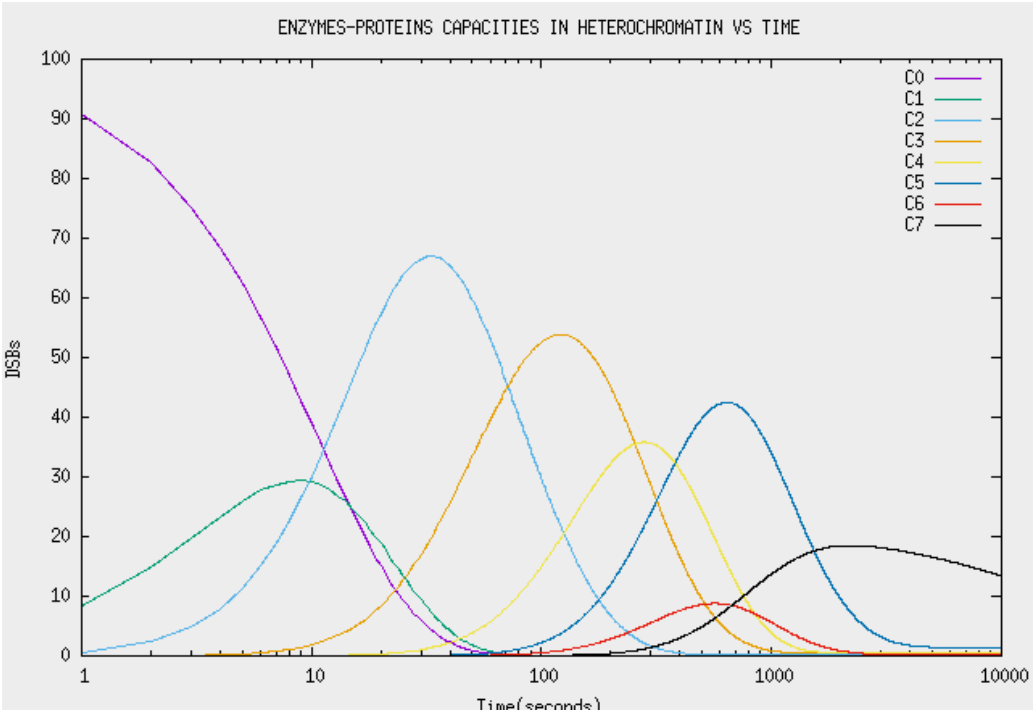

Figure 3.8: *The graph illustarates the quantities of 6 repair enzyme-proteins D=1Gy and a=25 that are used in NHEJ pathway, and the Remaining Double Strand Breaks in each step of Repair process. Denoted Ku70/Ku80 as C0, DNA-PKcs as C1, DNA-PK as C2, ABCDE cites as C3, PQ cites as C4, Artemis as C5, Polymerase as C6, XLF/XRCC4/Ligase as C7*

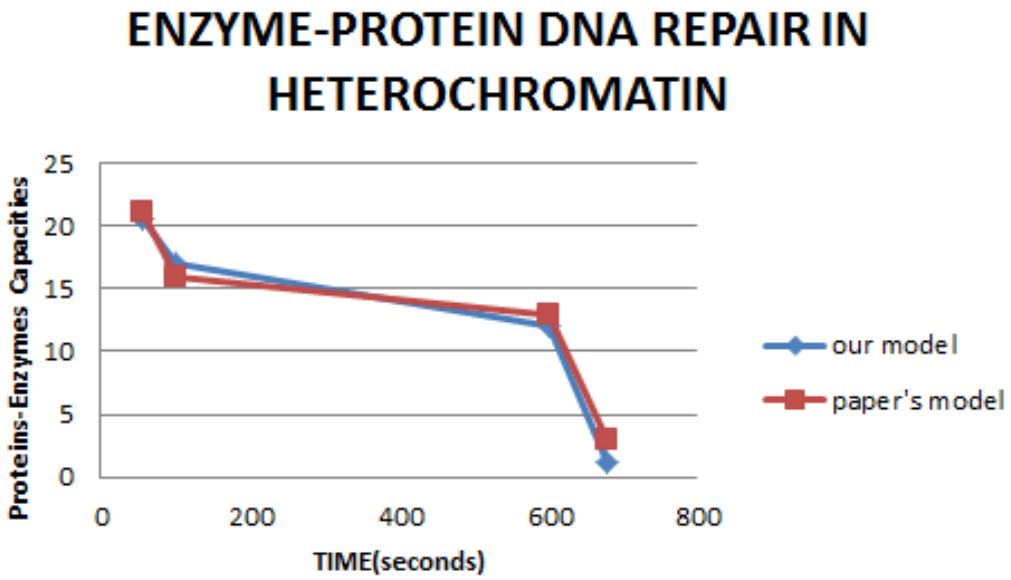

Figure 3.9: *Compared results of repaired proteins-enzymes in NHEJ in Heterochromatin pathway.The fiqure adopted from"The Non-homologous End-Joining(NHEJ) Mathematical Model for the Repair of Double-Strand Breaks:II.Application to Damage Induced by Ultrasoft X Rays and Low- Energy Electrons-Reza Taleei, Hooshang Nikjoo, Krishnaswami Sankaranarayanan-Radiation Research 179,000-000,(2013)."***[20]**

time(seconds)	Our Model	Paper's Model
55	20.5	21
100	17	16
600	12	13
680	1.25	3

Table 3.4: *The coeficient table has three columns, the first column indicates the time, second column indicates the paper's results and the third column indicates our outcomes coming out Runge-Kuta method.***[22]**

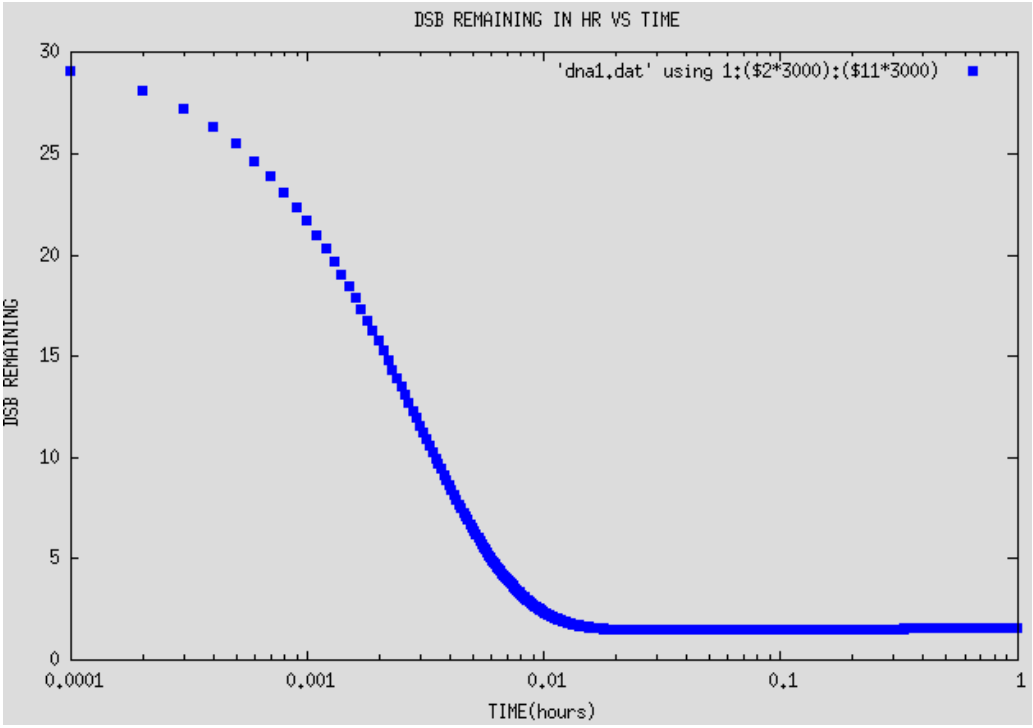

Figure 3.10: *The graph indicates the DSB remaing of NHEJ in Heterochromatin pathway. Dose=80 Gy, a=25, Hi=3000. The data added were table2.3 and the model executed until 1 hour because of stiffness*

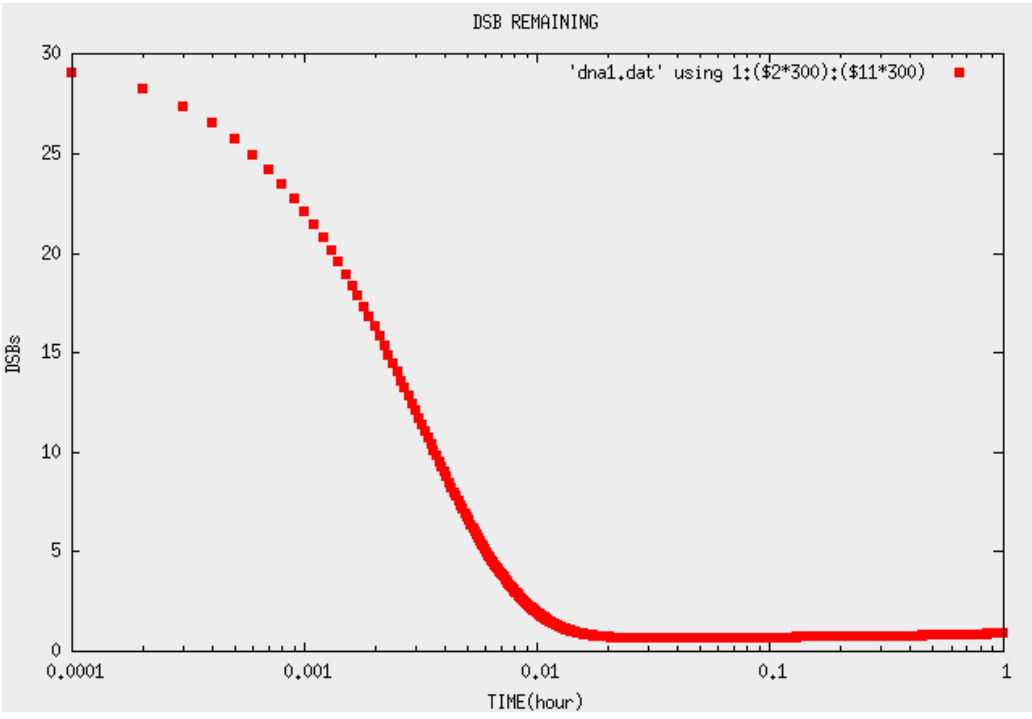

Figure 3.11: *The graph indicates the DSB remaing of NHEJ in Heterochromatin pathway. Dose=80 Gy, a=35, Hi=3000.The data added were table1.3 and the model executed until 1 hour because of stiffness*

The unrejoined fraction observed, fells very quickly. From the first 36 seconds the dam-

ages repaired until the 82% of the initial damages, because the fraction unrejoined is 0,18. Finaly, at t=60 minutes, it balanced to the 0,13 fraction unrejoined and the 87% repair of initial damages. Subsequently,the final steps of repair mechanisms are very slow since they repair only 5% in 59 minutes.

time(Seconds)	Our Results
0	1
36	0.18
180	0.14

Table 3.5: *The coeficient table has three columns, the first column indicates the time(minutes),and the second column indicates the fraction unrejoined*

Because the previous model tackle some serious stiff problems we fix our rate constants more appropriate. Firstly our model will be executed for ten hours and its repair will be more slow as it is in the reality. For this simulation we use the coefficients $k_1 = 250$, $k_2 = 100$, $k_3 = 50$, $k_4 = 20$, $k_5 = 15$, $k_6 = 5$, $k_7 = 3.6$, $k_8 = 8$, $k_9 = 1.5$, $k_{10} = 1.02$

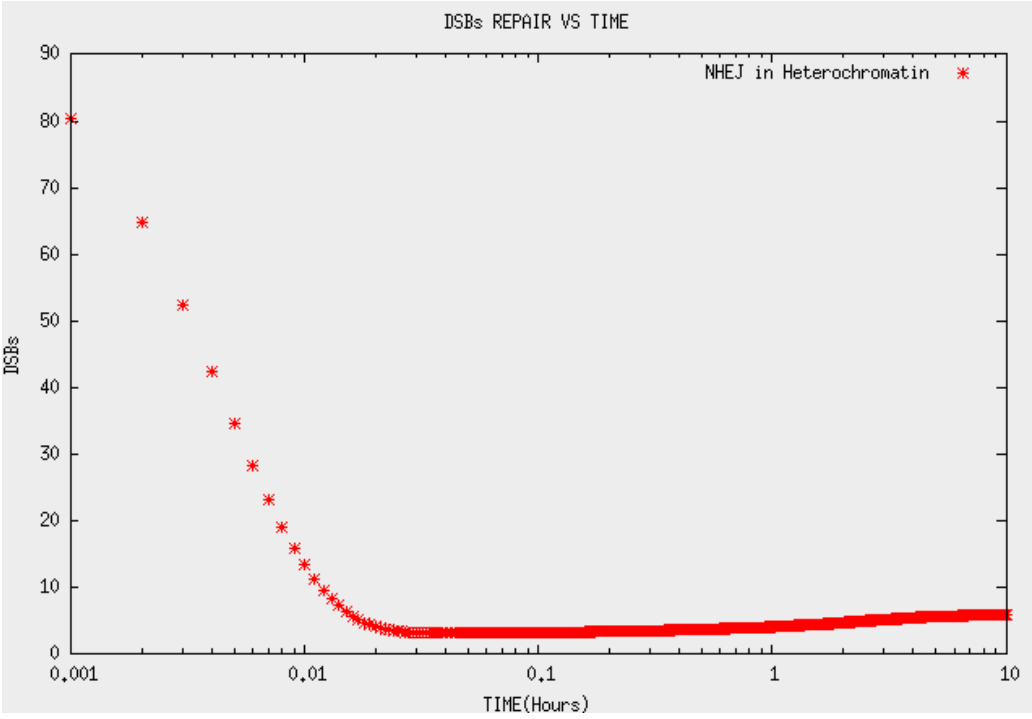

Figure 3.12: *DSB unrejoined in NHEJ repair process in Heterochromatin pathway of DSBs, D=80Gy, a=25.The coefficients utilized were* $k_1 = 250$, $k_2 = 100$, $k_3 = 50$, $k_4 = 20$, $k_5 = 15$, $k_6 = 5$, $k_7 = 3.6$, $k_8 = 8$, $k_9 = 1.5$, $k_{10} = 1.02$

3.3 Results of Third Model

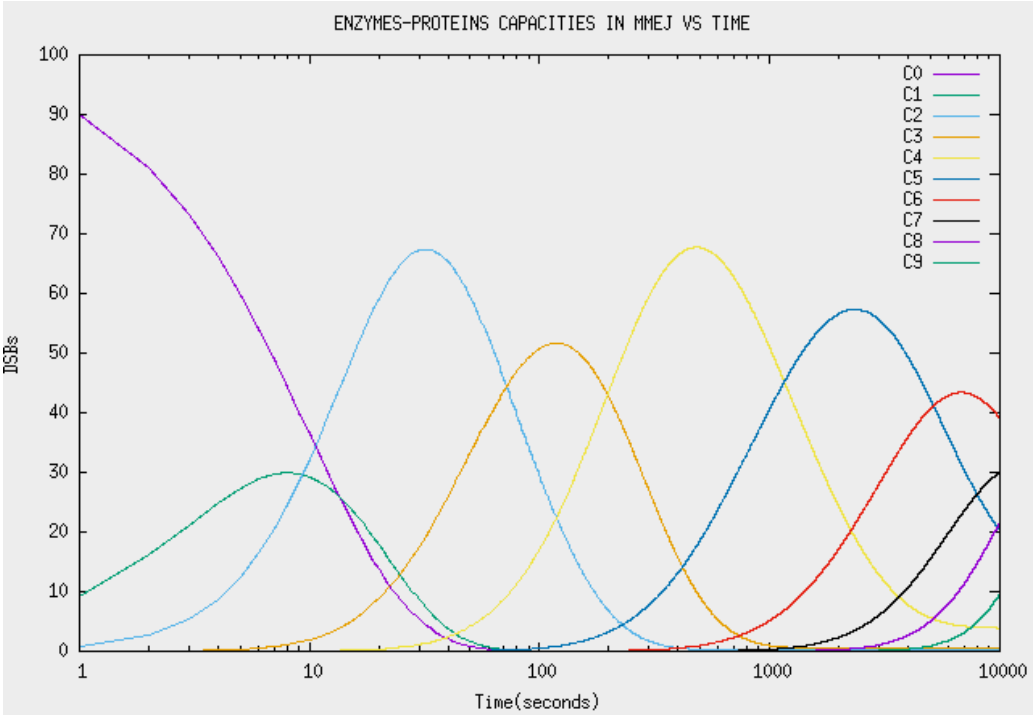

Figure 3.13: *The graph illustarates the quantities of 6 repair enzyme-proteins with D=1 Gy, a=25, that are used in NHEJ pathway, and the Remaining Double Strand Breaks in each step of Repair process. Denoted Ku70/Ku80 as C0, DNA-PKcs as C1, DNA-PK as C2, ABCDE cites as C3, PQ cites as C4, MRN as C5, PARP-1 as C6, FEN1 as C7, Polymerase as C8, XRCC1/LigaseIII as C9*

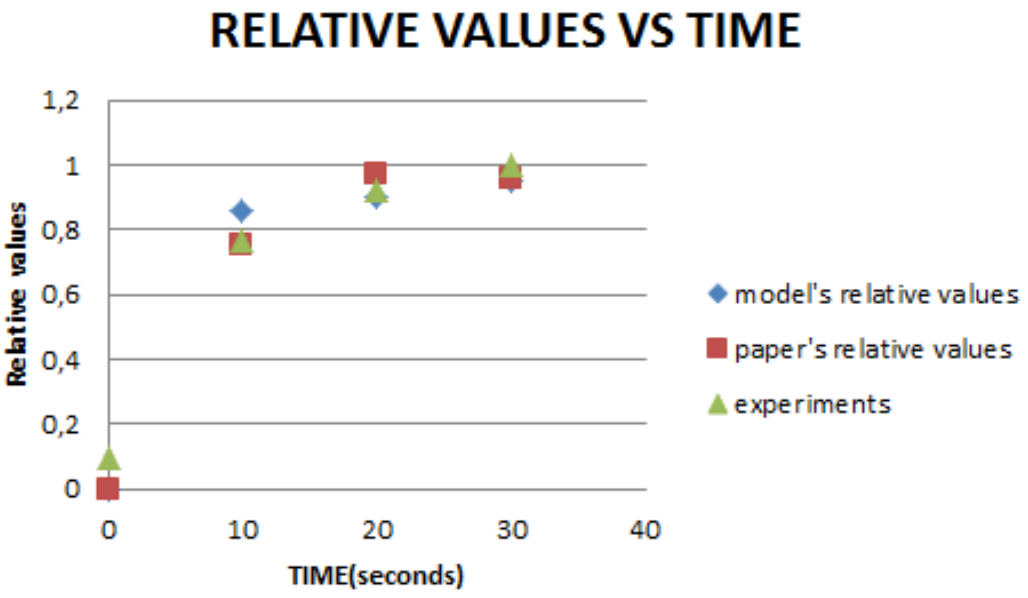

Figure 3.14: *Fitting graph between relative values of MMEJ model and experimental data of the paper [**14**](Figure5). The experimental data was referred to the initial DNA-PKcs recruitment in Xrs6 cells*

time(Seconds)	Our Model	Paper's Model	Experimental Data
0	0	0	0.1
10	0.86	0.75	0.77
20	0.90	0.97	0.92
30	0.95	0.96	1.0

Table 3.6: *The coeficient table has four columns, the first column indicates the time(seconds),the second column indicates our model's results for Ku70/80 and DNA-PKcs recruitment, coming out Runge-Kuta method [**22**], the third column indicates the paper's computational results[**14**] and the forth column indicates the experimental results (Fig.5)[**14**]*

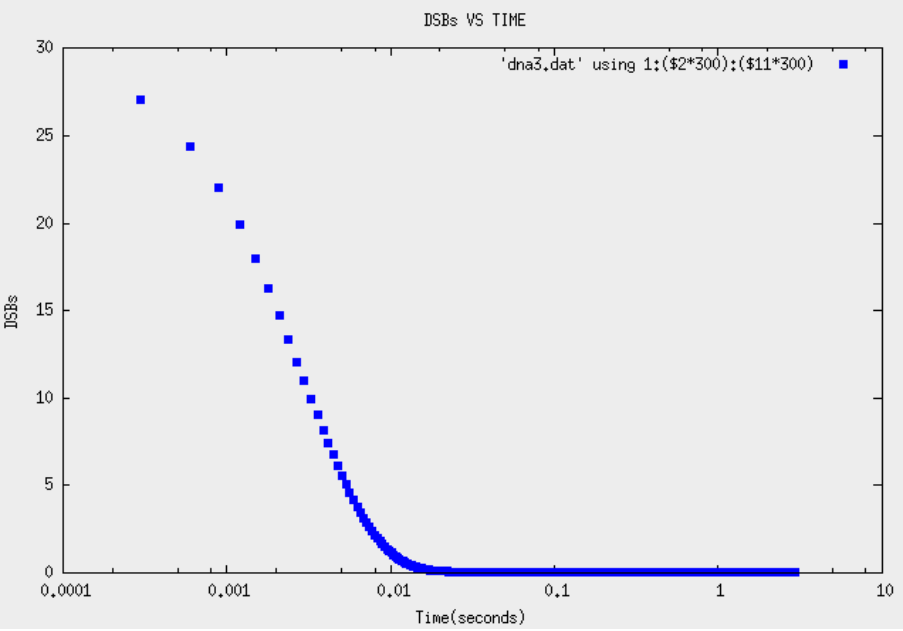

Figure 3.15: *DSB induced by D=1Gy, a=45 in MMEJ pathway, the time is calculated in hours. The 11 non-linear differential system is executed without stiffness until two hours then we tackle stiff problems.The coeffiecients used were from table1.4. As we observe the repair of the system happens rapidly until time, t=0.01 h, in other words in the first 36 second, then the repair stays stable to zero.*

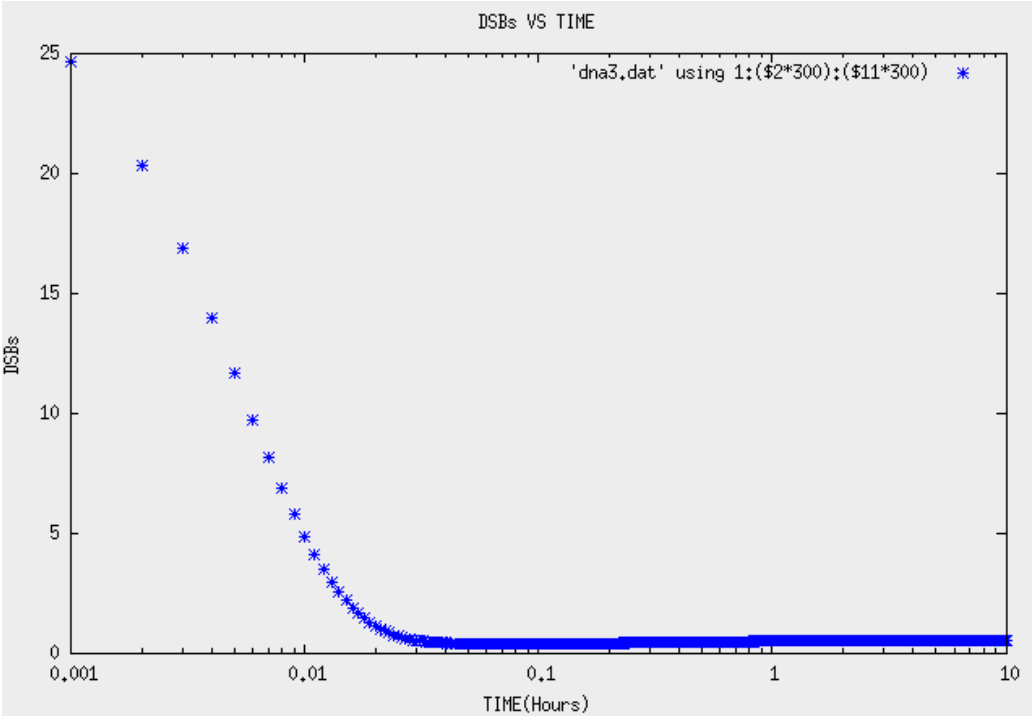

Figure 3.16: *In our effort to make the model more executable for ten hours, the constants changed and the DSB induced by D=30Gy, a=25 in MMEJ pathway, the time is calculated in hours. The 11 non-linear differential system is executed without stiffness until ten hours, and has 2% residues. For this simulation we use the coefficients* $k_1 = 200$, $k_2 = 100$, $k_3 = 50$, $k_4 = 20$, $k_{12} = 12.5$, $k_{13} = 10$, $k_{14} = 7.6$, $k_{15} = 5.00$, $k_{16} = 2.25$, $k_{17} = 1.55$

3.4 Conclusions

As the dissertation coming to an end our final step is to compare the three pathways of NHEJ with our own coefficients to see if the simulation is quite good to the biology logic. We will compare the simple NHEJ pathway towards to the NHEJ in Heterochromatin and then the simple NHEJ toward to the complex NHEJ(alternative NHEJ). The results of the below figures are quite good since the models have resonably behaviour to the repair and the remaining residues. The NHEJ in heterochromatin and complex damages are more difficult to be repaired than NHEJ pathway in simple damages. Also a general observation, the residues do not ovecome the 5% of the total initial damages. Finally, it is is very important and much more close to thegeneral theory to see the simulation until ten hours, since the DSB repair it is a lasting process.

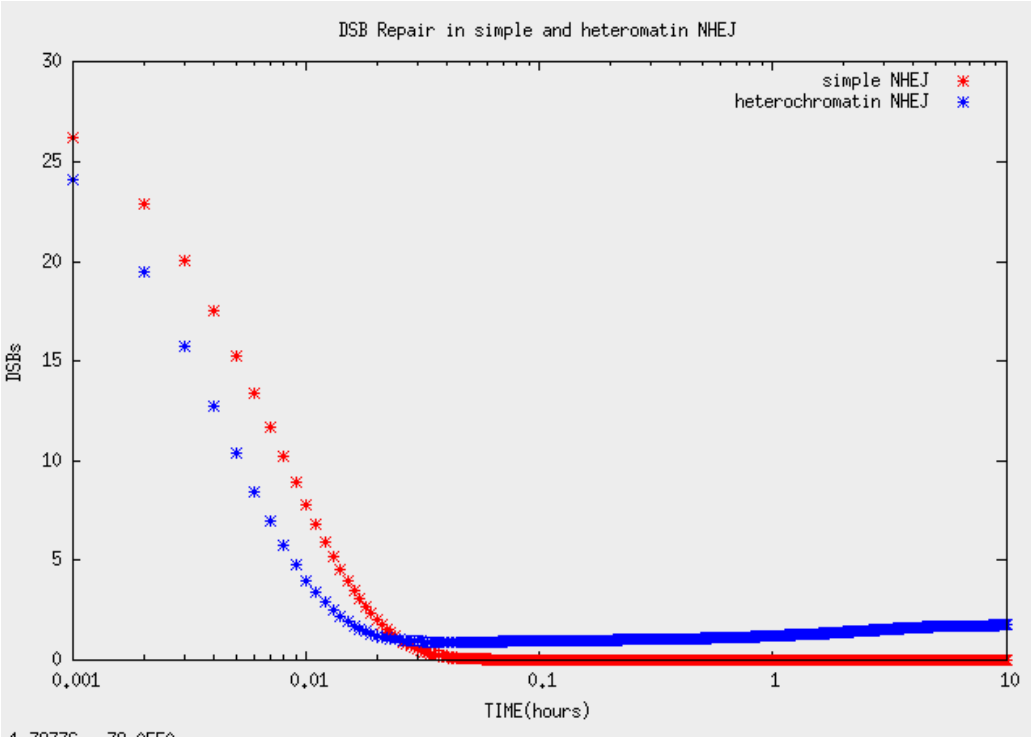

Figure 3.17: *The graph illustarates the comparison of NHEJ pathways. The simple towards to heterochromatin. Both of the models had Dose= 80, a=25 , E=3000. For the simulation in simple NHEJ we utilize the coefficients [4] of the original model .For the simulation of NHEJ in heterochromatin we utize the coefficients* $k_1 = 250$, $k_2 = 100$, $k_3 = 50$, $k_4 = 20$, $k_5 = 15$, $k_6 = 5$, $k_7 = 3.6$, $k_8 = 8$, $k_9 = 1.5$, $k_{10} = 1.02$

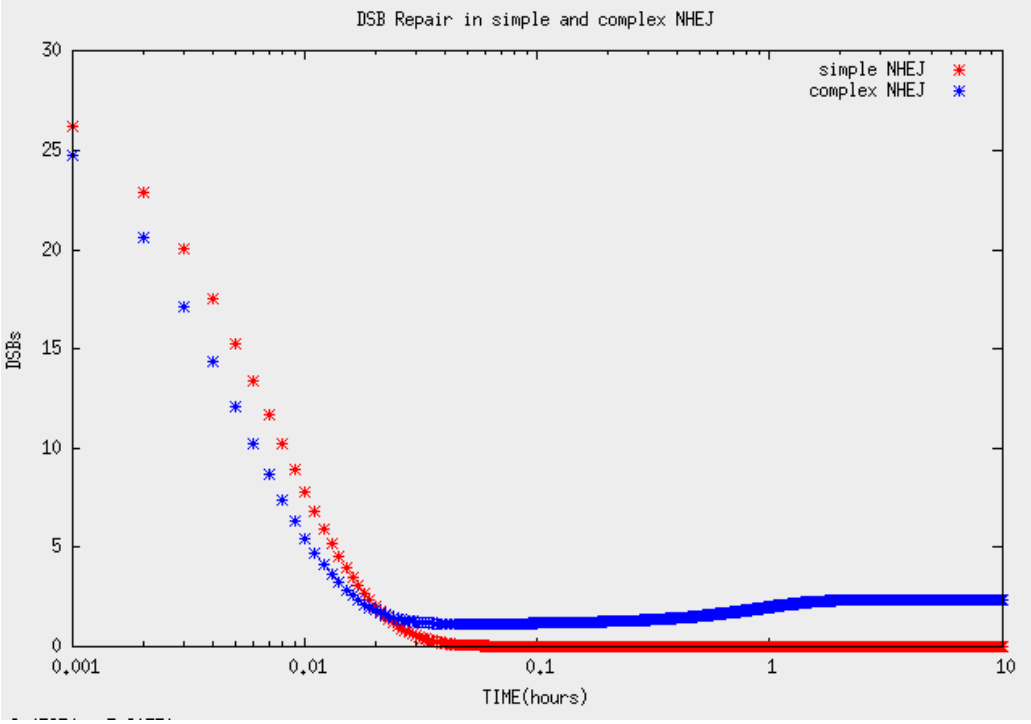

Figure 3.18: *The graph illustarates the comparison of NHEJ pathways. The simple towards to complex(alternative). Both of the models had Dose= 80, a=25 , E=3000. . For the simulation in simple NHEJ we utilize the coefficients [**4**] of the original model .For the simulation of NHEJ in heterochromatin we utize the coefficients* $k_1 = 200$, $k_2 = 100$, $k_3 = 50$, $k_4 = 20$, $k_{12} = 12.5$, $k_{13} = 10$, $k_{14} = 7.6$, $k_{15} = 5.00$, $k_{16} = 2.25$, $k_{17} = 1.55$

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